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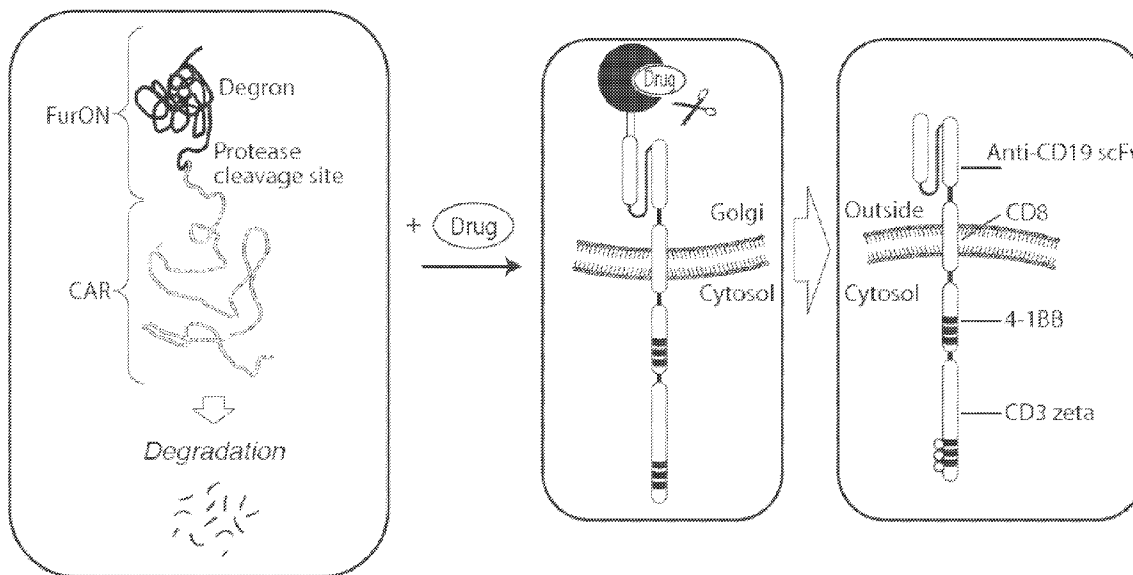
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(71) Demandeur/Applicant:  
NOVARTIS AG, CH  
(72) Inventeurs/Inventors:  
BRADNER, JAMES E., US;  
GOLOSOV, ANDREI, US;  
GOOLD, CARLETON PROCTOR, US;  
GUIMARAES, CARLE PATRICIA PINTO, US;  
HILD, MARC HORST PETER, US;  
MOTZ, GREGORY, US;

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(54) Title: COMPOSITIONS AND METHODS FOR SELECTIVE PROTEIN DEGRADATION

FIG. 11



(57) **Abrégé/Abstract:**

The invention provides compositions including a fusion polypeptide and methods for making a fusion polypeptide that includes a COF1/CRBN-binding polypeptide, COF2/CRBN-binding polypeptide, or COF3/CRBN-binding polypeptide and a heterologous polypeptide of interest.

(72) **Inventeurs(suite)/Inventors(continued)**: ROSS, NATHAN THOMAS, US; SOLOMON, JONATHAN M., US;  
BECKWITH, ROHAN ERIC JOHN, US; CARBONNEAU, SETH, US

(74) **Agent**: SMART & BIGGAR IP AGENCY CO.

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(71) Applicant: **NOVARTIS AG** [CH/CH]; Lichtstrasse 35, 4056 Basel (CH).

## (72) Inventor; and

(71) Applicant (for US only): **BRADNER, James, E.** [US/US]; Novartis Institutes for Biomedical Research, Inc., 250 Massachusetts Avenue, Cambridge, MA 02139 (US).(72) Inventors: **GOLOSOV, Andrei**; Novartis Institutes for BioMedical Research, Inc., 250 Massachusetts Avenue, Cambridge, MA 02139 (US). **GOOLD, Carleton, Proctor**; Novartis Institutes for BioMedical Research, Inc., 250 Massachusetts Avenue, Cambridge, MA 02139 (US).

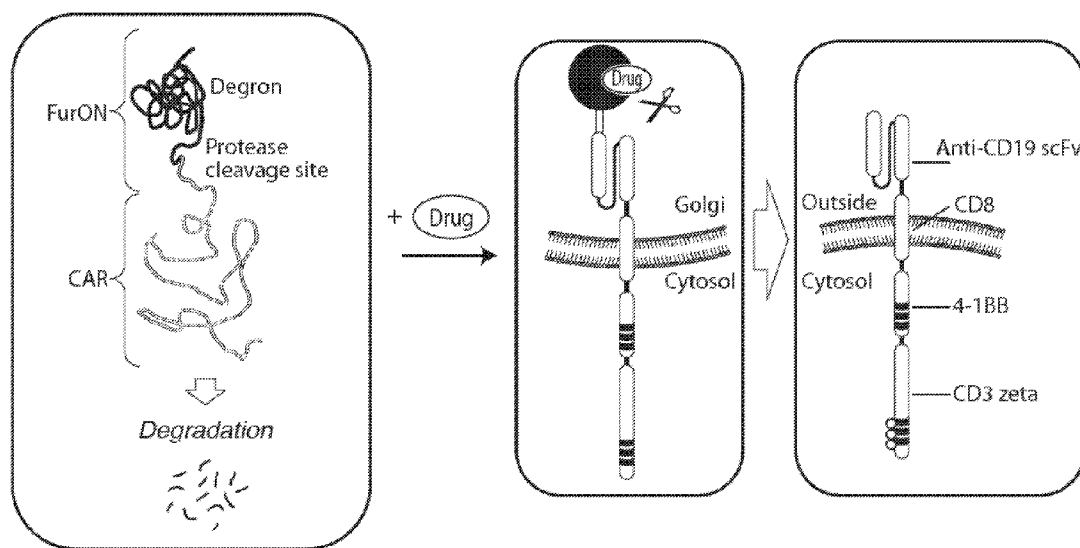
**GUIMARAES, Carle, Patricia Pinto**; Novartis Institutes for BioMedical Research, Inc., 250 Massachusetts Avenue, Cambridge, MA 02139 (US). **HILD, Marc Horst Peter**; Novartis Institutes for BioMedical Research, Inc., 250 Massachusetts Avenue, Cambridge, MA 02139 (US). **MOTZ, Gregory**; 83 Greene St., Quincy, MA 02170 (US). **ROSS, Nathan Thomas**; Novartis Institutes for BioMedical Research, Inc., 250 Massachusetts Avenue, Cambridge, MA 02139 (US). **SOLOMON, Jonathan, M.**; 47 Winslow Avenue, Sommerville, MA 02144 (US). **BECKWITH, Rohan, Eric John**; Novartis Institutes for BioMedical Research, Inc., 250 Massachusetts Avenue, Cambridge, MA 02139 (US).

(74) Agent: **COLLAZO, Diana M.** et al.; Lando & Anastasi, LLP, Riverfront Office Park, One Main Street, Suite 1100, Cambridge, MA 02142 (US).

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(54) Title: COMPOSITIONS AND METHODS FOR SELECTIVE PROTEIN DEGRADATION

FIG. 11



(57) Abstract: The invention provides compositions including a fusion polypeptide and methods for making a fusion polypeptide that includes a COF1/CRBN-binding polypeptide, COF2/CRBN-binding polypeptide, or COF3/CRBN-binding polypeptide and a heterologous polypeptide of interest.

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WO 2019/079569 A1

**WO 2019/079569 A1** 

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## DEMANDE OU BREVET VOLUMINEUX

LA PRÉSENTE PARTIE DE CETTE DEMANDE OU CE BREVET COMPREND PLUS D'UN TOME.

CECI EST LE TOME        1    DE    2  
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## JUMBO APPLICATIONS/PATENTS

THIS SECTION OF THE APPLICATION/PATENT CONTAINS MORE THAN ONE VOLUME

THIS IS VOLUME        1    OF    2  
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## COMPOSITIONS AND METHODS FOR SELECTIVE PROTEIN DEGRADATION

### RELATED APPLICATION

This application claims priority to U.S. Serial No. 62/574,188 filed October 18, 2017, the  
 5 contents of which are incorporated herein by reference in their entireties.

### SEQUENCE LISTING

The instant application contains a Sequence Listing which has been submitted electronically in  
 ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on  
 10 October 17, 2018, is named N2067-7136WO\_SL.txt and is 2,052,554 bytes in size.

### BACKGROUND OF THE INVENTION

Many therapeutic proteins have been developed as important medications for preventing or  
 treating diseases. Side effects can occur during or after the treatment, varying from a loss of drug efficacy  
 15 to serious toxicities. It is desirable to develop strategies to modulate the expression level of the  
 therapeutic proteins, e.g., to modulate the levels of the therapeutic proteins to increase efficacy and/or  
 decrease side effects.

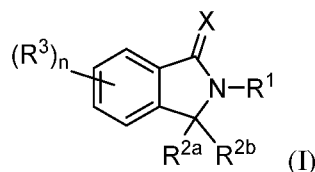
### SUMMARY OF THE INVENTION

20 The present disclosure provides, at least in part, a fusion polypeptide comprising a compound of  
 Formula (I) (COF1)/CRBN-binding polypeptide, a compound of Formula (II) (COF2)/CRBN-binding  
 polypeptide, or a compound of Formula (III) (COF3)/CRBN-binding polypeptide for targeted protein  
 inactivation. In some embodiments, the fusion polypeptide includes one or more COF1/CRBN-,  
 COF2/CRBN-, or COF3/CRBN-binding polypeptides and one or more heterologous polypeptides, e.g.,  
 25 polypeptides of interest. The COF1/CRBN-, COF2/CRBN-, or COF3/CRBN-binding polypeptide can be  
 operably linked to the heterologous polypeptide, e.g., via a linker. In some embodiments, in the presence  
 of COF1 or COF2 (such as thalidomide and derivatives thereof (e.g., lenalidomide, pomalidomide, and  
 thalidomide)), or in the presence of COF3 (e.g., a compound disclosed in Table 29) the COF1/CRBN-,  
 COF2/CRBN-, or COF3/CRBN-binding polypeptide alters the level and/or activity of the fusion  
 30 polypeptide; for example, increases degradation, e.g., proteosomal degradation, of the fusion polypeptide.  
 In some embodiments, the degradation of the fusion polypeptide is ubiquitin-dependent.

Without wishing to be bound by theory, in some embodiments, the COF1/CRBN-, COF2/CRBN-  
 , or COF3/CRBN-binding polypeptide provides an amino acid sequence and/or a structural motif that, in

the presence of COF1 or COF2 (such as thalidomide and derivatives thereof (e.g., lenalidomide, pomalidomide, and thalidomide)), or in the presence of COF3 (e.g., a compound disclosed in Table 29), results in a post-translational modification (e.g., ubiquitination) of the fusion polypeptide, resulting in a modified, e.g., ubiquitinated, fusion polypeptide. For example, one or more amino acids, e.g., lysine or methionine, in the fusion polypeptide can be ubiquitinated, in the presence of COF1, COF2, or COF3. In some embodiments, the ubiquitinated fusion polypeptide is selectively degraded. In some embodiments, the post-translational modification of the fusion polypeptide increases the degradation (e.g., an increased level and/or rate of degradation) of the fusion polypeptide. In some embodiments, the level and/or rate of degradation is increased by at least 1.5-, 2-, 3-, 4-, 5-, 10-, 20-, 30-, 40-, or 50-fold relative to the level and/or rate of degradation of a reference polypeptide, e.g., the fusion polypeptide in the absence of COF1, COF2, or COF3, the heterologous polypeptide, or a fusion of the heterologous polypeptide without the COF1/CRBN-, COF2/CRBN-, or COF3/CRBN-binding polypeptide, or with a moiety other than the COF1/CRBN-, COF2/CRBN-, or COF3/CRBN-binding polypeptide.

In one aspect, provided herein is a fusion polypeptide comprising a compound of Formula (I) (COF1)/CRBN-binding polypeptide and a heterologous polypeptide, wherein the compound of Formula (I) is:



or a pharmaceutically acceptable salt, ester, hydrate, solvate, or tautomer thereof, wherein:

X is O or S;

R<sup>1</sup> is C<sub>1</sub>-C<sub>6</sub> alkyl, C<sub>2</sub>-C<sub>6</sub> alkenyl, C<sub>2</sub>-C<sub>6</sub> alkynyl, C<sub>1</sub>-C<sub>6</sub> heteroalkyl, carbocyclyl, heterocyclyl, aryl, or heteroaryl, each of which is independently and optionally substituted by one or more R<sup>4</sup>;

each of R<sup>2a</sup> and R<sup>2b</sup> is independently hydrogen or C<sub>1</sub>-C<sub>6</sub> alkyl; or R<sup>2a</sup> and R<sup>2b</sup> together with the carbon atom to which they are attached form a carbonyl group or a thiocarbonyl group;

each of R<sup>3</sup> is independently C<sub>1</sub>-C<sub>6</sub> alkyl, C<sub>2</sub>-C<sub>6</sub> alkenyl, C<sub>2</sub>-C<sub>6</sub> alkynyl, C<sub>1</sub>-C<sub>6</sub> heteroalkyl, halo, cyano, -C(O)R<sup>A</sup>, -C(O)OR<sup>B</sup>, -OR<sup>B</sup>, -N(R<sup>C</sup>)(R<sup>D</sup>), -C(O)N(R<sup>C</sup>)(R<sup>D</sup>), -N(R<sup>C</sup>)C(O)R<sup>A</sup>, -S(O)<sub>x</sub>R<sup>E</sup>, -S(O)<sub>x</sub>N(R<sup>C</sup>)(R<sup>D</sup>), or -N(R<sup>C</sup>)S(O)<sub>x</sub>R<sup>E</sup>, wherein each alkyl, alkenyl, alkynyl, and heteroalkyl is independently and optionally substituted with one or more R<sup>6</sup>;

each R<sup>4</sup> is independently C<sub>1</sub>-C<sub>6</sub> alkyl, C<sub>2</sub>-C<sub>6</sub> alkenyl, C<sub>2</sub>-C<sub>6</sub> alkynyl, C<sub>1</sub>-C<sub>6</sub> heteroalkyl, halo, cyano, oxo, -C(O)R<sup>A</sup>, -C(O)OR<sup>B</sup>, -OR<sup>B</sup>, -N(R<sup>C</sup>)(R<sup>D</sup>), -C(O)N(R<sup>C</sup>)(R<sup>D</sup>), -N(R<sup>C</sup>)C(O)R<sup>A</sup>, -S(O)<sub>x</sub>R<sup>E</sup>, -S(O)<sub>x</sub>N(R<sup>C</sup>)(R<sup>D</sup>), -N(R<sup>C</sup>)S(O)<sub>x</sub>R<sup>E</sup>, carbocyclyl, heterocyclyl, aryl, or heteroaryl, wherein each alkyl,

alkenyl, alkynyl, heteroalkyl, carbocyclyl, heterocyclyl, aryl, and heteroaryl is independently and optionally substituted with one or more  $R^7$ ;

each of  $R^A$ ,  $R^B$ ,  $R^C$ ,  $R^D$ , and  $R^E$  is independently hydrogen or  $C_1$ - $C_6$  alkyl;

each  $R^6$  is independently  $C_1$ - $C_6$  alkyl, oxo, cyano,  $-OR^B$ ,  $-N(R^C)(R^D)$ ,  $-C(O)N(R^C)(R^D)$ , -

5  $N(R^C)C(O)R^A$ , aryl, or heteroaryl, wherein each aryl and heteroaryl is independently and optionally substituted with one or more  $R^8$ ;

each  $R^7$  is independently halo, oxo, cyano,  $-OR^B$ ,  $-N(R^C)(R^D)$ ,  $-C(O)N(R^C)(R^D)$ , or -  
 $N(R^C)C(O)R^A$ ;

each  $R^8$  is independently  $C_1$ - $C_6$  alkyl, cyano,  $-OR^B$ ,  $-N(R^C)(R^D)$ ,  $-C(O)N(R^C)(R^D)$ , or -  
 10  $N(R^C)C(O)R^A$ ;

n is 0, 1, 2, 3 or 4; and

x is 0, 1, or 2.

In some embodiments, the heterologous polypeptide is a heterologous mammalian polypeptide.

In some embodiments, the heterologous polypeptide is a heterologous bacterial polypeptide. In some  
 15 embodiments, the heterologous polypeptide is a heterologous viral polypeptide. In some embodiments, the heterologous polypeptide comprises an amino acid sequence from, or derived from, a mammalian polypeptide, a bacterial polypeptide, a viral polypeptide, a plant polypeptide, a yeast polypeptide, a fungi polypeptide, an archaeobacterial polypeptide, a fish, e.g., Zebrafish, polypeptide. In some embodiments, the heterologous polypeptide comprises a polypeptide in Table 2, e.g., a cytoplasmic and/or nuclear  
 20 polypeptide, or a transmembrane polypeptide as described in Table 2.

In some embodiments, the COF1/CRBN-binding polypeptide is fused to the heterologous polypeptide. In some embodiments, the COF1/CRBN-binding polypeptide and the heterologous polypeptide are linked by a peptide bond. In some embodiments, the COF1/CRBN-binding polypeptide and the heterologous polypeptide are linked by a bond other than a peptide bond. In some embodiments,  
 25 the heterologous polypeptide is linked directly to the COF1/CRBN-binding polypeptide. In some embodiments, the heterologous polypeptide is linked indirectly to the COF1/CRBN-binding polypeptide. In some embodiments, the COF1/CRBN-binding polypeptide and the heterologous polypeptide are operatively linked via a linker, e.g., a glycine-serine linker, e.g., a linker comprising the amino acid sequence of SEQ ID NO: 28. In some embodiments, the linker comprises an amino acid sequence  
 30 selected from the group consisting of SEQ ID NOs: 28, 37, 38, 39, and 99.

In some embodiments, the COF1/CRBN-binding polypeptide is linked to the C-terminus of the heterologous polypeptide. In some embodiments, the COF1/CRBN-binding polypeptide is linked to the N-terminus of the heterologous polypeptide. In some embodiments, the COF1/CRBN-binding polypeptide is at the middle of the heterologous polypeptide.

In some embodiments, the association of the COF1/CRBN-binding polypeptide with cereblon (CRBN) in the absence of COF1 is no more than, e.g., 0.01%, 0.1%, 1%, 5%, 10%, 15%, or 20%, of the association of the COF1/CRBN-binding polypeptide with CRBN in the presence of COF1, e.g., an excess of COF1, e.g., as measured by an assay described herein, e.g., immunoprecipitation. In some  
5   embodiments, the COF1/CRBN-binding polypeptide does not bind to CRBN in the absence of COF1. In some embodiments, the association of the fusion polypeptide with CRBN in the absence of COF1 is no more than, e.g., 0.01%, 0.1%, 1%, 5%, 10%, 15%, or 20%, of the association of the fusion polypeptide with CRBN in the presence of COF1, e.g., an excess of COF1, e.g., as measured by an assay described herein, e.g., immunoprecipitation. In some embodiments, the fusion polypeptide does not bind to CRBN  
10   in the absence of COF1. In some embodiments, the association or binding is as measured in a mammalian cell, e.g., a human cell.

In some embodiments, the ubiquitination of the heterologous polypeptide in the absence of COF1 is no more than, e.g., 0.01%, 0.1%, 1%, 10%, 20%, 30%, 40%, 50%, 60%, or 70%, of the ubiquitination of the heterologous polypeptide in the presence of COF1, e.g., an excess of COF1, e.g., as measured by an  
15   assay described herein. In some embodiments, the ubiquitination of the fusion polypeptide in the absence of COF1 is no more than, e.g., 0.01%, 0.1%, 1%, 10%, 20%, 30%, 40%, 50%, 60%, or 70%, of the ubiquitination of the fusion polypeptide in the presence of COF1, e.g., an excess of COF1, e.g., as measured by an assay described herein. In some embodiments, the heterologous polypeptide or the fusion polypeptide is ubiquitinated at one or more lysine or methionine residues in the presence of COF1. In  
20   some embodiments, the ubiquitination is as measured in a mammalian cell, e.g., a human cell.

In some embodiments, the degradation of the fusion polypeptide in the absence of COF1 is no more than, e.g., 0.01%, 0.1%, 1%, 10%, 20%, 30%, 40%, 50%, 60%, or 70% of the degradation of the fusion polypeptide in the presence of COF1, e.g., an excess of COF1, e.g., as measured by an assay described herein, e.g., a Western blot analysis or a flow cytometry analysis. In some embodiments, the  
25   degradation of the fusion polypeptide is mediated by ubiquitination in the presence of COF1. In some embodiments, the degradation of the fusion polypeptide is mediated by the lysosome. In some embodiments, the degradation is as measured in a mammalian cell, e.g., a human cell.

In some embodiments, the fusion polypeptide is a cell surface polypeptide. In some embodiments, the rate of recycling of the fusion polypeptide from the cell surface to an intracellular  
30   compartment in the absence of COF1 is no more than, e.g., 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% of the recycling of the fusion polypeptide from the cell surface to an intracellular compartment in the presence of COF1, e.g., an excess of COF1, e.g., as measured by an assay described herein. In some embodiments, the recycling is as measured in a mammalian cell, e.g., a human cell.

In some embodiments, the COF1/CRBN-binding polypeptide is between 10 and 95 amino acid residues in length, between 15 and 90 amino acid residues in length, between 20 and 85 amino acid residues in length, between 25 and 80 amino acid residues in length, between 30 and 75 amino acid residues in length, between 35 and 70 amino acid residues in length, between 40 and 65 amino acid residues in length, between 45 and 65 amino acid residues in length, between 50 and 65 amino acid residues in length, or between 55 and 65 amino acid residues in length.

In some embodiments, the COF1/CRBN-binding polypeptide comprises a beta turn. In some embodiments, the COF1/CRBN-binding polypeptide comprises a beta turn of IKZF1 or IKZF3 (e.g., human IKZF1 or IKZF3). In some embodiments, the COF1/CRBN-binding polypeptide comprises a beta hairpin. In some embodiments, the COF1/CRBN-binding polypeptide comprises a beta hairpin of IKZF1 or IKZF3 (e.g., human IKZF1 or IKZF3). In some embodiments, the COF1/CRBN-binding polypeptide comprises a beta strand. In some embodiments, the COF1/CRBN-binding polypeptide comprises a beta strand of IKZF1 or IKZF3 (e.g., human IKZF1 or IKZF3). In some embodiments, the COF1/CRBN-binding polypeptide comprises an alpha helix. In some embodiments, the COF1/CRBN-binding polypeptide comprises an alpha helix of IKZF1 or IKZF3 (e.g., human IKZF1 or IKZF3). In some embodiments, the COF1/CRBN-binding polypeptide comprises, from N-terminus to C-terminus, a first beta strand, a beta hairpin, a second beta strand, and a first alpha helix. In some embodiments, the COF1/CRBN-binding polypeptide comprises, from N-terminus to C-terminus, a first beta strand, a beta hairpin, a second beta strand, and a first alpha helix of IKZF1 or IKZF3 (e.g., human IKZF1 or IKZF3). In some embodiments, the COF1/CRBN-binding polypeptide comprises, from N-terminus to C-terminus, a first beta strand, a beta hairpin, a second beta strand, a first alpha helix, and a second alpha helix. In some embodiments, the COF1/CRBN-binding polypeptide comprises, from N-terminus to C-terminus, a first beta strand, a beta hairpin, a second beta strand, a first alpha helix, and a second alpha helix of IKZF1 or IKZF3 (e.g., human IKZF1 or IKZF3). In some embodiments, the beta hairpin and the second alpha helix are separated by no more than 60, 50, 40, or 30 amino acid residues.

In some embodiments, the COF1/CRBN-binding polypeptide comprises a COF1/CRBN-binding sequence from a naturally occurring polypeptide or a COF1/CRBN-binding variant thereof. In some embodiments, the COF1/CRBN-binding polypeptide comprises a COF1/CRBN-binding sequence from a naturally occurring IKZF polypeptide or a COF1/CRBN-binding variant thereof. In some embodiments, the COF1/CRBN-binding polypeptide comprises a COF1/CRBN-binding sequence from a naturally occurring IKZF1, IKZF2, IKZF3, IKZF4, or IKZF5, or a COF1/CRBN-binding variant thereof. In some embodiments, the COF1/CRBN-binding sequence comprises two or more discontinuous sequences from the naturally occurring polypeptide, e.g., a naturally occurring IKZF polypeptide, e.g., a naturally occurring IKZF1, IKZF2, IKZF3, IKZF4, or IKZF5.

In some embodiments, the COF1/CRBN-binding polypeptide comprises an IKZF polypeptide or a structural motif thereof.

In some embodiments, the IKZF polypeptide is an IKZF1 polypeptide, an IKZF3 polypeptide, an IKZF2 polypeptide having H141Q substitution (numbered according to SEQ ID NO: 21), or an IKZF4  
5 polypeptide having H188Q substitution (numbered according to SEQ ID NO: 22).

In some embodiments, the COF1/CRBN-binding polypeptide comprises sufficient amino acid sequence and/or a structural motif from IKZF (e.g., IKZF1 or IKZF3) that:

i) the association of the COF1/CRBN-binding polypeptide with CRBN in the absence of COF1 is no more than, e.g., 0.01%, 0.1%, 1%, 5%, 10%, 15%, or 20%, of the association of the COF1/CRBN-binding  
10 polypeptide with CRBN in the presence of COF1, e.g., an excess of COF1, e.g., as measured by an assay described herein, e.g., immunoprecipitation;

ii) the association of the fusion polypeptide with CRBN in the absence of COF1 is no more than, e.g., 0.01%, 0.1%, 1%, 5%, 10%, 15%, or 20% of the association of the fusion polypeptide with CRBN in the  
15 presence of COF1, e.g., an excess of COF1, e.g., as measured by an assay described herein, e.g., immunoprecipitation;

iii) the ubiquitination of the heterologous polypeptide in the absence of COF1 is no more than, e.g., 0.01%, 0.1%, 1%, 10%, 20%, 30%, 40%, 50%, 60%, or 70%, of the ubiquitination of the heterologous  
polypeptide in the presence of COF1, e.g., an excess of COF1, e.g., as measured by an assay described herein;

iv) the ubiquitination of the fusion polypeptide in the absence of COF1 is no more than, e.g., 0.01%, 0.1%, 1%, 10%, 20%, 30%, 40%, 50%, 60%, or 70%, of the ubiquitination of the fusion polypeptide in  
20 the presence of COF1, e.g., an excess of COF1, e.g., as measured by an assay described herein; or

v) the degradation of the fusion polypeptide in the absence of COF1 is no more than, e.g., 0.01%, 0.1%, 1%, 10%, 20%, 30%, 40%, 50%, 60%, or 70%, of the degradation of the fusion polypeptide in the  
25 presence of COF1, e.g., an excess of COF1, e.g., as measured by an assay described herein, e.g., a Western blot analysis or a flow cytometry analysis.

In some embodiments, the association, ubiquitination, and/or degradation is as measured in a mammalian cell, e.g., a human cell.

In some embodiments, the COF1/CRBN-binding polypeptide comprises about 10 to about 95  
30 amino acid residues, about 15 to about 90 amino acid residues, about 20 to about 85 amino acid residues, about 25 to about 80 amino acid residues, about 30 to about 75 amino acid residues, about 35 to about 70 amino acid residues, about 40 to about 65 amino acid residues, about 45 to about 65 amino acid residues, about 50 to about 65 amino acid residues, or about 55 to about 65 amino acid residues of IKZF1 or IKZF3.

In some embodiments, the COF1/CRBN-binding polypeptide comprises sufficient amino acid sequence and/or a structural motif from amino acid residues 136-180 of IKZF3 (numbered according to SEQ ID NO: 19) (e.g., sufficient amino acid sequence and/or a structural motif from amino acid residues 136-180 of SEQ ID NO: 19) that:

- 5 i) the association of the COF1/CRBN-binding polypeptide with CRBN in the absence of COF1 is no more than, e.g., 0.01%, 0.1%, 1%, 5%, 10%, 15%, or 20%, of the association of the COF1/CRBN-binding polypeptide with CRBN in the presence of COF1, e.g., an excess of COF1, e.g., as measured by an assay described herein, e.g., immunoprecipitation;
- 10 ii) the association of the fusion polypeptide with CRBN in the absence of COF1 is no more than, e.g., 0.01%, 0.1%, 1%, 5%, 10%, 15%, or 20% of the association of the fusion polypeptide with CRBN in the presence of COF1, e.g., an excess of COF1, e.g., as measured by an assay described herein, e.g., immunoprecipitation;
- 15 iii) the ubiquitination of the heterologous polypeptide in the absence of COF1 is no more than, e.g., 0.01%, 0.1%, 1%, 10%, 20%, 30%, 40%, 50%, 60%, or 70%, of the ubiquitination of the heterologous polypeptide in the presence of COF1, e.g., an excess of COF1, e.g., as measured by an assay described herein;
- iv) the ubiquitination of the fusion polypeptide in the absence of COF1 is no more than, e.g., 0.01%, 0.1%, 1%, 10%, 20%, 30%, 40%, 50%, 60%, or 70%, of the ubiquitination of the fusion polypeptide in the presence of COF1, e.g., an excess of COF1, e.g., as measured by an assay described herein; or
- 20 v) the degradation of the fusion polypeptide in the absence of COF1 is no more than, e.g., 0.01%, 0.1%, 1%, 10%, 20%, 30%, 40%, 50%, 60%, or 70%, of the degradation of the fusion polypeptide in the presence of COF1, e.g., an excess of COF1, e.g., as measured by an assay described herein, e.g., a Western blot analysis or a flow cytometry analysis.

25 In some embodiments, the association, ubiquitination, and/or degradation is as measured in a mammalian cell, e.g., a human cell.

In some embodiments, the COF1/CRBN-binding polypeptide comprises amino acid residues 136-180 of IKZF3 (numbered according to SEQ ID NO: 19) (e.g., the COF1/CRBN-binding polypeptide comprises the amino acid sequence of SEQ ID NO: 5) or a sequence that differs from amino acid residues 136-180 of IKZF3 (numbered according to SEQ ID NO: 19) by no more than 1, 2, 3, 4, 5, 10, 15, 20, 25, 30, 35, or 40 amino acid residues (e.g., a sequence that differs from amino acid residues 136-180 of SEQ ID NO: 19 by no more than 1, 2, 3, 4, 5, 10, 15, 20, 25, 30, 35, or 40 amino acid residues) (e.g., a sequence having no more than 1, 2, 3, 4, 5, 10, 15, 20, 25, 30, 35, or 40 amino acid substitutions from amino acid residues 136-180 of SEQ ID NO: 19).



In some embodiments, the COF1/CRBN-binding polypeptide comprises sufficient amino acid sequence and/or a structural motif from amino acid residues 136-170 of IKZF3 (numbered according to SEQ ID NO: 19) (e.g., sufficient amino acid sequence and/or a structural motif from amino acid residues 136-170 of SEQ ID NO: 19) that:

- 5 i) the association of the COF1/CRBN-binding polypeptide with CRBN in the absence of COF1 is no more than, e.g., 0.01%, 0.1%, 1%, 5%, 10%, 15%, or 20%, of the association of the COF1/CRBN-binding polypeptide with CRBN in the presence of COF1, e.g., an excess of COF1, e.g., as measured by an assay described herein, e.g., immunoprecipitation;
- 10 ii) the association of the fusion polypeptide with CRBN in the absence of COF1 is no more than, e.g., 0.01%, 0.1%, 1%, 5%, 10%, 15%, or 20% of the association of the fusion polypeptide with CRBN in the presence of COF1, e.g., an excess of COF1, e.g., as measured by an assay described herein, e.g., immunoprecipitation;
- 15 iii) the ubiquitination of the heterologous polypeptide in the absence of COF1 is no more than, e.g., 0.01%, 0.1%, 1%, 10%, 20%, 30%, 40%, 50%, 60%, or 70%, of the ubiquitination of the heterologous polypeptide in the presence of COF1, e.g., an excess of COF1, e.g., as measured by an assay described herein;
- iv) the ubiquitination of the fusion polypeptide in the absence of COF1 is no more than, e.g., 0.01%, 0.1%, 1%, 10%, 20%, 30%, 40%, 50%, 60%, or 70%, of the ubiquitination of the fusion polypeptide in the presence of COF1, e.g., an excess of COF1, e.g., as measured by an assay described herein; or
- 20 v) the degradation of the fusion polypeptide in the absence of COF1 is no more than, e.g., 0.01%, 0.1%, 1%, 10%, 20%, 30%, 40%, 50%, 60%, or 70%, of the degradation of the fusion polypeptide in the presence of COF1, e.g., an excess of COF1, e.g., as measured by an assay described herein, e.g., a Western blot analysis or a flow cytometry analysis.

25 In some embodiments, the association, ubiquitination, and/or degradation is as measured in a mammalian cell, e.g., a human cell.

In some embodiments, the COF1/CRBN-binding polypeptide comprises amino acid residues 136-170 of IKZF3 (numbered according to SEQ ID NO: 19) (e.g., the COF1/CRBN-binding polypeptide comprises the amino acid sequence of SEQ ID NO: 6) or a sequence that differs from amino acid residues 136-170 of IKZF3 (numbered according to SEQ ID NO: 19) by no more than 1, 2, 3, 4, 5, 10, 15, 20, 25, or 30 amino acid residues (e.g., a sequence that differs from amino acid residues 136-170 of SEQ ID NO: 19 by no more than 1, 2, 3, 4, 5, 10, 15, 20, 25, or 30 amino acid residues) (e.g., a sequence having no more than 1, 2, 3, 4, 5, 10, 15, 20, 25, or 30 amino acid substitutions from amino acid residues 136-170 of SEQ ID NO: 19).

In some embodiments, one, two, three or all of the following amino acid residues remain unaltered: glutamine at position 147, cysteine at position 148, glutamine at position 150, glycine at position 152, leucine at position 161, or leucine at position 162, numbered according to SEQ ID NO: 19.

In some embodiments, glutamine at position 147 remains unaltered, numbered according to SEQ ID NO:

19. In some embodiments, cysteine at position 148 remains unaltered, numbered according to SEQ ID NO: 19. In some embodiments, glutamine at position 150 remains unaltered, numbered according to SEQ ID NO: 19. In some embodiments, glycine at position 152 remains unaltered, numbered according to SEQ ID NO: 19. In some embodiments, leucine at position 161 remains unaltered, numbered according to SEQ ID NO: 19. In some embodiments, leucine at position 162 remains unaltered, numbered according to SEQ ID NO: 19.

In some embodiments, the COF1/CRBN-binding polypeptide comprises amino acid residues 136-139 of IKZF3 (numbered according to SEQ ID NO: 19), e.g., the COF1/CRBN-binding polypeptide comprises the amino acid sequence of SEQ ID NO: 40.

In some embodiments, the COF1/CRBN-binding polypeptide comprises amino acid residues 136-180 of IKZF3 (numbered according to SEQ ID NO: 19). In some embodiments, the COF1/CRBN-binding polypeptide comprises the amino acid sequence of SEQ ID NO: 5. In some embodiments, the COF1/CRBN-binding polypeptide comprises the amino acid sequence of SEQ ID NO: 77. In some embodiments, the COF1/CRBN-binding polypeptide consists of the amino acid sequence of SEQ ID NO: 5. In some embodiments, the COF1/CRBN-binding polypeptide consists of the amino acid sequence of SEQ ID NO: 5.

In some embodiments, the COF1/CRBN-binding polypeptide comprises amino acid residues 136-170 of IKZF3 (numbered according to SEQ ID NO: 19). In some embodiments, the COF1/CRBN-binding polypeptide comprises the amino acid sequence of SEQ ID NO: 6. In some embodiments, the COF1/CRBN-binding polypeptide comprises the amino acid sequence of SEQ ID NO: 78. In some embodiments, the COF1/CRBN-binding polypeptide consists of the amino acid sequence of SEQ ID NO: 6. In some embodiments, the COF1/CRBN-binding polypeptide consists of the amino acid sequence of SEQ ID NO: 78.

In some embodiments, the COF1/CRBN-binding polypeptide comprises sufficient amino acid sequence and/or a structural motif from amino acid residues 236-249 of IKZF3 (numbered according to SEQ ID NO: 19) (e.g., sufficient amino acid sequence and/or a structural motif from amino acid residues 236-249 of SEQ ID NO: 19) that:

i) the association of the COF1/CRBN-binding polypeptide with CRBN in the absence of COF1 is no more than, e.g., 0.01%, 0.1%, 1%, 5%, 10%, 15%, or 20%, of the association of the COF1/CRBN-binding

polypeptide with CRBN in the presence of COF1, e.g., an excess of COF1, e.g., as measured by an assay described herein, e.g., immunoprecipitation;

ii) the association of the fusion polypeptide with CRBN in the absence of COF1 is no more than, e.g., 0.01%, 0.1%, 1%, 5%, 10%, 15%, or 20% of the association of the fusion polypeptide with CRBN in the presence of COF1, e.g., an excess of COF1, e.g., as measured by an assay described herein, e.g., immunoprecipitation;

iii) the ubiquitination of the heterologous polypeptide in the absence of COF1 is no more than, e.g., 0.01%, 0.1%, 1%, 10%, 20%, 30%, 40%, 50%, 60%, or 70%, of the ubiquitination of the heterologous polypeptide in the presence of COF1, e.g., an excess of COF1, e.g., as measured by an assay described herein;

iv) the ubiquitination of the fusion polypeptide in the absence of COF1 is no more than, e.g., 0.01%, 0.1%, 1%, 10%, 20%, 30%, 40%, 50%, 60%, or 70%, of the ubiquitination of the fusion polypeptide in the presence of COF1, e.g., an excess of COF1, e.g., as measured by an assay described herein; or

v) the degradation of the fusion polypeptide in the absence of COF1 is no more than, e.g., 0.01%, 0.1%, 1%, 10%, 20%, 30%, 40%, 50%, 60%, or 70%, of the degradation of the fusion polypeptide in the presence of COF1, e.g., an excess of COF1, e.g., as measured by an assay described herein, e.g., a Western blot analysis or a flow cytometry analysis.

In some embodiments, the association, ubiquitination, and/or degradation is as measured in a mammalian cell, e.g., a human cell.

In some embodiments, the COF1/CRBN-binding polypeptide comprises amino acid residues 236-249 of IKZF3 (numbered according to SEQ ID NO: 19) (e.g., the COF1/CRBN-binding polypeptide comprises the amino acid sequence of SEQ ID NO: 11) or a sequence that differs from amino acid residues 236-249 of IKZF3 (numbered according to SEQ ID NO: 19) by no more than 1, 2, 3, 4, 5, 6, or 7 amino acid residues (e.g., a sequence that differs from amino acid residues 236-249 of SEQ ID NO: 19 by no more than 1, 2, 3, 4, 5, 6, or 7 amino acid residues) (e.g., a sequence having no more than 1, 2, 3, 4, 5, 6, or 7 amino acid substitutions from amino acid residues 236-249 of SEQ ID NO: 19).

In some embodiments, the COF1/CRBN-binding polypeptide comprises amino acid residues 236-249 of IKZF3 (numbered according to SEQ ID NO: 19). In some embodiments, the COF1/CRBN-binding polypeptide comprises the amino acid sequence of SEQ ID NO: 11.

In some embodiments, the COF1/CRBN-binding polypeptide comprises the amino acid sequence of SEQ ID NO: 91.

In some embodiments, the COF1/CRBN-binding polypeptide comprises sufficient amino acid sequence and/or a structural motif from amino acid residues 136-180 and 236-249 of IKZF3 (numbered

according to SEQ ID NO: 19) (e.g., sufficient amino acid sequence and/or a structural motif from amino acid residues 136-180 and 236-249 of SEQ ID NO: 19) that:

i) the association of the COF1/CRBN-binding polypeptide with CRBN in the absence of COF1 is no more than, e.g., 0.01%, 0.1%, 1%, 5%, 10%, 15%, or 20%, of the association of the COF1/CRBN-binding polypeptide with CRBN in the presence of COF1, e.g., an excess of COF1, e.g., as measured by an assay described herein, e.g., immunoprecipitation;

ii) the association of the fusion polypeptide with CRBN in the absence of COF1 is no more than, e.g., 0.01%, 0.1%, 1%, 5%, 10%, 15%, or 20% of the association of the fusion polypeptide with CRBN in the presence of COF1, e.g., an excess of COF1, e.g., as measured by an assay described herein, e.g., immunoprecipitation;

iii) the ubiquitination of the heterologous polypeptide in the absence of COF1 is no more than, e.g., 0.01%, 0.1%, 1%, 10%, 20%, 30%, 40%, 50%, 60%, or 70%, of the ubiquitination of the heterologous polypeptide in the presence of COF1, e.g., an excess of COF1, e.g., as measured by an assay described herein;

iv) the ubiquitination of the fusion polypeptide in the absence of COF1 is no more than, e.g., 0.01%, 0.1%, 1%, 10%, 20%, 30%, 40%, 50%, 60%, or 70%, of the ubiquitination of the fusion polypeptide in the presence of COF1, e.g., an excess of COF1, e.g., as measured by an assay described herein; or

v) the degradation of the fusion polypeptide in the absence of COF1 is no more than, e.g., 0.01%, 0.1%, 1%, 10%, 20%, 30%, 40%, 50%, 60%, or 70%, of the degradation of the fusion polypeptide in the presence of COF1, e.g., an excess of COF1, e.g., as measured by an assay described herein, e.g., a Western blot analysis or a flow cytometry analysis.

In some embodiments, the association, ubiquitination, and/or degradation is as measured in a mammalian cell, e.g., a human cell.

In some embodiments, the COF1/CRBN-binding polypeptide comprises a first sequence comprising amino acid residues 136-180 of IKZF3 (numbered according to SEQ ID NO: 19) (e.g., a first sequence comprising the amino acid sequence of SEQ ID NO: 5) or a first sequence that differs from amino acid residues 136-180 of IKZF3 (numbered according to SEQ ID NO: 19) by no more than 1, 2, 3, 4, 5, 10, 15, 20, 25, 30, 35, or 40 amino acid residues (e.g., a first sequence that differs from amino acid residues 136-180 of SEQ ID NO: 19 by no more than 1, 2, 3, 4, 5, 10, 15, 20, 25, 30, 35, or 40 amino acid residues); and a second sequence comprising amino acid residues 236-249 of IKZF3 (numbered according to SEQ ID NO: 19) (e.g., a second sequence comprising the amino acid sequence of SEQ ID NO: 11) or a second sequence that differs from amino acid residues 236-249 of IKZF3 (numbered according to SEQ ID NO: 19) by no more than 1, 2, 3, 4, 5, 6, or 7 amino acid residues (e.g., a second

sequence that differs from amino acid residues 236-249 of SEQ ID NO: 19 by no more than 1, 2, 3, 4, 5, 6, or 7 amino acid residues).

In some embodiments, the COF1/CRBN-binding polypeptide comprises amino acid residues 136-180 and 236-249 of IKZF3 (numbered according to SEQ ID NO: 19). In some embodiments, the COF1/CRBN-binding polypeptide comprises the amino acid sequence of SEQ ID NO: 1. In some  
 5 embodiments, the COF1/CRBN-binding polypeptide comprises the amino acid sequence of SEQ ID NO: 3. In some embodiments, the COF1/CRBN-binding polypeptide consists of the amino acid sequence of SEQ ID NO: 1. In some embodiments, the COF1/CRBN-binding polypeptide consists of the amino acid sequence of SEQ ID NO: 3.

10 In some embodiments, the COF1/CRBN-binding polypeptide comprises a first sequence comprising amino acid residues 136-180 of IKZF3 (numbered according to SEQ ID NO: 19) and a second sequence comprising the amino acid sequence of MALEKMALEKMALE (SEQ ID NO: 91). In some  
 15 embodiments, the COF1/CRBN-binding polypeptide comprises the amino acid sequence of SEQ ID NO: 14. In some embodiments, the COF1/CRBN-binding polypeptide comprises the amino acid sequence of SEQ ID NO: 85. In some embodiments, the COF1/CRBN-binding polypeptide consists of the amino acid sequence of SEQ ID NO: 14. In some embodiments, the COF1/CRBN-binding polypeptide consists of the amino acid sequence of SEQ ID NO: 85.

In some embodiments, the COF1/CRBN-binding polypeptide comprises a first sequence comprising amino acid residues 136-170 of IKZF3 (numbered according to SEQ ID NO: 19) and a second  
 20 sequence comprising the amino acid sequence of MALEKMALEKMALE (SEQ ID NO: 91). In some embodiments, the COF1/CRBN-binding polypeptide comprises the amino acid sequence of SEQ ID NO: 15. In some embodiments, the COF1/CRBN-binding polypeptide comprises the amino acid sequence of SEQ ID NO: 86. In some embodiments, the COF1/CRBN-binding polypeptide consists of the amino acid sequence of SEQ ID NO: 15. In some embodiments, the COF1/CRBN-binding polypeptide consists of  
 25 the amino acid sequence of SEQ ID NO: 86.

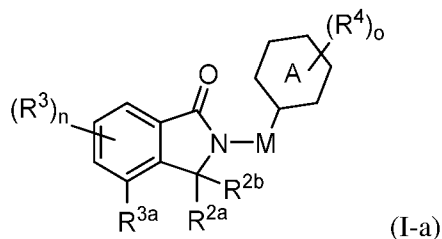
In some embodiments, the COF1/CRBN-binding polypeptide comprises at least one less lysine than the corresponding native sequence. In some embodiments, one or more lysine residues in the corresponding native sequence are replaced by a different amino acid, e.g., arginine. In some  
 30 embodiments, the COF1/CRBN-binding polypeptide comprises less than 1, 2, 3, 4, or 5 lysine residues. In some embodiments, the COF1/CRBN-binding polypeptide does not comprise a lysine residue. In some embodiments, the COF1/CRBN-binding polypeptide is not ubiquitinated, e.g., in the presence of COF1, e.g., as measured by an assay described herein, optionally wherein ubiquitination is as measured in a mammalian cell, e.g., a human cell.

In some embodiments, the COF1/CRBN-binding polypeptide comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 4, 41, 42, and 43. In some embodiments, the COF1/CRBN-binding polypeptide comprises the amino acid sequence of SEQ ID NO: 4. In some embodiments, the COF1/CRBN-binding polypeptide comprises the amino acid sequence of SEQ ID NO: 41. In some embodiments, the COF1/CRBN-binding polypeptide comprises the amino acid sequence of SEQ ID NO: 42. In some embodiments, the COF1/CRBN-binding polypeptide comprises the amino acid sequence of SEQ ID NO: 43.

In some embodiments, the COF1/CRBN-binding polypeptide comprises the amino acid sequence of SEQ ID NO: 2. In some embodiments, the COF1/CRBN-binding polypeptide comprises the amino acid sequence of SEQ ID NO: 4. In some embodiments, the COF1/CRBN-binding polypeptide consists of the amino acid sequence of SEQ ID NO: 2. In some embodiments, the COF1/CRBN-binding polypeptide consists of the amino acid sequence of SEQ ID NO: 4.

In some embodiments, the COF1 is an immunomodulatory imide drug (IMiD), or a pharmaceutically acceptable salt thereof.

In some embodiments, the COF1 has the structure of Formula (I-a):



or a pharmaceutically acceptable salt, ester, hydrate, or tautomer thereof, wherein:

Ring A is carbocyclyl, heterocyclyl, aryl, or heteroaryl, each of which is independently and optionally substituted with one or more R⁴;

M is absent, C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, or C₁-C₆ heteroalkyl, wherein each alkyl, alkenyl, alkynyl, and heteroalkyl is independently and optionally substituted with one or more R⁴;

each of R²ᵃ and R²ᵇ is independently hydrogen or C₁-C₆ alkyl; or R²ᵃ and R²ᵇ together with the carbon atom to which they are attached to form a carbonyl group or thiocarbonyl group;

R³ᵃ is hydrogen, C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, C₁-C₆ heteroalkyl, halo, cyano, -C(O)Rᵃ, -C(O)ORᵇ, -ORᵇ, -N(Rᶜ)(Rᵈ), -C(O)N(Rᶜ)(Rᵈ), -N(Rᶜ)C(O)Rᵃ, -S(O)ₓRᵉ, -S(O)ₓN(Rᶜ)(Rᵈ), or -N(Rᶜ)S(O)ₓRᵉ, wherein each alkyl, alkenyl, alkynyl, and heteroalkyl is independently and optionally substituted with one or more R⁶;

each of R³ is independently C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, C₁-C₆ heteroalkyl, halo, cyano, -C(O)Rᵃ, -C(O)ORᵇ, -ORᵇ, -N(Rᶜ)(Rᵈ), -C(O)N(Rᶜ)(Rᵈ), -N(Rᶜ)C(O)Rᵃ, -S(O)ₓRᵉ, -

$S(O)_xN(R^C)(R^D)$ , or  $-N(R^C)S(O)_xR^E$ , wherein each alkyl, alkenyl, alkynyl, and heteroalkyl is independently and optionally substituted with one or more  $R^6$ ;

each  $R^4$  is independently  $C_1$ - $C_6$  alkyl,  $C_2$ - $C_6$  alkenyl,  $C_2$ - $C_6$  alkynyl,  $C_1$ - $C_6$  heteroalkyl, halo, cyano, oxo,  $-C(O)R^A$ ,  $-C(O)OR^B$ ,  $-OR^B$ ,  $-N(R^C)(R^D)$ ,  $-C(O)N(R^C)(R^D)$ ,  $-N(R^C)C(O)R^A$ ,  $S(O)_xR^E$ ,  $-S(O)_xN(R^C)(R^D)$ ,  $-N(R^C)S(O)_xR^E$ , carbocyclyl, heterocyclyl, aryl, or heteroaryl, wherein each alkyl, alkenyl, alkynyl, carbocyclyl, heterocyclyl, aryl, and heteroaryl is independently and optionally substituted with one or more  $R^7$ ;

each of  $R^A$ ,  $R^B$ ,  $R^C$ ,  $R^D$ , and  $R^E$  is independently hydrogen or  $C_1$ - $C_6$  alkyl;

each  $R^6$  is independently  $C_1$ - $C_6$  alkyl, oxo, cyano,  $-OR^B$ ,  $-N(R^C)(R^D)$ ,  $-C(O)N(R^C)(R^D)$ ,  $-N(R^C)C(O)R^A$ , aryl, or heteroaryl, wherein each aryl and heteroaryl is independently and optionally substituted with one or more  $R^8$ ;

each  $R^7$  is independently halo, oxo, cyano,  $-OR^B$ ,  $-N(R^C)(R^D)$ ,  $-C(O)N(R^C)(R^D)$ , or  $-N(R^C)C(O)R^A$ ;

each  $R^8$  is independently  $C_1$ - $C_6$  alkyl, cyano,  $-OR^B$ ,  $-N(R^C)(R^D)$ ,  $-C(O)N(R^C)(R^D)$ , or  $-N(R^C)C(O)R^A$ ;

$n$  is 0, 1, 2, or 3;

$o$  is 0, 1, 2, 3, 4, or 5; and

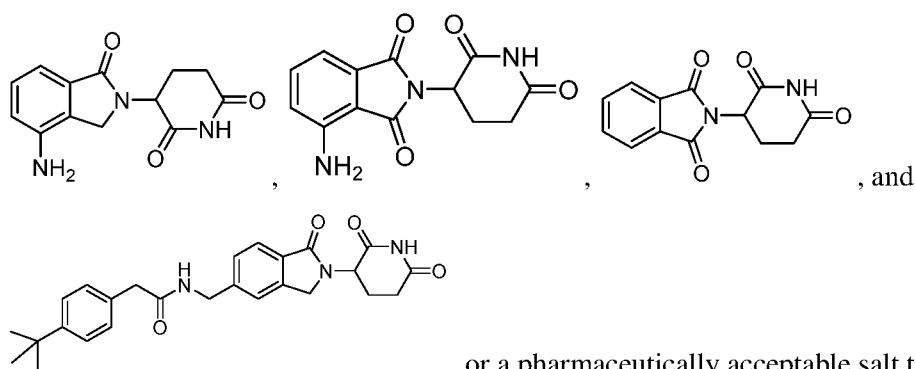
$x$  is 0, 1, or 2.

In some embodiments of Formula (I-a),  $X$  is O. In some embodiments,  $M$  is absent. In some embodiments, Ring A is heterocyclyl (e.g., a nitrogen-containing heterocyclyl, e.g., 2-(2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione). In some embodiments,  $R^4$  is oxo or  $OR^B$  (e.g.,  $-OCH_3$  or  $-OCH_2CH_3$ ) and  $o$  is 0, 1, or 2. In some embodiments, each of  $R^{2a}$  and  $R^{2b}$  is independently hydrogen, or  $R^{2a}$  and  $R^{2b}$  together with the carbon atom to which they are attached form a carbonyl group. In some embodiments,  $R^{3a}$  is heteroalkyl (e.g.,  $-CH_2NHC(O)CH_2$ ),  $-N(R^C)(R^D)$  (e.g.,  $-NH_2$ ), or  $-N(R^C)C(O)R^A$  (e.g.,  $-NHC(O)CH_3$ ). In some embodiments,  $n$  is 0.

In some embodiments, the COF1 is thalidomide or analogue, or a pharmaceutically acceptable salt thereof.

In some embodiments, the COF1 is selected from the group consisting of lenalidomide, pomalidomide, thalidomide, and 2-(4-(tert-butyl)phenyl)-N-((2-(2,6-dioxopiperidin-3-yl)-1-oxoisindolin-5-yl)methyl)acetamide, or a pharmaceutically acceptable salt thereof.

In some embodiments, the COF1 is selected from the group consisting of:



In some embodiments, the COF1 is lenalidomide or pomalidomide, or a pharmaceutically acceptable salt thereof. In some embodiments, the COF1 is lenalidomide or analogue, or a pharmaceutically acceptable salt thereof. In some embodiments, the COF1 is lenalidomide, or a pharmaceutically acceptable salt thereof.

In some embodiments, the fusion polypeptide further comprises a degradation domain, wherein the degradation domain is separated from the COF1/CRBN-binding polypeptide and the heterologous polypeptide by a heterologous protease cleavage site.

In some embodiments, the fusion polypeptide comprises, from N-terminus to C-terminus:

- i) the degradation domain, the heterologous protease cleavage site, the heterologous polypeptide, and the COF1/CRBN-binding polypeptide;
- ii) the degradation domain, the heterologous protease cleavage site, the COF1/CRBN-binding polypeptide, and the heterologous polypeptide;
- iii) the COF1/CRBN-binding polypeptide, the heterologous polypeptide, the heterologous protease cleavage site, and the degradation domain; or
- iv) the heterologous polypeptide, and the COF1/CRBN-binding polypeptide, the heterologous protease cleavage site, and the degradation domain.

In some embodiments, the fusion polypeptide comprises, from N-terminus to C-terminus, the degradation domain, the heterologous protease cleavage site, the heterologous polypeptide, and the COF1/CRBN-binding polypeptide.

In some embodiments, the degradation domain has a first state associated with a first level of expression of the fusion polypeptide and a second state associated with a second level of expression of the fusion polypeptide, wherein the second level is increased, e.g., by at least 2-, 3-, 4-, 5-, 10-, 20- or 30-fold over the first level in the presence of a stabilization compound.

In some embodiments, in the absence of the stabilization compound, the fusion polypeptide is degraded by a cellular degradation pathway, e.g., at least 50%, 60%, 70%, 80%, 90% or greater of the



fusion polypeptide is degraded, e.g., as measured by an assay described herein, e.g., a Western blot analysis or a flow cytometry analysis.

In some embodiments, the level of expression, and/or degradation is as measured in a mammalian cell, e.g., a human cell.

5 In some embodiments, in the presence of the stabilization compound:

i) the degradation domain assumes a conformation more resistant to cellular degradation relative to a conformation in the absence of the stabilization compound; or

ii) the conformation of the fusion polypeptide is more permissive to cleavage at the heterologous protease cleavage site relative to a conformation in the absence of the stabilization compound.

10 In some embodiments, the degradation domain is chosen from an estrogen receptor (ER) domain, an FKB protein (FKBP) domain, or a dihydrofolate reductase (DHFR) domain.

In some embodiments, the degradation domain is an estrogen receptor (ER) domain. In some embodiments, the degradation domain comprises an amino acid sequence that is at least 90, 95, 97, 98, 99, or 100% identical to SEQ ID NO: 46 or 48. In some embodiments, the degradation domain comprises  
15 the amino acid sequence of SEQ ID NO: 46. In some embodiments, the degradation domain comprises the amino acid sequence of SEQ ID NO: 48.

In some embodiments, the stabilization compound is bazedoxifene or 4-hydroxy tamoxifen (4-OHT), or a pharmaceutically acceptable salt thereof. In some embodiments, the degradation domain is an estrogen receptor (ER) domain and the stabilization compound is bazedoxifene, or a pharmaceutically  
20 acceptable salt thereof. In some embodiments, the degradation domain comprises the amino acid sequence of SEQ ID NO: 46 and the stabilization compound is bazedoxifene, or a pharmaceutically acceptable salt thereof.

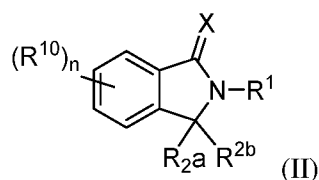
In some embodiments, the degradation domain is an FKB protein (FKBP) domain. In some embodiments, the degradation domain comprises an amino acid sequence that is at least 90, 95, 97, 98, 99, or 100% identical to SEQ ID NO: 50. In some embodiments, the degradation domain comprises the  
25 amino acid sequence of SEQ ID NO: 50. In some embodiments, the stabilization compound is Shield-1, or a pharmaceutically acceptable salt thereof.

In some embodiments, the degradation domain is a dihydrofolate reductase (DHFR) domain. In some embodiments, the degradation domain comprises an amino acid sequence that is at least 90, 95, 97, 98, 99, or 100% identical to SEQ ID NO: 51. In some embodiments, the degradation domain comprises  
30 the amino acid sequence of SEQ ID NO: 51. In some embodiments, the stabilization compound is trimethoprim, or a pharmaceutically acceptable salt thereof.

In some embodiments, the heterologous protease cleavage site is cleaved by a mammalian intracellular protease. In some embodiments, the heterologous protease cleavage site is cleaved by a

protease selected from the group consisting of furin, PCSK1, PCSK5, PCSK6, PCSK7, cathepsin B, Granzyme B, Factor XA, Enterokinase, genenase, sortase, precision protease, thrombin, TEV protease, and elastase 1. In some embodiments, the heterologous protease cleavage site comprises a sequence having a cleavage motif selected from the group consisting of RX(K/R)R consensus motif (X can be any amino acid; SEQ ID NO: 52), RXXX[KR]R consensus motif (X can be any amino acid; SEQ ID NO: 53), RRX consensus motif (SEQ ID NO : 54), I-E-P-D-X consensus motif (SEQ ID NO: 55), Ile-Glu/Asp-Gly-Arg (SEQ IDNO : 56), Asp-Asp-Asp-Asp-Lys (SEQ ID NO: 57), Pro-Gly-Ala-Ala-His-Tyr (SEQ ID NO: 58), LPXTG/A consensus motif (SEQ ID NO: 59), Leu-Glu-Val-Phe-Gln-Gly-Pro (SEQ ID NO: 60), Leu-Val-Pro-Arg-Gly-Ser (SEQ ID NO: 61), E-N-L-Y-F-Q-G (SEQ ID NO: 62), and [AGSV]-X (X can be any amino acid; SEQ ID NO: 63). In some embodiments, the heterologous protease cleavage site is cleaved by furin. In some embodiments, the heterologous protease cleavage site comprises a furin cleavage site selected from the group consisting of RTKR (SEQ ID NO: 123); GTGAEDPRPSRKRRSLGDVG (SEQ ID NO: 125); GTGAEDPRPSRKRR (SEQ ID NO: 127); LQWLEQQVAKRRTRK (SEQ ID NO: 129); GTGAEDPRPSRKRRSLGG (SEQ ID NO: 131); GTGAEDPRPSRKRRSLG (SEQ ID NO: 133); SLNLTESHNSRKRR (SEQ ID NO: 135); CKINGYPKRGRKRR (SEQ ID NO: 137); and SARNRQKR (SEQ ID NO: 34). In some embodiments, the heterologous protease cleavage site does not comprise the amino acid sequence of SARNRQKR (SEQ ID NO: 34). In some embodiments, the heterologous protease cleavage site comprises the furin cleavage site of GTGAEDPRPSRKRRSLGDVG (SEQ ID NO: 125). In some embodiments, the heterologous protease cleavage site is cleaved by a mammalian extracellular protease. In some embodiments, the mammalian extracellular protease is selected from the group consisting of Factor XA, Enterokinase, genenase, sortase, precision protease, thrombin, TEV protease, and elastase 1. In some embodiments, the heterologous protease cleavage site comprises an amino acid sequence selected from the group consisting of Ile-Glu/Asp-Gly-Arg (SEQ IDNO : 56), Asp-Asp-Asp-Asp-Lys (SEQ ID NO: 57), Pro-Gly-Ala-Ala-His-Tyr (SEQ ID NO: 58), LPXTG/A consensus motif (SEQ ID NO: 59), Leu-Glu-Val-Phe-Gln-Gly-Pro (SEQ ID NO: 60), Leu-Val-Pro-Arg-Gly-Ser (SEQ ID NO: 61), E-N-L-Y-F-Q-G (SEQ ID NO: 62), and [AGSV]-X (X can be any amino acid; SEQ ID NO: 63).

In one aspect, provided herein is a fusion polypeptide comprising a first domain and a second domain separated by a heterologous protease cleavage site, wherein the first domain comprises a degradation domain and the second domain comprises a compound of Formula (II) (COF2)/CRBN-binding polypeptide and a heterologous polypeptide, e.g., a heterologous mammalian, bacterial, or viral polypeptide, wherein the compound of Formula (II) is:



or a pharmaceutically acceptable salt, ester, hydrate, tautomer, or prodrug thereof, wherein:

X is O or S;

R¹ is C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, C₁-C₆ heteroalkyl, carbocyclyl, heterocyclyl, aryl, or heteroaryl, each of which is independently and optionally substituted by one or more R⁴;

each of R²ᵃ and R²ᵇ is independently hydrogen or C₁-C₆ alkyl; or R²ᵃ and R²ᵇ together with the carbon atom to which they are attached to form carbonyl group or thiocarbonyl group;

each of R¹⁰ is independently C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, C₁-C₆ heteroalkyl, halo, cyano, -C(O)Rᵃ, -C(O)ORᵇ, -ORᵇ, -N(Rᶜ)(Rᵈ), -C(O)N(Rᶜ)(Rᵈ), -N(Rᶜ)C(O)Rᵃ, -S(O)ₓRᵉ, -

S(O)ₓN(Rᶜ)(Rᵈ), or -N(Rᶜ)S(O)ₓRᵉ, or L-Tag; wherein each alkyl, alkenyl, alkynyl, and heteroalkyl is independently and optionally substituted with one or more R¹¹;

each R⁴ is independently C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, C₁-C₆ heteroalkyl, halo, cyano, oxo, C(O)Rᵃ, -C(O)ORᵇ, ORᵇ, -N(Rᶜ)(Rᵈ), -C(O)N(Rᶜ)(Rᵈ), -N(Rᶜ)C(O)Rᵃ, S(O)ₓRᵉ, -

S(O)ₓN(Rᶜ)(Rᵈ), -N(Rᶜ)S(O)ₓRᵉ, carbocyclyl, heterocyclyl, aryl, or heteroaryl, wherein each alkyl, alkenyl, alkynyl, heteroalkyl, carbocyclyl, heterocyclyl, aryl, and heteroaryl is independently and optionally substituted with one or more R⁷;

each of Rᵃ, Rᵇ, Rᶜ, Rᵈ, and Rᵉ is independently hydrogen or C₁-C₆ alkyl;

each R¹¹ is independently C₁-C₆ alkyl, halo, oxo, cyano, -ORᵇ, -N(Rᶜ)(Rᵈ), -C(O)N(Rᶜ)(Rᵈ), -N(Rᶜ)C(O)Rᵃ, aryl, or heteroaryl, wherein each aryl and heteroaryl is independently and optionally substituted with one or more R⁸;

each R⁷ is independently halo, oxo, cyano, -ORᵇ, -N(Rᶜ)(Rᵈ), -C(O)N(Rᶜ)(Rᵈ), or -N(Rᶜ)C(O)Rᵃ;

each R⁸ is independently C₁-C₆ alkyl, halo, cyano, -ORᵇ, -N(Rᶜ)(Rᵈ), -C(O)N(Rᶜ)(Rᵈ), or -N(Rᶜ)C(O)Rᵃ;

each L is independently C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, C₁-C₆ heteroalkyl, -C(O)Rᵃ¹, -C(O)ORᵇ¹, -ORᵇ¹, -N(Rᶜ¹)(Rᵈ¹), -C(O)N(Rᶜ¹)(Rᵈ¹), -N(Rᶜ¹)C(O)Rᵃ¹, -S(O)ₓRᵉ¹, -S(O)ₓN(Rᶜ¹)(Rᵈ¹), or -N(Rᶜ¹)S(O)ₓRᵉ¹, wherein each alkyl, alkenyl, alkynyl, and heteroalkyl is independently and optionally substituted with one or more R¹²;

each Tag is a targeting moiety capable of binding to a target protein;

each of Rᵃ¹, Rᵇ¹, Rᶜ¹, Rᵈ¹, and Rᵉ¹ is independently hydrogen, C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, C₁-C₆ heteroalkyl, carbocyclyl, heterocyclyl, aryl, or heteroaryl, wherein each alkyl, alkenyl,

alkynyl, heteroalkyl, carbocyclyl, heterocyclyl, aryl, and heteroaryl is independently and optionally substituted with one or more R<sup>12</sup>;

each R<sup>12</sup> is independently C<sub>1</sub>-C<sub>6</sub> alkyl, halo, cyano, carbocyclyl, or heterocyclyl;

n is 0, 1, 2, 3 or 4; and

5 x is 0, 1, or 2.

In some embodiments, the degradation domain has a first state associated with a first level of expression of the fusion polypeptide and a second state associated with a second level of expression of the fusion polypeptide, wherein the second level is increased, e.g., by at least 2-, 3-, 4-, 5-, 10-, 20- or 30-fold over the first level in the presence of a stabilization compound.

10 In some embodiments, in the absence of the stabilization compound, the fusion polypeptide is degraded by a cellular degradation pathway, e.g., at least 50%, 60%, 70%, 80%, 90% or greater of the fusion polypeptide is degraded, e.g., as measured by an assay described herein, e.g., a Western blot analysis or a flow cytometry analysis.

In some embodiments, the level of expression, and/or degradation is as measured in a mammalian  
15 cell, e.g., a human cell.

In some embodiments, in the presence of the stabilization compound:

i) the degradation domain assumes a conformation more resistant to cellular degradation relative to a conformation in the absence of the stabilization compound; or

ii) the conformation of the fusion polypeptide is more permissive to cleavage at the heterologous protease  
20 cleavage site relative to a conformation in the absence of the stabilization compound.

In some embodiments, the degradation domain is chosen from an estrogen receptor (ER) domain, an FKB protein (FKBP) domain, or a dihydrofolate reductase (DHFR) domain.

In some embodiments, the degradation domain is an estrogen receptor (ER) domain. In some  
25 embodiments, the degradation domain comprises an amino acid sequence that is at least 90, 95, 97, 98, 99, or 100% identical to SEQ ID NO: 46 or 48. In some embodiments, the degradation domain comprises the amino acid sequence of SEQ ID NO: 46. In some embodiments, the degradation domain comprises the amino acid sequence of SEQ ID NO: 48.

In some embodiments, the stabilization compound is bazedoxifene or 4-hydroxy tamoxifen (4-OHT), or a pharmaceutically acceptable salt thereof. In some embodiments, the degradation domain is an  
30 estrogen receptor (ER) domain and the stabilization compound is bazedoxifene, or a pharmaceutically acceptable salt thereof. In some embodiments, the degradation domain comprises the amino acid sequence of SEQ ID NO: 46 and the stabilization compound is bazedoxifene, or a pharmaceutically acceptable salt thereof.

In some embodiments, the degradation domain is an FKB protein (FKBP) domain. In some embodiments, the degradation domain comprises an amino acid sequence that is at least 90, 95, 97, 98, 99, or 100% identical to SEQ ID NO: 50. In some embodiments, the degradation domain comprises the amino acid sequence of SEQ ID NO: 50. In some embodiments, the stabilization compound is Shield-1,  
5 or a pharmaceutically acceptable salt thereof.

In some embodiments, the degradation domain is a dihydrofolate reductase (DHFR) domain. In some embodiments, the degradation domain comprises an amino acid sequence that is at least 90, 95, 97, 98, 99, or 100% identical to SEQ ID NO: 51. In some embodiments, the degradation domain comprises the amino acid sequence of SEQ ID NO: 51. In some embodiments, the stabilization compound is  
10 trimethoprim, or a pharmaceutically acceptable salt thereof.

In some embodiments, the heterologous protease cleavage site is cleaved by a mammalian intracellular protease. In some embodiments, the heterologous protease cleavage site is cleaved by a protease selected from the group consisting of furin, PCSK1, PCSK5, PCSK6, PCSK7, cathepsin B, Granzyme B, Factor XA, Enterokinase, genenase, sortase, precision protease, thrombin, TEV protease,  
15 and elastase 1. In some embodiments, the heterologous protease cleavage site comprises a sequence having a cleavage motif selected from the group consisting of RX(K/R)R consensus motif (X can be any amino acid; SEQ ID NO: 52), RXXX[KR]R consensus motif (X can be any amino acid; SEQ ID NO: 53), RRX consensus motif (SEQ ID NO : 54), I-E-P-D-X consensus motif (SEQ ID NO: 55), Ile-Glu/Asp-Gly-Arg (SEQ IDNO : 56), Asp-Asp-Asp-Asp-Lys (SEQ ID NO: 57), Pro-Gly-Ala-Ala-His-Tyr (SEQ ID  
20 NO: 58), LPXTG/A consensus motif (SEQ ID NO: 59), Leu-Glu-Val-Phe-Gln-Gly-Pro (SEQ ID NO: 60), Leu-Val-Pro-Arg-Gly-Ser (SEQ ID NO: 61), E-N-L-Y-F-Q-G (SEQ ID NO: 62), and [AGSV]-X (X can be any amino acid; SEQ ID NO: 63). In some embodiments, the heterologous protease cleavage site is cleaved by furin. In some embodiments, the heterologous protease cleavage site comprises a furin cleavage site selected from the group consisting of RTKR (SEQ ID NO: 123);  
25 GTGAEDPRPSRKRRSLGDVG (SEQ ID NO: 125); GTGAEDPRPSRKRR (SEQ ID NO: 127); LQWLEQQVAKRRTKR (SEQ ID NO: 129); GTGAEDPRPSRKRRSLGG (SEQ ID NO: 131); GTGAEDPRPSRKRRSLG (SEQ ID NO: 133); SLNLTESHNSRKRR (SEQ ID NO: 135); CKINGYPKRGRKRR (SEQ ID NO: 137); and SARNRQKR (SEQ ID NO: 34). In some embodiments, the heterologous protease cleavage site does not comprise the amino acid sequence of SARNRQKR (SEQ  
30 ID NO: 34). In some embodiments, the heterologous protease cleavage site comprises the furin cleavage site of GTGAEDPRPSRKRRSLGDVG (SEQ ID NO: 125). In some embodiments, the heterologous protease cleavage site is cleaved by a mammalian extracellular protease. In some embodiments, the mammalian extracellular protease is selected from the group consisting of Factor XA, Enterokinase, genenase, sortase, precision protease, thrombin, TEV protease, and elastase 1. In some embodiments,

the heterologous protease cleavage site comprises an amino acid sequence selected from the group consisting of Ile-Glu/Asp-Gly-Arg (SEQ ID NO : 56), Asp-Asp-Asp-Asp-Lys (SEQ ID NO: 57), Pro-Gly-Ala-Ala-His-Tyr (SEQ ID NO: 58), LPXTG/A consensus motif (SEQ ID NO: 59), Leu-Glu-Val-Phe-Gln-Gly-Pro (SEQ ID NO: 60), Leu-Val-Pro-Arg-Gly-Ser (SEQ ID NO: 61), E-N-L-Y-F-Q-G (SEQ ID NO: 62), and [AGSV]-X (X can be any amino acid; SEQ ID NO: 63).

In some embodiments, the degradation domain is fused to the heterologous protease cleavage site, which is further fused to the second domain.

In some embodiments, the fusion polypeptide comprises, from N-terminus to C-terminus:

- i) the degradation domain, the heterologous protease cleavage site, the heterologous polypeptide, and the COF2/CRBN-binding polypeptide;
- ii) the degradation domain, the heterologous protease cleavage site, the COF2/CRBN-binding polypeptide, and the heterologous polypeptide;
- iii) the COF2/CRBN-binding polypeptide, the heterologous polypeptide, the heterologous protease cleavage site, and the degradation domain; or
- iv) the heterologous polypeptide, and the COF2/CRBN-binding polypeptide, the heterologous protease cleavage site, and the degradation domain.

In some embodiments, the fusion polypeptide comprises, from N-terminus to C-terminus, the degradation domain, the heterologous protease cleavage site, the heterologous polypeptide, and the COF2/CRBN-binding polypeptide. In some embodiments, the fusion polypeptide comprises, from N-terminus to C-terminus, the degradation domain, the heterologous protease cleavage site, the COF2/CRBN-binding polypeptide, and the heterologous polypeptide. In some embodiments, the fusion polypeptide comprises, from N-terminus to C-terminus, the COF2/CRBN-binding polypeptide, the heterologous polypeptide, the heterologous protease cleavage site, and the degradation domain. In some embodiments, the fusion polypeptide comprises, from N-terminus to C-terminus, the heterologous polypeptide, and the COF2/CRBN-binding polypeptide, the heterologous protease cleavage site, and the degradation domain.

In some embodiments, the association of the COF2/CRBN-binding polypeptide with cereblon (CRBN) in the absence of COF2 is no more than, e.g., 0.01%, 0.1%, 1%, 5%, 10%, 15%, or 20%, of the association of the COF2/CRBN-binding polypeptide with CRBN in the presence of COF2, e.g., an excess of COF2, e.g., as measured by an assay described herein, e.g., immunoprecipitation. In some embodiments, the COF2/CRBN-binding polypeptide does not bind to CRBN in the absence of COF2. In some embodiments, the association of the fusion polypeptide with CRBN in the absence of COF2 is no more than, e.g., 0.01%, 0.1%, 1%, 5%, 10%, 15%, or 20%, of the association of the fusion polypeptide with CRBN in the presence of COF2, e.g., an excess of COF2, e.g., as measured by an assay described

herein, e.g., immunoprecipitation. In some embodiments, the fusion polypeptide does not bind to CRBN in the absence of COF2. In some embodiments, the association and/or binding is as measured in a mammalian cell, e.g., a human cell.

In some embodiments, the ubiquitination of the heterologous polypeptide in the absence of COF2 is no more than, e.g., 0.01%, 0.1%, 1%, 10%, 20%, 30%, 40%, 50%, 60%, or 70%, of the ubiquitination of the heterologous polypeptide in the presence of COF2, e.g., an excess of COF2, e.g., as measured by an assay described herein. In some embodiments, the ubiquitination of the fusion polypeptide in the absence of COF2 is no more than, e.g., 0.01%, 0.1%, 1%, 10%, 20%, 30%, 40%, 50%, 60%, or 70%, of the ubiquitination of the fusion polypeptide in the presence of COF2, e.g., an excess of COF2, e.g., as measured by an assay described herein. In some embodiments, the heterologous polypeptide or the fusion polypeptide is ubiquitinated at one or more lysine or methionine residues in the presence of COF2. In some embodiments, the ubiquitination is as measured in a mammalian cell, e.g., a human cell.

In some embodiments, the degradation of the fusion polypeptide in the absence of COF2 is no more than, e.g., 0.01%, 0.1%, 1%, 10%, 20%, 30%, 40%, 50%, 60%, or 70%, of the degradation of the fusion polypeptide in the presence of COF2, e.g., an excess of COF2, e.g., as measured by an assay described herein, e.g., a Western blot analysis or a flow cytometry analysis. In some embodiments, the degradation of the fusion polypeptide is mediated by ubiquitination in the presence of COF2. In some embodiments, the degradation is as measured in a mammalian cell, e.g., a human cell.

In some embodiments, the COF2/CRBN-binding polypeptide is between 10 and 95 amino acid residues in length, between 15 and 90 amino acid residues in length, between 20 and 85 amino acid residues in length, between 25 and 80 amino acid residues in length, between 30 and 75 amino acid residues in length, between 35 and 70 amino acid residues in length, between 40 and 65 amino acid residues in length, between 45 and 65 amino acid residues in length, between 50 and 65 amino acid residues in length, or between 55 and 65 amino acid residues in length.

In some embodiments, the COF2/CRBN-binding polypeptide comprises a beta turn. In some embodiments, the COF2/CRBN-binding polypeptide comprises a beta turn of IKZF1 or IKZF3 (e.g., human IKZF1 or IKZF3). In some embodiments, the COF2/CRBN-binding polypeptide comprises a beta hairpin. In some embodiments, the COF2/CRBN-binding polypeptide comprises a beta hairpin of IKZF1 or IKZF3 (e.g., human IKZF1 or IKZF3). In some embodiments, the COF2/CRBN-binding polypeptide comprises a beta strand. In some embodiments, the COF2/CRBN-binding polypeptide comprises a beta strand of IKZF1 or IKZF3 (e.g., human IKZF1 or IKZF3). In some embodiments, the COF2/CRBN-binding polypeptide comprises an alpha helix. In some embodiments, the COF2/CRBN-binding polypeptide comprises an alpha helix of IKZF1 or IKZF3 (e.g., human IKZF1 or IKZF3). In some embodiments, the COF2/CRBN-binding polypeptide comprises, from N-terminus to C-terminus, a first

beta strand, a beta hairpin, a second beta strand, and a first alpha helix. In some embodiments, the COF2/CRBN-binding polypeptide comprises, from N-terminus to C-terminus, a first beta strand, a beta hairpin, a second beta strand, and a first alpha helix of IKZF1 or IKZF3 (e.g., human IKZF1 or IKZF3). In some embodiments, the COF2/CRBN-binding polypeptide comprises, from N-terminus to C-terminus, a first beta strand, a beta hairpin, a second beta strand, a first alpha helix, and a second alpha helix. In some embodiments, the COF2/CRBN-binding polypeptide comprises, from N-terminus to C-terminus, a first beta strand, a beta hairpin, a second beta strand, a first alpha helix, and a second alpha helix of IKZF1 or IKZF3 (e.g., human IKZF1 or IKZF3). In some embodiments, the beta hairpin and the second alpha helix are separated by no more than 60, 50, 40, or 30 amino acid residues.

In some embodiments, the COF2/CRBN-binding polypeptide comprises a COF2/CRBN-binding sequence from a naturally occurring polypeptide or a COF2/CRBN-binding variant thereof. In some embodiments, the COF2/CRBN-binding polypeptide comprises a COF2/CRBN-binding sequence from a naturally occurring IKZF polypeptide or a COF2/CRBN-binding variant thereof. In some embodiments, the COF2/CRBN-binding polypeptide comprises a COF2/CRBN-binding sequence from a naturally occurring IKZF1, IKZF2, IKZF3, IKZF4, or IKZF5, or a COF2/CRBN-binding variant thereof. In some embodiments, the COF2/CRBN-binding sequence comprises two or more discontinuous sequences from the naturally occurring polypeptide, e.g., a naturally occurring IKZF polypeptide, e.g., a naturally occurring IKZF1, IKZF2, IKZF3, IKZF4, or IKZF5.

In some embodiments, the COF2/CRBN-binding polypeptide comprises an IKZF polypeptide or a structural motif thereof.

In some embodiments, the IKZF polypeptide is an IKZF1 polypeptide, an IKZF3 polypeptide, an IKZF2 polypeptide having H141Q substitution (numbered according to SEQ ID NO: 21), or an IKZF4 polypeptide having H188Q substitution (numbered according to SEQ ID NO: 22).

In some embodiments, the COF2/CRBN-binding polypeptide comprises sufficient amino acid sequence and/or a structural motif from IKZF (e.g., IKZF1 or IKZF3) that:

i) the association of the COF2/CRBN-binding polypeptide with CRBN in the absence of COF2 is no more than, e.g., 0.01%, 0.1%, 1%, 5%, 10%, 15%, or 20%, of the association of the COF2/CRBN-binding polypeptide with CRBN in the presence of COF2, e.g., an excess of COF2, e.g., as measured by an assay described herein, e.g., immunoprecipitation;

ii) the association of the fusion polypeptide with CRBN in the absence of COF2 is no more than, e.g., 0.01%, 0.1%, 1%, 5%, 10%, 15%, or 20% of the association of the fusion polypeptide with CRBN in the presence of COF2, e.g., an excess of COF2, e.g., as measured by an assay described herein, e.g., immunoprecipitation;



iii) the ubiquitination of the heterologous polypeptide in the absence of COF2 is no more than, e.g., 0.01%, 0.1%, 1%, 10%, 20%, 30%, 40%, 50%, 60%, or 70%, of the ubiquitination of the heterologous polypeptide in the presence of COF2, e.g., an excess of COF2, e.g., as measured by an assay described herein;

iv) the ubiquitination of the fusion polypeptide in the absence of COF2 is no more than, e.g., 0.01%, 0.1%, 1%, 10%, 20%, 30%, 40%, 50%, 60%, or 70%, of the ubiquitination of the fusion polypeptide in the presence of COF2, e.g., an excess of COF2, e.g., as measured by an assay described herein; or

v) the degradation of the fusion polypeptide in the absence of COF2 is no more than, e.g., 0.01%, 0.1%, 1%, 10%, 20%, 30%, 40%, 50%, 60%, or 70%, of the degradation of the fusion polypeptide in the presence of COF2, e.g., an excess of COF2, e.g., as measured by an assay described herein, e.g., a Western blot analysis or a flow cytometry analysis.

In some embodiments, the association, ubiquitination, and/or degradation is as measured in a mammalian cell, e.g., a human cell.

In some embodiments, the COF2/CRBN-binding polypeptide comprises about 10 to about 95 amino acid residues, about 15 to about 90 amino acid residues, about 20 to about 85 amino acid residues, about 25 to about 80 amino acid residues, about 30 to about 75 amino acid residues, about 35 to about 70 amino acid residues, about 40 to about 65 amino acid residues, about 45 to about 65 amino acid residues, about 50 to about 65 amino acid residues, or about 55 to about 65 amino acid residues of IKZF1 or IKZF3.

In some embodiments, the COF2/CRBN-binding polypeptide comprises sufficient amino acid sequence and/or a structural motif from amino acid residues 136-180 of IKZF3 (numbered according to SEQ ID NO: 19) (e.g., sufficient amino acid sequence and/or a structural motif from amino acid residues 136-180 of SEQ ID NO: 19) that:

i) the association of the COF2/CRBN-binding polypeptide with CRBN in the absence of COF2 is no more than, e.g., 0.01%, 0.1%, 1%, 5%, 10%, 15%, or 20%, of the association of the COF2/CRBN-binding polypeptide with CRBN in the presence of COF2, e.g., an excess of COF2, e.g., as measured by an assay described herein, e.g., immunoprecipitation;

ii) the association of the fusion polypeptide with CRBN in the absence of COF2 is no more than, e.g., 0.01%, 0.1%, 1%, 5%, 10%, 15%, or 20% of the association of the fusion polypeptide with CRBN in the presence of COF2, e.g., an excess of COF2, e.g., as measured by an assay described herein, e.g., immunoprecipitation;

iii) the ubiquitination of the heterologous polypeptide in the absence of COF2 is no more than, e.g., 0.01%, 0.1%, 1%, 10%, 20%, 30%, 40%, 50%, 60%, or 70%, of the ubiquitination of the

heterologous polypeptide in the presence of COF2, e.g., an excess of COF2, e.g., as measured by an assay described herein;

iv) the ubiquitination of the fusion polypeptide in the absence of COF2 is no more than, e.g., 0.01%, 0.1%, 1%, 10%, 20%, 30%, 40%, 50%, 60%, or 70%, of the ubiquitination of the fusion

polypeptide in the presence of COF2, e.g., an excess of COF2, e.g., as measured by an assay described herein; or

v) the degradation of the fusion polypeptide in the absence of COF2 is no more than, e.g., 0.01%, 0.1%, 1%, 10%, 20%, 30%, 40%, 50%, 60%, or 70%, of the degradation of the fusion polypeptide in the presence of COF2, e.g., an excess of COF2, e.g., as measured by an assay described herein, e.g., a

Western blot analysis or a flow cytometry analysis.

In some embodiments, the association, ubiquitination, and/or degradation is as measured in a mammalian cell, e.g., a human cell.

In some embodiments, the COF2/CRBN-binding polypeptide comprises amino acid residues 136-180 of IKZF3 (numbered according to SEQ ID NO: 19) (e.g., the COF2/CRBN-binding polypeptide comprises the amino acid sequence of SEQ ID NO: 5) or a sequence that differs from amino acid residues 136-180 of IKZF3 (numbered according to SEQ ID NO: 19) by no more than 1, 2, 3, 4, 5, 10, 15, 20, 25, 30, 35, or 40 amino acid residues (e.g., a sequence that differs from amino acid residues 136-180 of SEQ ID NO: 19 by no more than 1, 2, 3, 4, 5, 10, 15, 20, 25, 30, 35, or 40 amino acid residues) (e.g., a sequence having no more than 1, 2, 3, 4, 5, 10, 15, 20, 25, 30, 35, or 40 amino acid substitutions from amino acid residues 136-180 of SEQ ID NO: 19).

In some embodiments, the COF2/CRBN-binding polypeptide comprises sufficient amino acid sequence and/or a structural motif from amino acid residues 136-170 of IKZF3 (numbered according to SEQ ID NO: 19) (e.g., sufficient amino acid sequence and/or a structural motif from amino acid residues 136-170 of SEQ ID NO: 19) that:

i) the association of the COF2/CRBN-binding polypeptide with CRBN in the absence of COF2 is no more than, e.g., 0.01%, 0.1%, 1%, 5%, 10%, 15%, or 20%, of the association of the COF2/CRBN-binding polypeptide with CRBN in the presence of COF2, e.g., an excess of COF2, e.g., as measured by an assay described herein, e.g., immunoprecipitation;

ii) the association of the fusion polypeptide with CRBN in the absence of COF2 is no more than, e.g., 0.01%, 0.1%, 1%, 5%, 10%, 15%, or 20% of the association of the fusion polypeptide with CRBN in the presence of COF2, e.g., an excess of COF2, e.g., as measured by an assay described herein, e.g., immunoprecipitation;

iii) the ubiquitination of the heterologous polypeptide in the absence of COF2 is no more than, e.g., 0.01%, 0.1%, 1%, 10%, 20%, 30%, 40%, 50%, 60%, or 70%, of the ubiquitination of the

heterologous polypeptide in the presence of COF2, e.g., an excess of COF2, e.g., as measured by an assay described herein;

iv) the ubiquitination of the fusion polypeptide in the absence of COF2 is no more than, e.g., 0.01%, 0.1%, 1%, 10%, 20%, 30%, 40%, 50%, 60%, or 70%, of the ubiquitination of the fusion

polypeptide in the presence of COF2, e.g., an excess of COF2, e.g., as measured by an assay described herein; or

v) the degradation of the fusion polypeptide in the absence of COF2 is no more than, e.g., 0.01%, 0.1%, 1%, 10%, 20%, 30%, 40%, 50%, 60%, or 70%, of the degradation of the fusion polypeptide in the presence of COF2, e.g., an excess of COF2, e.g., as measured by an assay described herein, e.g., a

Western blot analysis or a flow cytometry analysis.

In some embodiments, the association, ubiquitination, and/or degradation is as measured in a mammalian cell, e.g., a human cell.

In some embodiments, the COF2/CRBN-binding polypeptide comprises amino acid residues 136-170 of IKZF3 (numbered according to SEQ ID NO: 19) (e.g., the COF2/CRBN-binding polypeptide comprises the amino acid sequence of SEQ ID NO: 6) or a sequence that differs from amino acid residues 136-170 of IKZF3 (numbered according to SEQ ID NO: 19) by no more than 1, 2, 3, 4, 5, 10, 15, 20, 25, or 30 amino acid residues (e.g., a sequence that differs from amino acid residues 136-170 of SEQ ID NO: 19 by no more than 1, 2, 3, 4, 5, 10, 15, 20, 25, or 30 amino acid residues) (e.g., a sequence having no more than 1, 2, 3, 4, 5, 10, 15, 20, 25, or 30 amino acid substitutions from amino acid residues 136-170 of SEQ ID NO: 19).

In some embodiments, one, two, three or all of the following amino acid residues remain unaltered: glutamine at position 147, cysteine at position 148, glutamine at position 150, glycine at position 152, leucine at position 161, or leucine at position 162, numbered according to SEQ ID NO: 19.

In some embodiments, glutamine at position 147 remains unaltered, numbered according to SEQ ID NO:

19. In some embodiments, cysteine at position 148 remains unaltered, numbered according to SEQ ID NO: 19. In some embodiments, glutamine at position 150 remains unaltered, numbered according to SEQ ID NO: 19. In some embodiments, glycine at position 152 remains unaltered, numbered according to SEQ ID NO: 19. In some embodiments, leucine at position 161 remains unaltered, numbered according to SEQ ID NO: 19. In some embodiments, leucine at position 162 remains unaltered, numbered according to SEQ ID NO: 19.

In some embodiments, the COF2/CRBN-binding polypeptide comprises amino acid residues 136-139 of IKZF3 (numbered according to SEQ ID NO: 19), e.g., the COF2/CRBN-binding polypeptide comprises the amino acid sequence of SEQ ID NO: 40.

In some embodiments, the COF2/CRBN-binding polypeptide comprises amino acid residues 136-180 of IKZF3 (numbered according to SEQ ID NO: 19). In some embodiments, the COF2/CRBN-binding polypeptide comprises the amino acid sequence of SEQ ID NO: 5. In some embodiments, the COF2/CRBN-binding polypeptide comprises the amino acid sequence of SEQ ID NO: 77. In some  
 5       embodiments, the COF2/CRBN-binding polypeptide consists of the amino acid sequence of SEQ ID NO: 5. In some embodiments, the COF2/CRBN-binding polypeptide consists of the amino acid sequence of SEQ ID NO: 5.

In some embodiments, the COF2/CRBN-binding polypeptide comprises amino acid residues 136-170 of IKZF3 (numbered according to SEQ ID NO: 19). In some embodiments, the COF2/CRBN-binding polypeptide comprises the amino acid sequence of SEQ ID NO: 6. In some embodiments, the COF2/CRBN-binding polypeptide comprises the amino acid sequence of SEQ ID NO: 78. In some  
 10       embodiments, the COF2/CRBN-binding polypeptide consists of the amino acid sequence of SEQ ID NO: 6. In some embodiments, the COF2/CRBN-binding polypeptide consists of the amino acid sequence of SEQ ID NO: 78.

In some embodiments, the COF2/CRBN-binding polypeptide comprises sufficient amino acid sequence and/or a structural motif from amino acid residues 236-249 of IKZF3 (numbered according to SEQ ID NO: 19) (e.g., sufficient amino acid sequence and/or a structural motif from amino acid residues 236-249 of SEQ ID NO: 19) that:

i) the association of the COF2/CRBN-binding polypeptide with CRBN in the absence of COF2 is  
 20       no more than, e.g., 0.01%, 0.1%, 1%, 5%, 10%, 15%, or 20%, of the association of the COF2/CRBN-binding polypeptide with CRBN in the presence of COF2, e.g., an excess of COF2, e.g., as measured by an assay described herein, e.g., immunoprecipitation;

ii) the association of the fusion polypeptide with CRBN in the absence of COF2 is no more than, e.g., 0.01%, 0.1%, 1%, 5%, 10%, 15%, or 20% of the association of the fusion polypeptide with CRBN in  
 25       the presence of COF2, e.g., an excess of COF2, e.g., as measured by an assay described herein, e.g., immunoprecipitation;

iii) the ubiquitination of the heterologous polypeptide in the absence of COF2 is no more than, e.g., 0.01%, 0.1%, 1%, 10%, 20%, 30%, 40%, 50%, 60%, or 70%, of the ubiquitination of the heterologous polypeptide in the presence of COF2, e.g., an excess of COF2, e.g., as measured by an assay  
 30       described herein;

iv) the ubiquitination of the fusion polypeptide in the absence of COF2 is no more than, e.g., 0.01%, 0.1%, 1%, 10%, 20%, 30%, 40%, 50%, 60%, or 70%, of the ubiquitination of the fusion polypeptide in the presence of COF2, e.g., an excess of COF2, e.g., as measured by an assay described herein; or

v) the degradation of the fusion polypeptide in the absence of COF2 is no more than, e.g., 0.01%, 0.1%, 1%, 10%, 20%, 30%, 40%, 50%, 60%, or 70%, of the degradation of the fusion polypeptide in the presence of COF2, e.g., an excess of COF2, e.g., as measured by an assay described herein, e.g., a Western blot analysis or a flow cytometry analysis.

5 In some embodiments, the association, ubiquitination, and/or degradation is as measured in a mammalian cell, e.g., a human cell.

In some embodiments, the COF2/CRBN-binding polypeptide comprises amino acid residues 236-249 of IKZF3 (numbered according to SEQ ID NO: 19) (e.g., the COF2/CRBN-binding polypeptide comprises the amino acid sequence of SEQ ID NO: 11) or a sequence that differs from amino acid  
10 residues 236-249 of IKZF3 (numbered according to SEQ ID NO: 19) by no more than 1, 2, 3, 4, 5, 6, or 7 amino acid residues (e.g., a sequence that differs from amino acid residues 236-249 of SEQ ID NO: 19 by no more than 1, 2, 3, 4, 5, 6, or 7 amino acid residues) (e.g., a sequence having no more than 1, 2, 3, 4, 5, 6, or 7 amino acid substitutions from amino acid residues 236-249 of SEQ ID NO: 19).

In some embodiments, the COF2/CRBN-binding polypeptide comprises amino acid residues 236-  
15 249 of IKZF3 (numbered according to SEQ ID NO: 19). In some embodiments, the COF2/CRBN-binding polypeptide comprises the amino acid sequence of SEQ ID NO: 11.

In some embodiments, the COF2/CRBN-binding polypeptide comprises the amino acid sequence of SEQ ID NO: 91.

In some embodiments, the COF2/CRBN-binding polypeptide comprises sufficient amino acid  
20 sequence and/or a structural motif from amino acid residues 136-180 and 236-249 of IKZF3 (numbered according to SEQ ID NO: 19) (e.g., sufficient amino acid sequence and/or a structural motif from amino acid residues 136-180 and 236-249 of SEQ ID NO: 19) that:

i) the association of the COF2/CRBN-binding polypeptide with CRBN in the absence of COF2 is no more than, e.g., 0.01%, 0.1%, 1%, 5%, 10%, 15%, or 20%, of the association of the COF2/CRBN-  
25 binding polypeptide with CRBN in the presence of COF2, e.g., an excess of COF2, e.g., as measured by an assay described herein, e.g., immunoprecipitation;

ii) the association of the fusion polypeptide with CRBN in the absence of COF2 is no more than, e.g., 0.01%, 0.1%, 1%, 5%, 10%, 15%, or 20% of the association of the fusion polypeptide with CRBN in the presence of COF2, e.g., an excess of COF2, e.g., as measured by an assay described herein, e.g.,  
30 immunoprecipitation;

iii) the ubiquitination of the heterologous polypeptide in the absence of COF2 is no more than, e.g., 0.01%, 0.1%, 1%, 10%, 20%, 30%, 40%, 50%, 60%, or 70%, of the ubiquitination of the heterologous polypeptide in the presence of COF2, e.g., an excess of COF2, e.g., as measured by an assay described herein;

iv) the ubiquitination of the fusion polypeptide in the absence of COF2 is no more than, e.g., 0.01%, 0.1%, 1%, 10%, 20%, 30%, 40%, 50%, 60%, or 70%, of the ubiquitination of the fusion polypeptide in the presence of COF2, e.g., an excess of COF2, e.g., as measured by an assay described herein; or

5 v) the degradation of the fusion polypeptide in the absence of COF2 is no more than, e.g., 0.01%, 0.1%, 1%, 10%, 20%, 30%, 40%, 50%, 60%, or 70%, of the degradation of the fusion polypeptide in the presence of COF2, e.g., an excess of COF2, e.g., as measured by an assay described herein, e.g., a Western blot analysis or a flow cytometry analysis.

In some embodiments, the association, ubiquitination, and/or degradation is as measured in a  
10 mammalian cell, e.g., a human cell.

In some embodiments, the COF2/CRBN-binding polypeptide comprises a first sequence comprising amino acid residues 136-180 of IKZF3 (numbered according to SEQ ID NO: 19) (e.g., a first sequence comprising the amino acid sequence of SEQ ID NO: 5) or a first sequence that differs from amino acid residues 136-180 of IKZF3 (numbered according to SEQ ID NO: 19) by no more than 1, 2, 3,  
15 4, 5, 10, 15, 20, 25, 30, 35, or 40 amino acid residues (e.g., a first sequence that differs from amino acid residues 136-180 of SEQ ID NO: 19 by no more than 1, 2, 3, 4, 5, 10, 15, 20, 25, 30, 35, or 40 amino acid residues); and a second sequence comprising amino acid residues 236-249 of IKZF3 (numbered according to SEQ ID NO: 19) (e.g., a second sequence comprising the amino acid sequence of SEQ ID NO: 11) or a second sequence that differs from amino acid residues 236-249 of IKZF3 (numbered  
20 according to SEQ ID NO: 19) by no more than 1, 2, 3, 4, 5, 6, or 7 amino acid residues (e.g., a second sequence that differs from amino acid residues 236-249 of SEQ ID NO: 19 by no more than 1, 2, 3, 4, 5, 6, or 7 amino acid residues).

In some embodiments, the COF2/CRBN-binding polypeptide comprises amino acid residues 136-180 and 236-249 of IKZF3 (numbered according to SEQ ID NO: 19). In some embodiments, the  
25 COF2/CRBN-binding polypeptide comprises the amino acid sequence of SEQ ID NO: 1. In some embodiments, the COF2/CRBN-binding polypeptide comprises the amino acid sequence of SEQ ID NO: 3. In some embodiments, the COF2/CRBN-binding polypeptide consists of the amino acid sequence of SEQ ID NO: 1. In some embodiments, the COF2/CRBN-binding polypeptide consists of the amino acid sequence of SEQ ID NO: 3.

30 In some embodiments, the COF2/CRBN-binding polypeptide comprises a first sequence comprising amino acid residues 136-180 of IKZF3 (numbered according to SEQ ID NO: 19) and a second sequence comprising the amino acid sequence of MALEKMALEKMALE (SEQ ID NO: 91). In some embodiments, the COF2/CRBN-binding polypeptide comprises the amino acid sequence of SEQ ID NO: 14. In some embodiments, the COF2/CRBN-binding polypeptide comprises the amino acid sequence of

SEQ ID NO: 85. In some embodiments, the COF2/CRBN-binding polypeptide consists of the amino acid sequence of SEQ ID NO: 14. In some embodiments, the COF2/CRBN-binding polypeptide consists of the amino acid sequence of SEQ ID NO: 85.

In some embodiments, the COF2/CRBN-binding polypeptide comprises a first sequence  
5 comprising amino acid residues 136-170 of IKZF3 (numbered according to SEQ ID NO: 19) and a second sequence comprising the amino acid sequence of MALEKMALEKMALE (SEQ ID NO: 91). In some embodiments, the COF2/CRBN-binding polypeptide comprises the amino acid sequence of SEQ ID NO: 15. In some embodiments, the COF2/CRBN-binding polypeptide comprises the amino acid sequence of SEQ ID NO: 86. In some embodiments, the COF2/CRBN-binding polypeptide consists of the amino acid  
10 sequence of SEQ ID NO: 15. In some embodiments, the COF2/CRBN-binding polypeptide consists of the amino acid sequence of SEQ ID NO: 86.

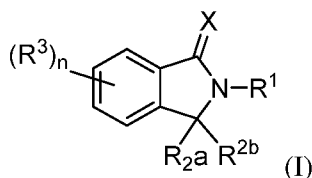
In some embodiments, the COF2/CRBN-binding polypeptide comprises at least one less lysine than the corresponding native sequence. In some embodiments, one or more lysine residues in the corresponding native sequence are replaced by a different amino acid, e.g., arginine. In some  
15 embodiments, the COF2/CRBN-binding polypeptide comprises less than 1, 2, 3, 4, or 5 lysine residues. In some embodiments, the COF2/CRBN-binding polypeptide does not comprise a lysine residue. In some embodiments, the COF2/CRBN-binding polypeptide is not ubiquitinated, e.g., in the presence of COF2, e.g., as measured by an assay described herein, optionally wherein ubiquitination is as measured in a mammalian cell, e.g., a human cell.

In some embodiments, the COF2/CRBN-binding polypeptide comprises an amino acid sequence  
20 selected from the group consisting of SEQ ID NOs: 4, 41, 42, and 43. In some embodiments, the COF2/CRBN-binding polypeptide comprises the amino acid sequence of SEQ ID NO: 4. In some embodiments, the COF2/CRBN-binding polypeptide comprises the amino acid sequence of SEQ ID NO: 41. In some embodiments, the COF2/CRBN-binding polypeptide comprises the amino acid sequence of  
25 SEQ ID NO: 42. In some embodiments, the COF2/CRBN-binding polypeptide comprises the amino acid sequence of SEQ ID NO: 43.

In some embodiments, the COF2/CRBN-binding polypeptide comprises the amino acid sequence of SEQ ID NO: 2. In some embodiments, the COF2/CRBN-binding polypeptide comprises the amino acid sequence of SEQ ID NO: 4. In some embodiments, the COF2/CRBN-binding polypeptide consists  
30 of the amino acid sequence of SEQ ID NO: 2. In some embodiments, the COF2/CRBN-binding polypeptide consists of the amino acid sequence of SEQ ID NO: 4.

In some embodiments, the COF2 is an immunomodulatory imide drug (IMiD), or a pharmaceutically acceptable salt thereof.

In some embodiments, the COF2 has the structure of Formula (I):



or a pharmaceutically acceptable salt, ester, hydrate, solvate, or tautomer thereof, wherein:

X is O or S;

R<sup>1</sup> is C<sub>1</sub>-C<sub>6</sub> alkyl, C<sub>2</sub>-C<sub>6</sub> alkenyl, C<sub>2</sub>-C<sub>6</sub> alkynyl, C<sub>1</sub>-C<sub>6</sub> heteroalkyl, carbocyclyl, heterocyclyl, aryl,  
 5 or heteroaryl, each of which is independently and optionally substituted by one or more R<sup>4</sup>;

each of R<sup>2a</sup> and R<sup>2b</sup> is independently hydrogen or C<sub>1</sub>-C<sub>6</sub> alkyl; or R<sup>2a</sup> and R<sup>2b</sup> together with the  
 carbon atom to which they are attached form a carbonyl group or a thiocarbonyl group;

each of R<sup>3</sup> is independently C<sub>1</sub>-C<sub>6</sub> alkyl, C<sub>2</sub>-C<sub>6</sub> alkenyl, C<sub>2</sub>-C<sub>6</sub> alkynyl, C<sub>1</sub>-C<sub>6</sub> heteroalkyl, halo,  
 cyano, -C(O)R<sup>A</sup>, -C(O)OR<sup>B</sup>, -OR<sup>B</sup>, -N(R<sup>C</sup>)(R<sup>D</sup>), -C(O)N(R<sup>C</sup>)(R<sup>D</sup>), -N(R<sup>C</sup>)C(O)R<sup>A</sup>, -S(O)<sub>x</sub>R<sup>E</sup>, -

10 S(O)<sub>x</sub>N(R<sup>C</sup>)(R<sup>D</sup>), or -N(R<sup>C</sup>)S(O)<sub>x</sub>R<sup>E</sup>, wherein each alkyl, alkenyl, alkynyl, and heteroalkyl is  
 independently and optionally substituted with one or more R<sup>6</sup>;

each R<sup>4</sup> is independently C<sub>1</sub>-C<sub>6</sub> alkyl, C<sub>2</sub>-C<sub>6</sub> alkenyl, C<sub>2</sub>-C<sub>6</sub> alkynyl, C<sub>1</sub>-C<sub>6</sub> heteroalkyl, halo,  
 cyano, oxo, -C(O)R<sup>A</sup>, -C(O)OR<sup>B</sup>, -OR<sup>B</sup>, -N(R<sup>C</sup>)(R<sup>D</sup>), -C(O)N(R<sup>C</sup>)(R<sup>D</sup>), -N(R<sup>C</sup>)C(O)R<sup>A</sup>, -S(O)<sub>x</sub>R<sup>E</sup>, -

15 S(O)<sub>x</sub>N(R<sup>C</sup>)(R<sup>D</sup>), -N(R<sup>C</sup>)S(O)<sub>x</sub>R<sup>E</sup>, carbocyclyl, heterocyclyl, aryl, or heteroaryl, wherein each alkyl,  
 alkenyl, alkynyl, heteroalkyl, carbocyclyl, heterocyclyl, aryl, and heteroaryl is independently and  
 optionally substituted with one or more R<sup>7</sup>;

each of R<sup>A</sup>, R<sup>B</sup>, R<sup>C</sup>, R<sup>D</sup>, and R<sup>E</sup> is independently hydrogen or C<sub>1</sub>-C<sub>6</sub> alkyl;

each R<sup>6</sup> is independently C<sub>1</sub>-C<sub>6</sub> alkyl, oxo, cyano, -OR<sup>B</sup>, -N(R<sup>C</sup>)(R<sup>D</sup>), -C(O)N(R<sup>C</sup>)(R<sup>D</sup>), -  
 N(R<sup>C</sup>)C(O)R<sup>A</sup>, aryl, or heteroaryl, wherein each aryl and heteroaryl is independently and optionally  
 20 substituted with one or more R<sup>8</sup>;

each R<sup>7</sup> is independently halo, oxo, cyano, -OR<sup>B</sup>, -N(R<sup>C</sup>)(R<sup>D</sup>), -C(O)N(R<sup>C</sup>)(R<sup>D</sup>), or -  
 N(R<sup>C</sup>)C(O)R<sup>A</sup>;

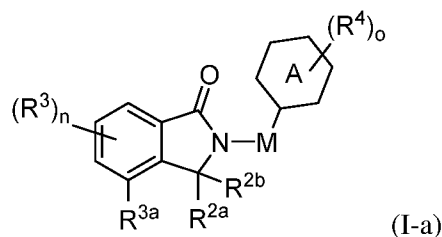
each R<sup>8</sup> is independently C<sub>1</sub>-C<sub>6</sub> alkyl, cyano, -OR<sup>B</sup>, -N(R<sup>C</sup>)(R<sup>D</sup>), -C(O)N(R<sup>C</sup>)(R<sup>D</sup>), or -  
 N(R<sup>C</sup>)C(O)R<sup>A</sup>;

25 n is 0, 1, 2, 3 or 4; and

x is 0, 1, or 2.

In some embodiments, the COF2 has the structure of Formula (I-a):





or a pharmaceutically acceptable salt, ester, hydrate, or tautomer thereof, wherein:

Ring A is carbocyclyl, heterocyclyl, aryl, or heteroaryl, each of which is independently and optionally substituted with one or more  $R^4$ ;

5 M is absent,  $C_1$ - $C_6$  alkyl,  $C_2$ - $C_6$  alkenyl,  $C_2$ - $C_6$  alkynyl, or  $C_1$ - $C_6$  heteroalkyl, wherein each alkyl, alkenyl, alkynyl, and heteroalkyl is independently and optionally substituted with one or more  $R^4$ ;

each of  $R^{2a}$  and  $R^{2b}$  is independently hydrogen or  $C_1$ - $C_6$  alkyl; or  $R^{2a}$  and  $R^{2b}$  together with the carbon atom to which they are attached to form a carbonyl group or thiocarbonyl group;

10  $R^{3a}$  is hydrogen,  $C_1$ - $C_6$  alkyl,  $C_2$ - $C_6$  alkenyl,  $C_2$ - $C_6$  alkynyl,  $C_1$ - $C_6$  heteroalkyl, halo, cyano, - $C(O)R^A$ , - $C(O)OR^B$ , - $OR^B$ , - $N(R^C)(R^D)$ , - $C(O)N(R^C)(R^D)$ , - $N(R^C)C(O)R^A$ , - $S(O)_xR^E$ , - $S(O)_xN(R^C)(R^D)$ , or - $N(R^C)S(O)_xR^E$ , wherein each alkyl, alkenyl, alkynyl, and heteroalkyl is independently and optionally substituted with one or more  $R^6$ ;

each of  $R^3$  is independently  $C_1$ - $C_6$  alkyl,  $C_2$ - $C_6$  alkenyl,  $C_2$ - $C_6$  alkynyl,  $C_1$ - $C_6$  heteroalkyl, halo, cyano, - $C(O)R^A$ , - $C(O)OR^B$ , - $OR^B$ , - $N(R^C)(R^D)$ , - $C(O)N(R^C)(R^D)$ , - $N(R^C)C(O)R^A$ , - $S(O)_xR^E$ , -  
15  $S(O)_xN(R^C)(R^D)$ , or - $N(R^C)S(O)_xR^E$ , wherein each alkyl, alkenyl, alkynyl, and heteroalkyl is independently and optionally substituted with one or more  $R^6$ ;

each  $R^4$  is independently  $C_1$ - $C_6$  alkyl,  $C_2$ - $C_6$  alkenyl,  $C_2$ - $C_6$  alkynyl,  $C_1$ - $C_6$  heteroalkyl, halo, cyano, oxo, - $C(O)R^A$ , - $C(O)OR^B$ , - $OR^B$ , - $N(R^C)(R^D)$ , - $C(O)N(R^C)(R^D)$ , - $N(R^C)C(O)R^A$ , - $S(O)_xR^E$ , -  
20  $S(O)_xN(R^C)(R^D)$ , - $N(R^C)S(O)_xR^E$ , carbocyclyl, heterocyclyl, aryl, or heteroaryl, wherein each alkyl, alkenyl, alkynyl, carbocyclyl, heterocyclyl, aryl, and heteroaryl is independently and optionally substituted with one or more  $R^7$ ;

each of  $R^A$ ,  $R^B$ ,  $R^C$ ,  $R^D$ , and  $R^E$  is independently hydrogen or  $C_1$ - $C_6$  alkyl;

each  $R^6$  is independently  $C_1$ - $C_6$  alkyl, oxo, cyano, - $OR^B$ , - $N(R^C)(R^D)$ , - $C(O)N(R^C)(R^D)$ , -  
25  $N(R^C)C(O)R^A$ , aryl, or heteroaryl, wherein each aryl and heteroaryl is independently and optionally substituted with one or more  $R^8$ ;

each  $R^7$  is independently halo, oxo, cyano, - $OR^B$ , - $N(R^C)(R^D)$ , - $C(O)N(R^C)(R^D)$ , or -  
 $N(R^C)C(O)R^A$ ;

each  $R^8$  is independently  $C_1$ - $C_6$  alkyl, cyano, - $OR^B$ , - $N(R^C)(R^D)$ , - $C(O)N(R^C)(R^D)$ , or -  
 $N(R^C)C(O)R^A$ ;

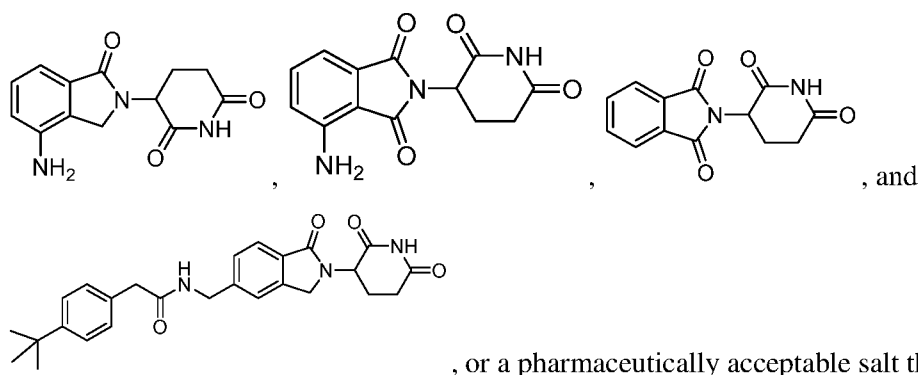
30 n is 0, 1, 2, or 3;

o is 0, 1, 2, 3, 4, or 5; and

x is 0, 1, or 2.

In some embodiments, the COF2 is thalidomide or analogue, or a pharmaceutically acceptable salt thereof. In some embodiments, the COF2 is selected from the group consisting of lenalidomide, pomalidomide, thalidomide, and 2-(4-(tert-butyl)phenyl)-N-((2-(2,6-dioxopiperidin-3-yl)-1-oxoisindolin-5-yl)methyl)acetamide, or a pharmaceutically acceptable salt thereof.

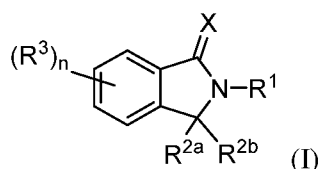
In some embodiments, the COF2 is selected from the group consisting of:



, or a pharmaceutically acceptable salt thereof.

In some embodiments, the COF2 is lenalidomide or pomalidomide, or a pharmaceutically acceptable salt thereof. In some embodiments, the COF2 is lenalidomide or analogue, or a pharmaceutically acceptable salt thereof. In some embodiments, the COF2 is lenalidomide, or a pharmaceutically acceptable salt thereof.

In some embodiments, the COF2 comprises the structure of Formula (I):



or a pharmaceutically acceptable salt, ester, hydrate, solvate, or tautomer thereof, wherein:

X is O or S;

$R^1$  is  $C_1$ - $C_6$  alkyl,  $C_2$ - $C_6$  alkenyl,  $C_2$ - $C_6$  alkynyl,  $C_1$ - $C_6$  heteroalkyl, carbocyclyl, heterocyclyl, aryl, or heteroaryl, each of which is independently and optionally substituted by one or more  $R^4$ ;

each of  $R^{2a}$  and  $R^{2b}$  is independently hydrogen or  $C_1$ - $C_6$  alkyl; or  $R^{2a}$  and  $R^{2b}$  together with the carbon atom to which they are attached form a carbonyl group or a thiocarbonyl group;

each of  $R^3$  is independently  $C_1$ - $C_6$  alkyl,  $C_2$ - $C_6$  alkenyl,  $C_2$ - $C_6$  alkynyl,  $C_1$ - $C_6$  heteroalkyl, halo, cyano,  $-C(O)R^A$ ,  $-C(O)OR^B$ ,  $-OR^B$ ,  $-N(R^C)(R^D)$ ,  $-C(O)N(R^C)(R^D)$ ,  $-N(R^C)C(O)R^A$ ,  $-S(O)_xR^E$ , -

$S(O)_xN(R^C)(R^D)$ , or  $-N(R^C)S(O)_xR^E$ , wherein each alkyl, alkenyl, alkynyl, and heteroalkyl is independently and optionally substituted with one or more  $R^6$ ;

each  $R^4$  is independently  $C_1$ - $C_6$  alkyl,  $C_2$ - $C_6$  alkenyl,  $C_2$ - $C_6$  alkynyl,  $C_1$ - $C_6$  heteroalkyl, halo, cyano, oxo,  $-C(O)R^A$ ,  $-C(O)OR^B$ ,  $-OR^B$ ,  $-N(R^C)(R^D)$ ,  $-C(O)N(R^C)(R^D)$ ,  $-N(R^C)C(O)R^A$ ,  $-S(O)_xR^E$ ,  $-S(O)_xN(R^C)(R^D)$ ,  $-N(R^C)S(O)_xR^E$ , carbocyclyl, heterocyclyl, aryl, or heteroaryl, wherein each alkyl, alkenyl, alkynyl, heteroalkyl, carbocyclyl, heterocyclyl, aryl, and heteroaryl is independently and optionally substituted with one or more  $R^7$ ;

each of  $R^A$ ,  $R^B$ ,  $R^C$ ,  $R^D$ , and  $R^E$  is independently hydrogen or  $C_1$ - $C_6$  alkyl;

each  $R^6$  is independently  $C_1$ - $C_6$  alkyl, oxo, cyano,  $-OR^B$ ,  $-N(R^C)(R^D)$ ,  $-C(O)N(R^C)(R^D)$ ,  $-N(R^C)C(O)R^A$ , aryl, or heteroaryl, wherein each aryl and heteroaryl is independently and optionally substituted with one or more  $R^8$ ;

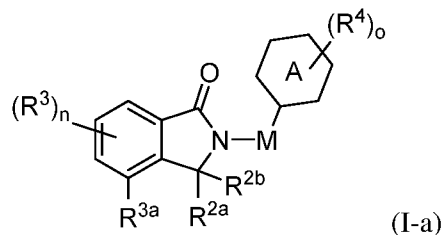
each  $R^7$  is independently halo, oxo, cyano,  $-OR^B$ ,  $-N(R^C)(R^D)$ ,  $-C(O)N(R^C)(R^D)$ , or  $-N(R^C)C(O)R^A$ ;

each  $R^8$  is independently  $C_1$ - $C_6$  alkyl, cyano,  $-OR^B$ ,  $-N(R^C)(R^D)$ ,  $-C(O)N(R^C)(R^D)$ , or  $-N(R^C)C(O)R^A$ ;

$n$  is 0, 1, 2, 3 or 4; and

$x$  is 0, 1, or 2.

In some embodiments, the COF2 comprises the structure of Formula (I-a):



or a pharmaceutically acceptable salt, ester, hydrate, or tautomer thereof, wherein:

Ring A is carbocyclyl, heterocyclyl, aryl, or heteroaryl, each of which is independently and optionally substituted with one or more  $R^4$ ;

M is absent,  $C_1$ - $C_6$  alkyl,  $C_2$ - $C_6$  alkenyl,  $C_2$ - $C_6$  alkynyl, or  $C_1$ - $C_6$  heteroalkyl, wherein each alkyl, alkenyl, alkynyl, and heteroalkyl is independently and optionally substituted with one or more  $R^4$ ;

each of  $R^{2a}$  and  $R^{2b}$  is independently hydrogen or  $C_1$ - $C_6$  alkyl; or  $R^{2a}$  and  $R^{2b}$  together with the carbon atom to which they are attached to form a carbonyl group or thiocarbonyl group;

$R^{3a}$  is hydrogen,  $C_1$ - $C_6$  alkyl,  $C_2$ - $C_6$  alkenyl,  $C_2$ - $C_6$  alkynyl,  $C_1$ - $C_6$  heteroalkyl, halo, cyano,  $-C(O)R^A$ ,  $-C(O)OR^B$ ,  $-OR^B$ ,  $-N(R^C)(R^D)$ ,  $-C(O)N(R^C)(R^D)$ ,  $-N(R^C)C(O)R^A$ ,  $-S(O)_xR^E$ ,  $-S(O)_xN(R^C)(R^D)$ , or  $-N(R^C)S(O)_xR^E$ , wherein each alkyl, alkenyl, alkynyl, and heteroalkyl is independently and optionally substituted with one or more  $R^6$ ;

each of  $R^3$  is independently  $C_1$ - $C_6$  alkyl,  $C_2$ - $C_6$  alkenyl,  $C_2$ - $C_6$  alkynyl,  $C_1$ - $C_6$  heteroalkyl, halo, cyano,  $-C(O)R^A$ ,  $-C(O)OR^B$ ,  $-OR^B$ ,  $-N(R^C)(R^D)$ ,  $-C(O)N(R^C)(R^D)$ ,  $-N(R^C)C(O)R^A$ ,  $-S(O)_xR^E$ , -

$S(O)_xN(R^C)(R^D)$ , or  $-N(R^C)S(O)_xR^E$ , wherein each alkyl, alkenyl, alkynyl, and heteroalkyl is independently and optionally substituted with one or more  $R^6$ ;

each  $R^4$  is independently  $C_1$ - $C_6$  alkyl,  $C_2$ - $C_6$  alkenyl,  $C_2$ - $C_6$  alkynyl,  $C_1$ - $C_6$  heteroalkyl, halo, cyano, oxo,  $-C(O)R^A$ ,  $-C(O)OR^B$ ,  $-OR^B$ ,  $-N(R^C)(R^D)$ ,  $-C(O)N(R^C)(R^D)$ ,  $-N(R^C)C(O)R^A$ ,  $S(O)_xR^E$ ,  $-S(O)_xN(R^C)(R^D)$ ,  $-N(R^C)S(O)_xR^E$ , carbocyclyl, heterocyclyl, aryl, or heteroaryl, wherein each alkyl, alkenyl, alkynyl, carbocyclyl, heterocyclyl, aryl, and heteroaryl is independently and optionally substituted with one or more  $R^7$ ;

each of  $R^A$ ,  $R^B$ ,  $R^C$ ,  $R^D$ , and  $R^E$  is independently hydrogen or  $C_1$ - $C_6$  alkyl;

each  $R^6$  is independently  $C_1$ - $C_6$  alkyl, oxo, cyano,  $-OR^B$ ,  $-N(R^C)(R^D)$ ,  $-C(O)N(R^C)(R^D)$ ,  $-N(R^C)C(O)R^A$ , aryl, or heteroaryl, wherein each aryl and heteroaryl is independently and optionally substituted with one or more  $R^8$ ;

each  $R^7$  is independently halo, oxo, cyano,  $-OR^B$ ,  $-N(R^C)(R^D)$ ,  $-C(O)N(R^C)(R^D)$ , or  $-N(R^C)C(O)R^A$ ;

each  $R^8$  is independently  $C_1$ - $C_6$  alkyl, cyano,  $-OR^B$ ,  $-N(R^C)(R^D)$ ,  $-C(O)N(R^C)(R^D)$ , or  $-N(R^C)C(O)R^A$ ;

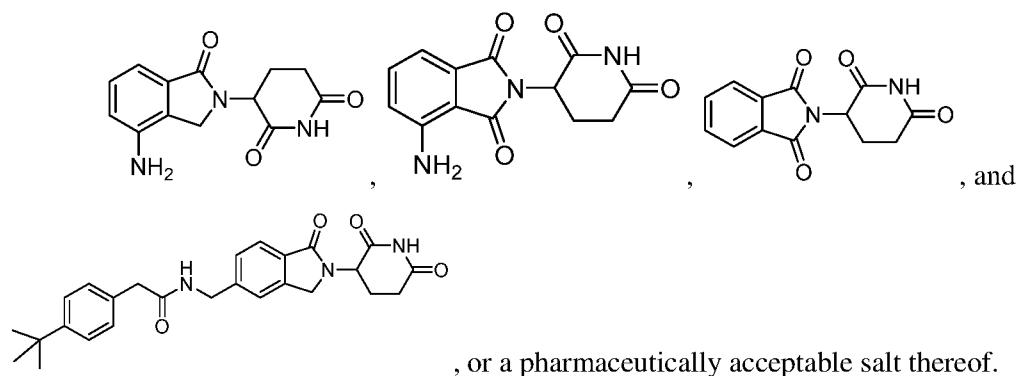
$n$  is 0, 1, 2, or 3;

$o$  is 0, 1, 2, 3, 4, or 5; and

$x$  is 0, 1, or 2.

In some embodiments, the COF2 comprises an immunomodulatory imide drug (IMiD), or a pharmaceutically acceptable salt thereof. In some embodiments, the COF2 comprises thalidomide or analogue, or a pharmaceutically acceptable salt thereof. In some embodiments, the COF2 comprises lenalidomide, pomalidomide, thalidomide, and 2-(4-(tert-butyl)phenyl)-N-((2-(2,6-dioxopiperidin-3-yl)-1-oxoisindolin-5-yl)methyl)acetamide, or a pharmaceutically acceptable salt thereof.

In some embodiments, the COF2 comprises a compound selected from the group consisting of:

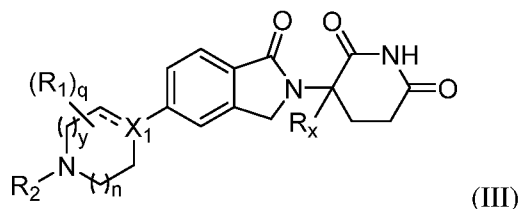


In some embodiments, the COF2 comprises lenalidomide or pomalidomide, or a pharmaceutically acceptable salt thereof. In some embodiments, the COF2 comprises lenalidomide or

analogue, or a pharmaceutically acceptable salt thereof. In some embodiments, the COF2 comprises lenalidomide, or a pharmaceutically acceptable salt thereof.

In some embodiments, the COF2 further comprises a ligand (e.g., wherein  $R^{10}$  in Formula (II) is L-Tag). In some embodiments,  $R^{10}$  in Formula (II) is L-Tag, L is a linker selected from a linker disclosed in International Patent Publication No. WO2017/024318 (e.g., Figures 28-31), and Tag is selected from a dTAG Targeting Ligand disclosed in International Patent Publication No. WO2017/024318 (e.g., Table T, pages 119-129). In some embodiments, the COF2 comprises IMiD (e.g., lenalidomide or pomalidomide, or a pharmaceutically acceptable salt thereof) and a ligand, wherein the IMiD (e.g., lenalidomide or pomalidomide, or a pharmaceutically acceptable salt thereof) is linked to the ligand, e.g., via a linker. In some embodiments, the COF2/CRBN-binding polypeptide binds to the ligand and wherein the binding between the COF2/CRBN-binding polypeptide and IMiD (e.g., lenalidomide or pomalidomide, or a pharmaceutically acceptable salt thereof) in the absence of the ligand is no more than 0.0001, 0.001, 0.01, 0.1, 1, or 10% of the binding between the COF2/CRBN-binding polypeptide and the ligand, e.g., the COF2/CRBN-binding polypeptide does not bind to IMiD (e.g., lenalidomide or pomalidomide, or a pharmaceutically acceptable salt thereof), optionally wherein the COF2/CRBN-binding polypeptide is selected from a dTAG disclosed in International Patent Publication No. WO2017/024318 (e.g., pages 36-65).

In one aspect, provided herein is a fusion polypeptide comprising a compound of Formula (III) (COF3)/CRBN-binding polypeptide and a heterologous polypeptide, wherein the compound of Formula (III) is:



or a pharmaceutically acceptable salt, ester, hydrate, solvate, or tautomer thereof, wherein:

$X_1$  is  $CR_3$ ;

----- is optionally a double bond when  $X_1$  is  $CR_3$  and  $R_3$  is absent;

each  $R_1$  is independently  $C_1$ - $C_6$  alkyl,  $C_1$ - $C_6$  haloalkyl,  $C_1$ - $C_6$  hydroxyalkyl, or halo, or

two  $R_1$  together with the carbon atoms to which they are attached form a 5- or 6- membered heterocyclyl ring, or

two  $R_1$ , when on adjacent atoms, together with the atoms to which they are attached form a  $C_6$ - $C_{10}$  aryl or 5- or 6-membered heteroaryl ring comprising 1 to 3 heteroatoms selected from O, N, and S;

R<sub>2</sub> is hydrogen, C<sub>1</sub>-C<sub>6</sub> alkyl, -C(O)C<sub>1</sub>-C<sub>6</sub> alkyl, -C(O)(CH<sub>2</sub>)<sub>0-3</sub>-C<sub>6</sub>-C<sub>10</sub> aryl, -C(O)O(CH<sub>2</sub>)<sub>0-3</sub>-C<sub>6</sub>-C<sub>10</sub>aryl, C<sub>6</sub>-C<sub>10</sub> aryl, or 5- or 6-membered heteroaryl comprising 1 to 3 heteroatoms selected from O, N, and S, C<sub>3</sub>-C<sub>8</sub> carbocyclyl, or 5- to 7-heterocyclyl comprising 1 to 3 heteroatoms selected from O, N, and S, wherein the alkyl is optionally substituted with one or more R<sub>4</sub>; and the aryl, heteroaryl, carbocyclyl, and heterocyclyl are optionally substituted with one or more R<sub>5</sub>, or

R<sub>1</sub> and R<sub>2</sub>, when on adjacent atoms, together with the atoms to which they are attached form a 5- or 6-membered heterocyclyl ring;

R<sub>3</sub> is hydrogen, or R<sub>3</sub> is absent when  $\text{-----}$  is a double bond;

each R<sub>4</sub> is independently selected from -C(O)OR<sub>6</sub>, -C(O)NR<sub>6</sub>R<sub>6</sub>, -NR<sub>6</sub>C(O)R<sub>6</sub>, halo, -OH, -NH<sub>2</sub>, cyano, C<sub>6</sub>-C<sub>10</sub> aryl, 5- or 6-membered heteroaryl comprising 1 to 4 heteroatoms selected from O, N, and S, C<sub>3</sub>-C<sub>8</sub> carbocyclyl, and 5- to 7-membered heterocyclyl ring comprising 1 to 3 heteroatoms selected from O, N, and S, wherein the aryl, heteroaryl, carbocyclyl, and heterocyclyl are optionally substituted with one or more R<sub>7</sub>;

each R<sub>5</sub> is independently selected from C<sub>1</sub>-C<sub>6</sub> alkyl, C<sub>2</sub>-C<sub>6</sub> alkenyl, C<sub>2</sub>-C<sub>6</sub> alkynyl, C<sub>1</sub>-C<sub>6</sub> alkoxy, C<sub>1</sub>-C<sub>6</sub> haloalkyl, C<sub>1</sub>-C<sub>6</sub> haloalkoxy, C<sub>1</sub>-C<sub>6</sub> hydroxyalkyl, halo, -OH, -NH<sub>2</sub>, cyano, C<sub>3</sub>-C<sub>7</sub> carbocyclyl, 5- to 7-membered heterocyclyl comprising 1 to 3 heteroatoms selected from O, N, and S, C<sub>6</sub>-C<sub>10</sub> aryl, and 5- or 6-membered heteroaryl comprising 1 to 3 heteroatoms selected from O, N, and S, or

two R<sub>5</sub>, when on adjacent atoms, together with the atoms to which they are attached form a C<sub>6</sub>-C<sub>10</sub> aryl or 5- or 6-membered heteroaryl comprising 1 to 3 heteroatoms selected from O, N, and S, optionally substituted with one or more R<sub>10</sub>, or

two R<sub>5</sub>, when on adjacent atoms, together with the atoms to which they are attached form a C<sub>5</sub>-C<sub>7</sub> carbocyclyl or 5- to 7-membered heterocyclyl comprising 1 to 3 heteroatoms selected from O, N, and S optionally substituted with one or more R<sub>10</sub>;

R<sub>6</sub> and R<sub>6</sub> are each independently hydrogen, C<sub>1</sub>-C<sub>6</sub> alkyl, or C<sub>6</sub>-C<sub>10</sub> aryl;

each R<sub>7</sub> is independently selected from C<sub>1</sub>-C<sub>6</sub> alkyl, C<sub>2</sub>-C<sub>6</sub> alkenyl, C<sub>2</sub>-C<sub>6</sub> alkynyl, C<sub>1</sub>-C<sub>6</sub> alkoxy, C<sub>1</sub>-C<sub>6</sub> haloalkyl, C<sub>1</sub>-C<sub>6</sub> haloalkoxy, -C(O)R<sub>8</sub>, -(CH<sub>2</sub>)<sub>0-3</sub>C(O)OR<sub>8</sub>, -C(O)NR<sub>8</sub>R<sub>9</sub>, -NR<sub>8</sub>C(O)R<sub>9</sub>, -NR<sub>8</sub>C(O)OR<sub>9</sub>, -S(O)<sub>p</sub>NR<sub>8</sub>R<sub>9</sub>, -S(O)<sub>p</sub>R<sub>12</sub>, (C<sub>1</sub>-C<sub>6</sub>)hydroxyalkyl, halo, -OH, -O(CH<sub>2</sub>)<sub>1-3</sub>CN, -NH<sub>2</sub>, cyano, -O(CH<sub>2</sub>)<sub>0-3</sub>-C<sub>6</sub>-C<sub>10</sub> aryl, adamantyl, -O(CH<sub>2</sub>)<sub>0-3</sub>-5- or 6-membered heteroaryl comprising 1 to 3 heteroatoms selected from O, N, and S, C<sub>6</sub>-C<sub>10</sub> aryl, monocyclic or bicyclic 5- to 10-membered heteroaryl comprising 1 to 3 heteroatoms selected from O, N, and S, C<sub>3</sub>-C<sub>7</sub> carbocyclyl, and 5- to 7-membered heterocyclyl comprising 1 to 3 heteroatoms selected from O, N, and S, wherein the alkyl is optionally substituted with one or more R<sub>11</sub>, and the aryl, heteroaryl, and heterocyclyl are optionally substituted with one or more substituents each independently selected from halogen, C<sub>1</sub>-C<sub>6</sub> alkyl, C<sub>1</sub>-C<sub>6</sub> haloalkyl, and C<sub>1</sub>-C<sub>6</sub> alkoxy, or

two R<sub>7</sub> together with the carbon atom to which they are attached form a =O), or

two R<sub>7</sub>, when on adjacent atoms, together with the atoms to which they are attached form a C<sub>6</sub>-C<sub>10</sub> aryl or 5- or 6-membered heteroaryl comprising 1 to 3 heteroatoms selected from O, N, and S, optionally substituted with one or more R<sub>10</sub>, or

5 two R<sub>7</sub> together with the atoms to which they are attached form a C<sub>5</sub>-C<sub>7</sub> carbocyclyl or a 5- to 7-membered heterocyclyl comprising 1 to 3 heteroatoms selected from O, N, and S, optionally substituted with one or more R<sub>10</sub>;

R<sub>8</sub> and R<sub>9</sub> are each independently hydrogen or C<sub>1</sub>-C<sub>6</sub> alkyl;

10 each R<sub>10</sub> is independently selected from C<sub>1</sub>-C<sub>6</sub> alkyl, C<sub>1</sub>-C<sub>6</sub> alkoxy, C<sub>1</sub>-C<sub>6</sub> haloalkyl, C<sub>1</sub>-C<sub>6</sub> haloalkoxy, C<sub>1</sub>-C<sub>6</sub> hydroxyalkyl, halo, -OH, -NH<sub>2</sub>, and cyano, or

two R<sub>10</sub> together with the carbon atom to which they are attached form a =O);

15 each R<sub>11</sub> is independently selected from cyano, C<sub>1</sub>-C<sub>6</sub> alkoxy, C<sub>6</sub>-C<sub>10</sub> aryl, and 5- to 7-membered heterocyclyl comprising 1 to 3 heteroatoms selected from O, N, and S, wherein each aryl and heterocyclyl is optionally substituted with one or more substituents each independently selected from C<sub>1</sub>-C<sub>6</sub> alkyl, C<sub>1</sub>-C<sub>6</sub> alkoxy, C<sub>1</sub>-C<sub>6</sub> haloalkyl, C<sub>1</sub>-C<sub>6</sub> haloalkoxy, C<sub>1</sub>-C<sub>6</sub> hydroxyalkyl, halo, -OH, -NH<sub>2</sub>, and cyano;

R<sub>12</sub> is C<sub>1</sub>-C<sub>6</sub> alkyl, C<sub>1</sub>-C<sub>6</sub> haloalkyl, C<sub>6</sub>-C<sub>10</sub> aryl, or 5- to 7-membered heterocyclyl comprising 1 to 3 heteroatoms selected from O, N, and S;

R<sub>x</sub> is hydrogen or deuterium;

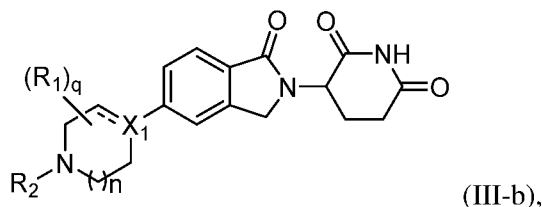
p is 0, 1, or 2;

20 n is 0, 1, or 2;

y is 1 or 2, wherein n + y ≤ 3; and

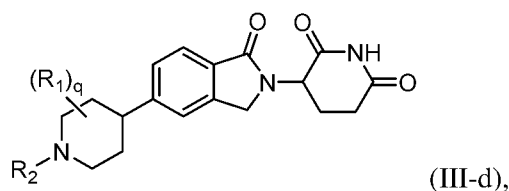
q is 0, 1, 2, 3, or 4.

In one embodiment, the compound of Formula (III) is a compound of Formula (III-b):



25 or a pharmaceutically acceptable salt, hydrate, solvate, prodrug, stereoisomer, and tautomer thereof, wherein X<sub>1</sub>, R<sub>1</sub>, R<sub>2</sub>, n, q, and subvariables thereof are defined as described above for Formula (III).

In one embodiment, the compound of Formula (III) is a compound of Formula (III-d):



or a pharmaceutically acceptable salt, hydrate, solvate, prodrug, stereoisomer, and tautomer thereof, wherein  $R_1$ ,  $R_2$ ,  $q$ , and subvariables thereof are defined as described above for Formula (III).

In one embodiment, the COF3/CRBN-binding polypeptide is fused to the heterologous polypeptide. In one embodiment, the COF3/CRBN-binding polypeptide and the heterologous polypeptide are linked by a peptide bond. In one embodiment, the COF3/CRBN-binding polypeptide and the heterologous polypeptide are linked by a bond other than a peptide bond. In one embodiment, the heterologous polypeptide is linked directly to the COF3/CRBN-binding polypeptide. In one embodiment, the heterologous polypeptide is linked indirectly to the COF3/CRBN-binding polypeptide. In one embodiment, the COF3/CRBN-binding polypeptide and the heterologous polypeptide are operatively linked via a linker, e.g., a glycine-serine linker, e.g., a linker comprising the amino acid sequence of SEQ ID NO: 28. In one embodiment, the COF3/CRBN-binding polypeptide is linked to the C-terminus of the heterologous polypeptide. In one embodiment, the COF3/CRBN-binding polypeptide is linked to the N-terminus of the heterologous polypeptide.

In one embodiment, the association of the fusion polypeptide with CRBN in the absence of COF3 is no more than, e.g., 0.01%, 0.1%, 1%, 5%, 10%, 15%, or 20%, of the association of the fusion polypeptide with CRBN in the presence of COF3, e.g., an excess of COF3, e.g., as measured by an assay described herein, e.g., immunoprecipitation. In one embodiment, the fusion polypeptide does not bind to CRBN in the absence of COF3. In one embodiment, the ubiquitination of the fusion polypeptide in the absence of COF3 is no more than, e.g., 0.01%, 0.1%, 1%, 10%, 20%, 30%, 40%, 50%, 60%, or 70%, of the ubiquitination of the fusion polypeptide in the presence of COF3, e.g., an excess of COF3, e.g., as measured by an assay described herein. In one embodiment, the fusion polypeptide is ubiquitinated at one or more lysine or methionine residues in the presence of COF3. In one embodiment, the degradation of the fusion polypeptide in the absence of COF3 is no more than, e.g., 0.01%, 0.1%, 1%, 10%, 20%, 30%, 40%, 50%, 60%, or 70% of the degradation of the fusion polypeptide in the presence of COF3, e.g., an excess of COF3, e.g., as measured by an assay described herein, e.g., a Western blot analysis or a flow cytometry analysis. In one embodiment, the degradation of the fusion polypeptide is mediated by ubiquitination in the presence of COF3. In one embodiment, the association, ubiquitination, and/or degradation is as measured in a mammalian cell, e.g., a human cell.

In one embodiment, the COF3/CRBN-binding polypeptide is between 10 and 95 amino acid residues in length, between 15 and 90 amino acid residues in length, between 20 and 85 amino acid



residues in length, between 25 and 80 amino acid residues in length, between 30 and 75 amino acid residues in length, between 35 and 70 amino acid residues in length, between 40 and 65 amino acid residues in length, between 45 and 65 amino acid residues in length, between 50 and 65 amino acid residues in length, or between 55 and 65 amino acid residues in length. In one embodiment, the  
 5 COF3/CRBN-binding polypeptide is 59 amino acid residues in length.

In one embodiment, the COF3/CRBN-binding polypeptide comprises a beta turn. In one embodiment, the COF3/CRBN-binding polypeptide comprises a beta hairpin. In one embodiment, the COF3/CRBN-binding polypeptide comprises a beta strand. In one embodiment, the COF3/CRBN-binding polypeptide comprises an alpha helix. In one embodiment, the COF3/CRBN-binding polypeptide  
 10 comprises, from N-terminus to C-terminus, a first beta strand, a beta hairpin, a second beta strand, and a first alpha helix. In one embodiment, the COF3/CRBN-binding polypeptide comprises, from N-terminus to C-terminus, a first beta strand, a beta hairpin, a second beta strand, a first alpha helix, and a second alpha helix. In one embodiment, the beta hairpin and the second alpha helix are separated by no more than 60, 50, 40, or 30 amino acid residues.

In one embodiment, the COF3/CRBN-binding polypeptide comprises a COF3/CRBN-binding sequence from a naturally occurring polypeptide or a COF3/CRBN-binding variant thereof. In one embodiment, the COF3/CRBN-binding polypeptide comprises a COF3/CRBN-binding sequence from a naturally occurring IKZF polypeptide or a COF3/CRBN-binding variant thereof. In one embodiment, the COF3/CRBN-binding polypeptide comprises a COF3/CRBN-binding sequence from a naturally  
 20 occurring IKZF2, or a COF3/CRBN-binding variant thereof. In one embodiment, the COF3/CRBN-binding polypeptide comprises two or more discontinuous sequences from a naturally occurring IKZF polypeptide, e.g., a naturally occurring IKZF2.

In one embodiment, the COF3/CRBN-binding polypeptide comprises amino acid residues 130-174 of IKZF2 (numbered according to SEQ ID NO: 21). In one embodiment, the COF3/CRBN-binding polypeptide comprises the amino acid sequence of SEQ ID NO: 113. In one embodiment, the COF3/CRBN-binding polypeptide comprises a sequence that differs from amino acid residues 130-174 of IKZF2 (numbered according to SEQ ID NO: 21) by no more than 1, 2, 3, 4, 5, 10, 15, 20, 25, 30, 35, or 40 amino acid residues. In one embodiment, the COF3/CRBN-binding polypeptide comprises a sequence that differs from amino acid residues 130-174 of SEQ ID NO: 21 by no more than 1, 2, 3, 4, 5, 10, 15, 20,  
 25 25, 30, 35, or 40 amino acid residues. In one embodiment, the COF3/CRBN-binding polypeptide comprise a sequence having no more than 1, 2, 3, 4, 5, 10, 15, 20, 25, 30, 35, or 40 amino acid substitutions from amino acid residues 130-174 of SEQ ID NO: 21.

In one embodiment, the COF3/CRBN-binding polypeptide comprises amino acid residues 230-243 of IKZF2 (numbered according to SEQ ID NO: 21). In one embodiment, the COF3/CRBN-binding

polypeptide comprises the amino acid sequence of SEQ ID NO: 114. In one embodiment, the COF3/CRBN-binding polypeptide comprises a sequence that differs from amino acid residues 230-243 of IKZF2 (numbered according to SEQ ID NO: 21) by no more than 1, 2, 3, 4, 5, or 10 amino acid residues. In one embodiment, the COF3/CRBN-binding polypeptide comprises a sequence that differs from amino acid residues 230-243 of SEQ ID NO: 21 by no more than 1, 2, 3, 4, 5, or 10 amino acid residues. In one embodiment, the COF3/CRBN-binding polypeptide comprises a sequence having no more than 1, 2, 3, 4, 5, or 10 amino acid substitutions from amino acid residues 230-243 of SEQ ID NO: 21.

In one embodiment, histidine at position 141 remains unaltered, numbered according to SEQ ID NO: 21.

In one embodiment, the COF3/CRBN-binding polypeptide comprises amino acid residues 130-174 of IKZF2 (numbered according to SEQ ID NO: 21). In one embodiment, the COF3/CRBN-binding polypeptide comprises the amino acid sequence of SEQ ID NO: 113. In one embodiment, the COF3/CRBN-binding polypeptide comprises amino acid residues 230-243 of IKZF2 (numbered according to SEQ ID NO: 21). In one embodiment, the COF3/CRBN-binding polypeptide comprises the amino acid sequence of SEQ ID NO: 114. In one embodiment, the COF3/CRBN-binding polypeptide comprises or consists of the amino acid sequence of SEQ ID NO: 109.

In some embodiments, the fusion polypeptide further comprises a degradation domain, wherein the degradation domain is separated from the COF3/CRBN-binding polypeptide and the heterologous polypeptide by a heterologous protease cleavage site.

In some embodiments, the fusion polypeptide comprises, from N-terminus to C-terminus:

- i) the degradation domain, the heterologous protease cleavage site, the heterologous polypeptide, and the COF3/CRBN-binding polypeptide;
- ii) the degradation domain, the heterologous protease cleavage site, the COF3/CRBN-binding polypeptide, and the heterologous polypeptide;
- iii) the COF3/CRBN-binding polypeptide, the heterologous polypeptide, the heterologous protease cleavage site, and the degradation domain; or
- iv) the heterologous polypeptide, and the COF3/CRBN-binding polypeptide, the heterologous protease cleavage site, and the degradation domain.

In some embodiments, the fusion polypeptide comprises, from N-terminus to C-terminus, the degradation domain, the heterologous protease cleavage site, the heterologous polypeptide, and the COF3/CRBN-binding polypeptide.

In some embodiments, the degradation domain has a first state associated with a first level of expression of the fusion polypeptide and a second state associated with a second level of expression of the

fusion polypeptide, wherein the second level is increased, e.g., by at least 2-, 3-, 4-, 5-, 10-, 20- or 30-fold over the first level in the presence of a stabilization compound.

In some embodiments, in the absence of the stabilization compound, the fusion polypeptide is degraded by a cellular degradation pathway, e.g., at least 50%, 60%, 70%, 80%, 90% or greater of the fusion polypeptide is degraded, e.g., as measured by an assay described herein, e.g., a Western blot analysis or a flow cytometry analysis.

In some embodiments, the level of expression, and/or degradation is as measured in a mammalian cell, e.g., a human cell.

In some embodiments, in the presence of the stabilization compound:

- i) the degradation domain assumes a conformation more resistant to cellular degradation relative to a conformation in the absence of the stabilization compound; or
- ii) the conformation of the fusion polypeptide is more permissive to cleavage at the heterologous protease cleavage site relative to a conformation in the absence of the stabilization compound.

In some embodiments, the degradation domain is chosen from an estrogen receptor (ER) domain, an FKB protein (FKBP) domain, or a dihydrofolate reductase (DHFR) domain.

In some embodiments, the degradation domain is an estrogen receptor (ER) domain. In some embodiments, the degradation domain comprises an amino acid sequence that is at least 90, 95, 97, 98, 99, or 100% identical to SEQ ID NO: 46 or 48. In some embodiments, the degradation domain comprises the amino acid sequence of SEQ ID NO: 46. In some embodiments, the degradation domain comprises the amino acid sequence of SEQ ID NO: 48.

In some embodiments, the stabilization compound is bazedoxifene or 4-hydroxy tamoxifen (4-OHT), or a pharmaceutically acceptable salt thereof. In some embodiments, the degradation domain is an estrogen receptor (ER) domain and the stabilization compound is bazedoxifene, or a pharmaceutically acceptable salt thereof. In some embodiments, the degradation domain comprises the amino acid sequence of SEQ ID NO: 46 and the stabilization compound is bazedoxifene, or a pharmaceutically acceptable salt thereof.

In some embodiments, the degradation domain is an FKB protein (FKBP) domain. In some embodiments, the degradation domain comprises an amino acid sequence that is at least 90, 95, 97, 98, 99, or 100% identical to SEQ ID NO: 50. In some embodiments, the degradation domain comprises the amino acid sequence of SEQ ID NO: 50. In some embodiments, the stabilization compound is Shield-1, or a pharmaceutically acceptable salt thereof.

In some embodiments, the degradation domain is a dihydrofolate reductase (DHFR) domain. In some embodiments, the degradation domain comprises an amino acid sequence that is at least 90, 95, 97, 98, 99, or 100% identical to SEQ ID NO: 51. In some embodiments, the degradation domain comprises

the amino acid sequence of SEQ ID NO: 51. In some embodiments, the stabilization compound is trimethoprim, or a pharmaceutically acceptable salt thereof.

In some embodiments, the heterologous protease cleavage site is cleaved by a mammalian intracellular protease. In some embodiments, the heterologous protease cleavage site is cleaved by a protease selected from the group consisting of furin, PCSK1, PCSK5, PCSK6, PCSK7, cathepsin B, Granzyme B, Factor XA, Enterokinase, genenase, sortase, precision protease, thrombin, TEV protease, and elastase 1. In some embodiments, the heterologous protease cleavage site comprises a sequence having a cleavage motif selected from the group consisting of RX(K/R)R consensus motif (X can be any amino acid; SEQ ID NO: 52), RXXX[KR]R consensus motif (X can be any amino acid; SEQ ID NO: 53), RRX consensus motif (SEQ ID NO : 54), I-E-P-D-X consensus motif (SEQ ID NO: 55), Ile-Glu/Asp-Gly-Arg (SEQ IDNO : 56), Asp-Asp-Asp-Asp-Lys (SEQ ID NO: 57), Pro-Gly-Ala-Ala-His-Tyr (SEQ ID NO: 58), LPXTG/A consensus motif (SEQ ID NO: 59), Leu-Glu-Val-Phe-Gln-Gly-Pro (SEQ ID NO: 60), Leu-Val-Pro-Arg-Gly-Ser (SEQ ID NO: 61), E-N-L-Y-F-Q-G (SEQ ID NO: 62), and [AGSV]-X (X can be any amino acid; SEQ ID NO: 63). In some embodiments, the heterologous protease cleavage site is cleaved by furin. In some embodiments, the heterologous protease cleavage site comprises a furin cleavage site selected from the group consisting of RTKR (SEQ ID NO: 123); GTGAEDPRPSRKRRSLGDVG (SEQ ID NO: 125); GTGAEDPRPSRKRR (SEQ ID NO: 127); LQWLEQQVAKRRTRK (SEQ ID NO: 129); GTGAEDPRPSRKRRSLGG (SEQ ID NO: 131); GTGAEDPRPSRKRRSLG (SEQ ID NO: 133); SLNLTESHNSRKRR (SEQ ID NO: 135); CKINGYPKRGRKRR (SEQ ID NO: 137); and SARNRQKR (SEQ ID NO: 34). In some embodiments, the heterologous protease cleavage site does not comprise the amino acid sequence of SARNRQKR (SEQ ID NO: 34). In some embodiments, the heterologous protease cleavage site comprises the furin cleavage site of GTGAEDPRPSRKRRSLGDVG (SEQ ID NO: 125). In some embodiments, the heterologous protease cleavage site is cleaved by a mammalian extracellular protease. In some embodiments, the mammalian extracellular protease is selected from the group consisting of Factor XA, Enterokinase, genenase, sortase, precision protease, thrombin, TEV protease, and elastase 1. In some embodiments, the heterologous protease cleavage site comprises an amino acid sequence selected from the group consisting of Ile-Glu/Asp-Gly-Arg (SEQ IDNO : 56), Asp-Asp-Asp-Asp-Lys (SEQ ID NO: 57), Pro-Gly-Ala-Ala-His-Tyr (SEQ ID NO: 58), LPXTG/A consensus motif (SEQ ID NO: 59), Leu-Glu-Val-Phe-Gln-Gly-Pro (SEQ ID NO: 60), Leu-Val-Pro-Arg-Gly-Ser (SEQ ID NO: 61), E-N-L-Y-F-Q-G (SEQ ID NO: 62), and [AGSV]-X (X can be any amino acid; SEQ ID NO: 63).

In certain embodiments of the foregoing aspects, the heterologous polypeptide is chosen from a cytoplasmic and/or nuclear polypeptide, or a transmembrane polypeptide, e.g., a heterologous polypeptide

in Table 2. In some embodiments, the cytoplasmic and/or nuclear polypeptide is selected from the group consisting of a component of the apoptosis pathway (e.g., Caspase 9), a component of a CRISPR/Cas system (e.g., Cas9), a transcription factor (e.g., MITF, c-Myc, STAT3, STAT5, NF-kappaB, beta-catenin, Notch, GLI, or c-JUN), Tet methylcytosine dioxygenase 2 (TET2), FKBP, and Tau. In some  
 5 embodiments, the transmembrane polypeptide is selected from the group consisting of CD62L, CCR1, CCR2, CCR5, CCR7, CCR10, CXCR2, CXCR3, CXCR4, CXCR6, CTLA4, PD1, BTLA, VISTA, CD137L, CD80, CD86, TIGIT, CD3, CD8, CD19, CD22, CD20, BCMA, and a chimeric antigen receptor (CAR). In some embodiments, the heterologous polypeptide is selected from the group consisting of a chimeric antigen receptor (CAR), a component of a CRISPR/Cas system (e.g., Cas9), CD8, CD19, and  
 10 CD22.

In some embodiments, the heterologous polypeptide is a chimeric antigen receptor (CAR). In some embodiments, the CAR comprises, in a N-terminal to C-terminal direction, an antigen binding domain, a transmembrane domain, and one or more intracellular signaling domains. In some  
 15 embodiments, the intracellular signaling domain comprises one or more primary signaling domains. In some embodiments, the intracellular signaling domain comprises one or more costimulatory signaling domains. In some embodiments, one of the one or more primary signaling domains comprises a CD3-zeta stimulatory domain. In some embodiments, one or more of the costimulatory signaling domains is an intracellular domain from a costimulatory protein selected from the group consisting of CD27, CD28, 4-1BB (CD137), OX40, GITR, CD30, CD40, ICOS, BAFFR, HVEM, ICAM-1, lymphocyte function-  
 20 associated antigen-1 (LFA-1), CD2, CDS, CD7, CD287, LIGHT, NKG2C, NKG2D, SLAMF7, NKp80, NKp30, NKp44, NKp46, CD160, B7-H3, and a ligand that specifically binds with CD83. In some embodiments, the one or more of said costimulatory signaling domains comprises the 4-1BB costimulatory domain. In some embodiments, the one or more of said costimulatory signaling domains comprises the CD28 costimulatory domain. In some embodiments, the antigen binding domain is an  
 25 scFv.

In some embodiments, the antigen binding domain binds an antigen selected from the group consisting of CD19; CD123; CD22; CD30; CD171; CS-1; C-type lectin-like molecule-1, CD33; epidermal growth factor receptor variant III (EGFRvIII); ganglioside G2 (GD2); ganglioside GD3; TNF receptor family member; B-cell maturation antigen; Tn antigen ((Tn Ag) or (GalNAc $\alpha$ -Ser/Thr));  
 30 prostate-specific membrane antigen (PSMA); Receptor tyrosine kinase-like orphan receptor 1 (ROR1); Fms-Like Tyrosine Kinase 3 (FLT3); Tumor-associated glycoprotein 72 (TAG72); CD38; CD44v6; Carcinoembryonic antigen (CEA); Epithelial cell adhesion molecule (EPCAM); B7H3 (CD276); KIT (CD117); Interleukin-13 receptor subunit alpha-2; Mesothelin; Interleukin 11 receptor alpha (IL-11Ra); prostate stem cell antigen (PSCA); Protease Serine 21; vascular endothelial growth factor receptor 2

(VEGFR2); Lewis(Y) antigen; CD24; Platelet-derived growth factor receptor beta (PDGFR-beta); Stage-specific embryonic antigen-4 (SSEA-4); CD20; Folate receptor alpha; Receptor tyrosine-protein kinase ERBB2 (Her2/neu); Mucin 1, cell surface associated (MUC1); epidermal growth factor receptor (EGFR); neural cell adhesion molecule (NCAM); Prostase; prostatic acid phosphatase (PAP); elongation factor 2 mutated (ELF2M); Ephrin B2; fibroblast activation protein alpha (FAP); insulin-like growth factor 1 receptor (IGF-I receptor), carbonic anhydrase IX (CAIX); Proteasome (Prosome, Macropain) Subunit, Beta Type, 9 (LMP2); glycoprotein 100 (gp100); oncogene polypeptide consisting of breakpoint cluster region (BCR) and Abelson murine leukemia viral oncogene homolog 1 (Abl) (bcr-abl); tyrosinase; ephrin type-A receptor 2 (EphA2); Fucosyl GM1; sialyl Lewis adhesion molecule (sLe); ganglioside GM3; transglutaminase 5 (TGS5); high molecular weight-melanoma-associated antigen (HMWMAA); o-acetyl-GD2 ganglioside (OAcGD2); Folate receptor beta; tumor endothelial marker 1 (TEM1/CD248); tumor endothelial marker 7-related (TEM7R); claudin 6 (CLDN6); thyroid stimulating hormone receptor (TSHR); G protein-coupled receptor class C group 5, member D (GPRC5D); chromosome X open reading frame 61 (CXORF61); CD97; CD179a; anaplastic lymphoma kinase (ALK); Polysialic acid; placenta-specific 1 (PLAC1); hexasaccharide portion of globoH glycosphingolipid (GloboH); mammary gland differentiation antigen (NY-BR-1); uroplakin 2 (UPK2); Hepatitis A virus cellular receptor 1 (HAVCR1); adrenoceptor beta 3 (ADRB3); pannexin 3 (PANX3); G protein-coupled receptor 20 (GPR20); lymphocyte antigen 6 complex, locus K 9 (LY6K); Olfactory receptor 51E2 (OR51E2); TCR Gamma Alternate Reading Frame Protein (TARP); Wilms tumor protein (WT1); Cancer/testis antigen 1 (NY-ESO-1); Cancer/testis antigen 2 (LAGE-1a); Melanoma-associated antigen 1 (MAGE-A1); ETS translocation-variant gene 6, located on chromosome 12p (ETV6-AML); sperm protein 17 (SPA17); X Antigen Family, Member 1A (XAGE1); angiopoietin-binding cell surface receptor 2 (Tie 2); melanoma cancer testis antigen-1 (MAD-CT-1); melanoma cancer testis antigen-2 (MAD-CT-2); Fos-related antigen 1; tumor protein p53 (p53); p53 mutant; prostatein; surviving; telomerase; prostate carcinoma tumor antigen-1, melanoma antigen recognized by T cells 1; Rat sarcoma (Ras) mutant; human Telomerase reverse transcriptase (hTERT); sarcoma translocation breakpoints; melanoma inhibitor of apoptosis (ML-IAP); ERG (transmembrane protease, serine 2 (TMPRSS2) ETS fusion gene); N-Acetyl glucosaminyl-transferase V (NA17); paired box protein Pax-3 (PAX3); Androgen receptor; Cyclin B1; v-myc avian myelocytomatosis viral oncogene neuroblastoma derived homolog (MYCN); Ras Homolog Family Member C (RhoC); Tyrosinase-related protein 2 (TRP-2); Cytochrome P450 1B1 (CYP1B1); CCCTC-Binding Factor (Zinc Finger Protein)-Like, Squamous Cell Carcinoma Antigen Recognized By T Cells 3 (SART3); Paired box protein Pax-5 (PAX5); proacrosin binding protein sp32 (OY-TES1); lymphocyte-specific protein tyrosine kinase (LCK); A kinase anchor protein 4 (AKAP-4); synovial sarcoma, X breakpoint 2 (SSX2); Receptor for Advanced Glycation Endproducts (RAGE-1); renal ubiquitous 1

(RU1); renal ubiquitous 2 (RU2); legumain; human papilloma virus E6 (HPV E6); human papilloma virus E7 (HPV E7); intestinal carboxyl esterase; heat shock protein 70-2 mutated (mut hsp70-2); CD79a; CD79b; CD72; Leukocyte-associated immunoglobulin-like receptor 1 (LAIR1); Fc fragment of IgA receptor (FCAR or CD89); Leukocyte immunoglobulin-like receptor subfamily A member 2 (LILRA2);  
 5 CD300 molecule-like family member f (CD300LF); C-type lectin domain family 12 member A (CLEC12A); bone marrow stromal cell antigen 2 (BST2); EGF-like module-containing mucin-like hormone receptor-like 2 (EMR2); lymphocyte antigen 75 (LY75); Glypican-3 (GPC3); Fc receptor-like 5 (FCRL5); and immunoglobulin lambda-like polypeptide 1 (IGLL1). In some embodiments, the antigen is chosen from CD19, CD22, BCMA, CD20, CD123, EGFRvIII, or mesothelin. In some embodiments, the  
 10 antigen is CD19. In some embodiments, the antigen is CD22. In some embodiments, the antigen is BCMA. In some embodiments, the antigen is CD20. In some embodiments, the antigen is CD123. In some embodiments, the antigen is EGFRvIII.

In one aspect, provided herein is a nucleic acid molecule encoding a fusion polypeptide disclosed  
 15 herein. In another aspect, provided herein is a vector comprising the nucleic acid molecule. In some embodiments, the vector is a viral vector. In some embodiments, the vector is a lentiviral vector. In another aspect, provided herein is a viral particle comprising the vector.

In another aspect, provided herein is a cell, e.g., a host cell, comprising a fusion polypeptide disclosed herein, a nucleic acid molecule disclosed herein, or a vector disclosed herein. In some  
 20 embodiments, the cell, e.g., host cell, is a mammalian cell, e.g., a human cell, e.g., a human effector cell, e.g., a human T cell or a human NK cell.

In some embodiments, the cell, e.g., host cell, is a CAR-expressing cell, e.g., a CAR-T cell. In some embodiments, the cell, e.g., host cell, comprises a component of a CRISPR/Cas system. In some  
 25 embodiments, the cell, e.g., host cell, is a human cancer cell, e.g., a human tumor cell.

In some embodiments, the cell, e.g., host cell, comprises a ubiquitin ligase complex, e.g., an E3  
 ubiquitin ligase complex, wherein the ubiquitin ligase complex comprises CRBN.

In some embodiments, the cell comprises a fusion polypeptide disclosed herein (e.g., a fusion polypeptide comprising a COF1/CRBN-binding polypeptide and a heterologous polypeptide), wherein  
 when the cell is contacted with COF1, e.g., an excess of COF1:

30 i) the association of the COF1/CRBN-binding polypeptide with CRBN is increased by at least, e.g., 10-, 50-, 100-, 1000-, or 10000-fold, compared to the association of the COF1/CRBN-binding polypeptide with CRBN when the cell is not contacted with COF1, e.g., as measured by an assay described herein, e.g., immunoprecipitation;

- ii) the association of the fusion polypeptide with CRBN is increased by at least, e.g., 10-, 50-, 100-, 1000-, or 10000-fold, compared to the association of the fusion polypeptide with CRBN when the cell is not contacted with COF1, e.g., as measured by an assay described herein, e.g., immunoprecipitation;
- iii) the ubiquitination of the heterologous polypeptide is increased by at least, e.g., 1.5-, 2-, 3-, 4-, 5-, 10-, 20-, 30-, 40-, or 50-fold, compared to the ubiquitination of the heterologous polypeptide when the cell is not contacted with COF1, e.g., as measured by an assay described herein;
- iv) the ubiquitination of the fusion polypeptide is increased by at least, e.g., 1.5-, 2-, 3-, 4-, 5-, 10-, 20-, 30-, 40-, or 50-fold, compared to the ubiquitination of the fusion polypeptide when the cell is not contacted with COF1, e.g., as measured by an assay described herein;
- v) the degradation of the fusion polypeptide is increased by at least, e.g., 1.5-, 2-, 3-, 4-, 5-, 10-, 20-, 30-, 40-, or 50-fold, compared to the degradation of the fusion polypeptide when the cell is not contacted with COF1, e.g., as measured by an assay described herein, e.g., a Western blot analysis or a flow cytometry analysis; or
- vi) the expression level of the fusion polypeptide is no more than, e.g., 0.01%, 0.1%, 1%, 10%, 20%, 30%, 40%, 50%, 60%, or 70%, of the expression level of the fusion polypeptide when the cell is not contacted with COF1, e.g., as measured by an assay described herein, e.g., a Western blot analysis or a flow cytometry analysis.

In some embodiments, the cell further comprises COF1, e.g., lenalidomide or pomalidomide, or a pharmaceutically acceptable salt thereof.

In some embodiments, the cell comprises a fusion polypeptide disclosed herein (e.g., a fusion polypeptide comprising a COF3/CRBN-binding polypeptide and a heterologous polypeptide), wherein when the cell is contacted with COF3, e.g., an excess of COF3:

- i) the association of the COF3/CRBN-binding polypeptide with CRBN is increased by at least, e.g., 10-, 50-, 100-, 1000-, or 10000-fold, compared to the association of the COF3/CRBN-binding polypeptide with CRBN when the cell is not contacted with COF3, e.g., as measured by an assay described herein, e.g., immunoprecipitation;
- ii) the association of the fusion polypeptide with CRBN is increased by at least, e.g., 10-, 50-, 100-, 1000-, or 10000-fold, compared to the association of the fusion polypeptide with CRBN when the cell is not contacted with COF3, e.g., as measured by an assay described herein, e.g., immunoprecipitation;
- iii) the ubiquitination of the heterologous polypeptide is increased by at least, e.g., 1.5-, 2-, 3-, 4-, 5-, 10-, 20-, 30-, 40-, or 50-fold, compared to the ubiquitination of the heterologous polypeptide when the cell is not contacted with COF3, e.g., as measured by an assay described herein;



iv) the ubiquitination of the fusion polypeptide is increased by at least, e.g., 1.5-, 2-, 3-, 4-, 5-, 10-, 20-, 30-, 40-, or 50-fold, compared to the ubiquitination of the fusion polypeptide when the cell is not contacted with COF3, e.g., as measured by an assay described herein;

v) the degradation of the fusion polypeptide is increased by at least, e.g., 1.5-, 2-, 3-, 4-, 5-, 10-, 20-, 30-, 40-, or 50-fold, compared to the degradation of the fusion polypeptide when the cell is not contacted with COF3, e.g., as measured by an assay described herein, e.g., a Western blot analysis or a flow cytometry analysis; or

vi) the expression level of the fusion polypeptide is no more than, e.g., 0.01%, 0.1%, 1%, 10%, 20%, 30%, 40%, 50%, 60%, or 70%, of the expression level of the fusion polypeptide when the cell is not contacted with COF3, e.g., as measured by an assay described herein, e.g., a Western blot analysis or a flow cytometry analysis.

In some embodiments, the cell further comprises COF3, e.g., a compound disclosed in Table 29, or a pharmaceutically acceptable salt thereof.

In some embodiments, the cell comprises a fusion polypeptide disclosed herein (e.g., a fusion polypeptide comprising a COF1/CRBN-, COF2/CRBN-, or COF3/CRBN-binding polypeptide, a heterologous polypeptide, and a degradation domain), wherein in the absence of a stabilization compound, the fusion polypeptide is degraded by a cellular degradation pathway, e.g., at least 50%, 60%, 70%, 80%, 90% or greater of the fusion polypeptide is degraded, e.g., as measured by an assay described herein, e.g., a Western blot analysis or a flow cytometry analysis.

In some embodiments, the fusion polypeptide further comprises a heterologous protease cleavage site. In some embodiments, the cell further comprises a protease capable of cleaving the heterologous protease cleavage site.

In some embodiments, the cell comprises a fusion polypeptide disclosed herein (e.g., a fusion polypeptide comprising a COF1/CRBN-, COF2/CRBN-, or COF3/CRBN-binding polypeptide, a heterologous polypeptide, and a degradation domain), wherein when the cell is contacted with a stabilization compound, e.g., an excess of a stabilization compound:

i) the degradation domain assumes a conformation more resistant to cellular degradation relative to a conformation in the absence of the stabilization compound;

ii) the conformation of the fusion polypeptide is more permissive to cleavage at the heterologous protease cleavage site relative to a conformation in the absence of the stabilization compound; or

iii) the expression level of the fusion polypeptide is increased by at least, e.g., 1.5-, 2-, 3-, 4-, 5-, 10-, 20-, 30-, 40-, or 50-fold, compared to the expression level of the fusion polypeptide when the cell is not contacted with the stabilization compound, e.g., as measured by an assay described herein, e.g., a Western blot analysis or a flow cytometry analysis.

In some embodiments, the cell further comprises a stabilization compound. In some embodiments, the stabilization compound is bazedoxifene, or a pharmaceutically acceptable salt thereof. In some embodiments, the degradation domain comprises the amino acid sequence of SEQ ID NO: 46.

In some embodiments, the cell comprises a fusion polypeptide disclosed herein (e.g., a fusion polypeptide comprising a COF1/CRBN-, COF2/CRBN-, or COF3/CRBN-binding polypeptide, a heterologous polypeptide, and a degradation domain), wherein when the cell is contacted with both a stabilization compound, e.g., an excess of a stabilization compound, and COF1, COF2, or COF3, e.g., an excess of COF1, COF2, or COF3:

i) the association of the COF1/CRBN-, COF2/CRBN-, or COF3/CRBN-binding polypeptide with CRBN is increased by at least, e.g., 10, 50, 100, 1000, or 10000 fold, compared to the association of the COF1/CRBN-, COF2/CRBN-, or COF3/CRBN-binding polypeptide with CRBN when the cell is only contacted with the stabilization compound but not COF1, COF2, or COF3, e.g., as measured by an assay described herein, e.g., immunoprecipitation;

ii) the association of the fusion polypeptide with CRBN is increased by at least, e.g., 10-, 50-, 100-, 1000-, or 10000-fold, compared to the association of the fusion polypeptide with CRBN when the cell is only contacted with the stabilization compound but not COF1, COF2, or COF3, e.g., as measured by an assay described herein, e.g., immunoprecipitation;

iii) the ubiquitination of the heterologous polypeptide is increased by at least, e.g., 1.5-, 2-, 3-, 4-, 5-, 10-, 20-, 30-, 40-, or 50-fold, compared to the ubiquitination of the heterologous polypeptide when the cell is only contacted with the stabilization compound but not COF1, COF2, or COF3, e.g., as measured by an assay described herein;

iv) the ubiquitination of the fusion polypeptide is increased by at least, e.g., 1.5-, 2-, 3-, 4-, 5-, 10-, 20-, 30-, 40-, or 50-fold, compared to the ubiquitination of the fusion polypeptide when the cell is only contacted with the stabilization compound but not COF1, COF2, or COF3, e.g., as measured by an assay described herein;

v) the degradation of the fusion polypeptide is increased by at least, e.g., 1.5-, 2-, 3-, 4-, 5-, 10-, 20-, 30-, 40-, or 50-fold, compared to the degradation of the fusion polypeptide when the cell is only contacted with the stabilization compound but not COF1, COF2, or COF3, e.g., as measured by an assay described herein, e.g., a Western blot analysis or a flow cytometry analysis; or

vi) the expression level of the fusion polypeptide is no more than, e.g., 0.01%, 0.1%, 1%, 10%, 20%, 30%, 40%, 50%, 60%, or 70%, of the expression level of the fusion polypeptide when the cell is only contacted with the stabilization compound but not COF1, COF2, or COF3, e.g., as measured by an assay described herein, e.g., a Western blot analysis or a flow cytometry analysis.

In some embodiments, the cell further comprises COF1 (e.g., lenalidomide or pomalidomide, or a pharmaceutically acceptable salt thereof), COF2 (e.g., lenalidomide or pomalidomide, or a pharmaceutically acceptable salt thereof), or COF3 (a compound disclosed in Table 29, or a pharmaceutically acceptable salt thereof).

5 In some embodiments, the heterologous polypeptide is a chimeric antigen receptor (CAR), optionally wherein the CAR comprises, in a N-terminal to C-terminal direction, an antigen binding domain, a transmembrane domain, and one or more intracellular signaling domains.

In one aspect, disclosed herein is a pharmaceutical composition comprising a fusion polypeptide disclosed herein or a cell disclosed herein, and a pharmaceutically acceptable carrier, excipient or  
10 stabilizer.

In one aspect, disclosed herein is a method of making a cell disclosed herein.

In one aspect, disclosed herein is a method of degrading a fusion polypeptide disclosed herein (e.g., a fusion polypeptide comprising a COF1/CRBN-binding polypeptide and a heterologous polypeptide (e.g., a CAR polypeptide)), comprising contacting the fusion polypeptide or a cell comprising  
15 said fusion polypeptide with COF1 (e.g., lenalidomide or pomalidomide, or a pharmaceutically acceptable salt thereof). In some embodiments, in the presence of COF1 (e.g., lenalidomide or pomalidomide, or a pharmaceutically acceptable salt thereof), the expression level of said fusion polypeptide is substantially decreased, e.g., by at least about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 60, 70, 80, 90, or 100 percent, relative to the expression level of said fusion polypeptide in the absence of COF1 (e.g., lenalidomide or  
20 pomalidomide, or a pharmaceutically acceptable salt thereof), e.g., as measured by an assay described herein, e.g., a Western blot analysis or a flow cytometry analysis. In some embodiments, the fusion polypeptide or the cell is contacted with COF1 (e.g., lenalidomide or pomalidomide, or a pharmaceutically acceptable salt thereof) *ex vivo*. In some embodiments, the fusion polypeptide or the cell is contacted with COF1 (e.g., lenalidomide or pomalidomide, or a pharmaceutically acceptable salt  
25 thereof) *in vivo*.

In one aspect, disclosed herein is a method of regulating the expression of a fusion polypeptide disclosed herein (e.g., a fusion polypeptide comprising a COF1/CRBN- or COF2/CRBN-binding polypeptide, a heterologous polypeptide (e.g., a CAR polypeptide), and a degradation domain), comprising:

30 i) contacting the fusion polypeptide or a cell comprising the fusion polypeptide with a stabilization compound, optionally wherein in the presence of the stabilization compound:

a) the degradation domain assumes a conformation more resistant to cellular degradation relative to a conformation in the absence of the stabilization compound;

b) the conformation of the fusion polypeptide is more permissive to cleavage at the heterologous protease cleavage site relative to a conformation in the absence of the stabilization compound; or

c) the expression level of the fusion polypeptide is increased by at least, e.g., 1.5-, 2-, 3-, 4-, 5-, 10-, 20-, 30-, 40-, or 50-fold, compared to the expression level of the fusion polypeptide in the absence of the stabilization compound, e.g., as measured by an assay described herein, e.g., a Western blot analysis or a flow cytometry analysis.

In some embodiments, the method further comprises, after step i):

ii) contacting the fusion polypeptide or a cell comprising the fusion polypeptide with COF1 (e.g., lenalidomide or pomalidomide, or a pharmaceutically acceptable salt thereof) or COF2 (e.g., lenalidomide or pomalidomide, or a pharmaceutically acceptable salt thereof), optionally wherein in the presence of COF1 or COF2, the expression level of the fusion polypeptide is substantially decreased, e.g., by at least about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 60, 70, 80, 90, or 100 percent, relative to the expression level of the fusion polypeptide after step i) and before step ii), e.g., as measured by an assay described herein, e.g., a Western blot analysis or a flow cytometry analysis.

In some embodiments, the stabilization compound is bazedoxifene, or a pharmaceutically acceptable salt thereof. In some embodiments, the degradation domain comprises the amino acid sequence of SEQ ID NO: 46.

In some embodiments, the fusion polypeptide or the cell is contacted with COF1 or COF2 (e.g., lenalidomide or pomalidomide, or a pharmaceutically acceptable salt thereof) and/or the stabilization compound *ex vivo*. In some embodiments, the fusion polypeptide or the cell is contacted with COF1 or COF2 (e.g., lenalidomide or pomalidomide, or a pharmaceutically acceptable salt thereof) and/or the stabilization compound *in vivo*.

In some embodiments, the heterologous polypeptide is a chimeric antigen receptor (CAR). In some embodiments, the CAR comprises, in a N-terminal to C-terminal direction, an antigen binding domain, a transmembrane domain, and one or more intracellular signaling domains.

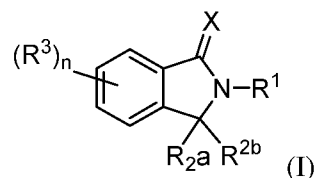
In one aspect, disclosed herein is a method of making a cell, comprising:

i) providing a cell comprising a nucleic acid molecule encoding a fusion polypeptide comprising a compound of formula 1 (COF1)/CRBN-binding polypeptide and a chimeric antigen receptor (CAR), optionally wherein the CAR comprises, in a N-terminal to C-terminal direction, an antigen binding domain, a transmembrane domain, and one or more intracellular signaling domains; and

ii) contacting the cell *ex vivo* with COF1, optionally wherein:

in the presence of COF1, the expression level of the fusion polypeptide is substantially decreased, e.g., by at least about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 60, 70, 80, 90, or 100 percent, relative to the expression level of the fusion polypeptide in the absence of COF1, e.g., as measured by an assay

described herein, e.g., a Western blot analysis or a flow cytometry analysis, wherein the compound of Formula (I) is:



or a pharmaceutically acceptable salt, ester, hydrate, solvate, or tautomer thereof, wherein:

5 X is O or S;

R¹ is C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, C₁-C₆ heteroalkyl, carbocyclyl, heterocyclyl, aryl, or heteroaryl, each of which is independently and optionally substituted by one or more R⁴;

each of R²ᵃ and R²ᵇ is independently hydrogen or C₁-C₆ alkyl; or R²ᵃ and R²ᵇ together with the carbon atom to which they are attached form a carbonyl group or a thiocarbonyl group;

10 each of R³ is independently C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, C₁-C₆ heteroalkyl, halo, cyano, -C(O)Rᵃ, -C(O)ORᵇ, -ORᵇ, -N(Rᶜ)(Rᵈ), -C(O)N(Rᶜ)(Rᵈ), -N(Rᶜ)C(O)Rᵃ, -S(O)ₓRᵉ, -S(O)ₓN(Rᶜ)(Rᵈ), or -N(Rᶜ)S(O)ₓRᵉ, wherein each alkyl, alkenyl, alkynyl, and heteroalkyl is independently and optionally substituted with one or more R⁶;

15 each R⁴ is independently C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, C₁-C₆ heteroalkyl, halo, cyano, oxo, -C(O)Rᵃ, -C(O)ORᵇ, -ORᵇ, -N(Rᶜ)(Rᵈ), -C(O)N(Rᶜ)(Rᵈ), -N(Rᶜ)C(O)Rᵃ, -S(O)ₓRᵉ, -S(O)ₓN(Rᶜ)(Rᵈ), -N(Rᶜ)S(O)ₓRᵉ, carbocyclyl, heterocyclyl, aryl, or heteroaryl, wherein each alkyl, alkenyl, alkynyl, heteroalkyl, carbocyclyl, heterocyclyl, aryl, and heteroaryl is independently and optionally substituted with one or more R⁷;

each of Rᵃ, Rᵇ, Rᶜ, Rᵈ, and Rᵉ is independently hydrogen or C₁-C₆ alkyl;

20 each R⁶ is independently C₁-C₆ alkyl, oxo, cyano, -ORᵇ, -N(Rᶜ)(Rᵈ), -C(O)N(Rᶜ)(Rᵈ), -N(Rᶜ)C(O)Rᵃ, aryl, or heteroaryl, wherein each aryl and heteroaryl is independently and optionally substituted with one or more R⁸;

each R⁷ is independently halo, oxo, cyano, -ORᵇ, -N(Rᶜ)(Rᵈ), -C(O)N(Rᶜ)(Rᵈ), or -N(Rᶜ)C(O)Rᵃ;

25 each R⁸ is independently C₁-C₆ alkyl, cyano, -ORᵇ, -N(Rᶜ)(Rᵈ), -C(O)N(Rᶜ)(Rᵈ), or -N(Rᶜ)C(O)Rᵃ;

n is 0, 1, 2, 3 or 4; and

x is 0, 1, or 2.

30 In some embodiments, after the cell is contacted with COF1 *ex vivo*, the proliferation of the cell is increased by at least, e.g., 1.2-, 1.5-, 2-, 5-, or 10-fold, relative to the proliferation of the cell prior to the contacting with COF1. In some embodiments, COF1 is lenalidomide or pomalidomide, or a

pharmaceutically acceptable salt thereof. In some embodiments, the COF1/CRBN-binding polypeptide comprises or consists of an amino acid sequence selected from the group consisting of SEQ ID NOs: 1-6, 11-15, 40, 41-43, 77, 78, 84-86, and 100 (e.g., the COF1/CRBN-binding polypeptide comprises or consists of the amino acid sequence of SEQ ID NO: 3).

In one aspect, provided herein is a method of treating a subject having a disease associated with expression of a tumor antigen, comprising:

i) contacting a cell disclosed herein (e.g., a cell comprising a fusion polypeptide comprising a COF1/CRBN-binding polypeptide and a heterologous polypeptide (e.g., a CAR polypeptide)) with COF1 *ex vivo*, optionally wherein:

in the presence of COF1, the expression level of the fusion polypeptide is decreased, e.g., by at least about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 60, 70, 80, 90, or 100 percent, relative to the expression level of the fusion polypeptide before the cell is contacted with COF1 *ex vivo*, and

ii) administering to the subject an effective amount of the cell, optionally wherein the method further comprises after step i) and prior to step ii):

reducing the amount of COF1 contacting the cell, e.g., inside and/or surrounding the cell, thereby treating the disease.

In some embodiments, the method further comprises after step ii):

iii) administering to the subject an effective amount of COF1, optionally wherein the administration of COF1 decreases, e.g., by at least about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 60, 70, 80, 90, or 100

percent, the expression level of the fusion polypeptide relative to the expression level of the fusion polypeptide after step ii) and prior to step iii), optionally wherein:

a) the subject has developed, is developing, or is anticipated to develop an adverse reaction,

b) the administration of COF1 is in response to an occurrence of an adverse reaction in the subject, or in response to an anticipation of an occurrence of an adverse reaction in the subject, and/or

c) the administration of COF1 reduces or prevents an adverse effect.

In some embodiments, the method further comprises after step iii):

iv) discontinuing the administration of COF1, optionally wherein discontinuing the administration of COF1 increases, e.g., by at least about 1.5-, 2-, 3-, 4-, 5-, 10-, 20-, 30-, 40-, or 50-fold, the expression level of the fusion polypeptide relative to the expression level of the fusion polypeptide after step iii) and

prior to step iv) (e.g., wherein discontinuing the administration of COF1 restores the expression level of the fusion polypeptide to the expression level after step ii) and prior to step iii)), optionally wherein:

a) the subject has relapsed, is relapsing, or is anticipated to relapse,

b) the discontinuation of the administration of COF1 is in response to a tumor relapse in the subject, or in response to an anticipation of a relapse in the subject, and/or

c) the discontinuation of the administration of COF1 treats or prevents a tumor relapse.

In some embodiments, the method further comprises after step iv):

v) repeating step iii) and/or iv),

thereby treating the disease.

5 In some embodiments, COF1 is lenalidomide or pomalidomide, or a pharmaceutically acceptable salt thereof, optionally wherein the COF1/CRBN-binding polypeptide comprises or consists of an amino acid sequence selected from the group consisting of SEQ ID NOs: 1-6, 11-15, 40, 41-43, 77, 78, 84-86, and 100 (e.g., the COF1/CRBN-binding polypeptide comprises or consists of the amino acid sequence of SEQ ID NO: 3). In some embodiments, COF1 is lenalidomide, or a pharmaceutically acceptable salt thereof, optionally wherein lenalidomide, or a pharmaceutically acceptable salt thereof, is administered at, e.g., 2.5 mg, 5 mg, 10 mg, 15 mg, or 25 mg per day.

In one aspect, provided herein is a method of treating a subject having a disease associated with expression of a tumor antigen, comprising:

15 i) administering to the subject an effective amount of a cell disclosed herein (e.g., a cell comprising a fusion polypeptide comprising a COF1/CRBN-binding polypeptide and a heterologous polypeptide (e.g., a CAR polypeptide)), optionally wherein the cell is contacted with COF1 *ex vivo* before administration, optionally wherein:  
in the presence of COF1, the expression level of the fusion polypeptide is decreased, e.g., by at least about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 60, 70, 80, 90, or 100 percent, relative to the expression  
20 level of the fusion polypeptide before the cell is contacted with COF1 *ex vivo*, optionally wherein after the cell is contacted with COF1 *ex vivo* and before the cell is administered to the subject, the amount of COF1 contacting the cell, e.g., inside and/or surrounding the cell, is reduced,  
thereby treating the disease.

In some embodiments, the cell is not contacted with COF1 *ex vivo* before administration.

25 In some embodiments, the method further comprises after step i):

ii) administering to the subject an effective amount of COF1, optionally wherein the administration of COF1 decreases, e.g., by at least about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 60, 70, 80, 90, or 100 percent, the expression level of the fusion polypeptide relative to the expression level of the fusion polypeptide after step i) and prior to step ii), optionally wherein:

30 a) the subject has developed, is developing, or is anticipated to develop an adverse reaction,  
b) the administration of COF1 is in response to an occurrence of an adverse reaction in the subject, or in response to an anticipation of an occurrence of an adverse reaction in the subject, and/or  
c) the administration of COF1 reduces or prevents an adverse effect.

In some embodiments, the method further comprises after step ii):

iii) discontinuing the administration of COF1, optionally wherein discontinuing the administration of COF1 increases, e.g., by at least about 1.5-, 2-, 3-, 4-, 5-, 10-, 20-, 30-, 40-, or 50-fold, the expression level of the fusion polypeptide relative to the expression level of the fusion polypeptide after step ii) and prior to step iii) (e.g., wherein discontinuing the administration of COF1 restores the expression level of the fusion polypeptide to the expression level after step i) and prior to step ii)), optionally wherein:

- a) the subject has relapsed, is relapsing, or is anticipated to relapse,
- b) the discontinuation of the administration of COF1 is in response to a tumor relapse in the subject, or in response to an anticipation of a relapse in the subject, and/or
- c) the discontinuation of the administration of COF1 treats or prevents a tumor relapse.

In some embodiments, the method further comprises after step iii):

iv) repeating step ii) and/or iii),  
thereby treating the disease.

In some embodiments, COF1 is lenalidomide or pomalidomide, or a pharmaceutically acceptable salt thereof, optionally wherein the COF1/CRBN-binding polypeptide comprises or consists of an amino acid sequence selected from the group consisting of SEQ ID NOs: 1-6, 11-15, 40, 41-43, 77, 78, 84-86, and 100 (e.g., the COF1/CRBN-binding polypeptide comprises or consists of the amino acid sequence of SEQ ID NO: 3). In some embodiments, COF1 is lenalidomide, or a pharmaceutically acceptable salt thereof, optionally wherein lenalidomide, or a pharmaceutically acceptable salt thereof, is administered at, e.g., 2.5 mg, 5 mg, 10 mg, 15 mg, or 25 mg per day.

In one aspect, provided herein is a method of treating a subject having a disease associated with expression of a tumor antigen, comprising:

- i) administering an effective amount of COF1 to the subject, wherein the subject comprises a cell disclosed herein (e.g., a cell comprising a fusion polypeptide comprising a COF1/CRBN-binding polypeptide and a heterologous polypeptide (e.g., a CAR polypeptide)), optionally wherein the administration of COF1 decreases, e.g., by at least about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 60, 70, 80, 90, or 100 percent, the expression level of the fusion polypeptide relative to the expression level of the fusion polypeptide before the administration of COF1, optionally wherein:
  - a) the subject has developed, is developing, or is anticipated to develop an adverse reaction,
  - b) the administration of COF1 is in response to an occurrence of an adverse reaction in the subject, or in response to an anticipation of an occurrence of an adverse reaction in the subject, and/or
  - c) the administration of COF1 reduces or prevents an adverse effect.

In some embodiments, the method further comprises after step i):

- ii) discontinuing the administration of COF1, optionally wherein discontinuing the administration of COF1 increases, e.g., by at least about 1.5-, 2-, 3-, 4-, 5-, 10-, 20-, 30-, 40-, or 50-fold, the expression



level of the fusion polypeptide relative to the expression level of the fusion polypeptide after step i) and prior to step ii) (e.g., wherein discontinuing the administration of COF1 restores the expression level of the fusion polypeptide to the expression level before the administration of COF1), optionally wherein:

- a) the subject has relapsed, is relapsing, or is anticipated to relapse,
- 5 b) the discontinuation of the administration of COF1 is in response to a tumor relapse in the subject, or in response to an anticipation of a relapse in the subject, and/or
- c) the discontinuation of the administration of COF1 treats or prevents a tumor relapse.

In some embodiments, the method further comprises after step ii):

- iii) repeating step i) and/or ii),
- 10 thereby treating the disease.

In some embodiments, COF1 is lenalidomide or pomalidomide, or a pharmaceutically acceptable salt thereof, optionally wherein the COF1/CRBN-binding polypeptide comprises or consists of an amino acid sequence selected from the group consisting of SEQ ID NOs: 1-6, 11-15, 40, 41-43, 77, 78, 84-86, and 100 (e.g., the COF1/CRBN-binding polypeptide comprises or consists of the amino acid sequence of

15 SEQ ID NO: 3). In some embodiments, COF1 is lenalidomide, or a pharmaceutically acceptable salt thereof, optionally wherein lenalidomide, or a pharmaceutically acceptable salt thereof, is administered at, e.g., 2.5 mg, 5 mg, 10 mg, 15 mg, or 25 mg per day.

In one aspect, provided herein is a method of treating a subject having a disease associated with expression of a tumor antigen, comprising:

- 20 i) administering to the subject:
  - (1) a stabilization compound, and
  - (2) an effective amount of a cell disclosed herein (e.g., a cell comprising a fusion polypeptide comprising a COF1/CRBN- or COF2/CRBN-binding polypeptide, a heterologous polypeptide (e.g., a CAR polypeptide), and a degradation domain), optionally wherein:
- 25 the expression level of the fusion polypeptide in the presence of the stabilization compound is e.g., at least about 1.5-, 2-, 3-, 4-, 5-, 10-, 20-, 30-, 40-, or 50-fold, higher than the expression level of the fusion polypeptide in the absence of the stabilization compound,
- thereby treating the disease.

In some embodiments, the method further comprises after step i):

- 30 ii) discontinuing the administration of the stabilization compound, optionally wherein discontinuing the administration of the stabilization compound reduces, e.g., at least about 1.5-, 2-, 3-, 4-, 5-, 10-, 20-, 30-, 40-, or 50-fold, the expression level of the fusion polypeptide relative to the expression of the fusion polypeptide after step i) and prior to step ii), optionally wherein:

a) the subject responded to the treatment of step i) (e.g., the subject has a complete response to the treatment of step i), the subject shows a shrinkage in tumor mass, the subject shows a decrease in tumor cells, or the treatment of step i) is effective in the subject), and/or

5 b) the discontinuation of the administration of the stabilization compound is in response to a response of the subject to the treatment of step i) (e.g., the subject has a complete response to the treatment of step i), the subject shows a shrinkage in tumor mass, the subject shows a decrease in tumor cells, or the treatment of step i) is effective in the subject).

In some embodiments, the method further comprises after step i):

10 iii) discontinuing the administration of the stabilization compound, optionally wherein discontinuing the administration of the stabilization compound reduces, e.g., at least about 1.5-, 2-, 3-, 4-, 5-, 10-, 20-, 30-, 40-, or 50-fold, the expression level of the fusion polypeptide relative to the expression of the fusion polypeptide after step i) and prior to step ii), optionally wherein:

a) the subject has developed, is developing, or is anticipated to develop an adverse reaction,  
 15 b) the discontinuation of the administration of the stabilization compound is in response to an occurrence of an adverse reaction in the subject, or in response to an anticipation of an occurrence of an adverse reaction in the subject, and/or  
 c) the discontinuation of the administration of the stabilization compound reduces or prevents an adverse effect.

In some embodiments, the method further comprises after step i):

20 iv) discontinuing the administration of the stabilization compound and administering to the subject an effective amount of COF1 or COF2, optionally wherein step iv) reduces, e.g., at least about 1.5-, 2-, 3-, 4-, 5-, 10-, 20-, 30-, 40-, or 50-fold, the expression level of the fusion polypeptide relative to the expression of the fusion polypeptide after step i) and prior to step iv), optionally wherein:

a) the subject has developed, is developing, or is anticipated to develop an adverse reaction,  
 25 b) step iv) is in response to an occurrence of an adverse reaction in the subject, or in response to an anticipation of an occurrence of an adverse reaction in the subject, and/or  
 c) step iv) reduces or prevents an adverse effect. In some embodiments, the adverse effect is an acute toxicity.

In some embodiments, the method further comprises after step iv):

30 v) discontinuing the administration of COF1 or COF2, e.g., after the amount of cells expressing the fusion polypeptide on the surface is smaller than a pre-defined value, for, e.g., 1 day, 5 days, 10 days, or 15 days.

In some embodiments, the method further comprises after step ii), iii), iv), or v):

vi) administering an effective amount of a stabilization compound, optionally wherein the administration of the stabilization compound increases, e.g., by at least about 1.5-, 2-, 3-, 4-, 5-, 10-, 20-, 30-, 40-, or 50-

fold, the expression level of the fusion polypeptide relative to the expression level of the fusion polypeptide after step ii), iii), iv), or v) and prior to step vi), optionally wherein:

- a) the subject has relapsed, is relapsing, or is anticipated to relapse,
- b) the administration of the stabilization compound is in response to a tumor relapse in the subject, or in response to an anticipation of a relapse in the subject, and/or
- c) the administration of the stabilization compound treats or prevents a tumor relapse.

In some embodiments, the method further comprises after step vi):

vii) repeating step iii), iv), v), or vi),  
thereby treating the disease.

