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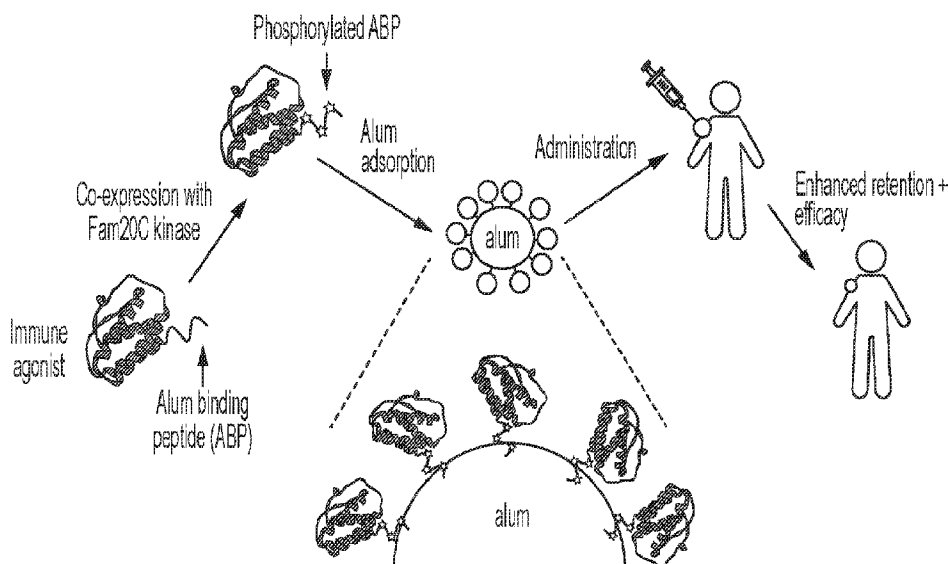


FIG. 1

(57) **Abrégé/Abstract:**

The present disclosure provides surprisingly useful fusion polypeptides including an immunomodulatory moiety and a metal-hydroxide binding moiety, as well as various related technologies, including methods of making and of using such fusion polypeptides.

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Abstract:

The present disclosure provides surprisingly useful fusion polypeptides including an immunomodulatory moiety and a metal-hydroxide binding moiety, as well as various related technologies, including methods of making and of using such fusion polypeptides.

FUSION POLYPEPTIDES

Cross Reference to Related Applications

[0001] This application claims priority to and the benefit of U.S. Provisional Patent Application No. 63/184,620, filed May 5, 2021, the entirety of which is incorporated herein by reference.

Background

[0002] Immunomodulatory polypeptides engineered to conjugate with metal hydroxides (*e.g.*, aluminum hydroxide) can show increased anti-tumor efficacy when delivered by intratumoral injection relative to unconjugated immunomodulatory polypeptides.

Summary

[0003] Various immunomodulatory polypeptides, such as certain cytokines, immune checkpoint modulators, *etc.*, have shown significant promise in the treatment of cancer, but significant challenges remain, including dose-limiting toxicities that have been reported for many of the potent cytokines. *See*, for example, Milling *et al.*, *Adv. Drug. Deliv. Rec.* 114:79, 2017). Recent work, as described for example, in International Patent Application , WO2020263399 (the “Fusion Protein Filing”) has described a particularly interesting approach for addressing such toxicity and/or otherwise improving therapeutic usefulness: fusing the relevant immunomodulatory polypeptide with another polypeptide characterized by an ability to complex with metal hydroxides. Without wishing to be bound by any particular theory, it is proposed that the metal hydroxide can act as a particulate scaffold able to persist at the site of injection for extended periods of time (*e.g.*, days to weeks), and that such complexation (*i.e.*, conjugation of the fusion polypeptide with the metal hydroxide) retains the fusion polypeptide (including its immunomodulatory polypeptide moiety) at the injection site (*e.g.*, intratumoral injection). Accordingly, the immunomodulatory polypeptide persists within the tumor microenvironment enhancing efficacy and systemic exposure is limited, reducing toxicity.

[0004] The Fusion Protein Filing specifically demonstrated that polypeptides amenable to phosphorylation can adsorb to alum much more strongly when in their

phosphorylated form (*i.e.*, where phosphate groups have replaced hydroxyl groups). Various alum-binding peptides (“ABPs”) were developed that included phosphorylation sites for the Fam20C kinase and that could be fused with other polypeptides (*e.g.*, immunomodulatory polypeptides). Fusion polypeptides were generated, in which an alum-binding polypeptide was linked to the N- or C- terminus of an immunomodulatory polypeptide, typically by way of a linker. The extent of phosphorylation of these fusion polypeptides was assessed when expressed alone or in combination with Fam20C kinase, and the phosphorylated polypeptides were characterized for their absorption to and release from alum in the presence of serum. Moreover, intratumoral persistence of fusion polypeptides complexed with alum was determined after intratumoral injection.

[0005] Fusion polypeptides with greater phosphorylation tended to be retained longer on alum in serum conditions. Of the ABPs analyzed, the polypeptide, GGGGSFQSEEQQGGSGGSEEGGMESEESNGGGSGGSEEGGGGSHHHHHH, referred to as ABP10, demonstrated the highest phosphorylation, with an increase of phosphorylation of approximately 4- to 6-fold when the protein was expressed with a wild-type (WT) kinase (*e.g.*, WT Fam20C kinase), compared to when the protein was expressed with a mutant kinase (*e.g.*, mutant Fam20C kinase). ABP10 consists of four SXE motifs, a prevalent phosphorylation site motif, separated by short spacer sequences. The Fusion Protein Filing demonstrated that immunomodulatory polypeptides (*e.g.*, interleukin-2 and interleukin-12) linked to ABP10 showed improved survival in a mouse model of melanoma compared to the immunomodulatory polypeptides without ABP10.

[0006] We have surprisingly found that improved metal-hydroxide binding polypeptides, and improved fusion polypeptides including them, can be developed. Among other things, we have developed metal-hydroxide binding polypeptides characterized by enhanced metal hydroxide (*e.g.*, alum) retention relative to an appropriate reference (*e.g.*, to ABP10). Alternatively or additionally, provided metal-hydroxide binding polypeptides are characterized by improved efficacy, as compared to an appropriate reference (*e.g.*, to ABP10), when administered to a subject with a tumor.

[0007] Among other things, the present disclosure provides particularly useful fusion polypeptides (**Figure 1**). Furthermore, the present disclosure demonstrates effectiveness of

the fusion polypeptides in treating a subject with a tumor; in some embodiments, effectiveness is demonstrated as monotherapy. Alternatively or additionally, in some embodiments, effectiveness is demonstrated in combination, for example, with immune-modulator therapy, chemotherapeutic agent, or surgical resection.

[0008] Among other things, the present disclosure provides certain technologies for production of provided fusion polypeptides and/or compositions that comprise them. In some embodiments, provided technologies achieve reproducible production of fusion peptide preparations, including specifically phosphorylated preparations. In some embodiments, provided technologies may include, for example, expression, purification, and/or analytical technologies. Moreover, the present disclosure provides desirable preparations of provided fusion polypeptides, including in some embodiments phosphorylated preparations and/or in some embodiments, preparations of fusion polypeptides (e.g., phosphorylated fusion polypeptides) complexed with a metal hydroxide.

[0009] Thus, among other things, the present disclosure identifies the source of a problem with certain metal-hydroxide binding polypeptides and/or fusion polypeptides that include them. For example, the present disclosure appreciates that manufacturing challenges can be associated with certain such polypeptides and/or fusions. Without wishing to be bound by any particular theory, the present disclosure notes that secondary phosphorylation on the polypeptide may contribute to and/or be responsible for certain such manufacturing challenges. Among other things, the present disclosure provides metal-hydroxide-binding polypeptides, and fusion polypeptides that include them, which demonstrate high levels of adsorption to metal hydroxides and also desirable manufacturing characteristics (e.g., one or more of reproducibility, consistency, reduced immunogenicity, etc.).

[0010] In one aspect, the present disclosure provides fusion polypeptides comprising: (a) an immunomodulatory polypeptide that comprises an immune agonist moiety; and (b) a metal-hydroxide binding polypeptide whose amino acid sequence includes a plurality of phosphorylation sites, so that it can adopt phosphorylated and unphosphorylated forms. In some embodiments, the fusion polypeptides, when exposed to a metal-hydroxide forms a complex therewith. In some embodiments, the metal hydroxide is aluminum hydroxide. In some embodiments, the complex forms more readily when the

metal-hydroxide-binding polypeptide is in a phosphorylated form than when it is in an unphosphorylated form.

[0011] In some embodiments, one or more of the phosphorylation sites is targeted by a Fam20C kinase. In some embodiments, the phosphorylation site is or comprises an S-X-E motif. In some embodiments, the plurality of phosphorylation sites comprises more than 4 S-X-E motifs. In some embodiments, the plurality of phosphorylation sites comprises 8 S-X-E motifs.

[0012] In some embodiments, at least two adjacent S-X-E motifs are separated by a spacer. In some embodiments, the spacer comprises at least one glycine residue. In some embodiments, the spacer comprises a plurality of glycine residues. In some embodiments, the spacer comprises at least four glycine residues. In some embodiments, the spacer has a sequence that comprises four glycine residues. In some embodiments, each SXE motif is separated from each adjacent S-X-E motif by a spacer.

[0013] In one aspect, the present disclosure provides method of treating a subject with a tumor, the method comprising a step of: treating the subject with a complex comprising: (a) fusion polypeptide comprising: (i) an immunomodulatory polypeptide that comprises an immune agonist moiety; and (ii) a metal-hydroxide binding peptide; and, (b) a metal hydroxide. In some embodiments, (a) and (b) are formulated together. In some embodiments, (a) and (b) are mixed prior to administration.

[0014] In some embodiments, the complex is administered by intratumoral injection. In some embodiments, the complex is administered by peritumoral injection. In some embodiments, the complex is administered to a tumor-draining lymph node or lymph nodes.

[0015] In some embodiments, the complex is administered in combination with a second therapeutic. In some embodiments, the second therapeutic is radiation. In some embodiments, the second therapeutic is surgical tumor resection. In some embodiments, the fusion polypeptide is administered prior to surgical tumor resection. In some embodiments, the second therapeutic is a chemotherapy. In some embodiments, the second therapeutic is an anti-tumor antibody. In some embodiments, the second therapeutic is a targeted therapy (e.g., BRAF inhibitor, MEK inhibitor, etc.). In some embodiments, the second therapeutic is an immune modulator. In some embodiments, the immune modulator is a checkpoint

inhibitor. In some embodiments, the checkpoint inhibitor is an antibody or a functional fragment thereof. In some embodiments, the antibody targets one or more of PD-1, PD-L1, CTLA-4, TIM3, TIGIT, and LAG3. In some embodiments, the antibody targets PD-1. In some embodiments, the antibody is a tumor-targeting CD3 bispecific antibody. In some embodiments, the immune modulator is a cell therapy. In some embodiments, the cell therapy is selected from the group consisting of: CAR-T cells, ex-vivo expanded TILs, and NK cells.

[0016] In one aspect, the present disclosure provides methods of treating a subject with a tumor comprising administering a fusion polypeptide comprising: (a) an immunomodulatory polypeptide that comprises an immune agonist moiety; and (b) a metal-hydroxide binding peptide, wherein the fusion polypeptide is formulated with a metal hydroxide; and wherein the subject has received or is receiving therapy with at least one additional therapeutic. In some embodiments, the fusion polypeptide and metal-hydroxide are formulated together, forming a complex. In some embodiments, the fusion polypeptide and metal-hydroxide are mixed prior to administration.

[0017] In some embodiments, the fusion polypeptide is administered by intratumoral injection. In some embodiments, the fusion polypeptide is administered by peritumoral injection. In some embodiments, the fusion polypeptide is administered to a tumor-draining lymph node or lymph nodes.

[0018] In some embodiments, the at least one additional therapeutic is radiation. In some embodiments, the at least one additional therapeutic is surgical tumor resection. In some embodiments, the fusion polypeptide is administered prior to surgical tumor resection. In some embodiments, the at least one additional therapeutic is a chemotherapy. In some embodiments, the at least one additional therapeutic is an anti-tumor antibody. In some embodiments, the second therapeutic is an immune modulator. In some embodiments, the immune modulator is a checkpoint inhibitor. In some embodiments, a checkpoint inhibitor inhibits MEK. In some embodiments, the checkpoint inhibitor is an antibody or a functional fragment thereof. In some embodiments, the antibody targets one or more of PD-1, PD-L1, CTLA-4, TIM3, TIGIT, and LAG3. In some embodiments, the antibody targets PD-1. In some embodiments, the antibody is a tumor-targeting CD3 bispecific antibody. In some

embodiments, the immune modulator is a cell therapy. In some embodiments, the cell therapy is selected from the group consisting of: CAR-T cells, ex-vivo expanded TILs, and NK cells.

[0019] In some embodiments, the immune agonist moiety of a fusion polypeptide disclosed herein comprises a first moiety or a functional fragment thereof. In some embodiments, the functional fragment is signaling competent. In some embodiments, the first moiety comprises an IL12 moiety or a functional fragment thereof. In some embodiments, the IL12 moiety comprises IL12B or a functional fragment thereof.

[0020] In some embodiments, the immune agonist moiety of a fusion polypeptide disclosed herein comprises a first and a second moiety or a functional fragment thereof. In some embodiments, the first moiety comprises an IL12 moiety or a functional fragment thereof. In some embodiments, the IL12 moiety comprises IL12B or a functional fragment thereof. In some embodiments, the second moiety comprises an IL12 moiety or a functional fragment thereof. In some embodiments, the second IL12 moiety comprises IL12A or a functional fragment thereof. In some embodiments, the first and second moieties or functional fragments thereof are linked via a first linker. In some embodiments, the first linker comprises a polypeptide. In some embodiments, the polypeptide comprises a (G₄S)₃ linker.

[0021] In some embodiments, an immunomodulatory polypeptide of a fusion polypeptide disclosed herein and a metal-hydroxide binding polypeptide are linked via a second linker. In some embodiments, the second linker comprises a polypeptide. In some embodiments, the polypeptide comprises the amino acid sequence, GGGGEGGGG. In some embodiments, the polypeptide comprises the amino acid sequence, GGGGSGGGG. In some embodiments, the metal-hydroxide binding polypeptide is linked directly to the c-terminus of the immunomodulatory polypeptide. In some embodiments, the metal-hydroxide binding polypeptide is linked via a second linker to the c-terminus of the immunomodulatory polypeptide.

[0022] In some embodiments, the present disclosure provides a method of manufacturing a phosphorylated form of fusion polypeptides disclosed herein by contacting the fusion polypeptide with a kinase. In some embodiments, contacting comprises co-

expressing the fusion polypeptide and a kinase. In some embodiments, the fusion polypeptide and kinase are co-expressed at a ratio of 2:1 to 100:1. In some embodiments, the ratio is 4:1. In some embodiments, the 4:1 ratio is achieved using two separate plasmids to express the fusion polypeptide and the kinase. In some embodiments, the two separate plasmids comprise promoters of differing strength to produce the ratio of 4:1. In some embodiments, the ratio is 8:1. In some embodiments, the 8:1 ratio is achieved using a single vector with two promoters to express the fusion polypeptide and the kinase. In some embodiments, the kinase is Fam20C.

[0023] In some embodiments, the step of co-expressing comprises expressing from promoters established to direct expression at the ratio. In some embodiments, the step of co-expressing comprises expressing from a bi-cistronic construct.

[0024] In some embodiments, the method further comprises a step of purifying the phosphorylated form. In some embodiments, the step of purifying comprises affinity chromatography.

[0025] In some embodiments, the fusion polypeptide is exposed to a metal-hydroxide to form a complex therewith. In some embodiments, the complex is prepared prior to administering to a subject. In some embodiments, a fusion polypeptide of the present disclosure is manufactured by contacting a phosphorylated form of a fusion polypeptide with a metal hydroxide.

[0026] In some embodiments, a complex comprises a fusion polypeptide of the present disclosure and a metal hydroxide. In some embodiments, the complex comprises an average of 2-8 phosphates per fusion polypeptide. In some embodiments, the complex is characterized as having greater than 95% metal hydroxide retention. In some embodiments, the complex comprises a ratio of 1:1 to 1:20 by mass of fusion polypeptide to metal hydroxide, *e.g.*, as defined by metal mass. In some embodiments, the ratio is 1:5 to 1:20 by mass of fusion polypeptide to metal hydroxide, *e.g.*, as defined by metal mass. In some embodiments, the ratio is 1:10 by mass of fusion polypeptide to metal hydroxide, *e.g.*, as defined by metal mass. In some embodiments, the ratio is 1:5 by mass of fusion polypeptide to metal hydroxide, *e.g.*, as defined by metal mass.

[0027] In some embodiments, the present disclosure provides a pharmaceutical composition comprising a fusion polypeptide as disclosed herein. In some embodiments, a pharmaceutical composition is formulated as a fusion polypeptide metal-hydroxide complex.

[0028] In some embodiments, the present disclosure provides methods of characterizing a preparation of a fusion polypeptide of the present disclosure by assessing degree of phosphorylation. In some embodiments, the degree of phosphorylation is assessed by determining the number of phosphates per protein. In some embodiments, the number of phosphates per protein is determined using a colorimetric assay. In some embodiments, the colorimetric assay is a malachite green assay.

[0029] In some embodiments, the present disclosure provides methods of characterizing a preparation of a fusion polypeptide of the present disclosure by assessing heterogeneity of the preparation. In some embodiments, heterogeneity of the preparation is assessed using analytical ion exchange.

[0030] In some embodiments, the present disclosure provides methods of characterizing a preparation of a fusion polypeptide of the present disclosure by assessing retention of the fusion polypeptide on the metal hydroxide. In some embodiments, retention is assessed using an *in vitro* retention assay.

[0031] In some embodiments, the present disclosure provides methods of characterizing a fusion polypeptide of the present disclosure by assessing signaling activity of the fusion polypeptide. In some embodiments, the signaling activity is assessed *in vitro*. In some embodiments, *in vitro* assessment utilizes a reporter assay.

[0032] In some embodiments, the present disclosure provides methods of characterizing a fusion polypeptide of the present disclosure by assessing purity of the preparation.

[0033] In some embodiments, the present disclosure provides methods of characterizing a fusion polypeptide of the present disclosure by assessing phosphate content. In some embodiments, assessing phosphate content comprises use of a malachite green assay. In some embodiments, assessing phosphate content comprises use of a high

performance liquid chromatography assay. In some embodiments, high performance liquid chromatography assay utilizes a SAX-10 column.

[0034] In some embodiments, the present disclosure provides methods of characterizing a fusion polypeptide of the present disclosure by assessing potency of the preparation. In some embodiments, potency is characterized by assessing immune moiety signaling. In some embodiments, immune moiety signaling is determined using a reporter assay. In some embodiments, potency is characterized by assessing IL12 signaling. In some embodiments, IL12 signaling is determined using a reporter assay.

[0035] In some embodiments, the present disclosure provides methods of characterizing a complex as disclosed herein comprising assessing retention of a fusion polypeptide of the present disclosure to the metal hydroxide. In some embodiments, assessing retention comprises use of a metal hydroxide retention assay.

[0036] In some embodiments, the present disclosure provides a method of characterizing a pharmaceutical composition as described herein comprising assessing one or more of: (a) the purity of the preparation; (b) phosphate content; (c) potency of the pharmaceutical composition; (d) retention of the fusion polypeptide to the metal hydroxide; (e) efficacy of treating a subject having a tumor; and (f) combination with a second therapeutic agent

Brief Description of the Drawing

[0037] **Figure 1** provides an exemplary schematic of fusion polypeptide metal-hydroxide complexes of the present disclosure. Fusion polypeptide metal-hydroxide complexes can be administered to a subject and result in enhanced retention and/or efficacy compared to an appropriate reference standard.

[0038] **Figure 2** provides a diagram of an exemplary fusion polypeptide of the present disclosure comprising a first (p40) and second (p35) IL12 immune agonist moieties and a metal-hydroxide binding polypeptide with a plurality of phosphorylation sites.

[0039] **Figures 3A-C** provide assessment of phosphorylation levels using a malachite green assay for exemplary human and mouse IL-12 constructs with various metal-

hydroxide binding polypeptides (*e.g.*, alum binding polypeptides). **Figure 3A** shows an exemplary graph illustrating that hIL12-ABP and mIL12-ABP polypeptides co-expressed with Fam20C (denoted as “-K”) had significantly higher phosphate levels compared to polypeptides without Fam20C co-expression or wild-type hIL12 or mIL12 lacking the ABP co-expressed with Fam20C. **Figure 3B** shows an exemplary graph illustrating results from mouse IL12-ABP variants that were expressed at 1 L scale in transient HEK with Fam20C co-expression and purified by sequential Ni-NTA and SEC chromatography. In the malachite green assay, mIL12-ABP10 again had the highest phosphorylation followed in order by ABP20-G4-GS20, ABP20-G4, and ABP20. **Figure 3C** provides exemplary metal-hydroxide binding polypeptide sequences.

[0040] **Figures 4A-4G** demonstrate an exemplary *in vivo* study of ABP10 verse exemplary metal-hydroxide binding polypeptides of the present disclosure. Mouse IL12-ABP polypeptides were tested in a dual flank B16F10 syngeneic tumor model in combination with systemic PD-1 blockade. Vehicle treatment was utilized as a control. **Figure 4A** shows an exemplary graph of tumor volume in mice treated with vehicle or PD-1 only. **Figure 4B** shows an exemplary graph of tumor volume in mice treated with vehicle or an mIL12 and PD-1 combination treatment. **Figure 4C** shows an exemplary graph of tumor volume in mice treated with vehicle or an mIL12/alum and PD-1 combination treatment. **Figure 4D** shows an exemplary graph of tumor volume in mice treated with vehicle or an mIL12-ABP10-k/alum and PD-1 combination treatment. **Figure 4E** shows an exemplary graph of tumor volume in mice treated with vehicle or an mIL12-ABP20-K/alum and PD-1 combination treatment. **Figure 4F** shows an exemplary graph of tumor volume in mice treated with vehicle or an mIL12-ABP20-G4-K/alum and PD-1 combination treatment. **Figure 4G** shows an exemplary graph of tumor volume in mice treated with vehicle or an mIL12-ABP20-G4-GS20-K/alum and PD-1 combination treatment.

[0041] **Figure 5** shows exemplary ABP sequences.

[0042] **Figure 6A** and **Figure 6B** demonstrate analysis of phosphorylation levels using a malachite green assay of exemplary, purified fusion polypeptides. All ABPs demonstrated significantly higher phosphorylation compared to negative controls (no

Fam20C or TBS). Runs 1 and 2 of **Figure 6A** are technical replicates of the same experiment.

[0043] **Figures 7A-7C** show purification of exemplary fusion polypeptides using sequential Ni-NTA affinity and size exclusion chromatography. All polypeptides ran as a single peak on SDS-PAGE and SEC, demonstrating there was no significant degradation or aggregation. **Figure 7A** shows results for mIL12-ABP10. **Figure 7B** shows results for mIL12-ABP20-G4-6x-GE. **Figure 7C** shows results for mIL12-ABP20-G4-8x-GE.

[0044] **Figure 8** provides assessment of alum retention for exemplary fusion polypeptides, mIL12-ABP10-K, mIL12-ABP20-G4-6x-GE-K, and mIL12-ABP20-G4-8x-GE-K, relative to control, mIL12. All three fusion polypeptides showed greater retention on alum compared to unmodified IL12, mIL12-ABP20-G4-6x-GE was eluted more quickly than both mIL12-ABP10 and mIL12-ABP20-G4-8x-GE.

[0045] **Figure 9A** and **Figure 9B** demonstrate exemplary purification of fusion polypeptides. To generate more homogeneously phosphorylated material, mIL12-ABP10-K (**Figure 9A**) and mIL12-ABP20-G4-8x-GE-K (**Figure 9B**) were further purified by anion exchange chromatography. Samples were eluted with a linear salt gradient and the second half of each elution peak was collected and pooled to eliminate early eluting fractions containing lower levels of phosphorylation.

[0046] **Figures 10A-10C** provide exemplary assessment of enriched pools of fusion polypeptide. Enriched pools of mIL12-ABP10 (**Figure 10A**) and mIL12-ABP20-G4-8x-GE (**Figure 10B**) after preparative anion exchange chromatography were compared to the original material by analytical ion exchange chromatography. Elution times for (mIL12-ABP10) and (mIL12-ABP20-G4-8x-GE) are summarized in (**Figure 10C**).

[0047] **Figure 11A** and **Figure 11B** demonstrate exemplary assessment of ion-exchange enriched fusion polypeptides. **Figure 11A** shows ion-exchange enriched mIL12-ABP10 and mIL12-ABP20-G4-8x-GE assessed in an alum retention assay along with a sample of mIL12-ABP20-G4-8x-GE prior to ion-exchange polishing. Polypeptides were complexed with a 10-fold mass excess of aluminum hydroxide as defined by metal mass then diluted in elution buffer containing 5 mM phosphate and 20% mouse serum for 24 hours before quantifying free polypeptide in the supernatant. Ion exchange purified mIL12-

ABP20-G4-8x-GE had the highest retention with 89% bound to alum at 24 hours compared to 86% for mIL12-ABP10 and 78% for the non-ion exchanged material demonstrating that the low phosphate fractions elute off the alum faster. **Figure 11B** shows that samples were bound to a 10-fold mass excess of aluminum hydroxide as defined by metal mass then eluted with 5 mM phosphate and 20% mouse serum for up to 48 hours. Ion exchange purified mIL12-ABP20-G4-8x-GE again had the highest retention with 82% bound to alum at 48 hours compared to 78% for mIL12-ABP10 and 17% for unmodified mIL12.

[0048] **Figure 12A** and **Figure 12B** demonstrate assessment of mIL12-ABP10 and mIL12-ABP20-G4-8x-GE in a HEK-Blue-IL12 reporter assay wherein SEAP is expressed in response to IL12 induced signaling. **Figure 12A** shows that mIL12-ABP10 was active in the assay with an EC₅₀ value of 3.6 ng/mL compared to control, mIL12, with an EC₅₀ value of 3.5 ng/mL. When bound to alum the EC₅₀ values shifted ~2-4 fold with EC₅₀ values of 8 ng/mL for mIL12-ABP10. **Figure 12B** shows that mIL12-ABP20-G4-8x-GE was active in the assay with an EC₅₀ value of 5.4 ng/mL, identical to control, mIL12, with an EC₅₀ value of 5.4 ng/mL. When bound to alum the EC₅₀ values shifted ~2-4 fold with EC₅₀ value of 22 ng/mL for mIL12-ABP20-G4-8x-GE.

[0049] **Figure 13** shows exemplary assessment for the activity of alum bound and eluted fractions of mIL12-ABP20-G4-8x-GE. A supernatant fraction, resuspended alum pellet, and a control sample of mIL12-ABP20-G4-8x-GE incubated overnight in elution buffer without alum were tested in the HEK-Blue-IL12 assay. A control sample without alum had a similar EC₅₀ to previous measurements at 7 ng/mL, a supernatant fraction had an EC₅₀ >1000 ng/mL, and an alum pellet was active in the assay with a potency shift of ~5x demonstrating that IL12 remains active while retained on alum for extended time.

[0050] **Figures 14A-14C** demonstrate efficacy of mIL12-ABP10-K/alum and mIL12-ABP20-G4-8x-GE-K/alum as a single shot monotherapy in a CT26 colorectal cancer model. **Figure 14A** shows an exemplary graph illustrating that a single intratumoral injection of 5 µg of either mIL12-ABP or mIL12-ABP20-G4-8x-GE fusion polypeptides complexed with 50 µg alum on Day 6 post tumor inoculation led to increased survival compared to vehicle treated animals. **Figures 14B** and **14C** show exemplary graphs illustrating that a single intratumoral injection of 5 µg of either mIL12-ABP or mIL12-

ABP20-G4-8x-GE fusion polypeptides complexed with 50 µg alum on Day 6 post tumor inoculation led to significant tumor delays and regressions compared to vehicle treated mice. Complete responses with no measurable tumor were observed in 4/10 mice treated with mIL12-ABP10 + alum and 5/10 mice treated with mIL12-ABP20-G4-8x-GE + alum.

[0051] **Figures 15A-15E** demonstrate exemplary fusion polypeptides, as a monotherapy and in combination with an anti-PD-1 antibody, delays refractory B16F10 tumors. 7.7 µg of either mIL12-ABP10 or mIL12-ABP20-G4-8x-GE complexed with 50 µg alum was injected intratumorally on Day 6 and 13 following tumor inoculation. In some animals, anti-PD1 antibody was administered intraperitoneally on Day 0, 3, 6, & 9 in combination with either mIL12-ABP10 or mIL12-ABP20-G4-8x-GE. Vehicle treatment or anti-PD-1 antibody as a monotherapy were used as controls. Tumor delay was further extended in groups receiving the combination treatment with systemic PD-1 blockade. In both the monotherapy and PD-1 combination groups, the median survival was longer in mice treated with mIL12-ABP20-G4-8x-GE compared to mIL12-ABP10. **Figure 15A** shows an exemplary graph of percent survival over time. **Figure 15B** shows an exemplary graph of tumor volume over time in animals treated with mIL12-ABP10-K/alum compared to vehicle. **Figure 15C** shows an exemplary graph of tumor volume over time in animals treated with mIL12-ABP20-G4-8x-GE/alum compared to vehicle. **Figure 15D** shows an exemplary graph of tumor volume over time in animals treated with mIL12-ABP10-K/alum and anti-PD-1 antibody compared to treatment with anti-PD-1 antibody alone. **Figure 15E** shows an exemplary graph of tumor volume over time in animals treated with mIL12-ABP20-G4-8x-GE-K/alum and anti-PD-1 antibody compared to treatment with anti-PD-1 antibody alone.

[0052] **Figure 16A** and **Figure 16B** demonstrate exemplary fusion polypeptide metal-hydroxide complex, mIL12-ABP20-G4-8x-GE-K/alum, reduces spontaneous metastases formation in an orthotopic 4T1 breast cancer model. Tumor-bearing mice were treated with a single intratumoral injection of 5 µg of mIL12-ABP polypeptides complexed with 50 µg alum on Day 7 post tumor inoculation. **Figure 16A** shows an exemplary graph illustrating that mIL12-ABP20-G4-8x-GE/alum treatment led to growth delay of the primary tumor compared to vehicle treatment. **Figure 16B** shows an exemplary graph illustrating

results from animals that were sacrificed on Day 28 and metastases were counted in the lung. 10/10 vehicle treated mice had at least one lung metastasis, while 6/10 of the mice in the mIL12-ABP20-G4-8x-GE/alum treated mice were metastases-free.

[0053] **Figure 17A** and **Figure 17B** provide exemplary results from purification of fusion polypeptide metal-hydroxide complex, mIL12-ABP20-G4-8x-GE to isolate fractions with different phosphorylation levels. **Figure 17A** show exemplary results for a fusion polypeptide that was purified by Ni-NTA chromatography and size exclusion chromatography, then run on a HiTrap Q Sepharose anion exchange column with a linear salt elution gradient. **Figure 17B** shows exemplary results from when individual elution fractions were collected and phosphate levels were measured by malachite green assay. Average phosphate levels per fraction ranged from 2 to 7 phosphate groups per fusion polypeptide. No Fam20C was utilized as a negative control. A pool of fractions 112-118 was utilized as a positive control.

