

MASKED ACTIVATABLE CYTOKINE CONSTRUCTS AND RELATED COMPOSITIONS AND METHODS

TECHNICAL FIELD

The present disclosure relates to the field of biotechnology, and more specifically,
5 to activatable cytokine constructs.

BACKGROUND

Cytokines are a family of naturally-occurring small proteins and glycoproteins produced and secreted by most nucleated cells in response to viral infection and/or other antigenic stimuli. Interferons are a subclass of cytokines. Interferons are presently
10 grouped into three major classes: interferon type I, interferon type II, and interferon type III. Interferons exert their cellular activities by binding to specific membrane receptors on a cell surface.

Interferon therapy has many clinical benefits. For example, interferons are known to up-regulate the immune system and also to have antiviral and anti-proliferative
15 properties. These biological properties have led to the clinical use of interferons as therapeutic agents for the treatment of viral infections and malignancies. Further, interferons are useful for recruiting a patient's innate immune system to identify and attack cancer cells. Accordingly, interferon therapy has been extensively used in cancer and antiviral therapy, including for the treatment of hepatitis, Kaposi sarcoma, hairy cell
20 leukemia, chronic myeloid leukemia (CML), follicular lymphoma, renal cell cancer (RCC), melanoma, and other disease states. However, systemic administration of interferons is accompanied by dose-dependent toxicities, including strong flu-like symptoms, neurological symptoms, hepatotoxicity, bone marrow suppression, and arrhythmia, among others. In a melanoma patient study, the combination of
25 Pembrolizumab and Pegylated IFNa led to an investigator-assessed objective response rate ("ORR") of 60.5%. The combination treatment was also associated with 49% of G3/G4 adverse events which required dose reduction of Pegylated IFNa (Davar et al., J. Clin. Oncol., 2018). These undesired side-effects have limited the dosage of interferon therapies and sometimes leads to discontinuation or delay of interferon treatment.

30 Interleukins are another subclass of cytokines. Interleukins regulate cell growth, differentiation, and motility. They are particularly important in stimulating immune

responses, such as inflammation. Interleukins have been used for treatment of cancer, autoimmune disorders, and other disorders. For example, interleukin-2 (IL2) is indicated for treatment of melanoma, graft-versus-host disease (GVHD), neuroblastoma, renal cell cancer (RCC), and is also considered useful for conditions including acute coronary syndrome, acute myeloid syndrome, atopic dermatitis, autoimmune liver diseases, basal cell carcinoma, bladder cancer, breast cancer, candidiasis, colorectal cancer, cutaneous T-cell lymphoma, endometriomas, HIV infection, ischemic heart disease, rheumatoid arthritis, nasopharyngeal adenocarcinoma, non-small cell lung cancer (NSCLC), ovarian cancer, pancreatic cancer, systemic lupus erythematosus, tuberculosis, and other disorders. Other interleukins, such as IL-6, IL-7, IL-12, and IL-21, among others, are potential treatments for cancers and other disorders. Interleukin therapy is often accompanied by undesired side effects, including flu-like symptoms, nausea, vomiting, diarrhea, low blood pressure, and arrhythmia, among others.

Thus, the need and desire for improved specificity and selectivity of cytokine therapy to the desired target is of great interest. Increased targeting of cytokine therapeutics to the disease site could reduce systemic mechanism-based toxicities and lead to broader therapeutic utility.

SUMMARY

The present disclosure provides activatable cytokine constructs (ACCs) that include: (a) a first monomer comprising a first peptide mask (PM1), a first mature cytokine protein (CP1), a first and a third cleavable moieties (CM1 and CM3), and a first dimerization domain (DD1), wherein the CM1 is positioned between the CP1 and the DD1, and the CM3 is positioned between the PM1 and the CP1; and (b) a second monomer comprising a second mature cytokine protein (CP2), a second cleavable moiety (CM2), and a second dimerization domain (DD2), wherein the CM2 is positioned between the CP2 and the DD2, where: the CM1, the CM2, and the CM3 function as a substrate for a protease; the DD1 and the DD2 bind to each other; and where the ACC is characterized by a reduction in at least one activity of the CP1 and/or CP2 as compared to a control level of the at least one activity of the CP1 and/or CP2. The protease(s) that cleave the CM1, CM2, and CM3 may be over-expressed in diseased tissue (e.g., tumor tissue) relative to healthy tissue. The ACC may be activated upon cleavage of the CM1,

CM2, and/or CM3 so that the cytokine may exert its activity in the diseased tissue (e.g., in a tumor microenvironment) while the cytokine activity is attenuated in the context of healthy tissue. Thus, the ACCs provided herein may provide reduced toxicity relative to traditional cytokine therapeutics, enable higher effective dosages of cytokine, and/or
5 increase the therapeutic window for the cytokine.

Provided herein are activatable cytokine constructs (ACC) that include a first monomer construct and a second monomer construct, wherein: (a) the first monomer construct comprises a first peptide mask (PM1), a first mature cytokine protein (CP1), a first and a third cleavable moieties (CM1 and CM3), and a first dimerization domain
10 (DD1), wherein the CM1 is positioned between the CP1 and the DD1, and the CM3 is positioned between the PM1 and the CP1; and (b) the second monomer construct comprises a second mature cytokine protein (CP2), a second cleavable moiety (CM2), and a second dimerization domain (DD2), wherein the CM2 is positioned between the CP2 and the DD2; wherein the DD1 and the DD2 bind each other thereby forming a
15 dimer of the first monomer construct and the second monomer construct; and wherein the ACC is characterized by having a reduced level of at least one CP1 and/or CP2 activity as compared to a control level of the at least one CP1 and/or CP2 activity.

In some embodiments, the second monomer construct further comprises a second peptide mask (PM2) and a fourth cleavable moiety (CM4), wherein the CM4 is
20 positioned between the PM2 and the CP2. In some embodiments, the first monomer construct comprises a first polypeptide that comprises the PM1, the CM3, the CP1, the CM1, and the DD1. In some embodiments, the second monomer construct comprises a second polypeptide that comprises the CP2, the CM2, and the DD2. In some
25 embodiments, the second monomer construct comprises a second polypeptide that comprises the PM2, the CM4, the CP2, the CM2, and the DD2.

In some embodiments, the PM1 comprises a sequence selected from the group consisting of SEQ ID NOs: 328, 329, 323, and 331-369, and the CP1 is an interferon; the PM1 comprises a sequence selected from the group consisting of SEQ ID NOs: 328, 329, 323, and 331-364, and the CP1 is an interferon α ; the PM1 comprises a sequence selected
30 from the group consisting of SEQ ID NOs: 331-360, 362-364, and the CP1 is an interferon β ; the PM1 comprises a sequence selected from the group consisting of SEQ

ID NOs: 331-360, 366-369, and the CP1 is an interferon γ ; the PM1 comprises a sequence selected from the group consisting of SEQ ID NOs: 370-374, and the CP1 is an IL-12; the PM1 comprises a sequence selected from the group consisting of SEQ ID NOs: 375-382, 469-477, 478, and the CP1 is an IL-15; the PM1 comprises a sequence
5 selected from the group consisting of SEQ ID NOs: 383-468, 469-478, and the CP1 is an IL-2; or the PM1 comprises a sequence selected from the group consisting of SEQ ID NOs: 478 and 479, and the CP1 is an IL-21. In some embodiments, the PM2 comprises a sequence selected from the group consisting of SEQ ID NOs: 328, 329, 323, and 331-369, and the CP2 is an interferon; the PM2 comprises a sequence selected from the group
10 consisting of SEQ ID NOs: 328, 329, 323, and 331-364, and the CP2 is an interferon α ; the PM2 comprises a sequence selected from the group consisting of SEQ ID NOs: 331-360, 362-364, and the CP2 is an interferon β ; the PM2 comprises a sequence selected from the group consisting of SEQ ID NOs: 331-360, 366-369, and the CP2 is an interferon γ ; the PM2 comprises a sequence selected from the group consisting of SEQ
15 ID NOs: 370-374, and the CP2 is an IL-12; the PM2 comprises a sequence selected from the group consisting of SEQ ID NOs: 375-382, 469-477, 478, and the CP2 is an IL-15; the PM2 comprises a sequence selected from the group consisting of the group consisting of SEQ ID NOs: 383-468, 469-478, and the CP2 is an IL-2; or the PM2 comprises a sequence selected from the group consisting of SEQ ID NOs: 478 and 479, and the CP2
20 is an IL-21. In some embodiments, the PM1 comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 328-329, 323, and 331-479. In some embodiments, the PM2 comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 328-329, 323, and 331-479.

In some embodiments, the DD1 and the DD2 are a pair selected from the group
25 consisting of: a pair of Fc domains, a sushi domain from an alpha chain of human IL-15 receptor (IL15R α) and a soluble IL-15; barnase and barnstar; a protein kinase A (PKA) and an A-kinase anchoring protein (AKAP); adapter/docking tag modules based on mutated RNase I fragments; an epitope and single domain antibody (sdAb); an epitope and single chain variable fragment (scFv); and soluble N-ethyl-maleimide sensitive factor
30 attachment protein receptors (SNARE) modules based on interactions of the proteins

syntaxin, synaptotagmin, synaptobrevin, and SNAP25, an antigen-binding domain and an epitope.

In some embodiments, the DD1 and the DD2 are a pair of Fc domains. In some embodiments, the pair of Fc domains is a pair of human Fc domains. In some
5 embodiments, the human Fc domains are human IgG1 Fc domains, human IgG2 Fc domains, human IgG3 Fc domains, or human IgG4 Fc domains. In some embodiments, the human Fc domains are human IgG4 Fc domains. In some embodiments, the human Fc domains each comprise a sequence that is at least 80% identical to SEQ ID NO: 3. In some embodiments, the human Fc domains each comprise a sequence that is at least 90%,
10 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 3. In some embodiments, the human Fc domains comprise SEQ ID NO: 3. In some embodiments, the DD1 and the DD2 comprise SEQ ID NOs: 318 and 319, respectively. In some embodiments, the DD1 and the DD2 are the same. In some embodiments, the human Fc domains include mutations to eliminate glycosylation and/or to reduce Fc-gamma receptor binding. In
15 some embodiments, the human Fc domains comprise the mutation N297Q, N297A, or N297G; in some embodiments the human Fc domains comprise a mutation at position 234 and/or 235, for example L235E, or L234A and L235A (in IgG1), or F234A and L235A (in IgG4); in some embodiments the human Fc domains are IgG2 Fc domains that comprise the mutations V234A, G237A, P238S, H268Q/A, V309L, A330S, or P331S, or
20 a combination thereof (all according to EU numbering).

Additional examples of engineered human Fc domains are known to those skilled in the art. Examples of Ig heavy chain constant region amino acids in which mutations in at least one amino acid leads to reduced Fc function include, but are not limited to, mutations in amino acid 228, 233, 234, 235, 236, 237, 239, 252, 254, 256, 265, 270, 297,
25 318, 320, 322, 327, 329, 330, and 331 of the heavy constant region (according to EU numbering). Examples of combinations of mutated amino acids are also known in the art, such as, but not limited to a combination of mutations in amino acids 234, 235, and 331, such as L234F, L235E, and P331S or a combination of amino acids 318, 320, and 322, such as E318A, K320A, and K322A.

30 Further examples of engineered Fc domains include F243L/R292P/Y300L/V305I/P396 IgG1; S239D/I332E IgG1; S239D/I332E/A330L

IgG1; S298A/E333A/K334A; in one heavy chain,
 L234Y/L235Q/G236W/S239M/H268D/D270E/S298A IgG1, and in the opposing heavy
 chain, D270E/K326D, A330M/K334E IgG; G236A/S239D/I332E IgG1; K326W/E333S
 IgG1; S267E/H268F/S324T IgG1; E345R/E430G/S440Y IgG1; N297A or N297Q or
 5 N297G IgG1; L235E IgG1; L234A/L235A IgG1; F234A/L235A IgG4;
 H268Q/V309L/A330S/P331S IgG2;
 V234A/G237A/P238S/H268A/V309L/A330S/P331S IgG2; M252Y/S254T/T256E IgG1;
 M428L/N434S IgG1; S267E/L328F IgG1; N325S/L328F IgG1, and the like. In some
 embodiments, the engineered Fc domain comprises one or more substitutions selected
 10 from the group consisting of N297A IgG1, N297Q IgG1, and S228P IgG4.

In some embodiments, the DD1 comprises an antigen-binding domain and the
 DD2 comprises a corresponding epitope. In some embodiments, the antigen-binding
 domain is an anti-His tag antigen-binding domain and wherein the DD2 comprises a His
 tag. In some embodiments, the antigen-binding domain is a single chain variable
 15 fragment (scFv). In some embodiments, the antigen-binding domain is a single domain
 antibody (sdAb). In some embodiments, at least one of the DD1 and the DD2 comprises a
 dimerization domain substituent selected from the group consisting of a non-polypeptide
 polymer and a small molecule. In some embodiments, the DD1 and the DD2 comprise
 non-polypeptide polymers covalently bound to each other. In some embodiments, the
 20 non-polypeptide polymer is a sulfur-containing polyethylene glycol, and wherein the
 DD1 and the DD2 are covalently bound to each other via one or more disulfide bonds. In
 some embodiments, at least one of the DD1 and the DD2 comprises a small molecule. In
 some embodiments, the small molecule is biotin. In some embodiments, the DD1
 comprises biotin and the DD2 comprises an avidin.

25 In some embodiments, the CP1 and the CP2 are mature cytokines. In some
 embodiments, each of the CP1 and the CP2 comprise a mature cytokine sequence and
 further comprise a signal peptide. A signal peptide is also referred to herein as a “signal
 sequence.” In some embodiments, the CP1 and/or the CP2 is/are each individually
 selected from the group consisting of: an interferon, an interleukin, GM-CSF, G-CSF,
 30 LIF, OSM, CD154, LT- β , TNF- α , TNF- β , 4-1BBL, APRIL, CD70, CD153, CD178,
 GITRL, LIGHT, OX40L, TALL-1, TRAIL, TWEAK, TRANCE, TGF- β 1, TGF- β 1,

TGF- β 3, Epo, Tpo, Flt-3L, SCF, M-CSF, and MSP, optionally wherein the CP1 and/or the CP2 is independently selected from IL-2, IL-7, IL-8, IL-10, IL-12, IL-15, IL-21, an IFN-alpha, an IFN beta, an IFN gamma, GM-CSF, TGF-beta, LIGHT, GITR-L, CD40L, CD27L, 4-1BB-L, OX40, and OX40L. In some embodiments, the CP1 and the CP2 are
5 the same. In some embodiments, the CP1 and the CP2 are different. In some embodiments, the CP1 and/or the CP2 is/are an interferon. In some embodiments, the CP1 and the CP2 both are an interferon. In some embodiments, the CP1 and the CP2 are different interferons. In some embodiments, the CP1 and the CP2 are the same interferon. In some embodiments, one of the CP1 or the CP2 is an interferon, and the other of CP1 or
10 CP2 is a cytokine other than an interferon. In some aspects, one or both cytokines are monomeric cytokines. In some aspects, one or both interferons are monomeric inteferons. In some aspects, either CP1 or CP2 is a monomeric interferon and the other CP1 or CP2 is a different cytokine. In some aspects, the CP1 and/or the CP2 include a mutant cytokine sequence. In some aspects, the CP1 and/or the CP2 include a universal
15 cytokine sequence. In some aspects, the CP1 and/or the CP2 include a truncated sequence that retains cytokine activity.

In some embodiments, the interferon(s) is/are a human wildtype mature interferon. In some embodiments, the interferon(s) may be type I and type II interferons, for example including, but not limited to interferon-alpha, interferon-beta, interferon-
20 gamma, interferon-omega, and interferon-tau. In some embodiments, the interferons is/are an interferon-alpha. In some embodiments, the interferon(s) is/are selected from the group consisting of: interferon alpha-2a, interferon alpha-2b, and interferon alpha-n3. In some embodiments, the interferon(s) is/are interferon alpha-2b. In some embodiments, the interferon(s) is/are a mutant interferon. In some embodiments, the interferon(s) is/are
25 a mutant interferon wherein an endogenous protease cleavage site has been rendered disfunctional by substitution, deletion, or insertion of one or more amino acids. In some embodiments, the interferon(s) is/are a universal cytokine molecule, e.g., having a hybrid sequence of different cytokine subtypes or a chimeric cytokine sequence or a humanized cytokine sequence. In some embodiments, the interferon(s) is/are a universal interferon
30 molecule. In some embodiments, the interferon(s) is/are a universal interferon alpha, e.g., a hybrid of interferon alpha 1 and interferon alpha 2a. In some embodiments, the

CP1 and/or the CP2 comprises a sequence that is at least 80% identical to SEQ ID NO: 1. In some embodiments, the CP1 and/or the CP2 comprises a sequence that is at least 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 1. In some embodiments, the CP1 and/or the CP2 comprises a sequence of SEQ ID NO: 1. In some embodiments, the
 5 interferon is an interferon beta. In some embodiments, the interferon beta is selected from the group consisting of interferon beta-1a, and interferon beta-1b. In some embodiments, the CP1 and/or the CP2 comprises an IFab domain. In some embodiments, the CP1 and/or the CP2 comprises an interleukin. In some embodiments, the interleukin is selected from the group consisting of IL-1 α , IL-1 β , IL-1RA, IL-18, IL-2, IL-4, IL-7, IL-
 10 9, IL-13, IL-15, IL-3, IL-5, IL-6, IL-11, IL-12, IL-10, IL-20, IL-14, IL-16, and IL-17.

In some embodiments, the CM1 and/or the CM2 comprise a total of about 3 amino acids to about 15 amino acids. In some embodiments, the CM1 and the CM2 comprise substrates for different proteases. In some embodiments, wherein the CM1 and the CM2 comprise substrates for the same protease. In some embodiments, the
 15 protease(s) is/are selected from the group consisting of: ADAM8, ADAM9, ADAM10, ADAM12, ADAM15, ADAM17/TACE, ADAMDEC1, ADAMTS1, ADAMTS4, ADAMTS5, BACE, Renin, Cathepsin D, Cathepsin E, Caspase 1, Caspase 2, Caspase 3, Caspase 4, Caspase 5, Caspase 6, Caspase 7, Caspase 8, Caspase 9, Caspase 10, Caspase 14, Cathepsin B, Cathepsin C, Cathepsin K, Cathepsin L, Cathepsin S, Cathepsin V/L2,
 20 Cathepsin X/Z/P, Cruzipain, Legumain, Otubain-2, KLK4, KLK5, KLK6, KLK7, KLK8, KLK10, KLK11, KLK13, KLK14, Meprin, Neprilysin, PSMA, BMP-1, matrix metalloproteinases (e.g., MMP-1, MMP-2, MMP-3, MMP-7, MMP-9, MMP-10, MMP-11, MMP-12, MMP-13, MMP-14, MMP-15, MMP-16, MMP-17, MMP-19, MMP-20, MMP-23, MMP-24, MMP-26, MMP-27), activated protein C, cathepsin A, cathepsin G,
 25 Chymase, FVIIa, FIXa, FXa, FXIa, FXIIa, Elastase, Granzyme B, Guanidinobenzoate, HtrA1, human neutrophil lyase, lactoferrin, marapsin, NS3/4A, PACE4, Plasmin, PSA, tPA, thrombin, tryptase, uPA, DESC1, DPP-4, FAP, Hepsin, Matriptase-2, MT-SP1/Matriptase, TMPRSS2, TMPRSS3, and TMPRSS4. In some embodiments, the protease(s) is/are selected from the group consisting of: uPA, legumain, MT-SP1,
 30 ADAM17, BMP-1, TMPRSS3, TMPRSS4, MMP-2, MMP-9, MMP-12, MMP-13, and MMP-14.

Suitable cleavable moieties have been disclosed in WO 2010/081173, WO 2015/048329, WO 2015/116933, WO 2016/118629, and WO 2020/118109, the disclosures of which are incorporated herein by reference in their entireties.

In some embodiments, the CM1 and/or the CM2 comprise a sequence selected from the group consisting of: LSGRSDNH (SEQ ID NO: 5), TGRGPSWV (SEQ ID NO: 6), PLTGRSGG (SEQ ID NO: 7), TARGPSFK (SEQ ID NO: 8), NTLSGRSENHSG (SEQ ID NO: 9), NTLSGRSGNHGS (SEQ ID NO: 10), TSTSGRSANPRG (SEQ ID NO: 11), TSGRSANP (SEQ ID NO: 12), VHMPLGFLGP (SEQ ID NO: 13), AVGLLAPP (SEQ ID NO: 14), AQNLLGMV (SEQ ID NO: 15), QNQALRMA (SEQ ID NO: 16), LAAPLGLL (SEQ ID NO: 17), STFPFGMF (SEQ ID NO: 18), ISSGLLSS (SEQ ID NO: 19), PAGLWLDP (SEQ ID NO: 20), VAGRSMRP (SEQ ID NO: 21), VVPEGRRS (SEQ ID NO: 22), ILPRSPAF (SEQ ID NO: 23), MVLGRSLL (SEQ ID NO: 24), QGRAITFI (SEQ ID NO: 25), SPRSIMLA (SEQ ID NO: 26), SMLRSMPL (SEQ ID NO: 27), ISSGLLSGRSDNH (SEQ ID NO: 28), AVGLLAPPGGLSGRSDNH (SEQ ID NO: 29), ISSGLLSSGGSGGSLSGRSDNH (SEQ ID NO: 30), LSGRSGNH (SEQ ID NO: 31), SGRSANPRG (SEQ ID NO: 32), LSGRSDDH (SEQ ID NO: 33), LSGRSDIH (SEQ ID NO: 34), LSGRSDQH (SEQ ID NO: 35), LSGRSDTH (SEQ ID NO: 36), LSGRSDYH (SEQ ID NO: 37), LSGRSDNP (SEQ ID NO: 38), LSGRSANP (SEQ ID NO: 39), LSGRSANI (SEQ ID NO: 40), LSGRSDNI (SEQ ID NO: 41), MIAPVAYR (SEQ ID NO: 42), RPSPMWAY (SEQ ID NO: 43), WATPRPMR (SEQ ID NO: 44), FRLLDWQW (SEQ ID NO: 45), ISSGL (SEQ ID NO: 46), ISSGLLS (SEQ ID NO: 47), ISSGLL (SEQ ID NO: 48), ISSGLLSGRSANPRG (SEQ ID NO: 49), AVGLLAPPTSGRSANPRG (SEQ ID NO: 50), AVGLLAPPSGRSANPRG (SEQ ID NO: 51), ISSGLLSGRSDDH (SEQ ID NO: 52), ISSGLLSGRSDIH (SEQ ID NO: 53), ISSGLLSGRSDQH (SEQ ID NO: 54), ISSGLLSGRSDTH (SEQ ID NO: 55), ISSGLLSGRSDYH (SEQ ID NO: 56), ISSGLLSGRSDNP (SEQ ID NO: 57), ISSGLLSGRSANP (SEQ ID NO: 58), ISSGLLSGRSANI (SEQ ID NO: 59), AVGLLAPPGGLSGRSDDH (SEQ ID NO: 60), AVGLLAPPGGLSGRSDIH (SEQ ID NO: 61), AVGLLAPPGGLSGRSDQH (SEQ ID NO: 62), AVGLLAPPGGLSGRSDTH (SEQ ID NO: 63), AVGLLAPPGGLSGRSDYH (SEQ ID NO: 64), AVGLLAPPGGLSGRSDNP (SEQ ID NO: 65), AVGLLAPPGGLSGRSANP (SEQ ID

NO: 66), AVGLLAPPGGLSGRSANI (SEQ ID NO: 67), ISSGLLSGRSDNI (SEQ ID NO: 68), AVGLLAPPGGLSGRSDNI (SEQ ID NO: 69), GLSGRSDNHGGAVGLLAPP (SEQ ID NO: 70), GLSGRSDNHGGVHMPLGFLGP (SEQ ID NO: 71), LSGRSDNHGGVHMPLGFLGP (SEQ ID NO: 72), ISSGLSS (SEQ ID NO: 73),
5 PVGYTSSL (SEQ ID NO: 74), DWLYWPGI (SEQ ID NO: 75), LKAAPRWA (SEQ ID NO: 76), GPSHLVLT (SEQ ID NO: 77), LPGAALPW (SEQ ID NO: 78), MGLFSEAG (SEQ ID NO: 79), SPLPLRVP (SEQ ID NO: 80), RMHLRSLG (SEQ ID NO: 81), LLAPSHRA (SEQ ID NO: 82), GPRSFGL (SEQ ID NO: 83), GPRSFG (SEQ ID NO: 84), SARGPSRW (SEQ ID NO: 85), GGWHTGRN (SEQ ID NO: 86), HTGRSGAL
10 (SEQ ID NO: 87), AARGPAIH (SEQ ID NO: 88), RGPAPNPM (SEQ ID NO: 89), SSRGPAYL (SEQ ID NO: 90), RGPATPIM (SEQ ID NO: 91), RGPA (SEQ ID NO: 92), GGQPSGMWGW (SEQ ID NO: 93), FPRPLGITGL (SEQ ID NO: 94), SPLTGRSG (SEQ ID NO: 95), SAGFSLPA (SEQ ID NO: 96), LAPLGLQRR (SEQ ID NO: 97), SGGPLGVR (SEQ ID NO: 98), PLGL (SEQ ID NO: 99), and SGRSDNI (SEQ ID NO: 100). In some embodiments, the CM comprises a sequence selected from the group consisting of: ISSGLLSGRSDNH (SEQ ID NO: 28), LSGRSDDH (SEQ ID NO: 33), ISSGLLSGRSDQH (SEQ ID NO: 54), SGRSDNI (SEQ ID NO: 100), and ISSGLLSGRSDNI (SEQ ID NO: 68). In some embodiments, the protease(s) is/are produced by a tumor in the subject, e.g., the protease(s) are produced in greater amounts
20 in the tumor than in healthy tissues of the subject. In some embodiments, the subject has been diagnosed or identified as having a cancer.

In some embodiments, the CP1 and the CM1 directly abut each other in the first monomer construct. In some embodiments, the CM1 and the DD1 directly abut each other in the first monomer construct. In some embodiments, the CP2 and the CM2
25 directly abut each other in the second monomer construct. In some embodiments, the CM2 and the DD2 directly abut each other in the second monomer construct. In some embodiments, the first monomer construct comprises the CP1 directly abutting the CM1, and the CM1 directly abutting the DD1, wherein the CM1 comprises a sequence that is selected from the group consisting of SEQ ID Nos 5-100. In some embodiments, the
30 second monomer construct comprises the CP2 directly abutting the CM2, and the CM2 directly abutting the DD2, wherein the CM2 comprises a sequence that is selected from

the group consisting of SEQ ID Nos 5-100. In some embodiments, the first monomer construct comprises the CP1 directly abutting the CM1, and the CM1 directly abutting the DD1, wherein the CM1 comprises a sequence that is no more than 13, 12, 11, 10, 9, 8, 7, 6, 5 or 4 amino acids in length. In some embodiments, the second monomer construct comprises the CP2 directly abutting the CM2, and the CM2 directly abutting the DD2, wherein the CM2 comprises a sequence that is no more than 13, 12, 11, 10, 9, 8, 7, 6, 5 or 4 amino acids in length. In some embodiments, the first and second monomer construct each are configured such that the cytokine (CM1 and CM2, respectively) directly abuts a cleavable moiety (CM1 and CM2, respectively) that is no more than 10, 9, 8, 7, 6, 5, or 4 amino acids in length, and the cleavable moiety directly abuts a dimerization domain (DD1 and DD2, respectively) that is the Fc region of a human IgG, wherein the N-terminus of the Fc region is the first cysteine residue (reading in the N- to C- direction) in the hinge region that participates in a disulfide linkage with a second Fc domain (e.g., Cysteine 226 of human IgG1, using EU numbering). In some aspects, the dimerization domain is an IgG Fc region wherein the upper hinge residues have been deleted. For example, the Fc is a variant wherein N-terminal sequences EPKSCDKTHT (SEQ ID NO: 330), ERK, ELKTPPLGDTTHT (SEQ ID NO: 365), or ESKYGPP (SEQ ID NO: 317) have been deleted.

In some embodiments, the first monomer construct comprises at least one linker. In some embodiments, the at least one linker is a linker L1 disposed between the PM1 and the CM3 and/or a linker L2 disposed between the CM3 and the CP1. In some embodiments, the second monomer construct comprises at least one linker. In some embodiments, the at least one linker is a linker L3 disposed between the PM2 and the CM4 and/or a linker L4 disposed between the CM4 and the CP2. In some embodiments, the first monomer construct comprises a linker L1 and the second monomer construct comprises a linker L3. In some embodiments, L1 and L3 are the same. In some embodiments, the first monomer construct comprises a linker L2 and the second monomer construct comprises a linker L4. In some embodiments, L2 and L4 are the same. In some embodiments, the first monomer construct comprises a linker between the CP1 and CM1 and/or a linker between the CM1 and the DD1. In some embodiments, the second monomer construct comprises a linker between the CP2 and the CM2 and/or a

linker between the CM2 and the DD2. In some embodiments, each linker has a total length of 1 amino acid to about 15 amino acids. In some embodiments, each linker has a total length of at least 5 amino acids.

In some embodiments, the first monomer construct comprises at least one linker,
 5 wherein each linker is independently selected from the group consisting of
 GSSGSGGSGG (SEQ ID NO: 210); GGGS (SEQ ID NO: 2); GGGSGGGS (SEQ ID
 NO: 211); GGGSGGGSGGGS (SEQ ID NO: 212); GGGGSGGGGSGGGGS (SEQ ID
 NO: 213); GGGGSGGGGSGGGGSGGGGSGGGGS (SEQ ID NO: 214);
 GGGGSGGGGS (SEQ ID NO: 215); GGGGS (SEQ ID NO: 216); GS; GGGGSGS
 10 (SEQ ID NO: 217); GGGGSGGGGSGGGGSGS (SEQ ID NO: 218);
 GGSLDPKGGGGS (SEQ ID NO: 219); PKSCDKTHTCPPCAPELLG (SEQ ID NO:
 220); SKYGPPCPPCAPEFLG (SEQ ID NO: 221); GKSSGSGSESKS (SEQ ID NO:
 222); GSTSGSGKSSEGKG (SEQ ID NO: 223); GSTSGSGKSSEGSGSTKG (SEQ ID
 NO: 224); GSTSGSGKPGSGEGSTKG (SEQ ID NO: 225); GSTSGSGKPGSSEGST
 15 (SEQ ID NO: 226); (GS)_n, (GGG)_n, (GSGGS)_n (SEQ ID NO: 227), (GGGS)_n (SEQ ID
 NO: 228), (GGGG)_n (SEQ ID NO: 216), wherein each n is an integer of at least one;
 GGSG (SEQ ID NO: 229); GGSGG (SEQ ID NO: 230); GSGSG (SEQ ID NO: 231);
 GSGGG (SEQ ID NO: 232); GGGSG (SEQ ID NO: 233); GSSSG (SEQ ID NO: 234);
 GGGGSGGGGSGGGGS (SEQ ID NO: 213); GGGGSGGGGSGGGGSGGGGS (SEQ
 20 ID NO: 214); and GSTSGSGKPGSSEGST (SEQ ID NO: 226). In some embodiments,
 the linker comprises a sequence of GGGS (SEQ ID NO: 2).

In some embodiments, the first monomer construct, comprises in an N- to C-
 terminal direction, the PM1, the CM3, the CP1, the CM1, and, linked directly or
 indirectly to the C-terminus of the CM1, the DD1. In some embodiments, the first
 25 polypeptide comprises in a C- to N-terminal direction, the PM1, the CM3, the CP1, the
 CM1, and, linked directly or indirectly to the N-terminus of the CM1, the DD1. In some
 embodiments, the second polypeptide comprises in a N- to C-terminal direction, the
 PM2, the CM4, the CP2, the CM2, and, linked directly or indirectly to the C-terminus of
 the CM2, the DD2. In some embodiments, the second polypeptide comprises in a C- to
 30 N-terminal direction, the PM2, the CM4, the CP2, the CM2, and, linked directly or
 indirectly to the CM2, the DD2.

In some embodiments, the first monomer construct comprises in an N- to C-terminal direction, the CP1, an optional linker, the CM1, an optional linker, and the DD1, wherein DD1 is an Fc region of an IgG, wherein the N-terminus of the Fc region is the first cysteine residue (reading in the N- to C- direction) in the hinge region that participates in a disulfide linkage with a second Fc domain (e.g., Cysteine 226 of human IgG1 or IgG4, using EU numbering), and wherein the CM1 and any linker(s) interposed between the CP1 and the N-terminal cysteine of DD1 (the “linking region”) have a combined total length of no more than 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, or 4 amino acids, preferably no more than 10 amino acids, especially preferably no more than 7 amino acids. In some such embodiments, the first monomer construct further comprises, in an N- to C- terminal direction, the PM1, an optional linker, the CM3, and an optional linker attached to the N-terminus of the CP1. In some embodiments, the second monomer construct comprises in an N- to C- terminal direction, the CP2, an optional linker, the CM2, an optional linker, and the DD2, wherein DD2 is an Fc region of an IgG, wherein the N-terminus of the Fc region is the first cysteine residue (reading in the N- to C- direction) in the hinge region that participates in a disulfide linkage with a second Fc domain (e.g., Cysteine 226 of human IgG1 or IgG4, using EU numbering), and wherein the CM2 and any linker(s) interposed between the CP2 and the N-terminal cysteine of the DD2 (the “linking region”) have a combined total length of no more than 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, or 4 amino acids, preferably no more than 10 amino acids, especially preferably no more than 7 amino acids. In some such embodiments, the second monomer construct further comprises, in an N- to C- terminal direction, the PM2, an optional linker, the CM4, and an optional linker attached to the N-terminus of the CP2. In some aspects, there is no linker or spacer between a peptide mask and a cleavable moiety. In some aspects, there is no linker or spacer between a cytokine protein and a cleavable moiety. In some aspects, there is no linker or spacer between a cleavable moiety and a dimerization domain.

In some embodiments, the ACC is a homodimer in which the first monomer construct and the second monomer construct are identical and comprise the amino acid sequence of SEQ ID NO: 321. In some embodiments, the first monomer construct and the second monomer construct each comprise an amino acid sequence that is at least

90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 321. In some embodiments, the first monomer construct and the second monomer construct each comprise, in an N- to C- terminal direction, SEQ ID NO: 323; an optional flexible linker of zero to 10 amino acids; a CM comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 41, SEQ ID NO: 68, and SEQ ID NO: 100; an optional flexible linker of zero to 10 amino acids; SEQ ID NO: 1; a second CM comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 41, SEQ ID NO: 68, and SEQ ID NO: 100; and a dimerization domain.

In some embodiments, the at least one CP1 and/or CP2 activity is a binding affinity of the CP1 and/or the CP2 for its cognate receptor as determined using surface plasmon resonance. For example, where the CP1 or CP2 is an interferon, the cognate receptor may be the interferon-alpha/beta receptor (IFNAR). In some embodiments, the at least one CP1 and/or CP2 activity is a level of proliferation of lymphoma cells. In some embodiments, the at least one CP1 and/or CP2 activity is the level of JAK/STAT/ISGF3 pathway activation in a lymphoma cell. In some embodiments, the at least one activity is a level of secreted alkaline phosphatase (SEAP) production in a lymphoma cell. In some embodiments, the ACC is characterized by at least a 2-fold reduction in at least one of the CP1 and the CP2 activity as compared to the control level. In some embodiments, the ACC is characterized by at least a 5-fold, 10-fold, 20-fold, 50-fold, 100-fold, 200-fold, 300-fold, 400-fold, 500-fold, 600-fold, 700-fold, 800-fold, 900-fold, 1000-fold, 1100-fold, 1200-fold, 1300-fold, 1400-fold, 1500-fold, 1600-fold, 1700-fold, 1800-fold, 1900-fold, 2000-fold, 3000-fold, or 4000-fold reduction in at least one CP1 and/or the CP2 activity as compared to the control level. In some embodiments, the ACC is characterized by at least a 5000-fold reduction in at least one activity of the CP1 and/or the CP2 as compared to the control level. In some embodiments, the control level of the at least one activity of the CP1 and/or CP2, is the activity of the CP1 and/or the CP2 in the ACC following exposure of the ACC to the protease(s). In some embodiments, the control level of the at least one CP1 and/or the CP2, is the corresponding the CP1 and/or the CP2 activity of a corresponding wildtype mature cytokine.

In some embodiments, the ACC is characterized by generating a cleavage product following exposure to the protease(s), wherein the cleavage product comprises the at least one activity of the CP1 and/or the CP2. In some embodiments, the at least one activity of the CP1 and/or the CP2 is anti-proliferation activity. In some embodiments, the control
5 level is an EC50 value of the wildtype mature cytokine, and wherein ratio of EC50 (cleavage product) to EC50 (wildtype control level) is less than about 10, or less than about 9, or less than about 8, or less than about 7, or less than about 6, or less than about 5, or less than about 4, or less than about 3, or less than about 2, or less than about 1.5, or equal to about 1. In some embodiments, the EC50 of the cleavage product is
10 approximately the same as the EC50 of the wildtype mature cytokine, demonstrating that the following cleavage, the activity of the CP1 and/or CP2 is fully recovered, or nearly fully recovered.

Provided herein are compositions comprising any one of the ACCs described herein. In some embodiments, the composition is a pharmaceutical composition. Also
15 provided herein are kits comprising at least one dose of any one of the compositions described herein.

Provided herein are methods of treating a subject in need thereof comprising administering to the subject a therapeutically effective amount of any one of the ACCs described herein or any one of the compositions described herein. In some embodiments,
20 the subject has been identified or diagnosed as having a cancer. In some non-limiting embodiments, the cancer is Kaposi sarcoma, hairy cell leukemia, chronic myeloid leukemia (CML), follicular lymphoma, renal cell cancer (RCC), melanoma, neuroblastoma, basal cell carcinoma, bladder cancer, breast cancer, colorectal cancer, cutaneous T-cell lymphoma, nasopharyngeal adenocarcinoma, non-small cell lung cancer
25 (NSCLC), ovarian cancer, pancreatic cancer. In some non-limiting embodiments, the cancer is a lymphoma. In some non-limiting embodiments, the lymphoma is Burkitt's lymphoma.

Provided herein are nucleic acids encoding a polypeptide that comprises the CP1 and the CM1 of any one of the ACCs described herein. In some embodiments, the
30 polypeptide further comprises any one of the DD1 described herein. In some embodiments, the polypeptide further comprises any one of the PM1 and the CM3

described herein. Also provided herein are nucleic acids encoding a polypeptide that comprises the CP2 and the CM2 of any one of the ACCs described herein. When the monomers are identical, then the present disclosure provides a single nucleic acid encoding the monomer that dimerizes to form ACC. In some embodiments, the
5 polypeptide further comprises any one of the DD2 described herein. In some embodiments, the polypeptide further comprises any one of the PM2 and the CM4 described herein. In certain embodiments, the first monomer construct and the second monomer construct comprise identical CP, CM, and DD components. In some of these embodiments, the first and second monomer constructs are encoded by the same
10 polypeptide (i.e., the same amino acid sequence). Often, when the first and second monomer constructs comprise the same amino acid sequence, they are encoded by the same nucleic acid (i.e., the same nucleic acid sequence). In some of these embodiments, the first and second monomer constructs are encoded by the same nucleic acid. Also provided herein are vectors comprising any one of the nucleic acids described herein. In
15 some embodiments, the vector is an expression vector. Also provided herein are cells comprising any one of the nucleic acids described herein or any one of the vectors described herein.

Provided herein are pairs of nucleic acids that together encode a polypeptide that comprises the CP1 and the CM1 of the first monomer construct and a polypeptide that
20 comprises the CP2 and the CM2 of the second monomer construct of any one of the ACCs described herein. Also provided herein are pairs of nucleic acids that together encode a polypeptide that comprises the PM1, the CM3, CP1 and the CM1 of the first monomer construct and a polypeptide that comprises the PM2, the CM4, the CP2 and the CM2 of the second monomer construct of any one of the ACCs described herein. Also
25 provided herein are pairs of vectors that together comprise any one of the pair of nucleic acids described herein. In some embodiments, the pair of vectors is a pair of expression vectors. Also provided herein are cells comprising any one of the pairs of nucleic acids described herein or any one of the pairs of vectors described herein. In other embodiments, the present invention provides a vector comprising the pair of
30 vectors.

Provided herein are methods of producing an ACC comprising: culturing any one of the cells described herein in a liquid culture medium under conditions sufficient to produce the ACC; and recovering the ACC from the cell or the liquid culture medium. In some embodiments, the method further comprises: isolating the ACC recovered from the cell or the liquid culture medium. In some embodiments, the method further comprises: formulating isolated ACC into a pharmaceutical composition.

Provided herein are ACCs produced by any one of the methods described herein. Also provided herein are compositions comprising any one the ACCs described herein. Also provided herein are compositions of any one of the compositions described herein, wherein the composition is a pharmaceutical composition. Also provided herein are kits comprising at least one dose of any one of the compositions described herein.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Methods and materials are described herein for use in the present invention; other, suitable methods and materials known in the art can also be used. The materials, methods, and examples are illustrative only and not intended to be limiting. All publications, patent applications, patents, sequences, database entries, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will control.

Other features and advantages of the invention will be apparent from the following detailed description and figures, and from the claims.

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

As used herein, the terms “about” and “approximately,” when used to modify an amount specified in a numeric value or range, indicate that the numeric value as well as reasonable deviations from the value known to the skilled person in the art. For example, $\pm 20\%$, $\pm 10\%$, or $\pm 5\%$, are within the intended meaning of the recited value where appropriate.

Concentrations, amounts, and other numerical data may be expressed or presented herein in a range format. It is to be understood that such a range format is used merely for convenience and brevity and thus should be interpreted flexibly to include not only the

numerical values explicitly recited as the limits of the range, but also to include all the individual numerical values or sub-ranges encompassed within that range as if each numerical value and sub-range is explicitly recited. As an illustration, a numerical range of “about 0.01 to 2.0” should be interpreted to include not only the explicitly recited
5 values of about 0.01 to about 2.0, but also include individual values and sub-ranges within the indicated range. Thus, included in this numerical range are individual values such as 0.5, 0.7, and 1.5, and sub-ranges such as from 0.5 to 1.7, 0.7 to 1.5, and from 1.0 to 1.5, etc. Furthermore, such an interpretation should apply regardless of the breadth of the range or the characteristics being described. Additionally, it is noted that all
10 percentages are in weight, unless specified otherwise.

In understanding the scope of the present disclosure, the terms “including” or “comprising” and their derivatives, as used herein, are intended to be open ended terms that specify the presence of the stated features, elements, components, groups, integers, and/or steps, but do not exclude the presence of other unstated features, elements,
15 components, groups, integers and/or steps. The foregoing also applies to words having similar meanings such as the terms “including”, “having” and their derivatives. The term “consisting” and its derivatives, as used herein, are intended to be closed terms that specify the presence of the stated features, elements, components, groups, integers, and/or steps, but exclude the presence of other unstated features, elements, components, groups,
20 integers and/or steps. The term “consisting essentially of,” as used herein, is intended to specify the presence of the stated features, elements, components, groups, integers, and/or steps as well as those that do not materially affect the basic and novel characteristic(s) of features, elements, components, groups, integers, and/or steps. It is understood that reference to any one of these transition terms (i.e. “comprising,” “consisting,” or
25 “consisting essentially”) provides direct support for replacement to any of the other transition term not specifically used. For example, amending a term from “comprising” to “consisting essentially of” or “consisting of” would find direct support due to this definition for any elements disclosed throughout this disclosure. Based on this definition, any element disclosed herein or incorporated by reference may be included in or
30 excluded from the claimed invention.

As used herein, a plurality of compounds, elements, or steps may be presented in

a common list for convenience. However, these lists should be construed as though each member of the list is individually identified as a separate and unique member. Thus, no individual member of such list should be construed as a *de facto* equivalent of any other member of the same list solely based on their presentation in a common group without indications to the contrary.

Furthermore, certain molecules, constructs, compositions, elements, moieties, excipients, disorders, conditions, properties, steps, or the like may be discussed in the context of one specific embodiment or aspect or in a separate paragraph or section of this disclosure. It is understood that this is merely for convenience and brevity, and any such disclosure is equally applicable to and intended to be combined with any other embodiments or aspects found anywhere in the present disclosure and claims, which all form the application and claimed invention at the filing date. For example, a list of constructs, molecules, method steps, kits, or compositions described with respect to a construct, composition, or method is intended to and does find direct support for embodiments related to constructs, compositions, formulations, and methods described in any other part of this disclosure, even if those method steps, active agents, kits, or compositions are not re-listed in the context or section of that embodiment or aspect.

Unless otherwise specified, a “nucleic acid sequence encoding a protein” includes all nucleotide sequences that are degenerate versions of each other and thus encode the same amino acid sequence.

The term “N-terminally positioned” when referring to a position of a first domain or sequence relative to a second domain or sequence in a polypeptide primary amino acid sequence means that the first domain or sequence is located closer to the N-terminus of the polypeptide primary amino acid sequence than the second domain or sequence. In some embodiments, there may be additional sequences and/or domains between the first domain or sequence and the second domain or sequence.

The term “C-terminally positioned” when referring to a position of a first domain or sequence relative to a second domain or sequence in a polypeptide primary amino acid sequence means that the first domain or sequence is located closer to the C-terminus of the polypeptide primary amino acid sequence than the second domain or sequence. In

some embodiments, there may be additional sequences and/or domains between the first domain or sequence and the second domain or sequence.

The term “exogenous” refers to any material introduced from or originating from outside a cell, a tissue, or an organism that is not produced by or does not originate from the same cell, tissue, or organism in which it is being introduced.

The term “transduced,” “transfected,” or “transformed” refers to a process by which an exogenous nucleic acid is introduced or transferred into a cell. A “transduced,” “transfected,” or “transformed” cell (e.g., mammalian cell) is one that has been transduced, transfected, or transformed with exogenous nucleic acid (e.g., a vector) that includes an exogenous nucleic acid encoding any of the activatable cytokine constructs described herein.

The term “nucleic acid” refers to a deoxyribonucleic acid (DNA) or ribonucleic acid (RNA), or a combination thereof, in either a single- or double-stranded form. Unless specifically limited, the term encompasses nucleic acids containing known analogues of natural nucleotides that have similar binding properties as the reference nucleotides. Unless otherwise indicated, a particular nucleic acid sequence also implicitly encompasses complementary sequences as well as the sequence explicitly indicated. In some embodiments of any of the nucleic acids described herein, the nucleic acid is DNA. In some embodiments of any of the nucleic acids described herein, the nucleic acid is RNA.

Modifications can be introduced into a nucleotide sequence by standard techniques known in the art, such as site-directed mutagenesis and polymerase chain reaction (PCR)-mediated mutagenesis. Conservative amino acid substitutions are ones in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in the art. These families include: amino acids with acidic side chains (e.g., aspartate and glutamate), amino acids with basic side chains (e.g., lysine, arginine, and histidine), non-polar amino acids (e.g., alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, and tryptophan), uncharged polar amino acids (e.g., glycine, asparagine, glutamine, cysteine, serine, threonine and tyrosine), hydrophilic amino acids (e.g., arginine, asparagine, aspartate, glutamine, glutamate, histidine, lysine, serine, and

threonine), hydrophobic amino acids (e.g., alanine, cysteine, isoleucine, leucine, methionine, phenylalanine, proline, tryptophan, tyrosine, and valine). Other families of amino acids include: aliphatic-hydroxy amino acids (e.g., serine and threonine), amide family (e.g., asparagine and glutamine), aliphatic family (e.g., alanine, valine, leucine and isoleucine), and aromatic family (e.g., phenylalanine, tryptophan, and tyrosine).

As used herein the phrase “specifically binds,” or “immunoreacts with” means that the activatable antigen-binding protein complex reacts with one or more antigenic determinants of the desired target antigen and does not react with other polypeptides, or binds at much lower affinity, e.g., about or greater than 10^{-6} M.

The term “treatment” refers to ameliorating at least one symptom of a disorder. In some embodiments, the disorder being treated is a cancer and to ameliorate at least one symptom of a cancer.

BRIEF DESCRIPTION OF DRAWINGS

Figs. 1-4 are schematics of illustrative activatable cytokine constructs.

Fig. 5 depicts the cleavage reaction of a cytokine construct without a peptide mask, IFN α -2b-hIgG4 Fc (with either cleavable moiety 1204dL or cleavable moiety 1490), and a protease (either uPA or MT-SP1), which generates monomeric mature IFN α -2b.

Figs. 6A-6C show activation of a cytokine construct (ProC440) by proteases uPA and MMP14. **Fig. 6B** depicts the ProC440 sequence (SEQ ID NO: 316) with MMP14 and uPA cleavable sites.

Figs. 7A-7B show the activity of a cytokine construct (ProC440) tested in vitro using IFN-responsive HEK293 cells (**Fig. 7A**) and Daudi cells (**Fig. 7B**).

Fig. 8 shows the sequence of a masked cytokine construct, ProC732 (SEQ ID NO: 321), with an optional signal sequence in italics (SEQ ID NO: 244), the masking peptide sequence in double-underline, the sequences of cleavable moieties in bold, and the sequence of the mature IFN α -2b underlined.

Fig. 9 shows the sequence of a masked cytokine construct with no cleavable moiety sequence between the cytokine and the dimerization domain, ProC733 (SEQ ID NO: 322), with an optional signal sequence in italics (SEQ ID NO: 244), the

masking peptide sequence in double-underline, the cleavable moiety sequence in bold, and the sequence of the mature IFNalpha-2b underlined.

Fig. 10A shows schematics of cytokine constructs ProC440, ProC732 and ProC733. **Fig. 10B** shows the activity of cytokine constructs (ProC440, ProC732 and ProC733) tested using IFN-responsive HEK293 cells. **Fig. 10C** shows the activity of cytokine construct ProC732 (also referred to as Pb-IFNalpha-2b), tested using IFN-responsive HEK293 cells.

Fig. 11A shows a schematic of the structure of cytokine construct ProC286, and the activity of ProC286 compared to the activity of Sylatron® (PEGylated interferon alpha-2b) in the Daudi apoptosis assay. ProC286 and Sylatron® showed similar levels of activity, indicating that ProC286 could be used as surrogate Sylatron® control to evaluate the tolerability of IFNalpha-2b in the hamster study. **Fig. 11B** depicts a schematic of the structure of cytokine construct ProC291 and the activity of ProC291 compared to the activity of Sylatron® in the Daudi apoptosis assay. ProC291 showed significantly reduced activity compared to Sylatron® and ProC286.

Fig. 12 shows the specific activity of IFNa-con (recombinant interferon alpha, a non-naturally occurring type-I interferon); the active cytokine cleavage product of ProC440 (ProC440+uPA); Sylatron® ("PEG-IFNa2b"); and ProC440, and anticipated toxic dosages in a dose-escalation study in vivo, e.g., at escalating doses of 0.08, 0.4, 2, 10, and 15 mg/kg ("mpk").

Figs. 13A-13C show body weight loss profiles of animals in response to different doses of cytokine constructs ProC286, ProC440, and ProC732 or control (human IgG4) in tolerability tests at different dosages in Syrian Gold Hamsters. Fig. 13A shows data for 2 mg/kg ("2 mpk") dosages; Fig. 13B shows data for 10 mg/kg ("10 mpk") dosages; and Fig. 13C shows data for 15 mg/kg ("15 mpk") dosages of each construct tested. **Fig. 13D** shows IFNa2b mediated toxicity in animals dosed with unmasked IFNa2b/Fc (ProC286) corresponding to increased ALP and increased therapeutic index of IFNa2b single mask (ProC440) and dual mask (ProC732).

Fig. 14 shows clinical chemistry analysis outcomes (Alkaline phosphatase, Alanine transaminase, and Aspartate transaminase) of Syrian Gold Hamsters in response

to different doses (2 mpk, 10 mpk, and 15 mpk) of cytokine constructs ProC286, ProC440, and ProC732 or control (human IgG4) in tolerability tests.

Fig. 15 shows hematology analysis outcomes (Reticulocyte, Neutrophil, and White Blood Cells (WBC) counts) in Syrian Gold Hamsters in response to different doses (2 mpk, 10 mpk, and 15 mpk) of cytokine constructs ProC286, ProC440, and ProC732 or control (human IgG4) in tolerability tests.

Fig. 16 depicts the effect of length of a Linking Region (LR) on the activities of IFN α -2b-Fc fusion proteins without a peptide mask, as determined from a Daudi apoptosis assay.

Fig. 17 depicts the structure of ACC ProC859 mouse cross-reactive interferon (top), the anti-proliferative effects of ACC ProC859 in a B16 mouse melanoma cell assay and the activity of ACC ProC859 in the IFN-responsive HEK293 assay.

Fig. 18 schematically illustrates a cytokine construct including a depiction of the linking region (LR) and mask linking region (MLR).

Fig. 19 shows activation-dependent effects of ProC732 on primary human cells. Top row indicates induction of IP-10 in myeloid cells in myeloid cells by activated ProC732, bottom row indicates induction of IFN- γ in T and NK lymphocytes.

Fig. 20 shows *in vivo* efficacy of masked IFN- α 2b (ProC732) at increasing concentrations in the Daudi xenograft model.

Fig. 21 shows *in vitro* activation of mouse splenocytes by IFN α -A/D (ProC1023) in compared to uPA-treated ProC1549 uncleavable masked construct.

Fig. 22 shows the effects of masked IFN- α 2b (ProC732) and ProC732 activated with uPA on hamster melanoma cell viability.

Fig. 23A shows anti-tumor activity of masked activatable IFN α A/D (ProC1023) at 10, 50, and 200 μ g. Fig. 23B shows *in vivo* activation of masked IFN α A/D relative (ProC1023) to an uncleavable masked IFN α A/D (ProC1549).

Figs. 24A-24B show immune memory in response to MC38 tumor cell rechallenge in mice previously treated with activatable IFN α A/D (200 micrograms ProC1023) (bottom, Fig. 24B) compared to MC38 tumor cell challenge in naïve control mice (top, Fig. 24A).

Fig. 25 shows activation-dependent induction of type I interferon signature comparisons using masked Pro-IFN-a2b (uncleaved ProC732), unmasked IFN-a2b (uPA-treated ProC732), or Peg-IFN-a2b (Sylatron®).

Fig. 26 shows pharmacokinetics of the dual masked INF-a2b (ProC732) and
5 ProC286, ProC440, and ProC659 in hamsters.

Fig. 27 shows anti-tumor activity of single masked activatable IFNa A/D (ProC859) at 20 µg intratumoral injection and 200 µg systemic administration compared to PBS control.

Fig. 28A shows the activity of dual masked ProC1023 compared to single masked
10 ProC859 in an IFNa reporter assay in B16 mouse melanoma cells. **Figs. 28B and 28C** show the activity of ProC1023 compared to ProC1549 in an IFNa reporter assay in B16 mouse melanoma cells.

Fig. 29 shows the activity of monomeric dual masked ProC1239 and dimeric dual masked ProC732 tested *in vitro* using IFN-responsive HEK293 cells.

Fig. 30 shows the activity of ProC732, ProC1550 and ProC1552 tested *in vitro*
15 using IFN-responsive HEK293 cells in an uncleaved state and after protease activation with either uPa or MTSP1.

Fig. 31 shows activity of recombinant IFNa2b, monomeric IFNa2b (ProC657),
activated homodimeric IFNa2b (ProC440 + uPA), and homodimeric IFNa2b (ProC440)
20 using IFN-responsive HEK293.

Fig. 32 shows anti-tumor activity of ProC440 (top) and peginterferon (bottom) at increasing doses.

Fig. 33 shows anti-tumor activity of dual masked activatable IFNa A/D (ProC1023) compared to dual masked non-activatable IFNa A/D (ProC1549) at 10 µg, 50
25 µg, and 200 µg.

Fig. 34A shows anti-tumor activity of ProC1023 at 10, 50, and 200 µg compared to PBS control. **Fig. 34B** shows anti-tumor activity of ProC1023 compared to non-activatable IFNa A/D (ProC1549) at 200 µg.

Fig. 35A shows MMP restores NSUB (ProC649) activity. **Fig. 35B** shows
30 conditional activation of ProC732 and ProC1299 by uPA. **Fig. 35C** shows IFNa2b (SEQ

ID NO: 1) compared to IFNaAD (SEQ ID NO: 481) and that ProC1301 is resistant to activation compared to ProC732.

Fig. 36 shows tumor growth suppression activity of ProC440, ProC732, and ProC1301 as a function of concentration.

5 **Figs. 37A-37C** show an assay of activation of ProC732 by tumor tissues (Fig. 37A) and results. Fluorescently labeled ProC732 was incubated on tumor tissue sections at 37°C. Recovered solution was then analyzed through capillary electrophoresis enabling quantification of active molecules (Fig. 37C) and using HEK-blue IFNA reporter model (Fig. 37B). Enzymatically inactive samples were used as control tissues.

10 **Figs. 38A-38C** show changes in bioactivity of ProC732 (Fig. 38A) and recombinant IFN- α 2b (Fig. 38B) molecules after incubation with tumor tissues analyzed by HEK-blue IFNA reporter model. Fold change of bioactivity of 10 ng/mL ProC732 or 1 ng/mL of recombinant IFN- α 2b was calculated relative to 0h values. Bioactivity of ProC732 and IFN- α 2b proteins incubated in the absence of tumor tissues for 24h (Fig. 38C). Each line connects an individual sample (concentration range 100-0.01 ng/mL) analyzed before and after 24h incubation.

15 **Figs. 39, 40A, and 40B** show pharmacokinetics of the masked IFN- α 2b and control molecules in non-human primates. Cynomolgus monkey (N=2 per group) were treated with a single dose subcutaneous administration of ProC732 at 0.03, 0.3, 3 or 15 mg/kg. Fig. 39 shows results where plasma samples were collected at indicated time points and analyzed for total ProC732 concentration. Fig. 40A shows concentrations of IP-10 in serum were measured by MSD V-plex assay. Fig. 40B shows concentrations of circulating Pb-IFN- α 2b and IP-10 plotted against each other at day 1 and day 7 after administration.

25 **Figs. 41A-41D** show binding of activated Pb-IFN- α 2b to interferon alpha receptors in vitro. Human IFNAR1, human IFNAR2, cyno IFNAR1 or cyno IFNAR2 proteins were captured on a chip coated with immobilized anti-human Fc. Concentrations of activated IFN- α 2b (ProC1640) ranging from 25 nM to 1.5625 μ M were flowed over the ligand-captured chip to generate multi-cycle kinetic sensorgrams.

30 **Figs. 42A-42D** show binding of single masked Pb-IFN- α 2b molecules to human IFNAR2. The ligands were captured on a chip coated with immobilized anti-human Fc

(Figs. 42A-42B) or anti-histidine antibodies (Figs. 42C-42D). Concentrations of IFN- α 2b (ProC1640) ranging from 25 nM to 1.5625 μ M were flowed over the ligand-captured chip to generate multi-cycle kinetic sensorgrams (Figs. 42A and 42C). Masked Pb-IFN- α 2b molecules (ProC440 – Fig. 42D, ProC1976 – Fig. 42B) at concentrations ranging
5 from 250 nM to 15.625 μ M were flowed over the ligand-captured chip to generate multi-cycle kinetic sensorgrams.

Figs. 43 and 44 show gene expression profile changes induced by ProC732 non-human primates based on concentration (Fig. 43). Cynomolgus monkey (N=2 per group) were treated with a single dose subcutaneous administration of ProC732 at 0.03, 0.3, 3 or
10 15 mg/kg. PBMC from treated animals were harvested and analyzed by bulk RNAseq. Genes were called differentially expressed if number of reads changes were >3 (Fig. 44).

Fig. 45 shows that ProC1023 preferentially activates immune cells in tumor tissues. Six days after the treatment tumors and tissues were harvested and analyzed by flow cytometry. Gated on viable CD45+CD3+ cells.

Fig. 46 shows that ProC732 is well tolerated after multidose administration. Male Syrian golden hamsters (N=5) were treated i.p. with three weekly administrations of ProC732 or unmasked Fc-IFN- α 2b (ProC286) fusion proteins and monitored for body weight changes and survival prior to dosing and twice a week post-dose.

Fig. 47 shows that masking of ProC732 attenuates cytokine/chemokine release in
20 non-human primates.

DETAILED DESCRIPTION

Provided herein are activatable cytokine constructs (ACCs) that exhibit a reduced level of at least one activity of the corresponding cytokine, but which, after exposure to an activation condition, yield a cytokine product having substantially restored activity.

25 Activatable cytokine constructs of the present invention may be designed to selectively activate upon exposure to diseased tissue, and not in normal tissue. As such, these compounds have the potential for conferring the benefit of a cytokine-based therapy, with potentially less of the toxicity associated with certain cytokine-based therapies.

Also provided herein are related intermediates, compositions, kits, nucleic acids, and recombinant cells, as well as related methods, including methods of using and methods of producing any of the activatable cytokine constructs described herein.

The inventors have surprisingly found that ACCs having the specific elements
5 and structural orientations described herein appear potentially effective in improving the safety and therapeutic index of cytokines in therapy, particularly for treating cancers. While cytokines are regulators of innate and adaptive immune system and have broad anti-tumor activity in pre-clinical models, their clinical success has been limited by systemic toxicity and poor systemic exposure to target tissues. The inventors have
10 surprisingly found that ACCs having the specific elements and structural orientations described herein appear to reduce the systemic toxicity associated with cytokine therapeutics and improve targeting and exposure to target issues. As such, the present disclosure provides a method of reducing target-mediated drug disposition (TMDD) of cytokine therapeutics by administering ACCs having the specific elements and structural
15 orientations described herein to a subject. As such, the invention solves the problem of sequestration of a significant fraction of the administered cytokine dose by normal tissues, which is a problem that limits the fraction of the dose available in the systemic circulation to reach the target tissues, e.g., cancerous tissue, in conventional cytokine therapeutics. The present cytokine constructs localizes target binding to tumor tissues,
20 thereby maintaining potency, reducing side effects, enabling new target opportunities, improving the therapeutic window for validated targets, creating a therapeutic window for undruggable targets, and providing multiple binding modalities. The present disclosure enables safe and effective systemic delivery, thereby avoiding the dose-dependent toxicities of conventional systemic cytokine therapies, and also avoids a
25 requirement for intra-tumoral injection. The present disclosure provides a means for imparting localized anti-viral activity, immunomodulatory activity, antiproliferative activity and pro-apoptotic activity. The inventors surprisingly found that dimerization of the first and second monomer constructs achieves high reduction of cytokine activity, and surprisingly discovered that the cytokine activity can be substantially reduced with very
30 high masking efficiency by the addition of a peptide mask at the other terminus of the activatable construct. See, e.g., Figs. 10A-10C.

Applicant's U.S. Provisional App. No. 63/008,542, filed April 10, 2020, and U.S. Provisional App. No. 63/161,889 filed March 16, 2021, which describe certain activatable cytokine constructs without an affinity peptide mask, are incorporated herein by reference in their entireties.

5 **Activatable Cytokine Constructs**

Activatable cytokine constructs (ACCs) of the present invention are dimer complexes comprising a first monomer construct and a second monomer construct. Dimerization of the monomeric components is facilitated by a pair of dimerization domains. In one aspect, each monomer construct includes a cytokine protein (CP), one or
10 more cleavable moieties (CM), a dimerization domain (DD), and a peptide mask (PM). The present inventors unexpectedly found that ACC structures comprising both a dimerization domain and a peptide mask have improved masking efficiency to minimize or eliminate off-target effects and undesired activity and/or toxic side effects of cytokines.

15 In a specific embodiment, the present invention provides an activatable cytokine construct (ACC) that includes a first monomer construct and a second monomer construct, wherein:

(a) the first monomer construct comprises a first peptide mask (PM1), a first mature cytokine protein (CP1), a first and third cleavable moieties (CM1 and CM2), and
20 a first dimerization domain (DD1),

 wherein the CM1 is positioned between the CP1 and the DD1 and the CM2 is positioned between the PM1 and the CP1; and

(b) the second monomer construct comprises a second mature cytokine protein (CP2), a second cleavable moiety (CM3), and a second dimerization domain (DD2),

25 wherein the CM3 is positioned between the CP2 and the DD2;

 wherein the DD1 and the DD2 bind each other thereby forming a dimer of the first monomer construct and the second monomer construct; and

 wherein the ACC is characterized by having a reduced level of at least one CP1 and/or CP2 activity as compared to a control level of the at least one CP1 and/or CP2
30 activity. In some embodiments, the second monomer construct further comprises a second peptide mask (PM2) and a fourth cleavable moiety (CM4) positioned between the

PM2 and the CP2. In some embodiments, the first monomer construct and the second monomer construct are identical and bind one another to form a homodimer. In other embodiments, at least one of the CP, CM, PM, or DD components in each of the first and second monomer constructs is not identical, and the first and second monomer constructs
5 bind one another to form a heterodimer.

The term “activatable” when used in reference to a cytokine construct, refers to a cytokine construct that exhibits a first level of one or more activities, whereupon exposure to a condition that causes cleavage of one or more cleavable moieties results in the generation of a cytokine construct that exhibits a second level of the one or more
10 activities, where the second level of activity is greater than the first level of activity. Non-limiting examples of an activities include any of the exemplary activities of a cytokine described herein or known in the art.

The term “mature cytokine protein” refers herein to a cytokine protein that lacks a signal sequence. A signal sequence is also referred to herein as a “signal peptide.” A
15 cytokine protein (CP) may be a mature cytokine protein or a cytokine protein with a signal peptide. Thus, the ACCs of the present disclosure may include a mature cytokine protein sequence in some aspects. In some aspects, the ACCs of the present disclosure may include a mature cytokine protein sequence and, additionally, a signal sequence. In some aspects, the ACCs of the present disclosure may include sequences disclosed
20 herein, including or lacking the signal sequences recited herein. In some embodiments, a signal sequence is selected from the group consisting of SEQ ID NO: 242, SEQ ID NO: 243, and SEQ ID NO: 244.

The terms “cleavable moiety” and “CM” are used interchangeably herein to refer to a peptide, the amino acid sequence of which comprises a substrate for a sequence-
25 specific protease. Cleavable moieties that are suitable for use as a CM include any of the protease substrates that are known the art. Exemplary cleavable moieties are described in more detail below.

The terms “peptide mask” and “PM” are used interchangeably herein to refer to an amino acid sequence of less than 50 amino acids that reduces or inhibits one or more
30 activities of a cytokine protein. The PM may bind to the cytokine and limit the interaction of the cytokine with its receptor. In some embodiments, the PM is no more

than 40 amino acids in length. In preferred embodiments, the PM is no more than 20 amino acids in length. In some embodiments, the PM is no more than 19, 18, 17, 16, or 15 amino acids in length. In some aspects, the PM has at least 13 amino acids (including any number from 13 to 49). In some aspects, the PM has at least 14 amino acids
5 (including any number from 14 to 49). In some aspects, the PM has at least 15 amino acids (including any number from 15 to 49). In certain aspects, the number of amino acids in the PM may be counted as those amino acids that bind to the cytokine protein. For example, the PM excludes large polypeptides. For example, the PM is not a latency associated peptide. For example, the PM is not a cytokine. For example, the PM is not a
10 receptor for a cytokine. For example, the PM is not a fragment of a receptor for a cytokine. In some aspects, the PM does not have an amino acid sequence that is at least 85% identical to a receptor for a cytokine. For example, the PM is not an albumin. For example, the PM excludes proteins or polypeptides having more than 50 amino acids. In some aspects, the PM excludes proteins or polypeptides having more than 25 amino
15 acids. In some aspects, the PM excludes proteins or polypeptides having more than 20 amino acids. In some aspects, the PM excludes proteins or polypeptides having more than 15 amino acids. In some aspects, the PM does not include amino acids forming flexible N-terminal or C-terminal tail regions.

The terms “dimerization domain” and “DD” are used interchangeably herein to
20 refer to one member of a pair of dimerization domains, wherein each member of the pair is capable of binding to the other via one or more covalent or non-covalent interactions. The first DD and the second DD may be the same or different. Exemplary DDs suitable for use as DD1 and or DD2 are described in more detail herein below.