In some embodiments, the method further comprises prior to step i):

viii) contacting the cell with a stabilization compound *ex vivo*, optionally wherein the expression level of the fusion polypeptide in the presence of the stabilization compound is e.g., at least about 1.5-, 2-, 3-, 4-, 5-, 10-, 20-, 30-, 40-, or 50-fold, higher than the expression level of the fusion polypeptide in the absence of the stabilization compound.

In some embodiments, the cell is not contacted with the stabilization compound *ex vivo* before administration. In some embodiments, the cell is not contacted with any of: the stabilization compound, COF1, or COF2 *ex vivo* before administration.

In some embodiments, the stabilization compound is bazedoxifene, or a pharmaceutically acceptable salt thereof, and optionally wherein the degradation domain comprises the amino acid sequence of SEQ ID NO: 46.

In some embodiments, COF1 is lenalidomide or pomalidomide, or a pharmaceutically acceptable salt thereof, optionally wherein the COF1/CRBN-binding polypeptide comprises or consists of an amino acid sequence selected from the group consisting of SEQ ID NOs: 1-6, 11-15, 40, 41-43, 77, 78, 84-86, and 100 (e.g., the COF1/CRBN-binding polypeptide comprises or consists of the amino acid sequence of SEQ ID NO: 3).

In some embodiments, COF2 is lenalidomide or pomalidomide, or a pharmaceutically acceptable salt thereof, optionally wherein the COF2/CRBN-binding polypeptide comprises or consists of an amino acid sequence selected from the group consisting of SEQ ID NOs: 1-6, 11-15, 40, 41-43, 77, 78, 84-86, and 100 (e.g., the COF1/CRBN-binding polypeptide comprises or consists of the amino acid sequence of SEQ ID NO: 3).

In some embodiments, COF1 or COF2 is lenalidomide, or a pharmaceutically acceptable salt thereof, optionally wherein lenalidomide, or a pharmaceutically acceptable salt thereof, is administered at, e.g., 2.5 mg, 5 mg, 10 mg, 15 mg, or 25 mg per day.

In one aspect, disclosed herein is a method of degrading a fusion polypeptide disclosed herein (e.g., a fusion polypeptide comprising a COF3/CRBN-binding polypeptide and a heterologous polypeptide (e.g., a CAR polypeptide)), comprising contacting the fusion polypeptide or a cell comprising said fusion polypeptide with COF3 (e.g., a compound disclosed in Table 29, or a pharmaceutically acceptable salt thereof). In one embodiment, in the presence of COF3, the expression level of said fusion polypeptide is substantially decreased, e.g., by at least about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 60, 70, 80, 90, or 100 percent, relative to the expression level of said fusion polypeptide in the absence of COF3, e.g., as measured by an assay described herein, e.g., a Western blot analysis or a flow cytometry analysis. In one embodiment, the fusion polypeptide or the cell is contacted with COF3 *ex vivo*. In one embodiment, the fusion polypeptide or the cell is contacted with COF3 *in vivo*.

In one aspect, disclosed herein is a method of regulating the expression of a fusion polypeptide disclosed herein (e.g., a fusion polypeptide comprising a COF3/CRBN-binding polypeptide, a heterologous polypeptide (e.g., a CAR polypeptide), and a degradation domain), comprising:

i) contacting the fusion polypeptide or a cell comprising the fusion polypeptide with a stabilization compound, optionally wherein in the presence of the stabilization compound:

a) the degradation domain assumes a conformation more resistant to cellular degradation relative to a conformation in the absence of the stabilization compound;

b) the conformation of the fusion polypeptide is more permissive to cleavage at the heterologous protease cleavage site relative to a conformation in the absence of the stabilization compound; or

c) the expression level of the fusion polypeptide is increased by at least, e.g., 1.5-, 2-, 3-, 4-, 5-, 10-, 20-, 30-, 40-, or 50-fold, compared to the expression level of the fusion polypeptide in the absence of the stabilization compound, e.g., as measured by an assay described herein, e.g., a Western blot analysis or a flow cytometry analysis.

In some embodiments, the method further comprises, after step i):

ii) contacting the fusion polypeptide or a cell comprising the fusion polypeptide with COF3 (e.g., a compound disclosed in Table 29, or a pharmaceutically acceptable salt thereof), optionally wherein in the presence of COF3, the expression level of the fusion polypeptide is substantially decreased, e.g., by at least about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 60, 70, 80, 90, or 100 percent, relative to the expression level of the fusion polypeptide after step i) and before step ii), e.g., as measured by an assay described herein, e.g., a Western blot analysis or a flow cytometry analysis.

In some embodiments, the fusion polypeptide or the cell is contacted with COF3 *ex vivo*. In some embodiments, the fusion polypeptide or the cell is contacted with COF3 *in vivo*. In some embodiments, the stabilization compound is bazedoxifene, or a pharmaceutically acceptable salt thereof. In some embodiments, the degradation domain comprises the amino acid sequence of SEQ ID NO: 46.

In one aspect, disclosed herein is a method of making a cell, comprising:

i) providing a cell comprising a nucleic acid molecule encoding a fusion polypeptide comprising a COF3/CRBN-binding polypeptide and a chimeric antigen receptor (CAR), optionally wherein the CAR comprises, in a N-terminal to C-terminal direction, an antigen binding domain, a transmembrane domain,  
5 and one or more intracellular signaling domains; and

ii) contacting the cell *ex vivo* with COF3, optionally wherein:

in the presence of COF3, the expression level of the fusion polypeptide is substantially decreased, e.g., by at least about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 60, 70, 80, 90, or 100 percent, relative to the expression level of the fusion polypeptide in the absence of COF3, e.g., as measured by an assay  
10 described herein, e.g., a Western blot analysis or a flow cytometry analysis.

In one aspect, provided herein is a method of treating a subject having a disease associated with expression of a tumor antigen, comprising:

i) contacting a cell disclosed herein (e.g., a cell comprising a fusion polypeptide comprising a COF3/CRBN-binding polypeptide and a heterologous polypeptide (e.g., a CAR polypeptide)) with COF3  
15 *ex vivo*, optionally wherein:

in the presence of COF3, the expression level of the fusion polypeptide is decreased, e.g., by at least about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 60, 70, 80, 90, or 100 percent, relative to the expression level of the fusion polypeptide before the cell is contacted with COF3 *ex vivo*, and

ii) administering to the subject an effective amount of the cell, optionally wherein the method further  
20 comprises after step i) and prior to step ii):

reducing the amount of COF3 contacting the cell, e.g., inside and/or surrounding the cell, thereby treating the disease.

In some embodiments, the method further comprises after step ii):

iii) administering to the subject an effective amount of COF3, optionally wherein the administration of  
25 COF3 decreases, e.g., by at least about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 60, 70, 80, 90, or 100 percent, the expression level of the fusion polypeptide relative to the expression level of the fusion polypeptide after step ii) and prior to step iii), optionally wherein:

a) the subject has developed, is developing, or is anticipated to develop an adverse reaction,

b) the administration of COF3 is in response to an occurrence of an adverse reaction in the subject, or in  
30 response to an anticipation of an occurrence of an adverse reaction in the subject, and/or

c) the administration of COF3 reduces or prevents an adverse effect.

In some embodiments, the method further comprises after step iii):

iv) discontinuing the administration of COF3, optionally wherein discontinuing the administration of COF3 increases, e.g., by at least about 1.5-, 2-, 3-, 4-, 5-, 10-, 20-, 30-, 40-, or 50-fold, the expression

level of the fusion polypeptide relative to the expression level of the fusion polypeptide after step iii) and prior to step iv) (e.g., wherein discontinuing the administration of COF3 restores the expression level of the fusion polypeptide to the expression level after step ii) and prior to step iii)), optionally wherein:

a) the subject has relapsed, is relapsing, or is anticipated to relapse,

5 b) the discontinuation of the administration of COF3 is in response to a tumor relapse in the subject, or in response to an anticipation of a relapse in the subject, and/or

c) the discontinuation of the administration of COF3 treats or prevents a tumor relapse.

In some embodiments, the method further comprises after step iv):

v) repeating step iii) and/or iv),

10 thereby treating the disease.

In some embodiments, COF3 is a compound disclosed in Table 29, or a pharmaceutically acceptable salt thereof. In some embodiments, the COF3/CRBN-binding polypeptide comprises or consists of the amino acid sequence of SEQ ID NO: 109, or an amino acid sequence with at least 80, 85, 90, or 95% identity thereto.

15 In one aspect, provided herein is a method of treating a subject having a disease associated with expression of a tumor antigen, comprising:

i) administering to the subject an effective amount of a cell disclosed herein (e.g., a cell comprising a fusion polypeptide comprising a COF3/CRBN-binding polypeptide and a heterologous polypeptide (e.g., a CAR polypeptide)), optionally wherein the cell is contacted with COF3 *ex vivo* before administration,

20 optionally wherein:

in the presence of COF3, the expression level of the fusion polypeptide is decreased, e.g., by at least about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 60, 70, 80, 90, or 100 percent, relative to the expression level of the fusion polypeptide before the cell is contacted with COF3 *ex vivo*, optionally wherein after the cell is contacted with COF3 *ex vivo* and before the cell is administered to the subject, the amount of COF3

25 contacting the cell, e.g., inside and/or surrounding the cell, is reduced, thereby treating the disease.

In some embodiments, the cell is not contacted with COF3 *ex vivo* before administration.

In some embodiments, the method further comprises after step i):

ii) administering to the subject an effective amount of COF3, optionally wherein the administration of COF3 decreases, e.g., by at least about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 60, 70, 80, 90, or 100 percent, the expression level of the fusion polypeptide relative to the expression level of the fusion polypeptide after step i) and prior to step ii), optionally wherein:

30 a) the subject has developed, is developing, or is anticipated to develop an adverse reaction,

- b) the administration of COF3 is in response to an occurrence of an adverse reaction in the subject, or in response to an anticipation of an occurrence of an adverse reaction in the subject, and/or
- c) the administration of COF3 reduces or prevents an adverse effect.

In some embodiments, the method further comprises after step ii):

- 5    iii) discontinuing the administration of COF3, optionally wherein discontinuing the administration of COF3 increases, e.g., by at least about 1.5-, 2-, 3-, 4-, 5-, 10-, 20-, 30-, 40-, or 50-fold, the expression level of the fusion polypeptide relative to the expression level of the fusion polypeptide after step ii) and prior to step iii) (e.g., wherein discontinuing the administration of COF3 restores the expression level of the fusion polypeptide to the expression level after step i) and prior to step ii)), optionally wherein:
  - 10    a) the subject has relapsed, is relapsing, or is anticipated to relapse,
  - b) the discontinuation of the administration of COF3 is in response to a tumor relapse in the subject, or in response to an anticipation of a relapse in the subject, and/or
  - c) the discontinuation of the administration of COF3 treats or prevents a tumor relapse.

In some embodiments, the method further comprises after step iii):

- 15    iv) repeating step ii) and/or iii),  
thereby treating the disease.

In some embodiments, COF3 is a compound disclosed in Table 29, or a pharmaceutically acceptable salt thereof. In some embodiments, the COF3/CRBN-binding polypeptide comprises or consists of the amino acid sequence of SEQ ID NO: 109, or an amino acid sequence with at least 80, 85,  
20    90, or 95% identity thereto.

In one aspect, provided herein is a method of treating a subject having a disease associated with expression of a tumor antigen, comprising:

- i) administering an effective amount of COF3 to the subject, wherein the subject comprises a cell disclosed herein (e.g., a cell comprising a fusion polypeptide comprising a COF3/CRBN-binding  
25    polypeptide and a heterologous polypeptide (e.g., a CAR polypeptide)), optionally wherein the administration of COF3 decreases, e.g., by at least about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 60, 70, 80, 90, or 100 percent, the expression level of the fusion polypeptide relative to the expression level of the fusion polypeptide before the administration of COF3, optionally wherein:
  - a) the subject has developed, is developing, or is anticipated to develop an adverse reaction,
  - 30    b) the administration of COF3 is in response to an occurrence of an adverse reaction in the subject, or in response to an anticipation of an occurrence of an adverse reaction in the subject, and/or
  - c) the administration of COF3 reduces or prevents an adverse effect.

In some embodiments, the method further comprises after step i):

ii) discontinuing the administration of COF3, optionally wherein discontinuing the administration of COF3 increases, e.g., by at least about 1.5-, 2-, 3-, 4-, 5-, 10-, 20-, 30-, 40-, or 50-fold, the expression level of the fusion polypeptide relative to the expression level of the fusion polypeptide after step i) and prior to step ii) (e.g., wherein discontinuing the administration of COF3 restores the expression level of the fusion polypeptide to the expression level before the administration of COF3), optionally wherein:

- a) the subject has relapsed, is relapsing, or is anticipated to relapse,
- b) the discontinuation of the administration of COF3 is in response to a tumor relapse in the subject, or in response to an anticipation of a relapse in the subject, and/or
- c) the discontinuation of the administration of COF3 treats or prevents a tumor relapse.

In some embodiments, the method further comprises after step ii):

iii) repeating step i) and/or ii),  
thereby treating the disease.

In some embodiments, COF3 is a compound disclosed in Table 29, or a pharmaceutically acceptable salt thereof. In some embodiments, the COF3/CRBN-binding polypeptide comprises or consists of the amino acid sequence of SEQ ID NO: 109, or an amino acid sequence with at least 80, 85, 90, or 95% identity thereto.

In one aspect, provided herein is a method of treating a subject having a disease associated with expression of a tumor antigen, comprising:

i) administering to the subject:

- (1) a stabilization compound, and
- (2) an effective amount of a cell disclosed herein (e.g., a cell comprising a fusion polypeptide comprising a COF3/CRBN-binding polypeptide, a heterologous polypeptide (e.g., a CAR polypeptide), and a degradation domain), optionally wherein:

the expression level of the fusion polypeptide in the presence of the stabilization compound is e.g., at least about 1.5-, 2-, 3-, 4-, 5-, 10-, 20-, 30-, 40-, or 50-fold, higher than the expression level of the fusion polypeptide in the absence of the stabilization compound,  
thereby treating the disease.

In some embodiments, the method further comprises after step i):

ii) discontinuing the administration of the stabilization compound, optionally wherein discontinuing the administration of the stabilization compound reduces, e.g., at least about 1.5-, 2-, 3-, 4-, 5-, 10-, 20-, 30-, 40-, or 50-fold, the expression level of the fusion polypeptide relative to the expression of the fusion polypeptide after step i) and prior to step ii), optionally wherein:



a) the subject responded to the treatment of step i) (e.g., the subject has a complete response to the treatment of step i), the subject shows a shrinkage in tumor mass, the subject shows a decrease in tumor cells, or the treatment of step i) is effective in the subject), and/or

5 b) the discontinuation of the administration of the stabilization compound is in response to a response of the subject to the treatment of step i) (e.g., the subject has a complete response to the treatment of step i), the subject shows a shrinkage in tumor mass, the subject shows a decrease in tumor cells, or the treatment of step i) is effective in the subject).

In some embodiments, the method further comprises after step i):

10 iii) discontinuing the administration of the stabilization compound, optionally wherein discontinuing the administration of the stabilization compound reduces, e.g., at least about 1.5-, 2-, 3-, 4-, 5-, 10-, 20-, 30-, 40-, or 50-fold, the expression level of the fusion polypeptide relative to the expression of the fusion polypeptide after step i) and prior to step ii), optionally wherein:

a) the subject has developed, is developing, or is anticipated to develop an adverse reaction,  
 15 b) the discontinuation of the administration of the stabilization compound is in response to an occurrence of an adverse reaction in the subject, or in response to an anticipation of an occurrence of an adverse reaction in the subject, and/or  
 c) the discontinuation of the administration of the stabilization compound reduces or prevents an adverse effect.

In some embodiments, the method further comprises after step i):

20 iv) discontinuing the administration of the stabilization compound and administering to the subject an effective amount of COF3, optionally wherein step iv) reduces, e.g., at least about 1.5-, 2-, 3-, 4-, 5-, 10-, 20-, 30-, 40-, or 50-fold, the expression level of the fusion polypeptide relative to the expression of the fusion polypeptide after step i) and prior to step iv), optionally wherein:

a) the subject has developed, is developing, or is anticipated to develop an adverse reaction,  
 25 b) step iv) is in response to an occurrence of an adverse reaction in the subject, or in response to an anticipation of an occurrence of an adverse reaction in the subject, and/or  
 c) step iv) reduces or prevents an adverse effect. In some embodiments, the adverse effect is an acute toxicity.

In some embodiments, the method further comprises after step iv):

30 v) discontinuing the administration of COF3, e.g., after the amount of cells expressing the fusion polypeptide on the surface is smaller than a pre-defined value, for, e.g., 1 day, 5 days, 10 days, or 15 days.

In some embodiments, the method further comprises after step ii), iii), iv), or v):

vi) administering an effective amount of a stabilization compound, optionally wherein the administration of the stabilization compound increases, e.g., by at least about 1.5-, 2-, 3-, 4-, 5-, 10-, 20-, 30-, 40-, or 50-

fold, the expression level of the fusion polypeptide relative to the expression level of the fusion polypeptide after step ii), iii), iv), or v) and prior to step vi), optionally wherein:

- a) the subject has relapsed, is relapsing, or is anticipated to relapse,
- b) the administration of the stabilization compound is in response to a tumor relapse in the subject, or in response to an anticipation of a relapse in the subject, and/or
- c) the administration of the stabilization compound treats or prevents a tumor relapse.

In some embodiments, the method further comprises after step vi):

vii) repeating step iii), iv), v), or vi),  
thereby treating the disease.

In some embodiments, the method further comprises prior to step i):

viii) contacting the cell with a stabilization compound *ex vivo*, optionally wherein the expression level of the fusion polypeptide in the presence of the stabilization compound is e.g., at least about 1.5-, 2-, 3-, 4-, 5-, 10-, 20-, 30-, 40-, or 50-fold, higher than the expression level of the fusion polypeptide in the absence of the stabilization compound.

In some embodiments, the cell is not contacted with the stabilization compound *ex vivo* before administration.

In some embodiments, the stabilization compound is bazedoxifene, or a pharmaceutically acceptable salt thereof, and optionally wherein the degradation domain comprises the amino acid sequence of SEQ ID NO: 46.

In some embodiments, COF3 is a compound disclosed in Table 29, or a pharmaceutically acceptable salt thereof. In some embodiments, the COF3/CRBN-binding polypeptide comprises or consists of the amino acid sequence of SEQ ID NO: 109, or an amino acid sequence with at least 80, 85, 90, or 95% identity thereto.

In some embodiments, the heterologous polypeptide of the fusion polypeptide is a chimeric antigen receptor (CAR), optionally wherein the CAR comprises, in a N-terminal to C-terminal direction, an antigen binding domain, a transmembrane domain, and one or more intracellular signaling domains.

In one aspect, this invention also provides a fusion polypeptide, nucleic acid molecule, vector, viral particle, cell, or pharmaceutical composition disclosed herein for use as a medicament. In one aspect, this invention also provides a fusion polypeptide, nucleic acid molecule, vector, viral particle, cell, or pharmaceutical composition disclosed herein for use in the treatment of a subject having a disease associated with expression of a tumor antigen.

In certain embodiments of the foregoing methods, the disease associated with expression of a tumor antigen is a cancer.

In some embodiments, the cancer is mesothelioma (e.g., malignant pleural mesothelioma), e.g., in a subject who has progressed on at least one prior standard therapy; lung cancer (e.g., non-small cell lung cancer, small cell lung cancer, squamous cell lung cancer, or large cell lung cancer); pancreatic cancer (e.g., pancreatic ductal adenocarcinoma, or metastatic pancreatic ductal adenocarcinoma (PDA), e.g., in a subject who has progressed on at least one prior standard therapy); esophageal adenocarcinoma, ovarian cancer (e.g., serous epithelial ovarian cancer, e.g., in a subject who has progressed after at least one prior regimen of standard therapy), breast cancer, colorectal cancer, bladder cancer or any combination thereof.

In some embodiments, the disease associated with expression of a tumor antigen is a hematological cancer, e.g., a hematological cancer chosen from a leukemia or lymphoma.

In some embodiments, the cancer is chosen from: chronic lymphocytic leukemia (CLL), mantle cell lymphoma (MCL), multiple myeloma, acute lymphoid leukemia (ALL), Hodgkin lymphoma, B-cell acute lymphoid leukemia (BALL), T-cell acute lymphoid leukemia (TALL), small lymphocytic leukemia (SLL), B cell prolymphocytic leukemia, blastic plasmacytoid dendritic cell neoplasm, Burkitt's lymphoma, diffuse large B cell lymphoma (DLBCL), DLBCL associated with chronic inflammation, chronic myeloid leukemia, myeloproliferative neoplasms, follicular lymphoma, pediatric follicular lymphoma, hairy cell leukemia, small cell- or a large cell-follicular lymphoma, malignant lymphoproliferative conditions, MALT lymphoma (extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue), Marginal zone lymphoma, myelodysplasia, myelodysplastic syndrome, non-Hodgkin lymphoma, plasmablastic lymphoma, plasmacytoid dendritic cell neoplasm, Waldenstrom macroglobulinemia, splenic marginal zone lymphoma, splenic lymphoma/leukemia, splenic diffuse red pulp small B-cell lymphoma, hairy cell leukemia-variant, lymphoplasmacytic lymphoma, a heavy chain disease, plasma cell myeloma, solitary plasmacytoma of bone, extraosseous plasmacytoma, nodal marginal zone lymphoma, pediatric nodal marginal zone lymphoma, primary cutaneous follicle center lymphoma, lymphomatoid granulomatosis, primary mediastinal (thymic) large B-cell lymphoma, intravascular large B-cell lymphoma, ALK+ large B-cell lymphoma, large B-cell lymphoma arising in HHV8-associated multicentric Castleman disease, primary effusion lymphoma, B-cell lymphoma, acute myeloid leukemia (AML), or unclassifiable lymphoma.

In some embodiments, the cancer is chosen from MCL, CLL, ALL, Hodgkin lymphoma, AML, or multiple myeloma.

In certain embodiments of the foregoing methods, the cell is autologous to said subject. In certain embodiments of the foregoing methods, the cell is allogenic to said subject. In some embodiments, the cell is a CAR-expressing cell, e.g., a CART cell.

In some embodiments, the subject was administered a cell expressing at least one fusion polypeptide disclosed herein, prior to administration of COF1, COF2, or COF3.

In one aspect, disclosed herein is a method of identifying a genetic element associated with a specific biological phenotype, e.g., a genetic element associated with the development and/or progression of a cancer, the method comprising the steps of:

i) modulating the expression of a fusion polypeptide disclosed herein (e.g., a fusion polypeptide comprising a COF1/CRBN- or COF3/CRBN-binding polypeptide and a heterologous polypeptide) in cells by exposing said cell to COF1 or COF3, e.g., lenalidomide, or a pharmaceutically acceptable salt thereof,

(ii) selecting for cells with a phenotype of interest, e.g., a phenotype associated with the development and/or progression of a cancer, and

(iii) identifying said fusion polypeptide that induces said phenotype of interest,

wherein exposure of said cell to COF1 or COF3, e.g., lenalidomide, or a pharmaceutically acceptable salt thereof, decreases, e.g., by at least about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 60, 70, 80, 90, or 100 percent, the expression level of said fusion polypeptide relative to the expression level of said fusion polypeptide prior to exposure to COF1 or COF3, e.g., lenalidomide, or a pharmaceutically acceptable salt thereof.

In one aspect, disclosed herein is a method of identifying a genetic element associated with a specific biological phenotype, e.g., a genetic element associated with the development and/or progression of a cancer, the method comprising the steps of:

i) modulating the expression of a fusion polypeptide disclosed herein (e.g., a fusion polypeptide comprising a COF1/CRBN-, COF2/CRBN-, or COF3/CRBN-binding polypeptide, a heterologous polypeptide, and a degradation domain) in cells by exposing said cell to a stabilization compound, e.g., bazedoxifene, or a pharmaceutically acceptable salt thereof, and then to COF1, COF2, or COF3, e.g., lenalidomide, or a pharmaceutically acceptable salt thereof,

(ii) selecting for cells with a phenotype of interest, e.g., a phenotype associated with the development and/or progression of a cancer, and

(iii) identifying said fusion polypeptide that induces said phenotype of interest,

wherein exposure of said cell to the stabilization compound, e.g., bazedoxifene, or a pharmaceutically acceptable salt thereof, increases, e.g., by at least about 1.5-, 2-, 3-, 4-, 5-, 10-, 20-, 30-, 40-, or 50-fold, the expression level of the fusion polypeptide relative to the expression level of the fusion polypeptide prior to exposure to the stabilization compound, e.g., bazedoxifene, or a pharmaceutically acceptable salt thereof, and wherein exposure of said cell to COF1, COF2, or COF3, e.g., lenalidomide, or a

pharmaceutically acceptable salt thereof, decreases, e.g., by at least about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 60, 70, 80, 90, or 100 percent, the expression level of said fusion polypeptide relative to the expression level of said fusion polypeptide after exposure to the stabilization compound and prior to exposure to COF1 or COF2 (such as thalidomide and derivatives thereof (e.g., lenalidomide, pomalidomide, and thalidomide)), or COF3 (e.g., a compound disclosed in Table 29).

In certain embodiments of the foregoing aspects, the heterologous polypeptide is a chimeric antigen receptor (CAR) polypeptide. In some embodiments, the CAR polypeptide comprises an amino acid sequence disclosed herein, e.g., an amino acid sequence disclosed in Table 3. In some embodiments, the CAR polypeptide is an anti-CD19 CAR polypeptide and comprises an amino acid sequence disclosed herein, e.g., an amino acid sequence disclosed in any of: Table 5, Table 6, Table 7, and Table 30. In some embodiments, the CAR polypeptide is an anti-CD123 CAR polypeptide and comprises an amino acid sequence disclosed herein, e.g., an amino acid sequence disclosed in any of: Table 8, Table 9, Table 10, Table 11, Table 12, Table 13, and Table 14. In some embodiments, the CAR polypeptide is an anti-BCMA CAR polypeptide and comprises an amino acid sequence disclosed herein, e.g., an amino acid sequence disclosed in any of: Table 15, Table 16, Table 17, Table 18, and Table 31. In some embodiments, the CAR polypeptide is an anti-CD22 CAR polypeptide and comprises an amino acid sequence disclosed herein, e.g., an amino acid sequence disclosed in any of: Table 19 and Table 20. In some embodiments, the CAR polypeptide is an anti-CD20 CAR polypeptide and comprises an amino acid sequence disclosed herein, e.g., an amino acid sequence disclosed in Table 32. In some embodiments, the CAR polypeptide is an anti-EGFRvIII CAR polypeptide and comprises an amino acid sequence disclosed herein, e.g., an amino acid sequence disclosed in Table 33. In some embodiments, the CAR polypeptide is an anti-mesothelin CAR polypeptide and comprises an amino acid sequence disclosed herein, e.g., an amino acid sequence disclosed in Table 34.

In certain embodiments of the foregoing aspects, the fusion polypeptide comprises an amino acid sequence disclosed herein, e.g., an amino acid sequence disclosed in Table 4 or Table 28.

In certain embodiments of the foregoing aspects, the COF1/CRBN-, COF2/CRBN-, or COF3/CRBN-binding polypeptide comprises an amino acid sequence disclosed herein, e.g., an amino acid sequence disclosed in Table 1.

In certain embodiments of the foregoing aspects, the degradation domain comprises an amino acid sequence disclosed herein, e.g., an amino acid sequence disclosed in Table 22.

In certain embodiments of the foregoing aspects, the heterologous protease cleavage site comprises an amino acid sequence disclosed herein, e.g., an amino acid sequence disclosed in Table 23.

### Brief Description of the DRAWINGS

**FIG. 1A** is a schematic of the HiD-tag IKZF3 136-180 and 236-249 degron fused to NanoLuciferase via a 16 glycine-serine linker. **FIG. 1B** is a graph showing the level of luminescence measured from HEK293T cells reverse transfected with 50 ng of pNL1.1CMV construct encoding NanoLuciferase linked to IKZF3 136-180 and 236-249. IKZF3 136-180 and 236-249 facilitated a reduction in luminescence in cells treated with 1  $\mu$ M, 10  $\mu$ M, or 100  $\mu$ M lenalidomide for 6 hours as compared to cells treated with DMSO only. MG132 treatment blocked lenalidomide-dependent degradation of NanoLuciferase.

**FIG. 2** is a Western blot showing that IKZF3 136-180 and 236-249 facilitated lenalidomide-dependent degradation of NanoLuciferase (IC<sub>50</sub> = 10 nM) in HEK293GT cells transfected with a pNL1.1CMV construct encoding IKZF3 136-180 and 236-249-tagged NanoLuciferase. Lenalidomide-dependent degradation was not observed in HEK293GT Cereblon (CRBN) KO cells that were similarly transfected. Treatment with a proteasome inhibitor, MG132, blocked the ability of IKZF3 136-180 and 236-249 to facilitate lenalidomide-dependent degradation.

**FIG. 3A** is a schematic depicting IKZF3 136-180 which contains two beta-sheets flanking a hairpin and an alpha-helix as well as IKZF3 236-249, which is predicted as an additional alpha-helix. Below the schematic is a diagram of the shortened versions of the IKZF3 136-180 degron, eliminating amino acids on the N and C-terminus (SEQ ID NOs: 3, 5, and 7-10, respectively, in order of appearance). **FIG. 3B** is a Western blot showing results from studies testing lenalidomide-dependent degradation of NanoLuciferase fused to various IKZF3-based degradation tags. The IKZF3-based degradation tags were fused to the N-terminus of NanoLuciferase, cloned into pNL1.1CMV vectors, and transfected into HEK293T cells. The transfected cells were treated with either DMSO or 10  $\mu$ M lenalidomide for 4 hours before analyzed by Western blot. Two exposures (a long and a short exposure) were shown for NanoLuciferase ("Nanoluc"). IKZF3 136-180 and 236-249, IKZF3 136-180 and 236-249 K245R, IKZF3 136-180 and 236-249 K245S, IKZF3 136-180 MALEK, and IKZF3 136-170 MALEK all facilitated lenalidomide-induced degradation, whereas IKZF3 140-170 MALEK, IKZF3 141-163 MALEK, and IKZF3 145-155 MALEK did not mediate lenalidomide induced degradation.

**FIGs. 4A and 4B** are Western blot graphs showing lenalidomide-dependent degradation of IKZF3 136-180-tagged NanoLuciferase (FIG. 4A) or IKZF3 136-170 MALEK-tagged NanoLuciferase (FIG. 4B) in HEK293T cells, with an IC<sub>50</sub> of approximately 100 nM with a 2-hour lenalidomide treatment in both cases. The tagged NanoLuciferase fusions were expressed using pNL1.1CMV constructs. **FIG. 4C** is a Western blot showing a time-course of lenalidomide-dependent degradation of IKZF3 136-180-tagged NanoLuciferase in HEK293T cells showing degradation as soon as 1 hour and

near complete degradation by 4 hours. The tagged NanoLuciferase fusion was expressed using a pNL1.1CMV construct.

**FIG. 5A** is a Western blot showing lenalidomide-dependent degradation of IKZF3 136-180 and 236-249-tagged melanogenesis associated transcription factor (MITF) (left panel) as well as IKZF3 136-180-tagged MITF (right panel). The tagged MITF fusions were transfected into HEK293T using pNL1.1CMV constructs. The degradation of IKZF3 136-180 and 236-249-tagged MITF shows an IC<sub>50</sub> of ~100 nM. This degradation depended on the activity of proteasome as the degradation was blocked by MG132 treatment. IKZF3 136-180 also mediated lenalidomide-dependent degradation, although to a lesser degree than IKZF3 136-180 and 236-249. **FIG. 5B** is a Western blot showing lenalidomide-dependent degradation of IKZF3 136-180 and 236-249-tagged MITF (left panel) as well as IKZF3 136-180-tagged MITF (right panel) after cells expressing these fusion proteins were treated with 10  $\mu$ M of lenalidomide for various amounts of time. Among the time points tested, the 4-hour treatment shows maximal amount of degradation.

**FIGS. 6A and 6B** are Western blot graphs showing lenalidomide-dependent degradation of MITF tagged with IKZF3 136-180 and 236-249 (FIG. 6A) or IKZF3 136-180 and 236-249 in which every lysine residue in the tag was mutated to arginine ("lysine free IKZF3 136-180 and 236-249") (FIG. 6B). HEK293T cells expressing the tagged MITF fusions using pNL1.1CMV constructs were treated with various concentrations of lenalidomide for 24 hours. The IC<sub>50</sub> is approximately 10 nM for IKZF3 136-180 and 236-249-tagged MITF (FIG. 6A) and is below 100 nM for lysine free IKZF3 136-180 and 236-249-tagged MITF (FIG. 6B). In both cases, lenalidomide-dependent degradation was dependent on proteasome as the degradation could be blocked by the proteasome inhibitor, MG132. This data suggests that MITF, rather than the IKZF3 degron tag, was being ubiquitinated. **FIG. 6C** is a Western blot showing lenalidomide-dependent degradation of lysine free IKZF3 136-180 and 236-249-tagged MITF. HEK293T cells expressing the tagged MITF fusion using a pNL1.1CMV construct was treated with 10  $\mu$ M lenalidomide for 2 hours, 4 hours, 8 hours, or 24 hours. **FIG. 6D** is a Western blot of IKZF3 136-180 and 236-249-tagged MITF (left panel) as well as lysine free IKZF3 136-180 and 236-249-tagged MITF (right panel). HEK293T cells expressing the tagged MITF fusions using the pNL1.1CMV constructs were treated with 10  $\mu$ M of either lenalidomide, pomalidomide, thalidomide, a negative control IMiD that can bind to CRBN, but not IKZF1 or IKZF3, or DMSO for 24 hours before the cells were subjected to Western blot analysis. Pomalidomide mediated the degradation of the tagged MITF to a slightly greater extent than lenalidomide, whereas thalidomide was much less effective in mediating such degradation.

**FIG. 7** is a Western blot showing lenalidomide-dependent degradation of IKZF3 136-180 Q147H-tagged MITF. HEK293T cells transfected with pNL1.1CMV constructs encoding the tagged

MITF fusions were treated with various lenalidomide doses for 24 hours. IKZF3 136-180 Q147H-tagged MITF did not show degradation in the presence of lenalidomide.

**FIG. 8** is a Western blot showing lenalidomide-dependent degradation of IKZF3 136-180 and 236-249-tagged avian myelocytomatosis viral oncogene (MYC) homolog with an IC<sub>50</sub> of approximately 10 nM. HEK293T cells expressing tagged MYC fusions using pNL1.1CMV constructs were treated with various lenalidomide doses for 4 hours.

**FIG. 9A** is a Western blot showing 4-hour lenalidomide-dependent degradation of C-terminally degron-tagged single-pass membrane proteins, CD3zeta, CD8/CD3zeta chimera, CD8, CD19, and CD22. Jurkat cells were infected with pNGX\_LV\_V002-CDx-IKZF3 136-180 and 236-249 construct virus, selected with G418, and treated with 10  $\mu$ M lenalidomide or DMSO. Shown in FIG. 9A is staining using an anti-V5 antibody (both a long 1 min exposure and a short 1 second exposure are shown) and an anti-beta-actin antibody. All of the constructs were expressed and degraded with 10  $\mu$ M lenalidomide treatment. The table in FIG. 9A shows the protein molecular weight (MW), number of cytosolic amino acid residues ("cytosolic AA"), and number of cytosolic lysines for each protein. Interestingly, degradation correlates better with the total number of cytoplasmic amino acids ("AA") than with the number of cytosolic lysine residues. **FIGs. 9B, 9C, and 9D** are Western blot graphs showing lenalidomide-dependent degradation of the C-terminally tagged CD19 (FIG. 9B), C-terminally tagged CD3zeta (FIG. 9C), and C-terminally tagged CD8/CD3zeta (FIG. 9D). Cells expressing IKZF3 136-180 and 236-249-tagged CD19, CD3zeta, or CD8/CD3zeta were treated with 10  $\mu$ M of lenalidomide for 6 hours or various lenalidomide doses for 24 hours. In FIG. 9B, degradation of IKZF3 136-180 and 236-249-tagged CD19 shows an IC<sub>50</sub> of approximate 100 nM and strong degradation was detected at 6 hours. The degradation of IKZF3 136-180 and 236-249-tagged CD3zeta shown in FIG. 9C is weaker than that of IKZF3 136-180 and 236-249-tagged CD19. The degradation of tagged CD3zeta was evident after cells were treated with 10  $\mu$ M of lenalidomide for 24 hours. The degradation of IKZF3 136-180 and 236-249-tagged CD8/CD3zeta shown in FIG. 9D is stronger than that of IKZF3 136-180 and 236-249-tagged CD3zeta.

**FIGs. 10A, 10B, 10C, and 10D** are a series of flow cytometry histograms comparing IKZF3 136-180 and 236-249-tagged CD19 cell surface expression on Jurkat cells that were treated with 1  $\mu$ M or 10  $\mu$ M lenalidomide for 1 hour (FIG. 10A), 6 hours (FIG. 10B), 16 hours (FIG. 10C), or 24 hours (FIG. 10D). Some cells were pre-treated with 10  $\mu$ M MG132 prior to treatment with 10  $\mu$ M lenalidomide. DMSO served as vehicle control. IKZF3 136-180 and 236-249 was fused to the C-terminus of CD19. **FIGs. 10E and 10F** are bar graphs showing the % CD19 positive cells (FIG. 10E) or mean fluorescence intensity (MFI) of CD19 positive cells (FIG. 10F) across all lenalidomide doses and time points tested. There was minimal degradation at 1 hour and minor degradation at 6 hours. The degradation was much



more evident at 16 and 24 hours in both the 1  $\mu$ M and 10  $\mu$ M treatment groups and this degradation could be partially blocked by the proteasome inhibitor MG132. There was approximately 50% reduction of CD19 positive cells at 16 hours in both the 1  $\mu$ M and 10  $\mu$ M treatment groups (FIG. 10E), and this reduction corresponded with a reduction in MFI (FIG. 10F).

**FIG. 11** is a schematic showing an exemplary fusion protein comprising a degradation domain (degron), protease cleavage site, and a second protein domain (a CAR), and the change in degradation of the fusion protein in the presence of a drug, e.g., stabilization compound.

**FIGs. 12A, 12B, and 12C** are schematics showing regulation of CAR molecules fused to FurON (FIG. 12A), HiID (FIG. 12B), or both FurON and HiID (FIG. 12C). As shown in FIG. 12A, a CAR fused to FurON can be turned on by administering a stabilization compound (e.g., a small molecule ligand that binds to and stabilizes the degradation domain, e.g., bazedoxifene (BZA)) or turned off by withdrawing the stabilization compound. As shown in FIG. 12B, a CAR fused to the HiID tag can be turned off by administering an IMiD compound (e.g., lenalidomide or pomalidomide) and turned on again by stopping the administration of the IMiD compound. As shown in FIG. 12C, a CAR fused to both FurON and the HiID tag can be turned on by administering the stabilization compound, turned off by discontinuing the stabilization compound and administering an IMiD compound, and turned on again by discontinuing the IMiD compound and administering the stabilization compound. Combining the FurON switch and the HiID switch adds additional layers of regulation to the expression and activity of a CAR molecule.

**FIGs. 13A, 13B, and 13C** are Western blot graphs showing lenalidomide-dependent degradation of CAR molecules. JNL cells expressing construct 765 (FurON\_CAR19) (FIG. 13A), construct 766 (FurON\_CAR19\_16GS\_HiID tag\_V5) (FIG. 13B), or construct 767 (FurON\_CAR19\_16GS\_HiID tag) (FIG. 13C) were incubated in the presence 10  $\mu$ M of lenalidomide (“+”) or DMSO (“-”) for 24 hours before Western blot analysis. All the samples received 1  $\mu$ M Bazedoxifene. “A” represents cells transduced with 275  $\mu$ L of viral supernatant. “B” represents cells transduced with 700  $\mu$ L of viral supernatant.

**FIGs. 14A, 14B, 14C, and 14D** are Western blot graphs showing lenalidomide-dependent degradation of CAR molecules. JNL cells expressing construct 771 (CAR19\_HiID tag\_V5) (FIG. 14A), construct 769 (CAR19\_16GS\_HiID tag) (FIG. 14B), construct 768 (CAR19\_16GS\_HiID tag\_V5) (FIG. 14C), or construct 770 (CAR19\_16GS\_HiID tag\_NoK) were incubated in the presence 10  $\mu$ M of lenalidomide (“+”) or DMSO (“-”) for 24 hours before Western blot analysis. “A” represents cells transduced with 275  $\mu$ L of viral supernatant. “B” represents cells transduced with 700  $\mu$ L of viral supernatant.

**FIGs. 15A and 15B** are Western blot graphs showing lenalidomide-dependent degradation of CAR molecules. JNL cells expressing construct 769 (CAR19\_16GS\_HiID tag) were incubated with 10

$\mu$ M of lenalidomide or DMSO for 2, 4, 8, 16 or 24 hours (FIG. 15A) or incubated with various doses of lenalidomide or DMSO for 24 hours (FIG. 15B) before Western blot analysis. FIG. 15A shows time-course of 10  $\mu$ M lenalidomide treatment. FIG. 15B shows a dose-response of lenalidomide at 24 hours.

**FIGs. 16A, 16B, 16C, 16D, 16E, 16F, and 16G** are a set of flow cytometry histograms showing surface CAR expression in the presence or absence of lenalidomide. Constructs tested include: construct 769 (CAR19\_16GS\_HiD tag) (FIG. 16A), construct 771 (CAR19\_HiD tag\_V5) (FIG. 16B), construct 6761 (CAR19\_16KGS\_HiD tag\_V5) (FIG. 16C), construct 768 (CAR19\_16GS\_HiD tag\_V5) (FIG. 16D), construct 770 (CAR19\_16GS\_HiD tag\_NoK) (FIG. 16E), construct 773 (HiD tag\_CAR19\_modSigPep) (FIG. 16F), and construct 774 (HiD tag\_CAR19) (FIG. 16G). JNL cells expressing the indicated constructs were incubated with or without 10  $\mu$ M lenalidomide for 24 hours and then subjected to flow cytometry analysis.

**FIGs. 17A, 17B, and 17C** are a set of flow cytometry histograms showing surface CAR expression regulated by lenalidomide and/or bazedoxifene (BZA). Constructs tested include: construct 765 (FurON\_CAR19) (FIG. 17A), construct 767 (FurON\_CAR19\_16GS\_HiD tag) (FIG. 17B), and construct 766 (FurON\_CAR19\_16GS\_HiD tag\_V5) (FIG. 17C). JNL cells expressing the indicated constructs were incubated in the presence or absence of lenalidomide and/or bazedoxifene (BZA) for 24 hours prior to flow cytometry analysis.

**FIGs. 18A, 18B, 18C, and 18D** are a set of flow cytometry histograms showing surface CAR expression in the presence or absence of various concentrations of lenalidomide. Constructs tested include: construct 769 (CAR19\_16GS\_HiD tag) (FIGs. 18A and 18C) and construct 770 (CAR19\_16GS\_HiD tag\_NoK) (FIGs. 18B and 18D). JNL cells expressing the indicated constructs were incubated in the presence or absence of lenalidomide for 4 hours (FIGs. 18A and 18B) or 20 hours (FIGs. 18C and 18D) prior to flow cytometry analysis. **FIGs. 18E and 18F** are bar graphs showing % CAR expression (FIG. 18E) or mean fluorescence intensity (FIG. 18F) for each cell line and each lenalidomide concentration tested.

**FIGs. 19A and 19B** are a series of bar graphs showing lenalidomide response comparisons between JNL target cell line treatment conditions, length of time of target cell line treatment, time of lenalidomide treatment, and number of cells. FIG. 19A is a set of graphs showing luminescence signals from a study where JNL cells expressing construct 769 (CAR19\_16GS\_HiD tag) (9000 or 12000 cells/well) were treated with 10  $\mu$ M lenalidomide for 4 hours or 24 hours and then incubated with Nalm6 cells, CD19-expressing K562 cells ("K562 +CD19"), K562 cells, or media (no cells) for 4 hours, 8 hours, or 20 hours. FIG. 19B is a set of graphs showing a subset of data from the study described in FIG. 19A: JNL cells expressing construct 769 (CAR19\_16GS\_HiD tag) (9000 cells/well) were treated with 10  $\mu$ M lenalidomide for 4 hours and then incubated with Nalm6 cells, CD19-expressing K562 cells ("K562

+CD19”), K562 cells, or media (no cells) for 20 hours. The y-axis in FIG. 19B shows luminescence signals after the background signals (signals from the media sample) were subtracted. In both FIGs. 19A and 19B, the two bars in each graph represent samples treated with DMSO (“DMSO”) and samples treated with lenalidomide (“Lenalidomide (10  $\mu$ M)”), respectively.

**FIGs. 20A and 20B** are a series of bar graphs showing lenalidomide response comparisons between JNL target cell treatment conditions, length of time of target cell treatment, time of lenalidomide treatment, and number of cells. FIG. 20A is a set of graphs showing luminescence signals from a study where JNL cells expressing construct 767 (FurON\_CAR19\_16GS\_HilD tag) (9000 or 12000 cells/well) were treated with 10  $\mu$ M lenalidomide for 4 hours or 24 hours and then incubated with Nalm6 cells, CD19-expressing K562 cells (“K562 +CD19”), K562 cells, or media (no cells) for 4 hours, 8 hours, or 20 hours. FIG. 20B is a set of graphs showing a subset of data from the study described in FIG. 20A: JNL cells expressing construct 767 (FurON\_CAR19\_16GS\_HilD tag) (9000 cells/well) were treated with 10  $\mu$ M lenalidomide for 4 hours and then incubated with Nalm6 cells, CD19-expressing K562 cells (“K562 +CD19”), K562 cells, or media (no cells) for 20 hours. The y-axis in FIG. 20B shows luminescence signals after the background signals (signals from the media sample) were subtracted. In both FIGs. 20A and 20B, the four bars in each graph represent samples treated with neither lenalidomide nor bazedoxifene (“DMSO>>DMSO”), samples treated with bazedoxifene but not lenalidomide (“DMSO>>BZA (1  $\mu$ M)”), samples treated with lenalidomide but not bazedoxifene (“Lenalidomide (10  $\mu$ M)>>DMSO”), and samples treated with both lenalidomide and bazedoxifene (“Lenalidomide (10  $\mu$ M)>>BZA (1  $\mu$ M)”), respectively.

**FIGs. 21A, 21B, 21C, and 21D** are graphs showing a dose-response effect of lenalidomide on an NFAT luciferase reporter across three treatment time points. JNL cells expressing construct 765 (FurON\_CAR19) (FIG. 21A), construct 767 (FurON\_CAR19\_16GS\_HilD tag) (FIG. 21B), construct 769 (CAR19\_16GS\_HilD tag) (FIG. 21C), or construct 770 (CAR19\_16GS\_HilD tag\_NoK) (FIG. 21D) were incubated with K562 target cells (“K562”) or K562 target cells expressing CD19 (“K562+CD19”). Lenalidomide was added 20 hours prior to adding the target cells (a 44-hour lenalidomide treatment, “20hr pre-target cells”), 4 hours prior to adding target cells (a 28-hour lenalidomide treatment, “4hr pre-target cells”), or 16 hours after adding target cells (an 8-hour lenalidomide treatment, “16hr post-target cells”). JNL cells expressing construct 765 (FurON\_CAR19) (FIG. 21A) or construct 767 (FurON\_CAR19\_16GS\_HilD tag) (FIG. 21B) were also treated with bazedoxifene. In each graph, raw luminescence is plotted against the indicated lenalidomide concentration.

**FIGs. 22A, 22B, 22C, and 22D** are graphs showing data from the study described in FIGs. 21A, 21B, 21C, and 21D, where JNL cells were treated with MG132 5 hours prior to K562 + CD19 target cell treatment, and were treated with lenalidomide 4 hours prior to K562 + CD19 target cell treatment. The

cells tested include: JNL cells expressing construct 765 (FurON\_CAR19) (FIG. 22A), construct 767 (FurON\_CAR19\_16GS\_HiLD tag) (FIG. 22B), construct 769 (CAR19\_16GS\_HiLD tag) (FIG. 22C), or construct 770 (CAR19\_16GS\_HiLD tag\_NoK) (FIG. 22D). The four bars in each graph represent samples treated with bazedoxifene (BZA), MG132, and lenalidomide ("BZA, MG132, Lenalidomide"), samples treated with bazedoxifene (BZA) and lenalidomide ("BZA, Lenalidomide"), samples treated with bazedoxifene (BZA) ("BZA"), and samples treated with DMSO only ("DMSO"), respectively. The y axis in each graph shows raw luminescence.

**FIG. 23** is a set of schematics showing HiLD-Tau fusion constructs. The 0N4R Tau isoform was used, which includes the C-terminal repeat domain exon but does not include the N-terminal exons. Lentiviral constructs were used, though all the constructs were introduced through lipofectamine transfection or nucleofection. Tau fusion products were expressed downstream of CAG or CMV promoters.

**FIGs. 24A and 24B** are graphs showing design and results from a study examining the recruitment of the E3 ligase CRBN to HiLD-Tau fusion proteins. FIG. 24A: Diagram of experiment. Lenalidomide recruits the E3 ligase Cereblon (CRBN) to the IKZF3 beta hairpin, leading to ubiquitination and degradation of the associated protein. To test that this recruitment occurred in HiLD-Tau fusions, HiLD-Tau-biotin ligase fusions were generated. In the presence of biotin, biotin ligase generates a reactive biotin species which covalently binds to nearby proteins. If lenalidomide is added, CRBN should be recruited to the HiLD-Tau fusion, and should be in range of biotin ligase mediated biotinylation. FIG. 24B: HEK293T cells were transfected with FLAG-tagged CRBN and HiLD-Tau-biotin ligase or Tau-biotin ligase fusions. 48 hours after transfection, cells were treated for 21 hours with 50  $\mu$ M biotin and either DMSO or 1  $\mu$ M lenalidomide. Cells were subsequently washed in PBS, and then lysed in ice-cold M-PER buffer and protease inhibitors. Approximately 1 million cells were estimated to be lysed, in a volume of 300  $\mu$ L. Western analysis of cell lysate is shown in lower blot, probed with anti-Tau (HT7) or anti-GAPDH antibodies. Biotinylated proteins were immunoprecipitated by incubating 20% of cell lysate with 50  $\mu$ L of streptavidin magnetic beads (Dynabeads M-280) for 30 minutes at room temperature. Biotinylated proteins were eluted from beads by boiling, and then analyzed by Western. Probing for FLAG signal on FLAG-CRBN, strong bands were observed only in immunoprecipitated material from HEK293T cells treated with lenalidomide and containing HiLD tags, but not in cells treated with DMSO, or in cells treated with lenalidomide but transfected with Tau constructs not containing the HiLD tag.

**FIGs. 25A, 25B and 25C** are graphs showing reduction of toxic Tau protein by inducible recruitment of the E3 ligase CRBN. HEK293T cells were transfected with HiLD-Tau (P301S)-YFP fusion constructs. Tau (P301S) is an aggregation-prone form of Tau, identified in patients with familial

neurodegenerative diseases. Upon overnight treatment with lenalidomide, YFP fluorescence was reduced in a dose-dependent fashion by lenalidomide, as seen in imaging of YFP fluorescence (FIG. 25A). Nine fields of view per condition are shown. FIG. 25B: YFP fluorescence intensity was quantified after lenalidomide treatment at various doses. FIG. 25C: Toxicity due to overexpression of the aggregation-prone Tau was noted, quantified by the number of cells, identified by segmentation of Hoechst dye fluorescence. Cell death was abrogated by lenalidomide treatment and reduction of Tau levels, indicating that lenalidomide inducible degradation can reveal cytoprotective action of targeted protein degradation of toxic proteins.

**FIGs. 26A, 26B, 26C, and 26D** are graphs showing quantification of Tau protein reduction and reduction of specific forms of Tau in HEK293T cells by inducible recruitment of CRBN. FIG. 26A: HEK293T cells were transfected with HilD-Tau (wild type) fusion constructs and treated with either lenalidomide, at varying doses, or DMSO. Top and bottom Western blots are representative of experiments repeated in triplicate. Intensity of Tau bands, from either a polyclonal anti-Tau antibody (Dako) or an antibody against phosphorylated forms of Tau (AT8) were quantified by normalization to anti-Actin band intensity. Transfection of a reduced amount of DNA in this experiment yielded a greater reduction of the phosphorylated form of Tau (1X DNA: 0.625 micrograms DNA transfected in 50  $\mu$ L Optimem media with 1.5  $\mu$ L lipofectamine 2000; 0.1X DNA = 0.0625 micrograms; into 24-well plates of HEK cells). This suggests that this system can measure the capacity of the E3 ligase mediated degradation of Tau. In experiments shown, lenalidomide was dosed 4 hours after transfection (for the higher DNA concentration transfection) or 24 hours after transfection (for the lower DNA concentration transfection). FIG. 26B: Left panels, Tau without a HilD tag was not reduced by lenalidomide treatment. Right panels, there was no reduction of Tau levels by lenalidomide treatment in HEK293T cells knocked out for Cereblon (CRBN). FIG. 26C: Quantification of dose response of lenalidomide treatment on YFP intensity in Cereblon (CRBN) knock out (KO) cells versus wild-type (WT) cells (same data for wild-type cells as shown in FIG. 26B). FIG. 26D: Co-treatment with the Neddylation inhibitor MLN4924 (1  $\mu$ M), including a 1 hour pretreatment with MLN4924, also prevented degradation of Tau. Altogether this data indicates that the E3 ligase function of CRBN is required for lenalidomide induced HilD-Tau fusion degradation.

**FIG. 27** is a set of graphs showing assessment of aggregation propensity of HilD-Tau (P301S)-YFP fusion, expressed in rodent cortical neurons. Rodent cortical neurons were nucleofected with HilD-Tau (P301S)-YFP fusion, and then subsequently incubated with insoluble Tau fractions isolated from a Tau transgenic mouse, generated in-house. Live YFP fluorescence was imaged using InCell 6000 Analyzer. Middle and bottom panels show zoom-in of neurons identified in the top panel. Tau aggregates, as shown by intense, punctate YFP fluorescence, are clearly visible.

**FIG. 28** is a set of graphs showing lenalidomide mediated degradation of HilD-Tau (P301S)-YFP expressed in rat neurons. Rodent cortical neurons were nucleofected with HilD-Tau (P301S)-YFP fusion, or Tau (P301S)-YFP fusion. Co-transfection with FLAG tagged CRBN was also tested (top rows). Beginning at 9 days in vitro, neurons were treated with indicated doses of lenalidomide. Neurons were imaged live for YFP fluorescence at indicated intervals. Lenalidomide treatment reduced YFP intensity over time relative to HilD-Tau (P301S)-YFP expressing neurons treated with DMSO or Tau (P301S)-YFP expressing neurons treated with lenalidomide. Degradation occurred either with or without co-transfection of human CRBN, indicating that the HilD-Tau fusion can be degraded by lenalidomide by either rodent or human CRBN.

**FIG. 29** is a set of graphs showing lenalidomide mediated degradation of HilD-Tau (P301S)-YFP expressed in rat neurons. A single-cell suspension of dissociated 63 days in vitro old human neurospheres, derived from embryonic stem cells, was nucleofected with HilD – Tau (P301S) – YFP. Neurospheres contain both neurons and neuronal progenitors. After 10 days in culture, neurons were treated with lenalidomide (at a total age of 73 days in vitro). Images show YFP fluorescence after 20 hours of lenalidomide treatment, at indicated dose. Lenalidomide substantially reduced YFP fluorescence intensity in a dose dependent fashion.

**FIGs. 30A and 30B** are a set of graphs showing lenalidomide mediated degradation of CAR19-16GS-HilDtag. FIG. 30A is a set of Western blot graphs of CAR19-HilDtag-transduced Jurkat cells treated with a single dose of lenalidomide over time. Samples from post-compound treatment or post-washout period were tested. FIG. 30B is a set of flow cytometry histograms analyzing the same samples used in the Western blot analysis. An anti-CD3zeta antibody was used in the Western blot analysis and CD19-PE conjugate was used in the flow cytometry analysis.

**FIGs. 31A, 31B, and 31C** are a set of flow cytometry histograms analyzing CAR expression under different conditions. FIG. 31A is a set of flow cytometry histograms showing CAR expression in primary T cells. The effect of lenalidomide on CAR19 expression at 24 hours is shown in FIG. 31B. The effect of lenalidomide on CAR19-HilD expression at 24 or 48 hours is shown in FIG. 31C.

**FIGs. 32A, 32B, and 32C** are a set of graphs showing % killing mediated by CART cells. FIG. 32A is a graph showing percent killing against CD19 negative cells. FIGs. 32B and 32C are graphs showing percent killing of CAR19 T cells (FIG. 32B) or CAR19-HilD T cells (FIG. 32C) against CD19 positive cells in the presence or absence of 1 $\mu$ M lenalidomide.

**FIGs. 33A and 33B** are graphs showing the levels of secreted IFN gamma and IL2, respectively, from T cells expressing CAR19 or CAR19-HilD in the presence or absence of 1 $\mu$ M lenalidomide. On the x-axis, the concentration of lenalidomide is shown in  $\mu$ M.

**FIG. 34** is a graph showing that lenalidomide abolishes the ability of CART19.HiLD to control tumor growth in vivo. Total flux of ROI is plotted against days post Nalm6 implant.

**FIG. 35** is a set of flow cytometry plots showing loss of CAR19-HiLD expression after lenalidomide treatment.

**FIG. 36** is a graph showing levels of tumor control in different treatment groups. Total flux of ROI is plotted against days post Nalm6 implant. Early injection of lenalidomide effectively abolished CART expression in mice treated with CART-HiLD, leading to absence of tumor control in this group. Later treatment of lenalidomide (day 5 post CART injection) also reduced the function of CARTs as shown by loss of tumor control in this group of mice.

**FIGs. 37A, 37B, 37C, 37D, and 37E** are graphs analyzing CAR expression in CD3+ cells from splenocytes. FIG. 37A is a graph showing CAR expression in CD3+ cells from splenocytes of mice treated with CART-HiLD (Group 1). FIGs. 37B, 37C, and 37D are graphs showing CAR expression in CD3+ cells from splenocytes of mice treated with CART-HiLD and lenalidomide (Group 2, Group 3, and Group 4, respectively). The peaks in FIGs. 37A-37D represent CD3 expression levels for individual mice. Group 1. CART19.HiLD (5x10<sup>6</sup>). Group 2. CART19-HiLD (5x10<sup>6</sup>) + Lena qd. Group 3. CART19-HiLD (5x10<sup>6</sup>) + Lena bid. Group 4. CART19.HiLD (5x10<sup>6</sup>) + Lena+5Day. FIG. 37E is a graph summarizing the data.

**FIGs. 38A, 38B, and 38C** are graphs showing impact of Compound I-112 on the expression and activity of CAR19-CARBtag. FIG. 38A is western blot of Jurkat NFAT luciferase (JNL) cells expressing CAR19-CARBtag treated with various doses of Compound I-112 or DMSO for 24 hours, showing a dose-responsive degradation of CAR19-CARBtag. FIG. 38B is a set of histograms showing flow cytometry analysis of CAR19 surface expression in JNL CAR19-CARBtag cells compared to untagged CAR19 cells after treatment with 10 $\mu$ M Compound I-112. FIG. 38C is a graph showing JNL assay results of JNL luciferase cells expressing CAR19-CARBtag treated with a dose-response of Compound I-112 for 15 hours followed by co-treatment with either K562 (CD19-) or Nalm6 (CD19+) cells with a readout of luciferase activity.

**FIG. 39** is western blot of HEK293T cells transiently transfected with CARBtag-MITF-FLAG and treated with either 10 $\mu$ M, 1 $\mu$ M, 0.1 $\mu$ M, or 0.01 $\mu$ M Compound I-112 or lenalidomide, or DMSO, showing I-112-specific degradation of the CARB-tagged MITF.

**FIGs. 40A and 40B** are graphs analyzing impact of lenalidomide on the expression and activity of BCMACAR-HiLDtag. FIG. 40A is a set of histograms showing flow cytometry analysis results of JNL cells infected with BCMACAR HiLD-tag treated with a dose-response of lenalidomide for 24 hours, showing a lenalidomide dose-dependent degradation of BCMACAR. FIG. 40B is a graph showing JNL assay results of Jurkat NFAT luciferase cells expressing BCMA-HiLDtag treated with a dose-response of

lenalidomide for 15 hours followed by co-treatment with KMS11 cells with a readout of luciferase activity.

### DETAILED DESCRIPTION

5 The present disclosure provides, at least in part, a fusion polypeptide comprising a compound of Formula (I) (COF1)/CRBN-binding polypeptide, a compound of Formula (II) (COF2)/CRBN-binding polypeptide, or a compound of Formula (III) (COF3)/CRBN-binding polypeptide for targeted protein inactivation. In some embodiments, the fusion polypeptide includes one or more COF1/CRBN-, COF2/CRBN-, or COF3/CRBN-binding polypeptides, and one or more heterologous polypeptides, e.g.,  
 10 heterologous mammalian, bacterial, or viral polypeptides, e.g., one or more polypeptides of interest. The COF1/CRBN-, COF2/CRBN-, or COF3/CRBN-binding polypeptide can be operably linked to the heterologous polypeptide, e.g., via a linker. In some embodiments, in the presence of COF1 or COF2 (such as thalidomide and derivatives thereof (e.g., lenalidomide, pomalidomide, and thalidomide)), or in the presence of COF3 (e.g., a compound disclosed in Table 29), the COF1/CRBN-, COF2/CRBN-, or  
 15 COF3/CRBN-binding polypeptide increases degradation, e.g., ubiquitination-mediated degradation, of the fusion polypeptide; and/or alters the level and/or activity of the fusion polypeptide. In some embodiments, the degradation of the fusion polypeptide is ubiquitin-dependent.

Without wishing to be bound by theory, in some embodiments, the COF1/CRBN-, COF2/CRBN-, or COF3/CRBN-binding polypeptide provides an amino acid sequence and/or a structural motif that, in  
 20 the presence of COF1 or COF2 (such as thalidomide and derivatives thereof (e.g., lenalidomide, pomalidomide, and thalidomide)), or in the presence of COF3 (e.g., a compound disclosed in Table 29), results in a post-translational modification (e.g., ubiquitination) of the fusion polypeptide, resulting in a modified, e.g., ubiquitinated, fusion polypeptide. For example, one or more amino acids, e.g., lysine or methionine, in the fusion polypeptide can be ubiquitinated, in the presence of COF1, COF2, or COF3. In  
 25 some embodiments, the ubiquitinated fusion polypeptide is selectively degraded. In some embodiments, the post-translational modification of the fusion polypeptide increases the degradation (e.g., an increased level and/or rate of degradation) of the fusion polypeptide (e.g., all or a part of the heterologous polypeptide). In some embodiments, the increase in the level and/or rate of degradation is at least 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%,  
 30 100%, 200%, 500%, 10 times, 100 times, 1,000 times, or higher than the level and/or rate of degradation of a reference protein, e.g., the fusion polypeptide in the absence of COF1, COF2, or COF3, the heterologous polypeptide, a fusion of the heterologous polypeptide without the COF1/CRBN-, COF2/CRBN-, or COF3/CRBN-binding polypeptide, or a fusion of the heterologous polypeptide with a moiety other than the COF1/CRBN-, COF2/CRBN-, or COF3/CRBN-binding polypeptide.



Without wishing to be bound by theory, degradation of the fusion polypeptide can include one, two or all of the following steps: (1) binding of COF1 or COF2 (e.g., thalidomide and derivatives thereof (e.g., lenalidome)), or COF3 (e.g., a compound disclosed in Table 29) to one or more subunits of a ubiquitin ligase complex (e.g., an E3 ubiquitin ligase complex), e.g., binding to CUL4, RBX1, DDB1 and/or CRBN, also known as CRL4(CRBN), typically, a DDB1-CRBN complex, thereby forming a COF1-ligase or COF2-ligase complex;

(2) the COF1-ligase, COF2-ligase, or COF3-ligase complex binds to and increases ubiquitination of one or more amino acids, e.g., lysine or methionine, in the fusion polypeptide, thereby forming a ubiquitinated fusion polypeptide, e.g., a mono- or a poly- ubiquitinated fusion polypeptide; and

(3) the ubiquitinated fusion polypeptide is targeted for degradation, e.g., the fusion polypeptide is selectively targeted, e.g., to a proteasome, for degradation.

In some embodiments, the COF1/CRBN- or COF2/CRBN-binding polypeptide comprises about 10 to about 95 amino acid residues, about 15 to about 90 amino acid residues, about 20 to about 85 amino acid residues, about 25 to about 80 amino acid residues, about 30 to about 75 amino acid residues, about 35 to about 70 amino acid residues, about 40 to about 65 amino acid residues, about 45 to about 65 amino acid residues, about 50 to about 65 amino acid residues, or about 55 to about 65 amino acid residues of IKZF1 (e.g., SEQ ID NO: 20) or IKZF3 (e.g., SEQ ID NO: 19).

In some embodiments, the COF3/CRBN-binding polypeptide comprises about 10 to about 95 amino acid residues, about 15 to about 90 amino acid residues, about 20 to about 85 amino acid residues, about 25 to about 80 amino acid residues, about 30 to about 75 amino acid residues, about 35 to about 70 amino acid residues, about 40 to about 65 amino acid residues, about 45 to about 65 amino acid residues, about 50 to about 65 amino acid residues, or about 55 to about 65 amino acid residues of IKZF2 (e.g., SEQ ID NO: 21).

In some embodiments, the COF1/CRBN- or COF2/CRBN-binding polypeptide comprises a beta turn (e.g., a beta turn of IKZF3). In some embodiments, the COF1/CRBN- or COF2/CRBN-binding polypeptide comprises a beta turn (e.g., a beta turn of IKZF3) and an alpha helix (e.g., an alpha helix of IKZF3). In some embodiments, the COF1/CRBN- or COF2/CRBN-binding polypeptide comprises amino acid residues 136 to 170 or 136 to 180 and/or 236-249 of IKZF3 (numbered according to SEQ ID NO: 19) or an amino acid sequence substantially identical thereto (e.g., least 85, 87, 90, 95, 97, 98, 99, or 100% identical thereto). In some embodiments, the COF1/CRBN- or COF2/CRBN-binding polypeptide comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 1-6, 11-15, 40, 41-43, 77, 78, 84-86, and 100 or an amino acid sequence substantially identical thereto (e.g., least 85, 87, 90, 95, 97, 98, 99, or 100% identical thereto).

In some embodiments, the COF3/CRBN-binding polypeptide comprises a beta turn (e.g., a beta turn of IKZF2). In some embodiments, the COF3/CRBN-binding polypeptide comprises a beta turn (e.g., a beta turn of IKZF2) and an alpha helix (e.g., an alpha helix of IKZF2). In some embodiments, the COF3/CRBN-binding polypeptide comprises amino acid residues 130-174 and/or 230-243 of IKZF2 (numbered according to SEQ ID NO: 21) or an amino acid sequence substantially identical thereto (e.g., least 85, 87, 90, 95, 97, 98, 99, or 100% identical thereto). In some embodiments, the COF3/CRBN-binding polypeptide comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 109, 113, and 114, or an amino acid sequence substantially identical thereto (e.g., least 85, 87, 90, 95, 97, 98, 99, or 100% identical thereto).

In some embodiments, the COF1/CRBN- or COF2/CRBN-binding polypeptide comprises a beta turn (e.g., a beta turn of IKZF1). In some embodiments, the COF1/CRBN- or COF2/CRBN-binding polypeptide comprises a beta turn (e.g., a beta turn of IKZF1) and an alpha helix (e.g., an alpha helix of IKZF1).

In some embodiments, the heterologous polypeptide of the fusion polypeptide is susceptible to a post-translational modification (e.g., ubiquitination at one or more residues) and degradation in the presence of COF1 or COF2 (e.g., thalidomide and derivatives thereof, e.g., lenalidomide, pomalidomide, and thalidomide), or in the presence of COF3 (e.g., a compound disclosed in Table 29).

Optionally, the COF1/CRBN-, COF2/CRBN-, or COF3/CRBN-binding polypeptide and the heterologous polypeptide can be operatively linked, e.g., via a linker, e.g., a glycine-serine linker (e.g., SEQ ID NO: 28, 37, 38, 39, or 99). For example, the fusion polypeptides can include three elements: a COF1/CRBN-, COF2/CRBN-, or COF3/CRBN-binding polypeptide, e.g., a portion of a degradation amino acid sequence (e.g., a degron), a heterologous polypeptide of interest to be degraded, and a linker separating the two. The heterologous polypeptide can be a cytosolic protein, a nuclear protein, a transmembrane protein (e.g., including one or more transmembrane domains), or a secreted protein. For example, heterologous polypeptides of interest can include, e.g., a chimeric antigen receptor (CAR), a CRISPR associated protein, CD8, CD19, CD22, a transcription factor (e.g., STAT3, STAT5, NF-kappaB, beta-catenin, Notch, GLI, or c-JUN), e.g., as described herein.

In some embodiments, the fusion polypeptide of this invention further comprises a degradation domain. In some embodiments, the degradation domain has a first state associated with a first level of expression of the fusion polypeptide and a second state associated with a second level of expression of the fusion polypeptide, wherein the second level is increased, e.g., by at least 2-, 3-, 4-, 5-, 10-, 20- or 30-fold over the first level in the presence of a stabilization compound. In some embodiments, the degradation domain is separated from the COF1/CRBN-binding polypeptide and the heterologous polypeptide by a heterologous cleavage site. In some embodiments, the degradation domain is separated from the

COF2/CRBN-binding polypeptide and the heterologous polypeptide by a heterologous cleavage site. In some embodiments, the degradation domain is separated from the COF3/CRBN-binding polypeptide and the heterologous polypeptide by a heterologous cleavage site.

In some embodiments, the fusion polypeptide comprises a first domain and a second domain, wherein the first domain comprises a degradation domain and the second domain comprises a COF1/CRBN-, COF2/CRBN-, or COF3/CRBN-binding polypeptide and a heterologous polypeptide. In some embodiments, the first domain and the second domain are separated by a heterologous cleavage site. Without wishing to be bound by theory, the expression level of the fusion polypeptide can be regulated by a stabilization compound and COF1, COF2, or COF3. In some embodiments, in the absence of the stabilization compound, the degradation domain is unable to acquire a proper conformation and is targeted for degradation by intracellular degradation pathways along with the rest of the fusion polypeptide. In some embodiments, in the presence of the stabilization compound, the degradation domain assumes a proper conformation and is less susceptible to degradation by intracellular degradation pathways. In some embodiments, in the presence of the stabilization compound, the proper folding of the degradation domain exposes the heterologous cleavage site, leaving to the cleavage of the heterologous cleavage site and the removal of the degradation domain from the rest of the fusion polypeptide. The level of the fusion polypeptide can be further regulated by COF1, COF2, or COF3 as described above.

In some embodiments, the degradation domain is chosen from an estrogen receptor (ER) domain, an FKB protein (FKBP) domain, or a dihydrofolate reductase (DHFR) domain. In some embodiments, the degradation domain is an estrogen receptor (ER) domain, e.g., the degradation domain comprises an amino acid sequence that is at least 90, 95, 97, 98, 99, or 100% identical to SEQ ID NO: 46 or 48, e.g., the degradation domain comprises the amino acid sequence of SEQ ID NO: 46. In some embodiments, the degradation domain is an estrogen receptor (ER) domain and the stabilization compound is bazedoxifene or 4-hydroxy tamoxifen (4-OHT). In some embodiments, the degradation domain is an FKB protein (FKBP) domain, e.g., the degradation domain comprises an amino acid sequence that is at least 90, 95, 97, 98, 99, or 100% identical to SEQ ID NO: 50, e.g., the degradation domain comprises the amino acid sequence of SEQ ID NO: 50. In some embodiments, the degradation domain is an FKB protein (FKBP) domain and the stabilization compound is Shield-1. In some embodiments, the degradation domain is a dihydrofolate reductase (DHFR) domain, e.g., the degradation domain comprises an amino acid sequence that is at least 90, 95, 97, 98, 99, or 100% identical to SEQ ID NO: 51, e.g., the degradation domain comprises the amino acid sequence of SEQ ID NO: 51. In some embodiments, the degradation domain is a dihydrofolate reductase (DHFR) domain and the stabilization compound is trimethoprim.

Accordingly, disclosed herein are fusion polypeptides that include a heterologous polypeptide, a COF1/CRBN-, COF2/CRBN-, or COF3/CRBN-binding polypeptide, and/or a degradation domain, e.g., polypeptides of interest for selective protein degradation, as well as nucleic acid molecules encoding the fusion polypeptides, vectors and cells, e.g., host cells, that include the aforesaid fusion polypeptides. The fusion polypeptides and related compositions disclosed herein can be used to activate or inactivate, e.g., degrade, a variety of target proteins for regulating therapies, e.g., secreted, cellular, or transmembrane therapies (e.g., CAR therapies), regulating gene expression (e.g., via regulating the expression and/or activity of a component of the CRISPR/CAS system), validating target, as well as screening libraries. Methods for selectively regulating (e.g., degrading) said fusion polypeptides for, e.g., treating a subject are additionally disclosed.

The compositions and methods disclosed herein offer novel and inventive features over art known regulation systems, including the fact that the COF1/CRBN-, COF2/CRBN-, or COF3/CRBN-binding polypeptide is acting at the protein level (as opposed to mRNA) and leads to active degradation of existing and newly made proteins in a cell (as opposed to blocking the production of a nascent protein). In addition, the COF1/CRBN-, COF2/CRBN-, or COF3/CRBN-binding polypeptide can have a short length and COF1, COF2, and COF3 are typically of low molecular weights.

Without wishing to be bound by theory, as described in Example 16, COF1 or COF2 (e.g., thalidomide and derivatives thereof (e.g., lenalidomide, pomalidomide, and thalidomide)) does not lead to, or does not substantially lead to degradation of a fusion polypeptide comprising a COF3/CRBN-binding polypeptide described herein (e.g., a fusion polypeptide comprising a CARB tag described herein, e.g., a fusion polypeptide comprising a CARB tag comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 109, 113, and 114). In some embodiments, the degradation of a fusion polypeptide comprising a COF3/CRBN-binding polypeptide described herein in the presence of COF1 or COF2 is no more than 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, or 20% of the degradation of said fusion polypeptide in the presence of COF3 under same conditions.

Similarly, COF3 (e.g., a compound disclosed in Table 29) does not lead to, or does not substantially lead to degradation of a fusion polypeptide comprising a COF1/CRBN- or COF2/CRBN-binding polypeptide described herein (e.g., a fusion polypeptide comprising a HiID tag described herein, e.g., a fusion polypeptide comprising a HiID tag comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 1-6, 11-15, 40, 41-43, 77, 78, 84-86, and 100). In some embodiments, the degradation of a fusion polypeptide comprising a COF1/CRBN- or COF2/CRBN-binding polypeptide described herein in the presence of COF3 is no more than 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, or 20% of the degradation of said fusion polypeptide in the presence of COF1 or COF2 under same conditions.

As a consequence, two target polypeptides, one tagged with a COF1/CRBN- or COF2/CRBN-binding polypeptide (e.g., a HilD tag described herein), the other tagged with a COF3/CRBN-binding polypeptide (e.g., a CARB tag described herein), can be regulated independently using COF1 or COF2 and COF3. For example, a cell expressing a HilD-tagged protein and a CARB-tagged protein can be manipulated to express only the HilD-tagged protein (e.g., by contacting the cell with COF3), express only the CARB-tagged protein (e.g., by contacting the cell with COF1 or COF2), or express neither protein (e.g., by contacting the cell with COF1 or COF2 and COF3).

### Definitions

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which the invention pertains.

As used herein, the term “compound of Formula (I) (COF1)/CRBN-binding polypeptide” refers to a polypeptide that binds to COF1, a polypeptide that binds to a complex of COF1 and CRBN, or a polypeptide that binds to CRBN in the presence of COF1. In some embodiments, the COF1/CRBN-binding polypeptide binds to COF1 with an affinity ( $K_D$ ) that is lower than  $10^{-3}$ ,  $10^{-4}$ ,  $10^{-5}$ ,  $10^{-6}$ ,  $10^{-7}$ , or  $10^{-8}$  M, e.g., as measured by a method recognized in the art, e.g., Biacore. In some embodiments, the COF1/CRBN-binding polypeptide binds to the complex of COF1 and CRBN with an affinity ( $K_D$ ) that is lower than  $10^{-3}$ ,  $10^{-4}$ ,  $10^{-5}$ ,  $10^{-6}$ ,  $10^{-7}$ , or  $10^{-8}$  M, e.g., as measured by a method recognized in the art, e.g., Biacore. In some embodiments, the COF1/CRBN-binding polypeptide binds to CRBN in the presence of COF1 with an affinity ( $K_D$ ) that is lower than  $10^{-3}$ ,  $10^{-4}$ ,  $10^{-5}$ ,  $10^{-6}$ ,  $10^{-7}$ , or  $10^{-8}$  M, e.g., as measured by a method recognized in the art, e.g., Biacore. In some embodiments, the COF1/CRBN-binding polypeptide, when present in a fusion polypeptide (e.g., operably linked to a heterologous polypeptide (e.g., a fusion polypeptide as described herein)), can result in an increase in ubiquitination of the fusion polypeptide. In some embodiments, the COF1/CRBN-binding polypeptide, when present in a fusion polypeptide (e.g., operably linked to a heterologous polypeptide (e.g., a fusion polypeptide as described herein)), can result in an increase in degradation of the fusion polypeptide. In some embodiments, the COF1/CRBN-binding polypeptide, when present in a fusion polypeptide (e.g., operably linked to a heterologous polypeptide (e.g., a fusion polypeptide as described herein)), can result in an increase in inactivation of the fusion polypeptide. In some embodiments, the increase in ubiquitination, degradation, and/or inactivation occurs in the presence of COF1 and one or more components of a ubiquitination ligase complex (e.g., CRBN). In some embodiments, the increase in ubiquitination, degradation, and/or inactivation is at least 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 100%, 200%, 500%, 10 times, 100 times, 1,000 times, or higher than

ubiquitination, degradation, and/or inactivation of a reference polypeptide, e.g., a reference fusion polypeptide with the COF1/CRBN-binding polypeptide in the absence of COF1, or a reference polypeptide without the COF1/CRBN-binding polypeptide. In some embodiments, the degradation of the fusion polypeptide containing the COF1/CRBN-binding polypeptide is ubiquitin-dependent. For example, one or more amino acids, e.g., lysine or methionine, in the fusion polypeptide with the COF1/CRBN-binding polypeptide are ubiquitinated, in the presence of COF1.

As used herein, the term “compound of Formula (II) (COF2)/CRBN-binding polypeptide” refers to a polypeptide that binds to COF2, a polypeptide that binds to a complex of COF2 and CRBN, or a polypeptide that binds to CRBN in the presence of COF2. In some embodiments, the COF2/CRBN-binding polypeptide binds to COF2 with an affinity ( $K_D$ ) that is lower than  $10^{-3}$ ,  $10^{-4}$ ,  $10^{-5}$ ,  $10^{-6}$ ,  $10^{-7}$ , or  $10^{-8}$  M, e.g., as measured by a method recognized in the art, e.g., Biacore. In some embodiments, the COF2/CRBN-binding polypeptide binds to the complex of COF2 and CRBN with an affinity ( $K_D$ ) that is lower than  $10^{-3}$ ,  $10^{-4}$ ,  $10^{-5}$ ,  $10^{-6}$ ,  $10^{-7}$ , or  $10^{-8}$  M, e.g., as measured by a method recognized in the art, e.g., Biacore. In some embodiments, the COF2/CRBN-binding polypeptide binds to CRBN in the presence of COF2 with an affinity ( $K_D$ ) that is lower than  $10^{-3}$ ,  $10^{-4}$ ,  $10^{-5}$ ,  $10^{-6}$ ,  $10^{-7}$ , or  $10^{-8}$  M, e.g., as measured by a method recognized in the art, e.g., Biacore. In some embodiments, the COF2/CRBN-binding polypeptide, when present in a fusion polypeptide (e.g., operably linked to a heterologous polypeptide (e.g., a fusion polypeptide as described herein)), can result in an increase in ubiquitination of the fusion polypeptide. In some embodiments, the COF2/CRBN-binding polypeptide, when present in a fusion polypeptide (e.g., operably linked to a heterologous polypeptide (e.g., a fusion polypeptide as described herein)), can result in an increase in degradation of the fusion polypeptide. In some embodiments, the COF2/CRBN-binding polypeptide, when present in a fusion polypeptide (e.g., operably linked to a heterologous polypeptide (e.g., a fusion polypeptide as described herein)), can result in an increase in inactivation of the fusion polypeptide. In some embodiments, the increase in ubiquitination, degradation, and/or inactivation occurs in the presence of COF2 and one or more components of a ubiquitination ligase complex (e.g., CRBN). In some embodiments, the increase in ubiquitination, degradation, and/or inactivation is at least 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 100%, 200%, 500%, 10 times, 100 times, 1,000 times, or higher than ubiquitination, degradation, and/or inactivation of a reference polypeptide, e.g., a reference fusion polypeptide with the COF2/CRBN-binding polypeptide in the absence of COF2, or a reference polypeptide without the COF2/CRBN-binding polypeptide. In some embodiments, the degradation of the fusion polypeptide containing the COF2/CRBN-binding polypeptide is ubiquitin-dependent. For example, one or more amino acids, e.g., lysine or methionine, in the fusion polypeptide with the COF2/CRBN-binding polypeptide are ubiquitinated, in the presence of COF2.

As used herein, the term “compound of Formula (III) (COF3)/CRBN-binding polypeptide” refers to a polypeptide that binds to COF3, a polypeptide that binds to a complex of COF3 and CRBN, or a polypeptide that binds to CRBN in the presence of COF3. In some embodiments, the COF3/CRBN-binding polypeptide binds to COF3 with an affinity ( $K_D$ ) that is lower than  $10^{-3}$ ,  $10^{-4}$ ,  $10^{-5}$ ,  $10^{-6}$ ,  $10^{-7}$ , or  $10^{-8}$  M, e.g., as measured by a method recognized in the art, e.g., Biacore. In some embodiments, the COF3/CRBN-binding polypeptide binds to the complex of COF3 and CRBN with an affinity ( $K_D$ ) that is lower than  $10^{-3}$ ,  $10^{-4}$ ,  $10^{-5}$ ,  $10^{-6}$ ,  $10^{-7}$ , or  $10^{-8}$  M, e.g., as measured by a method recognized in the art, e.g., Biacore. In some embodiments, the COF3/CRBN-binding polypeptide binds to CRBN in the presence of COF3 with an affinity ( $K_D$ ) that is lower than  $10^{-3}$ ,  $10^{-4}$ ,  $10^{-5}$ ,  $10^{-6}$ ,  $10^{-7}$ , or  $10^{-8}$  M, e.g., as measured by a method recognized in the art, e.g., Biacore. In some embodiments, the COF3/CRBN-binding polypeptide, when present in a fusion polypeptide (e.g., operably linked to a heterologous polypeptide (e.g., a fusion polypeptide as described herein)), can result in an increase in ubiquitination of the fusion polypeptide. In some embodiments, the COF3/CRBN-binding polypeptide, when present in a fusion polypeptide (e.g., operably linked to a heterologous polypeptide (e.g., a fusion polypeptide as described herein)), can result in an increase in degradation of the fusion polypeptide. In some embodiments, the COF3/CRBN-binding polypeptide, when present in a fusion polypeptide (e.g., operably linked to a heterologous polypeptide (e.g., a fusion polypeptide as described herein)), can result in an increase in inactivation of the fusion polypeptide. In some embodiments, the increase in ubiquitination, degradation, and/or inactivation occurs in the presence of COF3 and one or more components of a ubiquitination ligase complex (e.g., CRBN). In some embodiments, the increase in ubiquitination, degradation, and/or inactivation is at least 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 100%, 200%, 500%, 10 times, 100 times, 1,000 times, or higher than ubiquitination, degradation, and/or inactivation of a reference polypeptide, e.g., a reference fusion polypeptide with the COF3/CRBN-binding polypeptide in the absence of COF3, or a reference polypeptide without the COF3/CRBN-binding polypeptide. In some embodiments, the degradation of the fusion polypeptide containing the COF3/CRBN-binding polypeptide is ubiquitin-dependent. For example, one or more amino acids, e.g., lysine or methionine, in the fusion polypeptide with the COF3/CRBN-binding polypeptide are ubiquitinated, in the presence of COF3.

As used herein, “ubiquitination” refers to the addition of a ubiquitin molecule, e.g., a single ubiquitin (mono-ubiquitination) or more than one ubiquitin (e.g., a chain of ubiquitin molecules, or poly-ubiquitination). Ubiquitination can be performed by an enzyme machinery including one or more of a ubiquitin-activating enzyme (E1), a ubiquitin-conjugating enzyme (E2), and a ubiquitin ligase (E3).

As used herein, the term “CRBN” refers to a protein that in humans is encoded by the *CRBN* gene, or fragment or variant thereof (e.g., an amino acid sequence substantially identical thereto, e.g.,

least 85, 87, 90, 95, 97, 98, 99, or 100% identical thereto). Swiss-Prot accession number Q96SW2 provides exemplary human CRBN amino acid sequences.

As used herein, an “IKZF polypeptide” refers to an IKZF, or fragment or variant thereof (e.g., an amino acid sequence substantially identical thereto, e.g., least 85, 87, 90, 95, 97, 98, 99, or 100% identical thereto).

As used herein, the term “IKZF3” refers to a protein that in humans is encoded by the *IKZF3* gene. Swiss-Prot accession number Q9UKT9 provides exemplary human IKZF3 amino acid sequences. An exemplary human IKZF3 amino acid sequence is provided in SEQ ID NO: 19. The term “IKZF3 polypeptide” refers to IKZF3, or fragment or variant thereof (e.g., an amino acid sequence substantially identical thereto, e.g., least 85, 87, 90, 95, 97, 98, 99, or 100% identical thereto).

As used herein, the term “IKZF1” refers to a protein that in humans is encoded by the *IKZF1* gene. Swiss-Prot accession number Q13422 provides exemplary human IKZF1 amino acid sequences. An exemplary human IKZF1 amino acid sequence is provided in SEQ ID NO: 20. The term “IKZF1 polypeptide” refers to IKZF1, or fragment or variant thereof (e.g., an amino acid sequence substantially identical thereto, e.g., least 85, 87, 90, 95, 97, 98, 99, or 100% identical thereto).

As used herein, the term “IKZF2” refers to a protein that in humans is encoded by the *IKZF2* gene. Swiss-Prot accession number Q9UKS7 provides exemplary human IKZF2 amino acid sequences. An exemplary human IKZF2 amino acid sequence is provided in SEQ ID NO: 21. The term “IKZF2 polypeptide” refers to IKZF2, or fragment or variant thereof (e.g., an amino acid sequence substantially identical thereto, e.g., least 85, 87, 90, 95, 97, 98, 99, or 100% identical thereto).

As used herein, the term “IKZF4” refers to a protein that in humans is encoded by the *IKZF4* gene. Swiss-Prot accession number Q9H2S9 provides exemplary human IKZF4 amino acid sequences. An exemplary human IKZF4 amino acid sequence is provided in SEQ ID NO: 22. The term “IKZF4 polypeptide” refers to IKZF4, or fragment or variant thereof (e.g., an amino acid sequence substantially identical thereto, e.g., least 85, 87, 90, 95, 97, 98, 99, or 100% identical thereto).

As used herein, the term “IKZF5” refers to a protein that in humans is encoded by the *IKZF5* gene. Swiss-Prot accession number Q9H5V7 provides exemplary human IKZF5 amino acid sequences. An exemplary human IKZF5 amino acid sequence is provided in SEQ ID NO: 23. The term “IKZF5 polypeptide” refers to IKZF5, or fragment or variant thereof (e.g., an amino acid sequence substantially identical thereto, e.g., least 85, 87, 90, 95, 97, 98, 99, or 100% identical thereto).

As used herein, a “fusion polypeptide” or “chimeric polypeptide” refers to a polypeptide that includes two or more heterologous amino acid sequences and/or protein domains in a single, continuous polypeptide. In some embodiments, the two or more heterologous protein domains are covalently linked directly or indirectly, e.g., via a linker.



As used herein, the term “estrogen receptor (ER)” refers to a protein that in humans is encoded by the *ESR1* gene. Swiss-Prot accession number P03372 provides exemplary human estrogen receptor (ER) amino acid sequences. An “estrogen receptor (ER) domain” refers to estrogen receptor, or fragment or variant thereof (e.g., an amino acid sequence substantially identical thereto, e.g., least 85, 87, 90, 95, 97, 98, 99, or 100% identical thereto). Exemplary estrogen receptor (ER) domain amino acid sequences are provided in SEQ ID NOs: 44, 46, and 48. Exemplary estrogen receptor (ER) domain nucleotide sequences are provided in SEQ ID NOs: 45, 47, and 49.

As used herein, an “FKB protein (FKBP) domain” refers to FKBP, or fragment or variant thereof. An exemplary FKB protein (FKBP) domain amino acid sequence is provided in SEQ ID NO: 50.

As used herein, the term “dihydrofolate reductase (DHFR)” refers to a protein that in humans is encoded by the *DHFR* gene. Swiss-Prot accession number P00374 provides exemplary human dihydrofolate reductase (DHFR) amino acid sequences. A “dihydrofolate reductase (DHFR) domain” refers to DHFR, or fragment or variant thereof. An exemplary dihydrofolate reductase (DHFR) domain amino acid sequence is provided in SEQ ID NO: 51.

As used herein, the term “degradation domain” refers to a domain of a fusion polypeptide that assumes a stable conformation when expressed in the presence of a stabilization compound. Absent the stable conformation when expressed in a cell of interest, a large fraction of degradation domains (and, typically, any protein to which they are fused to) will be degraded by endogenous cellular machinery. Notably, a degradation domain is not a naturally occurring domain of a protein but is rather engineered to be unstable absent contact with the stabilization compound. Thus, a degradation domain is identifiable by the following characteristics: (1) it is not naturally occurring; (2) its expression is regulated co-translationally or post-translationally through increased or decreased degradation rates; (3) the rate of degradation is substantially decreased in the presence of a stabilization compound. In some embodiments, absent a stabilization compound, the degradation domain or other domain of the fusion polypeptide is not substantially detectable in or on the cell. In some embodiments, the degradation domain is in a destabilized state in the absence of a stabilization compound. In some embodiments, the degradation domain does not self-associate, e.g., does not homodimerize, in the absence of a stabilization compound. In some embodiments, the degradation domain is fused to a heterologous protease cleavage site, wherein in the presence of the stabilization compound, the cleavage of the heterologous protease cleavage site is more efficient than in the absence of the stabilization compound.

The degradation domain is not an aggregation domain as defined in PCT Application Number PCT/US2017/027778.

By “stabilization compound” or “stabilizing compound” is meant a compound that, when added to a cell expressing a degradation domain, stabilizes the degradation domain and any protein that is fused

to it, and decreases the rate at which it is subsequently degraded. Stabilization compounds or stabilizing compounds can be naturally occurring or synthetic.

By the term “heterologous polypeptide” is meant an amino acid sequence (e.g., a protein domain) that is different from a COF1/CRBN-, COF2/CRBN-, or COF3/CRBN-binding polypeptide (e.g., by at least one amino acid) and that is not an active luciferase domain or has a luciferase sequence. In some embodiments, the heterologous polypeptide is not a reporter polypeptide, e.g., a luciferase, a green fluorescent protein, or a  $\beta$ -galactosidase. In some embodiments, the heterologous polypeptide comprises an amino acid sequence from, or derived from, a mammalian polypeptide, a bacterial polypeptide, a viral polypeptide, a plant polypeptide, a yeast polypeptide, a fungi polypeptide, an archaeobacterial polypeptide, or a fish, e.g., Zebrafish, polypeptide. In some embodiments, the heterologous polypeptide comprises a polypeptide in Table 2, e.g., a cytoplasmic and/or nuclear polypeptide, a secretory polypeptide, or a transmembrane polypeptide as described in Table 2.

Furthermore, by “heterologous protease cleavage site” is meant a protease cleavage site that has a different origin than one or more protein domains to which it is fused (e.g., is not naturally fused to at least one of the other referenced domains)

By “protease” is meant a protein that cleaves another protein based on the presence of a cleavage site in the to-be-cleaved protein.

By “intracellular protease” is meant a protease that is natively expressed inside a cell of interest.

By “extracellular protease” is meant a protease that is natively expressed in an organism (e.g., a mammal) and secreted or exposed to the outside of cells (e.g., in the blood or the surface of the skin).

As used herein, the term “cleavage” refers to the breakage of covalent bonds, such as in the backbone of a nucleic acid molecule or the hydrolysis of peptide bonds. Cleavage can be initiated by a variety of methods, including, but not limited to, enzymatic or chemical hydrolysis of a phosphodiester bond. Both single-stranded cleavage and double-stranded cleavage are possible. Double-stranded cleavage can occur as a result of two distinct single-stranded cleavage events.

Additional terms are described herein below.

The term “a” and “an” refers to one or to more than one (i.e., to at least one) of the grammatical object of the article. By way of example, “an element” means one element or more than one element.

The term “about” when referring to a measurable value such as an amount, a temporal duration, and the like, is meant to encompass variations of  $\pm 20\%$  or in some instances  $\pm 10\%$ , or in some instances  $\pm 5\%$ , or in some instances  $\pm 1\%$ , or in some instances  $\pm 0.1\%$  from the specified value, as such variations are appropriate to perform the disclosed methods.

The term “antibody,” as used herein, refers to a protein, or polypeptide sequence derived from an immunoglobulin molecule which specifically binds with an antigen. Antibodies can be polyclonal or monoclonal, multiple or single chain, or intact immunoglobulins, and may be derived from natural sources or from recombinant sources. Antibodies can be tetramers of immunoglobulin molecules.

5 The term “antibody fragment” refers to at least one portion of an antibody, that retains the ability to specifically interact with (e.g., by binding, steric hindrance, stabilizing/destabilizing, spatial distribution) an epitope of an antigen. Examples of antibody fragments include, but are not limited to, Fab, Fab', F(ab')<sub>2</sub>, Fv fragments, scFv antibody fragments, disulfide-linked Fvs (sdFv), a Fd fragment consisting of the VH and CH1 domains, linear antibodies, single domain antibodies such as sdAb (either 10 VL or VH), camelid VHH domains, multi-specific antibodies formed from antibody fragments such as a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region, and an isolated CDR or other epitope binding fragments of an antibody. An antigen binding fragment can also be incorporated into single domain antibodies, maxibodies, minibodies, nanobodies, intrabodies, diabodies, triabodies, tetrabodies, v-NAR and bis-scFv (see, e.g., Hollinger and Hudson, Nature Biotechnology 15 23:1126-1136, 2005). Antigen binding fragments can also be grafted into scaffolds based on polypeptides such as a fibronectin type III (Fn3)(see U.S. Patent No.: 6,703,199, which describes fibronectin polypeptide minibodies).

The term “antibody heavy chain,” refers to the larger of the two types of polypeptide chains present in antibody molecules in their naturally occurring conformations, and which normally determines 20 the class to which the antibody belongs.

The term “antibody light chain,” refers to the smaller of the two types of polypeptide chains present in antibody molecules in their naturally occurring conformations. Kappa (κ) and lambda (λ) light chains refer to the two major antibody light chain isotypes.

The term “antigen,” “Ag,” or “antigen molecule” refers to a molecule that provokes an immune 25 response. This immune response may involve either antibody production, or the activation of specific immunologically-competent cells, or both. In some embodiments, an antigen is any macromolecule, including all proteins or peptides. In other embodiments, antigens are derived from recombinant or genomic DNA. Any DNA, which comprises nucleotide sequences or a partial nucleotide sequence encoding a protein that elicits an immune response therefore encodes an “antigen” as that term is used 30 herein.

An antigen need not be encoded solely by a full length nucleotide sequence of a gene. In embodiments, antigens include, but are not limited to, the use of partial nucleotide sequences of more than one gene and that these nucleotide sequences are arranged in various combinations to encode polypeptides that elicit the desired immune response. In an embodiment, an antigen need not be encoded

by a “gene” at all. In one embodiment, an antigen can be generated synthesized or can be derived from a biological sample, or might be macromolecule besides a polypeptide. Such a biological sample can include, but is not limited to a tissue sample, a tumor sample, a cell or a fluid with other biological components. In embodiments, antigens include, for example, carbohydrates (e.g., monosaccharides, disaccharides, oligosaccharides, and polysaccharides).

The term “antigen presenting cell” or “APC” refers to an immune system cell such as an accessory cell (e.g., a B-cell, a dendritic cell, and the like) that displays a foreign antigen complexed with major histocompatibility complexes (MHC's) on its surface. T-cells may recognize these complexes using their T-cell receptors (TCRs). APCs process antigens and present them to T-cells.

The term “Chimeric Antigen Receptor” or alternatively a “CAR” refers to a set of polypeptides, typically two in the simplest embodiments, which when in an immune effector cell, provides the cell with specificity for a target cell, typically a cancer cell, and with intracellular signal generation. In some embodiments, a CAR comprises at least an extracellular antigen binding domain, a transmembrane domain and a cytoplasmic signaling domain (also referred to herein as “an intracellular signaling domain”) comprising a functional signaling domain derived from a stimulatory molecule and/or costimulatory molecule as defined below. In some embodiments, the set of polypeptides are in the same polypeptide chain (e.g., comprise a chimeric fusion protein). In some embodiments, the set of polypeptides are not contiguous with each other, e.g., are in different polypeptide chains. In some embodiments, the set of polypeptides are not contiguous with each other, e.g., are in different polypeptide chains. In some embodiments, the set of polypeptides include a dimerization switch that, upon the presence of a dimerization molecule, can couple the fusion polypeptides to one another, e.g., can couple an antigen binding domain to an intracellular signaling domain. In one aspect, the stimulatory molecule is the zeta chain associated with the T cell receptor complex. In one aspect, the cytoplasmic signaling domain comprises a primary signaling domain (e.g., a primary signaling domain of CD3-zeta). In one aspect, the cytoplasmic signaling domain further comprises one or more functional signaling domains derived from at least one costimulatory molecule as defined below. In one aspect, the costimulatory molecule of the CAR is chosen from the costimulatory molecules described herein, e.g., 4-1BB (i.e., CD137), CD27, ICOS, and/or CD28. In one aspect, the CAR comprises a chimeric fusion protein comprising an extracellular antigen binding domain, a transmembrane domain and an intracellular signaling domain comprising a functional signaling domain derived from a stimulatory molecule. In one aspect, the CAR comprises a chimeric fusion protein comprising an extracellular antigen binding domain, a transmembrane domain and an intracellular signaling domain comprising a functional signaling domain derived from a costimulatory molecule and a functional signaling domain derived from a stimulatory molecule. In one aspect, the CAR comprises a chimeric fusion protein comprising an extracellular

antigen binding domain, a transmembrane domain and an intracellular signaling domain comprising two functional signaling domains derived from one or more costimulatory molecule(s) and a functional signaling domain derived from a stimulatory molecule. In one aspect, the CAR comprises a chimeric fusion protein comprising an extracellular antigen binding domain, a transmembrane domain and an intracellular signaling domain comprising at least two functional signaling domains derived from one or more costimulatory molecule(s) and a functional signaling domain derived from a stimulatory molecule. In one aspect the CAR comprises an optional leader sequence at the amino-terminus (N-ter) of the CAR fusion protein. In one aspect, the CAR further comprises a leader sequence at the N-terminus of the extracellular antigen binding domain, wherein the leader sequence is optionally cleaved from the antigen binding domain (e.g., a scFv) during cellular processing and localization of the CAR to the cellular membrane.

The term “cancer” refers to a disease characterized by the uncontrolled growth of aberrant cells. Cancer cells can spread locally or through the bloodstream and lymphatic system to other parts of the body. Examples of various cancers are described herein and include but are not limited to, breast cancer, prostate cancer, ovarian cancer, cervical cancer, skin cancer, pancreatic cancer, colorectal cancer, renal cancer, liver cancer, brain cancer, lymphoma, leukemia, lung cancer and the like. The terms “tumor” and “cancer” are used interchangeably herein, e.g., both terms encompass solid and liquid, e.g., diffuse or circulating, tumors. As used herein, the term “cancer” or “tumor” includes premalignant, as well as malignant cancers and tumors.

“CAR molecule”, depending on the context, refers to a CAR (e.g., a CAR polypeptide), a nucleic acid encoding a CAR, or both.

A CAR that comprises an antigen binding domain (e.g., a scFv, or TCR) that targets a specific tumor antigen X, such as those described herein, is also referred to as XCAR. For example, a CAR that comprises an antigen binding domain that targets CD19 or BCMA is referred to as CD19CAR or BCMACAR, respectively.

As used herein, the term “BCMA” refers to B-cell maturation antigen. BCMA (also known as TNFRSF17, BCM or CD269) is a member of the tumor necrosis receptor (TNFR) family and is predominantly expressed on terminally differentiated B cells, e.g., memory B cells, and plasma cells. Its ligand is called B-cell activator of the TNF family (BAFF) and a proliferation inducing ligand (APRIL). BCMA is involved in mediating the survival of plasma cells for maintaining long-term humoral immunity. The gene for BCMA is encoded on chromosome 16 producing a primary mRNA transcript of 994 nucleotides in length (NCBI accession NM\_001192.2) that encodes a protein of 184 amino acids (NP\_001183.2). A second antisense transcript derived from the BCMA locus has been described, which may play a role in regulating BCMA expression. (Laabi Y. et al., Nucleic Acids Res., 1994, 22:1147-

1154). Additional transcript variants have been described with unknown significance (Smirnova AS et al. Mol Immunol., 2008, 45(4):1179-1183. A second isoform, also known as TV4, has been identified (Uniprot identifier Q02223-2). As used herein, “BCMA” includes proteins comprising mutations, e.g., point mutations, fragments, insertions, deletions and splice variants of full length wild-type BCMA.

5 As used herein, the term “CD19” refers to the Cluster of Differentiation 19 protein, which is an antigenic determinant detectable on leukemia precursor cells. The human and murine amino acid and nucleic acid sequences can be found in a public database, such as GenBank, UniProt and Swiss-Prot. For example, the amino acid sequence of human CD19 can be found as UniProt/Swiss-Prot Accession No. P15391 and the nucleotide sequence encoding of the human CD19 can be found at Accession No. NM\_001178098. As used herein, “CD19” includes proteins comprising mutations, e.g., point mutations, fragments, insertions, deletions and splice variants of full length wild-type CD19.

CD19 is expressed on most B lineage cancers, including, e.g., acute lymphoblastic leukaemia, chronic lymphocyte leukaemia and non-Hodgkin lymphoma. Other cells with express CD19 are provided below in the definition of “disease associated with expression of CD19.” It is also an early marker of B cell progenitors. See, e.g., Nicholson et al. Mol. Immun. 34 (16-17): 1157-1165 (1997). In one aspect the antigen-binding portion of the CART recognizes and binds an antigen within the extracellular domain of the CD19 protein. In one aspect, the CD19 protein is expressed on a cancer cell.

As used herein, the term “CD20” refers to an antigenic determinant known to be detectable on B cells. Human CD20 is also called membrane-spanning 4-domains, subfamily A, member 1 (MS4A1). The human and murine amino acid and nucleic acid sequences can be found in a public database, such as GenBank, UniProt and Swiss-Prot. For example, the amino acid sequence of human CD20 can be found at Accession Nos. NP\_690605.1 and NP\_068769.2, and the nucleotide sequence encoding transcript variants 1 and 3 of the human CD20 can be found at Accession No. NM\_152866.2 and NM\_021950.3, respectively. In one aspect the antigen-binding portion of the CAR recognizes and binds an antigen within the extracellular domain of the CD20 protein. In one aspect, the CD20 protein is expressed on a cancer cell. As used herein, “CD20” includes proteins comprising mutations, e.g., point mutations, fragments, insertions, deletions and splice variants of full length wild-type CD20.

As used herein, the terms “CD22,” refers to an antigenic determinant known to be detectable on leukemia precursor cells. The human and murine amino acid and nucleic acid sequences can be found in a public database, such as GenBank, UniProt and Swiss-Prot. For example, the amino acid sequences of isoforms 1-5 human CD22 can be found at Accession Nos. NP 001762.2, NP 001172028.1, NP 001172029.1, NP 001172030.1, and NP 001265346.1, respectively, and the nucleotide sequence encoding variants 1-5 of the human CD22 can be found at Accession No. NM 001771.3, NM 001185099.1, NM

001185100.1, NM 001185101.1, and NM 001278417.1, respectively. In one aspect the antigen-binding portion of the CAR recognizes and binds an antigen within the extracellular domain of the CD22 protein. In one aspect, the CD22 protein is expressed on a cancer cell. As used herein, "CD22" includes proteins comprising mutations, e.g., point mutations, fragments, insertions, deletions and splice variants of full  
5 length wild-type CD22.

As used herein, the term "CD123" refers to an antigenic determinant known to be detectable on some malignant hematological cancer cells, e.g., leukemia cells. The human and murine amino acid and nucleic acid sequences can be found in a public database, such as GenBank, UniProt and Swiss-Prot. For example, the amino acid sequences of human CD123 can be found at Accession Nos. NP\_002174.1  
10 (isoform 1 precursor); NP\_001254642.1 (isoform 2 precursor), and the mRNA sequences encoding them can be found at Accession Nos. NM\_002183.3 (variant 1); NM\_001267713.1 (variant 2). In one aspect the antigen-binding portion of the CAR recognizes and binds an antigen within the extracellular domain of the CD123 protein. In one aspect, the CD123 protein is expressed on a cancer cell. As used herein, "CD123" includes proteins comprising mutations, e.g., point mutations, fragments, insertions, deletions  
15 and splice variants of full length wild-type CD123.

The portion of the CAR comprising an antibody or antibody fragment thereof may exist in a variety of forms where the antigen binding domain is expressed as part of a contiguous polypeptide chain including, for example, a single domain antibody fragment (sdAb), a single chain antibody (scFv), a humanized antibody, or bispecific antibody (Harlow et al., 1999, In: Using Antibodies: A Laboratory  
20 Manual, Cold Spring Harbor Laboratory Press, NY; Harlow et al., 1989, In: Antibodies: A Laboratory Manual, Cold Spring Harbor, New York; Houston et al., 1988, Proc. Natl. Acad. Sci. USA 85:5879-5883; Bird et al., 1988, Science 242:423-426). In one aspect, the antigen binding domain of a CAR composition of the invention comprises an antibody fragment. In a further aspect, the CAR comprises an antibody fragment that comprises a scFv.

25 The term "cognate antigen molecule" refers to any antigen described herein. In one embodiment, it refers to an antigen recognized, e.g., targeted, by a CAR molecule, e.g., any CAR described herein. In another embodiment, it refers to a cancer associated antigen described herein. In one embodiment, the cognate antigen molecule is a recombinant molecule.

The term "conservative sequence modifications" refers to amino acid modifications that do not  
30 significantly affect or alter the binding characteristics of the antibody or antibody fragment containing the amino acid sequence. Such conservative modifications include amino acid substitutions, additions and deletions. Modifications can be introduced into an antibody or antibody fragment of the invention by standard techniques known in the art, such as site-directed mutagenesis and PCR-mediated mutagenesis. Conservative amino acid substitutions are ones in which the amino acid residue is replaced with an amino

acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in the art. These families include amino acids with basic side chains (e.g., lysine, arginine, histidine), acidic side chains (e.g., aspartic acid, glutamic acid), uncharged polar side chains (e.g., glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine, tryptophan), nonpolar side chains (e.g., alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine), beta-branched side chains (e.g., threonine, valine, isoleucine) and aromatic side chains (e.g., tyrosine, phenylalanine, tryptophan, histidine). Thus, one or more amino acid residues within a polypeptide of interest (e.g., a CAR) described herein can be replaced with other amino acid residues from the same side chain family and the altered polypeptide of interest (e.g., a CAR) can be tested using the functional assays described herein.

The term “costimulatory molecule” refers to the cognate binding partner on a T cell that specifically binds with a costimulatory ligand, thereby mediating a costimulatory response by the T cell, such as, but not limited to, proliferation. Costimulatory molecules are cell surface molecules other than antigen receptors or their ligands that are required for an efficient immune response. Costimulatory molecules include, but are not limited to MHC class I molecule, TNF receptor proteins, Immunoglobulin-like proteins, cytokine receptors, integrins, signalling lymphocytic activation molecules (SLAM proteins), activating NK cell receptors, BTLA, a Toll ligand receptor, OX40, CD2, CD7, CD27, CD28, CD30, CD40, CDS, ICAM-1, LFA-1 (CD11a/CD18), 4-1BB (CD137), B7-H3, CDS, ICAM-1, ICOS (CD278), GITR, BAFRR, LIGHT, HVEM (LIGHTR), KIRDS2, SLAMF7, NKp80 (KLRP1), NKp44, NKp30, NKp46, CD19, CD4, CD8alpha, CD8beta, IL2R beta, IL2R gamma, IL7R alpha, ITGA4, VLA1, CD49a, ITGA4, IA4, CD49D, ITGA6, VLA-6, CD49f, ITGAD, CD11d, ITGAE, CD103, ITGAL, CD11a, LFA-1, ITGAM, CD11b, ITGAX, CD11c, ITGB1, CD29, ITGB2, CD18, LFA-1, ITGB7, NKG2D, NKG2C, TNFR2, TRANCE/RANKL, DNAM1 (CD226), SLAMF4 (CD244, 2B4), CD84, CD96 (Tactile), CEACAM1, CRTAM, Ly9 (CD229), CD160 (BY55), PSGL1, CD100 (SEMA4D), CD69, SLAMF6 (NTB-A, Ly108), SLAM (SLAMF1, CD150, IPO-3), BLAME (SLAMF8), SELPLG (CD162), LTBR, LAT, GADS, SLP-76, PAG/Cbp, CD19a, and a ligand that specifically binds with CD83. A costimulatory intracellular signaling domain refers to an intracellular portion of a costimulatory molecule. The intracellular signaling domain can comprise the entire intracellular portion, or the entire native intracellular signaling domain, of the molecule from which it is derived, or a functional fragment thereof. The intracellular signaling domain can comprise the entire intracellular portion, or the entire native intracellular signaling domain, of the molecule from which it is derived, or a functional fragment thereof.

“Derived from” as that term is used herein, indicates a relationship between a first and a second molecule. It generally refers to structural similarity between the first molecule and a second molecule and does not connote or include a process or source limitation on a first molecule that is derived from a second molecule. For example, in the case of an intracellular signaling domain that is derived from a



CD3zeta molecule, the intracellular signaling domain retains sufficient CD3zeta structure such that it has the required function, namely, the ability to generate a signal under the appropriate conditions. It does not connote or include a limitation to a particular process of producing the intracellular signaling domain, e.g., it does not mean that, to provide the intracellular signaling domain, one must start with a CD3zeta sequence and delete unwanted sequence, or impose mutations, to arrive at the intracellular signaling domain.

The phrase “disease associated with expression of a tumor antigen” as described herein includes, but is not limited to, a disease associated with expression of a tumor antigen as described herein or condition associated with cells which express a tumor antigen as described herein including, e.g., proliferative diseases such as a cancer or malignancy or a precancerous condition such as a myelodysplasia, a myelodysplastic syndrome or a preleukemia; or a noncancer related indication associated with cells which express a tumor antigen as described herein. In one embodiment, a cancer associated with expression of a tumor antigen as described herein is a hematological cancer. In one embodiment, a cancer associated with expression of a tumor antigen as described herein is a solid cancer. Further diseases associated with expression of a tumor antigen as described herein include, but not limited to, e.g., atypical and/or non-classical cancers, malignancies, precancerous conditions or proliferative diseases associated with expression of a tumor antigen as described herein. Non-cancer related indications associated with expression of a tumor antigen as described herein include, but are not limited to, e.g., autoimmune disease, (e.g., lupus), inflammatory disorders (allergy and asthma) and transplantation. In some embodiments, the tumor antigen-expressing cells express, or at any time expressed, mRNA encoding the tumor antigen. In an embodiment, the tumor antigen-expressing cells produce the tumor antigen protein (e.g., wild-type or mutant), and the tumor antigen protein may be present at normal levels or reduced levels. In an embodiment, the tumor antigen-expressing cells produced detectable levels of a tumor antigen protein at one point, and subsequently produced substantially no detectable tumor antigen protein.

The phrase “disease associated with expression of CD19” includes, but is not limited to, a disease associated with a cells that expresses CD19 (e.g., wild-type or mutant CD19) or condition associated with a cell which expresses, or at any time expressed, CD19 (e.g., wild-type or mutant CD19) including, e.g., proliferative diseases such as a cancer or malignancy or a precancerous condition such as a myelodysplasia, a myelodysplastic syndrome or a preleukemia; or a noncancer related indication associated with cells which express CD19. For the avoidance of doubt, a disease associated with expression of CD19 may include a condition associated with a cell which does not presently express CD19, e.g., because CD19 expression has been downregulated, e.g., due to treatment with a molecule targeting CD19, e.g., a CD19 CAR, but which at one time expressed CD19. In one aspect, a cancer

associated with expression of CD19 is a hematological cancer. In one aspect, the hematological cancer is a leukemia or a lymphoma. In one aspect, a cancer associated with expression of CD19 includes cancers and malignancies including, but not limited to, e.g., one or more acute leukemias including but not limited to, e.g., acute myeloid leukemia (AML), B-cell acute Lymphoid Leukemia (BALL), T-cell acute

5 Lymphoid Leukemia (TALL), acute lymphoid leukemia (ALL); one or more chronic leukemias including but not limited to, e.g., chronic myelogenous leukemia (CML), Chronic Lymphoid Leukemia (CLL). Additional cancers or hematologic conditions associated with expression of CD19 comprise, but are not limited to, e.g., B cell prolymphocytic leukemia, blastic plasmacytoid dendritic cell neoplasm, Burkitt's lymphoma, diffuse large B cell lymphoma, Follicular lymphoma, Hairy cell leukemia, small cell- or a

10 large cell-follicular lymphoma, malignant lymphoproliferative conditions, MALT lymphoma, mantle cell lymphoma (MCL), Marginal zone lymphoma, multiple myeloma, myelodysplasia and myelodysplastic syndrome, non-Hodgkin lymphoma, Hodgkin lymphoma, plasmablastic lymphoma, plasmacytoid dendritic cell neoplasm, Waldenstrom macroglobulinemia, myeloproliferative neoplasm; a histiocytic disorder (e.g., a mast cell disorder or a blastic plasmacytoid dendritic cell neoplasm); a mast cell disorder,

15 e.g., systemic mastocytosis or mast cell leukemia; B-cell prolymphocytic leukemia, plasma cell myeloma, and "preleukemia" which are a diverse collection of hematological conditions united by ineffective production (or dysplasia) of myeloid blood cells, and the like.

Further diseases associated with expression of CD19 expression include, but not limited to, e.g., atypical and/or non-classical cancers, malignancies, precancerous conditions or proliferative diseases

20 associated with expression of CD19. Non-cancer related indications associated with expression of CD19 include, but are not limited to, e.g., autoimmune disease, (e.g., lupus), inflammatory disorders (allergy and asthma) and transplantation. In some embodiments, the CD19-expressing cells express, or at any time expressed, CD19 mRNA. In an embodiment, the CD19-expressing cells produce a CD19 protein (e.g., wild-type or mutant), and the CD19 protein may be present at normal levels or reduced levels. In an

25 embodiment, the CD19-expressing cells produced detectable levels of a CD19 protein at one point, and subsequently produced substantially no detectable CD19 protein.

In some embodiments, the tumor antigen-expressing cells express, or at any time expressed, mRNA encoding the tumor antigen. In an embodiment, the tumor antigen-expressing cells produce the tumor antigen protein (e.g., wild-type or mutant), and the tumor antigen protein may be present at normal

30 levels or reduced levels. In an embodiment, the tumor antigen -expressing cells produced detectable levels of a tumor antigen protein at one point, and subsequently produced substantially no detectable tumor antigen protein. In other embodiments, the disease is a CD19-negative cancer, e.g., a CD19-negative relapsed cancer. In some embodiments, the tumor antigen (e.g., CD19)-expressing cell expresses, or at any time expressed, mRNA encoding the tumor antigen. In an embodiment, the tumor

antigen (e.g., CD19)-expressing cell produces the tumor antigen protein (e.g., wild-type or mutant), and the tumor antigen protein may be present at normal levels or reduced levels. In an embodiment, the tumor antigen (e.g., CD19)-expressing cell produced detectable levels of a tumor antigen protein at one point, and subsequently produced substantially no detectable tumor antigen protein.

5           The term “effector function” refers to a specialized function of a cell. Effector function of a T cell, for example, may be cytolytic activity or helper activity including the secretion of cytokines. The term “encoding” refers to the inherent property of specific sequences of nucleotides in a polynucleotide, such as a gene, a cDNA, or an mRNA, to serve as templates for synthesis of other polymers and macromolecules in biological processes having either a defined sequence of nucleotides (e.g., rRNA, 10 tRNA and mRNA) or a defined sequence of amino acids and the biological properties resulting therefrom. Thus, a gene, cDNA, or RNA, encodes a protein if transcription and translation of mRNA corresponding to that gene produces the protein in a cell or other biological system. Both the coding strand, the nucleotide sequence of which is identical to the mRNA sequence and is usually provided in sequence listings, and the non-coding strand, used as the template for transcription of a gene or cDNA, can be 15 referred to as encoding the protein or other product of that gene or cDNA.

The term “endogenous” refers to any material from or produced inside an organism, cell, tissue or system.

The term “exogenous” refers to any material introduced from or produced outside an organism, cell, tissue or system.

20           The term “expression” refers to the transcription and/or translation of a particular nucleotide sequence driven by a promoter

The term “4-1BB” refers to a member of the TNFR superfamily with an amino acid sequence provided as GenBank Acc. No. AAA62478.2, or the equivalent residues from a non-human species, e.g., mouse, rodent, monkey, ape and the like; and a “4-1BB costimulatory domain” is defined as amino acid 25 residues 214-255 of GenBank Acc. No. AAA62478.2, or the equivalent residues from a non-human species, e.g., mouse, rodent, monkey, ape and the like. In one aspect, the “4-1BB costimulatory domain” is the sequence provided as SEQ ID NO:158 or the equivalent residues from a non-human species, e.g., mouse, rodent, monkey, ape and the like.

The term “expression vector” refers to a vector comprising a recombinant polynucleotide 30 comprising expression control sequences operatively linked to a nucleotide sequence to be expressed. An expression vector comprises sufficient cis-acting elements for expression; other elements for expression can be supplied by the host cell or in an in vitro expression system. Expression vectors include all those known in the art, including cosmids, plasmids (e.g., naked or contained in liposomes) and viruses (e.g.,

lentiviruses, retroviruses, adenoviruses, and adeno-associated viruses) that incorporate the recombinant polynucleotide.

The term “homologous” or “identity” refers to the subunit sequence identity between two polymeric molecules, e.g., between two nucleic acid molecules, such as, two DNA molecules or two RNA molecules, or between two polypeptide molecules. When a subunit position in both of the two molecules is occupied by the same monomeric subunit; e.g., if a position in each of two DNA molecules is occupied by adenine, then they are homologous or identical at that position. The homology between two sequences is a direct function of the number of matching or homologous positions; e.g., if half (e.g., five positions in a polymer ten subunits in length) of the positions in two sequences are homologous, the two sequences are 50% homologous; if 90% of the positions (e.g., 9 of 10), are matched or homologous, the two sequences are 90% homologous.

The compositions and methods of the present invention encompass polypeptides and nucleic acids having the sequences specified, or sequences substantially identical or similar thereto, e.g., sequences at least 85%, 90%, 95% identical or higher to the sequence specified. In the context of an amino acid sequence, the term “substantially identical” is used herein to refer to a first amino acid that contains a sufficient or minimum number of amino acid residues that are i) identical to, or ii) conservative substitutions of aligned amino acid residues in a second amino acid sequence such that the first and second amino acid sequences can have a common structural domain and/or common functional activity. For example, amino acid sequences that contain a common structural domain having at least about 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity to a reference sequence, e.g., a sequence provided herein.

In the context of nucleotide sequence, the term “substantially identical” is used herein to refer to a first nucleic acid sequence that contains a sufficient or minimum number of nucleotides that are identical to aligned nucleotides in a second nucleic acid sequence such that the first and second nucleotide sequences encode a polypeptide having common functional activity, or encode a common structural polypeptide domain or a common functional polypeptide activity. For example, nucleotide sequences having at least about 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity to a reference sequence, e.g., a sequence provided herein.

The term “variant” refers to a polypeptide that has a substantially identical amino acid sequence to the naturally-occurring sequence, or are encoded by a substantially identical nucleotide sequence. In some embodiments, the variant is a functional variant.

The term “functional variant” refers to a polypeptide that has a substantially identical amino acid sequence to the naturally-occurring sequence, or are encoded by a substantially identical nucleotide sequence, and are capable of having one or more activities of the naturally-occurring sequence.

The term “COF1/CRBN-binding variant” of sequence X refers to a polypeptide that: (1) has a substantially identical amino acid sequence to sequence X, and (2) binds to COF1, binds to a complex of COF1 and CRBN, or binds to CRBN in the presence of COF1.

The term “COF2/CRBN-binding variant” of sequence X refers to a polypeptide that: (1) has a substantially identical amino acid sequence to sequence X, and (2) binds to COF2, binds to a complex of COF2 and CRBN, or binds to CRBN in the presence of COF2.

The term “COF3/CRBN-binding variant” of sequence X refers to a polypeptide that: (1) has a substantially identical amino acid sequence to sequence X, and (2) binds to COF3, binds to a complex of COF3 and CRBN, or binds to CRBN in the presence of COF3.

“Immune effector cell,” as that term is used herein, refers to a cell that is involved in an immune response, e.g., in the promotion of an immune effector response. Examples of immune effector cells include T cells, e.g., alpha/beta T cells and gamma/delta T cells, B cells, natural killer (NK) cells, natural killer T (NKT) cells, mast cells, and myeloid-derived phagocytes.

“Immune effector function or immune effector response,” as that term is used herein, refers to function or response, e.g., of an immune effector cell, that enhances or promotes an immune attack of a target cell. E.g., an immune effector function or response refers to a property of a T or NK cell that promotes killing or the inhibition of growth or proliferation, of a target cell. In the case of a T cell, primary stimulation and co-stimulation are examples of immune effector function or response.

The term “inhibition” or “inhibitor” includes a reduction in a certain parameter, e.g., an activity, of a given molecule, e.g., CD19, CD20, CD10, CD22, CD34, CD123, FLT-3, ROR1, CD79b, CD179b, mesothelin, or CD79a. For example, inhibition of an activity, e.g., an activity of CD20, CD10, CD19, CD22, CD34, CD123, FLT-3, ROR1, CD79b, CD179b, mesothelin, or CD79a, of at least 5%, 10%, 20%, 30%, 40%, or more is included by this term. Thus, inhibition need not be 100%. Activities for the inhibitors can be determined as described herein or by assays known in the art.

An “intracellular signaling domain,” as the term is used herein, refers to an intracellular portion of a molecule. The intracellular signaling domain can generate a signal that promotes an immune effector function of the CAR containing cell, e.g., a CART cell. Examples of immune effector function, e.g., in a CART cell, include cytolytic activity and helper activity, including the secretion of cytokines. In embodiments, the intracellular signaling domain is the portion of a protein which transduces the effector function signal and directs the cell to perform a specialized function. While the entire intracellular signaling domain can be employed, in many cases it is not necessary to use the entire chain. To the extent that a truncated portion of the intracellular signaling domain is used, such truncated portion may be used in place of the intact chain as long as it transduces the effector function signal. The term intracellular

signaling domain is thus meant to include any truncated portion of the intracellular signaling domain sufficient to transduce the effector function signal.

In an embodiment, the intracellular signaling domain can comprise a primary intracellular signaling domain. Exemplary primary intracellular signaling domains include those derived from the molecules responsible for primary stimulation, or antigen dependent stimulation. In an embodiment, the intracellular signaling domain can comprise a costimulatory intracellular domain. Exemplary costimulatory intracellular signaling domains include those derived from molecules responsible for costimulatory signals, or antigen independent stimulation. For example, in the case of a CART, a primary intracellular signaling domain can comprise a cytoplasmic sequence of a T cell receptor, and a costimulatory intracellular signaling domain can comprise cytoplasmic sequence from co-receptor or costimulatory molecule.

A primary intracellular signaling domain can comprise a signaling motif which is known as an immunoreceptor tyrosine-based activation motif or ITAM. Examples of ITAM containing primary cytoplasmic signaling sequences include, but are not limited to, those derived from CD3 zeta, FcR gamma, FcR beta, CD3 gamma, CD3 delta, CD3 epsilon, CD5, CD22, CD79a, CD79b, CD278 ("ICOS"), FcεRI, CD66d, CD32, DAP10 and DAP12.

The term "isolated" means altered or removed from the natural state. For example, a nucleic acid or a peptide naturally present in a living animal is not "isolated," but the same nucleic acid or peptide partially or completely separated from the coexisting materials of its natural state is "isolated." An isolated nucleic acid or protein can exist in substantially purified form, or can exist in a non-native environment such as, for example, a host cell.

As used herein, the term "mesothelin" refers to the 40-kDa protein, mesothelin, which is anchored at the cell membrane by a glycosylphosphatidyl inositol (GPI) linkage and an amino-terminal 31-kDa shed fragment, called megkaryocyte potentiating factor (MPF). Both fragments contain N-glycosylation sites. The term also refers to a soluble splice variant of the 40-kDa carboxyl-terminal fragment also called "soluble mesothelin/MPF-related". Preferably, the term refers to a human mesothelin of GenBank accession number AAH03512.1, and naturally cleaved portions thereof, e.g., as expressed on a cell membrane, e.g., a cancer cell membrane. As used herein, "mesothelin" includes proteins comprising mutations, e.g., point mutations, fragments, insertions, deletions and splice variants of full length wild-type mesothelin.

Unless otherwise specified, a "nucleotide sequence encoding an amino acid sequence" includes all nucleotide sequences that are degenerate versions of each other and that encode the same amino acid sequence. The phrase nucleotide sequence that encodes a protein or a RNA may also include introns to the extent that the nucleotide sequence encoding the protein may in some version contain an intron(s)

In the context of the present invention, the following abbreviations for the commonly occurring nucleic acid bases are used. “A” refers to adenosine, “C” refers to cytosine, “G” refers to guanosine, “T” refers to thymidine, and “U” refers to uridine.

The term “nucleic acid” or “polynucleotide” refers to deoxyribonucleic acids (DNA) or ribonucleic acid (RNA), or a combination of a DNA or RNA thereof, and polymers thereof in either single- or double-stranded form. The term “nucleic acid” includes a gene, cDNA or an mRNA. In one embodiment, the nucleic acid molecule is synthetic (e.g., chemically synthesized) or recombinant. Unless specifically limited, the term encompasses nucleic acids containing analogues or derivatives of natural nucleotides that have similar binding properties as the reference nucleic acid and are metabolized in a manner similar to naturally occurring nucleotides. Unless otherwise indicated, a particular nucleic acid sequence also implicitly encompasses conservatively modified variants thereof (e.g., degenerate codon substitutions), alleles, orthologs, SNPs, and complementary sequences as well as the sequence explicitly indicated. Specifically, degenerate codon substitutions may be achieved by generating sequences in which the third position of one or more selected (or all) codons is substituted with mixed-base and/or deoxyinosine residues (Batzner et al., *Nucleic Acid Res.* 19:5081 (1991); Ohtsuka et al., *J. Biol. Chem.* 260:2605-2608 (1985); and Rossolini et al., *Mol. Cell. Probes* 8:91-98 (1994)).

The terms “peptide,” “polypeptide,” and “protein” are used interchangeably, and refer to a compound comprised of amino acid residues covalently linked by peptide bonds. A protein or peptide must contain at least two amino acids, and no limitation is placed on the maximum number of amino acids that can comprise a protein’s or peptide’s sequence. Polypeptides include any peptide or protein comprising two or more amino acids joined to each other by peptide bonds. As used herein, the term refers to both short chains, which also commonly are referred to in the art as peptides, oligopeptides and oligomers, for example, and to longer chains, which generally are referred to in the art as proteins, of which there are many types. “Polypeptides” include, for example, biologically active fragments, substantially homologous polypeptides, oligopeptides, homodimers, heterodimers, variants of polypeptides, modified polypeptides, derivatives, analogs, fusion proteins, among others. A polypeptide includes a natural peptide, a recombinant peptide, or a combination thereof.

The term “operably linked” or “transcriptional control” refers to functional linkage between a regulatory sequence and a heterologous nucleic acid sequence resulting in expression of the latter. For example, a first nucleic acid sequence is operably linked with a second nucleic acid sequence when the first nucleic acid sequence is placed in a functional relationship with the second nucleic acid sequence. For instance, a promoter is operably linked to a coding sequence if the promoter affects the transcription or expression of the coding sequence. Operably linked DNA sequences can be contiguous with each other and, e.g., where necessary to join two protein coding regions, are in the same reading frame.

The term “parenteral” administration of an immunogenic composition includes, e.g., subcutaneous (s.c.), intravenous (i.v.), intramuscular (i.m.), or intrasternal injection, intratumoral, or infusion techniques.

The term “promoter” refers to a DNA sequence recognized by the synthetic machinery of the cell, or introduced synthetic machinery, required to initiate the specific transcription of a polynucleotide sequence.

The term “promoter/regulatory sequence” refers to a nucleic acid sequence which is required for expression of a gene product operably linked to the promoter/regulatory sequence. In some instances, this sequence may be the core promoter sequence and in other instances, this sequence may also include an enhancer sequence and other regulatory elements which are required for expression of the gene product. The promoter/regulatory sequence may, for example, be one which expresses the gene product in a tissue specific manner.

The term “signaling domain” refers to the functional portion of a protein which acts by transmitting information within the cell to regulate cellular activity via defined signaling pathways by generating second messengers or functioning as effectors by responding to such messengers.

The term “scFv” refers to a fusion protein comprising at least one antibody fragment comprising a variable region of a light chain and at least one antibody fragment comprising a variable region of a heavy chain, wherein the light and heavy chain variable regions are contiguously linked, e.g., via a synthetic linker, e.g., a short flexible polypeptide linker, and capable of being expressed as a single chain polypeptide, and wherein the scFv retains the specificity of the intact antibody from which it is derived. Unless specified, as used herein an scFv may have the VL and VH variable regions in either order, e.g., with respect to the N-terminal and C-terminal ends of the fusion polypeptide, the scFv may comprise VL-linker-VH or may comprise VH-linker-VL.

The portion of a CAR comprising an antibody or antibody fragment thereof may exist in a variety of forms where the antigen binding domain is expressed as part of a contiguous polypeptide chain including, for example, a single domain antibody fragment (sdAb), a single chain antibody (scFv) and a humanized antibody (Harlow et al., 1999, In: Using Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory Press, NY; Harlow et al., 1989, In: Antibodies: A Laboratory Manual, Cold Spring Harbor, New York; Houston et al., 1988, Proc. Natl. Acad. Sci. USA 85:5879-5883; Bird et al., 1988, Science 242:423-426). In one embodiment, the antigen binding domain of a CAR comprises an antibody fragment. In a further embodiment, the CAR comprises an antibody fragment that comprises a scFv. As used herein, the term “binding domain” or “antibody molecule” refers to a protein, e.g., an immunoglobulin chain or fragment thereof, comprising at least one immunoglobulin variable domain sequence. The term “binding domain” or “antibody molecule” encompasses antibodies and antibody



fragments. In an embodiment, an antibody molecule is a multispecific antibody molecule, e.g., it comprises a plurality of immunoglobulin variable domain sequences, wherein a first immunoglobulin variable domain sequence of the plurality has binding specificity for a first epitope and a second immunoglobulin variable domain sequence of the plurality has binding specificity for a second epitope.

5 In an embodiment, a multispecific antibody molecule is a bispecific antibody molecule. A bispecific antibody has specificity for no more than two antigens. A bispecific antibody molecule is characterized by a first immunoglobulin variable domain sequence which has binding specificity for a first epitope and a second immunoglobulin variable domain sequence that has binding specificity for a second epitope.

10 The term “stimulation,” refers to a primary response induced by binding of a stimulatory molecule (e.g., a TCR/CD3 complex or CAR) with its cognate ligand (e.g., antigen molecule), thereby mediating a signal transduction event, such as, but not limited to, signal transduction via the TCR/CD3 complex or signal transduction via the appropriate NK receptor or signaling domains of the CAR. Stimulation can mediate altered expression of certain molecules.

15 The term “stimulatory molecule,” refers to a molecule expressed by an immune cell (e.g., T cell, NK cell, B cell) that provides the cytoplasmic signaling sequence(s) that regulate activation of the immune cell in a stimulatory way for at least some aspect of the immune cell signaling pathway. In one aspect, the signal is a primary signal that is initiated by, for instance, binding of a TCR/CD3 complex with an MHC molecule loaded with peptide, and which leads to mediation of a T cell response, including, but not limited to, proliferation, activation, differentiation, and the like. A primary cytoplasmic signaling  
20 sequence (also referred to as a “primary signaling domain”) that acts in a stimulatory manner may contain a signaling motif which is known as immunoreceptor tyrosine-based activation motif or ITAM. Examples of an ITAM containing cytoplasmic signaling sequence that is of particular use in the invention includes, but is not limited to, those derived from CD3 zeta, common FcR gamma (FCER1G), Fc gamma RIIa., FcR beta (Fc Epsilon R1b), CD3 gamma, CD3 delta , CD3 epsilon , , CD79a, CD79b, DAP10, and  
25 DAP12. In a specific CAR of the invention, the intracellular signaling domain in any one or more CARS of the invention comprises an intracellular signaling sequence, e.g., a primary signaling sequence of CD3-zeta. In a specific CAR of the invention, the primary signaling sequence of CD3-zeta is the sequence provided as SEQ ID NO: 163, or the equivalent residues from a non-human species, e.g., mouse, rodent, monkey, ape and the like. In a specific CAR of the invention, the primary signaling sequence of CD3-zeta  
30 is the sequence as provided in SEQ ID NO: 166, or the equivalent residues from a non-human species, e.g., mouse, rodent, monkey, ape and the like.

The term “transfer vector” refers to a composition of matter which comprises an isolated nucleic acid and which can be used to deliver the isolated nucleic acid to the interior of a cell. Numerous vectors

are known in the art including, but not limited to, linear polynucleotides, polynucleotides associated with ionic or amphiphilic compounds, plasmids, and viruses. Thus, the term “transfer vector” includes an autonomously replicating plasmid or a virus. The term should also be construed to further include non-plasmid and non-viral compounds which facilitate transfer of nucleic acid into cells, such as, for example, a polylysine compound, liposome, and the like. Examples of viral transfer vectors include, but are not limited to, adenoviral vectors, adeno-associated virus vectors, retroviral vectors, lentiviral vectors, and the like.

The term “zeta” or alternatively “zeta chain”, “CD3-zeta” or “TCR-zeta” is defined as the protein provided as GenBank Acc. No. BAG36664.1, or the equivalent residues from a non-human species, e.g., mouse, rodent, monkey, ape and the like, and a “zeta stimulatory domain” or alternatively a “CD3-zeta stimulatory domain” or a “TCR-zeta stimulatory domain” is defined as the amino acid residues from the cytoplasmic domain of the zeta chain that are sufficient to functionally transmit an initial signal necessary for T cell activation. In one aspect the cytoplasmic domain of zeta comprises residues 52 through 164 of GenBank Acc. No. BAG36664.1 or the equivalent residues from a non-human species, e.g., mouse, rodent, monkey, ape and the like, that are functional orthologs thereof. In one aspect, the “zeta stimulatory domain” or a “CD3-zeta stimulatory domain” is the sequence provided as SEQ ID NO: 163 (mutant CD3 zeta). In one aspect, the “zeta stimulatory domain” or a “CD3-zeta stimulatory domain” is the sequence provided as SEQ ID NO: 166 (wild-type human CD3 zeta).

The term “constitutive” promoter refers to a nucleotide sequence which, when operably linked with a polynucleotide which encodes or specifies a gene product, causes the gene product to be produced in a cell under most or all physiological conditions of the cell.

The term “inducible” promoter refers to a nucleotide sequence which, when operably linked with a polynucleotide which encodes or specifies a gene product, causes the gene product to be produced in a cell substantially only when an inducer which corresponds to the promoter is present in the cell.

The term “tissue-specific” promoter refers to a nucleotide sequence which, when operably linked with a polynucleotide encodes or specified by a gene, causes the gene product to be produced in a cell substantially only if the cell is a cell of the tissue type corresponding to the promoter.

The terms “cancer associated antigen,” “tumor antigen,” “hyperproliferative disorder antigen,” and “antigen associated with a hyperproliferative disorder” interchangeably refer to antigens that are common to specific hyperproliferative disorders. In some embodiments, these terms refer to a molecule (typically a protein, carbohydrate or lipid) that is expressed on the surface of a cancer cell, either entirely or as a fragment (e.g., MHC/peptide), and which is useful for the preferential targeting of a pharmacological agent to the cancer cell. In some embodiments, a tumor antigen is a marker expressed by both normal cells and cancer cells, e.g., a lineage marker, e.g., CD19 on B cells. In some

embodiments, a tumor antigen is a cell surface molecule that is overexpressed in a cancer cell in comparison to a normal cell, for instance, 1-fold over expression, 2-fold overexpression, 3-fold overexpression or more in comparison to a normal cell. In some embodiments, a tumor antigen is a cell surface molecule that is inappropriately synthesized in the cancer cell, for instance, a molecule that contains deletions, additions or mutations in comparison to the molecule expressed on a normal cell. In some embodiments, a tumor antigen will be expressed exclusively on the cell surface of a cancer cell, entirely or as a fragment (e.g., MHC/peptide), and not synthesized or expressed on the surface of a normal cell. In certain aspects, the hyperproliferative disorder antigens of the present invention are derived from, cancers including but not limited to primary or metastatic melanoma, thymoma, lymphoma, sarcoma, lung cancer, liver cancer, non-Hodgkin lymphoma, Hodgkin lymphoma, leukemias, uterine cancer, cervical cancer, bladder cancer, kidney cancer and adenocarcinomas such as breast cancer, prostate cancer (e.g., castrate-resistant or therapy-resistant prostate cancer, or metastatic prostate cancer), ovarian cancer, pancreatic cancer, and the like, or a plasma cell proliferative disorder, e.g., asymptomatic myeloma (smoldering multiple myeloma or indolent myeloma), monoclonal gammopathy of undetermined significance (MGUS), Waldenstrom's macroglobulinemia, plasmacytomas (e.g., plasma cell dyscrasia, solitary myeloma, solitary plasmacytoma, extramedullary plasmacytoma, and multiple plasmacytoma), systemic amyloid light chain amyloidosis, and POEMS syndrome (also known as Crow-Fukase syndrome, Takatsuki disease, and PEP syndrome). In some embodiments, the CARs of the present invention include CARs comprising an antigen binding domain (e.g., antibody or antibody fragment) that binds to an MHC presented peptide. Normally, peptides derived from endogenous proteins fill the pockets of Major histocompatibility complex (MHC) class I molecules, and are recognized by T cell receptors (TCRs) on CD8 + T lymphocytes. The MHC class I complexes are constitutively expressed by all nucleated cells. In cancer, virus-specific and/or tumor-specific peptide/MHC complexes represent a unique class of cell surface targets for immunotherapy. TCR-like antibodies targeting peptides derived from viral or tumor antigens in the context of human leukocyte antigen (HLA)-A1 or HLA-A2 have been described (see, e.g., Sastry et al., J Virol. 2011 85(5):1935-1942; Sergeeva et al., Blood, 2011 117(16):4262-4272; Verma et al., J Immunol 2010 184(4):2156-2165; Willemsen et al., Gene Ther 2001 8(21) :1601-1608; Dao et al., Sci Transl Med 2013 5(176) :176ra33; Tassev et al., Cancer Gene Ther 2012 19(2):84-100). For example, TCR-like antibody can be identified from screening a library, such as a human scFv phage displayed library.

The term "flexible polypeptide linker" or "linker" as used refers to a peptide linker that comprises, or consists of, amino acids such as glycine and/or serine residues used alone or in combination, to link two polypeptides together, e.g., a COF1/CRBN-, COF2/CRBN-, or COF3/CRBN-binding polypeptide and a heterologous polypeptide, or a variable heavy and variable light chain regions.

In one embodiment, the flexible polypeptide linker is a Gly/Ser linker and comprises the amino acid sequence (Gly-Gly-Gly-Ser)<sub>n</sub> (SEQ ID NO: 173), where *n* is a positive integer equal to or greater than 1. For example, *n*=1, *n*=2, *n*=3, *n*=4, *n*=5, *n*=6, *n*=7, *n*=8, *n*=9 and *n*=10. In one embodiment, the flexible polypeptide linkers include, but are not limited to, (Gly<sub>4</sub> Ser)<sub>4</sub> (SEQ ID NO: 141) or (Gly<sub>4</sub> Ser)<sub>3</sub> (SEQ ID NO: 142). In another embodiment, the linkers include multiple repeats of (Gly<sub>2</sub>Ser), (GlySer) or (Gly<sub>3</sub>Ser) (SEQ ID NO: 143). Also included within the scope of the invention are linkers described in WO2012/138475, incorporated herein by reference).

As used herein, “transient” refers to expression of a non-integrated transgene for a period of hours, days or weeks, wherein the period of time of expression is less than the period of time for expression of the gene if integrated into the genome or contained within a stable plasmid replicon in the cell. In embodiments, a CAR molecule is transiently expressed in a cell, e.g., host cell, for a finite period of time or number of cell replications, e.g., less than 50 days (e.g., less than 40, 30, 25, 20, 15, 10, 5, 4, 3, 2 or fewer days). In one embodiment, transient expression is effected using an in vitro transcribed RNA.

As used herein, “stable” refers to expression of a transgene that is for a longer period than transient expression. In embodiments, the transgene is integrated into the genome of a cell, e.g., a host cell, or contained within a stable plasmid replicon in the cell. In one embodiment, a transgene is integrated into the cell genome using a gene delivery vector, e.g., a retroviral vector such as a lentivirus vector.

As used herein, the terms “treat”, “treatment” and “treating” refer to the reduction or amelioration of the progression, severity and/or duration of a proliferative disorder, or the amelioration of one or more symptoms (e.g., one or more discernible symptoms) of a proliferative disorder resulting from the administration of one or more therapies (e.g., one or more therapeutic agents, such as a CAR of the invention). In specific embodiments, the terms “treat”, “treatment” and “treating” refer to the amelioration of at least one measurable physical parameter of a proliferative disorder, such as growth of a tumor, not necessarily discernible by the patient. In other embodiments the terms “treat”, “treatment” and “treating” - refer to the inhibition of the progression of a proliferative disorder, either physically by, e.g., stabilization of a discernible symptom, physiologically by, e.g., stabilization of a physical parameter, or both. In other embodiments the terms “treat”, “treatment” and “treating” refer to the reduction or stabilization of tumor size or cancerous cell count. Treatment need not be 100%, and in some embodiments a reduction or delay in at least one symptom of the disease or disorder by at least 50%, 60%, 70%, 80%, 90%, 95%, or 99% is sufficient to be considered within these terms.

The term “subject” is intended to include living organisms in which an immune response can be elicited (e.g., mammals, e.g., humans). Examples of subjects include humans, monkeys, chimpanzees, dogs, cats, mice, rats, and transgenic species thereof. T cells can be obtained from a number of sources,

including peripheral blood mononuclear cells, bone marrow, lymph node tissue, cord blood, thymus tissue, tissue from a site of infection, ascites, pleural effusion, spleen tissue, and tumors.

The term “transfected” or “transformed” or “transduced” refers to a process by which exogenous nucleic acid is transferred or introduced into the host cell. A “transfected” or “transformed” or “transduced” cell is one which has been transfected, transformed or transduced with exogenous nucleic acid. The cell includes the primary subject cell and its progeny.

The term “specifically binds,” refers to an antibody, or a ligand, which recognizes and binds with a cognate binding partner protein present in a sample, but which antibody or ligand does not substantially recognize or bind other molecules in the sample.

Ranges: throughout this disclosure, various aspects of the invention can be presented in a range format. It should be understood that the description in range format is merely for convenience and brevity and should not be construed as an inflexible limitation on the scope of the invention. Accordingly, the description of a range should be considered to have specifically disclosed all the possible subranges as well as individual numerical values within that range. For example, description of a range such as from 1 to 6 should be considered to have specifically disclosed subranges such as from 1 to 3, from 1 to 4, from 1 to 5, from 2 to 4, from 2 to 6, from 3 to 6 etc., as well as individual numbers within that range, for example, 1, 2, 2.7, 3, 4, 5, 5.3, and 6. As another example, a range such as 95-99% identity, includes something with 95%, 96%, 97%, 98% or 99% identity, and includes subranges such as 96-99%, 96-98%, 96-97%, 97-99%, 97-98% and 98-99% identity. This applies regardless of the breadth of the range.

Definitions of specific functional groups and chemical terms are described in more detail below. The chemical elements are identified in accordance with the Periodic Table of the Elements, CAS version, *Handbook of Chemistry and Physics*, 75<sup>th</sup> Ed., inside cover, and specific functional groups are generally defined as described therein. Additionally, general principles of organic chemistry, as well as specific functional moieties and reactivity, are described in Thomas Sorrell, *Organic Chemistry*, University Science Books, Sausalito, 1999; Smith and March, *March's Advanced Organic Chemistry*, 5<sup>th</sup> Edition, John Wiley & Sons, Inc., New York, 2001; Larock, *Comprehensive Organic Transformations*, VCH Publishers, Inc., New York, 1989; and Carruthers, *Some Modern Methods of Organic Synthesis*, 3<sup>rd</sup> Edition, Cambridge University Press, Cambridge, 1987.

The term “alkyl,” as used herein, refers to a monovalent saturated, straight- or branched-chain hydrocarbon such as a straight or branched group of 1-12, 1-10, or 1-6 carbon atoms, referred to herein as C<sub>1</sub>-C<sub>12</sub> alkyl, C<sub>1</sub>-C<sub>10</sub> alkyl, and C<sub>1</sub>-C<sub>6</sub> alkyl, respectively. Examples of alkyl groups include, but are not limited to, methyl, ethyl, n-propyl, isopropyl, n-butyl, iso-butyl, sec-butyl, sec-pentyl, iso-pentyl, tert-butyl, n-pentyl, neopentyl, n-hexyl, sec-hexyl, and the like.

The terms “alkenyl” and “alkynyl” as used herein refer to unsaturated aliphatic groups analogous in length and possible substitution to the alkyls described above, but that contain at least one double or triple bond, respectively. Exemplary alkenyl groups include, but are not limited to,  $-\text{CH}=\text{CH}_2$  and  $-\text{CH}_2\text{CH}=\text{CH}_2$ .

5       The term “alkoxy” as used herein refers to a straight or branched chain saturated hydrocarbon containing 1-12 carbon atoms containing a terminal “O” in the chain, e.g.,  $-\text{O}(\text{alkyl})$ . Examples of alkoxy groups include, without limitation, methoxy, ethoxy, propoxy, butoxy, t-butoxy, or pentoxy groups.

10       The term “aryl” as used herein refers to a monocyclic, bicyclic or polycyclic hydrocarbon ring system, wherein at least one ring is aromatic. Representative aryl groups include fully aromatic ring systems, such as phenyl (e.g.,  $(\text{C}_6)$  aryl), naphthyl (e.g.,  $(\text{C}_{10})$  aryl), and anthracenyl (e.g.,  $(\text{C}_{14})$  aryl), and ring systems where an aromatic carbon ring is fused to one or more non-aromatic carbon rings, such as indanyl, phthalimidyl, naphthimidyl, or tetrahydronaphthyl, and the like.

15       The term “carbocyclyl” as used herein refers to monocyclic, or fused, spiro-fused, and/or bridged bicyclic or polycyclic hydrocarbon ring system containing 3-18 carbon atoms, wherein each ring is either completely saturated or contains one or more units of unsaturation, but where no ring is aromatic. Representative carbocyclyl groups include cycloalkyl groups (e.g., cyclopentyl, cyclobutyl, cyclopentyl, cyclohexyl and the like), and cycloalkenyl groups (e.g., cyclopentenyl, cyclohexenyl, cyclopentadienyl, and the like).

20       The term “carbonyl” as used herein refers to  $-\text{C}=\text{O}$ .

20       The term “cyano” as used herein refers to  $-\text{CN}$ .

The terms “halo” or “halogen” as used herein refer to fluorine (fluoro,  $-\text{F}$ ), chlorine (chloro,  $-\text{Cl}$ ), bromine (bromo,  $-\text{Br}$ ), or iodine (iodo,  $-\text{I}$ ).

25       The term “haloalkyl” as used herein refers to a monovalent saturated straight or branched alkyl chain wherein at least one carbon atom in the chain is substituted with one or more halogen atoms. In some embodiments, a haloalkyl group may comprise, e.g., 1-12, 1-10, or 1-6 carbon atoms, referred to herein as  $\text{C}_1\text{-C}_{12}$  haloalkyl,  $\text{C}_1\text{-C}_{10}$  haloalkyl, and  $\text{C}_1\text{-C}_6$  haloalkyl. Examples of haloalkyl groups include, but are not limited to, trifluoromethyl, difluoromethyl, pentafluoroethyl, trichloromethyl, etc.

30       The term “haloalkoxy” to a straight or branched chain saturated hydrocarbon containing 1-12 carbon atoms containing a terminal “O” in the chain, wherein at least one carbon atom in the chain is substituted with one or more halogens. Examples of haloalkoxy groups include, but are not limited to, trifluoromethoxy, difluoromethoxy, pentafluoroethoxy, trichloromethoxy, etc.

The term “heteroalkyl” as used herein refers to a monovalent saturated straight or branched alkyl chain wherein at least one carbon atom in the chain is replaced with a heteroatom, such as O, S, or N, provided that upon substitution, the chain comprises at least one carbon atom. In some embodiments, a

heteroalkyl group may comprise, e.g., 1-12, 1-10, or 1-6 carbon atoms, referred to herein as C<sub>1</sub>-C<sub>12</sub> heteroalkyl, C<sub>1</sub>-C<sub>10</sub> heteroalkyl, and C<sub>1</sub>-C<sub>6</sub> heteroalkyl. In certain instances, a heteroalkyl group comprises 1, 2, 3, or 4 independently selected heteroatoms in place of 1, 2, 3, or 4 individual carbon atoms in the alkyl chain. Representative heteroalkyl groups include -CH<sub>2</sub>NHC(O)CH<sub>3</sub>, -CH<sub>2</sub>CH<sub>2</sub>OCH<sub>3</sub>, -CH<sub>2</sub>CH<sub>2</sub>NHCH<sub>3</sub>, -CH<sub>2</sub>CH<sub>2</sub>N(CH<sub>3</sub>)CH<sub>3</sub>, and the like.

The terms “alkylene,” “alkenylene,” “alkynylene,” and “heteroalkylene” as used herein refer to a divalent radical of an alkyl, alkenyl, alkynyl, or heteroalkyl group, respectively. Any of a monovalent alkyl, alkenyl, alkynyl, or heteroalkyl group may be an alkylene, alkenylene, alkynylene, or heteroalkylene by abstraction of a second hydrogen atom from the alkyl, alkenyl, alkynyl, or heteroalkyl group.

The term “heteroaryl” as used herein refers to a monocyclic, bicyclic or polycyclic ring system wherein at least one ring is both aromatic and comprises a heteroatom; and wherein no other rings are heterocyclyl (as defined below). Representative heteroaryl groups include ring systems where (i) each ring comprises a heteroatom and is aromatic, e.g., imidazolyl, oxazolyl, thiazolyl, triazolyl, pyrrolyl, furanyl, thiophenyl, pyrazolyl, pyridinyl, pyrazinyl, pyridazinyl, pyrimidinyl, indolizinyl, purinyl, naphthyridinyl, and pteridinyl; (ii) each ring is aromatic or carbocyclyl, at least one aromatic ring comprises a heteroatom and at least one other ring is a hydrocarbon ring or e.g., indolyl, isoindolyl, benzothienyl, benzofuranyl, dibenzofuranyl, indazolyl, benzimidazolyl, benzthiazolyl, quinolyl, isoquinolyl, cinnolinyl, phthalazinyl, quinazolinyl, quinoxalinyl, carbazolyl, acridinyl, phenazinyl, phenothiazinyl, phenoxazinyl, pyrido[2,3-*b*]-1,4-oxazin-3(4H)-one, thiazolo-[4,5-*c*]-pyridinyl, 4,5,6,7-tetrahydrothieno[2,3-*c*]pyridinyl, 5,6-dihydro-4H-thieno[2,3-*c*]pyrrolyl, 4,5,6,7,8-tetrahydroquinolinyl and 5,6,7,8-tetrahydroisoquinolinyl; and (iii) each ring is aromatic or carbocyclyl, and at least one aromatic ring shares a bridgehead heteroatom with another aromatic ring, e.g., 4H-quinolizinyl. In certain embodiments, the heteroaryl is a monocyclic or bicyclic ring, wherein each of said rings contains 5 or 6 ring atoms where 1, 2, 3, or 4 of said ring atoms are a heteroatom independently selected from N, O, and S.

The term “heterocyclyl” as used herein refers to a monocyclic, or fused, spiro-fused, and/or bridged bicyclic and polycyclic ring systems where at least one ring is saturated or partially unsaturated (but not aromatic) and comprises a heteroatom. A heterocyclyl can be attached to its pendant group at any heteroatom or carbon atom that results in a stable structure and any of the ring atoms can be optionally substituted. Representative heterocyclyls include ring systems in which (i) every ring is non-aromatic and at least one ring comprises a heteroatom, e.g., tetrahydrofuranyl, tetrahydrothienyl, pyrrolidinyl, pyrrolidonyl, piperidinyl, pyrrolinyl, decahydroquinolinyl, oxazolidinyl, piperazinyl, dioxanyl, dioxolanyl, diazepinyl, oxazepinyl, thiazepinyl, morpholinyl, and quinuclidinyl; (ii) at least one ring is

non-aromatic and comprises a heteroatom and at least one other ring is an aromatic carbon ring, *e.g.*, 1,2,3,4-tetrahydroquinolinyl; and (iii) at least one ring is non-aromatic and comprises a heteroatom and at least one other ring is aromatic and comprises a heteroatom, *e.g.*, 3,4-dihydro-1H-pyrano[4,3-c]pyridinyl, and 1,2,3,4-tetrahydro-2,6-naphthyridinyl. In certain embodiments, the heterocyclyl is a monocyclic or bicyclic ring, wherein each of said rings contains 3-7 ring atoms where 1, 2, 3, or 4 of said ring atoms are a heteroatom independently selected from N, O, and S.

As described herein, compounds of the invention may contain “optionally substituted” moieties. In general, the term “substituted”, whether preceded by the term “optionally” or not, means that one or more hydrogens of the designated moiety are replaced with a suitable substituent. Unless otherwise indicated, an “optionally substituted” group may have a suitable substituent at each substitutable position of the group, and when more than one position in any given structure may be substituted with more than one substituent selected from a specified group, the substituent may be either the same or different at each position. Combinations of substituents envisioned under this invention are preferably those that result in the formation of stable or chemically feasible compounds. The term “stable”, as used herein, refers to compounds that are not substantially altered when subjected to conditions to allow for their production, detection, and, in certain embodiments, their recovery, purification, and use for one or more of the purposes disclosed herein.

The term “oxo” as used herein refers to =O.

The term “thiocarbonyl” as used herein refers to C=S.

As used herein, the term “pharmaceutically acceptable salt” refers to those salts which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of humans and lower animals without undue toxicity, irritation, allergic response and the like, and are commensurate with a reasonable benefit/risk ratio. Pharmaceutically acceptable salts are well known in the art. For example, Berge et al., describe pharmaceutically acceptable salts in detail in *J. Pharmaceutical Sciences*, 1977, 66, 1–19, incorporated herein by reference. Pharmaceutically acceptable salts of the compounds of this invention include those derived from suitable inorganic and organic acids and bases. Examples of pharmaceutically acceptable, nontoxic acid addition salts are salts of an amino group formed with inorganic acids such as hydrochloric acid, hydrobromic acid, phosphoric acid, sulfuric acid, and perchloric acid or with organic acids such as acetic acid, oxalic acid, maleic acid, tartaric acid, citric acid, succinic acid, or malonic acid or by using other methods known in the art such as ion exchange. Other pharmaceutically acceptable salts include adipate, alginate, ascorbate, aspartate, benzenesulfonate, benzoate, bisulfate, borate, butyrate, camphorate, camphorsulfonate, citrate, cyclopentanepropionate, digluconate, dodecylsulfate, ethanesulfonate, formate, fumarate, glucoheptonate, glycerophosphate, gluconate, hemisulfate, heptanoate, hexanoate, hydroiodide, 2-hydroxy-ethanesulfonate, lactobionate,



lactate, laurate, lauryl sulfate, malate, maleate, malonate, methanesulfonate, 2-naphthalenesulfonate, nicotinate, nitrate, oleate, oxalate, palmitate, pamoate, pectinate, persulfate, 3-phenylpropionate, phosphate, picrate, pivalate, propionate, stearate, succinate, sulfate, tartrate, thiocyanate, p-toluenesulfonate, undecanoate, valerate salts, and the like. Salts derived from appropriate bases include alkali metal, alkaline earth metal, ammonium and  $N^+(C_{1-4} \text{ alkyl})_4^-$  salts. Representative alkali or alkaline earth metal salts include sodium, lithium, potassium, calcium, magnesium, and the like. Further pharmaceutically acceptable salts include, when appropriate, nontoxic ammonium, quaternary ammonium, and amine cations formed using counterions such as halide, hydroxide, carboxylate, sulfate, phosphate, nitrate, lower alkyl sulfonate, and aryl sulfonate.

The term “solvate” refers to forms of the compound that are associated with a solvent, usually by a solvolysis reaction. This physical association may include hydrogen bonding. Conventional solvents include water, methanol, ethanol, acetic acid, DMSO, THF, diethyl ether, and the like. The compounds of Formula (I), Formula (I-a), and/or Formula (II) may be prepared, *e.g.*, in crystalline form, and may be solvated. Suitable solvates include pharmaceutically acceptable solvates and further include both stoichiometric solvates and non-stoichiometric solvates. In certain instances, the solvate will be capable of isolation, for example, when one or more solvent molecules are incorporated in the crystal lattice of a crystalline solid. “Solvate” encompasses both solution-phase and isolable solvates. Representative solvates include hydrates, ethanolates, and methanolates.

The term “hydrate” refers to a compound which is associated with water. Typically, the number of the water molecules contained in a hydrate of a compound is in a definite ratio to the number of the compound molecules in the hydrate. Therefore, a hydrate of a compound may be represented, for example, by the general formula  $R \cdot x \text{ H}_2\text{O}$ , wherein R is the compound and wherein x is a number greater than 0. A given compound may form more than one type of hydrates, including, *e.g.*, monohydrates (x is 1), lower hydrates (x is a number greater than 0 and smaller than 1, *e.g.*, hemihydrates ( $R \cdot 0.5 \text{ H}_2\text{O}$ )), and polyhydrates (x is a number greater than 1, *e.g.*, dihydrates ( $R \cdot 2 \text{ H}_2\text{O}$ ) and hexahydrates ( $R \cdot 6 \text{ H}_2\text{O}$ )).

It is to be understood that compounds that have the same molecular formula but differ in the nature or sequence of bonding of their atoms or the arrangement of their atoms in space are termed “isomers”. Isomers that differ in the arrangement of their atoms in space are termed “stereoisomers”.

Stereoisomers that are not mirror images of one another are termed “diastereomers” and those that are non-superimposable mirror images of each other are termed “enantiomers”. When a compound has an asymmetric center, for example, it is bonded to four different groups and a pair of enantiomers is possible. An enantiomer can be characterized by the absolute configuration of its asymmetric center and is described by the R- and S-sequencing rules of Cahn and Prelog, or by the manner in which the molecule rotates the plane of polarized light and designated as dextrorotatory or levorotatory (*i.e.*, as (+)

or (-)-isomers respectively). A chiral compound can exist as either individual enantiomer or as a mixture thereof. A mixture containing equal proportions of the enantiomers is called a “racemic mixture”.

The term “tautomers” refer to compounds that are interchangeable forms of a particular compound structure, and that vary in the displacement of hydrogen atoms and electrons. Thus, two structures may be in equilibrium through the movement of  $\pi$  electrons and an atom (usually H). For example, enols and ketones are tautomers because they are rapidly interconverted by treatment with either acid or base. Another example of tautomerism is the aci- and nitro- forms of phenylnitromethane that are likewise formed by treatment with acid or base.

Tautomeric forms may be relevant to the attainment of the optimal chemical reactivity and biological activity of a compound of interest.

Unless otherwise stated, structures depicted herein are also meant to include all isomeric (*e.g.*, enantiomeric, diastereomeric, and geometric (or conformational)) forms of the structure; for example, the R and S configurations for each asymmetric center, Z and E double bond isomers, and Z and E conformational isomers. Therefore, single stereochemical isomers as well as enantiomeric, diastereomeric, and geometric (or conformational) mixtures of the present compounds are within the scope of the invention. Unless otherwise stated, all tautomeric forms of the compounds of the invention are within the scope of the invention. Additionally, unless otherwise stated, structures depicted herein are also meant to include compounds that differ only in the presence of one or more isotopically enriched atoms. For example, compounds having the present structures including the replacement of hydrogen by deuterium or tritium, or the replacement of a carbon by a  $^{13}\text{C}$ - or  $^{14}\text{C}$ -enriched carbon are within the scope of this invention. In an embodiment, the hydrogen atoms present within any one of the compounds disclosed herein (for example, a compound of Formula (I)) are isotopically enriched in deuterium. Such compounds are useful, for example, as analytical tools, as probes in biological assays, or as therapeutic agents in accordance with the present invention.

Where a particular enantiomer is preferred, it may, in some embodiments be provided substantially free of the corresponding enantiomer, and may also be referred to as “optically enriched.” “Optically-enriched,” as used herein, means that the compound is made up of a significantly greater proportion of one enantiomer. In certain embodiments the compound is made up of at least about 90% by weight of a preferred enantiomer. In other embodiments the compound is made up of at least about 95%, 98%, or 99% by weight of a preferred enantiomer. Preferred enantiomers may be isolated from racemic mixtures by any method known to those skilled in the art, including chiral high pressure liquid chromatography (HPLC) and the formation and crystallization of chiral salts or prepared by asymmetric syntheses. See, for example, Jacques et al., *Enantiomers, Racemates and Resolutions* (Wiley Interscience, New York, 1981); Wilen, et al., *Tetrahedron* 33:2725 (1977); Eliel, E.L. *Stereochemistry of*

*Carbon Compounds* (McGraw-Hill, NY, 1962); Wilen, S.H. *Tables of Resolving Agents and Optical Resolutions* p. 268 (E.L. Eliel, Ed., Univ. of Notre Dame Press, Notre Dame, IN 1972).

These and other exemplary substituents are described in more detail in the **Detailed Description, Figures, Examples, and Claims**. The invention is not intended to be limited in any manner by the above exemplary listing of substituents.

### **COF1/CRBN-binding polypeptide, COF2/CRBN-binding polypeptide, or COF3/CRBN-binding polypeptide**

Disclosed herein are, *inter alia*, fusion polypeptides that include a compound of Formula (I) (COF1)/CRBN-binding polypeptide, a compound of Formula (II) (COF2)/CRBN-binding polypeptide, or a compound of Formula (III) (COF3)/CRBN-binding polypeptide. In embodiments, in the presence of COF1 or COF2 (e.g., thalidomide and derivatives thereof, e.g., lenalidomide, pomalidomide, and thalidomide), or in the presence of COF3 (e.g., a compound disclosed in Table 29), the COF1/CRBN-, COF2/CRBN-, or COF3/CRBN-binding polypeptide in the fusion polypeptide increases a post-translational modification and/or degradation of the fusion polypeptide. In some embodiments, post-translational modification can include ubiquitination (e.g., mono- or poly- ubiquitination) of one or more amino acid residues, e.g., one or more of lysine or methionine, in the fusion polypeptide (e.g., one or all of: all or a part of a heterologous polypeptide and/or the COF1/CRBN-, COF2/CRBN-, or COF3/CRBN-binding polypeptide).

In certain embodiments, one or more lysine residues of the fusion polypeptide (e.g., all or a part of a heterologous polypeptide and/or the COF1/CRBN-, COF2/CRBN-, or COF3/CRBN-binding polypeptide) are ubiquitinated. In some embodiments, one or more methionine residues of the fusion polypeptide (e.g., all or a part of a heterologous polypeptide and/or the COF1/CRBN-, COF2/CRBN-, or COF3/CRBN-binding polypeptide) are ubiquitinated (e.g., mono- or poly- ubiquitinated).

Without wishing to be bound by theory, in some embodiments, inactivation, e.g., degradation, of a fusion polypeptide described herein can include one, two, three or all of following steps, e.g., in a cell or a reaction mixture:

(1) association of the fusion polypeptide that comprises the COF1/CRBN-, COF2/CRBN-, or COF3/CRBN-binding polypeptide to one or more subunits (e.g., CRBN) of a ubiquitin ligase complex (e.g., an E3 ubiquitin ligase complex) in the presence of COF1 or COF2 (e.g., thalidomide and derivatives thereof (e.g., lenalidomide)) or in the presence of COF3 (e.g., a compound disclosed in Table 29);

(2) ubiquitination of the fusion polypeptide (e.g., ubiquitination at a heterologous polypeptide and/or the COF1/CRBN-, COF2/CRBN-, or COF3/CRBN-binding polypeptide), thereby providing a ubiquitinated fusion polypeptide; and

(3) degradation of the ubiquitinated fusion polypeptide.

In some embodiments, any COF1/CRBN-, COF2/CRBN-, or COF3/CRBN-binding polypeptide described herein increases a post-translational modification and/or degradation of the fusion polypeptide in the presence of COF1, COF2, or COF3, *e.g.*, relative to the modification and/or degradation in the absence of COF1, COF2, or COF3. In one embodiment, the COF1/CRBN-, COF2/CRBN-, or COF3/CRBN-binding polypeptide increases selective ubiquitination of the fusion polypeptide in the presence of COF1, COF2, or COF3, *e.g.*, relative to the ubiquitination in the absence of COF1, COF2, or COF3.

In some embodiments, a COF1/CRBN-, COF2/CRBN-, or COF3/CRBN-binding polypeptide is derived from an amino acid sequence and/or structural motif (*e.g.*, a domain) that binds to one or more components of a ubiquitin ligase complex (*e.g.*, the E3 ubiquitin ligase complex) in the presence of COF1, COF2, or COF3. In some embodiments, COF1 or COF2 is a thalidomide class of compounds (*e.g.*, lenalidomide, pomalidomide, and thalidomide), *e.g.*, as described herein. In some embodiments, COF3 is a compound disclosed in Table 29. In some embodiments, the COF1/CRBN-, COF2/CRBN-, or COF3/CRBN-binding polypeptide comprises a zinc finger domain (*e.g.*, a zinc finger 2 domain) or a portion thereof. In some embodiments, the COF1/CRBN-, COF2/CRBN-, or COF3/CRBN-binding polypeptide comprises a  $\beta$  turn. In some embodiments, the COF1/CRBN- or COF2/CRBN-binding polypeptide comprises a  $\beta$  turn of an Ikaros family of transcription factors, *e.g.*, IKZF1 or IKZF3, or a sequence substantially identical thereto (*e.g.*, at least 85%, 87, 90, 95, 97, 98, 99, or 100% identical thereto). In some embodiments, the COF1/CRBN- or COF2/CRBN-binding polypeptide comprises a  $\beta$  hairpin of an Ikaros family of transcription factors, *e.g.*, IKZF1 or IKZF3, or a sequence substantially identical thereto (*e.g.*, at least 85%, 87, 90, 95, 97, 98, 99, or 100% to a  $\beta$  hairpin of IKZF1 or IKZF3, *e.g.*, as described in Kronke, J. *et al.* (2014) *Science* 343(6168):301-5). In some embodiments, the COF3/CRBN-binding polypeptide comprises a  $\beta$  turn of IKZF2, or a sequence substantially identical thereto (*e.g.*, at least 85%, 87, 90, 95, 97, 98, 99, or 100% identical thereto). In some embodiments, the COF3/CRBN-binding polypeptide comprises a  $\beta$  hairpin of IKZF2, or a sequence substantially identical thereto (*e.g.*, at least 85%, 87, 90, 95, 97, 98, 99, or 100% identical thereto).

In some embodiments, the COF1/CRBN- or COF2/CRBN-binding polypeptide comprises about 10 to about 95 amino acid residues, about 15 to about 90 amino acid residues, about 20 to about 85 amino acid residues, about 25 to about 80 amino acid residues, about 30 to about 75 amino acid residues, about 35 to about 70 amino acid residues, about 40 to about 65 amino acid residues, about 45 to about 65 amino acid residues, about 50 to about 65 amino acid residues, or about 55 to about 65 amino acid residues of IKZF1 (*e.g.*, SEQ ID NO: 20) or IKZF3 (*e.g.*, SEQ ID NO: 19) or a sequence substantially identical thereto (*e.g.*, at least 85%, 87, 90, 95, 97, 98, 99, or 100% identical thereto). In some embodiments, the

COF1/CRBN- or COF2/CRBN-binding polypeptide comprises at least 10 amino acids, at least 15 amino acids, at least 20 amino acids, at least 25 amino acids, at least 30 amino acids, at least 35 amino acids, at least 40 amino acids, at least 45 amino acids, at least 50 amino acids, at least 55 amino acids, at least 60 amino acids, at least 65 amino acids, at least 70 amino acids, at least 75 amino acids, at least 80 amino acids, at least 85 amino acids, at least 90 amino acids, at least 90 amino acids, or at least 95 amino acids of IKZF1 (e.g., SEQ ID NO: 20) or IKZF3 (e.g., SEQ ID NO: 19), or a sequence substantially identical thereto (e.g., at least 85%, 87, 90, 95, 97, 98, 99, or 100% identical thereto). In some embodiments, the COF1/CRBN- or COF2/CRBN-binding polypeptide comprises or consists of the amino acid sequences selected from the group consisting of SEQ ID NOs: 1-6, 11-15, 40, 41-43, 77, 78, 84-86, and 100.

In some embodiments, the COF3/CRBN-binding polypeptide comprises about 10 to about 95 amino acid residues, about 15 to about 90 amino acid residues, about 20 to about 85 amino acid residues, about 25 to about 80 amino acid residues, about 30 to about 75 amino acid residues, about 35 to about 70 amino acid residues, about 40 to about 65 amino acid residues, about 45 to about 65 amino acid residues, about 50 to about 65 amino acid residues, or about 55 to about 65 amino acid residues of IKZF2 (e.g., SEQ ID NO: 21) or a sequence substantially identical thereto (e.g., at least 85%, 87, 90, 95, 97, 98, 99, or 100% identical thereto). In some embodiments, the COF3/CRBN-binding polypeptide comprises at least 10 amino acids, at least 15 amino acids, at least 20 amino acids, at least 25 amino acids, at least 30 amino acids, at least 35 amino acids, at least 40 amino acids, at least 45 amino acids, at least 50 amino acids, at least 55 amino acids, at least 60 amino acids, at least 65 amino acids, at least 70 amino acids, at least 75 amino acids, at least 80 amino acids, at least 85 amino acids, at least 90 amino acids, at least 90 amino acids, or at least 95 amino acids of IKZF2 (e.g., SEQ ID NO: 21), or a sequence substantially identical thereto (e.g., at least 85%, 87, 90, 95, 97, 98, 99, or 100% identical thereto). In some embodiments, the COF3/CRBN-binding polypeptide comprises or consists of the amino acid sequences selected from the group consisting of SEQ ID NOs: 109, 113, and 114.

In some embodiments, exemplary full-length sequences of IKZF1, IKZF2, IKZF3, IKZF4, and IKZF5 or fragment thereof are provided in Table 1.

**Table 1. Exemplary IKZF sequences, variants, or fragments**

| SEQ ID NO    | Comment                                                   | Sequence                                                     |
|--------------|-----------------------------------------------------------|--------------------------------------------------------------|
| SEQ ID NO: 1 | IKZF3 136-180 and 236-249 (with N-terminal methionine)    | MHKRSHTGERPFQCNQCGASFTQKGNLLRHIKLHTGEKPFKCHLCNTASAEARHIKAEMG |
| SEQ ID NO: 3 | IKZF3 136-180 and 236-249 (without N-terminal methionine) | HKRSHTGERPFQCNQCGASFTQKGNLLRHIKLHTGEKPKFKCHLCNTASAEARHIKAEMG |

|               |                                                                               |                                                              |
|---------------|-------------------------------------------------------------------------------|--------------------------------------------------------------|
| SEQ ID NO: 2  | Lysine-free IKZF3 136-180 and 236-249 variant (with N-terminal methionine)    | MHRRSHTGERPFQCNQCGASFTQRGNLLRHIRLHTGERPFRCHLCNTASAEARHIRAEMG |
| SEQ ID NO: 4  | Lysine-free IKZF3 136-180 and 236-249 variant (without N-terminal methionine) | HRRSHTGERPFQCNQCGASFTQRGNLLRHIRLHTGERPFRCHLCNTASAEARHIRAEMG  |
| SEQ ID NO: 77 | IKZF3 136-180 (with N-terminal methionine)                                    | MHKRSHTGERPFQCNQCGASFTQKGNLLRHIKLHTGEKPFKCHLCN               |
| SEQ ID NO: 5  | IKZF3 136-180 (without N-terminal methionine)                                 | HKRSHTGERPFQCNQCGASFTQKGNLLRHIKLHTGEKPFKCHLCN                |
| SEQ ID NO: 41 | Lysine-free IKZF3 136-180                                                     | HRRSHTGERPFQCNQCGASFTQRGNLLRHIRLHTGERPFRCHLCN                |
| SEQ ID NO: 78 | IKZF3 136-170 (with N-terminal methionine)                                    | MHKRSHTGERPFQCNQCGASFTQKGNLLRHIKLHTG                         |
| SEQ ID NO: 6  | IKZF3 136-170 (without N-terminal methionine)                                 | HKRSHTGERPFQCNQCGASFTQKGNLLRHIKLHTG                          |
| SEQ ID NO: 42 | Lysine-free IKZF3 136-170                                                     | HRRSHTGERPFQCNQCGASFTQRGNLLRHIRLHTG                          |
| SEQ ID NO: 79 | IKZF3 140-170 (with N-terminal methionine)                                    | MHTGERPFQCNQCGASFTQKGNLLRHIKLHTG                             |
| SEQ ID NO: 7  | IKZF3 140-170 (without N-terminal methionine)                                 | HTGERPFQCNQCGASFTQKGNLLRHIKLHTG                              |
| SEQ ID NO: 80 | IKZF3 140-169 (with N-terminal methionine)                                    | MHTGERPFQCNQCGASFTQKGNLLRHIKLHT                              |
| SEQ ID NO: 24 | IKZF3 140-169 (without N-terminal methionine)                                 | HTGERPFQCNQCGASFTQKGNLLRHIKLHT                               |
| SEQ ID NO: 81 | IKZF3 141-163 (with N-terminal methionine)                                    | MTGERPFQCNQCGASFTQKGNLLR                                     |
| SEQ ID NO: 8  | IKZF3 141-163 (without N-terminal methionine)                                 | TGERPFQCNQCGASFTQKGNLLR                                      |
| SEQ ID NO: 82 | IKZF3 145-170 (with N-terminal methionine)                                    | MPFQCNQCGASFTQKGNLLRHIKLHTG                                  |
| SEQ ID NO: 9  | IKZF3 145-170 (without N-terminal methionine)                                 | PFQCNQCGASFTQKGNLLRHIKLHTG                                   |
| SEQ ID NO: 83 | IKZF3 145-155 (with N-terminal methionine)                                    | MPFQCNQCGASF                                                 |
| SEQ ID NO: 10 | IKZF3 145-155 (without N-terminal methionine)                                 | PFQCNQCGASF                                                  |
| SEQ ID NO: 11 | IKZF3 236-249                                                                 | TASAEARHIKAEMG                                               |
| SEQ ID NO: 43 | Lysine-free IKZF3 236-249                                                     | TASAEARHIRAEMG                                               |
| SEQ ID NO: 12 | IKZF3 136-180 and 236-249 K245R (with N-terminal methionine)                  | MHKRSHTGERPFQCNQCGASFTQKGNLLRHIKLHTGEKPFKCHLCNTASAEARHIRAEMG |
| SEQ ID NO: 84 | IKZF3 136-180 and 236-249 K245R (without N-terminal methionine)               | HKRSHTGERPFQCNQCGASFTQKGNLLRHIKLHTGEKPFKCHLCNTASAEARHIRAEMG  |

|                |                                                                 |                                                                              |
|----------------|-----------------------------------------------------------------|------------------------------------------------------------------------------|
| SEQ ID NO: 13  | IKZF3 136-180 and 236-249 K245S (with N-terminal methionine)    | MHKRSHTGERPFQCNQCGASFTQKGNLLRHIKLHTGEKPFKCHLCNTASAEARHISAEMG                 |
| SEQ ID NO: 100 | IKZF3 136-180 and 236-249 K245S (without N-terminal methionine) | HKRSHTGERPFQCNQCGASFTQKGNLLRHIKLHTGEKPKFCHLCNTASAEARHISAEMG                  |
| SEQ ID NO: 14  | IKZF3 136-180 MALEK (with N-terminal methionine)                | MHKRSHTGERPFQCNQCGASFTQKGNLLRHIKLHTGEKPFKCHLCNMALEKMALEKMALE                 |
| SEQ ID NO: 85  | IKZF3 136-180 MALEK (without N-terminal methionine)             | HKRSHTGERPFQCNQCGASFTQKGNLLRHIKLHTGEKPKFCHLCNMALEKMALEKMALE                  |
| SEQ ID NO: 15  | IKZF3 136-170 MALEK (with N-terminal methionine)                | MHKRSHTGERPFQCNQCGASFTQKGNLLRHIKLHTGMALEKMALEKMALE                           |
| SEQ ID NO: 86  | IKZF3 136-170 MALEK (without N-terminal methionine)             | HKRSHTGERPFQCNQCGASFTQKGNLLRHIKLHTGMALEKMALEKMALE                            |
| SEQ ID NO: 16  | IKZF3 140-170 MALEK (with N-terminal methionine)                | MHTGERPFQCNQCGASFTQKGNLLRHIKLHTGMALEKMALEKMALE                               |
| SEQ ID NO: 87  | IKZF3 140-170 MALEK (without N-terminal methionine)             | HTGERPFQCNQCGASFTQKGNLLRHIKLHTGMALEKMALEKMALE                                |
| SEQ ID NO: 17  | IKZF3 141-163 MALEK (with N-terminal methionine)                | MTGERPFQCNQCGASFTQKGNLLRMALEKMALEKMALE                                       |
| SEQ ID NO: 88  | IKZF3 141-163 MALEK (without N-terminal methionine)             | TGERPFQCNQCGASFTQKGNLLRMALEKMALEKMALE                                        |
| SEQ ID NO: 18  | IKZF3 145-155 MALEK (with N-terminal methionine)                | MPFQCNQCGASFMALEKMALEKMALE                                                   |
| SEQ ID NO: 89  | IKZF3 145-155 MALEK (without N-terminal methionine)             | PFQCNQCGASFMALEKMALEKMALE                                                    |
| SEQ ID NO: 27  | IKZF3 136-180 Q147H (with N-terminal methionine)                | MHKRSHTGERPFHCNQCGASFTQKGNLLRHIKLHTGEKPFKCHLCN                               |
| SEQ ID NO: 90  | IKZF3 136-180 Q147H (without N-terminal methionine)             | HKRSHTGERPFHCNQCGASFTQKGNLLRHIKLHTGEKPKFCHLCN                                |
| SEQ ID NO: 109 | IKZF2 130-174 and 230-243                                       | HKRSHTGERPFHCNQCGASFTQKGNLLRHIKLHSGEKPKFCPFCSAGQVMSHHVPPMED                  |
| SEQ ID NO: 113 | IKZF2 130-174                                                   | HKRSHTGERPFHCNQCGASFTQKGNLLRHIKLHSGEKPKFCPFCS                                |
| SEQ ID NO: 114 | IKZF2 230-243                                                   | AGQVMSHHVPPMED                                                               |
| SEQ ID NO: 19  | IKZF3 full length                                               | MEDIQTNAELKSTQEVSVAESA AVLNDYSLTKSHEME NVDSGEGPANEDEDIGDDSMKVKDEYSERDENVLKSE |

|                  |                   |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |
|------------------|-------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|                  |                   | PMGNAEEPEIPYSYSREYNEYENIKLERHVVSFDSSRPT<br>SGKMNCDDVCGLSGISFNVLMVHKRSHTGERPFQCNQC<br>GASFTQKGNLLRHIKLHTGEKPFKCHLCNYACQRRDAL<br>TGHLRTHSVEKPYKCEFCGRSYKQRSSLEEHEKERCRTF<br>LQSTDPGDTASAEARHIKAEMGSERALVLDRLASNVA<br>KRKSSMPQKFIGEKRHCFDVNYNSSYMYEKESELIQTR<br>MMDQAINNAISYLGAELRPLVQTPPAPTSEMVPVISS<br>MYPIALTRAEMSNGAPQELEKKSIHLPEKSVPSERGLSP<br>NNSGHDSTDTDSNHEERQNHYYQNHMVLSRARNGMP<br>LLKEVPRSYELLKPPPICPRDSVKVINKEGEVMDVYRC<br>DHCRLVFLDYVMFTIHMGCCHGFRDPFECNMCGYRSHD<br>RYEFSSHIARGEHRALLK                                                                                                 |
| SEQ ID<br>NO: 20 | IKZF1 full length | MDADEGQDMSQVSGKESPPVSDTPDEGDEPMPIPEDLS<br>TTSGGQQSSKSDRVVASNVKQVETQSDEENGRACEMNG<br>EECAEDLRMLDASGEKMNGSHRDQGSALSQVGGIRL<br>PNGKLKCDICGIIICIGPNVLMVHKRSHTGERPFQCNQCG<br>ASFTQKGNLLRHIKLHSGEKPFKCHLCNYACRRRDALT<br>GHLRTHSVGKPHKCGYCGRSYKQRSSLEEHEKERNY<br>LESMGLPGTLYPVIKEETNHSEMAEDLCKIGSERSLVLD<br>RLASNVAKRKSSMPQKFLGDKGLSDTPYDSSASYEKEN<br>EMMKSHVMDQAINNAINYLGAESLRPLVQTPPGGSEV<br>VPVISPMYQLHKPLAEGTPRSNHSAQDSAVENTLLLSK<br>AKLVPSEREASPSNSCQDSTDTESSNEEQRSGLIYLTNH<br>IAPHARNGLSLKEEHRA YDLLRAASENSQDALRVVSTS<br>GEQMKVYKCEHCRVLFDHVMYTIHMGCCHGFRDPFEC<br>NMCGYHSQDRYEFSSHITRGEHRFHMS    |
| SEQ ID<br>NO: 21 | IKZF2 full length | METEADGYITCDNELSPEREHSNMAIDLTSSTPNGQHA<br>SPSHMTSTNSVKLEMQSDEECDRKPLSREDEIRGHDEG<br>SSLEEPLIESSEVADNRKVQELQGEGGIRLPNGKLKCDV<br>CGMVCIGPNVLMVHKRSHTGERPFHCNQCASFTQKG<br>NLLRHIKLHSGEKPFKCPFCSYACRRRDALTGHLRTHS<br>VGKPHKCNVYCGRSYKQRSSLEEHEKERNYLNQVSM<br>AAGQVMSSHVPPMEDCKEQEPIMDNNISLVPPERPAVI<br>EKLGTGNGMKRKSSTPQKFVGEKLMRFSYPDIHFDMNL<br>TYEKEAELMQSHMMDQAINNAITYLGAELHPLMQHP<br>PSTIAEVAPVISSAYSQVYHPNRIERPISRETADSHENNM<br>DGPISLIRPKSRPQEREASPSNSCLDSTDSESSHDDHQS<br>YQHPALNPKRKQSPAYMKEDVKALDTTKAPKGSCLKDI<br>YKVFNGEGEQIRAFKCEHCRVLFDHVMYTIHMGCCHG<br>YRDPLECNICGYRSQDRYEFSSHIVRGEHTFH |
| SEQ ID<br>NO: 22 | IKZF4 full length | MHTPPALPRRFQGGGRVVRTPGSHRQGKDNLERDPSGG<br>CVPDFLPQAQDSNHFIMESLFCESGDSLEKEFLGAPV<br>GPSVSTPNSQHSSPSRSLANSIKVEMYSDSESSRLLGPD<br>ERLLEKDDSVIVEDSLSEPLGYCDGSGPEPHSPGGIRLPN<br>GKLKCDVCGMVCIGPNVLMVHKRSHTGERPFHCNQC<br>ASFTQKGNLLRHIKLHSGEKPFKCPFCNYACRRRDALT<br>GHLRTHSVSSPTVGKPYKCNVYCGRSYKQSTLEEHEK<br>CHNYLQSLSTEALAGQPGDEIRDLEMVPSMLHSSS<br>ERPTFIDRLANSLTKRKRSTPQKFVGEKQMRFSLSLDPY<br>DVNSGGYEKDVVELVAHHSLEPGFGSSSLAFVGAHLRPL                                                                                                                                                                  |



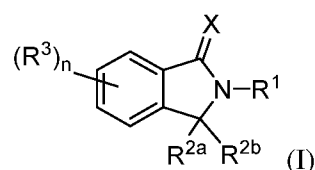
|                  |                   |                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |
|------------------|-------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|                  |                   | RLPPTNCISELTPVISSVYTQMQPLPGRLELPGSREAGEG<br>PEDLADGGPLL YRPRGPLTDPGASPSNGCQDSTDTESN<br>HEDRVAGVVS LPPQGPPTIVVGRHSPAYAKEDPK<br>PQEGLLRGTPGPSKEVLR VVGESGEPVKA FKCEHCRILF<br>LDHVMFTIHMGC HGRDPFECNICGYHSQDRYEFSSHI<br>VRGEHKVG                                                                                                                                                                                                                                                 |
| SEQ ID<br>NO: 23 | IKZF5 full length | MGEKKPEPLDFVKDFQEYLTQQTHHVN MISGSVSGDK<br>EAEALQGAGTDGDQNGLDHPSVEVSLDENSGMLVDGF<br>ERTFDGKLKCRYCNYASKGTARLIEHRIHTGEKPHRCH<br>LCPFASAYERHLEAHMRSHTGEKPYKCELC SFRCSDRS<br>NLSHRRRKHKMVPIKGRSSLSSKKMWGV LQKKT SN<br>LGYSRRALINLSPPSMVVQKPDYLNDFTHEIPNIQTDSY<br>ESMAKTTPTGGLPRDPQELMVDNPLNQLSTLAGQLSSL<br>PPENQNPASPDV VPCPDEKPFMIQQPSTQAVVSAVSASI<br>PQSSPTSPEPRPSHSQRNYS PVAGPSSEPSAHTSTPSIGN<br>SQPSTPAPALPVQDPQLLHHCQHCDMYFADNILYTIHM<br>GCHGYENPFQCNICGCKCKNKYDFACHFARGQHNQH |

### Degradation Compounds

Disclosed herein are, *inter alia*, degradation compounds that can, e.g., increase the ubiquitination and/or degradation of the fusion proteins including the degradation tag.

- 5 In some embodiments, the degradation compound comprises a member of the thalidomide class of compounds. In some embodiments, members of the thalidomide class of compounds include, but are not limited to, lenalidomide (CC-5013), pomalidomide (CC-4047 or ACTIMID), thalidomide, or salts or derivatives thereof. In some embodiments, the degradation compound can be a mixture of one, two, three, or more members of the thalidomide class of compounds. Thalidomide analogs and
- 10 immunomodulatory properties of thalidomide analogs are described in Boder and Stankiewicz, Recent Pat Endocr Metab Immune Drug Discov. 2011 Sep;5(3):192-6, which is hereby incorporated by reference in its entirety. The structural complex of thalidomide analogs and the E3 ubiquitin is described in Gandhi et al., Br J Haematol. 2014 Mar;164(6):811-21, which is hereby incorporated by reference in its entirety. The modulation of the E3 ubiquitin ligase by thalidomide analogs is described in Fischer et al., Nature.
- 15 2014 Aug 7;512(7512):49-53, which is hereby incorporated by reference in its entirety.

In some embodiments, the degradation compound comprises a compound of Formula (I):



or a pharmaceutically acceptable salt, ester, hydrate, solvate, or tautomer thereof, wherein:

X is O or S;

R<sup>1</sup> is C<sub>1</sub>-C<sub>6</sub> alkyl, C<sub>2</sub>-C<sub>6</sub> alkenyl, C<sub>2</sub>-C<sub>6</sub> alkynyl, C<sub>1</sub>-C<sub>6</sub> heteroalkyl, carbocyclyl, heterocyclyl, aryl, or heteroaryl, each of which is independently and optionally substituted by one or more R<sup>4</sup>;

each of R<sup>2a</sup> and R<sup>2b</sup> is independently hydrogen or C<sub>1</sub>-C<sub>6</sub> alkyl; or R<sup>2a</sup> and R<sup>2b</sup> together with the carbon atom to which they are attached form a carbonyl group or a thiocarbonyl group;

5 each of R<sup>3</sup> is independently C<sub>1</sub>-C<sub>6</sub> alkyl, C<sub>2</sub>-C<sub>6</sub> alkenyl, C<sub>2</sub>-C<sub>6</sub> alkynyl, C<sub>1</sub>-C<sub>6</sub> heteroalkyl, halo, cyano, -C(O)R<sup>A</sup>, -C(O)OR<sup>B</sup>, -OR<sup>B</sup>, -N(R<sup>C</sup>)(R<sup>D</sup>), -C(O)N(R<sup>C</sup>)(R<sup>D</sup>), -N(R<sup>C</sup>)C(O)R<sup>A</sup>, -S(O)<sub>x</sub>R<sup>E</sup>, -S(O)<sub>x</sub>N(R<sup>C</sup>)(R<sup>D</sup>), or -N(R<sup>C</sup>)S(O)<sub>x</sub>R<sup>E</sup>, wherein each alkyl, alkenyl, alkynyl, and heteroalkyl is independently and optionally substituted with one or more R<sup>6</sup>;

10 each R<sup>4</sup> is independently C<sub>1</sub>-C<sub>6</sub> alkyl, C<sub>2</sub>-C<sub>6</sub> alkenyl, C<sub>2</sub>-C<sub>6</sub> alkynyl, C<sub>1</sub>-C<sub>6</sub> heteroalkyl, halo, cyano, oxo, -C(O)R<sup>A</sup>, -C(O)OR<sup>B</sup>, -OR<sup>B</sup>, -N(R<sup>C</sup>)(R<sup>D</sup>), -C(O)N(R<sup>C</sup>)(R<sup>D</sup>), -N(R<sup>C</sup>)C(O)R<sup>A</sup>, -S(O)<sub>x</sub>R<sup>E</sup>, -S(O)<sub>x</sub>N(R<sup>C</sup>)(R<sup>D</sup>), -N(R<sup>C</sup>)S(O)<sub>x</sub>R<sup>E</sup>, carbocyclyl, heterocyclyl, aryl, or heteroaryl, wherein each alkyl, alkenyl, alkynyl, heteroalkyl, carbocyclyl, heterocyclyl, aryl, and heteroaryl is independently and optionally substituted with one or more R<sup>7</sup>;

each of R<sup>A</sup>, R<sup>B</sup>, R<sup>C</sup>, R<sup>D</sup>, and R<sup>E</sup> is independently hydrogen or C<sub>1</sub>-C<sub>6</sub> alkyl;

15 each R<sup>6</sup> is independently C<sub>1</sub>-C<sub>6</sub> alkyl, oxo, cyano, -OR<sup>B</sup>, -N(R<sup>C</sup>)(R<sup>D</sup>), -C(O)N(R<sup>C</sup>)(R<sup>D</sup>), -N(R<sup>C</sup>)C(O)R<sup>A</sup>, aryl, or heteroaryl, wherein each aryl and heteroaryl is independently and optionally substituted with one or more R<sup>8</sup>;

each R<sup>7</sup> is independently halo, oxo, cyano, -OR<sup>B</sup>, -N(R<sup>C</sup>)(R<sup>D</sup>), -C(O)N(R<sup>C</sup>)(R<sup>D</sup>), or -N(R<sup>C</sup>)C(O)R<sup>A</sup>;

20 each R<sup>8</sup> is independently C<sub>1</sub>-C<sub>6</sub> alkyl, cyano, -OR<sup>B</sup>, -N(R<sup>C</sup>)(R<sup>D</sup>), -C(O)N(R<sup>C</sup>)(R<sup>D</sup>), or -N(R<sup>C</sup>)C(O)R<sup>A</sup>;

n is 0, 1, 2, 3 or 4; and

x is 0, 1, or 2.