[0054] **Figure 18A** and **Figure 18B** provide exemplary assessment of alum retention on individual fractions of exemplary fusion polypeptide, mIL12-ABP20-G4-8x-GE. Fractions eluted earlier off the anion exchange resin and containing lower phosphorylation levels had lower retention on alum compared to later eluting fractions with higher phosphorylation levels. In **Figure 18A** free protein in supernatant over time quantified by ELISA was compared to theoretical concentration of 12,500 ng/mL if all protein was eluted. **Figures 18B** shows exemplary results illustrating that fraction 110 with 2.4 phosphates per polypeptide had <50% polypeptide retained on alum at 24 hours and fraction 111 with 3.7 phosphates per polypeptide had ~80% polypeptide retained on alum. Fractions 112 and 113 with 4.6 and 5.8 phosphates per polypeptide respectively were highly retained with >95% bound to alum at 24 hours. Fractions 114-117 with >6 phosphates per polypeptide had no measurable free polypeptide eluting off alum in the assay.

[0055] **Figures 19A-19D** provide exemplary assessment of signaling competency on individual fractions of exemplary fusion polypeptide, mIL12-ABP20-G4-8x-GE. Individual fractions were tested in the HEK-Blue-IL12 reporter assay either alone or complexed with a 10-fold mass excess of aluminum hydroxide as defined by metal mass. **Figure 19A** and **Figure 19B** illustrate that fractions 110 and 112 had similar peak activity levels with or

without alum and 2-3 fold shifts in EC₅₀ when bound to alum. **Figure 19C** and **Figure 19D** illustrate that fractions 114 and 117 had reduced peak activity when complexed with alum.

[0056] **Figure 20** summarizes phosphorylation levels, alum retention, and signaling competency of individual fractions of exemplary fusion polypeptide, mIL12-ABP20-G4-8x.

[0057] **Figures 21A-21C** demonstrate that human IL12-ABP material is active and retained on alum. **Figure 21A** shows an exemplary graph illustrating that human IL12-ABP20-G4-8x-GE is active in the HEK-Blue-IL12 assay with an EC₅₀ of 3.6 ng/mL alone or 8.2 ng/mL when complexed with aluminum hydroxide. **Figure 21B** shows an exemplary graph illustrating that, following incubation of the polypeptide/alum complex in elution buffer containing 1 mM phosphate and 20% mouse serum, the supernatant EC₅₀ shifts >300-fold suggesting minimal polypeptide elution after 24 hours, while the pellet maintained activity with a 5x EC₅₀ shift. **Figure 21C** shows an exemplary graph illustrating that in a separate alum retention assay, hIL12 or hIL12-ABP was bound to a 10-fold mass excess of aluminum hydroxide as defined by metal mass then diluted in elution buffer containing 1 mM phosphate and 20% mouse serum with free polypeptide detected by ELISA. Approximately 80% of the hIL12-ABP20-G4-8x-GE was retained on the alum at 24 hours compared to 0% for unmodified IL12.

[0058] **Figure 22** presents exemplary results of an assessment of an exemplary human IL12-ABP20-G4-8x-GE protein. Human IL12-ABP20-G4-8x-GE was separated by anion exchange chromatography and selected purification fractions were assessed for numbers of phosphate per protein using a malachite green assay, IL12 signaling potency using the HEK-Blue-IL12 assay, and aluminum hydroxide binding using an alum retention assay.

[0059] **Figure 23** presents exemplary results of an assessment of binding and retention of purified fractions of an exemplary human IL12-ABP20-G4-8x-GE on aluminum hydroxide. Human IL12-ABP20-G4-8x-GE purification fractions that eluted earlier off the anion exchange resin and containing lower phosphorylation levels had lower retention on aluminum hydroxide compared to later eluting fractions with higher phosphorylation levels.

[0060] **Figure 24** presents exemplary results of an assessment of the ability of purified fractions of an exemplary human IL12-ABP20-G4-8x-GE to induce IL12-based

signaling using a HEK-Blue-IL12 reporter assay either alone or complexed with a 10-fold mass excess of aluminum hydroxide as defined by metal mass.

[0061] **Figure 25** presents results of assessment of an exemplary canine-IL12-ABP20-G4-8x-GE. Canine-IL12-ABP20-G4-8x-GE was co-transfected in suspension HEK-293 cells with a human Fam20C-KDEL plasmid at a 4:1 mass ratio. Supernatants were harvested and canine-IL12-ABP20-G4-8x-GE was purified. Selected purification fractions were assessed for numbers of phosphate per protein using a malachite green assay, IL12 signaling potency using the HEK-Blue-IL12 assay, and aluminum hydroxide binding using an alum retention assay.

[0062] **Figure 26** presents results of an assessment of binding and retention of purified fractions of an exemplary canine-IL12-ABP20-G4-8x-GE on aluminum hydroxide. Canine-IL12-ABP20-G4-8x-GE purification fractions that eluted earlier off the anion exchange resin and containing lower phosphorylation levels had lower retention on aluminum hydroxide compared to later eluting fractions with higher phosphorylation levels.

[0063] **Figure 27** presents results of an assessment of the ability of purified fractions of an exemplary canine-IL12-ABP20-G4-8x-GE to induce IL12-based signaling using a HEK-Blue-IL12 reporter assay either alone or complexed with a 10-fold mass excess of aluminum hydroxide as defined by metal mass.

Definitions

[0064] **Administration:** As used herein, the term “administration” typically refers to the administration of a composition to a subject or system. Those of ordinary skill in the art will be aware of a variety of routes that may, in appropriate circumstances, be utilized for administration to a subject, for example a human. For example, in some embodiments, administration may be systemic or local. In some embodiments, administration may be enteral or parenteral. In some embodiments, administration may be by injection (e.g., intramuscular, intratumoral, intravenous, or subcutaneous injection). In some embodiments, injection may involve bolus injection, drip, perfusion, or infusion. In many embodiments, administration in accordance with the present disclosure is by intratumoral injection.

[0065] *Affinity*: As is known in the art, “affinity” is a measure of the tightness with which two or more binding partners associate with one another. Those skilled in the art are aware of a variety of assays that can be used to assess affinity, and will furthermore be aware of appropriate controls for such assays. In some embodiments, affinity is assessed in a quantitative assay. In some embodiments, affinity is assessed over a plurality of concentrations (*e.g.*, of one binding partner at a time). In some embodiments, affinity is assessed in the presence of one or more potential competitor entities (*e.g.*, that might be present in a relevant – *e.g.*, physiological – setting). In some embodiments, affinity is assessed relative to a reference (*e.g.*, that has a known affinity above a particular threshold [a “positive control” reference] or that has a known affinity below a particular threshold [a “negative control” reference]). In some embodiments, affinity may be assessed relative to a contemporaneous reference; in some embodiments, affinity may be assessed relative to a historical reference. Typically, when affinity is assessed relative to a reference, it is assessed under comparable conditions.

[0066] *Agent*: In general, the term “agent”, as used herein, is used to refer to an entity (*e.g.*, for example, a lipid, metal, nucleic acid, polypeptide, polysaccharide, small molecule, etc, or complex, combination, mixture or system [e.g., cell, tissue, organism] thereof), or phenomenon (*e.g.*, heat, electric current or field, magnetic force or field, etc). In appropriate circumstances, as will be clear from context to those skilled in the art, the term may be utilized to refer to an entity that is or comprises a cell or organism, or a fraction, extract, or component thereof. Alternatively or additionally, as context will make clear, the term may be used to refer to a natural product in that it is found in and/or is obtained from nature. In some instances, again as will be clear from context, the term may be used to refer to one or more entities that is man-made in that it is designed, engineered, and/or produced through action of the hand of man and/or is not found in nature. In some embodiments, an agent may be utilized in isolated or pure form; in some embodiments, an agent may be utilized in crude form. In some embodiments, potential agents may be provided as collections or libraries, for example that may be screened to identify or characterize active agents within them.

[0067] **Agonist:** Those skilled in the art will appreciate that the term “agonist” may be used to refer to an agent, condition, or event whose presence, level, degree, type, or form correlates with increased level or activity of another agent (i.e., the agonized agent or the target agent). In general, an agonist may be or include an agent of any chemical class including, for example, small molecules, polypeptides, nucleic acids, carbohydrates, lipids, metals, and/or any other entity that shows the relevant activating activity. In some embodiments, an agonist may be direct (in which case it exerts its influence directly upon its target); in some embodiments, an agonist may be indirect (in which case it exerts its influence by other than binding to its target; e.g., by interacting with a regulator of the target, so that level or activity of the target is altered).

[0068] **Amino acid:** in its broadest sense, as used herein, the term “amino acid” refers to a compound and/or substance that can be, is, or has been incorporated into a polypeptide chain, e.g., through formation of one or more peptide bonds. In some embodiments, an amino acid has the general structure $\text{H}_2\text{N}-\text{C}(\text{H})(\text{R})-\text{COOH}$. In some embodiments, an amino acid is a naturally-occurring amino acid. In some embodiments, an amino acid is a non-natural amino acid; in some embodiments, an amino acid is a D-amino acid; in some embodiments, an amino acid is an L-amino acid. “Standard amino acid” refers to any of the twenty standard L-amino acids commonly found in naturally occurring peptides. “Nonstandard amino acid” refers to any amino acid, other than the standard amino acids, regardless of whether it is prepared synthetically or obtained from a natural source. In some embodiments, an amino acid, including a carboxy- and/or amino-terminal amino acid in a polypeptide, can contain a structural modification as compared with the general structure above. For example, in some embodiments, an amino acid may be modified by methylation, amidation, acetylation, pegylation, glycosylation, phosphorylation, and/or substitution (e.g., of the amino group, the carboxylic acid group, one or more protons, and/or the hydroxyl group) as compared with the general structure. In some embodiments, such modification may, for example, alter the circulating half-life of a polypeptide containing the modified amino acid as compared with one containing an otherwise identical unmodified amino acid. In some embodiments, such modification does not significantly alter a relevant activity of a polypeptide containing the modified amino acid, as compared with one containing an otherwise identical unmodified amino acid. As will be clear from context, in

some embodiments, the term “amino acid” may be used to refer to a free amino acid; in some embodiments it may be used to refer to an amino acid residue of a polypeptide.

[0069] ***Animal:*** as used herein refers to any member of the animal kingdom. In some embodiments, "animal" refers to humans, of either sex and at any stage of development. In some embodiments, "animal" refers to non-human animals, at any stage of development. In certain embodiments, the non-human animal is a mammal (*e.g.*, a rodent, a mouse, a rat, a rabbit, a monkey, a dog, a cat, a horse, a sheep, cattle, a primate, and/or a pig). In some embodiments, animals include, but are not limited to, mammals, birds, reptiles, amphibians, fish, insects, and/or worms. In some embodiments, an animal may be a transgenic animal, genetically engineered animal, and/or a clone.

[0070] ***Antibody:*** As used herein, the term “antibody” refers to a polypeptide that includes canonical immunoglobulin sequence elements sufficient to confer specific binding to a particular target antigen. As is known in the art, intact antibodies as produced in nature are approximately 150 kD tetrameric agents comprised of two identical heavy chain polypeptides (about 50 kD each) and two identical light chain polypeptides (about 25 kD each) that associate with each other into what is commonly referred to as a “Y-shaped” structure. Each heavy chain is comprised of at least four domains (each about 110 amino acids long)— an amino-terminal variable (VH) domain (located at the tips of the Y structure), followed by three constant domains: CH1, CH2, and the carboxy-terminal CH3 (located at the base of the Y’s stem). A short region, known as the “switch”, connects the heavy chain variable and constant regions. The “hinge” connects CH2 and CH3 domains to the rest of the antibody. Two disulfide bonds in this hinge region connect the two heavy chain polypeptides to one another in an intact antibody. Each light chain is comprised of two domains – an amino-terminal variable (VL) domain, followed by a carboxy-terminal constant (CL) domain, separated from one another by another “switch”. Intact antibody tetramers are comprised of two heavy chain-light chain dimers in which the heavy and light chains are linked to one another by a single disulfide bond; two other disulfide bonds connect the heavy chain hinge regions to one another, so that the dimers are connected to one another and the tetramer is formed. Naturally-produced antibodies are also glycosylated, typically on the CH2 domain. Each domain in a natural antibody has a

structure characterized by an “immunoglobulin fold” formed from two beta sheets (e.g., 3-, 4-, or 5-stranded sheets) packed against each other in a compressed antiparallel beta barrel. Each variable domain contains three hypervariable loops known as “complement determining regions” (CDR1, CDR2, and CDR3) and four somewhat invariant “framework” regions (FR1, FR2, FR3, and FR4). When natural antibodies fold, the FR regions form the beta sheets that provide the structural framework for the domains, and the CDR loop regions from both the heavy and light chains are brought together in three-dimensional space so that they create a single hypervariable antigen binding site located at the tip of the Y structure. The Fc region of naturally-occurring antibodies binds to elements of the complement system, and also to receptors on effector cells, including for example effector cells that mediate cytotoxicity. As is known in the art, affinity and/or other binding attributes of Fc regions for Fc receptors can be modulated through glycosylation or other modification. In some embodiments, antibodies produced and/or utilized in accordance with the present invention include glycosylated Fc domains, including Fc domains with modified or engineered such glycosylation. For purposes of the present invention, in certain embodiments, any polypeptide or complex of polypeptides that includes sufficient immunoglobulin domain sequences as found in natural antibodies can be referred to and/or used as an “antibody”, whether such polypeptide is naturally produced (e.g., generated by an organism reacting to an antigen), or produced by recombinant engineering, chemical synthesis, or other artificial system or methodology. In some embodiments, an antibody is polyclonal; in some embodiments, an antibody is monoclonal. In some embodiments, an antibody has constant region sequences that are characteristic of mouse, rabbit, primate, or human antibodies. In some embodiments, antibody sequence elements are humanized, primatized, chimeric, etc, as is known in the art. Moreover, the term “antibody” as used herein, can refer in appropriate embodiments (unless otherwise stated or clear from context) to any of the art-known or developed constructs or formats for utilizing antibody structural and functional features in alternative presentation. For example, in some embodiments, an antibody utilized in accordance with the present invention is in a format selected from, but not limited to, intact IgA, IgG, IgE or IgM antibodies; bi- or multi- specific antibodies (*e.g.*, Zybodies®, etc); antibody fragments such as Fab fragments, Fab’ fragments, F(ab’)2 fragments, Fd’ fragments, Fd fragments, and isolated CDRs or sets thereof; single chain

Fvs; polypeptide-Fc fusions; single domain antibodies, alternative scaffolds or antibody mimetics (*e.g.*, anticalins, FN3 monobodies, DARPins, Affibodies, Affilins, Affimers, Affitins, Alphabodies, Avimers, Fynomers, Im7, VLR, VNAR, Trimab, CrossMab, Trident); nanobodies, binanobodies, F(ab')₂, Fab', di-sdFv, single domain antibodies, trifunctional antibodies, diabodies, and minibodies. etc. In some embodiments, relevant formats may be or include: Adnectins®; Affibodies®; Affilins®; Anticalins®; Avimers®; BiTE®s; cameloid antibodies; Centyrins®; ankyrin repeat proteins or DARPINs®; dual-affinity re-targeting (DART) agents; Fynomers®; shark single domain antibodies such as IgNAR; immune mobilizing monoclonal T cell receptors against cancer (ImmTACs); KALBITOR®s; MicroProteins; Nanobodies® minibodies; masked antibodies (*e.g.*, Probodies®); Small Modular ImmunoPharmaceuticals (“SMIPs™”); single chain or Tandem diabodies (TandAb®); TCR-like antibodies; Trans-bodies®; TrimerX®; VHHs. In some embodiments, an antibody may lack a covalent modification (*e.g.*, attachment of a glycan) that it would have if produced naturally. In some embodiments, an antibody may contain a covalent modification (*e.g.*, attachment of a glycan, a payload [*e.g.*, a detectable moiety, a therapeutic moiety, a catalytic moiety, etc], or other pendant group [*e.g.*, polyethylene glycol, etc.])

[0071] *Antibody fragment*: As used herein, an “antibody fragment” refers to a portion of an antibody or antibody agent as described herein, and typically refers to a portion that includes an antigen-binding portion or variable region thereof. An antibody fragment may be produced by any means. For example, in some embodiments, an antibody fragment may be enzymatically or chemically produced by fragmentation of an intact antibody or antibody agent. Alternatively, in some embodiments, an antibody fragment may be recombinantly produced (*i.e.*, by expression of an engineered nucleic acid sequence. In some embodiments, an antibody fragment may be wholly or partially synthetically produced. In some embodiments, an antibody fragment (particularly an antigen-binding antibody fragment) may have a length of at least about 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190 amino acids or more, in some embodiments at least about 200 amino acids.

[0072] ***Binding:*** It will be understood that the term “binding”, as used herein, typically refers to a non-covalent association between or among two or more entities. “Direct” binding involves physical contact between entities or moieties; indirect binding involves physical interaction by way of physical contact with one or more intermediate entities. Binding between two or more entities can typically be assessed in any of a variety of contexts – including where interacting entities or moieties are studied in isolation or in the context of more complex systems (*e.g.*, while covalently, electrostatically, or otherwise associated with a carrier entity and/or in a biological system or cell). Binding between two entities may be considered “specific” if, under the conditions assessed, the relevant entities are more likely to associate with one another than with other available binding partners.

[0073] ***Cancer:*** The terms "cancer", “malignancy”, "neoplasm", "tumor", and "carcinoma", are used herein to refer to cells that exhibit relatively abnormal, uncontrolled, and/or autonomous growth, so that they exhibit an aberrant growth phenotype characterized by a significant loss of control of cell proliferation. In some embodiments, a tumor may be or comprise cells that are precancerous (*e.g.*, benign), malignant, pre-metastatic, metastatic, and/or non-metastatic. The present disclosure specifically identifies certain cancers to which its teachings may be particularly relevant. In some embodiments, a relevant cancer may be characterized by a solid tumor. In some embodiments, a relevant cancer may be characterized by a hematologic tumor. In general, examples of different types of cancers known in the art include, for example, hematopoietic cancers including leukemias, lymphomas (Hodgkin’s and non-Hodgkin’s), myelomas and myeloproliferative disorders; sarcomas, melanomas, adenomas, carcinomas of solid tissue, squamous cell carcinomas of the mouth, throat, larynx, and lung, liver cancer, genitourinary cancers such as prostate, cervical, bladder, uterine, and endometrial cancer and renal cell carcinomas, bone cancer, pancreatic cancer, skin cancer, cutaneous or intraocular melanoma, cancer of the endocrine system, cancer of the thyroid gland, cancer of the parathyroid gland, head and neck cancers, breast cancer, gastro-intestinal cancers and nervous system cancers, benign lesions such as papillomas, and the like.

[0074] ***Characteristic sequence element:*** As used herein, the phrase “characteristic sequence element” refers to a sequence element found in a polymer (*e.g.*, in a polypeptide or

nucleic acid) that represents a characteristic portion of that polymer. In some embodiments, presence of a characteristic sequence element correlates with presence or level of a particular activity or property of the polymer. In some embodiments, presence (or absence) of a characteristic sequence element defines a particular polymer as a member (or not a member) of a particular family or group of such polymers. A characteristic sequence element typically comprises at least two monomers (e.g., amino acids or nucleotides). In some embodiments, a characteristic sequence element includes at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 20, 25, 30, 35, 40, 45, 50, or more monomers (e.g., contiguously linked monomers). In some embodiments, a characteristic sequence element includes at least first and second stretches of contiguous monomers spaced apart by one or more spacer regions whose length may or may not vary across polymers that share the sequence element.

[0075] *Chemotherapeutic Agent:* The term “chemotherapeutic agent”, has used herein has its art-understood meaning referring to one or more pro-apoptotic, cytostatic and/or cytotoxic agents, for example specifically including agents utilized and/or recommended for use in treating one or more diseases, disorders or conditions associated with undesirable cell proliferation. In many embodiments, chemotherapeutic agents are useful in the treatment of cancer. In some embodiments, a chemotherapeutic agent may be or comprise one or more alkylating agents, one or more anthracyclines, one or more cytoskeletal disruptors (e.g. microtubule targeting agents such as taxanes, maytansine and analogs thereof, of), one or more epothilones, one or more histone deacetylase inhibitors HDACs), one or more topoisomerase inhibitors (e.g., inhibitors of topoisomerase I and/or topoisomerase II), one or more kinase inhibitors, one or more nucleotide analogs or nucleotide precursor analogs, one or more peptide antibiotics, one or more platinum-based agents, one or more retinoids, one or more vinca alkaloids, and/or one or more analogs of one or more of the following (i.e., that share a relevant anti-proliferative activity). In some particular embodiments, a chemotherapeutic agent may be or comprise one or more of Actinomycin, All-trans retinoic acid, an Auiristatin, Azacitidine, Azathioprine, Bleomycin, Bortezomib, Carboplatin, Capecitabine, Cisplatin, Chlorambucil, Cyclophosphamide, Curcumin, Cytarabine, Daunorubicin, Docetaxel, Doxifluridine, Doxorubicin, Epirubicin, Epothilone, Etoposide, Fluorouracil, Gemcitabine, Hydroxyurea, Idarubicin, Imatinib, Irinotecan, Maytansine and/or analogs thereof (e.g. DM1) Mechlorethamine,

Mercaptopurine, Methotrexate, Mitoxantrone, a Maytansinoid, Oxaliplatin, Paclitaxel, Pemetrexed, Teniposide, Tioguanine, Topotecan, Valrubicin, Vinblastine, Vincristine, Vindesine, Vinorelbine, and combinations thereof. In some embodiments, a chemotherapeutic agent may be utilized in the context of an antibody-drug conjugate. In some embodiments, a chemotherapeutic agent is one found in an antibody-drug conjugate selected from the group consisting of: hLL1-doxorubicin, hRS7-SN-38, hMN-14-SN-38, hLL2-SN-38, hA20-SN-38, hPAM4-SN-38, hLL1-SN-38, hRS7-Pro-2-P-Dox, hMN-14-Pro-2-P-Dox, hLL2-Pro-2-P-Dox, hA20-Pro-2-P-Dox, hPAM4-Pro-2-P-Dox, hLL1-Pro-2-P-Dox, P4/D10-doxorubicin, gemtuzumab ozogamicin, brentuximab vedotin, trastuzumab emtansine, inotuzumab ozogamicin, glembatumomab vedotin, SAR3419, SAR566658, BIIB015, BT062, SGN-75, SGN-CD19A, AMG-172, AMG-595, BAY-94-9343, ASG-5ME, ASG-22ME, ASG-16M8F, MDX-1203, MLN-0264, anti-PSMA ADC, RG-7450, RG-7458, RG-7593, RG-7596, RG-7598, RG-7599, RG-7600, RG-7636, ABT-414, IMGN-853, IMGN-529, vorsetuzumab mafodotin, and lorvotuzumab mertansine. In some embodiments, a chemotherapeutic agent may be one described as utilized in an antibody-drug conjugate as described or discussed in one or more of Govindan et al, *TheScientificWorldJOURNAL* 10:2070, 2010, –2089). In some embodiments, a chemotherapeutic agent may be or comprise one or more of farnesyl-thiosalicylic acid (FTS), 4-(4-Chloro-2-methylphenoxy)-N-hydroxybutanamide (CMH), estradiol (E2), tetramethoxystilbene (TMS), δ -tocatrienol, salinomycin, or curcumin. *Combination Therapy*: As used herein, the term “combination therapy” refers to those situations in which a subject is simultaneously exposed to two or more therapeutic regimens (e.g., two or more therapeutic agents). In some embodiments, two or more agents may be administered simultaneously; in some embodiments, such agents may be administered sequentially; in some embodiments, such agents are administered in overlapping dosing regimens.

[0076] *Combination therapy*: As used herein, the term “combination therapy” refers to those situations in which a subject is simultaneously exposed to two or more therapeutic regimens (e.g., two or more therapeutic agents). In some embodiments, the two or more regimens may be administered simultaneously; in some embodiments, such regimens may be administered sequentially (e.g., all “doses” of a first regimen are administered prior to administration of any doses of a second regimen); in some

embodiments, such agents are administered in overlapping dosing regimens. In some embodiments, “administration” of combination therapy may involve administration of one or more agent(s) or modality(ies) to a subject receiving the other agent(s) or modality(ies) in the combination. For clarity, combination therapy does not require that individual agents be administered together in a single composition (or even necessarily at the same time), although in some embodiments, two or more agents, or active moieties thereof, may be administered together in a combination composition, or even in a combination compound (e.g., as part of a single chemical complex or covalent entity).

[0077] *Dosing regimen:* Those skilled in the art will appreciate that the term “dosing regimen” may be used to refer to a set of unit doses (typically more than one) that are administered individually to a subject, typically separated by periods of time. In some embodiments, a given therapeutic agent has a recommended dosing regimen, which may involve one or more doses. In some embodiments, a dosing regimen comprises a plurality of doses each of which is separated in time from other doses. In some embodiments, individual doses are separated from one another by a time period of the same length; in some embodiments, a dosing regimen comprises a plurality of doses and at least two different time periods separating individual doses. In some embodiments, all doses within a dosing regimen are of the same unit dose amount. In some embodiments, different doses within a dosing regimen are of different amounts. In some embodiments, a dosing regimen comprises a first dose in a first dose amount, followed by one or more additional doses in a second dose amount different from the first dose amount. In some embodiments, a dosing regimen comprises a first dose in a first dose amount, followed by one or more additional doses in a second dose amount same as the first dose amount. In some embodiments, a dosing regimen is correlated with a desired or beneficial outcome when administered across a relevant population (*i.e.*, is a therapeutic dosing regimen).

[0078] *Epitope:* as used herein, the term “epitope” refers to a moiety that is specifically recognized by an immunoglobulin (e.g., antibody or receptor) binding component. In some embodiments, an epitope is comprised of a plurality of chemical atoms or groups on an antigen. In some embodiments, such chemical atoms or groups are surface-exposed when the antigen adopts a relevant three-dimensional conformation. In some

embodiments, such chemical atoms or groups are physically near to each other in space when the antigen adopts such a conformation. In some embodiments, at least some such chemical atoms or groups are physically separated from one another when the antigen adopts an alternative conformation (e.g., is linearized).

[0079] ***Excipient:*** as used herein, refers to a non-therapeutic agent that may be included in a pharmaceutical composition, for example to provide or contribute to a desired consistency or stabilizing effect. Suitable pharmaceutical excipients include, for example, starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene, glycol, water, ethanol and the like.

[0080] ***Expression:*** As used herein, the term “expression” of a nucleic acid sequence refers to the generation of any gene product from the nucleic acid sequence. In some embodiments, a gene product can be a transcript. In some embodiments, a gene product can be a polypeptide. In some embodiments, expression of a nucleic acid sequence involves one or more of the following: (1) production of an RNA template from a DNA sequence (e.g., by transcription); (2) processing of an RNA transcript (e.g., by splicing, editing, etc); (3) translation of an RNA into a polypeptide or protein; and/or (4) post-translational modification of a polypeptide or protein.

[0081] ***Functional:*** As used herein, the term “functional” is used to refer to a form or fragment of an entity that exhibits a particular property and/or activity.

[0082] ***Fragment:*** A “fragment” of a material or entity as described herein has a structure that includes a discrete portion of the whole, but lacks one or more moieties found in the whole. In some embodiments, a fragment consists of such a discrete portion. In some embodiments, a fragment consists of or comprises a characteristic structural element or moiety found in the whole. In some embodiments, a polymer fragment comprises or consists of at least 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 210, 220, 230, 240, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475, 500 or more monomeric units (e.g., residues) as found in the whole polymer. In some embodiments, a polymer fragment comprises or consists of at least about 5%, 10%, 15%, 20%, 25%, 30%,

25%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or more of the monomeric units (e.g., residues) found in the whole polymer. The whole material or entity may in some embodiments be referred to as the “parent” of the fragment.

[0083] *Gene*: As used herein, the term “gene” refers to a DNA sequence in a chromosome that codes for a product (e.g., an RNA product and/or a polypeptide product). In some embodiments, a gene includes coding sequence (i.e., sequence that encodes a particular product); in some embodiments, a gene includes non-coding sequence. In some particular embodiments, a gene may include both coding (e.g., exonic) and non-coding (e.g., intronic) sequences. In some embodiments, a gene may include one or more regulatory elements that, for example, may control or impact one or more aspects of gene expression (e.g., cell-type-specific expression, inducible expression, etc.).

[0084] *Gene product or expression product*: As used herein, the term “gene product” or “expression product” generally refers to an RNA transcribed from the gene (pre- and/or post-processing) or a polypeptide (pre- and/or post-modification) encoded by an RNA transcribed from the gene.

[0085] *Genome*: As used herein, the term “genome” refers to the total genetic information carried by an individual organism or cell, represented by the complete DNA sequences of its chromosomes.

[0086] *Host cell*: as used herein, refers to a cell into which exogenous DNA (recombinant or otherwise) has been introduced. Persons of skill upon reading this disclosure will understand that such terms refer not only to the particular subject cell, but also to the progeny of such a cell. Because certain modifications may occur in succeeding generations due to either mutation or environmental influences, such progeny may not, in fact, be identical to the parent cell, but are still included within the scope of the term “*host cell*” as used herein. In some embodiments, host cells include prokaryotic and eukaryotic cells selected from any of the Kingdoms of life that are suitable for expressing an exogenous DNA (e.g., a recombinant nucleic acid sequence). Exemplary cells include those of prokaryotes and eukaryotes (single-cell or multiple-cell), bacterial cells (e.g., strains of *E. coli*, *Bacillus spp.*, *Streptomyces spp.*, etc.), mycobacteria cells, fungal cells, yeast cells (e.g.,

S. cerevisiae, *S. pombe*, *P. pastoris*, *P. methanolica*, etc.), plant cells, insect cells (e.g., SF-9, SF-21, baculovirus-infected insect cells, Trichoplusia ni, etc.), non-human animal cells, human cells, or cell fusions such as, for example, hybridomas or quadromas. In some embodiments, the cell is a human, monkey, ape, hamster, rat, or mouse cell. In some embodiments, the cell is eukaryotic and is selected from the following cells: CHO (e.g., CHO K1, DXB-1 CHO, Veggie-CHO), COS (e.g., COS-7), retinal cell, Vero, CV1, kidney (e.g., HEK293, 293 EBNA, MSR 293, MDCK, HaK, BHK), HeLa, HepG2, WI38, MRC 5, Colo205, HB 8065, HL-60, (e.g., BHK21), Jurkat, Daudi, A431 (epidermal), CV-1, U937, 3T3, L cell, C127 cell, SP2/0, NS-0, MMT 060562, Sertoli cell, BRL 3 A cell, HT1080 cell, myeloma cell, tumor cell, and a cell line derived from an aforementioned cell. In some embodiments, the cell comprises one or more viral genes.

[0087] ***Human:*** In some embodiments, a human is an embryo, a fetus, an infant, a child, a teenager, an adult, or a senior citizen.

[0088] ***“Improved,” “increased” or “reduced”:*** As used herein, these terms, or grammatically comparable comparative terms, indicate values that are relative to a comparable reference measurement. For example, in some embodiments, an assessed value achieved with an agent of interest may be “improved” relative to that obtained with a comparable reference agent. Alternatively or additionally, in some embodiments, an assessed value achieved in a subject or system of interest may be “improved” relative to that obtained in the same subject or system under different conditions (e.g., prior to or after an event such as administration of an agent of interest), or in a different, comparable subject (e.g., in a comparable subject or system that differs from the subject or system of interest in presence of one or more indicators of a particular disease, disorder or condition of interest, or in prior exposure to a condition or agent, etc). In some embodiments, comparative terms refer to statistically relevant differences (e.g., that are of a prevalence and/or magnitude sufficient to achieve statistical relevance). Those skilled in the art will be aware, or will readily be able to determine, in a given context, a degree and/or prevalence of difference that is required or sufficient to achieve such statistical significance.

[0089] ***In vitro***: The term “in vitro” as used herein refers to events that occur in an artificial environment, *e.g.*, in a test tube or reaction vessel, in cell culture, etc., rather than within a multi-cellular organism.

