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(54) Title: SHEDDASE-TARGETING CHIMERAS FOR PROTEOSTASIS MODULATION

(57) Abstract: The invention provides fusion molecules for inducing proximity between integral membrane proteins and integral membrane proteases (sheddases), thereby promoting targeted proteolysis (shedding) of the target membrane protein ectodomain. These fusion molecules, Sheddase-Targeting Chimera (SHEDTACs), contain a protease-targeting domain and a substrate-targeting domain that respectively bind to a sheddase and a target protein of interest (e.g., a cell surface receptor). Also provided in the invention are polynucleotides encoding the fusion molecules, related expression vectors and host cells, as well as methods using the fusion molecules to accelerate integral membrane proteolysis by local sheddases.

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SHEDDASE-TARGETING CHIMERAS FOR PROTEOSTASIS MODULATION

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] The subject patent application claims the benefit of priority to U.S. Provisional Patent Application No. 63/585,233 (filed September 26, 2023; now pending). The full disclosure of the priority application is incorporated herein by reference in its entirety and for all purposes.

BACKGROUND OF THE INVENTION

[0002] Current methods to modulate extracellular proteostasis mainly require target protein ubiquitylation or lysosomal trafficking. Examples of such technologies include Proteolysis-Targeting Chimeras (PROTACs), Lysosome-Targeting Chimeras (LYTACs), Antibody-based PROTACS (AbTACs, PROTABs, REULERs), and CytoKine Receptor-Targeting Chimeras (KineTACs), among others as reviewed in Ahn et al., Cell Chem. Biol. 28, 1072-1080 (2021); and Zhao et al., Signal Transduct. Target. Ther. 7, 113 (2022). See, e.g., Sakamoto et al., Proc. Natl. Acad. Sci. USA 98, 8554-9 (2001); Banik et al., Nature 584, 291-297 (2020); Ahn et al., Nat. Chem. Biol. 17, 937-946 (2021); Cotton et al., J. Am. Chem. Soc. 143, 593-598 (2021); Marei et al., Nature 610:182-189 (2022); Siepe et al., bioRxiv, 2022.10.31.514624 (2022) ; and Pance et al., Nat. Biotechnol. 41:273–281 (2022). Additional methods for degrading membrane proteins utilize proteases that are delivered to the cell surface where they proteolyze broad collections of membrane proteins, as described in Pedram et al., bioRxiv, 2022.05.20.492748 (2022), Romei et al., J. Biolo 299(5), 104685 (2023), and in US20220363778A1. Notably, all these methods are limited by the need for ubiquitylation, lysosomal trafficking, or the requirement for delivery of exogeneous proteases.

[0003] There is a need in the art for more efficient and dynamic methods for targeted modulation of membrane proteostasis. The present invention addresses this and other unmet needs in the art.

SUMMARY OF THE INVENTION

[0004] The invention provides fusion molecules that contain a protease-targeting domain and a covalently linked substrate-targeting domain. In these fusion molecules, also termed SHEDTACs (Sheddase-Targeting Chimeras) herein, the protease-targeting domain can specifically bind to an integral membrane protease or to an auxiliary membrane protein in a multimeric integral membrane protease complex, the substrate-targeting domain can specifically bind to a transmembrane substrate protein (also termed herein a target integral membrane protein) that is bound to the same phospholipid membrane, and the membrane bound protease can cleave the extracellular domain of the target transmembrane substrate protein. In some of these fusion molecules, the integral membrane protease to be targeted is a sheddase. In some embodiments, the sheddase to be targeted is a member of the A Disintegrin And Metalloprotease (ADAM) family of proteases, e.g., ADAM 10.

[0005] Some of the fusion molecules of the invention are intended to target a transmembrane substrate protein that is a cellular signaling receptor or a receptor ligand. Some transmembrane substrate proteins targeted by the fusion molecules of the invention are tumor-associated antigens. In some of the fusion molecules, the protease-targeting domain and the substrate-targeting domain are each an antibody or an antigen-binding fragment. In some of these embodiments, the protease-targeting domain and the substrate-targeting domain are each a single-domain molecule or a single chain variable fragment (scFv). For example, the protease-targeting domain and the substrate-targeting domain in some SHEDTACs of the invention are each a single domain antibody (sdAb). In some fusion molecules of the invention, the protease-targeting domain and the substrate-targeting domain are linked via a linker motif.

[0006] As some specifically exemplified embodiments, some SHEDTACs of the invention contain a protease-targeting domain that recognizes ADAM10 and a substrate-targeting domain that recognizes Lymphocyte Activation Gene 3 (LAG3, CD233) or programmed cell death protein 1 (PD-1; CD279). In some of these embodiments, the protease-targeting domain contains the amino acid sequence shown in any one of SEQ ID NOs:12-16, and the substrate-targeting domain contains the amino acid sequence shown in any one of SEQ ID NOs:17-21. Other than the protease-targeting domain and the substrate-targeting domain, some SHEDTACS of the invention can additionally contain a covalently

or non-covalently linked carrier moiety. In various embodiments, the carrier moiety extends serum half-life *in vivo* and is a carrier protein, a Fc domain, or a PEG molecule that is covalently linked to the protease-targeting domain or the substrate-targeting domain.

[0007] In a related aspect, the invention provides polynucleotides that encode the novel fusion molecules described herein. In some further related aspects, the invention provides vectors that contain one of such polynucleotides, as well as cells that harbor one or more of these vectors or the encoded polynucleotide molecules. In still another aspect, the invention provides pharmaceutical compositions that contain a SHEDTAC molecule disclosed herein or a polynucleotide encoding the SHEDTAC, and a pharmaceutically acceptable carrier.

[0008] In another aspect, the invention provides methods for cleaving the ectodomain of a membrane-bound protein on the surface of a cell. These methods entail first constructing a fusion molecule that contains a protease-targeting domain and a covalently linked substrate-targeting domain. The substrate-targeting domain and the protease-targeting domain specifically bind to the extracellular portion of the membrane-bound protein and a protease that is bound to the same membrane surface, respectively. Once the fusion molecule is generated, it is applied to contact with the cell that expresses the membrane-bound protein and the membrane-bound protease, leading to cleaving of the ectodomain of the membrane-bound protein. In some embodiments, the targeted membrane-bound protein is a cellular receptor or a receptor ligand, and the targeted protease is a sheddase. In some exemplified embodiments, the targeted cellular receptor is LAG3 or PD-1, and the targeted sheddase is ADAM 10. In some embodiments, the substrate-targeting domain and the protease-targeting domain are antibodies or antigen-binding fragments (e.g., VHH sdAbs) that specifically binds to the membrane-bound protein and the protease, respectively. In some embodiments, the fusion molecule can be contacted with the cell in vitro or ex vivo. In some other embodiments, the fusion molecule or a polynucleotide encoding the fusion protein can be administered to a subject, allowing in vivo contact between the fusion molecule and the cellular targets.

[0009] A further understanding of the nature and advantages of the present invention may be realized by reference to the remaining portions of the specification and claims.

DESCRIPTION OF THE DRAWINGS

[0010] Figure 1 shows a scheme of the function of Sheddase-Targeting Chimeras (SHEDTACs). (A) SHEDTACs recruit an integral membrane protease (sheddase) to a target substrate on the same-cell membrane (in *cis*). (A) Induced proximity accelerates substrate ectodomain cleavage by the protease, decoupling associated signaling events (C). (D-F) A similar process is indicated for SHEDTACs that catalyze proteolysis of target substrate complexes.

[0011] Figure 2 illustrates cleavage of target membrane proteins by SHEDTACs. (A) Substrate proteolysis catalyzed by SHEDTACs produces substrate fragments that include extracellular domains (ECD) and intracellular domains (ICD). (B) SHEDTAC-generated ICDs may translocate to the nucleus to initiate gene transcription. (C) SHEDTAC-generated ECDs may engage soluble ligands to affect downstream signaling processes. (D) SHEDTAC-generated ECDs may ligate same-cell (*cis*) receptors to initiate signaling. (E) SHEDTAC-generated ECDs may ligate (*trans*) receptors on neighboring cells to initiate signaling.

[0012] Figure 3 shows general configurations for protein SHEDTACs. (A) Generalized SHEDTAC format, consisting of a sheddase-targeting domain and a substrate-targeting domain. Modular targeting domains may be reconfigured as needed. (B) Reducing SDS-PAGE analysis of a representative SHEDTAC.

[0013] Figure 4 shows LAG3-SHEDTAC library construction and validation. (A) (top) Design of a focused LAG3-SHEDTAC library. (bottom) Reducing SDS-PAGE analysis indicating purified LAG3-SHEDTACs (used to treat cells in (B-D)). (B) Western blot analysis of cell pellets indicating levels of full-length LAG3 on cells following 24h treatment with 500nM LAG3-SHEDTACs. Band intensity is expressed as percent control of cells treated with vehicle in the absence of PMA/ionomycin, indicated by "-". (C) Western blot analysis of cell pellets and corresponding cell supernatants treated with 1μM BMGX0016, sampled every 10 minutes for 60 minutes, indicating time-dependent decreases in full-length (~70kDa) LAG3 and concomitant increases in soluble LAG3 (sLAG3, ~60kDa) ectodomain released into the growth medium by ADAM10. (D) quantification of data from (C) expressed as percent control of cell pellet at t=0, normalized to GAPDH band intensity.

[0014] Figure 5 shows that LAG3-SHEDTACs accelerate LAG3 ectodomain cleavage by ADAM10. (A) Flow cytometry contour plots indicating PBMC gating strategy for

CD3+ADAM10+ cells. (B) Reducing SDS-PAGE analysis indicating LAG3-SHEDTAC (left) and TEV-proteolyzed LAG3-SHEDTAC (right) used to treat cells in (C-E). Flow cytometry contour plots showing cell surface LAG3 abundance on CD3+ADAM10+ cells following 1h treatment with (C) vehicle, (D) LAG3-SHEDTACs, (E) TEV-proteolyzed LAG3-SHEDTACs. (F) Flow cytometry contour plots indicating dose-dependent reductions in detectable cell surface LAG3 levels following treatment with LAG3-SHEDTACs at various concentrations. (G) Western blot analysis of cell pellets indicating levels of full-length LAG3 (~70kDa) on cells following 24h treatment with vehicle or PMA ± LAG3-SHEDTACs. (H, I) Western blot analysis of cell supernatants indicating levels of soluble LAG3 (sLAG3, ~60kDa) ectodomain released into the growth medium by ADAM10, following 24h treatment with LAG3-SHEDTACs at various concentrations.

[0015] Figure 6 shows that protein depletion by SHEDTACs does not require proteasomes or lysosomes. Flow cytometry contour plots showing cell surface LAG3 abundance on CD3+ADAM10+ cells treated with LAG3-SHEDTACs (top) or TEV-digested LAG3-SHEDTAC (monospecific controls, bottom). Prior to treatment, cells were incubated for 2h at 37°C with (A) vehicle, (B) proteasome inhibitor (MG132, 10μM), or (C) lysosome inhibitor (Dynasore, 50μM).

[0016] Figure 7 shows that SHEDTAC-mediated LAG3 shedding from T cells enhances TCR signaling. (A) LAG3 suppresses T cell signaling through interactions with the TCR on T cells and MHCII on antigen presenting cells (APCs) (left). LAG3-SHEDTACs catalyze LAG3 ectodomain cleavage by the integral membrane protease ADAM10 to restore TCR signaling and induce a luciferase reporter (right). (B) Flow cytometry contour plots indicating LAG3 abundance on ADAM10(+) Jurkat reporter cells treated with isotype-SHEDTACs or LAG3-SHEDTACS. (C) dose-dependent luminescence increases following treatment with LAG3-SHEDTACs at various concentrations, indicating enhanced TCR signaling through LAG3 shedding. RLU = relative luminescence units

[0017] Figure 8 shows that SHEDTACs catalyze depletion of the immune checkpoint PD-1 from T cells. (A) T cell activation proceeds through T cell receptor (TCR) recognition of antigen-loaded major histocompatibility complex (MHC), followed by ligation of co-stimulatory CD28 to its B7 ligands CD80/86 on antigen presenting cells (APCs). The immune checkpoint PD1 suppresses T cell signaling by binding its B7 ligands on APCs. (B) PD1-SHEDTACs catalyze PD1 ectodomain shedding by the integral membrane protease

ADAM10 to restore T cell function. (C) Western blot analysis of PD1(+) PBMCs treated with vehicle or PMA/ionomycin to induce ADAM10/17 expression, signified by "+". SHEDTAC-treated samples also contain PMA-ionomycin. PD1 abundance is normalized to tubulin, expressed as percent of PMA-ionomycin induced control.

DETAILED DESCRIPTION OF THE INVENTION

I. Overview

[0018] The present invention provides novel compositions and related methods to proteolyze integral membrane proteins on demand by using endogenous proteases. In some embodiments, this is accomplished by using a multi-specific reagent that binds and induces proximity between an integral membrane protease (sheddase) and a target membrane protein. This multi-specific chimera recruits a sheddase to its target and has therefore been termed a Sheddase-Targeting Chimera (SHEDTAC). By virtue of this induced proximity, SHEDTACs catalyze ectodomain cleavage of a designated target protein (Figure 1). As demonstrated herein, SHEDTAC-derived cleavage products include a soluble extracellular domain (ECD) and a transmembrane domain which may be further processed into a soluble, intracellular domains (ICD). These ECD/ICD cleavage products may affect downstream processes, including gene transcription, soluble ligand complexation, or receptor ligation on the same cell (in *cis*) or on a distant cell (in *trans*) (Fig. 2, Panels B-E). As an exemplification, it is described herein that LAG3-targeting SHEDTACS were able to promote robust LAG3 shedding through induced proximity, and reverse LAG3 suppression of T cell function. Further, SHEDTACs described herein apparently are also able to cleave ECDs of membrane proteins that are not known to be sheddase substrates. This is demonstrated herein with SHEDTACs that target Programmed Cell Death-1 (PD-1) on T lymphocytes.

[0019] There are many technological advantages associated with SHEDTACs and their therapeutic applications as described herein. The fusion molecules can be readily employed as pharmaceuticals for their ability to therapeutically modulate membrane proteostasis and alleviate disease phenotypes. In contrast to currently known methods for depleting extracellular proteins, methods utilizing SHEDTACs for targeted proteolysis operate independent of the ubiquitylation or lysosomal trafficking, and do not require delivery of exogeneous proteases. Instead, these fusion proteins catalyze target protein ectodomain

cleavage by local, endogenous proteases (sheddases) (Figure 1). Moreover, SHEDTAC-derived cleavage products may affect downstream processes on a same cell (in *cis*) or distant cell (in *trans*) (Figure 2). This novel mechanism of action cannot be achieved by existing technologies and is entirely unique to SHEDTACs.

[0020] A further understanding of the nature and advantages of the present invention may be realized by reference to the remaining portions of the specification and claims.

II. Definitions

[0021] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by those of ordinary skill in the art to which this invention pertains. The following references provide one of skill with a general definition of many of the terms used in this invention: *Academic Press Dictionary of Science and Technology*, Morris (Ed.), Academic Press (1st ed., 1992); *Oxford Dictionary of Biochemistry and Molecular Biology*, Smith et al. (Eds.), Oxford University Press (revised ed., 2000); *Encyclopaedic Dictionary of Chemistry*, Kumar (Ed.), Anmol Publications Pvt. Ltd. (2002); *Dictionary of Microbiology and Molecular Biology*, Singleton et al. (Eds.), John Wiley & Sons (3rd ed., 2002); *Dictionary of Chemistry*, Hunt (Ed.), Routledge (1st ed., 1999); *Dictionary of Pharmaceutical Medicine*, Nahler (Ed.), Springer-Verlag Telos (1994); *Dictionary of Organic Chemistry*, Kumar and Anandand (Eds.), Anmol Publications Pvt. Ltd. (2002); and *A Dictionary of Biology (Oxford Paperback Reference)*, Martin and Hine (Eds.), Oxford University Press (4th ed., 2000). In addition, the following definitions are provided to assist the reader in the practice of the invention.

[0022] The singular terms "a," "an," and "the" include plural referents unless the context clearly indicates otherwise. Similarly, the word "or" is intended to include "and" unless the context clearly indicates otherwise.

[0023] As used herein, the term "amino acid" of a peptide refers to naturally occurring and synthetic amino acids, as well as amino acid analogs and amino acid mimetics that function in a manner similar to the naturally occurring amino acids. Naturally occurring amino acids are those encoded by the genetic code, as well as those amino acids that are later modified, e.g., hydroxyproline, γ-carboxyglutamate, and O-phosphoserine. Amino acid analogs refers to compounds that have the same basic chemical structure as a naturally occurring amino acid, i.e., a carbon that is bound to a hydrogen, a carboxyl group, an amino

group, and an R group, e.g., homoserine, norleucine, methionine sulfoxide, methionine methyl sulfonium. Such analogs have modified R groups (e.g., norleucine) or modified peptide backbones, but retain the same basic chemical structure as a naturally occurring amino acid.

[0024] 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 invention, yet open to the inclusion of unspecified elements, whether essential or not.

[0025] 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 of the invention.

[0026] 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.

[0027] The term “contacting” has its normal meaning and refers to combining two or more agents (e.g., polypeptides or small organic molecules), combining agents and cells, or combining two populations of different cells. Contacting can occur in vitro, e.g., mixing two polypeptides or mixing a protein with a population of cells in a test tube or growth medium. Contacting can also occur in a cell or in situ, e.g., contacting two polypeptides in a cell by coexpression in the cell of recombinant polynucleotides encoding the two polypeptides, or in a cell lysate.

[0028] For polypeptide sequences, “conservatively modified variants” refer to a variant which has conservative amino acid substitutions, amino acid residues replaced with other amino acid residue having a side chain with a similar charge. Families of amino acid residues having side chains with similar charges have been defined in the art. These families include amino acids with basic side chains (e.g., lysine, arginine, histidine), acidic side chains (e.g., aspartic acid, glutamic acid), uncharged polar side chains (e.g., glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (e.g., alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (e.g., threonine, valine, isoleucine) and aromatic side chains (e.g., tyrosine, phenylalanine, tryptophan, histidine).

[0029] The term “engineered cell” or “recombinant host cell” (or simply “host cell”) refers to a cell into which a recombinant expression vector has been introduced. It should be understood that such terms are intended to refer not only to the particular subject cell but to the progeny of such a cell. Because certain modifications may occur in succeeding generations due to either mutation or environmental influences, such progeny may not, in fact, be identical to the parent cell, but are still included within the scope of the term “host cell” as used herein.

[0030] A “fusion” protein or polypeptide refers to a polypeptide comprised of at least two polypeptides and a linking sequence or a linkage to operatively link the two polypeptides into one continuous polypeptide. The two polypeptides linked in a fusion polypeptide are typically derived from two independent sources, and therefore a fusion polypeptide comprises two linked polypeptides not normally found linked in nature.

[0031] "Heterologous", when used with reference to two polypeptides, indicates that the two are not found in the same cell or microorganism in nature. Allelic variations or naturally occurring mutational events do not give rise to a heterologous biomolecule or sequence as defined herein. A "heterologous" region of a vector construct is an identifiable segment of polynucleotide within a larger polynucleotide molecule that is not found in association with the larger molecule in nature. Thus, when the heterologous region encodes a mammalian gene, the gene will usually be flanked by polynucleotide that does not flank the mammalian genomic polynucleotide in the genome of the source organism.

[0032] The term "isolated" means a polypeptide or protein is removed from its natural surroundings. However, some of the components found with it may continue to be with an "isolated" protein. Thus, an "isolated polypeptide" is not as it appears in nature but may be substantially less than 100% pure protein.

[0033] The terms “identical” or percent “identity,” in the context of two or more nucleic acids or polypeptide sequences, refer to two or more sequences or subsequences that are the same. Two sequences are "substantially identical" if two sequences have a specified percentage of amino acid residues or nucleotides that are the same (*i.e.*, 60% identity, optionally 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 99% identity over a specified region, or, when not specified, over the entire sequence), when compared and aligned for maximum correspondence over a comparison window, or designated region as measured using one of the following sequence comparison algorithms or by manual alignment and visual

inspection. Optionally, the identity exists over a region that is at least about 50 nucleotides (or 10 amino acids) in length, or more preferably over a region that is 100 to 500 or 1000 or more nucleotides (or 20, 50, 200 or more amino acids) in length.

[0034] Lymphocyte Activation Gene 3 (LAG3, CD233) belongs to the immunoglobulin (Ig) superfamily and comprises a 503-amino acid type I transmembrane protein with four extracellular Ig-like domains, designated D1 to D4. LAG3 is expressed on activated T cells, natural killer cells, B cells and plasmacytoid dendritic cells. LAG3 plays a regulatory role in the immune system comparable to PD-1 and CTLA-4, generally consisting of inhibition of cell proliferation, immune function, cytokine secretion, and homeostasis. It functions by delivering inhibitory signals that regulate immune cell homeostasis, T cell activation, proliferation, cytokine production, cytolytic activity and other functions. LAG3 can be upregulated under various antigen stimulation conditions. LAG3 inhibitors can directly bind LAG3 molecules or their ligands, blocking the interaction between ligands and LAG3, and downregulating the inhibitory efficacy of LAG3 toward the immune system.

[0035] Methods of alignment of sequences for comparison are well known in the art. Optimal alignment of sequences for comparison can be conducted, *e.g.*, by the local homology algorithm of Smith and Waterman, Adv. Appl. Math. 2:482c, 1970; by the homology alignment algorithm of Needleman and Wunsch, J. Mol. Biol. 48:443, 1970; by the search for similarity method of Pearson and Lipman, Proc. Nat'l. Acad. Sci. USA 85:2444, 1988; by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, Madison, WI); or by manual alignment and visual inspection (see, e.g., Brent et al., *Current Protocols in Molecular Biology*, John Wiley & Sons, Inc. (ringbou ed., 2003)). Two examples of algorithms that are suitable for determining percent sequence identity and sequence similarity are the BLAST and BLAST 2.0 algorithms, which are described in Altschul et al., Nuc. Acids Res. 25:3389-3402, 1977; and Altschul et al., J. Mol. Biol. 215:403-410, 1990, respectively.

[0036] Other than percentage of sequence identity noted above, another indication that two nucleic acid sequences or polypeptides are substantially identical is that the polypeptide encoded by the first nucleic acid is immunologically cross reactive with the antibodies raised against the polypeptide encoded by the second nucleic acid, as described below. Thus, a polypeptide is typically substantially identical to a second polypeptide, for example, where

the two peptides differ only by conservative substitutions. Another indication that two nucleic acid sequences are substantially identical is that the two molecules or their complements hybridize to each other under stringent conditions, as described below. Yet another indication that two nucleic acid sequences are substantially identical is that the same primers can be used to amplify the sequence.

[0037] A "ligand" is a molecule that is recognized by a particular antigen, receptor or target molecule. Examples of ligands that can be employed in the practice of the present invention may include, but are not restricted to, agonists and antagonists for cell membrane receptors, toxins and venoms, viral epitopes, hormones, hormone receptors, polypeptides, peptides, enzymes, enzyme substrates, cofactors, drugs (e.g., opiates, steroids, etc.), lectins, sugars, polynucleotides, nucleic acids, oligosaccharides, proteins, and monoclonal antibodies.

[0038] “Linkage” refers to means of operably or functionally connecting two biomolecules (e.g., polypeptides or polynucleotides encoding two polypeptides), including, without limitation, recombinant fusion, covalent bonding, disulfide bonding, ionic bonding, hydrogen bonding, and electrostatic bonding. "Fused" refers to linkage by covalent bonding. A "linker" or "spacer" refers to a molecule or group of molecules that connects two biomolecules, and serves to place the two molecules in a preferred configuration with minimal steric hindrance.

[0039] The term "operably linked" when referring to a nucleic acid, refers to a linkage of polynucleotide elements in a functional relationship. A nucleic acid is "operably linked" when it is placed into a functional relationship with another nucleic acid sequence. For instance, a promoter or enhancer is operably linked to a coding sequence if it affects the transcription of the coding sequence. Operably linked means that the DNA sequences being linked are typically contiguous and, where necessary to join two protein coding regions, contiguous and in reading frame.

[0040] Programmed cell death protein 1 (PD-1; CD279) is a cell surface receptor that belongs to the immunoglobulin superfamily and is expressed on T cells and pro-B cells. PD-1 binds two ligands, PD-L1 and PD-L2, and plays an important role in regulating the immune system's response. It is an immune checkpoint and guards against autoimmunity through two mechanisms. First, it promotes apoptosis (programmed cell death) of antigen-specific T-cells in lymph nodes. Second, it reduces apoptosis in regulatory T cells (anti-

inflammatory, suppressive T cells). See, e.g., Francisco et al., Immunol. Rev. 2010; 236: 219–42; and Fife et al., Ann. N. Y. Acad. Sci. 2011; 1217: 45–59.Unless otherwise specified, the terms "polypeptide" and "peptide" are used interchangeably herein to refer to a polymer of amino acid residues. They encompass both short oligopeptides (e.g., peptides with less than about 25 residues) and longer polypeptide molecules (e.g., polymers of more than about 25 or 30 amino acid residues). Typically, the candidate peptide or polypeptide ligands used in the invention can comprise from about 4 amino acid residues to about 350 or more amino acid residues in length. In some embodiments, the peptides or polypeptides comprise from about 6 amino acid residues to about 60 amino acid residues in length. In some other embodiments, they can comprise from about 8 amino acid residues to about 40 amino acid residues in length. The peptides or polypeptides can include naturally occurring amino acid polymers and non-naturally occurring amino acid polymer, as well as amino acid polymers in which one or more amino acid residue is an artificial chemical mimetic of a corresponding naturally occurring amino acid. Unless otherwise indicated, a particular polypeptide sequence also implicitly encompasses conservatively modified variants thereof.

[0041] As used herein, the term "peptide mimetic" or "peptidomimetic" refers to a derivative compound of a reference peptide that biologically mimics the peptide's functions. Typically, the peptidomimetic derivative has at least 50%, at least 75% or at least 90% of the biological function of the reference polypeptide.

[0042] Unless otherwise noted, the term "receptor" broadly refers to a molecule that has an affinity for a given ligand. Receptors may-be naturally-occurring or manmade molecules. Also, they can be employed in their unaltered state or as aggregates with other species. Receptors may be attached, covalently or noncovalently, to a binding member, either directly or via a specific binding substance. A typical example of receptors which can be employed in the practice of the invention is cell surface signaling receptor.

[0043] The phrase "signal transduction pathway" or "signaling activities" (e.g., the GLP-1R mediated signaling) refers to at least one biochemical reaction, but more commonly a series of biochemical reactions, which result from interaction of a cell with a stimulatory compound or agent. Thus, the interaction of a stimulatory compound with a cell generates a "signal" that is transmitted through the signal transduction pathway, ultimately resulting in a cellular response.