As used herein, the term “linker” refers to a peptide, the amino acid sequence of
25 which is not a substrate for a protease. Exemplary linkers are described in more detail below.

As used herein, the term “linking region” or “LR” refers to the stretch of amino acid residues between the C-terminus of the cytokine and the amino acid residue that is N-terminally adjacent to the proximal point of interaction between the dimerization
30 domains (i.e., the linking region does not include the C-terminal amino acid of the cytokine or the N-terminal amino acid of the DD that forms the proximal point of

interaction to the DD of the corresponding second monomer). For example, when the DDs are a pair of Fc domains, the linking region is the stretch of amino acid residues between the C-terminus of the cytokine and the first N-terminal cysteine residue of the Fc that participates in the disulfide linkage with the second Fc domain (e.g., Cysteine 226 of an IgG1 or IgG4 Fc domain, according to EU numbering). When the dimerization domain is not a polypeptide, then the linking region is the stretch of amino acid residues following the C-terminus of the cytokine until the last amino acid. For example, when the DDs are a biotin-streptavidin pair, the linking region of the biotin-containing monomer is the stretch of amino acid residues between the C-terminus of the cytokine and the biotin molecule, and the linking region of the streptavidin-containing monomer is the stretch of amino acid residues between the C-terminus of the cytokine and the streptavidin molecule.

As used herein, the term “mask linking region” or “MLR” refers to the stretch of amino acid residues between a PM and a CP. The MLR spans from the N-terminus of a CP to the C-terminus of a PM. Thus, the MLR may include a PM, a PM and a linker, or a PM and two linkers. In some aspects, the MLR spans 15 to 22 amino acids. In some aspects, the MLR spans 16 to 21 amino acids. In some aspects, the MLR spans 17 to 20 amino acids. In some aspects, the MLR spans 18 to 20 amino acids. In some aspects, the MLR spans 15, 16, 17, 18, 19, 20, 21, or 22 amino acids.

As used herein, the term “masking efficiency” refers to the activity (e.g., EC₅₀) of the uncleaved ACC divided by the activity of a control cytokine, wherein the control cytokine may be either cleavage product of the ACC or the cytokine used as the CP of the ACC. An ACC having a reduced level of at least one CP1 and/or CP2 activity has a masking efficiency that is greater than 10. In some embodiments, the ACCs described herein have a masking efficiency that is greater than 10, greater than 100, greater than 1000, or greater than 5000.

As used herein, the term “spacer” refers herein to an amino acid residue or a peptide incorporated at a free terminus of the mature ACC, for example between the signal peptide and the N-terminus of the mature ACC. In some aspects, a spacer (or “header”) may contain glutamine (Q) residues. In some aspects, residues in the spacer minimize aminopeptidase and/or exopeptidase action to prevent cleavage of N-terminal

amino acids. Illustrative and non-limiting spacer amino acid sequences may comprise or consist of any of the following exemplary amino acid sequences: QGQSGS (SEQ ID NO: 246); GQSGS (SEQ ID NO: 247); QSGS (SEQ ID NO: 248); SGS; GS; S; QGQSGQG (SEQ ID NO: 249); GQSGQG (SEQ ID NO: 251); QSGQG (SEQ ID NO: 252); SGQG (SEQ ID NO: 253); GQG; QG; G; QGQSGQ (SEQ ID NO: 254); GQSGQ (SEQ ID NO: 255); QSGQ (SEQ ID NO: 256); QGQSG (SEQ ID NO: 257); QGQS (SEQ ID NO: 258); SGQ; GQ; and Q. In some embodiments, spacer sequences may be omitted.

As used herein, a polypeptide, such as a cytokine or an Fc domain, may be a wild-type polypeptide (e.g., a naturally-existing polypeptide) or a variant of the wild-type polypeptide. A variant may be a polypeptide modified by substitution, insertion, deletion and/or addition of one or more amino acids of the wild-type polypeptide, provided that the variant retains the basic function or activity of the wild-type polypeptide. In some examples, a variant may have altered (e.g., increased or decreased) function or activity comparing with the wild-type polypeptide. In some aspects, the variant may be a functional fragment of the wild-type polypeptide. The term "functional fragment" means that the sequence of the polypeptide (e.g., cytokine) may include fewer amino acids than the full-length polypeptide sequence, but sufficient polypeptide chain length to confer activity (e.g., cytokine activity).

The first and second monomer constructs may further comprise additional elements, such as, for example, one or more linkers, and the like. The additional elements are described below in more detail. The organization of the CP, CM, PM, and DD components in each of the first and second monomer constructs may be arranged in the same order in each monomer construct. The CP1, CM1, PM1, and DD1 components may be the same or different as compared to the corresponding CP2, CM2, PM2, and DD2, in terms of, for example, molecular weight, size, amino acid sequence of the CP, CM, and PM components (and the DD components in embodiments where the DD components are polypeptides), and the like. Thus, the resulting dimer may have symmetrical or asymmetrical monomer construct components.

In some embodiments, the first monomer construct comprises, from N- to C-terminus of the CP and CM components, the PM1, the CM3, the CP1, the CM1, and,

linked directly or indirectly (via a linker) to the C-terminus of the CM1, the DD1. In other embodiments, the first monomer construct comprises from C- to N- terminus of the CP and CM components, the PM1, the CM3, the CP1, the CM1, and, linked directly or indirectly (via a linker) to the N-terminus of the CM1, the DD1. In some embodiments, 5 the second monomer construct comprises, from N- to C- terminal terminus of the CP and CM components, the PM2, the CM4, the CP2, the PM2, the CM2, and, linked directly or indirectly (via a linker) to the C-terminus of the CM2, the DD2. In other embodiments, the second monomer construct comprises, from C- to N- terminus of the CP and CM components, the PM2, the CM4, the CP2, the PM2, the CM2, and, linked directly or 10 indirectly (via a linker) to the N-terminus of the CM2, the DD2. In one example, the first monomer construct comprises a first polypeptide that comprises the PM1, the CM3, the CP1, the CM1, and the DD1. In one example, the second monomer construct comprises a second polypeptide that comprises the CP2, the CM2, and the DD2. In another example, second monomer construct comprises a second polypeptide that comprises the 15 PM2, the CM4, the CP2, the CM2, and the DD2.

In some embodiments, the CP and DD components are linked by a linker that is not cleavable by a protease. For example, the CP and DD components may be linked by a non-cleavable substrate sequence (NSUB). In some embodiments, one of the first and second monomer constructs comprises a NSUB between the CP and DD, and the other 20 comprises a CM between the CP and DD. In some aspects, the linker may be an amino acid substrate sequence that includes glycine and serine residues, but is not susceptible to protease cleavage. Examples of non-cleavable linker sequences include those described in U.S. Patent No. 10,611,845B2, which is incorporated by reference herein by its entirety. In such cases, the CP and/or the DD may have a cleavage site for a protease.

25 Examples of the ACCs in the present disclosure can be presented by the following formulae (in the form of monomer 1/monomer 2, from the N-terminus to the C-terminus in each monomer)

PM1-CM3-CP1-CM1-DD1 / PM2-CM4-CP2-CM2-DD2

PM1-CM3-CP1-CM1-DD1 / CP2-CM2-DD2

30 DD1-CM1-CP1-CM3-PM1 / DD2-CM2-CP2-CM4-PM2

DD1-CM1-CP1-CM3-PM1 / DD2-CM2-CP2

The ACCs may comprise one or more linkers between the components. For example, the ACCs may comprise one or more linkers between PM and CP, and/or between CP and DD. Thus, as used herein and unless otherwise stated, each dash (-) between the ACC components represents either a direct linkage or linkage via one or more linkers.

In some aspects, when the ACC has an orientation of N-PM-CM1-CP-CM2-DD-C, then the entire span of amino acids from the N-terminus of the ACC to the N-terminal amino acid of the cytokine is 17 to 71 amino acids in length. In some aspects, when the ACC has an orientation of N-DD-CM1-CP-CM2-PM-C, then the entire span of amino acids from the C-terminus of the ACC to the C-terminal amino acid of the cytokine is 17 to 71 amino acids in length.

In certain embodiments, the first and second monomeric constructs are oriented such that the components in each member of the dimer are organized in the same order from N-terminus to C-terminus of the CP and CM components. A schematic of an illustrative ACC is provided in Fig. 1. With reference to Fig. 1, the ACC comprises, from N-terminus to C-terminus: (1) a first monomer construct **110** having a PM1 **119**, a CM3 **117**, a CP1 **115**, a CM1 **113**, and a DD1 **111**, and; (2) a second monomer construct **120** having optionally a PM2 **129** and a CM4 **127**, a CP2 **125**, a CM2 **123**, and a DD2 **121**; and (3) one or more covalent or non-covalent bonds ($\longleftrightarrow$) bonding the first monomer construct **110** to the second monomer construct **120**. The ACC may further comprise one or more of the optional linkers **112**, **114**, **116**, **118**, **122**, **124**, **126**, and **128** between the components. In one example, DD1 **111** and DD2 **121** are the same. In another example, DD1 **111** and DD2 **121** are different.

A schematic of a further illustrative ACC, with its components organized in the reverse orientation of the ACC is provided in Fig. 2. With reference to Fig. 2, the ACC comprises, from N-terminus to C-terminus of the CP and CM components: (1) a first monomer construct **210** having a DD1 **211**, a CM1 **213**, a CP1 **215**, a CM3 **217**, and a PM1 **219**; (2) a second monomer construct **220** having a DD2 **221**, a CM2 **223**, a CP2 **225**, and optionally a CM4 **227** and a PM2 **229**; and (3) one or more covalent or non-covalent bonds ($\longleftrightarrow$) bonding the first monomer construct **210** to the second monomer construct **220**. The ACC may further comprise one or more of the optional linkers **212**,

214, 216, 218, 222, 224, 226, and 228 between the components. In one example, DD1 211 and DD2 221 are the same. In another example, DD1 211 and DD2 221 are different.

A schematic of another illustrative ACC is provided in Fig. 3. With reference to Fig. 3, the ACC comprises, from N-terminus to C-terminus: (1) a first monomer construct 310 having a PM1 319, a CM3 317, a CP1 315, a CM1 313, and a DD1 311; and (2) a second monomer construct 320 having a CP2 325, a CM2 323, and a DD2 321, and optionally a PM2 329 and a CM4 327. DD1 311 and DD2 321 are binding partners, e.g., a ligand/receptor pair or an antigen/antigen-binding peptide pair, so that DD1 and DD2 are covalently or non-covalently bound together. The ACC may further comprise one or more of the optional linkers 312, 314, 316, 318, 322, 324, 326, and 328 between the components. In one example, DD1 311 and DD2 321 are the same. In another example, DD1 311 and DD2 321 are different.

In alternative aspects, one of the two moieties depicted as CP1 315 and CP2 325 is a truncated cytokine protein that lacks cytokine activity. For example, either CP1 or CP2 may be a truncated interferon alpha 2b having the first 151 amino acids of wild-type interferon alpha 2b. In alternative aspects, one of the two moieties depicted as CP1 315 and CP2 325 is a mutated cytokine protein that lacks cytokine activity. For example, either CP1 or CP2 may be a truncated interferon alpha 2b having a L130P mutation (e.g., SEQ ID NO: 329). In alternative aspects, one of the two moieties depicted as CP1 315 and CP2 325 is a polypeptide sequence that lacks cytokine activity, e.g., a signal moiety and/or a stub sequence. In alternative aspects, a first one of the two moieties depicted as CP1 315 and CP2 325 is a polypeptide sequence that binds with high affinity to a second one of the two moieties depicted as CP1 315 and CP2 325 and reduces the cytokine activity of the second moiety as compared to the control level of the second moiety.

A schematic of another illustrative ACC, with its components organized in the reverse orientation, is provided in Fig. 4. With reference to Fig. 4, the ACC comprises, from N-terminus to C-terminus of the CP and CM components: (1) a first monomer construct 410 having a DD1 411, a CM1 413, a CP1 415, a CM3 417, and a PM1 419; and (2) a second monomer construct 420 having a DD2 421, a CM2 423, a CP2 425, and optionally a CM4 427 and a PM2 429. DD1 411 and DD2 421 are binding partners, e.g.,

a ligand/receptor pair or an antigen/antigen-binding peptide pair, so that DD1 and DD2 are covalently or non-covalently bound together. The ACC may further comprise one or more of the optional linkers 412, 414, 416, 418, 422, 424, 426, and 428 between the components. In one example, DD1 411 and DD2 421 are the same. In another example, DD1 411 and DD2 421 are different.

The ACC structure was discovered to be highly effective at reducing activity of the mature cytokine protein components in a way that does not lead to substantially impaired cytokine activity after activation. The CP's activity in the ACC may be reduced by both the structure of the ACC (e.g., the dimer structure) and the peptide mask(s) in the ACC. In some embodiments, the activation condition for the ACCs described herein is exposure to one or more proteases that can dissociate the CP from both the DD and the PM. For example, the one or more proteases may cleave the CM between the CP and the PM and the CM between the CP and the DD. As demonstrated in the Examples, activation of the ACC resulted in substantial recovery of cytokine activity. The results suggest that conformation of the cytokine components was not irreversibly altered within the context of the ACC. Significantly, the inventors have discovered that the present structure, utilizing both a dimerization domain and one or more peptide masks that have specific binding affinity for the cytokine protein appears to result in a substantial masking effect achieved over use of either a peptide mask alone or a dimerization domain alone.

The ACC may employ any of a variety of mature cytokine proteins, cleavable moieties, peptide masks, and dimerization domains as the CP1, CP2, CM1, CM2, CM3, CM4, PM1, PM2, DD1, and DD2, respectively. For example, any of a variety of mature cytokine proteins that are known in the art or sequence and/or truncation variants thereof, may be suitable for use as either or both CP1 and CP2 components of the ACC. The mature cytokine proteins, CP1 and CP2 may be the same or different. In certain specific embodiments, CP1 and CP2 are the same. In other embodiments, CP1 and CP2 are different. The ACC may comprise additional amino acid residues at either or both N- and/or C-terminal ends of the CP1 and/or CP2.

In some embodiments, the CP1 and/or the CP2 may each independently comprise a mature cytokine protein selected from the group of: an interferon (such as, for example, an interferon alpha, an interferon beta, an interferon gamma, an interferon tau, and an

interferon omega), an interleukin (such as, for example, IL-1 α , IL-1 β , IL-1RA, IL-18, IL-2, IL-4, IL-7, IL-9, IL-13, IL-15, IL-3, IL-5, GM-CSF, IL-6, IL-11, IL-21), G-CSF, IL-12, LIF, OSM, IL-10, IL-20, IL-14, IL-16, IL-17, CD154, LT- β , TNF- α , TNF- β , 4-1BBL, APRIL, CD27, CD70, CD153, CD178, GITRL, LIGHT, OX40L, OX40, TALL-1, TRAIL, TWEAK, TRANCE, TGF- β 1, TGF- β 2, TGF- β 3, EPOo, TPO, Flt-3L, SCF, M-CSF, and MSP, and the like, as well as sequence and truncation variants thereof. In particular, an ACC for use in combination may contain IL-2, IL-7, IL-8, IL-10, IL-12, IL-15, IL-21, an IFN-alpha, an IFN beta, an IFN gamma, GM-CSF, TGF-beta, LIGHT, GTR-L, CD40L, CD27L, 4-1BB-L, OX40, OX40L. For example, sequences of such proteins include those in Table 11 and additional examples of the sequences can be obtained from ncbi.nlm.nih.gov/protein. Truncation variants that are suitable for use in the ACCs of the present invention include any N- or C- terminally truncated cytokine that retains a cytokine activity. Exemplary truncation variants employed in the present invention include any of the truncated cytokine polypeptides that are known in the art (see, e.g., Slutzki et al., *J. Mol. Biol.* 360:1019-1030, 2006, and US 2009/0025106), as well as cytokine polypeptides that are N- and/or C-terminally truncated by 1 to about 40 amino acids, 1 to about 35 amino acids, 1 to about 30 amino acids, 1 to about 25 amino acids, 1 to about 20 amino acids, 1 to about 15 amino acids, 1 to about 10 amino acids, 1 to about 8 amino acids, 1 to about 6 amino acids, 1 to about 4 amino acids, that retain a cytokine activity. In some of the foregoing embodiments, the truncated CP is an N-terminally truncated CP. In other embodiments, the truncated CP is a C-terminally truncated CP. In certain embodiments, the truncated CP is a C- and an N-terminally truncated CP.

In some embodiments, the CP1 and/or the CP2 each independently comprise an amino acid sequence that is at least 80% identical (e.g., at least 82%, at least 84%, at least 86%, at least 88%, at least 90%, at least 92%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical) to a cytokine reference sequence selected from the group consisting of: SEQ ID NO: 101, SEQ ID NO: 102, SEQ ID NO: 103, SEQ ID NO: 104, SEQ ID NO: 105, SEQ ID NO: 106, SEQ ID NO: 107, SEQ ID NO: 108, SEQ ID NO: 109, SEQ ID NO: 110, SEQ ID NO: 111, SEQ ID NO: 112, SEQ ID NO: 113, SEQ ID NO: 114, SEQ ID NO: 115, SEQ ID NO: 116, SEQ ID NO: 117,

SEQ ID NO: 118, SEQ ID NO: 119, SEQ ID NO: 12, SEQ ID NO: 121, SEQ ID NO: 122, SEQ ID NO: 123, SEQ ID NO: 124, SEQ ID NO: 125, SEQ ID NO: 126, SEQ ID NO: 127, SEQ ID NO: 128, SEQ ID NO: 129, SEQ ID NO: 130, SEQ ID NO: 131, SEQ ID NO: 132, SEQ ID NO: 133, SEQ ID NO: 134, SEQ ID NO: 135, SEQ ID NO: 136, SEQ ID NO: 137, SEQ ID NO: 138, SEQ ID NO: 139, SEQ ID NO: 140, SEQ ID NO: 141, SEQ ID NO: 142, SEQ ID NO: 143, SEQ ID NO: 144, SEQ ID NO: 145, SEQ ID NO: 146, SEQ ID NO: 147, SEQ ID NO: 148, SEQ ID NO: 149, SEQ ID NO: 150, SEQ ID NO: 151, SEQ ID NO: 152, SEQ ID NO: 153, SEQ ID NO: 154, SEQ ID NO: 155, SEQ ID NO: 156, SEQ ID NO: 157, SEQ ID NO: 158, SEQ ID NO: 159, SEQ ID NO: 160, SEQ ID NO: 161, SEQ ID NO: 162, SEQ ID NO: 163, SEQ ID NO: 164, SEQ ID NO: 165, SEQ ID NO: 166, SEQ ID NO: 167, SEQ ID NO: 168, SEQ ID NO: 169, SEQ ID NO: 170, SEQ ID NO: 171, SEQ ID NO: 172, SEQ ID NO: 173, SEQ ID NO: 174, SEQ ID NO: 175, SEQ ID NO: 176, SEQ ID NO: 177, SEQ ID NO: 178, SEQ ID NO: 179, SEQ ID NO: 180, SEQ ID NO: 181, SEQ ID NO: 182, SEQ ID NO: 183, SEQ ID NO: 184, SEQ ID NO: 185, SEQ ID NO: 186, SEQ ID NO: 187, SEQ ID NO: 188, SEQ ID NO: 189, SEQ ID NO: 190, SEQ ID NO: 191, SEQ ID NO: 192, SEQ ID NO: 193, SEQ ID NO: 194, SEQ ID NO: 195, SEQ ID NO: 196, SEQ ID NO: 197, SEQ ID NO: 198, SEQ ID NO: 199, SEQ ID NO: 200, SEQ ID NO: 201, SEQ ID NO: 202, SEQ ID NO: 203, SEQ ID NO: 204, SEQ ID NO: 205, SEQ ID NO: 206, SEQ ID NO: 207, SEQ ID NO: 208, and SEQ ID NO: 209. The percentage of sequence identity refers to the level of amino acid sequence identity between two or more peptide sequences when aligned using a sequence alignment program, e.g., the suite of BLAST programs, publicly available on the Internet at the NCBI website. See also Altschul et al., J. Mol. Biol. 215:403-10, 1990. In some aspects, the ACC includes an interferon alpha 2b mutant, for example, an interferon alpha 2b molecule having a mutation at position L130, e.g., L130P mutation relative to SEQ ID NO: 1 (e.g., SEQ ID NO: 329), as either CP1 or CP2. In some aspects, the ACC includes an interferon alpha 2b mutant having a mutation at position I24, F64, I60, I63, F64, W76, I116, L117, F123, or L128, or a combination thereof. For example, the interferon alpha 2b mutant may include mutations I116 to T, N, or R; L128 to N, H, or R; I24 to P or Q; L117H; or L128T, or a combination thereof. In some aspects, the interferon alpha 2b mutant may include mutations I24Q, I60T, F64A,

W76H, I116R, and L128N, or a subset thereof. In some aspects, the ACC includes as one of CP1 and CP2 a truncated interferon alpha 2b molecule that lacks cytokine activity. For example, the truncated interferon alpha 2b may consist of 151 or fewer amino acids of interferon alpha 2b, e.g., any one of amino acids in the wild-type interferon alpha 2b sequence from N to C-terminus: 1 to 151, 1 to 150, 1 to 149, 1 to 148, . . . 1 to 10, 1 to 9, 1 to 8, 1 to 7, 1 to 6, or 2 to 151, 3 to 151, 4 to 151, 5 to 150, 6 to 149, 7 to 148, 8 to 147, or any intervening sequence of amino acids or mutants thereof.

In certain specific embodiments, the CP1 and/or the CP2 comprise an interferon. Interferons that are suitable for use in the constructs of the present invention as CP1 and/or CP2 include, for example, an interferon-alpha, an interferon-beta, an interferon-gamma, an interferon-omega, and an interferon-tau. In some embodiments, when the interferon is an interferon alpha, it may be an interferon alpha-2a, an interferon alpha-2b, or an interferon alpha-n3. Further examples of interferon alpha include interferon alpha-1, interferon alpha-4, interferon alpha-5, interferon alpha-6, interferon alpha-7, interferon alpha-8, interferon alpha-10, interferon alpha-13, interferon alpha-14, interferon alpha-16, interferon alpha-17, and interferon alpha-21. In some embodiments, the interferon is a recombinant or purified interferon alpha. In certain embodiments, when the interferon is an interferon-beta, it is selected from the group consisting of an interferon beta-1a and an interferon beta-1b. In some embodiments, the CP1 and/or the CP2 comprises an IFab domain, which is a conserved protein domain found in interferon alpha or interferon beta. The IFab domain is responsible for the cytokine release and antiviral functions of interferons. Exemplary IFab sequences are provided in SEQ ID Nos: 482-491. In one example, the CP1 and the CP2 are different interferons. In another example, the CP1 and the CP2 are the same interferon.

In some embodiments, the CP1 and/or the CP2 exhibit(s) an interferon activity and include(s) an amino acid sequence that is at least 80% identical, at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, or at least 99% identical, or 100% identical to an interferon alpha reference sequence selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 101, SEQ ID NO: 102, SEQ ID NO: 103, SEQ ID NO: 104, and SEQ ID NO: 105. In

certain specific embodiments, the interferon alpha reference sequence is SEQ ID NO: 1 (human interferon alpha-2b). In some embodiments, the CP1 and/or the CP2 comprise a mature alpha interferon having an amino acid sequence selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 101, SEQ ID NO: 102, SEQ ID NO: 103, SEQ ID NO: 104, and SEQ ID NO: 105. In certain embodiments, the CP1 and/or the CP2 comprise a mature human alpha interferon having the amino acid sequence of SEQ ID NO: 1. In some of the above-described embodiments, the CP1 and the CP2 comprise the same amino acid sequence.

In other embodiments, the CP1 and/or the CP2 exhibit(s) an interferon activity and include(s) an amino acid sequence that is at least 80% identical, at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, or at least 99% identical, or 100% identical to an interferon beta reference sequence selected from the group consisting of SEQ ID NO: 106, SEQ ID NO: 107, SEQ ID NO: 108, and SEQ ID NO: 109. In certain embodiments, the interferon beta reference sequence is a human interferon beta reference sequence selected from the group consisting of SEQ ID NO: 106 and SEQ ID NO: 107. In some embodiments, the CP1 and/or the CP2 comprise a mature beta interferon having an amino acid sequence selected from the group consisting of SEQ ID NO: 106, SEQ ID NO: 107, SEQ ID NO: 108, and SEQ ID NO: 109. In some of the above-described embodiments, the CP1 and the CP2 comprise the same amino acid sequence.

In some embodiments, the CP1 and/or CP2 exhibit(s) an interferon activity and include(s) an amino acid sequence that is at least 80% identical, at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, or at least 99% identical, or 100% identical to an interferon omega reference sequence corresponding to SEQ ID NO: 110 (human interferon omega). In certain specific embodiments, the CP1 and/or CP2 comprise a mature human omega interferon having the amino acid sequence of SEQ ID NO: 110. In some of the above-described embodiments, the CP1 and the CP2 comprise the same amino acid sequence.

In some embodiments, the CP1 and/or the CP2 exhibit(s) an interleukin activity and include(s) an amino acid sequence that is at least 80% identical, at least 82%, at least 84%, at least 86%, at least 88%, at least 90%, at least 92%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical or 100% identical to an interleukin reference sequence selected from the group consisting of: SEQ ID NO: 111, SEQ ID NO: 112, SEQ ID NO: 113, SEQ ID NO: 114, SEQ ID NO: 115, SEQ ID NO: 116, SEQ ID NO: 117, SEQ ID NO: 118, SEQ ID NO: 119, SEQ ID NO: 12, SEQ ID NO: 121, SEQ ID NO: 122, SEQ ID NO: 123, SEQ ID NO: 124, SEQ ID NO: 125, SEQ ID NO: 126, SEQ ID NO: 127, SEQ ID NO: 128, SEQ ID NO: 129, SEQ ID NO: 130, SEQ ID NO: 131, SEQ ID NO: 132, SEQ ID NO: 133, SEQ ID NO: 134, SEQ ID NO: 135, SEQ ID NO: 136, SEQ ID NO: 137, SEQ ID NO: 138, SEQ ID NO: 139, SEQ ID NO: 140, SEQ ID NO: 143, SEQ ID NO: 144, SEQ ID NO: 145, SEQ ID NO: 146, SEQ ID NO: 151, SEQ ID NO: 152, SEQ ID NO: 153, SEQ ID NO: 154, SEQ ID NO: 155, SEQ ID NO: 156, SEQ ID NO: 157, SEQ ID NO: 158, SEQ ID NO: 159, and SEQ ID NO: 160. In some embodiments, CP1 and/or CP2 comprises a mature interleukin having an amino acid sequence selected from the group consisting of: SEQ ID NO: 111, SEQ ID NO: 112, SEQ ID NO: 113, SEQ ID NO: 114, SEQ ID NO: 115, SEQ ID NO: 116, SEQ ID NO: 117, SEQ ID NO: 118, SEQ ID NO: 119, SEQ ID NO: 12, SEQ ID NO: 121, SEQ ID NO: 122, SEQ ID NO: 123, SEQ ID NO: 124, SEQ ID NO: 125, SEQ ID NO: 126, SEQ ID NO: 127, SEQ ID NO: 128, SEQ ID NO: 129, SEQ ID NO: 130, SEQ ID NO: 131, SEQ ID NO: 132, SEQ ID NO: 133, SEQ ID NO: 134, SEQ ID NO: 135, SEQ ID NO: 136, SEQ ID NO: 137, SEQ ID NO: 138, SEQ ID NO: 139, SEQ ID NO: 140, SEQ ID NO: 143, SEQ ID NO: 144, SEQ ID NO: 145, SEQ ID NO: 146, SEQ ID NO: 151, SEQ ID NO: 152, SEQ ID NO: 153, SEQ ID NO: 154, SEQ ID NO: 155, SEQ ID NO: 156, SEQ ID NO: 157, SEQ ID NO: 158, SEQ ID NO: 159, and SEQ ID NO: 160. In some of the above-described embodiments, the CP1 and the CP2 comprise the same amino acid sequence.

In some embodiments, CP1 and/or CP2 exhibit(s) an interleukin activity and include(s) an amino acid sequence that is at least 80% identical, at least 82%, at least 84%, at least 86%, at least 88%, at least 90%, at least 92%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to an interleukin reference

sequence selected from the group consisting of SEQ ID NO: 111 (human IL-1 alpha), SEQ ID NO: 113 (human IL-1 beta), SEQ ID NO: 115 (human IL-1RA), SEQ ID NO: 117 (human IL-18), SEQ ID NO: 119 (human IL-2), SEQ ID NO: 121 (human IL-4), SEQ ID NO: 123 (human IL-7), SEQ ID NO: 125 (human IL-9), SEQ ID NO: 127 (human IL-13), SEQ ID NO: 129 (human IL-15), SEQ ID NO: 131 (human IL-3), SEQ ID NO: 133 (human IL-5), SEQ ID NO: 137 (human IL-6), SEQ ID NO: 139 (human IL-11), SEQ ID NO: 143 (human IL-12 alpha), SEQ ID NO: 144 (human IL-12 beta), SEQ ID NO: 151 (human IL-10), SEQ ID NO: 153 (human IL-20), SEQ ID NO: 155 (human IL-14), SEQ ID NO: 157 (human IL-16), and SEQ ID NO: 159 (human IL-17). In certain of these embodiments, CP1 and/or CP2 comprise an amino acid sequence from the group consisting of SEQ ID NO: 111 (human IL-1 alpha), SEQ ID NO: 113 (human IL-1 beta), SEQ ID NO: 115 (human IL-1RA), SEQ ID NO: 117 (human IL-18), SEQ ID NO: 119 (human IL-2), SEQ ID NO: 121, SEQ ID NO: 123 (human IL-7), SEQ ID NO: 125 (human IL-9), SEQ ID NO: 127 (human IL-13), SEQ ID NO: 129 (human IL-15), SEQ ID NO: 131 (human IL-3), SEQ ID NO: 133 (human IL-5), SEQ ID NO: 137 (human IL-6), SEQ ID NO: 139 (human IL-11), SEQ ID NO: 143 (human IL-12 alpha), SEQ ID NO: 144 (human IL-12 beta), SEQ ID NO: 151 (human IL-10), SEQ ID NO: 153 (human IL-20), SEQ ID NO: 155 (human IL-14), SEQ ID NO: 157 (human IL-16), and SEQ ID NO: 159 (human IL-17). In some of the above-described embodiments, the CP1 and the CP2 comprise the same amino acid sequence.

The number of amino acids in the sequence of the cytokine proteins employed may vary, depending on the specific cytokine protein employed. In some embodiments, the CP1 and/or the CP2 includes a total of about 10 amino acids to about 700 amino acids, about 10 amino acids to about 650 amino acids, about 10 amino acids to about 600 amino acids, about 10 amino acids to about 550 amino acids, about 10 amino acids to about 500 amino acids, about 10 amino acids to about 450 amino acids, about 10 amino acids to about 400 amino acids, about 10 amino acids to about 350 amino acids, about 10 amino acids to about 300 amino acids, about 10 amino acids to about 250 amino acids, about 10 amino acids to about 200 amino acids, about 10 amino acids to about 150 amino acids, about 10 amino acids to about 100 amino acids, about 10 amino acids to about 80 amino acids, about 10 amino acids to about 60 amino acids, about 10 amino acids to

[illegible]

[illegible]

amino acids, about 350 amino acids to about 600 amino acids, about 350 amino acids to about 550 amino acids, about 350 amino acids to about 500 amino acids, about 350 amino acids to about 450 amino acids, about 350 amino acids to about 400 amino acids, about 400 amino acids to about 700 amino acids, about 400 amino acids to about 650 amino acids, about 400 amino acids to about 600 amino acids, about 400 amino acids to about 550 amino acids, about 400 amino acids to about 500 amino acids, about 400 amino acids to about 450 amino acids, about 450 amino acids to about 700 amino acids, about 450 amino acids to about 650 amino acids, about 450 amino acids to about 600 amino acids, about 450 amino acids to about 550 amino acids, about 450 amino acids to about 500 amino acids, about 500 amino acids to about 700 amino acids, about 500 amino acids to about 650 amino acids, about 500 amino acids to about 600 amino acids, about 500 amino acids to about 550 amino acids, about 550 amino acids to about 700 amino acids, about 550 amino acids to about 650 amino acids, about 550 amino acids to about 600 amino acids, about 600 amino acids to about 700 amino acids, about 600 amino acids to about 650 amino acids, or about 650 amino acids to about 700 amino acids. In some embodiments, CP1 and/or the CP2 is a mature wildtype human cytokine protein.

Each monomer construct of the ACC may employ any of a variety of dimerization domains. Suitable DDs include both polymeric (e.g., a synthetic polymer, a polypeptide, a polynucleotide, and the like) and small molecule (non-polymeric moieties having a molecular weight of less than about 1 kilodalton, and sometimes less than about 800 daltons) types of moieties. The pair of DDs may be any pair of moieties that are known in the art to bind to each other.

For example, in some embodiments, the DD1 and the DD2 are members of a pair selected from the group of: a sushi domain from an alpha chain of human IL-15 receptor (IL15R α) and a soluble IL-15; barnase and barnstar; a protein kinase A (PKA) and an A-kinase anchoring protein (AKAP); adapter/docking tag molecules based on mutated RNase I fragments; a pair of antigen-binding domains (e.g., a pair of single domain antibodies); soluble N-ethyl-maleimide sensitive factor attachment protein receptors (SNARE); modules based on interactions of the proteins syntaxin, synaptotagmin, synaptobrevin, and SNAP25; a single domain antibody (sdAb) and corresponding

epitope; an antigen-binding domain (e.g., a single chain antibody such as a single chain variable fragment (scFv), a single domain antibody, and the like) and a corresponding epitope; coiled coil polypeptide structures (e.g., Fos-Jun coiled coil structures, acid/base coiled-coil helices, Glu-Lys coiled coil helices, leucine zipper structures), small molecule
5 binding pairs such as biotin and avidin or streptavidin, amine/aldehyde, lectin/carbohydrate; a pair of polymers that can bind each other, such as, for example, a pair of sulfur- or thiol-containing polymers (e.g., a pair of Fc domains, a pair of thiolized-human serum albumin polypeptides, and the like); and the like.

In some embodiments, the DD1 and DD2 are non-polypeptide polymers. The
10 non-polypeptide polymers may covalently bound to each other. In some examples, the non-polypeptide polymers may be a sulfur-containing polymer, e.g., sulfur-containing polyethylene glycol. In such cases, the DD1 and DD2 may be covalently bound to each other via one or more disulfide bonds.

When the pair of DD1 and DD2 are members of a pair of epitope and antigen-
15 binding domain, the epitope may be a naturally or non-naturally occurring epitope. Exemplary non-naturally occurring epitopes include, for example, a non-naturally occurring peptide, such as, for example, a poly-His peptide (e.g., a His tag, and the like).

In certain specific embodiments, the DD1 and the DD2 are a pair of Fc domains. As used herein, an "Fc domain" refers to a contiguous amino acid sequence of a single
20 heavy chain of an immunoglobulin, e.g., the CH2-CH3 domains of IgG, IgA, or IgD, or the CH2-CH3-CH4 domains of IgE or IgM. A pair of Fc domains associate together to form an Fc region of an immunoglobulin.

In some embodiments, the pair of Fc domains is a pair of human Fc domains (e.g., a pair of wildtype human Fc domains). In some embodiments, the human Fc
25 domains are human IgG1 Fc domains (e.g., wildtype human IgG1 Fc domains), human IgG2 Fc domains (e.g., wildtype human IgG2 Fc domains), human IgG3 Fc domains (e.g., wildtype human IgG3 Fc domains), or human IgG4 Fc domains (e.g., wildtype human IgG4 Fc domains). In some embodiments, the human Fc domains comprise a sequence that is at least 80% identical (e.g., at least 82%, at least 84%, at least 85%, at
30 least 86%, at least 88%, at least 90%, at least 92%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical) to SEQ ID NO: 3.

In some embodiments, the pair of Fc domains comprise a knob mutant and a hole mutant of a Fc domain. The knob and hole mutants may interact with each other to facilitate the dimerization. In some embodiments, the knob and hole mutants may comprise one or more amino acid modifications within the interface between two Fc domains (e.g., in the CH3 domain). In one example, the modifications comprise amino acid substitution T366W and optionally the amino acid substitution S354C in one of the antibody heavy chains, and the amino acid substitutions T366S, L368A, Y407V and optionally Y349C in the other one of the antibody heavy chains (numbering according to EU index of Kabat numbering system). Examples of the knob and hole mutants include Fc mutants of SEQ ID NOs: 318 and 319, as well as those described in U.S. Pat. Nos. 5,731,168; 7,695,936; and 10,683,368, which are incorporated herein by reference in their entireties. In some embodiments, the dimerization domains comprise a sequence that is at least 80% identical (e.g., at least 82%, at least 84%, at least 85%, at least 86%, at least 88%, at least 90%, at least 92%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical) to SEQ ID NOs: 318 and 319, respectively.

In some embodiments, DD1 and/or DD2 can further include a serum half-life extending moiety (e.g., polypeptides that bind serum proteins, such as immunoglobulin (e.g., IgG) or serum albumin (e.g., human serum albumin (HSA))). Examples of half-life extending moieties include hexa-hat GST (glutathione S-transferase) glutathione affinity, Calmodulin-binding peptide (CBP), Strep-tag, Cellulose Binding Domain, Maltose Binding Protein, S-Peptide Tag, Chitin Binding Tag, Immuno-reactive Epitopes, Epitope Tags, E2Tag, HA Epitope Tag, Myc Epitope, FLAG Epitope, AU1 and AU5 Epitopes, Glu-Glu Epitope, KT3 Epitope, IRS Epitope, Btag Epitope, Protein Kinase-C Epitope, and VSV Epitope.

In some embodiments, DD1 and/or DD2 each include a total of about 5 amino acids to about 250 amino acids, about 5 amino acids to about 200 amino acids, about 5 amino acids to about 180 amino acids, about 5 amino acids to about 160 amino acids, about 5 amino acids to about 140 amino acids, about 5 amino acids to about 120 amino acids, about 5 amino acids to about 100 amino acids, about 5 amino acids to about 80 amino acids, about 5 amino acids to about 60 amino acids, about 5 amino acids to about

40 amino acids, about 5 amino acids to about 20 amino acids, about 5 amino acids to about 10 amino acids, about 10 amino acids to about 250 amino acids, about 10 amino acids to about 200 amino acids, about 10 amino acids to about 180 amino acids, about 10 amino acids to about 160 amino acids, about 10 amino acids to about 140 amino acids, about 10 amino acids to about 120 amino acids, about 10 amino acids to about 100 amino acids, about 10 amino acids to about 80 amino acids, about 10 amino acids to about 60 amino acids, about 10 amino acids to about 40 amino acids, about 10 amino acids to about 20 amino acids, about 20 amino acids to about 250 amino acids, about 20 amino acids to about 200 amino acids, about 20 amino acids to about 180 amino acids, about 20 amino acids to about 160 amino acids, about 20 amino acids to about 140 amino acids, about 20 amino acids to about 120 amino acids, about 20 amino acids to about 100 amino acids, about 20 amino acids to about 80 amino acids, about 20 amino acids to about 60 amino acids, about 20 amino acids to about 40 amino acids, about 40 amino acids to about 250 amino acids, about 40 amino acids to about 200 amino acids, about 40 amino acids to about 180 amino acids, about 40 amino acids to about 160 amino acids, about 40 amino acids to about 140 amino acids, about 40 amino acids to about 120 amino acids, about 40 amino acids to about 100 amino acids, about 40 amino acids to about 80 amino acids, about 40 amino acids to about 60 amino acids, about 60 amino acids to about 250 amino acids, about 60 amino acids to about 200 amino acids, about 60 amino acids to about 180 amino acids, about 60 amino acids to about 160 amino acids, about 60 amino acids to about 140 amino acids, about 60 amino acids to about 120 amino acids, about 60 amino acids to about 100 amino acids, about 60 amino acids to about 80 amino acids, about 80 amino acids to about 250 amino acids, about 80 amino acids to about 200 amino acids, about 80 amino acids to about 180 amino acids, about 80 amino acids to about 160 amino acids, about 80 amino acids to about 140 amino acids, about 80 amino acids to about 120 amino acids, about 80 amino acids to about 100 amino acids, about 100 amino acids to about 250 amino acids, about 100 amino acids to about 200 amino acids, about 100 amino acids to about 180 amino acids, about 100 amino acids to about 160 amino acids, about 100 amino acids to about 140 amino acids, about 100 amino acids to about 120 amino acids, about 120 amino acids to about 250 amino acids, about 120 amino acids to about 200 amino acids, about 120 amino acids to about 180 amino acids, about 120 amino acids to about 160 amino acids, about 120 amino acids to about 140 amino acids, about 120 amino acids to about 120 amino acids, about 120 amino acids to about 100 amino acids, about 120 amino acids to about 80 amino acids, about 120 amino acids to about 60 amino acids, about 120 amino acids to about 40 amino acids, about 120 amino acids to about 20 amino acids, about 120 amino acids to about 10 amino acids, about 120 amino acids to about 5 amino acids, about 5 amino acids to about 10 amino acids, about 10 amino acids to about 20 amino acids, about 20 amino acids to about 40 amino acids, about 40 amino acids to about 80 amino acids, about 80 amino acids to about 160 amino acids, about 160 amino acids to about 320 amino acids, about 320 amino acids to about 640 amino acids, about 640 amino acids to about 1280 amino acids, about 1280 amino acids to about 2560 amino acids, about 2560 amino acids to about 5120 amino acids, about 5120 amino acids to about 10240 amino acids, about 10240 amino acids to about 20480 amino acids, about 20480 amino acids to about 40960 amino acids, about 40960 amino acids to about 81920 amino acids, about 81920 amino acids to about 163840 amino acids, about 163840 amino acids to about 327680 amino acids, about 327680 amino acids to about 655360 amino acids, about 655360 amino acids to about 1310720 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to about 23058430092136939520 amino acids, about 23058430092136939520 amino acids to about 46116860184273879040 amino acids, about 46116860184273879040 amino acids to about 92233720368547758080 amino acids, about 92233720368547758080 amino acids to about 184467440737095516160 amino acids, about 184467440737095516160 amino acids to about 368934881474191032320 amino acids, about 368934881474191032320 amino acids to about 737869762948382064640 amino acids, about 737869762948382064640 amino acids to about 1475739525896764129280 amino acids, about 1475739525896764129280 amino acids to about 2951479051793528258560 amino acids, about 2951479051793528258560 amino acids to about 5902958103587056517120 amino acids, about 5902958103587056517120 amino acids to about 11805916207174113034240 amino acids, about 11805916207174113034240 amino acids to about 23611832414348226068480 amino acids, about 23611832414348226068480 amino acids to about 47223664828696452136960 amino acids, about 47223664828696452136960 amino acids to about 94447329657392904273920 amino acids, about 94447329657392904273920 amino acids to about 188894659314785808547840 amino acids, about 188894659314785808547840 amino acids to about 377789318629571617095680 amino acids, about 377789318629571617095680 amino acids to about 755578637259143234191360 amino acids, about 755578637259143234191360 amino acids to about 1511157274518286468382720 amino acids, about 1511157274518286468382720 amino acids to about 3022314549036572936765440 amino acids, about 3022314549036572936765440 amino acids to about 6044629098073145873530880 amino acids, about 6044629098073145873530880 amino acids to about 12089258196146291747061760 amino acids, about 12089258196146291747061760 amino acids to about 24178516392292583494123520 amino acids, about 24178516392292583494123520 amino acids to about 4835703278458516698824704

amino acids to about 160 amino acids, about 120 amino acids to about 140 amino acids, about 140 amino acids to about 250 amino acids, about 140 amino acids to about 200 amino acids, about 140 amino acids to about 180 amino acids, about 140 amino acids to about 160 amino acids, about 160 amino acids to about 250 amino acids, about 160 amino acids to about 200 amino acids, about 160 amino acids to about 180 amino acids, about 180 amino acids to about 250 amino acids, about 180 amino acids to about 200 amino acids, or about 200 amino acids to about 250 amino acids. In some embodiments, DD1 and DD2 are each an Fc domain that comprises a portion of the hinge region that includes two cysteine residues, a CH2 domain, and a CH3 domain. In some

5 amino acids to about 200 amino acids, about 160 amino acids to about 180 amino acids, about 180 amino acids to about 250 amino acids, about 180 amino acids to about 200 amino acids, or about 200 amino acids to about 250 amino acids. In some embodiments, DD1 and DD2 are each an Fc domain that comprises a portion of the hinge region that includes two cysteine residues, a CH2 domain, and a CH3 domain. In some

10 embodiments, DD1 and DD2 are each an Fc domain whose N-terminus is the first cysteine residue (reading in the N- to C- direction) in the hinge region that participates in a disulfide linkage with a second Fc domain (e.g., Cysteine 226 of human IgG1 or IgG4, using EU numbering).

In some aspects, positioned between the CP and the DD, and/or between the CP and the PM components, either directly or indirectly (e.g., via a linker), is a cleavable moiety that comprises a substrate for a protease. In some embodiments, the CMs may each independently comprise a substrate for a protease selected from the group consisting of ADAM8, ADAM9, ADAM10, ADAM12, ADAM15, ADAM17/TACE, ADEMDEC1, ADAMTS1, ADAMTS4, ADAMTS5, BACE, Renin, Cathepsin D, Cathepsin E, Caspase

15 and the PM components, either directly or indirectly (e.g., via a linker), is a cleavable moiety that comprises a substrate for a protease. In some embodiments, the CMs may each independently comprise a substrate for a protease selected from the group consisting of ADAM8, ADAM9, ADAM10, ADAM12, ADAM15, ADAM17/TACE, ADEMDEC1, ADAMTS1, ADAMTS4, ADAMTS5, BACE, Renin, Cathepsin D, Cathepsin E, Caspase

20 1, Caspase 2, Caspase 3, Caspase 4, Caspase 5, Caspase 6, Caspase 7, Caspase 8, Caspase 9, Caspase 10, Caspase 14, Cathepsin A, Cathepsin B, Cathepsin C, Cathepsin G, Cathepsin K, Cathepsin L, Cathepsin S, Cathepsin V/L2, Cathepsin X/Z/P, Chymase, Cruzipain, DESC1, DPP-4, FAP, Legumain, Otubain-2, Elastase, FVIIa, FiXA, FXa, FXIa, FXIIa, Granzyme B, Guanidinobenzoate, Hepsin, HtrA1, Human Neutrophil

25 Elastase, KLK4, KLK5, KLK6, KLK7, KLK8, KLK10, KLK11, KLK13, KLK14, Lactoferrin, Marapsin, Matriptase-2, Meprin, MT-SP1/Matriptase, Neprilysin, NS3/4A, PACE4, Plasmin, PSMA, PSA, BMP-1, MMP1, MMP2, MMP3, MMP7, MMP8, MMP9, MMP10, MMP11, MMP12, MMP13, MMP14, MMP15, MMP16, MMP17, MMP19, MMP20, MMP23, MMP24, MMP26, MMP27, TMPRSS2, TMPRSS3,

30 TMPRSS4, tPA, Thrombin, Trypsin, and uPA, and any combination of two or more thereof.

In some embodiments of any of the ACCs described herein, the protease that cleaves any of the CMs described herein can be ADAM8, ADAM9, ADAM10, ADAM12, ADAM15, ADAM17/TACE, ADAMDEC1, ADAMTS1, ADAMTS4, ADAMTS5, BACE, Renin, Cathepsin D, Cathepsin E, Caspase 1, Caspase 2, Caspase 3, 5 Caspase 4, Caspase 5, Caspase 6, Caspase 7, Caspase 8, Caspase 9, Caspase 10, Caspase 14, Cathepsin B, Cathepsin C, Cathepsin K, Cathepsin L, Cathepsin S, Cathepsin V/L2, Cathepsin X/Z/P, Cruzipain, Legumain, Otubain-2, KLK4, KLK5, KLK6, KLK7, KLK8, KLK10, KLK11, KLK13, KLK14, Meprin, Neprilysin, PSMA, BMP-1, MMP-1, MMP-2, MMP-3, MMP-7, MMP-9, MMP-10, MMP-11, MMP-12, MMP-13, MMP-14, MMP-10 15, MMP-16, MMP-17, MMP-19, MMP-20, MMP-23, MMP-24, MMP-26, MMP-27, activated protein C, cathepsin A, cathepsin G, Chymase, FVIIa, FIXa, FXa, FXIa, FXIIa, Elastase, Granzyme B, Guanidinobenzoate, HtrA1, human neutrophil lyase, lactoferrin, marapsin, NS3/4A, PACE4, Plasmin, PSA, tPA, thrombin, tryptase, uPA, DESC1, DPP-4, FAP, Hepsin, Matrilysin-2, MT-SP1/Matrilysin, TMPRSS2, TMPRSS3, and 15 TMPRSS4, and any combination of two or more thereof.

In some embodiments of any of the ACCs described herein, the protease is selected from the group of: uPA, legumain, MT-SP1, ADAM17, BMP-1, TMPRSS3, TMPRSS4, MMP-2, MMP-9, MMP-12, MMP-13, and MMP-14.

Increased levels of proteases having known substrates have been reported in a 20 number of cancers. See, e.g., La Roca et al., *British J. Cancer* 90(7):1414-1421, 2004. Substrates suitable for use in the CMs components employed herein include those which are more prevalently found in cancerous cells and tissue. Thus, in certain embodiments, CMs each independently comprise a substrate for a protease that is more prevalently found in diseased tissue associated with a cancer. In some embodiments, the cancer is 25 selected from the group of: gastric cancer, breast cancer, osteosarcoma, and esophageal cancer. In some embodiments, the cancer is breast cancer. In some embodiments, the cancer is a HER2-positive cancer. In some embodiments, the cancer is Kaposi sarcoma, hairy cell leukemia, chronic myeloid leukemia (CML), follicular lymphoma, renal cell cancer (RCC), melanoma, neuroblastoma, basal cell carcinoma, cutaneous T-cell 30 lymphoma, nasopharyngeal adenocarcinoma, breast cancer, ovarian cancer, bladder cancer, BCG-resistant non-muscle invasive bladder cancer (NMIBC), endometrial

cancer, pancreatic cancer, non-small cell lung cancer (NSCLC), colorectal cancer, esophageal cancer, gallbladder cancer, glioma, head and neck carcinoma, uterine cancer, cervical cancer, or testicular cancer, and the like. In some of the above-described embodiments, the CM components comprise substrates for protease(s) that is/are more prevalent in tumor tissue

In some embodiments, CMs each independently include(s) a sequence selected from the group consisting of SEQ ID NO: 5 through SEQ ID NO: 100, as well as C-terminal and N-terminal truncation variants thereof.

In some embodiments, the CM includes a sequence selected from the group of: ISSGLLSGRSDNH (SEQ ID NO: 28), LSGRSDDH (SEQ ID NO: 33), ISSGLLSGRSDQH (SEQ ID NO: 54), and ISSGLLSGRSDNI (SEQ ID NO: 68).

In certain embodiments, CM1 and/or CM1 include(s) a sequence selected from the group of: AQNLLGMY (SEQ ID NO: 264), LSGRSDNHGGAVGLLAPP (SEQ ID NO: 265), VHMPLGFLGPGGLSGRSDNH (SEQ ID NO: 266), LSGRSDNHGGVHMPLGFLGP (SEQ ID NO: 267), LSGRSDNHGGSGGSISSGLLSS (SEQ ID NO: 268), ISSGLLSSGGSGGSLSGRSGNH (SEQ ID NO: 269), LSGRSDNHGGSGGSQLRMA (SEQ ID NO: 270), QNQALRMAGGSGGSLSGRSDNH (SEQ ID NO: 271), LSGRSGNHGGSGGSQLRMA (SEQ ID NO: 272), QNQALRMAGGSGGSLSGRSGNH (SEQ ID NO: 273), ISSGLLSGRSGNH (SEQ ID NO: 274), as well as C-terminal and N-terminal truncation variants thereof. Examples of CMs also include those described in U.S. Patent Application Publication Nos. US20160289324, US20190284283, and in publication numbers WO 2010/081173, WO 2015/048329, WO 2015/116933, WO 2016/118629, and WO 2020/118109, which are incorporated herein by reference in their entireties.

Truncation variants of the aforementioned amino acid sequences that are suitable for use in the CMs are any that retain the recognition site for the corresponding protease. These include C-terminal and/or N-terminal truncation variants comprising at least 3 contiguous amino acids of the above-described amino acid sequences, or at least 4, or at least 5, or at least 6, or at least 7 amino acids of the foregoing amino acid sequences that retain a recognition site for a protease. In certain embodiments, the truncation variant of

the above-described amino acid sequences is an amino acid sequence corresponding to any of the above, but that is C- and/or N-terminally truncated by 1 to about 10 amino acids, 1 to about 9 amino acids, 1 to about 8 amino acids, 1 to about 7 amino acids, 1 to about 6 amino acids, 1 to about 5 amino acids, 1 to about 4 amino acids, or 1 to about 3 amino acids, and which: (1) has at least three amino acid residues; and (2) retains a recognition site for a protease. In some of the foregoing embodiments, the truncated CM is an N-terminally truncated CM. In some embodiments, the truncated CM is a C-terminally truncated CM. In some embodiments, the truncated C is a C- and an N-terminally truncated CM.

In some embodiments of any of the activatable cytokine constructs described herein, the CM may comprise a total of about 3 amino acids to about 25 amino acids. In some embodiments, the CM may comprise a total of about 3 amino acids to about 25 amino acids, about 3 amino acids to about 20 amino acids, about 3 amino acids to about 15 amino acids, about 3 amino acids to about 10 amino acids, about 3 amino acids to about 5 amino acids, about 5 amino acids to about 25 amino acids, about 5 amino acids to about 20 amino acids, about 5 amino acids to about 15 amino acids, about 5 amino acids to about 10 amino acids, about 10 amino acids to about 25 amino acids, about 10 amino acids to about 20 amino acids, about 10 amino acids to about 15 amino acids, about 15 amino acids to about 25 amino acids, about 15 amino acids to about 20 amino acids, or about 20 amino acids to about 25 amino acids.

In some embodiments, the ACC may comprise multiple CMs that comprise substrates for different proteases. In some embodiments, the ACC may comprise multiple CMs that are substrates for the same protease. In one example, the CM(s) between each CP and PM may be substrates for the same protease as each other, and the CM(s) between each CP and DD may be substrates for the same protease as each other, but may be substrates for a different protease than the CM(s) between the CP and the PM. In another example, the CM(s) between the CP and the PM and the CM(s) between the CP and the DD may comprise substrates for the same protease. In another example, the CM(s) between the CP and the PM may comprise substrates for different proteases. In another example, the CM(s) between the CP and the PM may comprise substrates for the same protease. In another example, the CM(s) between the CP and the DD may comprise

substrates for different proteases. In another example, the CM(s) between the CP and the DD may comprise substrates for the same protease.

The first and second monomer constructs may comprise one or more additional components including one or more linkers, and the like. In some embodiments, the first monomer can include a linker disposed between the CP1 and the CM1. In some
5 embodiments, the CP1 and the CM1 directly abut each other in the first monomer. In some embodiments, the first monomer comprises a linker disposed between the CM1 and the DD1. In some embodiments, the CM1 and the DD1 directly abut each other in the first monomer. In some embodiments, the first monomer can include a linker disposed
10 between the CP1 and the CM3. In some embodiments, the CP1 and the CM3 directly abut each other in the first monomer. In some embodiments, the first monomer can include a linker disposed between the CP1 and the PM1. In some embodiments, the CP1 and the PM1 directly abut each other in the first monomer. In some embodiments, the linker has a total length of 1 amino acid to about 15 amino acids. In some embodiments,
15 the CM and any linkers disposed between the CP1 and DD1 have a combined total length of 3 to 15 amino acids, or 3 to 10 amino acids, or 3 to 7 amino acids.

In some embodiments, the second monomer comprises a linker disposed between the CP2 and the CM2. In some embodiments, the CP2 and the CM2 directly abut each other in the second monomer. In some embodiments, the second monomer comprises a
20 linker disposed between the CM2 and the DD2. In some embodiments, the CM2 (e.g., any of the cleavable moieties described herein) and the DD2 (e.g., any of the DDs described herein) directly abut each other in the second monomer. In some
embodiments, the second monomer can include a linker disposed between the CP2 and the CM4. In some embodiments, the CP2 and the CM4 directly abut each other in the
25 second monomer. In some embodiments, the second monomer can include a linker disposed between the CP2 and the PM2. In some embodiments, the CP2 and the PM2 directly abut each other in the second monomer. In some embodiments, the linker has a total length of 1 amino acid to about 15 amino acids. In some embodiments, the linker comprises a sequence of GGGS (SEQ ID NO: 2). In some embodiments, the CM and any
30 linkers disposed between the CP2 and DD2 have a combined total length of 3 to 15 amino acids, or 3 to 10 amino acids, or 3 to 7 amino acids.

In some embodiments, the first monomer and/or the second monomer can include a total of about 50 amino acids to about 800 amino acids, about 50 amino acids to about 750 amino acids, about 50 amino acids to about 700 amino acids, about 50 amino acids to about 650 amino acids, about 50 amino acids to about 600 amino acids, about 50 amino acids to about 550 amino acids, about 50 amino acids to about 500 amino acids, about 50 amino acids to about 450 amino acids, about 50 amino acids to about 400 amino acids, about 50 amino acids to about 350 amino acids, about 50 amino acids to about 300 amino acids, about 50 amino acids to about 250 amino acids, about 50 amino acids to about 200 amino acids, about 50 amino acids to about 150 amino acids, about 50 amino acids to about 100 amino acids, about 100 amino acids to about 800 amino acids, about 100 amino acids to about 750 amino acids, about 100 amino acids to about 700 amino acids, about 100 amino acids to about 650 amino acids, about 100 amino acids to about 600 amino acids, about 100 amino acids to about 550 amino acids, about 100 amino acids to about 500 amino acids, about 100 amino acids to about 450 amino acids, about 100 amino acids to about 400 amino acids, about 100 amino acids to about 350 amino acids, about 100 amino acids to about 300 amino acids, about 100 amino acids to about 250 amino acids, about 100 amino acids to about 200 amino acids, about 100 amino acids to about 150 amino acids, about 150 amino acids to about 800 amino acids, about 150 amino acids to about 750 amino acids, about 150 amino acids to about 700 amino acids, about 150 amino acids to about 650 amino acids, about 150 amino acids to about 600 amino acids, about 150 amino acids to about 550 amino acids, about 150 amino acids to about 500 amino acids, about 150 amino acids to about 450 amino acids, about 150 amino acids to about 400 amino acids, about 150 amino acids to about 350 amino acids, about 150 amino acids to about 300 amino acids, about 150 amino acids to about 250 amino acids, about 150 amino acids to about 200 amino acids, about 200 amino acids to about 800 amino acids, about 200 amino acids to about 750 amino acids, about 200 amino acids to about 700 amino acids, about 200 amino acids to about 650 amino acids, about 200 amino acids to about 600 amino acids, about 200 amino acids to about 550 amino acids, about 200 amino acids to about 500 amino acids, about 200 amino acids to about 450 amino acids, about 200 amino acids to about 400 amino acids, about 200 amino acids to about 350 amino acids, about 200 amino acids to about 300 amino acids,

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amino acids to about 700 amino acids, about 550 amino acids to about 650 amino acids, about 550 amino acids to about 600 amino acids, about 600 amino acids to about 800 amino acids, about 600 amino acids to about 750 amino acids, about 600 amino acids to about 700 amino acids, about 600 amino acids to about 650 amino acids, about 650 amino acids to about 800 amino acids, about 650 amino acids to about 750 amino acids, about 650 amino acids to about 700 amino acids, about 700 amino acids to about 800 amino acids, about 700 amino acids to about 750 amino acids, or about 750 amino acids to about 800 amino acids.

In some embodiments of any of the ACCs described herein, one or more linkers (e.g., flexible linkers) can be introduced into the activatable cytokine construct to provide flexibility at one or more of the junctions between domains, between moieties, between moieties and domains, or at any other junctions where a linker would be beneficial. In some embodiments, where the ACC is provided as a conformationally constrained construct, a flexible linker can be inserted to facilitate formation and maintenance of a structure in the uncleaved activatable cytokine construct. Any of the linkers described herein can provide the desired flexibility to facilitate the inhibition of the binding of a target (e.g., a receptor of a cytokine), or to facilitate cleavage of a CM by a protease. In some embodiments, linkers are included in the ACC that are all or partially flexible, such that the linker can include a flexible linker as well as one or more portions that confer less flexible structure to provide for a desired ACC. Some linkers may include cysteine residues, which may form disulfide bonds and reduce flexibility of the construct. It has been found that reducing the length of the linkers or linking region reduces the activity of the mature cytokine protein in ACCs (see, e.g., Fig. 16 showing data for ACCs without a peptide affinity mask). In most instances, linker length is determined by counting, in a N- to C- direction, the number of amino acids from the N-terminus of the linker adjacent to the C-terminal amino acid of the preceding component, to the C-terminus of the linker adjacent to the N-terminal amino acid of the following component (i.e., where the linker length does not include either the C-terminal amino acid of the preceding component or the N-terminal amino acid of the following component). In embodiments in which a linker is employed at the N-terminus of a DD that comprises an Fc domain, linker length is determined by counting the number of amino acids from the N-terminus of the linker

adjacent to the C-terminal amino acid of the preceding component to C-terminus of the linker adjacent to the first cysteine of an Fc hinge region that participates in the disulfide linkage with a second Fc domain (i.e., where the linker length does not include the C-terminal amino acid of the preceding component or the first cysteine of the Fc hinge region).

As apparent from the present disclosure and Fig. 18, ACCs of the present disclosure include a stretch of amino acids between the CP and the proximal point of interaction between the dimerization domains. That stretch of amino acids may be referred to as a Linking Region (LR) as defined above.

In some embodiments, additional amino acid sequences may be positioned N-terminally or C-terminally to any of the domains of any of the ACCs. Examples include, but are not limited to, targeting moieties (e.g., a ligand for a receptor of a cell present in a target tissue) and serum half-life extending moieties (e.g., polypeptides that bind serum proteins, such as immunoglobulin (e.g., IgG) or serum albumin (e.g., human serum albumin (HSA))).

In some embodiments of any of the activatable cytokine constructs described herein, the linker can include a total of about 1 amino acid to about 25 amino acids (e.g., about 1 amino acid to about 24 amino acids, about 1 amino acid to about 22 amino acids, about 1 amino acid to about 20 amino acids, about 1 amino acid to about 18 amino acids, about 1 amino acid to about 16 amino acids, about 1 amino acid to about 15 amino acids, about 1 amino acid to about 14 amino acids, about 1 amino acid to about 12 amino acids, about 1 amino acid to about 10 amino acids, about 1 amino acid to about 8 amino acids, about 1 amino acid to about 6 amino acids, about 1 amino acid to about 5 amino acids, about 1 amino acid to about 4 amino acids, about 1 amino acid to about 3 amino acids, about 1 amino acid to about 2 amino acids, about 2 amino acids to about 25 amino acids, about 2 amino acids to about 24 amino acids, about 2 amino acids to about 22 amino acids, about 2 amino acids to about 20 amino acids, about 2 amino acids to about 18 amino acids, about 2 amino acids to about 16 amino acids, about 2 amino acids to about 15 amino acids, about 2 amino acids to about 14 amino acids, about 2 amino acids to about 12 amino acids, about 2 amino acids to about 10 amino acids, about 2 amino acids to about 8 amino acids, about 2 amino acids to about 6 amino acids, about 2 amino acids

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about 24 amino acids, about 12 amino acids to about 22 amino acids, about 12 amino acids to about 20 amino acids, about 12 amino acids to about 18 amino acids, about 12 amino acids to about 16 amino acids, about 12 amino acids to about 15 amino acids, about 12 amino acids to about 14 amino acids, about 14 amino acids to about 25 amino acids, about 14 amino acids to about 24 amino acids, about 14 amino acids to about 22 amino acids, about 14 amino acids to about 20 amino acids, about 14 amino acids to about 18 amino acids, about 14 amino acids to about 16 amino acids, about 14 amino acids to about 15 amino acids, about 15 amino acids to about 25 amino acids, about 15 amino acids to about 24 amino acids, about 15 amino acids to about 22 amino acids, about 15 amino acids to about 20 amino acids, about 15 amino acids to about 18 amino acids, about 15 amino acids to about 16 amino acids, about 16 amino acids to about 25 amino acids, about 16 amino acids to about 24 amino acids, about 16 amino acids to about 22 amino acids, about 16 amino acids to about 20 amino acids, about 16 amino acids to about 18 amino acids, about 18 amino acids to about 25 amino acids, about 18 amino acids to about 24 amino acids, about 18 amino acids to about 22 amino acids, about 18 amino acids to about 20 amino acids, about 20 amino acids to about 25 amino acids, about 20 amino acids to about 24 amino acids, about 20 amino acids to about 22 amino acids, about 22 amino acid to about 25 amino acids, about 22 amino acid to about 24 amino acids, or about 24 amino acid to about 25 amino acids).

In some embodiments of any of the ACCs described herein, the linker includes a total of about 1 amino acid, about 2 amino acids, about 3 amino acids, about 4 amino acids, about 5 amino acids, about 6 amino acids, about 7 amino acids, about 8 amino acids, about 9 amino acids, about 10 amino acids, about 11 amino acids, about 12 amino acids, about 13 amino acids, about 14 amino acids, about 15 amino acids, about 16 amino acids, about 17 amino acids, about 18 amino acids, about 19 amino acids, about 20 amino acids, about 21 amino acids, about 22 amino acids, about 23 amino acids, about 24 amino acids, or about 25 amino acids.

Surprisingly, the applicant has discovered that ACCs that do not comprise any linkers between the CP and the DD exhibit the most significant reduction in cytokine activity relative to the wildtype mature cytokine, compared to ACCs that include linkers or additional sequences in the linking region. See, e.g., Fig. 16 (showing data for ACCs

without a peptide affinity mask). Further, a configuration in which there are no linkers between the CP and the DD still allows effective cleavage of a CM positioned between the CP and the DD. See e.g., Figs. 7A, 7B, 10A-10C. Thus, in some embodiments, the ACC does not comprise any linkers between the CP and the DD, and the CM between the CP and the DD comprises not more than 10, 9, 8, 7, 6, 5, 4, or 3 amino acids. In some embodiments the total number of amino acids in the LR comprises not more than 25 amino acids, e.g., not more than 25, 24, 23, 22, 21, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, or 3 amino acids, or 3 to 10 amino acids or 5 to 15 amino acids, or 7 to 12 amino acids, or any range or specific number of amino acids selected from the range encompassed by 3 to 25 amino acids.

In some embodiments of any of the ACCs described herein, a linker can be rich in glycine (Gly or G) residues. In some embodiments, the linker can be rich in serine (Ser or S) residues. In some embodiments, the linker can be rich in glycine and serine residues. In some embodiments, the linker has one or more glycine-serine residue pairs (GS) (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 or more GS pairs). In some embodiments, the linker has one or more Gly-Gly-Gly-Ser (GGGS) (SEQ ID NO: 2) sequences (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 or more GGGS sequences). In some embodiments, the linker has one or more Gly-Gly-Gly-Gly-Ser (GGGGS) (SEQ ID NO: 216) sequences (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 or more GGGGS sequences). In some embodiments, the linker has one or more Gly-Gly-Ser-Gly (GGSG) (SEQ ID NO: 229) sequences (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 or more GGSG sequences).

In some embodiments of any of the ACCs described herein, a linker includes any one of or a combination of one or more of: GSSGGSGSGG (SEQ ID NO: 210), GGGS (SEQ ID NO: 2), GGGSGGGS (SEQ ID NO: 211), GGGSGGGSGGGS (SEQ ID NO: 212), GGGGSGGGGSGGGGS (SEQ ID NO: 213), GGGGSGGGGSGGGGSGGGGSGGGGS (SEQ ID NO: 214), GGGGSGGGGS (SEQ ID NO: 215), GGGGS (SEQ ID NO: 216), GS, GGGGSGS (SEQ ID NO: 217), GGGGSGGGGSGGGGSGS (SEQ ID NO: 218), GGS LDPKGGGGS (SEQ ID NO: 219), PKSCDKTHTCPPCPAPELLG (SEQ ID NO: 220), SKYGPPCPPCPAPEFLG (SEQ ID NO: 221), GKSSGSGSES KS (SEQ ID NO: 222), GSTSGSGKSSEGKG (SEQ

ID NO: 223), GSTSGSGKSSEGSSTKG (SEQ ID NO: 224), and GSTSGSGKPGSGEGSTKG (SEQ ID NO: 225).

