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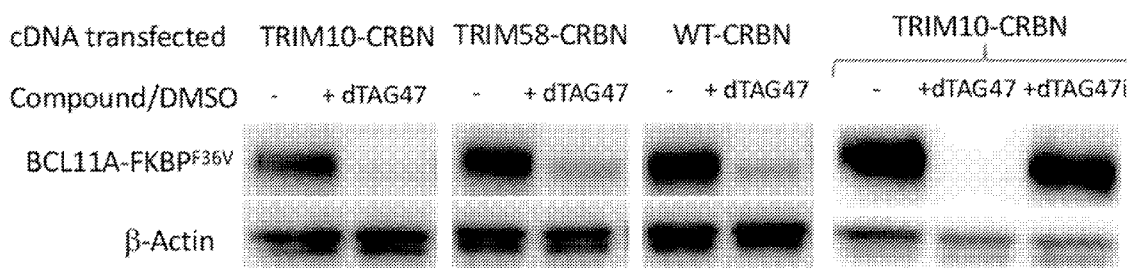
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(54) Title: COMPOSITIONS AND METHODS FOR TARGETED PROTEIN DEGRADATION

Fig. 3



Host: CRBN^{-/-} 293T cells

(57) Abstract: Described herein are compositions and methods for targeted erythroid-specific protein degradation. The compositions described comprise a binding domain for a erythroid protein of interest, e.g., BCL11A, and a binding domain for a erythroid enriched E3 ubiquitin ligase. Compositions and methods described can be used to promote fetal hemoglobin and treat hemoglobinopathy disorders.

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COMPOSITIONS AND METHODS FOR TARGETED PROTEIN DEGRADATION

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application includes a claim of priority under 35 U.S.C. 119(e) to U.S. provisional patent application No. 63/339,208, filed May 6, 2022, the entirety of which is hereby incorporated by reference.

SEQUENCE LISTING

[0002] The instant application contains a Sequence Listing that has been submitted in XML format via Patent Center and is hereby incorporated by reference in its entirety. Said XML copy, created on May 4, 2023, is named “701039-191700WOPT_SL2.xml” and is 93,396 bytes in size.

GOVERNMENT SUPPORT

[0003] This invention was made with government support under Grant Number HL032259, awarded by the National Institutes of Health. The Government has certain rights in the invention.

BACKGROUND

[0004] Targeted protein degradation (TPD) in cells is most commonly achieved with molecular glues or proteolysis-targeting chimeras (PROTACs). In the majority of instances, PROTACs leverage widely expressed E3 ubiquitin ligases (or their associated subunits), e.g. cereblon or von Hippel-Lindau (VHL) proteins, to recruit a protein of interest (POI) to the proteasome for degradation. In order to restrict TPD to specific cells, rather than risk TPD in many different cell types, it would be advantageous to leverage cell-restricted E3 ubiquitin ligases.

SUMMARY

[0005] The technology described herein relates, in part, to the discovery that certain ubiquitin ligases are enriched for expression in cells of the erythroid lineage. Where ubiquitination marks proteins for ubiquitin-mediated degradation, this discovery provides approaches for the targeted degradation of a desired target polypeptide in an erythroid-preferring manner. In one approach, a bispecific construct including binding domains for a target polypeptide and an

erythroid-enriched E3 ubiquitin ligase can act as a tether, bringing the target polypeptide into close proximity with the erythroid-enriched E3 ubiquitin ligase. Where the E3 ubiquitin ligase involved is expressed in an erythroid-enriched manner, such a construct will function to selectively channel the target polypeptide into the degradation pathway in erythroid cells. In another approach, the E3 ubiquitin ligase itself is fused to a molecule that specifically binds a desired target polypeptide. Similar to the first approach, tethering of the E3 ubiquitin ligase to a degradation target in this manner results in the ubiquitination and subsequent degradation of the tethered target. Compositions for targeting desired polypeptides for degradation and methods of using them are described herein. In various embodiments, the constructs described herein can be used therapeutically to target desired polypeptides expressed in erythroid cells for degradation. Non-limiting examples include the transcription factor BCL11A, which is involved in repression of fetal hemoglobin (HbF) expression in adult erythroid lineage cells. Targeting compositions and methods described herein can promote the degradation of BCL11A in erythroid cells, thereby promoting re-expression of HbF in those cells, which can provide therapeutic benefit for subjects suffering from β -hemoglobinopathies.

[0006] In one aspect, described herein is a fusion polypeptide comprising a binding domain that specifically binds an erythroid-enriched E3 ubiquitin ligase and a binding domain that specifically binds an erythroid-enriched polypeptide of interest.

[0007] In one embodiment of this and any other aspect described herein, the fusion polypeptide mediates degradation of the polypeptide of interest in erythroid cells.

[0008] In another embodiment of this and any other aspect described herein, the binding domain that specifically binds an erythroid-enriched E3 ubiquitin ligase specifically binds TRIM10 or TRIM58.

[0009] In another embodiment of this and any other aspect described herein, the binding domain that specifically binds an erythroid-enriched E3 ubiquitin ligase comprises an antibody or antigen-binding fragment thereof. In another embodiment of this and any other aspect described herein, the antibody or antigen-binding fragment thereof comprises an scFv, a single domain antibody or a nanobody.

[00010] In another embodiment of this and any other aspect described herein, the binding domain that specifically binds an erythroid-enriched polypeptide of interest comprises an antibody or antigen-binding fragment thereof. In another embodiment of this and any other

aspect described herein, the antibody or antigen-binding fragment thereof comprises an scFv, a single domain antibody or a nanobody.

[00011] In another embodiment of this and any other aspect described herein, the polypeptide of interest is selected from BCL11A, LRF and ZNF410.

[00012] In another embodiment of this and any other aspect described herein, the E3 ubiquitin ligase is TRIM10 or TRIM58.

[00013] In another embodiment of this and any other aspect described herein, the polypeptide of interest is BCL11A, and the binding domain that specifically binds the polypeptide of interest binds BCL11A at an epitope comprised by zinc-finger 6 (ZNF6) of the BCL11A polypeptide.

[00014] In another embodiment of this and any other aspect described herein, the polypeptide of interest is BCL11A, and the binding domain that specifically binds the polypeptide of interest binds BCL11A at an epitope comprised by zinc finger 23 (ZNF23) of the BCL11A polypeptide. With regard to nomenclature, as used herein, “ZNF 23” is shorthand for zinc fingers 2 and 3, or a polypeptide fragment of BCL11A including those zinc fingers, and “ZNF456” is shorthand for zinc fingers 4, 5 and 6, or a polypeptide fragment of BCL11A including those zinc fingers.

[00015] In another embodiment of this and any other aspect described herein, the polypeptide of interest is BCL11A, and the binding domain that specifically binds the polypeptide of interest has an amino acid sequence at least 90% identical to SEQ ID NO: 12. In another embodiment of this and any other aspect described herein, amino acid sequence variation relative to SEQ ID NO: 12 occurs at one or more of amino acids according to Table 2. In some embodiments, variation relative to SEQ ID NO: 12 occurs only at only one site, two sites, three sites, four sites, five sites, six sites, seven sites, eight sites, 9 sites, 10 sites, 11 sites or 12 sites as set out in Table 2.

[00016] In another embodiment of this and any other aspect described herein, the polypeptide of interest is BCL11A, and the binding domain that specifically binds BCL11A has an amino acid sequence at least 90% identical to SEQ ID NO: 34. In another embodiment of this and any other aspect described herein, amino acid sequence variation relative to SEQ ID NO: 34 occurs at one or more of amino acids at amino acid number 102 or 108. In another embodiment of this and any other aspect described herein, variation occurs only at amino acids 102 or 108 relative to SEQ ID NO: 34.

[00017] In another embodiment of this and any other aspect described herein, the polypeptide of interest is BCL11A, and the binding domain that specifically binds BCL11A comprises a

single-domain antibody in which: CDR1 has an amino acid sequence selected from SEQ ID NOs: 43-50; CDR2 has an amino acid sequence selected from SEQ ID NOs: 51-59; and CDR3 has an amino acid sequence selected from SEQ ID NOs 60-65.

[00018] In another embodiment of this and any other aspect described herein, the polypeptide of interest is BCL11A, and the binding domain that specifically binds BCL11A comprises a single-domain antibody in which: CDR1 has the amino acid sequence SIFVNNAM (SEQ ID NO: 37); CDR2 has the amino acid sequence ELVAAISASGGSTYY (SEQ ID NO: 38); and CDR3 has a sequence selected from ADQDVYPYEW (SEQ ID NO: 39), ADQDGYPYEW (SEQ ID NO: 40) and ADQDVYPYEYL (SEQ ID NO: 41).

[00019] In another embodiment of this and any other aspect described herein, the fusion polypeptide further comprises a cell-penetrating peptide.

[00020] In another embodiment of this and any other aspect described herein, the fusion polypeptide further comprises a linker peptide between the binding domain that specifically binds an erythroid-enriched E3 ubiquitin ligase and the binding domain that specifically binds an erythroid-enriched polypeptide of interest.

[00021] In another aspect, described herein is a nucleic acid comprising sequence encoding a fusion polypeptide as described herein.

[00022] In one embodiment of this and any other aspect described herein, the sequence encoding the fusion polypeptide is operatively linked to regulatory sequences that permit expression in erythroid cells.

[00023] In another aspect, described herein is a vector comprising a nucleic acid comprising sequence encoding a fusion polypeptide as described herein. In one embodiment of this and any other aspect described herein, the vector is a viral vector. In another embodiment of this and any other aspect described herein, the viral vector is an AAV vector.

[00024] In another aspect, described herein is a method of erythroid-specific, targeted degradation of a protein of interest, the method comprising introducing a fusion polypeptide as described herein, a nucleic acid comprising sequence encoding a fusion polypeptide as described herein or a vector comprising sequence encoding a fusion polypeptide as described herein to an erythroid cell.

[00025] In another aspect, described herein is a method of targeted degradation of BCL11A, the method comprising introducing a fusion polypeptide as described herein, a nucleic acid comprising sequence encoding a fusion polypeptide as described herein or a vector comprising sequence encoding a fusion polypeptide as described herein to a cell expressing BCL11A.

[00026] In one embodiment of this and any other aspect described herein, the targeted degradation of BCL11A is targeted to erythroid cells.

[00027] In another aspect, described herein is a method of promoting fetal hemoglobin (HbF) expression in adult erythroid cells, the method comprising introducing a fusion polypeptide as described herein, a nucleic acid comprising sequence encoding a fusion polypeptide as described herein or a vector comprising sequence encoding a fusion polypeptide as described herein to a cell, wherein the protein of interest is BCL11A, and wherein the fusion polypeptide promotes the degradation of BCL11A in erythroid cells, thereby promoting HbF expression.

[00028] In another aspect, described herein is a method of treating a hemoglobinopathy disorder, the method comprising introducing a fusion polypeptide as described herein, a nucleic acid comprising sequence encoding a fusion polypeptide as described herein or a vector comprising sequence encoding a fusion polypeptide as described herein to a subject in need thereof, wherein the protein of interest is BCL11A, and wherein the fusion polypeptide promotes the degradation of BCL11A in erythroid cells, thereby promoting HbF expression to treat the hemoglobinopathy disorder.

[00029] In another aspect, described herein is a fusion polypeptide comprising a TRIM10 or TRIM58 polypeptide fused to a binding domain that specifically binds a target polypeptide.

[00030] In one embodiment of this and any other aspect described herein, the binding domain that specifically binds a target polypeptide comprises an antigen-binding domain of an antibody.

In another embodiment of this and any other aspect described herein, the binding domain that specifically binds a target polypeptide comprises an scFv, a single domain antibody or a nanobody.

[00031] In another embodiment of this and any other aspect described herein, the target polypeptide is expressed in an erythroid cell.

[00032] In another embodiment of this and any other aspect described herein, the target polypeptide is selected from BCL11A, LRF and ZNF410.

[00033] In another embodiment of this and any other aspect described herein, the polypeptide of interest is BCL11A, and the binding domain that specifically binds the polypeptide of interest binds BCL11A at an epitope comprised by zinc-finger 6 (ZNF6) of the BCL11A polypeptide.

[00034] In another embodiment of this and any other aspect described herein, the polypeptide of interest is BCL11A, and the binding domain that specifically binds the polypeptide of

interest binds BCL11A at an epitope comprised by zinc finger 23 (ZNF23) of the BCL11A polypeptide.

[00035] In another embodiment of this and any other aspect described herein, the polypeptide of interest is BCL11A, and the binding domain that specifically binds the polypeptide of interest has an amino acid sequence at least 90% identical to SEQ ID NO: 12. In another embodiment of this and any other aspect described herein, amino acid sequence variation relative to SEQ ID NO: 12 occurs at one or more of amino acids according to Table 2. In some embodiments, variation relative to SEQ ID NO: 12 occurs only at only one site, two sites, three sites, four sites, five sites, six sites, seven sites, eight sites, 9 sites, 10 sites, 11 sites or 12 sites as set out in Table 2.

[00036] In another embodiment of this and any other aspect described herein, the polypeptide of interest is BCL11A, and the binding domain that specifically binds BCL11A has an amino acid sequence at least 90% identical to SEQ ID NO: 34. In another embodiment of this and any other aspect described herein, amino acid sequence variation relative to SEQ ID NO: 34 occurs at one or more of amino acids number 102 or 108. In another embodiment of this and any other aspect described herein, amino acid sequence variation relative to SEQ ID NO: 34 occurs only at amino acids number 102 or 108.

[00037] In another embodiment of this and any other aspect described herein, the polypeptide of interest is BCL11A, and the binding domain that specifically binds BCL11A comprises a single-domain antibody in which: CDR1 has an amino acid sequence selected from SEQ ID NOs: 43-50; CDR2 has an amino acid sequence selected from SEQ ID NOs: 51-59; and CDR3 has an amino acid sequence selected from SEQ ID NOs 60-65.

[00038] In another embodiment of this and any other aspect described herein, the polypeptide of interest is BCL11A, and the binding domain that specifically binds BCL11A comprises a single-domain antibody in which: CDR1 has the amino acid sequence SIFVNNAM (SEQ ID NO: 37);

CDR2 has the amino acid sequence ELVAAISASGGSTYY (SEQ ID NO: 38); and CDR3 has a sequence selected from ADQDVYPYEYW (SEQ ID NO: 39), ADQDGYPYEYW (SEQ ID NO: 40) and ADQDVYPYEYL (SEQ ID NO: 41).

[00039] In another embodiment of this and any other aspect described herein, the fusion polypeptide further comprises a cell-penetrating peptide.

[00040] In another embodiment of this and any other aspect described herein, the fusion polypeptide further comprises a linker peptide between the TRIM10 or TRIM58 polypeptide and the binding domain that specifically binds a target polypeptide.

[00041] In another aspect, described herein is a nucleic acid comprising sequence encoding a fusion polypeptide as described herein.

[00042] In one embodiment of this and any other aspect described herein, the sequence encoding the fusion polypeptide is operatively linked to regulatory sequences that permit expression in erythroid cells.

[00043] In another aspect, described herein is a vector comprising a nucleic acid comprising sequence encoding a fusion polypeptide as described herein. In one embodiment of this and any other aspect described herein, the vector is a viral vector. In another embodiment of this and any other aspect described herein, the viral vector is an AAV vector.

[00044] In another aspect, described herein is a method of erythroid-specific, targeted degradation of a protein of interest, the method comprising introducing a fusion polypeptide as described herein, a nucleic acid encoding a fusion polypeptide as described herein, or a vector comprising a nucleic acid encoding a fusion polypeptide as described herein to an erythroid cell.

[00045] In another aspect, described herein is a method of targeted degradation of BCL11A, the method comprising introducing a fusion polypeptide as described herein, a nucleic acid encoding a fusion polypeptide as described herein, or a vector comprising a nucleic acid encoding a fusion polypeptide as described herein to a cell expressing BCL11A.

[00046] In one embodiment of this and any other aspect described herein, the targeted degradation of BCL11A is targeted to erythroid cells.

[00047] In another aspect, described herein is a method of promoting fetal hemoglobin (HbF) expression in adult erythroid cells, the method comprising introducing a fusion polypeptide as described herein, a nucleic acid encoding a fusion polypeptide as described herein, or a vector comprising a nucleic acid encoding a fusion polypeptide as described herein to a cell, wherein the protein of interest is BCL11A, and wherein the fusion polypeptide promotes the degradation of BCL11A in erythroid cells, thereby promoting HbF expression.

[00048] In another aspect, described herein is a method of treating a hemoglobinopathy disorder, the method comprising introducing a fusion polypeptide as described herein, a nucleic acid encoding a fusion polypeptide as described herein, or a vector comprising a nucleic acid encoding a fusion polypeptide as described herein to a subject in need thereof, wherein the protein of interest is BCL11A, and wherein the fusion polypeptide promotes the degradation of BCL11A in erythroid cells, thereby promoting HbF expression to treat the hemoglobinopathy disorder.

[00049] Unless otherwise defined herein, scientific and technical terms used in connection with the present application shall have the meanings that are commonly understood by those of ordinary skill in the art to which this disclosure belongs. It should be understood that this invention is not limited to the particular methodology, protocols, and reagents, etc., described herein and as such can vary. The terminology used herein is for the purpose of describing particular embodiments only, and is not intended to limit the scope of the present invention, which is defined solely by the claims. Definitions of common terms in immunology, and molecular biology can be found in The Merck Manual of Diagnosis and Therapy, 19th Edition, published by Merck Sharp & Dohme Corp., 2011 (ISBN 978-0-911910-19-3); Robert S. Porter *et al.* (eds.), The Encyclopedia of Molecular Cell Biology and Molecular Medicine, published by Blackwell Science Ltd., 1999-2012 (ISBN 9783527600908); and Robert A. Meyers (ed.), Molecular Biology and Biotechnology: a Comprehensive Desk Reference, published by VCH Publishers, Inc., 1995 (ISBN 1-56081-569-8); Immunology by Werner Luttmann, published by Elsevier, 2006; Janeway's Immunobiology, Kenneth Murphy, Allan Mowat, Casey Weaver (eds.), Taylor & Francis Limited, 2014 (ISBN 0815345305, 9780815345305); Lewin's Genes XI, published by Jones & Bartlett Publishers, 2014 (ISBN-1449659055); Michael Richard Green and Joseph Sambrook, Molecular Cloning: A Laboratory Manual, 4th ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., USA (2012) (ISBN 1936113414); Davis *et al.*, Basic Methods in Molecular Biology, Elsevier Science Publishing, Inc., New York, USA (2012) (ISBN 044460149X); Laboratory Methods in Enzymology: DNA, Jon Lorsch (ed.) Elsevier, 2013 (ISBN 0124199542); Current Protocols in Molecular Biology (CPMB), Frederick M. Ausubel (ed.), John Wiley and Sons, 2014 (ISBN 047150338X, 9780471503385), Current Protocols in Protein Science (CPPS), John E. Coligan (ed.), John Wiley and Sons, Inc., 2005; and Current Protocols in Immunology (CPI) (John E. Coligan, ADA M Kruisbeek, David H Margulies, Ethan M Shevach, Warren Strobe, (eds.) John Wiley and Sons, Inc., 2003 (ISBN 0471142735, 9780471142737), the contents of which are all incorporated by reference herein in their entireties.

[00050] The terms “increased”, “increase”, “enhance”, “activate” are all used herein to refer to an increase by a statistically significant amount. In some embodiments, the terms “increased”, “increase”, “enhance”, or “activate” can mean an increase of at least 10% as compared to a reference level, for example an increase of at least about 20%, or at least about 30%, or at least about 40%, or at least about 50%, or at least about 60%, or at least about 70%, or at least about 80%, or at least about 90% or up to and including a 100% increase or

any increase between 10-100% as compared to a reference level, or at least about a 2-fold, or at least about a 3-fold, or at least about a 4-fold, or at least about a 5-fold or at least about a 10-fold increase, or any increase between 2-fold and 10-fold or greater as compared to a reference level.

[00051] The term “improve” or “improvement,” when applied to a score in a standardized scale or rating, e.g., for disease symptoms or severity, means a statistically significant, favorable change in the scale or rating on that scale.

[00052] The term “decrease”, “reduced”, “reduction”, or “inhibit” are all used herein to mean a decrease by a statistically significant amount. In some embodiments, “decrease”, “reduced”, “reduction”, or “inhibit” typically means a decrease by at least 10% as compared to a reference level, for example, a decrease of at least about 20%, or at least about 30%, or at least about 40%, or at least about 50%, or at least about 60%, or at least about 70%, or at least about 80%, or at least about 90%, or at least about 95% as compared to a reference level. As used herein, “reduction” or “inhibition” does not encompass a complete inhibition or reduction as compared to a reference level. “Complete inhibition” is a 100% inhibition as compared to a reference level.

[00053] As used herein, a “reference level” refers to the level or value for a given parameter against which one compares the level or value in a given sample or situation to determine whether the level or value has changed in a meaningful way. A reference level can be a level in or from a sample that is not treated to change the parameter. A reference level can alternatively be a level in or from a normal or otherwise unaffected sample. A reference level can alternatively be a level in or from a sample obtained from a subject at a prior time point, for example, prior to a given treatment.

[00054] As used herein, an “appropriate control” refers to an untreated, otherwise identical cell or population (e.g., a subject who was not administered an agent described herein, or was administered by only a subset of agents described herein, as compared to a non-control cell).

[00055] As used herein the term “comprising” or “comprises” is used in reference to compositions, methods, and respective component(s) thereof, that are essential to the method or composition, yet open to the inclusion of unspecified elements, whether essential or not.

[00056] As used herein the term “consisting essentially of” refers to those elements required for a given embodiment. The term permits the presence of elements that do not materially affect the basic and novel or functional characteristic(s) of that embodiment. The term “consisting of” refers to compositions, methods, and respective components thereof as

described herein, which are exclusive of any element not recited in that description of the embodiment.

[00057] The singular terms "a," "an," and "the" include plural referents unless context clearly indicates otherwise. Similarly, the word "or" is intended to include "and" unless the context clearly indicates otherwise. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of this disclosure, suitable methods and materials are described below. The abbreviation, "e.g." is derived from the Latin *exempli gratia*, and is used herein to indicate a non-limiting example. Thus, the abbreviation "e.g." is synonymous with the term "for example."

BRIEF DESCRIPTION OF THE FIGURES

[00058] **Figs 1A-1C show erythroid cell enriched expression of TRIM10 and TRIM48** (Fig. 1A) The modular structures of TRIM10 and TRIM58. (Fig. 1B) RNA expression of E3 ligases during cell maturation. (Fig. 1C) Heat-map depicting the relative expression of UBE20, TRIM10, and TRIM58 across cell types. The dots indicate erythroid cells.

[00059] **Fig 2 shows fusion of TRIM10/58 to the recognition domain of cereblon (CRBN).** Schematic showing the fusion proteins of TRIM10/58 with the CRBN recognition domain along with control constructs.

[00060] **Fig 3 shows degradation of BCL11A protein by TRIM10/58.** Western blot for BCL11A depicting fusions of TRIM10/58 with the recognition domain of CRBN were capable of degrading tagged BCL11A upon addition of dTAG47 PROTAC. WT-CRBN served as a positive control and inactive form of dTAG47 served as a negative control.

[00061] **Fig 4 shows degradation of BCL11A protein by TRIM10/58 in HUDEP-2 erythroid cells.** HUDEP-2 cells with CRBN or lacking CRBN were infected with lentiviruses expressing CRBN cDNA or TRIM10 fused with the recognition domain of CRBN and then dTAG47 PROTAC was added. BCL11A protein was degraded in the presence of dTAG47 in CRBN- cells rescued with CRBN cDNA and the fusion of TRIM10 with the recognition domain of CRBN leads to a reduction in BCL11A protein.

[00062] **Fig. 5 shows targeted protein degradation of BCL11A by TRIM10/58 in 293T cells.** Western blot for BCL11A of 293T cells transfected with constructs expressing either TRIM10 or TRIM58 fused to a BCL11A-specific nanobody (Nb19). The nanobody provides the recognition domain to direct the TRIM to BCL11A.

DETAILED DESCRIPTION

[00063] The technology described herein relates, in part, to the discovery that certain ubiquitin ligases are enriched for expression in cells of the erythroid lineage. In particular, it was discovered that the E3 ubiquitin ligases TRIM10 and TRIM58 are expressed in an erythroid-enriched or erythroid-specific manner. Where E3 ubiquitin ligases target proteins in the cells in which they are expressed for degradation via the ubiquitin-mediated proteasome pathway, the identification of erythroid-specific or erythroid enriched E3 ubiquitin ligases provides avenues for erythroid specific, targeted degradation of a protein of interest. The ability to target a protein of interest for degradation in an erythroid-enriched manner has therapeutic implications, in that, as but one example, hemoglobinopathies generally stem from defects in cells of the erythroid lineage, and targeted degradation of, e.g., BCL11A, which represses expression of fetal hemoglobin (HbF), can de-repress and promote the expression of HbF, providing a new source of active hemoglobin.

[00064] Described herein below are constructs for targeting proteins of interest for degradation in cells of the erythroid lineage, as well as formulations and uses of such constructs for the treatment of diseases or disorders including, but not limited to hemoglobinopathies. The following describes the various considerations for one of skill in the art to practice the described technology.

Ubiquitin-mediated protein degradation

[00065] The degradation of proteins occurs mainly through two major pathways: autophagy and the ubiquitin proteasome system (UPS), both of which are critical for maintaining cellular homeostasis (see, e.g., Dikic, I., *Ann. Rev. Biochem.* 86: 193-224 (2017)). The UPS is a cascade reaction, and an important mechanism for the degradation of misfolded and damaged proteins, as well as a regulated mechanism that ensures short lives of certain regulatory proteins, among others. Degradation of a protein via the ubiquitin pathway generally involves two discrete and successive steps: (a) covalent attachment of multiple ubiquitin molecules to the protein substrate — so called “ubiquitination” or “ubiquitylation;” and (b) degradation of the ubiquitin-targeted protein by the 26S proteasome complex, with the release of free and reusable ubiquitin. As used herein, “ubiquitination” or “ubiquitylation” refers to the post-translational modification of a protein by the covalent attachment (via an isopeptide bond) of one or more ubiquitin monomers.

[00066] The ubiquitin-proteasome system consists of several components that act in concert. One of these, ubiquitin, an evolutionarily conserved protein of 76 residues (human ubiquitin has the amino acid sequence:

MQIFVKTLTGKTITLEVEPSDTIENVKAKIQDKEGIPPDQQRLIFAGKQLEDGRTLSDYNIQKESTLHLVLRRLGG (SEQ ID NO: 1), is activated in its C-terminal Gly to a high-energy thiol-ester intermediate. This reaction is catalyzed by the ubiquitin-activating enzyme, E1. After ubiquitin activation, one of several ubiquitin-carrier proteins or ubiquitin-conjugating enzymes, referred to as E2s, transfers the activated ubiquitin moiety from E1 to a member of the ubiquitin-protein ligase family, E3, to which the substrate protein is bound. E3 catalyzes the last step in the conjugation process, covalent attachment of ubiquitin to the substrate protein. The first ubiquitin moiety is transferred to the ϵ -NH₂ group of a lysine residue of the protein substrate to generate an isopeptide bond. In successive reactions, a polyubiquitin chain is generated by processive transfer of additional activated ubiquitin moieties to a lysine residue (one of the most prominently used is Lys48, or K48, but each of K6, K11, K27, K29, K33, K63 and the N-terminal methionine 1 (Met1) are also functional sites in various ubiquitination reactions) of the previously conjugated ubiquitin molecule. The polyubiquitin chain serves as a recognition marker for the 26S proteasome. The binding of the substrate to E3 is generally specific and indicates that E3 ubiquitin ligases play a major role in recognition and selection of proteins for conjugation and subsequent degradation. The structure of the system appears to be hierarchical: a single E1 appears to carry out activation of ubiquitin required for all modifications. Several major species of E2 enzymes have been characterized in mammalian cells, each of which can act with one or more E3 enzymes.