[0044] As used herein, the term "variant" refers to a molecule (e.g., a polypeptide or an antibody fragment) that contains a sequence that is substantially identical to the sequence of a reference molecule. For example, the reference molecule can be an enzymatic polypeptide disclosed herein or a fusion thereof. The reference molecule can also be a polynucleotide encoding the polypeptide. In some embodiments, the variant can share at least 50%, at least 70%, at least 80%, at least 90%, at least 95% or more sequence identity with the reference molecule. In some other embodiments, the variant differs from the reference molecule by having one or more conservative amino acid substitutions. In some other embodiments, a variant of a reference molecule is a conservatively modified variant, e.g., a variant which has altered amino acid sequences (e.g., with one or more conservative amino acid substitutions) but substantially retains the biological activity of the reference molecule. Conservative amino acid substitutions are well known to one skilled in the art.

[0045] The term “vector” is intended to refer to a polynucleotide molecule capable of transporting another polynucleotide to which it has been linked. One type of vector is a “plasmid”, which refers to a circular double stranded DNA loop into which additional DNA segments may be ligated. Another type of vector is a viral vector, wherein additional DNA segments may be ligated into the viral genome. Certain vectors are capable of autonomous replication in a host cell into which they are introduced (e.g., bacterial vectors having a bacterial origin of replication and episomal mammalian vectors). Other vectors (e.g., non-episomal mammalian vectors) can be integrated into the genome of a host cell upon introduction into the host cell, and thereby are replicated along with the host genome. Moreover, certain vectors are capable of directing the expression of genes to which they are operatively linked. Such vectors are referred to herein as “recombinant expression vectors” (or simply, “expression vectors”).

[0046] The term “integral membrane protein” refers to proteins, polypeptides, peptidomimetics or conjugates thereof, characterized by their ability to span or traverse the lipid bilayer of biological membranes, which can be found in cells or organelles like the cell membrane, endoplasmic reticulum, Golgi apparatus, mitochondria, and others. Examples of integral membrane proteins include transporters, receptors, cytokines and enzymes.

[0047] An “integral membrane protease” is a type of integral membrane protein that is also a protease. Proteases, in general, are enzymes that catalyze the cleavage of peptide bonds in proteins, leading to the breakdown of proteins into smaller peptide fragments or

individual amino acids. Integral membrane proteases hydrolyze polypeptides located near, or embedded within, biological lipid membranes, such as those constituting cells and organelles, e.g. cell membrane, endoplasmic reticulum, Golgi apparatus, and mitochondria.

III. SHEDTACs for inducing proximity between sheddases and target proteins

[0048] The invention provides Sheddase-Targeting Chimeras (SHEDTACs), which by induced proximity between an integral membrane protein (substrate or target) and an integral membrane protease (sheddase), can catalyze ectodomain cleavage of designated target proteins. Typically, the SHEDTACs contain a protease-targeting domain and a substrate-targeting domain. The protease-targeting domain, e.g., an antibody or a ligand, is able to specifically bind to an endogenous or local protease. The substrate-targeting domain, e.g., an antibody or a ligand, specifically binds to a target membrane protein. Any molecules or compounds that can specifically bind to a target protein of interest and a corresponding sheddase can be used as the substrate-targeting domain and the protease-targeting domain, respectively. They can contain small molecules, amino acids, nucleotides, lipids, carbohydrates, antibody or antibody fragments, oligopeptides, oligonucleotides, polypeptides, polynucleotides, cytokines, chemokines, DNA/RNA aptamers, or combinations thereof. In some preferred embodiments, the protease-targeting domain and the substrate-targeting domain are covalently linked in the SHEDTACs. In some of these embodiments, a liker motif is used to fuse the protease-targeting domain to the substrate-targeting domain.

[0049] SHEDTACs may be constructed in diverse formats, as monomeric and multimeric fusions, using targeting molecules or moieties for multi-specific targeting to an integral membrane protease (sheddase) and a target substrate. General configurations for protein SHEDTACs are shown in Figure 3. Exemplifications are provided herein for SHEDTACs that direct the LAG3 or PD-1 membrane protein for proteolysis by an integral membrane sheddase, such as A Disintegrin And Metalloprotease (ADAM10). LAG3 is an immune checkpoint, which is a co-inhibitory receptor to suppress T cells activation and cytokines secretion, thereby ensuring a state of immune homeostasis. LAG3 exerts differential inhibitory impacts on various types of lymphocytes. As detailed in the Examples herein, a library of LAG3-ADAM10 engaging SHEDTACs was constructed and recombinantly expressed in Escherichia coli. This SHEDTAC contains a LAG3-binding

sdAb and a ADAM10-binding sdAb. The two targeting domains can be linked a linker motif, e.g., a GGGGS (SEQ ID NO:11) tandem repeat linker as exemplified herein. As shown in Figure 3, Panel B, this exemplary SHEDTAC has a molecular weight of ~34kDa. Using this LAG3-ADAM10 engaging SHEDTACs for illustration (Figure 3, Panel A), the substrate-targeting domain can be fused at its C-terminus to the N-terminus of the protease-targeting domain. In alternative embodiments, the protease-targeting domain can be fused at its C-terminus to the N-terminus of the substrate-targeting domain. In alternative embodiments, substrate-targeting domains or protease-targeting domains can be fused at their C-terminus or N-terminus with molecules that extend serum half-life in vivo, e.g., carrier proteins, albumin binding polypeptides, Fc domains, antibody fragments, polyethylene glycol (PEG) chains, nucleic acids, lipids, or carbohydrates. Besides LAG3-trargeting SHEDTACs, specific SHEDTACs that target PD-1 for cleavage by ADAM10 via induced proximity are also exemplified herein (Figure 8).

[0050] In some preferred embodiments, the protease-targeting domain and the substrate-targeting domain of the SHEDTACs are antibodies or antigen-binding fragments thereof. Any antibody sequences can be employed in the invention. In some embodiments, each of the protease-targeting domain and the substrate-targeting domain is an antibody fragment or antigen-binding fragment. Antibody fragments or antigen-binding fragments contain the antigen-binding portions of an intact antibody that retain capacity to bind the cognate antigen. Examples of such antibody fragments include (i) a Fab fragment, a monovalent fragment consisting of the V_L, V_H, C_L and C_{H1} domains; (ii) a $F(ab')_2$ fragment, a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region; (iii) a Fd fragment consisting of the V_H and C_{H1} domains; (iv) a Fv fragment consisting of the V_L and V_H domains of a single arm of an intact antibody; (v) disulfide stabilized Fvs (dsFvs) which have an interchain disulfide bond engineered between structurally conserved framework regions; (vi) a single domain antibody (sdAb) or sdAb which consists of a V_H domain (see, e.g., Ward et al., Nature 341:544-546, 1989); and (vii) an isolated complementarity determining region (CDR).

[0051] In some embodiments, antibodies employed for practicing the present invention are single chain antibodies. The term "single chain antibody" refers to a polypeptide comprising a V_H domain and/or a V_L domain in polypeptide linkage, generally linked via a spacer peptide, and which may comprise additional domains or amino acid sequences at the

amino- and/or carboxyl-termini. For example, a single-chain antibody may comprise a tether segment for linking to the encoding polynucleotide. As an example, a single chain variable region fragment (scFv) is a single-chain antibody. Compared to the V_L and V_H domains of the Fv fragment which are coded for by separate genes, a scFv has the two domains joined (e.g., via recombinant methods) by a synthetic linker. This enables them to be made as a single protein chain in which the V_L and V_H regions pair to form monovalent molecules. In some preferred embodiments, the protease-targeting domain and the substrate-targeting domain of the SHEDTACs are single domain antibodies such as VHH sdAb domains as exemplified herein. As specific exemplifications, the protease-targeting domain can be a ADAM10-binding sdAb that contains the amino acid sequence shown in any one of SEQ ID NOs:12-16, a substantially identical sequence, or a conservatively modified variant thereof, and the substrate-targeting domain can be a LAG3-binding sdAb or PD-1 binding sdAb that contains the amino acid sequence shown in any one of SEQ ID NOs:17-21, a substantially identical sequence, or a conservatively modified variant thereof.

[0052] Antibodies or antigen-binding fragments for practicing the invention can be produced by enzymatic or chemical modifications of the intact antibodies, or synthesized de novo using recombinant DNA methodologies, or identified using phage display libraries. Methods for generating these antibodies or antigen-binding molecules are all well known in the art. For example, single chain antibodies can be identified using phage display libraries or ribosome display libraries, gene shuffled libraries (see, e.g., McCafferty et al., Nature 348:552-554, 1990; and U.S. Pat. No. 4,946,778). In particular, scFv antibodies can be obtained using methods described in, e.g., Bird et al., Science 242:423-426, 1988; and Huston et al., Proc. Natl. Acad. Sci. USA 85:5879-5883, 1988. Fv antibody fragments can be generated as described in Skerra and Plückthun, Science 240:1038-41, 1988. Disulfide-stabilized Fv fragments (dsFvs) can be made using methods described in, e.g., Reiter et al., Int. J. Cancer 67:113-23, 1996. Similarly, single domain antibodies (sdAb) can be produced by recombinant methods as exemplified herein and/or methods known in the art. See, e.g., Ward et al., Nature 341:544-546, 1989; and Cai and Garen, Proc. Natl. Acad. Sci. USA 93:6280-85, 1996. Camelid single domain antibodies can be produced using methods well known in the art, e.g., Dumoulin et al., Nature Struct. Biol. 11:500–515, 2002; Ghahroudi et al., FEBS Letters 414:521–526, 1997; and Bond et al., J Mol Biol. 332:643-55, 2003. Other types of antigen-binding fragments (e.g., Fab, $F(ab')_2$ or Fd fragments) can also be readily

produced with routinely practiced immunology methods. See, e.g., Harlow & Lane, *Using Antibodies, A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 1998. In some embodiments, sdAb fragments used in the SHEDTACs of the invention can be produced via recombinant expression.

[0053] In some embodiments, the SHEDTACs of the invention can be further conjugated to a carrier moiety to enhance their stability and serum half-lives. The conjugation can be either covalent or non-covalent via any conventional methods. The "carrier moiety" ("carrier" or "carrier molecule") is typically a large, slowly metabolized macromolecule such as proteins; polysaccharides (such as latex functionalized SEPHAROSE™, agarose, cellulose, cellulose beads and the like); polymeric amino acids (such as polyglutamic acid, polylysine, and the like); amino acid copolymers; and inactive virus particles or attenuated bacteria, such as Salmonella. In various embodiments, the carrier moiety can be a carrier protein, an immunoglobulin, a Fc domain, a PEG molecule or other polymer. In some of these embodiments, the employed carrier moiety is the Fc domain of an IgG. Fusion with an Fc-domain provides a number of beneficial biological and pharmacological properties. For example, the presence of the Fc domain can markedly increase the plasma half-life of the hybrid molecule, which prolongs therapeutic activity, owing to its interaction with the salvage neonatal Fc-receptor, as well as to the slower renal clearance for larger sized molecules. The attached Fc domain also enables the molecules to interact with Fc-receptors (FcRs) found on immune cells, a feature that is particularly important for their use in oncological therapies and vaccines. In some other embodiments, the carrier moiety is a protein. Integral membrane proteins from, e.g., *E. coli* and other bacteria are useful conjugation partners. Especially useful carrier proteins are serum albumins, keyhole limpet hemocyanin (KLH), certain immunoglobulin molecules, thyroglobulin, ovalbumin, bovine serum albumin (BSA), tetanus toxoid (TT), and diphtheria toxoid (CRM). In some of these embodiments, the employed carrier moiety is a protein that can bind serum molecules, e.g., albumin. In some other embodiments, the carrier moiety can be a polymer other than a protein or polypeptide. Examples of such polymers include, e.g., carbohydrates such as dextran, mannose or mannan.

[0054] The various SHEDTACs of the invention can be generated in accordance with routinely practiced biological and/or chemical methods (e.g., recombination technology as exemplified herein). For SHEDTACs whose protease-targeting domain and substrate-

targeting domain are both polypeptides (e.g., sdAb domains), the method for generating the SHEDTAC fusion proteins is not subject to any particular limitation. The fusion proteins may be a fusion protein synthesized by chemical synthesis, or a recombinant fusion protein produced by a genetic engineering technique as exemplified herein. If the fusion protein of the invention is to be chemically synthesized, synthesis may be carried out by, for example, the Fmoc (fluorenylmethyloxycarbonyl) process or the tBoc (t-butyloxycarbonyl) process. In addition, peptide synthesizers available from, for example, Advanced ChemTech, PerkinElmer, Pharmacia, Protein Technology Instrument, Syntheceh-Vega, PerSeptive and Shimadzu Corporation may be used for chemical synthesis. If the SHEDTAC fusion protein is to be produced by a genetic engineering technique, production may be carried out using the conventional recombination techniques routinely practiced in the art. Such techniques are described, e.g., in Sambrook et al., *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Press, N.Y., (3rd ed., 2000); and Brent et al., *Current Protocols in Molecular Biology*, John Wiley & Sons, Inc. (ringbou ed., 2003). As detailed herein, the SHEDTAC fusion protein can be produced by inserting a polynucleotide (e.g., DNA) encoding the fusion protein into a suitable vector, followed by introducing the vector into an appropriate expression system, e.g., a pET23a vector for expression in E. coli as exemplified herein.

[0055] The SHEDTACs of the invention can be readily employed to specifically target a cellular membrane protein (e.g., a regulatory protein such as LAG3 or PD-1 as exemplified herein) for proteolytic cleavage in vivo by a corresponding endogenous sheddase (e.g., ADAM sheddase as exemplified herein). Typically, these applications involve contacting in vivo (e.g., by administration to a subject) or ex vivo a SHEDTAC described herein that specifically target a membrane protein for proteolysis and a sheddase enzyme that specifically proteolyze the target protein of interest. In some embodiments, the employed SHEDTAC is a protein. In some other embodiments, a polynucleotide sequence or an expression vector encoding the SHEDTAC protein is introduced (e.g., by administration to a subject) for in vivo expression.

[0056] In some embodiments, the membrane protein to be targeted for proteolysis is specifically or differentially expressed in a given cell or tissue type (e.g., LAG3 in $CD4^+$ and $CD8^+$ T cells, and PD-1 on T cells and B cells). For example, the target protein can be a regulatory receptor predominantly expressed on the surface of immune cells such as T cells.

In some embodiments, the target protein can be a membrane protein that is specifically or differentially expressed on a diseased tissue or cell such as a tumor cell.

IV. Proteases for degrading target proteins via induced proximity with SHEDTACs

[0057] The endogenous proteases to be targeted by the SHEDTACs of the invention can be any protease that is capable of proteolyzing a membrane bound protein of interest on the surface of a cell (e.g., a leukocyte). Typically, to allow proximity induced by a SHEDTAC, the protease is expressed by and bound to the membrane of the same cell. In some preferred embodiments, the membrane-bound protease is a sheddase as described herein.

[0058] Sheddases are membrane-bound enzymes that cleave extracellular portions of transmembrane proteins, releasing the soluble ectodomains from the cell surface. Many sheddases are members of the ADAM or aspartic protease (BACE) protein families. These enzymes can activate a transmembrane protein if it is a receptor (e.g., HER2), or cut off the part of the transmembrane protein which has already bound a ligand (e.g., in the case of EGFR), allowing this ligand to engage a receptor on another cell. Due to the nature of the mechanisms and functions of sheddase enzymes, they have been studied on the basis of discovering possible uses in medicine. Therapeutic uses of sheddases can encompass various diseases and disorders, e.g., brain disorders, e.g. Alzheimer's disease, Prion disease, Fragile X-syndrome and Huntington's disease. Additional medical uses can be applied to address immune disorders, e.g. psoriasis, systemic lupus erythematosus (SLE), rheumatoid arthritis, bacterial or viral infection, chronic tissue and organ inflammation and atherosclerosis. Additional medical uses can be found in areas of cancer, e.g. indications that include melanoma, non-small cell lung cancer (NSCLC), small cell lung cancer (SCLC), head and neck squamous cell carcinoma (HNSCC), classical Hodgkin lymphoma (cHL), primary mediastinal large B-cell lymphoma (PMBCL), urothelial carcinoma, microsatellite instability-high cancer, gastric cancer, esophageal cancer, cervical cancer, hepatocellular carcinoma, merkel cell carcinoma, renal cell carcinoma and endometrial carcinoma. Possible therapeutic substrates contained within these examples could include membrane-bound and soluble forms of proteins or their ligands that include amyloid precursor protein (APP), prion protein PrPc, PrPsc, Fragile X Mental Retardation Protein (FMRP), htt, N-cadherin, VE-cadherin, IL6, IL6R, CXCL16, CXCL1, EGF, EGFR, LAG3, Notch1, Delta,

HB-EFG, TGFa, Axl, TACI, CX3CL1/fractalkine, GM-CSF, CD23, JAM-A, JAM-C, IL8, TNFa, c-Met, IL10, including others listed in Table 1.

[0059] The SHEDTAC fusion constructs of the invention are intended to direct a target membrane protein of interest (e.g., a signaling receptor) to an endogenous sheddase for proteolysis, thereby eliciting desired cellular responses and/or therapeutic activities. Many sheddases known in the art can be targeted by the SHEDTACs of the invention. In some embodiments, the targeted sheddases are A Disintegrin And Metalloprotease (ADAM) proteases such as ADAM10 as exemplified herein. Other examples of ADAM proteases that can be targeted by the SHEDTACs of the invention include ADAM 1, ADAM2, ADAM3B, ADAM4, ADAM5, ADAM6, ADAM7, ADAM8, ADAM9, ADAM10, ADAM11, ADAM12, ADAM13, ADAM14, ADAM15, ADAM16, ADAM17, ADAM18, ADAM19, ADAM20, ADAM21, ADAM21, ADAM22, ADAM23, ADAM24, ADAM25, ADAM26, ADAM27, ADAM28, ADAM29, ADAM30, ADAM31, ADAM32, ADAM33, ADAM34, ADAM35, ADAM36, ADAM37, ADAM38, ADAM39, ADAM40 - ADAMTS1, ADAMTS2, ADAMTS3, ADAMTS4, ADAMTS5, ADAMTS6, ADAMTS7, ADAMTS8, ADAMTS9, ADAMTS10, ADAMTS11, ADAMTS12, ADAMTS13, ADAMTS14, ADAMTS15, ADAMTS16, ADAMTS17, ADAMTS18, ADAMTS19, ADAMTS20 . Some SHEDTACs of the invention are intended to target BACE proteases (aspartyl proteases). Examples of BACE proteases to be targeted include BACE1, BACE2 and etc. Some SHEDTACs of the invention are intended to target the Site-1 protease (serine protease), also known as SKI-1 or S1P. Some SHEDTACs of the invention can target the meprin β metalloprotease. In some other embodiments, the sheddase to be targeted by the SHEDTACs of the invention is a membrane-type matrix metalloproteinase (MT-MMP). Specific examples of MT-MMPs that can be targeted include MT1-MMP, MT2-MMP, MT3-MMP, MT4-MMP, MT5-MMP, and MT6-MMP. In some other embodiments, the sheddase to be targeted by the SHEDTACs of the invention is a pro-protein convertase (serine protease). Examples of pro-protein convertases that can be targeted include PCSK1/3, PCSK2, furin, PCSK4, PCSK5/6, PACE4, PCSK7 and PCSK9. In some embodiments, the sheddase to be targeted is a transmembrane serine protease. Specific examples of transmembrane serine proteases that can be targeted by the SHEDTACs of the invention include Matriptase, Matriptase-2, Matriptase-3, Polyserase-1, Corin, Hepsin, TMPRSS2, TMPRSS3, TMPRSS4, MSPL, Spinesin, Enteropeptidase, HAT, DESC1, TMPRSS11A, HAT-like 4,

and HAT-like 5. In some other embodiments, the sheddase to be targeted is a matrix metalloprotease (MMP). Specific examples of MMPs that can be targeted include MMP1, MMP2, MMP3, MMP7, MMP8, MMP9, MMP10, MMP11, MMP12, MMP13, MMP19, MMP20, MMP21, MMP23, MMP26, MMP27, and MMP28. In various other embodiments, the SHEDTACs of the invention are intended to direct target proteins for proteolysis by additional sheddases such as Legumain (δ -secretase), cysteine proteases, Cathepsin S and L, Rhomboid proteases (e.g., RHBDL1, RHBDL2, RHBDL3, and RHBDL4), the SPP/SPPL family of aspartyl proteases (e.g., SPPL3, SPP, SPPL2a, SPPL2b, and SPPL2c), and the Presenilin/ γ -secretase aspartyl protease (e.g., Presenilin-1 and Presenilin-2).

[0060] Other than targeting a protease directly, the SHEDTACs of the invention can also target the protease (e.g., sheddase) indirectly by binding to a molecule that interacts with the protease. Integral membrane proteases such as sheddases typically co-localize with auxiliary membrane proteins to form integral membrane protease complexes capable of proteolyzing membrane protein substrates. In some embodiments, SHEDTACs of the invention are intended to induce proximity between a target membrane protein and integral membrane protease complex. This can be achieved directly through binding of the SHEDTAC protease-targeting domain to the integral membrane protease as exemplified herein, or indirectly through binding of the SHEDTAC protease-targeting domain to auxiliary membrane proteins that constitute a multimeric integral membrane protease complex (e.g. Tspan1, Tspan2, Tspan3, Tspan4, Tspan5, Tspan6, Tspan7, Tspan8, Tspan9, Tspan10, Tspan11, Tspan12, Tspan13, Tspan14, Tspan15, Tspan16, Tspan17, Tspan18, Tspan19, Tspan20, Tspan21, Tspan22, Tspan23, Tspan24, Tspan25, Tspan26, Tspan27, Tspan28, Tspan29, Tspan30, Tspan31, Tspan32, Tspan33, FRMD8, iRhom1, iRhom2, nicastrin, PEN-2, APH-1, etc.)

V. Target proteins for proteolysis by sheddases brought in proximity via SHEDTACs

[0061] Many proteins that are "shed" from cellular phospholipid membranes represent sheddase targets. By association, these proteins are target proteins or substrates for proteolysis by sheddases that can be brought in proximity via SHEDTACs of the invention. In general, any known protein attached to a phospholipid membrane (e.g., transmembrane proteins) can be targeted, provided that a membrane-bound protease expressed on the same membrane recognizes the protein for proteolysis. Such candidate target proteins include all

computationally predicted and/or experimentally validated to localize to the cellular plasma membrane, which can be retrieved from the EMBL-EBI database (www.ebi.ac.uk/QuickGO/term/GO:0005886). When a colocalized integral membrane protease is available, these membrane proteins can all be targeted by a corresponding SHEDTAC molecule that can be generated in accordance with the invention. In some embodiments of the invention, the transmembrane substrate protein to be targeted by the SHEDTACs of the invention is a cellular signaling receptor or a receptor ligand. In some embodiments, the target transmembrane substrate protein is a tumor-associated antigen.

[0062] In some embodiments, the target protein is a known or suggested substrate of a sheddase. A non-exhaustive list of annotated sheddase targets has been compiled on the SheddomeDB. See, e.g., Tien et al., BMC Bioinformatics 18, 42 (2017). The substrate-targeting domain in the SHEDTACs of the invention can be a binding moiety (e.g., a single domain VHH sdAb or a single chain scFv fragment) that specifically recognizes any of these target proteins. Examples of known and potential sheddase substrates in humans are summarized in Table 1. In some embodiments, the target substrate to be engaged by the SHEDTACs for proteolysis is a protein whose depletion at the plasma membrane provides therapeutic benefit. Some specific examples of such target proteins include immune checkpoint receptors such as PD-1, CTLA4, TIM3 and TIGIT. In some embodiments, the substrate to be targeted for proteolysis is a membrane protein whose soluble extracellular domain provides therapeutic benefit once released from the membrane. Some specific examples of such target proteins include immune checkpoint receptors such as PD-1, CTLA4, TIM3, TIGIT, CD33-related Siglecs, HER2, EGFR, TNFRI/II, Notch1, Delta, APP. Additional sheddase substrates whose depletion at the membrane directly confers therapeutic benefit, or whose soluble extracellular or intracellular domain provides therapeutic benefit, are listed in Table 1.