Non-limiting examples of linkers can include a sequence that is at least 70% identical (e.g., at least 72%, at least 74%, at least 75%, at least 76%, at least 78%, at least 80%, at least 82%, at least 84%, at least 85%, at least 86%, at least 88%, at least 90%, at least 92%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical) to GGGS (SEQ ID NO: 2), GSSGSGGSGG (SEQ ID NO: 210), GGGGSGGGGSGGGGS (SEQ ID NO: 213), GGGGSGS (SEQ ID NO: 217), GGGGSGGGGSGGGGSGS (SEQ ID NO: 218), GGGGSGGGGSGGGGSGGGGS (SEQ ID NO: 214), GGS LDPKGGGGS (SEQ ID NO: 215), and GSTSGSGKPGSSEGST (SEQ ID NO: 226).

In some embodiments, the linker includes a sequence selected from the group of: GGS LDPKGGGGS (SEQ ID NO: 219), GGGGSGGGGSGGGGSGS (SEQ ID NO: 218), GGGGSGS (SEQ ID NO: 217), GS, (GS)_n, (GGG)_n, (GSGGS)_n (SEQ ID NO: 227) and (GGG)_n (SEQ ID NO: 228), GGSG (SEQ ID NO: 229), GGS GG (SEQ ID NO: 230), GSGSG (SEQ ID NO: 231), GSGGG (SEQ ID NO: 232), GGGSG (SEQ ID NO: 233), GSSSG (SEQ ID NO: 234), GGGGSGGGGSGGGGS (SEQ ID NO: 213), GGGGSGGGGSGGGGSGGGGS (SEQ ID NO: 214), GSTSGSGKPGSSEGST (SEQ ID NO: 226), (GGGG)_n (SEQ ID NO: 216), wherein n is an integer of at least one. In some embodiments, the linker includes a sequence selected from the group consisting of: GGS LDPKGGGGS (SEQ ID NO: 219), GGGGSGGGGSGGGGSGS (SEQ ID NO: 218), GGGGSGS (SEQ ID NO: 217), and GS. In some embodiments of any of the ACCs described herein, the linker includes a sequence selected from the group of: GGGGSGGGGSGGGGS (SEQ ID NO: 213), GGGGSGGGGSGGGGSGGGGS (SEQ ID NO: 214), and GSTSGSGKPGSSEGST (SEQ ID NO: 226). In some embodiments of any of the activatable cytokine constructs described herein, the linker includes a sequence selected from the group of: GGGGSGGGGSGGGGS (SEQ ID NO: 213) or GGGGS (SEQ ID NO: 216). In some embodiments, the linker comprises a sequence of GGGS (SEQ ID NO: 2). Additional examples of linkers include those listed in Table 11.

In some embodiments, an ACC can include one, two, three, four, five, six, seven, eight, nine, or ten linker sequence(s) (e.g., the same or different linker sequences of any

of the exemplary linker sequences described herein or known in the art). In some embodiments, a linker comprises sulfo-SIAB, SMPB, and sulfo-SMPB, wherein the linkers react with primary amines sulfhydryls.

In some embodiments of any of the ACCs described herein, the ACC is
5 characterized by a reduction in at least one activity of the CP1 and/or CP2 as compared to a control level of the at least one activity of the CP1 and/or CP2. In some embodiments, a control level can be the level of the activity for a recombinant CP1 and/or CP2 (e.g., a commercially available recombinant CP1 and/or CP2, a recombinant wildtype CP1 and/or CP2, and the like). In some embodiments, a control level can be the level of the
10 activity of a cleaved (activated) form of the ACC. In certain embodiments, a control level can be the level of the activity of a pegylated CP1 and/or CP2.

In some embodiments, the at least one activity is the binding affinity of the CP1 and/or the CP2 for its cognate receptor as determined using surface plasmon resonance (e.g., performed in phosphate buffered saline at 25 degrees Celsius). In certain
15 embodiments, the at least one activity is the level of proliferation of lymphoma cells. In other embodiments, the at least one activity is the level of JAK/STAT/ISGF3 pathway activation in a lymphoma cell. In some embodiments, the at least one activity is a level of SEAP production in a lymphoma cell. In a further embodiment, the at least one activity of the CP1 and/or CP2 is level of cytokine-stimulated gene induction using, for
20 example RNAseq methods (see, e.g., Zimmerer et al., *Clin. Cancer Res.* 14(18):5900-5906, 2008; Hilkens et al., *J. Immunol.* 171:5255-5263, 2003).

In some embodiments, the ACC is characterized by at least a 2-fold reduction in at least one CP1 and/or CP2 activity as compared to the control level of the at least one CP1 and/or CP2 activity. In some embodiments, the ACC is characterized by at least a 5-
25 fold reduction in at least one activity of the CP1 and/or CP2 as compared to the control level of the at least one activity of the CP1 and/or CP2. In some embodiments, the ACC is characterized by at least a 10-fold reduction in at least one activity of the CP1 and/or CP2 as compared to the control level of the at least one activity of the CP1 and/or CP2. In some embodiments, the ACC is characterized by at least a 20-fold reduction in at least
30 one activity of the CP1 and/or CP2 as compared to the control level of the at least one activity of the CP1 and/or CP2. In some embodiments, the ACC is characterized by at

least a 30-fold, 40-fold, 50-fold, 60-fold, 70-fold, 80-fold, 90-fold, 100-fold, 500-fold, 1000-fold, 2000-fold, 3000-fold, 5000-fold or 5,000-fold reduction in at least one activity of the CP1 and/or CP2 as compared to the control level of the at least one activity of the CP1 and/or CP2. In some embodiments, ACC is characterized by at least a 1- to 20-fold
5 reduction, a 200- to 2000-fold reduction, a 300- to 2000-fold reduction, a 400- to 2000-fold reduction, a 500- to 2000-fold reduction, a 1000- to 2000-fold reduction, a 1500- to 2000-fold reduction, a 100- to 1500-fold reduction, a 200- to 1500-fold reduction, a 300- to 1500-fold reduction, a 400- to 1500-fold reduction, a 500- to 1500-fold reduction, a 1000- to 1500-fold reduction, a 100- to 1000-fold reduction, a 200- to 1000-fold
10 reduction, a 300- to 1000-fold reduction, a 400- to 1000-fold reduction, a 500- to 1000-fold reduction, a 1000- to 5000-fold reduction, a 2000- to 5000-fold reduction, a 3000- to 5000-fold reduction, a 4000- to 5000-fold reduction, a 1000- to 4000-fold reduction, a 2000- to 4000-fold reduction, a 3000- to 4000-fold reduction, a 1000- to 3000-fold reduction, a 2000- to 3000-fold reduction, or a 1000- to 2000-fold reduction in at least
15 one activity of the CP1 and/or CP2 as compared to the control level of the at least one activity of the CP1 and/or CP2.

In some embodiments, the control level of the at least one activity of the CP1 and/or CP2 is the activity of the CP1 and/or CP2 released from the ACC following cleavage of the CMs by protease(s) (the "cleavage product"). In some embodiments, the
20 control level of the at least one activity of the CP1 and/or CP2 is the activity of a corresponding wildtype mature cytokine (e.g., recombinant wildtype mature cytokine).

In some embodiments, incubation of the ACC with the protease yields an activated cytokine product(s), where one or more activities of CP1 and/or CP2 of the activated cytokine product(s) is greater than the one or more activities of CP1 and/or CP2
25 of the intact ACC. In some embodiments, one or more activities of CP1 and/or CP2 of the activated cytokine product(s) is at least 1-fold greater than the one or more activities of CP1 and/or CP2 of the ACC. In some embodiments, one or more activities of CP1 and/or CP2 of the activated cytokine product(s) is at least 2-fold greater than the one or more activities of CP1 and/or CP2 of the ACC. In some embodiments, one or more
30 activities of CP1 and/or CP2 of the activated cytokine product(s) is at least 5-fold greater than the one or more activities of CP1 and/or CP2 of the ACC. In some embodiments,

one or more activities of CP1 and/or CP2 of the activated cytokine product(s) is at least 10-fold greater than the one or more activities of CP1 and/or CP2 of the ACC. In some embodiments, one or more activities of CP1 and/or CP2 of the activated cytokine product(s) is at least 20-fold greater than the one or more activities of CP1 and/or CP2 of the ACC. In some embodiments, one or more activities of CP1 and/or CP2 of the activated cytokine product(s) is at least 1- to 20-fold greater, a 200- to 2000-fold greater, a 300- to 2000-fold greater, a 400- to 2000-fold greater, a 500- to 2000-fold greater, a 1000- to 2000-fold greater, a 1500- to 2000-fold greater, a 100- to 1500-fold greater, a 200- to 1500-fold greater, a 300- to 1500-fold greater, a 400- to 1500-fold greater, a 500- to 1500-fold greater, a 1000- to 1500-fold greater, a 100- to 1000-fold greater, a 200- to 1000-fold greater, a 300- to 1000-fold greater, a 400- to 1000-fold greater, a 500- to 1000-fold greater, a 1000- to 5000-fold greater, a 2000- to 5000-fold greater, a 3000- to 5000-fold greater, a 4000- to 5000-fold greater, a 1000- to 4000-fold greater, a 2000- to 4000-fold greater, a 3000- to 4000-fold greater, a 1000- to 3000-fold greater, a 2000- to 3000-fold greater, or a 1000- to 2000-fold than the one or more activities of CP1 and/or CP2 of the ACC.

In some embodiments, an ACC can include a sequence that is at least 80% (e.g., at least 82%, at least 84%, at least 86%, at least 88%, at least 90%, at least 92%, at least 94%, at least 96%, at least 98%, at least 99%, or 100%) identical to SEQ ID NO: 321 or 322. In some embodiments, an ACC can be encoded by a nucleic acid including a sequence that is at least 80% (e.g., at least 82%, at least 84%, at least 86%, at least 88%, at least 90%, at least 92%, at least 94%, at least 96%, at least 98%, at least 99%, or 100%) identical to a nucleic acid encoding SEQ ID NOs: 321 or 322. In some aspects, an ACC may include such sequences but either without the signal sequences of those sequences. Signal sequences are not particularly limited. Some non-limiting examples of signal sequences include, e.g., SEQ ID NO: 244 and corresponding residues and nucleotides in the other sequences, or substituted with a signal sequence from another species or cell line. Other examples of signal sequences include MRAWIFFLLCLAGRALA (SEQ ID NO: 242) and MALTFALLVALLVLSCSSCSVG (SEQ ID NO: 243).

Various exemplary aspects of these activatable cytokine constructs are described below and can be used in any combination in the methods provided herein without limitation. Exemplary aspects of the activatable cytokine constructs and methods of making activatable cytokine constructs are described below.

5 In some embodiments, the CM is selected for use with a specific protease. The protease may be one produced by a tumor cell (e.g., the tumor cell may express greater amounts of the protease than healthy tissues). In some embodiments, the CM is a substrate for at least one protease selected from the group of an ADAM 17, a BMP-1, a cysteine protease such as a cathepsin, a HtrA1, a legumain, a matriptase (MT-SP1), a
10 matrix metalloprotease (MMP), a neutrophil elastase, a TMPRSS, such as TMPRSS3 or TMPRSS4, a thrombin, and a u-type plasminogen activator (uPA, also referred to as urokinase).

In some embodiments, a CM is a substrate for at least one matrix metalloprotease (MMP). Examples of MMPs include MMP1, MMP2, MMP3, MMP7, MMP8, MMP9,
15 MMP10, MMP11, MMP12, MMP13, MMP14, MMP15, MMP16, MMP17, MMP19, MMP20, MMP23, MMP24, MMP26, and MMP27. In some embodiments, the CM is a substrate for MMP9, MMP14, MMP1, MMP3, MMP13, MMP17, MMP11, and MMP19. In some embodiments, the CM is a substrate for MMP7. In some embodiments, the CM is a substrate for MMP9. In some embodiments, the CM is a substrate for MMP14. In
20 some embodiments, the CM is a substrate for two or more MMPs. In some embodiments, the CM is a substrate for at least MMP9 and MMP14. In some embodiments, the CM includes two or more substrates for the same MMP. In some embodiments, the CM includes at least two or more MMP9 substrates. In some embodiments, the CM includes at least two or more MMP14 substrates.

25 In some embodiments, a CM is a substrate for an MMP and includes the sequence ISSGLLS (SEQ ID NO: 19); QNQALRMA (SEQ ID NO: 16); AQNLLGMV (SEQ ID NO: 15); STFPFGMF (SEQ ID NO: 18); PVGYTSSL (SEQ ID NO: 74); DWLYWPGI (SEQ ID NO: 75); MIAPVAYR (SEQ ID NO: 42); RSPMWAY (SEQ ID NO: 43); WATPRPMR (SEQ ID NO: 44); FRLLDWQW (SEQ ID NO: 45); LKAAPRWA (SEQ
30 ID NO: 76); GPSHLVLT (SEQ ID NO: 77); LPGGLSPW (SEQ ID NO: 78); MGLFSEAG (SEQ ID NO: 79); SPLPLRVP (SEQ ID NO: 80); RMHLRSLG (SEQ ID

NO: 81); LAAPLGLL (SEQ ID NO: 17); AVGLLAPP (SEQ ID NO: 14); LLAPSHRA (SEQ ID NO: 82); PAGLWLDP (SEQ ID NO: 20); and/or ISSGLSS (SEQ ID NO: 73).

In some embodiments, a CM is a substrate for thrombin. In some embodiments, the CM is a substrate for thrombin and includes the sequence GPRSFG (SEQ ID NO: 83) or GPRSFG (SEQ ID NO: 84).

In some embodiments, a CM includes an amino acid sequence selected from the group of NTLSGRSENHSG (SEQ ID NO: 9); NTLSGRSGNHGS (SEQ ID NO: 10); TSTSGRSANPRG (SEQ ID NO: 11); TSGRSANP (SEQ ID NO: 12); VAGRSMRP (SEQ ID NO: 21); VVPEGRRS (SEQ ID NO: 22); ILPRSPAF (SEQ ID NO: 23);
10 MVLGRSLL (SEQ ID NO: 24); QGRAITFI (SEQ ID NO: 25); SPRSIMLA (SEQ ID NO: 26); and SMLRSMPL (SEQ ID NO: 27).

In some embodiments, a CM is a substrate for a neutrophil elastase. In some embodiments, a CM is a substrate for a serine protease. In some embodiments, a CM is a substrate for uPA. In some embodiments, a CM is a substrate for legumain. In some
15 embodiments, the CM is a substrate for matriptase. In some embodiments, the CM is a substrate for a cysteine protease. In some embodiments, the CM is a substrate for a cysteine protease, such as a cathepsin.

In some embodiments, a CM includes a sequence of ISSGLLSGRSDNH (SEQ ID NO: 28); ISSGLSSGGSGGSLSGRSDNH (SEQ ID NO: 30);
20 AVGLLAPPGGTSTSGRSANPRG (SEQ ID NO: 275);
TSTSGRSANPRGGGAVGLLAPP (SEQ ID NO: 276);
VHMPLGFLGPGGTSTSGRSANPRG (SEQ ID NO: 277);
TSTSGRSANPRGGGVHMPLGFLGP (SEQ ID NO: 278); AVGLLAPPGGLSGRSDNH (SEQ ID NO: 29); LSGRSDNHGGAVGLLAPP (SEQ ID NO: 70);
25 VHMPLGFLGPGGLSGRSDNH (SEQ ID NO: 266); LSGRSDNHGGVHMPLGFLGP (SEQ ID NO: 267); LSGRSDNHGGSGGSISSGLSS (SEQ ID NO: 268);
LSGRSGNHGGSGGSISSGLSS (SEQ ID NO: 279); ISSGLSSGGSGGSLSGRSGNH (SEQ ID NO: 269); LSGRSDNHGGSGGSQNQALRMA (SEQ ID NO: 270);
QNQALRMAGGSGGSLSGRSDNH (SEQ ID NO: 271);
30 LSGRSGNHGGSGGSQNQALRMA (SEQ ID NO: 272);

QNQUALRMAGGSGGSLSGRSGNH (SEQ ID NO: 273), and/or ISSGLLSGRSGNH (SEQ ID NO: 274).

In some embodiments, a CM comprises a sequence selected from the group consisting of SEQ ID NO: 5 through SEQ ID NO: 100. In some embodiments, the CM
5 comprises a sequence selected from the group of: ISSGLLSGRSDNH (SEQ ID NO: 28), LSGRSDDH (SEQ ID NO: 33), ISSGLLSGRSDQH (SEQ ID NO: 54), SGRSDNI (SEQ ID NO: 100), and ISSGLLSGRSDNI (SEQ ID NO: 68).

In some aspects, the ACC includes a first monomer comprising a CP1 selected from the group consisting of SEQ ID Nos: 1 and 101-209, a CM1 selected from the group
10 consisting of SEQ ID Nos: 5-100 and 264-308, a PM1 selected from the group consisting of SEQ ID Nos: 328-329, 323, and 331-479, a CM3 selected from the group consisting of SEQ ID Nos: 5-100 and 264-308, and a DD1 dimerized with a second monomer comprising a CP2 selected from the group consisting of SEQ ID Nos: 1 and 101-209, a
CM2 selected from the group consisting of SEQ ID Nos: 5-100 and 264-308, a PM2
15 selected from SEQ ID Nos: 328-329, 323, and 331-479, a CM3 selected from the group consisting of SEQ ID Nos: 5-100 and 264-308 and a DD2. In some aspects, the ACC may include, between CP1 and CM1, between CP1 and PM1, between CP1 and CM3, between PM1 and CM3, and/or between CM1 and DD1, a linker selected from the group consisting of SEQ ID Nos: 2 and 210-263, and between CP2 and CM2, between CP2 and
20 PM2, between CP2 and CM4, between PM2 and CM4, and/or between CM2 and DD2, a linker selected from the group consisting of SEQ ID Nos: 2 and 210-263. In some aspects, the PM1 is selected for use with the CP1 in accordance with Table 10, and the PM2 is selected for use with the CP2, in accordance with Table 10.

In some embodiments, the ACC includes a DD1 and/or a DD2 that has an amino
25 acid sequence that is at least 80% identical (e.g., at least 82%, at least 84%, at least 85%, at least 86%, at least 88%, at least 90%, at least 92%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical) to SEQ ID NO: 3 or SEQ ID NO: 4. In some embodiments, the ACC includes a DD1 that has an amino acid sequence that is at least 80% identical (e.g., at least 82%, at least 84%, at least 85%, at
30 least 86%, at least 88%, at least 90%, at least 92%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical) to SEQ ID NO: 318 or

SEQ ID NO: 319. In some embodiments, the ACC includes a DD2 that has an amino acid sequence that is at least 80% identical (e.g., at least 82%, at least 84%, at least 85%, at least 86%, at least 88%, at least 90%, at least 92%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical) to SEQ ID NO: 318 or
5 SEQ ID NO: 319.

One or both monomers of the ACC herein may comprise one or more peptide masks (PMs), which can interfere with the binding of the CP to its binding partner (e.g., receptors). In some embodiments, when an ACC is not activated, the PM in the ACC prevents the CP from target binding; but when the ACC is activated, the PM does not
10 substantially or significantly interfere with the CP's binding to its binding partner. In some embodiments, a PM is coupled to a CP by a CM and optionally one or more linkers described herein.

In some embodiments, a PM may interact with the CP, thus reducing or inhibiting the interaction between the CP and its binding partner. In some embodiments, the PM
15 may not specifically bind to the CP, but rather interfere with CP's binding to its binding partner through non-specific interactions such as steric hindrance. For example, the PM may be positioned in the uncleaved ACC such that the tertiary or quaternary structure of the ACC allows the PM to mask the CP through charge-based interaction, thereby holding the PM in place to interfere with binding partner access to the CP.

The structural properties of the PM may be selected according to factors such as
20 the minimum amino acid sequence required for interference with protein binding to target, the target protein-protein binding pair of interest, the size of the cytokine, the presence or absence of linkers, and the like.

The PMs may be identified and/or further optimized through a screening
25 procedure from a library of candidate ACC having variable PMs. For example, a CP and a CM can be selected to provide for a desired enzyme/target combination, and the amino acid sequence of the PM can be identified by the screening procedure described below to identify a PM that provides for a switchable phenotype. For example, a random peptide library (e.g., of peptides comprising about 2 to about 40 amino acids or more) may be
30 used in the screening methods disclosed herein to identify a suitable PM. In specific embodiments, PMs with specific binding affinity for a CP can be identified through a

screening procedure that includes providing a library of peptide scaffolds consisting of candidate PMs wherein each scaffold is made up of a transmembrane protein and the candidate PM. The library may then be contacted with an entire or portion of a protein such as a full length protein, a naturally occurring protein fragment, or a non-naturally occurring fragment containing a protein (also capable of binding the binding partner of interest), and identifying one or more candidate PMs having detectably bound protein. The screening may be performed by one more rounds of magnetic-activated sorting (MACS) or fluorescence-activated sorting (FACS), as well as determination of the binding affinity of PM towards the CP and subsequent determination of the masking efficiency, e.g., as described in US20200308243A1, which is incorporated herein by reference in its entirety.

In some embodiments, the PM is unique for the coupled CP. Examples of PMs include PMs that were specifically screened to bind a binding domain of the cytokine or protein fragment (e.g., affinity peptide masks). Methods for screening PMs to obtain PMs unique for the cytokine and those that specifically and/or selectively bind a binding domain of a binding partner/target are provided herein and can include protein display methods. Table 10 discloses exemplary PMs suitable for use with various exemplary CPs.

In some embodiments, when a CP is coupled to a PM and in the presence of a natural binding partner of the CP, there is no binding or substantially no binding of the CP to the binding partner, or no more than 0.001%, 0.01%, 0.1%, 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, or 50% binding of the CP to its binding partner, as compared to the binding of the CP not coupled to a PM, for at least 2, 4, 6, 8, 12, 28, 24, 30, 36, 48, 60, 72, 84, 96 hours, or 5, 10, 15, 30, 45, 60, 90, 120, 150, 180 days, or 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 months or greater when measured in a masking efficiency assay, e.g., as described in Example 1.

The PMs contemplated by this disclosure may range from 1-50 amino acids (e.g., at least 3, 4, 5, 6, 7, 8, 9, 10, 12, 15, 20, 30, or 40 amino acids, or no greater than 40, 30, 20, 15, 12, 10, 9, 8, 7, 6, 5, 4, or 3 amino acids). In some examples, the PMs may be from 8 to 15 amino acids in length.

The PMs may contain genetically encoded or genetically non-encoded amino acids. Examples of genetically non-encoded amino acids are but not limited to D-amino acids, β -amino acids, and γ -amino acids. In specific embodiments, the PMs contain no more than 50%, 40%, 30%, 20%, 15%, 10%, 5% or 1% of genetically non-encoded amino acids.

The binding affinity of the cytokine towards the target or binding partner when coupled to a PM may be at least 5, 10, 25, 50, 100, 250, 500, 1,000, 2,500, 5,000, 10,000, 50,000, 100,000, 500,000, 1,000,000, 5,000,000, 10,000,000, 50,000,000 or greater times lower than the binding affinity of the cytokine towards its binding partner when not coupled to a PM, or between 5-10, 10-100, 10-1,000, 10-10,000, 10-100,000, 10-1,000,000, 10-10,000,000, 100-1,000, 100-10,000, 100-100,000, 100-1,000,000, 100-10,000,000, 1,000-10,000, 1,000-100,000, 1,000-1,000,000, 1000-10,000,000, 10,000-100,000, 10,000-1,000,000, 10,000-10,000,000, 100,000-1,000,000, or 100,000-10,000,000 times lower than the binding affinity of the cytokine towards its binding partner when not coupled to a PM.

When the cytokine is coupled to a PM and is in the presence of the binding partner, specific binding of the cytokine to its binding partner may be reduced or inhibited, as compared to the specific binding of the cytokine not coupled to a PM to its binding partner. When compared to the binding of the cytokine not coupled to a PM to its binding partner, the cytokine's ability to bind the binding partner when coupled to a PM can be reduced by at least 50%, 60%, 70%, 80%, 90%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% and even 100% for at least 2, 4, 6, 8, 12, 28, 24, 30, 36, 48, 60, 72, 84, 96, hours, or 5, 10, 15, 30, 45, 60, 90, 120, 150, 180 days, or 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 months or greater when measured in vivo or in a masking efficiency assay, e.g., as shown in Example 1, an in vitro immunoabsorbant assay, e.g., as described in US20200308243A1.

The K_D of the PM towards the cytokine may be generally greater than the K_D of the cytokine towards the cytokine's binding partner. The K_D of the PM towards the cytokine may be at least 5, 10, 25, 50, 100, 250, 500, 1,000, 2,500, 5,000, 10,000, 100,000, 1,000,000 or even 10,000,000 times greater than the K_D of the cytokine towards its binding partner. Alternatively, the binding affinity of the PM towards the cytokine

may be generally lower than the binding affinity of the cytokine towards the cytokine's binding partner. The binding affinity of PM towards the cytokine may be at least 5, 10, 25, 50, 100, 250, 500, 1,000, 2,500, 5,000, 10,000, 100,000, 1,000,000 or 10,000,000 times lower than the binding affinity of the cytokine towards its binding partner.

5 In some embodiments, the PM comprises at least partial or complete amino acid sequence of a naturally occurring binding partner of the CP (e.g., a receptor of the CP). The PM may be a fragment of a naturally occurring binding partner. The fragment may retain no more than 95%, 90%, 80%, 75%, 70%, 60%, 50%, 40%, 30%, 25%, or 20% nucleic acid or amino acid sequence homology to the naturally occurring binding partner.

10 In some embodiments, the PM comprises an amino acid sequence that is not naturally occurring or does not contain the amino acid sequence of a naturally occurring binding partner or target protein. In certain embodiments the PM is not a natural binding partner of the CP. The PM may be a modified binding partner for the CP which contains amino acid changes that at least slightly decrease affinity and/or avidity of binding to the
15 CP. In some embodiments the PM contains no or substantially no nucleic acid or amino acid homology to the CP's natural binding partner. In other embodiments the PM is no more than 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, or 80% similar to the natural binding partner of the CP.

In some embodiments, the PM comprises an amino acid sequence that is at least
20 80% identical (e.g., at least 82%, at least 84%, at least 86%, at least 88%, at least 90%, at least 92%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical) to a sequence selected from SEQ ID Nos: 328-329, 323, and 331-479. An exemplary PM for use with a CP that is an interferon, preferably an IFN-alpha, can contain the consensus sequence: TDVDYYREWXXXXXXXXX (SEQ ID No:
25 361), where X is any amino acid.

In some embodiments, an ACC may comprise a pair of PM1 and CP1 or a pair of PM2 and CP2 listed in Table 10, which contains example PMs for use with specific exemplary cytokines. In some examples, the PM1 comprises a sequence selected from the group consisting of SEQ ID NOs: 328, 329, 323, and 331-369, and the CP1 is an
30 interferon; the PM1 comprises a sequence selected from the group consisting of SEQ ID NOs: 328, 329, 323, and 331-364, and the CP1 is an interferon alpha; the PM1 comprises

a sequence selected from the group consisting of SEQ ID NOs: 331-360, 362-364, and the CP1 is an interferon beta; the PM1 comprises a sequence selected from the group consisting of SEQ ID NOs: 331-360, 366-369, and the CP1 is an interferon gamma; the PM1 comprises a sequence selected from the group consisting of SEQ ID NOs: 370-374, and the CP1 is an IL-12; the PM1 comprises a sequence selected from the group consisting of SEQ ID NOs: 375-382, 469-477, 478, and the CP1 is an IL-15; the PM1 comprises a sequence selected from the group consisting of SEQ ID NOs: 383-468, 469-478, and the CP1 is an IL-2; or the PM1 comprises a sequence selected from the group consisting of SEQ ID NOs: 478 and 479, and the CP1 is an IL-21. In some examples, the PM2 comprises a sequence selected from the group consisting of SEQ ID NOs: 328, 329, 323, and 331-369, and the CP2 is an interferon; the PM2 comprises a sequence selected from the group consisting of SEQ ID NOs: 328, 329, 323, and 331-364, and the CP2 is an interferon alpha; the PM2 comprises a sequence selected from the group consisting of SEQ ID NOs: 331-360, 362-364, and the CP2 is an interferon beta; the PM2 comprises a sequence selected from the group consisting of SEQ ID NOs: 331-360, 366-369, and the CP2 is an interferon gamma; the PM2 comprises a sequence selected from the group consisting of SEQ ID NOs: 370-374, and the CP2 is an IL-12; the PM2 comprises a sequence selected from the group consisting of SEQ ID NOs: 375-382, 469-477, 478, and the CP2 is an IL-15; the PM2 comprises a sequence selected from the group consisting of SEQ ID NOs: 383-468, 469-478, and the CP2 is an IL-2; or the PM2 comprises a sequence selected from the group consisting of SEQ ID NOs: 478 and 479, and the CP2 is an IL-21.

In some embodiments, the PM may comprise an inactive cytokine. For example, the inactive cytokine may interact with the CP component in the ACC and interfere the interaction between the CP and its binding partner. In one example, the inactive cytokine may comprise a mutation, e.g., an IFN alpha-2b with L130P mutation (SEQ ID No: 329). In another example, the inactive cytokine may be a truncation of a wild type cytokine, e.g., IFN alpha-2b with amino acids 1-150 (SEQ ID No: 328).

In some embodiments, once uncoupled from the cytokine and in a free state, the PM may have a biological activity or a therapeutic effect, such as binding capability. For example, the free peptide can bind with the same or a different binding partner. In certain

embodiments the free PM (uncoupled PM) can exert a therapeutic effect, providing a secondary function to the compositions disclosed herein. In some embodiments, once uncoupled from the cytokine and in a free state, the PM may advantageously not exhibit biological activity. For example, in some embodiments the PM in a free state does not elicit an immune response in the subject.

Conjugation to Agents

This disclosure also provides methods and materials for including additional elements in any of the ACCs described herein including, for example, a targeting moiety to facilitate delivery to a cell or tissue of interest, an agent (e.g., a therapeutic agent, an antineoplastic agent), a toxin, or a fragment thereof.

In some embodiments of any of the ACCs described herein, the ACC can be conjugated to a cytotoxic agent, including, without limitation, a toxin (e.g., an enzymatically active toxin of bacterial, fungal, plant, or animal origin, or fragments thereof) or a radioactive isotope. In some embodiments of any of the ACCs described herein, the activatable cytokine construct can be conjugated to a cytotoxic agent including, without limitation, a toxin (e.g., an enzymatically active toxin of bacterial, fungal, plant, or animal origin, or fragments thereof), or a radioactive isotope.

Non-limiting exemplary cytotoxic agents that can be conjugated to any of the ACCs described herein include: dolastatins and derivatives thereof (e.g., auristatin E, AFP, monomethyl auristatin D (MMAD), monomethyl auristatin F (MMAF), monomethyl auristatin E (MMAE), desmethyl auristatin E (DMAE), auristatin F, desmethyl auristatin F (DMAF), dolastatin 16 (DmJ), dolastatin 16 (Dpv), auristatin derivatives (e.g., auristatin tyramine, auristatin quinolone), maytansinoids (e.g., DM-1, DM-4), maytansinoid derivatives, duocarmycin, alpha-amanitin, turbostatin, phenstatin, hydroxyphenstatin, spongistatin 5, spongistatin 7, halistatin 1, halistatin 2, halistatin 3, halocomstatin, pyrrolbenzimidazoles (PBI), cibrostatin6, doxaliform, cemadotin analogue (CemCH2-SH), Pseudomonas toxin A (PES8) variant, Pseudomonase toxin A (ZZ-PE38) variant, ZJ-101, anthracycline, doxorubicin, daunorubicin, bryostatin, camptothecin, 7-substituted camptothecin, 10, 11-difluoromethylenedioxycamptothecin, combretastatins, debromoaplysiatoxin, KahaMide-F, discodermolide, and Ecteinascidins.

Non-limiting exemplary enzymatically active toxins that can be conjugated to any of the ACCs described herein include: diphtheria toxin, exotoxin A chain from *Pseudomonas aeruginosa*, ricin A chain, abrin A chain, modeccin A chain, alpha-sarcin, *Aleurites fordii* proteins, dianthin proteins, *Phytolacca Americana* proteins (e.g., PAPI, PAPII, and PAP-8), momordica charantia inhibitor, curcin, crotins, saponaria officinalis inhibitor, geionin, mitogeliin, restrictocin, phenomycin, neomycin, and tricothecenes.

Non-limiting exemplary anti-neoplastics that can be conjugated to any of the ACCs described herein include: adriamycin, cerubidine, bleomycin, alkeran, velban, oncovin, fluorouracil, methotrexate, thiotepa, bisantrene, novantrone, thioguanine, procarbazine, and cytarabine.

Non-limiting exemplary antivirals that can be conjugated to any of the ACCs described herein include: acyclovir, vira A, and symmetrel.

Non-limiting exemplary antifungals that can be conjugated to any of the ACCs described herein include: nystatin.

Non-limiting exemplary conjugatable detection reagents that can be conjugated to any of the ACCs described herein include: fluorescein and derivatives thereof, fluorescein isothiocyanate (FITC).

Non-limiting exemplary antibacterials that can be conjugated to any of the activatable cytokine constructs described herein include: aminoglycosides, streptomycin, neomycin, kanamycin, amikacin, gentamicin, and tobramycin.

Non-limiting exemplary 3beta,16beta,17alpha-trihydroxycholest-5-en-22-one 16-O-(2-O-4-methoxybenzoyl-beta-D-xylopyranosyl)-(1-->3)-(2-O-acetyl-alpha-L-arabinopyranoside) (OSW-1) that can be conjugated to any of the activatable cytokine constructs described herein include: s-nitrobenzyloxycarbonyl derivatives of O6-benzylguanine, topoisomerase inhibitors, hemiasterlin, cephalotaxine, homoharringtonine, pyrrol benzodiazepine dimers (PBDs), functionalized pyrrol benzodiazepenes, calcicheamicins, podophyllotoxins, taxanes, and vinca alkaloids.

Non-limiting exemplary radiopharmaceuticals that can be conjugated to any of the activatable cytokine constructs described herein include: ^{123}I , ^{89}Zr , ^{125}I , ^{131}I , $^{99\text{m}}\text{Tc}$, ^{201}Tl , ^{62}Cu , ^{18}F , ^{68}Ga , ^{13}N , ^{15}O , ^{38}K , ^{82}Rb , ^{111}In , ^{133}Xe , ^{11}C , and $^{99\text{m}}\text{Tc}$ (Technetium).

Non-limiting exemplary heavy metals that can be conjugated to any of the ACCs described herein include: barium, gold, and platinum.

Non-limiting exemplary anti-mycoplasmals that can be conjugated to any of the ACCs described herein include: tylosine, spectinomycin, streptomycin B, ampicillin,
5 sulfanilamide, polymyxin, and chloramphenicol.

Those of ordinary skill in the art will recognize that a large variety of possible moieties can be conjugated to any of the activatable cytokine constructs described herein. Conjugation can include any chemical reaction that will bind the two molecules so long as the ACC and the other moiety retain their respective activities. Conjugation can
10 include many chemical mechanisms, e.g., covalent binding, affinity binding, intercalation, coordinate binding, and complexation. In some embodiments, the preferred binding is covalent binding. Covalent binding can be achieved either by direct condensation of existing side chains or by the incorporation of external bridging molecules. Many bivalent or polyvalent linking agents are useful in conjugating any of
15 the activatable cytokine constructs described herein. For example, conjugation can include organic compounds, such as thioesters, carbodiimides, succinimide esters, glutaraldehyde, diazobenzenes, and hexamethylene diamines. In some embodiments, the activatable cytokine construct can include, or otherwise introduce, one or more non-natural amino acid residues to provide suitable sites for conjugation.

20 In some embodiments of any of the ACCs described herein, an agent and/or conjugate is attached by disulfide bonds (e.g., disulfide bonds on a cysteine molecule) to the antigen-binding domain. Since many cancers naturally release high levels of glutathione, a reducing agent, glutathione present in the cancerous tissue microenvironment can reduce the disulfide bonds, and subsequently release the agent
25 and/or the conjugate at the site of delivery.

In some embodiments of any of the ACCs described herein, when the conjugate binds to its target in the presence of complement within the target site (e.g., diseased tissue (e.g., cancerous tissue)), the amide or ester bond attaching the conjugate and/or agent to the linker is cleaved, resulting in the release of the conjugate and/or agent in its
30 active form. These conjugates and/or agents when administered to a subject, will accomplish delivery and release of the conjugate and/or the agent at the target site (e.g.,

diseased tissue (e.g., cancerous tissue)). These conjugates and/or agents are particularly effective for the *in vivo* delivery of any of the conjugates and/or agents described herein.

In some embodiments, the linker is not cleavable by enzymes of the complement system. For example, the conjugate and/or agent is released without complement
5 activation since complement activation ultimately lyses the target cell. In such embodiments, the conjugate and/or agent is to be delivered to the target cell (e.g., hormones, enzymes, corticosteroids, neurotransmitters, or genes). Furthermore, the linker is mildly susceptible to cleavage by serum proteases, and the conjugate and/or agent is released slowly at the target site.

10 In some embodiments of any of the ACCs described herein, the conjugate and/or agent is designed such that the conjugate and/or agent is delivered to the target site (e.g., disease tissue (e.g., cancerous tissue)) but the conjugate and/or agent is not released.

In some embodiments of any of the ACCs described herein, the conjugate and/or agent is attached to an antigen-binding domain either directly or via a non-cleavable
15 linker. Exemplary non-cleavable linkers include amino acids (e.g., D-amino acids), peptides, or other organic compounds that may be modified to include functional groups that can subsequently be utilized in attachment to antigen-binding domains by methods described herein.

In some embodiments of any of the ACCs described herein, an ACC includes at
20 least one point of conjugation for an agent. In some embodiments, all possible points of conjugation are available for conjugation to an agent. In some embodiments, the one or more points of conjugation include, without limitation, sulfur atoms involved in disulfide bonds, sulfur atoms involved in interchain disulfide bonds, sulfur atoms involved in interchain sulfide bonds but not sulfur atoms involved in intrachain disulfide bonds,
25 and/or sulfur atoms of cysteine or other amino acid residues containing a sulfur atom. In such cases, residues may occur naturally in the protein construct structure or may be incorporated into the protein construct using methods including, without limitation, site-directed mutagenesis, chemical conversion, or mis-incorporation of non-natural amino acids.

30 This disclosure also provides methods and materials for preparing an ACC for conjugation. In some embodiments of any of the ACCs described herein, an ACC is

modified to include one or more interchain disulfide bonds. For example, disulfide bonds in the ACC can undergo reduction following exposure to a reducing agent such as, without limitation, TCEP, DTT, or β -mercaptoethanol. In some cases, the reduction of the disulfide bonds is only partial. As used herein, the term partial reduction refers to situations where an ACC is contacted with a reducing agent and a fraction of all possible sites of conjugation undergo reduction (e.g., not all disulfide bonds are reduced). In some embodiments, an activatable cytokine construct is partially reduced following contact with a reducing agent if less than 99%, (e.g., less than 98%, 97%, 96%, 95%, 90%, 85%, 80%, 75%, 70%, 65%, 60%, 55%, 50%, 45%, 40%, 35%, 30%, 25%, 20%, 15%, 10% or less than 5%) of all possible sites of conjugation are reduced. In some embodiments, the ACC having a reduction in one or more interchain disulfide bonds is conjugated to a drug reactive with free thiols.

This disclosure also provides methods and materials for conjugating a therapeutic agent to a particular location on an ACC. In some embodiments of any of the ACC described herein, an ACC is modified so that the therapeutic agents can be conjugated to the ACC at particular locations on the ACC. For example, an ACC can be partially reduced in a manner that facilitates conjugation to the ACC. In such cases, partial reduction of the ACC occurs in a manner that conjugation sites in the ACC are not reduced. In some embodiments, the conjugation site(s) on the ACC are selected to facilitate conjugation of an agent at a particular location on the protein construct. Various factors can influence the “level of reduction” of the ACC upon treatment with a reducing agent. For example, without limitation, the ratio of reducing agent to ACC, length of incubation, incubation temperature, and/or pH of the reducing reaction solution can require optimization in order to achieve partial reduction of the ACC with the methods and materials described herein. Any appropriate combination of factors (e.g., ratio of reducing agent to ACC, the length and temperature of incubation with reducing agent, and/or pH of reducing agent) can be used to achieve partial reduction of the ACC (e.g., general reduction of possible conjugation sites or reduction at specific conjugation sites).

An effective ratio of reducing agent to ACC can be any ratio that at least partially reduces the ACC in a manner that allows conjugation to an agent (e.g., general reduction

of possible conjugation sites or reduction at specific conjugation sites). In some embodiments, the ratio of reducing agent to ACC will be in a range from about 20:1 to 1:1, from about 10:1 to 1:1, from about 9:1 to 1:1, from about 8:1 to 1:1, from about 7:1 to 1:1, from about 6:1 to 1:1, from about 5:1 to 1:1, from about 4:1 to 1:1, from about 3:1 to 1:1, from about 2:1 to 1:1, from about 20:1 to 1:1.5, from about 10:1 to 1:1.5, from about 9:1 to 1:1.5, from about 8:1 to 1:1.5, from about 7:1 to 1:1.5, from about 6:1 to 1:1.5, from about 5:1 to 1:1.5, from about 4:1 to 1:1.5, from about 3:1 to 1:1.5, from about 2:1 to 1:1.5, from about 1.5:1 to 1:1.5, or from about 1:1 to 1:1.5. In some embodiments, the ratio is in a range of from about 5:1 to 1:1. In some embodiments, the ratio is in a range of from about 5:1 to 1.5:1. In some embodiments, the ratio is in a range of from about 4:1 to 1:1. In some embodiments, the ratio is in a range from about 4:1 to 1.5:1. In some embodiments, the ratio is in a range from about 8:1 to about 1:1. In some embodiments, the ratio is in a range of from about 2.5:1 to 1:1.

An effective incubation time and temperature for treating an ACC with a reducing agent can be any time and temperature that at least partially reduces the ACC in a manner that allows conjugation of an agent to an ACC (e.g., general reduction of possible conjugation sites or reduction at specific conjugation sites). In some embodiments, the incubation time and temperature for treating an ACC will be in a range from about 1 hour at 37 °C to about 12 hours at 37 °C (or any subranges therein).

An effective pH for a reduction reaction for treating an ACC with a reducing agent can be any pH that at least partially reduces the ACC in a manner that allows conjugation of the ACC to an agent (e.g., general reduction of possible conjugation sites or reduction at specific conjugation sites).

When a partially-reduced ACC is contacted with an agent containing thiols, the agent can conjugate to the interchain thiols in the ACC. An agent can be modified in a manner to include thiols using a thiol-containing reagent (e.g., cysteine or N-acetyl cysteine). For example, the ACC can be partially reduced following incubation with reducing agent (e.g., TEPC) for about 1 hour at about 37 °C at a desired ratio of reducing agent to ACC. An effective ratio of reducing agent to ACC can be any ratio that partially reduces at least two interchain disulfide bonds located in the ACC in a manner that

allows conjugation of a thiol-containing agent (e.g., general reduction of possible conjugation sites or reduction at specific conjugation sites).

In some embodiments of any of the ACCs described herein, an ACC is reduced by a reducing agent in a manner that avoids reducing any intrachain disulfide bonds. In
5 some embodiments of any of the ACCs described herein, an ACC is reduced by a reducing agent in a manner that avoids reducing any intrachain disulfide bonds and reduces at least one interchain disulfide bond.

In some embodiments of any of the ACCs described herein, the ACC can also include an agent conjugated to the ACC. In some embodiments, the conjugated agent is a
10 therapeutic agent.

In some embodiments, the agent (e.g., agent conjugated to an activatable cytokine construct) is a detectable moiety such as, for example, a label or other marker. For example, the agent is or includes a radiolabeled amino acid, one or more biotinyl moieties that can be detected by marked avidin (e.g., streptavidin containing a fluorescent marker
15 or enzymatic activity that can be detected by optical or calorimetric methods), one or more radioisotopes or radionuclides, one or more fluorescent labels, one or more enzymatic labels, and/or one or more chemiluminescent agents. In some embodiments, detectable moieties are attached by spacer molecules.

In some embodiments, the agent (e.g., cytotoxic agent conjugated to an
20 activatable cytokine construct) is linked to the ACC using a carbohydrate moiety, sulfhydryl group, amino group, or carboxylate group.

In some embodiments of any of the ACCs described herein conjugated to an agent, the agent (e.g., cytotoxic agent conjugated to an activatable cytokine construct) is conjugated to the ACC via a linker and/or a CM (also referred to as a cleavable
25 sequence). In some embodiments, the agent (e.g., cytotoxic agent conjugated to an activatable cytokine construct) is conjugated to a cysteine or a lysine in the ACC. In some embodiments, the agent (e.g., cytotoxic agent conjugated to an activatable cytokine construct) is conjugated to another residue of the ACC, such as those residues disclosed herein. In some embodiments, the linker is a thiol-containing linker. Some non-limiting
30 examples of the linker and/or CMs are provided in Table 1.

Table 1.

Types of Cleavable Sequences/CMs	Amino Acid Sequence
<u>Plasmin CMs</u>	
Pro-urokinase	PRFKIIGG (SEQ ID NO: 280)
	PRFRIIGG (SEQ ID NO: 281)
TGF β	SSRHRRALD (SEQ ID NO: 282)
Plasminogen	RKSSIIIRMRDVVL (SEQ ID NO: 283)
Staphylokinase	SSSFDKGKYKKGDDA (SEQ ID NO: 284)
	SSSFDKGKYKRGDDA (SEQ ID NO: 285)
<u>Factor Xa CMs</u>	
	IEGR (SEQ ID NO: 286)
	IDGR (SEQ ID NO: 287)
	GGSIDGR (SEQ ID NO: 288)
<u>MMP CMs</u>	
Gelatinase A	PLGLWA (SEQ ID NO: 289)
<u>Collagenase CMs</u>	
Calf skin collagen (α 1(I) chain)	GPQGIAGQ (SEQ ID NO: 290)
Calf skin collagen (α 2(I) chain)	GPQGLLGA (SEQ ID NO: 291)
Bovine cartilage collagen (α 1(II) chain)	GIAGQ (SEQ ID NO: 292)
Human liver collagen (α 1(III) chain)	GPLGIAGI (SEQ ID NO: 293)
Human α 2M	GPEGLRVG (SEQ ID NO: 294)
Human PZP	YGAGLGVV (SEQ ID NO: 295)
	AGLGVVER (SEQ ID NO: 296)
	AGLGISST (SEQ ID NO: 297)
Rat α 1M	EPQALAMS (SEQ ID NO: 298)
	QALAMSAI (SEQ ID NO: 299)
Rat α 2M	AAYHLVSQ (SEQ ID NO: 300)
	MDAFLESS (SEQ ID NO: 301)
Rat α 1I3(2J)	ESLPVVAV (SEQ ID NO: 302)
Rat α 1I3(27J)	SAPAVESE (SEQ ID NO: 303)
Human fibroblast collagenase	DVAQFVLT (SEQ ID NO: 304)
<u>(autolytic cleavages)</u>	VAQFVLT (SEQ ID NO: 305)

VAQFVLTE (SEQ ID NO: 306)

AQFVLTEG (SEQ ID NO: 307)

PVQPIGPQ (SEQ ID NO: 308)

Those of ordinary skill in the art will recognize that a large variety of possible moieties can be coupled to the ACCs of the disclosure. (*See, for example*, “Conjugate Vaccines”, Contributions to Microbiology and Immunology, J. M. Cruse and R. E.

5 Lewis, Jr (eds), Carger Press, New York, (1989), the entire contents of which are incorporated herein by reference). In general, an effective conjugation of an agent (e.g., cytotoxic agent) to an ACC can be accomplished by any chemical reaction that will bind the agent to the ACC while also allowing the agent and the ACC to retain functionality.

In some embodiments of any of the ACCs conjugated to an agent, a variety of
10 bifunctional protein-coupling agents can be used to conjugate the agent to the ACC including, without limitation, N-succinimidyl-3-(2-pyridyldithiol) propionate (SPDP), iminothiolane (IT), bifunctional derivatives of imidoesters (e.g., dimethyl adipimidate HCL), active esters (e.g., disuccinimidyl suberate), aldehydes (e.g., glutaraldehyde), bis-azido compounds (e.g., bis (p-azidobenzoyl) hexanediamine), bis-diazonium derivatives
15 (e.g., bis-(p-diazoniumbenzoyl)-ethylenediamine), diisocyanates (e.g., tolyene 2,6-diisocyanate), and bis-active fluorine compounds (e.g., 1,5-difluoro-2,4-dinitrobenzene). For example, a ricin immunotoxin can be prepared as described in Vitetta et al., Science 238: 1098 (1987). In some embodiments, a carbon-14-labeled 1-isothiocyanatobenzyl-3-methyldiethylene triaminepentaacetic acid (MX-DTPA) chelating agent can be used to
20 conjugate a radionucleotide to the ACC. (*See, e.g.*, WO94/11026).

Suitable linkers and CMs are described in the literature. (*See, for example*, Ramakrishnan, S. et al., Cancer Res. 44:201-208 (1984) describing use of MBS (M-maleimidobenzoyl-N-hydroxysuccinimide ester). *See also*, U.S. Patent No. 5,030,719, describing use of halogenated acetyl hydrazide derivative coupled to an ACC by way of
25 an oligopeptide linker. In some embodiments, suitable linkers include: (i) EDC (1-ethyl-3-(3-dimethylamino-propyl) carbodiimide hydrochloride; (ii) SMPT (4-succinimidylloxycarbonyl-alpha-methyl-alpha-(2-pyridyl-dithio)-toluene (Pierce Chem. Co., Cat. (21558G), (iii) SPDP (succinimidyl-6 [3-(2-pyridyldithio) propionamido]

hexanoate (Pierce Chem. Co., Cat #21651G); (iv) Sulfo-LC-SPDP (sulfosuccinimidyl 6
[3-(2-pyridyldithio)-propionamide] hexanoate (Pierce Chem. Co. Cat. #2165-G); and (v)
sulfo-NHS (N-hydroxysulfo-succinimide: Pierce Chem. Co., Cat. #24510) conjugated to
EDC. Additional linkers include, but are not limited to, SMCC, sulfo-SMCC, SPDB, or
5 sulfo-SPDB.

The linkers and CMs described above contain components that have different
attributes, thus leading to conjugates with differing physio-chemical properties. For
example, sulfo-NHS esters of alkyl carboxylates are more stable than sulfo-NHS esters of
aromatic carboxylates. NHS-ester containing linkers are less soluble than sulfo-NHS
10 esters. Further, the linker SMPT contains a sterically-hindered disulfide bond, and can
form conjugates with increased stability. Disulfide linkages, are in general, less stable
than other linkages because the disulfide linkage is cleaved *in vitro*, resulting in less
conjugate available. Sulfo-NHS, in particular, can enhance the stability of carbodimide
couplings. Carbodimide couplings (such as EDC) when used in conjunction with sulfo-
15 NHS, forms esters that are more resistant to hydrolysis than the carbodimide coupling
reaction alone.

In some embodiments of any of the ACCs, an agent can be conjugated to the ACC
using a modified amino acid sequence included in the amino acid sequence of the ACC.
By inserting conjugation-enabled amino acids at specific locations within the amino acid
20 sequence of the ACC, the protein construct can be designed for controlled placement
and/or dosage of the conjugated agent (e.g., cytotoxic agent). For example, the ACC can
be modified to include a cysteine amino acid residue at positions on the first monomer,
the second monomer, the third monomer, and/or the fourth monomer that provide
reactive thiol groups and does not negatively impact protein folding and/or assembly and
25 does not alter antigen-binding properties. In some embodiments, the ACC can be
modified to include one or more non-natural amino acid residues within the amino acid
sequence of the ACC to provide suitable sites for conjugation. In some embodiments, the
ACC can be modified to include enzymatically activatable peptide sequences within the
amino acid sequence of the ACC.

Nucleic Acids

Provided herein are nucleic acids including sequences that encode the first monomer construct (or the protein portion of the first monomer construct) (e.g., any of the first monomers constructs described herein) and the second monomer construct (or the protein portion of the second monomer construct) (e.g., any of the second monomer constructs described herein) of any of the ACCs described herein. In some embodiments, a pair of nucleic acids together encode the first monomer construct (or the protein portion of the first monomer construct) and the second monomer construct (or the protein portion of the second monomer construct). In some embodiments, the nucleic acid sequence encoding the first monomer construct (or the protein portion of the first monomer construct) is at least 70% identical (e.g., at least 72% identical, at least 74% identical, at least 76% identical, at least 78% identical, at least 80% identical, at least 82% identical, at least 84 % identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to the nucleic acid sequence encoding the second monomer construct (or the protein portion of the second monomer construct).

In some embodiments, the nucleic acid encoding the protein portion of a first monomer construct encodes a polypeptide comprising the PM1, CP1, CM1, and CM3 moieties. In some embodiments, the nucleic acid encoding the protein portion of a second monomer encodes a polypeptide comprising the CP2 and CM2 moieties. In some embodiments, the nucleic acid encoding the protein portion of a second monomer encodes a polypeptide comprising the CP2, CM2, PM2, and CM4 moieties. In some embodiments, a pair of nucleic acids together encode the protein portion of a first monomer construct and the protein portion of the second monomer construct, wherein the protein portions are then conjugated to the DD1 and DD2 moieties, respectively (in a subsequent conjugation step).

In some embodiments, the nucleic acid encoding the first monomer construct encodes a polypeptide comprising the DD1 moiety. In some embodiments, the nucleic acid encoding the second monomer construct encodes a polypeptide comprising the DD2 moiety.

Vectors

Provided herein are vectors and sets of vectors including any of the nucleic acids described herein. One skilled in the art will be capable of selecting suitable vectors or sets of vectors (e.g., expression vectors) for making any of the ACCs described herein, and using the vectors or sets of vectors to express any of the ACCs described herein. For example, in selecting a vector or a set of vectors, the cell must be considered because the vector(s) may need to be able to integrate into a chromosome of the cell and/or replicate in it. Exemplary vectors that can be used to produce an ACC are also described below.

As used herein, the term “vector” refers to a polynucleotide capable of inducing the expression of a recombinant protein (e.g., a first or second monomer) in a cell (e.g., any of the cells described herein). A “vector” is able to deliver nucleic acids and fragments thereof into a host cell, and includes regulatory sequences (e.g., promoter, enhancer, poly(A) signal). Exogenous polynucleotides may be inserted into the expression vector in order to be expressed. The term “vector” also includes artificial chromosomes, plasmids, retroviruses, and baculovirus vectors.

Methods for constructing suitable vectors that include any of the nucleic acids described herein, and suitable for transforming cells (e.g., mammalian cells) are well-known in the art. See, e.g., Sambrook et al., Eds. “Molecular Cloning: A Laboratory Manual,” 2nd Ed., Cold Spring Harbor Press, 1989 and Ausubel et al., Eds. “Current Protocols in Molecular Biology,” Current Protocols, 1993.

Non-limiting examples of vectors include plasmids, transposons, cosmids, and viral vectors (e.g., any adenoviral vectors (e.g., pSV or pCMV vectors), adeno-associated virus (AAV) vectors, lentivirus vectors, and retroviral vectors), and any Gateway® vectors. A vector can, for example, include sufficient cis-acting elements for expression; other elements for expression can be supplied by the host mammalian cell or in an *in vitro* expression system. Skilled practitioners will be capable of selecting suitable vectors and mammalian cells for making any of the ACCs described herein.

In some embodiments of any of the ACCs described herein, the ACC may be made biosynthetically using recombinant DNA technology and expression in eukaryotic or prokaryotic species.

In some embodiments, the vector includes a nucleic acid encoding the first monomer and the second monomer of any of the ACCs described herein. In some embodiments, the vector is an expression vector.

In some embodiments, a pair of vectors together include a pair of nucleic acids that together encode the first monomer and the second monomer of any of the ACCs described herein. In some embodiments, the pair of vectors is a pair of expression vectors.

Cells

Also provided herein are host cells including any of the vector or sets of vectors described herein including any of the nucleic acids described herein.

Any of the ACCs described herein can be produced by any cell (e.g., a mammalian cell). In some embodiments, a host cell is a mammalian cell (e.g., a human cell), a rodent cell (e.g., a mouse cell, a rat cell, a hamster cell, or a guinea pig cell), or a non-human primate cell.

Methods of introducing nucleic acids and vectors (e.g., any of the vectors or any of the sets of vectors described herein) into a cell are known in the art. Non-limiting examples of methods that can be used to introducing a nucleic acid into a cell include: lipofection, transfection, calcium phosphate transfection, cationic polymer transfection, viral transduction (e.g., adenoviral transduction, lentiviral transduction), nanoparticle transfection, and electroporation.

In some embodiments, the introducing step includes introducing into a cell a vector (e.g., any of the vectors or sets of vectors described herein) including a nucleic acid encoding the monomers that make up any of the ACCs described herein.

In some embodiments of any of the methods described herein, the cell can be a eukaryotic cell. As used herein, the term "eukaryotic cell" refers to a cell having a distinct, membrane-bound nucleus. Such cells may include, for example, mammalian (e.g., rodent, non-human primate, or human), insect, fungal, or plant cells. In some embodiments, the eukaryotic cell is a yeast cell, such as *Saccharomyces cerevisiae*. In some embodiments, the eukaryotic cell is a higher eukaryote, such as mammalian, avian, plant, or insect cells. Non-limiting examples of mammalian cells include Chinese hamster ovary (CHO) cells and human embryonic kidney cells (e.g., HEK293 cells).

In some embodiments, the cell contains the nucleic acid encoding the first monomer and the second monomer of any one of the ACCs described herein. In some embodiments, the cell contains the pair of nucleic acids that together encode the first monomer and the second monomer of any of the ACCs described herein.

5 **Methods of Producing Activatable Cytokine Constructs**

Provided herein are methods of producing any of the ACCs described herein that include: (a) culturing any of the recombinant host cells described herein in a liquid culture medium under conditions sufficient to produce the ACC; and (b) recovering the ACC from the host cell and/or the liquid culture medium.

10 Methods of culturing cells are well known in the art. Cells can be maintained *in vitro* under conditions that favor cell proliferation, cell differentiation and cell growth. For example, cells can be cultured by contacting a cell (e.g., any of the cells described herein) with a cell culture medium that includes the necessary growth factors and supplements sufficient to support cell viability and growth.

15 In some embodiments of any of the methods described herein, the method further includes isolating the recovered ACC. Non-limiting examples of methods of isolation include: ammonium sulfate precipitation, polyethylene glycol precipitation, size exclusion chromatography, ligand-affinity chromatography, ion-exchange chromatography (e.g., anion or cation), and hydrophobic interaction chromatography.

20 In some embodiments, the cells can produce a protein portion of a first monomer construct that includes the CP1, the CM1, the PM2, and the CM3, and a protein portion of a second monomer construct that includes the CP2, and the CM2, and optionally the PM2 and the CM4, and then the protein portions are subsequently conjugated to the DD1 and DD2 moieties, respectively.

25 Compositions and methods described herein may involve use of non-reducing or partially-reducing conditions that allow disulfide bonds to form between the dimerization domains to form and maintain dimerization of the ACCs.

30 In some embodiments of any of the methods described herein, the method further includes formulating the isolated ACC into a pharmaceutical composition. Various formulations are known in the art and are described herein. Any of the isolated ACCs described herein can be formulated for any route of administration (e.g., intravenous,

intratumoral, subcutaneous, intradermal, oral (e.g., inhalation), transdermal (e.g., topical), transmucosal, or intramuscular).

Also provided herein are ACCs produced by any of the methods described herein. Also provided are compositions (e.g., pharmaceutical compositions) that include any of the ACCs produced by any of the methods described herein. Also provided herein are kits that include at least one dose of any of the compositions (e.g., pharmaceutical compositions) described herein.

Methods of Treatment

Provided herein are methods of treating a disease (e.g., a cancer (e.g., any of the cancers described herein)) in a subject including administering a therapeutically effective amount of any of the ACCs described herein to the subject.

As used herein, the term “subject” refers to any mammal. In some embodiments, the subject is a feline (e.g., a cat), a canine (e.g., a dog), an equine (e.g., a horse), a rabbit, a pig, a rodent (e.g., a mouse, a rat, a hamster or a guinea pig), a non-human primate (e.g., a simian (e.g., a monkey (e.g., a baboon, a marmoset), or an ape (e.g., a chimpanzee, a gorilla, an orangutan, or a gibbon)), or a human. In some embodiments, the subject is a human.

In some embodiments, the subject has been previously identified or diagnosed as having the disease (e.g., cancer (e.g., any of the cancers described herein)).

As used herein, the term “treat” includes reducing the severity, frequency or the number of one or more (e.g., 1, 2, 3, 4, or 5) symptoms or signs of a disease (e.g., a cancer (e.g., any of the cancers described herein)) in the subject (e.g., any of the subjects described herein). In some embodiments where the disease is cancer, treating results in reducing cancer growth, inhibiting cancer progression, inhibiting cancer metastasis, or reducing the risk of cancer recurrence in a subject having cancer.

In some embodiments, the methods and uses of the present disclosure include any route of administration including intravenous, infusion, intratumoral, subcutaneous, intraperitoneal, intradermal, oral (e.g., inhalation), intranasal, transdermal (e.g., topical), transmucosal, and/or intramuscular.

In some embodiments of any of the methods described herein, the disease is a cancer. Also provided herein are methods of treating a subject in need thereof (e.g., any

of the exemplary subjects described herein or known in the art) that include administering to the subject a therapeutically effective amount of any of the ACCs described herein or any of the compositions (e.g., pharmaceutical compositions) described herein.

In some embodiments of these methods, the subject has been identified or
5 diagnosed as having a cancer. Non-limiting examples of cancer include: solid tumor, hematological tumor, sarcoma, osteosarcoma, glioblastoma, neuroblastoma, melanoma, rhabdomyosarcoma, Ewing sarcoma, osteosarcoma, B-cell neoplasms, multiple myeloma, a lymphoma (e.g., B-cell lymphoma, B-cell non-Hodgkin's lymphoma, Hodgkin's lymphoma, cutaneous T-cell lymphoma), a leukemia (e.g., hairy cell leukemia, chronic
10 lymphocytic leukemia (CLL), acute myeloid leukemia (AML), chronic myeloid leukemia (CML), acute lymphocytic leukemia (ALL)), myelodysplastic syndromes (MDS), Kaposi sarcoma, retinoblastoma, stomach cancer, urothelial carcinoma, lung cancer, renal cell carcinoma, gastric and esophageal cancer, pancreatic cancer, prostate cancer, brain cancer, colon cancer, bone cancer, lung cancer, breast cancer including triple negative
15 breast cancer (TNBC), colorectal cancer, ovarian cancer, nasopharyngeal adenocarcinoma, non-small cell lung carcinoma (NSCLC), squamous cell head and neck carcinoma, endometrial cancer, bladder cancer, cervical cancer, liver cancer, and hepatocellular carcinoma. In some embodiments, the cancer is a lymphoma. In some embodiments, the lymphoma is Burkitt's lymphoma. In some aspects, the subject has been identified or
20 diagnosed as having familial cancer syndromes such as Li Fraumeni Syndrome, Familial Breast-Ovarian Cancer (BRCA1 or BRCA2 mutations) Syndromes, and others. The disclosed methods are also useful in treating non-solid cancers. Exemplary solid tumors include malignancies (e.g., sarcomas, adenocarcinomas, and carcinomas) of the various organ systems, such as those of lung, breast, lymphoid, gastrointestinal (e.g., colon), and
25 genitourinary (e.g., renal, urothelial, or testicular tumors) tracts, pharynx, prostate, and ovary. Exemplary adenocarcinomas include colorectal cancers, renal-cell carcinoma, liver cancer, non-small cell carcinoma of the lung, and cancer of the small intestine.