E3 Ubiquitin Ligases

[00067] Hundreds of different E3 ubiquitin ligases have been identified. These are reviewed and classified in, e.g., Yang et al., *Molecular Biomedicine* 2: 23 (2021). Different types of E3 ubiquitin ligases have low sequence homology and large differences in composition. The tripartite motif (TRIM) protein family is a subset of the so-called RING-type E3 ligase subfamily. The TRIM family of E3 ubiquitin ligases is reviewed, e.g., by Esposito et al., *Biochem. Soc. Trans.* 45: 183-191 (2017). As discussed in the Examples herein, it was discovered that two E3 ubiquitin ligases, TRIM10 and TRIM58, are expressed in an erythroid-enriched manner. As used herein, the term “erythroid enriched” refers to the preferential expression and activity of the TRIM10 and TRIM58 E3 ubiquitin ligases in cells of the erythroid lineage. By preferential in this context is meant that TRIM10 and TRIM58

are most highly expressed in cells of this lineage, relative to their expression in other cell types or tissues. The erythroid-enriched expression of TRIM10 and TRIM58 renders these E3 ubiquitin ligase polypeptides useful for the targeted degradation of a protein of interest in an erythroid-enriched manner.

[00068] In some embodiments of any of the aspects, the E3 ubiquitin ligase is TRIM10.

TRIM10 is an E3 ubiquitin ligase that plays an essential role in the differentiation and survival of terminal erythroid cells. Sequences of TRIM10 are known for a number of species, e.g., human TRIM10 (NCBI GeneID: 10107), mRNA (e.g., Isoform 1: NCBI Ref Seq NM_006778.4, **SEQ ID NO: 2**) and polypeptide (e.g., Isoform 1: NCBI Ref Seq: NP_006769.2, **SEQ ID NO: 3**). TRIM10 can refer to human TRIM10, including naturally occurring variants, molecules, and alleles thereof. For example, SEQ ID NO: 2 is a mRNA sequence for TRIM10. Note: U's replaced with T's in this representation.

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1  cttctgacag tggacagctg acatatcacc actttccttc tactgtgagt gtctctggat
61  gggcagaaag gaatggccag cccctgggta tggatcatcta aggtcacctc tgaaatgctg
121 tgagcccctc ttccttcctc tcctctgcta tttcccatct ctgctgttgg caggagaata
181 gaaccctggc tgccagagat gcaagtgtgt gacgatatgg gtgctgggtc atatttagta
241 tgtgcctgtg tccagccatg tgcattgtgt ggtgtgtgag tgtgtgacct agcccttccc
301 ccgtggccaa gcagagagag tggccttgag gaagccatag cagcaggacc agcatggcct
361 ctgctgcctc tgtgaccagc ctggcagatg aagtcaactg ccccatctgt cagggtagcc
421 tgagggagcc ggtcactatc gactgcggcc acaacttctg ccgggcctgc cttaccgct
481 actgtgagat accaggccca gacctggagg agtcccctac ttgcccactc tgcaaagaac
541 ccttcctgctc tgggagcttc cggcccaact ggcagctggc taacgtgggtg gagaacattg
601 agcgcctcca gctggtgtcc aactggggtt tgggagagga ggatgtctgc caagagcacg
661 gagagaagat ctacttcttc tgtgaggatg atgagatgca gttgtgcgtg gtgtgccggg
721 aggctgggga gcacgctacc cacaccatgc gcttcctgga ggatgcagcg gctccctata
781 gggaacaaat ccataagtgt cttaaagtgc taagaaaaga gagagaggag attcaagaaa
841 tccagtcaag agaaaataaa aggatgcaag tcctcctgac tcaggtgtcc accaagagac
901 aacaggtgat ttctgagttc gcacacctga ggaagtttct agaggaacag cagagcatcc
961 tcttagcaca attggagagc caggatgggg acatcttgag gcaacgggat gaatttgatt
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1081 atgagaggcc agcaaggagg ctctgacgg acatcagaag cactctaata agatgtgaaa
1141 ccagaaagtg ccggaaaccg gtggctgtgt cgccagagct gggccagagg attcgggact
1201 ttccccagca ggccctcccg ctgcagaggg agatgaagat gtttctggaa aaactatgct
1261 ttgagttgga ctatgagcca gctcacatct ctctagacct tcagacttcc caccccaagc

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1321 tcctcttgtc cgaggaccac cagcgagctc agttctccta caaatggcag aactcaccag
 1381 acaacccccca acgttttgac cgggccacct gtgttctggc ccacactggc atcacagggg
 1441 ggagacacac gtgggtggtg agtatagacc tggcccatgg gggcagctgc accgtgggcg
 1501 tgggtgagcga ggatgtgcag cggaaggggg agcttcggct gcggccagag gaggggggtgt
 1561 gggctgtgag gctggcttggt ggcttcgtct cggctctggg ctcttcccc acacggctga
 1621 ccctgaagga gcagccccgg caggtgaggg tgtctcttga ctatgaggtg ggctgggtga
 1681 ccttcaccaa cgctgtcacc cgagagccca tctacacctt cactgcctcc ttcactagga
 1741 aggtcattcc cttctttggg ctctggggcc gaggggtccag tttctccctg agctcctgag
 1801 aaggagcagt tacctactct cctctaagta caggactcat atcaacccaa gtacatgtg
 1861 gacttgatcc ctggctgaat cacctggatg acttggaata gaaatgactg ctttagaaga
 1921 tgggatgggg ccgggtggta agggatagaa gagaggactc tcaatctact gatcaagtcc
 1981 tttccccaat gcccagtgga tggccagggt acctggggac tcaggctgct gccagttctg
 2041 ctccaccacca tccgtgcttg gcacagaagt agctgcatag aaagggcact ggatttgaag
 2101 tcagaagacc tgggttcttg aaccagcctg tcaaccagtt gtatgacttt aaacaaggca
 2161 tctcacctct tttcatcttg ttttcttcca ataagttag agttcatgta atcacattct
 2221 ctagaaccat ttagtttgtg ttaactatga accaagcagt gtgggtggcca ctggtgactt
 2281 gaaaatatag agaaaaaaaa aacctgctct atatctgaaa gagctcttgg gaagacagag
 2341 aacataaag aggaaattac agcacagtgt ggtgggtgtt acgggaagtc caacccagc
 2401 attatgggag ttcaggggaa ggggcatagc ccagcctgga aggagaggggt gagtggggga
 2461 tggctttctg aaaaggggtg tctaaaggat gcctatggc aatagggaaa agaggggaag
 2521 aagcatctta agaagaggaa acagcagaga actggctgga tgacctgtga ctctggagca
 2581 ctgggttgtc tccattgtca ttatgggcag atgtgtgcca tcccagccg acgccactca
 2641 ctgcctcctt cctcctgggtg ttgccacttc tgggtgagat taaggtgcag ggctggggg
 2701 caggaggaca taaggtatag ccataaatca taaccagggt accacactca accctagggg
 2761 aattgtcttc ctgatcagtt gattaccatc tgagggtcaag aaatgagata gtgggagcaa
 2821 atgggtcaca aatagctcag ctgtgggctc agaaaactgct aggtaaaaga attccagaag
 2881 gagggcagggt cataagttgg atgacctatg aactttagtc taaagaattg agactaccgt
 2941 aattgagact actgtagtga catctgagaa atgggatgga agagtgacca tgttttattt
 3001 tcttgttctt gtcactattg tattttattt tgcataatca tgcccttcac tgacagtctc
 3061 cttaacatca tctgtttact ctgctcagtg taaactacaa tgctctgtca tctccctact
 3121 gggctctcctg ggaggagggg agccatccag ggtgcaaact caaaggcaga gggcacagcg
 3181 tgcttaggcc caagcttaga attcaattga gaagttctgt tgttcactct tacctcagca
 3241 ggtagaaaag aggtggagat cagaagccag ggattagaga ttgaattgct ttcctggga
 3301 gtgtgcagta tctcattaaa atgttgata ttcaaaaaaa tacagacaca cacaagtgcc

3361 tatataatga taaaacatat ccaaagcacc caaacctgtg attccagaaa agtagatcct
 3421 atttttggtt atttttattg gcttttagctg tgtcttttga aaggactatt atcctctaaa
 3481 atgtatgtgt atgaagatcc tggcttgacg ctcgaggagct ttacacacct tgtgtcactt
 3541 tatcttggtg acgagccaag atcatgcaac cagttcatcc atgtctgacc caagagctct
 3601 taactgctat gctgcactgc ctcatcaga gatagatgcc tgcattgggtc ctggcgatta
 3661 ttttaatgct ggctacaccc ccacaggtga cttggattca gaaatacaat ttatatcttct
 3721 tcttttttaa ttgttttatt ttatttttct ttttaatagtt atatgtcagt gagaacaatt
 3781 tatatgtctt acactgagaa ataaaactgc tcataagtga aaaca

[00069] SEQ ID NO: 3 is an amino acid sequence of TRIM10.

1 masaasvtsl adevncpicq gtlrepvtid cghnfcracl tryceipgpd leesptcplc
 61 kepfrpgsfr pnwqlanvve nierlqlvst lglgeedvcq ehgekiyffc eddemqlcvv
 121 creagehath tmrfledaaa pyreqihkcl kclrkereei qeigsrenkr mqvlltqvst
 181 krqqvisefa hlrkfleeqq sillaqlesq dgdilrqrde fdllvageic rfsalieele
 241 eknerparel ltdirstlir cetrkcrkp vavspelgqri rdfpqqalpl qremkmflek
 301 lcfeldyepa hisldpqtsh pklllsedhq raqfsykwqn spdnpqrfd r atcvlahtgi
 361 tggrrhtwvvs idlahggsct vgvvsedvqr kgelrlrpee gvwavrlawg fvsalgsfpt
 421 rltlkeqprq vrvslidyevg wvtftnavtr epiytftasf trkvipffgl wgrgssfsls
 481 s

In some embodiments of any of the aspects, the E3 ubiquitin ligase is TRIM58. TRIM58 is an E3 ubiquitin ligase that plays a role during late erythropoiesis and erythroblast enucleation. Sequences of TRIM58 are known for a number of species, e.g., human TRIM58 (NCBI GeneID: 25893), mRNA (e.g., NCBI Ref Seq NM_015431.4, **SEQ ID NO: 4**) and polypeptide (e.g., NCBI Ref Seq: NP_056246.3, **SEQ ID NO: 5**). TRIM58 can refer to human TRIM58, including naturally occurring variants and alleles thereof. Antibodies that specifically bind TRIM58 are known; see, e.g., Novusbio Trim58 Antibody (2H3) H00025893-M02. One can generate monoclonal antibodies using standard procedures e.g., Corti et al, *Microbiol Spectr* 2 (2014), Huang et al. *Nat Protoc* 10, 1907-1915 (2013), and Pedrioli et al., *Trends Immunol.* 42: 1143-1158 (2021), which are incorporated herein by reference. Single domain antibody fragments or nanobodies can be screened using a fully *in vitro* platform based on yeast surface display as in McMahon et al *Nature* 25: 289-296 (2018).

[00070] SEQ ID NO: 4 is a mRNA sequence for TRIM58. Note: U's replaced with T's in this representation.

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1  gcagaccgcg aggggagacg gtgcggggcgg ccgggagcgc agccctccgg gaggcgggtc
61 atggcctggg cgccgcccgg ggagcggctg cgcgaggatg cgcggtgccc ggtgtgcctg
121 gatttcctgc aggagccggg cagcgtggac tgcggccaca gcttctgcct caggtgcatc
181 tccgagttct gcgagaagtc ggacggcgcg cagggcgggc tctacgcctg tccgcagtgc
241 cggggccccct tccggccctc gggctttcgc cccaaccggc agctggcggg cctggtggag
301 agcgtgcggc ggctgggggtt gggcgcgggg cccggggcgc ggcgatgcgc gcggcacggc
361 gaggacctga gccgcttctg cgaggaggac gaggcggcgc tgtgctgggt gtgcgacgcc
421 ggccccgagc acaggacgca ccgcacggcg ccgctgcagg aggccgcccgg cagctaccag
481 gtaaagctcc agatggctctt ggaacttatg aggaaagagt tggaggacgc cttgactcag
541 gaggccaacg tggggaaaaa gactgtcatt tggaaaggaga aagtggaaat gcagaggcag
601 cgcttcagat tggagtttga gaagcatcgt ggctttctgg cccaggagga gcaacggcag
661 ctgaggcggc tggaggcgga ggagcgagcg acgctgcaga gactgcggga gagcaagagc
721 cggctgggtcc agcagagcaa ggccctgaag gagctggcgg atgagctgca ggagaggtgc
781 cagcgcccgg ccctgggtct gctggagggt gtgagaggag tctgagcag aagtaaggct
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901 gggaggaggg agctcttaag gaagttccaa gtggatgtaa agctggatcc cgccacggcg
961 cacccgagtc tgctcttgac cgccgacctg cgcagtgtgc aggatggaga accatggagg
1021 gatgtcccca acaaccctga gcgatttgac acatggccct gcatcctggg tttgcagagc
1081 ttctcatcag ggaggcatta ctgggagggt ctggtgggag aaggagcaga gtggggttta
1141 ggggtctgtc aagacacact gccaaagaaag ggggaaacca cgccatctcc tggagaatgg
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1261 cctcttctcc aactggaaag tcctcgctgc attgggattt tcttgacta tgaagccggt
1321 gaaatttcat tctacaatgt cacagatgga tcttatactt acacattcaa ccaactcttc
1381 tctggtcttc ttcggcctta ctttttcatc tgtgatgcaa ctctcttat cttgccaccc
1441 acaacaatag cagggtcagg aaattgggca tccagggatc atttagatcc tgcttctgat
1501 gtaagagatg atcatctcta aaattctgtt cccaagatgc agtcctagcg tagcgaacgt
1561 tcctggagtg ggtgaagga tatcaatata ctaagtttta acagataccc catttaggtc
1621 agcacttgat tcgttggtgc tgtgaaatat gtccatggga caaaagaggg aatatgaaat
1681 atttgcatat gggaagatta tagagcataa taattttgta aatggagcaa tctcaacctc
1741 tatttctaga tcacattttc ttgatgtctt cttcaaatt aatgaccttg gattacataa
1801 ggatttctat gcattcatta taatttggtt ttctttcaa tatccttgta tttcaaactc
1861 tccatataag aattagacat ggcaattctt aaattgattc agaatggtct gatactattc
1921 cagtatcacc tccttaattc tgtttctcct cgttttcctg attttccttc tcattctctc
1981 cttccccgct ctgtctctct ctccctgtca ctctctctct cttgttctct attttttgtt
2041 tcttacctct tactgtttta cctgttgctt ctttctggat taatacattt agagccattc
2101 ctttataatg tcacatttcc tatgacttta ctcaattact tttaaatccc tttctattct
2161 gagcataaatt ttaagaattt acaaagctca ttcttctgaa tctaataatca ctaactccta
2221 gactttttcc gttttctttg gatacacttt aagtaggaat ttatcagaat tttcattcaa
2281 ctggttcttt aatgcagata tttactagtt ataagacctt aaggctgggt gcagtggctc
2341 acgcctgtaa tcccagcact ttgggagggt gaggcgggtg gatcacaagc tcaggagttc
2401 aagaccagcc tggccaacat ggtgaaaccc tgtctctact aaaaaaaaaa aaaaaataga
2461 aaaattagct gggcatgggt gcaggagcct gtaatcccag ctattctgga ggtggagaca
2521 ggagaattgc ttgaaccctg gaggcggagg ttgcagtgag ccaatatctc accactgtac
2581 tccagcccag tgcgagactc catctcaaaa aagaaaaaag acctcaaaac acacttctct
2641 ctctctttta gctgcttggt atggttccta tacatggaac aattatactg gcctcactgt
2701 gttatggtaa atatttaagg tcatatttga tattgctggt ttgaattcag cttttccatt
2761 taaatacatt ataatgatga tgatgaaatc atgataatat ttaacttatt tttaaagtat
2821 attctgtacc tttccaacaa aaagggttaa agtcattgaa ggctaacctt actgccttct
2881 ttgtatcact gtcttctaaa taattattat gtctgggtac agtggctcac gcctgtaatc
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3001 gctaacacag tgaaaccccc tctctactaa aaatacaaaa agaaattagc tgggcgtggt
3061 ggtgggtgcc tgttggtcca gctacttggg aggctgaggc aggagaatgg catgaacca
3121 ggaggcagag cttgtagtga gccgagatcg cgccactgca ctccagccgg ggcaacagag
3181 caagactcca tctcaaaaat aataaataaa ataaataaaa aataaataaa aataaataaa
3241 attacacaaa tgctaaaatg tttaaaatgg aatgcttca atgctaacca aatattaatt
3301 aatggcaaat tatttaacat tatctgataa taatctgcag aaggtttaat tttcctcctc
3361 aatttgaagt tcaagatggt tttctcttcc agggagattt ttcgactga catctttaac

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3421 ttaccttcca atcatattac taacgtagcc ttcttcctag attttttaaat tgtttgatca
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3721 gtgggaggga gaacacctcc acatgtcttc tactctctcc ataggatgga atgagtgtcc
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4021 cagcaaaatc ctgcacatcc ataccggttt cctggctttc cctctccttt cctctgaatg
4081 gtagtctttt atattcagct gtccacttga catcaaaata gacattttga actcaatttg
4141 cctaaaactt acccacaat ttctcccaa gtctctccct aactgcaaca acaaaaacca
4201 caggcttctc cctgtcactg gatggcaact ccattctttt gattgcttaa gccaggcatc
4261 cgattgagta ctttcttgat ttctccagcc cacatccagt ccatcgga gacctgttg
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4381 ggtcagaact ccatcctgcc cctctggatt atgactttcg tttcctcaca gtggtcctgc
4441 ttgggctcta ggcccttcca ctcccattct ctctacagca gctgggctga ttccttttagc
4501 acccaaggat atgttggcat cacagtgact tagataccat cacaaagacc tcccattcaa
4561 cttagagtga aagtcagaat cctcacagt aatccccagg ccctagagga tgtgaacccc
4621 caggccctag aggatctgaa ccccatccc tcctctgatt atctctccca ccccaacttc
4681 cctttgcatt ctgctccagc tgccctggcc tcatggctgg gtttccacca aagcaggcac
4741 ttcccatcac agggccattt ccccgctgt ggcttctgct tgacattccc ttttccctga
4801 tatccccctg actcattatt ccctttcttc cttaactctt ctgagatcca gcttctcagt
4861 gataccacac agccctactc cccccagagc ccatctagag ctacacttcc cagtcgccct
4921 tgccaggctc agtggaggct ctttgttccc catacagtac gtgtcgtcgt actatattgt
4981 taggcttatt taatttatgt atgttttgcc tttttgtgct aaatgtaa accacaagg
5041 gaggtatctt tgtctgttga caatgatata ttcaatgttt ctcaagcacc cccaatgctg
5101 gtttgtatgt ggttatcatt caatctgtat ttgttgaatg aataaatgat tgactatgtg
5161 gagagcaaaa

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[00071] SEQ ID NO: 5 is an amino acid sequence of TRIM58.

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1 mawappgerl redarcpvcl dflqepvsvd cghsfclrci sefckesdga qggvyacpqc
61 rgpfrpsgr pnrqlaglv svrrlglgag pgarrcarhg edlsrfceed eaalcwvcd
121 gpehrthrta plqeaagsyq vklqmalelm rkeledaltq eanvgkktvi wkekvmqrq
181 rfrlefekhr gflaqeeqrq lrrleaeera tlqrlresks rlvqqskalk eladelqerc
241 qrpalgllg vrgvlsrsk vtrleaenip melktaccip grrellrkfq vdvkldpata
301 hpsllltadl rsvqdgepwr dvpnnperfd twpcilglqs fssgrhywev lvgegaewgl
361 gvcqdtlprk gettpspeng vwalwllkgn eymvlaspsv pllqlesprc igifldyeag
421 eisfynvtdg syiytnqlf sgllrpyffi cdatplilpp ttiagsgnwa srhdldpasd
481 vrddhl

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[00072] As noted above, the erythroid-enriched nature of TRIM10 and TRIM58 establishes them as candidates for the targeting of a protein of interest for degradation in erythroid cells. In one embodiment, a binding agent that specifically binds TRIM10 or TRIM58 can be joined to a binding agent that specifically binds a protein of interest. Upon introduction to an erythroid cell, whether by expression therein or by more direct introduction, such a bispecific construct can bring the protein of interest into close proximity with the TRIM E3 ubiquitin ligase, resulting in ubiquitination of the protein of interest, thereby promoting the targeted

degradation of the protein of interest in an erythroid-enriched manner. In such an embodiment, the binding agent that binds either or both of the TRIM polypeptide and the protein of interest can be, for example, antibodies or antigen-binding fragments thereof. The binding agents can be joined, for example, by a chemical or peptide linker, e.g., a flexible peptide linker, including, but not limited to a serine/glycine-rich peptide linker.

[00073] In another embodiment, a TRIM10 or TRIM58 polypeptide can be directly fused, e.g., as a fusion polypeptide, to a binding agent that specifically binds a protein of interest. In such an embodiment, upon introduction to a cell expressing the polypeptide of interest, the polypeptide of interest is brought into close proximity with the TRIM E3 ubiquitin ligase, resulting in ubiquitination of the protein of interest, thereby promoting the targeted degradation of the protein of interest. In such an embodiment, the binding agent that binds the protein of interest can be, for example, an antibody or antigen-binding fragment thereof. The binding agents can be joined, for example, by a chemical or peptide linker, e.g., a flexible peptide linker, including, but not limited to a serine/glycine-rich peptide linker.

Erythroid targets for degradation

[00074] Any polypeptide expressed in an erythroid cell, whether erythroid specific, erythroid enriched, or simply expressed in an erythroid lineage cell, can be target for proteasome-mediated degradation using constructs as described herein. Non-limiting examples of erythroid-enriched factors include LRF, ZNF410, and BLC11A.

LRF

[00075] Leukemia/Lymphoma-Related Factor (LRF), also known as Zinc Finger and BTB Domain Containing 7A (ZBTB7A), Factor binding IST protein 1 (FBI-1), or Pokemon is a member of the POK family of transcription factors that possesses a POZ-domain at the N-terminus and Krüppel-like zinc finger at the c-terminus. See, e.g., Gupta et al., *Cancer Lett.* 483, 22-34 (2020), which is incorporated herein by reference in its entirety. LRF is involved in several fundamental biological processes including cell proliferation, differentiation, and development. It also participates in hematopoiesis and erythrocyte maturation, adipogenesis, chondrogenesis, cellular metabolism and alternative splicing of BCLXL, DNA repair, development of oligodendrocytes, osteoclast, and the unfolded protein response. LRF also negatively regulates SMAD4-DNA complex. Sequences of LRF are known for a number of species, e.g., human LRF/ZBTB7A (NCBI GeneID: 51341), mRNA (e.g., LRF variant 2 NCBI Ref Seq NM_001317990.2, **SEQ ID NO: 6**) and polypeptide (e.g., LRF: NCBI Ref

Seq: NP_001304919.1, **SEQ ID NO: 7**). Antibodies for specific detection or binding to LRF/ZBTB7A are available, for example, from R&D Systems, see Human/Mouse ZBTB7A/Pokemon Antibody (clone 466407) MAB3496; from Origen, see Pokemon (ZBTB7A) Mouse Monoclonal Antibody (clone BC17-2.9F), AM32711PU-N; from Novusbio, see ZBTB7A/Pokemon Antibody (clone 3T2J1) NBP3-15914; from Abcam, see Anti-ZBTB7A antibody (EPR13178B) ab175918; and from Invitrogen, see Pokemon (LRF) monoclonal antibody (13E9) 14-3309-82. LRF can refer to human LRF, including naturally occurring variants and alleles thereof. For example, SEQ ID NO: 6 is a transcript variant 2, mRNA of human LRF. Note: U's replaced with T's in this representation.