In some embodiments, X is O.

25 In some embodiments, R<sup>1</sup> is C<sub>1</sub>-C<sub>6</sub> alkyl, C<sub>2</sub>-C<sub>6</sub> alkenyl, C<sub>2</sub>-C<sub>6</sub> alkynyl, C<sub>1</sub>-C<sub>6</sub> heteroalkyl, carbocyclyl, heterocyclyl, aryl, or heteroaryl, each of which is independently and optionally substituted by 1-12 R<sup>4</sup> (e.g., 1 R<sup>4</sup>, 2 R<sup>4</sup>, 3 R<sup>4</sup>, 4 R<sup>4</sup>, 5 R<sup>4</sup>, 6 R<sup>4</sup>, 7 R<sup>4</sup>, 8 R<sup>4</sup>, 9 R<sup>4</sup>, 10 R<sup>4</sup>, 11 R<sup>4</sup>, or 12 R<sup>4</sup>). In some embodiments, R<sup>1</sup> is heterocyclyl. In some embodiments, R<sup>1</sup> is a 6-membered heterocyclyl or a 5-membered heterocyclyl. In some embodiments, R<sup>1</sup> is a 6-membered heterocyclyl or a 5-membered heterocyclyl, each of which is independently and optionally substituted by 1-6 R<sup>4</sup> (e.g., 1 R<sup>4</sup>, 2 R<sup>4</sup>, 3 R<sup>4</sup>, 4 R<sup>4</sup>, 5 R<sup>4</sup>, or 6 R<sup>4</sup>). In some embodiments, R<sup>1</sup> is a nitrogen-containing heterocyclyl. In some  
30 embodiments, R<sup>1</sup> is piperidiny (e.g., piperidine-2,6-dionyl).

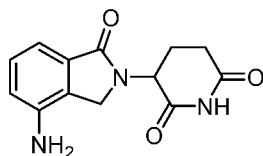
In some embodiments, each of R<sup>2a</sup> and R<sup>2b</sup> is independently hydrogen. In some embodiments, R<sup>2a</sup> and R<sup>2b</sup> together with the carbon to which they are attached form a carbonyl group.

In some embodiments, each of  $R^3$  is independently  $C_1$ - $C_6$  alkyl,  $C_2$ - $C_6$  alkenyl,  $C_2$ - $C_6$  alkynyl,  $C_1$ - $C_6$  heteroalkyl, halo, cyano,  $-C(O)R^A$ ,  $-C(O)OR^B$ ,  $-OR^B$ ,  $-N(R^C)(R^D)$ ,  $-C(O)N(R^C)(R^D)$ ,  $-N(R^C)C(O)R^A$ ,  $-S(O)_xR^E$ ,  $-S(O)_xN(R^C)(R^D)$ , or  $-N(R^C)S(O)_xR^E$ , wherein each alkyl, alkenyl, alkynyl, and heteroalkyl is independently and optionally substituted with 1-12  $R^6$  (e.g., 1  $R^6$ , 2  $R^6$ , 3  $R^6$ , 4  $R^6$ , 5  $R^6$ , 6  $R^6$ , 7  $R^6$ , 8  $R^6$ , 9  $R^6$ , 10  $R^6$ , 11  $R^6$ , or 12  $R^6$ ). In some embodiments,  $R^3$  is  $C_1$ - $C_6$  heteroalkyl,  $-N(R^C)(R^D)$  or  $-N(R^C)C(O)R^A$ . In some embodiments,  $R^3$  is  $C_1$ - $C_6$  heteroalkyl (e.g.,  $CH_2NHC(O)CH_2$ -phenyl-*t*-butyl),  $-N(R^C)(R^D)$  (e.g.,  $NH_2$ ), or  $-N(R^C)C(O)R^A$  (e.g.,  $NHC(O)CH_3$ ). In some embodiments,  $R^3$  is  $C_1$ - $C_6$  heteroalkyl optionally substituted with 1-6  $R^6$  (e.g., 1  $R^6$ , 2  $R^6$ , 3  $R^6$ , 4  $R^6$ , 5  $R^6$ , or 6  $R^6$ ).

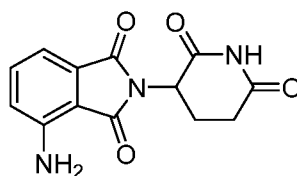
In some embodiments, each  $R^4$  is independently  $C_1$ - $C_6$  alkyl,  $C_2$ - $C_6$  alkenyl,  $C_2$ - $C_6$  alkynyl,  $C_1$ - $C_6$  heteroalkyl, halo, cyano, oxo,  $-C(O)R^A$ ,  $-C(O)OR^B$ ,  $-OR^B$ ,  $-N(R^C)(R^D)$ ,  $-C(O)N(R^C)(R^D)$ ,  $-N(R^C)C(O)R^A$ ,  $-S(O)_xR^E$ ,  $-S(O)_xN(R^C)(R^D)$ ,  $-N(R^C)S(O)_xR^E$ , carbocyclyl, heterocyclyl, aryl, or heteroaryl, wherein each alkyl, alkenyl, alkynyl, heteroalkyl, carbocyclyl, heterocyclyl, aryl, and heteroaryl is independently and optionally substituted with 1-12  $R^7$  (e.g., 1  $R^7$ , 2  $R^7$ , 3  $R^7$ , 4  $R^7$ , 5  $R^7$ , 6  $R^7$ , 7  $R^7$ , 8  $R^7$ , 9  $R^7$ , 10  $R^7$ , 11  $R^7$ , or 12  $R^7$ ).

In some embodiments, each  $R^6$  is independently  $C_1$ - $C_6$  alkyl, oxo, cyano,  $-OR^B$ ,  $-N(R^C)(R^D)$ ,  $-C(O)N(R^C)(R^D)$ ,  $-N(R^C)C(O)R^A$ , aryl, or heteroaryl, wherein each aryl and heteroaryl is independently and optionally substituted with 1-6  $R^8$  (e.g., 1  $R^8$ , 2  $R^8$ , 3  $R^8$ , 4  $R^8$ , 5  $R^8$ , or 6  $R^8$ ).

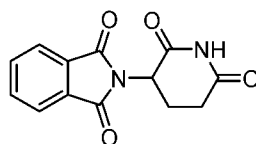
In an embodiment, X is O. In an embodiment,  $R^1$  is heterocyclyl (e.g., piperidine-2,6-dionyl). In an embodiment, each of  $R^{2a}$  and  $R^{2b}$  is independently hydrogen. In an embodiment, n is 1. In an embodiment,  $R^3$  is  $-N(R^C)(R^D)$  (e.g.,  $-NH_2$ ). In an embodiment, the degradation compound comprises lenalidomide, e.g., 3-(4-amino-1-oxoisindolin-2-yl)piperidine-2,6-dione, or a pharmaceutically acceptable salt thereof. In an embodiment, the degradation compound is lenalidomide, e.g., according to the following formula:



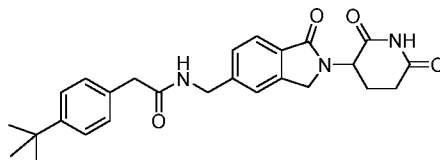
In an embodiment, X is O. In an embodiment,  $R^1$  is heterocyclyl (e.g., piperidinyl-2,6-dionyl). In some embodiments,  $R^{2a}$  and  $R^{2b}$  together with the carbon to which they are attached form a carbonyl group. In an embodiment, n is 1. In an embodiment,  $R^3$  is  $-N(R^C)(R^D)$  (e.g.,  $-NH_2$ ). In an embodiment, the degradation compound comprises pomalidomide, e.g., 4-amino-2-(2,6-dioxopiperidin-3-yl)isindoline-1,3-dione, or a pharmaceutically acceptable salt thereof. In an embodiment, the degradation compound is pomalidomide, e.g., according to the following formula:



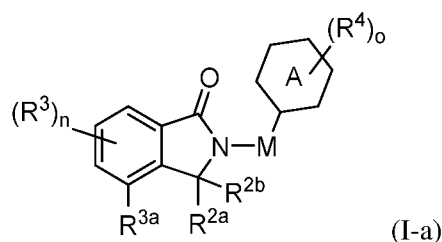
In an embodiment, X is O. In an embodiment, R<sup>1</sup> is heterocyclyl (e.g., piperidinyl-2,6-dionyl). In an embodiment, R<sup>2a</sup> and R<sup>2b</sup> together with the carbon to which they are attached form a carbonyl group. In an embodiment, n is 0. In an embodiment, the degradation compound comprises thalidomide, e.g., 2-(2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione, or a pharmaceutically acceptable salt thereof. In an embodiment, the degradation product is thalidomide, e.g., according to the following formula:



In an embodiment, X is O. In an embodiment, R<sup>1</sup> is heterocyclyl (e.g., piperidine-2,6-dionyl). In an embodiment, each of R<sup>2a</sup> and R<sup>2b</sup> is independently hydrogen. In an embodiment, n is 1. In an embodiment, R<sup>3</sup> is C<sub>1</sub>-C<sub>6</sub> heteroalkyl (e.g., CH<sub>2</sub>NHC(O)CH<sub>2</sub>-phenyl-t-butyl). In an embodiment, R<sup>3</sup> is C<sub>1</sub>-C<sub>6</sub> heteroalkyl substituted with 1 R<sup>6</sup> (e.g., CH<sub>2</sub>NHC(O)CH<sub>2</sub>-phenyl-t-butyl). In an embodiment, the degradation compound comprises 2-(4-(tert-butyl)phenyl)-N-((2-(2,6-dioxopiperidin-3-yl)-1-oxoisoindolin-5-yl)methyl)acetamide, or a pharmaceutically acceptable salt thereof. In an embodiment, the degradation compound has the structure as shown in the following formula:



In some embodiments, the degradation compound is a compound of Formula (I-a):



or a pharmaceutically acceptable salt, ester, hydrate, or tautomer thereof, wherein:

Ring A is carbocyclyl, heterocyclyl, aryl, or heteroaryl, each of which is independently and optionally substituted with one or more R<sup>4</sup>;

M is absent, C<sub>1</sub>-C<sub>6</sub> alkyl, C<sub>2</sub>-C<sub>6</sub> alkenyl, C<sub>2</sub>-C<sub>6</sub> alkynyl, or C<sub>1</sub>-C<sub>6</sub> heteroalkyl, wherein each alkyl, alkenyl, alkynyl, and heteroalkyl is independently and optionally substituted with one or more R<sup>4</sup>;

each of R<sup>2a</sup> and R<sup>2b</sup> is independently hydrogen or C<sub>1</sub>-C<sub>6</sub> alkyl; or R<sup>2a</sup> and R<sup>2b</sup> together with the carbon atom to which they are attached to form a carbonyl group or thiocarbonyl group;

R<sup>3a</sup> is hydrogen, C<sub>1</sub>-C<sub>6</sub> alkyl, C<sub>2</sub>-C<sub>6</sub> alkenyl, C<sub>2</sub>-C<sub>6</sub> alkynyl, C<sub>1</sub>-C<sub>6</sub> heteroalkyl, halo, cyano, -C(O)R<sup>A</sup>, -C(O)OR<sup>B</sup>, -OR<sup>B</sup>, -N(R<sup>C</sup>)(R<sup>D</sup>), -C(O)N(R<sup>C</sup>)(R<sup>D</sup>), -N(R<sup>C</sup>)C(O)R<sup>A</sup>, -S(O)<sub>x</sub>R<sup>E</sup>, -S(O)<sub>x</sub>N(R<sup>C</sup>)(R<sup>D</sup>), or -N(R<sup>C</sup>)S(O)<sub>x</sub>R<sup>E</sup>, wherein each alkyl, alkenyl, alkynyl, and heteroalkyl is independently and optionally substituted with one or more R<sup>6</sup>;

each of R<sup>3</sup> is independently C<sub>1</sub>-C<sub>6</sub> alkyl, C<sub>2</sub>-C<sub>6</sub> alkenyl, C<sub>2</sub>-C<sub>6</sub> alkynyl, C<sub>1</sub>-C<sub>6</sub> heteroalkyl, halo, cyano, -C(O)R<sup>A</sup>, -C(O)OR<sup>B</sup>, -OR<sup>B</sup>, -N(R<sup>C</sup>)(R<sup>D</sup>), -C(O)N(R<sup>C</sup>)(R<sup>D</sup>), -N(R<sup>C</sup>)C(O)R<sup>A</sup>, -S(O)<sub>x</sub>R<sup>E</sup>, -S(O)<sub>x</sub>N(R<sup>C</sup>)(R<sup>D</sup>), or -N(R<sup>C</sup>)S(O)<sub>x</sub>R<sup>E</sup>, wherein each alkyl, alkenyl, alkynyl, and heteroalkyl is independently and optionally substituted with one or more R<sup>6</sup>;

each R<sup>4</sup> is independently C<sub>1</sub>-C<sub>6</sub> alkyl, C<sub>2</sub>-C<sub>6</sub> alkenyl, C<sub>2</sub>-C<sub>6</sub> alkynyl, C<sub>1</sub>-C<sub>6</sub> heteroalkyl, halo, cyano, oxo, -C(O)R<sup>A</sup>, -C(O)OR<sup>B</sup>, -OR<sup>B</sup>, -N(R<sup>C</sup>)(R<sup>D</sup>), -C(O)N(R<sup>C</sup>)(R<sup>D</sup>), -N(R<sup>C</sup>)C(O)R<sup>A</sup>, S(O)<sub>x</sub>R<sup>E</sup>, -S(O)<sub>x</sub>N(R<sup>C</sup>)(R<sup>D</sup>), -N(R<sup>C</sup>)S(O)<sub>x</sub>R<sup>E</sup>, carbocyclyl, heterocyclyl, aryl, or heteroaryl, wherein each alkyl, alkenyl, alkynyl, carbocyclyl, heterocyclyl, aryl, and heteroaryl is independently and optionally substituted with one or more R<sup>7</sup>;

each of R<sup>A</sup>, R<sup>B</sup>, R<sup>C</sup>, R<sup>D</sup>, and R<sup>E</sup> is independently hydrogen or C<sub>1</sub>-C<sub>6</sub> alkyl;

each R<sup>6</sup> is independently C<sub>1</sub>-C<sub>6</sub> alkyl, oxo, cyano, -OR<sup>B</sup>, -N(R<sup>C</sup>)(R<sup>D</sup>), -C(O)N(R<sup>C</sup>)(R<sup>D</sup>), -N(R<sup>C</sup>)C(O)R<sup>A</sup>, aryl, or heteroaryl, wherein each aryl and heteroaryl is independently and optionally substituted with one or more R<sup>8</sup>;

each R<sup>7</sup> is independently halo, oxo, cyano, -OR<sup>B</sup>, -N(R<sup>C</sup>)(R<sup>D</sup>), -C(O)N(R<sup>C</sup>)(R<sup>D</sup>), or -N(R<sup>C</sup>)C(O)R<sup>A</sup>;

each R<sup>8</sup> is independently C<sub>1</sub>-C<sub>6</sub> alkyl, cyano, -OR<sup>B</sup>, -N(R<sup>C</sup>)(R<sup>D</sup>), -C(O)N(R<sup>C</sup>)(R<sup>D</sup>), or -N(R<sup>C</sup>)C(O)R<sup>A</sup>;

n is 0, 1, 2, or 3;

o is 0, 1, 2, 3, 4, or 5; and

x is 0, 1, or 2.

In some embodiments, X is O.

In some embodiments, M is C<sub>1</sub>-C<sub>6</sub> alkyl, C<sub>2</sub>-C<sub>6</sub> alkenyl, C<sub>2</sub>-C<sub>6</sub> alkynyl, or C<sub>1</sub>-C<sub>6</sub> heteroalkyl, wherein each alkyl, alkenyl, alkynyl, and heteroalkyl is independently and optionally substituted with 1-6 R<sup>4</sup> (e.g., 1 R<sup>4</sup>, 2 R<sup>4</sup>, 3 R<sup>4</sup>, 4 R<sup>4</sup>, 5 R<sup>4</sup>, or 6 R<sup>4</sup>). In some embodiments, M is absent.

In some embodiments, Ring A is carbocyclyl, heterocyclyl, aryl, or heteroaryl, each of which is independently and optionally substituted with 1-6 R<sup>4</sup> (e.g., 1 R<sup>4</sup>, 2 R<sup>4</sup>, 3 R<sup>4</sup>, 4 R<sup>4</sup>, 5 R<sup>4</sup>, or 6 R<sup>4</sup>). In some embodiments, Ring A is heterocyclyl. In some embodiments, Ring A is heterocyclyl, e.g., a 6-membered

heterocyclyl or a 5-membered heterocyclyl. In some embodiments, Ring A is a nitrogen-containing heterocyclyl. In some embodiments, Ring A is piperidinyl (e.g., piperidine-2,6-dionyl).

In some embodiments, M is absent and Ring A is heterocyclyl (e.g., piperidinyl, e.g., piperidine-2,6-dionyl).

5 In some embodiments, each of  $R^{2a}$  and  $R^{2b}$  is independently hydrogen. In some embodiments,  $R^{2a}$  and  $R^{2b}$  together with the carbon to which they are attached form a carbonyl group.

In some embodiments,  $R^{3a}$  is hydrogen,  $C_1$ - $C_6$  alkyl,  $C_2$ - $C_6$  alkenyl,  $C_2$ - $C_6$  alkynyl,  $C_1$ - $C_6$  heteroalkyl, halo, cyano,  $-C(O)R^A$ ,  $-C(O)OR^B$ ,  $-OR^B$ ,  $-N(R^C)(R^D)$ ,  $-C(O)N(R^C)(R^D)$ ,  $-N(R^C)C(O)R^A$ ,  $-S(O)_xR^E$ ,  $-S(O)_xN(R^C)(R^D)$ , or  $-N(R^C)S(O)_xR^E$ , wherein each alkyl, alkenyl, alkynyl, and heteroalkyl is  
10 independently and optionally substituted with 1-12  $R^6$  (e.g., 1  $R^6$ , 2  $R^6$ , 3  $R^6$ , 4  $R^6$ , 5  $R^6$ , 6  $R^6$ , 7  $R^6$ , 8  $R^6$ , 9  $R^6$ , 10  $R^6$ , 11  $R^6$ , or 12  $R^6$ ). In some embodiments,  $R^{3a}$  is hydrogen,  $-N(R^C)(R^D)$  or  $-N(R^C)C(O)R^A$ . In some embodiments,  $R^{3a}$  is hydrogen. In some embodiments,  $R^{3a}$  is  $-N(R^C)(R^D)$  (e.g.,  $-NH_2$ ). In some embodiments,  $R^{3a}$  is  $-N(R^C)C(O)R^A$  (e.g.,  $NHC(O)CH_3$ ).

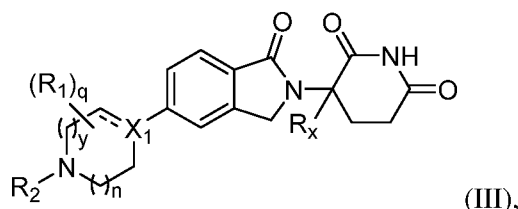
In some embodiments, each  $R^3$  is independently  $C_1$ - $C_6$  alkyl,  $C_2$ - $C_6$  alkenyl,  $C_2$ - $C_6$  alkynyl,  $C_1$ - $C_6$  heteroalkyl, halo, cyano,  $-C(O)R^A$ ,  $-C(O)OR^B$ ,  $-OR^B$ ,  $-N(R^C)(R^D)$ ,  $-C(O)N(R^C)(R^D)$ ,  $-N(R^C)C(O)R^A$ ,  $-S(O)_xR^E$ ,  $-S(O)_xN(R^C)(R^D)$ , or  $-N(R^C)S(O)_xR^E$ , wherein each alkyl, alkenyl, alkynyl, and heteroalkyl is  
15 independently and optionally substituted with 1-12  $R^6$  (e.g., 1  $R^6$ , 2  $R^6$ , 3  $R^6$ , 4  $R^6$ , 5  $R^6$ , 6  $R^6$ , 7  $R^6$ , 8  $R^6$ , 9  $R^6$ , 10  $R^6$ , 11  $R^6$ , or 12  $R^6$ ). In some embodiments,  $R^3$  is  $C_1$ - $C_6$  heteroalkyl (e.g.,  $CH_2NHC(O)CH_2$ -phenyl-t-butyl).

In some embodiments, each  $R^4$  is independently  $C_1$ - $C_6$  alkyl,  $C_2$ - $C_6$  alkenyl,  $C_2$ - $C_6$  alkynyl,  $C_1$ - $C_6$  heteroalkyl, halo, cyano, oxo,  $-C(O)R^A$ ,  $-C(O)OR^B$ ,  $-OR^B$ ,  $-N(R^C)(R^D)$ ,  $-C(O)N(R^C)(R^D)$ ,  $-N(R^C)C(O)R^A$ ,  $S(O)_xR^E$ ,  $-S(O)_xN(R^C)(R^D)$ ,  $-N(R^C)S(O)_xR^E$ , carbocyclyl, heterocyclyl, aryl, or heteroaryl, wherein each  
20 alkyl, alkenyl, alkynyl, carbocyclyl, heterocyclyl, aryl, and heteroaryl is independently and optionally substituted with 1-12  $R^7$  (e.g., 1  $R^7$ , 2  $R^7$ , 3  $R^7$ , 4  $R^7$ , 5  $R^7$ , 6  $R^7$ , 7  $R^7$ , 8  $R^7$ , 9  $R^7$ , 10  $R^7$ , 11  $R^7$ , or  
25 12  $R^7$ ).

In some embodiments, each  $R^6$  is independently  $C_1$ - $C_6$  alkyl, oxo, cyano,  $-OR^B$ ,  $-N(R^C)(R^D)$ ,  $-C(O)N(R^C)(R^D)$ ,  $-N(R^C)C(O)R^A$ , aryl, or heteroaryl, wherein each aryl and heteroaryl is independently and optionally substituted with 1-6  $R^8$  (e.g., 1  $R^8$ , 2  $R^8$ , 3  $R^8$ , 4  $R^8$ , 5  $R^8$ , or 6  $R^8$ ).

In some embodiments, n is 0 or 1. In some embodiments, n is 0. In some embodiments, n is 1.

30 In some embodiments, the degradation compound is a compound of Formula (III):



or a pharmaceutically acceptable salt, ester, hydrate, or tautomer thereof, wherein:

$X_1$  is  $CR_3$ ;

----- is optionally a double bond when  $X_1$  is  $CR_3$  and  $R_3$  is absent;

5 each  $R_1$  is independently  $C_1$ - $C_6$  alkyl,  $C_1$ - $C_6$  haloalkyl,  $C_1$ - $C_6$  hydroxyalkyl, or halo, or two  $R_1$  together with the carbon atoms to which they are attached form a 5- or 6- membered heterocyclyl ring, or

two  $R_1$ , when on adjacent atoms, together with the atoms to which they are attached form a  $C_6$ - $C_{10}$  aryl or 5- or 6-membered heteroaryl ring comprising 1 to 3 heteroatoms selected from O, N, and S;

10  $R_2$  is hydrogen,  $C_1$ - $C_6$  alkyl,  $-C(O)C_1$ - $C_6$  alkyl,  $-C(O)(CH_2)_{0.3}$ -  $C_6$ - $C_{10}$  aryl,  $-C(O)O(CH_2)_{0.3}$ - $C_6$ - $C_{10}$  aryl,  $C_6$ - $C_{10}$  aryl, or 5- or 6-membered heteroaryl comprising 1 to 3 heteroatoms selected from O, N, and S,  $C_3$ - $C_8$  carbocyclyl, or 5- to 7-membered heterocyclyl comprising 1 to 3 heteroatoms selected from O, N, and S, wherein the alkyl is optionally substituted with one or more  $R_4$ ; and the aryl, heteroaryl, carbocyclyl, and heterocyclyl are optionally substituted with one or more  $R_5$ , or

15  $R_1$  and  $R_2$ , when on adjacent atoms, together with the atoms to which they are attached form a 5- or 6-membered heterocyclyl ring;

$R_3$  is hydrogen, or  $R_3$  is absent when ----- is a double bond;

each  $R_4$  is independently selected from  $-C(O)OR_6$ ,  $-C(O)NR_6R_6$ ,  $-NR_6C(O)R_6$ , halo,  $-OH$ ,  $-NH_2$ , cyano,  $C_6$ - $C_{10}$  aryl, 5- or 6-membered heteroaryl comprising 1 to 4 heteroatoms selected from O, N, and S,  $C_3$ - $C_8$  carbocyclyl, and 5- to 7-membered heterocyclyl ring comprising 1 to 3 heteroatoms selected from O, N, and S, wherein the aryl, heteroaryl, carbocyclyl, and heterocyclyl are optionally substituted with one or more  $R_7$ ;

each  $R_5$  is independently selected from  $C_1$ - $C_6$  alkyl,  $C_2$ - $C_6$  alkenyl,  $C_2$ - $C_6$  alkynyl,  $C_1$ - $C_6$  alkoxy,  $C_1$ - $C_6$  haloalkyl,  $C_1$ - $C_6$  haloalkoxy,  $C_1$ - $C_6$  hydroxyalkyl, halo,  $-OH$ ,  $-NH_2$ , cyano,  $C_3$ - $C_7$  carbocyclyl, 5- to 7-membered heterocyclyl comprising 1 to 3 heteroatoms selected from O, N, and S,  $C_6$ - $C_{10}$  aryl, and 5- or 6-membered heteroaryl comprising 1 to 3 heteroatoms selected from O, N, and S, or

two  $R_5$ , when on adjacent atoms, together with the atoms to which they are attached form a  $C_6$ - $C_{10}$  aryl or 5- or 6-membered heteroaryl comprising 1 to 3 heteroatoms selected from O, N, and S, optionally substituted with one or more  $R_{10}$ , or

two R<sub>5</sub>, when on adjacent atoms, together with the atoms to which they are attached form a C<sub>5</sub>-C<sub>7</sub> carbocyclyl or 5- to 7-membered heterocyclyl comprising 1 to 3 heteroatoms selected from O, N, and S optionally substituted with one or more R<sub>10</sub>;

R<sub>6</sub> and R<sub>6</sub> are each independently hydrogen, C<sub>1</sub>-C<sub>6</sub> alkyl, or C<sub>6</sub>-C<sub>10</sub> aryl;

5 each R<sub>7</sub> is independently selected from C<sub>1</sub>-C<sub>6</sub> alkyl, C<sub>2</sub>-C<sub>6</sub> alkenyl, C<sub>2</sub>-C<sub>6</sub> alkynyl, C<sub>1</sub>-C<sub>6</sub> alkoxy, C<sub>1</sub>-C<sub>6</sub> haloalkyl, C<sub>1</sub>-C<sub>6</sub> haloalkoxy, -C(O)R<sub>8</sub>, -(CH<sub>2</sub>)<sub>0-3</sub>C(O)OR<sub>8</sub>, -C(O)NR<sub>8</sub>R<sub>9</sub>, -NR<sub>8</sub>C(O)R<sub>9</sub>, -NR<sub>8</sub>C(O)OR<sub>9</sub>, -S(O)<sub>p</sub>NR<sub>8</sub>R<sub>9</sub>, -S(O)<sub>p</sub>R<sub>12</sub>, (C<sub>1</sub>-C<sub>6</sub>)hydroxyalkyl, halo, -OH, -O(CH<sub>2</sub>)<sub>1-3</sub>CN, -NH<sub>2</sub>, cyano, -O(CH<sub>2</sub>)<sub>0-3</sub>- C<sub>6</sub>-C<sub>10</sub> aryl, adamantyl, -O(CH<sub>2</sub>)<sub>0-3</sub>-5- or 6-membered heteroaryl comprising 1 to 3 heteroatoms selected from O, N, and S, C<sub>6</sub>-C<sub>10</sub> aryl, monocyclic or bicyclic 5- to 10-membered heteroaryl  
10 comprising 1 to 3 heteroatoms selected from O, N, and S, C<sub>3</sub>-C<sub>7</sub> carbocyclyl, and 5- to 7-membered heterocyclyl comprising 1 to 3 heteroatoms selected from O, N, and S, wherein the alkyl is optionally substituted with one or more R<sub>11</sub>, and the aryl, heteroaryl, and heterocyclyl are optionally substituted with one or more substituents each independently selected from halogen, C<sub>1</sub>-C<sub>6</sub> alkyl, C<sub>1</sub>-C<sub>6</sub> haloalkyl, and C<sub>1</sub>-C<sub>6</sub> alkoxy, or

15 two R<sub>7</sub> together with the carbon atom to which they are attached form a =(O), or

two R<sub>7</sub>, when on adjacent atoms, together with the atoms to which they are attached form a C<sub>6</sub>-C<sub>10</sub> aryl or 5- or 6-membered heteroaryl comprising 1 to 3 heteroatoms selected from O, N, and S, optionally substituted with one or more R<sub>10</sub>, or

20 two R<sub>7</sub> together with the atoms to which they are attached form a C<sub>5</sub>-C<sub>7</sub> carbocyclyl or a 5- to 7-membered heterocyclyl comprising 1 to 3 heteroatoms selected from O, N, and S, optionally substituted with one or more R<sub>10</sub>;

R<sub>8</sub> and R<sub>9</sub> are each independently hydrogen or C<sub>1</sub>-C<sub>6</sub> alkyl;

each R<sub>10</sub> is independently selected from C<sub>1</sub>-C<sub>6</sub> alkyl, C<sub>1</sub>-C<sub>6</sub> alkoxy, C<sub>1</sub>-C<sub>6</sub> haloalkyl, C<sub>1</sub>-C<sub>6</sub> haloalkoxy, C<sub>1</sub>-C<sub>6</sub> hydroxyalkyl, halo, -OH, -NH<sub>2</sub>, and cyano, or

25 two R<sub>10</sub> together with the carbon atom to which they are attached form a =(O);

each R<sub>11</sub> is independently selected from cyano, C<sub>1</sub>-C<sub>6</sub> alkoxy, C<sub>6</sub>-C<sub>10</sub> aryl, and 5- to 7-membered heterocyclyl comprising 1 to 3 heteroatoms selected from O, N, and S, wherein each aryl and heterocyclyl is optionally substituted with one or more substituents each independently selected from C<sub>1</sub>-C<sub>6</sub> alkyl, C<sub>1</sub>-C<sub>6</sub> alkoxy, C<sub>1</sub>-C<sub>6</sub> haloalkyl, C<sub>1</sub>-C<sub>6</sub> haloalkoxy, C<sub>1</sub>-C<sub>6</sub> hydroxyalkyl, halo, -OH, -NH<sub>2</sub>, and cyano;

30 R<sub>12</sub> is C<sub>1</sub>-C<sub>6</sub> alkyl, C<sub>1</sub>-C<sub>6</sub> haloalkyl, C<sub>6</sub>-C<sub>10</sub> aryl, or 5- to 7-membered heterocyclyl comprising 1 to 3 heteroatoms selected from O, N, and S;

R<sub>x</sub> is hydrogen or deuterium;

p is 0, 1, or 2;

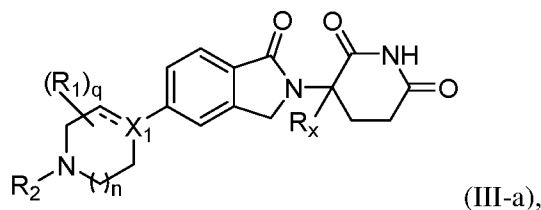
n is 0, 1, or 2;



y is 1 or 2, wherein  $n + y \leq 3$ ; and

q is 0, 1, 2, 3, or 4.

In some embodiments, the degradation compound of Formula (III) is a compound of Formula (III-a):



or a pharmaceutically acceptable salt, ester, hydrate, or tautomer thereof, wherein:

$X_1$  is  $CR_3$ ;

----- is optionally a double bond when  $X_1$  is  $CR_3$  and  $R_3$  is absent;

each  $R_1$  is independently  $C_1$ - $C_6$  alkyl,  $C_1$ - $C_6$  haloalkyl,  $C_1$ - $C_6$  hydroxyalkyl, or halo;

$R_2$  is hydrogen,  $C_1$ - $C_6$  alkyl,  $C_6$ - $C_{10}$  aryl, or 5- or 6-membered heteroaryl comprising 1 to 3 heteroatoms selected from O, N, and S,  $C_3$ - $C_8$  carbocyclyl, or 5- to 7-membered heterocyclyl comprising 1 to 3 heteroatoms selected from O, N, and S, wherein the alkyl is optionally substituted with one or more  $R_4$ ; and the aryl, heteroaryl, carbocyclyl, and heterocyclyl are optionally substituted with one or more  $R_5$ ;

$R_3$  is hydrogen, or  $R_3$  is absent when ----- is a double bond;

each  $R_4$  is independently selected from  $-C(O)OR_6$ ,  $-C(O)NR_6R_6$ ,  $-NR_6C(O)R_6$ ,  $C_6$ - $C_{10}$  aryl, 5- or 6-membered heteroaryl comprising 1 to 4 heteroatoms selected from O, N, and S,  $C_3$ - $C_8$  carbocyclyl, and 5- to 7-membered heterocyclyl ring comprising 1 to 3 heteroatoms selected from O, N, and S, wherein the aryl, heteroaryl, carbocyclyl, and heterocyclyl are optionally substituted with one or more  $R_7$ ;

each  $R_5$  is independently selected from  $C_1$ - $C_6$  alkyl,  $C_2$ - $C_6$  alkenyl,  $C_2$ - $C_6$  alkynyl,  $C_1$ - $C_6$  alkoxy,  $C_1$ - $C_6$  haloalkyl,  $C_1$ - $C_6$  haloalkoxy,  $C_1$ - $C_6$  hydroxyalkyl, halo,  $-OH$ ,  $-NH_2$ , cyano,  $C_3$ - $C_7$  carbocyclyl, 5- to 7-membered heterocyclyl comprising 1 to 3 heteroatoms selected from O, N, and S,  $C_6$ - $C_{10}$  aryl, and 5- or 6-membered heteroaryl comprising 1 to 3 heteroatoms selected from O, N, and S, or

two  $R_5$ , when on adjacent atoms, together with the atoms to which they are attached form a  $C_6$ - $C_{10}$  aryl or 5- or 6-membered heteroaryl comprising 1 to 3 heteroatoms selected from O, N, and S,

optionally substituted with one or more  $R_{10}$ , or

two  $R_5$ , when on adjacent atoms, together with the atoms to which they are attached form a  $C_5$ - $C_7$  carbocyclyl or 5- to 7-membered heterocyclyl comprising 1 to 3 heteroatoms selected from O, N, and S optionally substituted with one or more  $R_{10}$ ;

$R_6$  and  $R_6$  are each independently hydrogen, or  $C_1$ - $C_6$  alkyl;

each  $R_7$  is independently selected from  $C_1$ - $C_6$  alkyl,  $C_2$ - $C_6$  alkenyl,  $C_2$ - $C_6$  alkynyl,  $C_1$ - $C_6$  alkoxy,  $C_1$ - $C_6$  haloalkyl,  $C_1$ - $C_6$  haloalkoxy,  $-C(O)R_8$ ,  $-C(O)NR_8R_9$ ,  $-NR_8C(O)R_9$ ,  $-NR_8C(O)OR_9$ , ( $C_1$ - $C_6$ )hydroxyalkyl, halo,  $-OH$ ,  $-NH_2$ , cyano,  $C_6$ - $C_{10}$  aryl, 5- or 6-membered heteroaryl comprising 1 to 3 heteroatoms selected from O, N, and S,  $C_3$ - $C_7$  carbocyclyl, and 5- to 7-membered heterocyclyl comprising 1 to 3 heteroatoms selected from O, N, and S, or

two  $R_7$ , when on adjacent atoms, together with the atoms to which they are attached form a  $C_6$ - $C_{10}$  aryl or a 5- or 6-membered heteroaryl comprising 1 to 3 heteroatoms selected from O, N, and S, optionally substituted with one or more  $R_{10}$ , or

two  $R_7$  together with the atoms to which they are attached form a  $C_5$ - $C_7$  carbocyclyl or a 5- to 7-membered heterocyclyl comprising 1 to 3 heteroatoms selected from O, N, and S, optionally substituted with one or more  $R_{10}$ ;

$R_8$  and  $R_9$  are each independently hydrogen or  $C_1$ - $C_6$  alkyl;

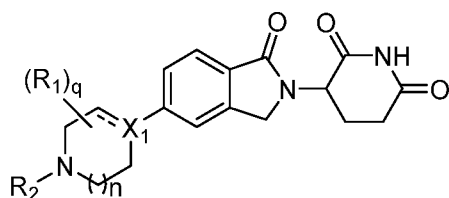
each  $R_{10}$  is independently selected from  $C_1$ - $C_6$  alkyl,  $C_1$ - $C_6$  alkoxy,  $C_1$ - $C_6$  haloalkyl,  $C_1$ - $C_6$  haloalkoxy,  $C_1$ - $C_6$  hydroxyalkyl, halo,  $-OH$ ,  $-NH_2$ , and cyano;

$R_x$  is hydrogen or deuterium;

$n$  is 1 or 2; and

$q$  is 0, 1, 2, 3, or 4.

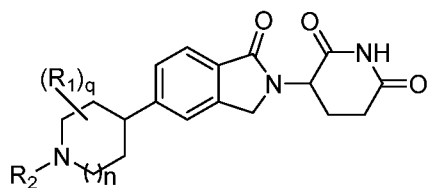
In an embodiment, the compound of Formula (III) is a compound of Formula (III-b):



(III-b), or a pharmaceutically acceptable salt, hydrate,

solvate, prodrug, stereoisomer, and tautomer thereof, wherein  $X_1$ ,  $R_1$ ,  $R_2$ ,  $n$ ,  $q$ , and subvariables thereof are defined as described for Formula (III).

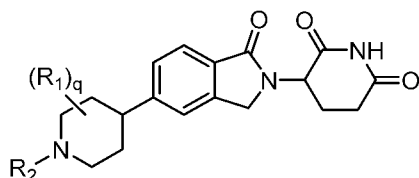
In an embodiment, the compound of Formula (III) is a compound of Formula (III-c):



(III-c), or a pharmaceutically acceptable salt, hydrate,

solvate, prodrug, stereoisomer, and tautomer thereof, wherein  $R_1$ ,  $R_2$ ,  $n$ ,  $q$ , and subvariables thereof are defined as described for Formula (III).

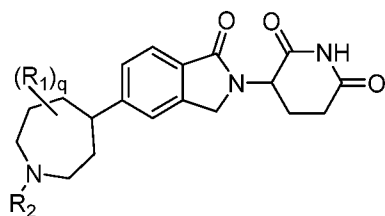
In an embodiment, the compound of Formula (III) is a compound of Formula (III-d):



(III-d), or a pharmaceutically acceptable salt, hydrate,

solvate, prodrug, stereoisomer, and tautomer thereof, wherein  $R_1$ ,  $R_2$ ,  $q$ , and subvariables thereof are defined as described for Formula (III).

In an embodiment, the compound of Formula (III) is a compound of Formula (III-e):



(III-e), or a pharmaceutically acceptable salt, hydrate, solvate,

prodrug, stereoisomer, and tautomer thereof, wherein  $R_1$ ,  $R_2$ ,  $q$ , and subvariables thereof are defined as described for Formula (III).

In some embodiments of Formula (III),  $X_1$  is CH and  $n$  is 1. In another embodiment,  $X_1$  is CH,  $n$  is 1, and  $q$  is 0.

In some embodiments of Formula (III),  $X_1$  is CH,  $n$  is 1, and  $q$  is 0 or 1. In another embodiment,  $X_1$  is CH,  $n$  is 1,  $q$  is 0 or 1, and  $R_1$  is  $C_1$ - $C_6$  alkyl. In another embodiment,  $X_1$  is CH,  $n$  is 1,  $q$  is 0 or 1,  $R_1$  is  $C_1$ - $C_6$  alkyl, and  $R_2$  is  $C_1$ - $C_6$  alkyl optionally substituted with one to three  $R_4$ . In another embodiment,  $X_1$  is CH,  $n$  is 1,  $q$  is 0 or 1,  $R_1$  is  $C_1$ - $C_6$  alkyl, and  $R_2$  is  $C_1$ - $C_6$  alkyl substituted with one to three  $R_4$ .

In another embodiment,  $X_1$  is CH,  $n$  is 1,  $q$  is 0, and  $R_2$  is  $C_1$ - $C_6$  alkyl optionally substituted with one to three  $R_4$ . In another embodiment,  $X_1$  is CH,  $n$  is 1,  $q$  is 0, and  $R_2$  is  $C_1$ - $C_6$  alkyl substituted with one to three  $R_4$ .

In some embodiments of the formulae above,  $X_1$  is CH,  $n$  is 1,  $q$  is 0 or 1,  $R_1$  is  $C_1$ - $C_6$  alkyl,  $R_2$  is  $C_1$ - $C_6$  alkyl optionally substituted with one to three  $R_4$ , and each  $R_4$  is independently selected from - $C(O)OR_6$ ,  $C_6$ - $C_{10}$  aryl, 5- or 6-membered heteroaryl comprising 1 to 3 heteroatoms selected from O, N, and S,  $C_3$ - $C_8$  carbocyclyl, and 5- to 7-membered heterocyclyl comprising 1 to 3 heteroatoms selected from O, N, and S, wherein the aryl, heteroaryl, carbocyclyl, and heterocyclyl are optionally substituted with one to three  $R_7$ .

In some embodiments of Formula (III),  $X_1$  is CH,  $n$  is 1,  $q$  is 0 or 1,  $R_1$  is  $C_1$ - $C_6$  alkyl,  $R_2$  is  $C_1$ - $C_6$  alkyl substituted with one to three  $R_4$ , and each  $R_4$  is independently selected from - $C(O)OR_6$ ,  $C_6$ - $C_{10}$  aryl, 5- or 6-membered heteroaryl comprising 1 to 3 heteroatoms selected from O, N, and S,  $C_3$ - $C_8$  carbocyclyl, and 5- to 7-membered heterocyclyl comprising 1 to 3 heteroatoms selected from O, N, and S, wherein the aryl, heteroaryl, carbocyclyl, and heterocyclyl groups are optionally substituted with one to three  $R_7$ .

In some embodiments of Formula (III),  $X_1$  is CH,  $n$  is 1,  $q$  is 0 or 1,  $R_1$  is  $C_1$ - $C_6$  alkyl,  $R_2$  is  $C_1$ - $C_6$  alkyl optionally substituted with one to three  $R_4$ , and each  $R_4$  is independently selected from  $C_6$ - $C_{10}$  aryl, 5- or 6-membered heteroaryl comprising 1 to 3 heteroatoms selected from O, N, and S,  $C_3$ - $C_8$  carbocyclyl, and 5- to 7-membered heterocyclyl comprising 1 to 3 heteroatoms selected from O, N, and S, wherein the aryl, heteroaryl, carbocyclyl, and heterocyclyl groups are optionally substituted with one to three  $R_7$ .

In some embodiments of Formula (III),  $X_1$  is CH,  $n$  is 1,  $q$  is 0 or 1,  $R_1$  is  $C_1$ - $C_6$  alkyl,  $R_2$  is  $C_1$ - $C_6$  alkyl substituted with one to three  $R_4$ , and each  $R_4$  is independently selected from  $C_6$ - $C_{10}$  aryl, 5- or 6-membered heteroaryl comprising 1 to 3 heteroatoms selected from O, N, and S,  $C_3$ - $C_8$  carbocyclyl, and 5- to 7-membered heterocyclyl comprising 1 to 3 heteroatoms selected from O, N, and S, wherein the aryl, heteroaryl, carbocyclyl, and heterocyclyl groups are optionally substituted with one to three  $R_7$ .

In some embodiments of Formula (III),  $X_1$  is CH,  $n$  is 1,  $q$  is 0, and  $R_2$  is  $C_6$ - $C_{10}$  aryl,  $C_3$ - $C_8$  carbocyclyl, or 5- to 7-membered heterocyclyl comprising 1 to 3 heteroatoms selected from O, N, and S, wherein the aryl, carbocyclyl, and heterocyclyl are optionally substituted with one to three  $R_5$ . In yet another embodiment,  $X_1$  is CH,  $n$  is 1,  $q$  is 0, and  $R_2$  is  $C_6$ - $C_{10}$  aryl,  $C_3$ - $C_8$  carbocyclyl, or 5- to 7-membered heterocyclyl comprising 1 to 3 heteroatoms selected from O, N, and S.

In some embodiments of Formula (III),  $X_1$  is CH,  $n$  is 1,  $q$  is 0, and  $R_2$  is  $C_6$ - $C_{10}$  aryl optionally substituted with one to three  $R_5$ . In another embodiment,  $X_1$  is CH,  $n$  is 1,  $q$  is 0, and  $R_2$  is 5- or 6-membered heteroaryl comprising 1 to 3 heteroatoms selected from O, N, and S optionally substituted with one to three  $R_5$ . In yet another embodiment,  $X_1$  is CH,  $n$  is 1,  $q$  is 0, and  $R_2$  is  $C_3$ - $C_8$  carbocyclyl optionally substituted with one to three  $R_5$ . In another embodiment,  $X_1$  is CH,  $n$  is 1,  $q$  is 0, and  $R_2$  is 5- to 7-membered heterocyclyl comprising 1 to 3 heteroatoms selected from O, N, and S, optionally substituted with one to three  $R_5$ .

In some embodiments of Formula (III),  $X_1$  is CH,  $n$  is 1,  $q$  is 0 or 1,  $R_1$  is  $C_1$ - $C_6$  alkyl, and  $R_2$  is  $C_6$ - $C_{10}$  aryl,  $C_3$ - $C_8$  carbocyclyl, or 5- to 7-membered heterocyclyl comprising 1 to 3 heteroatoms selected from O, N, and S, wherein the aryl, carbocyclyl, and heterocyclyl are optionally substituted with one to three  $R_5$ . In yet another embodiment,  $X_1$  is CH,  $n$  is 1,  $q$  is 0 or 1,  $R_1$  is  $C_1$ - $C_6$  alkyl, and  $R_2$  is  $C_6$ - $C_{10}$  aryl,  $C_3$ - $C_8$  carbocyclyl, or 5- to 7-membered heterocyclyl comprising 1 to 3 heteroatoms selected from O, N, and S.

In some embodiments of Formula (III),  $X_1$  is CH,  $n$  is 1,  $q$  is 0 or 1,  $R_1$  is  $C_1$ - $C_6$  alkyl, and  $R_2$  is  $C_6$ - $C_{10}$  aryl optionally substituted with one to three  $R_5$ . In another embodiment,  $X_1$  is CH,  $n$  is 1,  $q$  is 0, and  $R_2$  is 5- or 6-membered heteroaryl comprising 1 to 3 heteroatoms selected from O, N, and S optionally substituted with one to three  $R_5$ . In yet another embodiment,  $X_1$  is CH,  $n$  is 1,  $q$  is 0 or 1,  $R_1$  is  $C_1$ - $C_6$  alkyl, and  $R_2$  is  $C_3$ - $C_8$  carbocyclyl optionally substituted with one to three  $R_5$ . In another

embodiment,  $X_1$  is CH,  $n$  is 1,  $q$  is 0 or 1,  $R_1$  is  $C_1$ - $C_6$  alkyl, and  $R_2$  is 5- to 7-membered heterocyclyl comprising 1 to 3 heteroatoms selected from O, N, and S, optionally substituted with one to three  $R_5$ .

In some embodiments of Formula (III),  $X_1$  is CH,  $n$  is 1,  $q$  is 0, and  $R_2$  is  $C_1$ - $C_6$  alkyl optionally substituted with one to three  $R_4$ . In another embodiment  $X_1$  is CH,  $n$  is 1,  $q$  is 0, and  $R_2$  is  $C_1$ - $C_6$  alkyl substituted with one to three  $R_4$ .

In some embodiments of Formula (III),  $X_1$  is CH,  $n$  is 1,  $q$  is 0,  $R_2$  is  $C_1$ - $C_6$  alkyl optionally substituted with one to three  $R_4$ , and each  $R_4$  is independently selected from  $-C(O)OR_6$ ,  $C_6$ - $C_{10}$  aryl, 5- or 6-membered heteroaryl comprising 1 to 3 heteroatoms selected from O, N, and S,  $C_3$ - $C_8$  carbocyclyl, and 5- to 7-membered heterocyclyl comprising 1 to 3 heteroatoms selected from O, N, and S, wherein the aryl, heteroaryl, carbocyclyl, and heterocyclyl groups are optionally substituted with one to three  $R_7$ .

In some embodiments of Formula (III),  $X_1$  is CH,  $n$  is 1,  $q$  is 0,  $R_2$  is  $C_1$ - $C_6$  alkyl substituted with one to three  $R_4$ , and each  $R_4$  is independently selected from  $-C(O)OR_6$ ,  $C_6$ - $C_{10}$  aryl, 5- or 6-membered heteroaryl comprising 1 to 3 heteroatoms selected from O, N, and S,  $C_3$ - $C_8$  carbocyclyl, and 5- to 7-membered heterocyclyl comprising 1 to 3 heteroatoms selected from O, N, and S, wherein the aryl, heteroaryl, carbocyclyl, and heterocyclyl groups are optionally substituted with one to three  $R_7$ .

In some embodiments of Formula (III),  $X_1$  is CH,  $n$  is 1,  $q$  is 0,  $R_2$  is  $C_1$ - $C_6$  alkyl optionally substituted with one to three  $R_4$ , and each  $R_4$  is independently selected from halo,  $-OH$ ,  $C_6$ - $C_{10}$  aryl, 5- or 6-membered heteroaryl comprising 1 to 3 heteroatoms selected from O, N, and S,  $C_3$ - $C_8$  carbocyclyl, and 5- to 7-membered heterocyclyl comprising 1 to 3 heteroatoms selected from O, N, and S, wherein the aryl, heteroaryl, carbocyclyl, and heterocyclyl groups are optionally substituted with one to three  $R_7$ .

In some embodiments of Formula (III),  $X_1$  is CH,  $n$  is 1,  $q$  is 0,  $R_2$  is  $C_1$ - $C_6$  alkyl substituted with one to three  $R_4$ , and each  $R_4$  is independently selected from halo,  $-OH$ ,  $C_6$ - $C_{10}$  aryl, 5- or 6-membered heteroaryl comprising 1 to 3 heteroatoms selected from O, N, and S,  $C_3$ - $C_8$  carbocyclyl, and 5- to 7-membered heterocyclyl comprising 1 to 3 heteroatoms selected from O, N, and S, wherein the aryl, heteroaryl, carbocyclyl, and heterocyclyl groups are optionally substituted with one to three  $R_7$ .

In some embodiments of the formulae above,  $X_1$  is CH,  $n$  is 1,  $n_1$  is 1,  $q$  is 0,  $R_2$  is  $C_1$ - $C_6$  alkyl optionally substituted with one to three  $R_4$ , and each  $R_4$  is independently selected from halo,  $-OH$ ,  $C_6$ - $C_{10}$  aryl, 5- or 6-membered heteroaryl comprising 1 to 3 heteroatoms selected from O, N, and S,  $C_3$ - $C_8$  carbocyclyl, and 5- to 7-membered heterocyclyl comprising 1 to 3 heteroatoms selected from O, N, and S, wherein the aryl, heteroaryl, carbocyclyl, and heterocyclyl groups are optionally substituted with one to three  $R_7$ .

In some embodiments of Formula (III),  $X_1$  is CH,  $n$  is 1,  $n_1$  is 1,  $q$  is 0,  $R_2$  is  $C_1$ - $C_6$  alkyl substituted with one to three  $R_4$ , and each  $R_4$  is independently selected from halo,  $-OH$ ,  $C_6$ - $C_{10}$  aryl, 5- or 6-membered heteroaryl comprising 1 to 3 heteroatoms selected from O, N, and S,  $C_3$ - $C_8$  carbocyclyl, and

5- to 7-membered heterocyclyl comprising 1 to 3 heteroatoms selected from O, N, and S, wherein the aryl, heteroaryl, carbocyclyl, and heterocyclyl groups are optionally substituted with one to three R<sub>7</sub>.

In some embodiments of Formula (III), X<sub>1</sub> is CH, n is 1, q is 0, R<sub>2</sub> is C<sub>1</sub>-C<sub>6</sub> alkyl optionally substituted with one to three R<sub>4</sub>, and each R<sub>4</sub> is independently selected from C<sub>6</sub>-C<sub>10</sub> aryl, 5- or 6-membered heteroaryl comprising 1 to 3 heteroatoms selected from O, N, and S, C<sub>3</sub>-C<sub>8</sub> carbocyclyl, and 5- to 7-membered heterocyclyl comprising 1 to 3 heteroatoms selected from O, N, and S, wherein the aryl, heteroaryl, carbocyclyl, and heterocyclyl groups are optionally substituted with one to three R<sub>7</sub>.

In some embodiments of Formula (III), X<sub>1</sub> is CH, n is 1, q is 0, R<sub>2</sub> is C<sub>1</sub>-C<sub>6</sub> alkyl substituted with one to three R<sub>4</sub>, and each R<sub>4</sub> is independently selected from C<sub>6</sub>-C<sub>10</sub> aryl, 5- or 6-membered heteroaryl comprising 1 to 3 heteroatoms selected from O, N, and S, C<sub>3</sub>-C<sub>8</sub> carbocyclyl, and 5- to 7-membered heterocyclyl comprising 1 to 3 heteroatoms selected from O, N, and S, wherein the aryl, heteroaryl, carbocyclyl, and heterocyclyl groups are optionally substituted with one to three R<sub>7</sub>.

In some embodiments of Formula (III), X<sub>1</sub> is CH, n is 1, q is 0, R<sub>2</sub> is C<sub>1</sub>-C<sub>6</sub> alkyl optionally substituted with one to three R<sub>4</sub>, and each R<sub>4</sub> is independently selected from halo, -OH, phenyl, 5- or 6-membered heteroaryl comprising 1 to 3 heteroatoms selected from O, N, and S, C<sub>3</sub>-C<sub>8</sub> carbocyclyl, and 5- to 7-membered heterocyclyl comprising 1 to 3 heteroatoms selected from O, N, and S, wherein the aryl, heteroaryl, carbocyclyl, and heterocyclyl groups are optionally substituted with one to three R<sub>7</sub>.

In some embodiments of Formula (III), X<sub>1</sub> is CH, n is 1, q is 0, R<sub>2</sub> is C<sub>1</sub>-C<sub>6</sub> alkyl substituted with one to three R<sub>4</sub>, and each R<sub>4</sub> is independently selected from halo, -OH, phenyl, 5- or 6-membered heteroaryl comprising 1 to 3 heteroatoms selected from O, N, and S, C<sub>3</sub>-C<sub>8</sub> carbocyclyl, and 5- to 7-membered heterocyclyl comprising 1 to 3 heteroatoms selected from O, N, and S, wherein the aryl, heteroaryl, carbocyclyl, and heterocyclyl groups are optionally substituted with one to three R<sub>7</sub>.

In some embodiments of Formula (III), X<sub>1</sub> is CH, n is 1, n<sub>1</sub> is 1, q is 0, R<sub>2</sub> is C<sub>1</sub>-C<sub>6</sub> alkyl optionally substituted with one to three R<sub>4</sub>, and each R<sub>4</sub> is independently selected from halo, -OH, phenyl, 5- or 6-membered heteroaryl comprising 1 to 3 heteroatoms selected from O, N, and S, C<sub>3</sub>-C<sub>8</sub> carbocyclyl, and 5- to 7-membered heterocyclyl comprising 1 to 3 heteroatoms selected from O, N, and S, wherein the aryl, heteroaryl, carbocyclyl, and heterocyclyl groups are optionally substituted with one to three R<sub>7</sub>.

In some embodiments of Formula (III), X<sub>1</sub> is CH, n is 1, n<sub>1</sub> is 1, q is 0, R<sub>2</sub> is C<sub>1</sub>-C<sub>6</sub> alkyl substituted with one to three R<sub>4</sub>, and each R<sub>4</sub> is independently selected from halo, -OH, phenyl, 5- or 6-membered heteroaryl comprising 1 to 3 heteroatoms selected from O, N, and S, C<sub>3</sub>-C<sub>8</sub> carbocyclyl, and 5- to 7-membered heterocyclyl comprising 1 to 3 heteroatoms selected from O, N, and S, wherein the phenyl, heteroaryl, carbocyclyl, and heterocyclyl groups are optionally substituted with one to three R<sub>7</sub>.

In some embodiments of Formula (III), X<sub>1</sub> is CH, n is 1, q is 0, R<sub>2</sub> is C<sub>1</sub>-C<sub>6</sub> alkyl optionally substituted with one to three R<sub>4</sub>, and each R<sub>4</sub> is independently selected from phenyl, 5- or 6-membered

heteroaryl comprising 1 to 3 heteroatoms selected from O, N, and S, C<sub>3</sub>-C<sub>8</sub> carbocyclyl, and 5- to 7-membered heterocyclyl comprising 1 to 3 heteroatoms selected from O, N, and S, wherein the phenyl, heteroaryl, carbocyclyl, and heterocyclyl groups are optionally substituted with one to three R<sub>7</sub>.

In some embodiments of Formula (III), X<sub>1</sub> is CH, n is 1, q is 0, R<sub>2</sub> is C<sub>1</sub>-C<sub>6</sub> alkyl substituted with one to three R<sub>4</sub>, and each R<sub>4</sub> is independently selected from phenyl, 5- or 6-membered heteroaryl comprising 1 to 3 heteroatoms selected from O, N, and S, C<sub>3</sub>-C<sub>8</sub> carbocyclyl, and 5- to 7-membered heterocyclyl comprising 1 to 3 heteroatoms selected from O, N, and S, wherein the phenyl, heteroaryl, carbocyclyl, and heterocyclyl groups are optionally substituted with one to three R<sub>7</sub>.

In some embodiments of Formula (III), X<sub>1</sub> is CH, n is 1, n<sub>1</sub> is 1, q is 0, R<sub>2</sub> is C<sub>1</sub>-C<sub>6</sub> alkyl optionally substituted with one to three R<sub>4</sub>, and each R<sub>4</sub> is independently selected from phenyl, 5- or 6-membered heteroaryl comprising 1 to 3 heteroatoms selected from O, N, and S, C<sub>3</sub>-C<sub>8</sub> carbocyclyl, and 5- to 7-membered heterocyclyl comprising 1 to 3 heteroatoms selected from O, N, and S, wherein the phenyl, heteroaryl, carbocyclyl, and heterocyclyl groups are optionally substituted with one to three R<sub>7</sub>.

In some embodiments of Formula (III), X<sub>1</sub> is CH, n is 1, n<sub>1</sub> is 1, q is 0, R<sub>2</sub> is C<sub>1</sub>-C<sub>6</sub> alkyl substituted with one to three R<sub>4</sub>, and each R<sub>4</sub> is independently selected from phenyl, 5- or 6-membered heteroaryl comprising 1 to 3 heteroatoms selected from O, N, and S, C<sub>3</sub>-C<sub>8</sub> carbocyclyl, and 5- to 7-membered heterocyclyl comprising 1 to 3 heteroatoms selected from O, N, and S, wherein the phenyl, heteroaryl, carbocyclyl, and heterocyclyl groups are optionally substituted with one to three R<sub>7</sub>.

In some embodiments of Formula (III), X<sub>1</sub> is CH, n is 1, q is 0, R<sub>2</sub> is C<sub>1</sub>-C<sub>6</sub> alkyl optionally substituted with one to three R<sub>4</sub>, and each R<sub>4</sub> is independently selected from phenyl and 5- or 6-membered heteroaryl comprising 1 to 3 heteroatoms selected from O, N, and S, wherein the phenyl and heteroaryl groups are optionally substituted with one to three R<sub>7</sub>.

In some embodiments of Formula (III), X<sub>1</sub> is CH, n is 1, q is 0, R<sub>2</sub> is C<sub>1</sub>-C<sub>6</sub> alkyl substituted with one to three R<sub>4</sub>, and each R<sub>4</sub> is independently selected from phenyl and 5- or 6-membered heteroaryl comprising 1 to 3 heteroatoms selected from O, N, and S, C<sub>3</sub>-C<sub>8</sub> carbocyclyl, and 5- to 7-membered heterocyclyl comprising 1 to 3 heteroatoms selected from O, N, and S, wherein the phenyl and heteroaryl groups are optionally substituted with one to three R<sub>7</sub>.

In some embodiments of Formula (III), X<sub>1</sub> is CH, n is 1, n<sub>1</sub> is 1, q is 0, R<sub>2</sub> is C<sub>1</sub>-C<sub>6</sub> alkyl optionally substituted with one to three R<sub>4</sub>, and each R<sub>4</sub> is independently selected from phenyl and 5- or 6-membered heteroaryl comprising 1 to 3 heteroatoms selected from O, N, and S, wherein the phenyl and heteroaryl groups are optionally substituted with one to three R<sub>7</sub>.

In some embodiments of Formula (III), X<sub>1</sub> is CH, n is 1, n<sub>1</sub> is 1, q is 0, R<sub>2</sub> is C<sub>1</sub>-C<sub>6</sub> alkyl substituted with one to three R<sub>4</sub>, and each R<sub>4</sub> is independently selected from phenyl and 5- or 6-membered heteroaryl comprising 1 to 3 heteroatoms selected from O, N, and S, C<sub>3</sub>-C<sub>8</sub> carbocyclyl, and 5- to 7-

membered heterocyclyl comprising 1 to 3 heteroatoms selected from O, N, and S, wherein the phenyl and heteroaryl groups are optionally substituted with one to three R<sub>7</sub>.

In some embodiments of Formula (III), X<sub>1</sub> is CH, n is 1, q is 0, R<sub>2</sub> is C<sub>1</sub>-C<sub>6</sub> alkyl optionally substituted with one to three R<sub>4</sub>, and each R<sub>4</sub> is phenyl optionally substituted with one to three R<sub>7</sub>.

5 In some embodiments of Formula (III), X<sub>1</sub> is CH, n is 1, q is 0, R<sub>2</sub> is C<sub>1</sub>-C<sub>6</sub> alkyl substituted with one to three R<sub>4</sub>, and each R<sub>4</sub> is phenyl optionally substituted with one to three R<sub>7</sub>.

In some embodiments of Formula (III), X<sub>1</sub> is CH, n is 1, n<sub>1</sub> is 1, q is 0, R<sub>2</sub> is C<sub>1</sub>-C<sub>6</sub> alkyl optionally substituted with one to three R<sub>4</sub>, and each R<sub>4</sub> is phenyl optionally substituted with one to three R<sub>7</sub>.

10 In some embodiments of Formula (III), X<sub>1</sub> is CH, n is 1, n<sub>1</sub> is 1, q is 0, R<sub>2</sub> is C<sub>1</sub>-C<sub>6</sub> alkyl substituted with one to three R<sub>4</sub>, and each R<sub>4</sub> is phenyl optionally substituted with one to three R<sub>7</sub>.

In some embodiments of Formula (III), X<sub>1</sub> is CH and n is 2. In another embodiment, X<sub>1</sub> is CH, n is 2, and q is 0. In yet another embodiment, X<sub>1</sub> is CH, n is 2, and q is 0 or 1. In another embodiment, X<sub>1</sub> is CH, n is 2, q is 0 or 1, and R<sub>1</sub> is C<sub>1</sub>-C<sub>6</sub> alkyl.

15 In some embodiments of Formula (III), X<sub>1</sub> is CH, n is 2, q is 0 or 1, R<sub>1</sub> is C<sub>1</sub>-C<sub>6</sub> alkyl, and R<sub>2</sub> is C<sub>1</sub>-C<sub>6</sub> alkyl optionally substituted with one to three R<sub>4</sub>. In another embodiment, X<sub>1</sub> is CH, n is 2, q is 0 or 1, R<sub>1</sub> is C<sub>1</sub>-C<sub>6</sub> alkyl, and R<sub>2</sub> is C<sub>1</sub>-C<sub>6</sub> alkyl substituted with one to three R<sub>4</sub>.

In some embodiments of Formula (III), X<sub>1</sub> is CH, n is 2, q is 0, and R<sub>2</sub> is C<sub>1</sub>-C<sub>6</sub> alkyl optionally substituted with one to three R<sub>4</sub>. In another embodiment, X<sub>1</sub> is CH, n is 2, q is 0, and R<sub>2</sub> is C<sub>1</sub>-C<sub>6</sub> alkyl substituted with one to three R<sub>4</sub>.

20 In some embodiments of Formula (III), X<sub>1</sub> is CH, n is 2, q is 0 or 1, R<sub>1</sub> is C<sub>1</sub>-C<sub>6</sub> alkyl, R<sub>2</sub> is C<sub>1</sub>-C<sub>6</sub> alkyl optionally substituted with one to three R<sub>4</sub>, and each R<sub>4</sub> is independently selected from -C(O)OR<sub>6</sub>, C<sub>6</sub>-C<sub>10</sub> aryl, 5- or 6-membered heteroaryl comprising 1 to 3 heteroatoms selected from O, N, and S, C<sub>3</sub>-C<sub>8</sub> carbocyclyl, and 5- to 7-membered heterocyclyl comprising 1 to 3 heteroatoms selected from O, N, and S, wherein the aryl, heteroaryl, carbocyclyl, and heterocyclyl groups are optionally substituted with one to three R<sub>7</sub>.

25 In some embodiments of Formula (III), X<sub>1</sub> is CH, n is 2, q is 0 or 1, R<sub>1</sub> is C<sub>1</sub>-C<sub>6</sub> alkyl, R<sub>2</sub> is C<sub>1</sub>-C<sub>6</sub> alkyl substituted with one to three R<sub>4</sub>, and each R<sub>4</sub> is independently selected from -C(O)OR<sub>6</sub>, C<sub>6</sub>-C<sub>10</sub> aryl, 5- or 6-membered heteroaryl comprising 1 to 3 heteroatoms selected from O, N, and S, C<sub>3</sub>-C<sub>8</sub> carbocyclyl, and 5- to 7-membered heterocyclyl comprising 1 to 3 heteroatoms selected from O, N, and S, wherein the aryl, heteroaryl, carbocyclyl, and heterocyclyl groups are optionally substituted with one to three R<sub>7</sub>.

30 In some embodiments of Formula (III), X<sub>1</sub> is CH, n is 2, q is 0 or 1, R<sub>1</sub> is C<sub>1</sub>-C<sub>6</sub> alkyl, R<sub>2</sub> is C<sub>1</sub>-C<sub>6</sub> alkyl optionally substituted with one to three R<sub>4</sub>, and each R<sub>4</sub> is independently selected from C<sub>6</sub>-C<sub>10</sub> aryl, 5- or 6-membered heteroaryl comprising 1 to 3 heteroatoms selected from O, N, and S, C<sub>3</sub>-C<sub>8</sub> carbocyclyl,



and 5- to 7-membered heterocyclyl comprising 1 to 3 heteroatoms selected from O, N, and S, wherein the aryl, heteroaryl, carbocyclyl, and heterocyclyl groups are optionally substituted with one to three R<sub>7</sub>.

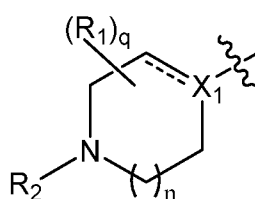
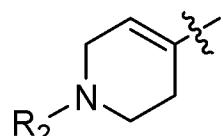
In some embodiments of Formula (III), X<sub>1</sub> is CH, n is 2, q is 0 or 1, R<sub>1</sub> is C<sub>1</sub>-C<sub>6</sub> alkyl, R<sub>2</sub> is C<sub>1</sub>-C<sub>6</sub> alkyl substituted with one to three R<sub>4</sub>, and each R<sub>4</sub> is independently selected from C<sub>6</sub>-C<sub>10</sub> aryl, 5- or 6-membered heteroaryl comprising 1 to 3 heteroatoms selected from O, N, and S, C<sub>3</sub>-C<sub>8</sub> carbocyclyl, and 5- to 7-membered heterocyclyl comprising 1 to 3 heteroatoms selected from O, N, and S, wherein the aryl, heteroaryl, carbocyclyl, and heterocyclyl groups are optionally substituted with one to three R<sub>7</sub>.

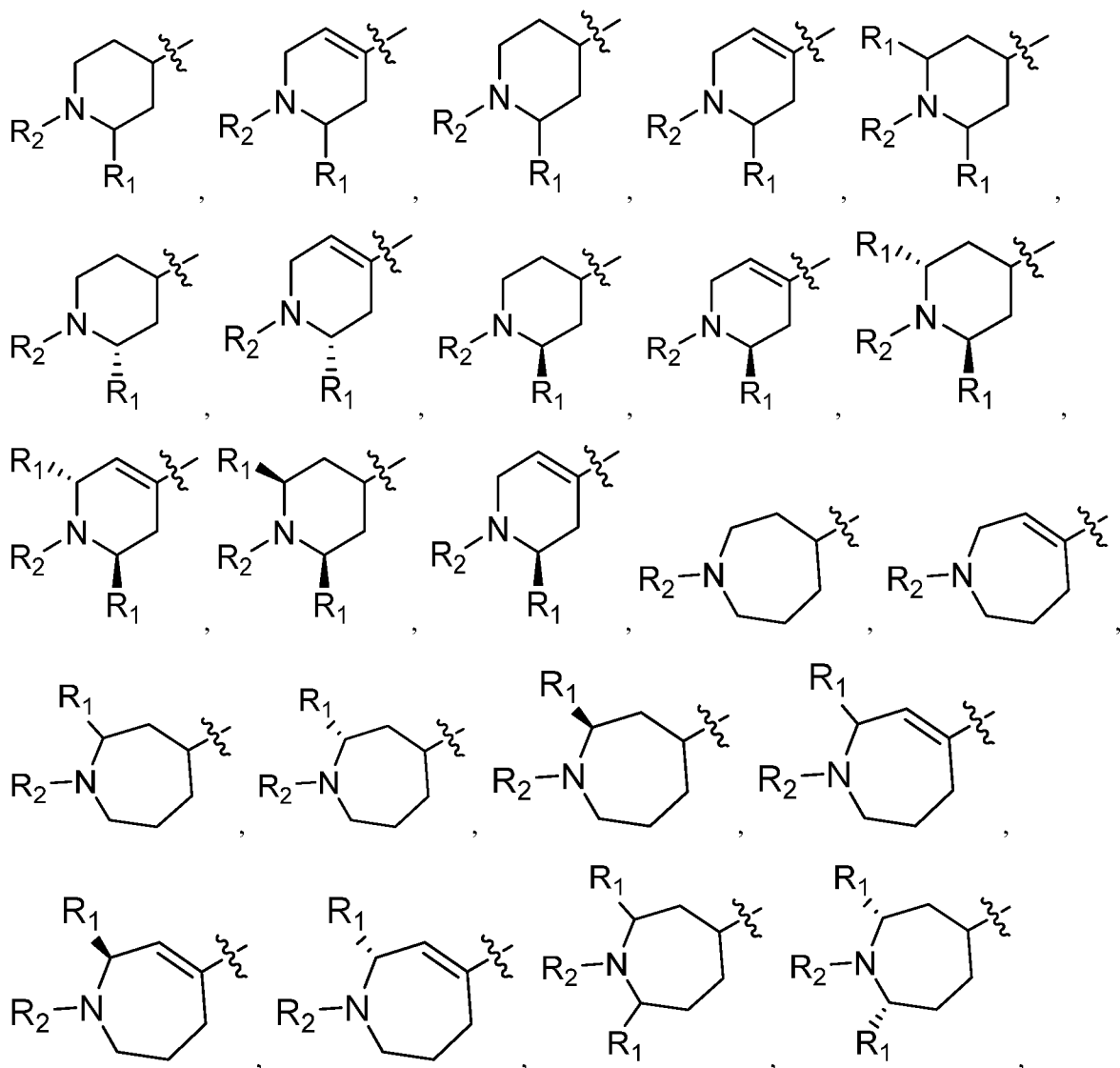
In some embodiments of Formula (III), X<sub>1</sub> is CH, n is 2, q is 0, and R<sub>2</sub> is C<sub>6</sub>-C<sub>10</sub> aryl, C<sub>3</sub>-C<sub>8</sub> carbocyclyl, or 5- to 7-membered heterocyclyl comprising 1 to 3 heteroatoms selected from O, N, and S, wherein the aryl, carbocyclyl, and heterocyclyl are optionally substituted with one to three R<sub>5</sub>. In yet another embodiment, X<sub>1</sub> is CH, n is 2, q is 0, and R<sub>2</sub> is C<sub>6</sub>-C<sub>10</sub> aryl, C<sub>3</sub>-C<sub>8</sub> carbocyclyl, or 5- to 7-membered heterocyclyl comprising 1 to 3 heteroatoms selected from O, N, and S.

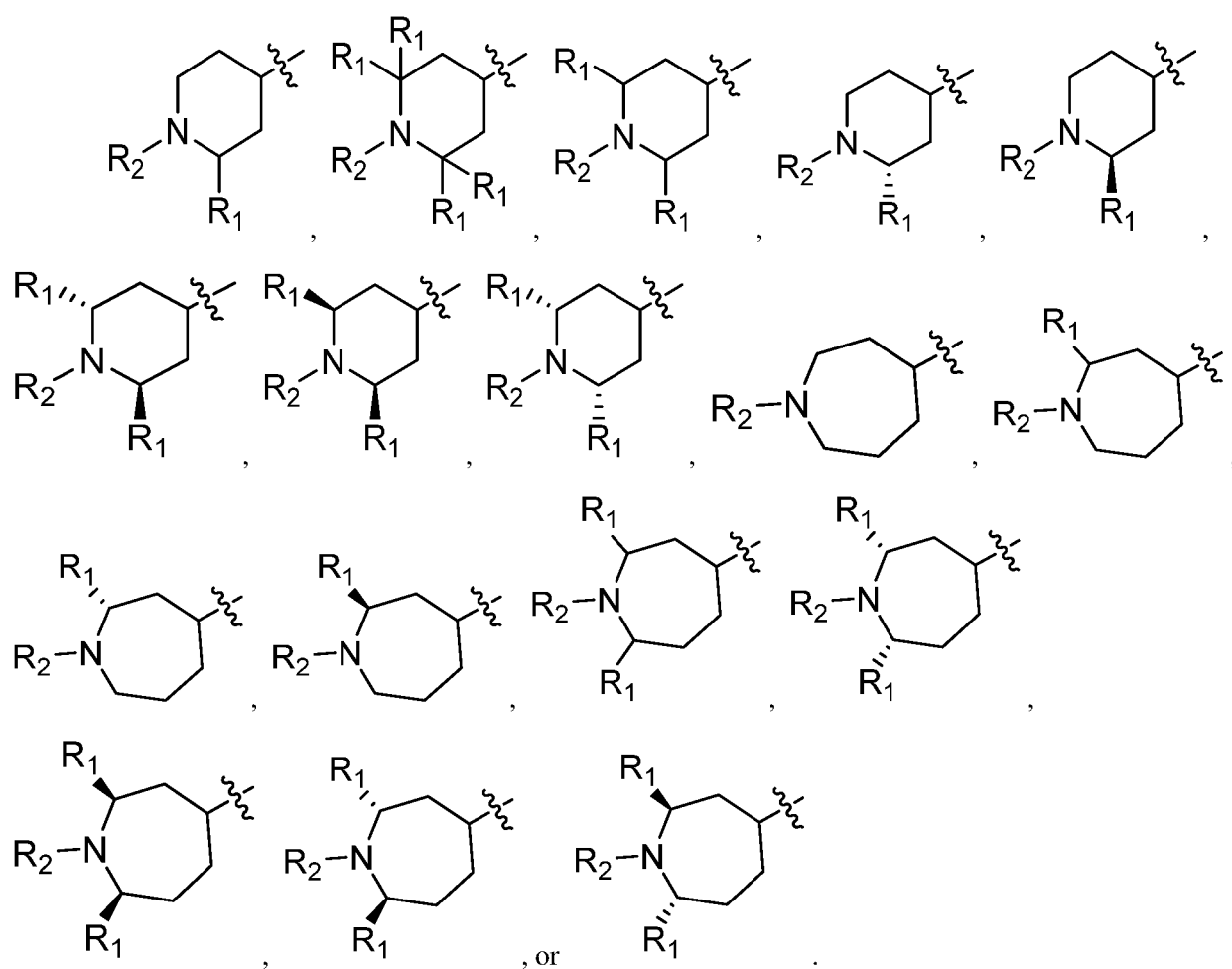
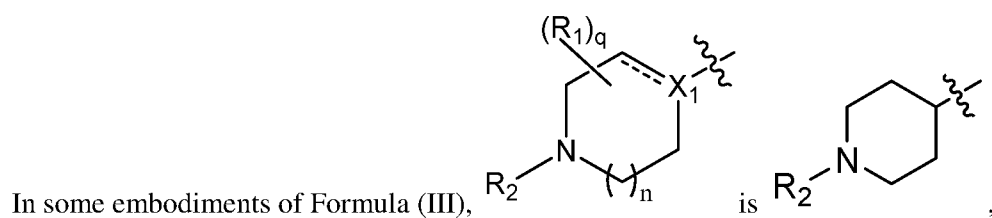
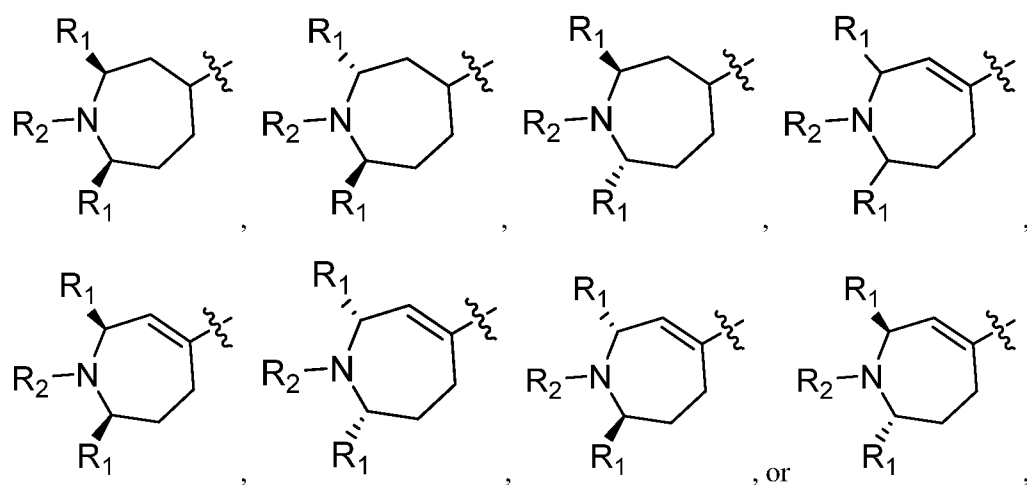
In some embodiments of Formula (III), X<sub>1</sub> is CH, n is 2, q is 0, and R<sub>2</sub> is C<sub>6</sub>-C<sub>10</sub> aryl optionally substituted with one to three R<sub>5</sub>. In another embodiment, X<sub>1</sub> is CH, n is 2, q is 0, and R<sub>2</sub> is 5- or 6-membered heteroaryl comprising 1 to 3 heteroatoms selected from O, N, and S optionally substituted with one to three R<sub>5</sub>. In yet another embodiment, X<sub>1</sub> is CH, n is 2, q is 0, and R<sub>2</sub> is C<sub>3</sub>-C<sub>8</sub> carbocyclyl optionally substituted with one to three R<sub>5</sub>. In another embodiment, X<sub>1</sub> is CH, n is 2, q is 0, and R<sub>2</sub> is 5- to 7-membered heterocyclyl comprising 1 to 3 heteroatoms selected from O, N, and S, optionally substituted with one to three R<sub>5</sub>.

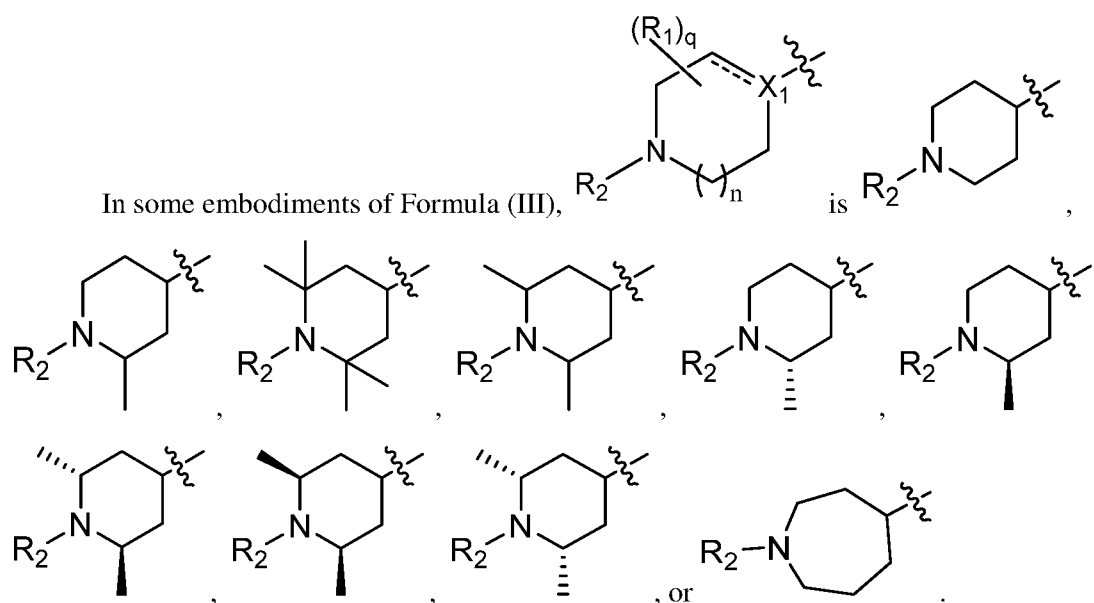
In some embodiments of Formula (III), X<sub>1</sub> is CH, n is 2, q is 0 or 1, R<sub>1</sub> is C<sub>1</sub>-C<sub>6</sub> alkyl, and R<sub>2</sub> is C<sub>6</sub>-C<sub>10</sub> aryl, C<sub>3</sub>-C<sub>8</sub> carbocyclyl, or 5- to 7-membered heterocyclyl comprising 1 to 3 heteroatoms selected from O, N, and S, wherein the aryl, carbocyclyl, and heterocyclyl are optionally substituted with one to three R<sub>5</sub>. In yet another embodiment, X<sub>1</sub> is CH, n is 2, q is 0 or 1, R<sub>1</sub> is C<sub>1</sub>-C<sub>6</sub> alkyl, and R<sub>2</sub> is C<sub>6</sub>-C<sub>10</sub> aryl, C<sub>3</sub>-C<sub>8</sub> carbocyclyl, or 5- to 7-membered heterocyclyl comprising 1 to 3 heteroatoms selected from O, N, and S.

In some embodiments of Formula (III), X<sub>1</sub> is CH, n is 2, q is 0 or 1, R<sub>1</sub> is C<sub>1</sub>-C<sub>6</sub> alkyl, and R<sub>2</sub> is C<sub>6</sub>-C<sub>10</sub> aryl optionally substituted with one to three R<sub>5</sub>. In another embodiment, X<sub>1</sub> is CH, n is 2, q is 0, and R<sub>2</sub> is 5- or 6-membered heteroaryl comprising 1 to 3 heteroatoms selected from O, N, and S optionally substituted with one to three R<sub>5</sub>. In yet another embodiment, X<sub>1</sub> is CH, n is 2, q is 0 or 1, R<sub>1</sub> is C<sub>1</sub>-C<sub>6</sub> alkyl, and R<sub>2</sub> is C<sub>3</sub>-C<sub>8</sub> carbocyclyl optionally substituted with one to three R<sub>5</sub>. In another embodiment, X<sub>1</sub> is CH, n is 2, q is 0 or 1, R<sub>1</sub> is C<sub>1</sub>-C<sub>6</sub> alkyl, and R<sub>2</sub> is 5- to 7-membered heterocyclyl comprising 1 to 3 heteroatoms selected from O, N, and S, optionally substituted with one to three R<sub>5</sub>.

In some embodiments of Formula (III),  is ,







A degradation compound may comprise one or more chiral centers or exist as one or more stereoisomers. In some embodiments, the degradation compound comprises a single chiral center and is a mixture of stereoisomers, e.g., an R stereoisomer and an S stereoisomer. In some embodiments, the mixture comprises a ratio of R stereoisomers to S stereoisomers, for example, about a 1:1 ratio of R stereoisomers to S stereoisomers (i.e., a racemic mixture). In some embodiments, the mixture comprises a ratio of R stereoisomers to S stereoisomers of about 51:49, about 52:48, about 53:47, about 54:46, about 55:45, about 60:40, about 65:35, about 70:30, about 75:25, about 80:20, about 85:15, about 90:10, about 95:5, or about 99:1. In some embodiments, the mixture comprises a ratio of S stereoisomers to R stereoisomers of about 51:49, about 52:48, about 53:47, about 54:46, about 55:45, about 60:40, about 65:35, about 70:30, about 75:25, about 80:20, about 85:15, about 90:10, about 95:5, or about 99:1. In some embodiments, the degradation compound is a single stereoisomer of Formula (I) or Formula (I-a), e.g., a single R stereoisomer or a single S stereoisomer.

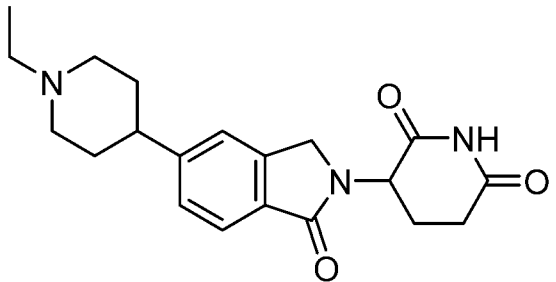
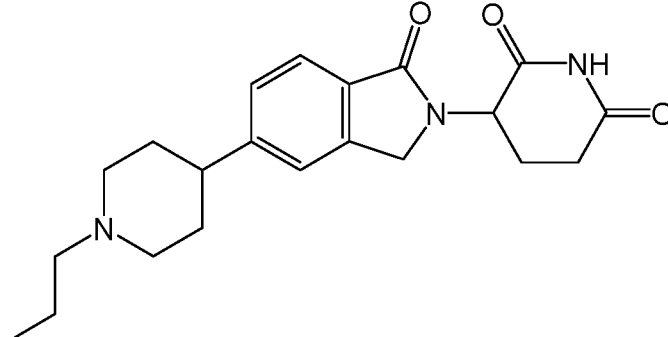
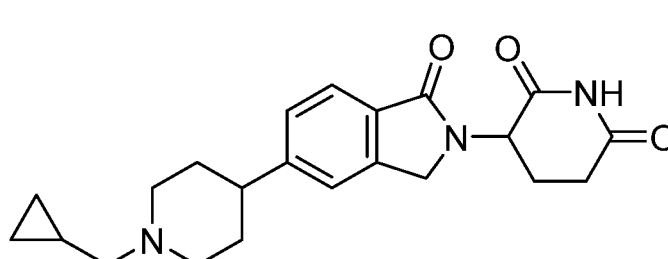
In some embodiments, the degradation compound (e.g., a compound of Formulas (I), (I-a), (III), (III-a), (III-b), (III-c), (III-d), or (III-e)) is not attached to a linker or attachment group. In some embodiments, the degradation compound (e.g., a compound of Formulas (I), (I-a), (III), (III-a), (III-b), (III-c), (III-d), or (III-e)) does not comprise another moiety, e.g., a ligand, a targeting agent, or a moiety capable of dimerization.

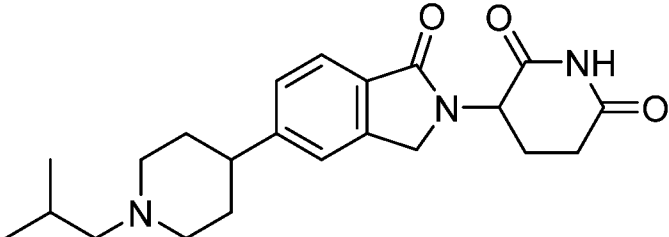
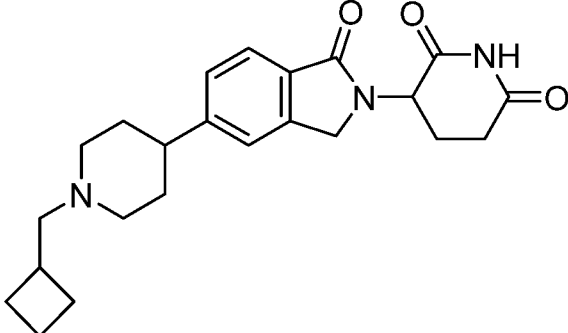
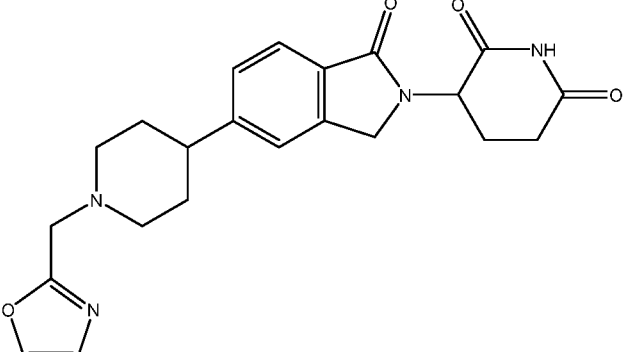
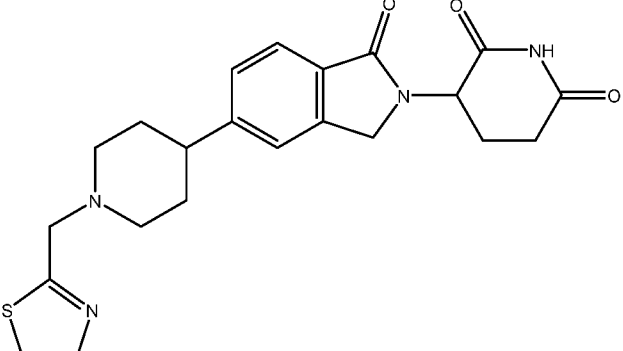
In an embodiment, the degradation compound is a compound of Formula (I) or a pharmaceutically acceptable salt thereof. In an embodiment, the degradation compound is a compound of Formula (I-a) or a pharmaceutically acceptable salt thereof. In an embodiment, the degradation compound is a compound of Formula (III) or a pharmaceutically acceptable salt thereof. In an

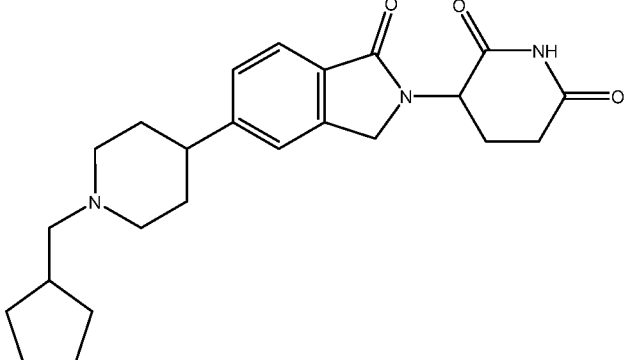
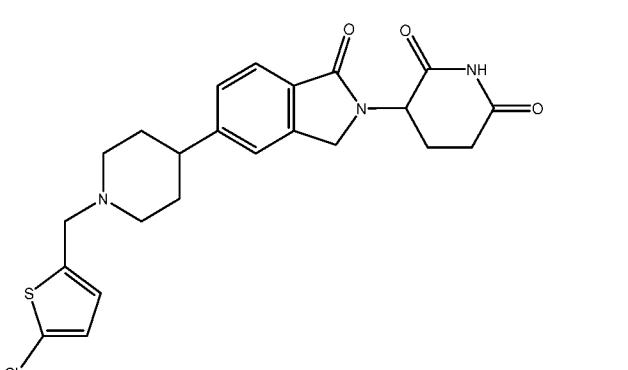
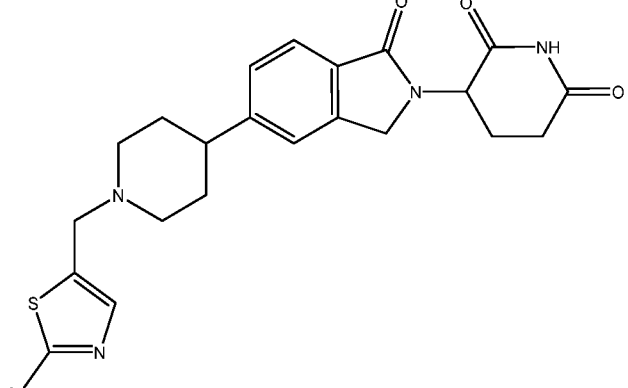
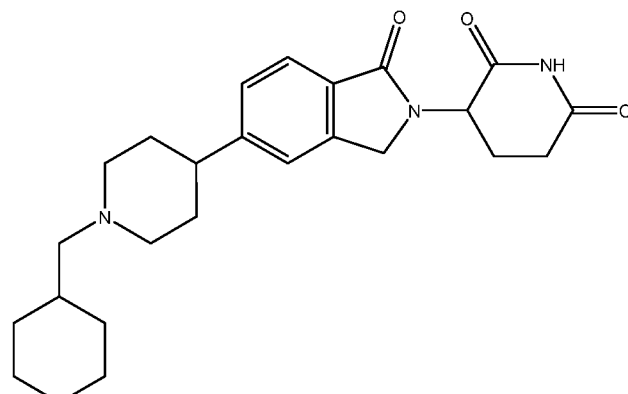
embodiment, the degradation compound is a compound of Formula (III-a) or a pharmaceutically acceptable salt thereof. In an embodiment, the degradation compound is a compound of Formula (III-b) or a pharmaceutically acceptable salt thereof. In an embodiment, the degradation compound is a compound of Formula (III-c) or a pharmaceutically acceptable salt thereof. In an embodiment, the degradation compound is a compound of Formula (III-d) or a pharmaceutically acceptable salt thereof. In an embodiment, the degradation compound is a compound of Formula (III-e) or a pharmaceutically acceptable salt thereof.

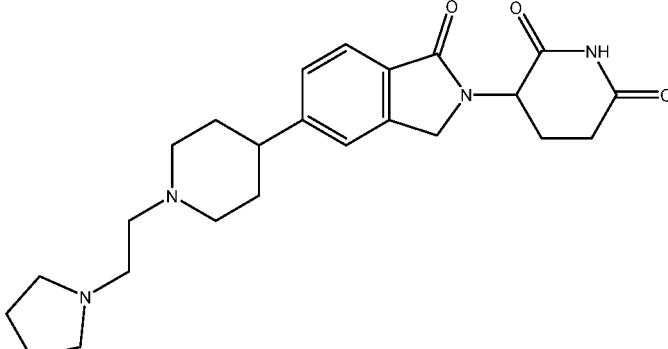
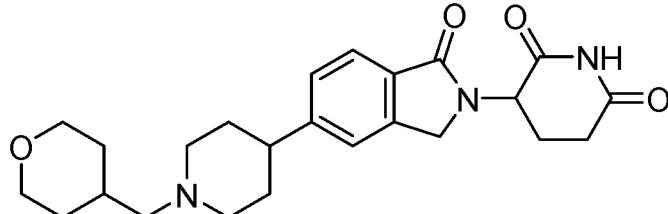
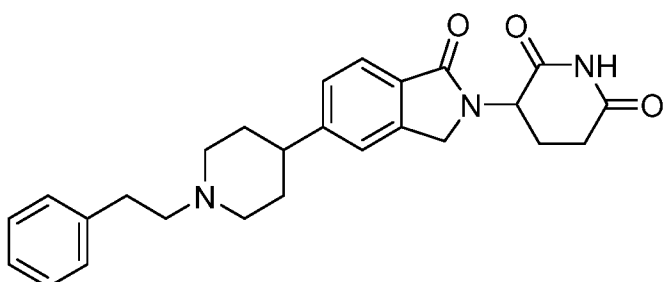
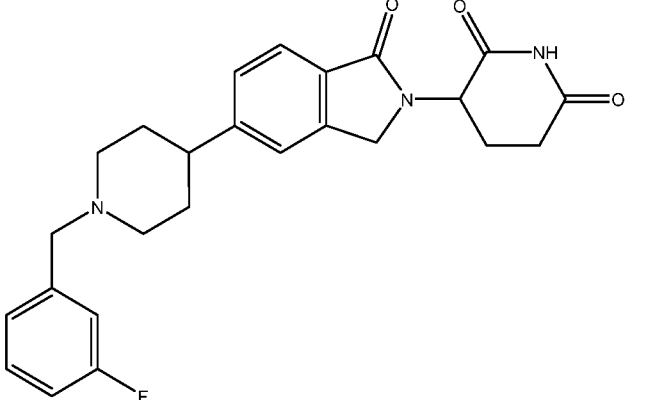
Exemplary degradation compounds of the disclosure (e.g., a compound of Formula (III), (III-a), (III-b), (III-c), (III-d), or (III-e) or a pharmaceutically acceptable salt thereof) include those in Table 29.

**Table 29. Exemplary degradation compounds**

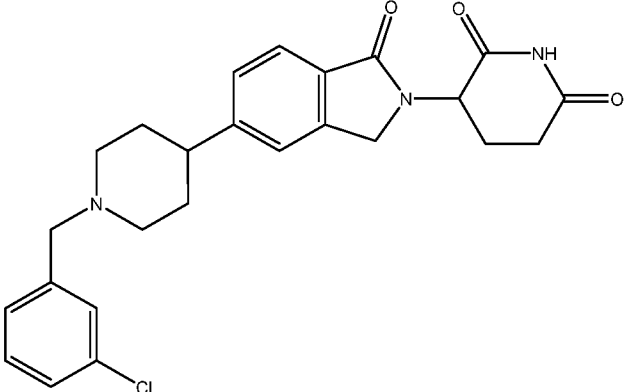
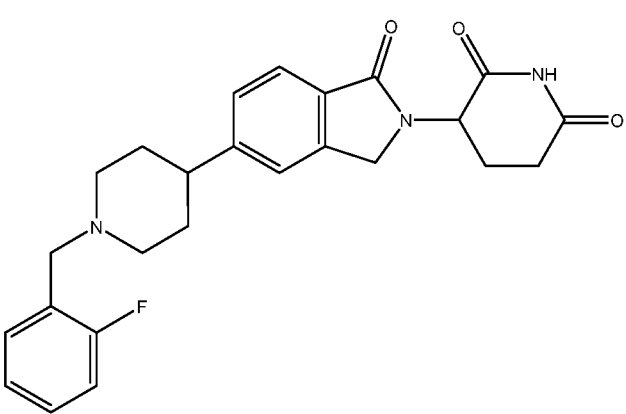
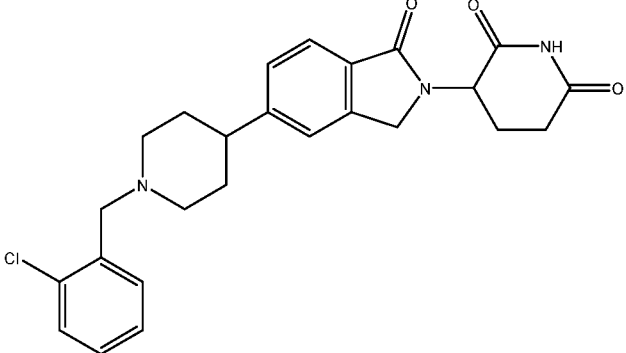
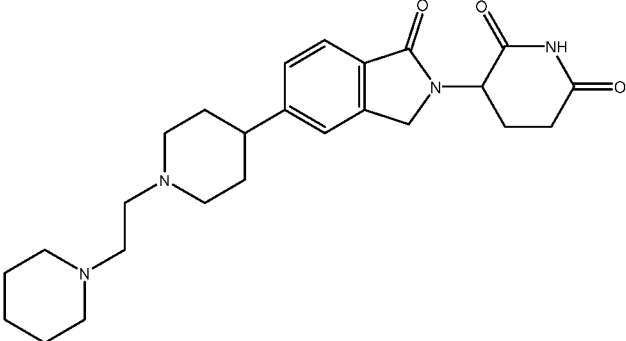
| Cmpd No.   | Structure                                                                           | Compound Name                                                                       |
|------------|-------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------|
| <b>I-1</b> |   | 3-(5-(1-ethylpiperidin-4-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione               |
| <b>I-2</b> |  | 3-(1-oxo-5-(1-propylpiperidin-4-yl)isoindolin-2-yl)piperidine-2,6-dione             |
| <b>I-3</b> |  | 3-(5-(1-(cyclopropylmethyl)piperidin-4-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione |

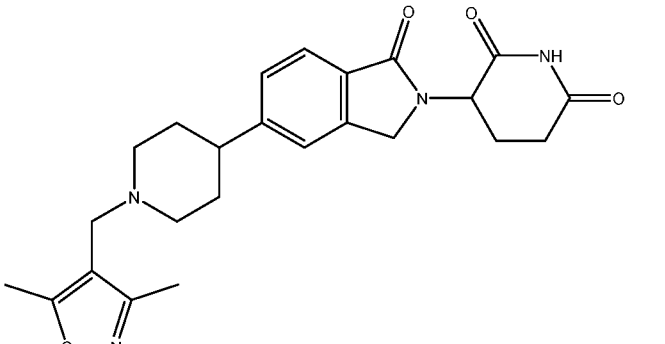
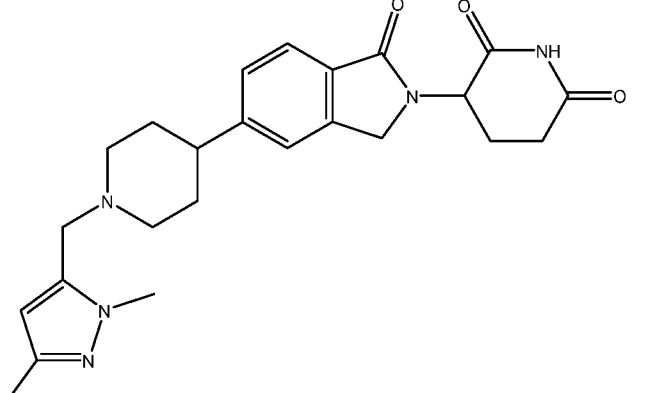
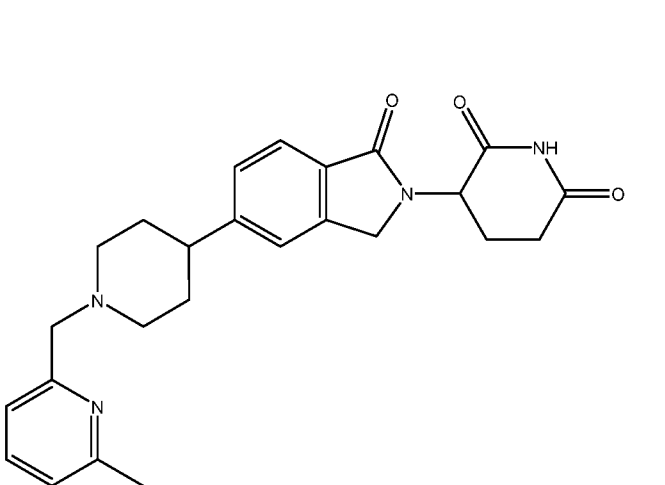
| Cmpd No.   | Structure                                                                           | Compound Name                                                                        |
|------------|-------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------|
| <b>I-4</b> |    | 3-(5-(1-isobutylpiperidin-4-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione             |
| <b>I-5</b> |    | 3-(5-(1-(cyclobutylmethyl)piperidin-4-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione   |
| <b>I-6</b> |   | 3-(5-(1-(oxazol-2-ylmethyl)piperidin-4-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione  |
| <b>I-7</b> |  | 3-(1-oxo-5-(1-(thiazol-2-ylmethyl)piperidin-4-yl)isindolin-2-yl)piperidine-2,6-dione |

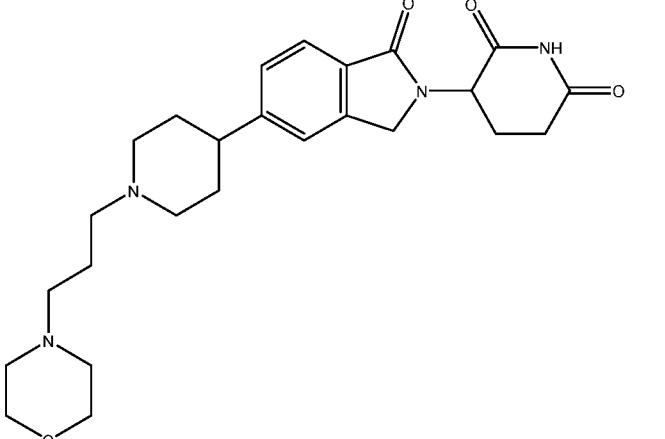
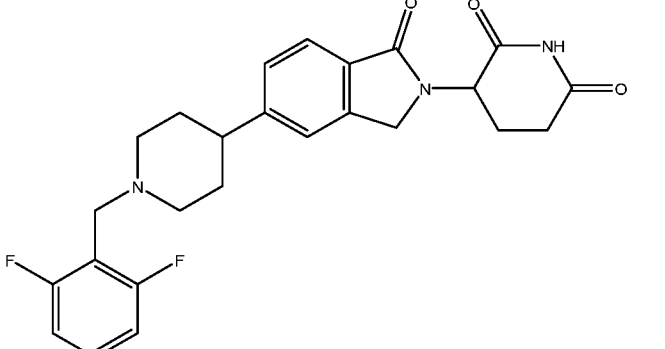
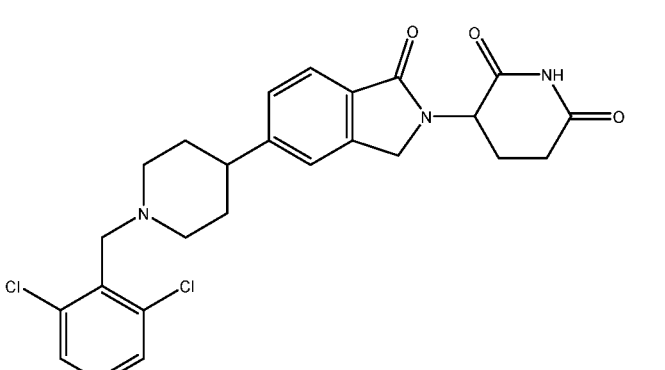
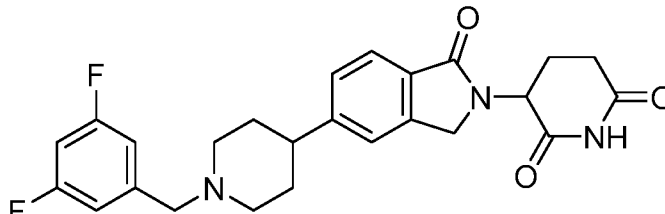
| Cmpd No.    | Structure                                                                           | Compound Name                                                                                   |
|-------------|-------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------|
| <b>I-8</b>  |    | 3-(5-(1-(cyclopentylmethyl)piperidin-4-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione             |
| <b>I-9</b>  |   | 3-(5-(1-((5-chlorothiophen-2-yl)methyl)piperidin-4-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione |
| <b>I-10</b> |  | 3-(5-(1-((2-chlorothiazol-5-yl)methyl)piperidin-4-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione  |
| <b>I-11</b> |  | 3-(5-(1-(cyclohexylmethyl)piperidin-4-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione              |

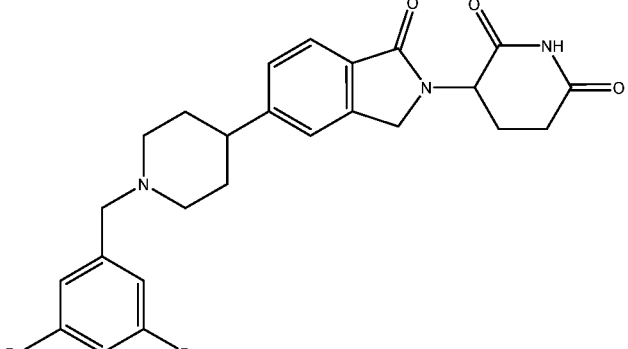
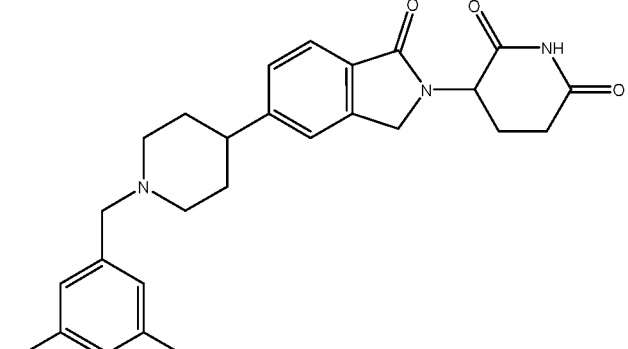
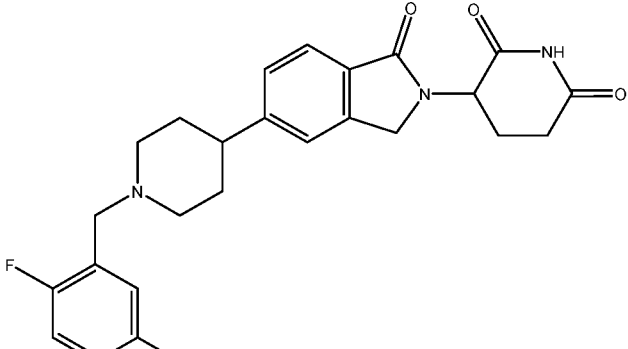
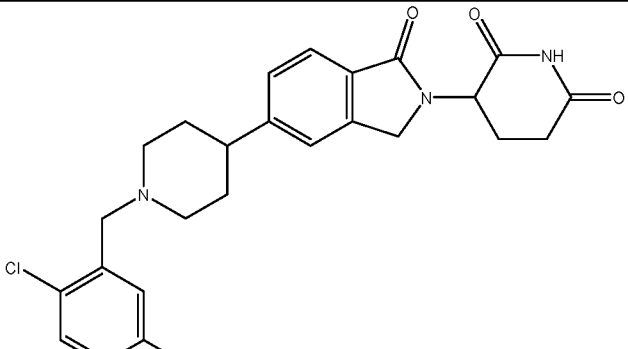
| Cmpd No.    | Structure                                                                           | Compound Name                                                                                       |
|-------------|-------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------|
| <b>I-12</b> |    | 3-(1-oxo-5-(1-(2-(pyrrolidin-1-yl)ethyl)piperidin-4-yl)isoindolin-2-yl)piperidine-2,6-dione         |
| <b>I-13</b> |    | 3-(1-oxo-5-(1-((tetrahydro-2H-pyran-4-yl)methyl)piperidin-4-yl)isoindolin-2-yl)piperidine-2,6-dione |
| <b>I-14</b> |   | 3-(1-oxo-5-(1-phenethylpiperidin-4-yl)isoindolin-2-yl)piperidine-2,6-dione                          |
| <b>I-15</b> |  | 3-(5-(1-(3-fluorobenzyl)piperidin-4-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione                    |

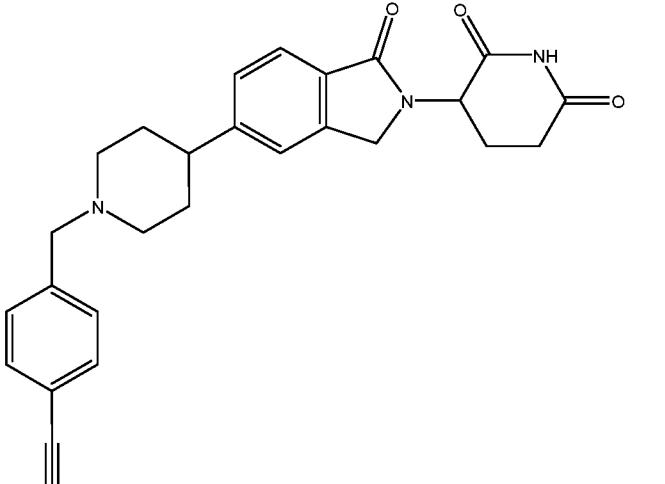
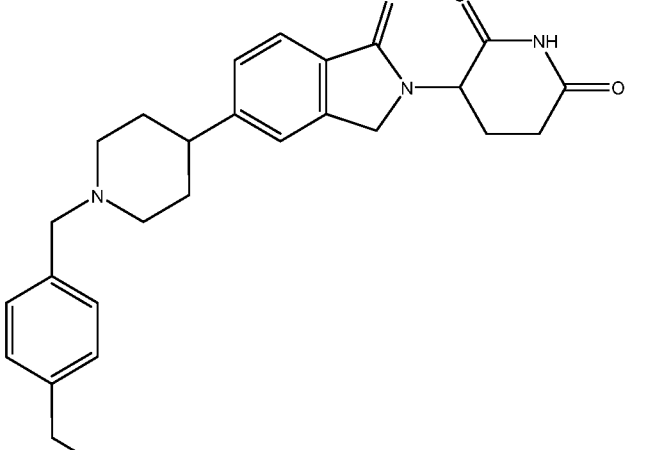
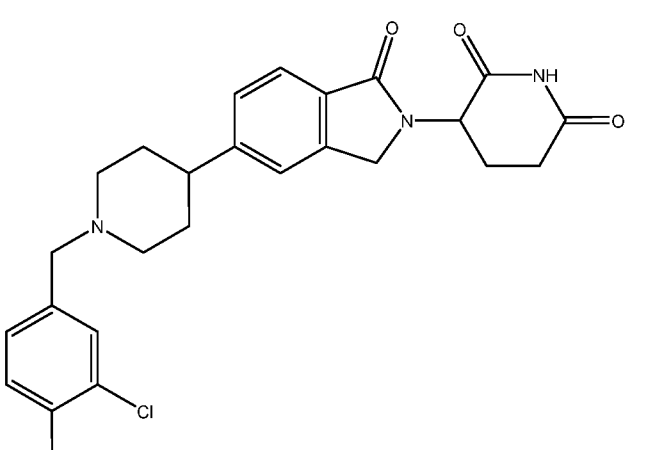


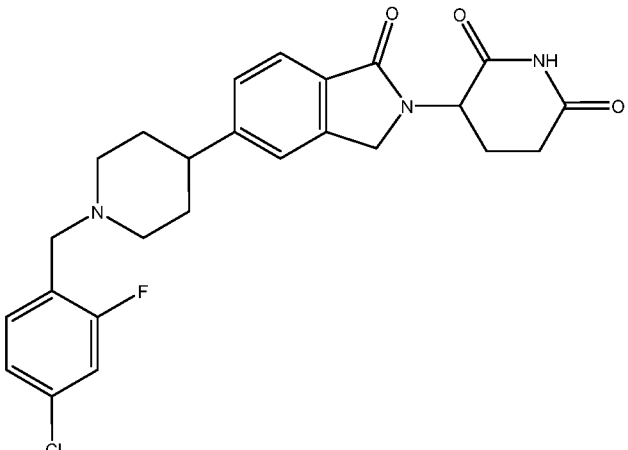
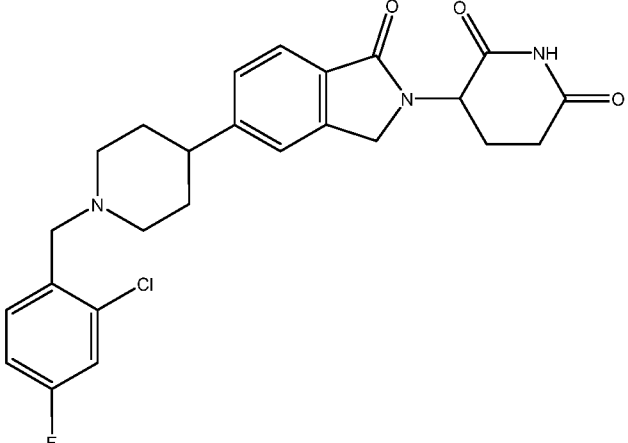
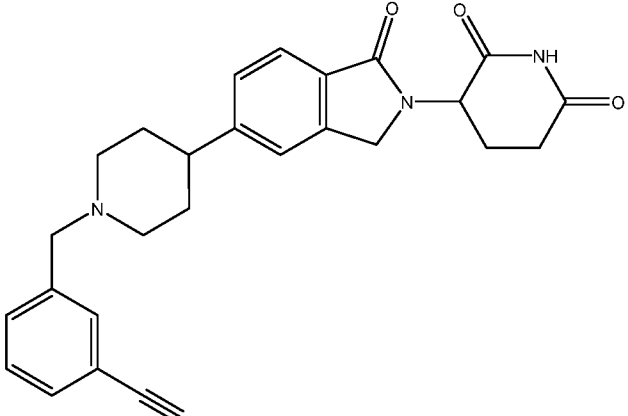
| Cmpd No.    | Structure                                                                           | Compound Name                                                                              |
|-------------|-------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------|
| <b>I-16</b> |    | 3-(5-(1-(3-chlorobenzyl)piperidin-4-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione           |
| <b>I-17</b> |   | 3-(5-(1-(2-fluorobenzyl)piperidin-4-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione           |
| <b>I-18</b> |  | 3-(5-(1-(2-chlorobenzyl)piperidin-4-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione           |
| <b>I-19</b> |  | 3-(1-oxo-5-(1-(2-(piperidin-1-yl)ethyl)piperidin-4-yl)isoindolin-2-yl)piperidine-2,6-dione |

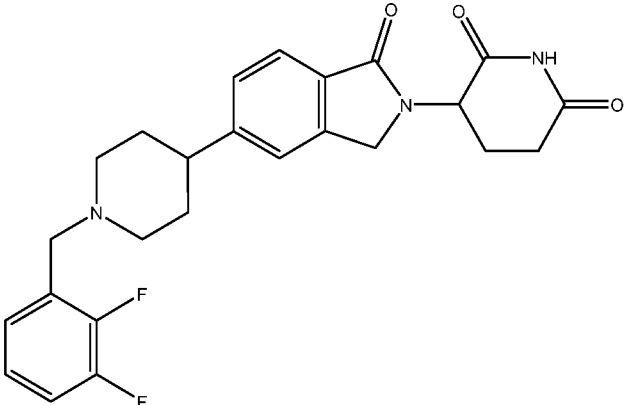
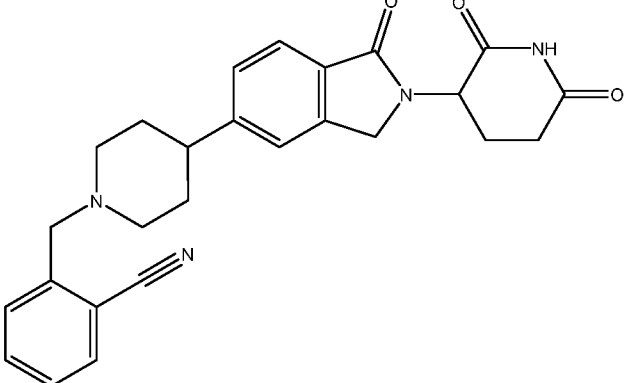
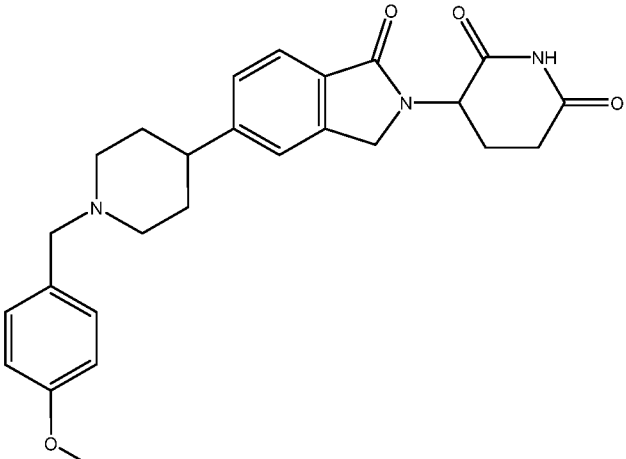
| Cmpd No.    | Structure                                                                           | Compound Name                                                                                          |
|-------------|-------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------|
| <b>I-20</b> |    | 3-(5-(1-((3,5-dimethylisoxazol-4-yl)methyl)piperidin-4-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione    |
| <b>I-21</b> |   | 3-(5-(1-((1,3-dimethyl-1H-pyrazol-5-yl)methyl)piperidin-4-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione |
| <b>I-22</b> |  | 3-(5-(1-((6-methylpyridin-2-yl)methyl)piperidin-4-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione         |

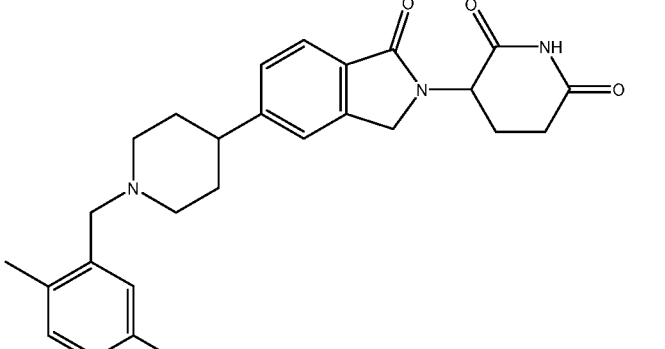
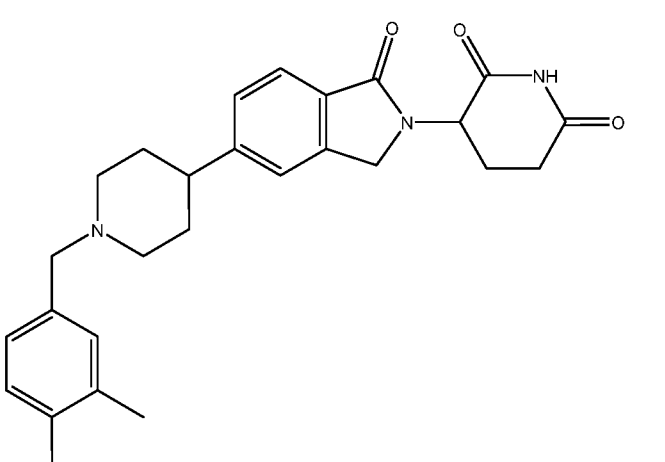
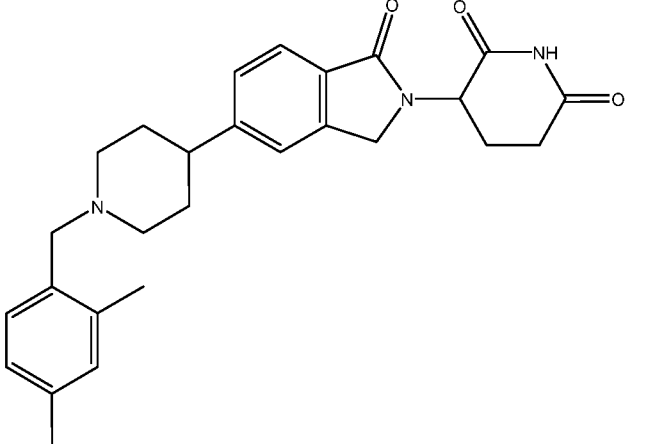
| Cmpd No.    | Structure                                                                           | Compound Name                                                                        |
|-------------|-------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------|
| <b>I-23</b> |    | 3-(5-(1-(3-morpholinopropyl)piperidin-4-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione |
| <b>I-24</b> |   | 3-(5-(1-(2,6-difluorobenzyl)piperidin-4-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione |
| <b>I-25</b> |  | 3-(5-(1-(2,6-dichlorobenzyl)piperidin-4-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione |
| <b>I-26</b> |  | 3-(5-(1-(3,5-difluorobenzyl)piperidin-4-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione |

| Cmpd No.    | Structure                                                                           | Compound Name                                                                             |
|-------------|-------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------|
| <b>I-27</b> |    | 3-(5-(1-(3,5-dibromobenzyl)piperidin-4-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione       |
| <b>I-28</b> |   | 3-(5-(1-(3-chloro-5-fluorobenzyl)piperidin-4-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione |
| <b>I-29</b> |  | 3-(5-(1-(2,5-difluorobenzyl)piperidin-4-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione      |
| <b>I-30</b> |  | 3-(5-(1-(2,5-dichlorobenzyl)piperidin-4-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione      |

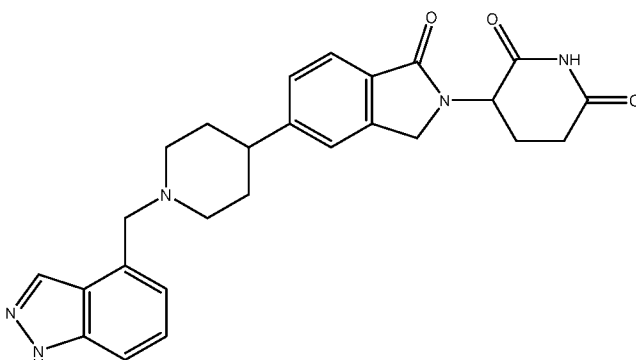
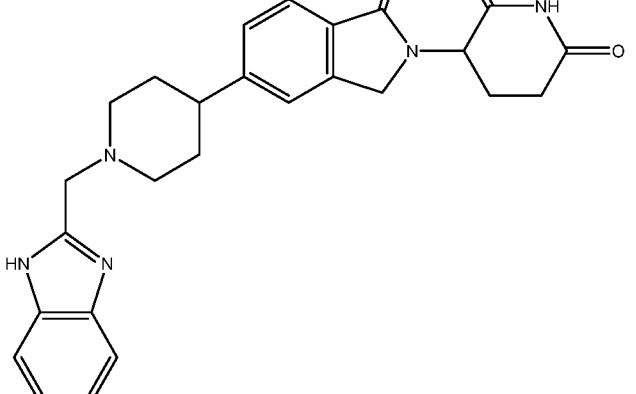
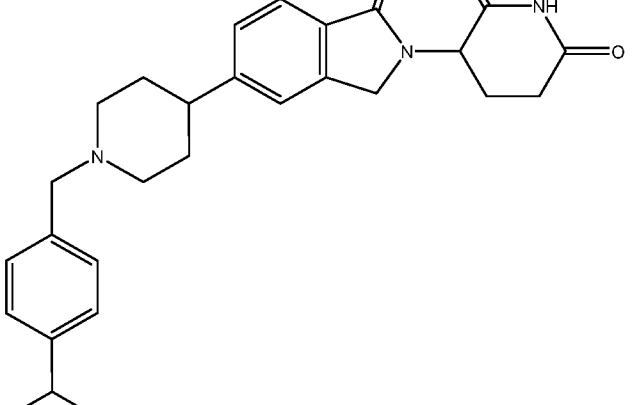
| Cmpd No.    | Structure                                                                           | Compound Name                                                                                                                                                                       |
|-------------|-------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>I-31</b> |    | 4-((4-(2-(2,6-dioxopiperidin-3-yl)-1-oxoisindolin-5-yl)piperidin-1-yl)methyl)benzonitrile<br>(or 3-(5-(1-(4-nitrilebenzyl)piperidin-4-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione) |
| <b>I-32</b> |   | 3-(5-(1-(4-(hydroxymethyl)benzyl)piperidin-4-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione                                                                                           |
| <b>I-33</b> |  | 3-(5-(1-(3,4-dichlorobenzyl)piperidin-4-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione                                                                                                |

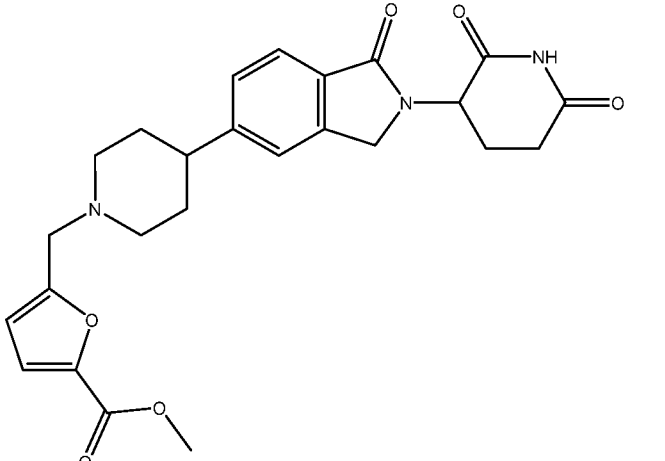
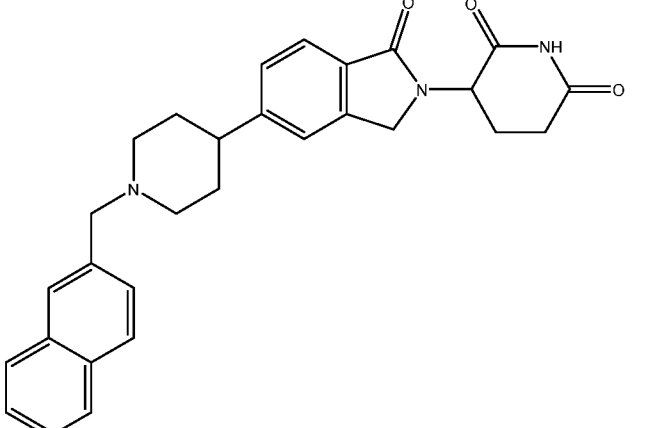
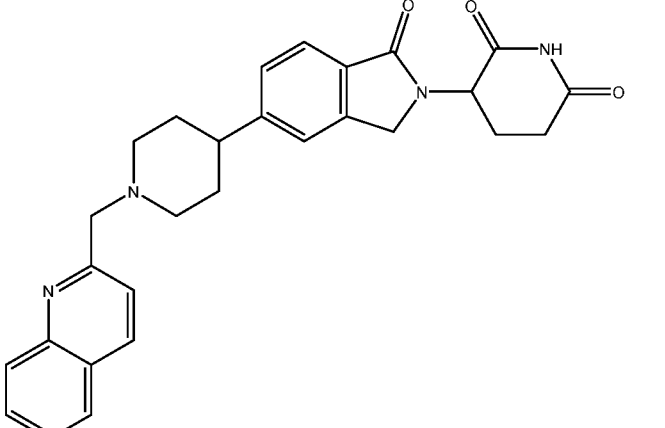
| Cmpd No.    | Structure                                                                           | Compound Name                                                                             |
|-------------|-------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------|
| <b>I-34</b> |    | 3-(5-(1-(4-chloro-2-fluorobenzyl)piperidin-4-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione |
| <b>I-35</b> |   | 3-(5-(1-(2-chloro-4-fluorobenzyl)piperidin-4-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione |
| <b>I-36</b> |  | 3-((4-(2-(2,6-dioxopiperidin-3-yl)-1-oxoisindolin-5-yl)piperidin-1-yl)methyl)benzonitrile |

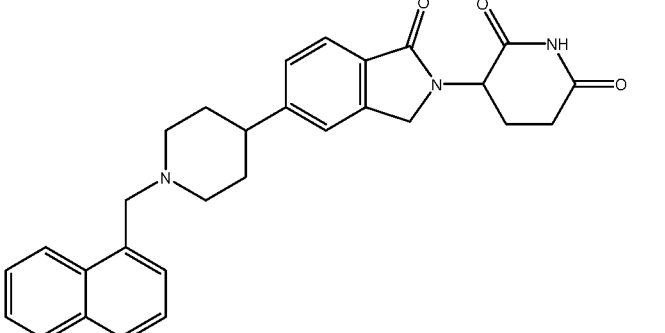
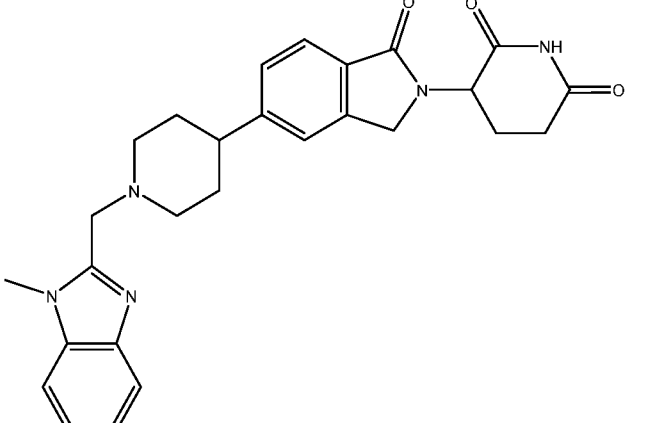
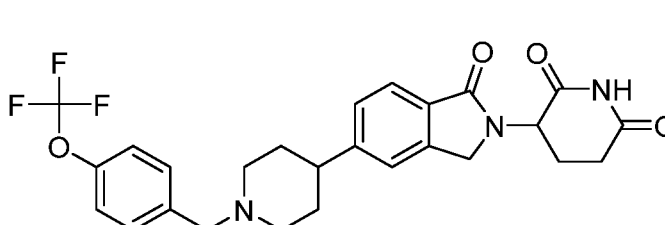
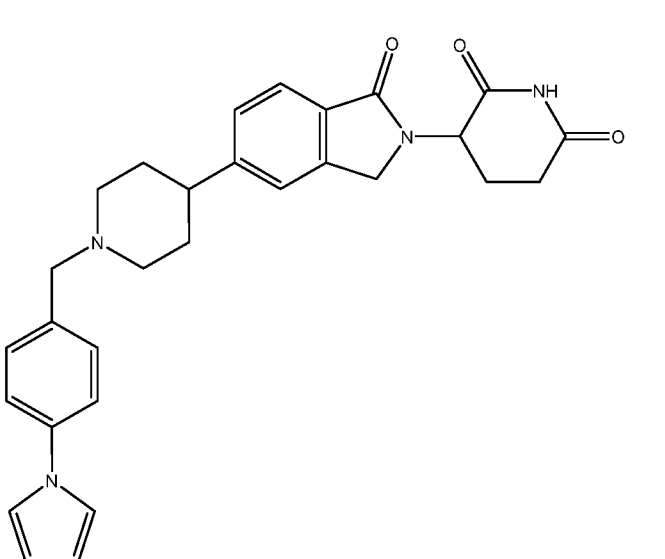
| Cmpd No.    | Structure                                                                           | Compound Name                                                                             |
|-------------|-------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------|
| <b>I-37</b> |    | 3-(5-(1-(2,3-difluorobenzyl)piperidin-4-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione      |
| <b>I-38</b> |   | 2-((4-(2-(2,6-dioxopiperidin-3-yl)-1-oxoisindolin-5-yl)piperidin-1-yl)methyl)benzonitrile |
| <b>I-39</b> |  | 3-(5-(1-(4-methoxybenzyl)piperidin-4-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione         |

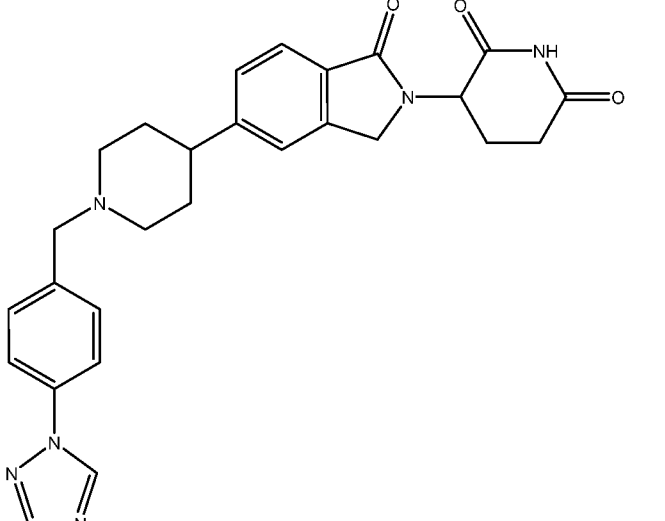
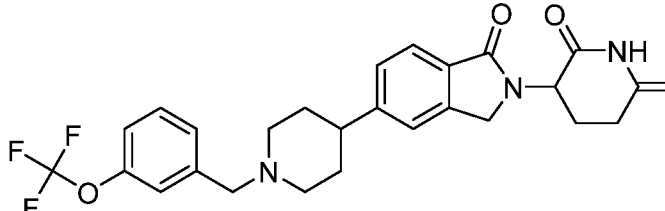
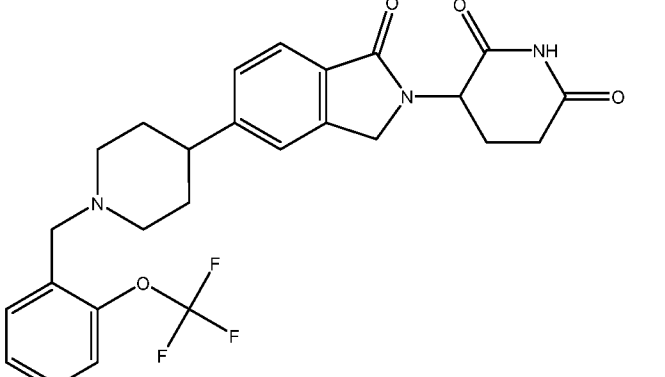
| Cmpd No.    | Structure                                                                           | Compound Name                                                                        |
|-------------|-------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------|
| <b>I-40</b> |    | 3-(5-(1-(2,5-dimethylbenzyl)piperidin-4-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione |
| <b>I-41</b> |   | 3-(5-(1-(3,4-dimethylbenzyl)piperidin-4-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione |
| <b>I-42</b> |  | 3-(5-(1-(2,4-dimethylbenzyl)piperidin-4-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione |

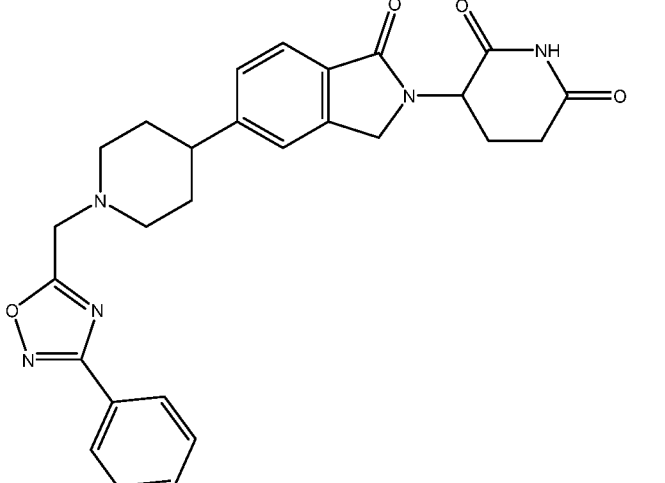
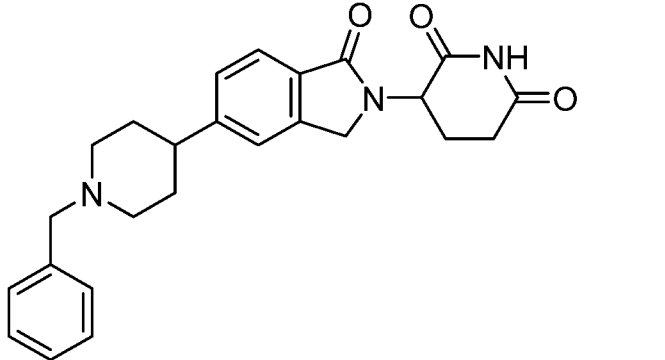
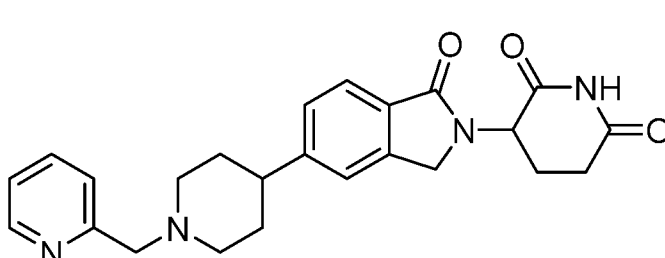
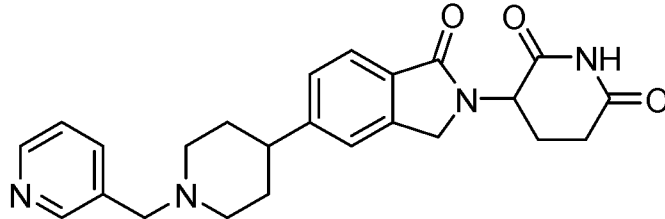


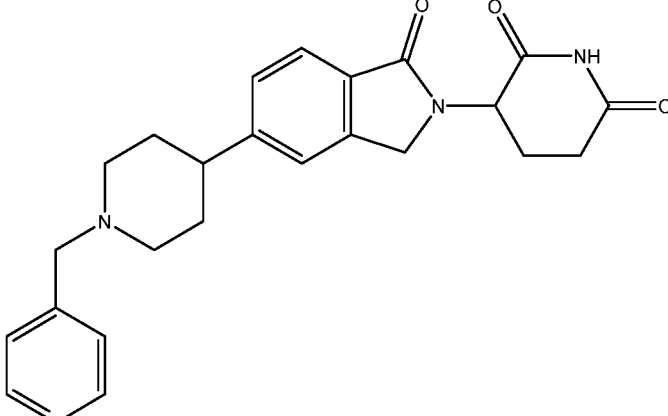
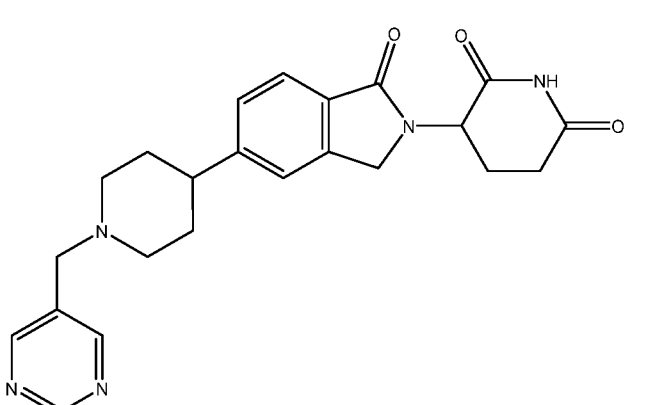
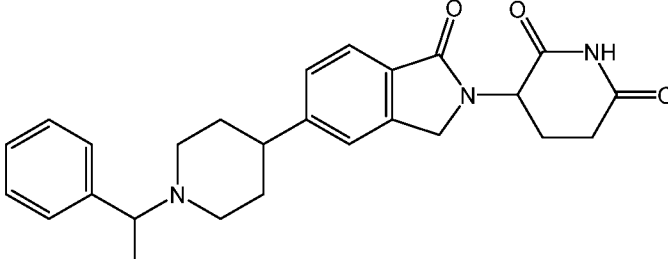
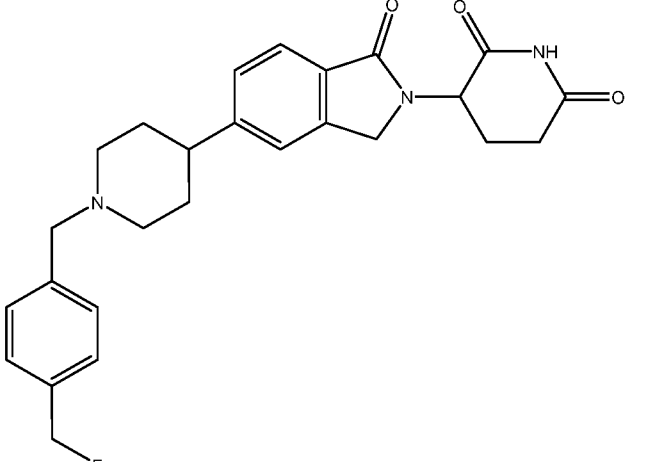
| Cmpd No.    | Structure                                                                           | Compound Name                                                                                      |
|-------------|-------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------|
| <b>I-43</b> |    | 3-(5-(1-((1H-indazol-4-yl)methyl)piperidin-4-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione          |
| <b>I-44</b> |   | 3-(5-(1-((1H-benzo[d]imidazol-2-yl)methyl)piperidin-4-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione |
| <b>I-45</b> |  | 3-(5-(1-(4-isopropylbenzyl)piperidin-4-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione                |

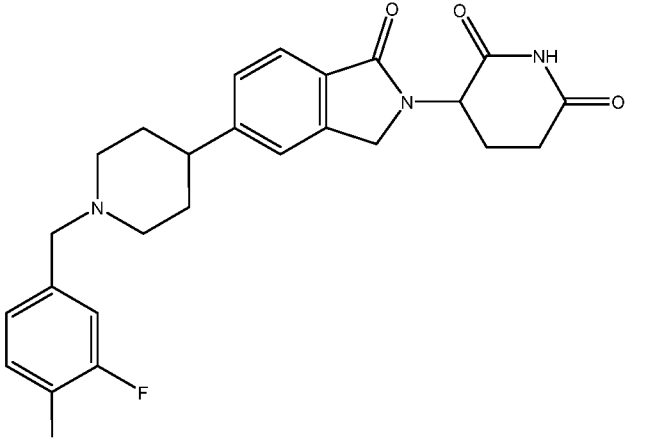
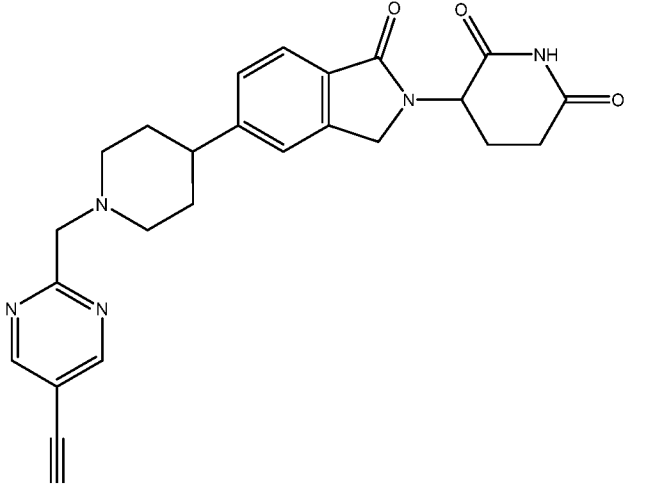
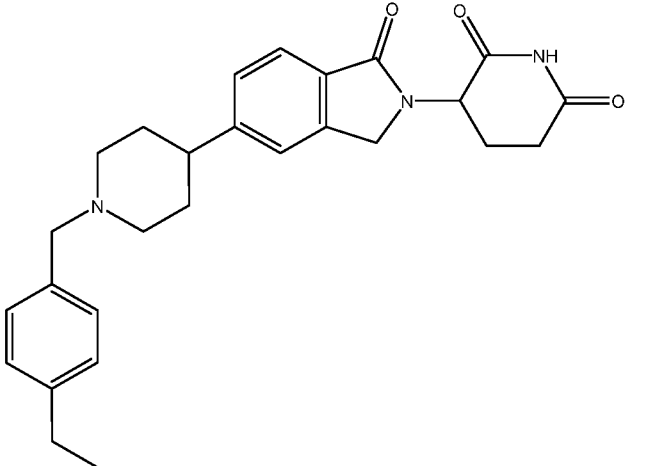
| Cmpd No.    | Structure                                                                           | Compound Name                                                                                            |
|-------------|-------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------|
| <b>I-46</b> |    | methyl 5-(((4-(2-(2,6-dioxopiperidin-3-yl)-1-oxoisindolin-5-yl)piperidin-1-yl)methyl)furan-2-carboxylate |
| <b>I-47</b> |   | 3-(5-(1-(naphthalen-2-ylmethyl)piperidin-4-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione                  |
| <b>I-48</b> |  | 3-(1-oxo-5-(1-(quinolin-2-ylmethyl)piperidin-4-yl)isindolin-2-yl)piperidine-2,6-dione                    |

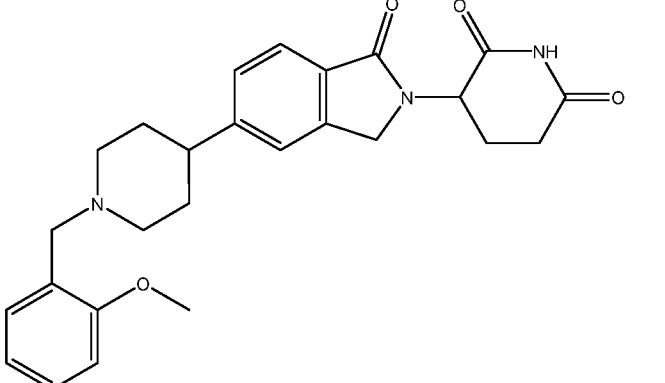
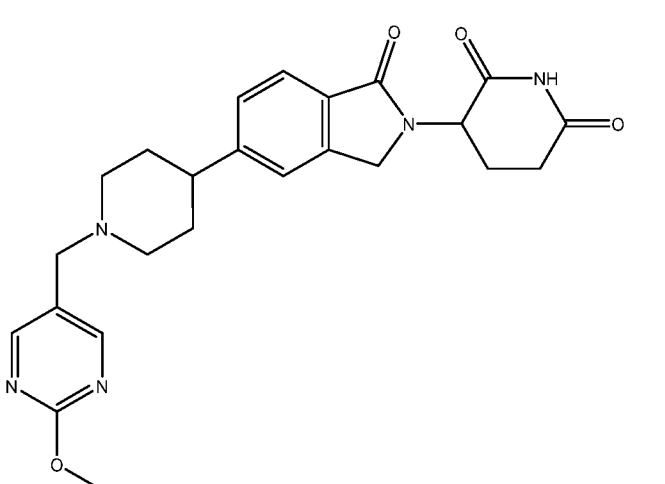
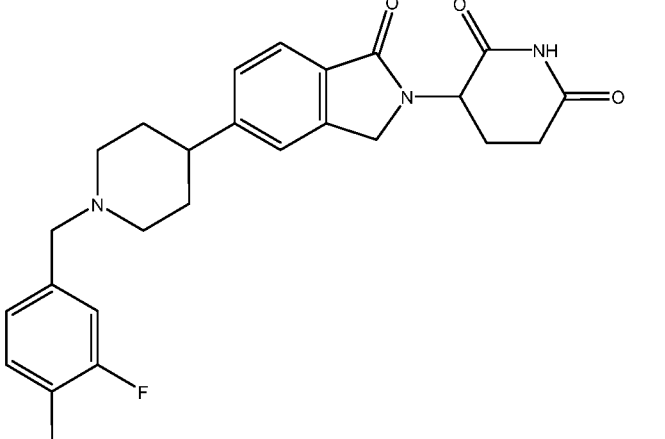
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|-------------|-------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------|
| <b>I-49</b> |    | 3-(5-(1-(naphthalen-1-ylmethyl)piperidin-4-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione                     |
| <b>I-50</b> |   | 3-(5-(1-((1-methyl-1H-benzo[d]imidazol-2-yl)methyl)piperidin-4-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione |
| <b>I-51</b> |  | 3-(1-oxo-5-(1-(4-(trifluoromethoxy)benzyl)piperidin-4-yl)isindolin-2-yl)piperidine-2,6-dione                |
| <b>I-52</b> |  | 3-(5-(1-(4-(1H-pyrrol-1-yl)benzyl)piperidin-4-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione                  |

| Cmpd No.    | Structure                                                                           | Compound Name                                                                                     |
|-------------|-------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------|
| <b>I-53</b> |    | 3-(5-(1-(4-(1H-1,2,4-triazol-1-yl)benzyl)piperidin-4-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione |
| <b>I-54</b> |   | 3-(1-oxo-5-(1-(3-(trifluoromethoxy)benzyl)piperidin-4-yl)isindolin-2-yl)piperidine-2,6-dione      |
| <b>I-55</b> |  | 3-(1-oxo-5-(1-(2-(trifluoromethoxy)benzyl)piperidin-4-yl)isindolin-2-yl)piperidine-2,6-dione      |

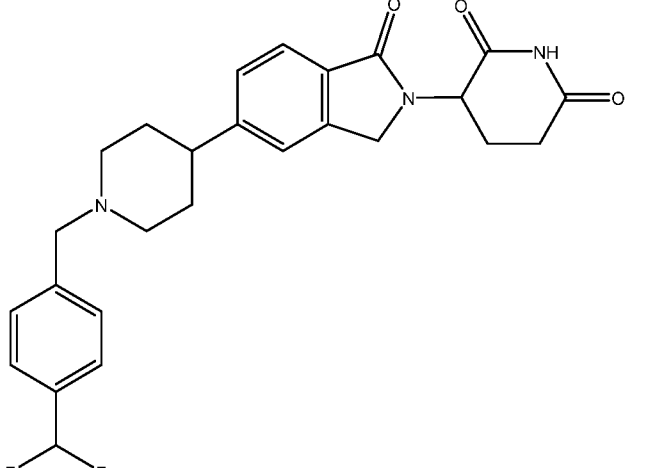
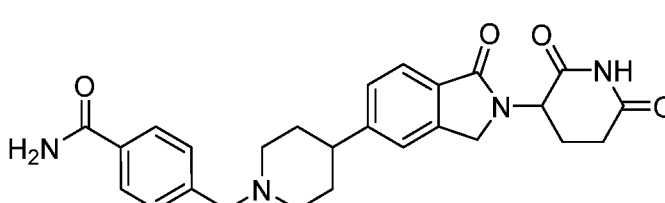
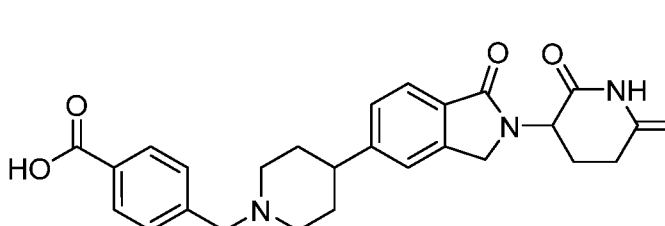
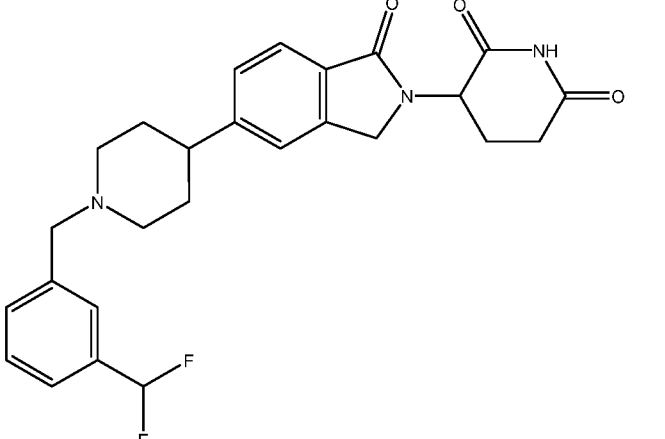
| Cmpd No.    | Structure                                                                           | Compound Name                                                                                            |
|-------------|-------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------|
| <b>I-56</b> |    | 3-(1-oxo-5-(1-((3-phenyl-1,2,4-oxadiazol-5-yl)methyl)piperidin-4-yl)isoindolin-2-yl)piperidine-2,6-dione |
| <b>I-57</b> |   | 3-(5-(1-benzylpiperidin-4-yl)-1-oxoisoindolin-2-yl)piperidine-2,6-dione                                  |
| <b>I-58</b> |  | 3-(1-oxo-5-(1-(pyridin-2-ylmethyl)piperidin-4-yl)isoindolin-2-yl)piperidine-2,6-dione                    |
| <b>I-59</b> |  | 3-(1-oxo-5-(1-(pyridin-3-ylmethyl)piperidin-4-yl)isoindolin-2-yl)piperidine-2,6-dione                    |

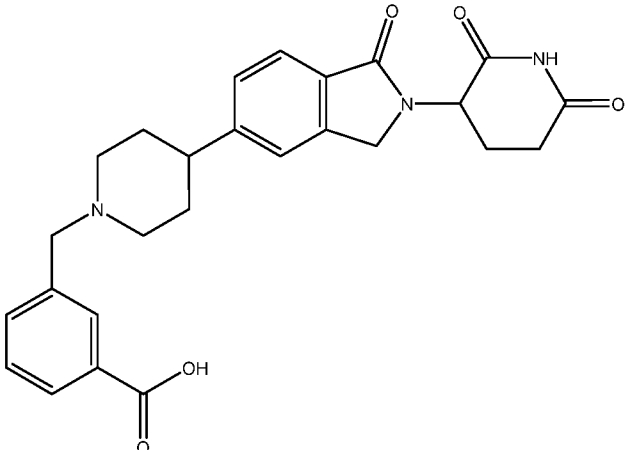
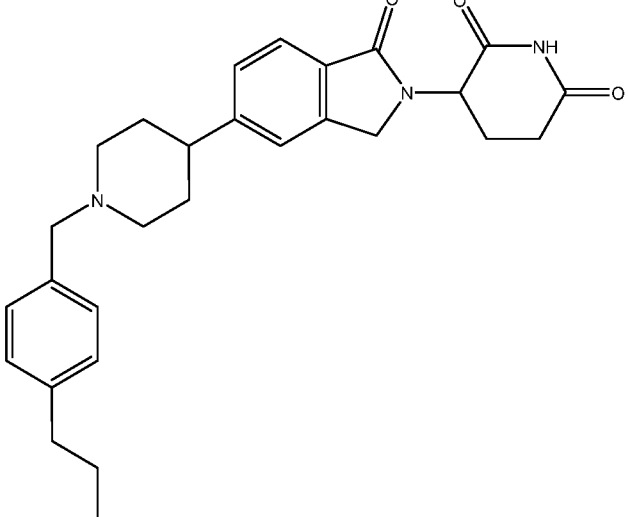
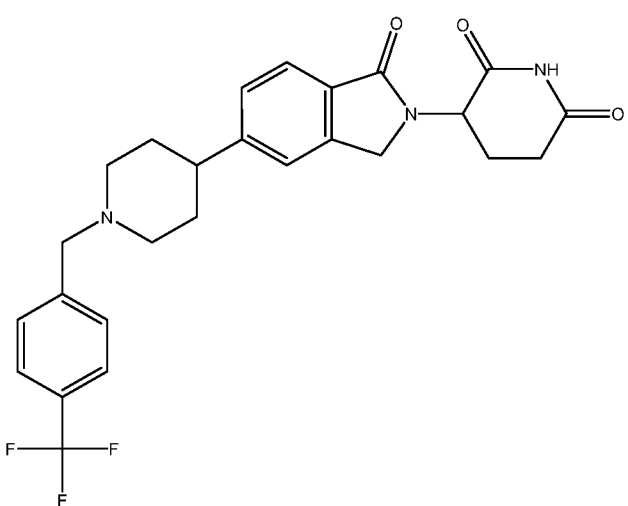
| Cmpd No.    | Structure                                                                           | Compound Name                                                                             |
|-------------|-------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------|
| <b>I-60</b> |    | 3-(1-oxo-5-(1-(pyridin-4-ylmethyl)piperidin-4-yl)isoindolin-2-yl)piperidine-2,6-dione     |
| <b>I-61</b> |   | 3-(1-oxo-5-(1-(pyrimidin-5-ylmethyl)piperidin-4-yl)isoindolin-2-yl)piperidine-2,6-dione   |
| <b>I-62</b> |  | 3-(1-oxo-5-(1-(1-phenylethyl)piperidin-4-yl)isoindolin-2-yl)piperidine-2,6-dione          |
| <b>I-63</b> |  | 3-(5-(1-(4-(fluoromethyl)benzyl)piperidin-4-yl)-1-oxoisoindolin-2-yl)piperidine-2,6-dione |

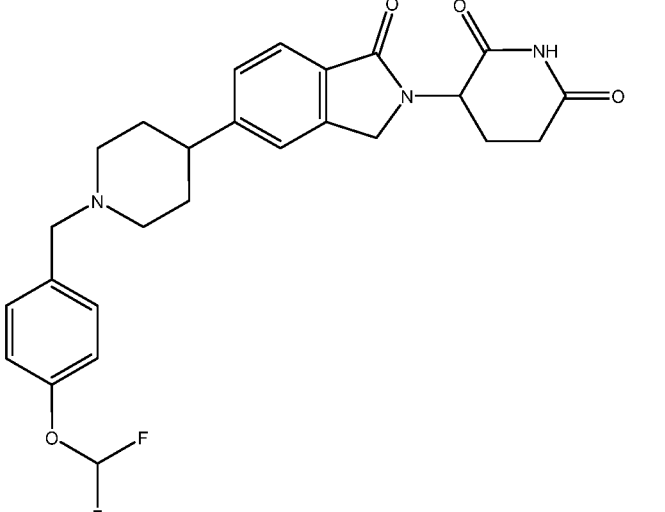
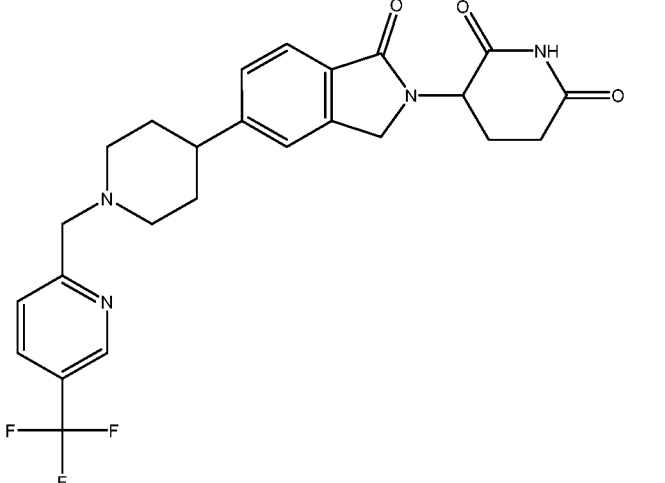
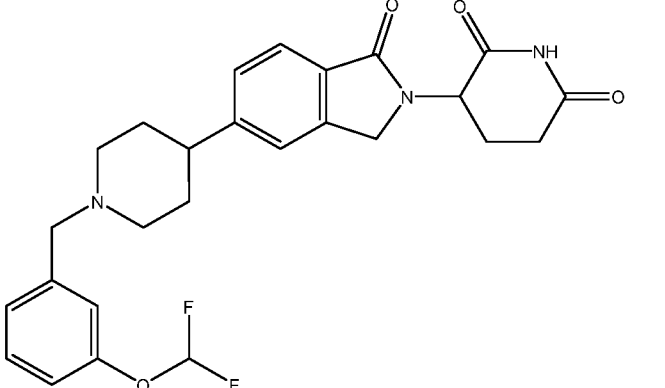
| Cmpd No.    | Structure                                                                           | Compound Name                                                                                          |
|-------------|-------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------|
| <b>I-64</b> |    | 3-(5-(1-(3,4-difluorobenzyl)piperidin-4-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione                   |
| <b>I-65</b> |   | 2-((4-(2-(2,6-dioxopiperidin-3-yl)-1-oxoisindolin-5-yl)piperidin-1-yl)methyl)pyrimidine-5-carbonitrile |
| <b>I-66</b> |  | 3-(5-(1-(4-ethylbenzyl)piperidin-4-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione                        |

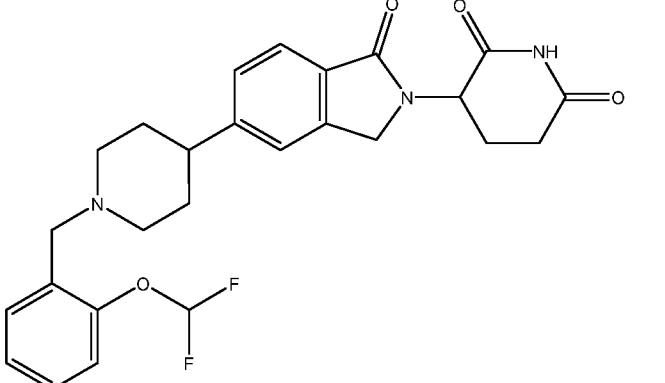
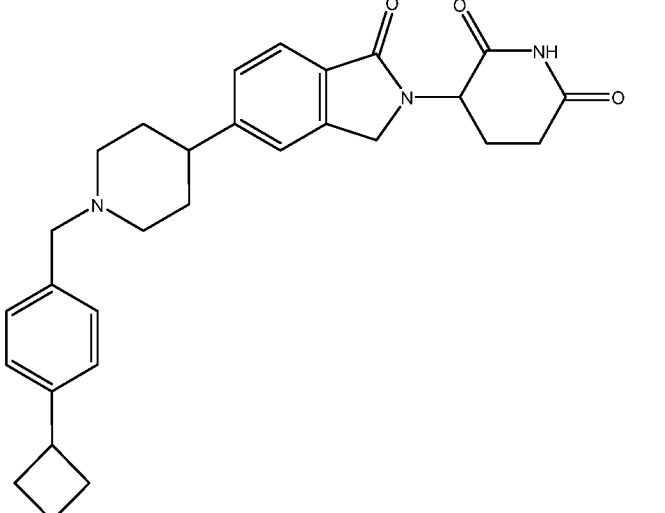
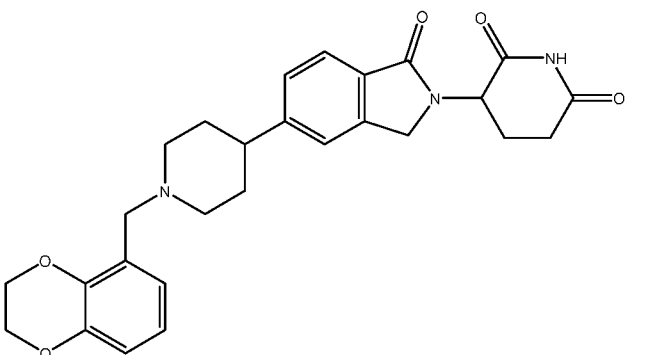
| Cmpd No.    | Structure                                                                           | Compound Name                                                                                     |
|-------------|-------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------|
| <b>I-67</b> |    | 3-(5-(1-(2-methoxybenzyl)piperidin-4-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione                 |
| <b>I-68</b> |   | 3-(5-(1-((2-methoxypyrimidin-5-yl)methyl)piperidin-4-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione |
| <b>I-69</b> |  | 3-(5-(1-(3-fluoro-4-methylbenzyl)piperidin-4-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione         |

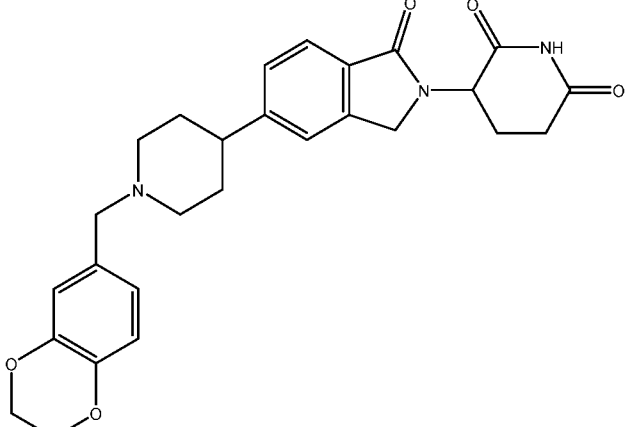
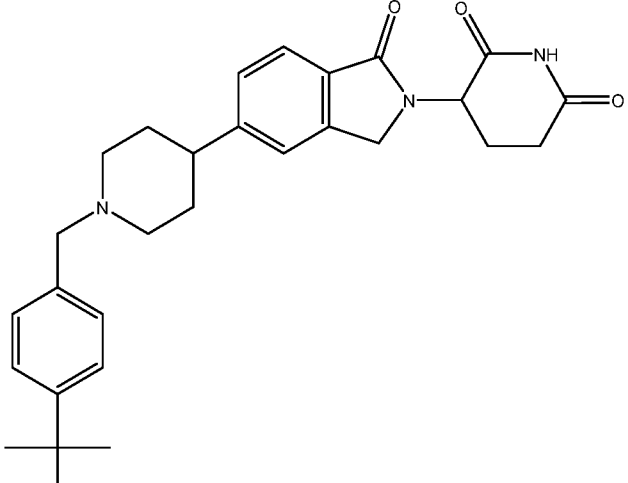
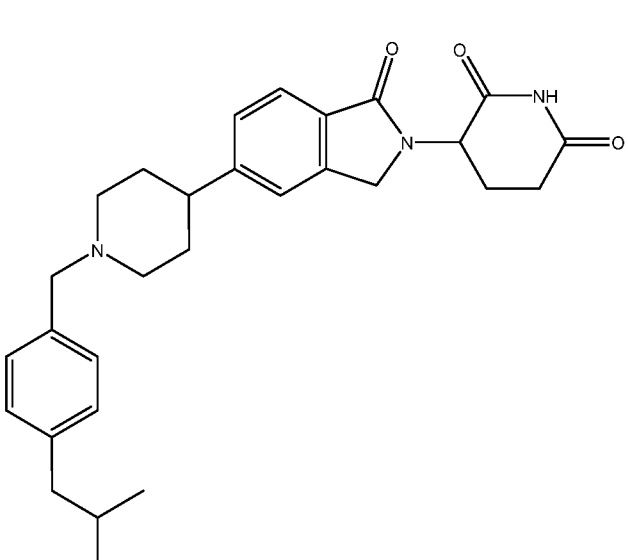


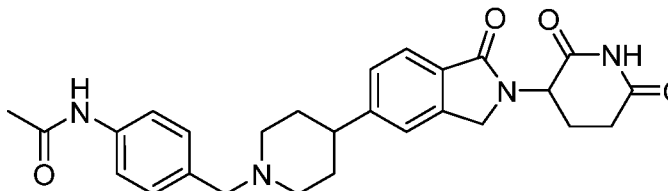
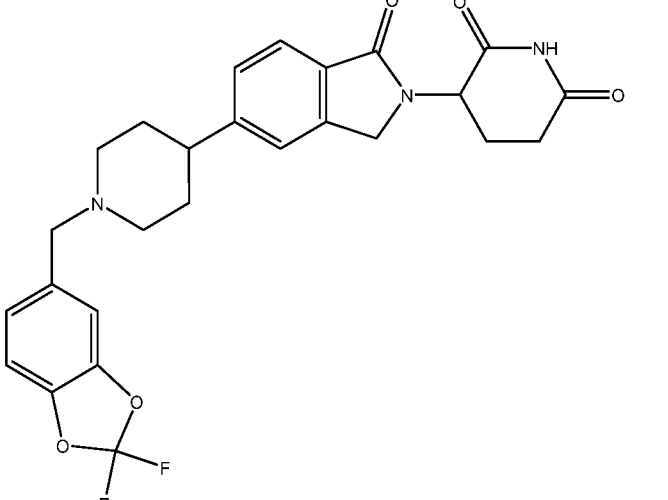
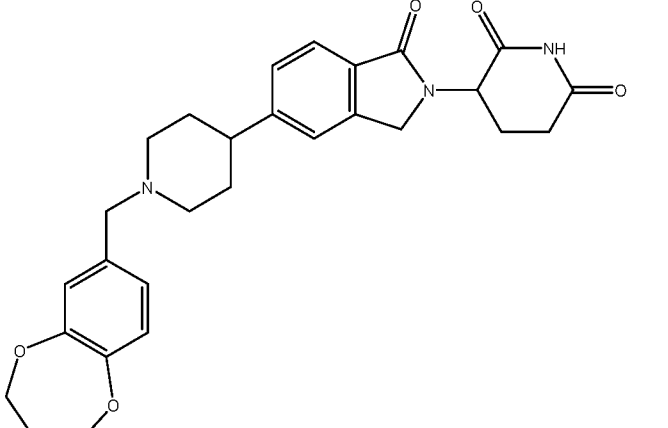
| Cmpd No.    | Structure                                                                           | Compound Name                                                                              |
|-------------|-------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------|
| <b>I-70</b> |    | 3-(5-(1-(4-(difluoromethyl)benzyl)piperidin-4-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione |
| <b>I-71</b> |   | 4-((4-(2-(2,6-dioxopiperidin-3-yl)-1-oxoisindolin-5-yl)piperidin-1-yl)methyl)benzamide     |
| <b>I-72</b> |  | 4-((4-(2-(2,6-dioxopiperidin-3-yl)-1-oxoisindolin-5-yl)piperidin-1-yl)methyl)benzoic acid  |
| <b>I-73</b> |  | 3-(5-(1-(3-(difluoromethyl)benzyl)piperidin-4-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione |

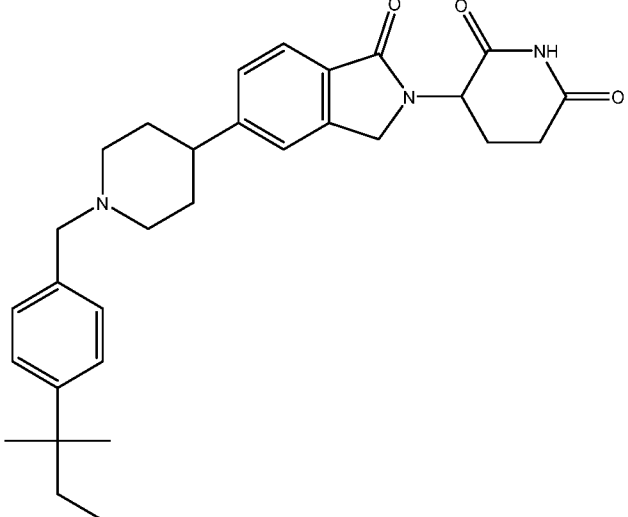
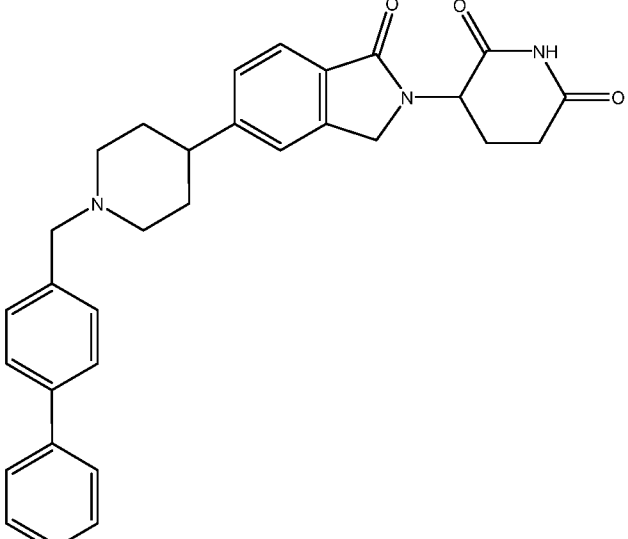
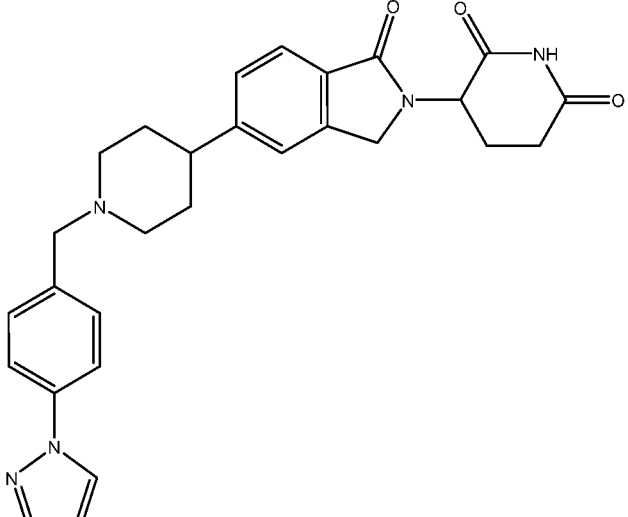
| Cmpd No.    | Structure                                                                           | Compound Name                                                                               |
|-------------|-------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------|
| <b>I-74</b> |    | 3-((4-(2-(2,6-dioxopiperidin-3-yl)-1-oxoisindolin-5-yl)piperidin-1-yl)methyl)benzoic acid   |
| <b>I-75</b> |   | 3-(1-oxo-5-(1-(4-propylbenzyl)piperidin-4-yl)isindolin-2-yl)piperidine-2,6-dione            |
| <b>I-76</b> |  | 3-(1-oxo-5-(1-(4-(trifluoromethyl)benzyl)piperidin-4-yl)isindolin-2-yl)piperidine-2,6-dione |

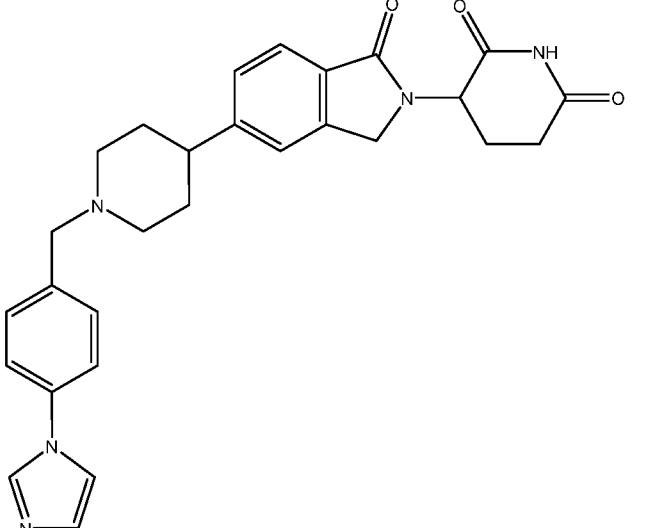
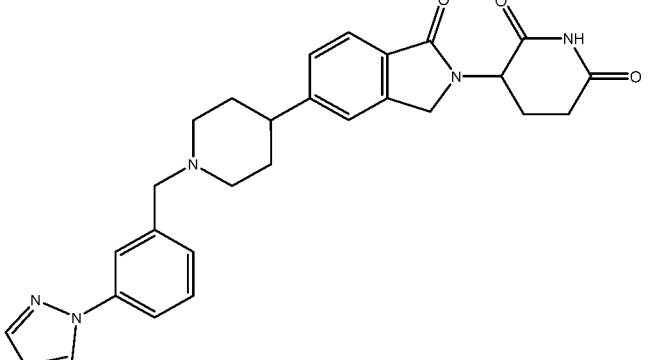
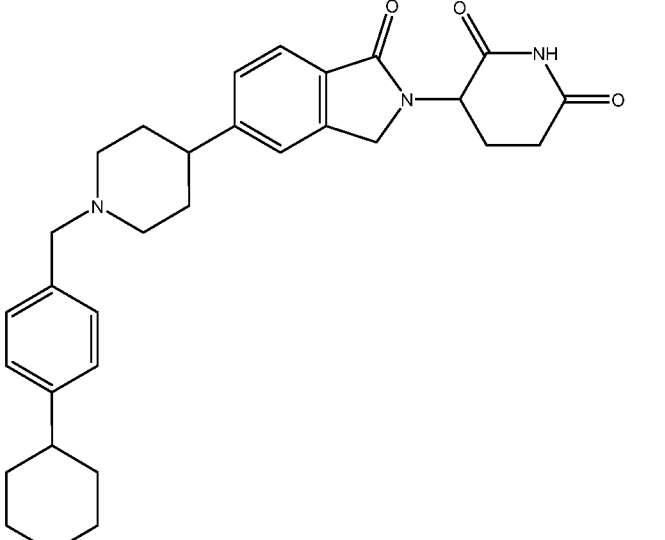
| Cmpd No.    | Structure                                                                           | Compound Name                                                                                             |
|-------------|-------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------|
| <b>I-77</b> |    | 3-(5-(1-(4-(difluoromethoxy)benzyl)piperidin-4-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione               |
| <b>I-78</b> |   | 3-(1-oxo-5-(1-((5-(trifluoromethyl)pyridin-2-yl)methyl)piperidin-4-yl)isindolin-2-yl)piperidine-2,6-dione |
| <b>I-79</b> |  | 3-(5-(1-(3-(difluoromethoxy)benzyl)piperidin-4-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione               |

| Cmpd No.    | Structure                                                                           | Compound Name                                                                                                 |
|-------------|-------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------|
| <b>I-80</b> |    | 3-(5-(1-(2-(difluoromethoxy)benzyl)piperidin-4-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione                   |
| <b>I-81</b> |   | 3-(5-(1-(4-cyclobutylbenzyl)piperidin-4-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione                          |
| <b>I-82</b> |  | 3-(5-(1-((2,3-dihydrobenzo[b][1,4]dioxin-5-yl)methyl)piperidin-4-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione |

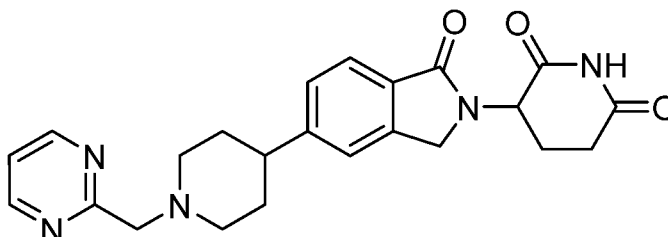
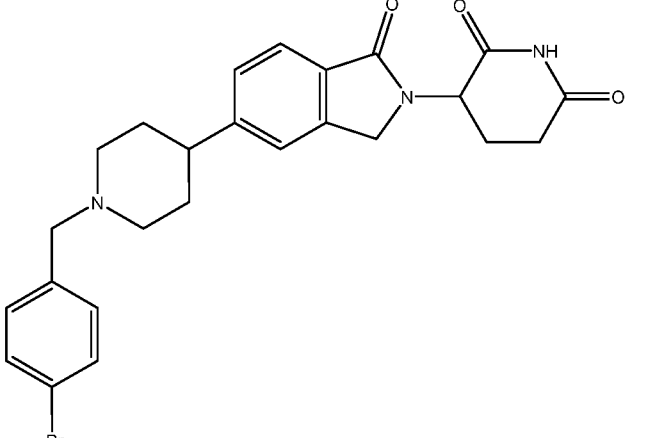
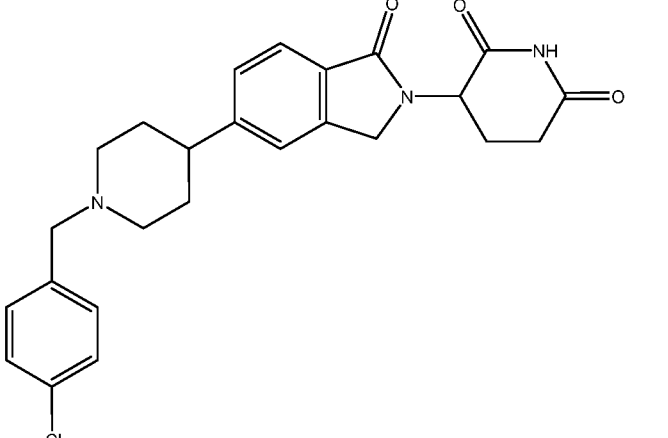
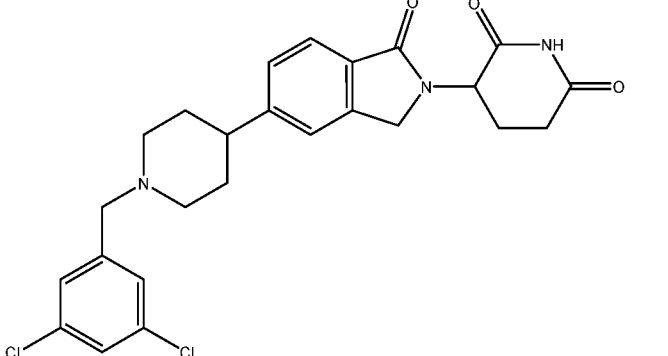
| Cmpd No.    | Structure                                                                           | Compound Name                                                                                                 |
|-------------|-------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------|
| <b>I-83</b> |    | 3-(5-(1-((2,3-dihydrobenzo[b][1,4]dioxin-6-yl)methyl)piperidin-4-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione |
| <b>I-84</b> |   | 3-(5-(1-(4-( <i>tert</i> -butyl)benzyl)piperidin-4-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione               |
| <b>I-85</b> |  | 3-(5-(1-(4-isobutylbenzyl)piperidin-4-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione                            |

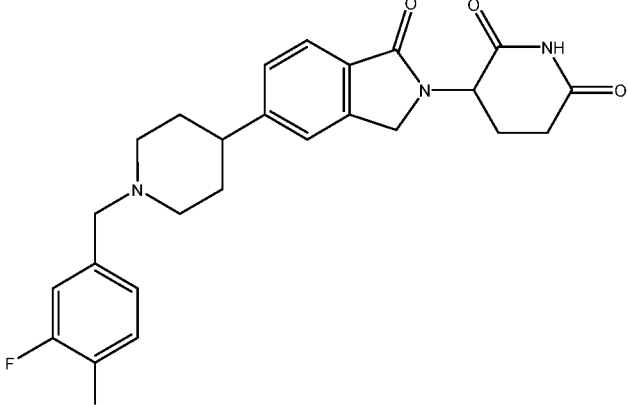
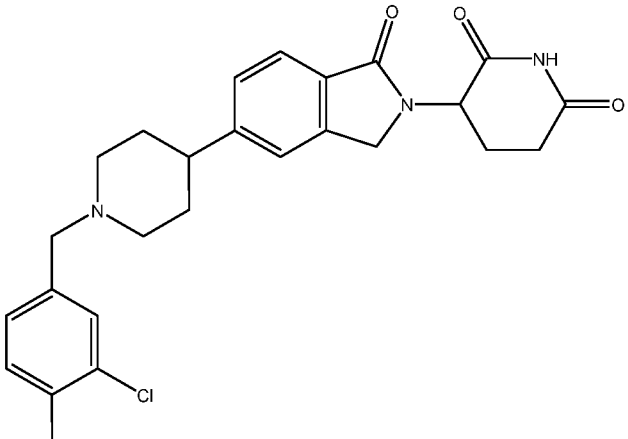
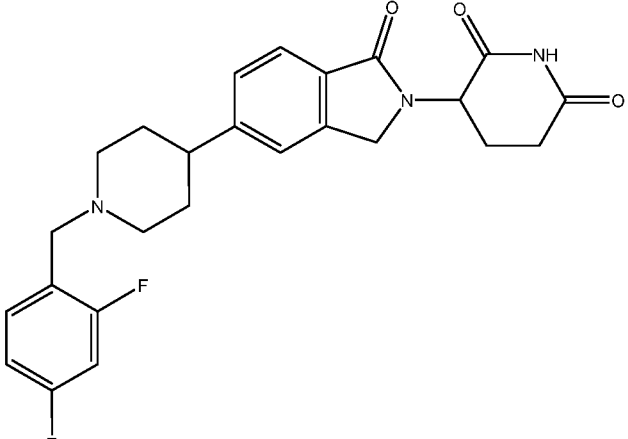
| Cmpd No.    | Structure                                                                           | Compound Name                                                                                                       |
|-------------|-------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------|
| <b>I-86</b> |    | N-(4-((4-(2-(2,6-dioxopiperidin-3-yl)-1-oxoisindolin-5-yl)piperidin-1-yl)methyl)phenyl)acetamide                    |
| <b>I-87</b> |   | 3-(5-(1-((2,2-difluorobenzo[d][1,3]dioxol-5-yl)methyl)piperidin-4-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione      |
| <b>I-88</b> |  | 3-(5-(1-((3,4-dihydro-2H-benzo[b][1,4]dioxepin-7-yl)methyl)piperidin-4-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione |

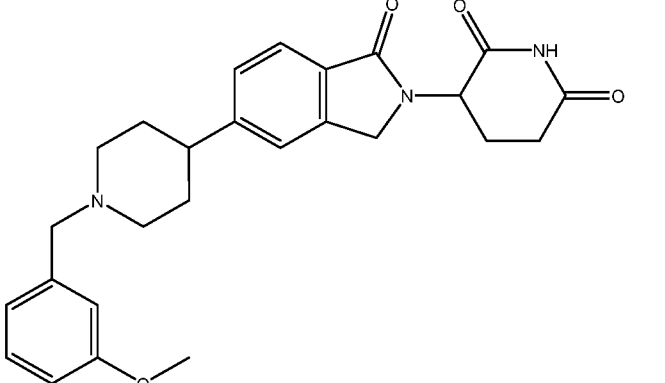
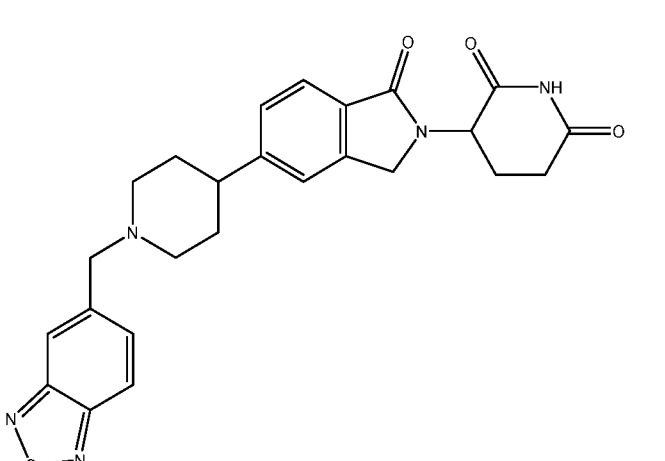
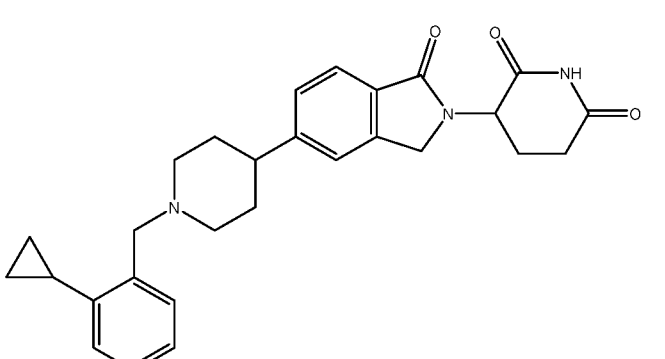
| Cmpd No.    | Structure                                                                           | Compound Name                                                                                     |
|-------------|-------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------|
| <b>I-89</b> |    | 3-(1-oxo-5-(1-(4-( <i>tert</i> -pentyl)benzyl)piperidin-4-yl)isoindolin-2-yl)piperidine-2,6-dione |
| <b>I-90</b> |   | 3-(5-(1-([1,1'-biphenyl]-4-ylmethyl)piperidin-4-yl)-1-oxoisoindolin-2-yl)piperidine-2,6-dione     |
| <b>I-91</b> |  | 3-(5-(1-(4-(1H-pyrazol-1-yl)benzyl)piperidin-4-yl)-1-oxoisoindolin-2-yl)piperidine-2,6-dione      |

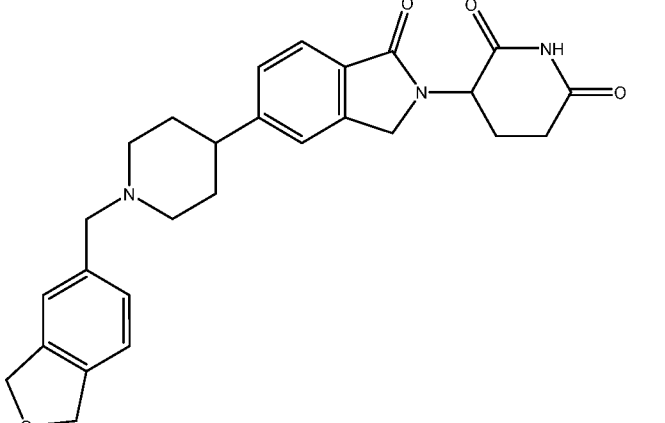
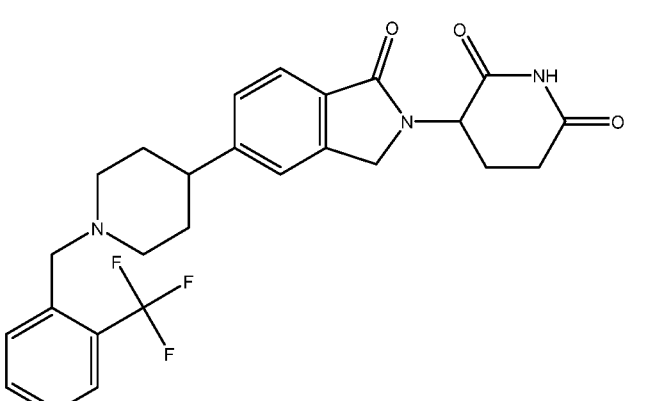
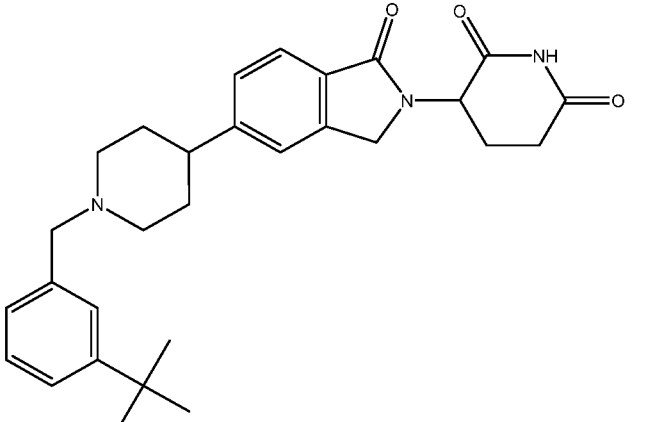
| Cmpd No.    | Structure                                                                           | Compound Name                                                                                |
|-------------|-------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------|
| <b>I-92</b> |    | 3-(5-(1-(4-(1H-imidazol-1-yl)benzyl)piperidin-4-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione |
| <b>I-93</b> |   | 3-(5-(1-(3-(1H-pyrazol-1-yl)benzyl)piperidin-4-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione  |
| <b>I-94</b> |  | 3-(5-(1-(4-cyclohexylbenzyl)piperidin-4-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione         |

