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(71) Applicant: AXELYF EHF. [IS/IS]; Laekjargata 2, 207, IS-220 Hafnarfjordur (IS).

(72) Inventor; and

(71) Applicant: WITTRUP, Karl, Dane [US/US]; c/o 76BIO, INC., 689 Fifth Avenue, 12th Floor, New York, NY 10022 (US).

(72) Inventors: LI, Heng; c/o 76BIO, INC., 689 Fifth Avenue, 12th Floor, New York, NY 10022 (US). POP, Marius, S.; c/o 76BIO, INC., 689 Fifth Avenue, 12th Floor, New York, NY 10022 (US). WILFONG, Christopher, M.; c/o 76BIO, INC., 689 Fifth Avenue, 12th Floor, New York, NY 10022 (US). WIJAYA, Juwina; c/o 76BIO, INC., 689 Fifth Avenue, 12th Floor, New York, NY 10022 (US). BENENATO, Kerry, E.; c/o 76BIO, INC., 689 Fifth Avenue, 12th Floor, New York, NY 10022 (US). LUNDE, Bradley; c/o ADIMAB, LLC, 7 Lucent Drive, Lebanon, NH 03766 (US). DESAI, Alec; c/o ADIMAB, LLC, 7 Lucent Drive, Lebanon, NH 03766 (US).

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(54) Title: SINGLE DOMAIN ANTIBODY BINDERS OF MYC

(57) Abstract: Disclosed herein are polypeptides comprising or consisting of anti-Myc single domain antibodies (sdAd), as well as bi-functional and dual-specific bifunctional polypeptides incorporating the anti-Myc sdAbs that induce degradation of a first and optionally a second biological target through the cellular proteasome mechanism. The disclosed bifunctional polypeptides generally comprise a first targeting moiety that includes the anti-Myc sdAb, and a second component that has ubiquitination function, and optionally a second targeting moiety targeting at least a second target protein. The binding of Myc by the first targeting moiety promotes ubiquitination of Myc by the second component, thereby labeling the Myc for proteasomal degradation. These bifunctional polypeptides may be used for the treatment of a disease in a subject, where the first and/or second targeting moieties bind a biological target(s) whose abnormal function and/or expression is associated with the disease.



SINGLE DOMAIN ANTIBODY BINDERS OF MYC

CROSS-REFERENCE TO RELATED APPLICATION

[0001] This application claims the benefit of U.S. Provisional Application Serial No. 63/597,283, filed November 8, 2023, the disclosure of which is hereby incorporated by reference in its entirety, including all figures, tables and amino acid or nucleic acid sequences.

REFERENCE TO SEQUENCE LISTING

[0002] The present application is being filed along with a Sequence Listing in electronic format. The Sequence Listing is provided as a file entitled Seq-List.xml, which was created on November 7, 2024, which is 924,266 bytes in size. The information in the electronic Sequence Listing is hereby incorporated by reference in its entirety.

FIELD

[0003] Aspects of the present disclosure related to novel polypeptides comprising single domain antibody binders of Myc (e.g., c-Myc). Aspects of the present disclosure also relate generally to bifunctional polypeptides that promote ubiquitin-mediated proteasomal degradation of desired biological target(s), for example Myc, and uses such as for the treatment of a disease associated with the biological target(s). Aspects of the present disclosure also relate generally to dual-specific bifunctional polypeptides that promote ubiquitin-mediated proteasomal degradation of desired biological targets, for example Myc and another target, and uses such as for the treatment of a disease associated with the biological targets.

BACKGROUND

[0004] Conventional therapeutics, such as small molecule and antibody inhibitors, operate by blocking or otherwise modulating the function of a therapeutic target (e.g., blocking an enzymatic or transcription promoting function). While many therapeutics belonging to this category have shown to be effective, confounding effects may arise as the therapeutic target is still present within the cellular milieu.

[0005] Molecular glues and proteolysis targeting chimeras (PROTACs) are alternative classes of therapeutics that involve small molecule compounds that recruit E3 ubiquitin ligases to a therapeutic target, thereby inducing proteolysis of the therapeutic target through the endogenous proteasomal degradation machinery. The physical degradation of the target drives

a therapeutic effect through the elimination of the dysfunctional and/or disease-associated target. However, design of these small molecule degraders involves significant screening and optimizations to identify chemical modalities that are compatible for binding to both the therapeutic target and E3 ubiquitin ligase. Identification of such small molecule degraders is particularly challenging for previously undrugged intracellular targets, especially proteins that lack enzymatic domains amenable to traditional small molecule screening and optimization.

[0006] Further, the use of antibody derived binding moieties as to bind to target proteins such as Myc in the intracellular space (e.g., intrabodies) can be hindered by the highly reducing conditions of the intracellular environment which can disrupt the disulfide bonds required for proper mAb/scFv folding.

[0007] Therefore, there is a need for target binding moieties that can function in the intracellular space, and can be employed in compounds designed to utilize a proteasomal degradation tactic to degrade target proteins, for example in the intracellular space.

SUMMARY OF THE DISCLOSURE

[0008] Disclosed herein are polypeptides comprising or consisting of an anti-Myc single domain antibody (sdAb), comprising or consisting of a V_H domain of an IgG antibody, or a V_{HH} , or V_H domain of a heavy chain antibody (HcAb). These anti-Myc sdAbs can be expressed intracellularly and can be employed as part of a designed to utilize a proteasomal degradation tactic to degrade Myc, and optionally one or more additional target proteins. Disclosed herein are bifunctional polypeptides comprising the anti-Myc sdAb that promote proteasome-mediated degradation of Myc. In some embodiments, the bifunctional polypeptide comprises a) an anti-Myc sdAb; and b) a ubiquitin-proteasome system recruiting domain (URD). In some embodiments, proximity of the bifunctional polypeptide to the target protein through binding of the targeting moiety induces ubiquitination of the target protein (Myc) via the URD, thereby promoting proteasome-mediated degradation of the target protein. Also disclosed herein are dual-specific bifunctional polypeptides that promote proteasome-mediated degradation of Myc and at least one second target protein. In some embodiments, the dual-specific bifunctional polypeptide comprises a) an anti-Myc sdAb (target binding domain) that is capable of binding to at least Myc; b) a second targeting moiety that is capable of binding to at least one second target protein; and c) a URD. Optionally, in some embodiments the polypeptides further comprise d) a first linker peptide; wherein the first linker peptide is positioned between the first targeting moiety and the URD; and/or optionally e) a second linker

peptide, wherein the second linker peptide is positioned between the second targeting moiety and the URD. In some embodiments, proximity of the dual-specific bifunctional polypeptide to either or both of the target proteins through binding of the targeting moiety induces ubiquitination of one or both of the target protein(s) via the URD, thereby promoting proteasome-mediated degradation of the target protein(s). In some embodiments, dual-specific bifunctional polypeptide is in a “contralateral” configuration in which the first and second target binding domains are on either side of the URD, i.e., one is on the C-terminal side of the URD, and one is on the N-terminal side of the URD. In some embodiments, dual-specific bifunctional polypeptide is in a “tandem” configuration in which the first and second target binding domains are both on the same side of the URD, i.e., both are on the C-terminal side of the URD, or both are on the N-terminal side of the URD. Optionally, in some embodiments a linker peptide (a first or a second linker peptide) is positioned between the two target binding domains, and/or between a targeting moiety and the URD. In some embodiments, the bifunctional polypeptide or dual-specific bifunctional polypeptide comprises one or more localization sequences to direct the polypeptide to a preferred subcellular compartment (e.g., nucleus).

[0009] Also disclosed herein are polynucleotides encoding for any of the polypeptides comprising or consisting of the anti-Myc sdAb, the bifunctional or dual-specific bifunctional polypeptides, or any portions thereof, provided herein.

[0010] Also disclosed herein are pharmaceutical compositions comprising any of the polypeptides comprising or consisting of the anti-Myc sdAb, or the bifunctional or dual-specific bifunctional polypeptides provided herein and one or more pharmaceutically acceptable excipients, carriers, or diluents.

[0011] Also disclosed herein are methods of treating a subject. In some embodiments, the methods comprise administering any of the polypeptides comprising or consisting of the anti-Myc sdAb, the bifunctional or dual-specific bifunctional polypeptides, polynucleotides, or pharmaceutical compositions provided herein to a subject in need thereof. For example, the methods may be for the treatment of a cancer.

[0012] Also disclosed herein are methods of reducing the amount of a target protein in a cell. In some embodiments, the methods comprise contacting the cell with any of polypeptides comprising or consisting of the anti-Myc sdAb, the bifunctional or dual-specific bifunctional polypeptides, polynucleotides, or pharmaceutical compositions provided herein. In some embodiments, the cell is in a subject, and the polypeptides comprising or consisting of the anti-

Myc sdAb, the bifunctional or dual-specific bifunctional polypeptide, polynucleotide, or pharmaceutical composition is administered to the subject. In some embodiments, the cell is contacted *ex vivo*, and after contacting the cell with the polypeptides comprising or consisting of the anti-Myc sdAb, the bifunctional or dual-specific bifunctional polypeptide, polynucleotide, or pharmaceutical composition, the cell is administered to a subject, optionally wherein the subject is also the source of the cell (e.g., in an adoptive cell therapy).

[0013] Some embodiments provided herein are described by way of the following provided numbered embodiments:

1. A polypeptide comprising or consisting of an anti-Myc single domain antibody (sdAb), comprising or consisting of a V_H domain of an IgG antibody, or a V_{HH}, or V_H domain of a heavy chain antibody (HcAb), wherein the V_H or V_{HH} domain comprises a V_H CDR3 comprising or consisting of any one of SEQ ID NOs: 179, 195, 211, 219, 251, 267 or 283, optionally wherein the V_H CDR3 sequence comprises 1, 2, 3, 4, 5, 6, 7, or more substitutions and/or 1, 2, 3, 4, 5, 6, 7, or more deletions or additions, and/or has at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to a sequence selected from any one of SEQ ID NOs: 179, 195, 211, 219, 251, 267 and 283.

2. The polypeptide of embodiment 1, wherein the sdAb, optionally a V_{HH} domain, comprises a CDR3 comprising or consisting of the amino acid sequence AX₁X₂X₃X₄X₅X₆X₇X₈X₉X₁₀X₁₁X₁₂EX₁₃DY (SEQ ID NO: 299), wherein X₁ is A, H, Y, I, or T; X₂ is L or I; X₃ is G, D or P; X₄ is D, Q, G or E; X₅ is Y, E or I; X₆ is G, L or S; X₇ is D, T, S or G; X₈ is A, G, F, or no amino acid; X₉ is I, G, or no amino acid; X₁₀ is D, G, T or L; X₁₁ is Y, A, F or G; X₁₂ is S, D or N; and X₁₃ is A, H, G or Y; or optionally a conservative substitution of any of the foregoing; wherein the CDR3 does not comprise or consist of any one of SEQ ID NOs: 143, 151, 159, 167, or 175.

3. The polypeptide of embodiment 1 or 2, wherein the sdAb, optionally a V_{HH} domain, comprises:

a CDR1 comprising or consisting of any one of SEQ ID NOs: 177, 185, 201, 209, 217, 225, 233, 241, 249, 257, 265, 273, 281, 289;

a CDR2 comprising or consisting of any one of SEQ ID NOs: 178, 186, 194, 202, 210, 218, 226, 234, 242, 250, 266, 274, 282 or 290; and

a CDR3 comprising or consisting of any one of SEQ ID Nos: 179, 195, 211, 219, 251, 267 or 283; optionally wherein the combination of CDR1, CDR2, and CDR3 is selected from one of the following combinations:

- a.) SEQ ID NOs: 177, 178, and 179;
- b.) SEQ ID NOs: 185, 186, and 187;
- c.) SEQ ID NOs: 177, 194, and 195;
- d.) SEQ ID NOs: 201, 202, and 179;
- e.) SEQ ID NOs: 209, 210, and 211;
- f.) SEQ ID NOs: 217, 218, and 219;
- g.) SEQ ID NOs: 225, 226, and 219;
- h.) SEQ ID NOs: 233, 234, and 219;
- i.) SEQ ID NOs: 241, 242, and 219;
- j.) SEQ ID NOs: 249, 250, and 251;
- k.) SEQ ID NOs: 257, 218, and 219;
- l.) SEQ ID NOs: 265, 266, and 267;
- m.) SEQ ID NOs: 273, 274, and 267;
- n.) SEQ ID NOs: 281, 282, and 283; and
- o.) SEQ ID NOs: 289, 290, and 283;

optionally wherein each CDR sequence independently comprises 1, 2, 3, 4, 5, 6, 7, or more substitutions and/or 1, 2, 3, 4, 5, 6, 7, or more deletions or additions, and/or has at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to a sequence selected from any one of SEQ ID NOs: 177-179, 185-186, 194-195, 201-202, 209-211, 217-219, 225-226, 233-234, 241-242, 249-251, 257, 265-267, 273-274, 281-283, or 289-290.

4. The polypeptide of any one of embodiments 1-3, wherein the sdAb, optionally a V_HH domain, comprises:

a CDR1 comprising or consisting of FX₁FX₂X₃X₄X₅MX₆ (SEQ ID NO: 297), wherein X₁ is T or D; X₂ is A, S, E, G or D; X₃ is D, T, G, E, V or S; X₄ is T, M, Q, A, H, Y, V or N; X₅ is D, P or A; and X₆ is G or S; or optionally a conservative substitution of any of the foregoing;

a CDR2 comprising or consisting of AIX₁X₂X₃X₄X₅X₆X₇YYADSVX₈G (SEQ ID NO: 298), wherein X₁ is S or no amino acid; X₂ is G, A, P, R or S; X₃ is S, D, T or G; X₄ is G, A, S, or E; X₅ is G, D, E, A or S; X₆ is N, V, A, S, E, T, D or H; X₇ is T or A, and X₈ is K or R; or optionally a conservative substitution of any of the foregoing; and

a CDR3 comprising or consisting of AX₁X₂X₃X₄X₅X₆X₇X₈X₉X₁₀X₁₁X₁₂EX₁₃DY (SEQ ID NO: 299), wherein X₁ is A, H, Y, I, or T; X₂ is L or I; X₃ is G, D or P; X₄ is D, Q, G or E; X₅ is Y, E or I; X₆ is G, L or S; X₇ is D, T, S or G; X₈ is A, G, F, or no amino acid; X₉ is I, G, or no amino acid; X₁₀ is D, G, T or L; X₁₁ is Y, A, F or G; X₁₂ is S, D or N; and X₁₃ is A, H, G or Y; or optionally a conservative substitution of any of the foregoing;

wherein the combination of CDR1, CDR2, and CDR3 is not one of the following combinations:

- a.) CDR1, CDR2 and CDR3 of mycV.0300 (SEQ ID NOs: 139, 141 and 143);
- b.) CDR1, CDR2 and CDR3 of mycV.1300 (SEQ ID NOs: 147, 149 and 151);
- c.) CDR1, CDR2 and CDR3 of mycV.1500 SEQ ID NOs: (155, 157 and 159);
- d.) CDR1, CDR2 and CDR3 of mycV.3700 SEQ ID NOs: (163, 165 and 167); and
- e.) CDR1, CDR2 and CDR3 of mycV.6600 SEQ ID NOs: (171, 173 and 175).

5. The polypeptide of any one of embodiments 1-4, wherein the sdAb, optionally a V_HH domain, comprises a V_H FR1 comprising or consisting of any one of SEQ ID NOs: 180, 188, 212, 228 or 284, a V_H FR2 comprising or consisting of SEQ ID NO: 181, a V_H FR3 comprising or consisting of any one of SEQ ID NOs: 182, 190, 278 or 286, and a V_H FR4 comprising or consisting of any one of SEQ ID NOs: 183 or 215, optionally wherein the combination of FR1, FR2, FR3, and FR4 is selected from one of the following combinations:

- a.) SEQ ID NOs: 180, 181, 182, and 183;
- b.) SEQ ID NOs: 188, 181, 182, and 183;
- c.) SEQ ID NOs: 212, 181, 182, and 215;
- d.) SEQ ID NOs: 188, 181, 182, and 215;
- e.) SEQ ID NOs: 228, 181, 182, and 215;
- f.) SEQ ID NOs: 188, 181, 278, and 183; and
- g.) SEQ ID NOs: 284, 181, 286, and 183;

optionally wherein each V_H framework region comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17 or more substitutions and/or 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11,

12, 13, 14, 15, 16, 17 or more deletions or additions, and/or has at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to a sequence selected from any one of SEQ ID NOs: 180-183, 188, 190, 212, 215, 228, 278, 284 or 286.

6. The polypeptide of any one of embodiments 1-5, wherein the sdAb, optionally a V_HH domain, comprises:

a FR1 comprising or consisting of the amino acid sequence X₁VX₂LX₃ESGGGLVQPGGSLRLSX₄AASG (SEQ ID NO: 300), wherein X₁ is E or G; X₂ is Q or R; X₃ is L or V; and X₄ is A or C; or optionally a conservative substitution of any of the foregoing; optionally wherein X₁ is E, X₂ is Q, X₃ is L, and X₄ is A or C;

a FR2 comprising or consisting of the amino acid sequence SEQ ID NO: 301;

a FR3 comprising or consisting of the amino acid sequence RFX₁ISRDN SKNTLYLQMNX₂LRAX₃DTAVYYX₄ (SEQ ID NO: 302), wherein X₁ is T or S, X₂ is S or G, X₃ is E or A, X₄ is A, V, or C; or optionally a conservative substitution of any of the foregoing; optionally wherein X₁ is R, X₂ is S, X₃ is E, and X₄ is A or C; and

a FR4 comprising or consisting of the amino acid sequence WGX₁GTQVTVSS (SEQ ID NO: 303), wherein X₁ is Q or R; or optionally a conservative substitution of any of the foregoing; optionally wherein X₁ is Q.

7. The polypeptide of any one of embodiments 1-6, wherein the sdAb, optionally a V_HH domain, comprises or consists of a sequence selected from any one of SEQ ID NOs: 184, 192, 200, 208, 216, 224, 232, 240, 248, 256, 264, 272, 280, 288 or 296, optionally wherein each CDR sequence independently comprises 1, 2, 3, 4, 5, 6, 7, or more substitutions and/or 1, 2, 3, 4, 5, 6, 7, or more deletions or additions, and/or has at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to a CDR sequence of any one of SEQ ID NOs: 177-179, 185-186, 194-195, 201, 202, 209-211, 217-219, 225-226, 233-234, 241-242, 249-251, 257, 265-267, 273-274, 281-283, or 289-290, and/or wherein the framework region comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17 or more substitutions and/or 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17 or more deletions or additions, and/or has at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the framework region of any one of SEQ ID NOs: 180-183, 188, 190, 212, 215, 228, 278, 284 or 286.

8. The polypeptide of any one of embodiments 1-7, wherein the sdAb, optionally a V_HH domain, comprises or consists of an amino acid sequence having at least 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to any one of SEQ ID NOs: 184, 192, 200, 208, 216, 224, 232, 240, 248, 256, 264, 272, 280, 288 or 296.

9. The polypeptide of any one of embodiments 1-8, wherein the sdAb, optionally a V_HH domain, comprises a CDR3 comprising or consisting of the amino acid sequence AALGDYGX₁AIDYSEADY (SEQ ID NO: 306), wherein X₁ is D or G; or optionally a conservative substitution of any of the foregoing; optionally wherein the CDR3 comprises or consists of any one of SEQ ID NOs: 179 or 195.

10. The polypeptide of embodiment 9, wherein the sdAb, optionally a V_HH domain, comprises:

a CDR1 comprising or consisting of any one of SEQ ID NOs: 177, 185, or 201;

a CDR2 comprising or consisting of any one of SEQ ID NOs: 178, 186, 194 or 202; and

a CDR3 comprising or consisting of any one of SEQ ID NOs: 179 or 195; optionally wherein the combination of CDR1, CDR2, and CDR3 is selected from one of the following combinations:

a.) SEQ ID NOs: 177, 178, and 179;

b.) SEQ ID NOs: 185, 186, and 179;

c.) SEQ ID NOs: 177, 194, and 195; and

d.) SEQ ID NOs: 201, 202, and 179;

optionally wherein each CDR sequence independently comprises 1, 2, 3, 4, 5, 6, 7, or more substitutions and/or 1, 2, 3, 4, 5, 6, 7, or more deletions or additions, and/or has at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to a sequence selected from any one of SEQ ID NOs: 177-179, 185-186, 194-195, or 201-202.

11. The polypeptide of any one of embodiments 9-10, wherein the sdAb, optionally a V_HH domain, comprises:

a CDR1 comprising or consisting of the amino acid sequence FTFX₁X₂X₃X₄MX₅ (SEQ ID NO: 304), wherein X₁ is A, S or E; X₂ is D, T, or G; X₃ is T, M or Q; X₄ is D or P; and X₅ is G or S; or optionally a conservative substitution of any of the foregoing;

a CDR2 comprising or consisting of the amino acid sequence AISX₁X₂X₃X₄X₅TYYADSVX₆G (SEQ ID NO: 305), wherein X₁ is G or A; X₂ is S or D; X₃ is G or A; X₄ is G, D, or E; X₅ is N, V or A; and X₆ is K or R; or optionally a conservative substitution of any of the foregoing; and

a CDR3 comprising or consisting of the amino acid sequence AALGDYGX₁AIDYSEADY (SEQ ID NO: 306), wherein X₁ is D or G; or optionally a conservative substitution of any of the foregoing;

wherein the combination of CDR1, CDR2 and CDR3 is not the combination of CDR1, CDR2 and CDR3 of mycV.0300 (SEQ ID NOs: 139, 141 and 143).

12. The polypeptide of any one of embodiments 9-11, wherein the sdAb, optionally a V_HH domain, comprises a V_H FR1 comprising or consisting of any one of SEQ ID NOs: 180 or 188, a V_H FR2 comprising or consisting of SEQ ID NO: 181, a V_H FR3 comprising or consisting of any one of SEQ ID NOs: 182 or 190, and a V_H FR4 comprising or consisting of SEQ ID NO: 183, optionally wherein the combination of FR1, FR2, FR3, and FR4 is selected from one of the following combinations:

- a.) SEQ ID NOs: 180, 181, 182, and 183;
- b.) SEQ ID NOs: 188, 181, 190, and 183; and
- c.) SEQ ID NOs: 188, 181, 182, and 183;

optionally wherein each V_H framework region comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17 or more substitutions and/or 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17 or more deletions or additions, and/or has at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to a sequence selected from any one of SEQ ID NOs: 180-183, 188 or 190.

13. The polypeptide of any one of embodiments 9-12, wherein the sdAb, optionally a V_HH domain, comprises or consists of a sequence selected from any one of SEQ ID NOs: 184, 192, 200, 208, optionally wherein each CDR sequence independently comprises 1, 2, 3, 4, 5, 6, 7, or more substitutions and/or 1, 2, 3, 4, 5, 6, 7, or more deletions or additions, and/or has at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to a CDR sequence of any one of SEQ ID NOs: 177-179, 185-186, 194-195, or 201-202, and/or wherein the framework region comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17 or more substitutions and/or 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17 or more deletions or additions, and/or has at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%,

99%, or 100% sequence identity to the framework region of any one of SEQ ID NOs: 180-183, 188 or 190.

14. The polypeptide of any one of embodiments 9-13, wherein the sdAb, optionally a V_HH domain, comprises or consists of an amino acid sequence having at least 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to any one of SEQ ID NOs: 184, 192, 200, 208.

15. The polypeptide of any one of embodiments 1-8, wherein the sdAb, optionally a V_HH domain, comprises a CDR3 comprising or consisting of the amino acid sequence AX₁LX₂QELTGASEHDY (SEQ ID NO: 309), wherein X₁ is H or Y; and X₂ is D or G; or optionally a conservative substitution of any of the foregoing; optionally wherein the CDR3 comprises or consists of SEQ ID NO: 211.

16. The polypeptide of embodiment 15, wherein the sdAb, optionally a V_HH domain, comprises:

a CDR1 comprising or consisting of SEQ ID NO: 209,

a CDR2 comprising or consisting of SEQ ID NO: 210, and

a CDR3 comprising or consisting of SEQ ID NO: 211;

optionally wherein each CDR sequence independently comprises 1, 2, 3, 4, 5, 6, 7, or more substitutions and/or 1, 2, 3, 4, 5, 6, 7, or more deletions or additions, and/or has at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to a sequence selected from any one of SEQ ID NOs: 209-211.

17. The polypeptide of any one of embodiments 15-16, wherein the sdAb, optionally a V_HH domain, comprises:

a CDR1 comprising or consisting of the amino acid sequence FTFX₁X₂X₃X₄MG (SEQ ID NO: 307), wherein X₁ is G or D; X₂ is D or E; X₃ is A or T; and X₄ is D or A; or optionally a conservative substitution of any of the foregoing;

a CDR2 comprising or consisting of the amino acid sequence AISASX₁X₂X₃TYYADSVKG (SEQ ID NO: 308), wherein X₁ is G or S, X₂ is D or A, and X₃ is X or E; or optionally a conservative substitution of any of the foregoing; and

a CDR3 comprising or consisting of the amino acid sequence AX₁LX₂QELTGASEHDY (SEQ ID NO: 309), wherein X₁ is H or Y; and X₂ is D or G; or optionally a conservative substitution of any of the foregoing;

wherein the combination of CDR1, CDR2 and CDR3 is not the combination of CDR1, CDR2 and CDR3 of mycV.1300 (SEQ ID NOs: 147, 149 and 151).

18. The polypeptide of any one of embodiments 15-17, wherein the sdAb, optionally a V_HH domain, comprises a V_H FR1 comprising or consisting of SEQ ID NO: 212, a V_H FR2 comprising or consisting of SEQ ID NO: 213 a V_H FR3 comprising or consisting of SEQ ID NO: 214, and a V_H FR4 comprising or consisting of SEQ ID NO: 215;

optionally wherein each V_H framework region comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17 or more substitutions and/or 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17 or more deletions or additions, and/or has at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to a sequence selected from any one of SEQ ID NOs: 212-215.

19. The polypeptide of any one of embodiments 15-18, wherein the sdAb, optionally a V_HH domain, comprises or consists of a sequence that is SEQ ID NO: 216, optionally wherein each CDR sequence independently comprises 1, 2, 3, 4, 5, 6, 7, or more substitutions and/or 1, 2, 3, 4, 5, 6, 7, or more deletions or additions, and/or has at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to a CDR sequence of any one of SEQ ID NOs: 209-211, and/or wherein the framework region comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17 or more substitutions and/or 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17 or more deletions or additions, and/or has at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the framework region of any one of SEQ ID NOs: 212-215.

20. The polypeptide of any one of embodiments 15-19, wherein the sdAb, optionally a V_HH domain, comprises or consists of an amino acid sequence having at least 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 216.

21. The polypeptide of any one of embodiments 1-8, wherein the sdAb, optionally a V_HH domain, comprises a CDR3 comprising or consisting of the amino acid sequence AIIDDILTX₁GTFDEGDY (SEQ ID NO: 312), wherein X₁ is G or D; or optionally a conservative substitution of any of the foregoing; optionally wherein the CDR3 comprises or consists of any one of SEQ ID NOs: 219 or 251.

22. The polypeptide of embodiment 21, wherein the sdAb, optionally a V_HH domain, comprises:

a CDR1 comprising or consisting of any one of SEQ ID NOs: 217, 225, 233, 241, 249 or 257;

a CDR2 comprising or consisting of any one of SEQ ID NOs: 218, 226, 234, 242 or 250, and

a CDR3 comprising or consisting of any one of SEQ ID NOs: 219 or 251; optionally wherein the combination of CDR1, CDR2, and CDR3 is selected from one of the following combinations:

- a.) SEQ ID NOs: 217, 218, and 219;
- b.) SEQ ID NOs: 225, 226, and 219;
- c.) SEQ ID NOs: 233, 234, and 219;
- d.) SEQ ID NOs: 241, 242, and 219;
- e.) SEQ ID NOs: 249, 250, and 251; and
- f.) SEQ ID NOs: 257, 218, and 219;

Optionally wherein each CDR sequence independently comprises 1, 2, 3, 4, 5, 6, 7, or more substitutions and/or 1, 2, 3, 4, 5, 6, 7, or more deletions or additions, and/or has at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to a sequence selected from any one of SEQ ID NOs: 217-219, 225-226, 233-234, 241-242, 249-251, and 257.

23. The polypeptide of any one of embodiments 21-22, wherein the sdAb, optionally a V_HH domain, comprises:

a CDR1 comprising or consisting of the amino acid sequence FX₁FX₂X₃X₄X₅MG (SEQ ID NO: 310), wherein X₁ is T or D; X₂ is A, E, D, S or G; X₃ is D, E or T; X₄ is T, H, Y, V or A; and X₅ is D, A or S; or optionally a conservative substitution of any of the foregoing;

a CDR2 comprising or consisting of the amino acid sequence AISX₁X₂X₃X₄X₅X₆YYADSVKG (SEQ ID NO: 311), wherein X₁ is G, P, R or A; X₂ is S or D, X₃ is G or A, X₄ is G, A, T or D; X₅ is S, T, A or V; and X₆ is T or A; or optionally a conservative substitution of any of the foregoing; and

a CDR3 comprising or consisting of the amino acid sequence AIIDDILTX₁GTFDEGDY (SEQ ID NO: 312), wherein X₁ is G or D; or optionally a conservative substitution of any of the foregoing;

wherein the combination of CDR1, CDR2 and CDR3 is not the combination of CDR1, CDR2 and CDR3 of mycV.1500 SEQ ID NOs: (155, 157 and 159).

24. The polypeptide of any one of embodiments 21-23, wherein the sdAb, optionally a V_HH domain, comprises a V_H FR1 comprising or consisting of any one of SEQ ID

NOs: 220 or 228, a V_H FR2 comprising or consisting of SEQ ID NO: 221, a V_H FR3 comprising or consisting of SEQ ID NO: 222, and a V_H FR4 comprising or consisting of SEQ ID NO: 223, optionally wherein the combination of FR1, FR2, FR3, and FR4 is selected from one of the following combinations:

a.) SEQ ID NOs: 220, 221, 222, and 223; and

b.) SEQ ID NOs: 228, 221, 222, and 223;

optionally wherein each V_H framework region comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17 or more substitutions and/or 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17 or more deletions or additions, and/or has at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to a sequence selected from any one of SEQ ID NOs: 220-223, or 228.

25. The polypeptide of any one of embodiments 21-24, wherein the sdAb, optionally a V_{HH} domain, comprises or consists of a sequence selected from any one of SEQ ID NOs: 224, 232, 240, 248, 256 or 264, optionally wherein each CDR sequence independently comprises 1, 2, 3, 4, 5, 6, 7, or more substitutions and/or 1, 2, 3, 4, 5, 6, 7, or more deletions or additions, and/or has at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to a CDR sequence of any one of SEQ ID NOs: 217-219, 225-226, 233-234, 241-242, 249-251, or 257; and/or wherein the framework region comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17 or more substitutions and/or 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17 or more deletions or additions, and/or has at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the framework region of any one of SEQ ID NOs: 220-223, or 228.

26. The polypeptide of any one of embodiments 21-25, wherein the sdAb, optionally a V_{HH} domain, comprises or consists of an amino acid sequence having at least 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to any one of SEQ ID NOs: 224, 232, 240, 248, 256 or 264.

27. The polypeptide of any one of embodiments 1-8, wherein the sdAb, optionally a V_{HH} domain, comprises a CDR3 comprising or consisting of SEQ ID NO: 267.

28. The polypeptide of embodiment 27, wherein the sdAb, optionally a V_{HH} domain, comprises:

a CDR1 comprising or consisting of any one of SEQ ID NOs: 265 or 273,

a CDR2 comprising or consisting of any one of SEQ ID NOs: 266 or 274, and

a CDR3 comprising or consisting of SEQ ID NO: 267; optionally wherein the combination of CDR1, CDR2, and CDR3 is selected from one of the following combinations:

a.) SEQ ID NOs: 265, 266, and 267; and

b.) SEQ ID NOs: 273, 274, and 267;

optionally wherein each CDR sequence independently comprises 1, 2, 3, 4, 5, 6, 7, or more substitutions and/or 1, 2, 3, 4, 5, 6, 7, or more deletions or additions, and/or has at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to a sequence selected from any one of SEQ ID NOs: 265-267, or 273-274.

29. The polypeptide of any one of embodiments 27-28, wherein the sdAb, optionally a V_HH domain, comprises:

a CDR1 comprising or consisting of the amino acid sequence FTFX₁X₂X₃DMG (SEQ ID NO: 313), wherein X₁ is D, G or E; X₂ is V, S or G; and X₃ is S, N, or Q; or optionally a conservative substitution of any of the foregoing;

a CDR2 comprising or consisting of the amino acid sequence AIX₁X₂X₃X₄X₅X₆TYADSVKG (SEQ ID NO: 314), wherein X₁ is no amino acid or S; X₂ is G or S; X₃ is T, G, or S; X₄ is E or G; X₅ is G, D or E; and X₆ is D, T or H; or optionally a conservative substitution of any of the foregoing; and

a CDR3 comprising or consisting of the amino acid sequence SEQ ID NO: 315; wherein the combination of CDR1, CDR2 and CDR3 is not the combination of CDR1, CDR2 and CDR3 of mycV.3700 SEQ ID NOs: (163, 165 and 167).

30. The polypeptide of any one of embodiments 27-29, wherein the sdAb, optionally a V_HH domain, comprises a V_H FR1 comprising or consisting of SEQ ID NO: 268, a V_H FR2 comprising or consisting of SEQ ID NO: 269, a V_H FR3 comprising or consisting of any one of SEQ ID NOs: 270 or 278, and a V_H FR4 comprising or consisting of SEQ ID NO: 271, optionally wherein the combination of FR1, FR2, FR3, and FR4 is selected from one of the following combinations:

a.) SEQ ID NOs: 268, 269, 270, and 271; and

b.) SEQ ID NOs: 268, 269, 278, and 271;

optionally wherein each V_H framework region comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17 or more substitutions and/or 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17 or more deletions or additions, and/or has at least 90%, 91%,

92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to a sequence selected from any one of SEQ ID NOs: 268-271, or 278.

31. The polypeptide of any one of embodiments 27-30, wherein the sdAb, optionally a V_HH domain, comprises or consists of a sequence selected from any one of SEQ ID NOs: 272 or 280, optionally wherein each CDR sequence independently comprises 1, 2, 3, 4, 5, 6, 7, or more substitutions and/or 1, 2, 3, 4, 5, 6, 7, or more deletions or additions, and/or has at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to a CDR sequence of any one of SEQ ID NOs: 265-267, or 273-274, and/or wherein the framework region comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17 or more substitutions and/or 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17 or more deletions or additions, and/or has at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the framework region of any one of SEQ ID NOs: 268-271, or 278.

32. The polypeptide of any one of embodiments 27-31, wherein the sdAb, optionally a V_HH domain, comprises or consists of an amino acid sequence having at least 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to any one of SEQ ID NOs: 272 or 280.

33. The polypeptide of any one of embodiments 1-8, wherein the sdAb, optionally a V_HH domain, comprises a CDR3 comprising or consisting of SEQ ID NO: 283.

34. The polypeptide of embodiment 33, wherein the sdAb, optionally a V_HH domain, comprises:

a CDR1 comprising or consisting of any one of SEQ ID NOs: 281 or 289,

a CDR2 comprising or consisting of any one of SEQ ID NOs: 282 or 290, and

a CDR3 comprising or consisting of SEQ ID NO: 283; optionally wherein the combination of CDR1, CDR2, and CDR3 is selected from one of the following combinations:

a.) SEQ ID NOs: 281, 282, and 283; and

b.) SEQ ID NOs: 289, 290, and 283;

optionally wherein each CDR sequence independently comprises 1, 2, 3, 4, 5, 6, 7, or more substitutions and/or 1, 2, 3, 4, 5, 6, 7, or more deletions or additions, and/or has at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to a sequence selected from any one of SEQ ID NOs: 281-283, and 289-290.

35. The polypeptide of any one of embodiments 33-34, wherein the sdAb, optionally a V_HH domain, comprises:

a CDR1 comprising or consisting of the amino acid sequence FTFX₁X₂X₃AMX₄ (SEQ ID NO: 316), wherein X₁ is S or D; X₂ is S, E or D; X₃ is Y or H, and X₄ is S or G; or optionally a conservative substitution of any of the foregoing;

a CDR2 comprising or consisting of the amino acid sequence AISX₁X₂X₃X₄X₅TYYADSVKG (SEQ ID NO: 317), wherein X₁ G or A; X₂ is S or D; X₃ is G or A; X₄ is G or S; and X₅ is S, V or T; or optionally a conservative substitution of any of the foregoing; and

a CDR3 comprising or consisting of the amino acid sequence SEQ ID NO: 318;

wherein the combination of CDR1, CDR2 and CDR3 is not the combination of CDR1, CDR2 and CDR3 of mycV.6600 SEQ ID NOs: (171, 173 and 175).

36. The polypeptide of any one of embodiments 33-35, wherein the sdAb, optionally a V_HH domain, comprises a V_H FR1 comprising or consisting of SEQ ID NO: 284, a V_H FR2 comprising or consisting of SEQ ID NO: 285, a V_H FR3 comprising or consisting of SEQ ID NO: 286, and a V_H FR4 comprising or consisting of SEQ ID NO: 287, optionally wherein the combination of FR1, FR2, FR3, and FR4 is 284, 285, 286, and 287;

optionally wherein each V_H framework region comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17 or more substitutions and/or 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17 or more deletions or additions, and/or has at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to a sequence selected from any one of SEQ ID NOs: 284, 285, 286, and 287.

37. The polypeptide of any one of embodiments 33-36, wherein the sdAb, optionally a V_HH domain, comprises or consists of a sequence selected from any one of SEQ ID NOs: 288 or 296, optionally wherein each CDR sequence independently comprises 1, 2, 3, 4, 5, 6, 7, or more substitutions and/or 1, 2, 3, 4, 5, 6, 7, or more deletions or additions, and/or has at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to a CDR sequence of any one of SEQ ID NOs: 281-283, and 289-290, and/or wherein the framework region comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17 or more substitutions and/or 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17 or more deletions or additions, and/or has at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the framework region of any one of SEQ ID NOs: 284, 285, 286, and 287.

38. The polypeptide of any one of embodiments 33-37, wherein the sdAb, optionally a V_HH domain, comprises or consists of an amino acid sequence having at least

80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to any one of SEQ ID NOs: 288 or 296.

39. The polypeptide of any one of embodiments 9-38, wherein the sdAb, optionally a V_HH domain, comprises:

a FR1 comprising or consisting of the amino acid sequence X₁VX₂LX₃ESGGGLVQPGGSLRLSX₄AASG (SEQ ID NO: 300), wherein X₁ is E or G; X₂ is Q or R; X₃ is L or V; and X₄ is A or C; or optionally a conservative substitution of any of the foregoing; optionally wherein X₁ is E, X₂ is Q, X₃ is L, and X₄ is A or C;

a FR2 comprising or consisting of the amino acid sequence SEQ ID NO: 301;

a FR3 comprising or consisting of the amino acid sequence RFX₁ISRDN SKNTLYLQMNX₂LRAX₃DTAVYYX₄ (SEQ ID NO: 302), wherein X₁ is T or S; X₂ is S or G; X₃ is E or A; X₄ is A, V, or C; or optionally a conservative substitution of any of the foregoing; optionally wherein X₁ is R, X₂ is S, X₃ is E, and X₄ is A or C; and

a FR4 comprising or consisting of the amino acid sequence WGX₁GTQVTVSS (SEQ ID NO: 303), wherein X₁ is Q or R; or optionally a conservative substitution of any of the foregoing; optionally wherein X₁ is Q.

40. The polypeptide of any one of embodiments 1-39, wherein the sdAb comprises a V_HH domain with a substitution at C22 of FR1 and/or C30 of FR3, optionally wherein the substitution is independently selected from a C to A and a C to V substitution.

41. The polypeptide of any one of embodiments 1-40, wherein the sdAb has a K_d value of less than 1×10^{-7} and optionally at least 1×10^{-10} , optionally wherein the K_d is 1.49×10^{-7} to 5.56×10^{-9} .

42. The polypeptide of any one of embodiments 1-41, wherein the sdAb has a Poly Specificity Reagent (PSR) score of less than 1, optionally less than 0.43, optionally less than 0.26.

43. The polypeptide of any one of embodiments 1-42, wherein the sdAb has a PSR score of at least 0.01, optionally at least 0.26, optionally at least 0.43.

44. The polypeptide of any one of embodiments 1-43, wherein the sdAb has a PSR score of 1 to 0.01, 0.43 to 0.01, or 0.26 to 0.01.

45. The polypeptide of any one of embodiments 1-44, wherein the sdAb has a melting temperature (T_M) of at least 40°C, optionally at least 45.5°C, optionally at least 64°C.

46. The polypeptide of any one of embodiments 1-45, wherein the sdAb has a T_M of less than 70°C, optionally wherein the T_M is 45°C to 65°C.

47. The polypeptide of any one of embodiments 1-46, wherein the sdAb binds, or binds at least in part, to at least a portion of amino acids 410-419 of MYC (SEQ ID NO: 1) and/or to a peptide consisting of SEQ ID NO: 769.

48. The polypeptide of any one of embodiments 1-47, wherein the sdAb does not comprise or consist of any one of SEQ ID NO: 138-176 and 641-768.

49. A polynucleotide encoding the polypeptide of any one of embodiments 1-48, optionally wherein the polynucleotide comprises or consists of any one of SEQ ID NOs: 626-640.

50. A bifunctional polypeptide that promotes proteasome-mediated degradation of at least one target protein, the bifunctional polypeptide comprising:

- a) a first targeting moiety comprising or consisting of the polypeptide any one of embodiments 1-48, wherein the polypeptide comprises or consists of the anti-Myc sdAb;
- b) a ubiquitin-proteasome system recruiting domain (URD),
- c) optionally a first linker peptide, optionally wherein the first linker peptide is positioned between the first targeting moiety and the URD,
- d) optionally one or more localization peptide sequences,

wherein at least one target protein is MYC, optionally c-MYC, and wherein proximity of the bifunctional polypeptide to at the least one target protein through binding of the targeting moiety induces ubiquitination of the at least one target protein via the URD, thereby promoting proteasome-mediated degradation of the at least one target protein.

51. The bifunctional polypeptide of embodiment 50, wherein the URD is derived from at least one E3 ubiquitin ligase.

52. The bifunctional polypeptide of any one of embodiments 50-51, wherein the URD is derived from a RING family E3 ubiquitin ligase, an H-box family E3 ubiquitin ligase or a viral analogue of an E3 ubiquitin ligase.

53. The bifunctional polypeptide of any one of embodiments 50-52, wherein the URD is selected from:

- a) the URD derived from a protein which localizes to the cytoplasm, optionally selected from FBXW7 beta and Keap1;

- b) the URD derived from a protein which localizes to the nucleus, optionally selected from FBXW7 alpha, RNF165, and SPOP, optionally SPOP.2; and
- c) the URD derived from a protein which localizes to the cytoplasm and the nucleus, optionally selected from beta-TRCP, CHIP, RNF114, RNF125, RNF138, RNF166, NHLRC1, CBL-b (Y363E), TRIM21, E6AP, DCAF1 VIF, RNF11, RNF111, RNF115, RNF12, RNF128, RNF149, RNF152, RNF165, RNF182, RNF20, RNF25, RNF4, RNF6, TRIM32, ZNRF1, ZNRF4;
- d) the URD derived from an E3 ligase selected from the group consisting of NHLRC1, RNF125, RNF165, RNF4, and ZNRF1, optionally NHLRC1, and RNF4 or
- e) a truncation or fragment of the URD that retains ubiquitin-proteasome recruiting activity, optionally NHLRC1.1, NHLRC1.6, NHLRC1.7, NHLRC1.8, RNF125.2, RNF165.1, RNF4.1, RNF4.2, RNF4.3, RNF4.4, RNF4.5, RNF4.6, RNF4.7, RNF4.8 or ZNRF1.1, optionally NHLRC1.1, or RNF4.1;

optionally wherein the protein is an E3 ubiquitin ligase.

54. The bifunctional polypeptide of any one of embodiments 50-51, wherein the URD is derived from a RING family E3 ubiquitin ligase, a cullin family E3 ubiquitin ligase, a homologous to E6AP carboxyl terminus (HECT) family E3 ubiquitin ligase, and a viral homolog of an E3 ubiquitin ligase.

55. The bifunctional polypeptide of any one of embodiments 50-51, wherein the URD is derived from an E3 ubiquitin ligase selected from the group consisting of CHIP, DCAF1, E6AP, FBXW7-alpha, FBXW7-beta, Keap1, NHLRC1, RNF4, RNF6, RNF11, RNF12, RNF20, RNF25, RNF111, RNF114, RNF115, RNF125, RNF128, RNF138, RNF149, RNF152, RNF165, RNF166, RNF182, SPOP, beta-TRCP, TRIM21, TRIM32, VIF, ZNRF1, ZNRF4, and CBL-b (Y363E), optionally wherein the portion is selected from the group consisting of CBLb.1, CHIP.1, DCAF1.1, E6AP.1, FBXW7a.1, FBXW7a.2, FBXW7a.3, FBXW7a.4, FBXW7a.5, FBXW7a.6, FBXW7a.7, FBXW7a.8, FBXW7a.9, FBXW7b, FBXW7b.2, FBXW7b.3, FBXW7b.4, Keap1.1, NHLRC1.1, NHLRC1.2, NHLRC1.3, NHLRC1.4, NHLRC1.5, RNF4.1, RNF6.1, RNF6.2, RNF11.1, RNF12.1, RNF12.2, RNF20.1, RNF25.1, RNF111.1, RNF114.1, RNF115.1, RNF125.1, RNF125.2, RNF125.3, RNF125.4, RNF125.5, RNF125.6, RNF125.7, RNF128.1, RNF138.1, RNF149.1, RNF152.1, RNF165.1, RNF166.1, RNF182.1, RNF182.2, SPOP.1, SPOP.2, SPOP.3, bTRCP.1, TRIM21.1, TRIM21.2, TRIM21.3, TRIM32.1, VIF.1, ZNRF1.1, and ZNRF4.1.

56. The bifunctional polypeptide of any one of embodiments 50-51, wherein the URD is derived from a monomeric RING family E3 ligase.

57. The bifunctional polypeptide of any one of embodiments 50-51, wherein the URD is derived from an E3 ligase selected from the group consisting of NHLRC1, RNF11, RNF111, RNF114, RNF115, RNF12, RNF125, RNF128, RNF138, RNF149, RNF152, RNF165, RNF166, RNF182, RNF20, RNF25, RNF4, RNF6, ZNRF1, ZNRF4, and CBL-b (Y363E), or optionally selected from the group consisting of RNF125, NHLRC1, RNF4, RNF6, RNF12, RNF138 and ZNRF1.

58. The bifunctional polypeptide of any one of embodiments 50-51, wherein the URD is selected from the group consisting of NHLRC1.1, NHLRC1.2, NHLRC1.3, NHLRC1.4, NHLRC1.5, RNF11.1, RNF111.1, RNF114.1, RNF115.1, RNF12.1, RNF12.2, RNF125.1, RNF125.2, RNF125.3, RNF125.4, RNF125.5, RNF125.6, RNF125.7, RNF128.1, RNF138.1, RNF149.1, RNF152.1, RNF165.1, RNF166.1, RNF182.1, RNF182.2, RNF20.1, RNF25.1, RNF4.1, RNF6.1, RNF6.2, ZNRF1.1, ZNRF4.1, and CBLb.1, optionally selected from the group consisting of NHLRC1.1, NHLRC1.2, NHLRC1.3, NHLRC1.4, NHLRC1.5, RNF125.1, RNF125.2, RNF125.3, RNF125.4, RNF125.5, RNF125.6, RNF125.7, RNF4.1, RNF6.1, RNF6.2, RNF12.1, RNF12.2, RNF138.1, RNF165.1, and ZNRF1.1.

59. The bifunctional polypeptide of any one of embodiments 50-51, wherein the URD is derived from a cullin family E3 ligase.

60. The bifunctional polypeptide of any one of embodiments 50-51, wherein the URD is derived from an E3 ligase selected from the group consisting of DCAF1, beta-TRCP, FBXW7-alpha, FBXW7-beta, Keap1, and SPOP, optionally selected from the group consisting of beta-TRCP, FBXW7-alpha, and FBXW7-beta.

61. The bifunctional polypeptide of any one of embodiments 50-51, wherein the URD is selected from the group consisting of DCAF1.1, bTRCP.1, FBXW7a.1, FBXW7a.2, FBXW7a.3, FBXW7a.4, FBXW7a.5, FBXW7a.6, FBXW7a.7, FBXW7a.8, FBXW7a.9, FBXW7b, FBXW7b.2, FBXW7b.3, FBXW7b.4, Keap1.1, SPOP.1, SPOP.2, and SPOP.3.

62. The bifunctional polypeptide of any one of embodiments 50-51, wherein the URD is derived from an E3 ligase NHLRC1, DCAF1, RNF125, RNF165, RNF4, and SPOP, optionally NHLRC1, RNF125, RNF165, RNF4, and SPOP, optionally NHLRC1, RNF125, RNF165, and RNF4, and/or wherein the URD is selected from the group consisting of NHLRC1.1, DCAF1.1, RNF125.2, RNF125.3, RNF125.4, RNF125.5, RNF125.6, RNF165.1, RNF4.1, SPOP.2, and SPOP.3, optionally NHLRC1.1, RNF125.2, RNF125.3, RNF125.4,

RNF125.5, RNF125.6, RNF165.1, RNF4.1, SPOP.2, and SPOP.3, optionally NHLRC1.1, RNF125.2, RNF165.1, and RNF4.1.

63. The bifunctional polypeptide of any one of embodiments 50-51, wherein the URD is derived from an E3 ligase selected from the group consisting of NHLRC1, RNF125, RNF165, RNF4, and ZNRF1, optionally NHLRC1, RNF125, RNF165, and RNF4, optionally wherein the URD comprises or consists of NHLRC1.1, RNF125.2, RNF165.1, RNF4.1, or ZNRF1.1, optionally wherein the URD comprises or consists of NHLRC1.1, RNF125.2, RNF165.1, or RNF4.1.

64. The bifunctional polypeptide of any one of embodiments 50-63, wherein the number of amino acids in the URD is, is about, or is less than, 80, 75, 70, 65, 60, 55, 50, 45, 40, 35, 30, 25, 20, 15, 10, 5, 4, 3, 2, or 1%, of the number of amino acids in the wild-type full-length protein from which it is derived, or a range defined by any two of the preceding values, optionally 15-80, 15-50, 25-80, 25-50, 30-60, 1-80, 2-80, 1-70, 2-70, 2-65%, optionally 15-50%, optionally 2-61%.

65. The bifunctional polypeptide of any one of embodiments 50-64, wherein the URD is less than or equal to 370, 369, 368, 367, 366, 365, 364, 363, 362, 361, 360, 300, 250, 200, 175, 170, 165, 160, 155, 150, 145, 140, 135, 130, 129, 128, 127, 126, 125, 124, 123, 120, 115, 110, 105, 100, 95, 90, 85, 80, 75, 70, 65, 60, 55, 50, 47, 46, 45, 44, 43, 42, 41, 40, 39, 38, 37, 36, 35, 34, 33, 32, 31, 30, 25, or 20 amino acids long, or a range defined by any two of the preceding values, optionally 20-370, 20-150, 20-100, 20-80, 30-150, 30-100, 50-150, or 50-100, optionally 20-100 amino acids long, optionally 40-130 or 44-126.

66. The bifunctional polypeptide of any one of embodiments 50-65, wherein the URD comprises or consists of an amino acid sequence having at least 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to any one of SEQ ID NO: 39-113, optionally any one of SEQ ID NO: 54-113, optionally any one of SEQ ID NO: 76, 88, 98, 104 and 111, optionally any one of SEQ ID NO: 76, 88, 98, and 104, or a truncation or fragment thereof that retains ubiquitin-proteasome recruiting activity.

67. The bifunctional polypeptide of any one of embodiments 50-66, further comprising one or more linker peptides, wherein the linker peptide is positioned between one or more of: the targeting moiety and the URD; a localization peptide and the targeting moiety; a localization peptide and the URD; and/or a localization peptide and an epitope tag, optionally an HA tag.

68. The bifunctional polypeptide of any one of embodiments 50-67, comprising a linker peptide positioned between the targeting moiety and the URD.

69. The bifunctional polypeptide of any one of embodiments 50-68, wherein the one or more linker peptides is independently 1, 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 or 30 amino acids in length, or a range defined by any two of the preceding values, optionally 0-30, 1-30, 2-30, 0-20, 1-20, 2-20, 0-15, 0-12, 0-10, 1-15, 1-12, 1-10, 2-15, 2-12, 2-10, 3-15, 3-12, or 3-10 amino acids in length, optionally 2-30 amino acids in length.

70. The bifunctional polypeptide any one of embodiments 50-69, wherein the at least one or more linker peptides comprise or consist of glycine and serine.

71. The bifunctional polypeptide any one of embodiments 50-70, wherein the one or more linker peptides comprises or consists of one or more alpha helixes, optionally flanked by one or more glycine and/or a serine residues.

72. The bifunctional polypeptide any one of embodiments 50-71, wherein the one or more linker peptides independently comprise or consist of the amino acid sequence of any one of SEQ ID NO: 345-383 (345-351, GS, and 353-383), optionally SEQ ID N: 347-352 (SEQ ID NO: 347-351 and GS).

73. The bifunctional polypeptide of any one of embodiments 50-72, further comprising one or more localization peptide sequences.

74. The bifunctional polypeptide of embodiment 73, wherein the one or more localization peptide sequences direct the bifunctional polypeptide to a subcellular compartment or compartments, optionally wherein the subcellular compartment is the nucleus and/or the cytoplasm.

75. The bifunctional polypeptide of embodiment 73 or 74, wherein the one or more localization peptide sequences direct the bifunctional polypeptide to a desired subcellular compartment or compartments, optionally wherein the one or more localization peptide sequences are selected or designed to direct the bifunctional polypeptide to a desired subcellular compartment or compartments.

76. The bifunctional polypeptide of any one of embodiments 73-75, wherein the one or more localization peptide sequences comprise or consist of a nuclear localization signal (NLS) peptide and/or a nuclear export signal (NES) peptide, optionally wherein the localization peptide comprises or consists of an amino acid sequence of any one of SEQ ID NO: 114-127.

77. The bifunctional polypeptide of any one of embodiments 73-76, wherein the one or more localization peptide sequences comprise or consist of an endogenous localization peptide sequence of the first targeting moiety and/or the URD.

78. The bifunctional polypeptide of embodiment 77, wherein the one or more localization peptide sequences comprise or consist of an endogenous localization peptide sequence of the URD.

79. The bifunctional polypeptide of embodiment 78, wherein the endogenous localization peptide sequence is from a URD selected from:

a URD derived from a protein which localizes to the cytoplasm, optionally selected from FBXW7 beta and Keap1;

a URD derived from a protein which localizes to the nucleus, optionally selected from FBXW7 alpha, RNF165, and SPOP; and

a URD derived from a protein which localizes to the cytoplasm and the nucleus, optionally selected from beta-TRCP, CHIP, RNF114, RNF125, RNF138, RNF166, NHLRC1, CBL-b (Y363E), TRIM21, E6AP, DCAF1 VIF, RNF11, RNF111, RNF115, RNF12, RNF128, RNF149, RNF152, RNF165, RNF182, RNF20, RNF25, RNF4, RNF6, TRIM32, ZNRF1, ZNRF4.

80. The bifunctional polypeptide of any one of embodiments 73-79, wherein the one or more localization peptide sequences comprise or consist of an endogenous localization peptide sequence of the first targeting moiety and/or the URD that is modified, wherein the modification alters the subcellular compartment or compartments to which the bifunctional polypeptide is directed as compared to the native endogenous localization peptide sequence.

81. The bifunctional polypeptide of embodiment 80, wherein the modification comprises or consists of a substitution, truncation or deletion of an endogenous localization peptide sequence.

82. The bifunctional polypeptide of embodiment 80 or 81, wherein:

- a. the native endogenous localization peptide sequence directs the polypeptide to the nucleus, and the modified endogenous localization peptide sequence directs the polypeptide to the cytoplasm;
- b. the native endogenous localization peptide sequence directs the polypeptide to the cytoplasm, and the modified endogenous localization peptide sequence directs the polypeptide to the nucleus;

- c. the native endogenous localization peptide sequence directs the polypeptide to the nucleus and the cytoplasm, and the modified endogenous localization peptide sequence directs the polypeptide to only the cytoplasm;
- d. the native endogenous localization peptide sequence directs the polypeptide to the nucleus and the cytoplasm, and the modified endogenous localization peptide sequence directs the polypeptide to only the nucleus;
- e. the native endogenous localization peptide sequence directs the polypeptide to the nucleus, and the modified endogenous localization peptide sequence directs the polypeptide to the nucleus and the cytoplasm; or
- f. the native endogenous localization peptide sequence directs the polypeptide to the cytoplasm, and the modified endogenous localization peptide sequence directs the polypeptide to the nucleus and the cytoplasm.

83. The bifunctional polypeptide of embodiment 73-82, wherein the one or more localization peptide sequences comprises or consists of an endogenous localization peptide sequence of the URD that is modified, wherein the native endogenous localization peptide sequence directs the polypeptide to the nucleus, and the modified endogenous localization peptide sequence directs the polypeptide to the cytoplasm.

84. The bifunctional polypeptide of any one of embodiments 80-83, wherein the modified endogenous localization peptide sequence comprises or consists of a modified SPOP URD endogenous localization peptide sequence, optionally wherein the URD is SPOP.3.

85. The bifunctional polypeptide of any one of embodiments 73-84, wherein the one or more localization peptide sequences comprise or consist of an exogenous localization peptide sequence.

86. The bifunctional polypeptide of any one of embodiments 73-85, wherein the one or more localization peptide sequences comprise or consist of an NLS peptide that is, or is derived from the NLS of MYC, simian virus 40 (SV40), SPOP, nucleoplasmin, 53BP1, or Hrp1.

87. The bifunctional polypeptide of any one of embodiments 73-86, wherein the one or more localization peptide sequences comprise or consist of an NLS or NES comprising or consisting of an amino acid sequence having at least 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to any one of SEQ ID NO: 114-127.

88. The bifunctional polypeptide of any one of embodiments 73-87, wherein the bifunctional polypeptide comprises or consists of two localization peptide sequences, optionally wherein the bifunctional polypeptide comprises or consists of 3, 4, or more localization peptide sequences, optionally wherein the bifunctional polypeptide comprises or consists of 2, 3, 4 or more copies of the same localization sequence in series.

89. The bifunctional polypeptide of any one of embodiments 73-88, comprising one or more localization peptide sequences located at a location selected from the group consisting of: the N-terminus of the bifunctional polypeptide; the C-terminus of the bifunctional polypeptide; internally within the bifunctional polypeptide; as part of the URD, optionally an endogenous portion of the URD; as a part of the first targeting moiety, optionally an endogenous portion of the first targeting moiety; and a combination of any of the foregoing.

90. The bifunctional polypeptide of any one of embodiments 73-89, wherein a first localization peptide sequence is located at the N-terminus of the bifunctional polypeptide and a second localization peptide sequence is located at the C-terminus of the bifunctional polypeptide.

91. The bifunctional polypeptide of any one of embodiments 73-90, further comprising at least one NLS peptide, wherein the at least one NLS peptide is located at the N-terminus of the bifunctional polypeptide, the C-terminus of the bifunctional polypeptide, is part of the URD, optionally an endogenous NLS of the URD, or is part of the first targeting moiety, optionally an endogenous NLS of the first targeting moiety.

92. The bifunctional polypeptide of embodiment 91, wherein a first NLS peptide is located at the N-terminus of the bifunctional polypeptide and a second NLS peptide is located at the C-terminus of the bifunctional polypeptide.

93. The bifunctional polypeptide of any one of embodiments 50-92, wherein the orientation of the targeting moiety relative to the URD is the same orientation as compared to the naturally occurring substrate recognition domain of the protein from which the URD is derived.

94. The bifunctional polypeptide of any one of embodiments 50-93, wherein the orientation of the first targeting moiety relative to the URD is the opposite orientation as compared to the naturally occurring substrate recognition domain of the protein from which the URD is derived.

95. The bifunctional polypeptide of any one of embodiments 50-94, wherein the targeting moiety is N-terminal relative to the URD.

96. The bifunctional polypeptide of any one of embodiments 50-94, wherein the targeting moiety is C-terminal relative to the URD.