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1  actgccgcct cccggccccct cggaggggagc cagcccagcc gcagccgcgcg ccaccgcccgc
61  cgcggggggcc gggccccctc gccgctgccc cgggaaggag gtctcggcgc ggaagatggc
121  cggcggcgtg gacggcccca tcgggatccc gttccccgac cacagcagcg acatcctgag
181  tgggctgaac gagcagcgga cgcagggcct gctgtgcgac gtggtgatcc tgggtggagg
241  ccgcgagttc cccacgcacc gctcgggtgct ggccgcctgc agccagtact tcaagaagct
301  gttcacgtcg ggcgcggtgg tggaccagca gaacgtgtac gagatcgact tcgtcagcgc
361  cgaggcgctc accgcgctca tggacttcgc ctacacggcc acgctcaccc tcagcacagc
421  caacgtgggt gacatcctca gcgcgcgccg cctgctggag atccccgcgc tgagccacgt
481  gtgcgcgcgac ctcttgacc gccagatcct ggcgcccgac gcgggcgcgc acgccgggca
541  gctggacctt gtagatcaaa ttgatcagcg caacctcctc cgcgccaagg agtacctcga
601  gttcttccag agcaacccca tgaacagcct gccccccgcg gccgcgcgcg ccgctgccag
661  cttcccgctg tccgcctttg gggcgctcca tgatgacctg gatgccacca aggaggccgt
721  ggcgcgcgct gtggccgcgc tggccgcggg cgactgcaac ggcttagact tctatgggcc
781  gggccccccg gccgagcggc ccccgacggg ggacggggac gagggcgaca gcaaccggg
841  tctgtggcca gagcgggatg aggacgcccc caccgggggt ctctttccgc cgcgggtggc
901  cccgcgcggc gccacgcaga acggccaacta cggccgcggc ggagaggagg aggcgcctc
961  gctgtcggag gcggcccccg agccgggcga ctctccgggc ttctgtcggg gagcggccga
1021  gggcgaggac ggggacgggc ccgacgtgga cgggctggcg gccagcacgc tgcgcagca
1081  gatgatgtca tcggtgggccc gggcgggggc cgcggcgggg gacagcgacg aggagtcgcg
1141  ggccgacgac aaggcgctca tggactacta cctgaagtac ttcagcggcg cccacgacgg
1201  gaaggtctac ccggcctggt cgcagaaggt ggagaagaag atccgagcca aggccttcca
1261  gaagtgcgcc atctgcgaga aggtcatcca gggcgccggc aagctgcgcg gacacatccg
1321  caccacacg ggcgagaagc cctacgagtg caacatctgc aaggtccgct tcaccaggca
1381  ggacaagctg aaggtgcaca tgcggaagca cacgggcgag aagccgtacc tgtgccagca
1441  gtgcggcgcc gcctttgcc acaactacga cctgaagaac cacatgcgcg tgcacacggg
1501  cctgcgcccc taccagtgcg acagctgctg caagaccttc gtccgctccg accacctgca
1561  cagacacctc aagaaagacg gctgcaacgg cgtccccctc gcgcgcggcc gcaagccccg
1621  cgtccggggc ggggcgcgcg accccagccc gggggccacc gcgacccccg gcgccccgc
1681  ccagcccagc tcccccgacg cccggcgcaa cggccaggag aagcacttta aggacgagga
1741  cgaggacgag gacgtggcca gccccgacgg cttgggcccg ttgaatgtag cgggcgcggg
1801  tggaggaggt gacagcggag gtggcccccg ggccgccacc gacggttaact tcacagccgg
1861  actcgcctaa aaacaaaaaa gagaaaaacag aaacccgaga aagagagaga cagagagaga
1921  gaaaaaaaat caccacccac cccccaaaaa acacaaaaaa agaaaatcta tctatataca
1981  gatatactata tctatatata tatatacaga tatatatata tgacgcgtca cagaatctag
2041  ggtagcgctt tctcagattt ccctcctttc tgacgttttt ctccctccgc aggggccccg
2101  gccctccctg gctccctttc cccccaccac cccatcgctg ggtttcgggg cttggtttgg
2161  ggttttttgt aggacacaag gaatccgaga ccccgcacag cccctgggc acccgcatg
2221  gggcgctggg cccgatccga ggcctgggc tggggggagg gtagacgtgg gggcgctggg
2281  gggggactgg ggtgggcttt taatttctc cctcgctgg tttctatgag tttttcagac
2341  aagaccttaa atgattttctg tctgctctga gcggacgtta aaatggggcc cgtcccccg
2401  accgcacccc tccttccctca gggcacttac taaggagggg gtctccctct ccctctcccc
2461  agtggcctcc ccgcctccaa ccctgcctgc ggccctcccc cgtcgcgccac cccacgtctc
2521  ctggccactg agacacaaac ctattttatt ctaggcctgg agaaaggaga tcggactggg
2581  gttcccggtg gggcgccagg atggctcctg ggggtgctcc tgccgccttc cttcacggca
2641  cttacaaccg gcgggacccc cagggaccac ccctcagggg cccccccac ccccgcccgg

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2701 tccacctaga cccccacgtt tggagattca aaactttctgt cttcgtcctc tcccccgagc
 2761 cccctctccc aaatttttaa agcacttttt agattttttt ttctctttcc tccttaaaaa
 2821 caaaatttat atatatatat atatatatat ataaataata tacttttccct cagaggagca
 2881 ggcaacagtg tgggataaac agagtcacga tcagaggaaac cccagggtct ggtgatggca
 2941 gggatggggg gagagagaga aaatccacaa attccaatgt cacaaaagca ataaaaacaaa
 3001 ctagaaaaaa aaaaggtttt acaaaatgaa aggaaggaaa aaaaaaaagg caaccaacca
 3061 cattagaagt cttggcactt tgtaacggaa cgggtactac actttatctt aattcttaat
 3121 ttaaaaaacat gtttacaaagt tacaaccaac ttctatgaaa agttgaaaag aaaaaaaaaa
 3181 aaaaaaaaag cgagcgagag agagagcgag agagagagcg agagcagaag aaattcctaa
 3241 aagtcgattt atttttgtac aaaataataa aaaaaaaaaa ccaccacaaa cgtagaatcc
 3301 acttctgttc cccaaaaagc gagaaggggg gttcaggagg aagccatcgc aggggacctg
 3361 ggagacgccc cgaggtgttt gtgcttcacc ccagacgtc agcctcgaag gcagactgt
 3421 ggggtgttcg tgcgtgtttc ccccgcctcc ccctttctgt cccctttttt ggttctgacg
 3481 tgaagaggtc ttagcgcccg cttctgtcca cggggtctct ccttcctcct ccctagctca
 3541 gggatggggc ttccagccgg agcaccgccg tccccatccg gcacccccca atcccccaac
 3601 acgcctgtcc ctccgcctg gccaccaagg agctggacct tggatgcgcc tacctgtctg
 3661 aggtgggtga caggggcccc ccacctccag ggccttagaa ccaccgcccc tctccccacc
 3721 ccaggcacc ctctttttac tcaaaggcac tgactgtaat ccagggggac tgggacctgc
 3781 ctcccccaa cctctggctc ccacaaggcc cgggtgtgac cgagccacag gccacggaca
 3841 ggggcccggg ttggggagac tatgtcgcca gatgccagga cgccctcacc ccgtttgcat
 3901 atgcaatgct agcatgggac cccgaaaata gacgtctctg tgactgaga cttcttgtca
 3961 atgccccacc ggcggggggg tgtctccctg ccccgaccc ccccatacc ccttctctgt
 4021 gacacacaca tcttctcgtc tctttttctt tcattgttaa agggaagctt ttaagaagg
 4081 caattttcat attgtttcta caggatggtt ttggttccct tcccttccca cccccctta
 4141 agcctgtcag cccctccaa atgtctcagg atccccctc tccctgggg ctgggtgaca
 4201 gcaccccggc tgcgttcaca cccagtgctc acagggcgag ctgttctgga gagaaaacca
 4261 tctgtcgtgg ctgagcgggg agcttgaaca cccaggccag ggacaccctt cccagctcc
 4321 cagagaggcc cctgagggg tgagccctct ttccacctc cctatccat gcacccctc
 4381 gcaataaaac caactctaaa atcacagctg tcgtcctagc cagtgggggc gaccggactt
 4441 ggggggtgga gccctctggg acttccgtag gaacaagggc tgcggccac cgcgacatt
 4501 acacagacct cggggattgc actaaaccc ctgtcctagc tccgactca gcttcgctg
 4561 tcctgcccgc ccactttgcc ttaactaccc gccgtcctg ggggccacag cctctgcatg
 4621 ggccagagc cgggaccccc ccagcccagc cccgccctc ccagactccg cgcaatcaca
 4681 tactgtatat agacgtgaat cgattttatt tttattctt aaattaaggt cgtgataaag
 4741 tgttgccaaa gatacctgct gaattctcgc gtttcaggaa acaaacaaac aaaaaaaaaat
 4801 gatatttgag gaggtcgtg ttgactccat atgaaaggac acagctcaaa gcttttttgt
 4861 ttggttgttt ggggtttttt gtgttttctt tttttgggg gtttttttt taactgcctg
 4921 gtacaaaaaa aaaaagagaa aaaaaaaaaa gaaaaacaat gcgaaattgt tatttccatt
 4981 ctcatggtga agttgcgtgg acgcgtgtgt gcgtgtgtgc aagagagcgg gagtgaagtc
 5041 caggctgggg ttgggggggt tcaggcgggg gcgcccgggg gccggggagg tggccggggc
 5101 ggagcccccg tctgcagtgc cccccagcct gccgggcca ggagagagag agaagcatct
 5161 ttgtactag ctgttgctgc tacctgcctc tgcccccca cgcccccg cttttgagat
 5221 taaggaaaaa aaaaaaaagt caaaaaagtt ttaaaaaatg aaaaaaaaaa attataaacc
 5281 agtgaatgta aaatgccgga gcaggcccg cctggcatgg gtgtggacct gcagccaggc
 5341 aggtcgcagc gggcgatacc aaagtctgcc ccccaccat tgtggccatg cagtccgtgc
 5401 actgtctttt tgcctccttc cgaggggggt ccccgagcct cttccagggt cttccctgg
 5461 aagtgggcgg ctgcagggaa ggtgggggac aggggtctt gcacgattca gacccggg
 5521 cgtggcagg agcggtcacc tcacaggtgg tgacactgag gcaggggcct cggggtgcc
 5581 cctcccgccc ggcaaccaga atggttgagg gcaagacaga gagaatgaaa ggaaaaacag
 5641 aagaaaaaaa aatattaaaa accaacaata aaagcaaaaa tcctattttt tgagaaagaa
 5701 agatatttat atttgcagtt ttatttttaa aagttattta agttgaagca gcttctctgg
 5761 aggtgggggg gggggggtgg tgggtggctg gcgcaggacg ggtcaggggc ctggaggctg
 5821 ggggtgcccc aggagctaca acctcagagt taagactagc tcgcattaaa tacatagatt
 5881 tacggggggg gggggggggg gccgggcca ggggtggag ggggccagg agaccccat
 5941 cctcgccgg ggtgcctgg aggtgtgga ccaggatccg atgccagggt cccgcccccc
 6001 accccacccc aggccagaa tcgaggtgcc ttggactttg gaggggccag gcctggtgaa
 6061 tggggggcgg ggccggccc tcagggtaca gagcacagac agatagacat tccagagact
 6121 gtattgagag tctttataaa gtgtgggaga ttaaaaaaaa aaaaaaactg ataaaaatgc
 6181 actttttggg agtggggagg gagaagcttt aaaagtaata aaaaacaaac aaaaacacaa
 6241 aagatgaaaa aacaaaaaaa ttcatttttc ttgtacataa aaaaaaaaaa agaaccacta
 6301 aacgcgcct gttacgacc

[00076] SEQ ID NO: 7 is an amino acid sequence of human LRF.

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1  maggvdgpig ipfpdhssdi lsglneqrtq gllcdvivilv egrefpthrs vlaacsqyfk
61  klftsgavvd qqnvyeidfv saealtalmd faytatltvs tanvgdilsa arlleipavs
121 hvcadlldrq ilaadagada gqldldvdqid qnlllrakey leffqsnpmn slppaaaaaa
181 asfpwsafga sddlddatke avaaavaava agdcngldfy gpgppaerpp tgdgdegdsn
241 pglwperded aptggfppp vappaatqng hygrggeeea aslseapep gdspgflsga
301 aegegdgdpd vdglaaastll qqmmssvgra gaaagdsdee sraddkgvmd yylkyfsgah
361 dgdvypawsq kvekkiraka fqkcpicekv iqqagklprh irthtgekpy ecnickvrft
421 rqdklkvhmr khtgekpylc qqcgaaafahn ydlknhmrvh tglrpyqcds ccktfvrtdh
481 lhrhlkkdgc ngvpsrrgrk prvrvggapdp spgatatpga paqpsspdar rngqekhfkd
541 edededvasp dglgrlnvag aggggdsggg pgaatdgnft agla

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[00077] In some embodiments, LRF can be targeted for degradation in erythroid cells via TRIM10 or TRIM58 in a manner directly analogous to that described herein for targeting of BCL11A in erythroid cells. Briefly, in one embodiment, a binding agent, e.g., an antibody or antigen-binding fragment thereof specific for TRIM10 or TRIM58 and a binding agent, e.g., an antibody or antigen-binding fragment thereof specific for LRF can be fused, optionally via a peptide linker. In another embodiment, TRIM10 or TRIM58 polypeptide itself can be fused to a binding agent, e.g., an antibody or antigen-binding fragment thereof specific for LRF. Introduction of either or both of these constructs to a cell, e.g., an erythroid cell, for example as discussed herein for BCL11A-targeting constructs, can target LRF for degradation in those cells.

ZNF410

[00078] Zinc Finger Protein 410 (ZNF410), also known as APA-1 is a transcription factor that contains five tandem canonical C2H2 zinc fingers. See, e.g., Lan et al., *Mol Cell*. 21: 239-254, (2021), which is incorporated herein by reference in its entirety. ZNF410 binds to the sequence motif 5'-CATCCCATAATA-3' (SEQ ID NO: 66) in regulatory elements and modulates transcription of target genes. In erythroid cells, ZNF410 directly binds and transcriptionally activates CHD4, a component of the NuRD complex, which leads to repression of fetal globin genes HBG1 and HBG2. Sequences of ZNF410 are known for a number of species, e.g., human ZNF410 (NCBI GeneID: 57862), mRNA (e.g., ZNF410 Isoform a: NCBI Ref Seq NM_001242924.2, SEQ ID NO: 8) and polypeptide (e.g., ZNF410 Isoform a: NCBI Ref Seq: NP_001229853.1, SEQ ID NO: 9). Antibodies for specific detection of or binding to ZNF410 are available, for example, from Creative Biolabs, see Mouse Anti-ZNF410 Recombinant Antibody (clone 1C7) CBMAB-0827-LY; Mouse Anti-ZNF410 Recombinant Antibody (clone 1A12) CBMAB-0826-LY; and Anti-ZNF410

monoclonal antibody (clone DCABH-17601. ZNF410 can refer to human ZNF410, including naturally occurring variants and alleles thereof. For example, SEQ ID NO: 8 is an mRNA sequence of human ZNF410. Note: U's replaced with T's in this representation.

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1  gtgtgtggac ggaattcggg accgactgac ggccggccgg cttcccgga ctggaaggtt
61  acattgatta cccacctagt acaacatctt acggaagag catagtattt cctagaggaa
121  tatgaacata acaggaaggt atcattggct ctgaattaaa tttgaacttg tcccctgaat
181  agctacaggt tttggaagct gaatcaatgt tatcagatga gttagaatcc aaaccagagc
241  tcctggtaca gtttggtcag aatacgtcca tcccattggg acaggggctt gtagaatcag
301  aagctaaaga tattacttgc ttgtccctcc tcccgtgac tgaagcctca gaatgcagtc
361  ggctaagtgt accagaacct gttccatgga gagaagagga tggaaagtca ggctgcagtg
421  acttgaatga tactacaaat cattctaact cctccaagga ggtcccttcc tcagctgttt
481  tgagaagcct tcgggtgaat gtgggtccag acggagagga gacgagagct cagactgtac
541  agaaatcccc ggagtttttg tccacttcag agtcttctag cttgttgcaa gatctacagc
601  caagtgatag cacttctttt attcttctta acctaacaag agcaggtctg ggctcttcag
661  ctgagcactt agtgtttgta caggatgagg cagaagattc agggaatgat ttcctctcca
721  gtgagagcac agacagtagc attccatggt tcctccgggt tcaggagtgt gccatgaca
781  gtttgattgc tgctactcgt gcacaactgg caaagaatgc aaaaaccagc agcaatggag
841  aaaaagtcca ctttggttct ggtgatgggc agtcaaaaaga ttctgggccc cttcctcaag
901  tggaaaagaa gctcaagtgt acagttgaag gttgtgaccg gacatttgta tggccagctc
961  actttaataa ccacctcaag actcatcgaa atgaccgctc cttcatctgt cctgcagaag
1021  gttgtgggaa aagcttctat gtgctgcaga ggctgaaggt gcacatgagg acccacaatg
1081  gagagaagcc ctttatgtgc catgagtctg gctgtggtaa gcagtttact acagctggaa
1141  acctgaagaa ccaccggcgc atccacacag gagagaaacc tttcctttgt gaagcccaag
1201  gatgtggccg ttcctttgct gagtattcta gcctccgaaa acatctgggtg gttcactcag
1261  gagagaagcc tcatcagtgc caagtctgtg ggaagacctt ctctcagagt ggaagcagga
1321  atgtgcatat gagaaagcat caccctgcagc tgggagcagc tgggagtcaa gagcaggagc
1381  aaactgctga gccactaatg ggcagtagtt tgcttgaaga ggcttcagta cccagtaaaa
1441  acctggtgtc tatgaattcc cagcccagcc ttggtggaga gtccttgaac ctaccaaata
1501  ccaattctat cctgggagtt gatgatgagg tgcttgctga aggatcccca cgttccctgt
1561  cttcagtgcc tgatgtgaca catcacctgg tgaccatgca gtcagggagg caatcatatg
1621  aagtttctgt cttaactgca gtaaatccac aagagagtct cgctccgttg cccaggctgg
1681  agtgagtggt tgcgttctcg gctcactgca acctctgcct cccaggttca agcgattctc
1741  ctgcctcagc ctcttgagta gctgctacta cagttactaa accaaggaga ttttaactgaa
1801  agacggacat gagcgtgggt gctgactcct ggaagagcaa ctctatctga tctcaaatg
1861  cgtataactg gaacaggatg ccttagccca caacagaacc agaatgaatc tttgaaggca
1921  caagactctg cttttgccac tcttctctt tctgtgtata gaagtggat gtaggagagc
1981  ttcttttcta actaccatct gatcagacaa ggaatgaagc aatgactgtg ggctgggaaa
2041  ctgtacctac ctctcttccc actgcaaat tctgggatag accaaaagtg aatttgatta
2101  tgtgttggtc gaagttcttc attctgactg ttgaggggag gttttccttt gaagagtttt
2161  catcccagac tcagctgtct tttcacatgg atgaaataat tcctgctacc aacaacagag
2221  cttcaccagg aagttgagtt ttcaagatgc cttgttgctt tgaagaaggg agtgatgtca
2281  attctcttgt tacattctcc ctttagcaac ctgagtaaga gactctctgc cactgggctg
2341  caaaaaaata aattacttga atctccctt ggcccaggct gaggtactat cttgtcctat
2401  acacttctac ttggtcactt tgctttcttc gttaatggaa catacagtag catgttaaga
2461  gggatttcat gtttttgttt ttttaaagtt aaaaattaca tgatgcagag attcaggttt
2521  tccttttaaa aaacaaaaca gccagtcag ttcttttggt cttgttttat acaaaatttg
2581  ttttcttaga gattaagaat ttaatctttc cttttaaaat agtgtattga tttaccctta
2641  ggattccata ccagtaaaac tgaaccgagg agtcttagga aacaacaaga acatcttcat
2701  ttcttaagcc caggtgatag ttactctgtc accacaaaaa aagacgcac tgagaaaatg
2761  gcaataaaaa cagatacttc tgaatttttc ca

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[00079] SEQ ID NO: 9 is an amino acid sequence of human ZNF410.

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1  mlsdeleskp ellvqfvqnt siplgqglve seakditcls llpvteasec srlmlpepvp
61  wreedgksgc sdlndttnhs nsskevpsa vlrsrlrvnv pdgeetraqt vqkspeflst
121  sesssllqdl qpsdstsfil lnltraglgs saehlvfvqd eaedsgndfl ssestdssip
181  wflrvqelah dsliaatraq laknaktssn genvhlgsd gqskdsglpl qveklklctv
241  egcdrtfvwp ahfkyhlkth rndrsficpa egcgksfyvl qrlkvhmrrh ngekpfmche

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301  sgcgkqftta  gnlknhrrih  tgekpflcea  qgcgrsfaey  sslrkhlvvh  sgekphqcqv
361  cgkftfsqsgs  rnvhmrkhhhl  qlgaagsqeq  eqtaeplmgs  slleeasvps  knlvsmnsqp
421  slggeslnlp  ntasilgvdd  evlaegsprs  lssvpdvthh  lvtmqsggrqs  yevsvltavn
481  pqueslaplpr  lecsqafsa  cnlclpgssd  spsas

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[00080] In some embodiments, ZNF410 can be targeted for degradation in erythroid cells via TRIM10 or TRIM58 in a manner directly analogous to that described herein for targeting of BCL11A in erythroid cells. Briefly, in one embodiment, a binding agent, e.g., an antibody or antigen-binding fragment thereof specific for TRIM10 or TRIM58 and a binding agent, e.g., an antibody or antigen-binding fragment thereof specific for ZNF410 can be fused, optionally via a peptide linker. In another embodiment, TRIM10 or TRIM58 polypeptide itself can be fused to a binding agent, e.g., an antibody or antigen-binding fragment thereof specific for ZNF410. Introduction of either or both of these constructs to a cell, e.g., an erythroid cell, for example as discussed herein for BCL11A-targeting constructs, can target ZNF410 for degradation in those cells.

BCL11A

[00081] BCL11A (B-cell lymphoma/leukemia 11A), also known as Evi9, CTIP1, or ZNF856 is a Kruppel-like sequence specific C2H2 type zinc-finger transcription factor located on chromosome 2. See, e.g., Liu et al., *Cell*. 173, 430-442 (2018) and Satterwhite et al., *Blood*. 98, 3413-3420 (2001), which are incorporated herein by reference in their entireties. BCL11A functions mainly as a transcriptional repressor that is involved in brain and hematopoietic system development, as well as fetal-to-adult hemoglobin switching. Sequences of BCL11A are known for a number of species, e.g., human BCL11A (NCBI GeneID: 53335), mRNA (e.g., BCL11A isoform 1 NCBI Ref Seq NM_00022893.4, **SEQ ID NO: 10**) and polypeptide (e.g., BCL11A isoform 1: NCBI Ref Seq: NP_075044.2, **SEQ ID NO: 11**). BCL11A can refer to human BCL11A, including naturally occurring variants and alleles thereof. For example, SEQ ID NO: 10 is a mRNA sequence for Isoform 1 of human BCL11A. Note: U's replaced with T's in this representation.

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1  gtctctgtcc  atccagactc  ctgacgttca  agttcgcagg  gacgtcacgt  ccgcacttga
61  acttgcagct  caggggggct  tttgccatth  ttttcatctc  tctctctctc  tctccctcta
121  tctctcttct  ctctctctcc  ctcttttttt  tttttttttt  tttttttttt  ttgcttaaaa
181  aaaagccatg  acggctctcc  cacaattcat  cttccctgcg  ccattcttgt  attatttcta
241  atttattttg  gatgtcaaaa  ggcactgatg  aagataatth  ctctggagtc  tccttctttc
301  taaccgggct  ctcccgatgt  gaaccgagcc  gtcgtccgcc  cgccgcgcgc  gccgcgcgcg
361  ccgcgcgcgc  ccccgagacc  caccatgtct  cgccgcaagc  aaggcaaacc  ccagcactta
421  agcaaacggg  aattctcgcc  cgagcctctt  gaagccattc  ttacagatga  tgaaccagac
481  cacggcccg  tgggagctcc  agaaggggat  catgacctcc  tcacctgtgg  gcagtgccag
541  atgaacttcc  cattggggga  cattcttatt  tttatcgagc  acaaacggaa  acaatgcaat
601  ggcagcctct  gcttagaaaa  agctgtggat  aagccacctt  ccccttcacc  aatcgagatg
661  aaaaaagcat  ccaatcccg  ggaggttggc  atccaggtca  cgccagagga  tgacgattgt
721  ttatcaacgt  catctagagg  aatttgcccc  aaacaggaac  acatagcaga  taaacttctg

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781 cactggaggg gcctctcctc ccctcgttct gcacatggag ctctaatccc cacgcctggg
841 atgagtgcag aatatgcccc gcagggtatt tgtaaagatg agcccagcag ctacacatgt
901 acaacttgca aacagccatt caccagtgcg tggtttctct tgcaacacgc acagaacact
961 catggattaa gaatctactt agaaagcgaa cacggaagtc ccctgacccc gcggttggt
1021 atcccttcag gactaggtgc agaatgtcct tcccagccac ctctccatgg gattcatatt
1081 gcagacaata acccctttta cctgctaaga ataccaggat cagtatcgag agaggcttcc
1141 ggctggcagc aagggcgctt tccacccact cccccctgt ttagtccacc accgagacat
1201 cacttggacc cccaccgcat agagcgcctg ggggcggaag agatggccct ggccaccat
1261 cacccgagtgc cttttgacag ggtgctgcgg ttgaatccaa tggctatgga gctcccgcc
1321 atggatttct ctaggagact tagagagctg gcagggaaca cgtctagccc accgctgtcc
1381 ccaggccggc ccagccctat gcaaaggtta ctgcaaccat tccagccagg tagcaagccg
1441 cccttcctgg cgacgcccc cctccctcct ctgcaatccg cccctcctcc gctccagccc
1501 ccggtcaagt ccaagtcatt cgagttctgc ggcaagacgt tcaaatttca gagcaacctg
1561 gtggtgcacc ggcgagccca cacgggcgag aagccctaca agtgcaacct gtgcgaccac
1621 gcggtgaccc aggccagcaa gctgaagcgc cacatgaaga cgcacatgca caaatcgtcc
1681 cccatgacgg tcaagtccga cgacggtctc tccaccgcca gctccccgga acccggcacc
1741 agcgacttgg tgggcagcgc cagcagcgcg ctcaagtccg tgggtggcaa gttcaagagc
1801 gagaacgacc ccaacctgat cccggagaac ggggacgagg aggaagagga ggacgacgag
1861 gaagaggaag aagaggagga agaggaggag gaggagctga cggagagcga gagggtggac
1921 tacggcttcg ggctgagcct ggaggcggcg cgccaccacg agaacagctc gcggggcgcg
1981 gtcgtgggcg tgggcgacga gagccgcgcc ctgcccgcg tcatgcaggg catggtgctc
2041 agctccatgc agcacttcag cgaggccttc caccaggtcc tgggcgagaa gcataagcgc
2101 ggccacctgg ccgaggccga gggccacagg gacacttgcg acgaagactc ggtggccggc
2161 gagtcggacc gcatagacga tggcactgtt aatggccgcg gctgctcccc gggcgagtcg
2221 gcctcggggg gcctgtccaa aaagctgctg ctgggcagcc ccagctcgct gagccccctc
2281 tctaagcgca tcaagctcga gaaggagttc gacctgcccc cggccgcgat gcccaacacg
2341 gagaacgtgt actcgagtg gctcgcgggc tacgcggcct ccaggcagct caaatatccc
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2461 gagaacggga gtttgcgctt ctccacaccg cccggggagc tggacggagg gatctcggg
2521 cgacgcggca cgggaagtgg agggagcagc ccccatatta gtggtccggg cccgggcagg
2581 ccagctcaa aagaggcgag acgcagcgac acttgtgagt actgtgggaa agtcttcaag
2641 aactgtagca atctcactgt ccacaggaga agccacacgg gcgaaaggcc ttataaatgc
2701 gagctgtgca actatgcctg tgcccagagt agcaagctca ccaggcacat gaaaacgcat
2761 ggccagggtg ggaaggacgt ttacaaatgt gaaatttgta agatgccttt tagcgtgtac
2821 agtaccctgg agaaacacat gaaaaaatgg cacagtgatc gagtggtgaa taatgatata
2881 aaaactgaat agaggtatat taatacccct ccctcactcc cacctgacac cccctttttc
2941 accactcccc ttccccatcg ccctccagcc ccactccctg taggattttt ttctagtccc
3001 atgtgattta aacaaacaaa caaacaaaca gaagtaacga agctaagaat atgagagtgc
3061 ttgtcaccag cacacctgtt ttttttcttt ttcttttctt ttttctttt tctttttttt
3121 tttttttcct ttatgttctc accgtttgaa tgcatgatct gtatggggca atactattgc
3181 attttacgca aactttgagc ctttctcttg tgcaataatt tacatgttgt gtatgttttt
3241 ttttaaactt agacagcatg tatggtatgt tatggctatt ttaaattgtc cctaattcgt
3301 tgctgagcaa acatgttgct gtttccagtt ccgttctgag agaaaaagag agagagagag
3361 aaaaagacca tgctgcatac attctgtaat acatatcatg tacagtttta ttttataacg
3421 tgaggaggaa aaacagtctt tggattaac cttctatagac agaatagata gcactgaaaa
3481 aaaatctcta tgagctaaat gtcgttctct aaagggttaa atgtatcaat tggaaaggaa
3541 gaaaaaaggc cttgaattga caaatataca gaaaaacaga acaagttaat tctatcattt
3601 ggttttaaaa tatgagtgcc ttggatctat taaaaccaca tcgatgggtc tttctacttg
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3721 taattctata ttgtatttct cacaacaatg gctacctaata aagatgacct attatgtcct
3781 agttaatcat catttttctt ttagtttaat tttataaaca aaactgatta taccagtata
3841 aaagctactt tgctcctggg gagagcttaa aagaaatggg ctgttttgcc caaagtttta
3901 ttttttttaa acaatgatta aattgaatgt gtaatgtgca aaagccctgg aacgcaatta
3961 aatacactag taaggagttc attttatgaa gatatttgct ttaataatgt ctttttaaaa
4021 atactggcac caaaagaaat agatccagat ctacttgggt gtcaagtgga caatcaaagt
4081 ataaacttta agaccttgta taccatattg aaagggaag gctgacaata aggtttgaca
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4321 tatatatgtc tgtgtccctt aaaagttgta cttcatttct tttccataca ctgtgtgcta
4381 tttgtgttaa catggaagag gattcattgt ttttattttt atttttttta ttttttcttt

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4441 tttattaagc tagcatctgc cccagttggt gttcaaatac cacttgactc tgcctgtgat
4501 atctgtatct tttctctaata cagagataca gaggttgagt ataaaaataaa cctgctcaga
4561 taggacaatt aagtgcactg tacaattttc ccagtttaca ggtctatact taagggaataa
4621 gttgcaagaa tgctgaaaaa aaattgaaca caatctcatt gaggagcatt ttttaaaaaac
4681 taaaaaaaaa aaaactttgc cagccattta cttgactatt gagcttactt acttgagcgc
4741 aacattgcaa gcgctgtgaa tggaaacaga atacacttaa catagaaatg aatgattgct
4801 ttcgcttcta cagtgcgaag atttttttgt acaaaaacttt tttaaatata aatgttaaga
4861 aaaatTTTTT ttaaaaaaca cttcattatg tttagggggg aactgcattt taggggtcca
4921 ttgtcttggt ggtgttacaa gacttggtat ccatttataaa atggtagtgg aaattctatg
4981 ccttgataac acaccgctct tcaggttgta aaaaaaaaaa acatacattg gggaaagggtt
5041 taagattata tagtacttaa atataggaaa atgcacactc atgttgattc ctatgctaaa
5101 atacatttat ggtctttttt ctgtatttct agaattggtat ttgaattaaa tgttcactca
5161 gtgttaggca ctatagtatt tatattgaag cttgtatttt taactgttgc ttgttctctt
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5281 atttttttaa tttggcagga taatatagtg caaattattt gtatgcttca aaaaaaaaaa
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5401 ggagcctgct agaattgtac atggatggct gtcataaggg ttgtacatat ctttttttgt
5461 tcctttttcc tgctgccata ctgtatgcag tactgcaagc taataacgtt ggtttgttat
5521 gtagtggtct tttgtccct ttccttctat caccctacat tccagcatct taccttcata
5581 tgcagtaaaa gaaagaaaga aaaaaaaagg aaaaaaaaaa aaaaaccaat gttttgcagt
5641 ttttttcatt gccaaaaact aaatggtgct ttatatattag attggaaaga atttcatatg
5701 caaagcatat taaagagaaa gccgcgttta gtcaataact ttttgtaaat ggcaatgcag
5761 aatattttgt tattggcctt ttctattcct gtaatgaaag ctgtttgtcg taacttgaaa
5821 ttttatcttt tactatggga gtcactattt attattgctt atgtgccctg ttcaaaacag
5881 aggcacttaa tttgatcttt tatttttctt tgtttttatt ttttttttta ttttagatgac
5941 caaaggatcat tacaacctgg ctttttattg tatttgtttc tggctttgt taagttctat
6001 tggaaaaacc actgtctgtg tttttttggc agttgtctgc attaacctgt tcatacaccc
6061 attttgtccc tttattgaaa aaataaaaaa aattaaagta ca

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[00082] SEQ ID NO: 11 is an amino acid sequence of Isoform 1 of human BCL11A.