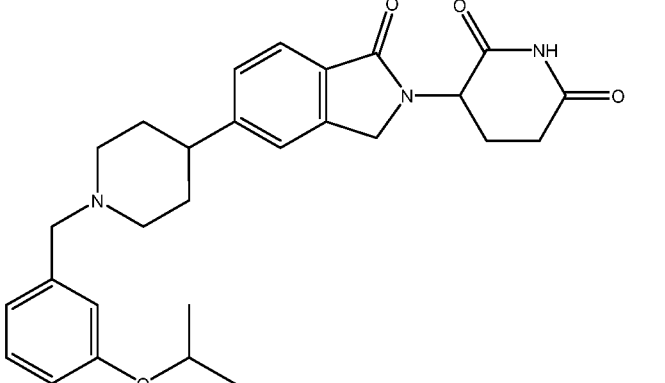
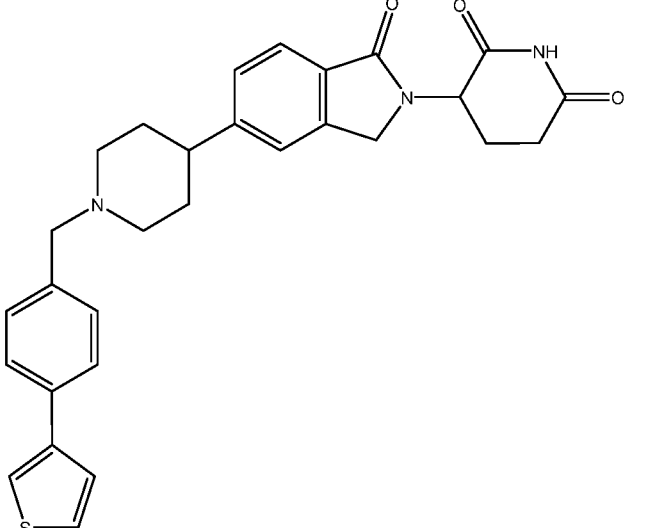
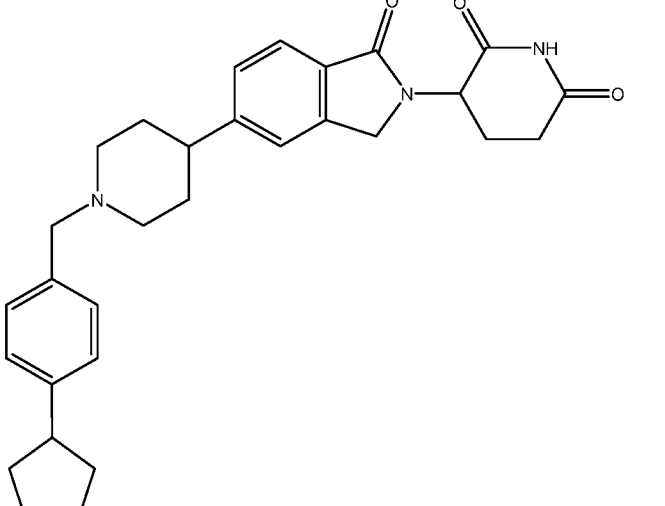


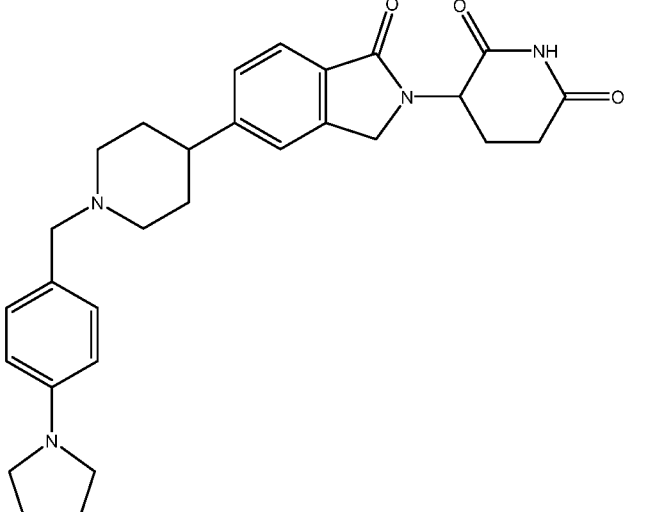
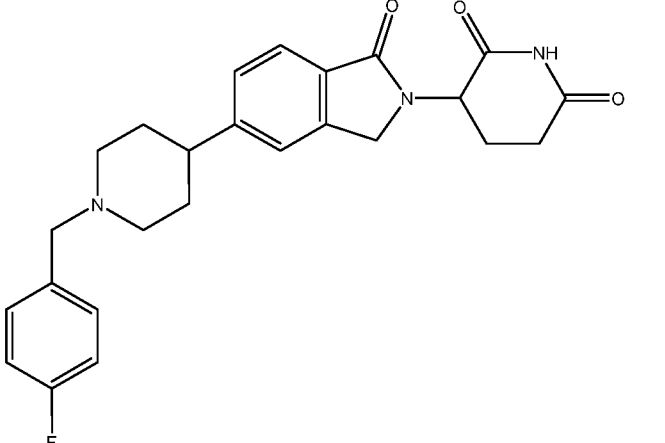
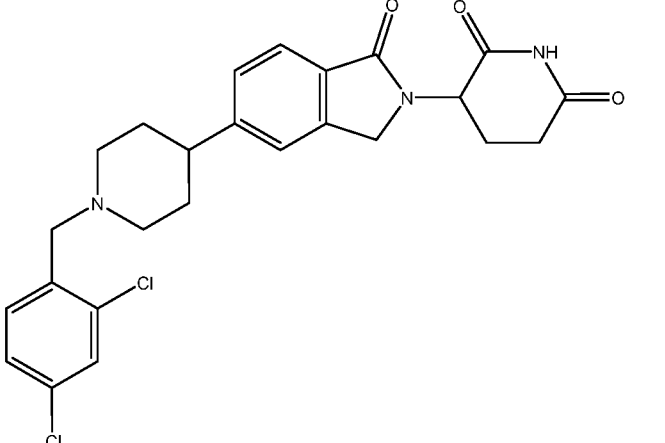
| Cmpd No.    | Structure                                                                           | Compound Name                                                                           |
|-------------|-------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------|
| <b>I-95</b> |    | 3-(1-oxo-5-(1-(pyrimidin-2-ylmethyl)piperidin-4-yl)isoindolin-2-yl)piperidine-2,6-dione |
| <b>I-96</b> |   | 3-(5-(1-(4-bromobenzyl)piperidin-4-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione         |
| <b>I-97</b> |  | 3-(5-(1-(4-chlorobenzyl)piperidin-4-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione        |
| <b>I-98</b> |  | 3-(5-(1-(3,5-dichlorobenzyl)piperidin-4-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione    |

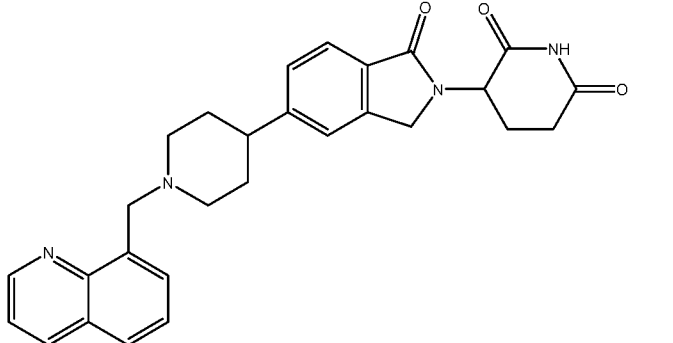
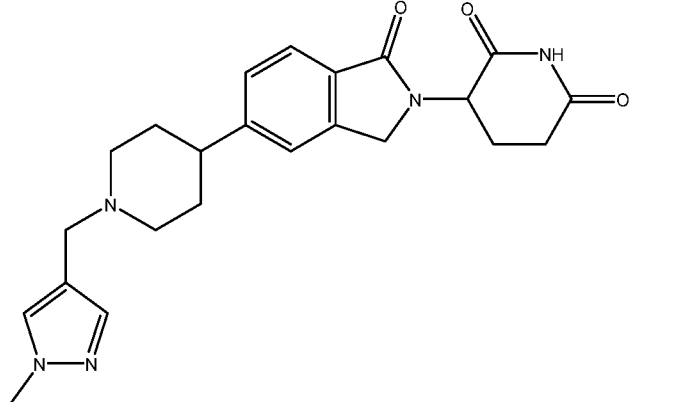
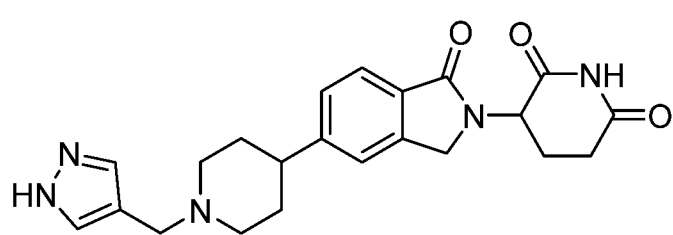
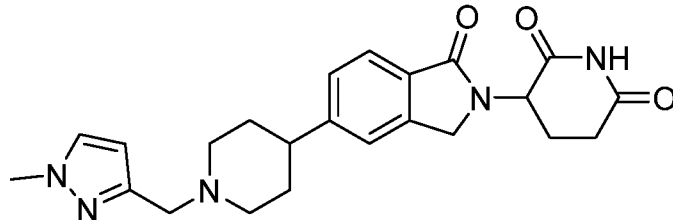
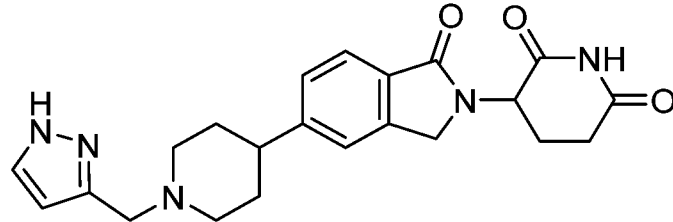
| Cmpd No.     | Structure                                                                           | Compound Name                                                                             |
|--------------|-------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------|
| <b>I-99</b>  |    | 3-(5-(1-(4-chloro-3-fluorobenzyl)piperidin-4-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione |
| <b>I-100</b> |   | 3-(5-(1-(3-chloro-4-fluorobenzyl)piperidin-4-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione |
| <b>I-101</b> |  | 3-(5-(1-(2,4-difluorobenzyl)piperidin-4-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione      |

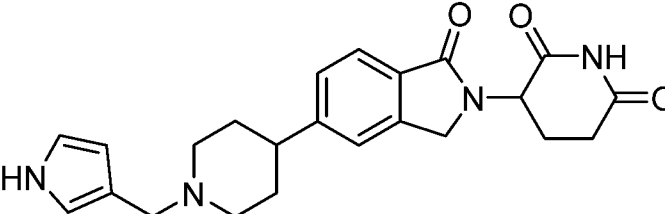
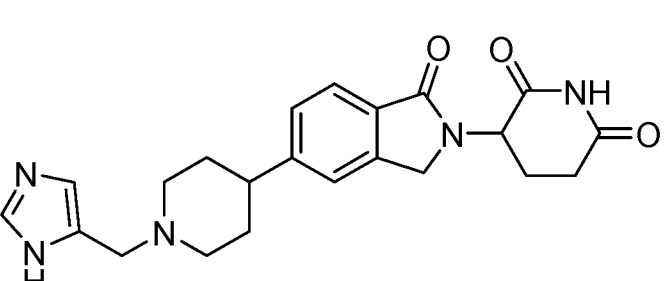
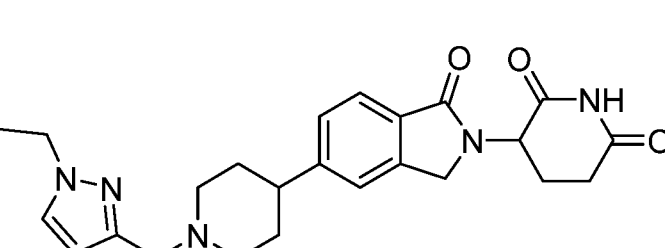
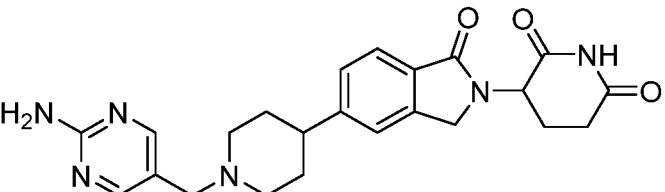
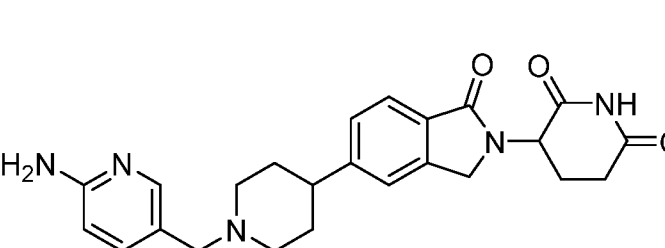
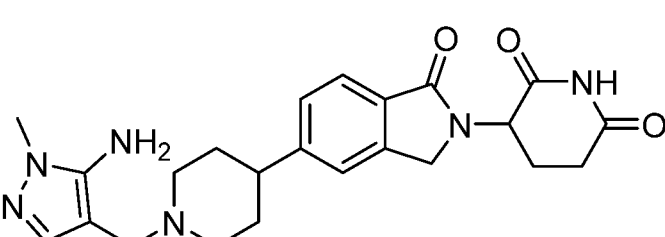
| Cmpd No.     | Structure                                                                           | Compound Name                                                                                         |
|--------------|-------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------|
| <b>I-102</b> |    | 3-(5-(1-(3-methoxybenzyl)piperidin-4-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione                     |
| <b>I-103</b> |   | 3-(5-(1-(benzo[c][1,2,5]oxadiazol-5-ylmethyl)piperidin-4-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione |
| <b>I-104</b> |  | 3-(5-(1-(2-cyclopropylbenzyl)piperidin-4-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione                 |

| Cmpd No.     | Structure                                                                           | Compound Name                                                                                           |
|--------------|-------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------|
| <b>I-105</b> |    | 3-(5-(1-((1,3-dihydroisobenzofuran-5-yl)methyl)piperidin-4-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione |
| <b>I-106</b> |   | 3-(1-oxo-5-(1-(2-(trifluoromethyl)benzyl)piperidin-4-yl)isindolin-2-yl)piperidine-2,6-dione             |
| <b>I-107</b> |  | 3-(5-(1-(3-( <i>tert</i> -butyl)benzyl)piperidin-4-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione         |

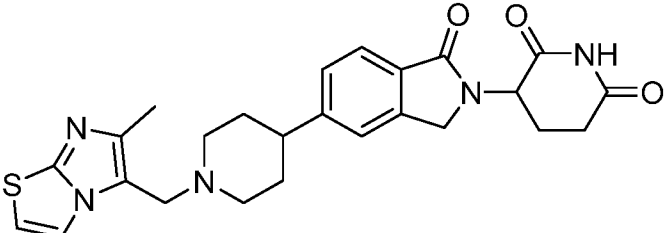
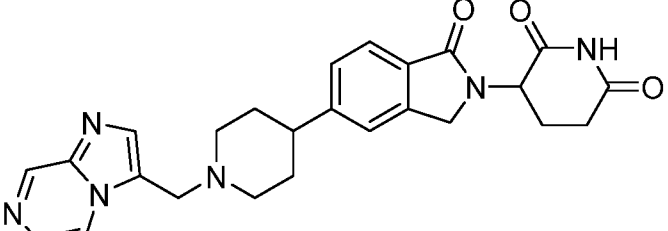
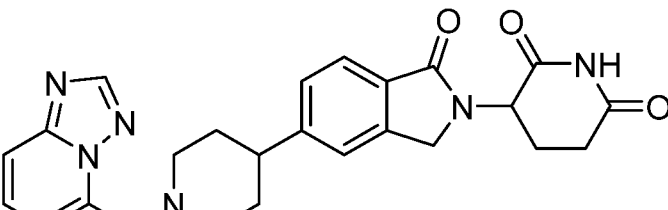
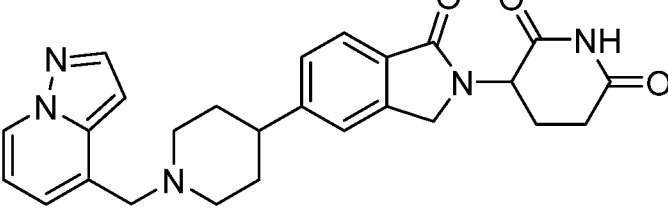
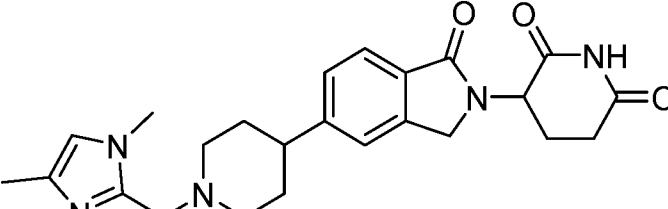
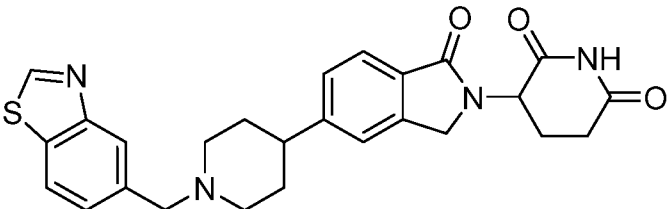
| Cmpd No.     | Structure                                                                           | Compound Name                                                                             |
|--------------|-------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------|
| <b>I-108</b> |    | 3-(5-(1-(3-isopropoxybenzyl)piperidin-4-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione      |
| <b>I-109</b> |   | 3-(1-oxo-5-(1-(4-(thiophen-3-yl)benzyl)piperidin-4-yl)isindolin-2-yl)piperidine-2,6-dione |
| <b>I-110</b> |  | 3-(5-(1-(4-cyclopentylbenzyl)piperidin-4-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione     |

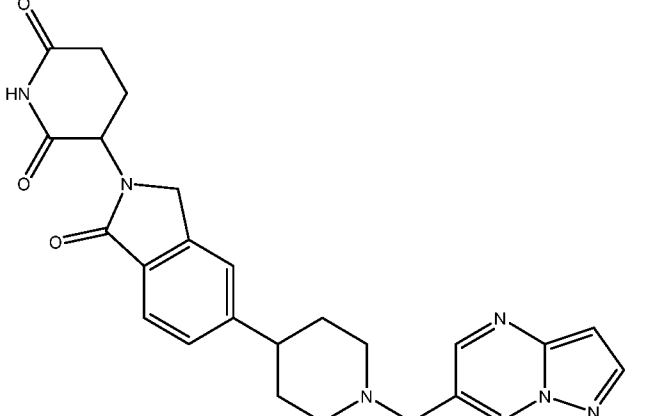
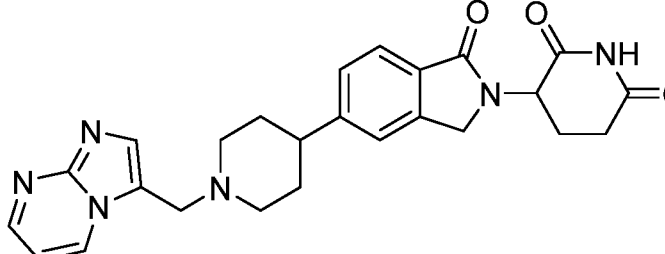
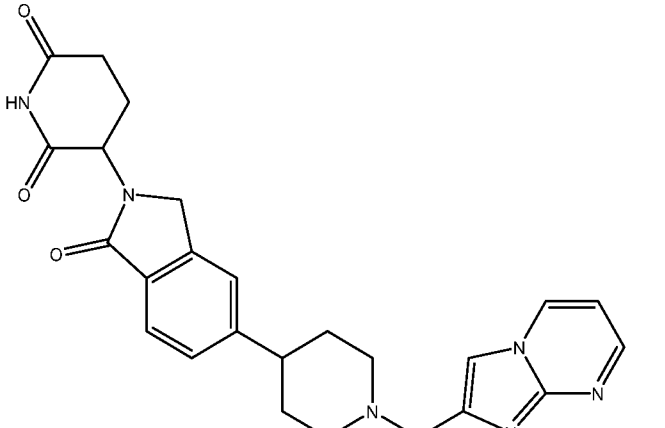
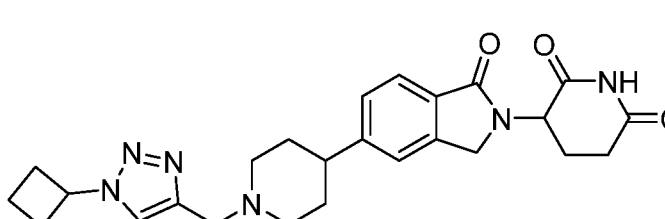
| Cmpd No.     | Structure                                                                           | Compound Name                                                                               |
|--------------|-------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------|
| <b>I-111</b> |    | 3-(1-oxo-5-(1-(4-(pyrrolidin-1-yl)benzyl)piperidin-4-yl)isindolin-2-yl)piperidine-2,6-dione |
| <b>I-112</b> |   | 3-(5-(1-(4-fluorobenzyl)piperidin-4-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione            |
| <b>I-113</b> |  | 3-(5-(1-(2,4-dichlorobenzyl)piperidin-4-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione        |

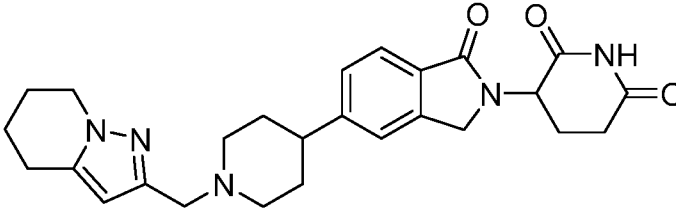
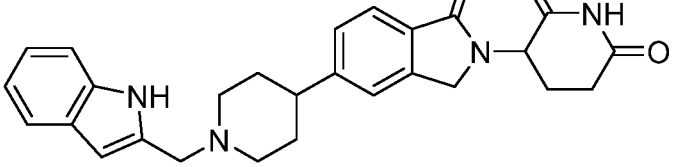
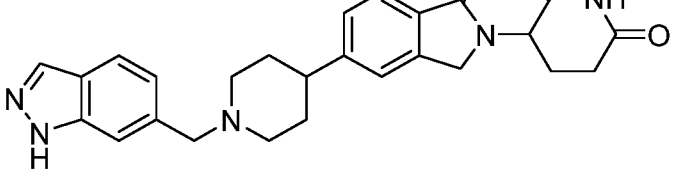
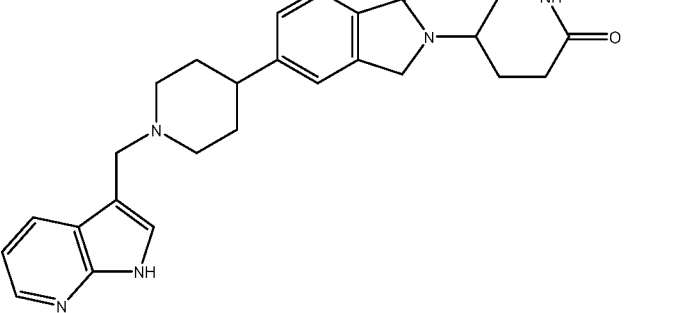
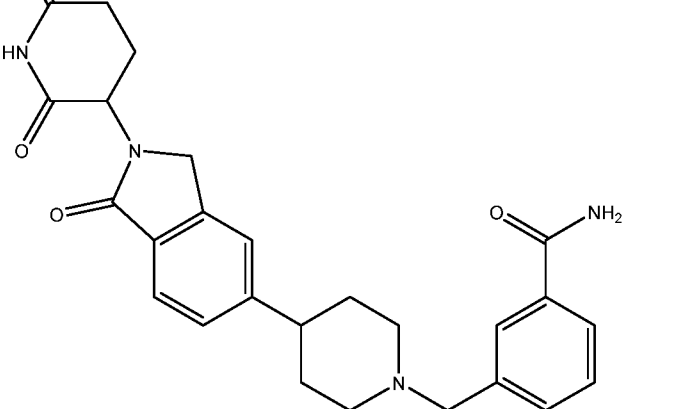
| Cmpd No.     | Structure                                                                           | Compound Name                                                                                      |
|--------------|-------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------|
| <b>I-114</b> |    | 3-(1-oxo-5-(1-(quinolin-8-ylmethyl)piperidin-4-yl)isoindolin-2-yl)piperidine-2,6-dione             |
| <b>I-115</b> |   | 3-(5-(1-((1-methyl-1H-pyrazol-4-yl)methyl)piperidin-4-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione |
| <b>I-116</b> |  | 3-(5-(1-((1H-pyrazol-4-yl)methyl)piperidin-4-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione          |
| <b>I-117</b> |  | 3-(5-(1-((1-methyl-1H-pyrazol-3-yl)methyl)piperidin-4-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione |
| <b>I-118</b> |  | 3-(5-(1-((1H-pyrazol-3-yl)methyl)piperidin-4-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione          |

| Cmpd No.     | Structure                                                                           | Compound Name                                                                                              |
|--------------|-------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------|
| <b>I-119</b> |    | 3-(5-(1-((1H-pyrrol-3-yl)methyl)piperidin-4-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione                   |
| <b>I-120</b> |    | 3-(5-(1-((1H-imidazol-5-yl)methyl)piperidin-4-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione                 |
| <b>I-121</b> |   | 3-(5-(1-((1-ethyl-1H-pyrazol-3-yl)methyl)piperidin-4-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione          |
| <b>I-122</b> |  | 3-(5-(1-((2-aminopyrimidin-5-yl)methyl)piperidin-4-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione            |
| <b>I-123</b> |  | 3-(5-(1-((6-aminopyridin-3-yl)methyl)piperidin-4-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione              |
| <b>I-124</b> |  | 3-(5-(1-((5-amino-1-methyl-1H-pyrazol-4-yl)methyl)piperidin-4-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione |

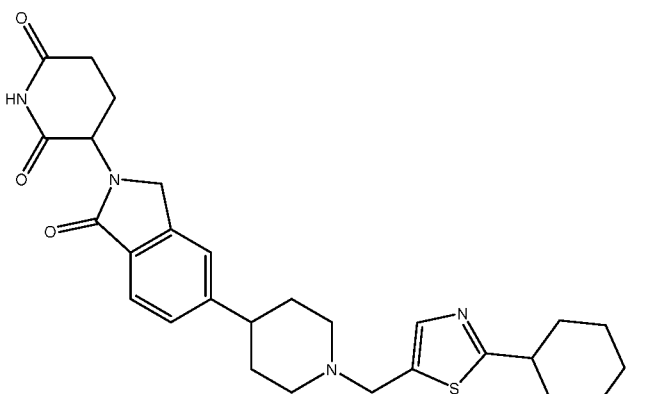
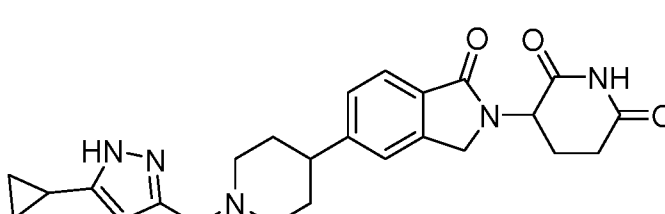
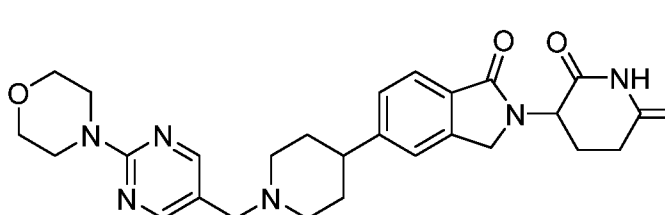
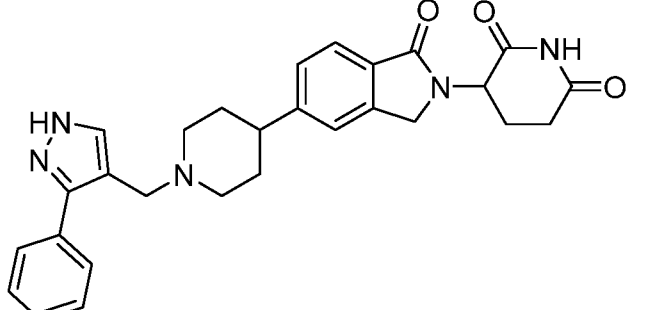


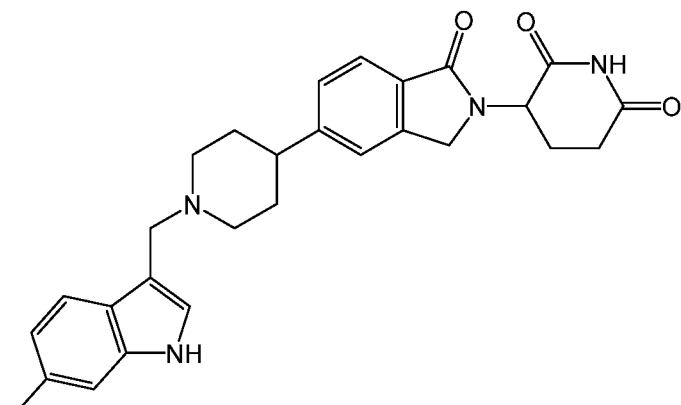
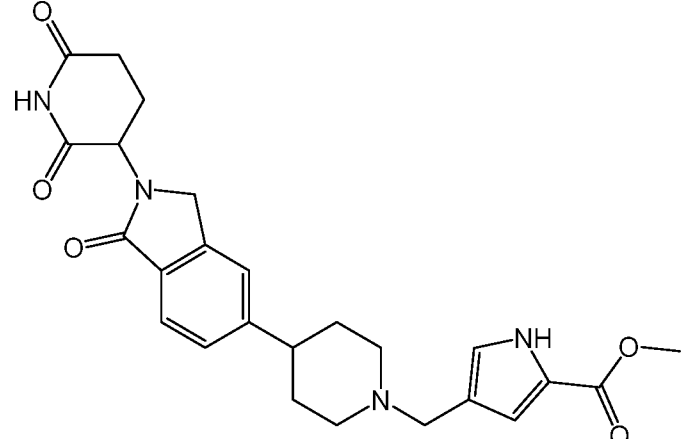
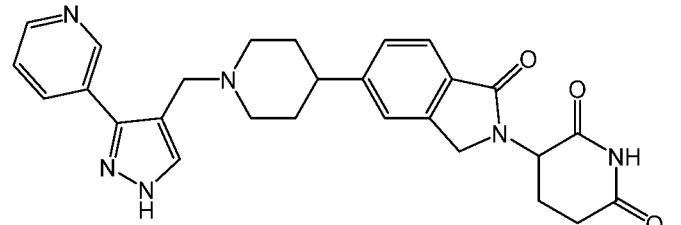
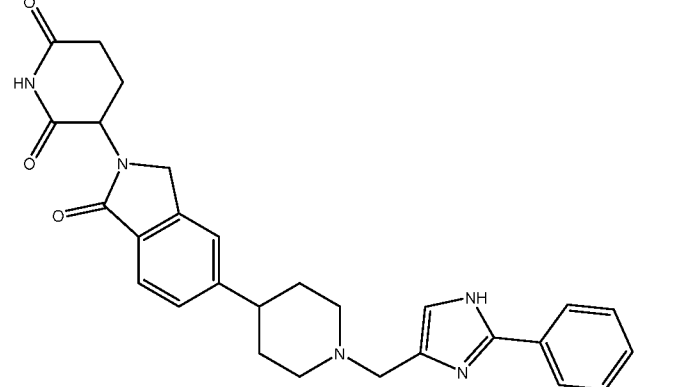
| Cmpd No. | Structure                                                                           | Compound Name                                                                                                |
|----------|-------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------|
| I-125    |    | 3-(5-(1-((6-methylimidazo[2,1-b]thiazol-5-yl)methyl)piperidin-4-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione |
| I-126    |    | 3-(5-(1-(imidazo[1,2-a]pyrazin-3-yl)methyl)piperidin-4-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione          |
| I-127    |   | 3-(5-(1-([1,2,4]triazolo[1,5-a]pyridin-5-yl)methyl)piperidin-4-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione  |
| I-128    |  | 3-(1-oxo-5-(1-(pyrazolo[1,5-a]pyridin-4-yl)methyl)piperidin-4-yl)isindolin-2-yl)piperidine-2,6-dione         |
| I-129    |  | 3-(5-(1-((1,4-dimethyl-1H-imidazol-2-yl)methyl)piperidin-4-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione      |
| I-130    |  | 3-(5-(1-(benzo[d]thiazol-5-yl)methyl)piperidin-4-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione                |

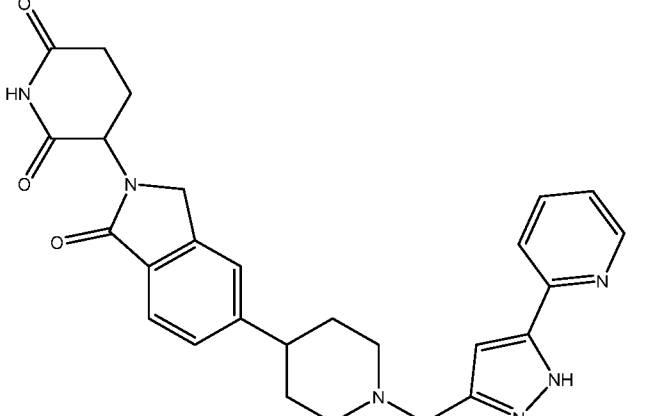
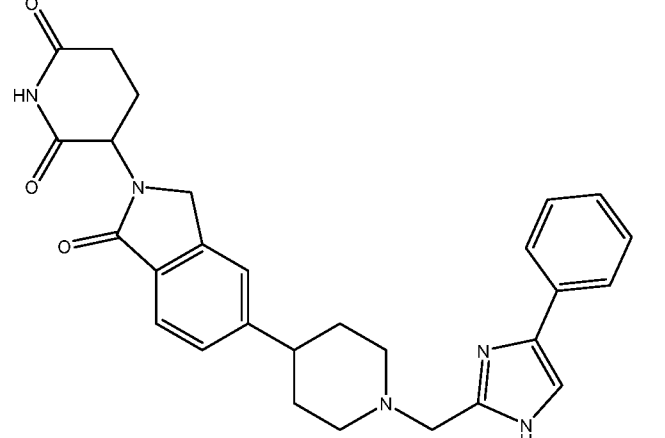
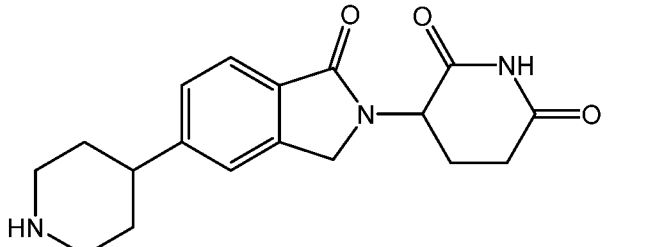
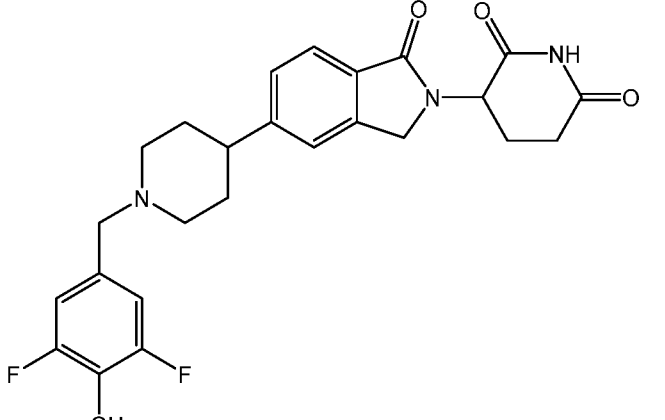
| Cmpd No.     | Structure                                                                           | Compound Name                                                                                                |
|--------------|-------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------|
| <b>I-131</b> |    | 3-(1-oxo-5-(1-(pyrazolo[1,5-a]pyrimidin-6-ylmethyl)piperidin-4-yl)isoindolin-2-yl)piperidine-2,6-dione       |
| <b>I-132</b> |   | 3-(5-(1-(imidazo[1,2-a]pyrimidin-3-ylmethyl)piperidin-4-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione         |
| <b>I-133</b> |  | 3-(5-(1-(imidazo[1,2-a]pyrimidin-2-ylmethyl)piperidin-4-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione         |
| <b>I-134</b> |  | 3-(5-(1-((1-cyclobutyl-1H-1,2,3-triazol-4-yl)methyl)piperidin-4-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione |

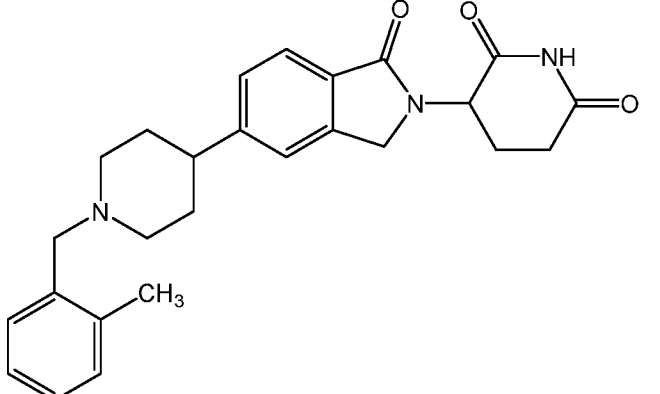
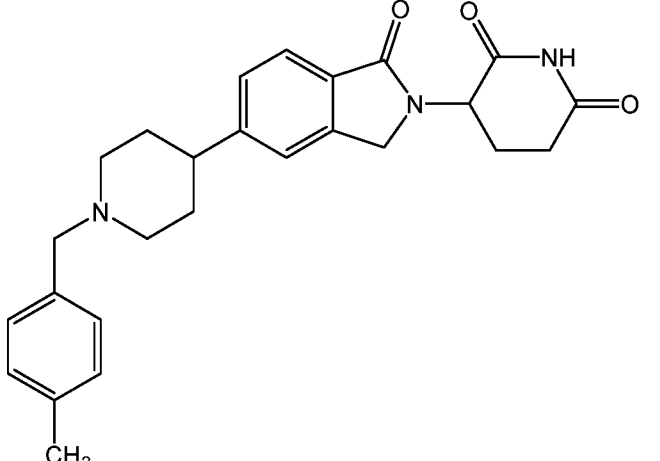
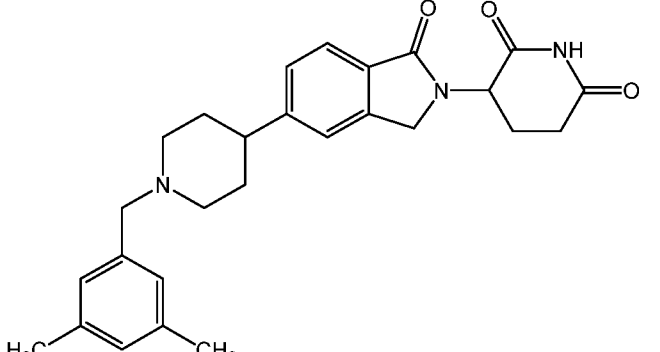
| Cmpd No. | Structure                                                                            | Compound Name                                                                                                            |
|----------|--------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------|
| I-135    |    | 3-(1-oxo-5-(1-((4,5,6,7-tetrahydropyrazolo[1,5-a]pyridin-2-yl)methyl)piperidin-4-yl)isoindolin-2-yl)piperidine-2,6-dione |
| I-136    |    | 3-(5-(1-((1H-indol-2-yl)methyl)piperidin-4-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione                                  |
| I-137    |   | 3-(5-(1-((1H-indazol-6-yl)methyl)piperidin-4-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione                                |
| I-138    |  | 3-(5-(1-((1H-pyrrolo[2,3-b]pyridin-3-yl)methyl)piperidin-4-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione                  |
| I-139    |  | 3-((4-(2-(2,6-dioxopiperidin-3-yl)-1-oxoisindolin-5-yl)piperidin-1-yl)methyl)benzamide                                   |

| Cmpd No.     | Structure | Compound Name                                                                                                      |
|--------------|-----------|--------------------------------------------------------------------------------------------------------------------|
| <b>I-140</b> |           | 3-(5-(1-((1H-pyrrolo[2,3-b]pyridin-6-yl)methyl)piperidin-4-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione            |
| <b>I-141</b> |           | 3-(5-(1-((3,4-dihydro-2H-benzo[b][1,4]thiazin-6-yl)methyl)piperidin-4-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione |
| <b>I-142</b> |           | 3-(1-oxo-5-(1-((2-(pyrrolidin-1-yl)pyrimidin-5-yl)methyl)piperidin-4-yl)isindolin-2-yl)piperidine-2,6-dione        |
| <b>I-143</b> |           | 3-(5-(1-((2-( <i>tert</i> -butyl)thiazol-4-yl)methyl)piperidin-4-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione      |
| <b>I-144</b> |           | 3-(1-oxo-5-(1-((2-(thiophen-2-yl)thiazol-5-yl)methyl)piperidin-4-yl)isindolin-2-yl)piperidine-2,6-dione            |

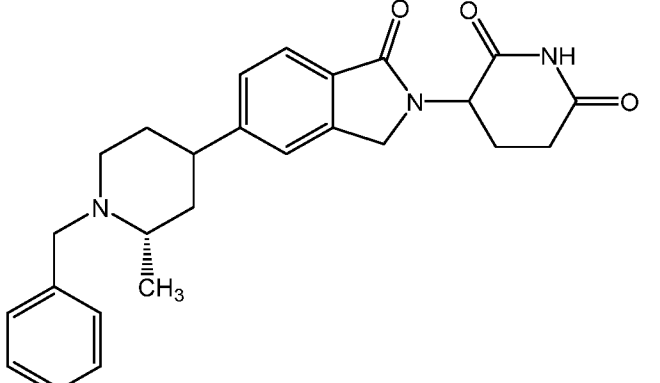
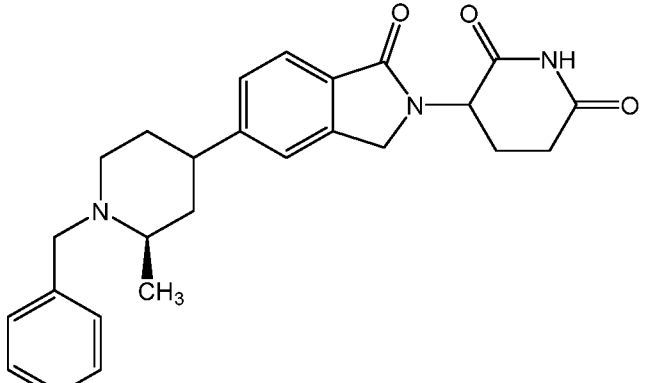
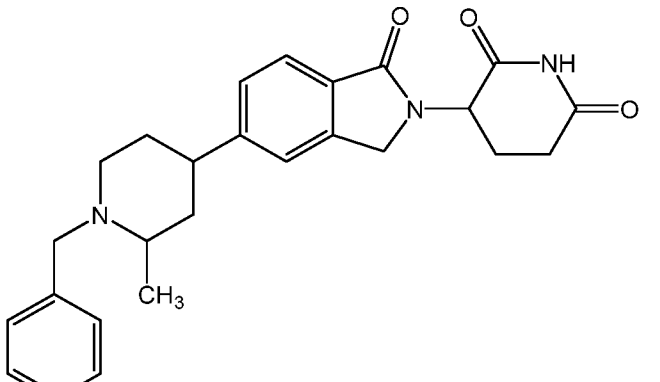
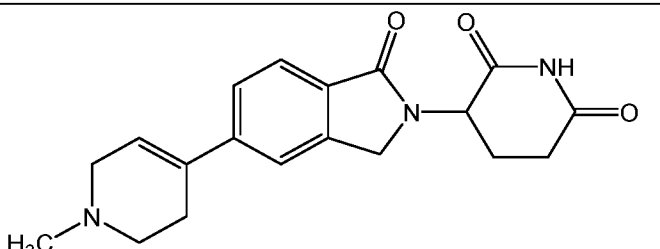
| Cmpd No.     | Structure                                                                           | Compound Name                                                                                           |
|--------------|-------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------|
| <b>I-145</b> |    | 3-(5-(1-((2-cyclohexylthiazol-5-yl)methyl)piperidin-4-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione      |
| <b>I-146</b> |    | 3-(5-(1-((5-cyclopropyl-1H-pyrazol-3-yl)methyl)piperidin-4-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione |
| <b>I-147</b> |   | 3-(5-(1-((2-morpholinopyrimidin-5-yl)methyl)piperidin-4-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione    |
| <b>I-148</b> |  | 3-(1-oxo-5-(1-((3-phenyl-1H-pyrazol-4-yl)methyl)piperidin-4-yl)isindolin-2-yl)piperidine-2,6-dione      |

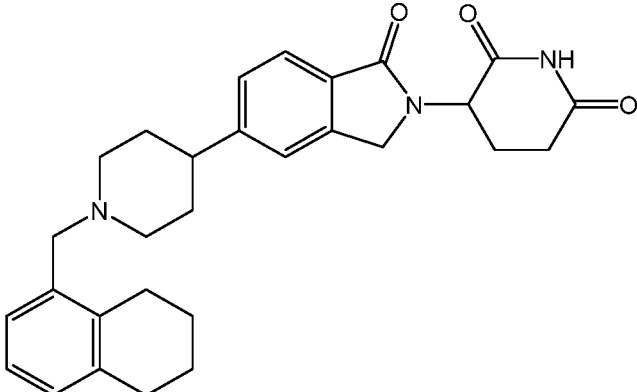
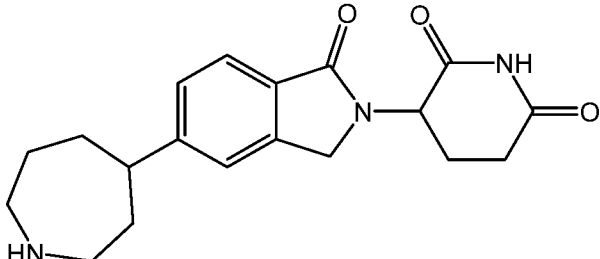
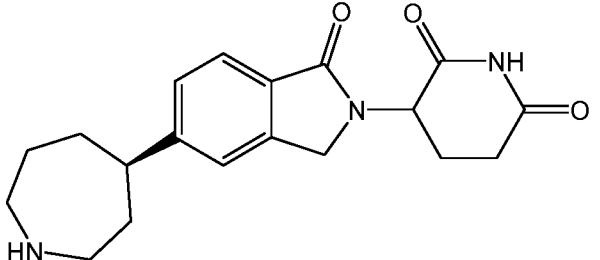
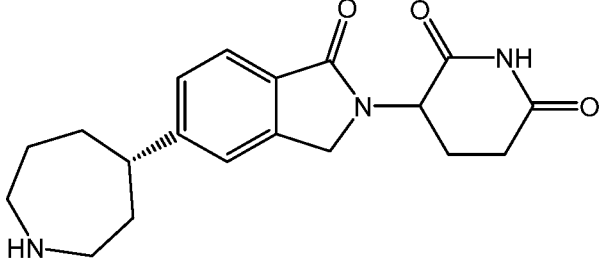
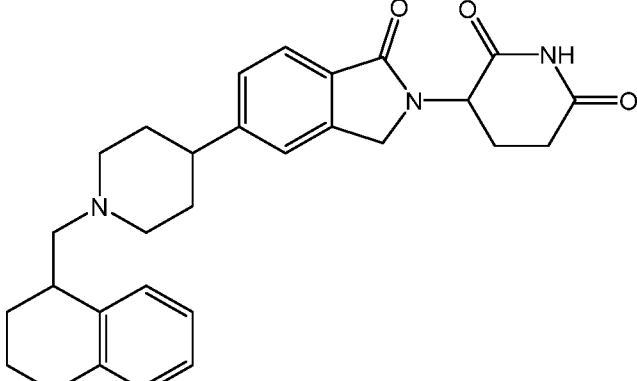
| Cmpd No.     | Structure                                                                           | Compound Name                                                                                                 |
|--------------|-------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------|
| <b>I-149</b> |    | 3-(5-(1-((6-methyl-1H-indol-3-yl)methyl)piperidin-4-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione              |
| <b>I-150</b> |   | methyl 4-((4-(2-(2,6-dioxopiperidin-3-yl)-1-oxoisindolin-5-yl)piperidin-1-yl)methyl)-1H-pyrrole-2-carboxylate |
| <b>I-151</b> |  | 3-(1-oxo-5-(1-((3-(pyridin-3-yl)-1H-pyrazol-4-yl)methyl)piperidin-4-yl)isoindolin-2-yl)piperidine-2,6-dione   |
| <b>I-152</b> |  | 3-(1-oxo-5-(1-((2-phenyl-1H-imidazol-4-yl)methyl)piperidin-4-yl)isoindolin-2-yl)piperidine-2,6-dione          |

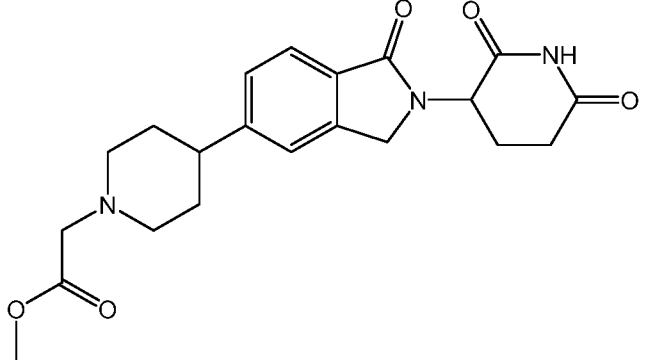
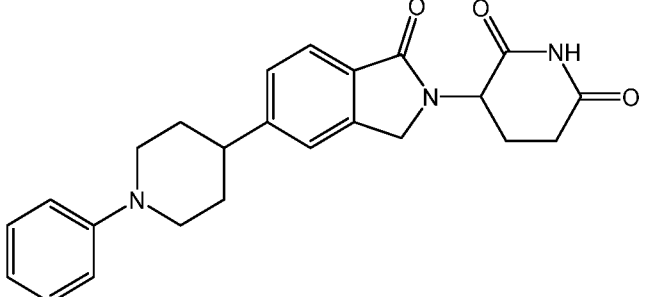
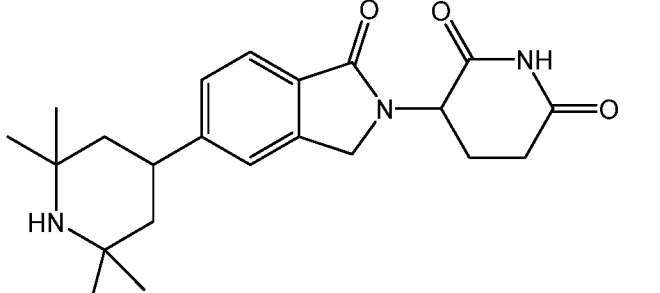
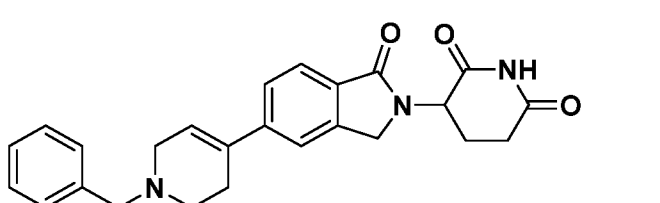
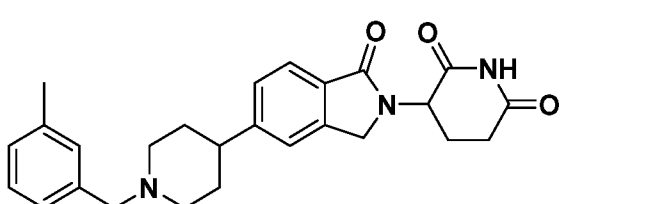
| Cmpd No.     | Structure                                                                           | Compound Name                                                                                               |
|--------------|-------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------|
| <b>I-153</b> |    | 3-(1-oxo-5-(1-((5-(pyridin-2-yl)-1H-pyrazol-3-yl)methyl)piperidin-4-yl)isoindolin-2-yl)piperidine-2,6-dione |
| <b>I-154</b> |   | 3-(1-oxo-5-(1-((4-phenyl-1H-imidazol-2-yl)methyl)piperidin-4-yl)isoindolin-2-yl)piperidine-2,6-dione        |
| <b>I-155</b> |  | 3-(1-oxo-5-(piperidin-4-yl)isoindolin-2-yl)piperidine-2,6-dione                                             |
| <b>I-156</b> |  | 3-(5-(1-(3,5-difluoro-4-hydroxybenzyl)piperidin-4-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione              |

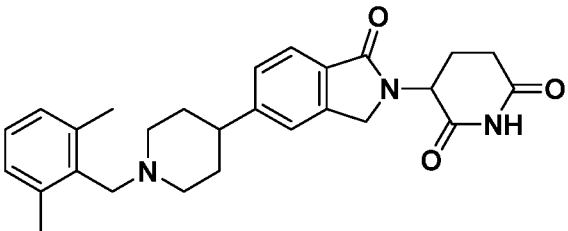
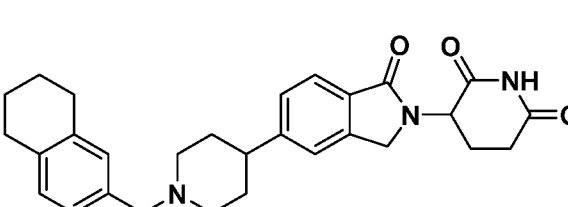
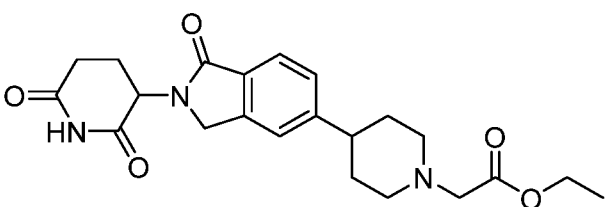
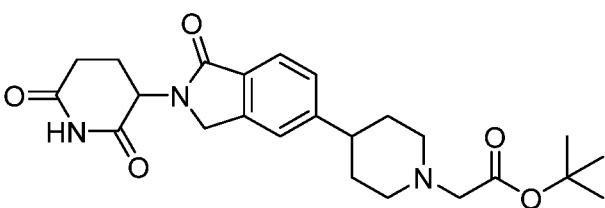
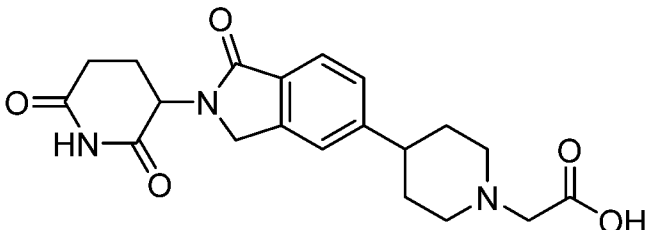
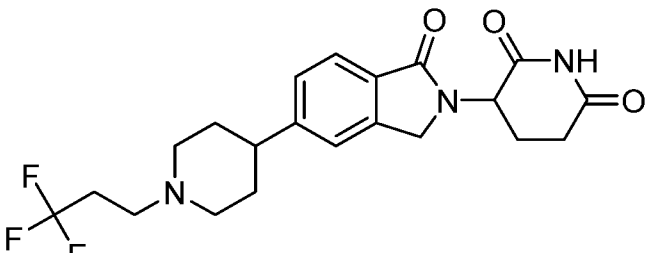
| Cmpd No.     | Structure                                                                           | Compound Name                                                                        |
|--------------|-------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------|
| <b>I-157</b> |    | 3-(5-(1-(2-methylbenzyl)piperidin-4-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione     |
| <b>I-158</b> |   | 3-(5-(1-(4-methylbenzyl)piperidin-4-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione     |
| <b>I-159</b> |  | 3-(5-(1-(3,5-dimethylbenzyl)piperidin-4-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione |

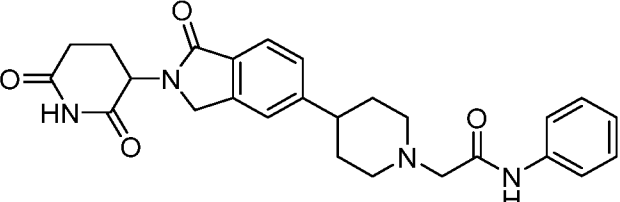
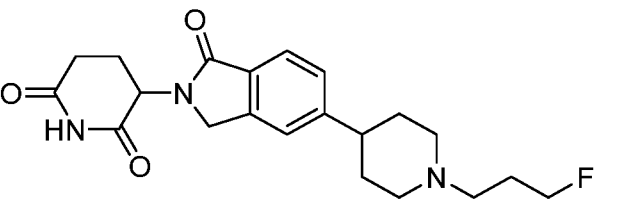
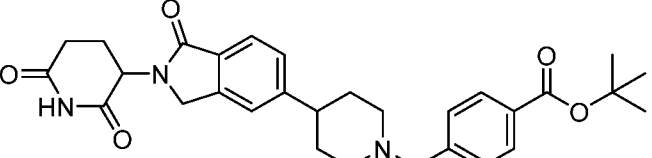
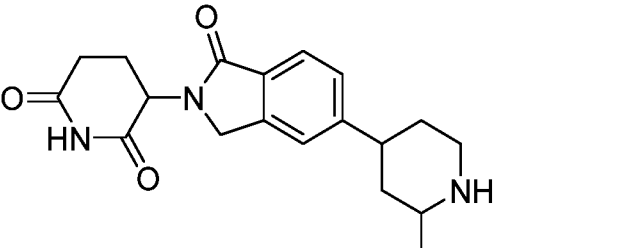
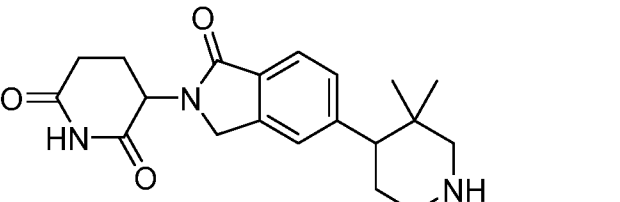
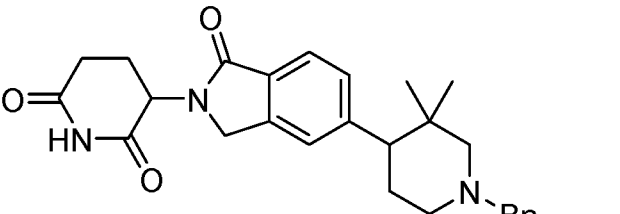
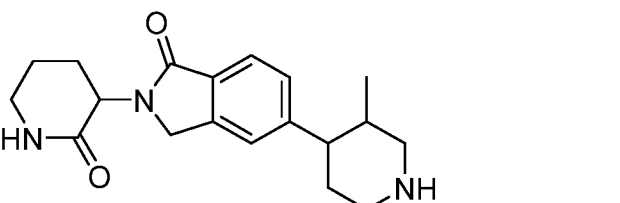


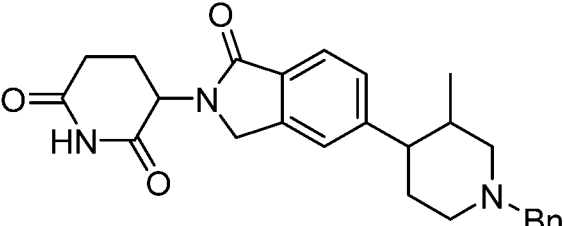
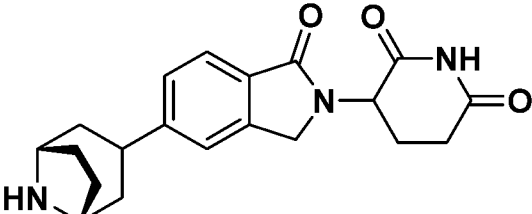
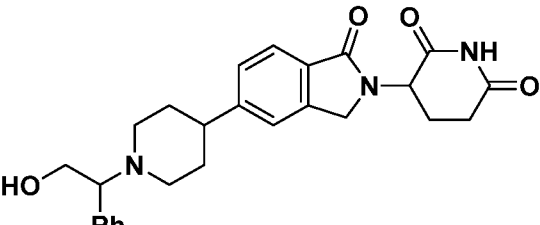
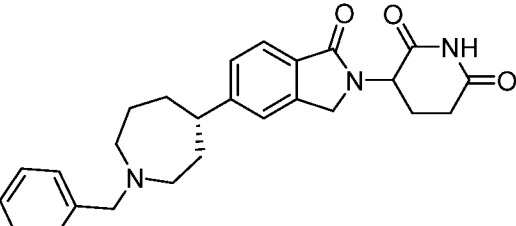
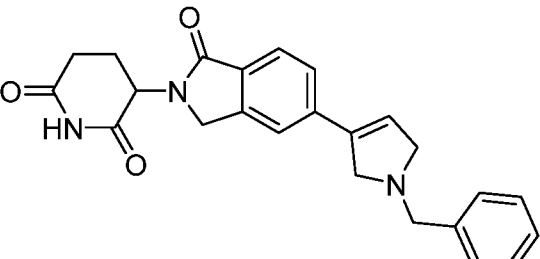
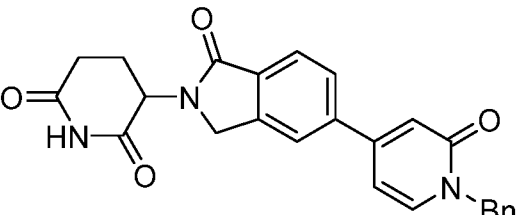
| Cmpd No.     | Structure                                                                           | Compound Name                                                                           |
|--------------|-------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------|
| <b>I-160</b> |    | 3-(5-((2S)-1-benzyl-2-methylpiperidin-4-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione    |
| <b>I-161</b> |   | 3-(5-((2R)-1-benzyl-2-methylpiperidin-4-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione    |
| <b>I-162</b> |  | 3-(5-(1-benzyl-2-methylpiperidin-4-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione         |
| <b>I-163</b> |  | 3-(5-(1-methyl-1,2,3,6-tetrahydropyridin-4-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione |

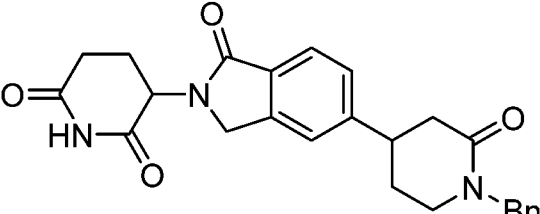
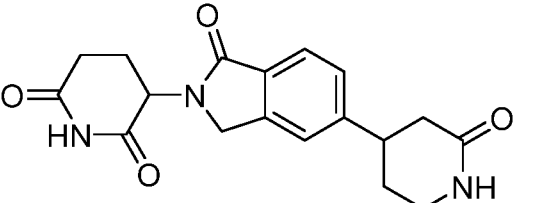
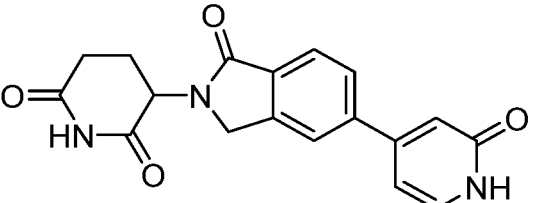
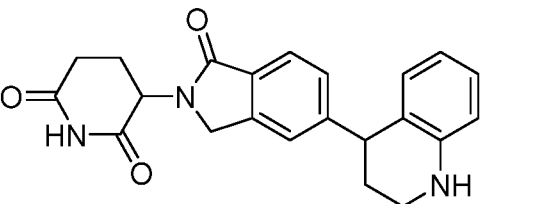
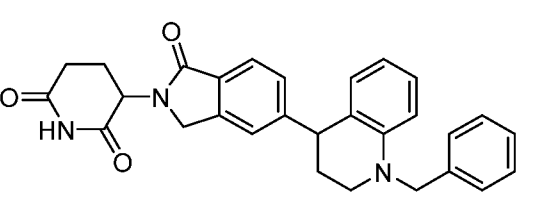
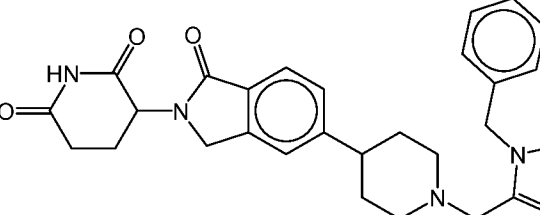
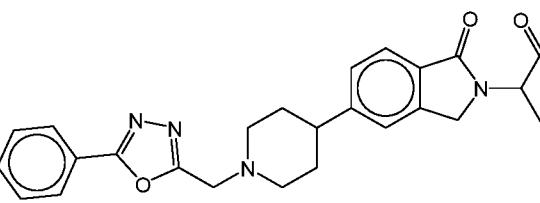
| Cmpd No. | Structure                                                                           | Compound Name                                                                                                |
|----------|-------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------|
| I-164    |    | 3-(1-oxo-5-(1-((5,6,7,8-tetrahydronaphthalen-1-yl)methyl)piperidin-4-yl)isoindolin-2-yl)piperidine-2,6-dione |
| I-165    |   | 3-(5-(azepan-4-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione                                                  |
| I-166    |  | 3-(5-((R)-azepan-4-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione                                              |
| I-167    |  | 3-(5-((S)-azepan-4-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione                                              |
| I-168    |  | 3-(1-oxo-5-(1-((1,2,3,4-tetrahydronaphthalen-1-yl)methyl)piperidin-4-yl)isoindolin-2-yl)piperidine-2,6-dione |

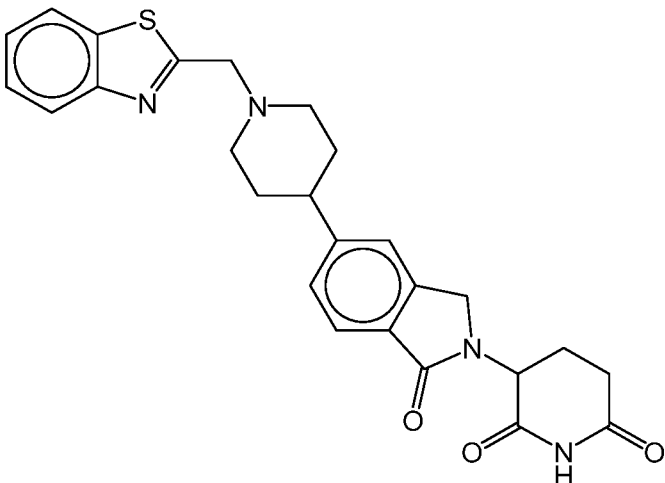
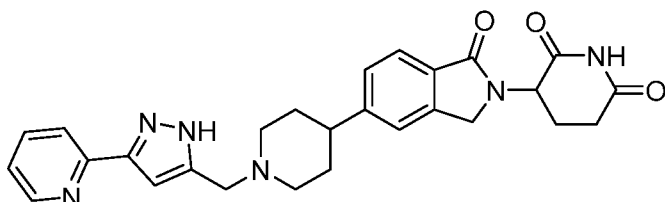
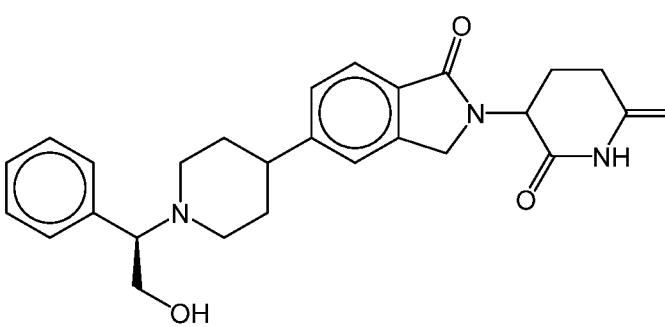
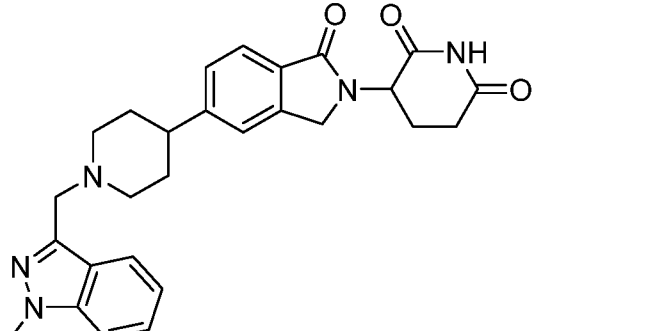
| Cmpd No.     | Structure                                                                           | Compound Name                                                                           |
|--------------|-------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------|
| <b>I-169</b> |    | methyl 2-(4-(2-(2,6-dioxopiperidin-3-yl)-1-oxoisindolin-5-yl)piperidin-1-yl)acetate     |
| <b>I-170</b> |   | 3-(1-oxo-5-(1-phenylpiperidin-4-yl)isindolin-2-yl)piperidine-2,6-dione                  |
| <b>I-171</b> |  | 3-(1-oxo-5-(2,2,6,6-tetramethylpiperidin-4-yl)isindolin-2-yl)piperidine-2,6-dione       |
| <b>I-172</b> |  | 3-(5-(1-benzyl-1,2,3,6-tetrahydropyridin-4-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione |
| <b>I-173</b> |  | 3-(5-(1-(3-methylbenzyl)piperidin-4-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione        |

| Cmpd No. | Structure                                                                           | Compound Name                                                                                               |
|----------|-------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------|
| I-174    |    | 3-(5-(1-(2,6-dimethylbenzyl)piperidin-4-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione                        |
| I-175    |    | 3-(1-oxo-5-(1-((5,6,7,8-tetrahydronaphthalen-2-yl)methyl)piperidin-4-yl)isindolin-2-yl)piperidine-2,6-dione |
| I-176    |   | ethyl 2-(4-(2-(2,6-dioxopiperidin-3-yl)-1-oxoisindolin-5-yl)piperidin-1-yl)acetate                          |
| I-177    |  | <i>tert</i> -butyl 2-(4-(2-(2,6-dioxopiperidin-3-yl)-1-oxoisindolin-5-yl)piperidin-1-yl)acetate             |
| I-178    |  | 2-(4-(2-(2,6-dioxopiperidin-3-yl)-1-oxoisindolin-5-yl)piperidin-1-yl)acetic acid                            |
| I-179    |  | 3-(1-oxo-5-(1-(3,3,3-trifluoropropyl)piperidin-4-yl)isindolin-2-yl)piperidine-2,6-dione                     |

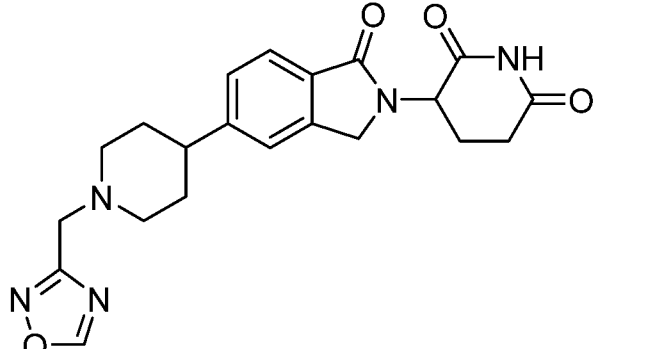
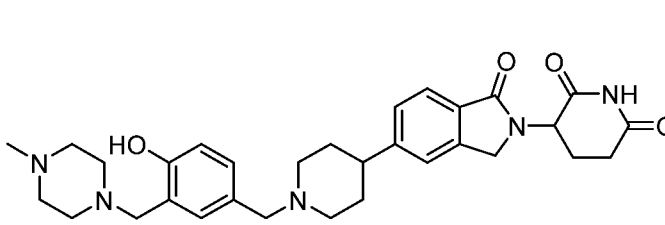
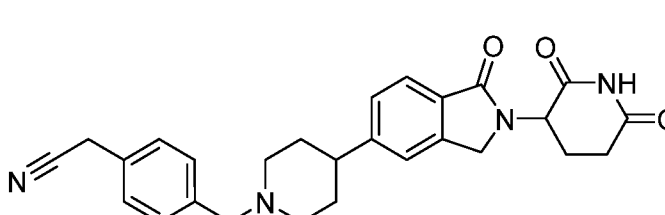
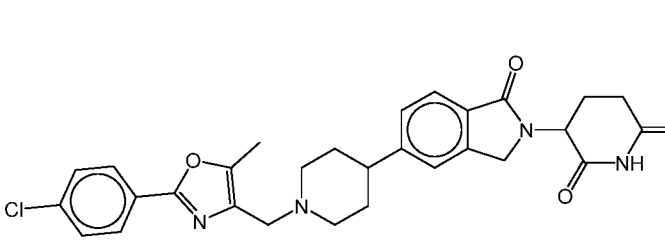
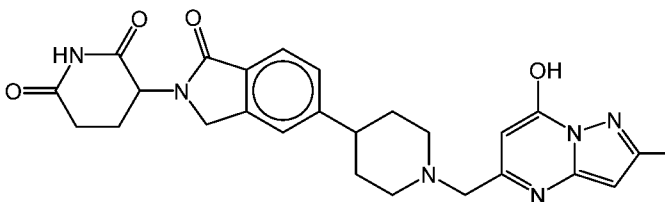
| Cmpd No.     | Structure                                                                           | Compound Name                                                                                            |
|--------------|-------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------|
| <b>I-180</b> |    | 2-(4-(2-(2,6-dioxopiperidin-3-yl)-1-oxoisindolin-5-yl)piperidin-1-yl)-N-phenylacetamide                  |
| <b>I-181</b> |    | 3-(5-(1-(3-fluoropropyl)piperidin-4-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione                         |
| <b>I-182</b> |    | <i>tert</i> -butyl 4-((4-(2-(2,6-dioxopiperidin-3-yl)-1-oxoisindolin-5-yl)piperidin-1-yl)methyl)benzoate |
| <b>I-183</b> |   | 3-(5-(2-methylpiperidin-4-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione                                   |
| <b>I-184</b> |  | 3-(5-(3,3-dimethylpiperidin-4-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione                               |
| <b>I-185</b> |  | 3-(5-(1-benzyl-3,3-dimethylpiperidin-4-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione                      |
| <b>I-186</b> |  | 5-(3-methylpiperidin-4-yl)-2-(2-oxopiperidin-3-yl)isoindolin-1-one                                       |

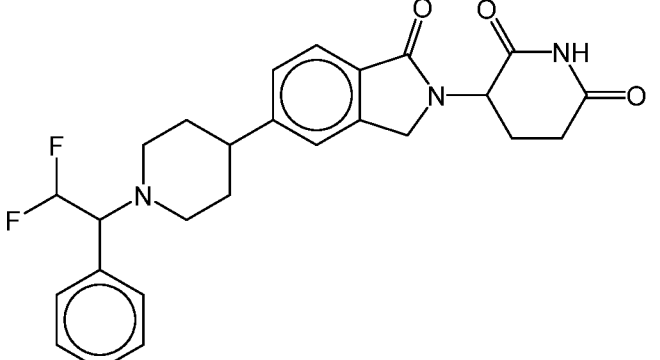
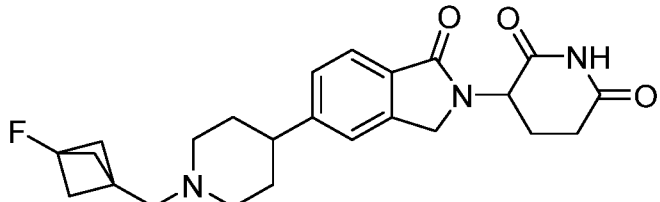
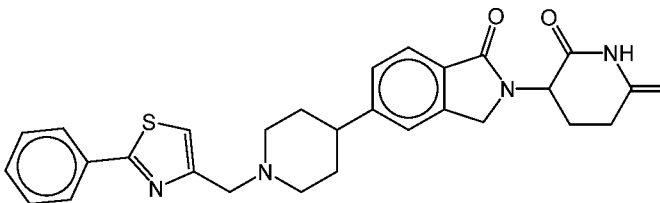
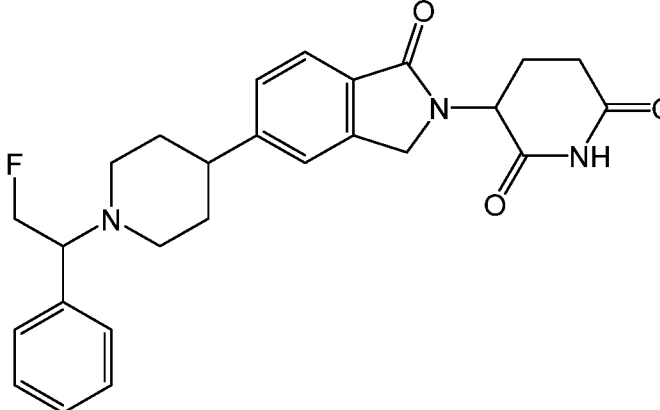
| Cmpd No. | Structure                                                                           | Compound Name                                                                             |
|----------|-------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------|
| I-187    |    | 3-(5-(1-benzyl-3-methylpiperidin-4-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione           |
| I-188    |    | 3-(5-(8-azabicyclo[3.2.1]octan-3-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione             |
| I-189    |   | 3-(5-(1-(2-hydroxy-1-phenylethyl)piperidin-4-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione |
| I-190    |  | 3-(5-((S)-1-benzylazepan-4-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione                   |
| I-191    |  | 3-(5-(1-benzyl-2,5-dihydro-1H-pyrrol-3-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione       |
| I-192    |  | 3-(5-(1-benzyl-2-oxo-1,2-dihydropyridin-4-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione    |

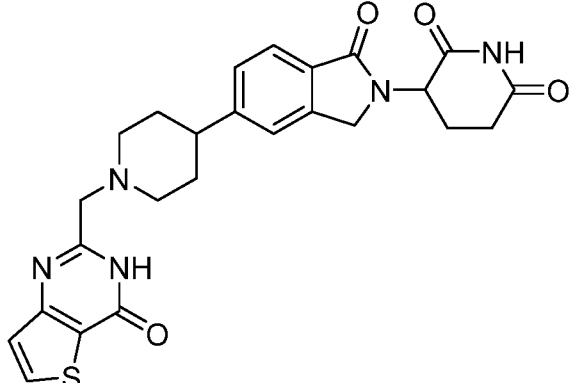
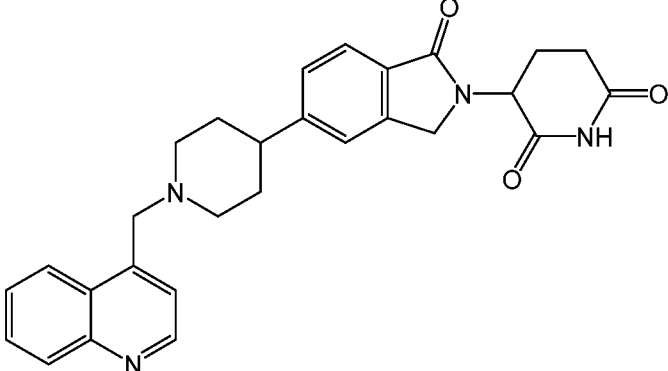
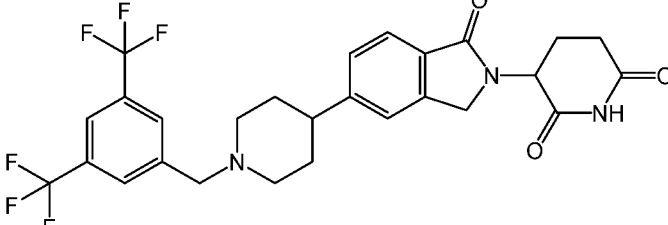
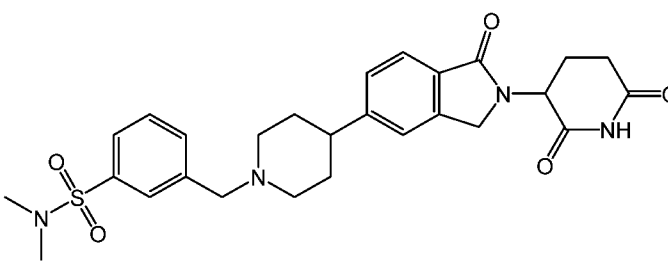
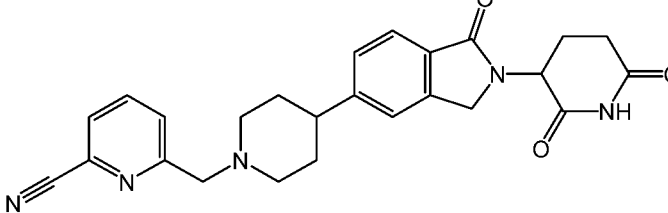
| Cmpd No.     | Structure                                                                           | Compound Name                                                                                            |
|--------------|-------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------|
| <b>I-193</b> |    | 3-(5-(1-benzyl-2-oxopiperidin-4-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione                             |
| <b>I-194</b> |    | 3-(1-oxo-5-(2-oxopiperidin-4-yl)isoindolin-2-yl)piperidine-2,6-dione                                     |
| <b>I-195</b> |   | 3-(1-oxo-5-(2-oxo-1,2-dihydropyridin-4-yl)isoindolin-2-yl)piperidine-2,6-dione                           |
| <b>I-196</b> |  | 3-(1-oxo-5-(1,2,3,4-tetrahydroquinolin-4-yl)isoindolin-2-yl)piperidine-2,6-dione                         |
| <b>I-197</b> |  | 3-(5-(1-benzyl-1,2,3,4-tetrahydroquinolin-4-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione                 |
| <b>I-198</b> |  | 3-(5-(1-((1-benzyl-1H-tetrazol-5-yl)methyl)piperidin-4-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione      |
| <b>I-199</b> |  | 3-(1-oxo-5-(1-((5-phenyl-1,3,4-oxadiazol-2-yl)methyl)piperidin-4-yl)isoindolin-2-yl)piperidine-2,6-dione |

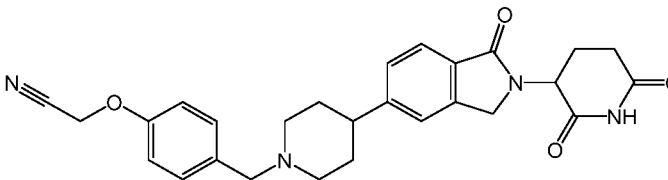
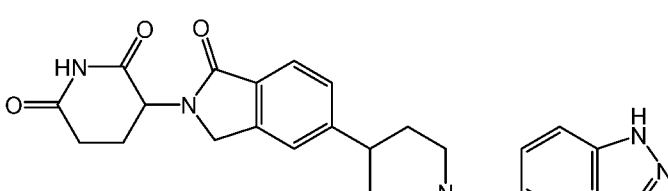
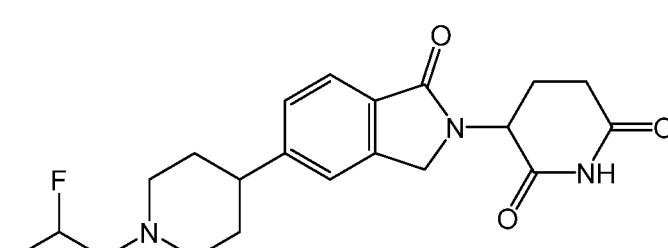
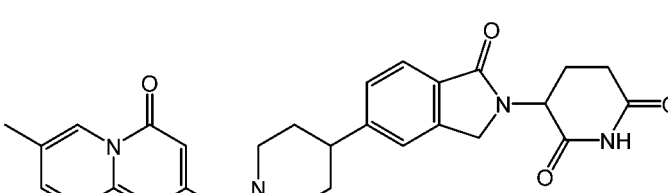
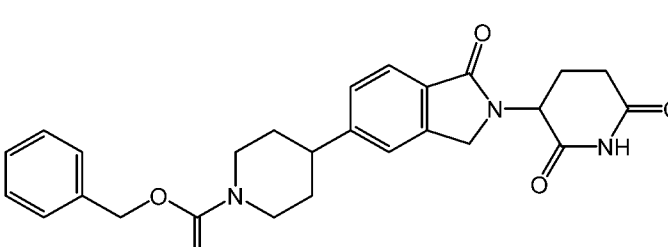
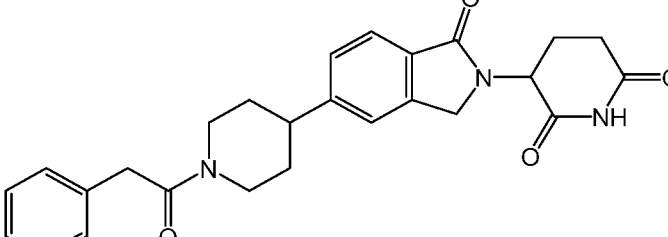
| Cmpd No.     | Structure                                                                           | Compound Name                                                                                              |
|--------------|-------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------|
| <b>I-200</b> |    | 3-(5-(1-(benzo[d]thiazol-2-ylmethyl)piperidin-4-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione               |
| <b>I-201</b> |   | 3-(1-oxo-5-(1-((3-(pyridin-2-yl)-1H-pyrazol-5-yl)methyl)piperidin-4-yl)isindolin-2-yl)piperidine-2,6-dione |
| <b>I-202</b> |  | 3-(5-(1-((R)-2-hydroxy-1-phenylethyl)piperidin-4-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione              |
| <b>I-203</b> |  | 3-(5-(1-((1-methyl-1H-indazol-3-yl)methyl)piperidin-4-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione         |

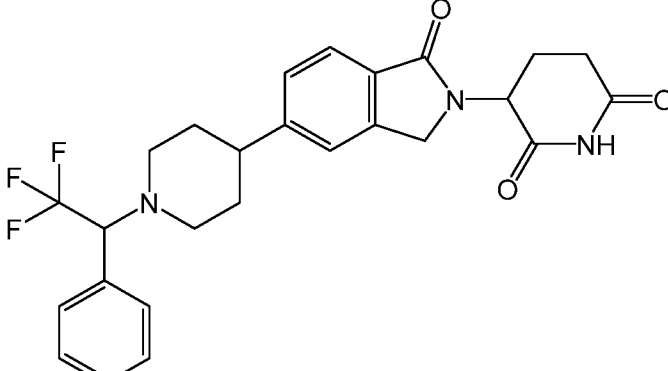
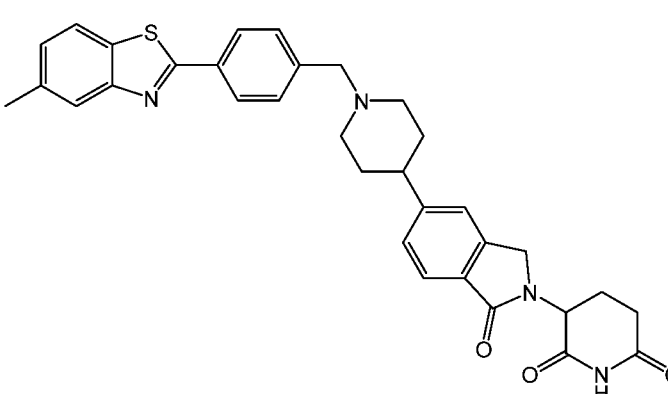
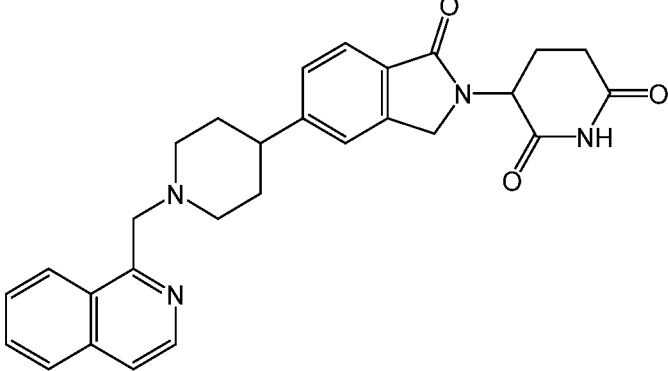
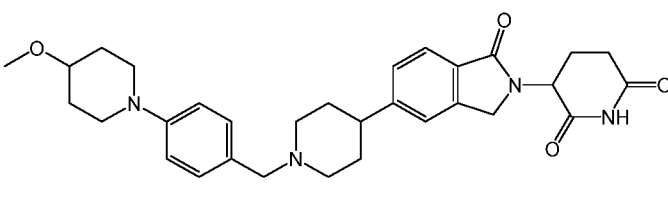


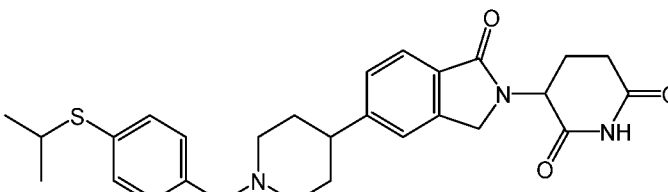
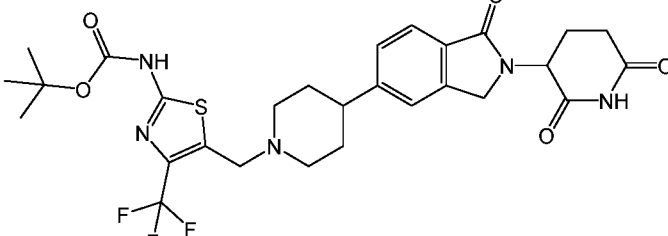
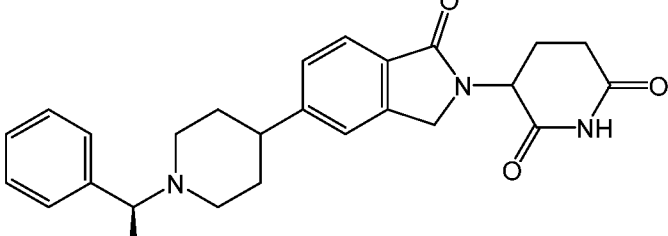
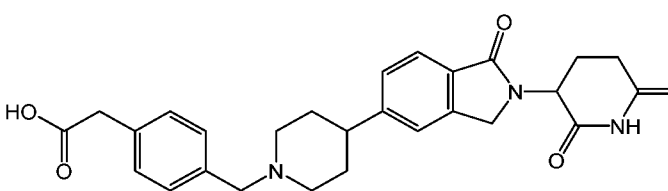
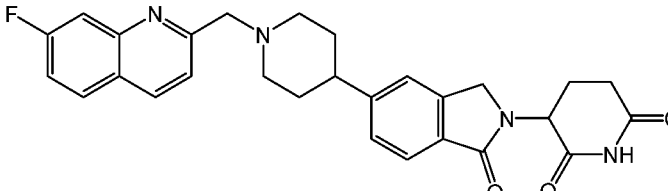
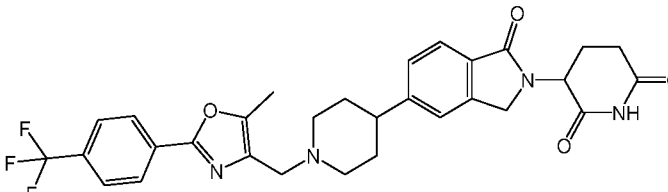
| Cmpd No.     | Structure                                                                           | Compound Name                                                                                                             |
|--------------|-------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------|
| <b>I-204</b> |    | 3-(5-(1-((1,2,4-oxadiazol-3-yl)methyl)piperidin-4-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione                            |
| <b>I-205</b> |    | 3-(5-(1-(4-hydroxy-3-((4-methylpiperazin-1-yl)methyl)benzyl)piperidin-4-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione      |
| <b>I-206</b> |   | 2-(4-((4-(2-(2,6-dioxopiperidin-3-yl)-1-oxoisindolin-5-yl)piperidin-1-yl)methyl)phenyl)acetonitrile                       |
| <b>I-207</b> |  | 3-(5-(1-((2-(4-chlorophenyl)-5-methyloxazol-4-yl)methyl)piperidin-4-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione          |
| <b>I-208</b> |  | 3-(5-(1-((7-hydroxy-2-methylpyrazolo[1,5-a]pyrimidin-5-yl)methyl)piperidin-4-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione |

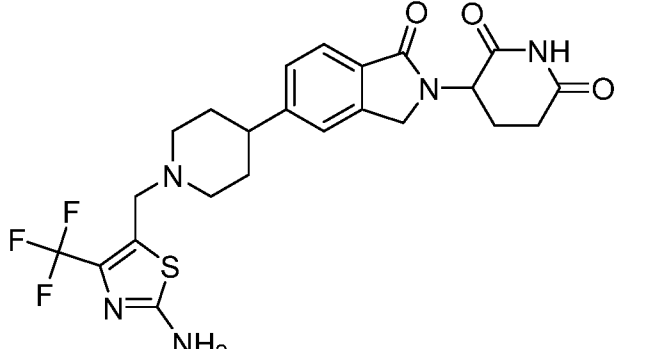
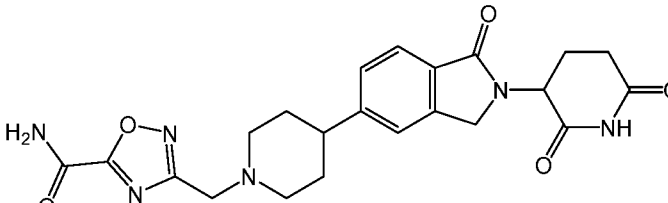
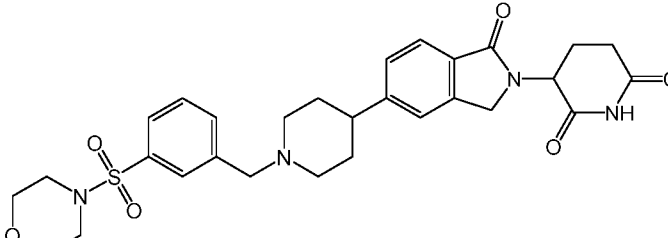
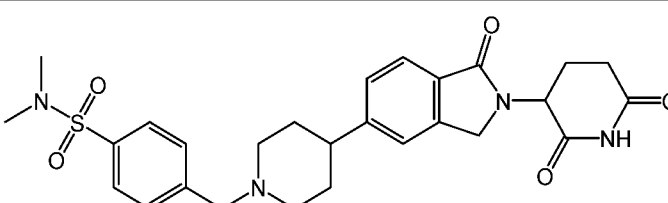
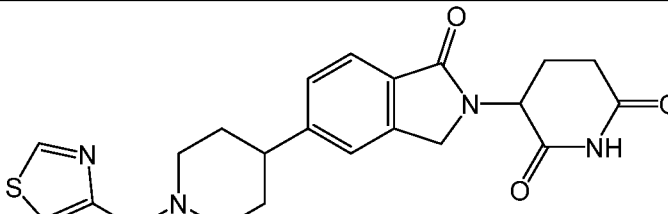
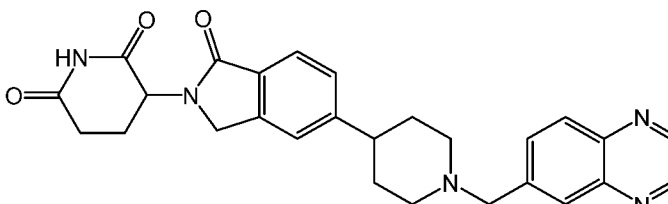
| Cmpd No.     | Structure                                                                           | Compound Name                                                                                               |
|--------------|-------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------|
| <b>I-209</b> |    | 3-(5-(1-(2,2-difluoro-1-phenylethyl)piperidin-4-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione                |
| <b>I-210</b> |    | 3-(5-(1-((3-fluorobicyclo[1.1.1]pentan-1-yl)methyl)piperidin-4-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione |
| <b>I-211</b> |   | 3-(1-oxo-5-(1-((2-phenylthiazol-4-yl)methyl)piperidin-4-yl)isindolin-2-yl)piperidine-2,6-dione              |
| <b>I-212</b> |  | 3-(5-(1-(2-fluoro-1-phenylethyl)piperidin-4-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione                    |

| Cmpd No.     | Structure                                                                           | Compound Name                                                                                                           |
|--------------|-------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------|
| <b>I-213</b> |    | 3-(1-oxo-5-(1-((4-oxo-3,4-dihydrothieno[3,2-d]pyrimidin-2-yl)methyl)piperidin-4-yl)isoindolin-2-yl)piperidine-2,6-dione |
| <b>I-214</b> |   | 3-(1-oxo-5-(1-(quinolin-4-ylmethyl)piperidin-4-yl)isoindolin-2-yl)piperidine-2,6-dione                                  |
| <b>I-215</b> |  | 3-(5-(1-(3,5-bis(trifluoromethyl)benzyl)piperidin-4-yl)-1-oxoisoindolin-2-yl)piperidine-2,6-dione                       |
| <b>I-216</b> |  | 3-((4-(2-(2,6-dioxopiperidin-3-yl)-1-oxoisoindolin-5-yl)piperidin-1-yl)methyl)-N,N-dimethylbenzenesulfonamide           |
| <b>I-217</b> |  | 6-((4-(2-(2,6-dioxopiperidin-3-yl)-1-oxoisoindolin-5-yl)piperidin-1-yl)methyl)picolinonitrile                           |

| Cmpd No.     | Structure                                                                           | Compound Name                                                                                                           |
|--------------|-------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------|
| <b>I-218</b> |    | 2-((4-((4-(2-(2,6-dioxopiperidin-3-yl)-1-oxoisindolin-5-yl)piperidin-1-yl)methyl)phenoxy)acetonitrile                   |
| <b>I-219</b> |    | 3-(5-(1-((1H-indazol-5-yl)methyl)piperidin-4-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione                               |
| <b>I-220</b> |   | 3-(5-(1-(2,2-difluoroethyl)piperidin-4-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione                                     |
| <b>I-221</b> |  | 3-(5-(1-((7-methyl-4-oxo-4H-pyrido[1,2-a]pyrimidin-2-yl)methyl)piperidin-4-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione |
| <b>I-222</b> |  | benzyl 4-(2-(2,6-dioxopiperidin-3-yl)-1-oxoisindolin-5-yl)piperidine-1-carboxylate                                      |
| <b>I-223</b> |  | 3-(1-oxo-5-(1-(2-phenylacetyl)piperidin-4-yl)isindolin-2-yl)piperidine-2,6-dione                                        |

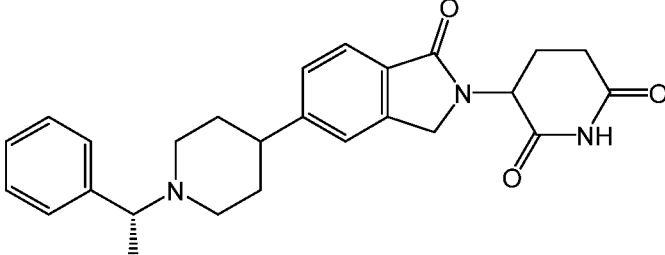
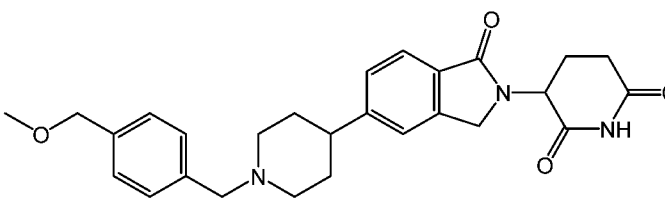
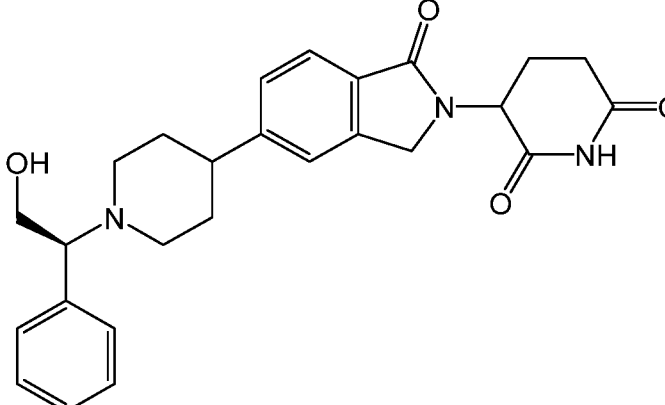
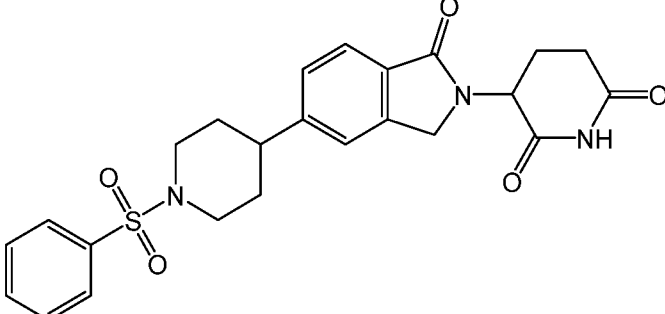
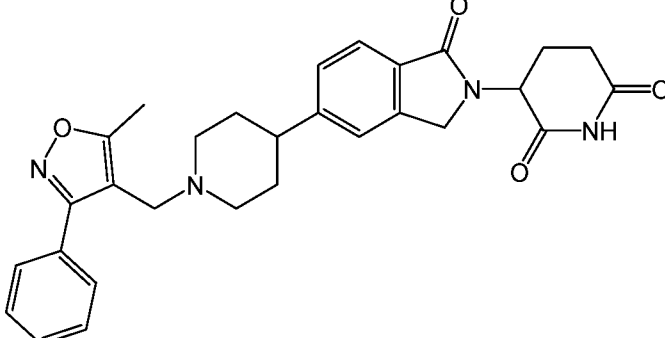
| Cmpd No.     | Structure                                                                           | Compound Name                                                                                            |
|--------------|-------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------|
| <b>I-224</b> |    | 3-(1-oxo-5-(1-(2,2,2-trifluoro-1-phenylethyl)piperidin-4-yl)isoindolin-2-yl)piperidine-2,6-dione         |
| <b>I-225</b> |   | 3-(5-(1-(4-(5-methylbenzo[d]thiazol-2-yl)benzyl)piperidin-4-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione |
| <b>I-226</b> |  | 3-(5-(1-(isoquinolin-1-ylmethyl)piperidin-4-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione                 |
| <b>I-227</b> |  | 3-(5-(1-(4-(4-methoxypiperidin-1-yl)benzyl)piperidin-4-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione      |

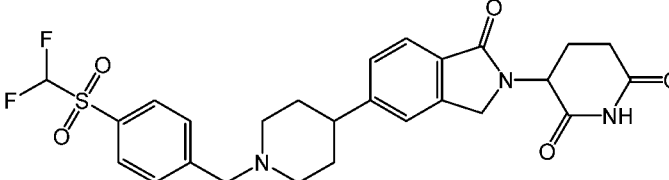
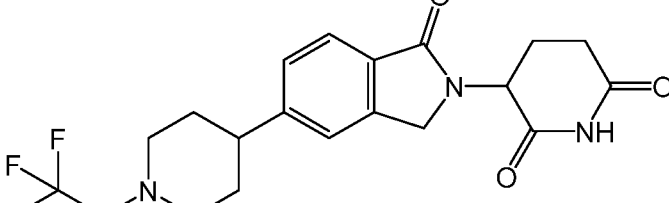
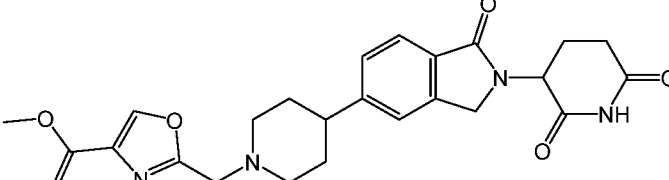
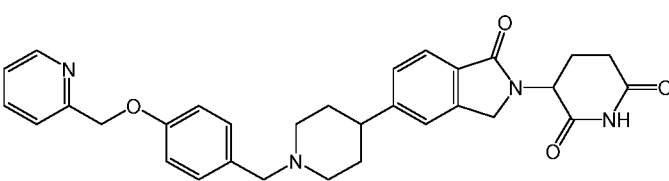
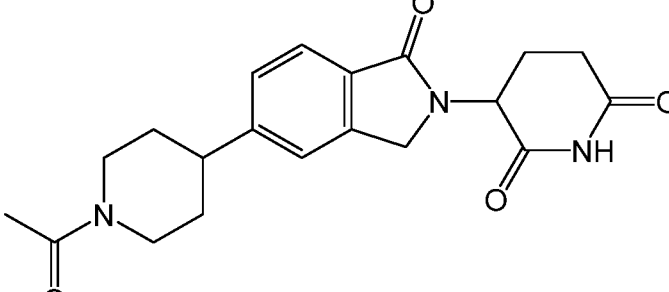
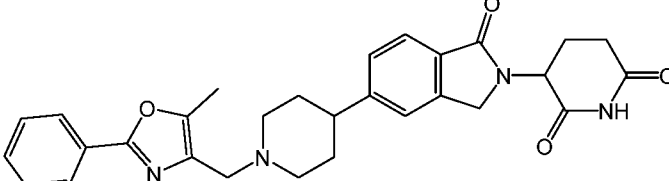
| Cmpd No.     | Structure                                                                           | Compound Name                                                                                                                               |
|--------------|-------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------|
| <b>I-228</b> |    | 3-(5-(1-(4-(isopropylthio)benzyl)piperidin-4-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione                                                   |
| <b>I-229</b> |    | <i>tert</i> -butyl (5-((4-(2-(2,6-dioxopiperidin-3-yl)-1-oxoisindolin-5-yl)piperidin-1-yl)methyl)-4-(trifluoromethyl)thiazol-2-yl)carbamate |
| <b>I-230</b> |   | 3-(1-oxo-5-(1-((S)-1-phenylethyl)piperidin-4-yl)isindolin-2-yl)piperidine-2,6-dione                                                         |
| <b>I-231</b> |  | 2-(4-((4-(2-(2,6-dioxopiperidin-3-yl)-1-oxoisindolin-5-yl)piperidin-1-yl)methyl)phenyl)acetic acid                                          |
| <b>I-232</b> |  | 3-(5-(1-((7-fluoroquinolin-2-yl)methyl)piperidin-4-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione                                             |
| <b>I-233</b> |  | 3-(5-(1-((5-methyl-2-(4-(trifluoromethyl)phenyl)oxazol-4-yl)methyl)piperidin-4-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione                 |

| Cmpd No.     | Structure                                                                           | Compound Name                                                                                                     |
|--------------|-------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------|
| <b>I-234</b> |    | 3-(5-(1-((2-amino-4-(trifluoromethyl)thiazol-5-yl)methyl)piperidin-4-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione |
| <b>I-235</b> |    | 3-((4-(2-(2,6-dioxopiperidin-3-yl)-1-oxoisindolin-5-yl)piperidin-1-yl)methyl)-1,2,4-oxadiazole-5-carboxamide      |
| <b>I-236</b> |   | 3-(5-(1-(3-(morpholinosulfonyl)benzyl)piperidin-4-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione                    |
| <b>I-237</b> |  | 4-((4-(2-(2,6-dioxopiperidin-3-yl)-1-oxoisindolin-5-yl)piperidin-1-yl)methyl)-N,N-dimethylbenzenesulfonamide      |
| <b>I-238</b> |  | 3-(1-oxo-5-(1-(thiazol-4-ylmethyl)piperidin-4-yl)isindolin-2-yl)piperidine-2,6-dione                              |
| <b>I-239</b> |  | 3-(1-oxo-5-(1-(quinoxalin-6-ylmethyl)piperidin-4-yl)isindolin-2-yl)piperidine-2,6-dione                           |

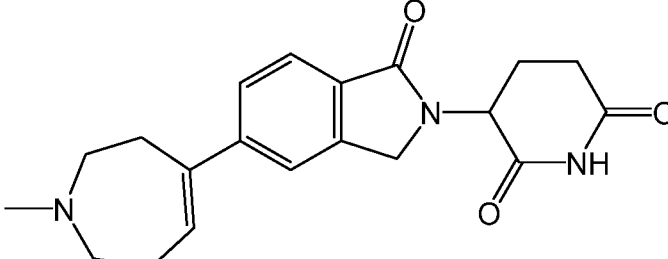
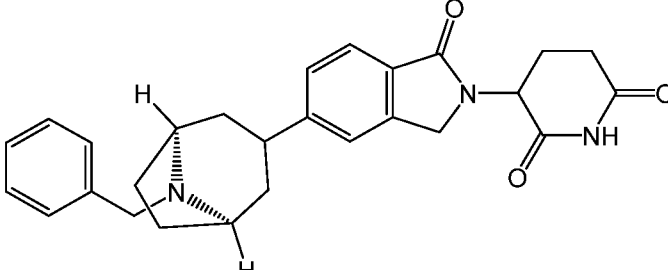
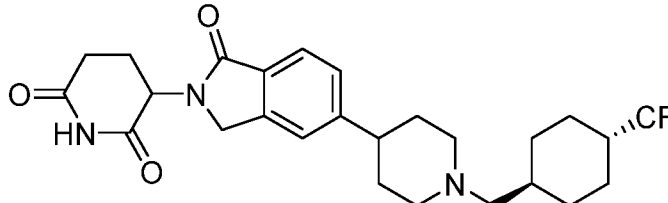
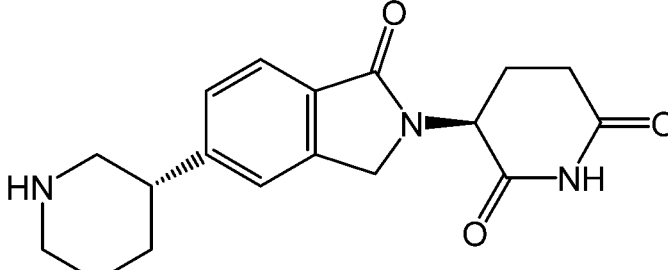
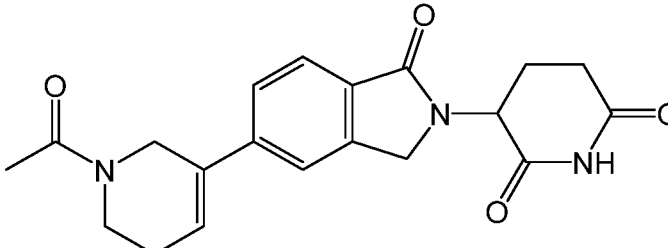
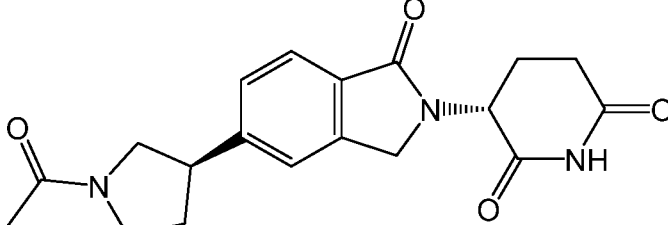
| Cmpd No.     | Structure | Compound Name                                                                                                                 |
|--------------|-----------|-------------------------------------------------------------------------------------------------------------------------------|
| <b>I-240</b> |           | 3-(5-(1-((2-(4-fluorophenyl)-5-methyloxazol-4-yl)methyl)piperidin-4-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione              |
| <b>I-241</b> |           | 3-(1-oxo-5-(1-((3-(m-tolyl)-1,2,4-oxadiazol-5-yl)methyl)piperidin-4-yl)isoindolin-2-yl)piperidine-2,6-dione                   |
| <b>I-242</b> |           | 3-(5-(1-(4-(tert-butyl)benzoyl)piperidin-4-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione                                       |
| <b>I-243</b> |           | 3-(1-oxo-5-(1-((5-(4-(trifluoromethyl)phenyl)-1,2,4-oxadiazol-3-yl)methyl)piperidin-4-yl)isoindolin-2-yl)piperidine-2,6-dione |
| <b>I-244</b> |           | 3-(5-(1-(4-((4-fluorobenzyl)oxy)benzyl)piperidin-4-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione                               |
| <b>I-245</b> |           | 3-(5-(1-((3-methylisoxazol-5-yl)methyl)piperidin-4-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione                               |
| <b>I-246</b> |           | 3-(5-(1-(isoxazol-3-yl)methyl)piperidin-4-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione                                        |

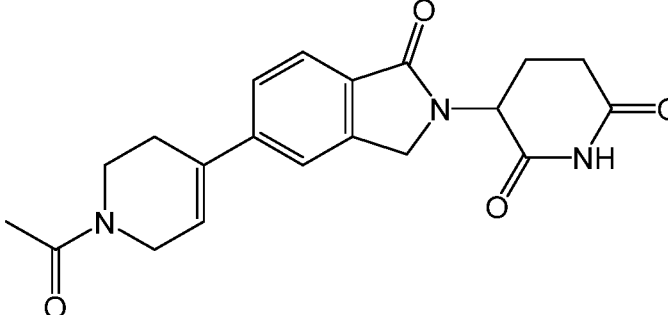
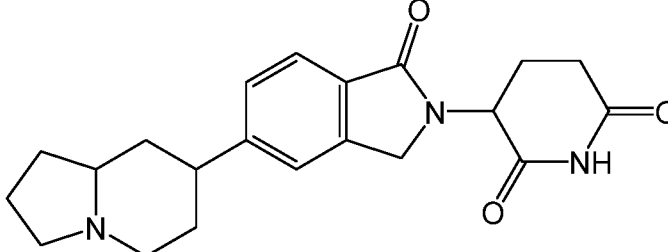
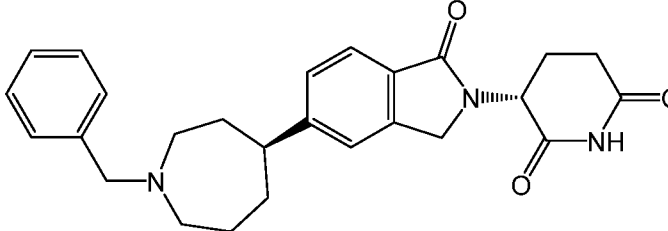
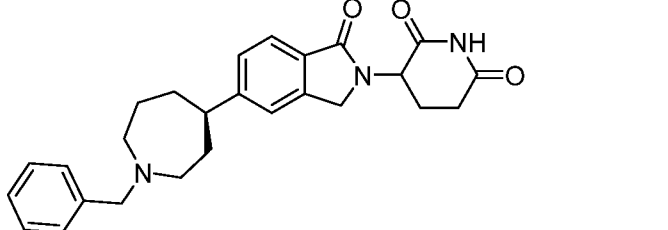
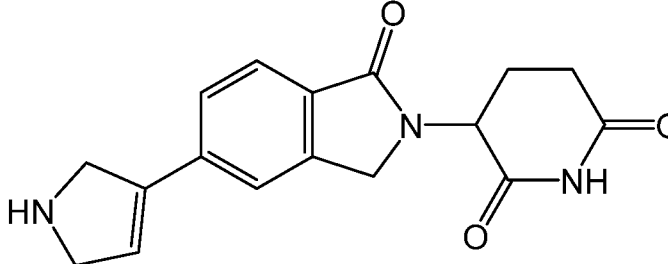
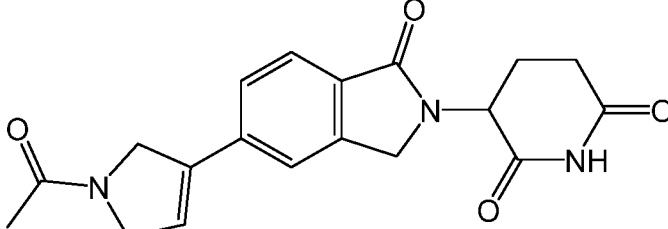


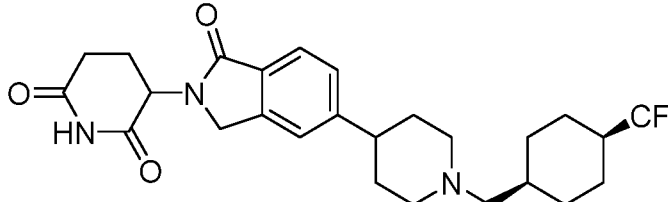
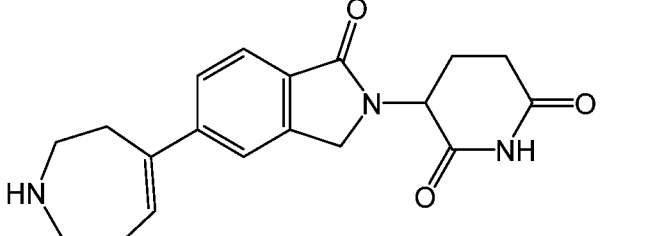
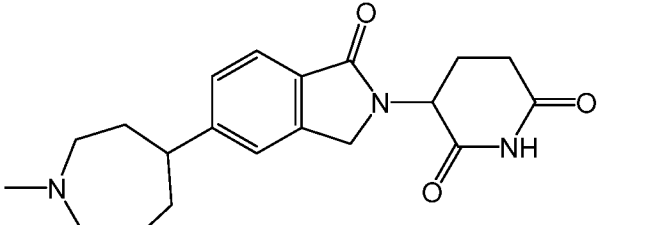
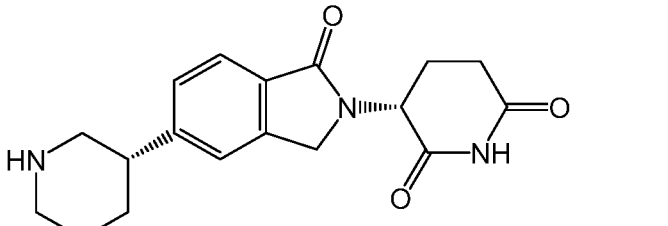
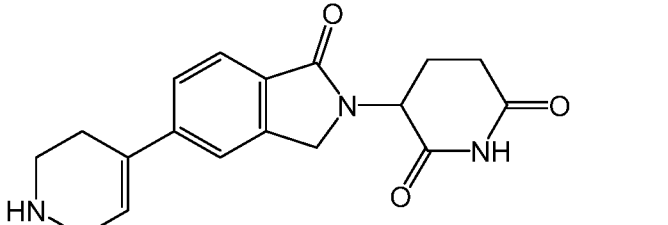
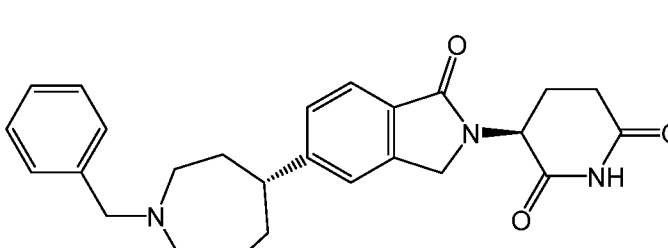
| Cmpd No. | Structure                                                                           | Compound Name                                                                                            |
|----------|-------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------|
| I-247    |    | 3-(1-oxo-5-(1-((R)-1-phenylethyl)piperidin-4-yl)isoindolin-2-yl)piperidine-2,6-dione                     |
| I-248    |    | 3-(5-(1-(4-(methoxymethyl)benzyl)piperidin-4-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione                |
| I-249    |   | 3-(5-(1-((S)-2-hydroxy-1-phenylethyl)piperidin-4-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione            |
| I-250    |  | 3-(1-oxo-5-(1-(phenylsulfonyl)piperidin-4-yl)isoindolin-2-yl)piperidine-2,6-dione                        |
| I-251    |  | 3-(5-(1-((5-methyl-3-phenylisoxazol-4-yl)methyl)piperidin-4-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione |

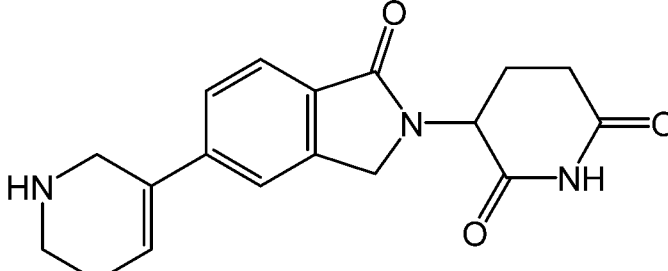
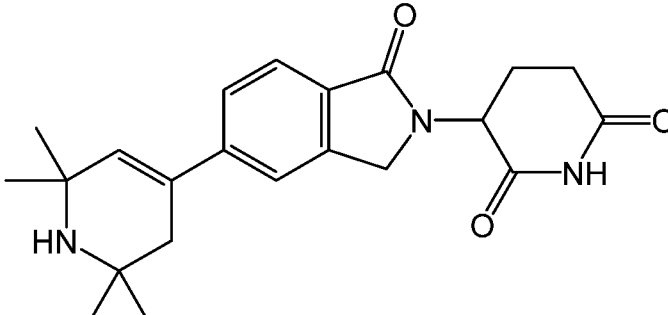
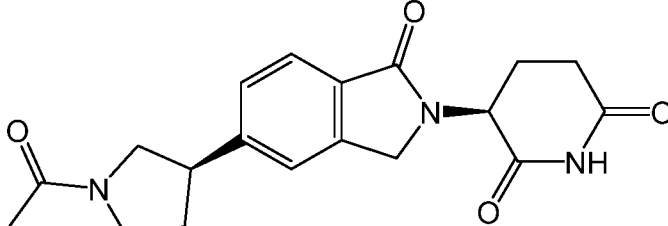
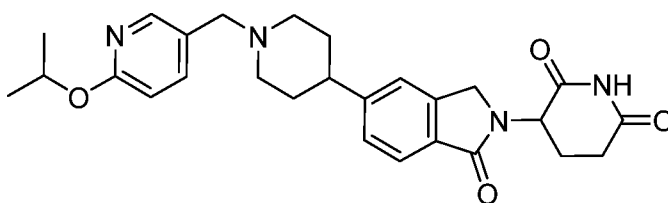
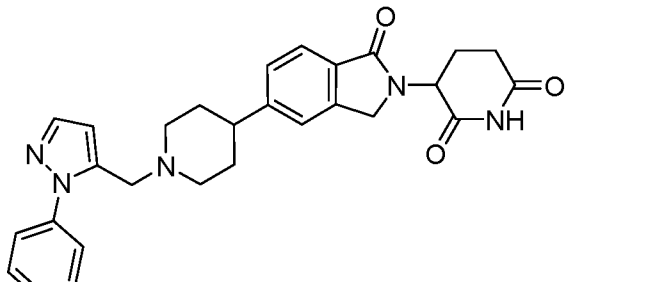
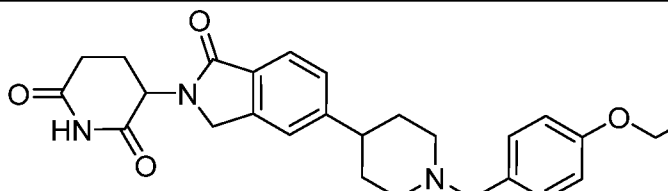
| Cmpd No.     | Structure                                                                           | Compound Name                                                                                             |
|--------------|-------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------|
| <b>I-252</b> |    | 3-(5-(1-(4-((difluoromethyl)sulfonyl)benzyl)piperidin-4-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione      |
| <b>I-253</b> |    | 3-(1-oxo-5-(1-(2,2,2-trifluoroethyl)piperidin-4-yl)isindolin-2-yl)piperidine-2,6-dione                    |
| <b>I-254</b> |   | methyl 2-((4-(2-(2,6-dioxopiperidin-3-yl)-1-oxoisindolin-5-yl)piperidin-1-yl)methyl)oxazole-4-carboxylate |
| <b>I-255</b> |  | 3-(1-oxo-5-(1-(4-(pyridin-2-ylmethoxy)benzyl)piperidin-4-yl)isindolin-2-yl)piperidine-2,6-dione           |
| <b>I-256</b> |  | 3-(5-(1-acetylpiperidin-4-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione                                    |
| <b>I-257</b> |  | 3-(5-(1-((5-methyl-2-phenyloxazol-4-yl)methyl)piperidin-4-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione    |

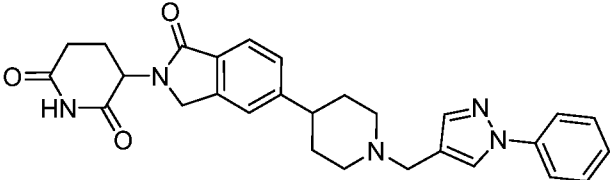
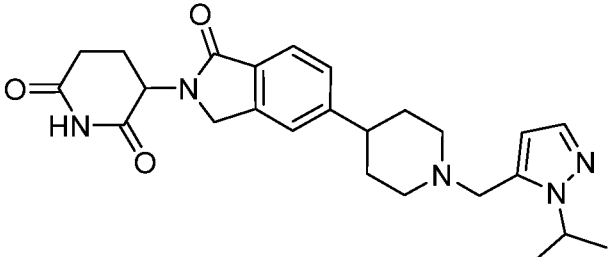
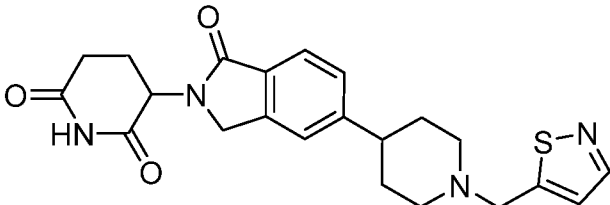
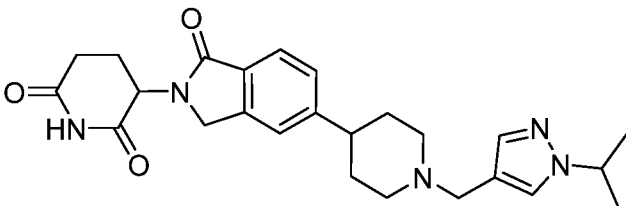
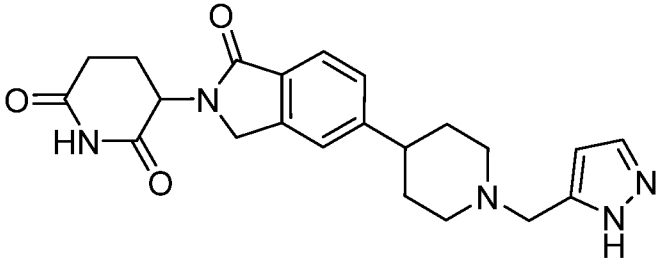
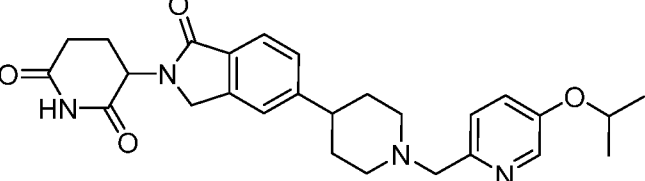
| Cmpd No.     | Structure | Compound Name                                                                                                        |
|--------------|-----------|----------------------------------------------------------------------------------------------------------------------|
| <b>I-258</b> |           | 3-(5-(1-((3-cyclohexylisoxazol-5-yl)methyl)piperidin-4-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione                  |
| <b>I-259</b> |           | 3-(1-oxo-5-(1-((2-oxo-2,3-dihydro-1H-benzo[d]imidazol-5-yl)methyl)piperidin-4-yl)isindolin-2-yl)piperidine-2,6-dione |
| <b>I-260</b> |           | 3-(5-(1-benzylpyrrolidin-3-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione                                              |
| <b>I-261</b> |           | (R)-3-(5-((R)-1-benzylazepan-4-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione                                          |
| <b>I-262</b> |           | (S)-3-(5-((S)-1-benzylazepan-4-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione                                          |
| <b>I-263</b> |           | 3-(5-(1-benzylazepan-4-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione                                                  |

| Cmpd No.     | Structure                                                                           | Compound Name                                                                                                         |
|--------------|-------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------|
| <b>I-264</b> |    | 3-(5-(1-methyl-2,3,6,7-tetrahydro-1H-azepin-4-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione                            |
| <b>I-265</b> |    | 3-(5-(8-benzyl-8-azabicyclo[3.2.1]octan-3-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione                                |
| <b>I-266</b> |   | <i>trans</i> -3-(1-oxo-5-(1-((4-(trifluoromethyl)cyclohexyl)methyl)piperidin-4-yl)isindolin-2-yl)piperidine-2,6-dione |
| <b>I-267</b> |  | (S)-3-(1-oxo-5-((S)-piperidin-3-yl)isindolin-2-yl)piperidine-2,6-dione                                                |
| <b>I-268</b> |  | 3-(5-(1-acetyl-1,2,5,6-tetrahydropyridin-3-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione                               |
| <b>I-269</b> |  | (R)-3-(5-((R)-1-acetylpyrrolidin-3-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione                                       |

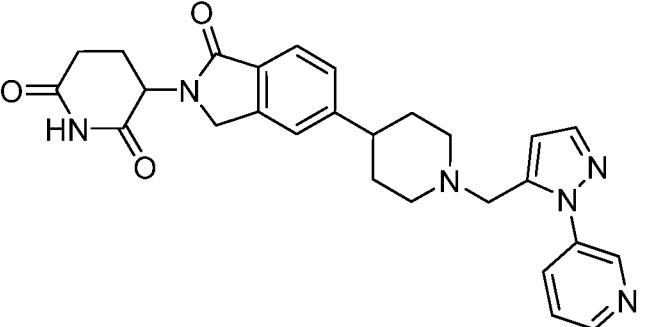
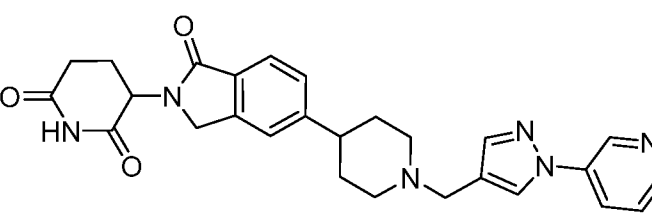
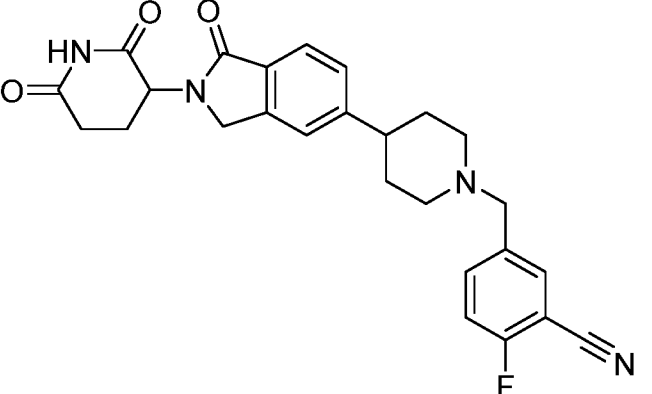
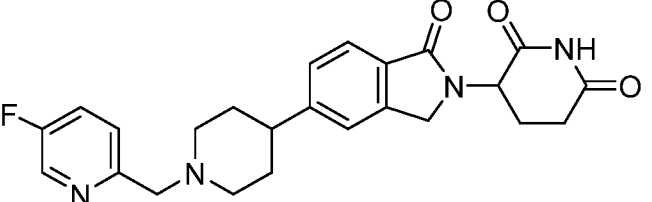
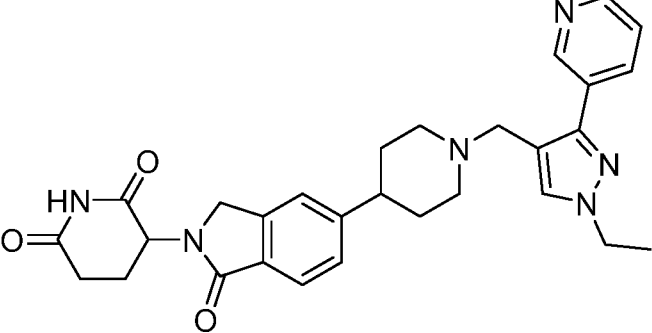
| Cmpd No.     | Structure                                                                           | Compound Name                                                                           |
|--------------|-------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------|
| <b>I-270</b> |    | 3-(5-(1-acetyl-1,2,3,6-tetrahydropyridin-4-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione |
| <b>I-271</b> |    | 3-(5-(octahydroindolizin-7-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione                 |
| <b>I-272</b> |   | (R)-3-(5-((S)-1-benzylazepan-4-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione             |
| <b>I-273</b> |  | 3-(5-((R)-1-benzylazepan-4-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione                 |
| <b>I-274</b> |  | 3-(5-(2,5-dihydro-1H-pyrrol-3-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione              |
| <b>I-275</b> |  | 3-(5-(1-acetyl-2,5-dihydro-1H-pyrrol-3-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione     |

| Cmpd No.     | Structure                                                                           | Compound Name                                                                                                        |
|--------------|-------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------|
| <b>I-276</b> |    | <i>cis</i> -3-(1-oxo-5-(1-((4-(trifluoromethyl)cyclohexyl)methyl)piperidin-4-yl)isoindolin-2-yl)piperidine-2,6-dione |
| <b>I-277</b> |    | 3-(1-oxo-5-(2,3,6,7-tetrahydro-1H-azepin-4-yl)isoindolin-2-yl)piperidine-2,6-dione                                   |
| <b>I-278</b> |   | 3-(5-(1-methylazepan-4-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione                                                  |
| <b>I-279</b> |  | (R)-3-(1-oxo-5-((S)-piperidin-3-yl)isoindolin-2-yl)piperidine-2,6-dione                                              |
| <b>I-280</b> |  | 3-(1-oxo-5-(1,2,3,6-tetrahydropyridin-4-yl)isoindolin-2-yl)piperidine-2,6-dione                                      |
| <b>I-281</b> |  | (S)-3-(5-((R)-1-benzylazepan-4-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione                                          |

| Cmpd No. | Structure                                                                           | Compound Name                                                                                        |
|----------|-------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------|
| I-282    |    | 3-(1-oxo-5-(1,2,5,6-tetrahydropyridin-3-yl)isoindolin-2-yl)piperidine-2,6-dione                      |
| I-283    |    | 3-(1-oxo-5-(2,2,6,6-tetramethyl-1,2,3,6-tetrahydropyridin-4-yl)isoindolin-2-yl)piperidine-2,6-dione  |
| I-284    |   | (S)-3-(5-((R)-1-acetylpyrrolidin-3-yl)-1-oxoisoindolin-2-yl)piperidine-2,6-dione                     |
| I-285    |  | 3-(5-(1-((6-isopropoxy)pyridin-3-yl)methyl)piperidin-4-yl)-1-oxoisoindolin-2-yl)piperidine-2,6-dione |
| I-286    |  | 3-(1-oxo-5-(1-((1-phenyl-1H-pyrazol-5-yl)methyl)piperidin-4-yl)isoindolin-2-yl)piperidine-2,6-dione  |
| I-287    |  | 3-(5-(1-(4-ethoxybenzyl)piperidin-4-yl)-1-oxoisoindolin-2-yl)piperidine-2,6-dione                    |

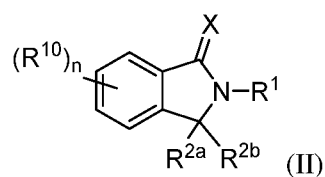
| Cmpd No.     | Structure                                                                           | Compound Name                                                                                         |
|--------------|-------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------|
| <b>I-288</b> |    | 3-(1-oxo-5-(1-((1-phenyl-1H-pyrazol-4-yl)methyl)piperidin-4-yl)isindolin-2-yl)piperidine-2,6-dione    |
| <b>I-289</b> |    | 3-(5-(1-((1-isopropyl-1H-pyrazol-5-yl)methyl)piperidin-4-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione |
| <b>I-290</b> |   | 3-(5-(1-(isothiazol-5-yl)methyl)piperidin-4-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione              |
| <b>I-291</b> |  | 3-(5-(1-((1-isopropyl-1H-pyrazol-4-yl)methyl)piperidin-4-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione |
| <b>I-292</b> |  | 3-(5-(1-((1H-pyrazol-5-yl)methyl)piperidin-4-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione             |
| <b>I-293</b> |  | 3-(5-(1-((5-isopropoxy)pyridin-2-yl)methyl)piperidin-4-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione   |



| Cmpd No.     | Structure                                                                           | Compound Name                                                                                                      |
|--------------|-------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------|
| <b>I-294</b> |    | 3-(1-oxo-5-(1-((1-(pyridin-3-yl)-1H-pyrazol-5-yl)methyl)piperidin-4-yl)isoindolin-2-yl)piperidine-2,6-dione        |
| <b>I-295</b> |    | 3-(1-oxo-5-(1-((1-(pyridin-3-yl)-1H-pyrazol-4-yl)methyl)piperidin-4-yl)isoindolin-2-yl)piperidine-2,6-dione        |
| <b>I-296</b> |   | 5-((4-(2-(2,6-dioxopiperidin-3-yl)-1-oxoisindolin-5-yl)piperidin-1-yl)methyl)-2-fluorobenzonitrile                 |
| <b>I-297</b> |  | 3-(5-(1-((5-fluoropyridin-2-yl)methyl)piperidin-4-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione                     |
| <b>I-298</b> |  | 3-(5-(1-((1-ethyl-3-(pyridin-3-yl)-1H-pyrazol-4-yl)methyl)piperidin-4-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione |

| Cmpd No. | Structure | Compound Name                                                                                                        |
|----------|-----------|----------------------------------------------------------------------------------------------------------------------|
| I-299    |           | 3-(5-(1-((6-methoxypyridin-2-yl)methyl)piperidin-4-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione                      |
| I-300    |           | 3-(5-(1-((3-((3S,5S)-adamantan-1-yl)-1H-pyrazol-5-yl)methyl)piperidin-4-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione |
| I-301    |           | 3-(5-(1-((6-isopropoxy-pyridin-2-yl)methyl)piperidin-4-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione                  |
| I-302    |           | 3-(5-(1-((1-benzyl-5-(pyridin-2-yl)-1H-pyrazol-3-yl)methyl)piperidin-4-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione  |
| I-303    |           | <i>trans</i> -3-(5-(1-((4-methoxycyclohexyl)methyl)piperidin-4-yl)-1-oxoisindolin-2-yl)piperidine-2,6-dione          |

In another aspect, the degradation compound is a compound of Formula (II):



or a pharmaceutically acceptable salt, ester, hydrate, tautomer, or prodrug thereof, wherein:

R<sup>1</sup> is C<sub>1</sub>-C<sub>6</sub> alkyl, C<sub>2</sub>-C<sub>6</sub> alkenyl, C<sub>2</sub>-C<sub>6</sub> alkynyl, C<sub>1</sub>-C<sub>6</sub> heteroalkyl, carbocyclyl, heterocyclyl, aryl, or heteroaryl, each of which is independently and optionally substituted by one or more R<sup>4</sup>;

each of R<sup>2a</sup> and R<sup>2b</sup> is independently hydrogen or C<sub>1</sub>-C<sub>6</sub> alkyl; or R<sup>2a</sup> and R<sup>2b</sup> together with the carbon atom to which they are attached to form carbonyl group or thiocarbonyl group;

5 each of R<sup>10</sup> is independently C<sub>1</sub>-C<sub>6</sub> alkyl, C<sub>2</sub>-C<sub>6</sub> alkenyl, C<sub>2</sub>-C<sub>6</sub> alkynyl, C<sub>1</sub>-C<sub>6</sub> heteroalkyl, halo, cyano, -C(O)R<sup>A</sup>, -C(O)OR<sup>B</sup>, -OR<sup>B</sup>, -N(R<sup>C</sup>)(R<sup>D</sup>), -C(O)N(R<sup>C</sup>)(R<sup>D</sup>), -N(R<sup>C</sup>)C(O)R<sup>A</sup>, -S(O)<sub>x</sub>R<sup>E</sup>, -S(O)<sub>x</sub>N(R<sup>C</sup>)(R<sup>D</sup>), or -N(R<sup>C</sup>)S(O)<sub>x</sub>R<sup>E</sup>, or L-Tag; wherein each alkyl, alkenyl, alkynyl, and heteroalkyl is independently and optionally substituted with one or more R<sup>11</sup>;

10 each R<sup>4</sup> is independently C<sub>1</sub>-C<sub>6</sub> alkyl, C<sub>2</sub>-C<sub>6</sub> alkenyl, C<sub>2</sub>-C<sub>6</sub> alkynyl, C<sub>1</sub>-C<sub>6</sub> heteroalkyl, halo, cyano, oxo, C(O)R<sup>A</sup>, -C(O)OR<sup>B</sup>, OR<sup>B</sup>, -N(R<sup>C</sup>)(R<sup>D</sup>), -C(O)N(R<sup>C</sup>)(R<sup>D</sup>), -N(R<sup>C</sup>)C(O)R<sup>A</sup>, S(O)<sub>x</sub>R<sup>E</sup>, -S(O)<sub>x</sub>N(R<sup>C</sup>)(R<sup>D</sup>), -N(R<sup>C</sup>)S(O)<sub>x</sub>R<sup>E</sup>, carbocyclyl, heterocyclyl, aryl, or heteroaryl, wherein each alkyl, alkenyl, alkynyl, heteroalkyl, carbocyclyl, heterocyclyl, aryl, and heteroaryl is independently and optionally substituted with one or more R<sup>7</sup>;

each of R<sup>A</sup>, R<sup>B</sup>, R<sup>C</sup>, R<sup>D</sup>, and R<sup>E</sup> is independently hydrogen or C<sub>1</sub>-C<sub>6</sub> alkyl;

15 each R<sup>11</sup> is independently C<sub>1</sub>-C<sub>6</sub> alkyl, halo, oxo, cyano, -OR<sup>B</sup>, -N(R<sup>C</sup>)(R<sup>D</sup>), -C(O)N(R<sup>C</sup>)(R<sup>D</sup>), -N(R<sup>C</sup>)C(O)R<sup>A</sup>, aryl, or heteroaryl, wherein each aryl and heteroaryl is independently and optionally substituted with one or more R<sup>8</sup>;

each R<sup>7</sup> is independently halo, oxo, cyano, -OR<sup>B</sup>, -N(R<sup>C</sup>)(R<sup>D</sup>), -C(O)N(R<sup>C</sup>)(R<sup>D</sup>), or -N(R<sup>C</sup>)C(O)R<sup>A</sup>;

20 each R<sup>8</sup> is independently C<sub>1</sub>-C<sub>6</sub> alkyl, halo, cyano, -OR<sup>B</sup>, -N(R<sup>C</sup>)(R<sup>D</sup>), -C(O)N(R<sup>C</sup>)(R<sup>D</sup>), or -N(R<sup>C</sup>)C(O)R<sup>A</sup>;

25 each L is independently C<sub>1</sub>-C<sub>6</sub> alkyl, C<sub>2</sub>-C<sub>6</sub> alkenyl, C<sub>2</sub>-C<sub>6</sub> alkynyl, C<sub>1</sub>-C<sub>6</sub> heteroalkyl, -C(O)R<sup>A1</sup>, -C(O)OR<sup>B1</sup>, -OR<sup>B1</sup>, -N(R<sup>C1</sup>)(R<sup>D1</sup>), -C(O)N(R<sup>C1</sup>)(R<sup>D1</sup>), -N(R<sup>C1</sup>)C(O)R<sup>A1</sup>, -S(O)<sub>x</sub>R<sup>E1</sup>, -S(O)<sub>x</sub>N(R<sup>C1</sup>)(R<sup>D1</sup>), or -N(R<sup>C1</sup>)S(O)<sub>x</sub>R<sup>E1</sup>, wherein each alkyl, alkenyl, alkynyl, and heteroalkyl is independently and optionally substituted with one or more R<sup>12</sup>;

each Tag is a targeting moiety capable of binding to a target protein;

30 each of R<sup>A1</sup>, R<sup>B1</sup>, R<sup>C1</sup>, R<sup>D1</sup>, and R<sup>E1</sup> is independently hydrogen, C<sub>1</sub>-C<sub>6</sub> alkyl, C<sub>2</sub>-C<sub>6</sub> alkenyl, C<sub>2</sub>-C<sub>6</sub> alkynyl, C<sub>1</sub>-C<sub>6</sub> heteroalkyl, carbocyclyl, heterocyclyl, aryl, or heteroaryl, wherein each alkyl, alkenyl, alkynyl, heteroalkyl, carbocyclyl, heterocyclyl, aryl, and heteroaryl is independently and optionally substituted with one or more R<sup>12</sup>;

each R<sup>12</sup> is independently C<sub>1</sub>-C<sub>6</sub> alkyl, halo, cyano, carbocyclyl, or heterocyclyl;

n is 0, 1, 2, 3 or 4; and

x is 0, 1, or 2.

In some embodiments, X is O.

In some embodiments,  $R^1$  is  $C_1$ - $C_6$  alkyl,  $C_2$ - $C_6$  alkenyl,  $C_2$ - $C_6$  alkynyl,  $C_1$ - $C_6$  heteroalkyl, carbocyclyl, heterocyclyl, aryl, or heteroaryl, each of which is independently and optionally substituted by 1-12  $R^4$  (e.g., 1  $R^4$ , 2  $R^4$ , 3  $R^4$ , 4  $R^4$ , 5  $R^4$ , 6  $R^4$ , 7  $R^4$ , 8  $R^4$ , 9  $R^4$ , 10  $R^4$ , 11  $R^4$ , or 12  $R^4$ ). In some embodiments,  $R^1$  is  $C_1$ - $C_6$  alkyl or heterocyclyl. In some embodiments,  $R^1$  is  $C_1$ - $C_6$  alkyl (e.g., methyl or ethyl) substituted by  $R^4$ . In some embodiments,  $R^1$  is  $C_1$ - $C_6$  alkyl (e.g., methyl or ethyl) substituted by 1-6  $R^4$ . In some embodiments,  $R^1$  is heterocyclyl. In some embodiments,  $R^1$  is a 6-membered heterocyclyl or a 5-membered heterocyclyl. In some embodiments,  $R^1$  is a 6-membered heterocyclyl or a 5-membered heterocyclyl optionally substituted with 1-6  $R^4$  (e.g., 1  $R^4$ , 2  $R^4$ , 3  $R^4$ , 4  $R^4$ , 5  $R^4$ , or 6  $R^4$ ). In some embodiments,  $R^1$  is a nitrogen-containing heterocyclyl. In some

embodiments,  $R^1$  is piperidinyl (e.g., piperidine-2,6-dionyl).

In some embodiments, each of  $R^{2a}$  and  $R^{2b}$  is independently hydrogen. In some embodiments,  $R^{2a}$  and  $R^{2b}$  together with the carbon to which they are attached form a carbonyl group.

In some embodiments, each  $R^{10}$  is independently  $C_1$ - $C_6$  alkyl,  $C_2$ - $C_6$  alkenyl,  $C_2$ - $C_6$  alkynyl,  $C_1$ - $C_6$  heteroalkyl, halo, cyano,  $-C(O)R^A$ ,  $-C(O)OR^B$ ,  $-OR^B$ ,  $-N(R^C)(R^D)$ ,  $-C(O)N(R^C)(R^D)$ ,  $-N(R^C)C(O)R^A$ ,  $-S(O)_xR^E$ ,  $-S(O)_xN(R^C)(R^D)$ , or  $-N(R^C)S(O)_xR^E$ , or L-Tag; wherein each alkyl, alkenyl, alkynyl, and heteroalkyl is independently and optionally substituted with 1-12  $R^{11}$  (e.g., 1  $R^{11}$ , 2  $R^{11}$ , 3  $R^{11}$ , 4  $R^{11}$ , 5  $R^{11}$ , 6  $R^{11}$ , 7  $R^{11}$ , 8  $R^{11}$ , 9  $R^{11}$ , 10  $R^{11}$ , 11  $R^{11}$ , or 12  $R^{11}$ ). In some embodiments,  $R^{10}$  is  $C_1$ - $C_6$  heteroalkyl,  $-N(R^C)(R^D)$  or  $-N(R^C)C(O)R^A$ . In some embodiments,  $R^{10}$  is  $C_1$ - $C_6$  heteroalkyl (e.g.,  $CH_2NHC(O)CH_2$ ),  $-N(R^C)(R^D)$  (e.g.,  $NH_2$ ), or  $-N(R^C)C(O)R^A$  (e.g.,  $NHC(O)CH_3$ ).

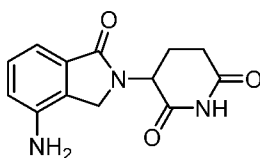
In some embodiments, each  $R^4$  is independently  $C_1$ - $C_6$  alkyl,  $C_2$ - $C_6$  alkenyl,  $C_2$ - $C_6$  alkynyl,  $C_1$ - $C_6$  heteroalkyl, halo, cyano, oxo,  $C(O)R^A$ ,  $-C(O)OR^B$ ,  $OR^B$ ,  $-N(R^C)(R^D)$ ,  $-C(O)N(R^C)(R^D)$ ,  $-N(R^C)C(O)R^A$ ,  $S(O)_xR^E$ ,  $-S(O)_xN(R^C)(R^D)$ ,  $-N(R^C)S(O)_xR^E$ , carbocyclyl, heterocyclyl, aryl, or heteroaryl, wherein each alkyl, alkenyl, alkynyl, heteroalkyl, carbocyclyl, heterocyclyl, aryl, and heteroaryl is independently and optionally substituted with 1-12  $R^7$  (e.g., 1  $R^7$ , 2  $R^7$ , 3  $R^7$ , 4  $R^7$ , 5  $R^7$ , 6  $R^7$ , 7  $R^7$ , 8  $R^7$ , 9  $R^7$ , 10  $R^7$ , 11  $R^7$ , or 12  $R^7$ ).

In some embodiments, each  $R^{11}$  is independently  $C_1$ - $C_6$  alkyl, halo, oxo, cyano,  $-OR^B$ ,  $-N(R^C)(R^D)$ ,  $-C(O)N(R^C)(R^D)$ ,  $-N(R^C)C(O)R^A$ , aryl, or heteroaryl, wherein each aryl and heteroaryl is independently and optionally substituted with 1-6  $R^8$  (e.g., 1  $R^8$ , 2  $R^8$ , 3  $R^8$ , 4  $R^8$ , 5  $R^8$ , or 6  $R^8$ ).

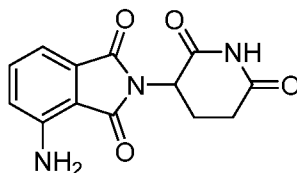
In some embodiments, each L is independently  $C_1$ - $C_6$  alkyl,  $C_2$ - $C_6$  alkenyl,  $C_2$ - $C_6$  alkynyl,  $C_1$ - $C_6$  heteroalkyl,  $-C(O)R^{A1}$ ,  $-C(O)OR^{B1}$ ,  $-OR^{B1}$ ,  $-N(R^{C1})(R^{D1})$ ,  $-C(O)N(R^{C1})(R^{D1})$ ,  $-N(R^{C1})C(O)R^{A1}$ ,  $-S(O)_xR^{E1}$ ,  $-S(O)_xN(R^{C1})(R^{D1})$ , or  $-N(R^{C1})S(O)_xR^{E1}$ , wherein each alkyl, alkenyl, alkynyl, and heteroalkyl is independently and optionally substituted with 1-12  $R^{12}$  (e.g., 1  $R^{12}$ , 2  $R^{12}$ , 3  $R^{12}$ , 4  $R^{12}$ , 5  $R^{12}$ , 6  $R^{12}$ , 7  $R^{12}$ , 8  $R^{12}$ , 9  $R^{12}$ , 10  $R^{12}$ , 11  $R^{12}$ , or 12  $R^{12}$ ).

In some embodiments, each of  $R^{A1}$ ,  $R^{B1}$ ,  $R^{C1}$ ,  $R^{D1}$ , and  $R^{E1}$  is independently hydrogen, C<sub>1</sub>-C<sub>6</sub> alkyl, C<sub>2</sub>-C<sub>6</sub> alkenyl, C<sub>2</sub>-C<sub>6</sub> alkynyl, C<sub>1</sub>-C<sub>6</sub> heteroalkyl, carbocyclyl, heterocyclyl, aryl, or heteroaryl, wherein each alkyl, alkenyl, alkynyl, heteroalkyl, carbocyclyl, heterocyclyl, aryl, and heteroaryl is independently and optionally substituted with 1-12  $R^{12}$  (e.g., 1  $R^{12}$ , 2  $R^{12}$ , 3  $R^{12}$ , 4  $R^{12}$ , 5  $R^{12}$ , 6  $R^{12}$ , 7  $R^{12}$ , 8  $R^{12}$ , 9  $R^{12}$ , 10  $R^{12}$ , 11  $R^{12}$ , or 12  $R^{12}$ ).

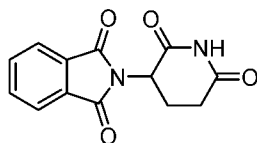
In an embodiment, X is O. In an embodiment,  $R^1$  is heterocyclyl (e.g., piperidine-2,6-dionyl). In an embodiment, each of  $R^{2a}$  and  $R^{2b}$  is independently hydrogen. In an embodiment, n is 1. In an embodiment,  $R^{10}$  is -N( $R^C$ )( $R^D$ ) (e.g., -NH<sub>2</sub>). In an embodiment, the degradation compound comprises lenalidomide, e.g., 3-(4-amino-1-oxoisindolin-2-yl)piperidine-2,6-dione, or a pharmaceutically acceptable salt thereof. In an embodiment, the degradation compound is lenalidomide, e.g., according to the following formula:



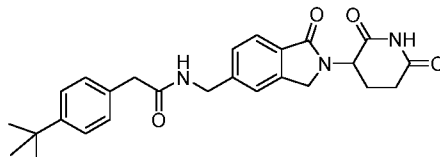
In an embodiment, X is O. In an embodiment,  $R^1$  is heterocyclyl (e.g., piperidinyl-2,6-dionyl). In some embodiments,  $R^{2a}$  and  $R^{2b}$  together with the carbon to which they are attached form a carbonyl group. In an embodiment, n is 1. In an embodiment,  $R^{10}$  is -N( $R^C$ )( $R^D$ ) (e.g., -NH<sub>2</sub>). In an embodiment, the degradation compound comprises pomalidomide, e.g., 4-amino-2-(2,6-dioxopiperidin-3-yl)isindoline-1,3-dione, or a pharmaceutically acceptable salt thereof. In an embodiment, the degradation compound is pomalidomide, e.g., according to the following formula:



In an embodiment, X is O. In an embodiment,  $R^1$  is heterocyclyl (e.g., piperidinyl-2,6-dionyl). In an embodiment,  $R^{2a}$  and  $R^{2b}$  together with the carbon to which they are attached form a carbonyl group. In an embodiment, n is 0. In an embodiment, the degradation compound comprises thalidomide, e.g., 2-(2,6-dioxopiperidin-3-yl)isindoline-1,3-dione, or a pharmaceutically acceptable salt thereof. In an embodiment, the degradation product is thalidomide, e.g., according to the following formula:



In an embodiment, X is O. In an embodiment, R<sup>1</sup> is heterocyclyl (e.g., piperidine-2,6-dionyl). In an embodiment, each of R<sup>2a</sup> and R<sup>2b</sup> is independently hydrogen. In an embodiment, n is 1. In an embodiment, R<sup>10</sup> is C<sub>1</sub>-C<sub>6</sub> heteroalkyl (e.g., CH<sub>2</sub>NHC(O)CH<sub>2</sub>-phenyl-t-butyl). In an embodiment, the degradation compound comprises 2-(4-(tert-butyl)phenyl)-N-((2-(2,6-dioxopiperidin-3-yl)-1-oxoisindolin-5-yl)methyl)acetamide, or a pharmaceutically acceptable salt thereof. In an embodiment, the degradation compound has the structure as shown in the following formula:



In some embodiments, the degradation compound (e.g., a compound of Formula (II)) is not attached to a linker or attachment group. In some embodiments, the degradation compound (e.g., a compound of Formula (II)) does not comprise another moiety, e.g., a ligand, a targeting agent, or a moiety capable of dimerization. In some embodiments, R<sup>10</sup> is not L-Tag.

In some embodiments, the degradation compound (e.g., a compound of Formula (II)) is attached to a linker or attachment group (e.g., at least one R<sup>10</sup> is L-Tag). In some embodiments, the degradation compound (e.g., a compound of Formula (II)) comprises another moiety, e.g., a ligand, a targeting agent, or a moiety capable of dimerization. In some embodiments, R<sup>10</sup> is L-Tag, and L is alkyl or heteroalkyl (e.g., a PEG chain). In some embodiments, L is a linker selected from a linker disclosed in International Patent Publication No. WO2017/024318 (e.g., Figures 28-31).

In some embodiments, R<sup>10</sup> is L-Tag, and Tag is a targeting moiety that is capable of binding or is bound to a target protein. A Tag may comprise a small molecule compound or an amino acid sequence (e.g., a peptide or polypeptide). In some embodiments, the Tag is a kinase inhibitor, a BET bromodomain-containing protein inhibitor, cytosolic signaling protein FKBP12 ligand, an HDAC inhibitor, a lysine methyltransferase inhibitor, an angiogenesis inhibitor, an immunosuppressive compound, or an aryl hydrocarbon receptor (AHR) inhibitor.

In certain embodiments, the Tag is a SERM (selective estrogen receptor modulator) or SERD (selective estrogen receptor degrader). Non-limiting examples of SERMs and SERDs are provided in International Patent Publication Nos. WO2014/191726, WO2013/090921, WO2014/203129, WO2014/205136, WO2014/205138, and WO 2014/203132; U.S. Patent Publication Nos. US2013/0178445 and US 2015/0005286; and U.S. Patent Nos. 9,078,871, 8,853,423, and 8,703,810.

Additional Tags include, for example, any moiety which binds to an endogenous protein (binds to a target protein). Exemplary Tags include Hsp90 inhibitors, kinase inhibitors, HDM2 and MDM2

inhibitors, compounds targeting human BET bromodomain-containing proteins, HDAC inhibitors, human lysine methyltransferase inhibitors, angiogenesis inhibitors, nuclear hormone receptor compounds, immunosuppressive compounds, and compounds targeting the aryl hydrocarbon receptor (AHR), among numerous others. Such small molecule Tags also include pharmaceutically acceptable salts, enantiomers, solvates and polymorphs of these compositions, as well as other small molecules that may bind to a target protein of interest.

In an embodiment, the Tag is an Ubc9 SUMO E2 ligase 5F6D targeting ligand, e.g., as described in Hewitt, W.M., et. al. (2016) *Angew.Chem.Int.Ed.Engl.*55: 5703-5707

In an embodiment, the Tag is a Tank1 targeting ligand, e.g., as described in Kirby, C.A. et al, (2012) *Acta Crystallogr. Sect.F* 68: 115-118; and Shultz, M.D., et al. (2013) *J.Med.Chem.*56: 7049-7059.

In an embodiment, the Tag is an SH2 domain of pp60 Src targeting ligand, e.g., as described in Gudrun Lange, et al., (2003) *J. Med. Chem.* 46, 5184-5195.

In an embodiment, the Tag is a Sec7 domain targeting ligand, e.g., as described in Huta, B.P., et al., (2016) *Chemmedchem* 11: 277.

In an embodiment, the Tag is a Saposin-B targeting ligand, e.g., as described in I. Nemcovicova and D. M. Zajonc *Acta Cryst.* (2014). D70, 851-862.

In an embodiment, the Tag is a protein S100-A7 2OWS targeting ligand, e.g., as described in Leon, R., Murray, et al., (2009) *Biochemistry* 48: 10591-10600.

In an embodiment, the Tag is a Phospholipase A2 targeting ligand, e.g., as described in Schevitz, R.W., et al., (1995) *Nat. Struct. Biol.* 2, 458-465.

In an embodiment, the Tag is a PHIP targeting ligand, e.g., as described in Krojer, T.; et al. *Chem. Sci.*2016, 7, 2322–2330.

In an embodiment, the Tag is a PDZ targeting ligand, e.g., as described in Mangesh Joshi, et al. *Angew. Chem. Int. Ed.* (2006) 45, 3790-3795.

In an embodiment, the Tag is a PARP15 targeting ligand, e.g., as described in Karlberg, T., et al., (2015) *J.Biol.Chem.*290: 7336-7344.

In an embodiment, the Tag is a PARP14 targeting ligand, e.g., as described in Andersson, C.D., et al.,(2012) *J.Med.Chem.* 55: 7706-7718.; Wahlberg, E., et al. (2012) *Nat.Biotechnol.* 30: 283-288.; Andersson, C.D., et al. (2012) *J.Med.Chem.*55: 7706-7718.

In an embodiment, the Tag is a MTH1 targeting ligand, e.g., as described in Helge Gad, et. al. *Nature*, (2014) 508, 215-221.

In an embodiment, the Tag is a mPGES-1 targeting ligand, , e.g., as described in Luz, J.G., et al., (2015) *J.Med.Chem.*58: 4727-4737.

In an embodiment, the Tag is a FLAP- 5-lipoxygenase-activating protein targeting ligand, e.g., as described Ferguson, A.D., et al (2007) *Science* 317: 510-512.

In an embodiment, the Tag is a FA Binding Protein targeting ligand, e.g., as described in Kuhn, B.; et al. *J. Med. Chem.* (2016) 59, 4087–4102.

5 In an embodiment, the Tag is a BCL2 targeting ligand, e.g., as described in Souers, A.J., et al. (2013) *Nat Med* 19: 202-208.

In an embodiment, the Tag is any small molecule or protein which can bind to a target protein and acted on or degraded by a ubiquitin ligase is a target protein. In some embodiments, the Tag is a dTAG Targeting Ligand disclosed in International Patent Publication No. WO2017/024318 (e.g., Table T,  
10 pages 119-129).

When R<sup>10</sup> is L-Tag, Tag is capable of binding to or is bound to a target protein. Exemplary target proteins include FK506 binding protein-12 (FKBP12), bromodomain-containing protein 4 (BRD4), CREB binding protein (CREBBP), or transcriptional activator BRG1 (SMARCA4). In some  
15 embodiments, the target protein comprises a hormone receptor e.g., estrogen-receptor protein, androgen receptor protein, retinoid x receptor (RXR) protein, or dihydrofolate reductase (DHFR), including bacterial DHFR. In some embodiments, the target protein comprises an amino acid sequence derived from a bacterial dehalogenase. In other embodiments, the target protein comprises amino acid sequences derived from 7,8-dihydro-8-oxoguanin triphosphatase, AFAD, Arachidonate 5- lipoxygenase activating protein, apolipoprotein, ASH1L, ATAD2, baculoviral IAP repeat- containing protein 2, BAZ1A, BAZ1B,  
20 BAZ2A, BAZ2B, Bcl-2, Bcl-xL, BRD1, BRD2, BRD3, BRD4, BRD5, BRD6, BRD7, BRD8, BRD9, BRD10, BRDT, BRPF1, BRPF3, BRWD3, CD209, CECR2, CREBBP, E3 ligase XIAP, EP300, FALZ, fatty acid binding protein from adipocytes 4 (FABP4), GCN5L2, GTPase k-RAS, HDAC6, hematopoietic prostaglandin D synthase, KIAA1240, lactoylglutathione lyase, LOC93349, Mcl-1, MLL, PA2GA, PB1, PCAF, peptidyl-prolyl cis-trans isomerase NIMA-interacting 1, PHIP, poly-ADP-ribose polymerase 14,  
25 poly-ADP-ribose polymerase 15, PRKCBP1, prosaposin, prostaglandin E synthase, retinal rod rhodopsin-sensitive cGMP 3',5-cyclic phosphodiesterase subunit delta, S100-A7, SMARCA2, SMARCA4, SP100, SP110, SP140, Src, Sumo-conjugating enzyme UBC9, superoxide dismutase, TAF1, TAF1L, tankyrase 1, tankyrase 2, TIF1a, TRIM28, TRIM33, TRIM66, WDR9, ZMYND11, or MLL4. In other embodiments, the target protein comprises an amino acid sequence derived from MDM2. In some embodiments, the  
30 target protein is a dTAG disclosed in International Patent Publication No. WO2017/024318 (e.g., pages 112-114).

In one embodiment, the target protein is derived from BRD2, BRD3, BRD4, or BRDT. In one embodiment, the target protein is a modified or mutant BRD2, BRD3, BRD4, or BRDT protein. In certain embodiments, the one or more mutations of BRD2 include a mutation of the Tryptophan (W) at



amino acid position 97, a mutation of the Valine (V) at amino acid position 103, a mutation of the Leucine (L) at amino acid position 110, a mutation of the W at amino acid position 370, a mutation of the V at amino acid position 376, or a mutation of the L at amino acid position 381.

In one embodiment, the target protein is derived from cytosolic signaling protein FKBP12. In certain embodiments, the target protein is a modified or mutant cytosolic signaling protein FKBP12. In certain embodiments, the modified or mutant cytosolic signaling protein FKBP12 contains one or more mutations that create an enlarged binding pocket for FKBP12 ligands. In certain embodiments, the one or more mutations include a mutation of the phenylalanine (F) at amino acid position 36 to valine (V) (F36V) (referred to interchangeably herein as FKBP12\* or FKBP\*).

In some embodiments, the degradation compound is a compound disclosed in U.S. Patent No. 7,973,057; U.S. Patent No. 8,546,430; U.S. Patent No. 8,716,315; International Patent Publication No. WO2017/059062; or International Patent Publication No. WO2017/024318; each of which is hereby incorporated by reference in its entirety.

## **Heterologous Polypeptides**

Provided herein are fusion polypeptides including a COF1/CRBN-, COF2/CRBN-, or COF3/CRBN-binding polypeptide and a heterologous polypeptide of interest. In some embodiments, the COF1/CRBN-, COF2/CRBN-, or COF3/CRBN-binding polypeptide and the heterologous polypeptide are separated by a linker (e.g., a glycine-serine linker). In some embodiments, the fusion polypeptide described herein comprises three elements: a COF1/CRBN-, COF2/CRBN-, or COF3/CRBN-binding polypeptide (e.g., a portion of an amino acid sequence of a degron as described herein), a heterologous polypeptide, and a linker separating the COF1/CRBN-, COF2/CRBN-, or COF3/CRBN-binding polypeptide and the heterologous polypeptide. In other embodiments, the fusion polypeptide described herein comprises two elements: a COF1/CRBN-, COF2/CRBN-, or COF3/CRBN-binding polypeptide (e.g., a portion of an amino acid sequence of a degron, e.g., as described herein) linked directly to a heterologous polypeptide. These elements can be arranged such that the COF1/CRBN-, COF2/CRBN-, or COF3/CRBN-binding polypeptide (e.g., a portion of an amino acid sequence of a degron, e.g., as described herein) is located at the N-terminus of the heterologous polypeptide of interest, at the C-terminus of the heterologous polypeptide of interest, or in the middle of the heterologous polypeptide of interest. In one embodiment, the heterologous polypeptide is a cytosolic and/or nuclear protein and the COF1/CRBN-, COF2/CRBN-, or COF3/CRBN-binding polypeptide is located N-terminal to the heterologous polypeptide. In one embodiment, the heterologous polypeptide is a transmembrane protein and the COF1/CRBN-, COF2/CRBN-, or COF3/CRBN-binding polypeptide is located C-terminal to the heterologous polypeptide.

In some embodiments, the fusion polypeptide further comprises a degradation domain. In some embodiments, the degradation domain is separated from the COF1/CRBN-, COF2/CRBN-, or COF3/CRBN-binding polypeptide and the heterologous polypeptide by a heterologous protease cleavage site.

The fusion polypeptides disclosed herein can include any heterologous polypeptide of interest. In some embodiments, the heterologous polypeptide can be a transmembrane protein (e.g., a transmembrane receptor). In certain embodiments, the heterologous polypeptide of interest can be, e.g., an ion channel-linked receptor, an enzyme-linked receptor (e.g., receptor tyrosine kinase, a tyrosine kinase associated receptor, a receptor-like tyrosine phosphatase, a receptor serine/threonine kinase; a receptor guanylyl cyclase, and a histidine kinase associated receptor), or a G protein coupled receptor. In some embodiments, the transmembrane protein is a chimeric antigen receptor, e.g., as described herein.

In another embodiment, the heterologous polypeptide is a secreted protein (e.g., a small secreted protein). In some embodiments, the heterologous polypeptide can be, e.g., an antibody, a nanobody, or a protein binding molecule in cell manufacturing. In some embodiments, the heterologous polypeptide can be a therapeutic or clinical protein (e.g., insulin, growth hormone, erythropoietin, or a therapeutic antibody). In certain embodiments, the protein can be toxic to a cell for manufacturing (e.g., bacterial toxins and proteases).

Table 2 includes a list of exemplary heterologous polypeptide for use in the fusion polypeptides disclosed herein. Additional heterologous polypeptide of interest include Chimeric Antigen T Cell Receptors as described in the section below.

**Table 2. Heterologous Polypeptides of Interest**

| <b>Cytoplasmic or Nuclear</b>       | <b>Transmembrane</b> | <b>Secreted</b>        |
|-------------------------------------|----------------------|------------------------|
| Apoptosis pathway (e.g., Caspase 9) | CD62L                | IL-12 p35              |
| TALENs                              | CCR1                 | IL-12 p40              |
| ZFN                                 | CCR2                 | IL-12 p70              |
| Meganuclease                        | CCR5                 | IL-15 or IL-15 complex |
| Cas9                                | CCR7                 | IL-2                   |
| MITF                                | CCR10                | IL-7                   |
| MYC                                 | CXCR2                | IL-18                  |
| STAT3                               | CXCR3                | IL-9                   |
| STAT5                               | CXCR4                | IL-21                  |
| NF-kappaB                           | CXCR6                | RANTES/CCL5            |
| Beta-catenin                        | CTLA4                | CCL2                   |

|                                         |                                                                                                                  |                                                                                                                    |
|-----------------------------------------|------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------|
| Notch                                   | PD1                                                                                                              | CCL1                                                                                                               |
| GLI                                     | BTLA                                                                                                             | CCL22                                                                                                              |
| c-JUN                                   | VISTA                                                                                                            | Heparanase                                                                                                         |
| Tet methylcytosine dioxygenase 2 (TET2) | CD137L                                                                                                           | matrix metalloproteinase (MMP)                                                                                     |
| FKBP                                    | CD80                                                                                                             | Cathepsin                                                                                                          |
| Tau                                     | CD86                                                                                                             | Antibody (e.g., anti-tumor antibody, e.g., Herceptin, or a checkpoint inhibitor antibody, e.g., anti-PD1 antibody) |
| Enzyme                                  | TIGIT                                                                                                            | Peptide (e.g., anti-tumor peptide, or protein hormone)                                                             |
| Scaffold protein                        | Chimeric Antigen Receptor (e.g., CAR that binds to CD19, CD22, CD20, BCMA, CD123, CD33, EGFRvIII, or Mesothelin) | IL-6 inhibitory peptide                                                                                            |
|                                         | CD3                                                                                                              | TGFbeta inhibitory peptide                                                                                         |
|                                         | CD8                                                                                                              |                                                                                                                    |
|                                         | CD19                                                                                                             |                                                                                                                    |
|                                         | CD22                                                                                                             |                                                                                                                    |
|                                         | CD20                                                                                                             |                                                                                                                    |
|                                         | BCMA                                                                                                             |                                                                                                                    |

### CAR Antigen Binding Domain

In one aspect, the CAR of the disclosure linked to a COF1/CRBN-, COF2/CRBN-, or COF3/CRBN-binding polypeptide and/or a degradation domain comprises a target-specific binding element otherwise referred to as an antigen binding domain. In one embodiment, the portion of the CAR comprising the antigen binding domain comprises an antigen binding domain that targets, *e.g.*, specifically binds to, an antigen, *e.g.*, antigen described herein, *e.g.*, CD19. In one embodiment, the antigen binding domain targets, *e.g.*, specifically binds to, human CD19.

In some embodiments, the heterologous polypeptide linked to a COF1/CRBN-, COF2/CRBN-, or COF3/CRBN-binding polypeptide and/or a degradation domain comprises a chimeric antigen receptor (CAR). In some embodiments, the CAR comprises an antigen binding domain (*e.g.*, an antibody or antibody fragment, a TCR, or a TCR fragment) that binds to a tumor antigen, a transmembrane domain, and an intracellular signaling domain (*e.g.*, an intracellular signaling domain comprising a costimulatory domain) and/or a primary signaling domain. CAR nucleic acid constructs, encoded proteins, containing vectors, host cells, pharmaceutical compositions, and methods of administration and treatment related to

the present disclosure are disclosed in detail in International Patent Application Publication No. WO2015142675, which is incorporated by reference in its entirety.

In some embodiments, the heterologous polypeptide is a chimeric antigen receptor (CAR), wherein the CAR comprises an antigen binding domain (e.g., antibody or antibody fragment, TCR or TCR fragment) that binds to a tumor-supporting antigen (e.g., a tumor-supporting antigen as described herein), a transmembrane domain (e.g., a transmembrane domain described herein), and an intracellular signaling domain (e.g., an intracellular signaling domain described herein) (e.g., an intracellular signaling domain comprising a costimulatory domain (e.g., a costimulatory domain described herein) and/or a primary signaling domain (e.g., a primary signaling domain described herein). In some embodiments, the tumor-supporting antigen is an antigen present on a stromal cell or a myeloid-derived suppressor cell (MDSC). In other aspects, the invention features polypeptides encoded by such nucleic acids and host cells containing such nucleic acids and/or polypeptides.

In some embodiments, a CAR molecule comprises at least one intracellular signaling domain selected from a CD137 (4-1BB) signaling domain, a CD28 signaling domain, a CD27 signaling domain, an ICOS signaling domain, a CD3zeta signal domain, or any combination thereof. In some embodiments, a CAR molecule comprises at least one intracellular signaling domain selected from one or more costimulatory molecule(s) selected from CD137 (4-1BB), CD28, CD27, or ICOS.

In some embodiments, a plurality of immune effector cells, e.g., the population of T regulatory-depleted cells, include a nucleic acid encoding a CAR that comprises a target-specific binding element otherwise referred to as an antigen binding domain. The choice of binding element depends upon the type and number of ligands that define the surface of a target cell. For example, the antigen binding domain may be chosen to recognize a ligand that acts as a cell surface marker on target cells associated with a particular disease state. Thus, examples of cell surface markers that may act as ligands for the antigen binding domain in a CAR described herein include those associated with viral, bacterial and parasitic infections, autoimmune disease and cancer cells.

Sequences of non-limiting examples of various components that can be part of a CAR molecule, e.g., a TA CAR or a BCA CAR described herein, are listed in **Table 3**, where “aa” stands for amino acids, and “na” stands for nucleic acids that encode the corresponding peptide.