Table 1. Known and Potential Sheddase Substrates

5HT1a	B3KXZ8	CNR1	GPR34	LEPR	OR4S1	Q6KAV0	SLCO5A1
6M1-16	B3KY39	CNR2	GPR35	LGALS3	OR4S2	Q6SV87	SLCO6A1
A0A023T695	B3KY43	CNRIP1	GPR37	LGALS4	OR4X1	Q6SV88	SLITRK1
A0A077CXZ9	B3KY56	CNST	GPR37L1	LGR4	OR4X2	Q6SV89	SLITRK2
A0A0A1HAV6	B3KY88	CNTN1	GPR39	LGR5	OR51A2	Q6SV90	SLITRK3
A0A0A1HAV9	B4DDB3	CNTN2	GPR4	LGR6	OR51A4	Q6SV91	SLITRK4
A0A0A6YYJ9	B4DDK0	CNTN3	GPR42	LGSN	OR51A7	Q6ULR6	SLITRK5
A0A0G2JMM	B4DDQ9	CNTN4	GPR45	LHCGR	OR51B2	Q6ULR8	SLITRK6

0							
A0A0G2JNH3	B4DDR6	CNTN5	GPR49	LHFPL2	OR51B4	Q6XGY1	SLMAP
A0A0G2R1G9	B4DDS1	CNTN6	GPR50	LHFPL3	OR51B5	Q6XYC3	SLURP2
A0A0G2R211	B4DE29	CNTNAP1	GPR52	LHFPL4	OR51B6	Q6ZMF6	SMAD3
A0A140GPP7	B4DE41	CNTNAP2	GPR55	LHFPL5	OR51C1P	Q6ZRS5	SMAD7
A0A140VJC8	B4DE51	CNTNAP3	GPR56	LIFR	OR51D1	Q6ZRZ8	SMAGP
A0A140VJE0	B4DE59	CNTNAP4	GPR6	LILRA1	OR51E1	Q6ZS12	SMAP1
A0A140VJE9	B4DE74	COA6	GPR61	LILRA2	OR51E2	Q6ZSC4	SMIM1
A0A140VJG8	B4DEL7	COBL	GPR62	LILRA3	OR51F1	Q6ZV11	SMIM23
A0A140VJH4	B4DEP8	COG3	GPR63	LILRA4	OR51F2	Q6ZV33	SMIM43
A0A140VJK5	B4DEQ8	COL13A1	GPR65	LILRA5	OR51G1	Q6ZVS2	SMIM9
A0A140VJN4	B4DER2	COL17A1	GPR68	LILRA6	OR51G2	Q6ZW62	SMO
A0A140VJS9	B4DEU7	COL23A1	GPR75	LILRB1	OR51H1	Q71SF7	SMPD1
A0A140VJU7	B4DEX6	COL25A1	GPR77	LILRB2	OR51I1	Q71U75	SMPD2
A0A140VJW8	B4DEZ4	COL26A1	GPR78	LILRB3	OR51I2	Q71UK3	SMPD3
A0A140VJX7	B4DF02	COL6A1	GPR82	LILRB4	OR51J1	Q7KYV2	SMPD4
A0A140VK09	B4DF56	COL6A2	GPR83	LILRB5	OR51L1	Q7KZ86	SMPDL3B
A0A140VK10	B4DFB3	COL6A3	GPR84	LIM2	OR51M1	Q7LD69	SMURF1
A0A140VK54	B4DFG7	COLEC10	GPR85	LIMA1	OR51Q1	Q7Z4F3	SMURF2
A0A140VK64	B4DFI2	COLEC11	GPR87	LIMD1	OR51S1	Q7Z4X9	SNAP23
A0A140VK65	B4DFJ9	COLEC12	GPR88	LIMD2	OR51T1	Q7Z528	SNAP25
A0A140VK97	B4DFM1	COLQ	GPRC5A	LIMS1	OR51V1	Q86SF1	SNAP29
A0A140VKC0	B4DFM5	COMMD4	GPRC5B	LIMS2	OR52A1	Q86SF4	SNAP47
A0A140VKD9	B4DFP0	COMT	GPRC5C	LIMS3	OR52A4P	Q86SG0	SNAP91
A0A158T700	B4DFX3	COPB1	GPRC5D	LIN7A	OR52A5	Q86SG9	SNCA
A0A172Q397	B4DFZ1	COPRS	GPRC6A	LIN7C	OR52B2	Q86SH1	SNCAIP
A0A172Q3A0	B4DG19	COQ8B	GPRIN1	LINGO1	OR52B4	Q86SH3	SNF8
A0A1B0QZF0	B4DG25	CORIN	GPRIN2	LIPE	OR52B6	Q86SI3	SNTB2
A0A1I9UVK8	B4DG68	CORO1A	GPRIN3	LIPH	OR52D1	Q86SI5	SNUPN
A0A1U9X791	B4DGD0	CP	GPSM1	LIPI	OR52E1	Q86SI8	SNX15
A0A1U9X7Q3	B4DGL3	CPAMD8	GPSM2	LITAF	OR52E2	Q86SJ4	SNX18
A0A1U9X7Q8	B4DH85	CPD	GPSM3	LLGL1	OR52E4	Q86T00	SNX20
A0A1U9X7Q9	B4DH92	CPE	GRAMD1A	LLGL2	OR52E5	Q86TS8	SNX27
A0A1U9X7R0	B4DHL2	CPEB3	GRAMD1B	LMAN2	OR52E6	Q86TZ7	SNX33
A0A1U9X7R1	B4DHN0	CPLX3	GRAMD1C	LMBR1L	OR52E8	Q86UG7	SNX4
A0A1U9X7U3	B4DHQ5	CPLX4	GRAMD2A	LMBRD2	OR52H1	Q86UG9	SNX5
A0A1U9X7Y3	B4DHU3	CPM	GRAP	LMO7	OR52I1	Q86UH0	SNX9
A0A1U9X7Y6	B4DI21	CPNE1	GRAP2	LNIR	OR52I2	Q86UH9	SOCS3
A0A1U9X7Z5	B4DI30	CPNE2	GRB10	LNPEP	OR52J3	Q86YF2	SOCS6
A0A1U9X7Z8	B4DI53	CPNE3	GRB14	LNX2	OR52K1	Q86YG3	SOCS7
A0A1U9X800	B4DIB1	CPNE4	GRB2	LOC112268384	OR52K2	Q8IW23	SOD1
A0A1U9X801	B4DID1	CPNE5	GRB7	LOC115098	OR52L1	Q8IWP3	SORBS1
A0A1U9X807	B4DIG3	CPNE6	GRIA1	LOC133308	OR52L2	Q8IXB0	SORBS2

A0A1U9X808	B4DII7	CPNE7	GRIA2	LOC392395	OR52L2P	Q8IXD9	SORCS2
A0A1U9X830	B4DIM6	CPNE8	GRIA3	LOC51057	OR52M1	Q8IXE2	SORCS3
A0A1U9X844	B4DIQ8	CPNE9	GRIA4	LOC57228	OR52N1	Q8IXE5	SORL1
A0A1U9X848	B4DJ65	CPO	GRID1	LOR	OR52N2	Q8IY78	SORT1
A0A1U9X849	B4DJB1	CPPED1	GRID2	LORICRIN	OR52N4	Q8IZT0	SOX11
A0A1U9X851	B4DJD6	CPS1	GRID2IP	LPAR1	OR52N5	Q8IZT1	SPA17
A0A1U9X853	B4DJM8	CPTP	GRIK1	LPAR2	OR52P1	Q8N0Z0	SPACA4
A0A1U9X855	B4DJP5	CR1	GRIK2	LPAR3	OR52R1	Q8N164	SPAM1
A0A1U9X857	B4DJS3	CR1L	GRIK4	LPAR4	OR52W1	Q8N1Z7	SPARC
A0A1U9X864	B4DJT9	CR2	GRIN1	LPAR5	OR52Z1P	Q8N2G2	SPART
A0A1U9X869	B4DJZ0	CRACR2A	GRIN2A	LPAR6	OR56A1	Q8N7J6	SPATA13
A0A1U9X874	B4DK83	CRB1	GRIN2B	LPCAT1	OR56A3	Q8N8W2	SPEF1
A0A1U9X877	B4DKD3	CRB2	GRIN2C	LPCAT2	OR56A4	Q8N9J3	SPG11
A0A1U9X880	B4DKF6	CRB3	GRIN3A	LPL	OR56A5	Q8NB64	SPHK1
A0A1U9X881	B4DKF7	CRCP	GRIN3B	LPO	OR56B1	Q8NCJ3	SPINT1
A0A1U9X883	B4DKT9	CRHR1	GRIP1	LPP	OR56B2P	Q8NG71	SPINT2
A0A1U9X884	B4DKW1	CRHR2	GRIP2	LPXN	OR56B4	Q8NG73	SPIRE1
A0A1U9X894	B4DKY3	CRIM1	GRIPAP1	LRATD2	OR5A1	Q8NG79	SPIRE2
A0A1U9X8A9	B4DL06	CRK	GRK2	LRBA	OR5A2	Q8NGA3	SPN
A0A1U9X8D6	B4DL16	CRKL	GRK3	LRCH4	OR5AC1	Q8NGE6	SPNS2
A0A1U9X8K1	B4DL19	CRLF1	GRK4	LRFN1	OR5AC2	Q8NGF2	SPPL2A
A0A1U9X8K6	B4DL31	CRLF2	GRK5	LRFN2	OR5AK2	Q8NGG9	SPPL2B
A0A1U9X8K7	B4DL33	CRLF3	GRK6	LRFN3	OR5AK3P	Q8NGP7	SPPL3
A0A1U9X8L1	B4DL49	CROCC	GRM1	LRFN4	OR5AL1	Q8NGU0	SPRED1
A0A1U9X8M9	B4DLA1	CRTAM	GRM2	LRFN5	OR5AN1	Q8NGV4	SPRED2
A0A1U9X8Y4	B4DLC3	CRTC1	GRM3	LRIG1	OR5AP2	Q8NGX7	SPRED3
A0A1U9X933	B4DLF0	CRYAB	GRM4	LRIG2	OR5AR1	Q8NGY8	SPRN
A0A1U9X938	B4DLH9	CSDE1	GRM5	LRIG3	OR5AS1	Q8NH14	SPRR1A
A0A1U9X943	B4DLJ5	CSF1	GRM6	LRIG3(NM_001136051)-ROS1	OR5AU1	Q8NH24	SPRR1B
A0A1U9X944	B4DLT8	CSF1R	GRM7	LRIG3(NM_153377)-ROS1	OR5B12	Q8NH27	SPRR2A
A0A1U9X946	B4DLY9	CSF2	GRM8	LRP1	OR5B17	Q8NH29	SPRR2B
A0A1U9X948	B4DLZ2	CSF2RA	GRN	LRP10	OR5B2	Q8NH36	SPRR2D
A0A1U9X951	B4DM00	CSF2RB	GRPR	LRP11	OR5B21	Q8NH44	SPRR2E
A0A1U9X953	B4DM20	CSF3R	GSDMA	LRP12	OR5B3	Q8NH47	SPRR2F
A0A1U9X954	B4DM81	CSK	GSDMB	LRP1B	OR5BS1P	Q8NH62	SPRR2G
A0A1U9X958	B4DMA7	CSMD2	GSDMB-1	LRP2	OR5C1	Q8NH68	SPRR3
A0A1U9X964	B4DMC7	CSMD3	GSDMC	LRP3	OR5D13	Q8NH75	SPRY1
A0A1U9X966	B4DMD5	CSNK1D	GSDMD	LRP4	OR5D14	Q8NH86	SPRY2
A0A1U9X967	B4DMI7	CSNK1G1	GSDME	LRP6	OR5D16	Q8NH96	SPRY4
A0A1U9X968	B4DMV9	CSNK1G2	GSDML	LRP8	OR5D18	Q8NHA5	SPTA1
A0A1U9X974	B4DN32	CSNK1G3	GSG1	LRPAP1	OR5D3P	Q8NHA7	SPTAN1
A0A1U9X976	B4DN59	CSNK2A1	GSG1L	LRRC15	OR5F1	Q8NHA9	SPTB
A0A1U9X978	B4DN77	CSNK2B	GSG1L2	LRRC19	OR5G3	Q8NHD6	SPTBN1
A0A1U9X980	B4DNA3	CSPG4	GSK3B	LRRC3	OR5H1	Q8NHX0	SPTBN2
A0A1U9X983	B4DNB9	CSPG5	GSN	LRRC32	OR5H14	Q8TCH7	SPTBN4

A0A1W2PQF6	B4DNE1	CST3	GSR	LRRC4	OR5H15	Q8TD27	SPTBN5
A0A1W2PQM1	B4DNE4	CST6	GUCA1B	LRRC45	OR5H2	Q8TDA6	SRA1
A0A1W2PRS3	B4DNH0	CSTA	GUCY2C	LRRC4B	OR5H6	Q8TDC5	SRC
A0A2R8YCY7	B4DNT6	CT83	GUCY2D	LRRC4C	OR5H8	Q8TDC6	SRGAP2
A0A2R8YDH4	B4DNU9	CTLA4	GUCY2F	LRRC53	OR5I1	Q8WUX3	SRI
A0A2R8YEY8	B4DNX6	CTNNA1	GYPA	LRRC7	OR5J2	Q95353	SRMS
A0A384MDP3	B4DNY6	CTNNAL1	GYPB	LRRC70	OR5K1	Q96FQ5	SRPK1
A0A384MDP7	B4DPA5	CTNND1	GYPC	LRRC8A	OR5K2	Q96G76	SRPX2
A0A384MDX2	B4DPF4	CTNND2	GYPE	LRRC8B	OR5K3	Q96GX3	SSH1
A0A384ME06	B4DPH1	CTNS	GZMA	LRRC8C	OR5K4	Q96KB4	SSNA1
A0A384ME54	B4DPH3	CTSB	GZMB	LRRC8D	OR5L1	Q96KE0	SSTR1
A0A384ME58	B4DPI4	CTSF	H0Y626	LRRC8E	OR5L2	Q96KY9	SSTR2
A0A384MEH9	B4DPK0	CTSG	H2BC11	LRRFIP1	OR5M1	Q96N83	SSTR3
A0A384MEK5	B4DPQ6	CTSK	H3BUA1	LRRK2	OR5M10	Q96PL7	SSTR4
A0A384MR00	B4DPZ5	CTSL	H6VQ59	LRRN4	OR5M11	Q96QL3	SSTR5
A0A384MR29	B4DQ16	CTSZ	H7C1H1	LRRTM1	OR5M3	Q9BXG1	ST14
A0A384MR38	B4DQ18	CTTN	H9NIL7	LRRTM2	OR5M8	Q9BXV3	ST6GALNAC6
A0A384MTL6	B4DQ54	CUBN	hA2aR	LRRTM3	OR5M9	Q9BZK2	STAB1
A0A384N5V6	B4DQ59	CUL1	HARBI1	LRRTM4	OR5P2	Q9BZT2	STAB2
A0A384N6F4	B4DQ91	CUL3	HAS1	LSAMP	OR5P3	Q9H2B1	STAC
A0A384N6H6	B4DQA0	CX3CL1	HAS2	LSP1	OR5R1	Q9H2C6	STAC2
A0A384NPH9	B4DQI9	CX3CR1	HAS3	LSR	OR5T1	Q9H2C7	STAMBP
A0A384NPK6	B4DQM1	CXADR	HAUS7	LST1	OR5T2	Q9H349	STAP2
A0A384NYN5	B4DQW5	CXCL10	HAVCR1	LST3	OR5T3	Q9H6J4	STAT2
A0A384P5C6	B4DQY6	CXCL12	HAVCR2	LTA	OR5V1	Q9HAB4	STAT3
A0A384P5Q5	B4DR44	CXCL16	HAX1	LTB	OR5W2	Q9HD50	STAU1
A0A3B3IT09	B4DR53	CXCL9	HBEGF	LTB4R	OR6A2	Q9N2J8	STAU2
A0A3B3IT50	B4DRB1	CXCR1	HCAR1	LTB4R2	OR6B1	Q9N2K0	STBD1
A0A3B3ITA1	B4DRH7	CXCR2	HCAR2	LTBR	OR6B2	Q9NR74	STC1
A0A3G8GE10	B4DRL4	CXCR3	HCAR3	LTF	OR6B3	Q9NRB8	STEAP1
A0A3G8GE18	B4DRX4	CXCR4	HCFC2	LTK	OR6C1	Q9NRU0	STEAP1B
A0A4D5RA86	B4DS29	CXCR5	hCG_1641511	LY49L	OR6C2	Q9NUS1	STEAP2
A0A4D5RA95	B4DS65	CXCR6	hCG_1646325	LY6D	OR6C3	Q9NX12	STEAP3
A0A4D5RAA2	B4DS89	CYB5R1	hCG_1648279	LY6E	OR6C4	Q9UEP2	STEAP4
A0A4D5RAA5	B4DSA0	CYBA	hCG_1774561	LY6G5B	OR6C6	Q9UHH0	STIM1
A0A4D5RAB7	B4DSB3	CYBRD1	hCG_1810791	LY6G5C	OR6C65	Q9UHH1	STIM2
A0A4D5RAD4	B4DSB6	CYLD	hCG_1821181	LY6G6	OR6C68	Q9UHH2	STING1
A0A4D5RAG0	B4DSD2	CYP2C18	hCG_1983280	LY6G6C	OR6C70	Q9UHV4	STK10
A0A4D5RAH1	B4DSE0	CYP2C19	hCG_1991735	LY6G6D	OR6C74	Q9UII7	STK16

A0A4D5RAH8	B4DSH4	CYP2C8	hCG_1995540	LY6G6E	OR6C75	Q9UJZ6	STK17A
A0A4D5RAI5	B4DSI4	CYP2C9	hCG_2007960	LY6G6F	OR6C76	Q9UJZ7	STK17B
A0A4D5RAJ5	B4DSJ6	CYP2W1	hCG_2009644	LY6G6F-LY6G6D	OR6F1	Q9UJZ8	STK26
A0A4D5RAU4	B4DSM3	CYP4A11	hCG_2024613	LY6H	OR6J1	Q9UK19	STK32A
A0A4D5RBL6	B4DSR9	CYP4F12	hCG_2033729	LY6K	OR6K2	Q9UK20	STK39
A0A4D5RBR8	B4DSW9	CYP4F2	hCG_2039413	LY6L	OR6K3	Q9UK68	STOM
A0A4D6J698	B4DT46	CYS1	hCG_2043376	LY6S	OR6K6	Q9UK70	STOML1
A0A583ZAW1	B4DT49	CYSLTR1	hCG_2044349	LY75	OR6M1	Q9UK74	STOML2
A0A583ZAW5	B4DT82	CYSLTR2	hCG_2044638	LY9	OR6N1	Q9UNB7	STOML3
A0A583ZB54	B4DTD6	CYSRT1	hCG_2044837	LY96	OR6N2	Q9Y5B2	STP
A0A583ZB56	B4DTF4	CYSTM1	hCG_20471	LYN	OR6P1	QRFPR	STRA6
A0A583ZBQ6	B4DTH8	CYTH1	hCG_39634	LYNX1	OR6Q1	QRICH1	STRN
A0A583ZBV8	B4DTI4	CYTH2	hCG_39899	LYPD1	OR6S1	QSOX2	STRN3
A0A583ZBV9	B4DTP0	CYTH3	hCG_39951	LYPD2	OR6T1	RAB10	STS
A0A583ZBW6	B4DTP4	CYTH4	hCG_40688	LYPD3	OR6V1	RAB11A	STX11
A0A583ZC13	B4DTR4	D2CGC9	hCG_40978	LYPD4	OR6X1	RAB11FIP2	STX17
A0A583ZC25	B4DTS6	D3KDA6	HCK	LYPD5	OR6Y1	RAB11FIP3	STX19
A0A5F9Z9Y6	B4DTV1	D4S234E	HCLS1	LYPD6	OR7A10	RAB11FIP4	STX1A
A0A5F9ZH88	B4DTX3	DAAM1	HCN1	LYPD6B	OR7A17	RAB12	STX1B
A0A5J6MBG1	B4DTZ6	DAB2	HCN3	LYPD8	OR7A2P	RAB13	STX2
A0A7T5V792	B4DU00	DAB2IP	HCP1	LYPLA1	OR7A5	RAB14	STX3
A0A7T6AMI0	B4DU18	DAGLA	HCRTR1	LYSMD3	OR7C1	RAB15	STX3A
A0MSJ5	B4DU31	DAGLB	HCRTR2	LYVE1	OR7C2	RAB17	STX4
A1BG	B4DUA8	DAPK1	HCST	LYZL6	OR7D2	RAB18	STX6
A4UU13	B4DUD6	DAPP1	HDAC11	LZTS1	OR7D4	RAB19	STX7
A6N9G8	B4DUD9	DAT1	HDAC3	LZTS2	OR7E24	RAB21	STX8
A7U7M4	B4DUH5	DBN1	HDAC6	M6PR	OR7G1	RAB22A	STXBP1
A8I0M1	B4DUK6	DBNL	HDLBP	MACF1	OR7G2	RAB23	STXBP2
A8K099	B4DUL1	DCBLD2	HEG1	MADCAM1	OR7G3	RAB24	STXBP3
A8K0D8	B4DUZ8	DCC	HEL-215	MADD	OR8A1	RAB25	STXBP5
A8K0E7	B4DV25	DCHS2	HEL-68	MAEA	OR8B12	RAB26	STXBP5L
A8K0F9	B4DV52	DCLK1	HEL-75	MAG	OR8B2	RAB27A	STYK1
A8K0G9	B4DV98	DCST1	HEL-S-10	MAGEA1	OR8B3	RAB27B	SUCLG2
A8K0H7	B4DVA8	DCSTAMP	HEL-S-101	MAGED1	OR8B4	RAB28	SUCNR1
A8K0J8	B4DVC4	DCT	HEL-S-102	MAGEE1	OR8B8	RAB29	SULF1
A8K0K0	B4DVC8	DCTN3	HEL-S-105	MAGI1	OR8D1	RAB2B	SULF2
A8K0L1	B4DVI9	DCUN1D3	HEL-S-123m	MAGI2	OR8D2	RAB31	SUMO1
A8K0L9	B4DVK7	DCXR	HEL-S-125m	MAGI3	OR8D4	RAB33A	SURF2
A8K0M3	B4DVN2	DDN	HEL-S-132P	MAGT1	OR8G1	RAB35	SURF4
A8K0N9	B4DVP5	DDOST	HEL-S-162eP	MAL	OR8G2	RAB37	SUSD2
A8K0U1	B4DVY0	DDR1	HEL-S-165mP	MAL2	OR8G2P	RAB38	SUSD3
A8K0W1	B4DW05	DDR2	HEL-S-17	MALL	OR8G3P	RAB39A	SUV39H1
A8K0Y1	B4DW32	DDX3X	HEL-S-171mP	MANBA	OR8G5	RAB39B	SV2A
A8K139	B4DW87	DDX50	HEL-S-172mP	MANSC4	OR8H1	RAB3A	SV2B

A8K177	B4DWH7	DEF6	HEL-S-18	MAP1B	OR8H2	RAB3B	SV2C
A8K191	B4DWM4	DEGS1	HEL-S-303	MAP2K1	OR8H3	RAB3C	SVIL
A8K1C7	B4DWQ9	DEHAL1	HEL-S-45	MAP2K2	OR8I2	RAB3D	SVIP
A8K1F6	B4DWR2	DENND1A	HEL-S-47e	MAP3K12	OR8J1	RAB3GAP2	SVOP
A8K1R9	B4DWV3	DENND2B	HEL-S-52	MAP3K5	OR8J3	RAB40A	SWAP70
A8K1X9	B4DWX0	DENND4C	HEL-S-7	MAP3K7	OR8K1	RAB40AL	SYAP1
A8K215	B4DX51	DES	HEL-S-70p	MAP4	OR8K3	RAB40B	SYCE1
A8K2C5	B4DXM7	DESC1	HEL-S-72p	MAP4K2	OR8K5	RAB40C	SYDE1
A8K2D2	B4DXN5	DFFA	HEL-S-79	MAP4K5	OR8S1	RAB41	SYDE2
A8K2E3	B4DXU9	DFNA5	HEL-S-7a	MAP7	OR8U1	RAB42	SYMPK
A8K2H4	B4DYD6	DGAT1	HEL-S-83	MAPK1	OR8U3	RAB44	SYN2
A8K2M4	B4DYE3	DGKA	HEL-S-89n	MAPK10	OR8U8	RAB4A	SYNC
A8K2M5	B4DYL5	DGKB	HEL-S-99n	MAPK3	OR8U9	RAB4B	SYNDIG1
A8K2P8	B4DZ13	DGKD	HEL113	MAPK8IP1	OR9A1P	RAB5A	SYNE1
A8K2T6	B4DZ71	DGKE	HEL114	MAPK9	OR9A2	RAB5B	SYNE2
A8K2T7	B4DZA3	DGKG	HEL163	MAPKAP1	OR9A4	RAB5C	SYNGAP1
A8K2V7	B4DZA5	DGKH	HEL70	MAPRE1	OR9G1	RAB6A	SYNGR1
A8K2W3	B4DZH9	DGKI	HEPH	MAPT	OR9G4	RAB7A	SYNJ2
A8K2Y6	B4DZK4	DGKK	HEPHL1	MARCHF1	OR9G9	RAB8A	SYNM
A8K2Z9	B4DZK8	DGKQ	HERC2	MARCHF2	OR9I1	RAB8B	SYNPO
A8K309	B4DZS6	DGKZ	HERVK_113	MARCHF7	OR9K2	RAB9A	SYP
A8K385	B4DZS7	DHH	HFE	MARCKS	OR9Q1	RAB9B	SYPL1
A8K396	B4E003	DHRS3	HFE2	MARCKSL1	OR9Q2	RABAC1	SYT1
A8K3D0	B4E028	DIAPH1	HGSNAT	MARCO	ORAI1	RABGGTA	SYT10
A8K3L9	B4E0B0	DIO1	HHIP	MARK1	ORAI3	RABGGTB	SYT11
A8K3T2	B4E0R8	DIO2	HHLA2	MARK2	ORMDL3	RAC1	SYT12
A8K3U5	B4E0R9	DIO3	HIC2	MARK3	OSBP	RAC2	SYT13
A8K3Z4	B4E0S0	DIP2A	HIF3A	MARVELD1	OSBP2	RAC3	SYT15
A8K410	B4E116	DIRAS1	HINT1	MARVELD2	OSBPL1A	RACGAP1	SYT17
A8K424	B4E140	DIRAS2	HIP1	MAS1	OSBPL2	RACK1	SYT2
A8K496	B4E1F8	DIRAS3	HIP1R	MAS1L	OSBPL3	RAET-1E	SYT3
A8K4E4	B4E1H7	DIS3L	HIPK3	MAST1	OSBPL6	RAET1E	SYT4
A8K4H5	B4E1J1	DISP2	HJV	MAST2	OSBPL7	RAET1G	SYT5
A8K4L6	B4E1T0	dJ402G11.9	HLA A	MASTL	OSCAR	RAET1L	SYT6
A8K4S1	B4E1U9	DKFZp434B0923	HLA B	MBL2	OSCP1	RAF1	SYT7
A8K4T1	B4E1V1	DKFZp451B1115	HLA C	MBLAC2	OTOA	RALA	SYT8
A8K556	B4E1Y7	DKFZp547I09 10	HLA-A	MBP	OTOF	RALB	SYT9
A8K582	B4E1Z0	DKFZp566D234	HLA-A*	MC1R	OTOG	RALBP1	SYTL1
A8K594	B4E236	DKFZp586I1919	HLA-A*01	MC2R	OTOP1	RALGAPA2	SYTL2
A8K5C5	B4E292	DKFZp586L0518	HLA-A*02	MC3R	OTOP2	RALGDS	SYTL3
A8K5P7	B4E2D8	DKFZp666G172	HLA-A*0204	MC4R	OTOP3	RALGPS1	SYTL4
A8K5Q0	B4E2G8	DKFZp666L2010	HLA-A*0226	MC5R	OXER1	RALGPS2	SYTL5
A8K5Q6	B4E2L9	DKFZp666O0110	HLA-A*33	MCAM	OXGR1	RAMP3	T2DPW6
A8K5U1	B4E2M1	DKFZp686A1195	HLA-A*68	MCC	OXTR	RANBP9	TAAR1
A8K5U6	B4E2S3	DKFZp686D0714	HLA-A24AK	MCEMP1	P0	RANGRF	TAAR2