Exemplary cancers described by the National Cancer Institute include: Acute Lymphoblastic Leukemia, Adult; Acute Lymphoblastic Leukemia, Childhood; Acute
30 Myeloid Leukemia, Adult; Adrenocortical Carcinoma; Adrenocortical Carcinoma, Childhood; AIDS-Related Lymphoma; AIDS-Related Malignancies; Anal Cancer;

Astrocytoma, Childhood Cerebellar; Astrocytoma, Childhood Cerebral; Bile
 Duct Cancer, Extrahepatic; Bladder Cancer; Bladder Cancer, Childhood; Bone Cancer,
 Osteosarcoma/Malignant Fibrous Histiocytoma; Brain Stem Glioma, Childhood; Brain
 Tumor, Adult; Brain Tumor, Brain Stem Glioma, Childhood; Brain Tumor, Cerebellar
 5 Astrocytoma, Childhood; Brain Tumor, Cerebral Astrocytoma/Malignant Glioma,
 Childhood; Brain Tumor, Ependymoma, Childhood; Brain Tumor, Medulloblastoma,
 Childhood; Brain Tumor, Supratentorial Primitive Neuroectodermal Tumors, Childhood;
 Brain Tumor, Visual Pathway and Hypothalamic Glioma, Childhood; Brain Tumor,
 Childhood (Other); Breast Cancer; Breast Cancer and Pregnancy; Breast Cancer,
 10 Childhood; Breast Cancer, Male; Bronchial Adenomas/Carcinoids, Childhood; Carcinoid
 Tumor, Childhood; Carcinoid Tumor, Gastrointestinal; Carcinoma, Adrenocortical;
 Carcinoma, Islet Cell; Carcinoma of Unknown Primary; Central Nervous System
 Lymphoma, Primary; Cerebellar Astrocytoma, Childhood; Cerebral
 Astrocytoma/Malignant Glioma, Childhood; Cervical Cancer; Childhood Cancers;
 15 Chronic Lymphocytic Leukemia; Chronic Myelogenous Leukemia; Chronic
 Myeloproliferative Disorders; Clear Cell Sarcoma of Tendon Sheaths; Colon Cancer;
 Colorectal Cancer, Childhood; Cutaneous T-Cell Lymphoma; Endometrial Cancer;
 Ependymoma, Childhood; Epithelial Cancer, Ovarian; Esophageal Cancer;
 Esophageal Cancer, Childhood; Ewing's Family of Tumors; Extracranial Germ Cell
 20 Tumor, Childhood; Extragenital Germ Cell Tumor; Extrahepatic Bile Duct Cancer;
 Eye Cancer, Intraocular Melanoma; Eye Cancer, Retinoblastoma; Gallbladder Cancer;
 Gastric (Stomach) Cancer; Gastric (Stomach) Cancer, Childhood; Gastrointestinal
 Carcinoid Tumor; Germ Cell Tumor, Extracranial, Childhood; Germ Cell Tumor,
 Extragenital; Germ Cell Tumor, Ovarian; Gestational Trophoblastic Tumor; Glioma,
 25 Childhood Brain Stem; Glioma, Childhood Visual Pathway and Hypothalamic; Hairy
 Cell Leukemia; Head and Neck Cancer; Hepatocellular (Liver) Cancer, Adult (Primary);
 Hepatocellular (Liver) Cancer, Childhood (Primary); Hodgkin's Lymphoma, Adult;
 Hodgkin's Lymphoma, Childhood; Hodgkin's Lymphoma During Pregnancy;
 Hypopharyngeal Cancer; Hypothalamic and Visual Pathway Glioma, Childhood;
 30 Intraocular Melanoma; Islet Cell Carcinoma (Endocrine Pancreas); Kaposi's Sarcoma;
 Kidney Cancer; Laryngeal Cancer; Laryngeal Cancer, Childhood; Leukemia, Acute

Lymphoblastic, Adult; Leukemia, Acute Lymphoblastic, Childhood; Leukemia, Acute
 Myeloid, Adult; Leukemia, Acute Myeloid, Childhood; Leukemia, Chronic
 Lymphocytic; Leukemia, Chronic Myelogenous; Leukemia, Hairy Cell; Lip and Oral
 Cavity Cancer; Liver Cancer, Adult (Primary); Liver Cancer, Childhood (Primary);
 5 Lung Cancer, Non-Small Cell; Lung Cancer, Small Cell; Lymphoblastic Leukemia,
 Adult Acute; Lymphoblastic Leukemia, Childhood Acute; Lymphocytic Leukemia,
 Chronic; Lymphoma, AIDS-Related; Lymphoma, Central Nervous System (Primary);
 Lymphoma, Cutaneous T-Cell; Lymphoma, Hodgkin's, Adult; Lymphoma, Hodgkin's,
 Childhood; Lymphoma, Hodgkin's During Pregnancy; Lymphoma, Non-Hodgkin's,
 10 Adult; Lymphoma, Non-Hodgkin's, Childhood; Lymphoma, Non-Hodgkin's During
 Pregnancy; Lymphoma, Primary Central Nervous System; Macroglobulinemia,
 Waldenstrom's; Male Breast Cancer; Malignant Mesothelioma, Adult; Malignant
 Mesothelioma, Childhood; Malignant Thymoma; Medulloblastoma, Childhood;
 Melanoma; Melanoma, Intraocular; Merkel Cell Carcinoma; Mesothelioma, Malignant;
 15 Metastatic Squamous Neck Cancer with Occult Primary; Multiple Endocrine Neoplasia
 Syndrome, Childhood; Multiple Myeloma/Plasma Cell Neoplasm; Mycosis Fungoides;
 Myelodysplastic Syndromes; Myelogenous Leukemia, Chronic; Myeloid Leukemia,
 Childhood Acute; Myeloma, Multiple; Myeloproliferative Disorders, Chronic; Nasal
 Cavity and Paranasal Sinus Cancer; Nasopharyngeal Cancer; Nasopharyngeal Cancer,
 20 Childhood; Neuroblastoma; Non-Hodgkin's Lymphoma, Adult; Non-Hodgkin's
 Lymphoma, Childhood; Non-Hodgkin's Lymphoma During Pregnancy; Non-Small Cell
 Lung Cancer; Oral Cancer, Childhood; Oral Cavity and Lip Cancer;
 Oropharyngeal Cancer; Osteosarcoma/Malignant Fibrous Histiocytoma of Bone;
 Ovarian Cancer, Childhood; Ovarian Epithelial Cancer; Ovarian Germ Cell Tumor;
 25 Ovarian Low Malignant Potential Tumor; Pancreatic Cancer; Pancreatic Cancer,
 Childhood; Pancreatic Cancer, Islet Cell; Paranasal Sinus and Nasal Cavity Cancer;
 Parathyroid Cancer; Penile Cancer; Pheochromocytoma; Pineal and Supratentorial
 Primitive Neuroectodermal Tumors, Childhood; Pituitary Tumor; Plasma Cell
 Neoplasm/Multiple Myeloma; Pleuropulmonary Blastoma; Pregnancy and Breast Cancer;
 30 Pregnancy and Hodgkin's Lymphoma; Pregnancy and Non-Hodgkin's Lymphoma;
 Primary Central Nervous System Lymphoma; Primary Liver Cancer, Adult; Primary

Liver Cancer, Childhood; Prostate Cancer; Rectal Cancer; Renal Cell (Kidney) Cancer; Renal Cell Cancer, Childhood; Renal Pelvis and Ureter, Transitional Cell Cancer; Retinoblastoma; Rhabdomyosarcoma, Childhood; Salivary Gland Cancer; Salivary Gland Cancer, Childhood; Sarcoma, Ewing's Family of Tumors; Sarcoma, Kaposi's; 5 Sarcoma (Osteosarcoma)/Malignant Fibrous Histiocytoma of Bone; Sarcoma, Rhabdomyosarcoma, Childhood; Sarcoma, Soft Tissue, Adult; Sarcoma, Soft Tissue, Childhood; Sezary Syndrome; Skin Cancer; Skin Cancer, Childhood; Skin Cancer (Melanoma); Skin Carcinoma, Merkel Cell; Small Cell Lung Cancer; Small Intestine Cancer; Soft Tissue Sarcoma, Adult; Soft Tissue Sarcoma, Childhood; 10 Squamous Neck Cancer with Occult Primary, Metastatic; Stomach (Gastric) Cancer; Stomach (Gastric) Cancer, Childhood; Supratentorial Primitive Neuroectodermal Tumors, Childhood; T-Cell Lymphoma, Cutaneous; Testicular Cancer; Thymoma, Childhood; Thymoma, Malignant; Thyroid Cancer; Thyroid Cancer, Childhood; Transitional Cell Cancer of the Renal Pelvis and Ureter; Trophoblastic Tumor, 15 Gestational; Unknown Primary Site, Cancer of, Childhood; Unusual Cancers of Childhood; Ureter and Renal Pelvis, Transitional Cell Cancer; Urethral Cancer; Uterine Sarcoma; Vaginal Cancer; Visual Pathway and Hypothalamic Glioma, Childhood; Vulvar Cancer; Waldenstrom's Macro globulinemia; and Wilms' Tumor.

Further exemplary cancers include diffuse large B-cell lymphoma (DLBCL) and 20 mantle cell lymphoma (MCL).

Metastases of the aforementioned cancers can also be treated or prevented in accordance with the methods described herein.

In some embodiments, these methods can result in a reduction in the number, severity, or frequency of one or more symptoms of the cancer in the subject (e.g., as 25 compared to the number, severity, or frequency of the one or more symptoms of the cancer in the subject prior to treatment).

In some embodiments of any of the methods described herein, the methods further include administering to a subject an additional therapeutic agent (e.g., one or more of the therapeutic agents listed in Table 2).

30 **Table 2. Additional Therapeutic Agents**

Antibody Trade Name (antibody name)	Target
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Raptiva™ (efalizumab)	CD11a
Arzerra™ (ofatumumab)	CD20
Bexxar™ (tositumomab)	CD20
Gazyva™ (obinutuzumab)	CD20
Ocrevus™ (ocrelizumab)	CD20
Rituxan™ (rituximab)	CD20
Zevalin™ (ibritumomab tiuxetan)	CD20
Adcetris™ (brentuximab vedotin)	CD30
Myelotarg™ (gemtuzumab)	CD33
Mylotarg™ (gemtuzumab ozogamicin)	CD33
(vadastuximab)	CD33
(vadastuximab talirine)	CD33
Campath™ (alemtuzumab)	CD52
Lemtrada™ (alemtuzumab)	CD52
Tactress™ (tamtuxetmab)	CD52
Soliris™ (eculizumab)	Complement C5
Ultomiris™ (ravulizumab)	Complement C5
(olendalizumab)	Complement C5
Yervoy™ (ipilimumab)	CTLA-4
(tremelimumab)	CTLA-4
Orencia™ (abatacept)	CTLA-4
Hu5c8	CD40L
(letolizumab)	CD40L
Rexomun™ (ertumaxomab)	CD3/Her2
Erbitux™ (cetuximab)	EGFR
Portrazza™ (necitumumab)	EGFR
Vectibix™ (panitumumab)	EGFR
CH806	EGFR
(depatuxizumab)	EGFR
(depatuxizumab mafodotin)	EGFR
(futuximab:modotuximab)	EGFR
ICR62 (imgatuzumab)	EGFR
(laprituximab)	EGFR
(losatuxizumab)	EGFR
(losatuxizumab vedotin)	EGFR
mAb 528	EGFR
(matuzumab)	EGFR
(nimotuzumab)	EGFR
(tomuzotuximab)	EGFR
(zalutumumab)	EGFR
MDX-447	EGFR/CD64
(adecatumumab)	EpCAM
Panorex™ (edrecolomab)	EpCAM
Vicinium™	EpCAM
Synagis™ (palivizumab)	F protein of RSV

ReoPro™ (abiximab)	Glycoprotein receptor IIb/IIIa
Herceptin™ (trastuzumab)	Her2
Herceptin™ Hylecta (trastuzumab; Hyaluronidase)	Her2
(trastuzumab deruxtecan)	Her2
(hertuzumab verdotin)	Her2
Kadcyla™ (trastuzumab emtansine)	Her2
(margetuximab)	Her2
(timigutuzumab)	Her2
Xolair™ (omalizumab)	IgE
(ligelizumab)	IgE
(figitumumab)	IGF1R
(teprotumumab)	IGF1R
Simulect™ (basiliximab)	IL2R
Zenapax™ (daclizumab)	IL2R
Zinbryta™ (daclizumab)	IL2R
Actemra™ (tocilizumab)	IL-6 receptor
Kevzara™ (sarilumab)	IL-6 receptor
(vobarilizumab)	IL-6 receptor
Stelara™ (ustekinumab)	IL-12/IL-23
Tysabri™ (natalizumab)	Integrin α 4
(abrilumab)	Integrin α 4
	Jagged 1 or Jagged 2
(fasinumab)	NGF
(fulranumab)	NGF
(tanezumab)	NGF
	Notch, e.g., Notch 1
Pidilizumab	Delta like-1 (PD-1 pathway inhibitor)
Opdivo® (nivolumab)	PD1
Keytruda® (pembrolizumab)	PD1
Libtayo® (cemiplimab)	PD1
BGB-A317 (tislelizumab)	PD1
PDR001 (spartalizumab)	PD1
JNJ-63723283 (cetrelimab)	PD1
TSR042 (dostarlimab)	PD1
AGEN2034 (balstilimab)	PD1
JS001 (toripalimab)	PD1
IOB308 (sintilimab)	PD1
BCD100 (prolgolimab)	PD1
CBT-501 (genolimzumab)	PD1
ABBV181 (budigalimab)	PD1
AK105	PD1
BI-754091	PD1
INCSHR-1210	PD1

MEDI0680	PD1
MGA012	PD1
SHR-1210	PD1
Imfinzi™ (durvalumab)	PD-L1
Tecentriq® (atezolizumab)	PD-L1
Bavencio® (avelumab)	PD-L1
KN035 (envafolimab)	PD-L1
BMS936559 (MDX1105)	PD-L1
BGBA 333	PD-L1
FAZ053	PD-L1
LY-3300054	PD-L1
SH-1316	PD-L1
AMP-224	PD-L2
(bavituximab)	Phosphatidylserine
huJ591	PSMA
RAV12	RAAG12
Prolia™ (denosumab)	RANKL
GC1008 (fresolimumab)	TGFbeta
Cimzia™ (Certolizumab Pegol)	TNFα
Remicade™ (infliximab)	TNFα
Humira™ (adalimumab)	TNFα
Simponi™ (golimumab)	TNFα
Enbrel™ (etanercept)	TNF-R
(mapatumumab)	TRAIL-R1
Avastin™ (bevacizumab)	VEGF
Lucentis™ (ranibizumab)	VEGF
(brolucizumab)	VEGF
(vanucizumab)	VEGF

Compositions/Kits

Also provided herein are compositions (e.g., pharmaceutical compositions) including any of the ACCs described herein and one or more (e.g., 1, 2, 3, 4, or 5)

- 5 pharmaceutically acceptable carriers (e.g., any of the pharmaceutically acceptable carriers described herein), diluents, or excipients.

In some embodiments, the compositions (e.g. pharmaceutical compositions) that include any of the ACCs described herein can be disposed in a sterile vial or a pre-loaded syringe.

- 10 In some embodiments, the compositions (e.g. pharmaceutical compositions) that include any of the ACCs described herein can be formulated for different routes of

administration (e.g., intravenous, subcutaneous, intramuscular, intraperitoneal, or intratumoral).

In some embodiments, any of the pharmaceutical compositions described herein can include one or more buffers (e.g., a neutral-buffered saline, a phosphate-buffered saline (PBS), amino acids (e.g., glycine), one or more carbohydrates (e.g., glucose, 5 mannose, sucrose, dextran, or mannitol), one or more antioxidants, one or more chelating agents (e.g., EDTA or glutathione), one or more preservatives, and/or a pharmaceutically acceptable carrier (e.g., bacteriostatic water, PBS, or saline).

As used herein, the phrase “pharmaceutically acceptable carrier” refers to any and 10 all solvents, dispersion media, coatings, antibacterial agents, antimicrobial agents, isotonic and absorption delaying agents, and the like, compatible with pharmaceutical administration. Suitable carriers include, but are not limited to: water, saline, ringer’s solutions, dextrose solution, and about 5% human serum albumin.

In some embodiments of any of the pharmaceutical compositions described 15 herein, any of the ACCs described herein are prepared with carriers that protect against rapid elimination from the body, e.g., sustained and controlled release formulations, including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, e.g., ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Methods for preparation 20 of such pharmaceutical compositions and formulations are apparent to those skilled in the art.

Also provided herein are kits that include any of the ACCs described herein, any of the compositions that include any of the ACCs described herein, or any of the pharmaceutical compositions that include any of the ACCs described herein. Also 25 provided are kits that include one or more second therapeutic agent(s) selected from Table 2 in addition to an ACC described herein. The second therapeutic agent(s) may be provided in a dosage administration form that is separate from the ACC. Alternatively, the second therapeutic agent(s) may be formulated together with the ACC.

Any of the kits described herein can include instructions for using any of the 30 compositions (e.g., pharmaceutical compositions) and/or any of the ACCs described herein. In some embodiments, the kits can include instructions for performing any of the

methods described herein. In some embodiments, the kits can include at least one dose of any of the compositions (e.g., pharmaceutical compositions) described herein. In some embodiments, the kits can provide a syringe for administering any of the pharmaceutical compositions described herein.

5 The present disclosure includes and finds support for and in any one or any combination of the following items:

1. An activatable cytokine construct (ACC) that includes a first monomer construct and a second monomer construct, wherein:
 - (a) the first monomer construct comprises a first peptide mask (PM1), a first mature
 10 cytokine protein (CP1), a first and a third cleavable moieties (CM1 and CM3), and a first dimerization domain (DD1), wherein the CM1 is positioned between the CP1 and the DD1 and the CM3 is positioned between the PM1 and the CP1; and
 - (b) the second monomer construct comprises a second mature cytokine protein (CP2), a second cleavable moiety (CM2), and a second dimerization domain (DD2), wherein
 15 the CM2 is positioned between the CP2 and the DD2;
 wherein the DD1 and the DD2 bind to each other thereby forming a dimer of the first monomer construct and the second monomer construct.
2. The ACC of item 1, wherein the second monomer construct further comprises a second peptide mask (PM2) and a fourth cleavable moiety (CM4), wherein the CM4
 20 is positioned between the PM2 and the CP2.
3. The ACC of item 1 or 2, wherein the first monomer construct comprises a first polypeptide that comprises the PM1, the CM3, the CP1, the CM1, and the DD1, optionally wherein the first monomer construct comprises a structural arrangement in a N- to C-terminal direction of PM1-CM3-CP1-CM1-DD1, wherein each dash (-)
 25 between the ACC components represents either a direct linkage or linkage via one or more linkers.
4. The ACC of any one or combination of items 1-3, wherein the second monomer construct comprises a second polypeptide that comprises the CP2, the CM2, and the DD2.
- 30 5. The ACC of item 2, wherein the second monomer construct comprises a second polypeptide that comprises the PM2, the CM4, the CP2, the CM2, and the DD2,

- optionally wherein the second monomer construct comprises a structural arrangement in a N- to C-terminal direction of PM2-CM4-CP2-CM2-DD2.
6. The ACC of any one or combination of items 1-5, wherein the CP1 and/or the CP2 is/are each individually selected from the group consisting of: an interferon, an interleukin, GM-CSF, G-CSF, LIF, OSM, CD154, LT- β , TNF- α , TNF- β , 4-1BBL, APRIL, CD70, CD153, CD178, GITRL, LIGHT, OX40L, TALL-1, TRAIL, TWEAK, TRANCE, TGF- β 1, TGF- β 1, TGF- β 3, Epo, Tpo, Flt-3L, SCF, M-CSF, and MSP, optionally wherein the CP1 and/or the CP2 is independently selected from IL-2, IL-7, IL-8, IL-10, IL-12, IL-15, IL-21, an IFN-alpha, an IFN beta, an IFN gamma, GM-CSF, TGF-beta, LIGHT, GITR-L, CD40L, CD27L, 4-1BB-L, OX40, and OX40L.
7. The ACC of any one or combination of items 1-6, wherein:
- the PM1 comprises a sequence selected from the group consisting of SEQ ID NOs: 328, 329, 323, and 331-369, and the CP1 is an interferon;
 - the PM1 comprises a sequence selected from the group consisting of SEQ ID NOs: 328, 329, 323, and 331-364, and the CP1 is an interferon alpha;
 - the PM1 comprises a sequence selected from the group consisting of SEQ ID NOs: 331-360, 362-364, and the CP1 is an interferon beta;
 - the PM1 comprises a sequence selected from the group consisting of SEQ ID NOs: 331-360, 366-369, and the CP1 is an interferon gamma;
 - the PM1 comprises a sequence selected from the group consisting of SEQ ID NOs: 370-374, and the CP1 is an IL-12;
 - the PM1 comprises a sequence selected from the group consisting of SEQ ID NOs: 375-382, 469-477, 478, and the CP1 is an IL-15;
 - the PM1 comprises a sequence selected from the group consisting of SEQ ID NOs: 383-468, 469-478, and the CP1 is an IL-2; or
 - the PM1 comprises a sequence selected from the group consisting of SEQ ID NOs: 478 and 479, and the CP1 is an IL-21.
8. The ACC of any one or combination of items 2-7, wherein:
- the PM2 comprises a sequence selected from the group consisting of SEQ ID NOs: 328, 329, 323, and 331-369, and the CP2 is an interferon;

- the PM2 comprises a sequence selected from the group consisting of SEQ ID NOs: 328, 329, 323, and 331-364, and the CP2 is an interferon alpha;
- the PM2 comprises a sequence selected from the group consisting of SEQ ID NOs: 331-360, 362-364, and the CP2 is an interferon beta;
- 5 the PM2 comprises a sequence selected from the group consisting of SEQ ID NOs: 331-360, 366-369, and the CP2 is an interferon gamma;
- the PM2 comprises a sequence selected from the group consisting of SEQ ID NOs: 370-374, and the CP2 is an IL-12;
- the PM2 comprises a sequence selected from the group consisting of SEQ ID NOs: 375-382, 469-477, 478, and the CP2 is an IL-15;
- 10 the PM2 comprises a sequence selected from the group consisting of SEQ ID NOs: 383-468, 469-478, and the CP2 is an IL-2; or
- the PM2 comprises a sequence selected from the group consisting of SEQ ID NOs: 478 and 479, and the CP2 is an IL-21.
- 15 9. The ACC of any one or combination of items 2-8, wherein PM1, PM2 or PM1 and PM2 comprises SEQ ID NO: 323.
10. The ACC of any one or combination of items 2-9, wherein PM1, PM2 or PM1 and PM2 comprises SEQ ID NO: 331.
11. The ACC of any one or combination of items 2-10, wherein PM1, PM2 or PM1 and
- 20 PM2 comprises SEQ ID NO: 332.
12. The ACC of any one or combination of items 2-11, comprising a mask linking region between PM1 and CP1 that comprises 15, 16, 17, 18, 19, 20, 21, or 22 amino acids.
13. The ACC of any one or combination of items 2-12, comprising a mask linking region between PM2 and CP2 that comprises 15, 16, 17, 18, 19, 20, 21, or 22 amino acids.
- 25 14. The ACC of any one or combination of items 1 to 13, wherein the DD1 and the DD2 are a pair selected from the group consisting of: a pair of Fc domains; a sushi domain from an alpha chain of human IL-15 receptor (IL15R α) and a soluble IL-15; barnase and barnstar; a PKA and an AKAP; adapter/docking tag modules based on mutated RNase I fragments; an epitope and sdAb; an epitope and scFv; and SNARE modules
- 30 based on interactions of the proteins syntaxin, synaptotagmin, synaptobrevin, and SNAP25; an antigen-binding domain and an epitope.

15. The ACC of item 14, wherein the DD1 and the DD2 are a pair of Fc domains.
16. The ACC of item 15, wherein the pair of Fc domains is a pair of human Fc domains.
17. The ACC of item 16, wherein the human Fc domains are human IgG1 Fc domains, human IgG2 Fc domains, human IgG3 Fc domains, or human IgG4 Fc domains.
- 5 18. The ACC of item 17, wherein the human Fc domains are human IgG4 Fc domains.
19. The ACC of item 16, wherein the human Fc domains comprise a sequence that is at least 80% identical to SEQ ID NO: 3.
20. The ACC of item 19, wherein the human Fc domains comprise a sequence that is at least 90% identical to SEQ ID NO: 3.
- 10 21. The ACC of item 20, wherein the human Fc domains comprise SEQ ID NO: 3.
22. The ACC of item 14, wherein the DD1 and the DD2 comprise SEQ ID NOs: 318 and 319, respectively.
23. The ACC of any one or combination of items 1-21, wherein the DD1 and the DD2 are the same.
- 15 24. The ACC of item 14, wherein the DD1 comprises an antigen-binding domain and DD2 comprises a corresponding epitope.
25. The ACC of item 24, wherein the antigen-binding domain is an anti-His tag antigen-binding domain and wherein the DD2 comprises a His tag.
26. The ACC of item 24, wherein the antigen-binding domain is a single chain variable
20 fragment (scFv).
27. The ACC of item 24, wherein the antigen-binding domain is a single domain antibody (sdAb).
28. The ACC of item 14, wherein at least one of the DD1 and the DD2 comprises a
25 dimerization domain substituent selected from the group consisting of a non-polypeptide polymer and a small molecule.
29. The ACC of item 28, wherein the DD1 and the DD2 comprise non-polypeptide polymers covalently bound to each other.
30. The ACC of item 29, wherein the non-polypeptide polymer is a sulfur-containing polyethylene glycol, and wherein the DD1 and the DD2 are covalently bound to each
30 other via one or more disulfide bonds.

31. The ACC of item 28, wherein at least one of the DD1 and the DD2 comprises a small molecule.
32. The ACC of item 31, wherein the small molecule is biotin.
33. The ACC of item 32, wherein DD1 comprises biotin and DD2 comprises an avidin.
- 5 34. The ACC of any one or combination of items 1-33, wherein the CP1 and the CP2 are mature cytokines.
35. The ACC of any one or combination of items 1-33, wherein the CP1 and the CP2 comprise a signal peptide.
36. The ACC of any one or combination of items 1-35, wherein the CP1 and the CP2 are
10 the same.
37. The ACC of any one or combination of items 1-35, wherein the CP1 and the CP2 are different.
38. The ACC of any one or combination of items 1-35, wherein the CP1 and/or the CP2 is/are an interferon.
- 15 39. The ACC of item 38, wherein the CP1 and the CP2 are an interferon.
40. The ACC of item 38, wherein the CP1 and the CP2 are different interferons.
41. The ACC of item 38, wherein the CP1 and the CP2 are the same interferon.
42. The ACC of any one one or combination of items 38-41, wherein the interferon(s) is/are a human wildtype mature interferon.
- 20 43. The ACC of any one one or combination of items 38-42, wherein the interferon(s) is/are selected from the group consisting of: interferon-alpha, interferon-beta, interferon-omega, and interferon-tau.
44. The ACC of item 43, wherein the interferons is/are an interferon-alpha.
45. The ACC of item 44, wherein the interferon(s) is/are selected from the group
25 consisting of: interferon alpha-2a, interferon alpha-2b, and interferon alpha-n3.
46. The ACC of item 45, wherein the interferon(s) is/are interferon alpha-2b.
47. The ACC of item 46, wherein the CP1 and/or the CP2 comprises a sequence that is at least 80% identical to SEQ ID NO: 1.
48. The ACC of item 47, wherein the CP1 and/or the CP2 comprises a sequence that is at
30 least 90% identical to SEQ ID NO: 1.

49. The ACC of item 48, wherein the CP1 and/or the CP2 comprises the sequence of
SEQ ID NO: 1.
50. The ACC of item 43, wherein the interferon is an interferon beta.
51. The ACC of item 50, wherein the interferon beta is selected from the group consisting
5 of interferon beta-1a, and interferon beta-1b.
52. The ACC of any one or combination of items 1-51, wherein the CP1 and/or the CP2
comprises an IFab domain.
53. The ACC of any one or combination of items 1-38, wherein the CP1 and/or the CP2
comprises an interleukin.
- 10 54. The ACC of item 53, wherein the interleukin is selected from the group consisting of
IL-1 α , IL-1 β , IL-1RA, IL-18, IL-2, IL-4, IL-7, IL-9, IL-13, IL-15, IL-3, IL-5, IL-6,
IL-11, IL-12, IL-10, IL-20, IL-21, IL-14, IL-16, and IL-17.
55. The ACC of any one or combination of items 1-54, wherein each of the CM1 and the
CM2 comprises a total of about 3 amino acids to about 15 amino acids.
- 15 56. The ACC of any one or combination of items 1-55, wherein one or more of the CM1,
the CM2, the CM3, and the CM4 comprise substrates for different proteases.
57. The ACC of any one or combination of items 1-56, wherein the CM1, the CM2, and
the CM3 comprise substrates for the same protease.
58. The ACC of any one or combination of items 1-57, wherein the protease(s) is/are
20 selected from the group consisting of: ADAM8, ADAM9, ADAM10, ADAM12,
ADAM15, ADAM17/TACE, ADAMDEC1, ADAMTS1, ADAMTS4, ADAMTS5,
BACE, Renin, Cathepsin D, Cathepsin E, Caspase 1, Caspase 2, Caspase 3, Caspase
4, Caspase 5, Caspase 6, Caspase 7, Caspase 8, Caspase 9, Caspase 10, Caspase 14,
Cathepsin B, Cathepsin C, Cathepsin K, Cathepsin L, Cathepsin S, Cathepsin V/L2,
25 Cathepsin X/Z/P, Cruzipain, Legumain, Otubain-2, KLK4, KLK5, KLK6, KLK7,
KLK8, KLK10, KLK11, KLK13, KLK14, Meprin, Neprilysin, PSMA, BMP-1,
MMP-1, MMP-2, MMP-3, MMP-7, MMP-9, MMP-10, MMP-11, MMP-12, MMP-
13, MMP-14, MMP-15, MMP-16, MMP-17, MMP-19, MMP-20, MMP-23, MMP-
24, MMP-26, MMP-27, activated protein C, cathepsin A, cathepsin G, Chymase,
30 FVIIa, FIXa, FXa, FXIa, FXIIa, Elastase, Granzyme B, Guanidinobenzoate, HtrA1,
human neutrophil lyase, lactoferrin, marapsin, NS3/4A, PACE4, Plasmin, PSA, tPA,

thrombin, tryptase, uPA, DESC1, DPP-4, FAP, Hepsin, Matriptase-2, MT-SP1/Matrilysin, TMPRSS2, TMPRSS3, and TMPRSS4.

59. The ACC of item 58, wherein the protease(s) is/are selected from the group consisting of: uPA, legumain, MT-SP1, ADAM17, BMP-1, TMPRSS3, TMPRSS4, MMP-2, 5 MMP-9, MMP-12, MMP-13, and MMP-14.
60. The ACC of any one or combination of item 1-57, wherein the CM1, CM2, CM3, and/or the CM4 comprise a sequence selected from the group consisting of:
- LSGRSDNH (SEQ ID NO: 5), TGRGPSWV (SEQ ID NO: 6), PLTGRSGG (SEQ ID NO: 7), TARGPSFK (SEQ ID NO: 8), NTLSGRSENHSG (SEQ ID NO: 9), 10 NTLSGRSGNHGS (SEQ ID NO: 10), TSTSGRSANPRG (SEQ ID NO: 11), TSGRSANP (SEQ ID NO: 12), VHMPGLGLP (SEQ ID NO: 13), AVGLLAPP (SEQ ID NO: 14), AQNLLGMV (SEQ ID NO: 15), QNQALRMA (SEQ ID NO: 16), LAAPLGLL (SEQ ID NO: 17), STFPFGMF (SEQ ID NO: 18), ISSGLLS (SEQ ID NO: 19), PAGLWLD (SEQ ID NO: 20), VAGRSMP (SEQ ID NO: 21), 15 VVPEGRRS (SEQ ID NO: 22), ILPRSPAF (SEQ ID NO: 23), MVLGRSLL (SEQ ID NO: 24), QGRAITFI (SEQ ID NO: 25), SPRSIMLA (SEQ ID NO: 26), SMLRSMPL (SEQ ID NO: 27), ISSGLLSGRSDNH (SEQ ID NO: 28), AVGLLAPPGLSGRSDNH (SEQ ID NO: 29), ISSGLLSGGSGGSLSGRSDNH (SEQ ID NO: 30), LSGRSGNH (SEQ ID NO: 31), SGRSANPRG (SEQ ID NO: 32), 20 LSGRSDDH (SEQ ID NO: 33), LSGRSDIH (SEQ ID NO: 34), LSGRSDQH (SEQ ID NO: 35), LSGRSDTH (SEQ ID NO: 36), LSGRSDYH (SEQ ID NO: 37), LSGRSDNP (SEQ ID NO: 38), LSGRSANP (SEQ ID NO: 39), LSGRSANI (SEQ ID NO: 40), LSGRSDNI (SEQ ID NO: 41), MIAPVAYR (SEQ ID NO: 42), RPSPMWAY (SEQ ID NO: 43), WATPRPMR (SEQ ID NO: 44), FRLLDWQW (SEQ ID NO: 45), ISSGL (SEQ ID NO: 46), ISSGLLS (SEQ ID NO: 47), ISSGLL (SEQ ID NO: 48), ISSGLLSGRSANPRG (SEQ ID NO: 49), 25 AVGLLAPPTSGRSANPRG (SEQ ID NO: 50), AVGLLAPPSGRSANPRG (SEQ ID NO: 51), ISSGLLSGRSDDH (SEQ ID NO: 52), ISSGLLSGRSDIH (SEQ ID NO: 53), ISSGLLSGRSDQH (SEQ ID NO: 54), ISSGLLSGRSDTH (SEQ ID NO: 55), 30 ISSGLLSGRSDYH (SEQ ID NO: 56), ISSGLLSGRSDNP (SEQ ID NO: 57), ISSGLLSGRSANP (SEQ ID NO: 58), ISSGLLSGRSANI (SEQ ID NO: 59),

- AVGLLAPPGGLSGRSDDH (SEQ ID NO: 60), AVGLLAPPGGLSGRSDIH (SEQ ID NO: 61), AVGLLAPPGGLSGRSDQH (SEQ ID NO: 62), AVGLLAPPGGLSGRSDTH (SEQ ID NO: 63), AVGLLAPPGGLSGRSDYH (SEQ ID NO: 64), AVGLLAPPGGLSGRSDNP (SEQ ID NO: 65),
- 5 AVGLLAPPGGLSGRSANP (SEQ ID NO: 66), AVGLLAPPGGLSGRSANI (SEQ ID NO: 67), ISSGLLSGRSDNI (SEQ ID NO: 68), AVGLLAPPGGLSGRSDNI (SEQ ID NO: 69), GLSGRSDNHGGAVGLLAPP (SEQ ID NO: 70), GLSGRSDNHGGVHMPLGFLGP (SEQ ID NO: 71), LSGRSDNHGGVHMPLGFLGP (SEQ ID NO: 72), ISSGLSS (SEQ ID NO: 73),
- 10 PVGYTSSL (SEQ ID NO: 74), DWLYWPGI (SEQ ID NO: 75), LKAAPRWA (SEQ ID NO: 76), GPSHLVLT (SEQ ID NO: 77), LPGGLSPW (SEQ ID NO: 78), MGLFSEAG (SEQ ID NO: 79), SPLPLRVP (SEQ ID NO: 80), RMHLRSLG (SEQ ID NO: 81), LLAPSHRA (SEQ ID NO: 82), GPRSFGL (SEQ ID NO: 83), GPRSFG (SEQ ID NO: 84), SARGPSRW (SEQ ID NO: 85), GGWHTGRN (SEQ ID NO: 86),
- 15 HTGRSGAL (SEQ ID NO: 87), AARGPAIH (SEQ ID NO: 88), RGPAFNPM (SEQ ID NO: 89), SSRGPAYL (SEQ ID NO: 90), RGPATPIM (SEQ ID NO: 91), RGPA (SEQ ID NO: 92), GGQPSGMWGW (SEQ ID NO: 93), FPRPLGITGL (SEQ ID NO: 94), SPLTGRSG (SEQ ID NO: 95), SAGFSLPA (SEQ ID NO: 96), LAPLGLQRR (SEQ ID NO: 97), SGGPLGVR (SEQ ID NO: 98), PLGL (SEQ ID NO: 99), and SGRSDNI (SEQ ID NO: 100).
- 20 61. The ACC of item 60, wherein the CM1, the CM2, the CM3 and/or the CM4 comprises a sequence selected from the group consisting of: ISSGLLSGRSDNH (SEQ ID NO: 28), LSGRSDDH (SEQ ID NO: 33), ISSGLLSGRSDQH (SEQ ID NO: 54), SGRSDNI (SEQ ID NO: 100), and ISSGLLSGRSDNI (SEQ ID NO: 68).
- 25 62. The ACC of any one or combination of items 1-61, wherein the protease(s) is/are produced by a tumor in a subject.
63. The ACC of item 62, wherein the subject has been diagnosed or identified as having a cancer.
64. The ACC of any one or combination of items 1-63, wherein the CP1 and the CM1
- 30 directly abut each other in the first monomer construct.

65. The ACC of any one or combination of items 1-64, wherein the CM1 and the DD1 directly abut each other in the first monomer construct.
66. The ACC of any one or combination of items 1-65, wherein the CP2 and the CM2 directly abut each other in the second monomer construct.
- 5 67. The ACC of any one or combination of items 1-66, wherein the CM2 and the DD2 directly abut each other in the second monomer construct.
68. The ACC of any one or combination of items 1-63, wherein the first monomer construct comprises at least one linker.
69. The ACC of item 68, wherein the at least one linker is a linker L1 disposed between
10 the PM1 and the CM3 and/or a linker L2 disposed between the CM3 and the CP1.
70. The ACC of item 68, wherein the second monomer construct comprises at least one linker.
71. The ACC of item 70, wherein the at least one linker is a linker L3 disposed between the PM2 and the CM4 and/or a linker L4 disposed between the CM4 and the CP2.
- 15 72. The ACC of item 71, wherein the first monomer construct comprises a linker L1 and the second monomer construct comprises a linker L3.
73. The ACC of item 72, wherein L1 and L3 are the same.
74. The ACC of item 71, wherein the first monomer construct comprises a linker L2 and the second monomer construct comprises a linker L4.
- 20 75. The ACC of item 74, wherein L2 and L4 are the same.
76. The ACC of any one or combination of items 68-75, wherein each linker has a total length of 1 amino acid to about 15 amino acids.
77. The ACC of item 76, wherein each linker has a total length of at least 5 amino acids.
78. The ACC of item 68, wherein the first monomer construct comprises at least one
25 linker, wherein each linker is independently selected from the group consisting of GSSGGSGGSGG (SEQ ID NO: 210); GGGS (SEQ ID NO: 2); GGGSGGGS (SEQ ID NO: 211); GGGSGGGSGGGS (SEQ ID NO: 212); GGGSGGGGSGGGGS (SEQ ID NO: 213); GGGSGGGGSGGGGSGGGGSGGGGS (SEQ ID NO: 214); GGGSGGGGS (SEQ ID NO: 215); GGGS (SEQ ID NO: 216); GS; GGGSGGS (SEQ ID NO: 217); GGGSGGGGSGGGGSGS (SEQ ID NO: 218);
30 GGS LDPKGGGS (SEQ ID NO: 219); PKSCDKTHTCPPAPPELLG (SEQ ID

- NO: 220); SKYGPPCPPCPAPEFLG (SEQ ID NO: 221); GKSSGSGSESKS (SEQ ID NO: 222); GSTSGSGKSSEGKG (SEQ ID NO: 223); GSTSGSGKSSEGSGSTKG (SEQ ID NO: 224); GSTSGSGKPGSGEGSTKG (SEQ ID NO: 225); GSTSGSGKPGSSEGST (SEQ ID NO: 226); (GS)_n, (GGS)_n, (GSGGS)_n (SEQ ID NO: 227), (GGGS)_n (SEQ ID NO: 228), (GGGGS)_n (SEQ ID NO: 216), wherein each n is an integer of at least one; GGSG (SEQ ID NO: 229); GGSGG (SEQ ID NO: 230); GSGSG (SEQ ID NO: 231); GSGGG (SEQ ID NO: 232); GGGSG (SEQ ID NO: 233); GSSSG (SEQ ID NO: 234); GGGGSGGGGSGGGGS (SEQ ID NO: 213); GGGGSGGGGSGGGGSGGGGS (SEQ ID NO: 214); GSTSGSGKPGSSEGST (SEQ ID NO: 226); SGGG (SEQ ID NO: 327); and SGGGG (SEQ ID NO: 492).
79. The ACC of item 78, wherein the linker comprises a sequence of GGGS (SEQ ID NO: 2).
80. The ACC of any one or combination of items 1-79, wherein the first monomer construct comprises in a N- to C- terminal direction, the PM1, the CM3, the CP1, the CM1, and the DD1.
81. The ACC of any one or combination of items 1-79, wherein the first polypeptide comprises in a C- to N-terminal direction, the PM1, the CM3, the CP1, the CM1, and the DD1.
82. The ACC of any one or combination of items 1-81, wherein the second polypeptide comprises in a N- to C-terminal direction, the CP2, CM2, and the DD2.
83. The ACC of any one or combination of items 1-82, wherein the second polypeptide comprises in a C- to N-terminal direction, the CP2, CM2, and the DD2.
84. The ACC of any one or combination of items 1-36, 38, 39, or 41-83, wherein the first monomer construct and the second monomer construct are the same.
85. The ACC of any one or combination of items 1-79, wherein the first monomer construct comprises in a N- to C- terminal direction, the PM1, an optional linker, the CM3, an optional linker, the CP1, the CM1, and the DD1, wherein the CP1 and the CM1 directly abut each other, wherein the CM1 and the DD1 directly abut each other, wherein the CM1 is a peptide of not more than 10 amino acids, wherein the second monomer construct is the same as the first monomer construct, and wherein the first

- and second monomer constructs are covalently bound to each other via at least two disulfide bonds.
86. The ACC of item 85, wherein the DD1 and the DD2 are each a human Fc domain having an N-terminus at Cysteine 216, as numbered according to EU numbering.
- 5 87. The ACC of item 85 or 86, wherein the CM1 is a peptide of not more than 7 amino acids.
88. The ACC of any one or combination of item 85-87, wherein the CP1 and the CP2 comprise an amino acid sequence that is at least 90% identical to SEQ ID NO: 1.
89. The ACC of any one or combination of items 1-88, wherein the ACC is characterized
10 by having a reduced level of at least one CP1 and/or CP2 activity as compared to a control level of at least one CP1 and/or CP2 activity.
90. The ACC of item 89, wherein the at least one of the CP1 and the CP2 activity is a level of proliferation of lymphoma cells.
91. The ACC of item 89, wherein the at least one of the CP1 and the CP2 activity is the
15 level of JAK/STAT/ISGF3 pathway activation in a lymphoma cell.
92. The ACC of item 89, wherein the at least one activity is a level of SEAP production in a lymphoma cell.
93. The ACC of item 89, wherein the ACC is characterized by at least a 2-fold reduction in at least one of the CP1 and the CP2 activity as compared to the control level.
- 20 94. The ACC of item 93, wherein the ACC is characterized by at least a 5-fold reduction in at least one CP1 and/or the CP2 activity as compared to the control level.
95. The ACC of item 94, wherein the ACC is characterized by at least a 10-fold reduction in at least one activity of the CP1 and/or the CP2 as compared to the control level.
96. The ACC of item 95, wherein the ACC is characterized by at least a 500-fold
25 reduction in at least one CP1 and/or the CP2 activity as compared to the control level.
97. The ACC of any one or combination of items 89-95, wherein the control level of the at least one activity of the CP1 and/or the CP2, is the activity of the CP1 and/or the CP2 in the ACC following exposure of the ACC to the protease(s).
98. The ACC of any one or combination of items 89-97, wherein the control level of the
30 at least one CP1 and/or CP2, is the corresponding CP1 and/or the CP2 activity of a corresponding wildtype mature cytokine.

99. The ACC of any one or combination of items 89-98, wherein the ACC is characterized by generating a cleavage product following exposure to the protease(s), wherein the cleavage product comprises the at least one activity of the CP1 and/or the CP2.
- 5 100. The ACC of item 99, wherein the at least one activity of the CP1 and/or the CP2 is anti-proliferation activity.
101. The ACC of item 100, wherein the control level is an EC50 value, and wherein ratio of EC50 (cleavage product) to EC50 (control level) is less than about 10, or less than about 9, or less than about 8, or less than about 7, or less than about 6, or less than about 5, or less than about 4, or less than about 3, or less than about 2, or less than about 1.5, or less than about 1.0.
- 10 102. The ACC of any one or combination of items 1-101, wherein the first monomer construct is characterized in that the CP1 and the DD1 are linked by a linking region of no more than 18 amino acids such that the linking region of no more than 18 amino acids includes the CM3.
- 15 103. The ACC of any one or combination of items 1-102, wherein the second monomer construct is characterized in that the CP2 and the DD2 are linked by a linking region of no more than 18 amino acids such that the linking region of no more than 18 amino acids includes the CM2.
- 20 104. The ACC of any one or combination of items 1-103, wherein the PM1 comprises less than 50 amino acids.
105. The ACC of any one or combination of items 2-104, wherein the PM2 comprises less than 50 amino acids.
106. The ACC of item 105, wherein each of PM1 and PM2 is less than 19 amino acids.
- 25 107. The ACC of any one or combination of items 1-103, wherein the PM1 is not a latency associated peptide and the PM2 is not a latency associated peptide.
108. The ACC of any one or combination of items 1-103, wherein the PM1 is not a receptor for a cytokine and the PM2 is not a receptor for a cytokine and/or does not have an amino acid sequence that is at least 85% identical to a receptor for a cytokine.

109. The ACC of any one or combination of items 1-103, wherein the PM1 is not a fragment of a receptor for a cytokine and/or does not have an amino acid sequence that is at least 85% identical to a receptor for a cytokine.
110. The ACC of any one or combination of items 1-103 and 109, wherein the PM2 is
5 not a fragment of a receptor for a cytokine.
111. The ACC of any one or combination of items 1-103, wherein the PM1 is not an albumin and the PM2 is not an albumin.
112. A composition comprising an ACC of any one or a combination of items 1-111.
113. The composition of item 112, wherein the composition is a pharmaceutical
10 composition.
114. A container, vial, syringe, injector pen, or kit comprising at least one dose of the composition of item 112 or 113.
115. A method of treating a subject in need thereof comprising administering to the subject a therapeutically effective amount of the ACC of any one of items 1-96 or the
15 composition of items 112 or 113.
116. The method of item 115, wherein the subject has been identified or diagnosed as having a cancer.
117. The method of item 116, wherein the cancer is a lymphoma.
118. The method of item 117, wherein the lymphoma is Burkitt's lymphoma.
- 20 119. A nucleic acid encoding a polypeptide that comprises the CP1 and the CM1 of the ACC of any one or a combination of items 1-111.
120. The nucleic acid of item 109, wherein the polypeptide further comprises the DD1 of any one or combination of items 1-21 or items 28-111.
121. A nucleic acid encoding a polypeptide that comprises the CP2 and the CM2 of the
25 ACC of any one or combination of items 1-111.
122. The nucleic acid of item 111, wherein the polypeptide further comprises the DD2 of any one or combination of items 1-21 or items 28-111.
123. A vector comprising the nucleic acid of any one or a combination of items 119-122.
- 30 124. The vector of item 123, wherein the vector is an expression vector.

125. A cell comprising the nucleic acid of any one or a combination of items 119-122 or the vector of item 123 or 124.
126. A pair of nucleic acids that together encode a polypeptide that comprises the CP1 and the CM1 of the first monomer construct and a polypeptide that comprises the CP2 and the CM2 of the second monomer construct of any one or combination of items 1-111.
127. A pair of vectors that together comprise the pair of nucleic acids of item 126.
128. The pair of vectors of item 127, wherein the pair of vectors is a pair of expression vectors.
129. A cell comprising the pair of nucleic acids of item 126 or the pair of vectors of items 127 or 128.
130. A method of producing an ACC comprising:
- culturing a cell of item 125 or 129 in a liquid culture medium under conditions sufficient to produce the ACC; and
 - recovering the ACC from the cell or the liquid culture medium.
131. The method of item 130, further comprising: isolating the ACC recovered from the cell or the liquid culture medium.
132. The method of item 131, further comprising: formulating isolated ACC into a pharmaceutical composition.
133. An ACC produced by the method of item 130.
134. A composition comprising an ACC of item 133.
135. The composition of item 134, wherein the composition is a pharmaceutical composition.
136. A container, vial, syringe, injector pen, or kit comprising at least one dose of the composition of item 134 or 135.
137. The ACC of any one or combination of items 89-101, wherein the at least one of the CP1 and the CP2 activity is a binding affinity of the CP1 and/or the CP2 for its cognate receptor as determined using surface plasmon resonance.
138. An activatable cytokine construct (ACC) comprising a first monomer construct and a second monomer construct, wherein:

- (a) the first monomer construct is a polypeptide comprising a first peptide mask (PM1), a first mature cytokine protein (CP1), a first and a third cleavable moieties (CM1 and CM3), and a first dimerization domain (DD1);
- (b) the second monomer construct is a polypeptide comprising a second peptide mask (PM2), a second mature cytokine protein (CP2), a second and a fourth cleavable moieties (CM2 and CM4), and a second dimerization domain (DD2);
- 5 (c) the first monomer construct comprises, in an N- to C- terminal direction, the PM1, the CM3, the CP1, the CM1, and the DD1, further wherein:
- (i) the PM1 comprises no more than 20 amino acids and binds to the CP1,
- 10 (ii) the CM1 and the DD1 directly abut each other,
- (iii) the CP1 and the CM1 directly abut each other,
- (iv) the CM1 comprises no more than 12 amino acids,
- (v) the CM1 and the CM3 each functions as a substrate for a protease, and
- (vi) the CP1 is a mature interferon;
- 15 (d) further wherein:
- (i) the second monomer construct is the same as the first monomer construct,
- (ii) the DD1 and the DD2 are a pair of human IgG Fc domains;
- (iii) the DD1 and the DD2 bind to each other via at least one disulfide bond, thereby forming a homodimer of the first monomer construct and the second monomer
- 20 construct; and
- (e) the ACC is characterized by having a reduced level of interferon activity as compared to a corresponding wildtype interferon or a corresponding pegylated interferon.
139. The ACC of item 138, wherein the CP1 is a mature interferon-alpha and the PM1
- 25 comprises a sequence that is at least 85% identical to SEQ ID NO: 323.
140. The ACC of item 138 or 139, wherein the CM1 and the CM3 each independently functions as a substrate of urokinase (uPa) and/or a matrix metalloproteinase (MMP).
141. The ACC of item 140, wherein the CM1 and the CM3 each independently functions as a substrate of urokinase (uPa) and/or MMP-14.
- 30 142. The ACC of any one or combination of items 138-141, wherein the mature interferon is a mature human interferon alpha.

143. The ACC of any one or combination of items 138-142, wherein the mature interferon alpha is mature interferon alpha-2b.
144. The ACC of any one or combination of items 138-143, wherein the mature interferon alpha is a truncated form of a wildtype mature interferon alpha-2b.
- 5 145. The ACC of any one or combination of items 138-144, wherein the mature interferon comprises a sequence that is at least 95% identical to SEQ ID NO: 1.
146. The ACC of any one or combination of items 138-145, wherein the mature interferon comprises the sequence of SEQ ID NO: 1.
147. The ACC of any one or combination of items 138-146, wherein the CM1 and the
10 CM3 each comprises no more than 8 amino acids.
148. The ACC of any one or combination of items 138-147, wherein the CM1 and the CM3 are the same.
149. The ACC of any one or combination of items 138-148, wherein the CM1 and the CM3 each comprises a sequence that is at least 85% identical to SEQ ID NO: 41.
- 15 150. The ACC of any one or combination of items 138-148, wherein the CM1 and the CM3 each comprises a sequence selected from the group consisting of SEQ ID NO: 41, SEQ ID NO: 68, and SEQ ID NO: 100.
151. The ACC of any one or combination of items 138-150, wherein the DD1 and the DD2 are a pair of human IgG4 Fc domains.
- 20 152. The ACC of item 151, wherein the DD1 and the DD2 are a pair of human IgG4 Fc domains truncated at N-terminus to Cysteine 226 as numbered by EU numbering.
153. The ACC of item 151 or 152, wherein the human IgG4 Fc domains comprise a S228P mutation as numbered by EU numbering.
154. The ACC of any one or combination of items 138-153, wherein the DD1 and the
25 DD2 each comprises a sequence that is at least 95% identical to SEQ ID NO: 3.
155. The ACC of any one or combination of items 138-154, wherein the DD1 and the DD2 each comprises the sequence of SEQ ID NO: 3.
156. The ACC of any one or combination of items 138-155, wherein the first and
30 second monomer constructs are covalently bound to each other via at least two disulfide bonds.

157. The ACC of item 156, wherein the first and second monomer constructs are covalently bound to each other via at least three disulfide bonds.
158. The ACC of any one or combination of items 138-157, wherein:
the first monomer construct further comprises a first signal sequence at the N-terminus,
5 and the second monomer construct further comprises a second signal sequence at the N-terminus.
159. The ACC of item 158, wherein the first and second signal sequences each comprises a sequence that is at least 95% identical to SEQ ID NO: 244.
160. The ACC of item 159, wherein the first and second signal sequences each
10 comprises the sequence of SEQ ID NO: 244.
161. The ACC of any one or combination of items 158-160, wherein:
the first monomer construct further comprises a first spacer positioned between the first signal sequence and the PM1, and the second monomer construct further comprises a second spacer positioned between the second signal sequence and the PM2.
- 15 162. The ACC of item 161, wherein the first and second spacers each comprises a sequence that is at least 80% identical to SEQ ID NO: 256.
163. The ACC of item 162, wherein the first and second spacers each comprises a sequence of SEQ ID NO: 256.
164. The ACC of any one or combination of items 138-163, further comprising a linker
20 L1 between the PM1 and the CM3, and/or a linker L2 between the CM3 and the CP1, wherein each of L1 and L2 independently comprises a sequence that is at least 80% identical to SEQ ID NO: 27 (wherein n=1), a sequence that is at least 80% identical to SEQ ID NO: 324, or is absent.
165. The ACC of item 164, wherein the L1 comprises the sequence SEQ ID NO: 27
25 (wherein n=1) and L2 comprises the sequence of SEQ ID NO: 324.
166. The ACC of any one or combination of items 138-165 comprising a linking region comprising no more than 12 amino acids.
167. The ACC of item 166, wherein the linking region comprises 7 to 12 amino acids.
168. The ACC of item 166, wherein the linking region comprises 7 amino acids.

169. The ACC of any one or combination of items 138-168, wherein the ACC is characterized by at least a 2000-fold reduction in interferon alpha activity as compared to wildtype interferon alpha.
170. The ACC of item 169, wherein the ACC is characterized by at least a 4000-fold
5 reduction in interferon alpha activity as compared to wildtype interferon alpha.
171. The ACC of item 170, wherein the ACC is characterized by at least a 5000-fold reduction in interferon alpha activity as compared to wildtype interferon alpha.
172. The ACC of any one or combination of items 138-168, wherein the ACC is characterized by at least a 2000-fold reduction in interferon alpha activity as
10 compared to pegylated interferon alpha.
173. The ACC of any one or combination of items 138-172, wherein the reduction in interferon activity is determined by comparing the EC50 of the ACC with the EC50 of the wildtype interferon or the pegylated interferon in an anti-proliferation assay in lymphoma cells.
- 15 174. The ACC of any one or combination of items 138-173, wherein the reduction in interferon activity is determined by comparing the EC50 of the ACC with the EC50 of the wildtype interferon or the pegylated interferon in an assay of induction of secreted embryonic alkaline phosphatase production in interferon-responsive HEK293 cells.
- 20 175. The ACC of any of items 138-174, wherein the ACC is further characterized by generating a cleavage product following exposure to the protease(s) for which CM1 and CM3 function as a substrate, wherein the ratio of the interferon activity of the corresponding wildtype interferon to the cleavage product is less than about 2.
176. The ACC of item 175, wherein the EC50 of the cleavage product is approximately
25 the same as the EC50 of the corresponding wildtype interferon.
177. The ACC of item 138, wherein the first and second monomer constructs each comprises a sequence that is at least 95% identical to SEQ ID NO: 321 or wherein each of the first and second monomer constructs comprises the sequence of SEQ ID NO: 321 or is identical to SEQ ID NO: 321, wherein the ACC is characterized by at
30 least a 1000-fold reduction in interferon activity as compared to wildtype interferon alpha-2b, and wherein the ACC is further characterized by generating a cleavage

product following exposure to uPA, wherein the cleavage product has approximately the same interferon activity as wildtype interferon alpha-2b, wherein interferon activity is measured in an anti-proliferation assay in lymphoma cells or in an assay of induction of secreted embryonic alkaline phosphatase production in interferon-responsive HEK293 cells.

178. An activatable cytokine construct (ACC) comprising a first monomer construct and a second monomer construct, wherein:

(a) the first monomer construct comprises a first peptide mask (PM1), a first mature cytokine protein (CP1), a first and a third cleavable moieties (CM1 and CM3), and a first dimerization domain (DD1);

(b) the second monomer construct is a polypeptide comprising a second peptide mask (PM2), a second mature cytokine protein (CP2), a second and a fourth cleavable moieties (CM2 and CM4), and a second dimerization domain (DD2);

(c) the first monomer construct is a polypeptide comprising, in an N- to C- terminal direction, the PM1, the CM3, the CP1, the CM1, and the DD1, further wherein:

(i) the PM1 comprises a sequence that is at least 85% identical to SEQ ID NO: 323,

(ii) the CM1 and the DD1 directly abut each other,

(iii) the CM1 comprises a sequence that is at least 85% identical to SEQ ID NO: 41, and

(iv) the CP1 comprises a sequence that is at least 85% identical to SEQ ID NO: 1;

(d) further wherein:

(i) the second monomer construct is the same as the first monomer construct,

(ii) the DD1 and DD2 are a pair of human IgG4 Fc domains;

(e) the DD1 and the DD2 covalently bind to each other via at least one disulfide bond, thereby forming a homodimer of the first monomer construct and the second monomer construct; and

(f) the ACC is characterized by having a reduced level of interferon alpha activity as compared to the interferon alpha activity of PEGylated interferon alpha-2b.

179. The ACC of item 178, wherein the PM2 comprises a sequence that is at least 85%, 90%, or 95% identical to one of SEQ ID NOs: 323, 331, or 332.

180. The ACC of item 138, wherein the first and second monomer constructs each comprises a sequence that is at least 95% identical to SEQ ID NO: 321 or wherein each of the first and second monomer constructs comprises the sequence of SEQ ID NO: 321 or is identical to SEQ ID NO: 321, wherein the ACC exhibits lower toxicity
5 in vivo compared to either wildtype interferon alpha-2b or PEGylated interferon alpha-2b.
181. A composition comprising the ACC of any one or combination of items 138-180.
182. The composition of item 181, where the composition is a pharmaceutical composition.
- 10 183. A container, vial, syringe, injector pen, or kit comprising at least one dose of the composition of item 181 or 182.
184. A method of treating a subject in need thereof comprising administering to the subject a therapeutically effective amount of the ACC of any one or combination of items 138-179 or the composition of item 181 or 182.
- 15 185. The method of item 184, wherein the subject has been identified or diagnosed as having a cancer.
186. A nucleic acid encoding a polypeptide that comprises the first monomer of the ACC of any one or combination of items 138-179.
187. A vector comprising the nucleic acid of item 186.
- 20 188. The vector of item 187, wherein the vector is an expression vector.
189. A mammalian cell comprising the nucleic acid of item 186 or the vector of item 187 or 188.
190. The mammalian cell of item 189, wherein the mammalian cell is an HEK293 cell or a CHO cell.
- 25 191. A method of manufacturing an ACC, the method comprising:
expressing the ACC in the mammalian cell of item 189 or 190; and
purifying the expressed ACC.
192. The ACC of any one or combination of items 138-180, wherein the CM1 and the CM3 each functions as a substrate for a protease that is over-expressed in a tumor
30 tissue.

193. The ACC of any one or combination of any preceding items, wherein PM1, PM2 or PM1 and PM2 comprises SEQ ID NO: 323.
194. The ACC of any one or combination of any preceding items, wherein PM1, PM2 or PM1 and PM2 comprises SEQ ID NO: 331.
- 5 195. The ACC of any one or combination of any preceding items, wherein PM1, PM2 or PM1 and PM2 comprises SEQ ID NO: 332.
196. The ACC of any one or combination of any preceding items, wherein the CP1 and the DD1 are linked by a linking region of no more than 15 amino acids such that the linking region of no more than 15 amino acids includes the CM3.
- 10 197. The ACC of any one or combination of any preceding items, wherein the second monomer construct is characterized in that the CP2 and the DD2 are linked by a linking region of no more than 15 amino acids such that the linking region of no more than 15 amino acids includes the CM2.
198. The ACC of any one or combination of any preceding items, wherein the PM1, the PM2, or the PM1 and the PM2 are not a latency associated peptide.
- 15 199. The ACC of any one or combination of any preceding items, wherein the PM1, the PM2, or the PM1 and the PM2 are not a cytokine.
200. The ACC of any one or combination of any preceding items, wherein the PM1, the PM2, or the PM1 and the PM2 are not a receptor for a cytokine and/or do not have an amino acid sequence that is at least 85% identical to a receptor for a cytokine.
- 20 201. The ACC of any one or combination of any preceding items, wherein the PM1, the PM2, or the PM1 and the PM2 are not a fragment of a receptor for a cytokine.
202. The ACC of any one or combination of any preceding items, wherein the PM1, the PM2, or the PM1 and the PM2 are not an albumin.
- 25 203. The ACC of any one or combination of any preceding items, wherein the PM1, the PM2, or the PM1 and the PM2 are less than 50, 45, 40, 35, 30, 25, 20, 15, or 10 amino acids.
204. The ACC of any one or combination of the preceding items, wherein the ACC is characterized in that it has at least one of the following characteristics:

- (i) a structural arrangement in an N- to C-terminal direction comprising:
PM1-CM3-CP1-CM1-DD1 and CP2-CM2-DD2, wherein DD1 and DD2
are dimerized;
 - (ii) a structural arrangement in an N- to C-terminal direction comprising:
5 PM1-CM3-CP1-CM1-DD1 and PM2-CM4-CP2-CM2-DD2, wherein DD1
and DD2 are dimerized;
 - (iii) wherein each of PM1 and PM2 is less than 40 amino acids;
 - (iv) wherein each of PM1 and PM2 is not a receptor for the CP1 and the CP2;
 - (v) wherein each of PM1 and PM2 does not have an amino acid sequence that
10 is at least 85% identical to a receptor for a cytokine;
 - (vi) wherein each of PM1 and PM2 is not a fragment of receptor for the CP1
and the CP2;
 - (vii) wherein each of PM1 and PM2 is between 13 and 49 amino acids;
 - (viii) wherein the first monomer construct is characterized in that the CP1 and
15 the DD1 are linked by a linking region of no more than 18 amino acids
such that the linking region of no more than 18 amino acids includes the
CM3;
 - (ix) when the ACC has an orientation of N-PM-CM1-CP-CM2-DD-C, then the
entire span of amino acids from the N-terminus of the ACC to the N-
20 terminal amino acid of the cytokine is 17 to 71 amino acids in length, and
when the ACC has an orientation of N-DD-CM1-CP-CM2-PM-C, then the
entire span of amino acids from the C-terminus of the ACC to the C-
terminal amino acid of the cytokine is 17 to 71 amino acids in length;
and/or
 - 25 (x) wherein the second monomer construct is characterized in that the CP2
and the DD2 are linked by a linking region of no more than 18 amino
acids such that the linking region of no more than 18 amino acids includes
the CM2.
205. The ACC of any one or combination of any preceding items, wherein the first
30 monomer construct has only one peptide mask.

206. The ACC of any one or combination of any preceding items, wherein the second monomer construct has only one peptide mask.

207. The ACC of any one or combination of any preceding items, wherein the first monomer construct has only one peptide mask and the second monomer construct has only one peptide mask.

208. A method of attenuating IP-10 release in a subject in need thereof comprising administering the ACC of any one or combination of any preceding items.

EXAMPLES

The invention is further described in the following examples, which do not limit the scope of the invention described in the claims.

Example 1: *In vitro* characterization of example cytokine constructs

An activatable cytokine construct ProC440 was prepared by recombinant methods. The 1st and 2nd monomer constructs of the ProC440 were identical, with each being a polypeptide having the amino acid sequence of SEQ ID NO: 316 and a signal sequence at its N-terminus. Each of the 1st and 2nd monomer constructs comprises, from N-terminus to C-terminus, a signal sequence (e.g., SEQ ID NO: 244), a mature cytokine protein that corresponds to human interferon alpha-2b (SEQ ID NO: 1), a cleavable moiety having the amino acid sequence of SEQ ID NO: 100, and a dimerization domain corresponding to human IgG4 Fc, truncated at Cys226 (according to EU numbering) and including an S228P mutation (SEQ ID NO: 3).

The polypeptide was prepared by transforming a host cell with a polynucleotide having the sequence of SEQ ID NO: 316, followed by cultivation of the resulting recombinant host cells. Dimerization of the resulting expressed polypeptides yielded the cytokine construct ProC440.

The activity of ProC440 was tested in vitro using IFN-responsive HEK293 cells and Daudi cells. See Figs. 7A and 7B, respectively.

IFN-responsive HEK293 cells were generated by stable transfection with the human STAT2 and IRF9 genes to obtain a fully active type I IFN signaling pathway. The cells also feature an inducible SEAP (secreted embryonic alkaline phosphatase) reporter gene under the control of the IFN α/β inducible ISG54 promoter. To maintain transgene expression, cells were cultured in DMEM GlutaMax media supplemented with

10% FBS, Pen/Strep, 30 µg/mL of blasticidin, 100 µg/ml of zeocin and 100 µg/mL of normocin. The addition of type I IFN to these cells activates the JAK/STAT/ISGF3 pathway and subsequently induces the production of SEAP which can be readily assessed in the supernatant using Quanti-Blue solution, a colorimetric detection for alkaline phosphatase activity.

The Daudi cell is a cell line of human B-cell lymphoblastic origin. Daudi cells were prepared at a concentration of 2×10^5 cells/mL in RPMI-1640 media supplemented with 10% FBS and 50 µL aliquots were pipetted into wells of a white flat-bottom 96-well plate (10K/well). The tested ProC440 or controls were diluted in RPMI 1640 media supplemented with 10% FBS. Duplicate five-fold serial dilutions were generated from which 50 µL was added to the each well. After 3 days of incubation at 37 °C, a viability kit was used to measure the levels of intracellular ATP as an indirect estimate of the number of viable cells remaining. 100 µL of cell-titer go was directly added to the plates which were then placed on an orbital shaker for 10 minutes. Following this incubation, the luminescent signal was directly measured using an Envision plate reader. Dose-response curves were generated and EC50 values were obtained by sigmoidal fit non-linear regression using Graph Pad Prism software.

In both of the assays using HEK293 cells and Daudi cells, the activity of ProC440 was reduced at least 1,000X as compared to Stem Cell IFNα-2b (human recombinant IFN-alpha2b, available from StemCell Technologies, Catalog #78077.1) (Figs. 7A and 7B). This indicates that the fusion of a cleavable dimerization domain corresponding to human IgG Fc provided steric masking to IFNα-2b in the ProC440 construct. Protease activation with uPa restored activity to a level comparable to the recombinant cytokine. EC50 values for ProC440, ProC440 + uPA, and Stem Cell IFNα-2b were computed from the IFNα/β assay results and are provided below in Table 3.

Table 3. EC50: IFNα/β Reporter Assay

	ProC440	ProC440 + uPA	Stem Cell IFNα-2b
EC50	7643	4.333	10.88

EC50 values for ProC440, ProC440 + uPA, and Stem Cell IFNα-2b were computed from the Daudi apoptosis assay results and are provided below in Table 4.

Table 4. EC50: Daudi Apoptosis Assay

	ProC440	ProC440 + uPA	Stem Cell IFN α -2b
EC50	264.2	0.1842	0.3530

Cleavage with uPa at the expected site in the cleavable moiety was confirmed by electrophoresis and Mass spectrometry analysis (Figs. 6A and 6B). The results suggest that the uPa protease was effective at cleaving the cleavable moieties in the ProC440 activatable cytokine construct. In addition to sensitivity to uPa activation, ProC440 was cleaved by MMP14 (Figs. 6A to 6C). Fig. 6A depicts the gel electrophoresis results; the left column shows ProC440 that has not been exposed to protease, the middle column shows ProC440 exposed to protease uPA, and the far right column shows ProC440 exposed to MMP14. Analysis by Mass spectrometry identified an MMP14 cleavage site at the C-terminal extremity of IFN α -2b, near the cleavable moiety (Fig. 6B). Protease activation with MMP14 also restored activity to a level comparable to the recombinant cytokine (Fig. 6C). The data indicate that ProC440 recovered full activity after cleavage of intrinsic and engineered cleavable moieties by proteases such as uPA or MMP14.

Activatable cytokine construct ProC732 was prepared by recombinant methods. The 1st and 2nd monomer constructs of this ACC were identical, with each being a polypeptide having the amino acid sequence shown in Fig. 8 (SEQ ID NO: 321 with an exemplary optional signal sequence). Each of the 1st and 2nd monomer constructs comprises, from N-terminus to C-terminus, a signal sequence, a spacer (QSGQ) sequence, an IFN α -2b masking peptide (SEQ ID NO: 323), a linker (SEQ ID NO: 324), a cleavable moiety having the amino acid sequence of SEQ ID NO: 41 (LSGRSDNI), a linker (SEQ ID NO: 227, wherein n=1), a mature cytokine protein that corresponds to human interferon alpha-2b (SEQ ID NO: 1), a cleavable moiety having the amino acid sequence of SEQ ID NO: 41, and a DD corresponding to human IgG4 S228P Fc, truncated to Cys226 (according to EU numbering) (SEQ ID NO: 3).

Another activatable cytokine construct, ProC733, was prepared by recombinant methods. The 1st and 2nd monomer constructs of this ACC were identical, with each being a polypeptide having the amino acid sequence shown in Fig. 9 (SEQ ID NO: 322

with an exemplary optional signal sequence). Each of the 1st and 2nd monomer constructs comprises, from N-terminus to C-terminus, a signal sequence, a spacer (e.g., QSGQ) sequence, an IFN α -2b masking peptide (SEQ ID NO: 323), a linker (SEQ ID NO: 324), a cleavable moiety having the amino acid sequence of SEQ ID NO: 41, a linker (SEQ ID NO: 227, wherein n=1), a mature cytokine protein that corresponds to human interferon alpha-2b (SEQ ID NO: 1), and a DD corresponding to human IgG4 S228P Fc, including the full hinge sequence (SEQ ID NO: 4). Because the ProC733 construct lacks a cleavable moiety between the cytokine sequence and the DD, it is only partially activatable, as discussed below.

The masked cytokine constructs ProC732 and ProC733 were prepared by transforming a host cell with polynucleotides encoding the sequence of SEQ ID NOs: 321 and 322, respectively, followed by cultivation of the resulting recombinant host cells. Dimerization of the resulting expressed polypeptides yielded the cytokine constructs ProC732 and ProC733, respectively.

The activity of ProC732, ProC733 and ProC440 was tested *in vitro* using IFN-responsive HEK293 cells as previously described. The activity of ProC732 and ProC733 was further reduced as compared to ProC440 (Figs. 10A-10C). This indicates that the addition of a peptide mask provided additional masking strength even though the cytokine activity was already significantly reduced in ProC440 by steric masking through the dimerization domains. Surprisingly, it appears that the addition of a masking peptide (PM) does not interfere with steric masking by the DD, nor does the DD appear to interfere with masking by the PM. Protease activation with uPa restored the activity of ProC732 to a level comparable to the level of ProC440 after protease activation with uPa. This indicates that ProC732, upon protease activation, recovered the full strength of activity of an unmasked IFN α -2b.