**Table 3. Sequences of various components of CAR (aa – amino acid sequence, na – nucleic acid sequence).**

| SEQ ID NO: | description | Sequence |
|------------|-------------|----------|
|------------|-------------|----------|

|                   |                       |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |
|-------------------|-----------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| SEQ ID NO:<br>144 | EF-1<br>promoter (na) | CGTGAGGCTCCGGTGCCCGTCAGTGGGCAGAGCGCACATC<br>GCCCACAGTCCCCGAGAAGTTGGGGGGAGGGGTTCGGCAAT<br>TGAACCGGTGCCTAGAGAAGGTGGCGCGGGGTAAACTGGG<br>AAAGTGATGTCGTGTACTGGCTCCGCCTTTTCCCGAGGGT<br>GGGGGAGAACCGTATATAAGTGCAGTAGTCGCCGTGAACG<br>TTCTTTTTTCGCAACGGGTTTGGCGCCAGAACACAGGTAAGT<br>GCCGTGTGTGGTTCCCGCGGGCCTGGCCTCTTTACGGGTTA<br>TGGCCCTTGCGTGCCTTGAATTACTTCCACCTGGCTGCAGT<br>ACGTGATTCTTGATCCCGAGCTTCGGGTGGAAGTGGGTGG<br>GAGAGTTCGAGGCCTTGCGCTTAAGGAGCCCCCTTCGCCTCG<br>TGCTTGAGTTGAGGCCTGGCCTGGGCGCTGGGGCCCGCCGC<br>TGCGAATCTGGTGGCACCTTCGCGCCTGTCTCGCTGCTTTC<br>GATAAGTCTCTAGCCATTTAAAATTTTTGATGACCTGCTGC<br>GACGCTTTTTTTCTGGCAAGATAGTCTTGTAATGCGGGCC<br>AAGATCTGCACACTGGTATTTTCGGTTTTTGGGGCCGCGGGC<br>GGCGACGGGGCCCGTGCGTCCCAGCGCACATGTTTCGGCGA<br>GGCGGGGCCTGCGAGCGCGGCCACCGAGAATCGGACGGGG<br>GTAGTCTCAAGCTGGCCGGCCTGCTCTGGTGCCTGGCCTCG<br>CGCCGCCGTGTATCGCCCCGCCCTGGGCGGCAAGGCTGGCC<br>CGGTCGGCACCAAGTTGCGTGAGCGGAAAGATGGCCGCTTC<br>CCGGCCCTGCTGCAGGGAGCTCAAAATGGAGGACGCGGGCG<br>CTCGGGAGAGCGGGCGGGTGAGTCACCCACACAAAGGAAA<br>AGGGCCTTTCCGTCTCAGCCGTCGCTTCATGTGACTCCAC<br>GGAGTACCGGGCGCCGTCCAGGCACCTCGATTAGTTCTCGA<br>GCTTTTGGAGTACGTCGTCTTTAGGTTGGGGGGAGGGGTTT<br>TATGCGATGGAGTTTCCCCACACTGAGTGGGTGGAGACTGA<br>AGTTAGGCCAGCTTGGCACTTGATGTAATTCTCCTTGGAAT<br>TTGCCCTTTTTGAGTTTGGATCTTGTTTCATTCTCAAGCCTC<br>AGACAGTGGTTCAAAGTTTTTTTCTTCCATTTTCAGGTGTCGT<br>GA |
| SEQ ID NO:<br>64  | Leader (aa)           | MALPVTALLLPLALLLHAARP                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |
| SEQ ID NO:<br>145 | Leader (na)           | ATGGCCCTGCCTGTGACAGCCCTGCTGCTGCCTCTGGCTCT<br>GCTGCTGCATGCCGCTAGACCC                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |
| SEQ ID NO:<br>146 | Leader (na)           | ATGGCCCTCCCTGTCACCGCCCTGCTGCTTCCGCTGGCTCTT<br>CTGCTCCACGCCGCTCGGCC                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       |
| SEQ ID NO:<br>147 | CD 8 hinge<br>(aa)    | TTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFAC<br>D                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |
| SEQ ID NO:<br>148 | CD8 hinge<br>(na)     | ACCACGACGCCAGCGCCGCGACCACCAACACCGGCGCCCA<br>CCATCGCGTCGCAGCCCCTGTCCCTGCGCCCAGAGGCGTGC<br>CGGCCAGCGGCGGGGGGCGCAGTGACACAGAGGGGGGCTGG<br>ACTTCGCCTGTGAT                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |
| SEQ ID NO:<br>149 | Ig4 hinge<br>(aa)     | ESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCV<br>VVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVV<br>SVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKISKAKGQPREP<br>QVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPE<br>NNYKTTTPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHE<br>ALHNHYTQKSLSLGLKGM                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                |
| SEQ ID NO:<br>150 | Ig4 hinge<br>(na)     | GAGAGCAAGTACGGCCCTCCCTGCCCCCTTGCCCTGCCCC<br>CGAGTTCCTGGGCGGACCCAGCGTGTTCTGTTCCCCCCCCA                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |

|                   |                            |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            |
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|                   |                            | AGCCCAAGGACACCCTGATGATCAGCCGGACCCCCGAGGT<br>GACCTGTGTGGTGGTGGACGTGTCCCAGGAGGACCCCGAG<br>GTCCAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCACA<br>ACGCCAAGACCAAGCCCCGGGAGGAGCAGTTCAATAGCAC<br>CTACCGGGTGGTGTCCGTGCTGACCGTGCTGCACCAGGACT<br>GGCTGAACGGCAAGGAATACAAGTGTAAGGTGTCCAACAA<br>GGGCCTGCCCAGCAGCATCGAGAAAACCATCAGCAAGGCC<br>AAGGGCCAGCCTCGGGAGCCCCAGGTGTACACCCTGCCCC<br>CTAGCCAAGAGGAGATGACCAAGAACCAGGTGTCCCTGAC<br>CTGCCTGGTGAAGGGCTTCTACCCCAGCGACATCGCCGTGG<br>AGTGGGAGAGCAACGGCCAGCCCGAGAACAACACTACAAGAC<br>CACCCCCCTGTGCTGGACAGCGACGGCAGCTTCTTCCTGT<br>ACAGCCGGCTGACCGTGGACAAGAGCCGGTGGCAGGAGGG<br>CAACGTCTTTAGCTGCTCCGTGATGCACGAGGCCCTGCACA<br>ACCACTACACCCAGAAGAGCCTGAGCCTGTCCCTGGGCAA<br>GATG                                                                                                                                                                                                                                                              |
| SEQ ID NO:<br>151 | IgD hinge<br>(aa)          | RWPESPKAQASSVPTAQPPAEGSLAKATTAPATTRNTGRGGE<br>EKKKEKEKEEQEERETKTPECPSHTQPLGVYLLTPAVQDLWL<br>RDKATFTCFVVGSDLKDAHLTWEVAGKVPTGGVEEGLLERH<br>SNGSQSQHSRLTLPRSLWNAGTSVTCTLNHPSLPPQRLMALRE<br>PAAQAPVKLSLNLASSDPPEAASWLLCEVSGFSPNILLMWL<br>EDQREVNTSGFAPARPPPQPGSTTFWAWSVLRVPAPPSPQPAT<br>YTCVVSHEDSRTLLNASRSLEVS YVTDH                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |
| SEQ ID NO:<br>152 | IgD hinge<br>(na)          | AGGTGGCCCGAAAGTCCCAAGGCCAGGCATCTAGTGTTT<br>CTACTGCACAGCCCCAGGCAGAAAGGCAGCCTAGCCAAAGC<br>TACTACTGCACCTGCCACTACGCGCAATACTGGCCGTGGCG<br>GGGAGGAGAAGAAAAAGGAGAAAGAGAAAGAAGAACAG<br>GAAGAGAGGGAGACCAAGACCCCTGAATGTCCATCCATA<br>CCCAGCCGCTGGGCGTCTATCTCTTGACTCCCGCAGTACAG<br>GACTTGTGGCTTAGAGATAAGGCCACCTTTACATGTTTCGT<br>CGTGGGCTCTGACCTGAAGGATGCCCATTTGACTTGGGAGG<br>TTGCCGGAAGGTACCCACAGGGGGGGTTGAGGAAGGGTT<br>GCTGGAGCGCCATTCCAATGGCTCTCAGAGCCAGCACTCAA<br>GACTCACCTTCCGAGATCCCTGTGGAACGCCGGGACCTCT<br>GTCACATGTACTCTAAATCATCCTAGCCTGCCCCACAGCG<br>TCTGATGGCCCTTAGAGAGCCAGCCGCCAGGCACCAAGTTA<br>AGCTTAGCCTGAATCTGCTCGCCAGTAGTGATCCCCCAGAG<br>GCCGCCAGCTGGCTCTTATGCGAAGTGTCCGGCTTTAGCCC<br>GCCCAACATCTTGCTCATGTGGCTGGAGGACCAGCGAGAA<br>GTGAACACCAGCGGCTTCGCTCCAGCCCGGCCCCCACCCCA<br>GCCGGGTTCTACCACATTCTGGGCCTGGAGTGTCTTAAGGG<br>TCCCAGCACCACTAGCCCCCAGCCAGCCACATACACCTGT<br>GTTGTGTCCCATGAAGATAGCAGGACCCTGCTAAATGCTTC<br>TAGGAGTCTGGAGGTTTCCTACGTGACTGACCATT |
| SEQ ID NO:<br>153 | GS<br>hinge/linker<br>(aa) | GGGSGGGGS                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |
| SEQ ID NO:<br>154 | GS<br>hinge/linker<br>(na) | GGTGGCGGAGGTTCTGGAGGTGGAGGTTCC                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             |

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| SEQ ID NO:<br>155 | CD8TM (aa)                            | IYIWAPLAGTCGVLLSLVITLYC                                                                                                                                                                                                                                                                                                                                                           |
| SEQ ID NO:<br>156 | CD8 TM (na)                           | ATCTACATCTGGGCGCCCTTGGCCGGGACTTGTGGGGTCCT<br>TCTCCTGTCACTGGTTATCACCCTTTACTGC                                                                                                                                                                                                                                                                                                      |
| SEQ ID NO:<br>157 | CD8 TM (na)                           | ATCTACATTTGGGCCCCCTCTGGCTGGTACTTGCGGGGTCCT<br>GCTGCTTTCACCTCGTGATCACTCTTTACTGT                                                                                                                                                                                                                                                                                                    |
| SEQ ID NO:<br>158 | 4-1BB<br>intracellular<br>domain (aa) | KRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCEL                                                                                                                                                                                                                                                                                                                                        |
| SEQ ID NO:<br>159 | 4-1BB<br>intracellular<br>domain (na) | AAACGGGGCAGAAAGAACTCCTGTATATATTCAAACAAC<br>CATTTATGAGACCAGTACAACTACTCAAGAGGAAGATGG<br>CTGTAGCTGCCGATTTCAGAGAAGAAGAAGGAGGATGT<br>GAACTG                                                                                                                                                                                                                                            |
| SEQ ID NO:<br>160 | 4-1BB<br>intracellular<br>domain (na) | AAGCGCGGTCGGAAGAAGCTGCTGTACATCTTTAAGCAAC<br>CCTTCATGAGGCCTGTGCAGACTACTCAAGAGGAGGACGG<br>CTGTTTCATGCCGTTCCAGAGGAGGAGGAAGGCGGCTGC<br>GAACTG                                                                                                                                                                                                                                         |
| SEQ ID NO:<br>161 | CD27 (aa)                             | QRRKYRSNKGESPVEPAEPCRYSCPREEEGSTIPIQEDYRKPEP<br>ACSP                                                                                                                                                                                                                                                                                                                              |
| SEQ ID NO:<br>162 | CD27 (na)                             | AGGAGTAAGAGGAGCAGGCTCCTGCACAGTGACTACATGA<br>ACATGACTCCCCGCCGCCCGGGCCACCCGCAAGCATTAC<br>CAGCCCTATGCCCCACCACGCGACTTCGCAGCCTATCGCTC<br>C                                                                                                                                                                                                                                             |
| SEQ ID NO:<br>163 | CD3-zeta<br>(aa)                      | RVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGR<br>DPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRR<br>GKGHDGLYQGLSTATKDTYDALHMQALPPR                                                                                                                                                                                                                                                          |
| SEQ ID NO:<br>164 | CD3-zeta<br>(na)                      | AGAGTGAAGTTTCAGCAGGAGCGCAGACGCCCCCGCGTACA<br>AGCAGGGCCAGAACCAGCTCTATAACGAGCTCAATCTAGG<br>ACGAAGAGAGGAGTACGATGTTTTGGACAAGAGACGTGGC<br>CGGGACCCTGAGATGGGGGGAAGCCGAGAAGGAAGAAC<br>CCTCAGGAAGGCCTGTACAATGAACTGCAGAAAGATAAGA<br>TGGCGGAGGCCTACAGTGAGATTGGGATGAAAGGCGAGCG<br>CCGGAGGGGCAAGGGGCACGATGGCCTTTACCAGGGTCTC<br>AGTACAGCCACCAAGGACACCTACGACGCCCTTCACATGC<br>AGGCCCTGCCCGCTCGC  |
| SEQ ID NO:<br>165 | CD3-zeta<br>(na)                      | CGCGTGAAATTCAGCCGCAGCGCAGATGCTCCAGCCTACA<br>AGCAGGGGCAGAACCAGCTCTACAACGAACTCAATCTTGG<br>TCGGAGAGAGGAGTACGACGTGCTGGACAAGCGGAGAGG<br>ACGGGACCCAGAAATGGGCGGGAAGCCGCGCAGAAAGAA<br>TCCCCAAGAGGGCCTGTACAACGAGCTCCAAAAGGATAAG<br>ATGGCAGAAGCCTATAGCGAGATTGGTATGAAAGGGGAAC<br>GCAGAAGAGGGCAAAGGCCACGACGGACTGTACCAGGGACT<br>CAGCACCGCCACCAAGGACACCTATGACGCTCTTCACATGC<br>AGGCCCTGCCCGCTCGG |
| SEQ ID NO:<br>166 | CD3-zeta<br>(aa)                      | RVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGR<br>DPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRR<br>GKGHDGLYQGLSTATKDTYDALHMQALPPR                                                                                                                                                                                                                                                          |
| SEQ ID NO:<br>167 | CD3-zeta<br>(na)                      | AGAGTGAAGTTTCAGCAGGAGCGCAGACGCCCCCGCGTACC<br>AGCAGGGCCAGAACCAGCTCTATAACGAGCTCAATCTAGG<br>ACGAAGAGAGGAGTACGATGTTTTGGACAAGAGACGTGGC                                                                                                                                                                                                                                                 |

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|                   |                                      | CGGGACCCTGAGATGGGGGGAAAGCCGAGAAGGAAGAAC<br>CCTCAGGAAGGCCTGTACAATGAACTGCAGAAAGATAAGA<br>TGGCGGAGGCCTACAGTGAGATTGGGATGAAAGGCGAGCG<br>CCGGAGGGGCAAGGGGCACGATGGCCTTTACCAGGGTCTC<br>AGTACAGCCACCAAGGACACCTACGACGCCCTTCACATGC<br>AGGCCCTGCCCCCTCGC                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                |
| SEQ ID NO:<br>168 | Linker (aa)                          | GGGGS                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       |
| SEQ ID NO:<br>154 | Linker (aa)                          | GGTGGCGGAGGTTCTGGAGGTGGAGGTTCC                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |
| SEQ ID NO:<br>169 | PD-1<br>extracellular<br>domain (aa) | Pgwfldspdrpwnpptsfpallvvtgednatftcsfntsfsfvlwyrmspsnqtdklaafpe<br>drsqpgqdcfrfvrtqlpngrdfhmsvvrarndsgtylclgaislapkaqikesraelrvterrae<br>vptahpspsprpagqfqlv                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |
| SEQ ID NO:<br>170 | PD-1<br>extracellular<br>domain (na) | Cccggatggtttctggactctccggatcgcccggtggaatcccccaacctctcaccggcactcttg<br>gttggtgactgagggcgataatcgaccttcacgtgctcgttctccaacacctccgaatcattcgtgct<br>gaactggtaccgcatgagcccgtaaacagaccgacaagctcgccgcgttccggaagatcggtg<br>tcgcaaccgggacaggttgcgttcccggtgactcaactgccgaatggcagagactccacat<br>gagcgtggtccgcgctaggcgaaacgactccgggacctacgtgctggagccatctcgtggcg<br>cctaaggcccaaatcaagagagcttgaggcggaactgagagtgaccgagcgagagctgag<br>gtgccaactgcacatccatccccatcgctcgccctcgccggcgagttcagaccctggtc                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             |
| SEQ ID NO:<br>171 | PD-1 CAR<br>(aa) with<br>signal      | Malpvtalllplallhaarppgwfldspdrpwnpptsfpallvvtgednatftcsfntsfsfvlw<br>yrmspsnqtdklaafpedrsqpgqdcfrfvrtqlpngrdfhmsvvrarndsgtylclgaislap<br>kaqikesraelrvterraevptahpspsprpagqfqlvttppaprpptpaptiasqplslrpeacr<br>paaggavhtrgldfacdiyiwaplagtcgvllslvitlyckrgrklllyifkqpfmrpvqtqee<br>dgscrfpeeeeggcelrvkfsrsadapaykqgqnqlynelnlgrreeydvldkrrgrdpemg<br>gkprknppqeglynelqkdkmaeyseigmkgerrrgkghdglyqlstatkdydalhmq<br>alppr                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |
| SEQ ID NO:<br>172 | PD-1 CAR<br>(na)                     | Atggccctcctgtcactgcctgcttctccccctcgcactctgctccacgcgcgtagaccaccc<br>ggatggtttctggactctccggatcgcccggtggaatcccccaacctctcaccggcactcttggtgt<br>gactgagggcgataatcgaccttcacgtgctcgttctccaacacctccgaatcattcgtgctgaac<br>tggtaccgcatgagcccgtaaacagaccgacaagctcgccgcgttccggaagatcggtcgc<br>aacccgggacaggttgcgttccgctgactcaactgccgaatggcagagactccacatgagc<br>gtggtccgcgctaggcgaaacgactccgggacctacgtgctggagccatctcgtggcgcccta<br>aggcccaaatcaagagagcttgaggcggaactgagagtgaccgagcgagagctgaggtg<br>ccaactgcacatccatccccatcgctcgccctcgccggcgagttcagaccctggtcacgaccac<br>tcggcgccgcgcccaccgactccggccccaactatcgcgagccagccctgctcgtgagggc<br>ggaagcatgccgcctgcccggaggtgctgtgcataccggggattggaacttcgcatgcgac<br>atctacatttgggtcctctcgccggaacttggtgctgctcctctgctcctggtcatcacctgtac<br>tgcaagcggggtcggaaaaagcttctgtacatttcaagcagccctcatgagggccgtgcaaacc<br>accagggaggagacggttgcctctgcccgttccccgaagaggagaaggaggttgcgagctg<br>cgcgtgaagttctccggagcgccgacgccccgcctataagcaggggcagaaccagctgtac<br>aacgaactgaacctgggacggcggaagagtacgatgtgctggacaagcgcgccggcgga<br>ccccgaaatggcggggaagcctagaagaagaaccctcaggaaggcctgtataacgagctgca<br>gaaggacaagatggcgaggcctactccgaaattgggatgaaggagagcggcgaggggga<br>aggggcacgacggcctgtaccaaggactgtccaccgccaccaaggacacatacgtgcctgc<br>acatgcaggccctccccctgc |
| SEQ ID NO:<br>173 | Linker (aa)                          | (Gly-Gly-Gly-Ser) <sub>n</sub> , where n = 1-10                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             |
| SEQ ID NO:<br>141 | Linker (aa)                          | (Gly <sub>4</sub> Ser) <sub>4</sub>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |



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| SEQ ID NO:<br>142 | Linker (aa)                          | (Gly4 Ser)3                                                                                                                                                                                                                                                                                                                                                                                                                                     |
| SEQ ID NO:<br>143 | Linker (aa)                          | (Gly3Ser)                                                                                                                                                                                                                                                                                                                                                                                                                                       |
| SEQ ID NO:<br>174 | polyA (na)                           | [a] <sub>50-5000</sub>                                                                                                                                                                                                                                                                                                                                                                                                                          |
| SEQ ID NO:<br>175 | PD1 CAR<br>(aa)                      | <u>Pgwfldspdrpwnpptfspallvvtegdnatftcsfsntsesfvlnwyrmspsnqtdklaafpe</u><br><u>drsqpgqdcfrvrtqlpngrdfhmsvvrarrndsgtylclgaislapkaqikeslraelrvterrae</u><br><u>vptahpspsprpagqfqlvtttppaprpptpaptiasqplslrpeacrpaaggavhtrgldfacdiy</u><br><u>iwaplagtegvllslvitlyckgrkkllyifkqpfmrpvqttqeedgscrfpeeeeggcelrv</u><br><u>kfsrsadapaykqggnqlynelnlgrreedydldkrrgrdpemggkprknpqeglynelqk</u><br><u>dkmaeayseigmkgerrrgkghdglyqglstatkdydalhmqalppr</u> |
| SEQ ID NO:<br>176 | ICOS<br>intracellular<br>domain (aa) | TKKKYSSSVHDPNGEYMFMRVNTAKKSRLTDVTL                                                                                                                                                                                                                                                                                                                                                                                                              |
| SEQ ID NO:<br>177 | ICOS<br>intracellular<br>domain (na) | ACAAAAAAGAAGTATTCATCCAGTGTGCACGACCCTAACG<br>GTGAATACATGTTTCATGAGAGCAGTGAACACAGCCAAAAA<br>ATCCAGACTCACAGATGTGACCCTA                                                                                                                                                                                                                                                                                                                              |
| SEQ ID NO:<br>178 | ICOS TM<br>domain (aa)               | TTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFAC<br>DFWLPIGCAAFVVVCILGCILICWL                                                                                                                                                                                                                                                                                                                                                                       |
| SEQ ID NO:<br>179 | ICOS TM<br>domain (na)               | ACCACGACGCCAGCGCCGCGACCACCAACACCGGCGCCCA<br>CCATCGCGTTCGCAGCCCCCTGTCCCTGCGCCCAGAGGCGTGC<br>CGGCCAGCGGCGGGGGGCGCAGTGCACACGAGGGGGGCTGG<br>ACTTCGCCTGTGATTTCTGGTTACCCATAGGATGTGCAGCC<br>TTTGTTGTAGTCTGCATTTTGGGATGCATACTTATTTGTTGG<br>CTT                                                                                                                                                                                                          |
| SEQ ID NO:<br>180 | CD28<br>intracellular<br>domain (aa) | RSKRSRLHSDYMNMTPRRPGPTRKHYPYAPPRDFAAYRS                                                                                                                                                                                                                                                                                                                                                                                                         |
| SEQ ID NO:<br>162 | CD28<br>intracellular<br>domain (na) | AGGAGTAAGAGGAGCAGGCTCCTGCACAGTGACTACATGA<br>ACATGACTCCCCGCCGCCCGGGCCCCACCCGCAAGCATTAC<br>CAGCCCTATGCCCCACCACGCGACTTCGCAGCCTATCGCTC<br>C                                                                                                                                                                                                                                                                                                         |

**Table 4. CAR modified with degadation tag and/or FurON**

| SEQ ID<br>NO      | Description                     | Amino acid sequences (signal peptide included)                                                                                         |
|-------------------|---------------------------------|----------------------------------------------------------------------------------------------------------------------------------------|
| SEQ ID<br>NO: 64  | Signal peptide (aa)             | MALPVTALLPLALLLHAARP                                                                                                                   |
| SEQ ID<br>NO: 140 | Modified signal<br>peptide (aa) | MALPVTALLPLALLLHAARPRSSLA                                                                                                              |
| SEQ ID<br>NO: 28  | 16GS linker (aa)                | GGGSGGGGTGGGSG                                                                                                                         |
| SEQ ID<br>NO: 99  | 16KGS linker (aa)               | KGGGSKGGGTKGGGSK                                                                                                                       |
| SEQ ID<br>NO: 29  | CAR19 (aa)                      | MALPVTALLPLALLLHAARPEIVMTQSPATLSLSPGERATL<br>SCRASQDISKYLNWYQQKPGQAPRLLIYHTSRLHSGIPARFS<br>GSGSGTDYTLTISSLQPEDFAVYFCQQGNTLPYTFGQGGTKLE |

|                  |                                                          |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |
|------------------|----------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|                  |                                                          | IKGGGGSGGGGSGGGGSQVQLQESGPGLVKPSSETLSLTCTV<br>SGVSLPDYGVSWIRQPPGKGLEWIGVIWGSETTYQSSLKS<br>RVTISKDNSKNQVSLKLSSVTAADTAVYYCAKHYYYGGSY<br>AMDYWGQGTLLTVSSTTTPAPRPPTPAPTASQPLSLRPEAC<br>RPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLY<br>CKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFEEEEGGC<br>ELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDR<br>RGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMK<br>GERRRGKGHGDLTYQGLSTATKDTYDALHMQALPPR                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |
| SEQ ID<br>NO: 92 | FurON_CAR19<br>(construct 765) (aa)                      | MALPVTALLPLALLLHAARPRSSLALSLTADQMVSAALLDA<br>EPPILYSEYDTPRPFSEASMMGLLTNLADREL VHMINWAKR<br>VPGFVDLALHDQVHLLCAWMEILMIGLVWRSMEHPGKLL<br>FAPNLLLDNRNQGKCEGGVEIFDMLLATSSRFRMMNLQGE<br>EFVCLKSIILLNSGVYTFLSSTLKSLEEKDHIHRVLDKITDTLI<br>HLMAGAGLTLLQQHQRLAQLLILSHIRHMSSKRMEHLYS<br>MKCKNVVPLSDLLLEMLDAHRLGTGAEDPRPSRKRRSLGD<br>VGEIVMTQSPATLSLSPGERATLSCRASQDISKYLNWYQQK<br>PGQAPRLLIYHTSRLHSGIPARFSGSGSGTDYTLTISSLQPEDF<br>AVYFCQQGNTLPYTFGQGTKLEIKGGGGSGGGGSGGGGSQ<br>VQLQESGPGLVKPSSETLSLTCTVSGVSLPDYGVSWIRQPPGK<br>GLEWIGVIWGSETTYQSSLKSRVTISKDNSKNQVSLKLSSV<br>TAADTAVYYCAKHYYYGGSYAMDYWGQGTLLTVSSTTTP<br>APRPPTPAPTASQPLSLRPEACRPAAGGAVHTRGLDFACDI<br>YIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPV<br>QTTQEEDGCSCRFEEEEGGCELRVKFSRSADAPAYQQGQN<br>QLYNELNLGRREEYDVLDRGRDPEMGGKPRRKNPQEGLY<br>NELQKDKMAEAYSEIGMKGERRRGKGHGDLTYQGLSTAT<br>KDTYDALHMQALPPR                                                                                                  |
| SEQ ID<br>NO: 93 | FurON_CAR19_16<br>GS_HilD tag_V5<br>(construct 766) (aa) | MALPVTALLPLALLLHAARPRSSLALSLTADQMVSAALLDA<br>EPPILYSEYDTPRPFSEASMMGLLTNLADREL VHMINWAKR<br>VPGFVDLALHDQVHLLCAWMEILMIGLVWRSMEHPGKLL<br>FAPNLLLDNRNQGKCEGGVEIFDMLLATSSRFRMMNLQGE<br>EFVCLKSIILLNSGVYTFLSSTLKSLEEKDHIHRVLDKITDTLI<br>HLMAGAGLTLLQQHQRLAQLLILSHIRHMSSKRMEHLYS<br>MKCKNVVPLSDLLLEMLDAHRLGTGAEDPRPSRKRRSLGD<br>VGEIVMTQSPATLSLSPGERATLSCRASQDISKYLNWYQQK<br>PGQAPRLLIYHTSRLHSGIPARFSGSGSGTDYTLTISSLQPEDF<br>AVYFCQQGNTLPYTFGQGTKLEIKGGGGSGGGGSGGGGSQ<br>VQLQESGPGLVKPSSETLSLTCTVSGVSLPDYGVSWIRQPPGK<br>GLEWIGVIWGSETTYQSSLKSRVTISKDNSKNQVSLKLSSV<br>TAADTAVYYCAKHYYYGGSYAMDYWGQGTLLTVSSTTTP<br>APRPPTPAPTASQPLSLRPEACRPAAGGAVHTRGLDFACDI<br>YIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPV<br>QTTQEEDGCSCRFEEEEGGCELRVKFSRSADAPAYQQGQN<br>QLYNELNLGRREEYDVLDRGRDPEMGGKPRRKNPQEGLY<br>NELQKDKMAEAYSEIGMKGERRRGKGHGDLTYQGLSTAT<br>KDTYDALHMQALPPRGGGGSGGGGTGGGGSGMHKRSHTG<br>ERPFQCNQCGASFTQKGNLLRHIKLHTGEKPFKCHLCNTAS<br>AEARHIKAEMGGKPIPNPLGLDST |

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| SEQ ID<br>NO: 32 | FurON_CAR19_16<br>GS_HilD tag<br>(construct 767) (aa) | MALPVTALLLPLALLLHAARPRSSLALSLTADQMVSAALLDA<br>EPPILYSEYDPTRPFSEASMMGLLTNLADRELVHMINWAKR<br>VPGFVDLALHDQVHLLLECAWMEILMIGLVWRSMEHPGKLL<br>FAPNLLLDNRNQGKCEGGVEIFDMLLATSSRFRMMNLQGE<br>EFVCLKSIILLNSGVYTFLSSTLKSLEEKDHIHRVLDKITDTLI<br>HLMAGAGLTQQQHQRQAQLLLILSHIRHMSSKRMEHLYS<br>MKCKNVVPLSDLLLEMLDAHRLGTGAEDPRPSRKRRSLGD<br>VGEIVMTQSPATLSLSPGERATLSCRASQDISKYLNWYQQK<br>PGQAPRLLIYHTSRLHSGIPARFSGSGSGTDYTLTISSLQPEDF<br>AVYFCQQGNTLPYTFGQGTKLEIKGGGGSGGGGSGGGGSQ<br>VQLQESGPGLVKPSETLSLTCTVSGVSLPDYGVSWIRQPPGK<br>GLEWIGVIWGSETTYQSSLSKSRVTISKDNSKNQVSLKLSSV<br>TAADTAVYYCAKHYYYGGSYAMDYWGQGLTVTVSSTTTP<br>APRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDI<br>YIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPV<br>QTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQN<br>QLYNELNLGRREEYDVLDRRGRDPEMGGKPRRKNPQEG<br>YNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTAT<br>KDTYDALHMQALPPRGGGSGGGGTGGGGSGMHKRSHTG<br>ERPFQCNQCGASFTQKGNLLRHIKLHTGEKPFKCHLCNTAS<br>AEARHIKAEMG |
| SEQ ID<br>NO: 33 | FurON_CAR19_16<br>GS_HilD tag_NoK<br>(aa)             | MALPVTALLLPLALLLHAARPRSSLALSLTADQMVSAALLDA<br>EPPILYSEYDPTRPFSEASMMGLLTNLADRELVHMINWAKR<br>VPGFVDLALHDQVHLLLECAWMEILMIGLVWRSMEHPGKLL<br>FAPNLLLDNRNQGKCEGGVEIFDMLLATSSRFRMMNLQGE<br>EFVCLKSIILLNSGVYTFLSSTLKSLEEKDHIHRVLDKITDTLI<br>HLMAGAGLTQQQHQRQAQLLLILSHIRHMSSKRMEHLYS<br>MKCKNVVPLSDLLLEMLDAHRLGTGAEDPRPSRKRRSLGD<br>VGEIVMTQSPATLSLSPGERATLSCRASQDISKYLNWYQQK<br>PGQAPRLLIYHTSRLHSGIPARFSGSGSGTDYTLTISSLQPEDF<br>AVYFCQQGNTLPYTFGQGTKLEIKGGGGSGGGGSGGGGSQ<br>VQLQESGPGLVKPSETLSLTCTVSGVSLPDYGVSWIRQPPGK<br>GLEWIGVIWGSETTYQSSLSKSRVTISKDNSKNQVSLKLSSV<br>TAADTAVYYCAKHYYYGGSYAMDYWGQGLTVTVSSTTTP<br>APRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDI<br>YIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPV<br>QTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQN<br>QLYNELNLGRREEYDVLDRRGRDPEMGGKPRRKNPQEG<br>YNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTAT<br>KDTYDALHMQALPPRGGGSGGGGTGGGGSGMHRRSHTG<br>ERPFQCNQCGASFTQKGNLLRHRLHTGERPFRCHLCNTASA<br>EARHIRAEMG  |
| SEQ ID<br>NO: 94 | CAR19_16GS_HilD<br>tag_V5 (construct<br>768) (aa)     | MALPVTALLLPLALLLHAARPEIVMTQSPATLSLSPGERATL<br>SCRASQDISKYLNWYQQKPGQAPRLLIYHTSRLHSGIPARFS<br>GSGSGTDYTLTISSLQPEDFAVYFCQQGNTLPYTFGQGTKLE<br>IKGGGGSGGGGSGGGGSQVQLQESGPGLVKPSETLSLTCTV<br>SGVSLPDYGVSWIRQPPGKGLEWIGVIWGSETTYQSSLSK<br>RVTISKDNSKNQVSLKLSSVTAADTAVYYCAKHYYYGGSY<br>AMDYWGQGLTVTVSSTTTPAPRPPTPAPTIASQPLSLRPEAC<br>RPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLY                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |

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|                  |                                                    | CKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEEGGC<br>ELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKR<br>RGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMK<br>GERRRGKGHDLGYQGLSTATKDTYDALHMQALPPRGGGG<br>SGGGGTGGGGSGMHKRSHTGERPFQCNQCGASFTQKGNLL<br>RHIKLHTGEKPFKCHLCNTASAEARHIKAEMGGKPIPNNLLG<br>LDST                                                                                                                                                                                                                                                                                                                                                   |
| SEQ ID<br>NO: 30 | CAR19_16GS_HiID<br>tag (construct 769)<br>(aa)     | MALPVTALLPLALLLHAARPEIVMTQSPATLSLSPGERATL<br>SCRASQDISKYLNWYQQKPGQAPRLLIYHTSRLHSGIPARFS<br>GSGSGTDYTLTISSLPEDFAVYFCQQGNTLPYTFGQGTKE<br>IKGGGSGGGGSGGGGSSQVQLQESGPGLVKPSSETLSLTCTV<br>SGVSLPDYGVSWIRQPPGKGLEWIGVIWGSETTYQSSLKS<br>RVTISKDNSKNQVSLKLSSVTAADTAVYYCAKHYYGGSY<br>AMDYWGQGTLLTVSSTTTPAPRPPTPAPTASQPLSLRPEAC<br>RPAAGGAVHTRGLDFACDIYWAPLAGTCGVLLSLVITLY<br>CKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEEGGC<br>ELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKR<br>RGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMK<br>GERRRGKGHDLGYQGLSTATKDTYDALHMQALPPRGGGG<br>SGGGGTGGGGSGMHKRSHTGERPFQCNQCGASFTQKGNLL<br>RHIKLHTGEKPFKCHLCNTASAEARHIKAEMG |
| SEQ ID<br>NO: 31 | CAR19_16GS_HiID<br>tag_NoK (construct<br>770) (aa) | MALPVTALLPLALLLHAARPEIVMTQSPATLSLSPGERATL<br>SCRASQDISKYLNWYQQKPGQAPRLLIYHTSRLHSGIPARFS<br>GSGSGTDYTLTISSLPEDFAVYFCQQGNTLPYTFGQGTKE<br>IKGGGSGGGGSGGGGSSQVQLQESGPGLVKPSSETLSLTCTV<br>SGVSLPDYGVSWIRQPPGKGLEWIGVIWGSETTYQSSLKS<br>RVTISKDNSKNQVSLKLSSVTAADTAVYYCAKHYYGGSY<br>AMDYWGQGTLLTVSSTTTPAPRPPTPAPTASQPLSLRPEAC<br>RPAAGGAVHTRGLDFACDIYWAPLAGTCGVLLSLVITLY<br>CKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEEGGC<br>ELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKR<br>RGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMK<br>GERRRGKGHDLGYQGLSTATKDTYDALHMQALPPRGGGG<br>SGGGGTGGGGSGMHRSHTGERPFQCNQCGASFTQKGNLL<br>RHIRLHTGERPFRCHLCNTASAEARHIRAEMG  |
| SEQ ID<br>NO: 95 | CAR19_HiID<br>tag_V5 (construct<br>771) (aa)       | MALPVTALLPLALLLHAARPEIVMTQSPATLSLSPGERATL<br>SCRASQDISKYLNWYQQKPGQAPRLLIYHTSRLHSGIPARFS<br>GSGSGTDYTLTISSLPEDFAVYFCQQGNTLPYTFGQGTKE<br>IKGGGSGGGGSGGGGSSQVQLQESGPGLVKPSSETLSLTCTV<br>SGVSLPDYGVSWIRQPPGKGLEWIGVIWGSETTYQSSLKS<br>RVTISKDNSKNQVSLKLSSVTAADTAVYYCAKHYYGGSY<br>AMDYWGQGTLLTVSSTTTPAPRPPTPAPTASQPLSLRPEAC<br>RPAAGGAVHTRGLDFACDIYWAPLAGTCGVLLSLVITLY<br>CKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEEGGC<br>ELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKR<br>RGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMK<br>GERRRGKGHDLGYQGLSTATKDTYDALHMQALPPRMHHR<br>SHTGERPFQCNQCGASFTQKGNLLRHIKLHTGEKPFKCHLC<br>NTASAEARHIKAEMGGKPIPNNLLGLDST   |

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| SEQ ID<br>NO: 96  | CAR19_16KGS_Hil<br>D tag_V5 (construct<br>6761) (aa)    | MALPVTALLLPLALLLHAARPEIVMTQSPATLSLSPGERATL<br>SCRASQDISKYLNWYQQKPGQAPRLLIYHTSRLHSGIPARFS<br>GSGSGTDYTLTISSLPEDFAVYFCQQGNTLPYTFGQGTKE<br>IKGGGSGGGGSGGGGSQVQLQESGPGLVKPSSETLSLTCTV<br>SGVSLPDYGVSWIRQPPGKGLEWIGVIWGSETTYQSSLKS<br>RVTISKDNSKNQVSLKLSSVTAADTAVYYCAKHYYGGSY<br>AMDYWGQGTLLTVSSTTTTPAPRPPTPAPTIASQPLSLRPEAC<br>RPAAGGAVHTRGLDFACDIYWAPLAGTCGVLLLSLVITLY<br>CKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGC<br>ELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDR<br>RGRDPPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMK<br>GERRRGKGHDLGYQGLSTATKDTYDALHMQALPPRKG<br>SKGGGTGKGGSKMHKRSHTGERPFQCNQCGASFTQKGNLL<br>RHIKLHTGEKPFKCHLCNTASAEARHIKAEMGGKPIPPLLGL<br>LDST |
| SEQ ID<br>NO: 97  | HilD<br>tag_CAR19_modSig<br>Pep (construct 773)<br>(aa) | MALPVTALLLPLALLLHAARPRSSLAHKRSHTGERPFQCNQ<br>CGASFTQKGNLLRHIKLHTGEKPFKCHLCNTASAEARHIKA<br>EMGGTGAEDPRPSRKRRSLGDVGEIVMTQSPATLSLSPGER<br>ATLSCRASQDISKYLNWYQQKPGQAPRLLIYHTSRLHSGIPA<br>RFSGSGSGTDYTLTISSLPEDFAVYFCQQGNTLPYTFGQGT<br>KLEIKGGGSGGGGSGGGGSQVQLQESGPGLVKPSSETLSLT<br>CTVSGVSLPDYGVSWIRQPPGKGLEWIGVIWGSETTYQSS<br>LKSRTISKDNSKNQVSLKLSSVTAADTAVYYCAKHYYG<br>GSYAMDYWGQGTLLTVSSTTTTPAPRPPTPAPTIASQPLSLRP<br>EACRPAAGGAVHTRGLDFACDIYWAPLAGTCGVLLLSLVIT<br>LYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEG<br>GCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDR<br>KRRGRDPPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIG<br>MKGERRRGKGHDLGYQGLSTATKDTYDALHMQALPPR       |
| SEQ ID<br>NO: 98  | HilD tag_CAR19<br>(construct 774) (aa)                  | MALPVTALLLPLALLLHAARPHKRSHTGERPFQCNQCGASF<br>TQKGNLLRHIKLHTGEKPFKCHLCNTASAEARHIKAEMGGT<br>GAEDPRPSRKRRSLGDVGEIVMTQSPATLSLSPGERATLSCR<br>ASQDISKYLNWYQQKPGQAPRLLIYHTSRLHSGIPARFSGSG<br>SGTDYTLTISSLPEDFAVYFCQQGNTLPYTFGQGTKEIKG<br>GGGSGGGGSGGGGSQVQLQESGPGLVKPSSETLSLTCTVSGV<br>SLPDYGVSWIRQPPGKGLEWIGVIWGSETTYQSSLKSRTI<br>SKDNSKNQVSLKLSSVTAADTAVYYCAKHYYGGSYAMD<br>YWGQGTLLTVSSTTTTPAPRPPTPAPTIASQPLSLRPEACRPA<br>AGGAVHTRGLDFACDIYWAPLAGTCGVLLLSLVITLYCKR<br>GRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRV<br>KFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDRGRD<br>PEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRR<br>GKGHDLGYQGLSTATKDTYDALHMQALPPR                |
| SEQ ID<br>NO: 112 | CAR19-16GS-<br>CARBtag (aa)                             | MALPVTALLLPLALLLHAARPEIVMTQSPATLSLSPGERATL<br>SCRASQDISKYLNWYQQKPGQAPRLLIYHTSRLHSGIPARFS<br>GSGSGTDYTLTISSLPEDFAVYFCQQGNTLPYTFGQGTKE<br>IKGGGSGGGGSGGGGSQVQLQESGPGLVKPSSETLSLTCTV<br>SGVSLPDYGVSWIRQPPGKGLEWIGVIWGSETTYQSSLKS<br>RVTISKDNSKNQVSLKLSSVTAADTAVYYCAKHYYGGSY<br>AMDYWGQGTLLTVSSTTTTPAPRPPTPAPTIASQPLSLRPEAC                                                                                                                                                                                                                                                                                                                            |

|                       |                                   |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |
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|                       |                                   | RPAAGGAVHTRGLDFACDIYWAPLAGTCGVLLLSLVITLY<br>CKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFEEEEGGC<br>ELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKR<br>RGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMK<br>GERRRGKGHGGLYQGLSTATKDTYDALHMQALPPRGGGG<br>SGGGGTGGGGSGHKRSHTGERPFHCNQCASFTQKGNLLR<br>HIKLHSGEKPFCPCFCSAGQVMSHHVPPMED                                                                                                                                                                                                                                                                                                                          |
| SEQ ID<br>NO:<br>1450 | BCMA CAR-16GS<br>linker-HilD (aa) | MALPVTALLPLALLLHAARPEVQLVESGGGLVQPGGSLRL<br>SCAVSGFALSNGMSWVRRAPGKGLEWVSGIVYSGSTYYA<br>ASVKGRFTISRDNSTNTLYLQMNSLRPEDTAIYYCSAHGGES<br>DVWGQGTITVTVSSASGGGGSGGRASGGGGSDIQLTQSPSSL<br>SASVGDRVTITCRASQSISSYLNWYQQKPKGAPKLLIYAASS<br>LQSGVPSRFSGSGSGTDFLTITSLQPEDFATYYCQQSYSTPY<br>TFGQGTKVEIKTTTPAPRPPTPAPTIASQPLSLRPEACRPAAG<br>GAVHTRGLDFACDIYWAPLAGTCGVLLLSLVITLYCKRGR<br>KKLLYIFKQPFMRPVQTTQEEDGCSCRFEEEEGGCELRVKF<br>SRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPE<br>MGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRG<br>KGHGGLYQGLSTATKDTYDALHMQALPPRGGGGSGGGGT<br>GGGGSGMHKRSHTGERPFQCNQCASFTQKGNLLRHILH<br>TGEKPFKCHLCNTASAEARHIKAEMG |
| SEQ ID<br>NO: 837     |                                   | MALEK                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |

In one aspect, an exemplary CAR constructs comprise an optional leader sequence (e.g., a leader sequence described herein), an extracellular antigen binding domain (e.g., an antigen binding domain described herein), a hinge (e.g., a hinge region described herein), a transmembrane domain (e.g., a transmembrane domain described herein), and an intracellular stimulatory domain (e.g., an intracellular stimulatory domain described herein). In one aspect, an exemplary CAR construct comprises an optional leader sequence (e.g., a leader sequence described herein), an extracellular antigen binding domain (e.g., an antigen binding domain described herein), a hinge (e.g., a hinge region described herein), a transmembrane domain (e.g., a transmembrane domain described herein), an intracellular costimulatory signaling domain (e.g., a costimulatory signaling domain described herein) and/or an intracellular primary signaling domain (e.g., a primary signaling domain described herein).

In one aspect, the CARs (e.g., CD19 CARs) of the invention comprise at least one intracellular signaling domain selected from the group of a CD137 (4-1BB) signaling domain, a CD28 signaling domain, a CD27 signaling domain, an ICOS signaling domain, a CD3zeta signal domain, and any combination thereof. In one aspect, the CARs comprise at least one intracellular signaling domain is from one or more costimulatory molecule(s) selected from CD137 (4-1BB), CD28, CD27, or ICOS.

## CAR Antigen Binding Domain

In one aspect, the CAR of the disclosure linked to a COF1/CRBN-, COF2/CRBN-, or COF3/CRBN-binding polypeptide, and/or a degradation domain comprises a target-specific binding element otherwise referred to as an antigen binding domain. In one embodiment, the portion of the CAR comprising the antigen binding domain comprises an antigen binding domain that targets, *e.g.*, specifically binds to, an antigen, *e.g.*, antigen described herein, *e.g.*, CD19. In one embodiment, the antigen binding domain targets, *e.g.*, specifically binds to, human CD19.

In some embodiments, a plurality of immune effector cells, *e.g.*, the population of T regulatory-depleted cells, include a nucleic acid encoding a CAR that comprises a target-specific binding element otherwise referred to as an antigen binding domain. The choice of binding element depends upon the type and number of ligands that define the surface of a target cell. For example, the antigen binding domain may be chosen to recognize a ligand that acts as a cell surface marker on target cells associated with a particular disease state. Thus, examples of cell surface markers that may act as ligands for the antigen binding domain in a CAR described herein include those associated with viral, bacterial and parasitic infections, autoimmune disease and cancer cells.

In one aspect, the portion of the CAR comprising the antigen binding domain comprises an antigen binding domain that targets a tumor antigen, *e.g.*, a tumor antigen described herein. In some embodiments, the antigen binding domain is chosen from: CD19; CD123; CD22; CD30; CD171; CS-1; C-type lectin-like molecule-1, CD33; epidermal growth factor receptor variant III (EGFRvIII); ganglioside G2 (GD2); ganglioside GD3; TNF receptor family member; B-cell maturation antigen (BCMA); Tn antigen ((Tn Ag) or (GalNAc $\alpha$ -Ser/Thr)); prostate-specific membrane antigen (PSMA); Receptor tyrosine kinase-like orphan receptor 1 (ROR1); Fms-Like Tyrosine Kinase 3 (FLT3); Tumor-associated glycoprotein 72 (TAG72); CD38; CD44v6; Carcinoembryonic antigen (CEA); Epithelial cell adhesion molecule (EPCAM); B7H3 (CD276); KIT (CD117); Interleukin-13 receptor subunit alpha-2; Mesothelin; Interleukin 11 receptor alpha (IL-11Ra); prostate stem cell antigen (PSCA); Protease Serine 21; vascular endothelial growth factor receptor 2 (VEGFR2); Lewis(Y) antigen; CD24; Platelet-derived growth factor receptor beta (PDGFR-beta); Stage-specific embryonic antigen-4 (SSEA-4); CD20; Folate receptor alpha; Receptor tyrosine-protein kinase ERBB2 (Her2/neu); Mucin 1, cell surface associated (MUC1); epidermal growth factor receptor (EGFR); neural cell adhesion molecule (NCAM); Prostase; prostatic acid phosphatase (PAP); elongation factor 2 mutated (ELF2M); Ephrin B2; fibroblast activation protein alpha (FAP); insulin-like growth factor 1 receptor (IGF-I receptor), carbonic anhydrase IX (CAIX); Proteasome (Prosome, Macropain) Subunit, Beta Type, 9 (LMP2); glycoprotein 100 (gp100); oncogene fusion protein consisting of breakpoint cluster region (BCR) and Abelson murine leukemia

viral oncogene homolog 1 (Abl) (bcr-abl); tyrosinase; ephrin type-A receptor 2 (EphA2); Fucosyl GM1; sialyl Lewis adhesion molecule (sLe); ganglioside GM3; transglutaminase 5 (TGS5); high molecular weight-melanoma-associated antigen (HMWMAA); o-acetyl-GD2 ganglioside (OAcGD2); Folate receptor beta; tumor endothelial marker 1 (TEM1/CD248); tumor endothelial marker 7-related (TEM7R);

5 claudin 6 (CLDN6); thyroid stimulating hormone receptor (TSHR); G protein-coupled receptor class C group 5, member D (GPRC5D); chromosome X open reading frame 61 (CXORF61); CD97; CD179a; anaplastic lymphoma kinase (ALK); Polysialic acid; placenta-specific 1 (PLAC1); hexasaccharide portion of globoH glycosphingolipid (GloboH); mammary gland differentiation antigen (NY-BR-1); uroplakin 2 (UPK2); Hepatitis A virus cellular receptor 1 (HAVCR1); adrenoceptor beta 3 (ADRB3); pannexin 3

10 (PANX3); G protein-coupled receptor 20 (GPR20); lymphocyte antigen 6 complex, locus K 9 (LY6K); Olfactory receptor 51E2 (OR51E2); TCR Gamma Alternate Reading Frame Protein (TARP); Wilms tumor protein (WT1); Cancer/testis antigen 1 (NY-ESO-1); Cancer/testis antigen 2 (LAGE-1a); Melanoma-associated antigen 1 (MAGE-A1); ETS translocation-variant gene 6, located on chromosome 12p (ETV6-AML); sperm protein 17 (SPA17); X Antigen Family, Member 1A (XAGE1); angiopoietin-

15 binding cell surface receptor 2 (Tie 2); melanoma cancer testis antigen-1 (MAD-CT-1); melanoma cancer testis antigen-2 (MAD-CT-2); Fos-related antigen 1; tumor protein p53 (p53); p53 mutant; prostein; surviving; telomerase; prostate carcinoma tumor antigen-1, melanoma antigen recognized by T cells 1; Rat sarcoma (Ras) mutant; human Telomerase reverse transcriptase (hTERT); sarcoma translocation breakpoints; melanoma inhibitor of apoptosis (ML-IAP); ERG (transmembrane protease, serine 2

20 (TMPRSS2) ETS fusion gene); N-Acetyl glucosaminyl-transferase V (NA17); paired box protein Pax-3 (PAX3); Androgen receptor; Cyclin B1; v-myc avian myelocytomatosis viral oncogene neuroblastoma derived homolog (MYCN); Ras Homolog Family Member C (RhoC); Tyrosinase-related protein 2 (TRP-2); Cytochrome P450 1B1 (CYP1B1); CCCTC-Binding Factor (Zinc Finger Protein)-Like, Squamous Cell Carcinoma Antigen Recognized By T Cells 3 (SART3); Paired box protein Pax-5 (PAX5);

25 proacrosin binding protein sp32 (OY-TES1); lymphocyte-specific protein tyrosine kinase (LCK); A kinase anchor protein 4 (AKAP-4); synovial sarcoma, X breakpoint 2 (SSX2); Receptor for Advanced Glycation Endproducts (RAGE-1); renal ubiquitous 1 (RU1); renal ubiquitous 2 (RU2); legumain; human papilloma virus E6 (HPV E6); human papilloma virus E7 (HPV E7); intestinal carboxyl esterase; heat shock protein 70-2 mutated (mut hsp70-2); CD79a; CD79b; CD72; Leukocyte-associated

30 immunoglobulin-like receptor 1 (LAIR1); Fc fragment of IgA receptor (FCAR or CD89); Leukocyte immunoglobulin-like receptor subfamily A member 2 (LILRA2); CD300 molecule-like family member f (CD300LF); C-type lectin domain family 12 member A (CLEC12A); bone marrow stromal cell antigen 2 (BST2); EGF-like module-containing mucin-like hormone receptor-like 2 (EMR2); lymphocyte antigen



75 (LY75); Glypican-3 (GPC3); Fc receptor-like 5 (FCRL5); and immunoglobulin lambda-like polypeptide 1 (IGLL1).

In one embodiment, the antigen binding domain binds to CD19. In another embodiment, the antigen binding domain binds to CD123. In another embodiment, the antigen binding domain binds to BCMA. In another embodiment, the antigen binding domain binds to CD20.

The antigen binding domain can be any domain that binds to the antigen including but not limited to a monoclonal antibody, a polyclonal antibody, a recombinant antibody, a human antibody, a humanized antibody, and a functional fragment thereof, including but not limited to a single-domain antibody such as a heavy chain variable domain (VH), a light chain variable domain (VL) and a variable domain (VHH) of camelid derived nanobody, and to an alternative scaffold known in the art to function as antigen binding domain, such as a recombinant fibronectin domain, a T cell receptor (TCR), or a fragment thereof, e.g., single chain TCR, and the like. In some instances, it is beneficial for the antigen binding domain to be derived from the same species in which the CAR will ultimately be used in. For example, for use in humans, it may be beneficial for the antigen binding domain of the CAR to comprise human or humanized residues for the antigen binding domain of an antibody or antibody fragment.

The antigen binding domain can be any domain that binds to the antigen including but not limited to a monoclonal antibody, a polyclonal antibody, a recombinant antibody, a human antibody, a humanized antibody, and a functional fragment thereof, including but not limited to a single-domain antibody such as a heavy chain variable domain (VH), a light chain variable domain (VL) and a variable domain (VHH) of camelid derived nanobody, and to an alternative scaffold known in the art to function as an antigen binding domain, such as a recombinant fibronectin domain, and the like. In some instances, it is beneficial for the antigen binding domain to be derived from the same species in which the CAR will ultimately be used in. For example, for use in humans, it may be beneficial for the antigen binding domain of the CAR to comprise human or humanized residues for the antigen binding domain of an antibody or antibody fragment. Thus, in one aspect, the antigen binding domain comprises a human antibody or an antibody fragment.

In one embodiment, the antigen binding domain comprises one, two, or three (e.g., all three) heavy chain CDRs, HC CDR1, HC CDR2 and HC CDR3, from an antibody described herein (e.g., an antibody described in WO2015/142675, US-2015-0283178-A1, US-2016-0046724-A1, US2014/0322212A1, US2016/0068601A1, US2016/0051651A1, US2016/0096892A1, US2014/0322275A1, or WO2015/090230, incorporated herein by reference), and/or one, two, or three (e.g., all three) light chain CDRs, LC CDR1, LC CDR2 and LC CDR3, from an antibody described herein (e.g., an antibody described in WO2015/142675, US-2015-0283178-A1, US-2016-0046724-A1,

US2014/0322212A1, US2016/0068601A1, US2016/0051651A1, US2016/0096892A1, US2014/0322275A1, or WO2015/090230, incorporated herein by reference). In one embodiment, the antigen binding domain comprises a heavy chain variable region and/or a variable light chain region of an antibody listed above.

5 In embodiments, the antigen binding domain is an antigen binding domain described in WO2015/142675, US-2015-0283178-A1, US-2016-0046724-A1, US2014/0322212A1, US2016/0068601A1, US2016/0051651A1, US2016/0096892A1, US2014/0322275A1, or WO2015/090230, incorporated herein by reference.

10 Exemplary target antigens that can be targeted using the CAR-expressing cells, include, but are not limited to, CD19, CD123, EGFRvIII, CD33, mesothelin, BCMA, and GFR ALPHA-4, among others, as described in, for example, WO2014/153270, WO 2014/130635, WO2016/028896, WO 2014/130657, WO2016/014576, WO 2015/090230, WO2016/014565, WO2016/014535, and WO2016/025880, each of which is herein incorporated by reference in its entirety.

#### 15 *Multispecific CAR*

In some embodiments, the CAR molecule is a multispecific, e.g., bispecific, CAR molecule having a first binding specificity for a first antigen, e.g., a B-cell epitope, and a second binding specificity for the same or a different antigen, e.g., a B cell epitope. In some embodiments, the bispecific CAR molecule has a first binding specificity for CD19 (e.g., the bispecific CAR molecule comprises an anti-  
20 CD19 CAR disclosed in Tables 5, 6, 7 and 30) and a second binding specificity for CD22 (e.g., the bispecific CAR molecule comprises an anti-CD22 CAR disclosed in Tables 19 and 20). In some embodiments, the bispecific CAR molecule has a first binding specificity for CD19 (e.g., the bispecific CAR molecule comprises an anti-CD19 CAR disclosed in Tables 5, 6, 7 and 30) and a second binding specificity for CD20 (e.g., the bispecific CAR molecule comprises an anti-CD20 CAR disclosed in Table  
25 32).

In one embodiment, the first and second binding specificity is an antibody molecule, e.g., an antibody binding domain (e.g., a scFv). Within each antibody molecule (e.g., scFv) of a bispecific CAR molecule, the VH can be upstream or downstream of the VL.

30 In some embodiments, the upstream antibody or antibody fragment (e.g., scFv) is arranged with its VH (VH<sub>1</sub>) upstream of its VL (VL<sub>1</sub>) and the downstream antibody or antibody fragment (e.g., scFv) is arranged with its VL (VL<sub>2</sub>) upstream of its VH (VH<sub>2</sub>), such that the overall bispecific CAR molecule has the arrangement VH<sub>1</sub>-VL<sub>1</sub>-VL<sub>2</sub>-VH<sub>2</sub>, from an N- to C-terminal orientation.

In some embodiments, the upstream antibody or antibody fragment (e.g., scFv) is arranged with its VL (VL<sub>1</sub>) upstream of its VH (VH<sub>1</sub>) and the downstream antibody or antibody fragment (e.g., scFv) is arranged with its VH (VH<sub>2</sub>) upstream of its VL (VL<sub>2</sub>), such that the overall bispecific CAR molecule has the arrangement VL<sub>1</sub>-VH<sub>1</sub>-VH<sub>2</sub>-VL<sub>2</sub>, from an N- to C-terminal orientation.

5 In some embodiments, the upstream antibody or antibody fragment (e.g., scFv) is arranged with its VL (VL<sub>1</sub>) upstream of its VH (VH<sub>1</sub>) and the downstream antibody or antibody fragment (e.g., scFv) is arranged with its VL (VL<sub>2</sub>) upstream of its VH (VH<sub>2</sub>), such that the overall bispecific CAR molecule has the arrangement VL<sub>1</sub>-VH<sub>1</sub>-VL<sub>2</sub>-VH<sub>2</sub>, from an N- to C-terminal orientation.

10 In yet some embodiments, the upstream antibody or antibody fragment (e.g., scFv) is arranged with its VH (VH<sub>1</sub>) upstream of its VL (VL<sub>1</sub>) and the downstream antibody or antibody fragment (e.g., scFv) is arranged with its VH (VH<sub>2</sub>) upstream of its VL (VL<sub>2</sub>), such that the overall bispecific CAR molecule has the arrangement VH<sub>1</sub>-VL<sub>1</sub>-VH<sub>2</sub>-VL<sub>2</sub>, from an N- to C-terminal orientation.

In any of the aforesaid configurations, optionally, a linker is disposed between the two antibodies or antibody fragments (e.g., scFvs), e.g., between VL<sub>1</sub> and VL<sub>2</sub> if the construct is arranged as VH<sub>1</sub>-VL<sub>1</sub>-VL<sub>2</sub>-VH<sub>2</sub>; between VH<sub>1</sub> and VH<sub>2</sub> if the construct is arranged as VL<sub>1</sub>-VH<sub>1</sub>-VH<sub>2</sub>-VL<sub>2</sub>; between VH<sub>1</sub> and VL<sub>2</sub> if the construct is arranged as VL<sub>1</sub>-VH<sub>1</sub>-VL<sub>2</sub>-VH<sub>2</sub>; or between VL<sub>1</sub> and VH<sub>2</sub> if the construct is arranged as VH<sub>1</sub>-VL<sub>1</sub>-VH<sub>2</sub>-VL<sub>2</sub>. In general, the linker between the two scFvs should be long enough to avoid mispairing between the domains of the two scFvs. The linker may be a linker as described herein. In some embodiments, the linker is a (Gly<sub>4</sub>-Ser)<sub>n</sub> linker, wherein n is 1, 2, 3, 4, 5, or 6. In some  
20 embodiments, the linker is (Gly<sub>4</sub>-Ser)<sub>n</sub>, wherein n = 1 (SEQ ID NO: 168), e.g., the linker has the amino acid sequence Gly<sub>4</sub>-Ser (SEQ ID NO: 168). In some embodiments, the linker is (Gly<sub>4</sub>-Ser)<sub>n</sub>, wherein n = 3 (SEQ ID NO: 142). In some embodiments, the linker is (Gly<sub>4</sub>-Ser)<sub>n</sub>, wherein n = 4 (SEQ ID NO: 141). In some embodiments, the linker comprises, e.g., consists of, the amino acid sequence: LAEAAAK (SEQ ID NO: 822).

25 In any of the aforesaid configurations, optionally, a linker is disposed between the VL and VH of the first scFv. Optionally, a linker is disposed between the VL and VH of the second scFv. In constructs that have multiple linkers, any two or more of the linkers can be the same or different. Accordingly, in some embodiments, a bispecific CAR comprises VLs, VHs, and optionally one or more linkers in an arrangement as described herein.

30 In some embodiments, each antibody molecule, e.g., each antigen binding domain (e.g., each scFv) comprises a linker between the VH and the VL regions. In some embodiments, the linker between the VH and the VL regions is a (Gly<sub>4</sub>-Ser)<sub>n</sub> linker, wherein n is 1, 2, 3, 4, 5, or 6. In some embodiments, the linker is (Gly<sub>4</sub>-Ser)<sub>n</sub>, wherein n = 1 (SEQ ID NO: 168), e.g., the linker has the amino acid sequence Gly<sub>4</sub>-Ser (SEQ ID NO: 168). In some embodiments, the linker is (Gly<sub>4</sub>-Ser)<sub>n</sub>, wherein n = 3 (SEQ ID NO:

142). In some embodiments, the linker is (Gly<sub>4</sub>-Ser)<sub>n</sub>, wherein n= 4 (SEQ ID NO: 141). In some embodiments, the VH and VL regions are connected without a linker.

Additional exemplary multispecific CAR molecules are disclosed on pages 26-39 of WO2018/067992, herein incorporated by reference.

5

### CD19 CAR

In other embodiments, the CAR-expressing cells can specifically bind to CD19, *e.g.*, can include a CAR molecule, or an antigen binding domain (*e.g.*, a humanized antigen binding domain) according to Table 3 of WO2014/153270, incorporated herein by reference.

10 In embodiments, the CAR molecule comprises an antigen binding domain that binds specifically to CD19 (CD19 CAR). In one embodiment, the antigen binding domain targets human CD19. In one embodiment, the antigen binding domain of the CAR has the same or a similar binding specificity as the FMC63 scFv fragment described in Nicholson et al. *Mol. Immun.* 34 (16-17): 1157-1165 (1997). In one embodiment, the antigen binding domain of the CAR includes the scFv fragment described in Nicholson  
15 et al. *Mol. Immun.* 34 (16-17): 1157-1165 (1997). A CD19 antibody molecule can be, *e.g.*, an antibody molecule (*e.g.*, a humanized anti-CD19 antibody molecule) described in WO2014/153270, which is incorporated herein by reference in its entirety. WO2014/153270 also describes methods of assaying the binding and efficacy of various CAR constructs.

In one aspect, the parental murine scFv sequence is the CAR19 construct provided in PCT  
20 publication WO2012/079000 (incorporated herein by reference). In one embodiment, the anti-CD19 binding domain is a scFv described in WO2012/079000.

In one embodiment, the CAR molecule comprises the fusion polypeptide sequence provided as SEQ ID NO: 12 in PCT publication WO2012/079000, and provided herein in Table 5, which provides an scFv fragment of murine origin that specifically binds to human CD19. Humanization of this mouse scFv  
25 may be desired for the clinical setting, where the mouse-specific residues may induce a human-anti-mouse antigen (HAMA) response in patients who receive CART19 treatment, *e.g.*, treatment with T cells transduced with the CAR19 construct.

In one embodiment, the CD19 CAR comprises an amino acid sequence provided as SEQ ID NO: 12 in PCT publication WO2012/079000. In embodiment, the amino acid sequence is

30 (MALPVTALLLPLALLLHAARP)diqmtqtsslsaslgdrvtiscrasqdiskylnwyyqqkpdgtvkllyhtsrhsgvp  
srfsgsgsgtdysltisnleqediayfcqqgntlpytfgggtkleitggggsgggsggggsevkqlqesgpglvapsqslsvtctvsgvslpdygvsw  
irqprrglewlgviwgsettyynsalksrliikdnksqvflkmnslqtddtaiyycahyyyggsyamdywgqgtsvtvssttpaprrpptpap  
tiasqplslrpeacrpaaggavhtrglfdacdiyiwaplagtcgvllslvitlyckrgrkkllyifkqpfmrvpvtqtteedgcsrpfpeeeeggcclrvkf

srsadapaykqggnqlynelnlgreeydvldkrrgrdpemggkprknpqeglynelqkdkmaeyseigmkgerrrgkghdglyqglstatk  
 dtydalhmqlppr (SEQ ID NO: 181), or a sequence substantially homologous thereto. The optional  
 sequence of the signal peptide is shown in capital letters and parenthesis.

In one embodiment, the amino acid sequence is:

5 Diqmtqtsslsaslgdrvtiscrasqdiskylnwyqqkpdgtvklliyhtsrhsgvpsrfsqsgsgtdysltisnleqediayfcqqgntl  
 pytfgggktleitggggsgggsggggsevkqlqesgpglvapsqslsvtctvsgvslpdygvswirpprkglewlgviwgsettyynsalksrtii  
 kdnsksqvflkmnslqtdtaiyycahyyyggsyamydwgqgtsvtvssttpaprpptaptiasqplsrpeacrpaaggavhtrglfacdiy  
 iwaplagtcgvllslvitlyckrgrklllyifkqpfmrpvqttqeedgcscrpfceeeeggcelrvkfsrsadapaykqggnqlynelnlgreeydvld  
 krrgrdpemggkprknpqeglynelqkdkmaeyseigmkgerrrgkghdglyqglstatk dtydalhmqlppr (SEQ ID NO:  
 10 182), or a sequence substantially homologous thereto.

In one embodiment, the CD19 CAR has the USAN designation TISAGENLEUCLEUCEL-T. In  
 embodiments, CTL019 is made by a gene modification of T cells is mediated by stable insertion via  
 transduction with a self-inactivating, replication deficient Lentiviral (LV) vector containing the CTL019  
 transgene under the control of the EF-1 alpha promoter. CTL019 can be a mixture of transgene positive  
 15 and negative T cells that are delivered to the subject on the basis of percent transgene positive T cells.

In other embodiments, the CD19 CAR comprises an antigen binding domain (e.g., a humanized  
 antigen binding domain) according to Table 3 of WO2014/153270, incorporated herein by reference.

Humanization of murine CD19 antibody is desired for the clinical setting, where the mouse-  
 specific residues may induce a human-anti-mouse antigen (HAMA) response in patients who receive  
 20 CART19 treatment, i.e., treatment with T cells transduced with the CAR19 construct. The production,  
 characterization, and efficacy of humanized CD19 CAR sequences is described in International  
 Application WO2014/153270 which is herein incorporated by reference in its entirety, including  
 Examples 1-5 (p. 115-159).

In some embodiments, CD19 CAR constructs are described in PCT publication WO  
 25 2012/079000, incorporated herein by reference, and the amino acid sequence of the murine CD19 CAR  
 and scFv constructs are shown in Table 5 below, or a sequence substantially identical to any of the  
 aforesaid sequences (e.g., at least 85%, 90%, 95% or more identical to any of the sequences described  
 herein).

**Table 5. CD19 CAR Constructs**

| SEQ ID NO         | Region                    | Sequence                                                                            |
|-------------------|---------------------------|-------------------------------------------------------------------------------------|
| CTL019            |                           |                                                                                     |
| SEQ ID NO:<br>183 | CTL019 Full<br>amino acid | MALPVTALLLPLALLLHAARPDIQMTQTTSSLSASLGDRV<br>TISCRAQDISKYLNWYQQKPDGTVKLLIYHTSRHSGVPS |

|                   |                                       |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |
|-------------------|---------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|                   | sequence                              | <p>RFSGSGSGTDYSLTISNLEQEDIATYFCQQGNTLPYTFGGG<br/> TKLEITGGGSGGGGSGGGGSEVKLQESGPGLVAPSQSL<br/> VTCTVSGVSLPDYGVSWIRQPPRKGLEWLGVWGETTY<br/> NSALKSRLTIHKDNSKSQVFLKMNSLQTDDETAIYYCAKHY<br/> YYGGSYAMDYWGQGTSTVVSSTTTPAPRPPTPAPTASQP<br/> LSLRPEACRPAAGGAVHTRGLDFACDIYWAPLAGTCGVL<br/> LLSLVITLYCKRGRKLLYIFKQPFMRPVQTTQEEDGCSCR<br/> FPEEEEGGCELRVKFSRSADAPAYKQGQNQLYNELNLGRR<br/> EEYDVLDRRRGRDPGEMGGKPRRKNPQEGLYNELQKDKM<br/> AEAYSEIGMKGERRRRGKGHDLGYQGLSTATKDTYDALH<br/> MQALPPR</p>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |
| SEQ ID NO:<br>184 | CTL019 Full<br>nucleotide<br>sequence | <p>ATGGCCTTACCAGTGACCGCCTTGCTCCTGCCGCTGGCC<br/> TTGCTGCTCCACGCCCGCCAGGCCGGACATCCAGATGAC<br/> ACAGACTACATCCTCCCTGTCTGCCTCTCTGGGAGACAG<br/> AGTCACCATCAGTTGCAGGGCAAGTCAGGACATTAGTA<br/> AATATTTAAATTGGTATCAGCAGAAACCAGATGGAAC<br/> GTAAACTCCTGATCTACCATAACATCAAGATTACACTCA<br/> GGAGTCCCATCAAGGTTTCAAGTGGCAGTGGGTCTGGAAC<br/> AGATTATTCTCTCACCATTAGCAACCTGGAGCAAGAAG<br/> ATATTGCCACTTACTTTTGCCAACAGGGTAATACGCTTC<br/> CGTACACGTTTCGGAGGGGGGACCAAGCTGGAGATCACA<br/> GGTGGCGGTGGCTCGGGCGGTGGTGGGTGGGTGGCGG<br/> CGGATCTGAGGTGAAACTGCAGGAGTCAGGACCTGGCC<br/> TGGTGGCGCCCTCACAGAGCCTGTCCGTCACATGCACTG<br/> TCTCAGGGGTCTCATTACCCGACTATGGTGTAAAGCTGGA<br/> TTCGCCAGCCTCCACGAAAGGGTCTGGAGTGGCTGGGA<br/> GTAATATGGGGTAGTGAAACCACATACTATAATTACGC<br/> TCTCAAATCCAGACTGACCATCATCAAGGACAACCTCCA<br/> AGAGCCAAGTTTTCTTAAAAATGAACAGTCTGCAAAC<br/> GATGACACAGCCATTTACTACTGTGCCAAACATTATTAC<br/> TACGGTGGTAGCTATGCTATGGACTACTGGGGCCAAGG<br/> AACCTCAGTCACCGTCTCCTCAACCACGACGCCAGCGCC<br/> GCGACCACCAACACCGGCGCCACCATCGCGTCGCAGC<br/> CCCTGTCCCTGCGCCAGAGGCGTGCCGGCCAGCGGCG<br/> GGGGGCGCAGTGCACACGAGGGGGCTGGACTTCGCCTG<br/> TGATATCTACATCTGGGCGCCCTTGCCGGGACTTGTGG<br/> GGTCCTTCTCCTGTCACTGGTTATCACCTTTACTGCAA<br/> ACGGGGCAGAAAGAAACTCCTGTATATATTCAAACAAC<br/> CATTTATGAGACCAGTACAAACTACTCAAGAGGAAGAT<br/> GGCTGTAGCTGCCGATTTCCAGAAGAAGAAGAGGAGG<br/> ATGTGAAGTGAAGTTCAGCAGGAGCGCAGACG<br/> CCCCCGGTACAAGCAGGGCCAGAACCAGCTCTATAAC<br/> GAGCTCAATCTAGGACGAAGAGAGGAGTACGATGTTTT<br/> GGACAAGAGACGTGGCCGGGACCCTGAGATGGGGGGA<br/> AAGCCGAGAAGGAAGAACCCTCAGGAAGGCCTGTACA<br/> ATGAACTGCAGAAAGATAAGATGGCGGAGGCCTACAGT<br/> GAGATTGGGATGAAAGGCGAGCGCCGGAGGGGCAAGG<br/> GGCACGATGGCCTTTACCAGGGTCTCAGTACAGCCACC<br/> AAGGACACCTACGACGCCCTTCACATGCAGGCCCTGCC<br/> CCCTCGC</p> |

|                   |                                                       |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |
|-------------------|-------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| SEQ ID NO:<br>185 | CTL019 scFv<br>domain                                 | DIQMTQTTSSLSASLGDRVTISCRASQDISKYLNWYQQKPD<br>GTVKLLIYHTSRLHSGVPSRFSGSGSGTDYSLTISNLEQEDI<br>ATYFCQQGNTLPYTFGGGTKLEITGGGSGGGGSGGGGSE<br>VKLQESGPGLVAPSQSLSVTCTVSGVSLPDYGVSWIRQPPR<br>KGLEWLGVWGSSETTYYNALKSRLLTIKDNSKSKVFLKM<br>NSLQTDDETAIYYCAKHYYYGGSYAMDYWGQGTSTVTVSS                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             |
| Humanized<br>CAR2 |                                                       |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |
| SEQ ID NO:<br>197 | CAR2 scFv<br>domain – aa<br>(Linker is<br>underlined) | EIVMTQSPATLSLSPGERATLSCRASQDISKYLNWYQQKPG<br>QAPRLLIYHTSRLHSGIPARFSGSGSGTDYTLTISSLQPEDF<br>AVYFCQQGNTLPYTFGQGTKEIKGGGSGGGGSGGGGSS<br>QVQLQESGPGLVKPSETLSLTCTVSGVSLPDYGVSWIRQPP<br>GKLEWIGVIWGSSETTYQSSLSKSRVTISKDNSKNQVSLK<br>LSSVTAADTAVYYCAKHYYYGGSYAMDYWGQGTSLTVTS<br>S                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |
| SEQ ID NO:<br>198 | CAR2 scFv<br>domain – nt                              | ATGGCCCTCCCTGTACCGCCCTGCTGCTTCCGCTGGCT<br>CTTCTGCTCCACGCCGCTCGGCCCGAAATTGTGATGACC<br>CAGTACCCCGCCACTCTTAGCCTTTCACCCGGTGAGCGC<br>GCAACCCTGTCTTGACAGAGCCTCCCAAGACATCTCAAA<br>ATACCTTAATTGGTATCAACAGAAGCCCGGACAGGCTC<br>CTCGCCTTCTGATCTACCACACCAGCCGGCTCATTCTG<br>GAATCCCTGCCAGGTTACAGCGGTAGCGGATCTGGGACC<br>GACTACACCCTCACTATCAGCTCACTGCAGCCAGAGGA<br>CTTCGCTGTCTATTTCTGTCAGCAAGGGAACACCCTGCC<br>CTACACCTTTGGACAGGGCACCAAGCTCGAGATTAAG<br>GTGGAGGTGGCAGCGGAGGAGGTGGGTCCGGCGGTGG<br>AGGAAGCCAGGTCCAACCTCCAAGAAAGCGGACCGGGTC<br>TTGTGAAGCCATCAGAAACTCTTTCACTGACTTGTACTG<br>TGAGCGGAGTGTCTCTCCCCGATTACGGGGTGTCTTGGA<br>TCAGACAGCCACCGGGGAAGGGTCTGGAATGGATTGGA<br>GTGATTTGGGGCTCTGAGACTACTTACTACCAATCATCC<br>CTCAAGTCACGCGTCACCATCTCAAAGGACAACCTCTAA<br>GAATCAGGTGTCACTGAAACTGTCATCTGTGACCGCAG<br>CCGACACCGCCGTGTACTATTGCGCTAAGCATTACTATT<br>ATGGCGGGAGCTACGCAATGGATTACTGGGGACAGGGT<br>ACTCTGGTCACCGTGTCCAGCCACCACCATCATCACCAT<br>CACCAT |
| SEQ ID NO:<br>199 | CAR 2 - Full<br>– aa                                  | MALPVTALLLPLALLLHAARPEIVMTQSPATLSLSPGERAT<br>LSCRASQDISKYLNWYQQKPGQAPRLLIYHTSRLHSGIPAR<br>FSGSGSGTDYTLTISSLQPEDFAVYFCQQGNTLPYTFGQGT<br>KLEIKGGGSGGGGSGGGGSSQVQLQESGPGLVKPSETLSL<br>TCTVSGVSLPDYGVSWIRQPPGKLEWIGVIWGSSETTYQ<br>SSLKSRVTISKDNSKNQVSLKLSSVTAADTAVYYCAKHYY<br>YGGSYAMDYWGQGTSLTVSSTTTTPAPRPPTPAPTIASQPLS<br>LRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLL<br>SLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFP<br>EEEEGGCELRVKFSRSADAPAYKQGQNQLYNELNLGRRE<br>EYDVLDRRRGRDPEMGGKPRRKNPQEGLYNELQKDKMA<br>EAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHM<br>QALPPR                                                                                                                                                                                                                                                                                                                                                                             |

|                   |                                |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |
|-------------------|--------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| SEQ ID NO:<br>200 | CAR 2 - Full<br>- nt           | ATGGCCCTCCCTGTACCGCCCTGCTGCTTCCGCTGGCT<br>CTTCTGCTCCACGCCGCTCGGCCCCGAAATTGTGATGACC<br>CAGTACCCCCGCACTCTTAGCCTTTCACCCGGTGAGCGC<br>GCAACCCTGTCTTGCAGAGCCTCCCAAGACATCTCAAA<br>ATACCTTAATTGGTATCAACAGAAGCCCCGACAGGCTC<br>CTCGCCTTCTGATCTACCACACCAGCCGGCTCCATTCTG<br>GAATCCCTGCCAGGTTTACGCGGTAGCGGATCTGGGACC<br>GACTACACCCTCACTATCAGCTCACTGCAGCCAGAGGA<br>CTTCGCTGTCTATTTCTGTCAGCAAGGGAACACCCTGCC<br>CTACACCTTTGGACAGGGCACCAAGCTCGAGATTAAG<br>GTGGAGGTGGCAGCGGAGGAGGTGGGTCCGGCGGTGG<br>AGGAAGCCAGGTCCAACCTCCAAGAAAGCGGACCGGGT<br>TTGTGAAGCCATCAGAAACTCTTTCCTGACTTGTACTG<br>TGAGCGGAGTGTCTCTCCCCGATTACGGGGTGTCTTGA<br>TCAGACAGCCACCGGGGAAGGGTCTGGAATGGATTGGA<br>GTGATTTGGGGCTCTGAGACTACTTACTACCAATCATCC<br>CTCAAGTCACGCGTCACCATCTCAAAGGACAACCTCTAA<br>GAATCAGGTGTCACTGAAACTGTCATCTGTGACCGCAG<br>CCGACACCGCCGTGTACTATTGCGCTAAGCATTACTATT<br>ATGGCGGGAGCTACGCAATGGATTACTGGGGACAGGGT<br>ACTCTGGTCACCGTGTCCAGCACCACTACCCAGCACCG<br>AGGCCACCCACCCCGGCTCCTACCATCGCTCCAGCCT<br>CTGTCCCTGCGTCCGGAGGCATGTAGACCCGCAGCTGGT<br>GGGGCCGTGCATACCCGGGGTCTTGACTTCGCTGCGAT<br>ATCTACATTTGGGCCCCCTCTGGCTGGTACTTGCGGGGTC<br>CTGCTGCTTTCCTCGTGATCACTCTTTACTGTAAGCGC<br>GGTCGGAAGAAGCTGCTGTACATCTTTAAGCAACCCTTC<br>ATGAGGCCTGTGCAGACTACTCAAGAGGAGGACGGCTG<br>TTCATGCCGTTCCAGAGGAGGAGGAAGGCGGCTGCG<br>AACTGCGCGTGAAATTCAGCCGCAGCGCAGATGCTCCA<br>GCCTACAAGCAGGGGCAGAACCCAGCTCTACAACGAACT<br>CAATCTTGGTTCGGAGAGAGGAGTACGACGTGCTGGACA<br>AGCGGAGAGGACGGGACCCAGAAATGGGCGGGAAGCC<br>GCGCAGAAAGAATCCCCAAGAGGGCCTGTACAACGAGC<br>TCCAAAAGGATAAGATGGCAGAAGCCTATAGCGAGATT<br>GGTATGAAAGGGGAACGCAGAAGAGGCAAAGGCCACG<br>ACGGACTGTACCAGGGACTCAGCACCGCCACCAAGGAC<br>ACCTATGACGCTCTTCACATGCAGGCCCTGCCGCTCGG |
| SEQ ID NO:<br>201 | CAR2 –<br>Soluble scFv –<br>aa | MALPVTALLLPLALLLHAARPEIVMTQSPATLSLSPGERAT<br>LSCRASQDISKYLNWYQQKPGQAPRLLIYHSTRHSGIPAR<br>FSGSGSGTDYTLTISSLQPEDFAVYFCQQGNTLPYTFGQGT<br>KLEIKGGGSGGGGSGGGGSGVQLQESGPGLVKPSSETLSL<br>TCTVSGVSLPDYGVSWIRQPPGKGLEWIGVIWGSETTYQ<br>SSLKSRVTISKDNSKNQVSLKLSSVTAADTAVYYCAKHYY<br>YGGSYAMDYWGQGTLLTVSSHHHHHHHH                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            |
| Murine<br>CART19  |                                |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |
| 221               | HCDR1<br>(Kabat)               | DYGVS                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            |
| 222               | HCDR2                          | VIWGSETTYNSALKS                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |



|                       |                  |                 |
|-----------------------|------------------|-----------------|
|                       | (Kabat)          |                 |
| 223                   | HCDR3<br>(Kabat) | HYYYGGSYAMDY    |
| 224                   | LCDR1<br>(Kabat) | RASQDISKYLN     |
| 225                   | LCDR2<br>(Kabat) | HTSRLHS         |
| 226                   | LCDR3<br>(Kabat) | QQGNTLPYT       |
| Humanized<br>CART19 a |                  |                 |
| 221                   | HCDR1<br>(Kabat) | DYGVS           |
| 227                   | HCDR2<br>(Kabat) | VIWGSETTYSSSLKS |
| 223                   | HCDR3<br>(Kabat) | HYYYGGSYAMDY    |
| 224                   | LCDR1<br>(Kabat) | RASQDISKYLN     |
| 225                   | LCDR2<br>(Kabat) | HTSRLHS         |
| 226                   | LCDR3<br>(Kabat) | QQGNTLPYT       |
| Humanized<br>CART19 b |                  |                 |
| 221                   | HCDR1<br>(Kabat) | DYGVS           |
| 228                   | HCDR2<br>(Kabat) | VIWGSETTYQSSLKS |
| 223                   | HCDR3<br>(Kabat) | HYYYGGSYAMDY    |
| 224                   | LCDR1<br>(Kabat) | RASQDISKYLN     |
| 225                   | LCDR2<br>(Kabat) | HTSRLHS         |
| 226                   | LCDR3<br>(Kabat) | QQGNTLPYT       |
| Humanized<br>CART19 c |                  |                 |
| 221                   | HCDR1<br>(Kabat) | DYGVS           |
| 229                   | HCDR2<br>(Kabat) | VIWGSETTYNSSLKS |
| 223                   | HCDR3<br>(Kabat) | HYYYGGSYAMDY    |
| 224                   | LCDR1<br>(Kabat) | RASQDISKYLN     |
| 225                   | LCDR2<br>(Kabat) | HTSRLHS         |
| 226                   | LCDR3            | QQGNTLPYT       |

|  |         |  |
|--|---------|--|
|  | (Kabat) |  |
|--|---------|--|

**Table 30. Additional CD19 CAR Constructs**

| SEQ ID NO       | Description                    |
|-----------------|--------------------------------|
| mCAR1           |                                |
| SEQ ID NO: 186  | mCAR1 scFv                     |
| SEQ ID NO: 187  | mCAR1 Full amino acid sequence |
| mCAR2           |                                |
| SEQ ID NO: 188  | mCAR2 scFv                     |
| SEQ ID NO: 189  | mCAR2 amino acid sequence      |
| SEQ ID NO: 190  | mCAR2 full amino acid sequence |
| mCAR3           |                                |
| SEQ ID NO: 191  | mCAR3 scFv                     |
| SEQ ID NO: 192  | mCAR3 full amino acid sequence |
| SSJ25-C1        |                                |
| SEQ ID NO: 193  | SSJ25-C1 VH sequence           |
| SEQ ID NO: 194  | SSJ25-C1 VL                    |
| Humanized CAR1  |                                |
| SEQ ID NO: 195  | CAR1 scFv domain               |
| SEQ ID NO: 196  | CAR 1 – Full – aa              |
| Humanized CAR3  |                                |
| SEQ ID NO: 202  | CAR3 scFv domain               |
| SEQ ID NO: 203  | CAR 3 – Full – aa              |
| Humanized CAR4  |                                |
| SEQ ID NO: 204  | CAR4 scFv domain               |
| SEQ ID NO: 205  | CAR 4 – Full – aa              |
| Humanized CAR5  |                                |
| SEQ ID NO: 206  | CAR5 scFv domain               |
| SEQ ID NO: 207  | CAR 5 – Full – aa              |
| Humanized CAR6  |                                |
| SEQ ID NO: 208  | CAR6 scFv domain               |
| SEQ ID NO: 209  | CAR6 – Full – aa               |
| Humanized CAR7  |                                |
| SEQ ID NO: 210  | CAR7 scFv domain               |
| SEQ ID NO: 211  | CAR 7 Full – aa                |
| Humanized CAR8  |                                |
| SEQ ID NO: 212  | CAR8 scFv domain               |
| SEQ ID NO: 213  | CAR 8 – Full – aa              |
| Humanized CAR9  |                                |
| SEQ ID NO: 214  | CAR9 scFv domain               |
| SEQ ID NO: 215  | CAR 9 – Full – aa              |
| Humanized CAR10 |                                |
| SEQ ID NO: 216  | CAR10 scFv domain              |
| SEQ ID NO: 215  | CAR 10 Full – aa               |
| Humanized CAR11 |                                |
| SEQ ID NO: 217  | CAR11 scFv domain              |

|                 |                    |
|-----------------|--------------------|
| SEQ ID NO: 218  | CAR 11 Full – aa   |
| Humanized CAR12 |                    |
| SEQ ID NO: 219  | CAR12 scFv domain  |
| SEQ ID NO: 220  | CAR 12 – Full – aa |

CD19 CAR constructs containing humanized anti-CD19 scFv domains are described in PCT publication WO 2014/153270, incorporated herein by reference.

The sequences of murine and humanized CDR sequences of the anti-CD19 scFv domains are shown in Table 6 for the heavy chain variable domains and in Table 7 for the light chain variable domains. In some embodiments, the HCDR1 of a murine or humanized CD19 binding domain is GVSLPDYGVSV (SEQ ID NO: 230).

**Table 6. Heavy Chain Variable Domain CDR (Kabat) SEQ ID NOs of CD19 Antibodies**

| Candidate          | HCDR1          | HCDR2          | HCDR3          |
|--------------------|----------------|----------------|----------------|
| murine_CART19      | SEQ ID NO: 221 | SEQ ID NO: 222 | SEQ ID NO: 223 |
| humanized_CART19 a | SEQ ID NO: 221 | SEQ ID NO: 227 | SEQ ID NO: 223 |
| humanized_CART19 b | SEQ ID NO: 221 | SEQ ID NO: 228 | SEQ ID NO: 223 |
| humanized_CART19 c | SEQ ID NO: 221 | SEQ ID NO: 229 | SEQ ID NO: 223 |

**Table 7. Light Chain Variable Domain CDR (Kabat) SEQ ID NOs of CD19 Antibodies**

| Candidate          | LCDR1          | LCDR2          | LCDR3          |
|--------------------|----------------|----------------|----------------|
| murine_CART19      | SEQ ID NO: 224 | SEQ ID NO: 225 | SEQ ID NO: 226 |
| humanized_CART19 a | SEQ ID NO: 224 | SEQ ID NO: 225 | SEQ ID NO: 226 |
| humanized_CART19 b | SEQ ID NO: 224 | SEQ ID NO: 225 | SEQ ID NO: 226 |
| humanized_CART19 c | SEQ ID NO: 224 | SEQ ID NO: 225 | SEQ ID NO: 226 |

Any known CD19 CAR, e.g., the CD19 antigen binding domain of any known CD19 CAR, in the art can be used in accordance with the present disclosure. For example, LG-740; CD19 CAR described in the US Pat. No. 8,399,645; US Pat. No. 7,446,190; Xu et al., Leuk Lymphoma. 2013 54(2):255-260(2012); Cruz et al., Blood 122(17):2965-2973 (2013); Brentjens et al., Blood, 118(18):4817-4828 (2011); Kochenderfer et al., Blood 116(20):4099-102 (2010); Kochenderfer et al., Blood 122 (25):4129-39(2013); and 16<sup>th</sup> Annu Meet Am Soc Gen Cell Ther (ASGCT) (May 15-18, Salt Lake City) 2013, Abst 10.

Exemplary CD19 CARs include CD19 CARs described herein, e.g., in one or more tables described herein, or an anti-CD19 CAR described in Xu et al. Blood 123.24(2014):3750-9; Kochenderfer et al. Blood 122.25(2013):4129-39, Cruz et al. Blood 122.17(2013):2965-73, NCT00586391, NCT01087294, NCT02456350, NCT00840853, NCT02659943, NCT02650999, NCT02640209,

NCT01747486, NCT02546739, NCT02656147, NCT02772198, NCT00709033, NCT02081937, NCT00924326, NCT02735083, NCT02794246, NCT02746952, NCT01593696, NCT02134262, NCT01853631, NCT02443831, NCT02277522, NCT02348216, NCT02614066, NCT02030834, NCT02624258, NCT02625480, NCT02030847, NCT02644655, NCT02349698, NCT02813837, NCT02050347, NCT01683279, NCT02529813, NCT02537977, NCT02799550, NCT02672501, NCT02819583, NCT02028455, NCT01840566, NCT01318317, NCT01864889, NCT02706405, NCT01475058, NCT01430390, NCT02146924, NCT02051257, NCT02431988, NCT01815749, NCT02153580, NCT01865617, NCT02208362, NCT02685670, NCT02535364, NCT02631044, NCT02728882, NCT02735291, NCT01860937, NCT02822326, NCT02737085, NCT02465983, NCT02132624, NCT02782351, NCT01493453, NCT02652910, NCT02247609, NCT01029366, NCT01626495, NCT02721407, NCT01044069, NCT00422383, NCT01680991, NCT02794961, or NCT02456207, each of which is incorporated herein by reference in its entirety.

### CD123 CAR

In other embodiments, the CAR-expressing cells can specifically bind to CD123, *e.g.*, can include a CAR molecule (*e.g.*, any of the CAR1 to CAR8), or an antigen binding domain according to Tables 1-2 of WO 2014/130635, incorporated herein by reference. The amino acid and nucleotide sequences encoding the CD123 CAR molecules and antigen binding domains (*e.g.*, including one, two, three VH CDRs; and one, two, three VL CDRs according to Kabat or Chothia), as specified in WO 2014/130635, are provided in Tables 8-14. Amino and nucleotide sequences identical and substantially identical to the aforesaid sequences provided in Tables 8-14 are specifically incorporated into the instant specification.

The CDRs for CD123 binding domains provided in Tables 8-14 are according to a combination of the Kabat and Chothia numbering scheme.

**Table 8. Heavy Chain Variable Domain CDRs**

| Candidate | HCDR1      | SEQ ID NO | HCDR2              | SEQ ID NO | HCDR3         | SEQ ID NO |
|-----------|------------|-----------|--------------------|-----------|---------------|-----------|
| CAR123-2  | GYTFTGYMH  | 231       | WINPNSGGTNYAQKFQ G | 234       | DMNILATVPFD I | 236       |
| CAR123-3  | GYIFTGYYIH | 232       | WINPNSGGTNYAQKFQ G | 234       | DMNILATVPFD I | 236       |
| CAR123-4  | GYTFTGYMH  | 231       | WINPNSGGTNYAQKFQ G | 234       | DMNILATVPFD I | 236       |
| CAR123-1  | GYTFTDYMH  | 233       | WINPNSGDTNYAQKFQ G | 235       | DMNILATVPFD I | 236       |

**Table 9. Light Chain Variable Domain CDRs**

| Candidate | LCDR1       | SEQ ID NO | LCDR2   | SEQ ID NO | LCDR3     | SEQ ID NO |
|-----------|-------------|-----------|---------|-----------|-----------|-----------|
| CAR123-2  | RASQSISSYLN | 237       | AAFSLQS | 239       | QQGDSVPLT | 241       |
| CAR123-3  | RASQSISSYLN | 237       | AASSLQS | 240       | QQGDSVPLT | 241       |
| CAR123-4  | RASQSISSYLN | 237       | AASSLQS | 240       | QQGDSVPLT | 241       |
| CAR123-1  | RASQSISTYLN | 238       | AASSLQS | 240       | QQGDSVPLT | 241       |

**Table 10. Heavy Chain Variable Domain CDR**

|          | HCDR1          | SEQ ID NO | HCDR2                 | SEQ ID NO | HCDR3      | SEQ ID NO |
|----------|----------------|-----------|-----------------------|-----------|------------|-----------|
| hzCAR123 | GYTFTSY<br>WMN | 242       | RIDPYDSETHYNQK<br>FKD | 243       | GNWDD<br>Y | 244       |

**5 Table 11. Light Chain Variable Domain CDR**

|          | LCDR1       | SEQ ID NO | LCDR2   | SEQ ID NO | LCDR3     | SEQ ID NO |
|----------|-------------|-----------|---------|-----------|-----------|-----------|
| hzCAR123 | RASKSISKDLA | 245       | SGSTLQS | 246       | QQHNKYPYT | 247       |

**Table 12: Exemplary CD123 CAR sequences**

| Name          | SEQ ID NO      |
|---------------|----------------|
| CAR123-2 NT   | SEQ ID NO: 248 |
| CAR123-2 AA   | SEQ ID NO: 249 |
| CAR123-2 scFv | SEQ ID NO: 250 |
| CAR123-2 VH   | SEQ ID NO: 251 |
| CAR123-2 VL   | SEQ ID NO: 252 |
| CAR123-3 NT   | SEQ ID NO: 253 |
| CAR123-3 AA   | SEQ ID NO: 254 |
| CAR123-3 scFv | SEQ ID NO: 255 |
| CAR123-3 VH   | SEQ ID NO: 256 |
| CAR123-3 VL   | SEQ ID NO: 257 |
| CAR123-4 NT   | SEQ ID NO: 258 |
| CAR123-4 AA   | SEQ ID NO: 259 |
| CAR123-4 scFv | SEQ ID NO: 260 |
| CAR123-4 VH   | SEQ ID NO: 261 |
| CAR123-4 VL   | SEQ ID NO: 262 |
| CAR123-1 NT   | SEQ ID NO: 263 |
| CAR123-1 AA   | SEQ ID NO: 264 |
| CAR123-1 scFv | SEQ ID NO: 265 |
| CAR123-1 VH   | SEQ ID NO: 266 |
| CAR123-1 VL   | SEQ ID NO: 267 |

**Table 13: Humanized CD123 CAR Sequences**

| <b>Name</b>     | <b>SEQ ID NO</b> |
|-----------------|------------------|
| hzCAR123-1 NT   | 268              |
| hzCAR123-1 AA   | 269              |
| hzCAR123-1 scFv | 270              |
| hzCAR123-1 VH   | 271              |
| hzCAR123-1 VL   | 272              |
| hzCAR123-2 NT   | 273              |
| hzCAR123-2 AA   | 274              |
| hzCAR123-2 scFv | 275              |
| hzCAR123-2 VH   | 271              |
| hzCAR123-2 VL   | 276              |
| hzCAR123-3 NT   | 277              |
| hzCAR123-3 AA   | 278              |
| hzCAR123-3 scFv | 279              |
| hzCAR123-3 VH   | 271              |
| hzCAR123-3 VL   | 280              |
| hzCAR123-4 NT   | 281              |
| hzCAR123-4 AA   | 282              |
| hzCAR123-4 scFv | 283              |
| hzCAR123-4 VH   | 271              |
| hzCAR123-4 VL   | 284              |
| hzCAR123-5 NT   | 285              |
| hzCAR123-5 AA   | 286              |
| hzCAR123-5 scFv | 287              |
| hzCAR123-5 VH   | 271              |
| hzCAR123-5 VL   | 272              |
| hzCAR123-6 NT   | 288              |
| hzCAR123-6 AA   | 289              |
| hzCAR123-6 scFv | 290              |
| hzCAR123-6 VH   | 271              |
| hzCAR123-6 VL   | 276              |
| hzCAR123-7 NT   | 291              |
| hzCAR123-7 AA   | 292              |
| hzCAR123-7 scFv | 293              |
| hzCAR123-7 VH   | 271              |
| hzCAR123-7 VL   | 280              |
| hzCAR123-8 NT   | 294              |
| hzCAR123-8 AA   | 295              |
| hzCAR123-8 scFv | 296              |
| hzCAR123-8 VH   | 271              |
| hzCAR123-8 VL   | 284              |
| hzCAR123-9 NT   | 297              |
| hzCAR123-9 AA   | 298              |
| hzCAR123-9 scFv | 299              |
| hzCAR123-9 VH   | 300              |
| hzCAR123-10 VL  | 272              |
| hzCAR123-10 NT  | 301              |

|                  |     |
|------------------|-----|
| hzCAR123-10 AA   | 302 |
| hzCAR123-10 scFv | 303 |
| hzCAR123-10 VH   | 300 |
| hzCAR123-10 VL   | 276 |
| hzCAR123-11 NT   | 304 |
| hzCAR123-11 AA   | 305 |
| hzCAR123-11 scFv | 306 |
| hzCAR123-11 VH   | 300 |
| hzCAR123-11 VL   | 280 |
| hzCAR123-12 NT   | 307 |
| hzCAR123-12 AA   | 308 |
| hzCAR123-12 scFv | 309 |
| hzCAR123-12 VH   | 300 |
| hzCAR123-12 VL   | 284 |
| hzCAR123-13 NT   | 310 |
| hzCAR123-13 AA   | 311 |
| hzCAR123-13 scFv | 312 |
| hzCAR123-13 VH   | 300 |
| hzCAR123-13 VL   | 272 |
| hzCAR123-14 NT   | 313 |
| hzCAR123-14 AA   | 314 |
| hzCAR123-14 scFv | 315 |
| hzCAR123-14 VH   | 300 |
| hzCAR123-14 VL   | 276 |
| hzCAR123-15 NT   | 316 |
| hzCAR123-15 AA   | 317 |
| hzCAR123-15 scFv | 318 |
| hzCAR123-15 VH   | 300 |
| hzCAR123-15 VL   | 280 |
| hzCAR123-16 NT   | 319 |
| hzCAR123-16 AA   | 320 |
| hzCAR123-16 scFv | 321 |
| hzCAR123-16 VH   | 300 |
| hzCAR123-16 VL   | 284 |
| hzCAR123-17 NT   | 322 |
| hzCAR123-17 AA   | 323 |
| hzCAR123-17 scFv | 324 |
| hzCAR123-17 VH   | 325 |
| hzCAR123-17 VL   | 272 |
| hzCAR123-18 NT   | 326 |
| hzCAR123-18 AA   | 327 |
| hzCAR123-18 scFv | 328 |
| hzCAR123-18 VH   | 325 |
| hzCAR123-18 VL   | 276 |
| hzCAR123-19 NT   | 329 |
| hzCAR123-19 AA   | 330 |
| hzCAR123-19 scFv | 331 |
| hzCAR123-19 VH   | 325 |
| hzCAR123-19 VL   | 280 |

|                  |     |
|------------------|-----|
| hzCAR123-20 NT   | 332 |
| hzCAR123-20 AA   | 333 |
| hzCAR123-20 scFv | 334 |
| hzCAR123-20 VH   | 325 |
| hzCAR123-20 VL   | 284 |
| hzCAR123-21 NT   | 335 |
| hzCAR123-21 AA   | 336 |
| hzCAR123-21 scFv | 337 |
| hzCAR123-21 VH   | 325 |
| hzCAR123-21 VL   | 272 |
| hzCAR123-22 NT   | 338 |
| hzCAR123-22 AA   | 339 |
| hzCAR123-22 scFv | 340 |
| hzCAR123-22 VH   | 325 |
| hzCAR123-22 VL   | 276 |
| hzCAR123-23 NT   | 341 |
| hzCAR123-23 AA   | 342 |
| hzCAR123-23 scFv | 343 |
| hzCAR123-23 VH   | 325 |
| hzCAR123-23 VL   | 280 |
| hzCAR123-24 NT   | 344 |
| hzCAR123-24 AA   | 345 |
| hzCAR123-24 scFv | 346 |
| hzCAR123-24 VH   | 325 |
| hzCAR123-24 VL   | 284 |
| hzCAR123-25 NT   | 347 |
| hzCAR123-25 AA   | 348 |
| hzCAR123-25 scFv | 349 |
| hzCAR123-25 VH   | 350 |
| hzCAR123-25 VL   | 272 |
| hzCAR123-26 NT   | 351 |
| hzCAR123-26 AA   | 352 |
| hzCAR123-26 scFv | 353 |
| hzCAR123-26 VH   | 350 |
| hzCAR123-26 VL   | 276 |
| hzCAR123-27 NT   | 354 |
| hzCAR123-27 AA   | 355 |
| hzCAR123-27 scFv | 356 |
| hzCAR123-27 VH   | 350 |
| hzCAR123-27 VL   | 280 |
| hzCAR123-28 NT   | 357 |
| hzCAR123-28 AA   | 358 |
| hzCAR123-28 scFv | 359 |
| hzCAR123-28 VH   | 350 |
| hzCAR123-28 VL   | 284 |
| hzCAR123-29 NT   | 360 |
| hzCAR123-29 AA   | 361 |
| hzCAR123-29 scFv | 362 |
| hzCAR123-29 VH   | 350 |



|                  |     |
|------------------|-----|
| hzCAR123-29 VL   | 272 |
| hzCAR123-30 NT   | 363 |
| hzCAR123-30 AA   | 364 |
| hzCAR123-30 scFv | 365 |
| hzCAR123-30 VH   | 350 |
| hzCAR123-30 VL   | 276 |
| hzCAR123-31 NT   | 366 |
| hzCAR123-31 AA   | 367 |
| hzCAR123-31 scFv | 368 |
| hzCAR123-31 VH   | 350 |
| hzCAR123-31 VL   | 280 |
| hzCAR123-32 NT   | 369 |
| hzCAR123-32 AA   | 370 |
| hzCAR123-32 scFv | 371 |
| hzCAR123-32 VH   | 350 |
| hzCAR123-32 VL   | 284 |

In embodiments, a CAR molecule described herein comprises a scFv that specifically binds to CD123, and does not contain a leader sequence, e.g., the amino acid sequence SEQ ID NO: 64. Table 14 below provides amino acid and nucleotide sequences for CD123 scFv sequences that do not contain a leader sequence SEQ ID NO: 64.

5

**Table 14. CD123 CAR scFv sequences**

| Name                 | SEQ ID NO |
|----------------------|-----------|
| CAR123-2 scFv – NT   | 372       |
| CAR123-2 scFv – AA   | 373       |
| CAR123-2 ORF-free NT | 374       |
| CAR123-3 scFv – NT   | 375       |
| CAR123-3 scFv – AA   | 376       |
| CAR123-4 scFv – NT   | 377       |
| CAR123-4 scFv – AA   | 378       |
| CAR123-1 scFv – AA   | 379       |
| hzCAR123-1 scFv      | 380       |
| hzCAR123-2 scFv      | 381       |
| hzCAR123-3 scFv      | 382       |
| hzCAR123-4 scFv      | 383       |
| hzCAR123-5 scFv      | 384       |
| hzCAR123-6 scFv      | 385       |
| hzCAR123-7 scFv      | 386       |
| hzCAR123-8 scFv      | 387       |
| hzCAR123-9 scFv      | 388       |
| hzCAR123-10 scFv     | 389       |
| hzCAR123-11 scFv     | 390       |
| hzCAR123-12 scFv     | 391       |
| hzCAR123-13 scFv     | 392       |
| hzCAR123-14 scFv     | 393       |

|                  |     |
|------------------|-----|
| hzCAR123-15 scFv | 394 |
| hzCAR123-16 scFv | 395 |
| hzCAR123-17 scFv | 396 |
| hzCAR123-18 scFv | 397 |
| hzCAR123-19 scFv | 398 |
| hzCAR123-20 scFv | 399 |
| hzCAR123-21 scFv | 400 |
| hzCAR123-22 scFv | 401 |
| hzCAR123-23 scFv | 402 |
| hzCAR123-24 scFv | 403 |
| hzCAR123-25 scFv | 404 |
| hzCAR123-26 scFv | 405 |
| hzCAR123-27 scFv | 406 |
| hzCAR123-28 scFv | 407 |
| hzCAR123-29 scFv | 408 |
| hzCAR123-30 scFv | 409 |
| hzCAR123-31 scFv | 410 |
| hzCAR123-32 scFv | 411 |

In other embodiments, the CAR-expressing cells can specifically bind to CD123, e.g., can include a CAR molecule (e.g., any of the CAR123-1 or CAR123-4 and hzCAR123-1 to hzCAR123-32), or an antigen binding domain according to Tables 2, 6, and 9 of WO2016/028896, incorporated herein by reference. The amino acid and nucleotide sequences encoding the CD123 CAR molecules and antigen binding domains (e.g., including one, two, three VH CDRs; and one, two, three VL CDRs according to Kabat or Chothia), as specified in WO2016/028896, are incorporated herein by reference in their entireties.

#### 10 *EGFRvIII CAR*

In other embodiments, the CAR-expressing cells can specifically bind to EGFRvIII, e.g., can include a CAR molecule, or an antigen binding domain according to Table 2 or SEQ ID NO:11 of WO 2014/130657, incorporated herein by reference. Exemplary amino acid and nucleotide sequences encoding the EGFRvIII CAR molecules and antigen binding domains (e.g., including one, two, three VH CDRs; and one, two, three VL CDRs according to Kabat or Chothia) are provided in WO 2014/130657. Exemplary anti-EGFRvIII CAR sequences may comprise a CDR, a variable region, an scFv, or a full-length CAR sequence of a sequence disclosed in Table 33 (or a sequence at least about 85%, 90%, 95%, 99% or more identical thereto, and/or having one, two, three or more substitutions, insertions, deletions, or modifications).

**Table 33. EGFRvIII CAR sequences.**

| Name                         | SEQ ID NO:      |
|------------------------------|-----------------|
| <b>CAR 1</b>                 |                 |
| CAR1 scFv domain             | SEQ ID NO: 1451 |
| CAR1 scFv domain nt          | SEQ ID NO: 1452 |
| CAR1 Soluble scFv - nt       | SEQ ID NO: 1453 |
| CAR1 Soluble scFv – aa       | SEQ ID NO: 1454 |
| CAR 1 – Full – nt lentivirus | SEQ ID NO: 1455 |
| CAR 1 – Full – aa            | SEQ ID NO: 1456 |
| <b>CAR 2</b>                 |                 |
| CAR2 scFv domain             | SEQ ID NO: 1457 |
| CAR2 scFv domain – nt        | SEQ ID NO: 1458 |
| CAR2 – Soluble scFv – nt     | SEQ ID NO: 1459 |
| CAR2 – Soluble scFv – aa     | SEQ ID NO: 1460 |
| CAR 2 - Full – nt            | SEQ ID NO: 1461 |
| CAR 2 - Full – aa            | SEQ ID NO: 1462 |
| <b>CAR 3</b>                 |                 |
| CAR3 scFv domain             | SEQ ID NO: 1463 |
| CAR3 scFv domain nt          | SEQ ID NO: 1464 |
| CAR 3 - Soluble scFv – nt    | SEQ ID NO: 1465 |
| CAR 3 - Soluble scFv – aa    | SEQ ID NO: 1466 |
| CAR 3 – Full – nt            | SEQ ID NO: 1467 |
| CAR 3 – Full – aa            | SEQ ID NO: 1468 |
| <b>CAR 4</b>                 |                 |
| CAR4 scFv domain             | SEQ ID NO: 1469 |
| CAR4 scFv domain nt          | SEQ ID NO: 1470 |
| CAR4 – Soluble scFv – nt     | SEQ ID NO: 1471 |
| CAR4 – Soluble scFv -aa      | SEQ ID NO: 1472 |
| CAR 4 – Full – nt            | SEQ ID NO: 1473 |
| CAR 4 – Full – aa            | SEQ ID NO: 1474 |
| <b>CAR 5</b>                 |                 |
| CAR5 scFv domain             | SEQ ID NO: 1475 |
| CAR5 scFv domain nt          | SEQ ID NO: 1476 |
| CAR5 – Soluble scFv – nt     | SEQ ID NO: 1477 |
| CAR5 – Soluble scFv -aa      | SEQ ID NO: 1478 |
| CAR 5 – Full – nt            | SEQ ID NO: 1479 |
| CAR 5 – Full – aa            | SEQ ID NO: 1480 |
| <b>CAR 6</b>                 |                 |
| CAR6 scFv domain             | SEQ ID NO: 1481 |
| CAR6 scFv domain nt          | SEQ ID NO: 1482 |
| CAR6 – Soluble scFv – nt     | SEQ ID NO: 1483 |
| CAR6 – Soluble scFv – aa     | SEQ ID NO: 1484 |
| CAR6 –Full – nt              | SEQ ID NO: 1485 |
| CAR6 –Full – aa              | SEQ ID NO: 1486 |
| <b>CAR 7</b>                 |                 |
| CAR7 scFv domain             | SEQ ID NO: 1487 |
| CAR7 scFv domain nt          | SEQ ID NO: 1488 |
| CAR7 - Soluble scFv – nt     | SEQ ID NO: 1489 |
| CAR7 - Soluble scFv – aa     | SEQ ID NO: 1490 |
| CAR 7 Full – nt              | SEQ ID NO: 1491 |

|                           |                                |
|---------------------------|--------------------------------|
| CAR 7 Full – aa           | SEQ ID NO: 1492                |
| <b>CAR 8</b>              |                                |
| CAR8 scFv domain          | SEQ ID NO: 1493                |
| CAR8 scFv domain nt       | SEQ ID NO: 1494                |
| CAR8 - Soluble scFv – nt  | SEQ ID NO: 1495                |
| CAR8 - Soluble scFv – aa  | SEQ ID NO: 1496                |
| CAR 8 – Full – nt         | SEQ ID NO: 1497                |
| CAR 8 – Full – aa         | SEQ ID NO: 1498                |
| <b>CAR 9</b>              | Mouse anti-EGFRvIII clone 3C10 |
| CAR9 scFv domain          | SEQ ID NO: 1499                |
| CAR9 scFv domain nt       | SEQ ID NO: 1500                |
| CAR9 - Soluble scFv – nt  | SEQ ID NO: 1501                |
| CAR9 - Soluble scFv – aa  | SEQ ID NO: 1502                |
| CAR 9 – Full – nt         | SEQ ID NO: 1503                |
| CAR 9 – Full – aa         | SEQ ID NO: 1504                |
| <b>CAR10</b>              | Anti-EGFRvIII clone 139        |
| CAR10 scFv domain         | SEQ ID NO: 1505                |
| CAR9 scFv domain nt       | SEQ ID NO: 1506                |
| CAR10 - Soluble scFv – nt | SEQ ID NO: 1507                |
| CAR10 - Soluble scFv – aa | SEQ ID NO: 1508                |
| CAR 10 Full – nt          | SEQ ID NO: 1509                |
| CAR 10 Full – aa          | SEQ ID NO: 1510                |

### CD33 CAR

In other embodiments, the CAR-expressing cells can specifically bind to CD33, *e.g.*, can include a CAR molecule (*e.g.*, any of CAR33-1 to CAR-33-9), or an antigen binding domain according to Table 2 or 9 of WO2016/014576, incorporated herein by reference. Exemplary amino acid and nucleotide sequences encoding the CD33 CAR molecules and antigen binding domains (*e.g.*, including one, two, three VH CDRs; and one, two, three VL CDRs according to Kabat or Chothia) are provided in WO2016/014576.

### 10 Mesothelin CAR

In some embodiments, the CAR-expressing cells can specifically bind to mesothelin, *e.g.*, can include a CAR molecule, or an antigen binding domain according to Tables 2-3 of WO 2015/090230, incorporated herein by reference. Exemplary amino acid and nucleotide sequences encoding the mesothelin CAR molecules and antigen binding domains (*e.g.*, including one, two, three VH CDRs; and one, two, three VL CDRs according to Kabat or Chothia) are provided in WO 2015/090230. Exemplary anti-mesothelin CAR sequences may comprise a CDR, a variable region, an scFv, or a full-length CAR sequence of a sequence disclosed in Table 34 (or a sequence at least about 85%, 90%, 95%, 99% or more

identical thereto, and/or having one, two, three or more substitutions, insertions, deletions, or modifications).

**Table 34. Mesothelin CAR sequences.** Amino acid sequences of human scFvs and CARs that bind to mesothelin (bold underline is the leader sequence). In the case of the scFvs, the remaining amino acids are the heavy chain variable region and light chain variable regions, with each of the HC CDRs (HC CDR1, HC CDR2, HC CDR3) and LC CDRs (LC CDR1, LC CDR2, LCCDR3) underlined. In the case of the CARs, the further remaining amino acids are the remaining amino acids of the CARs.

| SEQ ID NO:      | Description                                                                    | Amino Acid Sequence                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            |
|-----------------|--------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| SEQ ID NO: 1511 | M1 (ScFv domain)                                                               | QVQLQQSGAEVKKPGASVKVSKASGYTFTGYMHWVRQAPGQGLEWMGRINPNSSGGTNY<br>AOKFQGRVTMTDTSISTAYMELSRRLSDDTAVYYCARGRYGMDVWGQGTMTVTVSSGGG<br>GSGGGSGGGSGGGGSEIVLTQSPATLSLSPGERATISCRASQSVSSNFAWYQQRPGQA<br>PRLLIYDASNRATGIPPRFSGSGSGTDFTLTISSELPEDFAAYYCHQRSNWLYTFGQGTK<br>VDIK                                                                                                                                                                                                                                                                                               |
| SEQ ID NO: 1512 | M1 (full)<br>>ZA53-<br>27BC<br>(M1<br>ZA53-<br>27BC<br>R001-<br>A11<br>126161) | <b><u>MALPVTALLPLALLHAARP</u></b> QVQLQQSGAEVKKPGASVKVSKASGYTFTGYMHWVRQ<br>APGQGLEWMGRINPNSSGGTNYAOKFQGRVTMTDTSISTAYMELSRRLSDDTAVYYCARG<br>RYYGMDVWGQGTMTVTVSSGGGGSGGGSGGGSGGGGSEIVLTQSPATLSLSPGERATIS<br>CRASQSVSSNFAWYQQRPGQAPRLLIYDASNRATGIPPRFSGSGSGTDFTLTISSELPED<br>FAAYYCHQRSNWLYTFGQGTKVDIKTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAV<br>HTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEED<br>GCSCRFPEEEEEGGCELRVKFSRSADAPAYKQGQNLQYNELNLGRREEYDVLDRGRDPE<br>MGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRRGKGDGLYQGLSTATKDTYDAL<br>HMQALPPR              |
| SEQ ID NO: 1513 | M2 (ScFv domain)                                                               | QVQLVQSGAEVKKPGASVKVSKASGYTFTGYMHWVRQAPGQGLEWMGWINPNSSGGTNY<br>AOKFQGRVTMTDTSISTAYMELSRRLSDDTAVYYCARDLRRTVVTPRAYYGMDVWGQGT<br>TVTVSSGGGGSGGGSGGGSGGGSGGGSDIQLTQSPSTLSASVGDRTTITCQASQDISNSLN<br>WYQQKAGKAPKLLIYDASTLETGVP SRFSGSGSGTDFTSF TISSLPED IATYYCQ QHDNL<br>PLTFGQGTKVEIK                                                                                                                                                                                                                                                                               |
| SEQ ID NO: 1514 | M2 (full)<br>>FA56-<br>26RC<br>(M2<br>FA56-<br>26RC<br>R001-<br>A10<br>126162) | <b><u>MALPVTALLPLALLHAARP</u></b> QVQLVQSGAEVKKPGASVKVSKASGYTFTGYMHWVRQ<br>APGQGLEWMGWINPNSSGGTNYAOKFQGRVTMTDTSISTAYMELSRRLSDDTAVYYCARD<br>LRRTVVTPRAYYGMDVWGQGT TVTVSSGGGGSGGGSGGGSGGGSDIQLTQSPSTLSA<br>SVGDRTTITCQASQDISNSLNWYQQKAGKAPKLLIYDASTLETGVP SRFSGSGSGTDFTSF<br>TISSLPED IATYYCQ QHDNLPLTFGQGTKVEIKTTTPAPRPPTPAPTIASQPLSLRPEA<br>CRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMR<br>PVQTTQEEDGCSCRFPEEEEEGGCELRVKFSRSADAPAYKQGQNLQYNELNLGRREEYDVL<br>DKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRRGKGDGLYQGLST<br>ATKDTYDALHMQALPPR |
| SEQ ID NO: 1515 | M3 (ScFv domain)                                                               | QVQLVQSGAEVKKPGAPVKVSKASGYTFTGYMHWVRQAPGQGLEWMGWINPNSSGGTNY<br>AOKFQGRVTMTDTSISTAYMELSRRLSDDTAVYYCARGEWDGSEYDYWGQGTTLTVTVSS<br>GGGGSGGGSGGGSGGGSGGGSDIVLTQTPSSLSASVGDRTTITCRASQSINTYLNWYQHKG<br>GKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSELPEDFATYYCQ QSFSP LTFGGG<br>TKLEIK                                                                                                                                                                                                                                                                                       |
| SEQ ID NO: 1516 | M3<br>>VA58-<br>21LC<br>(M3<br>VA58-<br>21LC                                   | <b><u>MALPVTALLPLALLHAARP</u></b> QVQLVQSGAEVKKPGAPVKVSKASGYTFTGYMHWVRQ<br>APGQGLEWMGWINPNSSGGTNYAOKFQGRVTMTDTSISTAYMELSRRLSDDTAVYYCARG<br>EWDGSEYDYWGQGTTLTVTVSSGGGGSGGGSGGGSGGGSDIVLTQTPSSLSASVGDRT<br>TITCRASQSINTYLNWYQHKGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSELP<br>EDFATYYCQ QSFSP LTFGGGKLEIKTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGG<br>AVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQE                                                                                                                                                          |

|                       |                                                                      |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                |
|-----------------------|----------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|                       | R001-A1<br>126163)                                                   | EDGCSCRFPEEEEEGGCELRVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDRRGRD<br>PEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYD<br>ALHMQALPPR                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |
| SEQ ID<br>NO:<br>1517 | M4 (ScFv<br>domain)                                                  | <u>QVQLVESGGGLVQPGGSLRLSCAASGFTFSSYWMHWVRQVPGKGLVWVSRINTDGSITTY</u><br><u>ADSV</u> <u>EG</u> <u>RFT</u> <u>IS</u> <u>R</u> <u>DN</u> <u>AK</u> <u>NT</u> <u>LY</u> <u>L</u> <u>Q</u> <u>M</u> <u>N</u> <u>S</u> <u>L</u> <u>R</u> <u>D</u> <u>D</u> <u>D</u> <u>T</u> <u>A</u> <u>V</u> <u>Y</u> <u>Y</u> <u>C</u> <u>V</u> <u>G</u> <u>G</u> <u>H</u> <u>W</u> <u>A</u> <u>V</u> <u>W</u> <u>G</u> <u>Q</u> <u>G</u> <u>T</u> <u>T</u> <u>V</u> <u>T</u> <u>V</u> <u>S</u> <u>S</u> <u>G</u> <u>G</u> <u>G</u> <u>G</u> <u>S</u> <u>G</u> <u>S</u> <u>G</u> <u>S</u> <u>G</u> <u>S</u> <u>D</u> <u>I</u> <u>Q</u> <u>M</u> <u>T</u> <u>Q</u> <u>S</u> <u>P</u> <u>S</u> <u>T</u> <u>L</u> <u>S</u> <u>A</u> <u>S</u> <u>V</u> <u>G</u> <u>D</u> <u>R</u> <u>V</u> <u>T</u> <u>I</u> <u>T</u> <u>C</u> <u>R</u> <u>A</u> <u>S</u> <u>Q</u> <u>S</u> <u>I</u> <u>S</u> <u>D</u> <u>R</u> <u>L</u> <u>A</u> <u>W</u> <u>Y</u> <u>Q</u> <u>Q</u> <u>K</u> <u>P</u> <u>G</u> <u>K</u> <u>A</u> <u>P</u> <u>K</u> <u>L</u> <u>I</u> <u>Y</u> <u>K</u> <u>A</u> <u>S</u> <u>S</u> <u>L</u> <u>E</u> <u>S</u> <u>G</u> <u>V</u> <u>P</u> <u>S</u> <u>R</u> <u>F</u> <u>S</u> <u>G</u> <u>S</u> <u>G</u> <u>S</u> <u>G</u> <u>T</u> <u>E</u> <u>F</u> <u>T</u> <u>L</u> <u>T</u> <u>I</u> <u>S</u> <u>S</u> <u>L</u> <u>Q</u> <u>P</u> <u>D</u> <u>D</u> <u>F</u> <u>A</u> <u>V</u> <u>Y</u> <u>C</u> <u>Q</u> <u>Q</u> <u>Y</u> <u>G</u> <u>H</u> <u>L</u> <u>P</u> <u>M</u> <u>Y</u> <u>T</u> <u>F</u> <u>G</u> <u>Q</u> <u>G</u> <u>T</u> <u>K</u> <u>V</u> <u>E</u> <u>I</u> <u>K</u>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |
| SEQ ID<br>NO:<br>1518 | M4<br>>DP37-<br>07IC<br>(M4<br>DP37-<br>07IC<br>R001-C6<br>126164)   | <u>MALPVTALLPLALLHAARP</u> <u>QVQLVESGGGLVQPGGSLRLSCAASGFTFSSYWMHWVRQ</u><br><u>V</u> <u>P</u> <u>G</u> <u>K</u> <u>L</u> <u>V</u> <u>W</u> <u>V</u> <u>S</u> <u>R</u> <u>I</u> <u>N</u> <u>T</u> <u>D</u> <u>G</u> <u>S</u> <u>I</u> <u>T</u> <u>T</u> <u>Y</u> <u>A</u> <u>D</u> <u>S</u> <u>V</u> <u>E</u> <u>G</u> <u>R</u> <u>F</u> <u>T</u> <u>I</u> <u>S</u> <u>R</u> <u>D</u> <u>N</u> <u>A</u> <u>K</u> <u>N</u> <u>T</u> <u>L</u> <u>Y</u> <u>L</u> <u>Q</u> <u>M</u> <u>N</u> <u>S</u> <u>L</u> <u>R</u> <u>D</u> <u>D</u> <u>D</u> <u>T</u> <u>A</u> <u>V</u> <u>Y</u> <u>Y</u> <u>C</u> <u>V</u> <u>G</u> <u>G</u><br><u>H</u> <u>W</u> <u>A</u> <u>V</u> <u>W</u> <u>G</u> <u>Q</u> <u>G</u> <u>T</u> <u>T</u> <u>V</u> <u>T</u> <u>V</u> <u>S</u> <u>S</u> <u>G</u> <u>G</u> <u>G</u> <u>S</u> <u>G</u> <u>G</u> <u>G</u> <u>S</u> <u>G</u> <u>G</u> <u>G</u> <u>S</u> <u>D</u> <u>I</u> <u>Q</u> <u>M</u> <u>T</u> <u>Q</u> <u>S</u> <u>P</u> <u>S</u> <u>T</u> <u>L</u> <u>S</u> <u>A</u> <u>S</u> <u>V</u> <u>G</u> <u>D</u> <u>R</u> <u>V</u> <u>T</u> <u>I</u> <u>T</u> <u>C</u> <u>R</u> <u>A</u><br><u>S</u> <u>Q</u> <u>S</u> <u>I</u> <u>S</u> <u>D</u> <u>R</u> <u>L</u> <u>A</u> <u>W</u> <u>Y</u> <u>Q</u> <u>Q</u> <u>K</u> <u>P</u> <u>G</u> <u>K</u> <u>A</u> <u>P</u> <u>K</u> <u>L</u> <u>I</u> <u>Y</u> <u>K</u> <u>A</u> <u>S</u> <u>S</u> <u>L</u> <u>E</u> <u>S</u> <u>G</u> <u>V</u> <u>P</u> <u>S</u> <u>R</u> <u>F</u> <u>S</u> <u>G</u> <u>S</u> <u>G</u> <u>S</u> <u>G</u> <u>T</u> <u>E</u> <u>F</u> <u>T</u> <u>L</u> <u>T</u> <u>I</u> <u>S</u> <u>S</u> <u>L</u> <u>Q</u> <u>P</u> <u>D</u> <u>D</u> <u>F</u> <u>A</u><br><u>V</u> <u>Y</u> <u>C</u> <u>Q</u> <u>Q</u> <u>Y</u> <u>G</u> <u>H</u> <u>L</u> <u>P</u> <u>M</u> <u>Y</u> <u>T</u> <u>F</u> <u>G</u> <u>Q</u> <u>G</u> <u>T</u> <u>K</u> <u>V</u> <u>E</u> <u>I</u> <u>K</u> <u>T</u> <u>T</u> <u>T</u> <u>P</u> <u>A</u> <u>P</u> <u>R</u> <u>P</u> <u>P</u> <u>T</u> <u>P</u> <u>A</u> <u>P</u> <u>T</u> <u>I</u> <u>A</u> <u>S</u> <u>Q</u> <u>P</u> <u>L</u> <u>S</u> <u>R</u> <u>P</u> <u>E</u> <u>A</u> <u>C</u> <u>R</u> <u>P</u> <u>A</u> <u>A</u> <u>G</u> <u>G</u> <u>A</u> <u>V</u> <u>H</u> <u>T</u><br><u>R</u> <u>G</u> <u>L</u> <u>D</u> <u>F</u> <u>A</u> <u>C</u> <u>D</u> <u>I</u> <u>Y</u> <u>I</u> <u>W</u> <u>A</u> <u>P</u> <u>L</u> <u>A</u> <u>G</u> <u>T</u> <u>C</u> <u>G</u> <u>V</u> <u>L</u> <u>L</u> <u>L</u> <u>S</u> <u>L</u> <u>V</u> <u>I</u> <u>T</u> <u>L</u> <u>Y</u> <u>C</u> <u>K</u> <u>R</u> <u>G</u> <u>R</u> <u>K</u> <u>L</u> <u>L</u> <u>Y</u> <u>I</u> <u>F</u> <u>K</u> <u>Q</u> <u>P</u> <u>F</u> <u>M</u> <u>R</u> <u>P</u> <u>V</u> <u>Q</u> <u>T</u> <u>T</u> <u>Q</u> <u>E</u> <u>E</u> <u>D</u> <u>G</u> <u>C</u><br><u>S</u> <u>C</u> <u>R</u> <u>F</u> <u>P</u> <u>E</u> <u>E</u> <u>E</u> <u>E</u> <u>G</u> <u>G</u> <u>C</u> <u>E</u> <u>L</u> <u>R</u> <u>V</u> <u>K</u> <u>F</u> <u>S</u> <u>R</u> <u>S</u> <u>A</u> <u>D</u> <u>A</u> <u>P</u> <u>A</u> <u>Y</u> <u>K</u> <u>Q</u> <u>G</u> <u>Q</u> <u>N</u> <u>Q</u> <u>L</u> <u>Y</u> <u>N</u> <u>E</u> <u>L</u> <u>N</u> <u>L</u> <u>G</u> <u>R</u> <u>R</u> <u>E</u> <u>E</u> <u>Y</u> <u>D</u> <u>V</u> <u>L</u> <u>D</u> <u>K</u> <u>R</u> <u>R</u> <u>G</u> <u>R</u> <u>D</u> <u>P</u> <u>E</u> <u>M</u> <u>G</u><br><u>G</u> <u>K</u> <u>P</u> <u>R</u> <u>R</u> <u>K</u> <u>N</u> <u>P</u> <u>Q</u> <u>E</u> <u>G</u> <u>L</u> <u>Y</u> <u>N</u> <u>E</u> <u>L</u> <u>Q</u> <u>K</u> <u>D</u> <u>K</u> <u>M</u> <u>A</u> <u>E</u> <u>A</u> <u>Y</u> <u>S</u> <u>E</u> <u>I</u> <u>G</u> <u>M</u> <u>K</u> <u>G</u> <u>E</u> <u>R</u> <u>R</u> <u>R</u> <u>G</u> <u>K</u> <u>G</u> <u>H</u> <u>D</u> <u>G</u> <u>L</u> <u>Y</u> <u>Q</u> <u>G</u> <u>L</u> <u>S</u> <u>T</u> <u>A</u> <u>T</u> <u>K</u> <u>D</u> <u>T</u> <u>Y</u> <u>D</u> <u>A</u> <u>L</u> <u>H</u> <u>M</u><br><u>Q</u> <u>A</u> <u>L</u> <u>P</u> <u>P</u> <u>R</u>                                                                                         |
| SEQ ID<br>NO:<br>1519 | M5 (ScFv<br>domain)                                                  | <u>QVQLVQSGAEVEKPGASVKVSCASGYTFDYYMHWVRQAPGQGLEWMGWINPNSGGTNY</u><br><u>A</u> <u>Q</u> <u>K</u> <u>F</u> <u>Q</u> <u>G</u> <u>R</u> <u>V</u> <u>T</u> <u>M</u> <u>T</u> <u>R</u> <u>D</u> <u>T</u> <u>S</u> <u>I</u> <u>S</u> <u>T</u> <u>A</u> <u>Y</u> <u>M</u> <u>E</u> <u>L</u> <u>S</u> <u>R</u> <u>L</u> <u>R</u> <u>S</u> <u>D</u> <u>D</u> <u>T</u> <u>A</u> <u>V</u> <u>Y</u> <u>Y</u> <u>C</u> <u>A</u> <u>S</u> <u>G</u> <u>W</u> <u>D</u> <u>F</u> <u>D</u> <u>Y</u> <u>W</u> <u>G</u> <u>Q</u> <u>G</u> <u>T</u> <u>L</u> <u>V</u> <u>T</u> <u>V</u> <u>S</u> <u>S</u> <u>G</u> <u>G</u> <u>G</u> <u>S</u><br><u>G</u> <u>G</u> <u>G</u> <u>S</u> <u>G</u> <u>G</u> <u>G</u> <u>S</u> <u>G</u> <u>G</u> <u>G</u> <u>S</u> <u>D</u> <u>I</u> <u>V</u> <u>M</u> <u>T</u> <u>Q</u> <u>S</u> <u>P</u> <u>S</u> <u>S</u> <u>L</u> <u>S</u> <u>A</u> <u>S</u> <u>V</u> <u>G</u> <u>D</u> <u>R</u> <u>V</u> <u>T</u> <u>I</u> <u>T</u> <u>C</u> <u>R</u> <u>A</u> <u>S</u> <u>Q</u> <u>S</u> <u>I</u> <u>R</u> <u>Y</u> <u>Y</u> <u>L</u> <u>S</u> <u>W</u> <u>Y</u> <u>Q</u> <u>Q</u> <u>K</u> <u>P</u> <u>G</u> <u>K</u> <u>A</u> <u>P</u> <u>K</u><br><u>L</u> <u>L</u> <u>I</u> <u>Y</u> <u>T</u> <u>A</u> <u>S</u> <u>I</u> <u>L</u> <u>Q</u> <u>N</u> <u>G</u> <u>V</u> <u>P</u> <u>S</u> <u>R</u> <u>F</u> <u>S</u> <u>G</u> <u>S</u> <u>G</u> <u>S</u> <u>G</u> <u>T</u> <u>D</u> <u>F</u> <u>T</u> <u>L</u> <u>T</u> <u>I</u> <u>S</u> <u>S</u> <u>L</u> <u>Q</u> <u>P</u> <u>E</u> <u>D</u> <u>F</u> <u>A</u> <u>T</u> <u>Y</u> <u>Y</u> <u>C</u> <u>L</u> <u>Q</u> <u>T</u> <u>Y</u> <u>T</u> <u>T</u> <u>P</u> <u>D</u> <u>F</u> <u>G</u> <u>P</u> <u>G</u> <u>T</u> <u>K</u> <u>V</u> <u>E</u> <u>I</u> <u>K</u>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            |
| SEQ ID<br>NO:<br>1520 | M5<br>>XP31-<br>20LC<br>(M5<br>XP31-<br>20LC<br>R001-B4<br>126165)   | <u>MALPVTALLPLALLHAARP</u> <u>QVQLVQSGAEVEKPGASVKVSCASGYTFDYYMHWVRQ</u><br><u>A</u> <u>P</u> <u>G</u> <u>Q</u> <u>G</u> <u>L</u> <u>E</u> <u>W</u> <u>M</u> <u>G</u> <u>I</u> <u>N</u> <u>P</u> <u>N</u> <u>S</u> <u>G</u> <u>G</u> <u>T</u> <u>N</u> <u>Y</u> <u>A</u> <u>Q</u> <u>K</u> <u>F</u> <u>Q</u> <u>G</u> <u>R</u> <u>V</u> <u>T</u> <u>M</u> <u>T</u> <u>R</u> <u>D</u> <u>T</u> <u>S</u> <u>I</u> <u>S</u> <u>T</u> <u>A</u> <u>Y</u> <u>M</u> <u>E</u> <u>L</u> <u>S</u> <u>R</u> <u>L</u> <u>R</u> <u>S</u> <u>D</u> <u>D</u> <u>T</u> <u>A</u> <u>V</u> <u>Y</u> <u>Y</u> <u>C</u> <u>A</u> <u>S</u><br><u>W</u> <u>D</u> <u>F</u> <u>D</u> <u>Y</u> <u>W</u> <u>G</u> <u>Q</u> <u>G</u> <u>T</u> <u>L</u> <u>V</u> <u>T</u> <u>V</u> <u>S</u> <u>S</u> <u>G</u> <u>G</u> <u>G</u> <u>S</u> <u>G</u> <u>G</u> <u>G</u> <u>S</u> <u>G</u> <u>G</u> <u>G</u> <u>S</u> <u>D</u> <u>I</u> <u>V</u> <u>M</u> <u>T</u> <u>Q</u> <u>S</u> <u>P</u> <u>S</u> <u>S</u> <u>L</u> <u>S</u> <u>A</u> <u>S</u> <u>V</u> <u>G</u> <u>D</u> <u>R</u> <u>V</u> <u>T</u> <u>I</u> <u>T</u> <u>C</u> <u>R</u><br><u>A</u> <u>S</u> <u>Q</u> <u>S</u> <u>I</u> <u>R</u> <u>Y</u> <u>Y</u> <u>L</u> <u>S</u> <u>W</u> <u>Y</u> <u>Q</u> <u>Q</u> <u>K</u> <u>P</u> <u>G</u> <u>K</u> <u>A</u> <u>P</u> <u>K</u> <u>L</u> <u>L</u> <u>I</u> <u>Y</u> <u>T</u> <u>A</u> <u>S</u> <u>I</u> <u>L</u> <u>Q</u> <u>N</u> <u>G</u> <u>V</u> <u>P</u> <u>S</u> <u>R</u> <u>F</u> <u>S</u> <u>G</u> <u>S</u> <u>G</u> <u>S</u> <u>G</u> <u>T</u> <u>D</u> <u>F</u> <u>T</u> <u>L</u> <u>T</u> <u>I</u> <u>S</u> <u>S</u> <u>L</u> <u>Q</u> <u>P</u> <u>E</u> <u>D</u> <u>F</u><br><u>A</u> <u>T</u> <u>Y</u> <u>Y</u> <u>C</u> <u>L</u> <u>Q</u> <u>T</u> <u>Y</u> <u>T</u> <u>T</u> <u>P</u> <u>D</u> <u>F</u> <u>G</u> <u>P</u> <u>G</u> <u>T</u> <u>K</u> <u>V</u> <u>E</u> <u>I</u> <u>K</u> <u>T</u> <u>T</u> <u>T</u> <u>P</u> <u>A</u> <u>P</u> <u>R</u> <u>P</u> <u>P</u> <u>T</u> <u>P</u> <u>A</u> <u>P</u> <u>T</u> <u>I</u> <u>A</u> <u>S</u> <u>Q</u> <u>P</u> <u>L</u> <u>S</u> <u>R</u> <u>P</u> <u>E</u> <u>A</u> <u>C</u> <u>R</u> <u>P</u> <u>A</u> <u>A</u> <u>G</u> <u>G</u> <u>A</u> <u>V</u> <u>H</u> <u>T</u><br><u>R</u> <u>G</u> <u>L</u> <u>D</u> <u>F</u> <u>A</u> <u>C</u> <u>D</u> <u>I</u> <u>Y</u> <u>I</u> <u>W</u> <u>A</u> <u>P</u> <u>L</u> <u>A</u> <u>G</u> <u>T</u> <u>C</u> <u>G</u> <u>V</u> <u>L</u> <u>L</u> <u>L</u> <u>S</u> <u>L</u> <u>V</u> <u>I</u> <u>T</u> <u>L</u> <u>Y</u> <u>C</u> <u>K</u> <u>R</u> <u>G</u> <u>R</u> <u>K</u> <u>L</u> <u>L</u> <u>Y</u> <u>I</u> <u>F</u> <u>K</u> <u>Q</u> <u>P</u> <u>F</u> <u>M</u> <u>R</u> <u>P</u> <u>V</u> <u>Q</u> <u>T</u> <u>T</u> <u>Q</u> <u>E</u> <u>E</u> <u>D</u> <u>G</u> <u>C</u><br><u>S</u> <u>C</u> <u>R</u> <u>F</u> <u>P</u> <u>E</u> <u>E</u> <u>E</u> <u>E</u> <u>G</u> <u>G</u> <u>C</u> <u>E</u> <u>L</u> <u>R</u> <u>V</u> <u>K</u> <u>F</u> <u>S</u> <u>R</u> <u>S</u> <u>A</u> <u>D</u> <u>A</u> <u>P</u> <u>A</u> <u>Y</u> <u>K</u> <u>Q</u> <u>G</u> <u>Q</u> <u>N</u> <u>Q</u> <u>L</u> <u>Y</u> <u>N</u> <u>E</u> <u>L</u> <u>N</u> <u>L</u> <u>G</u> <u>R</u> <u>R</u> <u>E</u> <u>E</u> <u>Y</u> <u>D</u> <u>V</u> <u>L</u> <u>D</u> <u>K</u> <u>R</u> <u>R</u> <u>G</u> <u>R</u> <u>D</u> <u>P</u> <u>E</u> <u>M</u> <u>G</u><br><u>K</u> <u>P</u> <u>R</u> <u>R</u> <u>K</u> <u>N</u> <u>P</u> <u>Q</u> <u>E</u> <u>G</u> <u>L</u> <u>Y</u> <u>N</u> <u>E</u> <u>L</u> <u>Q</u> <u>K</u> <u>D</u> <u>K</u> <u>M</u> <u>A</u> <u>E</u> <u>A</u> <u>Y</u> <u>S</u> <u>E</u> <u>I</u> <u>G</u> <u>M</u> <u>K</u> <u>G</u> <u>E</u> <u>R</u> <u>R</u> <u>R</u> <u>G</u> <u>K</u> <u>G</u> <u>H</u> <u>D</u> <u>G</u> <u>L</u> <u>Y</u> <u>Q</u> <u>G</u> <u>L</u> <u>S</u> <u>T</u> <u>A</u> <u>T</u> <u>K</u> <u>D</u> <u>T</u> <u>Y</u> <u>D</u> <u>A</u> <u>L</u> <u>H</u> <u>M</u><br><u>Q</u> <u>A</u> <u>L</u> <u>P</u> <u>P</u> <u>R</u>                                                                                                    |
| SEQ ID<br>NO:<br>1521 | M6 (ScFv<br>domain)                                                  | <u>QVQLVQSGAEVKKPGASVKVSCASGYTFSSYMHWVRQAPGQGLEWMGIINPSSGGSTSY</u><br><u>A</u> <u>Q</u> <u>K</u> <u>F</u> <u>Q</u> <u>G</u> <u>R</u> <u>V</u> <u>T</u> <u>M</u> <u>T</u> <u>R</u> <u>D</u> <u>T</u> <u>S</u> <u>T</u> <u>S</u> <u>T</u> <u>V</u> <u>M</u> <u>E</u> <u>L</u> <u>S</u> <u>S</u> <u>L</u> <u>R</u> <u>S</u> <u>E</u> <u>D</u> <u>T</u> <u>A</u> <u>V</u> <u>Y</u> <u>Y</u> <u>C</u> <u>A</u> <u>R</u> <u>Y</u> <u>R</u> <u>L</u> <u>I</u> <u>A</u> <u>V</u> <u>A</u> <u>G</u> <u>D</u> <u>Y</u> <u>Y</u> <u>Y</u> <u>G</u> <u>M</u> <u>D</u> <u>V</u> <u>W</u> <u>G</u> <u>Q</u> <u>G</u> <u>T</u><br><u>M</u> <u>V</u> <u>T</u> <u>V</u> <u>S</u> <u>S</u> <u>G</u> <u>G</u> <u>G</u> <u>S</u> <u>G</u> <u>G</u> <u>G</u> <u>S</u> <u>G</u> <u>G</u> <u>G</u> <u>S</u> <u>D</u> <u>I</u> <u>Q</u> <u>M</u> <u>T</u> <u>Q</u> <u>S</u> <u>P</u> <u>S</u> <u>S</u> <u>V</u> <u>S</u> <u>A</u> <u>S</u> <u>V</u> <u>G</u> <u>D</u> <u>R</u> <u>V</u> <u>T</u> <u>I</u> <u>T</u> <u>C</u> <u>R</u> <u>A</u> <u>S</u> <u>Q</u> <u>G</u> <u>V</u> <u>G</u> <u>R</u> <u>W</u> <u>L</u> <u>A</u><br><u>W</u> <u>Y</u> <u>Q</u> <u>Q</u> <u>K</u> <u>P</u> <u>G</u> <u>T</u> <u>A</u> <u>P</u> <u>K</u> <u>L</u> <u>L</u> <u>I</u> <u>Y</u> <u>A</u> <u>A</u> <u>S</u> <u>T</u> <u>L</u> <u>Q</u> <u>S</u> <u>G</u> <u>V</u> <u>P</u> <u>S</u> <u>R</u> <u>F</u> <u>S</u> <u>G</u> <u>S</u> <u>G</u> <u>S</u> <u>G</u> <u>T</u> <u>D</u> <u>F</u> <u>T</u> <u>L</u> <u>T</u> <u>I</u> <u>N</u> <u>N</u> <u>L</u> <u>Q</u> <u>P</u> <u>E</u> <u>D</u> <u>F</u> <u>A</u> <u>T</u> <u>Y</u> <u>Y</u> <u>C</u> <u>Q</u> <u>Q</u> <u>A</u> <u>N</u> <u>S</u> <u>F</u><br><u>P</u> <u>L</u> <u>T</u> <u>F</u> <u>G</u> <u>G</u> <u>G</u> <u>T</u> <u>R</u> <u>L</u> <u>E</u> <u>I</u> <u>K</u>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |
| SEQ ID<br>NO:<br>1522 | M6<br>>FE10-<br>06ID<br>(M6<br>46FE10-<br>06ID<br>R001-A4<br>126166) | <u>MALPVTALLPLALLHAARP</u> <u>QVQLVQSGAEVKKPGASVKVSCASGYTFSSYMHWVRQ</u><br><u>A</u> <u>P</u> <u>G</u> <u>Q</u> <u>G</u> <u>L</u> <u>E</u> <u>W</u> <u>M</u> <u>G</u> <u>I</u> <u>N</u> <u>P</u> <u>S</u> <u>G</u> <u>G</u> <u>S</u> <u>T</u> <u>S</u> <u>Y</u> <u>A</u> <u>Q</u> <u>K</u> <u>F</u> <u>Q</u> <u>G</u> <u>R</u> <u>V</u> <u>T</u> <u>M</u> <u>T</u> <u>R</u> <u>D</u> <u>T</u> <u>S</u> <u>T</u> <u>S</u> <u>T</u> <u>V</u> <u>M</u> <u>E</u> <u>L</u> <u>S</u> <u>S</u> <u>L</u> <u>R</u> <u>S</u> <u>E</u> <u>D</u> <u>T</u> <u>A</u> <u>V</u> <u>Y</u> <u>Y</u> <u>C</u> <u>A</u> <u>R</u><br><u>R</u> <u>L</u> <u>I</u> <u>A</u> <u>V</u> <u>A</u> <u>G</u> <u>D</u> <u>Y</u> <u>Y</u> <u>Y</u> <u>G</u> <u>M</u> <u>D</u> <u>V</u> <u>W</u> <u>G</u> <u>Q</u> <u>G</u> <u>T</u> <u>M</u> <u>V</u> <u>T</u> <u>V</u> <u>S</u> <u>S</u> <u>G</u> <u>G</u> <u>G</u> <u>S</u> <u>G</u> <u>G</u> <u>G</u> <u>S</u> <u>G</u> <u>G</u> <u>G</u> <u>S</u> <u>D</u> <u>I</u> <u>Q</u> <u>M</u> <u>T</u> <u>Q</u> <u>S</u> <u>P</u> <u>S</u> <u>S</u> <u>V</u> <u>S</u> <u>A</u><br><u>S</u> <u>V</u> <u>G</u> <u>D</u> <u>R</u> <u>V</u> <u>T</u> <u>I</u> <u>T</u> <u>C</u> <u>R</u> <u>A</u> <u>S</u> <u>Q</u> <u>G</u> <u>V</u> <u>G</u> <u>R</u> <u>W</u> <u>L</u> <u>A</u> <u>W</u> <u>Y</u> <u>Q</u> <u>Q</u> <u>K</u> <u>P</u> <u>G</u> <u>T</u> <u>A</u> <u>P</u> <u>K</u> <u>L</u> <u>L</u> <u>I</u> <u>Y</u> <u>A</u> <u>A</u> <u>S</u> <u>T</u> <u>L</u> <u>Q</u> <u>S</u> <u>G</u> <u>V</u> <u>P</u> <u>S</u> <u>R</u> <u>F</u> <u>S</u> <u>G</u> <u>S</u> <u>G</u> <u>S</u> <u>G</u> <u>T</u> <u>D</u> <u>F</u> <u>T</u> <u>L</u><br><u>T</u> <u>I</u> <u>N</u> <u>N</u> <u>L</u> <u>Q</u> <u>P</u> <u>E</u> <u>D</u> <u>F</u> <u>A</u> <u>T</u> <u>Y</u> <u>Y</u> <u>C</u> <u>Q</u> <u>Q</u> <u>A</u> <u>N</u> <u>S</u> <u>F</u> <u>P</u> <u>L</u> <u>T</u> <u>F</u> <u>G</u> <u>G</u> <u>G</u> <u>T</u> <u>R</u> <u>L</u> <u>E</u> <u>I</u> <u>K</u> <u>T</u> <u>T</u> <u>T</u> <u>P</u> <u>A</u> <u>P</u> <u>R</u> <u>P</u> <u>P</u> <u>T</u> <u>P</u> <u>A</u> <u>P</u> <u>T</u> <u>I</u> <u>A</u> <u>S</u> <u>Q</u> <u>P</u> <u>L</u> <u>S</u> <u>R</u> <u>P</u> <u>E</u> <u>A</u><br><u>C</u> <u>R</u> <u>P</u> <u>A</u> <u>A</u> <u>G</u> <u>G</u> <u>A</u> <u>V</u> <u>H</u> <u>T</u> <u>R</u> <u>G</u> <u>L</u> <u>D</u> <u>F</u> <u>A</u> <u>C</u> <u>D</u> <u>I</u> <u>Y</u> <u>I</u> <u>W</u> <u>A</u> <u>P</u> <u>L</u> <u>A</u> <u>G</u> <u>T</u> <u>C</u> <u>G</u> <u>V</u> <u>L</u> <u>L</u> <u>L</u> <u>S</u> <u>L</u> <u>V</u> <u>I</u> <u>T</u> <u>L</u> <u>Y</u> <u>C</u> <u>K</u> <u>R</u> <u>G</u> <u>R</u> <u>K</u> <u>L</u> <u>L</u> <u>Y</u> <u>I</u> <u>F</u> <u>K</u> <u>Q</u> <u>P</u> <u>F</u> <u>M</u> <u>R</u><br><u>P</u> <u>V</u> <u>Q</u> <u>T</u> <u>T</u> <u>Q</u> <u>E</u> <u>E</u> <u>D</u> <u>G</u> <u>C</u> <u>S</u> <u>C</u> <u>R</u> <u>F</u> <u>P</u> <u>E</u> <u>E</u> <u>E</u> <u>E</u> <u>G</u> <u>G</u> <u>C</u> <u>E</u> <u>L</u> <u>R</u> <u>V</u> <u>K</u> <u>F</u> <u>S</u> <u>R</u> <u>S</u> <u>A</u> <u>D</u> <u>A</u> <u>P</u> <u>A</u> <u>Y</u> <u>K</u> <u>Q</u> <u>G</u> <u>Q</u> <u>N</u> <u>Q</u> <u>L</u> <u>Y</u> <u>N</u> <u>E</u> <u>L</u> <u>N</u> <u>L</u> <u>G</u> <u>R</u> <u>R</u> <u>E</u> <u>E</u> <u>Y</u> <u>D</u> <u>V</u> <u>L</u><br><u>D</u> <u>K</u> <u>R</u> <u>R</u> <u>G</u> <u>R</u> <u>D</u> <u>P</u> <u>E</u> <u>M</u> <u>G</u> <u>G</u> <u>K</u> <u>P</u> <u>R</u> <u>R</u> <u>K</u> <u>N</u> <u>P</u> <u>Q</u> <u>E</u> <u>G</u> <u>L</u> <u>Y</u> <u>N</u> <u>E</u> <u>L</u> <u>Q</u> <u>K</u> <u>D</u> <u>K</u> <u>M</u> <u>A</u> <u>E</u> <u>A</u> <u>Y</u> <u>S</u> <u>E</u> <u>I</u> <u>G</u> <u>M</u> <u>K</u> <u>G</u> <u>E</u> <u>R</u> <u>R</u> <u>R</u> <u>G</u> <u>K</u> <u>G</u> <u>H</u> <u>D</u> <u>G</u> <u>L</u> <u>Y</u> <u>Q</u> <u>G</u> <u>L</u> <u>S</u> <u>T</u><br><u>A</u> <u>T</u> <u>K</u> <u>D</u> <u>T</u> <u>Y</u> <u>D</u> <u>A</u> <u>L</u> <u>H</u> <u>M</u> <u>Q</u> <u>A</u> <u>L</u> <u>P</u> <u>P</u> <u>R</u> |
| SEQ ID<br>NO:<br>1523 | M7 (ScFv<br>domain)                                                  | <u>QVQLVQSGGGVVPGRSLRLSCAASGFTFSSYAMHWVRQAPGKGLEWVAVISYDGSNKYY</u><br><u>A</u> <u>D</u> <u>S</u> <u>V</u> <u>K</u> <u>G</u> <u>R</u> <u>F</u> <u>T</u> <u>I</u> <u>S</u> <u>R</u> <u>D</u> <u>N</u> <u>S</u> <u>K</u> <u>N</u> <u>T</u> <u>L</u> <u>Y</u> <u>L</u> <u>Q</u> <u>M</u> <u>N</u> <u>S</u> <u>L</u> <u>R</u> <u>A</u> <u>E</u> <u>D</u> <u>T</u> <u>A</u> <u>V</u> <u>Y</u> <u>Y</u> <u>C</u> <u>A</u> <u>R</u> <u>W</u> <u>K</u> <u>V</u> <u>S</u> <u>S</u> <u>S</u> <u>S</u> <u>P</u> <u>A</u> <u>F</u> <u>D</u> <u>Y</u> <u>W</u> <u>G</u> <u>Q</u> <u>G</u> <u>T</u> <u>L</u> <u>V</u> <u>T</u> <u>V</u><br><u>S</u> <u>G</u> <u>G</u> <u>G</u> <u>S</u> <u>G</u> <u>G</u> <u>G</u> <u>S</u> <u>G</u> <u>G</u> <u>G</u> <u>S</u> <u>G</u> <u>G</u> <u>G</u> <u>S</u> <u>E</u> <u>I</u> <u>V</u> <u>L</u> <u>T</u> <u>Q</u> <u>S</u> <u>P</u> <u>A</u> <u>T</u> <u>L</u> <u>S</u> <u>L</u> <u>S</u> <u>P</u> <u>G</u> <u>E</u> <u>R</u> <u>A</u> <u>I</u> <u>L</u> <u>S</u> <u>C</u> <u>R</u> <u>A</u> <u>S</u> <u>Q</u> <u>S</u> <u>V</u> <u>Y</u> <u>T</u> <u>K</u> <u>Y</u> <u>L</u> <u>G</u> <u>W</u> <u>Y</u> <u>Q</u><br><u>K</u> <u>P</u> <u>G</u> <u>Q</u> <u>A</u> <u>P</u> <u>R</u> <u>L</u> <u>L</u> <u>I</u> <u>Y</u> <u>D</u> <u>A</u> <u>S</u> <u>T</u> <u>R</u> <u>A</u> <u>T</u> <u>G</u> <u>I</u> <u>P</u> <u>D</u> <u>R</u> <u>F</u> <u>S</u> <u>G</u> <u>S</u> <u>G</u> <u>S</u> <u>G</u> <u>T</u> <u>D</u> <u>F</u> <u>T</u> <u>L</u> <u>T</u> <u>I</u> <u>N</u> <u>R</u> <u>L</u> <u>E</u> <u>P</u> <u>E</u> <u>D</u> <u>F</u> <u>A</u> <u>V</u> <u>Y</u> <u>Y</u> <u>C</u> <u>Q</u> <u>H</u> <u>Y</u> <u>G</u> <u>G</u> <u>S</u> <u>P</u> <u>L</u> <u>I</u> <u>T</u><br><u>F</u> <u>G</u> <u>Q</u> <u>G</u> <u>T</u> <u>R</u> <u>L</u> <u>E</u> <u>I</u> <u>K</u>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |

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| SEQ ID NO: 1524 | M7<br>>VE12-01CD<br>(M7<br>VE12-01CD<br>R001-A5<br>126167)    | <u><b>MALPVTALLPLALLHAARP</b></u> QVQLVQSGGGVVPGRSLRLSCAASGFTFSSYAMHWVRQAPGKGLEWVAVISYDGSNKYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCARWKVSSSSPAFDYWGQGLVTVSSGGGGSGGGSGGGSGGGGSEIVLTQSPATLSLSPGERAILSCRASQSVYTKYLGWYQQKPGQAPRLLIYDASTRATGIPDRFSGSGSGTDFTLTINRLEPEDFAVYYCQHYGGSPITFGQGTREIKTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFP EEEEEGGCEL RVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVL DKKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRRGK GHDGLYQGLSTATKDTYDALHMQALPPR               |
| SEQ ID NO: 1525 | M8 (ScFv domain)                                              | QVQLQQSGAEVKKPGASVKV SCKTSGYPFTGYSLHWVRQAPGQGLEWMGWINPNSSGGTNYAQKFQGRVTMTRDTSISTAYMELSR LRSDDTAVYYCARDHYGGNSLFYWGQGLTVTVSSGGGGSGGGSGGGSGGGSGGGSDIQLTQSPSSISASVGD TVSITCRASQDSGTWLA WYQQKPGKAPNLLMYDASTLEDGVP SRFSGSASGTEFTLT VNRLQPEDSATYYCQQYNSYPLTFGGG TKVDIK                                                                                                                                                                                                                                                                             |
| SEQ ID NO: 1526 | M8<br>>LE13-05XD<br>(M8<br>LE13-05XD<br>R001-E5<br>126168)    | <u><b>MALPVTALLPLALLHAARP</b></u> QVQLQQSGAEVKKPGASVKV SCKTSGYPFTGYSLHWVRQAPGQGLEWMGWINPNSSGGTNYAQKFQGRVTMTRDTSISTAYMELSR LRSDDTAVYYCARDHYGGNSLFYWGQGLTVTVSSGGGGSGGGSGGGSGGGSGGGSDIQLTQSPSSISASVGD TVSITCRASQDSGTWLA WYQQKPGKAPNLLMYDASTLEDGVP SRFSGSASGTEFTLT VNRLQPEDSATYYCQQYNSYPLTFGGG TKVDIKTTTPAPRPPTPAPT IASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFP EEEEEGGCEL RVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVL DKKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRRGK GHDGLYQGLSTATKDTYDALHMQALPPR    |
| SEQ ID NO: 1527 | M9 (ScFv domain)                                              | QVQLVQSGAEVKKPGASVEV SCKASGYTFTSYMHWVRQAPGQGLEWMGIIINPSSGGSTGYAQKFQGRVTMTRDTSISTVHME LSSLRSED TAVYYCARGGYSSSSDAFDIWGQGTMTVSSGGGGSGGGSGGGSGGGSGGGSDIQMTQSPPSLSASVGD RVTITCRASQDISSALAWYQQKPGTPPKLLIYDASSLES GVP SRFSGSASGTEFTLTIS SLQPEDFATYYCQQFSSYPLTFGGTRLEIK                                                                                                                                                                                                                                                                             |
| SEQ ID NO: 1528 | M9<br>>BE15-00SD<br>(M9<br>BE15-00SD<br>R001-A3<br>126169)    | <u><b>MALPVTALLPLALLHAARP</b></u> QVQLVQSGAEVKKPGASVEV SCKASGYTFTSYMHWVRQAPGQGLEWMGIIINPSSGGSTGYAQKFQGRVTMTRDTSISTVHME LSSLRSED TAVYYCARGGYSSSSDAFDIWGQGTMTVTVSSGGGGSGGGSGGGSGGGSGGGSDIQMTQSPPSLSASVGD RVTITCRASQDISSALAWYQQKPGTPPKLLIYDASSLES GVP SRFSGSASGTEFTLTIS SLQPEDFATYYCQQFSSYPLTFGGTRLEIKTTTPAPRPPTPAPT IASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFP EEEEEGGCEL RVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVL DKKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRRGK GHDGLYQGLSTATKDTYDALHMQALPPR  |
| SEQ ID NO: 1529 | M10 (ScFv domain)                                             | QVQLVQSGAEVKKPGASVKV SCKASGYTFTSYGISWVRQAPGQGLEWMGWISAYNGNTNYAQKLQGRVTMTTDTSTSTAYMELSR LRSDDTAVYYCARVAGGIY YYYGMDVWGQGTITVSSGGGGSGGGSGGGSGGGSGGGSDIVMTQTPDSLAVSLGERATISCKSSH SVLYNRNNKNYLAWYQQKPGQPPKLLFYWASTRKSGVPDRFSGSGSGTDFTLTIS SLQPEDFATYFCQQQTFPLTFGQGTREIN                                                                                                                                                                                                                                                                          |
| SEQ ID NO: 1530 | M10<br>>RE16-05MD<br>(M10<br>RE16-05MD<br>R001-D10<br>126170) | <u><b>MALPVTALLPLALLHAARP</b></u> QVQLVQSGAEVKKPGASVKV SCKASGYTFTSYGISWVRQAPGQGLEWMGWISAYNGNTNYAQKLQGRVTMTTDTSTSTAYMELSR LRSDDTAVYYCARVAGGIY YYYGMDVWGQGTITVSSGGGGSGGGSGGGSGGGSGGGSDIVMTQTPDSLAVSLGERATISCKSSH SVLYNRNNKNYLAWYQQKPGQPPKLLFYWASTRKSGVPDRFSGSGSGTDFTLTIS SLQPEDFATYFCQQQTFPLTFGQGTREINTTTPAPRPPTPAPT IASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFP EEEEEGGCEL RVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVL DKKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRRGK GHDGLYQGLSTATKDTYDALHMQALPPR |

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| SEQ ID<br>NO:<br>1531 | M11<br>(ScFv<br>domain)                                              | QVQLQQSGAEVKKPGASVKVSKASGYTFTGYMHWVRQAPGQGLEWMGWINPNSSGGTNY<br>AQNFGQGRVTMTRDTSISTAYMELRRLRSDDTAVYYCASGWDYWGQGLTVTVSSGGGGGS<br>GGGGSGGGSGGGGSDIRMTQSPSSLSASVGDRTITCRASQSIRYYLSWYQQKPGKAPK<br>LLIYTASILQNGVPSRFSGSGSGTDFTLTISLQPEDFATYYCLQTYTTPDFGPGTKVEI<br>K                                                                                                                                                                                                                                                                                                       |
| SEQ ID<br>NO:<br>1532 | M11<br>>NE10-<br>19WD<br>(M11<br>NE10-<br>19WD<br>R001-G2<br>126171) | <u><b>MALPVTALLPLALLHAARP</b></u> QVQLQQSGAEVKKPGASVKVSKASGYTFTGYMHWVRQ<br>APGQGLEWMGWINPNSSGGTNYAQNFGQGRVTMTRDTSISTAYMELRRLRSDDTAVYYCASG<br>WDFDYWGQGLTVTVSSGGGGSGGGGSGGGGSDIRMTQSPSSLSASVGDRTITCR<br>ASQSIRYYLSWYQQKPGKAPKLLIYTASILQNGVPSRFSGSGSGTDFTLTISLQPEDFA<br>TYYCLQTYTTPDFGPGTKVEIKTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTR<br>GLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCS<br>CRFPEEEEGGCELRVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDRRGRDPEMGG<br>KPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRRGKGGHDGLYQGLSTATKDTYDALHMQ<br>ALPPR                        |
| SEQ ID<br>NO:<br>1533 | M12<br>(ScFv<br>domain)                                              | QVQLVQSGAEVKKPGASVKVSKASGYTFTGYMHWVRQAPGQGLEWMGRINPNSSGGTNY<br>AQKFQGRVTMTTDTSTSTAYMELRSLRSDDTAVYYCARTTTSYAFDIWGQGTMTVTVSSGG<br>GGSGGGSGGGGSDIQLTQSPSTLSASVGDRTITCRASQSISTWLAWYQQKPGK<br>APNLLIYKASTLESGVPSRFSGSGSGTEFTLTISLQPDDEFATYYCQQYNTYSPYTFGQG<br>TKLEIK                                                                                                                                                                                                                                                                                                     |
| SEQ ID<br>NO:<br>1534 | M12<br>>DE12-<br>14RD<br>(M12<br>DE12-<br>14RD<br>R001-G9<br>126172) | <u><b>MALPVTALLPLALLHAARP</b></u> QVQLVQSGAEVKKPGASVKVSKASGYTFTGYMHWVRQ<br>APGQGLEWMGRINPNSSGGTNYAQKFQGRVTMTTDTSTSTAYMELRSLRSDDTAVYYCART<br>TTSYAFDIWGQGTMTVTVSSGGGGSGGGGSGGGGSDIQLTQSPSTLSASVGDRTIT<br>TCRASQSISTWLAWYQQKPGKAPNLLIYKASTLESGVPSRFSGSGSGTEFTLTISLQPD<br>DEFATYYCQQYNTYSPYTFGQGTKLEIKTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGG<br>AVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQE<br>EDGCSCRFPPEEEEGGCELRVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDRRGRD<br>PEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRRGKGGHDGLYQGLSTATKDTYD<br>ALHMQALPPR                |
| SEQ ID<br>NO:<br>1535 | M13<br>(ScFv<br>domain)                                              | QVQLVQSGGGLVKPGGSLRLSCEASGFIFSDYYMGWIRQAPGKGLEWVSYIGRSGSSMY<br>ADSVKGRFTFSRDNAKNSLYLQMNSLRAEDTAVYYCAASPVAATEDFQHWGQGLTVTVS<br>SGGGSGGGSGGGGSDIVMTQTPATLSLSPGERATLSCRASQSVTSNYLAWYQQ<br>KPGQAPRLLLF <del>G</del> ASTRATGIPDRFSGSGSGTDFTLTINRLEPEDFAMYYCQQYGSAPVTF<br>GQGTKLEIK                                                                                                                                                                                                                                                                                       |
| SEQ ID<br>NO:<br>1536 | M13<br>>TE13-<br>19LD<br>(M13<br>TE13-<br>19LD<br>R002-C3<br>126173) | <u><b>MALPVTALLPLALLHAARP</b></u> QVQLVQSGGGLVKPGGSLRLSCEASGFIFSDYYMGWIRQ<br>APGKGLEWVSYIGRSGSSMYADSVKGRFTFSRDNAKNSLYLQMNSLRAEDTAVYYCAAS<br>PVVAATEDFQHWGQGLTVTVSSGGGGSGGGGSGGGGSDIVMTQTPATLSLSPGER<br>ATLSCRASQSVTSNYLAWYQQKPGQAPRLLLF <del>G</del> ASTRATGIPDRFSGSGSGTDFTLTINR<br>LEPEDFAMYYCQQYGSAPVTFGQGTKLEIKTTTPAPRPPTPAPTIASQPLSLRPEACRPA<br>AGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQT<br>TQEEDGCSCRFPPEEEEGGCELRVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDRR<br>GRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRRGKGGHDGLYQGLSTATKD<br>TYDALHMQALPPR |
| SEQ ID<br>NO:<br>1537 | M14<br>(ScFv<br>domain)                                              | QVQLVQSGAEVRAPGASVKISCKASGFTFRGYYIHWVRQAPGQGLEWMGIINPSGGSRAY<br>AQKFQGRVTMTRDTSISTVYMELSSLSRSDDTAMYYCARTASCGGDCYYLDYWGQGLTVTV<br>SSGGGGSGGGGSDIQTQSPPTLSASVGDRTITCRASENVNIWLAWYQQ<br>KPGKAPKLLIYKSSSLASGVPSRFSGSGSGAEFTLTISLQPDDEFATYYCQQYQSYPLTF<br>GGGKVDIK                                                                                                                                                                                                                                                                                                       |
| SEQ ID<br>NO:<br>1538 | M14<br>>BS83-<br>95ID<br>(M14                                        | <u><b>MALPVTALLPLALLHAARP</b></u> QVQLVQSGAEVRAPGASVKISCKASGFTFRGYYIHWVRQ<br>APGQGLEWMGIINPSGGSRAYAQKFQGRVTMTRDTSISTVYMELSSLSRSDDTAMYYCART<br>ASCGGDCYYLDYWGQGLTVTVSSGGGGSGGGGSDIQTQSPPTLSASVGD<br>RVTITCRASENVNIWLAWYQQKPGKAPKLLIYKSSSLASGVPSRFSGSGSGAEFTLTISL<br>LQPDDEFATYYCQQYQSYPLTFGGGKVDIKTTTPAPRPPTPAPTIASQPLSLRPEACRPA                                                                                                                                                                                                                                     |



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|                       | BS83-95ID<br>R001-E8<br><br>126174)                             | AGGAVHTRGLDFACDIYIWAPLAGTCGVLLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQT<br>TQEEDGCSCRFPEEEEEGGCELRVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDRR<br>GRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRRGKGDGLYQGLSTATKD<br>TYDALHMQALPPR                                                                                                                                                                                                                                                                                                                                          |
| SEQ ID<br>NO:<br>1539 | M15<br>(ScFv<br>domain)                                         | <u>QVQLVQSGGGLVQPGRSLRLSCAASGFTFDDYAMHWVRQAPGKGLEWVSGISWNSGSGIGY</u><br><u>ADSVKGRFTISRDNANKNSLYLQMNSLRAEDTAVYYCAKD</u> GSSSWSWGYFDYWGQGTILTV<br>SSGGGSGGGGSGGGGSSSELTQDPAVSVALGQTVRTTCQGDALRSYYASWYQQKPGQAP<br>MLVIYGKNNRPSGIPDRFSGSDSGDTASLTITGAQAEDEADYYCNSRDSSGYPVFGTGT<br>VTVL                                                                                                                                                                                                                                                                     |
| SEQ ID<br>NO:<br>1540 | M15<br>>HS86-<br>94XD<br>(M15<br>HS86-<br>94XD<br>NT<br>127553) | <u><b>MALPVTALLPLALLHAARP</b></u> QVQLVQSGGGLVQPGRSLRLSCAASGFTFDDYAMHWVRQ<br>APGKGLEWVSGISWNSGSGIGYADSVKGRFTISRDNANKNSLYLQMNSLRAEDTAVYYCAKD<br>GSSSWSWGYFDYWGQGTILTVSSGGGSGGGGSGGGGSSSELTQDPAVSVALGQTVRTTC<br>QGDALRSYYASWYQQKPGQAPMLVIYGKNNRPSGIPDRFSGSDSGDTASLTITGAQAEDE<br>ADYYCNSRDSSGYPVFGTGTGTVTLTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAV<br>HTRGLDFACDIYIWAPLAGTCGVLLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEED<br>GCSCRFPEEEEEGGCELRVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDRRGRDPE<br>MGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRRGKGDGLYQGLSTATKDTYDAL<br>HMQALPPR  |
| SEQ ID<br>NO:<br>1541 | M16<br>(ScFv<br>domain)                                         | EVQLVESGGGLVQPGRSLRLSCAASGFTFDDYAMHWVRQAPGKGLEWVSGISWNSGSTGY<br>ADSVKGRFTISRDNANKNSLYLQMNSLRAEDTALYYCAKDSSSWYGGGSAFDIWGQGTMTV<br>VSSGGGSGGGGSGGGGSSSELTQEPAVSVALGQTVRITCQGDLSRSYYASWYQQKPGQA<br>PVLVIFGRSRRPSGIPDRFSGSSSGNTASLIITGAQAEDEADYYCNSRDNTANHYVFGTG<br>TKLTVL                                                                                                                                                                                                                                                                                  |
| SEQ ID<br>NO:<br>1542 | M16<br>>XS87-<br>99RD<br>(M16<br>XS87-<br>99RD<br>NT<br>127554) | <u><b>MALPVTALLPLALLHAARP</b></u> EVQLVESGGGLVQPGRSLRLSCAASGFTFDDYAMHWVRQ<br>APGKGLEWVSGISWNSGSTGYADSVKGRFTISRDNANKNSLYLQMNSLRAEDTALYYCAKD<br>SSSWYGGGSAFDIWGQGTMTVSSGGGSGGGGSGGGGSSSELTQEPAVSVALGQTVRIT<br>CQGDLSRSYYASWYQQKPGQAPVLVIFGRSRRPSGIPDRFSGSSSGNTASLIITGAQAEDE<br>EADYYCNSRDNTANHYVFGTGTKLTVLTTPAPRPPTPAPTIASQPLSLRPEACRPAAGG<br>AVHTRGLDFACDIYIWAPLAGTCGVLLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQE<br>EDGCSCRFPEEEEEGGCELRVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDRRGRD<br>PEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRRGKGDGLYQGLSTATKDTYD<br>ALHMQALPPR |
| SEQ ID<br>NO:<br>1543 | M17<br>(ScFv<br>domain)                                         | EVQLVESGGGLVQPGRSLRLSCAASGFTFDDYAMHWVRQAPGKGLEWVSGISWNSGSTGY<br>ADSVKGRFTISRDNANKNSLYLQMNSLRAEDTALYYCAKDSSSWYGGGSAFDIWGQGTMTV<br>VSSGGGSGGGGSGGGGSSSELTQDPAVSVALGQTVRITCQGDLSRSYYASWYQQKPGQA<br>PVLVIYGKNNRPSGIPDRFSGSSSGNTASLTITGAQAEDEADYYCNSRGSSGNHYVFGTG<br>TKVTVL                                                                                                                                                                                                                                                                                  |
| SEQ ID<br>NO:<br>1544 | M17<br>>NS89-<br>94MD<br>(M17<br>NS89-<br>94MD<br>NT<br>127555) | <u><b>MALPVTALLPLALLHAARP</b></u> EVQLVESGGGLVQPGRSLRLSCAASGFTFDDYAMHWVRQ<br>APGKGLEWVSGISWNSGSTGYADSVKGRFTISRDNANKNSLYLQMNSLRAEDTALYYCAKD<br>SSSWYGGGSAFDIWGQGTMTVSSGGGSGGGGSGGGGSSSELTQDPAVSVALGQTVRIT<br>CQGDLSRSYYASWYQQKPGQAPVLVIYGKNNRPSGIPDRFSGSSSGNTASLTITGAQAEDE<br>EADYYCNSRGSSGNHYVFGTGTGTVTLTTPAPRPPTPAPTIASQPLSLRPEACRPAAGG<br>AVHTRGLDFACDIYIWAPLAGTCGVLLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQE<br>EDGCSCRFPEEEEEGGCELRVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDRRGRD<br>PEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRRGKGDGLYQGLSTATKDTYD<br>ALHMQALPPR |
| SEQ ID<br>NO:<br>1545 | M18<br>(ScFv<br>domain)                                         | <u>QVQLVQSGGGLVQPGSLRLSCAASGFTFSSYWMHWVRQAPGKGLVWVSRINSDGSSTSY</u><br><u>ADSVKGRFTISRDNANKNTLYLQMNSLRAEDTAVYYCVRTGWVGSYYYYMDVWGKTTTVTV</u><br>SSGGGSGGGGSGGGGSGGGGSEIVLTQSPGTLSPGERATLSCRASQSVSSNYLAWYQ<br>QKPGQPPRLLIYDVSTRATGIPARFSGGSGTDFTLTISSELEDFAVYYCQQRSNWPPW<br>TFGQGTKVEIK                                                                                                                                                                                                                                                                    |

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| SEQ ID NO: 1546 | M18<br>>DS90-09HD (M18 DS90-09HD R003-A05 127556)        | <u><b>MALPVTALLLPALALLHAARP</b></u> QVQLVQSGGGLVQPGGSLRLSCAASGFTFSSYWMHWVRQAPGKGLVWVSRINSDGSSTSYADSVKGRFTISRDNANKNTLYLQMNSLRAEDTAVYYCVRTGWVGSYYYYMDVWGKTTTVTVSSGGGSGGGGSGGGGSGGGGSEIVLTQSPGTLISLSPGERATLSCRASQSVSSNYLAWYQQKPGQPPRLLIYDVSTRATGIPARFSGGGSGTDFTLTISLLEPEDFAVYYCQQRSNWPPWTFGQGTKVEIKTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYKQGQNLQYLNELNLGRREEYDVLDRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRRGKGHGDLGYQGLSTATKDTYDALHMQALPPR |
| SEQ ID NO: 1547 | M19 (ScFv domain)                                        | QVQLVQSGGGVVPGRSLRLSCAASGFTFSSYGMHWVRQAPGKGLEWVAVISYDGSNKYYADSVKGRFTISRDNANKNTLYLQMNSLRAEDTAVYYCAKGYSSRYYYGMDVWGQGTITVTVSSGGGSGGGGSGGGGSGGGGSEIVMTQSPATLSLSPGERAILSCRASQSVYTKYLGWYQQKPGQAPRLLIYDASTRATGIPDRFSGSGSGTDFTLTINRLEPEDFAVYYCQHYGGSPPLITFGQGTKVDIK                                                                                                                                                                                                                                                                       |
| SEQ ID NO: 1548 | M19<br>>TS92-04BD (M19 TS92-04BD R003-C06 127557)        | <u><b>MALPVTALLLPALALLHAARP</b></u> QVQLVQSGGGVVPGRSLRLSCAASGFTFSSYGMHWVRQAPGKGLEWVAVISYDGSNKYYADSVKGRFTISRDNANKNTLYLQMNSLRAEDTAVYYCAKGYSSRYYYGMDVWGQGTITVTVSSGGGSGGGGSGGGGSGGGGSEIVMTQSPATLSLSPGERAILSCRASQSVYTKYLGWYQQKPGQAPRLLIYDASTRATGIPDRFSGSGSGTDFTLTINRLEPEDFAVYYCQHYGGSPPLITFGQGTKVDIKTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYKQGQNLQYLNELNLGRREEYDVLDRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRRGKGHGDLGYQGLSTATKDTYDALHMQALPPR  |
| SEQ ID NO: 1549 | M20 (ScFv domain)                                        | QVQLVQSGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSAISGSGGSTYYADSVKGRFTISRDNANKNTLYLQMNSLRAEDTAVYYCAKREAAAGHDWYFDLWGRGTLTVTVSSGGGSGGGGSGGGGSGGGGSDIRVTQSPSSLSASVGDRTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSYSLPLTFGQGTKVEIK                                                                                                                                                                                                                                                                         |
| SEQ ID NO: 1550 | M20 (full)<br>>JS93-08WD (M20 JS93-08WD R003-E07 127558) | <u><b>MALPVTALLLPALALLHAARP</b></u> QVQLVQSGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSAISGSGGSTYYADSVKGRFTISRDNANKNTLYLQMNSLRAEDTAVYYCAKREAAAGHDWYFDLWGRGTLTVTVSSGGGSGGGGSGGGGSGGGGSDIRVTQSPSSLSASVGDRTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSYSLPLTFGQGTKVEIKTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYKQGQNLQYLNELNLGRREEYDVLDRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRRGKGHGDLGYQGLSTATKDTYDALHMQALPPR    |
| SEQ ID NO: 1551 | M21 (ScFv domain)                                        | QVQLVQSWAEVKKPGASVKVSCASGYTFTSYMHWVRQAPGQGLEWMGIINPSSGGSTSYAQKFQGRVTMTRDTSTSTVYMELSNLRSEDVAVYYCARSPRVTITGYFDYWGQGTITVTVSSGGGSGGGGSGGGGSGGGGSDIQLTQSPSTLSASVGDRTITCRASQSISSWLAWYQQKPGKAPKLLIYKASSLESQSGVPSRFSGSGSGTEFTTLTISSLQPDFFATYYCQQYSSYPLTFGGGTRLEIK                                                                                                                                                                                                                                                                         |
| SEQ ID NO: 1552 | M21 (full CAR)                                           | <u><b>MALPVTALLLPALALLHAARP</b></u> QVQLVQSWAEVKKPGASVKVSCASGYTFTSYMHWVRQAPGQGLEWMGIINPSSGGSTSYAQKFQGRVTMTRDTSTSTVYMELSNLRSEDVAVYYCARSPRVTITGYFDYWGQGTITVTVSSGGGSGGGGSGGGGSGGGGSDIQLTQSPSTLSASVGDRTITCRASQSISSWLAWYQQKPGKAPKLLIYKASSLESQSGVPSRFSGSGSGTEFTTLTISSLQPDFFATYYCQQYSSYPLTFGGGTRLEIKTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYKQGQNLQYLNELNLGRREEYDVLDRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRRGKGHGDLGYQGLSTATKDTYDALHMQALPPR    |

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| SEQ ID<br>NO:<br>1553 | M22<br>(ScFv<br>domain) | QVQLVQSGAEVRRPGASVKISCRASGDTSTRHYIHWLRQAPGQGPEWMGVINPTTGPATG<br>SPAYAQMLQGRVTMTRDTSTRTVYMELRSLRFEDTAVYYCARSVVGRSAPYYFDYWGQGT<br>LVTVSSGGGGSGGGGSGGGGSDIQMTQSPSSLSASVGDRTITCRASQGISDYSA<br>WYQQKPGKAPKLLIYA <u>ASTLQSGVPSRFSGSGSGTDFTLTISY</u> LQSEDFATYYC <u>QQYYSY</u><br>PLTFGGGTKVDIK                                                                                                                                                                                                                                                                                              |
| SEQ ID<br>NO:<br>1554 | M22 (full<br>CAR)       | <u><b>MALPVTALLPLALLHAARP</b></u> QVQLVQSGAEVRRPGASVKISCRASGDTSTRHYIHWLRQ<br>APGQGPEWMGVINPTTGPATGSPAYAQMLQGRVTMTRDTSTRTVYMELRSLRFEDTAVYY<br>CARSVVGRSAPYYFDYWGQGT LVTVSSGGGGSGGGGSGGGGSDIQMTQSPSSLSA<br>SVGDRTITCRASQGISDYSAWYQQKPGKAPKLLIYA <u>ASTLQSGVPSRFSGSGSGTDFTL</u><br><u>TISY</u> LQSEDFATYYC <u>QQYYSY</u> PLTFGGGTKVDIKTTTPAPRPPTPAPTIASQPLSLRPEA<br>CRPA<br>AGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQT<br>TQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYKQGQNQLYNELNLGRREEDVLDKRR<br>GRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKD<br>TYDALHMQALPPR |
| SEQ ID<br>NO:<br>1555 | M23<br>(ScFv<br>domain) | QVQLQQSGAEVKKPGASVKVSKASGYTFTNYYMHWRQAPGQGLEWMGIINPSGGYTTY<br>AQKFQGRLTMTTRDTSTSTVYMELSSLRSED TAVYYCARIRSCGGDCYYFDNWGQGT LVT<br>SSGGGGSGGGGSGGGGSDIQLTQSPSTLSASVGDRTITCRASENVNIWLAWYQQ<br>KPGKAPKLLIYKSSSLASGVPSRFSGSGSGAEFTLTISLQPD F ATYYC <u>QQYQSY</u> PLTF<br>GGGTKVDIK                                                                                                                                                                                                                                                                                                          |
| SEQ ID<br>NO:<br>1556 | M23 (full<br>CAR)       | <u><b>MALPVTALLPLALLHAARP</b></u> QVQLQQSGAEVKKPGASVKVSKASGYTFTNYYMHWRQ<br>APGQGLEWMGIINPSGGYTTYAQKFQGRLTMTTRDTSTSTVYMELSSLRSED TAVYYCARI<br>RSCGGDCYYFDNWGQGT LVTVSSGGGGSGGGGSGGGGSDIQLTQSPSTLSASVGD<br>RVTITCRASENVNIWLAWYQQKPGKAPKLLIYKSSSLASGVPSRFSGSGSGAEFTLTISL<br>LQPD F ATYYC <u>QQYQSY</u> PLTFGGGTKVDIKTTTPAPRPPTPAPTIASQPLSLRPEACRPA<br>AGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQT<br>TQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYKQGQNQLYNELNLGRREEDVLDKRR<br>GRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKD<br>TYDALHMQALPPR                       |
| SEQ ID<br>NO:<br>1557 | M24<br>(ScFv<br>domain) | QITLKESGPALVKPTQTLTLTCTFSGFSLSLSTAGVHVGWIRQPPGKALEWLALISWADDDR<br>YRPSLRSLRDITRVTSKDQVVLMTNMQPEDTATYYCALQGF DGYEANWGPGLTVTVSSG<br>GGGGGGGGGGGGSDIVMTQSPSSLSASAGDRVTITCRASRGISSALAWYQQKPG<br>KPPKLLIYDASSLESGVPSRFSGSGSGTDFTLTIDSLEPEDFATYYC <u>QQSYSTPWTF</u> GGQ<br>TKVDIK                                                                                                                                                                                                                                                                                                           |
| SEQ ID<br>NO:<br>1558 | M24 (full<br>CAR)       | <u><b>MALPVTALLPLALLHAARP</b></u> QITLKESGPALVKPTQTLTLTCTFSGFSLSLSTAGVHVGI<br>RQPPGKALEWLALISWADDKRYRPSLRSLRDITRVTSKDQVVLMTNMQPEDTATYYCAL<br>QGF DGYEANWGPGLTVTVSSGGGGSGGGGSGGGGSDIVMTQSPSSLSASAGDRVT<br>ITCRASRGISSALAWYQQKPGKPPKLLIYDASSLESGVPSRFSGSGSGTDFTLTIDSLEP<br>EDFATYYC <u>QQSYSTPWTF</u> GGQTKVDIKTTTPAPRPPTPAPTIASQPLSLRPEACRPA<br>AGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQT<br>TQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYKQGQNQLYNELNLGRREEDVLDKRR<br>GRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKD<br>TYDALHMQALPPR                           |
| SEQ ID<br>NO:<br>1559 | Ss1 (scFv<br>domain)    | QVQLQQSGPELEKPGASVKISCKASGYSFTGYTMNWVKQSHGKSLEWIGLITPYNGASS<br>YNQKFRGKATLTVDKSSSTAYMDLLSLTSEDSAVYFCARGGYDGRGFDYWGQGT TVTVS<br>SGGGGGGGGGGGSDIELTQSPAIMASASPGEKVTMTCSASSSVSYMHWYQQKSGTSP<br>KRWIYDTSKLASGVPGRFSGSGSGNSYSLTISSVEAEDDATYYC <u>QQWSGY</u> PLTFGAGTK<br>LEI                                                                                                                                                                                                                                                                                                               |
| SEQ ID<br>NO:<br>1560 | Ss1 (full<br>CAR)       | <u><b>MALPVTALLPLALLHAARP</b></u> QVQLQQSGPELEKPGASVKISCKASGYSFTGYTMNWVK<br>QSHGKSLEWIGLITPYNGASSYNQKFRGKATLTVDKSSSTAYMDLLSLTSEDSAVYFCA<br>RGGYDGRGFDYWGQGT TVTVSSGGGGSGGGGSDIELTQSPAIMASASPGEKVTMT<br>CSASSSVSYMHWYQQKSGTSPKRWIYDTSKLASGVPGRFSGSGSGNSYSLTISSVEAED                                                                                                                                                                                                                                                                                                                    |

|  |  |                                                                                                                                                              |
|--|--|--------------------------------------------------------------------------------------------------------------------------------------------------------------|
|  |  | DATYYCQQWSGYPLTFGAGTKLEITTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAV<br>HTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEE<br>DGCSCRFPEEEEGGCELRVKFSRSADAPA |
|--|--|--------------------------------------------------------------------------------------------------------------------------------------------------------------|

**BCMA CAR**

In other embodiments, the CAR-expressing cells can specifically bind to BCMA, e.g., can include a CAR molecule, or an antigen binding domain according to Table 1 or 16, SEQ ID NO: 271 or SEQ ID NO: 273 of WO2016/014565, incorporated herein by reference. The amino acid and nucleotide sequences encoding the BCMA CAR molecules and antigen binding domains (*e.g.*, including one, two, three VH CDRs; and one, two, three VL CDRs according to Kabat or Chothia), as specified in WO2016/014565, are provided in Tables 15-18 herein.

- 10 **Table 15.** Heavy Chain Variable Domain CDRs according to the Kabat numbering scheme (Kabat et al. (1991), "Sequences of Proteins of Immunological Interest," 5<sup>th</sup> Ed. Public Health Service, National Institutes of Health, Bethesda, MD)

| Candidate | HCDR1 | SEQ ID NO | HCDR2              | SEQ ID NO | HCDR3           | SEQ ID NO |
|-----------|-------|-----------|--------------------|-----------|-----------------|-----------|
| 139109    | NHGMS | 412       | GIVYSGSTYYAA SVKG  | 431       | HGGESDV         | 453       |
| 139103    | NYAMS | 413       | GISRSGENTYYA DSVKG | 432       | SPAHYYGGMD V    | 454       |
| 139105    | DYAMH | 414       | GISWNSGSIGYA DSVKG | 433       | HSFLAY          | 455       |
| 139111    | NHGMS | 412       | GIVYSGSTYYAA SVKG  | 431       | HGGESDV         | 453       |
| 139100    | NFGIN | 415       | WINPKNNNTNY AQKFQG | 434       | GPYYYQSYMD V    | 456       |
| 139101    | SDAMT | 416       | VISGSGGTYYA DSVKG  | 435       | LDSSGYYYARG PRY | 457       |
| 139102    | NYGIT | 417       | WISAYNGNTNY AQKFQG | 436       | GPYYYMDV        | 458       |
| 139104    | NHGMS | 412       | GIVYSGSTYYAA SVKG  | 431       | HGGESDV         | 453       |
| 139106    | NHGMS | 412       | GIVYSGSTYYAA SVKG  | 431       | HGGESDV         | 453       |
| 139107    | NHGMS | 412       | GIVYSGSTYYAA SVKG  | 431       | HGGESDV         | 453       |
| 139108    | DYYMS | 418       | YISSSGSTIYYAD SVKG | 437       | ESGDGMDV        | 459       |
| 139110    | DYYMS | 418       | YISSSGNTIYYAD SVKG | 438       | STMVREDY        | 460       |
| 139112    | NHGMS | 412       | GIVYSGSTYYAA SVKG  | 431       | HGGESDV         | 453       |

|                            |             |     |                         |     |                          |     |
|----------------------------|-------------|-----|-------------------------|-----|--------------------------|-----|
| 139113                     | NHGMS       | 412 | GIVYSGSTYYAA<br>SVKG    | 431 | HGGESDV                  | 453 |
| 139114                     | NHGMS       | 412 | GIVYSGSTYYAA<br>SVKG    | 431 | HGGESDV                  | 453 |
| 149362                     | SSYYY<br>WG | 419 | SIYYSGSAYYNP<br>SLKS    | 439 | HWQEWPAFDI               | 461 |
| 149363                     | TSGMC<br>VS | 420 | RIDWDEDKFYST<br>SLKT    | 440 | SGAGGTSATAF<br>DI        | 462 |
| 149364                     | SYSMN       | 421 | SISSSSYIYYAD<br>SVKG    | 441 | TIAAVYAFDI               | 463 |
| 149365                     | DYYMS       | 418 | YISSSGSTIYYAD<br>SVKG   | 437 | DLRGAFDI                 | 464 |
| 149366                     | SHYIH       | 422 | MINPSGGVTAYS<br>QTLQG   | 442 | EGSGSGWYFDF              | 465 |
| 149367                     | SGGYY<br>WS | 423 | YIYYSGSTYYNP<br>SLKS    | 443 | AGIAARLRGAF<br>DI        | 466 |
| 149368                     | SYAIS       | 424 | GIPIFGTANYAQ<br>KFQG    | 444 | RGGYQLLRWD<br>VGLLRSAFDI | 467 |
| 149369                     | SNSAA<br>WN | 425 | RTYYRSKWYSF<br>Y AISLKS | 445 | SSPEGLFLYWF<br>DP        | 468 |
| BCMA_EB<br>B-C1978-<br>A4  | SYAMS       | 426 | AISGSGGSTYYA<br>DSVKG   | 446 | VEGSGSLDY                | 469 |
| BCMA_EB<br>B-C1978-<br>G1  | RYPMS       | 427 | GISDSGVSTYYA<br>DSAKG   | 447 | RAGSEASDI                | 470 |
| BCMA_EB<br>B-C1979-C1      | SYAMS       | 426 | AISGSGGSTYYA<br>DSVKG   | 446 | ATYKRELRIYY<br>GMDV      | 471 |
| BCMA_EB<br>B-C1978-C7      | SYAMS       | 426 | AISGSGGSTYYA<br>DSVKG   | 446 | ATYKRELRIYY<br>GMDV      | 471 |
| BCMA_EB<br>B-C1978-<br>D10 | DYAMH       | 414 | GISWNSGSIGYA<br>DSVKG   | 433 | VGKAVPDV                 | 472 |
| BCMA_EB<br>B-C1979-<br>C12 | DYAMH       | 414 | SINWKGNSLAY<br>GDSVKG   | 448 | HQGVAYYNYA<br>MDV        | 473 |
| BCMA_EB<br>B-C1980-<br>G4  | SYAMS       | 426 | AISGSGGSTYYA<br>DSVKG   | 446 | VVRDGMDV                 | 474 |
| BCMA_EB<br>B-C1980-<br>D2  | SYAMS       | 426 | AISGSGGSTYYA<br>DSVKG   | 446 | IPQTGTFDY                | 475 |
| BCMA_EB<br>B-C1978-<br>A10 | SYAMS       | 426 | AISGSGGSTYYA<br>DSVKG   | 446 | ANYKRELRIYY<br>GMDV      | 476 |
| BCMA_EB<br>B-C1978-<br>D4  | SYAMS       | 426 | AISGSGGSTYYA<br>DSVKG   | 446 | ALVGATGAFDI              | 477 |

|                           |       |     |                       |     |                      |     |
|---------------------------|-------|-----|-----------------------|-----|----------------------|-----|
| BCMA_EB<br>B-C1980-<br>A2 | SYAMS | 426 | AISGSGGSTYYA<br>DSVKG | 446 | WFGEGFDP             | 478 |
| BCMA_EB<br>B-C1981-C3     | SYAMS | 426 | AISGSGGSTYYA<br>DSVKG | 446 | VGVDSSGYRD<br>YYGMDV | 479 |
| BCMA_EB<br>B-C1978-<br>G4 | SYAMS | 426 | AISGSGGSTYYA<br>DSVKG | 446 | MGWSSGYLGA<br>FDI    | 480 |
| A7D12.2                   | NFGMN | 428 | WINTYTGESYFA<br>DDFKG | 449 | GEIYYGYDGGF<br>AY    | 481 |
| C11D5.3                   | DYSIN | 429 | WINTETREPAYA<br>YDFRG | 450 | DYSYAMDY             | 482 |
| C12A3.2                   | HYSMN | 430 | RINTESGVPIYAD<br>DFKG | 451 | DYLYSLDF             | 483 |
| C13F12.1                  | HYSMN | 430 | RINTETGEPLYA<br>DDFKG | 452 | DYLYSCDY             | 484 |

**Table 16.** Light Chain Variable Domain CDRs according to the Kabat numbering scheme (Kabat et al. (1991), "Sequences of Proteins of Immunological Interest," 5<sup>th</sup> Ed. Public Health Service, National Institutes of Health, Bethesda, MD)

| Candidate | LCDR1                | SEQ<br>ID<br>NO | LCDR2       | SEQ<br>ID NO | LCDR3          | SEQ ID<br>NO |
|-----------|----------------------|-----------------|-------------|--------------|----------------|--------------|
| 139109    | RASQSISSYLN          | 237             | AASSLQ<br>S | 240          | QQSYSTPYT      | 545          |
| 139103    | RASQSISSSFLA         | 485             | GASRRAT     | 516          | QQYHSSPSW<br>T | 546          |
| 139105    | RSSQSLLHSNGYN<br>YLD | 486             | LGSNRA<br>S | 517          | MQALQTPY<br>T  | 547          |
| 139111    | KSSQSLLRNDGKT<br>PLY | 487             | EVSNRFS     | 518          | MQNIQFPS       | 548          |
| 139100    | RSSQSLLHSNGYN<br>YLN | 488             | LGSKRAS     | 519          | MQALQTPY<br>T  | 547          |
| 139101    | RASQSISSYLN          | 237             | GASTLAS     | 520          | QQSYKRAS       | 549          |
| 139102    | RSSQSLLYSNGYN<br>YVD | 489             | LGSNRA<br>S | 517          | MQGRQFPYS      | 550          |
| 139104    | RASQSVSSNLA          | 490             | GASTRAS     | 521          | QQYGSSLT       | 551          |
| 139106    | RASQSVSSKLA          | 491             | GASIRAT     | 522          | QQYGSSSWT      | 552          |
| 139107    | RASQSVGSTNLA         | 492             | DASNRA<br>T | 523          | QQYGSSPPW<br>T | 553          |
| 139108    | RASQSISSYLN          | 237             | AASSLQ<br>S | 240          | QQSYTLA        | 554          |
| 139110    | KSESLEHNSGKT<br>YLN  | 493             | EVSNRDS     | 524          | MQGTHWPG<br>T  | 555          |
| 139112    | QASEDINKFLN          | 494             | DASTLQ<br>T | 525          | QQYESLPLT      | 556          |

|                        |                      |     |             |     |                    |     |
|------------------------|----------------------|-----|-------------|-----|--------------------|-----|
| 139113                 | RASQSVGSNLA          | 495 | GASTRA<br>T | 526 | QQYNDWLP<br>VT     | 557 |
| 139114                 | RASQSIGSSSLA         | 496 | GASSRA<br>S | 527 | QQYAGSPPF<br>T     | 558 |
| 149362                 | KASQDIDDAMN          | 497 | SATSPVP     | 528 | LQHDFNPLT          | 559 |
| 149363                 | RASQDIYNNLA          | 498 | AANKSQ<br>S | 529 | QHYYRFPYS          | 560 |
| 149364                 | RSSQSLHSHNGYN<br>YLD | 486 | LGSNRA<br>S | 517 | MQALQTPY<br>T      | 547 |
| 149365                 | GGNNIGTKSVH          | 499 | DDSVRP<br>S | 530 | QVWDSSE<br>HV      | 561 |
| 149366                 | SGDGLSKKYVS          | 500 | RDKERP<br>S | 531 | QAWDDTTV<br>V      | 562 |
| 149367                 | RASQGIRNWLA          | 501 | AASNQ<br>S  | 532 | QKYNAPFT           | 563 |
| 149368                 | GGNNIGSKSVH          | 502 | GKNNRP<br>S | 533 | SSRDSSGDH<br>LRV   | 564 |
| 149369                 | QGDSLGNYYAT          | 503 | GTNNRP<br>S | 534 | NSRDSSGHH<br>LL    | 565 |
| BCMA_EBB-<br>C1978-A4  | RASQSVSSAYLA         | 504 | GASTRA<br>T | 526 | QHYGSSFNG<br>SSLFT | 566 |
| BCMA_EBB-<br>C1978-G1  | RASQSVSNSLA          | 505 | DASSRA<br>T | 535 | QQFGTSSGL<br>T     | 567 |
| BCMA_EBB-<br>C1979-C1  | RASQSVSSSFLA         | 506 | GASSRA<br>T | 536 | QQYHSSPSW<br>T     | 546 |
| BCMA_EBB-<br>C1978-C7  | RASQSVSTTFLA         | 507 | GSSNRA<br>T | 537 | QQYHSSPSW<br>T     | 546 |
| BCMA_EBB-<br>C1978-D10 | RASQSISSYLN          | 237 | AASSLQ<br>S | 240 | QQSYSTPYS          | 568 |
| BCMA_EBB-<br>C1979-C12 | RATQSIGSSFLA         | 508 | GASQRA<br>T | 538 | QHYESSPSW<br>T     | 569 |
| BCMA_EBB-<br>C1980-G4  | RASQSVSSSYLA         | 509 | GASSRA<br>T | 536 | QQYGSPPRF<br>T     | 570 |
| BCMA_EBB-<br>C1980-D2  | RASQSVSSSYLA         | 509 | GASSRA<br>T | 536 | QHYGSSPSW<br>T     | 571 |
| BCMA_EBB-<br>C1978-A10 | RASQRVASNYLA         | 510 | GASSRA<br>T | 536 | QHYDSSPSW<br>T     | 572 |
| BCMA_EBB-<br>C1978-D4  | RASQSLSSNFLA         | 511 | GASNW<br>AT | 539 | QYYGTSPM<br>YT     | 573 |
| BCMA_EBB-<br>C1980-A2  | RSSQSLHSHNGYN<br>YLD | 486 | LGSNRA<br>S | 517 | MQALQTPLT          | 574 |
| BCMA_EBB-<br>C1981-C3  | RASQSVSSSYLA         | 509 | GTSSRA<br>T | 540 | QHYGNSPPK<br>FT    | 575 |
| BCMA_EBB-<br>C1978-G4  | RASQSVASSFLA         | 512 | GASGRA<br>T | 541 | QHYGGSPRL<br>T     | 576 |
| A7D12.2                | RASQDVNTAVS          | 513 | SASYRY<br>T | 542 | QQHYSTPW<br>T      | 577 |
| C11D5.3                | RASESVSVIGAHLI<br>H  | 514 | LASNLE<br>T | 543 | LQSRIFPRT          | 578 |

|          |                     |     |             |     |           |     |
|----------|---------------------|-----|-------------|-----|-----------|-----|
| C12A3.2  | RASESVTILGSHLI<br>Y | 515 | LASNVT<br>T | 544 | LQSRTIPRT | 579 |
| C13F12.1 | RASESVTILGSHLI<br>Y | 515 | LASNVT<br>T | 544 | LQSRTIPRT | 579 |

**Table 17.** Amino Acid and Nucleic Acid Sequences of exemplary anti-BCMA scFv domains and BCMA CAR molecules. The amino acid sequences variable heavy chain and variable light chain sequences for each scFv is also provided.

| Name/<br>Description              | SEQ<br>ID<br>NO: | Sequence                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |
|-----------------------------------|------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>139109</b>                     |                  |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |
| <b>139109- aa<br/>ScFv domain</b> | 580              | EVQLVESGGGLVQPGGSLRLSCAVSGFALSNHGMSWVRRAPGKGLEWVSG<br>IVYSGSTYYAASVKGRFTISRDNSTNTLYLQMNSLRPEDTAIYYCSAHGG<br>ESDVWGQGTITVTVSSASGGGSGGRASGGGSDIQLTQSPSSLSASVGDR<br>VTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSG<br>TDFTLTISSLQPEDFATYYCQQSYSTPYTFGQGTKVEIK                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |
| <b>139109- nt<br/>ScFv domain</b> | 581              | GAAGTGCAATTGGTGGGAATCAGGGGGAGGACTTGTGCAGCCTGGAGGATC<br>GCTGAGACTGTGATGTGCCGTGTCCGGCTTTGCCCTGTCCAACACGGGA<br>TGTCTTGGGTCCGCCGCGCGCTGGAAAGGGCCTCGAATGGGTGTCCGGT<br>ATTGTGTACAGCGGTAGCACCTACTATGCCGCATCCGTGAAGGGGAGATT<br>CACCATCAGCCGGGACAACCTCCAGGAACACTCTGTACCTCCAATGAATT<br>CGCTGAGGCCAGAGGACACTGCCATCTACTACTGCTCCGCGCATGGCGGA<br>GAGTCCGACGTCTGGGGACAGGGGACCACCGTGACCGTGTCTAGCGCGTC<br>CGGCGGAGGCGGCAGCGGGGTCGGGCATCAGGGGGCGGCGGATCGGACA<br>TCCAGCTCACCCAGTCCCCGAGCTCGCTGTCCGCCTCCGTGGGAGATCGG<br>GTCACCATCACGTGCCGCGCCAGCCAGTCGATTTCTCCTACCTGAACCTG<br>GTACCAACAGAAGCCCGGAAAAGCCCCGAAGCTTCTCATCTACGCCGCT<br>CGAGCCTGCAGTCAGGAGTGCCTTACCGGTTCTCCGGCTCCGGTTCCGGT<br>ACTGATTTACCCCTGACCATTCTCCTCCCTGCAACCGGAGGACTTCGCTAC<br>TTACTACTGCCAGCAGTCGTACTCCACCCCTACACTTTCGGACAAGGCA<br>CCAAGGTGCAATCAAG |
| <b>139109- aa<br/>VH</b>          | 582              | EVQLVESGGGLVQPGGSLRLSCAVSGFALSNHGMSWVRRAPGKGLEWVSG<br>IVYSGSTYYAASVKGRFTISRDNSTNTLYLQMNSLRPEDTAIYYCSAHGG<br>ESDVWGQGTITVTVSS                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |
| <b>139109- aa<br/>VL</b>          | 583              | DIQLTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYA<br>ASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSYSTPYTFGQ<br>GTKVEIK                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |
| <b>139109- aa<br/>Full CAR</b>    | 584              | MALPVTALLLPLALLHAARPEVQLVESGGGLVQPGGSLRLSCAVSGFAL<br>SNHGMSWVRRAPGKGLEWVSGIVYSGSTYYAASVKGRFTISRDNSTNTLY<br>LQMNSLRPEDTAIYYCSAHGGESDVWGQGTITVTVSSASGGGSGGRASGG<br>GGSDIQLTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLL<br>IYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSYSTPYT<br>FGQGTKVEIKTTTPAPRPPTPAPTIIASQPLSLRPEACRPAAGGAVHTRGL<br>DFACDIYIWAPLAGTCGVLLSLVITLYCKRGRKLLYIFKQPFMRPVQT<br>TQEEDGCSCRFEEEEGGCELRVKFSRSADAPAYKQGQNQLYNEINLGRR<br>EEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQDKMAEAYSEIGMKGE<br>RRRGKGDGLYQGLSTATKDTYDALHMQALPPR                                                                                                                                                                                                                                                                |



|                                |     |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |
|--------------------------------|-----|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>139109- nt<br/>Full CAR</b> | 585 | ATGGCCCTCCCTGTCACCGCCCTGCTGCTTCCGCTGGCTCTTCTGCTCCA<br>CGCCGCTCGGCCCGAAGTGCAATTGGTGGAATCAGGGGGAGGACTTGTGC<br>AGCCTGGAGGATCGCTGAGACTGTATGTGCCGTGTCCGGCTTTGCCCTG<br>TCCAACCACGGGATGTCTTGGGTCCGCCGCGCGCTGGAAAGGGCCTCGA<br>ATGGGTGTCCGGTATTGTGTACAGCGGTAGCACCTACTATGCCGCATCCG<br>TGAAGGGGAGATTACCATCAGCCGGGACAACCTCCAGGAACACTCTGTAC<br>CTCCAAATGAATTCGCTGAGGCCAGAGGACACTGCCATCTACTACTGCTC<br>CGCGCATGGCGGAGAGTCCGACGTCTGGGGACAGGGGACCACCGTGACCG<br>TGTCTAGCGCGTCCGGCGGAGGCGGCAGCGGGGGTCCGGGCATCAGGGGGC<br>GCGGATCGGACATCCAGCTCACCCAGTCCCCGAGCTCGCTGTCCGCCTC<br>CGTGGGAGATCGGGTCACCATCACGTGCCGCGCCAGCCAGTCGATTTCTC<br>CCTACCTGAACTGGTACCAACAGAAGCCCGGAAAAGCCCCGAAGCTTCTC<br>ATCTACGCCGCTCGAGCCTGCAGTCAGGAGTGCCCTCACGGTTCTCCGG<br>CTCCGGTTCCGGTACTGATTTACCCCTGACCATTCTCTCCCTGCAACCGG<br>AGGACTTCGCTACTTACTACTGCCAGCAGTCGTACTCCACCCCTACACT<br>TTCGGACAAGGCACCAAGGTCGAAATCAAGACCACTACCCAGCACCGAG<br>GCCACCCACCCCGGCTCCTACCATCGCCTCCCAGCCTCTGTCCCTGCGTC<br>CGGAGGCATGTAGACCCGAGCTGGTGGGGCCGTGCATACCCGGGGTCTT<br>GACTTCGCCTGCGATATCTACATTTGGGGCCCTCTGGCTGGTACTTGCGG<br>GGTCTGTCTGCTTTCACTCGTGATCACTCTTTACTGTAAGCGCGGTGCGA<br>AGAAGCTGCTGTACATCTTTAAGCAACCCTTCATGAGGCCTGTGCAGACT<br>ACTCAAGAGGAGGACGGCTGTTTCATGCCGGTTCAGAGGAGGAGGAAGG<br>CGGCTGCGAACTGCGCGTGAAATTCAGCCGCAGCGCAGATGCTCCAGCCT<br>ACAAGCAGGGGCAGAACCAGCTCTACAACGAACTCAATCTTGGTTCGGAGA<br>GAGGAGTACGACGTGCTGGACAAGCGGAGAGGACGGGACCCAGAAATGGG<br>CGGGAAGCCGCGCAGAAAGAATCCCCAAGAGGGCCTGTACAACGAGCTCC<br>AAAAGGATAAGATGGCAGAAGCCTATAGCGAGATTGGTATGAAAGGGGAA<br>CGCAGAAGAGGCAAAGGCCACGACGGACTGTACCAGGGACTCAGCACCGC<br>CACCAAGGACACCTATGACGCTCTTCACATGCAGGCCCTGCCGCCTCGG |
|--------------------------------|-----|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|

**Table 31.** Amino Acid and Nucleic Acid Sequences of exemplary anti-BCMA scFv domains and BCMA CAR molecules . The amino acid sequences variable heavy chain and variable light chain sequences for each scFv is also provided.

| <b>Name/ Description</b> | <b>SEQ ID NO:</b> |
|--------------------------|-------------------|
| 139103                   |                   |
| 139103- aa ScFv domain   | 586               |
| 139103- nt ScFv domain   | 587               |
| 139103- aa VH            | 588               |
| 139103- aa VL            | 589               |
| 139103- aa Full CAR      | 590               |
| 139103- nt Full CAR      | 591               |
| 139105                   |                   |
| 139105- aa ScFv domain   | 592               |
| 139105- nt ScFv domain   | 593               |
| 139105- aa VH            | 594               |
| 139105- aa VL            | 595               |
| 139105- aa Full CAR      | 596               |
| 139105- nt Full CAR      | 597               |

|                        |     |
|------------------------|-----|
| 139111                 |     |
| 139111- aa ScFv domain | 598 |
| 139111- nt ScFv domain | 599 |
| 139111- aa VH          | 600 |
| 139111- aa VL          | 601 |
| 139111- aa Full CAR    | 602 |
| 139111- nt Full CAR    | 603 |
| 139100                 |     |
| 139100- aa ScFv domain | 604 |
| 139100- nt ScFv domain | 605 |
| 139100- aa VH          | 606 |
| 139100- aa VL          | 607 |
| 139100- aa Full CAR    | 608 |
| 139100- nt Full CAR    | 609 |
| 139101                 |     |
| 139101- aa ScFv domain | 610 |
| 139101- nt ScFv domain | 611 |
| 139101- aa VH          | 612 |
| 139101- aa VL          | 613 |
| 139101- aa Full CAR    | 614 |
| 139101- nt Full CAR    | 615 |
| 139102                 |     |
| 139102- aa ScFv domain | 616 |
| 139102- nt ScFv domain | 617 |
| 139102- aa VH          | 618 |
| 139102- aa VL          | 619 |
| 139102- aa Full CAR    | 620 |
| 139102- nt Full CAR    | 621 |
| 139104                 |     |
| 139104- aa ScFv domain | 622 |
| 139104- nt ScFv domain | 623 |
| 139104- aa VH          | 624 |
| 139104- aa VL          | 625 |
| 139104- aa Full CAR    | 626 |
| 139104- nt Full CAR    | 627 |
| 139106                 |     |
| 139106- aa ScFv domain | 628 |
| 139106- nt ScFv domain | 629 |
| 139106- aa VH          | 630 |
| 139106- aa VL          | 631 |
| 139106- aa Full CAR    | 632 |
| 139106- nt Full CAR    | 633 |
| 139107                 |     |
| 139107- aa ScFv domain | 634 |
| 139107- nt ScFv domain | 635 |
| 139107- aa VH          | 636 |
| 139107- aa VL          | 637 |
| 139107- aa Full CAR    | 638 |
| 139107- nt Full CAR    | 639 |

|                        |     |
|------------------------|-----|
| 139108                 |     |
| 139108- aa ScFv domain | 640 |
| 139108- nt ScFv domain | 641 |
| 139108- aa VH          | 642 |
| 139108- aa VL          | 643 |
| 139108- aa Full CAR    | 644 |
| 139108- nt Full CAR    | 645 |
| 139110                 |     |
| 139110- aa ScFv domain | 646 |
| 139110- nt ScFv domain | 647 |
| 139110- aa VH          | 648 |
| 139110- aa VL          | 649 |
| 139110- aa Full CAR    | 650 |
| 139110- nt Full CAR    | 651 |
| 139112                 |     |
| 139112- aa ScFv domain | 652 |
| 139112- nt ScFv domain | 653 |
| 139112- aa VH          | 654 |
| 139112- aa VL          | 655 |
| 139112- aa Full CAR    | 656 |
| 139112- nt Full CAR    | 657 |
| 139113                 |     |
| 139113- aa ScFv domain | 658 |
| 139113- nt ScFv domain | 659 |
| 139113- aa VH          | 630 |
| 139113- aa VL          | 660 |
| 139113- aa Full CAR    | 661 |
| 139113- nt Full CAR    | 662 |
| 139114                 |     |
| 139114- aa ScFv domain | 663 |
| 139114- nt ScFv domain | 664 |
| 139114- aa VH          | 582 |
| 139114- aa VL          | 665 |
| 139114- aa Full CAR    | 666 |
| 139114- nt Full CAR    | 667 |
| 149362                 |     |
| 149362-aa ScFv domain  | 668 |
| 149362-nt ScFv domain  | 669 |
| 149362-aa VH           | 670 |
| 149362-aa VL           | 671 |
| 149362-aa Full CAR     | 672 |
| 149362-nt Full CAR     | 673 |
| 149363                 |     |
| 149363-aa ScFv domain  | 674 |
| 149363-nt ScFv domain  | 675 |
| 149363-aa VH           | 676 |
| 149363-aa VL           | 677 |
| 149363-aa Full CAR     | 678 |

|                                    |     |
|------------------------------------|-----|
| 149363-nt Full CAR                 | 679 |
| 149364                             |     |
| 149364-aa ScFv domain              | 680 |
| 149364-nt ScFv domain              | 681 |
| 149364-aa VH                       | 682 |
| 149364-aa VL                       | 683 |
| 149364-aa Full CAR                 | 684 |
| 149364-nt Full CAR                 | 685 |
| 149365                             |     |
| 149365-aa ScFv domain              | 686 |
| 149365-nt ScFv domain              | 687 |
| 149365-aa VH                       | 688 |
| 149365-aa VL                       | 689 |
| 149365-aa Full CAR                 | 690 |
| 149365-nt Full CAR                 | 691 |
| 149366                             |     |
| 149366-aa ScFv domain              | 692 |
| 149366-nt ScFv domain              | 693 |
| 149366-aa VH                       | 694 |
| 149366-aa VL                       | 695 |
| 149366-aa Full CAR                 | 696 |
| 149366-nt Full CAR                 | 697 |
| 149367                             |     |
| 149367-aa ScFv domain              | 698 |
| 149367-nt ScFv domain              | 699 |
| 149367-aa VH                       | 700 |
| 149367-aa VL                       | 701 |
| 149367-aa Full CAR                 | 702 |
| 149367-nt Full CAR                 | 703 |
| 149368                             |     |
| 149368-aa ScFv domain              | 704 |
| 149368-nt ScFv domain              | 705 |
| 149368-aa VH                       | 706 |
| 149368-aa VL                       | 707 |
| 149368-aa Full CAR                 | 708 |
| 149368-nt Full CAR                 | 709 |
| 149369                             |     |
| 149369-aa ScFv domain              | 710 |
| 149369-nt ScFv domain              | 711 |
| 149369-aa VH                       | 712 |
| 149369-aa VL                       | 713 |
| 149369-aa Full CAR                 | 714 |
| 149369-nt Full CAR                 | 715 |
| BCMA_EBB-C1978-A4                  |     |
| BCMA_EBB-C1978-A4 – aa ScFv domain | 716 |
| BCMA_EBB-C1978-A4 – nt ScFv domain | 717 |
| BCMA_EBB-C1978-A4 – aa VH          | 718 |
| BCMA_EBB-C1978-A4 – aa VL          | 719 |
| BCMA_EBB-C1978-A4 – aa Full CART   | 720 |

|                                     |     |
|-------------------------------------|-----|
| BCMA_EBB-C1978-A4 – nt Full CART    | 721 |
| BCMA_EBB-C1978-G1                   |     |
| BCMA_EBB-C1978-G1 – aa ScFv domain  | 722 |
| BCMA_EBB-C1978-G1 – nt ScFv domain  | 723 |
| BCMA_EBB-C1978-G1 – aa VH           | 724 |
| BCMA_EBB-C1978-G1 – aa VL           | 725 |
| BCMA_EBB-C1978-G1 – aa Full CART    | 726 |
| BCMA_EBB-C1978-G1 – nt Full CART    | 727 |
| BCMA_EBB-C1979-C1                   |     |
| BCMA_EBB-C1979-C1 – aa ScFv domain  | 728 |
| BCMA_EBB-C1979-C1 – nt ScFv domain  | 729 |
| BCMA_EBB-C1979-C1 – aa VH           | 730 |
| BCMA_EBB-C1979-C1 – aa VL           | 731 |
| BCMA_EBB-C1979-C1 – aa Full CART    | 732 |
| BCMA_EBB-C1979-C1 – nt Full CART    | 733 |
| BCMA_EBB-C1978-C7                   |     |
| BCMA_EBB-C1978-C7 – aa ScFv domain  | 734 |
| BCMA_EBB-C1978-C7 – nt ScFv domain  | 735 |
| BCMA_EBB-C1978-C7 – aa VH           | 736 |
| BCMA_EBB-C1978-C7 – aa VL           | 737 |
| BCMA_EBB-C1978-C7 – aa Full CART    | 738 |
| BCMA_EBB-C1978-C7 – nt Full CART    | 739 |
| BCMA_EBB-C1978-D10                  |     |
| BCMA_EBB-C1978-D10 – aa ScFv domain | 740 |
| BCMA_EBB-C1978-D10- nt ScFv domain  | 741 |
| BCMA_EBB-C1978-D10 – aa VH          | 742 |
| BCMA_EBB-C1978-D10- aa VL           | 743 |
| BCMA_EBB-C1978-D10 – aa Full CART   | 744 |
| BCMA_EBB-C1978-D10 – nt Full CART   | 745 |
| BCMA_EBB-C1979-C12                  |     |
| BCMA_EBB-C1979-C12- aa ScFv domain  | 746 |
| BCMA_EBB-C1979-C12 – nt ScFv domain | 747 |
| BCMA_EBB-C1979-C12 – aa VH          | 748 |
| BCMA_EBB-C1979-C12 – aa VL          | 749 |
| BCMA_EBB-C1979-C12 – aa Full CART   | 750 |
| BCMA_EBB-C1979-C12 – nt Full CART   | 751 |
| BCMA_EBB-C1980-G4                   |     |
| BCMA_EBB- C1980-G4- aa ScFv domain  | 752 |
| BCMA_EBB- C1980-G4- nt ScFv domain  | 753 |
| BCMA_EBB- C1980-G4- aa VH           | 754 |
| BCMA_EBB- C1980-G4- aa VL           | 755 |
| BCMA_EBB- C1980-G4- aa Full CART    | 756 |
| BCMA_EBB- C1980-G4- nt Full CART    | 757 |
| BCMA_EBB-C1980-D2                   |     |
| BCMA_EBB- C1980-D2- aa ScFv domain  | 758 |
| BCMA_EBB- C1980-D2- nt ScFv domain  | 759 |
| BCMA_EBB- C1980-D2- aa VH           | 760 |
| BCMA_EBB- C1980-D2- aa VL           | 761 |
| BCMA_EBB- C1980-D2- aa Full CART    | 762 |

|                                     |     |
|-------------------------------------|-----|
| BCMA_EBB- C1980-D2- nt Full CART    | 763 |
| BCMA_EBB-C1978-A10                  |     |
| BCMA_EBB- C1978-A10- aa ScFv domain | 764 |
| BCMA_EBB- C1978-A10- nt ScFv domain | 765 |
| BCMA_EBB- C1978-A10- aa VH          | 766 |
| BCMA_EBB- C1978-A10- aa VL          | 767 |
| BCMA_EBB- C1978-A10- aa Full CART   | 768 |
| BCMA_EBB- C1978-A10- nt Full CART   | 769 |
| BCMA_EBB-C1978-D4                   |     |
| BCMA_EBB- C1978-D4- aa ScFv domain  | 770 |
| BCMA_EBB- C1978-D4- nt ScFv domain  | 771 |
| BCMA_EBB- C1978-D4- aa VH           | 772 |
| BCMA_EBB- C1978-D4- aa VL           | 773 |
| BCMA_EBB- C1978-D4- aa Full CART    | 774 |
| BCMA_EBB- C1978-D4- nt Full CART    | 775 |
| BCMA_EBB-C1980-A2                   |     |
| BCMA_EBB- C1980-A2- aa ScFv domain  | 776 |
| BCMA_EBB- C1980-A2- nt ScFv domain  | 777 |
| BCMA_EBB- C1980-A2- aa VH           | 778 |
| BCMA_EBB- C1980-A2- aa VL           | 779 |
| BCMA_EBB- C1980-A2- aa Full CART    | 780 |
| BCMA_EBB- C1980-A2- nt Full CART    | 781 |
| BCMA_EBB-C1981-C3                   |     |
| BCMA_EBB- C1981-C3- aa ScFv domain  | 782 |
| BCMA_EBB- C1981-C3- nt ScFv domain  | 783 |
| BCMA_EBB- C1981-C3- aa VH           | 784 |
| BCMA_EBB- C1981-C3- aa VL           | 785 |
| BCMA_EBB- C1981-C3- aa Full CART    | 786 |
| BCMA_EBB- C1981-C3- nt Full CART    | 787 |
| BCMA_EBB-C1978-G4                   |     |
| BCMA_EBB- C1978-G4- aa ScFv domain  | 788 |
| BCMA_EBB- C1978-G4- nt ScFv domain  | 789 |
| BCMA_EBB- C1978-G4- aa VH           | 790 |
| BCMA_EBB- C1978-G4- aa VL           | 791 |
| BCMA_EBB- C1978-G4- aa Full CART    | 792 |
| BCMA_EBB- C1978-G4- nt Full CART    | 793 |

**Table 18.** Amino acid sequences of exemplary BCMA binding domains

| SEQ ID NO      | Description                   |
|----------------|-------------------------------|
| ER26           |                               |
| SEQ ID NO: 794 | J6M0 VH                       |
| SEQ ID NO: 795 | J6M0 VL                       |
| SEQ ID NO: 796 | Anti-BCMA heavy chain of ER26 |
| SEQ ID NO: 797 | Anti-BCMA light chain of ER26 |
| BQ76           |                               |
| SEQ ID NO: 798 | 17A5 VH                       |
| SEQ ID NO: 799 | 17A5 VL                       |

|                |                               |
|----------------|-------------------------------|
| SEQ ID NO: 800 | Anti-BCMA heavy chain of BQ76 |
| SEQ ID NO: 801 | Anti-BCMA light chain of BQ76 |
| BU76           |                               |
| SEQ ID NO: 802 | C11D5 VH                      |
| SEQ ID NO: 803 | C11D5 VL                      |
| SEQ ID NO: 804 | Anti-BCMA heavy chain of BU76 |
| SEQ ID NO: 805 | Anti-BCMA light chain of BU76 |
| EE11           |                               |
| SEQ ID NO: 806 | 83A10 VH                      |
| SEQ ID NO: 807 | 83A10 VL                      |
| SEQ ID NO: 808 | Anti-BCMA scFv-Fc of EE11     |
| EM90           |                               |
| SEQ ID NO: 809 | Comment light chain of EM90   |
| SEQ ID NO: 810 | Anti-BCMA heavy chain of EM90 |

Additional exemplary BCMA-targeting sequences that can be used in the anti-BCMA CAR constructs are disclosed in WO 2017/021450, WO 2017/011804, WO 2017/025038, WO 2016/090327, WO 2016/130598, WO 2016/210293, WO 2016/090320, WO 2016/014789, WO 2016/094304, WO 2016/154055, WO 2015/166073, WO 2015/188119, WO 2015/158671, US 9,243,058, US 8,920,776, US 9,273,141, US 7,083,785, US 9,034,324, US 2007/0049735, US 2015/0284467, US 2015/0051266, US 2015/0344844, US 2016/0131655, US 2016/0297884, US 2016/0297885, US 2017/0051308, US 2017/0051252, US 2017/0051252, WO 2016/020332, WO 2016/087531, WO 2016/079177, WO 2015/172800, WO 2017/008169, US 9,340,621, US 2013/0273055, US 2016/0176973, US 2015/0368351, US 2017/0051068, US 2016/0368988, US 2015/0232557, herein incorporated by reference in their entireties.