[0090] ***In vivo***: as used herein refers to events that occur within a multi-cellular organism, such as a human and a non-human animal. In the context of cell-based systems, the term may be used to refer to events that occur within a living cell (as opposed to, for example, *in vitro* systems).

[0091] ***Isolated***: as used herein, refers to a substance and/or entity that has been (1) separated from at least some of the components with which it was associated when initially produced (whether in nature and/or in an experimental setting), and/or (2) designed, produced, prepared, and/or manufactured by the hand of man. Isolated substances and/or entities may be separated from about 10%, about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, or more than about 99% of the other components with which they were initially associated. In some embodiments, isolated agents are about 80%, about 85%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, or more than about 99% pure. As used herein, a substance is “*pure*” if it is substantially free of other components. In some embodiments, as will be understood by those skilled in the art, a substance may still be considered “*isolated*” or even “*pure*”, after having been combined with certain other components such as, for example, one or more carriers or excipients (*e.g.*, buffer, solvent, water, etc.); in such embodiments, percent isolation or purity of the substance is calculated without including such carriers or excipients. To give but one example, in some embodiments, a biological polymer such as a polypeptide or polynucleotide that occurs in nature is considered to be “*isolated*” when, a) by virtue of its origin or source of derivation is not associated with some or all of the components that accompany it in its native state in nature; b) it is substantially free of other polypeptides or nucleic acids of the same species from the species that produces it in nature; c) is expressed by or is otherwise in association with components from a cell or other expression system that is not of the species that produces it in nature. Thus, for instance, in some

embodiments, a polypeptide that is chemically synthesized or is synthesized in a cellular system different from that which produces it in nature is considered to be an "*isolated*" polypeptide. Alternatively or additionally, in some embodiments, a polypeptide that has been subjected to one or more purification techniques may be considered to be an "*isolated*" polypeptide to the extent that it has been separated from other components a) with which it is associated in nature; and/or b) with which it was associated when initially produced.

[0092] **Linker:** as used herein, is used to refer to that portion of a multi-element agent that connects different elements to one another. For example, those of ordinary skill in the art appreciate that a polypeptide whose structure includes two or more functional or organizational moieties or domains often includes a stretch of amino acids between such moieties or domains that links them to one another. In some embodiments, a polypeptide comprising a linker element has an overall structure of the general form S1-L-S2, wherein S1 and S2 may be the same or different and represent two moieties or domains associated with one another by the linker. In some embodiments, a polypeptide linker is at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100 or more amino acids in length. In some embodiments, a linker is characterized in that it tends not to adopt a rigid three-dimensional structure, but rather provides flexibility to the polypeptide. A variety of different linker elements that can appropriately be used when engineering polypeptides (*e.g.*, fusion polypeptides) known in the art (*see e.g.*, Holliger *et al.*, *Proc. Natl. Acad. Sci. USA* 90:6444, 1993; Poljak *et al.* *Structure* 2:1121, 1994).

[0093] **Modulator:** The term "modulator" is used to refer to an entity whose presence or level in a system in which an activity of interest is observed correlates with a change in level and/or nature of that activity as compared with that observed under otherwise comparable conditions when the modulator is absent. In some embodiments, a modulator is an activator, in that activity is increased in its presence as compared with that observed under otherwise comparable conditions when the modulator is absent. In some embodiments, a modulator is an antagonist or inhibitor, in that activity is reduced in its presence as compared with otherwise comparable conditions when the modulator is absent. In some embodiments, a modulator interacts directly with a target entity whose activity is of

interest. In some embodiments, a modulator interacts indirectly (i.e., directly with an intermediate agent that interacts with the target entity) with a target entity whose activity is of interest. In some embodiments, a modulator affects level of a target entity of interest; alternatively or additionally, in some embodiments, a modulator affects activity of a target entity of interest without affecting level of the target entity. In some embodiments, a modulator affects both level and activity of a target entity of interest, so that an observed difference in activity is not entirely explained by or commensurate with an observed difference in level

[0094] ***Moiety:*** Those skilled in the art will appreciate that a “moiety” is a defined chemical group or entity with a particular structure and/or activity, as described herein. Typically, a “moiety” is part of, less than the entirety of, a molecule or entity.

[0095] ***Mutant:*** As used herein, the term “mutant” refers to an entity that shows significant structural identity with a reference entity but differs structurally from the reference entity in the presence or level of one or more chemical moieties as compared with the reference entity. In many embodiments, a mutant also differs functionally from its reference entity. In general, whether a particular entity is properly considered to be a “mutant” of a reference entity is based on its degree of structural identity with the reference entity. As will be appreciated by those skilled in the art, any biological or chemical reference entity has certain characteristic structural elements. A mutant, by definition, is a distinct chemical entity that shares one or more such characteristic structural elements. To give but a few examples, a small molecule may have a characteristic core structural element (e.g., a macrocycle core) and/or one or more characteristic pendent moieties so that a mutant of the small molecule is one that shares the core structural element and the characteristic pendent moieties but differs in other pendent moieties and/or in types of bonds present (single vs double, E vs Z, etc.) within the core, a polypeptide may have a characteristic sequence element comprised of a plurality of amino acids having designated positions relative to one another in linear or three-dimensional space and/or contributing to a particular biological function, a nucleic acid may have a characteristic sequence element comprised of a plurality of nucleotide residues having designated positions relative to one another in linear or three-dimensional space. For example, a mutant polypeptide may differ

from a reference polypeptide as a result of one or more differences in amino acid sequence and/or one or more differences in chemical moieties (e.g., carbohydrates, lipids, etc.) covalently attached to the polypeptide backbone. In some embodiments, a mutant polypeptide shows an overall sequence identity with a reference polypeptide that is at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, or 99%. Alternatively or additionally, in some embodiments, a mutant polypeptide does not share at least one characteristic sequence element with a reference polypeptide. In some embodiments, the reference polypeptide has one or more biological activities. In some embodiments, a mutant polypeptide shares one or more of the biological activities of the reference polypeptide. In some embodiments, a mutant polypeptide lacks one or more of the biological activities of the reference polypeptide. In some embodiments, a mutant polypeptide shows a reduced level of one or more biological activities as compared with the reference polypeptide.

[0096] ***Operably linked***: as used herein, refers to a juxtaposition wherein the components described are in a relationship permitting them to function in their intended manner. A control element "*operably linked*" to a functional element is associated in such a way that expression and/or activity of the functional element is achieved under conditions compatible with the control element. In some embodiments, "*operably linked*" control elements are contiguous (e.g., covalently linked) with the coding elements of interest; in some embodiments, control elements act in *trans* to or otherwise at a from the functional element of interest.

[0097] ***Patient***: As used herein, the term "patient" refers to any organism to which a provided composition is or may be administered, *e.g.*, for experimental, diagnostic, prophylactic, cosmetic, and/or therapeutic purposes. Typical patients include animals (*e.g.*, mammals such as mice, rats, rabbits, non-human primates, and/or humans). In some embodiments, a patient is a human. In some embodiments, a patient is suffering from or susceptible to one or more disorders or conditions. In some embodiments, a patient displays one or more symptoms of a disorder or condition. In some embodiments, a patient has been diagnosed with one or more disorders or conditions. In some embodiments, the disorder or condition is or includes cancer, or presence of one or more tumors. In some embodiments,

the patient is receiving or has received certain therapy to diagnose and/or to treat a disease, disorder, or condition.

[0098] *Pharmaceutical composition:* As used herein, the term “pharmaceutical composition” refers to an active agent, formulated together with one or more pharmaceutically acceptable carriers. In some embodiments, active agent is present in unit dose amount appropriate for administration in a therapeutic regimen that shows a statistically significant probability of achieving a predetermined therapeutic effect when administered to a relevant population. In some embodiments, pharmaceutical compositions may be specially formulated for administration in solid or liquid form, including those adapted for a particular route of administration, *e.g.*, as described herein..

[0099] *Pharmaceutically acceptable:* As used herein, the phrase “pharmaceutically acceptable” is used to refer to an agent or entity that, within the scope of sound medical judgment, is suitable for use in contact with tissues of human beings and/or animals without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio.

[0100] *Pharmaceutically acceptable carrier:* As used herein, the term “pharmaceutically acceptable carrier” means a pharmaceutically-acceptable material, composition or vehicle, such as a liquid or solid filler, diluent, excipient, or solvent encapsulating material, involved in carrying or transporting the subject compound from one organ, or portion of the body, to another organ, or portion of the body. Each carrier must be “acceptable” in the sense of being compatible with the other ingredients of the formulation and not injurious to the patient. Some examples of materials which can serve as pharmaceutically-acceptable carriers include: sugars, such as lactose, glucose and sucrose; starches, such as corn starch and potato starch; cellulose, and its derivatives, such as sodium carboxymethyl cellulose, ethyl cellulose and cellulose acetate; powdered tragacanth; malt; gelatin; talc; excipients, such as cocoa butter and suppository waxes; oils, such as peanut oil, cottonseed oil, safflower oil, sesame oil, olive oil, corn oil and soybean oil; glycols, such as propylene glycol; polyols, such as glycerin, sorbitol, mannitol and polyethylene glycol; esters, such as ethyl oleate and ethyl laurate; agar; buffering agents, such as magnesium hydroxide and aluminum hydroxide; alginic acid; pyrogen-free water; isotonic saline;

Ringer's solution; ethyl alcohol; pH buffered solutions; polyesters, polycarbonates and/or polyanhydrides; and other non-toxic compatible substances employed in pharmaceutical formulations.

[0101] *Pharmaceutically acceptable salt:* The term “pharmaceutically acceptable salt”, as used herein, refers to salts of such compounds that are appropriate for use in pharmaceutical contexts, *i.e.*, salts which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of humans and lower animals without undue toxicity, irritation, allergic response and the like, and are commensurate with a reasonable benefit/risk ratio. Pharmaceutically acceptable salts are well known in the art. For example, S. M. Berge, et al. describes pharmaceutically acceptable salts in detail in *J. Pharmaceutical Sciences*, 66: 1-19 (1977). In some embodiments, pharmaceutically acceptable salts include, but are not limited to, nontoxic acid addition salts, which are salts of an amino group formed with inorganic acids such as hydrochloric acid, hydrobromic acid, phosphoric acid, sulfuric acid and perchloric acid or with organic acids such as acetic acid, maleic acid, tartaric acid, citric acid, succinic acid or malonic acid or by using other methods used in the art such as ion exchange. In some embodiments, pharmaceutically acceptable salts include, but are not limited to, adipate, alginate, ascorbate, aspartate, benzenesulfonate, benzoate, bisulfate, borate, butyrate, camphorate, camphorsulfonate, citrate, cyclopentanepropionate, digluconate, dodecylsulfate, ethanesulfonate, formate, fumarate, glucoheptonate, glycerophosphate, gluconate, hemisulfate, heptanoate, hexanoate, hydroiodide, 2-hydroxy-ethanesulfonate, lactobionate, lactate, laurate, lauryl sulfate, malate, maleate, malonate, methanesulfonate, 2-naphthalenesulfonate, nicotinate, nitrate, oleate, oxalate, palmitate, pamoate, pectinate, persulfate, 3-phenylpropionate, phosphate, picrate, pivalate, propionate, stearate, succinate, sulfate, tartrate, thiocyanate, *p*-toluenesulfonate, undecanoate, valerate salts, and the like. Representative alkali or alkaline earth metal salts include sodium, lithium, potassium, calcium, magnesium, and the like. In some embodiments, pharmaceutically acceptable salts include, when appropriate, nontoxic ammonium, quaternary ammonium, and amine cations formed using counterions such as halide, hydroxide, carboxylate, sulfate, phosphate, nitrate, alkyl having from 1 to 6 carbon atoms, sulfonate and aryl sulfonate.

[0102] ***Polypeptide:*** As used herein refers to a polymeric chain of amino acids. In some embodiments, a polypeptide has an amino acid sequence that occurs in nature. In some embodiments, a polypeptide has an amino acid sequence that does not occur in nature. In some embodiments, a polypeptide has an amino acid sequence that is engineered in that it is designed and/or produced through action of the hand of man. In some embodiments, a polypeptide may comprise or consist of natural amino acids, non-natural amino acids, or both. In some embodiments, a polypeptide may comprise or consist of only natural amino acids or only non-natural amino acids. In some embodiments, a polypeptide may comprise D-amino acids, L-amino acids, or both. In some embodiments, a polypeptide may comprise only D-amino acids. In some embodiments, a polypeptide may comprise only L-amino acids. In some embodiments, a polypeptide may include one or more pendant groups or other modifications, e.g., modifying or attached to one or more amino acid side chains, at the polypeptide's N-terminus, at the polypeptide's C-terminus, or any combination thereof. In some embodiments, such pendant groups or modifications may be selected from the group consisting of acetylation, amidation, lipidation, methylation, pegylation, etc., including combinations thereof. In some embodiments, a polypeptide may be cyclic, and/or may comprise a cyclic portion. In some embodiments, a polypeptide is not cyclic and/or does not comprise any cyclic portion. In some embodiments, a polypeptide is linear. In some embodiments, a polypeptide may be or comprise a stapled polypeptide. In some embodiments, the term "polypeptide" may be appended to a name of a reference polypeptide, activity, or structure; in such instances it is used herein to refer to polypeptides that share the relevant activity or structure and thus can be considered to be members of the same class or family of polypeptides. For each such class, the present specification provides and/or those skilled in the art will be aware of exemplary polypeptides within the class whose amino acid sequences and/or functions are known; in some embodiments, such exemplary polypeptides are reference polypeptides for the polypeptide class or family. In some embodiments, a member of a polypeptide class or family shows significant sequence homology or identity with, shares a common sequence motif (e.g., a characteristic sequence element) with, and/or shares a common activity (in some embodiments at a comparable level or within a designated range) with a reference polypeptide of the class; in some embodiments with all polypeptides within the class). For example, in some embodiments, a

member polypeptide shows an overall degree of sequence homology or identity with a reference polypeptide that is at least about 30-40%, and is often greater than about 50%, 60%, 70%, 80%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more and/or includes at least one region (e.g., a conserved region that may in some embodiments be or comprise a characteristic sequence element) that shows very high sequence identity, often greater than 90% or even 95%, 96%, 97%, 98%, or 99%. Such a conserved region usually encompasses at least 3-4 and often up to 20 or more amino acids; in some embodiments, a conserved region encompasses at least one stretch of at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 or more contiguous amino acids. In some embodiments, a relevant polypeptide may comprise or consist of a fragment of a parent polypeptide. In some embodiments, a useful polypeptide as may comprise or consist of a plurality of fragments, each of which is found in the same parent polypeptide in a different spatial arrangement relative to one another than is found in the polypeptide of interest (e.g., fragments that are directly linked in the parent may be spatially separated in the polypeptide of interest or vice versa, and/or fragments may be present in a different order in the polypeptide of interest than in the parent), so that the polypeptide of interest is a derivative of its parent polypeptide.

[0103] ***Predetermined:*** By predetermined is meant deliberately selected, for example as opposed to randomly occurring or achieved.

[0104] ***Pure:*** As used herein, an agent or entity is “pure” if it is substantially free of other components. For example, a preparation that contains more than about 90% of a particular agent or entity is typically considered to be a pure preparation. In some embodiments, an agent or entity is at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% pure.

[0105] ***Recombinant:*** as used herein, is intended to refer to polypeptides that are designed, engineered, prepared, expressed, created, manufactured, and/or or isolated by recombinant means, such as polypeptides expressed using a recombinant expression vector transfected into a host cell; polypeptides isolated from a recombinant, combinatorial human polypeptide library; polypeptides isolated from an animal (e.g., a mouse, rabbit, sheep, fish, etc) that is transgenic for or otherwise has been manipulated to express a gene or genes, or gene components that encode and/or direct expression of the polypeptide or one or more

component(s), portion(s), element(s), or domain(s) thereof; and/or polypeptides prepared, expressed, created or isolated by any other means that involves splicing or ligating selected nucleic acid sequence elements to one another, chemically synthesizing selected sequence elements, and/or otherwise generating a nucleic acid that encodes and/or directs expression of the polypeptide or one or more component(s), portion(s), element(s), or domain(s) thereof. In some embodiments, one or more of such selected sequence elements is found in nature. In some embodiments, one or more of such selected sequence elements is designed *in silico*. In some embodiments, one or more such selected sequence elements results from mutagenesis (e.g., *in vivo* or *in vitro*) of a known sequence element, e.g., from a natural or synthetic source such as, for example, in the germline of a source organism of interest (e.g., of a human, a mouse, etc).

[0106] **Reference standard:** As used herein describes a standard or control relative to which a comparison is performed. For example, in some embodiments, an agent, animal, individual, population, sample, sequence or value of interest is compared with a reference or control agent, animal, individual, population, sample, sequence or value. In some embodiments, a reference or control is tested and/or determined substantially simultaneously with the testing or determination of interest. In some embodiments, a reference or control is a historical reference or control, optionally embodied in a tangible medium. Typically, as would be understood by those skilled in the art, a reference or control is determined or characterized under comparable conditions or circumstances to those under assessment. Those skilled in the art will appreciate when sufficient similarities are present to justify reliance on and/or comparison to a particular possible reference or control.

[0107] **Specific binding:** As used herein, the term “specific binding” refers to an ability to discriminate between possible binding partners in the environment in which binding is to occur. A binding agent that interacts with one particular target when other potential targets are present is said to “*bind specifically*” to the target with which it interacts. In some embodiments, specific binding is assessed by detecting or determining degree of association between the binding agent and its partner; in some embodiments, specific binding is assessed by detecting or determining degree of dissociation of a binding agent-partner complex; in some embodiments, specific binding is assessed by detecting or

determining ability of the binding agent to compete an alternative interaction between its partner and another entity. In some embodiments, specific binding is assessed by performing such detections or determinations across a range of concentrations.

[0108] *Specific:* The term “specific”, when used herein with reference to an agent having an activity, is understood by those skilled in the art to mean that the agent discriminates between potential target entities or states. For example, in some embodiments, an agent is said to bind “specifically” to its target if it binds preferentially with that target in the presence of one or more competing alternative targets. In many embodiments, specific interaction is dependent upon the presence of a particular structural feature of the target entity (e.g., an epitope, a cleft, a binding site). It is to be understood that specificity need not be absolute. In some embodiments, specificity may be evaluated relative to that of the binding agent for one or more other potential target entities (e.g., competitors). In some embodiments, specificity is evaluated relative to that of a reference specific binding agent. In some embodiments specificity is evaluated relative to that of a reference non-specific binding agent. In some embodiments, the agent or entity does not detectably bind to the competing alternative target under conditions of binding to its target entity. In some embodiments, binding agent binds with higher on-rate, lower off-rate, increased affinity, decreased dissociation, and/or increased stability to its target entity as compared with the competing alternative target(s).

[0109] *Specificity:* As is known in the art, “specificity” is a measure of the ability of a particular ligand to distinguish its binding partner from other potential binding partners.

[0110] *Subject:* As used herein, the term “subject” refers an organism, typically a mammal (e.g., a human, in some embodiments including prenatal human forms). In some embodiments, a subject is suffering from a relevant disease, disorder or condition. In some embodiments, a subject is susceptible to a disease, disorder, or condition. In some embodiments, a subject displays one or more symptoms or characteristics of a disease, disorder or condition. In some embodiments, a subject does not display any symptom or characteristic of a disease, disorder, or condition. In some embodiments, a subject is someone with one or more features characteristic of susceptibility to or risk of a disease,

disorder, or condition. In some embodiments, a subject is a patient. In some embodiments, a subject is an individual to whom diagnosis and/or therapy is and/or has been administered.

[0111] *Therapeutic agent:* As used herein, the phrase “therapeutic agent” refers to an agent that, when administered to a subject, has a therapeutic effect and/or elicits a desired biological and/or pharmacological effect. In some embodiments, a therapeutic agent is any substance that can be used to alleviate, ameliorate, relieve, inhibit, prevent, delay onset of, reduce severity of, and/or reduce incidence of one or more symptoms or features of a disease, disorder, and/or condition.

[0112] *Therapeutically effective amount:* As used herein, the term “therapeutically effective amount” means an amount of a substance (*e.g.*, a therapeutic agent, composition, and/or formulation) that elicits a desired biological response when administered as part of a therapeutic regimen. In some embodiments, a therapeutically effective amount of a substance is an amount that is sufficient, when administered to a subject suffering from or susceptible to a disease, disorder, and/or condition, to treat, diagnose, prevent, and/or delay the onset of the disease, disorder, and/or condition. As will be appreciated by those of ordinary skill in this art, the effective amount of a substance may vary depending on such factors as the desired biological endpoint, the substance to be delivered, the target cell or tissue, *etc.* For example, the effective amount of compound in a formulation to treat a disease, disorder, and/or condition is the amount that alleviates, ameliorates, relieves, inhibits, prevents, delays onset of, reduces severity of and/or reduces incidence of one or more symptoms or features of the disease, disorder, and/or condition. In some embodiments, a therapeutically effective amount is administered in a single dose; in some embodiments, multiple unit doses are required to deliver a therapeutically effective amount.

[0113] *Transformation:* as used herein, refers to any process by which exogenous DNA is introduced into a host cell. Transformation may occur under natural or artificial conditions using various methods well known in the art. Transformation may rely on any known method for the insertion of foreign nucleic acid sequences into a prokaryotic or eukaryotic host cell. In some embodiments, a particular transformation methodology is selected based on the host cell being transformed and may include, but is not limited to, viral infection, electroporation, mating, lipofection. In some embodiments, a “transformed” cell

is stably transformed in that the inserted DNA is capable of replication either as an autonomously replicating plasmid or as part of the host chromosome. In some embodiments, a transformed cell transiently expresses introduced nucleic acid for limited periods of time.

[0114] ***Treatment:*** As used herein, the term “treatment” (also “treat” or “treating”) refers to administration of a therapy that partially or completely alleviates, ameliorates, relieves, inhibits, delays onset of, reduces severity of, and/or reduces incidence of one or more symptoms, features, and/or causes of a particular disease, disorder, and/or condition. In some embodiments, such treatment may be of a subject who does not exhibit signs of the relevant disease, disorder and/or condition and/or of a subject who exhibits only early signs of the disease, disorder, and/or condition. Alternatively or additionally, such treatment may be of a subject who exhibits one or more established signs of the relevant disease, disorder and/or condition. In some embodiments, treatment may be of a subject who has been diagnosed as suffering from the relevant disease, disorder, and/or condition. In some embodiments, treatment may be of a subject known to have one or more susceptibility factors that are statistically correlated with increased risk of development of the relevant disease, disorder, and/or condition. Thus, in some embodiments, treatment may be prophylactic; in some embodiments, treatment may be therapeutic.

[0115] ***Tumor:*** As used herein, the term “tumor” refers to an abnormal growth of cells or tissue. In some embodiments, a tumor may comprise cells that are precancerous (e.g., benign), malignant, pre-metastatic, metastatic, and/or non-metastatic. In some embodiments, a tumor is associated with, or is a manifestation of, a cancer. In some embodiments, a tumor may be a disperse tumor or a liquid tumor. In some embodiments, a tumor may be a solid tumor.

[0116] ***Variant:*** As used herein in the context of molecules, e.g., nucleic acids, proteins, or small molecules, the term “variant” refers to a molecule that shows significant structural identity with a reference molecule but differs structurally from the reference molecule, e.g., in the presence or absence or in the level of one or more chemical moieties as compared to the reference entity. In some embodiments, a variant also differs functionally from its reference molecule. In general, whether a particular molecule is properly

considered to be a “variant” of a reference molecule is based on its degree of structural identity with the reference molecule. As will be appreciated by those skilled in the art, any biological or chemical reference molecule has certain characteristic structural elements. A variant, by definition, is a distinct molecule that shares one or more such characteristic structural elements but differs in at least one aspect from the reference molecule. To give but a few examples, a polypeptide may have a characteristic sequence element comprised of a plurality of amino acids having designated positions relative to one another in linear or three-dimensional space and/or contributing to a particular structural motif and/or biological function; a nucleic acid may have a characteristic sequence element comprised of a plurality of nucleotide residues having designated positions relative to one another in linear or three-dimensional space. In some embodiments, a variant polypeptide or nucleic acid may differ from a reference polypeptide or nucleic acid as a result of one or more differences in amino acid or nucleotide sequence and/or one or more differences in chemical moieties (*e.g.*, carbohydrates, lipids, phosphate groups) that are covalently components of the polypeptide or nucleic acid (*e.g.*, that are attached to the polypeptide or nucleic acid backbone). In some embodiments, a variant polypeptide or nucleic acid shows an overall sequence identity with a reference polypeptide or nucleic acid that is at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, or 99%. In some embodiments, a variant polypeptide or nucleic acid does not share at least one characteristic sequence element with a reference polypeptide or nucleic acid. In some embodiments, a reference polypeptide or nucleic acid has one or more biological activities. In some embodiments, a variant polypeptide or nucleic acid shares one or more of the biological activities of the reference polypeptide or nucleic acid. In some embodiments, a variant polypeptide or nucleic acid lacks one or more of the biological activities of the reference polypeptide or nucleic acid. In some embodiments, a variant polypeptide or nucleic acid shows a reduced level of one or more biological activities as compared to the reference polypeptide or nucleic acid. In some embodiments, a polypeptide or nucleic acid of interest is considered to be a “variant” of a reference polypeptide or nucleic acid if it has an amino acid or nucleotide sequence that is identical to that of the reference but for a small number of sequence alterations at particular positions. Typically, fewer than about 20%, about 15%, about 10%, about 9%, about 8%, about 7%, about 6%, about 5%, about 4%, about 3%, or about 2% of the residues in a

variant are substituted, inserted, or deleted, as compared to the reference. In some embodiments, a variant polypeptide or nucleic acid comprises about 10, about 9, about 8, about 7, about 6, about 5, about 4, about 3, about 2, or about 1 substituted residues as compared to a reference. Often, a variant polypeptide or nucleic acid comprises a very small number (*e.g.*, fewer than about 5, about 4, about 3, about 2, or about 1) number of substituted, inserted, or deleted, functional residues (*i.e.*, residues that participate in a particular biological activity) relative to the reference. In some embodiments, a variant polypeptide or nucleic acid comprises not more than about 5, about 4, about 3, about 2, or about 1 addition or deletion, and, in some embodiments, comprises no additions or deletions, as compared to the reference. In some embodiments, a variant polypeptide or nucleic acid comprises fewer than about 25, about 20, about 19, about 18, about 17, about 16, about 15, about 14, about 13, about 10, about 9, about 8, about 7, about 6, and commonly fewer than about 5, about 4, about 3, or about 2 additions or deletions as compared to the reference. In some embodiments, a reference polypeptide or nucleic acid is one found in nature. In some embodiments, a reference polypeptide or nucleic acid is a human polypeptide or nucleic acid.

[0117] *Vector*: as used herein, refers to a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked. One type of vector is a "*plasmid*", which refers to a circular double stranded DNA loop into which additional DNA segments may be ligated. Another type of vector is a viral vector, wherein additional DNA segments may be ligated into the viral genome. Certain vectors are capable of autonomous replication in a host cell into which they are introduced (*e.g.*, bacterial vectors having a bacterial origin of replication and episomal mammalian vectors). Other vectors (*e.g.*, non-episomal mammalian vectors) can be integrated into the genome of a host cell upon introduction into the host cell, and thereby are replicated along with the host genome. Moreover, certain vectors are capable of directing the expression of genes to which they are operatively linked. Such vectors are referred to herein as "*expression vectors*."

[0118] *Wild-type*: As used herein, the term "wild-type" has its art-understood meaning and refers to a form of an entity (*e.g.*, a polypeptide or nucleic acid) that has a structure and/or activity as found in nature in a "normal" (as contrasted with mutant,

diseased, altered) state or context. In some embodiments, more than one “wild type” form of a particular polypeptide or nucleic acid may exist in nature, for example as “alleles” of a particular gene or normal variants of a particular polypeptide. In some embodiments, that form (or those forms) of a particular polypeptide or nucleic acid that is most commonly observed in a population (*e.g.*, in a human population) is the “wild type” form.

Detailed Description of Certain Embodiments

Fusion polypeptide-metal hydroxide complexes

[0119] Immune-modulating therapeutic approaches can be useful in treating a variety of disease states, including cancer. Established immune-modulating therapeutic approaches include, but are not limited to, monoclonal antibodies, T cell therapy, immune checkpoint modulators, and immunomodulatory polypeptides. The present disclosure provides technologies that, among other things, include fusion polypeptides comprising an immunomodulatory polypeptide and a metal-hydroxide-binding polypeptide, as well as technologies to produce and/or utilize such fusion polypeptides (for example, Figure 2). In some embodiments, provided fusion polypeptides utilize a metal- hydroxide-binding polypeptide that adopts at least first and second states, one of which shows materially higher metal-hydroxide binding (*e.g.*, when included in a fusion polypeptide as described herein) than the other. In some embodiments, such first and second states differ from one another by substitution of one or more hydroxyl moieties (*e.g.*, by phosphorylation).

[0120] In some embodiments, provided fusion polypeptides display one or more improved structural or functional characteristics relative to a reference polypeptide as described in the Fusion Protein Filing; in some such embodiments, the reference polypeptide includes ABP10.

[0121] Among other things, the present disclosure provides a surprising discovery that improved (*e.g.*, relative to a reference described in the Fusion Protein Filing, which reference may be or comprise ABP10) metal-hydroxide binding polypeptides can be developed. For example, in some embodiments, the present disclosure provides metal-hydroxide binding polypeptides characterized by enhanced metal hydroxide (*e.g.*, alum)

conjugation (*e.g.*, enhanced rate and/or extent of association and/or decreased rate and/or extent of release) relative to such appropriate reference (*e.g.*, alone and/or when included in a fusion polypeptide, for example as described and/or exemplified herein). Alternatively or additionally, in some embodiments, provided metal-hydroxide binding polypeptides are characterized by improved intratumoral retention (*e.g.*, after intratumoral injection) efficacy, as compared to such appropriate reference (*e.g.*, alone and/or when included in a fusion polypeptide, for example as described and/or exemplified herein).

[0122] In some embodiments, a fusion polypeptide in accordance with the present disclosure is characterized by improved tumor retention and/or efficacy as compared with an appropriate comparable reference fusion polypeptide that includes ABP10.

[0123] In some embodiments, the present disclosure provides technologies for developing, providing, and/or utilizing improved metal-hydroxide binding polypeptides and/or fusion polypeptides that include them; in some embodiments, improvement comprises and/or involves optimizing phosphate content (*e.g.*, of a metal-hydroxide-binding polypeptide and/or of a fusion polypeptide that comprises it).

[0124] Among other things, the present disclosure surprisingly teaches that certain metal-hydroxide-binding polypeptides, and/or one or more fusion polypeptides that include them, are characterized by one or more in improved manufacturing features (*e.g.*, are amenable to more facile and/or reproducible manufacturing).

[0125] Among other things, the present disclosure provides particularly useful fusion polypeptides. Furthermore, the present disclosure demonstrates effectiveness of provided fusion polypeptides in treating a subject with a tumor. Still further, the present disclosure surprisingly teaches that, in some embodiments, a particular fusion polypeptide is useful as a monotherapy and/or that, in some embodiments, a particular fusion polypeptide is particularly useful in combination therapy (*e.g.*, in combination with an immune modulator such as an immune checkpoint inhibitor such as, for example, an anti-PD-1 agent such as an anti-PD-1 antibody).

[0126] Alternatively or additionally, in some embodiments, the present disclosure provides certain technologies for production and/or characterization of provided fusion

polypeptides, compositions that comprise them, and/or components within them (*e.g.*, immunomodulatory polypeptides, metal-hydroxide-binding polypeptides, linkers, *etc.*).