ProC733 contains an affinity peptide mask attached to IFN α -2b via a cleavable moiety, with the C-terminus of IFN α -2b fused directly to human IgG Fc (without a cleavable moiety interposed between the cytokine and the Fc region). Protease activation with uPa restored the activity of ProC733 to a level comparable to the level of unactivated ProC440. This further indicates that in addition to the steric masking provided by the cleavable human IgG Fc, or constraint by IFN α -2b dimerization, a

cleavable affinity peptide mask provides additional masking strength to IFN α -2b. EC50 values for ProC440, ProC440 + uPa, ProC732, ProC732 + uPa, ProC733, and ProC733 + uPa were computed from the IFN α / β assay results and are provided below in Table 5.

Table 5. EC50: IFN α / β Reporter Assay

	ProC440	ProC440 + uPA	ProC732	ProC732 + uPA	ProC733	ProC733 + uPA
EC50	0.6344	0.0004	40.69	0.0005	21.83	0.2977

5 The masking efficiencies of ACCs in a HEK reporter assay (as measured by comparing the EC50 of the uncleaved ACC to the EC50 of the cleaved ACC) were as follows:

ProC440: 1358X

ProC732: 7380X

10 ProC733: 60X

Thus, high levels of masking efficiency (e.g., > 5,000x) can be achieved in ACCs that include both a peptide mask and steric masking through dimerization domains as shown, for example, in Fig. 10C.

As shown in Figs. 42A-42D, each of the peptide masks (Fig. 42A (no peptide mask) vs. Fig. 42B (peptide masked)) and the Fc masks (Fig. 42C (no Fc mask) vs. 42D (Fc masked)) affect binding of the ACC to the receptor. In view of the data, synergistic activity has been obtained through the use of the dual masking structure of the ACCs of the present disclosure. The activity of recombinant IFN α 2b, monomeric IFN α 2b/Fc, activated homodimeric IFN α 2b/Fc, and homodimeric IFN α 2b/Fc was tested *in vitro* using IFN-responsive HEK293 cells as previously described. Recombinant IFN α 2b, monomeric IFN α 2b/Fc, activated homodimeric IFN α 2b/Fc, and homodimeric IFN α 2b/Fc were prepared as described above. The activity of homodimeric IFN α 2b/Fc was substantially reduced compared to recombinant IFN α 2b, but was rescued by protease activation to a level commensurate with recombinant IFN α 2b (Fig. 31). Fig. 31 also shows that monomeric IFN α 2b/Fc exhibited activity at an approximate midpoint between the activity observed for activated and unactivated homodimeric IFN α 2b/Fc.

Additionally, ProC440 shows substantially reduced activity compared to uPA treated ProC440 (Fig. 35A). The same molecule, but with a NSUB substrate has restored activity in response to MMP indicating the presence of a cryptic cleavage site (Fig. 35A). The activity of both ProC732 and ProC1299 (deletion of L161) was rescued by uPA (Fig. 35B). Deletion of L161 (in the MMP14 cleavage site) prevents activation of ProC1301 (NSUB substrate) even in the presence of MMP14 or uPA (Fig. 35C).

Example 2: *In vivo* tolerability of cytokine constructs

Human IFN α -2b cross-reacts with hamster IFN α receptor and has been previously shown to be active in hamster (Altrock et al, Journal of Interferon Research, 1986). To assess the tolerability of IFN α -2b-containing cytokine constructs, Syrian Gold Hamsters were dosed with a starting dose of 0.4 mg/kg. Animals received one dose of test article and kept on study up to 7 days post dose, unless non-tolerated toxicities were identified. The starting dose (0.4mpk) represents an equivalent dose of IFN α -con (recombinant interferon alpha, a non-naturally occurring type-I interferon manufactured by Amgen under the name Infergen®) expected to induce body weight lost, decreased food consumption and bone marrow suppression in a hamster (125gr). If the starting dose was tolerated, animals were moved up to a “medium dose” of 2 mg/kg and received three doses of test article unless not tolerated. If tolerated, animals were moved up to a “high dose” of 10 mg/kg and received three doses of test article unless not tolerated. If tolerated, animals were moved up to a “higher dose” of 15 mg/kg. At each stage, if the test dose was not tolerated, the animal was moved down to the next lower dose. If the starting dose was not tolerated, the animal was moved down to a “lower dose” of 0.08 mg/kg. Animals were also dosed with the unmasked IFN α -2b Fc fusion constructs ProC286. As a negative control, animals were dosed with a human IgG4.

ProC286 (ChIgG4 5AA 1204DNI α L IFN α 2b) was also prepared by recombinant methods. The 1st and 2nd monomer constructs were identical, with each being a polypeptide having the amino acid sequence of SEQ ID NO: 326 and a signal sequence at its N-terminus. Each of the 1st and 2nd monomer constructs comprises, from N-terminus to C-terminus, a signal sequence, a DD corresponding to human IgG4 S228P Fc including the ESKYGPP (SEQ ID NO: 317) hinge sequence (SEQ ID NO:4), a linker (SEQ ID NO:327), a cleavable moiety having the amino acid sequence of SEQ ID

NO:100, a linker (SEQ ID NO: 228), and a mature cytokine protein that corresponds to human interferon alpha-2b (SEQ ID NO:1).

ProC291 (NhIgG4 5AA 1204DNI_{DL} IFN α 2b) was also prepared by recombinant methods. The 1st and 2nd monomer constructs were identical, with each being a
5 polypeptide having the amino acid sequence of SEQ ID NO: 496 and a signal sequence at its N-terminus. Each of the 1st and 2nd monomer constructs comprises, from N-terminus to C-terminus, a signal sequence, a mature cytokine protein that corresponds to human interferon alpha-2b (SEQ ID NO: 1), a linker (SEQ ID NO: 492), a CM (SEQ ID NO: 100), a linker (GGGS), and a human IgG4 Fc region including the ESKYGPP (SEQ ID
10 NO: 317) hinge sequence (SEQ ID NO: 4).

The activity of ProC286 and ProC291 were compared to the activity of Sylatron® (PEG-IFN-alpha2b) in the Daudi apoptosis assay (Figs. 11A-11B). In this assay, ProC286 and Sylatron® show similar levels of activity as shown in Fig. 11A. This indicates that ProC286 has similar activity to commercially-available pegylated IFN-
15 alpha2b, and could be used as surrogate Sylatron control to evaluate the tolerability of IFN α -2b in the hamster study. ProC291 showed reduced activity compared to ProC286 and Sylatron®, indicating that the structural orientation of the IFN N-terminal to the Fc was important for reduction in activity. That is, when the DD is a pair of Fc domains, positioning the cytokine N-terminal to the DD (as in ProC291) may provide greater
20 reduction of cytokine activity than when the cytokine is positioned C-terminal to the DD (as in ProC286).

Animal were dosed on day 1 with the indicated doses on Figs. 13A-13C. Animals were kept on study for one week, unless a non-tolerated dose (DLT) was reached. Clinical observations, body weights & temperature were measured prior to dosing, and at
25 6h, 24h, 72h, 7d post-dose for each animal. Blood samples for Hematology and Chemistry analysis were collected at 72h, 7d post-dose for each animal. Hematology and Chemistry analysis were performed right after sampling. For the Hematology analysis, blood smear, differential white blood cell count, hematocrit, hemoglobin, mean corpuscular hemoglobin, mean corpuscular volume, platelet count, red blood cell
30 (erythrocyte) count, red blood cell distribution width, reticulocyte count and white blood cell (leukocyte) count were evaluated. The clinical chemistry panel included

measurement of alanine aminotransferase, albumin, albumin/globulin ratio, alkaline phosphatase, aspartate aminotransferase, calcium, chloride, cholesterol, creatine kinase, creatine, gamma glutamyltransferase, globulin, glucose, inorganic phosphorus, potassium, sodium, total bilirubin, total protein, triglycerides, urea, nitrogen, and C-reactive protein.

5 The evidence of toxicities in the tolerability study are summarized in Figs. 13A-13C, 14, and 15.

Overall, animals dosed with the ProC286 constructs showed on average 5 % body weight loss when dosed at 2mpk (i.e., 2 mg/kg), and 15% body weight loss when dosed at 10mpk and 15mpk (Figs. 13A-13C). One animal dosed with ProC286 at 15mpk showed
10 20% body weight loss at 7 days post-dose (end of study). This is considered a non-tolerated dose. In contrast, animals dosed with ProC440 and ProC732 at 2mpk and 10mpk did not show body weight loss (Figs. 13A-13B). Animals dosed with ProC440 at 15mpk showed on average 5% body weight loss (Figs. 13A-13C). Animals dosed with ProC732 at 15mpk showed no body weight loss (Fig. 13C). This indicates that the
15 masking of IFN α -2b to its receptor in the context of ProC440 limits IFN α -2b mediated bodyweight loss. Animals dosed with unmasked IFN α 2b/Fc at 15mpk showed elevated ALP (and increased ALP detected at 0.4 mpk) compared to animals dosed with masked and dual masked IFN α 2b/Fc. The results indicate that the masked IFN α 2b/Fc is well tolerated up to 15 mg/kg in the hamster toxicity model.

20 Without wishing to be bound by theory, it is believed that positioning the interferon N-terminal of the DD and using a relatively short LR inhibits cytokine activity in the context of ProC440, reducing the toxicity of the interferon in comparison to PEGylated IFN α -2b (Sylatron®) or ProC286. Unexpectedly, the addition of a peptide affinity mask at the N-terminus of the cytokine in the context of ProC732 fully abrogates
25 IFN α -2b mediated bodyweight lost at a dose of at least 15mpk. The use of both a cleavable peptide mask and a cleavable dimerization domain thus lowers toxicity and allows dosing at higher levels, potentially resulting in an improved therapeutic window for this cytokine therapeutic.

In terms of clinical chemistry, animals dosed with ProC286 showed significant
30 elevation of Alkaline Phosphatase (ALP) at all doses (0.4mpk, 2mpk, 10mpk and 15mpk), 7 days post-dose (end of study) (Fig. 14). No significant increase of ALP was

measured when animals were dosed with 10mpk or 15mpk of ProC440 or ProC732 (Fig. 14). Elevation of ALT is a marker of liver toxicity. IFN α -2b has been shown to induce liver toxicities. This indicates that the masking of IFN α -2b from binding to its receptor in the context of ProC440 and ProC732 limit IFN α -2b mediated liver toxicities.

5 In terms of hematology, 3 days post-dose and 7 days post-dose (end of study), animals dosed with ProC286 at 2mpk, 10mpk and 15mpk showed significant reduction level of Reticulocyte count, Neutrophyle count and White Blood Cells (WBC) count (Fig. 15). These reductions are reminiscent of IFN α -2b mediated bone-marrow toxicities. Three days post-dose, animals dosed with ProC440 and ProC732 showed reduction level
10 of Reticulocyte count, Neutrophyle count and White Blood Cells (WBC) count (Fig. 15). Overall, the reduction level of hematopoietic cells observed in animals dosed with ProC440 and ProC732 is not as significant as the reduction levels observed in animals dosed with ProC286. At 7 days post-dose (end of study), in animals dosed with ProC732 and ProC440, the overall level of Reticulocyte count, Neutrophyle count and White
15 Blood Cells (WBC) count is back to normal levels, or to a similar level that what observed in animals dosed with the negative control IgG4 (Fig. 15). In animals dosed with ProC286, the level of Reticulocyte count, Neutrophyle count and White Blood Cells (WBC) count remains low. This indicates that the masking of IFN α -2b to its receptor in the context of ProC440 and ProC732 limit IFN α -2b mediated bone marrow toxicities.

20 **Example 3. *In vitro* anti-proliferative effect of cytokine constructs with linkers of various lengths on cancer cells**

The anti-proliferative effects of IFN α -2b-hIgG4 Fc fusion constructs with varying linker lengths or without a linker between the IFN α -2b and the hIgG4 Fc were tested *in vitro* using Daudi cells. The test was performed using the Daudi cell assay described in
25 Example 1.

The fusion proteins tested in this experiment include, in an N- to C-terminal direction, the mature IFN α -2b cytokine sequence, an optional linker and/or cleavable moiety, and the Fc domain of human IgG4 of SEQ ID NO: 4 (including the full hinge region such that the N-terminus of the Fc sequence begins with the amino acid sequence
30 ESKYGPPCPPC...) (SEQ ID NO: 500) (first eleven amino acids of SEQ ID NO:4). The ESKYGPP (SEQ ID NO: 317; first seven amino acids of SEQ ID NO:4) sequence

contributes 7 amino acids to the “linking region” of these constructs. The first construct (Linking Region = 7) construct has no linker or cleavable moiety; its sequence in the N- to C-terminal direction consists of SEQ ID NO: 1 fused to SEQ ID NO: 4. The second construct (Linking Region = 12) construct has a 5 amino acid linker SGGGG (SEQ ID NO: 492) and no CM; its sequence in the N- to C-terminal direction consists of SEQ ID NO: 1 fused to SEQ ID NO: 492 fused to SEQ ID NO: 4. The third construct (Linking Region = 18) includes a 7 amino acid CM (SGRSDNI) (SEQ ID NO: 100) and a 4 amino acid linker GGGS (SEQ ID NO: 228); its sequence in the N- to C-terminal direction consists of SEQ ID NO: 1 fused to SEQ ID NO: 100 fused to SEQ ID NO: 2 fused to SEQ ID NO: 4. The fourth construct (Linking Region = 23) includes a 5 amino acid linker, a 7 amino acid CM, and a 4 amino acid linker; its sequence in the N- to C-terminal direction consists of SEQ ID NO: 1 fused to SEQ ID NO: 492 fused to SEQ ID NO: 100 fused to SEQ ID NO: 2 fused to SEQ ID NO: 4. The fifth construct (Linking Region = 24) includes a 13 amino acid CM (ISSGLLSGRSDNI) (SEQ ID NO: 68) and a 4 amino acid linker; its sequence in the N- to C-terminal direction consists of SEQ ID NO: 1 fused to SEQ ID NO: 68 fused to SEQ ID NO: 2 fused to SEQ ID NO: 4.

Fig. 16 shows the activities of the above ACCs in Daudi cells. The ACCs tested in this example did not have a peptide affinity mask attached thereto. The data indicates that the length of the flexible linkers and the length of the Linking Region (LR) between the cytokine and the Fc domain had an impact on the activity of the (uncleaved) ACCs. Constructs with zero linkers, or short linkers, and a correspondingly short LR display reduced cytokine activity, whereas constructs with longer linkers and thus a longer LR have a higher level of cytokine activity.

Example 4. *In vitro* characterization of additional activatable cytokine constructs

Additional activatable cytokine constructs without a peptide mask were also prepared by recombinant methods. The 1st and 2nd monomer constructs of these ACCs were identical. Each of the 1st and 2nd monomer constructs comprises, from N-terminus to C-terminus, a signal sequence, a mature cytokine protein that corresponds to human interferon alpha-2b (SEQ ID NO: 1), a cleavable moiety (CM) having the amino acid sequence of SEQ ID NO: 100 (SGRSDNI), and a dimerization domain corresponding to human IgG4 S228P Fc (comprising SEQ ID NO: 3). In addition, these ACCs include or

not a linker having the amino acid sequence SGGGG (SEQ ID NO: 492) between the CP and the CM. These ACCs include or not a linker having the amino acid sequence GGGS (SEQ ID NO: 228) between the CM and DD. These ACCs also contain or not portions of the hinge of the DD that are N-terminal to Cysteine 226 (by EU numbering). These

5 additional activable cytokines constructs are described in Table 6.

Table 6: Activable cytokines having different lengths of the linking region

Name	Alternative Name	Linker between CP and CM	Linker between CM and DD	Fc Hinge N-terminal residues	Linking Region Length
ProC288	IFNa2b 1204DNI 0AA Fc	SGGGG (SEQ ID NO: 492)	absent	absent	12
ProC289	IFNa2b 1204DNI 3AA Fc	SGGGG (SEQ ID NO: 492)	absent	GPP	15
ProC290	IFNa2b 1204DNI 7AA Fc	SGGGG (SEQ ID NO: 492)	absent	ESKYGPP (SEQ ID NO: 317) (first seven amino acids of SEQ ID NO:4)	19
ProC291	IFNa2b 1204DNI 11AA Fc	SGGGG (SEQ ID NO: 492)	GGGS (SEQ ID NO: 2)	ESKYGPP (SEQ ID NO: 317) (first seven amino acids of SEQ ID NO:4)	23
ProC440	N IFNa2b 0 1204DNIIdL 0AA Fc	absent	absent	absent	7
ProC441	N IFNa2b 0 1204DNIIdL 3AA Fc	absent	absent	GPP	10
ProC442	N IFNa2b 0 1204DNIIdL 7AA Fc	absent	absent	ESKYGPP (SEQ ID NO: 317) (first seven amino acids of SEQ ID NO:4)	14
ProC443	N IFNa2b 0 1204DNIIdL 11AA Fc	absent	GGGS (SEQ ID NO: 2)	ESKYGPP (SEQ ID NO: 317)	18

				(first seven amino acids of SEQ ID NO:4)	
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The activity of ProC440, an ACC with no flexible linkers and an IgG4 Fc region truncated to Cys226 (i.e., comprising a linking region of 7 amino acids), and the activity of additional ACCs containing various flexible linkers and Fc region sequences (i.e., comprising linking regions having more than 7 amino acids) was tested *in vitro* using IFN-responsive HEK293 cells and Daudi cells as previously described. In both assays, the activity (e.g., anti-proliferative effects) of ProC440 was reduced as compared to all other ACCs with longer linking regions, which contain various additional sequences between the cytokine and the first amino acid that binds the DD to the corresponding second monomer (i.e., Cys226 of IgG4 by EU numbering). EC50 values for the ACCs were computed from the IFN α / β assay results and are provided below in Table 7.

Table 7. EC50: IFN α / β Reporter Assay

	Pro C288	Pro C289	Pro C290	Pro C291	Pro C440	Pro C441	Pro C442	Pro C443
EC50	34.34	17.93	10.33	8.743	41.37	6.28	6.637	1.687

EC50 values for the ACCs were computed from the Daudi apoptosis assay results and are provided below in Table 8.

Table 8. EC50: Daudi Apoptosis Assay

	Pro C288	Pro C289	Pro C290	Pro C291	Pro C440	Pro C441	Pro C442	Pro C443
EC50	112.8	64.55	23.04	13.39	2078	1053	642.9	478

The data in Tables 7-8 also shows that the activity of the (uncleaved) ACCs could be modulated by varying the length of the Linking Region.

The ACCs tested in this Example 4 do not comprise a peptide mask. Based on the experimental results reported herein comparing ProC440 with ProC732, the activity of the uncleaved ACCs may be further decreased by adding a cleavable moiety and peptide mask to the N-terminus of the cytokine construct. Likewise, based on the data herein

comparing ProC440 and ProC732, ACCs further comprising a CM and a PM at the N-terminus may have increased masking efficiency compared to ACCs that do not comprise a PM.

Example 5. Universal cytokine constructs

5 A universal activatable cytokine construct was prepared by recombinant methods described herein. The universal ACC has a universal interferon sequence (ProC859) having activity on both human and mouse cells as shown in Fig. 17. The universal ACC is a dimer. The 1st and 2nd monomer constructs of this ACC were identical, with each being a polypeptide having the amino acid sequence of SEQ ID NO: 480 with a signal
10 sequence at its N-terminus. Each of the 1st and 2nd monomer constructs comprises, from N-terminus to C-terminus, a signal sequence, a mature cytokine protein that corresponds to a universal interferon molecule that is a hybrid of IFN alpha 1 and IFN alpha 2a (SEQ ID NO: 481), a cleavable moiety having the amino acid sequence of SEQ ID NO: 100, and a dimerization domain corresponding to human IgG Fc (SEQ ID NO: 3).

15 The activity of the universal cytokine construct was tested *in vitro* using IFN-responsive HEK293 cells and B16 mouse melanoma cells. The activity of ProC859 was reduced at least 150X as compared to mouse IFNa4. Protease activation with uPa restored activity to a level that is comparable to mouse IFNa4 as shown in Fig. 17 (bottom panel). EC50 values for ACC ProC859, ACC ProC859 + uPA, and mouse
20 IFNa4 were computed from the assay results and are provided in Fig. 17 (bottom panel).

EC50: B16 IFN α / β Reporter Assay

	ProC859 (ACC)	ProC859 (ACC) + uPA	IFNa4
EC50	293.7	1.951	1.966

An ACC with universal IFN and a peptide mask according to the present disclosure may be prepared by recombinant methods described herein. The peptide
25 masks are coupled to the universal interferon to further reduce the cytokine activity of the ACC compared to ProC859. The 1st and 2nd monomer constructs of this ACC are identical, with each being a polypeptide having the amino acid sequence. Each of the 1st

and 2nd monomer constructs comprises, from N-terminus to C-terminus, a signal sequence (for example, one of SEQ ID NOs: 242-244), a masking peptide (e.g., any one PM selected from the group consisting of SEQ ID NOs: 323, 328-479), an optional linker (e.g., any one selected from SEQ ID NO:2, or SEQ ID Nos: 210-263), a cleavable moiety (e.g., any one selected from SEQ ID NOs: 5-100, and 264-279), an optional linker (e.g., any one selected from SEQ ID NOs: 2, 210-263, 324, 325, 327), a mature cytokine protein that corresponds to a universal interferon molecule that is a hybrid of IFN alpha 1 and IFN alpha 2a (SEQ ID NO: 481), a cleavable moiety having the amino acid sequence of SEQ ID NO: 100, and a dimerization domain corresponding to human IgG Fc (SEQ ID NO: 3). The activity of the universal ACC is tested in vitro using IFN-responsive HEK293 cells and B16 mouse melanoma cells. Based on the experimental results reported herein comparing ProC440 with ProC732, it is expected that the presence of the affinity mask (PM) will further decrease the cytokine activity of the uncleaved ACC relative to ProC859, but will permit full recovery of cytokine activity when the CMs are cleaved by protease, thereby further reducing toxicity and improving the therapeutic window.

Without wishing to be bound by theory, based on the results presented herein, the inventors envisage that use of an affinity mask (PM) at the N-terminus of a cytokine in addition to the use of a DD with a relatively short LR at the C-terminus of the cytokine will provide significant masking of cytokine activity for cytokines in addition to the interferon-alpha cytokines exemplified in the foregoing specific examples. As described above, the invention described herein encompasses activatable cytokine constructs that include various cytokine proteins discussed herein. As non-limiting examples, the CP used in the ACCs of the invention may be any of those listed in SEQ ID NOs: 101 to 209, and variants thereof. In particular, monomeric cytokines are suited to use in the ACCs described herein. Based on the results provided herein, it is believed that the ACCs of the invention will exhibit reduced cytokine activity relative to the corresponding wildtype cytokine, and that upon cleavage of the ACC by the relevant protease(s), the cleavage product will recover cytokine activity similar to that of the corresponding wildtype cytokine.

Example 6: Tolerability of cytokine constructs in non-human primates

In prior studies using conventional half-life-extended IFN-a2b (pegylated IFN-a2b -reference PEG-IFN BLA #99-148, RMS #1039494 Study 95028) (PEG-IFN, PEG-INTRON™) in cynomolgus monkeys, it was reported that a single subcutaneous dose of 117,721 ug/m² (approximately 9.8 mg/kg) was associated with transient inappetence, hypothermia, hypotension, and death of multiple animals. In those prior studies using pegylated IFN-a2b, it was reported that the no-observed-adverse-effect level (NOAEL) was ~4.9 mg/kg in males (58,860 µg/m²) and ~2.5 mg/kg in females (29,440 µg/m²).

In this Example, to assess the tolerability of the peptide masked IFN α -2b ACC construct (ProC732) in non-human primates, cynomolgus monkeys were dosed via subcutaneous administration at doses of 0.03, 0.30, 3.0 and 15 mg/kg of body weight. The monkeys (N=2 per group, mixed gender) received one dose of masked ACC and were kept on study up to 14-28 days post dose. Plasma concentrations were measured over time as shown in Fig. 39.

All animals were observed cage-side at least twice a day (am / pm) for signs of ill health, morbidity, mortality, injury, viability, and availability/accessibility of food and water. Whole blood was collected, in appropriate anticoagulant tubes, at approximately 48 hours postdose (Day 3) and on Days 7, 15, 22, and 29.

Hematology parameters included the following: White blood cell count; Mean cell volume; Absolute differential leukocyte count; Mean cell hemoglobin; Red blood cell count; Mean cell hemoglobin concentration; Hemoglobin; Platelet count; Hematocrit; Red cell distribution width; Reticulocyte count.

Clinical chemistry parameters included: Sodium; Potassium; Chloride; Alkaline phosphatase; Alanine aminotransferase; Aspartate aminotransferase; Glucose; Blood urea nitrogen; Creatinine; Gamma-glutamyl transferase; Total cholesterol; Triglycerides; Total protein; Albumin; Globulin (calculated); Albumin/globulin ratio (calculated); Calcium; Inorganic phosphorus; Total bilirubin.

Cytokines evaluated included the following: IL-2; IP-10 (CXCL 10); IL-6; IL-12p70; IFN-gamma; IL-10. The concentration of IP-10 in plasma was measured by MSD V-PLEX assay (Meso Scale Diagnostics, Rockville, Maryland) as shown in Fig. 40A and plotted against ProC732 plasma concentration as shown in Fig. 40B.

Additionally, a fraction of whole blood samples were shipped overnight to the analytical laboratory for analysis by flow cytometry within 24 to 36 hours post-collection. FAC evaluations included the following: Viability/ CD20/ CD45/ CD14/ CD3/ CD159a/ CD4/ Ki67/ CD8/ PD-L1.

5 Overall, animals treated with the ProC732 at all dose levels demonstrated no clinical signs of adverse effect. There were no signs of inappetence, hypothermia, hypotension, or body weight loss. Clinical biochemistry and hematology analysis performed at day 3, day 7, day 15 postdose indicated no deviations from prespecified normal ranges for all parameters with a single exception: a single animal treated with 15
10 mg/kg of ProC732 demonstrated elevated AST parameter 3 days after the drug administration. In cynomolgus monkey peptide masked IFN α -2b ACC construct (ProC732, also referred to as Pb-IFN-alpha2b) demonstrated linear pharmacokinetics and extended half-life, and was well tolerated at doses up to 15 mg/kg. The results are summarized in the following table:

	0.03 mg/kg	0.3 mg/kg	3 mg/kg	15 mg/kg
Adverse events	-	-	-	-
AST elevation	-	-	-	+/-
WBC reduction	-	-	-	+/-
Ki67 increase in NK, T-cells	-	-	+	+
IP-10 elevated	-	-	+	++

15

Overall, these results indicated increased tolerability of the conditionally masked IFN-a2b of the present disclosure up to the highest tested dose of 15 mg/kg in both males and females when compared to conventional half-life-extended IFN-a2b.

20 **Example 7: *In vitro* Characterization of CXCL10 and IFN-gamma induced release by PBMC**

CXCL-10 and IFN-gamma induced release in healthy donor peripheral blood mononuclear cells (PBMC) was assessed *in vitro* using untreated PBMC and PBMC treated with peptide masked IFN α -2b ACC construct (ProC732) or ProC732 activated by

in vitro protease treatment for 5 hours in the presence of Brefeldin A at doses of 1 ng/ml (Fig. 19, top row) or 10 ng/ml (Fig. 19, bottom row). Cells were stained for CD3/CD19/CD14, fixed/permeabilized, and stained for intracellular expression of CXCL10 and IFN-gamma. Gating was based on viable monocytes (Fig. 19, top row) or viable CD19-negative lymphocytes (Fig. 19, bottom row). These results show that ProC732 induces release of CXCL10 and IFN-gamma from PBMC in an activation dependent manner.

Example 8: *in vivo* tumor suppression by masked IFN α -2b

Tumor suppressive effect of the masked IFN α -2b ACC construct (ProC732) was assessed *in vivo* using Beige/SCID mice implanted subcutaneously with 10×10^6 Daudi cells grown in serum-free medium (1:1 Matrigel). Tumor measurements were recorded twice weekly over the duration of the study as indicated on the x-axis of the graph shown in Fig. 20. Mice (n=8 per group) were treated with PBS (control) or ProC732 at doses of 0.02 mg/kg, 0.1 mg/kg, and 0.5 mg/kg once a week for five weeks. Treatment was initiated once average tumor volume reached approximately 200 mm³. As shown in Fig. 20, a dose-dependent reduction in mean tumor volume was observed for mice treated with conditionally active ProC732. ProC732 induced complete regression at doses as low as 0.1 mg/kg out to study day 20 and a dose of 0.5 mg/kg induced complete regression for the duration of the study.

Fig. 36 shows ProC732 and ProC1301 inhibited tumor volume growth in a dose-dependent manner. ProC440, ProC732, and ProC1301 showed complete tumor suppression at a dose of 0.1 mg/kg.

Example 9: *In vitro* activation of mouse splenocytes by IFN α A/D

The ability of masked activatable IFN α A/D (ProC1023) or uncleavable IFN α A/D to stimulate release of IP-10 in mouse splenocytes was tested *in vitro*. IFN α A/D (SEQ ID NO: 481) was prepared as described in Rehberg et al. (Rehberg, et al. Specific molecular activities of recombinant and hybrid leukocyte interferons. J Biol Chem. 1982;257:11497-502), which is incorporated by reference herein in its entirety. Control uncleavable IFN α A/D was constructed by replacing protease cleavage sites with an uncleavable linker sequence. Masked activatable IFN α A/D was activated *in vitro* by incubation with uPA at a 1:250 molar ratio. Mouse splenocytes were treated with

masked activatable IFN α A/D, with and without protease treatment, or uncleavable IFN α A/D for 24 hours. IP-10 levels were determined with a standard ELISA assay using conventional methods.

As shown in Fig. 21, uPA-treated masked activatable IFN α A/D induced release of mouse splenocyte IP-10. Both control uncleavable IFN α A/D and masked activatable IFN α A/D without uPA treatment showed similar levels of IP-10 stimulation, which were well below the concentration of IP-10 detected for uPA-treated masked activatable IFN α A/D. The results in Fig. 21 show that stimulation of mouse splenocyte IP-10 release is conditionally dependent on activation of masked IFN α A/D.

Example 10: Effect of masked IFN- α 2b on *in vitro* hamster cell viability

Cell viability of hamster melanoma RPMI1846 cells in response to treatment with masked activatable IFN α -2b ACC construct (ProC732) compared to treatment with ProC732 activated with uPA protease was assessed *in vitro*. RPMI1846 cells were treated with unmasked and masked ACC at increasing concentrations as shown in Fig. 22. The viability of RPMI1846 cells was determined using a CellTiter Glo assay (Promega, USA) according to standard assay protocol.

As shown in Fig. 22, hamster melanoma RPMI1846 cells were able to tolerate substantially higher doses of masked activatable ProC732 compared to unmasked ProC286. Mask moieties on ProC732 caused a substantial rightward shift in cell viability as a function of the cytokine construct concentration. These results demonstrate sensitivity of hamster cells to effects of human IFN- α 2b and improved *in vitro* cell tolerability for ProC732.

ProC1301 (SEQ ID NO: 240) showed activation resistance to MMP14 and uPA. Hamster melanoma RPMI1846 cell viability was reduced upon activation of ProC732 with uPA (Fig. 22). The activation dependent reduction in cell viability was not observed in activation resistant ProC1301 (Fig. 35C).

Example 11: *In vivo* characterization of conditionally active INF α -A/D

Dual masked INF α -A/D ProC1023 and its modified version with potentially reduced cleavability ProC1549 were prepared as described in Example 1.

The antitumor activity of the masked IFN α -A/D was tested *in vivo* using the MC38 tumor model. Mice (N=5 per group) were implanted subcutaneously with 1.5×10^6

MC38 cells in serum-free medium. Body weights and tumor measurements were recorded twice weekly for the duration of the study. When the average tumor volume reached 80 mm³, mice were dosed two times per week by subcutaneous injections of masked IFNa-A/D (ProC1023), or masked uncleavable IFNa-A/D (ProC1549) one time
5 per week intraperitoneally at the indicated dose levels.

Masked IFNa-A/D demonstrated antitumor activity in the 50-200 ug dose level. Administration of 50 ug resulted in significant tumor growth inhibition, while administration of 200 ug also resulted in rejection of the tumors by 60% of the animals Fig. 23A. Antitumor effect of the masked IFNa-A/D (ProC1023) was dependent on
10 proteolytic activation, because the uncleavable construct (ProC1549) did not mediate similar responses Fig. 23B.

Masked IFNa2b reduced tumor volume at increasing doses. Masked IFNa2b was prepared as described above. Masked IFNa2b/Fc prevented tumor progression at a dose of 0.02 mg/kg and induced tumor regression at a dose of 0.1 mg/kg (Fig. 32). As shown
15 in Fig. 32, masked IFNa2b/Fc exhibited antitumor activity similar to peginterferon.

The antitumor activity of the masked IFNa-A/D was tested as described above with doses on days 1, 4, 8, 11, and 15. Tumor volume was assessed at times indicated in the graph of Fig. 27.

Additionally, masked IFNa2b showed anti-tumor activity at 20 µg and 200 µg
20 compared to control (Fig. 33). The antitumor activity of the masked IFNa-A/D was tested as described above with doses on days 1, 4, 8, 11, and 15. Tumor volume was assessed at times indicated in the graph of Fig. 27.

As shown in Fig. 33, dual masked IFNa AD reduced tumor volume compared to a non-cleavable version at doses of 10, 50, and 200 µg (Fig. 33).

As shown in Fig. 34A, Pro IFNa A/D (ProC1023) inhibited tumor volume growth in a dose-dependent manner. The inhibition requires activation as shown in Fig. 34B), where IFNa A/D NSUB (ProC1549) at 200 µg showed reduced antitumor activity compared to Pro IFNa A/D (ProC1023) at the same dose.

Example 12. Immune memory in IFNa-A/D treated mice.

Naïve mice (N=5; Fig. 24A) or mice that rejected MC38 tumor after IFNa-A/D treatment with a 200 microgram dose of ProC1023 (N=3; Fig. 24B) were re-challenged
30

with 1.5×10^6 MC38 cells at day 56 after initial treatment. Tumor growth was monitored twice weekly. Mice that rejected tumor after treatment with 200 ug/dose IFNa-A/D were re-challenged with MC38 tumor 56 days after the initial treatment. The mice were not administered any treatment during the re-challenge period. After the challenge, MC38
 5 tumors progressively grew in all five control animals (Fig. 24A), however only one out of three previously IFNa-A/D-treated mice developed the tumor, and the tumor in that mouse exhibited significantly slower growth consistent with the formation of antitumor immune memory in these mice that had been previously treated the 200 micrograms dose of ProC1023 (Fig. 24B).

10 The results indicate that masked IFNa-A/D suppresses MC38 tumor growth in activation-dependent, immune mediated manner.

Example 13: Activation-dependent induction of type I interferon signature by unmasked IFN-a2b

Dual masked INFa-a2b (SEQ ID NO: 321, ProC732) was activated by treatment
 15 with uPA as described previously. Pegylated IFN-a2b (Merck, USA) was purchased from a vendor.

PBMCs from four healthy donors were purchased from a vendor as a cryopreserved, single-cell suspensions with at least 80% viability after thawing. PBMCs from each donor were treated in vitro with 1 ug/mL (high dose) of masked IFN-a2b
 20 (uncleaved ProC732), or 10 ng/mL of masked IFN-a2b (uncleaved ProC732), unmasked IFN-a2b (uPA-treated ProC732), or Peg-IFN-a2b (Sylatron® - Merck, USA) for 24 hours. Bulk mRNA from treated cells was subjected to paired-end 150c RNAseq high-throughput sequencing. Unique gene hit counts were calculated by using Subread package v.1.5.2. Using DESeq2, a comparison of gene expression between the indicated
 25 groups of samples was performed. The Wald test was used to generate p-values and log2 fold changes. Genes with an adjusted p-value < 0.05 and absolute log2 fold change > 1 were called as differentially expressed genes for each comparison.

Table 9. Pair-wise comparison of gene expression profiles

	Untreated	ProC732	ProC732 high dose	ProC732 + uPA	Sylatron (Peg-IFN-a2b)
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Untreated		↑ 1 ↓ 0	↑ 248 ↓ 36	↑ 418 ↓ 77	↑ 480 ↓ 86
ProC732			↑ 8 ↓ 1	↑ 71 ↓ 10	↑ 125 ↓ 17
ProC732 high dose				↑ 1 ↓ 0	↑ 0 ↓ 0
ProC732 + uPA					↑ 0 ↓ 0

Treatment of PBMCs with masked IFN- α 2b did not result in gene expression changes, while activated IFN- α 2b consistently upregulated and downregulated large number of genes in all four donors (**Fig. 25**). The results demonstrate statistically significant increases in the expression of 418 genes, whereas 77 genes were downregulated (**Table 9**). Gene ontology analysis revealed a pattern associated with activation of type I interferon signaling, including enhanced expression of known targets of IFN- α 2b such as CXCL10, TRAIL and 2'OAS. Treatment with pegylated IFN- α 2b induced and suppressed similar number of genes in all donors. Direct comparison between expression profile of PBMC treated with activated IFN- α 2b and Peg-IFN- α 2b revealed no difference between two treatments.

The results are consistent with activation dependent induction of interferon signaling in primary human immune cells by unmasked IFN- α 2b. Minimal changes between gene expression induced by high dose of masked IFN- α 2b and the unmasked interferon indicate that dual masking reduced signaling potential of the cytokine without creating new interactions with the receptor.

Example 14: Pharmacokinetic properties of masked IFN- α 2b

Dual masked INFa- α 2b (ProC732), steric masked IFN- α 2b (SEQ ID NO: 316, ProC440), its uncleavable control (ProC659), or Fc-IFN- α 2b fusion molecule (ProC286) were administered to golden Syrian hamsters as described previously. Blood samples were obtained at 6, 24, 72 hours or 7 days after administration. Concentrations of IFN- α 2b were measured using ELISA (Mabtech, USA). Non-compartmental pharmacokinetic analysis was performed using WinNonlin software (Certara, USA).

Pharmacokinetic profiles of all tested molecules demonstrate increased serum concentrations proportional to the administered dose (**Fig. 26**). At each dose level, drug exposure was comparable between masked IFN-a2b and control proteins. Non-compartmental analysis revealed average circulation half-life of 4.3 days – ranging 1.98 to 6.38 days (**Table 10**).

The results indicate linear pharmacokinetic properties of IFN-a2b in vivo and extended half-life compared to published data for unmodified IFN-a2b (2.3 hours) and Peg-IFN-a2b conjugated with a 12kDa PEG molecule (4.3 hours).

Pharmacokinetics profiles of all tested molecules indicate increased serum concentrations proportional to the administered dosages.

Table 10. Summary of non-compartmental analysis of IFN-a2b pharmacokinetics

Test Article	Dose mg/kg	Tmax day	Cmax ng/mL	AUClast day*ng/mL	HL_Lambda_z (half life) day
ProC286	0.4	0.25	2913	10371	4.3499
ProC286	2	1	8225	30936	
ProC286	2	1	8863	39595	
ProC286	2	1	7685	29731	
ProC286	10	3	19443	104368	
ProC286	10	1	37673	79286	
ProC286	10	1	24036	118866	
ProC286	15	0.25	41340	187894	1.9774
ProC286	15	1	63075	250384	
ProC286	15	1	74989	259900	
ProC286	15	3	45546	219676	
ProC440	0.4	1	401	1791	4.3952
ProC440	2	1	4718	18986	
ProC440	2	1	7137	27274	
ProC440	2	1	8968	40329	
ProC440	10	1	36860	161885	
ProC440	10	1	31851	152170	
ProC440	15	0.25	53422	214393	5.1186
ProC440	15	1	44331	226428	
ProC440	15	0.25	37551	122954	4.5772
ProC440	15	1	18738	109485	
ProC659	0.4	1	686	3143	5.0481
ProC659	2	3	9842	48705	
ProC659	2	0.25	12284	44567	

ProC659	2	0.25	15715	36674	5.7591
ProC659	10	1	51601	303538	
ProC659	10	1	57389	315392	
ProC659	10	1	51022	241447	
ProC732	10	1	21019	128288	
ProC732	10	1	34498	182458	
ProC732	10	1	34191	179881	
ProC732	15	0.25	33121	186676	6.3841
ProC732	15	1	54723	164326	
ProC732	15	1	27760	157575	
ProC732	15	1	33898	177802	

Unmasked, single and dual masked IFNa2b, and NSUB control IFNa2b were administered to golden Syrian hamsters as described previously. Blood samples were obtained at 6, 24, 72 hours or 7 days after administration. Concentrations of IFNa2b were measured using ELISA (Mabtech, USA).

Pharmacokinetic profiles of all tested molecules demonstrate increased concentrations proportional to the administered dose (Fig. 26).

Example 15: *In vitro* characterization of example Universal cytokine constructs

A universal activatable cytokine construct was prepared by recombinant methods described herein. The universal ACC has a universal interferon sequence (ProC1023) having activity on both human and mouse cells. The universal ACC is a dimer. The 1st and 2nd monomer constructs of this ACC were identical, with each being a polypeptide having the amino acid sequence of SEQ ID NO: 235 with a signal sequence at its N-terminus. Each of the 1st and 2nd monomer constructs comprises, from N-terminus to C-terminus a signal sequence, a spacer (QSGQ) sequence (SEQ ID NO: 256), an IFNalpha-2b masking peptide (TDVDYYREWSWTQVS) (SEQ ID NO: 323), a linker (GSSGGS) (SEQ ID NO: 324), a cleavable moiety having the amino acid sequence (LSGRSDNI) (SEQ ID NO: 41), a linker ((GS)_n, (GGS)_n, (GSGGS)_n (SEQ ID NO: 227), wherein n=1), a mature cytokine protein that corresponds to a universal interferon molecule that is a hybrid of IFN alpha 1 and IFN alpha 2a (SEQ ID NO: 481), a cleavable moiety having the amino acid sequence (LSGRSDNI) (SEQ ID NO: 41), and a DD corresponding to human IgG4 S228P Fc, truncated to Cys226 (according to EU numbering) (SEQ ID NO: 3).

Another universal cytokine construct, ProC1549, was prepared by recombinant methods. The 1st and 2nd monomer constructs of this ACC were identical, with each being a polypeptide having the amino acid sequence of ProC1549 (having an exemplary optional signal sequence). Each of the 1st and 2nd monomer constructs comprises, from
 5 N-terminus to C-terminus, a signal sequence, a spacer (e.g., QSGQ) sequence, an IFNalpha-2b masking peptide (TDVDYYREWSWTQVS) (SEQ ID NO: 323), a linker (GSSGGS) (SEQ ID NO: 324), a non-cleavable moiety having the amino acid sequence of SEQ ID NO: 211, a linker ((GS)_n, (GGS)_n, (GSSGGS)_n (SEQ ID NO: 227), wherein n=1), a mature cytokine protein that corresponds to a universal interferon molecule that is
 10 a hybrid of IFN alpha 1 and IFN alpha 2a (SEQ ID NO: 481), a non-cleavable moiety having the amino acid sequence (GGSGGGGS) (SEQ ID NO: 501), and a DD corresponding to human IgG4 S228P Fc, including the full hinge sequence (SEQ ID NO: 3). Because the ProC1549 construct lacks cleavable moieties between the masking peptide and the cytokine, as well between the cytokine sequence and the DD, it is not
 15 activatable, as discussed below.

Another universal activatable cytokine construct, ProC859, was prepared by recombinant methods described herein. ProC859 has a universal interferon sequence having activity on both human and mouse cells. ProC859 is a dimer. The 1st and 2nd monomer constructs of this ProC859 were identical, with each being a polypeptide
 20 having the amino acid sequence of ProC859 with a signal sequence at its N-terminus. Each of the 1st and 2nd monomer constructs comprises, from N-terminus to C-terminus, a signal sequence, a mature cytokine protein that corresponds to a universal interferon molecule that is a hybrid of IFN alpha 1 and IFN alpha 2a (SEQ ID NO: 481), a cleavable moiety having the amino acid sequence (SGRSDNI) (SEQ ID NO: 100), and a
 25 dimerization domain corresponding to human IgG Fc (SEQ ID NO: 3). Unlike ProC1023, ProC859 does not comprise a peptide masking moiety.

The activity of the universal cytokine constructs ProC1023 and proC859 was tested *in vitro* using B16 mouse melanoma cells. The activity of ProC1023 was further reduced as compared to ProC859 (Fig. 28A). This indicates that the addition of a peptide
 30 mask provided additional masking strength even though the cytokine activity was already significantly reduced in ProC859 by steric masking through the dimerization domains.

Surprisingly, it appears that the addition of a masking peptide (PM) does not interfere with steric masking by the DD, nor does the DD appear to interfere with masking by the PM. Protease activation with uPa restored the activity of ProC1023 to a level comparable to the level of ProC859 after protease activation with uPa. This indicates that ProC1023, upon protease activation, recovered the full strength of activity of an unmasked universal IFN α .

The masking efficiencies of ACCs in a HEK reporter assay (as measured by comparing the EC₅₀ of the uncleaved ACC to the EC₅₀ of the cleaved ACC) were as follows:

ProC1023: 1387X
ProC859: 700X

The activity of the universal cytokine constructs ProC1023 and ProC1549 was tested *in vitro* using B16 mouse melanoma cells. In the un-activated state, ProC1023 and ProC1549 showed similar reduction of signaling activity (Figs. 28B and 28C). Upon Protease activation with either uPa or MMP14, activity of the non-cleavable ProC1549 remains low and similar to ProC1549 without protease activation, while activity of ProC1023 was significantly increased after protease activation as compare ProC1023 and ProC1549 without protease activation (Figs. 28B and 28C). This indicates that ProC1549 is resistant to Protease activation, and it can be used as a control to demonstrate protease-dependent activation of universal activatable cytokine constructs.

Example 16: *In vitro* characterization of additional heterodimeric ACCs

ACC ProC1239 (Pro-IFN 49CS 1204 IFN α 2b 0 1204 0 G4 Knob Stub Hole) was also prepared by recombinant methods. The 1st monomer construct of this ACC is a polypeptide having the amino acid sequence of ProC1239 Arm 1 and a signal sequence at its N-terminus. The 1st monomer construct of this ACC comprises, from N-terminus to C-terminus a signal sequence, a spacer (QSGQ) sequence (SEQ ID NO: 256), an IFN α -2b masking peptide (TDVDYYREWSWTQVS) (SEQ ID NO: 323), a linker (GSSGGS) (SEQ ID NO: 324), a cleavable moiety having the amino acid sequence (LSGRSDNI) (SEQ ID NO: 41), a linker ((GS)_n, (GGS)_n, (GSSGGS)_n (SEQ ID NO: 227), wherein n=1), a mature cytokine protein that corresponds to human interferon

alpha-2b (SEQ ID NO: 1), a cleavable moiety having the amino acid sequence (LSGRSDNI) (SEQ ID NO: 41), and a DD corresponding to human IgG Fc with a knob mutation, truncated to Cys226 (according to EU numbering) (SEQ ID NO: 318). The 2nd monomer construct of this ACC is a polypeptide having the amino acid sequence of ProC1239 Arm 2 and a signal sequence at its N-terminus. The 2nd monomer construct has, from N-terminus to C-terminus, a signal sequence, a stub moiety (SDNI) (SEQ ID NO: 320), and a dimerization domain corresponding to human IgG Fc with a hole mutation (SEQ ID NO: 319).

The activity of ProC1239 and ProC732 was tested in vitro using IFN-responsive HEK293 cells as previously described. The activity of ProC1239 was moderately reduced as compared to ProC732 (Fig. 29).

Example 17: *In vitro* characterization of additional ACCs with various cleavable linkers

Additional activatable cytokine constructs with varying cleavable linker were also prepared by recombinant methods. The 1st and 2nd monomer constructs of these ACCs were identical. Each of the 1st and 2nd monomer constructs comprises, from N-terminus to C-terminus, a signal sequence, a spacer (QSGQ) sequence (SEQ ID NO: 256), an IFNalpha-2b masking peptide (TDVDYYREWSWTQVS) (SEQ ID NO: 323), a linker (GSSGGS) (SEQ ID NO: 324), a cleavable moiety, a linker ((GS)_n, (GGS)_n, (GSGGS)_n (SEQ ID NO: 227), wherein n=1), a mature cytokine protein that corresponds to human interferon alpha-2b (SEQ ID NO: 1), a cleavable moiety, and a DD corresponding to human IgG4 S228P Fc, truncated to Cys226 (according to EU numbering) (SEQ ID NO: 3). The various cleavable linkers used in the ACCs are described in the following table:

Activable cytokines having different cleavable linkers between

Name	Alternative Name	CM between CP and PM	CM between CP and DD
ProC732	Pro-IFN 49CS 1204 IFNa2b 0 1204 0 (-ESKYGPP (SEQ ID NO: 317)) G4	LSGRSDNI (SEQ ID NO: 41)	LSGRSDNI (SEQ ID NO: 41)
ProC1550	Pro-IFN 49CS 1205 IFNa2b 0 1205 0 (-ESKYGPP (SEQ ID NO: 317)) G4	LSGRSNI (SEQ ID NO: 315)	LSGRSNI (SEQ ID NO: 315)

ProC1552	Pro-IFN 49CS 559 IFNa2b 0 559 0 (-ESKYGPP (SEQ ID NO: 317)) G4	QNQALRMA (SEQ ID NO: 16)	QNQALRMA (SEQ ID NO: 16)
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The activity of ProC732, ProC1550 and ProC1552 were tested *in vitro* using IFN-responsive HEK293 cells as previously described. Upon protease activation with either uPa or MTSP1, all activable cytokine constructs showed a similar increased of activity, indicating that all activated cytokines constructs recover the same level of activity upon protease treatment as shown in Fig. 30.

Example 18: Binding of activated Pb-IFN-a2b to interferon alpha receptors *in vitro*

Pb-INF-a2b was activated *in vitro* with uPA, and the active fraction was purified by chromatography (ProC1640). Interferon alpha receptor 1 human (ProC1822) and cyno (ProC1824), as well as IFNAR2 human (ProC1823) and cyno (ProC1825) were expressed as recombinant proteins and purified. Binding was performed *in vitro* using the surface plasmon resonance approach. The ligands were captured on a chip coated with immobilized anti-human Fc or anti-histidine antibodies. Regeneration conditions to permit multi-cycle kinetic measurements were established. Different concentrations of analytes were flowed over the ligand-captured chip to generate multi-cycle kinetic sensorgrams that were analyzed to obtain the kinetic rate constants and the affinity constant using a 1:1 binding model.

ProC1640 binds to human and cyno IFNAR1, however affinity and specificity of the interaction could not be determined with current method due to extremely slow dissociation of the molecules. Binding of the activated fraction of the IFN-a2b to human IFNAR2 and cyno IFNAR2 was detected. As shown in Figs. 41A-41D, ProC732 binds to human and cynomolgus monkey interferon alpha receptor IFNAR2 with similar affinity. Fig. 41A shows human IFNAR1 response over time. Fig. 41B shows cynomolgus monkey IFNAR1 response over time. Fig. 41C shows human IFNAR2 response over time. Fig. 41D shows cynomolgus monkey IFNAR2 response over time.

Affinity to hIFNAR2 was 2.7 nM, cyno – 9.3 nM as shown in the following table:
Summary of binding studies with IFN-a2b molecules

Ligand	Analyte	ka (1/Ms)	kd (1/s)	KD (nM)
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ProC1823	ProC1640	4.374E+06	1.195E-02	2.731
ProC1825	ProC1640	2.674E+06	2.501E-02	9.353
ProC1823	ProC1976	8.985E+04	1.175E-02	130.8
ProC1718	ProC1640	5.177E+06	1.214E-02	2.344
ProC440	ProC1718	2.055E+05	2.077E-02	101.1

For confirmatory studies, binding of the activated Pb-IFN-a2b (ProC1640) to Fc-tagged dimeric IFNAR2 (ProC1718) was analyzed. The K_d of the interaction of ProC1640 with ProC1718 was 2.3 nM.

5 Therefore, human IFN-a2b binds to human and cynomolgus monkey IFNAR2 with similar affinity. Format and valency of the ligand did not affect measurement results.

Example 19: Binding of single masked Pb-IFN-a2b molecules to IFNAR2

Binding of masked Pb-IFN-a2b to human IFNAR2 was performed as described
 10 above. Direct comparison of the peptide masked IFN-a2b (ProC1976) with its unmasked version (ProC1640) demonstrated ~50x affinity differential (130.8 nM vs 2.7 nM, respectively). Furthermore, sterically masked molecule (ProC440) binds to IFNAR2 with significantly reduced affinity (K_D = 101.1 nM) compared to the unmasked molecule. As shown in Figs. 42A-42D, each of the peptide masks (Fig. 42A (no peptide mask) vs. Fig.
 15 42B (peptide masked)) and the Fc masks (Fig. 42C (no Fc mask) vs. 42D (Fc masked)) affect binding of the ACC to the receptor. In view of the data, synergistic activity has been obtained through the use of the dual masking structure of the ACCs of the present disclosure. Therefore, both affinity and steric masking decreases binding of the IFN-a2b to IFNAR2.

Example 20: Activation of ACCs by tumor tissues

20 Fluorescently labeled ProC732 was incubated with enzymatically active tumor samples or low-activity control tissues at 37°C as shown in Fig. 37A as described in (Howng, B, Winter, MB, LePage, C, et al. Novel Ex Vivo Zymography Approach for Assessment of Protease Activity in Tissues with Activatable Antibodies. *Pharmaceutics*
 25 2021;13:1390). Proteins recovered after 2 or 16 hours of incubation were analyzed for activation status (capillary electrophoresis) and bioactivity (HEK-blue reporter assay).

Recovered solution was then analyzed through capillary electrophoresis enabling quantification of active molecules or low-activity control tissue (Fig. 37B) or using HEK-blue IFNA reporter model (Fig. 37C). Enzymatically inactive samples were used as control tissues. The results demonstrate the activation of ProC732 in the tumor microenvironment.

Incubation with breast carcinoma tumor samples but not low-activity control tissues resulted in appearance of protein products corresponding to molecules expected to be generated after release of steric (Fc fragment) and affinity (CS49 peptide) masks (Fig. 37B). Release of the peptide mask was detected earlier while separation of the Fc mask was more pronounced at the later time point. Pb-IFN-a2b samples incubated with the breast carcinoma tissues, but not control tissues demonstrated increased potency in the IFN pathway activation assay (Fig. 37C). 16h incubation resulted in higher potency as compared to 2h.

The observation is consistent with time-dependent release of the steric and peptide masks from the Pb-IFN-a2b molecule, and therefore, proteolytic activation of Pb-IFN-a2b by tumor tissues.

Example 21: Changes in bioactivity of the interferon molecules after incubation with tumor tissues.

Fully masked Pb-INF-a2b (ProC732) or *in vitro* activated (ProC1640) IFN-a2b proteins were incubated with tumor samples. Proteins recovered after 2, 6 or 24 hours of incubation were analyzed for bioactivity using HEK-blue reporter assay.

Incubation with enzymatically active tumor tissues resulted in activation and enhanced bioactivity of Pb-IFN-a2b. On contrary, incubation with tumor tissues reduced bioactivity of the unmasked interferon, potentially by proteolytic degradation of the molecule. Bioactivity of control samples of both Pb-IFN-a2b and unmasked IFN-a2b did not change upon incubation in the absence of tumor. As shown in Figs. 38A-38C, ProC732 or recombinant IFN-a2b were incubated on TNBC and head and neck ("H&N") tumor tissue sections or in tumor-free glass area at 37°C. Recovered solutions were then analyzed by HEK-blue IFNA reporter model. Figs. 38A and 38B show the fold change of bioactivity of 10 ng/mL ProC732 or 1 ng/mL of recombinant IFN-a2b calculated relative to 0 hour values. Fig. 38C shows bioactivity of ProC732 and IFN-a2b proteins incubated

in the absence of tumor tissues for 24h. Each line connects an individual sample (concentration range 100-0.01 ng/mL) analyzed before and after 24h incubation.

The results suggest that exposure to tumor tissue could degrade unmasked interferon molecules in vitro. Masked Pb-IFN-a2b retains and enhances its bioactivity after tumor exposure.

Example 22: Pharmacokinetics of the masked INF-a2b and control molecules in non-human primates

To understand PK/PD properties of the Pb-IFN-a2b in cynomolgus monkey, animals (N=2 per group) were treated with a single dose subcutaneous administration of Pb-IFN-a2b at 0.03, 0.3, 3 or 15 mg/kg. Plasma samples were collected at indicated time points and analyzed for total Pb-IFN-a2b concentration. Concentrations of IP-10 in the serum were measured by the MesoScale Discovery MSD V-plex assay.

Administration of ProC732 resulted in dose-dependent increase in plasma concentrations of the drug starting from the first measurement at 24h after administration (Fig. 39). Plasma concentrations of the Pb-IFN-a2b were maintained for at least 2 weeks after the administration.

Elevated serum concentrations of IP-10 were detected in treated animals as early as 24h after the administration (Fig. 40A). Magnitude of the increase was correlated with dose level; 15 and 3 mg/kg administrations resulted in IP-10 concentrations above 8 and 4 ng/mL respectively. Seven days after the administration, serum levels of IP-10 came back to the physiological concentration in all animals except the monkeys treated with the highest dose level. Concentrations of circulating Pb-IFN-a2b and IP-10 plotted against each other at day 1 and day 7 after administration (Fig. 40B).

The results are consistent with extended half-life of Pb-IFN-a2b in non-human primates. Transient increase in IP-10 after treatment with high dose of Pb-IFN-a2b indicates that the molecule can activate the type I IFN signaling pathway in non-human primates then given at high dose levels.

Example 23: Gene expression profile changes induced by Pb-IFN-a2b non-human primates

Cynomolgus monkeys were treated with Pb-IFN-a2b as described previously. PBMC were isolated from whole blood at 24h after the administration. Gene expression

profile changes induced by ProC732 in cynomolgus monkeys were analyzed.

Cynomolgus monkey (N=2 per group) were treated with a single dose subcutaneous administration of ProC732 at 0.03, 0.3, 3 or 15 mg/kg. Bulk mRNA from isolated cells was subjected to paired-end 150c RNAseq high-throughput sequencing. Unique gene hit counts were calculated by using Subread package v.1.5.2. Using DESeq2, a comparison of gene expression between the indicated groups of samples was performed. The Wald test was used to generate p-values and log2 fold changes. Genes with an adjusted p-value < 0.05 and absolute fold change > 3 were called as differentially expressed genes for each comparison.

Administration of Pb-IFN-a2b at all dose levels was associated with upregulation of 35 genes in circulating leucocytes (Fig. 43). Additional numbers of genes (3, 12, and 47) were upregulated by increased dose level of administered Pb-IFN-a2b (0.3, 3, and 15 mg/kg), respectively. Many of the upregulated genes belong to the group identified as ISG, or interferon-stimulated genes, known to be induced by type I interferons. Analysis of individual upregulated genes indicated dose-dependent pattern of induction where most evident changes were associated with the top dose level of the Pb-IFN-a2b. Fig. 44 shows the dose-dependent changes in gene expression. Genes were called differentially expressed if number of reads changes were >3.

The results are consistent with in vivo activation of type I IFN signaling by Pb-IFN-a2b.

Example 24: In vivo characterization of Pb-IFNa-A/D

The antitumor activity of the masked IFNa-A/D (ProC1023) was tested in vivo using MC38 tumor model. Mice (N=10 per group) were be implanted subcutaneously with 1.5×10^6 MC38 cells in serum-free medium. Body weights and tumor measurements were recorded twice weekly for the duration of the study. When the average tumor volume has reached 80 mm^3 , mice were dosed with the indicated amounts of ProC1023 by a single subcutaneous injection. Previously we established that masked IFNa-A/D demonstrate antitumor activity in the 50-200 ug dose level. Administration of 50 ug resulted in significant tumor growth inhibition, while administration of 200 ug also resulted in rejection of the tumors by 60% of the animals. In this experiment, animals were euthanized 6 days after the administration and tumors, tumor-draining lymph nodes,

and spleens were collected and processed into single-cell suspensions. Composition and activation of tumor immune infiltrate was analyzed by flow cytometry performed with total cells and gated on viable CD45+CD3+ subsets.

In mice treated with Pb-IFN α -A/D, CD8+ T cell subset in tumor
5 microenvironment (TME), but not in peripheral tissues demonstrated enhanced activation, including production of effector molecules associated with tumoricidal activity (Fig. 45). Granzyme B is an effector molecule of cytotoxic T cells that could be induced by type I interferon signaling. Administration of Pb-IFN α -A/D was associated with significant increase in frequency of Granzyme B positive and CD69 positive CD8+
10 T cells in tumors, while there was no major changes in peripheral tissues, including tumor-draining lymph nodes.

Thus, the data show that Pb-IFN α -A/D mediates immune activation in tumor but not in periphery. The pattern of immune activation was generally consistent with published effects of type I interferon. The tumor-preferred manner of immune activation
15 shows activation of the ACCs by tumors through proteolytic cleavage. The observation is in agreement with immune-mediated mechanism of MC38 tumor growth suppression by Pb-IFN α -A/D.

Example 25: *In vivo* tolerability of the Pb-IFN-a2b

Human IFN α -2b cross-reacts with hamster IFN α receptor and has been previously
20 shown to be active in hamsters (Altrock et al, Journal of Interferon Research, 1986). Improved tolerability of the ProC732 compared to unmasked IFN-a2b-Fc fusion (ProC286) or single (sterically) masked IFN-a2b (ProC440) in hamsters after single administration was shown in Example 2. In this Example 25, tolerability of the Pb-IFN α -2b in Syrian Gold Hamsters was determined after multiple administrations.

25 Animals (N=5 per group) were dosed with ProC732 15 or 30 mg/kg dose level or unmasked IFN-a2b-Fc fusion protein (ProC286) at 7.5 or 15 mg/kg dose level, i.p. once weekly for total 3 administrations. Clinical observations, body weights & temperature were measured prior to dosing, and twice weekly thereafter. Animals dosed with the unmasked IFN molecules (ProC286) showed significant body weight loss as early as 3
30 days after first administration (Fig. 46). The first animal dosed with 15 mg/kg ProC286 was euthanized at day 10 due to excessive weight loss (>25%) and all the other animals

were either euthanized due to excessive weight loss, inactivity and lethargy or were found dead between days 11 and 19. Similar observations were made for the animals treated with lower (7.5 mg/kg) dose of the unmasked INF-a2b. In contrast, none of the animals treated with ProC732 up to 30 mg/kg demonstrated significant loss of weight or morbidity.

The results are in agreement with increased safety of Pb-IFN-a2b due to the use of the dual masking structure used in the present disclosure.

Example 26: Reduced cytokine and chemokine release in cynomolgus monkey treated with single dose administration of the Pb-IFN-a2b

To understand effect of masking of the Pb-IFN-a2b in cynomolgus monkeys, animals (N=2 per group) were treated with a single dose subcutaneous administration of Pb-IFN-a2b (ProC732) at 1 mg/kg of unmasked control molecule ProC286 at 1 or 0.1 mg/kg. Plasma samples were collected at indicated time points and analyzed for IP-10, MIP-1b and IL-12p70 concentrations using the multiplex MSD V-plex assay.

As shown in Fig. 47, administration of ProC732 resulted in elevation of the plasma IP-10 and MIP-1b level 6 hours after the administration. Plasma concentrations of all measured molecules were higher in animals treated with unmasked molecule at high and low dose level. 24 hours after the administration only slight elevation of IP-10 was noted in animals treated with ProC732. On contrary, animals received 1 mg/kg of ProC286 demonstrated highly elevated IP-10 levels. IP-10 elevation observed 24 hours after administration of 0.1 mg/kg ProC286 was greater than such induced by 10-fold higher dose of ProC732.

The results indicate attenuated induction of biomarkers of type I interferon response in non-human primates treated with ProC732 as compared to unmasked cytokine-Fc fusion. The observation is in agreement with masking effects.