A8K5W4	B4E2T0	DKFZp686F17268	HLA-B	MCF2L	P0DOX2	RAP1A	TAAR3P
A8K651	B4E2V5	DKFZp686H1993	HLA-B*	MCHR1	P0DOX3	RAP1B	TAAR5
A8K653	B4E2W5	DKFZp686I11137	HLA-B*1513	MCHR2	P0DOX4	RAP1BL	TAAR6
A8K6I8	B4E2W9	DKFZp686M05161	HLA-B*3512	MCOLN1	P0DOX5	RAP1GAP2	TAAR8
A8K6Q3	B4E2X4	DKFZp686M088	HLA-B*3908	MCOLN3	P0DOX6	RAP2A	TAAR9
A8K6Q8	B4E304	DKFZp686N10220	HLA-B*44new	MDFIC	P0DOX7	RAP2B	TAB2
A8K6T3	B4E310	DKFZp686O00215	HLA-B35	MDGA1	P0DOX8	RAP2C	TAB3
A8K6W1	B4E316	DKFZp686O088	HLA-B44	MDGA2	P2RX1	RAPGEF1	TACC2
A8K7F0	B4E336	DKFZp686P18250	HLA-B57	MDM2	P2RX2	RAPGEF2	TACI
A8K7J9	B4E385	DKFZp761E0417	HLA-Bw62.1	ME1	P2RX3	RAPGEF3	TACR1
A8K7N8	B4E398	DKFZp761I0921	HLA-Bw62.3	ME2	P2RX4	RAPGEF4	TACR2
A8K7Y1	B4E3I1	DKFZp779L0468	HLA-Bw62.4	ME3	P2RX5	RAPGEF5	TACR3
A8K7Z2	B4E3I5	DKFZp779P0659	HLA-Bw62.5	ME4	P2RX6	RAPGEF6	TACSTD2
A8K841	B4E3N0	DKFZp781G125	HLA-C	MEGF10	P2RX7	RAPGEFL1	TAMALIN
A8K861	B4E3Q1	DKFZp781L0846	HLA-C*03	MEGF11	P2RY1	RAPH1	TAOK3
A8K8E4	B4E3Q9	DLC1	HLA-C*12NEW	MELK	P2RY10	RAPSN	TAP1
A8K8M6	B4GALT1	DLG1	HLA-Cw	MELTF	P2RY11	RARA	TAP2-G
A8K8U3	B7Z1D1	DLG2	HLA-Cw-1503	MEN1	P2RY12	RASA1	TAPBPL
A8K8V7	B7Z1D7	DLG3	HLA-Cw*070x	MEP1A	P2RY13	RASA3	TARM1
A8K8W7	B7Z1E3	DLG4	HLA-Cw*12042	MEP1B	P2RY14	RASA4	TAS1R1
A8K920	B7Z1H9	DLG5	HLA-E	MERTK	P2RY2	RASA4B	TAS1R2
A8K9B9	B7Z1L9	DLGAP1	HLA-F	MESD	P2RY4	RASAL1	TAS1R3
A8K9G0	B7Z1Q9	DLGAP2	HLA-G	MET	P2RY6	RASD1	TAS2R1
A8K9G2	B7Z1R6	DLGAP3	HLA-H	METAP2	P2RY8	RASD2	TAS2R10
A8K9M7	B7Z1W1	DLGAP4	HLAC	MFAP3	P2X4	RASGEF1A	TAS2R13
A8K9S2	B7Z204	DLL1	HLTF	MFAP3L	P3.58	RASGEF1B	TAS2R14
A8KA29	B7Z236	DLL3	HM13	MFGE8	P4HB	RASGEF1C	TAS2R16
A8KA85	B7Z288	DLL4	HMCN1	MFRP	PACC1	RASGRF1	TAS2R19
A8KAB0	B7Z2A4	DMD	HMCN2	MFSD10	PACSIN1	RASGRF2	TAS2R20
A8KAF0	B7Z2C7	DMPK	HMGB1	MFSD2A	PACSIN2	RASGRP1	TAS2R3
A8KAH7	B7Z2D1	DMTN	HMMR	MFSD2B	PACSIN3	RASGRP2	TAS2R30
A8KAM8	B7Z2E4	DNAAF1	HMOX2	MFSD4B	PAFAH1B2	RASGRP3	TAS2R31
A8KAN9	B7Z2G0	DNAAF4	HNRNPF	MFSD5	PAG1	RASGRP4	TAS2R33
A8KAP5	B7Z2G5	DNAI2	HOMER1	MFSD6	PAK1	RASL10A	TAS2R36
A9CB80	B7Z2I3	DNAJA3	HOMER2	MGAM	PAK2	RASL10B	TAS2R38
A9X165	B7Z2I6	DNAJB4	HOMER3	MGC13034	PAK3	RASL12	TAS2R39
A9XTE5	B7Z2M1	DNAJC1	Homo sapiens (human) hsa-miR-124-3p	MGC39518	PAK5	RASSF2	TAS2R4
AAK1	B7Z2R3	DNAJC13	Hosa(Adygei)-T2R10	MGLL	PALLD	RASSF3	TAS2R40
AAMP	B7Z2S7	DNAJC5	Hosa(Adygei)-T2R3	MGRA	PALM	RBSN	TAS2R41
ABCA1	B7Z2V3	DNAJC6	Hosa(Adygei)-T2R38	MGRC	PALM2	RCC2	TAS2R42

ABCA12	B7Z313	DNAJC9	Hosa(Adygei) -T2R4	MGRE	PALM3	RCCD1	TAS2R43
ABCA13	B7Z336	DNAL4	Hosa(Adygei) -T2R46	MGRF	PALS1	RCE1	TAS2R45
ABCA2	B7Z375	DNER	Hosa(Adygei) -T2R49	MGRG1	PALS2	RDH	TAS2R46
ABCA3	B7Z379	DNM1	Hosa(Adygei) -T2R50	MGRG2	PANX1	RDH12	TAS2R49
ABCA4	B7Z3F8	DNM1L	Hosa(Adygei) -T2R55	MGRH	PANX2	RDH8	TAS2R5
ABCA5	B7Z3G2	DNM2	Hosa(Adygei) -T2R7	MGRN1	PANX3	RDX	TAS2R50
ABCA6	B7Z3H7	DNM3	Hosa(Biaka)-T2R1	MGST1	PAPLN	RECK	TAS2R60
ABCA7	B7Z3H9	DOC2B	Hosa(Biaka)-T2R10	MGST2	PAPPA2	REEP2	TAS2R7
ABCA8	B7Z3L5	DOCK1	Hosa(Biaka)-T2R13	MHC	PAQR5	RELL1	TAS2R8
ABCB1	B7Z3M9	DOCK2	Hosa(Biaka)-T2R16	MHC class I HLA-A	PAQR6	RELL2	TAS2R9
ABCB11	B7Z3P8	DOCK3	Hosa(Biaka)-T2R2	MIB1	PAQR7	RELN	TAX1BP3
ABCB4	B7Z3V1	DOCK4	Hosa(Biaka)-T2R3	MIB2	PAQR8	RELT	TBC1D10A
ABCB5	B7Z3V7	DOCK5	Hosa(Biaka)-T2R38	MICA	PAQR9	REM1	TBC1D10B
ABCB6	B7Z3W4	DOCK8	Hosa(Biaka)-T2R4	MICAL1	PARD3	REM2	TBC1D10C
ABCC1	B7Z3W8	DOK3	Hosa(Biaka)-T2R40	MICAL3	PARD3B	REN	TBC1D2
ABCC10	B7Z435	DOK7	Hosa(Biaka)-T2R43	MICALL2	PARD6A	REPS1	TBC1D24
ABCC11	B7Z437	DOM3Z	Hosa(Biaka)-T2R46	MICB	PARD6B	REPS2	TBC1D3
ABCC2	B7Z470	dopamine D4 receptor	Hosa(Biaka)-T2R47	MIEN1	PARD6G	RER1	TBC1D30
ABCC3	B7Z473	DPEP1	Hosa(Biaka)-T2R48	MIF	PARK7	RERG	TBC1D3B
ABCC5	B7Z487	DPEP3	Hosa(Biaka)-T2R49	MIGA1	PARM1	RESDA1	TBC1D3C
ABCC6	B7Z4B6	DPP4	Hosa(Biaka)-T2R5	MIGA2	PARP14	RET	TBC1D3D
ABCC8	B7Z4C0	DPYSL2	Hosa(Biaka)-T2R50	MILR1	PARVA	RET/PTC2	TBC1D3E
ABCC9	B7Z4E9	DRAM2	Hosa(Biaka)-T2R55	MINAR1	PARVB	RFFL	TBC1D3F
ABCG1	B7Z4M3	DRD2	Hosa(Biaka)-T2R7	MIP	PARVG	RFTN1	TBC1D3G
ABCG2	B7Z4N2	DRD3	Hosa(Biaka)-T2R8	MISP	PATJ	RFTN2	TBC1D3H
ABCG4	B7Z4Q2	DRD4	Hosa(Japanese)-T2R1	MLANA	PAWR	RGL1	TBC1D3I
ABCG8	B7Z4Q5	DRD5	Hosa(Japanese)-T2R10	MLC1	PCDH-psi1	RGL3	TBC1D3K
abeta-42-oligomer_human	B7Z4R1	DRP2	Hosa(Japanese)-T2R13	MLEC	PCDH1	RGL4	TBC1D3L
ABHD12	B7Z4S4	DSC1	Hosa(Japanese)-T2R14	MLIP	PCDH10	RGMA	TBC1D5
ABHD17A	B7Z508	DSC2	Hosa(Japanese)-T2R16	MLKL	PCDH11X	RGMB	TBCD
ABHD17B	B7Z533	DSC3	Hosa(Japanese)-T2R38	MLLT4	PCDH11Y	RGP1	TBXA2R
ABHD17C	B7Z5A7	DSCAM	Hosa(Japanese)-T2R39	MLNR	PCDH12	RGR	TCAF1
ABHD2	B7Z5G7	DSCAML1	Hosa(Japanese)-T2R40	MMD	PCDH15	RGS1	TCAF2
ABHD3	B7Z5G8	DSG1	Hosa(Japanese)-T2R45	MME	PCDH17	RGS10	TCAF2C
ABRA	B7Z5H8	DSG2	Hosa(Japanese)-T2R48	MMEL1	PCDH18	RGS11	TCBA1

ABTB1	B7Z5I3	DSG3	Hosa(Japanese)-T2R49	MMGT1	PCDH19	RGS12	TCHH
ACCN3	B7Z5J8	DSG4	Hosa(Japanese)-T2R5	MMP14	PCDH20	RGS13	TCHP
ACE	B7Z5K3	DSP	Hosa(Japanese)-T2R50	MMP15	PCDH7	RGS14	TCIM
ACE2	B7Z5S3	DST	Hosa(Japanese)-T2R55	MMP16	PCDH8	RGS16	TCIRG1
ACHE	B7Z5V2	DSTYK	Hosa(Japanese)-T2R56	MMP17	PCDH9	RGS17	TCN2
ACIN1	B7Z5V3	DTNA	Hosa(Japanese)-T2R8	MMP2	PCDHA1	RGS18	TCTN2
ACKR1	B7Z5W1	DTNB	HPCA	MMP24	PCDHA10	RGS19	TCTN3
ACKR2	B7Z5Y4	DTNBP1	HPGD	MMP25	PCDHA11	RGS2	TDG
ACKR3	B7Z610	DUOX1	HPN	MOG	PCDHA12	RGS20	TDGF1
ACKR4	B7Z670	DUOXA1	HPSE2	MORF4L2	PCDHA13	RGS21	TDGF1P3
ACP1	B7Z691	DUOXA2	HRG	MOSMO	PCDHA2	RGS3	TDP1
ACP3	B7Z6C3	DUSP15	HRH1	MOSPD2	PCDHA3	RGS4	TEC
ACP4	B7Z6F0	DUSP22	HRH2	MPDZ	PCDHA4	RGS5	TECTA
ACSBG1	B7Z6K5	DUSP3	HRH3	MPHOSPH8	PCDHA5	RGS6	TECTB
ACSL3	B7Z6L3	DVL1	HRH4	MPIG6B	PCDHA6	RGS7	TEK
ACSL4	B7Z6W6	DVL2	HRNR	MPL	PCDHA7	RGS7BP	TENM1
ACSL5	B7Z6W8	DXO	HS3ST3B1	MPP1	PCDHA8	RGS8	TENM2
ACSL6	B7Z761	DYNAP	HS6ST1	MPP2	PCDHA9	RGS9	TENM3
ACTB	B7Z7C7	DYNC1LI1	HSD17B10	MPP3	PCDHAC1	RH50	TENM4
ACTG1	B7Z7U7	DYNC2H1	HSD17B8	MPP4	PCDHAC2	RHAG	TENT4B
ACTL6A	B7Z7Y6	DYNLL1	HSP90AA1	MPP5	PCDHB1	RHBDF2	TERT
ACTN1	B7Z831	DYNLL2	HSP90AA2P	MPP6	PCDHB10	RHBDL1	TES
ACTN2	B7Z853	DYSF	HSP90AA4P	MPP7	PCDHB11	RHBDL2	TESC
ACTN3	B7Z856	dystrophin	HSP90AB1	MPZ	PCDHB12	RHBG	TEX101
ACTN4	B7Z8J8	DYTN	HSP90AB2P	MPZL1	PCDHB13	RHCE	TF
ACVR1B	B7Z8P6	DYX1C1	HSP90AB3P	MPZL2	PCDHB14	RHCG	TFCP2
ACY3	B7Z8R6	E5KR06	HSP90AB4P	MPZL3	PCDHB15	RHD	TFPI
ADA	B7Z8R8	E9PAM4	HSP90B1	MR1	PCDHB16	RHD/RHCE	TFR2
ADAM10	B7Z8T5	E9PNW4	HSPA13	MRAP	PCDHB18P	RhDCw	TFRC
ADAM11	B7Z8W5	EBI2	HSPA5	MRAP2	PCDHB2	RhDTI	TGFA
ADAM12	B7Z946	EBI3	HSPA8	MRAS	PCDHB3	RHEB	TGFB1
ADAM15	B7Z952	ECE1	HSPB1	MRC1	PCDHB4	RHEBL1	TGFB3
ADAM17	B7Z954	ECE2	HSPD1	MREG	PCDHB5	RHEX	TGFBI
ADAM18	B7Z966	ECEL1	HTR1A	MRGPRD	PCDHB6	RhIVb(J)	TGFBR2
ADAM19	B7Z985	ECRG4	HTR1B	MRGPRE	PCDHB7	RHO	TGFBR3
ADAM2	B7Z999	ECSCR	HTR1D	MRGPRF	PCDHB8	RHOA	TGFBR3L
ADAM20	B7Z9A5	ECT2	HTR1E	MRGPRG	PCDHB9	RHOB	TGM1
ADAM21	B7Z9B1	EDA	HTR1F	MRGPRX1	PCDHGA1	RHOBTB1	TGM3
ADAM22	B7Z9B9	EDA2R	HTR2A	MRGPRX2	PCDHGA10	RHOBTB2	TGM5
ADAM23	B7Z9G8	EDAR	HTR2B	MRGPRX3	PCDHGA11	RHOC	TGOLN2
ADAM28	B7Z9H2	EDG1	HTR2C	MRGPRX4	PCDHGA12	RHOD	TH
ADAM29	B7Z9M4	EDG2	HTR3A	MRP	PCDHGA2	RHOF	THBD
ADAM30	B7Z9Q3	EDG4	HTR3B	MRP3	PCDHGA3	RHOG	THBS1
ADAM32	B7Z9T4	EDG5	HTR3C	MRPL42	PCDHGA4	RHOH	THEM4
ADAM7	B7Z9U4	EDNRA	HTR3D	MRPL44	PCDHGA5	RHOJ	thrombin receptor
ADAM8	B7Z9V4	EDNRB	HTR3E	MRPL58	PCDHGA6	RHOQ	THSD7A
ADAM9	B7Z9V5	EEF1A1	HTR4	MS4A1	PCDHGA7	RHOU	THSD7B

ADAMTSL1	B7Z9Z0	EEF1AKMT4-ECE2	HTR4B	MS4A10	PCDHGA8	RHOV	THY1
ADAP1	B7ZAD2	EEF2	HTR5A	MS4A12	PCDHGA9	RHPN2	TIAM1
ADAP2	B7ZAD4	EEPD1	HTR6	MS4A13	PCDHGB1	RIC8A	TICAM2
ADCY1	B7ZAE6	EFNA1	HTR7	MS4A14	PCDHGB2	RIC8B	Tie-2
ADCY10	B7ZAE8	EFNA2	HTRA1	MS4A15	PCDHGB3	RIF1	TIE1
ADCY2	B7ZAP7	EFNA3	Human Notch 3	MS4A18	PCDHGB4	RIGI	TIGIT
ADCY3	B7ZAP8	EFNA4	HUT11	MS4A3	PCDHGB5	RILPL1	TIMD4
ADCY4	B7ZAZ3	EFNA4-EFNA3	HVCN1	MS4A4A	PCDHGB6	RIMBP2	TIRAP
ADCY5	B7ZB79	EFNA5	hxCT	MS4A5	PCDHGB7	RIMS1	TJAP1
ADCY6	BACE1	EFNB1	HYAL2	MS4A6A	PCDHGC3	RIMS2	TJP1
ADCY7	BACE2	EFNB2	HYAL3	MS4A6E	PCDHGC4	RIMS3	TJP2
ADCY8	BAG4	EFNB3	HYCC1	MS4A7	PCDHGC5	RIMS4	TJP3
ADCY9	BAIAP2	EFR3A	HYCC2	MS4A8	PCDHJ	RIN1	TLCD1
ADCYAP1R1	BAIAP2L1	EFR3B	HYLS1	MSLN	PCSK6	RIPK1	TLCD2
ADD1	BAIAP2L2	EFS	I3L3M4	MSMO1	PCSK9	RIPK2	TLCD3A
ADD2	BAIAP3	EGF	IA4	MSN	PCTK1	RIPOR2	TLN1
ADD3	BAMBI	EGFR	ICAM1	MSR1	PDAP1	RIT1	TLN2
ADFP	BARGIN	EHBP1	ICAM2	MSRA	PDCD1	RIT2	TLR10
ADGRA1	BASP1	EHD1	ICAM3	MST1R	PDCD10	RNASEK	TLR2
ADGRA2	BBIP1	EHD2	ICAM4	MTCL1	PDCD1LG2	RND1	TLR3
ADGRA3	BBS1	EHD3	ICAM5	MTDH	PDCD6IP	RND2	TLR4
ADGRB1	BBS2	EHD4	ICOS	MTFR1	PDE2A	RND3	TLR5
ADGRB2	BBS4	EIF2B1	ICOSLG	MTM1	PDE4A	RNF10	TLR7
ADGRB3	BBS5	EIF5	IDE	MTMR1	PDE4B	RNF114	TLR8
ADGRD1	BBS7	EL52	IFI6	MTMR2	PDE4D	RNF144A	TLR9
ADGRE1	BBS9	ELAPOR1	IFIT5	MTMR6	PDE6A	RNF146	TM2D1
ADGRE2	BCAM	ELAPOR2	IFITM1	MTMR9	PDE6B	RNF167	TM2D3
ADGRE3	BCAP31	ELMO1	IFITM10	MTNR1A	PDE6C	RNF34	TM4SF1
ADGRE4P	BCAR1	EMB	IFITM2	MTNR1B	PDE6G	RNF43	TM4SF13
ADGRE5	BCHE	EMCN	IFITM3	MTSS2	PDE6H	RNF5	TM4SF20
ADGRF1	BCL10	EMP1	IFITM5	MTTP	PDE9A	RNH1	TM4SF5
ADGRF5	BCL3	EMP2	IFNAR1	MTUS1	PDGFB	RNPEP	TM7SF2
ADGRG1	BCORL1	EMP3	IFNAR2	MUC1	PDGFC	ROBO1	TM7SF3
ADGRG2	BCR	EMR2	IFNGR1	MUC12	PDGFRA	ROBO2	TM9SF2
ADGRG3	BDKRB1	EMR3	IFNGR2	MUC13	PDGFRB	ROBO3	TMBIM1
ADGRG5	BDKRB2	ENAH	IFNLR1	MUC15	PDIA6	ROBO4	TMBIM6
ADGRG6	BEST1	ENDOU	IGDCC3	MUC16	PDLIM4	ROCK1	TMC1
ADGRL1	BEST2	ENG	IGDCC4	MUC17	PDPK1	ROCK2	TMC2
ADGRL2	BEST3	ENHO	IGF1R	MUC19	PDPN	ROM1	TMC3
ADGRL3	BEST4	ENO1	IGF2R	MUC2	PDS5A	ROR1	TMC4
ADGRL4	BFAR	ENO2	IGFBP2	MUC20	PDXP	ROR2	TMC5
ADH1A	BFSP1	ENO3	IGFLR1	MUC21	PDYN	ROS1	TMC6
ADH1B	BFSP2	ENOX1	IgG VH	MUC3A	PDZD11	RP11-301I17.1	TMC7
ADH1C	BGN	ENOX2	IGHA1	MUC3B	PDZD2	RP2	TMC8
ADH7	BICD2	ENPEP	IGHA2	MUC4	PDZD7	RPE65	TMED1
ADI1	BIN1	ENPP1	IGHD	MUC5AC	PDZK1	RPGRIP1L	TMEFF1
ADIPOR1	BIN2	ENPP2	IGHD1-1	MUC5B	PDZK1P1	RPH3A	TMEM100
ADIPOR2	BLK	ENPP3	IGHE	MUC6	Pe1Fe3	RPL36A	TMEM102

ADK	BLNK	ENPP4	IGHG1	MUC7	PEAR1	RPL36A-HNRNPH2	TMEM106A
ADORA1	BLR1	ENPP5	IGHG2	MUCL1	PECAM1	RPL36AL	TMEM11
ADORA2A	BLTP1	ENPP6	IGHG3	MUCL3	PENK	RPS27A	TMEM114
ADORA2B	BLTP2	ENPP7	IGHG4	MUPCDH	PERP	RPS3	TMEM117
ADORA3	BLVRB	ENTHD1	IGHJ1	MUSK	PEX19	RPSA	TMEM119
ADRA1A	BMF	ENTPD1	IGHM	MVB12B	PFKM	RPSA2	TMEM120A
ADRA1B	BMP2	ENTPD2	IGHV	MXRA8	PGAP6	RPTN	TMEM123
ADRA1D	BMPR1B	ENTPD3	IGHV1-18	MYADM	PGM5	RRAD	TMEM126A
ADRA2A	BMPR2	ENTPD6	IGHV1-2	MYCBP2	PGR	RRAS	TMEM127
ADRA2B	BMX	ENTPD8	IGHV1-24	MYCBPAP	PGRMC1	RRAS2	TMEM131L
ADRA2C	BOC	ENTREP1	IGHV1-3	MYD88	PHACTR2	RRH	TMEM132A
ADRB1	BORCS5	EP3-I	IGHV1-38-4	MYH10	PHB	RRP12	TMEM147
ADRB2	BRAF	EP3f	IGHV1-45	MYH9	PHB1	RRP8	TMEM150A
ADRB3	BRCA1	EPB41	IGHV1-46	MYLIP	PHB2	RS1	TMEM150B
ADRM1	BRI3	EPB41L1	IGHV1-58	MYLK	PHEX	RSC1A1	TMEM150C
ADTRP	BRPF1	EPB41L2	IGHV1-69	MYMK	PHF7	RTBDN	TMEM161B
AFAP1L2	BRS3	EPB41L3	IGHV1-69-2	MYMX	PHKA1	RTKN2	TMEM163
AFDN	BSG	EPB41L4B	IGHV1-69D	MYO10	PHKA2	RTL8C	TMEM16A
AGAP2	BSND	EPB41L5	IGHV1-8	MYO16	PHKB	RTN2	TMEM16K
AGER	BST1	EPB42	IGHV2-26	MYO1A	PHLDA3	RTN3	TMEM17
AGPAT2	BST2	EPCAM	IGHV2-5	MYO1B	PHLDB1	RTN4	TMEM170B
AGPAT3	BTBD17	EPGN	IGHV2-70	MYO1C	PHLDB2	RTN4R	TMEM174
AGRN	BTC	EPHA1	IGHV2-70D	MYO1D	PHLPP1	RTN4RL1	TMEM179B
AGTR1	BTK	EPHA10	IGHV3-11	MYO1E	PHLPP2	RTN4RL2	TMEM182
AGTR2	BTLA	EPHA2	IGHV3-13	MYO1F	PHPT1	RTP1	TMEM184A
AGTRAP	BTN1A1	EPHA3	IGHV3-15	MYO1G	PI3	RTP2	TMEM198
AHCYL1	BTN2A1	EPHA4	IGHV3-16	MYO1H	PI4K2A	RXFP1	TMEM2
AHNAK	BTN2A2	EPHA5	IGHV3-20	MYO6	PI4K2B	RXFP2	TMEM202
AHNAK2	BTN2A3P	EPHA6	IGHV3-21	Myo6-008	PI4KA	RXFP3	TMEM204
AIF1	BTN3A1	EPHA7	IGHV3-23	MYO7A	PIANP	RXFP4	TMEM219
AIF1L	BTN3A2	EPHA8	IGHV3-30	MYOF	PICALM	RXYLT1	TMEM231
AIFM2	BTN3A3	EPHB1	IGHV3-30-3	MYOT	PICK1	RYK	TMEM235
AIG1	BTNL10P	EPHB2	IGHV3-30-5	MYPN	PIEZO1	RYR1	TMEM240
AIP	BTNL2	EPHB2 variant protein	IGHV3-33	MYZAP	PIEZO2	RYR2	TMEM249
AJAP1	BTNL3	EPHB3	IGHV3-35	Na+/H+ exchanger isoform NHE-2	PIG59	RYR3	TMEM25
AJM1	BTNL8	EPHB4	IGHV3-38	Na+/H+ exchanger isoform NHE-3	PIGN	S100A12	TMEM262
AJUBA	BTNL9	EPHB6	IGHV3-38-3	NAA35	PIGR	S100A13	TMEM266
AKAP10	BVES	EPM2A	IGHV3-43	NAALAD2	PIGU	S100A16	TMEM30A
AKAP11	C10orf90	EPN1	IGHV3-43D	NAALADL1	PIGY	S100A3	TMEM30B
AKAP12	C11orf24	EPN2	IGHV3-48	NACAIS4	PIK3AP1	S100A6	TMEM43
AKAP5	C15orf29	EPN3	IGHV3-49	NAE1	PIK3C2A	S100A8	TMEM47
AKAP6	C15orf62	EPOR	IGHV3-53	NAIF1	PIK3C2B	S100A9	TMEM50B
AKAP7	C1orf210	EPPIN	IGHV3-64	NAIP	PIK3C2G	S100G	TMEM59
AKAP9	C1QBP	EPPK1	IGHV3-64D	NALCN	PIK3CA	S100P	TMEM63A
AKT1	C1QTNF1	EPRS1	IGHV3-66	NALF1	PIK3CB	S1PR1	TMEM63B