97. The bifunctional polypeptide of any one of embodiments 50-96, further comprising an epitope tag, optionally a hemagglutinin (HA) tag, optionally wherein the HA tag comprises or consists of the amino acid sequence of SEQ ID NO: 384, wherein the epitope tag can be used to visualize the dual-specific bifunctional polypeptide.

98. The bifunctional polypeptide of any one of embodiments 50-96, wherein the bifunctional polypeptide does not comprise an epitope tag, optionally a hemagglutinin (HA) tag, optionally wherein the bifunctional polypeptide does not comprise an HA tag comprising or consisting of the amino acid sequence of SEQ ID NO: 384.

99. The bifunctional polypeptide of any one of embodiments 50-98, wherein the bifunctional polypeptide is less than or equal to 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, or 800 amino acids long, or a range defined by any two of the preceding values, optionally 50-800, 50-650, 50-500, 50-400, 70-800, 70-650, 70-500, 70-400, 70-300, 70-200, 80-800, 80-650, 80-500, 80-400, 80-300, or 80-200 amino acids long, optionally 90-550, 150-550, or 80-450 amino acids long.

100. The bifunctional polypeptide of any one of embodiments 50-99, wherein the URD is not derived from CHIP, optionally wherein the URD does not comprise or consist of CHIP.1, optionally wherein the URD does not comprise or consist of SEQ ID NO: 54.

101. A polynucleotide encoding a bifunctional polypeptide comprising or consisting of the bifunctional polypeptide of any one of embodiments 50-100.

102. The bifunctional polypeptide of any one of embodiments 50-100, wherein the at least one target protein comprises MYC, optionally c-MYC.

103. The bifunctional polypeptide of embodiment 102, wherein the bifunctional polypeptide comprises a localization peptide sequence which directs the bifunctional polypeptide to the nucleus of a cell, optionally wherein the localization peptide sequence comprises or consists of the NLS sequence selected from the group of the NLS sequences of MYC, SPOP, Hrp1, SV40, 53BP1, and nucleoplasmin, optionally MYC, SPOP, Hrp1, optionally wherein the localization peptide sequence comprises or consists of a NLS sequence selected from the group of the NLS sequence of SPOP, optionally SPNLS.2, MYC, optionally mycNLS, and Hrp1, optionally hrpNLS.

104. The bifunctional polypeptide of embodiment 103, wherein the NLS comprises or consists of a sequence selected from any one of SEQ ID NOs: 114-127, optionally wherein the NLS comprises or consists of a sequence selected from any one of SEQ ID NOs: 114, 122 and 116.

105. The bifunctional polypeptide of any one of embodiments 102-104, wherein the URD is the URD of NHLRC1, RNF125, RNF165, RNF4, or ZNRF1, or a truncation or fragment thereof that retains ubiquitin-proteasome recruiting activity, optionally wherein the URD comprises or consists of NHLRC1.1, RNF125.2, RNF165.1, RNF4.1, or ZNRF1.1, optionally wherein the URD comprises or consists of NHLRC1.1 RNF125.2, RNF165.1, or RNF4.1.

106. The bifunctional polypeptide of any one of embodiments 102-105, wherein the URD comprises or consists of a sequence selected from any one of SEQ ID NOs: 76-80, 87-93, 98, 104 and 111, optionally wherein the URD comprises or consists of a sequence selected from any one of SEQ ID NOs: 76, 88, 98, 104, and 111, or optionally SEQ ID NOs: 76, 88, 98, and 104.

107. The bifunctional polypeptide of any one of embodiments 102-106, wherein the first targeting moiety comprises or consists of the polypeptide of any one of embodiments 1-48, wherein the polypeptide comprises or consists of the anti-Myc sdAb.

108. The bifunctional polypeptide of embodiment 107, wherein the first targeting moiety comprises or consists of a V_{HH} domain.

109. The bifunctional polypeptide of any one of embodiments 102-108, wherein the bifunctional polypeptide comprises or consists of an amino acid sequence having at least 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to any one of SEQ ID NOs: 428-561, optionally wherein the sequence lacks the HA tag sequence (SEQ ID NO: 384).

110. The bifunctional polypeptide of any one of embodiments 102-109, wherein the bifunctional polypeptide comprises or consists of an amino acid sequence having at least 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to any one of the SEQ ID NOs in Table 27, optionally excluding any one of the SEQ ID NOs listed as having a MYC degradation Grade of D, optionally wherein the sequence lacks the HA tag sequence (SEQ ID NO: 384).

111. The bifunctional polypeptide of any one of embodiments 102-110, wherein the bifunctional polypeptide comprises or consists of an amino acid sequence of any one of the

SEQ ID NOs in Table 27 listed as having a MYC degradation Grade of A, and/or MYC degradation Grade of B, optionally wherein the sequence lacks the HA tag sequence (SEQ ID NO: 384).

112. The bifunctional polypeptide of any one of embodiments 102-111, wherein the bifunctional polypeptide does not comprise or consist of an amino acid sequence of any one of the SEQ ID NOs in Table 27 listed as having a MYC degradation Grade of C and/or D, optionally wherein the sequence lacks the HA tag sequence (SEQ ID NO: 384).

113. The bifunctional polypeptide of any one of embodiments 102-112, wherein the URD is not derived from CHIP, optionally wherein the URD does not comprise or consist of CHIP.1, optionally wherein the URD does not comprise or consist of SEQ ID NO: 54.

114. A polynucleotide encoding a bifunctional polypeptide comprising or consisting of the bifunctional polypeptide of any one of embodiments 102-113.

115. The bifunctional polypeptide of any one of embodiments 50-113, wherein the bifunctional polypeptide is a dual-specific bifunctional polypeptide further comprising:

- a. a second targeting moiety that is capable of binding to at least one second target protein, optionally an intracellular target protein,
- b. optionally a second linker peptide, wherein the second linker peptide is positioned between the second targeting moiety and the URD or between the first targeting moiety and the second targeting moiety, and

wherein proximity of the dual-specific bifunctional polypeptide to at least one target protein through binding of the first targeting moiety and/or the second targeting moiety induces ubiquitination of the at least one target protein via the URD, thereby promoting proteasome-mediated degradation of the at least one target protein.

116. The dual-specific bifunctional polypeptide of embodiment 115, wherein the first targeting moiety binds to and/or is designed to bind a first target protein and the second targeting moiety binds to and/or is designed to bind a second target protein.

117. The dual-specific bifunctional polypeptide of embodiment 115, wherein the first targeting moiety and the second targeting moiety bind to and/or are designed to bind to the same target protein.

118. The dual-specific bifunctional polypeptide of embodiment 117, wherein the first targeting moiety binds to and/or is capable of binding to a first binding site of the target protein and the second targeting moiety binds to and/or is capable of binding to a second binding site of the target protein.

119. The dual-specific bifunctional polypeptide of embodiment 117, wherein the first targeting moiety and second targeting moiety bind to and/or are capable of binding to the same binding site of the target protein, wherein the dual-specific bifunctional polypeptide engages and/or is capable of engaging at least two molecules of the at least one target protein.

120. The dual-specific bifunctional polypeptide of any one of embodiments 115-119, wherein the second targeting moiety is less than or equal to 260, 259, 258, 257, 256, 255, 254, 253, 252, 251, 250, 245, 240, 235, 230, 225, 220, 215, 210, 205, 200, 195, 190, 185, 180, 175, 170, 165, 160, 155, 150, 145, 140, 135, 130, 129, 128, 127, 126, 125, 124, 123, 122, 121, 120, 119, 118, 117, 116, 115, 114, 113, 112, 110, 109, 108, 107, 106, 105, 104, 103, 102, 101, 100, 99, 98, 97, 96, 95, 94, 93, 92, 91, 90, 89, 88, 87, 86, 85, 84, 83, 82, 81, 80, 75, 70, 65, 60, 55, 54, 53, 52, 51, 50, 49, 48, 47, 46, 45, 44, 43, 42, 41, 40, 39, 38, 37, 36, 35, 34, 33, 32, 31, 30, 29, 28, 27, 26, 25, 24, 23, 22, 21, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, or 10 amino acids long, or a range defined by any two of the preceding values, optionally 10-260, 10-200, 10-130, 25-260, 25-200, 25-130, 35-260, 35-200, 35-130, 50-260, 50-200, or 50-130 amino acids long.

121. The dual-specific bifunctional polypeptide of any one of embodiments 115-120, wherein the second targeting moiety comprises or consists of an endogenous binding partner of the at least one target protein, antibody, Fab, F(ab')₂, Fab', scFv, single domain antibody (sdAb), V_H domain, V_L domain, V_{HH}, V_{NAR}, diabody, intrabody, DARPIn, monobody, affibody, avimer, or any fragment or derivative thereof.

122. The dual-specific bifunctional polypeptide of any one of embodiments 115-121, wherein the second targeting moiety comprises or consists of an endogenous protein binding partner of the target protein, a fragment and/or a derivative thereof.

123. The dual-specific bifunctional polypeptide of embodiment 122, wherein the second targeting moiety is, or is less than, 100, 99, 98, 97, 96, 95, 94, 93, 92, 91, 90, 89, 88, 87, 86, 85, 84, 83, 82, 81, 80, 75, 70, 65, 60, 55, 54, 53, 52, 51, 50, 49, 48, 47, 46, 45, 44, 43, 42, 41, 40, 39, 38, 37, 36, 35, 34, 33, 32, 31, 30, 29, 28, 27, 26, 25, 24, 23, 22, 21, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, or 10 amino acids long, or a range defined by any two of the preceding values, optionally 10-100, 10-25, 14-100, 14-25, 30-100, 30-50, 30-45, or 80-100 amino acids long.

124. The dual-specific bifunctional polypeptide of any one of embodiments 115-123, wherein the second targeting moiety comprises or consists of an sdAb, optionally comprising or consisting of either a V_H domain or a V_L domain of an IgG antibody, or a V_{HH}, V_H, or V_{NAR}

domain of a heavy chain antibody (HcAb), optionally wherein the sdAb comprises or consists of a V_HH or V_L domain.

125. The dual-specific bifunctional polypeptide of embodiment 124, wherein the second targeting moiety is, or is less than, 170, 165, 160, 155, 150, 145, 140, 135, 130, 129, 128, 127, 126, 125, 124, 123, 122, 121, 120, 119, 118, 117, 116, 115, 114, 113, 112, 110, 109, 108, 107, 106, 105, 104, 103, 102, 101, 100, 99, 98, 97, 96, 95, 94, 93, 92, 91, or 90, amino acids long, or a range defined by any two of the preceding values, optionally 90-150, 90-130, 110-150, 110-130, 110-125, or 114-124 amino acids long.

126. The dual-specific bifunctional polypeptide of embodiment 115-125, wherein the second targeting moiety comprises or consists of an scFv, optionally wherein the targeting moiety is, or is less than, 260, 259, 258, 257, 256, 255, 254, 253, 252, 251, 250, 245, 240, 235, 230, 225, 220, 215, 210, 205, 200, 195, or 190 amino acids long, or a range defined by any two of the preceding values, optionally 190-260, 200-260, or 230-260 amino acids long.

127. The dual-specific bifunctional polypeptide of any of embodiments 115-126, wherein the at least one second target protein is selected from MYC, optionally c-MYC, beta catenin 1 (CTNNB1) and proliferating cell nuclear antigen (PCNA).

128. The dual-specific bifunctional polypeptide of any one of embodiments 115-127, wherein the at least one second target protein is a wild-type and/or a mutant form of the target protein, optionally wherein the targeting moiety preferentially or selectively binds the mutant form of the target protein relative to the wild-type form of the target protein.

129. The dual-specific bifunctional polypeptide of embodiment 128, wherein the mutant form of the target protein is CTNNB1 (SEQ ID NO: 2) comprising one or more mutations selected from: D32Y; D32N; D32V; D32G; S33Y; S33L; S33F; S33C; S33P; G34V; G34E; I35S; H36P; S37C; S37P; S37P and D207G; T41A; T41I; T42_K49del; S45F; and Y670X wherein X indicates termination; S45C; S45F; S45P; S45Y; and S45del.

130. The dual-specific bifunctional polypeptide of any one of embodiments 115-129, wherein the second targeting moiety bind, or bind at least in part, to at least a portion of:

- a) the DNA binding domain of MYC;
- b) the armadillo domain of CTNNB1; or
- c) the p21 binding domain of PCNA.

131. The dual-specific bifunctional polypeptide of any one of embodiments 115-130, wherein the second targeting moiety binds, or binds at least in part, to at least a portion of the target protein selected from the portion consisting of:

- a) amino acids 54-406 of MYC (SEQ ID NO: 1),
- b) amino acids 354-406 of MYC (SEQ ID NO: 1),
- c) amino acids 408-437 of MYC (SEQ ID NO: 1),
- d) amino acids 410-419 of MYC (SEQ ID NO: 1),
- e) amino acids 150-663 of CTNNB1 (SEQ ID NO: 2),
- f) amino acids 15-29 of CTNNB1 (SEQ ID NO: 2),
- g) amino acids 249-265, 292-306, 379-390, 415-429, 462-470, or 505-519, or any combination thereof, of CTNNB1 (SEQ ID NO: 2), and
- h) amino acids 38-48, 123-129, or 251-257 or any combination thereof, of PCNA (SEQ ID NO: 3).

132. The dual-specific bifunctional polypeptide of any one of embodiments 115-131, wherein the second targeting moiety is selected from Omomyc, TCF4, TCF4.1, TCF4.2, TCF4.3, TCF4.4, TCF4.5, TCF4.6, TCF4.7, TCF4.8, TCF4.9, TCF4.10, TCF4.11, TCF4.12, TCF4.13, TCF4.14, TCF4.15, TCF4.16, con1, con1.1, con1.2, p21, p21.1, p21.2, p21.3, p21.4, p21.5, p21.6, p21.7, p21.8, p21.9, mycV.0300, mycV.1300, mycV.1500, mycV.3700, mycV.6600, mycV.1516, mycV.1515, mycV.1514, mycV.1513, mycV.1512, mycV.1511, mycV.1510, mycV.1509, mycV.1508, mycV.1507, mycV.1506, mycV.1505, mycV.1504, mycV.1503, mycV.1502, mycV.1501, mycV.0302, mycV.0305, mycV.0306, mycV.0307, mycV.1308, mycV.1517, mycV.1518, mycV.1519, mycV.1520, mycV.1522, mycV.1524, mycV.3706, mycV.3708, mycV.6605, mycV.6608, or a fragment thereof that is capable of binding to the target protein; optionally wherein the second targeting moiety is selected from Omomyc, TCF4, TCF4.1, TCF4.2, TCF4.3, TCF4.4, TCF4.5, TCF4.6, TCF4.7, TCF4.8, TCF4.9, TCF4.10, TCF4.11, TCF4.12, TCF4.13, TCF4.14, TCF4.15, TCF4.16, con1, con1.1, con1.2, p21, p21.1, p21.2, p21.3, p21.4, p21.5, p21.6, p21.7, p21.8, p21.9, mycV.0302, mycV.0305, mycV.0306, mycV.0307, mycV.1308, mycV.1517, mycV.1518, mycV.1519, mycV.1520, mycV.1522, mycV.1524, mycV.3706, mycV.3708, mycV.6605, and mycV.6608; optionally wherein the targeting moiety does not comprise full length or unmodified con1 or p21.

133. The dual-specific bifunctional polypeptide of any one of embodiments 115-132, wherein the second targeting moiety comprises or consists of an amino acid sequence having at least 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to any one of SEQ ID NO: 136, 137, 145, 153, 161, 169, 184, 192, 200, 208, 216, 224, 232, 240, 248, 256, 264, 272, 280, 288, 296, 319-344, 641, 649, 657,

665, 673, 681, 689, 697, 705, 713, 721, 729, 737, 745, 753, and 761; optionally any one of SEQ ID NO: 184, 192, 200, 208, 216, 224, 232, 240, 248, 256, 264, 272, 280, 288, 296, and 319-344.

134. The dual-specific bifunctional polypeptide of any one of embodiments 115-133, wherein the dual-specific bifunctional polypeptide comprises or consists of an amino acid sequence having at least 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to any one of SEQ ID NOs: 562-577, optionally wherein the sequence lacks the HA tag sequence (SEQ ID NO: 384).

135. The dual-specific bifunctional polypeptide of any one of embodiments 115-134, wherein the dual-specific bifunctional polypeptide comprises or consists of an amino acid sequence having at least 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to any one of the SEQ ID NOs in Table 29 listed as having a degradation Grade of A for at least one of the target proteins, degradation Grade of B for at least one of the target proteins, degradation Grade of C for at least one of the target proteins, and/or degradation Grade of D for at least one of the target proteins, optionally excluding any one of the SEQ ID NOs listed as having a degradation Grade of D for at least one of the target proteins, optionally wherein the sequence lacks the HA tag sequence (SEQ ID NO: 384).

136. The dual-specific bifunctional polypeptide of any one of embodiments 115-135, wherein the dual-specific bifunctional polypeptide comprises or consists of an amino acid sequence of any one of the SEQ ID NOs in Table 29 listed as having degradation Grade of A for at least one of the target proteins, and/or degradation Grade of B for at least one of the target proteins, optionally wherein the sequence lacks the HA tag sequence (SEQ ID NO: 384).

137. The dual-specific bifunctional polypeptide of any one of embodiments 115-135, wherein the dual-specific bifunctional polypeptide comprises or consists of an amino acid sequence of any one of the SEQ ID NOs in Table 29 listed as having a degradation Combined Grade of A/A or A, optionally wherein the sequence lacks the HA tag sequence (SEQ ID NO: 384).

138. The dual-specific bifunctional polypeptide of any one of embodiments 115-135, wherein the dual-specific bifunctional polypeptide comprises or consists of an amino acid sequence of any one of the SEQ ID NOs in Table 29 listed as having a degradation Combined Grade of A/B or B/A, optionally wherein the sequence lacks the HA tag sequence (SEQ ID NO: 384).

139. The dual-specific bifunctional polypeptide of any one of embodiments 115-135, wherein the dual-specific bifunctional polypeptide comprises or consists of an amino acid sequence of any one of the SEQ ID NOs in Table 29 listed as having a degradation Combined Grade of B/B or B, optionally wherein the sequence lacks the HA tag sequence (SEQ ID NO: 384).

140. The dual-specific bifunctional polypeptide of any one of embodiments 115-139, wherein the dual-specific bifunctional polypeptide does not comprise or consist of an amino acid sequence of any one of the SEQ ID NOs in Table 29 listed as having a degradation Grade of C and/or D for at least one of the target proteins, optionally wherein the sequence lacks the HA tag sequence (SEQ ID NO: 384).

141. The dual-specific bifunctional polypeptide of any one of embodiments 115-140, wherein the URD is the URD of NHLRC1, RNF125, RNF165, or RNF4, or a truncation or fragment thereof that retains ubiquitin-proteasome recruiting activity, optionally wherein the URD comprises or consists of NHLRC1.1, RNF125.2, RNF165.1, or RNF4.1.

142. The dual-specific bifunctional polypeptide of any one of embodiments 115-141, wherein the URD comprises or consists of an amino acid sequence having at least 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to any one of SEQ ID NO: 76-80, 87-93, 98, and 104, optionally any one of SEQ ID NO: 76, 88, 98, and 104, or a truncation or fragment thereof that retains ubiquitin-proteasome recruiting activity.

143. The dual-specific bifunctional polypeptide of any one of embodiments 115-142, further comprising one or more linker peptides, wherein the linker peptide is positioned between one or more of: the first targeting moiety and the URD; the second targeting moiety and the URD; the first targeting moiety and the second targeting moiety; a localization peptide and the first targeting moiety; a localization peptide and the second targeting moiety; a localization peptide and the URD; and/or a localization peptide and an epitope tag, optionally an HA tag.

144. The dual-specific bifunctional polypeptide of any one of embodiments 115-143, comprising a first linker peptide positioned between the first targeting moiety and the URD or between the second targeting moiety and the URD, and a second linker peptide positioned between the second targeting moiety and the URD or between the first targeting moiety and the second targeting moiety, optionally wherein the first linker peptide is positioned between

the first targeting moiety and the URD and the second linker peptide is positioned between the second targeting moiety and the URD.

145. The dual-specific bifunctional polypeptide of any one of embodiments 115-144, wherein the one or more linker peptides is independently 1, 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 or 30 amino acids in length, or a range defined by any two of the preceding values, optionally 0-30, 1-30, 2-30, 0-20, 1-20, 2-20, 0-15, 0-12, 0-10, 1-15, 1-12, 1-10, 2-15, 2-12, 2-10, 3-15, 3-12, or 3-10 amino acids in length, optionally 2-30 amino acids in length.

146. The dual-specific bifunctional polypeptide of any one of embodiments 115-145, wherein the at least one or more linker peptides comprise or consist of glycine and serine.

147. The dual-specific bifunctional polypeptide of any one of embodiments 115-146, wherein the one or more linker peptides comprises or consists of one or more alpha helixes, optionally flanked by one or more glycine and/or a serine residues.

148. The dual-specific bifunctional polypeptide of any one of embodiments 115-147, wherein the one or more linker peptides independently comprise or consist of the amino acid sequence of any one of SEQ ID NO: 345-383 (345-351, GS, and 353-383), optionally SEQ ID NO: 347-352 (SEQ ID NO: 347-351 and GS).

149. The dual-specific bifunctional polypeptide of any one of embodiments 115-148, further comprising one or more localization peptide sequences.

150. The dual-specific bifunctional polypeptide of embodiment 149, wherein the one or more localization peptide sequences direct the dual-specific bifunctional polypeptide to a subcellular compartment or compartments, optionally wherein the subcellular compartment is the nucleus and/or the cytoplasm.

151. The dual-specific bifunctional polypeptide of embodiment 149 or 150, wherein the one or more localization peptide sequences direct the dual-specific bifunctional polypeptide to a desired subcellular compartment or compartments, optionally wherein the one or more localization peptide sequences are selected or designed to direct the dual-specific bifunctional polypeptide to a desired subcellular compartment or compartments.

152. The dual-specific bifunctional polypeptide of any one of embodiments 149-151, wherein the one or more localization peptide sequences comprise or consist of a nuclear localization signal (NLS) peptide and/or a nuclear export signal (NES) peptide, optionally wherein the localization peptide comprises or consists of an amino acid sequence of any one of SEQ ID NO: 114-127.

153. The dual-specific bifunctional polypeptide of any one of embodiments 149-152, wherein the one or more localization peptide sequences comprise or consist of an endogenous localization peptide sequence of the first and/or second targeting moiety and/or the URD.

154. The dual-specific bifunctional polypeptide of any one of embodiments 149-153, wherein the one or more localization peptide sequences comprise or consist of an endogenous localization peptide sequence of the first and/or second targeting moiety and/or the URD that is modified, wherein the modification alters the subcellular compartment or compartments to which the dual-specific bifunctional polypeptide is directed as compared to the native endogenous localization peptide sequence.

155. The dual-specific bifunctional polypeptide of embodiment 154, wherein the modification comprises or consists of a substitution, truncation or deletion of an endogenous localization peptide sequence.

156. The dual-specific bifunctional polypeptide of embodiment 154 or 155, wherein:

- a. the native endogenous localization peptide sequence directs the polypeptide to the nucleus, and the modified endogenous localization peptide sequence directs the polypeptide to the cytoplasm;
- b. the native endogenous localization peptide sequence directs the polypeptide to the cytoplasm, and the modified endogenous localization peptide sequence directs the polypeptide to the nucleus;
- c. the native endogenous localization peptide sequence directs the polypeptide to the nucleus and the cytoplasm, and the modified endogenous localization peptide sequence directs the polypeptide to only the cytoplasm;
- d. the native endogenous localization peptide sequence directs the polypeptide to the nucleus and the cytoplasm, and the modified endogenous localization peptide sequence directs the polypeptide to only the nucleus;
- e. the native endogenous localization peptide sequence directs the polypeptide to the nucleus, and the modified endogenous localization peptide sequence directs the polypeptide to the nucleus and the cytoplasm; or
- f. the native endogenous localization peptide sequence directs the polypeptide to the cytoplasm, and the modified endogenous localization peptide sequence directs the polypeptide to the nucleus and the cytoplasm.

157. The dual-specific bifunctional polypeptide of any one of embodiments 149-156, wherein the dual-specific bifunctional polypeptide comprises or consists of two localization

peptide sequences, optionally wherein the dual-specific bifunctional polypeptide comprises or consists of 3, 4, or more localization peptide sequences, optionally wherein the wherein the dual-specific bifunctional polypeptide comprises or consists of 2, 3, 4 or more copies of the same localization sequence in series.

158. The dual-specific bifunctional polypeptide of any one of embodiments 149-157, comprising one or more localization peptide sequences located at a location selected from the group consisting of: the N-terminus of the dual-specific bifunctional polypeptide; the C-terminus of the dual-specific bifunctional polypeptide; internally within the dual-specific bifunctional polypeptide; as part of the URD, optionally an endogenous portion of the URD; as a part of the first and/or second targeting moiety, optionally an endogenous portion of the first and/or second targeting moiety; and a combination of any of the foregoing.

159. The dual-specific bifunctional polypeptide of any one of embodiments 149-158, wherein a first localization peptide sequence is located at the N-terminus of the dual-specific bifunctional polypeptide and a second localization peptide sequence is located at the C-terminus of the dual-specific bifunctional polypeptide.

160. The dual-specific bifunctional polypeptide of any one of embodiments 149-159, further comprising at least one NLS peptide, wherein the at least one NLS peptide is located at the N-terminus of the dual-specific bifunctional polypeptide, the C-terminus of the dual-specific bifunctional polypeptide, is part of the URD, optionally an endogenous NLS of the URD, or is part of the first and/or second targeting moiety, optionally an endogenous NLS of the first and/or second targeting moiety.

161. The dual-specific bifunctional polypeptide of embodiment 149-160, wherein a first NLS peptide is located at the N-terminus of the dual-specific bifunctional polypeptide and a second NLS peptide is located at the C-terminus of the dual-specific bifunctional polypeptide.

162. The dual-specific bifunctional polypeptide of any one of embodiments 115-161, wherein the orientation of the first targeting moiety relative to the URD is the same orientation as compared to the naturally occurring substrate recognition domain of the protein from which the URD is derived.

163. The dual-specific bifunctional polypeptide of any one of embodiments 115-161, wherein the orientation of the first targeting moiety relative to the URD is the opposite orientation as compared to the naturally occurring substrate recognition domain of the protein from which the URD is derived.

164. The dual-specific bifunctional polypeptide of any one of embodiments 115-162, wherein the orientation of the second targeting moiety relative to the URD is the same orientation as compared to the naturally occurring substrate recognition domain of the protein from which the URD is derived.

165. The dual-specific bifunctional polypeptide of any one of embodiments 115-162, wherein the orientation of the second targeting moiety relative to the URD is the opposite orientation as compared to the naturally occurring substrate recognition domain of the protein from which the URD is derived.

166. The dual-specific bifunctional polypeptide of any one of embodiments 115-165, wherein the first targeting moiety is N-terminal relative to the URD and the second targeting moiety is C-terminal relative to the URD.

167. The dual-specific bifunctional polypeptide of any one of embodiments 115-166, wherein the first targeting moiety is C-terminal relative to the URD, and the second targeting moiety is N-terminal relative to the URD.

168. The dual-specific bifunctional polypeptide of any one of embodiments 115-165, wherein the first and the second targeting moieties are N-terminal relative to the URD.

169. The dual-specific bifunctional polypeptide of any one of embodiments 115-165, wherein the first and the second targeting moieties are C-terminal relative to the URD.

170. The dual-specific bifunctional polypeptide of any one of embodiments 115-169, further comprising an epitope tag, optionally a hemagglutinin (HA) tag, optionally wherein the HA tag comprises or consists of the amino acid sequence of SEQ ID NO: 384, wherein the epitope tag can be used to visualize the dual-specific bifunctional polypeptide.

171. The dual-specific bifunctional polypeptide of any one of embodiments 115-169, wherein the dual-specific bifunctional polypeptide does not comprise an epitope tag, optionally a hemagglutinin (HA) tag, optionally wherein the dual-specific bifunctional polypeptide does not comprise an HA tag comprising or consisting of the amino acid sequence of SEQ ID NO: 384.

172. The dual-specific bifunctional polypeptide of any one of embodiments 115-171, wherein the dual-specific bifunctional polypeptide is less than or equal to 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950, 1000, 1050, 1100, or 1150, amino acids long, or a range defined by any two of the preceding values, optionally 120-400 or 130-370 amino acids long.

173. The dual-specific bifunctional polypeptide of any one of embodiments 115-172, wherein the dual-specific bifunctional polypeptide comprises or consists of an amino acid sequence having at least 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the peptide of any one of SEQ ID NO: 562-577.

174. The dual-specific bifunctional polypeptide of any one of embodiments 115-173, wherein the at least one second target protein comprises MYC, optionally c-MYC.

175. The dual-specific bifunctional polypeptide of embodiment 174, wherein the second targeting moiety binds, or binds at least in part, to at least a portion of:

- a.) the DNA binding domain of MYC;
- b.) amino acids 54-406 of MYC (SEQ ID NO: 1);
- c.) amino acids 354-406 of MYC (SEQ ID NO: 1);
- d.) amino acids 408-437 of MYC (SEQ ID NO: 1); and
- e.) amino acids 410-419 of MYC (SEQ ID NO: 1).

176. The dual-specific bifunctional polypeptide of any one of embodiments 174-175, wherein the bifunctional polypeptide comprises a localization peptide sequence which directs the bifunctional polypeptide to the nucleus of a cell, optionally wherein the localization peptide sequence comprises or consists of a NLS sequence selected from the group of the NLS sequences of MYC, SPOP, Hrp1, SV40, 53BP1, and nucleoplasmin, optionally MYC, SPOP, Hrp1, optionally wherein the localization peptide sequence comprises or consists of a NLS sequence selected from the group of the NLS sequence of MYC, optionally mycNLS, SPOP, optionally spNLS.2, and Hrp1.

177. The dual-specific bifunctional polypeptide of embodiment 176, wherein the NLS comprises or consists of a sequence selected from any one of SEQ ID NOs: 114-127, optionally wherein the NLS comprises or consists of a sequence selected from any one of SEQ ID NOs: 122, 114, and 116.

178. The dual-specific bifunctional polypeptide of any one of embodiments 174-177, wherein the URD is the URD of NHLRC1, RNF125, RNF165, and RNF4, or a truncation or fragment thereof that retains ubiquitin-proteasome recruiting activity, optionally NHLRC1.1, RNF125.2, RNF165.1, or RNF4.1.

179. The dual-specific bifunctional polypeptide of any one of embodiment 174-178, wherein the URD comprises or consists of a sequence selected from any one of SEQ ID NOs: 76-80, 87-93, 98, and 104, optionally any one of SEQ ID NO: 76, 88, 98, and 104.

180. The dual-specific bifunctional polypeptide of any one of embodiments 174-179, wherein the second targeting moiety comprises or consists of an endogenous protein binding partner of the target protein, a fragment and/or a derivative thereof.

181. The dual-specific bifunctional polypeptide of any one of embodiments 174-180, wherein the second targeting moiety is Omomyc, or a fragment thereof that is capable of binding to the target protein, optionally comprising or consisting of an amino acid sequence having at least 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 136.

182. The dual-specific bifunctional polypeptide of any one of embodiments 174-179, wherein the second targeting moiety comprises or consists of a sdAb, optionally comprising or consisting of either a V_H domain or a V_L domain of an IgG antibody, or a V_{HH}, V_H, or V_{NAR} domain of a heavy chain antibody (HcAb), optionally wherein the sdAb comprises or consists of a V_{HH} domain.

183. The dual-specific bifunctional polypeptide of embodiment 182, wherein the second targeting moiety comprises or consists of a sdAb, optionally a V_{HH} domain, comprising a CDR1 selected from any one of SEQ ID NOs: 139, 147, 163, and 171, a CDR2 selected from any one of SEQ ID NOs: 141, 149, 157, and 165, and a CDR3 selected from any one of SEQ ID NOs: 143, 151, 159, 167, and 175; optionally wherein the combination of CDR1, CDR2, and CDR3 is SEQ ID NOs: 155, 157 and 159;

optionally wherein each CDR sequence independently comprises 1, 2, 3, 4, 5, 6, 7, or more substitutions and/or 1, 2, 3, 4, 5, 6, 7, or more deletions or additions, and/or has at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to a sequence selected from any one of SEQ ID NOs: 139, 147, 163, 171, 141, 149, 157, 165, 143, 151, 159, 167, and 175.

184. The dual-specific bifunctional polypeptide of embodiment 183, wherein the sdAb, optionally a V_{HH} domain, of the second targeting moiety comprises a V_H FR1 selected from any one of SEQ ID NOs: 138 and 170, a V_H FR2 of SEQ ID NO: 140, a V_H FR3 selected from any one of SEQ ID NOs: 142, 158, and 174, and a V_H FR4 selected from any one of SEQ

ID NOs: 144 and 152, optionally wherein the combination of FR1, FR2, FR3 and FR4 is SEQ ID NOs: 154, 156, 158, and 160;

optionally wherein each V_H framework region comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17 or more substitutions and/or 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17 or more deletions or additions, and/or has at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to a sequence selected from any one of SEQ ID NOs: 138, 170, 140, 142, 158, 174, 144, and 152.

185. The dual-specific bifunctional polypeptide of any one of embodiments 182-184, wherein the second targeting moiety comprises or consists of a sdAb, optionally a V_HH domain, sequence selected from any one of SEQ ID NOs: 137, 145, 153, 161, 169, 641, 649, 657, 665, 673, 681, 689, 697, 705, 713, 721, 729, 737, 745, 753, and 761, optionally wherein each CDR sequence independently comprises 1, 2, 3, 4, 5, 6, 7, or more substitutions and/or 1, 2, 3, 4, 5, 6, 7, or more deletions or additions, and/or has at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to a CDR sequence of any one of SEQ ID NOs: 137, 145, 153, 161, 169, 641, 649, 657, 665, 673, 681, 689, 697, 705, 713, 721, 729, 737, 745, 753, and 761, and/or wherein the framework region comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17 or more substitutions and/or 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17 or more deletions or additions, and/or has at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the framework region of any one of SEQ ID NOs: 137, 145, 153, 161, 169, 641, 649, 657, 665, 673, 681, 689, 697, 705, 713, 721, 729, 737, 745, 753, and 761.

186. The dual-specific bifunctional polypeptide of embodiment 182, wherein the second targeting moiety comprises or consists of a sdAb, wherein the sdAb comprises or consists of a V_HH domain.

187. The dual-specific bifunctional polypeptide of embodiment 182 or 186, wherein the second targeting moiety comprises or consists of the polypeptide of any one of embodiments 1-48, wherein the polypeptide comprises or consists of the anti-Myc sdAb.

188. The dual-specific bifunctional polypeptide of any one of embodiments 182-187, wherein the bifunctional polypeptide comprises or consists of an amino acid sequence having at least 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to any one of SEQ ID NOs: 562, 565, 567, and 570-577, optionally wherein the sequence lacks the HA tag sequence (SEQ ID NO: 384).

189. The dual-specific bifunctional polypeptide of any one of embodiments 174-188, wherein the bifunctional polypeptide comprises or consists of an amino acid sequence having at least 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to any one of the SEQ ID NOs in Table 30, optionally excluding any one of the SEQ ID NOs listed as having a MYC degradation Grade of D, optionally wherein the sequence lacks the HA tag sequence (SEQ ID NO: 384).

190. The dual-specific bifunctional polypeptide of any one of embodiments 174-189, wherein the bifunctional polypeptide comprises or consists of an amino acid sequence of any one of the SEQ ID NOs in Table 30 listed as having a MYC degradation Grade of A, and/or MYC degradation Grade of B, optionally wherein the sequence lacks the HA tag sequence (SEQ ID NO: 384).

191. The dual-specific bifunctional polypeptide of any one of embodiments 174-190, wherein the bifunctional polypeptide does not comprise or consist of an amino acid sequence of any one of the SEQ ID NOs in Table 30 listed as having a MYC degradation Grade of C and/or D, optionally wherein the sequence lacks the HA tag sequence (SEQ ID NO: 384).

192. The dual-specific bifunctional polypeptide of any one of embodiments 174-191, wherein the URD is not derived from CHIP, optionally wherein the URD does not comprise or consist of CHIP.1, optionally wherein the URD does not comprise or consist of SEQ ID NO: 54.

193. A polynucleotide encoding a dual-specific bifunctional polypeptide comprising or consisting of the dual-specific bifunctional polypeptide of any one of embodiments 174-192.

194. The dual-specific bifunctional polypeptide of any one of embodiments 115-173, wherein the at least one second target protein comprises CTNNB1.

195. The dual-specific bifunctional polypeptide of embodiment 194, wherein the CTNNB1 protein is a mutant form of CTNNB1 (SEQ ID NO: 2) comprising one or more mutations selected from: D32Y; D32N; D32V; D32G; S33Y; S33L; S33F; S33C; S33P; G34V; G34E; I35S; H36P; S37C; S37P; S37P and D207G; T41A; T41I; T42_K49del; S45F and Y670X wherein X indicates termination; S45C; S45F; S45P; S45Y; and S45del.

196. The dual-specific bifunctional polypeptide of any one of embodiments 194-195, wherein the bifunctional polypeptide comprises a localization peptide sequence which directs the bifunctional polypeptide to the nucleus of a cell, optionally wherein the localization peptide sequence comprises or consists of a NLS sequence selected from the group of the NLS sequences of MYC, SPOP, Hrp1, SV40, 53BP1, and nucleoplasmin, optionally wherein the

localization peptide sequence comprises or consists of a NLS sequence selected from the group of the NLS sequence of MYC, optionally mycNLS, SPOP, optionally spNLS.2, and Hrp1.

197. The dual-specific bifunctional polypeptide of embodiment 195, wherein the NLS comprises or consists of a sequence selected from any one of SEQ ID NOs: 114-127, optionally wherein the NLS comprises or consists of a sequence selected from any one of SEQ ID NOs: 122, 114 and 116.

198. The dual-specific bifunctional polypeptide of any one of embodiments 194-197, wherein the URD is the URD of NHLRC1, RNF125, RNF165, and RNF4, optionally NHLRC1, RNF125, and RNF4, or a truncation or fragment thereof that retains ubiquitin-proteasome recruiting activity, optionally NHLRC1.1, RNF125.2, or RNF4.1.

199. The dual-specific bifunctional polypeptide of any one of embodiment 194-198, wherein the URD comprises or consists of a sequence selected from any one of SEQ ID NOs: 76-80, 87-93, and 104, optionally wherein the URD comprises or consists of a sequence selected from any one of SEQ ID NOs: 76, 88 and 104.

200. The dual-specific bifunctional polypeptide of any one of embodiments 194-199, wherein the second targeting moiety binds, or binds at least in part, to at least a portion of:

- a) the armadillo domain of CTNNB1,
- b) amino acids 150-663 of CTNNB1 (SEQ ID NO: 2),
- c) amino acids 15-29 of CTNNB1 (SEQ ID NO: 2), and
- d) amino acids 249-265, 292-306, 379-390, 415-429, 462-470, or 505-519, or any combination thereof, of CTNNB1 (SEQ ID NO: 2).

201. The dual-specific bifunctional polypeptide of any one of embodiments 194-200, wherein the second targeting moiety comprises or consists of an endogenous protein binding partner of the target protein, a fragment and/or a derivative thereof.

202. The dual-specific bifunctional polypeptide of any one of embodiments 194-201, wherein the second targeting moiety is TCF4.1, TCF4.2, TCF4.3, TCF4.4, TCF4.5, TCF4.6, TCF4.7, TCF4.8, TCF4.9, TCF4.10, TCF4.11, TCF4.12, TCF4.13, TCF4.14, TCF4.15, or TCF4.16, or a fragment thereof that is capable of binding to the target protein, optionally TCF4.6.

203. The dual-specific bifunctional polypeptide of any one of embodiments 194-202, wherein the second targeting moiety comprises or consists of an amino acid sequence having at least 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%,

99%, or 100% sequence identity to any one of SEQ ID NOs: 319-333, optionally SEQ ID NO: 330.

204. The dual-specific bifunctional polypeptide of any one of embodiments 194-203, wherein the dual-specific bifunctional polypeptide comprises or consists of an amino acid sequence having at least 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to any one of SEQ ID NO: 563, 564, 566, 568, and 569, optionally wherein the sequence lacks the HA tag sequence (SEQ ID NO: 384).

205. The dual-specific bifunctional polypeptide of any one of embodiments 194-204, wherein the bifunctional polypeptide comprises or consists of an amino acid sequence having at least 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to any one of the SEQ ID NOs in Table 31, optionally excluding any one of the SEQ ID NOs listed as having a degradation Grade of D for at least one of the target proteins, optionally wherein the sequence lacks the HA tag sequence (SEQ ID NO: 384).

206. The dual-specific bifunctional polypeptide of any one of embodiments 194-205, wherein the bifunctional polypeptide comprises or consists of an amino acid sequence of any one of the SEQ ID NOs in Table 31 listed as having a degradation Grade of A for at least one target protein, and/or a degradation Grade of B for at least one target protein, optionally wherein the sequence lacks the HA tag sequence (SEQ ID NO: 384).

207. The dual-specific bifunctional polypeptide of any one of embodiments 194-205, wherein the dual-specific bifunctional polypeptide comprises or consists of an amino acid sequence of any one of the SEQ ID NOs in Table 31 listed as having a degradation Combined Grade of A/A or A, optionally wherein the sequence lacks the HA tag sequence (SEQ ID NO: 384).

208. The dual-specific bifunctional polypeptide of any one of embodiments 194-205, wherein the dual-specific bifunctional polypeptide comprises or consists of an amino acid sequence of any one of the SEQ ID NOs in Table 31 listed as having a degradation Combined Grade of A/B or B/A, optionally wherein the sequence lacks the HA tag sequence (SEQ ID NO: 384).

209. The dual-specific bifunctional polypeptide of any one of embodiments 194-205, wherein the dual-specific bifunctional polypeptide comprises or consists of an amino acid sequence of any one of the SEQ ID NOs in Table 31 listed as having a degradation Combined

Grade of B/B or B, optionally wherein the sequence lacks the HA tag sequence (SEQ ID NO: 384).

210. The dual-specific bifunctional polypeptide of any one of embodiments 194-209, wherein the bifunctional polypeptide does not comprise or consist of an amino acid sequence of any one of the SEQ ID NOs in Table 31 listed as having a degradation Grade of C and/or D for at least one of the target proteins, optionally wherein the sequence lacks the HA tag sequence (SEQ ID NO: 384).

211. The bifunctional polypeptide of any one of embodiments 194-210, wherein the URD is not derived from beta-TRCP, optionally wherein the URD does not comprise or consist of bTRCP.1, optionally wherein the URD does not comprise or consist of SEQ ID NOs: 57.

212. A polynucleotide encoding a bifunctional polypeptide comprising or consisting of the bifunctional polypeptide of any one of embodiments 194-211.

213. The dual-specific bifunctional polypeptide of any one of embodiments 115-173, wherein the at least one target protein comprises PCNA.

214. The dual-specific bifunctional polypeptide of embodiment 213, wherein the bifunctional polypeptide comprises a localization peptide sequence which directs the bifunctional polypeptide to the nucleus of a cell, optionally wherein the localization peptide sequence comprises or consists of a NLS sequence selected from the group of the NLS sequences of MYC, SPOP, Hrp1, SV40, 53BP1, and nucleoplasmin, optionally wherein the localization peptide sequence comprises or consists of a NLS sequence selected from the group of the NLS sequence of MYC, optionally mcyNLS and/or 3xmycNLS, SPOP, Hrp1, SV40; optionally wherein the localization peptide sequence comprises or consists of a NLS sequence selected from the group of the NLS sequence of MYC, optionally mcyNLS, and SPOP, optionally spNLS.2.

215. The dual-specific bifunctional polypeptide of embodiment 214, wherein the NLS comprises or consists of a sequence selected from any one of SEQ ID NOs: 114-127, optionally wherein the NLS comprises or consists of a sequence selected from any one of SEQ ID NOs: 115-116, and 118-122.

216. The dual-specific bifunctional polypeptide of any one of embodiments 213-215, wherein the URD is the of NHLRC1, RNF125, RNF165, and RNF4, optionally NHLRC1, RNF125, and RNF4, or a truncation or fragment thereof that retains ubiquitin-proteasome recruiting activity, optionally NHLRC1.1, RNF125.2, or RNF4.1.

217. The dual-specific bifunctional polypeptide of any one of embodiments 213-216, wherein the URD comprises or consists of a sequence selected from any one of SEQ ID NOs: 76-80, 87-93, and 104, optionally wherein the URD comprises or consists of a sequence selected from any one of SEQ ID NOs: 76, 88 and 104.

218. The dual-specific bifunctional polypeptide of any one of embodiments 213-217, wherein the second targeting moiety binds, or binds at least in part, to at least a portion of amino acids 38-48, 123-129, or 251-257 or any combination thereof, of PCNA (SEQ ID NO: 3):

219. The dual-specific bifunctional polypeptide of any one of embodiments 213-218, wherein the second targeting moiety comprises or consists of an endogenous protein binding partner of the target protein, a fragment and/or a derivative thereof.

220. The dual-specific bifunctional polypeptide of any one of embodiments 213-219, wherein the second targeting moiety comprises or consists of con1.1, con1.2, p21.1, p21.2, p21.3, p21.4, p21.5, p21.6, p21.7, p21.8, p21.9, or a fragment thereof that is capable of binding to the target protein.

221. The dual-specific bifunctional polypeptide of any one of embodiment 213-220, wherein the first and/or second targeting moiety comprises or consists of an amino acid sequence having at least 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to any one of SEQ ID NOs: 334-344.

222. The dual-specific bifunctional polypeptide of any one of embodiments 213-221, wherein the URD is not derived from SPOP, optionally wherein the URD does not comprise or consist of SPOP.1, SPOP.2, or SPOP.3, optionally wherein the URD does not comprise or consist of a sequence selected from any one of SEQ ID NOs: 72-74.

223. A polynucleotide encoding a dual-specific bifunctional polypeptide comprising or consisting of the bifunctional polypeptide of any one of embodiments 213-222.

224. The dual-specific bifunctional polypeptide of embodiment 115, wherein the dual-specific bifunctional polypeptide comprises or consists of an amino acid sequence having at least 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to any one of SEQ ID NO: 562-577, optionally wherein the sequence lacks the HA tag sequence (SEQ ID NO: 384).

225. A polypeptide comprising or consisting of an amino acid sequence having at least 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to any one of SEQ ID NO: 184, 192, 200, 208, 216, 224, 232,

240, 248, 256, 264, 272, 280, 288, 296, and 385-577, optionally wherein the sequence lacks the HA tag sequence (SEQ ID NO: 384).

226. A polynucleotide encoding for the polypeptide of embodiment 225.

227. The polynucleotide of any one of embodiments 49, 101, 114, 193, 212, or 226, wherein the polynucleotide is a DNA or an RNA, optionally an mRNA or a circular RNA, optionally wherein the RNA, mRNA or circular RNA comprises one or more modified nucleosides, optionally wherein up to 100% of the nucleotides comprising uracil are replaced with pseudouridine and/or N1-methylpseudouridine.

228. The polynucleotide of any one of embodiments 226-227, wherein the polynucleotide is packaged in a lipid nanoparticle, a polymeric nanoparticle, an extracellular vesicle, optionally an exosome, or a viral vector, optionally a replicating viral vector or a non-replicating viral vector, optionally an adenovirus, adeno-associated virus, lentivirus, or retrovirus vector.

229. A pharmaceutical composition comprising the polypeptide comprising or consisting of the anti-Myc sdAb, bifunctional polypeptide, dual-specific bifunctional polypeptide, polypeptide, or polynucleotide of any one of embodiments 1-228 and one or more pharmaceutically acceptable excipients, carriers, or diluents.

230. The pharmaceutical composition embodiment 229, wherein the composition is formulated for intravenous, intraperitoneal, intra-arterial, subcutaneous, intramuscular, intrathecal, intratumoral, inhalation, or intracranial administration.

231. The polypeptide comprising or consisting of the anti-Myc sdAb, bifunctional polypeptide, dual-specific bifunctional polypeptide, polypeptide, polynucleotide, or pharmaceutical composition of any one of embodiments 1-230 for use in the treatment of a cancer in a patient in need thereof.

232. The polypeptide comprising or consisting of the anti-Myc sdAb, bifunctional polypeptide, dual-specific bifunctional polypeptide, polypeptide, polynucleotide, or pharmaceutical composition of any one of embodiments 1-231 for use in the manufacture of a medicament, optionally for the treatment of cancer.

233. A method of treating a subject comprising administering the polypeptide comprising or consisting of the anti-Myc sdAb, bifunctional polypeptide, dual-specific bifunctional polypeptide, polypeptide, polynucleotide, or pharmaceutical composition of any one of embodiments 1-232 to a subject in need thereof, optionally wherein the subject has a cancer.

234. A method of reducing the amount of a target protein in a cell comprising contacting the cell with the polypeptide comprising or consisting of the anti-Myc sdAb, bifunctional polypeptide, dual-specific bifunctional polypeptide, polypeptide, polynucleotide, or pharmaceutical composition of any one of embodiments 1-232, optionally wherein the target protein is selected from MYC, (optionally c-MYC), CTNNB1, and/or PCNA.

235. The method of embodiment 233 or 234, wherein said cell is in a subject, and said contacting comprises administering the polypeptide comprising or consisting of the anti-Myc sdAb, bifunctional polypeptide, dual-specific bifunctional polypeptide, polypeptide, polynucleotide, or pharmaceutical composition of any one of embodiments 1-232 to the subject, optionally wherein the subject has cancer.

236. The method of embodiment 235, wherein the administering is intravenous, intraperitoneal, intra-arterial, subcutaneous, intramuscular, intrathecal, intratumoral, inhalation, or intracranial administration.

237. The method of any one of embodiments 234 or 235, wherein the cell is *ex vivo*, and said contacting comprises contacting the polypeptide comprising or consisting of the anti-Myc sdAb, bifunctional polypeptide, dual-specific bifunctional polypeptide, polypeptide, polynucleotide, or pharmaceutical composition of any one of embodiments 1-232 to the cell *ex vivo*, optionally in an adoptive cell therapy.

238. The method of embodiment 237, further comprising administering the cell to a subject after the contacting step, optionally wherein the subject is also the source of the cell.

239. The polypeptide comprising or consisting of the anti-Myc sdAb, bifunctional polypeptide, dual-specific bifunctional polypeptide, polypeptide, polynucleotide, pharmaceutical composition or method of any one of embodiments 1-238, wherein the polypeptide, bifunctional polypeptide, or dual-specific bifunctional polypeptide promotes proteasome-mediated degradation of the target protein when measured in one or more assays described in the Exemplary Assays for assessing proteasomal-mediated degradation, for example, by HiBiT tag assay and/or Western blot, optionally wherein the proteasome-mediated degradation is inhibited when the cells comprising the target protein and the dual-specific bifunctional polypeptide are treated with a proteasome inhibitor.

240. The polypeptide comprising or consisting of the anti-Myc sdAb, bifunctional polypeptide, dual-specific bifunctional polypeptide, polypeptide, polynucleotide, pharmaceutical composition or method of embodiment 239, wherein treatment of the cells comprising the target protein and the polypeptide, bifunctional polypeptide, or dual-specific

bifunctional polypeptide with a proteasome inhibitor reduces the amount of degradation of the target protein by at least 50%, 60%, 70%, 80%, 90%, 95% or 100% as compared to cells not treated with the proteasome inhibitor.

241. The bifunctional polypeptide, dual-specific bifunctional polypeptide, polypeptide, polynucleotide, pharmaceutical composition, or method of any one of embodiments 50-240, wherein the polypeptide, bifunctional polypeptide, or dual-specific bifunctional polypeptide does not comprise a URD derived from CHIP, SPOP, TRIM21 or beta-TRCP.

242. A compound comprising the polypeptide of any one of embodiments 1-48, the polypeptide comprising or consisting of the anti-Myc sdAb, and a therapeutic compound and/or drug and/or detectable label conjugated to the polypeptide.

243. Any one of the preceding embodiments, wherein the conservative substitution is a conservative substitution from one of the following eight groups, wherein each group contains amino acids that are conservative substitutions for one another: 1) Alanine (A), Glycine (G); 2) Aspartic acid (D), Glutamic acid (E); 3) Asparagine (N), Glutamine (Q); 4) Arginine (R), Lysine (K); 5) Isoleucine (I), Leucine (L), Methionine (M), Valine (V); 6) Phenylalanine (F), Tyrosine (Y), Tryptophan (W); 7) Serine (S), Threonine (T); and 8) Cysteine (C), Methionine (M).

BRIEF DESCRIPTION OF THE DRAWINGS

[0014] In addition to the features described above, additional features and variations will be readily apparent from the following descriptions of the drawings and exemplary embodiments. It is to be understood that these drawings depict typical embodiments and are not intended to be limiting in scope.

[0015] FIGS. 1A, 1B and 1C depict embodiments of a schematic describing proteasome-mediated degradation of a target protein using a bifunctional (FIG. 1A) or dual-specific bifunctional polypeptide (FIGS. 1B and 1C), which comprises a first and optionally a second targeting domain specific for a first and optionally second target protein and a URD comprising an E3 ligase or component, fragment, or truncation thereof that recruits the ubiquitination complex to ubiquitinate one (FIG. 1A) or both (FIGS. 1B and 1C) of the target protein(s) for proteasomal degradation. FIG. 1B depicts an embodiment of a “contralateral” configuration of a dual-specific bifunctional polypeptide in which the first and second targeting domains are located on either side of the URD. FIG. 1C depicts an embodiment of a “tandem”

configuration of a dual-specific functional polypeptide in which the first and second targeting domains are located on the same side (both on the N-terminal side or both on the C-terminal side of the URD).

[0016] FIGS. 2A, 2B and 2C depict embodiments of a schematic for different ordering combinations of the components that comprise the bifunctional or dual-specific bifunctional polypeptides of some embodiments disclosed herein. FIG. 2A depicts embodiments of a mono-specific bifunctional polypeptide comprising a single targeting moiety that is capable of binding to at least one target protein (e.g., Myc). FIG. 2B depicts embodiments of a “contralateral” configuration in which the dual-specific bifunctional polypeptides comprise a first targeting moiety (target binding domain) (N-TBD or C-TBD) that is capable of binding to at least one target protein (e.g., Myc); a second targeting moiety (C-TBD or N-TBD) that is capable of binding to at least one target protein (e.g. Myc or a different target protein); and a URD. FIG. 2C depicts embodiments of a “tandem” configuration in which the dual-specific bifunctional polypeptide is in a “tandem” configuration in which the first and second target binding domains are both on the same side of the URD, i.e., both are on the C-terminal side of the URD, or both are on the N-terminal side of the URD. In some embodiments the bifunctional or dual-specific bifunctional polypeptide further comprises one or more linker peptides. In some embodiments when in a “contralateral” configuration the dual-specific bifunctional polypeptide further comprises a first linker peptide (N-linker or C-linker), wherein the first linker peptide is positioned between the first targeting moiety and the URD; and/or a second linker peptide (C-linker or N-linker), wherein the second linker peptide is positioned between the second targeting moiety and the URD. In some embodiments, there is no linker between the first and/or second targeting moiety and the URD. In some embodiments when a “tandem” configuration is used (see, e.g., FIG. 2C), a linker peptide (a first or a second linker peptide) is positioned between the two target binding domains, and/or a target binding domain and the URD. In some embodiments of the tandem configuration one or both of the linkers is not present (not shown). The linker(s), if present, may provide flexibility in positioning between the URD and target binding moiety. In some embodiments, the bifunctional or dual-specific bifunctional polypeptides may further comprise a localization sequence, (e.g., a nuclear localization signal (NLS)), which may be at the N-terminus, C-terminus, or both the N-terminus and C-terminus, or internally (not shown) within the bifunctional polypeptide (e.g., if the URD and/or target binder comprises an NLS within its sequence), if the target protein localizes to the nucleus of a cell. In some embodiments, the localization sequence(s) (e.g., N-

NLS, and/or C-NLS) may be separated from other components by a linker sequence (not shown).

[0017] FIG. 3 depicts an embodiment of subcellular localization of SPOP variant URD mono-specific bifunctional polypeptides to the nucleus and the cytoplasm.

[0018] FIG. 4 depicts an embodiment of subcellular localization of NHLRC1 containing mono-specific bifunctional polypeptides with and without an exogenous NLS sequence.

[0019] FIG. 5 depicts an embodiment of subcellular localization of VL12.3 containing mono-specific bifunctional polypeptides with URDs containing endogenous NLS or NES signals.

[0020] FIG. 6 is a table disclosing a summary of an embodiment of an experiment examining expression of various exemplary dual-specific bifunctional polypeptides. Provided is a description of elements listed in a N- to C-terminal direction, a summary of the observed subcellular localization of HA staining and the respective SEQ ID NOs.

[0021] FIGS. 7A-7C depict an embodiment of HA staining of the dual-specific bifunctional polypeptides disclosed in FIG. 6.

[0022] FIG. 8 depicts an embodiment of various exemplary bifunctional or dual-specific bifunctional polypeptides listing the target proteins ("Targets"), description of elements listed in a N- to C-terminal direction, and respective SEQ ID NOs.

DETAILED DESCRIPTION OF THE DISCLOSURE

[0023] Antibodies targeting Myc such as 9E10 are known in the art. However, antibodies or binding portions thereof which require a disulfide bond to maintain proper folding of the polypeptide for target binding typically do not function in the highly reducing conditions of the intracellular environment. Therefore, the anti-Myc sdAbs disclosed herein, are designed to function in the intracellular environment. This allows these anti-Myc sdAbs to be expressed intracellularly and utilized as Myc-binding moieties on their own, or as portions of other compounds or polypeptides (e.g., antibody-drug conjugates, detectably labeled probes, diabodies, etc.), for example the bifunctional polypeptides disclosed herein. While these anti-Myc sdAbs have the advantage of functioning in highly reducing environments such as the intracellular environment, they are not limited to use in the intracellular environment.

[0024] In some embodiments, the anti-Myc sdAbs disclosed herein are part of a bifunctional polypeptide that uses the ubiquitin-proteasome system to degrade Myc and optionally other target protein(s). The ubiquitin-proteasome system is the major and essential mechanism by which eukaryotic cells regulate protein abundance and clear misfolded or damaged proteins

through proteolytic degradation. This regulation is vital for many cellular functions, such as regulating the cell cycle and gene expression. Proteins are marked for degradation and recognized by the proteasomal complex when they are polyubiquitinated at lysine residues. This polyubiquitination is mediated by recognition of the target proteins by an E3 ubiquitin ligase, which catalyzes the transfer of a ubiquitin subunit from an E2 ubiquitin-conjugating enzyme to the protein target. As ubiquitin also contains ubiquitination sites, the protein target can be polyubiquitinated, thereby marking it for degradation by the proteasome. It is estimated that there are over 600 unique E3 ubiquitin ligases encoded by the human genome, each having varying specificities to target proteins. Therefore, each E3 ubiquitin ligase generally comprises a substrate recognition domain specific for one or more targets and a ubiquitin-proteasome system recruiting domain (URD) that binds to an E2 ubiquitin-conjugating enzyme to enable ubiquitination of the target.

[0025] As disclosed herein, the ubiquitin-proteasome system may be exploited for the directed degradation of desired protein targets such as Myc. For example, many diseases are associated with the abnormal function and/or expression of certain proteins (e.g., cancer caused by dysfunctional expression or localization of oncogenes). Degradation of said proteins can have a therapeutic effect in treating associated diseases, and the ubiquitin-proteasome system offers a naturally occurring process for effecting said degradation. Furthermore, degradation of disease-associated proteins may be more effective than inhibition of the protein, for example, using a small molecule or antibody composition, as in these cases, the protein is still present within the cell and may perform various biological functions at interfaces other than the inhibited domain, for example scaffolding functions, or contribute to pathology as a result of accumulation, aggregation or mislocalization.