Table 11. Example sequences

SEQ ID NO.	NAME	SEQUENCE
1	Human Interferon-alpha-2b	CDLPQTHSLGSRRTLMLLAQMRRISLFSCLKDRHDFGFP QEEFGNQFQKAETIPVLHEMIQQIFNLFSTKDSSAAWDET

		LLDKFYTELYQQNLNDLEACVIQGVGVTTETPLMKEDSILA VRKYFQRITLYLKEKKYSPCAWEVVRAEIMRSFSLSTNL QESLRSKE
2	Linker	GGGS
3	Human IgG4, S228P truncated Fc Region	CPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVD VSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRV VSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKG QPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVE WESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQ QGNVFSCSVMHEALHNHYTQKSLSLS
4	Human IgG4 Fc region, with S228P mutation and full hinge	ESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEV TCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQF NSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEK TISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFY PSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTV DKSRWQQGNVFSCSVMHEALHNHYTQKSLSLS
5	CM	LSGRSDNH
6	CM	TGRGPSWV
7	CM	PLTGRSGG
8	CM	TARGPSFK
9	CM	NTLSGRSENHSG
10	CM	NTLSGRSGNHGS
11	CM	TSTSGRSANPRG
12	CM	TSGRSANP
13	CM	VHMPLGFLGP
14	CM	AVGLLAPP
15	CM	AQNLLGMV
16	CM	QNQALRMA
17	CM	LAAPLGLL
18	CM	STFPFGMF
19	CM	ISSGLLSS
20	CM	PAGLWLDP

21	CM	VAGRSMRP
22	CM	VVPEGRRS
23	CM	ILPRSPAF
24	CM	MVLGRSLL
25	CM	QGRAITFI
26	CM	SPRSIMLA
27	CM	SMLRSMPL
28	CM	ISSGLLSGRSDNH
29	CM	AVGLLAPPGGLSGRSDNH
30	CM	ISSGLLSSGGSGGSLSGRSDNH
31	CM	LSGRSGNH
32	CM	SGRSANPRG
33	CM	LSGRSDDH
34	CM	LSGRSDIH
35	CM	LSGRSDQH
36	CM	LSGRSDTH
37	CM	LSGRSDYH
38	CM	LSGRSDNP
39	CM	LSGRSANP
40	CM	LSGRSANI
41	CM	LSGRSDNI
42	CM	MIAPVAYR
43	CM	RPSPMWAY
44	CM	WATPRPMR
45	CM	FRLLDWQW
46	CM	ISSGL
47	CM	ISSGLLS
48	CM	ISSGLL
49	CM	ISSGLLSGRSANPRG
50	CM	AVGLLAPPTSGRSANPRG

51	CM	AVGLLAPPSGRSANPRG
52	CM	ISSGLLSGRSDDH
53	CM	ISSGLLSGRSDIH
54	CM	ISSGLLSGRSDQH
55	CM	ISSGLLSGRSDTH
56	CM	ISSGLLSGRSDYH
57	CM	ISSGLLSGRSDNP
58	CM	ISSGLLSGRSANP
59	CM	ISSGLLSGRSANI
60	CM	AVGLLAPPGGLSGRSDDH
61	CM	AVGLLAPPGGLSGRSDIH
62	CM	AVGLLAPPGGLSGRSDQH
63	CM	AVGLLAPPGGLSGRSDTH
64	CM	AVGLLAPPGGLSGRSDYH
65	CM	AVGLLAPPGGLSGRSDNP
66	CM	AVGLLAPPGGLSGRSANP
67	CM	AVGLLAPPGGLSGRSANI
68	CM	ISSGLLSGRSDNI
69	CM	AVGLLAPPGGLSGRSDNI
70	CM	GLSGRSDNHGGAVGLLAPP
71	CM	GLSGRSDNHGGVHMPLGFLGP
72	CM	LSGRSDNHGGVHMPLGFLGP
73	CM	ISSGLSS
74	CM	PVGYTSSL
75	CM	DWLYWPGI
76	CM	LKAAPRWA
77	CM	GPSHLVLT
78	CM	LPGGLSPW
79	CM	MGLFSEAG
80	CM	SPLPLRVP

81	CM	RMHLRSLG
82	CM	LLAPSHRA
83	CM	GPRSFG
84	CM	GPRSFG
85	CM	SARGPSRW
86	CM	GGWHTGRN
87	CM	HTGRSGAL
88	CM	AARGPAIH
89	CM	RGPAFNPM
90	CM	SSRGPAYL
91	CM	RGPATPIM
92	CM	RGPA
93	CM	GGQPSGMWGW
94	CM	FPRPLGITGL
95	CM	SPLTGRSG
96	CM	SAGFSLPA
97	CM	LAPLGLQRR
98	CM	SGGPLGVR
99	CM	PLGL
100	CM	SGRSDNI
101	Human Interferon alpha-2a	CDLPQTHSLGSRRTLMLLAQMRKISLFSLKDRHDFGFP QEEFGNQFQKAETIPVLHEMIQQIFNLFSTKDSSAAWDET LLDKFYTELYQQNLNDLEACVIQGVGVTTETPLMKEDSILA VRKYFQRITLYLKEKKYSPCAWEVVRAEIMRSFSLSTNL QESLRSKE
102	Rat Interferon alpha-2	CDLPHTHNLRNKRAFTLLAQMRRLSPVSCLKDRKDFGF PLEKVDGQQIQKAQAIPVLHELTQQILSLFTSKESSTAWD ASLLDSFCNDLQQQLSGLQACLMQQVGVQESPLTQEDS LLAVREYFHRITVYLREKKHSPCAWEVVRAEVWRALSSS ANLLGRLREERNES

103	Mouse Interferon alpha-2	CDLPHTYNLRNKRALKVLAQMRRLPFLSCLKDRQDFGF PLEKVDNQQIQKAQAIPVLRDLTQQTLNLFSTKASSAAW NATLLDSFCN DLHQQLNDLQ TCLMQQVGVO EPPLTQEDAL LAVRKYFHRITVYLREKKHS PCAWEVVRAE VWRALSSSVN LLPRLSEEKE
104	Human Interferon Alpha-2b	CDLPQTHSLGSRRTLMLLAQMRRLSFLSCLKDRHDFGFP QEEFGNQFQKAETIPVLHEMIQQIFNLFSTKDSSAAWDET LLDKFYTELYQQNLNDLEACVIQGVGVGTETPLMKEDSILA VRKYFQRITLYLKEKKYSPCAWEVVRAEIMRSFSLSTNL QESLRSKE
105	Human Interferon Alpha-n3	CDLPQTHSLGSRRTLMLLAQMRKISFLSCLKDRHDFGFP QEEFGNQFQKAETIPVLHEMIQQIFNLFSTKDSSAAWDET LLDKFYTELYQQNLNDLEACVIQGVGVGTETPLMKEDSILA VRKYFQRITLYLKEKKYSPCAWEVVRAEIMRSFSLSTNL QESLRSKECDLPQTHSLGSRRTLMLLAQMRRLSFLSCLK DRHDFGFPQEEFGNQFQKAETIPVLHEMIQQIFNLFSTKD SSAAWDETLLDKFYTELYQQNLNDLEACVIQGVGVGTETPL MNEDSILAVRKYFQRITLYLKEKKYSPCAWEVVRAEIMR SFSLSTNLQESLRSKECDLPQTHSLGSRRTLMLLAQMRRI SFLSCLKDRRDFGFPQEEFGNQFQKAETIPVLHEMIQQIF NLFSTKDSSAAWDETLLDKFYTELYQQNLNDLEACVIQGV GVGTETPLMNEDSILAVRKYFQRITLYLKEKKYSPCAWEV VRAEIMRSFSLSTNLQESLRSKE
106	Human Interferon beta-1a	MSYNLLGFLQRSSNFQCQKLLWQLNGRLEYCLKDRMNF DIPEEIKQLQQFQKEDAALTIYEMLNIFAIQRDSSSTGW NETIVENLLANVYHQINHLKTVLEEKLEKEDFTRGKLMS SLHLKRYYYGRILHYLKAKEYSHCAWTIVRVEILRNIFYFIN RLTGYLNR
107	Human Interferon beta-1b	SYNLLGFLQRSSNFQSQKLLWQLNGRLEYCLKDRMNF IPEEIKQLQQFQKEDAALTIYEMLNIFAIQRDSSSTGW NETIVENLLANVYHQINHLKTVLEEKLEKEDFTRGKLMS

		SLHLKRYYYGRILHYLKAKEYSHCAWTIVRVEILRNIFYFI NRLTGYLRLN
108	Mouse Interferon- Beta	MNNRWILHAAFLLCFSTTALSINYKQLQLQERTNIRKCCQ ELLEQLNGKINLTYRADFKIPMEMTEKMQKSYTAFAIQE MLQNVFLVFRNNFSSTGWNETIVVRLLDLHQQTIVFLK TVLEEKQEERLTWEMSSTALHLKSYWVRVQRYLKLKMK YNSYAWMVVRAEIFRNFLIIRRLTRNFQN
109	Rat Interferon-Beta	MANRWTLHIAFLLCFSTTALSIDYKQLQFRQSTSIRTCQK LLRQLNGRLNLSYRTDFKIPMEVMHPSQMEKSYTAFAIQ VMLQNVFLVFRSNFSSTGWNETIVESLLDELHQQTLELEI ILKEKQEERLTWVTSTTTLGLKSYWVRVQRYLKDKKYN SYAWMVVRAEVRNFSIHLRLNRNFQN
110	Human Interferon Omega	MCDLPQNHGLLSRNTLVLLHQMRRISPFLCLKDRRDFRF PQEMVKGSQQLQKAHVMSVLHEMLQQIFSLFHTERSAA WNMTLLDQLHTGLHQQLQHLETCLLQVVGEGESAGAIS SPALTLRRYFQGIRVYLKEKKYSDCAWEVVRMEIMKSL FLSTNMQERLRSKDRDLGSS
111	Human IL-1 alpha	MAKVPMFEDLKNCYSENEEDSSSIDHLSLNQKSFYHVS YGPLHEGCMDQSVLSISETSKTSKLTFKESMVVVATNG KVLKKRRRLSLQSITDDDLAIAANDSEEEIHKPRSAFSLF NVKYNFMRIIKYEFILNDALNQSIIRANDQYLTAALHN LDEAVKFDMGAYKSSKDDAKITVILRISKTLQYVTAQDE DQPVLLKEMPEIPKTTITGSETNLLFFWETHGTKNYFTSVA HPNLFIAATKQDYWVCLAGGPPSITDFQILENQA
112	Mouse IL-1 alpha	MAKVPDFEDLKNCYSENEYSSAIDHLSLNQKSFYDAS YGSLHETCTDQFVSLRTSETSKMSNFTFKESRVTVSATSS NGKILKKRRLSFSETFTEDDLQSITHDLEETIQPRSAFYTY QSDLRYKLMKLVQKFVMNDSLNQTIYQDVDPKHLYLST TWLNDLQQEVKFDMYAYSSGGDDSKYPVTLKISDSQLF VSAQGEDQPVLLKELPETPKLITGSETDLIFFWKSINSKN YFTSAAYPELFIAATKEQSRVHLARGLPSTMTDFQIS

113	Human IL-1 beta	MAEVP ELASEMMA YYSGNEDDLFFEADGPKQMKCSFQ DL DLCPLDGGIQLRISDHHYSKGFRQAASVVVAMDKLR KMLVPCPQTFQENDLSTFFPFIFEEEPFFDTWDNEAYVH DAPVRSLNCTLRDSQQKSLVMSGPYELKALHLQGQDME QQVVF SMSFVQGEESNDKIPVALGLKEKNLYLSCVLKD DKPTLQLESVDPKNYPKKKMEKRFVFNKIEINNKLFEFES AQFPNWIYSTSQAENMPVFLGGTKGGQDITDFTMQFVSS
114	Mouse IL-1 beta	MATVPELNCEMPFPFDS DENDLFFEVDGPQKMKGCFQTF DLGCPDESIQLQISQQHINKSFRQAVSLIVAVEKLWQLPV SFPWTFQDEDMSTFFSFIFEEEPILCDSWDDDDNLLVCDV PIRQLHYRLRDEQQKSLVLSDPYELKALHLNGQNINQQV IFSMSFVQGEPSNDKIPVALGLKGKNLYLSCVMKDGTP LQLESVDPKQYPKKKMEKRFVFNKIEVKSKVEFESA EFP NWIYSTSQA EHKPVFLGNNSGQDIIDFTMESVSS
115	Human IL-1RA	MEICRGLRSHLITLLLFLFHSETICRPSGRKSSKMQA FRIW DVNQKTFYLRNNQLVAGYLQGPVNL EEKIDVVP IEPH ALFLGIHGGKMCLSCVKS GDETRLQLEAVNITD LSEN RK QDKRFAFIRSDSGPTTSFESAACPGWFLCTAMEADQPV S LTNMPDEGVMVTKFYFQEDE
116	Mouse IL-1RA	MEICWGPYSHLISLLLILFHSEAACRPSGKR PCKMQAFR IWDTNQKTFYLRNNQLIAGYLQGPNIKLEEKIDMVPIDL HSVFLGIHGGKLCLSCAKSGDDIKLQLEEVNITD LSKNKE EDKRFTFIRSEKGP TTSFESAACPGWFLCTTLEADRPVSL TNTPEEPLIVTKFYFQEDQ
117	Human IL-18	MAAEPVEDNCINFVAMKFIDNTLYFIAEDDENLESDYFG KLESKLSVIRNLNDQVLFIDQGNRPLFEDMTDSDCRDNA PRTIFIISMYKDSQPRGMAVTISVKCEKISTLSCENKIISFK EMNPPDNIKDTKSDIIFQRSVP GHDNKMQFESSSYEGYF LACEKERDLFKLILKKEDELGDRSIMFTVQNE D
118	Mouse IL-18	MAAMSEDS CVNFKEMMFIDNTLYFIPEENG DLESDNFG RLHCTTAVIRNINDQVLFVDKRQPVFEDMTDIDQSASEP

		QTRLIHYMYKDSEVRGLAVTL SVKDSKMSTLSCKNKIISF EEMDPPENIDDIQSDLIFFQKRVP GHNKMEFESSLYEGHF LACQKEDDAFKLILKKK DENGDKSVMFTLTNLHQS
119	Human IL-2	MYRMQLLSICIALSLALVTNSAPTSSSTKKTQLQLEHLLL DLQMILNGINNYKNPKLTRMLTFKFYMPKKATELKHLQ CLEEELKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELK GSETTFMCEYADETATIVEFLNRWITFCQSIIS
120	Mouse IL-2	MYSMQLASCVTLTLVLLVNSAPTSSSTSSSTAEEAQQQQQ QQQQQQQHLEQLLMDLQELLSRMENYRNKLPRMLTF KFYLPKQATELKDLQCLEDELGPLRHVLDLTQSKSFQLE DAENFISNIRVTVVKLKGS DNTFECQFDDESATVVDFLR RWIAFCQSIISTSPQ
121	Human IL-4	MGLTSQLLPPLFFLLACAGNFVHG HKCDITLQEIIKTLNS LTEQKTLCTELTVTDIFAASKNTTEKETFCRAATVLRQF YSHHEKDTRCLGATAQQFH RHKQLIRFLKRLDRNLWGL AGLNSCPVKEANQSTLENFLERLKTIMREKYSKCSS
122	Mouse IL-4	MGLNPQLVVILLFFLECTRSHIHGCDKNHLREIIGILNEVT GEGTPCTEMDVPNVLTATKNTTESELVCRASKVLRIFYL KHGKTPCLKKNSSVLMELQRLFRAFRCLDSSISCTMNES KSTSLKDFLES LKSIMQMDYS
123	Human IL-7	MFHVSFRYIFGLPPLILVLLPVASSDCDIEGKD GKQYESV LMVSIDQLLD SMKEIGSNCLNNEFNFFKRHICDANKEGM FLFRAARKLRQFLKMNSTGDFDLHLLKVSEGTTILLNCT GQVKGRKPAALGEAQPTKSLEENKSLKEQKKLNDLCFL KRLLEIKTCWNKILMGTKEH
124	Mouse IL-7	MFHVSFRYIFGIPPLILVLLPVTSS ECHIKDKEGKAYESVL MISIDELDKMTGTDSNCPNNEPNFFRKHVCDDTKEAAFL NRAARKLKQFLKMNI SEEFNVHLLTVSQGTQTLVNCTS KEEKNVKEQKKNDACFLKRLLEIKTCWNKILKGS I

125	Human IL-9	MLLAMVLTSAALLCSVAGQGCPFLAGILDINFLINKMQE DPASKCHCSANVTSCCLCLGIPSDNCTRPCFSERLSQMTNT TMQTRYPLIFSRVKKSVEVLKNNKCPYFSCEQPCNQTTA GNALTFLKSLLEIFQKEKMRGMRGKI
126	Mouse IL-9	MLVTYILASVLLFSSVLGQRCSTTWGIRDNTNYLIENLKD DPPSKCSCSGNVTSCCLSVPTDDCTTPCYREGLLQLTN ATQKSRLLPVFHRVKRIVEVLKNITCPSFSCEKPCNQTM AGNTLSFLKSLLGTFQKTEMQRQKSRP
127	Human IL-13	MHPLLNPLLLALGLMALLTTVIALTCLGGFASPGPVPPS TALRELIEELVNITQNQKAPLCNGSMVWSINLTAGMYCA ALESLINVSGCSAIEKTQRMLSGFCPHKVSAGQFSSLHVR DTKIEVAQFVKDLLLHLKKLFREGFRN
128	Mouse IL-13	MALWVTAVLALACLGGLAAPGPVPRSVSLPLTLKELIEE LSNITQDQTPLCNGSMVWSVDLAAGGFCVALDSLNTNISN CNAIYRTQRILHGLCNRKAPTTVSSLPDTKIEVAHFITKL LSYTKQLFRHGPF
129	Human IL-15	MRISKPHLRSISIQCYLCLLLNSHFLTEAGIHVFILGCFSA GLPKTEANWVNVISDLKKIEDLIQSMHIDATLYTESDVH PSCKVTAMKCFLELQVISLESGDASIHDTVENLIILANN SLSSNGNVTESGCKECEEELEEKNIKEFLQSFVHIVQMFIN TS
130	Mouse IL-15	MKILKPYMRNTSISCYLCFLLNSHFLTEAGIHVFILGCVS VGLPKTEANWIDVRYDLEKIESLIQSIHIDTTLYTDSDFHP SCKVTAMNCFLELQVILHEYSNMTLNETVRNVLYLAN STLSSNKNVAESGCKECEEELEKTFTEFLQSFIRIVQMFIN TS
131	Human IL-3	MSRLPVLLLLQLLVRPGLQAPMTQTTPLKTSWVNCSNM IDEIITHLKQPPLPLDFNNLNGEDQDILMENNLRPNLE AFNRAVKSLQNASAIESILKNLLPCLPLATAAPTRHPIHK DGDWNEFRRLTFYKLTLENAQAQQTTLAIF

132	Mouse IL-3	MVLASSTTSIHTMLLLLLMLFHLGLQASISGRDTHRLTRT LNCSSIVKEIIGKLPEPELKTDDDEGPSLRNKSFRRVNLSKF VESQGEVDPEDRYVIKSNLQKLNCCLPSTANDSALPGVF IRDLDDFRKKLRFYMVHLNDLETVLTSRPPQPASGSVSP NRGTVEC
133	Human IL-5	MRMLLHLSLLALGAAYVYAIPTEIPTSALVKETLALLST HRTLIIANETLRIPVPVHKNHQLCTEEIFQGIGTLESQTVQ GGTVERLFKNLSLIKKYIDGQKKKCGEERRRVNQFLDYL QEFLGVMNTEWIIES
134	Mouse IL-5	MRRMLLHLSVLTLSVCVWATAMEIPMSTVVKETLTQLSA HRALLTSNETMRLPVPTHKNHQLCIGEIFQGLDILKNQT VRGGTVEMLFQNLSLIKKYIDRQKEKCGEERRRTRQFLD YLQEFLGVMSTEWAMEG
135	Human GM-CSF	MWLQSLLLLGTVACISAPARSPSPSTQPWEHVNAIQEA RRLNLNRDTAAEMNETVEVISEMFDLQEPTCLQTRLEL YKQGLRGS�TKLKGPLTMMASHYKQHCPPTPETSCATQI ITFESFKENLKDFLLVIPFDCWEPVQE
136	Mouse GM-CSF	MWLQNLLFLGIVVYSLSAPTRSPITVTRPWKHVEAIKEA LNLLDDMPVTLNEEVEVVSNEFSFKKLTVCVQTRLKIFEQ GLRGNFTKLKGALNMTASYYYQTYCPPTPETDCETQVTT YADFIDSLKTFLTDIPFECKKPGQK
137	Human IL-6	MNSFSTSAFGPVAFSLGLLLVLPAAFPAPVPPGEDSKDV AAPHRQPLTSSERIDKQIRYILDGISALRKETCNKSNMCE SSKEALAENNLNLPKMAEKDGCQSGFNEETCLVKIITG LLEFEVYLEYLQNRFESSEEQARAVQMSTKVLIQFLQKK AKNLDAITTPDPTTNASLLTKLQAQNQWLQDMTTHLILR SFKEFLQSSLRALRQM
138	Mouse IL-6	MKFLSARDFHPVAFGLMLVTTTAFPTSQVRRGDFTEDT TPNRPVYTTSQVGGILITHVLWEIVEMRKELCNGNSDCM NNDDALAENNLKLPEIQRNDGCYQTGYNQEICLLKISSG

		LLEYHSYLEYMKNNLKDNKKDKARVLQRDTETLIHIFN QEVKDLHKIVLPTPISNALLTDKLESQKEWLRTKTIQFIL KSLEEFLKVTLRSTRQT
139	Human IL-11	MNCVCRLVLVVLVSLWPDТАVAPGPPPGPPRVSPDPRAEL DSTVLLTRSLADTRQLAAQLRDKFPADGDHNLDSLPTL AMSAGALGALQLPGVLTRLRADLLSYLRHVQWLRRAG GSSLKTLEPELGTЛQARLDRLRLRLQLLMSRLALPQPPPD PPAPPLAPPSSAWGGIRAAHAILGGLHLTLDWAVRGLLL LKTRL
140	Mouse IL-11	MNCVCRLVLVVLVSLWPDREVAPGPPAGSPRVSSDPRAD LDSAVLLTRSLADTRQLAAQMRDKFPADGDHSLDSLР TLAMSAGTLGSLQLPGVLTRLRVDLMSYLRHVQWLRR AGGPSLKTLEPELGAЛQARLERLLRLQLLMSRLALPQA APDQQVPLGPPASAWGSIRAAHAILGGLHLTLDWAVRG LLLLKTRL
141	Human G-CSF	MAGPATQSPMKLMALQLLLWHSALWTVQEATPLGPAS SLPQSFLKCLEQVRKIQGDGAALQEKLVSECATYKLCH PEELVLLGHSLGIPWAPLSSCPSQALQLAGCLSQЛHSGLF LYQGLLQALEGISPELGPTLDTLQLDVADFATTIWQQME ELGMAPALQPTQGAMPAFASAFQRRAGGVLVASHLQSF LEVSYRVLRHLAQP
142	Mouse G-CSF	MAQLSAQRRMKLMALQLLLWQSALWSGREAVPLVTVS ALPPSLPLPRSFLKSLQVRKIQASGSVLEQLCATYKL CHPEELVLLGHSLGIPKASLSGCSSQALQQTQCLSQЛHSG LCLYQGLLQALSGISPALAPTLDLLQLDVANFATTIWQQ MENLGVAPTVOPTQSAMPAFTSAFQRRAGGVLAISYLQ GFLETARLALHHLA
143	Human IL-12 alpha	MCPARSLLLVA TLVLLDHLSLARNLPVATPDPGMFPCЛH HSQNLLRAVSNMLQKARQTLEFYPTSEEIDHEDITKDK TSTVEACLPELTKNESCLNSRETSFITNGSCLASRKTSF MMALCLSSIEDLKMYQVEFKTMNAKLLMDPKRQIFLD

		QNMLAVIDELMQALNFNSETVPQKSSLEEPDFYKTKIKL CILLHAFRIRAVTIDRVMSYLNAS
144	Human IL-12 beta	MCHQQLVISWFSLVFLASPLVAIWELKKDVYVVELDWY PDAPGEMVVLTCDTPEEDGITWTLTDSSEVLGSGKTLTI QVKEFGDAGQYTCHKGGEVLSHSLLLLHKKEDGIWSTD ILKDQKEPKNKTFLRCEAKNYSGRFTCWWTITISTDLTF SVKSSRGSSDPQGVTCGAATLSAERVGRDNKEYEYSVE CQEDSACPAAEESLPIEVMVDAVHKLKYENYTSSFFIRDI IKPDPPKNLQKPLKNSRQVEVSWEYPDTWSTPHSYFSL TFCVQVQGKSKREKKDRVFTDKTSATVICRKNASISVRA QDRYYSSSWSEWASVPCS
145	Mouse IL-12 beta	MCPQKLTISWFAIVLLVSPLMAMWELEKDVYVVEVDW TPDAPGETVNLTCDTPEEDDITWTSQDRHGVIGSGKTLTI TVKEFLDAGQYTCHKGGETLSHSHLLLHKKENGWSTEI LKNFKNKTFKCEAPNYSGRFTCSWLVRNMDLKFNIK SSSSSPDSRAVTCGMASLSAEKVTLDQRDYEKYSVSCQE DVTCPATAEETLPIELALEARQQNKYENYSTSFFIRDIKPD PPKNLQMKPLKNSQVEVSWEYPDSWSTPHSYFSLKFFV RIQRKKEKMKETEEGCNQKGAFLVEKTSTEVQCKGGNV CVQAQDRYYNSSCSKWACVPCRVR
146	Mouse IL-12 alpha	MCQSRYLFLATLALLNHLSLARVIPVSGPARCLSQSRN LLKTTDDMVKTAREKLKHYSCTAEDIDHEDITRDQTSTL KTCLPLELHKNESCLATRETSSTTRGSCLPPQKTSMMT LCLGSIYEDLKMYQTEFQAINAALQNHNHQHIILDKGML VAIDELMQSLNHNGETLRQKPPVGEADPYRVKMKLCIL LHAFSTRVVTINRVMGYLSSA
147	Human LIF	MKVLAAGVVPLLLVLHWKHGAGSPLPITPVNATCAIRH PCHNNLMNQIRSQAQLNGSANALFIYYTAQGEPFPNN LDKLCGPNVTDFFPFHANGTEKAKLVELYRIVVYLGTS GNITRDQKILNPSALSLSKLNATADILRGLLSNVLCRLC

		SKYHVGHVDTVYGPDTSGKDV FQKKKLG CQLLGKYKQ IIAVLAQAF
148	Mouse LIF	MKVLAAGIVPLLLLVLHWKHGAGSPLPITPVNATCAIRH PCHGNLMNQIKNQLAQLNGSANALFISYYTAQGEFPNN VEKLCAPNMTDFPSFHGNGTEKTKLVELYRMVAYLSAS LTNITRDQKVLNPTAVSLQVKLNATIDVMRGLLSNVLCR LCNKYRVGHVDVPPVPDHSDEAFQRKKLG CQLLGTY KQVISVVVQAF
149	Human OSM	MGVLLTQRTLLSLVLALLFPSMASMAAIGSCSKEYRVLL GQLQKQTDLMQDTSRLDPYIRIQGLDVPKLREHCRERP GAFPSEETLRGLGRRGFLQTLNATLGCVLHRLADLEQRL PKAQDLERSGLNIEDLEKLQMARPNILGLRNNIYCMAQL LDNSDTAEPTKAGRGASQPPTPTPASDAFQRKLEGCRFL HGYHRFMHSVGRVFSKWGESPNRSRRHSPHQALRKG VRRTRPSRK GKRLMTRGQLPR
150	Mouse OSM	MQTRLLRTLTLSTLSLLILSMALANRGCSNSSSQLLSQLQ NQANLTGNTESLLEPYIRLQNLNTPDLRAACTQHSVAFP SEDTLRQLSKPHFLSTVYTTLDRVLYQLDALRQKFLKTP AFPKLDSARHNILGIRNNVFCMARLLNHSLEIPEPTQDS GASRSTTTDPVFNTKIGSCGFLWGYHRFMGSVGRVFRE WDDGSTRSRRSPLARRKGTRRIRVRHKGTRRIRVRRK GTRRIWVRRKGSRKIRPSRSTQSPTRA
151	Human IL-10	MHSSALLCCLVLLTGVRASPGQGTQSENSCTHFPGNLPN MLRDLRDAFSRVKTFQMKDQLDNLLLKESLLEDFKGY LGCQALSEMIQFYLEEVMQAENQDPDIKAHVNSLGEN LKTLLRLRLRRCHRFLPCENKSKAVEQVKNAFNKLQEKGI YKAMSEFDIFINYIEAYMTMKIRN
152	Mouse IL-10	MPGSALLCCLLLLTGMRISRGQYSREDNNCTHFPVGQSH MLLELR TAFS QVKTFQTKDQLDNILLTDSLMQDFKGYL GCQALSEMIQFYLV EVMQA EKHGPEIKEHLNSLGEKLG

		TLRMRLRRCHRFLPCENKSKAVEQVKSDFNKLQDQGVY KAMNEFDIFINCIEAYMMIKMKS
153	Human IL-20	MKASSLAFSLLSAAFYLLWTPSTGLKTLNLGSCVIATNL QEIRNGFSEIRGSVQAKDGNIDIRILRRTESLQDTKPANRC CLLRHLLRLYLDRVFKNYQTPDHYTLRKISSLANSLTIK KDLRLCHAHMTCHCGEEAMKKYSQILSHFEKLEPQAAV VKALGELDILLQWMEETE
154	Mouse IL-20	MKGFGFLAFGLFSAVGFLWTPLTGLKTLHLGSCVITANL QAIQKEFSEIRDSVQAEDTNIDIRILRTTESLKDILSLDRC CFLRHLVRFYLDREVFKVYQTPDHHTLRKISSLANSLTIK KDLSVCHSHMACHCGEEAMEKYNQILSHFIELELQAAV VKALGELGILLRWMEEML
155	Human IL-14	MKNQDKKNGAAKQSNPKSSPGQPEAGPEGAQERPSQA APAVEAEGPGSSQAPRKPEGAQARTAQSGALRDVSEELS RQLEDILSTYCVNNDQGGPGEDGAQGEPAPEDAESRT YVARNGEPEPTPVVNGEKEPSKGPNTTEIRQSDEVGDR DHRRPQEKKKAKGLGKEITLLMQTLNLTSTPEEKLAALC KKYAEELLEHRNSQKQMKLLQKKQSQLVQEKDHLRGE HSKAVLARSKLESCLRELQRHNRSLKEEGVQRAREEEEEK RKEVTSHFQVTLNDIQLQMEQHNERNNSKLRQENMELAE RLKKLIEQYELREEHIDKVFHKDLQQQLVDAKLQQAQ EMLKEAEERHQREKDFLLKEAVESQRMCELMKQQETH LKQQLALYTEKFEEFQNTLSKSSEVFTTFKQEMEKMTKK IKKLEKETTMYSRWESSNKALLEMAEETVRDKELEG LQVKIQRLEKLCRALQTERNDLNKRVQDLSAGGQGSLT DSGPERRPEGPGAQAPSSPRVTEAPCYPGAPSTEASGQT GPQEPTSARA
156	Mouse IL-14	MKNQDKKNGPAKHSNSKSGSPGQREAGPEGAHGRPRQT APGAEAEGSTSQAPGKTEGARAKAAQPGALCDVSEELS RQLEDILSTYCVNNDQGGPAEEGAQGEPTPEDEKESRT YAARNGEPEPGIPVVNGEKETSKGEPGTEEIRASDEVGD

		RDHRRPQEKKKAKGLGKEITLLMQTLNLTSTPEEKLAAL CKKYAELLEHRNSQKQMKLLQKKQSQLVQEKDHLRG EHSKAVLARSKLESLCRELQRHNRSLKEEGVQRAREEEE KRKEVTSHFQVTLNDIQLQMEQHNERNNSKLRQENMELA ERLKKLIEQYELREEHIDKVFKHKDLQQQLVDAKLQQA QEMLKEAEERHQREKEFLLKEAVESQRMCELMKQQET HLKQQLALYTEKFEEFQNTLSKSSEVFTTFKQEMEKMTK KIKKLEKETTMYSRWESSNKALLEMAEEKTVRDKELE GLQVKIQRLEKLCRALQTERNDLNKRVDLTAGGITDIG SERRPEATTASKEQGVESPGAQPASSPRATDAPCCSGAPS TG TAGQTGPGEPTPATA
157	Human IL-16	MESHSRAGKSRKSAKFRSISRSLMLCNAKTSDDGSSPDE KYPDPFEISLAQGKEGIFHSSVQLADTSEAGPSSVPDLAL ASEAAQLQAAGNDRGKTCRRIFFMKESSTASSREKPGKL EAQSSNFLFPKACHQRARSNSTSVNPYCTREIDFPMTKK SAAPTDRQPYSLCSNRKSLSQQLDCPAGKAAGTSRPTRS LSTAQLVQPSGGLQASVISNIVLMKGQAKGLGFSIVGGK DSIYGPIGIYVKTIFAGGAAAADGRLQEGDEILELNGESM AGLTHQDALQKFKQAKKGLLTLTVRTRLTAPPSLCSHLS PPLCRSLSSSTCITKDSSSFALESAPSAPISTAKPNYRIMVEV SLQKEAGVGLGIGLCSVPYFQCISGIFVHTLSPGSAHL GRLRCGDEIVEISDSPVHCLTLNEVYTLSHCDPGPVPIIV SRHPDPQVSEQQLKEAVAQAVENTKFGKERHQWSLEGV KRLESSWHGRPTLEKEREKNSAPPHRAQKVMIRSSSDS SYMSGSPGGSPGSGSAEKPSSDVIDSTHSPSLPLAREPVV LSIASSRLPQESPLPESRDSHPPLRLKKSFEIVRKPMSSKP KPPPRKYFKSDSDPQKSLEERENSSCSSGHTPPTCGQEAR ELLPLLLPQEDTAGRSPSASAGCPGPGIGPQTKSSTEGEP GWRRASPVQTQTSPIKHPLLKRQARMDYSFDTTAEDPWV RISDCIKNLFSPIMSENHGHMPLQPNASLNEEGTQGHDP GTPPKLDTANGTPKVYKSADSSTVKKGPPVAPKPAWFR

		QSLKGLRNRASDPRGLPDPALSTQPAPASREHLGSHIRAS SSSSSIRQRISSEFETFGSSQLPDKGAQRLSLQPSSGEAAKP LGKHEEGRFSGLLGRGAAPTLVPQQPEQVLSSGSPAASE ARDPGVSESPPPGRQPNQKTLPPGPDPLRLLLSTQAEESQ GPVLKMPSQRARSFPLTRSQSCETKLLDEKTSKLYSISSQ VSSAVMKSLCLPSSISCAQTPCIPKEGASPTSSSNEDSAA NGSAETSALDTGFSNLSELREYTEGLTEAKEDDDGDHS SLQSGQSVISLLSSEELKKLIEEVKVLDEATLKQLDGIHV TILHKEEGAGLGFSLAGGADLENKVITVHRVFPNGLASQ EGTIQKGNEVLSINGKSLKGTTHHDALAILRQAREPRQA VIVTRKLTPEAMPDLNSSTDASAASASAASDVSVESTAEA TVCTVTLEKMSAGLGFSLGEGKGS LHGDKPLTINRIFKG AASEQSETVQPGDEILQLGGTAMQGLTRFEAWNIIKALP DGPVTIVIRRKSLQSKETTAAGDS
158	Mouse IL-16	MEPHGHSGKSRKSTKFRSISRSLILCNAKTSDDGSSPDEK YPDPFETSLCQGKEGFFHSSMQLADTFEAGLSNIPDLALA SDSAQLAAAGSDRGKHCRKMFFMKESSSTSSKEKSGKP EAQSSSFLFPKACHQRTRSNTSVNPYSAGEIDFPMTKKS AAPTDROPYSLCSNRKSLSQQLDYPILGTARPTRSLSTAQ LGQLSGGLQASVISNIVLMKGQAKGLGFSIVGGKDSIYG PIGIYVKSIFAGGAAAADGRLQEGDEILELNGESMAGLT HQDALQKFKQAKKGLLTLTVRTRLTPPSLCSHLSPLLC RSLSSSTCGAQDSSPFSLESPASPASTAKPNYRIMVEVSL KKEAGVGLGIGLCSIPYFQCISGIFVHTLSPGSVAHLDGR LRCGDEIVEINDSPVHCLTLNEVYTILSHCDPGVPVHIVSR HPDPQVSEQQKEAVAQAVEGVKFGKDRHQWSLEGVK RLESSWHGRPTLEKEREKHSAPPHRAQKIMVRSSSDSS YMSGSPGGSPCSAGAEPQPSEREGSTHSPSLSPGEEQEP PGVPSRPQQESPPLPESLERESHPLRLKKSFEILVRKPTSS KPKPPPRKYFKNDSEPQKKLEEKEKVTDPGHTLPTCSQ ETRELLPLLLQEDTAGRAPCTAACCPGPAASTQTSSSTEG

		ESRRSASPETPASPGKHPLLRQARM DY SFDITAEDPWV RISDCIKNLFSPIMSENHSHTPLQPNTSLGEEDGTQGCPEG GLSKMDAANGAPRVYKSADGSTVKKGPPVAPKPAWFR QSLKGLRNRAPDPRRPPEVASAIQPTVSRDPPGPQPQAS SSIRQRISSEFENFGSSQLPDRGVQRLSLQPSSGETTKFPGK QDGGRFSGLLGQGATVTAKHRQTEVESMSTTFPNSSEV RDPGLPESPPPGQRPSTKALSPDPLLRLTTQSED TQGGP LKMP SQRAR SFPLTRTQSCETKLLDEKASKLYSISSQLSS AVMKSLLCLPSSVSCGQITCIPKERVSPKSPCNNSSAAEG FGEAMASDTGFSNLSELREYSEGLTEPGETEDR NHCSS QAGQSVISLLSAEELEK LIEEVRVLDEATLKQLDSIHVTIL HKEEGAGLGFSLAGGADLENKVITVHRVFPNGLASQEG TIQKGNEVLSINGKSLKGATHNDALAILRQARDPRQAVI VTRRTTVEATHDLNSSTD SAASASAASDISVESKEATVC TVTLEKTSAGLGFSLEGKGSLHGDKPLTINRIFKGTEQG EMVQPGDEILQLAGTAVQGLTRFEAWNVIKALPDGPVTI VIRRTSLQCKQTTASADS
159	Human IL-17	MTPGKTSLSVLLLLLSLEAIVKAGITIPRNP GCPNSEDKNF PRTVMVNLNIHNRNTNTNPKRSSDY YNRSTSPWNLHRN EDPERYPSVIWEAKCRHLGCINADGNVDYH MNSVPIQQ EILVLRREPPHCPNSFRLEKILVSVGCTCVTPIVHHVA
160	Mouse IL-17	MSPGRASSVSLMLLLLLSLAATVKAAAHPQSSACP NTEA KDFLQNVKVN LKVFNSLGAKVSSRRPSDY LNRSTSPWT LHRNEDPD RYPSVIWEAQCRHQRCVNAEGKLDH HMNS VLIQQEILVLKREPESCPFTFRVEKMLVGVGCTCVASIVR QAA
161	Human CD154	MIETYNQTS PRSAATGLPISMKIFMYLLTVFLITQMIGSA LFAVYLHRRLDKIEDERNLHEDFVFMKTIQRCNTGERSL SLLNCEEIKSQFEGFVKDIMLNKEETKKENSFEMQKGDQ NPQIAAHVISEASSKTTSVLQWAEKGYYTMSNNLV TLEN GKQLTVKRQGLYYIYAQVTFCSNREASSQAPFIASLCLK

		SPGRFERILLRAANTHSSAKPCGQQSIHLGGVFELQPGAS VFVNVTDPSQVSHGTGFTSFGLLKL
162	Mouse CD154	MIETYSQSPRSVATGLPASMKIFMYLLTVFLITQMIGSV LFAVYLHRRLDKVEEEVNLHEDFVFIKKLKRCNKGECSL SLLNCEEMRRQFEDLVKDITLNKEEKKENSFEMQRGDE DPQIAAHVVSEANSNAASVLQWAKKGYTMTKSNLVML ENGKQLTVKREGLYYVYTQVTFCNREPSSQRPFIVGLW LKPSSGSERILLKAANTHSSSQLCEQQSVHLGGVFELQA GASVFVNVTEASQVIHRVGFSSFGLLKL
163	Human LT-beta	MGALGLEGRGRLQGRGSLLLAVAGATSLVTLALLAVPI TVLAVLALVPQDQGGLVTETADPGAQAQQGLGFQKLPE EEPETDLSPGLPA AHLIGAPLKGQGLGWETTKEQAFLTS GTQFSDAEGALPQDGLYYLYCLVGYRGRAPPGGGDPQ GRSVTLRSSLYRAGGAYGPGTPELLEGAETVTPVLDPA RRQGYGPLWYTSVGFGLVQLRRGERVYVNISHPDMV DFARGKTFFGAVMVG
164	Mouse LT-beta	MGTRGLQGLGGRPQGRGCLLLAVAGATSLVTLALLAVPI TVLAVLALVPQDQGRRVEKIIIGSGAQAQKRLDDSKPSCI LPSPSSLSETPDPRHPQRSNASRNLASTSQGPVAQSSRE ASAWMTILSPAADSTPDGPVQQLPKGEPETDLNPELPA HLIGAWMSGQGLSWEASQEEAFLRSGAQFSPHGLALP QDGVYYLYCHVGYRGRTPPAGRSRARSLLRSALYRAG GAYGRGSPELLEGAETVTPVVDPIGYGSLWYTSVGFGG LAQLRSGERVYVNISHPDMVDYRRGKTFFGAVMVG
165	Human TNF-alpha	STESMIRDVELAEEALPKKTGGPQGSRRCLFLSLFSFLIV AGATTFLCLLHFGVIGPQREEFPRDLISPLAQAVRSSSR TPSDKPVAHVVANPQAEQQLQWLNRRANALLANGVEL RDNQLVVPSEGLYLIYSQVLFKGQGCPSHVLTHHTISRI AVSYQTKVNLLSAIKSPCQRETPEGAEAKPWYEPIYLG VFQLEKGDRLSAEINRPDYLDFAESGQVYFGIHAL

166	Mouse TNF-alpha	NHQVEEQLEWLSQRANALLANGMDLKDNLVVPADGL YLVYSQVLFKGQGPCDYVLLTHTVSRFAISYQEKVNLLS AVKSPCPKDTPEGAELKPWYEPIYLGGVFQLEKGDQLSA EVNLPKYLDFAESGQVYFGVIAL
167	Human TNF-beta	MTPPERLFLPRVCGTTLHLLLLGLLLVLPGAQGLPGVG LTPSAAQTARQHPKMH LAHSTLKPAAHLIGDPSKQNSLL WRANTDRAFLQDGFSLSNNSLLVPTSGIYFVYSQVVFSG KAYSPKATSSPLYLAHEVQLFSSQYPFHVPLLSSQKMVY PGLQEPWLHSMYHGAAAFQLTQGDQLSTHTDGIPHLVLS PSTVFFGAFAL
168	Human 4-1BBL	MEYASDASLDPEAPWPPAPRARACRVLPWALVAGLLLL LLAAACAVFLACPWAVSGARASPGSAASPRREGPELS PDDPAGLLDLRQGMFAQLVAQNVLLIDGPLSWYSDPGL AGVSLTGGLSYKEDTKELVVAKAGVYVFFQLELRRVV AGEGSGSVSLALHLQPLRSAAGAAALALTVDLPPASSEA RNSAFGFQGRLLHLSAGQRLGVHLHTEARARHAWQLT QGATVLGLFRVTPEIPAGLPSRSE
169	Mouse 4-1BBL	MDQHTLDVEDTADARHPAGTSCPSDAALLRDTGLLADA ALLSDTVRPTNAALPTDAAYPAVNVRDREAAWPPALNF CSRHPKLYGLVALVLLLLIAACVPIFTRTEPRPALTITSP NLGTRENNADQVTPVSHIGCPNTTQQGSPVFAKLLAKN QASLCNTTLNWHWSQDGAGSSYLSQGLRYEEDKKELVVD SPGLYYVFLELKLSPFTNTGHKVQGWVSLVLQAKPQV DDFDNLALTVELFPCSMENKLVDRSWSQLLLLKAGHRL SVGLRAYLHGAQDAYRDWELSYPNNTTSFGLFLVKPDNP WE
170	Human APRIL	AVLTQKQKKQHSVLHLVPINATSKDDSDVTEVMWQPA LRRGRGLQAQGYGVRIQDAGVYLLYSQVLFQDVTFTM GQVVSREGQGRQETLFRCSMPSHPDRAYNCSYAGVF HLHQGDILSVIIPRARAKLNLSPHGTFLGFVKL

171	Mouse APRIL	MPASSPGHMGGSVREPALSVALWLSWGAVLGAVTCAV ALLIQQTEQLQSLRREVSRLQSRGGPSQKQGERPWQSLWE QSPDVLEAWKDGAKSRRRRRAVLTKHKKKHSVLHLVP VNITSKADSDVTEVMWQPVLRRGRGLEAQGDIVRVWD TGIYLLYSQVLFHDVTFTMGQVVSREGQGRRETLFRCIR SMPSDPDRAYNSCYSAGVFHLHQGDIITVKIPRANAKLS LSPHGTFLGFVKL
172	Human CD70	MPEEGSGCSVRRRPYGCVLRAALVPLVAGLVICLVVCIQ RFAQAQQQLPLESLGWDVAELQLNHTGPQQDPRLYWQ GGPALGRSFLHGPELDKGQLRIHRDGIYMVHIQVTLAICS STTASRHHPTTLAVGICSPASRSISLLRLSFHQGCTIASQR LTPLARGDTLCTNLGTLLPSRNTDETFFGVQWVRP
173	Mouse CD70	MPEEGRPCPWVRWSGTAFQRQWPWLLL VVFITVFCCW FHCSGLLSKQQQRLLHPEPHTAELQLNLTVPRKDPTLR WGAGPALGRSFTHGPELEEGHLRIHQDGLYRLHIQVTLA NCSSPGSTLQHRATLAVGICSPA AHGISLLRGRFGQDCTV ALQRLTYLVHGDVLCNLTLPLPSRNADETFFGVQWIC P
174	Human CD153	MDPGLQQALNGMAPPGDTAMHVPAGSVASHLGTTSR YFYLTATLALCLVFTVATIMVLVVQRTDSIPNSPDNVPL KGGNCSEDLCLKRAPFKKSWAYLQVAKHLNKTCLSW NKDGILHGVRYQDGNLVIQFPGLYFIICQLQFLVQCPNNS VDLKLELLINKHIKKQALVTVCESGMQTKHVYQNL SQF LLDYLQVNTTISVNVDTFQYIDTSTFPLENVLSIFLYSNSD
175	Mouse CD153	MEPGLQQAGSCGAPSPDPAMQVQPGSVASPW RSTRPW STSRSYFYLTSTALVCLVVAVAILVLVVQKKDSTPNTTE KAPLKGGNCSEDLFCTLKSTPSKKSWAYLQVSKHLNNT KLSWNEDGTIHLIYQDGNLIVQFPGLYFIVCQLQFLVQ CSNHSVDLTLQLLINSKIKKQTLVTVCESGVQSKNIYQNL SQFLLHYLQVNSTISVRVDNFQYVDNTFPLDNVLSVFL YSSSD

176	Human CD178	MQQPFNYPYPQIYWVDSSASSPWAPPGTVLPCPTSVPRR PGQRRPPPPPPPPPLPPPPPPPLPPLPLPPLKKRGNHSTGL CLLVMFFMVLVALVGLGLGMFQLFHLQKELAELEST QMHTASSLEKQIGHPSPPPEKKELRKVAHLTGKSNRSRM PLEWEDTYGIVLLSGVKYKKGGLVINETGLYFVYSKVYF RGQSCNNLPLSHKVYMRNSKYPQDLVMMEGKMMSYC TTGQMWARSSYLGAVFNLTSADHLYVNVSELSLVNFEE SQTFEGLYKL
177	Mouse CD178	MQQPMNYPQIYFWVDSSATSSWAPPGSVFPCPCGPRG PDQRRPPPPPPPVSPPLPPPSQPLPLPPLTPLKKKDHNTNLW LPVVFFMVLVALVGMGLGMYQLFHLQKELAELEFTN QSLKVSSFQKQIANPSTPSEKKEPRVAHLTGPNHRSRSL EWEDTYGTALISGVKYKKGGLVINETGLYFVYSKVYFR GQSCNNQPLNHKVYMRNSKYPEDLVLMEEKRLNYCTT GQIWAHSSYLGAVFNLTSADHLYVNISQLSLINFEESEKTF FGLYKL
178	Human GITRL	MTLHPSPITCEFLFSTALISPKMCLSHLENMPLSHSRTQG AQRSSWKLWLFCISVMLLFLCSFSWLIFLQLETAKEPC MAKFGPLPSKWQMASSEPPCVNKVSDWKLEILQNGLYL IYGQVAPNANYNDVAPFEVRLYKNKDMIQTLTNKSKIQ NVGGTYELHVGDTIDLIFNSEHQVLKNNTYWGILLANP QFIS
179	Mouse GITRL	MEEMPLRESSPQRAERCKKSWLLCIVALLMLLCSLGT IYTSLKPTAIESCMVKFELSSSKWHMTSPKPHCVNTTSD GKLKILQSGTYLIYGQVIPVDKKYIKDNAPFVVQIYKKN DVLQTLMNDFQILPIGGVYELHAGDNIYLFNSKDHIQK TNTYWGILMPDLPFIS
180	Human LIGHT	MEESVVRPSVFVVDGQTDIPFTRLGRSHRRQSCSVARVG LGLLLLLMGAGLAVQGWFLQLHWRLGEMVTRLPDGP AGSWEQLIQERRSHEVNPAHLTGANSSLTGSGGPLLW ETQLGLAFLRGLSYHDGALVVTKAGYYYIYSKVQLGGV

		GCPLGLASTITHGLYKRTPRYPEELELLVSQQSPCGRATS SSRVWWDSSFLGGVVHLEAGEKVVVRLDERLVRLRD GTRSYFGAFMV
181	Mouse LIGHT	MESVVQPSVFVVDGQTDIPFRRLEQNHRRRRCGTVQVS LALVLLLGAGLATQGWFLRLHQRLGDIVAHLPDGGKG SWEKLIQDQRSHQANPAAHLTGANASLIGIGGPLLWETR LGLAFLRGLTYHDGALVTMEPGYYYVYSKVQLSGVGCP QGLANGLPITHGLYKRTSRYPKELELLVSRRSPCGRANS SRVWWDSSFLGGVVHLEAGEEVVVRVPGNRLVRPRDG TRSYFGAFMV
182	Human OX40L	MERVQPLEENVGNAARPRFRNKLLLVASVIQGLGLLLC FTYICLHFSALQVSHRYPRIQSIKVQFTEYKKEKGFI LTSQ KEDEIMKVQNNSVIINCDFYLISLKGYSQEVNISLHYQ KDEEPLFQLKKVRSVNSLMVASLTYKDKVYLVNTTNT SLDDFHVNGGELILIHQNPGEFCVL
183	Mouse OX40L	MEGEGVQPLDENLENGSRPRFKWKKTLRLVVSIGKAG MLLCFIYVCLQLSSSPAKDPPIQRLRGAVTRCEDGQLFIS SYKNEYQTMEVQNNSVVIKCDGLYIHLKGSFFQEVKID LHFREDHNPISIPMLNDGRIVFTVVASLAFKDKVYLT NAPDTLCEHLQINDGELIVVQLTPGYCAPEGSYHSTVNQ VP
184	Human TALL-1	MDDSTEREQSRLTSLKKREEMKLKECVSILPRKESPSV RSSKD GKLLAATLLLALLSCCLTVVSFYQVAALQGD LAS LRAELQGHHA EKLPAGAGAPKAGLEEAPAVTAGLKIFEP PAPGEGNSSQNSRNKRAVQGPEETVTQDCLQIADSETP TIQKGSYTFVPWLLSFKRGSALEEKENKILVKETGYFFIY GQVLYTDKTYAMGH LIQRKKVHVFGDELSLVTLFRCIQ NMPETLPNNSCYSAGIAKLEEGDELQLAIPRENAQISLDG DVTFFGALKLL

185	Mouse TALL-1	MAMAFCPKDQYWDSSRKSCVSCALTCSQRSQRTCTDFC KFINCRKEQGYYDHLLGACVSCDSTCTQHPQQCAHFC EKRPQRSQANLQPELGRPQAGEVEVRSDNSGRHQGSEHG PGLRLSSDQLTLYCTLGVCLCAIFCCFLVALASFLRRRGE PLPSQPAGPRGSQANSPPHAHRPVTEACDEVTASPQPVET CSFCFPERSSPTQESAPRSLGIHGFAGTAAPQPCMRATVG GLGVLRASGTGDARPAT
186	Human TRAIL	MAMMEVQGGPSLGQTCVLIVIFTVLLQSLCVAVTYVYF TNELKQMQDKYSKSGIACFLKEDDSYWDPNDEESMNSP CWQVKWQLRQLVRKMILRTSEETISTVQEKQQNISPLVR ERGPQRVA AHITGTRGRSNTLSSPNSKNEKALGRKINSW ESSRSGHSFLSNLHLRNGELVIHEKGFYIYSQTYFRFQE EIKENTKNDKQMVQYIYKYTSYPDPILLMK SARNSCWS KDAEYGLYSIYQGGIFELKENDRIFVSVTNEHLIDMDHE ASFFGAFLVG
187	Mouse TRAIL	MPSSGALKDLSFSQHFRMMVICIVLLQVLLQAVSVAVTY MYFTNEMKQLQDNYSKIGLACFSKTDEDFWDSTDGEIL NRPCLQVKRQLYQLIEEVTLRTFQDTISTVPEKQLSTPPL PRGGRPQKVA AHITGITRRSNSALIPISKDGKTLGQKIES WESSRKGHSFLNHVLFNRNGELVIEQEGLYIYSQTYFRF QEAEDASKMVSKDKVRTKQLVQYIYKYTSYPDPIVLMK SARNSCWSRDAEYGLYSIYQGGIFELKKNDRIFVSVTNE HLMDLDQEASFFGAFLIN
188	Human TWEAK	MAARRSQRRRGRRGEPGTALLVPLALGLGLALACLGLL LAVVSLGSRASLSAQEPAQEELVAEEDQDPSELNPQTEE SQDPAPFLNRLVRPRRSAPKGRKTRARRAIAAHYEVHPR PGQDGAQAGVDGTVSGWEEARINSSSPLRYNRQIGEFIV TRAGLYYLYCQVHFDEGKAVYKLKDLLVDGVLALRCL EEFSATAASSLGPQLRLCQVSGLLALRPGSSLRIRTL PWA HLKAAPFLTYFGLFQVH

189	Mouse TWEAK	MASAWPRSLPQILVLGFGLVLMRAAAGEQAPGTSPCSS GSSWSADLDKCMDCASCPARPHSDFCLGCAAAPPAHFR LLWPILGGALSLVLVLALVSSFLVWRRCCRREKFTTPIEE TGGEPCPGVALIQ
190	Human TRANCE	MRRASRDYTKYLRGSEEMGGGPGAPHEGPLHAPPPAP HQPPAASRSMFVALLGLGLGQVVCSVALFFYFRAQMDP NRISEDGTHCIYRILRLHENADFQDTTLESQDTKLIPDSCR RIKQAFQGA VQKELQHIVGSQH IRAEKAMVDG SWLDLA KR SKLEAQPFAHLTINATDIPSGSHKVSLSWYHDRGWA KISNMTFSNGKLIVNQDGFYYLYANICFRHHETSGDLAT EYLQLMVYVTKTSIKIPSSHTLMKGGSTKYWSGNSEFHF YSINVGGFFKLRS GEEISIEVSNPSLLDPDQDATYFGAFK VRDID
191	Mouse TRANCE	MRRASRDYGYKYLRSSEEMGSGPGVPHEGPLHPAPSAPA PAPPPAASRSMFLALLGLGLGQVVCSIALFLYFRAQMDP NRISEDSTHCFYRILRLHENADLQDSTLESED TLPDSCRR MKQAFQGA VQKELQHIVGPQRFS GAPAMMEG SWLDVA QRGKPEAQPFAHLTINAASIPSGSHKVTLSWYHDRGWA KISNMTLSNGKL RVNQDGFYYLYANICFRHHETSGSVPT DYLQLMVYVVKTSIKIPSSHNLMKGGSTKNWSGNSEFH FYSINVGGFFKL RAGEEISIQVSNPSLLDPDQDATYFGAF KVQDID
192	Human TGF-beta1	MPPSGLRLLLLLLPLLWLLVLT PGRPAAGLSTCKTIDME LVKRKRIEAI RQILSKLRLASPPSQGEVPPGPLPEAVLAL YNSTRDRVAGESAEPEPEPEADYYAKEVTRVLMVETHN EIYDKFKQSTHSIYMFNTSELREAVPEPVLLSRAELRLL RLKLKVEQHVELYQKYSNNSWRYLSNRLLAPSDSPEWL SFDVTGVVRQWLSRGGEIEGFRLSAHCSCDSRDNTLQV DINGFTTGRRGDLATIHGMNRPFLLLMATPLERAQHLQS SRHRRALDTNYCFSSTEKNCCVRQLYIDFRKDLGWKWI HEPKGYHANFCLGPCPYIWSLDTQYSKVLALYNQHNPG

		ASAAPCCVPQALEPLPIVYYVGRKPKVEQLSNMIVRSCKCS
193	Mouse TGF-beta1	MPPSGLRLLPLLLPLPWLLVLTTPGRPAAGLSTCKTIDMEL VKRKRIEAIARGQILSKLRLASPPSQGEVPPGPLPEAVLAL YNSTRDRVAGESADPEPEPEADYYAKEVTRVLMVDRNN AIYEKTKDISHSIYMFNTSDIREAVPEPPLLSRAELRLQR LKSSVEQHVELYQKYSNNSWRYLGNRLLTPTDTPEWLS FDVTGVVRQWLNQGDGIQGFSAHCSCDSKDNKLHVE INGISPKRRGDLGTIHD MNRPFLLLMATPLERAQHLHSSR HRRALDTNYCFSSTEKNCCVRQLYIDFRKDLGWKWIHE PKGYPHANFCLGPCPYIWSLDTQYSKVLALYNQHNP GAS ASPCCVPQALEPLPIVYYVGRKPKVEQLSNMIVRSCKCS
194	Human TGF-beta2	MHYCVLSAFLILHLVTVALSLSTCSTLMDQFMRKRIEA IRGQILSKLKLTSPPEDYPEPEEVPPEVISIYNSTRDLLQEK ASRRAAACERERSDEEYYAKEVYKIDMPPFFPSENAIPPT FYRPFYFRIVRFDVSAMEKNASNLVKAEFRVRLQNPKAR VPEQRIELYQILKSKDLTSPTQRYIDSKVVKTRAEGEWLS FDVTD AVHEWLHHKDRNLGFKISLHCPCTFVPSNNYII PNKSEELEARFAGIDGTSTYTSGDQKTIKSTRKKNSGKTP HLLLMLLPSYRLESQQTNRKKRALDAAYCFRNVQDNC CLRPLYIDFKRDLGWKWIHEPKGYNANFCAGACPYLWS SDTQHSRVLSLYNTINPEASASPCCVSQDLEPLTILYYIGK TPKIEQLSNMIVKSCKCS
195	Mouse TGF-beta2	MHYCVLSTFLLLHLVPVALSLSTCSTLMDQFMRKRIEA IRGQILSKLKLTSPPEDYPEPDEVPEVISIYNSTRDLLQEK ASRRAAACERERSDEEYYAKEVYKIDMPSHLPSENAIPP TFYRPFYFRIVRFDVSTMEKNASNLVKAEFRVRLQNPKA RVAEQRIELYQILKSKDLTSPTQRYIDSKVVKTRAEGEW LSFDVTD AVQEWLHHKDRNLGFKISLHCPCTFVPSNNY IIPNKSEELEARFAGIDGTSTYASGDQKTIKSTRKKTSGKT PHLLLMLLPSYRLESQSSRRKKRALDAAYCFRNVQDN

		CCLRPLYIDFKRDLGWKWIHEPKGYNANFCAGACPYLW SSDTQHTKVLSTYNTINPEASASPCCVSQDLEPLTILYYIG NTPKIEQLSNMIVKSCKCS
196	Human TGF-beta3	MKMHLQRALVVLALLNFATVSLSLSTCTTLDFGHIKKK RVEAIRGQILSKLRLTSPPEPTVMTHVPYQVLALYNSTRE LLEEMHGEREEGCTQENTESEYYAKEIHKFDMIQGLAEH NELAVCPKGITSKVFRFNVSSVEKNRTNLFRAEFRVLRV PNPSSKRNEQRIELFQILRPDEHIAKQRYIGGKNLPTRG TAEWLSFDVTDTVREWLLRRESNLGLEISIHCPCHTFQPN GDILENIHEVMEIKFKGVDNEDDHGRGDLGRLKKQKDH HNPHLILMMIPPHRLDNPQGQGGQRKKRALDTNYCFRNL EENCCVRPLYIDFRQDLGWKWWHEPKGYANFCSGPCP YLRSA DTTHSTVLGLYNTLNPEASASPCCVPQDLEPLTIL YYVGRTPKVEQLSNMVKSKCS
197	Mouse TGF-beta3	MHLQRALVVLALLNLATISLSLSTCTTLDFGHIKKRVE AIRGQILSKLRLTSPPEPSVMTHVPYQVLALYNSTRELLE EMHGEREEGCTQETSESEYYAKEIHKFDMIQGLAEHNEL AVCPKGITSKVFRFNVSSVEKNGTNLFRAEFRVLRV PNPSSKRTEQRIELFQILRPDEHIAKQRYIGGKNLPTRGTA EWL SFDVTDTVREWLLRRESNLGLEISIHCPCHTFQPN GDILENVHEVMEIKFKGVDNEDDHGRGDLGRLKKQKDH HNPHLILMMIPPHRLDSPGQGSQRKKRALDTNYCFRN LEENCVRPLYIDFRQDLGWKWWHEPKGYANFCSGPCPY LRSADTTHSTVLGLYNTLNPEASASPCCVPQDLEPLTIL YYVGRTPKVEQLSNMVKSKCS
198	Human EPO	MGVHECPAWLWLLSLSLPLGLPVLGAPPRLICDSRVL ERYLLEAKEAENITTGCAEHCSLNENITVPDTKVN FYAWKRMEVGQQAVEVWQGLALLSEAVLRGQALLVN SSQPWEPLQLHVDKAVSGLRSLTLLRALGAQKEAIS PPDAASAAPLRTITADTFRKLFRVYSNFLRGKLKLYT GEACRTGDR

199	Mouse EPO	MGVPERPTLLLLLSLLLIPLGLPVLCAPPRLICDSRVLERY ILEAKEAENVTMGCAEGPRLSENITVPDTKVNIFYAWKR MEVEEQAIEVWQGLSLLSEAILQAQALLANSSQPPETLQ LHIDKAISGLRSLTSLLRVLGAQKELMSPPDITPPAPLRT LTVDTFCKLFRVYANFLRGKCLKLYTGEVCRRGDR
200	Human TPO	MELTELLLVVMLLLTARLTLSPPAPPACDLRVLSKLLRD SHVLHSRLSQCEVHPLPTPVLLPAVDFSLGEWKTMEE TKAQDILGAVTLLLEGVMAARGQLGPTCLSSLLGQLSG QVRLLL GALQSLLGTQLPPQGRRTAHKDPNAIFLSFQHL LRGKVRFLMLVGGSTLCVRRAPPTTAVPSRTSLVLTNE LPNRTSGLLETNFTASARTTGSGLLKWQQGFRAKIPGLL NQTSRSLDQIPGYLNRIHELLNGTRGLFPGPSRRTL GAPD ISSGTSDTGSLPPNLQPGYSPSPHTPPTGQYTLFPLPPTLPT PVVQLHPLLDPDSAPTPTPTSPLLNTSYTHSQNLSQEG
201	Mouse TPO	MELTDLLLAAMLLAVARLTLSPPVAPACDPRLLNKLLR DSHLLHSRLSQCPDVPDPLSIPVLLPAVDFSLGEWKQTQE QSKAQDILGAVSLLLEGVMAARGQLEPSCLSLLGQLSG QVRLLL GALQGLLGTQLPLQGRRTAHKDPNALFSLQQ LLRGKVRFLLLVEGPTLCVRRTLPTTAVPSSTSQLLTLNK FPNRTSGLLETNFSVTARTAGPGLLSRLQGFRVKITPGQL NQTSRSPVQISGYLNRTGHPVNGTHGLFAGTSLQTLEAS DISPGAFNKGSLAFNLQGGLPSPSLAPDGHTPFPPSPALP TTHGSPQLHPLFPDPSTTMPNSTAPHPVTMYPHPRNLSQ ET
202	Human FLT-3L	MTVLAPAWSPTTYLLLLLLLSSGLSGTQDCSFQHSPISD FAVKIRELSDYLLQDYPVTVASNLQDEELCGGLWRLVL AQRWMERLKT VAGSKMQGLLERVNTEIHFVTKCAFQPP PSCLRFVQTNISRLQETSEQLVALKPWITRQNFRCLEL QCQPDSSTLPPPWSRPLEATAPTAPQPPLLLLLLLLPVGL LLLA AAWCLHWQRTRRRTPRPGEQVPPVPSQDLLLLVE H

203	Mouse FLT-3L	MTVLAPAWSPNSSLLLLLLLLSPCLRGTDPDCYFSSHPISS NFKVKFRELTDHLLKDYPVTVAVNQLQDEKHCKALWSLF LAQRWIEQLKTVAGSKMQTLLEDVNTEIHFVTSCTFQPL PECLRFVQTNISHLLKDTCTQLLALKPCIGKACQNFSRCL EVQCQPDSSSTLLPPRSPIALEATELPEPRPRQLLLLLLLLL PLTLVLLAAAWGLRWQRARRRGELHPGVPLPSHP
204	Human SCF	MKKTQTWILTCIYLQLLLFNPLVKTEGICRNRVTNNVKD VTKLVANLPKDYMITLKYPGMDVLPSCWISSEMVVQL SDSLTDLLDKFSNISEGLSNYSIIDKLVNIVDDLVECVKE NSSKDLKKSFKSPEPRLFTPEEFFRIFNRSIDAFKDFVVAS ETSDCVVSSTLSPEKDSRVSVTKPFMLPPVAASSLRNDSS SSNRKAKNPPGDSSLHWAAMALPALFSLIIGFAFGALYW KKRQPSLTRA VENIQINEEDNEISMLQEKEREFQEV
205	Mouse SCF	MKKTQTWIITCIYLQLLLFNPLVKTKEICGNPVTDNVKDI TKLVANLPNDYMITLNYVAGMDVLPSCWL RDMVIQL SLSLTTLLDKFSNISEGLSNYSIIDKLGKIVDDL VLCMEEN APKNIKESPKRPETRSFTPEEFFSIFNRSIDAFKDFMVASD TSDCVLSSTLGPEKDSRVSVTKPFMLPPVAASSLRNDSSS SNRKAAPEDSGLQWTAMALPALISLVIGFAFGALYW KKKQSSLTRA VENIQINEEDNEISMLQKQKEREFQEV
206	Human M-CSF	MTAPGAAGRCPPTTWLGSLLLLVCLLASRSITEEVSEYC SHMIGSGHLQSLQRLIDSQMETSQITFEFVDQEQLKDPV CYLKKAFLLVQDIMEDTMRFRDNTPN AIAIVQLQELSLR LKSCFTKDYEEDHKACVRTFYETPLQLLEKVKNVFNET KNLLDKDWNIFSKNCNNSFAECSSQDVVTKPDCNCLYP KAIPSSDPASVSPHQPLAPSMAPVAGLTWEDSEGTEGSSL LPGEQPLHTVDPGSAKQRPPRSTCQSFEPPETPVVKDSTI GGSPQPRPSVGAFNPGMEDILDSAMGTNWVPEEASGEA SEIPVPQGTELSPSRPGGSMQTEPARPSNFLSASSPLPAS AKGQQPADVTGTALPRVGPVRPTGQDWNHTPQKTDHP SALLRDPPEPGSPRISSLRPQGLSNPSTLSAQPLSRSHSS

		GSVLPLGELEGRRSTRDRRSPAEEGGPASEGAARPLPRF NSVPLTDTGHERQSEGSFSPQLQESVFHLLVPSVILVLLA VGGLLFYRWRRRSHQEPQRADSPLEQPEGSPLTQDDRQ VELPV
207	Mouse M-CSF	MTARGAAGRCPSSTWLGSRLLLVCLLMSRSIAKEVSEHC SHMIGNGHLKVLQQLIDSQMETSCQIAFEFVDQEQLDDP VCYLKKAFFLVQDIIDETMRFKDNTPNANATERLQELSN NLNSCFTKDYEEQNKACVRTFHETPLQLEKIKNFFNET KNLLEKDOWNIFTKNCNNSFAKCSSRDVVTKPDCNCLYP KATPSSDPASASPHQPPAPSMAPLAGLAWDDSQRTGESS LLPSELPLRIEDPGSAKQRPPRSTCQTLESTEQPNHGDRL TEDSQPHPSAGGPVPGVEDILESSLGTNWVLEEASGEAS EGFLTQEAKFSPSTPVGGSIQAETDRPRALSASFPKSTED QKPVDITDRPLTEVNPMRPIGQTQNNTPKTDGTSTLRE DHQEPGSPHIATPNPQRVSN SATPVAQLLLPKSHSWGIVL PLGELEGKRSTRDRRSPAEEGGSASEGAARPVARFNSIP LTDTGHEQHEGSSDPQIPESVFHLLVPGIILVLLTVGGL LFYKWKWRSHRDPQTL DSSVGRPEDSSLTQDEDRQVEL PV
208	Human MSP	MGWLPLLLLLLTQCLGVPQGQRSPLNDFQVLRGTELQHLL HAVVPGPWQEDVADAEECAGRCGPLMDCRAFHYNVSS HGCQLLPWTQHSPHTRLRRSGRCDLFQKKDYVRTCIMN NGVG YRGTMATTVGGLPCQAWSHKFPNDH KYTPTLRN GLEENFCRNPDGDPGGPW CYTTDPAVR FQSCGIKSCREA ACVWCNGEEYRGAVDRTESGRECQRWDLQHPHQHPFE PGKFLDQGLDDNYCRNPDG SERPW CYTTDPQIEREFCDL PRCGSEAQPRQEATTVSCFRGKGEGYRG TANTTTAGVP CQRWDAQIPHQHRFTPEKYACKDLRENFCRNPDGSEAP WCFTLRPGMRAAF CYQIRRCTDDVRPQDCYHGAGEQY RGTVSKTRKGVQCQRWSAETPHKPQFTFTSEPHAQLEE NFCRNPDGDSHGPPWCYTMDPRTPFDYCALRRCADDDQPP