AKT2	C1QTNF5	EPS15	IGHV3-7	NALF2	PIK3CD	S1PR2	TMEM63C
AKTIP	C2CD2L	EPS8	IGHV3-72	NAPA	PIK3CG	S1PR3	TMEM65
ALCAM	C2CD5	EPS8L1	IGHV3-73	NAPEPLD	PIK3IP1	S1PR4	TMEM67
ALDH3A1	C2orf88	EPS8L2	IGHV3-74	NBC	PIK3R1	S1PR5	TMEM74
ALDH3B1	C3	EPS8L3	IGHV3-9	NBEA	PIK3R5	SAMD10	TMEM77
ALG10B	C3AR1	EQTN	IGHV4-28	NBEAL2	PIK3R6	SAMD12	TMEM87A
ALK	C4A	ERAS	IGHV4-30-2	Nbla02942	PILRA	SAMHD1	TMEM88
ALKAL1	C4B	ERBB2	IGHV4-30-4	Nbla04261	PILRB	SAPCD2	TMEM88B
ALKAL2	C4BPA	ERBB3	IGHV4-31	NCAM1	PIM1	SAPS3	TMEM8B
ALOX12	C4BPB	ERBB4	IGHV4-34	NCAM2	PIP4K2A	SARG	TMEM95
ALOX15	C5AR1	ERBIN	IGHV4-38-2	NCEH1	PIP4K2B	SCAMP1	TMEM97
ALOX15B	C5AR2	ERC1	IGHV4-39	NCK1	PIP4K2C	SCAMP5	TMEM98
ALPG	C6orf134	ERC2	IGHV4-4	NCKAP1	PIP4P1	SCARA5	TMIGD1
ALPI	C6orf25	EREG	IGHV4-59	NCKAP1L	PIP4P2	SCARB1	TMIGD3
ALPK2	C6orf89	ERLIN2	IGHV4-61	NCL	PIP5K1A	SCARB2	tmp_locus_29
ALPL	C9JQL5	ERMAP	IGHV5-10-1	NCMAP	PIP5K1B	SCARF1	TMPRSS11A
ALPP	C9orf88	ERRFI1	IGHV5-51	NCOA1	PIP5K1C	SCART1	TMPRSS11B
AMACR	C9orf90	ERVFC1	IGHV6-1	NCR1	PIP5KL1	SCEL	TMPRSS11D
AMBP	CA11	ERVFRD-1	IGHV7-4-1	NCR2	PIPSL	SCFD1	TMPRSS11E
AMER1	CA12	ERVH48-1	IGHV7-81	NCR3	PIRT	SCHIP1	TMPRSS11F
AMER2	CA14	ERVK-10	IGHV8-51-1	NCR3LG1	PITPNM1	SCIMP	TMPRSS12
AMER3	CA2	ERVK-18	IGKC	NCS1	PJA2	SCN2A	TMPRSS13
AMHR2	CA4	ERVK-19	IGKJ1	NCSTN	PKD1	SCN3A	TMPRSS2
AMIGO1	CA9	ERVK-21	IGKV1-12	NDC1	PKD1L1	SCN7A	TMPRSS4
AMIGO2	CABP1	ERVK-24	IGKV1-13	NDE1	PKD1L3	SCN8A	TMPRSS5
AMN	CABP2	ERVK-25	IGKV1-16	NDRG1	PKD2	SCN9A	TMPRSS6
AMN1	CABP7	ERVK-5	IGKV1-17	NDRG4	PKD2L1	SCNN1A	TMPRSS7
AMOT	CADM1	ERVK-6	IGKV1-27	NDUFC2	PKHD1	SCNN1B	TMPRSS9
AMOTL1	CADM2	ERVK-7	IGKV1-33	NDUFS7	PKN1	SCNN1D	TMUB1
AMOTL2	CADM3	ERVK-8	IGKV1-37	NECAB2	PKN2	SCNN1G	TMX3
AMPH	CALCR	ERVK-9	IGKV1-39	NECAP1	PKP1	SCRIB	TNF
ANGPT1	CALCRL	ERVK13-1	IGKV1-5	NECAP2	PKP2	SCTR	TNFA
ANK1	CALD1	ERVMER34-1	IGKV1-6	NECTIN1	PKP3	SCUBE1	TNFAIP8L3
ANK2	CALHM1	ERVPABLB-1	IGKV1-8	NECTIN2	PKP4	SCUBE3	TNFRSF10A
ANK3	CALHM3	ERVS71-1	IGKV1-9	NECTIN3	PLA2G2A	SDC1	TNFRSF10B
ANKH	CALN1	ERVW-1	IGKV1D-12	NECTIN4	PLA2G2F	SDC2	TNFRSF10C
ANKRD13	CALR	ESAM	IGKV1D-13	NEDD4	PLA2G3	SDC3	TNFRSF10D
ANKRD13A	CALY	ESR1	IGKV1D-16	NEDD4L	PLA2G4C	SDC4	TNFRSF11A
ANKRD13B	CAMK1G	ESYT1	IGKV1D-17	NEDD9	PLA2G4E	SDCBP2	TNFRSF11B
ANKRD13D	CAMK2D	ESYT2	IGKV1D-33	NEGR1	PLA2G4F	SDE2	TNFRSF12A
ANKRD20A1	CAMKV	ESYT3	IGKV1D-37	NELFE	PLA2G5	SDF4	TNFRSF13B
ANKRD24	CANT1	ETS2	IGKV1D-39	NEO1	PLA2G6	SDHB	TNFRSF13C
ANKRD27	CAP1	ETV6	IGKV1D-42	NETO1	PLA2R1	SDK1	TNFRSF14
ANKRD45	CAP2	EVA1A	IGKV1D-43	NETO2	PLAAT3	SDK2	TNFRSF17
ANKS1B	CAPN1	EVC	IGKV1D-8	NEU1	PLAC4	SDR16C5	TNFRSF18
ANKS4B	CAPN10	EVC2	IGKV2-24	NEU3	PLAU	SECTM1	TNFRSF19
ANO1	CAPN2	EVI2B	IGKV2-28	NEU4	PLAUR	SELE	TNFRSF1A
ANO10	CAPN3	EVPL	IGKV2-29	NEURL1	PLB1	SELENOK	TNFRSF1B
ANO2	CAPNS1	EWSR1	IGKV2-30	NEXN	PLCD1	SELENOS	TNFRSF21

ANO3	CAPNS2	EXO1	IGKV2-40	NF1	PLCD3	SELL	TNFRSF25
ANO4	CAPRIN2	EXOC1	IGKV2D-24	NF2	PLCD4	SELP	TNFRSF4
ANO5	CAPS	EXOC2	IGKV2D-26	NFAM1	PLCE1	SELPLG	TNFRSF6
ANO6	CARD11	EXOC3	IGKV2D-28	Nfasc	PLCG1	SEMA4A	TNFRSF8
ANO7	CARD14	EXOC4	IGKV2D-29	NFE2L2	PLCG2	SEMA4B	TNFRSF9
ANO8	CARD9	EXOC6	IGKV2D-30	NFKBIA	PLCH1	SEMA4C	TNFSF10
ANO9	CARMIL1	EXOC7	IGKV2D-40	NFX1	PLCH2	SEMA4D	TNFSF11
ANOS1	CARMIL2	EXOC8	IGKV3-11	NGFR	PLD1	SEMA4F	TNFSF12
ANPEP	CARMIL3	EXTL3	IGKV3-15	NHERF1	PLD2	SEMA4G	TNFSF13B
ANTXR1	CASK	EZR	IGKV3-20	NHERF2	PLEC	SEMA5A	TNFSF14
ANTXR2	CASP1	EZR-ROS1	IGKV3-7	NHERF4	PLEK	SEMA6A	TNFSF15
ANTXRL	CASP14	F10	IGKV3D-11	NHS	PLEK2	SEMA6B	TNFSF18
ANXA1	CASP4	F11	IGKV3D-15	NIBAN1	PLEKHA1	SEMA6C	TNFSF4
ANXA13	CASQ1	F11R	IGKV3D-20	NIBAN2	PLEKHA2	SEMA6D	TNFSF6
ANXA2P2	CASR	F12	IGKV3D-7	NID2	PLEKHA4	SEMA7A	TNFSF8
ANXA3	CASS4	F1JZ08	IGKV4-1	NIN	PLEKHG4	SEPHS1	TNFSF9
ANXA4	CATIP	F2	IGKV5-2	NINJ1	PLEKHG4B	SEPTIN12	TNIK
ANXA5	CAV1	F2R	IGKV6-21	NINJ2	PLEKHG5	SEPTIN2	TNK2
ANXA6	CAV2	F2RL1	IGKV6D-21	NIPA1	PLEKHG6	SEPTIN5	TNKS1BP1
ANXA8	CAV3	F2RL2	IGKV6D-41	NIPA2	PLEKHH2	SEPTIN6	TNLG7A
ANXA8L1	caveolin-2	F2RL3	IGLC1	NIPSNAP1	PLEKHM3	SEPTIN7	TNS2
ANXA9	CAVIN1	F2YGG7	IGLC2	NISCH	PLEKHN1	SERINC1	TOLLIP
AOC1	CAVIN2	F3	IGLC3	NKAIN1	PLEKHO1	SERINC3	TOM1
AOC2	CAVIN3	F5	IGLC6	NKAIN2	PLET1	SERINC5	TOMT
AOC3	CAVIN4	F7	IGLC7	NKAIN3	PLG	SERPINA12	TOPBP1
AP1G1	CBARP	F8	IGLJ1	NKAIN4	PLGRKT	SERPINA5	TP53I13
AP1M1	CBL	F9	IGLV	NKD1	PLIN2	SERPINB10	TPBG
AP1S1	CBLB	FABP5	IGLV1-36	NKD2	PLIN4	SERPINB12	TPBGL
AP1S2	CBLC	FADD	IGLV1-40	NKG7	PLK4	SERPINB2	TPM1
AP1S3	CBLIF	FADS2	IGLV1-44	NKTR	PLP	SERPINB3	TPO
AP2M1	CBLN1	FAIM2	IGLV1-47	NLGN1	PLP1	SERPINB4	TPRA1
APBA1	CC2D1A	FAK	IGLV1-50	NLGN2	PLP2	SERPINB5	TPSG1
APBA2	CCBP2	FAM107A	IGLV1-51	NLGN3	PLPP1	SERPINB6	TPTE2
APBA3	CCDC124	FAM120A	IGLV10-54	NLGN4X	PLPP2	SERPINC1	TRA
APBB1	CCDC25	FAM126A	IGLV11-55	NLGN4Y	PLPP3	SERPINE1	TRABD2A
APBB1IP	CCDC70	FAM168B	IGLV2-11	NLN	PLPP4	SEZ6	TRABD2B
APC	CCDC78	FAM171A1	IGLV2-14	NLRC4	PLPP5	SEZ6L2	TRADD
APCDD1	CCDC8	FAM174B	IGLV2-18	NLRP10	PLPP6	SFRP1	TRAF1
APCDD1L	CCDC88A	FAM62A	IGLV2-23	NLRP6	PLPPR1	SGIP1	TRAF3IP2
APLNR	CCKAR	FANCG	IGLV2-33	NLRX1	PLPPR4	SGK1	TRAF3IP3
APLP1	CCKBR	FAP	IGLV2-8	NMBR	PLPPR5	SGMS1	TRAF4
APLP2	CCN2	FARP1	IGLV3-1	NMDAR2C	PLS1	SGMS2	TRAF6
APOA1	CCNT2	FAS	IGLV3-10	NME1	PLS3	SGPP1	TRAF7
APOB	CCNY	FASLG	IGLV3-12	NMRK2	PLSCR1	SH2B1	TRAJ31
APOBR	CCNYL1	FASN	IGLV3-16	NMT1	PLSCR2	SH2B2	TRAJ42
APOE	CCNYL2	FAT1	IGLV3-19	NMT2	PLSCR3	SH2B3	TRAK2
APOLD1	CCNYL3	FAT2	IGLV3-21	NMUR1	PLSCR4	SH2D3C	TRAPPC14
APP	CCR-5	FAT3	IGLV3-22	NMUR2	PLSCR5	SH3BGRL	TRAPPC4
APPL1	CCR1	FAT4	IGLV3-25	NOD1	PLVAP	SH3BGRL3	TRARG1

APPL2	CCR10	FBP2	IGLV3-27	NOD2	PLXDC1	SH3BP1	TRAT1
AQP1	CCR2	FBXO2	IGLV3-32	NOS1	PLXNA1	SH3BP4	TRB
AQP10	CCR3	FBXO45	IGLV3-9	NOS1AP	PLXNA2	SH3D19	TRBV10-1
AQP11	CCR4	FBXW7-AS1	IGLV4-3	NOS2	PLXNA3	SH3D21	TRBV10-2
AQP2	CCR5	FCAMR	IGLV4-60	NOS3	PLXNA4	SH3GL2	TRBV10-3
AQP3	CCR6	FCAR	IGLV4-69	NOSTRIN	PLXNA4B	SH3KBP1	TRBV11-1
AQP4	CCR7	FCER1A	IGLV5-37	NOTCH1	PLXNB1	SH3TC2	TRBV11-2
AQP5	CCR8	FCER2	IGLV5-39	NOTCH2	PLXNB2	SH3YL1	TRBV11-3
AQP6	CCR9	FCGR1A	IGLV5-45	NOTCH3	PLXNB3	SHANK1	TRBV12-3
AQP7	CCRL1	FCGR1BP	IGLV5-48	NOTCH4	PLXNC1	SHANK2	TRBV12-4
AQP7B	CCRL2	FCGR2A	IGLV5-52	NOXA1	PLXND1	SHANK3	TRBV12-5
AQP7P3	CD101	FCGR2B	IGLV6-57	NOXO1	PMEL	SHB	TRBV13
AQP8	CD109	FCGR2C	IGLV7-43	NPAP1	PMEPA1	SHC1	TRBV14
AQP9	CD133	FCGR3B	IGLV7-46	NPBWR1	PMP22	SHC2	TRBV15
AR	CD14	FCGRT	IGLV8-61	NPBWR2	PNISR	SHC3	TRBV16
ARAP1	CD151	FCHO1	IGLV9-49	NPC1	PNN	SHC4	TRBV17
ARAP3	CD160	FCHO2	IGSF11	NPC1L1	PNOC	SHH	TRBV18
ARC	CD163	FCHSD2	IGSF21	NPDC1	PNPLA2	SHROOM1	TRBV19
ARF1	CD163L1	FCMR	IGSF23	NPFFR1	PODXL	SHROOM2	TRBV2
ARF3	CD164	FCN1	IGSF5	NPFFR2	PODXL2	SHROOM3	TRBV20-1
ARF4	CD177	FCN2	IGSF6	NPHS1	POLE	SHROOM4	TRBV20OR9-2
ARF5	CD180	FCN3	IGSF8	NPHS2	PON2	SI	TRBV23-1
ARF6	CD19	FCRL1	IGSF9	NPSR1	POPDC2	SIDT1	TRBV24-1
ARFGAP2	CD1A	FCRL2	IGSF9B	NPTN	POPDC3	SIDT2	TRBV25-1
ARFIP2	CD1B	FCRL3	IHH	NPY1R	POTED	SIGIRR	TRBV27
ARHGAP10	CD1C	FCRL4	IL10RA	NPY2R	POU2F3	SIGLEC1	TRBV28
ARHGAP12	CD1D	FCRL5	IL10RB	NPY4R	PPAN-P2RY11	SIGLEC10	TRBV29-1
ARHGAP15	CD1E	FCRL6	IL11RA	NPY4R2	PPAP2A	SIGLEC11	TRBV3-1
ARHGAP17	CD2	FER	IL12B	NPY5R	PPFIA2	SIGLEC12	TRBV30
ARHGAP18	CD200	FER1L5	IL12RB1	NPY6R	PPFIBP1	SIGLEC14	TRBV4-1
ARHGAP19	CD200R1	FERMT1	IL12RB2	NR2A	PPIL2	SIGLEC15	TRBV4-2
ARHGAP21	CD200R1L	FERMT2	IL13	NRAC	PPIP5K1	SIGLEC16	TRBV4-3
ARHGAP25	CD207	FES	IL13RA1	NRAS	PPL	SIGLEC5	TRBV5-1
ARHGAP33	CD209	FEZ1	IL13RA2	NRCAM	PPM1A	SIGLEC6	TRBV5-3
ARHGAP35	cd21	FFAR1	IL15RA	NRG1	PPP1CA	SIGLEC7	TRBV5-4
ARHGAP44	CD22	FFAR2	IL16	NRG2	PPP1CC	SIGLEC8	TRBV5-5
ARHGAP45	CD226	FFAR3	IL17A	NRG3	PPP1R12A	SIGLEC9	TRBV5-6
ARHGAP5	CD24	FFAR4	IL17RA	NRG4	PPP1R13B	SIGMAR1	TRBV5-7
ARHGDIA	CD244	FGA	IL17RB	NRN1	PPP1R16A	SIPA1	TRBV5-8
ARHGDIG	CD247	FGB	IL17RC	NRN1L	PPP1R16B	SIPA1L1	TRBV6-1
ARHGEF1	CD248	FGD2	IL17RD	NRP1	PPP1R9B	SIPA1L3	TRBV6-2
ARHGEF11	CD27	FGD5	IL17RE	NRP2	PPP2CA	SIRPA	TRBV6-3
ARHGEF18	CD274	FGF10	IL18R1	NRROS	PPP2R1A	SIRPB1	TRBV6-4
ARHGEF2	CD276	FGF13	IL18RAP	NRSN2	PPP2R5A	SIRPB2	TRBV6-5
ARHGEF25	CD28	FGF6	IL1R1	NRXN1	PPP3CA	SIRPD	TRBV6-6
ARHGEF28	CD2AP	FGF7B	IL1R2	NRXN2	PPP3CB	SIRPG	TRBV6-7
ARHGEF39	CD300A	FGF8	IL1RAP	NRXN3	PPP3R1	SIRT2	TRBV6-8
ARHGEF4	CD300C	FGFBP1	IL1RAPL1	NSF	PPP4C	SKAP1	TRBV6-9
ARHGEF40	CD300E	FGFR1	IL1RAPL2	NSG1	PPP5C	SKAP2	TRBV7-1

ARHGEF5	CD300H	FGFR2	IL1RL1	NSMF	PPP6R3	SLA	TRBV7-2
ARHGEF7	CD300LB	FGFR2-AHCYL1	IL1RL2	NT5E	PRAM1	SLA2	TRBV7-3
ARHH	CD300LD	FGFR2-BICC1	IL1RN	NTM	PRAME	SLAMF1	TRBV7-4
ARID1B	CD300LF	FGFR3	IL20RA	NTN4	PRC1	SLAMF6	TRBV7-6
ARID2	CD300LG	FGFR4	IL20RB	NTNG1	PRCD	SLAMF7	TRBV7-7
ARID4A	CD302	FGFRL1	IL21R	NTNG2	PRCP	SLAMF9	TRBV7-8
ARL13A	CD320	FGG	IL22RA1	NTRK1	PRDX3	SLC10A1	TRBV7-9
ARL13B	CD33	FGR	IL27RA	NTRK2	PREX1	SLC10A2	TRBV9
ARL14EP	CD34	FHIT	IL2RA	NTRK3	PREX2	SLC10A4	TRDC
ARL4A	CD36	FHL1	IL2RB	NTSR1	PRF1	SLC10A6	TRDN
ARL4C	CD37	FILIP1	IL2RG	NTSR2	PRICKLE3	SLC10A7	TREH
ARL4D	CD38	FIMP	IL31RA	NUBP1	PRIMA1	SLC11A1	TREM1
ARL6IP5	CD3E	FKRP	IL36G	NUBPL	PRKAA1	SLC11A2	TREM2
ARL8A	CD3G	FKSG41	IL3RA	NUCB2	PRKACA	SLC12A1	TREML1
ARMC12	CD4	FKSG47	IL4I1	NUMA1	PRKACB	SLC12A2	TREML2
ARPC1A	CD40	FKSG83	IL4R	NUMB	PRKAR1A	SLC12A3	TREML4
ARPC2	CD40LG	FLAD1	IL5RA	NUP35	PRKAR1B	SLC12A4	TRGC1
ARRB1	CD44	FLCN	IL6ST	O00557	PRKAR2A	SLC12A5	TRGC2
ARRB2	CD46	FLG	IL7R	O14549	PRKAR2B	SLC12A6	TRGV1
ARRDC1	CD47	FLG2	IL8RB	O43898	PRKCA	SLC12A7	TRGV2
ARRDC2	CD48	FLJ00114	IL9R	O60354	PRKCB	SLC12A9	TRGV3
ARRDC3	CD5	FLJ00233	ILDR1	O95883	PRKCD	SLC13A1	TRGV4
ARRDC4	CD52	FLJ14466	ILK	OAS3	PRKCE	SLC13A2	TRGV5
ARRDC5	CD53	FLJ33641	IMUP	OBSCN	PRKCG	SLC13A3	TRGV8
ARSA	CD55	FLJ44653	ING2	OCEL1	PRKCH	SLC13A4	TRGV9
ART1	CD58	FLNA	INPP4A	OCLN	PRKCI	SLC13A5	TRHDE
ART3	CD59	FLNB	INPP5A	OCRL	PRKCQ	SLC14A1	TRHR
ART4	CD5L	FLNC	INPP5B	ODZ1	PRKCZ	SLC14A1 JK	TRIB3
ARVCF	CD6	FLOT1	INPP5D	OGT	PRKD1	SLC14A2	TRIM16
ASAH2	CD63	FLOT2	INPP5E	OLFM4	PRKD2	SLC15A1	TRIM23
ASAP2	CD68	FLRT1	INPP5F	olfr89	PRKD3	SLC15A2	TRIM36
ASB3	CD69	FLRT2	INPP5J	OLR1	PRKG1	SLC15A4	TRIM4
ASGR1	CD7	FLRT3	INPP5K	OMG	PRKG2	SLC16A1	TRIM72
ASGR2	CD70	FLT1	INPPL1	OPALIN	PRLHR	SLC16A10	TRIP10
ASIC1	CD72	FLT3	INSC	OPCML	PRLR	SLC16A11	TRIP6
ASIC2	CD74	FLT3LG	intg_am_b2_human	OPN1LW	PRMT8	SLC16A12	TRO
ASIC3	CD80	FLT4	IPCEF1	OPN1MW	PRND	SLC16A13	TRPA1
ASIC4	CD81	FLVCR1	IQCE	OPN1MW2	PRNP	SLC16A14	TRPC1
ASIC5	CD82	FLVCR2	IQCJ-SCHIP1	OPN1MW3	PROCR	SLC16A2	TRPC3
ASPH	CD83	FMN1	IQGAP1	OPN1SW	PROKR1	SLC16A3	TRPC4
ASPM	CD84	FMN2	IQGAP2	OPN3	PROKR2	SLC16A4	TRPC4AP
ASPSCR1	CD86	FMNL1	IQGAP3	OPN4	PROM1	SLC16A5	TRPC5
ASTL	CD8A	FMNL3	IQSEC3	OPN5	PROM2	SLC16A6	TRPC6
ASTN1	CD8B	FMR1	IRAG2	OPRD1	PROS1	SLC16A7	TRPC7
ATAD1	CD8B2	FN1	IRAK1	OPRK1	PRPH	SLC16A8	TRPM1
ATAD3B	CD9	FNBP1	IRAK2	OPRL1	PRR7	SLC16A9	TRPM2
ATAT1	CD93	FNBP1L	IRAK3	OPRM1	PRRC2A	SLC17A1	TRPM3
ATF4	CD96	FNDC4	IRAK4	OR10A2	PRRG1	SLC17A2	TRPM4

ATGR2	CD99	FNDC5	IRGM	OR10A3	PRRG2	SLC17A3	TRPM5
ATIC	CD99L2	FNTA	IRS1	OR10A4	PRRG4	SLC17A4	TRPM6
ATP10A	CDAN1	FOLH1	IRS2	OR10A5	PRRT1	SLC17A5	TRPM7
ATP10B	CDC42	FOLH1B	IRS4	OR10A6	PRRT2	SLC17A6	TRPM8
ATP10D	CDC42BPB	FOLR1	ISLR2	OR10A7	PRSS12	SLC17A7	TRPV1
ATP11A	CDC42EP1	FOLR2	ITCH	OR10AC1	PRSS21	SLC17A8	TRPV2
ATP11B	CDC42EP2	FOLR3	ITFG1	OR10AD1	PRSS27	SLC18A2	TRPV3
ATP11C	CDC42EP3	FOSL1	ITGAE	OR10AG1	PRSS41	SLC19A1	TRPV4
ATP12A	CDC42EP4	FP3184	ITGAL	OR10C1	PRSS42P	SLC19A2	TRPV5
ATP13A4	CDC42EP5	FPR1	ITGAM	OR10D3	PRSS55	SLC19A3	TRPV6
ATP13A5	CDC42SE1	FPR2	ITGB1BP1	OR10D4P	PRSS8	SLC1A1	TSC1
ATP1A1	CDC42SE2	FPR3	ITGB2	OR10G2	PRTG	SLC1A2	TSC22D1
ATP1A3	CDCA4	FPRL1	ITGBL1	OR10G3	PRTN3	SLC1A3	TSG101
ATP1A4	CDCP1	FPRL2	ITIH4	OR10G4	PRX	SLC1A4	TSHR
ATP23	CDCrel-1	FRAS1	ITK	OR10G6	PS1TP5BP1	SLC1A5	TSHZ3
ATP2A2	CDH1	FREM2	ITLN1	OR10G7	PSAP	SLC1A6	TSPAN1
ATP2B1	CDH10	FREQ	ITM2A	OR10G8	PSCA	SLC1A7	TSPAN12
ATP2B2	CDH11	FRK	ITM2B	OR10G9	PSD	SLC20A1	TSPAN13
ATP2B2 variant protein	CDH16	FRMD1	ITM2C	OR10H1	PSD2	SLC20A2	TSPAN14
ATP2B3	CDH17	FRMD6	ITPK1	OR10H2	PSD3	SLC21A8	TSPAN15
ATP2B4	CDH18	FRMD7	ITPR1	OR10H3	PSD4	SLC22A1	TSPAN2
ATP2C1	CDH19	FRMD8	ITPR2	OR10H4	PSEN1	SLC22A11	TSPAN31
ATP2C2	CDH2	FRMD8P1	ITPR3	OR10H5	PSG1	SLC22A12	TSPAN33
ATP4A	CDH22	FRMPD1	ITPRID2	OR10J1	PSG3	SLC22A13	TSPAN4
ATP5F1A	CDH23	FRMPD2	ITPRIP	OR10J3	PSG4	SLC22A14	TSPAN5
ATP5F1B	CDH24	FRMPD3	ITSN1	OR10J4	PSG6	SLC22A15	TSPAN7
ATP5PO	CDH26	FRRS1L	ITSN2	OR10J5	PSG7	SLC22A16	TSPAN9
ATP6AP1	CDH4	FRS2	IVL	OR10J6P	PSG8	SLC22A17	TSPEAR
ATP6AP2	CDH5	FRS3	IYD	OR10K1	PSG9	SLC22A18	TSPO2
ATP6B1	CDH6	FSCN1	IZUMO1	OR10K2	PSKH1	SLC22A2	TTC17
ATP6V0A1	CDH7	FSCN3	IZUMO1R	OR10P1	PSMD10	SLC22A23	TTC7A
ATP6V0A2	CDH8	FSD1	IZUMO3	OR10Q1	PSTPIP1	SLC22A24	TTC7B
ATP6V0A4	CDH9	FSHR	J7LCD9	OR10R2	PSTPIP2	SLC22A3	TTC8
ATP6V0B	CDHR1	FSHR1	JADE1	OR10S1	PTAFR	SLC22A4	TTLL12
ATP6V0C	CDHR2	FTCD	JAG1	OR10T2	PTC	SLC22A5	TTLL5
ATP6V0D2	CDHR4	FTO	JAG2	OR10V1	PTCD3	SLC22A6	TTMP
ATP6V1A	CDHR5	FURIN	JAK1	OR10W1	PTCH1	SLC22A7	TTYH1
ATP6V1B1	CDIPT	FUT1	JAK2	OR10X1	PTCH2	SLC22A8	TTYH2
ATP6V1B2	CDK14	FXYD1	JAK3	OR10Z1	PTCHD1	SLC22A9	TTYH3
ATP6V1C1	CDK16	FXYD3	JAM2	OR11A1	PTCHD3	SLC23A1	TUB
ATP6V1D	CDK5	FXYD4	JAM3	OR11G2	PTEN	SLC23A2	TUBA1A
ATP6V1E1	CDK5R1	FXYD6	JAML	OR11H1	PTGDR	SLC23A3	TULP1
ATP6V1F	CDK5R2	FXYD7	JCAD	OR11H12	PTGDR2	SLC24A1	TULP3
ATP6V1G1	CDK7	FYB	JK	OR11H2	PTGER1	SLC24A2	TUSC3
ATP6V1G3	CDKL5	FYB1	JMJD6	OR11H4	PTGER2	SLC24A3	TWF1
ATP6V1H	CDON	FYB2	JOSD1	OR11H6	PTGER3	SLC24A4	TXK
ATP7A	CDSN	FYN	JPH1	OR11H7	PTGER4	SLC25A11	TXNDC15
ATP7B	CDV3	FZD1	JPH2	OR11L1	PTGFR	SLC25A13	TYK2
ATP8A1	CDW52	FZD10	JPH3	OR12D1	PTGIR	SLC25A14	TYRO3