[0026] Several groups have reported on the structure and preclinical activity of various macromolecule degrader formats, including fusion proteins comprising a full-length or truncated E3 ligase and macromolecular targeting constructs. However, general design principles for such macromolecule degraders are not yet well established, comparatively few of the hundreds of naturally occurring E3 ligases have been explored in this context, and degradation of intracellular targets using macromolecule degraders has been demonstrated for only a limited number of target proteins. Furthermore, there is little precedent for the optimization of drug-like properties for such molecules, and none have yet advanced to the stage of clinical testing. Examples of previous macromolecule degraders have been explored in: Wang *et al.*, “The state of the art of PROTAC technologies for drug discovery,” *Eur J.*

Med. Chem. (2022) 235:114290; Lim *et al.*, “bioPROTACs as versatile modulators of intracellular therapeutic targets including proliferating cell nuclear antigen (PCNA),” *PNAS* (2020) 117(11):5791-5800; Hatakeyama *et al.* “Targeted destruction of c-Myc by an engineered ubiquitin ligase suppresses cell transformation and tumor formation,” *Cancer Res.* (2005) 65(17):7874-9; Portnoff *et al.* “Ubiquibodies, synthetic E3 ubiquitin ligases endowed with unnatural substrate specificity for targeted protein silencing,” *J. Biol. Chem.* (2014) 289(11):7844-55; Liao *et al.* “A PROTAC peptide induces durable β -catenin degradation and suppresses Wnt-dependent intestinal cancer,” *Cell Discov.* (2020) 6:35; Liu *et al.* Targeted degradation of b-catenin by chimeric F-box fusion proteins. *Biochem. and Biophys. Res. Comm.* (2004) 313:1023-1029; Cong *et al.*, “A protein knockdown strategy to study the function of β -catenin in tumorigenesis,” *BMC Molecular Bio.* (2003) 4:10; and Shu *et al.* “Eradication of pathogenic β -catenin by Skp1/Cullin/F box ubiquitination machinery,” *PNAS* (2003) 100(22):12729-12734; each of which is hereby expressly incorporated by reference in its entirety. Localization of degraders to the correct subcellular compartment may be important for the ability to degrade a target protein, e.g., if the target is localized to the nucleus, or if degradation of target protein only in the nucleus but not cytoplasm is desired. It is noteworthy that apparently none of these references address, or even consider, this potentially important aspect of degrader design. Further, it is noted that none of these degrader formats include dual-specific bifunctional polypeptides as disclosed herein.

[0027] Accordingly, provided herein are several anti-Myc sdAbs, and polypeptides comprising or consisting thereof. Also disclosed are bifunctional, optionally dual-specific, polypeptides that promote proteasome-mediated degradation of a target protein, and uses thereof, such as for the treatment of a disease. These bifunctional polypeptides generally comprise a first component that is able to bind to a first target protein with specificity (e.g., an anti-Myc sdAb), a second component that is able to recruit a ubiquitination complex to mark the target protein for proteasomal degradation by ubiquitination, and optionally, a third component that is able to bind to a second target protein with specificity (e.g., Myc or a second target protein). An exemplary schematic for the function of a mono-specific bifunctional polypeptide comprising a single targeting moiety as disclosed herein is depicted in FIG. 1A. An exemplary schematic for the function of dual-specific bifunctional polypeptides disclosed herein may be seen in FIG. 1B (contralateral) and 1C (tandem). FIG. 2A, 2B and 2C depict some non-limiting embodiments of mono-specific (FIG. 2A) dual-specific (FIG. 2B and 2C) bifunctional polypeptides with various combinations of targeting moieties and URD, and

optionally localization sequences (e.g., NLS) and linkers (the depiction is in the conventional N-terminal to C-terminal (left to right) orientation). In some embodiments, the first and second targeting domains are on either side of the URD (see, e.g., FIG 2B) in a “contralateral” configuration. In some embodiments the first and second target binding domains are on the same side of the URD (both on the N-terminal side, or both on the C-terminal side) (see, e.g., FIG. 2C) in a “tandem” configuration. In some embodiments shown in FIG. 2A, 2B, or 2C a localization sequence (e.g., NLS) is present at both the N- and C-terminal ends. In some embodiments not shown each of the localization signals (e.g., NLS) shown can represent one, two, three or more copies of the same or a different NLS sequence. In some embodiments, the localization sequence(s) (e.g., N-NLS, and/or C-NLS) may be separated from other components by a linker sequence (not shown).

[0028] In some embodiments provided herein are mono- or dual-specific bifunctional polypeptides which are engineered (e.g., by modification of an endogenous localization peptide sequence, by adding localization peptide sequences, and/or by selecting a component which has an endogenous localization sequence) to direct the bifunctional polypeptide to a particular subcellular compartment, e.g., the cytoplasm, the nucleus, or both. Further, in some embodiments provided herein are polypeptides comprising or consisting of a portion derived from a an E3 ubiquitin ligase or viral homolog thereof. In some embodiments, the portion has ubiquitin-proteasome recruiting activity and may be designated as a ubiquitin recruiting domain (URD). In some embodiments provided herein are dual-specific bifunctional polypeptides comprising these portions.

Anti-Myc Single Domain Antibodies (sdAb)

[0029] Disclosed herein are polypeptides comprising or consisting of an anti-Myc sdAb. In some embodiments, the anti-Myc sdAb, comprises or consists of a V_H domain of an IgG antibody, or a V_HH, or V_H domain of a heavy chain antibody (HcAb). Sequences of exemplary anti-Myc sdAbs are shown in Table 1A.

Table 1A: Exemplary anti-Myc sdAb Sequences

SEQ ID NO	Description	Sequence
177	mycV.0302 CDR1	FTFADMDMG
178	mycV.0302 CDR2	AISGSGGNTYYADSVKG
179	mycV.0302 CDR3	AALGDYGDIDYSEADY
180	mycV.0302 FR1	EVQLVESGGGLVQPGGSLRLSAAASG
181	mycV.0302 FR2	WFRQAPGKEREGVS

SEQ ID NO	Description	Sequence
182	mycV.0302 FR3	RFTISRDN SKNTLYLQMNSLRAEDTAVYYA
183	mycV.0302 FR4	WGQGTQVTVSS
184	mycV.0302 full	EVQLVESGGGLVQP GGSRLSAAASGFTFADMDMGW FRQAPGKEREGVSAISGSGGNTYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYAAALGDYGD AIDYSE ADYWGQGTQVTVSS
185	mycV.0305 CDR1	FTFSTQPM S
186	mycV.0305 CDR2	AISASGDVTYYADSVKG
187	mycV.0305 CDR3	AALGDYGD AIDYSEADY
188	mycV.0305 FR1	EVQLLES G GGLVQP GGSRLSAAASG
189	mycV.0305 FR2	WFRQAPGKEREGVS
190	mycV.0305 FR3	RFTISRDN SKNTLYLQMNSLRAEDTAVYYA
191	mycV.0305 FR4	WGQGTQVTVSS
192	mycV.0305 full	EVQLLES G GGLVQP GGSRLSAAASGFTFSTQPM SWFR QAPGKEREGVSAISASGDVTYYADSVKGRFTISRDN SK NTLYLQMNSLRAEDTAVYYAAALGDYGD AIDYSEAD YWGQGTQVTVSS
193	mycV.0306 CDR1	FTFADMDMG
194	mycV.0306 CDR2	AISGSGGNTYYADSVRG
195	mycV.0306 CDR3	AALGDYGG AIDYSEADY
196	mycV.0306 FR1	EVQLLES G GGLVQP GGSRLSAAASG
197	mycV.0306 FR2	WFRQAPGKEREGVS
198	mycV.0306 FR3	RFTISRDN SKNTLYLQMNSLRAEDTAVYYA
199	mycV.0306 FR4	WGQGTQVTVSS
200	mycV.0306 full	EVQLLES G GGLVQP GGSRLSAAASGFTFADMDMGW FRQAPGKEREGVSAISGSGGNTYYADSVRGRFTISRDN SKNTLYLQMNSLRAEDTAVYYAAALGDYGG AIDYSE ADYWGQGTQVTVSS
201	mycV.0307 CDR1	FTFEGQDMG
202	mycV.0307 CDR2	AISGDAEATYYADSVKG
203	mycV.0307 CDR3	AALGDYGD AIDYSEADY
204	mycV.0307 FR1	EVQLLES G GGLVQP GGSRLSAAASG
205	mycV.0307 FR2	WFRQAPGKEREGVS
206	mycV.0307 FR3	RFTISRDN SKNTLYLQMNSLRAEDTAVYYA
207	mycV.0307 FR4	WGQGTQVTVSS
208	mycV.0307 full	EVQLLES G GGLVQP GGSRLSAAASGFTFEGQDMGWF RQAPGKEREGVSAISGDAEATYYADSVKGRFTISRDN S KNTLYLQMNSLRAEDTAVYYAAALGDYGD AIDYSEA DYWGQGTQVTVSS
209	mycV.1308 CDR1	FTFDETAMG
210	mycV.1308 CDR2	AISASSAETYYADSVKG
211	mycV.1308 CDR3	AYLGQELTGASEHDY
212	mycV.1308 FR1	EVRLLS G GGLVQP GGSRLSAAASG
213	mycV.1308 FR2	WFRQAPGKEREGVS

SEQ ID NO	Description	Sequence
214	mycV.1308 FR3	RFTISRDN SKNTLYLQMNSLRAEDTAVYYA
215	mycV.1308 FR4	WGRGTQVTVSS
216	mycV.1308 full	EVRLLES GGGLVQPGGSLRLSAAASGFTFDETAMGWF RQAPGKEREGVSAISASSAETYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYAAAYLGQELTGASEHDY WGRGTQVTVSS
217	mycV.1517 CDR1	FTFEDHAMG
218	mycV.1517 CDR2	AISGDAATTYYADSVKG
219	mycV.1517 CDR3	AIIDDILTGGTFDEGDY
220	mycV.1517 FR1	EVQLLES GGGLVQPGGSLRLSAAASG
221	mycV.1517 FR2	WFRQAPGKEREGVS
222	mycV.1517 FR3	RFTISRDN SKNTLYLQMNSLRAEDTAVYYV
223	mycV.1517 FR4	WGRGTQVTVSS
224	mycV.1517 full	EVQLLES GGGLVQPGGSLRLSAAASGFTFEDHAMGWF RQAPGKEREGVSAISGDAATTYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYVAIIDDILTGGTFDEGDY WGRGTQVTVSS
225	mycV.1518 CDR1	FTFDEYAMG
226	mycV.1518 CDR2	AISPSGDTAYYADSVKG
227	mycV.1518 CDR3	AIIDDILTGGTFDEGDY
228	mycV.1518 FR1	GVQLLES GGGLVQPGGSLRLSAAASG
229	mycV.1518 FR2	WFRQAPGKEREGVS
230	mycV.1518 FR3	RFTISRDN SKNTLYLQMNSLRAEDTAVYYV
231	mycV.1518 FR4	WGRGTQVTVSS
232	mycV.1518 full	GVQLLES GGGLVQPGGSLRLSAAASGFTFDEYAMGWF RQAPGKEREGVSAISPSGDTAYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYVAIIDDILTGGTFDEGDY WGRGTQVTVSS
233	mycV.1519 CDR1	FTFSTVAMG
234	mycV.1519 CDR2	AISRSGDTTYADSVKG
235	mycV.1519 CDR3	AIIDDILTGGTFDEGDY
236	mycV.1519 FR1	EVQLLES GGGLVQPGGSLRLSAAASG
237	mycV.1519 FR2	WFRQAPGKEREGVS
238	mycV.1519 FR3	RFTISRDN SKNTLYLQMNSLRAEDTAVYYV
239	mycV.1519 FR4	WGRGTQVTVSS
240	mycV.1519 full	EVQLLES GGGLVQPGGSLRLSAAASGFTFSTVAMGWF RQAPGKEREGVSAISRSGDTTYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYVAIIDDILTGGTFDEGDY WGRGTQVTVSS
241	mycV.1520 CDR1	FTFDEYSMG
242	mycV.1520 CDR2	AISASGDTVYYADSVKG
243	mycV.1520 CDR3	AIIDDILTGGTFDEGDY
244	mycV.1520 FR1	EVQLLES GGGLVQPGGSLRLSAAASG
245	mycV.1520 FR2	WFRQAPGKEREGVS

SEQ ID NO	Description	Sequence
246	mycV.1520 FR3	RFTISRDNSKNTLYLQMNSLRAEDTAVYYV
247	mycV.1520 FR4	WGRGTQVTVSS
248	mycV.1520 full	EVQLLES GGGLVQP GGSRLRLSAAASGFTFDEYS MGWF RQAPGKEREGVSAISASGDVTYYADSVKGRFTISRDNS KNTLYLQMNSLRAEDTAVYYVAIIDDILTGGTFDEGDY WGRGTQVTVSS
249	mycV.1522 CDR1	FTFAEADMG
250	mycV.1522 CDR2	AISGDAAVTYYADSVKG
251	mycV.1522 CDR3	AIIDDILTDGTFDEGDY
252	mycV.1522 FR1	EVQLLES GGGLVQP GGSRLRLSAAASG
253	mycV.1522 FR2	WFRQAPGKEREGVS
254	mycV.1522 FR3	RFTISRDNSKNTLYLQMNSLRAEDTAVYYV
255	mycV.1522 FR4	WGRGTQVTVSS
256	mycV.1522 full	EVQLLES GGGLVQP GGSRLRLSAAASGFTFAEADMGW F RQAPGKEREGVSAISGDAAVTYYADSVKGRFTISRDNS KNTLYLQMNSLRAEDTAVYYVAIIDDILTDGTFDEGDY WGRGTQVTVSS
257	mycV.1524 CDR1	FDFGDHAMG
258	mycV.1524 CDR2	AISGDAATTYYADSVKG
259	mycV.1524 CDR3	AIIDDILTGGTFDEGDY
260	mycV.1524 FR1	EVQLLES GGGLVQP GGSRLRLSAAASG
261	mycV.1524 FR2	WFRQAPGKEREGVS
262	mycV.1524 FR3	RFTISRDNSKNTLYLQMNSLRAEDTAVYYV
263	mycV.1524 FR4	WGRGTQVTVSS
264	mycV.1524 full	EVQLLES GGGLVQP GGSRLRLSAAASGFD FGDHAMGW F RQAPGKEREGVSAISGDAATTYYADSVKGRFTISRDNS KNTLYLQMNSLRAEDTAVYYVAIIDDILTGGTFDEGDY WGRGTQVTVSS
265	mycV.3706 CDR1	FTFGSNDMG
266	mycV.3706 CDR2	AISSGGDTTYADSVKG
267	mycV.3706 CDR3	AILPGELSLGNEYDY
268	mycV.3706 FR1	EVQLLES GGGLVQP GGSRLRLSAAASG
269	mycV.3706 FR2	WFRQAPGKEREGVS
270	mycV.3706 FR3	RFTISRDNSKNTLYLQMNSLRAEDTAVYYA
271	mycV.3706 FR4	WGQGTQVTVSS
272	mycV.3706 full	EVQLLES GGGLVQP GGSRLRLSAAASGFTFGSNDMGW F RQAPGKEREGVSAISSGGDTTYADSVKGRFTISRDNS KNTLYLQMNSLRAEDTAVYYAAILPGELSLGNEYDYW GQGTQVTVSS
273	mycV.3708 CDR1	FTFEGQDMG
274	mycV.3708 CDR2	AISGSGEHTYYADSVKG
275	mycV.3708 CDR3	AILPGELSLGNEYDY

SEQ ID NO	Description	Sequence
276	mycV.3708 FR1	EVQLLES GGGLVQPGGSLRLSAAASG
277	mycV.3708 FR2	WFRQAPGKERE GVS
278	mycV.3708 FR3	RFSISRDN SKNTLYLQMNSLRAEDTAVYYA
279	mycV.3708 FR4	WGQGTQVT VSS
280	mycV.3708 full	EVQLLES GGGLVQPGGSLRLSAAASGFTFEGQDMGWF RQAPGKERE GVS AISGSGEHTYYADSVKGRFSISRDN S KNTLYLQMNSLRAEDTAVYYAAILPGELSLGNEYDYW GQGTQVT VSS
281	mycV.6605 CDR1	FTFDEHAMG
282	mycV.6605 CDR2	AISGDASVTYYADSVKG
283	mycV.6605 CDR3	ATLDEYS GF GGASEADY
284	mycV.6605 FR1	EVQLLES GGGLVQPGGSLRLSCAASG
285	mycV.6605 FR2	WFRQAPGKERE GVS
286	mycV.6605 FR3	RFTISRDN SKNTLYLQMNSLRAEDTAVYYC
287	mycV.6605 FR4	WGQGTQVT VSS
288	mycV.6605 full	EVQLLES GGGLVQPGGSLRLSCAASGFTFSDHAMGWF RQAPGKERE GVS AISASGGTTYADSVKGRFTISRDN S KNTLYLQMNSLRAEDTAVYYCATLDEYS GF GGASEA DYWGQGTQVT VSS
289	mycV.6608 CDR1	FTFSDHAMG
290	mycV.6608 CDR2	AISASGGTTYADSVKG
291	mycV.6608 CDR3	ATLDEYS GF GGASEADY
292	mycV.6608 FR1	EVQLLES GGGLVQPGGSLRLSCAASG
293	mycV.6608 FR2	WFRQAPGKERE GVS
294	mycV.6608 FR3	RFTISRDN SKNTLYLQMNSLRAEDTAVYYC
295	mycV.6608 FR4	WGQGTQVT VSS
296	mycV.6608 full	EVQLLES GGGLVQPGGSLRLSCAASGFTFDEHAMGWF RQAPGKERE GVS AISGDASVTYYADSVKGRFTISRDN S KNTLYLQMNSLRAEDTAVYYCATLDEYS GF GGASEA DYWGQGTQVT VSS

[0030] In the above table CDR refers to a complementarity-determining region of the sdAb, and FR refers to a framework region of the sdAb, while “full” is the entire anti-Myc sdAb sequence. Note that not every sequence in the above table is unique, as some exemplary anti-Myc sdAbs share one or more sequences. For example, all of the FR2 sequences are identical even though a separate SEQ ID NO is provided for each FR2 sequence. Table 1B below provides a list of SEQ ID NOs which have identical sequences.

Table 1B: List of SEQ ID NOs having identical sequences

Name	SEQ ID NO	Sequence
mycV.0300 CDR1	139	FTFADTDMG
mycV.1500 CDR1	155	
mycV.0302 CDR1	185	FTFADMMDMG
mycV.0306 CDR1	193	
mycV.0300 CDR2	141	AISGSGGNTYYADSVKG
mycV.0302 CDR2	178	
mycV.0305 CDR2	194	AISASGDVTTYADSVKG
mycV.1520 CDR2	242	
mycV.1517 CDR2	218	AISGDAATTYYADSVKG
mycV.1524 CDR2	258	
mycV.0300 CDR3	143	AALGDYGDAIDYSEADY
mycV.0302 CDR3	179	
mycV.0305 CDR3	187	
mycV.0307 CDR3	203	
mycV.1500 CDR3	159	AIIDDILTGGTFDEGDY
mycV.1517 CDR3	219	
mycV.1518 CDR3	227	
mycV.1519 CDR3	235	
mycV.1520 CDR3	243	
mycV.1524 CDR3	259	
mycV.3700 CDR3	167	AILPGELS--LGNEYDY
mycV.3706 CDR3	267	
mycV.3708 CDR3	275	
mycV.6600 CDR3	175	ATLDEYSGFGGASEADY
mycV.6605 CDR3	283	
mycV.6608 CDR3	291	
mycV.0307 FR1	204	EVQLLESGGGLVQPGGSLRLSAAASG
mycV.1300 FR1	146	
mycV.1500 FR1	154	

mycV.1517 FR1	220	
mycV.1519 FR1	236	
mycV.1520 FR1	244	
mycV.1522 FR1	252	
mycV.1524 FR1	260	
mycV.3700 FR1	162	
mycV.3706 FR1	268	
mycV.3708 FR1	276	
mycV.6600 FR1	169	EVQLLESGGGLVQPGGSLRLSCAASG
mycV.6605 FR1	284	
mycV.6608 FR1	292	
mycV.0300 FR2	140	WFRQAPGKEREGVS
mycV.0302 FR2	181	
mycV.0305 FR2	189	
mycV.0306 FR2	197	
mycV.0307 FR2	205	
mycV.1300 FR2	148	
mycV.1308 FR2	213	
mycV.1500 FR2	156	
mycV.1517 FR2	221	
mycV.1518 FR2	229	
mycV.1519 FR2	237	
mycV.1520 FR2	245	
mycV.1522 FR2	254	
mycV.1524 FR2	261	
mycV.3700 FR2	164	
mycV.3706 FR2	269	
mycV.3708 FR2	277	
mycV.6600 FR2	172	
mycV.6605 FR2	285	

mycV.6608 FR2	293	
mycV.0300 FR3	142	RFTISRDN SKNTLYLQMNSLRAEDTAVYYA
mycV.0302 FR3	182	
mycV.0306 FR3	198	
mycV.0307 FR3	206	
mycV.1300 FR3	150	
mycV.1308 FR3	214	
mycV.3700 FR3	166	
mycV.3706 FR3	270	
mycV.1500 FR3	158	RFTISRDN SKNTLYLQMNSLRAEDTAVYYV
mycV.1517 FR3	222	
mycV.1518 FR3	230	
mycV.1519 FR3	238	
mycV.1520 FR3	246	
mycV.1522 FR3	254	
mycV.1524 FR3	262	
mycV.6600 FR3	174	RFTISRDN SKNTLYLQMNSLRAEDTAVYYC
mycV.6605 FR3	286	
mycV.6608 FR3	294	
mycV.0300 FR4	144	WGQGTQVTVSS
mycV.0302 FR4	183	
mycV.0305 FR4	191	
mycV.0306 FR4	199	
mycV.0307 FR4	207	
mycV.3700 FR4	168	
mycV.3706 FR4	271	
mycV.3708 FR4	279	
mycV.6600 FR4	176	
mycV.6605 FR4	287	
mycV.6608 FR4	295	

mycV.1300 FR4	152	WGRGTQVTVSS
mycV.1308 FR4	215	
mycV.1500 FR4	160	
mycV.1517 FR4	223	
mycV.1518 FR4	231	
mycV.1519 FR4	239	
mycV.1520 FR4	247	
mycV.1522 FR4	255	
mycV.1524 FR4	263	

[0031] The above exemplary anti-Myc sdAbs are affinity optimized antibodies of five parental anti-Myc sdAbs, the sequences of which are provided in Table 2 below.

Table 2: Parental anti-Myc sdAb sequences

SEQ ID NO	Description	Sequence
137	mycV.0300 full	EVQLLESGGGLVQPGGSLRLSAAASGFTFADTDMGW FRQAPGKEREVSAISGSGGNTYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYAAALGDYGDAIDYSE ADYWGQGTQVTVSS
138	mycV.0300 FR1	EVQLLESGGGLVQPGGSLRLSAAASG
139	mycV.0300 CDR1	FTFADTDMG
140	mycV.0300 FR2	WFRQAPGKEREVSAIS
141	mycV.0300 CDR2	SGSGGNTYYADSVKG
142	mycV.0300 FR3	RFTISRDN SKNTLYLQMNSLRAEDTAVYYA
143	mycV.0300 CDR3	AALGDYGDAIDYSEADY
144	mycV.0300 FR4	WGQGTQVTVSS
145	mycV.1300 full	EVQLLESGGGLVQPGGSLRLSAAASGFTFGDADMGW FRQAPGKEREVSAISASGDSTYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYAAHLQELTGASEHD YWGRGTQVTVSS
146	mycV.1300 FR1	EVQLLESGGGLVQPGGSLRLSAAASG
147	mycV.1300 CDR1	FTFGDADMG
148	mycV.1300 FR2	WFRQAPGKEREVSAIS
149	mycV.1300 CDR2	ASASGDSTYYADSVKG
150	mycV.1300 FR3	RFTISRDN SKNTLYLQMNSLRAEDTAVYYA
151	mycV.1300 CDR3	AHLQELTGASEHDY
152	mycV.1300 FR4	WGRGTQVTVSS

SEQ ID NO	Description	Sequence
153	mycV.1500 full	EVQLLESGGGLVQPGGSLRLSAAASGFTFADTDMGW FRQAPGKEREGLVSAISGSGGSTYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYVAIIDDILTGGTFDEG DYWGRGTQVTVSS
154	mycV.1500 FR1	EVQLLESGGGLVQPGGSLRLSAAASG
155	mycV.1500 CDR1	FTFADTDMG
156	mycV.1500 FR2	WFRQAPGKEREGLV
157	mycV.1500 CDR2	AISGSGGSTYYADSVKG
158	mycV.1500 FR3	RFTISRDN SKNTLYLQMNSLRAEDTAVYYV
159	mycV.1500 CDR3	AIIDDILTGGTFDEGDY
160	mycV.1500 FR4	WGRGTQVTVSS
161	mycV.3700 full	EVQLLESGGGLVQPGGSLRLSAAASGFTFDVSDMGWF RQAPGKEREGLVSAIGTEGDTYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYAAILPGELSLGNEYDY WGQGTQVTVSS
162	mycV.3700 FR1	EVQLLESGGGLVQPGGSLRLSAAASG
163	mycV.3700 CDR1	FTFDVSDMG
164	mycV.3700 FR2	WFRQAPGKEREGLV
165	mycV.3700 CDR2	AI-GTEGDTYYADSVKG
166	mycV.3700 FR3	RFTISRDN SKNTLYLQMNSLRAEDTAVYYA
167	mycV.3700 CDR3	AILPGELSLGNEYDY
168	mycV.3700 FR4	WGQGTQVTVSS
169	mycV.6600 full	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWF RQAPGKEREGLVSAISGSGGSTYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCATLDEYSGFGGASEA DYWGQGTQVTVSS
170	mycV.6600 FR1	EVQLLESGGGLVQPGGSLRLSCAASG
171	mycV.6600 CDR1	FTFSSYAMS
172	mycV.6600 FR2	WFRQAPGKEREGLV
173	mycV.6600 CDR2	AISGSGGSTYYADSVKG
174	mycV.6600 FR3	RFTISRDN SKNTLYLQMNSLRAEDTAVYYC
175	mycV.6600 CDR3	ATLDEYSGFGGASEADY
176	mycV.6600 FR4	WGQGTQVTVSS

[0032] The exemplary anti-Myc sdAbs disclosed in Table 1 have improved affinity for the target protein c-Myc as compared to the parental anti-Myc sdAb from which they are derived as evidenced by the K_d values calculated for each. The K_d values for the exemplary anti-Myc sdAbs and the parental anti-Myc sdAbs are provided in Table 3. “Octet sdAb K_d Human Myc Peptide Monovalent (M)” refers to the affinity of the anti-Myc sdAb against human c-Myc antigen peptide as described in more detail in Example 4. “Octet sdAb K_d Human Myc-Fc

Monovalent (M)” refers to the affinity of the anti-Myc sdAb against human c-Myc antigen-Fc fusion as described in more detail in Example 4.

Table 3: Kd Values of exemplary and parental anti-Myc sdAb

Anti-Myc sdAb	Octet sdAb Kd Human Myc Peptide Monovalent (M)	Octet sdAb Kd Human Myc-Fc Monovalent (M)
<u>mycV.0300 (Parental)</u>	<u>5.86E-08</u>	<u>4.92E-08</u>
mycV.0302	5.56E-09	3.41E-09
mycV.0305	1.16E-08	1.18E-08
mycV.0306	5.15E-09	3.78E-09
mycV.0307	1.16E-08	6.90E-09
<u>mycV.1300 (Parental)</u>	<u>9.18E-08</u>	<u>5.03E-08</u>
mycV.1308	1.12E-08	5.31E-09
<u>mycV.1500 (Parental)</u>	<u>1.49E-07</u>	<u>1.40E-07</u>
mycV.1517	4.67E-08	3.61E-08
mycV.1518	1.61E-08	1.81E-08
mycV.1519	5.36E-08	3.97E-08
mycV.1520	4.05E-08	2.70E-08
mycV.1522	3.12E-08	1.76E-08
mycV.1524	3.52E-08	2.76E-08
<u>mycV.3700 (Parental)</u>	<u>2.37E-08</u>	<u>2.04E-08</u>
mycV.3706	3.80E-09	1.61E-09
mycV.3708	4.60E-09	2.05E-09
<u>mycV.6600 (Parental)</u>	<u>1.09E-07</u>	<u>1.22E-07</u>
mycV.6605	1.00E-08	9.46E-09
mycV.6608	1.01E-08	9.47E-09

[0033] By aligning all of the parental and exemplary anti-Myc sdAb sequences, the following exemplary consensus sequences were prepared. Tables 4-9 are alignments of each of CDR1, CDR2, CDR3, FR1, FR3, and FR4 of all of the exemplary and parental anti-Myc sdAbs listed in Tables 1A and 2 above, with the resulting consensus sequences for each. The sequence of FR2 was identical across all anti-Myc sdAbs and therefore no table is provided for the FR2 consensus sequence.

Table 4: Consensus CDR1

Name	Sequence	SEQ ID NO
mycV.0300 CDR1	FTFADTDMG	139
mycV.0302 CDR1	FTFADMDMG	177
mycV.0305 CDR1	FTFSTQPMs	185
mycV.0306 CDR1	FTFADMDMG	193
mycV.0307 CDR1	FTFEGQDMG	201
mycV.1300 CDR1	FTFGDADMg	147
mycV.1308 CDR1	FTFDEtAMG	209
mycV.1500 CDR1	FTFADTDMG	155
mycV.1517 CDR1	FTFEDHAMG	217
mycV.1518 CDR1	FTFDEYAMG	225
mycV.1519 CDR1	FTFSTVAMG	233
mycV.1520 CDR1	FTFDEYSMG	241
mycV.1522 CDR1	FTFAEADMg	249
mycV.1524 CDR1	FDFGDHAMG	257
mycV.3700 CDR1	FTFDVSDMG	163
mycV.3706 CDR1	FTFGSNDMG	265
mycV.3708 CDR1	FTFEGQDMG	273
mycV.6600 CDR1	FTFSSYAMS	171
mycV.6605 CDR1	FTFDEHAMG	281
mycV.6608 CDR1	FTFSDHAMG	289
Consensus CDR1	FX ₁ FX ₂ X ₃ X ₄ X ₅ MX ₆	297
X residues	X ₁ = T or D; X ₂ = A, S, E, G or D; X ₃ = D, T, G, E, V or S; X ₄ = T, M, Q, A, H, Y, V or N; X ₅ = D, P or A; X ₆ = G or S	

Table 5: Consensus CDR2 with parental

Name	Sequence	SEQ ID NO
mycV.0300 CDR2	AlSGSGGNTYYADSVKG	141
mycV.0302 CDR2	AlSGSGGNTYYADSVKG	178

Name	Sequence	SEQ ID NO
mycV.0305 CDR2	AISASGDVTYYADSVKG	186
mycV.0306 CDR2	AISGSGGNTYYADSVRG	194
mycV.0307 CDR2	AISGDAEATYYADSVKG	202
mycV.1300 CDR2	AISASGDSTYYADSVKG	149
mycV.1308 CDR2	AISASSAETYYADSVKG	210
mycV.1500 CDR2	AISGSGGSTYYADSVKG	157
mycV.1517 CDR2	AISGDAATTYYADSVKG	218
mycV.1518 CDR2	AISPSGDTAYYADSVKG	226
mycV.1519 CDR2	AISRSGDTTYYADSVKG	234
mycV.1520 CDR2	AISASGDVTYYADSVKG	242
mycV.1522 CDR2	AISGDAAVTYYADSVKG	250
mycV.1524 CDR2	AISGDAATTYYADSVKG	258
mycV.3700 CDR2	AI-GTEGDTYYADSVKG	165
mycV.3706 CDR2	AISSGGDTTYYADSVKG	266
mycV.3708 CDR2	AISGSGEHTYYADSVKG	274
mycV.6600 CDR2	AISGSGGSTYYADSVKG	173
mycV.6605 CDR2	AISGDASVTYYADSVKG	282
mycV.6608 CDR2	AISASGGTTYYADSVKG	290
Consensus CDR2	AI $X_1X_2X_3X_4X_5X_6X_7$ YYADSV X_8 G	298
X residues	X_1 = S or no amino acid; X_2 = G, A, P, R or S; X_3 = S, D, T or G; X_4 = G, A, S, or E; X_5 = G, D, E, A or S; X_6 = N, V, A, S, E, T, D or H; X_7 = T or A; X_8 = K or R	

Table 6: Consensus CDR3

Name	Sequence	SEQ ID NO
mycV.0300 CDR3	AALGDYGDAIDYSEADY	143
mycV.0302 CDR3	AALGDYGDAIDYSEADY	179
mycV.0305 CDR3	AALGDYGDAIDYSEADY	187
mycV.0306 CDR3	AALGDYGGAIIDYSEADY	195

Name	Sequence	SEQ ID NO
mycV.0307 CDR3	AALGDYGD A IDYSEADY	203
mycV.1300 CDR3	AHLDQ E ELT--GASEHDY	151
mycV.1308 CDR3	AYLGQ E ELT--GASEHDY	211
mycV.1500 CDR3	AIIDDILTGGTFDEGDY	159
mycV.1517 CDR3	AIIDDILTGGTFDEGDY	219
mycV.1518 CDR3	AIIDDILTGGTFDEGDY	227
mycV.1519 CDR3	AIIDDILTGGTFDEGDY	235
mycV.1520 CDR3	AIIDDILTGGTFDEGDY	243
mycV.1522 CDR3	AIIDDILTDGTFDEGDY	251
mycV.1524 CDR3	AIIDDILTGGTFDEGDY	259
mycV.3700 CDR3	AILPGELS--LGNEYDY	167
mycV.3706 CDR3	AILPGELS--LGNEYDY	267
mycV.3708 CDR3	AILPGELS--LGNEYDY	275
mycV.6600 CDR3	ATLDEYSGFGGASEADY	175
mycV.6605 CDR3	ATLDEYSGFGGASEADY	283
mycV.6608 CDR3	ATLDEYSGFGGASEADY	291
Consensus CDR3	AX ₁ X ₂ X ₃ X ₄ X ₅ X ₆ X ₇ X ₈ X ₉ X ₁₀ X ₁₁ X ₁₂ EX ₁₃ DY	299
X residues	X ₁ = A, H, Y, I, or T; X ₂ = L or I; X ₃ = G, D or P; X ₄ = D, Q, G or E; X ₅ = Y, E or I; X ₆ = G, L or S; X ₇ = D, T, S or G; X ₈ = A, G, F, or no amino acid; X ₉ = I, G, or no amino acid; X ₁₀ = D, G, T or L; X ₁₁ = Y, A, F or G; X ₁₂ = S, D or N; X ₁₃ = A, H, G or Y	

Table 7: Consensus FR1

Name	Sequence	SEQ ID NO
mycV.0300 FR1	EVQLLES Q GGGLVQPGGSLRLSAAASG	138
mycV.0302 FR1	EVQLVES Q GGGLVQPGGSLRLSAAASG	180
mycV.0305 FR1	EVQLLES Q GGGLVQPGGSLRLSAAASG	188
mycV.0306 FR1	EVQLLES Q GGGLVQPGGSLRLSAAASG	196

mycV.0307 FR1	EVQLLES GGGLVQPGGSLRLS AA ASG	204
mycV.1300 FR1	EVQLLES GGGLVQPGGSLRLS AA ASG	146
mycV.1308 FR1	EVRLLES GGGLVQPGGSLRLS AA ASG	212
mycV.1500 FR1	EVQLLES GGGLVQPGGSLRLS AA ASG	154
mycV.1517 FR1	EVQLLES GGGLVQPGGSLRLS AA ASG	220
mycV.1518 FR1	GVQLLES GGGLVQPGGSLRLS AA ASG	228
mycV.1519 FR1	EVQLLES GGGLVQPGGSLRLS AA ASG	236
mycV.1520 FR1	EVQLLES GGGLVQPGGSLRLS AA ASG	244
mycV.1522 FR1	EVQLLES GGGLVQPGGSLRLS AA ASG	252
mycV.1524 FR1	EVQLLES GGGLVQPGGSLRLS AA ASG	260
mycV.3700 FR1	EVQLLES GGGLVQPGGSLRLS AA ASG	162
mycV.3706 FR1	EVQLLES GGGLVQPGGSLRLS AA ASG	268
mycV.3708 FR1	EVQLLES GGGLVQPGGSLRLS AA ASG	276
mycV.6600 FR1	EVQLLES GGGLVQPGGSLRLS CA ASG	170
mycV.6605 FR1	EVQLLES GGGLVQPGGSLRLS CA ASG	284
mycV.6608 FR1	EVQLLES GGGLVQPGGSLRLS CA ASG	292
Consensus FR1	X₁ V X₂ L X₃ ESGGGLVQPGGSLRLS X₄ AASG	300
X residues	X ₁ = E or G; X ₂ = Q or R; X ₃ = L or V; X ₄ = A or C	

Table 8: Consensus FR3

Name	Sequence	SEQ ID NO
mycV.0300 FR3	RF T ISRDNSKNTLYLQMN SLRAED TAVYY A	142
mycV.0302 FR3	RF T ISRDNSKNTLYLQMN SLRAED TAVYY A	182
mycV.0305 FR3	RF T ISRDNSKNTLYLQMN GLRAAD TAVYY A	190
mycV.0306 FR3	RF T ISRDNSKNTLYLQMN SLRAED TAVYY A	198
mycV.0307 FR3	RF T ISRDNSKNTLYLQMN SLRAED TAVYY A	206
mycV.1300 FR3	RF T ISRDNSKNTLYLQMN SLRAED TAVYY A	150
mycV.1308 FR3	RF T ISRDNSKNTLYLQMN SLRAED TAVYY A	214
mycV.1500 FR3	RF T ISRDNSKNTLYLQMN SLRAED TAVYY V	158

mycV.1517 FR3	RFTISRDN SKNTLYLQMN SLRAED TAVYY V	222
mycV.1518 FR3	RFTISRDN SKNTLYLQMN SLRAED TAVYY V	230
mycV.1519 FR3	RFTISRDN SKNTLYLQMN SLRAED TAVYY V	238
mycV.1520 FR3	RFTISRDN SKNTLYLQMN SLRAED TAVYY V	246
mycV.1522 FR3	RFTISRDN SKNTLYLQMN SLRAED TAVYY V	254
mycV.1524 FR3	RFTISRDN SKNTLYLQMN SLRAED TAVYY V	262
mycV.3700 FR3	RFTISRDN SKNTLYLQMN SLRAED TAVYY A	166
mycV.3706 FR3	RFTISRDN SKNTLYLQMN SLRAED TAVYY A	270
mycV.3708 FR3	RFSISRDN SKNTLYLQMN SLRAED TAVYY A	278
mycV.6600 FR3	RFTISRDN SKNTLYLQMN SLRAED TAVYY C	174
mycV.6605 FR3	RFTISRDN SKNTLYLQMN SLRAED TAVYY C	286
mycV.6608 FR3	RFTISRDN SKNTLYLQMN SLRAED TAVYY C	294
Consensus FR3	RFX ₁ ISRDN SKNTLYLQMN X₂ LRA X₃ DTAVYY X₄	302
X residues	X ₁ = T or S; X ₂ = S or G; X ₃ = E or A; X ₄ = A, V or C	

Table 9: Consensus FR4

Name	Sequence	SEQ ID NO
mycV.0300 FR4	WG Q GTQVT V SS	144
mycV.0302 FR4	WG Q GTQVT V SS	183
mycV.0305 FR4	WG Q GTQVT V SS	191
mycV.0306 FR4	WG Q GTQVT V SS	199
mycV.0307 FR4	WG Q GTQVT V SS	207
mycV.1300 FR4	WGRGTQVT V SS	152
mycV.1308 FR4	WGRGTQVT V SS	215
mycV.1500 FR4	WGRGTQVT V SS	160
mycV.1517 FR4	WGRGTQVT V SS	223
mycV.1518 FR4	WGRGTQVT V SS	231
mycV.1519 FR4	WGRGTQVT V SS	239
mycV.1520 FR4	WGRGTQVT V SS	247

mycV.1522 FR4	WGRGTQVTVSS	255
mycV.1524 FR4	WGRGTQVTVSS	263
mycV.3700 FR4	WGQGTQVTVSS	168
mycV.3706 FR4	WGQGTQVTVSS	271
mycV.3708 FR4	WGQGTQVTVSS	279
mycV.6600 FR4	WGQGTQVTVSS	176
mycV.6605 FR4	WGQGTQVTVSS	287
mycV.6608 FR4	WGQGTQVTVSS	295
Consensus FR4	WGX ₁ GTQVTVSS	303
X residues	X ₁ = Q or R	

[0034] Similar exemplary consensus sequences were prepared for each of CDR1-3 for each family of anti-Myc sdAbs (mycV.0300, mycV.1300, mycV.1500, mycV.3700, and mycV.6600) using the parental anti-Myc sdAb and exemplary variant anti-Myc sdAbs derived from the parental sequences. Because each of the CDR3 sequences for the mycV.3700 family were identical, and each of the CDR3 sequences for the mycV.6600 family were identical, no table is presented for the CDR3 consensus sequence for either family. Tables 10-22 provide the resulting consensus sequences.

Table 10: mycV.0300 Consensus CDR1

Name	Sequence	SEQ ID NO
mycV.0300 CDR1	FTFADTDMG	139
mycV.0302 CDR1	FTFADMDMG	177
mycV.0305 CDR1	FTFSTQPMs	185
mycV.0306 CDR1	FTFADMDMG	193
mycV.0307 CDR1	FTFEGQDMG	201
mycV.0300 consensus CDR1	FTFX ₁ X ₂ X ₃ X ₄ MX ₅	304
X residues	X ₁ = A, S or E; X ₂ = D, T, or G; X ₃ = T, M or Q; X ₄ = D or P; and X ₅ = G or S	

Table 11: mycV.0300 Consensus CDR2

Name	Sequence	SEQ ID NO
mycV.0300 CDR2	AIS GSGGNT YYADSVKG	141
mycV.0302 CDR2	AIS GSGGNT YYADSVKG	178
mycV.0305 CDR2	AIS ASGDV TYADSVKG	186
mycV.0306 CDR2	AIS GSGGNT YYADSVRG	194
mycV.0307 CDR2	AIS GDAEAT YYADSVKG	202
mcyV.0300 consensus CDR2	AIS X₁X₂X₃X₄X₅ YYADSV X₆ G	305
X residues	X ₁ = G or A; X ₂ is S or D; X ₃ = G or A; X ₄ = G, D, or E; X ₅ = N, V or A; X ₆ = K or R	

Table 12: mycV.0300 Consensus CDR3

Name	Sequence	SEQ ID NO
mycV.0300 CDR3	AALGDYGD AIDY SEADY	143
mycV.0302 CDR3	AALGDYGD AIDY SEADY	179
mycV.0305 CDR3	AALGDYGD AIDY SEADY	187
mycV.0306 CDR3	AALGDYGD GAIDY SEADY	195
mycV.0307 CDR3	AALGDYGD AIDY SEADY	203
mcyV.0300 consensus CDR3	AALGDYG X₁ AIDYSEADY	306
X residues	X ₁ = D or G	

Table 13: mycV.1300 Consensus CDR1

Name	Sequence	SEQ ID NO
mycV.1300 CDR1	FTF GD ADMG	147
mycV.1308 CDR1	FTF DE TAMG	209
mcyV.1300 consensus CDR1	FTF X₁X₂X₃X₄ MG	307
X residues	X ₁ = G or D; X ₂ = D or E; X ₃ = A or T; X ₄ = D or A	

Table 14: mycV.1300 Consensus CDR2

Name	Sequence	SEQ ID NO
mycV.1300 CDR2	AISAS GDS TYYADSVKG	149
mycV.1308 CDR2	AISAS SAE TYYADSVKG	210
mcyV.1300 consensus CDR2	AISAS X₁X₂X₃ TYYADSVKG	308
X residues	X ₁ = G or S; X ₂ = D or A; X ₃ = X or E	

Table 15: mycV.1300 Consensus CDR3

Name	Sequence	SEQ ID NO
mycV.1300 CDR3	AHLDQELT--GASEHDY	151
mycV.1308 CDR3	AYLGQELT--GASEHDY	211
mcyV.1300 consensus CDR3	AX ₁ LX ₂ QELTGASEHDY	309
X residues	X ₁ = H or Y; and X ₂ = D or G	

Table 16: mycV.1500 Consensus CDR1

Name	Sequence	SEQ ID NO
mycV.1500 CDR1	FTFADTDMG	155
mycV.1517 CDR1	FTFEDHAMG	217
mycV.1518 CDR1	FTFDEYAMG	225
mycV.1519 CDR1	FTFSTVAMG	233
mycV.1520 CDR1	FTFDEYSMG	241
mycV.1522 CDR1	FTFAEADMG	249
mycV.1524 CDR1	FDFGDHAMG	265
mcyV.1500 consensus CDR1	FX ₁ FX ₂ X ₃ X ₄ X ₅ MG	310
X residues	X ₁ = T or D; X ₂ = A, E, D, S or G; X ₃ = D, E or T; X ₄ = T, H, Y, V or A; X ₅ = D, A or S	

Table 17: mycV.1500 Consensus CDR2

Name	Sequence	SEQ ID NO
mycV.1500 CDR2	AIS GSGGST YYADSVKG	157
mycV.1517 CDR2	AIS GDAATT YYADSVKG	218
mycV.1518 CDR2	AIS PSGDTA YYADSVKG	226
mycV.1519 CDR2	AIS RSGDTT YYADSVKG	234
mycV.1520 CDR2	AIS ASGDVT YYADSVKG	242
mycV.1522 CDR2	AIS GDAAVT YYADSVKG	250
mycV.1524 CDR2	AIS GDAATT YYADSVKG	266
mcyV.1500 consensus CDR2	AIS X₁X₂X₃X₄X₅X₆ YYADSVKG	311
X residues	X ₁ = G, P, R or A; X ₂ = S or D; X ₃ = G or A; X ₄ = G, A, T or D; X ₅ = S, T, A or V; X ₆ = T or A	

Table 18: mycV.1500 Consensus CDR3

Name	Sequence	SEQ ID NO
mycV.1500 CDR3	AIIDDILT G GFDEGDY	159
mycV.1517 CDR3	AIIDDILT G GFDEGDY	219
mycV.1518 CDR3	AIIDDILT G GFDEGDY	227
mycV.1519 CDR3	AIIDDILT G GFDEGDY	235
mycV.1520 CDR3	AIIDDILT G GFDEGDY	243
mycV.1522 CDR3	AIIDDILT D GFDEGDY	251
mycV.1524 CDR3	AIIDDILT G GFDEGDY	267
mcyV.1500 consensus CDR3	AIIDDILT X₁ GFDEGDY	312
X residues	X ₁ = G or D	

Table 19: mycV.3700 Consensus CDR1

Name	Sequence	SEQ ID NO
mycV.3700 CDR1	FTFDVSDMG	163
mycV.3706 CDR1	FTFGSNDMG	265
mycV.3708 CDR1	FTFEGQDMG	273
mcyV.3700 consensus CDR1	FTFX ₁ X ₂ X ₃ DMG	313
X residues	X ₁ = D, G or E; X ₂ = V, S or G; X ₃ = S, N, or Q	

Table 20: mycV.3700 Consensus CDR2

Name	Sequence	SEQ ID NO
mycV.3700 CDR2	AI-GTEGDTYYADSVKG	165
mycV.3706 CDR2	AISSGGDTYYADSVKG	266
mycV.3708 CDR2	AISGSGEHTYYADSVKG	274
mcyV.3700 consensus CDR2	AIX ₁ X ₂ X ₃ X ₄ X ₅ X ₆ TYADSVKG	314
X residues	X ₁ = no amino acid or S; X ₂ = G or S; X ₃ = T, G, or S; X ₄ = E or G; X ₅ = G, D or E; X ₆ = D, T or H	

Table 21: mycV.6600 Consensus CDR1

Name	Sequence	SEQ ID NO
mycV.6600 CDR1	FTFSSYAMS	171
mycV.6605 CDR1	FTFDEHAMG	281
mycV.6608 CDR1	FTFSDHAMG	289
mcyV.6600 consensus CDR1	FTFX ₁ X ₂ X ₃ AMX ₄	316
X residues	X ₁ = S or D; X ₂ = S, E or D; X ₃ = Y or H; X ₄ = S or G	

Table 22: mycV.6600 Consensus CDR2

Name	Sequence	SEQ ID NO
mycV.6600 CDR2	AIS GSGGS TTYADSVKG	173
mycV.6605 CDR2	AIS GDASV TTYADSVKG	282
mycV.6608 CDR2	AIS ASGGT TTYADSVKG	290
mcyV.6600 consensus CDR2	AIS X₁X₂X₃X₄X₅ TTYADSVKG	317
X residues	X ₁ = G or A; X ₂ = S or D; X ₃ = G or A; X ₄ = G or S; X ₅ = S, V or T	

[0035] In some embodiments, the CDR, FR, full and consensus sequences provided in the preceding tables can be used to define polypeptides comprising or consisting of an anti-Myc sdAb.

[0036] In some embodiments, a consensus sequence has variable residues having one of the amino acid specified, or optionally “a conservative substitution of any of the foregoing.” For example, variable residues X₁X₂X₃X₄ and X₅ in SEQ ID NO: 317 as shown in Table 22, comprise the amino acid specified for the X_x residues as shown in the table, or optionally a conservative substitution of any of the amino acids specified for that X_x residue. For example, Table 22 specifies that X₁ in SEQ ID NO: 317 may comprise G or A, and thus in some embodiments also contemplated is a conservative substitution of G or a conservative substitution for A. Similarly, X₂ may be S or D, and optionally in some embodiments a conservative substitution for S, or a conservative substitution for D. The same applies for each of X₃, X₄ and X₅ in SEQ ID NO: 317, as well as the other consensus sequences provided in the preceding tables and elsewhere herein. In some embodiments, the following eight groups each contain amino acids that are conservative substitutions for one another: 1) Alanine (A), Glycine (G); 2) Aspartic acid (D), Glutamic acid (E); 3) Asparagine (N), Glutamine (Q); 4) Arginine (R), Lysine (K); 5) Isoleucine (I), Leucine (L), Methionine (M), Valine (V); 6) Phenylalanine (F), Tyrosine (Y), Tryptophan (W); 7) Serine (S), Threonine (T); and 8) Cysteine (C), Methionine (M) (*see*, for example, Creighton, T. E., “Proteins - Structures and Molecular Properties,” W. H. Freeman & Co. Ltd., 1984).

Exemplary Anti-Myc Single Domain Antibodies (sdAb)

[0037] Some embodiments disclosed herein are a polypeptide comprising or consisting of an anti-Myc sdAb.

[0038] In some embodiments, including any of the foregoing, the anti-Myc sdAb comprises or consists of a V_H domain of an IgG antibody, or a V_HH, or V_H domain of a heavy chain antibody (HcAb), wherein the V_H or V_HH domain comprises a V_H CDR3 comprising or consisting of any one of SEQ ID NOs: 179, 195, 211, 219, 251, 267 or 283. In some embodiments, the V_H CDR3 sequence comprises 1, 2, 3, 4, 5, 6, 7, or more substitutions and/or 1, 2, 3, 4, 5, 6, 7, or more deletions or additions, and/or has at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to a sequence selected from any one of SEQ ID NOs: 179, 195, 211, 219, 251, 267 and 283. In some embodiments, the substitution(s) are conservative substitution(s). In some embodiments, the sdAb, optionally a V_HH domain, comprises a CDR3 comprising or consisting of the amino acid sequence AX₁X₂X₃X₄X₅X₆X₇X₈X₉X₁₀X₁₁X₁₂EX₁₃DY (SEQ ID NO: 299), wherein X₁ is A, H, Y, I, or T; X₂ is L or I; X₃ is G, D or P; X₄ is D, Q, G or E; X₅ is Y, E or I; X₆ is G, L or S; X₇ is D, T, S or G; X₈ is A, G, F, or no amino acid; X₉ is I, G, or no amino acid; X₁₀ is D, G, T or L; X₁₁ is Y, A, F or G; X₁₂ is S, D or N; and X₁₃ is A, H, G or Y; or optionally a conservative substitution of any of the foregoing; wherein the CDR3 does not comprise or consist of any one of SEQ ID NOs: 143, 151, 159, 167, or 175.

[0039] In some embodiments, including any of the foregoing, the sdAb, optionally a V_HH domain, comprises: a CDR1 comprising or consisting of any one of SEQ ID NOs: 177, 185, 201, 209, 217, 225, 233, 241, 249, 257, 265, 273, 281, 289; a CDR2 comprising or consisting of any one of SEQ ID NOs: 178, 186, 194, 202, 210, 218, 226, 234, 242, 250, 266, 274, 282 or 290; and a CDR3 comprising or consisting of any one of SEQ ID Nos: 179, 195, 211, 219, 251, 267 or 283. In some embodiments, the combination of CDR1, CDR2, and CDR3 is selected from one of the following combinations: a) SEQ ID NOs: 177, 178, and 179; b) SEQ ID NOs: 185, 186, and 187; c) SEQ ID NOs: 177, 194, and 195; d) SEQ ID NOs: 201, 202, and 179; e) SEQ ID NOs: 209, 210, and 211; f) SEQ ID NOs: 217, 218, and 219; g) SEQ ID NOs: 225, 226, and 219; h) SEQ ID NOs: 233, 234, and 219; i) SEQ ID NOs: 241, 242, and 219; j) SEQ ID NOs: 249, 250, and 251; k) SEQ ID NOs: 257, 218, and 219; l) SEQ ID NOs: 265, 266, and 267; m) SEQ ID NOs: 273, 274, and 267; n) SEQ ID NOs: 281, 282, and 283; and o) SEQ ID NOs: 289, 290, and 283. In some embodiments, each CDR sequence independently comprises 1, 2, 3, 4, 5, 6, 7, or more substitutions and/or 1, 2, 3, 4, 5, 6, 7, or more deletions or additions,

and/or has at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to a sequence selected from any one of SEQ ID NOs: 177-179, 185-186, 194-195, 201-202, 209-211, 217-219, 225-226, 233-234, 241-242, 249-251, 257, 265-267, 273-274, 281-283, or 289-290. In some embodiments, the substitution(s) are conservative substitution(s).

[0040] In some embodiments, including any of the foregoing, the sdAb, optionally a V_HH domain, comprises: a CDR1 comprising or consisting of FX₁FX₂X₃X₄X₅MX₆ (SEQ ID NO: 297), wherein X₁ is T or D; X₂ is A, S, E, G or D; X₃ is D, T, G, E, V or S; X₄ is T, M, Q, A, H, Y, V or N; X₅ is D, P or A; and X₆ is G or S; or optionally a conservative substitution of any of the foregoing; a CDR2 comprising or consisting of AIX₁X₂X₃X₄X₅X₆X₇YYADSVX₈G (SEQ ID NO: 298), wherein X₁ is S or no amino acid; X₂ is G, A, P, R or S; X₃ is S, D, T or G; X₄ is G, A, S, or E; X₅ is G, D, E, A or S; X₆ is N, V, A, S, E, T, D or H; X₇ is T or A; and X₈ is K or R; or optionally a conservative substitution of any of the foregoing; and a CDR3 comprising or consisting of AX₁X₂X₃X₄X₅X₆X₇X₈X₉X₁₀X₁₁X₁₂EX₁₃DY (SEQ ID NO: 299), wherein X₁ is A, H, Y, I, or T; X₂ is L or I; X₃ is G, D or P; X₄ is D, Q, G or E; X₅ is Y, E or I; X₆ is G, L or S; X₇ is D, T, S or G; X₈ is A, G, F, or no amino acid; X₉ is I, G, or no amino acid; X₁₀ is D, G, T or L; X₁₁ is Y, A, F or G; X₁₂ is S, D or N; and X₁₃ is A, H, G or Y; or optionally a conservative substitution of any of the foregoing; wherein the combination of CDR1, CDR2, and CDR3 is not one of the following combinations: a.) CDR1, CDR2 and CDR3 of mycV.0300 (SEQ ID NOs: 139, 141 and 143); b.) CDR1, CDR2 and CDR3 of mycV.1300 (SEQ ID NOs: 147, 149 and 151); c.) CDR1, CDR2 and CDR3 of mycV.1500 SEQ ID NOs: (155, 157 and 159); d.) CDR1, CDR2 and CDR3 of mycV.3700 SEQ ID NOs: (163, 165 and 167); and e.) CDR1, CDR2 and CDR3 of mycV.6600 SEQ ID NOs: (171, 173 and 175). Note that in the sequence listing submitted herewith, SEQ ID NO: 297 is blank, as sequence listing rules do not permit an amino acid sequence with fewer than four “specifically defined” amino acids, and SEQ ID NO: 297, (FX₁FX₂X₃X₄X₅MX₆) has only three “specifically defined” amino acids at positions 1, 3 and 8. In SEQ ID NO: 297, X₆ is either G or S (see Table 4 above). For purposes of the sequence listing, SEQ ID NO: 297 is represented by two sequences, SEQ ID NO: 772 (FX₁FX₂X₃X₄X₅MG) and SEQ ID NO: 773 (FX₁FX₂X₃X₄X₅MS), which represent the sequences wherein X₆ is specified as G or S, respectively, with X₁, X₂, X₃, X₄, and X₅ defined as in SEQ ID NO: 297 (see Table 4 above). Therefore, SEQ ID NO: 297 is the same as the combined sequences of SEQ ID NOs 772 and 773, and the combination of SEQ ID NOs: 772 and 773 can replace SEQ ID NO: 297, optionally wherein the G or S at the position of X₆ of SEQ ID NO: 772 and 773 is substituted with a conservative substitution of G or S.

[0041] In some embodiments, including any of the foregoing, the sdAb, optionally a V_HH domain, comprises a V_H FR1 comprising or consisting of any one of SEQ ID NOs: 180, 188, 212, 228 or 284, a V_H FR2 comprising or consisting of SEQ ID NO: 181, a V_H FR3 comprising or consisting of any one of SEQ ID NOs: 182, 190, 278 or 286, and a V_H FR4 comprising or consisting of any one of SEQ ID NOs: 183 or 215, optionally wherein the combination of FR1, FR2, FR3, and FR4 is selected from one of the following combinations: a.) SEQ ID NOs: 180, 181, 182, and 183; b.) SEQ ID NOs: 188, 181, 182, and 183; c.) SEQ ID NOs: 212, 181, 182, and 215; d.) SEQ ID NOs: 188, 181, 182, and 215; e.) SEQ ID NOs: 228, 181, 182, and 215; f.) SEQ ID NOs: 188, 181, 278, and 183; and g.) SEQ ID NOs: 284, 181, 286, and 183. In some embodiments, each V_H framework region comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17 or more substitutions and/or 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17 or more deletions or additions, and/or has at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to a sequence selected from any one of SEQ ID NOs: 180-183, 188, 190, 212, 215, 228, 278, 284 or 286. In some embodiments, the substitution(s) are conservative substitution(s).

[0042] In some embodiments, including any of the foregoing, the sdAb, optionally a V_HH domain, comprises: a FR1 comprising or consisting of the amino acid sequence X₁VX₂LX₃ESGGGLVQPGGSLRLSX₄AASG (SEQ ID NO: 300), wherein X₁ is E or G, X₂ is Q or R, X₃ is L or V, and X₄ is A or C; or optionally a conservative substitution of any of the foregoing; optionally wherein X₁ is E; X₂ is Q; X₃ is L; and X₄ is A or C; a FR2 comprising or consisting of the amino acid sequence SEQ ID NO: 301; a FR3 comprising or consisting of the amino acid sequence RFX₁ISRDN SKNTLYLQMNX₂LRAX₃DTAVYYX₄ (SEQ ID NO: 302), wherein X₁ is T or S; X₂ is S or G; X₃ is E or A; X₄ is A, V, or C; or optionally a conservative substitution of any of the foregoing; optionally wherein X₁ is R; X₂ is S; X₃ is E; and X₄ is A or C; and a FR4 comprising or consisting of the amino acid sequence WGX₁GTQVTVSS (SEQ ID NO: 303), wherein X₁ is Q or R; or optionally a conservative substitution of any of the foregoing; optionally wherein X₁ is Q.

[0043] In some embodiments, including any of the foregoing, the sdAb, optionally a V_HH domain, comprises or consists of a sequence selected from any one of SEQ ID NOs: 184, 192, 200, 208, 216, 224, 232, 240, 248, 256, 264, 272, 280, 288 or 296. In some embodiments, each CDR sequence independently comprises 1, 2, 3, 4, 5, 6, 7, or more substitutions and/or 1, 2, 3, 4, 5, 6, 7, or more deletions or additions, and/or has at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to a CDR sequence of any one of SEQ ID

NOs: 177-179, 185-186, 194-195, 201, 202, 209-211, 217-219, 225-226, 233-234, 241-242, 249-251, 257, 265-267, 273-274, 281-283, or 289-290, and/or wherein the framework region comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17 or more substitutions and/or 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17 or more deletions or additions, and/or has at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the framework region of any one of SEQ ID NOs: 180-183, 188, 190, 212, 215, 228, 278, 284 or 286. In some embodiments, the substitution(s) are conservative substitution(s).