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1 msrrkqgkpq hlskrefspe pleailtdde pdhgplgape gdhdlltccg cqmfnplgdi
61 lifiehkrkq cngslcleka vdkppspspi emkkaaspve vgivtpeddd dclstssrgi
121 cpkqehiadk llhwrglssp rsahgalipt pgmsaeyapq gickdepssy tcttckqpf
181 sawflqlhaq nthglriyle sehgspltpv vgipsglgae cpsqpplhgi hiadnnpfnl
241 lripqsvsre asglaegrfp ptpplfsppp rhhldphrie rlgaemala thhpsafdrv
301 lrlnpmamep pamdfsrrlr elagntsspp lspgrpspmq rllqpffqgs kppflatppl
361 pplqsappps qppvksksce fcgktfkfqs nlvvhrrsht gekpykcnlc dhactqaskl
421 krhmktmhk sspmtvksdd glstasspep gtsdlvgsas salksvvakf ksendpnlip
481 engdeeeeee deeeeeeeee eeelteser vdygfglsle aarhhenssr gavvgvgdes
541 ralpdvmqgm vlssmqhfse afhqvlgekh krghlaeaeg hrtdcdedsv agesdriddg
601 tvnrgrcspg esasgglskk lllgspssls pfskriklek efdlppaamp ntenvysqwl
661 agyaasrqlk dpflsfqdsr qspfassseh ssengslrfs tppgeldggi sgrsgtgsgg
721 stphissgpg grpskegrrr sdtceycgkv fkncsnltvh rrshtgerpy kcelcnlyaca
781 qsskltrhmk thgqvdkdvy kceickmpfs vstlekhmk kwhsdrvlmn dikte

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[00083] Alternative splicing of BCL11a leads to four isoforms containing 1, 3, or 6 C2H2 zinc-finger domains required for DNA-binding. Isoform 1, also known as BCL11A-XL, contains 6 C2H2 zinc-finger domains, and is the most abundant isoform in erythroid cells. Zinc finger domains are located at the following amino acid sequences:

Zinc finger domain 1 amino acids: 170-193 (SEQ ID NO: 67: ytcttckqpftsawflqlhaqnth);

Zinc finger domain 2 amino acids: 377-399 (SEQ ID NO: 68: kscefcgktfkfqsnlvvhrrsh);

Zinc finger domain 3 amino acids: 405-429 (SEQ ID NO: 69: ykcnlcdhactqasklkrhmkthmh);

Zinc finger domain 4 amino acids: 742-764 (SEQ ID NO: 70: dtceycgkvfkncsnltvhrsh);

Zinc finger domain 5 amino acids: 770-792 (SEQ ID NO: 71: ykcelcnyacaqsskltrhmkth);

Zinc finger domain 6 amino acids: 800-823 (SEQ ID NO: 72: ykceickmpfsvstylekhmkkw).

[00084] BCL11A can be targeted for degradation in erythroid cells, for example, through introduction of a bispecific construct as described herein that includes an antibody or antigen-binding fragment thereof that specifically binds BCL11A and a binding element that specifically binds TRIM10 or TRIM58. In one embodiment, the binding element that specifically binds TRIM10 or TRIM58 can be an antibody or antigen-binding fragment thereof. Any of a number of ways to introduce the construct to erythroid cells can be used, including, but not limited to introduction of the construct or a nucleic acid encoding it via lipid nanoparticles, introduction (e.g., as a fusion) via cell-penetrating peptides, or introduction of a vector, e.g., a viral vector, among others, encoding the construct.

[00085] BCL11A can also be targeted for degradation, for example, through introduction of a fusion construct as described herein that includes an antibody or antigen-binding fragment thereof that specifically binds BCL11A fused to a TRIM10 or TRIM58 polypeptide. The fusion construct can be introduced to erythroid cells in any of a number of ways, including, but not limited to introduction of the construct or a nucleic acid encoding it via lipid nanoparticles, introduction (e.g., as a fusion) via cell-penetrating peptides, or introduction of a vector, e.g., a viral vector, among others, encoding the construct.

ANTIBODIES

[00086] As discussed above, in various embodiments, described herein are antibodies or antigen-binding fragments thereof that specifically bind to BCL11A or to a TRIM10 or TRIM58 polypeptide. There are a wide variety of antibodies and constructs based upon them, but each generally includes an antigen-binding structure that includes so-called complementarity determining regions (CDRs) of an immunoglobulin polypeptide separated by so-called “framework regions” of the immunoglobulin. The CDRs are highly variable between antibodies that bind different antigens, while the framework regions tend to be more conserved. Most naturally-occurring antibodies include six CDRs, with three contributed by a so-called heavy chain variable domain, V_H, and three contributed by a so-called light chain variable domain, V_L. In these antibodies, the V_H and V_L domains form a complex in which residues in

the CDRs of each chain are configured to make contact with an epitope on a given antigen, thereby conferring binding specificity for that epitope of that antigen. While the majority of naturally-occurring antibodies include the V_H/V_L, six CDR configuration, several classes of animals, including camelids and cartilaginous fishes, produce antibodies that include only a V_H domain, with three CDRs. As discussed further below, the discovery of such antibodies, and the recognition that they can bind to target antigens with specificity and avidity closely comparable to that of antibodies with six CDRs has spawned a movement to isolate so-called single domain antibodies that are highly specific for target antigens. The movement has developed approaches for the generation or selection of single domain antibodies based on immunization of camelid species, selection from libraries generated from pre-immune and immune camelid genes, humanized camelid-based antibodies, as well as approaches for the selection of human single domain antibodies from libraries of pre-immune or immune V region human genes. Among the benefits of single domain antibodies are their smaller size, which makes it easier to introduce them to cells, better suited to the packaging constraints of, e.g., viral vectors and better suited as fusion partners in bi- or multifunctional constructs. The following provides additional detail in regard to antibodies generally and single domain antibodies more specifically.

[00087] As used herein, the term “antibody” refers to IgG, IgM, IgA, IgD or IgE molecules or antigen-binding fragments thereof (including, but not limited to, a Fab, F(ab')₂, Fv, disulphide linked Fv, scFv, single domain antibody, closed conformation multispecific antibody, disulphide-linked scfv, diabody), whether derived from any species that naturally produces an antibody, or created by recombinant DNA technology; whether isolated from serum, B-cells, hybridomas, transfectomas, yeast or bacteria.

[00088] As described herein, an “antigen” is a molecule that is bound by a binding site on an antibody agent. Typically, antigens are bound by antibody ligands and are capable of raising an antibody response *in vivo*. An antigen can be a polypeptide, protein, nucleic acid or other molecule or portion thereof. The term “antigenic determinant” refers to an epitope on the antigen recognized by an antigen-binding molecule, and more particularly, by the antigen-binding site of said molecule. An epitope is that portion of an antigen molecule with which an antibody makes direct physical contact via its antigen-binding site when the antibody specifically binds the antigen.

[00089] As used herein, the term “antibody reagent” refers to a polypeptide that includes at least one immunoglobulin variable domain or immunoglobulin variable domain sequence and which specifically binds a given antigen. An antibody reagent can comprise an antibody or a

polypeptide comprising an antigen-binding domain of an antibody. In some embodiments, an antibody reagent can comprise a monoclonal antibody or a polypeptide comprising an antigen-binding domain of a monoclonal antibody. For example, an antibody can include a heavy (H) chain variable region (abbreviated herein as V_H), and a light (L) chain variable region (abbreviated herein as V_L). In another example, an antibody includes two heavy (H) chain variable regions and two light (L) chain variable regions, as occurs, for example, in an IgG immunoglobulin. The term "antibody reagent" encompasses antigen-binding fragments of antibodies (e.g., single chain antibodies, Fab and sFab fragments, F(ab')₂, Fd fragments, Fv fragments, scFv, and single domain antibodies (sdAb) fragments (see, e.g. de Wildt et al., Eur J. Immunol. 1996; 26(3):629-39; which is incorporated by reference herein in its entirety)) as well as complete antibodies. An antibody can have the structural features of IgA, IgG, IgE, IgD, IgM (as well as subtypes and combinations thereof). Antibodies can be from any source, including mouse, rabbit, pig, rat, and primate (human and non-human primate) and primatized antibodies. Antibodies also include midibodies, humanized antibodies, chimeric antibodies, and the like.

[00090] As noted above, the V_H and V_L regions can be further subdivided into regions of hypervariability, termed "complementarity determining regions" ("CDR"), interspersed with regions that are more conserved, termed "framework regions" ("FR"). The extent of the framework region and CDRs has been precisely defined (see, Kabat, E. A., et al. (1991) Sequences of Proteins of Immunological Interest, Fifth Edition, U.S. Department of Health and Human Services, NIH Publication No. 91-3242, and Chothia, C. et al. (1987) J. Mol. Biol. 196:901-917; which are incorporated by reference herein in their entireties). The term "complementarity determining region" or "CDR" refers to variable regions in antibody polypeptides and contains the amino acid sequences that mediate specific binding to antigenic targets. These CDR regions account for the basic specificity of the antibody or antigen-binding fragment thereof for a particular antigenic determinant structure. Such regions are also referred to as "hypervariable regions." Each V_H and V_L is typically composed of three CDRs and four FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4. Single domain antibodies have three CDR regions, each non-contiguous with the others (termed CDR1, CDR2, CDR3) and separated by framework regions.

[00091] The terms "antigen-binding fragment" or "antigen-binding domain", which are used interchangeably herein are used to refer to one or more fragments of a full length antibody that retain the ability to specifically bind to a target of interest. Examples of binding fragments encompassed within the term "antigen-binding fragment" of a full length antibody include (i)

a Fab fragment, a monovalent fragment consisting of the V_L , V_H , C_L and $CH1$ domains; (ii) a $F(ab')_2$ fragment, a bivalent fragment including two Fab fragments linked by a disulfide bridge at the hinge region; (iii) an Fd fragment consisting of the V_H and $CH1$ domains; (iv) an Fv fragment consisting of the V_L and V_H domains of a single arm of an antibody, (v) a dAb fragment (Ward et al., (1989) Nature 341:544-546; which is incorporated by reference herein in its entirety), which consists of a V_H or V_L domain; and (vi) an isolated complementarity determining region (CDR) that retains specific antigen-binding functionality.

[00092] In some embodiments, a target ligand-binding recognition domain is a single-domain antibody. By the term "single-domain antibody" or "sdAb", it is meant an antibody fragment comprising a single protein domain that specifically binds a target antigen. A single domain antibody requires only three CDRs to specifically bind its target antigen. Single domain antibodies can comprise any variable fragment, including V_L , V_H , V_{HH} (camelid), and V_{NAR} (shark), and can be naturally- occurring or produced by recombinant technologies. For example, V_H , V_L , V_{HH} , and V_{NAR} domains can be generated by techniques well known in the art (Holt, et al., 2003; Jespers, et al., 2004a; Jespers, et al., 2004b; Tanha, et al., 2001 ; Tanha, et al., 2002; Tanha, et al., 2006 ; Revets, et al., 2005 ; Holliger, et al., 2005 ; Harmsen, et al., 2007 ; Liu, et al., 2007 ; Dooley, et al., 2003 ; Nuttall, et al., 2001 ; Nuttall, et al., 2000 ; Hoogenboom, 2005; Arbabi-Ghahroudi et al., 2008). In the recombinant DNA technology approach, libraries of sdAbs can be constructed in a variety of ways, "displayed" in a variety of formats such as phage display, yeast display, ribosome display, and subjected to selection to isolate binders to the targets of interest (panning). Examples of libraries include immune libraries derived from llama, shark or human immunized with the target antigen; non-immune/naïve libraries derived from non-immunized llama, camel, shark or human; or synthetic or semi-synthetic libraries such as V_H , V_L , V_{HH} or V_{NAR} libraries. In one embodiment, the sdAb can be a heavy variable domain (V_H). The term includes single domain antibodies as initially identified by selection/isolation of clones, as well as affinity-matured versions prepared by mutagenesis of isolated candidates.

[00093] In some embodiments, the target ligand-binding recognition domain is a nanobody. A "nanobody" (Nb) is a single variable domain (V_{HH}) single domain antibody generally derived, whether via immunization or via recombinant techniques, from for example, camelids, alpacas, llamas, and sharks. Nanobodies generally comprise a single amino acid chain that can be considered to comprise four framework regions and three complementarity determining regions. The term "camelids" refers to old world camelids (*Camelus bactrianus* and *Camelus dromedarius*) and new world camelids (for example, *Lama paccos*, *Lama glama*, *Lama*

guanicoe and *Lama vicugna*). The small size and unique biophysical properties of Nbs exceed conventional antibody fragments for the recognition of uncommon or hidden epitopes and for binding into cavities or active sites of protein targets. Further, Nbs can be designed as multi-specific and multivalent antibodies or attached to reporter molecules. Certain Nbs and Nb variants can survive the gastro-intestinal system and Nbs can easily be manufactured. Therefore, Nbs can be used in many applications including drug discovery and therapy, but also as a versatile and valuable tool for purification, functional study and crystallization of proteins.

[00094] As used herein, the term “specific binding” refers to a physical interaction between two molecules, compounds, cells and/or particles wherein the first entity binds to the second, target entity with greater specificity and affinity than it binds to a third entity which is a non-target. A reagent specific for a given target is one that exhibits specific binding for that target under the conditions of the assay being utilized. The specificity of an antibody or antibody fragment thereof can be determined based on affinity and/or avidity. The affinity, represented by the equilibrium constant for the dissociation (K_D) of an antigen with an antigen-binding protein, is a measure of the binding strength between an antigenic determinant and an antigen-binding site on the antigen-binding protein, such as an antibody or antigen-binding fragment thereof: the less the value of the K_D , the stronger the binding strength between an antigenic determinant and the antigen-binding molecule. Alternatively, the affinity can also be expressed as the affinity constant (K_A), which is $1/K_D$). Accordingly, an antibody or antigen-binding fragment thereof as described herein is said to be “specific for” or to “specifically bind” or “selectively bind” a first target or antigen compared to a second target or antigen when it binds to the first antigen with an affinity (as described above, and suitably expressed, for example as a K_D value) that is at least 1000 times, 10000 times or more better than the affinity with which said amino acid sequence or polypeptide binds to another given polypeptide. Generally, a molecule that “specifically binds,” “selectively binds” or “is specific for” a given target will bind with a K_D of 10^{-5} M (10000 nM) or less, e.g., 10^{-6} M, 10^{-7} M, 10^{-8} M, 10^{-9} M, 10^{-10} M, 10^{-11} M, 10^{-12} M, or less. Specific binding can be influenced by, for example, the affinity and avidity of the polypeptide agent and the concentration of polypeptide agent. The person of ordinary skill in the art can determine appropriate conditions under which polypeptide agents as described herein selectively bind the target using any suitable methods, such as titration of a polypeptide agent in a suitable cell binding assay.

[00095] It should be understood in this context that the specific binding is mediated by the CDRs of the antibody polypeptide, as opposed to any other portion of the antibody polypeptide.

Antibody dissociation constants and affinities can be determined, for example, by a surface plasmon resonance based assay (such as the BIAcore assay described in PCT Application Publication No. WO2005/012359); Forte Bio OctetTM analysis, enzyme-linked immunosorbent assay (ELISA); and competition assays (e.g., RIA's), for example.

[00096] As used herein, "avidity" is a measure of the strength of binding between an antigen-binding molecule (such as an antibody or antibody fragment thereof described herein) and the pertinent antigen. Avidity is related to both the affinity between an antigenic determinant and its antigen binding site on the antigen-binding molecule, and the number of pertinent binding sites present on the antigen-binding molecule. Typically, antigen-binding proteins (such as an antibody or portion of an antibody as described herein) will bind to their cognate or specific antigen with a dissociation constant (K_D) of 10^{-5} to 10^{-12} moles/liter or less, such as 10^{-7} to 10^{-12} moles/liter or less, or 10^{-8} to 10^{-12} moles/liter (i.e., with an association constant (K_A) of 10^5 to 10^{12} liter/moles or more, such as 10^7 to 10^{12} liter/moles or 10^8 to 10^{12} liter/moles). Any K_D value greater than 10^{-4} mol/liter (or any K_A value lower than 10^4 M⁻¹) is generally considered to indicate non-specific binding. The K_D for biological interactions which are considered meaningful (e.g., specific) are typically in the range of 10^{-10} M (0.1 nM) to 10^{-5} M (10000 nM). The stronger an interaction, the lower is its K_D . For example, a binding site on an antibody or portion thereof described herein will bind to the desired antigen with an affinity less than 500 nM, such as less than 200 nM, or less than 10 nM, such as less than 500 pM. Specific binding of an antigen-binding protein to an antigen or antigenic determinant can be determined in any suitable manner, including, for example, Scatchard analysis and/or competitive binding assays, such as radioimmunoassays (RIA), enzyme immunoassays (EIA) and sandwich competition assays, and the different variants thereof known in the art; as well as other techniques as known in the art and/or mentioned herein. The person of ordinary skill in the art can determine appropriate conditions under which polypeptide agents as described herein selectively bind the targets using any suitable methods, such as titration of a polypeptide agent in a suitable binding assay.

[00097] As used herein, the term "selectively inhibits" means that an agent inhibits, as that term is used herein, the function or activity of a given target but does not substantially inhibit the function or activity of a relevant non-target. Thus, for example, an antibody polypeptide that selectively inhibits the binding or function of BCL11A will not substantially inhibit the binding or function of the structurally-related BCL11B polypeptide.

[00098] As used herein, the term "target" refers to a biological molecule (e.g., peptide, polypeptide, protein, nucleic acid, lipid, carbohydrate, etc.) to which a polypeptide domain

which has a binding site can selectively bind. The target can be, for example, an intracellular target (e.g., an intracellular protein target) or a cell surface target (e.g., a membrane protein, a receptor protein). Exemplary “target” biological molecules for the purposes of the methods and compositions described herein include BCL11A. In some embodiments, a “target” can be one which it is wished to specifically degrade or inhibit, which can be the case when BCL11A is a target. In other instances, a “target” can be one which it is wished to bind and tether to another moiety, which can be the case when the target is, e.g., a TRIM10 or TRIM58 polypeptide.

[00099] As used herein, an antibody reagent (e.g., an antibody or antigen-binding domain thereof) that specifically binds to BCL11A, binds BCL11A with a dissociation constant (K_D) of 10^{-6} M or less, 10^{-7} M or less, 10^{-8} M or less, 10^{-9} M or less, 10^{-10} M or less, 10^{-11} M or less, or 10^{-12} M or less and binds to that target at least 100x, 1000x, or 10,000x more strongly than it binds to an off-target protein or distinct cell-surface or intracellular marker. An antibody reagent that specifically binds BCL11A will bind BCL11B, if at all, with a K_D at least 100X, or at least 1000X greater than the K_D with which it binds to BCL11A.

[000100] Similarly, an antibody reagent that specifically binds to TRIM10 or to TRIM58 binds to TRIM10 or TRIM58 with a dissociation constant (K_D) of 10^{-6} M or less, 10^{-7} M or less, 10^{-8} M or less, 10^{-9} M or less, 10^{-10} M or less, 10^{-11} M or less, or 10^{-12} M or less and binds to that target at least 100x, 1000x, or 10,000x more strongly than it binds to an off-target protein or distinct cell-surface or intracellular marker.

[000101] Additionally, and as described herein, a recombinant antibody or antigen-binding domain thereof can be further optimized to decrease potential immunogenicity, while maintaining functional activity, e.g., for therapy in humans. In this regard, functional activity means a polypeptide capable of displaying one or more known functional activities associated with an antibody or antibody reagent thereof as described herein. Such functional activities include, e.g. the ability to specifically bind to a target. One approach for decreasing potential immunogenicity of an antibody or antigen-binding fragment thereof is referred to as “humanizing” the antibody or antigen-binding fragment thereof. The term “humanized antibody” refers to forms of antibodies (or an antigen-binding fragment thereof) that contain sequences from non-human (e.g., murine) antibodies as well as human antibodies. Such antibodies are chimeric antibodies which contain minimal sequence derived from non-human immunoglobulin. In general, a humanized antibody or antigen-binding fragment will ideally comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the hypervariable loops correspond to those of a non-human immunoglobulin and all or substantially all of the framework (FR) regions are those of a human

immunoglobulin sequence. The humanized antibody optionally also will comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin (Jones et al., Nature 321 :522-525 (1986); Riechmann et al., Nature 332:323-329 (1988); and Presta, Curr. Op. Struct. Biol 2:593-596 (1992)). The constant region, can if desired, include one or more modifications that modify or disrupt interaction of the human or humanized antibody with an Fc receptor. Humanization can essentially be performed following the method of Winter and co-workers (Jones et al., Nature 321: 522-525 (1986); Riechmann et al., Nature 332: 323-3'27 (1988); Verhoeyen et al., Science 239: 1534-1536 (1988)), by substituting rodent, camelid or shark framework sequences with the corresponding sequences of a human antibody.

[000102] As discussed above, antibody polypeptides are provided herein that specifically bind to BCL11A and do not substantially cross-react with BCL11B. Non-limiting examples include the following nanobodies that target different zinc finger domains of the BCL11A polypeptide.

Nanobodies that target BCL11A ZNF6

[000103] The following provides amino acid sequence for nanobodies that specifically bind human BCL11A.