In embodiments, additional exemplary BCMA CAR constructs are generated using the VH and VL sequences from PCT Publication WO2012/0163805 (the contents of which are hereby incorporated by reference in its entirety). Exemplary BCMA CAR constructs and their corresponding DNA sequences are shown in Table 17.

#### CD22 CAR

In other embodiments, the CAR-expressing cells can specifically bind to CD22, *e.g.*, can include a CAR molecule, or an antigen binding domain (*e.g.*, a humanized antigen binding domain) according to WO2016/164731, incorporated herein by reference.

In embodiments, the CAR molecule comprises an antigen binding domain that binds specifically to CD22 (CD22 CAR). In one embodiment, the antigen binding domain targets human CD22. In one embodiment, the antigen binding domain includes a single chain Fv sequence as described herein.

The sequences of human CD22 CAR are provided below. In some embodiments, a human CD22 CAR is CAR22-65.

Human CD22 CAR scFv sequence (VH-(G4S)3-VL)

EVQLQQSGPGLVKPSQTLSTLCAISGDSMLSNSDTWNWIRQSPSRGLEWLGRTYHRSTWYDDYASSVRGR  
 5 VSINVDTSKNQYSLQLNAVTPEDTGVYYCARVRLQDGNWSDAFDVWGQGTMTVSSGGGGSGGGGSGGG  
 GSQSALTQPASASGSPGQSVTISCTGTSSDVGGYNYVSWYQQHPGKAPKLMYDVSNRPSGVSNRFSGSK  
 SGNTASLTISGLQAEDEADYYCSSYTSSSTLYVFGTGTQLTVL (SEQ ID NO: 811)

Human CD22 CAR heavy chain variable region

EVQLQQSGPGLVKPSQTLSTLCAISGDSMLSNSDTWNWIRQSPSRGLEWLGRTYHRSTWYDDYA  
 10 SSVRGRVSINVDTSKNQYSLQLNAVTPEDTGVYYCARVRLQDGNWSDAFDVWGQGTMTVVS  
 S (SEQ ID NO: 812)

Human CD22 CAR light chain variable region

QSALTQPASASGSPGQSVTISCTGTSSDVGGYNYVSWYQQHPGKAPKLMYDVSNRPSGVSNRF  
 15 SGSKSGNTASLTISGLQAEDEADYYCSSYTSSSTLYVFGTGTQLTVL (SEQ ID NO: 813)

**Table 19.** Heavy Chain Variable Domain CDRs of CD22 CAR (CAR22-65)

| Candidate         | HCDR1        | SEQ ID NO: | HCDR2               | SEQ ID NO: | HCDR3          | SEQ ID NO: |
|-------------------|--------------|------------|---------------------|------------|----------------|------------|
| CAR22-65 Combined | GDSMLSNSDTWN | 814        | RTYHRSTWYDDYAS SVRG | 816        | VRLQDGNWSDAFDV | 817        |
| CAR22-65 Kabat    | SNSDTWN      | 815        | RTYHRSTWYDDYAS SVRG | 816        | VRLQDGNWSDAFDV | 817        |

**Table 20.** Light Chain Variable Domain CDRs of CD22 CAR (CAR22-65). The LC CDR sequences in this table have the same sequence under the Kabat or combined definitions.

| Candidate         | LCDR1          | SEQ ID NO: | LCDR2   | SEQ ID NO: | LCDR3       | SEQ ID NO: |
|-------------------|----------------|------------|---------|------------|-------------|------------|
| CAR22-65 Combined | TGTSSDVGGYNYVS | 818        | DVSNRPS | 819        | SSYTSSSTLYV | 820        |

In some embodiments, the antigen binding domain comprises a HC CDR1, a HC CDR2, and a HC CDR3 of any heavy chain binding domain amino acid sequences listed in **Table 19**. In embodiments, the antigen binding domain further comprises a LC CDR1, a LC CDR2, and a LC CDR3. In embodiments, the antigen binding domain comprises a LC CDR1, a LC CDR2, and a LC CDR3 amino acid sequences listed in **Table 20**.



In some embodiments, the antigen binding domain comprises one, two or all of LC CDR1, LC CDR2, and LC CDR3 of any light chain binding domain amino acid sequences listed in **Table 20**, and one, two or all of HC CDR1, HC CDR2, and HC CDR3 of any heavy chain binding domain amino acid sequences listed in **Table 19**.

5 In some embodiments, the CDRs are defined according to the Kabat numbering scheme, the Chothia numbering scheme, or a combination thereof.

Additional anti-CD20 scFv sequences are provided below:

Human CD22 CAR scFv sequence (VH-(G4S)-VL)

EVQLQQSGPGLVKPSQTLSTCAISGDSMLSNSDTWNWIRQSPSRGLEWLGRTYHRSTWYDDYA  
10 SSVRGRVSINVDTSKNQYSLQLNAVTPEDTGVYYCARVRLQDGNSWSDAFDVWGQGTMTVVS  
SGGGGSQSALTQPASASGSPGQSVTISCTGTSSDVGGYNYVSWYQQHPGKAPKLMYDVSNRPS  
GVSNRFSGSKSGNTASLTISGLQAEDEADYYCSSYTSSSTLYVFGTGTQLTVL (SEQ ID NO: 1447)

Human CD22 CAR scFv sequence (VL-(G4S)<sup>3</sup>-VH)

QSALTQPASASGSPGQSVTISCTGTSSDVGGYNYVSWYQQHPGKAPKLMYDVSNRPSGVSNRF  
15 SGSKSGNTASLTISGLQAEDEADYYCSSYTSSSTLYVFGTGTQLTVLGGGGSGGGGSGGGGSEVQ  
LQQSGPGLVKPSQTLSTCAISGDSMLSNSDTWNWIRQSPSRGLEWLGRTYHRSTWYDDYASSV  
RGRVSINVDTSKNQYSLQLNAVTPEDTGVYYCARVRLQDGNSWSDAFDVWGQGTMTVSS  
(SEQ ID NO: 1448)

Human CD22 CAR scFv sequence (VL-(G4S)-VH)

20 QSALTQPASASGSPGQSVTISCTGTSSDVGGYNYVSWYQQHPGKAPKLMYDVSNRPSGVSNRF  
SGSKSGNTASLTISGLQAEDEADYYCSSYTSSSTLYVFGTGTQLTVLGGGGSEVQLQQSGPGLVK  
PSQTLSTCAISGDSMLSNSDTWNWIRQSPSRGLEWLGRTYHRSTWYDDYASSVRGRVSINVDTS  
KNQYSLQLNAVTPEDTGVYYCARVRLQDGNSWSDAFDVWGQGTMTVSS (SEQ ID NO:  
1449)

25 The order in which the VL and VH domains appear in the scFv can be varied (i.e., VL-VH, or VH-VL orientation), and where any of one, two, three or four copies of the “G4S” (SEQ ID NO: 168) subunit, in which each subunit comprises the sequence GGGGS (SEQ ID NO: 168) (e.g., (G4S)<sub>3</sub> (SEQ ID NO: 142) or (G4S)<sub>4</sub> (SEQ ID NO: 141)), can connect the variable domains to create the entirety of the scFv domain. Alternatively, the CAR construct can include, for example, a linker including the sequence  
30 GSTSGSGKPGSGEGSTKG (SEQ ID NO: 821). Alternatively, the CAR construct can include, for example, a linker including the sequence LAEAAAK (SEQ ID NO: 822). In an embodiment, the CAR construct does not include a linker between the VL and VH domains.

These clones all contained a Q/K residue change in the signal domain of the co-stimulatory domain derived from CD3zeta chain.

In some embodiments, the CAR molecule described herein is a bispecific CAR molecule. In one embodiment, the bispecific CAR molecule comprises a first binding specificity to CD19, e.g., a VL1-VH1 binding specificity to CD19, and a second binding specificity to CD22, e.g., a VL2-VH2 or VH2-VL1 binding specificity to CD22. In one embodiment, the first and second binding specificity are in a contiguous polypeptide chain, e.g., a single chain. In some embodiments, the first and second binding specificities, optionally, comprise a linker as described herein. In some embodiments, the bispecific CAR molecule comprises a CD19-binding domain comprising an amino acid sequence disclosed in Table 5 and Table 30.

In one embodiment, the bispecific CAR molecule comprises a first binding specificity to CD22, e.g., a VL2-VH2 or VH2-VL1 binding specificity to CD22, and a second binding specificity to CD19, e.g., a VL1-VH1 binding specificity to CD19. In one embodiment, the first and second binding specificity are in a contiguous polypeptide chain, e.g., a single chain. In some embodiments, the first and second binding specificities, optionally, comprise a linker as described herein.

In some embodiments, the linker is a  $(\text{Gly}_4\text{-Ser})_n$  linker, wherein  $n$  is 1, 2, 3, 4, 5, or 6. In some embodiments, the linker is  $(\text{Gly}_4\text{-Ser})_n$ , wherein  $n = 1$  (SEQ ID NO: 168), e.g., the linker has the amino acid sequence Gly<sub>4</sub>-Ser (SEQ ID NO: 168). In some embodiments, the linker is  $(\text{Gly}_4\text{-Ser})_n$ , wherein  $n = 3$  (SEQ ID NO: 142). In some embodiments, the linker is  $(\text{Gly}_4\text{-Ser})_n$ , wherein  $n = 4$  (SEQ ID NO: 141). In some embodiments, the linker comprises, e.g., consists of, the amino acid sequence: LAEAAAK (SEQ ID NO: 822).

#### *CD20 CAR*

In some embodiments, the CAR-expressing cell described herein is a CD20 CAR-expressing cell (e.g., a cell expressing a CAR that binds to human CD20). In some embodiments, the CD20 CAR-expressing cell includes an antigen binding domain according to WO2016/164731 and PCT/US2017/055627, incorporated herein by reference. Exemplary CD20-binding sequences or CD20 CAR sequences are disclosed in, e.g., Tables 1-5 of PCT/US2017/055627, incorporated herein by reference. In some embodiments, the CD20-binding sequences or CD20 CAR comprises a CDR, variable region, scFv, or full-length sequence of a CD20 CAR disclosed in PCT/US2017/055627 or WO2016/164731, incorporated herein by reference. Exemplary anti-CD20 CAR sequences may comprise a CDR, a variable region, an scFv, or a full-length CAR sequence of a sequence disclosed in Table 32 (or a sequence at least about 85%, 90%, 95%, 99% or more identical thereto, and/or having one, two, three or more substitutions, insertions, deletions, or modifications).

**Table 32.** CD20 CAR sequences.

| <b>SEQ ID NO</b>                            | <b>Ab region</b>               |
|---------------------------------------------|--------------------------------|
| <b>CD20-C3H2</b>                            |                                |
| SEQ ID NO: 838 (Kabat)                      | HCDR1                          |
| SEQ ID NO: 839 (Kabat)                      | HCDR2                          |
| SEQ ID NO: 840 (Kabat)                      | HCDR3                          |
| SEQ ID NO: 841 (Chothia)                    | HCDR1                          |
| SEQ ID NO: 842 (Chothia)                    | HCDR2                          |
| SEQ ID NO: 843 (Chothia)                    | HCDR3                          |
| SEQ ID NO: 844 (IMGT)                       | HCDR1                          |
| SEQ ID NO: 845 (IMGT)                       | HCDR2                          |
| SEQ ID NO: 846 (IMGT)                       | HCDR3                          |
| SEQ ID NO: 847 (Combined Chothia and Kabat) | HCDR1                          |
| SEQ ID NO: 848 (Combined Chothia and Kabat) | HCDR2                          |
| SEQ ID NO: 849 (Combined Chothia and Kabat) | HCDR3                          |
| SEQ ID NO: 850                              | VH                             |
| SEQ ID NO: 851                              | DNA VH                         |
| SEQ ID NO: 852 (Kabat)                      | LCDR1                          |
| SEQ ID NO: 853 (Kabat)                      | LCDR2                          |
| SEQ ID NO: 854 (Kabat)                      | LCDR3                          |
| SEQ ID NO: 855 (Chothia)                    | LCDR1                          |
| SEQ ID NO: 856 (Chothia)                    | LCDR2                          |
| SEQ ID NO: 857 (Chothia)                    | LCDR3                          |
| SEQ ID NO: 858 (IMGT)                       | LCDR1                          |
| SEQ ID NO: 859 (IMGT)                       | LCDR2                          |
| SEQ ID NO: 860 (IMGT)                       | LCDR3                          |
| SEQ ID NO: 861 (Combined Chothia and Kabat) | LCDR1                          |
| SEQ ID NO: 862 (Combined Chothia and Kabat) | LCDR2                          |
| SEQ ID NO: 863 (Combined Chothia and Kabat) | LCDR3                          |
| SEQ ID NO: 864                              | VL                             |
| SEQ ID NO: 865                              | DNA VL                         |
| SEQ ID NO: 866                              | Linker                         |
| SEQ ID NO: 867                              | scFv (VH-linker-VL)            |
| SEQ ID NO: 868                              | DNA scFv (VH-linker-VL)        |
| SEQ ID NO: 869                              | Full CAR amino acid sequence   |
| SEQ ID NO: 870                              | Full CAR nucleic acid sequence |
| <b>CD20-C5H1</b>                            |                                |
| SEQ ID NO: 871 (Kabat)                      | HCDR1                          |
| SEQ ID NO: 872 (Kabat)                      | HCDR2                          |
| SEQ ID NO: 873 (Kabat)                      | HCDR3                          |
| SEQ ID NO: 874 (Chothia)                    | HCDR1                          |
| SEQ ID NO: 875 (Chothia)                    | HCDR2                          |
| SEQ ID NO: 876 (Chothia)                    | HCDR3                          |
| SEQ ID NO: 877 (IMGT)                       | HCDR1                          |
| SEQ ID NO: 878 (IMGT)                       | HCDR2                          |
| SEQ ID NO: 879 (IMGT)                       | HCDR3                          |
| SEQ ID NO: 880 (Combined Chothia and Kabat) | HCDR1                          |
| SEQ ID NO: 881 (Combined Chothia and Kabat) | HCDR2                          |

|                                             |                                |
|---------------------------------------------|--------------------------------|
| SEQ ID NO: 882 (Combined Chothia and Kabat) | HCDR3                          |
| SEQ ID NO: 883                              | VH                             |
| SEQ ID NO: 884                              | DNA VH                         |
| SEQ ID NO: 885 (Kabat)                      | LCDR1                          |
| SEQ ID NO: 886 (Kabat)                      | LCDR2                          |
| SEQ ID NO: 887 (Kabat)                      | LCDR3                          |
| SEQ ID NO: 888 (Chothia)                    | LCDR1                          |
| SEQ ID NO: 889 (Chothia)                    | LCDR2                          |
| SEQ ID NO: 890 (Chothia)                    | LCDR3                          |
| SEQ ID NO: 891 (IMGT)                       | LCDR1                          |
| SEQ ID NO: 892 (IMGT)                       | LCDR2                          |
| SEQ ID NO: 893 (IMGT)                       | LCDR3                          |
| SEQ ID NO: 894 (Combined Chothia and Kabat) | LCDR1                          |
| SEQ ID NO: 895 (Combined Chothia and Kabat) | LCDR2                          |
| SEQ ID NO: 896 (Combined Chothia and Kabat) | LCDR3                          |
| SEQ ID NO: 897                              | VL                             |
| SEQ ID NO: 898                              | DNA VL                         |
| SEQ ID NO: 899                              | Linker                         |
| SEQ ID NO: 900                              | scFv (VH-linker-VL)            |
| SEQ ID NO: 901                              | DNA scFv (VH-linker-VL)        |
| SEQ ID NO: 902                              | Full CAR amino acid sequence   |
| SEQ ID NO: 903                              | Full CAR nucleic acid sequence |
| <b>CD20-C2H1</b>                            |                                |
| SEQ ID NO: 904 (Kabat)                      | HCDR1                          |
| SEQ ID NO: 905 (Kabat)                      | HCDR2                          |
| SEQ ID NO: 906 (Kabat)                      | HCDR3                          |
| SEQ ID NO: 907 (Chothia)                    | HCDR1                          |
| SEQ ID NO: 908 (Chothia)                    | HCDR2                          |
| SEQ ID NO: 909 (Chothia)                    | HCDR3                          |
| SEQ ID NO: 910 (IMGT)                       | HCDR1                          |
| SEQ ID NO: 911 (IMGT)                       | HCDR2                          |
| SEQ ID NO: 912 (IMGT)                       | HCDR3                          |
| SEQ ID NO: 913 (Combined Chothia and Kabat) | HCDR1                          |
| SEQ ID NO: 914 (Combined Chothia and Kabat) | HCDR2                          |
| SEQ ID NO: 915 (Combined Chothia and Kabat) | HCDR3                          |
| SEQ ID NO: 916                              | VH                             |
| SEQ ID NO: 917                              | DNA VH                         |
| SEQ ID NO: 918 (Kabat)                      | LCDR1                          |
| SEQ ID NO: 919 (Kabat)                      | LCDR2                          |
| SEQ ID NO: 920 (Kabat)                      | LCDR3                          |
| SEQ ID NO: 921 (Chothia)                    | LCDR1                          |
| SEQ ID NO: 922 (Chothia)                    | LCDR2                          |
| SEQ ID NO: 923 (Chothia)                    | LCDR3                          |
| SEQ ID NO: 924 (IMGT)                       | LCDR1                          |
| SEQ ID NO: 925 (IMGT)                       | LCDR2                          |
| SEQ ID NO: 926 (IMGT)                       | LCDR3                          |
| SEQ ID NO: 927 (Combined Chothia and Kabat) | LCDR1                          |
| SEQ ID NO: 928 (Combined Chothia and Kabat) | LCDR2                          |
| SEQ ID NO: 929 (Combined Chothia and Kabat) | LCDR3                          |

|                                             |                                |
|---------------------------------------------|--------------------------------|
| SEQ ID NO: 930                              | VL                             |
| SEQ ID NO: 931                              | DNA VL                         |
| SEQ ID NO: 932                              | Linker                         |
| SEQ ID NO: 933                              | scFv (VH-linker-VL)            |
| SEQ ID NO: 934                              | DNA scFv (VH-linker-VL)        |
| SEQ ID NO: 935                              | Full CAR amino acid sequence   |
| SEQ ID NO: 936                              | Full CAR nucleic acid sequence |
| <b>CD20-C2H2</b>                            |                                |
| SEQ ID NO: 937 (Kabat)                      | HCDR1                          |
| SEQ ID NO: 938 (Kabat)                      | HCDR2                          |
| SEQ ID NO: 939 (Kabat)                      | HCDR3                          |
| SEQ ID NO: 940 (Chothia)                    | HCDR1                          |
| SEQ ID NO: 941 (Chothia)                    | HCDR2                          |
| SEQ ID NO: 942 (Chothia)                    | HCDR3                          |
| SEQ ID NO: 943 (IMGT)                       | HCDR1                          |
| SEQ ID NO: 944 (IMGT)                       | HCDR2                          |
| SEQ ID NO: 945 (IMGT)                       | HCDR3                          |
| SEQ ID NO: 946 (Combined Chothia and Kabat) | HCDR1                          |
| SEQ ID NO: 947 (Combined Chothia and Kabat) | HCDR2                          |
| SEQ ID NO: 948 (Combined Chothia and Kabat) | HCDR3                          |
| SEQ ID NO: 949                              | VH                             |
| SEQ ID NO: 950                              | DNA VH                         |
| SEQ ID NO: 951 (Kabat)                      | LCDR1                          |
| SEQ ID NO: 952 (Kabat)                      | LCDR2                          |
| SEQ ID NO: 953 (Kabat)                      | LCDR3                          |
| SEQ ID NO: 954 (Chothia)                    | LCDR1                          |
| SEQ ID NO: 955 (Chothia)                    | LCDR2                          |
| SEQ ID NO: 956 (Chothia)                    | LCDR3                          |
| SEQ ID NO: 957 (IMGT)                       | LCDR1                          |
| SEQ ID NO: 958 (IMGT)                       | LCDR2                          |
| SEQ ID NO: 959 (IMGT)                       | LCDR3                          |
| SEQ ID NO: 960 (Combined Chothia and Kabat) | LCDR1                          |
| SEQ ID NO: 961 (Combined Chothia and Kabat) | LCDR2                          |
| SEQ ID NO: 962 (Combined Chothia and Kabat) | LCDR3                          |
| SEQ ID NO: 963                              | VL                             |
| SEQ ID NO: 964                              | DNA VL                         |
| SEQ ID NO: 965                              | Linker                         |
| SEQ ID NO: 966                              | scFv (VH-linker-VL)            |
| SEQ ID NO: 967                              | DNA scFv (VH-linker-VL)        |
| SEQ ID NO: 968                              | Full CAR amino acid sequence   |
| SEQ ID NO: 969                              | Full CAR nucleic acid sequence |
| <b>CD20-C2H3</b>                            |                                |
| SEQ ID NO: 970 (Kabat)                      | HCDR1                          |
| SEQ ID NO: 971 (Kabat)                      | HCDR2                          |
| SEQ ID NO: 972 (Kabat)                      | HCDR3                          |
| SEQ ID NO: 973 (Chothia)                    | HCDR1                          |
| SEQ ID NO: 974 (Chothia)                    | HCDR2                          |
| SEQ ID NO: 975 (Chothia)                    | HCDR3                          |
| SEQ ID NO: 976 (IMGT)                       | HCDR1                          |

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|----------------------------------------------|--------------------------------|
| SEQ ID NO: 977 (IMGT)                        | HCDR2                          |
| SEQ ID NO: 978 (IMGT)                        | HCDR3                          |
| SEQ ID NO: 979 (Combined Chothia and Kabat)  | HCDR1                          |
| SEQ ID NO: 980 (Combined Chothia and Kabat)  | HCDR2                          |
| SEQ ID NO: 981 (Combined Chothia and Kabat)  | HCDR3                          |
| SEQ ID NO: 982                               | VH                             |
| SEQ ID NO: 983                               | DNA VH                         |
| SEQ ID NO: 984 (Kabat)                       | LCDR1                          |
| SEQ ID NO: 985 (Kabat)                       | LCDR2                          |
| SEQ ID NO: 986 (Kabat)                       | LCDR3                          |
| SEQ ID NO: 987 (Chothia)                     | LCDR1                          |
| SEQ ID NO: 988 (Chothia)                     | LCDR2                          |
| SEQ ID NO: 989 (Chothia)                     | LCDR3                          |
| SEQ ID NO: 990 (IMGT)                        | LCDR1                          |
| SEQ ID NO: 991 (IMGT)                        | LCDR2                          |
| SEQ ID NO: 992 (IMGT)                        | LCDR3                          |
| SEQ ID NO: 993 (Combined Chothia and Kabat)  | LCDR1                          |
| SEQ ID NO: 994 (Combined Chothia and Kabat)  | LCDR2                          |
| SEQ ID NO: 995 (Combined Chothia and Kabat)  | LCDR3                          |
| SEQ ID NO: 996                               | VL                             |
| SEQ ID NO: 997                               | DNA VL                         |
| SEQ ID NO: 998                               | Linker                         |
| SEQ ID NO: 999                               | scFv (VH-linker-VL)            |
| SEQ ID NO: 1000                              | DNA scFv (VH-linker-VL)        |
| SEQ ID NO: 1001                              | Full CAR amino acid sequence   |
| SEQ ID NO: 1002                              | Full CAR nucleic acid sequence |
| <b>CD20-C2H4</b>                             |                                |
| SEQ ID NO: 1003 (Kabat)                      | HCDR1                          |
| SEQ ID NO: 1004 (Kabat)                      | HCDR2                          |
| SEQ ID NO: 1005 (Kabat)                      | HCDR3                          |
| SEQ ID NO: 1006 (Chothia)                    | HCDR1                          |
| SEQ ID NO: 1007 (Chothia)                    | HCDR2                          |
| SEQ ID NO: 1008 (Chothia)                    | HCDR3                          |
| SEQ ID NO: 1009 (IMGT)                       | HCDR1                          |
| SEQ ID NO: 1010 (IMGT)                       | HCDR2                          |
| SEQ ID NO: 1011 (IMGT)                       | HCDR3                          |
| SEQ ID NO: 1012 (Combined Chothia and Kabat) | HCDR1                          |
| SEQ ID NO: 1013 (Combined Chothia and Kabat) | HCDR2                          |
| SEQ ID NO: 1014 (Combined Chothia and Kabat) | HCDR3                          |
| SEQ ID NO: 1015                              | VH                             |
| SEQ ID NO: 1016                              | DNA VH                         |
| SEQ ID NO: 1017 (Kabat)                      | LCDR1                          |
| SEQ ID NO: 1018 (Kabat)                      | LCDR2                          |
| SEQ ID NO: 1019 (Kabat)                      | LCDR3                          |
| SEQ ID NO: 1020 (Chothia)                    | LCDR1                          |
| SEQ ID NO: 1021 (Chothia)                    | LCDR2                          |
| SEQ ID NO: 1022 (Chothia)                    | LCDR3                          |
| SEQ ID NO: 1023 (IMGT)                       | LCDR1                          |
| SEQ ID NO: 1024 (IMGT)                       | LCDR2                          |

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|----------------------------------------------|--------------------------------|
| SEQ ID NO: 1025 (IMGT)                       | LCDR3                          |
| SEQ ID NO: 1026 (Combined Chothia and Kabat) | LCDR1                          |
| SEQ ID NO: 1027 (Combined Chothia and Kabat) | LCDR2                          |
| SEQ ID NO: 1028 (Combined Chothia and Kabat) | LCDR3                          |
| SEQ ID NO: 1029                              | VL                             |
| SEQ ID NO: 1030                              | DNA VL                         |
| SEQ ID NO: 1031                              | Linker                         |
| SEQ ID NO: 1032                              | scFv (VH-linker-VL)            |
| SEQ ID NO: 1033                              | DNA scFv (VH-linker-VL)        |
| SEQ ID NO: 1034                              | Full CAR amino acid sequence   |
| SEQ ID NO: 1035                              | Full CAR nucleic acid sequence |
| <b>CD20-C3H1</b>                             |                                |
| SEQ ID NO: 1036 (Kabat)                      | HCDR1                          |
| SEQ ID NO: 1037 (Kabat)                      | HCDR2                          |
| SEQ ID NO: 1038 (Kabat)                      | HCDR3                          |
| SEQ ID NO: 1039 (Chothia)                    | HCDR1                          |
| SEQ ID NO: 1040 (Chothia)                    | HCDR2                          |
| SEQ ID NO: 1041 (Chothia)                    | HCDR3                          |
| SEQ ID NO: 1042 (IMGT)                       | HCDR1                          |
| SEQ ID NO: 1043 (IMGT)                       | HCDR2                          |
| SEQ ID NO: 1044 (IMGT)                       | HCDR3                          |
| SEQ ID NO: 1045 (Combined Chothia and Kabat) | HCDR1                          |
| SEQ ID NO: 1046 (Combined Chothia and Kabat) | HCDR2                          |
| SEQ ID NO: 1047 (Combined Chothia and Kabat) | HCDR3                          |
| SEQ ID NO: 1048                              | VH                             |
| SEQ ID NO: 1049                              | DNA VH                         |
| SEQ ID NO: 1050 (Kabat)                      | LCDR1                          |
| SEQ ID NO: 1051 (Kabat)                      | LCDR2                          |
| SEQ ID NO: 1052 (Kabat)                      | LCDR3                          |
| SEQ ID NO: 1053 (Chothia)                    | LCDR1                          |
| SEQ ID NO: 1054 (Chothia)                    | LCDR2                          |
| SEQ ID NO: 1055 (Chothia)                    | LCDR3                          |
| SEQ ID NO: 1056 (IMGT)                       | LCDR1                          |
| SEQ ID NO: 1057 (IMGT)                       | LCDR2                          |
| SEQ ID NO: 1058 (IMGT)                       | LCDR3                          |
| SEQ ID NO: 1059 (Combined Chothia and Kabat) | LCDR1                          |
| SEQ ID NO: 1060 (Combined Chothia and Kabat) | LCDR2                          |
| SEQ ID NO: 1061 (Combined Chothia and Kabat) | LCDR3                          |
| SEQ ID NO: 1062                              | VL                             |
| SEQ ID NO: 1063                              | DNA VL                         |
| SEQ ID NO: 1064                              | Linker                         |
| SEQ ID NO: 1065                              | scFv (VH-linker-VL)            |
| SEQ ID NO: 1066                              | DNA scFv (VH-linker-VL)        |
| SEQ ID NO: 1067                              | Full CAR amino acid sequence   |
| SEQ ID NO: 1068                              | Full CAR nucleic acid sequence |
| <b>CD20-C3H3</b>                             |                                |
| SEQ ID NO: 1069 (Kabat)                      | HCDR1                          |
| SEQ ID NO: 1070 (Kabat)                      | HCDR2                          |
| SEQ ID NO: 1071 (Kabat)                      | HCDR3                          |

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|----------------------------------------------|--------------------------------|
| SEQ ID NO: 1072 (Chothia)                    | HCDR1                          |
| SEQ ID NO: 1073 (Chothia)                    | HCDR2                          |
| SEQ ID NO: 1074 (Chothia)                    | HCDR3                          |
| SEQ ID NO: 1075 (IMGT)                       | HCDR1                          |
| SEQ ID NO: 1076 (IMGT)                       | HCDR2                          |
| SEQ ID NO: 1077 (IMGT)                       | HCDR3                          |
| SEQ ID NO: 1078                              | VH                             |
| SEQ ID NO: 1079                              | DNA VH                         |
| SEQ ID NO: 1080 (Kabat)                      | LCDR1                          |
| SEQ ID NO: 1081 (Kabat)                      | LCDR2                          |
| SEQ ID NO: 1082 (Kabat)                      | LCDR3                          |
| SEQ ID NO: 1083 (Chothia)                    | LCDR1                          |
| SEQ ID NO: 1084 (Chothia)                    | LCDR2                          |
| SEQ ID NO: 1085 (Chothia)                    | LCDR3                          |
| SEQ ID NO: 1086 (IMGT)                       | LCDR1                          |
| SEQ ID NO: 1087 (IMGT)                       | LCDR2                          |
| SEQ ID NO: 1088 (IMGT)                       | LCDR3                          |
| SEQ ID NO: 1089 (Combined Chothia and Kabat) | LCDR1                          |
| SEQ ID NO: 1090 (Combined Chothia and Kabat) | LCDR2                          |
| SEQ ID NO: 1091 (Combined Chothia and Kabat) | LCDR3                          |
| SEQ ID NO: 1092                              | VL                             |
| SEQ ID NO: 1093                              | DNA VL                         |
| SEQ ID NO: 1094                              | Linker                         |
| SEQ ID NO: 1095                              | scFv (VH-linker-VL)            |
| SEQ ID NO: 1096                              | DNA scFv (VH-linker-VL)        |
| SEQ ID NO: 1097                              | Full CAR amino acid sequence   |
| SEQ ID NO: 1098                              | Full CAR nucleic acid sequence |
| <b>CD20-C3H4</b>                             |                                |
| SEQ ID NO: 1099 (Kabat)                      | HCDR1                          |
| SEQ ID NO: 1100 (Kabat)                      | HCDR2                          |
| SEQ ID NO: 1101 (Kabat)                      | HCDR3                          |
| SEQ ID NO: 1102 (Chothia)                    | HCDR1                          |
| SEQ ID NO: 1103 (Chothia)                    | HCDR2                          |
| SEQ ID NO: 1104 (Chothia)                    | HCDR3                          |
| SEQ ID NO: 1105 (IMGT)                       | HCDR1                          |
| SEQ ID NO: 1106 (IMGT)                       | HCDR2                          |
| SEQ ID NO: 1107 (IMGT)                       | HCDR3                          |
| SEQ ID NO: 1108 (Combined Chothia and Kabat) | HCDR1                          |
| SEQ ID NO: 1109 (Combined Chothia and Kabat) | HCDR2                          |
| SEQ ID NO: 1110 (Combined Chothia and Kabat) | HCDR3                          |
| SEQ ID NO: 1111                              | VH                             |
| SEQ ID NO: 1112                              | DNA VH                         |
| SEQ ID NO: 1113 (Kabat)                      | LCDR1                          |
| SEQ ID NO: 1114 (Kabat)                      | LCDR2                          |
| SEQ ID NO: 1115 (Kabat)                      | LCDR3                          |
| SEQ ID NO: 1116 (Chothia)                    | LCDR1                          |
| SEQ ID NO: 1117 (Chothia)                    | LCDR2                          |
| SEQ ID NO: 1118 (Chothia)                    | LCDR3                          |
| SEQ ID NO: 1119 (IMGT)                       | LCDR1                          |



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|----------------------------------------------|--------------------------------|
| SEQ ID NO: 1120 (IMGT)                       | LCDR2                          |
| SEQ ID NO: 1121 (IMGT)                       | LCDR3                          |
| SEQ ID NO: 1122 (Combined Chothia and Kabat) | LCDR1                          |
| SEQ ID NO: 1123 (Combined Chothia and Kabat) | LCDR2                          |
| SEQ ID NO: 1124 (Combined Chothia and Kabat) | LCDR3                          |
| SEQ ID NO: 1125                              | VL                             |
| SEQ ID NO: 1126                              | DNA VL                         |
| SEQ ID NO: 1127                              | Linker                         |
| SEQ ID NO: 1128                              | scFv (VH-linker-VL)            |
| SEQ ID NO: 1129                              | DNA scFv (VH-linker-VL)        |
| SEQ ID NO: 1130                              | Full CAR amino acid sequence   |
| SEQ ID NO: 1131                              | Full CAR nucleic acid sequence |
| <b>CD20-C5H2</b>                             |                                |
| SEQ ID NO: 1132 (Kabat)                      | HCDR1                          |
| SEQ ID NO: 1133 (Kabat)                      | HCDR2                          |
| SEQ ID NO: 1134 (Kabat)                      | HCDR3                          |
| SEQ ID NO: 1135 (Chothia)                    | HCDR1                          |
| SEQ ID NO: 1136 (Chothia)                    | HCDR2                          |
| SEQ ID NO: 1137 (Chothia)                    | HCDR3                          |
| SEQ ID NO: 1138 (IMGT)                       | HCDR1                          |
| SEQ ID NO: 1139 (IMGT)                       | HCDR2                          |
| SEQ ID NO: 1140 (IMGT)                       | HCDR3                          |
| SEQ ID NO: 1141 (Combined Chothia and Kabat) | HCDR1                          |
| SEQ ID NO: 1142 (Combined Chothia and Kabat) | HCDR2                          |
| SEQ ID NO: 1143 (Combined Chothia and Kabat) | HCDR3                          |
| SEQ ID NO: 1144                              | VH                             |
| SEQ ID NO: 1145                              | DNA VH                         |
| SEQ ID NO: 1146 (Kabat)                      | LCDR1                          |
| SEQ ID NO: 1147 (Kabat)                      | LCDR2                          |
| SEQ ID NO: 1148 (Kabat)                      | LCDR3                          |
| SEQ ID NO: 1149 (Chothia)                    | LCDR1                          |
| SEQ ID NO: 1150 (Chothia)                    | LCDR2                          |
| SEQ ID NO: 1151 (Chothia)                    | LCDR3                          |
| SEQ ID NO: 1152 (IMGT)                       | LCDR1                          |
| SEQ ID NO: 1153 (IMGT)                       | LCDR2                          |
| SEQ ID NO: 1154 (IMGT)                       | LCDR3                          |
| SEQ ID NO: 1155 (Combined Chothia and Kabat) | LCDR1                          |
| SEQ ID NO: 1156 (Combined Chothia and Kabat) | LCDR2                          |
| SEQ ID NO: 1157 (Combined Chothia and Kabat) | LCDR3                          |
| SEQ ID NO: 1158                              | VL                             |
| SEQ ID NO: 1159                              | DNA VL                         |
| SEQ ID NO: 1160                              | Linker                         |
| SEQ ID NO: 1161                              | scFv (VH-linker-VL)            |
| SEQ ID NO: 1162                              | DNA scFv (VH-linker-VL)        |
| SEQ ID NO: 1163                              | Full CAR amino acid sequence   |
| SEQ ID NO: 1164                              | Full CAR nucleic acid sequence |
| <b>CD20-C5H3</b>                             |                                |
| SEQ ID NO: 1165 (Kabat)                      | HCDR1                          |
| SEQ ID NO: 1166 (Kabat)                      | HCDR2                          |

|                                              |                                |
|----------------------------------------------|--------------------------------|
| SEQ ID NO: 1167 (Kabat)                      | HCDR3                          |
| SEQ ID NO: 1168 (Chothia)                    | HCDR1                          |
| SEQ ID NO: 1169 (Chothia)                    | HCDR2                          |
| SEQ ID NO: 1170 (Chothia)                    | HCDR3                          |
| SEQ ID NO: 1171 (IMGT)                       | HCDR1                          |
| SEQ ID NO: 1172 (IMGT)                       | HCDR2                          |
| SEQ ID NO: 1173 (IMGT)                       | HCDR3                          |
| SEQ ID NO: 1174 (Combined Chothia and Kabat) | HCDR1                          |
| SEQ ID NO: 1175 (Combined Chothia and Kabat) | HCDR2                          |
| SEQ ID NO: 1176 (Combined Chothia and Kabat) | HCDR3                          |
| SEQ ID NO: 1177                              | VH                             |
| SEQ ID NO: 1178                              | DNA VH                         |
| SEQ ID NO: 1179 (Kabat)                      | LCDR1                          |
| SEQ ID NO: 1180 (Kabat)                      | LCDR2                          |
| SEQ ID NO: 1181 (Kabat)                      | LCDR3                          |
| SEQ ID NO: 1182 (Chothia)                    | LCDR1                          |
| SEQ ID NO: 1183 (Chothia)                    | LCDR2                          |
| SEQ ID NO: 1184 (Chothia)                    | LCDR3                          |
| SEQ ID NO: 1185 (IMGT)                       | LCDR1                          |
| SEQ ID NO: 1186 (IMGT)                       | LCDR2                          |
| SEQ ID NO: 1187 (IMGT)                       | LCDR3                          |
| SEQ ID NO: 1188 (Combined Chothia and Kabat) | LCDR1                          |
| SEQ ID NO: 1189 (Combined Chothia and Kabat) | LCDR2                          |
| SEQ ID NO: 1190 (Combined Chothia and Kabat) | LCDR3                          |
| SEQ ID NO: 1191                              | VL                             |
| SEQ ID NO: 1192                              | DNA VL                         |
| SEQ ID NO: 1193                              | Linker                         |
| SEQ ID NO: 1194                              | scFv (VH-linker-VL)            |
| SEQ ID NO: 1195                              | DNA scFv (VH-linker-VL)        |
| SEQ ID NO: 1196                              | Full CAR amino acid sequence   |
| SEQ ID NO: 1197                              | Full CAR nucleic acid sequence |
| <b>CD20-C5H4</b>                             |                                |
| SEQ ID NO: 1198 (Kabat)                      | HCDR1                          |
| SEQ ID NO: 1199 (Kabat)                      | HCDR2                          |
| SEQ ID NO: 1200 (Kabat)                      | HCDR3                          |
| SEQ ID NO: 1201 (Chothia)                    | HCDR1                          |
| SEQ ID NO: 1202 (Chothia)                    | HCDR2                          |
| SEQ ID NO: 1203 (Chothia)                    | HCDR3                          |
| SEQ ID NO: 1204 (IMGT)                       | HCDR1                          |
| SEQ ID NO: 1205 (IMGT)                       | HCDR2                          |
| SEQ ID NO: 1206 (IMGT)                       | HCDR3                          |
| SEQ ID NO: 1207 (Combined Chothia and Kabat) | HCDR1                          |
| SEQ ID NO: 1208 (Combined Chothia and Kabat) | HCDR2                          |
| SEQ ID NO: 1209 (Combined Chothia and Kabat) | HCDR3                          |
| SEQ ID NO: 1210                              | VH                             |
| SEQ ID NO: 1211                              | DNA VH                         |
| SEQ ID NO: 1212 (Kabat)                      | LCDR1                          |
| SEQ ID NO: 1213 (Kabat)                      | LCDR2                          |
| SEQ ID NO: 1214 (Kabat)                      | LCDR3                          |

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|----------------------------------------------|--------------------------------|
| SEQ ID NO: 1215 (Chothia)                    | LCDR1                          |
| SEQ ID NO: 1216 (Chothia)                    | LCDR2                          |
| SEQ ID NO: 1217 (Chothia)                    | LCDR3                          |
| SEQ ID NO: 1218 (IMGT)                       | LCDR1                          |
| SEQ ID NO: 1219 (IMGT)                       | LCDR2                          |
| SEQ ID NO: 1220 (IMGT)                       | LCDR3                          |
| SEQ ID NO: 1221 (Combined Chothia and Kabat) | LCDR1                          |
| SEQ ID NO: 1222 (Combined Chothia and Kabat) | LCDR2                          |
| SEQ ID NO: 1223 (Combined Chothia and Kabat) | LCDR3                          |
| SEQ ID NO: 1224                              | VL                             |
| SEQ ID NO: 1225                              | DNA VL                         |
| SEQ ID NO: 1226                              | Linker                         |
| SEQ ID NO: 1227                              | scFv (VH-linker-VL)            |
| SEQ ID NO: 1228                              | DNA scFv (VH-linker-VL)        |
| SEQ ID NO: 1229                              | Full CAR amino acid sequence   |
| SEQ ID NO: 1230                              | Full CAR nucleic acid sequence |
| <b>CD20-C8H1</b>                             |                                |
| SEQ ID NO: 1231 (Kabat)                      | HCDR1                          |
| SEQ ID NO: 1232 (Kabat)                      | HCDR2                          |
| SEQ ID NO: 1233 (Kabat)                      | HCDR3                          |
| SEQ ID NO: 1234 (Chothia)                    | HCDR1                          |
| SEQ ID NO: 1235 (Chothia)                    | HCDR2                          |
| SEQ ID NO: 1236 (Chothia)                    | HCDR3                          |
| SEQ ID NO: 1237 (IMGT)                       | HCDR1                          |
| SEQ ID NO: 1238 (IMGT)                       | HCDR2                          |
| SEQ ID NO: 1239 (IMGT)                       | HCDR3                          |
| SEQ ID NO: 1240 (Combined Chothia and Kabat) | HCDR1                          |
| SEQ ID NO: 1241 (Combined Chothia and Kabat) | HCDR2                          |
| SEQ ID NO: 1242 (Combined Chothia and Kabat) | HCDR3                          |
| SEQ ID NO: 1243                              | VH                             |
| SEQ ID NO: 1244                              | DNA VH                         |
| SEQ ID NO: 1245 (Kabat)                      | LCDR1                          |
| SEQ ID NO: 1246 (Kabat)                      | LCDR2                          |
| SEQ ID NO: 1247 (Kabat)                      | LCDR3                          |
| SEQ ID NO: 1248 (Chothia)                    | LCDR1                          |
| SEQ ID NO: 1249 (Chothia)                    | LCDR2                          |
| SEQ ID NO: 1250 (Chothia)                    | LCDR3                          |
| SEQ ID NO: 1251 (IMGT)                       | LCDR1                          |
| SEQ ID NO: 1252 (IMGT)                       | LCDR2                          |
| SEQ ID NO: 1253 (IMGT)                       | LCDR3                          |
| SEQ ID NO: 1254 (Combined Chothia and Kabat) | LCDR1                          |
| SEQ ID NO: 1255 (Combined Chothia and Kabat) | LCDR2                          |
| SEQ ID NO: 1256 (Combined Chothia and Kabat) | LCDR3                          |
| SEQ ID NO: 1257                              | VL                             |
| SEQ ID NO: 1258                              | DNA VL                         |
| SEQ ID NO: 1259                              | Linker                         |
| SEQ ID NO: 1260                              | scFv (VH-linker-VL)            |
| SEQ ID NO: 1261                              | DNA scFv (VH-linker-VL)        |
| SEQ ID NO: 1262                              | Full CAR amino acid sequence   |

|                                              |                                |
|----------------------------------------------|--------------------------------|
| SEQ ID NO: 1263                              | Full CAR nucleic acid sequence |
| <b>CD20-C8H2</b>                             |                                |
| SEQ ID NO: 1264 (Kabat)                      | HCDR1                          |
| SEQ ID NO: 1265 (Kabat)                      | HCDR2                          |
| SEQ ID NO: 1266 (Kabat)                      | HCDR3                          |
| SEQ ID NO: 1267 (Chothia)                    | HCDR1                          |
| SEQ ID NO: 1268 (Chothia)                    | HCDR2                          |
| SEQ ID NO: 1269 (Chothia)                    | HCDR3                          |
| SEQ ID NO: 1270 (IMGT)                       | HCDR1                          |
| SEQ ID NO: 1271 (IMGT)                       | HCDR2                          |
| SEQ ID NO: 1272 (IMGT)                       | HCDR3                          |
| SEQ ID NO: 1273 (Combined Chothia and Kabat) | HCDR1                          |
| SEQ ID NO: 1274 (Combined Chothia and Kabat) | HCDR2                          |
| SEQ ID NO: 1275 (Combined Chothia and Kabat) | HCDR3                          |
| SEQ ID NO: 1276                              | VH                             |
| SEQ ID NO: 1277                              | DNA VH                         |
| SEQ ID NO: 1278 (Kabat)                      | LCDR1                          |
| SEQ ID NO: 1279 (Kabat)                      | LCDR2                          |
| SEQ ID NO: 1280 (Kabat)                      | LCDR3                          |
| SEQ ID NO: 1281 (Chothia)                    | LCDR1                          |
| SEQ ID NO: 1282 (Chothia)                    | LCDR2                          |
| SEQ ID NO: 1283 (Chothia)                    | LCDR3                          |
| SEQ ID NO: 1284 (IMGT)                       | LCDR1                          |
| SEQ ID NO: 1285 (IMGT)                       | LCDR2                          |
| SEQ ID NO: 1286 (IMGT)                       | LCDR3                          |
| SEQ ID NO: 1287 (Combined Chothia and Kabat) | LCDR1                          |
| SEQ ID NO: 1288 (Combined Chothia and Kabat) | LCDR2                          |
| SEQ ID NO: 1289 (Combined Chothia and Kabat) | LCDR3                          |
| SEQ ID NO: 1290                              | VL                             |
| SEQ ID NO: 1291                              | DNA VL                         |
| SEQ ID NO: 1292                              | Linker                         |
| SEQ ID NO: 1293                              | scFv (VH-linker-VL)            |
| SEQ ID NO: 1294                              | DNA scFv (VH-linker-VL)        |
| SEQ ID NO: 1295                              | Full CAR amino acid sequence   |
| SEQ ID NO: 1296                              | Full CAR nucleic acid sequence |
| <b>CD20-C8H3</b>                             |                                |
| SEQ ID NO: 1297 (Kabat)                      | HCDR1                          |
| SEQ ID NO: 1298 (Kabat)                      | HCDR2                          |
| SEQ ID NO: 1299 (Kabat)                      | HCDR3                          |
| SEQ ID NO: 1300 (Chothia)                    | HCDR1                          |
| SEQ ID NO: 1301 (Chothia)                    | HCDR2                          |
| SEQ ID NO: 1302 (Chothia)                    | HCDR3                          |
| SEQ ID NO: 1303 (IMGT)                       | HCDR1                          |
| SEQ ID NO: 1304 (IMGT)                       | HCDR2                          |
| SEQ ID NO: 1305 (IMGT)                       | HCDR3                          |
| SEQ ID NO: 1306 (Combined Chothia and Kabat) | HCDR1                          |
| SEQ ID NO: 1307 (Combined Chothia and Kabat) | HCDR2                          |
| SEQ ID NO: 1308 (Combined Chothia and Kabat) | HCDR3                          |
| SEQ ID NO: 1309                              | VH                             |

|                                              |                                |
|----------------------------------------------|--------------------------------|
| SEQ ID NO: 1310                              | DNA VH                         |
| SEQ ID NO: 1311 (Kabat)                      | LCDR1                          |
| SEQ ID NO: 1312 (Kabat)                      | LCDR2                          |
| SEQ ID NO: 1313 (Kabat)                      | LCDR3                          |
| SEQ ID NO: 1314 (Chothia)                    | LCDR1                          |
| SEQ ID NO: 1315 (Chothia)                    | LCDR2                          |
| SEQ ID NO: 1316 (Chothia)                    | LCDR3                          |
| SEQ ID NO: 1317 (IMGT)                       | LCDR1                          |
| SEQ ID NO: 1318 (IMGT)                       | LCDR2                          |
| SEQ ID NO: 1319 (IMGT)                       | LCDR3                          |
| SEQ ID NO: 1320 (Combined Chothia and Kabat) | LCDR1                          |
| SEQ ID NO: 1321 (Combined Chothia and Kabat) | LCDR2                          |
| SEQ ID NO: 1322 (Combined Chothia and Kabat) | LCDR3                          |
| SEQ ID NO: 1323                              | VL                             |
| SEQ ID NO: 1324                              | DNA VL                         |
| SEQ ID NO: 1325                              | Linker                         |
| SEQ ID NO: 1326                              | scFv (VH-linker-VL)            |
| SEQ ID NO: 1327                              | DNA scFv (VH-linker-VL)        |
| SEQ ID NO: 1328                              | Full CAR amino acid sequence   |
| SEQ ID NO: 1329                              | Full CAR nucleic acid sequence |
| <b>CD20-C8H4</b>                             |                                |
| SEQ ID NO: 1330 (Kabat)                      | HCDR1                          |
| SEQ ID NO: 1331 (Kabat)                      | HCDR2                          |
| SEQ ID NO: 1332 (Kabat)                      | HCDR3                          |
| SEQ ID NO: 1333 (Chothia)                    | HCDR1                          |
| SEQ ID NO: 1334 (Chothia)                    | HCDR2                          |
| SEQ ID NO: 1335 (Chothia)                    | HCDR3                          |
| SEQ ID NO: 1336 (IMGT)                       | HCDR1                          |
| SEQ ID NO: 1337 (IMGT)                       | HCDR2                          |
| SEQ ID NO: 1338 (IMGT)                       | HCDR3                          |
| SEQ ID NO: 1339 (Combined Chothia and Kabat) | HCDR1                          |
| SEQ ID NO: 1340 (Combined Chothia and Kabat) | HCDR2                          |
| SEQ ID NO: 1341 (Combined Chothia and Kabat) | HCDR3                          |
| SEQ ID NO: 1342                              | VH                             |
| SEQ ID NO: 1343                              | DNA VH                         |
| SEQ ID NO: 1344 (Kabat)                      | LCDR1                          |
| SEQ ID NO: 1345 (Kabat)                      | LCDR2                          |
| SEQ ID NO: 1346 (Kabat)                      | LCDR3                          |
| SEQ ID NO: 1347 (Chothia)                    | LCDR1                          |
| SEQ ID NO: 1348 (Chothia)                    | LCDR2                          |
| SEQ ID NO: 1349 (Chothia)                    | LCDR3                          |
| SEQ ID NO: 1350 (IMGT)                       | LCDR1                          |
| SEQ ID NO: 1351 (IMGT)                       | LCDR2                          |
| SEQ ID NO: 1352 (IMGT)                       | LCDR3                          |
| SEQ ID NO: 1353 (Combined Chothia and Kabat) | LCDR1                          |
| SEQ ID NO: 1354 (Combined Chothia and Kabat) | LCDR2                          |
| SEQ ID NO: 1355 (Combined Chothia and Kabat) | LCDR3                          |
| SEQ ID NO: 1356                              | VL                             |
| SEQ ID NO: 1357                              | DNA VL                         |

|                 |                                |
|-----------------|--------------------------------|
| SEQ ID NO: 1358 | Linker                         |
| SEQ ID NO: 1359 | scFv (VH-linker-VL)            |
| SEQ ID NO: 1360 | DNA scFv (VH-linker-VL)        |
| SEQ ID NO: 1361 | Full CAR amino acid sequence   |
| SEQ ID NO: 1362 | Full CAR nucleic acid sequence |
| <b>CD20-C2</b>  |                                |
| SEQ ID NO: 1363 | VH                             |
| SEQ ID NO: 1364 | DNA VH                         |
| SEQ ID NO: 1365 | VL                             |
| SEQ ID NO: 1366 | DNA VL                         |
| <b>CD20-C3</b>  |                                |
| SEQ ID NO: 1367 | VH                             |
| SEQ ID NO: 1368 | DNA VH                         |
| SEQ ID NO: 1369 | VL                             |
| SEQ ID NO: 1370 | DNA VL                         |
| <b>CD20-C5</b>  |                                |
| SEQ ID NO: 1371 | VH                             |
| SEQ ID NO: 1372 | DNA VH                         |
| SEQ ID NO: 1373 | VL                             |
| SEQ ID NO: 1374 | DNA VL                         |
| <b>CD20-C6</b>  |                                |
| SEQ ID NO: 1375 | VH                             |
| SEQ ID NO: 1376 | DNA VH                         |
| SEQ ID NO: 1377 | VL                             |
| SEQ ID NO: 1378 | DNA VL                         |
| <b>CD20-C7</b>  |                                |
| SEQ ID NO: 1379 | VH                             |
| SEQ ID NO: 1380 | DNA VH                         |
| SEQ ID NO: 1381 | VL                             |
| SEQ ID NO: 1382 | DNA VL                         |
| <b>CD20-C8</b>  |                                |
| SEQ ID NO: 1383 | VH                             |
| SEQ ID NO: 1384 | DNA VH                         |
| SEQ ID NO: 1385 | VL                             |
| SEQ ID NO: 1386 | DNA VL                         |
| <b>CD20-3m</b>  |                                |
| SEQ ID NO: 1387 | VH                             |
| SEQ ID NO: 1388 | DNA VH                         |
| SEQ ID NO: 1389 | VL                             |
| SEQ ID NO: 1390 | DNA VL                         |
| SEQ ID NO: 1391 | Linker                         |
| SEQ ID NO: 1392 | scFv (VH-linker-VL)            |
| <b>CD20-3J</b>  |                                |
| SEQ ID NO: 1393 | VH                             |
| SEQ ID NO: 1394 | DNA VH                         |
| SEQ ID NO: 1395 | VL                             |
| SEQ ID NO: 1396 | DNA VL                         |
| SEQ ID NO: 1397 | Linker                         |
| SEQ ID NO: 1398 | scFv (VH-linker-VL)            |

|                           |                         |
|---------------------------|-------------------------|
| <b>CD20-3H5k1</b>         |                         |
| SEQ ID NO: 1399           | VH                      |
| SEQ ID NO: 1400           | DNA VH                  |
| SEQ ID NO: 1401           | VL                      |
| SEQ ID NO: 1402           | DNA VL                  |
| SEQ ID NO: 1403           | Linker                  |
| SEQ ID NO: 1404           | scFv (VH-linker-VL)     |
| <b>CD20-3H5k3</b>         |                         |
| SEQ ID NO: 1405           | VH                      |
| SEQ ID NO: 1406           | DNA VH                  |
| SEQ ID NO: 1407           | VL                      |
| SEQ ID NO: 1408           | DNA VL                  |
| SEQ ID NO: 1409           | Linker                  |
| SEQ ID NO: 1410           | scFv (VH-linker-VL)     |
| <b>CD20-Ofa</b>           |                         |
| SEQ ID NO: 1411 (Kabat)   | HCDR1                   |
| SEQ ID NO: 1412 (Kabat)   | HCDR2                   |
| SEQ ID NO: 1413 (Kabat)   | HCDR3                   |
| SEQ ID NO: 1414 (Chothia) | HCDR1                   |
| SEQ ID NO: 1415 (Chothia) | HCDR2                   |
| SEQ ID NO: 1416 (Chothia) | HCDR3                   |
| SEQ ID NO: 1417 (IMGT)    | HCDR1                   |
| SEQ ID NO: 1418 (IMGT)    | HCDR2                   |
| SEQ ID NO: 1419 (IMGT)    | HCDR3                   |
| SEQ ID NO: 1420           | VH                      |
| SEQ ID NO: 1421           | DNA VH                  |
| SEQ ID NO: 1422 (Kabat)   | LCDR1                   |
| SEQ ID NO: 1423 (Kabat)   | LCDR2                   |
| SEQ ID NO: 1424 (Kabat)   | LCDR3                   |
| SEQ ID NO: 1425 (Chothia) | LCDR1                   |
| SEQ ID NO: 1426 (Chothia) | LCDR2                   |
| SEQ ID NO: 1427 (Chothia) | LCDR3                   |
| SEQ ID NO: 1428 (IMGT)    | LCDR1                   |
| SEQ ID NO: 1429 (IMGT)    | LCDR2                   |
| SEQ ID NO: 1430 (IMGT)    | LCDR3                   |
| SEQ ID NO: 1431           | VL                      |
| SEQ ID NO: 1432           | DNA VL                  |
| SEQ ID NO: 1433           | Linker                  |
| SEQ ID NO: 1434           | scFv (VH-linker-VL)     |
| SEQ ID NO: 1435           | DNA scFv (VH-linker-VL) |
| <b>CD20-3</b>             |                         |
| SEQ ID NO: 1436           | VH                      |
| SEQ ID NO: 1437           | VL                      |
| SEQ ID NO: 1438           | Linker                  |
| SEQ ID NO: 1439           | scFv (VH-linker-VL)     |
| <b>CD20-8aBBz</b>         |                         |
| SEQ ID NO: 1440           | VH                      |
| SEQ ID NO: 1441           | DNA VH                  |
| SEQ ID NO: 1442           | VL                      |

|                 |                         |
|-----------------|-------------------------|
| SEQ ID NO: 1443 | DNA VL                  |
| SEQ ID NO: 1444 | Linker                  |
| SEQ ID NO: 1445 | scFv (VH-linker-VL)     |
| SEQ ID NO: 1446 | DNA scFv (VH-linker-VL) |

*CLL-1 CAR*

In other embodiments, the CAR-expressing cells can specifically bind to CLL-1, *e.g.*, can include a CAR molecule, or an antigen binding domain according to Table 2 of WO2016/014535, incorporated herein by reference. Exemplary amino acid and nucleotide sequences encoding the CLL-1 CAR molecules and antigen binding domains (*e.g.*, including one, two, three VH CDRs; and one, two, three VL CDRs according to Kabat or Chothia) are provided in WO2016/014535.

*GFR ALPHA-4*

In other embodiments, the CAR-expressing cells can specifically bind to GFR ALPHA-4, *e.g.*, can include a CAR molecule, or an antigen binding domain according to Table 2 of WO2016/025880, incorporated herein by reference. Exemplary amino acid and nucleotide sequences encoding the GFR ALPHA-4 CAR molecules and antigen binding domains (*e.g.*, including one, two, three VH CDRs; and one, two, three VL CDRs according to Kabat or Chothia) are provided in WO2016/025880.

In one embodiment, the antigen binding domain of any of the CAR molecules described herein (*e.g.*, any of CD19, CD123, EGFRvIII, CD33, mesothelin, BCMA, and GFR ALPHA-4) comprises one, two, or three (*e.g.*, all three) heavy chain CDRs, HC CDR1, HC CDR2 and HC CDR3, from an antibody listed above, and/or one, two, or three (*e.g.*, all three) light chain CDRs, LC CDR1, LC CDR2 and LC CDR3, from an antigen binding domain listed above. In one embodiment, the antigen binding domain comprises a heavy chain variable region and/or a variable light chain region of an antibody listed or described above.

In one aspect, the anti-tumor antigen binding domain is a fragment, *e.g.*, a single chain variable fragment (scFv). In one aspect, the anti-cancer associated antigen as described herein binding domain is a Fv, a Fab, a (Fab')<sub>2</sub>, or a bi-functional (*e.g.* bi-specific) hybrid antibody (*e.g.*, Lanzavecchia et al., Eur. J. Immunol. 17, 105 (1987)). In one aspect, the antibodies and fragments thereof of the invention binds a cancer associated antigen as described herein protein with wild-type or enhanced affinity.

In some instances, scFvs can be prepared according to method known in the art (see, for example, Bird et al., (1988) Science 242:423-426 and Huston et al., (1988) Proc. Natl. Acad. Sci. USA 85:5879-5883). ScFv molecules can be produced by linking VH and VL regions together using flexible polypeptide linkers. The scFv molecules comprise a linker (*e.g.*, a Ser-Gly linker) with an optimized



length and/or amino acid composition. The linker length can greatly affect how the variable regions of a scFv fold and interact. In fact, if a short polypeptide linker is employed (e.g., between 5-10 amino acids) intrachain folding is prevented. Interchain folding is also required to bring the two variable regions together to form a functional epitope binding site. For examples of linker orientation and size see, e.g.,  
 5 Hollinger et al. 1993 Proc Natl Acad. Sci. U.S.A. 90:6444-6448, U.S. Patent Application Publication Nos. 2005/0100543, 2005/0175606, 2007/0014794, and PCT publication Nos. WO2006/020258 and WO2007/024715, is incorporated herein by reference.

In another aspect, the antigen binding domain is a T cell receptor ("TCR"), or a fragment thereof, for example, a single chain TCR (scTCR). Methods to make such TCRs are known in the art. See, e.g.,  
 10 Willemsen RA et al, Gene Therapy 7: 1369-1377 (2000); Zhang T et al, Cancer Gene Ther 11: 487-496 (2004); Aggen et al, Gene Ther. 19(4):365-74 (2012) (references are incorporated herein by its entirety). For example, scTCR can be engineered that contains the V $\alpha$  and V $\beta$  genes from a T cell clone linked by a linker (e.g., a flexible peptide). This approach is very useful to cancer associated target that itself is intracellular, however, a fragment of such antigen (peptide) is presented on the surface of the cancer cells  
 15 by MHC.

#### *Additional exemplary antigen binding domains and CARs*

In one embodiment, an antigen binding domain against GD2 is an antigen binding portion, e.g., CDRs, of an antibody described in, e.g., Mujoo et al., Cancer Res. 47(4):1098-1104 (1987); Cheung et al.,  
 20 Cancer Res 45(6):2642-2649 (1985), Cheung et al., J Clin Oncol 5(9):1430-1440 (1987), Cheung et al., J Clin Oncol 16(9):3053-3060 (1998), Handgretinger et al., Cancer Immunol Immunother 35(3):199-204 (1992). In some embodiments, an antigen binding domain against GD2 is an antigen binding portion of an antibody selected from mAb 14.18, 14G2a, ch14.18, hu14.18, 3F8, hu3F8, 3G6, 8B6, 60C3, 10B8, ME36.1, and 8H9, see e.g., WO2012033885, WO2013040371, WO2013192294, WO2013061273,  
 25 WO2013123061, WO2013074916, and WO201385552. In some embodiments, an antigen binding domain against GD2 is an antigen binding portion of an antibody described in US Publication No.: 20100150910 or PCT Publication No.: WO 2011160119.

In one embodiment, an antigen binding domain against the Tn antigen, the sTn antigen, a Tn-O-glycopeptide antigen, or a sTn-O-glycopeptide antigen is an antigen binding portion, e.g., CDRs, of an  
 30 antibody described in, e.g., US 2014/0178365, US8,440,798, EP 2083868 A2, Brooks et al., PNAS 107(22):10056-10061 (2010), and Stone et al., OncoImmunology 1(6):863-873(2012).

In one embodiment, an antigen binding domain against PSMA is an antigen binding portion, e.g., CDRs, of an antibody described in, e.g., Parker et al., Protein Expr Purif 89(2):136-145 (2013), US

20110268656 (J591 ScFv); Frigerio et al, European J Cancer 49(9):2223-2232 (2013) (scFvD2B); WO 2006125481 (mAbs 3/A12, 3/E7 and 3/F11) and single chain antibody fragments (scFv A5 and D7).

In one embodiment, an antigen binding domain against CD97 is an antigen binding portion, e.g., CDRs, of an antibody described in, e.g., US6,846,911; de Groot et al., J Immunol 183(6):4127-4134 (2009); or an antibody from R&D:MAB3734.

In one embodiment, an antigen binding domain against TAG72 is an antigen binding portion, e.g., CDRs, of an antibody described in, e.g., Hombach et al., Gastroenterology 113(4):1163-1170 (1997); and Abcam ab691.

In one embodiment, an antigen binding domain against CD44v6 is an antigen binding portion, e.g., CDRs, of an antibody described in, e.g., Casucci et al., Blood 122(20):3461-3472 (2013).

In one embodiment, an antigen binding domain against CEA is an antigen binding portion, e.g., CDRs, of an antibody described in, e.g., Chmielewski et al., Gastroenterology 143(4):1095-1107 (2012).

In one embodiment, an antigen binding domain against EPCAM is an antigen binding portion, e.g., CDRs, of an antibody selected from MT110, EpCAM-CD3 bispecific Ab (see, e.g., clinicaltrials.gov/ct2/show/NCT00635596); Edrecolomab; 3622W94; ING-1; and adecatumumab (MT201).

In one embodiment, an antigen binding domain against KIT is an antigen binding portion, e.g., CDRs, of an antibody described in, e.g., US7915391, US20120288506, and several commercial catalog antibodies.

In one embodiment, an antigen binding domain against IL-13Ra2 is an antigen binding portion, e.g., CDRs, of an antibody described in, e.g., WO2008/146911, WO2004087758, several commercial catalog antibodies, and WO2004087758.

In one embodiment, an antigen binding domain against CD171 is an antigen binding portion, e.g., CDRs, of an antibody described in, e.g., Hong et al., J Immunother 37(2):93-104 (2014).

In one embodiment, an antigen binding domain against PSCA is an antigen binding portion, e.g., CDRs, of an antibody described in, e.g., Morgenroth et al., Prostate 67(10):1121-1131 (2007) (scFv 7F5); Nejatollahi et al., J of Oncology 2013(2013), article ID 839831 (scFv C5-II); and US Pat Publication No. 20090311181.

In one embodiment, an antigen binding domain against MAD-CT-2 is an antigen binding portion, e.g., CDRs, of an antibody described in, e.g., PMID: 2450952; US7635753.

In one embodiment, an antigen binding domain against Folate receptor alpha is an antigen binding portion, e.g., CDRs, of the antibody IMG853, or an antibody described in US20120009181; US4851332, LK26: US5952484.

In one embodiment, an antigen binding domain against ERBB2 (Her2/neu) is an antigen binding portion, e.g., CDRs, of the antibody trastuzumab, or pertuzumab.

In one embodiment, an antigen binding domain against MUC1 is an antigen binding portion, e.g., CDRs, of the antibody SAR566658.

5 In one embodiment, the antigen binding domain against EGFR is antigen binding portion, e.g., CDRs, of the antibody cetuximab, panitumumab, zalutumumab, nimotuzumab, or matuzumab.

In one embodiment, an antigen binding domain against NCAM is an antigen binding portion, e.g., CDRs, of the antibody clone 2-2B: MAB5324 (EMD Millipore)

10 In one embodiment, an antigen binding domain against CAIX is an antigen binding portion, e.g., CDRs, of the antibody clone 303123 (R&D Systems).

In one embodiment, an antigen binding domain against Fos-related antigen 1 is an antigen binding portion, e.g., CDRs, of the antibody 12F9 (Novus Biologicals).

In one embodiment, an antigen binding domain against SSEA-4 is an antigen binding portion, e.g., CDRs, of antibody MC813 (Cell Signaling), or other commercially available antibodies.

15 In one embodiment, an antigen binding domain against PDGFR-beta is an antigen binding portion, e.g., CDRs, of an antibody Abcam ab32570.

In one embodiment, an antigen binding domain against ALK is an antigen binding portion, e.g., CDRs, of an antibody described in, e.g., Mino-Kenudson et al., Clin Cancer Res 16(5):1561-1571 (2010).

20 In one embodiment, an antigen binding domain against polysialic acid is an antigen binding portion, e.g., CDRs, of an antibody described in, e.g., Nagae et al., J Biol Chem 288(47):33784-33796 (2013).

In one embodiment, an antigen binding domain against PLAC1 is an antigen binding portion, e.g., CDRs, of an antibody described in, e.g., Ghods et al., Biotechnol Appl Biochem 2013 doi:10.1002/bab.1177.

25 In one embodiment, an antigen binding domain against GloboH is an antigen binding portion of the antibody VK9; or an antibody described in, e.g., Kudryashov V et al, Glycoconj J.15(3):243-9 (1998), Lou et al., Proc Natl Acad Sci USA 111(7):2482-2487 (2014) ; MBr1: Bremer E-G et al. J Biol Chem 259:14773-14777 (1984).

30 In one embodiment, an antigen binding domain against NY-BR-1 is an antigen binding portion, e.g., CDRs of an antibody described in, e.g., Jager et al., Appl Immunohistochem Mol Morphol 15(1):77-83 (2007).

In one embodiment, an antigen binding domain against sperm protein 17 is an antigen binding portion, e.g., CDRs, of an antibody described in, e.g., Song et al., Target Oncol 2013 Aug 14 (PMID: 23943313); Song et al., Med Oncol 29(4):2923-2931 (2012).

In one embodiment, an antigen binding domain against TRP-2 is an antigen binding portion, e.g., CDRs, of an antibody described in, e.g., Wang et al, J Exp Med. 184(6):2207-16 (1996).

In one embodiment, an antigen binding domain against CYP1B1 is an antigen binding portion, e.g., CDRs, of an antibody described in, e.g., Maecker et al, Blood 102 (9): 3287-3294 (2003).

5 In one embodiment, an antigen binding domain against RAGE-1 is an antigen binding portion, e.g., CDRs, of the antibody MAB5328 (EMD Millipore).

In one embodiment, an antigen binding domain against human telomerase reverse transcriptase is an antigen binding portion, e.g., CDRs, of the antibody cat no: LS-B95-100 (Lifespan Biosciences)

10 In one embodiment, an antigen binding domain against intestinal carboxyl esterase is an antigen binding portion, e.g., CDRs, of the antibody 4F12: cat no: LS-B6190-50 (Lifespan Biosciences).

In one embodiment, an antigen binding domain against mut hsp70-2 is an antigen binding portion, e.g., CDRs, of the antibody Lifespan Biosciences: monoclonal: cat no: LS-C133261-100 (Lifespan Biosciences).

15 In one embodiment, an antigen binding domain against MAD-CT-2 is an antigen binding portion, e.g., CDRs, of an antibody described in, e.g., PMID: 2450952; US7635753.

In one embodiment, the antigen binding domain comprises one, two, or three (e.g., all three) heavy chain CDRs, HC CDR1, HC CDR2 and HC CDR3, from an antibody listed above, and/or one, two, or three (e.g., all three) light chain CDRs, LC CDR1, LC CDR2 and LC CDR3, from an antibody listed above. In one embodiment, the antigen binding domain comprises a heavy chain variable region and/or a  
20 variable light chain region of an antibody listed above.

In some embodiments, the antigen binding domain of a CAR targets a tumor antigen that is an antigen expressed on a myeloid tumor (either a surface antigen or presented by MHC), and a cell comprising such a CAR recognizes a myeloid tumor antigen.

25 In an embodiment, the myeloid tumor antigen is an antigen that is preferentially or specifically expressed on the surface of a myeloid tumor cell.

In one embodiment, the antigen-binding domain of a CAR can be chosen such that a myeloid tumor population is targeted. Alternatively, when targeting of more than one type of myeloid tumor is desired, an antigen binding domain that targets a myeloid tumor antigen that is expressed by more than one, e.g., all, of the myeloid tumors to be targeted can be selected.

30 A CAR can target the following additional tumor antigens: CD123, CD34, Flt3, CD33 and CLL-1. In embodiments, the tumor antigen is selected from CD123, CD33 and CLL-1. In some embodiments, the tumor antigen is CD123. In some embodiments, the tumor antigen is CD33. In some embodiments, the tumor antigen is CD34. In some embodiments, the tumor antigen is Flt3. In embodiments, the tumor antigen is CLL-1. In embodiments, the antigen binding domain targets the human antigen.

In one aspect, the antigen-binding domain of a CAR binds to CD123, e.g., human CD123. Any known CD123 binding domain may be used in the invention. In one embodiment, an antigen binding domain against CD123 is an antigen binding portion, e.g., CDRs or VH and VL, of an antibody, antigen-binding fragment or CAR described in, e.g., PCT publication WO2014/130635, incorporated herein by reference. In one embodiment, an antigen binding domain against CD123 is an antigen binding portion, e.g., CDRs or VH and VL, of an antibody, antigen-binding fragment or CAR described in, e.g., PCT publication WO/2016/028896, incorporated herein by reference. In one embodiment, an antigen binding domain against CD123 is an antigen binding portion, e.g., CDRs, of an antibody, antigen-binding fragment, or CAR described in, e.g., PCT publication WO1997/024373, WO2008/127735 (e.g., a CD123 binding domain of 26292, 32701, 37716 or 32703), WO2014/138805 (e.g., a CD123 binding domain of CSL362), WO2014/138819, WO2013/173820, WO2014/144622, WO2001/66139, WO2010/126066 (e.g., the CD123 binding domain of any of Old4, Old5, Old17, Old19, New102, or Old6), WO2014/144622, WO2016/028896, or US2009/0252742, incorporated herein by reference. In embodiments, the antigen binding domain is or is derived from a murine anti-human CD123 binding domain. In embodiments, the antigen binding domain is a humanized antibody or antibody fragment, e.g., scFv domain. In an embodiment, the antigen binding domain is a human antibody or antibody fragment that binds to human CD123. In embodiments, the antigen binding domain is an scFv domain which includes a light chain variable region (VL) and a heavy chain variable region (VH). The VL and VH may attached by a linker described herein, e.g., comprising the sequence GGGGSGGGGSGGGGS (SEQ ID NO: 142), and may be in any orientation, e.g., VL-linker-VH, or VH-linker-VL.

In some embodiments, the antigen binding domain of a CAR targets a B-Cell antigen. In an embodiment, the B cell antigen is an antigen that is preferentially or specifically expressed on the surface of the B cell. The antigen can be expressed on the surface of any one of the following types of B cells: progenitor B cells (e.g., pre-B cells or pro-B cells), early pro-B cells, late pro-B cells, large pre-B cells, small pre-B cells, immature B cells, e.g., naïve B cells, mature B cells, plama B cells, plasmablasts, memory B cells, B-1 cells, B-2 cells, marginal-zone B cells, follicular B cells, germinal center B cells, or regulatory B cells (Bregs).

The present disclosure provides CARs that can target the following antigens: CD19; CD123; CD22; CD30; CD171; CS-1; C-type lectin-like molecule-1, CD33; epidermal growth factor receptor variant III (EGFRvIII); ganglioside G2 (GD2); ganglioside GD3; TNF receptor family member; B-cell maturation antigen; Tn antigen ((Tn Ag) or (GalNAc $\alpha$ -Ser/Thr)); prostate-specific membrane antigen (PSMA); Receptor tyrosine kinase-like orphan receptor 1 (ROR1); Fms-Like Tyrosine Kinase 3 (FLT3); Tumor-associated glycoprotein 72 (TAG72); CD38; CD44v6; Carcinoembryonic antigen (CEA); Epithelial cell adhesion molecule (EPCAM); B7H3 (CD276); KIT (CD117); Interleukin-13 receptor

subunit alpha-2; Mesothelin; Interleukin 11 receptor alpha (IL-11Ra); prostate stem cell antigen (PSCA);  
 Protease Serine 21; vascular endothelial growth factor receptor 2 (VEGFR2); Lewis(Y) antigen; CD24;  
 Platelet-derived growth factor receptor beta (PDGFR-beta); Stage-specific embryonic antigen-4 (SSEA-  
 4); CD20; Folate receptor alpha; Receptor tyrosine-protein kinase ERBB2 (Her2/neu); Mucin 1, cell  
 5 surface associated (MUC1); epidermal growth factor receptor (EGFR); neural cell adhesion molecule  
 (NCAM); Prostase; prostatic acid phosphatase (PAP); elongation factor 2 mutated (ELF2M); Ephrin B2;  
 fibroblast activation protein alpha (FAP); insulin-like growth factor 1 receptor (IGF-I receptor), carbonic  
 anhydrase IX (CAIX); Proteasome (Prosome, Macropain) Subunit, Beta Type, 9 (LMP2); glycoprotein  
 100 (gp100); oncogene fusion protein consisting of breakpoint cluster region (BCR) and Abelson murine  
 10 leukemia viral oncogene homolog 1 (Abl) (bcr-abl); tyrosinase; ephrin type-A receptor 2 (EphA2);  
 Fucosyl GM1; sialyl Lewis adhesion molecule (sLe); ganglioside GM3; transglutaminase 5 (TGS5); high  
 molecular weight-melanoma-associated antigen (HMWMAA); o-acetyl-GD2 ganglioside (OAcGD2);  
 Folate receptor beta; tumor endothelial marker 1 (TEM1/CD248); tumor endothelial marker 7-related  
 (TEM7R); claudin 6 (CLDN6); thyroid stimulating hormone receptor (TSHR); G protein-coupled  
 15 receptor class C group 5, member D (GPRC5D); chromosome X open reading frame 61 (CXORF61);  
 CD97; CD179a; anaplastic lymphoma kinase (ALK); Polysialic acid; placenta-specific 1 (PLAC1);  
 hexasaccharide portion of globoH glycosphingolipid (GloboH); mammary gland differentiation antigen (NY-  
 BR-1); uroplakin 2 (UPK2); Hepatitis A virus cellular receptor 1 (HAVCR1); adrenoceptor beta 3  
 (ADRB3); pannexin 3 (PANX3); G protein-coupled receptor 20 (GPR20); lymphocyte antigen 6  
 20 complex, locus K 9 (LY6K); Olfactory receptor 51E2 (OR51E2); TCR Gamma Alternate Reading Frame  
 Protein (TARP); Wilms tumor protein (WT1); Cancer/testis antigen 1 (NY-ESO-1); Cancer/testis antigen  
 2 (LAGE-1a); Melanoma-associated antigen 1 (MAGE-A1); ETS translocation-variant gene 6, located on  
 chromosome 12p (ETV6-AML); sperm protein 17 (SPA17); X Antigen Family, Member 1A (XAGE1);  
 angiopoietin-binding cell surface receptor 2 (Tie 2); melanoma cancer testis antigen-1 (MAD-CT-1);  
 25 melanoma cancer testis antigen-2 (MAD-CT-2); Fos-related antigen 1; tumor protein p53 (p53); p53  
 mutant; prostatein; surviving; telomerase; prostate carcinoma tumor antigen-1, melanoma antigen  
 recognized by T cells 1; Rat sarcoma (Ras) mutant; human Telomerase reverse transcriptase (hTERT);  
 sarcoma translocation breakpoints; melanoma inhibitor of apoptosis (ML-IAP); ERG (transmembrane  
 protease, serine 2 (TMPRSS2) ETS fusion gene); N-Acetyl glucosaminyl-transferase V (NA17); paired  
 30 box protein Pax-3 (PAX3); Androgen receptor; Cyclin B1; v-myc avian myelocytomatosis viral oncogene  
 neuroblastoma derived homolog (MYCN); Ras Homolog Family Member C (RhoC); Tyrosinase-related  
 protein 2 (TRP-2); Cytochrome P450 1B1 (CYP1B1); CCCTC-Binding Factor (Zinc Finger Protein)-  
 Like, Squamous Cell Carcinoma Antigen Recognized By T Cells 3 (SART3); Paired box protein Pax-5  
 (PAX5); proacrosin binding protein sp32 (OY-TES1); lymphocyte-specific protein tyrosine kinase

(LCK); A kinase anchor protein 4 (AKAP-4); synovial sarcoma, X breakpoint 2 (SSX2); Receptor for Advanced Glycation Endproducts (RAGE-1); renal ubiquitous 1 (RU1); renal ubiquitous 2 (RU2); legumain; human papilloma virus E6 (HPV E6); human papilloma virus E7 (HPV E7); intestinal carboxyl esterase; heat shock protein 70-2 mutated (mut hsp70-2); CD79a; CD79b; CD72; Leukocyte-associated immunoglobulin-like receptor 1 (LAIR1); Fc fragment of IgA receptor (FCAR or CD89); Leukocyte immunoglobulin-like receptor subfamily A member 2 (LILRA2); CD300 molecule-like family member f (CD300LF); C-type lectin domain family 12 member A (CLEC12A); bone marrow stromal cell antigen 2 (BST2); EGF-like module-containing mucin-like hormone receptor-like 2 (EMR2); lymphocyte antigen 75 (LY75); Glypican-3 (GPC3); Fc receptor-like 5 (FCRL5); immunoglobulin lambda-like polypeptide 1 (IGLL1); TNF receptor family member; Fms-Like Tyrosine Kinase 3 (FLT3); CD10, CD19, CD20, CD21, CD22, CD23, CD24, CD25, CD37, CD38, CD53, CD72, CD73, CD74, CD75, CD77, CD79a, CD79b, CD80, CD81, CD82, CD83, CD84, CD85, ROR1, BCMA, CD86, and CD179b. Other B cell antigens that can be targeted by a CAR described herein include: CD1a, CD1b, CD1c, CD1d, CD2, CD5, CD6, CD9, CD11a, CD11b, CD11c, CD17, CD18, CD26, CD27, CD29, CD30, CD31, CD32a, CD32b, CD35, CD38, CD39, CD40, CD44, CD45, CD45RA, CD45RB, CD45RC, CD45RO, CD46, CD47, CD48, CD49b, CD49c, CD49d, CD50, CD52, CD54, CD55, CD58, CD60a, CD62L, CD63, CD63, CD68, CD69, CD70, CD85E, CD85I, CD85J, CD92, CD95, CD97, CD98, CD99, CD100, CD102, CD108, CD119, CD120a, CD120b, CD121b, CD122, CD124, CD125, CD126, CD130, CD132, CD137, CD138, CD139, CD147, CD148, CD150, CD152, CD162, CD164, CD166, CD167a, CD170, CD175, CD175s, CD180, CD184, CD185, CD192, CD196, CD197, CD200, CD205, CD210a, CD210b, CD212, CD213a1, CD213a2, CD215, CD217, CD218a, CD218b, CD220, CD221, CD224, CD225, CD226, CD227, CD229, CD230, CD232, CD252, CD253, CD257, CD258, CD261, CD262, CD263, CD264, CD267, CD268, CD269, CD270, CD272, CD274, CD275, CD277, CD279, CD283, CD289, CD290, CD295, CD298, CD300a, CD300c, CD305, CD306, CD307a, CD307b, CD307c, CD307d, CD307e, CD314, CD315, CD316, CD317, CD319, CD321, CD327, CD328, CD329, CD338, CD351, CD352, CD353, CD354, CD355, CD357, CD358, CD360, CD361, CD362, and CD363.

In another embodiment, the antigen targeted by the CAR is chosen from CD19, BCMA, CD20, CD22, FcRn5, FcRn2, CS-1 and CD138. In an embodiment, the antigen targeted by the CAR is CD19. In an embodiment, the antigen targeted by the CAR is CD20. In an embodiment, the antigen targeted by the CAR is CD22. In an embodiment, the antigen targeted by the CAR is BCMA. In an embodiment, the antigen targeted by the CAR is FcRn5. In an embodiment, the antigen targeted by the CAR is FcRn2. In an embodiment, the antigen targeted by the CAR is CS-1. In an embodiment, the antigen targeted by the CAR is CD138.

In one embodiment, the antigen-binding domain of a CAR, e.g., the CAR expressed by a cell of the invention (e.g., a cell that also expresses a CAR), can be chosen such that a preferred B cell population is targeted. For example, in an embodiment where targeting of B regulatory cells is desired, an antigen binding domain is selected that targets an antigen that is expressed on regulatory B cells and not on other B cell populations, e.g., plasma B cells and memory B cells. Cell surface markers expressed on regulatory B cells include: CD19, CD24, CD25, CD38, or CD86, or markers described in He et al., 2014, *J Immunology Research*, Article ID 215471. When targeting of more than one type of B cells is desired, an antigen binding domain that targets an antigen that is expressed by all of the B cells to be targeted can be selected.

### CAR Transmembrane domain

With respect to the transmembrane domain, in various embodiments, a CAR can be designed to comprise a transmembrane domain that is attached to the extracellular domain of the CAR. A transmembrane domain can include one or more additional amino acids adjacent to the transmembrane region, e.g., one or more amino acid associated with the extracellular region of the protein from which the transmembrane was derived (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 up to 15 amino acids of the extracellular region) and/or one or more additional amino acids associated with the intracellular region of the protein from which the transmembrane protein is derived (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 up to 15 amino acids of the intracellular region). In one aspect, the transmembrane domain is one that is associated with one of the other domains of the CAR. In some instances, the transmembrane domain can be selected or modified by amino acid substitution to avoid binding of such domains to the transmembrane domains of the same or different surface membrane proteins, e.g., to minimize interactions with other members of the receptor complex. In one aspect, the transmembrane domain is capable of homodimerization with another CAR on the cell surface of a CAR-expressing cell. In a different aspect, the amino acid sequence of the transmembrane domain may be modified or substituted so as to minimize interactions with the binding domains of the native binding partner present in the same CART.

The transmembrane domain may be derived either from a natural or from a recombinant source. Where the source is natural, the domain may be derived from any membrane-bound or transmembrane protein. In one aspect the transmembrane domain is capable of signaling to the intracellular domain(s) whenever the CAR has bound to a target. A transmembrane domain of particular use in this invention may include at least the transmembrane region(s) of e.g., the alpha, beta or zeta chain of the T-cell receptor, CD28, CD27, CD3 epsilon, CD45, CD4, CD5, CD8, CD9, CD16, CD22, CD33, CD37, CD64, CD80, CD86, CD134, CD137, CD154. In some embodiments, a transmembrane domain may include at least the transmembrane region(s) of, e.g., KIR2DS2, OX40, CD2, CD27, LFA-1 (CD11a, CD18), ICOS



(CD278), 4-1BB (CD137), GITR, CD40, BAFFR, HVEM (LIGHTR), SLAMF7, NKp80 (KLRP1), NKp44, NKp30, NKp46, CD160, CD19, IL2R beta, IL2R gamma, IL7R  $\alpha$ , ITGA1, VLA1, CD49a, ITGA4, IA4, CD49D, ITGA6, VLA-6, CD49f, ITGAD, CD11d, ITGAE, CD103, ITGAL, CD11a, LFA-1, ITGAM, CD11b, ITGAX, CD11c, ITGB1, CD29, ITGB2, CD18, LFA-1, ITGB7, TNFR2, DNAM1  
 5 (CD226), SLAMF4 (CD244, 2B4), CD84, CD96 (Tactile), CEACAM1, CRTAM, Ly9 (CD229), CD160 (BY55), PSGL1, CD100 (SEMA4D), SLAMF6 (NTB-A, Ly108), SLAM (SLAMF1, CD150, IPO-3), BLAME (SLAMF8), SELPLG (CD162), LTBR, PAG/Cbp, NKG2D, NKG2C.

In some instances, the transmembrane domain can be attached to the extracellular region of the CAR, e.g., the antigen binding domain of the CAR, via a hinge, e.g., a hinge from a human protein. For  
 10 example, in one embodiment, the hinge can be a human Ig (immunoglobulin) hinge, e.g., an IgG4 hinge, or a CD8a hinge. In one embodiment, the hinge or spacer comprises (e.g., consists of) the amino acid sequence of SEQ ID NO: 147. In one aspect, the transmembrane domain comprises (e.g., consists of) a transmembrane domain of SEQ ID NO: 155.

In one aspect, the hinge or spacer comprises an IgG4 hinge. For example, in one embodiment,  
 15 the hinge or spacer comprises a hinge of the amino acid sequence  
 ESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVE  
 VHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQ  
 VYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTV  
 DKSRWQEGNVFSCSVMHEALHNHYTQKSLSLGLKGM (SEQ ID NO: 149). In some embodiments,  
 20 the hinge or spacer comprises a hinge encoded by a nucleotide sequence of  
 GAGAGCAAGTACGGCCCTCCCTGCCCCCCTTGCCCTGCCCCGAGTTCCTGGGCGGACCCAG  
 CGTGTTCTGTTCCTGTTCCCCCAAGCCCAAGGACACCCTGATGATCAGCCGGACCCCGAGGTGA  
 CCTGTGTGGTGGTGGACGTGTCCCAGGAGGACCCCGAGGTCCAGTTCAACTGGTACGTGGAC  
 GCGGTGGAGGTGCACAACGCCAAGACCAAGCCCCGGGAGGAGCAGTTCAATAGCACCTACC  
 25 GGGTGGTGTCCGTGCTGACCGTGCTGCACCAGGACTGGCTGAACGGCAAGGAATACAAGTG  
 TAAGGTGTCCAACAAGGGCCTGCCCAGCAGCATCGAGAAAACCATCAGCAAGGCCAAGGGC  
 CAGCCTCGGGAGCCCCAGGTGTACACCCTGCCCCCTAGCCAAGAGGAGATGACCAAGAACC  
 AGGTGTCCCTGACCTGCCTGGTGAAGGGCTTCTACCCAGCGACATCGCCGTGGAGTGGGAG  
 AGCAACGGCCAGCCCGAGAACAACACTACAAGACCACCCCCCTGTGCTGGACAGCGACGGCA  
 30 GCTTCTTCCTGTACAGCCGGCTGACCGTGGACAAGAGCCGGTGGCAGGAGGGCAACGTCTTT  
 AGCTGCTCCGTGATGCACGAGGCCCTGCACAACCACTACACCCAGAAGAGCCTGAGCCTGTC  
 CCTGGGCAAGATG (SEQ ID NO: 150).

In one aspect, the hinge or spacer comprises an IgD hinge. For example, in one embodiment, the hinge or spacer comprises a hinge of the amino acid sequence

RWPESPKAQASSVPTAQPPAEGSLAKATTAPATTRNTGRGGEEKKKEKEKEEQEERETKTPECP  
 SHTQPLGVYLLTPAVQDLWLRDKATFTCFVVGSDLKDAHLTWEVAGKVPTGGVEEGLLERHSN  
 GSQSQHSRLTLPRSLWNAGTSVTCTLNHPSPQRLMALREPAAQAPVKLSLNLLASSDPPEAAS  
 WLLCEVSGFSPNILLMWLEDQREVNTSGFAPARPPPQPGSTTFWAWSVLRVPAPPSPQPATYTC  
 5 VVSHEDSRLLNASRSLEVSYVTDH (SEQ ID NO: 151). In some embodiments, the hinge or spacer  
 comprises a hinge encoded by a nucleotide sequence of  
 AGGTGGCCCGAAAGTCCCAAGGCCAGGCATCTAGTGTTCTACTGCACAGCCCCAGGCAG  
 AAGGCAGCCTAGCCAAAGCTACTACTGCACCTGCCACTACGCGCAATACTGGCCGTGGCGG  
 GGAGGAGAAGAAAAAGGAGAAAGAGAAAGAAGAACAGGAAGAGAGGGAGACCAAGACCC  
 10 CTGAATGTCCATCCCATAACCCAGCCGCTGGGCGTCTATCTCTTGACTCCCGCAGTACAGGAC  
 TTGTGGCTTAGAGATAAGGCCACCTTTACATGTTTTCGTCGTGGGCTCTGACCTGAAGGATGC  
 CCATTTGACTTGGGAGGTTGCCGAAAGGTACCCACAGGGGGGGTTGAGGAAGGGTTGCTG  
 GAGCGCCATTCCAATGGCTCTCAGAGCCAGCACTCAAGACTCACCTTCCGAGATCCCTGTG  
 GAACGCCGGGACCTCTGTACATGTACTCTAAATCATCCTAGCCTGCCCCACAGCGTCTGA  
 15 TGGCCCTTAGAGAGCCAGCCGCCCAGGCACCAGTTAAGCTTAGCCTGAATCTGCTCGCCAGT  
 AGTGATCCCCCAGAGGCCGCCAGCTGGCTCTTATGCGAAGTGTCCGGCTTTAGCCCGCCCAA  
 CATCTTGCTCATGTGGCTGGAGGACCAGCGAGAAGTGAACACCAGCGGCTTCGCTCCAGCCC  
 GGCCCCACCCCAGCCGGGTTCTACCACATTCTGGGCCTGGAGTGTCTTAAGGGTCCCAGCA  
 CCACCTAGCCCCCAGCCAGCCACATACACCTGTGTTGTGTCCCATGAAGATAGCAGGACCCT  
 20 GCTAAATGCTTCTAGGAGTCTGGAGGTTTCCTACGTGACTGACCATT (SEQ ID NO: 152).

In one aspect, the transmembrane domain may be recombinant, in which case it will comprise predominantly hydrophobic residues such as leucine and valine. In one aspect a triplet of phenylalanine, tryptophan and valine can be found at each end of a recombinant transmembrane domain.

Optionally, a short oligo- or polypeptide linker, between 2 and 10 amino acids in length may form  
 25 the linkage between the transmembrane domain and the cytoplasmic region of the CAR. A glycine-serine doublet provides a particularly suitable linker. For example, in one aspect, the linker comprises the amino acid sequence of GGGGSGGGGS (SEQ ID NO: 153). In some embodiments, the linker is encoded by a nucleotide sequence of GGTGGCGGAGGTTCTGGAGGTGGAGGTTCC (SEQ ID NO: 154).

In one aspect, the hinge or spacer comprises a KIR2DS2 hinge.

30

## Cytoplasmic domain

The cytoplasmic domain or region of the CAR includes an intracellular signaling domain. An intracellular signaling domain is generally responsible for activation of at least one of the normal effector functions of the immune cell in which the CAR has been introduced.

5        Examples of intracellular signaling domains for use in a CAR described herein include the cytoplasmic sequences of the T cell receptor (TCR) and co-receptors that act in concert to initiate signal transduction following antigen receptor engagement, as well as any derivative or variant of these sequences and any recombinant sequence that has the same functional capability.

10        It is known that signals generated through the TCR alone are insufficient for full activation of the T cell and that a secondary and/or costimulatory signal is also required. Thus, T cell activation can be said to be mediated by two distinct classes of cytoplasmic signaling sequences: those that initiate antigen-dependent primary activation through the TCR (primary intracellular signaling domains) and those that act in an antigen-independent manner to provide a secondary or costimulatory signal (secondary cytoplasmic domain, e.g., a costimulatory domain).

15        A primary signaling domain regulates primary activation of the TCR complex either in a stimulatory way, or in an inhibitory way. Primary intracellular signaling domains that act in a stimulatory manner may contain signaling motifs which are known as immunoreceptor tyrosine-based activation motifs or ITAMs.

20        Examples of ITAM containing primary intracellular signaling domains that are of particular use in the invention include those of TCR zeta, FcR gamma, FcR beta, CD3 gamma, CD3 delta, CD3 epsilon, CD5, CD22, CD79a, CD79b, CD278 (also known as "ICOS"), FcεRI, DAP10, DAP12, and CD66d. In one embodiment, a CAR of the invention comprises an intracellular signaling domain, e.g., a primary signaling domain of CD3-zeta, e.g., a CD3-zeta sequence described herein.

25        In one embodiment, a primary signaling domain comprises a modified ITAM domain, e.g., a mutated ITAM domain which has altered (e.g., increased or decreased) activity as compared to the native ITAM domain. In one embodiment, a primary signaling domain comprises a modified ITAM-containing primary intracellular signaling domain, e.g., an optimized and/or truncated ITAM-containing primary intracellular signaling domain. In an embodiment, a primary signaling domain comprises one, two, three, four or more ITAM motifs.

30

## Costimulatory Signaling Domain

The intracellular signalling domain of the CAR can comprise the CD3-zeta signaling domain by itself or it can be combined with any other desired intracellular signaling domain(s) useful in the context

of a CAR of the invention. For example, the intracellular signaling domain of the CAR can comprise a CD3 zeta chain portion and a costimulatory signaling domain. The costimulatory signaling domain refers to a portion of the CAR comprising the intracellular domain of a costimulatory molecule. In one embodiment, the intracellular domain is designed to comprise the signaling domain of CD3-zeta and the signaling domain of CD28. In one aspect, the intracellular domain is designed to comprise the signaling domain of CD3-zeta and the signaling domain of ICOS.

A costimulatory molecule can be a cell surface molecule other than an antigen receptor or its ligands that is required for an efficient response of lymphocytes to an antigen. Examples of such molecules include CD27, CD28, 4-1BB (CD137), OX40, CD30, CD40, PD-1, ICOS, lymphocyte function-associated antigen-1 (LFA-1), CD2, CD7, LIGHT, NKG2C, B7-H3, and a ligand that specifically binds with CD83, and the like. For example, CD27 costimulation has been demonstrated to enhance expansion, effector function, and survival of human CART cells in vitro and augments human T cell persistence and antitumor activity in vivo (Song et al. Blood. 2012; 119(3):696-706). Further examples of such costimulatory molecules include CDS, ICAM-1, GITR, BAFFR, HVEM (LIGHTR), SLAMF7, NKp80 (KLRF1), NKp30, NKp44, NKp46, CD160, CD19, CD4, CD8alpha, CD8beta, IL2R beta, IL2R gamma, IL7R alpha, ITGA4, VLA1, CD49a, ITGA4, IA4, CD49D, ITGA6, VLA-6, CD49f, ITGAD, CD11d, ITGAE, CD103, ITGAL, CD11a, LFA-1, ITGAM, CD11b, ITGAX, CD11c, ITGB1, CD29, ITGB2, CD18, LFA-1, ITGB7, TNFR2, TRANCE/RANKL, DNAM1 (CD226), SLAMF4 (CD244, 2B4), CD84, CD96 (Tactile), CEACAM1, CRTAM, Ly9 (CD229), CD160 (BY55), PSGL1, CD100 (SEMA4D), CD69, SLAMF6 (NTB-A, Ly108), SLAM (SLAMF1, CD150, IPO-3), BLAME (SLAMF8), SELPLG (CD162), LTBR, LAT, GADS, SLP-76, NKG2D, NKG2C and PAG/Cbp.

The intracellular signaling sequences within the cytoplasmic portion of the CAR may be linked to each other in a random or specified order. Optionally, a short oligo- or polypeptide linker, for example, between 2 and 10 amino acids (e.g., 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acids) in length may form the linkage between intracellular signaling sequence. In one embodiment, a glycine-serine doublet can be used as a suitable linker. In one embodiment, a single amino acid, e.g., an alanine, a glycine, can be used as a suitable linker.

In one aspect, the intracellular signaling domain is designed to comprise two or more, e.g., 2, 3, 4, 5, or more, costimulatory signaling domains. In an embodiment, the two or more, e.g., 2, 3, 4, 5, or more, costimulatory signaling domains, are separated by a linker molecule, e.g., a linker molecule described herein. In one embodiment, the intracellular signaling domain comprises two costimulatory signaling domains. In some embodiments, the linker molecule is a glycine residue. In some embodiments, the linker is an alanine residue.

In one aspect, the intracellular signaling domain is designed to comprise the signaling domain of CD3-zeta and the signaling domain of CD28. In one aspect, the intracellular signaling domain is designed to comprise the signaling domain of CD3-zeta and the signaling domain of 4-1BB. In one aspect, the signaling domain of 4-1BB is a signaling domain of SEQ ID NO: 158. In one aspect, the signaling domain of CD3-zeta is a signaling domain of SEQ ID NO: 163.

In one aspect, the intracellular signaling domain is designed to comprise the signaling domain of CD3-zeta and the signaling domain of CD27. In one aspect, the signaling domain of CD27 comprises an amino acid sequence of QRRKYRSNKGESPVPAEPCRYSCPREEEGSTIPIQEDYRKPEPACSP (SEQ ID NO: 161). In one aspect, the signalling domain of CD27 is encoded by a nucleic acid sequence of AGGAGTAAGAGGAGCAGGCTCCTGCACAGTGACTACATGAACATGACTCCCCGCCGCCCGGCCCCACCCGCAAGCATTACCAGCCCTATGCCCCACCACGCGACTTCGCAGCCTATCGCTCC (SEQ ID NO: 162).

In one aspect, the CAR-expressing cell described herein can further comprise a second CAR, e.g., a second CAR that includes a different antigen binding domain, e.g., to the same target or a different target (e.g., a target other than a cancer associated antigen described herein or a different cancer associated antigen described herein, e.g., CD19, CD33, CLL-1, CD34, FLT3, or folate receptor beta). In one embodiment, the second CAR includes an antigen binding domain to a target expressed the same cancer cell type as the cancer associated antigen. In one embodiment, the CAR-expressing cell comprises a first CAR that targets a first antigen and includes an intracellular signaling domain having a costimulatory signaling domain but not a primary signaling domain, and a second CAR that targets a second, different, antigen and includes an intracellular signaling domain having a primary signaling domain but not a costimulatory signaling domain. While not wishing to be bound by theory, placement of a costimulatory signaling domain, e.g., 4-1BB, CD28, ICOS, CD27 or OX-40, onto the first CAR, and the primary signaling domain, e.g., CD3 zeta, on the second CAR can limit the CAR activity to cells where both targets are expressed. In one embodiment, the CAR expressing cell comprises a first cancer associated antigen CAR that includes an antigen binding domain that binds a target antigen described herein, a transmembrane domain and a costimulatory domain and a second CAR that targets a different target antigen (e.g., an antigen expressed on that same cancer cell type as the first target antigen) and includes an antigen binding domain, a transmembrane domain and a primary signaling domain. In another embodiment, the CAR expressing cell comprises a first CAR that includes an antigen binding domain that binds a target antigen described herein, a transmembrane domain and a primary signaling domain and a second CAR that targets an antigen other than the first target antigen (e.g., an antigen expressed on the same cancer cell type as the first target antigen) and includes an antigen binding domain to the antigen, a transmembrane domain and a costimulatory signaling domain.

In another aspect, the disclosure features a population of CAR-expressing cells, e.g., CART cells. In some embodiments, the population of CAR-expressing cells comprises a mixture of cells expressing different CARs. For example, in one embodiment, the population of CART cells can include a first cell expressing a CAR having an antigen binding domain to a cancer associated antigen described herein, and  
 5 a second cell expressing a CAR having a different antigen binding domain, e.g., an antigen binding domain to a different a cancer associated antigen described herein, e.g., an antigen binding domain to a cancer associated antigen described herein that differs from the cancer associate antigen bound by the antigen binding domain of the CAR expressed by the first cell. As another example, the population of CAR-expressing cells can include a first cell expressing a CAR that includes an antigen binding domain  
 10 to a cancer associated antigen described herein, and a second cell expressing a CAR that includes an antigen binding domain to a target other than a cancer associate antigen as described herein. In one embodiment, the population of CAR-expressing cells includes, e.g., a first cell expressing a CAR that includes a primary intracellular signaling domain, and a second cell expressing a CAR that includes a secondary signaling domain.

In another aspect, the disclosure features a population of cells wherein at least one cell in the population expresses a CAR having an antigen binding domain to a cancer associated antigen described herein, and a second cell expressing another agent, e.g., an agent which enhances the activity of a CAR-expressing cell. For example, in one embodiment, the agent can be an agent which inhibits an inhibitory molecule. Inhibitory molecules, e.g., PD-1, can, in some embodiments, decrease the ability of a CAR-  
 20 expressing cell to mount an immune effector response. Examples of inhibitory molecules include PD-1, PD-L1, CTLA4, TIM3, CEACAM (CEACAM-1, CEACAM-3, and/or CEACAM-5), LAG3, VISTA, BTLA, TIGIT, LAIR1, CD160, 2B4, CD80, CD86, B7-H3 (CD276), B7-H4 (VTCN1), HVEM (TNFRSF14 or CD270), KIR, A2aR, MHC class I, MHC class II, GAL9, adenosine, and TGF (e.g., TGFbeta). In one embodiment, the agent which inhibits an inhibitory molecule comprises a first  
 25 polypeptide, e.g., an inhibitory molecule, associated with a second polypeptide that provides a positive signal to the cell, e.g., an intracellular signaling domain described herein. In one embodiment, the agent comprises a first polypeptide, e.g., of an inhibitory molecule such as PD-1, PD-L1, CTLA4, TIM3, CEACAM (CEACAM-1, CEACAM-3, and/or CEACAM-5), LAG3, VISTA, BTLA, TIGIT, LAIR1, CD160, 2B4 and TGF beta, or a fragment of any of these, and a second polypeptide which is an  
 30 intracellular signaling domain described herein (e.g., comprising a costimulatory domain (e.g., 41BB, CD27, OX40 or CD28, e.g., as described herein) and/or a primary signaling domain (e.g., a CD3 zeta signaling domain described herein). In one embodiment, the agent comprises a first polypeptide of PD-1 or a fragment thereof, and a second polypeptide of an intracellular signaling domain described herein (e.g., a CD28 signaling domain described herein and/or a CD3 zeta signaling domain described herein).

## Regulatory Polypeptides of Interest

In some embodiments, the heterologous polypeptide of interest linked to a COF1/CRBN-, COF2/CRBN-, or COF3/CRBN-binding polypeptide and/or a degradation domain is a regulatory protein.

5 Provided herein are regulatory polypeptides and regulatory polypeptide encoding sequences useful in genetic control circuits, cells, and methods for identifying, selecting or making a cell or cell line capable of producing high yields of a product, e.g., a recombinant or therapeutic polypeptide. In general, regulatory polypeptides regulate expression of the product, e.g., a recombinant or therapeutic polypeptide. In some embodiments, the regulatory polypeptide is a gene-editing polypeptide. In some embodiments, 10 the regulatory polypeptide encoding sequence is under the transcriptional control of a control element which activates transcription of the regulatory polypeptide encoding sequence dependent on one or more conditions. In some embodiments, a regulatory polypeptide binds to the control element, e.g., promoter element, operably linked to the recombinant or therapeutic polypeptide encoding sequence. In some embodiments, binding of the regulatory polypeptide to a control element inhibits transcription of the 15 operably linked recombinant or therapeutic polypeptide encoding sequence. In some embodiments, a regulatory polypeptide binds to a sequence encoding an untranslated region of the transcript of the recombinant or therapeutic polypeptide. In some embodiments, binding of the regulatory polypeptide to an untranslated region of the transcript of the recombinant or therapeutic polypeptide inhibits translation of the recombinant or therapeutic polypeptide encoding sequence. In some embodiments, a regulatory 20 polypeptide binds to the coding sequence of the recombinant or therapeutic polypeptide encoding sequence. In some embodiments, binding of the regulatory polypeptide to the coding sequence of the recombinant or therapeutic polypeptide inhibits transcription, translation, or transcription and translation of the recombinant or therapeutic polypeptide encoding sequence.

It is contemplated that the present disclosure is not specific to a particular regulatory polypeptide. 25 Exemplary regulatory polypeptides include but are not limited to: Cas9 molecules, TALE molecules, and zinc finger molecules. In some embodiments, the regulatory polypeptide is a Cas-related protein known in the art. In some embodiments, the regulatory polypeptide is a protein from a type I, II, or III CRISPR/Cas system (e.g. as described in K. S. Makarova et al., Nat. Rev. Microbiol. 9, 467 (2011); K. S. Makarova, N. V. Grishin, S. A. Shabalina, Y. I. Wolf, E. V. Koonin, Biol. Direct 1, 7 (2006); or K. S. Makarova, L. Aravind, Y. I. Wolf, E. V. Koonin, Biol. Direct 6, 38 (2011)). 30

In some embodiments, the regulatory polypeptide is a Cas9 molecule. Regulatory polypeptides that are Cas9 molecules require one or more (e.g., one, two, three, four or more) suitable gRNAs to inhibit expression of a recombinant or therapeutic polypeptide.

In some embodiments, the regulatory polypeptide is a TALE molecule.

In some embodiments, the regulatory polypeptide is a zinc finger molecule.

In some embodiments, the regulatory polypeptide is an endogenous regulator of the first control element, e.g., the first promoter element. In an embodiment, the endogenous gene encoding the regulatory polypeptide is inactive, e.g., has been knocked out or mutated to produce a loss of function.

5

### **Cas9 Molecules and Other Components of the CRISPR/CAS System**

In some embodiments, the heterologous polypeptide of interest linked to a COF1/CRBN-, COF2/CRBN-, or COF3/CRBN-binding polypeptide and/or a degradation domain is a Cas9 molecule, a Cas12 molecule, a Cas13 molecule, or another component of the CRISPR/CAS system (e.g., a ribonucleoprotein (RNP) molecule). For gene therapies using the CRISPR/CAS system, one important consideration is to limit side effects caused by the off-target activity of a Cas molecule (e.g., a Cas9 molecule). Fusing a degron, e.g., a COF1/CRBN-, COF2/CRBN-, or COF3/CRBN-binding polypeptide described herein, e.g., the HiLD tag or CARB tag described herein, to a component of the CRISPR/CAS system (e.g., a Cas9 molecule or a RNP molecule) helps to generate a gene therapy where the activity of the CRISPR/CAS system can be regulated by a degradation compound described herein, e.g., in the event of side effects.

Cas9 molecules to be used in the genetic control circuits, cells, and methods of the present disclosure may comprise polypeptides originating in a variety of species. In addition, one or more domains from a Cas9 molecule in one species may be combined with one or more domains from a Cas9 molecule in another species, e.g., in a fusion protein. Additional Cas9 polypeptide comprising species include: *Acidovorax avenae*, *Actinobacillus pleuropneumoniae*, *Actinobacillus succinogenes*, *Actinobacillus suis*, *Actinomyces* sp., *cycliphilus denitrificans*, *Aminomonas paucivorans*, *Bacillus cereus*, *Bacillus smithii*, *Bacillus thuringiensis*, *Bacteroides* sp., *Blastopirellula marina*, *Bradyrhizobium* sp., *Brevibacillus laterosporus*, *Campylobacter coli*, *Campylobacter jejuni*, *Campylobacter lari*, *Candidatus Puniceispirillum*, *Clostridium cellulolyticum*, *Clostridium perfringens*, *Corynebacterium accolens*, *Corynebacterium diphtheria*, *Corynebacterium matruchotii*, *Dinoroseobacter shibae*, *Eubacterium dolichum*, *gamma proteobacterium*, *Gluconacetobacter diazotrophicus*, *Haemophilus parainfluenzae*, *Haemophilus sputorum*, *Helicobacter canadensis*, *Helicobacter cinaedi*, *Helicobacter mustelae*, *Ilyobacter polytropus*, *Kingella kingae*, *Lactobacillus crispatus*, *Listeria ivanovii*, *Listeria monocytogenes*, *Listeriaceae bacterium*, *Methylocystis* sp., *Methylosinus trichosporium*, *Mobiluncus mulieris*, *Neisseria bacilliformis*, *Neisseria cinerea*, *Neisseria flavescens*, *Neisseria lactamica*, *Neisseria meningitidis*, *Neisseria* sp., *Neisseria wadsworthii*, *Nitrosomonas* sp., *Parvibaculum lavamentivorans*, *Pasteurella multocida*, *Phascolarctobacterium succinatutens*, *Ralstonia syzygii*, *Rhodopseudomonas palustris*, *Rhodovulum* sp., *Simonsiella muelleri*, *Sphingomonas* sp., *Sporolactobacillus vineae*,



*Staphylococcus lugdunensis*, *Streptococcus* sp., *Subdoligranulum* sp., *Tistrella mobilis*, *Treponema* sp., or *Verminephrobacter eiseniae*.

Cas12 molecules (e.g., Cas12a, Cas12b, and Cas12c) have been disclosed, e.g., in Chen et al., Science. 2018 Apr 27;360(6387):436-439 and Shmakov et al., Nat Rev Microbiol. 2017 Mar;15(3):169-182, herein incorporated by reference in their entireties. CRISPR-Cas12a (Cpf1) proteins are RNA-guided enzymes that bind DNA and generate targeted, double-stranded DNA breaks. Like CRISPR-Cas9, Cas12 is also a useful tool in genome editing. Additional Cas molecules that are useful for gene editing include, but not limited to, Cas13, e.g., Cas13a, Cas13b, and Cas13c, as disclosed in, e.g., WO2017219027 and Shmakov et al., Nat Rev Microbiol. 2017 Mar;15(3):169-182, herein incorporated by reference in their entireties. In some embodiments, the heterologous polypeptide of interest is Cas12. In some embodiments, the heterologous polypeptide of interest is Cas13.

#### Cas9 Structure and Activity

Crystal structures are available for naturally occurring Cas9 polypeptides (Jinek et al., Science, 343(6176):1247997, 2014) and for *S. pyogenes* Cas9 with a guide RNA (e.g., a synthetic fusion of crRNA and tracrRNA) (Nishimasu et al., Cell, 156:935-949, 2014; and Anders et al., Nature, 2014, doi: 10.1038/nature13579).

In an embodiment, a Cas9 molecule or Cas9 polypeptide comprises one or more of the following domains: a RuvC-like domain and an HNH-like domain. In an embodiment, a Cas9 molecule or Cas9 polypeptide is a dCas9 molecule or dCas9 polypeptide and the dCas9 molecule or dCas9 polypeptide comprises a RuvC-like domain, e.g., a RuvC-like domain that lacks nuclease activity, and/or an HNH-like domain, e.g., an HNH-like domain that lacks nuclease activity.

In an embodiment, the Cas9 molecule or Cas9 polypeptide can include more than one RuvC-like domain (e.g., one, two, three or more RuvC-like domains). In an embodiment, a RuvC-like domain comprises one or more mutations that alter its activity, such that the RuvC domain does not cleave DNA or has reduced DNA cleaving activity. In an embodiment, a RuvC-like domain is at least 5, 6, 7, 8 amino acids in length but not more than 20, 19, 18, 17, 16 or 15 amino acids in length. In an embodiment, the Cas9 molecule or Cas9 polypeptide comprises an N-terminal RuvC-like domain of about 10 to 20 amino acids, e.g., about 15 amino acids in length.

In an embodiment, the Cas9 molecule or Cas9 polypeptide can include more than one HNH-like domain (e.g., one, two, three or more HNH-like domains). In an embodiment, an HNH-like domain comprises one or more mutations that alter its activity, such that the HNH-like domain does not cleave DNA or has reduced DNA cleaving activity. In an embodiment, an HNH-like domain is at least 15, 20, 25

amino acids in length but not more than 40, 35 or 30 amino acids in length, e.g., 20 to 35 amino acids in length, e.g., 25 to 30 amino acids in length.

In embodiments, Cas9 molecules or Cas9 polypeptides have the ability to interact with a gRNA molecule, and in conjunction with the gRNA molecule localize to a core target domain, but are incapable of cleaving the target nucleic acid, or incapable of cleaving at efficient rates. Cas9 molecules having no, or no substantial, cleavage activity are referred to herein as dCas9 molecules or dCas9 polypeptides. For example, a dCas9 molecule or dCas9 polypeptide can lack cleavage activity or have substantially less, e.g., less than 20, 10, 5, 1 or 0.1 % of the cleavage activity of a reference Cas9 molecule or Cas9 polypeptide, as measured by assays known in the art or assays described herein.

#### Targeting and PAMs

A Cas9 molecule or Cas9 polypeptide is a polypeptide that can interact with a guide RNA (gRNA) molecule and, in concert with the gRNA molecule, localizes to a site which comprises a target domain and PAM sequence.

In an embodiment, the ability of a Cas9 molecule or Cas9 polypeptide to interact with a target nucleic acid is PAM sequence dependent. A PAM sequence is a sequence in the target nucleic acid. Cas9 molecules from different bacterial species can recognize different sequence motifs (e.g., PAM sequences). Cas9 molecules can be engineered to alter the PAM specificity of the Cas9 molecule. Exemplary naturally occurring Cas9 molecules are described in Chylinski *et al.*, RNA BIOLOGY 2013 10:5, 727-737.

#### Alterations in Cas9 Structure

In some embodiments, one or more mutation(s) can be present, e.g., in one or more RuvC-like domain, e.g., an N-terminal RuvC-like domain; an HNH-like domain; a region outside the RuvC-like domains and the HNH-like domain, of the Cas9 molecule or Cas9 polypeptide. In some embodiments, a mutation(s) is present in a RuvC-like domain, e.g., an N-terminal RuvC-like domain. In some embodiments, a mutation(s) is present in an HNH-like domain. In some embodiments, mutations are present in both a RuvC-like domain, e.g., an N-terminal RuvC-like domain and an HNH-like domain.

In an embodiment, a Cas9 molecule or Cas9 polypeptide, e.g., an dCas9 molecule or dCas9 polypeptide, comprises an amino acid sequence:

having 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% homology with; differs at no more than, 2, 5, 10, 15, 20, 30, or 40% of the amino acid residues when compared with;

differs by at least 1, 2, 5, 10 or 20 amino acids, but by no more than 100, 80, 70, 60, 50, 40 or 30

amino acids from; or

is identical to any Cas9 molecule sequence described herein, or a naturally occurring Cas9 molecule sequence, e.g., a Cas9 molecule from a species listed herein or described in Chylinski *et al.*, RNA BIOLOGY 2013 10:5, 727-737; Hou *et al.*, PNAS Early Edition 2013, 1-6;. In an embodiment, the  
 5 Cas9 molecule or Cas9 polypeptide comprises one or more of the following activities: a helicase activity; or the ability, together with a gRNA molecule, to localize to a target nucleic acid. In an embodiment, the Cas9 molecule or Cas9 polypeptide does not comprise a nickase activity or a double stranded cleavage activity (e.g., an endonuclease and/or exonuclease activity).

Exemplary mutations that may be made in the RuvC domain or HNH domain with reference to  
 10 the *S. pyogenes* sequence include: D10A, E762A, H840A, N854A, N863A and/or D986A.

Exemplary Cas9 polypeptide and Cas9 domain sequences can be found in Tables 50-54 of WO2015/157070.

#### dCas9 Polypeptides

15 In an embodiment, the heterologous polypeptide of interest linked to a COF1/CRBN-, COF2/CRBN-, or COF3/CRBN-binding polypeptide and/or a degradation domain is a dCas9 molecule, e.g., a dCas9 polypeptide comprising one or more differences in a RuvC domain and/or in an HNH domain as compared to a reference Cas9 molecule, and the dCas9 molecule or dCas9 polypeptide does not cleave a nucleic acid, or cleaves with significantly less efficiency than does wildtype, e.g., when  
 20 compared with wild type in a cleavage assay, e.g., as described herein, cuts with less than 50, 25, 10, or 1% of a reference Cas9 molecule, as measured by an assay described herein.

Mutating key residues in both DNA cleavage domains of the Cas9 protein (e.g. the D10A and H840A mutations) results in the generation of a catalytically inactive Cas9 (dCas9 which is also known as dead Cas9) molecule. An enzymatically inactive Cas9, e.g., dCas9, complexes with a gRNA and  
 25 localizes to the DNA sequence specified by that gRNA's targeting domain; however, it does not cleave the target DNA. An enzymatically inactive (e.g., dCas9) Cas9 molecule can block transcription when recruited to early regions in the coding sequence. Additional repression can be achieved by fusing a transcriptional repression domain (for example KRAB, SID or ERD) to the enzymatically inactive Cas9, e.g., dCas9, and recruiting it to the target sequence, e.g., within 1000bp of sequence 3' of the start codon  
 30 or within 500 bp of a control element, e.g., promoter element, e.g., 5' of the start codon of a gene. Targeting DNase I hypersensitive sites (DHSs) of the promoter (e.g., by making gRNAs complementary to the DHSs) may be an additional strategy for gene repression, e.g., inhibition of a recombinant or therapeutic polypeptide encoding sequence, because these regions are more likely to be accessible to the

enzymatically inactive Cas9, e.g., dCas9, and are also likely to harbor sites for endogenous transcription factors. While not wishing to be bound by theory, it is contemplated herein that blocking the binding site of an endogenous transcription factor or RNA polymerase would aid in down-regulating gene expression, e.g., expression of a recombinant or therapeutic polypeptide encoding sequence. In an embodiment, one or more enzymatically inactive Cas9, e.g., dCas9, molecules may be used to block binding of one or more endogenous transcription factors. In another embodiment, an enzymatically inactive Cas9, e.g., dCas9, molecule can be fused to an effector domain, e.g., a repression domain, an activation domain, a methylation enzyme, etc. Fusion of the enzymatically inactive Cas9, e.g., dCas9, to an effector domain enables recruitment of the effector to any DNA site specified by the gRNA. Altering chromatin status can result in decreased expression of the target gene. One or more enzymatically inactive Cas9, e.g., dCas9, molecules fused to one or more chromatin modifying proteins may be used to alter chromatin status.

In an embodiment, a gRNA molecule can be targeted to a control element (e.g., promoter element), e.g., the control element operably linked to a recombinant or therapeutic polypeptide encoding sequence. In an embodiment a gRNA molecule can be targeted to a sequence encoding a recombinant or therapeutic polypeptide.

### **TALE Molecules**

In some embodiments, the heterologous polypeptide of interest linked to a COF1/CRBN-, COF2/CRBN-, or COF3/CRBN-binding polypeptide and/or a degradation domain is a transcription activator-like effector (TALE) molecule or TALE polypeptide. A molecule or TALE polypeptide, as that term is used herein, refers to a molecule or polypeptide comprising multiple TALE DNA-binding repeat domains (TALE DBDs) that can home or localize to a nucleic acid position specified by the TALE DBDs. TALE molecule and TALE polypeptide, as those terms are used herein, refer to naturally occurring TALE molecules and to engineered, altered, or modified TALE molecules or TALE polypeptides that differ, e.g., by at least one amino acid residue, from a reference sequence, e.g., the most similar naturally occurring TALE molecule known in the art.

TALE DBD, as that term is used herein, refers to a 33-35 amino acid motif, including two hypervariable residues (i.e. a repeat variable di-residue, RVD) at positions 12 and 13 of the motif. The RVD of a TALE DNA-binding domain (DBD) specifies the DNA base-pair or base-pairs to which a TALE DBD has binding affinity. When TALE DBDs are combined in arrays within a TALE molecule or TALE polypeptide, the order of TALE DBDs (and their RVD) determine the DNA sequence to which a TALE molecule or TALE polypeptide has binding affinity. Naturally occurring TALE polypeptides and TALE DBDs are produced by *Xanthomonas* bacteria.

Repeat variable di-residue (RVD), as that term is used herein, refers to the two hypervariable amino acid residues at positions 12 and 13 of a TALE DBD. The RVD determines the DNA base-pair affinity of a TALE DBD. All possible combinations of RVDs and their respective base-pair affinities are known in the art. See, e.g., Cong L., et al. Nat Commun. 2012 Jul 24; (3):968; Juillerat A., et al. Sci Rep. 5 2015 Jan 30; 5():8150; Miller J. C. et al. Nat Methods 12, 465–471 (2015); Streubel J., et al. Nat Biotechnol 30, 593–595 (2012); and Yang J. et al. Cell Res 24, 628–631 (2014), incorporated herein by reference in their entireties. All possible RVDs are contemplated for use with the repressor polypeptides, e.g., TALE molecules, described herein.

TALE DBD array, as that term is used herein, refers to the identities and order of TALE DBDs, 10 e.g., the RVDs of each TALE DBD, within a TALE molecule or TALE polypeptide. The TALE DBD array determines the sequence specific binding affinity of a TALE molecule or TALE polypeptide.

In some embodiments, the repressor polypeptide is a TALE molecule or TALE polypeptide. TALE DBDs and TALE polypeptide from any species of *Xanthomonas* can be used in the genetic control circuits, cells, and methods for identifying, selecting, or making a cell or cell line capable of producing 15 high yields of a product, e.g., a recombinant or therapeutic polypeptide, described herein. In some embodiments, the repressor polypeptide is a naturally occurring TALE molecule or TALE polypeptide. In some embodiments, the repressor polypeptide is an engineered TALE molecule or TALE polypeptide, i.e. a TALE molecule or TALE polypeptide that differs by one or more amino acids from a naturally occurring TALE molecule or TALE polypeptide or from another engineered TALE molecule or TALE 20 polypeptide known in the art.

In some embodiments, an engineered TALE molecule or TALE polypeptide comprises an amino acid sequence:

having 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% homology with; 25 differs at no more than, 2, 5, 10, 15, 20, 30, or 40% of the amino acid residues when compared with;

differs by at least 1, 2, 5, 10 or 20 amino acids, but by no more than 100, 80, 70, 60, 50, 40 or 30 amino acids from; or

is identical to any TALE molecule sequence described herein, or a naturally occurring TALE molecule sequence, e.g., a TALE molecule from a species listed herein or described in a publication 30 referenced herein.

In some embodiments, a TALE molecule localizes to the target DNA sequence specified by that TALE molecules' TALE DBD array. In some embodiments, TALE molecule can block transcription when recruited to early regions in a coding sequence, e.g., the coding sequence of a recombinant or therapeutic polypeptide. In some embodiments, a TALE molecule can block transcription when recruited

to a control element, e.g., a promoter element, operably linked to a recombinant or therapeutic polypeptide encoding sequence. In some embodiments, additional repression can be achieved by fusing a transcriptional repression domain (for example KRAB, SID or ERD) to the TALE molecule, enabling recruitment of the effector to any DNA site specified by the TALE DBD array.

In some embodiments, a TALE molecule comprises two or more (e.g. 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, or more) TALE DBDs.

In some embodiments, the TALE DBD array of a repressor polypeptide, e.g., TALE molecule, specifies a target DNA sequence. In some embodiments, the target sequence specified by the TALE DBD array is comprised within a control element, e.g., promoter element, operably linked to a recombinant or therapeutic polypeptide encoding sequence. In some embodiments, the target sequence specified by the TALE DBD array is comprised with a recombinant or therapeutic polypeptide encoding sequence.

Exemplary naturally occurring and engineered TALE polypeptide sequences and methods for design and testing of TALE polypeptides for use with genetic control circuits, cells, and methods for identifying, selecting, or making a cell or cell line capable of producing high yields of a product, e.g., a recombinant or therapeutic polypeptide, described herein can be found in the art, e.g., in Zhang F, et al. *Nat Biotechnol.* 2011; 29:149–153; Geissler R, et al. *PLoS One.* 2011; 6:e19509; Garg A, et al. *Nucleic Acids Res.* 2012; Bultmann S, et al. *Nucleic Acids Res.* 2012; 40:5368–5377; Cermak T, et al. Efficient design and assembly of custom TALEN and other TAL effector-based constructs for DNA targeting. *Nucleic Acids Res.* 2011; 39:e82; Cong L, et al. *Nat Commun.* 2012; 3:968; and Miller JC, et al. *Nat Biotechnol.* 2011; 29:143–148, herein incorporated by reference in their entireties.

### **Zinc Finger Molecules**

In some embodiments, the heterologous polypeptide of interest linked to a COF1/CRBN-, COF2/CRBN-, or COF3/CRBN-binding polypeptide and/or a degradation domain is a zinc finger molecule. A zinc finger molecule, as that term is used herein, refers to a molecule or polypeptide comprising multiple zinc finger domains (ZFDs). A zinc finger molecule has affinity to a specific DNA sequence determined by the identity and order of the ZFDs the zinc finger molecule comprises.

A zinc finger domain (ZFD), as that term is used herein, refers to any of a family of polypeptides that bind DNA in a sequence specific manner and require a zinc ion ligand to bind DNA. Many families of ZFDs have been studied and characterized (see, e.g., Krishna, SS., et al. *Nucl. Acids Res.* (2003) 31 (2): 532-550). The disclosure contemplates zinc finger molecules that may comprise ZFDs of any type or origin known to those of skill in the art. Without intending to be limited to any particular type of ZFD, the disclosure contemplates zinc finger molecules comprising Cys<sub>2</sub>His<sub>2</sub> ZFDs, which are the most

prevalent and well-studied ZFDs in the art. Cys<sub>2</sub>His<sub>2</sub> ZFDs comprise two beta strands that form an anti-parallel beta sheet and an alpha helix. Positions -1, 1, 2, 3, 5, and 6 of the alpha helix are known to specify DNA sequence specific binding by interacting with DNA base pairs. In an embodiment, a Cys<sub>2</sub>His<sub>2</sub> ZFD may have specific binding affinity for a 3 base pair target sequence. In an embodiment, a Cys<sub>2</sub>His<sub>2</sub> ZFD may specifically interact with an additional base pair adjacent to the target sequence in a context specific manner, i.e. dependent upon the presence and identity of adjacent ZFDs within a zinc finger molecule.

A zinc finger domain array, or ZFD array, as that term is used herein, refers to the identities and order of ZFDs, within a zinc finger molecule or zinc finger polypeptide. The ZFD array determines the sequence specific binding affinity of a zinc finger molecule or zinc finger polypeptide.

In some embodiments, the repressor polypeptide is a zinc finger molecule or zinc finger polypeptide. ZFDs and zinc finger polypeptides from any species (e.g., a mammalian species, e.g., humans) can be used in the genetic control circuits, cells, and methods for identifying, selecting, or making a cell or cell line capable of producing high yields of a product, e.g., a recombinant or therapeutic polypeptide, described herein. In some embodiments, the repressor polypeptide is a naturally occurring zinc finger molecule or zinc finger polypeptide. In some embodiments, the repressor polypeptide is an engineered zinc finger molecule or zinc finger polypeptide, i.e. a zinc finger molecule or zinc finger polypeptide that differs by one or more amino acids from a naturally occurring zinc finger molecule or zinc finger polypeptide or from another engineered zinc finger molecule or zinc finger polypeptide known in the art.

In some embodiments, an engineered zinc finger molecule or zinc finger polypeptide comprises an amino acid sequence:

having 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% homology with;  
differs at no more than, 2, 5, 10, 15, 20, 30, or 40% of the amino acid residues when compared

with;

differs by at least 1, 2, 5, 10 or 20 amino acids, but by no more than 100, 80, 70, 60, 50, 40 or 30 amino acids from; or

is identical to any zinc finger molecule sequence described herein, or a naturally occurring zinc finger molecule sequence, e.g., a zinc finger molecule from a species listed herein or described in a publication referenced herein.

In some embodiments, a zinc finger molecule localizes to the target DNA sequence specified by that zinc finger molecules' ZFD array. In some embodiments, a zinc finger molecule can block transcription when recruited to early regions in a coding sequence, e.g., the coding sequence of a recombinant or therapeutic polypeptide. In some embodiments, a zinc finger molecule can block

transcription when recruited to a control element, e.g., a promoter element, operably linked to a recombinant or therapeutic polypeptide encoding sequence. In some embodiments, additional repression can be achieved by fusing a transcriptional repression domain (for example KRAB, SID or ERD) to the zinc finger molecule, enabling recruitment of the effector to any DNA site specified by the ZFD array.

In some embodiments, a zinc finger molecule comprises two or more (e.g. 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, or more) ZFDs. In some embodiments, a ZFD array can be constructed from ZFDs with known target sequence affinities to create a zinc finger molecule or zinc finger polypeptide with a desired specific target sequence.

In some embodiments, the ZFD array of a repressor polypeptide, e.g., zinc finger molecule, specifies a target DNA sequence. In some embodiments, the target sequence specified by the ZFD array is comprised within a control element, e.g., promoter element, operably linked to a recombinant or therapeutic polypeptide encoding sequence. In some embodiments, the target sequence specified by the ZFD array is comprised with a recombinant or therapeutic polypeptide encoding sequence.

Exemplary naturally occurring and engineered zinc finger polypeptide sequences and methods for design and testing of zinc finger polypeptides for use with genetic control circuits, cells, and methods for identifying, selecting, or making a cell or cell line capable of producing high yields of a product, e.g., a recombinant or therapeutic polypeptide, described herein can be found in the art, e.g., in Wolfe SA, et al. *Annu Rev Biophys Biomol Struct.* 2000; 29:183–212; Pabo CO, et al. *Annu Rev Biochem.* 2001; 70:313–340; Greisman HA, Pabo CO. *Science.* 1997; 275:657–661; Isalan M, et al., *Proc Natl Acad Sci U S A.* 1997; 94:5617–5621; Wolfe SA, et al. *J Mol Biol.* 1999; 285:1917–1934, herein incorporated by reference in their entireties.

Methods of designing ZFDs and ZFD arrays to bind specific target DNA sequences can be found in the art, e.g., in Maeder ML, et al. *Mol Cell.* 2008; 31:294–301; Sander JD, et al., *Nat Methods.* 2011; 8:67–69; and Meng X, et al. *Nat Biotechnol.* 2008; 26:695–701, herein incorporated by reference in their entireties.

### Degradation domains

In some embodiments, the fusion polypeptide of this invention further comprises a degradation domain. In some embodiments, the degradation domain has a first state and a second state, e.g., states of stabilization/destabilization, or states of folding/misfolding. The first state is associated with, causes, or mediates expression of the fusion polypeptide at a first rate or level and the second state is associated with, causes, or mediates expression of the fusion polypeptide at a second rate or level. In some embodiments, the second state has a level or rate that is greater, e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, or 30



fold greater, than the rate or level of the first state. In some embodiments, the second state is associated with, maintained by, or caused by the presence of a stabilization compound. In some embodiments, the presence of the stabilization compound can be associated with, cause, or mediate the transformation of a first folding state to a second folding state, e.g., from misfolded to more properly folded state, e.g., a first state susceptible to degradation to a second state less susceptible to degradation than the first state; or from a first folding state that has a first level of degradation to a second folding state what has a second, lessor, level of degradation, e.g., in a cell of interest.

In an embodiment, addition of a stabilization compound to a plurality of cells, e.g., host cells or cells comprising fusion polypeptides described herein, causes a transformation of a sub-plurality of cells from the first state to the second state, e.g., states of stabilization/destabilization, or states of folding/misfolding as described herein. In an embodiment, in the absence of the stabilization compound, less than 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1% of the cells in the plurality comprise the second state, and greater than or equal to 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99% of the cells in the plurality comprise the first state. In an embodiment, in the presence of the stabilization compound, greater than or equal to 20, 30, 40, 50, 60, 70, 80, 90, or 95% of the cells in the plurality comprise the second state, and less than 20, 15, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1% of the cells in the plurality comprise the first state. Determination of the percentages of cells in a plurality comprising a state can be made using methods described throughout the specification.

In one embodiment, the degradation domain is separated from the rest of the fusion polypeptide by a heterologous protease cleavage site.

Without wishing to be bound by theory, in some embodiments, the degradation domain is unstable and/or unable to fold into a stable conformation in the absence of a stabilization compound. This misfolded/unfolded degradation domain can be degraded by intracellular degradation pathway along with the rest of the fusion polypeptide. In the presence of the stabilization compound, the degradation domain assumes a proper conformation and is less susceptible to intercellular degradation pathways. Thus, the expression level of the fusion polypeptide can be regulated by the presence or absence of the stabilization compound.

In some embodiments, the proper folding of the degradation domain exposes the heterologous protease cleavage site, leading to the cleavage of the heterologous protease cleavage site and the removal of the degradation domain from the rest of the fusion polypeptide.

Methods of generating degradation domains that are selectively stable in the presence of a stabilization compound are well known in the art and discussed further below. Several such domain-stabilization compound pairs have been generated to date and are featured in the present invention. These include degradation domains based on FKBP (e.g., using a “Shield” stabilization compound) as described

in: A Rapid, Reversible, and Tunable Method to Regulate Protein Function in Living Cells Using Synthetic Small Molecules." Banaszynski, L. A.; Chen, L.-C.; Maynard-Smith, L. A.; Ooi, A. G. L.; Wandless, T. J. *Cell*, 2006, 126, 995-1004; domains based on DHFR (e.g., using trimethoprim as a stabilization compound) as described in A general chemical method to regulate protein stability in the mammalian central nervous system. Iwamoto, M.; Björklund, T.; Lundberg, C.; Kirik, D.; Wandless, T. J. *Chemistry & Biology*, 2010, 17, 981-988; and domains based on estrogen receptor alpha (e.g., where 4OHT is used as a stabilization compound) as described in Destabilizing domains derived from the human estrogen receptor Y Miyazaki, H Imoto, L-c Chen, TJ Wandless J. Am. Chem. Soc. 2012, 134, 3942-3945. Each of these references is incorporated by reference in its entirety.

The present disclosure encompasses degradation domains derived from any naturally occurring protein. Preferably, fusion polypeptides of the invention will include a degradation domain for which there is no ligand natively expressed in the cell compartments of interest. For example, if the fusion polypeptide is designed for expression in T cells, it is preferable to select a degradation domain for which there is no naturally occurring ligand present in T cells. Thus, the degradation domain, when expressed in the cell of interest, will only be stabilized in the presence of an exogenously added compound. Notably, this property can be engineered by either engineering the degradation domain to no longer bind a natively expressed ligand (in which case the degradation domain will only be stable in the presence of a synthetic compound) or by expressing the degradation domain in a compartment where the natively expressed ligand does not occur (e.g., the degradation domain can be derived from a species other than the species in which the fusion polypeptide will be expressed).

Degradation domain –stabilization compound pairs can be derived from any naturally occurring or synthetically developed protein. Stabilization compounds can be any naturally occurring or synthetic compounds. In certain embodiments, the stabilization compounds will be existing prescription or over-the-counter medicines. Examples of proteins that can be engineered to possess the properties of a degradation domain are set forth in Table 21 below along with a corresponding stabilization compound.

In some embodiments, the degradation domain is derived from a protein listed in Table 21.

In some embodiments, the degradation domain is derived from an estrogen receptor (ER). In some embodiments, the degradation domain comprises an amino acid sequence selected from SEQ ID NO: 46 or a sequence having at least 90%, 95%, 97%, 98%, or 99% identity thereto, or SEQ ID NO: 48 or a sequence having at least 90%, 95%, 97%, 98%, or 99% identity thereto. In some embodiments, the degradation domain comprises the amino acid sequence of SEQ ID NO: 46 or 48. When the degradation domain is derived from an estrogen receptor, the stabilization compound can be selected from Bazedoxifene or 4-hydroxy tamoxifen (4-OHT). In some embodiments, the stabilization compound is Bazedoxifene. Tamoxifen and Bazedoxifene are FDA approved drugs, and thus are safe to use in human.

In some embodiments, the degradation domain is derived from an FKB protein (FKBP). In some embodiments, the degradation domain comprises the amino acid sequence of SEQ ID NO: 50 or a sequence having at least 90%, 95%, 97%, 98%, or 99% identity thereto. In some embodiments, the degradation domain comprises the amino acid sequence of SEQ ID NO: 50. When the degradation domain is derived from a FKBP, the stabilization compound can be Shield-1.

In some embodiments, the degradation domain is derived from dihydrofolate reductase (DHFR). In some embodiments, the degradation domain comprises the amino acid sequence of SEQ ID NO: 51 or a sequence having at least 90%, 95%, 97%, 98%, or 99% identity thereto. In some embodiments, the degradation domain comprises the amino acid sequence of SEQ ID NO: 51. When the degradation domain is derived from a DHFR, the stabilization compound can be Trimethoprim.

In some embodiments, the degradation domain is not derived from an FKB protein, estrogen receptor, or DHFR.

**Table 21. Exemplary proteins for generating degradation domains**

| Type                                | Activity of drug         | Drug examples                                                                   |
|-------------------------------------|--------------------------|---------------------------------------------------------------------------------|
| <i><b>Oxidoreductases</b></i>       |                          |                                                                                 |
| Aldehyde dehydrogenase              | Inhibitor                | Disulfiram                                                                      |
| Monoamine oxidases (MAOs)           | MAO-A inhibitor          | Tranylcypromine, moclobemide                                                    |
|                                     | MAO-B inhibitor          | Tranylcypromine                                                                 |
| Cyclooxygenases (COXs)              | COX1 inhibitor           | Acetylsalicylic acid, profens, acetaminophen and dipyron (as arachidonylamides) |
|                                     | COX2 inhibitor           | Acetylsalicylic acid, profens, acetaminophen and dipyron (as arachidonylamides) |
| Vitamin K epoxide reductase         | Inhibitor                | Warfarin, phenprocoumon                                                         |
| Aromatase                           | Inhibitor                | Exemestane                                                                      |
| Lanosterol demethylase (fungal)     | Inhibitor                | Azole antifungals                                                               |
| Lipoxygenases                       | Inhibitor                | Mesalazine                                                                      |
|                                     | 5-lipoxygenase inhibitor | Zileuton                                                                        |
| Thyroidal peroxidase                | Inhibitor                | Thiouracils                                                                     |
| Iodothyronine-5' deiodinase         | Inhibitor                | Propylthiouracil                                                                |
| Inosine monophosphate dehydrogenase | Inhibitor                | Mycophenolate mofetil                                                           |
| HMG-CoA reductase                   | Inhibitor                | Statins                                                                         |
| $\alpha$ -5-Testosterone reductase  | Inhibitor                | Finasteride, dutasteride                                                        |
| Dihydrofolate reductase (bacterial) | Inhibitor                | Trimethoprim                                                                    |
| Dihydrofolate reductase (human)     | Inhibitor                | Methotrexate, pemetrexed                                                        |
| Dihydrofolate reductase (parasitic) | Inhibitor                | Proguanil                                                                       |

|                                                          |                                |                                |
|----------------------------------------------------------|--------------------------------|--------------------------------|
| Dihydroorotate reductase                                 | Inhibitor                      | Leflunomide                    |
| Enoyl reductase (mycobacterial)                          | Inhibitor                      | Isoniazid                      |
| Squalene epoxidase (fungal)                              | Inhibitor                      | Terbinafin                     |
| $\Delta$ -14 reductase (fungal)                          | Inhibitor                      | Amorolfin                      |
| Xanthine oxidase                                         | Inhibitor                      | Allopurinol                    |
| 4-Hydroxyphenylpyruvate dioxygenase                      | Inhibitor                      | Nitisinone                     |
| Ribonucleoside diphosphate reductase                     | Inhibitor                      | Hydroxycarbamide               |
| <b><i>Transferases</i></b>                               |                                |                                |
| Protein kinase C                                         | Inhibitor                      | Miltefosine                    |
| Bacterial peptidyl transferase                           | Inhibitor                      | Chloramphenicol                |
| Catecholamine- <i>O</i> -methyltransferase               | Inhibitor                      | Entacapone                     |
| RNA polymerase (bacterial)                               | Inhibitor                      | Ansamycins                     |
| Reverse transcriptases (viral)                           | Competitive inhibitors         | Zidovudine                     |
|                                                          | Allosteric inhibitors          | Efavirenz                      |
| DNA polymerases                                          | Inhibitor                      | Acyclovir, suramin             |
| GABA transaminase                                        | Inhibitor                      | Valproic acid, vigabatrin      |
| Tyrosine kinases                                         | PDGFR/ABL/KIT inhibitor        | Imatinib                       |
|                                                          | EGFR inhibitor                 | Erlotinib                      |
|                                                          | $\beta$ -VEGFR2/PDGFR/KIT/FLT3 | Sunitinib                      |
|                                                          | $\beta$ -VEGFR2/PDGFR/RAF      | Sorafenib                      |
| Glycinamide ribonucleotide formyl transferase            | Inhibitor                      | Pemetrexed                     |
| Phosphoenolpyruvate transferase (MurA, bacterial)        | Inhibitor                      | Fosfomycin                     |
| Human cytosolic branched-chain aminotransferase (hBCATc) | Inhibitor                      | Gabapentin                     |
| <b><i>Hydrolases (proteases)</i></b>                     |                                |                                |
| Aspartyl proteases (viral)                               | HIV protease inhibitor         | Saquinavir, indinavir          |
| <b><i>Hydrolases (serine proteases)</i></b>              |                                |                                |
| Unspecific                                               | Unspecific inhibitors          | Aprotinine                     |
| Bacterial serine protease                                | Direct inhibitor               | $\beta$ -lactams               |
| Bacterial serine protease                                | Indirect inhibitor             | Glycopeptides                  |
| Bacterial lactamases                                     | Direct inhibitor               | Sulbactam                      |
| Human antithrombin                                       | Activator                      | Heparins                       |
| Human plasminogen                                        | Activator                      | Streptokinase                  |
| Human coagulation factor                                 | Activator                      | Factor IX complex, Factor VIII |
| Human factor Xa                                          | Inhibitor                      | Fondaparinux                   |
| <b><i>Hydrolases (metalloproteases)</i></b>              |                                |                                |
| Human ACE                                                | Inhibitor                      | Captopril                      |
| Human HRD                                                | Inhibitor                      | Cilastatin                     |
| Human carboxypeptidase A (Zn)                            | Inhibitor                      | Penicillamine                  |
| Human enkephalinase                                      | Inhibitor                      | Racecadotril                   |
| <b><i>Hydrolases (other)</i></b>                         |                                |                                |

|                                                 |                                                            |                                      |
|-------------------------------------------------|------------------------------------------------------------|--------------------------------------|
| 26S proteasome                                  | Inhibitor                                                  | Bortezomib                           |
| Esterases                                       | AChE inhibitor                                             | Physostigmine                        |
|                                                 | AChE reactivators                                          | Obidoxime                            |
|                                                 | PDE inhibitor                                              | Caffeine                             |
|                                                 | PDE3 inhibitor                                             | Amrinon, milrinone                   |
|                                                 | PDE4 inhibitor                                             | Papaverine                           |
|                                                 | PDE5 inhibitor                                             | Sildenafil                           |
|                                                 | HDAC inhibitor                                             | Valproic acid                        |
|                                                 | HDAC3/HDAC7 inhibitor                                      | Carbamezepine                        |
| Glycosidases (viral)                            | $\alpha$ -glycosidase inhibitor                            | Zanamivir, oseltamivir               |
| Glycosidases (human)                            | $\alpha$ -glycosidase inhibitor                            | Acarbose                             |
| Lipases                                         | Gastrointestinal lipases inhibitor                         | Orlistat                             |
| Phosphatases                                    | Calcineurin inhibitor                                      | Cyclosporin                          |
|                                                 | Inositol polyphosphate phosphatase inhibitor               | Lithium ions                         |
| GTPases                                         | Rac1 inhibitor                                             | 6-Thio-GTP (azathioprine metabolite) |
| Phosphorylases                                  | Bacterial C55-lipid phosphate dephosphorylase inhibitor    | Bacitracin                           |
| <b><i>Lyases</i></b>                            |                                                            |                                      |
| DOPA decarboxylase                              | Inhibitor                                                  | Carbidopa                            |
| Carbonic anhydrase                              | Inhibitor                                                  | Acetazolamide                        |
| Histidine decarboxylase                         | Inhibitor                                                  | Tritoqualine                         |
| Ornithine decarboxylase                         | Inhibitor                                                  | Eflornithine                         |
| Soluble guanylyl cyclase                        | Activator                                                  | Nitric acid esters, molsidomine      |
| <b><i>Isomerases</i></b>                        |                                                            |                                      |
| Alanine racemase                                | Inhibitor                                                  | D-Cycloserine                        |
| DNA gyases (bacterial)                          | Inhibitor                                                  | Fluoroquinolones                     |
| Topoisomerases                                  | Topoisomerase I inhibitor                                  | Irinotecan                           |
|                                                 | Topoisomerase II inhibitor                                 | Etoposide                            |
| 8,7 isomerase (fungal)                          | Inhibitor                                                  | Amorolfine                           |
| <b><i>Ligases (also known as synthases)</i></b> |                                                            |                                      |
| Dihydropteroate synthase                        | Inhibitor                                                  | Sulphonamides                        |
| Thymidylate synthase (fungal and human)         | Inhibitor                                                  | Fluorouracil                         |
| Thymidylate synthase (human)                    | Inhibitor                                                  | Methotrexate, pemetrexed             |
| Phosphofructokinase                             | Inhibitor                                                  | Antimony compounds                   |
| mTOR                                            | Inhibitor                                                  | Rapamycin                            |
| Haem polymerase (Plasmodium)                    | Inhibitor                                                  | Quinoline antimalarials              |
| $\beta$ -1,3--D-glucansynthase (fungi)          | Inhibitor                                                  | Caspofungin                          |
| Glucosylceramide synthase                       | Inhibitor                                                  | Miglustat                            |
| <b>Substrate</b>                                | <b>Drug substance</b>                                      |                                      |
| Asparagine                                      | Asparaginase                                               |                                      |
| Urate                                           | Rasburicase (a urate oxidase)                              |                                      |
| VAMP-synaptobrevin, SNAP25, Syntaxin            | Light chain of the botulinum neurotoxin (Zn-endopeptidase) |                                      |
| <b>Type</b>                                     | <b>Activity of drug</b>                                    | <b>Drug examples</b>                 |

## DEMANDE OU BREVET VOLUMINEUX

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CONTAINING PAGES    1    TO    316

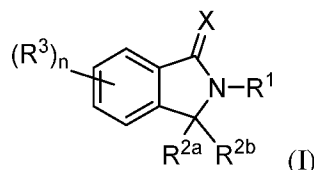
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What is claimed is:

1. A fusion polypeptide comprising a compound of Formula (I) (COF1)/CRBN (cereblon)-binding polypeptide and a heterologous polypeptide, e.g., a heterologous mammalian, bacterial, or viral polypeptide, wherein the compound of Formula (I) is:



or a pharmaceutically acceptable salt, ester, hydrate, solvate, or tautomer thereof, wherein:

X is O or S;

R<sup>1</sup> is C<sub>1</sub>-C<sub>6</sub> alkyl, C<sub>2</sub>-C<sub>6</sub> alkenyl, C<sub>2</sub>-C<sub>6</sub> alkynyl, C<sub>1</sub>-C<sub>6</sub> heteroalkyl, carbocyclyl, heterocyclyl, aryl, or heteroaryl, each of which is independently and optionally substituted by one or more R<sup>4</sup>;

each of R<sup>2a</sup> and R<sup>2b</sup> is independently hydrogen or C<sub>1</sub>-C<sub>6</sub> alkyl; or R<sup>2a</sup> and R<sup>2b</sup> together with the carbon atom to which they are attached form a carbonyl group or a thiocarbonyl group;

each of R<sup>3</sup> is independently C<sub>1</sub>-C<sub>6</sub> alkyl, C<sub>2</sub>-C<sub>6</sub> alkenyl, C<sub>2</sub>-C<sub>6</sub> alkynyl, C<sub>1</sub>-C<sub>6</sub> heteroalkyl, halo, cyano, -C(O)R<sup>A</sup>, -C(O)OR<sup>B</sup>, -OR<sup>B</sup>, -N(R<sup>C</sup>)(R<sup>D</sup>), -C(O)N(R<sup>C</sup>)(R<sup>D</sup>), -N(R<sup>C</sup>)C(O)R<sup>A</sup>, -S(O)<sub>x</sub>R<sup>E</sup>, -S(O)<sub>x</sub>N(R<sup>C</sup>)(R<sup>D</sup>), or -N(R<sup>C</sup>)S(O)<sub>x</sub>R<sup>E</sup>, wherein each alkyl, alkenyl, alkynyl, and heteroalkyl is independently and optionally substituted with one or more R<sup>6</sup>;

each R<sup>4</sup> is independently C<sub>1</sub>-C<sub>6</sub> alkyl, C<sub>2</sub>-C<sub>6</sub> alkenyl, C<sub>2</sub>-C<sub>6</sub> alkynyl, C<sub>1</sub>-C<sub>6</sub> heteroalkyl, halo, cyano, oxo, -C(O)R<sup>A</sup>, -C(O)OR<sup>B</sup>, -OR<sup>B</sup>, -N(R<sup>C</sup>)(R<sup>D</sup>), -C(O)N(R<sup>C</sup>)(R<sup>D</sup>), -N(R<sup>C</sup>)C(O)R<sup>A</sup>, -S(O)<sub>x</sub>R<sup>E</sup>, -S(O)<sub>x</sub>N(R<sup>C</sup>)(R<sup>D</sup>), -N(R<sup>C</sup>)S(O)<sub>x</sub>R<sup>E</sup>, carbocyclyl, heterocyclyl, aryl, or heteroaryl, wherein each alkyl, alkenyl, alkynyl, heteroalkyl, carbocyclyl, heterocyclyl, aryl, and heteroaryl is independently and optionally substituted with one or more R<sup>7</sup>;

each of R<sup>A</sup>, R<sup>B</sup>, R<sup>C</sup>, R<sup>D</sup>, and R<sup>E</sup> is independently hydrogen or C<sub>1</sub>-C<sub>6</sub> alkyl;

each R<sup>6</sup> is independently C<sub>1</sub>-C<sub>6</sub> alkyl, oxo, cyano, -OR<sup>B</sup>, -N(R<sup>C</sup>)(R<sup>D</sup>), -C(O)N(R<sup>C</sup>)(R<sup>D</sup>), -N(R<sup>C</sup>)C(O)R<sup>A</sup>, aryl, or heteroaryl, wherein each aryl and heteroaryl is independently and optionally substituted with one or more R<sup>8</sup>;

each R<sup>7</sup> is independently halo, oxo, cyano, -OR<sup>B</sup>, -N(R<sup>C</sup>)(R<sup>D</sup>), -C(O)N(R<sup>C</sup>)(R<sup>D</sup>), or -N(R<sup>C</sup>)C(O)R<sup>A</sup>;

each R<sup>8</sup> is independently C<sub>1</sub>-C<sub>6</sub> alkyl, cyano, -OR<sup>B</sup>, -N(R<sup>C</sup>)(R<sup>D</sup>), -C(O)N(R<sup>C</sup>)(R<sup>D</sup>), or -N(R<sup>C</sup>)C(O)R<sup>A</sup>;

n is 0, 1, 2, 3 or 4; and

x is 0, 1, or 2.

2. The fusion polypeptide of claim 1, wherein the COF1/CRBN-binding polypeptide is fused to the heterologous polypeptide.
3. The fusion polypeptide of claim 1 or 2, wherein the COF1/CRBN-binding polypeptide and the heterologous polypeptide are linked by a peptide bond.
4. The fusion polypeptide of claim 1 or 2, wherein the COF1/CRBN-binding polypeptide and the heterologous polypeptide are linked by a bond other than a peptide bond.
5. The fusion polypeptide of any one of claims 1-4, wherein the heterologous polypeptide is linked directly to the COF1/CRBN-binding polypeptide.
6. The fusion polypeptide of any one of claims 1-4, wherein the heterologous polypeptide is linked indirectly to the COF1/CRBN-binding polypeptide.
7. The fusion polypeptide of any one of claims 1-6, wherein the COF1/CRBN-binding polypeptide and the heterologous polypeptide are operatively linked via a linker, e.g., a glycine-serine linker, e.g., a linker comprising the amino acid sequence of SEQ ID NO: 28.
8. The fusion polypeptide of any one of claims 1-7, wherein the COF1/CRBN-binding polypeptide is linked to the C-terminus or N-terminus of the heterologous polypeptide.
9. The fusion polypeptide of any one of claims 1-8, wherein the association of the COF1/CRBN-binding polypeptide with cereblon (CRBN) in the absence of COF1 is no more than, e.g., 0.01%, 0.1%, 1%, 5%, 10%, 15%, or 20%, of the association of the COF1/CRBN-binding polypeptide with CRBN in the presence of COF1, e.g., an excess of COF1, e.g., as measured by an assay described herein, e.g., immunoprecipitation.
10. The fusion polypeptide of any one of claims 1-9, wherein the COF1/CRBN-binding polypeptide does not bind to CRBN in the absence of COF1.
11. The fusion polypeptide of any one of claims 1-10, wherein the association of the fusion polypeptide with CRBN in the absence of COF1 is no more than, e.g., 0.01%, 0.1%, 1%, 5%, 10%, 15%, or 20%, of the association of the fusion polypeptide with CRBN in the presence of COF1, e.g., an excess of COF1, e.g., as measured by an assay described herein, e.g., immunoprecipitation.



12. The fusion polypeptide of any one of claims 1-11, wherein the fusion polypeptide does not bind to CRBN in the absence of COF1.

13. The fusion polypeptide of any one of claims 1-12, wherein the ubiquitination of the heterologous polypeptide in the absence of COF1 is no more than, e.g., 0.01%, 0.1%, 1%, 10%, 20%, 30%, 40%, 50%, 60%, or 70%, of the ubiquitination of the heterologous polypeptide in the presence of COF1, e.g., an excess of COF1, e.g., as measured by an assay described herein.

14. The fusion polypeptide of any one of claims 1-13, wherein the ubiquitination of the fusion polypeptide in the absence of COF1 is no more than, e.g., 0.01%, 0.1%, 1%, 10%, 20%, 30%, 40%, 50%, 60%, or 70%, of the ubiquitination of the fusion polypeptide in the presence of COF1, e.g., an excess of COF1, e.g., as measured by an assay described herein.

15. The fusion polypeptide of claim 13 or 14, wherein the heterologous polypeptide or the fusion polypeptide is ubiquitinated at one or more lysine or methionine residues in the presence of COF1.

16. The fusion polypeptide of any one of claims 1-15, wherein the degradation of the fusion polypeptide in the absence of COF1 is no more than, e.g., 0.01%, 0.1%, 1%, 10%, 20%, 30%, 40%, 50%, 60%, or 70% of the degradation of the fusion polypeptide in the presence of COF1, e.g., an excess of COF1, e.g., as measured by an assay described herein, e.g., a Western blot analysis or a flow cytometry analysis.

17. The fusion polypeptide of claim 16, wherein the degradation of the fusion polypeptide is mediated by ubiquitination in the presence of COF1.

18. The fusion polypeptide of any one of claims 9-17, wherein the association, ubiquitination, and/or degradation is as measured in a mammalian cell, e.g., a human cell.

19. The fusion polypeptide of any one of claims 1-18, wherein the COF1/CRBN-binding polypeptide is between 10 and 95 amino acid residues in length, between 15 and 90 amino acid residues in length, between 20 and 85 amino acid residues in length, between 25 and 80 amino acid residues in length, between 30 and 75 amino acid residues in length, between 35 and 70 amino acid residues in length, between 40 and 65 amino acid residues in length, between 45 and 65 amino acid residues in length, between 50 and 65 amino acid residues in length, or between 55 and 65 amino acid residues in length.

20. The fusion polypeptide of any one of claims 1-19, wherein the COF1/CRBN-binding polypeptide comprises a beta turn.
21. The fusion polypeptide of any one of claims 1-20, wherein the COF1/CRBN-binding polypeptide comprises a beta hairpin.
22. The fusion polypeptide of any one of claims 1-21, wherein the COF1/CRBN-binding polypeptide comprises a beta strand.
23. The fusion polypeptide of any one of claims 1-22, wherein the COF1/CRBN-binding polypeptide comprises an alpha helix.
24. The fusion polypeptide of any one of claims 1-23, wherein the COF1/CRBN-binding polypeptide comprises, from N-terminus to C-terminus, a first beta strand, a beta hairpin, a second beta strand, and a first alpha helix.
25. The fusion polypeptide of any one of claims 1-24, wherein the COF1/CRBN-binding polypeptide comprises, from N-terminus to C-terminus, a first beta strand, a beta hairpin, a second beta strand, a first alpha helix, and a second alpha helix.
26. The fusion polypeptide of claim 25, wherein the beta hairpin and the second alpha helix are separated by no more than 60, 50, 40, or 30 amino acid residues.
27. The fusion polypeptide of any one of claims 1-26, wherein the COF1/CRBN-binding polypeptide comprises a COF1/CRBN-binding sequence from a naturally occurring polypeptide or a COF1/CRBN-binding variant thereof, e.g., a naturally occurring IKZF polypeptide or a COF1/CRBN-binding variant thereof, e.g., a naturally occurring IKZF1, IKZF2, IKZF3, IKZF4, IKZF5, or a COF1/CRBN-binding variant thereof.
28. The fusion polypeptide of claim 27, wherein the COF1/CRBN-binding sequence comprises two or more discontinuous sequences from the naturally occurring polypeptide, e.g., a naturally occurring IKZF polypeptide, e.g., a naturally occurring IKZF1, IKZF2, IKZF3, IKZF4, or IKZF5.
29. The fusion polypeptide of any one of claims 1-28, wherein the COF1/CRBN-binding polypeptide comprises an IKZF polypeptide or a structural motif thereof.

30. The fusion polypeptide of claim 29, wherein the IKZF polypeptide is an IKZF1 polypeptide, an IKZF3 polypeptide, an IKZF2 polypeptide having H141Q substitution (numbered according to SEQ ID NO: 21), or an IKZF4 polypeptide having H188Q substitution (numbered according to SEQ ID NO: 22).

31. The fusion polypeptide of any one of claims 1-30, wherein the COF1/CRBN-binding polypeptide comprises sufficient amino acid sequence and/or a structural motif from IKZF (e.g., IKZF1 or IKZF3) that:

- i) the association of the COF1/CRBN-binding polypeptide with CRBN in the absence of COF1 is no more than, e.g., 0.01%, 0.1%, 1%, 5%, 10%, 15%, or 20%, of the association of the COF1/CRBN-binding polypeptide with CRBN in the presence of COF1, e.g., an excess of COF1, e.g., as measured by an assay described herein, e.g., immunoprecipitation;
- ii) the association of the fusion polypeptide with CRBN in the absence of COF1 is no more than, e.g., 0.01%, 0.1%, 1%, 5%, 10%, 15%, or 20% of the association of the fusion polypeptide with CRBN in the presence of COF1, e.g., an excess of COF1, e.g., as measured by an assay described herein, e.g., immunoprecipitation;
- iii) the ubiquitination of the heterologous polypeptide in the absence of COF1 is no more than, e.g., 0.01%, 0.1%, 1%, 10%, 20%, 30%, 40%, 50%, 60%, or 70%, of the ubiquitination of the heterologous polypeptide in the presence of COF1, e.g., an excess of COF1, e.g., as measured by an assay described herein;
- iv) the ubiquitination of the fusion polypeptide in the absence of COF1 is no more than, e.g., 0.01%, 0.1%, 1%, 10%, 20%, 30%, 40%, 50%, 60%, or 70%, of the ubiquitination of the fusion polypeptide in the presence of COF1, e.g., an excess of COF1, e.g., as measured by an assay described herein; or
- v) the degradation of the fusion polypeptide in the absence of COF1 is no more than, e.g., 0.01%, 0.1%, 1%, 10%, 20%, 30%, 40%, 50%, 60%, or 70%, of the degradation of the fusion polypeptide in the presence of COF1, e.g., an excess of COF1, e.g., as measured by an assay described herein, e.g., a Western blot analysis or a flow cytometry analysis.

32. The fusion polypeptide of claim 31, wherein the association, ubiquitination, and/or degradation is as measured in a mammalian cell, e.g., a human cell.

33. The fusion polypeptide of any one of claims 1-32, wherein the COF1/CRBN-binding polypeptide comprises about 10 to about 95 amino acid residues, about 15 to about 90 amino acid residues, about 20 to about 85 amino acid residues, about 25 to about 80 amino acid residues, about 30 to about 75 amino

acid residues, about 35 to about 70 amino acid residues, about 40 to about 65 amino acid residues, about 45 to about 65 amino acid residues, about 50 to about 65 amino acid residues, or about 55 to about 65 amino acid residues of IKZF1 or IKZF3.

34. The fusion polypeptide of any one of claims 1-33, wherein the COF1/CRBN-binding polypeptide comprises sufficient amino acid sequence and/or a structural motif from amino acid residues 136-180 of IKZF3 (numbered according to SEQ ID NO: 19) (e.g., sufficient amino acid sequence and/or a structural motif from amino acid residues 136-180 of SEQ ID NO: 19) that:

- i) the association of the COF1/CRBN-binding polypeptide with CRBN in the absence of COF1 is no more than, e.g., 0.01%, 0.1%, 1%, 5%, 10%, 15%, or 20%, of the association of the COF1/CRBN-binding polypeptide with CRBN in the presence of COF1, e.g., an excess of COF1, e.g., as measured by an assay described herein, e.g., immunoprecipitation;
- ii) the association of the fusion polypeptide with CRBN in the absence of COF1 is no more than, e.g., 0.01%, 0.1%, 1%, 5%, 10%, 15%, or 20% of the association of the fusion polypeptide with CRBN in the presence of COF1, e.g., an excess of COF1, e.g., as measured by an assay described herein, e.g., immunoprecipitation;
- iii) the ubiquitination of the heterologous polypeptide in the absence of COF1 is no more than, e.g., 0.01%, 0.1%, 1%, 10%, 20%, 30%, 40%, 50%, 60%, or 70%, of the ubiquitination of the heterologous polypeptide in the presence of COF1, e.g., an excess of COF1, e.g., as measured by an assay described herein;
- iv) the ubiquitination of the fusion polypeptide in the absence of COF1 is no more than, e.g., 0.01%, 0.1%, 1%, 10%, 20%, 30%, 40%, 50%, 60%, or 70%, of the ubiquitination of the fusion polypeptide in the presence of COF1, e.g., an excess of COF1, e.g., as measured by an assay described herein; or
- v) the degradation of the fusion polypeptide in the absence of COF1 is no more than, e.g., 0.01%, 0.1%, 1%, 10%, 20%, 30%, 40%, 50%, 60%, or 70%, of the degradation of the fusion polypeptide in the presence of COF1, e.g., an excess of COF1, e.g., as measured by an assay described herein, e.g., a Western blot analysis or a flow cytometry analysis.

35. The fusion polypeptide of claim 34, wherein the association, ubiquitination, and/or degradation is as measured in a mammalian cell, e.g., a human cell.

36. The fusion polypeptide of any one of claims 1-35, wherein the COF1/CRBN-binding polypeptide comprises amino acid residues 136-180 of IKZF3 (numbered according to SEQ ID NO: 19) (e.g., the COF1/CRBN-binding polypeptide comprises the amino acid sequence of SEQ ID NO: 5) or a sequence that differs from amino acid residues 136-180 of IKZF3 (numbered according to SEQ ID NO: 19) by no

more than 1, 2, 3, 4, 5, 10, 15, 20, 25, 30, 35, or 40 amino acid residues (e.g., a sequence that differs from amino acid residues 136-180 of SEQ ID NO: 19 by no more than 1, 2, 3, 4, 5, 10, 15, 20, 25, 30, 35, or 40 amino acid residues) (e.g., a sequence having no more than 1, 2, 3, 4, 5, 10, 15, 20, 25, 30, 35, or 40 amino acid substitutions from amino acid residues 136-180 of SEQ ID NO: 19).

37. The fusion polypeptide of any one of claims 1-33, wherein the COF1/CRBN-binding polypeptide comprises sufficient amino acid sequence and/or a structural motif from amino acid residues 136-170 of IKZF3 (numbered according to SEQ ID NO: 19) (e.g., sufficient amino acid sequence and/or a structural motif from amino acid residues 136-170 of SEQ ID NO: 19) that:

- i) the association of the COF1/CRBN-binding polypeptide with CRBN in the absence of COF1 is no more than, e.g., 0.01%, 0.1%, 1%, 5%, 10%, 15%, or 20%, of the association of the COF1/CRBN-binding polypeptide with CRBN in the presence of COF1, e.g., an excess of COF1, e.g., as measured by an assay described herein, e.g., immunoprecipitation;
- ii) the association of the fusion polypeptide with CRBN in the absence of COF1 is no more than, e.g., 0.01%, 0.1%, 1%, 5%, 10%, 15%, or 20% of the association of the fusion polypeptide with CRBN in the presence of COF1, e.g., an excess of COF1, e.g., as measured by an assay described herein, e.g., immunoprecipitation;
- iii) the ubiquitination of the heterologous polypeptide in the absence of COF1 is no more than, e.g., 0.01%, 0.1%, 1%, 10%, 20%, 30%, 40%, 50%, 60%, or 70%, of the ubiquitination of the heterologous polypeptide in the presence of COF1, e.g., an excess of COF1, e.g., as measured by an assay described herein;
- iv) the ubiquitination of the fusion polypeptide in the absence of COF1 is no more than, e.g., 0.01%, 0.1%, 1%, 10%, 20%, 30%, 40%, 50%, 60%, or 70%, of the ubiquitination of the fusion polypeptide in the presence of COF1, e.g., an excess of COF1, e.g., as measured by an assay described herein; or
- v) the degradation of the fusion polypeptide in the absence of COF1 is no more than, e.g., 0.01%, 0.1%, 1%, 10%, 20%, 30%, 40%, 50%, 60%, or 70%, of the degradation of the fusion polypeptide in the presence of COF1, e.g., an excess of COF1, e.g., as measured by an assay described herein, e.g., a Western blot analysis or a flow cytometry analysis.

38. The fusion polypeptide of claim 37, wherein the association, ubiquitination, and/or degradation is as measured in a mammalian cell, e.g., a human cell.

39. The fusion polypeptide of any one of claims 1-33, 37, or 38, wherein the COF1/CRBN-binding polypeptide comprises amino acid residues 136-170 of IKZF3 (numbered according to SEQ ID NO: 19) (e.g., the COF1/CRBN-binding polypeptide comprises the amino acid sequence of SEQ ID NO: 6) or a

sequence that differs from amino acid residues 136-170 of IKZF3 (numbered according to SEQ ID NO: 19) by no more than 1, 2, 3, 4, 5, 10, 15, 20, 25, or 30 amino acid residues (e.g., a sequence that differs from amino acid residues 136-170 of SEQ ID NO: 19 by no more than 1, 2, 3, 4, 5, 10, 15, 20, 25, or 30 amino acid residues) (e.g., a sequence having no more than 1, 2, 3, 4, 5, 10, 15, 20, 25, or 30 amino acid substitutions from amino acid residues 136-170 of SEQ ID NO: 19).

40. The fusion polypeptide of any one of claims 36-39, wherein one, two, three or all of the following amino acid residues remain unaltered: glutamine at position 147, cysteine at position 148, glutamine at position 150, glycine at position 152, leucine at position 161, or leucine at position 162, numbered according to SEQ ID NO: 19.

41. The fusion polypeptide of claim 40, wherein glutamine at position 147 remains unaltered, numbered according to SEQ ID NO: 19.

42. The fusion polypeptide of claim 40, wherein cysteine at position 148 remains unaltered, numbered according to SEQ ID NO: 19.

43. The fusion polypeptide of claim 40, wherein glycine at position 152 remains unaltered, numbered according to SEQ ID NO: 19.

44. The fusion polypeptide of any one of claims 1-43, wherein the COF1/CRBN-binding polypeptide comprises amino acid residues 136-139 of IKZF3 (numbered according to SEQ ID NO: 19), e.g., the COF1/CRBN-binding polypeptide comprises the amino acid sequence of SEQ ID NO: 40.

45. The fusion polypeptide of any one of claims 1-44, wherein the COF1/CRBN-binding polypeptide comprises amino acid residues 136-180 of IKZF3 (numbered according to SEQ ID NO: 19), e.g., the COF1/CRBN-binding polypeptide comprises or consists of the amino acid sequence of SEQ ID NO: 5 or 77.

46. The fusion polypeptide of any one of claims 1-44, wherein the COF1/CRBN-binding polypeptide comprises amino acid residues 136-170 of IKZF3 (numbered according to SEQ ID NO: 19), e.g., the COF1/CRBN-binding polypeptide comprises or consists of the amino acid sequence of SEQ ID NO: 6 or 78.

47. The fusion polypeptide of any one of claims 1-46, wherein the COF1/CRBN-binding polypeptide comprises sufficient amino acid sequence and/or a structural motif from amino acid residues 236-249 of IKZF3 (numbered according to SEQ ID NO: 19) (e.g., sufficient amino acid sequence and/or a structural motif from amino acid residues 236-249 of SEQ ID NO: 19) that:

- i) the association of the COF1/CRBN-binding polypeptide with CRBN in the absence of COF1 is no more than, e.g., 0.01%, 0.1%, 1%, 5%, 10%, 15%, or 20%, of the association of the COF1/CRBN-binding polypeptide with CRBN in the presence of COF1, e.g., an excess of COF1, e.g., as measured by an assay described herein, e.g., immunoprecipitation;
- ii) the association of the fusion polypeptide with CRBN in the absence of COF1 is no more than, e.g., 0.01%, 0.1%, 1%, 5%, 10%, 15%, or 20% of the association of the fusion polypeptide with CRBN in the presence of COF1, e.g., an excess of COF1, e.g., as measured by an assay described herein, e.g., immunoprecipitation;
- iii) the ubiquitination of the heterologous polypeptide in the absence of COF1 is no more than, e.g., 0.01%, 0.1%, 1%, 10%, 20%, 30%, 40%, 50%, 60%, or 70%, of the ubiquitination of the heterologous polypeptide in the presence of COF1, e.g., an excess of COF1, e.g., as measured by an assay described herein;
- iv) the ubiquitination of the fusion polypeptide in the absence of COF1 is no more than, e.g., 0.01%, 0.1%, 1%, 10%, 20%, 30%, 40%, 50%, 60%, or 70%, of the ubiquitination of the fusion polypeptide in the presence of COF1, e.g., an excess of COF1, e.g., as measured by an assay described herein; or
- v) the degradation of the fusion polypeptide in the absence of COF1 is no more than, e.g., 0.01%, 0.1%, 1%, 10%, 20%, 30%, 40%, 50%, 60%, or 70%, of the degradation of the fusion polypeptide in the presence of COF1, e.g., an excess of COF1, e.g., as measured by an assay described herein, e.g., a Western blot analysis or a flow cytometry analysis.

48. The fusion polypeptide of claim 47, wherein the association, ubiquitination, and/or degradation is as measured in a mammalian cell, e.g., a human cell.

49. The fusion polypeptide of any one of claims 1-48, wherein the COF1/CRBN-binding polypeptide comprises amino acid residues 236-249 of IKZF3 (numbered according to SEQ ID NO: 19) (e.g., the COF1/CRBN-binding polypeptide comprises the amino acid sequence of SEQ ID NO: 11) or a sequence that differs from amino acid residues 236-249 of IKZF3 (numbered according to SEQ ID NO: 19) by no more than 1, 2, 3, 4, 5, 6, or 7 amino acid residues (e.g., a sequence that differs from amino acid residues 236-249 of SEQ ID NO: 19 by no more than 1, 2, 3, 4, 5, 6, or 7 amino acid residues) (e.g., a sequence having no more than 1, 2, 3, 4, 5, 6, or 7 amino acid substitutions from amino acid residues 236-249 of SEQ ID NO: 19).

50. The fusion polypeptide of any one of claims 1-49, wherein the COF1/CRBN-binding polypeptide comprises amino acid residues 236-249 of IKZF3 (numbered according to SEQ ID NO: 19), e.g., the COF1/CRBN-binding polypeptide comprises the amino acid sequence of SEQ ID NO: 11.

51. The fusion polypeptide of any one of claims 1-49, wherein the COF1/CRBN-binding polypeptide comprises the amino acid sequence of MALEKMALEKMALE (SEQ ID NO: 91).

52. The fusion polypeptide of any one of claims 1-51, wherein the COF1/CRBN-binding polypeptide comprises sufficient amino acid sequence and/or a structural motif from amino acid residues 136-180 and 236-249 of IKZF3 (numbered according to SEQ ID NO: 19) (e.g., sufficient amino acid sequence and/or a structural motif from amino acid residues 136-180 and 236-249 of SEQ ID NO: 19) that:

- i) the association of the COF1/CRBN-binding polypeptide with CRBN in the absence of COF1 is no more than, e.g., 0.01%, 0.1%, 1%, 5%, 10%, 15%, or 20%, of the association of the COF1/CRBN-binding polypeptide with CRBN in the presence of COF1, e.g., an excess of COF1, e.g., as measured by an assay described herein, e.g., immunoprecipitation;
- ii) the association of the fusion polypeptide with CRBN in the absence of COF1 is no more than, e.g., 0.01%, 0.1%, 1%, 5%, 10%, 15%, or 20% of the association of the fusion polypeptide with CRBN in the presence of COF1, e.g., an excess of COF1, e.g., as measured by an assay described herein, e.g., immunoprecipitation;
- iii) the ubiquitination of the heterologous polypeptide in the absence of COF1 is no more than, e.g., 0.01%, 0.1%, 1%, 10%, 20%, 30%, 40%, 50%, 60%, or 70%, of the ubiquitination of the heterologous polypeptide in the presence of COF1, e.g., an excess of COF1, e.g., as measured by an assay described herein;
- iv) the ubiquitination of the fusion polypeptide in the absence of COF1 is no more than, e.g., 0.01%, 0.1%, 1%, 10%, 20%, 30%, 40%, 50%, 60%, or 70%, of the ubiquitination of the fusion polypeptide in the presence of COF1, e.g., an excess of COF1, e.g., as measured by an assay described herein; or
- v) the degradation of the fusion polypeptide in the absence of COF1 is no more than, e.g., 0.01%, 0.1%, 1%, 10%, 20%, 30%, 40%, 50%, 60%, or 70%, of the degradation of the fusion polypeptide in the presence of COF1, e.g., an excess of COF1, e.g., as measured by an assay described herein, e.g., a Western blot analysis or a flow cytometry analysis.

53. The fusion polypeptide of claim 52, wherein the association, ubiquitination, and/or degradation is as measured in a mammalian cell, e.g., a human cell.



54. The fusion polypeptide of any one of claims 1-53, wherein the COF1/CRBN-binding polypeptide comprises a first sequence comprising amino acid residues 136-180 of IKZF3 (numbered according to SEQ ID NO: 19) (e.g., a first sequence comprising the amino acid sequence of SEQ ID NO: 5) or a first sequence that differs from amino acid residues 136-180 of IKZF3 (numbered according to SEQ ID NO: 19) by no more than 1, 2, 3, 4, 5, 10, 15, 20, 25, 30, 35, or 40 amino acid residues (e.g., a first sequence that differs from amino acid residues 136-180 of SEQ ID NO: 19 by no more than 1, 2, 3, 4, 5, 10, 15, 20, 25, 30, 35, or 40 amino acid residues); and a second sequence comprising amino acid residues 236-249 of IKZF3 (numbered according to SEQ ID NO: 19) (e.g., a second sequence comprising the amino acid sequence of SEQ ID NO: 11) or a second sequence that differs from amino acid residues 236-249 of IKZF3 (numbered according to SEQ ID NO: 19) by no more than 1, 2, 3, 4, 5, 6, or 7 amino acid residues (e.g., a second sequence that differs from amino acid residues 236-249 of SEQ ID NO: 19 by no more than 1, 2, 3, 4, 5, 6, or 7 amino acid residues).

55. The fusion polypeptide of any one of claims 1-54, wherein the COF1/CRBN-binding polypeptide comprises amino acid residues 136-180 and 236-249 of IKZF3 (numbered according to SEQ ID NO: 19), e.g., the COF1/CRBN-binding polypeptide comprises or consists of the amino acid sequence of SEQ ID NO: 1 or 3.

56. The fusion polypeptide of any one of claims 1-55, wherein the COF1/CRBN-binding polypeptide comprises or consists of the amino acid sequence of SEQ ID NO: 1 or 3.

57. The fusion polypeptide of any one of claims 1-56, wherein the COF1/CRBN-binding polypeptide comprises a first sequence comprising amino acid residues 136-180 of IKZF3 (numbered according to SEQ ID NO: 19) and a second sequence comprising the amino acid sequence of MALEKMALEKMALE (SEQ ID NO: 91), e.g., the COF1/CRBN-binding polypeptide comprises or consists of the amino acid sequence of SEQ ID NO: 14 or 85.

58. The fusion polypeptide of any one of claims 1-56, wherein the COF1/CRBN-binding polypeptide comprises a first sequence comprising amino acid residues 136-170 of IKZF3 (numbered according to SEQ ID NO: 19) and a second sequence comprising the amino acid sequence of MALEKMALEKMALE (SEQ ID NO: 91), e.g., the COF1/CRBN-binding polypeptide comprises or consists of the amino acid sequence of SEQ ID NO: 15 or 86.

59. The fusion polypeptide of any one of claims 1-58, wherein the COF1/CRBN-binding polypeptide comprises at least one less lysine than the corresponding native sequence.

60. The fusion polypeptide of claim 59, wherein one or more lysine residues in the corresponding native sequence are replaced by a different amino acid, e.g., arginine.

61. The fusion polypeptide of claim 59 or 60, wherein the COF1/CRBN-binding polypeptide comprises less than 1, 2, 3, 4, or 5 lysine residues.

62. The fusion polypeptide of claim 61, wherein the COF1/CRBN-binding polypeptide does not comprise a lysine residue.

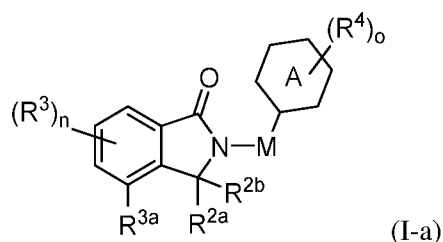
63. The fusion polypeptide of any one of claims 1-62, wherein the COF1/CRBN-binding polypeptide is not ubiquitinated, e.g., in the presence of COF1, e.g., as measured by an assay described herein, optionally wherein ubiquitination is as measured in a mammalian cell, e.g., a human cell.