[0044] In some embodiments, including any of the foregoing, the sdAb, optionally a V_{HH} domain, comprises or consists of an amino acid sequence having at least 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to any one of SEQ ID NOs: 184, 192, 200, 208, 216, 224, 232, 240, 248, 256, 264, 272, 280, 288 or 296.

A. Exemplary mcV.0300 Variant Anti-Myc sdAb

[0045] In some embodiments, including any of the exemplary anti-Myc sdAbs disclosed above and elsewhere herein, the sdAb, optionally a V_{HH} domain, comprises a CDR3 comprising or consisting of the amino acid sequence AALGDYGX₁AIDYSEADY (SEQ ID NO: 306), wherein X₁ is D or G; or optionally a conservative substitution of any of the foregoing. In some embodiments, the CDR3 comprises or consists of any one of SEQ ID NOs: 179 or 195. In some embodiments, the sdAb, optionally a V_{HH} domain, comprises: a CDR1 comprising or consisting of any one of SEQ ID NOs: 177, 185, or 201; a CDR2 comprising or consisting of any one of SEQ ID NOs: 178, 186, 194 or 202; and a CDR3 comprising or consisting of any one of SEQ ID NOs: 179 or 195. In some embodiments, the combination of CDR1, CDR2, and CDR3 is selected from one of the following combinations: a.) SEQ ID NOs: 177, 178, and 179; b.) SEQ ID NOs: 185, 186, and 179; c.) SEQ ID NOs: 177, 194, and 195; and d.) SEQ ID NOs: 201, 202, and 179. In some embodiments, each CDR sequence independently comprises 1, 2, 3, 4, 5, 6, 7, or more substitutions and/or 1, 2, 3, 4, 5, 6, 7, or more deletions or additions, and/or has at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to a sequence selected from any one of SEQ ID NOs: 177-179, 185-186, 194-195, or 201-202. In some embodiments, the substitution(s) are conservative substitution(s). In some embodiments, the sdAb, optionally a V_{HH} domain, comprises: a CDR1 comprising or consisting of the amino acid sequence FTFX₁X₂X₃X₄MX₅ (SEQ ID NO: 304), wherein X₁ is A, S or E; X₂ is D, T, or G; X₃ is T, M or Q; X₄ is D or P; and X₅ is G or S; or optionally a conservative substitution of any of the foregoing; a CDR2

comprising or consisting of the amino acid sequence AISX₁X₂X₃X₄X₅TYYADSVX₆G (SEQ ID NO: 305), wherein X₁ is G or A; X₂ is S or D; X₃ is G or A; X₄ is G, D, or E; X₅ is N, V or A; and X₆ is K or R; or optionally a conservative substitution of any of the foregoing; and a CDR3 comprising or consisting of the amino acid sequence AALGDYGX₁AIDYSEADY (SEQ ID NO: 306), wherein X₁ is D or G; or optionally a conservative substitution of any of the foregoing; wherein the combination of CDR1, CDR2 and CDR3 is not the combination of CDR1, CDR2 and CDR3 of mycV.0300 (SEQ ID NOS: 139, 141 and 143). In some embodiments, the sdAb, optionally a V_HH domain, comprises a V_H FR1 comprising or consisting of any one of SEQ ID NOS: 180 or 188, a V_H FR2 comprising or consisting of SEQ ID NO: 181, a V_H FR3 comprising or consisting of any one of SEQ ID NOS: 182 or 190, and a V_H FR4 comprising or consisting of SEQ ID NO: 183, optionally wherein the combination of FR1, FR2, FR3, and FR4 is selected from one of the following combinations: a.) SEQ ID NOS: 180, 181, 182, and 183; b.) SEQ ID NOS: 188, 181, 190, and 183; and c.) SEQ ID NOS: 188, 181, 182, and 183. In some embodiments, each V_H framework region comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17 or more substitutions and/or 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17 or more deletions or additions, and/or has at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to a sequence selected from any one of SEQ ID NOS: 180-183, 188 or 190. In some embodiments, the substitution(s) are conservative substitution(s). In some embodiments, the sdAb, optionally a V_HH domain, comprises or consists of a sequence selected from any one of SEQ ID NOS: 184, 192, 200, 208, optionally wherein each CDR sequence independently comprises 1, 2, 3, 4, 5, 6, 7, or more substitutions and/or 1, 2, 3, 4, 5, 6, 7, or more deletions or additions, and/or has at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to a CDR sequence of any one of SEQ ID NOS: 177-179, 185-186, 194-195, or 201-202, and/or wherein the framework region comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17 or more substitutions and/or 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17 or more deletions or additions, and/or has at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the framework region of any one of SEQ ID NOS: 180-183, 188 or 190. In some embodiments, the substitution(s) are conservative substitution(s). In some embodiments, the sdAb, optionally a V_HH domain, comprises or consists of an amino acid sequence having at least 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to any one of SEQ ID NOS: 184, 192, 200, 208.

B. Exemplary mycV.1300 Variant Anti-Myc sdAb

[0046] In some embodiments, including any of the exemplary anti-Myc sdAbs disclosed above and elsewhere herein, the sdAb, optionally a V_HH domain, comprises a CDR3 comprising or consisting of the amino acid sequence AX₁LX₂QELTGASEHDY (SEQ ID NO: 309), wherein X₁ is H or Y; and X₂ is D or G; or optionally a conservative substitution of any of the foregoing. In some embodiments, the CDR3 comprises or consists of SEQ ID NO: 211. In some embodiments, the sdAb, optionally a V_HH domain, comprises: a CDR1 comprising or consisting of SEQ ID NO: 209, a CDR2 comprising or consisting of SEQ ID NO: 210, and a CDR3 comprising or consisting of SEQ ID NO: 211. In some embodiments, each CDR sequence independently comprises 1, 2, 3, 4, 5, 6, 7, or more substitutions and/or 1, 2, 3, 4, 5, 6, 7, or more deletions or additions, and/or has at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to a sequence selected from any one of SEQ ID NOs: 209-211. In some embodiments, the substitution(s) are conservative substitution(s). In some embodiments, the sdAb, optionally a V_HH domain, comprises: a CDR1 comprising or consisting of the amino acid sequence FTFX₁X₂X₃X₄MG (SEQ ID NO: 307), wherein X₁ is G or D; X₂ is D or E; X₃ is A or T; and X₄ is D or A; or optionally a conservative substitution of any of the foregoing; a CDR2 comprising or consisting of the amino acid sequence AISASX₁X₂X₃TYADSVKG (SEQ ID NO: 308), wherein X₁ is G or S; X₂ is D or A; and X₃ is X or E; or optionally a conservative substitution of any of the foregoing; and a CDR3 comprising or consisting of the amino acid sequence AX₁LX₂QELTGASEHDY (SEQ ID NO: 309), wherein X₁ is H or Y; and X₂ is D or G; or optionally a conservative substitution of any of the foregoing; wherein the combination of CDR1, CDR2 and CDR3 is not the combination of CDR1, CDR2 and CDR3 of mycV.1300 (SEQ ID NOs: 147, 149 and 151). In some embodiments, the sdAb, optionally a V_HH domain, comprises a V_H FR1 comprising or consisting of SEQ ID NO: 212, a V_H FR2 comprising or consisting of SEQ ID NO: 213 a V_H FR3 comprising or consisting of SEQ ID NO: 214, and a V_H FR4 comprising or consisting of SEQ ID NO: 215. In some embodiments, each V_H framework region comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17 or more substitutions and/or 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17 or more deletions or additions, and/or has at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to a sequence selected from any one of SEQ ID NOs: 212-215. In some embodiments, the sdAb, optionally a V_HH domain, comprises or consists of a sequence that is SEQ ID NO: 216. In some embodiments, each CDR sequence independently comprises 1, 2, 3, 4, 5, 6, 7, or more substitutions and/or 1, 2, 3, 4, 5,

6, 7, or more deletions or additions, and/or has at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to a CDR sequence of any one of SEQ ID NOs: 209-211, and/or wherein the framework region comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17 or more substitutions and/or 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17 or more deletions or additions, and/or has at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the framework region of any one of SEQ ID NOs: 212-215. In some embodiments, the substitution(s) are conservative substitution(s). In some embodiments, the sdAb, optionally a V_HH domain, comprises or consists of an amino acid sequence having at least 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 216. In some embodiments, the substitution(s) are conservative substitution(s).

C. Exemplary mcyV.1500 Variant Anti-Myc sdAb

In some embodiments, including any of the exemplary anti-Myc sdAbs disclosed above and elsewhere herein, the sdAb, optionally a V_HH domain, comprises a CDR3 comprising or consisting of the amino acid sequence AIIDDILTX₁GTFDEGDY (SEQ ID NO: 312), wherein X₁ is G or D; or optionally a conservative substitution of any of the foregoing. In some embodiments, the CDR3 comprises or consists of any one of SEQ ID NOs: 219 or 251. In some embodiments, the sdAb, optionally a V_HH domain, comprises: a CDR1 comprising or consisting of any one of SEQ ID NOs: 217, 225, 233, 241, 249 or 257; a CDR2 comprising or consisting of any one of SEQ ID NOs: 218, 226, 234, 242 or 250, and a CDR3 comprising or consisting of any one of SEQ ID NOs: 219 or 251. In some embodiments, the combination of CDR1, CDR2, and CDR3 is selected from one of the following combinations: a.) SEQ ID NOs: 217, 218, and 219; b.) SEQ ID NOs: 225, 226, and 219; c.) SEQ ID NOs: 233, 234, and 219; d.) SEQ ID NOs: 241, 242, and 219; e.) SEQ ID NOs: 249, 250, and 251; and f.) SEQ ID NOs: 257, 218, and 219. In some embodiments, each CDR sequence independently comprises 1, 2, 3, 4, 5, 6, 7, or more substitutions and/or 1, 2, 3, 4, 5, 6, 7, or more deletions or additions, and/or has at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to a sequence selected from any one of SEQ ID NOs: 217-219, 225-226, 233-234, 241-242, 249-251, and 257. In some embodiments, the substitution(s) are conservative substitution(s). In some embodiments, the sdAb, optionally a V_HH domain, comprises: a CDR1 comprising or consisting of the amino acid sequence FX₁FX₂X₃X₄X₅MG (SEQ ID NO: 310), wherein X₁ is T or D; X₂ is A, E, D, S or G; X₃ is D, E or T; X₄ is T, H, Y, V or A; and X₅ is D, A or S; or optionally a conservative substitution

of any of the foregoing; a CDR2 comprising or consisting of the amino acid sequence AISX₁X₂X₃X₄X₅X₆YYADSVKG (SEQ ID NO: 311), wherein X₁ is G, P, R or A; X₂ is S or D; X₃ is G or A; X₄ is G, A, T or D; X₅ is S, T, A or V; and X₆ is T or A; or optionally a conservative substitution of any of the foregoing; and a CDR3 comprising or consisting of the amino acid sequence AIIDDILTX₁GTFDEGDY (SEQ ID NO: 312), wherein X₁ is G or D; or optionally a conservative substitution of any of the foregoing; wherein the combination of CDR1, CDR2 and CDR3 is not the combination of CDR1, CDR2 and CDR3 of mycV.1500 SEQ ID NOs: (155, 157 and 159). In some embodiments, the sdAb, optionally a V_HH domain, comprises a V_H FR1 comprising or consisting of any one of SEQ ID NOs: 220 or 228, a V_H FR2 comprising or consisting of SEQ ID NO: 221, a V_H FR3 comprising or consisting of SEQ ID NO: 222, and a V_H FR4 comprising or consisting of SEQ ID NO: 223, optionally wherein the combination of FR1, FR2, FR3, and FR4 is selected from one of the following combinations: a) SEQ ID NOs: 220, 221, 222, and 223; and b) SEQ ID NOs: 228, 221, 222, and 223. In some embodiments, each V_H framework region comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17 or more substitutions and/or 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17 or more deletions or additions, and/or has at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to a sequence selected from any one of SEQ ID NOs: 220-223, or 228. In some embodiments, the substitution(s) are conservative substitution(s). In some embodiments, the sdAb, optionally a V_HH domain, comprises or consists of a sequence selected from any one of SEQ ID NOs: 224, 232, 240, 248, 256 or 264, optionally wherein each CDR sequence independently comprises 1, 2, 3, 4, 5, 6, 7, or more substitutions and/or 1, 2, 3, 4, 5, 6, 7, or more deletions or additions, and/or has at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to a CDR sequence of any one of SEQ ID NOs: 217-219, 225-226, 233-234, 241-242, 249-251, or 257; and/or wherein the framework region comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17 or more substitutions and/or 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17 or more deletions or additions, and/or has at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the framework region of any one of SEQ ID NOs: 220-223, or 228. In some embodiments, the substitution(s) are conservative substitution(s). In some embodiments, the sdAb, optionally a V_HH domain, comprises or consists of an amino acid sequence having at least 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to any one of SEQ ID NOs: 224, 232, 240, 248, 256 or 264.

D. Exemplary mycV.3700 Variant Anti-Myc sdAb

[0047] In some embodiments, including any of the exemplary anti-Myc sdAbs disclosed above and elsewhere herein, the sdAb, optionally a V_HH domain, comprises a CDR3 comprising or consisting of SEQ ID NO: 267. In some embodiments, the sdAb, optionally a V_HH domain, comprises: a CDR1 comprising or consisting of any one of SEQ ID NOs: 265 or 273, a CDR2 comprising or consisting of any one of SEQ ID NOs: 266 or 274, and a CDR3 comprising or consisting of SEQ ID NO: 267. In some embodiments, the combination of CDR1, CDR2, and CDR3 is selected from one of the following combinations: a) SEQ ID NOs: 265, 266, and 267; and b) SEQ ID NOs: 273, 274, and 267. In some embodiments, each CDR sequence independently comprises 1, 2, 3, 4, 5, 6, 7, or more substitutions and/or 1, 2, 3, 4, 5, 6, 7, or more deletions or additions, and/or has at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to a sequence selected from any one of SEQ ID NOs: 265-267, or 273-274. In some embodiments, the substitution(s) are conservative substitution(s). In some embodiments, the sdAb, optionally a V_HH domain, comprises: a CDR1 comprising or consisting of the amino acid sequence FTFX₁X₂X₃DMG (SEQ ID NO: 313), wherein X₁ is D, G or E; X₂ is V, S or G; and X₃ is S, N, or Q; or optionally a conservative substitution of any of the foregoing; a CDR2 comprising or consisting of the amino acid sequence AIX₁X₂X₃X₄X₅X₆TYADSVKG (SEQ ID NO: 314), wherein X₁ is no amino acid or S; X₂ is G or S; X₃ is T, G, or S; X₄ is E or G; X₅ is G, D or E; and X₆ is D, T or H; or optionally a conservative substitution of any of the foregoing; and a CDR3 comprising or consisting of the amino acid sequence SEQ ID NO: 315; wherein the combination of CDR1, CDR2 and CDR3 is not the combination of CDR1, CDR2 and CDR3 of mycV.3700 SEQ ID NOs: (163, 165 and 167). In some embodiments, the sdAb, optionally a V_HH domain, comprises a V_H FR1 comprising or consisting of SEQ ID NO: 268, a V_H FR2 comprising or consisting of SEQ ID NO: 269, a V_H FR3 comprising or consisting of any one of SEQ ID NOs: 270 or 278, and a V_H FR4 comprising or consisting of SEQ ID NO: 271, optionally wherein the combination of FR1, FR2, FR3, and FR4 is selected from one of the following combinations: a) SEQ ID NOs: 268, 269, 270, and 271; and b) SEQ ID NOs: 268, 269, 278, and 271. In some embodiments, each V_H framework region comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17 or more substitutions and/or 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17 or more deletions or additions, and/or has at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to a sequence selected from any one of SEQ ID NOs: 268-271, or 278. In some embodiments, the substitution(s) are conservative

substitution(s). In some embodiments, the sdAb, optionally a V_HH domain, comprises or consists of a sequence selected from any one of SEQ ID NOs: 272 or 280. In some embodiments, each CDR sequence independently comprises 1, 2, 3, 4, 5, 6, 7, or more substitutions and/or 1, 2, 3, 4, 5, 6, 7, or more deletions or additions, and/or has at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to a CDR sequence of any one of SEQ ID NOs: 265-267, or 273-274, and/or wherein the framework region comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17 or more substitutions and/or 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17 or more deletions or additions, and/or has at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the framework region of any one of SEQ ID NOs: 268-271, or 278. In some embodiments, In some embodiments, the substitution(s) are conservative substitution(s). In some embodiments, the sdAb, optionally a V_HH domain, comprises or consists of an amino acid sequence having at least 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to any one of SEQ ID NOs: 272 or 280. In some embodiments, In some embodiments, the substitution(s) are conservative substitution(s).

E. Exemplary mcV.6600 Variant Anti-Myc sdAb

[0048] In some embodiments, including any of the exemplary anti-Myc sdAbs disclosed above and elsewhere herein, the sdAb, optionally a V_HH domain, comprises a CDR3 comprising or consisting of SEQ ID NO: 283. In some embodiments, the sdAb, optionally a V_HH domain, comprises: a CDR1 comprising or consisting of any one of SEQ ID NOs: 281 or 289, a CDR2 comprising or consisting of any one of SEQ ID NOs: 282 or 290, and a CDR3 comprising or consisting of SEQ ID NO: 283. In some embodiments, the combination of CDR1, CDR2, and CDR3 is selected from one of the following combinations: SEQ ID NOs: 281, 282, and 283; and b) SEQ ID NOs: 289, 290, and 283. In some embodiments, each CDR sequence independently comprises 1, 2, 3, 4, 5, 6, 7, or more substitutions and/or 1, 2, 3, 4, 5, 6, 7, or more deletions or additions, and/or has at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to a sequence selected from any one of SEQ ID NOs: 281-283, and 289-290. In some embodiments, In some embodiments, the substitution(s) are conservative substitution(s). In some embodiments, the sdAb, optionally a V_HH domain, comprises: a CDR1 comprising or consisting of the amino acid sequence FTFX₁X₂X₃AMX₄ (SEQ ID NO: 316), wherein X₁ is S or D, X₂ is S, E or D; X₃ is Y or H; and X₄ is S or G; or optionally a conservative substitution of any of the foregoing; a CDR2 comprising or consisting

of the amino acid sequence AISX₁X₂X₃X₄X₅TYADSVKG (SEQ ID NO: 317), wherein X₁ G or A; X₂ is S or D; X₃ is G or A; X₄ is G or S; and X₅ is S, V or T; or optionally a conservative substitution of any of the foregoing; and a CDR3 comprising or consisting of the amino acid sequence SEQ ID NO: 318; wherein the combination of CDR1, CDR2 and CDR3 is not the combination of CDR1, CDR2 and CDR3 of mycV.6600 SEQ ID NOs: (171, 173 and 175). In some embodiments, the sdAb, optionally a V_HH domain, comprises a V_H FR1 comprising or consisting of SEQ ID NO: 284, a V_H FR2 comprising or consisting of SEQ ID NO: 285, a V_H FR3 comprising or consisting of SEQ ID NO: 286, and a V_H FR4 comprising or consisting of SEQ ID NO: 287. In some embodiments, the combination of FR1, FR2, FR3, and FR4 is 284, 285, 286, and 287. In some embodiments, each V_H framework region comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17 or more substitutions and/or 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17 or more deletions or additions, and/or has at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to a sequence selected from any one of SEQ ID NOs: 284, 285, 286, and 287. In some embodiments, In some embodiments, the substitution(s) are conservative substitution(s). In some embodiments, the sdAb, optionally a V_HH domain, comprises or consists of a sequence selected from any one of SEQ ID NOs: 288 or 296. In some embodiments, each CDR sequence independently comprises 1, 2, 3, 4, 5, 6, 7, or more substitutions and/or 1, 2, 3, 4, 5, 6, 7, or more deletions or additions, and/or has at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to a CDR sequence of any one of SEQ ID NOs: 281-283, and 289-290, and/or wherein the framework region comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17 or more substitutions and/or 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17 or more deletions or additions, and/or has at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the framework region of any one of SEQ ID NOs: 284, 285, 286, and 287. In some embodiments, In some embodiments, the substitution(s) are conservative substitution(s). In some embodiments, the sdAb, optionally a V_HH domain, comprises or consists of an amino acid sequence having at least 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to any one of SEQ ID NOs: 288 or 296. In some embodiments, In some embodiments, the substitution(s) are conservative substitution(s).

F. Further Embodiments of Exemplary Anti-Myc sdAb

[0049] In some embodiments, including any of the exemplary anti-Myc sdAbs and variant anti-Myc sdAbs disclosed above and elsewhere herein, the sdAb, optionally a V_HH domain,

comprises: a FR1 comprising or consisting of the amino acid sequence $X_1VX_2LX_3ESGGGLVQPGGSLRLSX_4AASG$ (SEQ ID NO: 300), wherein X_1 is E or G, X_2 is Q or R; X_3 is L or V; and X_4 is A or C; or optionally a conservative substitution of any of the foregoing; optionally wherein X_1 is E; X_2 is Q; X_3 is L; and X_4 is A or C; a FR2 comprising or consisting of the amino acid sequence SEQ ID NO: 301; a FR3 comprising or consisting of the amino acid sequence $RFX_1ISRDN SKNTLYLQMNX_2LRAX_3DTAVYYX_4$ (SEQ ID NO: 302), wherein X_1 is T or S; X_2 is S or G; X_3 is E or A; X_4 is A, V, or C; or optionally a conservative substitution of any of the foregoing; optionally wherein X_1 is R; X_2 is S; X_3 is E; and X_4 is A or C; and a FR4 comprising or consisting of the amino acid sequence $WGX_1GTQVTVSS$ (SEQ ID NO: 303), wherein X_1 is Q or R; or optionally a conservative substitution of any of the foregoing; optionally wherein X_1 is Q. In some embodiments, the sdAb comprises a V_{HH} domain with a substitution at C22 of FR1 and/or C30 of FR3, optionally wherein the substitution is independently selected from a C to A and a C to V substitution. In some embodiments, the sdAb has a K_d value of less than 1×10^{-7} and optionally at least 1×10^{-10} , optionally wherein the K_d is 1.49×10^{-7} to 5.56×10^{-9} . In some embodiments, the K_d value is calculated using human c-Myc, optionally as described for values of Octet sdAb K_d Human Myc Peptide Monovalent (M) in Example 4. In some embodiments, the sdAb has a Poly Specificity Reagent (PSR) score of less than 1, optionally less than 0.43, optionally less than 0.26. In some embodiments, the sdAb has a PSR score of at least 0.01, optionally at least 0.26, optionally at least 0.43. In some embodiments, the sdAb has a PSR score of 1 to 0.01, 0.43 to 0.01, or 0.26 to 0.01. In some embodiments, the sdAb has a melting temperature (T_M) of at least 40°C , optionally at least 45.5°C , optionally at least 64°C . In some embodiments, the sdAb has a T_M of less than 70°C , optionally wherein the T_M is 45°C to 65°C . In some embodiments, any of the foregoing measures are calculated as detailed in Example 4. In some embodiments, the sdAb binds, or binds at least in part, to at least a portion of amino acids 410-419 of MYC (SEQ ID NO: 1). In some embodiments, the sdAb binds, or binds at least in part, to at least a portion of a peptide consisting of SEQ ID NO: 769. In some embodiments, the sdAb binds, or binds at least in part, to at least a portion of a peptide consisting of SEQ ID NO: 770. In some embodiments, the sdAb binds, or binds at least in part, to at least a portion of a peptide consisting of SEQ ID NO: 771. In some embodiments, the sdAb binds, or binds at least in part, to at least a portion of amino acids 410-419 of MYC (SEQ ID NO: 1), and/or one or more of SEQ ID NOs: 769-771. In some embodiments, the sdAb does not comprise or consist of any one of SEQ ID NO: 138-176 and 641-768.

[0050] In some embodiments where the amino acid sequence differs from a recited reference sequence, the difference is the result of the inclusion of one or more modifications relative to the reference sequence. In some embodiments, the one or more modifications comprise a substitution, insertion and/or deletion. In some embodiments, the substitution is a conservative substitution. In some embodiments, the % sequence identity is calculated over the entirety of the reference sequence.

[0051] In some embodiments, a polypeptide comprising or consisting of an anti-Myc sdAb disclosed above and elsewhere herein can be utilized as a myc-binding moiety in a larger polypeptide, compound or conjugate. In some embodiments, a polypeptide comprising or consisting of an anti-Myc sdAb disclosed herein is conjugated to a therapeutic compound such as a drug (e.g., a chemotherapeutic drug or agent). In some embodiments, a polypeptide comprising or consisting of an anti-Myc sdAb disclosed herein is conjugated to a detectable label (e.g., a radioisotope, fluorescent molecule, etc.).

[0052] Also disclosed herein are polynucleotides encoding for any of the polypeptides comprising or consisting of the anti-Myc sdAb disclosed above and elsewhere herein.

Portions / URDs of E3 Ubiquitin Ligases and Viral Homologs

[0053] Disclosed herein are polypeptides comprising or consisting of a portion derived from a protein selected from the group consisting of a RING family E3 ubiquitin ligase, a cullin family E3 ubiquitin ligase, a homologous to E6AP carboxyl terminus (HECT) family E3 ubiquitin ligase, and a viral homolog of an E3 ubiquitin ligase. In some embodiments the viral homolog is VIF of HIV-1. In some embodiments, the portion comprises an amino acid substitution (optionally a conservative substitution), addition, and/or deletion to the protein. In some embodiments, the deletion is not an end terminal deletion or truncation of the protein. In some embodiments, the polypeptide and/or the portion is a non-natural polypeptide. In some embodiments, the portion has ubiquitin-proteasome recruiting activity. In some embodiments, the portion is a URD that has ubiquitin-proteasome recruiting activity. In some embodiments the portion of E3 ligase or viral homolog is fused to a polypeptide. In some embodiments, the fusion polypeptide comprises a linker, localization sequence (e.g., NLS), first and/or second targeting moiety, and/or other polypeptide disclosed herein.

[0054] In some embodiments, the portion is derived from an E3 ubiquitin ligase selected from the group consisting of CHIP, DCAF1, E6AP, FBXW7-alpha (also referred to herein as "FBXW7a"), FBXW7-beta (also referred to herein as "FBXW7b"), Keap1, NHLRC1, RNF4,

RNF6, RNF11, RNF12, RNF20, RNF25, RNF111, RNF114, RNF115, RNF125, RNF128, RNF138, RNF149, RNF152, RNF165, RNF166, RNF182, SPOP, beta-TRCP, TRIM21, TRIM32, VIF, ZNRF1, ZNRF4, and CBL-b (Y363E). Table 23 below discloses representative sequences of the preceding. In some embodiments, the portion is selected from the group consisting of CBLb.1, CHIP.1, DCAF1.1, E6AP.1, FBXW7a.1, FBXW7a.2, FBXW7a.3, FBXW7a.4, FBXW7a.5, FBXW7a.6, FBXW7a.7, FBXW7a.8, FBXW7a.9, FBXW7b.1, FBXW7b.2, FBXW7b.3, FBXW7b.4, Keap1.1, NHLRC1.1, NHLRC1.2, NHLRC1.3, NHLRC1.4, NHLRC1.5, RNF4.1, RNF6.1, RNF6.2, RNF11.1, RNF12.1, RNF12.2, RNF20.1, RNF25.1, RNF111.1, RNF114.1, RNF115.1, RNF125.1, RNF125.2, RNF125.3, RNF125.4, RNF125.5, RNF125.6, RNF125.7, RNF128.1, RNF138.1, RNF149.1, RNF152.1, RNF165.1, RNF166.1, RNF182.1, RNF182.2, SPOP.1, SPOP.2, SPOP.3, bTRCP.1, TRIM21.1, TRIM21.2, TRIM21.3, TRIM32.1, VIF.1, ZNRF1.1, and ZNRF4.1. Table 24 below discloses representative sequences of the preceding.

[0055] In some embodiments, the portion is derived from a monomeric RING family E3 ligase. In some embodiments, the portion is derived from a monomeric RING family E3 ligase selected from the group consisting of NHLRC1, RNF11, RNF111, RNF114, RNF115, RNF12, RNF125, RNF128, RNF138, RNF149, RNF152, RNF165, RNF166, RNF182, RNF20, RNF25, RNF4, RNF6, ZNRF1, ZNRF4, and CBL-b (Y363E). In some embodiments, the portion is derived from a monomeric RING family E3 ligase selected from the group consisting of RNF125, NHL, RC1, RNF4, RNF6, RNF12, RNF138 and ZNRF1. In some embodiments, the portion is selected from the group consisting of NHLRC1.1, NHLRC1.2, NHLRC1.3, NHLRC1.4, NHLRC1.5, RNF11.1, RNF111.1, RNF114.1, RNF115.1, RNF12.1, RNF12.2, RNF125.1, RNF125.2, RNF125.3, RNF125.4, RNF125.5, RNF125.6, RNF125.7, RNF128.1, RNF138.1, RNF149.1, RNF152.1, RNF165.1, RNF166.1, RNF182.1, RNF182.2, RNF20.1, RNF25.1, RNF4.1, RNF6.1, RNF6.2, ZNRF1.1, ZNRF4.1, and CBLb.1. In some embodiments, the portion is selected from the group consisting of NHLRC1.1, NHLRC1.2, NHLRC1.3, NHLRC1.4, NHLRC1.5, RNF125.1, RNF125.2, RNF125.3, RNF125.4, RNF125.5, RNF125.6, RNF125.7, RNF4.1, RNF6.1, RNF6.2, RNF12.1, RNF12.2, RNF138.1, and ZNRF1.1.

[0056] In some embodiments, the portion is derived from a cullin family E3 ligase. In some embodiments, the portion is derived from a cullin family E3 ligase selected from the group consisting of DCAF1, beta-TRCP, FBXW7-alpha, FBXW7-beta, Keap1, and SPOP. In some embodiments, the portion is derived from a cullin family E3 ligase selected from the group

consisting of beta-TRCP, FBXW7-alpha, and FBXW7-beta. In some embodiments, the portion is selected from the group consisting of DCAF1.1, bTRCP.1, FBXW7a.1, FBXW7a.2, FBXW7a.3, FBXW7a.4, FBXW7a.5, FBXW7a.6, FBXW7a.7, FBXW7a.8, FBXW7a.9, FBXW7b.1, FBXW7b.2, FBXW7b.3, FBXW7b.4, Keap1.1, SPOP.1, SPOP.2, and SPOP.3.

[0057] In some embodiments, the number of amino acids in the portion is, is about, or is less than, 80, 75, 70, 65, 60, 65, 50, 45, 40, 35, 30, 25, 20, 15, 10, 5, 4, 3, 2, or 1%, of the number of amino acids in the wild-type full-length protein (e.g. sequences disclosed in Table 23), or a range defined by any two of the preceding values, optionally 15-80, 15-50, 25-80, 25-50, 30-60, 1-80, 2-80, 1-70, 2-70, 2-65%. In some embodiments, the number of amino acids in the portion is, or is about 15-50% of the number of amino acids in the wild-type full-length protein. In some embodiments, the number of amino acids in the portion is, or is about, 2-61% of the number of amino acids in the wild-type full-length protein. In some embodiments, the portion is, is about, is less than or equal to 370, 369, 368, 367, 366, 365, 364, 363, 362, 361, 360, 300, 250, 200, 175, 170, 165, 160, 155, 150, 145, 140, 135, 130, 129, 128, 127, 126, 125, 124, 123, 120, 115, 110, 105, 100, 95, 90, 85, 80, 75, 70, 65, 60, 55, 50, 47, 46, 45, 44, 43, 42, 41, 40, 39, 38, 37, 36, 35, 34, 33, 32, 31, 30, 25, or 20 amino acids long, or a range defined by any two of the preceding values. In some embodiments, the portion is, or is about, 20-370, 20-150, 20-100, 20-80, 30-150, 30-100, 50-150 amino acids long. In some embodiments, the portion is, or is about, 50-100 or 20-100 amino acids long. In some embodiments, the portion is, or is about, 40-130 or 44-126 amino acids long.

[0058] In some embodiments, the portion comprises or consists of an amino acid sequence having at least 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to any one of SEQ ID NOs: 39-113, optionally SEQ ID NOs: 54-113, optionally wherein the portion has ubiquitin-proteasome recruiting activity. In some embodiments, the portion comprises or consists of an amino acid sequence having at least 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to any one of SEQ ID NOs: 76-106 and 111-113, optionally wherein the portion has ubiquitin-proteasome recruiting activity. In some embodiments, the portion comprises or consists of an amino acid sequence having at least 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to any one of SEQ ID NOs: 76-80, 85-93, 95, 104-106, and 111, optionally wherein the portion has ubiquitin-proteasome recruiting activity. In some embodiments, the portion consists of an amino acid sequence selected from any one of SEQ ID NOs: 76-80, 85-

93, 95, 104-106, and 111. In some embodiments, the URD comprises or consists of an amino acid sequence having at least 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to any one of SEQ ID NO: 39-113. In some embodiments, the URD comprises or consists of an amino acid sequence having at least 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to any one of any one of SEQ ID NO: 54-113. In some embodiments, the URD comprises or consists of an amino acid sequence having at least 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to any one of SEQ ID NO: 76, 88, 98, 104 and 111. In some embodiments, the URD comprises or consists of an amino acid sequence having at least 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to any one of SEQ ID NO: 76, 88, 98, and 104. In some embodiments, the URD comprises or consists of a truncation or fragment of one of the foregoing that retains ubiquitin-proteasome recruiting activity. In some embodiments where the amino acid sequence differs from a recited reference sequence, the difference is the result of the inclusion of one or more modifications relative to the reference sequence. In some embodiments, the one or more modifications comprise a substitution, insertion and/or deletion. In some embodiments, the substitution is a conservative substitution. In some embodiments, the % sequence identity is calculated over the entirety of the reference sequence.

[0059] In some embodiments, the portion comprises an amino acid substitution, addition, and/or deletion to the protein. In some embodiments, the deletion is not an end terminal deletion or truncation of the protein. In some embodiments, the polypeptide and/or the portion is a non-natural polypeptide. In some embodiments, the portion is a URD that has ubiquitin-proteasome recruiting activity.

[0060] In some embodiments, disclosed herein are bifunctional polypeptides, optionally dual-specific, comprising the polypeptide comprising or consisting of the portion derived from a protein selected from the group consisting of a RING family E3 ubiquitin ligase, a cullin family E3 ubiquitin ligase, a homologous to E6AP carboxyl terminus (HECT) family E3 ubiquitin ligase, and a viral homolog of an E3 ubiquitin ligase, as described in the paragraphs above and elsewhere herein, wherein the bifunctional polypeptide comprises: a) the portion of the protein and, b) a first and optionally a second targeting moiety (also referred to as a target “binder” or “binding moiety”) that is capable of binding to a first (e.g., Myc) and optionally a second target protein, optionally the same target protein, optionally an intracellular target

protein(s). In some embodiment the first and/or second targeting moiety binds selectively or specifically to the target protein(s). In some embodiments, the first targeting moiety, and optionally the second targeting moiety, comprises or consists of an anti-Myc sdAb disclosed herein. In some embodiments, the bifunctional polypeptides, optionally dual-specific, promote proteasome-mediated degradation of a target protein or proteins. In some embodiments, proximity of the bifunctional polypeptide to the target protein(s) through binding of the targeting moiety induces ubiquitination of the target protein(s) via the URD, thereby promoting proteasome-mediated degradation of the target protein(s). In some embodiments one or both of the target protein(s) is an intracellular target protein. In some embodiments, the bifunctional polypeptide that promotes proteasome-mediated degradation of a target protein(s), comprises or consisting of the polypeptide of comprising or consisting of the portion derived from a protein selected from the group consisting of a RING family E3 ubiquitin ligase, a cullin family E3 ubiquitin ligase, a homologous to E6AP carboxyl terminus (HECT) family E3 ubiquitin ligase, and a viral homolog of an E3 ubiquitin ligase, as described in the paragraphs above and elsewhere herein. In some embodiments of the bifunctional polypeptide that promotes proteasome-mediated degradation of a target protein(s), the URD consists of the URD derived from a protein selected from the group consisting of a RING family E3 ubiquitin ligase, a cullin family E3 ubiquitin ligase, a homologous to E6AP carboxyl terminus (HECT) family E3 ubiquitin ligase, and a viral homolog of an E3 ubiquitin ligase, as described in the paragraphs above and elsewhere herein. In some embodiment the first and/or second targeting moiety binds selectively or specifically to the target protein(s).

[0061] Any combination of the targeting moieties and the URD disclosed herein may be used in the construction of the bifunctional polypeptide.

Table 23: Representative E3 ligases and viral homologs for derivation of portions

SEQ ID NO	E3 Ligase	Uniprot ID	Orientation (C-N)
7	CHIP human full length	Q9UNE7	SRD-URD
15	RNF114 human full length	Q9Y508	URD-SRD
17	RNF125 human full length	Q96EQ8	URD-SRD
19	RNF138 human full length	Q8WVD3	URD-SRD
23	RNF166 human full length	Q96A37	URD-SRD

25	NHLRC1 human full length	Q6VVB1	URD-SRD
26	CBL-b (Y363E) human full length	Q13191	SRD-URD
27	TRIM21 human full length	P19474	URD-SRD
28	E6AP human full length	Q05086	SRD-URD
29	FBXW7 alpha human full length	Q969H0	URD-SRD
30	FBXW7 beta human full length	Q969H0	URD-SRD
31	beta-TRCP human full length	Q9Y297	URD-SRD
32	Keap1 human full length	Q14145	URD-SRD
33	SPOP human full length	O43791	SRD-URD
34	DCAF1 human full length	Q9Y4B6	URD-SRD
8	RNF4 human full length	P78317	SRD-URD
9	RNF6 human full length	Q9Y252	SRD-URD
10	RNF11 human full length	Q9Y3C5	SRD-URD
11	RNF12 human full length	Q9NVW2	SRD-URD
12	RNF20 human full length	Q5VTR2	SRD-URD
13	RNF25 human full length	Q96BH1	URD-SRD
14	RNF111 human full length	Q6ZNA4	SRD-URD
16	RNF115 human full length	Q9Y4L5	SRD-URD
18	RNF128 human full length	Q8TEB7	SRD-URD
20	RNF149 human full length	Q8NC42	SRD-URD
21	RNF152 human full length	Q8N8N0	URD-SRD
22	RNF165 human full length	Q6ZSG1	SRD-URD
24	RNF182 human full length	Q8N6D2	URD-SRD
35	TRIM32 human full length	Q13049	URD-SRD
36	VIF HIV-1 full length	P12504	SRD-URD
37	ZNRF1 human full length	Q8ND25	SRD-URD
38	ZNRF4 human full length	Q8WWF5	SRD-URD

Table 24: Exemplary Portions / URDs derived from E3 ligases and viral homologs

SEQ ID NO	Name	E3 Family	Uniprot ID of Parental Protein
54	CHIP.1	U-box	Q9UNE7

SEQ ID NO	Name	E3 Family	Uniprot ID of Parental Protein
55	DCAF1.1	H-box	Q9Y4B6
56	E6AP.1	HECT	Q05086
57	bTRCP.1	F-box/Cul1	Q9Y297
58	FBXW7a.1	F-box/Cul1	Q969H0
59	FBXW7a.2	F-box/Cul1	Q969H0
60	FBXW7a.3	F-box/Cul1	Q969H0
61	FBXW7a.4	F-box/Cul1	Q969H0
62	FBXW7a.5	F-box/Cul1	Q969H0
63	FBXW7a.6	F-box/Cul1	Q969H0
64	FBXW7a.7	F-box/Cul1	Q969H0
65	FBXW7a.8	F-box/Cul1	Q969H0
66	FBXW7a.9	F-box/Cul1	Q969H0
67	FBXW7b.1	F-box/Cul1	Q969H0
68	FBXW7b.2	F-box/Cul1	Q969H0
69	FBXW7b.3	F-box/Cul1	Q969H0
70	FBXW7b.4	F-box/Cul1	Q969H0
71	Keap1.1	BTB/Cul3	Q14145
72	SPOP.1	BTB/Cul3	O43791
73	SPOP.2	BTB/Cul3	O43791
74	SPOP.3	BTB/Cul3	O43791
75	VIF.1	BC-Box/Cul5	P12504
76	NHLRC1.1	RING	Q6VVB1
77	NHLRC1.2	RING	Q6VVB1
78	NHLRC1.3	RING	Q6VVB1
79	NHLRC1.4	RING	Q6VVB1
80	NHLRC1.5	RING	Q6VVB1
81	RNF11.1	RING	Q9Y3C5
82	RNF111.1	RING	Q6ZNA4
83	RNF114.1	RING	Q9Y508
84	RNF115.1	RING	Q9Y4L5

SEQ ID NO	Name	E3 Family	Uniprot ID of Parental Protein
85	RNF12.1	RING	Q9NVW2
86	RNF12.2	RING	Q9NVW2
87	RNF125.1	RING	Q96EQ8
88	RNF125.2	RING	Q96EQ8
89	RNF125.3	RING	Q96EQ8
90	RNF125.4	RING	Q96EQ8
91	RNF125.5	RING	Q96EQ8
92	RNF125.6	RING	Q96EQ8
93	RNF125.7	RING	Q96EQ8
94	RNF128.1	RING	Q8TEB7
95	RNF138.1	RING	Q8WVD3
96	RNF149.1	RING	Q8NC42
97	RNF152.1	RING	Q8N8N0
98	RNF165.1	RING	Q6ZSG1
99	RNF166.1	RING	Q96A37
100	RNF182.1	RING	Q8N6D2
101	RNF182.2	RING	Q8N6D2
102	RNF20.1	RING	Q5VTR2
103	RNF25.1	RING	Q96BH1
104	RNF4.1	RING	P78317
105	RNF6.1	RING	Q9Y252
106	RNF6.2	RING	Q9Y252
107	TRIM21.1	RING	P19474
108	TRIM21.2	RING	P19474
109	TRIM21.3	RING	P19474
110	TRIM32.1	RING	Q13049
111	ZNRF1.1	RING	Q8ND25
112	ZNRF4.1	RING	Q8WWF5
113	CBLb.1	RING	Q13191

[0062] In some embodiments, the component of the dual-specific bifunctional polypeptide that is capable of recruiting the ubiquitination complex is generally composed of a URD. In some embodiments, the URD may be derived from an E3 ubiquitin ligase, such as a human E3 ubiquitin ligase. However, in some embodiments, the URD does not strictly need to be derived from an E3 ubiquitin ligase, and may otherwise be derived from other sources, such as viral analogues of E3 ubiquitin ligases that have ubiquitin complex recruitment function (for example, the VIF protein of HIV). The URD may be derived from any of the diverse family of E3 ubiquitin ligases based on factors such as size, localization in the cell (e.g., cytoplasm and/or nucleus), and orientation of the URD domain within the E3 ubiquitin ligase (i.e., if the URD appears N-terminally or C-terminally to the substrate recognition domain). In some embodiments, exemplary URD domains may include, but are not limited to, U-box, RING, HECT, F-box (Cul1-SKP1), BTB (Cul3), and H-box (Cul4-DDB1) domains. In some embodiments, exemplary URDs may be derived from E3 ubiquitin ligases including but not limited to CHIP (also termed STUB1), RNF114, RNF125, RNF138, RNF166, NHLRC1, CBL-b (Y363E), TRIM21, E6AP, FBXW7 alpha, FBXW7 beta, beta-TRCP, Keap1, SPOP, or DCAF1. It is envisioned that any truncation or fragment of these E3 ubiquitin ligases that retain ubiquitin-proteasome recruiting activity may be used as a URD in the embodiments herein. In some embodiments, the URD is derived from a RING domain (e.g., RNF114, RNF125, RNF138, RNF166, NHLRC1, CBL-b (Y363E)) or an H-box (Cul4-DDB1) domain (e.g., DCAF1), or any truncation or fragment thereof that retains ubiquitin-proteasome recruiting activity.

[0063] The URD should be understood as a protein, or fragment or truncation thereof, that can function to recruit a ubiquitination complex (e.g., can recruit an E2 ubiquitin conjugating enzyme). In some embodiments, the URD is derived from an E3 ubiquitin ligase. In some embodiments, the URD is derived from CHIP, RNF114, RNF125, RNF138, RNF166, NHLRC1, CBL-b (Y363E), TRIM21, E6AP, FBXW7 alpha, FBXW7 beta, beta-TRCP, Keap1, SPOP, or DCAF1, or a truncation or fragment thereof that retains ubiquitin-proteasome recruiting activity. In some embodiments, the URD is derived from CHIP, RNF114, RNF125 (e.g., RNF125.2), RNF138, RNF166, NHLRC1, CBL-b (Y363E), TRIM21, E6AP, FBXW7 alpha, FBXW7 beta, beta-TRCP, Keap1, SPOP (e.g., SPOP.2 or SPOP.3), or DCAF1, or a truncation or fragment thereof that retains ubiquitin-proteasome recruiting activity. In some embodiments, the URD is selected from a RING domain and an H-box (Cul4-DDB1) domain, or a truncation or fragment thereof that retains ubiquitin-proteasome recruiting activity. In

some embodiments, the RING domain is RNF114, RNF125, RNF138, RNF166, NHLRC1, CBL-b (Y363E), or TRIM21. In some embodiments, the H-box domain is DCAF1. In some embodiments, the URD is selected from: the URD derived from a protein which localizes to the cytoplasm; the URD derived from a protein which localizes to the nucleus; and the URD derived from a protein which localizes to the cytoplasm and the nucleus; or a truncation or fragment of the URD that retains ubiquitin-proteasome recruiting activity. In some embodiments, the protein which localizes to the cytoplasm is FBXW7 beta. In some embodiments, the protein which localizes to the nucleus is selected from FBXW7 alpha and SPOP. In some embodiments, the protein which localizes to the cytoplasm and the nucleus is selected from CHIP, RNF114, RNF125, RNF138, RNF166, NHLRC1, CBL-b (Y363E), TRIM21, E6AP, and DCAF1. In some embodiments, the URD is derived from an E3 ligase selected from the group consisting of NHLRC1, DCAF1, RNF125, RNF165, RNF4, and SPOP. In some embodiments, the URD is derived from an E3 ligase selected from the group consisting of optionally NHLRC1, RNF125, RNF165, RNF4 and SPOP. In some embodiments, the URD is derived from an E3 ligase selected from the group consisting of optionally NHLRC1, RNF125, RNF165, and RNF4. In some embodiments, the URD is selected from the group consisting of NHLRC1.1, DCAF1.1, RNF125.2, RNF125.3, RNF125.4, RNF125.5, RNF125.6, RNF165.1, RNF4.1, SPOP.2, and SPOP.3. In some embodiments, the URD is selected from the group consisting of NHLRC1.1, RNF125.2, RNF125.3, RNF125.4, RNF125.5, RNF125.6, RNF165.1, RNF4.1, SPOP.2, and SPOP.3. In some embodiments, the URD is selected from the group consisting of NHLRC1.1, RNF125.2, RNF165.1, and RNF4.1. In some embodiments, the protein is an E3 ubiquitin ligase. In some embodiments, the URD is, is about, is at least, is at least about, is not more than, or is not more than about, 370, 369, 368, 367, 366, 365, 364, 363, 362, 361, 360, 300, 250, 200, 175, 170, 165, 160, 155, 150, 145, 140, 135, 130, 129, 128, 127, 126, 125, 124, 123, 120, 115, 110, 105, 100, 95, 90, 85, 80, 75, 70, 65, 60, 55, 50, 47, 46, 45, 44, 43, 42, 41, 40, 39, 38, 37, 36, 35, 34, 33, 32, 31, 30, 25, or 20 amino acids long, or a range defined by any two of the preceding values, for example, 370-20, 370-120, 120-20, 80-20, or 360-150 amino acids long. In some embodiments, the URD comprises or consists of an amino acid sequence having at least 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to any one of SEQ ID NO: 39-53, or a truncation or fragment thereof that retains ubiquitin-proteasome recruiting activity. In some embodiments, the URD comprises or consists of an amino acid sequence having at least 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%,

94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to any one of SEQ ID NO: 54-113, or a truncation or fragment thereof that retains ubiquitin-proteasome recruiting activity. In some embodiments, the URD comprises or consists of an amino acid sequence having at least 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to any one of SEQ ID NO: 73, 74, 76, 88-92, 98, and 104, or a truncation or fragment thereof that retains ubiquitin-proteasome recruiting activity. In some embodiments, the URD comprises or consists of an amino acid sequence having at least 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to any one of SEQ ID NO: 76, 88, 98, or a truncation or fragment thereof that retains ubiquitin-proteasome recruiting activity. In some embodiments where the amino acid sequence differs from a recited reference sequence, the difference is the result of the inclusion of one or more modifications relative to the reference sequence. In some embodiments, the one or more modifications comprise a substitution, insertion and/or deletion. In some embodiments, the substitution is a conservative substitution. In some embodiments, the % sequence identity is calculated over the entirety of the reference sequence.

[0064] In some embodiments, the URD is selected from: a URD derived from a protein which localizes to the cytoplasm, optionally selected from FBXW7 beta and Keap1; a URD derived from a protein which localizes to the nucleus, optionally selected from FBXW7 alpha, RNF165, and SPOP; and a URD derived from a protein which localizes to the cytoplasm and the nucleus, optionally selected from beta-TRCP, CHIP, RNF114, RNF125, RNF138, RNF166, NHLRC1, CBL-b (Y363E), TRIM21, E6AP, DCAF1 VIF, RNF11, RNF111, RNF115, RNF12, RNF128, RNF149, RNF152, RNF165, RNF182, RNF20, RNF25, RNF4, RNF6, TRIM32, ZNRF1, ZNRF4.

[0065] Table 24 depicts various E3 ubiquitin ligases and respective URDs embodied herein. In some embodiments of the polypeptide comprising or consisting of a portion derived from a protein, the portion comprises an exemplary URD described below and/or in Table 24. In some embodiments of the dual-specific bifunctional polypeptide, the dual-specific bifunctional polypeptide comprises an exemplary URD described below and/or in Table 24. It is envisioned that alternative portions, fragments, or truncations of the E3 ubiquitin ligase proteins having ubiquitin-proteasome recruiting activity may also be used.

[0066] Additional URDs are disclosed in PCT/US2023/066619, filed May 4, 2023, and PCT/US2023/76886, filed October 13, 2023, each of which is herein incorporated by reference in its entirety.

Localization Sequences

[0067] Localization of bifunctional polypeptides to the correct subcellular compartment may be important for the ability to degrade a target protein, e.g., if the target is localized to the nucleus, or if degradation of target protein only in the nucleus but not cytoplasm is desired. In some embodiments provided herein are polypeptides, or bifunctional polypeptides, optionally dual-specific, which are engineered (e.g., by modification of an endogenous localization peptide sequence, by adding localization peptide sequences, and/or by selecting a component which has an endogenous localization sequence) to direct the polypeptide or bifunctional polypeptide to a particular subcellular compartment, e.g., the cytoplasm, the nucleus, or both.

[0068] In some embodiments, the polypeptide comprising a portion derived from an E3 ligase or viral homolog thereof comprises one or more localization peptide sequences. In some embodiments, the bifunctional polypeptide comprises one or more localization peptide sequences. In some embodiments, the polypeptide comprising a portion, or the bifunctional polypeptide comprises one or more localization peptide sequences that direct the polypeptide or bifunctional polypeptide to a subcellular compartment or compartments, optionally wherein the subcellular compartment is the nucleus and/or the cytoplasm. In some embodiments, the one or more localization peptide sequences direct the polypeptide or bifunctional polypeptide to a desired subcellular compartment or compartments, optionally wherein the one or more localization peptide sequences are selected or designed to direct the polypeptide or bifunctional polypeptide to a desired subcellular compartment or compartments. In some embodiments, the one or more localization peptide sequences comprise or consist of a nuclear localization signal (NLS) peptide and/or a nuclear export signal (NES) peptide. In some embodiments, the bifunctional polypeptide further comprises a nuclear localization signal (NLS) peptide. In some embodiments, the NLS peptide is located at the N-terminus of the bifunctional polypeptide, the C-terminus of the bifunctional polypeptide, or internally within the bifunctional polypeptide. In some embodiments, the NLS signal is an NLS that naturally occurs in one or more of the components of the bifunctional polypeptide, such as an NLS that is naturally found in the first and/or optional second targeting moiety and/or URD. For example, the first and/or optional second targeting moiety and/or URD of a bifunctional polypeptide may comprise an NLS within their sequence. In some embodiments the NLS is an NLS that is engineered into the bifunctional polypeptide, for example, added N-terminally, C-terminally, or internally within a component of the bifunctional polypeptide, such as the first and/or optional second targeting moiety and/or URD. In some embodiments, the NLS peptide comprises or consists of the amino

acid sequence of SEQ ID NO: 51. However, other suitable NLS peptides generally known in the art may also be used. In some embodiments the localization sequence is a sequence from Table 25 or 26.

[0069] In some embodiments, the one or more localization peptide sequences comprise or consist of an endogenous localization peptide sequence of the first and/or optional second targeting moiety and/or URD. In some embodiments, the one or more localization peptide sequences comprise or consist of an endogenous localization peptide sequence of the URD, for example the localization sequences disclosed in Table 26. In some embodiments, the URD is selected from: a URD derived from a protein which localizes to the cytoplasm, optionally selected from FBXW7 beta and Keap1; a URD derived from a protein which localizes to the nucleus, optionally selected from FBXW7 alpha, RNF165, and SPOP; and a URD derived from a protein which localizes to the cytoplasm and the nucleus, optionally selected from beta-TRCP, CHIP, RNF114, RNF125, RNF138, RNF166, NHLRC1, CBL-b (Y363E), TRIM21, E6AP, DCAF1 VIF, RNF11, RNF111, RNF115, RNF12, RNF128, RNF149, RNF152, RNF165, RNF182, RNF20, RNF25, RNF4, RNF6, TRIM32, ZNRF1, ZNRF4.

[0070] In some embodiments, the one or more localization peptide sequences comprise or consist of an endogenous localization peptide sequence of the URD and/or first and/or optional second targeting moiety that is modified, wherein the modification alters the subcellular compartment or compartments to which the bifunctional polypeptide, optionally dual-specific, is directed as compared to the native endogenous localization peptide sequence. In some embodiments, the modification comprises or consists of a substitution, truncation or deletion of an endogenous localization peptide sequence. In some embodiments:

- b. the native endogenous localization peptide sequence directs the polypeptide to the nucleus, and the modified endogenous localization peptide sequence directs the polypeptide to the cytoplasm;
- c. the native endogenous localization peptide sequence directs the polypeptide to the cytoplasm, and the modified endogenous localization peptide sequence directs the polypeptide to the nucleus;
- d. the native endogenous localization peptide sequence directs the polypeptide to the nucleus and the cytoplasm, and the modified endogenous localization peptide sequence directs the polypeptide to only the cytoplasm;

- e. the native endogenous localization peptide sequence directs the polypeptide to the nucleus and the cytoplasm, and the modified endogenous localization peptide sequence directs the polypeptide to only the nucleus;
- f. the native endogenous localization peptide sequence directs the polypeptide to the nucleus, and the modified endogenous localization peptide sequence directs the polypeptide to the nucleus and the cytoplasm; or
- g. the native endogenous localization peptide sequence directs the polypeptide to the cytoplasm, and the modified endogenous localization peptide sequence directs the polypeptide to the nucleus and the cytoplasm.

[0071] In some embodiments, the one or more localization peptide sequences comprises or consists of an endogenous localization peptide sequence of the URD that is modified, wherein the native endogenous localization peptide sequence directs the polypeptide to the nucleus, and the modified endogenous localization peptide sequence directs the polypeptide to the cytoplasm. In some embodiments, the modified endogenous localization peptide sequence comprises or consists of a modified SPOP URD endogenous localization peptide sequence, optionally wherein the URD is SPOP.3.

[0072] In some embodiments, the one or more localization peptide sequences comprise or consist of an exogenous localization peptide sequence. In some embodiments, the one or more localization peptide sequences comprise or consist of an NLS peptide that is or is a derivative of the NLS of MYC, simian virus 40 (SV40), SPOP, nucleoplasmin, 53BP1, Hrp1. In some embodiments, the one or more localization peptide sequences comprise or consists of an NLS sequence disclosed in Table 25. In some embodiments, the one or more localization peptide sequences comprise or consist of an NLS comprising or consisting of an amino acid sequence having at least 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to any one of SEQ ID NOs: 114-127, or SEQ ID NOs: 114-122. In some embodiments where the amino acid sequence differs from a recited reference sequence, the difference is the result of the inclusion of one or more modifications relative to the reference sequence. In some embodiments, the one or more modifications comprise a substitution, insertion and/or deletion. In some embodiments, the substitution is a conservative substitution. In some embodiments, the % sequence identity is calculated over the entirety of the reference sequence.

[0073] In some embodiments, the polypeptide comprising a portion derived from an E3 ligase or viral homolog thereof comprises 2, 3, 4, or more localization peptide sequences,

optionally wherein the polypeptide comprises 2, 3, 4 or more copies of the same localization sequence in series. In some embodiments, the bifunctional polypeptide, optionally dual-specific, comprises 2, 3, 4, or more localization peptide sequences, optionally wherein the bifunctional polypeptide comprises 2, 3, 4 or more copies of the same localization sequence in series. In some embodiments, the polypeptide or the bifunctional polypeptide, optionally dual-specific, comprises one or more localization peptide sequences located at a location selected from the group consisting of: the N-terminus of the bifunctional polypeptide; the C-terminus of the bifunctional polypeptide; internally within the bifunctional polypeptide; as part of the URD, optionally an endogenous portion of the URD; as a part of the first and/or optional second targeting moiety, optionally an endogenous portion of the URD; and a combination of any of the foregoing. In some embodiments, a first localization peptide sequence is located at the N-terminus of the polypeptide or the bifunctional polypeptide and a second localization peptide sequence is located at the C-terminus of the polypeptide or the bifunctional polypeptide.

Table 25: Exemplary NLS sequences

SEQ ID NO	Name	Parental Protein	Uniprot ID of Parental Protein
114	spNLS.2	SPOP	O43791
115	spNLS	SPOP	O43791
116	hrpNLS	Hrp1	Q99383
117	bpNLS	53BP1	Q12888
118	nlpNLS	Nucleoplasmin	P05221
119	3x(svNLS)	Large T antigen (SV40)	P03070
120	3x(mycNLS)	MYC	P01106
121	svNLS	Large T antigen (SV40)	P03070
122	mycNLS	MYC	P01106

Table 26: Exemplary URD endogenous NLS/NES sequences

SEQ ID NO	Name	NLS or NES	E3 Family	Parental protein	Uniprot ID of Parental Protein
123	CHIP.NLS	NLS	U-box	CHIP	Q9UNE7
124	SPOP.NLS	NLS	BTB/Cul3	SPOP	O43791
125	RNF165.NLS	NLS	RING	RNF165	Q6ZSG1

SEQ ID NO	Name	NLS or NES	E3 Family	Parental protein	Uniprot ID of Parental Protein
126	FBXW7a.NLS	NLS	F-box/Cul1	FBXW7a	Q969H0
127	NHLRC1.NLS	NLS	RING	NHLRC1	Q6VVB1
128	NHLRC1.NES	NES	RING	NHLRC1	Q6VVB1
129	E6AP.NES	NES	HECT	E6AP	Q05086
130	Keap1.NES	NES	BTB/Cul3	Keap1	Q14145
131	RNF12.NES	NES	RING	RNF12	Q9NVW2
132	RNF138.NES	NES	RING	RNF138	Q8WVD3
133	RNF166.NES	NES	RING	RNF166	Q96A37
134	TRIM21.NES	NES	RING	TRIM21	P19474
135	TRIM32.NES	NES	RING	TRIM32	Q13049

[0074] In some embodiments, the NLS peptide comprises or consists of the amino acid sequence of SEQ ID NOs: 51 or 1897-1903. In some embodiments, the NLS peptide is derived from MYC NLS, SV40 NLS, 3XMYC NLS, 3xSV40 NLS, SPOP NLS, nucleoplasmin NLS, 53BP1 NLS, or Hrp1 NLS (SEQ ID NOs: 51, 1897-1903). In some embodiments, other suitable NLS peptides generally known in the art are contemplated. Some URDSs derived from E3 ubiquitin ligases contain an endogenous NLS, for example, SPOP and SPOP.2. In some embodiments, the dual-specific bifunctional polypeptide contains a URD with an endogenous NLS. In some embodiments, the dual-specific bifunctional polypeptide contains a URD with an endogenous NLS and an exogenous NLS. In some embodiments, the dual-specific bifunctional polypeptide contains a URD with a truncated or deleted endogenous NLS (e.g., SPOP.3) and an exogenous NLS.

[0075] Additional localization sequences are disclosed in PCT/US2023/066619, filed May 4, 2023, and PCT/US2023/76886, filed October 13, 2023, each of which is herein incorporated by reference in its entirety.