[0127] In some embodiments, provided technologies achieve reproducible production of fusion peptide preparations, specifically including phosphorylated preparations and/or metal-hydroxide-complexed preparations. In some embodiments, provided technologies may include, for example, expression, purification, and/or analytical technologies. Moreover, in some embodiments, the present disclosure provides desirable preparations of provided fusion polypeptides, including in some embodiments phosphorylated preparations and/or in some embodiments, preparations of fusion polypeptides (*e.g.*, phosphorylated fusion polypeptides) complexed with a metal hydroxide.

[0128] **Table 1** provides exemplary amino acid sequences of polypeptides described herein.

Table 1: Exemplary Amino Acid Sequences

SEQ ID NO:	Polypeptide	Sequence
1	Mouse IL12B	MWELEKDVYVVEVDWTPDAPGETVNLTCDTPEEDDITWTSD QRHGVIGSGKTLTITVKEFLDAGQYTCHKGGETLSHSHLLLH KKENGIWSTEILKNFKNKTFKCEAPNYSGRFTCSWL VQRNM DLKFNIKSSSSSPDSRAVTCGMASLSAEKVTL DQRDYEKYSVS CQEDVTCPTAEETLPIELALEARQQNKYENYSTSFFIRDIKPD PKNLQMKPLKNSQVEVSWEYPDSWSTPHSYFSLKFFVRIQRK KEKMKETEEGCNQKGAFLVEKTSTEVQCKGGNVCVQAQDR YYNSSCSKWACVPCRVS
2	Mouse IL12A	RVIPVSGPARCLSQSRNLLKTTDDMVKTAREKLKHYSCTAED IDHEDITRDQTSTLKTCLPLELHKNESCLATRETSSTTRGSCLP PQKTSMMTLCLGSIYEDLKMYQTEFQAINAALQNHNHQQII LDKGMVLVAIDELMQSLNHNGETLRQKPPVGEADPYRVKMKL CILLHAFSTRVVTINRVMGYLSSA
3	(G4S) ₃ Linker	GGGGSGGGSGGGGS
4	Mouse IL12 (IL12B-linker-IL12A)	MWELEKDVYVVEVDWTPDAPGETVNLTCDTPEEDDITWTSD QRHGVIGSGKTLTITVKEFLDAGQYTCHKGGETLSHSHLLLH KKENGIWSTEILKNFKNKTFKCEAPNYSGRFTCSWL VQRNM DLKFNIKSSSSSPDSRAVTCGMASLSAEKVTL DQRDYEKYSVS CQEDVTCPTAEETLPIELALEARQQNKYENYSTSFFIRDIKPD PKNLQMKPLKNSQVEVSWEYPDSWSTPHSYFSLKFFVRIQRK

		KEKMKETEEGCNQKGAFLEKTSSTEVQCKGGNVCVQAQDR YNNSSCSKWACVPCRVRSGGGGSGGGGSGGGGSRVIPVSGP ARCLSQSRNLLKTTDDMVKTAREKLKHYSCTAEDIDHEDITR DQTSTLKTCLPLELHKNESCLATRETSSTTRGSCLPQKTSLM MTLCLGSIYEDLKMYQTEFQAINAALQNHNHQQIILDKGMLV AIDELMQSLNHNGETLRQKPPVGEADPYRVKMKLCILLHAFS TRVVTINRVMGYLSSA
5	mIL12-His (no ABP)	MWELEKDVYVVEVDWTPDAPGETVNLTCDTPEEDDITWTSD QRHGVIGSGKTLTITVKEFLDAGQYTCHKGGETLSHSHLLLH KKENGIWSTEILKNFKNKTFKCEAPNYSGRFTCSWLVRQNM DLKFNIKSSSSSPDSRAVTCGMASLSAEKVTLQDRDYEKYSVS CQEDVTCPTAEETLPIELALEARQQNKYENYSTSFFIRDIIKPD PKNLQMKPLKNSQVEVSWEYPDSWSTPHSYFSLKFFVRIQRK KEKMKETEEGCNQKGAFLEKTSSTEVQCKGGNVCVQAQDR YNNSSCSKWACVPCRVRSGGGGSGGGGSGGGGSRVIPVSGP ARCLSQSRNLLKTTDDMVKTAREKLKHYSCTAEDIDHEDITR DQTSTLKTCLPLELHKNESCLATRETSSTTRGSCLPQKTSLM MTLCLGSIYEDLKMYQTEFQAINAALQNHNHQQIILDKGMLV AIDELMQSLNHNGETLRQKPPVGEADPYRVKMKLCILLHAFS TRVVTINRVMGYLSSAGGGGSHHHHHH
6	mIL12- ABP10	MWELEKDVYVVEVDWTPDAPGETVNLTCDTPEEDDITWTSD QRHGVIGSGKTLTITVKEFLDAGQYTCHKGGETLSHSHLLLH KKENGIWSTEILKNFKNKTFKCEAPNYSGRFTCSWLVRQNM DLKFNIKSSSSSPDSRAVTCGMASLSAEKVTLQDRDYEKYSVS CQEDVTCPTAEETLPIELALEARQQNKYENYSTSFFIRDIIKPD PKNLQMKPLKNSQVEVSWEYPDSWSTPHSYFSLKFFVRIQRK KEKMKETEEGCNQKGAFLEKTSSTEVQCKGGNVCVQAQDR YNNSSCSKWACVPCRVRSGGGGSGGGGSGGGGSRVIPVSGP ARCLSQSRNLLKTTDDMVKTAREKLKHYSCTAEDIDHEDITR DQTSTLKTCLPLELHKNESCLATRETSSTTRGSCLPQKTSLM MTLCLGSIYEDLKMYQTEFQAINAALQNHNHQQIILDKGMLV AIDELMQSLNHNGETLRQKPPVGEADPYRVKMKLCILLHAFS TRVVTINRVMGYLSSAGGGGSFQSEEQQGGGSGGSEEGGMES EESNGGGSGGSEEGGGGSHHHHHH
7	mIL12- ABP20	MWELEKDVYVVEVDWTPDAPGETVNLTCDTPEEDDITWTSD QRHGVIGSGKTLTITVKEFLDAGQYTCHKGGETLSHSHLLLH KKENGIWSTEILKNFKNKTFKCEAPNYSGRFTCSWLVRQNM DLKFNIKSSSSSPDSRAVTCGMASLSAEKVTLQDRDYEKYSVS CQEDVTCPTAEETLPIELALEARQQNKYENYSTSFFIRDIIKPD PKNLQMKPLKNSQVEVSWEYPDSWSTPHSYFSLKFFVRIQRK KEKMKETEEGCNQKGAFLEKTSSTEVQCKGGNVCVQAQDR YNNSSCSKWACVPCRVRSGGGGSGGGGSGGGGSRVIPVSGP ARCLSQSRNLLKTTDDMVKTAREKLKHYSCTAEDIDHEDITR DQTSTLKTCLPLELHKNESCLATRETSSTTRGSCLPQKTSLM MTLCLGSIYEDLKMYQTEFQAINAALQNHNHQQIILDKGMLV AIDELMQSLNHNGETLRQKPPVGEADPYRVKMKLCILLHAFS

		TRVVTINRVMGYLSSAGGGGSGGGGSEEESEEESEEEGGGGS HHHHHH
8	mIL12- ABP20-G4	MWELEKDVYVVEVDWTPDAPGETVNLTCDTPEEDDITWTSD QRHGVIGSGKTLTITVKEFLDAGQYTCHKGGETLSHSHLLLH KKENGIWSTEILKNFKNKTFKCEAPNYSGRFTCSWL VQRNM DLKFNIKSSSSSPDSRAVTCGMASLSAEKVTL DQRDYEKYSVS CQEDVTCPTAEETLPIELALEARQQNKYENYSTSFFIRDIKDPD PKNLQMKPLKNSQVEVSWEYPDSWSTPHSYFSLKFFVRIQRK KEKMKETEEGCNQKGAFLVEKTSTEVQCKGGNVCVQAQDR YYNSSCSKWACVPCRVRSGGGGSGGGGSGGGGSRVIPVSGP ARCLSQSRNLLKTTDDMVKTAREKLKHYSCTAEDIDHEDITR DQTSTLKTCLPLELHKNESCLATRETSSTTRGSCLP PQKTSLM MTLCLGSIYEDLKMYQTEFQAINAALQNH NHQQIILDKGMLV AIDELMQSLNHNGETLRQKPPVGEADPYRVKMKLCILLHAFS TRVVTINRVMGYLSSAGGGGSGGGGSEEGGGGSEEGGGGSE EGGGGSEEGGGGSHHHHHH
9	mIL12- ABP20-G4- GS20	MWELEKDVYVVEVDWTPDAPGETVNLTCDTPEEDDITWTSD QRHGVIGSGKTLTITVKEFLDAGQYTCHKGGETLSHSHLLLH KKENGIWSTEILKNFKNKTFKCEAPNYSGRFTCSWL VQRNM DLKFNIKSSSSSPDSRAVTCGMASLSAEKVTL DQRDYEKYSVS CQEDVTCPTAEETLPIELALEARQQNKYENYSTSFFIRDIKDPD PKNLQMKPLKNSQVEVSWEYPDSWSTPHSYFSLKFFVRIQRK KEKMKETEEGCNQKGAFLVEKTSTEVQCKGGNVCVQAQDR YYNSSCSKWACVPCRVRSGGGGSGGGGSGGGGSRVIPVSGP ARCLSQSRNLLKTTDDMVKTAREKLKHYSCTAEDIDHEDITR DQTSTLKTCLPLELHKNESCLATRETSSTTRGSCLP PQKTSLM MTLCLGSIYEDLKMYQTEFQAINAALQNH NHQQIILDKGMLV AIDELMQSLNHNGETLRQKPPVGEADPYRVKMKLCILLHAFS TRVVTINRVMGYLSSAGGGGSGGGGSGGGGSGGGGSEEGGG GSEEGGGGSEEGGGGSEEGGGGSHHHHHH
10	mIL12- ABP20-G4- 6x	MWELEKDVYVVEVDWTPDAPGETVNLTCDTPEEDDITWTSD QRHGVIGSGKTLTITVKEFLDAGQYTCHKGGETLSHSHLLLH KKENGIWSTEILKNFKNKTFKCEAPNYSGRFTCSWL VQRNM DLKFNIKSSSSSPDSRAVTCGMASLSAEKVTL DQRDYEKYSVS CQEDVTCPTAEETLPIELALEARQQNKYENYSTSFFIRDIKDPD PKNLQMKPLKNSQVEVSWEYPDSWSTPHSYFSLKFFVRIQRK KEKMKETEEGCNQKGAFLVEKTSTEVQCKGGNVCVQAQDR YYNSSCSKWACVPCRVRSGGGGSGGGGSGGGGSRVIPVSGP ARCLSQSRNLLKTTDDMVKTAREKLKHYSCTAEDIDHEDITR DQTSTLKTCLPLELHKNESCLATRETSSTTRGSCLP PQKTSLM MTLCLGSIYEDLKMYQTEFQAINAALQNH NHQQIILDKGMLV AIDELMQSLNHNGETLRQKPPVGEADPYRVKMKLCILLHAFS TRVVTINRVMGYLSSAGGGGSGGGGSEEGGGGSEEGGGGSE EGGGGSEEGGGGSEEGGGGSEEGGGGSHHHHHH

11	mIL12- ABP20-G4- 8x	MWELEKDVYVVEVDWTPDAPGETVNLTCDTPEEDDITWTSD QRHGVIGSGKTLTITVKEFLDAGQYTCHKGGETLSHSHLLLH KKENGIWSTEILKNFKNKTFKCEAPNYSGRFTCSWL VQRNM DLKFNIKSSSSSPDSRAVTCGMASLSAEKVTL DQRDYEKYSVS CQEDVTCPTAEETLPIELALEARQQNKYENYSTSFFIRDIKPD PKNLQMKPLKNSQVEVSWEYPDSWSTPHSYFSLKFFVRIQRK KEKMKETEEGCNQKGAFLVEKTSTEVQCKGGNVCVQAQDR YYNSSCSKWACVPCRVRSGGGGSGGGGSGGGGSRVIPVSGP ARCLSQSRNLLKTTDDMVKTAREKLKHYSCTAEDIDHEDITR DQTSTLKTCLPLELHKNESCLATRETSSTTRGSCLP PQTSLM MTLCLGSIYEDLKMYQTEFQAINAALQNH NHQQIILDKGMLV AIDELMQSLNHNGETLRQKPPVGEADPYRVKMKLCILLHAFS TRVVTINRVMGYLSSAGGGGSGGGGSEEGGGGSEEGGGGSE EGGGGSEEGGGGSEEGGGGSEEGGGGSEEGGGGSEEGGGGS HHHHHH
12	mIL12- ABP20-6x	MWELEKDVYVVEVDWTPDAPGETVNLTCDTPEEDDITWTSD QRHGVIGSGKTLTITVKEFLDAGQYTCHKGGETLSHSHLLLH KKENGIWSTEILKNFKNKTFKCEAPNYSGRFTCSWL VQRNM DLKFNIKSSSSSPDSRAVTCGMASLSAEKVTL DQRDYEKYSVS CQEDVTCPTAEETLPIELALEARQQNKYENYSTSFFIRDIKPD PKNLQMKPLKNSQVEVSWEYPDSWSTPHSYFSLKFFVRIQRK KEKMKETEEGCNQKGAFLVEKTSTEVQCKGGNVCVQAQDR YYNSSCSKWACVPCRVRSGGGGSGGGGSGGGGSRVIPVSGP ARCLSQSRNLLKTTDDMVKTAREKLKHYSCTAEDIDHEDITR DQTSTLKTCLPLELHKNESCLATRETSSTTRGSCLP PQTSLM MTLCLGSIYEDLKMYQTEFQAINAALQNH NHQQIILDKGMLV AIDELMQSLNHNGETLRQKPPVGEADPYRVKMKLCILLHAFS TRVVTINRVMGYLSSAGGGGSGGGGSEEESEEESEEESEEESEE GGGGSHHHHHH
13	mIL12- ABP20-8x	MWELEKDVYVVEVDWTPDAPGETVNLTCDTPEEDDITWTSD QRHGVIGSGKTLTITVKEFLDAGQYTCHKGGETLSHSHLLLH KKENGIWSTEILKNFKNKTFKCEAPNYSGRFTCSWL VQRNM DLKFNIKSSSSSPDSRAVTCGMASLSAEKVTL DQRDYEKYSVS CQEDVTCPTAEETLPIELALEARQQNKYENYSTSFFIRDIKPD PKNLQMKPLKNSQVEVSWEYPDSWSTPHSYFSLKFFVRIQRK KEKMKETEEGCNQKGAFLVEKTSTEVQCKGGNVCVQAQDR YYNSSCSKWACVPCRVRSGGGGSGGGGSGGGGSRVIPVSGP ARCLSQSRNLLKTTDDMVKTAREKLKHYSCTAEDIDHEDITR DQTSTLKTCLPLELHKNESCLATRETSSTTRGSCLP PQTSLM MTLCLGSIYEDLKMYQTEFQAINAALQNH NHQQIILDKGMLV AIDELMQSLNHNGETLRQKPPVGEADPYRVKMKLCILLHAFS TRVVTINRVMGYLSSAGGGGSGGGGSEEESEEESEEESEEESEE SEEESEEGGGGSHHHHHH

14	mIL12- ABP20-G4- SEEE	MWELEKDVYVVEVDWTPDAPGETVNLTCDTPEEDDITWTSD QRHGVIGSGKTLTITVKEFLDAGQYTCHKGGETLSHSHLLLH KKENGIWSTEILKNFKNKTKFLKCEAPNYSGRFTCSWLVRQNM DLKFNIKSSSSSPDSRAVTCGMASLSAEKVTLQDRDYEKYSVS CQEDVTCPTAEETLPIELALEARQQNKYENYSTSFFIRDIKPD PKNLQMKPLKNSQVEVSWEYPDSWSTPHSYFSLKFFVRIQRK KEKMKETEEGCNQKGAFLVEKTSTEVQCKGGNVCVQAQDR YNNSSCSKWACVPCRVRSGGGGSGGGGSGGGGSRVIPVSGP ARCLSQSRNLLKTTDDMVKTAREKLKHYSCTAEDIDHEDITR DQTSTLKTCLPLELHKNESCLATRETSSTTRGSCLPQKTSLM MTLCLGSIYEDLKMYQTEFQAINAALQNHNHQQIILDKGMLV AIDELMQSLNHNGETLRQKPPVGEADPYRVKMKLCILLHAFS TRVVTINRVMGYLSSAGGGGSGGGGSEEEGGGSEEEGGGSEE EGGGSEEEGGGSHHHHHH
15	mIL12- ABP20-G4- SEEA	MWELEKDVYVVEVDWTPDAPGETVNLTCDTPEEDDITWTSD QRHGVIGSGKTLTITVKEFLDAGQYTCHKGGETLSHSHLLLH KKENGIWSTEILKNFKNKTKFLKCEAPNYSGRFTCSWLVRQNM DLKFNIKSSSSSPDSRAVTCGMASLSAEKVTLQDRDYEKYSVS CQEDVTCPTAEETLPIELALEARQQNKYENYSTSFFIRDIKPD PKNLQMKPLKNSQVEVSWEYPDSWSTPHSYFSLKFFVRIQRK KEKMKETEEGCNQKGAFLVEKTSTEVQCKGGNVCVQAQDR YNNSSCSKWACVPCRVRSGGGGSGGGGSGGGGSRVIPVSGP ARCLSQSRNLLKTTDDMVKTAREKLKHYSCTAEDIDHEDITR DQTSTLKTCLPLELHKNESCLATRETSSTTRGSCLPQKTSLM MTLCLGSIYEDLKMYQTEFQAINAALQNHNHQQIILDKGMLV AIDELMQSLNHNGETLRQKPPVGEADPYRVKMKLCILLHAFS TRVVTINRVMGYLSSAGGGGSGGGGSEEAGGGSEEAGGGSE EAGGGSEEAGGGSHHHHHH
16	mIL12- ABP20-G4- SEEQ	MWELEKDVYVVEVDWTPDAPGETVNLTCDTPEEDDITWTSD QRHGVIGSGKTLTITVKEFLDAGQYTCHKGGETLSHSHLLLH KKENGIWSTEILKNFKNKTKFLKCEAPNYSGRFTCSWLVRQNM DLKFNIKSSSSSPDSRAVTCGMASLSAEKVTLQDRDYEKYSVS CQEDVTCPTAEETLPIELALEARQQNKYENYSTSFFIRDIKPD PKNLQMKPLKNSQVEVSWEYPDSWSTPHSYFSLKFFVRIQRK KEKMKETEEGCNQKGAFLVEKTSTEVQCKGGNVCVQAQDR YNNSSCSKWACVPCRVRSGGGGSGGGGSGGGGSRVIPVSGP ARCLSQSRNLLKTTDDMVKTAREKLKHYSCTAEDIDHEDITR DQTSTLKTCLPLELHKNESCLATRETSSTTRGSCLPQKTSLM MTLCLGSIYEDLKMYQTEFQAINAALQNIINIIQQIILDKGMLV AIDELMQSLNHNGETLRQKPPVGEADPYRVKMKLCILLHAFS TRVVTINRVMGYLSSAGGGGSGGGGSEEQGGGSEEQGGGSE EQGGGSEEQGGGSHHHHHH
17	mIL12- ABP10-GS	MWELEKDVYVVEVDWTPDAPGETVNLTCDTPEEDDITWTSD QRHGVIGSGKTLTITVKEFLDAGQYTCHKGGETLSHSHLLLH KKENGIWSTEILKNFKNKTKFLKCEAPNYSGRFTCSWLVRQNM DLKFNIKSSSSSPDSRAVTCGMASLSAEKVTLQDRDYEKYSVS

		CQEDVTCPTAEETLPIELALEARQQNKYENYSTSFFIRDIKPD PKNLQMKPLKNSQVEVSWEYPDSWSTPHSYFSLKFFVRIQRK KEKMKETEEGCNQKGAFLEKTSTEVQCKGGNVCVQAQDR YYNSSCSKWACVPCRVRSGGGGSGGGGSGGGGSRVIPVSGP ARCLSQSRNLLKTTDDMVKTAREKLKHYSCTAEDIDHEDITR DQTSTLKTCLPLELHKNESCLATRETSSTTRGSCLPQKTSLM MTLCLGSIYEDLKMYQTEFQAINAALQNHNHQQIILDKGMLV AIDELMQSLNHNGETLRQKPPVGEADPYRVKMKLCILLHAFS TRVVTINRVMGYLSSAGGGGSGGSEEGGGGGSGGSEEGGGG SEEGGGGGSGGSEEGGGGHHHHH
18	mIL12- ABP10-GE	MWELEKDVYVVEVDWTPDAPGETVNLTCDTPEEDDITWTSD QRHGVIGSGKTLTITVKEFLDAGQYTCHKGGETLSHSHLLLH KKENGIWSTEILKNFKNKTFLEKCEAPNYSGRFTCSWLVRQNM DLKFNIKSSSSSPDSRAVTCGMASLSAEKVTLQDRDYEKYSVS CQEDVTCPTAEETLPIELALEARQQNKYENYSTSFFIRDIKPD PKNLQMKPLKNSQVEVSWEYPDSWSTPHSYFSLKFFVRIQRK KEKMKETEEGCNQKGAFLEKTSTEVQCKGGNVCVQAQDR YYNSSCSKWACVPCRVRSGGGGSGGGGSGGGGSRVIPVSGP ARCLSQSRNLLKTTDDMVKTAREKLKHYSCTAEDIDHEDITR DQTSTLKTCLPLELHKNESCLATRETSSTTRGSCLPQKTSLM MTLCLGSIYEDLKMYQTEFQAINAALQNHNHQQIILDKGMLV AIDELMQSLNHNGETLRQKPPVGEADPYRVKMKLCILLHAFS TRVVTINRVMGYLSSAGGGGEGGSEEGGGGGEGGSEEGGGG SEEGGGGGEGGSEEGGGGHHHHH
19	mIL12- ABP20-G8- GS	MWELEKDVYVVEVDWTPDAPGETVNLTCDTPEEDDITWTSD QRHGVIGSGKTLTITVKEFLDAGQYTCHKGGETLSHSHLLLH KKENGIWSTEILKNFKNKTFLEKCEAPNYSGRFTCSWLVRQNM DLKFNIKSSSSSPDSRAVTCGMASLSAEKVTLQDRDYEKYSVS CQEDVTCPTAEETLPIELALEARQQNKYENYSTSFFIRDIKPD PKNLQMKPLKNSQVEVSWEYPDSWSTPHSYFSLKFFVRIQRK KEKMKETEEGCNQKGAFLEKTSTEVQCKGGNVCVQAQDR YYNSSCSKWACVPCRVRSGGGGSGGGGSGGGGSRVIPVSGP ARCLSQSRNLLKTTDDMVKTAREKLKHYSCTAEDIDHEDITR DQTSTLKTCLPLELHKNESCLATRETSSTTRGSCLPQKTSLM MTLCLGSIYEDLKMYQTEFQAINAALQNHNHQQIILDKGMLV AIDELMQSLNHNGETLRQKPPVGEADPYRVKMKLCILLHAFS TRVVTINRVMGYLSSAGGGGEGGGGSEEGGGSGGGGSEEGG SGGGGSEEGGGSGGGGSEEGGGGHHHHH
20	mIL12- ABP20-G8- GE	MWELEKDVYVVEVDWTPDAPGETVNLTCDTPEEDDITWTSD QRHGVIGSGKTLTITVKEFLDAGQYTCHKGGETLSHSHLLLH KKENGIWSTEILKNFKNKTFLEKCEAPNYSGRFTCSWLVRQNM DLKFNIKSSSSSPDSRAVTCGMASLSAEKVTLQDRDYEKYSVS CQEDVTCPTAEETLPIELALEARQQNKYENYSTSFFIRDIKPD PKNLQMKPLKNSQVEVSWEYPDSWSTPHSYFSLKFFVRIQRK KEKMKETEEGCNQKGAFLEKTSTEVQCKGGNVCVQAQDR YYNSSCSKWACVPCRVRSGGGGSGGGGSGGGGSRVIPVSGP

		ARCLSQSRNLLKTTDDMVKTAREKLKHYSCTAEDIDHEDITR DQTSTLKTCLPLELHKNESCLATRETSSTTRGSCLPQKTSLM MTLCLGSIYEDLKMYQTEFQAINAALQNHNHQQIILDKGMLV AIDELMQSLNHNGETLRQKPPVGEADPYRVKMKLCILLHAFS TRVVTINRVMGYLSSAGGGGEGGGGSEEGGGEGGGGSEEGG GEGGGGSEEGGGEGGGGSEEGGGGHHHHHH
21	mIL12- ABP20-G4- 6x-GE	MWELEKDVYVVEVDWTPDAPGETVNLTCDTPEEDDITWTSD QRHGVIGSGKTLTITVKEFLDAGQYTCHKGGETLSHSHLLLH KKENGIWSTEILKNFKNKTFKCEAPNYSGRFTCSWLVRQNM DLKFNIKSSSSSPDSRAVTCGMASLSAEKVTLQDRDYEKYSVS CQEDVTCPTAEETLPIELALEARQQNKYENYSTSFFIRDIKPD PKNLQMKPLKNSQVEVSWEYPDSWSTPHSYFSLKFFVRIQRK KEKMKETEEGCNQKGAFLVEKTSTEVQCKGGNVCVQAQDR YYNSSCSKWACVPCRVRSGGGGSGGGGSGGGGSRVIPVSGP ARCLSQSRNLLKTTDDMVKTAREKLKHYSCTAEDIDHEDITR DQTSTLKTCLPLELHKNESCLATRETSSTTRGSCLPQKTSLM MTLCLGSIYEDLKMYQTEFQAINAALQNHNHQQIILDKGMLV AIDELMQSLNHNGETLRQKPPVGEADPYRVKMKLCILLHAFS TRVVTINRVMGYLSSAGGGGEGGGGSEEGGGGSEEGGGGSE EGGGGSEEGGGGSEEGGGGSEEGGGGHHHHHH
22	mIL12- ABP20-G4- 8x-GE	MWELEKDVYVVEVDWTPDAPGETVNLTCDTPEEDDITWTSD QRHGVIGSGKTLTITVKEFLDAGQYTCHKGGETLSHSHLLLH KKENGIWSTEILKNFKNKTFKCEAPNYSGRFTCSWLVRQNM DLKFNIKSSSSSPDSRAVTCGMASLSAEKVTLQDRDYEKYSVS CQEDVTCPTAEETLPIELALEARQQNKYENYSTSFFIRDIKPD PKNLQMKPLKNSQVEVSWEYPDSWSTPHSYFSLKFFVRIQRK KEKMKETEEGCNQKGAFLVEKTSTEVQCKGGNVCVQAQDR YYNSSCSKWACVPCRVRSGGGGSGGGGSGGGGSRVIPVSGP ARCLSQSRNLLKTTDDMVKTAREKLKHYSCTAEDIDHEDITR DQTSTLKTCLPLELHKNESCLATRETSSTTRGSCLPQKTSLM MTLCLGSIYEDLKMYQTEFQAINAALQNHNHQQIILDKGMLV AIDELMQSLNHNGETLRQKPPVGEADPYRVKMKLCILLHAFS TRVVTINRVMGYLSSAGGGGEGGGGSEEGGGGSEEGGGGSE EGGGGSEEGGGGSEEGGGGSEEGGGGSEEGGGGSEEGGGGH HHHHH
23	Human IL12B	IWELKKDVYVVELDWYDPDAPGEMVVLTCDTPEEDGITWTLD QSSEVLGSGKTLTIQVKEFGDAGQYTCHKGGEVLSHSLLLLH KKEDGIWSTDILKDQKEPKNKTFLRCEAKNYSGRFTCWWLTT ISTDLTFSVKSSRGSSDPQGVTCGAATLSAERVGRDNKEYEYS VECQEDSACPAAEESLPIEVMVDAVHKLKYENYTSSFFIRDIK PDPPKNLQLKPLKNSRQVEVSWEYPDTWSTPHSYFSLTFCVQ VQGKSKREKKDRVFTDKTSATVICRKNASISVRAQDRYYSSS WSEWASVPCS
24	Human IL12A	RNLPVATPDPMFPC LHHSQNLLRAVSNMLQKARQTLEFYPC TSEEIDHEDITKDKTSTVEACLPLELTKNESCLNSRETSFITNGS CLASRKTSFMMALCLSSIYEDLKMYQVEFKTMNAKLLMDPK

		RQIFLDQNMLAVIDELMQALNFNSETVPQKSSLEEPDFYKTKIKLCILLHAFRIRAVTIDRVMSYLNAS
25	Human IL12 (IL12B-linker-IL12A)	IWELKKDVYVVELDWYPDAPGEMVVLTCDTPEEDGITWTLD QSSEVLGSGKTLTIQVKEFGDAGQYTCHKGGEVLSHSLLLLH KKEDGIWSTDILKDQKEPKNKTFRLRCEAKNYSGRFTCWWLTT ISTDLTFSVKSSRGSSDPQGVTCGAATLSAERVGRDNKEYEYS VEQEDSACPAAEESLPIEVMVDAVHKLKYENYTSSFFIRDIIK PDPPKNLQLKPLKNSRQVEVSWEYPDTWSTPHSYFSLTFCVQ VQGKSKREKKDRVFTDKTSATVICRKNASISVRAQDRYSSS WSEWASVPCSGGGGSGGGGSGGGGSRNLPVATPDPGMFPC HHSQNLLRAVSNNMLQKARQTLEFYPTCTSEEIDHEDITKDKTST VEACLPLELTKNESCLNSRETSFITNGSCLASRKTSFMMALCL SSIIYEDLKMYQVEFKTMNAKLLMDPKRQIFLDQNMLAVIDEL MQALNFNSETVPQKSSLEEPDFYKTKIKLCILLHAFRIRAVTID RVMSYLNAS
26	hIL12-His (no ABP)	IWELKKDVYVVELDWYPDAPGEMVVLTCDTPEEDGITWTLD QSSEVLGSGKTLTIQVKEFGDAGQYTCHKGGEVLSHSLLLLH KKEDGIWSTDILKDQKEPKNKTFRLRCEAKNYSGRFTCWWLTT ISTDLTFSVKSSRGSSDPQGVTCGAATLSAERVGRDNKEYEYS VEQEDSACPAAEESLPIEVMVDAVHKLKYENYTSSFFIRDIIK PDPPKNLQLKPLKNSRQVEVSWEYPDTWSTPHSYFSLTFCVQ VQGKSKREKKDRVFTDKTSATVICRKNASISVRAQDRYSSS WSEWASVPCSGGGGSGGGGSGGGGSRNLPVATPDPGMFPC HHSQNLLRAVSNNMLQKARQTLEFYPTCTSEEIDHEDITKDKTST VEACLPLELTKNESCLNSRETSFITNGSCLASRKTSFMMALCL SSIIYEDLKMYQVEFKTMNAKLLMDPKRQIFLDQNMLAVIDEL MQALNFNSETVPQKSSLEEPDFYKTKIKLCILLHAFRIRAVTID RVMSYLNASGGGGSHHHHHH
27	hIL12- ABP10-His	IWELKKDVYVVELDWYPDAPGEMVVLTCDTPEEDGITWTLD QSSEVLGSGKTLTIQVKEFGDAGQYTCHKGGEVLSHSLLLLH KKEDGIWSTDILKDQKEPKNKTFRLRCEAKNYSGRFTCWWLTT ISTDLTFSVKSSRGSSDPQGVTCGAATLSAERVGRDNKEYEYS VEQEDSACPAAEESLPIEVMVDAVHKLKYENYTSSFFIRDIIK PDPPKNLQLKPLKNSRQVEVSWEYPDTWSTPHSYFSLTFCVQ VQGKSKREKKDRVFTDKTSATVICRKNASISVRAQDRYSSS WSEWASVPCSGGGGSGGGGSGGGGSRNLPVATPDPGMFPC HHSQNLLRAVSNNMLQKARQTLEFYPTCTSEEIDHEDITKDKTST VEACLPLELTKNESCLNSRETSFITNGSCLASRKTSFMMALCL SSIIYEDLKMYQVEFKTMNAKLLMDPKRQIFLDQNMLAVIDEL MQALNFNSETVPQKSSLEEPDFYKTKIKLCILLHAFRIRAVTID RVMSYLNASGGGGSFQSEEQGGGSGGSEEGGMESEESNGG GSGGSEEGGGGSHHHHHH
28	hIL12- ABP20-His	IWELKKDVYVVELDWYPDAPGEMVVLTCDTPEEDGITWTLD QSSEVLGSGKTLTIQVKEFGDAGQYTCHKGGEVLSHSLLLLH KKEDGIWSTDILKDQKEPKNKTFRLRCEAKNYSGRFTCWWLTT ISTDLTFSVKSSRGSSDPQGVTCGAATLSAERVGRDNKEYEYS