64. The fusion polypeptide of any one of claims 1-63, wherein the COF1/CRBN-binding polypeptide comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 4, 41, 42, and 43.

65. The fusion polypeptide of any one of claims 1-63, wherein the COF1/CRBN-binding polypeptide comprises or consists of the amino acid sequence of SEQ ID NO: 2 or 4.

66. The fusion polypeptide of any one of claims 1-65, wherein the COF1 is an immunomodulatory imide drug (IMiD), or a pharmaceutically acceptable salt thereof.

67. The fusion polypeptide of any one of claims 1-65, wherein the COF1 has the structure of Formula (I-a):



or a pharmaceutically acceptable salt, ester, hydrate, or tautomer thereof, wherein:

Ring A is carbocyclyl, heterocyclyl, aryl, or heteroaryl, each of which is independently and optionally substituted with one or more R⁴;

M is absent, C<sub>1</sub>-C<sub>6</sub> alkyl, C<sub>2</sub>-C<sub>6</sub> alkenyl, C<sub>2</sub>-C<sub>6</sub> alkynyl, or C<sub>1</sub>-C<sub>6</sub> heteroalkyl, wherein each alkyl, alkenyl, alkynyl, and heteroalkyl is independently and optionally substituted with one or more R<sup>4</sup>;

each of R<sup>2a</sup> and R<sup>2b</sup> is independently hydrogen or C<sub>1</sub>-C<sub>6</sub> alkyl; or R<sup>2a</sup> and R<sup>2b</sup> together with the carbon atom to which they are attached to form a carbonyl group or thiocarbonyl group;

R<sup>3a</sup> is hydrogen, C<sub>1</sub>-C<sub>6</sub> alkyl, C<sub>2</sub>-C<sub>6</sub> alkenyl, C<sub>2</sub>-C<sub>6</sub> alkynyl, C<sub>1</sub>-C<sub>6</sub> heteroalkyl, halo, cyano, -C(O)R<sup>A</sup>, -C(O)OR<sup>B</sup>, -OR<sup>B</sup>, -N(R<sup>C</sup>)(R<sup>D</sup>), -C(O)N(R<sup>C</sup>)(R<sup>D</sup>), -N(R<sup>C</sup>)C(O)R<sup>A</sup>, -S(O)<sub>x</sub>R<sup>E</sup>, -S(O)<sub>x</sub>N(R<sup>C</sup>)(R<sup>D</sup>), or -N(R<sup>C</sup>)S(O)<sub>x</sub>R<sup>E</sup>, wherein each alkyl, alkenyl, alkynyl, and heteroalkyl is independently and optionally substituted with one or more R<sup>6</sup>;

each of R<sup>3</sup> is independently C<sub>1</sub>-C<sub>6</sub> alkyl, C<sub>2</sub>-C<sub>6</sub> alkenyl, C<sub>2</sub>-C<sub>6</sub> alkynyl, C<sub>1</sub>-C<sub>6</sub> heteroalkyl, halo, cyano, -C(O)R<sup>A</sup>, -C(O)OR<sup>B</sup>, -OR<sup>B</sup>, -N(R<sup>C</sup>)(R<sup>D</sup>), -C(O)N(R<sup>C</sup>)(R<sup>D</sup>), -N(R<sup>C</sup>)C(O)R<sup>A</sup>, -S(O)<sub>x</sub>R<sup>E</sup>, -S(O)<sub>x</sub>N(R<sup>C</sup>)(R<sup>D</sup>), or -N(R<sup>C</sup>)S(O)<sub>x</sub>R<sup>E</sup>, wherein each alkyl, alkenyl, alkynyl, and heteroalkyl is independently and optionally substituted with one or more R<sup>6</sup>;

each R<sup>4</sup> is independently C<sub>1</sub>-C<sub>6</sub> alkyl, C<sub>2</sub>-C<sub>6</sub> alkenyl, C<sub>2</sub>-C<sub>6</sub> alkynyl, C<sub>1</sub>-C<sub>6</sub> heteroalkyl, halo, cyano, oxo, -C(O)R<sup>A</sup>, -C(O)OR<sup>B</sup>, -OR<sup>B</sup>, -N(R<sup>C</sup>)(R<sup>D</sup>), -C(O)N(R<sup>C</sup>)(R<sup>D</sup>), -N(R<sup>C</sup>)C(O)R<sup>A</sup>, S(O)<sub>x</sub>R<sup>E</sup>, -S(O)<sub>x</sub>N(R<sup>C</sup>)(R<sup>D</sup>), -N(R<sup>C</sup>)S(O)<sub>x</sub>R<sup>E</sup>, carbocyclyl, heterocyclyl, aryl, or heteroaryl, wherein each alkyl, alkenyl, alkynyl, carbocyclyl, heterocyclyl, aryl, and heteroaryl is independently and optionally substituted with one or more R<sup>7</sup>;

each of R<sup>A</sup>, R<sup>B</sup>, R<sup>C</sup>, R<sup>D</sup>, and R<sup>E</sup> is independently hydrogen or C<sub>1</sub>-C<sub>6</sub> alkyl;

each R<sup>6</sup> is independently C<sub>1</sub>-C<sub>6</sub> alkyl, oxo, cyano, -OR<sup>B</sup>, -N(R<sup>C</sup>)(R<sup>D</sup>), -C(O)N(R<sup>C</sup>)(R<sup>D</sup>), -N(R<sup>C</sup>)C(O)R<sup>A</sup>, aryl, or heteroaryl, wherein each aryl and heteroaryl is independently and optionally substituted with one or more R<sup>8</sup>;

each R<sup>7</sup> is independently halo, oxo, cyano, -OR<sup>B</sup>, -N(R<sup>C</sup>)(R<sup>D</sup>), -C(O)N(R<sup>C</sup>)(R<sup>D</sup>), or -N(R<sup>C</sup>)C(O)R<sup>A</sup>;

each R<sup>8</sup> is independently C<sub>1</sub>-C<sub>6</sub> alkyl, cyano, -OR<sup>B</sup>, -N(R<sup>C</sup>)(R<sup>D</sup>), -C(O)N(R<sup>C</sup>)(R<sup>D</sup>), or -N(R<sup>C</sup>)C(O)R<sup>A</sup>;

n is 0, 1, 2, or 3;

o is 0, 1, 2, 3, 4, or 5; and

x is 0, 1, or 2.

68. The fusion polypeptide of claim 67, wherein X is O.

69. The fusion polypeptide of claim 67 or 68, wherein M is absent.

70. The fusion polypeptide of any one of claims 67-69, wherein Ring A is heterocyclyl (e.g., a nitrogen-containing heterocyclyl, e.g., 2-(2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione).

71. The fusion polypeptide of any one of claims 67-70, wherein  $R^4$  is oxo or  $OR^B$  (e.g.,  $-OCH_3$  or  $-OCH_2CH_3$ ) and  $o$  is 0, 1, or 2.

72. The fusion polypeptide of any one of claims 67-71, wherein each of  $R^{2a}$  and  $R^{2b}$  is independently hydrogen, or  $R^{2a}$  and  $R^{2b}$  together with the carbon atom to which they are attached form a carbonyl group.

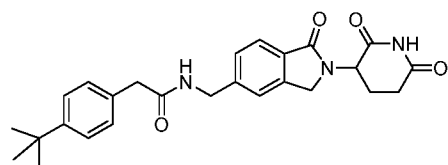
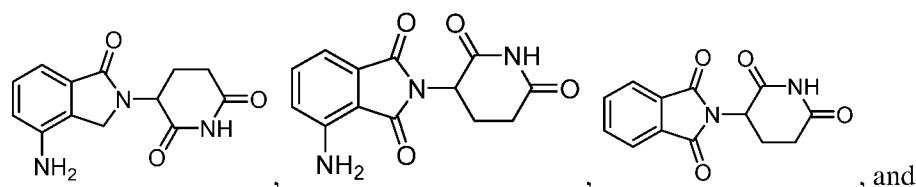
73. The fusion polypeptide of any one of claims 67-72, wherein  $R^{3a}$  is heteroalkyl (e.g.,  $-CH_2NHC(O)CH_2$ ),  $-N(R^C)(R^D)$  (e.g.,  $-NH_2$ ), or  $-N(R^C)C(O)R^A$  (e.g.,  $-NHC(O)CH_3$ ).

74. The fusion polypeptide of any one of claims 67-73, wherein  $n$  is 0.

75. The fusion polypeptide of any one of claims 1-74, wherein the COF1 is thalidomide or analogue, or a pharmaceutically acceptable salt thereof.

76. The fusion polypeptide of any one of claims 1-74, wherein the COF1 is selected from the group consisting of lenalidomide, pomalidomide, thalidomide, and 2-(4-(tert-butyl)phenyl)-N-((2-(2,6-dioxopiperidin-3-yl)-1-oxoisindolin-5-yl)methyl)acetamide, or a pharmaceutically acceptable salt thereof.

77. The fusion polypeptide of any one of claims 1-74, wherein the COF1 is selected from the group consisting of:



, or a pharmaceutically acceptable salt thereof.

78. The fusion polypeptide of any one of claims 1-74, wherein the COF1 is lenalidomide or pomalidomide, or a pharmaceutically acceptable salt thereof.

79. The fusion polypeptide of any one of claims 1-74, wherein the COF1 is lenalidomide or analogue, or a pharmaceutically acceptable salt thereof.

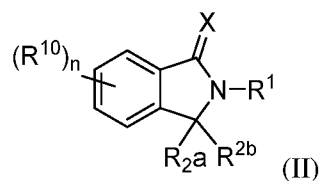
80. The fusion polypeptide of any one of claims 1-79, wherein the fusion polypeptide further comprises a degradation domain, wherein the degradation domain is separated from the COF1/CRBN-binding polypeptide and the heterologous polypeptide by a heterologous protease cleavage site.

81. The fusion polypeptide of claim 80, wherein the fusion polypeptide comprises, from N-terminus to C-terminus:

- i) the degradation domain, the heterologous protease cleavage site, the heterologous polypeptide, and the COF1/CRBN-binding polypeptide;
- ii) the degradation domain, the heterologous protease cleavage site, the COF1/CRBN-binding polypeptide, and the heterologous polypeptide;
- iii) the COF1/CRBN-binding polypeptide, the heterologous polypeptide, the heterologous protease cleavage site, and the degradation domain; or
- iv) the heterologous polypeptide, and the COF1/CRBN-binding polypeptide, the heterologous protease cleavage site, and the degradation domain.

82. The fusion polypeptide of claim 80, wherein the fusion polypeptide comprises, from N-terminus to C-terminus, the degradation domain, the heterologous protease cleavage site, the heterologous polypeptide, and the COF1/CRBN-binding polypeptide.

83. A fusion polypeptide comprising a first domain and a second domain separated by a heterologous protease cleavage site, wherein the first domain comprises a degradation domain and the second domain comprises a compound of Formula (II) (COF2)/CRBN (cereblon)-binding polypeptide and a heterologous polypeptide, e.g., a heterologous mammalian, bacterial, or viral polypeptide, wherein the compound of Formula (II) is:



or a pharmaceutically acceptable salt, ester, hydrate, tautomer, or prodrug thereof, wherein:

X is O or S;

R<sup>1</sup> is C<sub>1</sub>-C<sub>6</sub> alkyl, C<sub>2</sub>-C<sub>6</sub> alkenyl, C<sub>2</sub>-C<sub>6</sub> alkynyl, C<sub>1</sub>-C<sub>6</sub> heteroalkyl, carbocyclyl, heterocyclyl, aryl, or heteroaryl, each of which is independently and optionally substituted by one or more R<sup>4</sup>;

each of  $R^{2a}$  and  $R^{2b}$  is independently hydrogen or  $C_1$ - $C_6$  alkyl; or  $R^{2a}$  and  $R^{2b}$  together with the carbon atom to which they are attached to form carbonyl group or thiocarbonyl group;

each of  $R^{10}$  is independently  $C_1$ - $C_6$  alkyl,  $C_2$ - $C_6$  alkenyl,  $C_2$ - $C_6$  alkynyl,  $C_1$ - $C_6$  heteroalkyl, halo, cyano,  $-C(O)R^A$ ,  $-C(O)OR^B$ ,  $-OR^B$ ,  $-N(R^C)(R^D)$ ,  $-C(O)N(R^C)(R^D)$ ,  $-N(R^C)C(O)R^A$ ,  $-S(O)_xR^E$ ,  $-S(O)_xN(R^C)(R^D)$ , or  $-N(R^C)S(O)_xR^E$ , or L-Tag; wherein each alkyl, alkenyl, alkynyl, and heteroalkyl is independently and optionally substituted with one or more  $R^{11}$ ;

each  $R^4$  is independently  $C_1$ - $C_6$  alkyl,  $C_2$ - $C_6$  alkenyl,  $C_2$ - $C_6$  alkynyl,  $C_1$ - $C_6$  heteroalkyl, halo, cyano, oxo,  $C(O)R^A$ ,  $-C(O)OR^B$ ,  $OR^B$ ,  $-N(R^C)(R^D)$ ,  $-C(O)N(R^C)(R^D)$ ,  $-N(R^C)C(O)R^A$ ,  $S(O)_xR^E$ ,  $-S(O)_xN(R^C)(R^D)$ ,  $-N(R^C)S(O)_xR^E$ , carbocyclyl, heterocyclyl, aryl, or heteroaryl, wherein each alkyl, alkenyl, alkynyl, heteroalkyl, carbocyclyl, heterocyclyl, aryl, and heteroaryl is independently and optionally substituted with one or more  $R^7$ ;

each of  $R^A$ ,  $R^B$ ,  $R^C$ ,  $R^D$ , and  $R^E$  is independently hydrogen or  $C_1$ - $C_6$  alkyl;

each  $R^{11}$  is independently  $C_1$ - $C_6$  alkyl, halo, oxo, cyano,  $-OR^B$ ,  $-N(R^C)(R^D)$ ,  $-C(O)N(R^C)(R^D)$ ,  $-N(R^C)C(O)R^A$ , aryl, or heteroaryl, wherein each aryl and heteroaryl is independently and optionally substituted with one or more  $R^8$ ;

each  $R^7$  is independently halo, oxo, cyano,  $-OR^B$ ,  $-N(R^C)(R^D)$ ,  $-C(O)N(R^C)(R^D)$ , or  $-N(R^C)C(O)R^A$ ;

each  $R^8$  is independently  $C_1$ - $C_6$  alkyl, halo, cyano,  $-OR^B$ ,  $-N(R^C)(R^D)$ ,  $-C(O)N(R^C)(R^D)$ , or  $-N(R^C)C(O)R^A$ ;

each L is independently  $C_1$ - $C_6$  alkyl,  $C_2$ - $C_6$  alkenyl,  $C_2$ - $C_6$  alkynyl,  $C_1$ - $C_6$  heteroalkyl,  $-C(O)R^{A1}$ ,  $-C(O)OR^{B1}$ ,  $-OR^{B1}$ ,  $-N(R^{C1})(R^{D1})$ ,  $-C(O)N(R^{C1})(R^{D1})$ ,  $-N(R^{C1})C(O)R^{A1}$ ,  $-S(O)_xR^{E1}$ ,  $-S(O)_xN(R^{C1})(R^{D1})$ , or  $-N(R^{C1})S(O)_xR^{E1}$ , wherein each alkyl, alkenyl, alkynyl, and heteroalkyl is independently and optionally substituted with one or more  $R^{12}$ ;

each Tag is a targeting moiety capable of binding to a target protein;

each of  $R^{A1}$ ,  $R^{B1}$ ,  $R^{C1}$ ,  $R^{D1}$ , and  $R^{E1}$  is independently hydrogen,  $C_1$ - $C_6$  alkyl,  $C_2$ - $C_6$  alkenyl,  $C_2$ - $C_6$  alkynyl,  $C_1$ - $C_6$  heteroalkyl, carbocyclyl, heterocyclyl, aryl, or heteroaryl, wherein each alkyl, alkenyl, alkynyl, heteroalkyl, carbocyclyl, heterocyclyl, aryl, and heteroaryl is independently and optionally substituted with one or more  $R^{12}$ ;

each  $R^{12}$  is independently  $C_1$ - $C_6$  alkyl, halo, cyano, carbocyclyl, or heterocyclyl;

n is 0, 1, 2, 3 or 4; and

x is 0, 1, or 2.

84. The fusion polypeptide of any one of claims 80-83, wherein the degradation domain has a first state associated with a first level of expression of the fusion polypeptide and a second state associated with a

second level of expression of the fusion polypeptide, wherein the second level is increased, e.g., by at least 2-, 3-, 4-, 5-, 10-, 20- or 30-fold over the first level in the presence of a stabilization compound.

85. The fusion polypeptide of claim 84, wherein in the absence of the stabilization compound, the fusion polypeptide is degraded by a cellular degradation pathway, e.g., at least 50%, 60%, 70%, 80%, 90% or greater of the fusion polypeptide is degraded, e.g., as measured by an assay described herein, e.g., a Western blot analysis or a flow cytometry analysis.

86. The fusion polypeptide of claim 84 or 85, wherein the level of expression, and/or degradation is as measured in a mammalian cell, e.g., a human cell.

87. The fusion polypeptide of any one of claims 84-86, wherein in the presence of the stabilization compound:

- i) the degradation domain assumes a conformation more resistant to cellular degradation relative to a conformation in the absence of the stabilization compound; or
- ii) the conformation of the fusion polypeptide is more permissive to cleavage at the heterologous protease cleavage site relative to a conformation in the absence of the stabilization compound.

88. The fusion polypeptide of any one of claims 80-87, wherein the degradation domain is chosen from an estrogen receptor (ER) domain, an FKB protein (FKBP) domain, or a dihydrofolate reductase (DHFR) domain.

89. The fusion polypeptide of claim 88, wherein the degradation domain is an estrogen receptor (ER) domain.

90. The fusion polypeptide of claim 89, wherein the degradation domain comprises an amino acid sequence that is at least 90, 95, 97, 98, 99, or 100% identical to SEQ ID NO: 46 or 48.

91. The fusion polypeptide of claim 90, wherein the degradation domain comprises the amino acid sequence of SEQ ID NO: 46.

92. The fusion polypeptide of any one of claims 89-91, wherein the stabilization compound is bazedoxifene or 4-hydroxy tamoxifen (4-OHT), or a pharmaceutically acceptable salt thereof.

93. The fusion polypeptide of claim 88, wherein the degradation domain is an FKB protein (FKBP) domain.

94. The fusion polypeptide of claim 93, wherein the degradation domain comprises an amino acid sequence that is at least 90, 95, 97, 98, 99, or 100% identical to SEQ ID NO: 50.

95. The fusion polypeptide of claim 93 or 94, wherein the stabilization compound is Shield-1, or a pharmaceutically acceptable salt thereof.

96. The fusion polypeptide of claim 88, wherein the degradation domain is a dihydrofolate reductase (DHFR) domain.

97. The fusion polypeptide of claim 96, wherein the degradation domain comprises an amino acid sequence that is at least 90, 95, 97, 98, 99, or 100% identical to SEQ ID NO: 51.

98. The fusion polypeptide of claim 96 or 97, wherein the stabilization compound is trimethoprim, or a pharmaceutically acceptable salt thereof.

99. The fusion polypeptide of any one of claims 80-98, wherein the heterologous protease cleavage site is cleaved by a mammalian intracellular protease.

100. The fusion polypeptide of claim 99, wherein the heterologous protease cleavage site is cleaved by a protease selected from the group consisting of furin, PCSK1, PCSK5, PCSK6, PCSK7, cathepsin B, Granzyme B, Factor XA, Enterokinase, genenase, sortase, precision protease, thrombin, TEV protease, and elastase 1.

101. The fusion polypeptide of claim 99 or 100, wherein the heterologous protease cleavage site comprises a sequence having a cleavage motif selected from the group consisting of RX(K/R)R consensus motif (X can be any amino acid; SEQ ID NO: 52), RXXX[KR]R consensus motif (X can be any amino acid; SEQ ID NO: 53), RRX consensus motif (SEQ ID NO : 54), I-E-P-D-X consensus motif (SEQ ID NO: 55), Ile-Glu/Asp-Gly-Arg (SEQ IDNO : 56), Asp-Asp-Asp-Asp-Lys (SEQ ID NO: 57), Pro-Gly-Ala-Ala-His-Tyr (SEQ ID NO: 58), LPXTG/A consensus motif (SEQ ID NO: 59), Leu-Glu-Val-Phe-Gln-Gly-Pro (SEQ ID NO: 60), Leu-Val-Pro-Arg-Gly-Ser (SEQ ID NO: 61), E-N-L-Y-F-Q-G (SEQ ID NO: 62), and [AGSV]-X (X can be any amino acid; SEQ ID NO: 63).



102. The fusion polypeptide of claim 99, wherein the heterologous protease cleavage site is cleaved by furin.

103. The fusion polypeptide of claim 102, wherein the heterologous protease cleavage site comprises a furin cleavage site selected from the group consisting of RTKR (SEQ ID NO: 123); GTGAEDPRPSRKRRSLGDVG (SEQ ID NO: 125); GTGAEDPRPSRKRR (SEQ ID NO: 127); LQWLEQQVAKRRTRK (SEQ ID NO: 129); GTGAEDPRPSRKRRSLGG (SEQ ID NO: 131); GTGAEDPRPSRKRRSLG (SEQ ID NO: 133); SLNLTESHNSRKKR (SEQ ID NO: 135); CKINGYPKRGRKRR (SEQ ID NO: 137); and SARNRQKR (SEQ ID NO: 34).

104. The fusion polypeptide of claim 102, wherein the heterologous protease cleavage site comprises the furin cleavage site of GTGAEDPRPSRKRRSLGDVG (SEQ ID NO: 125).

105. The fusion polypeptide of any one of claims 80-98, wherein the heterologous protease cleavage site is cleaved by a mammalian extracellular protease.

106. The fusion polypeptide of claim 105, wherein the mammalian extracellular protease is selected from the group consisting of Factor XA, Enterokinase, genenase, sortase, precision protease, thrombin, TEV protease, and elastase 1.

107. The fusion polypeptide of claim 106, wherein the heterologous protease cleavage site comprises an amino acid sequence selected from the group consisting of Ile-Glu/Asp-Gly-Arg (SEQ IDNO : 56), Asp-Asp-Asp-Asp-Lys (SEQ ID NO: 57), Pro-Gly-Ala-Ala-His-Tyr (SEQ ID NO: 58), LPXTG/A consensus motif (SEQ ID NO: 59), Leu-Glu-Val-Phe-Gln-Gly-Pro (SEQ ID NO: 60), Leu-Val-Pro-Arg-Gly-Ser (SEQ ID NO: 61), E-N-L-Y-F-Q-G (SEQ ID NO: 62), and [AGSV]-X (X can be any amino acid; SEQ ID NO: 63).

108. The fusion polypeptide of any one of claims 83-107, wherein the degradation domain is fused to the heterologous protease cleavage site, which is further fused to the second domain.

109. The fusion polypeptide of any one of claims 83-108, comprising, from N-terminus to C-terminus:

- i) the degradation domain, the heterologous protease cleavage site, the heterologous polypeptide, and the COF2/CRBN-binding polypeptide;
- ii) the degradation domain, the heterologous protease cleavage site, the COF2/CRBN-binding polypeptide, and the heterologous polypeptide;

- iii) the COF2/CRBN-binding polypeptide, the heterologous polypeptide, the heterologous protease cleavage site, and the degradation domain; or
- iv) the heterologous polypeptide, and the COF2/CRBN-binding polypeptide, the heterologous protease cleavage site, and the degradation domain.

110. The fusion polypeptide of any one of claims 83-108, comprising, from N-terminus to C-terminus, the degradation domain, the heterologous protease cleavage site, the heterologous polypeptide, and the COF2/CRBN-binding polypeptide.

111. The fusion polypeptide of any one of claims 83-110, wherein the association of the COF2/CRBN-binding polypeptide with cereblon (CRBN) in the absence of COF2 is no more than, e.g., 0.01%, 0.1%, 1%, 5%, 10%, 15%, or 20%, of the association of the COF2/CRBN-binding polypeptide with CRBN in the presence of COF2, e.g., an excess of COF2, e.g., as measured by an assay described herein, e.g., immunoprecipitation.

112. The fusion polypeptide of any one of claims 83-111, wherein the COF2/CRBN-binding polypeptide does not bind to CRBN in the absence of COF2.

113. The fusion polypeptide of any one of claims 83-112, wherein the association of the fusion polypeptide with CRBN in the absence of COF2 is no more than, e.g., 0.01%, 0.1%, 1%, 5%, 10%, 15%, or 20%, of the association of the fusion polypeptide with CRBN in the presence of COF2, e.g., an excess of COF2, e.g., as measured by an assay described herein, e.g., immunoprecipitation.

114. The fusion polypeptide of any one of claims 83-113, wherein the fusion polypeptide does not bind to CRBN in the absence of COF2.

115. The fusion polypeptide of any one of claims 83-114, wherein the ubiquitination of the heterologous polypeptide in the absence of COF2 is no more than, e.g., 0.01%, 0.1%, 1%, 10%, 20%, 30%, 40%, 50%, 60%, or 70%, of the ubiquitination of the heterologous polypeptide in the presence of COF2, e.g., an excess of COF2, e.g., as measured by an assay described herein.

116. The fusion polypeptide of any one of claims 83-115, wherein the ubiquitination of the fusion polypeptide in the absence of COF2 is no more than, e.g., 0.01%, 0.1%, 1%, 10%, 20%, 30%, 40%, 50%, 60%, or 70%, of the ubiquitination of the fusion polypeptide in the presence of COF2, e.g., an excess of COF2, e.g., as measured by an assay described herein.

117. The fusion polypeptide of claim 115 or 116, wherein the heterologous polypeptide or the fusion polypeptide is ubiquitinated at one or more lysine or methionine residues in the presence of COF2.

118. The fusion polypeptide of any one of claims 83-117, wherein the degradation of the fusion polypeptide in the absence of COF2 is no more than, e.g., 0.01%, 0.1%, 1%, 10%, 20%, 30%, 40%, 50%, 60%, or 70%, of the degradation of the fusion polypeptide in the presence of COF2, e.g., an excess of COF2, e.g., as measured by an assay described herein, e.g., a Western blot analysis or a flow cytometry analysis.

119. The fusion polypeptide of claim 118, wherein the degradation of the fusion polypeptide is mediated by ubiquitination in the presence of COF2.

120. The fusion polypeptide of any one of claims 111-119, wherein the association, ubiquitination, and/or degradation is as measured in a mammalian cell, e.g., a human cell.

121. The fusion polypeptide of any one of claims 83-120, wherein the COF2/CRBN-binding polypeptide is between 10 and 95 amino acid residues in length, between 15 and 90 amino acid residues in length, between 20 and 85 amino acid residues in length, between 25 and 80 amino acid residues in length, between 30 and 75 amino acid residues in length, between 35 and 70 amino acid residues in length, between 40 and 65 amino acid residues in length, between 45 and 65 amino acid residues in length, between 50 and 65 amino acid residues in length, or between 55 and 65 amino acid residues in length.

122. The fusion polypeptide of any one of claims 83-121, wherein the COF2/CRBN-binding polypeptide comprises a beta turn.

123. The fusion polypeptide of any one of claims 83-122, wherein the COF2/CRBN-binding polypeptide comprises a beta hairpin.

124. The fusion polypeptide of any one of claims 83-123, wherein the COF2/CRBN-binding polypeptide comprises a beta strand.

125. The fusion polypeptide of any one of claims 83-124, wherein the COF2/CRBN-binding polypeptide comprises an alpha helix.

126. The fusion polypeptide of any one of claims 83-125, wherein the COF2/CRBN-binding polypeptide comprises, from N-terminus to C-terminus, a first beta strand, a beta hairpin, a second beta strand, and a first alpha helix.

127. The fusion polypeptide of any one of claims 83-126, wherein the COF2/CRBN-binding polypeptide comprises, from N-terminus to C-terminus, a first beta strand, a beta hairpin, a second beta strand, a first alpha helix, and a second alpha helix.

128. The fusion polypeptide of claim 127, wherein the beta hairpin and the second alpha helix are separated by no more than 60, 50, 40, or 30 amino acid residues.

129. The fusion polypeptide of any one of claims 83-128, wherein the COF2/CRBN-binding polypeptide comprises a COF2/CRBN-binding sequence from a naturally occurring polypeptide or a COF2/CRBN-binding variant thereof, e.g., a naturally occurring IKZF polypeptide or a COF2/CRBN-binding variant thereof, e.g., a naturally occurring IKZF1, IKZF2, IKZF3, IKZF4, or IKZF5 a COF2/CRBN-binding variant thereof.

130. The fusion polypeptide of claim 129, wherein the COF2/CRBN-binding sequence comprises two or more discontinuous sequences from the naturally occurring polypeptide, e.g., a naturally occurring IKZF polypeptide, e.g., a naturally occurring IKZF1, IKZF2, IKZF3, IKZF4, or IKZF5.

131. The fusion polypeptide of any one of claims 83-130, wherein the COF2/CRBN-binding polypeptide comprises an IKZF polypeptide or a structural motif thereof.

132. The fusion polypeptide of claim 131, wherein the IKZF polypeptide is an IKZF1 polypeptide, an IKZF3 polypeptide, an IKZF2 polypeptide having H141Q substitution (numbered according to SEQ ID NO: 21), or an IKZF4 polypeptide having H188Q substitution (numbered according to SEQ ID NO: 22).

133. The fusion polypeptide of any one of claims 83-132, wherein the COF2/CRBN-binding polypeptide comprises sufficient amino acid sequence and/or a structural motif from IKZF (e.g., IKZF1 or IKZF3) that:

i) the association of the COF2/CRBN-binding polypeptide with CRBN in the absence of COF2 is no more than, e.g., 0.01%, 0.1%, 1%, 5%, 10%, 15%, or 20%, of the association of the COF2/CRBN-

binding polypeptide with CRBN in the presence of COF2, e.g., an excess of COF2, e.g., as measured by an assay described herein, e.g., immunoprecipitation;

ii) the association of the fusion polypeptide with CRBN in the absence of COF2 is no more than, e.g., 0.01%, 0.1%, 1%, 5%, 10%, 15%, or 20% of the association of the fusion polypeptide with CRBN in the presence of COF2, e.g., an excess of COF2, e.g., as measured by an assay described herein, e.g., immunoprecipitation;

iii) the ubiquitination of the heterologous polypeptide in the absence of COF2 is no more than, e.g., 0.01%, 0.1%, 1%, 10%, 20%, 30%, 40%, 50%, 60%, or 70%, of the ubiquitination of the heterologous polypeptide in the presence of COF2, e.g., an excess of COF2, e.g., as measured by an assay described herein;

iv) the ubiquitination of the fusion polypeptide in the absence of COF2 is no more than, e.g., 0.01%, 0.1%, 1%, 10%, 20%, 30%, 40%, 50%, 60%, or 70%, of the ubiquitination of the fusion polypeptide in the presence of COF2, e.g., an excess of COF2, e.g., as measured by an assay described herein; or

v) the degradation of the fusion polypeptide in the absence of COF2 is no more than, e.g., 0.01%, 0.1%, 1%, 10%, 20%, 30%, 40%, 50%, 60%, or 70%, of the degradation of the fusion polypeptide in the presence of COF2, e.g., an excess of COF2, e.g., as measured by an assay described herein, e.g., a Western blot analysis or a flow cytometry analysis.

134. The fusion polypeptide of claim 133, wherein the association, ubiquitination, and/or degradation is as measured in a mammalian cell, e.g., a human cell.

135. The fusion polypeptide of any one of claims 83-134, wherein the COF2/CRBN-binding polypeptide comprises about 10 to about 95 amino acid residues, about 15 to about 90 amino acid residues, about 20 to about 85 amino acid residues, about 25 to about 80 amino acid residues, about 30 to about 75 amino acid residues, about 35 to about 70 amino acid residues, about 40 to about 65 amino acid residues, about 45 to about 65 amino acid residues, about 50 to about 65 amino acid residues, or about 55 to about 65 amino acid residues of IKZF1 or IKZF3.

136. The fusion polypeptide of any one of claims 83-135, wherein the COF2/CRBN-binding polypeptide comprises sufficient amino acid sequence and/or a structural motif from amino acid residues 136-180 of IKZF3 (numbered according to SEQ ID NO: 19) (e.g., sufficient amino acid sequence and/or a structural motif from amino acid residues 136-180 of SEQ ID NO: 19) that:

i) the association of the COF2/CRBN-binding polypeptide with CRBN in the absence of COF2 is no more than, e.g., 0.01%, 0.1%, 1%, 5%, 10%, 15%, or 20%, of the association of the COF2/CRBN-

binding polypeptide with CRBN in the presence of COF2, e.g., an excess of COF2, e.g., as measured by an assay described herein, e.g., immunoprecipitation;

ii) the association of the fusion polypeptide with CRBN in the absence of COF2 is no more than, e.g., 0.01%, 0.1%, 1%, 5%, 10%, 15%, or 20% of the association of the fusion polypeptide with CRBN in the presence of COF2, e.g., an excess of COF2, e.g., as measured by an assay described herein, e.g., immunoprecipitation;

iii) the ubiquitination of the heterologous polypeptide in the absence of COF2 is no more than, e.g., 0.01%, 0.1%, 1%, 10%, 20%, 30%, 40%, 50%, 60%, or 70%, of the ubiquitination of the heterologous polypeptide in the presence of COF2, e.g., an excess of COF2, e.g., as measured by an assay described herein;

iv) the ubiquitination of the fusion polypeptide in the absence of COF2 is no more than, e.g., 0.01%, 0.1%, 1%, 10%, 20%, 30%, 40%, 50%, 60%, or 70%, of the ubiquitination of the fusion polypeptide in the presence of COF2, e.g., an excess of COF2, e.g., as measured by an assay described herein; or

v) the degradation of the fusion polypeptide in the absence of COF2 is no more than, e.g., 0.01%, 0.1%, 1%, 10%, 20%, 30%, 40%, 50%, 60%, or 70%, of the degradation of the fusion polypeptide in the presence of COF2, e.g., an excess of COF2, e.g., as measured by an assay described herein, e.g., a Western blot analysis or a flow cytometry analysis.

137. The fusion polypeptide of claim 136, wherein the association, ubiquitination, and/or degradation is as measured in a mammalian cell, e.g., a human cell.

138. The fusion polypeptide of any one of claims 83-137, wherein the COF2/CRBN-binding polypeptide comprises amino acid residues 136-180 of IKZF3 (numbered according to SEQ ID NO: 19) (e.g., the COF2/CRBN-binding polypeptide comprises the amino acid sequence of SEQ ID NO: 5) or a sequence that differs from amino acid residues 136-180 of IKZF3 (numbered according to SEQ ID NO: 19) by no more than 1, 2, 3, 4, 5, 10, 15, 20, 25, 30, 35, or 40 amino acid residues (e.g., a sequence that differs from amino acid residues 136-180 of SEQ ID NO: 19 by no more than 1, 2, 3, 4, 5, 10, 15, 20, 25, 30, 35, or 40 amino acid residues) (e.g., a sequence having no more than 1, 2, 3, 4, 5, 10, 15, 20, 25, 30, 35, or 40 amino acid substitutions from amino acid residues 136-180 of SEQ ID NO: 19).

139. The fusion polypeptide of any one of claims 83-137, wherein the COF2/CRBN-binding polypeptide comprises sufficient amino acid sequence and/or a structural motif from amino acid residues 136-170 of IKZF3 (numbered according to SEQ ID NO: 19) (e.g., sufficient amino acid sequence and/or a structural motif from amino acid residues 136-170 of SEQ ID NO: 19) that:

- i) the association of the COF2/CRBN-binding polypeptide with CRBN in the absence of COF2 is no more than, e.g., 0.01%, 0.1%, 1%, 5%, 10%, 15%, or 20%, of the association of the COF2/CRBN-binding polypeptide with CRBN in the presence of COF2, e.g., an excess of COF2, e.g., as measured by an assay described herein, e.g., immunoprecipitation;
- ii) the association of the fusion polypeptide with CRBN in the absence of COF2 is no more than, e.g., 0.01%, 0.1%, 1%, 5%, 10%, 15%, or 20% of the association of the fusion polypeptide with CRBN in the presence of COF2, e.g., an excess of COF2, e.g., as measured by an assay described herein, e.g., immunoprecipitation;
- iii) the ubiquitination of the heterologous polypeptide in the absence of COF2 is no more than, e.g., 0.01%, 0.1%, 1%, 10%, 20%, 30%, 40%, 50%, 60%, or 70%, of the ubiquitination of the heterologous polypeptide in the presence of COF2, e.g., an excess of COF2, e.g., as measured by an assay described herein;
- iv) the ubiquitination of the fusion polypeptide in the absence of COF2 is no more than, e.g., 0.01%, 0.1%, 1%, 10%, 20%, 30%, 40%, 50%, 60%, or 70%, of the ubiquitination of the fusion polypeptide in the presence of COF2, e.g., an excess of COF2, e.g., as measured by an assay described herein; or
- v) the degradation of the fusion polypeptide in the absence of COF2 is no more than, e.g., 0.01%, 0.1%, 1%, 10%, 20%, 30%, 40%, 50%, 60%, or 70%, of the degradation of the fusion polypeptide in the presence of COF2, e.g., an excess of COF2, e.g., as measured by an assay described herein, e.g., a Western blot analysis or a flow cytometry analysis.

140. The fusion polypeptide of claim 139, wherein the association, ubiquitination, and/or degradation is as measured in a mammalian cell, e.g., a human cell.

141. The fusion polypeptide of any one of claims 83-137, 139, or 140, wherein the COF2/CRBN-binding polypeptide comprises amino acid residues 136-170 of IKZF3 (numbered according to SEQ ID NO: 19) (e.g., the COF2/CRBN-binding polypeptide comprises the amino acid sequence of SEQ ID NO: 6) or a sequence that differs from amino acid residues 136-170 of IKZF3 (numbered according to SEQ ID NO: 19) by no more than 1, 2, 3, 4, 5, 10, 15, 20, 25, or 30 amino acid residues (e.g., a sequence that differs from amino acid residues 136-170 of SEQ ID NO: 19 by no more than 1, 2, 3, 4, 5, 10, 15, 20, 25, or 30 amino acid residues) (e.g., a sequence having no more than 1, 2, 3, 4, 5, 10, 15, 20, 25, or 30 amino acid substitutions from amino acid residues 136-170 of SEQ ID NO: 19).

142. The fusion polypeptide of any one of claims 138-141, wherein one, two, three or all of the following amino acid residues remain unaltered: glutamine at position 147, cysteine at position 148,

glutamine at position 150, glycine at position 152, leucine at position 161, or leucine at position 162, numbered according to SEQ ID NO: 19.

143. The fusion polypeptide of claim 142, wherein glutamine at position 147 remains unaltered, numbered according to SEQ ID NO: 19.

144. The fusion polypeptide of claim 142, wherein cysteine at position 148 remains unaltered, numbered according to SEQ ID NO: 19.

145. The fusion polypeptide of claim 142, wherein glycine at position 152 remains unaltered, numbered according to SEQ ID NO: 19.

146. The fusion polypeptide of any one of claims 83-145, wherein the COF2/CRBN-binding polypeptide comprises amino acid residues 136-139 of IKZF3 (numbered according to SEQ ID NO: 19), e.g., the COF2/CRBN-binding polypeptide comprises the amino acid sequence of SEQ ID NO: 40.

147. The fusion polypeptide of any one of claims 83-146, wherein the COF2/CRBN-binding polypeptide comprises amino acid residues 136-180 of IKZF3 (numbered according to SEQ ID NO: 19), e.g., the COF2/CRBN-binding polypeptide comprises or consists of the amino acid sequence of SEQ ID NO: 5 or 77.

148. The fusion polypeptide of any one of claims 83-146, wherein the COF2/CRBN-binding polypeptide comprises amino acid residues 136-170 of IKZF3 (numbered according to SEQ ID NO: 19), e.g., the COF2/CRBN-binding polypeptide comprises or consists of the amino acid sequence of SEQ ID NO: 6 or 78.

149. The fusion polypeptide of any one of claims 83-148, wherein the COF2/CRBN-binding polypeptide comprises sufficient amino acid sequence and/or a structural motif from amino acid residues 236-249 of IKZF3 (numbered according to SEQ ID NO: 19) (e.g., sufficient amino acid sequence and/or a structural motif from amino acid residues 236-249 of SEQ ID NO: 19) that:

- i) the association of the COF2/CRBN-binding polypeptide with CRBN in the absence of COF2 is no more than, e.g., 0.01%, 0.1%, 1%, 5%, 10%, 15%, or 20%, of the association of the COF2/CRBN-binding polypeptide with CRBN in the presence of COF2, e.g., an excess of COF2, e.g., as measured by an assay described herein, e.g., immunoprecipitation;



- ii) the association of the fusion polypeptide with CRBN in the absence of COF2 is no more than, e.g., 0.01%, 0.1%, 1%, 5%, 10%, 15%, or 20% of the association of the fusion polypeptide with CRBN in the presence of COF2, e.g., an excess of COF2, e.g., as measured by an assay described herein, e.g., immunoprecipitation;
- iii) the ubiquitination of the heterologous polypeptide in the absence of COF2 is no more than, e.g., 0.01%, 0.1%, 1%, 10%, 20%, 30%, 40%, 50%, 60%, or 70%, of the ubiquitination of the heterologous polypeptide in the presence of COF2, e.g., an excess of COF2, e.g., as measured by an assay described herein;
- iv) the ubiquitination of the fusion polypeptide in the absence of COF2 is no more than, e.g., 0.01%, 0.1%, 1%, 10%, 20%, 30%, 40%, 50%, 60%, or 70%, of the ubiquitination of the fusion polypeptide in the presence of COF2, e.g., an excess of COF2, e.g., as measured by an assay described herein; or
- v) the degradation of the fusion polypeptide in the absence of COF2 is no more than, e.g., 0.01%, 0.1%, 1%, 10%, 20%, 30%, 40%, 50%, 60%, or 70%, of the degradation of the fusion polypeptide in the presence of COF2, e.g., an excess of COF2, e.g., as measured by an assay described herein, e.g., a Western blot analysis or a flow cytometry analysis.

150. The fusion polypeptide of claim 149, wherein the association, ubiquitination, and/or degradation is as measured in a mammalian cell, e.g., a human cell.

151. The fusion polypeptide of any one of claims 83-150, wherein the COF2/CRBN-binding polypeptide comprises amino acid residues 236-249 of IKZF3 (numbered according to SEQ ID NO: 19) (e.g., the COF2/CRBN-binding polypeptide comprises the amino acid sequence of SEQ ID NO: 11) or a sequence that differs from amino acid residues 236-249 of IKZF3 (numbered according to SEQ ID NO: 19) by no more than 1, 2, 3, 4, 5, 6, or 7 amino acid residues (e.g., a sequence that differs from amino acid residues 236-249 of SEQ ID NO: 19 by no more than 1, 2, 3, 4, 5, 6, or 7 amino acid residues) (e.g., a sequence having no more than 1, 2, 3, 4, 5, 6, or 7 amino acid substitutions from amino acid residues 236-249 of SEQ ID NO: 19).

152. The fusion polypeptide of any one of claims 83-151, wherein the COF2/CRBN-binding polypeptide comprises amino acid residues 236-249 of IKZF3 (numbered according to SEQ ID NO: 19), e.g., the COF2/CRBN-binding polypeptide comprises the amino acid sequence of SEQ ID NO: 11.

153. The fusion polypeptide of any one of claims 83-152, wherein the COF2/CRBN-binding polypeptide comprises the amino acid sequence of MALEKMALEKMALE (SEQ ID NO: 91).

154. The fusion polypeptide of any one of claims 83-153, wherein the COF2/CRBN-binding polypeptide comprises sufficient amino acid sequence and/or a structural motif from amino acid residues 136-180 and 236-249 of IKZF3 (numbered according to SEQ ID NO: 19) (e.g., sufficient amino acid sequence and/or a structural motif from amino acid residues 136-180 and 236-249 of SEQ ID NO: 19) that:

- i) the association of the COF2/CRBN-binding polypeptide with CRBN in the absence of COF2 is no more than, e.g., 0.01%, 0.1%, 1%, 5%, 10%, 15%, or 20%, of the association of the COF2/CRBN-binding polypeptide with CRBN in the presence of COF2, e.g., an excess of COF2, e.g., as measured by an assay described herein, e.g., immunoprecipitation;
- ii) the association of the fusion polypeptide with CRBN in the absence of COF2 is no more than, e.g., 0.01%, 0.1%, 1%, 5%, 10%, 15%, or 20% of the association of the fusion polypeptide with CRBN in the presence of COF2, e.g., an excess of COF2, e.g., as measured by an assay described herein, e.g., immunoprecipitation;
- iii) the ubiquitination of the heterologous polypeptide in the absence of COF2 is no more than, e.g., 0.01%, 0.1%, 1%, 10%, 20%, 30%, 40%, 50%, 60%, or 70%, of the ubiquitination of the heterologous polypeptide in the presence of COF2, e.g., an excess of COF2, e.g., as measured by an assay described herein;
- iv) the ubiquitination of the fusion polypeptide in the absence of COF2 is no more than, e.g., 0.01%, 0.1%, 1%, 10%, 20%, 30%, 40%, 50%, 60%, or 70%, of the ubiquitination of the fusion polypeptide in the presence of COF2, e.g., an excess of COF2, e.g., as measured by an assay described herein; or
- v) the degradation of the fusion polypeptide in the absence of COF2 is no more than, e.g., 0.01%, 0.1%, 1%, 10%, 20%, 30%, 40%, 50%, 60%, or 70%, of the degradation of the fusion polypeptide in the presence of COF2, e.g., an excess of COF2, e.g., as measured by an assay described herein, e.g., a Western blot analysis or a flow cytometry analysis.

155. The fusion polypeptide of claim 154, wherein the association, ubiquitination, and/or degradation is as measured in a mammalian cell, e.g., a human cell.

156. The fusion polypeptide of any one of claims 83-155, wherein the COF2/CRBN-binding polypeptide comprises a first sequence comprising amino acid residues 136-180 of IKZF3 (numbered according to SEQ ID NO: 19) (e.g., a first sequence comprising the amino acid sequence of SEQ ID NO: 5) or a first sequence that differs from amino acid residues 136-180 of IKZF3 (numbered according to SEQ ID NO: 19) by no more than 1, 2, 3, 4, 5, 10, 15, 20, 25, 30, 35, or 40 amino acid residues (e.g., a first sequence that differs from amino acid residues 136-180 of SEQ ID NO: 19 by no more than 1, 2, 3, 4, 5, 10, 15, 20, 25, 30, 35, or 40 amino acid residues); and a second sequence comprising amino acid

residues 236-249 of IKZF3 (numbered according to SEQ ID NO: 19) (e.g., a second sequence comprising the amino acid sequence of SEQ ID NO: 11) or a second sequence that differs from amino acid residues 236-249 of IKZF3 (numbered according to SEQ ID NO: 19) by no more than 1, 2, 3, 4, 5, 6, or 7 amino acid residues (e.g., a second sequence that differs from amino acid residues 236-249 of SEQ ID NO: 19 by no more than 1, 2, 3, 4, 5, 6, or 7 amino acid residues).

157. The fusion polypeptide of any one of claims 83-156, wherein the COF2/CRBN-binding polypeptide comprises amino acid residues 136-180 and 236-249 of IKZF3 (numbered according to SEQ ID NO: 19), e.g., the COF2/CRBN-binding polypeptide comprises or consists of the amino acid sequence of SEQ ID NO: 1 or 3.

158. The fusion polypeptide of any one of claims 83-157, wherein the COF2/CRBN-binding polypeptide comprises or consists of the amino acid sequence of SEQ ID NO: 1 or 3.

159. The fusion polypeptide of any one of claims 83-158, wherein the COF2/CRBN-binding polypeptide comprises a first sequence comprising amino acid residues 136-180 of IKZF3 (numbered according to SEQ ID NO: 19) and a second sequence comprising the amino acid sequence of MALEKMALEKMALE (SEQ ID NO: 91), e.g., the COF2/CRBN-binding polypeptide comprises or consists of the amino acid sequence of SEQ ID NO: 14 or 85.

160. The fusion polypeptide of any one of claims 83-159, wherein the COF2/CRBN-binding polypeptide comprises a first sequence comprising amino acid residues 136-170 of IKZF3 (numbered according to SEQ ID NO: 19) and a second sequence comprising the amino acid sequence of MALEKMALEKMALE (SEQ ID NO: 91), e.g., the COF2/CRBN-binding polypeptide comprises or consists of the amino acid sequence of SEQ ID NO: 15 or 86.

161. The fusion polypeptide of any one of claims 83-160, wherein the COF2/CRBN-binding polypeptide comprises at least one less lysine than the corresponding native sequence.

162. The fusion polypeptide of claim 161, wherein one or more lysine residues in the corresponding native sequence are replaced by a different amino acid, e.g., arginine.

163. The fusion polypeptide of claim 161 or 162, wherein the COF2/CRBN-binding polypeptide comprises less than 1, 2, 3, 4, or 5 lysine residues.

164. The fusion polypeptide of claim 163, wherein the COF2/CRBN-binding polypeptide does not comprise a lysine residue.

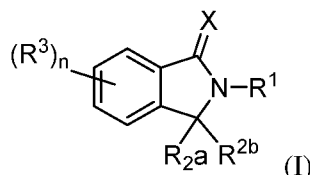
165. The fusion polypeptide of any one of claims 83-164, wherein the COF2/CRBN-binding polypeptide is not ubiquitinated, e.g., in the presence of COF2, e.g., as measured by an assay described herein, optionally wherein ubiquitination is as measured in a mammalian cell, e.g., a human cell.

166. The fusion polypeptide of any one of claims 83-165, wherein the COF2/CRBN-binding polypeptide comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 4, 41, 42, and 43.

167. The fusion polypeptide of any one of claims 83-165, wherein the COF2/CRBN-binding polypeptide comprises or consists of the amino acid sequence of SEQ ID NO: 2 or 4.

168. The fusion polypeptide of any one of claims 83-167, wherein the COF2 is an immunomodulatory imide drug (IMiD), or a pharmaceutically acceptable salt thereof.

169. The fusion polypeptide of any one of claims 83-167, wherein the COF2 has the structure of Formula (I):



or a pharmaceutically acceptable salt, ester, hydrate, solvate, or tautomer thereof, wherein:

X is O or S;

R¹ is C<sub>1</sub>-C<sub>6</sub> alkyl, C<sub>2</sub>-C<sub>6</sub> alkenyl, C<sub>2</sub>-C<sub>6</sub> alkynyl, C<sub>1</sub>-C<sub>6</sub> heteroalkyl, carbocyclyl, heterocyclyl, aryl, or heteroaryl, each of which is independently and optionally substituted by one or more R⁴;

each of R²ᵃ and R²ᵇ is independently hydrogen or C<sub>1</sub>-C<sub>6</sub> alkyl; or R²ᵃ and R²ᵇ together with the carbon atom to which they are attached form a carbonyl group or a thiocarbonyl group;

each of R³ is independently C<sub>1</sub>-C<sub>6</sub> alkyl, C<sub>2</sub>-C<sub>6</sub> alkenyl, C<sub>2</sub>-C<sub>6</sub> alkynyl, C<sub>1</sub>-C<sub>6</sub> heteroalkyl, halo, cyano, -C(O)R<sup>A</sup>, -C(O)OR<sup>B</sup>, -OR<sup>B</sup>, -N(R<sup>C</sup>)(R<sup>D</sup>), -C(O)N(R<sup>C</sup>)(R<sup>D</sup>), -N(R<sup>C</sup>)C(O)R<sup>A</sup>, -S(O)<sub>x</sub>R<sup>E</sup>, -S(O)<sub>x</sub>N(R<sup>C</sup>)(R<sup>D</sup>), or -N(R<sup>C</sup>)S(O)<sub>x</sub>R<sup>E</sup>, wherein each alkyl, alkenyl, alkynyl, and heteroalkyl is independently and optionally substituted with one or more R⁶;

each R⁴ is independently C<sub>1</sub>-C<sub>6</sub> alkyl, C<sub>2</sub>-C<sub>6</sub> alkenyl, C<sub>2</sub>-C<sub>6</sub> alkynyl, C<sub>1</sub>-C<sub>6</sub> heteroalkyl, halo, cyano, oxo, -C(O)R<sup>A</sup>, -C(O)OR<sup>B</sup>, -OR<sup>B</sup>, -N(R<sup>C</sup>)(R<sup>D</sup>), -C(O)N(R<sup>C</sup>)(R<sup>D</sup>), -N(R<sup>C</sup>)C(O)R<sup>A</sup>, -S(O)<sub>x</sub>R<sup>E</sup>, -

$S(O)_xN(R^C)(R^D)$ ,  $-N(R^C)S(O)_xR^E$ , carbocyclyl, heterocyclyl, aryl, or heteroaryl, wherein each alkyl, alkenyl, alkynyl, heteroalkyl, carbocyclyl, heterocyclyl, aryl, and heteroaryl is independently and optionally substituted with one or more  $R^7$ ;

each of  $R^A$ ,  $R^B$ ,  $R^C$ ,  $R^D$ , and  $R^E$  is independently hydrogen or  $C_1$ - $C_6$  alkyl;

each  $R^6$  is independently  $C_1$ - $C_6$  alkyl, oxo, cyano,  $-OR^B$ ,  $-N(R^C)(R^D)$ ,  $-C(O)N(R^C)(R^D)$ ,  $-N(R^C)C(O)R^A$ , aryl, or heteroaryl, wherein each aryl and heteroaryl is independently and optionally substituted with one or more  $R^8$ ;

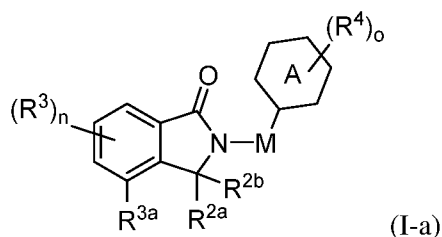
each  $R^7$  is independently halo, oxo, cyano,  $-OR^B$ ,  $-N(R^C)(R^D)$ ,  $-C(O)N(R^C)(R^D)$ , or  $-N(R^C)C(O)R^A$ ;

each  $R^8$  is independently  $C_1$ - $C_6$  alkyl, cyano,  $-OR^B$ ,  $-N(R^C)(R^D)$ ,  $-C(O)N(R^C)(R^D)$ , or  $-N(R^C)C(O)R^A$ ;

$n$  is 0, 1, 2, 3 or 4; and

$x$  is 0, 1, or 2.

170. The fusion polypeptide of any one of claims 83-167, wherein the COF2 has the structure of Formula (I-a):



or a pharmaceutically acceptable salt, ester, hydrate, or tautomer thereof, wherein:

Ring A is carbocyclyl, heterocyclyl, aryl, or heteroaryl, each of which is independently and optionally substituted with one or more  $R^4$ ;

M is absent,  $C_1$ - $C_6$  alkyl,  $C_2$ - $C_6$  alkenyl,  $C_2$ - $C_6$  alkynyl, or  $C_1$ - $C_6$  heteroalkyl, wherein each alkyl, alkenyl, alkynyl, and heteroalkyl is independently and optionally substituted with one or more  $R^4$ ;

each of  $R^{2a}$  and  $R^{2b}$  is independently hydrogen or  $C_1$ - $C_6$  alkyl; or  $R^{2a}$  and  $R^{2b}$  together with the carbon atom to which they are attached to form a carbonyl group or thiocarbonyl group;

$R^{3a}$  is hydrogen,  $C_1$ - $C_6$  alkyl,  $C_2$ - $C_6$  alkenyl,  $C_2$ - $C_6$  alkynyl,  $C_1$ - $C_6$  heteroalkyl, halo, cyano,  $-C(O)R^A$ ,  $-C(O)OR^B$ ,  $-OR^B$ ,  $-N(R^C)(R^D)$ ,  $-C(O)N(R^C)(R^D)$ ,  $-N(R^C)C(O)R^A$ ,  $-S(O)_xR^E$ ,  $-S(O)_xN(R^C)(R^D)$ , or  $-N(R^C)S(O)_xR^E$ , wherein each alkyl, alkenyl, alkynyl, and heteroalkyl is independently and optionally substituted with one or more  $R^6$ ;

each of  $R^3$  is independently  $C_1$ - $C_6$  alkyl,  $C_2$ - $C_6$  alkenyl,  $C_2$ - $C_6$  alkynyl,  $C_1$ - $C_6$  heteroalkyl, halo, cyano,  $-C(O)R^A$ ,  $-C(O)OR^B$ ,  $-OR^B$ ,  $-N(R^C)(R^D)$ ,  $-C(O)N(R^C)(R^D)$ ,  $-N(R^C)C(O)R^A$ ,  $-S(O)_xR^E$ , -

$S(O)_xN(R^C)(R^D)$ , or  $-N(R^C)S(O)_xR^E$ , wherein each alkyl, alkenyl, alkynyl, and heteroalkyl is independently and optionally substituted with one or more  $R^6$ ;

each  $R^4$  is independently  $C_1$ - $C_6$  alkyl,  $C_2$ - $C_6$  alkenyl,  $C_2$ - $C_6$  alkynyl,  $C_1$ - $C_6$  heteroalkyl, halo, cyano, oxo,  $-C(O)R^A$ ,  $-C(O)OR^B$ ,  $-OR^B$ ,  $-N(R^C)(R^D)$ ,  $-C(O)N(R^C)(R^D)$ ,  $-N(R^C)C(O)R^A$ ,  $S(O)_xR^E$ ,  $-S(O)_xN(R^C)(R^D)$ ,  $-N(R^C)S(O)_xR^E$ , carbocyclyl, heterocyclyl, aryl, or heteroaryl, wherein each alkyl, alkenyl, alkynyl, carbocyclyl, heterocyclyl, aryl, and heteroaryl is independently and optionally substituted with one or more  $R^7$ ;

each of  $R^A$ ,  $R^B$ ,  $R^C$ ,  $R^D$ , and  $R^E$  is independently hydrogen or  $C_1$ - $C_6$  alkyl;

each  $R^6$  is independently  $C_1$ - $C_6$  alkyl, oxo, cyano,  $-OR^B$ ,  $-N(R^C)(R^D)$ ,  $-C(O)N(R^C)(R^D)$ ,  $-N(R^C)C(O)R^A$ , aryl, or heteroaryl, wherein each aryl and heteroaryl is independently and optionally substituted with one or more  $R^8$ ;

each  $R^7$  is independently halo, oxo, cyano,  $-OR^B$ ,  $-N(R^C)(R^D)$ ,  $-C(O)N(R^C)(R^D)$ , or  $-N(R^C)C(O)R^A$ ;

each  $R^8$  is independently  $C_1$ - $C_6$  alkyl, cyano,  $-OR^B$ ,  $-N(R^C)(R^D)$ ,  $-C(O)N(R^C)(R^D)$ , or  $-N(R^C)C(O)R^A$ ;

$n$  is 0, 1, 2, or 3;

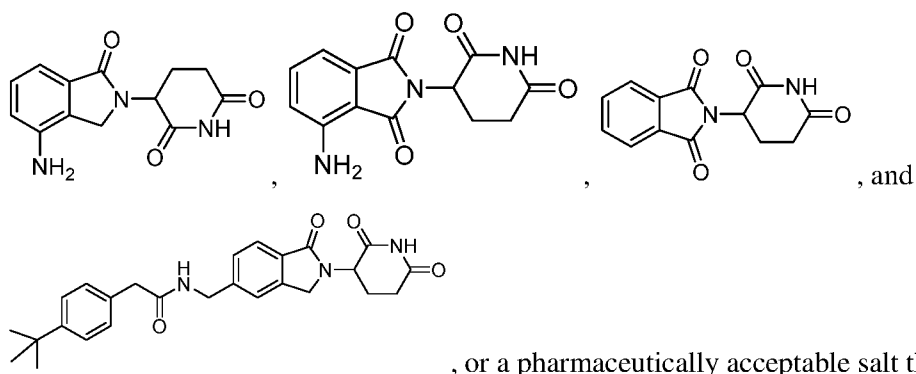
$o$  is 0, 1, 2, 3, 4, or 5; and

$x$  is 0, 1, or 2.

171. The fusion polypeptide of any one of claims 83-167, wherein the COF2 is thalidomide or analogue, or a pharmaceutically acceptable salt thereof.

172. The fusion polypeptide of any one of claims 83-167, wherein the COF2 is selected from the group consisting of lenalidomide, pomalidomide, thalidomide, and 2-(4-(tert-butyl)phenyl)-N-((2-(2,6-dioxopiperidin-3-yl)-1-oxoisindolin-5-yl)methyl)acetamide, or a pharmaceutically acceptable salt thereof.

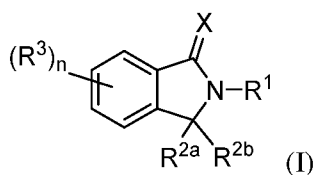
173. The fusion polypeptide of any one of claims 83-167, wherein the COF2 is selected from the group consisting of:



174. The fusion polypeptide of any one of claims 83-167, wherein the COF2 is lenalidomide or pomalidomide, or a pharmaceutically acceptable salt thereof.

175. The fusion polypeptide of any one of claims 83-167, wherein the COF2 is lenalidomide or analogue, or a pharmaceutically acceptable salt thereof.

176. The fusion polypeptide of any one of claims 83-167, wherein the COF2 comprises the structure of Formula (I):



or a pharmaceutically acceptable salt, ester, hydrate, solvate, or tautomer thereof, wherein:

$X$  is O or S;

$R^1$  is  $C_1$ - $C_6$  alkyl,  $C_2$ - $C_6$  alkenyl,  $C_2$ - $C_6$  alkynyl,  $C_1$ - $C_6$  heteroalkyl, carbocyclyl, heterocyclyl, aryl, or heteroaryl, each of which is independently and optionally substituted by one or more  $R^4$ ;

each of  $R^{2a}$  and  $R^{2b}$  is independently hydrogen or  $C_1$ - $C_6$  alkyl; or  $R^{2a}$  and  $R^{2b}$  together with the carbon atom to which they are attached form a carbonyl group or a thiocarbonyl group;

each of  $R^3$  is independently  $C_1$ - $C_6$  alkyl,  $C_2$ - $C_6$  alkenyl,  $C_2$ - $C_6$  alkynyl,  $C_1$ - $C_6$  heteroalkyl, halo, cyano,  $-C(O)R^A$ ,  $-C(O)OR^B$ ,  $-OR^B$ ,  $-N(R^C)(R^D)$ ,  $-C(O)N(R^C)(R^D)$ ,  $-N(R^C)C(O)R^A$ ,  $-S(O)_xR^E$ ,  $-S(O)_xN(R^C)(R^D)$ , or  $-N(R^C)S(O)_xR^E$ , wherein each alkyl, alkenyl, alkynyl, and heteroalkyl is independently and optionally substituted with one or more  $R^6$ ;

each  $R^4$  is independently  $C_1$ - $C_6$  alkyl,  $C_2$ - $C_6$  alkenyl,  $C_2$ - $C_6$  alkynyl,  $C_1$ - $C_6$  heteroalkyl, halo, cyano, oxo,  $-C(O)R^A$ ,  $-C(O)OR^B$ ,  $-OR^B$ ,  $-N(R^C)(R^D)$ ,  $-C(O)N(R^C)(R^D)$ ,  $-N(R^C)C(O)R^A$ ,  $-S(O)_xR^E$ ,  $-S(O)_xN(R^C)(R^D)$ ,  $-N(R^C)S(O)_xR^E$ , carbocyclyl, heterocyclyl, aryl, or heteroaryl, wherein each alkyl,

alkenyl, alkynyl, heteroalkyl, carbocyclyl, heterocyclyl, aryl, and heteroaryl is independently and optionally substituted with one or more  $R^7$ ;

each of  $R^A$ ,  $R^B$ ,  $R^C$ ,  $R^D$ , and  $R^E$  is independently hydrogen or  $C_1$ - $C_6$  alkyl;

each  $R^6$  is independently  $C_1$ - $C_6$  alkyl, oxo, cyano,  $-OR^B$ ,  $-N(R^C)(R^D)$ ,  $-C(O)N(R^C)(R^D)$ ,  $-N(R^C)C(O)R^A$ , aryl, or heteroaryl, wherein each aryl and heteroaryl is independently and optionally substituted with one or more  $R^8$ ;

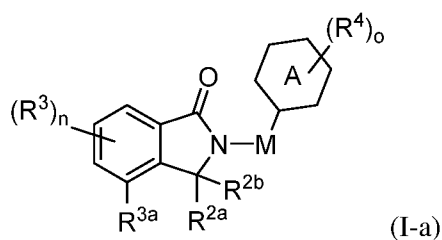
each  $R^7$  is independently halo, oxo, cyano,  $-OR^B$ ,  $-N(R^C)(R^D)$ ,  $-C(O)N(R^C)(R^D)$ , or  $-N(R^C)C(O)R^A$ ;

each  $R^8$  is independently  $C_1$ - $C_6$  alkyl, cyano,  $-OR^B$ ,  $-N(R^C)(R^D)$ ,  $-C(O)N(R^C)(R^D)$ , or  $-N(R^C)C(O)R^A$ ;

$n$  is 0, 1, 2, 3 or 4; and

$x$  is 0, 1, or 2.

177. The fusion polypeptide of any one of claims 83-167, wherein the COF2 comprises the structure of Formula (I-a):



or a pharmaceutically acceptable salt, ester, hydrate, or tautomer thereof, wherein:

Ring A is carbocyclyl, heterocyclyl, aryl, or heteroaryl, each of which is independently and optionally substituted with one or more  $R^4$ ;

M is absent,  $C_1$ - $C_6$  alkyl,  $C_2$ - $C_6$  alkenyl,  $C_2$ - $C_6$  alkynyl, or  $C_1$ - $C_6$  heteroalkyl, wherein each alkyl, alkenyl, alkynyl, and heteroalkyl is optionally substituted with one or more  $R^4$ ;

each of  $R^{2a}$  and  $R^{2b}$  is independently hydrogen or  $C_1$ - $C_6$  alkyl; or  $R^{2a}$  and  $R^{2b}$  together with the carbon atom to which they are attached to form a carbonyl group or thiocarbonyl group;

$R^{3a}$  is hydrogen,  $C_1$ - $C_6$  alkyl,  $C_2$ - $C_6$  alkenyl,  $C_2$ - $C_6$  alkynyl,  $C_1$ - $C_6$  heteroalkyl, halo, cyano,  $-C(O)R^A$ ,  $-C(O)OR^B$ ,  $-OR^B$ ,  $-N(R^C)(R^D)$ ,  $-C(O)N(R^C)(R^D)$ ,  $-N(R^C)C(O)R^A$ ,  $-S(O)_xR^E$ ,  $-S(O)_xN(R^C)(R^D)$ , or  $-N(R^C)S(O)_xR^E$ , wherein each alkyl, alkenyl, alkynyl, and heteroalkyl is independently and optionally substituted with one or more  $R^6$ ;

each of  $R^3$  is independently  $C_1$ - $C_6$  alkyl,  $C_2$ - $C_6$  alkenyl,  $C_2$ - $C_6$  alkynyl,  $C_1$ - $C_6$  heteroalkyl, halo, cyano,  $-C(O)R^A$ ,  $-C(O)OR^B$ ,  $-OR^B$ ,  $-N(R^C)(R^D)$ ,  $-C(O)N(R^C)(R^D)$ ,  $-N(R^C)C(O)R^A$ ,  $-S(O)_xR^E$ ,  $-S(O)_xN(R^C)(R^D)$ , or  $-N(R^C)S(O)_xR^E$ , wherein each alkyl, alkenyl, alkynyl, and heteroalkyl is independently and optionally substituted with one or more  $R^6$ ;



each  $R^4$  is independently  $C_1$ - $C_6$  alkyl,  $C_2$ - $C_6$  alkenyl,  $C_2$ - $C_6$  alkynyl,  $C_1$ - $C_6$  heteroalkyl, halo, cyano, oxo,  $-C(O)R^A$ ,  $-C(O)OR^B$ ,  $-OR^B$ ,  $-N(R^C)(R^D)$ ,  $-C(O)N(R^C)(R^D)$ ,  $-N(R^C)C(O)R^A$ ,  $S(O)_xR^E$ ,  $-S(O)_xN(R^C)(R^D)$ ,  $-N(R^C)S(O)_xR^E$ , carbocyclyl, heterocyclyl, aryl, or heteroaryl, wherein each alkyl, alkenyl, alkynyl, carbocyclyl, heterocyclyl, aryl, and heteroaryl is independently and optionally substituted with one or more  $R^7$ ;

each of  $R^A$ ,  $R^B$ ,  $R^C$ ,  $R^D$ , and  $R^E$  is independently hydrogen or  $C_1$ - $C_6$  alkyl;

each  $R^6$  is independently  $C_1$ - $C_6$  alkyl, oxo, cyano,  $-OR^B$ ,  $-N(R^C)(R^D)$ ,  $-C(O)N(R^C)(R^D)$ ,  $-N(R^C)C(O)R^A$ , aryl, or heteroaryl, wherein each aryl and heteroaryl is independently and optionally substituted with one or more  $R^8$ ;

each  $R^7$  is independently halo, oxo, cyano,  $-OR^B$ ,  $-N(R^C)(R^D)$ ,  $-C(O)N(R^C)(R^D)$ , or  $-N(R^C)C(O)R^A$ ;

each  $R^8$  is independently  $C_1$ - $C_6$  alkyl, cyano,  $-OR^B$ ,  $-N(R^C)(R^D)$ ,  $-C(O)N(R^C)(R^D)$ , or  $-N(R^C)C(O)R^A$ ;

$n$  is 0, 1, 2, or 3;

$o$  is 0, 1, 2, 3, 4, or 5; and

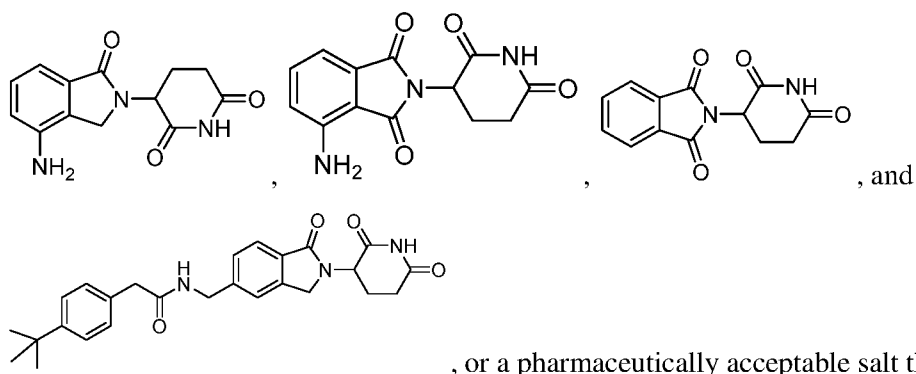
$x$  is 0, 1, or 2.

178. The fusion polypeptide of any one of claims 83-167, wherein the COF2 comprises an immunomodulatory imide drug (IMiD), or a pharmaceutically acceptable salt thereof.

179. The fusion polypeptide of any one of claims 83-167, wherein the COF2 comprises thalidomide or analogue, or a pharmaceutically acceptable salt thereof.

180. The fusion polypeptide of any one of claims 83-167, wherein the COF2 comprises lenalidomide, pomalidomide, thalidomide, and 2-(4-(tert-butyl)phenyl)-N-((2-(2,6-dioxopiperidin-3-yl)-1-oxoisindolin-5-yl)methyl)acetamide, or a pharmaceutically acceptable salt thereof.

181. The fusion polypeptide of any one of claims 83-167, wherein the COF2 comprises a compound selected from the group consisting of:



, or a pharmaceutically acceptable salt thereof.

182. The fusion polypeptide of any one of claims 83-167, wherein the COF2 comprises lenalidomide or pomalidomide, or a pharmaceutically acceptable salt thereof.

183. The fusion polypeptide of any one of claims 83-167, wherein the COF2 comprises lenalidomide or analogue, or a pharmaceutically acceptable salt thereof.

184. The fusion polypeptide of any one of claims 176-183, wherein the COF2 further comprises a ligand (e.g., wherein R<sup>10</sup> in Formula (II) is L-Tag).

185. The fusion polypeptide of any one of claims 176-184, wherein R<sup>10</sup> in Formula (II) is L-Tag, L is a linker selected from a linker disclosed in International Patent Publication No. WO2017/024318 (e.g., Figures 28-31), and Tag is selected from a dTAG Targeting Ligand disclosed in International Patent Publication No. WO2017/024318 (e.g., Table T, pages 119-129).

186. The fusion polypeptide of any one of claims 83-184, wherein the COF2 comprises IMiD (e.g., lenalidomide or pomalidomide, or a pharmaceutically acceptable salt thereof) and a ligand, wherein the IMiD (e.g., lenalidomide or pomalidomide, or a pharmaceutically acceptable salt thereof) is linked to the ligand, e.g., via a linker.

187. The fusion polypeptide of any one of claims 184-186, wherein the COF2/CRBN-binding polypeptide binds to the ligand and wherein the binding between the COF2/CRBN-binding polypeptide and IMiD (e.g., lenalidomide or pomalidomide, or a pharmaceutically acceptable salt thereof) in the absence of the ligand is no more than 0.0001, 0.001, 0.01, 0.1, 1, or 10% of the binding between the COF2/CRBN-binding polypeptide and the ligand, e.g., the COF2/CRBN-binding polypeptide does not bind to IMiD (e.g., lenalidomide or pomalidomide, or a pharmaceutically acceptable salt thereof),

optionally wherein the COF2/CRBN-binding polypeptide is selected from a dTAG disclosed in International Patent Publication No. WO2017/024318 (e.g., pages 36-65).

188. The fusion polypeptide of any one of claims 1-187, wherein the heterologous polypeptide is chosen from a cytoplasmic and/or nuclear polypeptide, or a transmembrane polypeptide, e.g., a heterologous polypeptide in Table 2.

189. The fusion polypeptide of claim 188, wherein the cytoplasmic and/or nuclear polypeptide is selected from the group consisting of a component of the apoptosis pathway (e.g., Caspase 9), a component of a CRISPR/Cas system (e.g., Cas9), a transcription factor (e.g., MITF, c-Myc, STAT3, STAT5, NF-kappaB, beta-catenin, Notch, GLI, or c-JUN), Tet methylcytosine dioxygenase 2 (TET2), FKBP, and Tau.

190. The fusion polypeptide of claim 188, wherein the transmembrane polypeptide is selected from the group consisting of CD62L, CCR1, CCR2, CCR5, CCR7, CCR10, CXCR2, CXCR3, CXCR4, CXCR6, CTLA4, PD1, BTLA, VISTA, CD137L, CD80, CD86, TIGIT, CD3, CD8, CD19, CD22, CD20, BCMA, and a chimeric antigen receptor (CAR).

191. The fusion polypeptide of claim 188, wherein the heterologous polypeptide is selected from the group consisting of a chimeric antigen receptor (CAR), a component of a CRISPR/Cas system (e.g., Cas9), CD8, CD19, and CD22.

192. The fusion polypeptide of claim 191, wherein the heterologous polypeptide is a chimeric antigen receptor (CAR).

193. The fusion polypeptide of claim 192, wherein said CAR comprises, in a N-terminal to C-terminal direction, an antigen binding domain, a transmembrane domain, and one or more intracellular signaling domains.

194. The fusion polypeptide of claim 193, wherein said intracellular signaling domain comprises one or more primary signaling domains.

195. The fusion polypeptide of claim 193 or 194, wherein said intracellular signaling domain comprises one or more costimulatory signaling domains.

196. The fusion polypeptide of claim 194, wherein one of said one or more primary signaling domains comprises a CD3-zeta stimulatory domain.

197. The fusion polypeptide of claim 195, wherein one or more of said costimulatory signaling domains is an intracellular domain from a costimulatory protein selected from the group consisting of CD27, CD28, 4-1BB (CD137), OX40, GITR, CD30, CD40, ICOS, BAFFR, HVEM, ICAM-1, lymphocyte function-associated antigen-1 (LFA-1), CD2, CDS, CD7, CD287, LIGHT, NKG2C, NKG2D, SLAMF7, NKp80, NKp30, NKp44, NKp46, CD160, B7-H3, and a ligand that specifically binds with CD83.

198. The fusion polypeptide of claim 197, wherein said one or more of said costimulatory signaling domains comprises the 4-1BB costimulatory domain.

199. The fusion polypeptide of claim 197 or 198, wherein said one or more of said costimulatory signaling domains comprises the CD28 costimulatory domain.

200. The fusion polypeptide of any one of claims 193-199, wherein said antigen binding domain is an scFv.

201. The fusion polypeptide of any one of claims 193-200, wherein said antigen binding domain binds an antigen selected from the group consisting of CD19; CD123; CD22; CD30; CD171; CS-1; C-type lectin-like molecule-1, CD33; epidermal growth factor receptor variant III (EGFRvIII); ganglioside G2 (GD2); ganglioside GD3; TNF receptor family member; B-cell maturation antigen; Tn antigen ((Tn Ag) or (GalNAc $\alpha$ -Ser/Thr)); prostate-specific membrane antigen (PSMA); Receptor tyrosine kinase-like orphan receptor 1 (ROR1); Fms-Like Tyrosine Kinase 3 (FLT3); Tumor-associated glycoprotein 72 (TAG72); CD38; CD44v6; Carcinoembryonic antigen (CEA); Epithelial cell adhesion molecule (EPCAM); B7H3 (CD276); KIT (CD117); Interleukin-13 receptor subunit alpha-2; Mesothelin; Interleukin 11 receptor alpha (IL-11Ra); prostate stem cell antigen (PSCA); Protease Serine 21; vascular endothelial growth factor receptor 2 (VEGFR2); Lewis(Y) antigen; CD24; Platelet-derived growth factor receptor beta (PDGFR-beta); Stage-specific embryonic antigen-4 (SSEA-4); CD20; Folate receptor alpha; Receptor tyrosine-protein kinase ERBB2 (Her2/neu); Mucin 1, cell surface associated (MUC1); epidermal growth factor receptor (EGFR); neural cell adhesion molecule (NCAM); Prostase; prostatic acid phosphatase (PAP); elongation factor 2 mutated (ELF2M); Ephrin B2; fibroblast activation protein alpha (FAP); insulin-like growth factor 1 receptor (IGF-I receptor), carbonic anhydrase IX (CAIX); Proteasome (Prosome, Macropain) Subunit, Beta Type, 9 (LMP2); glycoprotein 100 (gp100); oncogene polypeptide consisting of breakpoint cluster region (BCR) and Abelson murine

leukemia viral oncogene homolog 1 (Abl) (bcr-abl); tyrosinase; ephrin type-A receptor 2 (EphA2); Fucosyl GM1; sialyl Lewis adhesion molecule (sLe); ganglioside GM3; transglutaminase 5 (TGS5); high molecular weight-melanoma-associated antigen (HMWMAA); o-acetyl-GD2 ganglioside (OAcGD2); Folate receptor beta; tumor endothelial marker 1 (TEM1/CD248); tumor endothelial marker 7-related (TEM7R); claudin 6 (CLDN6); thyroid stimulating hormone receptor (TSHR); G protein-coupled receptor class C group 5, member D (GPRC5D); chromosome X open reading frame 61 (CXORF61); CD97; CD179a; anaplastic lymphoma kinase (ALK); Polysialic acid; placenta-specific 1 (PLAC1); hexasaccharide portion of globoH glycosphingolipid (GloboH); mammary gland differentiation antigen (NY-BR-1); uroplakin 2 (UPK2); Hepatitis A virus cellular receptor 1 (HAVCR1); adrenoceptor beta 3 (ADRB3); pannexin 3 (PANX3); G protein-coupled receptor 20 (GPR20); lymphocyte antigen 6 complex, locus K 9 (LY6K); Olfactory receptor 51E2 (OR51E2); TCR Gamma Alternate Reading Frame Protein (TARP); Wilms tumor protein (WT1); Cancer/testis antigen 1 (NY-ESO-1); Cancer/testis antigen 2 (LAGE-1a); Melanoma-associated antigen 1 (MAGE-A1); ETS translocation-variant gene 6, located on chromosome 12p (ETV6-AML); sperm protein 17 (SPA17); X Antigen Family, Member 1A (XAGE1); angiopoietin-binding cell surface receptor 2 (Tie 2); melanoma cancer testis antigen-1 (MAD-CT-1); melanoma cancer testis antigen-2 (MAD-CT-2); Fos-related antigen 1; tumor protein p53 (p53); p53 mutant; prostein; surviving; telomerase; prostate carcinoma tumor antigen-1, melanoma antigen recognized by T cells 1; Rat sarcoma (Ras) mutant; human Telomerase reverse transcriptase (hTERT); sarcoma translocation breakpoints; melanoma inhibitor of apoptosis (ML-IAP); ERG (transmembrane protease, serine 2 (TMPRSS2) ETS fusion gene); N-Acetyl glucosaminyl-transferase V (NA17); paired box protein Pax-3 (PAX3); Androgen receptor; Cyclin B1; v-myc avian myelocytomatosis viral oncogene neuroblastoma derived homolog (MYCN); Ras Homolog Family Member C (RhoC); Tyrosinase-related protein 2 (TRP-2); Cytochrome P450 1B1 (CYP1B1); CCCTC-Binding Factor (Zinc Finger Protein)-Like, Squamous Cell Carcinoma Antigen Recognized By T Cells 3 (SART3); Paired box protein Pax-5 (PAX5); proacrosin binding protein sp32 (OY-TES1); lymphocyte-specific protein tyrosine kinase (LCK); A kinase anchor protein 4 (AKAP-4); synovial sarcoma, X breakpoint 2 (SSX2); Receptor for Advanced Glycation Endproducts (RAGE-1); renal ubiquitous 1 (RU1); renal ubiquitous 2 (RU2); legumain; human papilloma virus E6 (HPV E6); human papilloma virus E7 (HPV E7); intestinal carboxyl esterase; heat shock protein 70-2 mutated (mut hsp70-2); CD79a; CD79b; CD72; Leukocyte-associated immunoglobulin-like receptor 1 (LAIR1); Fc fragment of IgA receptor (FCAR or CD89); Leukocyte immunoglobulin-like receptor subfamily A member 2 (LILRA2); CD300 molecule-like family member f (CD300LF); C-type lectin domain family 12 member A (CLEC12A); bone marrow stromal cell antigen 2 (BST2); EGF-like module-containing mucin-like hormone receptor-like 2 (EMR2); lymphocyte antigen 75 (LY75); Glypican-3 (GPC3); Fc receptor-like 5 (FCRL5); and immunoglobulin lambda-like polypeptide 1 (IGLL1).

202. The fusion polypeptide of any one of claims 193-200, wherein said antigen is chosen from CD19, CD22, BCMA, CD20, CD123, EGFRvIII, or mesothelin.
203. The fusion polypeptide of any one of claims 193-200, wherein said antigen is CD19.
204. The fusion polypeptide of any one of claims 193-200, wherein said antigen is CD22.
205. The fusion polypeptide of any one of claims 193-200, wherein said antigen is BCMA.
206. The fusion polypeptide of any one of claims 193-200, wherein said antigen is CD20.
207. The fusion polypeptide of any one of claims 193-200, wherein said antigen is CD123.
208. The fusion polypeptide of any one of claims 193-200, wherein said antigen is EGFRvIII.
209. A nucleic acid molecule encoding the fusion polypeptide of any one of claims 1-208.
210. A vector comprising the nucleic acid molecule of claim 209.
211. The vector of claim 210, wherein said vector is a viral vector.
212. The vector of claim 210, wherein said vector is a lentiviral vector.
213. A viral particle comprising the vector of any one of claims 210-212.
214. A cell, e.g., a host cell, comprising the fusion polypeptide of any one of claims 1-208, the nucleic acid molecule of claim 209, or the vector of any one of claims 210-212.
215. The cell of claim 214, wherein said cell, e.g., host cell, is a mammalian cell, e.g., a human cell, e.g., a human effector cell, e.g., a human T cell or a human NK cell.
216. The cell of claim 214 or 215, wherein said cell, e.g., host cell, is a CAR-expressing cell, e.g., a CAR-T cell.

217. The cell of claim 214 or 215, wherein said cell, e.g., host cell, comprises a component of a CRISPR/Cas system.

218. The cell of any one of claims 214-217, wherein said cell, e.g., host cell, is a human cancer cell, e.g., a human tumor cell.

219. The cell of any one of claims 214-218, wherein said cell, e.g., host cell, comprises a ubiquitin ligase complex, e.g., an E3 ubiquitin ligase complex, wherein the ubiquitin ligase complex comprises CRBN.

220. The cell of any one of claims 214-219, wherein the cell comprises the fusion polypeptide of any one of claims 1-79 or 188-208 or the cell comprises a nucleic acid molecule encoding the fusion polypeptide of any one of claims 1-79 or 188-208, wherein when the cell is contacted with COF1, e.g., an excess of COF1:

- i) the association of the COF1/CRBN-binding polypeptide with CRBN is increased by at least, e.g., 10-, 50-, 100-, 1000-, or 10000-fold, compared to the association of the COF1/CRBN-binding polypeptide with CRBN when the cell is not contacted with COF1, e.g., as measured by an assay described herein, e.g., immunoprecipitation;
- ii) the association of the fusion polypeptide with CRBN is increased by at least, e.g., 10-, 50-, 100-, 1000-, or 10000-fold, compared to the association of the fusion polypeptide with CRBN when the cell is not contacted with COF1, e.g., as measured by an assay described herein, e.g., immunoprecipitation;
- iii) the ubiquitination of the heterologous polypeptide is increased by at least, e.g., 1.5-, 2-, 3-, 4-, 5-, 10-, 20-, 30-, 40-, or 50-fold, compared to the ubiquitination of the heterologous polypeptide when the cell is not contacted with COF1, e.g., as measured by an assay described herein;
- iv) the ubiquitination of the fusion polypeptide is increased by at least, e.g., 1.5-, 2-, 3-, 4-, 5-, 10-, 20-, 30-, 40-, or 50-fold, compared to the ubiquitination of the fusion polypeptide when the cell is not contacted with COF1, e.g., as measured by an assay described herein;
- v) the degradation of the fusion polypeptide is increased by at least, e.g., 1.5-, 2-, 3-, 4-, 5-, 10-, 20-, 30-, 40-, or 50-fold, compared to the degradation of the fusion polypeptide when the cell is not contacted with COF1, e.g., as measured by an assay described herein, e.g., a Western blot analysis or a flow cytometry analysis; or
- vi) the expression level of the fusion polypeptide is no more than, e.g., 0.01%, 0.1%, 1%, 10%, 20%, 30%, 40%, 50%, 60%, or 70%, of the expression level of the fusion polypeptide when the cell is not contacted with COF1, e.g., as measured by an assay described herein, e.g., a Western blot analysis or a flow cytometry analysis.

221. The cell of claim 214-220, wherein said cell further comprises COF1, e.g., lenalidomide or pomalidomide, or a pharmaceutically acceptable salt thereof.

222. The cell of any one of claims 214-219, wherein the cell comprises the fusion polypeptide of any one of claims 80-82, 84-107, or 188-208 or the cell comprises a nucleic acid molecule encoding the fusion polypeptide of any one of claims 80-82, 84-107, or 188-208, wherein in the absence of a stabilization compound, the fusion polypeptide is degraded by a cellular degradation pathway, e.g., at least 50%, 60%, 70%, 80%, 90% or greater of the fusion polypeptide is degraded, e.g., as measured by an assay described herein, e.g., a Western blot analysis or a flow cytometry analysis.

223. The cell of any one of claims 214-219, wherein the cell comprises the fusion polypeptide of any one of claims 83-208 or the cell comprises a nucleic acid molecule encoding the fusion polypeptide of any one of claims 83-208, wherein in the absence of a stabilization compound, the fusion polypeptide is degraded by a cellular degradation pathway, e.g., at least 50%, 60%, 70%, 80%, 90% or greater of the fusion polypeptide is degraded, e.g., as measured by an assay described herein, e.g., a Western blot analysis or a flow cytometry analysis.

224. The cell of claim 222 or 223, further comprising a protease capable of cleaving said heterologous protease cleavage site.

225. The cell of any one of claims 222-224, when the cell is contacted with a stabilization compound, e.g., an excess of a stabilization compound:

- i) the degradation domain assumes a conformation more resistant to cellular degradation relative to a conformation in the absence of the stabilization compound;
- ii) the conformation of the fusion polypeptide is more permissive to cleavage at the heterologous protease cleavage site relative to a conformation in the absence of the stabilization compound; or
- iii) the expression level of the fusion polypeptide is increased by at least, e.g., 1.5-, 2-, 3-, 4-, 5-, 10-, 20-, 30-, 40-, or 50-fold, compared to the expression level of the fusion polypeptide when the cell is not contacted with the stabilization compound, e.g., as measured by an assay described herein, e.g., a Western blot analysis or a flow cytometry analysis.

226. The cell of any one of claims 222-225, further comprising a stabilization compound.



227. The cell of any one of claims 222-226, wherein the stabilization compound is bazedoxifene, or a pharmaceutically acceptable salt thereof, and optionally wherein the degradation domain comprises the amino acid sequence of SEQ ID NO: 46.

228. The cell of any one of claims 222 or 224-227, wherein when the cell is contacted with both a stabilization compound, e.g., an excess of a stabilization compound, and COF1, e.g., an excess of COF1:

- i) the association of the COF1/CRBN-binding polypeptide with CRBN is increased by at least, e.g., 10-, 50-, 100-, 1000-, or 10000-fold, compared to the association of the COF1/CRBN-binding polypeptide with CRBN when the cell is only contacted with the stabilization compound but not COF1, e.g., as measured by an assay described herein, e.g., immunoprecipitation;
- ii) the association of the fusion polypeptide with CRBN is increased by at least, e.g., 10-, 50-, 100-, 1000-, or 10000-fold, compared to the association of the fusion polypeptide with CRBN when the cell is only contacted with the stabilization compound but not COF1, e.g., as measured by an assay described herein, e.g., immunoprecipitation;
- iii) the ubiquitination of the heterologous polypeptide is increased by at least, e.g., 1.5-, 2-, 3-, 4-, 5-, 10-, 20-, 30-, 40-, or 50-fold, compared to the ubiquitination of the heterologous polypeptide when the cell is only contacted with the stabilization compound but not COF1, e.g., as measured by an assay described herein;
- iv) the ubiquitination of the fusion polypeptide is increased by at least, e.g., 1.5-, 2-, 3-, 4-, 5-, 10-, 20-, 30-, 40-, or 50-fold, compared to the ubiquitination of the fusion polypeptide when the cell is only contacted with the stabilization compound but not COF1, e.g., as measured by an assay described herein;
- v) the degradation of the fusion polypeptide is increased by at least, e.g., 1.5-, 2-, 3-, 4-, 5-, 10-, 20-, 30-, 40-, or 50-fold, compared to the degradation of the fusion polypeptide when the cell is only contacted with the stabilization compound but not COF1, e.g., as measured by an assay described herein, e.g., a Western blot analysis or a flow cytometry analysis; or
- vi) the expression level of the fusion polypeptide is no more than, e.g., 0.01%, 0.1%, 1%, 10%, 20%, 30%, 40%, 50%, 60%, or 70%, of the expression level of the fusion polypeptide when the cell is only contacted with the stabilization compound but not COF1, e.g., as measured by an assay described herein, e.g., a Western blot analysis or a flow cytometry analysis.

229. The cell of any one of claims 222 or 224-228, further comprising COF1, e.g., lenalidomide or pomalidomide, or a pharmaceutically acceptable salt thereof.

230. The cell of any one of claims 223-229, wherein when the cell is contacted with both a stabilization compound, e.g., an excess of a stabilization compound, and COF2, e.g., an excess of COF2:

- i) the association of the COF2/CRBN-binding polypeptide with CRBN is increased by at least, e.g., 10-, 50-, 100-, 1000-, or 10000-fold, compared to the association of the COF2/CRBN-binding polypeptide with CRBN when the cell is only contacted with the stabilization compound but not COF2, e.g., as measured by an assay described herein, e.g., immunoprecipitation;
- ii) the association of the fusion polypeptide with CRBN is increased by at least, e.g., 10-, 50-, 100-, 1000-, or 10000-fold, compared to the association of the fusion polypeptide with CRBN when the cell is only contacted with the stabilization compound but not COF2, e.g., as measured by an assay described herein, e.g., immunoprecipitation;
- iii) the ubiquitination of the heterologous polypeptide is increased by at least, e.g., 1.5-, 2-, 3-, 4-, 5-, 10-, 20-, 30-, 40-, or 50-fold, compared to the ubiquitination of the heterologous polypeptide when the cell is only contacted with the stabilization compound but not COF2, e.g., as measured by an assay described herein;
- iv) the ubiquitination of the fusion polypeptide is increased by at least, e.g., 1.5-, 2-, 3-, 4-, 5-, 10-, 20-, 30-, 40-, or 50-fold, compared to the ubiquitination of the fusion polypeptide when the cell is only contacted with the stabilization compound but not COF2, e.g., as measured by an assay described herein;
- v) the degradation of the fusion polypeptide is increased by at least, e.g., 1.5-, 2-, 3-, 4-, 5-, 10-, 20-, 30-, 40-, or 50-fold, compared to the degradation of the fusion polypeptide when the cell is only contacted with the stabilization compound but not COF2, e.g., as measured by an assay described herein, e.g., a Western blot analysis or a flow cytometry analysis; or
- vi) the expression level of the fusion polypeptide is no more than, e.g., 0.01%, 0.1%, 1%, 10%, 20%, 30%, 40%, 50%, 60%, or 70%, of the expression level of the fusion polypeptide when the cell is only contacted with the stabilization compound but not COF2, e.g., as measured by an assay described herein, e.g., a Western blot analysis or a flow cytometry analysis.

231. The cell of any one of claims 223-230, further comprising COF2, e.g., lenalidomide or pomalidomide, or a pharmaceutically acceptable salt thereof.

232. The cell of any one of claims 214-231, wherein the heterologous polypeptide is a chimeric antigen receptor (CAR), optionally wherein the CAR comprises, in a N-terminal to C-terminal direction, an antigen binding domain, a transmembrane domain, and one or more intracellular signaling domains.

233. A pharmaceutical composition comprising the fusion polypeptide of any one of claims 1-208 or the cell of any one of claims 214-232, and a pharmaceutically acceptable carrier, excipient or stabilizer.

234. A method of making the cell of any one of claims 214-232, said method comprising providing a cell, e.g., an immune effector cell, with the nucleic acid molecule of claim 209, the vector of any one of claims 210-212, or the viral particle of claim 213.

235. A method of degrading a fusion polypeptide, comprising contacting the fusion polypeptide of any one of claims 1-79 or 188-208 or a cell comprising said fusion polypeptide with COF1 (e.g., lenalidomide or pomalidomide, or a pharmaceutically acceptable salt thereof), optionally wherein in the presence of COF1 (e.g., lenalidomide or pomalidomide, or a pharmaceutically acceptable salt thereof), the expression level of said fusion polypeptide is substantially decreased, e.g., by at least about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 60, 70, 80, 90, or 100 percent, relative to the expression level of said fusion polypeptide in the absence of COF1 (e.g., lenalidomide or pomalidomide, or a pharmaceutically acceptable salt thereof), e.g., as measured by an assay described herein, e.g., a Western blot analysis or a flow cytometry analysis.

236. The method of claim 235, wherein the fusion polypeptide or the cell is contacted with COF1 (e.g., lenalidomide or pomalidomide, or a pharmaceutically acceptable salt thereof) *ex vivo*.

237. The method of claim 235, wherein the fusion polypeptide or the cell is contacted with COF1 (e.g., lenalidomide or pomalidomide, or a pharmaceutically acceptable salt thereof) *in vivo*.

238. A method of regulating the expression of a fusion polypeptide, comprising:

i) contacting the fusion polypeptide of any one of claims 80-82, 84-107, or 188-208 or a cell comprising said fusion polypeptide with a stabilization compound, optionally wherein in the presence of the stabilization compound:

a) the degradation domain assumes a conformation more resistant to cellular degradation relative to a conformation in the absence of the stabilization compound;

b) the conformation of the fusion polypeptide is more permissive to cleavage at the heterologous protease cleavage site relative to a conformation in the absence of the stabilization compound; or

c) the expression level of the fusion polypeptide is increased by at least, e.g., 1.5-, 2-, 3-, 4-, 5-, 10-, 20-, 30-, 40-, or 50-fold, compared to the expression level of the fusion polypeptide in the absence of the stabilization compound, e.g., as measured by an assay described herein, e.g., a Western blot analysis or a flow cytometry analysis.

239. The method of claim 238, further comprising, after step i):

ii) contacting the fusion polypeptide of any one of claims 83-208 or a cell comprising said fusion polypeptide with COF1 (e.g., lenalidomide or pomalidomide, or a pharmaceutically acceptable salt thereof), optionally wherein in the presence of COF1 (e.g., lenalidomide or pomalidomide, or a pharmaceutically acceptable salt thereof), the expression level of the fusion polypeptide is substantially decreased, e.g., by at least about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 60, 70, 80, 90, or 100 percent, relative to the expression level of the fusion polypeptide after step i) and before step ii), e.g., as measured by an assay described herein, e.g., a Western blot analysis or a flow cytometry analysis.

240. The method of claim 238 or 239, wherein the stabilization compound is bazedoxifene, or a pharmaceutically acceptable salt thereof, and optionally wherein the degradation domain comprises the amino acid sequence of SEQ ID NO: 46.

241. The method of any one of claims 238-240, wherein the fusion polypeptide or the cell is contacted with COF1 (e.g., lenalidomide or pomalidomide, or a pharmaceutically acceptable salt thereof) and/or the stabilization compound *ex vivo*.

242. The method of any one of claims 238-240, wherein the fusion polypeptide or the cell is contacted with COF1 (e.g., lenalidomide or pomalidomide, or a pharmaceutically acceptable salt thereof) and/or the stabilization compound *in vivo*.

243. A method of regulating the expression of a fusion polypeptide, comprising:

i) contacting the fusion polypeptide of any one of claims 83-208 or a cell comprising said fusion polypeptide with a stabilization compound, optionally wherein in the presence of the stabilization compound:

a) the degradation domain assumes a conformation more resistant to cellular degradation relative to a conformation in the absence of the stabilization compound;

b) the conformation of the fusion polypeptide is more permissive to cleavage at the heterologous protease cleavage site relative to a conformation in the absence of the stabilization compound; or

c) the expression level of the fusion polypeptide is increased by at least, e.g., 1.5-, 2-, 3-, 4-, 5-, 10-, 20-, 30-, 40-, or 50-fold, compared to the expression level of the fusion polypeptide in the absence of the stabilization compound, e.g., as measured by an assay described herein, e.g., a Western blot analysis or a flow cytometry analysis.

244. The method of claim 243, further comprising, after step i):

ii) contacting the fusion polypeptide of any one of claims 83-208 or a cell comprising said fusion polypeptide with COF2 (e.g., lenalidomide or pomalidomide, or a pharmaceutically acceptable salt thereof), optionally wherein in the presence of COF2 (e.g., lenalidomide or pomalidomide, or a pharmaceutically acceptable salt thereof), the expression level of the fusion polypeptide is substantially decreased, e.g., by at least about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 60, 70, 80, 90, or 100 percent, relative to the expression level of the fusion polypeptide after step i) and before step ii), e.g., as measured by an assay described herein, e.g., a Western blot analysis or a flow cytometry analysis.

245. The method of claim 243 or 244, wherein the stabilization compound is bazedoxifene, or a pharmaceutically acceptable salt thereof, and optionally wherein the degradation domain comprises the amino acid sequence of SEQ ID NO: 46.

246. The method of any one of claims 243-245, wherein the fusion polypeptide or the cell is contacted with COF2 (e.g., lenalidomide or pomalidomide, or a pharmaceutically acceptable salt thereof) and/or the stabilization compound *ex vivo*.

247. The method of any one of claims 243-245, wherein the fusion polypeptide or the cell is contacted with COF2 (e.g., lenalidomide or pomalidomide, or a pharmaceutically acceptable salt thereof) and/or the stabilization compound *in vivo*.

248. The method of any one of claims 234-247, wherein the heterologous polypeptide is a chimeric antigen receptor (CAR), optionally wherein the CAR comprises, in a N-terminal to C-terminal direction, an antigen binding domain, a transmembrane domain, and one or more intracellular signaling domains.

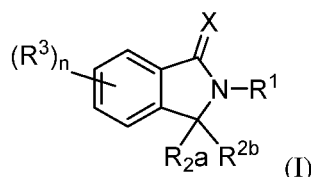
249. A method of making a cell, comprising:

i) providing a cell comprising a nucleic acid molecule encoding a fusion polypeptide comprising a compound of formula 1 (COF1)/CRBN-binding polypeptide and a chimeric antigen receptor (CAR), optionally wherein the CAR comprises, in a N-terminal to C-terminal direction, an antigen binding domain, a transmembrane domain, and one or more intracellular signaling domains; and

ii) contacting the cell *ex vivo* with COF1, optionally wherein:

in the presence of COF1, the expression level of the fusion polypeptide is substantially decreased, e.g., by at least about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 60, 70, 80, 90, or 100 percent, relative to the expression level of the fusion polypeptide in the absence of COF1, e.g., as measured by an assay

described herein, e.g., a Western blot analysis or a flow cytometry analysis, wherein the compound of Formula (I) is:



or a pharmaceutically acceptable salt, ester, hydrate, solvate, or tautomer thereof, wherein:

X is O or S;

R<sup>1</sup> is C<sub>1</sub>-C<sub>6</sub> alkyl, C<sub>2</sub>-C<sub>6</sub> alkenyl, C<sub>2</sub>-C<sub>6</sub> alkynyl, C<sub>1</sub>-C<sub>6</sub> heteroalkyl, carbocyclyl, heterocyclyl, aryl, or heteroaryl, each of which is independently and optionally substituted by one or more R<sup>4</sup>;

each of R<sup>2a</sup> and R<sup>2b</sup> is independently hydrogen or C<sub>1</sub>-C<sub>6</sub> alkyl; or R<sup>2a</sup> and R<sup>2b</sup> together with the carbon atom to which they are attached form a carbonyl group or a thiocarbonyl group;

each of R<sup>3</sup> is independently C<sub>1</sub>-C<sub>6</sub> alkyl, C<sub>2</sub>-C<sub>6</sub> alkenyl, C<sub>2</sub>-C<sub>6</sub> alkynyl, C<sub>1</sub>-C<sub>6</sub> heteroalkyl, halo, cyano, -C(O)R<sup>A</sup>, -C(O)OR<sup>B</sup>, -OR<sup>B</sup>, -N(R<sup>C</sup>)(R<sup>D</sup>), -C(O)N(R<sup>C</sup>)(R<sup>D</sup>), -N(R<sup>C</sup>)C(O)R<sup>A</sup>, -S(O)<sub>x</sub>R<sup>E</sup>, -S(O)<sub>x</sub>N(R<sup>C</sup>)(R<sup>D</sup>), or -N(R<sup>C</sup>)S(O)<sub>x</sub>R<sup>E</sup>, wherein each alkyl, alkenyl, alkynyl, and heteroalkyl is independently and optionally substituted with one or more R<sup>6</sup>;

each R<sup>4</sup> is independently C<sub>1</sub>-C<sub>6</sub> alkyl, C<sub>2</sub>-C<sub>6</sub> alkenyl, C<sub>2</sub>-C<sub>6</sub> alkynyl, C<sub>1</sub>-C<sub>6</sub> heteroalkyl, halo, cyano, oxo, -C(O)R<sup>A</sup>, -C(O)OR<sup>B</sup>, -OR<sup>B</sup>, -N(R<sup>C</sup>)(R<sup>D</sup>), -C(O)N(R<sup>C</sup>)(R<sup>D</sup>), -N(R<sup>C</sup>)C(O)R<sup>A</sup>, -S(O)<sub>x</sub>R<sup>E</sup>, -S(O)<sub>x</sub>N(R<sup>C</sup>)(R<sup>D</sup>), -N(R<sup>C</sup>)S(O)<sub>x</sub>R<sup>E</sup>, carbocyclyl, heterocyclyl, aryl, or heteroaryl, wherein each alkyl, alkenyl, alkynyl, heteroalkyl, carbocyclyl, heterocyclyl, aryl, and heteroaryl is independently and optionally substituted with one or more R<sup>7</sup>;

each of R<sup>A</sup>, R<sup>B</sup>, R<sup>C</sup>, R<sup>D</sup>, and R<sup>E</sup> is independently hydrogen or C<sub>1</sub>-C<sub>6</sub> alkyl;

each R<sup>6</sup> is independently C<sub>1</sub>-C<sub>6</sub> alkyl, oxo, cyano, -OR<sup>B</sup>, -N(R<sup>C</sup>)(R<sup>D</sup>), -C(O)N(R<sup>C</sup>)(R<sup>D</sup>), -N(R<sup>C</sup>)C(O)R<sup>A</sup>, aryl, or heteroaryl, wherein each aryl and heteroaryl is independently and optionally substituted with one or more R<sup>8</sup>;

each R<sup>7</sup> is independently halo, oxo, cyano, -OR<sup>B</sup>, -N(R<sup>C</sup>)(R<sup>D</sup>), -C(O)N(R<sup>C</sup>)(R<sup>D</sup>), or -N(R<sup>C</sup>)C(O)R<sup>A</sup>;

each R<sup>8</sup> is independently C<sub>1</sub>-C<sub>6</sub> alkyl, cyano, -OR<sup>B</sup>, -N(R<sup>C</sup>)(R<sup>D</sup>), -C(O)N(R<sup>C</sup>)(R<sup>D</sup>), or -N(R<sup>C</sup>)C(O)R<sup>A</sup>;

n is 0, 1, 2, 3 or 4; and

x is 0, 1, or 2.

250. The method of claim 249, wherein after the cell is contacted with COF1 *ex vivo*, the proliferation of the cell is increased by at least, e.g., 1.2-, 1.5-, 2-, 5-, or 10-fold, relative to the proliferation of the cell prior to the contacting with COF1.

251. The method of claim 250, wherein COF1 is lenalidomide or pomalidomide, or a pharmaceutically acceptable salt thereof.

252. The method of claim 249 or 250, wherein the COF1/CRBN-binding polypeptide comprises or consists of an amino acid sequence selected from the group consisting of SEQ ID NOs: 1-6, 11-15, 40, 41-43, 77, 78, 84-86, and 100 (e.g., the COF1/CRBN-binding polypeptide comprises or consists of the amino acid sequence of SEQ ID NO: 3).

253. A method of treating a subject having a disease associated with expression of a tumor antigen, comprising:

i) contacting a cell comprising the fusion polypeptide of any one of claims 1-79 or 188-208 with COF1 *ex vivo*, optionally wherein:

in the presence of COF1, the expression level of the fusion polypeptide is decreased, e.g., by at least about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 60, 70, 80, 90, or 100 percent, relative to the expression level of the fusion polypeptide before the cell is contacted with COF1 *ex vivo*, and

ii) administering to the subject an effective amount of the cell, optionally wherein the method further comprises after step i) and prior to step ii):

reducing the amount of COF1 contacting the cell, e.g., inside and/or surrounding the cell, thereby treating the disease.

254. The method of claim 253, further comprising after step ii):

iii) administering to the subject an effective amount of COF1, optionally wherein the administration of COF1 decreases, e.g., by at least about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 60, 70, 80, 90, or 100 percent, the expression level of the fusion polypeptide relative to the expression level of the fusion polypeptide after step ii) and prior to step iii), optionally wherein:

a) the subject has developed, is developing, or is anticipated to develop an adverse reaction,

b) the administration of COF1 is in response to an occurrence of an adverse reaction in the subject, or in response to an anticipation of an occurrence of an adverse reaction in the subject, and/or

c) the administration of COF1 reduces or prevents an adverse effect.

255. The method of claim 254, further comprising after step iii):

iv) discontinuing the administration of COF1, optionally wherein discontinuing the administration of COF1 increases, e.g., by at least about 1.5-, 2-, 3-, 4-, 5-, 10-, 20-, 30-, 40-, or 50-fold, the expression level of the fusion polypeptide relative to the expression level of the fusion polypeptide after step iii)

and prior to step iv) (e.g., wherein discontinuing the administration of COF1 restores the expression level of the fusion polypeptide to the expression level after step ii) and prior to step iii)), optionally wherein:

- a) the subject has relapsed, is relapsing, or is anticipated to relapse,
- b) the discontinuation of the administration of COF1 is in response to a tumor relapse in the subject, or in response to an anticipation of a relapse in the subject, and/or
- c) the discontinuation of the administration of COF1 treats or prevents a tumor relapse.

256. The method of claim 255, further comprising after step iv):

- v) repeating step iii) and/or iv),
- thereby treating the disease.

257. A method of treating a subject having a disease associated with expression of a tumor antigen, comprising:

- i) administering to the subject an effective amount of a cell comprising the fusion polypeptide of any one of claims 1-79 or 188-208, optionally wherein the cell is contacted with COF1 *ex vivo* before administration, optionally wherein:

in the presence of COF1, the expression level of the fusion polypeptide is decreased, e.g., by at least about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 60, 70, 80, 90, or 100 percent, relative to the expression level of the fusion polypeptide before the cell is contacted with COF1 *ex vivo*, optionally wherein after the cell is contacted with COF1 *ex vivo* and before the cell is administered to the subject, the amount of COF1 contacting the cell, e.g., inside and/or surrounding the cell, is reduced,

thereby treating the disease.

258. The method of claim 257, wherein the cell is not contacted with COF1 *ex vivo* before administration.

259. The method of claim 257 or 258, further comprising after step i):

- ii) administering to the subject an effective amount of COF1, optionally wherein the administration of COF1 decreases, e.g., by at least about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 60, 70, 80, 90, or 100 percent, the expression level of the fusion polypeptide relative to the expression level of the fusion polypeptide after step i) and prior to step ii), optionally wherein:
  - a) the subject has developed, is developing, or is anticipated to develop an adverse reaction,
  - b) the administration of COF1 is in response to an occurrence of an adverse reaction in the subject, or in response to an anticipation of an occurrence of an adverse reaction in the subject, and/or



c) the administration of COF1 reduces or prevents an adverse effect.

260. The method of claim 259, further comprising after step ii):

iii) discontinuing the administration of COF1, optionally wherein discontinuing the administration of COF1 increases, e.g., by at least about 1.5-, 2-, 3-, 4-, 5-, 10-, 20-, 30-, 40-, or 50-fold, the expression level of the fusion polypeptide relative to the expression level of the fusion polypeptide after step ii) and prior to step iii) (e.g., wherein discontinuing the administration of COF1 restores the expression level of the fusion polypeptide to the expression level after step i) and prior to step ii)), optionally wherein:

- a) the subject has relapsed, is relapsing, or is anticipated to relapse,
- b) the discontinuation of the administration of COF1 is in response to a tumor relapse in the subject, or in response to an anticipation of a relapse in the subject, and/or
- c) the discontinuation of the administration of COF1 treats or prevents a tumor relapse.

261. The method of claim 260, further comprising after step iii):

iv) repeating step ii) and/or iii),

thereby treating the disease.

262. A method of treating a subject having a disease associated with expression of a tumor antigen, comprising:

i) administering an effective amount of COF1 to the subject, wherein the subject comprises a cell comprising the fusion polypeptide of any one of claims 1-79 or 188-208, optionally wherein the administration of COF1 decreases, e.g., by at least about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 60, 70, 80, 90, or 100 percent, the expression level of the fusion polypeptide relative to the expression level of the fusion polypeptide before the administration of COF1, optionally wherein:

- a) the subject has developed, is developing, or is anticipated to develop an adverse reaction,
- b) the administration of COF1 is in response to an occurrence of an adverse reaction in the subject, or in response to an anticipation of an occurrence of an adverse reaction in the subject, and/or
- c) the administration of COF1 reduces or prevents an adverse effect.

263. The method of claim 262, further comprising after step i):

ii) discontinuing the administration of COF1, optionally wherein discontinuing the administration of COF1 increases, e.g., by at least about 1.5-, 2-, 3-, 4-, 5-, 10-, 20-, 30-, 40-, or 50-fold, the expression level of the fusion polypeptide relative to the expression level of the fusion polypeptide after step i) and prior to step ii) (e.g., wherein discontinuing the administration of COF1 restores the expression level of the fusion polypeptide to the expression level before the administration of COF1), optionally wherein:

- a) the subject has relapsed, is relapsing, or is anticipated to relapse,
- b) the discontinuation of the administration of COF1 is in response to a tumor relapse in the subject, or in response to an anticipation of a relapse in the subject, and/or
- c) the discontinuation of the administration of COF1 treats or prevents a tumor relapse.

264. The method of claim 263, further comprising after step ii):

- iii) repeating step i) and/or ii),
- thereby treating the disease.

265. The method of any one of claims 253-264, wherein COF1 is lenalidomide or pomalidomide, or a pharmaceutically acceptable salt thereof, optionally wherein the COF1/CRBN-binding polypeptide comprises or consists of an amino acid sequence selected from the group consisting of SEQ ID NOs: 1-6, 11-15, 40, 41-43, 77, 78, 84-86, and 100 (e.g., the COF1/CRBN-binding polypeptide comprises or consists of the amino acid sequence of SEQ ID NO: 3).

266. The method of any one of claims 259-265, wherein COF1 is lenalidomide, or a pharmaceutically acceptable salt thereof, optionally wherein lenalidomide, or a pharmaceutically acceptable salt thereof, is administered at, e.g., 2.5 mg, 5 mg, 10 mg, 15 mg, or 25 mg per day.

267. A method of treating a subject having a disease associated with expression of a tumor antigen, comprising:

- i) administering to the subject:

- (1) a stabilization compound, and

- (2) an effective amount of a cell comprising the fusion polypeptide of any one of claims 80-82, 84-107, or 188-208, or an effective amount of a cell comprising the fusion polypeptide of any one of claims 83-208, optionally wherein:

the expression level of the fusion polypeptide in the presence of the stabilization compound is e.g., at least about 1.5-, 2-, 3-, 4-, 5-, 10-, 20-, 30-, 40-, or 50-fold, higher than the expression level of the fusion polypeptide in the absence of the stabilization compound, thereby treating the disease.

268. The method of claim 267, further comprising after step i):

- ii) discontinuing the administration of the stabilization compound, optionally wherein discontinuing the administration of the stabilization compound reduces, e.g., at least about 1.5-, 2-, 3-, 4-, 5-, 10-, 20-, 30-,

40-, or 50-fold, the expression level of the fusion polypeptide relative to the expression of the fusion polypeptide after step i) and prior to step ii), optionally wherein:

- a) the subject responded to the treatment of step i) (e.g., the subject has a complete response to the treatment of step i), the subject shows a shrinkage in tumor mass, the subject shows a decrease in tumor cells, or the treatment of step i) is effective in the subject), and/or
- b) the discontinuation of the administration of the stabilization compound is in response to a response of the subject to the treatment of step i) (e.g., the subject has a complete response to the treatment of step i), the subject shows a shrinkage in tumor mass, the subject shows a decrease in tumor cells, or the treatment of step i) is effective in the subject).

269. The method of claim 267, further comprising after step i):

iii) discontinuing the administration of the stabilization compound, optionally wherein discontinuing the administration of the stabilization compound reduces, e.g., at least about 1.5-, 2-, 3-, 4-, 5-, 10-, 20-, 30-, 40-, or 50-fold, the expression level of the fusion polypeptide relative to the expression of the fusion polypeptide after step i) and prior to step ii), optionally wherein:

- a) the subject has developed, is developing, or is anticipated to develop an adverse reaction,
- b) the discontinuation of the administration of the stabilization compound is in response to an occurrence of an adverse reaction in the subject, or in response to an anticipation of an occurrence of an adverse reaction in the subject, and/or
- c) the discontinuation of the administration of the stabilization compound reduces or prevents an adverse effect.

270. The method of claim 267, further comprising after step i):

iv) discontinuing the administration of the stabilization compound and administering to the subject an effective amount of COF1 or COF2, optionally wherein step iv) reduces, e.g., at least about 1.5-, 2-, 3-, 4-, 5-, 10-, 20-, 30-, 40-, or 50-fold, the expression level of the fusion polypeptide relative to the expression of the fusion polypeptide after step i) and prior to step iv), optionally wherein:

- a) the subject has developed, is developing, or is anticipated to develop an adverse reaction,
- b) step iv) is in response to an occurrence of an adverse reaction in the subject, or in response to an anticipation of an occurrence of an adverse reaction in the subject, and/or
- c) step iv) reduces or prevents an adverse effect.

271. The method of claim 270, wherein the adverse effect is an acute toxicity.

272. The method of claim 270 or 271, further comprising after step iv):

v) discontinuing the administration of COF1 or COF2, e.g., after the amount of cells expressing the fusion polypeptide on the surface is smaller than a pre-defined value, for, e.g., 1 day, 5 days, 10 days, or 15 days.

273. The method of any one of claims 268-272, further comprising after step ii), iii), iv), or v):

- vi) administering an effective amount of a stabilization compound, optionally wherein the administration of the stabilization compound increases, e.g., by at least about 1.5-, 2-, 3-, 4-, 5-, 10-, 20-, 30-, 40-, or 50-fold, the expression level of the fusion polypeptide relative to the expression level of the fusion polypeptide after step ii), iii), iv), or v) and prior to step vi), optionally wherein:
- a) the subject has relapsed, is relapsing, or is anticipated to relapse,
  - b) the administration of the stabilization compound is in response to a tumor relapse in the subject, or in response to an anticipation of a relapse in the subject, and/or
  - c) the administration of the stabilization compound treats or prevents a tumor relapse.

274. The method of claim 273, further comprising after step vi):

- vii) repeating step iii), iv), v), or vi),  
thereby treating the disease.

275. The method of any one of claims 267-274, further comprising prior to step i):

- viii) contacting the cell with a stabilization compound *ex vivo*, optionally wherein the expression level of the fusion polypeptide in the presence of the stabilization compound is e.g., at least about 1.5-, 2-, 3-, 4-, 5-, 10-, 20-, 30-, 40-, or 50-fold, higher than the expression level of the fusion polypeptide in the absence of the stabilization compound.

276. The method of any one of claims 267-274, wherein the cell is not contacted with the stabilization compound *ex vivo* before administration.

277. The method of claim 276, wherein the cell is not contacted with any of: the stabilization compound, COF1, or COF2 *ex vivo* before administration.

278. The method of any one of claims 267-277, wherein the stabilization compound is bazedoxifene, or a pharmaceutically acceptable salt thereof, and optionally wherein the degradation domain comprises the amino acid sequence of SEQ ID NO: 46.

279. The method of any one of claims 270-278, wherein COF1 is lenalidomide or pomalidomide, or a pharmaceutically acceptable salt thereof, optionally wherein the COF1/CRBN-binding polypeptide comprises or consists of an amino acid sequence selected from the group consisting of SEQ ID NOs: 1-6, 11-15, 40, 41-43, 77, 78, 84-86, and 100 (e.g., the COF1/CRBN-binding polypeptide comprises or consists of the amino acid sequence of SEQ ID NO: 3).

280. The method of any one of claims 270-278, wherein COF2 is lenalidomide or pomalidomide, or a pharmaceutically acceptable salt thereof, optionally wherein the COF2/CRBN-binding polypeptide comprises or consists of an amino acid sequence selected from the group consisting of SEQ ID NOs: 1-6, 11-15, 40, 41-43, 77, 78, 84-86, and 100 (e.g., the COF1/CRBN-binding polypeptide comprises or consists of the amino acid sequence of SEQ ID NO: 3).

281. The method of any one of claims 270-280, wherein COF1 or COF2 is lenalidomide, or a pharmaceutically acceptable salt thereof, optionally wherein lenalidomide, or a pharmaceutically acceptable salt thereof, is administered at, e.g., 2.5 mg, 5 mg, 10 mg, 15 mg, or 25 mg per day.

282. The method of any one of claims 253-281, wherein the heterologous polypeptide of the fusion polypeptide is a chimeric antigen receptor (CAR), optionally wherein the CAR comprises, in a N-terminal to C-terminal direction, an antigen binding domain, a transmembrane domain, and one or more intracellular signaling domains.

283. The fusion polypeptide, nucleic acid molecule, vector, viral particle, cell, or pharmaceutical composition of any one of claims 1-233 for use as a medicament.

284. The fusion polypeptide, nucleic acid molecule, vector, viral particle, cell, or pharmaceutical composition of any one of claims 1-233 for use in the treatment of a subject having a disease associated with expression of a tumor antigen, e.g., for use in the method of any one of claims 253-282.

285. The method or composition for use of any one of claims 253-284, wherein the disease associated with expression of a tumor antigen is a cancer.

286. The method or composition for use of claim 285, wherein the cancer is mesothelioma (e.g., malignant pleural mesothelioma), e.g., in a subject who has progressed on at least one prior standard therapy; lung cancer (e.g., non-small cell lung cancer, small cell lung cancer, squamous cell lung cancer, or large cell lung cancer); pancreatic cancer (e.g., pancreatic ductal adenocarcinoma, or metastatic

pancreatic ductal adenocarcinoma (PDA), e.g., in a subject who has progressed on at least one prior standard therapy); esophageal adenocarcinoma, ovarian cancer (e.g., serous epithelial ovarian cancer, e.g., in a subject who has progressed after at least one prior regimen of standard therapy), breast cancer, colorectal cancer, bladder cancer or any combination thereof.

287. The method or composition for use of any one of claims 253-284, wherein the disease associated with expression of a tumor antigen is a hematological cancer, e.g., a hematological cancer chosen from a leukemia or lymphoma.

288. The method or composition for use of claim 287, wherein the cancer is chosen from: chronic lymphocytic leukemia (CLL), mantle cell lymphoma (MCL), multiple myeloma, acute lymphoid leukemia (ALL), Hodgkin lymphoma, B-cell acute lymphoid leukemia (BALL), T-cell acute lymphoid leukemia (TALL), small lymphocytic leukemia (SLL), B cell prolymphocytic leukemia, blastic plasmacytoid dendritic cell neoplasm, Burkitt's lymphoma, diffuse large B cell lymphoma (DLBCL), DLBCL associated with chronic inflammation, chronic myeloid leukemia, myeloproliferative neoplasms, follicular lymphoma, pediatric follicular lymphoma, hairy cell leukemia, small cell- or a large cell-follicular lymphoma, malignant lymphoproliferative conditions, MALT lymphoma (extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue), Marginal zone lymphoma, myelodysplasia, myelodysplastic syndrome, non-Hodgkin lymphoma, plasmablastic lymphoma, plasmacytoid dendritic cell neoplasm, Waldenstrom macroglobulinemia, splenic marginal zone lymphoma, splenic lymphoma/leukemia, splenic diffuse red pulp small B-cell lymphoma, hairy cell leukemia-variant, lymphoplasmacytic lymphoma, a heavy chain disease, plasma cell myeloma, solitary plasmacytoma of bone, extraosseous plasmacytoma, nodal marginal zone lymphoma, pediatric nodal marginal zone lymphoma, primary cutaneous follicle center lymphoma, lymphomatoid granulomatosis, primary mediastinal (thymic) large B-cell lymphoma, intravascular large B-cell lymphoma, ALK+ large B-cell lymphoma, large B-cell lymphoma arising in HHV8-associated multicentric Castleman disease, primary effusion lymphoma, B-cell lymphoma, acute myeloid leukemia (AML), or unclassifiable lymphoma.

289. The method or composition for use of claim 287, wherein the cancer is chosen from MCL, CLL, ALL, Hodgkin lymphoma, AML, or multiple myeloma.

290. The method or composition for use of any one of claims 253-289, wherein said cell is autologous to said subject.

291. The method or composition for use of any one of claims 253-289, wherein said cell is allogenic to said subject.

292. The method or composition for use of any one of claims 253-291, wherein said cell is a CAR-expressing cell, e.g., a CART cell.

293. The method or composition for use of any one of claims 253-292, wherein the subject was administered a cell expressing at least one fusion polypeptide of any one of claims 1-208, prior to administration of COF1 or COF2.

294. A method of identifying a genetic element associated with a specific biological phenotype, e.g., a genetic element associated with the development and/or progression of a cancer, the method comprising the steps of:

- i) modulating the expression of the fusion polypeptide of any one of claims 1-79 or 188-208 in cells by exposing said cell to COF1, e.g., lenalidomide, or a pharmaceutically acceptable salt thereof,
  - (ii) selecting for cells with a phenotype of interest, e.g., a phenotype associated with the development and/or progression of a cancer, and
  - (iii) identifying said fusion polypeptide that induces said phenotype of interest,
- wherein exposure of said cell to COF1, e.g., lenalidomide, or a pharmaceutically acceptable salt thereof, decreases, e.g., by at least about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 60, 70, 80, 90, or 100 percent, the expression level of said fusion polypeptide relative to the expression level of said fusion polypeptide prior to exposure to COF1, e.g., lenalidomide, or a pharmaceutically acceptable salt thereof.

295. A method of identifying a genetic element associated with a specific biological phenotype, e.g., a genetic element associated with the development and/or progression of a cancer, the method comprising the steps of:

- i) modulating the expression of the fusion polypeptide of any one of claims 80-82, 84-107, or 188-208 or the expression of the fusion polypeptide of any one of claims 83-208, in cells by exposing said cell to a stabilization compound, e.g., bazedoxifene, or a pharmaceutically acceptable salt thereof, and then to COF1 or COF2, e.g., lenalidomide, or a pharmaceutically acceptable salt thereof,
  - (ii) selecting for cells with a phenotype of interest, e.g., a phenotype associated with the development and/or progression of a cancer, and
  - (iii) identifying said fusion polypeptide that induces said phenotype of interest,
- wherein exposure of said cell to the stabilization compound, e.g., bazedoxifene, or a pharmaceutically acceptable salt thereof, increases, e.g., by at least about 1.5-, 2-, 3-, 4-, 5-, 10-, 20-, 30-, 40-, or 50-fold,

296. A fusion polypeptide comprising a compound of Formula (III) (COF3)/CRBN (cereblon)-binding polypeptide and a heterologous polypeptide, e.g., a heterologous mammalian, bacterial, or viral polypeptide, wherein the compound of Formula (III) is:



each R<sub>4</sub> is independently selected from -C(O)OR<sub>6</sub>, -C(O)NR<sub>6</sub>R<sub>6'</sub>, -NR<sub>6</sub>C(O)R<sub>6'</sub>, halo, -OH, -NH<sub>2</sub>, cyano, C<sub>6</sub>-C<sub>10</sub> aryl, 5- or 6-membered heteroaryl comprising 1 to 4 heteroatoms selected from O, N, and S, C<sub>3</sub>-C<sub>8</sub> carbocyclyl, and 5- to 7-membered heterocyclyl ring comprising 1 to 3 heteroatoms



selected from O, N, and S, wherein the aryl, heteroaryl, carbocyclyl, and heterocyclyl are optionally substituted with one or more R<sub>7</sub>;

each R<sub>5</sub> is independently selected from C<sub>1</sub>-C<sub>6</sub> alkyl, C<sub>2</sub>-C<sub>6</sub> alkenyl, C<sub>2</sub>-C<sub>6</sub> alkynyl, C<sub>1</sub>-C<sub>6</sub> alkoxy, C<sub>1</sub>-C<sub>6</sub> haloalkyl, C<sub>1</sub>-C<sub>6</sub> haloalkoxy, C<sub>1</sub>-C<sub>6</sub> hydroxyalkyl, halo, -OH, -NH<sub>2</sub>, cyano, C<sub>3</sub>-C<sub>7</sub> carbocyclyl, 5- to 7-membered heterocyclyl comprising 1 to 3 heteroatoms selected from O, N, and S, C<sub>6</sub>-C<sub>10</sub> aryl, and 5- or 6-membered heteroaryl comprising 1 to 3 heteroatoms selected from O, N, and S, or

two R<sub>5</sub>, when on adjacent atoms, together with the atoms to which they are attached form a C<sub>6</sub>-C<sub>10</sub> aryl or 5- or 6-membered heteroaryl comprising 1 to 3 heteroatoms selected from O, N, and S, optionally substituted with one or more R<sub>10</sub>, or

two R<sub>5</sub>, when on adjacent atoms, together with the atoms to which they are attached form a C<sub>5</sub>-C<sub>7</sub> carbocyclyl or 5- to 7-membered heterocyclyl comprising 1 to 3 heteroatoms selected from O, N, and S optionally substituted with one or more R<sub>10</sub>;

R<sub>6</sub> and R<sub>6</sub> are each independently hydrogen, C<sub>1</sub>-C<sub>6</sub> alkyl, or C<sub>6</sub>-C<sub>10</sub> aryl;

each R<sub>7</sub> is independently selected from C<sub>1</sub>-C<sub>6</sub> alkyl, C<sub>2</sub>-C<sub>6</sub> alkenyl, C<sub>2</sub>-C<sub>6</sub> alkynyl, C<sub>1</sub>-C<sub>6</sub> alkoxy, C<sub>1</sub>-C<sub>6</sub> haloalkyl, C<sub>1</sub>-C<sub>6</sub> haloalkoxy, -C(O)R<sub>8</sub>, -(CH<sub>2</sub>)<sub>0-3</sub>C(O)OR<sub>8</sub>, -C(O)NR<sub>8</sub>R<sub>9</sub>, -NR<sub>8</sub>C(O)R<sub>9</sub>, -NR<sub>8</sub>C(O)OR<sub>9</sub>, -S(O)<sub>p</sub>NR<sub>8</sub>R<sub>9</sub>, -S(O)<sub>p</sub>R<sub>12</sub>, (C<sub>1</sub>-C<sub>6</sub>)hydroxyalkyl, halo, -OH, -O(CH<sub>2</sub>)<sub>1-3</sub>CN, -NH<sub>2</sub>, cyano, -O(CH<sub>2</sub>)<sub>0-3</sub>- C<sub>6</sub>-C<sub>10</sub> aryl, adamantyl, -O(CH<sub>2</sub>)<sub>0-3</sub>-5- or 6-membered heteroaryl comprising 1 to 3 heteroatoms selected from O, N, and S, C<sub>6</sub>-C<sub>10</sub> aryl, monocyclic or bicyclic 5- to 10-membered heteroaryl comprising 1 to 3 heteroatoms selected from O, N, and S, C<sub>3</sub>-C<sub>7</sub> carbocyclyl, and 5- to 7-membered heterocyclyl comprising 1 to 3 heteroatoms selected from O, N, and S, wherein the alkyl is optionally substituted with one or more R<sub>11</sub>, and the aryl, heteroaryl, and heterocyclyl are optionally substituted with one or more substituents each independently selected from halogen, C<sub>1</sub>-C<sub>6</sub> alkyl, C<sub>1</sub>-C<sub>6</sub> haloalkyl, and C<sub>1</sub>-C<sub>6</sub> alkoxy, or

two R<sub>7</sub> together with the carbon atom to which they are attached form a =O, or

two R<sub>7</sub>, when on adjacent atoms, together with the atoms to which they are attached form a C<sub>6</sub>-C<sub>10</sub> aryl or 5- or 6-membered heteroaryl comprising 1 to 3 heteroatoms selected from O, N, and S, optionally substituted with one or more R<sub>10</sub>, or

two R<sub>7</sub> together with the atoms to which they are attached form a C<sub>5</sub>-C<sub>7</sub> carbocyclyl or a 5- to 7-membered heterocyclyl comprising 1 to 3 heteroatoms selected from O, N, and S, optionally substituted with one or more R<sub>10</sub>;

R<sub>8</sub> and R<sub>9</sub> are each independently hydrogen or C<sub>1</sub>-C<sub>6</sub> alkyl;

each R<sub>10</sub> is independently selected from C<sub>1</sub>-C<sub>6</sub> alkyl, C<sub>1</sub>-C<sub>6</sub> alkoxy, C<sub>1</sub>-C<sub>6</sub> haloalkyl, C<sub>1</sub>-C<sub>6</sub> haloalkoxy, C<sub>1</sub>-C<sub>6</sub> hydroxyalkyl, halo, -OH, -NH<sub>2</sub>, and cyano, or

two R<sub>10</sub> together with the carbon atom to which they are attached form a =O;

each  $R_{11}$  is independently selected from cyano,  $C_1$ - $C_6$  alkoxy,  $C_6$ - $C_{10}$  aryl, and 5- to 7-membered heterocyclyl comprising 1 to 3 heteroatoms selected from O, N, and S, wherein each aryl and heterocyclyl is optionally substituted with one or more substituents each independently selected from  $C_1$ - $C_6$  alkyl,  $C_1$ - $C_6$  alkoxy,  $C_1$ - $C_6$  haloalkyl,  $C_1$ - $C_6$  haloalkoxy,  $C_1$ - $C_6$  hydroxyalkyl, halo, -OH, -NH<sub>2</sub>, and cyano;

$R_{12}$  is  $C_1$ - $C_6$  alkyl,  $C_1$ - $C_6$  haloalkyl,  $C_6$ - $C_{10}$  aryl, or 5- to 7-membered heterocyclyl comprising 1 to 3 heteroatoms selected from O, N, and S;

$R_x$  is hydrogen or deuterium;

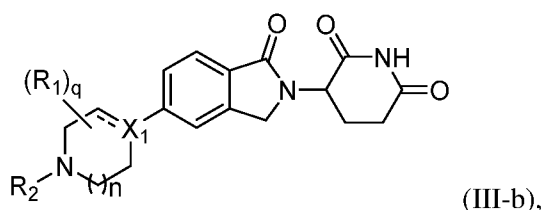
$p$  is 0, 1, or 2;

$n$  is 0, 1, or 2;

$y$  is 1 or 2, wherein  $n + y \leq 3$ ; and

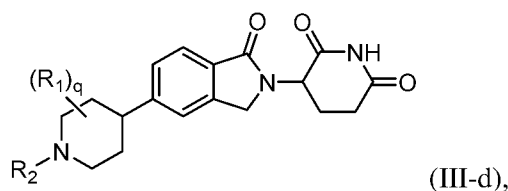
$q$  is 0, 1, 2, 3, or 4.

297. The fusion polypeptide of claim 296, wherein the compound of Formula (III) is a compound of Formula (III-b):



or a pharmaceutically acceptable salt, hydrate, solvate, prodrug, stereoisomer, and tautomer thereof, wherein  $X_1$ ,  $R_1$ ,  $R_2$ ,  $n$ ,  $q$ , and subvariables thereof are defined as described for Formula (III) in claim 296.

298. The fusion polypeptide of claim 296 or 297, wherein the compound of Formula (III) is a compound of Formula (III-d):



or a pharmaceutically acceptable salt, hydrate, solvate, prodrug, stereoisomer, and tautomer thereof, wherein  $R_1$ ,  $R_2$ ,  $q$ , and subvariables thereof are defined as described for Formula (III) in claim 296.

299. The fusion polypeptide of any one of claims 296-298, wherein:

(i) the COF3/CRBN-binding polypeptide is fused to the heterologous polypeptide,

- (ii) the COF3/CRBN-binding polypeptide and the heterologous polypeptide are linked by a peptide bond,
- (iii) the COF3/CRBN-binding polypeptide and the heterologous polypeptide are linked by a bond other than a peptide bond,
- (iv) the heterologous polypeptide is linked directly to the COF3/CRBN-binding polypeptide,
- (v) the heterologous polypeptide is linked indirectly to the COF3/CRBN-binding polypeptide,
- (vi) the COF3/CRBN-binding polypeptide and the heterologous polypeptide are operatively linked via a linker, e.g., a glycine-serine linker, e.g., a linker comprising the amino acid sequence of SEQ ID NO: 28, or
- (vii) the COF3/CRBN-binding polypeptide is linked to the C-terminus or N-terminus of the heterologous polypeptide.

300. The fusion polypeptide of any one of claims 296-299, wherein:

- (i) the association of the fusion polypeptide with CRBN in the absence of COF3 is no more than, e.g., 0.01%, 0.1%, 1%, 5%, 10%, 15%, or 20%, of the association of the fusion polypeptide with CRBN in the presence of COF3, e.g., an excess of COF3, e.g., as measured by an assay described herein, e.g., immunoprecipitation, optionally wherein the fusion polypeptide does not bind to CRBN in the absence of COF3;
- (ii) the ubiquitination of the fusion polypeptide in the absence of COF3 is no more than, e.g., 0.01%, 0.1%, 1%, 10%, 20%, 30%, 40%, 50%, 60%, or 70%, of the ubiquitination of the fusion polypeptide in the presence of COF3, e.g., an excess of COF3, e.g., as measured by an assay described herein, optionally wherein the fusion polypeptide is ubiquitinated at one or more lysine or methionine residues in the presence of COF3; or
- (iii) the degradation of the fusion polypeptide in the absence of COF3 is no more than, e.g., 0.01%, 0.1%, 1%, 10%, 20%, 30%, 40%, 50%, 60%, or 70% of the degradation of the fusion polypeptide in the presence of COF3, e.g., an excess of COF3, e.g., as measured by an assay described herein, e.g., a Western blot analysis or a flow cytometry analysis, optionally wherein the degradation of the fusion polypeptide is mediated by ubiquitination in the presence of COF3; optionally wherein the association, ubiquitination, and/or degradation is as measured in a mammalian cell, e.g., a human cell.

301. The fusion polypeptide of any one of claims 296-300, wherein the COF3/CRBN-binding polypeptide is between 10 and 95 amino acid residues in length, between 15 and 90 amino acid residues in length, between 20 and 85 amino acid residues in length, between 25 and 80 amino acid residues in length, between 30 and 75 amino acid residues in length, between 35 and 70 amino acid residues in

length, between 40 and 65 amino acid residues in length, between 45 and 65 amino acid residues in length, between 50 and 65 amino acid residues in length, or between 55 and 65 amino acid residues in length.

302. The fusion polypeptide of any one of claims 296-301, wherein the COF3/CRBN-binding polypeptide comprises a beta turn, a beta hairpin, a beta strand, or an alpha helix, optionally wherein the COF3/CRBN-binding polypeptide comprises, from N-terminus to C-terminus, a first beta strand, a beta hairpin, a second beta strand, and a first alpha helix, optionally wherein the COF3/CRBN-binding polypeptide comprises, from N-terminus to C-terminus, a first beta strand, a beta hairpin, a second beta strand, a first alpha helix, and a second alpha helix, optionally wherein the beta hairpin and the second alpha helix are separated by no more than 60, 50, 40, or 30 amino acid residues.

303. The fusion polypeptide of any one of claims 296-302, wherein the COF3/CRBN-binding polypeptide comprises a COF3/CRBN-binding sequence from a naturally occurring polypeptide or a COF3/CRBN-binding variant thereof, e.g., a naturally occurring IKZF polypeptide or a COF3/CRBN-binding variant thereof, e.g., a naturally occurring IKZF2, or a COF3/CRBN-binding variant thereof, optionally wherein the COF3/CRBN-binding polypeptide comprises two or more discontinuous sequences from a naturally occurring IKZF polypeptide, e.g., a naturally occurring IKZF2.

304. The fusion polypeptide of any one of claims 296-303, wherein the COF3/CRBN-binding polypeptide comprises amino acid residues 130-174 of IKZF2 (numbered according to SEQ ID NO: 21) (e.g., the COF3/CRBN-binding polypeptide comprises the amino acid sequence of SEQ ID NO: 113) or a sequence that differs from amino acid residues 130-174 of IKZF2 (numbered according to SEQ ID NO: 21) by no more than 1, 2, 3, 4, 5, 10, 15, 20, 25, 30, 35, or 40 amino acid residues (e.g., a sequence that differs from amino acid residues 130-174 of SEQ ID NO: 21 by no more than 1, 2, 3, 4, 5, 10, 15, 20, 25, 30, 35, or 40 amino acid residues) (e.g., a sequence having no more than 1, 2, 3, 4, 5, 10, 15, 20, 25, 30, 35, or 40 amino acid substitutions from amino acid residues 130-174 of SEQ ID NO: 21).

305. The fusion polypeptide of any one of claims 296-304, wherein the COF3/CRBN-binding polypeptide comprises amino acid residues 230-243 of IKZF2 (numbered according to SEQ ID NO: 21) (e.g., the COF3/CRBN-binding polypeptide comprises the amino acid sequence of SEQ ID NO: 114) or a sequence that differs from amino acid residues 230-243 of IKZF2 (numbered according to SEQ ID NO: 21) by no more than 1, 2, 3, 4, 5, or 10 amino acid residues (e.g., a sequence that differs from amino acid residues 230-243 of SEQ ID NO: 21 by no more than 1, 2, 3, 4, 5, or 10 amino acid

residues) (e.g., a sequence having no more than 1, 2, 3, 4, 5, or 10 amino acid substitutions from amino acid residues 230-243 of SEQ ID NO: 21).

306. The fusion polypeptide of claim 304 or 305, wherein histidine at position 141 remains unaltered, numbered according to SEQ ID NO: 21.

307. The fusion polypeptide of any one of claims 296-306, wherein:

(i) the COF3/CRBN-binding polypeptide comprises amino acid residues 130-174 of IKZF2 (numbered according to SEQ ID NO: 21), e.g., the COF3/CRBN-binding polypeptide comprises the amino acid sequence of SEQ ID NO: 113; and/or

(ii) the COF3/CRBN-binding polypeptide comprises amino acid residues 230-243 of IKZF2 (numbered according to SEQ ID NO: 21), e.g., the COF3/CRBN-binding polypeptide comprises the amino acid sequence of SEQ ID NO: 114.

308. The fusion polypeptide of any one of claims 296-307, wherein the COF3/CRBN-binding polypeptide comprises or consists of the amino acid sequence of SEQ ID NO: 109.

309. The fusion polypeptide of any one of claims 296-308, wherein the heterologous polypeptide is chosen from a cytoplasmic and/or nuclear polypeptide, or a transmembrane polypeptide, e.g., a heterologous polypeptide in Table 2.

310. The fusion polypeptide of claim 309, wherein the cytoplasmic and/or nuclear polypeptide is selected from the group consisting of a component of the apoptosis pathway (e.g., Caspase 9), a component of a CRISPR/Cas system (e.g., Cas9), a transcription factor (e.g., MTF, c-Myc, STAT3, STAT5, NF-kappaB, beta-catenin, Notch, GLI, or c-JUN), Tet methylcytosine dioxygenase 2 (TET2), FKBP, and Tau.

311. The fusion polypeptide of claim 309, wherein the transmembrane polypeptide is selected from the group consisting of CD62L, CCR1, CCR2, CCR5, CCR7, CCR10, CXCR2, CXCR3, CXCR4, CXCR6, CTLA4, PD1, BTLA, VISTA, CD137L, CD80, CD86, TIGIT, CD3, CD8, CD19, CD22, CD20, BCMA, and a chimeric antigen receptor (CAR).

312. The fusion polypeptide of claim 309, wherein the heterologous polypeptide is selected from the group consisting of a chimeric antigen receptor (CAR), a component of a CRISPR/Cas system (e.g.,

Cas9), CD8, CD19, and CD22, optionally wherein the heterologous polypeptide is a chimeric antigen receptor (CAR).

313. The fusion polypeptide of claim 312, wherein the CAR comprises an antigen binding domain, a transmembrane domain, and an intracellular signaling domain (e.g., a primary signaling domain or a costimulatory signaling domain), optionally wherein the primary signaling domain comprises a CD3-zeta stimulatory domain and/or the costimulatory signaling domain comprises an intracellular domain from a costimulatory protein selected from the group consisting of CD27, CD28, 4-1BB (CD137), OX40, GITR, CD30, CD40, ICOS, BAFFR, HVEM, ICAM-1, lymphocyte function-associated antigen-1 (LFA-1), CD2, CDS, CD7, CD287, LIGHT, NKG2C, NKG2D, SLAMF7, NKp80, NKp30, NKp44, NKp46, CD160, B7-H3, and a ligand that specifically binds with CD83.

314. The fusion polypeptide of claim 312, wherein the antigen binding domain binds an antigen selected from the group consisting of CD19; CD123; CD22; CD30; CD171; CS-1; C-type lectin-like molecule-1, CD33; epidermal growth factor receptor variant III (EGFRvIII); ganglioside G2 (GD2); ganglioside GD3; TNF receptor family member; B-cell maturation antigen; Tn antigen ((Tn Ag) or (GalNAc $\alpha$ -Ser/Thr)); prostate-specific membrane antigen (PSMA); Receptor tyrosine kinase-like orphan receptor 1 (ROR1); Fms-Like Tyrosine Kinase 3 (FLT3); Tumor-associated glycoprotein 72 (TAG72); CD38; CD44v6; Carcinoembryonic antigen (CEA); Epithelial cell adhesion molecule (EPCAM); B7H3 (CD276); KIT (CD117); Interleukin-13 receptor subunit alpha-2; Mesothelin; Interleukin 11 receptor alpha (IL-11Ra); prostate stem cell antigen (PSCA); Protease Serine 21; vascular endothelial growth factor receptor 2 (VEGFR2); Lewis(Y) antigen; CD24; Platelet-derived growth factor receptor beta (PDGFR-beta); Stage-specific embryonic antigen-4 (SSEA-4); CD20; Folate receptor alpha; Receptor tyrosine-protein kinase ERBB2 (Her2/neu); Mucin 1, cell surface associated (MUC1); epidermal growth factor receptor (EGFR); neural cell adhesion molecule (NCAM); Prostase; prostatic acid phosphatase (PAP); elongation factor 2 mutated (ELF2M); Ephrin B2; fibroblast activation protein alpha (FAP); insulin-like growth factor 1 receptor (IGF-I receptor), carbonic anhydrase IX (CAIX); Proteasome (Prosome, Macropain) Subunit, Beta Type, 9 (LMP2); glycoprotein 100 (gp100); oncogene polypeptide consisting of breakpoint cluster region (BCR) and Abelson murine leukemia viral oncogene homolog 1 (Abl) (bcr-abl); tyrosinase; ephrin type-A receptor 2 (EphA2); Fucosyl GM1; sialyl Lewis adhesion molecule (sLe); ganglioside GM3; transglutaminase 5 (TGS5); high molecular weight-melanoma-associated antigen (HMWMAA); o-acetyl-GD2 ganglioside (OAcGD2); Folate receptor beta; tumor endothelial marker 1 (TEM1/CD248); tumor endothelial marker 7-related (TEM7R); claudin 6 (CLDN6); thyroid stimulating hormone receptor (TSHR); G protein-coupled receptor class C group 5, member D (GPCR5D); chromosome X open reading frame 61 (CXORF61); CD97; CD179a; anaplastic

lymphoma kinase (ALK); Polysialic acid; placenta-specific 1 (PLAC1); hexasaccharide portion of globoH glycosphingolipid (GloboH); mammary gland differentiation antigen (NY-BR-1); uroplakin 2 (UPK2); Hepatitis A virus cellular receptor 1 (HAVCR1); adrenoceptor beta 3 (ADRB3); pannexin 3 (PANX3); G protein-coupled receptor 20 (GPR20); lymphocyte antigen 6 complex, locus K 9 (LY6K); Olfactory receptor 51E2 (OR51E2); TCR Gamma Alternate Reading Frame Protein (TARP); Wilms tumor protein (WT1); Cancer/testis antigen 1 (NY-ESO-1); Cancer/testis antigen 2 (LAGE-1a); Melanoma-associated antigen 1 (MAGE-A1); ETS translocation-variant gene 6, located on chromosome 12p (ETV6-AML); sperm protein 17 (SPA17); X Antigen Family, Member 1A (XAGE1); angiopoietin-binding cell surface receptor 2 (Tie 2); melanoma cancer testis antigen-1 (MAD-CT-1); melanoma cancer testis antigen-2 (MAD-CT-2); Fos-related antigen 1; tumor protein p53 (p53); p53 mutant; prostein; surviving; telomerase; prostate carcinoma tumor antigen-1, melanoma antigen recognized by T cells 1; Rat sarcoma (Ras) mutant; human Telomerase reverse transcriptase (hTERT); sarcoma translocation breakpoints; melanoma inhibitor of apoptosis (ML-IAP); ERG (transmembrane protease, serine 2 (TMPRSS2) ETS fusion gene); N-Acetyl glucosaminyl-transferase V (NA17); paired box protein Pax-3 (PAX3); Androgen receptor; Cyclin B1; v-myc avian myelocytomatosis viral oncogene neuroblastoma derived homolog (MYCN); Ras Homolog Family Member C (RhoC); Tyrosinase-related protein 2 (TRP-2); Cytochrome P450 1B1 (CYP1B1); CCCTC-Binding Factor (Zinc Finger Protein)-Like, Squamous Cell Carcinoma Antigen Recognized By T Cells 3 (SART3); Paired box protein Pax-5 (PAX5); proacrosin binding protein sp32 (OY-TES1); lymphocyte-specific protein tyrosine kinase (LCK); A kinase anchor protein 4 (AKAP-4); synovial sarcoma, X breakpoint 2 (SSX2); Receptor for Advanced Glycation Endproducts (RAGE-1); renal ubiquitous 1 (RU1); renal ubiquitous 2 (RU2); legumain; human papilloma virus E6 (HPV E6); human papilloma virus E7 (HPV E7); intestinal carboxyl esterase; heat shock protein 70-2 mutated (mut hsp70-2); CD79a; CD79b; CD72; Leukocyte-associated immunoglobulin-like receptor 1 (LAIR1); Fc fragment of IgA receptor (FCAR or CD89); Leukocyte immunoglobulin-like receptor subfamily A member 2 (LILRA2); CD300 molecule-like family member f (CD300LF); C-type lectin domain family 12 member A (CLEC12A); bone marrow stromal cell antigen 2 (BST2); EGF-like module-containing mucin-like hormone receptor-like 2 (EMR2); lymphocyte antigen 75 (LY75); Glypican-3 (GPC3); Fc receptor-like 5 (FCRL5); and immunoglobulin lambda-like polypeptide 1 (IGLL1), optionally wherein the antigen binding domain binds an antigen chosen from CD19, CD22, BCMA, CD20, CD123, EGFRvIII, or mesothelin.

315. The fusion polypeptide of any one of claims 296-314, wherein the fusion polypeptide further comprises a degradation domain, wherein the degradation domain is separated from the COF3/CRBN-binding polypeptide and the heterologous polypeptide by a heterologous protease cleavage site.

316. The fusion polypeptide of claim 315, wherein the fusion polypeptide comprises, from N-terminus to C-terminus:

- i) the degradation domain, the heterologous protease cleavage site, the heterologous polypeptide, and the COF3/CRBN-binding polypeptide;
- ii) the degradation domain, the heterologous protease cleavage site, the COF3/CRBN-binding polypeptide, and the heterologous polypeptide;
- iii) the COF3/CRBN-binding polypeptide, the heterologous polypeptide, the heterologous protease cleavage site, and the degradation domain; or
- iv) the heterologous polypeptide, and the COF3/CRBN-binding polypeptide, the heterologous protease cleavage site, and the degradation domain.

317. The fusion polypeptide of claim 315 or 316, wherein the degradation domain has a first state associated with a first level of expression of the fusion polypeptide and a second state associated with a second level of expression of the fusion polypeptide, wherein the second level is increased, e.g., by at least 2-, 3-, 4-, 5-, 10-, 20- or 30-fold over the first level in the presence of a stabilization compound, optionally wherein:

in the absence of the stabilization compound, the fusion polypeptide is degraded by a cellular degradation pathway, e.g., at least 50%, 60%, 70%, 80%, 90% or greater of the fusion polypeptide is degraded, e.g., as measured by an assay described herein, e.g., a Western blot analysis or a flow cytometry analysis; or

in the presence of the stabilization compound:

- i) the degradation domain assumes a conformation more resistant to cellular degradation relative to a conformation in the absence of the stabilization compound; or
- ii) the conformation of the fusion polypeptide is more permissive to cleavage at the heterologous protease cleavage site relative to a conformation in the absence of the stabilization compound.

318. The fusion polypeptide of any one of claims 315-317, wherein the degradation domain is chosen from an estrogen receptor (ER) domain, an FKB protein (FKBP) domain, or a dihydrofolate reductase (DHFR) domain.

319. The fusion polypeptide of any one of claims 315-318, wherein the heterologous protease cleavage site is cleaved by a mammalian intracellular protease selected from the group consisting of furin, PCSK1, PCSK5, PCSK6, PCSK7, cathepsin B, Granzyme B, Factor XA, Enterokinase, genenase, sortase, precision protease, thrombin, TEV protease, and elastase 1.



320. The fusion polypeptide of any one of claims 315-318, wherein the heterologous protease cleavage site is cleaved by a mammalian extracellular protease selected from the group consisting of Factor XA, Enterokinase, genenase, sortase, precision protease, thrombin, TEV protease, and elastase 1.

321. A nucleic acid molecule encoding the fusion polypeptide of any one of claims 296-320.

322. A vector comprising the nucleic acid molecule of claim 321, optionally wherein the vector is a viral vector, e.g., a lentiviral vector, a retroviral vector, or an adenoviral vector.

323. A cell, e.g., a host cell, comprising the fusion polypeptide of any one of claims 296-320, the nucleic acid molecule of claim 321, or the vector of claim 322, optionally wherein the cell is a T cell, a NK cell, or a tumor cell.

324. A pharmaceutical composition comprising the fusion polypeptide of any one of claims 296-320 or the cell of claim 323, and a pharmaceutically acceptable carrier, excipient or stabilizer.

325. A method of making the cell of claim 323, said method comprising providing a cell, e.g., an immune effector cell, with the nucleic acid molecule of claim 321 or the vector of claim 322.

326. A method of degrading a fusion polypeptide, comprising contacting the fusion polypeptide of any one of claims 296-320 or a cell comprising said fusion polypeptide with COF3, optionally wherein in the presence of COF3, the expression level of said fusion polypeptide is substantially decreased, e.g., by at least about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 60, 70, 80, 90, or 100 percent, relative to the expression level of said fusion polypeptide in the absence of COF3, e.g., as measured by an assay described herein, e.g., a Western blot analysis or a flow cytometry analysis, optionally wherein: the fusion polypeptide or the cell is contacted with COF3 *ex vivo*, or the fusion polypeptide or the cell is contacted with COF3 *in vivo*.

327. A method of regulating the expression of a fusion polypeptide, comprising:

i) contacting the fusion polypeptide of any one of claims 315-320 or a cell comprising said fusion polypeptide with a stabilization compound, optionally wherein in the presence of the stabilization compound:

a) the degradation domain assumes a conformation more resistant to cellular degradation relative to a conformation in the absence of the stabilization compound;

b) the conformation of the fusion polypeptide is more permissive to cleavage at the heterologous protease cleavage site relative to a conformation in the absence of the stabilization compound; or  
 c) the expression level of the fusion polypeptide is increased by at least, e.g., 1.5-, 2-, 3-, 4-, 5-, 10-, 20-, 30-, 40-, or 50-fold, compared to the expression level of the fusion polypeptide in the absence of the stabilization compound, e.g., as measured by an assay described herein, e.g., a Western blot analysis or a flow cytometry analysis,  
 optionally wherein the fusion polypeptide or the cell is contacted with the stabilization compound *ex vivo* or *in vivo*.

328. The method of claim 327, further comprising, after step i):

ii) contacting the fusion polypeptide of any one of claims 315-320 or a cell comprising said fusion polypeptide with COF3, optionally wherein in the presence of COF3, the expression level of the fusion polypeptide is substantially decreased, e.g., by at least about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 60, 70, 80, 90, or 100 percent, relative to the expression level of the fusion polypeptide after step i) and before step ii), e.g., as measured by an assay described herein, e.g., a Western blot analysis or a flow cytometry analysis,  
 optionally wherein the fusion polypeptide or the cell is contacted with COF3 *ex vivo* or *in vivo*.

329. A method of making a cell, comprising:

i) providing a cell, e.g., an immune effector cell, with a nucleic acid molecule encoding the fusion polypeptide of any one of claims 296-320; and  
 ii) contacting the cell *ex vivo* with COF3, optionally wherein:  
 in the presence of COF3, the expression level of the fusion polypeptide is substantially decreased, e.g., by at least about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 60, 70, 80, 90, or 100 percent, relative to the expression level of the fusion polypeptide in the absence of COF3, e.g., as measured by an assay described herein, e.g., a Western blot analysis or a flow cytometry analysis.

330. A method of treating a subject having a disease associated with expression of a tumor antigen, comprising:

i) contacting a cell comprising the fusion polypeptide of any one of claims 296-314 with COF3 *ex vivo*, optionally wherein:  
 in the presence of COF3, the expression level of the fusion polypeptide is decreased, e.g., by at least about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 60, 70, 80, 90, or 100 percent, relative to the expression level of the fusion polypeptide before the cell is contacted with COF3 *ex vivo*, and

ii) administering to the subject an effective amount of the cell, optionally wherein the method further comprises after step i) and prior to step ii):

reducing the amount of COF3 contacting the cell, e.g., inside and/or surrounding the cell, thereby treating the disease.

331. The method of claim 330, further comprising after step ii):

iii) administering to the subject an effective amount of COF3, optionally wherein the administration of COF3 decreases, e.g., by at least about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 60, 70, 80, 90, or 100 percent, the expression level of the fusion polypeptide relative to the expression level of the fusion polypeptide after step ii) and prior to step iii), optionally wherein:

- a) the subject has developed, is developing, or is anticipated to develop an adverse reaction,
- b) the administration of COF3 is in response to an occurrence of an adverse reaction in the subject, or in response to an anticipation of an occurrence of an adverse reaction in the subject, and/or
- c) the administration of COF3 reduces or prevents an adverse effect.

332. The method of claim 331, further comprising after step iii):

iv) discontinuing the administration of COF3, optionally wherein discontinuing the administration of COF3 increases, e.g., by at least about 1.5-, 2-, 3-, 4-, 5-, 10-, 20-, 30-, 40-, or 50-fold, the expression level of the fusion polypeptide relative to the expression level of the fusion polypeptide after step iii) and prior to step iv) (e.g., wherein discontinuing the administration of COF3 restores the expression level of the fusion polypeptide to the expression level after step ii) and prior to step iii)), optionally wherein:

- a) the subject has relapsed, is relapsing, or is anticipated to relapse,
- b) the discontinuation of the administration of COF3 is in response to a tumor relapse in the subject, or in response to an anticipation of a relapse in the subject, and/or
- c) the discontinuation of the administration of COF3 treats or prevents a tumor relapse.

333. The method of claim 332, further comprising after step iv):

v) repeating step iii) and/or iv), thereby treating the disease.

334. A method of treating a subject having a disease associated with expression of a tumor antigen, comprising:

i) administering to the subject an effective amount of a cell comprising the fusion polypeptide of any one of claims 296-314, optionally wherein the cell is contacted with COF3 *ex vivo* before administration, optionally wherein:

in the presence of COF3, the expression level of the fusion polypeptide is decreased, e.g., by at least about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 60, 70, 80, 90, or 100 percent, relative to the expression level of the fusion polypeptide before the cell is contacted with COF3 *ex vivo*, optionally wherein after the cell is contacted with COF3 *ex vivo* and before the cell is administered to the subject, the amount of COF3 contacting the cell, e.g., inside and/or surrounding the cell, is reduced, thereby treating the disease.

335. The method of claim 334, wherein the cell is not contacted with COF3 *ex vivo* before administration.

336. The method of claim 334 or 335, further comprising after step i):

ii) administering to the subject an effective amount of COF3, optionally wherein the administration of COF3 decreases, e.g., by at least about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 60, 70, 80, 90, or 100 percent, the expression level of the fusion polypeptide relative to the expression level of the fusion polypeptide after step i) and prior to step ii), optionally wherein:

- a) the subject has developed, is developing, or is anticipated to develop an adverse reaction,
- b) the administration of COF3 is in response to an occurrence of an adverse reaction in the subject, or in response to an anticipation of an occurrence of an adverse reaction in the subject, and/or
- c) the administration of COF3 reduces or prevents an adverse effect.

337. The method of claim 336, further comprising after step ii):

iii) discontinuing the administration of COF3, optionally wherein discontinuing the administration of COF3 increases, e.g., by at least about 1.5-, 2-, 3-, 4-, 5-, 10-, 20-, 30-, 40-, or 50-fold, the expression level of the fusion polypeptide relative to the expression level of the fusion polypeptide after step ii) and prior to step iii) (e.g., wherein discontinuing the administration of COF3 restores the expression level of the fusion polypeptide to the expression level after step i) and prior to step ii)), optionally wherein:

- a) the subject has relapsed, is relapsing, or is anticipated to relapse,
- b) the discontinuation of the administration of COF3 is in response to a tumor relapse in the subject, or in response to an anticipation of a relapse in the subject, and/or
- c) the discontinuation of the administration of COF3 treats or prevents a tumor relapse.

338. The method of claim 337, further comprising after step iii):

iv) repeating step ii) and/or iii),  
thereby treating the disease.

339. A method of treating a subject having a disease associated with expression of a tumor antigen, comprising:

- i) administering an effective amount of COF3 to the subject, wherein the subject comprises a cell comprising the fusion polypeptide of any one of claims 296-314, optionally wherein the administration of COF3 decreases, e.g., by at least about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 60, 70, 80, 90, or 100 percent, the expression level of the fusion polypeptide relative to the expression level of the fusion polypeptide before the administration of COF3, optionally wherein:
  - a) the subject has developed, is developing, or is anticipated to develop an adverse reaction,
  - b) the administration of COF3 is in response to an occurrence of an adverse reaction in the subject, or in response to an anticipation of an occurrence of an adverse reaction in the subject, and/or
  - c) the administration of COF3 reduces or prevents an adverse effect.

340. The method of claim 339, further comprising after step i):

- ii) discontinuing the administration of COF3, optionally wherein discontinuing the administration of COF3 increases, e.g., by at least about 1.5-, 2-, 3-, 4-, 5-, 10-, 20-, 30-, 40-, or 50-fold, the expression level of the fusion polypeptide relative to the expression level of the fusion polypeptide after step i) and prior to step ii) (e.g., wherein discontinuing the administration of COF3 restores the expression level of the fusion polypeptide to the expression level before the administration of COF3), optionally wherein:
  - a) the subject has relapsed, is relapsing, or is anticipated to relapse,
  - b) the discontinuation of the administration of COF3 is in response to a tumor relapse in the subject, or in response to an anticipation of a relapse in the subject, and/or
  - c) the discontinuation of the administration of COF3 treats or prevents a tumor relapse.

341. The method of claim 340, further comprising after step ii):

- iii) repeating step i) and/or ii),  
thereby treating the disease.

342. A method of treating a subject having a disease associated with expression of a tumor antigen, comprising:

- i) administering to the subject:
  - (1) a stabilization compound, and

(2) an effective amount of a cell comprising the fusion polypeptide of any one of claims 315-320, or an effective amount of a cell comprising the fusion polypeptide of any one of claims 315-320, optionally wherein:

the expression level of the fusion polypeptide in the presence of the stabilization compound is e.g., at least about 1.5-, 2-, 3-, 4-, 5-, 10-, 20-, 30-, 40-, or 50-fold, higher than the expression level of the fusion polypeptide in the absence of the stabilization compound, thereby treating the disease.

343. The method of claim 342, further comprising after step i):

ii) discontinuing the administration of the stabilization compound, optionally wherein discontinuing the administration of the stabilization compound reduces, e.g., at least about 1.5-, 2-, 3-, 4-, 5-, 10-, 20-, 30-, 40-, or 50-fold, the expression level of the fusion polypeptide relative to the expression of the fusion polypeptide after step i) and prior to step ii), optionally wherein:

- a) the subject responded to the treatment of step i) (e.g., the subject has a complete response to the treatment of step i), the subject shows a shrinkage in tumor mass, the subject shows a decrease in tumor cells, or the treatment of step i) is effective in the subject), and/or
- b) the discontinuation of the administration of the stabilization compound is in response to a response of the subject to the treatment of step i) (e.g., the subject has a complete response to the treatment of step i), the subject shows a shrinkage in tumor mass, the subject shows a decrease in tumor cells, or the treatment of step i) is effective in the subject).

344. The method of claim 342, further comprising after step i):

iii) discontinuing the administration of the stabilization compound, optionally wherein discontinuing the administration of the stabilization compound reduces, e.g., at least about 1.5-, 2-, 3-, 4-, 5-, 10-, 20-, 30-, 40-, or 50-fold, the expression level of the fusion polypeptide relative to the expression of the fusion polypeptide after step i) and prior to step ii), optionally wherein:

- a) the subject has developed, is developing, or is anticipated to develop an adverse reaction,
- b) the discontinuation of the administration of the stabilization compound is in response to an occurrence of an adverse reaction in the subject, or in response to an anticipation of an occurrence of an adverse reaction in the subject, and/or
- c) the discontinuation of the administration of the stabilization compound reduces or prevents an adverse effect.

345. The method of claim 342, further comprising after step i):

iv) discontinuing the administration of the stabilization compound and administering to the subject an effective amount of COF3, optionally wherein step iv) reduces, e.g., at least about 1.5-, 2-, 3-, 4-, 5-, 10-, 20-, 30-, 40-, or 50-fold, the expression level of the fusion polypeptide relative to the expression of the fusion polypeptide after step i) and prior to step iv), optionally wherein:

- a) the subject has developed, is developing, or is anticipated to develop an adverse reaction,
  - b) step iv) is in response to an occurrence of an adverse reaction in the subject, or in response to an anticipation of an occurrence of an adverse reaction in the subject, and/or
  - c) step iv) reduces or prevents an adverse effect,
- optionally wherein the adverse effect is an acute toxicity.

346. The method of claim 345, further comprising after step iv):

- v) discontinuing the administration of COF3, e.g., after the amount of cells expressing the fusion polypeptide on the surface is smaller than a pre-defined value, for, e.g., 1 day, 5 days, 10 days, or 15 days.

347. The method of any one of claims 343-346, further comprising after step ii), iii), iv), or v):

- vi) administering an effective amount of a stabilization compound, optionally wherein the administration of the stabilization compound increases, e.g., by at least about 1.5-, 2-, 3-, 4-, 5-, 10-, 20-, 30-, 40-, or 50-fold, the expression level of the fusion polypeptide relative to the expression level of the fusion polypeptide after step ii), iii), iv), or v) and prior to step vi), optionally wherein:
  - a) the subject has relapsed, is relapsing, or is anticipated to relapse,
  - b) the administration of the stabilization compound is in response to a tumor relapse in the subject, or in response to an anticipation of a relapse in the subject, and/or
  - c) the administration of the stabilization compound treats or prevents a tumor relapse.

348. The method of claim 347, further comprising after step vi):

- vii) repeating step iii), iv), v), or vi),
- thereby treating the disease.

349. The method of any one of claims 342-348, further comprising prior to step i):

- viii) contacting the cell with a stabilization compound *ex vivo*, optionally wherein the expression level of the fusion polypeptide in the presence of the stabilization compound is e.g., at least about 1.5-, 2-, 3-, 4-, 5-, 10-, 20-, 30-, 40-, or 50-fold, higher than the expression level of the fusion polypeptide in the absence of the stabilization compound.

350. The method of any one of claims 342-348, wherein the cell is not contacted with the stabilization compound *ex vivo* before administration.

351. The method of any one of claims 330-350, wherein the disease associated with expression of a tumor antigen is a cancer.

352. The method of claim 351, wherein the cancer is mesothelioma (e.g., malignant pleural mesothelioma), e.g., in a subject who has progressed on at least one prior standard therapy; lung cancer (e.g., non-small cell lung cancer, small cell lung cancer, squamous cell lung cancer, or large cell lung cancer); pancreatic cancer (e.g., pancreatic ductal adenocarcinoma, or metastatic pancreatic ductal adenocarcinoma (PDA), e.g., in a subject who has progressed on at least one prior standard therapy); esophageal adenocarcinoma, ovarian cancer (e.g., serous epithelial ovarian cancer, e.g., in a subject who has progressed after at least one prior regimen of standard therapy), breast cancer, colorectal cancer, bladder cancer or any combination thereof.

353. The method of claim 351, wherein the cancer is a hematological cancer chosen from: chronic lymphocytic leukemia (CLL), mantle cell lymphoma (MCL), multiple myeloma, acute lymphoid leukemia (ALL), Hodgkin lymphoma, B-cell acute lymphoid leukemia (BALL), T-cell acute lymphoid leukemia (TALL), small lymphocytic leukemia (SLL), B cell prolymphocytic leukemia, blastic plasmacytoid dendritic cell neoplasm, Burkitt's lymphoma, diffuse large B cell lymphoma (DLBCL), DLBCL associated with chronic inflammation, chronic myeloid leukemia, myeloproliferative neoplasms, follicular lymphoma, pediatric follicular lymphoma, hairy cell leukemia, small cell- or a large cell-follicular lymphoma, malignant lymphoproliferative conditions, MALT lymphoma (extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue), Marginal zone lymphoma, myelodysplasia, myelodysplastic syndrome, non-Hodgkin lymphoma, plasmablastic lymphoma, plasmacytoid dendritic cell neoplasm, Waldenstrom macroglobulinemia, splenic marginal zone lymphoma, splenic lymphoma/leukemia, splenic diffuse red pulp small B-cell lymphoma, hairy cell leukemia-variant, lymphoplasmacytic lymphoma, a heavy chain disease, plasma cell myeloma, solitary plasmocytoma of bone, extraosseous plasmocytoma, nodal marginal zone lymphoma, pediatric nodal marginal zone lymphoma, primary cutaneous follicle center lymphoma, lymphomatoid granulomatosis, primary mediastinal (thymic) large B-cell lymphoma, intravascular large B-cell lymphoma, ALK+ large B-cell lymphoma, large B-cell lymphoma arising in HHV8-associated multicentric Castleman disease, primary effusion lymphoma, B-cell lymphoma, acute myeloid leukemia (AML), or unclassifiable lymphoma.