Exemplary Bifunctional Degraders Targeting Myc

[0076] The following characteristics may apply to embodiments of the bifunctional polypeptides disclosed herein. One exemplary target protein embodied herein is MYC proto-oncogene, bHLH transcription factor (Myc; c-Myc). In some embodiments, the Myc is a mutant form. An example sequence for MYC is provided as SEQ ID NO: 1 (Uniprot #P01106).

Myc is a major oncogene involved in the pathology of many types of cancer, including solid tumors (including but not limited to hepatocellular carcinoma, liver metastases, and colorectal carcinoma) and hematological malignancies. In embodiments disclosed herein, the bifunctional polypeptide comprises a targeting moiety (also referred to as a “binder” or “binding moiety”) that is capable of binding to Myc. In some embodiments, the bifunctional polypeptide promotes proteasome-mediated degradation of at least one target protein. In some embodiments, the at least one target protein is Myc, optionally c-Myc. In some embodiments, the bifunctional polypeptide comprises: a first targeting moiety comprising or consisting of an anti-Myc sdAb disclosed above under the heading “Anti-Myc Single Domain Antibodies (sdAb)” and elsewhere herein and a URD. In some embodiments, proximity of the bifunctional polypeptide to at the least one target protein (e.g., Myc, c-Myc) through binding of the targeting moiety induces ubiquitination of the at least one target protein via the URD, thereby promoting proteasome-mediated degradation of the at least one target protein. In some embodiments, the bifunctional polypeptide further comprises a first linker peptide, optionally wherein the first linker peptide is positioned between the first targeting moiety and the URD. In some embodiments, the bifunctional polypeptide further comprises one or more localization peptide sequences. In some embodiment the first targeting moiety binds selectively or specifically to the target protein Myc, preferably c-Myc. In some embodiments, the first targeting moiety comprises or consists of a V_{HH} , V_H , or V_{NAR} domain of a heavy chain antibody (HcAb). In some embodiments, the first targeting moiety comprises or consists of an anti-Myc sdAb, comprising or consisting of a V_H domain of an IgG antibody, or a V_{HH} , or V_H domain of a heavy chain antibody (HcAb). In some embodiments, the first targeting moiety comprises or consists of a V_{HH} domain. In some embodiments, the first targeting moiety comprises or consists of a sdAb and the first targeting moiety is, or is less than, 170, 165, 160, 155, 150, 145, 140, 135, 130, 129, 128, 127, 126, 125, 124, 123, 122, 121, 120, 119, 118, 117, 116, 115, 114, 113, 112, 110, 109, 108, 107, 106, 105, 104, 103, 102, 101, 100, 99, 98, 97, 96, 95, 94, 93, 92, 91, or 90, amino acids long, or a range defined by any two of the preceding values, optionally 90-150, 90-130, 110-150, 110-130, 110-125, or 114-124 amino acids long.

[0077] In some embodiments, the first targeting moiety that is capable of binding to Myc may be capable of binding, or binding at least in part, to at least a portion of Myc, such as the DNA binding domain of Myc. In some embodiments, the first targeting moiety that is capable of binding to Myc is capable of binding, or binding at least in part, to at least a portion of amino acids 54-406, amino acids 354-406 (the DNA binding domain of MYC that binds to Omomyc;

as seen in PDB: 1NKP), or amino acids 408-437, of MYC (as represented as SEQ ID NO: 1). In some embodiments, the first targeting moiety that is capable of binding to Myc is capable of binding, or binding at least in part, to at least a portion of amino acids 410-419 of MYC (as represented as SEQ ID NO: 1), the binding epitope of antibody 9E10 (as seen in PDB: 2OR9). The bifunctional polypeptides disclosed herein that are specific for Myc may be used for the treatment of a cancer, for example, a cancer exhibiting elevated levels of Myc, for example solid tumors (including but not limited to hepatocellular carcinoma, liver metastases, and colorectal carcinoma) and hematological malignancies.

[0078] In some embodiments, bifunctional polypeptide comprises a localization peptide sequence which directs the bifunctional polypeptide to the nucleus of a cell. In some embodiments, the localization peptide sequence comprises or consists of the NLS sequence selected from the group of the NLS sequences of MYC, SPOP, Hrp1, SV40, 53BP1, and nucleoplasmin. In some embodiments, the localization peptide sequence comprises or consists of the NLS sequence selected from the group of the NLS sequences of MYC, SPOP, Hrp1. In some embodiments, the localization peptide sequence comprises or consists of a NLS sequence selected from the group of the NLS sequence of SPOP, optionally SPNLS.2, MYC, optionally mycNLS, and Hrp1, optionally hrpNLS. In some embodiments, the NLS comprises or consists of a sequence selected from any one of SEQ ID NOs: 114-127. In some embodiments, the NLS comprises or consists of a sequence selected from any one of SEQ ID NOs: 114, 122 and 116.

[0079] In some embodiments of the bifunctional polypeptide, the URD is the URD of NHLRC1, RNF125, RNF165, RNF4, or ZNRF1, or a truncation or fragment thereof that retains ubiquitin-proteasome recruiting activity. In some embodiments, the URD comprises or consists of NHLRC1.1, RNF125.2, RNF165.1, RNF4.1, or ZNRF1.1. In some embodiments, the URD comprises or consists of NHLRC1.1 RNF125.2, RNF165.1, or RNF4.1. In some embodiments, the URD comprises or consists of a sequence selected from any one of SEQ ID NOs: 76-80, 87-93, 98, 104 and 111. In some embodiments, the URD comprises or consists of a sequence selected from any one of SEQ ID NOs: 76, 88, 98, 104, and 111, In some embodiments, the URD comprises or consists of a sequence selected from any one of SEQ ID NOs: 76, 88, 98, and 104.

[0080] FIG. 8 discloses embodiments of bifunctional polypeptides, with SEQ ID NOs 562-577 being dual-specific bifunctional polypeptides. The description identifies the elements of the polypeptide in the N- to C-terminal direction. In some embodiments, the bifunctional

polypeptide targeting Myc is one of the embodiments described in FIG. 8. In some embodiments, the bifunctional polypeptide comprises the embodiments in FIG. 8 sharing one or more elements or features. In some embodiments the shared element or feature is the target. In some embodiments the shared element or feature is the URD name (e.g., those in Table 24). In some embodiments the shared element or feature is protein from which the URD is derived (e.g., those listed in Table 23). In some embodiments the shared element or feature is one or more of the following: the target; the protein from which the URD is derived; the URD name; the type of targeting moiety (e.g., sdAb); the targeting moiety name (e.g., those in Table 28; note that “full” is not included in the names in FIG. 8); the NLS name (e.g. those in Tables 25 and 26); the linker name (e.g., those in Table 32); the position of the URD relative to the targeting moiety (upstream or downstream); and both the URD name and targeting moiety name. In some embodiments, the HA tag sequence (SEQ ID NO: 384) is excluded. In some embodiments of the bifunctional polypeptide the first targeting moiety comprises or consists of an anti-Myc sdAb disclosed above under the heading “Anti-Myc Single Domain Antibodies (sdAb)” and elsewhere herein. In some embodiments, the first targeting moiety comprises or consists of a V_HH domain. In some embodiments, the bifunctional polypeptide comprises or consists of an amino acid sequence having at least 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to any one of SEQ ID NOs: 428-561, optionally wherein the sequence lacks the HA tag sequence (SEQ ID NO: 384).

[0081] Table 27 provided below lists the results of HiBiT™ degradation studies utilizing exemplary embodiments of bifunctional polypeptides disclosed herein. The tables list a “Grade” of A, B, C or D for the level of degradation of each of the targeted proteins. Details regarding the experimental protocol are provided in the Examples section below. In some embodiments, the bifunctional polypeptide comprises or consists of an amino acid sequence having at least 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to any one of the SEQ ID NOs in Table 27, optionally excluding any one of the SEQ ID NOs listed as having a MYC degradation Grade of D, optionally wherein the sequence lacks the HA tag sequence (SEQ ID NO: 384). In some embodiments, the bifunctional polypeptide comprises or consists of an amino acid sequence of any one of the SEQ ID NOs in Table 27 listed as having a MYC degradation Grade of A, optionally wherein the sequence lacks the HA tag sequence (SEQ ID NO: 384). In some embodiments, the bifunctional polypeptide comprises or consists of an amino acid sequence of

any one of the SEQ ID NOs in Table 27 listed as having a MYC degradation Grade of A or B, optionally wherein the sequence lacks the HA tag sequence (SEQ ID NO: 384). In some embodiments, the bifunctional polypeptide does not comprise or consist of an amino acid sequence of any one of the SEQ ID NOs in Table 27 listed as having a MYC degradation Grade of C and/or D, optionally wherein the sequence lacks the HA tag sequence (SEQ ID NO: 384). In some embodiments of the bifunctional polypeptide the URD is not derived from CHIP, optionally wherein the URD does not comprise or consist of CHIP.1, optionally wherein the URD does not comprise or consist of SEQ ID NO: 54.

[0082] Also disclosed herein is a polynucleotide encoding any of the bifunctional polypeptides targeting Myc disclosed above, and elsewhere herein.

Table 27: Exemplary bifunctional polypeptides with sdAb targeting Myc

Description	MYC Grade	SEQ ID NO
HA-spNLS.2-mycV.0302-(GS) ₂ -RNF4.1	A	430
HA-mycNLS-mycV.0302-NHLRC1.1	A	433
spNLS.2-mycV.0302-(GS) ₂ -RNF4.1	A	436
mycNLS-mycV.0302-RNF165.1	A	437
HA-mycNLS-mycV.0305-(GS) ₂ -NHLRC1.1	A	442
HA-spNLS.2-mycV.0306-(GS) ₂ -RNF4.1	A	448
HA-mycNLS-mycV.0306-NHLRC1.1	A	451
HA-spNLS.2-mycV.1517-(GS) ₂ -RNF4.1	A	464
HA-spNLS.2-mycV.1517-RNF4.1	A	465
HA-mycNLS-mycV.1517-(GS) ₂ -NHLRC1.1	A	466
spNLS.2-mycV.1517-RNF4.1	A	470
mycNLS-mycV.1517-RNF165.1	A	471
HA-spNLS.2-mycV.1518-(GS) ₂ -RNF4.1	A	475
HA-spNLS.2-mycV.1518-RNF4.1	A	476
HA-mycNLS-mycV.1518-NHLRC1.1	A	478
HA-spNLS.2-mycV.1519-(GS) ₂ -RNF4.1	A	483
HA-mycNLS-mycV.1519-(GS) ₂ -NHLRC1.1	A	485
HA-mycNLS-mycV.1519-NHLRC1.1	A	486
spNLS.2-mycV.1519-RNF4.1	A	489
mycNLS-mycV.1519-RNF165.1	A	490
mycNLS-mycV.1519-(GS) ₂ -NHLRC1.1	A	491
spNLS.2-mycV.1519-(GS) ₂ -RNF4.1	A	492
HA-spNLS.2-mycV.1519-GS-RNF4.1	A	493

Description	MYC Grade	SEQ ID NO
HA-spNLS.2-mycV.1519-(GS) ₃ -RNF4.1	A	494
HA-spNLS.2-mycV.1519-(GS) ₃ -RNF4.1	A	495
HA-spNLS.2-mycV.1519-(GS) ₃ -RNF4.1	A	496
HA-spNLS.2-mycV.1519-(GS) ₃ -RNF4.1	A	497
HA-spNLS.2-mycV.1520-(GS) ₂ -RNF4.1	A	500
HA-spNLS.2-mycV.1522-(GS) ₂ -RNF4.1	A	508
HA-spNLS.2-mycV.1524-(GS) ₂ -RNF4.1	A	516
HA-spNLS.2-mycV.1524-RNF4.1	A	517
HA-mycNLS-mycV.1524-(GS) ₂ -NHLRC1.1	A	518
HA-mycNLS-mycV.1524-NHLRC1.1	A	519
HA-spNLS.2-mycV.3706-(GS) ₂ -RNF4.1	A	524
HA-spNLS.2-mycV.3708-(GS) ₂ -RNF4.1	A	540
HA-mycNLS-mycV.6608-(GS) ₂ -NHLRC1.1	A	558
HA-RNF125.2-mycV.0302-hrpNLS	B	429
HA-spNLS.2-mycV.0302-RNF4.1	B	431
HA-mycNLS-mycV.0302-(GS) ₂ -NHLRC1.1	B	432
HA-RNF125.2-(GS) ₂ -mycV.0305-hrpNLS	B	438
HA-spNLS.2-mycV.0305-(GS) ₂ -RNF4.1	B	440
HA-mycNLS-mycV.0305-NHLRC1.1	B	443
HA-RNF125.2-(GS) ₂ -mycV.0306-hrpNLS	B	446
HA-RNF125.2-mycV.0306-hrpNLS	B	447
HA-spNLS.2-mycV.0306-RNF4.1	B	449
HA-mycNLS-mycV.0306-(GS) ₂ -NHLRC1.1	B	450
HA-spNLS.2-mycV.1308-(GS) ₂ -RNF4.1	B	456
HA-mycNLS-mycV.1308-(GS) ₂ -NHLRC1.1	B	458
HA-mycNLS-mycV.1308-NHLRC1.1	B	459
HA-RNF125.2-(GS) ₂ -mycV.1517-hrpNLS	B	462
HA-mycNLS-mycV.1517-NHLRC1.1	B	467
HA-mycNLS-mycV.1517-(GS) ₂ -ZNR1.1	B	468
HA-mycNLS-mycV.1517-ZNR1.1	B	469
HA-mycNLS-mycV.1518-ZNR1.1	B	480
HA-spNLS.2-mycV.1519-RNF4.1	B	484
HA-mycNLS-mycV.1519-ZNR1.1	B	488
HA-RNF125.2-mycV.1520-hrpNLS	B	499
HA-spNLS.2-mycV.1520-RNF4.1	B	501
HA-mycNLS-mycV.1520-(GS) ₂ -NHLRC1.1	B	502
HA-mycNLS-mycV.1520-NHLRC1.1	B	503

Description	MYC Grade	SEQ ID NO
HA-mycNLS-mycV.1520-ZNRF1.1	B	505
HA-RNF125.2-(GS) ₂ -mycV.1522-hrpNLS	B	506
HA-RNF125.2-mycV.1522-hrpNLS	B	507
HA-spNLS.2-mycV.1522-RNF4.1	B	509
HA-mycNLS-mycV.1522-(GS) ₂ -NHLRC1.1	B	510
HA-mycNLS-mycV.1522-NHLRC1.1	B	511
HA-mycNLS-mycV.1522-(GS) ₂ -ZNRF1.1	B	512
HA-mycNLS-mycV.1522-ZNRF1.1	B	513
HA-RNF125.2-(GS) ₂ -mycV.1524-hrpNLS	B	514
HA-RNF125.2-mycV.1524-hrpNLS	B	515
HA-mycNLS-mycV.1524-(GS) ₂ -ZNRF1.1	B	520
HA-mycNLS-mycV.3706-NHLRC1.1	B	527
HA-RNF125.2-(GS) ₂ -mycV.6605-hrpNLS	B	546
HA-RNF125.2-mycV.6605-hrpNLS	B	547
HA-spNLS.2-mycV.6605-(GS) ₂ -RNF4.1	B	548
HA-mycNLS-mycV.6605-(GS) ₂ -NHLRC1.1	B	550
HA-mycNLS-mycV.6605-NHLRC1.1	B	551
HA-RNF125.2-(GS) ₂ -mycV.6608-hrpNLS	B	554
HA-RNF125.2-mycV.6608-hrpNLS	B	555
HA-spNLS.2-mycV.6608-(GS) ₂ -RNF4.1	B	556
HA-mycNLS-mycV.6608-NHLRC1.1	B	559
HA-RNF125.2-(GS) ₂ -mycV.0302-hrpNLS	C	428
HA-mycNLS-mycV.0302-(GS) ₂ -ZNRF1.1	C	434
HA-mycNLS-mycV.0302-ZNRF1.1	C	435
HA-spNLS.2-mycV.0305-RNF4.1	C	441
HA-mycNLS-mycV.0306-(GS) ₂ -ZNRF1.1	C	452
HA-mycNLS-mycV.1308-ZNRF1.1	C	461
HA-RNF125.2-mycV.1517-hrpNLS	C	463
HA-mycNLS-mycV.1518-(GS) ₂ -NHLRC1.1	C	477
HA-mycNLS-mycV.1518-(GS) ₂ -ZNRF1.1	C	479
HA-RNF125.2-(GS) ₂ -mycV.1520-hrpNLS	C	498
HA-mycNLS-mycV.1520-(GS) ₂ -ZNRF1.1	C	504
HA-mycNLS-mycV.3706-(GS) ₂ -NHLRC1.1	C	526
HA-mycNLS-mycV.3707-(GS) ₂ -NHLRC1.1	C	534
HA-mycNLS-mycV.3708-(GS) ₂ -NHLRC1.1	C	542
HA-mycNLS-mycV.3708-NHLRC1.1	C	543
HA-spNLS.2-mycV.6605-RNF4.1	C	549

Description	MYC Grade	SEQ ID NO
HA-mycNLS-mycV.6605-(GS) ₂ -ZNR1.1	C	552
HA-RNF125.2-mycV.0305-hrpNLS	D	439
HA-mycNLS-mycV.0305-(GS) ₂ -ZNR1.1	D	444
HA-mycNLS-mycV.0305-ZNR1.1	D	445
HA-mycNLS-mycV.0306-ZNR1.1	D	453
HA-RNF125.2-(GS) ₂ -mycV.1308-hrpNLS	D	454
HA-RNF125.2-mycV.1308-hrpNLS	D	455
HA-spNLS.2-mycV.1308-RNF4.1	D	457
HA-mycNLS-mycV.1308-(GS) ₂ -ZNR1.1	D	460
HA-RNF125.2-(GS) ₂ -mycV.1518-hrpNLS	D	473
HA-RNF125.2-mycV.1518-hrpNLS	D	474
HA-RNF125.2-(GS) ₂ -mycV.1519-hrpNLS	D	481
HA-RNF125.2-mycV.1519-hrpNLS	D	482
HA-mycNLS-mycV.1519-(GS) ₂ -ZNR1.1	D	487
HA-mycNLS-mycV.1524-ZNR1.1	D	521
HA-RNF125.2-(GS) ₂ -mycV.3706-hrpNLS	D	522
HA-RNF125.2-mycV.3706-hrpNLS	D	523
HA-spNLS.2-mycV.3706-RNF4.1	D	525
HA-mycNLS-mycV.3706-(GS) ₂ -ZNR1.1	D	528
HA-mycNLS-mycV.3706-ZNR1.1	D	529
HA-RNF125.2-(GS) ₂ -mycV.3707-hrpNLS	D	530
HA-RNF125.2-mycV.3707-hrpNLS	D	531
HA-spNLS.2-mycV.3707-(GS) ₂ -RNF4.1	D	532
HA-spNLS.2-mycV.3707-RNF4.1	D	533
HA-mycNLS-mycV.3707-NHLRC1.1	D	535
HA-mycNLS-mycV.3707-(GS) ₂ -ZNR1.1	D	536
HA-mycNLS-mycV.3707-ZNR1.1	D	537
HA-RNF125.2-(GS) ₂ -mycV.3708-hrpNLS	D	538
HA-RNF125.2-mycV.3708-hrpNLS	D	539
HA-spNLS.2-mycV.3708-RNF4.1	D	541
HA-mycNLS-mycV.3708-(GS) ₂ -ZNR1.1	D	544
HA-mycNLS-mycV.3708-ZNR1.1	D	545
HA-mycNLS-mycV.6605-ZNR1.1	D	553
HA-spNLS.2-mycV.6608-RNF4.1	D	557
HA-mycNLS-mycV.6608-(GS) ₂ -ZNR1.1	D	560
HA-mycNLS-mycV.6608-ZNR1.1	D	561
spNLS.2-mycV.1517-(GS) ₂ -RNF4.1	Not tested	472

Dual-Specific Bifunctional Polypeptides: General Characteristics

[0083] The following characteristics may apply to embodiments of the dual-specific bifunctional polypeptides disclosed herein. In some embodiments, the dual-specific bifunctional polypeptide comprises the any one of the bifunctional polypeptides targeting Myc disclosed above and elsewhere herein, where the dual-specific bifunctional polypeptide further comprises a second targeting moiety that is capable of binding to at least one second target protein, optionally an intracellular target protein. In some embodiments of the dual-specific bifunctional polypeptide, proximity of the dual-specific bifunctional polypeptide to at least one target protein through binding of the first targeting moiety and/or the second targeting moiety induces ubiquitination of the at least one target protein via the URD, thereby promoting proteasome-mediated degradation of the at least one target protein. In some embodiments, the first and the second target proteins can be or are bound by the dual-specific bifunctional polypeptide simultaneously, in some embodiments only one of the first and second target proteins can be or are bound by the dual-specific bifunctional polypeptide at a time. In some embodiments, dual-specific bifunctional polypeptide further comprises a second linker peptide, wherein the second linker peptide is positioned between the second targeting moiety and the URD or between the first targeting moiety and the second targeting moiety.

[0084] In some embodiments, the first targeting moiety binds to and/or is designed to bind a first target protein and the second targeting moiety binds to and/or is designed to bind a second target protein. In some embodiments, the first targeting moiety and the second targeting moiety bind to and/or are designed to bind to the same target protein, Myc. In some embodiments, the first targeting moiety binds to and/or is capable of binding to a first binding site of the target protein and the second targeting moiety binds to and/or is capable of binding to a second binding site of the target protein Myc. In some embodiments, the first targeting moiety and second targeting moiety bind to and/or are capable of binding to the same binding site of the target protein, wherein the dual-specific bifunctional polypeptide engages and/or is capable of engaging at least two molecules of the at least one target protein Myc. In some embodiments, the first and second targeting moieties are two copies of the same class of targeting, an anti-Myc sdAb, or two copies of the same anti-Myc sdAb. In some embodiments, the first and second targeting moieties target the same protein (Myc), but are not the same class of targeting moieties (e.g., an anti-Myc sdAb and an endogenous binding partner of the target protein such

as omomyc). In some embodiments, the first targeting moiety targets Myc and the second targeting moiety targets different target protein.

[0085] In some embodiment the first and/or second targeting moiety binds selectively or specifically to the target protein(s). In some embodiments, the second targeting moiety is, or is less than, 260, 259, 258, 257, 256, 255, 254, 253, 252, 251, 250, 245, 240, 235, 230, 225, 220, 215, 210, 205, 200, 195, 190, 185, 180, 175, 170, 165, 160, 155, 150, 145, 140, 135, 130, 129, 128, 127, 126, 125, 124, 123, 122, 121, 120, 119, 118, 117, 116, 115, 114, 113, 112, 110, 109, 108, 107, 106, 105, 104, 103, 102, 101, 100, 99, 98, 97, 96, 95, 94, 93, 92, 91, 90, 89, 88, 87, 86, 85, 84, 83, 82, 81, 80, 75, 70, 65, 60, 55, 54, 53, 52, 51, 50, 49, 48, 47, 46, 45, 44, 43, 42, 41, 40, 39, 38, 37, 36, 35, 34, 33, 32, 31, 30, 29, 28, 27, 26, 25, 24, 23, 22, 21, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, or 10 amino acids long, or a range defined by any two of the preceding values, optionally 10-260, 10-200, 10-130, 25-260, 25-200, 25-130, 35-260, 35-200, 35-130, 50-260, 50-200, or 50-130 amino acids long. In some embodiments, the second targeting moiety comprises or consists of an endogenous binding partner of the target protein, an antibody, Fab, F(ab')₂, Fab', scFv, single domain antibody (sdAb), V_H domain, V_L domain, V_HH, V_{NAR}, diabody, intrabody, DARPin, monobody, affibody, avimer, or any binding fragment or derivative thereof.

[0086] In some embodiments, the second targeting moiety comprises or consists of an endogenous protein binding partner of the target protein, a fragment and/or a derivative thereof. In some embodiments, the second targeting moiety is, or is less than, 100, 99, 98, 97, 96, 95, 94, 93, 92, 91, 90, 89, 88, 87, 86, 85, 84, 83, 82, 81, 80, 75, 70, 65, 60, 55, 54, 53, 52, 51, 50, 49, 48, 47, 46, 45, 44, 43, 42, 41, 40, 39, 38, 37, 36, 35, 34, 33, 32, 31, 30, 29, 28, 27, 26, 25, 24, 23, 22, 21, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, or 10 amino acids long, or a range defined by any two of the preceding values. In some embodiments, the second targeting moiety is 10-100, 10-25, 14-100, 14-25, 30-100, 30-50, 30-45, or 80-100 amino acids long.

[0087] In some embodiments, the second targeting moiety comprises or consists of a sdAb. In some embodiments, the second targeting moiety comprises or consists of either a V_H domain or a V_L domain of an IgG antibody. In some embodiments, the second targeting moiety comprises or consists of a V_HH, V_H, or V_{NAR} domain of a heavy chain antibody (HcAb). In some embodiments, the second targeting moiety comprises or consists of a V_HH or V_L domain. In some embodiments, the second targeting moiety comprises or consists of a sdAb and the second targeting moiety is, or is less than, 170, 165, 160, 155, 150, 145, 140, 135, 130, 129, 128, 127, 126, 125, 124, 123, 122, 121, 120, 119, 118, 117, 116, 115, 114, 113, 112, 110, 109,

108, 107, 106, 105, 104, 103, 102, 101, 100, 99, 98, 97, 96, 95, 94, 93, 92, 91, or 90, amino acids long, or a range defined by any two of the preceding values, optionally 90-150, 90-130, 110-150, 110-130, 110-125, or 114-124 amino acids long. In some embodiments, the second targeting moiety comprises or consists of an scFv. In some embodiments, the second targeting moiety comprises or consists of an scFv second targeting moiety is, or is less than, 260, 259, 258, 257, 256, 255, 254, 253, 252, 251, 250, 245, 240, 235, 230, 225, 220, 215, 210, 205, 200, 195, or 190 amino acids long, or a range defined by any two of the preceding values, optionally 190-260, 200-260, or 230-260 amino acids long.

Exemplary Second Targets of Dual-Specific Bifunctional Polypeptides

[0088] In some embodiments, the second target protein of the dual-specific bifunctional polypeptide (the first target protein being Myc) is generally a protein that is involved in disease pathology, for example, cancer. In some embodiments, the dual-specific bifunctional polypeptide comprises a second targeting moiety that is capable of binding (optionally selectively or specifically) to target protein selected from Myc, (optionally c-Myc), beta catenin 1 (CTNNB1), and proliferating cell nuclear antigen (PCNA). In some embodiments, the target protein is a wild-type and/or a mutant form of the target protein, optionally wherein the targeting moiety preferentially or selectively binds the mutant form of the target protein relative to the wild-type form of the target protein. In some embodiments, the mutant form of the target protein is CTNNB1 (SEQ ID NO: 2) comprising one or more mutations selected from: D32Y; D32N; D32V; D32G; S33Y; S33L; S33F; S33C; S33P; G34V; G34E; I35S; H36P; S37C; S37P; S37P and D207G; T41A; T41I; T42_K49del; S45F and Y670X wherein X indicates termination; S45C; S45F; S45P; S45Y; and S45del.

[0089] In some embodiments of the dual-specific bifunctional polypeptide, the second targeting moiety binds, or binds at least in part, to at least a portion of: a) the DNA binding domain of MYC; b) the armadillo domain of CTNNB1, or c) the p21 binding domain of PCNA. In some embodiments, the second targeting moiety binds, or binds at least in part, to at least a portion of the target protein selected from the portion consisting of:

- a) amino acids 54-406 of MYC (SEQ ID NO: 1),
- b) amino acids 354-406 of MYC (SEQ ID NO: 1),
- c) amino acids 408-437 of MYC (SEQ ID NO: 1),
- d) amino acids 410-419 of MYC (SEQ ID NO: 1),
- e) amino acids 150-663 of CTNNB1 (SEQ ID NO: 2),

- f) amino acids 15-29 of CTNNB1 (SEQ ID NO: 2),
- g) amino acids 249-265, 292-306, 379-390, 415-429, 462-470, or 505-519, or any combination thereof, of CTNNB1 (SEQ ID NO: 2), and
- h) amino acids 38-48, 123-129, or 251-257 or any combination thereof, of PCNA (SEQ ID NO: 3).

[0090] In some embodiments of the dual-specific bifunctional polypeptide, the second targeting moiety is selected from Omomyc, TCF4, TCF4.1, TCF4.2, TCF4.3, TCF4.4, TCF4.5, TCF4.6, TCF4.7, TCF4.8, TCF4.9, TCF4.10, TCF4.11, TCF4.12, TCF4.13, TCF4.14, TCF4.15, TCF4.16, con1.1, con1.2, p21.1, p21.2, p21.3, p21.4, p21.5, p21.6, p21.7, p21.8, p21.9, mycV.0300, mycV.1300, mycV.1500, mycV.3700, mycV.6600, mycV.1516, mycV.1515, mycV.1514, mycV.1513, mycV.1512, mycV.1511, mycV.1510, mycV.1509, mycV.1508, mycV.1507, mycV.1506, mycV.1505, mycV.1504, mycV.1503, mycV.1502, mycV.1501, , mycV.0302, mycV.0305, mycV.0306, mycV.0307, mycV.1308, mycV.1517, mycV.1518, mycV.1519, mycV.1520, mycV.1522, mycV.1524, mycV.3706, mycV.3708, mycV.6605, mycV.6608, or a fragment thereof that is capable of binding to the target protein. In some embodiments, the second targeting moiety does not comprise full length or unmodified con1 or p21. Exemplary sequences of second targeting moieties are found in Table 28. In some embodiments, the second targeting moiety comprises or consists comprises or consists of an amino acid sequence having at least 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to any one of SEQ ID NO: 136, 137, 145, 153, 161, 169, 184, 192, 200, 208, 216, 224, 232, 240, 248, 256, 264, 272, 280, 288, 296, 319-344, 641, 649, 657, 665, 673, 681, 689, 697, 705, 713, 721, 729, 737, 745, 753, and 761. In some embodiments, the second targeting moiety comprises or consists comprises or consists of an amino acid sequence having at least 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to any one of SEQ ID NO: 184, 192, 200, 208, 216, 224, 232, 240, 248, 256, 264, 272, 280, 288, 296, and 319-344. In some embodiments where the amino acid sequence differs from a recited reference sequence, the difference is the result of the inclusion of one or more modifications relative to the reference sequence. In some embodiments, the one or more modifications comprise a substitution, insertion and/or deletion. In some embodiments, the substitution is a conservative substitution. In some embodiments, the % sequence identity is calculated over the entirety of the reference sequence.

[0091] FIG. 8 discloses embodiments of dual-specific bifunctional polypeptides at SEQ ID NOs 562-577. The description identifies the elements of the polypeptide in the N- to C-terminal direction. In some embodiments, the dual-specific bifunctional polypeptide is or comprises one of the embodiments described in FIG. 8 at SEQ ID NOs: 562-577. In some embodiments, the dual-specific bifunctional polypeptide comprises one of the embodiments at SEQ ID NOs: 385-561 and further comprising a second targeting moiety. In some embodiments, the dual-specific bifunctional polypeptide comprises the embodiments in FIG. 8 sharing one or more elements or features. In some embodiments the shared element or feature is the target(s). In some embodiments the shared element or feature is the URD name (e.g., those in Table 24). In some embodiments the shared element or feature is protein from which the URD is derived (e.g., those listed in Table 23). In some embodiments the shared element or feature is one or more of the following: the target; the protein from which the URD is derived; the URD name; the type of targeting moiety (e.g., sdAb, scFv, polypeptide binder); the targeting moiety name (e.g., those in Table 28; note that “full” is not included in the names in FIG. 8); the NLS name (e.g. those in Tables 25 and 26); the linker name (e.g., those in Table 32); the position of the URD relative to the targeting moiety (upstream or downstream); and both the URD name and targeting moiety name. In some embodiments, the HA tag sequence (SEQ ID NO: 384) is excluded.

[0092] In some embodiments, the dual-specific bifunctional polypeptide comprises or consists of an amino acid sequence having at least 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to any one of SEQ ID NOs: 562-577. In some embodiments, the dual-specific bifunctional polypeptide comprises or consists of an amino acid sequence of any one of SEQ ID NOs: 562-577. In some embodiments, the dual-specific bifunctional polypeptide comprises or consists of an amino acid sequence having at least 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to any one of SEQ ID NOs: 562-577, optionally wherein the sequence lacks the HA tag sequence (SEQ ID NO: 384). In some embodiments where the amino acid sequence differs from a recited reference sequence, the difference is the result of the inclusion of one or more modifications relative to the reference sequence. In some embodiments, the one or more modifications comprise a substitution, insertion and/or deletion. In some embodiments, the substitution is a conservative substitution. In some embodiments, the % sequence identity is calculated over the entirety of the reference sequence.

[0093] Table 29 below lists the results of HiBiT™ degradation studies utilizing exemplary embodiments of dual-specific bifunctional polypeptides disclosed herein. The tables list a “Grade” of A, B, C or D for the level of degradation of each of the targeted proteins. For dual-specific bifunctional polypeptides where the first and the second targeting moieties target the same protein, only a single grade is provided, a grade for the second target is listed as “n.a.” signifying that it is not applicable. Details regarding the experimental protocol are provided in the Examples section below. In some embodiments, the dual-specific bifunctional polypeptide comprises or consists of an amino acid sequence having at least 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to any one of the SEQ ID NOs in Table 29 disclosing Grade A degradation of at least one of the target proteins, Grade B degradation of at least one of the target proteins, Grade C degradation of at least one of the target proteins, and/or Grade D degradation of at least one of the target proteins, optionally wherein the sequence lacks the HA tag sequence (SEQ ID NO: 384). In some embodiments, the dual-specific bifunctional polypeptide comprises or consists of an amino acid sequence having at least 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to any one of the SEQ ID NOs in Table 29 disclosing Grade A degradation of at least one of the target proteins, optionally wherein the sequence lacks the HA tag sequence (SEQ ID NO: 384). In some embodiments, the dual-specific bifunctional polypeptide comprises or consists of an amino acid sequence of any one of the SEQ ID NOs in Table 29 disclosing Grade A degradation of at least one of the target proteins, and/or Grade B degradation of at least one of the target proteins, optionally wherein the sequence lacks the HA tag sequence (SEQ ID NO: 384). In some embodiments, the dual-specific bifunctional polypeptide comprises or consists of an amino acid sequence of any one of the SEQ ID NOs in Table 29 disclosing a Combined Grade A/A or A, optionally wherein the sequence lacks the HA tag sequence (SEQ ID NO: 384). In some embodiments, the dual-specific bifunctional polypeptide comprises or consists of an amino acid sequence of any one of the SEQ ID NOs in Table 29 disclosing a Combined Grade A/B or B/A, optionally wherein the sequence lacks the HA tag sequence (SEQ ID NO: 384). In some embodiments, the dual-specific bifunctional polypeptide comprises or consists of an amino acid sequence of any one of the SEQ ID NOs in Table 29 disclosing a Combined Grade B/B or B, optionally wherein the sequence lacks the HA tag sequence (SEQ ID NO: 384). In some embodiments, the dual-specific bifunctional polypeptide comprises or consists of an amino acid sequence of any one of the SEQ ID NOs in Table 29 disclosing Grade A degradation of both target proteins,

optionally wherein the sequence lacks the HA tag sequence (SEQ ID NO: 384). In some embodiments, the dual-specific bifunctional polypeptide does not comprise or consist of an amino acid sequence of any one of the SEQ ID NOs in Table 29 disclosing Grade C and/or D degradation of at least one of the target proteins, optionally wherein the sequence lacks the HA tag sequence (SEQ ID NO: 384). In some embodiments, the dual-specific bifunctional polypeptide further shares one or more elements of features as described above and elsewhere herein. In some embodiments where the amino acid sequence differs from a recited reference sequence, the difference is the result of the inclusion of one or more modifications relative to the reference sequence. In some embodiments, the one or more modifications comprise a substitution, insertion and/or deletion. In some embodiments, the substitution is a conservative substitution. In some embodiments, the % sequence identity is calculated over the entirety of the reference sequence.

Table 28: Exemplary targeting moieties

Target	Class	Name	SEQ ID NO
MYC	sdAb	mycV.0302 full	184
MYC	sdAb	mycV.0305 full	192
MYC	sdAb	mycV.0306 full	200
MYC	sdAb	mycV.0307 full	208
MYC	sdAb	mycV.1308 full	216
MYC	sdAb	mycV.1517 full	224
MYC	sdAb	mycV.1518 full	232
MYC	sdAb	mycV.1519 full	240
MYC	sdAb	mycV.1520 full	248
MYC	sdAb	mycV.1522 full	256
MYC	sdAb	mycV.1524 full	264
MYC	sdAb	mycV.3706 full	272
MYC	sdAb	mycV.3708 full	280
MYC	sdAb	mycV.6605 full	288
MYC	sdAb	mycV.6608 full	296
MYC	sdAb	mycV.0300 full	137
MYC	sdAb	mycV.1300 full	145

Target	Class	Name	SEQ ID NO
MYC	sdAb	mycV.1500 full	135
MYC	sdAb	mycV.3700 full	161
MYC	sdAb	mycV.6600 full	169
MYC	sdAb	mycV.1516 full	641
MYC	sdAb	mycV.1515 full	649
MYC	sdAb	mycV.1514 full	657
MYC	sdAb	mycV.1513 full	665
MYC	sdAb	mycV.1512 full	673
MYC	sdAb	mycV.1511 full	681
MYC	sdAb	mycV.1510 full	689
MYC	sdAb	mycV.1509 full	697
MYC	sdAb	mycV.1508 full	705
MYC	sdAb	mycV.1507 full	713
MYC	sdAb	mycV.1506 full	721
MYC	sdAb	mycV.1505 full	729
MYC	sdAb	mycV.1504 full	737
MYC	sdAb	mycV.1503 full	745
MYC	sdAb	mycV.1502 full	753
MYC	sdAb	mycV.1501 full	761
MYC	peptide	OMOMYC	136
CTNNB1	peptide	TCF4.1	319
CTNNB1	peptide	TCF4.10	320
CTNNB1	peptide	TCF4.11	321
CTNNB1	peptide	TCF4.12	322
CTNNB1	peptide	TCF4.14	323
CTNNB1	peptide	TCF4.15	324
CTNNB1	peptide	TCF4.16	325
CTNNB1	peptide	TCF4.2	326
CTNNB1	peptide	TCF4.3	327
CTNNB1	peptide	TCF4.4	328
CTNNB1	peptide	TCF4.5	329

Target	Class	Name	SEQ ID NO
CTNNB1	peptide	TCF4.6	330
CTNNB1	peptide	TCF4.7	331
CTNNB1	peptide	TCF4.8	332
CTNNB1	peptide	TCF4.9	333
PCNA	peptide	con1.1	334
PCNA	peptide	con1.2	335
PCNA	peptide	p21.1	336
PCNA	peptide	p21.2	337
PCNA	peptide	p21.3	338
PCNA	peptide	p21.4	339
PCNA	peptide	p21.5	340
PCNA	peptide	p21.6	341
PCNA	peptide	p21.7	342
PCNA	peptide	p21.8	343
PCNA	peptide	p21.9	344

Table 29: Exemplary dual-specific bifunctional polypeptides targeting MYC and CTNNB1 and corresponding exemplary target protein degradation grade

Targets	Description	MYC Grade	CTNNB1 Grade	Combined Grade	SEQ ID NO
MYC MYC	mycNLS-mycV.1519-(GS) ₂ -NHLRC1.1-(GS) ₂ -mycV.1519	A	n.a.	A	562
MYC CTNNB1	mycNLS-mycV.1519-(GS) ₂ -NHLRC1.1-(GS) ₂ -TCF4.6	A	A	A/A	563
CTNNB1 MYC	mycNLS-TCF4.6-(GS) ₃ -NHLRC1.1-(GS) ₂ -mycV.1519	A	A	A/A	564
MYC MYC	spNLS.2-mycV.1519-(GS) ₂ -RNF4.1-(GS) ₂ -mycV.1519	A	n.a.	A	565
MYC CTNNB1	spNLS.2-mycV.1519-(GS) ₂ -RNF4.1-(GS) ₂ -TCF4.6	A	A	A/A	566
MYC MYC	mycV.1524-(GS) ₂ -RNF125.2-(GS) ₂ -mycV.1524-hrpNLS	A	n.a.	A	567

Targets	Description	MYC Grade	CTNNB1 Grade	Combined Grade	SEQ ID NO
CTNNB1 MYC	TCF4.6-(GS) ₂ -RNF125.2-(GS) ₂ -mycV.1524-hrpNLS	A	A	A/A	568
MYC CTNNB1	mycV.1524-(GS) ₂ -RNF125.2-(GS) ₂ -TCF4.6-hrpNLS	A	A	A/A	569
MYC MYC	spNLS.2-mycV.1500-(GS) ₂ -RNF4.1-(GS) ₂ -mycV.1519	A	n.a.	A	570
MYC MYC	spNLS.2-mycV.1519-(GS) ₂ -RNF4.1-(GS) ₂ -mycV.0302	A	n.a.	A	572
MYC MYC	mycNLS-mycV.1519-(GS) ₂ -NHLRC1.1-(GS) ₂ -mycV.0302	A	n.a.	A	573
MYC MYC	mycNLS-mycV.1500-RNF165.1-(GS) ₃ -mycV.0302	A	n.a.	A	574
MYC MYC	mycNLS-mycV.0302-RNF165.1-(GS) ₃ -mycV.1500	A	n.a.	A	575
MYC MYC	spNLS.2-mycV.0302-(GS) ₂ -RNF4.1-(GS) ₂ -mycV.1519	A	n.a.	A	576
MYC MYC	mycNLS-mycV.0302-(GS) ₂ -NHLRC1.1-(GS) ₂ -mycV.1519	A	n.a.	A	577
MYC MYC	spNLS.2-mycV.1519-(GS) ₂ -RNF4.1-(GS) ₂ -mycV.1500	B	n.a.	B	571

Exemplary Dual-Specific Bifunctional Degraders Targeting Myc

[0094] In some embodiments, the at least one second target protein is Myc (c-Myc). In some embodiments, the Myc is a mutant form. An example sequence for Myc is provided as SEQ ID NO: 1 (Uniprot #P01106). In some embodiments, the dual-specific bifunctional polypeptide comprises a second targeting moiety that is capable of binding to Myc. Some examples of second targeting moieties that target Myc include Omomyc (SEQ ID NO: 4), although other suitable targeting moieties that bind to Myc generally known in the art may also be used. In some embodiments, the second targeting moiety that is capable of binding to Myc may be capable of binding, or binding at least in part, to at least a portion of Myc, such as the DNA binding domain of Myc. In some embodiments, the second targeting moiety that is capable of binding to Myc is capable of binding, or binding at least in part, to at least a portion of amino acids 54-406, amino acids 354-406 (the DNA binding domain of MYC that binds to Omomyc; as seen in PDB: 1NKP), amino acids 408-437, or amino acids 410-419 (binding epitope of

antibody 9E10; as seen in PDB: 2OR9) of MYC (as represented as SEQ ID NO: 1). The dual-specific bifunctional polypeptides disclosed herein that are specific for Myc may be used for the treatment of a cancer, for example, a cancer exhibiting elevated levels of Myc, for example solid tumors (including but not limited to hepatocellular carcinoma, liver metastases, and colorectal carcinoma) and hematological malignancies.

[0095] In some embodiments, the bifunction polypeptide targeting Myc comprises a localization peptide sequence which directs the dual-specific bifunctional polypeptide to the nucleus of a cell. In some embodiments, the localization peptide sequence comprises or consists of the NLS sequence selected from the group of the NLS sequences of MYC, SPOP, Hrp1, SV40, 53BP1, and nucleoplasmin. In some, the localization peptide sequence comprises or consists of a NLS sequence selected from the group of the NLS sequence of SPOP, optionally SPNLS.2, MYC, optionally mycNLS, and Hrp1, optionally hrpNLS. In some embodiments, the NLS comprises or consists of a sequence selected from any one of SEQ ID NOs: 114-127. In some embodiments, the NLS comprises or consists of a sequence selected from any one of SEQ ID NOs: 122, 114 and 116.

[0096] In some embodiments, the dual-specific bifunctional polypeptide targeting Myc comprises a URD derived from a URD selected from the group consisting of NHLRC1, RNF125, RNF165, and RNF4. In some embodiments, the URD is a truncation or fragment thereof that retains ubiquitin-proteasome recruiting activity. In some embodiments, the URD comprises or consists of NHLRC1.1, RNF125.2, RNF165.1, or RNF4.1. In some embodiments, the URD comprises or consists of a sequence selected from any one of SEQ ID NOs: 76-80, 87-93, 98, and 104. In some embodiments, the URD comprises or consists of a sequence selected from any one of SEQ ID NO: 76, 88, 98, and 104.

[0097] In some embodiments, the dual-specific bifunctional polypeptide targeting Myc comprises a second targeting moiety that comprises or consists of a targeting moiety disclosed in Table 28 as targeting Myc. In some embodiments, the second targeting moiety comprises or consists of an endogenous protein binding partner of the target protein, a fragment and/or a derivative thereof. In some embodiments, the second targeting moiety comprises or consists of Omomyc, or a fragment thereof that is capable of binding to the target protein. In some embodiments, the second targeting moiety comprises or consists of an amino acid sequence having at least 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 136. In some embodiments where the amino acid sequence differs from a recited reference sequence, the difference is the result

of the inclusion of one or more modifications relative to the reference sequence. In some embodiments, the one or more modifications comprise a substitution, insertion and/or deletion. In some embodiments, the substitution is a conservative substitution. In some embodiments, the % sequence identity is calculated over the entirety of the reference sequence.

[0098] In some embodiments, the dual-specific bifunctional polypeptide targeting Myc comprises a second targeting moiety that comprises or consists of a targeting moiety disclosed in Table 28 as targeting Myc. In some embodiments, the second targeting moiety comprises or consists of a sdAb, optionally comprising or consisting of either a V_H domain or a V_L domain of an IgG antibody, or a V_{HH}, V_H, or V_{NAR} domain of a heavy chain antibody (HcAb). In some embodiments, the sdAb comprises or consists of a V_{HH} domain. In some embodiments, the second targeting moiety comprises or consists of a sdAb, optionally a V_{HH} domain, comprising a CDR1 selected from any one of SEQ ID NOs: 139, 147, 163, and 171, a CDR2 selected from any one of SEQ ID NOs: 141, 149, 157, and 165, and a CDR3 selected from any one of SEQ ID NOs: 143, 151, 159, 167, and 175. In some embodiments, the combination of CDR1, CDR2, and CDR3 is 1936, 157 and 159. In some embodiments, each CDR sequence independently comprises 1, 2, 3, 4, 5, 6, 7, or more substitutions (optionally conservative substitutions) and/or 1, 2, 3, 4, 5, 6, 7, or more deletions or additions, and/or has at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to a sequence selected from any one of SEQ ID NOs: 139, 147, 163, 171, 141, 149, 157, 165, 143, 151, 159, 167, and 175. In some embodiments, the sdAb, optionally a V_{HH} domain, comprises a V_H FR1 selected from any one of SEQ ID NOs: 138 and 170, a V_H FR2 of SEQ ID NO: 140, a V_H FR3 selected from any one of SEQ ID NOs: 142, 158, and 174, and a V_H FR4 selected from any one of SEQ ID NOs: 144 and 152. In some embodiments, the combination of FR1, FR2, FR3 and FR4 is 154, 156, 158, and 160. In some embodiments, each V_H framework region comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17 or more substitutions (optionally conservative substitutions) and/or 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17 or more deletions or additions, and/or has at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to a sequence selected from any one of SEQ ID NOs: 138, 170, 140, 142, 158, 174, 144, and 152. In some embodiments, the second targeting moiety comprises or consists of a sdAb, optionally a V_{HH} domain, sequence selected from any one of SEQ ID NOs: 137, 145, 153, 161, 169, 641, 649, 657, 665, 673, 681, 689, 697, 705, 713, 721, 729, 737, 745, 753, and 761. In some embodiments, each CDR sequence independently comprises 1, 2, 3, 4, 5, 6, 7, or more substitutions (optionally conservative substitutions) and/or 1, 2, 3, 4, 5, 6, 7, or more

deletions or additions, and/or has at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to a CDR sequence of any one of SEQ ID NOs: 137, 145, 153, 161, 169, 641, 649, 657, 665, 673, 681, 689, 697, 705, 713, 721, 729, 737, 745, 753, and 761, and/or wherein the framework region comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17 or more substitutions (optionally conservative substitutions) and/or 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17 or more deletions or additions, and/or has at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the framework region of any one of SEQ ID NOs: 137, 145, 153, 161, 169, 641, 649, 657, 665, 673, 681, 689, 697, 705, 713, 721, 729, 737, 745, 753, and 761. In some embodiments where the amino acid sequence differs from a recited reference sequence, the difference is the result of the inclusion of one or more modifications relative to the reference sequence. In some embodiments, the one or more modifications comprise a substitution, insertion and/or deletion. In some embodiments, the substitution is a conservative substitution. In some embodiments, the % sequence identity is calculated over the entirety of the reference sequence.

[0099] In some embodiments, the dual-specific bifunctional polypeptide targeting Myc comprises a second targeting moiety comprising or consisting of a sdAb, wherein the sdAb comprises or consists of a V_HH domain. In some embodiments, the second targeting moiety comprises or consists of an anti-Myc sdAb described above under the heading “Anti-Myc Single Domain Antibodies (sdAb)”, and elsewhere herein. In some embodiments, the dual-specific bifunctional polypeptide comprises or consists of an amino acid sequence having at least 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to any one of SEQ ID NOs: 562, 565, 567, and 570-577, optionally wherein the sequence lacks the HA tag sequence (SEQ ID NO: 384). In some embodiments, the dual-specific bifunctional polypeptide comprising a second targeting moiety targeting Myc comprises or consists of an amino acid sequence having at least 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to any one of the SEQ ID NOs in Table 30, optionally excluding any one of the SEQ ID NOs having a Grade of D degradation of Myc and/or another target protein, optionally wherein the sequence lacks the HA tag sequence (SEQ ID NO: 384). In some embodiments, the dual-specific bifunctional polypeptide comprises or consists of an amino acid sequence of any one of the SEQ ID NOs in Table 30 having a Combined Grade A degradation of MYC, and/or a Combined Grade B degradation of MYC, optionally wherein the sequence lacks the HA tag sequence (SEQ ID NO: 384). In some embodiments, the dual-specific bifunctional

polypeptide comprises or consists of an amino acid sequence of any one of the SEQ ID NOs in Table 30 having a Combined Grade of A, optionally wherein the sequence lacks the HA tag sequence (SEQ ID NO: 384). In some embodiments, the dual-specific bifunctional polypeptide comprises or consists of an amino acid sequence of any one of the SEQ ID NOs in Table 30 having a Combined Grade of B, optionally wherein the sequence lacks the HA tag sequence (SEQ ID NO: 384). In some embodiments, the dual-specific bifunctional polypeptide does not comprise or consist of an amino acid sequence of any one of the SEQ ID NOs in Table 30 having a combined of Grade C and/or D, optionally wherein the sequence lacks the HA tag sequence (SEQ ID NO: 384). In some embodiments where the amino acid sequence differs from a recited reference sequence, the difference is the result of the inclusion of one or more modifications relative to the reference sequence. In some embodiments, the one or more modifications comprise a substitution, insertion and/or deletion. In some embodiments, the substitution is a conservative substitution. In some embodiments, the % sequence identity is calculated over the entirety of the reference sequence.

[0100] In some embodiments, the dual-specific bifunctional polypeptide comprising a second targeting moiety targeting Myc comprises embodiments further sharing one or more elements or features. In some embodiments the shared element or feature is the URD name. In some embodiments the shared element or feature is protein from which the URD is derived. In some embodiments the shared element or feature is one or more of the following: the protein from which the URD is derived; the URD name; the type of targeting moiety; the targeting moiety name; the NLS name; the linker name; the position of the URD relative to the targeting moiety (upstream or downstream); and both the URD name and targeting moiety name. In some embodiments, the HA tag sequence (SEQ ID NO: 384) is excluded.

[0101] In some embodiments of the dual-specific bifunctional polypeptide comprising a second targeting moiety targeting Myc the URD is not derived from CHIP. In some embodiments, the URD does not comprise or consist of CHIP.1. In some embodiments, the URD does not comprise or consist of SEQ ID NO: 54.

[0102] In some embodiments, disclosed herein is a polynucleotide encoding any of the dual-specific bifunctional polypeptides having a first and second targeting moiety targeting Myc disclosed above, or elsewhere herein.

Table 30: Exemplary dual-specific bifunctional polypeptides comprising a first and second targeting moiety targeting MYC and corresponding exemplary target protein degradation grade

Targets	Description	MYC Grade	CTNNB1 Grade	Combined Grade	SEQ ID NO
MYC MYC	mycNLS-mycV.1519-(GS) ₂ -NHLRC1.1-(GS) ₂ -mycV.1519	A	n.a.	A	562
MYC MYC	spNLS.2-mycV.1519-(GS) ₂ -RNF4.1-(GS) ₂ -mycV.1519	A	n.a.	A	565
MYC MYC	mycV.1524-(GS) ₂ -RNF125.2-(GS) ₂ -mycV.1524-hrpNLS	A	n.a.	A	567
MYC MYC	spNLS.2-mycV.1500-(GS) ₂ -RNF4.1-(GS) ₂ -mycV.1519	A	n.a.	A	570
MYC MYC	spNLS.2-mycV.1519-(GS) ₂ -RNF4.1-(GS) ₂ -mycV.0302	A	n.a.	A	572
MYC MYC	mycNLS-mycV.1519-(GS) ₂ -NHLRC1.1-(GS) ₂ -mycV.0302	A	n.a.	A	573
MYC MYC	mycNLS-mycV.1500-RNF165.1-(GS) ₃ -mycV.0302	A	n.a.	A	574
MYC MYC	mycNLS-mycV.0302-RNF165.1-(GS) ₃ -mycV.1500	A	n.a.	A	575
MYC MYC	spNLS.2-mycV.0302-(GS) ₂ -RNF4.1-(GS) ₂ -mycV.1519	A	n.a.	A	576
MYC MYC	mycNLS-mycV.0302-(GS) ₂ -NHLRC1.1-(GS) ₂ -mycV.1519	A	n.a.	A	577
MYC MYC	spNLS.2-mycV.1519-(GS) ₂ -RNF4.1-(GS) ₂ -mycV.1500	B	n.a.	B	571

Exemplary Dual-Specific Bifunctional Degraders Targeting Myc and CTNNB1

[0103] In some embodiments, the at least one second target protein is beta catenin 1 (CTNNB1). In some embodiments, the CTNNB1 is a mutant form. An example sequence for CTNNB1 is provided as SEQ ID NO: 2 (Uniprot #P35222). CTNNB1 is involved in the pathology of various solid tumors (including but not limited to liver metastases, colorectal carcinoma, or other gastrointestinal cancers) and hematological malignancies. In some embodiments disclosed herein, the dual-specific bifunctional polypeptide comprises a second targeting moiety that is capable of binding to CTNNB1. Some examples of targeting moieties that target CTNNB1 include TCF4 (SEQ ID NO: 6; Uniprot #Q9NQB0), and amino acids 10-

54 of TCF4 (SEQ ID NO: 5), although other suitable CTNNB1 targeting moieties generally known in the art may also be used. In some embodiments, the second targeting moiety that is capable of binding to CTNNB1 may be capable of binding, or binding at least in part, to at least a portion of CTNNB1, such as the armadillo domain of CTNNB1. In some embodiments, the second targeting moiety that is capable of binding to CTNNB1 is capable of binding, or binding at least in part, to at least a portion of amino acids 150-663 (the armadillo domain of CTNNB1; as seen in PDB: 2GL7), amino acids 15-29 (binding epitope of antibody BC2), or amino acids 249-265, 292-306, 379-390, 415-429, 462-470, or 505-519, (which are residues in the armadillo domain that interact with TCF4, E-cadherin, Axin, APC, TCF/LEF family transcription factors and/or SOX9) or any combination thereof, of CTNNB1 (as represented as SEQ ID NO: 2). The dual-specific bifunctional polypeptides disclosed herein comprising a second targeting moiety targeting CTNNB1 may be used for the treatment of a cancer, for example, a cancer involving nuclear expression of beta-catenin and/or harboring mutations or alterations in the Wnt/beta-catenin signaling pathway, for example, solid tumors (including but not limited to liver metastases, colorectal carcinoma, or other gastrointestinal cancers) and hematological malignancies.

[0104] In some embodiments, the CTNNB1 target protein is a mutant form of CTNNB1 (SEQ ID NO: 2) comprising one or more mutations selected from: D32Y; D32N; D32V; D32G; S33Y; S33L; S33F; S33C; S33P; G34V; G34E; I35S; H36P; S37C; S37P; S37P and D207G; T41A; T41I; T42_K49del; S45F and Y670X wherein X indicates termination; S45C; S45F; S45P; S45Y; and S45del.

[0105] In some embodiments, the dual-specific bifunctional polypeptide comprising a second targeting moiety targeting CTNNB1 comprises a localization peptide sequence which directs the dual-specific bifunctional polypeptide to the nucleus of a cell. In some embodiments, the localization peptide sequence comprises or consists of a NLS sequence selected from the group of the NLS sequences of MYC, SPOP, Hrp1, SV40, 53BP1, and nucleoplasmin. In some embodiments, the localization peptide sequence comprises or consists of a NLS sequence selected from the group of the NLS sequence of MYC, optionally mycNLS, SPOP, optionally spNLS.2, and Hrp1, optionally hrpNLS. In some embodiments, the NLS comprises or consists of a sequence selected from any one of SEQ ID NOs: 114-127. In some embodiments, the NLS comprises or consists of a sequence selected from any one of SEQ ID NOs: 122, 114 and 116.

[0106] In some embodiments, the dual-specific bifunctional polypeptide comprising a second targeting moiety targeting CTNNB1 comprises a URD derived from a URD selected from the group consisting of NHLRC1, RNF125, RNF165, and RNF4, or a truncation or fragment thereof that retains ubiquitin-proteasome recruiting activity. In some embodiments, the URD comprises or consists of NHLRC1.1, RNF125.2, RNF165.1, or RNF4.1. In some embodiments, the URD is a truncation or fragment thereof that retains ubiquitin-proteasome recruiting activity. In some embodiments, the URD comprises or consists of a sequence selected from any one of SEQ ID NOs: 6-80, 87-93, 98, and 104. In some embodiments, the URD comprises or consists of a sequence selected from any one of SEQ ID NOs: 76, 88, 98, and 104.

[0107] In some embodiments, the dual-specific bifunctional polypeptide comprising a second targeting moiety targeting CTNNB1 comprises a second targeting moiety wherein the second targeting moiety comprises or consists of a targeting moiety disclosed in Table 28 as targeting CTNNB1. In some embodiments, the second targeting moiety comprises or consists of an endogenous protein binding partner of the target protein, a fragment and/or a derivative thereof. In some embodiments, the second targeting moiety comprises or consists of TCF4.1, TCF4.2, TCF4.3, TCF4.4, TCF4.5, TCF4.6, TCF4.7, TCF4.8, TCF4.9, TCF4.10, TCF4.11, TCF4.12, TCF4.13, TCF4.14, TCF4.15, or TCF4.16, or a fragment thereof that is capable of binding to the target protein. In some embodiments, the second targeting moiety comprises or consists of an amino acid sequence having at least 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to any one of SEQ ID NOs: 319-333, optionally SEQ ID NO: 330. In some embodiments where the amino acid sequence differs from a recited reference sequence, the difference is the result of the inclusion of one or more modifications relative to the reference sequence. In some embodiments, the one or more modifications comprise a substitution, insertion and/or deletion. In some embodiments, the substitution is a conservative substitution. In some embodiments, the % sequence identity is calculated over the entirety of the reference sequence.