		VECQEDSACPAAEESLPIEVMVDAVHKLKYENYTSSFFIRDIK PDPPKNLQLKPLKNSRQVEVSWEYPDTWSTPHSYFSLTFCVQ VQGKSKREKKDRVFTDKTSATVICRKNASISVRAQDRYSSS WSEWASVPCSGGGGSGGGGSGGGGSRNLPVATPDPGMFPCL HHSQNLLRAVSNMLQKARQTLEFYPTCTSEEIDHEDITKDKTST VEACLPLELTKNESCLNSRETSFITNGSCLASRKTSFMMALCL SSIIYEDLKMYQVEFKTMNAKLLMDPKRQIFLDQNMLAVIDEL MQALNFNSETVPQKSSLEEPDFYKTKIKLCILLHAFRIRAVTID RVMSYLNASGGGGSGGGGSEEESEEESEEEGGGGSHHHHHH
29	hIL12- ABP20-G4- His	IWELKKDVYVVELDWYPDAPGEMVVLTCDTPEEDGITWTLD QSSEVLGSGKTLTIQVKEFGDAGQYTCHKGGEVLSHSLLLLH KKEDGIWSTDILKDQKEPKNKTFRLCEAKNYSGRFTCWWT ISTDLTFSVKSSRGSSDPQGVTCGAATLSAERVRGDNKEYEYS VECQEDSACPAAEESLPIEVMVDAVHKLKYENYTSSFFIRDIK PDPPKNLQLKPLKNSRQVEVSWEYPDTWSTPHSYFSLTFCVQ VQGKSKREKKDRVFTDKTSATVICRKNASISVRAQDRYSSS WSEWASVPCSGGGGSGGGGSGGGGSRNLPVATPDPGMFPCL HHSQNLLRAVSNMLQKARQTLEFYPTCTSEEIDHEDITKDKTST VEACLPLELTKNESCLNSRETSFITNGSCLASRKTSFMMALCL SSIIYEDLKMYQVEFKTMNAKLLMDPKRQIFLDQNMLAVIDEL MQALNFNSETVPQKSSLEEPDFYKTKIKLCILLHAFRIRAVTID RVMSYLNASGGGGSGGGGSEEGGGGSEEGGGGSEEGGGGSE EGGGGSHHHHHH
30	hIL12- ABP20-G4- 8x-GE (mature polypeptide)	IWELKKDVYVVELDWYPDAPGEMVVLTCDTPEEDGITWTLD QSSEVLGSGKTLTIQVKEFGDAGQYTCHKGGEVLSHSLLLLH KKEDGIWSTDILKDQKEPKNKTFRLCEAKNYSGRFTCWWT ISTDLTFSVKSSRGSSDPQGVTCGAATLSAERVRGDNKEYEYS VECQEDSACPAAEESLPIEVMVDAVHKLKYENYTSSFFIRDIK PDPPKNLQLKPLKNSRQVEVSWEYPDTWSTPHSYFSLTFCVQ VQGKSKREKKDRVFTDKTSATVICRKNASISVRAQDRYSSS WSEWASVPCSGGGGSGGGGSGGGGSRNLPVATPDPGMFPCL HHSQNLLRAVSNMLQKARQTLEFYPTCTSEEIDHEDITKDKTST VEACLPLELTKNESCLNSRETSFITNGSCLASRKTSFMMALCL SSIIYEDLKMYQVEFKTMNAKLLMDPKRQIFLDQNMLAVIDEL MQALNFNSETVPQKSSLEEPDFYKTKIKLCILLHAFRIRAVTID RVMSYLNASGGGGEGGGGSEEGGGGSEEGGGGSEEGGGGSE EGGGGSEEGGGGSEEGGGGSEEGGGGSEEGGGGS
31	Signal polypeptide	MYRMQLLSICIALSLALVTNS
32	hIL12- ABP20-G4- 8x-GE w/ signal polypeptide	MYRMQLLSICIALSLALVTNSIWELKKDVYVVELDWYPDAPG EMVVLTCDTPEEDGITWTLDQSSEVLGSGKTLTIQVKEFGDA GQYTCHKGGEVLSHSLLLLHKKEDGIWSTDILKDQKEPKNK FLRCEAKNYSGRFTCWWTISTDLTFSVKSSRGSSDPQGVTC GAATLSAERVRGDNKEYEYSVECQEDSACPAAEESLPIEVMV DAVHKLKYENYTSSFFIRDIKPDPPKNLQLKPLKNSRQVEVS WEYPDTWSTPHSYFSLTFCVQVQGKSKREKKDRVFTDKTSAT

		VICRKNASISVRAQDRYYSSSWSEWASVPCSGGGGSGGGGSG GGGSRNLPVATPDPMFPC LHHSQNLLRAVSNMLQKARQTL EFYPCTSEEIDHEDITKDKTSTVEACLPLELTKNESCLNSRETS FITNGSCLASRKTSFMMALCLSSIIYEDLKMYQVEFKTMNAKL LMDPKRQIFLDQNMLAVIDELMQALNFNSETVPQKSSLEEPD FYKTKIKLCILLHAFRIRAVTIDRVMSYLNASGGGGEGGGGSE EGGGGSEEGGGGSEEGGGGSEEGGGGSEEGGGGSEEGGGGS EEGGGGSEEGGGGS
33	hIL12- ABP10 (mature polypeptide)	IWELKKDVYVVELDWYPDAPGEMVVLTCDTPEEDGITWTLD QSSEVLGSGKTLTIQVKEFGDAGQYTCHKGGEVLSHSLLLLH KKEDGIWSTDILKDQKEPKNKTF LRCEAKNYSGRFTCWWLTT ISTDLTFSVKSSRGSSDPQGVTCGAATLSAERV RGDNKEYEYS VEQCEDSACPAAEESLPIEVMVD AVHKLKYENYTSSFFIRDIK PDPPKNLQLKPLKNSRQVEVSWEYPTDWTSTPHSYFSLTFCVQ VQGKSKREKKDRVFTDKTSATVICRKNASISVRAQDRYYSSS WSEWASVPCSGGGGSGGGGSGGGGSRNLPVATPDPMFPC LHHSQNLLRAVSNMLQKARQTL EFYPCTSEEIDHEDITKDKTST VEACLPLELTKNESCLNSRETSFITNGSCLASRKTSFMMALCL SSIIYEDLKMYQVEFKTMNAKLLMDPKRQIFLDQNMLAVIDEL MQALNFNSETVPQKSSLEEPDFYKTKIKLCILLHAFRIRAVTID RVMSYLNASGGGGSFQSEEQQGGGSGGSEEGGMESEESNGG GSGGSEEGGGGS
34	hIL12- ABP10 w/ signal polypeptide	MYRMQLLSIALSLALVTNSIWELKKDVYVVELDWYPDAPG EMVVLTCDTPEEDGITWTLDQSSEVLGSGKTLTIQVKEFGDA GQYTCHKGGEVLSHSLLLLHKKEDGIWSTDILKDQKEPKNK TF LRCEAKNYSGRFTCWWLTTISTDLTFSVKSSRGSSDPQGVTC GAATLSAERV RGDNKEYEYSVEQCEDSACPAAEESLPIEVMV DAVHKLKYENYTSSFFIRDIKPDPPKNLQLKPLKNSRQVEVS WEYPTDWTSTPHSYFSLTFCVQVQGKSKREKKDRVFTDKTSAT VICRKNASISVRAQDRYYSSSWSEWASVPCSGGGGSGGGGSG GGGSRNLPVATPDPMFPC LHHSQNLLRAVSNMLQKARQTL EFYPCTSEEIDHEDITKDKTSTVEACLPLELTKNESCLNSRETS FITNGSCLASRKTSFMMALCLSSIIYEDLKMYQVEFKTMNAKL LMDPKRQIFLDQNMLAVIDELMQALNFNSETVPQKSSLEEPD FYKTKIKLCILLHAFRIRAVTIDRVMSYLNASGGGGSFQSEEQ QGGGSGGSEEGGMESEESNGGGSGGSEEGGGGS
35	hIL12- ABP20	IWELKKDVYVVELDWYPDAPGEMVVLTCDTPEEDGITWTLD QSSEVLGSGKTLTIQVKEFGDAGQYTCHKGGEVLSHSLLLLH KKEDGIWSTDILKDQKEPKNKTF LRCEAKNYSGRFTCWWLTT ISTDLTFSVKSSRGSSDPQGVTCGAATLSAERV RGDNKEYEYS VEQCEDSACPAAEESLPIEVMVD AVHKLKYENYTSSFFIRDIK PDPPKNLQLKPLKNSRQVEVSWEYPTDWTSTPHSYFSLTFCVQ VQGKSKREKKDRVFTDKTSATVICRKNASISVRAQDRYYSSS WSEWASVPCSGGGGSGGGGSGGGGSRNLPVATPDPMFPC LHHSQNLLRAVSNMLQKARQTL EFYPCTSEEIDHEDITKDKTST VEACLPLELTKNESCLNSRETSFITNGSCLASRKTSFMMALCL

		SSIIYEDLKMYQVEFKTMNAKLLMDPKRQIFLDQNMLAVIDEL MQALNFNSETVPQKSSLEEPDFYKTKIKLCILLHAFRIRAVTID RVMSYLNASGGGGSGGGGSEEESEEESEEESEEGGGGS
36	hIL12- ABP20-G4	IWELKKDVYVVELDWYPDAPGEMVVLTCDTPEEDGITWTLD QSSEVLGSGKTLTIQVKEFGDAGQYTCHKGGEVLSHSLLLLH KKEDGIWSTDILKDQKEPKNKTFRLCEAKNYSGRFTCWWLTT ISTDLTFSVKSSRGSSDPQGVTCGAATLSAERVGRDNKEYEYS VECQEDSACPAAEESLPIEVMVDAVHKLKYENYTSSFFIRDIK PDPPKNLQLKPLKNSRQVEVSWEYPDTWSTPHSYFSLTFCVQ VQGKSKREKKDRVFTDKTSATVICRKNASISVRAQDRYSSS WSEWASVPCSGGGGSGGGGSGGGGSRNLPVATPDPGMFPC HHSQNLLRAVSNNMLQKARQTLEFYPTCTSEEDHEDITKDKTST VEACLPLELTKNESCLNSRETSFITNGSCLASRKTSFMMALCL SSIIYEDLKMYQVEFKTMNAKLLMDPKRQIFLDQNMLAVIDEL MQALNFNSETVPQKSSLEEPDFYKTKIKLCILLHAFRIRAVTID RVMSYLNASGGGGSGGGGSEEGGGGSEEGGGGSEEGGGGSE EGGGGS
37	hIL12- ABP20-G4- GS20	IWELKKDVYVVELDWYPDAPGEMVVLTCDTPEEDGITWTLD QSSEVLGSGKTLTIQVKEFGDAGQYTCHKGGEVLSHSLLLLH KKEDGIWSTDILKDQKEPKNKTFRLCEAKNYSGRFTCWWLTT ISTDLTFSVKSSRGSSDPQGVTCGAATLSAERVGRDNKEYEYS VECQEDSACPAAEESLPIEVMVDAVHKLKYENYTSSFFIRDIK PDPPKNLQLKPLKNSRQVEVSWEYPDTWSTPHSYFSLTFCVQ VQGKSKREKKDRVFTDKTSATVICRKNASISVRAQDRYSSS WSEWASVPCSGGGGSGGGGSGGGGSRNLPVATPDPGMFPC HHSQNLLRAVSNNMLQKARQTLEFYPTCTSEEDHEDITKDKTST VEACLPLELTKNESCLNSRETSFITNGSCLASRKTSFMMALCL SSIIYEDLKMYQVEFKTMNAKLLMDPKRQIFLDQNMLAVIDEL MQALNFNSETVPQKSSLEEPDFYKTKIKLCILLHAFRIRAVTID RVMSYLNASGGGGSGGGGSGGGGSGGGGSEEGGGGSEEGG GGSEEGGGGSEEGGGGS
38	hIL12- ABP20-G4- 6x	IWELKKDVYVVELDWYPDAPGEMVVLTCDTPEEDGITWTLD QSSEVLGSGKTLTIQVKEFGDAGQYTCHKGGEVLSHSLLLLH KKEDGIWSTDILKDQKEPKNKTFRLCEAKNYSGRFTCWWLTT ISTDLTFSVKSSRGSSDPQGVTCGAATLSAERVGRDNKEYEYS VECQEDSACPAAEESLPIEVMVDAVHKLKYENYTSSFFIRDIK PDPPKNLQLKPLKNSRQVEVSWEYPDTWSTPHSYFSLTFCVQ VQGKSKREKKDRVFTDKTSATVICRKNASISVRAQDRYSSS WSEWASVPCSGGGGSGGGGSGGGGSRNLPVATPDPGMFPC HHSQNLLRAVSNNMLQKARQTLEFYPTCTSEEDHEDITKDKTST VEACLPLELTKNESCLNSRETSFITNGSCLASRKTSFMMALCL SSIIYEDLKMYQVEFKTMNAKLLMDPKRQIFLDQNMLAVIDEL MQALNFNSETVPQKSSLEEPDFYKTKIKLCILLHAFRIRAVTID RVMSYLNASGGGGSGGGGSEEGGGGSEEGGGGSEEGGGGSE EGGGGSEEGGGGSEEGGGGS

39	hIL12- ABP20-G4- 8x	IWELKKDVYVVVELDWYPDAPGEMVVLTCDTPEEDGITWTLD QSSEVLGSGKTLTIQVKEFGDAGQYTCHKGGEVLSHSLLLLH KKEDGIWSTDILKDQKEPKNKTFRLCEAKNYSGRFTCWWLTT ISTDLTFSVKSSRGSSDPQGVTCGAATLSAERVRGDNKEYEYS VEQEDSACPAAEESLPIEVMVDAVHKLKYENYTSSFFIRDIK PDPPKNLQLKPLKNSRQVEVSWEYPDTWSTPHSYFSLTFCVQ VQGKSKREKKDRVFTDKTSATVICRKNASISVRAQDRYSSS WSEWASVPCSGGGGSGGGGSGGGGSRNLPVATPDPGMFPC HHSQNLLRAVSNMLQKARQTLEFYPTCTSEEIDHEDITKDKTST VEACLPLELTKNESCLNSRETSFITNGSCLASRKTSFMMALCL SSIIYEDLKMYQVEFKTMNAKLLMDPKRQIFLDQNMALVIDEL MQALNFNSETVPQKSSLEEDFYKTKIKLCILLHAFRIRAVTID RVMSYLNASGGGGSGGGGSEEGGGGSEEGGGGSEEGGGGSE EGGGGSEEGGGGSEEGGGGSEEGGGGSEEGGGGSEEGGGGS
40	hIL12- ABP20-6x	IWELKKDVYVVVELDWYPDAPGEMVVLTCDTPEEDGITWTLD QSSEVLGSGKTLTIQVKEFGDAGQYTCHKGGEVLSHSLLLLH KKEDGIWSTDILKDQKEPKNKTFRLCEAKNYSGRFTCWWLTT ISTDLTFSVKSSRGSSDPQGVTCGAATLSAERVRGDNKEYEYS VEQEDSACPAAEESLPIEVMVDAVHKLKYENYTSSFFIRDIK PDPPKNLQLKPLKNSRQVEVSWEYPDTWSTPHSYFSLTFCVQ VQGKSKREKKDRVFTDKTSATVICRKNASISVRAQDRYSSS WSEWASVPCSGGGGSGGGGSGGGGSRNLPVATPDPGMFPC HHSQNLLRAVSNMLQKARQTLEFYPTCTSEEIDHEDITKDKTST VEACLPLELTKNESCLNSRETSFITNGSCLASRKTSFMMALCL SSIIYEDLKMYQVEFKTMNAKLLMDPKRQIFLDQNMALVIDEL MQALNFNSETVPQKSSLEEDFYKTKIKLCILLHAFRIRAVTID RVMSYLNASGGGGSGGGGSEEESEEESEEESEEESEEESEEGGGGS
41	hIL12- ABP20-8x	IWELKKDVYVVVELDWYPDAPGEMVVLTCDTPEEDGITWTLD QSSEVLGSGKTLTIQVKEFGDAGQYTCHKGGEVLSHSLLLLH KKEDGIWSTDILKDQKEPKNKTFRLCEAKNYSGRFTCWWLTT ISTDLTFSVKSSRGSSDPQGVTCGAATLSAERVRGDNKEYEYS VEQEDSACPAAEESLPIEVMVDAVHKLKYENYTSSFFIRDIK PDPPKNLQLKPLKNSRQVEVSWEYPDTWSTPHSYFSLTFCVQ VQGKSKREKKDRVFTDKTSATVICRKNASISVRAQDRYSSS WSEWASVPCSGGGGSGGGGSGGGGSRNLPVATPDPGMFPC HHSQNLLRAVSNMLQKARQTLEFYPTCTSEEIDHEDITKDKTST VEACLPLELTKNESCLNSRETSFITNGSCLASRKTSFMMALCL SSIIYEDLKMYQVEFKTMNAKLLMDPKRQIFLDQNMALVIDEL MQALNFNSETVPQKSSLEEDFYKTKIKLCILLHAFRIRAVTID RVMSYLNASGGGGSGGGGSEEESEEESEEESEEESEEESEEG GGGS
42	hIL12- ABP20-G4- SEEE	IWELKKDVYVVVELDWYPDAPGEMVVLTCDTPEEDGITWTLD QSSEVLGSGKTLTIQVKEFGDAGQYTCHKGGEVLSHSLLLLH KKEDGIWSTDILKDQKEPKNKTFRLCEAKNYSGRFTCWWLTT ISTDLTFSVKSSRGSSDPQGVTCGAATLSAERVRGDNKEYEYS

		VECQEDSACPAAEESLPIEVMVDAVHKLKYENYTSSFFIRDIK PDPPKNLQLKPLKNSRQVEVSWEYPDTWSTPHSYFSLTFCVQ VQGKSKREKKDRVFTDKTSATVICRKNASISVRAQDRYSSS WSEWASVPCSGGGGSGGGGSGGGGSRNLPVATPDPGMFPC HHSQNLLRAVSNNMLQKARQTLEFYPTCTSEEIDHEDITKDKTST VEACLPLELTKNESCLNSRETSFITNGSCLASRKTSFMMALCL SSIEDLKMYQVEFKTMNAKLLMDPKRQIFLDQNMLAVIDEL MQALNFNSETVPQKSSLEEPDFYKTKIKLCILLHAFRIRAVTID RVMSYLNASGGGGSGGGGSEEEGGGSEEEGGGSEEEGGGSEE EGGGS
43	hIL12- ABP20-G4- SEEA	IWELKKDVYVVELDWYPDAPGEMVVLTCDTPEEDGITWTLD QSSEVLGSGKTLTIQVKEFGDAGQYTCHKGGEVLSHSLLLLH KKEDGIWSTDILKDQKEPKNKTFRLCEAKNYSGRFTCWWTLT ISTDLTFSVKSSRGSSDPQGVTCGAATLSAERVRGDNKEYEYS VECQEDSACPAAEESLPIEVMVDAVHKLKYENYTSSFFIRDIK PDPPKNLQLKPLKNSRQVEVSWEYPDTWSTPHSYFSLTFCVQ VQGKSKREKKDRVFTDKTSATVICRKNASISVRAQDRYSSS WSEWASVPCSGGGGSGGGGSGGGGSRNLPVATPDPGMFPC HHSQNLLRAVSNNMLQKARQTLEFYPTCTSEEIDHEDITKDKTST VEACLPLELTKNESCLNSRETSFITNGSCLASRKTSFMMALCL SSIEDLKMYQVEFKTMNAKLLMDPKRQIFLDQNMLAVIDEL MQALNFNSETVPQKSSLEEPDFYKTKIKLCILLHAFRIRAVTID RVMSYLNASGGGGSGGGGSEEAGGGSEEAGGGSEEAGGGSE EAGGGS
44	hIL12- ABP20-G4- SEEQ	IWELKKDVYVVELDWYPDAPGEMVVLTCDTPEEDGITWTLD QSSEVLGSGKTLTIQVKEFGDAGQYTCHKGGEVLSHSLLLLH KKEDGIWSTDILKDQKEPKNKTFRLCEAKNYSGRFTCWWTLT ISTDLTFSVKSSRGSSDPQGVTCGAATLSAERVRGDNKEYEYS VECQEDSACPAAEESLPIEVMVDAVHKLKYENYTSSFFIRDIK PDPPKNLQLKPLKNSRQVEVSWEYPDTWSTPHSYFSLTFCVQ VQGKSKREKKDRVFTDKTSATVICRKNASISVRAQDRYSSS WSEWASVPCSGGGGSGGGGSGGGGSRNLPVATPDPGMFPC HHSQNLLRAVSNNMLQKARQTLEFYPTCTSEEIDHEDITKDKTST VEACLPLELTKNESCLNSRETSFITNGSCLASRKTSFMMALCL SSIEDLKMYQVEFKTMNAKLLMDPKRQIFLDQNMLAVIDEL MQALNFNSETVPQKSSLEEPDFYKTKIKLCILLHAFRIRAVTID RVMSYLNASGGGGSGGGGSEEQGGGSEEQGGGSEEQGGGSE EQGGGS
45	hIL12- ABP10-GS	IWELKKDVYVVELDWYPDAPGEMVVLTCDTPEEDGITWTLD QSSEVLGSGKTLTIQVKEFGDAGQYTCHKGGEVLSHSLLLLH KKEDGIWSTDILKDQKEPKNKTFRLCEAKNYSGRFTCWWTLT ISTDLTFSVKSSRGSSDPQGVTCGAATLSAERVRGDNKEYEYS VECQEDSACPAAEESLPIEVMVDAVHKLKYENYTSSFFIRDIK PDPPKNLQLKPLKNSRQVEVSWEYPDTWSTPHSYFSLTFCVQ VQGKSKREKKDRVFTDKTSATVICRKNASISVRAQDRYSSS WSEWASVPCSGGGGSGGGGSGGGGSRNLPVATPDPGMFPC

		HHSQNLLRAVSNMLQKARQTLEFYPTCTSEEDHEDITKDKTST VEACLPLELTKNESCLNSRETSFITNGSCLASRKTSFMMALCL SSIIYEDLKMYQVEFKTMNAKLLMDPKRQIFLDQNMLAVIDEL MQALNFNSETVPQKSSLEEPDFYKTKIKLCILLHAFRIRAVTID RVMSYLNASGGGGSGGSEEGGGGGSGGSEEGGGGGSEEGGGG GGGGSEEGGGGS
46	hIL12- ABP10-GE	IWELKKDVYVVELDWYPDAPGEMVVLTCDTPEEDGITWTLD QSSEVLGSGKTLTIQVKEFGDAGQYTCHKGGEVLSHSLLLLH KKEDGIWSTDILKDQKEPKNKTFRLCEAKNYSGRFTCWWTLT ISTDLTFSVKSSRGSSDPQGVTCGAATLSAERVRGDNKEYEYS VEQEDSACPAAEESLPIEVMVDAVHKLKYENYTSSFFIRDIK PDPPKNLQLKPLKNSRQVEVSWEYPDTWSTPHSYFSLTFCVQ VQGKSKREKKDRVFTDKTSATVICRKNASISVRAQDRYSSS WSEWASVPCSGGGGSGGGGSGGGGSRNLPVATPDPMFPC HHSQNLLRAVSNMLQKARQTLEFYPTCTSEEDHEDITKDKTST VEACLPLELTKNESCLNSRETSFITNGSCLASRKTSFMMALCL SSIIYEDLKMYQVEFKTMNAKLLMDPKRQIFLDQNMLAVIDEL MQALNFNSETVPQKSSLEEPDFYKTKIKLCILLHAFRIRAVTID RVMSYLNASGGGGEGGSEEGGGGGEGGSEEGGGGGSEEGGGG GEGGSEEGGGGS
47	hIL12- ABP20-G8- GS	IWELKKDVYVVELDWYPDAPGEMVVLTCDTPEEDGITWTLD QSSEVLGSGKTLTIQVKEFGDAGQYTCHKGGEVLSHSLLLLH KKEDGIWSTDILKDQKEPKNKTFRLCEAKNYSGRFTCWWTLT ISTDLTFSVKSSRGSSDPQGVTCGAATLSAERVRGDNKEYEYS VEQEDSACPAAEESLPIEVMVDAVHKLKYENYTSSFFIRDIK PDPPKNLQLKPLKNSRQVEVSWEYPDTWSTPHSYFSLTFCVQ VQGKSKREKKDRVFTDKTSATVICRKNASISVRAQDRYSSS WSEWASVPCSGGGGSGGGGSGGGGSRNLPVATPDPMFPC HHSQNLLRAVSNMLQKARQTLEFYPTCTSEEDHEDITKDKTST VEACLPLELTKNESCLNSRETSFITNGSCLASRKTSFMMALCL SSIIYEDLKMYQVEFKTMNAKLLMDPKRQIFLDQNMLAVIDEL MQALNFNSETVPQKSSLEEPDFYKTKIKLCILLHAFRIRAVTID RVMSYLNASGGGGEGGGGSEEGGGSGGGGSEEGGGSGGGGS EEGGGSGGGGSEEGGGGS
48	hIL12- ABP20-G8- GE	IWELKKDVYVVELDWYPDAPGEMVVLTCDTPEEDGITWTLD QSSEVLGSGKTLTIQVKEFGDAGQYTCHKGGEVLSHSLLLLH KKEDGIWSTDILKDQKEPKNKTFRLCEAKNYSGRFTCWWTLT ISTDLTFSVKSSRGSSDPQGVTCGAATLSAERVRGDNKEYEYS VEQEDSACPAAEESLPIEVMVDAVHKLKYENYTSSFFIRDIK PDPPKNLQLKPLKNSRQVEVSWEYPDTWSTPHSYFSLTFCVQ VQGKSKREKKDRVFTDKTSATVICRKNASISVRAQDRYSSS WSEWASVPCSGGGGSGGGGSGGGGSRNLPVATPDPMFPC HHSQNLLRAVSNMLQKARQTLEFYPTCTSEEDHEDITKDKTST VEACLPLELTKNESCLNSRETSFITNGSCLASRKTSFMMALCL

		SSIIYEDLKMYQVEFKTMNAKLLMDPKRQIFLDQNMLAVIDEL MQALNFNSETVPQKSSLEEPDFYKTKIKLCILLHAFRIRAVTID RVMSYLNASGGGGEGGGGSEEGGGEGGGGSEEGGGEGGGG SEEGGGEGGGGSEEGGGGS
49	hIL12- ABP20-G4- GE	IWELKKDVYVVELDWYPDAPGEMVVLTCDTPEEDGITWTLD QSSEVLGSGKTLTIQVKEFGDAGQYTCHKGGEVLSHSLLLLH KKEDGIWSTDILKDQKEPKNKTFRLCEAKNYSGRFTCWWLTT ISTDLTFSVKSSRGSSDPQGVTCGAATLSAERVGRDNKEYEYS VEQEDSACPAAEESLPIEVMVDAVHKLKYENYTSSFFIRDIK PDPPKNLQLKPLKNSRQVEVSWEYPDTWSTPHSYFSLTFCVQ VQGKSKREKKDRVFTDKTSATVICRKNASISVRAQDRYSSS WSEWASVPCSGGGGSGGGGSGGGGSRNLPVATPDPMFPC HHSQNLLRAVSNNMLQKARQTLEFYPTCTSEEDHEDITKDKTST VEACLPLELTKNESCLNSRETSFITNGSCLASRKTSFMMALCL SSIIYEDLKMYQVEFKTMNAKLLMDPKRQIFLDQNMLAVIDEL MQALNFNSETVPQKSSLEEPDFYKTKIKLCILLHAFRIRAVTID RVMSYLNASGGGGEGGGGSEEGGGGSEEGGGGSEEGGGGSE EGGGGS
50	hIL12- ABP20-G4- 6x-GE	IWELKKDVYVVELDWYPDAPGEMVVLTCDTPEEDGITWTLD QSSEVLGSGKTLTIQVKEFGDAGQYTCHKGGEVLSHSLLLLH KKEDGIWSTDILKDQKEPKNKTFRLCEAKNYSGRFTCWWLTT ISTDLTFSVKSSRGSSDPQGVTCGAATLSAERVGRDNKEYEYS VEQEDSACPAAEESLPIEVMVDAVHKLKYENYTSSFFIRDIK PDPPKNLQLKPLKNSRQVEVSWEYPDTWSTPHSYFSLTFCVQ VQGKSKREKKDRVFTDKTSATVICRKNASISVRAQDRYSSS WSEWASVPCSGGGGSGGGGSGGGGSRNLPVATPDPMFPC HHSQNLLRAVSNNMLQKARQTLEFYPTCTSEEDHEDITKDKTST VEACLPLELTKNESCLNSRETSFITNGSCLASRKTSFMMALCL SSIIYEDLKMYQVEFKTMNAKLLMDPKRQIFLDQNMLAVIDEL MQALNFNSETVPQKSSLEEPDFYKTKIKLCILLHAFRIRAVTID RVMSYLNASGGGGEGGGGSEEGGGGSEEGGGGSEEGGGGSE EGGGGSEEGGGGSEEGGGGS
82	ABP20-G4	GGGGSGGGGSEEGGGGSEEGGGGSEEGGGGSEEGGGGS
83	ABP20-G4- 6x	GGGGSGGGGSEEGGGGSEEGGGGSEEGGGGSEEGGGGSEEG GGGSEEGGGGS
84	ABP20-G4- 8x	GGGGSGGGGSEEGGGGSEEGGGGSEEGGGGSEEGGGGSEEG GGGSEEGGGGSEEGGGGSEEGGGGS
85	ABP20-6x	GGGGSGGGGSEEESEEESEEESEEESEEESEEGGGGS
86	ABP20-8x	GGGGSGGGGSEEESEEESEEESEEESEEESEEESEEGGGGS
87	ABP20-G4- SEEE	GGGGSGGGGSEEEGGGSEEEGGGSEEEGGGSEEEGGGS
88	ABP20-G4- SEEA	GGGGSGGGGSEEAAGGGSEEAAGGGSEEAAGGGSEEAAGGS