SEQ ID NO: 12- NB14

QVQLVESGGG LVQAGGSLRL SCAASGSIFS FYAMGWYRQA PGKEREVAS ITWRDDSTYY (60)
ADSVKGRFTI SRDIAKNTVY LQMNSLKPED TAVYYCAADT DDYTVEMDYW GQGTQVTSS
(120)

SEQ ID NO: 13- NB15

QVQLVESGGG LVQAGGSLRL SCAASGSTFS DYAMGWYRQA PGKERELVAV ITASDDITYY (60)
ADSVKGRFTI SRDIAKNTVY LQMNSLKPED TAVYYCAA AV TPG-QDL DYW GQGTQVTSS
(119)

SEQ ID NO: 14- NB53

QVQLVESGGG LVQAGGSLRL SCAASGSTFS GYAMGWYRQA PGKERELVAA ITSSGASTYY (60)
ADSVKGRFTI SRDIAKNTVY LQMNSLKPED TAVYYCAALD EGY-LDYDSW GQGTQVTSS
(119)

SEQ ID NO: 15- NB61

QVQLVESGGG LVQAGGSLRL SCAASGFIFD SYAMGWYRQA PGKERELVAA ITSSGSSTYY (60)
ADSVKGRFTI SRDIAKNTVY LQMNSLKPED TAVYYCAALD --Y-VIDGYW GQGTQVTSS
(117)

SEQ ID NO: 16- NB19 (Nb6101-M45D-19)

RVQLVESGGG LVQAGGSLRL SCAADGFDFK SYAMGWYRQA PGREDELVAA ITASGDYTTY (60)
ADSVKGRFTI SRDIAKNTVY LQMNSLKPDD TAVYYCAALD --Y-VAEGYW GQGTQVTSS
(117)

SEQ ID NO: 17- NB5344-N74D

QVQLVKSGGG LVQAGDSLRL SCAASGSTFS GYAMGWYRQA PGKERELVAA ITSSGASTYY (60)
 ADSVRGRFTI SRDNAKNTVY LQMNSLKPED TAVYYCAALD EGY-LDYDSW GQGTQVTVSS
 (119)

SEQ ID NO: 18- NB6101

RVQLVESGGG LVQAGGSLRL SCAASGFIFD SYAMGWYRQA PGKEMELVAA ITSSGSSTYY (60)
 ADSVKGRFTI SRDNAKNTVY LQMNSLKPED TAVYYCAALD --Y-VIDGYW GQGTQVTVSS
 (117)

SEQ ID NO: 19- NB6101-M45D

RVQLVESGGG LVQAGGSLRL SCAASGFIFD SYAMGWYRQA PGKEDELVAA ITSSGSSTYY (60)
 ADSVKGRFTI SRDNAKNTVY LQMNSLKPED TAVYYCAALD --Y-VIDGYW GQGTQVTVSS
 (117)

SEQ ID NO: 20- NB1453

QVQLVESGGG LVQAGGSLHL SCAVSGSIFS FYAMGWYRQA PGKEREVAS ITRRDDSTYY (60)
 ADSVEGRFAI SRDNAKNTVY LQMNSLKPED TAVYYCAADT DDYTVEMDYW GQGTQVTVSS
 (120)

SEQ ID NO: 21- NB1459

QVQLVESGGG LVQAEGSLRL SCAASGSIFS FYAMGWYRQA PGKEREVAS ITWRDDSTYY (60)
 ADSVTGRFTI SRDNAKNTVY LQMNSLKPED TAVYYCAADT DDYSVEMDYW GQGTQVTVSS
 (120)

SEQ ID NO: 22- NB1431

QVQLVESGGG LVQAGGCLRL SCAASGSIFS FYAMGWYRQA PGNEREVAS ITWRDDSTYY (60)
 ADSVKGRFTI SRDNAKNTVY LQMNSLKPED TAVYYCAADT DDYTVEMDYW GQGTHVTVSS
 (120)

SEQ ID NO: 23- NB1407

QVQLVESGGD LVQAGASLRL SCAASGSIFS FYAMGWYRQA PGKEREVAS ITWRDDSTYY (60)
 ADSVKGRFTI SRDNARNTVY LQMNNLKPED TAVYYCAADT DDYTVEMDYW GQGTQVTVSS
 (120)

SEQ ID NO: 24- NB1481

QVQLVESGGG LVQAGGSLRL SCAASGSIFS FYAMGWYRQA PGKEREVAF ITWRDDSTYY (60)
 ADSVKGRFRI SRDNAKNTVY LQMNSLKPED TAVYYCAADT DDYTVEMDYW GQGTQVTVSS
 (120)

SEQ ID NO: 25- NB1419

QVQLVESGGD LVQAGGSLRL SCAASGSIFS FYAMGWYRQA PGKEREVAS ITWRDDSTYY (60)
 ADSVKGRFTI SRDNAKNTVY LQMNNLKPED TAVYYCAADT DDYTVEMDYW GQGTQVTVSS
 (120)

SEQ ID NO: 26- NB1487

QVQLVESGGG LVQAGGSLRL SCAASGSIFS FYAMGWYRQA PGKEREVAS ITWRDDSTYY (60)

ADSVMGFRFTI SRDNAKNTVY LQMNSLKPED TAVYYCAADT DDYTVEMDYW GQGTQVTVSS
(120)

SEQ ID NO: 27- NB6101-M45D-14

QVQLVKSGGG LVQAGDSLRL SCAASGSTFS GYAMGWYRQA PGKERELVAA ITSSGASTYY (60)
ADSVRGRFTI SRDNAKNTVY LQMNSLKPED TAVYYCAALD EGY-LDYDSW GQGTQVTVSS
(119)

SEQ ID NO: 28- NB6130

QVQFVESGGG FVQAGGSLRL SCAASGFIFD SYAMGWYSQA PGKERELVAA ITSSGSSTYY (60)
ADSVKGRFTI SRDNAKNTVY LQMNSLKPED TAVYYCAALD --Y-VIDGYW GQGTQVTVSS
(117)

SEQ ID NO: 29- NB6127

QVQLVESGGG LVQAGGSLRL SCAASGFIFD SYSMGWYRQA PGKEGELVAA ITSSGSSTYY (60)
ADSVNGRFTI SRDNAKNTVY LQMNSLKPED TAVYYCAALD --Y-VIDGYW GQGTQVTVSS
(117)

SEQ ID NO: 30- NB6160

QVQLVESGGG LVQAGGSLRL SCAASGFIFD SYAMGWYRQA PGKEGELVAA ITSSGSSTYY (60)
ADSVKGRFTI SRDNAKNTVY LQMNSLKPED TAVYYCAALD --Y-VIDGYW GQGTQVSVSS
(117)

SEQ ID NO: 31- NB6102

QVQLVESGGG LVQAGGSLRL SCAASGFIFD SYAMGWYRQA PGKEGELVAA ITSSGSSTYY (60)
ADSVKGRFTI SRDNAKNTVY LQMNSLKPED TAVYYCAALD --Y-VIDGYW GQGTQVTVSS
(117)

SEQ ID NO: 32- NB6101-M45D-26

RVQLVESGGG LVQAGGSLRL SCAADGFDFS SYAMGWYRQA PGTQDELVAA ITASGSYTTY (60)
SDSVKGRFTI SRDNAKNTVY LQMNSLKPDD TAVYYCAALS --Y-VAEGYW GQGTQVTVSS
(117)

SEQ ID NO: 33- NB6101-M45D-20

RVQLVESGGG LVQAGGSLRL SCAADGFDFK SYAMGWYRQA PGYEDELVAA ITASGSYTTY (60)
SDSVKGRFTI SRDNAKNTVY LQMNSLKPED TAVYYCAALS --Y-VAEGYW GQGTQVTVSS
(117)

Table 1: CDR's for Nanobodies directed to ZNF6

Nanobody	CDR1	CDR2	CDR3
Nb14	GSIFS FYAM SEQ ID NO: 43	EFVAS ITWRDDSTYY SEQ ID NO: 51	ADT DDYTVEMDYW SEQ ID NO: 60
Nb15	GSTFS DYAM SEQ ID NO: 44	ELVAV ITASDDITYY SEQ ID NO: 52	AAV TPG-QDLGYW SEQ ID NO: 61
Nb53	GSTFS GYAM SEQ ID NO: 45	ELVAA ITSSGASTYY SEQ ID NO: 53	ALD EGY-LDYDSW SEQ ID NO: 62

Nb61	GFIFD SYAM SEQ ID NO: 46	ELVAA ITSSGSSTYY SEQ ID NO: 54	ALD --Y-VIDGYW SEQ ID NO: 63
Nb19 (Nb6101- M45D-19)	GFDFK SYAM SEQ ID NO: 47	ELVAA ITASGDYTY SEQ ID NO: 55	ALD --Y-VAEGYW SEQ ID NO: 64
Nb5344- N74D	GSTFS GYAM SEQ ID NO: 45	ELVAA ITSSGASTYY SEQ ID NO: 53	ALD EGY-LDYDSW SEQ ID NO: 62
Nb6101	GFIFD SYAM SEQ ID NO: 46	ELVAA ITSSGSSTYY SEQ ID NO: 54	ALD --Y-VIDGYW SEQ ID NO: 63
Nb6101- M45D	GFIFD SYAM SEQ ID NO: 46	ELVAA ITSSGSSTYY SEQ ID NO: 54	ALD --Y-VIDGYW SEQ ID NO: 63
Nb1453	GSIFS FYAM SEQ ID NO: 43	EFVAS ITRRDDSTYY SEQ ID NO: 56	ADT DDYTVEMDYW SEQ ID NO: 60
Nb1459	GSIFS FYAM SEQ ID NO: 43	EFVAS ITWRDDSTYY SEQ ID NO: 57	ADT DDYSVEMDYW SEQ ID NO: 65
Nb1431	GSIFS FYAM SEQ ID NO: 43	EFVAS ITWRDDSTYY SEQ ID NO: 57	ADT DDYTVEMDYW SEQ ID NO: 60
Nb1407	GSIFS FYAM SEQ ID NO: 43	EFVAS ITWRDDSTYY SEQ ID NO: 57	ADT DDYTVEMDYW SEQ ID NO: 60
Nb1481	GSIFS FYAM SEQ ID NO: 43	EFVAF ITWRDDSTYY SEQ ID NO: 58	ADT DDYTVEMDYW SEQ ID NO: 60
Nb1419	GSIFS FYAM SEQ ID NO: 43	EFVAS ITWRDDSTYY SEQ ID NO: 57	ADT DDYTVEMDYW SEQ ID NO: 60
Nb1487	GSIFS FYAM SEQ ID NO: 43	EFVAS ITWRDDSTYY SEQ ID NO: 57	ADT DDYTVEMDYW SEQ ID NO: 60
Nb6101- M45D-14	GSTFS GYAM SEQ ID NO: 45	ELVAA ITSSGASTYY SEQ ID NO: 53	ALD EGY-LDYDSW SEQ ID NO: 62
Nb6130	GFIFD SYAM SEQ ID NO: 46	ELVAA ITSSGSSTYY SEQ ID NO: 54	ALD --Y-VIDGYW SEQ ID NO: 63
Nb6127	GFIFD SYAM SEQ ID NO: 48	ELVAA ITSSGSSTYY SEQ ID NO: 54	ALD --Y-VIDGYW SEQ ID NO: 63
Nb6160	GFIFD SYAM SEQ ID NO: 46	ELVAA ITSSGSSTYY SEQ ID NO: 54	ALD --Y-VIDGYW SEQ ID NO: 63
Nb6102	GFIFD SYAM SEQ ID NO: 46	ELVAA ITSSGSSTYY SEQ ID NO: 54	ALD --Y-VIDGYW SEQ ID NO: 63
Nb6101- M45D-26	GFDFS SYAM SEQ ID NO: 49	ELVAA ITASGSYTY SEQ ID NO: 59	ALS --Y-VAEGYW SEQ ID NO: 64
Nb6101- M45D-20	GFDFK SYAM SEQ ID NO: 50	ELVAA ITASGSYTY SEQ ID NO: 59	ALS --Y-VAEGYW SEQ ID NO: 64

Nanobodies directed to ZNF23 “extended region”

SEQ ID NO: 34- 2D9 / Consensus

QVQLVESGGG LVQAGGSLRL SCAASGSIFV NNAMGWYRQA PGKERELVAA ISASGGSTYY (60)
ADSVKGRFTI SRDIAKNTVY LQMNSLKPED TAVYYCAADQ DVYPYEWGQ GTQVTVSS (118)

SEQ ID NO: 35- V102G

QVQLVESGGG LVQAGGSLRL SCAASGSIFV NNAMGWYRQA PGKERELVAA ISASGGSTYY (60)
ADSVKGRFTI SRDIAKNTVY LQMNSLKPED TAVYYCAADQ DGYPYEWGQ GTQVTVSS (118)

SEQ ID NO: 36- W108L

QVQLVESGGG LVQAGGSLRL SCAASGSIFV NNAMGWYRQA PGKERELVAA ISASGGSTYY (60)
ADSVKGRFTI SRDIAKNTVY LQMNSLKPED TAVYYCAADQ DVYPYELGQ GTQVTVSS (118)

CDR's for Nanobodies directed to ZNF23 extended region

SEQ ID NO: 37- 2D9 / V102G / W108L CDR 1
SIFVNNAM

SEQ ID NO: 38- 2D9 / V102G / W108L CDR 2
ELVAAISASGGSTYY

SEQ ID NO: 39- 2D9 CDR 3
ADQDVYPYEYW

SEQ ID NO: 40- 2D9 CDR 3
ADQDGYPYEYW

SEQ ID NO: 41- 2D9 CDR 3
ADQDVYPYEYL

Nanobodies directed to BCL11A ZNF4

SEQ ID NO: 42- Nb12
QVQLVESGGG SVQPGGSLTL SCTASGFPLS MWGMTWLRQA PGKGLERVSG IASDSTNTYY (60)
ADSVKGRFAI SRDANKNKL F LQMNSLKPED TAVYFCARKW TNWDLKGQGT QVTVSS (116)

[000104] As a means of describing structure involved in or necessary for binding to BCL11A, the nanobody NB14 of SEQ ID NO: 12 is used as a reference herein. It should be understood that other nanobodies described herein can be used as a reference in a similar manner. The following Table 2 shows the amino acid sequence of NB14, with variations at each site that can permit specific binding of the nanobody to the same epitope of BCL11A. In some embodiments, variation relative to SEQ ID NO: 12 occurs only at the sites listed in Table 2.

Table 2: BCL11A ZNF6 nanobody amino acid variations. Compared to Nb14 (SEQ ID NO: 12).

Amino Acid #	Nb14 amino acid	Potential site specific mutations
1	Q	R;
2	V	M
4	L	F
6	E	K
10	G	D
11	L	F
15	G	E; D
16	G	A; D
17	S	C
19	R	H
24	A	V
25	S	D
27	S	F; Y

28	I	T; D
30	S	D; K
31	F	D; G; S
33	A	S
38	R	S
43	K	N; T; Y; R
44	E	Q
45	R	G; M; D;
46	E	Q
47	F	L
50	S	F; V; A
53	W	R; A; S
54	R	S
55	D	G
56	D	A; S
57	S	I; Y; E
61	A	S; H
62	D	P
65	K	E; T; M; R; N
69	T	A; R
76	K	R
85	S	N; R
89	E	D
99	D	A; L
100	T	V; D; S
101	D	E; S; T; V; --
102	D	V; P; G; --
103	Y	A; S; K; G
104	T	E; Q; S; --
105	V	G; D; Q; L
106	E	Y; D; I; A
107	M	W; L; Y; D; E
108	D	S; G
109	Y	Q; W; S
111	G	T
112	Q	G
113	G	T; V
114	T	Q
115	Q	H; V
116	V	S; T
117	T	S; V

HEMOGLOBINOPATHIES

[000105] Fetal hemoglobin (HbF) is a tetramer of two adult α -globin polypeptides and two fetal β -like γ -globin polypeptides. During gestation, the duplicated γ -globin genes constitute the predominant genes transcribed from the β -globin locus. Following birth, γ -globin becomes progressively replaced by adult β -globin, a process referred to as the "fetal switch." In humans, the developmental switch from production of predominantly fetal hemoglobin or HbF ($\alpha_2\gamma_2$) to production of adult hemoglobin or HbA ($\alpha_2\beta_2$) begins at about 28 to 34 weeks of gestation and continues shortly after birth at which point HbA becomes predominant. This switch results primarily from decreased transcription of the gamma-globin genes and increased transcription of beta-globin genes. On average, the blood of a normal adult contains only about 2% HbF, though residual HbF levels have a variance of over 20 fold in healthy adults (Atweh, Semin. Hematol. 38(4):367-73 (2001)).

[000106] Hemoglobinopathies encompass a number of anemias of genetic origin in which there are insufficient amounts of hemoglobin capable of carrying oxygen in red blood cells (RBCs). These disorders include genetic defects that result in the production of abnormal hemoglobins with a concomitant impaired ability to maintain oxygen concentration. Some such disorders involve the failure to produce normal β -globin in sufficient amounts, while others involve the failure to produce normal β -globin entirely. Those disorders specifically associated with the β -globin protein are referred to generally as β -hemoglobinopathies. For example, β -thalassemias result from a partial or complete defect in the expression of the β -globin gene, leading to deficient or absent HbA. Sickle cell anemia or sickle cell disease (SCD) results from a point mutation in the β -globin structural gene, leading to the production of an abnormal hemoglobin (HbS) that results in deformed (sickled) RBCs. HbS RBCs are more fragile than normal RBCs and undergo hemolysis more readily, leading eventually to anemia (Atweh, Semin. Hematol. 38(4):367-73 (2001)).

[000107] The search for treatment aimed at reduction of globin chain imbalance in patients with β -hemoglobinopathies has focused on the manipulation of fetal hemoglobin ($\alpha_2\gamma_2$; HbF). The important therapeutic potential of such approaches is indicated by observations of the mild phenotype of individuals with co-inheritance of both homozygous β -thalassemia and hereditary persistence of fetal hemoglobin (HPFH), as well as by those patients with homozygous β -thalassemia who synthesize no adult hemoglobin, but in whom a reduced requirement for transfusions is observed in the presence of increased concentrations of fetal hemoglobin. Furthermore, it has been observed that certain populations of adult patients with

β chain abnormalities have higher than normal levels of fetal hemoglobin (HbF), and have been observed to have a milder clinical course of disease than patients with normal adult levels of HbF. For example, a group of Saudi Arabian sickle-cell anemia patients who express 20-30% HbF have only mild clinical manifestations of the disease (Pembrey, et al., Br. J. Haematol. 40: 415-429 (1978)). Thus, it is now accepted that β -hemoglobinopathies, such as sickle cell anemia and the β -thalassemias, can be ameliorated by increased HbF production. (Reviewed in Jane and Cunningham Br. J. Haematol. 102: 415-422 (1998) and Bunn, N. Engl. J. Med. 328: 129-131 (1993)).

[000108] As used herein, treating or reducing a risk of developing a hemoglobinopathy in a subject means to ameliorate at least one symptom of hemoglobinopathy. In one aspect, the methods described herein feature methods of treating, e.g., reducing severity or progression of, a hemoglobinopathy in a subject. In another aspect, the methods can also be used to reduce a risk of developing a hemoglobinopathy in a subject, delaying the onset of symptoms of a hemoglobinopathy in a subject, or increasing the longevity of a subject having a hemoglobinopathy. In one aspect, the methods can include selecting a subject on the basis that they have, or are at risk of developing a hemoglobinopathy, but do not yet have symptoms of a hemoglobinopathy. Selection of a subject can include detecting symptoms of a hemoglobinopathy, a blood test, genetic testing, or clinical recordings. If the results of the test(s) indicate that the subject has a hemoglobinopathy, the methods can also include administering a composition as described herein, thereby treating, or reducing the risk of developing, a hemoglobinopathy in the subject. As non-limiting examples, a subject with a diagnosis of SCD with genotype HbSS, HbS/ β^0 thalassemia, HbSD, or HbSO, and/or HbF <10% by electrophoresis is indicated for treatment using compositions and methods as described herein. By the phrase "risk of developing disease" is meant the relative probability that a subject will develop a hemoglobinopathy in the future as compared to a control subject or population (e.g., a healthy subject or population). For example, an individual carrying the genetic mutation associated with SCD, an A to T mutation of the β -globin gene, and whether the individual is heterozygous or homozygous for that mutation increases that individual's risk. Methods that promote the de-repression or re-expression of fetal hemoglobin in an individual diagnosed with or suffering from a hemoglobinopathy can be effective for treatment of the disease or disorder.

[000109] As used herein, the term "hemoglobinopathy" refers to a condition involving the presence of an abnormal hemoglobin molecule or insufficient levels of hemoglobin capable

of carrying oxygen in the blood and releasing the oxygen in tissues of the body. The term refers to a condition involving any defect in the structure, function or amount of any hemoglobin of an individual, and includes defects in the primary, secondary, tertiary or quaternary structure of hemoglobin caused by any mutation, such as deletion mutations or substitution mutations in the coding regions of a globin gene, or mutations in, or deletions of, the promoters or enhancers of such genes that cause a reduction in the amount of hemoglobin produced as compared to a normal or standard condition. The term further includes any decrease in the amount or effectiveness of hemoglobin, whether normal or abnormal, caused by external factors such as disease, chemotherapy, toxins, poisons, or the like.

[000110] The term “sickle cell disease” or “SCD” is defined herein to include any symptomatic anemic condition which results from sickling of red blood cells. Manifestations of SCD include: anemia; pain; and/or organ dysfunction, such as renal failure, retinopathy, acute-chest syndrome, ischemia, priapism, and stroke. The term refers to a variety of clinical problems attendant upon SCD, especially in those subjects who are homozygotes for the sickle cell substitution in HbS. Among the constitutional manifestations referred to herein by use of the term of SCD are delay of growth and development, an increased tendency to develop serious infections, particularly due to pneumococcus, marked impairment of splenic function, preventing effective clearance of circulating bacteria, with recurrent infarcts and eventual destruction of splenic tissue. Also involved in SCD are acute episodes of musculoskeletal pain, which affect primarily the lumbar spine, abdomen, and femoral shaft, and which are similar in mechanism and in severity. In adults, such attacks commonly manifest as mild or moderate bouts of short duration every few weeks or months interspersed with agonizing attacks lasting 5 to 7 days that strike on average about once a year. Among events known to trigger such crises are acidosis, hypoxia, and dehydration, all of which potentiate intracellular polymerization of HbS (J. H. Jandl, Blood: Textbook of Hematology, 2nd Ed., Little, Brown and Company, Boston, 1996, pages 544-545).

[000111] As used herein, “THAL” or “thalassemia” refers to a hereditary disorder characterized by defective production of hemoglobin. In one embodiment, the term encompasses hereditary anemias that occur due to mutations affecting the synthesis of hemoglobins. In other embodiments, the term includes any symptomatic anemia resulting from thalassemic conditions such as severe or β -thalassemia, thalassemia major, thalassemia intermedia, α -thalassemias such as hemoglobin H disease. β -thalassemias are caused by a mutation in the β -globin chain, and can occur in a major or minor form. In the major form of

β -thalassemia, children are normal at birth, but develop anemia during the first year of life. The mild form of β -thalassemia produces small red blood cells. Alpha-thalassemias are caused by deletion of a gene or genes from the globin chain.

[000112] Where HbF is functional for carrying and delivering oxygen to tissues, the re-induction or de-repression of HbF expression provides an avenue for treating β -hemoglobinopathies. One approach as described herein targets BCL11A, which is responsible for repression of HbF expression. In various embodiments, an antibody or nucleic acid or vector encoding an antibody that specifically binds BCL11A and does not substantially cross-react with BCL11B, can be used to de-repress expression of the HbF subunit genes.

Inhibition of BCL11A

[000113] In various embodiments, the activity of BCL11A is inhibited or decreased, e.g., by targeted protein degradation. By “decreases BCL11A activity” or “inhibits BCL11A activity” is meant that the amount of functional activity of BCL11A is at least 5% lower in a cell or cell population treated with the compositions and methods described herein, than a comparable, control cell or population, wherein BCL11A is not targeted for degradation. BCL11A activity in a treated population is at least 10% lower, at least 20% lower, at least 30% lower, at least 40% lower, at least 50% lower, at least 60% lower, at least 70% lower, at least 80% lower, at least 90% lower, at least 1-fold lower, at least 2-fold lower, at least 5-fold lower, at least 10 fold lower, at least 100 fold lower, at least 1000-fold lower, or less relative to a control treated population in which BCL11A is not targeted for degradation. At a minimum, BCL11A activity can be assayed by determining the amount of BCL11A at the protein level, using techniques standard in the art. Alternatively, or in addition, BCL11A activity can be determined using a reporter construct, wherein the reporter construct is sensitive to BCL11A activity.

[000114] Alternatively, or in addition, BCL11A activity can be assayed by measuring fetal hemoglobin expression at the mRNA or protein level following treatment targeting BCL11A for degradation. Increased expression of endogenous HbF in adult cells treated or contacted with a construct targeting BCL11A for degradation is indicative of de-repression, and therefore reduced activity of BCL11A.

Treatment of hemoglobinopathies by targeting BCL11A

[000115] BCL11A activity and/or expression has been shown to repress expression of fetal hemoglobin isoforms. Thus, as noted above, inhibition or degradation of BCL11A removes or reduces this repression and permits fetal hemoglobin isoforms to be re-induced, for example, in an adult. Increasing expression of the γ -globin genes can ameliorate hemoglobinopathies. Thus, in some embodiments, targeted degradation of BCL11A via introduction of a targeted protein degradation construct as described herein can treat a hemoglobinopathy.

[000116] In connection with contacting a cell with a targeted protein degradation construct aimed at BCL11A, “increasing the fetal hemoglobin levels” in a cell indicates that HbF is at least 5% higher in populations treated with the construct than in a comparable, control population, wherein no BCL11A targeting construct is present. It is preferred that the percentage of HbF expression in a BCL11A targeting construct-treated population is at least 10% higher, at least 20% higher, at least 30% higher, at least 40% higher, at least 50% higher, at least 60% higher, at least 70% higher, at least 80% higher, at least 90% higher, at least 1-fold higher, at least 2-fold higher, at least 5-fold higher, at least 10 fold higher, at least 100 fold higher, at least 1000-fold higher, or more than a control treated population of comparable size and culture conditions. The term “control treated population” is used herein to describe a population of cells that has been treated with identical media, viral induction, nucleic acid sequences, temperature, confluency, flask size, pH, etc., without the BCL11A targeting construct. In one embodiment, any method known in the art can be used to measure an increase in fetal hemoglobin expression, e. g. Western Blot analysis of fetal γ -globin protein and PCR quantification of mRNA encoding fetal hemoglobin (e.g., HBG1 or HBG2 mRNAs).

[000117] It should be understood that complete inhibition of BCL11A activity is not required to derepress HbF expression enough for therapeutic benefit. As discussed above, HbF makes up about 2% of hemoglobin in adult humans, although the percentage varies. In one embodiment, derepression of HbF expression such that the amount of HbF expressed in adult erythroid cells is increased to at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% or more of the total hemoglobin can provide therapeutic benefit. In another embodiment, an increase in HbF expression by at least 5-fold, at least 10-fold, at least 15-fold, at least 20-fold,

at least 25-fold, at least 30-fold, at least 35-fold, at least 40-fold, at least 45-fold, at least 50 fold or more relative to baseline without a BCL11A inhibitor can provide therapeutic benefit.

Fusion Proteins

[000118] In some embodiments, an antibody or antigen-binding fragment thereof, e.g., a single domain antibody or nanobody, is fused to another polypeptide sequence to provide additional functionality to the antibody or antigen-binding fragment thereof. As discussed herein above, fusion of an antibody or antigen-binding fragment thereof that binds TRIM10 or TRIM58 to an antibody or antigen-binding fragment thereof that binds an erythroid-specific or erythroid-enriched polypeptide can provide an avenue for targeted protein degradation of the erythroid-specific or erythroid enriched polypeptide in erythroid cells. Alternatively, a TRIM10 or TRIM58 polypeptide can be fused to an antibody or antigen-binding fragment thereof that specifically binds an erythroid-specific or erythroid-enriched polypeptide to provide a targeted protein degradation construct for that erythroid-specific or erythroid-enriched polypeptide.

[000119] One of ordinary skill in the art, given sequences encoding TRIM10, TRIM58, or encoding an antibody or antigen-binding fragment thereof that specifically binds TRIM10 or TRIM58, can generate a fusion construct that fuses the TRIM10 or TRIM58 polypeptide or TRIM10 or TRIM58-specific antibody sequence to a given fusion partner, e.g., antibody that binds a target for targeted protein degradation where the sequence for the fusion partner is known. Fusion can optionally include a peptide linker between the fusion partners. In one embodiment, the linker can be a serine/glycine rich linker. By way of example, sequences for TRIM10, TRIM58, and nanobodies that specifically bind BCL11A are provided herein.

Expression and Delivery of Targeted Protein Degradation Constructs

[000120] In various embodiments, fusion proteins as described herein that target a protein of interest, e.g., an erythroid protein of interest, for degradation can be expressed from a vector as a recombinant polypeptide.