[0108] In some embodiments, the dual-specific bifunctional polypeptide targeting CTNNB1 comprises or consists of an amino acid sequence having at least 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to any one of SEQ ID NOs: 563, 564, 566, 568, and 569, optionally wherein the sequence lacks the HA tag sequence (SEQ ID NO: 384). In some embodiments, the dual-specific bifunctional

polypeptide comprises or consists of an amino acid sequence having at least 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to any one of the SEQ ID NOs in Table 31, optionally excluding any one of the SEQ ID NOs having a Grade of D degradation of CTNNB1 and/or MYC, optionally wherein the sequence lacks the HA tag sequence (SEQ ID NO: 384). In some embodiments, the dual-specific bifunctional polypeptide comprises or consists of an amino acid sequence of any one of the SEQ ID NOs in Table 31 having a Grade A degradation of CTNNB1, and/or a Grade B degradation of CTNNB1, optionally wherein the sequence lacks the HA tag sequence (SEQ ID NO: 384). In some embodiments, the dual-specific bifunctional polypeptide comprises or consists of an amino acid sequence of any one of the SEQ ID NOs in Table 31 having a Combined Grade of A and/or A/A, optionally wherein the sequence lacks the HA tag sequence (SEQ ID NO: 384). In some embodiments, the dual-specific bifunctional polypeptide comprises or consists of an amino acid sequence of any one of the SEQ ID NOs in Table 31 having a Combined Grade of A/B and/or B/A, optionally wherein the sequence lacks the HA tag sequence (SEQ ID NO: 384). In some embodiments, the dual-specific bifunctional polypeptide does not comprise or consist of an amino acid sequence of any one of the SEQ ID NOs in Table 31 having Grades C and/or D for either target protein, optionally wherein the sequence lacks the HA tag sequence (SEQ ID NO: 384). In some embodiments where the amino acid sequence differs from a recited reference sequence, the difference is the result of the inclusion of one or more modifications relative to the reference sequence. In some embodiments, the one or more modifications comprise a substitution, insertion and/or deletion. In some embodiments, the substitution is a conservative substitution. In some embodiments, the % sequence identity is calculated over the entirety of the reference sequence.

[0109] In some embodiments, the dual-specific bifunctional polypeptide targeting CTNNB1 comprises embodiments further sharing one or more elements or features. In some embodiments the shared element or feature is the URD name. In some embodiments the shared element or feature is protein from which the URD is derived. In some embodiments the shared element or feature is one or more of the following: the protein from which the URD is derived; the URD name; the type of targeting moiety; the targeting moiety name; the NLS name; the linker name; the position of the URD relative to the targeting moiety (upstream or downstream); and both the URD name and targeting moiety name. In some embodiments, the HA tag sequence (SEQ ID NO: 384) is excluded.

[0110] In some embodiments of the dual-specific bifunctional polypeptide targeting CTNNB1 the URD is not derived from beta-TRCP. In some embodiments, the URD does not comprise or consist of bTRCP.1. In some embodiments, the URD does not comprise or consist of SEQ ID NOs: 57.

[0111] In some embodiments, disclosed herein is a polynucleotide encoding any of the dual-specific bifunctional polypeptides targeting MYC and CTNNB1 disclosed above, or elsewhere herein.

Table 31: Exemplary dual-specific bifunctional polypeptides targeting MYC and CTNNB1 and corresponding exemplary target protein degradation grade

Targets	Description	MYC Grade	CTNNB1 Grade	Combined Grade	SEQ ID NO
MYC CTNNB1	mycNLS-mycV.1519-(GS) ₂ -NHLRC1.1-(GS) ₂ -TCF4.6	A	A	A/A	563
CTNNB1 MYC	mycNLS-TCF4.6-(GS) ₃ -NHLRC1.1-(GS) ₂ -mycV.1519	A	A	A/A	564
MYC CTNNB1	spNLS.2-mycV.1519-(GS) ₂ -RNF4.1-(GS) ₂ -TCF4.6	A	A	A/A	566
CTNNB1 MYC	TCF4.6-(GS) ₂ -RNF125.2-(GS) ₂ -mycV.1524-hrpNLS	A	A	A/A	568
MYC CTNNB1	mycV.1524-(GS) ₂ -RNF125.2-(GS) ₂ -TCF4.6-hrpNLS	A	A	A/A	569

Exemplary Dual-Specific Bifunctional Degraders Targeting Myc and PCNA

[0112] In some embodiments, the at least one second target protein is proliferating cell nuclear antigen (PCNA). In some embodiments, the PCNA is a mutant form. An example sequence for PCNA is provided as SEQ ID NO: 3 (Uniprot # P12004). PCNA is a co-factor for DNA polymerase delta and is involved in DNA synthesis and DNA repair. PCNA is implicated in the pathology of various solid tumor cancers (including but not limited to colorectal cancer, breast cancer, lung cancer, and liver cancer) and hematological malignancies. In some embodiments disclosed herein, the dual-specific bifunctional polypeptide comprises a second targeting moiety that is capable of binding to PCNA. Some examples of targeting moieties that target PCNA include those listed in Table 28 listed as targeting PCNA. In some embodiments, other suitable PCNA targeting moieties generally known in the art are used. The dual-specific

bifunctional polypeptides disclosed herein comprising a second targeting moiety targeting PCNA may be used for the treatment of a cancer, for example, solid tumor cancers (including but not limited to colorectal cancer, breast cancer, lung cancer, and liver cancer) and hematological malignancies.

[0113] In some embodiments, the dual-specific bifunctional polypeptide comprising a second targeting moiety targeting PCNA comprises a localization peptide sequence which directs the dual-specific bifunctional polypeptide to the nucleus of a cell. In some embodiments, the localization peptide sequence comprises or consists of a NLS sequence selected from the group of the NLS sequences of MYC, SPOP, Hrp1, SV40, 53BP1, and nucleoplasmin. In some embodiments, the localization peptide sequence comprises or consists of a NLS sequence selected from the group of the NLS sequence of MYC, optionally mcynLS and/or 3xmycNLS, SPOP, Hrp1, SV40, optionally svNLS or 3xsvNLS, and nucleoplasmin. In some embodiments, the NLS comprises or consists of a sequence selected from any one of SEQ ID NOs: 114-127. In some embodiments, the NLS comprises or consists of a sequence selected from any one of SEQ ID NOs: 115-116 and 118-122.

[0114] In some embodiments, the dual-specific bifunctional polypeptide comprising a second targeting moiety targeting PCNA comprises a URD derived from a URD selected from the group consisting of DCAF1, NHLRC1, RNF125, or SPOP. In some embodiments, the URD comprises or consists of DCAF1.1, NHLRC1.1, RNF125.2, RNF125.3, RNF125.4, RNF125.5, RNF125.6, SPOP.2 or SPOP.3. In some embodiments, the URD comprises or consists of optionally NHLRC1.1 or RNF125.2. In some embodiments, the URD is truncation or fragment thereof that retains ubiquitin-proteasome recruiting activity. In some embodiments, the URD comprises or consists of a sequence selected from any one of SEQ ID NOs: 39-113. In some embodiments, the URD comprises or consists of a sequence selected from any one of SEQ ID NOs: 55, 73, 74, 76 and 88-92. In some embodiments, the URD comprises or consists of a sequence selected from any one of SEQ ID NOs: 76 and 88.

[0115] In some embodiments, the dual-specific bifunctional polypeptide targeting PCNA comprises a second targeting moiety that comprises or consists of a targeting moiety disclosed in Table 28 as targeting PCNA. In some embodiments, the second targeting moiety binds, or binds at least in part, to at least a portion of amino acids 38-48, 123-129, or 251-257 or any combination thereof, of PCNA (SEQ ID NO: 3). In some embodiments, the second targeting moiety comprises or consists of an endogenous protein binding partner of the target protein, a fragment and/or a derivative thereof. In some embodiments, the second targeting moiety

comprises or consists of con1, con1.1, con1.2, p21, p21.1, p21.2, p21.3, p21.4, p21.5, p21.6, p21.7, p21.8, p21.9, or a fragment thereof that is capable of binding to the target protein. In some embodiments, the second targeting moiety does not comprise full length or unmodified con1 or p21. In some embodiments, the second targeting moiety comprises or consists of an amino acid sequence having at least 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to any one of SEQ ID NOs: 334-344. In some embodiments where the amino acid sequence differs from a recited reference sequence, the difference is the result of the inclusion of one or more modifications relative to the reference sequence. In some embodiments, the one or more modifications comprise a substitution, insertion and/or deletion. In some embodiments, the substitution is a conservative substitution. In some embodiments, the % sequence identity is calculated over the entirety of the reference sequence.

[0116] In some embodiments of the dual-specific bifunctional polypeptide targeting PCNA the URD is not derived from SPOP. In some embodiments, the URD does not comprise or consist of SPOP.1, SPOP.2, or SPOP.3. In some embodiments, the URD does not comprise or consist of a sequence selected from any one of SEQ ID NOs: 72-74.

[0117] In some embodiments, disclosed herein is a polynucleotide encoding any of the dual-specific bifunctional polypeptides targeting Myc and PCNA disclosed above, or elsewhere herein.

Additional Structural Features of the Bispecific Polypeptides

[0118] The following features may be generally applicable to the disclosed embodiments of the bifunctional polypeptides and dual-specific bifunctional polypeptides herein.

Linker Peptides

[0119] In some embodiments, the bifunctional polypeptide or the dual-specific bifunctional polypeptide disclosed above and herein further comprise one or more linker peptides. In some embodiments, the linker peptide is a length that allows for ubiquitination of the target protein via the URD when the target protein is bound by the first and/or second targeting moiety. Any suitable linker peptide generally known in the art may be used in embodiments herein. In some embodiments, the linker peptide is positioned between one or more of: the first and the second targeting moieties; the first and/or second targeting moiety and the URD; the NLS and the first and/or second targeting moiety; the NLS and the URD; and/or the NLS and an epitope tag, optionally an HA tag. In some embodiments, the linker peptide is positioned between the first

and/or second targeting moiety and the URD. In some embodiments, the linker peptide is 0 (i.e., there is no linker), 1, 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 or 30 amino acids in length, or a range defined by any two of the preceding values. In some embodiments, the linker peptide is 0-30, 1-30, 2-30, 0-20, 1-20, 2-20, 0-15, 0-12, 0-10, 1-15, 1-12, 1-10, 2-15, 2-12, 2-10, 3-15, 3-12, or 3-10 amino acids in length, optionally 2-30 amino acids in length. In some embodiments, the linker peptide comprises or consists of glycine and serine. In some embodiments, the linker peptide comprises or consists of one or more alpha helixes, optionally flanked by one or more glycine and/or a serine residues. In some embodiments, the linker peptide comprises or consists of the amino acid sequence of any one of SEQ ID NOs: 345-383. In some embodiments, the linker peptide comprises or consists of the amino acid sequence of any one of SEQ ID NOs: 347-352. Note that in the sequence listing submitted herewith, SEQ ID NO: 352 is blank because the sequence listing rules do not permit an amino acid sequence of fewer than four specifically defined amino acids, and the sequence of SEQ ID NO: 352 is "GS," which is only two amino acids long. Therefore, "SEQ ID NO: 352" can be replaced with "GS" herein. For the same reason, SEQ ID NOs: 345-383 is the same as SEQ ID NOs: 345-351, GS, and 353-383. Likewise, SEQ ID NOs: 347-352 is the same as SEQ ID NOs: 347-351 and GS. Exemplary linker sequences are disclosed in Table 32.

Table 32: Exemplary linker sequences

SEQ ID NO	Linker Name		SEQ ID NO	Linker Name
345	12xGS		365	L1H24L1
346	9xGS		366	L3H16L3
347	6xGS		367	L2H16L3
348	5xGS		368	L1H16L3
349	4xGS		369	L3H16L2
350	3xGS		370	L2H16L2
351	2xGS		371	L1H16L2
352	1xGS		372	L3H16L1
353	3xGGGS+GS		373	L2H16L1
354	3xGGGS		374	L1H16L1
355	2xGGGS		375	L3H8L3
356	GGGS		376	L2H8L3
357	L3H24L3		377	L1H8L3
358	L2H24L3		378	L3H8L2
359	L1H24L3		379	L2H8L2
360	L3H24L2		380	L1H8L2
361	L2H24L2		381	L3H8L1
362	L1H24L2		382	L2H8L1
363	L3H24L1		383	L1H8L1
364	L2H24L1			

Orientation of Elements of Polypeptides

[0120] In some embodiments, the bifunctional polypeptide or the dual-specific bifunctional polypeptide disclosed above and herein comprises a first targeting moiety, an optional second targeting moiety, and a URD. The orientation of the URD and SRD of exemplary proteins from which URDs can be derived is provided in Table 23, with “URD-SRD” indicating the URD is upstream of the SRD, and “SRD-URD” indicating the SRD is upstream of the URD, when viewed in the standard N- to C-terminal direction. In some embodiments, the orientation of the first and/or second targeting moiety relative to the URD is the same orientation as compared to the naturally occurring substrate recognition domain (SRD) of the protein from

which the URD is derived. In some embodiments, the orientation of the first and/or second targeting moiety relative to the URD is the opposite orientation as compared to the naturally occurring substrate recognition domain of the protein from which the URD is derived. In some embodiments, the first and/or second targeting moiety is N-terminal relative to the URD. In some embodiments, the first and/or second targeting moiety is C-terminal relative to the URD. In some embodiments of a dual-specific bifunctional polypeptide, both the first and the second targeting moiety are N-terminal relative to the URD. In some embodiments, a dual-specific bifunctional polypeptide, both the first and the second targeting moiety are C-terminal relative to the URD.

Tag peptides

[0121] In some embodiments, the bifunctional polypeptide or the dual-specific bifunctional polypeptide disclosed above and herein further comprises an epitope tag. In some embodiments, the epitope tag is a hemagglutinin (HA) tag. In some embodiments, the HA tag comprises or consists of the amino acid sequence of SEQ ID NO: 384. In some embodiments, the bifunctional polypeptide or dual-specific bifunctional polypeptide does not comprise an epitope tag. In some embodiments, the bifunctional polypeptide or dual-specific bifunctional polypeptide does not comprise a hemagglutinin (HA) tag. In some embodiments, the bifunctional polypeptide or dual-specific bifunctional polypeptide does not comprise an HA tag comprising or consisting of the amino acid sequence of SEQ ID NO: 384. In some embodiments, the epitope tag can be used to visualize the bifunctional polypeptide or dual-specific bifunctional polypeptide. Typically, bifunctional polypeptides or dual-specific bifunctional polypeptides comprising an epitope tag will be used for experimentation and visualization purposes. In some embodiments, the bifunctional polypeptide or dual-specific bifunctional polypeptides intended for treatment do not have an epitope tag. In some embodiments, the bifunctional polypeptide or dual-specific bifunctional polypeptides intended for treatment have an epitope tag.

Overall size of the bifunctional and dual-specific bifunctional polypeptides

[0122] In some embodiments, the bifunctional polypeptide is less than or equal to 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, or 800 amino acids long, or a range defined by any two of the preceding values, optionally 50-800, 50-650, 50-500, 50-400, 70-800, 70-650, 70-500, 70-400,

70-300, 70-200, 80-800, 80-650, 80-500, 80-400, 80-300, or 80-200 amino acids long, optionally 90-550, 150-550, or 80-450 amino acids long. In some embodiments, the dual-specific bifunctional polypeptide is, is about, is at least, is at least about, is not more than, or is not more than about, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950, 1000, 1050, 1100, or 1150 amino acids long, or a range defined by any two of the preceding values, for example, 60-1150 amino acids, 60-800 amino acids, 60-400 amino acids, 400-1150 amino acids, 400-800 amino acids, 600-1150 amino acids, or 100-700 amino acids long, optionally 120-400 or 130-370 amino acids long.

Binding characteristics

[0123] In some embodiments, the first and/or second targeting moiety and/or dual-specific bifunctional polypeptide of the present disclosure have an equilibrium dissociation constant (K_d) of less (that is superior binding) than about 10^{-7} or 10^{-8} M, for example, less than about 10^{-9} M or 10^{-10} M, in some embodiments, less than about 10^{-11} M, 10^{-12} M, or 10^{-13} M. In some embodiments, the first and/or second targeting moiety comprises or consists of a sdAb that has a K_d value of less than 1×10^{-7} and optionally at least 1×10^{-10} . In some embodiments, the K_d is 1.22×10^{-7} to 9.47×10^{-9} . In some embodiments, the first and/or second targeting moiety and/or dual-specific bifunctional polypeptide of the present disclosure have an association rate constant of at least (that is superior binding rate) about 10^{+2} or 10^{+3} 1/Ms, for example, at least about 10^{+5} 1/Ms or 10^{+6} 1/Ms, in some embodiments, at least about 10^{+7} 1/Ms, 10^{+8} 1/Ms, or 10^{+9} 1/Ms. In some embodiments, the first and/or second targeting moiety comprises or consists of a sdAb that has a k_{on} value of at least $1 \times 10^{+4}$ 1/Ms and optionally at least $1 \times 10^{+5}$ 1/Ms, optionally wherein the k_{on} is $6.79 \times 10^{+4}$ 1/Ms to $4.04 \times 10^{+5}$ 1/Ms. In some embodiments, the first and/or second targeting moiety and/or dual-specific bifunctional polypeptide of the present disclosure have a dissociation rate constant of less (that is superior binding) than about 10^{-1} or 10^{-2} 1/s, for example, less than about 10^{-3} 1/s or 10^{-5} 1/s, in some embodiments, less than about 10^{-6} 1/s, 10^{-7} 1/s, or 10^{-8} 1/s. In some embodiments, the first and/or second targeting moiety comprises or consists of a sdAb that has a k_{off} value of less than 1×10^{-2} and optionally less than 1×10^{-4} , optionally wherein the k_{off} is 1.74×10^{-2} to 8.67×10^{-4} . In some embodiments, any of the preceding values (e.g., K_d , k_{on} , k_{off}) is calculated using human c-Myc, optionally as described for values of Octet sdAb K_d Human Myc Peptide Monovalent (M) in Example 4. In some embodiments, first and/or second targeting moiety and/or dual-specific bifunctional

polypeptide has K_d , k_{on} , and/or k_{off} value selected from a value in Table 36, or a range defined by any two of the values presented therein for a given parameter. In some embodiments, the first and/or second targeting moiety binds selectively to the target protein. In some embodiments, the first and/or second targeting moiety binds specifically to the target protein.

Exemplary Polypeptides Defined by Sequence

[0124] Disclosed herein are embodiments of polypeptides comprising or consisting of an amino acid sequence having at least 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to 184, 192, 200, 208, 216, 224, 232, 240, 248, 256, 264, 272, 280, 288, 296, and 385-577. In some embodiments, the polypeptide or dual-specific bifunctional polypeptide is a non-natural polypeptide. In some embodiments, the % sequence identity is calculated over the entirety of the reference sequence.

Modifications

[0125] In some embodiments of the polypeptide or dual-specific bifunctional polypeptide disclosed herein wherein the amino acid sequence differs from the recited reference sequence the difference is the result of the inclusion of one or more modifications relative to the reference sequence. In some embodiments, the one or more modifications comprise a substitution, insertion and/or deletion. In some embodiments, the substitution is a conservative substitution. In some embodiments, the % sequence identity is calculated over the entirety of the reference sequence.

Exemplary Polynucleotides

[0126] Also disclosed herein in some embodiments, are polynucleotides encoding for a polypeptide having at least 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to any of polypeptides, anti-Myc sdAbs, bifunctional polypeptides, or the dual-specific bifunctional polypeptides disclosed herein. In some embodiments, the polynucleotide is a DNA or an RNA. In some embodiments, the RNA is an mRNA or a circular RNA. In some embodiments, the polynucleotide is packaged in a lipid nanoparticle or a viral vector. In some embodiments, the viral vector is an adenovirus, adeno-associated virus, lentivirus, or retrovirus vector. In some embodiments, the % sequence identity is calculated over the entirety of the reference sequence.

[0127] In some embodiments, the DNA the RNA, mRNA or circular RNA comprises one or more modified nucleosides. In some embodiments, up to 100% of the nucleotides comprising uracil are replaced with pseudouridine and/or N1-methylpseudouridine. In some embodiments, the polynucleotide is packaged in a lipid nanoparticle, a polymeric nanoparticle, an extracellular vesicle, optionally an exosome, or a viral vector, optionally a replicating viral vector or a non-replicating viral vector, optionally an adenovirus, adeno-associated virus, lentivirus, or retrovirus vector. Methods of making lipid nanoparticles for RNA delivery are known in the art. In some embodiments, the lipid nanoparticle is made from: an ionizable cationic lipid, a PEGylated lipid, a phospholipid, and cholesterol. In some embodiments, a neutral ionizable amino lipid is used. Lipid nanoparticles for RNA delivery are disclosed in, for example: US11338044B2; US20180153822A1; US20220249699A1; WO2022251953A1; Mitchell *et al.*, “Engineering precision nanoparticles for drug delivery,” *Nature Rev. Drug Discovery* (2021) 20:101-124; Kulkarni *et al.*, “Lipid Nanoparticles Enabling Gene Therapies: From Concepts to Clinical Utility,” *Nucleic Acid Therapeutics* (2018) 28(3):146-157; Hou *et al.* “Lipid nanoparticles for mRNA delivery,” *Nature Reviews Materials* (2021) 6:1078-1094; each of which is incorporated herein by reference in its entirety.

Exemplary Pharmaceutical Compositions, Uses, and Methods

[0128] Also disclosed herein in some embodiments are pharmaceutical compositions comprising any of the polypeptides, anti-Myc sdAbs, bifunctional polypeptides, dual-specific bifunctional polypeptides and/or the polynucleotides disclosed herein and one or more pharmaceutically acceptable excipients, carriers, or diluents. Disclosed herein are pharmaceutical compositions comprising any of the polypeptides, anti-Myc sdAbs, bifunctional polypeptides, dual-specific bifunctional polypeptides and/or the polynucleotides disclosed herein and one or more pharmaceutically acceptable excipients, carriers, or diluents. In some embodiments, the composition is formulated for intravenous, intraperitoneal, intra-arterial, subcutaneous, intramuscular, intrathecal, intratumoral, inhalation, or intracranial administration.

[0129] In some embodiments, the anti-Myc sdAb, bifunctional polypeptide, dual-specific bifunctional polypeptide, polypeptide, polynucleotide, or pharmaceutical composition disclosed herein is for use in the treatment of a cancer in a patient in need thereof. In some embodiments, the anti-Myc sdAb, bifunctional polypeptide, dual-specific bifunctional polypeptide, polypeptide, polynucleotide, or pharmaceutical composition disclosed herein is

for use in the manufacture of a medicament. In some embodiments, the medicament is for the treatment of cancer.

[0130] Disclosed herein in some embodiments, is a method of treating a subject comprising administering the anti-Myc sdAb, bifunctional polypeptide, dual-specific bifunctional polypeptide, polypeptide, polynucleotide, or pharmaceutical composition disclosed herein to a subject in need thereof. In some embodiments, the subject has a cancer.

[0131] Disclosed herein in some embodiments, is a method of reducing the amount of a target protein in a cell comprising contacting the cell with the anti-Myc sdAb, bifunctional polypeptide, dual-specific bifunctional polypeptide, polypeptide, polynucleotide, or pharmaceutical composition disclosed herein. In some embodiments, the target protein is selected from Myc, (optionally c-Myc), CTNNB1, and/or PCNA. In some embodiments, the cell is in a subject, and the contacting comprises administering the anti-Myc sdAb, bifunctional polypeptide, dual-specific bifunctional polypeptide, polypeptide, polynucleotide, or pharmaceutical composition to the subject. In some embodiments, the subject has cancer. In some embodiments, the administering is intravenous, intraperitoneal, intra-arterial, subcutaneous, intramuscular, intrathecal, intra-tumoral, or intracranial administration. In some embodiments, the cell is *ex vivo*, and said contacting comprises contacting the polypeptide comprising or consisting of the anti-Myc sdAb, bifunctional polypeptide, dual-specific bifunctional polypeptide, polypeptide, polynucleotide, or pharmaceutical composition to the cell *ex vivo*, optionally in an adoptive cell therapy. In some embodiments, the method further comprises administering the cell to a subject after the contacting step, optionally wherein the subject is also the source of the cell.

[0132] In some embodiments, in the polypeptide comprising or consisting of the anti-Myc sdAb, bifunctional polypeptide, dual-specific bifunctional polypeptide, polypeptide, polynucleotide, or pharmaceutical composition or method disclosed herein the bifunctional polypeptide, or dual-specific bifunctional polypeptide promotes proteasome-mediated degradation of a target protein when measured in one or more assays described in and Exemplary Assay for assessing proteasomal-mediated degradation of target protein disclosed herein. In some embodiments, the assay is a HiBiT™ tag assay. In some embodiments, the assay is a Western blot, optionally wherein the proteasome-mediated degradation is inhibited when the cells comprising the target protein and the bifunctional polypeptide or dual-specific bifunctional polypeptide are treated with a proteasome inhibitor. In some embodiments, the treatment with the proteasome inhibitor reduces the amount of degradation of the target protein

by at least 50%, 60%, 70%, 80%, 90%, 95% or 100% as compared to cells not treated with the proteasome inhibitor.

Exemplary Assays for assessing proteasomal-mediated degradation of target protein(s)

[0133] The following is an embodiment of exemplary assays for determining if a bifunctional polypeptide or dual-specific bifunctional polypeptide promotes proteasomal-mediated degradation of a target protein(s) and therefore that the URD retains ubiquitin-proteasome recruiting activity, and/or that the targeting moiety binds the target protein.

[0134] As used herein, a URD is a domain derived from a ubiquitin-proteasome system component that has the physical property of recruiting the ubiquitin-proteasome system machinery and the functional property of enabling the polyubiquitination and degradation of the recruited target(s) of interest.

[0135] In an exemplary screening assay, the target(s) of interest (either endogenous and/or exogenous target) is engineered to harbor the HiBiT™ tag (Promega) in a relevant cell line. The cell line is transfected with a plasmid encoding the bifunctional polypeptide, optionally dual-specific, designed to promote proteasome-mediated degradation of the target(s) of interest. 24 hours post transfection, doxycycline is added to induce expression of the bifunctional polypeptide. The HiBiT™ assay is performed the next day according to manufacturer instructions. Loss of HiBiT™ signal is indicative that the bifunctional polypeptide promotes proteasome-mediated degradation of the target(s), and that the URD retains ubiquitin-proteasome recruiting activity and/or that the targeting moiety binds the target. A construct that leads to loss of HiBiT™ signal can be subjected to further confirmation in the assay described below. Additional information regarding the HiBiT™ system can be found in Dixon et al. NanoLuc Complementation Reporter Optimized for Accurate Measurement of Protein Interactions in Cells. ACS Chem Biol. (2016) 11(2):400-8, which is hereby expressly incorporated by reference in its entirety.

[0136] An exemplary assay for confirmation of proteasome-mediated degradation activity of the bifunctional polypeptide, optionally dual-specific, is described. This assay can be used in addition to the assay described above, or independent of the above assay. After a suitable time to allow proteasome-mediated degradation of the target(s) of interest (e.g., 24 to 72 hours), lysate of cells expressing the target(s) of interest and the bifunctional polypeptide of interest, and control cells that express the target(s) of interest but not the bifunctional polypeptide of interest, are subjected to SDS-PAGE, transferred onto a suitable membrane, and probed with

antibodies that are specific and selective for the target(s) of interest (Western Blot). Loss of target protein is indicative that the bifunctional polypeptide promotes proteasome-mediated degradation of the target, and that the URD retains ubiquitin-proteasome recruiting activity. In some embodiments, to demonstrate that the target loss is proteasome dependent, cells expressing the target and the bifunctional polypeptide of interest are treated with a proteasome inhibitor such as bortezomib, MG-132, or lactacystin. The cells expressing the target(s) and the bifunctional polypeptide of interest in the presence of the proteasome inhibitor will show a partial (e.g., at least 50%, 60%, 70%, 80%, 90%, or 95%) or total (100%) reduction in amount of degradation as compared to cells not treated with the proteasome inhibitor, which indicates that the loss of the target is proteasome-dependent, which is indicative that the bifunctional polypeptide promotes proteasome-mediated degradation of the target(s), and that the URD retains ubiquitin-proteasome recruiting activity. For example, if the cells expressing the target(s) and the bifunctional polypeptide of interest in the absence of a proteasome inhibitor reduce the target protein by 90% relative to control cells that express the target(s) of interest but not the bifunctional polypeptide of interest, but only by 9% in the presence of a proteasome inhibitor, the proteasome inhibitor reduced the amount of degradation by 90% $((90\%-9\%)/90\% = 90\%)$.

[0137] Additional target proteins, target binding domains, linker sequences, and other disclosures related to components, features, uses, etc. of bispecific polypeptides are disclosed in PCT/US2023/066619, filed May 4, 2023, and PCT/US2023/76886, filed October 13, 2023, each of which is herein incorporated by reference in its entirety.

Terms

[0138] In the following detailed description, reference is made to the accompanying drawings, which form a part hereof. In the drawings, similar symbols typically identify similar components, unless context dictates otherwise. The illustrative embodiments described in the detailed description, drawings, and claims are not meant to be limiting. Other embodiments may be utilized, and other changes may be made, without departing from the spirit or scope of the subject matter presented herein. It will be readily understood that the aspects of the present disclosure, as generally described herein, and illustrated in the Figures, can be arranged, substituted, combined, separated, and designed in a wide variety of different configurations, all of which are explicitly contemplated herein.

[0139] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as is commonly understood by one of skill in the art to which the claimed subject matter belongs when read in light of the current disclosure. It is to be understood that the foregoing general description and the following detailed description are exemplary and explanatory only and are not restrictive of any subject matter claimed.

[0140] The section headings used herein are for organizational purposes only and are not to be construed as limiting the subject matter described.

[0141] The articles “a” and “an” are used herein to refer to one or to more than one (for example, at least one) of the grammatical object of the article. By way of example, “an element” means one element or more than one element.

[0142] By “about” is meant a quantity, level, value, number, frequency, percentage, dimension, size, amount, weight or length that is approximately the recited value, which can vary by as much as 30, 25, 20, 15, 10, 9, 8, 7, 6, 5, 4, 3, 2 or 1% of the recited value. Where it is not clear from the context what is encompassed by “about,” it will mean the value recited +/- 10%.

[0143] Throughout this specification, unless the context requires otherwise, the words “comprise,” “comprises,” and “comprising” will be understood to imply the inclusion of a stated step or element or group of steps or elements but not the exclusion of any other step or element or group of steps or elements. By “consisting of” is meant including, and limited to, whatever follows the phrase “consisting of.” Thus, the phrase “consisting of” indicates that the listed elements are required or mandatory, and that no other elements may be present. By “consisting essentially of” is meant including any elements listed after the phrase and limited to other elements that do not interfere with or contribute to the activity or action specified in the disclosure for the listed elements. Thus, the phrase “consisting essentially of” indicates that the listed elements are required or mandatory, but that other elements are optional and may or may not be present depending upon whether or not they materially affect the activity or action of the listed elements.

[0144] The terms “individual”, “subject”, or “patient” as used herein have their plain and ordinary meaning as understood in light of the specification, and mean a human or a non-human mammal, e.g., a dog, a cat, a mouse, a rat, a cow, a sheep, a pig, a goat, a non-human primate, or a bird, e.g., a chicken, as well as any other vertebrate or invertebrate. The term “mammal” is used in its usual biological sense. Thus, it specifically includes, but is not limited to, primates,

including simians (chimpanzees, apes, monkeys) and humans, cattle, horses, sheep, goats, swine, rabbits, dogs, cats, rodents, rats, mice, guinea pigs, or the like.

[0145] As used herein, the term “isolated” has its plain and ordinary meaning as understood in light of the specification, and refers to a substance and/or entity that has been (1) separated from at least some of the components with which it was associated when initially produced (whether in nature and/or in an experimental setting), and/or (2) produced, prepared, and/or manufactured by the hand of man. Isolated substances and/or entities may be separated from equal to, about, at least, at least about, not more than, or not more than about, 10%, about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90%, about 95%, about 98%, about 99%, substantially 100%, or 100% of the other components with which they were initially associated (or ranges including and/or spanning the aforementioned values). In some embodiments, isolated agents are, are about, are at least, are at least about, are not more than, or are not more than about 80%, about 85%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, substantially 100%, or 100% pure (or ranges including and/or spanning the aforementioned values). As used herein, a substance that is “isolated” may be “pure” (e.g., substantially free of other components). As used herein, the term “isolated cell” may refer to a cell not contained in a multi-cellular organism or tissue.

[0146] As used herein, “in vivo” is given its plain and ordinary meaning as understood in light of the specification and refers to the performance of a method inside living organisms, usually animals, mammals, including humans, and plants, as opposed to a tissue extract or dead organism.

[0147] As used herein, “ex vivo” is given its plain and ordinary meaning as understood in light of the specification and refers to the performance of a method outside a living organism with little alteration of natural conditions.

[0148] As used herein, “in vitro” is given its plain and ordinary meaning as understood in light of the specification and refers to the performance of a method outside of biological conditions, e.g., in a petri dish or test tube.

[0149] The terms “nucleic acid” or “nucleic acid molecule” as used herein have their plain and ordinary meaning as understood in light of the specification, and refer to polynucleotides, such as deoxyribonucleic acid (DNA) or ribonucleic acid (RNA), oligonucleotides, those that appear in a cell naturally, fragments generated by the polymerase chain reaction (PCR), and fragments generated by any of ligation, scission, endonuclease action, and exonuclease action.

Nucleic acid molecules can be composed of monomers that are naturally occurring nucleotides (such as DNA and RNA), or analogs of naturally occurring nucleotides (e.g., enantiomeric forms of naturally occurring nucleotides), or a combination of both. Modified nucleotides can have alterations in sugar moieties and/or in pyrimidine or purine base moieties. Sugar modifications include, for example, replacement of one or more hydroxyl groups with halogens, alkyl groups, amines, and azido groups, or sugars can be functionalized as ethers or esters. Moreover, the entire sugar moiety can be replaced with sterically and electronically similar structures, such as aza-sugars and carbocyclic sugar analogs. Examples of modifications in a base moiety include alkylated purines and pyrimidines, acylated purines or pyrimidines, or other well-known heterocyclic substitutes. Nucleic acid monomers can be linked by phosphodiester bonds or analogs of such linkages. Analogs of phosphodiester linkages include phosphorothioate, phosphorodithioate, phosphoroselenoate, phosphorodiselenoate, phosphoroanilothioate, phosphoranilidate, or phosphoramidate. The term “nucleic acid molecule” also includes so-called “peptide nucleic acids,” which comprise naturally occurring or modified nucleic acid bases attached to a polyamide backbone. Nucleic acids can be either single stranded or double stranded. “Oligonucleotide” can be used interchangeably with nucleic acid and can refer to either double stranded or single stranded DNA or RNA. A nucleic acid or nucleic acids can be contained in a nucleic acid vector or nucleic acid construct (e.g., plasmid, virus, retrovirus, lentivirus, bacteriophage, cosmid, fosmid, phagemid, bacterial artificial chromosome (BAC), yeast artificial chromosome (YAC), or human artificial chromosome (HAC)) that can be used for amplification and/or expression of the nucleic acid or nucleic acids in various biological systems. Typically, the vector or construct will also contain elements including but not limited to promoters, enhancers, terminators, inducers, ribosome binding sites, translation initiation sites, start codons, stop codons, polyadenylation signals, origins of replication, cloning sites, multiple cloning sites, restriction enzyme sites, epitopes, reporter genes, selection markers, antibiotic selection markers, targeting sequences, peptide purification tags, or accessory genes, or any combination thereof.

[0150] A nucleic acid or nucleic acid molecule can comprise one or more sequences encoding different peptides, polypeptides, or proteins. These one or more sequences can be joined in the same nucleic acid or nucleic acid molecule adjacently, or with extra nucleic acids in between, e.g. linkers, repeats or restriction enzyme sites, or any other sequence that is, is about, is at least, is at least about, is not more than, or is not more than about, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10,

11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 150, 200, or 300 bases long, or any length in a range defined by any two of the aforementioned lengths. The term “downstream” on a nucleic acid as used herein has its plain and ordinary meaning as understood in light of the specification and refers to a sequence being after the 3'-end of a previous sequence, on the strand containing the encoding sequence (sense strand) if the nucleic acid is double stranded. The term “upstream” on a nucleic acid as used herein has its plain and ordinary meaning as understood in light of the specification and refers to a sequence being before the 5'-end of a subsequent sequence, on the strand containing the encoding sequence (sense strand) if the nucleic acid is double stranded. The term “grouped” on a nucleic acid as used herein has its plain and ordinary meaning as understood in light of the specification and refers to two or more sequences that occur in proximity either directly or with extra nucleic acids in between, e.g. linkers, repeats, or restriction enzyme sites, or any other sequence that is, is about, is at least, is at least about, is not more than, or is not more than about, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 150, 200, or 300 bases long, or any length in a range defined by any two of the aforementioned lengths, but generally not with a sequence in between that encodes for a functioning or catalytic polypeptide, protein, or protein domain.

[0151] The nucleic acids described herein comprise nucleobases. Primary, canonical, natural, or unmodified bases are adenine, cytosine, guanine, thymine, and uracil. Other nucleobases include but are not limited to purines, pyrimidines, modified nucleobases, 5-methylcytosine, pseudouridine, dihydrouridine, inosine, 7-methylguanosine, hypoxanthine, xanthine, 5,6-dihydrouracil, 5-hydroxymethylcytosine, 5-bromouracil, isoguanine, isocytosine, aminoallyl bases, dye-labeled bases, fluorescent bases, or biotin-labeled bases. In some embodiments, the uridine of the RNA, mRNA or circular RNA is pseudouridine. In some embodiments, the uridine of the RNA, mRNA or circular RNA is N1-methylpseudouridine. In some embodiments, the uracil of the RNA, mRNA or circular RNA is a mixture of pseudouridine and N1-methylpseudouridine. In some embodiments, up to 100% of the nucleotides comprising uracil are replaced with pseudouridine and/or N1-methylpseudouridine.

[0152] The terms “peptide”, “polypeptide”, and “protein” as used herein have their plain and ordinary meaning as understood in light of the specification and refer to macromolecules comprised of amino acids linked by peptide bonds. The numerous functions of peptides, polypeptides, and proteins are known in the art, and include but are not limited to enzymes, structure, transport, defense, hormones, or signaling. Peptides, polypeptides, and proteins are

often, but not always, produced biologically by a ribosomal complex using a nucleic acid template, although chemical syntheses are also available. By manipulating the nucleic acid template, peptide, polypeptide, and protein mutations such as substitutions, deletions, truncations, additions, duplications, or fusions of more than one peptide, polypeptide, or protein can be performed. These fusions of more than one peptide, polypeptide, or protein can be joined in the same molecule adjacently, or with extra amino acids in between, e.g. linkers, repeats, epitopes, or tags, or any other sequence that is, is about, is at least, is at least about, is not more than, or is not more than about, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 150, 200, or 300 bases long, or any length in a range defined by any two of the aforementioned lengths. The term “downstream” on a polypeptide as used herein has its plain and ordinary meaning as understood in light of the specification and refers to a sequence being after the C-terminus of a previous sequence. The term “upstream” on a polypeptide as used herein has its plain and ordinary meaning as understood in light of the specification and refers to a sequence being before the N-terminus of a subsequent sequence.

[0153] A polypeptide or amino acid sequence “derived from” a designated protein refers to the origin of the polypeptide. Preferably, the polypeptide has an amino acid sequence that is essentially identical to that of a polypeptide encoded in the sequence, or a portion thereof, or which is immunologically identifiable with a polypeptide encoded in the sequence. This terminology also includes a polypeptide expressed from a designated nucleic acid sequence. Peptide sequences having at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% homology to any one of the peptide sequences disclosed herein and having the same or similar functional properties are envisioned. The percent homology may be determined according to amino acid substitutions, deletions, or additions between two peptide sequences. Peptide sequences having some percent homology to any one of the peptide sequences disclosed herein may be produced and tested by one skilled in the art through conventional methods.

[0154] The term “artificial” or “non-natural” denotes that the polypeptide or portion of a polypeptide in question has a sequence that is not present, in the noted state, in nature. In the present context the polypeptides have been altered from their native state, so that their sequences are no longer those found in wild-type proteins. Where a portion derived from a naturally occurring protein (e.g., a URD or NLS) is a fragment of the naturally occurring protein which does not contain an altered sequence (other than being less than the complete

sequence of the naturally occurring protein), a “non-natural” polypeptide comprising the derived portion does not encompass the naturally occurring protein from which the portion is derived. That is to say that a “non-natural” polypeptide comprising the portion must contain additional amino acids which are not found in the natural protein.

[0155] As disclosed herein, sequences having a % identity to any of the sequences disclosed herein are envisioned and may be used. The terms “% identity” refer to the percentage of units (i.e., amino acids or nucleotides) that are the same between two or more sequences relative to the length of the sequence. When the two or more sequences being compared are the same length, the % identity will be respective to that length. When two or more sequences being compared are different lengths, deletions and/or insertions may be introduced to obtain the best alignment. In some embodiments, these sequences may include peptide sequences, nucleic acid sequences, CDR sequences, variable region sequences, or heavy or light chain sequences. In some embodiments, any sequence having at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to any of the sequences disclosed herein may be used. In some embodiments, any sequence having at least 0, 1, 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, or 50 substitutions, deletions, or additions relative to any of the sequences disclosed herein may be used. The changes in sequences may apply to, for example, single amino acids, single nucleic acid bases, or nucleic acid codons; however, differences in longer stretches of sequences are also envisioned. As applied to sequences associated with antibodies or binding regions thereof, these differences in sequences may apply to antigen-binding regions (e.g., CDRs) or regions that do not bind to antigens or are only secondary to antigen binding (e.g., framework regions).

[0156] As disclosed herein, sequences having a % homology to any of the sequences disclosed herein are envisioned and may be used. The term “% homology” refers to the degree of conservation between two sequences when considering their three-dimensional structure. For example, homology between two protein sequences may be dependent on structural motifs, such as beta strands, alpha helices, and other folds, as well as their distribution throughout the sequence. Homology may be determined through structural determination, either empirically or *in silico*. In some embodiments, any sequence having at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence homology to any of the sequences disclosed herein may be used. In some

embodiments, any sequence having at least 0, 1, 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, or 50 substitutions, deletions, or additions relative to any of the sequences disclosed herein, which may or may not affect the overall % homology, may be used.

[0157] As applied herein, sequences having a certain % similarity to any of the sequence disclosed herein are envisioned and may be used. In some embodiments, these sequences may include peptide sequences, nucleic acid sequences, CDR sequences, variable region sequences, or heavy or light chain sequences. As understood in the art with respect to peptide sequences, “similarity” refers to the comparison of amino acids based on their properties, including but not limited to size, polarity, charge, pK, aromaticity, hydrogen bonding properties, or presence of functional groups (e.g., hydroxyl, thiol, amine, carboxyl, and the like). The term “% similarity” refers to the percentage of units (e.g., amino acids) that are the same between two or more sequences relative to the length of the sequence. When the two or more sequences being compared are the same length, the % similarity will be respective that length. When two or more sequences being compared are different lengths, deletions and/or insertions may be introduced to obtain the best alignment. The similarity of two amino acids may dictate whether a certain substitution is conservative or non-conservative. Methods of determining the conservativeness of an amino acid substitution are generally known in the art and may involve substitution matrices. Commonly used substitution matrices include BLOSUM45, BLOSUM62, BLOSUM80, PAM100, PAM120, PAM160, PAM200, PAM250, but other substitution matrices or approaches may be used as considered appropriate by the skilled person. A certain substitution matrix may be preferential over the others when considering aspects such as stringency, conservation and/or divergence of related sequences (e.g., within the same species or broader), and length of the sequences in question. As used herein, a peptide sequence having a certain % similarity to another sequence will have up to that % of amino acids that are either identical or an acceptable substitution as governed by the method of similarity determination used. In some embodiments, a sequence having at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence similarity to any of the sequences disclosed herein may be used. In some embodiments, any sequence having at least 0, 1, 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, or 50 similar substitutions relative to any of the sequences disclosed herein may be used. As applied to sequences associated with antibodies or

binding regions thereof, these similar substitutions may apply to antigen-binding regions (i.e., CDRs) or regions that do not bind to antigens or are only secondary to antigen binding (i.e., framework regions). In some embodiments, the percent identity is over the CDR and/or FR regions noted herein. In such situations, the percent identity of the CDR or FR can be identified separately from the rest of the protein or nucleic acid sequence. Thus, two CDRs or FRs can have a specified percentage of amino acid residues or nucleotides that are the same (for example, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% sequence identity over a specified region, or, when not specified, over the entire sequence of a reference sequence), while allowing for the remainder of the protein to either stay 100% identical to the comparison protein, or while also allowing the remainder of the protein to also have variation by a specified percent identity.

[0158] The term “consensus sequence” as used herein with regard to sequences has its plain and ordinary meaning as understood in light of the specification, and refers to the generalized sequence representing all of the different combinations of permissible amino acids at each location of a group of sequences. A consensus sequence may provide insight into the conserved regions of related sequences where the unit (e.g., amino acid or nucleotide) is the same in most or all of the sequences, and regions that exhibit divergence between sequences. In the case of sequences associated with antibodies or binding regions thereof, the consensus sequence of a CDR may indicate amino acids that are important or dispensable for antigen binding. It is envisioned that consensus sequences may be prepared with any of the sequences provided herein, and the resultant various sequences derived from the consensus sequence can be validated to have similar effects as the template sequences.

[0159] The term “purity” of any given substance, compound, or material as used herein has its plain and ordinary meaning as understood in light of the specification and refers to the actual abundance of the substance, compound, or material relative to the expected abundance. For example, the substance, compound, or material may be at least 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100% pure, including all decimals in between. Purity may be affected by unwanted impurities, including but not limited to nucleic acids, DNA, RNA, nucleotides, proteins, polypeptides, peptides, amino acids, lipids, cell membrane, cell debris, small molecules, degradation products, solvent, carrier, vehicle, or contaminants, or any combination thereof. In some embodiments, the substance, compound, or material is substantially free of host cell proteins, host cell nucleic acids, plasmid DNA, contaminating viruses, proteasomes, host cell culture components, process related components, mycoplasma, pyrogens, bacterial

endotoxins, and adventitious agents. Purity can be measured using technologies including but not limited to electrophoresis, SDS-PAGE, capillary electrophoresis, PCR, rtPCR, qPCR, chromatography, liquid chromatography, gas chromatography, thin layer chromatography, enzyme-linked immunosorbent assay (ELISA), spectroscopy, UV-visible spectrometry, infrared spectrometry, mass spectrometry, nuclear magnetic resonance, gravimetry, or titration, or any combination thereof.

[0160] The term “yield” of any given substance, compound, or material as used herein has its plain and ordinary meaning as understood in light of the specification and refers to the actual overall amount of the substance, compound, or material relative to the expected overall amount. For example, the yield of the substance, compound, or material is, is about, is at least, is at least about, is not more than, or is not more than about, 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100% of the expected overall amount, including all decimals in between. Yield may be affected by the efficiency of a reaction or process, unwanted side reactions, degradation, quality of the input substances, compounds, or materials, or loss of the desired substance, compound, or material during any step of the production.

[0161] The terms “effective amount” or “effective dose” as used herein have their plain and ordinary meaning as understood in light of the specification, and refer to that amount of a recited composition or compound that results in an observable effect. Actual dosage levels of active ingredients in an active composition of the presently disclosed subject matter can be varied so as to administer an amount of the active composition or compound that is effective to achieve the desired response for a particular subject and/or application. The selected dosage level will depend upon a variety of factors including, but not limited to, the activity of the composition, formulation, route of administration, combination with other drugs or treatments, severity of the condition being treated, and the physical condition and prior medical history of the subject being treated. In some embodiments, a minimal dose is administered, and dose is escalated in the absence of dose-limiting toxicity to a minimally effective amount. Determination and adjustment of an effective dose, as well as evaluation of when and how to make such adjustments, are contemplated herein.

[0162] The terms “function” and “functional” as used herein have their plain and ordinary meaning as understood in light of the specification, and refer to a biological, enzymatic, or therapeutic function.

[0163] The term “inhibit” as used herein has its plain and ordinary meaning as understood in light of the specification, and may refer to the reduction or prevention of a biological activity.

The reduction can be by a percentage that is, is about, is at least, is at least about, is not more than, or is not more than about, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100%, or an amount that is within a range defined by any two of the aforementioned values. As used herein, the term “delay” has its plain and ordinary meaning as understood in light of the specification, and refers to a slowing, postponement, or deferment of a biological event, to a time which is later than would otherwise be expected. The delay can be a delay of a percentage that is, is about, is at least, is at least about, is not more than, or is not more than about, 0%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, or an amount within a range defined by any two of the aforementioned values. The terms inhibit and delay may not necessarily indicate a 100% inhibition or delay. A partial inhibition or delay may be realized.

[0164] As used herein, the terms “treating” or “treatment” have their plain and ordinary meaning as understood in light of the specification, and refer to an approach for obtaining beneficial or desired results in a subject's condition, including clinical results. Beneficial or desired clinical results can include, but are not limited to, alleviation or amelioration of one or more symptoms or conditions, diminishment of the extent of a disease, stabilizing (i.e., not worsening) the state of disease, prevention of a disease's transmission or spread, delaying or slowing of disease progression, amelioration or palliation of the disease state, diminishment of the recurrence of disease, and remission, whether partial or total and whether detectable or undetectable. “Treating” and “treatment” as used herein also include prophylactic treatment. Treatment methods comprise administering to a subject a therapeutically effective amount of an active agent. The administering step may consist of a single administration or may comprise a series of administrations. The compositions are administered to the subject in an amount and for a duration sufficient to treat the subject. The length of the treatment period depends on a variety of factors, such as the severity of the condition, the age and genetic profile of the subject, the concentration of active agent, the activity of the compositions used in the treatment, or a combination thereof. It will also be appreciated that the effective dosage of an agent used for the treatment or prophylaxis may increase or decrease over the course of a particular treatment or prophylaxis regime. Changes in dosage may result and become apparent by standard diagnostic assays known in the art. In some instances, chronic administration may be required.

[0165] “Tumor,” as used herein, plain and ordinary meaning as understood in light of the specification, and refers to all neoplastic cell growth and proliferation, whether malignant or benign, and all pre-cancerous and cancerous cells and tissues. The terms “cancer,” “cancerous,”

“cell proliferative disorder,” “proliferative disorder” and “tumor” are not mutually exclusive as referred to herein. The term “neoplasia” encompasses the term tumor.

[0166] The terms “cancer” and “cancerous” plain and ordinary meaning as understood in light of the specification, and refer to or describe the physiological condition in mammals that is typically characterized by unregulated cell growth. Examples of cancer include, but are not limited to, carcinoma, lymphoma, blastoma, sarcoma, and leukemia or lymphoid malignancies. More particular examples of such cancers include lung cancer including small-cell lung cancer, non-small cell lung cancer and lung adenocarcinomas with neuroendocrine features; neuroendocrine prostate cancer, melanoma, gliomas, low-grade gliomas and glioblastoma, medullary thyroid cancer, carcinoid tumors, neuroendocrine tumors in the pancreas, bladder cancer, testicular cancer squamous cell cancer (e.g. epithelial squamous cell cancer), neuroendocrine neoplasms, such as neuroendocrine tumors of unknown primary, neuroendocrine neoplasms of the small bowel, carotid body, adrenal gland, colorectal gynecological organ, abdomen, esophagus, GI tract, bile duct, nervous system, appendix, liver, anal, thymus, ileocecal junction, head and neck, breast, peritoneum and retroperitoneum, kidney, thyroid, stomach, bone,; adenocarcinomas, such as adenocarcinoma of the lung and squamous carcinoma of the lung, cancer of the peritoneum, hepatocellular cancer, gastric or stomach cancer including gastrointestinal cancer, pancreatic cancer, glioblastoma, cervical cancer, ovarian cancer, liver cancer, bladder cancer, cancer of the urinary tract, hepatoma, breast cancer, colon cancer, rectal cancer, colorectal cancer, endometrial or uterine carcinoma, salivary gland carcinoma, kidney or renal cancer, prostate cancer, vulval cancer, thyroid cancer, bone cancer, hepatic carcinoma, anal carcinoma, penile carcinoma, melanoma, multiple myeloma and B-cell lymphoma, brain, as well as head and neck cancer, and associated metastases. The term cancer includes adult and pediatric solid cancers. In some embodiments, the cancer can be a solid tumor. In some embodiments, the cancer is a highly fibrotic tumor or cancer. In some embodiments, the cancer is a desmoplasia.

[0167] As used herein, the term “therapeutic target” refers to a gene or gene product that, upon modulation of its activity (e.g., by modulation of expression, biological activity, and the like), can provide for modulation of the disease phenotype. As used throughout, “modulation” is meant to refer to an increase or a decrease in the indicated phenomenon (e.g., modulation of a biological activity refers to an increase in a biological activity or a decrease in a biological activity).

[0168] The term “administering” includes oral administration, topical contact, administration as a suppository, intravenous, intraperitoneal, intramuscular, intralesional, intra-tumoral, intrathecal, intranasal, or subcutaneous administration, or the implantation of a slow-release device, e.g., a mini-osmotic pump, to a subject. Administration is by any route, including parenteral and transmucosal (e.g., buccal, sublingual, palatal, gingival, nasal, vaginal, rectal, or transdermal). Parenteral administration includes, e.g., intravenous, intramuscular, intra-arteriole, intradermal, subcutaneous, intraperitoneal, intra-tumoral, intraventricular, and intracranial. Other modes of delivery include, but are not limited to, the use of liposomal formulations, intravenous infusion, transdermal patches, etc. By “co-administer” it is meant that a first compound described herein is administered at the same time, just prior to, or just after the administration of a second compound described herein.

[0169] As used herein, “pharmaceutically acceptable” has its plain and ordinary meaning as understood in light of the specification and refers to carriers, excipients, and/or stabilizers that are nontoxic to the cell or mammal being exposed thereto at the dosages and concentrations employed or that have an acceptable level of toxicity. A “pharmaceutically acceptable” “diluent,” “excipient,” and/or “carrier” as used herein have their plain and ordinary meaning as understood in light of the specification and are intended to include any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like, compatible with administration to humans, cats, dogs, or other vertebrate hosts. Typically, a pharmaceutically acceptable diluent, excipient, and/or carrier is a diluent, excipient, and/or carrier approved by a regulatory agency of a Federal, a state government, or other regulatory agency, or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, including humans as well as non-human mammals, such as cats and dogs. The term diluent, excipient, and/or carrier can refer to a diluent, adjuvant, excipient, or vehicle with which the pharmaceutical formulation is administered. Such pharmaceutical diluent, excipient, and/or carriers can be sterile liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin. Water, saline solutions and aqueous dextrose and glycerol solutions can be employed as liquid diluents, excipients, and/or carriers, particularly for injectable solutions. Suitable pharmaceutical diluents and/or excipients include sugars, starch, glucose, fructose, lactose, sucrose, maltose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, salts, sodium chloride, dried skim milk, glycerol, propylene, glycol, water, ethanol and the like. A non-limiting example of a physiologically acceptable carrier is an

aqueous pH buffered solution. The physiologically acceptable carrier may also comprise one or more of the following: antioxidants, such as ascorbic acid, low molecular weight (less than about 10 residues) polypeptides, proteins, such as serum albumin, gelatin, immunoglobulins, hydrophilic polymers such as polyvinylpyrrolidone, amino acids, carbohydrates such as glucose, mannose, or dextrans, chelating agents such as EDTA, sugar alcohols such as glycerol, erythritol, threitol, arabitol, xylitol, ribitol, mannitol, sorbitol, galactitol, fucitol, iditol, inositol, isomalt, maltitol, or lactitol, salt-forming counterions such as sodium, and nonionic surfactants such as TWEEN®, polyethylene glycol (PEG), and PLURONICS®. The formulation, if desired, can also contain minor amounts of wetting, bulking, emulsifying agents, or pH buffering agents. These formulations can take the form of solutions, suspensions, emulsion, sustained release formulations and the like. The formulation should suit the mode of administration.

[0170] Additional excipients with desirable properties include but are not limited to preservatives, adjuvants, stabilizers, solvents, buffers, diluents, solubilizing agents, detergents, surfactants, chelating agents, antioxidants, alcohols, ketones, aldehydes, ethylenediaminetetraacetic acid (EDTA), citric acid, salts, sodium chloride, sodium bicarbonate, sodium phosphate, sodium borate, sodium citrate, potassium chloride, potassium phosphate, magnesium sulfate sugars, dextrose, fructose, mannose, lactose, galactose, sucrose, sorbitol, mannitol, cellulose, serum, amino acids, alanine, arginine, asparagine, aspartic acid, cysteine, glutamine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, valine, polysorbate 20, polysorbate 40, polysorbate 60, polysorbate 80, sodium deoxycholate, sodium taurodeoxycholate, magnesium stearate, octylphenol ethoxylate, benzethonium chloride, thimerosal, gelatin, esters, ethers, 2-phenoxyethanol, urea, or vitamins, or any combination thereof. Some excipients may be in residual amounts or contaminants from the process of manufacturing, including but not limited to serum, albumin, ovalbumin, antibiotics, inactivating agents, formaldehyde, glutaraldehyde, β -propiolactone, gelatin, cell debris, nucleic acids, peptides, amino acids, or growth medium components or any combination thereof. The amount of the excipient may be found in the formulation at a percentage that is, is about, is at least, is at least about, is not more than, or is not more than about, 0%, 0.1%, 0.2%, 0.3%, 0.4%, 0.5%, 0.6%, 0.7%, 0.8%, 0.9%, 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 100% w/w or any percentage by weight in a range defined by any two of the aforementioned numbers.

[0171] The term “pharmaceutically acceptable salts” has its plain and ordinary meaning as understood in light of the specification and includes relatively non-toxic, inorganic and organic acid, or base addition salts of compositions or excipients, including without limitation, analgesic agents, therapeutic agents, other materials, and the like. Examples of pharmaceutically acceptable salts include those derived from mineral acids, such as hydrochloric acid and sulfuric acid, and those derived from organic acids, such as ethanesulfonic acid, benzenesulfonic acid, p-toluenesulfonic acid, and the like. Examples of suitable inorganic bases for the formation of salts include the hydroxides, carbonates, and bicarbonates of ammonia, sodium, lithium, potassium, calcium, magnesium, aluminum, zinc, and the like. Salts may also be formed with suitable organic bases, including those that are non-toxic and strong enough to form such salts. For example, the class of such organic bases may include but are not limited to mono-, di-, and trialkylamines, including methylamine, dimethylamine, and triethylamine; mono-, di-, or trihydroxyalkylamines including mono-, di-, and triethanolamine; amino acids, including glycine, arginine and lysine; guanidine; N-methylglucosamine; N-methylglucamine; L-glutamine; N-methylpiperazine; morpholine; ethylenediamine; N-benzylphenethylamine; trihydroxymethyl aminoethane.

[0172] The term “ubiquitin-proteasome recruiting activity” as used herein has its plain and ordinary meaning as understood in light of the specification and refers to the biological function of binding with proteins involved in the ubiquitination pathway to affect the ubiquitination of a target protein. More specifically in the embodiments disclosed herein, this generally applies to the ability of a peptide to recruit a ubiquitinated E2 ubiquitin-conjugating enzyme and catalyze the transfer of the ubiquitin from the E2 ubiquitin-conjugate enzyme to the target protein. The peptide having this ubiquitin-proteasome recruiting activity will therefore generally be a “ubiquitin-proteasome system recruiting domain (URD)”, where the URD has the ability to recruit a ubiquitin E2 ubiquitin-conjugating enzyme and catalyze the transfer of the ubiquitin from the E2 ubiquitin-conjugate enzyme to the target protein. In some embodiments provided herein, the URD is a portion, truncation, or fragment of a naturally occurring E3 ubiquitin ligase, where the portion, truncation, or fragment of the E3 ubiquitin ligase exhibits the ubiquitin-proteasome recruiting activity. The E3 ubiquitin ligase can be obtained from any source (e.g., from any eukaryote or archaea) but, in some embodiments, is preferably a human E3 ubiquitin ligase. Nevertheless, the URD may also be derived from a protein that is not a E3 ubiquitin ligase but has a similar function, such as a viral analogue of an E3 ubiquitin ligase (e.g., VIF from HIV). Different portions, truncations, or fragments of an

E3 ubiquitin ligase or analogue may exhibit a ubiquitin-proteasome recruiting activity, and it would be within the capabilities of a skilled person to determine a certain portion, truncation, or fragment of an E3 ubiquitin ligase or analogue that is preferable for their uses (e.g., exhibiting better E2 ubiquitin-conjugating enzyme binding activity, reducing off-target or off-mechanism protein-protein interactions, and/or more efficiently catalyzing ubiquitination of a target protein). It is envisioned that the URDs or the portions, truncations, or fragments of the URDs and/or E3 ubiquitin ligases disclosed herein can be reasonably modified while still retaining their ubiquitin-proteasome recruiting activity, for example, by including or excluding amino acids from the N-terminus or C-terminus of the URD or the portion, truncation, or fragment of the URD and/or E3 ubiquitin ligase or performing amino acid substitutions to enhance various aspects of the URD and/or the portion, truncation, or fragment of the URD and/or E3 ubiquitin ligase such as protein folding, expression, or catalytic activity, optionally within the context of the dual-specific bifunctional polypeptide.