ATP8A2	CEACAM1	FZD2	JPH4	OR12D2	PTGIS	SLC25A3	TYROBP
ATP8B1	CEACAM20	FZD3	JPT2	OR12D3	PTGS2	SLC25A31	UAP1
ATP8B2	CEACAM21	FZD4	JTB	OR13A1	PTH1R	SLC25A4	UBA52
ATP8B3	CEACAM3	FZD5	JUP	OR13C2	PTH2R	SLC25A5	UBAC1
ATP8B4	CEACAM4	FZD6	K7N7A8	OR13C3	PTHR1	SLC26A1	UBAP1
ATP9A	CEACAM5	FZD7	K9J958	OR13C4	PTK2	SLC26A10P	UBB
ATP9B	CEACAM6	FZD8	K9J962	OR13C5	PTK2B	SLC26A11	UBC
ATRAID	CEACAM7	FZD9	K9J963	OR13C6P	PTK6	SLC26A2	UBE2B
ATRN	CEACAM8	G3CIS4	KANK1	OR13C7	PTK7	SLC26A3	UBE2C
ATXN10	CEBPE	G4V2I7	KANSL2	OR13C8	PTN	SLC26A4	UBE2D3
ATXN3	CELSR1	G4V2I8	KARS1	OR13C9	PTOV1	SLC26A5	UBE2QL1
AURKA	CELSR2	G4V2I9	KATNB1	OR13D1	PTP4A1	SLC26A6	UBL3
AVPR1	CELSR3	G6F-LY6G6D	KATNBL1	OR13F1	PTP4A2	SLC26A7	UBQLN1
AVPR1A	CEMIP	G6PD	KAZN	OR13G1	PTP4A3	SLC26A8	UBQLN2
AVPR1B	CEMIP2	GAA	KCMF1	OR13H1	PTPN1	SLC26A9	UBR4
AVPR2	CEP112	GAB2	KCNA10	OR13J1	PTPN13	SLC27A1	UCHL1
AXIN1	CEP295	GABARAP	KCNAB2	OR14A16	PTPN2	SLC27A2	UGT1A1
AXIN2	CEP55	GABBR1	KCNE1	OR14A2	PTPN20	SLC27A4	UGT8
AXL	CERCAM	GABBR2	KCNE2	OR14C36	PTPN22	SLC27A5	ULBP1
AZGP1	CERK	GABRA1	kcne2-kcnh2_human	OR14I1	PTPN3	SLC27A6	ULBP2
B0AZM0	CFAP126	GABRA2	KCNE3	OR14J1	PTPN4	SLC28A1	ULBP3
B0AZM5	CFAP157	GABRA3	KCNE4	OR14K1	PTPN5	SLC28A2	UMOD
B0AZU8	CFAP410	GABRA4	KCNE5	OR14L1	PTPN7	SLC28A3	UMODL1
B0AZV7	CFAP418	GABRA5	KCNH2	OR1A1	PTPRA	SLC29A1	UNC13A
B2LR44	CFAP65	GABRA6	KCNH3	OR1A2	PTPRB	SLC29A2	UNC13B
B2LR45	CFAP95	GABRB1	KCNH5	OR1B1	PTPRC	SLC29A3	UNC13C
B2R5Q9	CFB	GABRB2	KCNH6	OR1C1	PTPRCAP	SLC29A4	UNC5A
B2R5S2	CFC1	GABRB3	KCNH7	OR1D2	PTPRD	SLC2A1	UNC5B
B2R615	CFL1	GABRD	KCNH8	OR1D4	PTPRE	SLC2A10	UNC5C
B2R621	CFTR	GABRE	KCNJ1	OR1D5	PTPRF	SLC2A11	UNC5D
B2R674	CGAS	GABRG1	KCNJ10	OR1E1	PTPRG	SLC2A11-a	UNC79
B2R693	CGN	GABRG2	KCNJ11	OR1E2	PTPRH	SLC2A11-c	UNC80
B2R6A3	CHD1L	GABRG3	KCNJ12	OR1E3	PTPRJ	SLC2A12	UNC93A
B2R6F5	CHIC1	GABRP	kcnj12x	OR1E5	PTPRK	SLC2A13	UNQ9373
B2R6J2	CHIC2	GABRQ	KCNJ13	OR1F1	PTPRM	SLC2A14	UPK1A
B2R6Q2	CHL1	GABRR1	KCNJ14	OR1F12	PTPRN	SLC2A2	UPK1B
B2R6V6	CHMP1A	GABRR2	KCNJ15	OR1F12P	PTPRN2	SLC2A3	UPK2
B2R6V7	CHMP1B	GABRR3	KCNJ16	OR1F2P	PTPRO	SLC2A4	UPK3A
B2R708	CHMP2A	GAD1	KCNJ17	OR1G1	PTPRR	SLC2A5	UPK3B
B2R728	CHMP2B	GAD2	KCNJ18	OR1I1	PTPRS	SLC2A6	USH1C
B2R734	CHMP3	gag	KCNJ2	OR1J1	PTPRT	SLC2A7	USH1G
B2R736	CHMP4A	GAL3ST1	KCNJ3	OR1J2	PTPRU	SLC2A8	USP14
B2R776	CHMP4B	GALR1	KCNJ4	OR1J4	PTPRZ1	SLC2A9	USP21
B2R782	CHMP4BP1	GALR2	KCNJ5	OR1K1	PVR	SLC30A1	USP4
B2R785	CHMP4C	GALR3	KCNJ6	OR1L1	PVRIG	SLC30A10	USP50
B2R7C7	CHMP5	gamrh	KCNJ9	OR1L3	PXDNL	SLC30A2	USP6
B2R7P1	CHMP6	GAP43	KCNK1	OR1L4	PXK	SLC30A3	USP6NL
B2R7S2	CHMP7	GAPDH	KCNK10	OR1L6	PXN	SLC30A4	USP8
B2R7S8	CHP1	GAPT	KCNK13	OR1L8	Q05CQ2	SLC30A5	UT-B1

B2R7U1	CHP2	GAPVD1	KCNK15	OR1M1	Q08G24	SLC30A8	UTP20
B2R7U3	CHRDL1	GAREM1	KCNK16	OR1N1	Q09LL4	SLC31A1	UTRN
B2R7Y7	CHRM1	GAS1	KCNK17	OR1N2	Q0GM92	SLC31A2	UTS2R
B2R7Z4	CHRM2	GAS2L2	KCNK18	OR1P1	Q0GM93	SLC32A1	V9H0H3
B2R807	CHRM3	GAS7	KCNK2	OR1Q1	Q0MVN7	SLC33A1	V9H162
B2R876	CHRM4	GAS8	KCNK3	OR1S1	Q15515	SLC34A1	VAMP1
B2R879	CHRM5	GASK1A	KCNK4	OR1S2	Q29767	SLC34A2	VAMP2
B2R888	CHRNA1	GBA2	KCNK5	OR2A1	Q29853	SLC34A2-ROS1	VAMP3
B2R8C7	chrna1-chrne_human	GBP1	KCNK7	OR2A12	Q29945	SLC34A3	VAMP4
B2R8G3	chrna1-chrng_human	GBP4	KCNK9	OR2A14	Q29987	SLC35A1	VAMP5
B2R8G9	CHRNA10	GCA	KCNMA1	OR2A2	Q2TSD3	SLC35G1	VAMP7
B2R8H4	CHRNA2	GCG	KCNN1	OR2A25	Q2TTR7	SLC35G2	VAMP8
B2R8M3	chrna2-chrnb2_human	GCGR	KCNN2	OR2A4	Q30171	SLC36A1	VANGL1
B2R8P5	chrna2-chrnb4_human	GCSAM	KCNN3	OR2A42	Q3C1V9	SLC36A2	VANGL2
B2R8T0	CHRNA3	GDE1	KCNN4	OR2A5	Q3YAA2	SLC36A4	VAPA
B2R8W8	chrna3-chrna5-chrnb2_human	GDF5	KCNQ2	OR2A7	Q4ZIL0	SLC38A1	VAPB
B2R8Z5	chrna3-chrna5-chrnb4_human	GDNFR3	KCNQ3	OR2AE1	Q506J9	SLC38A2	VASN
B2R944	chrna3-chrna6-chrnb2-chrnb3_human	GDPD2	KCNQ4	OR2AG1	Q52R92	SLC38A3	VASP
B2R951	chrna3-chrna6-chrnb4_human	GDPD5	KCNQ5	OR2AG2	Q52R93	SLC38A4	VAV1
B2R9H8	chrna3-chrnb2_human	GEM	KCNT1	OR2AJ1	Q52R94	SLC38A5	VAV2
B2R9H9	chrna3-chrnb4_human	GFI1B	KCNT2	OR2AK2	Q53EM0	SLC38A6	VCAM1
B2R9J8	CHRNA4	GFRA1	KCNU1	OR2AP1	Q53EM9	SLC39A1	VCL
B2R9M7	chrna4-chrna5-chrnb2_human	GFRA2	KCTD12	OR2AT4	Q53ES0	SLC39A10	VDAC1
B2R9P0	chrna4-chrnb4_human	GFRA3	KCTD16	OR2B11	Q53EX3	SLC39A11	VEGFR1
B2R9S2	CHRNA5	GFRA4	KCTD3	OR2B2	Q53EZ5	SLC39A12	VEPH1
B2R9U4	CHRNA6	GFRAL	KCTD7	OR2B3	Q53F36	SLC39A14	VEZT
B2RA30	CHRNA7	GFRalpha-1	KCTD8	OR2B6	Q53F42	SLC39A2	VH3
B2RAH2	chrna7_human	GGT1	KDR	OR2B8	Q53FA1	SLC39A3	VHL
B2RAJ1	CHRNA7-2	GGT2P	KEL	OR2B8P	Q53FB0	SLC39A4	VIL1
B2RAP6	chrna7-beta2_human	GGT3P	KIAA0319	OR2C1	Q53FG2	SLC39A5	VIL2
B2RAQ4	CHRNA9	GGT5	KIAA0319L	OR2C3	Q53FL7	SLC39A6	VIM
B2RBD0	chrna9_human	GGT7	KIAA0811	OR2D2	Q53FM3	SLC39A8	VIPR1
B2RBF7	chrna9-chrna10_huma	GHR	KIAA1529	OR2D3	Q53FP6	SLC39A9	VIPR2

	n						
B2RBZ9	CHRNB1	GHRHR	KIAA1549	OR2F1	Q53FV0	SLC3A1	VLDLR
B2RC44	CHRNB2	GHSR	KIAA1614	OR2F2	Q53FV8	SLC3A2	VMP1
B2RC75	CHRNB3	GIPR	KIAA1755	OR2G2	Q53FX5	SLC40A1	VN1R1
B2RC95	CHRNB4	GJA1	KIF14	OR2G3	Q53G76	SLC41A1	VN1R17P
B2RCB8	CHRND	GLDC	KIF17	OR2G6	Q53G99	SLC41A2	VN1R2
B2RCH0	CHRNE	GLDN	KIF18A	OR2H1	Q53GC2	SLC41A3	VN1R3
B2RCJ5	CHRNG	GLG1	KIFAP3	OR2H2	Q53GK6	SLC43A1	VN1R4
B2RCL0	CIB2	GLIPR1	KIR-K36	OR2I1P	Q53GP0	SLC43A2	VN1R5
B2RCM3	CIP2A	GLIPR1L1	KIR-K39	OR2J1	Q53GZ4	SLC43A3	VPS28
B2RCN5	CISH	GLO1	KIR-K65	OR2J2	Q53H72	SLC44A1	VPS35L
B2RCT7	CIZ1	GLP1R	KIR2DL1	OR2J3	Q53HA8	SLC44A2	VPS37B
B2RCU1	CKAP4	GLP2R	KIR2DL2	OR2K2	Q53HB6	SLC44A3	VPS4A
B2RCW8	CKAP5	GLRA1	KIR2DL3	OR2L13	Q53HM0	SLC44A4	VPS4B
B2RCZ4	CKB	GLRA2	KIR2DL4	OR2L2	Q53HQ0	SLC44A5	VSIG1
B2RD89	CLASP2	GLRA3	KIR2DL5A	OR2L3	Q53HW4	SLC45A3	VSIG2
B2RDY9	CLCA1	GLRB	KIR2DL5B	OR2L5	Q53HW6	SLC46A1	VSIR
B2RE49	CLCA2	GLUL	KIR2DP1	OR2L8	Q59E85	SLC46A2	VSTM1
B2RE65	CLCA3P	GLYCAM1	KIR2DS1	OR2M2	Q59EA3	SLC47A1	VSTM4
B3GNT3	CLCA4	GM2A	KIR2DS2	OR2M3	Q59EB0	SLC47A2	VSTM5
B3KMQ8	CLCN1	GMIP	KIR2DS3	OR2M4	Q59ED3	SLC48A1	VTCN1
B3KMS7	CLCN2	GML	KIR2DS4	OR2M5	Q59EE1	SLC4A1	VXN
B3KMT6	CLCN3	GNA13	KIR2DS5	OR2M7	Q59EF6	SLC4A10	W6EAD8
B3KMT8	CLCN5	GNAS	KIR3DL1	OR2M7 D	Q59EH9	SLC4A11	WARS2
B3KMV5	CLCNKA	GNAT3	KIR3DL2	OR2M7 N	Q59EQ1	SLC4A1AP	WAS
B3KN27	CLCNKB	GNB5	KIR3DL3	OR2M7 O	Q59ER6	SLC4A2	WASF2
B3KND1	CLDN1	GNRHR	KIR3DP1	OR2S2	Q59ER8	SLC4A3	WASHC2A
B3KNF4	CLDN10	GNRHR2	KIR3DS1	OR2T1	Q59ES7	SLC4A4	WASHC2C
B3KNM3	CLDN11	GOLGA4	KIR3DX1	OR2T10	Q59EV2	SLC4A5	WASL
B3KNP6	CLDN12	GOLGA7B	KIRREL1	OR2T11	Q59EV7	SLC4A7	WDFY3
B3KNS3	CLDN14	GOLM1	KIRREL2	OR2T12	Q59EV9	SLC4A8	WDPCP
B3KNX4	CLDN15	GOLPH3	KIRREL3	OR2T2	Q59EW6	SLC4A9	WDR1
B3KNZ0	CLDN16	GOPC	KISS1	OR2T27	Q59EY4	SLC50A1	WDR13
B3KNZ3	CLDN17	GOT2	KISS1R	OR2T29	Q59F17	SLC51A	WDR19
B3KP11	CLDN18	GP1BA	KIT	OR2T3	Q59F30	SLC51B	WDR6
B3KP22	CLDN19	GP1BB	KITLG	OR2T32	Q59F54	SLC52A1	WDR73
B3KP57	CLDN2	GP2	KL	OR2T33	Q59F74	SLC52A2	WEE2
B3KP78	CLDN20	GP5	KLB	OR2T34	Q59F93	SLC52A3	WHRN
B3KPB8	CLDN22	GP6	KLC2	OR2T35	Q59FC4	SLC5A1	WLS
B3KPD2	CLDN23	GP9	KLF9	OR2T4	Q59FD5	SLC5A10	WNK2
B3KPI2	CLDN24	GPA33	KLHDC8B	OR2T5	Q59FG4	SLC5A11	WNT1
B3KPI8	CLDN25	GPAM	KLHL41	OR2T6	Q59FH4	SLC5A12	WNT3
B3KQH8	CLDN3	GPBAR1	KLHL7	OR2T7	Q59FK9	SLC5A2	WNT4
B3KQH9	CLDN34	GPBP1	KLK6	OR2T8	Q59FL8	SLC5A3	WNT5A
B3KQM8	CLDN4	GPC1	KLK7	OR2V1	Q59FM4	SLC5A4	WNT5B
B3KQN7	CLDN5	GPC2	KLKB1	OR2V2	Q59FM9	SLC5A5	WNT6
B3KQS1	CLDN6	GPC3	KLRB1	OR2W1	Q59FR6	SLC5A6	WNT7A
B3KQV4	CLDN7	GPC4	KLRC1	OR2W3	Q59FT4	SLC5A7	WNT7B
B3KQX7	CLDN8	GPC5	KLRC2	OR2W5P	Q59FZ9	SLC5A8	WWC1

B3KR01	CLDN9	GPC6	KLRC3	OR2W6P	Q59G08	SLC5A9	WWOX
B3KR10	CLDND1	gpcr	KLRC4	OR2Y1	Q59G84	SLC6A1	WWP1
B3KR29	CLDND2	GPCR40	KLRD1	OR2Z1	Q59G95	SLC6A11	WWTR1
B3KR81	CLEC10A	GPD1L	KLRF1	OR3A1	Q59GA0	SLC6A12	X2J7C8
B3KRC4	CLEC12A	GPER	KLRF2	OR3A2	Q59GA4	SLC6A13	XCR1
B3KRI1	CLEC12B	GPER1	KLRG1	OR3A3	Q59GA6	SLC6A14	XG
B3KRM3	CLEC14A	GPHN	KLRK1	OR3A4	Q59GC1	SLC6A15	XK
B3KRT0	CLEC17A	GPI	KMT2E	OR3A4P	Q59GD1	SLC6A17	XKR3
B3KRT6	CLEC1A	GPIHBP1	KNCN	OR4A15	Q59GD7	SLC6A18	XKR4
B3KRU1	CLEC1B	GPM6A	KNG1	OR4A16	Q59GE5	SLC6A19	XKR5
B3KRV7	CLEC2A	GPM6B	KNSTRN	OR4A4	Q59GF1	SLC6A2	XKR6
B3KRY5	CLEC2B	GPNMB	KNTC1	OR4A47	Q59GH3	SLC6A20	XKR7
B3KS23	CLEC2D	GPR101	KPG_008	OR4A4P	Q59GI7	SLC6A3	XKR8
B3KSJ0	CLEC4A	GPR107	KPG_009	OR4A5	Q59GJ2	SLC6A4	XKR9
B3KSK8	CLEC4C	GPR116	KPG_011	OR4A8	Q59GL5	SLC6A5	XKRX
B3KSM9	CLEC4D	GPR119	KRAS	OR4B1	Q59GL7	SLC6A6	XLKD1
B3KSY7	CLEC4E	GPR12	KREMEN1	OR4C11	Q59GM4	SLC6A7	XPC
B3KSZ3	CLEC4F	GPR132	KREMEN2	OR4C12	Q59GM5	SLC6A8	XPNPEP2
B3KSZ7	CLEC4G	GPR135	KRIT1	OR4C13	Q59GR1	SLC6A9	XPO6
B3KT73	CLEC4M	GPR137B	KRT1	OR4C15	Q59GU9	SLC7A1	XPR1
B3KTM0	CLEC5A	GPR139	KRT10	OR4C16	Q59GW0	SLC7A10	XRCC5
B3KTU1	CLEC6A	GPR141	KRT14	OR4C3	Q59GX2	SLC7A11	XRN1
B3KTU5	CLEC7A	GPR142	KRT16	OR4C45	Q59H00	SLC7A13	YBX1
B3KTV8	CLECL1P	GPR143	KRT17	OR4C46	Q59H01	SLC7A14	YES1
B3KU27	CLECSF5	GPR146	KRT2	OR4C5	Q59H05	SLC7A2	YIPF1
B3KUB8	CLIC1	GPR148	KRT75	OR4C6	Q59H32	SLC7A3	YIPF3
B3KUG1	CLIC2	GPR149	KRT77	OR4D1	Q59H34	SLC7A4	YIPF4
B3KUI0	CLIC4	GPR15	KSR1	OR4D10	Q59H35	SLC7A5	YKT6
B3KUQ8	CLIC5	GPR150	KSR2	OR4D11	Q59H41	SLC7A6	YRDC
B3KUU3	CLIC6	GPR151	KTN1	OR4D2	Q59H46	SLC7A7	YTHDC1
B3KV27	CLINT1	GPR152	L1CAM	OR4D5	Q59H69	SLC7A8	YWHAE
B3KV59	CLIP3	GPR153	LAG3	OR4D6	Q59HA9	SLC7A9	YWHAH
B3KVJ0	CLMP	GPR154	LAIR1	OR4D9	Q59HC2	SLC8A1	ZACN
B3KVN0	CLN3	GPR157	LAIR2	OR4E1	Q59HC3	SLC8A2	ZAN
B3KVN7	CLNS1A	GPR158	LAMA2	OR4E2	Q59HE0	SLC8A3	ZAP70
B3KVN8	CLPTM1	GPR160	LAMP1	OR4F14	Q59HF3	SLC8B1	ZBTB33
B3KVP9	CLRN1	GPR161	LAMP2	OR4F15	Q59HF9	SLC9A1	ZBTB42
B3KVQ4	CLRN2	GPR162	LAMP3	OR4F16	Q59HG0	SLC9A2	ZC4H2
B3KVV2	CLSTN1	GPR17	LAMP5	OR4F17	Q59HG2	SLC9A3	ZDHHC17
B3KVX5	CLSTN2	GPR171	LAMTOR1	OR4F21	Q59HG8	SLC9A3R2	ZDHHC2
B3KW38	CLSTN3	GPR173	LAMTOR2	OR4F3	Q5JB43	SLC9A4	ZDHHC20
B3KW46	CLTRN	GPR174	LAMTOR3	OR4F4	Q5U025	SLC9A5	ZDHHC21
B3KW93	CMKLR1	GPR176	LANCL2	OR4F5	Q5U0C9	SLC9A6	ZDHHC22
B3KWC7	CMKLR2	GPR179	LANCL3	OR4F6	Q5U0D0	SLC9A7	ZDHHC4
B3KWF2	CMTM6	GPR18	LAPTM4B	OR4K1	Q5U0F2	SLC9A8	ZDHHC5
B3KWG3	CMYA5	GPR182	LAPTM5	OR4K13	Q5U0F3	SLC9A9	ZFYVE19
B3KWK3	CNFN	GPR183	LARGE	OR4K14	Q5U0K3	SLC9B1	ZFYVE27
B3KWN4	CNGA1	GPR19	LARGE1	OR4K15	Q69F39	SLC9B2	ZGPAT
B3KWY5	CNGA2	GPR20	LAT	OR4K17	Q6IEV1	SLC9C1	ZMAT3

B3KX12	CNGA3	GPR21	lat1	OR4K2	Q6IEW6	SLC9C2	ZMYND10
B3KX18	CNGA4	GPR22	LAT2	OR4K3	Q6IEX5	SLCO1A2	ZMYND19
B3KX31	CNGB1	GPR24	LAX1	OR4K5	Q6IEZ2	SLCO1B1	ZNF397
B3KX35	CNGB3	GPR25	LCE1D	OR4L1	Q6IF01	SLCO1B3	ZNF607
B3KX58	CNKSR1	GPR26	LCK	OR4M1	Q6IFA1	SLCO1B3-SLCO1B7	ZNF804A
B3KX87	CNKSR2	GPR27	LCP1	OR4M2	Q6IFB0	SLCO1B7	ZNRF1
B3KXA2	CNKSR3	GPR3	LCP2	OR4N2	Q6IFE0	SLCO1C1	ZNRF2
B3KXI1	CNNM1	GPR30	LCT	OR4N4	Q6IFI9	SLCO2A1	ZNRF3
B3KXJ3	CNNM2	GPR31	LDB2	OR4N5	Q6IFM2	SLCO2B1	ZP1
B3KXK0	CNNM3	GPR32	LDLR	OR4P4	Q6IFM7	SLCO3A1	ZP2
	CNNM4	GPR32P1	LDLRAD3	OR4Q2	Q6IFR1	SLCO4A1	ZP3
	CNOT2	GPR33	LDLRAP1	OR4Q3	Q6IFR3	SLCO4C1	ZP4
							ZYX

VI. Polynucleotide and expression vectors

[0063] In addition to the SHEDTAC fusion proteins disclosed above, the invention also provides polynucleotide sequences that encode such fusions, expression constructs for expressing the fusion proteins, multimeric fusion protein combinations, and host cells that harbor the polynucleotides or expression constructs. The polynucleotide sequences of the invention can be any polynucleotide having a nucleotide sequence that encodes the fusion protein of the invention, although DNA is preferred. The recombinant constructs or expression vectors of the invention harbor a polynucleotide sequence of the invention that encodes a SHEDTAC fusion polypeptide. The recombinant constructs of the invention may be obtained by ligating (inserting) the polynucleotide (DNA) of the invention into a suitable vector. More specifically, the recombinant vector may be obtained by cleaving purified polynucleotide (DNA) with a suitable restriction enzyme, then inserting the cleaved polynucleotide to a restriction enzyme site or multicloning site on a suitable vector, and ligating the polynucleotide to the vector. The vector for inserting the polynucleotide sequence is not subject to any particular limitation, provided it is capable of replication in an appropriate host. The expression vectors of the invention are not subject to any particular limitation, and may be, for example, bacteriophages, plasmids, cosmids or phagemids. Examples of recombinant bacteriophage or phagemid vectors include that based on a filamentous phage such as M13. Plasmid vectors include those based on plasmids from, e.g., E. coli (e.g., pET23a, pBR322, pBR325, pUC118 and pUC119), plasmids from Bacillus subtilis (e.g., pUB110 and pTP5), and plasmids from yeasts (e.g., YEp13, YEp24 and YCp50). The expression vectors can also include animal viruses such as retroviruses, vaccinia viruses and insect viruses (e.g., baculoviruses).

[0064] In some embodiments, the vector for expressing the SHEDTAC fusion proteins of the invention is a viral vector. Various viral expression vectors can be used in the invention. For example, lentiviral vectors are suitable for introducing into and expressing in the host cell the combinatorial library of candidate binding partners. Lentiviral vectors are retroviral vectors that are able to transduce or infect both dividing and non-dividing cells and typically produce high viral titers. Examples of lentiviral based vectors that may be employed and modified for the invention include, e.g., pLV2, pLVX-Puro, pLVX-IRES-Neo, pLVX-IRES-Hyg, and pLVX-IRES-Puro. The various lentiviral vectors with cloned candidate ligand sequences can be introduced into an appropriate host cell for expressing the candidate ligand library. For example, the HEK293 cell line, the HEK293T cell line, and the TF-1 cell line are all suitable for the invention. Many other packaging cell lines well known in the art (e.g., Lenti-X 293T cell line) may also be employed for expressing the combinatorial library in the invention. In addition to lentiviral based vectors and host cells, other retroviral based vectors and expression systems may also be employed in the practice of the methods of the invention. These include MMLV based vectors pQCXIN, pQCXIQ and pQCXIH, and compatible producer cell lines such as HEK 293 based packaging cell lines GP2-293, EcoPack 2-293 and AmphoPack 293, as well as NIH/3T3-based packaging cell line RetroPack PT67.