		SILDPPDQVQFEKCGKRVDRLDQRRSKLRVVGGHPGNSP WTVSLRNRRQGHFCGGS LVKEQWILTARQCFSSCHMPL TGYEVWLGTLFQNPQHGEPSLQRVPVAKMVCGPSGSQ L VLLK LERSVT LNQRVALICLPPEWYVVP PGTKCEIAGWG ETKGTGNDTVLNVALLNVISNQECNIHRGRVRESEMC TEGL LAPVGACEGDYGGPLACFTHNCWVLEGIIIPNRVC ARSRWPAVFTRVS VFVDWIHKVMRLG
209	Mouse MSP	MGLPLPLLQSSLLLMLLLRLSAASTNLNWQCPRIPYAAS RDFSVKYVVP SFSAGGRVQATAAYEDSTNSAVFVATRN HLHVLGPD LQFIENLT TGPIGNPGCQTCASC GP GPHGPPK D TDTLVLVMEPGLPALVSCGSTLQGR CFLHELEPRGKAL HLAAPACLF SANNNKPEACTDCVASPLGTRVT VVEQGH ASYFYVASSLDPELAASFSPRSVSIRRLKSDTSGFQPGFPS LSVL PKYLASYLIK YVYSFHSGDFVYFLT VQPI SVTSPPS ALHTRLVRLNAVEPEIGDYREL VLDCHFAPKRRRRGAPE GTQPY PVLQAAHSAPVDAKLAVELSISEGQEVLFGVFVT VKDGGSGMGPNSVVCAPPIYHLNIIIEGVEYCCHSSNSS SLLSRGLDFFQTPSFCPNPPGGEASGPSSRCHYFPLMVHA SFTRVDL FNGLLG SVKVTALHVTRLGNVTVAHMGTV D GRVLQVEIARSLNYLLYVS NFSLGSSGQP VHRDVSRLGN DLLFASGDQVFKVPIQGPGCRHFLTCWRCLRAQRFMGC GWC GDRCDRQKECPGSWQQDHCPPEISEFYPHSGPLRG TTRLTLCGSNFYLRPDDV VPEGTHQITVGQSPCRLLPKDS SSPRPGSLKEFIQELECELEPLVTQAVGTTNISLVITNMPA GKHFRVEGISVQEGFSFVEPVLT SIKPDFGPRAGGTYLTL EGQSLSVGTSRAVLVNGTQCRLEQVNEEQILCVTPPGAG TARVPLHLQIGGAEVPGSWTFHYKEDPIVLDISPKCGYS GSHIMIHGQHLTSAWHFTLSFHDGQSTVESRCAGQFVEQ QORRCRLPEYVVRNPQG WATGNLSVWGDGAAGFTLPG FRFLPPPSPLRAGLVELKPEEHSVKVEYVGLGAVADCVT VNMTVGGEVCQHELRGDVVICPLPPSLQLGKDGVP LQV

		CVDGGCHILSQVVRSSPGRASQRILLIALLVLILLVAVLA VALIFNSRRRKQLGAHSLSP TTLSDINDTASGAPNHEES SESRDGTSPVLLRTESIRLQDLDRMLLAEVKDVLPHEQV VIHTDQVIGKGHFGVVYHGEYTDGAQNQTHCAIKSLSRI TEVQEVEAFLREGLLMRGLHHPNILALIGIMLPPEGLPRV LLPYMRHGDLLHFIRSPQRNPTVKDLVSFGLQVACGME YLAEQKFVHRDLAARNCMLDESFTVKVADFG LARGVL DKEYYSVRQHRHARLPVKWMALES LQTYRFTTKSDVW SFGVLLWELLTRGAPPYPHIDPFDLSHFLAQGRRLPQPEY CPDSLYHVMLRCWEADPAARPTFRALVLEVKQVVASLL GDHYVQLTAA YVNVGPRAVDDGSPPEQVQPS PQHCRS TSKPRPLSEPPLPT
210	Linker	GSSGGSGGSGG
211	Linker	GGGSGGGS
212	Linker	GGGSGGGS GGGS
213	Linker	GGGSGGGS GGGS
214	Linker	GGGSGGGS GGGS GGGS GGGS
215	Linker	GGGSGGGS
216	Linker	(GGGS)n
217	Linker	GGGSGS
218	Linker	GGGSGGGS GGGS
219	Linker	GGSLDPKGGGS
220	Linker	PKSCDKTHTCPPCPAPELLG
221	Linker	SKYGPPCPPCPAPEFLG
222	Linker	GKSSGSGSES KS
223	Linker	GSTSGSGKSSEGKG
224	Linker	GSTSGSGKSSEGSGSTKG
225	Linker	GSTSGSGKPGSGEGSTKG
226	Linker	GSTSGSGKPGSSEGST
227	Linker	(GSGGS)n

228	Linker	(GGGS)n
229	Linker	GGSG
230	Linker	GGSGG
231	Linker	GSGSG
232	Linker	GSGGG
233	Linker	GGGSG
234	Linker	GSSSG
245	Linker	GPQGTAQ
250	Linker	YGAGLGW
264	CM	AQNLLGMY
265	CM	LSGRSDNHGGAVGLLAPP
266	CM	VHMPLGFLGPGGLSGRSDNH
267	CM	LSGRSDNHGGVHMPLGFLGP
268	CM	LSGRSDNHGGSGGSISSGLLSS
269	CM	ISSGLLSSGGSGGSLSGRSGNH
270	CM	LSGRSDNHGGSGGSQLRMA
271	CM	QNQALRMAGGSGGSLSGRSDNH
272	CM	LSGRSGNHGGSGGSQLRMA
273	CM	QNQALRMAGGSGGSLSGRSGNH
274	CM	ISSGLLSGRSGNH
275	CM	AVGLLAPPGGTSTSGRSANPRG
276	CM	TSTSGRSANPRGGGAVGLLAPP
277	CM	VHMPLGFLGPGGTSTSGRSANPRG
278	CM	TSTSGRSANPRGGGVHMPLGFLGP
279	CM	LSGRSGNHGGSGGSISSGLLSS
280	Cleavable Sequence	PRFKIIGG
281	Cleavable Sequence	PRFRIIGG
282	Cleavable Sequence	SSRHRRALD
283	Cleavable Sequence	RKSSIIIRMRDVVL
284	Cleavable Sequence	SSSFDKGKYKKGDDA

285	Cleavable Sequence	SSSFDKGKYKRGDDA
286	Cleavable Sequence	IEGR
287	Cleavable Sequence	IDGR
288	Cleavable Sequence	GGSIDGR
289	Cleavable Sequence	PLGLWA
290	Cleavable Sequence	GPQGIAGQ
291	Cleavable Sequence	GPQGLLGA
292	Cleavable Sequence	GIAGQ
293	Cleavable Sequence	GPLGIAGI
294	Cleavable Sequence	GPEGLRVG
295	Cleavable Sequence	YGAGLGVV
296	Cleavable Sequence	AGLGVER
297	Cleavable Sequence	AGLISST
298	Cleavable Sequence	EPQALAMS
299	Cleavable Sequence	QALAMSAI
300	Cleavable Sequence	AAYHLVSQ
301	Cleavable Sequence	MDAFLESS
302	Cleavable Sequence	ESLPVVAV
303	Cleavable Sequence	SAPAVESE
304	Cleavable Sequence	DVAQFVLT
305	Cleavable Sequence	VAQFVLT
306	Cleavable Sequence	VAQFVLTE
307	Cleavable Sequence	AQFVLTEG
308	Cleavable Sequence	PVQPIGPQ
309	IFN- α 2b-1204dL-hlgG4, with signal sequence	<i>METDTLLLVVLLVWPGSTGCDLPQTHSLGSRRTLMLLA</i> <i>QMRRISLFSCLKDRHDFGFPQEEFGNQFQKAETIPVLHE</i> <i>MIQQIFNLFSTKDSSAAWDETLLDKFYTELYQQNLNDEA</i> <i>CVIQGVGVTTETPLMKEDSILAVRKYFQRITLYLKEKKYS</i> <i>PCAWEVVRAEIMRSFSLSTNLQESLRSKESGRSDNIGGGS</i> <i>ESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEV</i>

		TCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQF NSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEK TISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFY PSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSRLTV DKSRWQQGNVFCFSVMHEALHNHYTQKSLSLS
310	IFN- α -1204dL- hIgG4 (polynucleotide)	ATGGAACCGACACACTGCTGCTGTGGGTGCTGCTTT TGTGGGTGCCAGGATCCACAGGCTGTGATCTGCCTCA AACGCATTTCATTGGGGTCCAGGCGCACGCTTATGTTG CTTGACAGATGAGGAGAATATCACTTTTCTCTTGCTT GAAGGACCGCCACGATTTTGGCTTTCCGCAGGAAGAG TTCGGTAACCAAGTTCCAAAAGGCAGAGACAATCCCCG TTTTGCATGAGATGATCCAACAGATCTTTAACCTGTTT TCAACCAAGGATAGCAGCGCAGCGTGGGATGAGACA CTGCTTGACAAGTTTTACACCGAGCTCTATCAGCAACT TAATGATCTCGAAGCCTGCGTAATTCAAGGAGTAGGC GTTACAGAGACACCTTTGATGAAGGAGGATTCCATCC TTGCAGTAAGAAAATACTTCCAGAGGATCACCTCTA CCTCAAAGAAAAGAAATACTCCCCATGCGCGTGGGAA GTAGTGCGAGCTGAAATAATGCGGAGCTTTTCTTTGTC AACTAATCTCCAAGAATCTCTGAGAAGCAAGGAGTCA GGTAGGTCTGATAATATCGGGGGAGGTTCTGAATCTA AGTACGGCCCTCCTTGTCTCCATGTCCTGCTCCAGAG TTTCTCGGAGGCCCCCTCCGTGTTCTGTTTCTCCAAA GCCTAAGGACACCCTGATGATCAGCAGAACCCTGAA GTGACCTGCGTGGTGGTCGACGTTTCACAAGAGGACC CCGAGGTGCAGTTCAATTGGTACGTGGACGGCGTGGA AGTGCACAACGCCAAGACCAAGCCTAGAGAGGAACA GTTCAACAGCACCTACAGAGTGGTGTCCGTGCTGACC GTGCTGCACCAGGATTGGCTGAACGGCAAAGAGTACA AGTGCAAGGTGTCCAACAAGGGCCTGCCTAGCAGCAT CGAGAAAACCATCAGCAAGGCCAAGGGCCAGCCAAG

		GGAACCCAGGTTTACACACTGCCACCTAGCCAAGAG GAAATGACCAAGAACCAGGTGTCCCTGACCTGCCTGG TCAAGGGCTTTTACCCCTCCGATATCGCCGTGGAATG GGAGAGCAATGGCCAGCCTGAGAACAACTACAAGAC CACACCTCCTGTGCTGGACAGCGACGGCTCATTCTTCC TGTACAGCAGACTGACCGTGGACAAGAGCAGATGGC AGCAGGGCAACGTGTTTACAGCTGCAGCGTGATGCACGA GGCCCTGCACAACCACTACACCCAGAAGTCTCTGAGC CTGAGCTGA
311	IFN- α 2b-1490DNI- hIgG4, with signal sequence	<i>METDILLWVLLWVPGSTG</i> CDLPQTHSLGSRRTLMLLA QMRRISLFSLKDRHDFGFPQEFGNQFQKAETIPVLHE MIQQIFNLFSTKDSSAAWDETLLDKFYTELYQQLNDLEA CVIQGVGVTTETPLMKEDSILAVRKYFQRITLYLKEKKYS PCAWEVVRAEIMRSFSLSTNLQESLRSKEISSGLLSGRSD NIGGGSESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMI SRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTK PREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGL PSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCL VKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLY SRLTVDKSRWQQGNVFSQSVMEALHNHYTQKSLSLS
312	IFN- α 2b-1490DNI- hIgG4 (polynucleotide)	ATGGAAACCGACACACTGCTGCTGTGGGTGCTGCTTT TGTGGGTGCCAGGATCCACAGGCTGTGATCTGCCTCA AACGCATTCATTGGGGTCCAGGCGCACGCTTATGTTG CTTGACAGATGAGGAGAATATCACTTTTCTCTTGCTT GAAGGACCGCCACGATTTTGGCTTTCCGCAGGAAGAG TTCGGTAACCAGTTCCAAAAGGCAGAGACAATCCCCG TTTTGCATGAGATGATCCAACAGATCTTTAACCTGTTT TCAACCAAGGATAGCAGCGCAGCGTGGGATGAGACA CTGCTTGACAAGTTTTACACCGAGCTCTATCAGCAACT TAATGATCTCGAAGCCTGCGTAATTCAAGGAGTAGGC GTTACAGAGACACCTTTGATGAAGGAGGATTCCATCC

		TTGCAGTAAGAAAATACTTCCAGAGGATCACCCCTCTA CCTCAAAGAAAAGAAATACTCCCCATGCGCGTGGGAA GTAGTGCAGCTGAAATAATGCGGAGCTTTTCTTTGTC AACTAATCTCCAAGAATCTCTGAGAAGCAAGGAGATT AGTTCTGGCCTGCTGTCAGGTAGGTCTGATAATATCG GGGAGGTTCTGAATCTAAGTACGGCCCTCCTTGTCCT CCATGTCTGCTCCAGAGTTTCTCGGAGGCCCTCCGT GTTCTGTTTCTCCAAAGCCTAAGGACACCCTGATGA TCAGCAGAACCCCTGAAGTGACCTGCGTGGTGGTCA CGTTTCACAAGAGGACCCCGAGGTGCAGTTCAATTGG TACGTGGACGGCGTGGAAGTGACACAACGCCAAGACC AAGCCTAGAGAGGAACAGTTCAACAGCACCTACAGA GTGGTGTCCGTGCTGACCGTGCTGCACCAGGATTGGC TGAACGGCAAAGAGTACAAGTGCAAGGTGTCCAACA AGGGCCTGCCTAGCAGCATCGAGAAAACCATCAGCAA GGCCAAGGGCCAGCCAAGGGAACCCAGGTTTACAC ACTGCCACCTAGCCAAGAGGAAATGACCAAGAACCA GGTGTCCCTGACCTGCCTGGTCAAGGGCTTTTACCCCT CCGATATCGCCGTGGAATGGGAGAGCAATGGCCAGCC TGAGAACAACTACAAGACCACACCTCCTGTGCTGGAC AGCGACGGCTCATTCTTCCTGTACAGCAGACTGACCG TGGACAAGAGCAGATGGCAGCAGGGCAACGTGTTCA GCTGCAGCGTGATGCACGAGGCCCTGCACAACCACTA CACCCAGAAGTCTCTGAGCCTGAGCTGA
316	ProC440	CDLPQTHSLGSRRITMLLAQMRRISLFSLKDRHDFGFP QEEFGNQFQKAETIPVLHEMIQQIFNLFSTKDSSAAWDET LLDKFYTELYQQLENDLEACVIQGVGVTTETPLMKEDSILA VRKYFQRITLYLKEKKYSPCAWEVVRAEIMRSFSLSTNL QESLRSKESGRSDNICPPCPAPEFLGGPSVFLFPPKPKDTL MISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAK TKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNK

		GLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLT CLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFF LYSRLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSL S
318	human IgG Fc with a knob mutation	CPPCPAPEFEGGPSVFLFPPKPKDTLMISRTPEVTCVVVD VSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRV VSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKG QPREPQVYTLPPCQEEMTKNQVSLWCLVKGFYPSDIAVE WESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQ EGNVFSCSVMHEALHNHYTQKSLSLSLG
319	human IgG Fc with a hole mutation	CPPCPAPEFEGGPSVFLFPPKPKDTLMISRTPEVTCVVVD VSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRV VSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKG QPREPQVCTLPPSQEEMTKNQVSLSCAVKGFYPSDIAVE WESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQ EGNVFSCSVMHEALHNRFYQKSLSLSLG
320	stub moiety	SDNI
321	ProC732	QSGQTDVDYYREWSWTQVSGSSGGSLSGRSDNIGSGGS CDLPQTHSLGSRRTLMLLAQMRRISLFSLKDRHDFGFP QEEFGNQFQKAETIPVLHEMIQQIFNLFSTKDSSAAWDET LLDKFYTELYQQLEACVIQGVGVTTETPLMKEDSILA VRKYFQRITLYLKEKKYSPCAWEVVRAEIMRSFSLSTNL QESLRSKELSGRSDNICPPCPAPEFLGGPSVFLFPPKPKDT LMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNA KTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSN KGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSL TCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSF FLYSRLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLS LS
322	ProC733	QSGQTDVDYYREWSWTQVSGSSGGSLSGRSDNIGSGGS CDLPQTHSLGSRRTLMLLAQMRRISLFSLKDRHDFGFP

		QEEFGNQFQKAETIPVLHEMIQQIFNLFSTKDSSAAWDET LLDKFYTELYQQQLNDLEACVIQGVGVTE TPLMKEDSILA VRKYFQRITLYLKEKKYSPCAWEVVRAEIMRSFSLSTNL QESLRSKEESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTL MISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAK TKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNK GLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLT CLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFF LYSRLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSL S
323	IFNa-2b masking peptide	TDVDYYREWSWTQVS
324	Linker	GSSGGS
325	Linker	ESKY
326	ProC286	ESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEV TCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQF NSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEK TISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFY PSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTV DKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSSGGGGS GRSDNIGGGSCDLPQTHSLGSRRTLMLLAQMRRISLFSC LKDRHDFGFPQEEFGNQFQKAETIPVLHEMIQQIFNLFST KDSSAAWDETLLDKFYTELYQQQLNDLEACVIQGVGVTE TPLMKEDSILAVRKYFQRITLYLKEKKYSPCAWEVVRAE IMRSFSLSTNLQESLRSKE
327	Linker	SGGG
328	Truncated IFNa-2b (amino acid 1-150)	CDLPQTHSLGSRRTLMLLAQMRRISLFCLKDRHDFGFP QEEFGNQFQKAETIPVLHEMIQQIFNLFSTKDSSAAWDET LLDKFYTELYQQQLNDLEACVIQGVGVTE TPLMKEDSILA VRKYFQRITLYLKEKKYSPCAWEVVRAEIMRS

329	IFNa-2b L130P mutant	CDLPQTHSLGSRRTLMLLAQMRRISLFSCCLKDRHDFGFP QEEFGNQFQKAETIPVLHEMIQQIFNLFSTKDSSAAWDET LLDKFYTELYQQNDLEACVIQGVGVTTETPLMKEDSILA VRKYFQRITLYPKEKKYSPCAWEVVRAEIMRSFSLSTNL QESLRSKE
331 to 479	PM sequences	(See Table 12 below for sequences)
480	ProC859 sequence	CDLPQTHSLGSRRTLMLLAQMRKISLFSCCLKDRHDFGFP QEEFGNQFQKAETIPVLHEMIQQIFNLFTTKDSSAAWDE DLLDKFCTELYQQNDLEACVMQEERVGETPLMNVDSI LAVKKYFRRITLYLTEKKYSPCAWEVVRAEIMRSLSLST NLQERLRRKELSGRSDNICPPCPAPEFLGGPSVFLFPPKPK DTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHN AKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVS NKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVS LTCLVKGFYPSDIAVEWESNGQPENNYKTTPVLDSGGS FFLYSRLTVDKSRWQQGNVFSQSVMEALHNHYTQKSLSLS
481	Universal IFN-alpha A/D sequence	CDLPQTHSLGSRRTLMLLAQMRKISLFSCCLKDRHDFGFP QEEFGNQFQKAETIPVLHEMIQQIFNLFTTKDSSAAWDE DLLDKFCTELYQQNDLEACVMQEERVGETPLMNVDSI LAVKKYFRRITLYLTEKKYSPCAWEVVRAEIMRSLSLST NLQERLRRKE
482	Interferon beta, Chain A, human (1AU1)	MSYNLLGFLQRSSNFQCQKLLWQLNGRLEYCLKDRMN FDIPEEIKQLQQFQKEDAALTIYEMLQNIFAIF RQDSSSTGWNETIVENLLANVYHQINHLKTVLEEKLEKE DFTRGKLMSSLHLKRYYGRIHLHYLKAKEYSH CAWTIVRVEILRNIFYFINRLTGYLNRN
483	IFNB_CHICK Q90873.1	MTANHQSPGMHSILLLLLLPAITTTTFCNHLRHQDANFS WKSLLQLQNTAPPPPQPCPQQDVTFFPETL

		LKSKDKKQAAITTLRILQHILFNMLSSPHTPKHWIDRTRH SLLNQIQHYIHHLEQCFVNQGTRSQRRGPRN AHL SINKYFRSIHNFLQHNNYSACTWDHVRLQARDCFR HVDTLIQWMKSRAPLTASSKRLNTQ
484	IFNA3_CANLF O97945.1	MALPCSFSVALVLLSCHSLCCLACHLPDTHSLRNWVRLT LLGQMRRLSASSCDHYTTDFAFPKELFDDGQR LQEAQALS VVHVMTQKVHFLFCTNTSSAPWNMTLLEEL CSGLSEQLDDLDACPLQEAGLAETPLMHEDST LRTYFQRISLYLQDRNHSPCAWEMVRAEIGRSFFSLTILQ ERVRRRK
485	IFN_ANAPL P51526.1	MPGPSAPPPPAIYSALALLLLTPPANAFSCSPLRLHDSAF AWDSLQLLRNMAPSPTQPCPQQHAPCSFP DTLLDTNDTQQAHTALHLLQHLLFDLSSPSTPAHWLH TARHDLLNQHQHHIHLERCFPADAARLHRRG PRNLHLSINKYFGCIQHFLQNHTYSPCAWDHVRLEAHAC FQRIHRLTRTMR
486	IFNAH_BOVIN P49878.1	MAPAWSFLLALLLLSCNAICSLGCHLPHTHSLPNRRVLT LLRQLRRVSPSSCLQDRNDFAFPQEALGGSQ LQKAQAISVLHEVTQHTFQLFSTEGSAAAWDESLLDKLR AALDQQLTDLQACLRQEEGLRGAPLLKEDAS LAVRKYFHRLTLYLREKRHNPCAWEVVRAEVMRAFSSS TNLQERFRRKD
487	IFNA1_CHICK P42165.1	MAVPASPQHPRGYGILLTLLLKALATTASACNHLRPQD ATFSDSLQLLRDMAPTLPQLCPQHNASCSF NDTILDTSNTRQADKTTHDILQHLLFKILSSPSTPAHWNDS QRQSLNRIHRYTQHLEQCLDSSDTRSRTR WPRNLHLTIKKHFSLHTFLQDNDYSACAWEHVRLQAR AWFLHHNLGTNTRT
488	IFNA_FELCA P35849.1	MALPSSFLVALVALGCNSVCSLGCGLPQTHGLLNRRALT LLGQMRRLPASSCQKDRNDFAFPQDVFGGDQ

		SHKAQALSVVHVTNQKIFHFFCTEASSSAAWNNTTLLEEF CTGLDRQLTRLEACVLQEVEEGEAPLTNEDI HPEDSILRNYFQRLSLYLQEKKYSPCAWEIVRAEIMRSLY YSSTALQKRLRSEK
489	interferon-beta-1 [Sus scrofa] AAA31056.1	MANKCILQIALLMCFSTTALSMSYDVLRYQQRSSNLAC QKLLGQLPGTPQYCLEDRMNFEVPPEIMQPPQ FQKEDAVLIHHEMLQQIFGILRRNFSSTGWNETVIKTILVE LDGQMDDLETILEEIMEEENFPRGDMTIL HLKKYYLSILQYLKSKEYRSCAWTVVQVEILRNFSFLNR LTDYLRN
490	IFNB2_BOVIN P01576.1	MTHRCLLQMVLLLCFSTTALSRSYSLRFFQQRSLALCQ KLLRQLPSTPQHCLARMDFQMPEEMKQAAQ FQKEDAILVIYEMLQQIFNILTRDFSSTGWSETHIEDLLEEL YEQMNHLEPIQKEIMQKQNSTMGDTTVL HLRKYYFNLVQYLKSKEYNRCAWTVVRVQILRNFSFLT RLTGYLE
491	A Chain A, INTERFERON- ALPHA 2B 1RH2	CDLPQTHSLGSRRTLMLLAQMRRISLFSLCKDRHDFGFP QEEFGNQFQKAETIPVLHEMIQQIFNLFSTK DSSAAWDETLLDKFYTELYQQNLNDEACVIQGVGTET PLMNEDSILAVRKYFQRITLYLKEKKYSPCAW EVRRAEIMRSFSLSTNLQESLRSKE
492	Linker	SGGGG
493	ProC288	CDLPQTHSLGSRRTLMLLAQMRRISLFSLCKDRHDFGFP QEEFGNQFQKAETIPVLHEMIQQIFNLFSTKDSSAAWDET LLDKFYTELYQQNLNDEACVIQGVGTETPLMKEDSILA VRKYFQRITLYLKEKKYSPCAWEVVRRAEIMRSFSLSTNL QESLRSKESGGGGSGRSDNICPPCPAPEFLGGPSVFLFPP KPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVE VHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKC KVSNGKLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKN QVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLD

		DGSFFLYSRLTVDKSRWQQGNVFSCSVMEALHNHYTQ KSLSLS
494	ProC289	CDLPQTHSLGSRRTLMMLLAQMRRISLFSLKDRHDFGFP QEEFGNQFQKAETIPVLHEMIQQIFNLFSTKDSSAAWDET LLDKFYTELYQQNDLEACVIQGVGVTTETPLMKEDSILA VRKYFQRITLYLKEKKYSPCAWEVVRAEIMRSFSLSTNL QESLRSKESGGGGSGRSDNIGPP CPP CPAPEFLGGPSVFL FPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVD GVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGK EYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEE MTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTP PVLDSGDSFFLYSRLTVDKSRWQQGNVFSCSVMEALH NHYTQKSLSLS
495	ProC290	CDLPQTHSLGSRRTLMMLLAQMRRISLFSLKDRHDFGFP QEEFGNQFQKAETIPVLHEMIQQIFNLFSTKDSSAAWDET LLDKFYTELYQQNDLEACVIQGVGVTTETPLMKEDSILA VRKYFQRITLYLKEKKYSPCAWEVVRAEIMRSFSLSTNL QESLRSKESGGGGSGRSDNIESKY GPP CPP CPAPEFLGGP SVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNW YVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWL NGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPS QEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK TTPPVLDSGDSFFLYSRLTVDKSRWQQGNVFSCSVME ALHNHYTQKSLSLS
496	ProC291	CDLPQTHSLGSRRTLMMLLAQMRRISLFSLKDRHDFGFP QEEFGNQFQKAETIPVLHEMIQQIFNLFSTKDSSAAWDET LLDKFYTELYQQNDLEACVIQGVGVTTETPLMKEDSILA VRKYFQRITLYLKEKKYSPCAWEVVRAEIMRSFSLSTNL QESLRSKESGGGGSGRSDNIGGGSESKY GPP CPP CPAPEF LGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEV QFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLH

		QDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVY TLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPE NNYKTTTPPVLDSDGSFFLYSRLTVDKSRWQQGNVFSCS VMHEALHNHYTQKSLSLS
497	ProC441	CDLPQTHSLGSRRTLMLLAQMRRISLFSLKDRHDFGFP QEEFGNQFQKAETIPVLHEMIQQIFNLFSTKDSSAAWDET LLDKFYTELYQQNDLEACVIQGVGVGTETPLMKEDSILA VRKYFQRITLYLKEKKYSPCAWEVVRAEIMRSFSLSTNL QESLRSKESGRSDNIGPPCPPCPAPEFLGGPSVFLFPPKPK DTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHN A
498	ProC442	CDLPQTHSLGSRRTLMLLAQMRRISLFSLKDRHDFGFP QEEFGNQFQKAETIPVLHEMIQQIFNLFSTKDSSAAWDET LLDKFYTELYQQNDLEACVIQGVGVGTETPLMKEDSILA VRKYFQRITLYLKEKKYSPCAWEVVRAEIMRSFSLSTNL QESLRSKESGRSDNIESKYGPPCPPCPAPEFLGGPSVFLFP PKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGV EVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEY KCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMT KNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVL DSDGSFFLYSRLTVDKSRWQQGNVFSCSVMHEALHNHY TQKSLSLS
499	ProC443	CDLPQTHSLGSRRTLMLLAQMRRISLFSLKDRHDFGFP QEEFGNQFQKAETIPVLHEMIQQIFNLFSTKDSSAAWDET LLDKFYTELYQQNDLEACVIQGVGVGTETPLMKEDSILA VRKYFQRITLYLKEKKYSPCAWEVVRAEIMRSFSLSTNL QESLRSKESGRSDNIGGGSESKYGPPCPPCPAPEFLGGPS VFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWY VDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLN GKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQ EEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKT

		TPPVLDSDGSFFLYSRLTVDKSRWQQGNVFSCSVMHEA LHNHYTQKSLSLS
235	ProC1023	QSGQTDVDYYREWSWTQVSGSSGGSLSGRSDNIGSGGS CDLPQTHSLGSRRTLMLLAQMRKISLFSCLKDRHDFGFP QEEFGNQFQKAETIPVLHEMIQQIFNLFTTKDSSAAWDE DLLDKFCTELYQQNDLEACVMQEERVGETPLMNVDSI LAVKKYFRRITLYLTEKKYSPCAWEVVRAEIMRSLSLSTN LQERLRRKELSGRSDNICPPCPAPEFLGGPSVFLFPPKPKD TLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNA KTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSN KGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSL TCLVKGFYPSDIAVEWESNGQPENNYKTTTPPVLDSDGSF FLYSRLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLS LS
236	ProC1549	QSGQTDVDYYREWSWTQVSGSSGGSGGGSGGGSGSGG SCDLPQTHSLGSRRTLMLLAQMRKISLFSCLKDRHDFGF PQEEFGNQFQKAETIPVLHEMIQQIFNLFTTKDSSAAWDE DLLDKFCTELYQQNDLEACVMQEERVGETPLMNVDSI LAVKKYFRRITLYLTEKKYSPCAWEVVRAEIMRSLSLST NLQERRRKEGGSGGGGSCPPCPAPEFLGGPSVFLFPPKPK DTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHN AKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVS NKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVS LTCLVKGFYPSDIAVEWESNGQPENNYKTTTPPVLDSDGS FFLYSRLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSL SLS
237	ProC659 (non- cleavable steric masked IFN-a2b)	CDLPQTHSLGSRRTLMLLAQMRRISLFSCLKDRHDFGFP QEEFGNQFQKAETIPVLHEMIQQIFNLFTTKDSSAAWDET LLDKFYTELYQQNDLEACVIQGVGTETPLMKEDSILA VRKYFQRITLYLKEKKYSPCAWEVVRAEIMRFSLSLSTNL QESLRSKEGGSGGGSCPPCPAPEFLGGPSVFLFPPKPKDT

		LMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNA KTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSN KGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSL TCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSF FLYSRLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLS LS
238	ProC1239 Arm 1	QSGQTDVDYYREWSWTQVSGSSGGSLSGRSDNIGSGGS CDLPQTHSLGSRRTLMLLAQMRRISLFSCLKDRHDFGFP QEEFGNQFQKAETIPVLHEMIQQIFNLFSTKDSSAAWDET LLDKFYTELYQQNDLEACVIQGVGVTTETPLMKEDSILA VRKYFQRITLYLKEKKYSPCAWEVVRAEIMRSFSLSTNL QESLRSKELSGRSDNICPPCPAPEFEGGPSVFLFPPKPKDT LMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNA KTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSN KGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSL WCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGS FFLYSRLTVDKSRWQEGNVFCFSVMHEALHNHYTQKSL SLSLGK
239	ProC1239 Arm 2	SDNICPPCPAPEFEGGPSVFLFPPKPKDTLMISRTPEVTCV VVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNST YRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISK AKGQPREPQVCTLPPSQEEMTKNQVSLSCAVKGFYPSDI AVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSR WQEGNVFCFSVMHEALHNRFTQKSLSLSLG
240	ProC1301	QSGQTDVDYYREWSWTQVSGSSGGS GGGSGGGSGSGG SCDLPQTHSLGSRRTLMLLAQMRRISLFSCLKDRHDFGF PQEEFGNQFQKAETIPVLHEMIQQIFNLFSTKDSSAAWDE TLLDKFYTELYQQNDLEACVIQGVGVTTETPLMKEDSIL AVRKYFQRITLYLKEKKYSPCAWEVVRAEIMRSFSLSTN LQESRSKEGGSGGGGSCPPCPAPEFLGGGPSVFLFPPKPKD TLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNA

		KTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSN KGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSL TCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSF FLYSRLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLS LS
241	CM	GLSGRSDNHG
242	Signal sequence	MRAWIFFLLCLAGRALA
243	Signal sequence	MALTFALLVALLVLSCKSSCSVG
244	Signal sequence	METDTLLLWVLLLWVPGSTG
246	spacer	QGQSGS
247	spacer	GQSGS
248	spacer	QSGS
249	spacer	QGQSGQG
251	spacer	GQSGQG
252	spacer	QSGQG
253	spacer	SGQG
254	spacer	QGQSGQ
255	spacer	GQSGQ
256	spacer	QSGQ
257	spacer	QGQSG
258	spacer	QQS
259	ProC1640	DNIGSGGSCDLPQTHSLGSRRTLMLLAQMRRISLFCLK DRHDFGFPQEEFGNQFQKAETIPVLHEMIQQIFNLFSTKD SSAAWDETLLDKFYTELYQQLNDLEACVIQGVGTETP LMKEDSILAVRKYFQRITLYLKEKKYSPCAWEVVRAEIM RSFSLSTNLQESLRSKELSGRS
260	ProC1822	KNLKSPQKVEVDIIDDNFILRWNRSDSVGNVTFSDYQ KTGMDNWKLSGCQNITSTKCNFSSLKLNVEEIKLRIRA EKENTSSWYEVDSFTPFKAQIGPPEVHLEAEDKAIVIH SPGTKDSVMWALDGLSFTYSLVIWKNSSGVEERIENIYS

		RHKIYKLSPETTYCLKVKAALLTSWKIGVYSPVHCIKTT VENELPPPENIEVSVQNNQNYVLKWDYTYANMTFQVQW LHAFLKRNPNGHLYKWKQIPDCENVKTTQCVFPQNVFQ KGIYLLRVQASDGNNTSFWSEEIKFDTEIQAFLLPVFNIR SLSDSFHIYIGAPKQSGNTPVIQDYPLIYEIIFWENTSNAE RKIIEKKTDTVVPNLKPLTVYCVKARAHTMDEKLNKSS VFSDAVCEKTKPGNTSKPKSCDKTHTCPPCPAPELLGGP SVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNW YVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWL NGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPP SREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNY KTTTPVLDSGDGSFFLYSKLTVDKSRWQQGNVFSQVMHE EALHNHYTQKSLSLSPG
261	ProC1823	ISYDSPDYTDESCTFKISLRNFRSILSWELKNHSIVPTHYT LLYTIMSKPEDLKVVKNCANTTRSFCDLTDEWRSTHEA YVTVLEGFSGNTTLFSCSHNFWLAIDMSFEPPEFEIVGFT NHINVMVKFPSIVEEELQFDLSLVIEEQSEGIVKKHKPEIK GNMSGNFTYIIDKLIPNTNYCVSVYLEHSDEQAVIKSPLK CTLLPPGQESESAESAAPKPKSCDKTHTCPPCPAPELLGGPS VFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWY VDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLN GKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSR EEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKT TPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSQVMHEA LHNHYTQKSLSLSPG
262	ProC1824	KNLKSPQKVEVDVIDDNFILRWNRSEESVGNVTFSDYQ KPEMDNWIKLPGCQNMTSTKCNFSSLKLNIIYDEIKLRIR AEKENTSSWCEVDSFTPFRAQIGPPEVHLEAEDKAIVY ISPPGTEDSVMWALDRSSFTYSLVIWKNSSSVEERIENIYS RHKISKLSPETTYCLKVKAALLTSRKIGVYGPVHCIKTTV ENELPPPENIEVIVQNNQNYVLKWDYTYANMTFQVQWLH

		AFLKRKPGNHLYKWKQIPDCENVTTTQCVPFPPNTFQKGI YLLRVQASDGNNTSFWSEEIKFDTEIQASLLPPVFENIRSLS DSLHISIGAPKWSENKPVIQDYPLIYEILFWENTSKAERKI IKKKTDTVIPNLKPLTVYCVKARAHSMDEKLNKSSVFS VVCEETKSGNTSKPKSCDKTHTCPPCPAPELLGGPSVFLF PPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDG VEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKE YKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREE MTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTP PVLDSGDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALH NHYTQKSLSLSPG
263	ProC1825	ISHDLPDYTSECTFKISLRNFRSILSWELKNHSIVATHYK LLYTIMSKPEDLKIVKNCANTTRSFCDLTDEWRSTHEAY VTSLEGFSGNTTLFNC SHNFWLDIDMSFEPPEFEIVGFTN HINVIVKFPSIVEEELQFDLSLVIEEQSEGIVKKHKPTIKG NMSGNFTYIIDKLIPNTNYCVSVYFDHNDQAVIKSPLKC TLLQPGQESESAESA KPKSCDKTHTCPPCPAPELLGGPSV FLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYV DGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNG KEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRE EMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTT PPVLDSGDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEAL HNHYTQKSLSLSPG
313	ProC1718	ISYDSPDYTDESCCTFKISLRNFRSILSWELKNHSIVPTHYT LLYTIMSKPEDLKVVKNCANTTRSFCDLTDEWRSTHEA YVTVLEGFSGNTTLFSC SHNFWLAIDMSFEPPEFEIVGFT NHINVMVKFPSIVEEELQFDLSLVIEEQSEGIVKKHKPEIK GNMSGNFTYIIDKLIPNTNYCVSVYLEHSDEQAVIKSPLK CTLLPPGQESESAESA KGGGGSHHHHHHHH
314	ProC1976	DNIGSGGSCDLPQTHSLGSRRTLMLLAQMRRLSFLSCLK DRHDFGFPQEEFGNQFQKAETIPVLHEMIQQIFNLSTKD

		SSAAWDETLLDKFYTELYQQNDLEACVIQGVGTETP LMKEDSILAVRKYFQRITLYLKEKKYSPCAWEVVRAEIM RSFSLSTNLQESLRSKELSGRS
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Table 12. Examples of Masking Peptides (PMs) Correlated with Appropriate Cytokines

Cytokine that may be coupled with the PM	PM Sequence	SEQ ID NO.
IFN	IAYLEYEHLHMAYG	331
IFN	TDVDYYREWCWTQVS	332
IFN	FPLNTFDLVHELLSR	333
IFN	FLNDIHRFLHWTDLN	334
IFN	PYTFVEQVEYWLHAT	335
IFN	ACVIHFLDRISNILE	336
IFN	FCYIAAFSAMQRQSC	337
IFN	PLYLPEIGWMFGLPT	338
IFN	TVLVIPDLHYLYVDR	339
IFN	FINNVETALDTIYNL	340
IFN	SAKHLHPGRLPPMTK	341
IFN	ATMYAYLERLEAILS	342
IFN	IYPLDALLRHLNSLC	343
IFN	CFPTVVWRELYNLYG	344
IFN	NLDFYLNHLYNTLAG	345
IFN	DFINSMRSHLQSSDQ	346
IFN	EPKCSFCSPLIVPSP	347
IFN	PNCIESFLSSIHDLS	348
IFN	TDNALFLETVQHYLY	349
IFN	CYPSISWLFADAPRN	350
IFN	ELTQLLNALVDVRNC	351
IFN	LLSSFVETMSSILTC	352

IFN	YLLRLPSLEELWGPS	353
IFN	ATCYIINHWWERYII	354
IFN	IAYLEYEHLHMAY	355
IFN	RVTCDDYYYGFGCNKFGRPA	356
IFN	MLAVVGAAALVLVAGAPWVLPAAAGGENLKPP ENIDVYIIDDNYTLKWSSHGESMGSVTFSAEYRT KDEAKWLKVPECQHNTTTTKCEFSLLDTNVYIKT QFRVRAEEGNSTSSWNEVDPFIPFYTAHMSPPPEV RLEAEDKAILVHISPPGQDGNMWALEKPSFSYTI RIWQKSSSDKKTINSTYYVEKIPELLPETTYCLEV KAHPSLKKHSNYSTVQCISTTVANKMPVPGNLQ VDAQGKSYVLKWDYIASADVLFRAQWLPGYSK SSSGSRSDKWKPIPTCANVQTTHCVFSQDTVYTG TFFLHVQASEGNHTSFWSSEKFIDSQKHILPPPPVI TVTAMSDTLLVYVNCQDSTCDGLNYEIIIFWENTS NTKISMEKDGPEFTLKNLQPLTVYCVQARVLFR ALLNKTSNFSEKLCEKTRPGSFST	357
IFN	MLLSQNAFIFRSLNLVLMVYISLVFGISYDSPDYT DESCTFKISLRNFRSILSWELKNHSIVPTHYTLTY TIMSKPEDLKVVKNCAANTTRSFCDLTDEWRSTH EAYVTVLEGFSGNTTLFSCSHNFWLAIDMSFEPP EFEIVGFTNHINVMVKFPSIVEEELQFDLSLVIEEQ SEGIVKKHKPEIKGNMSGNFTYIIDKLIPNTNYCV SVYLEHSDEQAVIKSPLKCTLLPPGQESESAESA	358
IFN	MMVVLLGATTLLVLVAVAPWVLSAAAGGKNLKS PQKVEVDIIDDNFILRWNRSDSVGNVTFSEFDYQ KTGMDNWIKLSGCQNITSTKCNFSSLKLNVEEII KLRIRAEKENTSSWYEVDSTPFRKAQIGPPEVH LEAEDKAIVIHISPGTKDSVMWALDGLSFTYSLVI WKNSSGVEERIEINISRHKIYKLSPETTYCLKVK	359

	AALLTSWKIGVYSPVHCKTTVENELPPPENIEVS VQNQNYVLKWDYTYANMTFQVQWLHAFKRN PGNHLYKWKQIPDCENVKTTQCVFPQNVFQKGI YLLRVQASDGNNTSFWSEEIKFDTEIQAFLLPPVF NIRSLSDSFHIYIGAPKQSGNTPVIQDYPLIYEIIFW ENTSNAERKIIKKTDVTPNLKPLTVYCVKARA HTMDEKLNKSSVFSDAVCEKTKPGNTSK	
IFN	MRSRCTVSAVGLLSLCLVVSASLETITPSAFDGY PDEPCTINITIRNSRLILSWELNKS GPPANYTLW YTVMSKDENLTKVKNCSDTTKSSCDVTDKWLE GMESYVVAIVIVHRGDLTVCRCSDYIVPANAPLE PPEFEIVGFTDHINVTMEFPPVTSKIIQEKMKTPF VIKEQIGDSVRKKHEPKVNNVTGNFTFVLRDLLP KTNYCVSLYFDDDDPAIKSPLKCIVLQPGQESGLSE SA	360
IFN- α	TDVDYYREWXXXXXXXXX (X may be any amino acid)	361
IFN- α and IFN- β	GSGTDVDYYREWSWTQV	362
IFN- α and IFN- β	GSGTDVDYYREWSWTQVS	363
IFN- α and IFN- β	TDVDYYREWSWTQV	364
IFN- α and IFN- β	TDVDYYREWSWTQVS	323
IFN- γ	ALTSTEDPEPPSPVPPTNVLIKSYNLNPVVCWEY QNMSQTPIFTVQVKVYSGSWTDSCTNISDHCCNI YEQIMYPDVSAWARVKAKVGQKESDYARSKEF LMCLKGKVGPPGLEIRRKKEEQLSVLVFHPEVV VNGESQGTMF GDGSTCYTFDYTVYVEHNRSGEI	366

	LHTKHTVEKEECNETLCELNISVSTLDSRYCLSV DGISSFQVRTEKSKDVCIPPFHDDRKDS	
IFN- γ	ASSPDSFSQLAAPLNPRHLHLYNDEQILTWEPS PSS NDPRPVVYQVEYSFIDGSWHRLLEPNCTDITETK CDLTGGGRLKLFHPFTVFLRVRAKRGNLTSKW VGLEPFQHYENVTVGPPKNISVTPGKGSLVIHFSP PFDVFHGATFQYLVHYWEKSETQQEQVEGPFKS NSIVLGNLKPVRVYCLQTEAQLILKNKKIRPHGL LSNVSCHETTANASARLQQVILPLGIFALLLGLT GACFTLFLKYQSRVKYWFQAPPNIPEQIEEYLKD PDQFILEVLKDGSPKEDSWDSVSIHSSPEKERDD VLQTP	367
IFN- γ	EMGTADLGPSSVPTPTNVTIESYNMNPVYWEYQ IMPQVPVFTVEVKNYGVKNSEWIDACINISHHC NISDHVGDPSNSLWVRVKARVGQKESAYAKSEE FAVCRDGGKIGPPKLDIRKEEKQIMIDIFHPSVFN GDEQEVDYDPETTCYIRVYNVYVRMNGSEIQYK ILTQKEDDCDEIQCQLAIPVSSLNSQYCVSAEGVL HVWGVTTTEKSKEVCITIFNSSIKG	368
IFN- γ	SQLPAPQHPKIRLYNAEQVLSWEPVALSNSTRPV VYQVQFKYTDSKWFTADIMSIGVNCTQITATECD FTAASPSAGFPMDFNVTLRLRAELGALHSAWVT MPWFQHYRNVTVGPPENIEVTPGEGSLIIRFSSPF DIADTSTAFFCYVHYWEKGGIQQVKGPFRSNSI SLDNLKPSRVYCLQVQAQLLWNKSNIFRVGHLS NISCYETMADASTELQQ	369
IL-12	CRTSECCFQDPPYPDADSGSASGPRDLRCYRISSD RYECSWQYEGPTAGVSHFLRCCLSSGRCCYFAA GSATRLQFSDQAGVSVLYTVTLWVESWARNQTE	370

	KSPEVTLQLYNSVKYEPPLGDIKVSCLAGQLRME WETPDNQVGAEVQFRHRTSPSPWKLGDGCPQD DDTESCLCPLEMNVAQEFQLRRRQLGSQGSSWS KWSSPVCVPPENPPQPQVRFSVEQLGQDGRRLT LKEQPTQLELPEGCQGLAPGTEVTYRLQLHMLSC PCKAKATRTLHLGKMPYLSGAAYNVAVISSNQF GPGLNQTHIPADTHTEPVALNISVGTNGTTMY WPARAQSMTYCIEWQPVGQDGGGLATCSLTAPQ DPDPAGMATYSWSRESGAMGQEKCYITIFASA HPEKLTWSTVLSTYHFGGNASAAAGTPHHVSVK NHSLSVSVDWAPSLSTCPGVLKEYVVRCRDE DSKQVSEHPVQPTETQVTL SGLRAGVAYTVQVR ADTAWLRGVWSQPQRFSIEVQVSDWLIF FASLGS FLSILLVGVLGYLGLNRAARHLCPLPTPC AS S AIEFPGGKETW QWINP VDF QEEASLQEAL VVEMS WDKGERTEPLEKTELPEGAP ELALDTELSLEDGDRCKAKM	
IL-12	KIDACKRGDVTVKPSHVILLGSTVNITCSLKPRQ GCFHYSRRNKLILYKFDRRINFHHGHSLSNSQVTG LPLGTTLFVCKLACINSDEIQICGAEIFVGVAPEQP QNLSCIQKGEQGT VACTWERGRDTHLYTEYTLQ LSGPKNLTWQKQCKDIYCDYLD FGINLTPESPES NFTAKVTAVNSLGSSSSLPSTFTFLDIVRPLPPWD IRIKFQKASVSRCTLYWRDEGLVLLNRLRYRPSN SRLWNMVNVT KAKGRHDLLDLKPFTEYEFQISS KLHLYKGSWSDWSESLRAQTPEEEPTGMLDVW YMKRHIDYSRQQISLFWKNLSVSEARGKILHYQ VTLQELTG GKAMTQNITGHTSWTTVIPRTGNWA VAVSAANSKGSSLPTRINIMNLCEAGLLAPRQVS ANSEGMDNILVTWQPPRKDPSAVQEYVVEWREL HPGGDTQVPLNWLRSRPYNVSALISENIKSYICY	371

	EIRVYALSGDQGGCSSILGNSKHKAPLSGPHINAI TEEKGSILISWNSIPVQEQMGCLLHYRIYWKERD SNSQPQLCEIPYRVSQNSHPINSLQPRVTYVLWM TALTAAGESSHGNREFCLQGKANWMAFVAPSI CIAIIMVGIFSTHYFQQKVFVLLAALRPQWCSREI PDPANSTCAKKYPIAEEKTQLPLDRLLIDWPTPE DPEPLVISEVLHQVTPVFRHPPCSNWPQREKGIQ GHQASEKDMMHSASSPPPPRALQAESRQLVDLY KVLESRGSDPKPENPACPWTVLPAGDLPTHDGY LPSNIDDLPSHEAPLADSLEELEPQHISLSVFPSSS LHPLTFSCGDKLTLDQLKMRCDSLML	
IL-12	NIDVCKLGTVTVQPAPVIPLGSAANISCSLNPQKG CSHYPPSSNELILLKFVNDVLVENLHGKKVHDHT GHSSTFQVTNLSLGMTLFVCKLNCNSQKKPPVP VCGVEISVGVAPEPPQNISCVQEGENGTVACSWN SGKVTYLKTNYTLQLSGPNNLTCQKQCFSDNRQ NCNRLDLGINLSPDLAESRFIVRVTAINDLGNSSS LPHTFTFLDIVIPLPPWDIRINFLNASGSRGTLQWE DEGQVVLNQLRYQPLNSTSWNMVNATNAKGKY DLRDLRPFTEYEFQISSKLHLSGGSWSNWSESLR TRTPEEPPVGILDIWYMKQDIDYDRQQISLFWKS LNPSEARGKILHYQVTLQEVTKKTTLQNTTRHTS WTRVIPRTGAWTASVSAANSKGASAPTHINIVDL CGTGLLAPHQVSAKSENMDNILVTWQPPKKADS AVREYIVEWRALQPGSITKFPPHWRIPPDMNSA LISENIKPYICYEIRVHALSESQGGCSSIRGDSKHK APVSGPHITAITEKKERLFISWTHIPFPEQRGCILH YRIYWKERDSTAQPELCEIQYRRSQNSHPISLQP RVTYVLWMTAVTAAGESPQGNREFCPQGKAN WKAFVISSICIAIITVGTFISIRYFRQKAFTLLSTLKP QWYSRTIPDPANSTWVKKYPILEEKIQLPTDNLL	372

	MAWPTPEEPEPLIIHEVLYHMIPVVRQPYYFKRG QGFQGYSTSKQDAMYIANPQATGTLTAETRQLV NLYKVLESRDPSKLANLTSPLTVTPVNYLPSHE GYLPSNIEDLSPHEADPTDSFDLEHQHISLSIFASS SLRPLIFGGERLTLDRLKMGYDSLMSNEA	
IL-12	QLGASGPGDGCCVEKTSFPEGASGSPLGPRNLSC YRVSKTDYECSWQYDGPEDNVSHVLWCCFVPP NHTHTGQERCRYFSSGPDRTVQFWEQDGIPVLS KVNFWVESRLGNRTMKSQKISQYLYNWTKTTPP LGHIKVSQSHRQLRMDWNVSEEAGAEVQFRRR MPTTNWTLGDCGPQVNSGSGVLGDIRGSMSESC LCPSENMAQEIQIRRRRLSSGAPGGPWSDWSMP VCVPPEVLPQALVPRGS	373

IL-12	QLGASGPGDGCCVEKTSFPEGASGSPLGPRNLSC YRVSKTDYECSWQYDGPEDNVSHVLWCCFVPP NHTHTGQERCRYFSSGPDRTVQFWEQDGIPVLS KVNFWVESRLGNRTMKSQKISQYLYNWTKTTPP LGHIKVSQSHRQLRMDWNVSEEAGAEVQFRRR MPTTNWTLGDCGPQVNSGSGVLGDIRGSMSESC LCPSENMAQEIQIRRRRLSSGAPGGPWSDW SMP VCVPEVLPQAKIKFLVEPLNQGGRRRLTMQGG SPQLAVPEGCRGRPGAQVKKHLVLRMLSCRCQ AQTSTVPLGKKLNLSGATYDLNVLA KTRFGRS TIQKWHLPAQELTETRALNVSVGGNMTSMQWA AQAPGTTYCLEWQPWFQHRNHTHCTLIVPEEED PAKMVTHSWSSKPTLEQEECYRITVFASKNPKNP MLWATVLSSYYFGGNASRAGTPRHVSVRNQTG DSVSVEWTASQLSTCPGVLTQYVVRC EAEDGA WESEWLVPPTKTQVTLDGLRSRVMYKVQVRAD TARLPGAWSHQPQRFSEVQISRLSIIFASLG SFASV LLVGSLGYIGLNRAAWHLCPPLPTPCGSTAVEFP GSQGKQAWQWCNPEDFPEVLYPRDALVVEMPG DRGDGTESQA APECALDTRRPLETQRQRQVQA LSEARRLGLAREDCPRGDLAHVTLP LLLGGVTQ GASVLDDLWRTHKTAEPGPPTLGQEA	374
IL-15	AVNGTSQFTCFYNSRANISCVWSQDGALQDTSC QVHAWPDRRRWNQTCELLPVSQASWACNLILG APDSQKLTTVDIVTLRVLCREGVRWRVMAIQDF KPFENLRMAPISLQVVHVETHRCNISWEISQAS HYFERHLEFEARTLSPGHTWEEAPLLTLKQKQE WICLETLTPDTQYEFQVRVKPLQGEFTTWSPWSQ PLAFRTKPAALGKDT	375

IL-15	ITCPPPMSVEHADIWVKSYSLSYRERYICNSGFKR KAGTSSLTECVLNKATNVAHWTTPSLKCIRDPAL VHQRPAAPP	376
IL-15	ITCPPPMSVEHADIWVKSYSLSYRERYICNSGFKR KAGTSSLTECVLNKATNVAHWTTPSLKCIRDP	377
IL-15	ITCPPPMSVEHADIWVKSYSLSYRERYICNSGFKR KAGTSSLTECVLNKATNVAHWTTPSLKCIR	378
IL-15	ITCPPPMSVEHADIWVKSYSLSYRERYICNSGFKR KAGTSSLTECVLNKATNVAHWTTPSLKCIRDPAL VHQRPAAPPSTVTTAGVTPQPESLSPSGKEPAASSP SSNNTAATTAIVPGSQLMPSKSPSTGTTEISSHE SSHGTPSQTTAKNWELTASASHQPPGVYPQGH SDTT	379
IL-15	ITCPPPMSVEHADIWVKSYSLSYRERYICNSGFKR KAGTSSLTECVLNKATNVAHWTTPSLKCIRDPAL VHQRPAAPPSTVTTAGVTPQPESLSPSGKEPAAS	380
IL-15	ITCPPPMSVEHADIWVKSYSLSYRERYICNSGFKR KAGTSSLTECVLNKATNVAHWTTPSLKCIRDPAL VHQRPAAPP	381
IL-15	MAPRRARGCRTLGLPALLLLLLLRPPATRGITCPP PMSVEHADIWVKSYSLSYRERYICNSGFKRKAG TSSLTECVLNKATNVAHWTTPSLKCIRDPALVHQ RPAPPSTVTTAGVTPQPESLSPSGKEPAASSPSSN NTAATTAIVPGSQLMPSKSPSTGTTEISSHESH GTPSQTTAKNWELTASASHQPPGVYPQGHSDTT VAISTSTVLLCGLSAVSLACYLKSRQTPPLASVE MEAMEALPVTWGTSSRDEDLNCSHHL	382
IL-2	AVKNCSHLECFYNSRANVSCMWSHEEALNVTT CHVHAKSNLRHWNKTCELTLVRQASWACNLIL GSFPESQSLTSVDLLDINVVCWEEKGWRRVKTC	383

	DFHPFDNLRLVAPHSLQVLHIDTQRCNISWKVSQ VSHYIEPYLEFEARRRLLGHSWEDASVLSLKQRQ QWLFLEMLIPSTSYEVQVRVKAQRNNTGTW SPW SQPLTFRTRPADPMKE	
IL-2	AVNGTSQFTCFYNSRANISCVWSQDGALQDTSC QVHAWPDRRRWNQTCELLPVSQASWACNLILG APDSQKLTTVDIVTLRVLCREGVRWRVMAIQDF KPFENLRLMAPISLQVVHVETHRCNISWEISQAS HYFERHLEFEARTLSPGHTWEEAPLLTLKQKQE WICL ETLTPDTQYEFQVRVKPLQGEFTTWSPWSQPLAF RTKPAALGKDT	384
IL-2	AVNGTSQFTCFYNSRANISCVWSQDGALQDTSC QVHAWPDRRRWNQTCELLPVSQASWACNLILG APDSQKLTTVDIVTLRVLCREGVRWRVMAIQDF KPFENLRLMAPISLQVVHVETHRCNISWEISQAS HYFERHLEFEARTLSPGHTWEEAPLLTLKQKQE WICLETLTPDTQYEF QVRVKPLQ	385
IL-2	AVNGTSQFTCFYNSRANISCVWSQDGALQDTSC QVHAWPDRRRWNQTCELLPVSQASWACNLILG APDSQKLTTVDIVTLRVLCREGVRWRVMAIQDF	386
IL-2	AVNGTSQFTCFYNSRANISCVWSQDGALQDTSC QVHAWPDRRRWNQTCELLPVSQASWACNLILG APDSQKLTTVDIVTLRVLCREGVRWRVMAIQDF KPFENLRLMAPISLQVVHVETHRCNISWEISQAS HYFERHLEFEARTLSPGHTWEEAPLLTLKQKQE WICLETLTPDTQYEFQVRVKPLQGEFTTWSPWSQ PLAFRTKPAALGKD	387

IL-2	AVNGTSQFTCFYNSRANISCVWSQDGALQDTSC QVHAWPDRRRWNQTCELLPVSQASWACNLILG APDSQKLTTVDIVTLRVLCREGVRWRVMAIQDF KPFENLRMAPISLQVVHVETHRSNISWEISQASH YFERHLEFEARTLSPGHTWEEAPLLTLKQKQEWI SLETLTPDTQYEFQVRVKPLQGEFTTWSPWSQPL AFRTKPAALGKD	388
IL-2	AVNGTSQFTCFYNSRANISCVWSQDGALQDTSC QVHAWPDRRRWNQTCELLPVSQASWACNLILG APDSQKLTTVDIVTLRVLCREGVRWRVMAIQDF KPFENLRMAPISLQVVHVETHRSNISWEISQASH YFEDHLEFEARTLSPGHTWEEAPLLTLKWKQEW ISLATLTPDTQYEFQVRVKPLQGEFTTWSPWSQP LAFRTKPAALGKD	389
IL-2	CGGHQYERRGGC	390
IL-2	CGGHYFERHGGC	391
IL-2	CQKLTTVDIC	392
IL-2	CSFHQYERHEGC	393
IL-2	CSGHQYERREGC	394
IL-2	CSGHYFERHEGC	395
IL-2	CSHYFERC	396
IL-2	DIVMTQSPLSLPVTGPASISCRSSQSLLHSNGY NYLDWYLQKPGQSPQLLIYLGSNRASGVDPDRFS GSGSGTDFTLKLISRVEAEDVGVIYCMQALQTP LTFGGGTKVEIKR	397
IL-2	DIVMTQTPLSSPVTLGQPASISCRSSQSLVHSDGN TYLSWLQQRPGQPPRLLIYKISNRFSGVDPDRFSGS GAGTDFTLKISRVEAEDVGVIYCMQATQFPTFG QGTKVEIKR	398

IL-2	DIVMTQTPLSSPVTLGQPASISCRSSQSLVHSDGN TYLSWLQQRPGQPPRLLIYKISNRFSGVPDRFSGS GAGTDFTLKISRVEAEDVGVYYCMQTSQFPTFG QGTKVEIKR	399
IL-2	DIVMTQTPLSSPVTLGQPASISCRSSQSLVHSDGN TYLSWLQQRPGQPPRLLIYKISNRFSGVPDRFSGS GAGTDFTLKISRVEAEDVGVYYCMQTTQFPTFG QGTKVEIKR	400
IL-2	DIVMTQTPLSSPVTLGQPASISCRSSQSLVHSDGN TYLSWLQQRPGQPPRLLIYKISNRFSGVPDRFSGS GAGTDFTLKISRVEAEDVGVYYCMQVTQFPTFG QGTKVEIKR	401
IL-2	EIVLTQSPGTLSSLSPGERATLSCRASQSVSSSYLA WYQQKPGQAPRLLIYGASSRATGIPDRFSGSGSG TDFTLISRLEPEDFAVYYCQYGGSSPLTFGGGT KVEIKR	402
IL-2	ELCDDDPPEIPHATFKAMAYKEGTILNCECKRGF RRIKSGSLYMLCTGNSSHSSWDNQCQCTSSATR NTTKQVTPQPEEQKERKTTEMQSPMQPVDQASL PGHCREPPPWENEATERIYHFVVGQMVYYQCVQ GYRALHRGPAESVCKMTHGKTRWTQPQLICTGE METSQFPGEEKPQASPEGRPESETSCLVTTTDFQI QTEMAATMETSIFTTEYQ	403
IL-2	ELCDDDPPEIPHATFKAMAYKEGTILNCECKRGF RRIKSGSLYMLCTGNSSHSSWDNQCQCTSSATR NTTKQVTPQPEEQKERKTTEMQSPMQPVDQASL PGHCREPPPWENEATERIYHFVVGQMVYYQCVQ GYRALHRGPAESVCKMTHGKTRWTQPQLICTGE METSQFPGEEKPQASPEGRPESETSC	404

IL-2	ELCDDDPPEIPHATFKAMAYKEGTILNCECKRGF RRIKSGSLYMLCTGNSSHSSWDNQCQCTSSATR NTTKQVTPQPEEQKERKTTEMQSPMQPVDQASL PGHCREPPPWENEATERIYHFVVGQMVYYQCVQ GYRALHRGPAESVCKMTHGKTRWTQPQLICTGE METSQFPGEEKP	405
IL-2	ELCDDDPPEIPHATFKAMAYKEGTILNCECKRGF RRIKSGSVYMLCTGNSSHSSWDNQCQCTSSATR NTTKQVTPQPEEQKERKTTEMQSPMQPVDQASL PGHCREPPPWENEATERIYHFVVGQMVYYQCVQ GYRALHRGPAESVCKMTHGKTRWTQPQLICTGE METSQFPGEEKPQASPEGRPESETSCLVTTTDFQI QTEMAATMETSIFTTEYQ	406
IL-2	ELCDDDPPEIPHATFKAMAYKEGTILNCECKRGF RRIKSGSVYMLCTGNSSHSSWDNQCQCTSSATR NTTKQVTPQPEEQKERKTTEMQSPMQPVDQASL PGHCREPPPWENEATERIYHFVVGQMVYYQCVQ GYRALHRGPAESVCKMTHGKTRWTQPQLICTGE METSQFPGEEKPQASPEGRPESETSC	407
IL-2	ELCDDDPPEIPHATFKAMAYKEGTILNCECKRGF RRIKSGSVYMLCTGNSSHSSWDNQCQCTSSATR NTTKQVTPQPEEQKERKTTEMQSPMQPVDQASL PGHCREPPPWENEATERIYHFVVGQMVYYQCVQ GYRALHRGPAESVCKMTHGKTRWTQPQLICTGE METSQFPGEEKP	408
IL-2	ELCDDDPPEIPHATFKAMAYKEGTMLNCECKRG FRRIKELVYMLCTGNSSHSSWDNQCQCTSSATR NTTKQVTPQPEEQKERKTTEMQSPMQPVDQASL PGHCREPPPWENEATERIYHFVVGQMVYYQCVQ GYRALHRGPAESVCKMTHGKTRWTQPQLICTGE	409

	METSQFPGEEKPQASPEGRPESETSCLVTTTDFQI QTEMAATMETSIFTTEYQ	
IL-2	ELCDDDPPEIPHATFKAMAYKEGTMLNCECKRG FRRIKELVYMLCTGNSSHSSWDNQCQCTSSATR NTTKQVTPQPEEQKERKTTEMQSPMQPVDQASL PGHCREPPPWENEATERIYHFVVGQMVYYQCVQ GYRALHRGPAESVCKMTHGKTRWTQPQLICTGE METSQFPGEEKPQASPEGRPESETSC	410
IL-2	ELCDDDPPEIPHATFKAMAYKEGTMLNCECKRG FRRIKELVYMLCTGNSSHSSWDNQCQCTSSATR NTTKQVTPQPEEQKERKTTEMQSPMQPVDQASL PGHCREPPPWENEATERIYHFVVGQMVYYQCVQ GYRALHRGPAESVCKMTHGKTRWTQPQLICTGE METSQFPGEEKP	411
IL-2	ELCDDDPPEIPHATFKAMAYKEGTMLNCECKRG FRRIKSGSLYMLCTGNSSHSSWDNQCQCTS	412
IL-2	ELCDDDPPEIPHATFKAMAYKEGTMLNCECKRG FRRIKSGSLYMLCTGNSSHSSWDNQCQCTSSATR NTTKQVTPQPEEQKERKTTEMQSPMQPVDQASL PGHCREPPPWENEATERIYHFVVGQMVYYQCVQ GYRALHRGPAESVCKMTHGKTRWTQPQLICTGE	413
IL-2	ELCDDDPPEIPHATFKAMAYKEGTMLNCECKRG FRRIKSGSLYMLCTGNSSHSSWDNQCQCTSSATR NTTKQVTPQPEEQKERKTTEMQSPMQPVDQASL PGHCREPPPWENEATERIYHFVVGQMVYYQCVQ GYRALHRGPAESVCKMTHGKTRWTQPQLICTGE METSQFPGEEKPQASPEGRPESETSCLVTTTDFQI QTEMAATMETSIFTTEYQ	414
IL-2	ELCDDDPPEIPHATFKAMAYKEGTMLNCECKRG FRRIKSGSLYMLCTGNSSHSSWDNQCQCTSSATR	415

	NTTKQVTPQPEEQKERKTTEMQSPMQPVDQASL PGHCREPPPWENEATERIYHFVVGQMVYYQCVQ GYRALHRGPAESVCKMTHGKTRWTQPQLICTGE METSQFPGEEKPQASPEGRPESETSC	
IL-2	ELCDDDPPEIPHATFKAMAYKEGTMLNCECKRG FRIKSGSLYMLCTGNSSHSSWDNQCQCTSSATR NTTKQVTPQPEEQKERKTTEMQSPMQPVDQASL PGHCREPPPWENEATERIYHFVVGQMVYYQCVQ GYRALHRGPAESVCKMTHGKTRWTQPQLICTGE METSQFPGEEKP	416
IL-2	ELCDDDPPEIPHATFKAMAYKEGTMLNCECKRG FRIKSGSLYMLCTGNSSHSSWDNQCQCTSSATR NTTKQVTPQPEEQKERKTTEMQSPMQPVDQASL PGHCREPPPWENEATERIYHFVVGQMVYYQCVQ GYRALHRGPAESVCKMTHGKTRWTQPQLICTGE METSQFPGEEKPQASPEGRPESETSCLVTTTDFQI QTEMAATMETSIFTTEYQ	417
IL-2	ELCDDDPPEIPHATFKAMAYKEGTMLNCECKRG FRIKSGSLYMLCTGNSSHSSWDNQCQCTSSATR NTTKQVTPQPEEQKERKTTEMQSPMQPVDQASL PGHCREPPPWENEATERIYHFVVGQMVYYQCVQ GYRALHRGPAESVCKMTHGKTRWTQPQLICTG	418
IL-2	ELCDDDPPEIPHATFKAMAYKEGTMLNCECKRG FRIKSGSLYMLCTGNSSHSSWDNQCQCTSSATR NTTKQVTPQPEEQKERKTTEMQSPMQPVDQASL PGHCREPPPWENEATERIYHFVVGQMVYYQCVQ GYRALHRGPAESVCKMTHGKTRWTQPQLICT	419
IL-2	ELCDDDPPEIPHATFKAMAYKEGTMLNCECKRG FRIKSGSLYMLCTGNSSHSSWDNQCQCTSSATR NTTKQVTPQPEEQKERKTTEMQSPMQPVDQASL PGHCREPPPWENEATERIYHFVVGQMVYYQCVQ	420