[0173] The ubiquitin-proteasome recruiting activity of a certain URD and/or portion, truncation, or fragment of a URD and/or E3 ubiquitin ligase may be quantified or verified through methods generally known in the art. For example, activity may be observed by observing (a) loss of bioluminescent signal from a HiBiT™-tagged intracellular target protein, (b) loss of target protein signature and/or presence of polyubiquitin chain in target protein by mass spectrometry, (c) ubiquitination of the target protein in cell or in recombinant in vitro assays, or (d) detecting ubiquitination of target protein by immunoprecipitation, upon introduction of a URD-containing polypeptide specific for a known target protein. These exemplary approaches are explored in Franklin et al. A High-Throughput Assay for Monitoring Ubiquitination in Real Time. *Front. Chem.* (2019) 7:816, Choo & Zhang. Detection of protein ubiquitination. *J Vis. Exp.* (2009) 30:1293, Udeshi et al. Large-scale identification of ubiquitination sites by mass spectrometry. *Nat Protoc.* (2013) 8(10):1950-60, and Riching et al. Quantitative Live-Cell Kinetic Degradation and Mechanistic Profiling of PROTAC Mode of Action. *ACS Chem Biol.* (2018) 13(9):2758-2770, each of which are expressly incorporated by reference in its entirety. In some embodiments, a URD that retains ubiquitin-proteasome recruiting activity has polyubiquitination activity against a target bound by its targeting moiety when measured in an assay disclosed in one of the preceding references. In some embodiments, a URD that retains ubiquitin-proteasome recruiting activity has polyubiquitination activity against a target bound by its targeting moiety when measured in an assay described in Exemplary Assays for assessing proteasomal-mediated degradation and/or Example 1. In some

embodiments, a URD that retains ubiquitin-proteasome recruiting activity has polyubiquitination activity against a target bound by its targeting moiety when measured by Western blot (e.g., measuring the abundance of the target in cells). In some embodiments, a URD that retains ubiquitin-proteasome recruiting activity has polyubiquitination activity against a target bound by its targeting moiety when measured by Western blot, but where the target is partially or totally preserved when the cells comprising the URD and target are treated with a proteasome inhibitor.

[0174] The term “antibody” includes, but is not limited to, genetically engineered or otherwise modified forms of immunoglobulins, such as intrabodies, chimeric antibodies, fully human antibodies, humanized antibodies, antibody fragments, scFv, and heteroconjugate antibodies (for example, bispecific antibodies, diabodies, triabodies, tetrabodies, and nanobodies, etc.). The term “antibody” includes minibodies and diabodies. The term “antibody” includes a polypeptide of the immunoglobulin family or a polypeptide comprising fragments of an immunoglobulin that is capable of noncovalently, reversibly, and in a specific manner binding a corresponding antigen. An exemplary antibody structural unit comprises a tetramer. In some embodiments, a full length antibody can be composed of two identical pairs of polypeptide chains, each pair having one “light” and one “heavy” chain (connected through a disulfide bond). The recognized immunoglobulin genes include the kappa, lambda, alpha, gamma, delta, epsilon, hinge, and mu constant region genes, as well as the myriad immunoglobulin variable region genes. For full length chains, the light chains are classified as either kappa or lambda. For full length chains, the heavy chains are classified as gamma, mu, alpha, delta, or epsilon, which in turn define the immunoglobulin classes, IgG, IgM, IgA, IgD, and IgE, respectively. The N-terminus of each chain defines a variable region of about 100 to 110 or more amino acids primarily responsible for antigen recognition. The terms variable light chain (V_L) and variable heavy chain (V_H) refer to these regions of light and heavy chains respectively. As used in this application, an “antibody” encompasses all variations of antibody and fragments thereof. Thus, within the scope of this concept are full length antibodies, chimeric antibodies, humanized antibodies, single chain antibodies (scFv), Fab, Fab', and multimeric versions of these fragments (for example, $F(ab')_2$) with the same binding specificity. In some embodiments, the antibody binds specifically to a desired target.

[0175] The term "complementarity-determining domains" or "complementarity-determining regions ("CDRs") interchangeably refer to the hypervariable regions of V_L and V_H . The CDRs are the target molecule-binding site of the antibody chains that harbors specificity for such

target molecule. In some embodiments, there are three CDRs (CDR1-3, numbered sequentially from the N-terminus) in each V_L and/or V_H, constituting about 15-20% of the variable domains. The CDRs are structurally complementary to the epitope of the target molecule and are thus directly responsible for the binding specificity. The remaining stretches of the V_L or V_H, the so-called FRs, exhibit less variation in amino acid sequence (Kuby, Immunology, 4th ed., Chapter 4. W.H. Freeman & Co., New York, 2000).

[0176] The positions of the CDRs and framework regions can be determined using various well known definitions in the art. In some embodiments, the polypeptide is numbered from the beginning of the polypeptide signal sequence. In some embodiments, the polypeptide is numbered according from the beginning of the polypeptide and not including the signal sequence.

[0177] An "antibody variable light chain" or an "antibody variable heavy chain" as used herein have their plain and ordinary meaning as understood in light of the specification, and refers to a polypeptide comprising the V_L or V_H, respectively. The endogenous V_L is encoded by the gene segments V (variable) and J (junctional), and the endogenous V_H by V, D (diversity), and J. Each of V_L or V_H includes the CDRs as well as the framework regions. In this application, antibody variable light chains and/or antibody variable heavy chains may, from time to time, be collectively referred to as "antibody chains." These terms encompass antibody chains containing mutations that do not disrupt the basic structure of V_L or V_H, as one skilled in the art will readily recognize. In some embodiments, full length heavy and/or light chains are contemplated. In some embodiments, only the variable region of the heavy and/or light chains are contemplated as being present.

[0178] Antibodies can exist as intact immunoglobulins or as a number of fragments produced by digestion with various peptidases as is known in the art. While various antibody fragments are defined in terms of the digestion of an intact antibody, one of skill will appreciate that such fragments may be synthesized *de novo* either chemically or by using recombinant DNA methodology. Thus, the term "antibody," as used herein, has its plain and ordinary meaning as understood in light of the specification, and also includes antibody fragments either produced by the modification of whole antibodies, or those synthesized *de novo* using recombinant DNA methodologies (for example, single chain Fv) or those identified using phage display libraries (see, for example, McCafferty, J. et al., "Phage antibodies: filamentous phage displaying antibody variable domains," Nature, Vol. 348, No. 66301, pp. 552-554, 1990). For preparation of monoclonal or polyclonal antibodies, any technique known in the art can be used.

Techniques for the production of single chain antibodies (U.S. Pat. No. 4,946,778) can be adapted to produce antibodies to polypeptides of the present disclosure. Also, transgenic mice, or other organisms such as other mammals, may be used to express fully human monoclonal antibodies. Furthermore, *E. Coli* or yeast may be used to express and manufacture recombinant antibodies and antibody fragments. Alternatively, phage display and yeast display technologies can be used to identify high affinity binders to selected antigens. Alternatively, antibodies can be produced through B-cell screening technologies from human hosts (Pedrioli A., Oxenius A., Single B cell technologies for monoclonal antibody discovery, Trends in Immunology, (2021) volume 42, issue 12, pp. 1143-1158). Furthermore, antibodies can be derived from immunization of camelid animals or screening of camelid phage libraries.

[0179] Methods for humanizing or primatizing non-human antibodies are well known in the art. Generally, a humanized antibody has one or more amino acid residues introduced into it from a source which is non-human. These non-human amino acid residues are often referred to as import residues, which are typically taken from an import variable domain. In some embodiments, the terms “donor” and “acceptor” sequences can be employed. Humanization can be essentially performed following the method of Winter and co-workers by substituting rodent CDRs or CDR sequences for the corresponding sequences of a human antibody. Accordingly, such humanized antibodies are chimeric antibodies (U.S. Pat. No. 4,816,567), wherein substantially less than an intact human variable domain has been substituted by the corresponding sequence from a non-human species. In practice, humanized antibodies are typically human antibodies in which some complementarity determining region ("CDR") residues and possibly some framework ("FR") residues are substituted by residues from analogous sites in rodent antibodies.

[0180] The term “antibody fragment” includes but is not limited to one or more antigen binding fragments of antibodies alone or in combination with other molecules, including, but not limited to Fab', F(ab')₂, Fab, Fv, rIgG (reduced IgG), scFv fragments, single domain fragments (nanobodies), peptibodies, nanobodies), minibodies, and diabodies. The term “scFv” refers to a single chain Fv (“fragment variable”) antibody in which the variable domains of the heavy chain and of the light chain of a traditional two chain antibody have been joined to form one chain.

[0181] A "diabody" comprises a first polypeptide chain which comprises a heavy (VH) chain variable domain connected to a light chain variable domain (VL) on the first polypeptide chain (VH-VL) connected by a peptide linker that is too short to allow pairing between the two

domains on the first polypeptide chain and a second polypeptide chain comprising a light chain variable domain (VL) linked to a heavy chain variable domain VH on the second polypeptide chain (VL-VH) connected by a peptide linker that is too short to allow pairing between the two domains on the second polypeptide chain. The short linkages force chain pairing between the complementary domains of the first and the second polypeptide chains and promotes the assembly of a dimeric molecule with two functional antigen binding sites. Therefore, a peptide linker may be any suitable length that promotes such assembly, for example, between 5 and 20 amino acids in length. A “cys-diabody” is a diabody with one or more than one N-terminal cysteines.

[0182] In some embodiments, the targeting moiety includes all varieties of antibodies, including binding fragments thereof. Further included are constructs that include 1, 2, 3, 4, 5, and/or 6 CDRs. In some embodiments, these CDRs can be distributed between their appropriate framework regions in a traditional antibody. In some embodiments, the CDRs can be contained within a heavy and/or light chain variable region. In some embodiments, the CDRs can be within a heavy chain and/or a light chain. In some embodiments, the CDRs can be within a single peptide chain.

[0183] The terms “specific” “specifically” “selective” “selectively” and the like when used in the context of describing the interaction between a target protein and a targeting moiety, refers to a binding reaction that is determinative of the presence of the target protein in a heterogeneous population of proteins and other biologics, for example, in a biological sample, for example, a blood, serum, plasma or tissue sample. Thus, under designated immunoassay conditions, in some embodiments, the targeting moieties (e.g., protein binding partners, antibodies) with a particular binding specificity bind to a particular target protein at least two times the background and do not substantially bind in a significant amount to other non-target molecules present in the sample. Specific binding to a targeting moiety under such conditions may require the targeting moiety to have been selected for its specificity for a particular target protein. A variety of assay formats may be used to select targeting moieties specifically reactive with a particular target protein. For example, solid-phase ELISA immunoassays are routinely used to select antibodies specifically immunoreactive with a protein (*see*, for example, Harlow, E. & Lane D., “Using Antibodies, A Laboratory Manual,” Cold Spring Harbor Laboratory Press, 1998, for a description of immunoassay formats and conditions that can be used to determine specific immunoreactivity). Typically, a specific or selective binding reaction will

produce a signal at least twice over the background signal and more typically at least than 10 to 100 times over the background.

[0184] The term "equilibrium dissociation constant (K_d , M)" refers to the dissociation rate constant (k_d , time^{-1}) divided by the association rate constant (k_a , $\text{time}^{-1} \text{ M}^{-1}$). Equilibrium dissociation constants can be measured using any known method in the art. In some embodiments, the targeting moieties and/or bifunctional polypeptide of the present disclosure generally will have an equilibrium dissociation constant of less (that is superior binding) than about 10^{-7} or 10^{-8} M, for example, less than about 10^{-9} M or 10^{-10} M, in some embodiments, less than about 10^{-11} M, 10^{-12} M, or 10^{-13} M.

[0185] The term "conservative" modification, substitution, or variant applies to both amino acid and nucleic acid sequences. With respect to particular nucleic acid sequences, conservatively modified variants refers to those nucleic acids which encode identical or essentially identical amino acid sequences, or where the nucleic acid does not encode an amino acid sequence, to essentially identical sequences. Because of the degeneracy of the genetic code, a large number of functionally identical nucleic acids encode any given protein. For instance, the codons GCA, GCC, GCG and GCU all encode the amino acid alanine. Thus, at every position where an alanine is specified by a codon, the codon can be altered to any of the corresponding codons described without altering the encoded polypeptide. Such nucleic acid variations are "silent variations," which are one species of conservatively modified variations. Every nucleic acid sequence herein which encodes a polypeptide also describes every possible silent variation of the nucleic acid. One of skill will recognize that each codon in a nucleic acid (except AUG, which is ordinarily the only codon for methionine, and TGG, which is ordinarily the only codon for tryptophan) can be modified to yield a functionally identical molecule. Accordingly, each silent variation of a nucleic acid that encodes a polypeptide is implicit in each described sequence. As to amino acid sequences, one of skill will recognize that individual substitutions, deletions or additions to a nucleic acid, peptide, polypeptide, or protein sequence which alters, adds or deletes a single amino acid or a small percentage of amino acids in the encoded sequence is a "conservative" modification, substitution or variant where the alteration results in the substitution of an amino acid with a chemically similar amino acid. Conservative substitution tables providing functionally similar amino acids are well known in the art. Such conservatively modified variants are in addition to and do not exclude polymorphic variants, interspecies homologs, and alleles of the present disclosure.

[0186] The following eight groups each contain amino acids that are conservative substitutions for one another: 1) Alanine (A), Glycine (G); 2) Aspartic acid (D), Glutamic acid (E); 3) Asparagine (N), Glutamine (Q); 4) Arginine (R), Lysine (K); 5) Isoleucine (I), Leucine (L), Methionine (M), Valine (V); 6) Phenylalanine (F), Tyrosine (Y), Tryptophan (W); 7) Serine (S), Threonine (T); and 8) Cysteine (C), Methionine (M) (*see*, for example, Creighton, T. E., “Proteins - Structures and Molecular Properties,” W. H. Freeman & Co. Ltd., 1984).

[0187] The term “% w/w” or “% wt/wt” as used herein has its plain and ordinary meaning as understood in light of the specification and refers to a percentage expressed in terms of the weight of the ingredient or agent over the total weight of the composition multiplied by 100. The term “% v/v” or “% vol/vol” as used herein has its plain and ordinary meaning as understood in the light of the specification and refers to a percentage expressed in terms of the liquid volume of the compound, substance, ingredient, or agent over the total liquid volume of the composition multiplied by 100.

EXAMPLES

[0188] Some aspects of the embodiments discussed above are disclosed in further detail in the following examples, which are not in any way intended to limit the scope of the present disclosure. Those in the art will appreciate that many other embodiments also fall within the scope of the invention, as it is described herein above and in the claims.

Example 1: HiBiT™ assay for proteasomal-mediated degradation of target protein(s)

[0189] To obtain a preliminary assessment of the effectiveness of bifunctional polypeptide and dual-specific bifunctional polypeptides, HepG2 cells were transfected with plasmids for transiently expressing HiBiT™-tagged target protein and for expressing the corresponding bifunctional polypeptide degrader at the time of plating. Doxycycline was added 24 hours post-transfection to induce expression of the bifunctional polypeptide degrader from the doxycycline-inducible system. HiBiT™ signal was measured the following day using Nano-Glo HiBiT™ lytic detection system according to manufacturer’s protocol. Table 33 summarizes the assay conditions.

[0190] Table 33: Assay Conditions

Assay #	Targets	Cell line	Target Expression	SEQ ID NO
1	MYC/MYC MYC/CTNNB1	HepG2	Exogenous plasmid	All those listed in Tables 27, 29, 30 and 31

[0191] The results of the HiBiT™ assays are summarized in Tables 27, 29, 30, and 31 above. Average values of the percentage of target protein remaining relative to control was determined. A grade for the percent of degradation is provided based on the following cutoffs: A > 50% degradation (< 50% remaining); B ≥ 20% - ≤ 50% degradation (≥ 50% - ≤ 80% remaining); C ≥ 2% - <20% degradation (> 80% - ≤ 98 remaining); and D < 2% degradation (> 98% remaining) relative to control. “n.a.” means not applicable where both targeting moieties target the same target protein, and “not tested” or “” indicates that the degradation of the target protein was not tested. For dual-specific degraders where both targeting moieties are directed to the same target protein, the “Combined Grade” is a single letter.

Example 2: Subcellular localization of mono-specific bifunctional polypeptide degraders

[0192] Cells were seeded and transfected as described in Example 1. 48 hours post-plating, cells were fixed in 4% paraformaldehyde solution for 10 minutes, rinsed once in PBS pH 7.4, and permeabilized in 0.1% triton x-100 for 15 minutes at room temperature. To stain for mono-specific bifunctional polypeptide degraders, cells were incubated overnight with HA antibody in PBS containing 1% BSA and 0.1% triton x-100 to label the HA tag of the mono-specific bifunctional polypeptide degraders. The next day, cells were rinsed thrice with PBS and stained with fluorophore-tagged secondary antibody. Nucleus was counterstained with Hoescht33342. Cells were imaged on an inverted microscope with an LED fluorescence source.

[0193] Several NLS sequences were tested in various configurations. The following table provides a description of the NLS sequences tested.

Table 34: Exemplary NLS sequences used in Example 2

Name	Parental Protein	Uniprot ID of Parental Protein	SEQ ID NO
spNLS.2	SPOP	O43791	114
spNLS	SPOP	O43791	115

hrpNLS	Hrp1	Q99383	116
bpNLS	53BP1	Q12888	117
nlpNLS	Nucleoplasmin	P05221	118
3x(svNLS)	SV40	P03070	119
3x(mycNLS)	MYC	P01106	120
svNLS	SV40	P03070	121
mycNLS	MYC	P01106	122

[0194] The results are summarized in the following Table, where “Nuc” is predominantly nucleus, “Cyt” is predominantly cytoplasm, and “Mix” is both nucleus and cytoplasm. HA is the hemagglutinin epitope tag. The URDs and linkers are described elsewhere herein.

Table 35: Subcellular localization of exemplary polypeptides

URD Class	SEQ ID NO	Description	Location
SPOP	578	HA-con1.1-SPOP.2	Nuc
	609	HA-con1.1-SPOP.3	Cyt
	610	HA-con1.1-SPOP.3-mycNLS	Nuc
	611	HA-con1.1-SPOP.3-3xmycNLS	Nuc
	612	HA-con1.1-SPOP.3-svNLS	Nuc
	613	HA-con1.1-SPOP.3-3xsvNLS	Nuc
	614	HA-con1.1-SPOP.3-nlpNLS	Nuc
	615	HA-con1.1-SPOP.3-bpNLS	Nuc
	616	HA-con1.1-SPOP.3-hrpNLS	Nuc
NHLRC1	581	HA-mycNLS-TCF4.2-(GS) ₆ -NHLRC1.1	Nuc
	580	HA-TCF4.2-(GS) ₆ -NHLRC1.1	Mix
	582	HA-TCF4.2-(GS) ₆ -NHLRC1.1-spNLS	Nuc
	600	HA-nlpNLS-mycV.1500-d-NHLRC1.1	Nuc
	595	HA-mycNLS-mycV.1500-GGGS-NHLRC1.1	Mix
RNF125	590	HA-3xsvNLS-RNF125.2-(GGGS) ₂ -mycV.1500	Mix
	591	HA-nlpNLS-RNF125.2-(GGGS) ₁ -mycV.1500	Nuc
	592	HA-mycNLS-RNF125.2-GGGS-mycV.1500	Nuc

URD Class	SEQ ID NO	Description	Location
	593	HA-nlpNLS-RNF125.2-GGGS-mycV.1500	Mix
	594	HA-hrpNLS-RNF125.2-GGGS-mycV.1500	Mix
	602	HA-spNLS.2-RNF125.2-(GGGS) ₁ -mycV.1500	Mix
	603	HA-RNF125.2-(GGGS) ₁ -mycV.1500-mycNLS	Mix
	604	HA-RNF125.2-(GGGS) ₁ -mycV.1500-nlpNLS	Mix
	605	HA-RNF125.2-(GGGS) ₁ -mycV.1500-hrpNLS	Nuc
	606	HA-RNF125.2-(GGGS) ₁ -mycV.1500-spNLS.2	Mix
RNF4	596	HA-nlpNLS-mycV.1500-GGGS-RNF4.1	Nuc
	598	HA-nlpNLS-mycV.1500-d-RNF4.1	Nuc
	601	HA-spNLS.2-mycV.1500-GGGS1-RNF4.1	Nuc
RNF12.2	599	HA-nlpNLS-mycV.1500-d-RNF12.2	Nuc
	597	HA-nlpNLS-mycV.1500-GGGS-RNF12.2	Nuc
	607	HA-mycNLS-mycV.1500-GGGS-ZNRF1.1	Nuc
	608	HA-nlpNLS-mycV.1500-GGGS-ZNRF1.1	Nuc
CHIP	589	VL12.3-(GS) ₃ -CHIP.1-mycNLS-HA	Nuc
FBXW7a	584	HA-FBXW7a.1-(GS) ₃ -VL12.3	Nuc
SPOP	587	VL12.3-(GS) ₃ -SPOP.1-mycNLS-HA	Nuc
VIF	588	VL12.3-(GS) ₃ -VIF.1-mycNLS-HA	Mix
FBXW7b	585	HA-FBXW7b.1-(GS) ₃ -VL12.3	Cyt
ctrl	586	HA-mycNLS-VL12.3	Mix
ctrl	583	HA-VL12.3	Mix

[0195] The SPOP URD is derived from an E3 ubiquitin ligase. SPOP.2 and SPOP.3 variant URDs are truncated regions of the full-length SPOP URD. Compared to SPOP.2, SPOP.3 has a truncation of 14 residues at the C-terminus. The additional 14 C-terminal residues of SPOP.2 function as an endogenous nuclear localization signal (NLS). As summarized in Table 19 above, subcellular localization was observed for mono-specific bifunctional polypeptides with a con1 targeting moiety and either a SPOP.2 URD (HA-con1.1-SPOP.2, SEQ ID NO: 3236) or SPOP.3 URD (HA-con1.1-SPOP.3, SEQ ID NO: 3237). Truncation of the 14 residues changes the subcellular localization of SPOP from the nucleus (SPOP.2) to the cytoplasm (SPOP.3).

Microscopy data confirms that the SPOP.2 mono-specific bifunctional polypeptide is localized in the nucleus whereas the SPOP.3 mono-specific bifunctional polypeptide is localized in the cytoplasm (FIG. 3). This demonstrates the ability to direct polypeptides to subcellular compartments by modifying/deleting endogenous localization sequences.

[0196] NHLRC1 is a RING E3 ubiquitin ligase with a bioinformatically-predicted nuclear localization and export signal (NLS and NES, Table 26). Mono-specific bifunctional polypeptides with a TCF4 variant targeting moiety showed expression throughout the cells that is not exclusive to the nucleus (SEQ ID NO: 2417). Incorporation of mycNLS or spNLS.2 in the sequence of the mono-specific bifunctional polypeptides (SEQ ID NO: 2401 and 2426, respectively) resulted in nuclear localization (FIG. 4). This demonstrates the ability to alter localization of polypeptides to subcellular compartments of interest by incorporating a localization sequence specific to such subcellular compartment of interest.

[0197] VL12.3 targeting moiety (SEQ ID NO: 57) showed localization throughout the cells when expressed as a standalone intrabody (FIG. 5). Mono-specific bifunctional polypeptides comprising VL12.3 targeting moiety and URDs derived from either FBXW7a or FBW7b, E3 ligases with known nuclear and cytoplasmic localization (Yeh *et al.* “FBXW7: a critical tumor suppressor of human cancers,” *Molecular Cancer* (2018) 17:115), respectively directed the mono-specific bifunctional polypeptide exclusively the nucleus (SEQ ID NO: 2544) or to the cytoplasm (SEQ ID NO: 2592). This highlights the ability to design mono-specific bifunctional polypeptides to desired subcellular localization through URD selection.

Example 3: Subcellular localization of dual-specific bifunctional polypeptide degraders

[0198] Cell assays were prepared as described in Example 2.

[0199] Several exemplary dual-specific bifunctional polypeptides were expressed and analyzed for the amount of peptide expression as well as whether the expression was localized to the cytosol, nucleus, or both. The dual-specific bifunctional polypeptide of SEQ ID NO: 2203 has an N-terminal mycNLS sequence, but no C-terminal NLS sequence. Various NLS sequences were added to the C-terminus. The results show that adding additional NLS sequences to the C-terminus of can improve nuclear localization, The results of the experiment are summarized in FIG. 6, and exemplary photomicrographs are shown in FIG. 7A-7C.

Example 4. Development and Characterization of c-Myc sdABs

[0200] Anti-c-Myc single domain antibodies were developed and characterized.

Materials and methods

[0201] N-terminally and C-terminally biotinylated c-Myc peptide antigens were synthesized by Anaspec and c-Myc-Fc antigens including human, cynomolgus, and mouse were produced as N-terminal Fc fusions. All human c-Myc antigens were the portion of c-Myc with the sequence: AEEQKLISEEDLLRKRREQLKHKLEQLRNS (SEQ ID NO: 769). The cynomolgus c-Myc antigen was the portion of cynomolgus c-Myc with the sequence AEEQKLISEKDLLRKRREQLKHKLEQLRNS (SEQ ID NO: 770). The mouse c-Myc antigen was the portion of murine c-Myc with the sequence ADEHKLITSEKDLLRKRREQLKHKLEQLRNS (SEQ ID NO: 771). c-Myc-Fc antigens were biotinylated using the EZ-Link Sulfo-NHS-Biotinylation Kit from Thermo. Goat F(ab')₂ anti-human IgG (HC-488), ExtrAvidin-PE (EA-PE) and Streptavidin-AF633 (SA-633) were obtained from Jackson Immuno Research, Sigma, and Molecular Probes, respectively. Streptavidin MicroBeads and MACS LC separation columns were purchased from Miltenyi Biotec.

Antigen Quality Assessment

[0202] Prior to starting selections c-Myc peptide antigens were assessed for quality and suitability for selections. Quality assessments included Octet BLI kinetics to 9E10 control antibody, size exclusion chromatography, and non-specific binding checks to yeast heavy chain-only antibody libraries.

[0203] Antigens were initially run on size exclusion chromatography to confirm that antigens are not aggregated and have suitable quality for selection (> 90% main peak). Antigens were run on an HPLC equipped with a UV detector and TSK mAb HTP, SW3000, or SWAggregate SEC column (Tosoh Bioscience, King of Prussia, PA, USA) along with a set of molecular weight standards.

[0204] Antigen binding to the benchmark antibody, 9E10, was assessed by Octet Biolayer Interferometry (BLI). All data were acquired at 25 °C with an Octet HTX instrument (Sartorius, Bohemia, NY, USA) at an orbital shaking speed of 1000 rpm. Reagents were formulated in a running buffer (PBSF) of phosphate buffered saline with 0.1% IgG-free bovine serum albumin. For assessment of 9E10 binding to c-Myc-Fc antigens, 9E10 (100 nM) was captured to anti-human IgG Fc quantitation (AHQ) sensors (Sartorius, Bohemia, NY, USA) to a response level of 0.5-1.5 nm, the remaining unoccupied binding sites on the AHQ biosensors were blocked with an inert human antibody or inert biotinylated protein (for biotinylated antigens) (0.5 mg/mL for 10 min), and then allowed to stand in PBSF for a minimum of 15 minutes before

proceeding to the kinetic measurements. The kinetic measurements began with a short (60 s) baseline dip into PBSF before exposing (180 s) the 9E10-loaded sensors to human, cyno or mouse c-Myc-Fc (100 nM). This was immediately followed by a dip (180 s) into PBSF to measure the rate of dissociation of the sample 9E10/c-Myc complex. To assess 9E10 binding to c-Myc peptides, the biotinylated c-Myc peptides were captured to streptavidin (SA) biosensors (Sartorius, Bohemia, NY, USA) to a response level of 0.5-1.5 nm, and then allowed to stand in PBSF for a minimum of 15 minutes before proceeding to the kinetic measurements. The kinetic measurements began with a short (60 s) baseline dip into PBSF before exposing (180 s) the c-Myc peptide-loaded sensors to 9E10 (100 nM). This was immediately followed by a dip (180 s) into PBSF to measure the rate of dissociation of the 9E10/c-Myc peptide complex. All kinetics data were y-axis aligned, and inter-step corrected and then fit to a 1:1 binding model using the ForteBio Data Analysis Software version 11.1.3.10.

[0205] Non-specific binding to heavy-chain only antibody (HCAb) libraries was assessed prior to selection to ensure that c-Myc antigens do not show non-specific interaction to the yeast HCAb libraries. Briefly, yeast cells (~ 2 million cells/HCAb library) were incubated with 0.1 mL of 25 nM biotinylated Myc-Fc-antigen for 30 min at 30°C in wash buffer (phosphate-buffered saline (PBS)/0.1% bovine serum albumin (BSA)). For incubations with biotinylated peptide antigens, yeast cells (~ 2 million cells/HCAb library) were incubated with either 0.1 mL of a 500 nM mixture of N- and C-terminally biotinylated peptides or a 100 nM mixture of N- and C-terminally biotinylated peptides preincubated with 25 nM Streptavidin Microbeads to create a tetrameric peptide antigen for 30 min at 30°C in wash buffer (phosphate-buffered saline (PBS)/0.1% bovine serum albumin (BSA)). Following incubation, cells were washed twice with 200 µL of cold PBSF and the pellet was re-suspended in 100 µL of secondary labeling mix (SA-633, anti-human HC-488, and propidium iodide). The mix incubated on ice for 15 minutes and was then washed twice with 200 µL ice-cold PBSF. The cells were resuspended in 50 µL of ice-cold PBSF and analyzed on a FACSCanto (BD Biosciences) using an HTS sample injector. Flow cytometry data was analyzed and compared to no antigen control samples to assess for increased non-specific binding in the presence of antigen.

Initial HCAb Selection

[0206] Five naïve HCAb synthetic yeast libraries each of ~10⁹ diversity were propagated as previously described (see, e.g., Y. Xu et al, Addressing polyspecificity of antibodies selected from an in vitro yeast presentation system: a FACS-based, high-throughput selection and analytical tool. *PEDS* **26.10**, 663-70 (2013); WO2009036379; WO2010105256; and

WO2012009568; each of which are hereby incorporated by reference herein in their entirety and for all purposes). For the first two rounds of selection, a magnetic bead sorting technique utilizing the Miltenyi MACS system was performed, as previously described (see, e.g., Siegel et al, High efficiency recovery and epitope-specific sorting of an scFv yeast display library." *J Immunol Methods* **286(1-2)**, 141-153 (2004); which is hereby incorporated by reference herein in its entirety and for all purposes). Briefly, yeast cells ($\sim 10^{10}$ cells/HCAb library) were incubated with 3 mL of 25 nM biotinylated Myc-Fc-antigen for 30 min at 30°C in wash buffer (phosphate-buffered saline (PBS)/0.1% bovine serum albumin (BSA)). For incubations with biotinylated antigens, yeast cells ($\sim 10^{10}$ cells/HCAb library) were incubated with either 3 mL of a 500 nM mixture of N- and C-terminally biotinylated peptides or a 100 nM mixture of N- and C-terminally biotinylated peptides preincubated with 25 nM Streptavidin Microbeads to create a tetrameric peptide antigen for 30 min at 30°C in wash buffer (phosphate-buffered saline (PBS)/0.1% bovine serum albumin (BSA)). After washing once with 40 mL ice-cold wash buffer, the cell pellet was resuspended in 40 mL wash buffer, and Streptavidin MicroBeads (500 μ L) were added to the yeast, except for the tetrameric peptide antigen, and incubated for 15 min at 4°C. Next, the yeast were pelleted, resuspended in 20 mL wash buffer, and loaded onto a Miltenyi LS column. The column was washed 3 times with 5 mL wash buffer, then the column was removed from the magnetic field and the yeast were eluted with 5 mL of growth media and grown overnight. The following rounds of selection were performed using flow cytometry. Approximately 2×10^7 yeast were pelleted, washed three times with wash buffer, and incubated at 30°C with either 25 nM biotinylated c-Myc Fc antigen, 500 nM of a mix of N- and C-terminally biotinylated c-Myc peptides, 100 nM of c-Myc peptides tetramerized using SA-633, or with a poly-specificity depletion reagent (PSR) to remove non-specific antibodies from the selection. For the PSR depletion, the libraries were incubated with a 1:10 dilution of biotinylated PSR reagent as previously described (see, e.g., Y. Xu et al, Addressing polyspecificity of antibodies selected from an in vitro yeast presentation system: a FACS-based, high-throughput selection and analytical tool. *PEDS* **26.10**, 663-70 (2013); which is hereby incorporated by reference herein in its entirety and for all purposes). Yeast were then washed twice with wash buffer and stained with HC:488 (diluted 1:200), propidium iodide (1:100), and either SA-633 (diluted 1:500) or EAPE (diluted 1:50) secondary reagents for 15 min at 4°C. After washing twice with wash buffer, the cell pellets were resuspended in 0.3 mL wash buffer and transferred to strainer-capped sort tubes. Sorting was performed using a FACS ARIA sorter (BD Biosciences) and sort gates were determined to select for antibodies with

desired characteristics. Selection rounds were repeated and used decreasing c-Myc antigen concentrations until a population with all the desired characteristics was obtained. After the final round of sorting, yeast cells were plated and individual colonies were picked for characterization.

CDRH1 and CDRH2 batch shuffle

[0207] CDRH1 and CDRH2 batch shuffle diversification protocol was used during the primary discovery phase for further discovery and improvement of antibodies.

[0208] CDR H1 and CDR H2 batch diversification protocol: Pools of CDR H3s from naïve selection outputs were extracted from the yeast and transformed into a premade plasmid vector with CDR H1 and CDR H2 diversity of 2×10^5 . Additionally, this vector also contained limited diversities at positions C22 and C92 (Kabat) where the cysteines could be replaced by I, T, A, S, G, or V in order to remove the C22-C92 disulfide. Selections were performed with one round of MACS and two rounds of FACS as described in the naïve discovery. In the different FACS rounds the libraries were assessed for c-Myc and PSR binding. Affinity pressure was applied by antigen titration using either the biotinylated c-Myc peptides or the biotinylated c-Myc-Fc antigen. Selection rounds were repeated until a population with the desired characteristics was obtained.

Antibody Optimization

[0209] Optimization of antibodies was performed by introducing diversities into the heavy chain variable regions as described below.

[0210] CDRH1 and CDRH2 selection: The CDRH3 of a single antibody was recombined into a premade library with CDRH1 and CDRH2 variants including diversification at positions C22 and C92 (Kabat numbering; corresponding to residue 22 of FR1 and residue 30 of FR3 sequences presented herein) for a total library diversity of 2×10^7 and selections were performed with one round of MACS and two rounds of FACS as described in the naïve discovery.

[0211] During optimization, the libraries were assessed for PSR binding and affinity pressure in the different FACS rounds. Affinity pressure was generally applied by titrating the c-Myc biotinylated peptide antigens. In some instances, the final selection round included a parental HCAb pre-complexing pressure, this was performed by pre-complexing the c-Myc-Fc antigen with parental HCAb to select for antibodies with better affinity than the parent.

Antibody production and purification

[0212] Yeast clones were grown to saturation and then induced for 48 h at 30°C with shaking. After induction, yeast cells were pelleted and the supernatants were harvested for purification. HCABs were purified using a Protein A column and eluted with acetic acid, pH 2.0 into a neutralization buffer. Single domain antibody (sdAb) fragments were generated by papain digestion and purified using FcXL resin to remove cleaved and uncleaved Fc fragments followed by purification of the sdAb using a Praesto AC resin and elution with acetic acid, pH 2.0 into a neutralization buffer.

Biolayer interferometry (BLI) binding and kinetic measurements of c-Myc

[0213] BLI kinetic and epitope binning measurements were acquired at 25 °C with an Octet HTX instrument (Sartorius, Bohemia, NY, USA) at an orbital shaking speed of 1000 rpm. Reagents were formulated in a running buffer (PBSF) of phosphate buffered saline with 0.1% IgG-free bovine serum albumin.

[0214] For assessment of HCAB/IgG binding to c-Myc-Fc antigens, HCABs/IgGs (100 nM) were captured to anti-human IgG Fc capture (AHC) sensors (Sartorius, Bohemia, NY, USA) to a response level of 0.5-1.5 nm, and then allowed to stand in PBSF for a minimum of 15 minutes before proceeding to the kinetic measurements. The kinetic measurements began with a short (60 s) baseline dip into PBSF before exposing (180 s) the IgG/HCAB-loaded sensors to human, cyno or mouse c-Myc-Fc (100 nM). This was immediately followed by a dip (180-600 s) into PBSF to measure the rate of dissociation of the sample HCAB/c-Myc complex.

[0215] To assess monovalent binding of sdAbs to human, cyno, or mouse, c-Myc-Fc antigens, c-Myc-Fc antigen (100 nM) was captured to anti-human IgG Fc capture (AHC) sensors (Sartorius, Bohemia, NY, USA) to a response level of 0.5-1.5 nm, and then allowed to stand in PBSF for a minimum of 15 minutes before proceeding to the kinetic measurements. The kinetic measurements began with a short (60 s) baseline dip into PBSF before exposing (180 s) the c-Myc-Fc-loaded sensors to sample sdAbs (100-300 nM). This was immediately followed by a dip (180-600 s) into PBSF to measure the rate of dissociation of the sample sdAb/c-Myc complex. Table 36 shows the results of the assessment of sdAb binding to human Myc-Fc.

Table 36. Human Myc-Fc on AHC Sensor, sdAb in Solution (300 nM) Monovalent

Name	Octet sdAb Kd Human Myc-Fc (M) Monovalent	kon (1/Ms)	koff (1/s)	Response (nm)
mycV.0300	4.92E ⁻⁰⁸	1.59E ⁺⁰⁵	7.82E ⁻⁰³	0.28
mycV.0302	3.41E ⁻⁰⁹	2.17E ⁺⁰⁵	7.42E ⁻⁰⁴	0.35
mycV.0305	1.18E ⁻⁰⁸	1.01E ⁺⁰⁵	1.19E ⁻⁰³	0.33
mycV.0306	3.78E ⁻⁰⁹	1.70E ⁺⁰⁵	6.43E ⁻⁰⁴	0.35
mycV.0307	6.90E ⁻⁰⁹	1.26E ⁺⁰⁵	8.67E ⁻⁰⁴	0.33
mycV.1300	5.03E ⁻⁰⁸	4.04E ⁺⁰⁵	2.03E ⁻⁰²	0.26
mycV.1308	5.31E ⁻⁰⁹	1.90E ⁺⁰⁵	1.01E ⁻⁰³	0.36
mycV.1500	1.40E ⁻⁰⁷	1.24E ⁺⁰⁵	1.74E ⁻⁰²	0.20
mycV.1517	3.61E ⁻⁰⁸	7.55E ⁺⁰⁴	2.73E ⁻⁰³	0.28
mycV.1518	1.81E ⁻⁰⁸	6.79E ⁺⁰⁴	1.23E ⁻⁰³	0.32
mycV.1519	3.97E ⁻⁰⁸	8.27E ⁺⁰⁴	3.28E ⁻⁰³	0.30
mycV.1520	2.70E ⁻⁰⁸	1.24E ⁺⁰⁵	3.35E ⁻⁰³	0.30
mycV.1522	1.76E ⁻⁰⁸	1.24E ⁺⁰⁵	2.18E ⁻⁰³	0.33
mycV.1524	2.76E ⁻⁰⁸	1.07E ⁺⁰⁵	2.96E ⁻⁰³	0.30
mycV.3700	2.04E ⁻⁰⁸	9.31E ⁺⁰⁴	1.90E ⁻⁰³	0.30
mycV.3706	1.61E ⁻⁰⁹	1.24E ⁺⁰⁵	2.00E ⁻⁰⁴	0.35
mycV.3708	2.05E ⁻⁰⁹	1.01E ⁺⁰⁵	2.08E ⁻⁰⁴	0.35
mycV.6600	1.22E ⁻⁰⁷	6.86E ⁺⁰⁴	8.39E ⁻⁰³	0.21
mycV.6605	9.46E ⁻⁰⁹	8.31E ⁺⁰⁴	7.86E ⁻⁰⁴	0.30
mycV.6608	9.47E ⁻⁰⁹	9.11E ⁺⁰⁴	8.63E ⁻⁰⁴	0.32

[0216] As can be seen in Table 36, mycV variants bind human Myc-Fc with a K_d of between 1.22E⁻⁰⁷ and 9.47E⁻⁰⁹, a k_{on} of between 6.79E⁺⁰⁴ and 4.04E⁺⁰⁵, and a K_{off} of between 1.74E⁻⁰² and 8.67E⁻⁰⁴.

[0217] Table 37 shows the results of the assessment of sdAb binding to Mouse Myc-Fc.

Table 37. Octet Image: Mouse Myc-Fc on AHC Sensor, sdAb in Solution (100 nM)

Monovalent

Name	Octet sdAb Kd Mouse Myc-Fc (M) Monovalent	kon (1/Ms)	koff (1/s)	Response (nm)
mycV.0300	6.61E ⁻⁰⁸	2.96E ⁺⁰⁵	1.96E ⁻⁰²	0.15
mycV.0302	2.94E ⁻⁰⁹	4.09E ⁺⁰⁵	1.20E ⁻⁰³	0.25
mycV.0305	1.28E ⁻⁰⁸	1.81E ⁺⁰⁵	2.31E ⁻⁰³	0.20
mycV.0306	3.38E ⁻⁰⁹	3.16E ⁺⁰⁵	1.07E ⁻⁰³	0.26
mycV.0307	5.62E ⁻⁰⁹	2.85E ⁺⁰⁵	1.60E ⁻⁰³	0.23
mycV.1300	-	-	-	0.13
mycV.1308	2.86E ⁻⁰⁹	3.19E ⁺⁰⁵	9.13E ⁻⁰⁴	0.28
mycV.1500	2.41E ⁻⁰⁸	1.91E ⁺⁰⁵	4.59E ⁻⁰³	0.18
mycV.1517	4.91E ⁻⁰⁹	1.78E ⁺⁰⁵	8.77E ⁻⁰⁴	0.19
mycV.1518	2.78E ⁻⁰⁹	1.09E ⁺⁰⁵	3.04E ⁻⁰⁴	0.21
mycV.1519	6.45E ⁻⁰⁹	1.44E ⁺⁰⁵	9.32E ⁻⁰⁴	0.22
mycV.1520	3.14E ⁻⁰⁹	2.31E ⁺⁰⁵	7.26E ⁻⁰⁴	0.27
mycV.1522	2.59E ⁻⁰⁹	2.51E ⁺⁰⁵	6.50E ⁻⁰⁴	0.25
mycV.1524	3.82E ⁻⁰⁹	1.84E ⁺⁰⁵	7.04E ⁻⁰⁴	0.24
mycV.3700	4.34E ⁻⁰⁹	2.05E ⁺⁰⁵	8.89E ⁻⁰⁴	0.26
mycV.3706	5.35E ⁻¹⁰	1.85E ⁺⁰⁵	9.91E ⁻⁰⁵	0.32
mycV.3708	7.12E ⁻¹⁰	1.40E ⁺⁰⁵	9.94E ⁻⁰⁵	0.31
mycV.6600	-	-	-	0.10
mycV.6605	7.05E ⁻⁰⁹	1.80E ⁺⁰⁵	1.27E ⁻⁰³	0.25
mycV.6608	7.20E ⁻⁰⁹	1.80E ⁺⁰⁵	1.30E ⁻⁰³	0.26

[0218] As can be seen in Table 37, mycV variants bind Mouse Myc-Fc with a K_d of between 1.09E⁻⁰⁷ and 4.15E⁻¹⁰, a k_{on} of between 1.22E⁺⁰⁵ and 4.18E⁺⁰⁵, and a K_{off} of between 2.49E⁻⁰² and 7.27E⁻⁰⁵.

[0219] Table 38 shows the results of the assessment of sdAb binding to Cyno Myc-Fc.

Table 38. Cyno Myc-Fc on AHC Sensor, sdAb in Solution, Monovalent

Name	Octet sdAb Kd Cyno Myc-Fc (M) Monovalent	kon (1/Ms)	koff (1/s)	Response (nm)
mycV.0300	1.17E ⁻⁰⁷	2.13E ⁺⁰⁵	2.49E ⁻⁰²	0.11
mycV.0302	2.39E ⁻⁰⁹	4.18E ⁺⁰⁵	1.00E ⁻⁰³	0.23
mycV.0305	8.34E ⁻⁰⁹	2.20E ⁺⁰⁵	1.84E ⁻⁰³	0.19
mycV.0306	4.67E ⁻⁰⁹	2.95E ⁺⁰⁵	1.38E ⁻⁰³	0.17
mycV.0307	5.49E ⁻⁰⁹	2.48E ⁺⁰⁵	1.36E ⁻⁰³	0.21
mycV.1300	-	-	-	0.13
mycV.1308	2.91E ⁻⁰⁹	3.10E ⁺⁰⁵	9.02E ⁻⁰⁴	0.26
mycV.1500	1.73E ⁻⁰⁸	2.44E ⁺⁰⁵	4.22E ⁻⁰³	0.17
mycV.1517	4.93E ⁻⁰⁹	1.55E ⁺⁰⁵	7.63E ⁻⁰⁴	0.18
mycV.1518	2.58E ⁻⁰⁹	1.22E ⁺⁰⁵	3.15E ⁻⁰⁴	0.21
mycV.1519	5.78E ⁻⁰⁹	1.34E ⁺⁰⁵	7.72E ⁻⁰⁴	0.21
mycV.1520	3.44E ⁻⁰⁹	2.05E ⁺⁰⁵	7.04E ⁻⁰⁴	0.24
mycV.1522	2.55E ⁻⁰⁹	2.34E ⁺⁰⁵	5.96E ⁻⁰⁴	0.23
mycV.1524	3.53E ⁻⁰⁹	2.09E ⁺⁰⁵	7.40E ⁻⁰⁴	0.23
mycV.3700	8.74E ⁻⁰⁹	1.97E ⁺⁰⁵	1.72E ⁻⁰³	0.15
mycV.3706	4.15E ⁻¹⁰	1.75E ⁺⁰⁵	7.27E ⁻⁰⁵	0.21
mycV.3708	1.02E ⁻⁰⁹	1.61E ⁺⁰⁵	1.64E ⁻⁰⁴	0.21
mycV.6600	-	-	-	0.07
mycV.6605	1.06E ⁻⁰⁸	1.23E ⁺⁰⁵	1.31E ⁻⁰³	0.17
mycV.6608	9.87E ⁻⁰⁹	1.46E ⁺⁰⁵	1.45E ⁻⁰³	0.18

[0220] As can be seen in Table 38, mycV variants bind Cyno Myc-Fc with a K_d of between 1.09E⁻⁰⁷ and 4.15E⁻¹⁰, a k_{on} of between 1.22E⁺⁰⁵ and 4.18E⁺⁰⁵, and a K_{off} of between 2.49E⁻⁰² and 7.27E⁻⁰⁵.

[0221] Finally, to assess sdAb binding to c-Myc peptides, the C-terminally biotinylated human c-Myc antigen (SEQ ID NO: 769) was captured to streptavidin (SA) biosensors (Sartorius, Bohemia, NY, USA) to a response level of 0.5-1.5 nm, and then allowed to stand in PBSF for a minimum of 15 minutes before proceeding to the kinetic measurements. The kinetic

measurements began with a short (60 s) baseline dip into PBSF before exposing (180 s) the c-Myc-loaded sensors to sample sdAbs (300 nM). This was immediately followed by a dip (180-600 s) into PBSF to measure the rate of dissociation of the sample sdAb/c-Myc complex. All kinetics data was y-axis aligned, and inter-step corrected and then fit to a 1:1 binding model using the ForteBio Data Analysis Software version 11.1.3.10. Table 39, shows the results of the assessment of sdAb binding to c-Myc peptides.

Table 39. Octet sdAb Kd, Human Myc Peptide on Streptavidin Sensor, Monovalent

Name	Octet sdAb Kd Human Myc Peptide (M) Monovalent	kon (1/Ms)	koff (1/s)	Response (nm)
mycV.0300	5.86E ⁻⁰⁸	6.07E ⁺⁰⁴	3.56E ⁻⁰³	0.73
mycV.0302	5.56E ⁻⁰⁹	7.13E ⁺⁰⁴	3.96E ⁻⁰⁴	1.10
mycV.0305	1.16E ⁻⁰⁸	4.07E ⁺⁰⁴	4.71E ⁻⁰⁴	0.76
mycV.0306	5.15E ⁻⁰⁹	6.14E ⁺⁰⁴	3.16E ⁻⁰⁴	1.01
mycV.0307	1.16E ⁻⁰⁸	5.23E ⁺⁰⁴	6.06E ⁻⁰⁴	0.73
mycV.1300	9.18E ⁻⁰⁸	1.31E ⁺⁰⁵	1.21E ⁻⁰²	0.67
mycV.1308	1.12E ⁻⁰⁸	5.52E ⁺⁰⁴	6.20E ⁻⁰⁴	1.20
mycV.1500	1.49E ⁻⁰⁷	5.96E ⁺⁰⁴	8.90E ⁻⁰³	0.50
mycV.1517	4.67E ⁻⁰⁸	3.68E ⁺⁰⁴	1.72E ⁻⁰³	0.47
mycV.1518	1.61E ⁻⁰⁸	3.67E ⁺⁰⁴	5.90E ⁻⁰⁴	0.65
mycV.1519	5.36E ⁻⁰⁸	3.23E ⁺⁰⁴	1.73E ⁻⁰³	0.66
mycV.1520	4.05E ⁻⁰⁸	5.68E ⁺⁰⁴	2.30E ⁻⁰³	0.63
mycV.1522	3.12E ⁻⁰⁸	6.24E ⁺⁰⁴	1.95E ⁻⁰³	0.63
mycV.1524	3.52E ⁻⁰⁸	4.86E ⁺⁰⁴	1.71E ⁻⁰³	0.61
mycV.3700	2.37E ⁻⁰⁸	4.78E ⁺⁰⁴	1.13E ⁻⁰³	0.68
mycV.3706	3.80E ⁻⁰⁹	5.26E ⁺⁰⁴	2.00E ⁻⁰⁴	0.85
mycV.3708	4.60E ⁻⁰⁹	4.35E ⁺⁰⁴	2.00E ⁻⁰⁴	0.72
mycV.6600	1.09E ⁻⁰⁷	3.82E ⁺⁰⁴	4.18E ⁻⁰³	0.58
mycV.6605	1.00E ⁻⁰⁸	4.13E ⁺⁰⁴	4.14E ⁻⁰⁴	0.63
mycV.6608	1.01E ⁻⁰⁸	4.67E ⁺⁰⁴	4.72E ⁻⁰⁴	0.81

[0222] As can be seen in Table 39, mycV variants bind human myc peptide with a K_d of between $1.09E^{-07}$ and $5.56E^{-09}$, a k_{on} of between $3.23E^{+04}$ and $1.31E^{+05}$, and a K_{off} of between $1.21E^{-02}$ and $6.06E^{-04}$.

Epitope binning (sample IgG in solution)

[0223] The control IgGs or HCABs (100 nM) were captured (0.5-1.5 nm) to AHC sensors, the remaining unoccupied binding sites on the AHC biosensors were blocked with an inert human antibody (0.5 mg/mL for 10 min), and then allowed to equilibrate in PBSF for a minimum of 30 min. To assess any cross interactions between proteins on the sensor surface and the secondary HCABs, the loaded and blocked sensors were exposed (90-180 s) to the sample HCABs (300 nM) prior to the binning analysis. After a short baseline step (60 s) in PBSF, the loaded biosensor tips were exposed (180 s) to human c-Myc-Fc (100 nM) and then exposed (180 s) to the sample HCABs (300 nM). The data was y-axis aligned, and inter-step corrected using the ForteBio Data Analysis Software version 11.1.3.10. Additional binding by the sample HCAB indicates an unoccupied epitope (non-competitor), while no binding indicates epitope blocking (competitor).

PSR binding

[0224] Poly-Specificity Reagent (PSR) binding assays were performed as described in, e.g., Xu et al. *Protein Eng Des Sel*, 26(10):663-670 (2013); which is hereby incorporated by reference herein in its entirety and for all purposes. Monoclonal heavy chain-only antibodies presented on yeast, two million HCAB-presenting yeast were transferred into a 96-well assay plate and pelleted at $2400 \times g$ for 3 min to remove supernatant. The pellet was resuspended in 50 μ L of a freshly prepared 1:10 dilution of stock biotinylated PSRs and incubated on ice for 20 minutes. The cells were washed twice with 200 μ L of cold PBSF and the pellet was resuspended in 50 μ L of secondary labeling mix (Extravidin-R-PE, anti-human HC-488, and propidium iodide). The mix incubated on ice for 20 minutes and was then washed twice with 200 μ L ice-cold PBSF. The cells were resuspended in 50 μ L of ice-cold PBSF and analyzed on a FACSCanto (BD Biosciences) using an HTS sample injector. Flow cytometry data was analyzed for mean fluorescence intensity (MFI) in the R-PE channel and was normalized for background binding using appropriate controls. Numerous methods to measure the presentation or display of antibodies or antibody fragments on the surface of yeast have been described previously, all of which are consistent with this protocol (Blaise et al., *Gene*, 342(2):211-8 (2004), Boder and Wittrup, *Nat Biotechnol.*, 15(6):553-7 (1997), Kuroda and Ueda, *Biotechnol Lett.*, 33(1):1-9 (2011), Orcutt and Wittrup, *Springer Protocols: Antibody Engineering*, 1:207-

233 (2010), Rakestraw et al., Protein Eng Des Sel., 24(6):525-30 (2011), Sazinsky et al., Proc Natl Acad Sci U S A., 105(51):20167-72 (2008), Tasumi et al., Proc Natl Acad Sci U S A., 106(31):12891-6 (2009); each of which are hereby incorporated by reference herein in their entirety and for all purposes. A PSR score < 0.1 is considered clean, a PSR score > 0.10 and < 0.33 is considered low, > 0.33 and < 0.66 is considered medium, and > 0.66 and < 1.00 is considered high. Table 40 shows the results of the PSR binding assay.

Table 40. Poly-Specificity Reagent (PSR) binding assay

Name	PSR Score
mycV.0300	0.20
mycV.0302	0.43
mycV.0305	0.38
mycV.0306	0.41
mycV.0307	0.34
mycV.1300	0.34
mycV.1308	0.42
mycV.1500	0.11
mycV.1517	0.19
mycV.1518	0.15
mycV.1519	0.26
mycV.1520	0.15
mycV.1522	0.16
mycV.1524	0.21
mycV.3700	0.14
mycV.3706	0.35
mycV.3708	0.33
mycV.6600	0.01
mycV.6605	0.11
mycV.6608	0.15

[0225] As can be seen in Table 40, mycV variants of between 0.01 and 0.43.

Hydrophobic interaction chromatography (HIC) retention time

[0226] The method for measuring HIC retention time (RT) was described previously (Estep et al., MAbs. 2015, 7(3): 553–561; Jain et al, Bioinformatics, 33(23), 2017, 3758-3766 ; which are hereby incorporated by reference herein in their entirety and for all purposes). In brief, 5.0 ug of each sample (1.0 mg/ml) was spiked into mobile phase A solution (1.8 M ammonium sulfate, 0.1 M sodium phosphate, pH 6.5) to achieve an ammonium sulfate concentration of about 1 M prior to analysis. A Sepax Proteomix HIC butyl-NP5 column (Sigma Aldrich) was used with a linear gradient (of mobile phase A and mobile phase B solution (0.1 M sodium phosphate, pH 6.5) and protein was detected with UV absorbance (280 nm). The flow rate was 1.0 mL/min and the gradient time was 7.5 min. A standard IgG1 antibody sample corresponding to the variable regions of adalimumab was run periodically to assess assay reproducibility and used to normalize the measured RTs. A high throughput (HT) version of the experiment was adapted. The flow rate was increased to 2.0 mL/min and the gradient time shortened to 2.63 min. To correlate data between the different methods, the measured RTs of several standard IgG1 samples were used to normalize and convert RTs from the HT experiment to the standard (low throughput) experiment RTs. A HIC retention time of < 10.5 minutes is considered clean/low, ≥ 10.5 minutes and < 11.5 minutes is considered medium, and ≥ 11.5 minutes is considered high. Table 41 shows the HIC retention time, in minutes, of the tested mycV variants.

Table 41. mycV Variant HIC Retention Time

Name	HIC Retention Time (Minutes)
mycV.0300	9.3
mycV.0302	9.6
mycV.0305	10.2
mycV.0306	9.8
mycV.0307	9.5
mycV.1300	10.2
mycV.1308	10.7
mycV.1500	9.6
mycV.1517	10.4
mycV.1518	10.2
mycV.1519	10.1

mycV.1520	10.1
mycV.1522	9.3
mycV.1524	10.4
mycV.3700	10.1
mycV.3706	9.8
mycV.3708	9.8
mycV.6600	9.5
mycV.6605	10.1
mycV.6608	9.9

[0227] As can be seen in Table 41, mycV variants had a HIC retention time of between 9.3 and 10.4 minutes.

Melting temperature measurement

[0228] The melting temperature, T_m , of each sdAb (HCAb-derived) was measured with differential scanning fluorimetry (DSF). DSF measures protein unfolding by monitoring changes in fluorescence of a dye that binds preferentially to unfolded protein as a function of temperature. To conduct DSF assays, 10 μ L of 20 $\times$ Sypro Orange was added to 20 μ L of 0.2-1 mg/mL sdAb solution. A RT-PCR instrument (BioRad CFX96 RT PCR) was used to ramp the sample plate temperature from 40 to 95° C at 0.5° C. increments, with 2 min allowed for equilibration at each temperature. The negative of the first derivative for the raw data is used to extract T_m . Table 42 shows the melting temperature of the tested mycV variants.

Table 42. mycV Variant Melting Temperature

Name	Melting Temperature
mycV.0300	53.5
mycV.0302	53.0
mycV.0305	48.0
mycV.0306	49.5
mycV.0307	-
mycV.1300	47.0
mycV.1308	46.0
mycV.1500	47.8
mycV.1517	53.5

mycV.1518	-
mycV.1519	45.5
mycV.1520	54.0
mycV.1522	48.5
mycV.1524	54.0
mycV.3700	50.0
mycV.3706	49.0
mycV.3708	50.0
mycV.6600	56.5
mycV.6605	64.5
mycV.6608	58.5

[0229] As can be seen in Table 42, the tested mycV variants had a melting temperature of between 45.5° and 64.5° C.

Example 5. Dual-specific bifunctional polypeptides that direct proteasomal-mediated degradation for treatment of cancer

[0230] A patient is selected who has a cancer. The cancer may be a brain cancer, breast cancer, colorectal cancer, kidney cancer, liver cancer, lung cancer, pancreatic cancer, bladder cancer, stomach cancer, or a hematological malignancy, or another cancer not listed here. The cancer may be non-responsive to standard of care treatments. The cancer may be primary and/or metastatic.

[0231] Any of the bifunctional polypeptides, or dual-specific bifunctional polypeptides, polynucleotides that encode for the bifunctional polypeptides, or dual-specific bifunctional polypeptides, or a pharmaceutical composition that comprises the bifunctional polypeptides, or dual-specific bifunctional polypeptide or polynucleotide and is targeted to a protein target associated with cancer (e.g., Myc, CTNNB1, or PCNA) is administered to the patient. The bifunctional polypeptides, or dual-specific bifunctional polypeptide, polynucleotide, or pharmaceutical composition is administered enterally, parenterally, intravenously, intratumorally, intramuscularly, intra-arterially, intradermally, subcutaneously, intraperitoneally, intraventricularly, intrathecally, or intracranially. In some embodiments, the bifunctional polypeptides, or dual-specific bifunctional polypeptide, polynucleotide, or pharmaceutical composition is administered as at least one dose at an amount of 1, 10, 100,

1000 ng, or 1, 10, 50, 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000 µg, or 1, 10, 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000 mg, or any amount within a range defined by any two of the aforementioned amounts, or any other amount appropriate for optimal efficacy in humans. In some embodiments, the bifunctional polypeptides, or dual-specific bifunctional polypeptide, polynucleotide, or pharmaceutical composition is administered in a number of doses sufficient to achieve an improvement in the disease pathology in the patient. In some embodiments, the bifunctional polypeptides, or dual-specific bifunctional polypeptide, polynucleotide, or pharmaceutical composition is administered in 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, or 150 doses, or any number of doses within a range defined by any two of the aforementioned number of doses, for example, 1-150 doses, 1-10 doses, 1-50 doses, 50-100 doses, or 50-150 doses. In some embodiments, the bifunctional polypeptides, or dual-specific bifunctional polypeptide, polynucleotide, or pharmaceutical composition is administered in a single dose (for example, when using a gene therapy or neoadjuvant therapy approach). In the case of multiple doses, the doses can be administered every 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 24, 36, or 48 days or weeks or any time within a range defined by any two of the aforementioned times, for example, 1 day to 48 weeks, 1-48 days, 1-48 weeks, or 10-30 days.