[0065] In the expression constructs of the invention, the polynucleotide encoding the SHEDTAC fusion is generally ligated downstream from the promoter in a suitable vector in such a way as to be expressible. For example, if the host during transformation is an animal cell, preferred promoters include promoters from SV40, retrovirus promoters, metallothionein promoters, heat shock promoters, cytomegalovirus promoters and the SRα promoter. If the host is a genus Escherichia organism, preferred promoters include the tetracycline promoter, the Trp promoter, the T7 promoter, the lac promoter, the recA promoter, the λ promoter and the lpp promoter. If the host is a genus Bacillus organism, preferred promoters include the SPO1 promoter, the SPO2 promoter and the penP promoter. If the host is a yeast, preferred promoters include the PHO5 promoter, the PGK promoter, the GAP promoter, the ADH1 promoter and the GAL promoter. If the host is an insect cell, preferred promoters include the polyhedrin promoter and the P10 promoter.

[0066] In addition to the above, the recombinant vector used in the invention may contain, if desired, an enhancer, a splicing signal, a poly(A) addition signal, a ribosome

binding sequence (SD sequence), a selective marker and the like. Examples of selective markers include the tetracycline resistance gene, the carbencillin resistance gene, the dihydrofolate reductase gene, the ampicillin resistance gene and the neomycin resistance gene. The recombinant vector of the invention may additionally include a polynucleotide having a nucleotide sequence encoding an amino acid sequence for enhancing translation and/or a polynucleotide having a nucleotide sequence encoding a peptide sequence for purification. For example, the vectors can employ a translational enhancer element (TEE) sequence (see, e.g., Batten et al., FEBS Lett. 580:2591-7, 2006).

[0067] The invention further provides host cells that express the SHEDTAC fusion polypeptides described herein. The host cells are genetically engineered (transduced, transformed or transfected) with the recombinant constructs or expression vectors disclosed herein for production of the fusion protein or examination of its activity. To generate such cells, a recombinant construct which harbors and expresses a SHEDTAC fusion sequence can be introduced into a suitable host. The engineered host cells can be cultured in conventional nutrient media modified as appropriate for activating promoters, selecting transformants or amplifying particular genes such as the fusion gene encoding a SHEDTAC fusion polypeptide. The culture conditions for particular host cells selected for expression, such as temperature, pH and the like, will be readily apparent to the ordinarily skilled artisan. In some embodiments, the SHEDTAC fusion sequence is stably integrated into the chromosome of the host cells. With such host cells, the SHEDTAC sequence and its expression are substantially maintained in successive generations of cells. They are distinguished from host cells which transiently express the fusion polypeptide as detailed herein.

[0068] As noted above, the host cell for production or expression of a SHEDTAC construct of the invention, for example, can be a higher eukaryotic cell, such as a mammalian cell, or a lower eukaryotic cell, such as a yeast cell, or the host cell can be a prokaryotic cell, such as a bacterial cell. The selection of an appropriate host is within the scope of those skilled in the art and also exemplified in the Examples herein. Preferably, the host cell employed is suitable for expression of the SHEDTAC fusion protein. Representative examples of appropriate host cells suitable for practicing the present invention include, but need not be limited to, bacterial cells, such as E. coli, Streptomyces, Salmonella tvphimurium; fungal cells, such as yeast; insect cells, such as Drosophila S2 and

Spodoptera Sf9; animal cells, such as CHO, COS or 293 cells; adenoviruses; plant cells, or any suitable cell already adapted to in vitro propagation or so established de novo.

[0069] Introduction of the vector or expression construct into the host cell can be affected by a variety of methods with which those skilled in the art will be familiar, including but not limited to, for example, calcium phosphate transfection, DEAE-Dextran mediated transfection, or electroporation (see, e.g., Brent et al., *Current Protocols in Molecular Biology*, John Wiley & Sons, Inc. (ringbou ed., 2003)). Expression and, if desired, purification, of a SHEDTAC fusion polypeptide in a transfected or transformed host cell can be carried out in accordance with any of the routinely practiced methods in the art, e.g., Sambrook et al., *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Press, N.Y., (3rd ed., 2000); and Brent et al., *Current Protocols in Molecular Biology*, John Wiley & Sons, Inc. (ringbou ed., 2003). Typically, to produce the fusion protein of the invention, the host cell harboring the expression vector is cultured under appropriate conditions that allow the polynucleotide (DNA) encoding the fusion protein to be expressed, thereby inducing formation and accumulation of the fusion polypeptide, then isolating and purifying the fusion polypeptide. The fusion protein expressed in the host cell can be readily isolated and purified. Specifically, when the fusion protein of the invention accumulated within cultured bacteria or within cultured cells, following the completion of cultivation, an extract of the fusion protein of the invention may be obtained by a conventional method such as centrifugation or filtration after using a conventional technique (e.g., detergents, ultrasound, lysozymes, freezing and thawing) to disrupt the bacteria or cells. When the SHEDTAC fusion polypeptide accumulates in the periplasmic space, following the completion of cultivation, an extract containing the target protein may be obtained by a conventional method such as osmotic shock. When the fusion protein of the invention accumulates in the culture broth, following the completion of cultivation, a culture supernatant containing the inventive fusion protein may be obtained by using a conventional method such as centrifugation or filtration to separate the culture supernatant from the bacteria or cells.

VIII. Therapeutic applications and pharmaceutical compositions

[0070] The Sheddase-Targeting Chimeras (SHEDTACs) described herein can find many therapeutic or prophylactic applications. These fusion molecules can be employed for targeted proteolysis of many membrane-bound proteins for modulating cellular signaling

events and/or for treating diseases. In some embodiments, the compositions and methods described herein are employed to proteolyze a surface antigen that is associated with progression of cancers or tumors. These include tumor antigens associated with cardiac cancer (e.g., sarcoma, myxoma, rhabdomyoma, fibroma, lipoma and teratoma); lung cancer (e.g., bronchogenic carcinoma, alveolar carcinoma, bronchial adenoma, sarcoma, lymphoma, chondromatous hamartoma, mesothelioma); various gastrointestinal cancer (e.g., cancers of esophagus, stomach, pancreas, colon, small bowel, and large bowel); genitourinary tract cancer (e.g., kidney, bladder and urethra, prostate, testis; liver cancer (e.g., hepatoma, cholangiocarcinoma, hepatoblastoma, angiosarcoma, hepatocellular adenoma, hemangioma); bone cancer (e.g., osteogenic sarcoma, fibrosarcoma, malignant fibrous histiocytoma, chondrosarcoma, Ewing's sarcoma, malignant lymphoma, multiple myeloma, malignant giant cell tumor chordoma, osteochondroma, benign chondroma, chondroblastoma, chondromyxofibroma, osteoid osteoma and giant cell tumors); cancers of the nervous system (e.g., of the skull, meninges, brain, and spinal cord); gynecological cancers (e.g., uterus, cervix, ovaries, vulva, vagina); hematologic cancer (e.g., cancers relating to blood, Hodgkin's disease, non-Hodgkin's lymphoma); skin cancer (e.g., malignant melanoma, basal cell carcinoma, squamous cell carcinoma, Karposi's sarcoma, moles dysplastic nevi, lipoma, angioma, dermatofibroma, keloids, psoriasis); and cancers of the adrenal glands (e.g., neuroblastoma).

[0071] The SHEDTAC fusion molecules described herein (e.g., LAG3- or PD-1-targeting SHEDTACs) can be used in either prophylactic or therapeutic applications (e.g., cancer immunotherapies). In prophylactic applications, pharmaceutical compositions or medicaments are administered to a subject susceptible to, or otherwise at risk of, developing a disease or condition (*i.e.*, melanoma) in an amount sufficient to eliminate or reduce the risk, lessen the severity, or delay the outset of the disease, including biochemical, histologic and/or behavioral symptoms of the disease, its complications and intermediate pathological phenotypes presenting during development of the disease. In therapeutic applications, compositions or drugs are administered to a patient suspected of, or already suffering from such a disease in an amount sufficient to cure, or at least partially arrest, the symptoms of the disease (biochemical, histologic and/or behavioral), including its complications and intermediate pathological phenotypes in development of the disease. Often, the therapeutic benefit is monitored and repeated dosages are given if the benefit starts to wane.

[0072] The SHEDTAC fusion molecules for use in the methods of the invention should be administered to a subject in an amount that is sufficient to achieve the desired therapeutic effect (e.g., eliminating or ameliorating symptoms associated with a relevant condition). An amount adequate to accomplish therapeutic or prophylactic treatment is defined as a therapeutically- or prophylactically-effective dose. Thus, a therapeutically- or prophylactically-effective dose or efficacious dose of the functional derivative effector polypeptide is employed in the pharmaceutical compositions of the invention. In both prophylactic and therapeutic regimes, agents are usually administered in several dosages until a sufficient benefit has been achieved. Actual dosage levels of the active ingredients in the pharmaceutical compositions of the present invention can be varied so as to obtain an amount of the active ingredient which is effective to achieve the desired therapeutic response for a particular subject, composition, and mode of administration, without being toxic to the subject. The selected dosage level depends upon a variety of pharmacokinetic factors including the activity of the particular compositions of the present invention employed, the route of administration, the time of administration, and the rate of excretion of the particular compound being employed. It also depends on the duration of the treatment, other drugs, compounds and/or materials used in combination with the particular compositions employed, the age, gender, weight, condition, general health and prior medical history of the subject being treated, and like factors. Methods for determining optimal dosages are described in the art, e.g., Remington: The Science and Practice of Pharmacy, Mack Publishing Co., 20th ed., 2000. Typically, a pharmaceutically effective dosage would be between about 0.001 and 100 mg/kg body weight of the subject to be treated.

[0073] Typically, the SHEDTACs of the invention are formulated in pharmaceutical compositions for the therapeutic or prophylactic applications disclosed herein. The pharmaceutical compositions can be prepared in various forms, such as granules, tablets, pills, suppositories, capsules, suspensions, salves, lotions and the like. The pharmaceutical compositions typically contain a therapeutically effective amount of the active effector molecule (e.g., LAG3- or PD-1-targeting SHEDTAC of the invention). The concentration of therapeutically active compound in the formulation may vary from about 0.1-100% by weight. In addition to the active effector polypeptide, the compositions may contain pharmaceutically acceptable carriers and other ingredients known to facilitate administration and/or enhance uptake. Formulations for parenteral administration may, for example, contain

excipients, sterile water, or saline, polyalkylene glycols such as polyethylene glycol, oils of vegetable origin, or hydrogenated napthalenes. Biocompatible, biodegradable lactide polymer, lactide/glycolide copolymer, or polyoxyethylene-polyoxypropylene copolymers may be used to control the release of the compounds. Other potentially useful parenteral delivery systems for molecules of the invention include ethylene-vinyl acetate copolymer particles, osmotic pumps, implantable infusion systems, and liposomes. Formulations for inhalation may contain excipients, for example, lactose, or may be aqueous solutions containing, e.g., polyoxyethylene-9-lauryl ether, glycocholate and deoxycholate, or may be oily solutions for administration in the form of nasal drops, or as a gel. Therapeutic formulations are prepared by any methods well known in the art of pharmacy. The therapeutic formulations can be delivered by any effective means which could be used for treatment. See, e.g., *Goodman & Gilman's The Pharmacological Bases of Therapeutics*, Hardman et al., eds., McGraw-Hill Professional (10th ed., 2001); *Remington: The Science and Practice of Pharmacy*, Gennaro (ed.), Lippincott Williams & Wilkins (20th ed., 2003); and *Pharmaceutical Dosage Forms and Drug Delivery Systems*, Ansel et al. (eds.), Lippincott Williams & Wilkins (7th ed., 1999). Pharmaceutical compositions are preferably manufactured under GMP conditions.

[0074] The pharmaceutical compositions are usually administered to the subjects on multiple occasions. Intervals between single dosages can be daily, weekly, monthly or yearly. Intervals can also be irregular as indicated by measuring blood levels of the derivative effector polypeptide and the other therapeutic agents used in the subject. In some methods, dosage is adjusted to achieve a plasma concentration of 1–1000 μg/ml, and in some methods 25-300 μg/ml or 10-100 μg/ml. Alternatively, the therapeutic agents can be administered as a sustained release formulation, in which case less frequent administration is required. Dosage and frequency vary depending on the half-life of the effector polypeptide and the other drugs in the subject. The dosage and frequency of administration can vary depending on whether the treatment is prophylactic or therapeutic. In prophylactic applications, a relatively low dosage is administered at relatively infrequent intervals over a long period of time. Some subjects may continue to receive treatment for the rest of their lives. In therapeutic applications, a relatively high dosage at relatively short intervals is sometimes required until progression of the disease is reduced or terminated, and preferably

until the subject shows partial or complete amelioration of symptoms of disease. Thereafter, the subject can be administered a prophylactic regime.

[0075] The invention also provides kits for carrying out the therapeutic applications disclosed herein. For example, the invention provides therapeutic kits for immunotherapies in the treatment of subjects afflicted with tumors, e.g., melanoma. The therapeutic kits of the invention typically comprise as active agent one or more of the described SHEDTACs. The kits can optionally contain suitable pharmaceutically acceptable carriers or excipients for administering the active agents. The pharmaceutically acceptable carrier or excipient suitable for the kits can be coatings, isotonic and absorption delaying agents, binders, adhesives, lubricants, disintergrants, coloring agents, flavoring agents, sweetening agents, absorbants, detergents, and emulsifying agents. Other reagents that can be included in the kits include antioxidants, vitamins, minerals, proteins, fats, and carbohydrates. The therapeutic kits can further include packaging material for packaging the reagents and a notification in or on the packaging material. The kits can additionally include appropriate instructions for use and labels indicating the intended use of the contents of the kit. The instructions can be present on any written material or recorded material supplied on or with the kit or which otherwise accompanies the kit.

EXAMPLES

[0076] The following examples are provided to further illustrate the invention but not to limit its scope. Other variants of the invention will be readily apparent to one of ordinary skill in the art and are encompassed by the appended claims.

Example 1. Construction, expression and purification of LAG3- and PD-1-targeting SHEDTACs

[0077] Construction and validation of LAG3-targeting SHEDTACs are shown in Figure 4. In brief, a total of 30 SHEDTACs that target membrane protein LAG3 and ADAM protease ADAM10 were generated. The LAG3-targeting domain and the ADAM10-targeting domain in these SHEDTAC constructs are both VHH sdAb domains (variable domains of heavy chain of heavy chain-only antibody). To construct these SHEDTACs, 3 sdAb domains targeting LAG3 and 5 sdAb domains targeting ADAM10 are used. Each sdAb has a different sequence and targets a different epitope on LAG3 or ADAM10. Among

the 30 SHEDTACs, 15 have the ADAM10-targeting domain at the N-terminus and the LAG3-targeting domain at the C-terminus, with the other 15 having the ADAM10-targeting domain at the C-terminus and the LAG3-targeting domain at the N-terminus. The 15 constructs in each configuration represent all possible LAG3/ADAM10 sdAb pairings (3x5=15). As explained below, a linker sequence is used to connect the 2 targeting domains in the SHEDTACs. PD-1 targeting SHEDTACs detailed in Example 4 were similarly produced using the protocols as described herein for LAG3-targeting SHEDTACs.

Table 3. sdAb domains targeting different epitopes on protease or membrane proteins

sdAb clone	Reactivity	SEQ ID NO:	Reference
32-G6	ADAM10	12	EP2514767A1
33-C6	ADAM10	13	EP2514767A1
39-B1	ADAM10	14	EP2514767A1
39-C1	ADAM10	15	EP2514767A1
39-D1	ADAM10	16	EP2514767A1
W3396-R2-13	LAG3	17	WO2019179365
AS20592VH10	LAG3	18	WO2019185040
F023700842	LAG3	19	KR20190116546A
AS15193	PD-1	20	WO2019137541
NB52	PD-1	21	WO2019104860

[0078]

[0079] SEQ ID NO:12: ADAM10 targeting sdAb 1 (32-G6)

EVQLVESGGGLVQPGGSLRLSCAASGFTFDDYAIGWFRQAPGKEREGVSCISSSDGR TYYDDSVKGRFTISSDNAKNTVYLQMNSLKPEDTAVYYCAADRLAYGLDPNFYDY WGQGTQVTVSS

[0080] SEQ ID NO:13: ADAM10 targeting sdAb 2 (33-C6)

EVQLVESGGGLVQAGDSLRLSCAASGRTFSSYYMGWFRQAPGKEREFVAHISLSGG NTEYADSVKGRFTITRDNAGNTVYLQMNSLKPEDTGVYCCAASPSLRSAWQYWGQ GTQVTVSS

[0081] SEQ ID NO:14: ADAM10 targeting sdAb 3 (39-B1)

EVQLVESGGGLVQAGGSLRLSCARSGRISNINIMAWYRQAPGKTRDMVAAIIGDSTN YADSVKGRFTISRDNAKNTVYLHMNRLKPEDTGVYYCKISGVDWGQGTQVTVSS

[0082] SEQ ID NO:15: ADAM10 targeting sdAb 4 (39-C1)

KVQLVESGGGLVQAGGSLRLSCAASGNIFINNAVGWYRQAPGKQREMVAAMLSGGS TNYADSVKGRFTISRDNAKNTVYLQMNSLKPEDTAVYYCNVQVNGTWARWGQCTQ VTVSS

[0083] SEQ ID NO:16: ADAM10 targeting sdAb 5 (39-D1)
KVQLVESGGGLVQAGGSLRLSCAASGNIFINNAVGWYRQAPGKQREMVAAMLSGGS
TNYADSVKGRFTISRDNAKNTVYLQMNSLKPEDTAVYYCNVQVNGTWARWGQCTQ
VTVSS

[0084] SEQ ID NO:17: LAG3 targeting sdAb 1 (W3396-R2-13)
QVQLVESGGGVVQPGGSLRLSCAASGLTLSQYTMGWFRQAPGKERELVAAIHWTS
SVTDYADSVRGRFTISRDDSKNTGYLQMNSLRAEDTAVYYCAATWYYTHRGSFDY
WGQGTLVTVSS

[0085] SEQ ID NO:18: LAG3 targeting sdAb 2 (AS20592VH10)
EVQLVESGGGLVQPGGSLRLSCAASGYIISSYCMGWFRQAPGKGLEGVAAIDSDGG
TSYADSVKGRFTISKDNSKNTLYLQMNSLRAEDTAMYYCAADFCWVDEDRHLYEY
NSWGQGTLVTVSS

[0086] SEQ ID NO:19: LAG3 targeting sdAb 3 (F023700842)
EVQLVESGGGVVQPGGSLRLSCAASGRTFSDYVMGWFRQAPGKEREFVAAISESGG
RTHYADSVKGRFTISRDNSKNTLYLQMNSLRPEDTALYYCATTLLWWTSEYAPIKA
NDYDYWGQGTLVTVSS

[0087] SEQ ID NO:20: PD-1 targeting sdAb 1 (AS15193)
EVQLVESGGGSAQAGGSLRLSCVVSGNIYNRNFMGWFRQAPGKVREGVAAIYTGT
SRTYYADSVKGRFTISQDNAKNTVYLQMNSLKPEDTAMYYCAADLRDGFWDTGV
WNTWGQGTQVTVSSSLCTPSRGTQVTVSS

[0088] SEQ ID NO:21: PD-1 targeting sdAb 2 (NB52)
EVQLVESGGGSVQSGGSLRLTCAASGYTYSNYYMGWFRQAPGKEREGVASIDTLG
YTRYADSVKGRFTISRDNDKNTLYLQMSSLQPEDTAMYYCAAPRSPYYRGQTFWE
GAYNYWGQGTQVTVSSSLCTPSRGTQVTVSS

[0089] As listed in Table 3, sequences of the 8 sdAb domains used to construct the LAG3/ADAM10-targeting or PD-1/ADAM10-targeting SHEDTACs are all known in the art. Specifically, sequences and activities of the 5 ADAM10-targeting sdAb domains are described in European patent application EP2514767A1 (Clones 32-G6, 33-C6, 39-B1, 39-C1 and 39-D1). The 3 LAG3-targeting sdAb domains are respectively described in WO2019179365 (Clone W3396-R2-13), WO2019185040 (Clone AS20592VH10) and KR20190116546A (Clone F023700842). Genes corresponding to each sdAb were synthesized as gene fragments (Twist Biosciences), each containing a 5' adapter nucleotide

sequence (atggttaaattttctgaagtgcagttagttgaatct) (SEQ ID NO:1) and a 3' adapter nucleotide sequence (acgcaggtgactgtgagctca) (SEQ ID NO:2). Synthetic genes are amplified using primers that anneal to these 5' or 3' adapter regions using Q5® Hot Start High-Fidelity DNA Polymerase (New England Biolabs, M0493S) according to the manufacturer's protocol. Genes encoding N-terminal LAG3-SHEDTAC domains are amplified as "A" fragments using A.fwd and A.rev, while genes encoding C-terminal LAG3-SHEDTAC domains are amplified as "B" fragments using B.fwd and B.rev. N-terminal and C-terminal domains are separated by a linker region with amino acid sequence "GGGGSGGGGSGGGGS*ENLYFQ|G*GGGGSGGGGSGGGGS" (SEQ ID NO:3), containing a TEV-cleavage site (ENLYFQG; SEQ ID NO:4) indicated in italicized where the cut site is denoted by " | ". In parallel, a pET23a vector backbone (Novagen) was amplified as a "C" fragment using primers C.fwd and C.rev. A, B, and C DNA fragments are assembled using NEBuilder® HiFi DNA Assembly (New England Biolabs, E2621S) according to the manufacturer's protocol.

[0090] A.fwd: atggttaaattttctgaagtgcagttag (SEQ ID NO:5)

[0091] A.rev: gccttgaaagtacaagttttcgctgccgcctcctccgctgccgccgccgccgctgccgcctcctcctgagctcacagtcacctgcgt (SEQ ID NO:6)

[0092] B.fwd: gaaacttgtactttcaaggcggcggcggcggcagcggcggcggcggcagcggcggcggcggcagcatggttaaattttctgaagtgcagttag (SEQ ID NO:7)

[0093] B.rev: tgagctcacagtcacctgcgt (SEQ ID NO:8)

[0094] C.fwd: acgcaggtgactgtgagctcacatcatcaccatcaccattaa-tgagatccg (SEQ ID NO:9)

[0095] C.rev: ctaactgcacttcagaaaatttaaccatatgacctcctcttaaa-gttaaacaaaattatttctag (SEQ ID NO:10)

[0096] The coding sequence for each LAG3-SHEDTAC are transformed into E. coli strain BL21(DE3) (Invitrogen). E. coli harboring the pET23-LAG3-SHEDTAC plasmid are cultured overnight in sterile 15mm glass culture tubes containing 5ml of Luria Broth supplemented with 100µg/ml of carbenicillin. The following day, cells are diluted 1:100 into a sterile 500ml baffled glass flask containing 100ml of Terrific Broth supplemented with

100µg/ml carbenicillin, and are grown at 37°C with shaking at 200RPM. Once cells reached an optical density (OD) of approximately 0.75, the culture was cooled to 18°C and protein expression was induced by the addition of Isopropyl ß-D-1-thiogalactopyranoside (IPTG) at a final concentration of 1mM. Following 16h expression at 18°C, 200RPM, cells are centrifuged at 5000 relative centrifugal force (RCF) and the cell pellet resuspended in 50ml of Dulbecco's Phosphate-Buffered Saline (DPBS) containing 1mg/ml lysozyme. Cells are lysed by stirring at room temperature for 30min, cooled on ice, and further lysed using a sonicator (Fisher model FB50, amplitude 90, 3min continuous sonication). Following sonication, the 50ml cell lysate was centrifuged for 30min at 10,000 RCF, 4°C. The resulting supernatant was applied to nickel-nitrilotriacetic acid (Ni-NTA) resin and soluble LAG3-SHEDTAC (containing a C-terminal-hexahistidine affinity tag) was eluted with DPBS containing 500mM imidazole. Purified protein was buffer-exchanged into DPBS, boiled in reducing sodium dodecyl sulfate and analyzed by polyacrylamide gel electrophoresis.

Example 2. Activities of LAG3-SHEDTAC on proteolysis of cell surface LAG3

[0097] 30 LAG3-SHEDTACs (Figure 4, Panel A) were tested for their ability to accelerate LAG3 ectodomain cleavage from PBMCs by the sheddase ADAM10. The data in Figure 4 (Panel B) indicate the majority of LAG3-SHEDTACs tested accelerated LAG3 cleavage by ADAM10 following 24h treatment, as determined by diminished chemiluminescence intensity corresponding to intact LAG3. It should be noted that a subset of LAG3-SHEDTACs (BMGX0008, BMGX0009, BMGX0010, BMGX0012, BMGX0013, BMGX0016, BMGX0021, BMGX0023, BMGX0026) reduced LAG3 signal abundance by ≥75%. The LAG3-SHEDTAC BMGX0016 was used to demonstrate time-dependent effects on LAG3 abundance resulting from treatment with LAG3-SHEDTACs (Figure 4, Panels C-D). These data indicate LAG3-SHEDTACs rapidly accelerate LAG3 proteolysis by ADAM10, reducing surface LAG3 levels by ~50% after treatment for 30 minutes, and by ~75% after treatment for 1h. These data additionally indicate concomitant increases in soluble LAG3 ectodomain (sLAG3), supporting the mechanism of ectodomain release from the cell membrane. In good agreement with these data showing rapid proteolysis, it was found that treatment of CD3+ADAM10+ PBMCs with LAG3-SHEDTACs largely diminished detectable levels of cell surface LAG3 after 1h of treatment, as determined by flow cytometry (Figure 5, Panels A-F). To control for artificial signal reduction that could

occur through competitive epitope binding between LAG3-SHEDTAC targeting domains and flow cytometry detection antibodies, LAG3-SHEDTACs are proteolyzed and then applied to cells as two freely soluble constituent domains. By severing the linker region of the two targeting domains, TEV proteolysis additionally controls for the critical requirement of induced proximity between ADAM10 and LAG3 by intact LAG3-SHEDTACs. Indeed, cells treated with TEV-proteolyzed LAG3-SHEDTAC displayed cell surface LAG3 levels comparable to the vehicle control (Figure 5, Panels B, E), demonstrating the requirement for simultaneous and bispecific engagement of LAG3 and ADAM10. LAG3 cleavage by ADAM10 was accelerated by LAG3-SHEDTAC in a dose dependent manner following 1h of treatment as determined by flow cytometry (Figure 5, Panel F). Induction of ADAM10/ADAM17 activity by the addition of Phorbol-12-myristate-13-acetate and ionomycin was observed by western blot, indicating a band intensity corresponding to 80% of signal produced by cells treated with vehicle (Figure 5, Panel G). Levels of detectable LAG3 are further reduced by the addition of LAG3-SHEDTAC to within 21% of the control, in good agreement with LAG3 reduction as determined by flow cytometry. Primary LAG3 sequence analysis indicates transmembrane and cytosolic domains that together comprise ~10kDa of LAG3 (data not shown). It is therefore expected that a soluble LAG3 (sLAG3) ectodomain would be detected at a molecular weight that is ~10kDa lower than intact LAG3 (Figure 5, Panel H). Indeed, cell supernatants analyzed by western blot revealed a LAG3 fragment with molecular weight that is ~10kDa lower than full-length LAG3, consistent with the dose dependent production of sLAG3 ectodomain (Figure 5, Panel I).