FIG. 1A

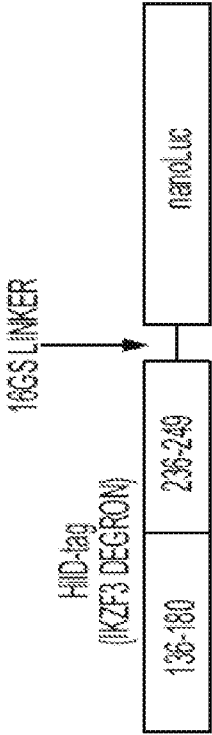


FIG. 1B

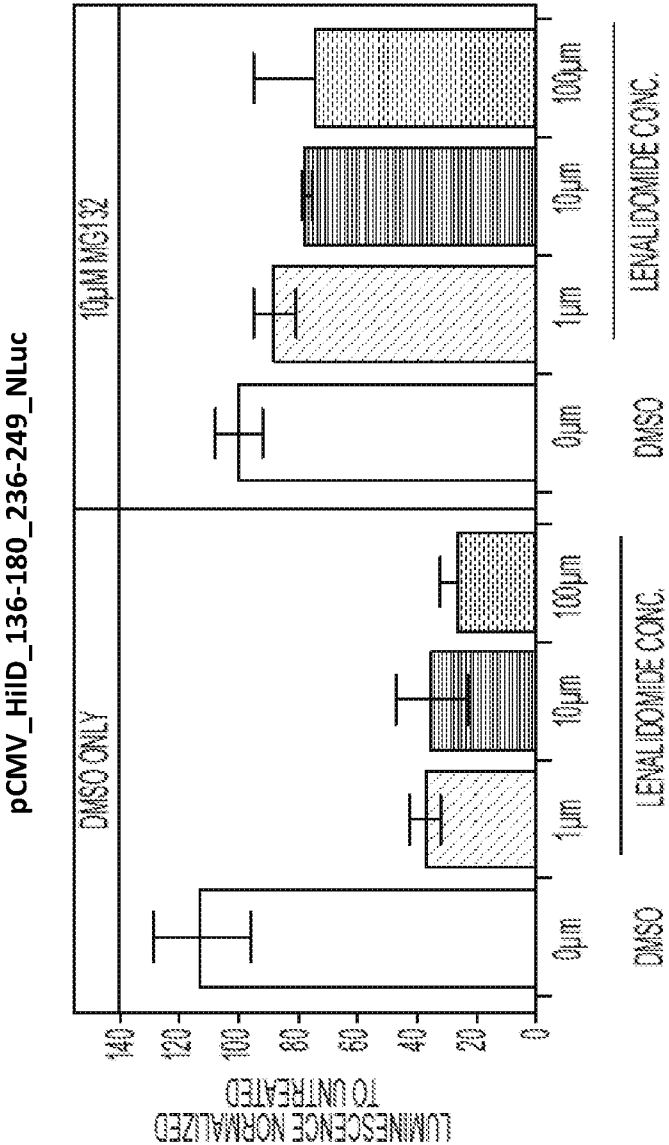


FIG. 2

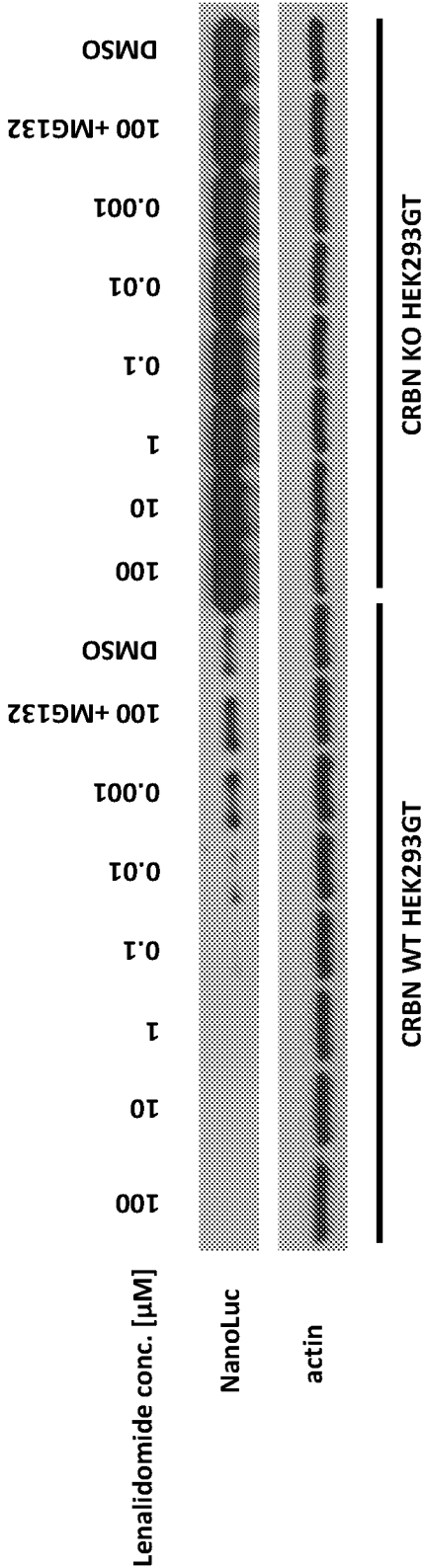


FIG. 3A

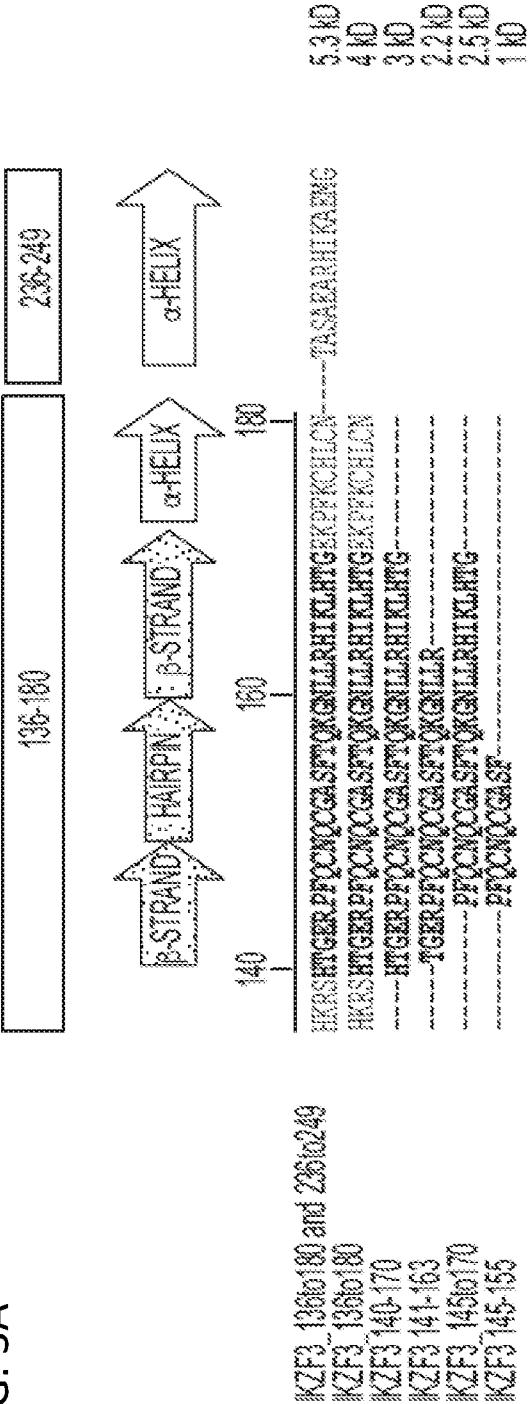


FIG. 3B

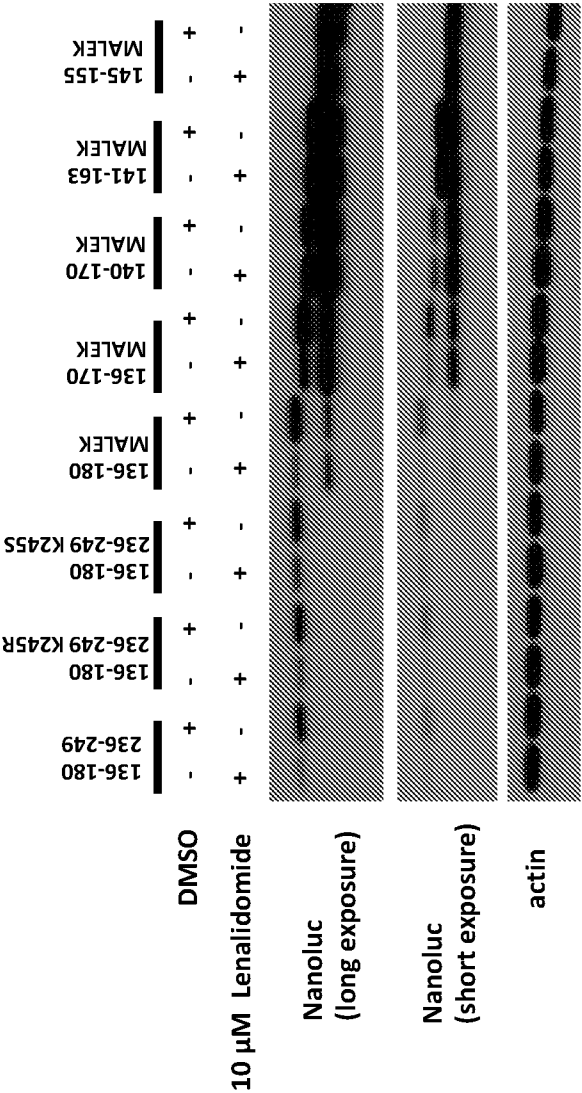


FIG. 4B

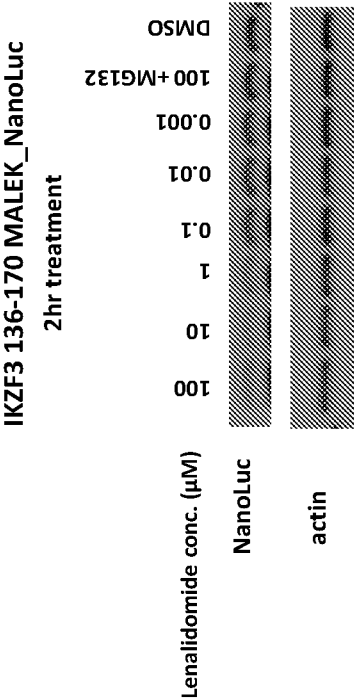


FIG. 4A

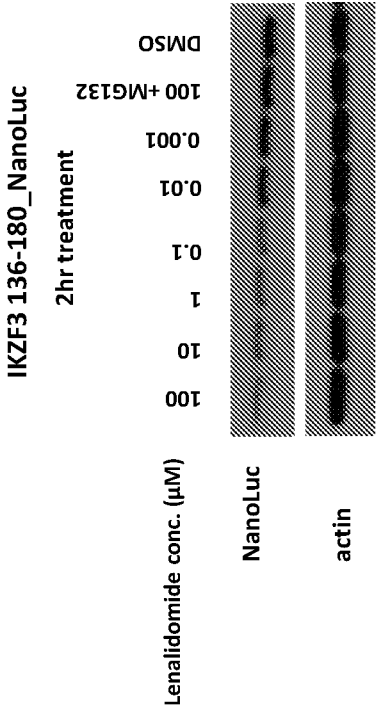


FIG. 4C

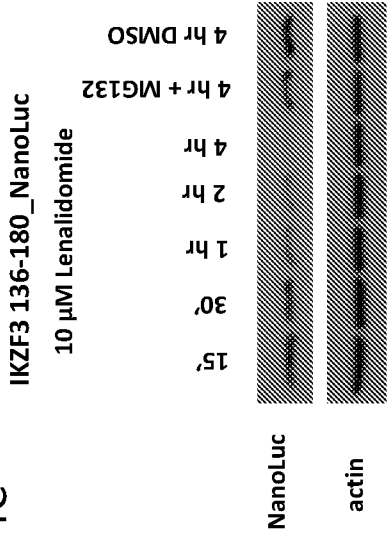


FIG. 5A

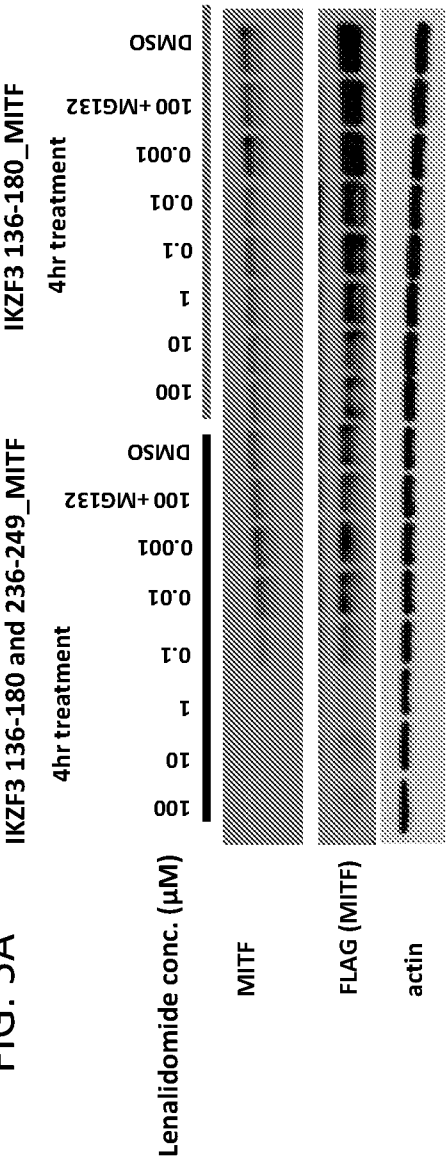


FIG. 5B

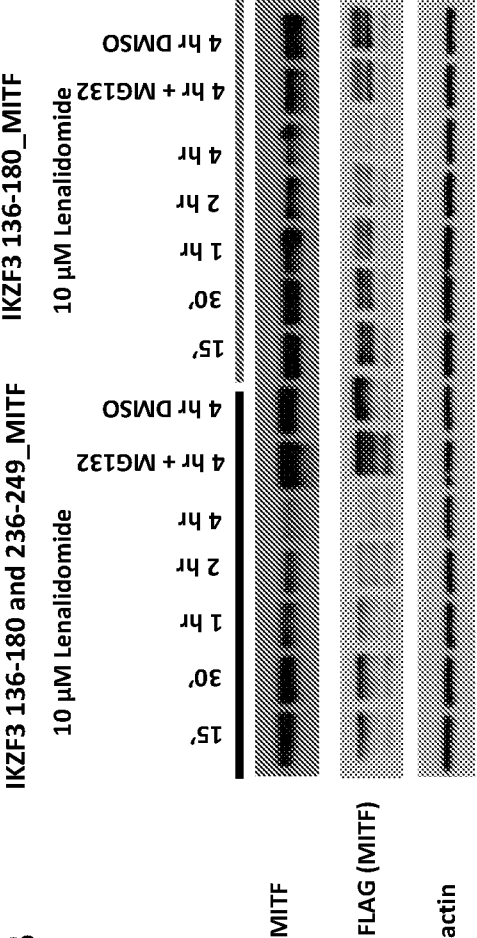


FIG. 6A

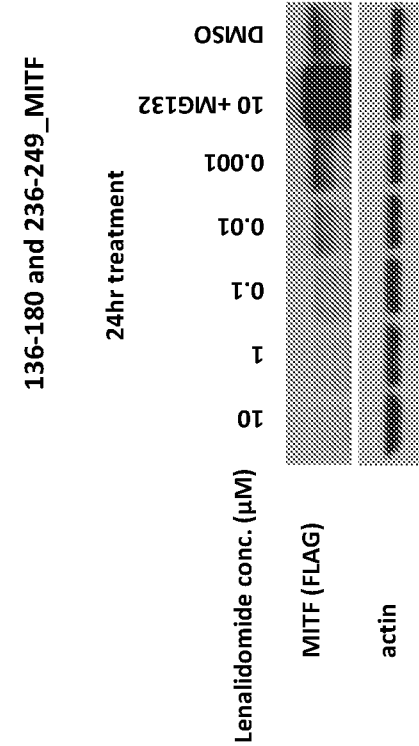


FIG. 6B

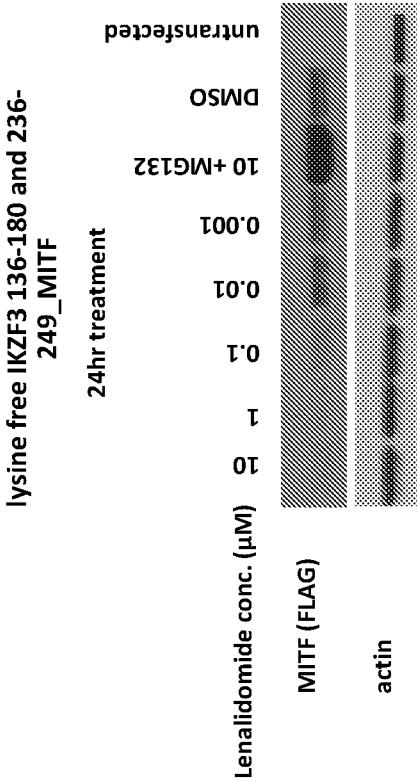


FIG. 6C

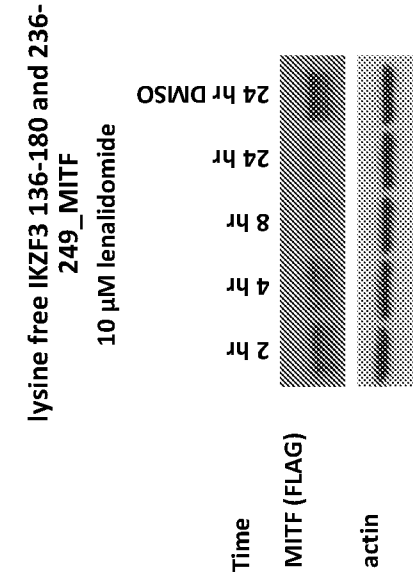


FIG. 6D

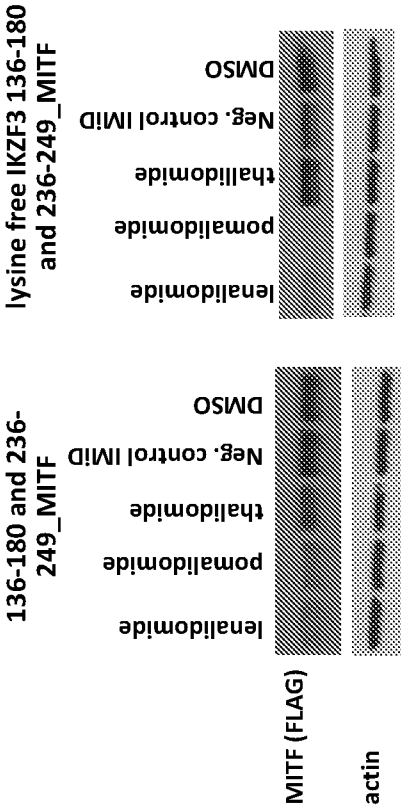


FIG. 7

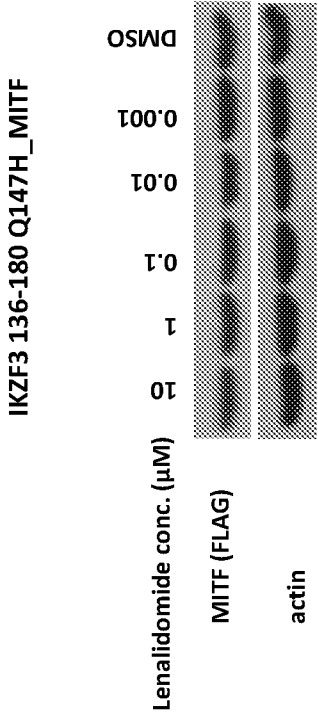


FIG. 8

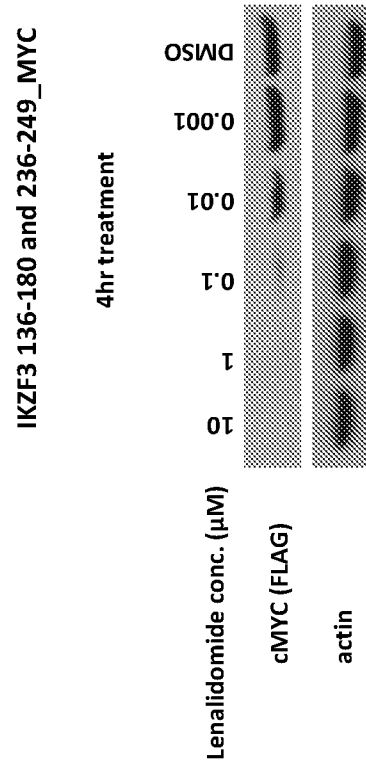
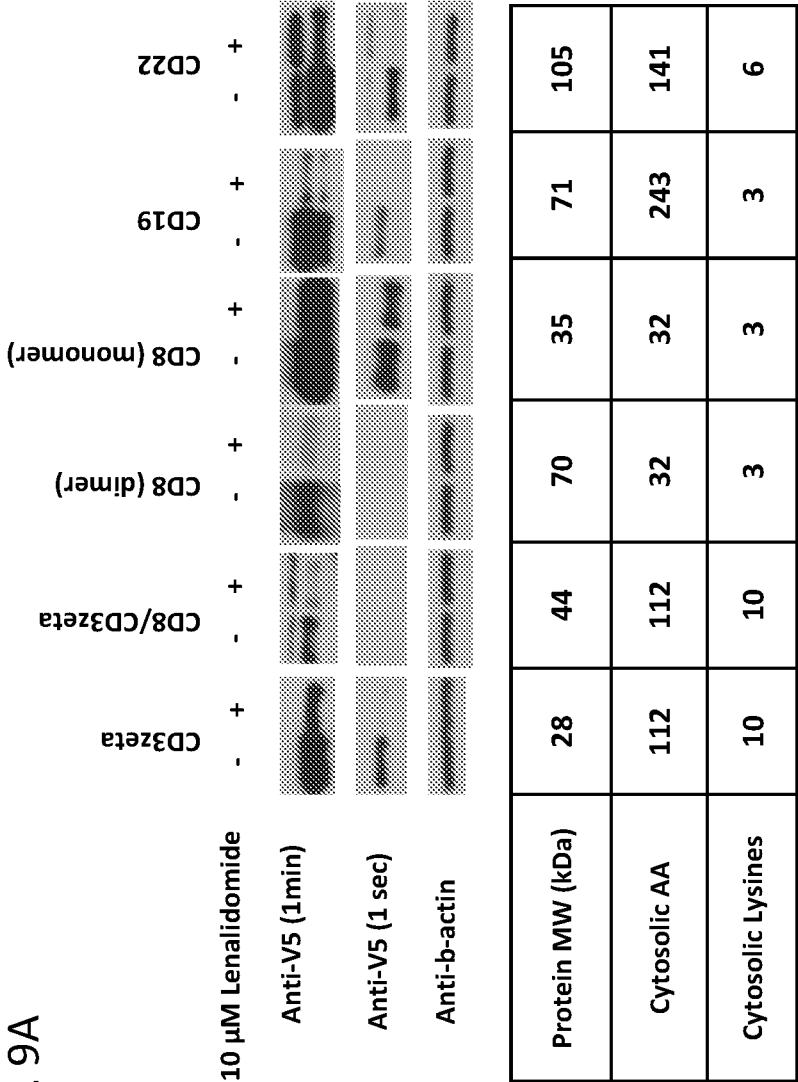




FIG. 9A



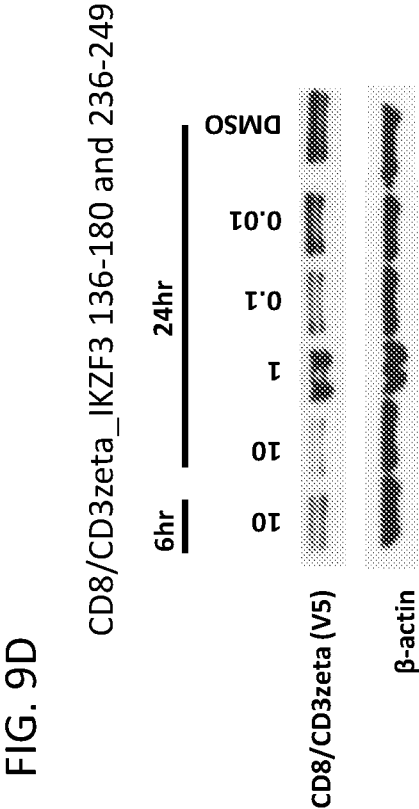
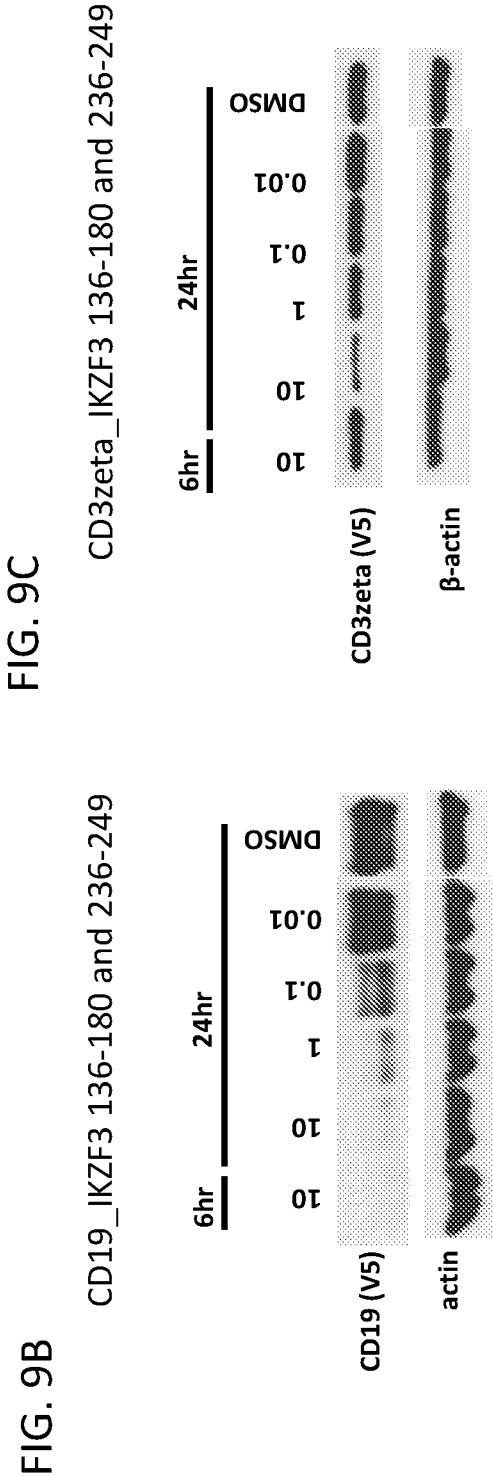


FIG. 10A

CD19\_IKZF3 136-180 and 236-249  
1hr Treatment

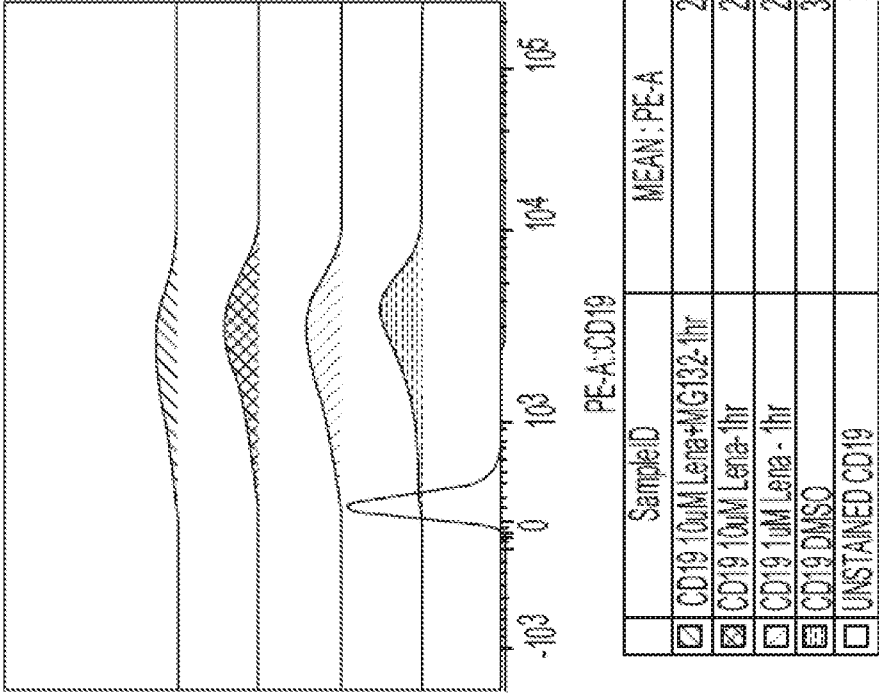


FIG. 10B

CD19\_IKZF3 136-180 and 236-249  
6hr Treatment

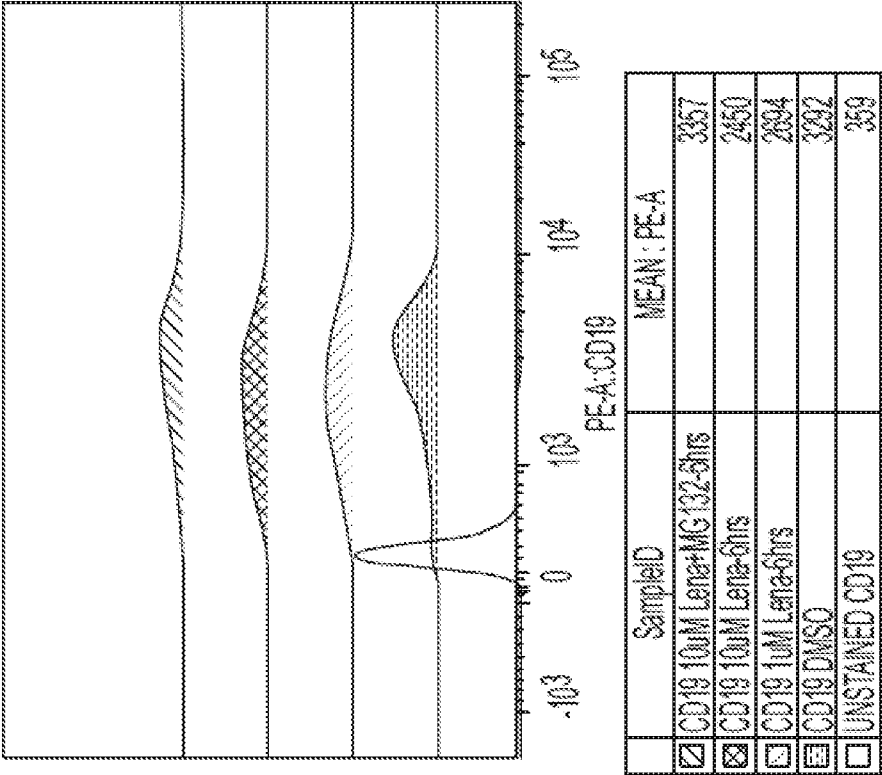


FIG. 10C

CD19\_IKZF3 136-180 and 236-249  
16hr Treatment

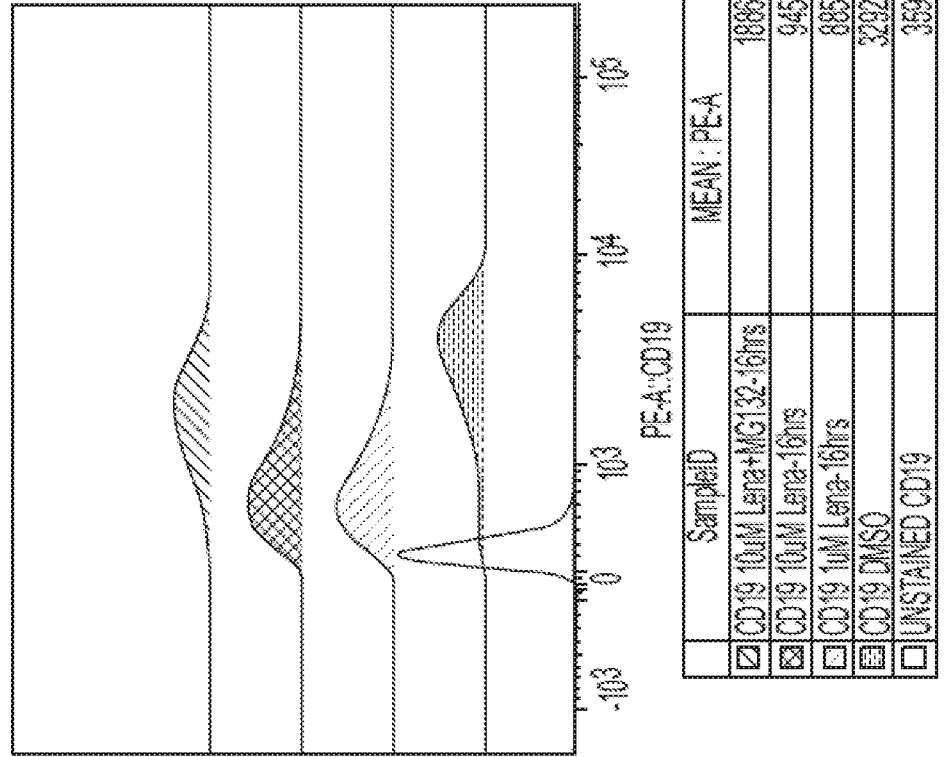
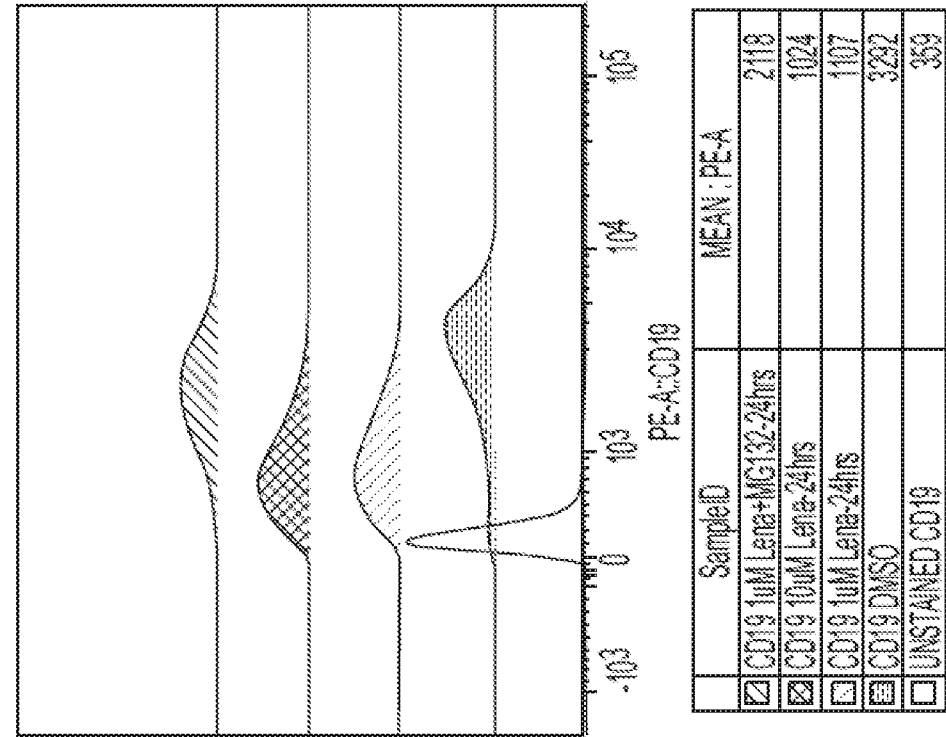


FIG. 10D

CD19\_IKZF3 136-180 and 236-249  
24hr Treatment



CD19\_IKZF3 136-180 and 236-249

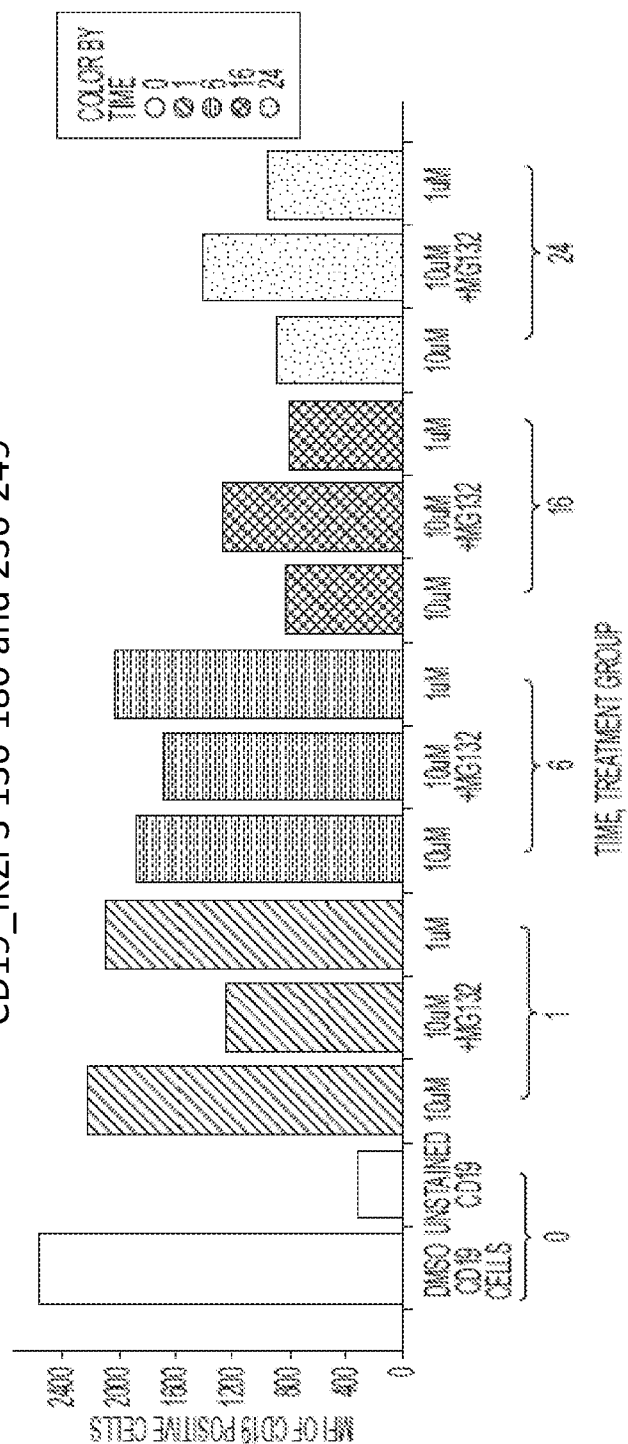
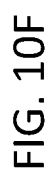


FIG. 11

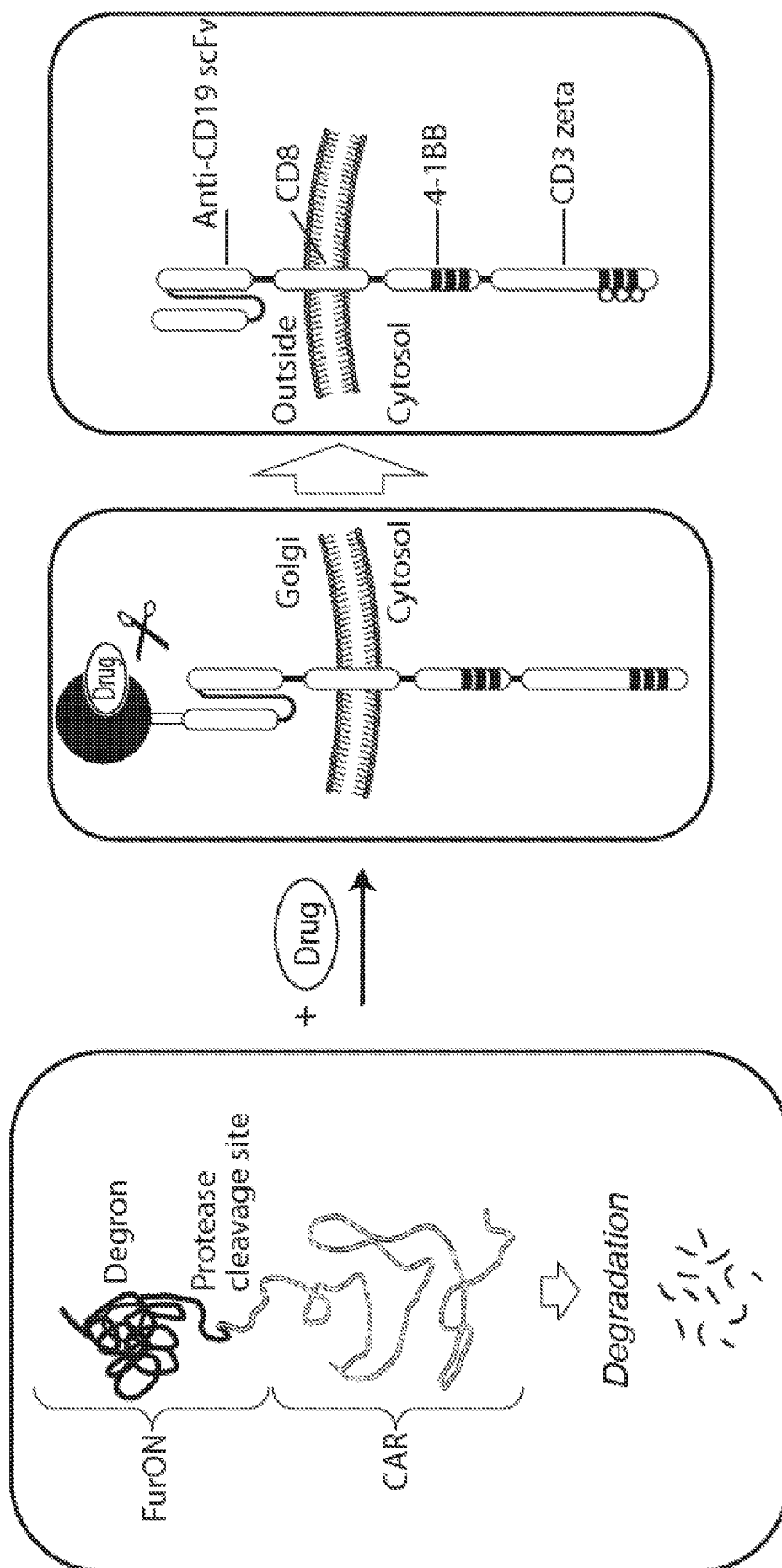


FIG. 12A

FurON CARTs

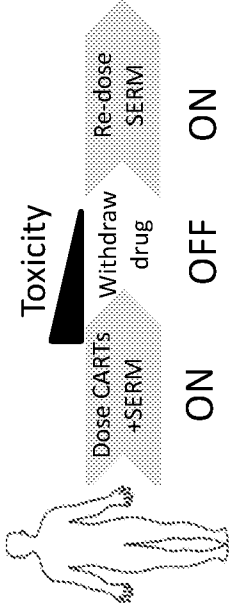
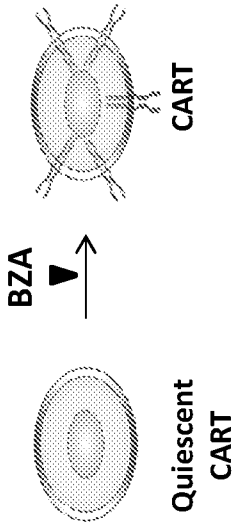


FIG. 12B

HiID CARTs

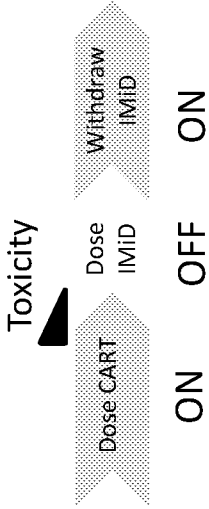
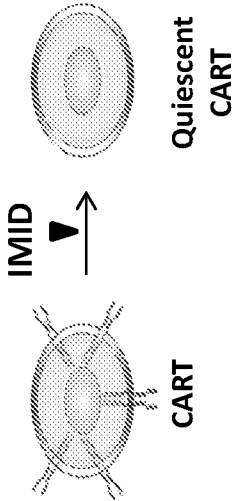
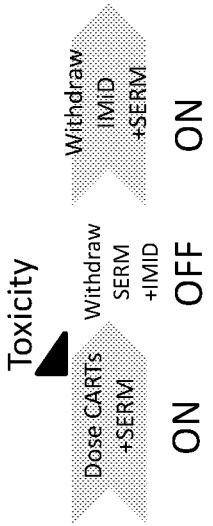
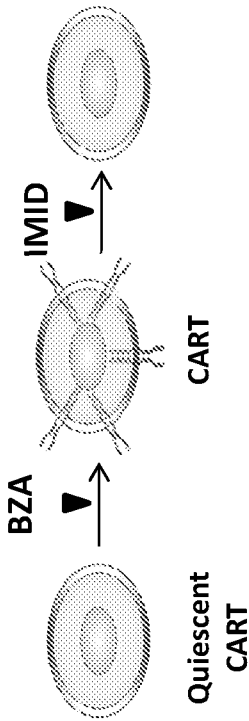


FIG. 12C

FurON/HiID CARTs



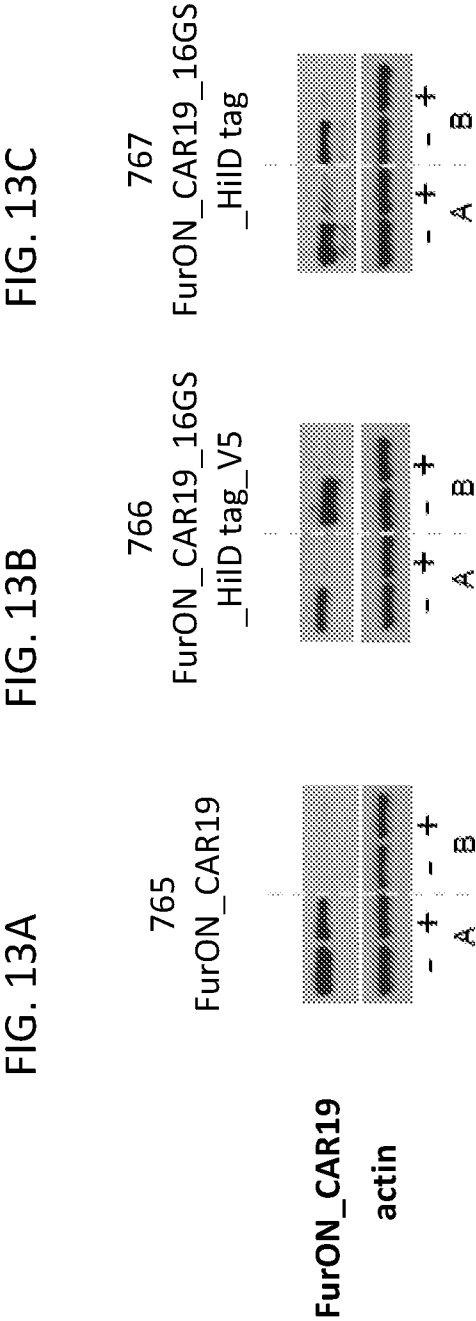




FIG. 14A

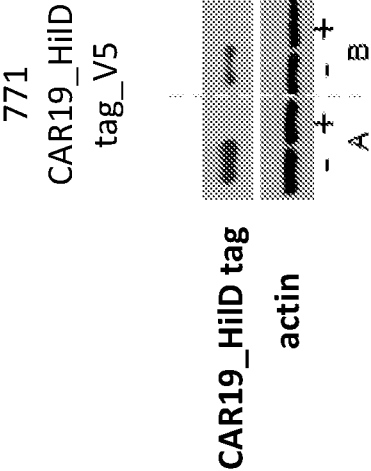


FIG. 14B

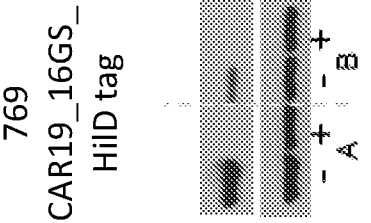


FIG. 14C

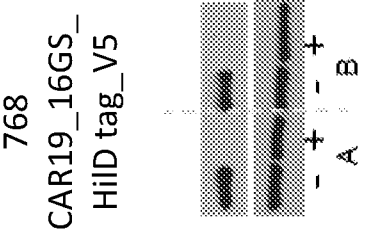


FIG. 14D

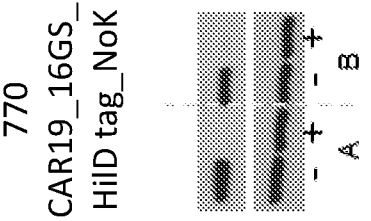


FIG. 15A

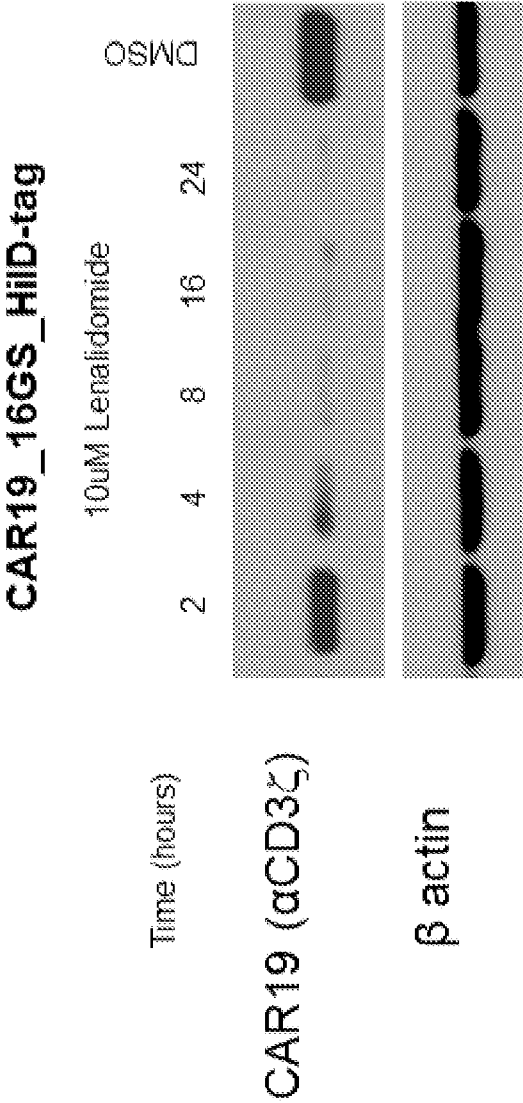


FIG. 15B

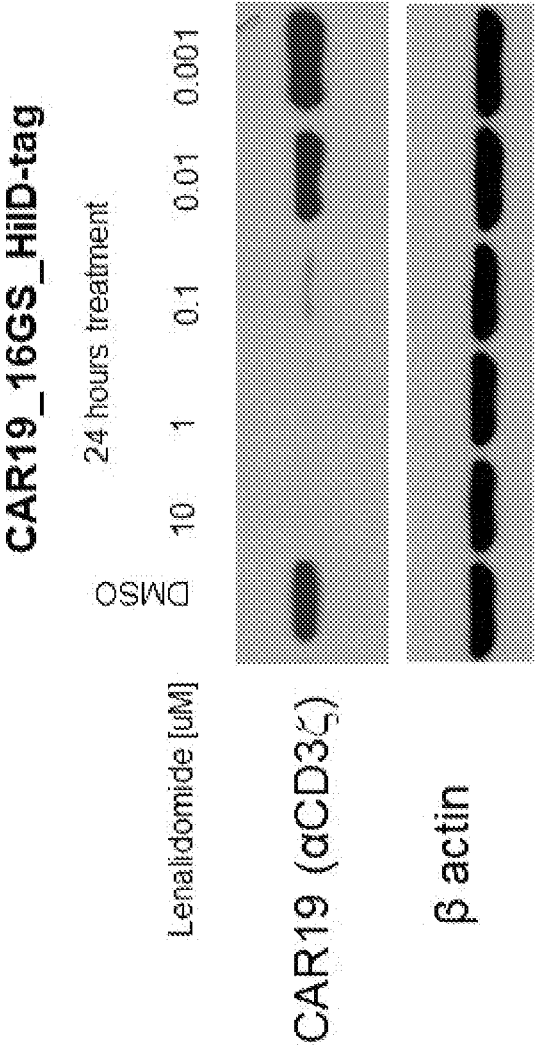


FIG. 16A

769 (CAR19\_16GS\_HiID tag)

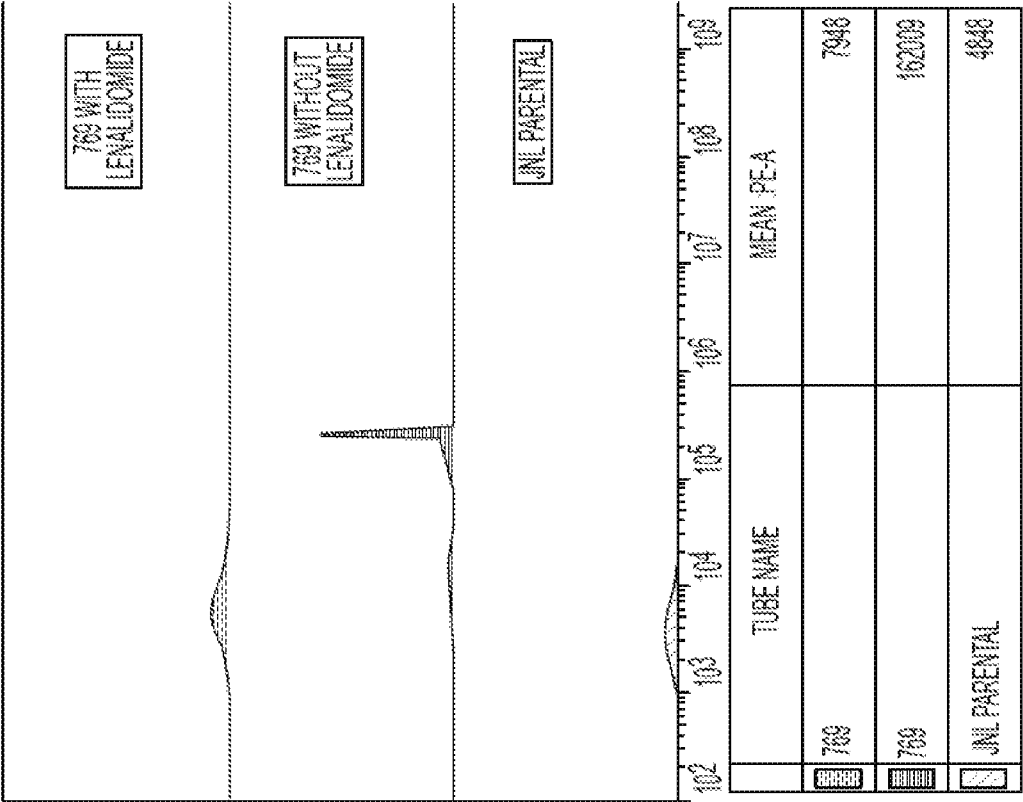


FIG. 16B

771 (CAR19\_HiID tag\_V5)

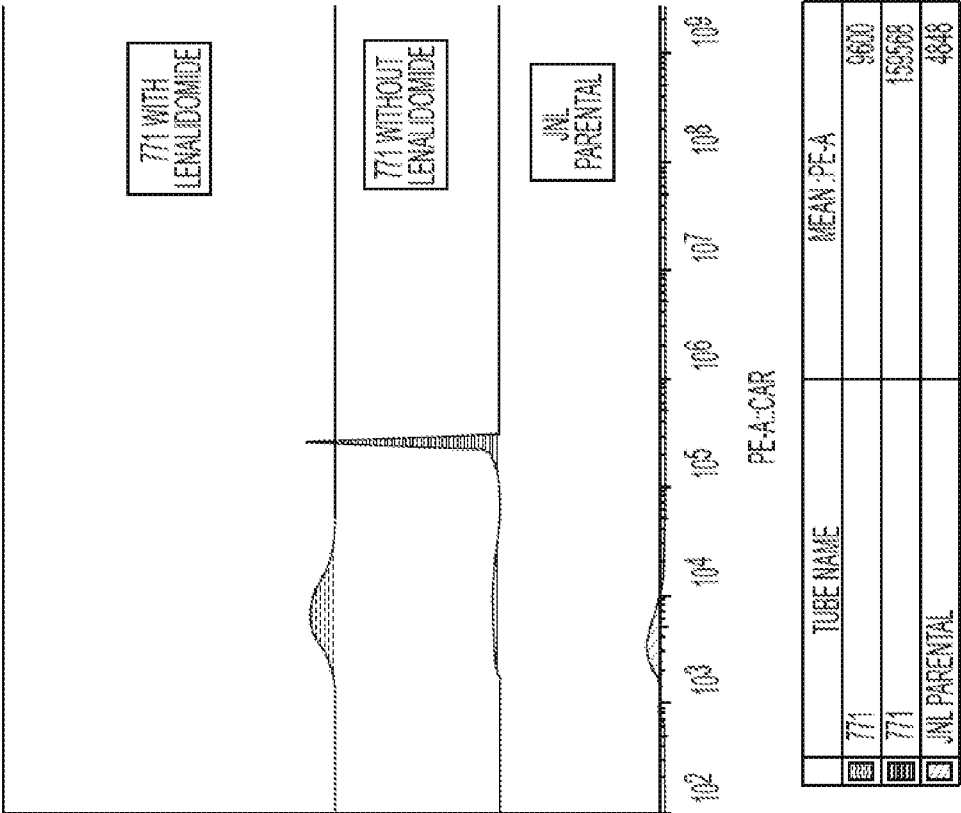


FIG. 16D

768 (CAR19\_16GS\_HiID tag\_V5)

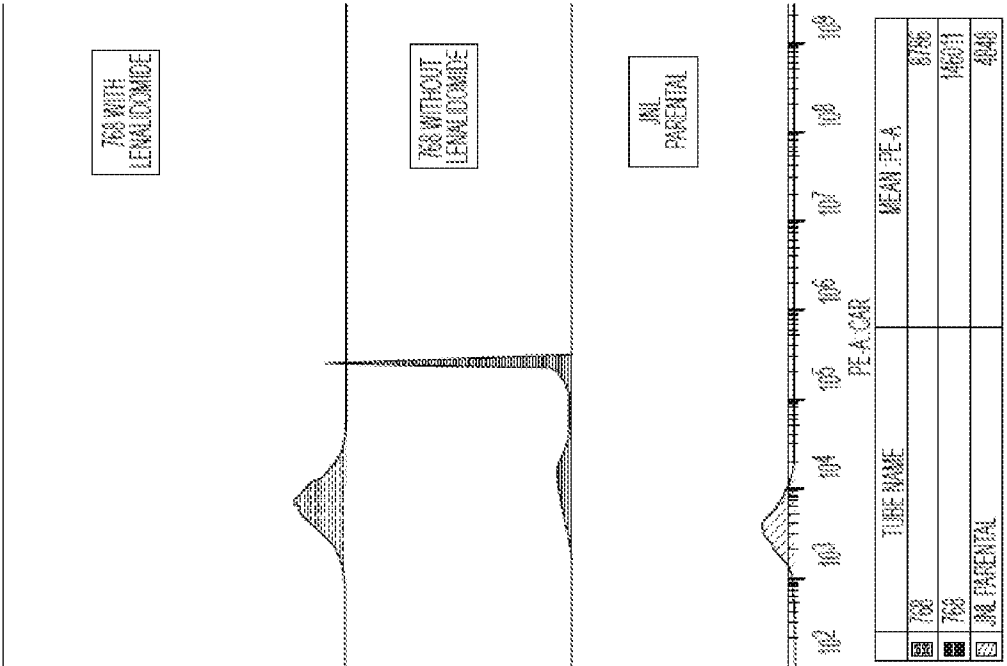


FIG. 16C

6761 (CAR19\_16KGS\_HiID tag\_V5)

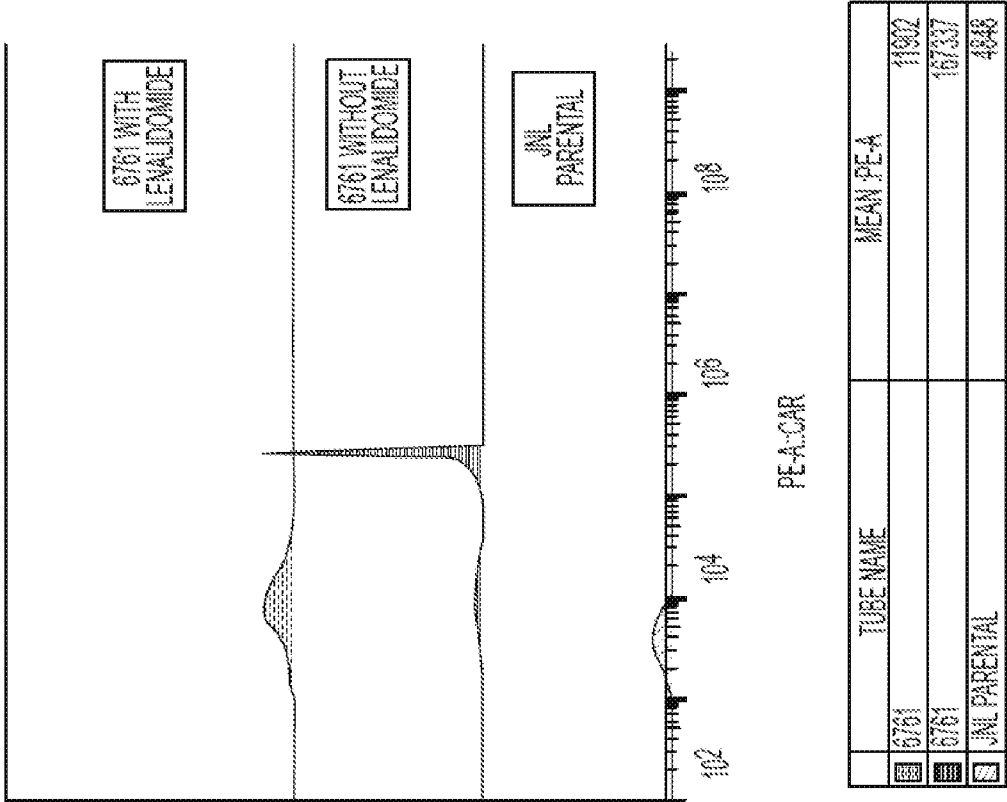


FIG. 16E

770 (CAR19\_16GS\_HiID tag\_NoK)

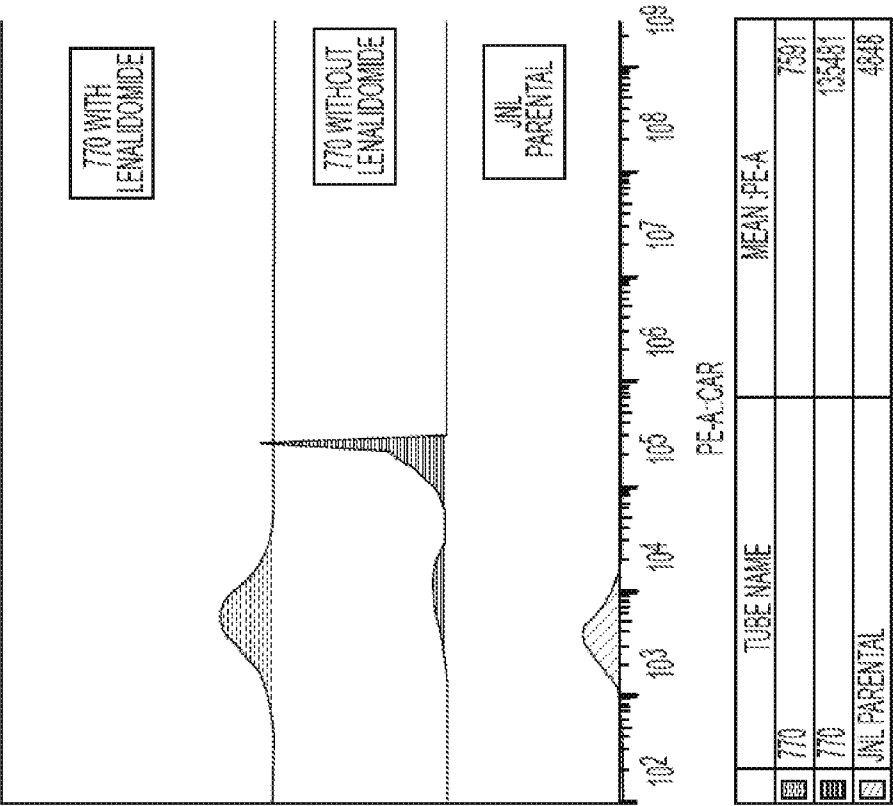


FIG. 16F

773 (HiID tag\_CAR19\_modSigPep)

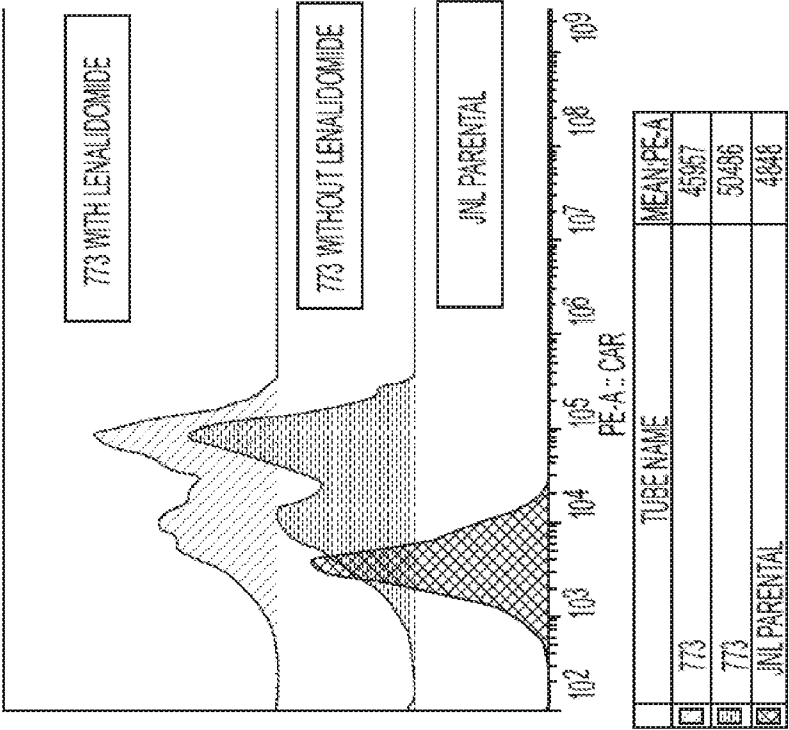


FIG. 16G

774 (HiID tag\_CAR19)

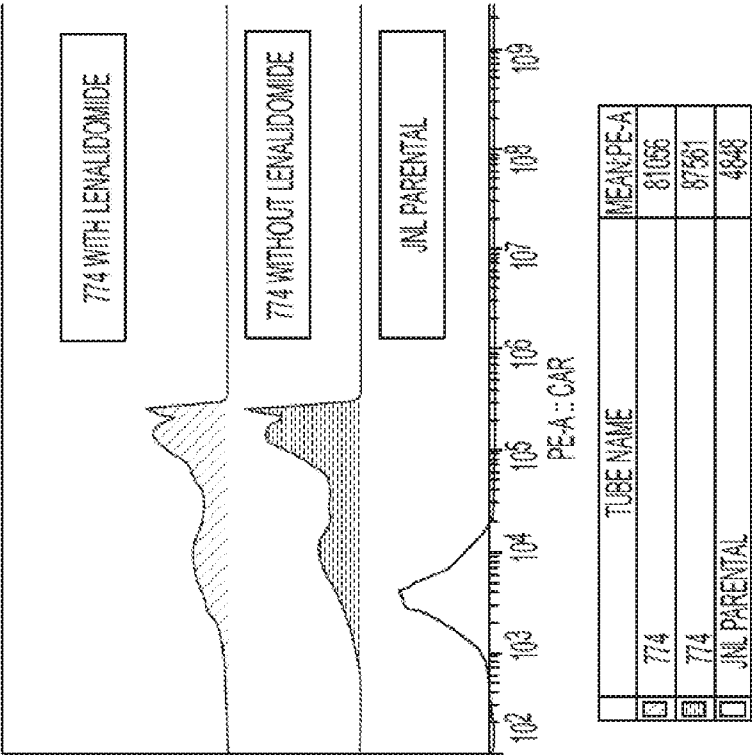


FIG. 17A

765 (FurON\_CAR19)

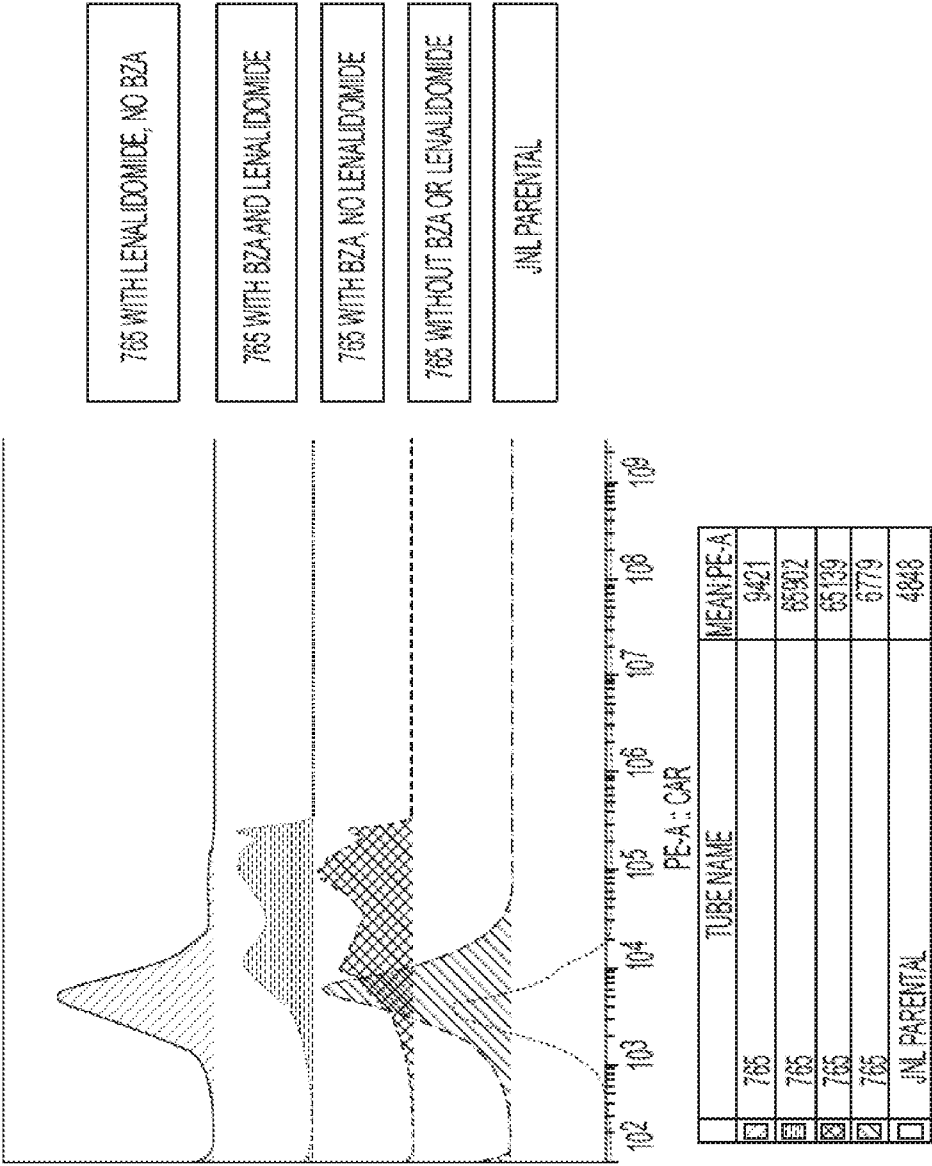


FIG. 17B

767 (FurON\_CAR19\_16GS\_HiID tag)

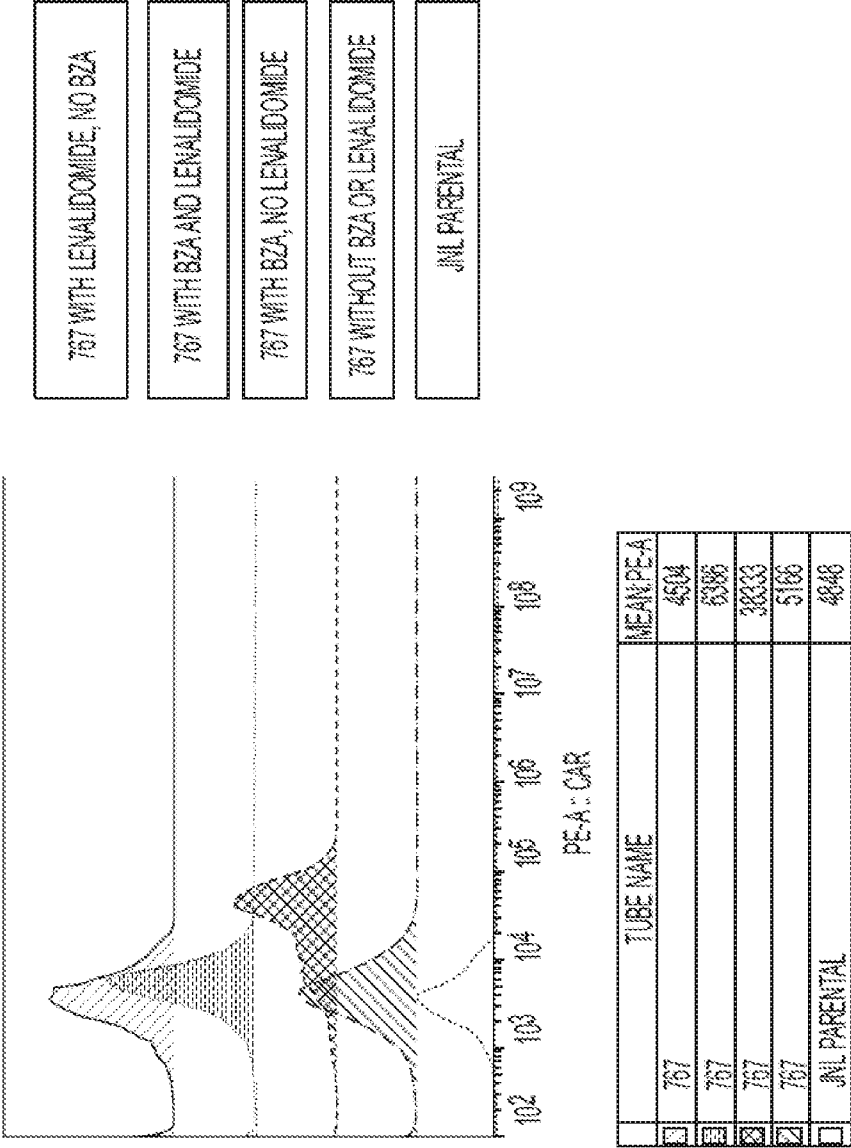




FIG. 17C

766 (FurON\_CAR19\_16GS\_HiID tag\_V5)

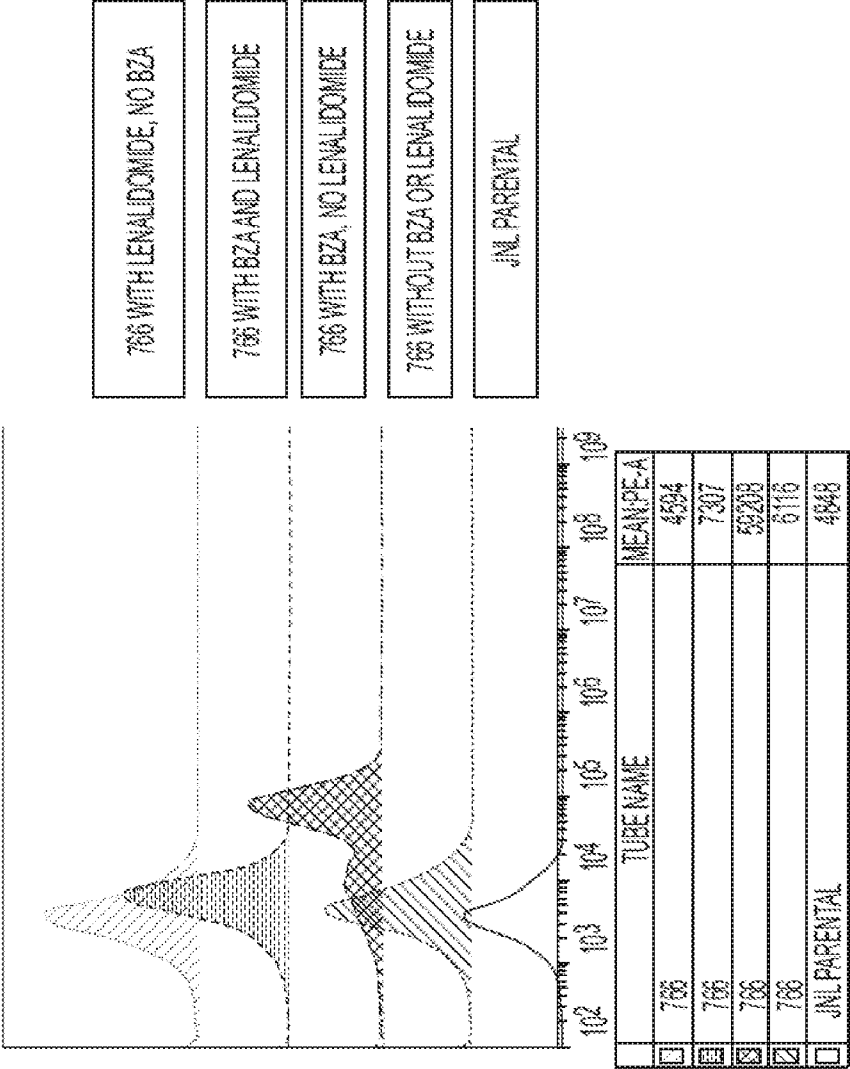


FIG. 18A

769 (CAR19\_16GS\_HiID tag)  
4-hour treatment

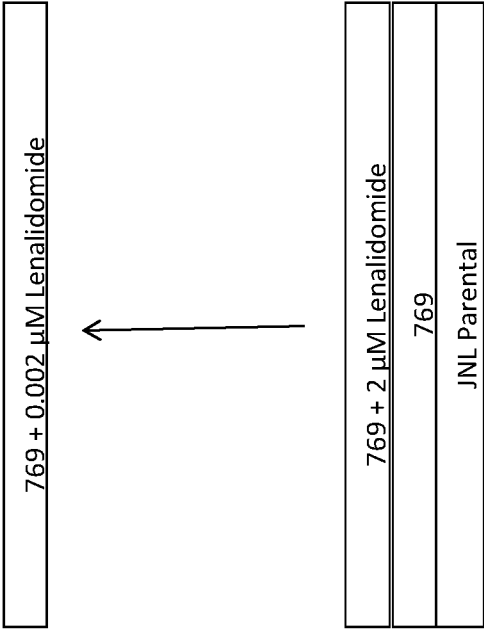
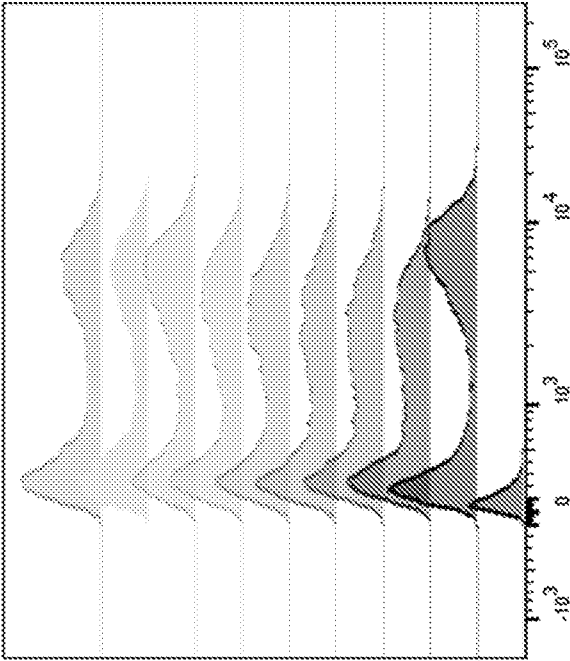


FIG. 18B

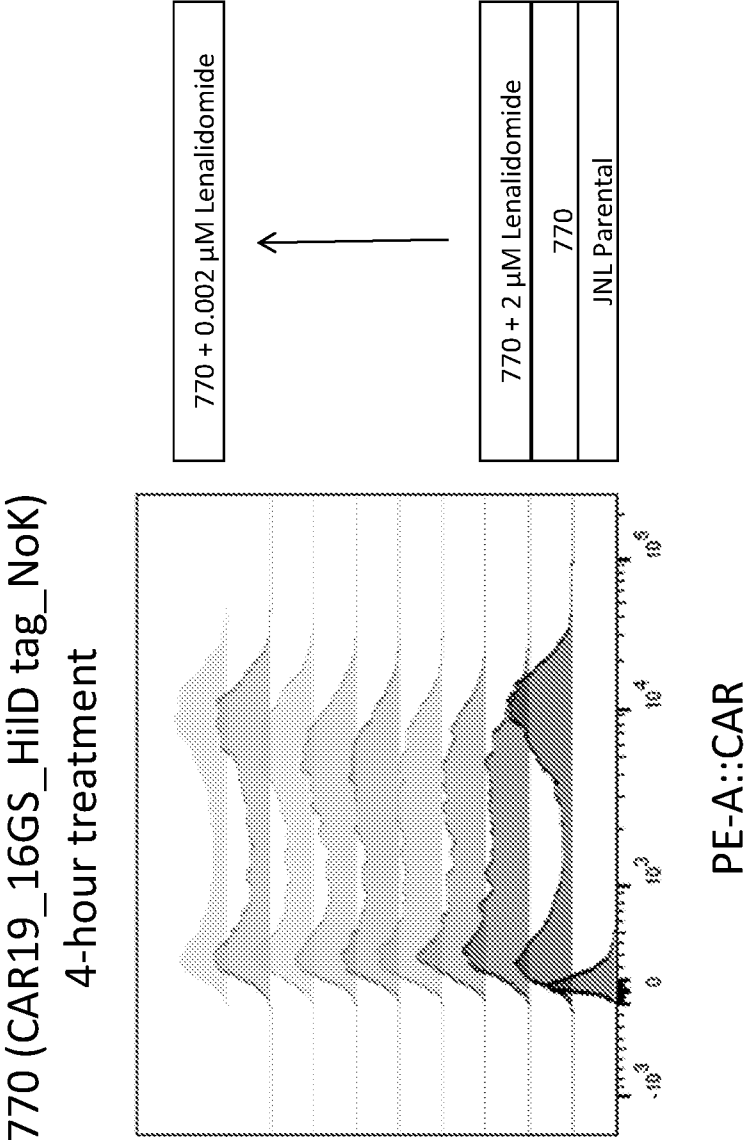


FIG. 18C

769 (CAR19\_16GS\_HiID tag)  
20-hour treatment

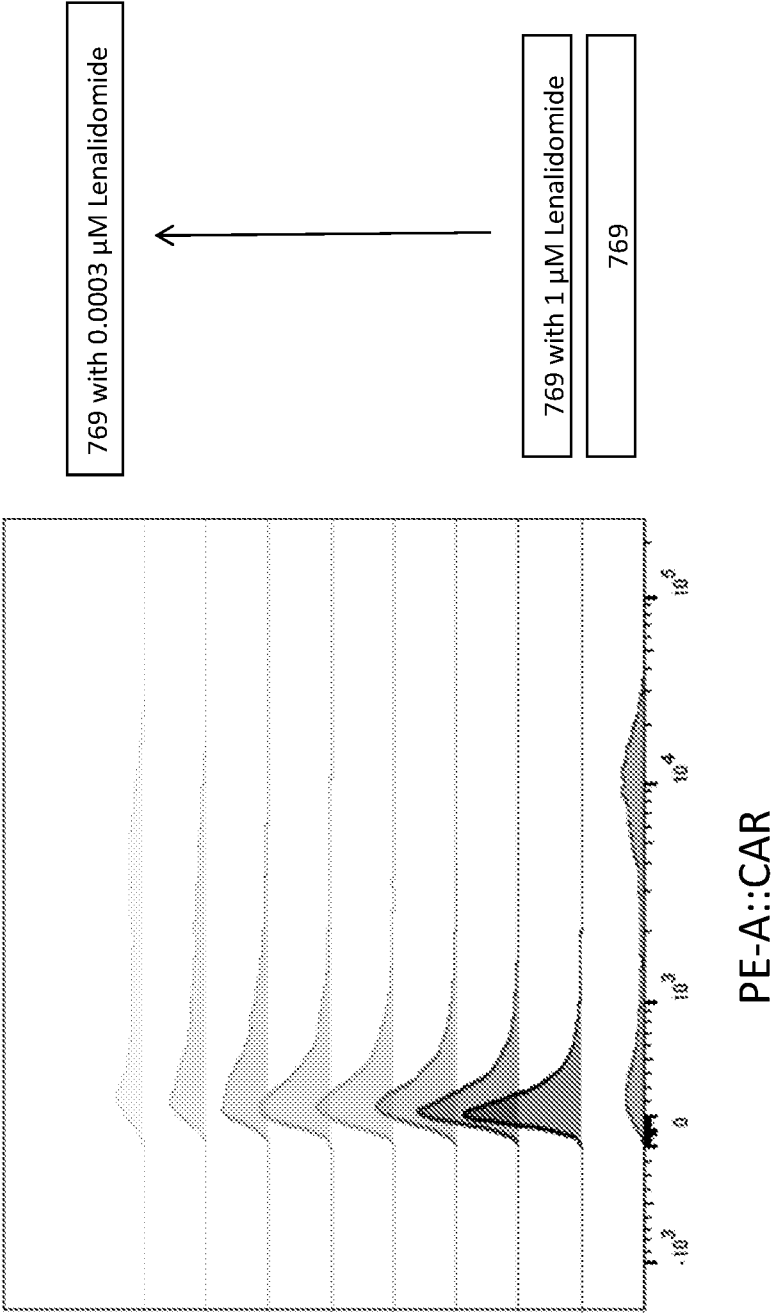


FIG. 18D

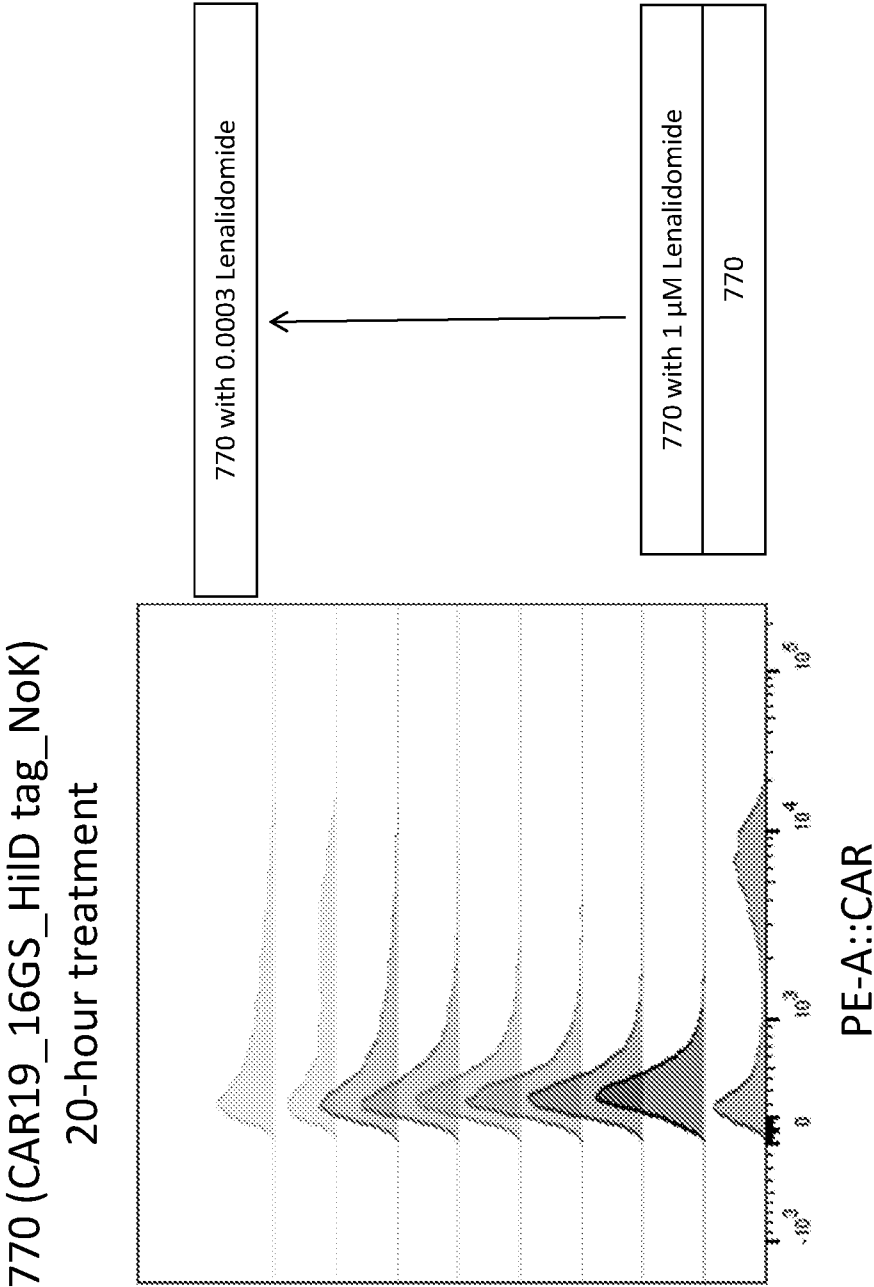


FIG. 18E

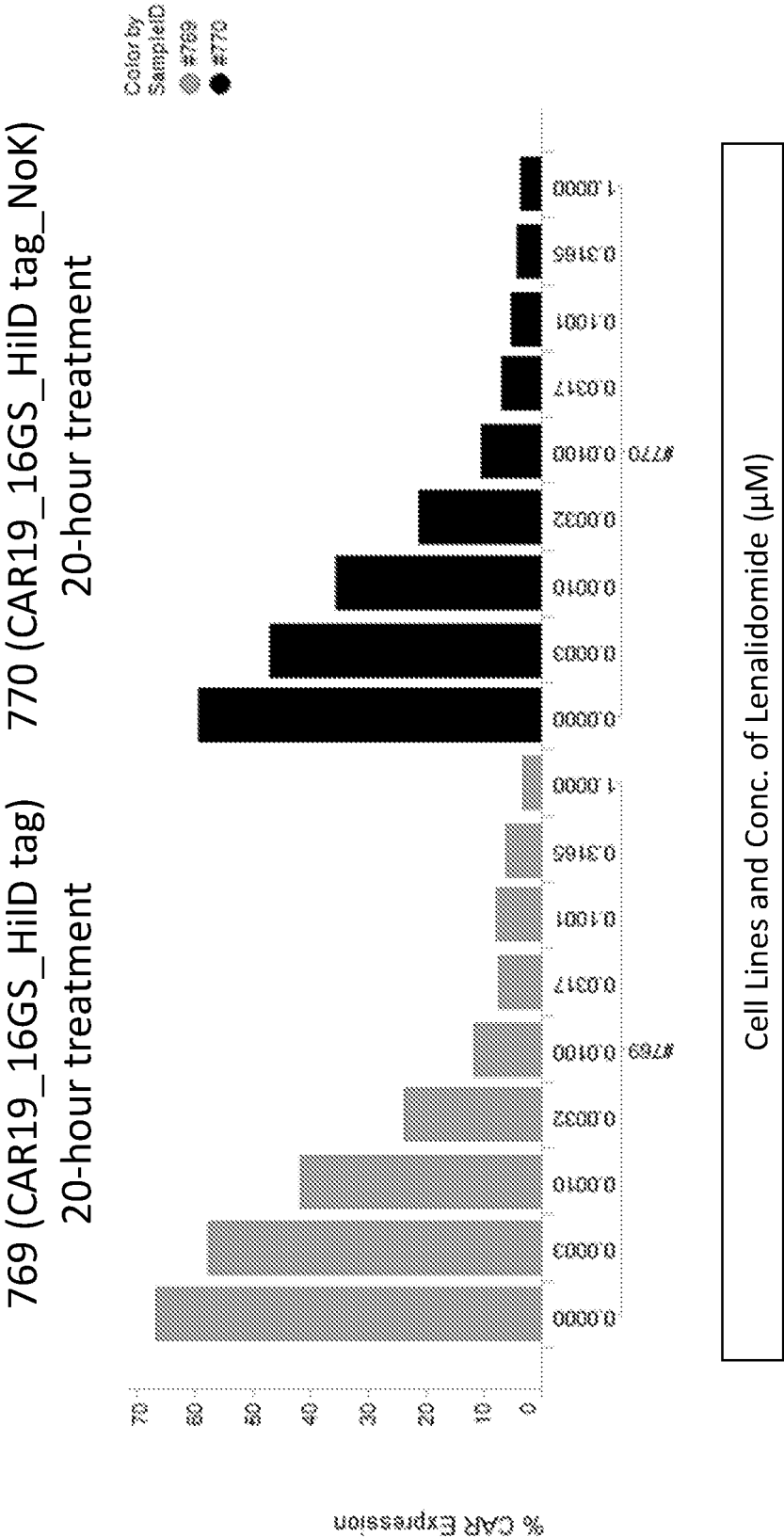


FIG. 18F

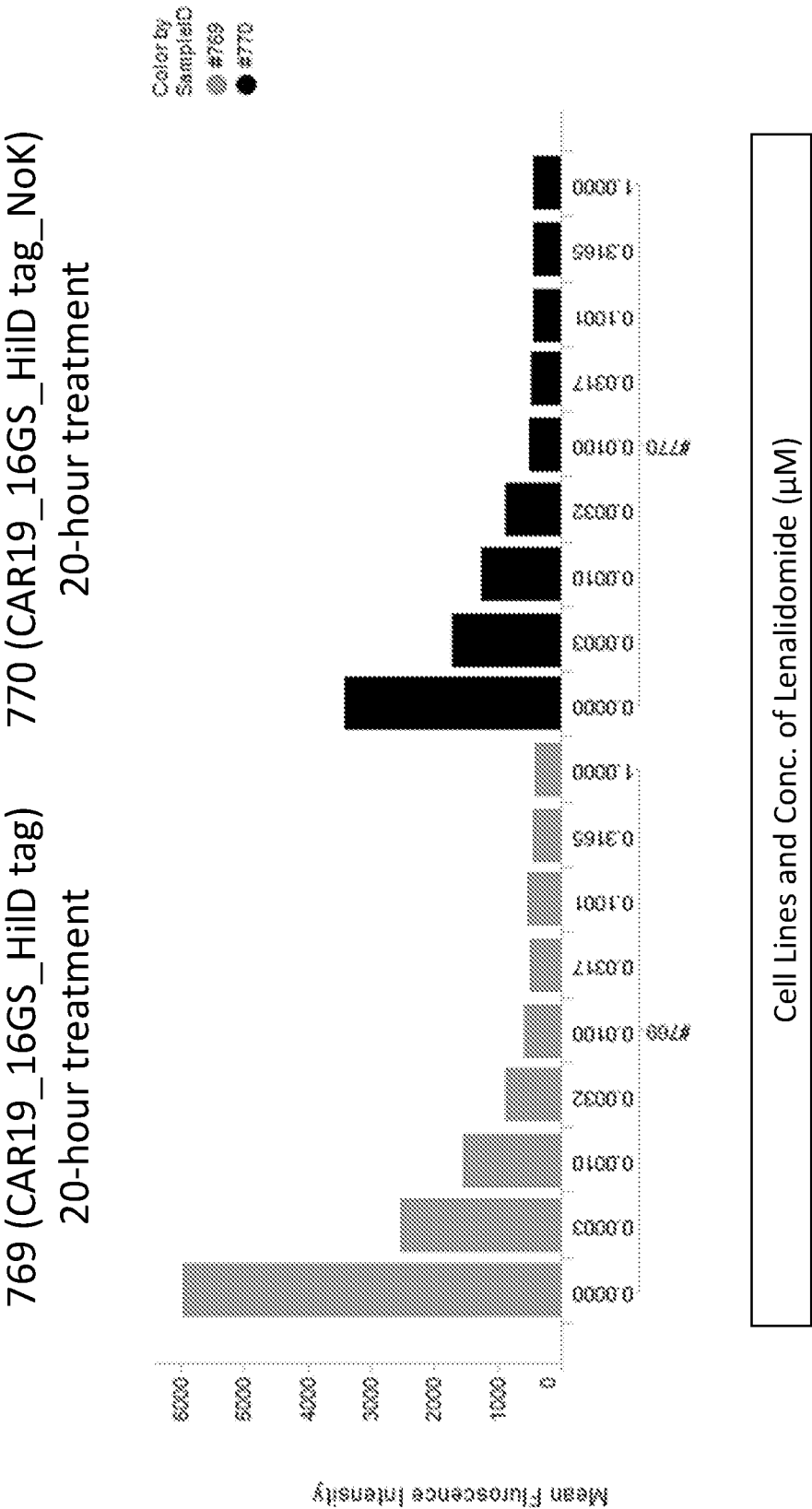


FIG. 19A

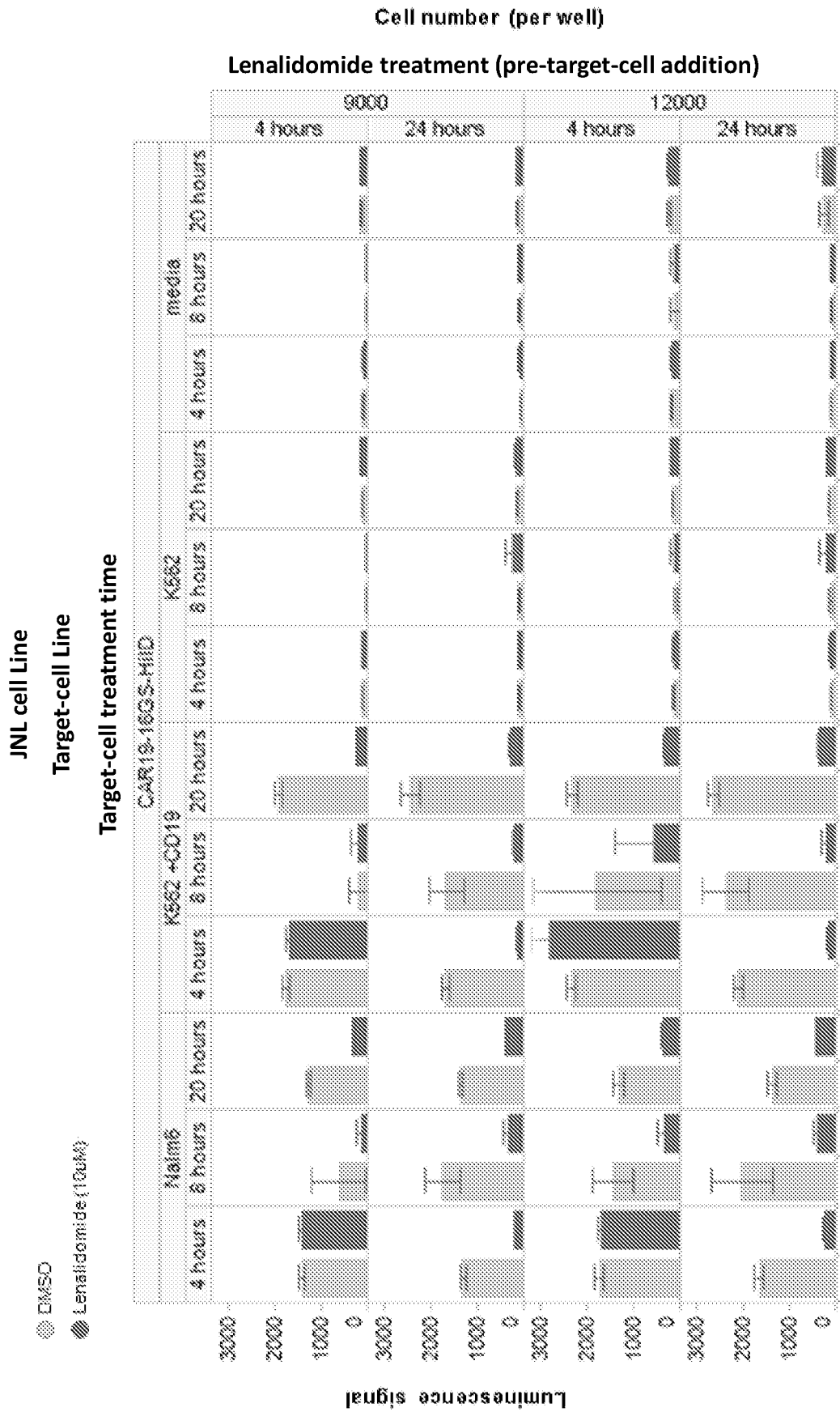




FIG. 19B

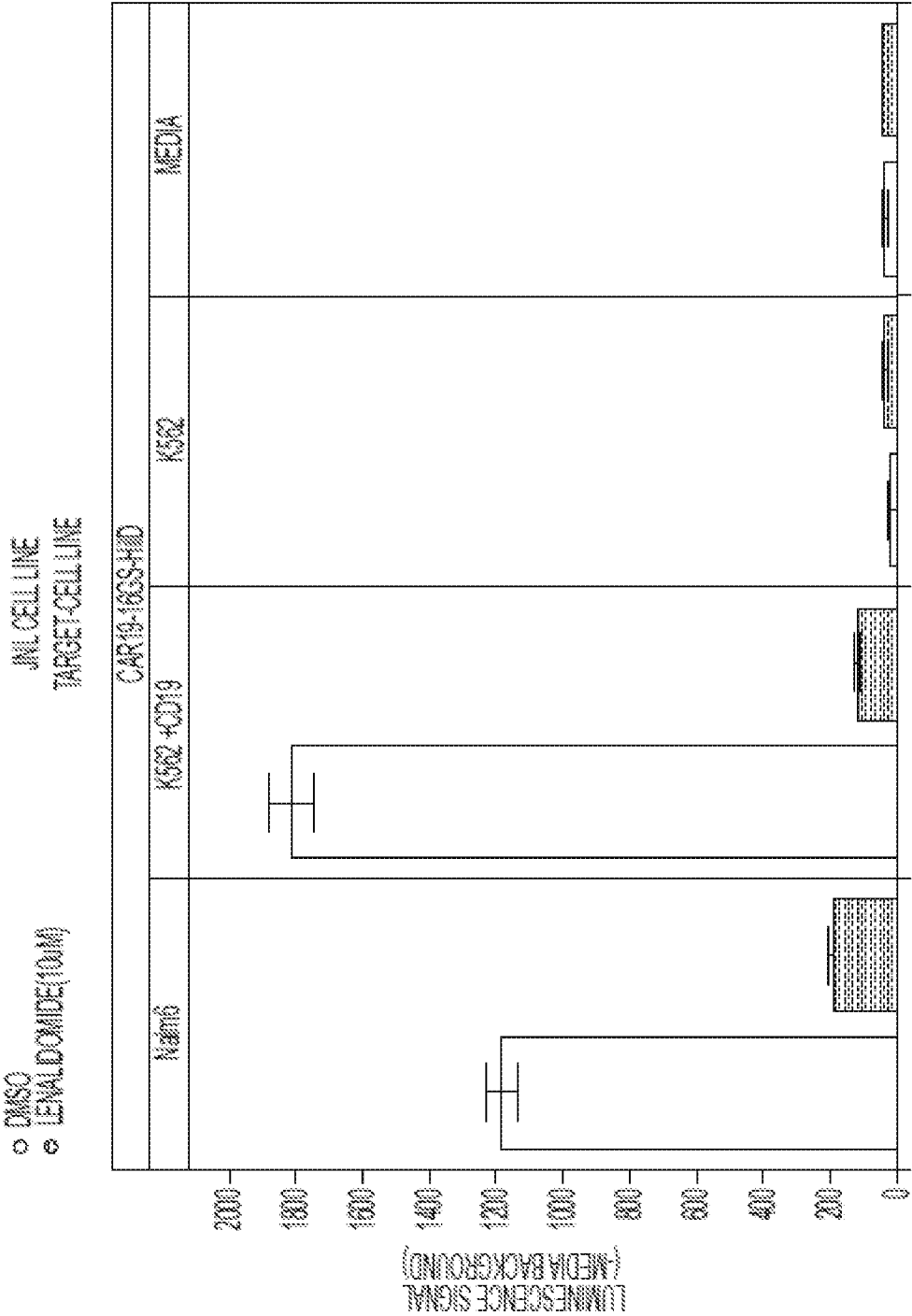


FIG. 20A

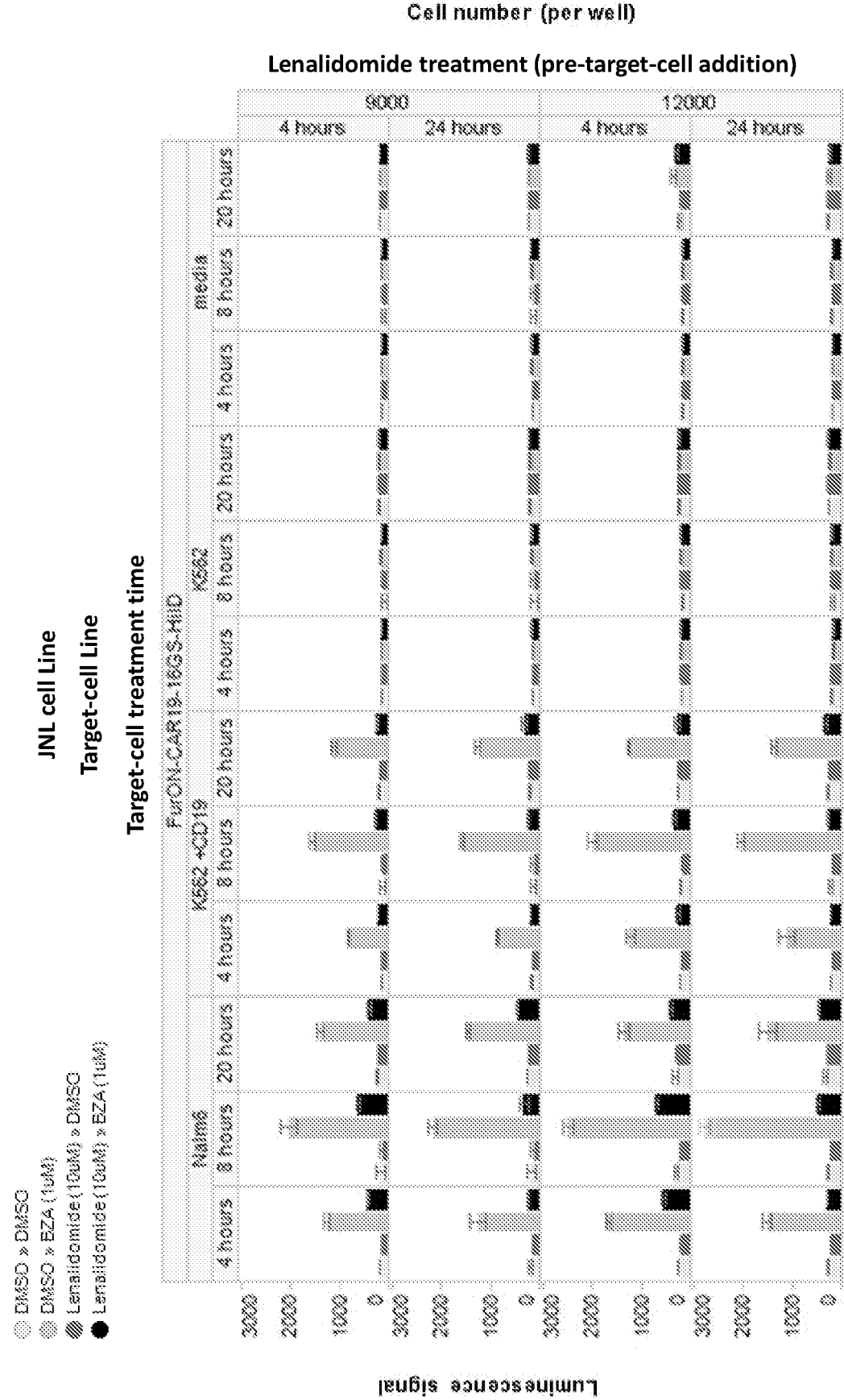


FIG. 20B

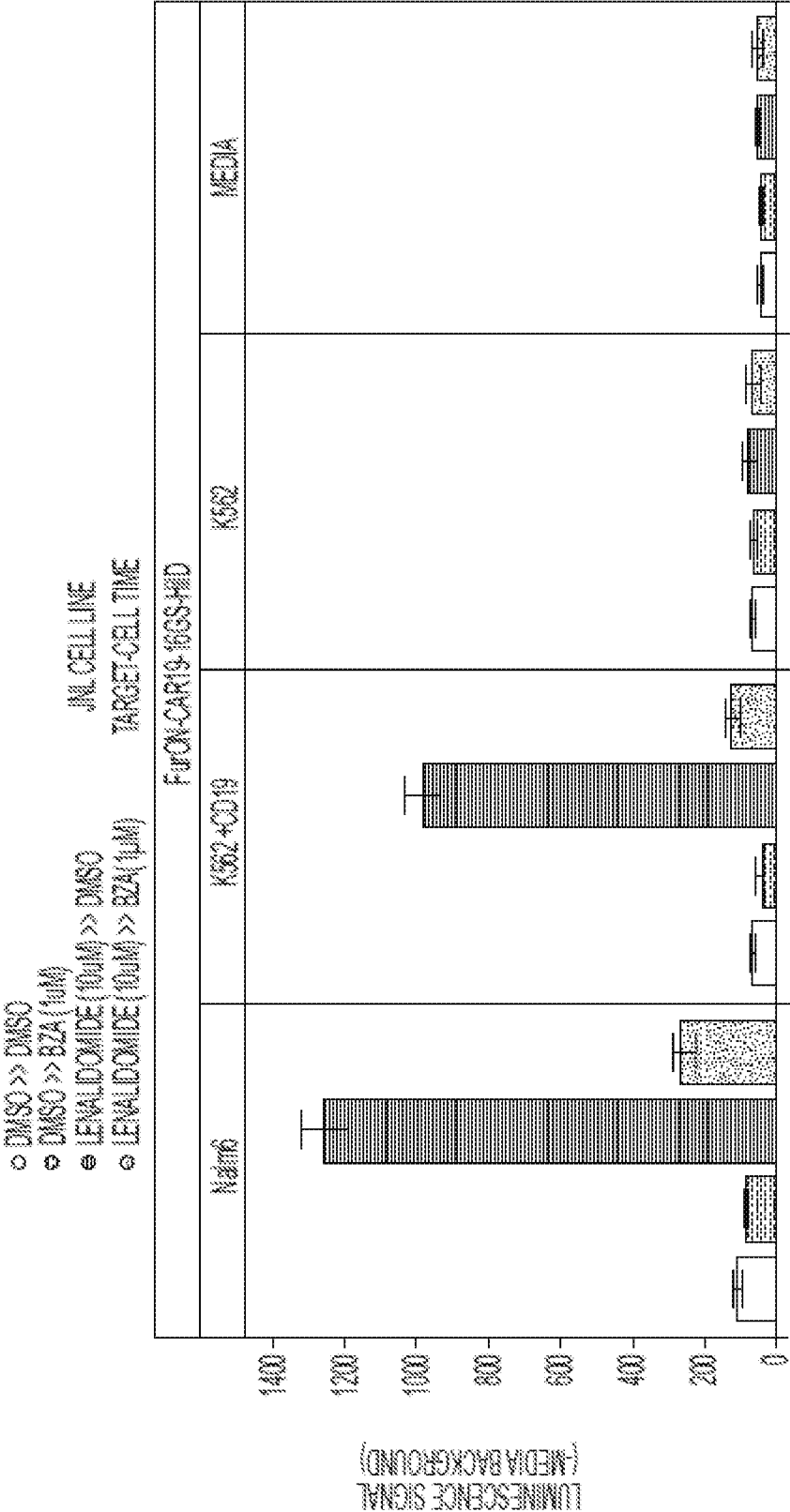


FIG. 21A

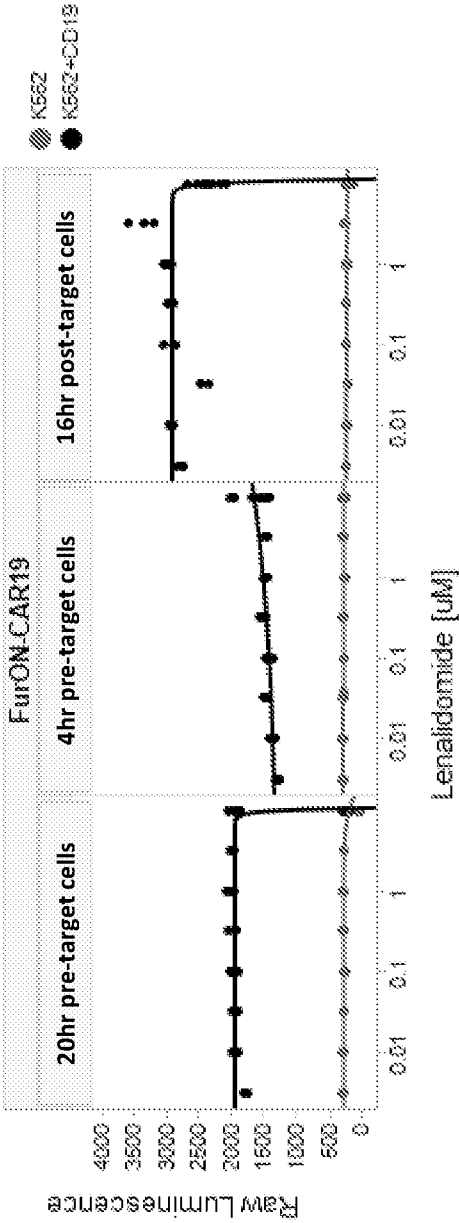


FIG. 21B

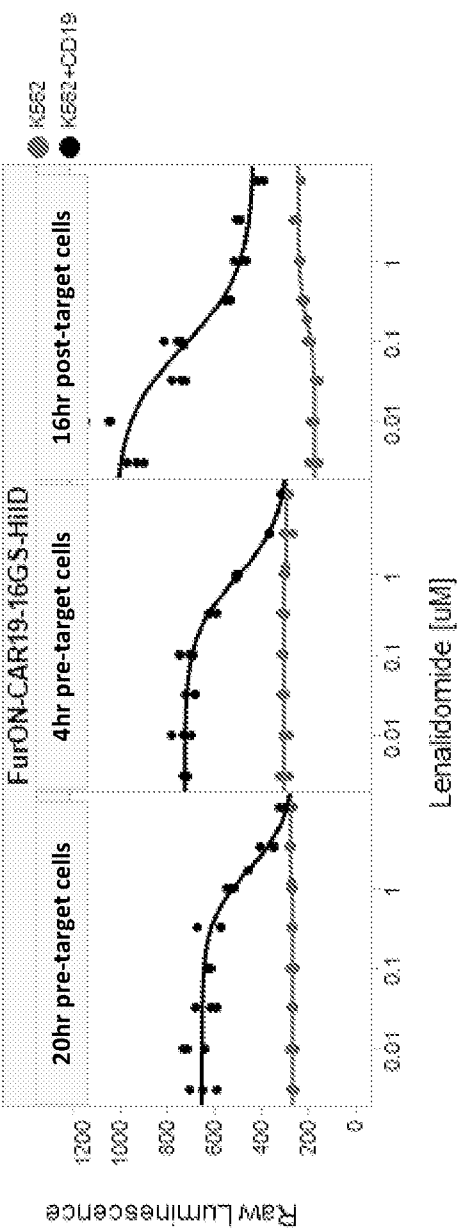


FIG. 21C

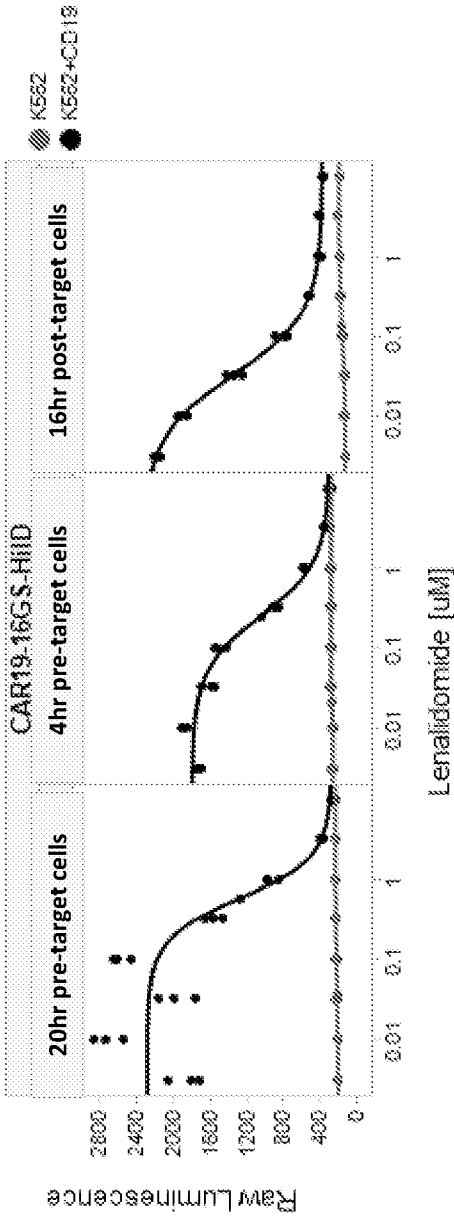


FIG. 21D

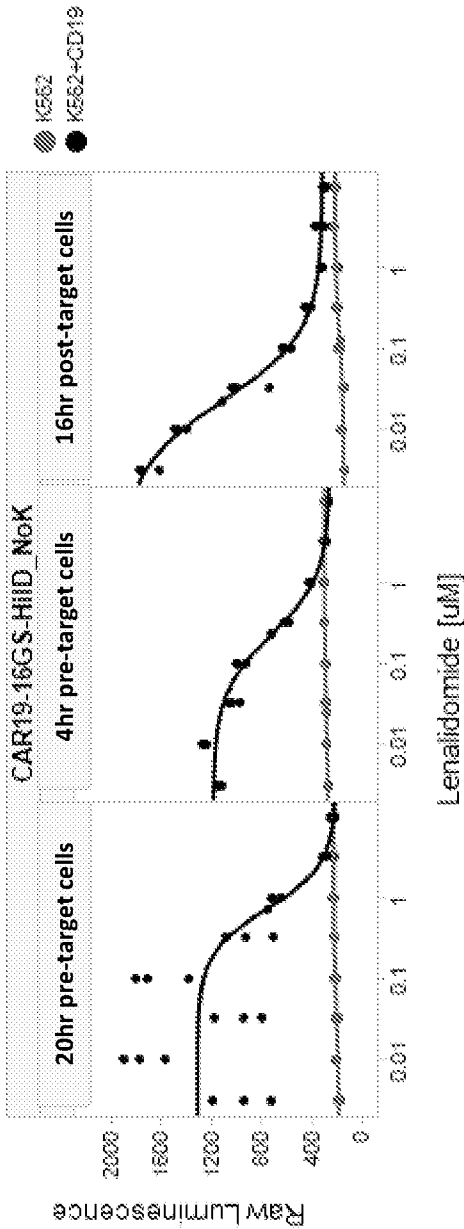


FIG. 22A

FIG. 22B

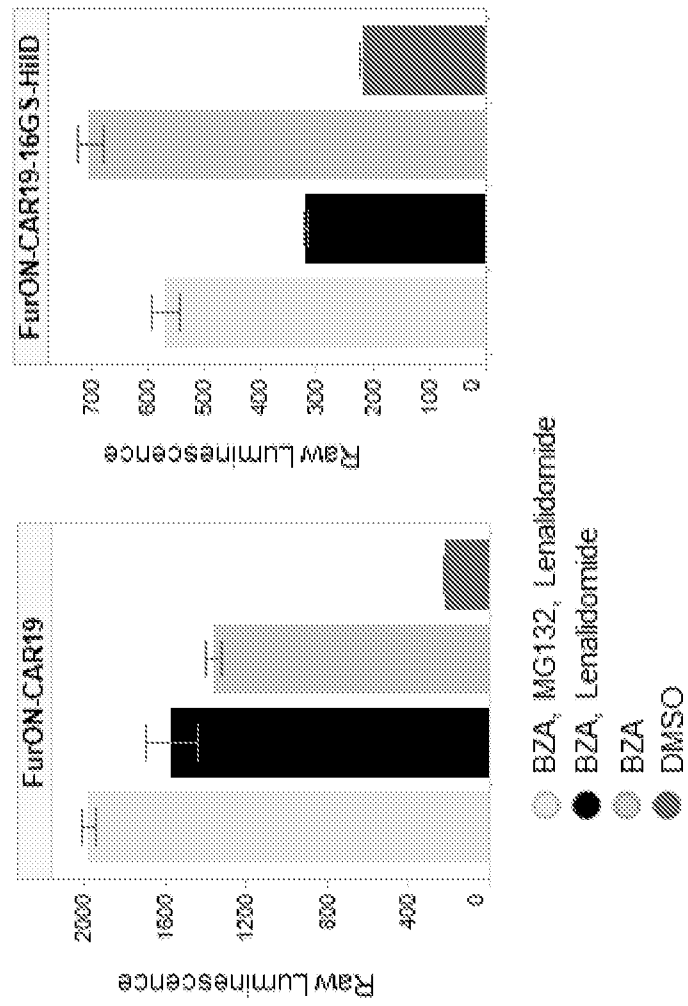


FIG. 22C

FIG. 22D

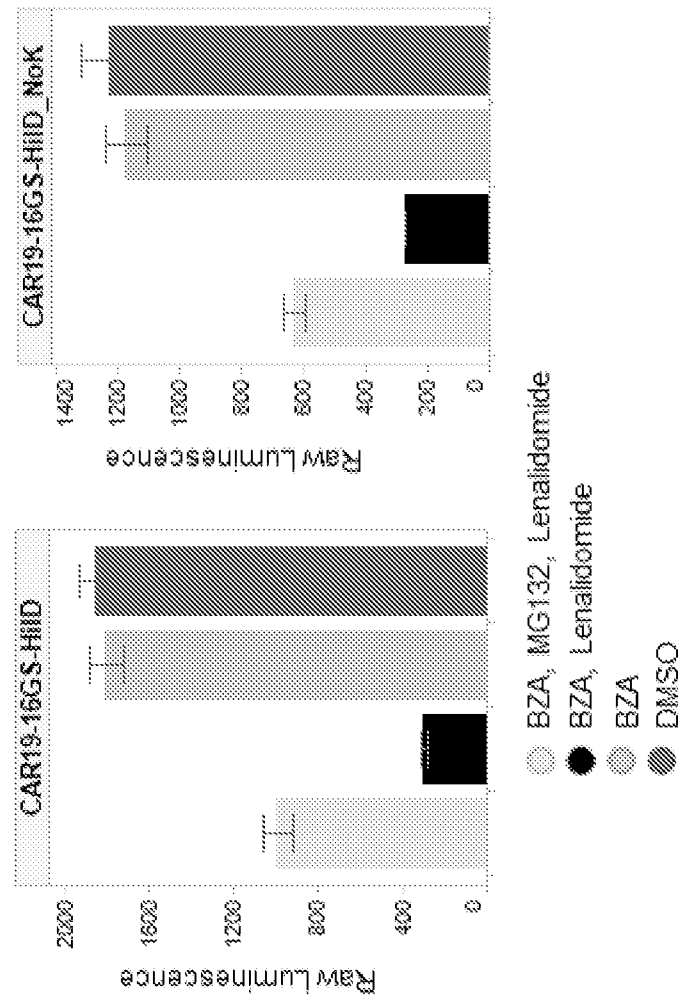
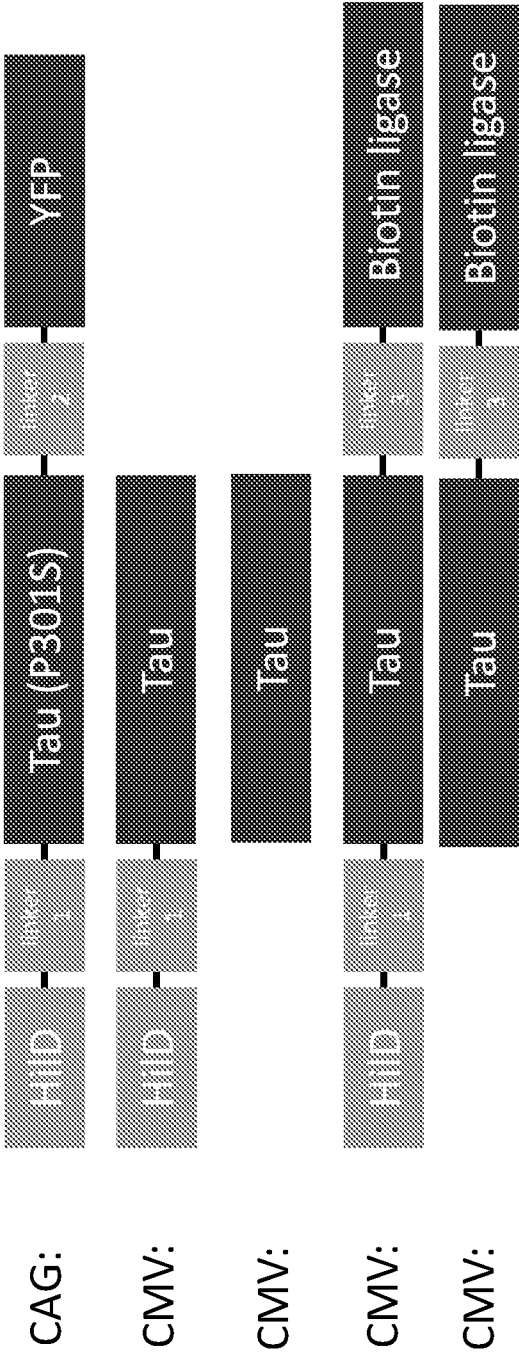


FIG. 23

Promoter



Tau isoform used: 0N4R

Linker 1: GGGGSGGGGTGGGGSG (SEQ ID NO: 28)

Linker 2: SGSETPGTSESATPES (SEQ ID NO: 37)

Linker 3: *Either*: 16xGS: GGGGSGGGGTGGGGSG (SEQ ID NO: 28)

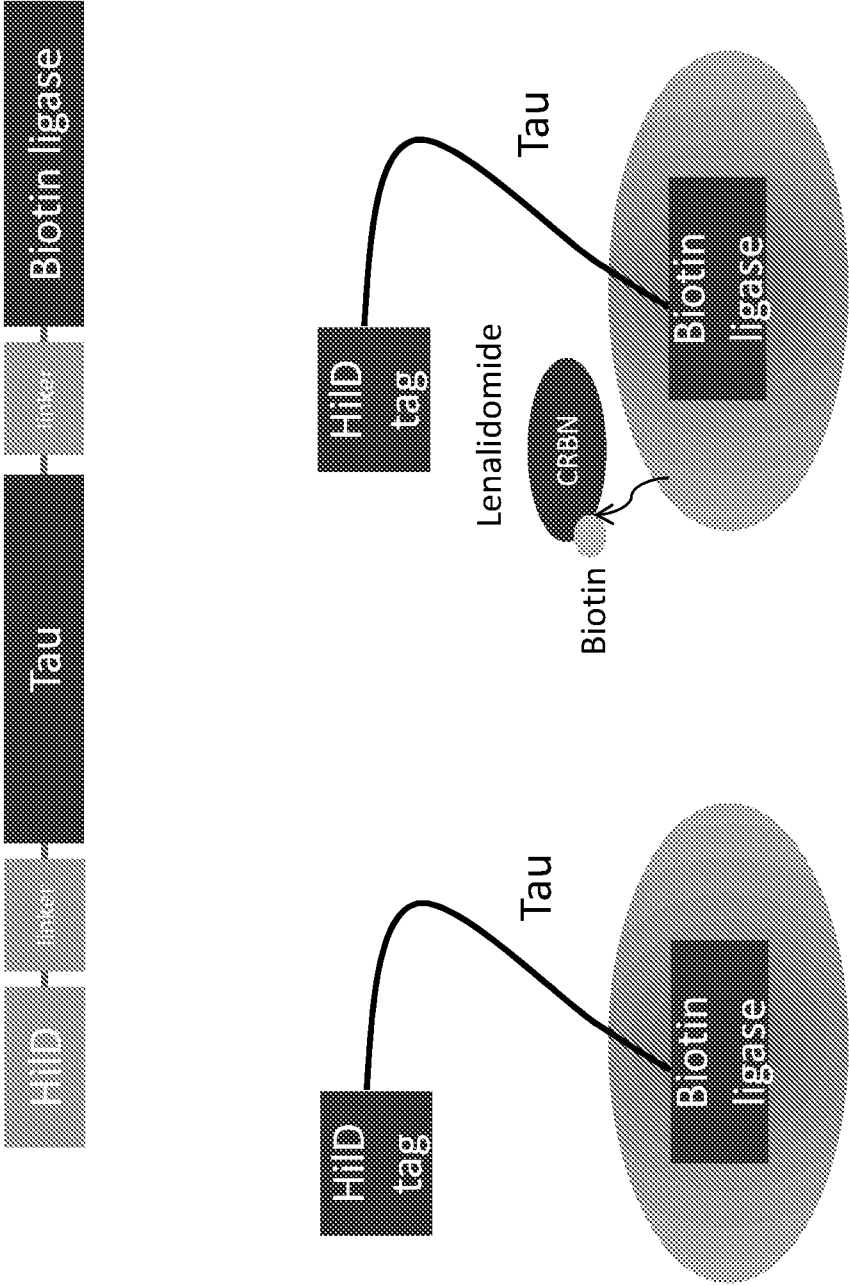
33xGS: GGGGSGGGGTGGGGSGGGGGTGGGGSGGGGGTG (SEQ ID NO: 38)

“XTEN”: SGSETPGTSESATPES (SEQ ID NO: 37)

16xGS – V5: GGGGSGGGGTGGGGSGGKPIPNPLLGLASTGSG (SEQ ID NO: 39)



FIG. 24A



Biotin ligase reference: Kim et al.,  
“An improved smaller biotin ligase for BiID proximity labeling,” Mol Biol Cell, 2016

FIG. 24B

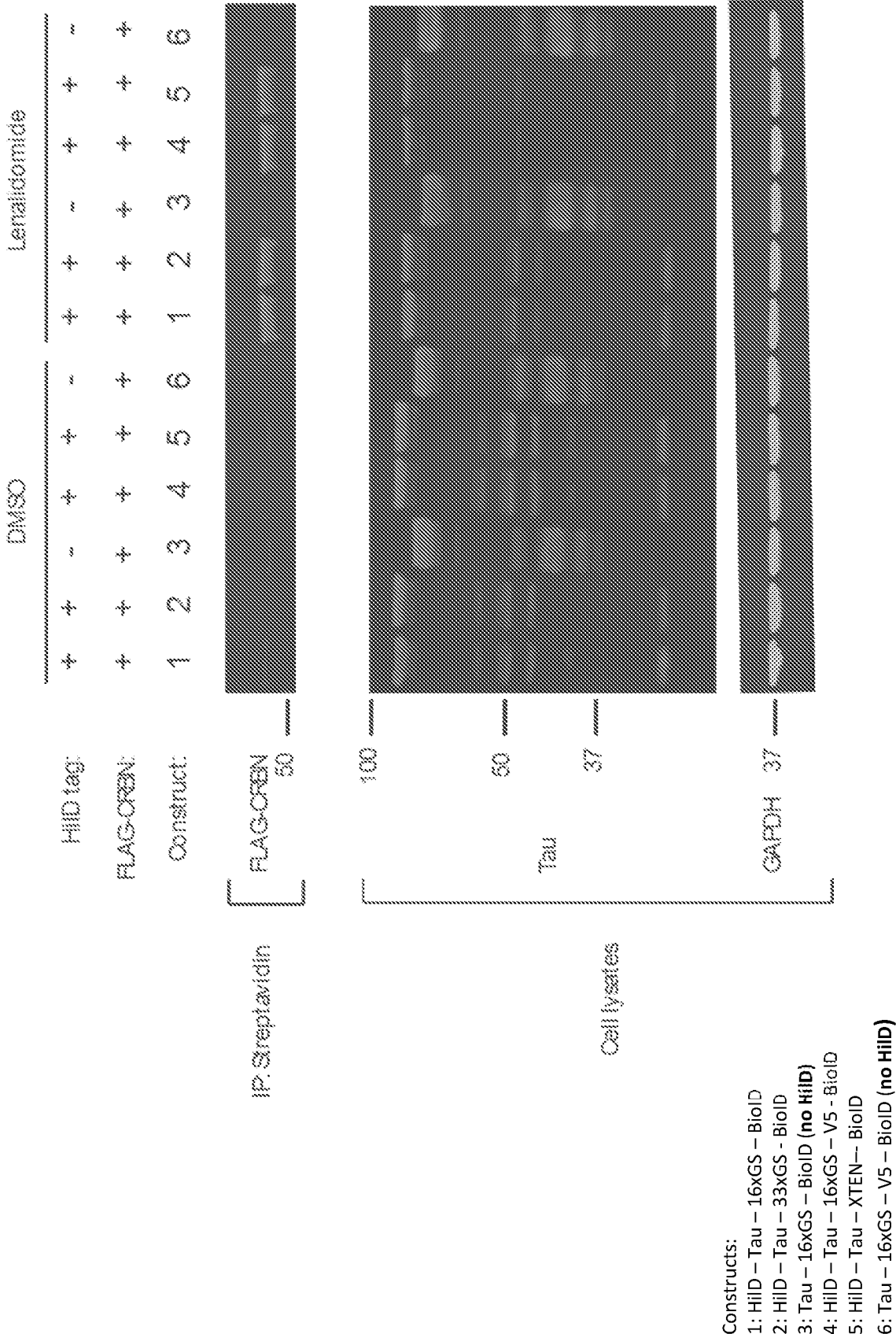
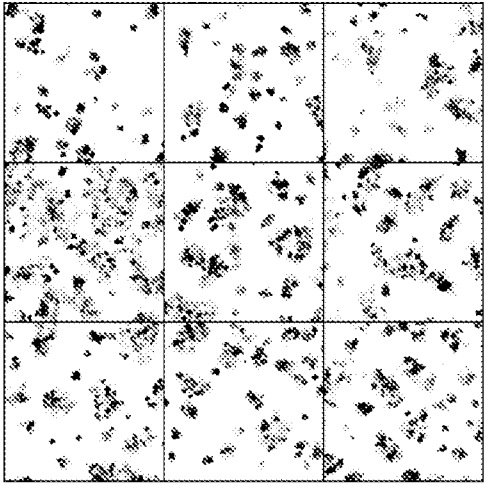
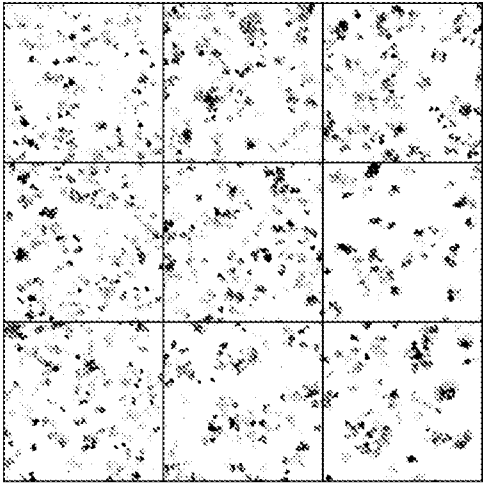


FIG. 25A

18 hr treatment, HEK293 cells  
Tau-YFP signal.  
Showing 9 fields of view each condition  
Triplicate wells/condition



DMSO



1 μM  
lenalidomide

FIG. 25B

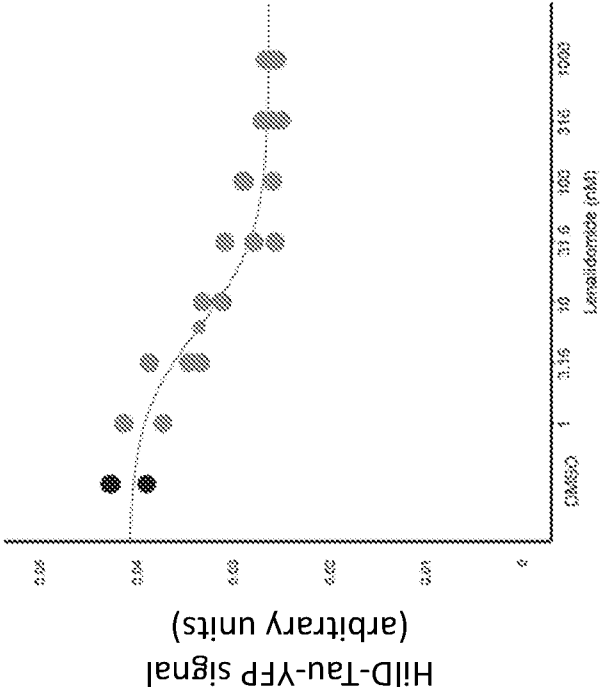
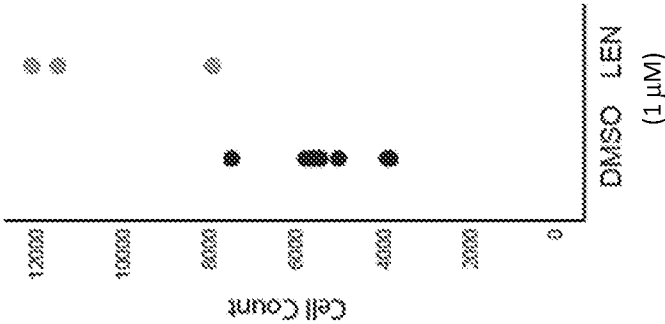


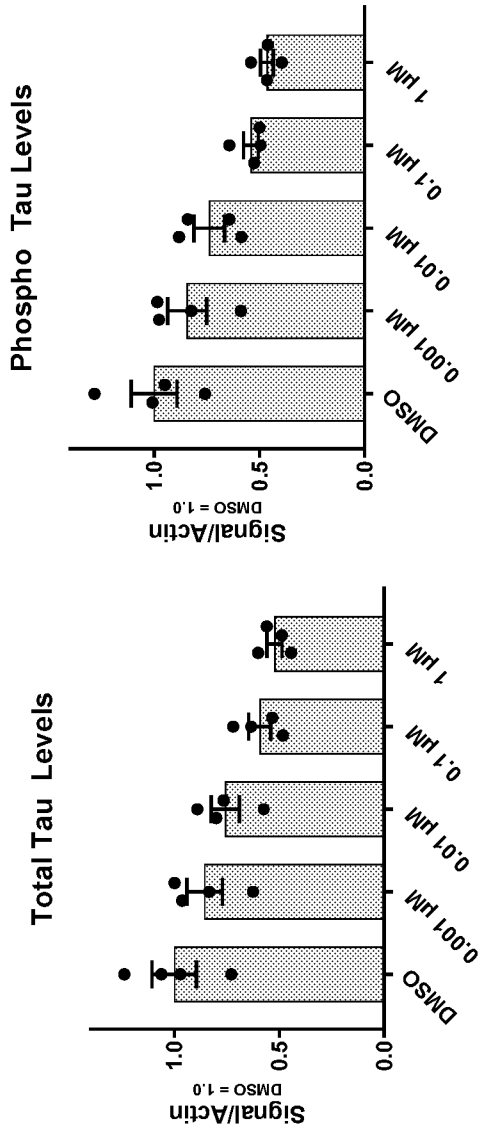
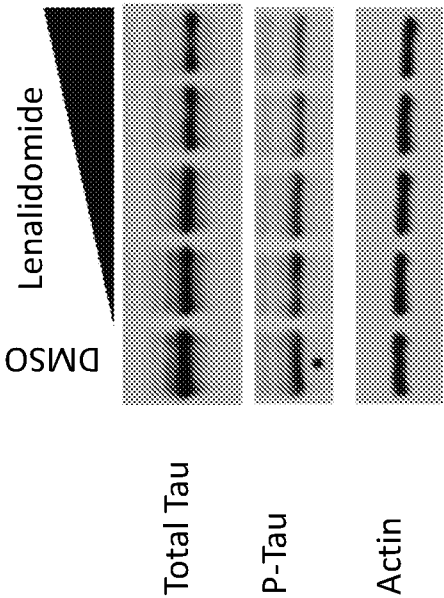
FIG. 25C



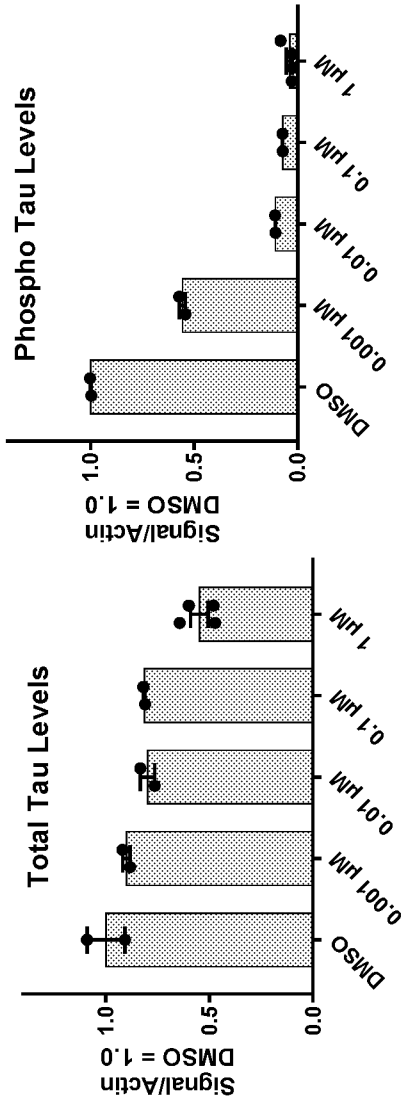
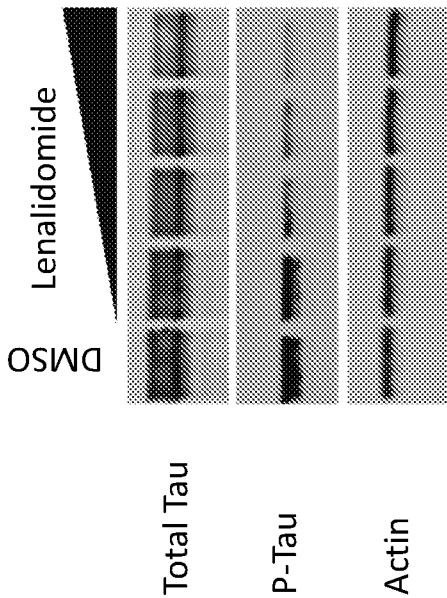
lenalidomide dosed @ +24 h transfection

18 hr lenalidomide treatment, HEK293 cells

1X DNA Transfection



0.1X DNA Transfection



1X: lenalidomide dosed @ + 4h transfection  
0.1X: lenalidomide dosed @ +24 h transfection

FIG. 26A

FIG. 26B

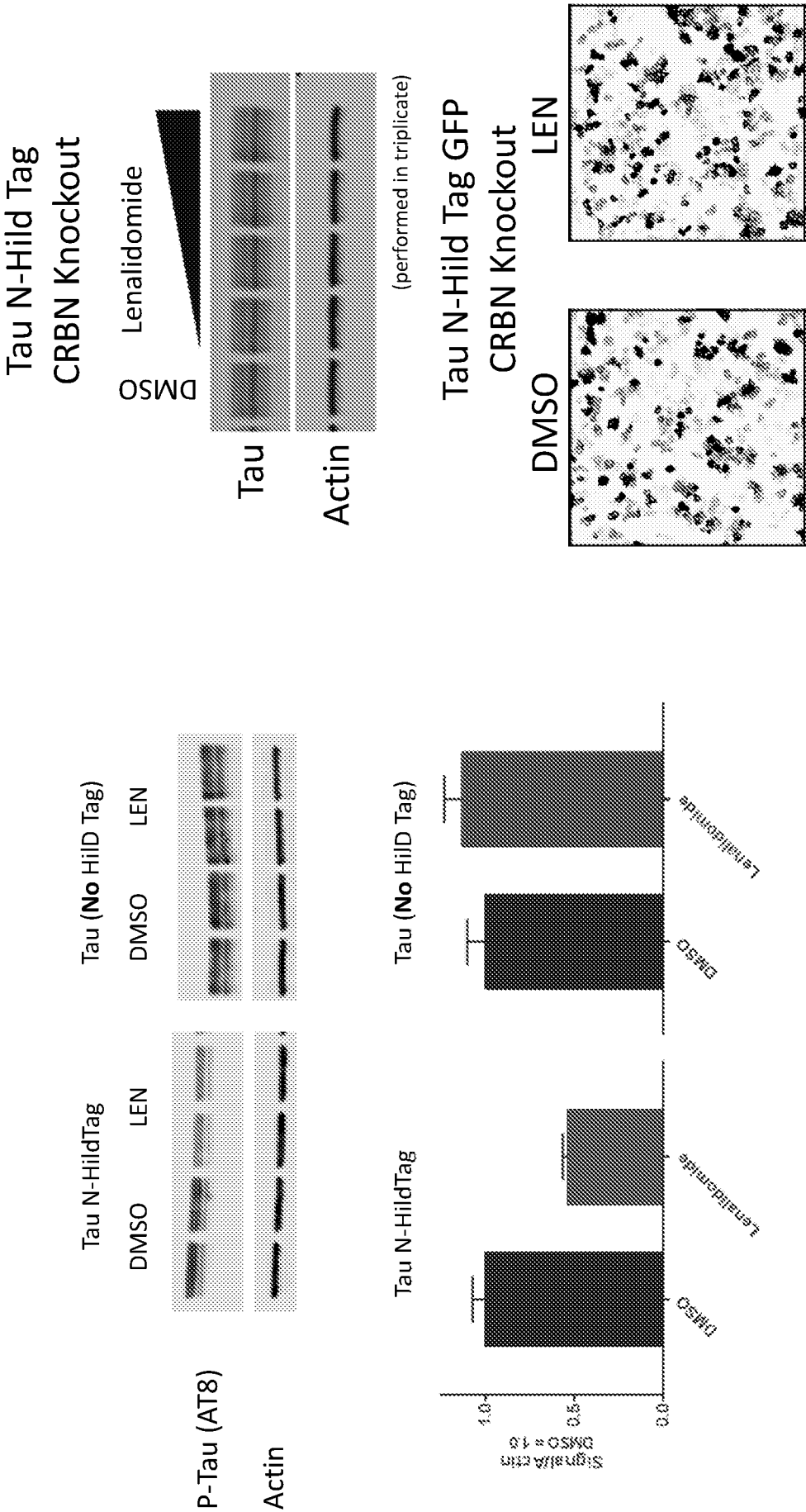


FIG. 26C

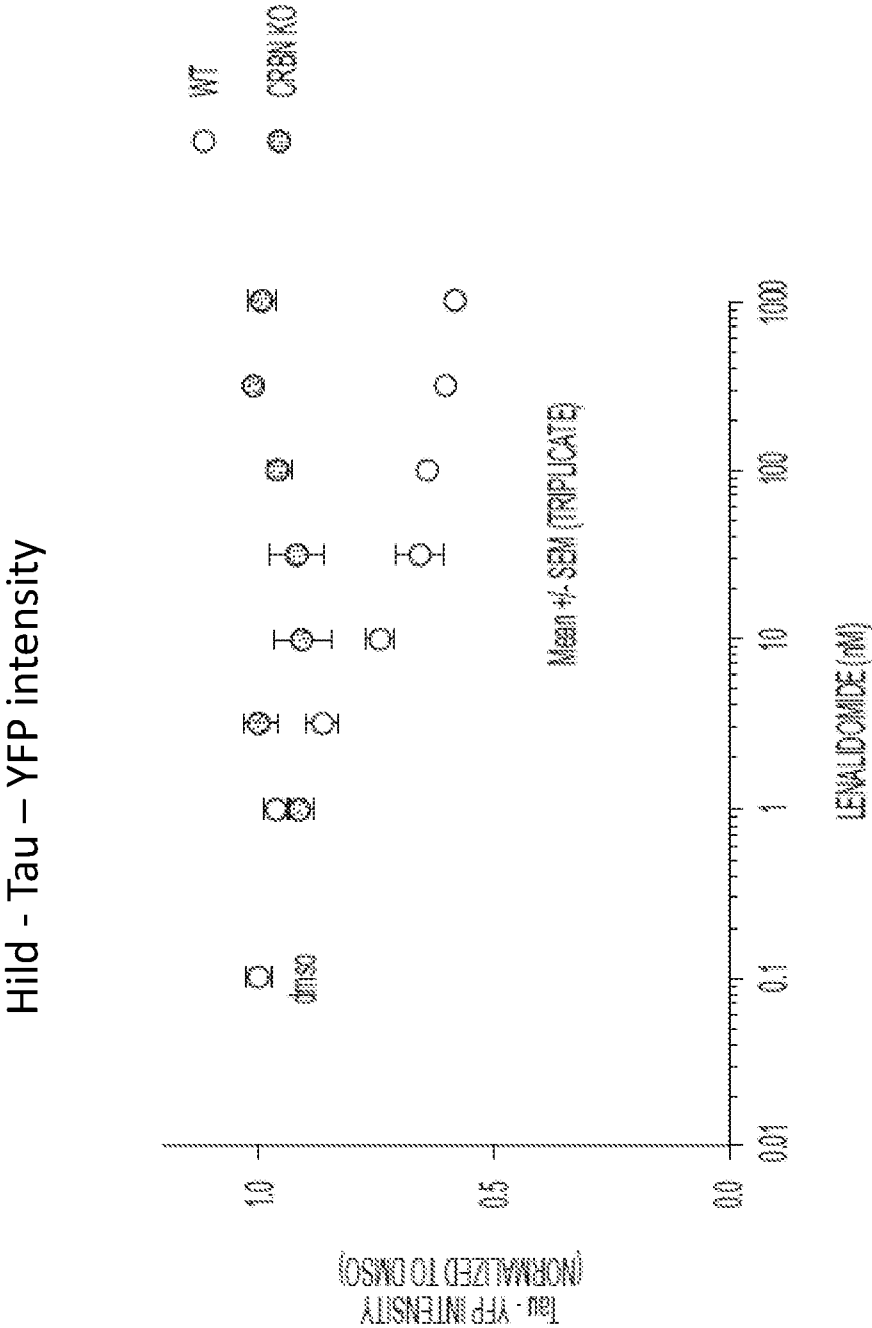
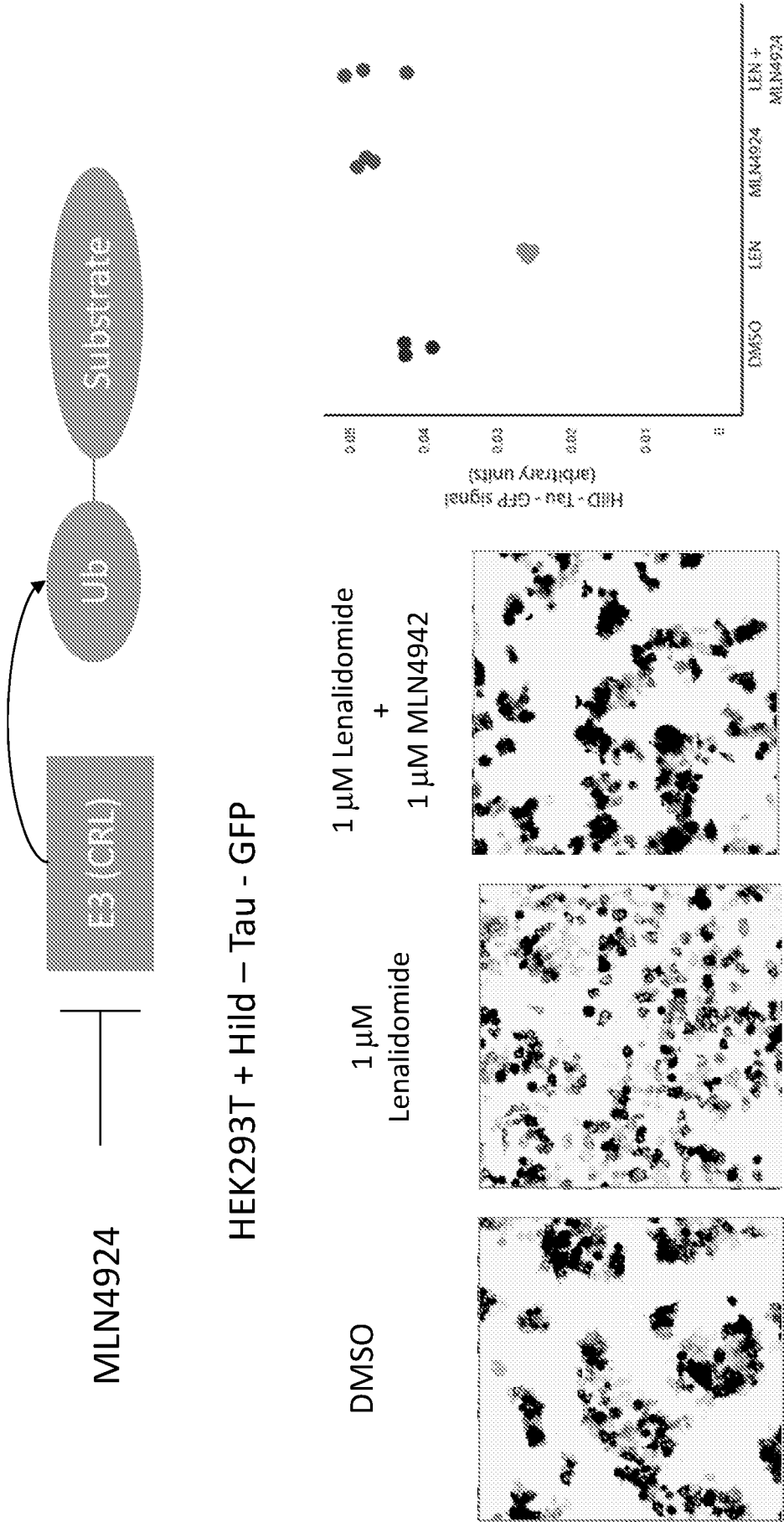


FIG. 26D



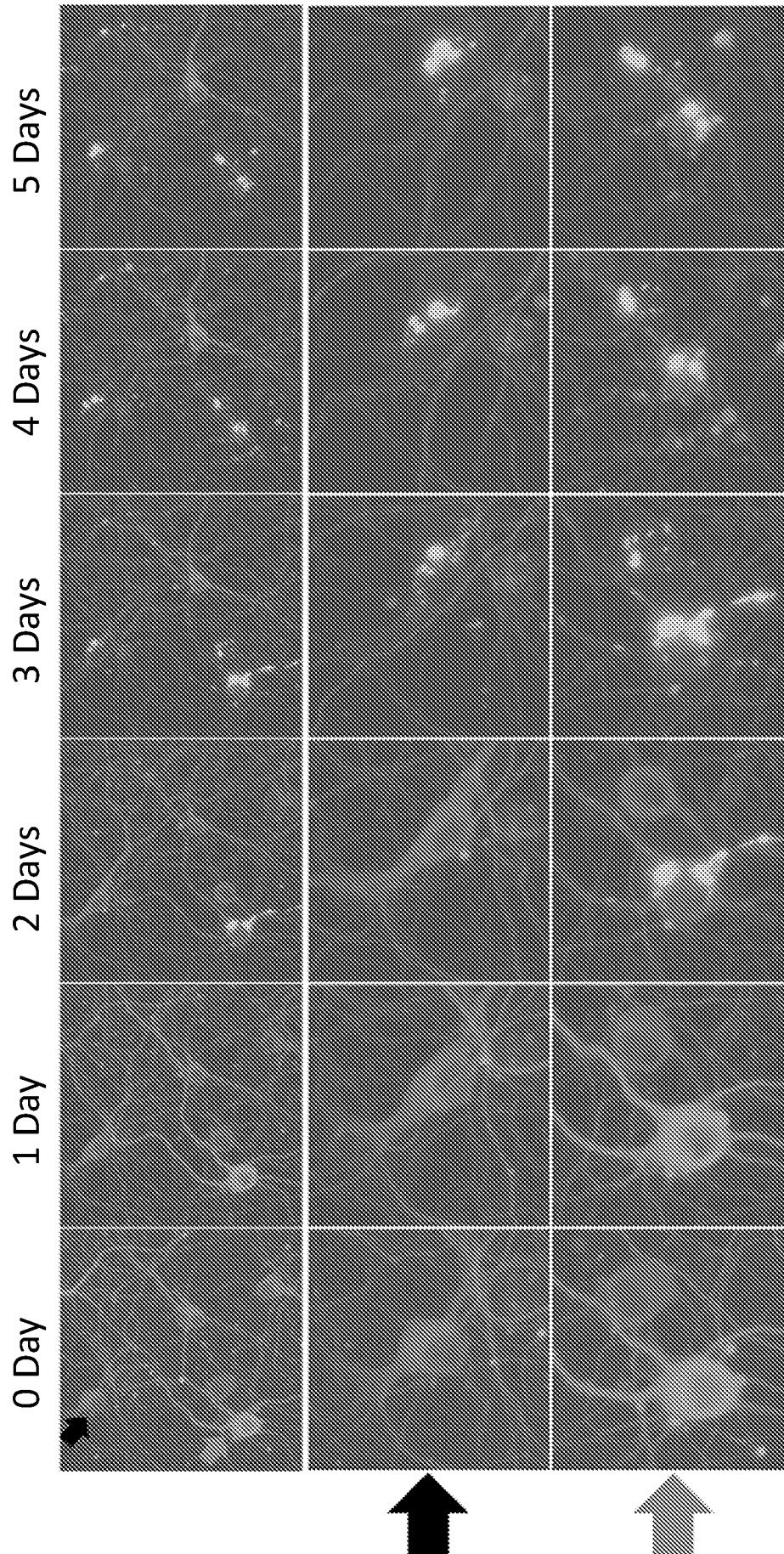


FIG. 27



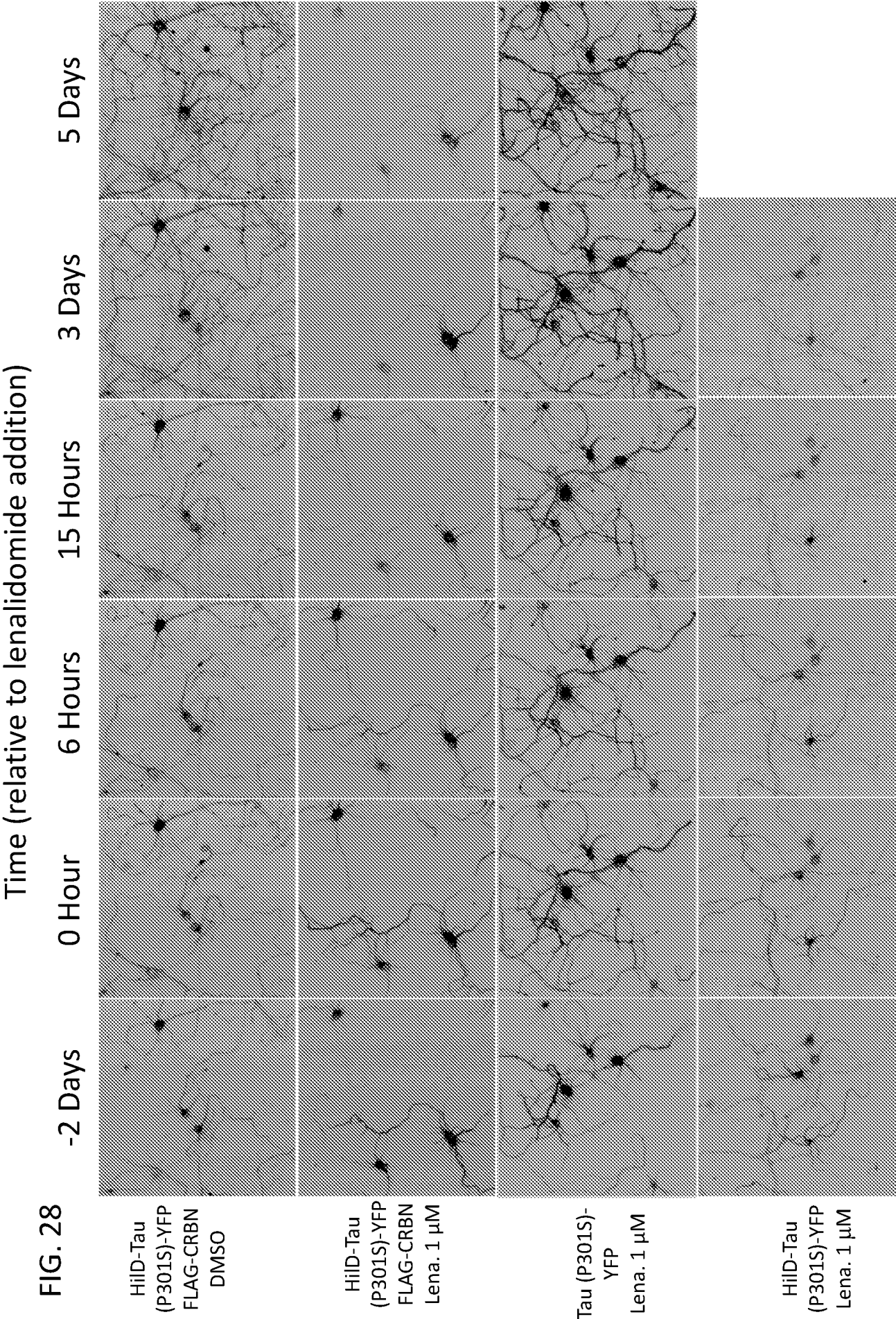


FIG. 29

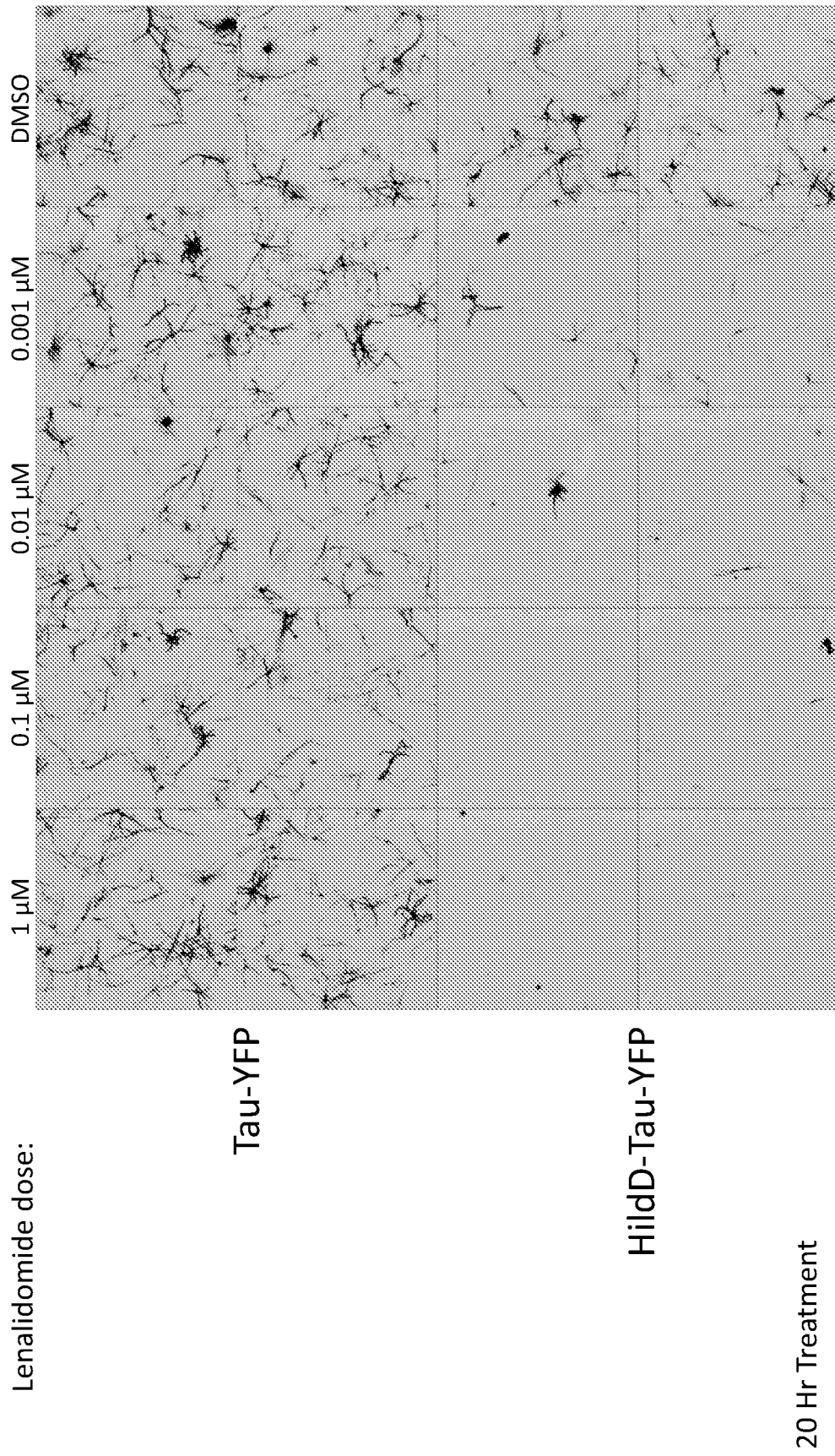


FIG. 30A

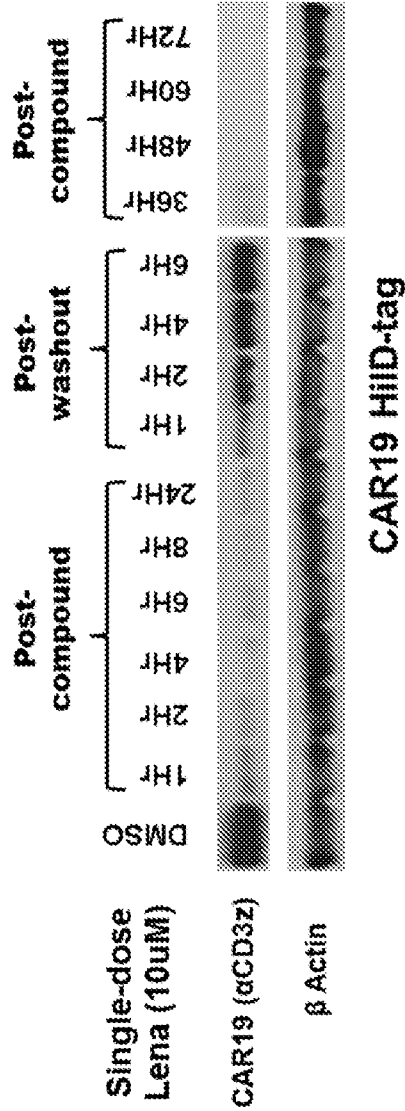


FIG. 30B

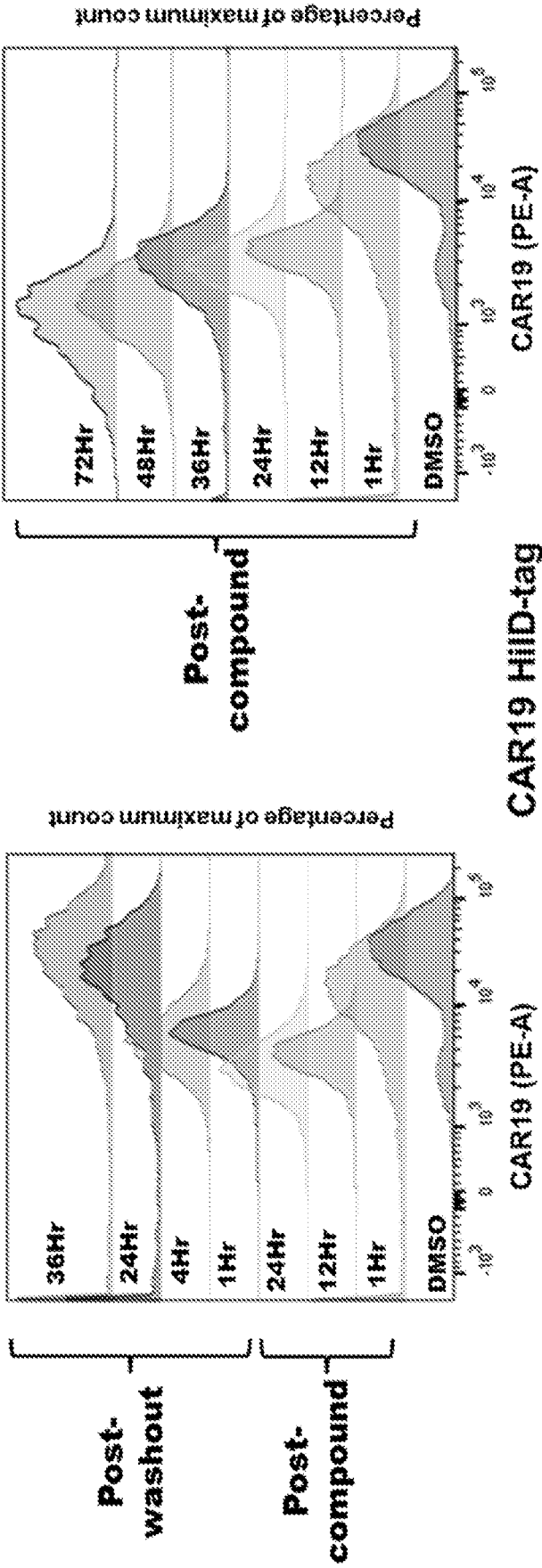
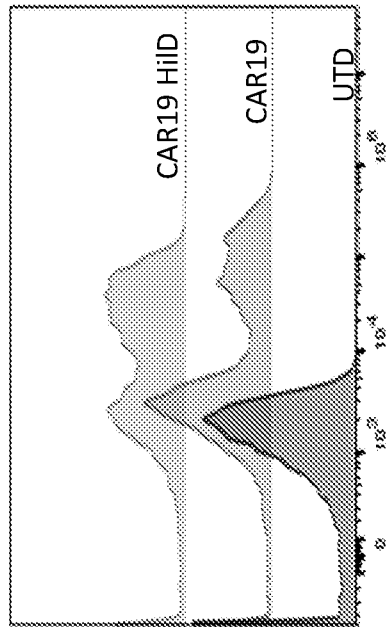


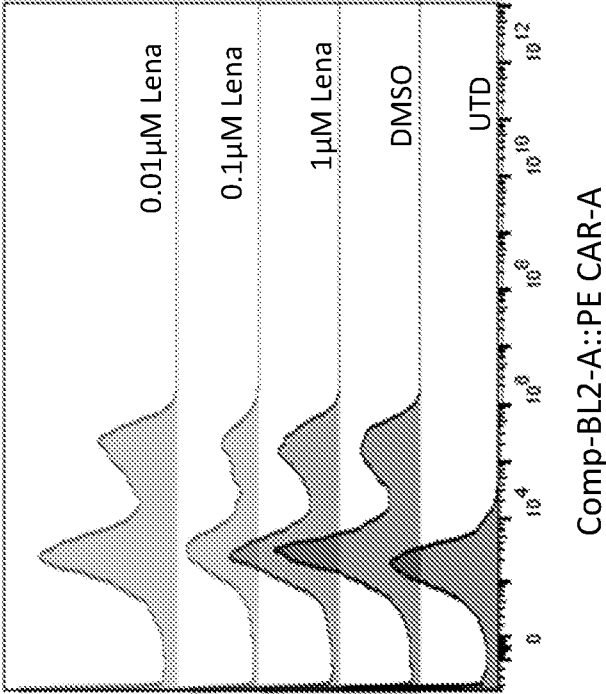
FIG. 31A



Comp-BL2-A::PE CAR-A

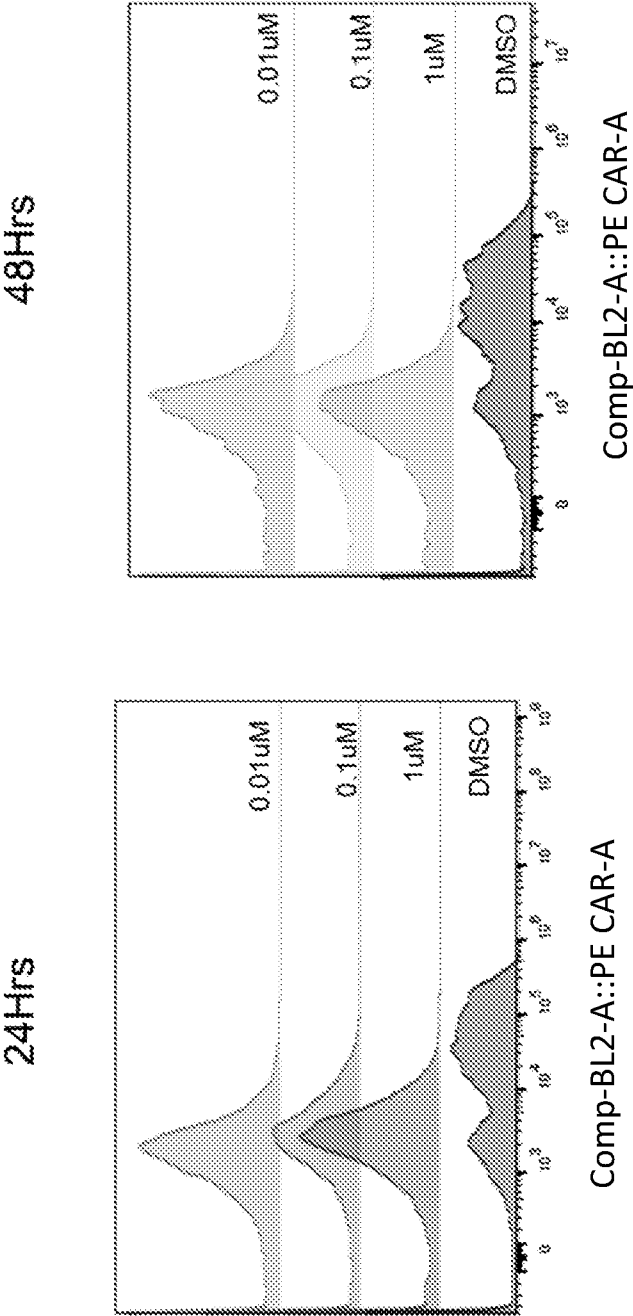
| Sample ID   | % CD3 CAR | CD3 CAR MFI | Fold Expansion |
|-------------|-----------|-------------|----------------|
| UTD         | 0.93      | 1888        | 238            |
| CAR 19      | 37.9      | 39034       | 288            |
| CAR 19 HILD | 50.1      | 21318       | 288            |

FIG. 31B



| Samples           | NT    |
|-------------------|-------|
| CAR19 Lena 0.01µM | 68995 |
| CAR19 Lena 0.1µM  | 68607 |
| CAR19 Lena 1µM    | 68872 |
| CAR19 DMSO        | 67172 |
| UTD               | 8141  |

FIG. 31C



| Conc. Of Leno In uM | 24Hrs |       | 48Hrs |       |
|---------------------|-------|-------|-------|-------|
|                     | %CAR  | MFI   | %CAR  | MFI   |
| 0.01                | 14.2  | 10260 | 1.46  | 1267  |
| 0.1                 | 14.6  | 10134 | 1.33  | 1271  |
| 1                   | 11    | 4971  | 1.02  | 1129  |
| DMSO                | 57    | 50823 | 45.8  | 20914 |

FIG. 32A

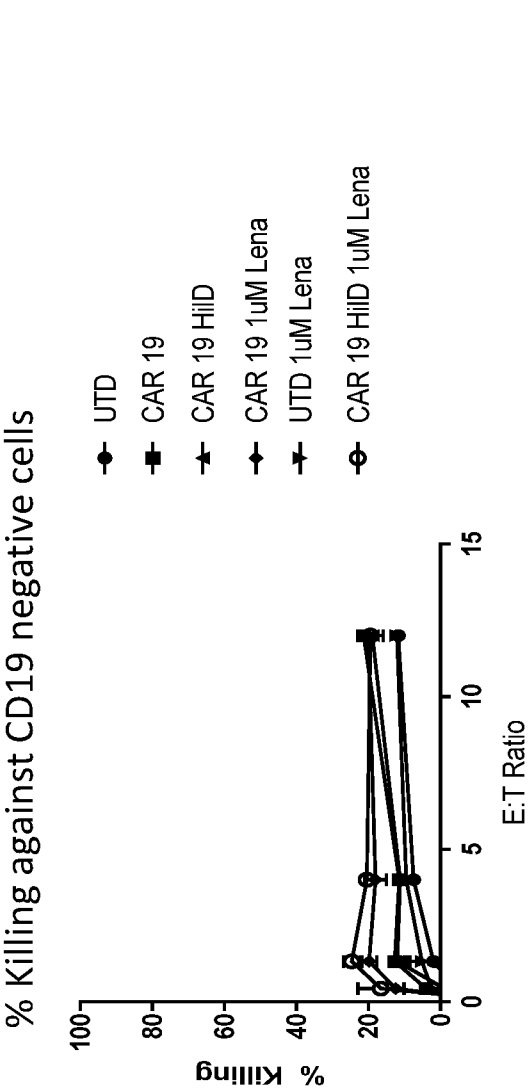


FIG. 32B

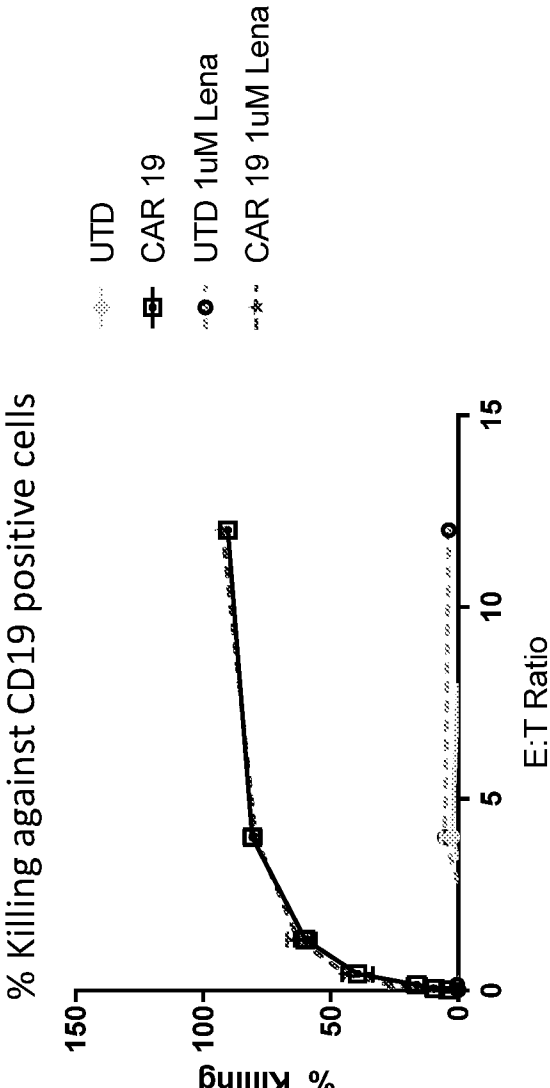


FIG. 32C

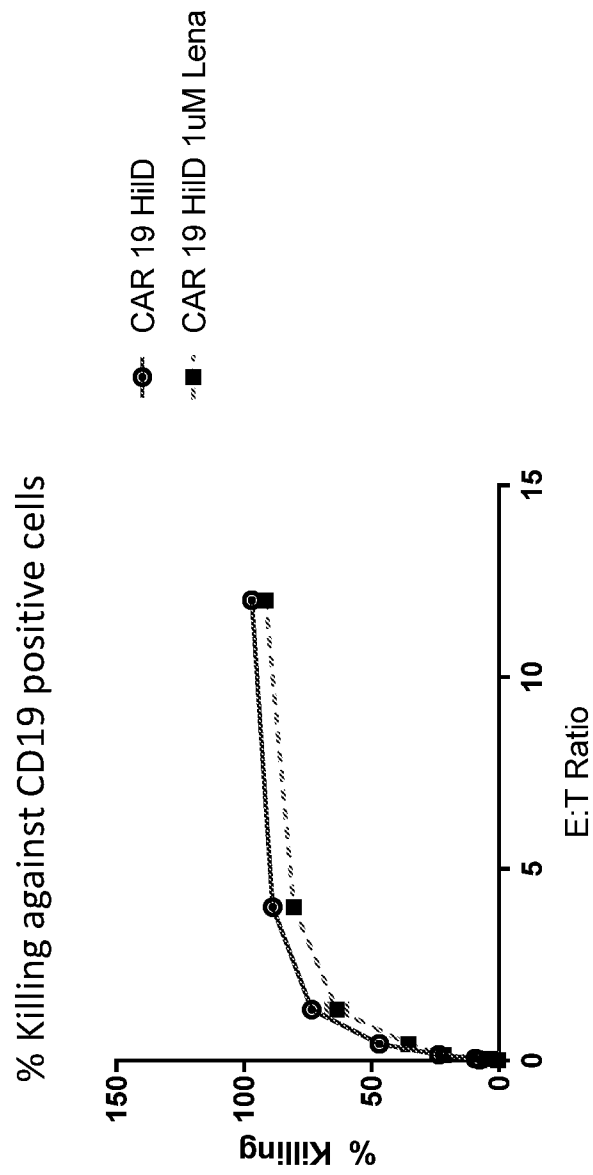




FIG. 33A

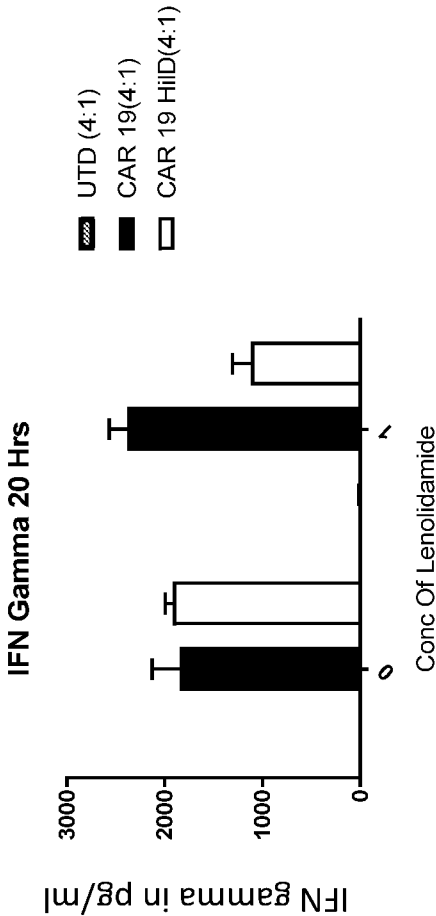


FIG. 33B

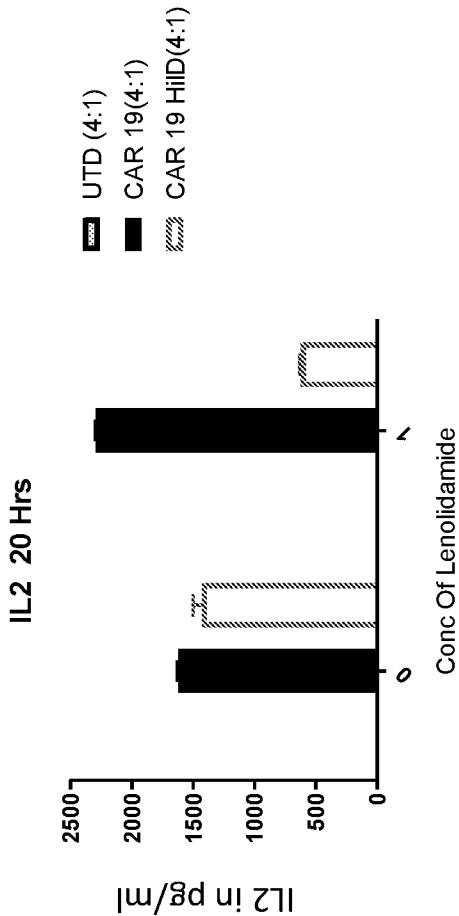


FIG. 34

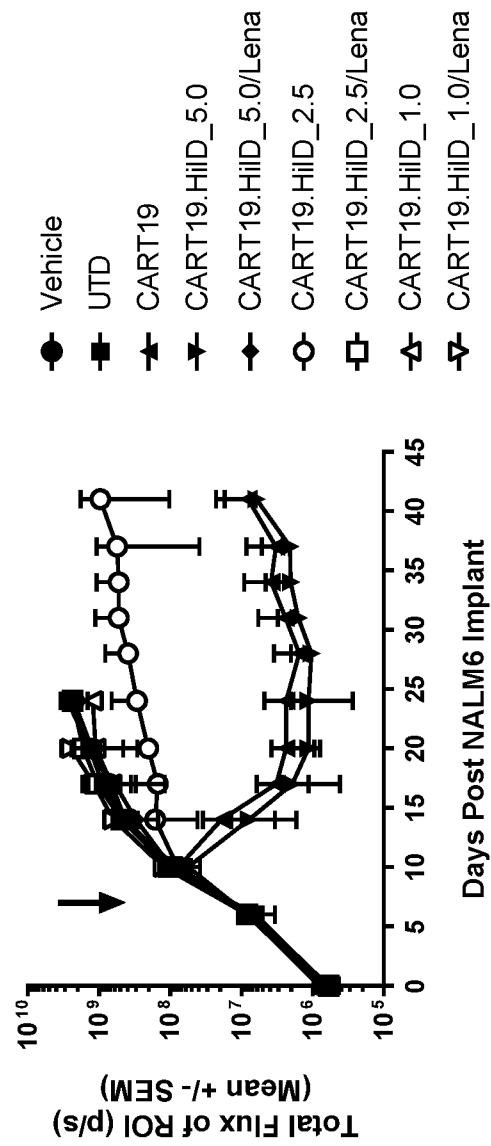


FIG. 35

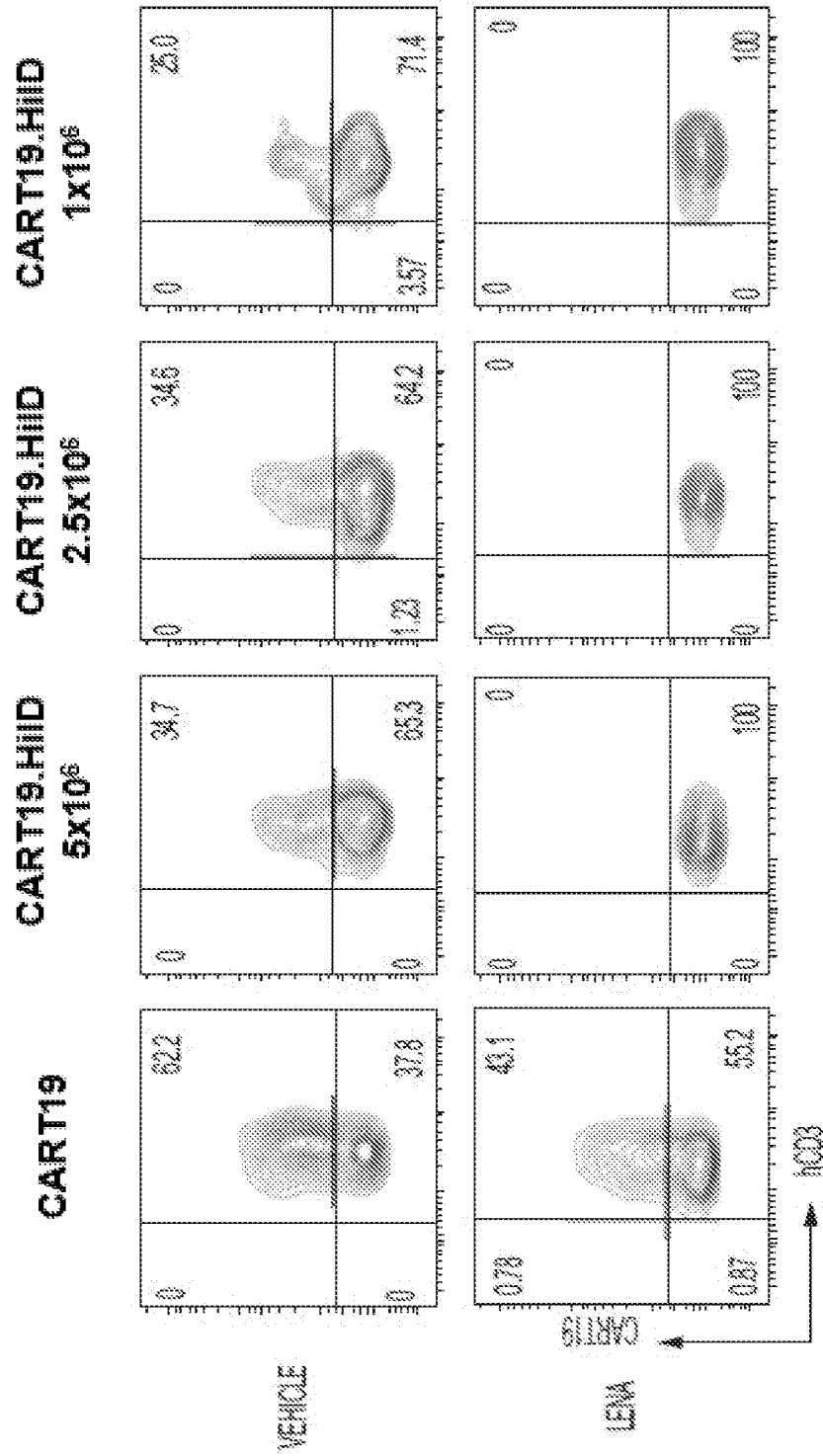


FIG. 36

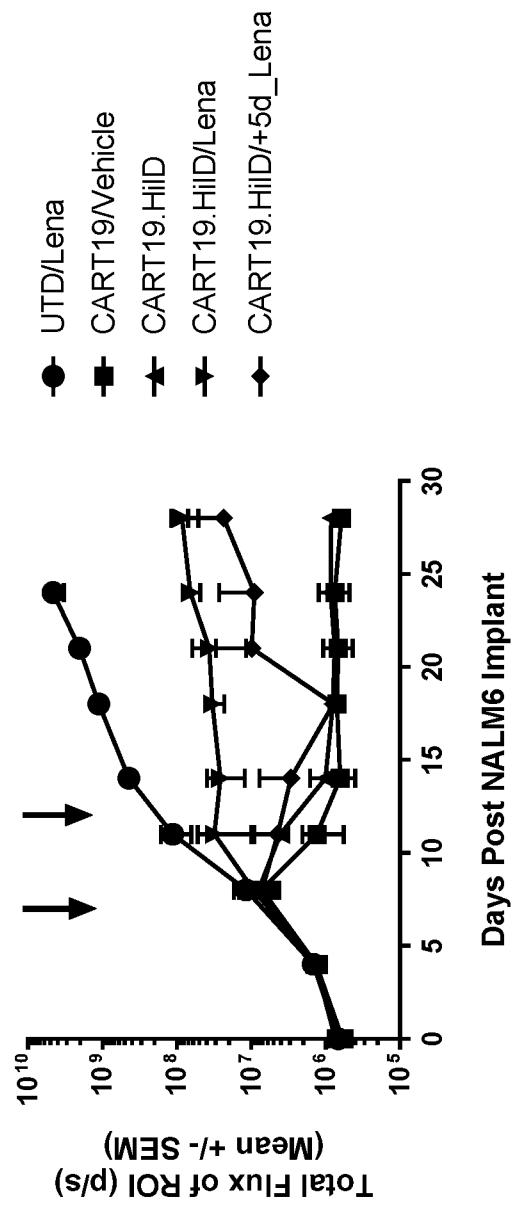


FIG. 37A

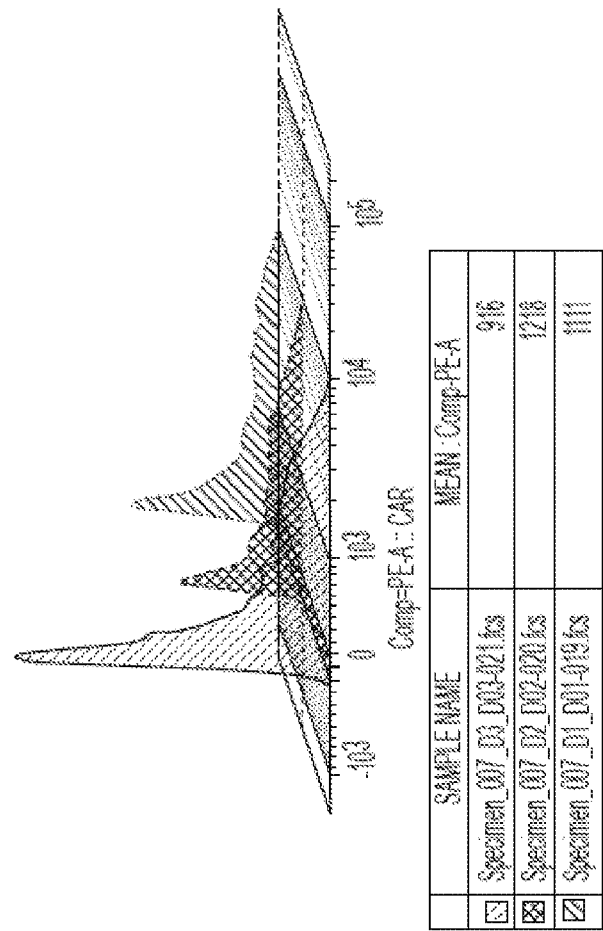


FIG. 37B

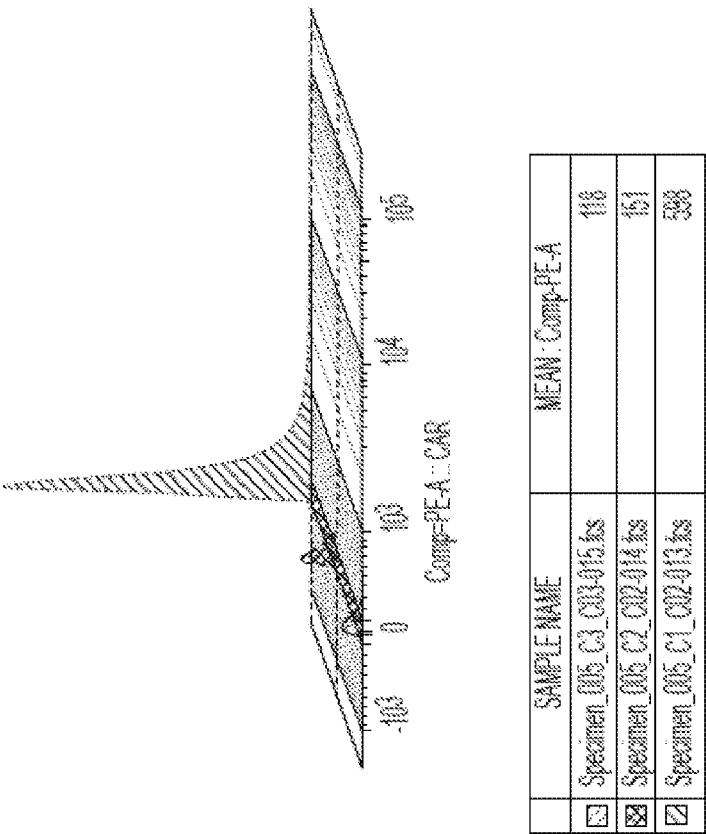


FIG. 37C

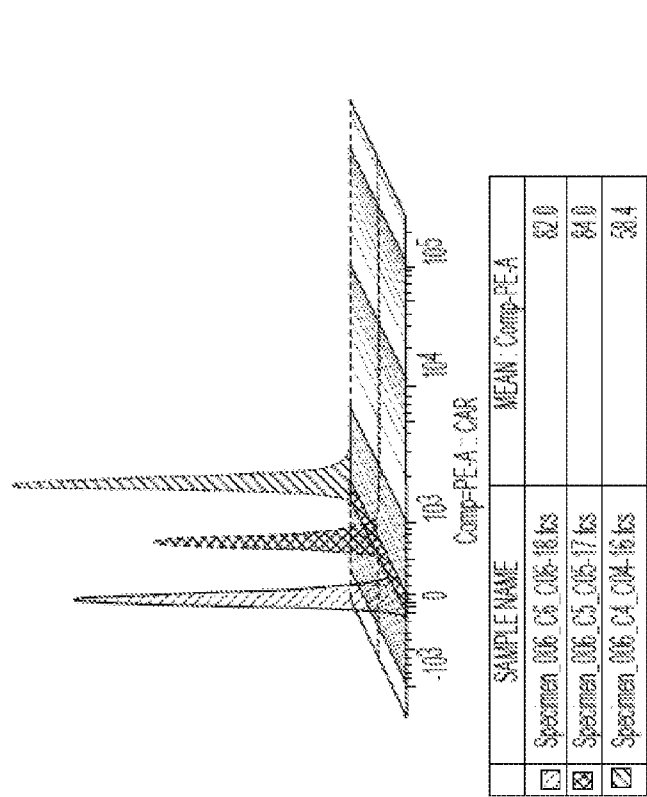


FIG. 37D

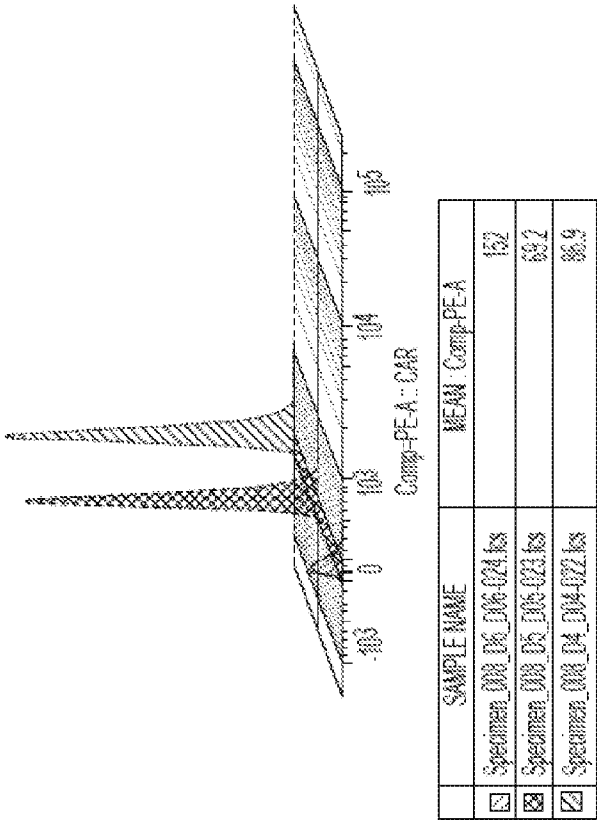


FIG. 37E

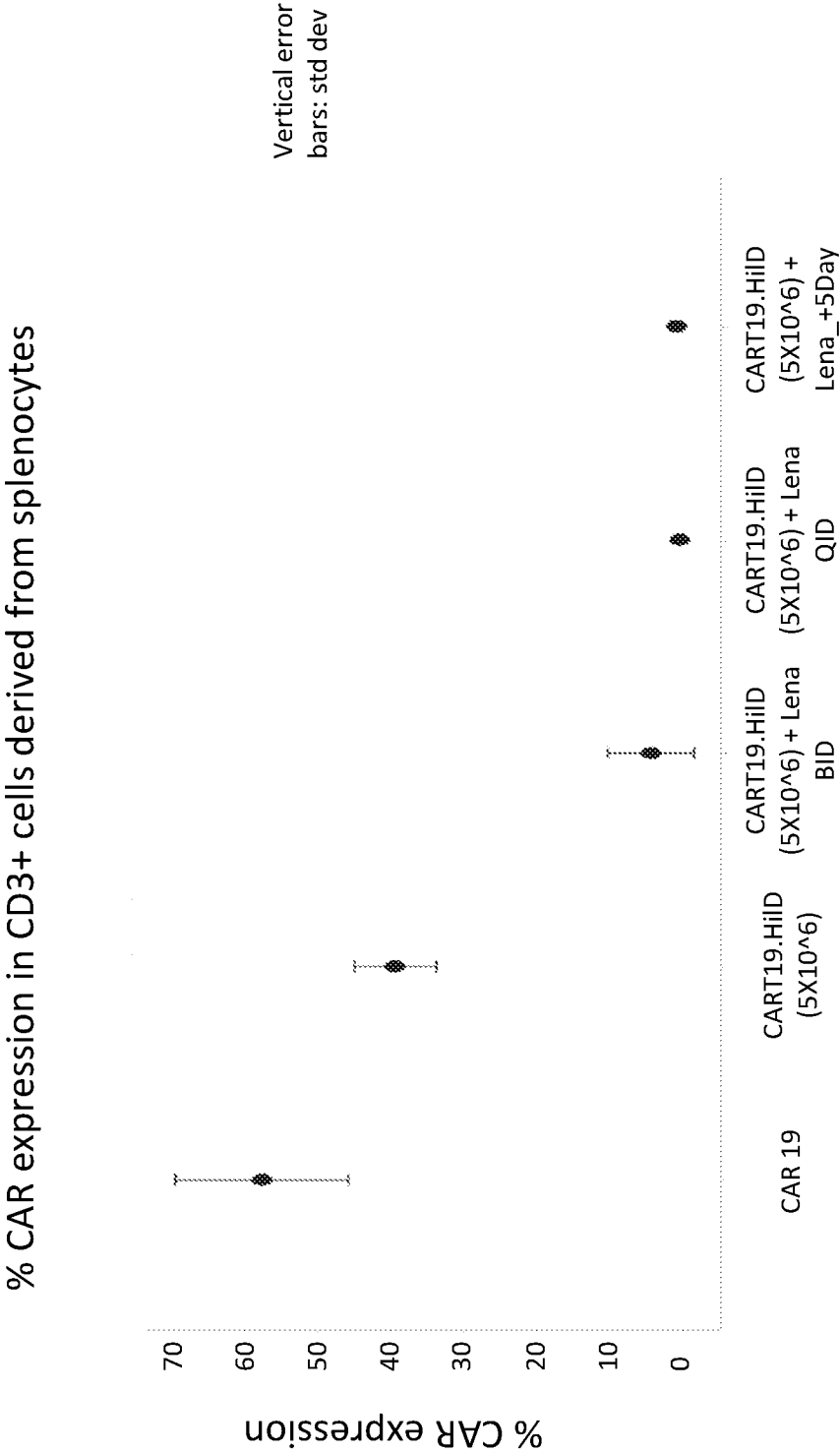


FIG. 38A

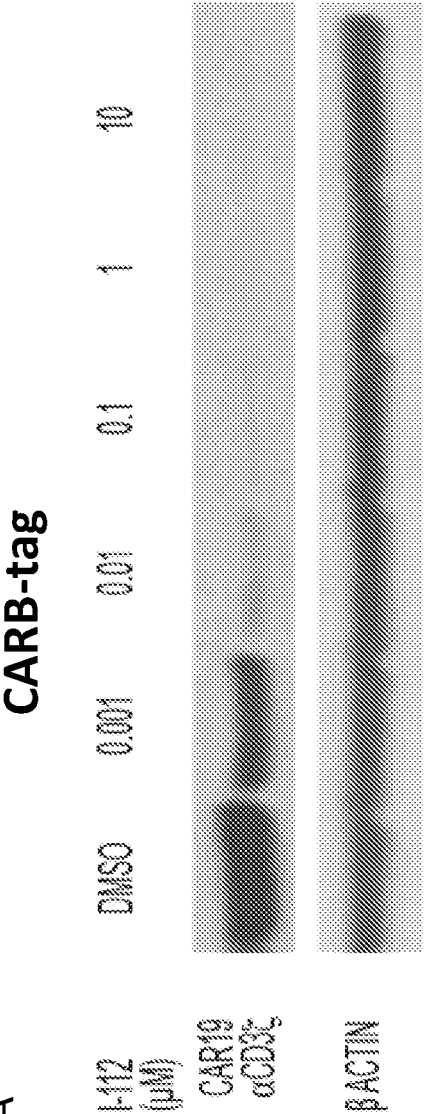


FIG. 38B

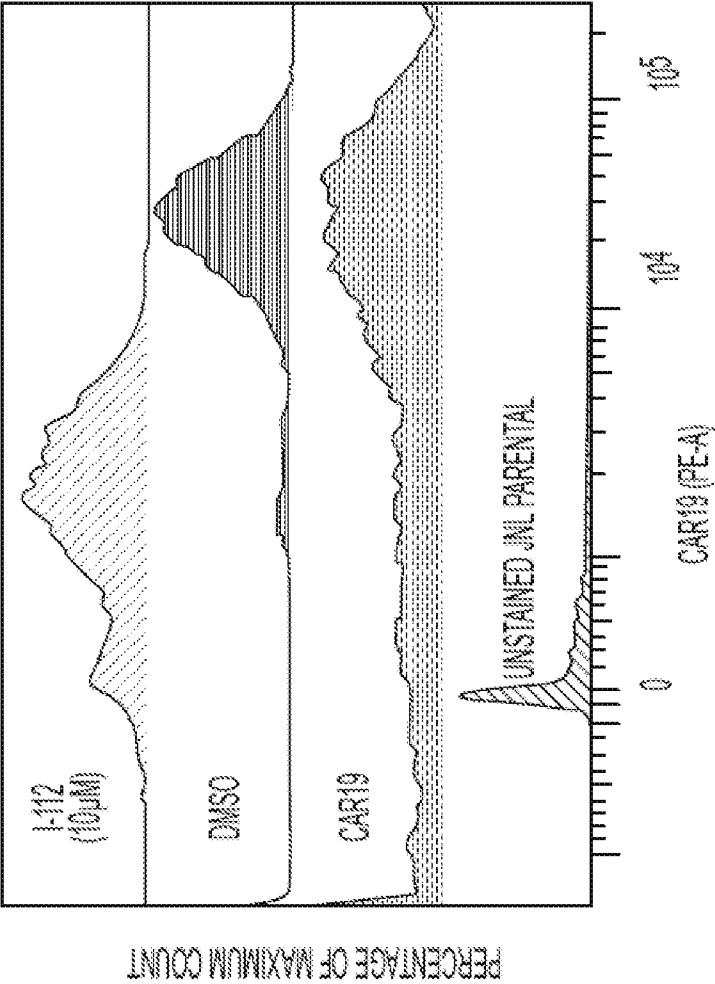




FIG. 38C

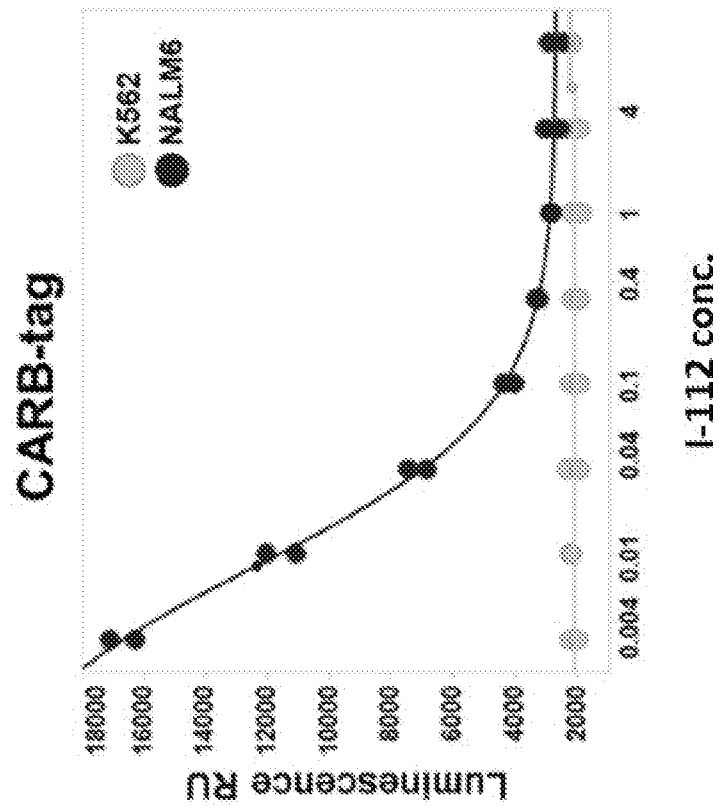
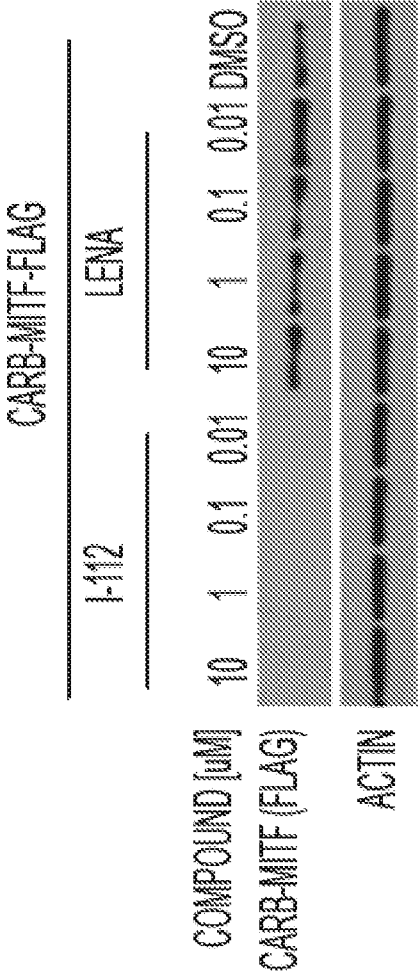


FIG. 39



BCMA HiID-tag

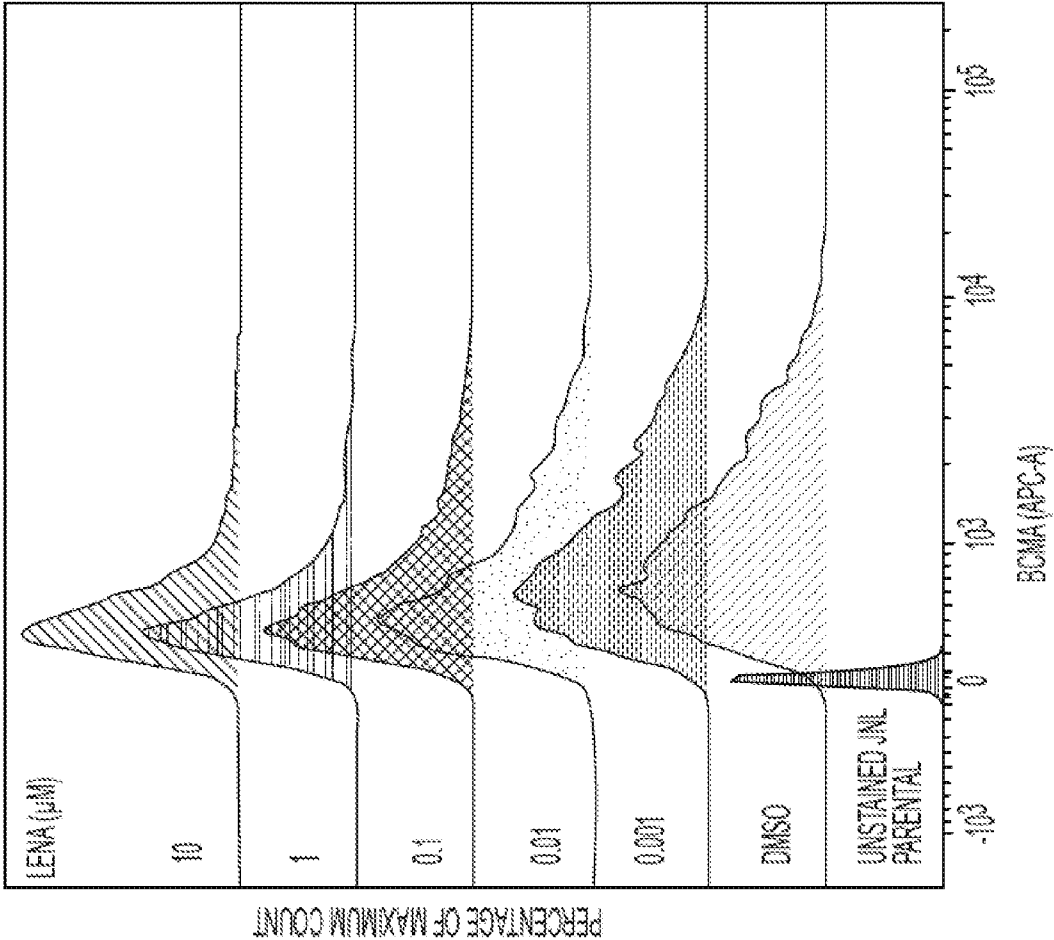


FIG. 40A

FIG. 40B

## BCMA HiID-tag

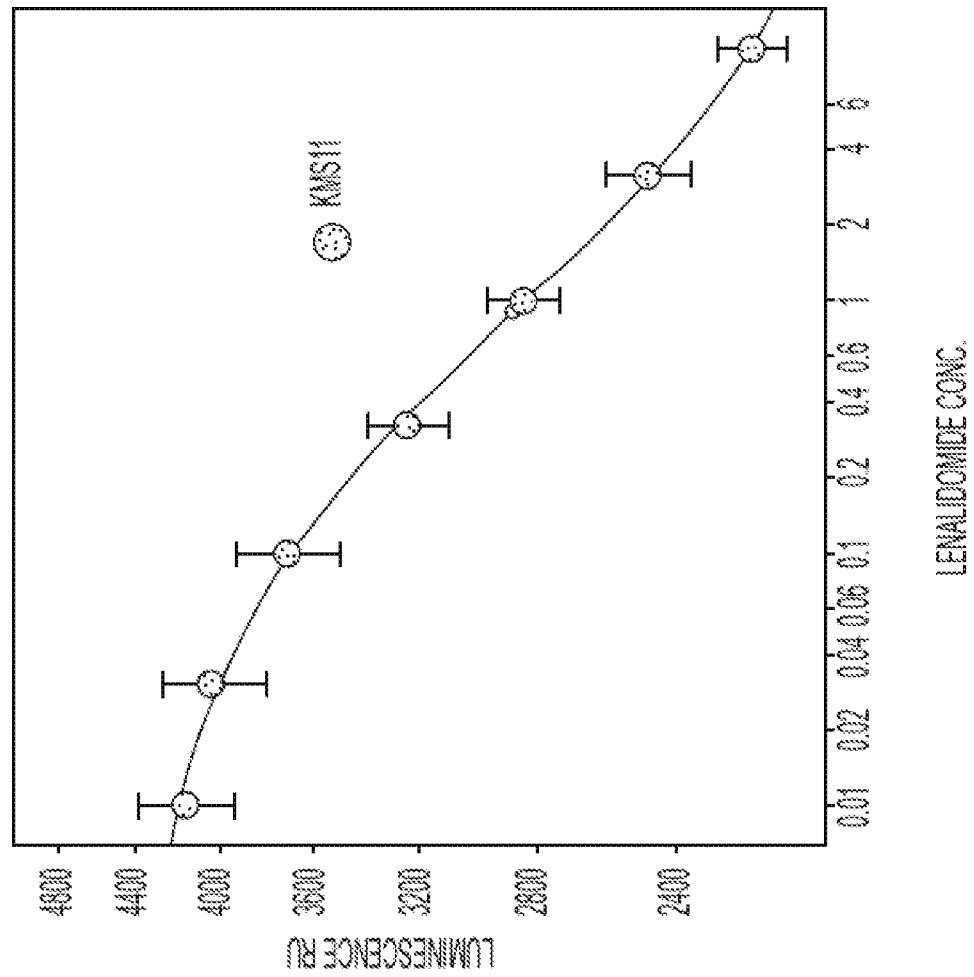


FIG. 11