89	ABP20-G4- SEEQ	GGGSGGGGSEEQGGGSEEQGGGSEEQGGGSEEQGGGS
90	ABP10	GGGGSFQSEEQGGGSGGSEEGGMESEESNGGGSGGSEEGG GGS
91	ABP10-GS	GGGSGGSEEGGGGGSGGSEEGGGGSEEGGGGGSGGSEEGG GG
92	ABP10-GE	GGGGEKGSEEGGGGEGGSEEGGGGSEEGGGGEGGSEEGG GG
93	ABP20-G8- GS	GGGGEKGKGSEEGGGSGGGGSEEGGGSGGGGSEEGGGSGGG GSEEGGGG
94	ABP20-G8- GE	GGGGEKGKGSEEGGGEGGGGSEEGGGEGGGGSEEGGGEGG GSEEGGGG
95	ABP20-G4- GE	GGGGEKGKGSEEGGGGSEEGGGGSEEGGGGSEEGGGG
96	ABP20-G4- 6x-GE	GGGGEKGKGSEEGGGGSEEGGGGSEEGGGGSEEGGGGSEEG GGGSEEGGGG
97	ABP20-G4- 8x-GE	GGGGEKGKGSEEGGGGSEEGGGGSEEGGGGSEEGGGGSEEG GGGSEEGGGGSEEGGGGSEEGGGG
98	His	HHHHHH
100	Canine IL12B	IWELEKDVYVVELDWHDPAPGEMVVLTCHTPEEDDITWTS QSSEVLGSGKTLTIQVKEFGDAGQYTCHKGGKVLRSLLLIHK KEDGIWSTDILKEQKESKNKIFLKCEAKNYSGRFTCWWLTAIS TDLKFSVKSSRGFSDPQGVTCGAVTLAERVVRVDNRDYKKYT VECQEGSACPSAEESLPIEVVVDIAHKLKYENYTSSFFIRDIKP DPPTNLQLKPLKNSRHVEVSWEYPDTWSTPHSYFSLTFCVQA QGKNNREKKDRLCVDKTSAKVVCHKDAKIRVQARDRYSS WSDWASVSCS
101	Canine IL12A	RSLPTASPGIFQCLNHSQNLLRAVSNTLQKARQTLELYSCTS EEIDHEDITKDKTSTVEACLPLELTMNESCLASREISLITNGSCL ASGKASFMTVLCLSSYIEDLKMYQMEFKAMNAKLLMDPKRQ IFLDQNMLTAIDELLQALNFNSVTVPPQKSSLEEDFYKTKIKLC ILLHAFRIRAVTIDRMMSYLNSS
102	Canine IL12 (IL12B- linker- IL12A)	IWELEKDVYVVELDWHDPAPGEMVVLTCHTPEEDDITWTS QSSEVLGSGKTLTIQVKEFGDAGQYTCHKGGKVLRSLLLIHK KEDGIWSTDILKEQKESKNKIFLKCEAKNYSGRFTCWWLTAIS TDLKFSVKSSRGFSDPQGVTCGAVTLAERVVRVDNRDYKKYT VECQEGSACPSAEESLPIEVVVDIAHKLKYENYTSSFFIRDIKP DPPTNLQLKPLKNSRHVEVSWEYPDTWSTPHSYFSLTFCVQA QGKNNREKKDRLCVDKTSAKVVCHKDAKIRVQARDRYSS WSDWASVSCSGGGGSGGGGSGGGGSRSLPTASPGIFQCLN HSQNLLRAVSNTLQKARQTLELYSCTSEEDHEDITKDKTSTV EACLPLELTMNESCLASREISLITNGSCLASGKASFMTVLCLSS IYEDLKMYQMEFKAMNAKLLMDPKRQIFLDQNMLTAIDELL QALNFNSVTVPPQKSSLEEDFYKTKIKLCILLHAFRIRAVTIDR MMSYLNSS

[illegible]

[illegible]

111	Porcine IL12A	RSLPATTAGPGMFKCLNHSQNLLKAVSNTLQKAKQTLEFYSC TSEEIDHEDITKDKTSTVEACLPLELATNESCLAARETSLITNG NCLTSGKTSFMTTLCSSYEDLKMYHVEFQAMNAKLLMDP KRQIFLDQNMLTAITELMQALNFNSETVPQKPSLEELDFYKTK IKLCILLHAFRIRAVTIDRMMSYLNSS
112	Porcine IL12 (IL12B-linker-IL12A)	IWELEKNVYVVELDWYPNAPGEMVVLTCNTPEEDGITWTS QSSEVLGTGKTLTIHVKEFGDAGQYTCRKGGAVLSQSLLLLH KKEDGIWSTDILKDQKEPKNKSFLKCEAKNYSGRFTCWWT AISTDLKFSVKSSRGSTDPRGVTCGTATLSEDLGEYKKYRVEC QEGSACPAAEESLPIEVVLEAVHKLKYENYTSFFIRDIKPDPP KNLQLNPLKNSRHVEISWEYPDTWSTPHSYFSLMFGVQVQGK NKREKDKLFTDQISAKVTCHKDANIRVQARDRYSSSSWSE WASVSCNGGGGSGGGGSGGGGSRSLPATTAGPGMFKCLNHS QNLLKAVSNTLQKAKQTLEFYSC TSEEIDHEDITKDKTSTVEA CLPLELATNESCLAARETSLITNGNCLTSGKTSFMTTLCSSY EDLKMYHVEFQAMNAKLLMDPKRQIFLDQNMLTAITELMQA LNFNSETVPQKPSLEELDFYKTKIKLCILLHAFRIRAVTIDRM SYLNSS
113	Porcine IL12-ABP20-G4-8x-GE (Untagged, mature protein)	IWELEKNVYVVELDWYPNAPGEMVVLTCNTPEEDGITWTS QSSEVLGTGKTLTIHVKEFGDAGQYTCRKGGAVLSQSLLLLH KKEDGIWSTDILKDQKEPKNKSFLKCEAKNYSGRFTCWWT AISTDLKFSVKSSRGSTDPRGVTCGTATLSEDLGEYKKYRVEC QEGSACPAAEESLPIEVVLEAVHKLKYENYTSFFIRDIKPDPP KNLQLNPLKNSRHVEISWEYPDTWSTPHSYFSLMFGVQVQGK NKREKDKLFTDQISAKVTCHKDANIRVQARDRYSSSSWSE WASVSCNGGGGSGGGGSGGGGSRSLPATTAGPGMFKCLNHS QNLLKAVSNTLQKAKQTLEFYSC TSEEIDHEDITKDKTSTVEA CLPLELATNESCLAARETSLITNGNCLTSGKTSFMTTLCSSY EDLKMYHVEFQAMNAKLLMDPKRQIFLDQNMLTAITELMQA LNFNSETVPQKPSLEELDFYKTKIKLCILLHAFRIRAVTIDRM SYLNSSGGGGEGGGGSEEGGGGSEEGGGGSEEGGGGSEEGG GGSEEGGGGSEEGGGGSEEGGGGSEEGGGGSEEGGGGSEEGG
114	Equine IL12B	IWELEKDVYVVELDWYPDAPGEMVVLTCNTPEEEGITWTS QSNEVLGSGKTLTIQVKEFGDAGWYTCHKGGEVLSSHLLH KKEDGIWSTDILKDQKESKNKTLKCEAKNYSGRFTCWWT AISTDLKFSVKSSRGSSDPRGVTCGAATLSAERVSVDREYKK YTVECQEGSACPAAEESLPIEVVDVAVHKLKYENYTSGFIRDI IKPDPPKNLQLKPLKNSRQVEVSWEYPETWSTPHSYFSLTFSIQ VQGKNKKERKDRLFMDETSATVTCHKDGQIRVQARDRYSS SWSEWASVSCS
115	Equine IL12A	RNLPTATPGPMFQCLNHSQNLLRTVSNTLQKARQTLEFYSC TSEEIDHEDITKDKSSTVAACLPLELAPNESCLASREISFITNGS CLTPGKASSMMTLCSSYEDLKMYQVEFKAMNAKLLIDPQR QIFLDENMLTAIDKLMQALNFNSETVPQKPSLEGLDFYKTKV KLCILLHAFRIRAVTINRMMSGYLNAS

116	Equine IL12 (IL12B-linker-IL12A)	IWELEKDVYVVELDWYPDAPGEMVVLTCNTPEEEGITWTS QSNEVLGSGKTLTIQVKEFGDAGWYTCHKGGEVLSSHLLH KKEDGIWSTDILKDQKESKNKTKFLKCEAKNYSGRFTCWWT AISTDLKFSVKSSRGSSDPRGVTCGAATLSAERSVDDREYKK YTVECQEGSACPAEESLPIEIVVDAVHKLKYENYTSGFFIRDI IKPDPPKNLQKPLKNSRQVEVSWEYPETWSTPHSYFSLTFSIQ VQGKNKKERKDRLFMDETSATVTCHKDQGIRVQARDRIYSS SWSEWASVSCSGGGGSGGGGSGGGGSRNLPTATPGPGMFQC LNHSQNLLRTVSNLTLQKARQTLEFYSCSTSEIDHEDITKDKSS TVAAACLPLELAPNESCLASREISFITNGSCLTPGKASSMMTLCL SSIEDLKMYQVEFKAMNAKLLIDPQRQIFLDENMLTAIDKL MQALNFNSETVPQKPSLEGLDFYKTKVKLCILLHAFRIRAVTI NRMMGYLNAS
117	Equine IL12- ABP20-G4- 8x-GE (Untagged, mature protein)	IWELEKDVYVVELDWYPDAPGEMVVLTCNTPEEEGITWTS QSNEVLGSGKTLTIQVKEFGDAGWYTCHKGGEVLSSHLLH KKEDGIWSTDILKDQKESKNKTKFLKCEAKNYSGRFTCWWT AISTDLKFSVKSSRGSSDPRGVTCGAATLSAERSVDDREYKK YTVECQEGSACPAEESLPIEIVVDAVHKLKYENYTSGFFIRDI IKPDPPKNLQKPLKNSRQVEVSWEYPETWSTPHSYFSLTFSIQ VQGKNKKERKDRLFMDETSATVTCHKDQGIRVQARDRIYSS SWSEWASVSCSGGGGSGGGGSGGGGSRNLPTATPGPGMFQC LNHSQNLLRTVSNLTLQKARQTLEFYSCSTSEIDHEDITKDKSS TVAAACLPLELAPNESCLASREISFITNGSCLTPGKASSMMTLCL SSIEDLKMYQVEFKAMNAKLLIDPQRQIFLDENMLTAIDKL MQALNFNSETVPQKPSLEGLDFYKTKVKLCILLHAFRIRAVTI NRMMGYLNASGGGGEGGGGSEEGGGGSEEGGGGSEEGGGG SEEGGGGSEEGGGGSEEGGGGSEEGGGGSEEGGGG

[0129] **Table 2** provides exemplary nucleotide sequences encoding polypeptides described herein.

Table 2: Exemplary Nucleotide Sequences

SEQ ID NO:	Polypeptide	Sequence
51	mIL12-His (no ABP)	ATGTGGGAACCTCGAAAAGGATGTGTACGTGGTGGAAAGTCG ACTGGACTCCTGATGCCCCGGGAGAGACAGTGAACCTGAC CTGTGACACTCCCGAAGAGGACGACATTACTTGGACCAGC GATCAGCGGCACGGCGTGATCGGATCCGGCAAAACCTGA CCATCACCGTGAAAGAATTCTTGACGCGGGACAGTACAC TTGCCACAAGGGCGGCGAAACCTCAGCCATTCCACCTTT TGCTCCACAAGAAGGAAAACGGAATCTGGTCCACCGAAAT TCTGAAGAACTTCAAGAACAAGACCTTCTGAAGTGCGAG GCGCCCAACTACTCGGGAAGGTTACATGCTCCTGGCTGGT

		GCAGAGGAACATGGATCTGAAGTTCAACATCAAGAGCTCC TCCTCCTCCCCGGATTCAAGAGCCGTGACTTGCGGGATGGC ATCCCTGAGCGCCGAGAAAGTCACTCTGGACCAGAGAGAC TACGAGAAGTACTCCGTGTCGTGCCAGGAGGACGTGACCT GTCCTACCGCCGAAGAGACTCTGCCATTGAGCTGGCTCTG GAAGCCCGCCAGCAGAACAATAACGAAAATACTCCACGT CATTCTTTATCCGGGACATCATCAAGCCCGACCCCTCCTAAG AACCTCCAGATGAAGCCCCCTCAAGAACAGCCAGGTGAGG TGTCTTGGGAATACCCGGACTCCTGGAGCACCCACACTCC TACTTCTCGCTGAAGTTTTTCGTGCGGATCCAGCGGAAGAA AGAGAAGATGAAAGAGACTGAAGAGGGGTGCAACCAGAA GGGCGCTTTCCTGGTGGAAAAGACCTCGACCGAAGTGCAG TGCAAAGGGGGCAACGTCTGCGTGCAAGCCCAAGACCGCT ACTACAACCTCCTCCTGCTCCAAATGGGCATGCGTGCCGTGT AGAGTGCGGTCCGGTGGTGGAGGATCCGGAGGGGGGTGGAT CAGGGGGAGGCGGATCTAGGGTCAATCCGGTGTCCGGTCC TGCTCGCTGCCTGAGTCAGTCCCGGAATCTGCTTAAGACTA CTGACGATATGGTCAAGACTGCCCCGCGAGAAGCTGAAGCA CTACTCCTGCACCGCCGAGGACATTGACCACGAAGATATC ACCCGGGACCAAAACCAGCACCCCTCAAGACTTGCTGCCAC TTGAACTGCACAAGAACGAGAGCTGCCTGGCAACCAGAGA AACCAGCTCAACCACACGCGGATCATGTCTGCCCCCGCAA AAGACCTCCCTGATGATGACTCTCTGTCTGGGAAGCATCTA CGAAGATCTGAAGATGTATCAGACCGAATTCCAGGCCATC AACGCGGCGCTGCAGAACCACAATCATCAGCAGATTATCC TGGATAAGGGCATGCTGGTTCGCCATCGACGAGCTGATGCA GTCCTTGAATCACAAACGGCGAAACGCTCCGCCAAAAGCCG CCAGTGGGCGAAGCGGACCCCTATCGCGTGAAGATGAAGC TCTGCATCCTGCTGCATGCCTTTTCGACCCGGGTGGTCACC ATCAACAGAGTGATGGGATACCTGAGCTCAGCTGGCGGTG GTGGATCTCACCACCATCACCATCAT
52	mIL12- ABP10	ATGTGGGAACCTCGAAAAGGATGTGTACGTGGTGGAAAGTCG ACTGGACTCCTGATGCCCCGGGAGAGACAGTGAACCTGAC CTGTGACACTCCCGAAGAGGACGACATTACTTGGACCAGC GATCAGCGGCACGGCGTGATCGGATCCGGCAAAACCCTGA CCATCACCGTGAAAGAATTCTTGACGCGGGACAGTACAC TTGCCACAAGGGCGGCGAAACCCTCAGCCATTCACACCTTT TGCTCCACAAGAAGGAAAACGGAATCTGGTCCACCGAAAT TCTGAAGAACTTCAAGAACAAGACCTTCTGAAAGTGCGAG GCGCCCAACTACTCGGGAAGGTTACATGCTCCTGGCTGGT GCAGAGGAACATGGATCTGAAGTTCAACATCAAGAGCTCC TCCTCCTCCCCGGATTCAAGAGCCGTGACTTGCGGGATGGC ATCCCTGAGCGCCGAGAAAGTCACTCTGGACCAGAGAGAC TACGAGAAGTACTCCGTGTCGTGCCAGGAGGACGTGACCT GTCCTACCGCCGAAGAGACTCTGCCATTGAGCTGGCTCTG GAAGCCCGCCAGCAGAACAATAACGAAAATACTCCACGT

		CATTCTTTATCCGGGACATCATCAAGCCCGACCCCTCCTAAG AACCTCCAGATGAAGCCCCTCAAGAACAGCCAGGTCGAGG TGTCTTGGGAATACCCGGACTCCTGGAGCACCCACACTCC TACTTCTCGCTGAAGTTTTTCGTGCGGATCCAGCGGAAGAA AGAGAAGATGAAAGAGACTGAAGAGGGGTGCAACCAGAA GGGCGCTTTCCTGGTGGAAAAGACCTCGACCGAAGTGCAG TGCAAAGGGGGCAACGTCTGCGTGCAAGCCCAAGACCGCT ACTACAACCTCCTCCTGCTCCAAATGGGCATGCGTGCCGTGT AGAGTGCGGTCCGGTGGTGGAGGATCCGGAGGGGGTGGAT CAGGGGGAGGCGGATCTAGGGTCATTCCGGTGTCCGGTCC TGCTCGCTGCCTGAGTCAGTCCCGGAATCTGCTTAAGACTA CTGACGATATGGTCAAGACTGCCCCGCGAGAAGCTGAAGCA CTACTCCTGCACCGCCGAGGACATTGACCACGAAGATATC ACCCGGGACCAAACCAGCACCCCTCAAGACTTGCCCTGCCAC TTGAACTGCACAAGAACGAGAGCTGCCTGGCAACCAGAGA AACCAGCTCAACCACACGCGGATCATGTCTGCCCCCGCAA AAGACCTCCCTGATGATGACTCTCTGTCTGGGAAGCATCTA CGAAGATCTGAAGATGTATCAGACCGAATTCCAGGCCATC AACGCGGCGCTGCAGAACCACAATCATCAGCAGATTATCC TGGATAAGGGCATGCTGGTCGCCATCGACGAGCTGATGCA GTCCTTGAATCACAAACGGCGAAACGCTCCGCCAAAAGCCG CCAGTGGGCGAAGCGGACCCTTATCGCGTGAAGATGAAGC TCTGCATCCTGCTGCATGCCTTTTCGACCCGGGTGGTCACC ATCAACAGAGTGATGGGATACCTGAGCTCAGCTGGGGGCG GAGGCTCCTTCCAAAGCGAAGAACAGCAGGGAGGGGGGCTC CGGAGGTAGCGAAGAGGGAGGCATGGAGAGCGAGGAAAG CAATGGAGGCGGATCAGGCGGATCCGAAGAAGGCGGTGGT GGATCTCACCACCATCACCATCAT
53	mIL12- ABP20	ATGTGGGAACCTCGAAAAGGATGTGTACGTGGTGGAAAGTCG ACTGGACTCCTGATGCCCCGGGAGAGACAGTGAACCTGAC CTGTGACACTCCCGAAGAGGACGACATTACTTGGACCAGC GATCAGCGGCACGGCGTGATCGGATCCGGCAAAACCCTGA CCATCACCGTGAAAGAATTCTTGACGCGGGACAGTACAC TTGCCACAAGGGCGGCGAAACCCTCAGCCATTCCCACCTTT TGCTCCACAAGAAGGAAAACGGAATCTGGTCCACCGAAAT TCTGAAGAACTTCAAGAACAAAGACCTTCTGAAGTGCGAG GCGCCCAACTACTCGGGAAGGTTACATGCTCCTGGCTGGT GCAGAGGAACATGGATCTGAAGTTCAACATCAAGAGCTCC TCCTCCTCCCCGGATTCAAGAGCCGTGACTTGCGGGATGGC ATCCCTGAGCGCCGAGAAAGTCACTCTGGACCAGAGAGAC TACGAGAAGTACTCCGTGTCGTGCCAGGAGGACGTGACCT GTCCTACCGCCGAAGAGACTCTGCCCATGAGCTGGCTCTG GAAGCCCGCCAGCAGAACAAATACGAAAACCTACTCCACGT CATTCTTTATCCGGGACATCATCAAGCCCGACCCCTCCTAAG AACCTCCAGATGAAGCCCCTCAAGAACAGCCAGGTCGAGG TGTCTTGGGAATACCCGGACTCCTGGAGCACCCACACTCC

		TACTTCTCGCTGAAGTTTTTCGTGCGGATCCAGCGGAAGAA AGAGAAGATGAAAGAGACTGAAGAGGGGTGCAACCAGAA GGGCGCTTTCCTGGTGGAAAAGACCTCGACCGAAGTGCAG TGCAAAGGGGGCAACGTCTGCGTGCAAGCCCAAGACCGCT ACTACAACCTCCTCCTGCTCCAAATGGGCATGCGTGCCGTGT AGAGTGCGGTCCGGTGGTGGAGGATCCGGAGGGGGTGGAT CAGGGGGAGGCGGATCTAGGGTCAATCCGGTGTCCGGTCC TGCTCGCTGCCTGAGTCAGTCCCGGAATCTGCTTAAGACTA CTGACGATATGGTCAAGACTGCCCCGCGAGAAGCTGAAGCA CTACTCCTGCACCGCCGAGGACATTGACCACGAAGATATC ACCCGGGACCAAACCAGCACCTCAAGACTTGCCCTGCCAC TTGAACTGCACAAGAACGAGAGCTGCCTGGCAACCAGAGA AACCAGCTCAACCACACGCGGATCATGTCTGCCCCCGCAA AAGACCTCCCTGATGATGACTCTCTGTCTGGGAAGCATCTA CGAAGATCTGAAGATGTATCAGACCGAATTCCAGGCCATC AACGCGGCGCTGCAGAACCACAATCATCAGCAGATTATCC TGGATAAGGGCATGCTGGTCGCCATCGACGAGCTGATGCA GTCCTTGAATCACACGGCGAAACGCTCCGCCAAAAGCCG CCAGTGGGCGAAGCGGACCCTTATCGCGTGAAGATGAAGC TCTGCATCCTGCTGCATGCCTTTTCGACCCGGGTGGTCACC ATCAACAGAGTGATGGGATACCTGAGCTCAGCTGGAGGCG GAGGCTCAGGAGGTGGCGGCAGCGAGGAGAGCGAAGAAT CCGAGGAGTCCGAGGAAGGCGGTGGTGGATCTCACCACCA TCACCATCAT
54	mIL12- ABP20-G4	ATGTGGGAACCTCGAAAAGGATGTGTACGTGGTGGAAAGTCG ACTGGACTCCTGATGCCCCGGGAGAGACAGTGAACCTGAC CTGTGACACTCCCGAAGAGGACGACATTACTTGGACCAGC GATCAGCGGCACGGCGTGATCGGATCCGGCAAAACCCTGA CCATCACCGTGAAAGAATTCTTGACGCGGGACAGTACAC TTGCCACAAGGGCGGCGAAACCCTCAGCCATTCCACCTTT TGCTCCACAAGAAGGAAAACGGAATCTGGTCCACCGAAAT TCTGAAGAACTTCAAGAACAAGACCTTCCTGAAGTGCGAG GCGCCCAACTACTCGGGAAGGTTACATGCTCCTGGCTGGT GCAGAGGAACATGGATCTGAAGTTCAACATCAAGAGCTCC TCCTCCTCCCCGATTCAAGAGCCGTGACTTGCGGGATGGC ATCCCTGAGCGCCGAGAAAGTCACTCTGGACCAGAGAGAC TACGAGAAGTACTCCGTGTCTGTGCCAGGAGGACGTGACCT GTCCTACCGCCGAAGAGACTCTGCCCATGAGCTGGCTCTG GAAGCCCGCCAGCAGAACAAATACGAAAATACTCCACGT CATTCTTTATCCGGGACATCATCAAGCCCGACCTTCCTAAG AACCTCCAGATGAAGCCCTCAAGAACAGCCAGGTCGAGG TGTCTTGGGAATAACCGGACTCCTGGAGCACCCACACTCC TACTTCTCGCTGAAGTTTTTCGTGCGGATCCAGCGGAAGAA AGAGAAGATGAAAGAGACTGAAGAGGGGTGCAACCAGAA GGGCGCTTTCCTGGTGGAAAAGACCTCGACCGAAGTGCAG TGCAAAGGGGGCAACGTCTGCGTGCAAGCCCAAGACCGCT

		ACTACAACTCCTCCTGCTCCAAATGGGCATGCGTGCCGTGT AGAGTGCGGTCCGGTGGTGGAGGATCCGGAGGGGGTGGAT CAGGGGGAGGCGGATCTAGGGTCATTCCGGTGTCCGGTCC TGCTCGCTGCCTGAGTCAGTCCCGGAATCTGCTTAAGACTA CTGACGATATGGTCAAGACTGCCCCGCGAGAAGCTGAAGCA CTACTCCTGCACCGCCGAGGACATTGACCACGAAGATATC ACCCGGGACCAAACCAGCACCCCTCAAGACTTGCCCTGCCAC TTGAACTGCACAAGAACGAGAGCTGCCTGGCAACCAGAGA AACCAGCTCAACCACACGCGGATCATGTCTGCCCCCGCAA AAGACCTCCCTGATGATGACTCTCTGTCTGGGAAGCATCTA CGAAGATCTGAAGATGTATCAGACCGAATTCCAGGCCATC AACGCGGCGCTGCAGAACCACAATCATCAGCAGATTATCC TGGATAAGGGCATGCTGGTCGCCATCGACGAGCTGATGCA GTCCTTGAATCACAACGGCGAAACGCTCCGCCAAAAGCCG CCAGTGGGCGAAGCGGACCCTTATCGCGTGAAGATGAAGC TCTGCATCCTGCTGCATGCCTTTTCGACCCGGGTGGTCACC ATCAACAGAGTGATGGGATACCTGAGCTCAGCTGGCGGAG GGGGCTCAGGAGGAGGCGGAAGCGAAGAGGGGGGTGGTG GATCCGAGGAGGGAGGAGGAGGCTCGGAAGAGGGAGGCG GAGGATCGGAGGAGGGCGGTGGTGGATCTCACCACCATCA CCATCAT
55	mIL12- ABP20-G4- GS20	ATGTGGGAGCTGGAGAAGGATGTGTACGTGGTGGAGGTGG ACTGGACCCCTGACGCTCCTGGAGAAACCGTGAACCTTGAC GTGCGACACCCCTGAGGAAGATGACATCACCTGGACCTCC GACCAAAGACACGGCGTGATCGGCTCCGGAAAGACCCTGA CCATCACCGTGAAGGAGTTCCTGGATGCCGGGCAGTACAC TTGCCACAAGGGGGGGCGAAACGCTGTCCCACTCCCATCTG CTGCTGCACAAGAAAGAAAACGGGATCTGGTCCACCGAGA TTCTCAAGAACTTCAAGAACAAGACCTTCCTTAAGTGCAG GCCCCCACTACTCCGGCCGCTTCACTTGCTCCTGGCTGGT GCAGCGCAACATGGACCTGAAGTTCAATATCAAGTCATCC TCCTCGTCGCCTGATTGAGAGCAGTCACTTGCGGGATGGC GTCCCTGAGCGCTGAAAAGGTCACCCTGGATCAGCGGGAC TACGAGAAGTACTCCGTGTCCTGCCAAGAGGACCTGACCT GTCCGACCGCCGAAGAACTTTGCCCATCGAATTGGCCCTC GAAGCCAGGCAGCAGAATAAGTACGAGAACTACTCCACTT CGTTCTTCATTCGGGATATCATCAAGCCGGACCCACCGAAG AACCTCCAGATGAAGCCCCCTCAAGAACAGCCAGGTGGAAG TGTCGTGGGAGTACCCGGACAGCTGGAGCACCCACATAG CTACTTCTCACTGAAATTCTTCGTGCGGATCCAGCGCAAGA AGGAGAAGATGAAGGAAACCGAAGAAGGGTGCAACCAGA AGGGCGCCTTCTTGGTGGAAAAGACCAGCACCGAGGTGCA GTGCAAAGGCGGCAACGTCTGCGTGCAAGCCCAGGATAGA TACTACAACTCCTCGTGCTCCAAATGGGCATGCGTGCCATG TAGAGTGCGCTCCGGTGGTGGAGGATCAGGCGGTGGAGGC TCAGGAGGAGGGGGATCGAGGGTCATTCCGGTGTCCGGTC

		CTGCCCCGATGCCTGTCACAATCCCGGAACCTTCTCAAGACT ACCGACGACATGGTCAAGACCGCTCGGGAGAACTGAAGC ACTACTCCTGCACCGCCGAGGACATCGACCACGAAGATAT TACTCGGGATCAAACCTTCCACTCTCAAACTTGCCTCCCGC TCGAACTCCACAAGAACGAGTCCTGCTTAGCGACCAGGGA AACTAGCTCCACAACGCGGGGGTTCATGTCTGCCACCCCAA AAGACCTCCCTCATGATGACCCTGTGCCTTGGCTCCATCTA TGAGGACCTGAAGATGTATCAGACCGAATTTTCAGGCCATC AACGCGGCACTGCAGAACCACAACCATCAGCAGATTATCC TGGATAAGGGGATGCTGGTCGCAATCGACGAGCTCATGCA GTCCCTGAACCACAATGGGGAACTCTGAGGCAGAAGCCC CCTGTGGGAGAAGCGGATCCCTACCGCGTGAAGATGAAGC TATGTATCCTGCTGCATGCCTTTTCCACCCGGGTCTGACT ATCAATCGCGTGATGGGCTACCTTAGCTCAGCGGGTGGAG GAGGCAGTGGAGGCGGTGGAAGCGGAGGAGGAGGAAGCG GTGGAGGGGGTTCCGAGGAAGGAGGGGGCGGATCTGAGG AAGGCGGAGGAGGCTCAGAAGAGGGTGGAGGCGGATCCG AAGAGGGAGGCGGAGGTTTCGCACCATCACCACCACCAC
56	mIL12- ABP20-G4- 6x	ATGTGGGAACCTCGAAAAGGATGTGTACGTGGTGGAAAGTCG ACTGGACTCCTGATGCCCCGGGAGAGACAGTGAACCTGAC CTGTGACACTCCCGAAGAGGACGACATTACTTGGACCAGC GATCAGCGGCACGGCGTGATCGGATCCGGCAAAACCTGA CCATCACCGTGAAAGAATTCTTGACGCGGGACAGTACAC TTGCCACAAGGGCGGCGAAACCCTCAGCCATTCCCACCTTT TGCTCCACAAGAAGGAAAACGGAATCTGGTCCACCGAAAT TCTGAAGAACTTCAAGAACAAGACCTTCTGAAGTGCGAG GCGCCCAACTACTCGGGAAGGTTACATGCTCCTGGCTGGT GCAGAGGAACATGGATCTGAAGTTCAACATCAAGAGCTCC TCCTCCTCCCCGGATTCAAGAGCCGTGACTTGCGGGATGGC ATCCCTGAGCGCCGAGAAAGTCACTCTGGACCAGAGAGAC TACGAGAAGTACTCCGTGTCGTGCCAGGAGGACGTGACCT GTCCTACCGCCGAAGAGACTCTGCCCATTGAGCTGGCTCTG GAAGCCCGCCAGCAGAACAAATACGAAAATACTCCACGT CATTCTTTATCCGGGACATCATCAAGCCCGACCTCCTAAG AACCTCCAGATGAAGCCCCTCAAGAACAGCCAGGTCGAGG TGTCTTGGAATACCCGGACTCCTGGAGCACCCACACTCC TACTTCTCGCTGAAGTTTTTTCGTGCGGATCCAGCGGAAGAA AGAGAAGATGAAAGAGACTGAAGAGGGGTGCAACCAGAA GGGCGCTTTCCTGGTGGAAAAGACCTCGACCGAAGTGCAG TGCAAAGGGGGCAACGTCTGCGTGCAAGCCCAAGACCGCT ACTACAACTCCTCCTGCTCCAAATGGGCATGCGTGCCGTGT AGAGTGCGGTCCGGTGGTGGAGGATCCGGAGGGGGTGGAT CAGGGGGAGGCGGATCTAGGGTTCATTCCGGTGTCCGGTCC TGCTCGCTGCCTGAGTCAGTCCCGGAATCTGCTTAAGACTA CTGACGATATGGTCAAGACTGCCCCGCGAGAAGCTGAAGCA CTACTCCTGCACCGCCGAGGACATTGACCACGAAGATATC