Example 3. Additional studies on the activities of LAG3-targeting SHEDTACs

[0098] Induced proximity between an integral membrane protease and a membrane protein substrate represents a novel modality to modulate proteostasis that is distinct from technologies that rely on proteosomes and lysosomes (e.g. PROTACs, LYTACs, and others). To demonstrate this point, PMBCs were pre-treated for 2h with MG132 (www.cell.com/cell-chemical-biology/fulltext/S1074-5521(15)00193-3) or Dynasore (pubmed.ncbi.nlm.nih.gov/16740485/), inhibitors of the proteasome and lysosome, respectively (Figure 6). Cells were subsequently treated with LAG3-SHEDTACs or equimolar, TEV-proteolyzed controls. In all cases, robust induction of LAG3 shedding (>90%) was observed across all treatment groups, relative to TEV-digested controls (Figure

6, Panels A-C). LAG3 suppresses T cell function by binding to MHC class II molecules, which leads to decreased TCR signaling, reduced cytokine production, and limited proliferation. It is known that LAG3 shedding by ADAM10 terminates these inhibitory effects and restores T cell function.

[0099] To demonstrate the functional enhancements of LAG3-SHEDTAC to TCR, a commercial LAG3/MHCII blockade co-culture assay was employed (Promega JA1111). Luciferase reporter T cells were co-cultured with antigen-loaded antigen presenting cells (APCs) and T cell activation was determined by increased promoter-mediated luminescence (Figure 7, Panel A). LAG3-SHEDTACs accelerate LAG3 shedding from luciferase reporter cells by ADAM10 (Figure 7, Panel B) to afford a dose-dependent luminescence increase, indicating enhanced TCR signaling is achieved through accelerated LAG3 shedding (Figure 7, Panel C). Collectively, these data demonstrated a model of LAG3 cleavage by ADAM10 that is accelerated through proximity that is efficiently induced by LAG3-SHEDTACs.

Example 4. SHEDTACs targeting proteins not known to be sheddase substrates

[00100] SHEDTACs represent a promising platform technology that can be reconfigured to induce shedding of broad membrane protein targets. One might be motivated to use SHEDTACs for protein substrates that are known to be shed. One the other hand, it is unclear whether SHEDTACs are capable of inducing ectodomain shedding of membrane proteins that are otherwise not shed (not known to be substrates of sheddases or do not shed naturally. One such protein is the immune checkpoint receptor Programmed Cell Death-1 (PD-1) on T lymphocytes. Soluble forms of PD-1 have been measured in serum and are attributed to alternative gene splicing (www.ncbi.nlm.nih.gov/pmc/articles/PMC7710690/) (pubmed.ncbi.nlm.nih.gov/16171790/) (pubmed.ncbi.nlm.nih.gov/16171790/).

[00101] It is counter intuitive that inducing proximity between PD-1 and a sheddases (e.g. ADAM10) would afford PD-1 shedding. To investigate this possibility, 8 SHEDTACs (BMGX221-228) targeting PD-1 or ADAM10, termed "PD1-SHEDTACs" were generated. These reagents were composed of two separate sdAb domains targeting PD-1 (SEQ IDs:20-21), and five separate sdAb domains targeting ADAM10 (SEQ IDs:12-16) separated by a linker (SEQ ID:3). Specifically, PD1-SHEDTACs BMGX221-225 respectively contain at the N-terminus an ADAM10 binding sdAb shown in SEQ ID NOs:12-16, plus at the C-terminus a PD-1 binding sdAb shown in SEQ ID NO:20. PD1-SHEDTACs BMGX226-228

respectively contain at the N-terminus an ADAM10 binding sdAb shown in SEQ ID NOs:12-14, plus at the C-terminus a PD-1 binding sdAb shown in SEQ ID NO:21.

[00102] Upon expression and purification of the PD1-SHEDTACs, they were examined for effects on cell surface PD-1 levels. Specification, PBMCs were stimulated with PMA/ionomycin to induce ADAM10/17 activity. PD1-SHEDTACs were applied to cells for 24h at 37˚C in cell culture medium before analysis by western blot. Western blot analysis revealed increased PD1 expression by PMA/ionomycin that was robustly diminished by the addition PD1-SHEDTACs spanning multiple epitopes on PD-1 and ADAM10. These data suggest that SHEDTACs described in the present disclosure represent a generalized approach that (i) can be applied to proteins that are not obviously known as sheddase substrates and (ii) maintains high efficiency despite targeting diverse epitopes on the protease and/or target.

Example 5. Experimental methods

[00103] *Cell culture and LAG3 induction*: To induce LAG3 expression 6-well tissue culture plates (Falcon, 353046) are coated with 10µg of sterile anti-human CD3 antibody (Biolegend, 300438) in 2ml of Dulbecco's Phosphate Buffered Saline (ThermoFisher, J61196.AP, PBS)) per well for 24h at 4˚C. Coated plates are washed once with 2ml of PBS and peripheral blood mononuclear cells (PBMCs) are seeded into each well at a density of $1x10^6$ cells per ml in 2ml of 0.22µm sterile-filtered T complete growth medium, consisting of RPMI 1640 growth medium (gibco, 11875-093) supplemented with 10% v/v heat-inactivated fetal bovine serum (gibco, 10438-026), 55µM beta-mercaptoethanol, 1mM sodium pyruvate (gibco, 11360-070), MEM non-essential amino acids (gibco, 11140-050), 10mM HEPES buffer (gibco, 15630-080), 2mM L-glutamine (gibco, 25030-081), and 100 units/ml of penicillin/streptomycin (gibco, 15140-122). T cells are activated by the addition of 4µg sterile anti-human CD28 antibody (Biolegend, 302934) per well (2µg/ml final) and cells are cultured for 72h at 37˚C in 5% CO_2 atmosphere and 95% relative humidity.

[00104] *Flow cytometry:* Following 72h activation, cells are pooled into a sterile 50ml centrifuge tube (Genesee Scientific, 21-108) and pelleted by centrifugation at 350g for 5min at room temperature. Pelleted cells are resuspended in T complete growth medium to a density of $5x10^6$ cells per ml and seeded into a 96-well U-bottom tissue culture plate (Falcon, 353077) at $0.5x10^6$ cells per well in a 100µl final volume. Cells are treated by directly adding 10µl of a 1µM stock of SHEDTAC in PBS, or Tobacco etch virus (TEV)-

proteolyzed SHEDTAC for a final SHEDTAC concentration of 100nM. Cells are incubated with SHEDTACs for 1h at 37˚C in 5% CO_2 atmosphere and 95% relative humidity without shaking. Following treatment, cells are pelleted by centrifugation at 350g for 5min at room temperature and resuspended in 200µl of sort buffer, consisting of 0.22µm sterile-filtered PBS containing 1% w/v bovine serum albumin (Prometheus, 25-529C) and 1mM ethylenediaminetetraacetic acid (EDTA, Invitrogen, AM9260G). Cells are similarly pelleted and resuspended in 200µl sort buffer for two total washes. Washed PBMCs are suspended in 50µl of sort buffer containing 1:400 dilutions of APC anti-human CD156c (ADAM10) antibody (Biolegend, 352706); Brilliant Violet 421™ anti-human CD223 (LAG-3) antibody (Biolegend, 369314); and BD Pharmingen™ FITC Mouse anti-human CD3 antibody (BD Biosciences, 555332) and incubated for 1h at 4˚C. Stained cells are washed twice with sort buffer and analyzed on a ZE5 Cell Analyzer (Bio-Rad) for mean fluorescence intensities corresponding to cell surface CD3, ADAM10 and LAG3.

[00105] *Western Blot:* Following 72h activation, cells are plated into 96-well U-bottom plates as described above under *Flow cytometry*. Cells are treated by directly adding 10µl of a 1µM stock of SHEDTAC serially diluted 2-fold in PBS. Cells are incubated with SHEDTACs for 24h at 37˚C in 5% CO_2 atmosphere and 95% relative humidity without shaking. Following treatment, cells are pelleted by centrifugation at 1,000g for 5min at room temperature and cell pellet and culture medium are collected and analyzed for LAG3 or soluble LAG3 (sLAG3). The entire cell pellet or 80µl of cell culture medium was boiled in reducing SDS buffer, resolved by SDS-PAGE (Bio-Rad 4568096) and transferred to a nitrocellulose membrane (Bio-Rad 1620233) in 20mM Tris, 2mM glycine and 20% methanol using an XCell II Blot Module (Thermo Fisher) powered at 35V for 120min at room temperature. The membrane was blocked at 4˚C overnight in 5% w/v BSA in PBS + 0.1% v/v Tween-20 (PBST). The following day, the membrane was incubated for 1h with a 5ml volume of polyclonal goat IgG anti-human LAG3 (R&D Systems, AF2319) and rat anti-human GAPDH (Biolegend, 607902) each diluted 1:1000 in PBS containing 0.1% w/v BSA in PBST. The membrane was washed in PBST 3x15min and subsequently incubated for 1h with a 5ml volume of donkey anti-goat IgG-HRP (Santa Cruz, SC-2020) diluted 1:2000 in PBST, and mouse-anti rat IgG2a-AF647 (Southern Biotech, 3065-31) diluted 1:1000 in PBST. The membrane was washed 3x15min in PBST and stained with 3ml of SuperSignal Plus PICO West (Thermo Fisher). Chemiluminescence was immediately measured using a

ChemiDoc MP Gel imaging system (Bio-Rad) at 60s exposure. Fluorescence at 647nm was similarly measured using the factory filter set and a 30s exposure.

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[00106] Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be readily apparent to one of ordinary skill in the art in light of the teachings of this invention that certain changes and modifications may be made thereto without departing from the spirit or scope of the appended claims.

[00107] All publications, databases, GenBank sequences, patents, and patent applications cited in this specification are herein incorporated by reference as if each was specifically and individually indicated to be incorporated by reference.

WE CLAIM:

1. A fusion molecule, comprising a protease-targeting domain and a covalently linked substrate-targeting domain, wherein the protease-targeting domain specifically binds to an integral membrane protease or to an auxiliary membrane protein in a multimeric integral membrane protease complex, and the substrate-targeting domain specifically binds to a transmembrane substrate protein that is bound to the same phospholipid membrane; wherein the integral membrane protease bound by the fusion molecule can cleave the extracellular domain of the transmembrane substrate protein bound by the same fusion molecule.

2. The fusion molecule of claim 1, wherein the integral membrane protease is a sheddase.

3. The fusion molecule of claim 2, wherein the sheddase is a member of the A Disintegrin And Metalloprotease (ADAM) family of proteases.

4. The fusion molecule of claim 2, wherein the sheddase is ADAM 10.

5. The fusion molecule of claim 1, wherein the transmembrane substrate protein is a cellular signaling receptor or a receptor ligand.

6. The fusion molecule of claim 1, wherein the transmembrane substrate protein is a tumor-associated antigen.

7. The fusion molecule of claim 1, wherein the protease-targeting domain and the substrate-targeting domain are each an antibody or an antigen-binding fragment.

8. The fusion molecule of claim 7, wherein the protease-targeting domain and the substrate-targeting domain are each a single-domain molecule or a single chain variable fragment (scFv).

9. The fusion molecule of claim 7, wherein the protease-targeting domain and the substrate-targeting domain are each a sdAb.

10. The fusion molecule of claim 1, wherein the protease-targeting domain and the substrate-targeting domain are linked via a linker motif.

11. The fusion molecule of claim 1, wherein the protease-targeting domain recognizes ADAM10, and the substrate-targeting domain recognizes LAG3 or PD-1.

12. The fusion molecule of claim 11, wherein the protease-targeting domain comprises the amino acid sequence shown in any one of SEQ ID NOs:12-16, and the substrate-targeting domain comprises the amino acid sequence shown in any one of SEQ ID NOs:17-21.

13. The fusion molecule of claim 1, further comprising a covalently linked carrier moiety.

14. The fusion molecule of claim 13, wherein the carrier moiety is a carrier protein, a Fc domain, or a PEG molecule.

15. A polynucleotide that encodes the fusion molecule of claim 1.

16. A vector comprising the polynucleotide of claim 15.

17. A cell harboring the vector of claim 16.

18. A pharmaceutical composition comprising the fusion molecule of claim 1 or the polynucleotide of claim 15, and a pharmaceutically acceptable carrier.

19. A method for cleaving the ectodomain of a target integral membrane protein, comprising (1) constructing a fusion molecule that comprises a protease-targeting domain and a covalently linked substrate-targeting domain, wherein the substrate-targeting domain specifically binds to the extracellular portion of the target integral membrane protein, and the protease-targeting domain specifically binds to an integral membrane protease or an auxiliary membrane protein in a multimeric integral membrane protease complex that is bound to the same cell surface, and wherein the integral membrane protease can cleave the extracellular domain of the target integral membrane protein, and (2)

contacting the fusion molecule with the cell; thereby cleaving the ectodomain of the target integral membrane protein.

20. The method of claim 19, wherein the target integral membrane protein is a cellular receptor or a receptor ligand, and the integral membrane protease is a sheddase.

21. The method of claim 20, wherein the cellular receptor is LAG3 or PD-1, and the sheddase is ADAM 10.

22. The method of claim 19, wherein the substrate-targeting domain and the protease-targeting domain are antibodies or antigen-binding fragments that specifically binds to the membrane-bound protein and the protease, respectively.

23. The method of claim 19, wherein the substrate-targeting domain and the protease-targeting domain are each a VHH sdAb.

24. The method of claim 19, wherein the cell is present in a subject, and the fusion molecule is contacted with the cell by administering to the subject a pharmaceutical composition that comprises the fusion molecule or a polynucleotide encoding the fusion molecule.

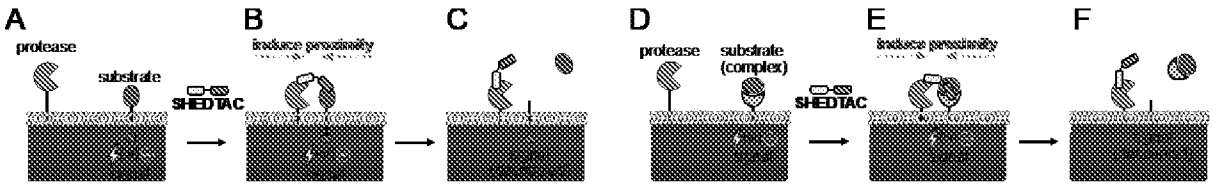


FIG. 1

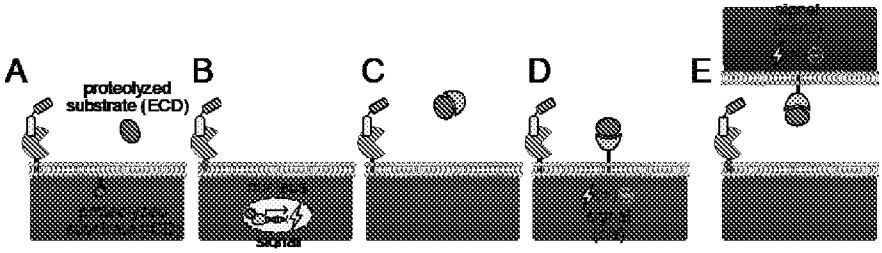


FIG. 2

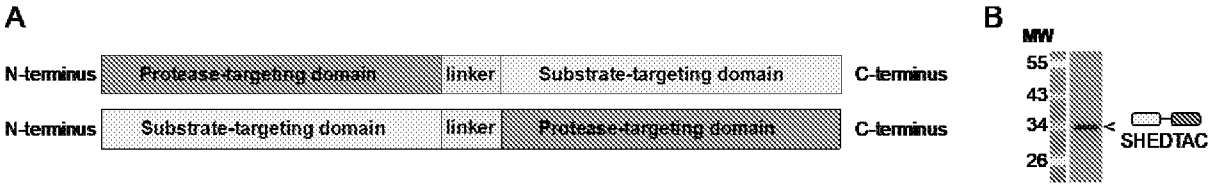
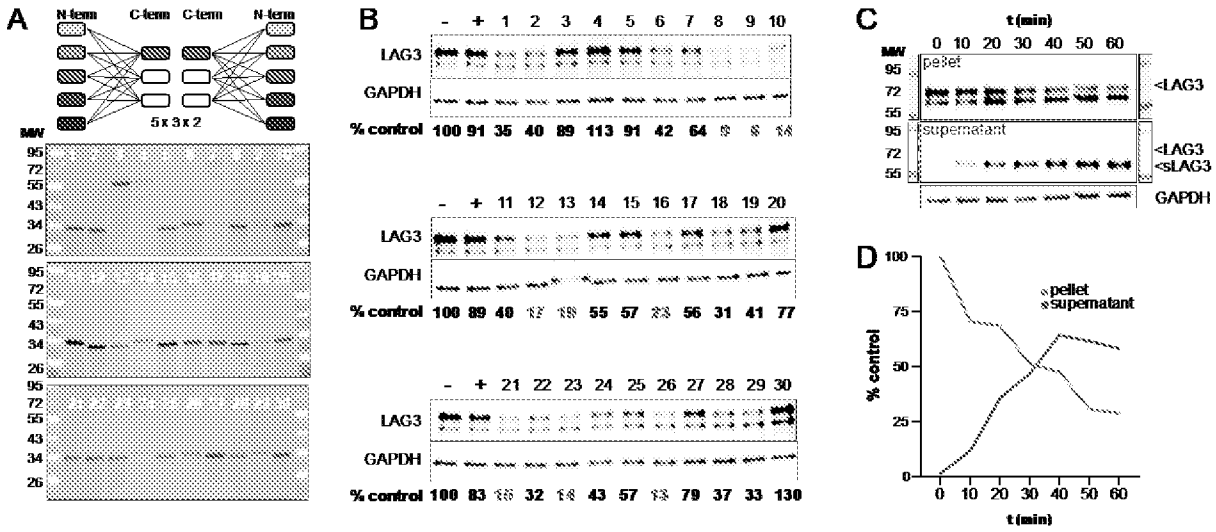


FIG. 3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GAPDH
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GAPDH
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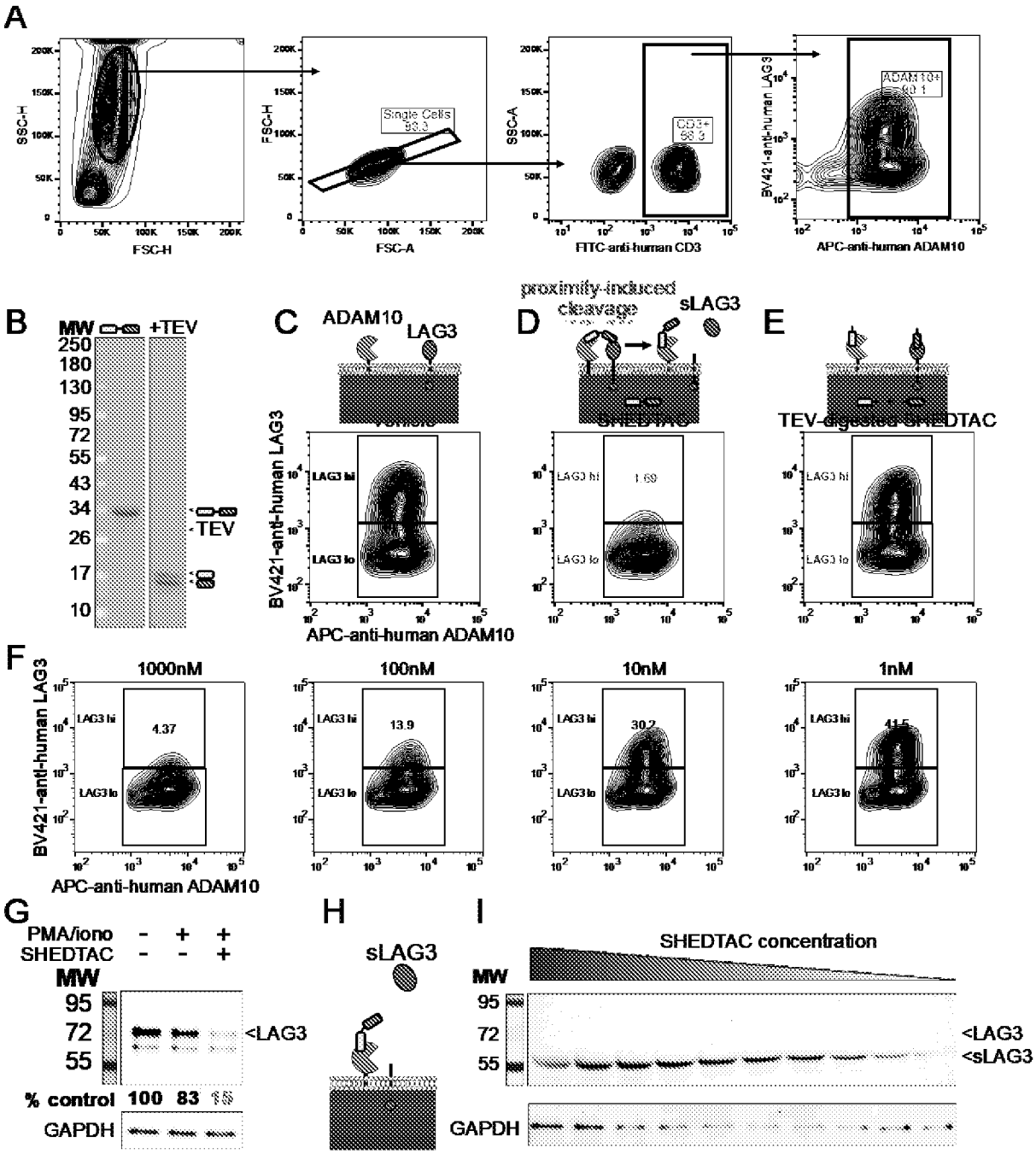
t (min)

FIG. 4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TEV
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LAG3 lo
APC-anti-human ADAM10
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100nM
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FIG. 5

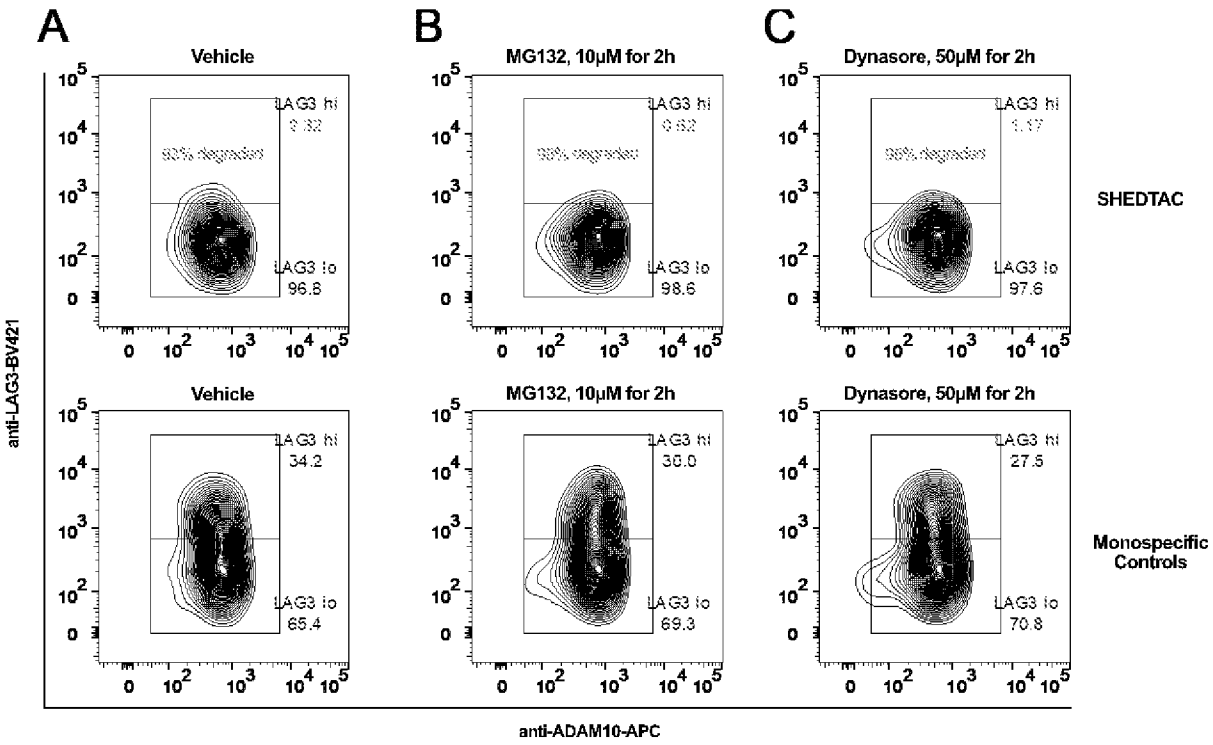

FIG. 6

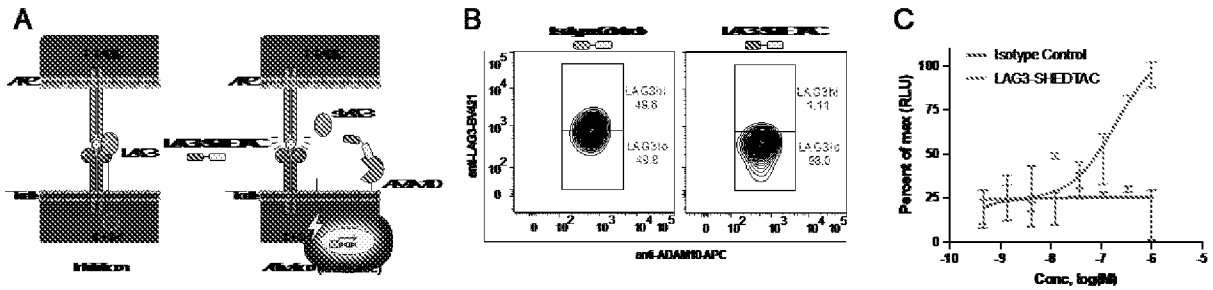

FIG. 7

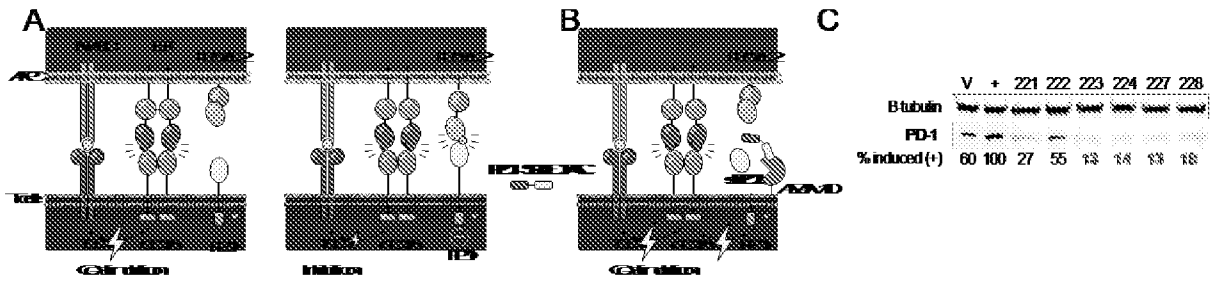

FIG. 8