[0232] After administration of the bifunctional polypeptides, or dual-specific bifunctional polypeptide, polynucleotide, or pharmaceutical composition, the patient exhibits a prolongment of progression-free survival (PFS), a prolongment of overall survival (OS), a reduction in cancer burden (e.g., tumor size and number), or an amelioration of symptoms such as symptoms of pain, discomfort, or inflammation.

[0233] In some embodiments, the bifunctional polypeptides, or dual-specific bifunctional polypeptide, polynucleotide, or pharmaceutical composition is administered as a supplement to a standard of care therapy for a cancer. In some embodiments, the standard of care treatment may include but is not limited to surgery, radiation, chemotherapy, targeted therapy, or immunotherapy. For example, the bifunctional polypeptides, or dual-specific bifunctional polypeptide, polynucleotide, or pharmaceutical composition may be administered in combination with anti-PD-1, anti-PD-L1, anti-CTLA4, anti-LAG3, or adoptive cell therapy. In some embodiments, the combination of the standard of care therapy and the bifunctional polypeptides, or dual-specific bifunctional polypeptide, polynucleotide, or pharmaceutical composition has a greater positive effect in reducing cancer burden or ameliorating symptoms compared to the standard of care therapy alone.

[0234] In at least some of the previously described embodiments, one or more elements used in an embodiment can interchangeably be used in another embodiment unless such a replacement is not technically feasible. It will be appreciated by those skilled in the art that various other omissions, additions and modifications may be made to the methods and structures described above without departing from the scope of the claimed subject matter. All such modifications and changes are intended to fall within the scope of the subject matter, as defined by the appended claims.

[0235] With respect to the use of substantially any plural and/or singular terms herein, those having skill in the art can translate from the plural to the singular and/or from the singular to the plural as is appropriate to the context and/or application. The various singular/plural permutations may be expressly set forth herein for sake of clarity.

[0236] It will be understood by those within the art that, in general, terms used herein, and especially in the appended claims (e.g., bodies of the appended claims) are generally intended as “open” terms (e.g., the term “including” should be interpreted as “including but not limited to,” the term “having” should be interpreted as “having at least,” the term “includes” should be interpreted as “includes but is not limited to,” etc.). It will be further understood by those within the art that if a specific number of an introduced claim recitation is intended, such an intent will be explicitly recited in the claim, and in the absence of such recitation no such intent is present. For example, as an aid to understanding, the following appended claims may contain usage of the introductory phrases “at least one” and “one or more” to introduce claim recitations. However, the use of such phrases should not be construed to imply that the introduction of a claim recitation by the indefinite articles “a” or “an” limits any particular claim containing such introduced claim recitation to embodiments containing only one such recitation, even when the same claim includes the introductory phrases “one or more” or “at least one” and indefinite articles such as “a” or “an” (e.g., “a” and/or “an” should be interpreted to mean “at least one” or “one or more”); the same holds true for the use of definite articles used to introduce claim recitations. In addition, even if a specific number of an introduced claim recitation is explicitly recited, those skilled in the art will recognize that such recitation should be interpreted to mean at least the recited number (e.g., the bare recitation of “two recitations,” without other modifiers, means at least two recitations, or two or more recitations). Furthermore, in those instances where a convention analogous to “at least one of A, B, and C, etc.” is used, in general such a construction is intended in the sense one having skill in the art would understand the convention (e.g., “a system having at least one of A, B, and C” would

include but not be limited to systems that have A alone, B alone, C alone, A and B together, A and C together, B and C together, and/or A, B, and C together, etc.). In those instances where a convention analogous to “at least one of A, B, or C, etc.” is used, in general such a construction is intended in the sense one having skill in the art would understand the convention (e.g., “a system having at least one of A, B, or C” would include but not be limited to systems that have A alone, B alone, C alone, A and B together, A and C together, B and C together, and/or A, B, and C together, etc.). It will be further understood by those within the art that virtually any disjunctive word and/or phrase presenting two or more alternative terms, whether in the description, claims, or drawings, should be understood to contemplate the possibilities of including one of the terms, either of the terms, or both terms. For example, the phrase “A or B” will be understood to include the possibilities of “A” or “B” or “A and B.”

[0237] In addition, where features or aspects of the disclosure are described in terms of Markush groups, those skilled in the art will recognize that the disclosure is also thereby described in terms of any individual member or subgroup of members of the Markush group.

[0238] As will be understood by one skilled in the art, for any and all purposes, such as in terms of providing a written description, all ranges disclosed herein also encompass any and all possible sub-ranges and combinations of sub-ranges thereof. Any listed range can be easily recognized as sufficiently describing and enabling the same range being broken down into at least equal halves, thirds, quarters, fifths, tenths, etc. As a non-limiting example, each range discussed herein can be readily broken down into a lower third, middle third and upper third, etc. As will also be understood by one skilled in the art all language such as “up to,” “at least,” “greater than,” “less than,” and the like include the number recited and refer to ranges which can be subsequently broken down into sub-ranges as discussed above. Finally, as will be understood by one skilled in the art, a range includes each individual member. Thus, for example, a group having 1-3 articles refers to groups having 1, 2, or 3 articles. Similarly, a group having 1-5 articles refers to groups having 1, 2, 3, 4, or 5 articles, and so forth.

[0239] While various aspects and embodiments have been disclosed herein, other aspects and embodiments will be apparent to those skilled in the art. The various aspects and embodiments disclosed herein are for purposes of illustration and are not intended to be limiting, with the true scope and spirit being indicated by the following claims.

[0240] All references cited herein, including but not limited to published and unpublished applications, patents, and literature references, are incorporated herein by reference in their entirety and are hereby made a part of this specification. To the extent publications and patents

or patent applications incorporated by reference contradict the disclosure contained in the specification, the specification is intended to supersede and/or take precedence over any such contradictory material.

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[0270] WO 2014/193632.

WHAT IS CLAIMED IS:

1. A polypeptide comprising or consisting of an anti-Myc single domain antibody (sdAb), comprising or consisting of a V_H domain of an IgG antibody, or a V_{HH}, or V_H domain of a heavy chain antibody (HcAb), wherein the V_H or V_{HH} domain comprises a V_H CDR3 comprising or consisting of any one of SEQ ID NOs: 179, 195, 211, 219, 251, 267 or 283, optionally wherein the V_H CDR3 sequence comprises 1, 2, 3, 4, 5, 6, 7, or more substitutions and/or 1, 2, 3, 4, 5, 6, 7, or more deletions or additions, and/or has at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to a sequence selected from any one of SEQ ID NOs: 179, 195, 211, 219, 251, 267 and 283.

2. A polynucleotide encoding the polypeptide of claim 1, optionally wherein the polynucleotide comprises or consists of any one of SEQ ID NOs: 626-640.

3. A bifunctional polypeptide that promotes proteasome-mediated degradation of at least one target protein, the bifunctional polypeptide comprising:

- a) a first targeting moiety comprising or consisting of the polypeptide of claim 1, wherein the polypeptide comprises or consists of the anti-Myc sdAb;
- b) a ubiquitin-proteasome system recruiting domain (URD),
- c) optionally a first linker peptide, optionally wherein the first linker peptide is positioned between the first targeting moiety and the URD,
- d) optionally one or more localization peptide sequences,

wherein at least one target protein is MYC, optionally c-MYC, and wherein proximity of the bifunctional polypeptide to at the least one target protein through binding of the targeting moiety induces ubiquitination of the at least one target protein via the URD, thereby promoting proteasome-mediated degradation of the at least one target protein.

4. The bifunctional polypeptide of claim 3, wherein the at least one target protein is selected from MYC, (optionally c-MYC), CTNNB1, and/or PCNA.

5. The bispecific polypeptide of claim 4, wherein the bispecific polypeptide is a dual-specific bifunctional polypeptide and wherein the second target protein comprises MYC, optionally c-MYC.

6. The bispecific polypeptide of claim 4, wherein the bispecific polypeptide is a dual-specific bifunctional polypeptide and wherein the second target protein comprises CTNNB1.

7. The bispecific polypeptide of claim 4, wherein the bispecific polypeptide is a dual-specific bifunctional polypeptide and wherein the at least one target protein comprises PCNA.

8. A polynucleotide encoding a bifunctional polypeptide comprising or consisting of the bifunctional polypeptide of claim 3.

9. A polynucleotide encoding a bifunctional polypeptide comprising or consisting of the bifunctional polypeptide of claim 4.

10. A polynucleotide encoding a dual-specific bifunctional polypeptide comprising or consisting of the dual-specific bifunctional polypeptide of claim 5.

11. A polynucleotide encoding a bifunctional polypeptide comprising or consisting of the bifunctional polypeptide of claim 6.

12. A polynucleotide encoding a dual-specific bifunctional polypeptide comprising or consisting of the bifunctional polypeptide of claim 7.

13. A polypeptide comprising or consisting of an amino acid sequence having at least 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to any one of SEQ ID NO: 184, 192, 200, 208, 216, 224, 232, 240, 248, 256, 264, 272, 280, 288, 296, and 385-577, optionally wherein the sequence lacks the HA tag sequence (SEQ ID NO: 384).

14. A polynucleotide encoding for the polypeptide of claim 13.
15. A pharmaceutical composition comprising the polypeptide comprising or consisting of the anti-Myc sdAb, bifunctional polypeptide, dual-specific bifunctional polypeptide, polypeptide, or polynucleotide of any one of claims 1-14 and one or more pharmaceutically acceptable excipients, carriers, or diluents.
16. A method of treating a subject comprising administering the polypeptide comprising or consisting of the anti-Myc sdAb, bifunctional polypeptide, dual-specific bifunctional polypeptide, polypeptide, polynucleotide, or pharmaceutical composition of any one of claims 1-14 to a subject in need thereof, optionally wherein the subject has a cancer.
17. A method of reducing the amount of a target protein in a cell comprising contacting the cell with the polypeptide comprising or consisting of the anti-Myc sdAb, bifunctional polypeptide, dual-specific bifunctional polypeptide, polypeptide, polynucleotide, or pharmaceutical composition of any one of claims 1-14, optionally wherein the target protein is selected from MYC, (optionally c-MYC), CTNNB1, and/or PCNA.
18. A compound comprising the polypeptide of claim 1, the polypeptide comprising or consisting of the anti-Myc sdAb, and a therapeutic compound and/or drug and/or detectable label conjugated to the polypeptide.

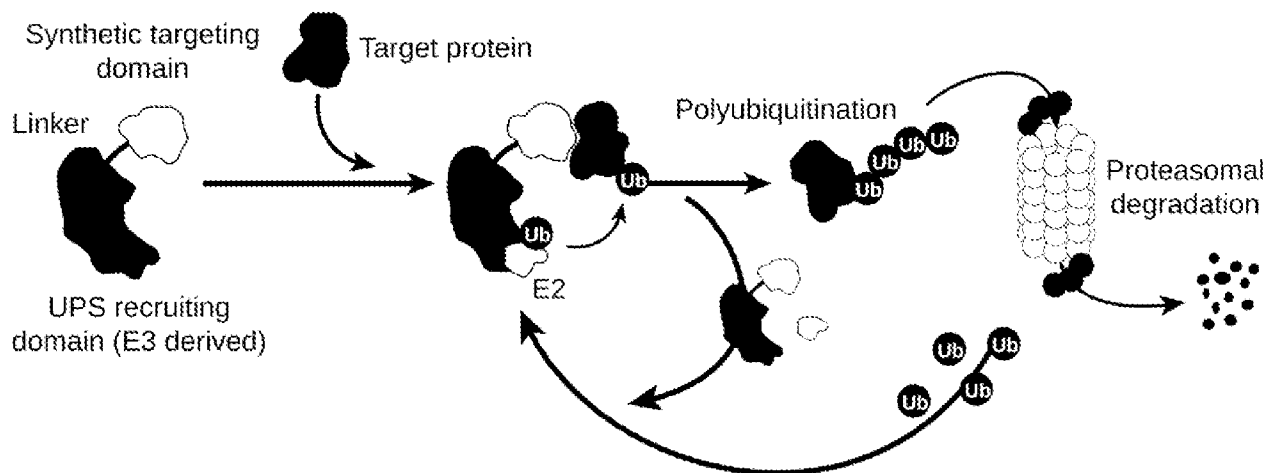


FIG. 1A

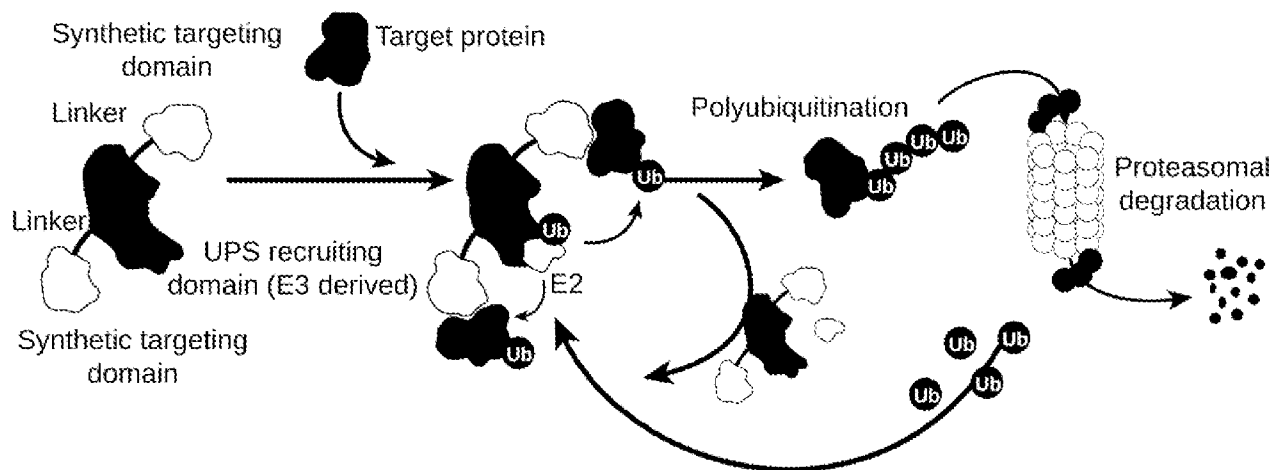


FIG. 1B

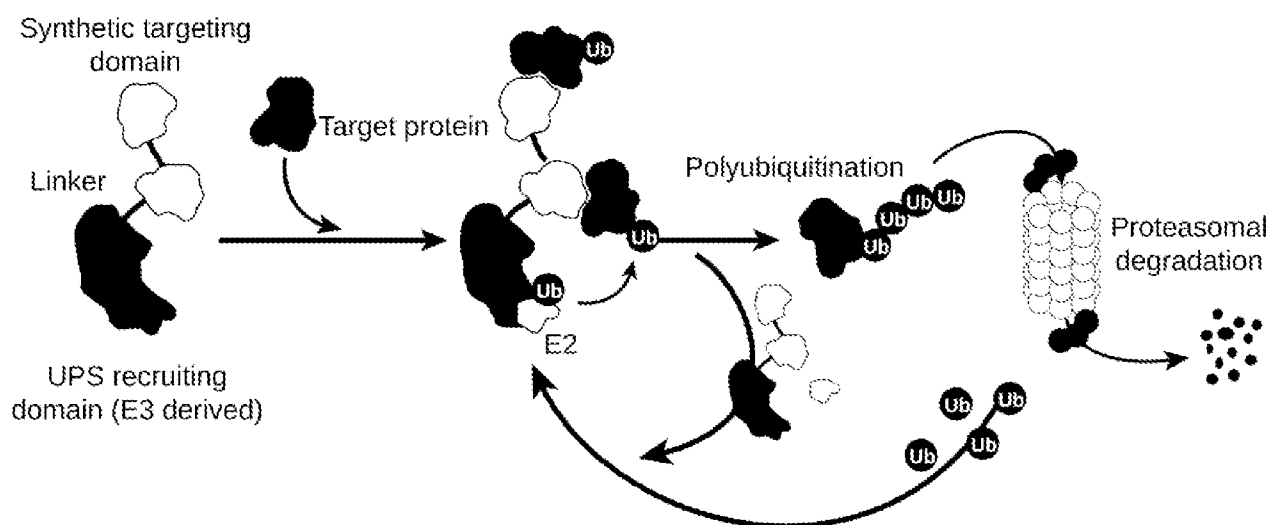


FIG. 1C

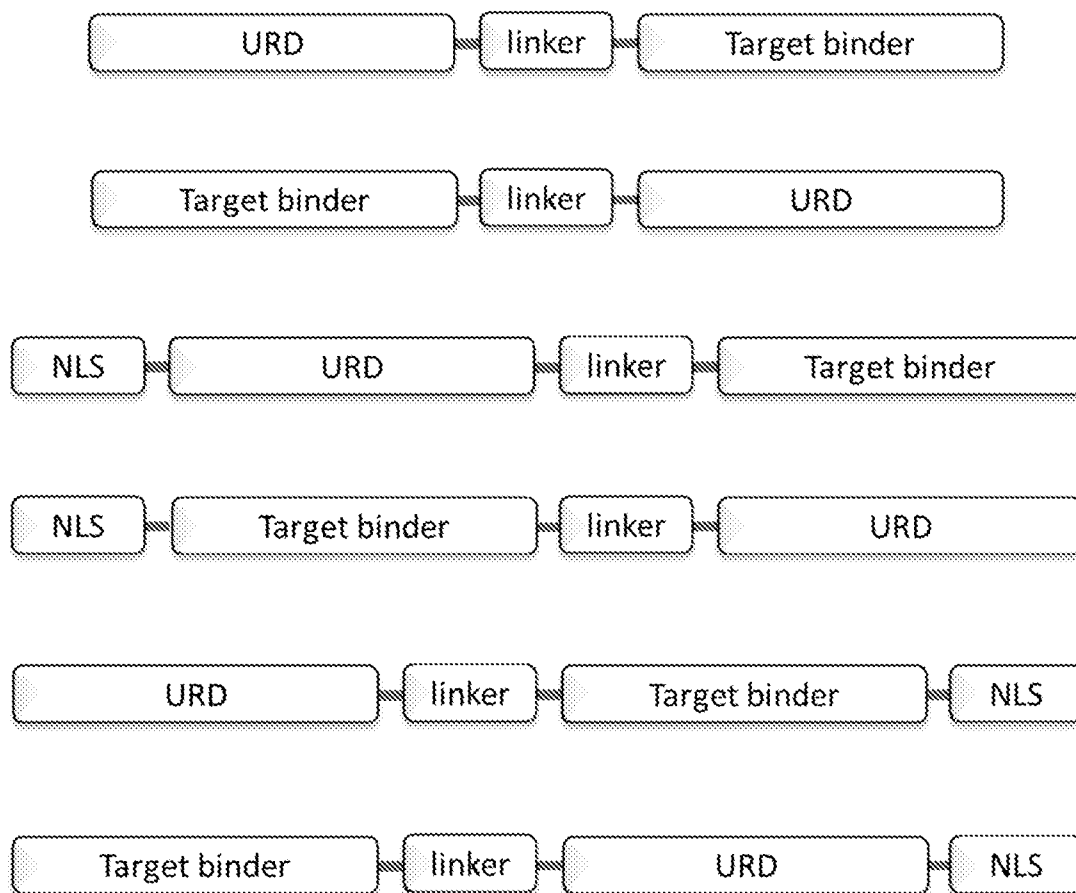


FIG. 2A

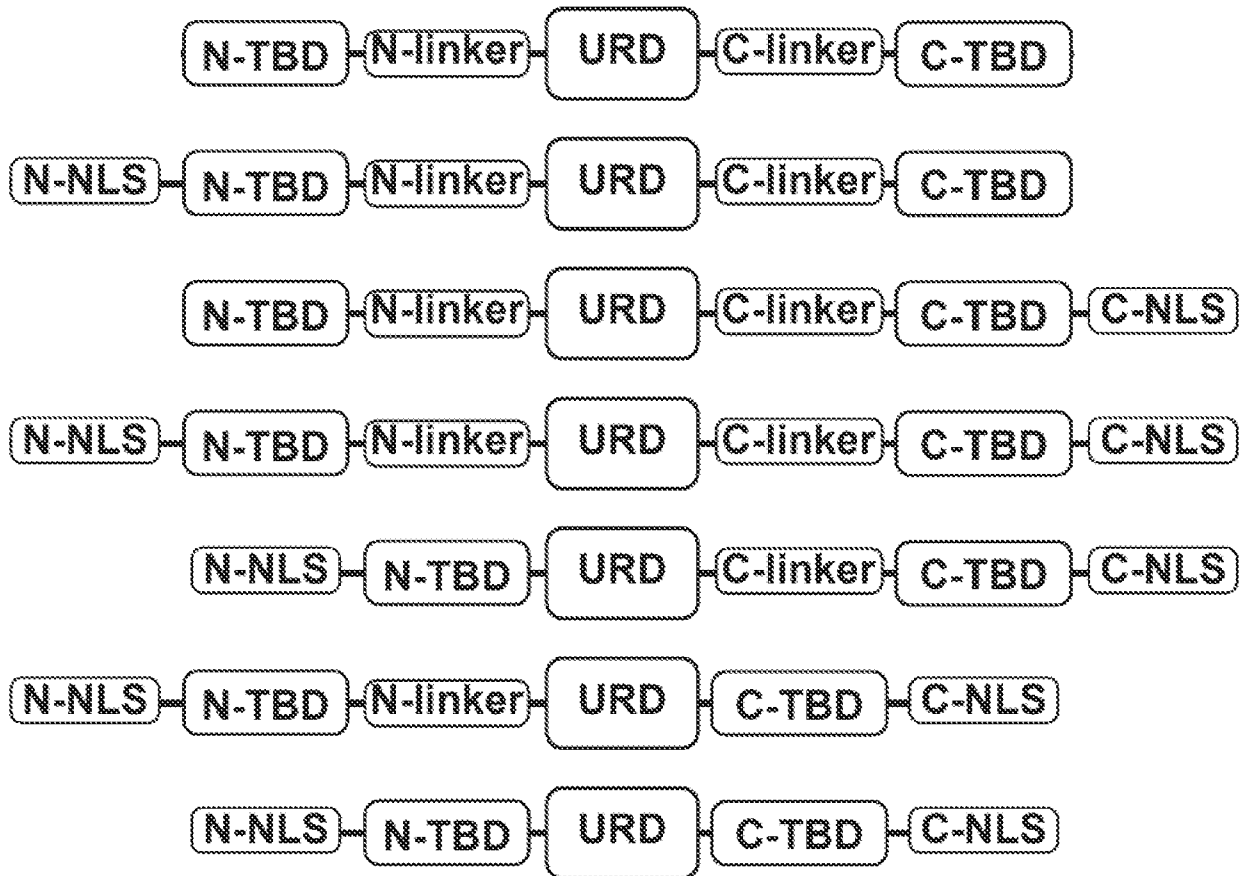


FIG. 2B

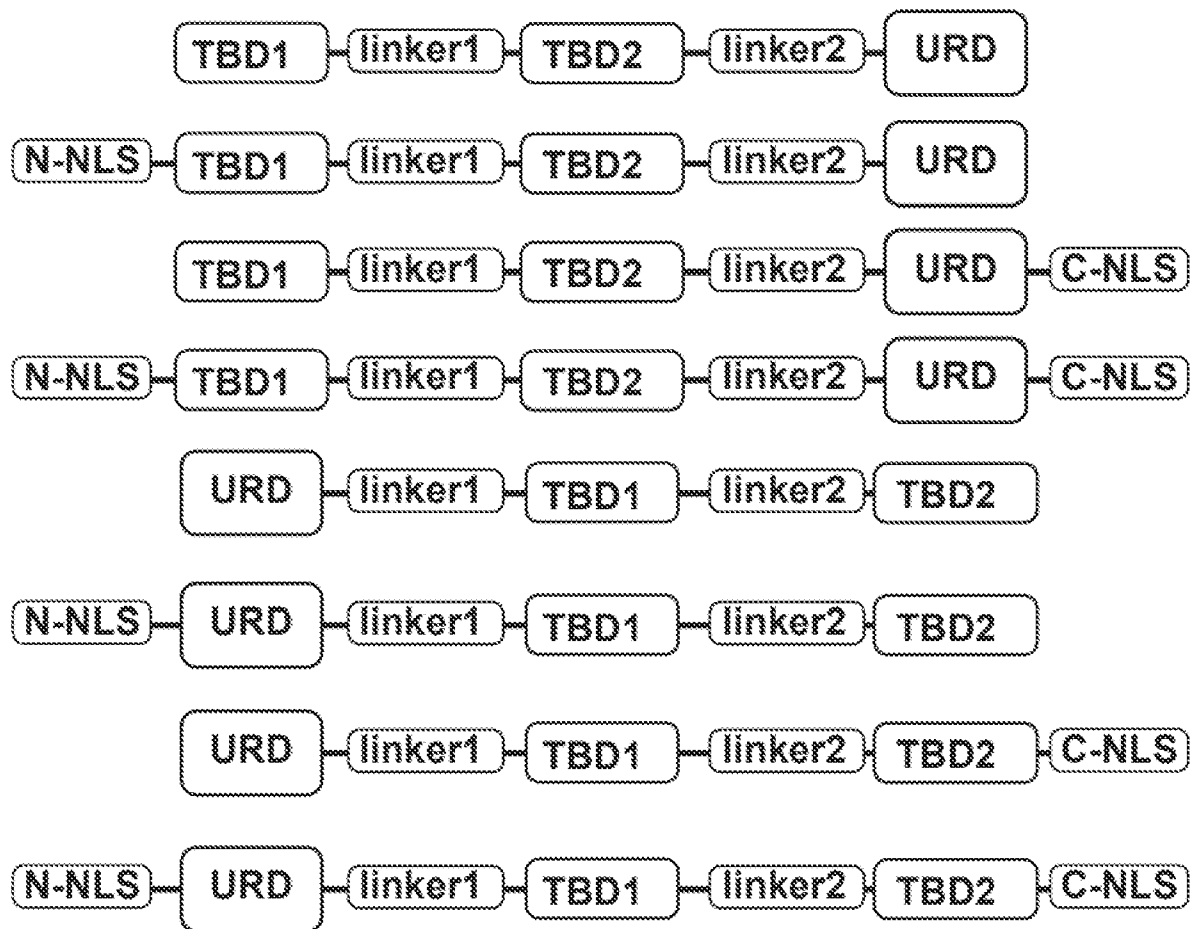
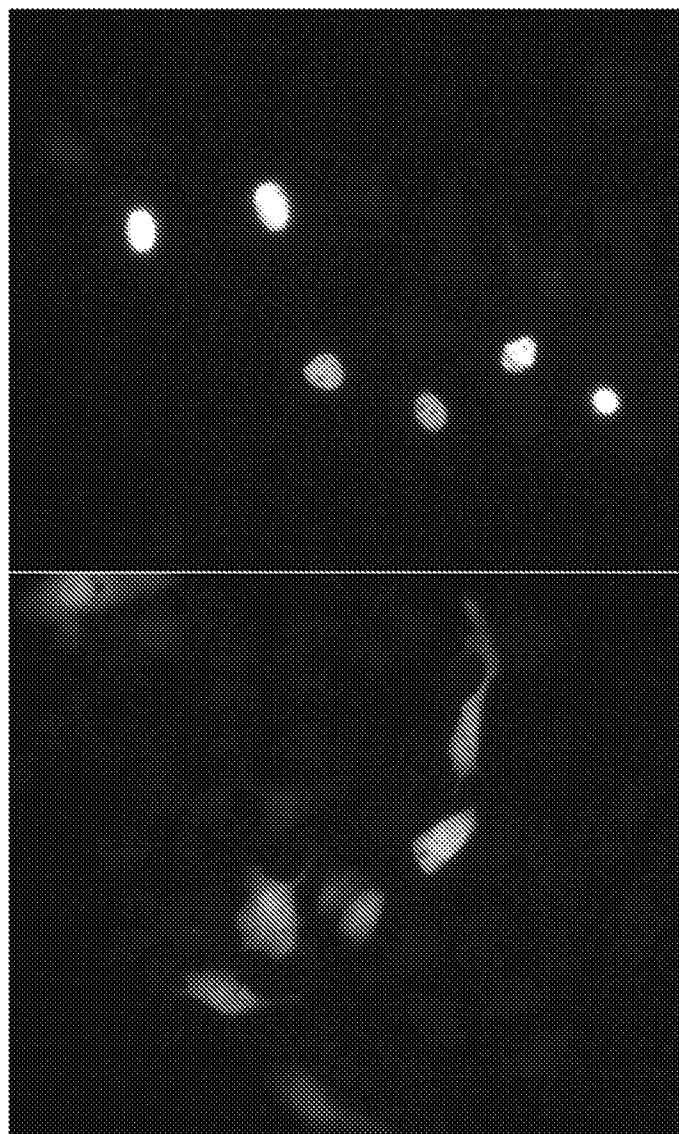


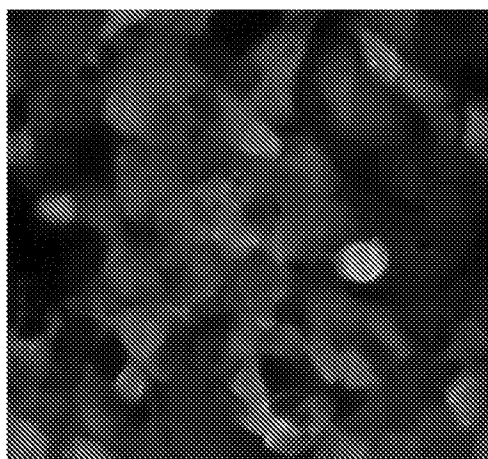
FIG. 2C



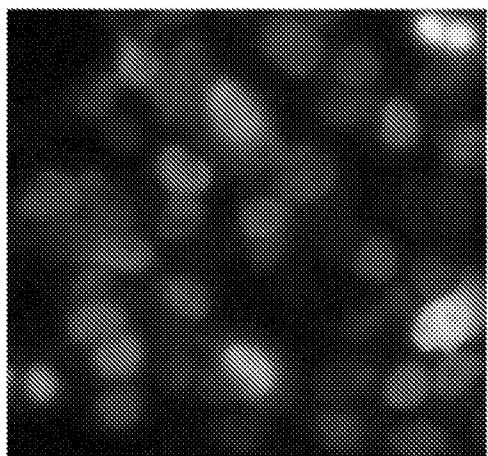
SEQ ID NO:
578
SPOP.2

SEQ ID NO:
579
SPOP.3

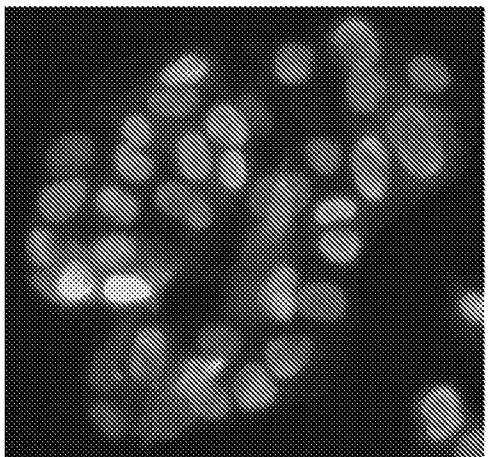
FIG. 3



SEQ ID NO:
580
No NLS



SEQ ID NO:
581
mycNLS



SEQ ID NO:
582
spNLS.2

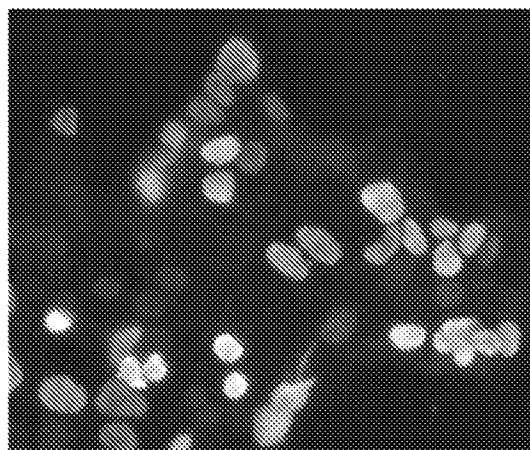
FIG. 4



SEQ ID NO:

583

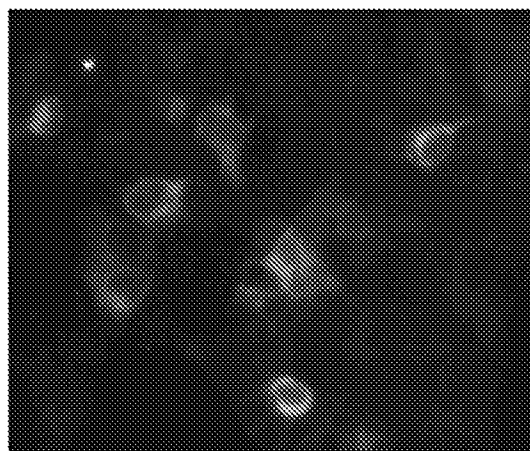
No NLS



SEQ ID NO:

584

FBXWa.1 URD



SEQ ID NO:

585

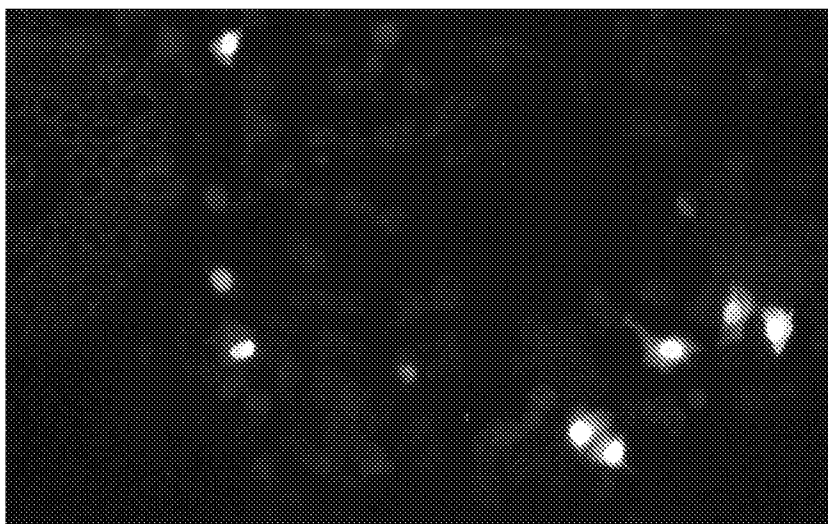
FBXWb.1 URD

FIG. 5

Description	HA staining	SEQ ID NO
HA-mycNLS-TCF4.1-(GS) ₃ -RNF125.2-(GS) ₃ -con1	cytosolic & nuclear	617
HA-mycNLS-TCF4.1-(GS) ₃ -RNF125.2-(GS) ₃ -con1-mycNLS	cytosolic & nuclear	618
HA-mycNLS-TCF4.1-(GS) ₃ -RNF125.2-(GS) ₃ -con1-3xmycNLS	Mix	619
HA-mycNLS-TCF4.1-(GS) ₃ -RNF125.2-(GS) ₃ -con1-svNLS	both but mostly nuclear	620
HA-mycNLS-TCF4.1-(GS) ₃ -RNF125.2-(GS) ₃ -con1-3xsvNLS	nuclear	621
HA-mycNLS-TCF4.1-(GS) ₃ -RNF125.2-(GS) ₃ -con1-nlpNLS	both but mostly nuclear	622
HA-mycNLS-TCF4.1-(GS) ₃ -RNF125.2-(GS) ₃ -con1-bpNLS	cytosolic & nuclear	623
HA-mycNLS-TCF4.1-(GS) ₃ -RNF125.2-(GS) ₃ -con1-hrpNLS	mostly nuclear, some cytosolic	624
HA-mycNLS-TCF4.1-(GS) ₃ -RNF125.2-(GS) ₃ -con1-spNLS	cytosolic & nuclear	625

FIG. 6

9/17



SEQ ID NO: 617

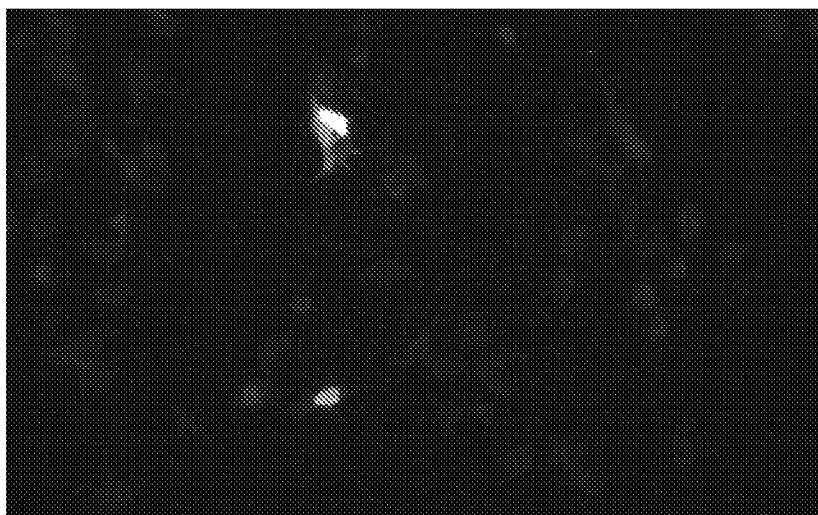


SEQ ID NO: 618

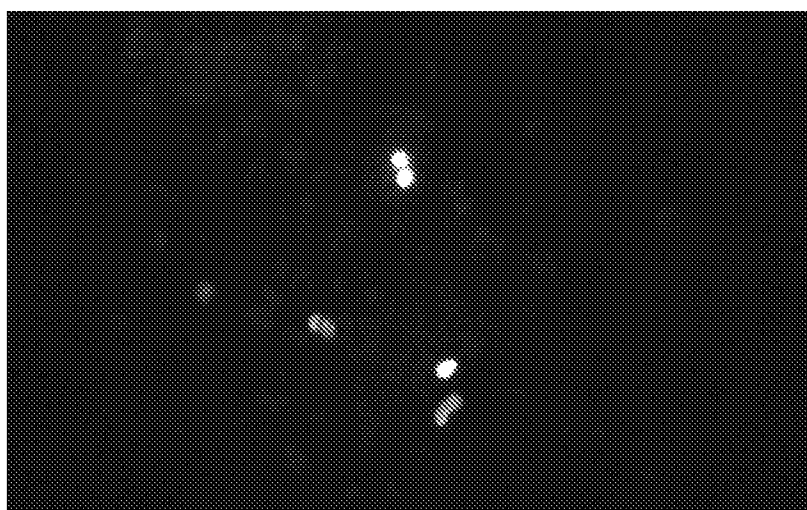


SEQ ID NO: 619

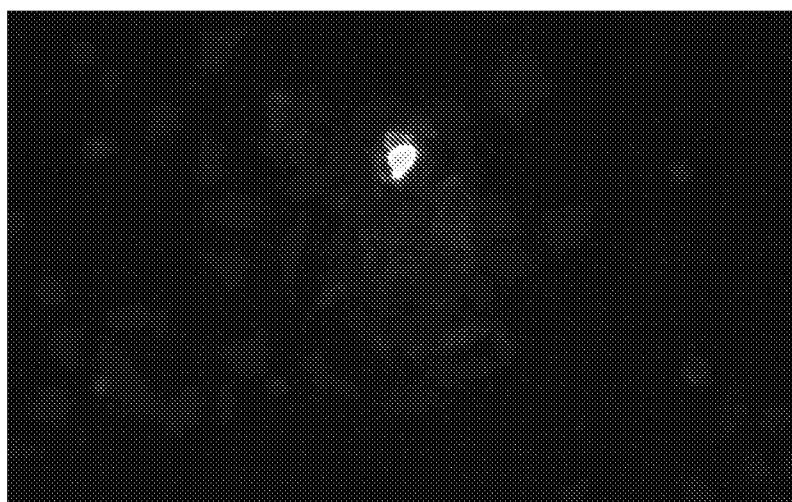
FIG. 7A



SEQ ID NO: 620



SEQ ID NO: 621



SEQ ID NO: 622

FIG. 7B



SEQ ID NO: 623



SEQ ID NO: 624



SEQ ID NO: 625

FIG. 7C

SEQ ID NO	Target	Description
385	MYC	HA-RNF125.2-(GS) ₂ -mycV.0300-hrpNLS
386	MYC	HA-RNF125.2-mycV.0300-hrpNLS
387	MYC	HA-spNLS.2-mycV.0300-(GS) ₂ -RNF4.1
388	MYC	HA-spNLS.2-mycV.0300-RNF4.1
389	MYC	HA-mycNLS-mycV.0300-(GS) ₂ -NHLRC1.1
390	MYC	HA-mycNLS-mycV.0300-NHLRC1.1
391	MYC	HA-mycNLS-mycV.0300-(GS) ₂ -ZNR1.1
392	MYC	HA-mycNLS-mycV.0300-ZNR1.1
393	MYC	HA-RNF125.2-(GS) ₂ -mycV.1300-hrpNLS
394	MYC	HA-RNF125.2-mycV.1300-hrpNLS
395	MYC	HA-spNLS.2-mycV.1300-(GS) ₂ -RNF4.1
396	MYC	HA-spNLS.2-mycV.1300-RNF4.1
397	MYC	HA-mycNLS-mycV.1300-(GS) ₂ -NHLRC1.1
398	MYC	HA-mycNLS-mycV.1300-NHLRC1.1
399	MYC	HA-mycNLS-mycV.1300-(GS) ₂ -ZNR1.1
400	MYC	HA-mycNLS-mycV.1300-ZNR1.1
401	MYC	HA-RNF125.2-(GS) ₂ -mycV.1500-hrpNLS
402	MYC	HA-RNF125.2-mycV.1500-hrpNLS
403	MYC	HA-spNLS.2-mycV.1500-(GS) ₂ -RNF4.1
404	MYC	HA-spNLS.2-mycV.1500-RNF4.1
405	MYC	HA-mycNLS-mycV.1500-(GS) ₂ -NHLRC1.1
406	MYC	HA-mycNLS-mycV.1500-NHLRC1.1
407	MYC	HA-mycNLS-mycV.1500-(GS) ₂ -ZNR1.1
408	MYC	HA-mycNLS-mycV.1500-ZNR1.1
409	MYC	mycNLS-mycV.1500-RNF165.1
410	MYC	mycV.1500-RNF165.1
411	MYC	HA-mycNLS-mycV.1500-(GS) ₃ -RNF165.1
412	MYC	HA-RNF125.2-(GS) ₂ -mycV.3700-hrpNLS
413	MYC	HA-RNF125.2-mycV.3700-hrpNLS
414	MYC	HA-spNLS.2-mycV.3700-(GS) ₂ -RNF4.1
415	MYC	HA-spNLS.2-mycV.3700-RNF4.1
416	MYC	HA-mycNLS-mycV.3700-(GS) ₂ -NHLRC1.1

FIG. 8

417	MYC	HA-mycNLS-mycV.3700-NHLRC1.1
418	MYC	HA-mycNLS-mycV.3700-(GS) ₂ -ZNR1.1
419	MYC	HA-mycNLS-mycV.3700-ZNR1.1
420	MYC	HA-RNF125.2-(GS) ₂ -mycV.6600-hrpNLS
421	MYC	HA-RNF125.2-mycV.6600-hrpNLS
422	MYC	HA-spNLS.2-mycV.6600-(GS) ₂ -RNF4.1
423	MYC	HA-spNLS.2-mycV.6600-RNF4.1
424	MYC	HA-mycNLS-mycV.6600-(GS) ₂ -NHLRC1.1
425	MYC	HA-mycNLS-mycV.6600-NHLRC1.1
426	MYC	HA-mycNLS-mycV.6600-(GS) ₂ -ZNR1.1
427	MYC	HA-mycNLS-mycV.6600-ZNR1.1
428	MYC	HA-RNF125.2-(GS) ₂ -mycV.0302-hrpNLS
429	MYC	HA-RNF125.2-mycV.0302-hrpNLS
430	MYC	HA-spNLS.2-mycV.0302-(GS) ₂ -RNF4.1
431	MYC	HA-spNLS.2-mycV.0302-RNF4.1
432	MYC	HA-mycNLS-mycV.0302-(GS) ₂ -NHLRC1.1
433	MYC	HA-mycNLS-mycV.0302-NHLRC1.1
434	MYC	HA-mycNLS-mycV.0302-(GS) ₂ -ZNR1.1
435	MYC	HA-mycNLS-mycV.0302-ZNR1.1
436	MYC	spNLS.2-mycV.0302-(GS) ₂ -RNF4.1
437	MYC	mycNLS-mycV.0302-RNF165.1
438	MYC	HA-RNF125.2-(GS) ₂ -mycV.0305-hrpNLS
439	MYC	HA-RNF125.2-mycV.0305-hrpNLS
440	MYC	HA-spNLS.2-mycV.0305-(GS) ₂ -RNF4.1
441	MYC	HA-spNLS.2-mycV.0305-RNF4.1
442	MYC	HA-mycNLS-mycV.0305-(GS) ₂ -NHLRC1.1
443	MYC	HA-mycNLS-mycV.0305-NHLRC1.1
444	MYC	HA-mycNLS-mycV.0305-(GS) ₂ -ZNR1.1
445	MYC	HA-mycNLS-mycV.0305-ZNR1.1
446	MYC	HA-RNF125.2-(GS) ₂ -mycV.0306-hrpNLS
447	MYC	HA-RNF125.2-mycV.0306-hrpNLS
448	MYC	HA-spNLS.2-mycV.0306-(GS) ₂ -RNF4.1
449	MYC	HA-spNLS.2-mycV.0306-RNF4.1
450	MYC	HA-mycNLS-mycV.0306-(GS) ₂ -NHLRC1.1

FIG. 8 (continued)

451	MYC	HA-mycNLS-mycV.0306-NHLRC1.1
452	MYC	HA-mycNLS-mycV.0306-(GS) ₂ -ZNR1.1
453	MYC	HA-mycNLS-mycV.0306-ZNR1.1
454	MYC	HA-RNF125.2-(GS) ₂ -mycV.1308-hrpNLS
455	MYC	HA-RNF125.2-mycV.1308-hrpNLS
456	MYC	HA-spNLS.2-mycV.1308-(GS) ₂ -RNF4.1
457	MYC	HA-spNLS.2-mycV.1308-RNF4.1
458	MYC	HA-mycNLS-mycV.1308-(GS) ₂ -NHLRC1.1
459	MYC	HA-mycNLS-mycV.1308-NHLRC1.1
460	MYC	HA-mycNLS-mycV.1308-(GS) ₂ -ZNR1.1
461	MYC	HA-mycNLS-mycV.1308-ZNR1.1
462	MYC	HA-RNF125.2-(GS) ₂ -mycV.1517-hrpNLS
463	MYC	HA-RNF125.2-mycV.1517-hrpNLS
464	MYC	HA-spNLS.2-mycV.1517-(GS) ₂ -RNF4.1
465	MYC	HA-spNLS.2-mycV.1517-RNF4.1
466	MYC	HA-mycNLS-mycV.1517-(GS) ₂ -NHLRC1.1
467	MYC	HA-mycNLS-mycV.1517-NHLRC1.1
468	MYC	HA-mycNLS-mycV.1517-(GS) ₂ -ZNR1.1
469	MYC	HA-mycNLS-mycV.1517-ZNR1.1
470	MYC	spNLS.2-mycV.1517-RNF4.1
471	MYC	mycNLS-mycV.1517-RNF165.1
472	MYC	spNLS.2-mycV.1517-(GS) ₂ -RNF4.1
473	MYC	HA-RNF125.2-(GS) ₂ -mycV.1518-hrpNLS
474	MYC	HA-RNF125.2-mycV.1518-hrpNLS
475	MYC	HA-spNLS.2-mycV.1518-(GS) ₂ -RNF4.1
476	MYC	HA-spNLS.2-mycV.1518-RNF4.1
477	MYC	HA-mycNLS-mycV.1518-(GS) ₂ -NHLRC1.1
478	MYC	HA-mycNLS-mycV.1518-NHLRC1.1
479	MYC	HA-mycNLS-mycV.1518-(GS) ₂ -ZNR1.1
480	MYC	HA-mycNLS-mycV.1518-ZNR1.1
481	MYC	HA-RNF125.2-(GS) ₂ -mycV.1519-hrpNLS
482	MYC	HA-RNF125.2-mycV.1519-hrpNLS
483	MYC	HA-spNLS.2-mycV.1519-(GS) ₂ -RNF4.1
484	MYC	HA-spNLS.2-mycV.1519-RNF4.1

FIG. 8 (continued)

485	MYC	HA-mycNLS-mycV.1519-(GS) ₂ -NHLRC1.1
486	MYC	HA-mycNLS-mycV.1519-NHLRC1.1
487	MYC	HA-mycNLS-mycV.1519-(GS) ₂ -ZNR1.1
488	MYC	HA-mycNLS-mycV.1519-ZNR1.1
489	MYC	spNLS.2-mycV.1519-RNF4.1
490	MYC	mycNLS-mycV.1519-RNF165.1
491	MYC	mycNLS-mycV.1519-(GS) ₂ -NHLRC1.1
492	MYC	spNLS.2-mycV.1519-(GS) ₂ -RNF4.1
493	MYC	HA-spNLS.2-mycV.1519-GS-RNF4.1
494	MYC	HA-spNLS.2-mycV.1519-(GS) ₃ -RNF4.1
495	MYC	HA-spNLS.2-mycV.1519-(GS) ₃ -RNF4.1
496	MYC	HA-spNLS.2-mycV.1519-(GS) ₃ -RNF4.1
497	MYC	HA-spNLS.2-mycV.1519-(GS) ₃ -RNF4.1
498	MYC	HA-RNF125.2-(GS) ₂ -mycV.1520-hrpNLS
499	MYC	HA-RNF125.2-mycV.1520-hrpNLS
500	MYC	HA-spNLS.2-mycV.1520-(GS) ₂ -RNF4.1
501	MYC	HA-spNLS.2-mycV.1520-RNF4.1
502	MYC	HA-mycNLS-mycV.1520-(GS) ₂ -NHLRC1.1
503	MYC	HA-mycNLS-mycV.1520-NHLRC1.1
504	MYC	HA-mycNLS-mycV.1520-(GS) ₂ -ZNR1.1
505	MYC	HA-mycNLS-mycV.1520-ZNR1.1
506	MYC	HA-RNF125.2-(GS) ₂ -mycV.1522-hrpNLS
507	MYC	HA-RNF125.2-mycV.1522-hrpNLS
508	MYC	HA-spNLS.2-mycV.1522-(GS) ₂ -RNF4.1
509	MYC	HA-spNLS.2-mycV.1522-RNF4.1
510	MYC	HA-mycNLS-mycV.1522-(GS) ₂ -NHLRC1.1
511	MYC	HA-mycNLS-mycV.1522-NHLRC1.1
512	MYC	HA-mycNLS-mycV.1522-(GS) ₂ -ZNR1.1
513	MYC	HA-mycNLS-mycV.1522-ZNR1.1
514	MYC	HA-RNF125.2-(GS) ₂ -mycV.1524-hrpNLS
515	MYC	HA-RNF125.2-mycV.1524-hrpNLS
516	MYC	HA-spNLS.2-mycV.1524-(GS) ₂ -RNF4.1
517	MYC	HA-spNLS.2-mycV.1524-RNF4.1
518	MYC	HA-mycNLS-mycV.1524-(GS) ₂ -NHLRC1.1

FIG. 8 (continued)

519	MYC	HA-mycNLS-mycV.1524-NHLRC1.1
520	MYC	HA-mycNLS-mycV.1524-(GS) ₂ -ZNRF1.1
521	MYC	HA-mycNLS-mycV.1524-ZNRF1.1
522	MYC	HA-RNF125.2-(GS) ₂ -mycV.3706-hrpNLS
523	MYC	HA-RNF125.2-mycV.3706-hrpNLS
524	MYC	HA-spNLS.2-mycV.3706-(GS) ₂ -RNF4.1
525	MYC	HA-spNLS.2-mycV.3706-RNF4.1
526	MYC	HA-mycNLS-mycV.3706-(GS) ₂ -NHLRC1.1
527	MYC	HA-mycNLS-mycV.3706-NHLRC1.1
528	MYC	HA-mycNLS-mycV.3706-(GS) ₂ -ZNRF1.1
529	MYC	HA-mycNLS-mycV.3706-ZNRF1.1
530	MYC	HA-RNF125.2-(GS) ₂ -mycV.3707-hrpNLS
531	MYC	HA-RNF125.2-mycV.3707-hrpNLS
532	MYC	HA-spNLS.2-mycV.3707-(GS) ₂ -RNF4.1
533	MYC	HA-spNLS.2-mycV.3707-RNF4.1
534	MYC	HA-mycNLS-mycV.3707-(GS) ₂ -NHLRC1.1
535	MYC	HA-mycNLS-mycV.3707-NHLRC1.1
536	MYC	HA-mycNLS-mycV.3707-(GS) ₂ -ZNRF1.1
537	MYC	HA-mycNLS-mycV.3707-ZNRF1.1
538	MYC	HA-RNF125.2-(GS) ₂ -mycV.3708-hrpNLS
539	MYC	HA-RNF125.2-mycV.3708-hrpNLS
540	MYC	HA-spNLS.2-mycV.3708-(GS) ₂ -RNF4.1
541	MYC	HA-spNLS.2-mycV.3708-RNF4.1
542	MYC	HA-mycNLS-mycV.3708-(GS) ₂ -NHLRC1.1
543	MYC	HA-mycNLS-mycV.3708-NHLRC1.1
544	MYC	HA-mycNLS-mycV.3708-(GS) ₂ -ZNRF1.1
545	MYC	HA-mycNLS-mycV.3708-ZNRF1.1
546	MYC	HA-RNF125.2-(GS) ₂ -mycV.6605-hrpNLS
547	MYC	HA-RNF125.2-mycV.6605-hrpNLS
548	MYC	HA-spNLS.2-mycV.6605-(GS) ₂ -RNF4.1
549	MYC	HA-spNLS.2-mycV.6605-RNF4.1
550	MYC	HA-mycNLS-mycV.6605-(GS) ₂ -NHLRC1.1
551	MYC	HA-mycNLS-mycV.6605-NHLRC1.1
552	MYC	HA-mycNLS-mycV.6605-(GS) ₂ -ZNRF1.1

FIG. 8 (continued)

553	MYC	HA-mycNLS-mycV.6605-ZNRF1.1
554	MYC	HA-RNF125.2-(GS) ₂ -mycV.6608-hrpNLS
555	MYC	HA-RNF125.2-mycV.6608-hrpNLS
556	MYC	HA-spNLS.2-mycV.6608-(GS) ₂ -RNF4.1
557	MYC	HA-spNLS.2-mycV.6608-RNF4.1
558	MYC	HA-mycNLS-mycV.6608-(GS) ₂ -NHLRC1.1
559	MYC	HA-mycNLS-mycV.6608-NHLRC1.1
560	MYC	HA-mycNLS-mycV.6608-(GS) ₂ -ZNRF1.1
561	MYC	HA-mycNLS-mycV.6608-ZNRF1.1
562	MYC MYC	mycNLS-mycV.1519-(GS) ₂ -NHLRC1.1-(GS) ₂ -mycV.1519
563	MYC CTNNB1	mycNLS-mycV.1519-(GS) ₂ -NHLRC1.1-(GS) ₂ -TCF4.6
564	CTNNB1 MYC	mycNLS-TCF4.6-(GS) ₃ -NHLRC1.1-(GS) ₂ -mycV.1519
565	MYC MYC	spNLS.2-mycV.1519-(GS) ₂ -RNF4.1-(GS) ₂ -mycV.1519
566	MYC CTNNB1	spNLS.2-mycV.1519-(GS) ₂ -RNF4.1-(GS) ₂ -TCF4.6
567	MYC MYC	mycV.1524-(GS) ₂ -RNF125.2-(GS) ₂ -mycV.1524-hrpNLS
568	CTNNB1 MYC	TCF4.6-(GS) ₂ -RNF125.2-(GS) ₂ -mycV.1524-hrpNLS
569	MYC CTNNB1	mycV.1524-(GS) ₂ -RNF125.2-(GS) ₂ -TCF4.6-hrpNLS
570	MYC MYC	spNLS.2-mycV.1500-(GS) ₂ -RNF4.1-(GS) ₂ -mycV.1519
571	MYC MYC	spNLS.2-mycV.1519-(GS) ₂ -RNF4.1-(GS) ₂ -mycV.1500
572	MYC MYC	spNLS.2-mycV.1519-(GS) ₂ -RNF4.1-(GS) ₂ -mycV.0302
573	MYC MYC	mycNLS-mycV.1519-(GS) ₂ -NHLRC1.1-(GS) ₂ -mycV.0302
574	MYC MYC	mycNLS-mycV.1500-RNF165.1-(GS) ₃ -mycV.0302
575	MYC MYC	mycNLS-mycV.0302-RNF165.1-(GS) ₃ -mycV.1500
576	MYC MYC	spNLS.2-mycV.0302-(GS) ₂ -RNF4.1-(GS) ₂ -mycV.1519
577	MYC MYC	mycNLS-mycV.0302-(GS) ₂ -NHLRC1.1-(GS) ₂ -mycV.1519

FIG. 8 (continued)

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2024/055071

A. CLASSIFICATION OF SUBJECT MATTER

INV. C07K16/32 C07K14/435
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CN 106 831 992 B (UNIV NANCHANG) 16 June 2020 (2020-06-16) example 1; sequence 1 -----	1, 2, 18
X	WO 2022/072255 A1 (MERCK SHARP & DOHME [US]; SEVY ALEXANDER MARIO [US] ET AL.) 7 April 2022 (2022-04-07) page 2, line 25 - line 29 ----- - / - -	13 - 15

☒ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

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"O" document referring to an oral disclosure, use, exhibition or other means

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"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

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Date of the actual completion of the international search

12 February 2025

Date of mailing of the international search report

25/02/2025

Name and mailing address of the ISA/
European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040.
Fax: (+31-70) 340-3016

Authorized officer

Pflug, Alexander

INTERNATIONAL SEARCH REPORT

International application No

PCT/US2024/055071

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	SHUHUI LIM ET AL: "bioPROTACs as versatile modulators of intracellular therapeutic targets including proliferating cell nuclear antigen (PCNA)", PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES (PNAS), vol. 117, no. 11, 2 March 2020 (2020-03-02), pages 5791-5800, XP055766456, ISSN: 0027-8424, DOI: 10.1073/pnas.1920251117 abstract; figure 1 -----	3-12,16, 17
X	HATAKEYAMA SHIGETSUGU ET AL: "Targeted Destruction of c-Myc by an Engineered Ubiquitin Ligase Suppresses Cell Transformation and Tumor Formation", CANCER RESEARCH, vol. 65, no. 17, 1 September 2005 (2005-09-01), pages 7874-7879, XP093239707, ISSN: 0008-5472, DOI: 10.1158/0008-5472.CAN-05-1581 Retrieved from the Internet: URL:https://cancerres.aacrjournals.org/content/65/17/7874.full-text.pdf> abstract; figure 4 -----	3-12,16, 17
A,P	WO 2023/215839 A1 (76BIO INC [US]) 9 November 2023 (2023-11-09) tables 10,11,30 -----	1-18
A,P	WO 2024/081913 A1 (76BIO INC [US]) 18 April 2024 (2024-04-18) tables 5,8,19 -----	1-18

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2024/055071

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:
 - a. ☒ forming part of the international application as filed.
 - b. ☐ furnished subsequent to the international filing date for the purposes of international search (Rule 13*ter*.1(a)).

☐ accompanied by a statement to the effect that the sequence listing does not go beyond the disclosure in the international application as filed.
2. ☐ With regard to any nucleotide and/or amino acid sequence disclosed in the international application, this report has been established to the extent that a meaningful search could be carried out without a WIPO Standard ST.26 compliant sequence listing.
3. Additional comments:

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2024/055071

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
CN 106831992	B	16-06-2020	NONE

WO 2022072255	A1	07-04-2022	EP 4221750 A1 09-08-2023
			US 2023365667 A1 16-11-2023
			WO 2022072255 A1 07-04-2022

WO 2023215839	A1	09-11-2023	NONE

WO 2024081913	A1	18-04-2024	NONE