		ACCCGGGACCAAACCAGCACCCCTCAAGACTTGCCTGCCAC TTGAACTGCACAAGAACGAGAGCTGCCTGGCAACCAGAGA AACCAGCTCAACCACACGCGGATCATGTCTGCCCCCGCAA AAGACCTCCCTGATGATGACTCTCTGTCTGGGAAGCATCTA CGAAGATCTGAAGATGTATCAGACCGAATTCCAGGCCATC AACGCGGCGCTGCAGAACCACAATCATCAGCAGATTATCC TGGATAAGGGCATGCTGGTCGCCATCGACGAGCTGATGCA GTCCTTGAATCACAACGGCGAAACGCTCCGCCAAAAGCCG CCAGTGGGCGAAGCGGACCCCTTATCGCGTGAAGATGAAGC TCTGCATCCTGCTGCATGCCTTTTCGACCCGGGTGGTCACC ATCAACAGAGTGATGGGATACCTGAGCTCAGCTGGCGGTG GTGGATCCGGGGGTGGAGGTTCCGAGGAGGGTGGAGGAG GCAGCGAAGAGGGCGGAGGAGGATCGGAAGAGGGAGGAG GCGGAAGCGAAGAAGGGGGTGGCGGATCAGAGGAGGGAG GGGGAGGTTCCGAAGAAGGAGGAGGGGGATCGCATCATC ACCACCACCAC
57	mIL12- ABP20-G4- 8x	ATGTGGGAACTCGAAAAGGATGTGTACGTGGTGGAAAGTCG ACTGGACTCCTGATGCCCCGGGAGAGACAGTGAACCTGAC CTGTGACACTCCCGAAGAGGACGACATTACTTGGACCAGC GATCAGCGGCACGGCGTGATCGGATCCGGCAAAACCTGA CCATCACCGTGAAAGAATTCTTGACGCGGGACAGTACAC TTGCCACAAGGGCGGCGAAACCTCAGCCATTCCACCTTT TGCTCCACAAGAAGGAAAACGGAATCTGGTCCACCGAAAT TCTGAAGAACTTCAAGAACAAGACCTTCTGAAGTGCGAG GCGCCCAACTACTCGGGAAGGTTACATGCTCCTGGCTGGT GCAGAGGAACATGGATCTGAAGTTCAACATCAAGAGCTCC TCCTCCTCCCCGGATTCAAGAGCCGTGACTTGCGGGATGGC ATCCCTGAGCGCCGAGAAAGTCACTCTGGACCAGAGAGAC TACGAGAAGTACTCCGTGTCGTGCCAGGAGGACGTGACCT GTCCTACCGCCGAAGAGACTCTGCCCATTGAGCTGGCTCTG GAAGCCCGCCAGCAGAACAAATACGAAACTACTCCACGT CATTCTTTATCCGGGACATCATCAAGCCCGACCTCCTAAG AACCTCCAGATGAAGCCCCTCAAGAACAGCCAGGTCGAGG TGTCTGGGAATACCCGGACTCCTGGAGCACCCACACTCC TACTTCTCGCTGAAGTTTTTCGTGCGGATCCAGCGGAAGAA AGAGAAGATGAAAGAGACTGAAGAGGGGTGCAACCAGAA GGGCGCTTTCCTGGTGGAAAAGACCTCGACCGAAGTGCAG TGCAAAGGGGGCAACGTCTGCGTGCAAGCCCAAGACCGCT ACTACAACTCCTCCTGCTCCAAATGGGCATGCGTGCCGTGT AGAGTGCGGTCCGGTGGTGGAGGATCCGGAGGGGGTGGAT CAGGGGGAGGCGGATCTAGGGTCATTCCGGTGTCCGGTCC TGCTCGCTGCCTGAGTCAGTCCCGGAATCTGCTTAAGACTA CTGACGATATGGTCAAGACTGCCCCGCGAGAAGCTGAAGCA CTACTCCTGCACCGCCGAGGACATTGACCACGAAGATATC ACCCGGGACCAAACCAGCACCCCTCAAGACTTGCCTGCCAC TTGAACTGCACAAGAACGAGAGCTGCCTGGCAACCAGAGA

		AACCAGCTCAACCACACGCGGATCATGTCTGCCCCCGCAA AAGACCTCCCTGATGATGACTCTCTGTCTGGGAAGCATCTA CGAAGATCTGAAGATGTATCAGACCGAATTCCAGGCCATC AACGCGGCGCTGCAGAACCACAATCATCAGCAGATTATCC TGGATAAGGGCATGCTGGTCGCCATCGACGAGCTGATGCA GTCCTTGAATCACAACGGCGAAACGCTCCGCCAAAAGCCG CCAGTGGGCGAAGCGGACCCCTTATCGCGTGAAGATGAAGC TCTGCATCCTGCTGCATGCCTTTTCGACCCGGGTGGTCACC ATCAACAGAGTGATGGGATACCTGAGCTCAGCTGGAGGCG GTGGATCGGGAGGAGGGGGATCGGAAGAAGGGGGCGGAG GTTTCAGAGGAAGGAGGTGGCGGATCCGAGGAAGGGGGAG GAGGCTCTGAGGAGGGTGGTGGCGGAAGCGAAGAGGGAG GCGGAGGATCCGAAGAGGGTGGCGGGGGATCAGAGGAGG GAGGGGGAGGATCAGAAGAGGGCGGTGGTGGATCGCACC ATCATCACCACCAC
58	mlL12- ABP20-6x	ATGTGGGAACCTCGAAAAGGATGTGTACGTGGTGGAAAGTCG ACTGGACTCCTGATGCCCCGGGAGAGACAGTGAACCTGAC CTGTGACACTCCCGAAGAGGACGACATTACTTGGACCAGC GATCAGCGGCACGGCGTGATCGGATCCGGCAAAACCTGA CCATCACCGTGAAAGAATTCTTGACGCGGGACAGTACAC TTGCCACAAGGGCGGGCGAACCCTCAGCCATTCCCACTTT TGCTCCACAAGAAGGAAAACGGAATCTGGTCCACCGAAAT TCTGAAGAACTTCAAGAACAAAGACCTTCTGAAGTGCGAG GCGCCCAACTACTCGGGAAGGTTACATGCTCCTGGCTGGT GCAGAGGAACATGGATCTGAAGTTCAACATCAAGAGCTCC TCCTCCTCCCCGGATTCAAGAGCCGTGACTTGCGGGATGGC ATCCCTGAGCGCCGAGAAAAGTCACTCTGGACCAGAGAGAC TACGAGAAGTACTCCGTGTCGTGCCAGGAGGACGTGACCT GTCCTACCGCCGAAGAGACTCTGCCCATTGAGCTGGCTCTG GAAGCCCGCCAGCAGAACAAATACGAAAATACTCCACGT CATTCTTTATCCGGGACATCATCAAGCCCGACCTCCTAAG AACCTCCAGATGAAGCCCCTCAAGAACAGCCAGGTGAGG TGTCTTGGGAATACCCGGACTCCTGGAGCACCCACACTCC TACTTCTCGCTGAAGTTTTTCGTGCGGATCCAGCGGAAGAA AGAGAAGATGAAAGAGACTGAAGAGGGGTGCAACCAGAA GGGCGCTTTCCTGGTGGAAAAGACCTCGACCGAAGTGCAG TGCAAAGGGGGCAACGTCTGCGTGCAAGCCCAAGACCGCT ACTACAACTCCTCCTGCTCCAAATGGGCATGCGTGCCGTGT AGAGTGCGGTCCGGTGGTGGAGGATCCGGAGGGGGTGGAT CAGGGGGAGGCGGATCTAGGGTTCATTCCGGTGTCCGGTCC TGCTCGCTGCCTGAGTCAGTCCCGGAATCTGCTTAAGACTA CTGACGATATGGTCAAGACTGCCC GCGAGAAGCTGAAGCA CTACTCCTGCACCGCCGAGGACATTGACCACGAAGATATC ACCCGGGACCAAACCAGCACCTCAAGACTTGCTGCCAC TTGAACTGCACAAGAACGAGAGCTGCCTGGCAACCAGAGA AACCAGCTCAACCACACGCGGATCATGTCTGCCCCCGCAA

		AAGACCTCCCTGATGATGACTCTCTGTCTGGGAAGCATCTA CGAAGATCTGAAGATGTATCAGACCGAATTCCAGGCCATC AACGCGGCGCTGCAGAACCACAATCATCAGCAGATTATCC TGGATAAGGGCATGCTGGTCGCCATCGACGAGCTGATGCA GTCCTTGAATCACAACGGCGAAACGCTCCGCCAAAAGCCG CCAGTGGGCGAAGCGGACCCTTATCGCGTGAAGATGAAGC TCTGCATCCTGCTGCATGCCTTTTCGACCCGGGTGGTCACC ATCAACAGAGTGATGGGATACCTGAGCTCAGCTGGAGGCG GTGGCTCTGGTGGCGGAGGATCCGAGGAATCGGAAGAGTC CGAAGAGTCGGAGGAAAGCGAAGAGTCAGAAGAAGGGGG CGGAGGTTACACCACCACCATCATCAC
59	mIL12- ABP20-8x	ATGTGGGAACCTCGAAAAGGATGTGTACGTGGTGGAAAGTCG ACTGGACTCCTGATGCCCCGGGAGAGACAGTGAACCTGAC CTGTGACACTCCCGAAGAGGACGACATTACTTGGACCAGC GATCAGCGGCACGGCGTGATCGGATCCGGCAAAACCCTGA CCATCACCGTGAAAGAATTCTTGACGCGGGACAGTACAC TTGCCACAAGGGCGGGCGAAACCCTCAGCCATTCCACCTTT TGCTCCACAAGAAGGAAAACGGAATCTGGTCCACCGAAAT TCTGAAGAACTTCAAGAACAAGACCTTCTGAAGTGCGAG GCGCCCAACTACTCGGGAAGGTTACATGCTCCTGGCTGGT GCAGAGGAACATGGATCTGAAGTTCAACATCAAGAGCTCC TCCTCCTCCCCGGATTCAAGAGCCGTGACTTGCGGGATGGC ATCCCTGAGCGCCGAGAAAGTCACTCTGGACCAGAGAGAC TACGAGAAGTACTCCGTGTCGTGCCAGGAGGACGTGACCT GTCCTACCGCCGAAGAGACTCTGCCCATTTGAGCTGGCTCTG GAAGCCCGCCAGCAGAACAAATACGAAAATACTCCACGT CATTCTTTATCCGGGACATCATCAAGCCCGACCCTCCTAAG AACCTCCAGATGAAGCCCCTCAAGAACAGCCAGGTGAGG TGTCTGGGAATACCCGGACTCCTGGAGCACCCACACTCC TACTTCTCGCTGAAGTTTTTCGTGCGGATCCAGCGGAAGAA AGAGAAGATGAAAGAGACTGAAGAGGGGTGCAACCAGAA GGGCGCTTTCCTGGTGGAAAAGACCTCGACCGAAGTGCAG TGCAAAGGGGGCAACGTCTGCGTGCAAGCCCAAGACCGCT ACTACAACTCCTCCTGCTCCAAATGGGCATGCGTGCCGTGT AGAGTGCGGTCCGGTGGTGGAGGATCCGGAGGGGGTGGAT CAGGGGGAGGCGGATCTAGGGTCATTCCGGTGTCCGGTCC TGCTCGCTGCCTGAGTCAGTCCCGGAATCTGCTTAAGACTA CTGACGATATGGTCAAGACTGCCCGCGAGAAGCTGAAGCA CTACTCCTGCACCGCCGAGGACATTGACCACGAAGATATC ACCCGGGACCAAACCAGCACCTCAAGACTTGCCCTGCCAC TTGAACTGCACAAGAACGAGAGCTGCCTGGCAACCAGAGA AACCAGCTCAACCACACGCGGATCATGTCTGCCCCCGCAA AAGACCTCCCTGATGATGACTCTCTGTCTGGGAAGCATCTA CGAAGATCTGAAGATGTATCAGACCGAATTCCAGGCCATC AACGCGGCGCTGCAGAACCACAATCATCAGCAGATTATCC TGGATAAGGGCATGCTGGTCGCCATCGACGAGCTGATGCA

		GTCCTTGAATCACAAACGGCGAAACGCTCCGCCAAAAGCCG CCAGTGGGCGAAGCGGACCCTTATCGCGTGAAGATGAAGC TCTGCATCCTGCTGCATGCCTTTTCGACCCGGGTGGTCACC ATCAACAGAGTGATGGGATACCTGAGCTCAGCTGGGGGTG GAGGTTTCGGGTGGCGGAGGATCCGAGGAATCGGAAGAGTC CGAAGAGTCGGAAGAATCCGAGGAAAGCGAAGAGTCAGA GGAATCTGAGGAGGGTGGTGGCGGAAGCCATCATCACCAC CACCAC
60	mIL12- ABP20-G4- SEEE	ATGTGGGAACTCGAAAAGGATGTGTACGTGGTGGAAAGTCG ACTGGACTCCTGATGCCCCGGGAGAGACAGTGAACCTGAC CTGTGACACTCCCGAAGAGGACGACATTACTTGGACCAGC GATCAGCGGCACGGCGTGATCGGATCCGGCAAAACCTGA CCATCACCGTGAAAGAATTCCTTGACGCGGGACAGTACAC TTGCCACAAGGGCGGGCGAAACCCTCAGCCATTCCACCTTT TGCTCCACAAGAAGGAAAACGGAATCTGGTCCACCGAAAT TCTGAAGA ACTTCAAGAACAGACCTTCTGAAGTGCGAG GCGCCCAACTACTCGGGAAGGTTACATGCTCCTGGCTGGT GCAGAGGAACATGGATCTGAAGTTCAACATCAAGAGCTCC TCCTCCTCCCCGGATTCAAGAGCCGTGACTTGCGGGATGGC ATCCCTGAGCGCCGAGAAAGTCACTCTGGACCAGAGAGAC TACGAGAAGTACTCCGTGTCGTGCCAGGAGGACGTGACCT GTCCTACCGCCGAAGAGACTCTGCCATTGAGCTGGCTCTG GAAGCCCGCCAGCAGAACAAATACGAAA ACTACTCCACGT CATTCTTTATCCGGGACATCATCAAGCCCGACCCTCCTAAG AACCTCCAGATGAAGCCCCTCAAGAACAGCCAGGTGAGG TGTCTTGGGAATACCCGGACTCCTGGAGCACCCACACTCC TACTTCTCGCTGAAGTTTTTCGTGCGGATCCAGCGGAAGAA AGAGAAGATGAAAGAGACTGAAGAGGGGTGCAACCAGAA GGGCGCTTTCCTGGTGGAAAAGACCTCGACCGAAGTGCAG TGCAAAGGGGGCAACGTCTGCGTGCAAGCCCAAGACCGCT ACTACA ACTCCTCCTGCTCCAAATGGGCATGCGTGCCGTGT AGAGTGCGGTCCGGTGGTGGAGGATCCGGAGGGGGTGGAT CAGGGGGAGGCGGATCTAGGGTCATTCCGGTGTCCGGTCC TGCTCGCTGCCTGAGTCACTCCCGGAATCTGCTTAAGACTA CTGACGATATGGTCAAGACTGCCCGCGAGAAGCTGAAGCA CTACTCCTGCACCGCCGAGGACATTGACCACGAAGATATC ACCCGGGACCAAACCAGCACCTCAAGACTTGCCCTGCCAC TTGAACTGCACAAGAACGAGAGCTGCCTGGCAACCAGAGA AACCAGCTCAACCACACGCGGATCATGTCTGCCCCCGCAA AAGACCTCCCTGATGATGACTCTCTGTCTGGGAAGCATCTA CGAAGATCTGAAGATGTATCAGACCGAATTCCAGGCCATC AACGCGGCGCTGCAGAACCACAATCATCAGCAGATTATCC TGGATAAGGGCATGCTGGTCCGATCGACGAGCTGATGCA GTCCTTGAATCACAAACGGCGAAACGCTCCGCCAAAAGCCG CCAGTGGGCGAAGCGGACCCTTATCGCGTGAAGATGAAGC TCTGCATCCTGCTGCATGCCTTTTCGACCCGGGTGGTCACC

		ATCAACAGAGTGATGGGATACCTGAGCTCAGCTGGAGGCG GAGGATCGGGAGGGGGAGGATCCGAGGAAGAAGGGGGTG GCAGCGAAGAAGAGGGAGGAGGGTCTGAAGAGGAGGGCG GTGGCTCAGAGGAAGAGGGTGGCGGTTTCGCACCATCACCA CCACCAT
61	mIL12- ABP20-G4- SEEA	ATGTGGGAACCTCGAAAAGGATGTGTACGTGGTGGAAAGTCG ACTGGACTCCTGATGCCCCGGGAGAGACAGTGAACCTGAC CTGTGACACTCCCGAAGAGGACGACATTACTTGGACCAGC GATCAGCGGCACGGCGTGATCGGATCCGGCAAAACCTGA CCATCACCGTGAAAGAATTCTTGACGCGGGACAGTACAC TTGCCACAAGGGCGGGCGAAACCTCAGCCATTCCACCTTT TGCTCCACAAGAAGGAAAACGGAATCTGGTCCACCGAAAT TCTGAAGAACTTCAAGAACAAGACCTTCTGAAGTGCGAG GCGCCCAACTACTCGGGAAGGTTACATGCTCCTGGCTGGT GCAGAGGAACATGGATCTGAAGTTCAACATCAAGAGCTCC TCCTCCTCCCCGGATTCAAGAGCCGTGACTTGCGGGATGGC ATCCCTGAGCGCCGAGAAAGTCACTCTGGACCAGAGAGAC TACGAGAAGTACTCCGTGTCGTGCCAGGAGGACGTGACCT GTCCTACCGCCGAAGAGACTCTGCCATTGAGCTGGCTCTG GAAGCCCGCCAGCAGAACAAATACGAAAATACTCCACGT CATTCTTTATCCGGGACATCATCAAGCCCGACCTCCTAAG AACCTCCAGATGAAGCCCCTCAAGAACAGCCAGGTGAGG TGTCTTGGGAATACCCGGACTCCTGGAGCACCCACACTCC TACTTCTCGCTGAAGTTTTTCGTGCGGATCCAGCGGAAGAA AGAGAAGATGAAAGAGACTGAAGAGGGGTGCAACCAGAA GGGCGCTTTCCTGGTGGAAAAGACCTCGACCGAAGTGCAG TGCAAAGGGGGCAACGTCTGCGTGCAAGCCCAAGACCGCT ACTACAACCTCCTCCTGCTCCAATGGGCATGCGTGCCGTGT AGAGTGCGGTCCGGTGGTGGAGGATCCGGAGGGGGTGGAT CAGGGGGAGGCGGATCTAGGGTCATTCCGGTGTCCGGTCC TGCTCGCTGCCTGAGTCAGTCCCGGAATCTGCTTAAGACTA CTGACGATATGGTCAAGACTGCCCCGCGAGAAGCTGAAGCA CTACTCCTGCACCGCCGAGGACATTGACCACGAAGATATC ACCCGGGACCAACCAGCACCTCAAGACTTGCTGCCAC TTGAACTGCACAAGAACGAGAGCTGCCTGGCAACCAGAGA AACCAGCTCAACCACACGCGGATCATGTCTGCCCCCGCAA AAGACCTCCCTGATGATGACTCTCTGTCTGGGAAGCATCTA CGAAGATCTGAAGATGTATCAGACCGAATTCCAGGCCATC AACGCGGCGCTGCAGAACCACAATCATCAGCAGATTATCC TGGATAAGGGCATGCTGGTCCGATCGACGAGCTGATGCA GTCCTTGAATCACAAACGGCGAAACGCTCCGCCAAAAGCCG CCAGTGGGCGAAGCGGACCTTATCGCGTGAAGATGAAGC TCTGCATCCTGCTGCATGCCTTTTCGACCCGGGTGGTCACC ATCAACAGAGTGATGGGATACCTGAGCTCAGCTGGAGGAG GGGGATCCGGTGGAGGCGGTTCCGAGGAAGCAGGCGGTGG ATCGGAAGAGGCTGGGGGTGGAAGCGAAGAGGCCGGTGG

		CGGATCAGAGGAGGCTGGCGGAGGATCGCACCACCATCAC CACCAT
62	mIL12- ABP20-G4- SEEQ	ATGTGGGAAC TCGAAAAGGATGTGTACGTGGTGGAAAGTCG ACTGGACTCCTGATGCCCCGGGAGAGACAGTGAACCTGAC CTGTGACACTCCCGAAGAGGACGACATTACTTGGACCAGC GATCAGCGGCACGGCGTGATCGGATCCGGCAAAACCTGA CCATCACCGTGAAAGAATTCCTTGACGCGGGACAGTACAC TTGCCACAAGGGCGGGCGAAACCTCAGCCATTCCACCTTT TGCTCCACAAGAAGGAAAACGGAATCTGGTCCACCGAAAT TCTGAAGA ACTTCAAGAACAAGACCTTCCTGAAGTGCGAG GCGCCCAACTACTCGGGAAGGTTACATGCTCCTGGCTGGT GCAGAGGAACATGGATCTGAAGTTCAACATCAAGAGCTCC TCCTCCTCCCCGGATTCAAGAGCCGTGACTTGCGGGGATGGC ATCCCTGAGCGCCGAGAAAGTCACTCTGGACCAGAGAGAC TACGAGAAGTACTCCGTGTCGTGCCAGGAGGACGTGACCT GTCCTACCGCCGAAGAGACTCTGCCCATTGAGCTGGCTCTG GAAGCCCGCCAGCAGAACAAATACGAAA ACTACTCCACGT CATTCTTTATCCGGGACATCATCAAGCCCGACCCTCCTAAG AACCTCCAGATGAAGCCCCTCAAGAACAGCCAGGTGAGG TGTCTGGGAATAACCCGGACTCCTGGAGCACCCACACTCC TACTTCTCGCTGAAGTTTTTCGTGCGGATCCAGCGGAAGAA AGAGAAGATGAAAGAGACTGAAGAGGGGTGCAACCAGAA GGGCGCTTTCCTGGTGGAAAAGACCTCGACCGAAGTGCAG TGCAAAGGGGGCAACGTCTGCGTGCAAGCCCAAGACCGCT ACTACA ACTCCTCCTGCTCCAAATGGGCATGCGTGCCGTGT AGAGTGCGGTCCGGTGGTGGAGGATCCGGAGGGGGTGGAT CAGGGGGAGGCGGATCTAGGGTCATTCCGGTGTCCGGTCC TGCTCGCTGCCTGAGTCAGTCCCGGAATCTGCTTAAGACTA CTGACGATATGGTCAAGACTGCCCCGCGAGAAGCTGAAGCA CTACTCCTGCACCGCCGAGGACATTGACCACGAAGATATC ACCCGGGACCAAACCAGCACCTCAAGACTTGCTGCCAC TTGAACTGCACAAGAACGAGAGCTGCCTGGCAACCAGAGA AACCAGCTCAACCACACGCGGATCATGTCTGCCCCCGCAA AAGACCTCCTGATGATGACTCTCTGTCTGGGAAGCATCTA CGAAGATCTGAAGATGTATCAGACCGAATTCCAGGCCATC AACGCGGCGCTGCAGAACCACAATCATCAGCAGATTATCC TGGATAAGGGCATGCTGGTTCGCCATCGACGAGCTGATGCA GTCCTTGAATCACAACGGCGAAACGCTCCGCCAAAAGCCG CCAGTGGGCGAAGCGGACCCTTATCGCGTGAAGATGAAGC TCTGCATCCTGCTGCATGCCTTTTCGACCCGGGTGGTCACC ATCAACAGAGTGATGGGATACCTGAGCTCAGCTGGAGGCG GTGGATCGGGAGGTGGTGGCAGCGAAGAACAGGGTGGAG GGTCTGAGGAACAGGGCGGAGGATCCGAGGAGCAAGGGG GTGGCTCGGAAGAACAAGGAGGAGGGTCGCACCATCATCA CCACCAC

63	mIL12- ABP10-GS	ATGTGGGAAC TCGAAAAGGATGTGTACGTGGTGGAAAGTCG ACTGGACTCCTGATGCCCCGGGAGAGACAGTGAACCTGAC CTGTGACACTCCCGAAGAGGACGACATTACTTGGACCAGC GATCAGCGGCACGGCGTGATCGGATCCGGCAAAACCTGA CCATCACCGTGAAAGAATTCTTGACGCGGGACAGTACAC TTGCCACAAGGGCGGCGAAACCTCAGCCATTCCCACCTTT TGCTCCACAAGAAGGAAAACGGAATCTGGTCCACCGAAAT TCTGAAGA ACTTCAAGAACAAAGACCTTCCTGAAGTGCGAG GCGCCCAACTACTCGGGAAGGTTACATGCTCCTGGCTGGT GCAGAGGAACATGGATCTGAAGTTCAACATCAAGAGCTCC TCCTCCTCCCCGGATTCAAGAGCCGTGACTTGCGGGATGGC ATCCCTGAGCGCCGAGAAAGTCACTCTGGACCAGAGAGAC TACGAGAAGTACTCCGTGTCGTGCCAGGAGGACGTGACCT GTCCTACCGCCGAAGAGACTCTGCCATTGAGCTGGCTCTG GAAGCCCGCCAGCAGAACAAATACGAAA ACTACTCCACGT CATTCTTTATCCGGGACATCATCAAGCCCGACCTCCTAAG AACCTCCAGATGAAGCCCCCTCAAGAACAGCCAGGTGAGG TGTCTTGGGAATAACCCGGACTCCTGGAGCACCCACACTCC TACTTCTCGCTGAAGTTTTTCGTGCGGATCCAGCGGAAGAA AGAGAAGATGAAAGAGACTGAAGAGGGGTGCAACCAGAA GGGCGCTTTCCTGGTGGAAAAGACCTCGACCGAAGTGCA TGCAAAGGGGGCAACGTCTGCGTGCAAGCCCAAGACCGCT ACTACA ACTCCTCCTGCTCCAAATGGGCATGCGTGCCGTGT AGAGTGCGGTCCGGTGGTGGAGGATCCGGAGGGGGTGGAT CAGGGGGAGGCGGATCTAGGGTCATTCCGGTGTCCGGTCC TGCTCGCTGCCTGAGTCAGTCCCGGAATCTGCTTAAGACTA CTGACGATATGGTCAAGACTGCCC GCGAGAAGCTGAAGCA CTACTCCTGCACCGCCGAGGACATTGACCACGAAGATATC ACCCGGGACCAAACCAGCACCTCAAGACTTGCTGCCAC TTGAACTGCACAAGAACGAGAGCTGCCTGGCAACCAGAGA AACCAGCTCAACCACACGCGGATCATGTCTGCCCCCGCAA AAGACCTCCCTGATGATGACTCTCTGTCTGGGAAGCATCTA CGAAGATCTGAAGATGTATCAGACCGAATTCCAGGCCATC AACGCGGCGCTGCAGAACCACAATCATCAGCAGATTATCC TGGATAAGGGCATGCTGGTTCGCCATCGACGAGCTGATGCA GTCCTTGAATCACAACGGCGAAACGCTCCGCCAAAAGCCG CCAGTGGGCGAAGCGGACCTTATCGCGTGAAGATGAAGC TCTGCATCCTGCTGCATGCCTTTTCGACCCGGGTGGTCACC ATCAACAGAGTGATGGGATACCTGAGCTCAGCTGGCGGAG GGGGCTCAGGAGGATCCGAGGAAGGGGGCGGCGGCGGAT CAGGCGGCTCCGAAGAGGGCGGAGGCGGAAGCGAAGAGG GTGGCGGCGGAGGGTCCGGCGGTTCCGAGGAGGGTGGAGG CGGCCACCACCATCACCACCAC
64	mIL12- ABP10-GE	ATGTGGGAAC TCGAAAAGGATGTGTACGTGGTGGAAAGTCG ACTGGACTCCTGATGCCCCGGGAGAGACAGTGAACCTGAC CTGTGACACTCCCGAAGAGGACGACATTACTTGGACCAGC

		GATCAGCGGCACGGCGTGATCGGATCCGGCAAAACCCCTGA CCATCACCGTGAAAGAATTCCTTGACGCGGGACAGTACAC TTGCCACAAGGGCGGCGAAACCCTCAGCCATTCCCACCTTT TGCTCCACAAGAAGGAAAACGGAATCTGGTCCACCGAAAT TCTGAAGA ACTTCAAGAACAAAGACCTTCCTGAAGTGCGAG GCGCCCAACTACTCGGGAAGGTTTACATGCTCCTGGCTGGT GCAGAGGAACATGGATCTGAAGTTCAACATCAAGAGCTCC TCCTCCTCCCCGGATTCAAGAGCCGTGACTTGCGGGATGGC ATCCCTGAGCGCCGAGAAAGTCACTCTGGACCAGAGAGAC TACGAGAAGTACTCCGTGTCGTGCCAGGAGGACGTGACCT GTCCTACCGCCGAAGAGACTCTGCCCATTGAGCTGGCTCTG GAAGCCCGCCAGCAGAACAAATACGAAA ACTACTCCACGT CATTCTTTATCCGGGACATCATCAAGCCCGACCCTCCTAAG AACCTCCAGATGAAGCCCTCAAGAACAGCCAGGTGAGG TGTCTGGGAATAACCGGACTCCTGGAGCACCCACACTCC TACTTCTCGCTGAAGTTTTTCGTGCGGATCCAGCGGAAGAA AGAGAAGATGAAAGAGACTGAAGAGGGGTGCAACCAGAA GGGCGCTTTCCTGGTGGAAAAGACCTCGACCGAAGTGCAG TGCAAAGGGGGCAACGTCTGCGTGCAAGCCCAAGACCGCT ACTACA ACTCCTCCTGCTCCAAATGGGCATGCGTGCCGTGT AGAGTGCGGTCCGGTGGTGGAGGATCCGGAGGGGGTGGAT CAGGGGGAGGCGGATCTAGGGTCATTCCGGTGTCCGGTCC TGCTCGCTGCCTGAGTCAGTCCCGGAATCTGCTTAAGACTA CTGACGATATGGTCAAGACTGCCC GCGAGAAGCTGAAGCA CTACTCCTGCACCGCCGAGGACATTGACCACGAAGATATC ACCGGGACCAAACCAGCACCTCAAGACTTGCCCTGCCAC TTGAACTGCACAAGAACGAGAGCTGCCTGGCAACCAGAGA AACCAGCTCAACCACACGCGGATCATGTCTGCCCCCGCAA AAGACCTCCCTGATGATGACTCTCTGTCTGGGAAGCATCTA CGAAGATCTGAAGATGTATCAGACCGAATTCCAGGCCATC AACGCGGCGCTGCAGAACCACAATCATCAGCAGATTATCC TGGATAAGGGCATGCTGGTCCGATCGACGAGCTGATGCA GTCCTTGAATCACAACGGCGAAACGCTCCGCCAAAAGCCG CCAGTGGGCGAAGCGGACCCTTATCGCGTGAAGATGAAGC TCTGCATCCTGCTGCATGCCTTTTCGACCCGGGTGGTCACC ATCAACAGAGTGATGGGATACCTGAGCTCAGCTGGCGGAG GGGGCGAGGGAGGTTCCGAAGAAGGGGGAGGGGGCGGAG AGGGAGGATCCGAGGAAGGCGGTGGCGGATCGGAAGAAG GTGGCGGCGGTGGAGAAGGGGGCTCGGAAGAGGGCGGCG GAGGCCACCACCATCACCACCAC
65	mIL12- ABP20-G8- GS	ATGTGGGA ACTCGAAAAGGATGTGTACGTGGTGGAAAGTCG ACTGGACTCCTGATGCCCCGGGAGAGACAGTGAACCTGAC CTGTGACACTCCCGAAGAGGACGACATTACTTGGACCAGC GATCAGCGGCACGGCGTGATCGGATCCGGCAAAACCCCTGA CCATCACCGTGAAAGAATTCCTTGACGCGGGACAGTACAC TTGCCACAAGGGCGGCGAAACCCTCAGCCATTCCCACCTTT