[000121] Sequences encoding a fusion protein as described herein can be contained in or expressed by a desired vector. Many such vectors useful for transferring exogenous genes into target mammalian cells are available. As used herein, a vector can be viral or non-viral. The term “vector” encompasses any genetic element that is capable of replication when associated with the proper control elements and that can transfer gene sequences to cells. A vector can include, but is not limited to, an expression vector, a plasmid, phage, transposon, cosmid, artificial chromosome, virus, virion, etc.

[000122] An expression vector can direct expression of a polypeptide (e.g., a fusion protein for targeted degradation of an erythroid-enriched or erythroid specific protein) from nucleic acid sequences contained therein linked to transcriptional regulatory sequences on the vector. The sequences expressed will often, but not necessarily, be heterologous to the cell. An expression vector can comprise additional elements, for example, the expression vector may have two replication systems, thus allowing it to be maintained in two organisms, for example in human or mammalian cells for expression and in a prokaryotic host for cloning and amplification. Expression refers to the cellular processes involved in producing RNA and/or proteins and as appropriate, secreting proteins, including where applicable, but not limited to, for example, transcription, transcript processing, translation and protein folding, modification and processing. "Expression products" include RNA transcribed from a gene and polypeptides obtained by translation of mRNA transcribed from a gene or gene construct.

[000123] Thus, in some embodiments, provided herein is a vector comprising a nucleic acid encoding a fusion protein for targeted degradation of an erythroid-enriched or erythroid specific protein as described herein. Typically, where introduction of the sequence encoding the fusion protein is desired, the vector is a viral vector which is an adeno-associated virus (AAV), a retrovirus, bovine papilloma virus, an adenovirus vector, a lentiviral vector, a vaccinia virus or a polyoma virus, among others.

[000124] In some embodiments, the vector is an AAV vector. As used herein, the term "AAV vector" means a vector derived from an adeno-associated virus serotype, including without limitation, AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, and mutated forms thereof. AAV vectors can have one or more of the AAV wild-type genes deleted in whole or part, preferably the rep and/or cap genes, but retain functional flanking ITR sequences.

[000125] Retroviruses can be chosen as gene delivery vectors due to their ability to integrate their genes into the host genome, transferring a large amount of foreign genetic material, infecting a broad spectrum of species and cell types and for being packaged in special cell lines. In order to construct a retroviral vector, a nucleic acid encoding a gene of interest is inserted into the viral genome in the place of certain viral sequences to produce a virus that is replication-defective. In order to produce virions, a packaging cell line is constructed containing the gag, pol, and/or env genes but without the LTR and/or packaging components. When a recombinant plasmid containing a cDNA, together with the retroviral LTR and packaging sequences is introduced into this cell line (by calcium phosphate precipitation for example), the packaging sequence allows the RNA transcript of the recombinant plasmid to

be packaged into viral particles, which are then secreted into the culture medium. The medium containing the recombinant retroviruses is then collected, optionally concentrated, and used for gene transfer. Retroviral vectors are able to infect a broad variety of cell types. Lentiviruses are complex retroviruses, which, in addition to the common retroviral genes gag, pol, and env, contain other genes with regulatory or structural function. The higher complexity enables the virus to modulate its life cycle, as in the course of latent infection. Some examples of lentivirus include the Human Immunodeficiency Viruses (HIV 1, HIV 2) and the Simian Immunodeficiency Virus (SIV). Lentiviral vectors have been generated by multiply attenuating the HIV virulence genes, for example, the genes env, vif, vpr, vpu and nef are deleted making the vector biologically safe. Lentiviral vectors are known in the art, see, e.g. U.S. Pat. Nos. 6,013,516 and 5,994,136, both of which are incorporated herein by reference. In general, the vectors are plasmid-based or virus-based, and are configured to carry the essential sequences for incorporating foreign nucleic acid, for selection and for transfer of the nucleic acid into a host cell. The gag, pol and env genes of viral vectors of interest also are known in the art. Thus, the relevant genes are cloned into the selected vector and then used to transform the target cell of interest. Recombinant lentivirus capable of infecting a non-dividing cell wherein a suitable host cell is transfected with two or more vectors carrying the packaging functions, namely gag, pol and env, as well as rev and tat is described in U.S. Pat. No. 5,994,136, incorporated herein by reference. This describes a first vector that can provide a nucleic acid encoding a viral gag and a pol gene and another vector that can provide a nucleic acid encoding a viral env to produce a packaging cell. Introducing a vector providing a heterologous gene into that packaging cell yields a producer cell which releases infectious viral particles carrying the foreign gene of interest. The env preferably is an amphotropic envelope protein which allows transduction of cells of human and other species. Typically, the nucleic acid molecule or the vector as described herein includes "expression control sequences", which refers collectively to promoter sequences, polyadenylation signals, transcription termination sequences, upstream regulatory domains, origins of replication, internal ribosome entry sites ("IRES"), enhancers, and the like, which collectively provide for the replication, transcription and translation of a coding sequence in a recipient cell. Not all of these control sequences need always be present so long as the selected coding sequence is capable of being replicated, transcribed and translated in an appropriate host cell. A promoter sequence is a nucleotide region comprising a DNA regulatory sequence, wherein the regulatory sequence is typically derived from a gene which is capable of binding RNA polymerase and initiating transcription of a downstream (3'-

direction) coding sequence. Transcription promoters can include inducible promoters (where expression of a polynucleotide sequence operably linked to the promoter is induced by an analyte, cofactor, regulatory protein, etc.), repressible promoters (where expression of a polynucleotide sequence operably linked to the promoter is induced by an analyte, cofactor, regulatory protein, etc.), and constitutive promoters. In some embodiments, the promoter can be a promoter for an erythroid-enriched or erythroid-specific gene, e.g., a hemoglobin gene.

[000126] In some embodiments, provided herein is a host cell transformed with a nucleic acid molecule encoding a fusion protein for targeted degradation of an erythroid-enriched or erythroid specific protein as described herein. The term "transformation" means the introduction of a "foreign" (i.e. extrinsic or extracellular) gene, DNA or RNA sequence to a host cell, so that the host cell will express the introduced gene or sequence to produce a desired substance, typically a protein or enzyme coded by the introduced gene or sequence. A host cell that receives and expresses introduced DNA or RNA has been "transformed." Where the sequence was introduced via a viral vector, the host cell can also be said to have been "transduced" with the sequence.

[000127] In instances, e.g., where the polypeptide will be used *in vivo*, e.g., therapeutically (or where mammalian post-translational modifications are or may be beneficial) it can be advantageous to produce the fusion polypeptide in mammalian cell culture, in order to, for example, produce a protein with mammalian-type post translational modifications, such as glycosylation patterns. In such embodiments, the host cells can be isolated from a mammalian subject selected from a group consisting of: a human, a horse, a dog, a cat, a mouse, a rat, a cow, a pig and a sheep. In some embodiments, the host cell is a human cell. In some embodiments, the host cell is a cell in culture. The cells can be obtained directly from a mammal (preferably human), or from a commercial source, or from tissue, or in the form for instance of cultured cells, prepared on site or purchased from a commercial cell source and the like. The cells can come from any organ including but not limited to the blood or lymphatic system, e.g., an hematopoietic cell, an erythroid cell or erythroid precursor, from muscles, any organ, gland, the skin, brain, lung, liver, kidney, etc.. In some embodiments, the cells are selected from the group consisting of epithelial cells, neural cells, epidermal cells, keratinocytes, hematopoietic cells, melanocytes, chondrocytes, hepatocytes, B-cells, T-cells, erythrocytes, macrophages, monocytes, fibroblasts, muscle cells, vascular smooth muscle cells, splenocytes, pancreatic β cells, among others.

[000128] While introduction of nucleic acid encoding the fusion proteins as described herein to prokaryotic or mammalian cells can be used to prepare and isolate the polypeptides or fusion polypeptides for various uses, introduction to mammalian cells, e.g., via viral vectors can also be used therapeutically. In therapeutic embodiments, expression from a vector, e.g., a viral vector, e.g., an AAV vector as discussed above, can be used to introduce sequence encoding the fusion protein into a cell expressing the target protein (including, but not limited to BCL11A, ZNF410 LRF) to thereby promote degradation of the target protein. Such cells include, for example, erythroid cells or erythroid progenitor cells in which inhibition of BCL11A can induce expression of HbF.

[000129] In some embodiments, the host cell is a stem cell. As used herein, the term "stem cell" refers to an undifferentiated cell that can be induced to proliferate. The stem cell is capable of self-maintenance or self-renewal, meaning that with each cell division, one daughter cell will also be a stem cell. Stem cells can be obtained from embryonic, post-natal, juvenile, or adult tissue. Stem cells can be pluripotent or multipotent. The term "progenitor cell," as used herein, refers to an undifferentiated cell derived from a stem cell, and is not itself a stem cell. Some progenitor cells can produce progeny that are capable of differentiating into more than one cell type. Stem cells include pluripotent stem cells, which can form cells of any of the body's tissue lineages: mesoderm, endoderm and ectoderm. Therefore, for example, stem cells can be selected from a human embryonic stem (ES) cell; a human inner cell mass (ICM)/epiblast cell; a human primitive ectoderm cell, a human primitive endoderm cell; a human primitive mesoderm cell; and a human primordial germ (EG) cell. Stem cells also include multipotent stem cells, which can form multiple cell lineages that constitute an entire tissue or tissues, such as but not limited to hematopoietic stem cells or neural precursor cells. Stem cells also include totipotent stem cells, which can form an entire organism. In some embodiment, the stem cell is a mesenchymal stem cell. The term "mesenchymal stem cell" or "MSC" is used interchangeably for adult cells which are not terminally differentiated, which can divide to yield cells that are either stem cells, or which, irreversibly differentiate to give rise to cells of a mesenchymal cell lineage, e.g., adipose, osseous, cartilaginous, elastic and fibrous connective tissues, myoblasts) as well as to tissues other than those originating in the embryonic mesoderm (e.g., neural cells) depending upon various influences from bioactive factors such as cytokines. In some embodiments, the stem cell is a partially differentiated or differentiating cell. In some embodiments, the stem cell is an induced pluripotent stem cell (iPSC), which has been reprogrammed or de-differentiated. Stem cells can be obtained from embryonic, fetal or adult tissues.

[000130] In the context of cell ontogeny, the adjective "differentiated", or "differentiating" is a relative term. A "differentiated cell" is a cell that has progressed further down the developmental pathway than the cell it is being compared with. Thus, stem cells can differentiate to lineage-restricted precursor cells (such as a hematopoietic progenitor cell), which in turn can differentiate into other types of precursor cells further down the pathway (such as an erythrocyte precursor), and then to an end-stage differentiated cell, such as an erythrocyte, which plays a characteristic role in a certain tissue type, and may or may not retain the capacity to proliferate further.

[000131] If so desired, viral vectors can also be targeted, e.g. to an erythroid cell or erythroid progenitor cell by manipulating the viral capsid to comprise or display a ligand for a myeloid cell-specific cell-surface molecule as known in the art.

[000132] "Hematopoietic stem or progenitor cell" as the term is used herein, refers to cells of a stem cell lineage that give rise to all the blood cell types including the myeloid (monocytes and macrophages, neutrophils, basophils, eosinophils, erythrocytes, megakaryocytes/platelets, dendritic cells), and the lymphoid lineages (T-cells, B-cells, NK-cells). A "cell of the erythroid lineage" indicates that the cell being contacted with a vector is a cell that undergoes erythropoiesis such that upon final differentiation it forms an erythrocyte or red blood cell (RBC). Such cells belong to one of three lineages, erythroid, lymphoid, and myeloid, originating from bone marrow hematopoietic progenitor cells. Upon exposure to specific growth factors and other components of the hematopoietic microenvironment, hematopoietic progenitor cells can mature through a series of intermediate differentiation cellular types, all intermediates of the erythroid lineage, into RBCs. Thus, cells of the "erythroid lineage", as the term is used herein, comprise hematopoietic progenitor cells, rubriblasts, prorubricytes, erythroblasts, metarubricytes, reticulocytes, and erythrocytes.

[000133] In some embodiments, the hematopoietic stem or progenitor cell has at least one of the cell surface markers characteristic of hematopoietic progenitor cells: CD34+, CD59+, Thy1/CD90+, CD38lo/-, and C-kit/CD117+. Preferably, the hematopoietic progenitor cells have several of these markers.

[000134] In some embodiments, the hematopoietic progenitor cells of the erythroid lineage have the cell surface marker characteristic of the erythroid lineage: CD71 and Ter119.

[000135] It is contemplated that a construct or vector encoding a fusion protein as described herein can be introduced to target cells *ex vivo*, which are then re-introduced to a subject, either with or without expansion or selection for transformed cells prior to re-introduction. In some embodiments, the cells can be autologous to the subject. In others, the cells can be allogeneic

to the subject. In such embodiments, it is optional to enrich a population of target cells, whether from peripheral blood or, for example, from bone marrow, for target hematopoietic cells or target erythroid progenitor or erythroid cells. Such enrichment can be performed on the basis of cell surface expression of hematopoietic progenitor cell markers or erythroid progenitor cell or erythroid cell markers, e.g., as noted above. In one embodiment of this aspect, and all other aspects, the hematopoietic progenitor is a cell of the erythroid lineage. Methods of isolating hematopoietic progenitor cells are well known in the art, e.g., by flow cytometric purification of CD34⁺ or CD133⁺ cells, microbeads conjugated with antibodies against CD34 or CD133, markers of hematopoietic progenitor cells. Commercial kits are also available, e.g., MACS® Technology CD34 MicroBead Kit, human, and CD34 MultiSort Kit, human, and STEMCELL™ Technology EasySep™ Mouse Hematopoietic Progenitor Cell Enrichment Kit. Similar approaches can be applied to isolate other sub-populations based on the specific markers they express. Cells transduced or transformed with vectors as described herein can be cultured for expansion and/or subjected to selection for expression of the exogenous sequence prior to (re)introduction to a subject. In other embodiments, such expansion or selection is not performed, and cells contacted with a vector are (re)introduced to a subject after contacting *in vitro*.

[000136] In other embodiments, the construct or vector, e.g., a viral vector (e.g., an AAV vector, among others) can be introduced to a subject to thereby deliver an expression construct to cells *in vivo*. Such delivery can inhibit the function of the protein targeted for degradation in the target hematopoietic progenitor, erythroid progenitor or erythroid cells. When the fusion protein introduced targets BCL11A, such expression can thereby increase HbF expression in the cells and/or their progeny.

[000137] In other embodiments, the degradation-targeting fusion protein can be produced, e.g., as a further fusion with a cell-penetrating peptide to thereby facilitate introduction of the degradation-targeting fusion protein to a target cell.

[000138] In some embodiments, the hematopoietic stem or progenitor cells described herein are derived from isolated pluripotent stem cells. An advantage of using iPSCs is that the cells can be derived from the same subject to which the progenitor cells are to be administered. That is, a somatic cell can be obtained from a subject, reprogrammed to an induced pluripotent stem cell, and then re-differentiated into a hematopoietic progenitor cell to be administered to the subject (e.g., autologous cells). Since the progenitors are essentially derived from an autologous source, the risk of engraftment rejection or allergic responses is reduced compared to the use of cells from another subject or group of subjects. In some embodiments, the

hematopoietic progenitors are derived from non-autologous sources. In addition, the use of iPSCs negates the need for cells obtained from an embryonic source. Thus, in one embodiment, the stem cells used in the disclosed methods are not embryonic stem cells.

[000139] Although differentiation is generally irreversible under physiological contexts, several methods have been recently developed to reprogram somatic cells to induced pluripotent stem cells. Exemplary methods are known to those of skill in the art and are described briefly herein below.

[000140] As used herein, the term “reprogramming” refers to a process that alters or reverses the differentiation state of a differentiated cell (e.g., a somatic cell). Stated another way, reprogramming refers to a process of driving the differentiation of a cell backwards to a more undifferentiated or more primitive type of cell. It should be noted that placing many primary cells in culture can lead to some loss of fully differentiated characteristics. Thus, simply culturing such cells included in the term differentiated cells does not render these cells non-differentiated cells (e.g., undifferentiated cells) or pluripotent cells. The transition of a differentiated cell to pluripotency requires a reprogramming stimulus beyond the stimuli that lead to partial loss of differentiated character in culture. Reprogrammed cells also have the characteristic of the capacity of extended passaging without loss of growth potential, relative to primary cell parents, which generally have capacity for only a limited number of divisions in culture.

[000141] The cell to be reprogrammed can be either partially or terminally differentiated prior to reprogramming. In some embodiments, reprogramming encompasses complete reversion of the differentiation state of a differentiated cell (e.g., a somatic cell) to a pluripotent state or a multipotent state. In some embodiments, reprogramming encompasses complete or partial reversion of the differentiation state of a differentiated cell (e.g., a somatic cell) to an undifferentiated cell (e.g., an embryonic-like cell). Reprogramming can result in expression of particular genes by the cells, the expression of which further contributes to reprogramming. In certain embodiments described herein, reprogramming of a differentiated cell (e.g., a somatic cell) causes the differentiated cell to assume an undifferentiated state (e.g., is an undifferentiated cell). The resulting cells are referred to as “reprogrammed cells,” or “induced pluripotent stem cells (iPSCs or iPS cells).”

[000142] Reprogramming can involve alteration, e.g., reversal, of at least some of the heritable patterns of nucleic acid modification (e.g., methylation), chromatin condensation, epigenetic changes, genomic imprinting, etc., that occur during cellular differentiation. Reprogramming is distinct from simply maintaining the existing undifferentiated state of a cell that is already

pluripotent or maintaining the existing less than fully differentiated state of a cell that is already a multipotent cell (e.g., a hematopoietic stem cell). Reprogramming is also distinct from promoting the self-renewal or proliferation of cells that are already pluripotent or multipotent, although the compositions and methods described herein can also be of use for such purposes, in some embodiments.

[000143] The specific approach or method used to generate pluripotent stem cells from somatic cells (broadly referred to as “reprogramming”) is not critical to the claimed invention. Thus, any method that re-programs a somatic cell to the pluripotent phenotype would be appropriate for use in the methods described herein. Reprogramming can be achieved by introducing a combination of nucleic acids encoding stem cell-associated genes including, for example Oct-4 (also known as Oct-3/4 or Pou5f1), Sox1, Sox2, Sox3, Sox 15, Sox 18, NANOG, Klf1, Klf2, Klf4, Klf5, NR5A2, c-Myc, l-Myc, n-Myc, Rem2, Tert, and LIN28. In one embodiment, reprogramming using the methods and compositions described herein can further comprise introducing one or more of Oct-3/4, a member of the Sox family, a member of the Klf family, and a member of the Myc family to a somatic cell. In one embodiment, the methods and compositions described herein further comprise introducing one or more of each of Oct 4, Sox2, Nanog, c-MYC and Klf4 for reprogramming. As noted above, the exact method used for reprogramming is not necessarily critical to the methods and compositions described herein. However, where cells differentiated from the reprogrammed cells are to be used in, *e.g.*, human therapy, in one embodiment the reprogramming is not effected by a method that alters the genome. Thus, in such embodiments, reprogramming is achieved, *e.g.*, without the use of viral or plasmid vectors.

[000144] Somatic cells, as that term is used herein, refer to any cells forming the body of an organism, excluding germline cells. Every cell type in the mammalian body—apart from the sperm and ova, the cells from which they are made (gametocytes) and undifferentiated stem cells—is a differentiated somatic cell. For example, internal organs, skin, bones, blood, and connective tissue are all made up of differentiated somatic cells.

[000145] Additional somatic cell types for use with the compositions and methods described herein include: a fibroblast (*e.g.*, a primary fibroblast), a muscle cell (*e.g.*, a myocyte), a cumulus cell, a neural cell, a mammary cell, an hepatocyte and a pancreatic islet cell. In some embodiments, the somatic cell is a primary cell line or is the progeny of a primary or secondary cell line. In some embodiments, the somatic cell is obtained from a human sample, *e.g.*, a hair follicle, a blood sample, a biopsy (*e.g.*, a skin biopsy or an adipose biopsy), a swab sample (*e.g.*, an oral swab sample), and is thus a human somatic cell.

[000146] When reprogrammed cells are used for generation of hematopoietic progenitor cells to be used in the therapeutic treatment of disease, it is desirable, but not required, to use somatic cells isolated from the patient being treated. For example, somatic cells involved in diseases, and somatic cells participating in therapeutic treatment of diseases and the like can be used. In some embodiments, a method for selecting the reprogrammed cells from a heterogeneous population comprising reprogrammed cells and somatic cells they were derived or generated from can be performed by any known means. For example, a drug resistance gene or the like, such as a selectable marker gene can be used to isolate the reprogrammed cells using the selectable marker as an index.

[000147] Reprogrammed somatic cells as disclosed herein can express any number of pluripotent cell markers, including: alkaline phosphatase (AP); ABCG2; stage specific embryonic antigen-1 (SSEA-1); SSEA-3; SSEA-4; TRA-1-60; TRA-1-81; Tra-2-49/6E; ERas/ECAT5, E-cadherin; fibroblast growth factor 4 (Fgf4), Cripto, Dax1; zinc finger protein 296 (Zfp296); N-acetyltransferase-1 (Nat1); (ES cell associated transcript 1 (ECAT1); ESG1/DPPA5/ECAT2; ECAT3; ECAT6; ECAT7; ECAT8; ECAT9; ECAT10; ECAT15-1; ECAT15-2; Fthl17; Sal14; undifferentiated embryonic cell transcription factor (Utf1); Rex1; p53; G3PDH; telomerase, including TERT; silent X chromosome genes; Dnmt3a; Dnmt3b; TRIM28; F-box containing protein 15 (Fbx15); Nanog/ECAT4; Oct3/4; Sox2; Klf4; c-Myc; Esrrb; TDGF1; GABRB3; Zfp42, FoxD3; GDF3; CYP25A1; developmental pluripotency-associated 2 (DPPA2); T-cell lymphoma breakpoint 1 (Tcl1); DPPA3/Stella; DPPA4; other general markers for pluripotency, etc. Other markers can include Dnmt3L; Sox15; Stat3; Grb2; β -catenin, and Bmi1. Such cells can also be characterized by the down-regulation of markers characteristic of the somatic cell from which the induced pluripotent stem cell is derived.

[000148] To confirm the induction of pluripotent stem cells for use with the methods described herein, isolated clones can be tested for the expression of a stem cell marker. Such expression in a cell derived from a somatic cell identifies the cells as induced pluripotent stem cells. Stem cell markers can be selected from the non-limiting group including SSEA3, SSEA4, CD9, Nanog, Fbx15, Ecat1, Esg1, Eras, Gdf3, Fgf4, Cripto, Dax1, Zfp296, Slc2a3, Rex1, Utf1, and Nat1. In one embodiment, a cell that expresses Oct4 or Nanog is identified as pluripotent. Methods for detecting the expression of such markers can include, for example, RT-PCR and immunological methods that detect the presence of the encoded polypeptides, such as Western blots or flow cytometric analyses. In some embodiments, detection does not involve only RT-PCR, but also includes detection of protein markers. Intracellular markers

may be best identified via RT-PCR, while cell surface markers are readily identified, e.g., by immunocytochemistry.

[000149] The pluripotent stem cell character of isolated cells can be confirmed by tests evaluating the ability of the iPSCs to differentiate to cells of each of the three germ layers. As one example, teratoma formation in nude mice can be used to evaluate the pluripotent character of the isolated clones. The cells are introduced to nude mice and histology and/or immunohistochemistry is performed on a tumor arising from the cells. The growth of a tumor comprising cells from all three germ layers, for example, further indicates that the cells are pluripotent stem cells.

[000150] In some embodiments, cell-penetration peptides or cell-penetrating peptides (CPPs) can be used as a transmembrane drug delivery agent for delivery of degradation-targeting fusion polypeptide as described herein. CPPs are a class of small cationic peptides of at least 10, or at least 11, or at least 12, or at least 13, or at least 14, or at least 15, or at least 15, or at least 20, or at least 25, or at least 30 amino acids that can be used as transmembrane drug delivery agents through various forms of endocytosis for compounds including drugs, imaging agents, oligonucleotides, peptides and proteins. CPPs are also known as “protein transduction domains.” CPPs include but are not limited to the peptides Tat (e.g., HIV-derived CPP TAT(48-60)) and penetratin. Addition of a CPP to a fusion protein provides an option for introducing the fusion protein to a target cell.

[000151] Delivery of a fusion protein as described herein, or nucleic acids encoding them can include the use of lipid complexes or lipid nanoparticles complexed or loaded with the fusion protein or nucleic acid encoding same. As used herein, the term “nanoparticle” refers to particles that are on the order of about 10^{-9} or one to several billionths of a meter. The term “nanoparticle” includes nanospheres; nanorods; nanoshells; and nanoprisms; these nanoparticles may be part of a nanonetwork. The term “nanoparticles” also encompasses liposomes and lipid particles having the size of a nanoparticle. Non-limiting examples of lipid-based nanoparticles include, but are not limited to: a solid lipid nanoparticle (SLN; e.g., a nanoparticle comprising a single outer phospholipid layer and an inner core comprising a lipophilic substance, such as a therapeutic agent); a nanostructured lipid carrier (NLC; e.g., which comprises a mixture of solid crystalline lipids and liquid lipids); a microemulsion or a nanoemulsion, e.g., comprising a liquid lipid droplet; a cubosome (e.g., a liquid crystalline nano-structure formed from the cubic phase of lipids, such as monooleate, or any other amphiphilic macromolecules with the property to be dispersed into particles; such cubosomes can further comprise a stabilizer); a non-lamellar lipid nanoparticle, referring to a

nanoparticle that does not comprise a lipid bilayer, but rather comprises non-lamellar liquid crystalline structures, such as cubic, hexagonal, and sponge phases (such a non-lamellar lipid nanoparticle can be particularly useful for controlled release formulations, e.g., for delivering inhaled drugs); or any combination thereof, or any other known structures in the art such as an ethasome, which is a lipid vesicular carrier comprising a relatively high percentage of ethanol. In some embodiments, the lipid-based nanoparticle comprises at least one phospholipid, at least one charged lipid, cholesterol, at least one membrane protein, and/or at least one nucleic acid or polypeptide construct for delivery to a cell. In some embodiments, the polypeptide or nucleic acid construct is linked to the at least one phospholipid, the at least one charged lipid, the cholesterol, or the at least one membrane protein of the lipid-based nanoparticle. See e.g., Naseri et al., *Adv. Pharm. Bull.* 2015 Sep, 5(3): 305–313; Montenegro et al., *Journal of Drug Delivery Science and Technology*, Volume 32, Part B, April 2016, Pages 100-112; Barriga et al., *Angew Chem Int Ed Engl.* 2019 Mar 4, 58(10):2958-2978; Chang et al., *Advances in Colloid and Interface Science*, Volume 222, August 2015, Pages 135-147; Abdulbaqi et al., *Int J Nanomedicine.* 2016 May 25, 11:2279-304; the contents of which are incorporated herein by reference in their entireties.

Administration & Efficacy

[000152] As used herein, the terms "administering," "introducing" and "transplanting" are used interchangeably in the context of the placement of an agent, e.g. a targeted protein degradation fusion protein or composition thereof as described herein into a subject, by a method or route which results in at least partial localization of the introduced agent (i.e., a fusion protein as described herein) at a desired site, such as an hematopoietic progenitor cell, erythroid progenitor cell or erythroid cell, among others, such that a desired effect(s) is produced. Where cells are introduced, the cells, e.g. hematopoietic progenitor cells, or their differentiated progeny can be administered by any appropriate route which results in delivery to a desired location in the subject where at least a portion of the implanted cells or components of the cells remain viable.