	GYRALHRGPAESVCKMTHGKTRWTQPQLICTGE METSQFPGEEKPQASPEGRPESETSCLVTTTDFQI QTEMAATMETS	
IL-2	ELCDDDPPEIPHATFKAMAYKEGTMLNCECKRG FRRIKSGSLYMLCTGSSSHSSWDNQCQCTSSATR STTKQVTPQPEEQKERKTTEMQSPMQPVDQASL PGHCREPPPWENEATERIYHFVVGQMVYYQCVQ GYRALHRGPAESVCKMTHGKTRWTQPQLICTGE	421
IL-2	ELCDDDPPEIPHATFKAMAYKEGTMLNCECKRG FRRIKSGSVYMLCTGNSSHSSWDNQCQCTSSATR NTTKQVTPQPEEQKERKTTEMQSPMQPVDQASL PGHCREPPPWENEATERIYHFVVGQMVYYQCVQ GYRALHRGPAESVCKMTHGKTRWTQPQLICTGE METSQFPGEEKPQASPEGRPESETSCLVTTTDFQI QTEMAATMETSIFTTEYQ	422
IL-2	ELCDDDPPEIPHATFKAMAYKEGTMLNCECKRG FRRIKSGSVYMLCTGNSSHSSWDNQCQCTSSATR NTTKQVTPQPEEQKERKTTEMQSPMQPVDQASL PGHCREPPPWENEATERIYHFVVGQMVYYQCVQ GYRALHRGPAESVCKMTHGKTRWTQPQLICTGE METSQFPGEEKPQASPEGRPESETSC	423
IL-2	ELCDDDPPEIPHATFKAMAYKEGTMLNCECKRG FRRIKSGSVYMLCTGNSSHSSWDNQCQCTSSATR NTTKQVTPQPEEQKERKTTEMQSPMQPVDQASL PGHCREPPPWENEATERIYHFVVGQMVYYQCVQ GYRALHRGPAESVCKMTHGKTRWTQPQLICTGE METSQFPGEEKP	424

IL-2	ELCLYDPPEIPHATFKAMAYKEGTILNCECKRGF RRIKSGSLYMLCTGNSSHSSWDNQCQCTSSATR NTTKQVTPQPEEQKERKTTEMQSPMQPVDQASL PGHCREPPPWENEATERIYHFVVGQMVYYQCVQ GYRALHRGPAESVCKMTHGKTRWTQPQLICTGE METSQFPGEEKPQASPEGRPESETSCLVTTTDFQI QTEMAATMETSIFTTEYQ	425
IL-2	ELCLYDPPEIPHATFKAMAYKEGTILNCECKRGF RRIKSGSLYMLCTGNSSHSSWDNQCQCTSSATR NTTKQVTPQPEEQKERKTTEMQSPMQPVDQASL PGHCREPPPWENEATERIYHFVVGQMVYYQCVQ GYRALHRGPAESVCKMTHGKTRWTQPQLICTGE METSQFPGEEKPQASPEGRPESETSC	426
IL-2	ELCLYDPPEIPHATFKAMAYKEGTILNCECKRGF RRIKSGSVYMLCTGNSSHSSWDNQCQCTSSATR NTTKQVTPQPEEQKERKTTEMQSPMQPVDQASL PGHCREPPPWENEATERIYHFVVGQMVYYQCVQ GYRALHRGPAESVCKMTHGKTRWTQPQLICTGE METSQFPGEEKPQASPEGRPESETSCLVTTTDFQI QTEMAATMETSIFTTEYQ	427
IL-2	ELCLYDPPEIPHATFKAMAYKEGTILNCECKRGF RRIKSGSVYMLCTGNSSHSSWDNQCQCTSSATR NTTKQVTPQPEEQKERKTTEMQSPMQPVDQASL PGHCREPPPWENEATERIYHFVVGQMVYYQCVQ GYRALHRGPAESVCKMTHGKTRWTQPQLICTGE METSQFPGEEKPQASPEGRPESETSC	428
IL-2	ELCLYDPPEIPHATFKAMAYKEGTILNCECKRGF RRIKSGSVYMLCTGNSSHSSWDNQCQCTSSATR NTTKQVTPQPEEQKERKTTEMQSPMQPVDQASL PGHCREPPPWENEATERIYHFVVGQMVYYQCVQ	429

	GYRALHRGPAESVCKMTHGKTRWTQPQLICTGE METSQFPGEEKP	
IL-2	ELCLYDPPEIPHATFKAMAYKEGTMLNCECKRG FRRIKSGSLYMLCTGNSSHSSWDNQCQCTSSATR NTTKQVTPQPEEQKERKTTEMQSPMQPVDQASL PGHCREPPPWENEATERIYHFVVGQMVYYQCVQ GYRALHRGPAESVCKMTHGKTRWTQPQLICTGE METSQFPGEEKPQASPEGRPESETSCLVTTTDFQI QTEMAATMETSIFTTEYQ	430
IL-2	ELCLYDPPEIPHATFKAMAYKEGTMLNCECKRG FRRIKSGSLYMLCTGNSSHSSWDNQCQCTSSATR NTTKQVTPQPEEQKERKTTEMQSPMQPVDQASL PGHCREPPPWENEATERIYHFVVGQMVYYQCVQ GYRALHRGPAESVCKMTHGKTRWTQPQLICTGE METSQFPGEEKPQASPEGRPESETSC	431
IL-2	ELCLYDPPEIPHATFKAMAYKEGTMLNCECKRG FRRIKSGSLYMLCTGNSSHSSWDNQCQCTSSATR NTTKQVTPQPEEQKERKTTEMQSPMQPVDQASL PGHCREPPPWENEATERIYHFVVGQMVYYQCVQ GYRALHRGPAESVCKMTHGKTRWTQPQLICTGE METSQFPGEEKP	432
IL-2	ELCLYDPPEIPHATFKAMAYKEGTMLNCECKRG FRRIKSGSVYMLCTGNSSHSSWDNQCQCTSSATR NTTKQVTPQPEEQKERKTTEMQSPMQPVDQASL PGHCREPPPWENEATERIYHFVVGQMVYYQCVQ GYRALHRGPAESVCKMTHGKTRWTQPQLICTGE METSQFPGEEKPQASPEGRPESETSCLVTTTDFQI QTEMAATMETSIFTTEYQ	433

IL-2	ELCLYDPPEIPHATFKAMAYKEGTMLNCECKRG FRRIKSGSVYMLCTGNSSHSSWDNQCQCTSSATR NTTKQVTPQPEEQKERKTTEMQSPMQPVDQASL PGHCREPPPWENEATERIYHFVVGQMVYYQCVQ GYRALHRGPAESVCKMTHGKTRWTQPQLICTGE METSQFPGEEKPQASPEGRPESETSC	434
IL-2	ELCLYDPPEIPHATFKAMAYKEGTMLNCECKRG FRRIKSGSVYMLCTGNSSHSSWDNQCQCTSSATR NTTKQVTPQPEEQKERKTTEMQSPMQPVDQASL PGHCREPPPWENEATERIYHFVVGQMVYYQCVQ GYRALHRGPAESVCKMTHGKTRWTQPQLICTGE METSQFPGEEKP	435
IL-2	ELCLYDPPEVPNATFKALSYKNGTILNCECKRGF RRLKELVYMRCLGNSWSSNCQCTS	436
IL-2	ELCLYDPPEVPNATFKALSYKNGTILNCECKRGF RRLKELVYMRCLGNSWSSNCQCTSNSHDKSRKQ VTAQLEHQKEQQTTTDMQKPTQSMHQENLTGH CREPPWKHEDSKRIYHFVEGQSVHYECIPGYKA LQRGPAISICKMKCGKTGWTQPQLTCVDEREHH RFLASEESQGSRNSSPESETSCPITTTDFPQPTETT AMTETFVLTMEYK	437
IL-2	ELCLYDPPEVPNATFKALSYKNGTILNCECKRGF RRLKELVYMRCLGNSWSSNCQCTSNSHDKSRKQ VTAQLEHQKEQQTTTDMQKPTQSMHQENLTGH CREPPWKHEDSKRIYHFVEGQSVHYECIPGYKA LQRGPAISICKMKCGKTGWTQPQLTCVDEREHH RFLASEESQGSRNS SPESETSCPITTTDFPQPTETTAMTETFVLTMEYK	438

IL-2	ELCLYDPPEVPNATFKALSYKNGTILNCECKRGF RRLKELVYMRCLGNSWSSNCQCTSSATRNTTKQ VTPQPEEQKERKTTEMQSPMQPVDQASLPGHCR EPPPWENEATERIYHFVVGQMVYYQCVQGYRA LHRGPAESVCKMTHGKTRWTQPQLICTGEMETS QFPGEEKPQASPEGRPESETSCLVTTTDFQIQTEM AATMETSIFTTEYQ	439
IL-2	EVQLVQSGAEVKKPGESLKISCKGSGYSFTSYWI GWVRQMPGKGLEWMGIIYPGDSDRYSPSFQGG VTISADKSISTAYLQWSSLKASDTAMYYPARQQ VAGMLDYWGQGTTVTVSS	440
IL-2	GMLSLAVNGTSQFTCFYNSRANISCVWSQDGL QDTSCQVHAWPDRRRWNQTCELLPVSQASWAC NLILGAPDSQKLTTVDIVTLRVLCREGVRWRVM AIQDFKPFENLRMAPISLQVVHVETHRCNISWEI SQASHYFERHLEFEARTLSPGHTWEEAPLLTLKQ KQEWICLETLTPDTQYEFQVRVKPLQAFRTLTGH	441
IL-2	GMLSLELCDDDPPEIPHATFKAMAYKEGTMLNC ECKRGFRRIKSGSLYMLCTGSSSHSSWDNQCQCT SSATRSTTKQVTPQPEEQKERKTTEMQSPMQPV DQASLPGHCREPPPWENEATERIYHFVVGQMVY YQCVQGYRALHRGPAESVCKMTHGKTRWTQPQ LICTGE	442
IL-2	GMLSLELCDDDPPEIPHATFKAMAYKEGTMLNC ECKRGFRRIKSGSLYMLCTGSSSHSSWDNQCQCT SSATRSTTKQVTPQPEEQKERKTTEMQSPMQPV DQASLPGHCREPPPWENEATERIYHFVVGQMVY YQCVQGYRALHRGPAESVCKMTHGKTRWTQPQ LICTGEAS	443

IL-2	GMLSLELCDDDPPEIPHATFKAMAYKEGTM LNC ECKRGFRRIKSGSLYMLCTGSSSSHSSWDNQCQCT SSATRSTTKQVTPQPEEQKERKTTEMQSPMQPV DQASLPGHCREPPPWENEATERIYHFVVGQMVY YQCVQGYRALHRGPAESVCKMTHGKTRWTQPQ LICTGEASGGGGHHHHHH	444
IL-2	GMLSLELCDDDPPEIPHATFKAMAYKEGTM LNC ECKRGFRRIKSGSLYMLCTGSSSSHSSWDNQCQCT SSATRSTTKQVTPQPEEQKERKTTEMQSPMQPV DQASLPGHCREPPPWENEATERIYHFVVGQMVY YQCVQGYRALHRGPAESVCKMTHGKTRWTQPQ LICTGEAS	445
IL-2	GMLSLELCDDDPPEIPHATFKAMAYKEGTM LNC ECKRGFRRIKSGSLYMLCTGSSSSHSSWDNQCQCT SSATRSTTKQVTPQPEEQKERKTTEMQSPMQPV DQASLPGHCREPPPWENEATERIYHFVVGQMVY YQCVQGYRALHRGPAESVCKMTHGKTRWTQPQ LICTGEASGGGGHHHHHH	446
IL-2	LNTTILTPNGNEDTTADFFLTMTPTDSL SVSTLPL PEVQCFVFVNVEYMNCTWNSSSEPQPTNL TLHYW YKNSDNDKVQKCSHYLFSEEITSGCQLQKKEIHL YQTFVVQLQDPREPRRQATQMLKLQNLVIPWAP ENLTLHKLSQSLELNWNNRFLNHCLEHLVQYR TDWDHSWTEQSVDYRHKFSLPSVDGQKRYTFR VRSRFNPLCGSAQHWSEWSHPIHWGSNTSKENP FLFALEAV	447
IL-2	MDSYLLMWGLLTFIMVPGCQAELCDDDPPEIPH ATFKAMAYKEGTM LNC ECKRGFRRIKSGSLYML CTGNSSHSSWDNQCQCTSSATRNTTKQVTPQPE EQKERKTTEMQSPMQPVDQASLPGHCREPPPW NEATERIYHFVVGQMVYYQCVQGYRALHRGPA	448

	ESVCKMTHGKTRWTQPQLICTGEMETSQFPGEE KPQASPEGRPESETSCLVTTTDFQIQTEMAATME TSIFTTEYQVAVAGCVFLLISVLLLSGLTWQRRQ RKSRRTI	
IL-2	MLSLELCDDDPPEIPHATFKAMAYKEGTMLNCE CKRGFRRIKSGSLYMLCTGSSSHSSWDNQCQCTS SATRSTTKQVTPQPEEQKERKTTEMQSPMQPVD QASLPGHCREPPPWENEATERIYHFVVGQMVYY QCVQGYRALHRGPAESVCKMTHGKTRWTQPQL ICTGE	449
IL-2	QKLTTVDI	450
IL-2	QVQLVESGGGVVQPGRSLRLSCAASGFTFSIYGM HWVRQAPGKGLEWVTVIWYDGSNEYADSVK GRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAR EDWLGEADYGMDVWGQGTTTVTVSS	451
IL-2	QVQLVESGGGVVQPGRSLRLSCAASGFTFSIYGM HWVRQAPGKGLEWVTVIWYDGSNEYADSVK GRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAR DGEQWRGFDYWGQGTTTVTVSS	452
IL-2	QVQLVESGGGVVQPGRSLRLSCAASGFTFSIYGM HWVRQAPGKGLEWVTVIWYDGSNEYADSVK GRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAR DQEQWRLAFDYWGQGTTTVTVSS	453
IL-2	QVQLVESGGGVVQPGRSLRLSCAASGFTFSIYGM HWVRQAPGKGLEWVTVIWYDGSNEYADSVK GRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAR GAVAGTGRDYYYYGMDVWGQGTTTVTVSS	454
IL-2	QVQLVESGGGVVQPGRSLRLSCAASGFTFSIYGM HWVRQAPGKGLEWVTVIWYDGSNEYADSVK	455

	GRFTISRDN SKNTLYLQMNSLRAEDTAVYYCAR GSYYDSSGYYYGEDFDYWGQGTTTVTVSS	
IL-2	QVQLVESGGGVVQPGRSLRLSCAASGFTFSIYGM HWVRQAPGKGLEWVTVIWYDGSNEY YADSVK GRFTISRDN SKNTLYLQMNSLRAEDTAVYYCAR EWELEDYGM DVWGQGTTTVTVSS	456
IL-2	QVQLVESGGGVVQPGRSLRLSCAASGFTFSIYGM HWVRQAPGKGLEWVTVIWYDGSNEY YADSVK GRFTISRDN SKNTLYLQMNSLRAEDTAVYYCAR DNWGSDAFDIWQGQGTTTVTVSS	457
IL-2	QVQLVESGGGVVQPGRSLRLSCAASGFTFSIYGM HWVRQAPGKGLEWVTVIWYDGSNEY YADSVK GRFTISRDN SKNTLYLQMNSLRAEDTAVYYCAR DDWFG EADYGM DVWGQGTTTVTVSS	458
IL-2	QVQLVESGGGVVQPGRSLRLSCAASGFTFSIYGM HWVRQAPGKGLEWVTVIWYDGSNEY YADSVK GRFTISRDN SKNTLYLQMNSLRAEDTAVYYCAR RISITPFDYWGQGTTTVTVSS	459
IL-2	QVQLVESGGGVVQPGRSLRLSCAASGFTFSIYGM HWVRQAPGKGLEWVTVIWYDGSNEY YADSVK GRFTISRDN SKNTLYLQMNSLRAEDTAVYYCAR DDFWSDY PFDYWGQGTTTVTVSS	460
IL-2	QVQLVESGGGVVQPGRSLRLSCAASGFTFSIYGM HWVRQAPGKGLEWVTVIWYDGSNEY YADSVK GRFTISRDN SKNTLYLQMNSLRAEDTAVYYCAR EWFGEADYGM DVWGQGTTTVTVSS	461
IL-2	QVQLVESGGGVVQPGRSLRLSCAASGFTFSIYGM HWVRQAPGKGLEWVTVIWYDGSNEY YADSVK GRFTISRDN SKNTLYLQMNSLRAEDTAVYYCAR GSYYDSSGYYYFGEDFDYWGQGTTTVTVSS	462

IL-2	QVQLVESGGGVVQPGRSLRLSCAASGFTFSIYGM HWVRQAPGKGLEWVTVIWYDGSNEYADSVK GRFTISRDN SKNTLYLQMNSLRAEDTAVYYCAR GTVAGTGRDY YYYGMDVWGQGTTVTVSS	463
IL-2	QVQLVESGGGVVQPGRSLRLSCAASGFTFSY G MHWVRQAPGKGLEWVA VIWYDGSNKYYADSV KGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCA REDFDSHYGMDVWGQGTTVTVSS	464
IL-2	SHYFER	465
IL-2	TLPLPEVQCFVFNVEYMNCTWNSSSEPQPTNLT LHYWYKNSDNDKVQKCSHYLFSEEITSGCQLQKK EIHLYQTFV VQLQDPREPRRQATQMLKLQNLVIP WAPENLTLHKLS ESQLELNWNNRFLNHCLEHLV QYRTDWDH SWTEQSV DYRHKFSLPSVDGQKRY TFRVRSR FNPLCGSAQHWSEWSHPIHWGSNT	466
IL-2	TLPLPEVQCFVFNVEYMNCTWNSSSEPQPTNLT LHYWYKNSDNDKVQKCSHYLFSEEITSGCQLQKK EIHLYQTFV VQLQDPREPRRQATQMLKLQNLVI	467
IL-2	WSSKVLMSANEDIKADLILTSTAPEHLSAPTLPL PEVQCFVFNIEYMNCTWNSSSEPQATNLT LHYR YKVSDNNTFQEC SHYLF SKEITSGCQIQKEDIQLY QTFV VQLQDPQKPQRRAVQKLN LQNLVIPRAPE NLTL SNLSESQLELRWKS RHIKERCLQYLVQYRS NRDRSWTE LIVNHEPRFSLPSVDELKRYTFRVRS RYNPICGSSQQW SKWSQPVHWGSHTVEENPSLF ALEA	468

IL-2 and IL-15	AVNGTSQFTCFYNSRANISCVWSQDGALQDTSC QVHAWPDRRRWNQTCELLPVSQASWACNLILG APDSQKLTTVDIVTLRVLCREGVRWRVMAIQDF KPFENLRLMAPISLQVVHVETHRCNISWEISQAS HYFERHLEFEARTLSPGHTWEEAPLLTLKQKQE WICLETLPDTQYEFQVRVKPLQGEFTTWSPWSQ PLAFRTKPAALGKDTGGGGSGGGSGGGSGSS GLSSGGSGGSLSGRSDNHGGGGSGGGGSLNTTI LTPNGNEDTTADFFLTMTDLSVSTLPLPEVQ CFVFNVEYMNCTWNSSSEPQPTNLTLYWYKNS DNDKVKCSHYLFSEEITSGCQLQKKEIHLQYTFV VQLQDPREPRRQATQMLKLQNLVIPWAPENLTL HKLSQSLELNWNNRFLNHCLEHLVQYRTDWD HSWTEQSVDYRHKFSLPSVDGQKRYTFRVRSRF NPLCGSAQHWSEWSHPIHWGSNTSKENPFLFAL EA	469
IL-2 and IL-15	AVNGTSQFTCFYNSRANISCVWSQDGALQDTSC QVHAWPDRRRWNQTCELLPVSQASWACNLILG APDSQKLTTVDIVTLRVLCREGVRWRVMAIQDF KPFENLRLMAPISLQVVHVETHRCNISWEISQAS HYFERHLEFEARTLSPGHTWEEAPLLTLKQKQE WICLETLPDTQYEFQVRVKPLQGEFTTWSPWSQ PLAFRTKPAALGKDTGGGGSGGGSGGGSGSS GLSSGGSGGSLSGRSDNHGGGGSGGGGSAVNG TSQFTCFYNSRANISCVWSQDGALQDTSCQVHA WPDRRRWNQTCELLPVSQASWACNLILGAPDSQ KLTTVDIVTLRVLCREGVRWRVMAIQDFKPFEN LRLMAPISLQVVHVETHRCNISWEISQASHYFER HLEFEARTLSPGHTWEEAPLLTLKQKQEWICLET LTPDTQYEFQVRVKPLQGEFTTWSPWSQPLAFRT KPAALGKDT	470

IL-2 and IL-15	AVNGTSQFTCFYNSRANISCVWSQDGALQDTSC QVHAWPDRRRWNQTCELLPVSQASWACNLILG APESQKLTTVDIVTLRVLCREGVRWRVMAIQDF KPFENLRMLAPISLQVVHVETHRCNISWEISQAS HYFERHLEFEARTLSPGHTWEEAPLLTLKQKQE WICLETLTPDTQYEFQVRVKPLQGEFTTWSPWSQ PLAFRTKPAALGKDT	471
IL-2 and IL-15	AVNGTSQFTCFYNSRANISCVWSQDGALQDTSC QVHAWPDRRRWNQTCELLPVSQASWACNLILG APDHQKLTTVDIVTLRVLCREGVRWRVMAIQDF KPFENLRMLAPISLQVVHVETHRCNISWEISQAS HYFERHLEFEARTLSPGHTWEEAPLLTLKQKQE WICLETLTPDTQYEFQVRVKPLQGEFTTWSPWSQ PLAFRTKPAALGKDT	472
IL-2 and IL-15	AVNGTSQFTCFYNSRANISCVWSQDGALQDTSC QVHAWPDRRRWNQTCELLPVSQASWACNLILG APDSQKLTTQDIVTLRVLCREGVRWRVMAIQDF KPFENLRMLAPISLQVVHVETHRCNISWEISQAS HYFERHLEFEARTLSPGHTWEEAPLLTLKQKQE WICLETLTPDTQYEFQVRVKPLQGEFTTWSPWSQ PLAFRTKPAALGKDT	473
IL-2 and IL-15	AVNGTSQFTCFYNSRANISCVWSQDGALQDTSC QVHAWPDRRRWNQTCELLPVSQASWACNLILG APDSQKLTTFDIVTLRVLCREGVRWRVMAIQDF KPFENLRMLAPISLQVVHVETHRCNISWEISQAS HYFERHLEFEARTLSPGHTWEEAPLLTLKQKQE WICLETLTPDTQYEFQVRVKPLQGEFTTWSPWSQ PLAFRTKPAALGKDT	474

IL-2 and IL-15	AVNGTSQFTCFYNSRANISCVWSQDGALQDTSC QVHAWPDRRRWNQTCELLPVSQASWACNLILG APDSQKLTTVDIVTLRVLCREGVRWRVMAIQDF KPFENLRMAPISLQVVHVETHRCNISWEISQAS HYFQRHLEFEARTLSPGHTWEEAPLLTLKQKQE WICLETLTPDTQYEFQVRVKPLQGEFTTWSPWSQ PLAFRTKPAALGKDT	475
IL-2 and IL-15	AVNGTSQFTCFYNSRANISCVWSQDGALQDTSC QVHAWPDRRRWNQTCELLPVSQASWACNLILG APDSQKLTTVDIVTLRVLCREGVRWRVMAIQDF KPFENLRMAPISLQVVHVETHRCNISWEISQAS HYFQRRLEFEARTLSPGHTWEEAPLLTLKQKQE WICLETLTPDTQYEFQVRVKPLQGEFTTWSPWSQ PLAFRTKPAALGKDT	476
IL-2 and IL-15	AVNGTSQFTCFYNSYANISCVWSQDGALQDTSC QVHAWPDRRRWNQTCELLPVSQASWACNLILG APDSQKLTTVDIVTLRVLCREGVRWRVMAIQDF KPFENLRMAPISLQVVHVETHRCNISWEISQAS HYFERHLEFEARTLSPGHTWEEAPLLTLKQKQE WICLETLTPDTQYEFQVRVKPLQGEFTTWSPWSQ PLAFRTKPAALGKDT	477
IL-2, IL-15, and IL-21	LNTTILTPNGNEDTTADFFLTMTPTDSLVSSTLPL PEVQCFVFVNVEYMNCTWNSSSEPQPTNLTLHYW YKNSDNDKVQKCSHYLFSEEITSGCQLQKKEIHL YQTFVVQLQDPREPRRQATQMLKLQNLVIPWAP ENLTLHKLSESQLELNWNNRFLNHCLEHLVQYR TDWDHSWTEQSVDIRHKFSLPSVDGQKRYTFR VRSRFNPLCGSAQHWSEWSHPIHWGSNTSKENP FLFALEA	478

IL-21	CPDLVCYTDYLTQTVICILEMWNLHPSTLTLTWQ DQYEELKDEATSCSLHRSAHNATHATYTCHMDV FHFMAADDIFSVNITDQSGNYSQECGSFLLAESIKP APPFNVTVTFSGQYNISWRSDYEDPAFYMLKGK LQYELQYRNRGDPWAVSPRRKLISVDSRSVSLLP LEFRKDSSYELQVRAGPMPGSSYQGTWSEWSDP VIFQTQSEELKE	479
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OTHER EMBODIMENTS

It is to be understood that while the invention has been described in conjunction with the detailed description thereof, the foregoing description is intended to illustrate
 5 and not limit the scope of the invention, which is defined by the scope of the appended claims. Other aspects, advantages, and modifications are within the scope of the following claims.

WHAT IS CLAIMED IS:

1. An activatable cytokine construct (ACC) that includes a first monomer construct and a second monomer construct, wherein:
 - (a) the first monomer construct comprises a first peptide mask (PM1), a first mature cytokine protein (CP1), a first and a third cleavable moieties (CM1 and CM3), and a first dimerization domain (DD1), wherein the CM1 is positioned between the CP1 and the DD1 and the CM3 is positioned between the PM1 and the CP1; and
 - (b) the second monomer construct comprises a second mature cytokine protein (CP2), a second cleavable moiety (CM2), and a second dimerization domain (DD2), wherein the CM2 is positioned between the CP2 and the DD2; wherein the DD1 and the DD2 bind to each other thereby forming a dimer of the first monomer construct and the second monomer construct, the ACC is characterized in that it has at least one of the following characteristics:
 - (i) a structural arrangement in an N- to C-terminal direction comprising: PM1-CM3-CP1-CM1-DD1 and CP2-CM2-DD2, wherein DD1 and DD2 are dimerized;
 - (ii) wherein the first monomer construct is characterized in that the CP1 and the DD1 are linked by a linking region of no more than 18 amino acids such that the linking region of no more than 18 amino acids includes the CM3;
 - (iii) wherein the second monomer construct is characterized in that the CP2 and the DD2 are linked by a linking region of no more than 18 amino acids such that the linking region of no more than 18 amino acids includes the CM2;
 - (iv) wherein each of PM1 and PM2 is less than 40 amino acids;
 - (v) wherein each of PM1 and PM2 is between 13 and 49 amino acids; and/or
 - (vi) wherein each of PM1 and PM2 is not a receptor for the CP1 and the CP2 and wherein each of PM1 and PM2 is not a fragment of receptor for the CP1 and the CP2.

2. The ACC of claim 1, wherein the second monomer construct further comprises a second peptide mask (PM2) and a fourth cleavable moiety (CM4), wherein the CM4 is positioned between the PM2 and the CP2.
3. The ACC of claim 1 or 2, wherein the first monomer construct comprises a first polypeptide that comprises the PM1, the CM3, the CP1, the CM1, and the DD1.
4. The ACC of any one or combination of claims 1-3, wherein the second monomer construct comprises a second polypeptide that comprises the CP2, the CM2, and the DD2.
5. The ACC of claim 2, wherein the second monomer construct comprises a second polypeptide that comprises the PM2, the CM4, the CP2, the CM2, and the DD2.
6. The ACC of any one or combination of claims 1-5, wherein the CP1 and/or the CP2 is/are each individually selected from the group consisting of: an interferon, an interleukin, GM-CSF, G-CSF, LIF, OSM, CD154, LT- β , TNF- α , TNF- β , 4-1BBL, APRIL, CD70, CD153, CD178, GITRL, LIGHT, OX40L, TALL-1, TRAIL, TWEAK, TRANCE, TGF- β 1, TGF- β 1, TGF- β 3, Epo, Tpo, Flt-3L, SCF, M-CSF, and MSP, optionally wherein the CP1 and/or the CP2 is independently selected from IL-2, IL-7, IL-8, IL-10, IL-12, IL-15, IL-21, an IFN-alpha, an IFN beta, an IFN gamma, GM-CSF, TGF-beta, LIGHT, GITR-L, CD40L, CD27L, 4-1BB-L, OX40, and OX40L.
7. The ACC of any one or combination of claims 1-6, wherein:
the PM1 comprises a sequence selected from the group consisting of SEQ ID NOs: 328, 329, 323, and 331-369, and the CP1 is an interferon;
the PM1 comprises a sequence selected from the group consisting of SEQ ID NOs: 328, 329, 323, and 331-364, and the CP1 is an interferon alpha;

the PM1 comprises a sequence selected from the group consisting of SEQ ID NOs: 331-360, 362-364, and the CP1 is an interferon beta;

the PM1 comprises a sequence selected from the group consisting of SEQ ID NOs: 331-360, 366-369, and the CP1 is an interferon gamma;

the PM1 comprises a sequence selected from the group consisting of SEQ ID NOs: 370-374, and the CP1 is an IL-12;

the PM1 comprises a sequence selected from the group consisting of SEQ ID NOs: 375-382, 469-477, 478, and the CP1 is an IL-15;

the PM1 comprises a sequence selected from the group consisting of SEQ ID NOs: 383-468, 469-478, and the CP1 is an IL-2; or

the PM1 comprises a sequence selected from the group consisting of SEQ ID NOs: 478 and 479, and the CP1 is an IL-21.

8. The ACC of any one or combination of claims 2-7, wherein:

the PM2 comprises a sequence selected from the group consisting of SEQ ID NOs: 328, 329, 323, and 331-369, and the CP2 is an interferon;

the PM2 comprises a sequence selected from the group consisting of SEQ ID NOs: 328, 329, 323, and 331-364, and the CP2 is an interferon alpha;

the PM2 comprises a sequence selected from the group consisting of SEQ ID NOs: 331-360, 362-364, and the CP2 is an interferon beta;

the PM2 comprises a sequence selected from the group consisting of SEQ ID NOs: 331-360, 366-369, and the CP2 is an interferon gamma;

the PM2 comprises a sequence selected from the group consisting of SEQ ID NOs: 370-374, and the CP2 is an IL-12;

the PM2 comprises a sequence selected from the group consisting of SEQ ID NOs: 375-382, 469-477, 478, and the CP2 is an IL-15;

the PM2 comprises a sequence selected from the group consisting of SEQ ID NOs: 383-468, 469-478, and the CP2 is an IL-2; or

the PM2 comprises a sequence selected from the group consisting of SEQ ID NOs: 478 and 479, and the CP2 is an IL-21.

9. The ACC of any one or combination of claims 2-8, wherein PM1, PM2, or PM1 and PM2 comprises SEQ ID NO: 323.
10. The ACC of any one or combination of claims 2-9, wherein PM1, PM2, or PM1 and PM2 comprises SEQ ID NO: 331.
11. The ACC of any one or combination of claims 2-10, wherein PM1, PM2, or PM1 and PM2 comprises SEQ ID NO: 332.
12. The ACC of any one or combination of claims 2-11, comprising a mask linking region between PM1 and CP1 that comprises 15, 16, 17, 18, 19, 20, 21, or 22 amino acids.
13. The ACC of any one or combination of claims 2-12, comprising a mask linking region between PM2 and CP2 that comprises 15, 16, 17, 18, 19, 20, 21, or 22 amino acids.
14. The ACC of any one or combination of claims 1 to 13, wherein the DD1 and the DD2 are a pair selected from the group consisting of: a pair of Fc domains; a sushi domain from an alpha chain of human IL-15 receptor (IL15R α) and a soluble IL-15; barnase and barnstar; a PKA and an AKAP; adapter/docking tag modules based on mutated RNase I fragments; an epitope and sdAb; an epitope and scFv; and SNARE modules based on interactions of the proteins syntaxin, synaptotagmin, synaptobrevin, and SNAP25; an antigen-binding domain and an epitope.
15. The ACC of claim 14, wherein the DD1 and the DD2 are a pair of Fc domains.
16. The ACC of claim 15, wherein the pair of Fc domains is a pair of human Fc domains.
17. The ACC of claim 16, wherein the human Fc domains are human IgG1 Fc domains, human IgG2 Fc domains, human IgG3 Fc domains, or human IgG4 Fc domains.

18. The ACC of claim 17, wherein the human Fc domains are human IgG4 Fc domains.
19. The ACC of claim 16, wherein the human Fc domains comprise a sequence that is at least 80% identical to SEQ ID NO: 3.
20. The ACC of claim 19, wherein the human Fc domains comprise a sequence that is at least 90% identical to SEQ ID NO: 3.
21. The ACC of claim 20, wherein the human Fc domains comprise SEQ ID NO: 3.
22. The ACC of claim 14, wherein the DD1 and the DD2 comprise SEQ ID NOs: 318 and 319, respectively.
23. The ACC of any one or combination of claims 1-21, wherein the DD1 and the DD2 are the same.
24. The ACC of claim 14, wherein the DD1 comprises an antigen-binding domain and DD2 comprises a corresponding epitope.
25. The ACC of claim 24, wherein the antigen-binding domain is an anti-His tag antigen-binding domain and wherein the DD2 comprises a His tag.
26. The ACC of claim 24, wherein the antigen-binding domain is a single chain variable fragment (scFv).
27. The ACC of claim 24, wherein the antigen-binding domain is a single domain antibody (sdAb).

28. The ACC of claim 14, wherein at least one of the DD1 and the DD2 comprises a dimerization domain substituent selected from the group consisting of a non-polypeptide polymer and a small molecule.
29. The ACC of claim 28, wherein the DD1 and the DD2 comprise non-polypeptide polymers covalently bound to each other.
30. The ACC of claim 29, wherein the non-polypeptide polymer is a sulfur-containing polyethylene glycol, and wherein the DD1 and the DD2 are covalently bound to each other via one or more disulfide bonds.
31. The ACC of claim 28, wherein at least one of the DD1 and the DD2 comprises a small molecule.
32. The ACC of claim 31, wherein the small molecule is biotin.
33. The ACC of claim 32, wherein DD1 comprises biotin and DD2 comprises an avidin.
34. The ACC of any one or combination of claims 1-33, wherein the CP1 and the CP2 are mature cytokines.
35. The ACC of any one or combination of claims 1-33, wherein the CP1 and the CP2 comprise a signal peptide.
36. The ACC of any one or combination of claims 1-35, wherein the CP1 and the CP2 are the same.
37. The ACC of any one or combination of claims 1-35, wherein the CP1 and the CP2 are different.

38. The ACC of any one or combination of claims 1-35, wherein the CP1 and/or the CP2 is/are an interferon.
39. The ACC of claim 38, wherein the CP1 and the CP2 are an interferon.
40. The ACC of claim 38, wherein the CP1 and the CP2 are different interferons.
41. The ACC of claim 38, wherein the CP1 and the CP2 are the same interferon.
42. The ACC of any one one or combination of claims 38-41, wherein the interferon(s) is/are a human wildtype mature interferon.
43. The ACC of any one one or combination of claims 38-42, wherein the interferon(s) is/are selected from the group consisting of: interferon-alpha, interferon-beta, interferon-omega, and interferon-tau.
44. The ACC of claim 43, wherein the interferons is/are an interferon-alpha.
45. The ACC of claim 44, wherein the interferon(s) is/are selected from the group consisting of: interferon alpha-2a, interferon alpha-2b, and interferon alpha-n3.
46. The ACC of claim 45, wherein the interferon(s) is/are interferon alpha-2b.
47. The ACC of claim 46, wherein the CP1 and/or the CP2 comprises a sequence that is at least 80% identical to SEQ ID NO: 1.
48. The ACC of claim 47, wherein the CP1 and/or the CP2 comprises a sequence that is at least 90% identical to SEQ ID NO: 1.
49. The ACC of claim 48, wherein the CP1 and/or the CP2 comprises the sequence of SEQ ID NO: 1.

50. The ACC of claim 43, wherein the interferon is an interferon beta.
51. The ACC of claim 50, wherein the interferon beta is selected from the group consisting of interferon beta-1a, and interferon beta-1b.
52. The ACC of any one or combination of claims 1-51, wherein the CP1 and/or the CP2 comprises an IFab domain.
53. The ACC of any one or combination of claims 1-38, wherein the CP1 and/or the CP2 comprises an interleukin.
54. The ACC of claim 53, wherein the interleukin is selected from the group consisting of IL-1 α , IL-1 β , IL-1RA, IL-18, IL-2, IL-4, IL-7, IL-9, IL-13, IL-15, IL-3, IL-5, IL-6, IL-11, IL-12, IL-10, IL-20, IL-21, IL-14, IL-16, and IL-17.
55. The ACC of any one or combination of claims 1-54, wherein each of the CM1 and the CM2 comprises a total of about 3 amino acids to about 15 amino acids.
56. The ACC of any one or combination of claims 1-55, wherein one or more of the CM1, the CM2, the CM3, and the CM4 comprise substrates for different proteases.
57. The ACC of any one or combination of claims 1-56, wherein the CM1, the CM2, and the CM3 comprise substrates for the same protease.
58. The ACC of any one or combination of claims 1-57, wherein the protease(s) is/are selected from the group consisting of: ADAM8, ADAM9, ADAM10, ADAM12, ADAM15, ADAM17/TACE, ADAMDEC1, ADAMTS1, ADAMTS4, ADAMTS5, BACE, Renin, Cathepsin D, Cathepsin E, Caspase 1, Caspase 2, Caspase 3, Caspase 4, Caspase 5, Caspase 6, Caspase 7, Caspase 8, Caspase 9,

Caspase 10, Caspase 14, Cathepsin B, Cathepsin C, Cathepsin K, Cathepsin L, Cathepsin S, Cathepsin V/L2, Cathepsin X/Z/P, Cruzipain, Legumain, Otubain-2, KLK4, KLK5, KLK6, KLK7, KLK8, KLK10, KLK11, KLK13, KLK14, Meprin, Neprilysin, PSMA, BMP-1, MMP-1, MMP-2, MMP-3, MMP-7, MMP-9, MMP-10, MMP-11, MMP-12, MMP-13, MMP-14, MMP-15, MMP-16, MMP-17, MMP-19, MMP-20, MMP-23, MMP-24, MMP-26, MMP-27, activated protein C, cathepsin A, cathepsin G, Chymase, FVIIa, FIXa, FXa, FXIa, FXIIa, Elastase, Granzyme B, Guanidinobenzoate, HtrA1, human neutrophil lyase, lactoferrin, marapsin, NS3/4A, PACE4, Plasmin, PSA, tPA, thrombin, tryptase, uPA, DESC1, DPP-4, FAP, Hepsin, Matrilysin-2, MT-SP1/Matrilysin, TMPRSS2, TMPRSS3, and TMPRSS4.

59. The ACC of claim 58, wherein the protease(s) is/are selected from the group consisting of: uPA, legumain, MT-SP1, ADAM17, BMP-1, TMPRSS3, TMPRSS4, MMP-2, MMP-9, MMP-12, MMP-13, and MMP-14.
60. The ACC of any one or combination of claim 1-57, wherein the CM1, CM2, CM3, and/or the CM4 comprise a sequence selected from the group consisting of: LSGRSDNH (SEQ ID NO: 5), TGRGPSWV (SEQ ID NO: 6), PLTGRSGG (SEQ ID NO: 7), TARGPSFK (SEQ ID NO: 8), NTLSGRSENHSG (SEQ ID NO: 9), NTLSGRSGNHGS (SEQ ID NO: 10), TSTSGRSANPRG (SEQ ID NO: 11), TSGRSANP (SEQ ID NO: 12), VHMLPLGFLGP (SEQ ID NO: 13), AVGLLAPP (SEQ ID NO: 14), AQNLLGMV (SEQ ID NO: 15), QNQALRMA (SEQ ID NO: 16), LAAPLGLL (SEQ ID NO: 17), STFPFGMF (SEQ ID NO: 18), ISSGLLSS (SEQ ID NO: 19), PAGLWLDL (SEQ ID NO: 20), VAGRSMRP (SEQ ID NO: 21), VVPEGRRS (SEQ ID NO: 22), ILPRSPAF (SEQ ID NO: 23), MVLGRSLL (SEQ ID NO: 24), QGRAITFI (SEQ ID NO: 25), SPRSIMLA (SEQ ID NO: 26), SMLRSMPL (SEQ ID NO: 27), ISSGLLSGRSDNH (SEQ ID NO: 28), AVGLLAPPGGLSGRSDNH (SEQ ID NO: 29), ISSGLSSGGSGGLSGRSDNH (SEQ ID NO: 30), LSGRSGNH (SEQ ID NO: 31), SGRSANPRG (SEQ ID NO: 32), LSGRSDDH (SEQ ID NO: 33),

LSGRSDIH (SEQ ID NO: 34), LSGRSDQH (SEQ ID NO: 35), LSGRSDTH (SEQ ID NO: 36), LSGRSDYH (SEQ ID NO: 37), LSGRSDNP (SEQ ID NO: 38), LSGRSANP (SEQ ID NO: 39), LSGRSANI (SEQ ID NO: 40), LSGRSDNI (SEQ ID NO: 41), MIAPVAYR (SEQ ID NO: 42), RPSPMWAY (SEQ ID NO: 43), WATPRPMR (SEQ ID NO: 44), FRLLDWQW (SEQ ID NO: 45), ISSGL (SEQ ID NO: 46), ISSGLLS (SEQ ID NO: 47), ISSGLL (SEQ ID NO: 48), ISSGLLSGRSANPRG (SEQ ID NO: 49), AVGLLAPPTSGRSANPRG (SEQ ID NO: 50), AVGLLAPPSGRSANPRG (SEQ ID NO: 51), ISSGLLSGRSDDH (SEQ ID NO: 52), ISSGLLSGRSDIH (SEQ ID NO: 53), ISSGLLSGRSDQH (SEQ ID NO: 54), ISSGLLSGRSDTH (SEQ ID NO: 55), ISSGLLSGRSDYH (SEQ ID NO: 56), ISSGLLSGRSDNP (SEQ ID NO: 57), ISSGLLSGRSANP (SEQ ID NO: 58), ISSGLLSGRSANI (SEQ ID NO: 59), AVGLLAPPGGLSGRSDDH (SEQ ID NO: 60), AVGLLAPPGGLSGRSDIH (SEQ ID NO: 61), AVGLLAPPGGLSGRSDQH (SEQ ID NO: 62), AVGLLAPPGGLSGRSDTH (SEQ ID NO: 63), AVGLLAPPGGLSGRSDYH (SEQ ID NO: 64), AVGLLAPPGGLSGRSDNP (SEQ ID NO: 65), AVGLLAPPGGLSGRSANP (SEQ ID NO: 66), AVGLLAPPGGLSGRSANI (SEQ ID NO: 67), ISSGLLSGRSDNI (SEQ ID NO: 68), AVGLLAPPGGLSGRSDNI (SEQ ID NO: 69), GLSGRSDNHGGAVGLLAPP (SEQ ID NO: 70), GLSGRSDNHGGVHMPLGFLGP (SEQ ID NO: 71), LSGRSDNHGGVHMPLGFLGP (SEQ ID NO: 72), ISSGLSS (SEQ ID NO: 73), PVGYTSSL (SEQ ID NO: 74), DWLYWPGI (SEQ ID NO: 75), LKAAPRWA (SEQ ID NO: 76), GPSHLVLT (SEQ ID NO: 77), LPGGLSPW (SEQ ID NO: 78), MGLFSEAG (SEQ ID NO: 79), SPLPLRVP (SEQ ID NO: 80), RMHLRSLG (SEQ ID NO: 81), LLAPSHRA (SEQ ID NO: 82), GPRSFG (SEQ ID NO: 83), GPRSFG (SEQ ID NO: 84), SARGPSRW (SEQ ID NO: 85), GGWHTGRN (SEQ ID NO: 86), HTGRSGAL (SEQ ID NO: 87), AARGPAIH (SEQ ID NO: 88), RGPAFNPM (SEQ ID NO: 89), SSRGPAYL (SEQ ID NO: 90), RGPATPIM (SEQ ID NO: 91), RGPA (SEQ ID NO: 92), GGQPSGMWGW (SEQ ID NO: 93), FPRPLGITGL (SEQ ID NO: 94), SPLTGRSG (SEQ ID NO: 95), SAGFSLPA (SEQ ID NO: 96), LAPLGLQRR (SEQ ID NO: 97),

SGGPLGVR (SEQ ID NO: 98), PLGL (SEQ ID NO: 99), and SGRSDNI (SEQ ID NO: 100).

61. The ACC of claim 60, wherein the CM1, the CM2, the CM3 and/or the CM4 comprises a sequence selected from the group consisting of: ISSGLLSGRSDNH (SEQ ID NO: 28), LSGRSDDH (SEQ ID NO: 33), ISSGLLSGRSDQH (SEQ ID NO: 54), SGRSDNI (SEQ ID NO: 100), and ISSGLLSGRSDNI (SEQ ID NO: 68).
62. The ACC of any one or combination of claims 1-61, wherein the protease(s) is/are produced by a tumor in a subject.
63. The ACC of claim 62, wherein the subject has been diagnosed or identified as having a cancer.
64. The ACC of any one or combination of claims 1-63, wherein the CP1 and the CM1 directly abut each other in the first monomer construct.
65. The ACC of any one or combination of claims 1-64, wherein the CM1 and the DD1 directly abut each other in the first monomer construct.
66. The ACC of any one or combination of claims 1-65, wherein the CP2 and the CM2 directly abut each other in the second monomer construct.
67. The ACC of any one or combination of claims 1-66, wherein the CM2 and the DD2 directly abut each other in the second monomer construct.
68. The ACC of any one or combination of claims 1-63, wherein the first monomer construct comprises at least one linker.

69. The ACC of claim 68, wherein the at least one linker is a linker L1 disposed between the PM1 and the CM3 and/or a linker L2 disposed between the CM3 and the CP1.
70. The ACC of claim 68, wherein the second monomer construct comprises at least one linker.
71. The ACC of claim 70, wherein the at least one linker is a linker L3 disposed between the PM2 and the CM4 and/or a linker L4 disposed between the CM4 and the CP2.
72. The ACC of claim 71, wherein the first monomer construct comprises a linker L1 and the second monomer construct comprises a linker L3.
73. The ACC of claim 72, wherein L1 and L3 are the same.
74. The ACC of claim 71, wherein the first monomer construct comprises a linker L2 and the second monomer construct comprises a linker L4.
75. The ACC of claim 74, wherein L2 and L4 are the same.
76. The ACC of any one or combination of claims 68-75, wherein each linker has a total length of 1 amino acid to about 15 amino acids.
77. The ACC of claim 76, wherein each linker has a total length of at least 5 amino acids.
78. The ACC of claim 68, wherein the first monomer construct comprises at least one linker, wherein each linker is independently selected from the group consisting of GSSGGSGGGG (SEQ ID NO: 210); GGGS (SEQ ID NO: 2); GGGSGGGG (SEQ ID NO: 211); GGGSGGGSGGGG (SEQ ID NO: 212);

GGGGSGGGSGGGGS (SEQ ID NO: 213);
 GGGGSGGGSGGGSGGGSGGGGS (SEQ ID NO: 214); GGGGSGGGGS
 (SEQ ID NO: 215); GGGGS (SEQ ID NO: 216); GS; GGGSGS (SEQ ID NO:
 217); GGGGSGGGSGGGSGGS (SEQ ID NO: 218); GGS LDPKGGGS (SEQ
 ID NO: 219); PKSCDKTHTCPPCPAPELLG (SEQ ID NO: 220);
 SKYGPPCPAPAEFLG (SEQ ID NO: 221); GKSSGSGSESKS (SEQ ID NO:
 222); GSTSGSGKSSEGKG (SEQ ID NO: 223); GSTSGSGKSSEGSGSTKG
 (SEQ ID NO: 224); GSTSGSGKPGSGEGSTKG (SEQ ID NO: 225);
 GSTSGSGKPGSSEGST (SEQ ID NO: 226); (GS)_n, (GGS)_n, (GSGGS)_n (SEQ
 ID NO: 227), (GGGS)_n (SEQ ID NO: 228), (GGGGS)_n (SEQ ID NO: 216),
 wherein each n is an integer of at least one; GGS G (SEQ ID NO: 229); GGS GG
 (SEQ ID NO: 230); GSGSG (SEQ ID NO: 231; GSGGG (SEQ ID NO: 232);
 GGGSG (SEQ ID NO: 233); GSSSG (SEQ ID NO: 234);
 GGGGSGGGSGGGGS (SEQ ID NO: 213); GGGGSGGGSGGGSGGGGS
 (SEQ ID NO: 214); GSTSGSGKPGSSEGST (SEQ ID NO: 226); SGGG (SEQ
 ID NO: 327); and SGGGG (SEQ ID NO: 492).

79. The ACC of claim 78, wherein the linker comprises a sequence of GGGGS (SEQ ID NO: 2).
80. The ACC of any one or combination of claims 1-79, wherein the first monomer construct comprises in a N- to C- terminal direction, the PM1, the CM3, the CP1, the CM1, and the DD1.
81. The ACC of any one or combination of claims 1-79, wherein the first polypeptide comprises in a C- to N-terminal direction, the PM1, the CM3, the CP1, the CM1, and the DD1.
82. The ACC of any one or combination of claims 1-81, wherein the second polypeptide comprises in a N- to C-terminal direction, the CP2, CM2, and the DD2.

83. The ACC of any one or combination of claims 1-82, wherein the second polypeptide comprises in a C- to N-terminal direction, the CP2, CM2, and the DD2.
84. The ACC of any one or combination of claims 1-36, 38, 39, or 41-83, wherein the first monomer construct and the second monomer construct are the same, and wherein each of the first monomer construct and the second monomer construct has only one peptide mask.
85. The ACC of any one or combination of claims 1-79, wherein the first monomer construct comprises in a N- to C- terminal direction, the PM1, an optional linker, the CM3, an optional linker, the CP1, the CM1, and the DD1, wherein the CP1 and the CM1 directly abut each other, wherein the CM1 and the DD1 directly abut each other, wherein the CM1 is a peptide of not more than 10 amino acids, wherein the second monomer construct is the same as the first monomer construct, and wherein the first and second monomer constructs are covalently bound to each other via at least two disulfide bonds.
86. The ACC of claim 85, wherein the DD1 and the DD2 are each a human Fc domain having an N-terminus at Cysteine 216, as numbered according to EU numbering.
87. The ACC of claim 85 or 86, wherein the CM1 is a peptide of not more than 7 amino acids.
88. The ACC of any one or combination of claim 85-87, wherein the CP1 and the CP2 comprise an amino acid sequence that is at least 90% identical to SEQ ID NO: 1.

89. The ACC of any one or combination of claims 1-88, wherein the ACC is characterized by having a reduced level of at least one CP1 and/or CP2 activity as compared to a control level of at least one CP1 and/or CP2 activity.
90. The ACC of claim 89, wherein the at least one of the CP1 and the CP2 activity is a level of proliferation of lymphoma cells.
91. The ACC of claim 89, wherein the at least one of the CP1 and the CP2 activity is the level of JAK/STAT/ISGF3 pathway activation in a lymphoma cell.
92. The ACC of claim 89, wherein the at least one activity is a level of SEAP production in a lymphoma cell.
93. The ACC of claim 89, wherein the ACC is characterized by at least a 2-fold reduction in at least one of the CP1 and the CP2 activity as compared to the control level.
94. The ACC of claim 93, wherein the ACC is characterized by at least a 5-fold reduction in at least one CP1 and/or the CP2 activity as compared to the control level.
95. The ACC of claim 94, wherein the ACC is characterized by at least a 10-fold reduction in at least one activity of the CP1 and/or the CP2 as compared to the control level.
96. The ACC of claim 95, wherein the ACC is characterized by at least a 500-fold reduction in at least one CP1 and/or the CP2 activity as compared to the control level.

97. The ACC of any one or combination of claims 89-95, wherein the control level of the at least one activity of the CP1 and/or the CP2, is the activity of the CP1 and/or the CP2 in the ACC following exposure of the ACC to the protease(s).
98. The ACC of any one or combination of claims 89-97, wherein the control level of the at least one CP1 and/or CP2, is the corresponding CP1 and/or the CP2 activity of a corresponding wildtype mature cytokine.
99. The ACC of any one or combination of claims 89-98, wherein the ACC is characterized by generating a cleavage product following exposure to the protease(s), wherein the cleavage product comprises the at least one activity of the CP1 and/or the CP2.
100. The ACC of claim 99, wherein the at least one activity of the CP1 and/or the CP2 is anti-proliferation activity.
101. The ACC of claim 100, wherein the control level is an EC50 value, and wherein ratio of EC50 (cleavage product) to EC50 (control level) is less than about 10, or less than about 9, or less than about 8, or less than about 7, or less than about 6, or less than about 5, or less than about 4, or less than about 3, or less than about 2, or less than about 1.5, or less than about 1.0.
102. The ACC of any one or combination of claims 1-101, wherein the first monomer construct is characterized in that the CP1 and the DD1 are linked by a linking region of no more than 12 amino acids such that the linking region of no more than 12 amino acids includes the CM3.
103. The ACC of any one or combination of claims 1-102, wherein the second monomer construct is characterized in that the CP2 and the DD2 are linked by a linking region of no more than 12 amino acids such that the linking region of no more than 12 amino acids includes the CM2.

104. The ACC of any one or combination of claims 1-103, wherein the PM1 comprises less than 50 amino acids.
105. The ACC of any one or combination of claims 2-104, wherein the PM2 comprises less than 50 amino acids.
106. The ACC of claim 105, wherein each of PM1 and PM2 is less than 19 amino acids.
107. The ACC of any one or combination of claims 1-103, wherein the PM1 is not a latency associated peptide and the PM2 is not a latency associated peptide.
108. The ACC of any one or combination of claims 1-103, wherein the PM1 is not a cytokine and the PM2 is not a cytokine.
109. The ACC of any one or combination of claims 1-103, wherein the PM1 is not a receptor for a cytokine and is not a fragment of a receptor for a cytokine.
110. The ACC of any one or combination of claims 1-103 and 109, wherein the PM2 is not a receptor for a cytokine and is not a fragment of a receptor for a cytokine.
111. The ACC of any one or combination of claims 1-103, wherein the PM1 is not an albumin and the PM2 is not an albumin.
112. A composition comprising an ACC of any one or a combination of claims 1-111.
113. The composition of claim 112, wherein the composition is a pharmaceutical composition.
114. A container, vial, syringe, injector pen, or kit comprising at least one dose of the composition of claim 112 or 113.
115. A method of treating a subject in need thereof comprising administering to the subject a therapeutically effective amount of the ACC of any one of claims 1-96 or the composition of claims 112 or 113.

116. The method of claim 115, wherein the subject has been identified or diagnosed as having a cancer.
117. The method of claim 116, wherein the cancer is a lymphoma.
118. The method of claim 117, wherein the lymphoma is Burkitt's lymphoma.
119. A nucleic acid encoding a polypeptide that comprises the CP1 and the CM1 of the ACC of any one or a combination of claims 1-111.
120. The nucleic acid of claim 109, wherein the polypeptide further comprises the DD1 of any one or combination of claims 1-21 or claims 28-111.
121. A nucleic acid encoding a polypeptide that comprises the CP2 and the CM2 of the ACC of any one or combination of claims 1-111.
122. The nucleic acid of claim 111, wherein the polypeptide further comprises the DD2 of any one or combination of claims 1-21 or claims 28-111.
123. A vector comprising the nucleic acid of any one or a combination of claims 119-122.
124. The vector of claim 123, wherein the vector is an expression vector.
125. A cell comprising the nucleic acid of any one or a combination of claims 119-122 or the vector of claim 123 or 124.
126. A pair of nucleic acids that together encode a polypeptide that comprises the CP1 and the CM1 of the first monomer construct and a polypeptide that comprises the CP2 and the CM2 of the second monomer construct of any one or combination of claims 1-111.

127. A pair of vectors that together comprise the pair of nucleic acids of claim 126.
128. The pair of vectors of claim 127, wherein the pair of vectors is a pair of expression vectors.
129. A cell comprising the pair of nucleic acids of claim 126 or the pair of vectors of claims 127 or 128.
130. A method of producing an ACC comprising:
- a. culturing a cell of claim 125 or 129 in a liquid culture medium under conditions sufficient to produce the ACC; and
 - b. recovering the ACC from the cell or the liquid culture medium.
131. The method of claim 130, further comprising: isolating the ACC recovered from the cell or the liquid culture medium.
132. The method of claim 131, further comprising: formulating isolated ACC into a pharmaceutical composition.
133. An ACC produced by the method of claim 130.
134. A composition comprising an ACC of claim 133.
135. The composition of claim 134, wherein the composition is a pharmaceutical composition.
136. A container, vial, syringe, injector pen, or kit comprising at least one dose of the composition of claim 134 or 135.

137. The ACC of any one or combination of claims 89-101, wherein the at least one of the CP1 and the CP2 activity is a binding affinity of the CP1 and/or the CP2 for its cognate receptor as determined using surface plasmon resonance.
138. An activatable cytokine construct (ACC) comprising a first monomer construct and a second monomer construct, wherein:
- (a) the first monomer construct is a polypeptide comprising a first peptide mask (PM1), a first mature cytokine protein (CP1), a first and a third cleavable moieties (CM1 and CM3), and a first dimerization domain (DD1);
 - (b) the second monomer construct is a polypeptide comprising a second peptide mask (PM2), a second mature cytokine protein (CP2), a second and a fourth cleavable moieties (CM2 and CM4), and a second dimerization domain (DD2);
 - (c) the first monomer construct comprises, in an N- to C- terminal direction, the PM1, the CM3, the CP1, the CM1, and the DD1, further wherein:
 - (i) the PM1 comprises no more than 20 amino acids and binds to the CP1,
 - (ii) the CM1 and the DD1 directly abut each other,
 - (iii) the CP1 and the CM1 directly abut each other,
 - (iv) the CM1 comprises no more than 12 amino acids,
 - (v) the CM1 and the CM3 each functions as a substrate for a protease, and
 - (vi) the CP1 is a mature interferon;
 - (d) further wherein:
 - (i) the second monomer construct is the same as the first monomer construct,
 - (ii) the DD1 and the DD2 are a pair of human IgG Fc domains;
 - (iii) the DD1 and the DD2 bind to each other via at least one disulfide bond, thereby forming a homodimer of the first monomer construct and the second monomer construct; and
 - (e) the ACC is characterized by having a reduced level of interferon activity as compared to a corresponding wildtype interferon or a corresponding pegylated interferon.

139. The ACC of claim 138, wherein the CP1 is a mature interferon-alpha and the PM1 comprises a sequence that is at least 85% identical to SEQ ID NO: 323.
140. The ACC of claim 138 or 139, wherein the CM1 and the CM3 each independently functions as a substrate of urokinase (uPa) and/or a matrix metalloproteinase (MMP).
141. The ACC of claim 140, wherein the CM1 and the CM3 each independently functions as a substrate of urokinase (uPa) and/or MMP-14.
142. The ACC of any one or combination of claims 138-141, wherein the mature interferon is a mature human interferon alpha.
143. The ACC of any one or combination of claims 138-142, wherein the mature interferon alpha is mature interferon alpha-2b.
144. The ACC of any one or combination of claims 138-143, wherein the mature interferon alpha is a truncated form of a wildtype mature interferon alpha-2b.
145. The ACC of any one or combination of claims 138-144, wherein the mature interferon comprises a sequence that is at least 95% identical to SEQ ID NO: 1.
146. The ACC of any one or combination of claims 138-145, wherein the mature interferon comprises the sequence of SEQ ID NO: 1.
147. The ACC of any one or combination of claims 138-146, wherein the CM1 and the CM3 each comprises no more than 8 amino acids.
148. The ACC of any one or combination of claims 138-147, wherein the CM1 and the CM3 are the same.

149. The ACC of any one or combination of claims 138-148, wherein the CM1 and the CM3 each comprises a sequence that is at least 85% identical to SEQ ID NO: 41.
150. The ACC of any one or combination of claims 138-148, wherein the CM1 and the CM3 each comprises a sequence selected from the group consisting of SEQ ID NO: 41, SEQ ID NO: 68, and SEQ ID NO: 100.
151. The ACC of any one or combination of claims 138-150, wherein the DD1 and the DD2 are a pair of human IgG4 Fc domains.
152. The ACC of claim 151, wherein the DD1 and the DD2 are a pair of human IgG4 Fc domains truncated at N-terminus to Cysteine 226 as numbered by EU numbering.
153. The ACC of claim 151 or 152, wherein the human IgG4 Fc domains comprise a S228P mutation as numbered by EU numbering.
154. The ACC of any one or combination of claims 138-153, wherein the DD1 and the DD2 each comprises a sequence that is at least 95% identical to SEQ ID NO: 3.
155. The ACC of any one or combination of claims 138-154, wherein the DD1 and the DD2 each comprises the sequence of SEQ ID NO: 3.
156. The ACC of any one or combination of claims 138-155, wherein the first and second monomer constructs are covalently bound to each other via at least two disulfide bonds.
157. The ACC of claim 156, wherein the first and second monomer constructs are covalently bound to each other via at least three disulfide bonds.
158. The ACC of any one or combination of claims 138-157, wherein:

the first monomer construct further comprises a first signal sequence at the N-terminus,
and the second monomer construct further comprises a second signal sequence at
the N-terminus.

159. The ACC of claim 158, wherein the first and second signal sequences each
comprises a sequence that is at least 95% identical to SEQ ID NO: 244.
160. The ACC of claim 159, wherein the first and second signal sequences each
comprises the sequence of SEQ ID NO: 244.
161. The ACC of any one or combination of claims 158-160, wherein:
the first monomer construct further comprises a first spacer positioned between
the first signal sequence and the PM1, and the second monomer construct further
comprises a second spacer positioned between the second signal sequence and the
PM2, and wherein each of the first monomer construct and the second monomer
construct has only one peptide mask.
162. The ACC of claim 161, wherein the first and second spacers each comprises a
sequence that is at least 80% identical to SEQ ID NO: 256.
163. The ACC of claim 162, wherein the first and second spacers each comprises a
sequence of SEQ ID NO: 256.
164. The ACC of any one or combination of claims 138-163, further comprising a
linker L1 between the PM1 and the CM3, and/or a linker L2 between the CM3
and the CP1, wherein each of L1 and L2 independently comprises a sequence that
is at least 80% identical to SEQ ID NO: 27 (wherein n=1), a sequence that is at
least 80% identical to SEQ ID NO: 324, or is absent.
165. The ACC of claim 164, wherein the L1 comprises the sequence SEQ ID NO: 27
(wherein n=1) and L2 comprises the sequence of SEQ ID NO: 324.

166. The ACC of any one or combination of claims 138-165 comprising a linking region comprising no more than 12 amino acids.
167. The ACC of claim 166, wherein the linking region comprises 7 to 12 amino acids.
168. The ACC of claim 166, wherein the linking region comprises 7 amino acids.
169. The ACC of any one or combination of claims 138-168, wherein the ACC is characterized by at least a 2000-fold reduction in interferon alpha activity as compared to wildtype interferon alpha.
170. The ACC of claim 169, wherein the ACC is characterized by at least a 4000-fold reduction in interferon alpha activity as compared to wildtype interferon alpha.
171. The ACC of claim 170, wherein the ACC is characterized by at least a 5000-fold reduction in interferon alpha activity as compared to wildtype interferon alpha.
172. The ACC of any one or combination of claims 138-168, wherein the ACC is characterized by at least a 2000-fold reduction in interferon alpha activity as compared to pegylated interferon alpha.
173. The ACC of any one or combination of claims 138-172, wherein the reduction in interferon activity is determined by comparing the EC50 of the ACC with the EC50 of the wildtype interferon or the pegylated interferon in an anti-proliferation assay in lymphoma cells.
174. The ACC of any one or combination of claims 138-173, wherein the reduction in interferon activity is determined by comparing the EC50 of the ACC with the EC50 of the wildtype interferon or the pegylated interferon in an assay of

induction of secreted embryonic alkaline phosphatase production in interferon-responsive HEK293 cells.

175. The ACC of any of claims 138-174, wherein the ACC is further characterized by generating a cleavage product following exposure to the protease(s) for which CM1 and CM3 function as a substrate, wherein the ratio of the interferon activity of the corresponding wildtype interferon to the cleavage product is less than about 2.
176. The ACC of claim 175, wherein the EC50 of the cleavage product is approximately the same as the EC50 of the corresponding wildtype interferon.
177. The ACC of claim 138, wherein the first and second monomer constructs each comprises a sequence that is at least 95% identical to SEQ ID NO: 321 or wherein each of the first and second monomer constructs comprises the sequence of SEQ ID NO: 321, wherein the ACC is characterized by at least a 1000-fold reduction in interferon activity as compared to wildtype interferon alpha-2b, and wherein the ACC is further characterized by generating a cleavage product following exposure to uPA, wherein the cleavage product has approximately the same interferon activity as wildtype interferon alpha-2b, wherein interferon activity is measured in an anti-proliferation assay in lymphoma cells or in an assay of induction of secreted embryonic alkaline phosphatase production in interferon-responsive HEK293 cells.
178. An activatable cytokine construct (ACC) comprising a first monomer construct and a second monomer construct, wherein:
 - (a) the first monomer construct comprises a first peptide mask (PM1), a first mature cytokine protein (CP1), a first and a third cleavable moieties (CM1 and CM3), and a first dimerization domain (DD1);

(b) the second monomer construct is a polypeptide comprising a second peptide mask (PM2), a second mature cytokine protein (CP2), a second and a fourth cleavable moieties (CM2 and CM4), and a second dimerization domain (DD2);
(c) the first monomer construct is a polypeptide comprising, in an N- to C-terminal direction, the PM1, the CM3, the CP1, the CM1, and the DD1, further wherein:

(i) the PM1 comprises a sequence that is at least 85% identical to SEQ ID NO: 323,

(ii) the CM1 and the DD1 directly abut each other,

(iii) the CM1 comprises a sequence that is at least 85% identical to SEQ ID NO: 41, and

(iv) the CP1 comprises a sequence that is at least 85% identical to SEQ ID NO: 1;

(d) further wherein:

(i) the second monomer construct is the same as the first monomer construct,

(ii) the DD1 and DD2 are a pair of human IgG4 Fc domains;

(e) the DD1 and the DD2 covalently bind to each other via at least one disulfide bond, thereby forming a homodimer of the first monomer construct and the second monomer construct; and

(f) the ACC is characterized by having a reduced level of interferon alpha activity as compared to the interferon alpha activity of PEGylated interferon alpha-2b.

179. The ACC of claim 178, wherein the PM2 comprises a sequence that is at least 85%, 90%, or 95% identical to one of SEQ ID NOs: 323, 331, or 332.

180. The ACC of claim 138, wherein the first and second monomer constructs each comprises a sequence that is at least 95% identical to SEQ ID NO: 321 or wherein each of the first and second monomer constructs comprises the sequence of SEQ ID NO: 321, wherein the ACC exhibits lower toxicity in vivo compared to either wildtype interferon alpha-2b or PEGylated interferon alpha-2b.

181. A composition comprising the ACC of any one or combination of claims 138-180.
182. The composition of claim 181, where the composition is a pharmaceutical composition.
183. A container, vial, syringe, injector pen, or kit comprising at least one dose of the composition of claim 181 or 182.
184. A method of treating a subject in need thereof comprising administering to the subject a therapeutically effective amount of the ACC of any one or combination of claims 138-179 or the composition of claim 181 or 182.
185. The method of claim 184, wherein the subject has been identified or diagnosed as having a cancer.
186. A nucleic acid encoding a polypeptide that comprises the first monomer of the ACC of any one or combination of claims 138-179.
187. A vector comprising the nucleic acid of claim 186.
188. The vector of claim 187, wherein the vector is an expression vector.
189. A mammalian cell comprising the nucleic acid of claim 186 or the vector of claim 187 or 188.
190. The mammalian cell of claim 189, wherein the mammalian cell is an HEK293 cell or a CHO cell.
191. A method of manufacturing an ACC, the method comprising:

expressing the ACC in the mammalian cell of claim 189 or 190; and
purifying the expressed ACC.

192. The ACC of any one or combination of claims 138-180, wherein the CM1 and the CM3 each functions as a substrate for a protease that is over-expressed in a tumor tissue.