[000153] Modes of administration include injection, infusion and instillation. "Injection" includes, without limitation, intravenous, intramuscular, intra-arterial, intrathecal, intraventricular, intracapsular, intraorbital, intracardiac, intradermal, intraperitoneal, transtracheal, subcutaneous, subcuticular, intraarticular, sub capsular, subarachnoid, intraspinal, intracerebro spinal, and intrasternal injection and infusion.

[000154] In one embodiment, the agent (e.g., fusion protein or composition described herein) as described herein is administered systemically. The phrases “systemic administration,” “administered systemically”, “peripheral administration” and “administered peripherally” as used herein refer to the administration of a formulation (whether including cells expressing a fusion protein or composition described herein, or including nucleic acid or a vector encoding a fusion protein or composition described herein, or including an isolated fusion protein or composition described herein) other than directly into a target site, tissue, or organ, such that it enters, instead, the subject’s circulatory system and, thus, is subject to metabolism and other like processes.

[000155] When provided prophylactically, an agent (e.g., a fusion protein or composition described herein) can be administered to a subject in advance of any symptom of a hemoglobinopathy, e.g., perinatally, prior to the switch or prior to the completion of the switch from fetal γ -globin to predominantly β -globin. Accordingly, the prophylactic administration of, e.g., a modified hematopoietic progenitor cell population can serve to prevent a hemoglobinopathy, as described herein.

[000156] When provided therapeutically, the agent is provided at (or after) the onset of a symptom or indication of a hemoglobinopathy, e.g., upon the onset of sickle cell disease or thalassemia.

[000157] In one embodiment, the term “effective amount” as used herein refers to the amount of an agent (e.g., a fusion protein or composition as described herein) needed to alleviate at least one or more symptom or marker of a hemoglobinopathy, and relates to a sufficient amount of a composition to provide the desired effect, e.g., treat a subject having a hemoglobinopathy. The term “therapeutically effective amount” therefore refers to an amount of an agent that is sufficient to promote a particular effect when administered to a typical subject, such as one who has or is at risk for a hemoglobinopathy. An effective amount as used herein would also include an amount sufficient to prevent or delay the development of a symptom of the disease, alter the course of a symptom disease (for example but not limited to, slow the progression of a symptom of the disease), or reverse a symptom of the disease. It is understood that for any given case, an appropriate “effective amount” can be determined by one of ordinary skill in the art using routine experimentation.

[000158] The efficacy of a treatment comprising an agent (e.g., a fusion protein or composition described herein) as described herein for the treatment of a hemoglobinopathy can be determined by the skilled clinician. However, a treatment is considered “effective

treatment," as the term is used herein, if any one or more of the signs or symptoms of a hemoglobinopathy is altered in a beneficial and/or statistically significant manner. For the avoidance of doubt, an improvement of at least 10% or more in a given sign, symptom or marker after treatment is considered effective treatment, and preferably improvement by at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95% or more. Effective treatment expressly includes an increase in levels of HbF as that term is defined herein. Efficacy can also be measured by failure of an individual to worsen as assessed by hospitalization or need for medical interventions (e.g., progression of the disease is halted or at least slowed). Methods of measuring these indicators are known to those of skill in the art and/or described herein.

[000159] Effective amounts, toxicity, and therapeutic efficacy can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, *e.g.*, for determining the LD₅₀ (the dose lethal to 50% of the population) and the ED₅₀ (the dose therapeutically effective in 50% of the population). The dosage can vary depending upon the dosage form employed and the route of administration utilized. The dose ratio between toxic and therapeutic effects is the therapeutic index and can be expressed as the ratio LD₅₀/ED₅₀. Compositions and methods that exhibit large therapeutic indices are preferred. A therapeutically effective dose can be estimated initially from cell culture assays. Also, an effective dose can be formulated in an appropriate animal model. The effects of any particular dosage can be monitored by a suitable bioassay, including, but not limited to measurement of HbF expression. The dosage can be determined by a physician and adjusted, as necessary, to suit observed effects of the treatment.

[000160] The agents described herein can be formulated, in some embodiments, with one or more additional therapeutic agents currently used to prevent or treat hemoglobinopathy, for example, hydroxyurea. The effective amount of such other agents depend on the amount of the agent/compositions provided herein in the formulation, the type of disorder or treatment, and other factors discussed above. These are generally used in the same dosages and with administration routes as used herein before or about from 1 to 99% of the heretofore employed dosages.

[000161] The dosage ranges for the agents or pharmaceutical compositions provided herein depend upon the potency, and encompass amounts large enough to produce the desired effect. The dosage should not be so large as to cause unacceptable adverse side effects. Generally, the dosage will vary with the age, condition, and sex of the patient and can be determined by one of skill in the art. The dosage can also be adjusted by the individual physician in the event of

any complication. In some embodiments, the dosage ranges from 0.001 mg/kg body weight to 100 mg/kg body weight. In some embodiments, the dose range is from 5 µg/kg body weight to 100 µg/kg body weight. For systemic administration, subjects can be administered a therapeutic amount, such as, *e.g.*, 0.1 mg/kg, 0.5 mg/kg, 1.0 mg/kg, 2.0 mg/kg, 2.5 mg/kg, 5 mg/kg, 7.5 mg/kg, 10 mg/kg, 15 mg/kg, 20 mg/kg, 25 mg/kg, 30 mg/kg, 40 mg/kg, 50 mg/kg, or more. These doses can be administered by one or more separate administrations, or by continuous infusion. For repeated administrations over several days or longer, depending on the condition, the treatment is sustained until, for example, the hemoglobinopathy is treated, as measured by the methods described above or known in the art.

[000162] However, other dosage regimens can be useful. The duration of a therapy using the methods described herein will continue for as long as medically indicated or until a desired therapeutic effect (*e.g.*, those described herein) is achieved. In certain embodiments, the administration of the pharmaceutical composition described herein is continued for 1 month, 2 months, 4 months, 6 months, 8 months, 10 months, 1 year, 2 years, 3 years, 4 years, 5 years, 10 years, 20 years, or for a period of years up to the lifetime of the subject.

[000163] As will be appreciated by one of skill in the art, appropriate dosing regimens for a given composition can comprise a single administration or multiple ones. Subsequent doses may be given repeatedly at time periods, for example, about two weeks or greater up through the entirety of a subject's life, *e.g.*, to provide a sustained therapeutic or preventative effect. The precise dose to be employed in the formulation will also depend on the route of administration and should be decided according to the judgment of the practitioner and each patient's circumstances. Ultimately, the practitioner or physician will decide the amount of the agent or composition thereof to administer to particular subjects.

[000164] The treatment as described herein ameliorates one or more symptoms associated with, *e.g.*, a β -globin disorder by increasing the amount of fetal hemoglobin in the individual. Symptoms typically associated with a hemoglobinopathy include, for example, anemia, tissue hypoxia, organ dysfunction, abnormal hematocrit values, ineffective erythropoiesis, abnormal reticulocyte (erythrocyte) count, abnormal iron load, the presence of ring sideroblasts, splenomegaly, hepatomegaly, impaired peripheral blood flow, dyspnea, increased hemolysis, jaundice, anemic pain crises, acute chest syndrome, splenic sequestration, priapism, stroke, hand-foot syndrome, and pain such as angina pectoris.

[000165] The levels of BCL11A or fetal hemoglobin (HbF) can be determined by methods known in the art. For example, PCR, Western blotting, immunological methods, flow cytometric analyses, ELISA. Accordingly, the activity of BCL11A can be determined by

methods known in the art, *e.g.*, a chromatin occupancy assay, binding assays, pull-down assays, RT-PCR of fetal hemoglobin levels, animal models, etc. BCL11A activity can be assayed by measuring fetal hemoglobin expression at the mRNA or protein level following treatment with a polypeptide, nucleic acid molecule, vector, nanoparticle or other agent providing a BCL11A-specific single domain antibody or construct as described herein.

[000166] In some embodiments of any of the aspects, the level or activity of BCL11A is decreased by at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or more as compared to an appropriate control. In some embodiments, a decreased level or activity of BCL11A, in a cell of the subject, increases the level and/or activity of fetal hemoglobin (HbF) by at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or more as compared to an appropriate control.

[000167] The technology provided herein can further be defined by the following numbered paragraphs.

[000168] Paragraph 1: A fusion polypeptide comprising a binding domain that specifically binds an erythroid-enriched E3 ubiquitin ligase and a binding domain that specifically binds an erythroid-enriched polypeptide of interest.

[000169] Paragraph 2: The fusion polypeptide of paragraph 1 which mediates degradation of the polypeptide of interest in erythroid cells.

[000170] Paragraph 3: The fusion polypeptide of any of the preceding paragraphs, wherein the binding domain that specifically binds an erythroid-enriched E3 ubiquitin ligase specifically binds TRIM10 or TRIM58.

[000171] Paragraph 4: The fusion polypeptide of any of the preceding paragraphs, wherein the binding domain that specifically binds an erythroid-enriched E3 ubiquitin ligase comprises an antibody or antigen-binding fragment thereof.

[000172] Paragraph 5: The fusion polypeptide of any of the preceding paragraphs, wherein the antibody or antigen-binding fragment thereof comprises an scFv, a single domain antibody or a nanobody.

[000173] Paragraph 6: The fusion polypeptide of any of the preceding paragraphs, wherein the binding domain that specifically binds an erythroid-enriched polypeptide of interest comprises an antibody or antigen-binding fragment thereof.

[000174] Paragraph 7: The fusion polypeptide of any of the preceding paragraphs, wherein the antibody or antigen-binding fragment thereof comprises an scFv, a single domain antibody or a nanobody.

[000175] Paragraph 8: The fusion polypeptide of any of the preceding paragraphs, wherein the polypeptide of interest is selected from BCL11A, LRF and ZNF410.

[000176] Paragraph 9: The fusion polypeptide of any of the preceding paragraphs, wherein the E3 ubiquitin ligase is TRIM10 or TRIM58.

[000177] Paragraph 10: The fusion polypeptide of any of the preceding paragraphs, wherein the polypeptide of interest is BCL11A, and the binding domain that specifically binds the polypeptide of interest binds BCL11A at an epitope comprised by zinc-finger 6 (ZNF6) of the BCL11A polypeptide.

[000178] Paragraph 11: The fusion polypeptide of any of the preceding paragraphs, wherein the polypeptide of interest is BCL11A, and the binding domain that specifically binds the polypeptide of interest binds BCL11A at an epitope comprised by zinc finger 23 (ZNF23) of the BCL11A polypeptide.

[000179] Paragraph 12: The fusion polypeptide of any of the preceding paragraphs, wherein the polypeptide of interest is BCL11A, and the binding domain that specifically binds the polypeptide of interest has an amino acid sequence at least 90% identical to SEQ ID NO: 12.

[000180] Paragraph 13: The fusion polypeptide of any of the preceding paragraphs, wherein amino acid sequence variation relative to SEQ ID NO: 12 occurs at one or more of amino acids according to Table 2.

[000181] Paragraph 14: The fusion polypeptide of any of the preceding paragraphs, wherein the polypeptide of interest is BCL11A, and the binding domain that specifically binds BCL11A has an amino acid sequence at least 90% identical to SEQ ID NO: 34.

[000182] Paragraph 15: The fusion polypeptide of any of the preceding paragraphs, wherein amino acid sequence variation relative to SEQ ID NO: 34 occurs at one or more of amino acids at amino acid number 102 or 108.

[000183] Paragraph 16: The fusion polypeptide of any of the preceding paragraphs, wherein the polypeptide of interest is BCL11A, and the binding domain that specifically binds BCL11A comprises a single-domain antibody in which:

CDR1 has an amino acid sequence selected from SEQ ID NOs: 43-50,

CDR2 has an amino acid sequence selected from SEQ ID NOs: 51-59; and

CDR3 has an amino acid sequence selected from SEQ ID NOs 60-65.

[000184] Paragraph 17: The fusion polypeptide of any of the preceding paragraphs, wherein the polypeptide of interest is BCL11A, and the binding domain that specifically binds BCL11A comprises a single-domain antibody in which:

CDR1 has the amino acid sequence SIFVNNAM (SEQ ID NO: 37);

CDR2 has the amino acid sequence ELVAAISASGGSTYY (SEQ ID NO: 38);
and

CDR3 has a sequence selected from ADQDVYPYEYW (SEQ ID NO: 39),
ADQDGYPYEYW (SEQ ID NO: 40), and ADQDVYPYEYL (SEQ ID NO:
41).

[000185] Paragraph 18: The fusion polypeptide of any of the preceding paragraphs which further comprises a cell-penetrating peptide.

[000186] Paragraph 19: The fusion polypeptide of any of the preceding paragraphs, further comprising a linker peptide between the binding domain that specifically binds an erythroid-enriched E3 ubiquitin ligase and the binding domain that specifically binds an erythroid-enriched polypeptide of interest.

[000187] Paragraph 20: A nucleic acid comprising sequence encoding a fusion polypeptide of any of the preceding paragraphs.

[000188] Paragraph 21: A nucleic acid of any of the preceding paragraphs, wherein the sequence encoding the fusion polypeptide is operatively linked to regulatory sequences that permit expression in erythroid cells.

[000189] Paragraph 22: A vector comprising a nucleic acid of any of the preceding paragraphs.

[000190] Paragraph 23: The vector of any of the preceding paragraphs, which is a viral vector.

[000191] Paragraph 24: The viral vector of any of the preceding paragraphs, which is an AAV vector.

[000192] Paragraph 25: A method of erythroid-specific, targeted degradation of a protein of interest, the method comprising introducing a fusion polypeptide of any of the preceding paragraphs, a nucleic acid of any of the preceding paragraphs, or a vector of any of the preceding paragraphs to an erythroid cell.

[000193] Paragraph 26: A method of targeted degradation of BCL11A, the method comprising introducing a fusion polypeptide of any of the preceding paragraphs, a nucleic acid of any of the preceding paragraphs, or a vector of any of the preceding paragraphs to a cell expressing BCL11A.

[000194] Paragraph 27: The method of any of the preceding paragraphs, wherein the targeted degradation of BCL11A is targeted to erythroid cells.

[000195] Paragraph 28: A method of promoting fetal hemoglobin (HbF) expression in adult erythroid cells, the method comprising introducing a fusion polypeptide of any of the preceding paragraphs, a nucleic acid of any of the preceding paragraphs, or a vector of any of the preceding paragraphs to a cell, wherein the protein of interest is BCL11A, and wherein the fusion polypeptide promotes the degradation of BCL11A in erythroid cells, thereby promoting HbF expression.

[000196] Paragraph 29: A method of treating a hemoglobinopathy disorder, the method comprising introducing a fusion polypeptide of any of the preceding paragraphs, a nucleic acid of any of the preceding paragraphs, or a vector of any of the preceding paragraphs to a subject in need thereof, wherein the protein of interest is BCL11A, and wherein the fusion polypeptide promotes the degradation of BCL11A in erythroid cells, thereby promoting HbF expression to treat the hemoglobinopathy disorder.

[000197] Paragraph 30: A fusion polypeptide comprising a TRIM10 or TRIM58 polypeptide fused to a binding domain that specifically binds a target polypeptide.

[000198] Paragraph 31: The fusion polypeptide of claim 30, wherein the binding domain that specifically binds a target polypeptide comprises an antigen-binding domain of an antibody.

[000199] Paragraph 32: The fusion polypeptide of any of the preceding paragraphs, wherein the binding domain that specifically binds a target polypeptide comprises an scFv, a single domain antibody or a nanobody.

[000200] Paragraph 33: The fusion polypeptide of any of the preceding paragraphs, wherein the target polypeptide is expressed in an erythroid cell.

[000201] Paragraph 34: The fusion polypeptide of any of the preceding paragraphs, wherein the target polypeptide is selected from BCL11A, LRF and ZNF410.

[000202] Paragraph 35: The fusion polypeptide of any of the preceding paragraphs, wherein the polypeptide of interest is BCL11A, and the binding domain that specifically binds the polypeptide of interest binds BCL11A at an epitope comprised by zinc-finger 6 (ZNF6) of the BCL11A polypeptide.

[000203] Paragraph 36: The fusion polypeptide of any of the preceding paragraphs, wherein the polypeptide of interest is BCL11A, and the binding domain that specifically binds the polypeptide of interest binds BCL11A at an epitope comprised by zinc finger 23 (ZNF23) of the BCL11A polypeptide.

[000204] Paragraph 37: The fusion polypeptide of any of the preceding paragraphs, wherein the polypeptide of interest is BCL11A, and the binding domain that specifically binds the polypeptide of interest has an amino acid sequence at least 90% identical to SEQ ID NO: 12.

[000205] Paragraph 38: The fusion polypeptide of claim 37, wherein amino acid sequence variation relative to SEQ ID NO: 12 occurs at one or more of amino acids according to Table 2.

[000206] Paragraph 39: The fusion polypeptide of any of the preceding paragraphs, wherein the polypeptide of interest is BCL11A, and the binding domain that specifically binds BCL11A has an amino acid sequence at least 90% identical to SEQ ID NO: 34.

[000207] Paragraph 40: The fusion polypeptide of any of the preceding paragraphs, wherein amino acid sequence variation relative to SEQ ID NO: 34 occurs at one or more of amino acids number 102 or 108.

[000208] Paragraph 41: The fusion polypeptide of any of the preceding paragraphs, wherein the polypeptide of interest is BCL11A, and the binding domain that specifically binds BCL11A comprises a single-domain antibody in which:

CDR1 has an amino acid sequence selected from SEQ ID NOs: 43-50,

CDR2 has an amino acid sequence selected from SEQ ID NOs: 51-59; and

CDR3 has an amino acid sequence selected from SEQ ID NOs 60-65.

[000209] Paragraph 42: The fusion polypeptide of any of the preceding paragraphs, wherein the polypeptide of interest is BCL11A, and the binding domain that specifically binds BCL11A comprises a single-domain antibody in which:

CDR1 has the amino acid sequence SIFVNNAM (SEQ ID NO: 37);

CDR2 has the amino acid sequence ELVAAISASGGSTYY (SEQ ID NO: 38);

and

CDR3 has a sequence selected from ADQDVYPYEYW (SEQ ID NO: 39), ADQDGYPYEYW (SEQ ID NO: 40), and ADQDVYPYEYL (SEQ ID NO: 41).

[000210] Paragraph 43: The fusion polypeptide of any of the preceding paragraphs which further comprises a cell-penetrating peptide.

[000211] Paragraph 44: The fusion polypeptide of any of the preceding paragraphs, further comprising a linker peptide between the TRIM10 or TRIM58 polypeptide and the binding domain that specifically binds a target polypeptide.

[000212] Paragraph 45: A nucleic acid comprising sequence encoding a fusion polypeptide of any one of any of the preceding paragraphs.

[000213] Paragraph 46: The nucleic acid of any of the preceding paragraphs, wherein the sequence encoding the fusion polypeptide is operatively linked to regulatory sequences that permit expression in erythroid cells.

[000214] Paragraph 47: A vector comprising a nucleic acid of any of the preceding paragraphs.

[000215] Paragraph 48: The vector of any of the preceding paragraphs, which is a viral vector.

[000216] Paragraph 49: The viral vector of claim 48, which is an AAV vector.

[000217] Paragraph 50: A method of erythroid-specific, targeted degradation of a protein of interest, the method comprising introducing a fusion polypeptide of any of the preceding paragraphs, a nucleic acid of any of the preceding paragraphs, or a vector of any of the preceding paragraphs to an erythroid cell.

[000218] Paragraph 51: A method of targeted degradation of BCL11A, the method comprising introducing a fusion polypeptide of any of the preceding paragraphs, a nucleic acid of any of the preceding paragraphs, or a vector of any of the preceding paragraphs to a cell expressing BCL11A.

[000219] Paragraph 52: The method of any of the preceding paragraphs, wherein the targeted degradation of BCL11A is targeted to erythroid cells.

[000220] Paragraph 53: A method of promoting fetal hemoglobin (HbF) expression in adult erythroid cells, the method comprising introducing a fusion polypeptide of any of the preceding paragraphs, a nucleic acid of any of the preceding paragraphs, or a vector of any of the preceding paragraphs to a cell, wherein the protein of interest is BCL11A, and wherein the fusion polypeptide promotes the degradation of BCL11A in erythroid cells, thereby promoting HbF expression.

[000221] Paragraph 54: A method of treating a hemoglobinopathy disorder, the method comprising introducing a fusion polypeptide of any of the preceding paragraphs, a nucleic acid of claim 45 or 46, or a vector of any of the preceding paragraphs to a subject in need thereof, wherein the protein of interest is BCL11A, and wherein the fusion polypeptide promotes the degradation of BCL11A in erythroid cells, thereby promoting HbF expression to treat the hemoglobinopathy disorder.

EXAMPLES

Example 1:

[000222] As described herein, the invention comes in part from investigation into erythroid specific E3 ubiquitin ligases. Analysis of RNA expression of E3 ligases during erythroid cell maturations revealed specific expression of TRIM10 and TRIM58 during erythroid cell maturation (FIG. 1B). TRIM10 and TRIM58 are members of the tripartite motif family that

includes three zinc-binding domains, a RING, a B-box type 1 and a B-box type 2, and a coiled-coil region (FIG 1A). Furthermore, TRIM10 and TRIM58 were found to be enriched in erythroid cells as they are highly expressed in that cell type but expressed at much lower levels across other cell types. Known erythroid cell specific E3 ligase UBE2O was used as a positive control (FIG. 1C).

[000223] In order to utilize erythroid specific E3 ubiquitin ligases for targeted protein degradation, proteolysis-targeting chimeras were created using TRIM10 or TRIM58. More specifically, TRIM10 or TRIM58 were fused to the recognition domain of cereblon (CRBN), then CRBN^{-/-} HEK293T cells were co-transfected with the E3 ligase construct and BCL11A tagged with FKBP, which binds to dTAG47 PROTAC (a heterobifunctional molecule including a thalidomide derivative and an FKBP12^{F36V}-selective ligand) (Fig. 2) Following transfection, dTAG47 was added to cells for 48 hrs and cells were collected via fluorescent activated cell sorting (FACS). To determine if TRIM-CRBN fusion can degrade BCL11A-FKBP, Western blot analysis with BCL11A antibody was used. Results indicate that fusions of TRIM10/58 with the recognition domain of CRBN are capable of degrading tagged BCL11A upon addition of dTAG47 PROTAC. Wild-type CRBN (WT-CRBN) cDNA transfected into CRBN^{-/-} 293T cells was used as a positive control. An inactive form of dTAG47 (dTAG47i) is inactive for degradation and showed no effects, indicating that degradation is dependent on recruitment through the CRBN recognition domain (Fig. 3).

[000224] Next, the degradation of BCL11A protein by TRIM10/58 in HUDEP-2 (erythroid cells) were tested. HUDEP-2 cells were tagged with FKBP, which permits the use of dTAG47 PROTAC for recruitment to CRBN for degradation. As a control, HUDEP-2 cells lacking CRBN were generated by CRISPR/Cas9 editing. CRBN⁺ and CRBN⁻ cells were infected with lentiviruses expressing CRBN cDNA or TRIM10 fused with the recognition domain of CRBN and cells were cultured with and without dTAG47 PROTAC. Degradation of BCL11A was analyzed by Western blot using BCL11A antibody. The fusion of TRIM10 with the recognition domain of CRBN led to a reduction in BCL11A. In CRBN⁻ cells, degradation of BCL11A does not occur (Fig. 4). These results indicate that when recruited to BCL11A by the recognition domain of CRBN, TRIM10 is capable of degrading BCL11A. In other experiments, no degradation is seen with these cells in the presence of dTAG47.

[000225] Finally, it was investigated whether BCL11A could be targeted for degradation by TRIM10/58 using BCL11A nanobodies. In these experiments, 293T cells were transfected with constructs expressing either TRIM10 or TRIM58 fused to a BCL11A-specific nanobody. Analysis by Western blot with BCL11A allowed for detection of endogenous BCL11A. As a

control, mutant versions of TRIM10 or TRIM58 (C16A) in which a critical, conserved residue of TRIM proteins is mutated were used. Cells were transfected and assayed 2 days later. Results show marked reduction in BCL11A in cells treated with TRIM10-Nb19 (BCL11A) fusion. Thus, TRIM10 and to a lesser extent TRIM58 are able to direct degradation of wild-type, untagged BCL11A protein.

CLAIMS

1. A fusion polypeptide comprising a binding domain that specifically binds an erythroid-enriched E3 ubiquitin ligase and a binding domain that specifically binds an erythroid-enriched polypeptide of interest.
2. The fusion polypeptide of claim 1 which mediates degradation of the polypeptide of interest in erythroid cells.
3. The fusion polypeptide of claim 1 or claim 2, wherein the binding domain that specifically binds an erythroid-enriched E3 ubiquitin ligase specifically binds TRIM10 or TRIM58.
4. The fusion polypeptide of any one of claims 1-3, wherein the binding domain that specifically binds an erythroid-enriched E3 ubiquitin ligase comprises an antibody or antigen-binding fragment thereof.
5. The fusion polypeptide of claim 4, wherein the antibody or antigen-binding fragment thereof comprises an scFv, a single domain antibody or a nanobody.
6. The fusion polypeptide of any one of claims 1-5, wherein the binding domain that specifically binds an erythroid-enriched polypeptide of interest comprises an antibody or antigen-binding fragment thereof.
7. The fusion polypeptide of claim 6, wherein the antibody or antigen-binding fragment thereof comprises an scFv, a single domain antibody or a nanobody.
8. The fusion polypeptide of any one of claims 1-7, wherein the polypeptide of interest is selected from BCL11A, LRF and ZNF410.
9. The fusion polypeptide of any one of claims 1-8, wherein the E3 ubiquitin ligase is TRIM10 or TRIM58.
10. The fusion polypeptide of any one of claims 1-9, wherein the polypeptide of interest is BCL11A, and the binding domain that specifically binds the polypeptide of interest binds BCL11A at an epitope comprised by zinc-finger 6 (ZNF6) of the BCL11A polypeptide.
11. The fusion polypeptide of any one of claims 1-9, wherein the polypeptide of interest is BCL11A, and the binding domain that specifically binds the polypeptide of interest binds BCL11A at an epitope comprised by zinc finger 23 (ZNF23) of the BCL11A polypeptide.
12. The fusion polypeptide of any one of claims 1-10, wherein the polypeptide of interest is BCL11A, and the binding domain that specifically binds the polypeptide of interest has an amino acid sequence at least 90% identical to SEQ ID NO: 12.

13. The fusion polypeptide of claim 12, wherein amino acid sequence variation relative to SEQ ID NO: 12 occurs at one or more of amino acids according to Table 2.
14. The fusion polypeptide of any one of claims 1-9 or 11, wherein the polypeptide of interest is BCL11A, and the binding domain that specifically binds BCL11A has an amino acid sequence at least 90% identical to SEQ ID NO: 34.
15. The fusion polypeptide of claim 14, wherein amino acid sequence variation relative to SEQ ID NO: 34 occurs at one or more of amino acids at amino acid number 102 or 108.
16. The fusion polypeptide of any one of claims 1-10 or 12-13, wherein the polypeptide of interest is BCL11A, and the binding domain that specifically binds BCL11A comprises a single-domain antibody in which:
 - CDR1 has an amino acid sequence selected from SEQ ID NOs: 43-50,
 - CDR2 has an amino acid sequence selected from SEQ ID NOs: 51-59; and
 - CDR3 has an amino acid sequence selected from SEQ ID NOs 60-65.
17. The fusion polypeptide of any one of claims 1-9, 11 or 14-15, wherein the polypeptide of interest is BCL11A, and the binding domain that specifically binds BCL11A comprises a single-domain antibody in which:
 - CDR1 has the amino acid sequence SIFVNNAM (SEQ ID NO: 37);
 - CDR2 has the amino acid sequence ELVAAISASGGSTYY (SEQ ID NO: 38);and
 - CDR3 has a sequence selected from ADQDVYPYEYW (SEQ ID NO: 39), ADQDGYPYEYW (SEQ ID NO: 40), and ADQDVYPYEYL (SEQ ID NO: 41).
18. The fusion polypeptide of any one of claims 1-17 which further comprises a cell-penetrating peptide.
19. The fusion polypeptide of any one of claims 1-18, further comprising a linker peptide between the binding domain that specifically binds an erythroid-enriched E3 ubiquitin ligase and the binding domain that specifically binds an erythroid-enriched polypeptide of interest.
20. A nucleic acid comprising sequence encoding a fusion polypeptide of any one of claims 1-19.
21. A nucleic acid of claim 20, wherein the sequence encoding the fusion polypeptide is operatively linked to regulatory sequences that permit expression in erythroid cells.
22. A vector comprising a nucleic acid of claim 20 or 21.

23. The vector of claim 22, which is a viral vector.
24. The viral vector of claim 23, which is an AAV vector.
25. A method of erythroid-specific, targeted degradation of a protein of interest, the method comprising introducing a fusion polypeptide of any one of claims 1-19, a nucleic acid of claim 20 or 21 or a vector of any one of claims 22-24 to an erythroid cell.
26. A method of targeted degradation of BCL11A, the method comprising introducing a fusion polypeptide of any one of claims 1-19, a nucleic acid of claim 20 or 21, or a vector of any one of claims 22-24 to a cell expressing BCL11A.
27. The method of claim 26, wherein the targeted degradation of BCL11A is targeted to erythroid cells.
28. A method of promoting fetal hemoglobin (HbF) expression in adult erythroid cells, the method comprising introducing a fusion polypeptide of any one of claims 1-19, a nucleic acid of claim 20 or 21, or a vector of any one of claims 22-24 to a cell, wherein the protein of interest is BCL11A, and wherein the fusion polypeptide promotes the degradation of BCL11A in erythroid cells, thereby promoting HbF expression.
29. A method of treating a hemoglobinopathy disorder, the method comprising introducing a fusion polypeptide of any one of claims 1-19, a nucleic acid of claim 22 or 21, or a vector of any one of claims 22-24 to a subject in need thereof, wherein the protein of interest is BCL11A, and wherein the fusion polypeptide promotes the degradation of BCL11A in erythroid cells, thereby promoting HbF expression to treat the hemoglobinopathy disorder.
30. A fusion polypeptide comprising a TRIM10 or TRIM58 polypeptide fused to a binding domain that specifically binds a target polypeptide.
31. The fusion polypeptide of claim 30, wherein the binding domain that specifically binds a target polypeptide comprises an antigen-binding domain of an antibody.
32. The fusion polypeptide of claim 30 or 31, wherein the binding domain that specifically binds a target polypeptide comprises an scFv, a single domain antibody or a nanobody.
33. The fusion polypeptide of any one of claims 30-32, wherein the target polypeptide is expressed in an erythroid cell.
34. The fusion polypeptide of any one of claims 30-33, wherein the target polypeptide is selected from BCL11A, LRF and ZNF410.

35. The fusion polypeptide of any one of claims 30-34, wherein the polypeptide of interest is BCL11A, and the binding domain that specifically binds the polypeptide of interest binds BCL11A at an epitope comprised by zinc-finger 6 (ZNF6) of the BCL11A polypeptide.
36. The fusion polypeptide of any one of claims 30-34, wherein the polypeptide of interest is BCL11A, and the binding domain that specifically binds the polypeptide of interest binds BCL11A at an epitope comprised by zinc finger 23 (ZNF23) of the BCL11A polypeptide.
37. The fusion polypeptide of any one of claims 30-35, wherein the polypeptide of interest is BCL11A, and the binding domain that specifically binds the polypeptide of interest has an amino acid sequence at least 90% identical to SEQ ID NO: 12.
38. The fusion polypeptide of claim 37, wherein amino acid sequence variation relative to SEQ ID NO: 12 occurs at one or more of amino acids according to Table 2.
39. The fusion polypeptide of any one of claims 30-34 or 36, wherein the polypeptide of interest is BCL11A, and the binding domain that specifically binds BCL11A has an amino acid sequence at least 90% identical to SEQ ID NO: 34.
40. The fusion polypeptide of claim 39, wherein amino acid sequence variation relative to SEQ ID NO: 34 occurs at one or more of amino acids number 102 or 108.
41. The fusion polypeptide of any one of claims 30-35 or 37-38, wherein the polypeptide of interest is BCL11A, and the binding domain that specifically binds BCL11A comprises a single-domain antibody in which:
 - CDR1 has an amino acid sequence selected from SEQ ID NOs: 43-50,
 - CDR2 has an amino acid sequence selected from SEQ ID NOs: 51-59; and
 - CDR3 has an amino acid sequence selected from SEQ ID NOs 60-65.
42. The fusion polypeptide of any one of claims 30-34, 36 or 39-40, wherein the polypeptide of interest is BCL11A, and the binding domain that specifically binds BCL11A comprises a single-domain antibody in which:
 - CDR1 has the amino acid sequence SIFVNNAM (SEQ ID NO: 37);
 - CDR2 has the amino acid sequence ELVAAISASGGSTYY (SEQ ID NO: 38);
 and
 - CDR3 has a sequence selected from ADQDVYPYEYW (SEQ ID NO: 39), ADQDGYPYEYW (SEQ ID NO: 40), and ADQDVYPYEYL (SEQ ID NO: 41).

43. The fusion polypeptide of any one of claims 30-42 which further comprises a cell-penetrating peptide.
44. The fusion polypeptide of any one of claims 30-43, further comprising a linker peptide between the TRIM10 or TRIM58 polypeptide and the binding domain that specifically binds a target polypeptide.
45. A nucleic acid comprising sequence encoding a fusion polypeptide of any one of claims 30-44.
46. The nucleic acid of claim 45, wherein the sequence encoding the fusion polypeptide is operatively linked to regulatory sequences that permit expression in erythroid cells.
47. A vector comprising a nucleic acid of claim 45 or 46.
48. The vector of claim 47, which is a viral vector.
49. The viral vector of claim 48, which is an AAV vector.
50. A method of erythroid-specific, targeted degradation of a protein of interest, the method comprising introducing a fusion polypeptide of any one of claims 30-44, a nucleic acid of claim 45 or 46, or a vector of any one of claims 47-49 to an erythroid cell.
51. A method of targeted degradation of BCL11A, the method comprising introducing a fusion polypeptide of any one of claims 30-44, a nucleic acid of claim 45 or 46, or a vector of any one of claims 47-49 to a cell expressing BCL11A.
52. The method of claim 51, wherein the targeted degradation of BCL11A is targeted to erythroid cells.
53. A method of promoting fetal hemoglobin (HbF) expression in adult erythroid cells, the method comprising introducing a fusion polypeptide of any one of claims 30-44, a nucleic acid of claim 45 or 46, or a vector of any one of claims 47-49 to a cell, wherein the protein of interest is BCL11A, and wherein the fusion polypeptide promotes the degradation of BCL11A in erythroid cells, thereby promoting HbF expression.
54. A method of treating a hemoglobinopathy disorder, the method comprising introducing a fusion polypeptide of any one of claims 30-44, a nucleic acid of claim 45 or 46, or a vector of any one of claims 47-49 to a subject in need thereof, wherein the protein of interest is BCL11A, and wherein the fusion polypeptide promotes the degradation of BCL11A in erythroid cells, thereby promoting HbF expression to treat the hemoglobinopathy disorder.

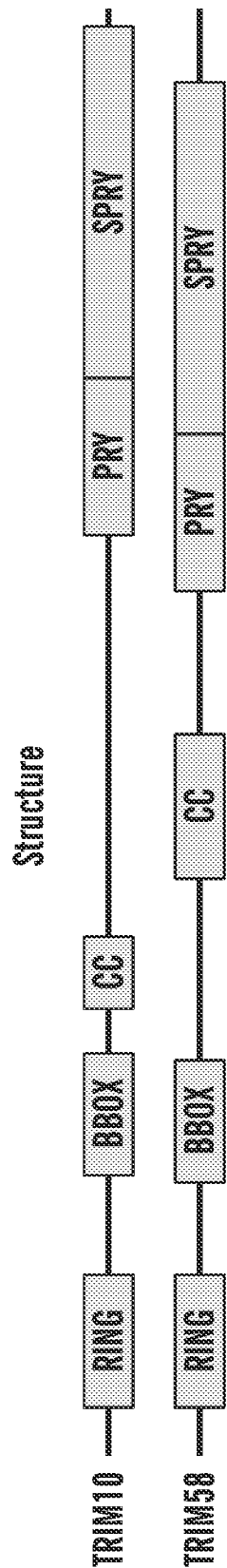
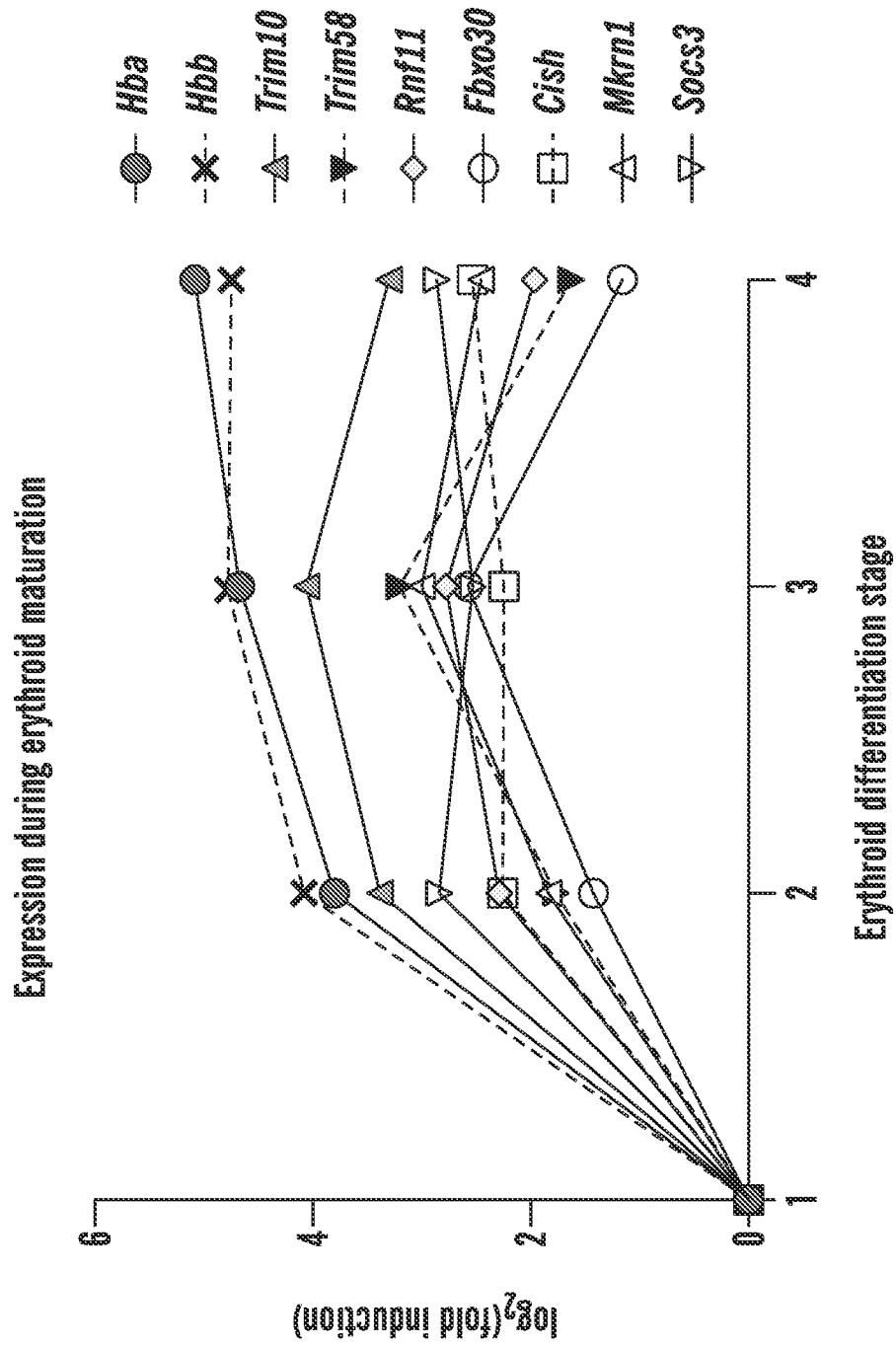


FIG. 1A

**FIG. 1B**

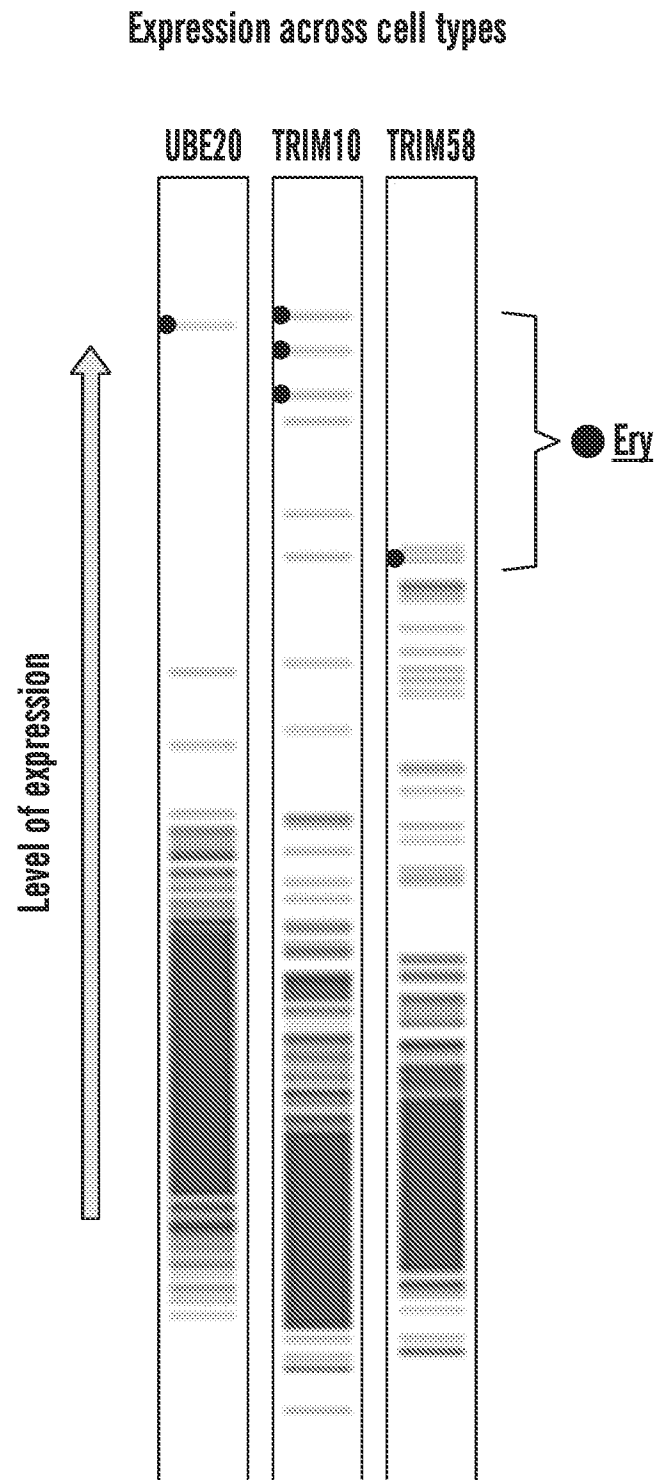
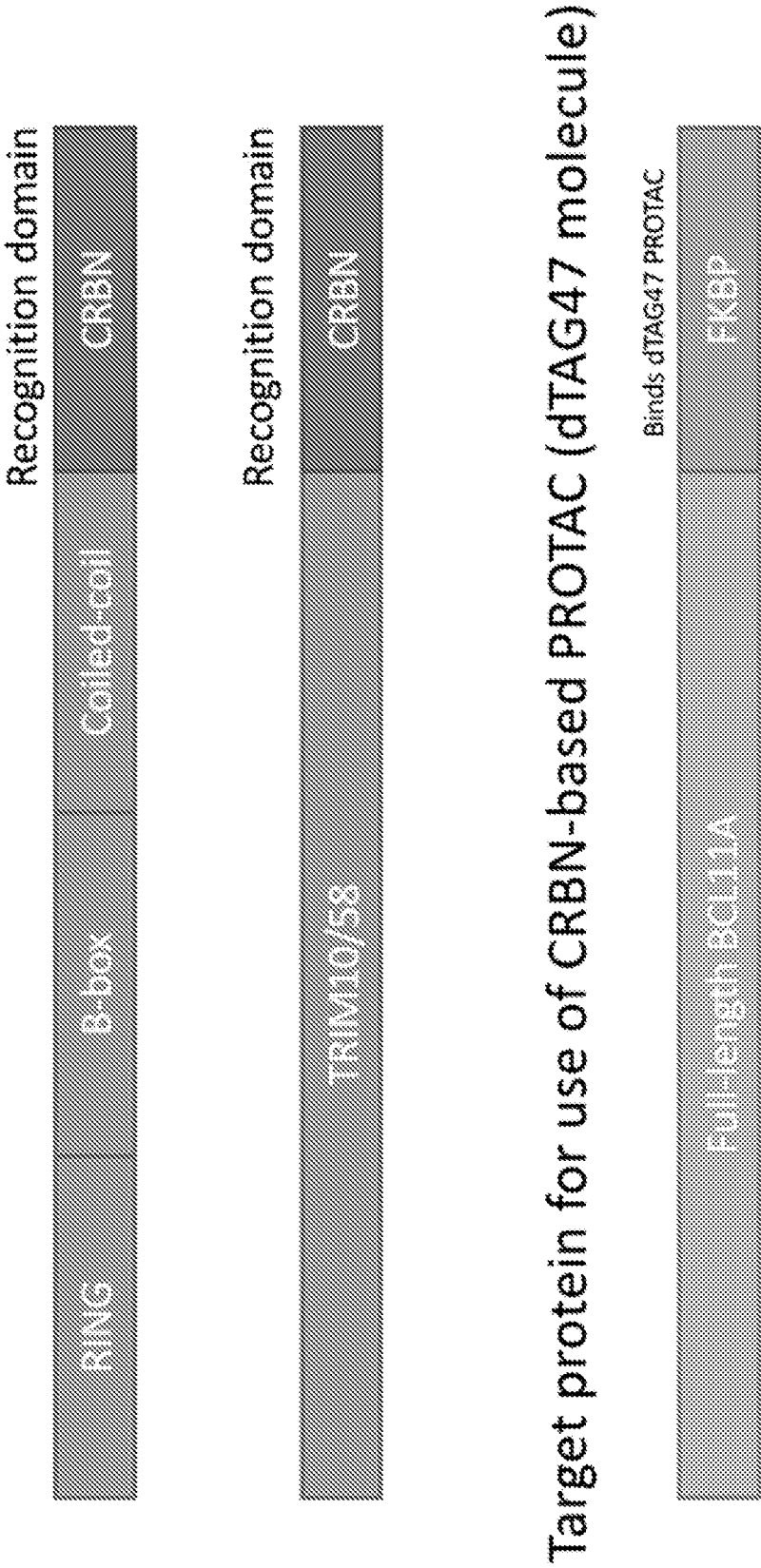
**FIG. 1C**

Fig. 2



Target protein for use of CRBN-based PROTAC (dTAG47 molecule)

Fig. 3

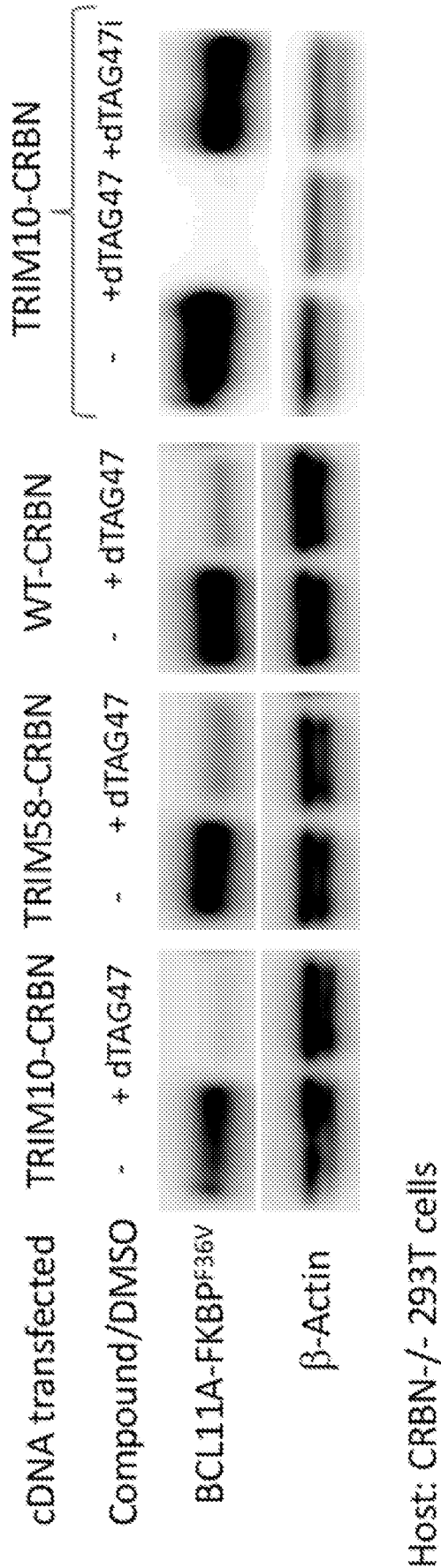
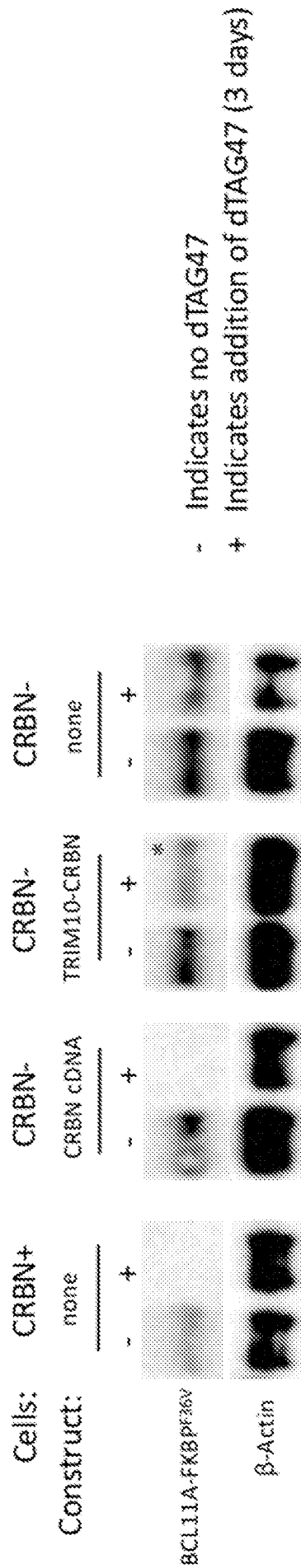


Fig. 4



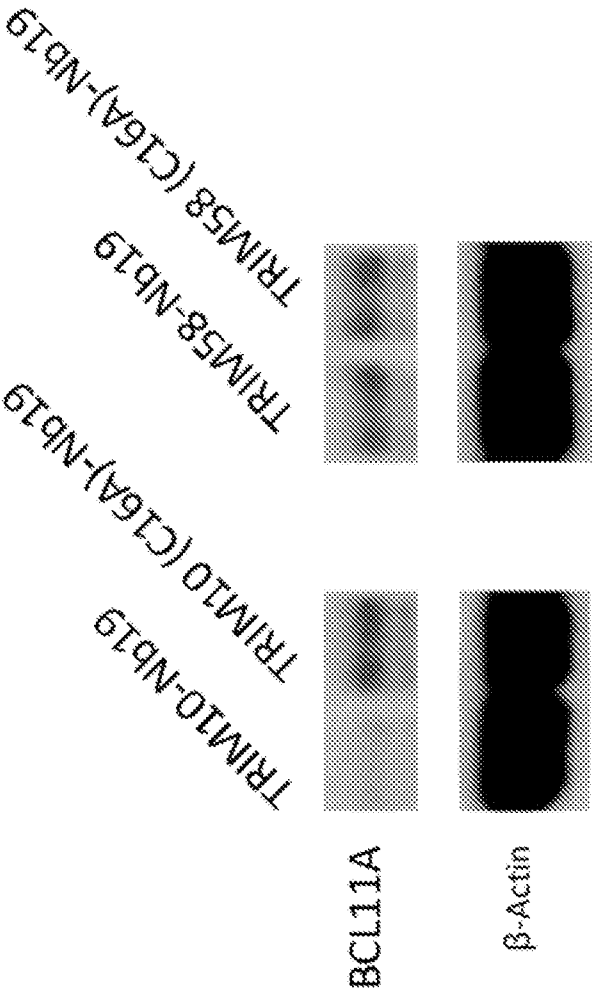


Fig. 5

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 23/66674

A. CLASSIFICATION OF SUBJECT MATTER

IPC - INV. C07K 14/47, A61P 7/06, C07K 19/00 (2023.01)
ADD. A61K 35/28 (2023.01)

CPC - INV. C07K 14/4702, A61P 7/06

ADD. C07K 2319/95, C07K 2317/56, C07K 2317/565, C07K 2319/569, A61K 35/28

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)
See Search History document

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

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

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 2018/0179527 A1 (CHILDREN'S MEDICAL CENTER CORPORATION) 28 June 2018 (28.06.2018). Especially para [0134], claims 1, 9.	1-3, 30-32
Y	LIU et al. TRIM58 suppresses the tumor growth in gastric cancer by inactivation of beta-catenin signaling via ubiquitination. Cancer Biol Ther. 2020, Vol 2, No 3, Pages 203-212. Especially pg 203 col 1 para 2, pg 206 col 1 para 2.	1-3, 30-32

☐ 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

"D" document cited by the applicant in the international application

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

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

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

"P" document published prior to the international filing date but later than the priority date claimed

"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

"&" document member of the same patent family

Date of the actual completion of the international search

8 August 2023

Date of mailing of the international search report

SEP 07 2023

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Kari Rodriguez

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 23/66674

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 13ter.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

International application No.

PCT/US 23/66674

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☒ Claims Nos.: 4-29, 33-54
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.