		TGCTCCACAAGAAGGAAAACGGAATCTGGTCCACCGAAAT TCTGAAGAACTTCAAGAACAAGACCTTCCTGAAGTGCGAG GCGCCCAACTACTCGGGAAGGTTACATGCTCCTGGCTGGT GCAGAGGAACATGGATCTGAAGTTCAACATCAAGAGCTCC TCCTCCTCCCCGATTCAAGAGCCGTGACTTGCGGGATGGC ATCCCTGAGCGCCGAGAAAGTCACTCTGGACCAGAGAGAC TACGAGAAGTACTCCGTGTCGTGCCAGGAGGACGTGACCT GTCCTACCGCCGAAGAGACTCTGCCCATTGAGCTGGCTCTG GAAGCCCGCCAGCAGAACAATAACGAAAATACTCCACGT CATTCTTTATCCGGGACATCATCAAGCCCGACCCTCCTAAG AACCTCCAGATGAAGCCCCCTCAAGAACAGCCAGGTGAGG TGTCTTGGGAATACCCGGACTCCTGGAGCACCCACACTCC TACTTCTCGCTGAAGTTTTTCGTGCGGATCCAGCGGAAGAA AGAGAAGATGAAAGAGACTGAAGAGGGGTGCAACCAGAA GGGCGCTTTCCTGGTGGAAAAGACCTCGACCGAAGTGCA TGCAAAGGGGGCAACGTCTGCGTGCAAGCCCAAGACCGCT ACTACAACCTCCTCCTGCTCCAAATGGGCATGCGTGCCGTGT AGAGTGCGGTCCGGTGGTGGAGGATCCGGAGGGGGTGGAT CAGGGGGAGGCGGATCTAGGGTCATTCCGGTGTCCGGTCC TGCTCGCTGCCTGAGTCAGTCCCGGAATCTGCTTAAGACTA CTGACGATATGGTCAAGACTGCCCCGCGAGAAGCTGAAGCA CTACTCCTGCACCGCCGAGGACATTGACCACGAAGATATC ACCCGGGACCAAACCAGCACCTCAAGACTTGCCCTGCCAC TTGAACTGCACAAGAACGAGAGCTGCCTGGCAACCAGAGA AACCAGCTCAACCACACGCGGATCATGTCTGCCCCCGCAA AAGACCTCCCTGATGATGACTCTCTGTCTGGGAAGCATCTA CGAAGATCTGAAGATGTATCAGACCGAATTCCAGGCCATC AACGCGGCGCTGCAGAACCACAATCATCAGCAGATTATCC TGGATAAGGGCATGCTGGTCCCATCGACGAGCTGATGCA GTCCTTGAATCACAACGGCGAAACGCTCCGCCAAAAGCCG CCAGTGGGCGAAGCGGACCCTTATCGCGTGAAGATGAAGC TCTGCATCCTGCTGCATGCCTTTTCGACCCGGGTGGTCACC ATCAACAGAGTGATGGGATACCTGAGCTCAGCTGGCGGAG GGGGCGAAGGGGGCGGCGGATCGGAAGAGGGGCGGCGGCT CCGGAGGCGGAGGATCGGAGGAGGGAGGGGGAAGCGGTG GAGGGGGATCTGAGGAAGGCGGCGGAAGCGGAGGGGGCG GATCCGAGGAGGGCGGTGGCGGACACCACCACCATCACCA C
66	mIL12- ABP20-G8- GE	ATGTGGGAACCTCGAAAAGGATGTGTACGTGGTGGAAAGTCG ACTGGACTCCTGATGCCCCGGGAGAGACAGTGAACCTGAC CTGTGACACTCCCGAAGAGGACGACATTACTTGGACCAGC GATCAGCGGCACGGCGTGATCGGATCCGGCAAAACCCTGA CCATCACCGTGAAAGAATTCTTGACGCGGGACAGTACAC TTGCCACAAGGGCGGCGAAACCCTCAGCCATTCCCACCTTT TGCTCCACAAGAAGGAAAACGGAATCTGGTCCACCGAAAT TCTGAAGAACTTCAAGAACAAGACCTTCCTGAAGTGCGAG

		GCGCCCAACTACTCGGGAAGGTTACATGCTCCTGGCTGGT GCAGAGGAACATGGATCTGAAGTTCAACATCAAGAGCTCC TCCTCCTCCCCGGATTCAAGAGCCGTGACTTGCGGGATGGC ATCCCTGAGCGCCGAGAAAGTCACTCTGGACCAGAGAGAC TACGAGAAGTACTCCGTGTCGTGCCAGGAGGACGTGACCT GTCCTACCGCCGAAGAGACTCTGCCCATTGAGCTGGCTCTG GAAGCCCGCCAGCAGAACAAATACGAAACTACTCCACGT CATTCTTTATCCGGGACATCATCAAGCCCGACCCTCCTAAG AACCTCCAGATGAAGCCCCTCAAGAACAGCCAGGTGAGG TGTCTGGGAATACCCGGACTCCTGGAGCACCCACACTCC TACTTCTCGCTGAAGTTTTTCGTGCGGATCCAGCGGAAGAA AGAGAAGATGAAAGAGACTGAAGAGGGGTGCAACCAGAA GGGCGCTTTCCTGGTGGAAAAGACCTCGACCGAAGTGCAG TGCAAAGGGGGCAACGTCTGCGTGCAAGCCCAAGACCGCT ACTACAACTCCTCCTGCTCCAAATGGGCATGCGTGCCGTGT AGAGTGCGGTCCGGTGGTGGAGGATCCGGAGGGGGTGGAT CAGGGGGAGGCGGATCTAGGGTCATTCCGGTGTCCGGTCC TGCTCGCTGCCTGAGTCAGTCCCGGAATCTGCTTAAGACTA CTGACGATATGGTCAAGACTGCCCCGCGAGAAGCTGAAGCA CTACTCCTGCACCGCCGAGGACATTGACCACGAAGATATC ACCCGGGACCAAACCAGCACCCCTCAAGACTTGCTGCCAC TTGAACTGCACAAGAACGAGAGCTGCCTGGCAACCAGAGA AACCAGCTCAACCACACGCGGATCATGTCTGCCCCCGCAA AAGACCTCCCTGATGATGACTCTCTGTCTGGGAAGCATCTA CGAAGATCTGAAGATGTATCAGACCGAATTCCAGGCCATC AACGCGGCGCTGCAGAACCACAATCATCAGCAGATTATCC TGGATAAGGGCATGCTGGTTCGCCATCGACGAGCTGATGCA GTCCTTGAATCACAACGGCGAAACGCTCCGCCAAAAGCCG CCAGTGGGCGAAGCGGACCCTTATCGCGTGAAGATGAAGC TCTGCATCCTGCTGCATGCCTTTTCGACCCGGGTGGTCACC ATCAACAGAGTGATGGGATACCTGAGCTCAGCTGGCGGAG GGGGCGAAGGCGGTGGCGGTTCGAGGAGGGCGGAGGAG AAGGGGGAGGGGGTTTCAGAGGAGGGAGGTGGAGAGGGTG GCGGCCGCGAGCGAAGAAGGCGGAGGCGAAGGAGGTGGCG GCAGCGAGGAAGGAGGAGGGGGCCACCATCACCACCACC AT
67	mL12- ABP20-G4- 6x-GE	ATGTGGGAACCTCGAAAAGGATGTGTACGTGGTGGAAAGTCG ACTGGACTCCTGATGCCCCGGGAGAGACAGTGAACCTGAC CTGTGACACTCCCGAAGAGGACGACATTACTTGGACCAGC GATCAGCGGCACGGCGTGATCGGATCCGGCAAAACCTGA CCATCACCGTGAAAGAATTCTTGACGCGGGACAGTACAC TTGCCACAAGGGCGGCGAAACCCTCAGCCATTCCCACCTTT TGCTCCACAAGAAGGAAAACGGAATCTGGTCCACCAGAAAT TCTGAAGAACTTCAAGAACAAGACCTTCTGAAAGTGCGAG GCGCCCAACTACTCGGGAAGGTTACATGCTCCTGGCTGGT GCAGAGGAACATGGATCTGAAGTTCAACATCAAGAGCTCC

		TCCTCCTCCCCGGATTCAAGAGCCGTGACTTGCGGGATGGC ATCCCTGAGCGCCGAGAAAGTCACTCTGGACCAGAGAGAC TACGAGAAGTACTCCGTGTCGTGCCAGGAGGACGTGACCT GTCCTACCGCCGAAGAGACTCTGCCCATTGAGCTGGCTCTG GAAGCCCGCCAGCAGAACAATAACGAAAATACTCCACGT CATTCTTTATCCGGGACATCATCAAGCCCGACCCCTCCTAAG AACCTCCAGATGAAGCCCCTCAAGAACAGCCAGGTGAGG TGTCTTGGGAATACCCGGACTCCTGGAGCACCCACACTCC TACTTCTCGCTGAAGTTTTTCGTGCGGATCCAGCGGAAGAA AGAGAAGATGAAAGAGACTGAAGAGGGGTGCAACCAGAA GGGCGCTTTCCTGGTGGAAAAGACCTCGACCGAAGTGCAG TGCAAAGGGGGCAACGTCTGCGTGCAAGCCCAAGACCGCT ACTACAACCTCCTCCTGCTCCAAATGGGCATGCGTGCCGTGT AGAGTGCGGTCCGGTGGTGGAGGATCCGGAGGGGGGTGGAT CAGGGGGAGGCGGATCTAGGGTCAATTCCGGTGTCCGGTCC TGCTCGCTGCCTGAGTCAGTCCCGGAATCTGCTTAAGACTA CTGACGATATGGTCAAGACTGCCCCGCGAGAAGCTGAAGCA CTACTCCTGCACCGCCGAGGACATTGACCACGAAGATATC ACCCGGGACCAAAACCAGCACCCCTCAAGACTTGCTGCCAC TTGAACTGCACAAGAACGAGAGCTGCCTGGCAACCAGAGA AACCAGCTCAACCACACGCGGATCATGTCTGCCCCCGCAA AAGACCTCCCTGATGATGACTCTCTGTCTGGGAAGCATCTA CGAAGATCTGAAGATGTATCAGACCGAATTCCAGGCCATC AACGCGGCGCTGCAGAACCACAATCATCAGCAGATTATCC TGGATAAGGGCATGCTGGTGCCTATCGACGAGCTGATGCA GTCCTTGAATCACAACGGCGAAACGCTCCGCCAAAAGCCG CCAGTGGGCGAAGCGGACCCTTATCGCGTGAAGATGAAGC TCTGCATCCTGCTGCATGCCTTTTCGACCCGGGTGGTCACC ATCAACAGAGTGATGGGATACCTGAGCTCAGCTGGAGGCG GTGGAGAGGGCGGTGGCGGATCGGAAGAAGGAGGTGGCG GATCCGAGGAAGGGGGAGGAGGCTCTGAGGAGGGTGGTG GCGGAAGCGAAGAGGGAGGCGGAGGATCCGAAGAGGGTG GCGGGGGATCAGAGGAGGGAGGGGGAGGACATCACCACC ACCATCAT
68	mL12- ABP20-G4- 8x-GE	ATGTGGGAACCTCGAAAAGGATGTGTACGTGGTGGAAAGTCG ACTGGACTCCTGATGCCCCGGGAGAGACAGTGAACCTGAC CTGTGACACTCCCGAAGAGGACGACATTACTTGGACCAGC GATCAGCGGCACGGCGTGATCGGATCCGGCAAAACCCCTGA CCATCACCGTGAAAGAATTCTTGACGCGGGACAGTACAC TTGCCACAAGGGCGGCGAAACCCCTCAGCCATTCACCTTT TGCTCCACAAGAAGGAAAACGGAATCTGGTCCACCAGAAAT TCTGAAGAACTTCAAGAACAAGACCTTCTGAAAGTGCGAG GCGCCCAACTACTCGGGAAGGTTACATGCTCCTGGCTGGT GCAGAGGAACATGGATCTGAAGTTCAACATCAAGAGCTCC TCCTCCTCCCCGGATTCAAGAGCCGTGACTTGCGGGATGGC ATCCCTGAGCGCCGAGAAAGTCACTCTGGACCAGAGAGAC

		TACGAGAAGTACTCCGTGTCGTGCCAGGAGGACGTGACCT GTCCTACCGCCGAAGAGACTCTGCCATTGAGCTGGCTCTG GAAGCCCGCCAGCAGAACAAATACGAAACTACTCCACGT CATTCTTTATCCGGGACATCATCAAGCCCGACCCTCCTAAG AACCTCCAGATGAAGCCCCTCAAGAACAGCCAGGTCGAGG TGTCTTGGGAATACCCGGACTCCTGGAGCACCCACACTCC TACTTCTCGCTGAAGTTTTTCGTGCGGATCCAGCGGAAGAA AGAGAAGATGAAAGAGACTGAAGAGGGGTGCAACCAGAA GGGCGCTTTCCTGGTGGAAAAGACCTCGACCGAAGTGCAG TGCAAAGGGGGCAACGTCTGCGTGCAAGCCCAAGACCGCT ACTACAACTCCTCCTGCTCCAAATGGGCATGCGTGCCGTGT AGAGTGCGGTCCGGTGGTGGAGGATCCGGAGGGGGTGGAT CAGGGGGAGGCGGATCTAGGGTCAATCCGGTGTCCGGTCC TGCTCGCTGCCTGAGTCAGTCCCGGAATCTGCTTAAGACTA CTGACGATATGGTCAAGACTGCCCCGCGAGAAGCTGAAGCA CTACTCCTGCACCGCCGAGGACATTGACCACGAAGATATC ACCCGGGACCAAACCAGCACCTCAAGACTTGCTGCCAC TTGAACTGCACAAGAACGAGAGCTGCCTGGCAACCAGAGA AACCAGCTCAACCACACGCGGATCATGTCTGCCCCCGCAA AAGACCTCCCTGATGATGACTCTCTGTCTGGGAAGCATCTA CGAAGATCTGAAGATGTATCAGACCGAATTCCAGGCCATC AACGCGGCGCTGCAGAACCACAATCATCAGCAGATTATCC TGGATAAGGGCATGCTGGTCCGATCGACGAGCTGATGCA GTCCTTGAATCACAACGGCGAAACGCTCCGCCAAAAGCCG CCAGTGGGCGAAGCGGACCCTTATCGCGTGAAGATGAAGC TCTGCATCCTGCTGCATGCCTTTTCGACCCGGGTGGTCACC ATCAACAGAGTGATGGGATACCTGAGCTCAGCTGGAGGCG GTGGAGAGGGTGGAGGCGGTTCGGAAGAAGGGGGCGGAG GTTACAGAGGAAGGAGGTGGCGGATCCGAGGAAGGGGGAG GAGGCTCTGAGGAGGGTGGTGGCGGAAGCGAAGAGGGAG GCGGAGGATCCGAAGAGGGTGGCGGGGGATCAGAGGAGG GAGGGGGAGGATCAGAAGAGGGCGGTGGTGGACACCACC ACCATCATCAC
69	hIL12-His (no ABP)	ATCTGGGAGCTGAAGAAGGACGTCTATGTGGTGGAACTTG ACTGGTACCCGGATGCCCTGGAGAAATGGTCTGTGCTGAC GTGCGACACCCCGAAGAGGACGGCATTACTTGGACCCTG GACCAGTCCTCCGAAGTGCTCGGGAGCGGAAAGACCCTGA CCATCCAAGTCAAGGAATTCCGGCGATGCCGGACAGTACAC CTGTCAACAAGGGCGGAGAAGTGCTGAGCCACTCCCTGCTG CTGCTGCACAAGAAGGAAGATGGCATCTGGTCCACCGACA TCCTGAAAGACCAGAAGGAGCCGAAGAACAAGACTTTCT CCGCTGCGAAGCCAAGAACTACTCCGGCCGGTTTACTTGTT GGTGGCTGACCACCATATCAACGGATCTCACCTTCTCCGTG AAATCGTCCCGGGGAAGCTCGGACCCCCAAGGAGTGA GCTT GCGGGGCAGCGACCTTGTCCGCCGAACGCGTCAGAGGGGA CAACAAGGAATACGAGTACTCCGTGGAGTGCCAAGAGGAC

		TCCGCATGTCCGGCTGCTGAAGAGTCGCTGCCGATTGAGGT CATGGTGGACGCCGTGCACAAGCTGAAATACGAGAATTAC ACCTCCTCCTTCTTCATCCGGGACATCATTAAAGCCCGACCC TCCTAAGAACCTCCAGCTGAAGCCTCTGAAGAACTCCCGCC AAGTGGAAGTGTCTGGGAGTACCCCGACACTTGGTCCAC CCCCATTCTGATTTTCTCACTGACTTTCTGCGTCCAAGTGCA GGGGAAATCCAAGAGAGAGAAGAAAGACCGGGTGTTCAC TGATAAGACTAGCGCGACCGTGATCTGTAGAAAGAACGCG TCTATTTCCGTGCGCGCCCAAGATCGCTACTACTCCTCCTC GTGGTCCGAATGGGCCTCAGTGCCATGTAGCGGTGGTGGC GGATCCGGTGGAGGCGGATCGGGTGGAGGGGGATCCCGGA ACCTCCCGGTGGCCACTCCTGACCCTGGAATGTTCCCGTGC CTGCACCATAGCCAGAATCTGCTGAGGGCCCGTGTGAATA TGTTACAGAAAGCTCGGCAGACCCTCGAGTTTTACCCCTGC ACCTCGGAAGAGATCGATCACGAGGACATCACTAAGGACA AGACCTCCACAGTGGAAGCGTGCTGCCACTCGAACTGAC CAAGAACGAATCCTGCCTGAACTCCCGGGAAACCTCGTTC ATCACTAACGGCTCTTGCTTGGCGTCGCGCAAGACCTCATT CATGATGGCCCTTTGCCTCTCATCCATCTACGAAGATCTGA AAATGTACCAGGTCGAGTTCAAGACCATGAACGCTAAGTT GCTGATGGATCCAAAGCGCCAGATTTTCTGGACCAGAAC ATGCTGGCCGTGATCGACGAGCTCATGCAGGCCTTGAAGTT TAACAGCGAAACCGTGCCGCAGAAGAGTAGCCTCGAGGAA CCCGACTTCTACAAGACTAAGATTAAGCTGTGCATTCTCCT GCACGCCTTCCGGATTAGGGCCGTCACCATCGACCGCGTCA TGTCATACCTGAACGCATCCGGTGGCGGTGGAAGCCACCA TCACCACCACCAT
70	hIL12- ABP10-His	ATCTGGGAGCTGAAGAAGGACGTCTATGTGGTGGAACTTG ACTGGTACCCGGATGCCCCCTGGAGAAATGGTCGTGCTGAC GTGCGACACCCCCGAAGAGGACGGCATTACTTGGACCCTG GACCAGTCCTCCGAAGTGCTCGGGAGCGGAAAGACCCTGA CCATCCAAGTCAAGGAATTCCGGCGATGCCGGACAGTACAC CTGTCACAAGGGCGGAGAAGTGCTGAGCCACTCCCTGCTG CTGCTGCACAAGAAGGAAGATGGCATCTGGTCCACCGACA TCCTGAAAGACCAGAAGGAGCCGAAGAACAAGACTTTCTCT CCGCTGCGAAGCCAAGAAGTACTCCGGCCGGTTTACTTGTT GGTGGCTGACCACCATATCAACGGATCTCACCTTCTCCGTG AAATCGTCCCGGGGAAGCTCGGACCCCCAAGGAGTGACTT GCGGGGCAGCGACCTTGTCCGCCGAACGCGTCAGAGGGGA CAACAAGGAATACGAGTACTCCGTGGAGTGCCAAGAGGAC TCCGCATGTCCGGCTGCTGAAGAGTCGCTGCCGATTGAGGT CATGGTGGACGCCGTGCACAAGCTGAAATACGAGAATTAC ACCTCCTCCTTCTTCATCCGGGACATCATTAAAGCCCGACCC TCCTAAGAACCTCCAGCTGAAGCCTCTGAAGAACTCCCGCC AAGTGGAAGTGTCTGGGAGTACCCCGACACTTGGTCCAC CCCCATTCTGATTTTCTCACTGACTTTCTGCGTCCAAGTGCA

		GGGGAATCCAAGAGAGAGAAGAAAGACCGGGTGTTCAC TGATAAGACTAGCGCGACCGTGATCTGTAGAAAGAACGCG TCTATTTCCGTGCGCGCCCAAGATCGCTACTACTCCTCCTC GTGGTCCGAATGGGCCTCAGTGCCATGTAGCGGTGGTGGC GGATCCGGTGGAGGCGGATCGGGTGGAGGGGGATCCCGGA ACCTCCCGGTGGCCACTCCTGACCCTGGAATGTTCCCGTGC CTGCACCATAGCCAGAATCTGCTGAGGGCCGTGTCAATA TGTTACAGAAAGCTCGGCAGACCCTCGAGTTTTACCCCTGC ACCTCGGAAGAGATCGATCACGAGGACATCACTAAGGACA AGACCTCCACAGTGGAAGCGTGCTGCCACTCGAACTGAC CAAGAACGAATCCTGCCTGAACTCCCGGGAAACCTCGTTC ATCACTAACGGCTCTTGCTTGGCGTCGCGCAAGACCTCATT CATGATGGCCCTTTGCCTCTCATCCATCTACGAAGATCTGA AAATGTACCAGGTCGAGTTCAAGACCATGAACGCTAAGTT GCTGATGGATCCAAAGCGCCAGATTTTCTGGACCAGAAC ATGCTGGCCGTGATCGACGAGCTCATGCAGGCCTTGAACCT TAACAGCGAAACCGTGCCGCAGAAGAGTAGCCTCGAGGAA CCCGACTTCTACAAGACTAAGATTAAGCTGTGCATTCTCCT GCACGCCTTCCGGATTAGGGCCGTCACCATCGACCGCGTCA TGTCATACCTGAACGCATCCGGGGGTGGAGGAAGCTTCCA ATCGGAGGAGCAGCAGGGAGGAGGATCGGGCGGATCCGA GGAAGGCGGAATGGAGAGCGAAGAAAGCAACGGCGGTGG TTCCGGAGGATCCGAAGAGGGTGGCGGTGGAAGCCACCAT CACCACCACCAT
71	hIL12- ABP20-His	ATCTGGGAGCTGAAGAAGGACGTCTATGTGGTGGAACTTG ACTGGTACCCGGATGCCCCCTGGAGAAATGGTCGTGCTGAC GTGCGACACCCCCGAAGAGGACGGCATTACTTGGACCCTG GACCAGTCCTCCGAAGTGCTCGGGAGCGGAAAGACCCTGA CCATCCAAGTCAAGGAATTTCGGCGATGCCGGACAGTACAC CTGTCACAAGGGCGGAGAAGTGCTGAGCCACTCCCTGCTG CTGCTGCACAAGAAGGAAGATGGCATCTGGTCCACCGACA TCCTGAAAGACCAGAAGGAGCCGAAGAACAAGACTTTCTCCT CCGCTGCGAAGCCAAGAATACTCCGGCCGGTTTACTTGTT GGTGGCTGACCACCATATCAACGGATCTCACCTTCTCCGTG AAATCGTCCCGGGGAAGCTCGGACCCCCAAGGAGTGACTT GCGGGGCAGCGACCTTGTCCGCCGAACGCGTCAGAGGGGA CAACAAGGAATACGAGTACTCCGTGGAGTGCCAAGAGGAC TCCGCATGTCCGGCTGCTGAAGAGTCGCTGCCGATTGAGGT CATGGTGGACGCCGTGCACAAGCTGAAATACGAGAATTAC ACCTCCTCCTTCTTCATCCGGGACATCATTAAAGCCCGACCC TCCTAAGAACCTCCAGCTGAAGCCTCTGAAGAACTCCCGCC AAGTGGAAGTGTCCTGGGAGTACCCCGACACTTGGTCCAC CCCCATTCTGATTTCTCACTGACTTTCTGCGTCCAAGTGCA GGGGAATCCAAGAGAGAGAAGAAAGACCGGGTGTTCAC TGATAAGACTAGCGCGACCGTGATCTGTAGAAAGAACGCG TCTATTTCCGTGCGCGCCCAAGATCGCTACTACTCCTCCTC

		GTGGTCCGAATGGGCCTCAGTGCCATGTAGCGGTGGTGGC GGATCCGGTGGAGGCGGATCGGGTGGAGGGGGATCCCGGA ACCTCCCGGTGGCCACTCCTGACCCTGGAATGTTCCCGTGC CTGCACCATAGCCAGAATCTGCTGAGGGCCGTGTCAATA TGTTACAGAAAGCTCGGCAGACCCTCGAGTTTTACCCCTGC ACCTCGGAAGAGATCGATCACGAGGACATCTACTAAGGACA AGACCTCCACAGTGGAAGCGTGCCTGCCACTCGAACTGAC CAAGAACGAATCCTGCCTGAACTCCCGGGAAACCTCGTTC ATCTAATACGGCTCTTGCTTGGCGTCGCGCAAGACCTCATT CATGATGGCCCTTTGCCTCTCATCCATCTACGAAGATCTGA AAATGTACCAGGTCGAGTTCAAGACCATGAACGCTAAGTT GCTGATGGATCCAAAGCGCCAGATTTTCTGGAACAGAAC ATGCTGGCCGTGATCGACGAGCTCATGCAGGCCTTGAACCT TAACAGCGAAACCGTGCCGCAGAAGAGTAGCCTCGAGGAA CCCGACTTCTACAAGACTAAGATTAAGCTGTGCATTCTCCT GCACGCCTTCCGGATTAGGGCCGTCACCATCGACCGCGTCA TGTCATACTGAACGCATCCGGAGGCGGAGGGTCTGGGGGG CGGGGGATCCGAAGAGTCCGAAGAATCCGAGGAATCCGAA GAAGGTGGCGGTGGAAGCCACCATCACCACCACCAT
72	hIL12- ΔBP20-G4- His	ATCTGGGAGCTGAAGAAGGACGTCTATGTGGTGGAACTTG ACTGGTACCCGGATGCCCCCTGGAGAAATGGTCGTGCTGAC GTGCGACACCCCCGAAGAGGACGGCATTACTTGGACCCTG GACCAGTCCTCCGAAGTGCTCGGGAGCGGAAAGACCCTGA CCATCCAAGTCAAGGAATTTCGGCGATGCCGGACAGTACAC CTGTCACAAGGGCGGAGAAGTGCTGAGCCACTCCCTGCTG CTGCTGCACAAGAAGGAAGATGGCATCTGGTCCACCGACA TCCTGAAAGACCAGAAGGAGCCGAAGAACAAGACTTTCTCCT CCGCTGCGAAGCCAAGAATACTCCGGCCGGTTTACTTGTT GGTGGCTGACCACCATATCAACGGATCTCACCTTCTCCGTG AAATCGTCCCGGGGAAGCTCGGACCCCCAAGGAGTGACTT GCGGGGCAGCGACCTTGTCCGCCGAACGCGTCAGAGGGGA CAACAAGGAATACGAGTACTCCGTGGAGTGCCAAGAGGAC TCCGCATGTCCGGCTGCTGAAGAGTCGCTGCCGATTGAGGT CATGGTGGACGCCGTGCACAAGCTGAAATACGAGAATTAC ACCTCCTCCTTCTTCATCCGGGACATCATTAAAGCCGACCC TCCTAAGAACCTCCAGCTGAAGCCTCTGAAGAACTCCCGCC AAGTGGAAGTGTCCTGGGAGTACCCCGACACTTGGTCCAC CCCCATTCTGATTTCTCACTGACTTTCTGCGTCCAAGTGCA GGGGAAATCCAAGAGAGAGAAGAAAGACCGGGTGTTCAC TGATAAGACTAGCGCGACCGTGATCTGTAGAAAGAACGCG TCTATTTCCGTGCGCGCCCAAGATCGCTACTACTCCTCCTC GTGGTCCGAATGGGCCTCAGTGCCATGTAGCGGTGGTGGC GGATCCGGTGGAGGCGGATCGGGTGGAGGGGGATCCCGGA ACCTCCCGGTGGCCACTCCTGACCCTGGAATGTTCCCGTGC CTGCACCATAGCCAGAATCTGCTGAGGGCCGTGTCAATA TGTTACAGAAAGCTCGGCAGACCCTCGAGTTTTACCCCTGC

		ACCTCGGAAGAGATCGATCACGAGGACATCACTAAGGACA AGACCTCCACAGTGGAAGCGTGCCTGCCACTCGAACTGAC CAAGAACGAATCCTGCCTGAACTCCCGGGAAACCTCGTTC ATCACTAACGGCTCTTGCTTGGCGTCGCGCAAGACCTCATT CATGATGGCCCTTTGCCTCTCATCCATCTACGAAGATCTGA AAATGTACCAGGTCGAGTTCAAGACCATGAACGCTAAGTT GCTGATGGATCCAAAGCGCCAGATTTTCTGGAACAGAAC ATGCTGGCCGTGATCGACGAGCTCATGCAGGCCTTGAACCT TAACAGCGAAACCGTGCCGCAGAAGAGTAGCCTCGAGGAA CCCGACTTCTACAAGACTAAGATTAAGCTGTGCATTCTCCT GCACGCCTTCCGGATTAGGGCCGTCACCATCGACCGCGTCA TGTCATACCTGAACGCATCCGGGGGAGGAGGATCGGGAGG TGGTGGCAGCGAAGAGGGCGGAGGGGGTTTCAGAGGAAGG CGGTGGAGGGTCCGAAGAGGGTGGTGGTGGGTCCGAGGAG GGTGGCGGTGGAAGCCACCATCACCACCACCAT
73	hIL12- ABP20-G4- His	ATCTGGGAGCTGAAGAAGGACGTCTATGTGGTGGAACTTG ACTGGTACCCGGATGCCCCCTGGAGAAATGGTCGTGCTGAC GTGCGACACCCCCGAAGAGGACGGCATTACTTGGACCCTG GACCAGTCCTCCGAAGTGCTCGGGAGCGGAAAGACCCTGA CCATCCAAGTCAAGGAATTCCGGCGATGCCGGACAGTACAC CTGTCAACAAGGGCGGAGAAAGTGCTGAGCCACTCCCTGCTG CTGCTGCACAAGAAGGAAGATGGCATCTGGTCCACCGACA TCCTGAAAGACCAGAAGGAGCCGAAGAACAAGACTTTCTCT CCGCTGCGAAGCCAAGAATACTCCGGCCGGTTTACTTGTT GGTGGCTGACCACCATATCAACGGATCTCACCTTCTCCGTG AAATCGTCCCGGGGAAGCTCGGACCCCCAAGGAGTGACTT GCGGGGCAGCGACCTTGTCCGCCGAACGCGTCAGAGGGGA CAACAAGGAATACGAGTACTCCGTGGAGTGCCAAGAGGAC TCCGCATGTCCGGCTGCTGAAGAGTCGCTGCCGATTGAGGT CATGGTGGACGCCGTGCACAAGCTGAAATACGAGAATTAC ACCTCCTCCTTCTTCATCCGGGACATCATTAAAGCCCGACCC TCCTAAGAACCTCCAGCTGAAGCCTCTGAAGAATACTCCGCC AAGTGGAAGTGTCTGGGAGTACCCCGACACTTGGTCCAC CCCCATTCTGATTTCTCACTGACTTTCTGCGTCCAAGTGCA GGGGAAATCCAAGAGAGAGAAGAAAGACCGGGTGTTAC TGATAAGACTAGCGCGACCGTGATCTGTAGAAAGAACGCG TCTATTTCCGTGCGCGCCCAAGATCGCTACTACTCCTCCTC GTGGTCCGAATGGGCCTCAGTGCCATGTAGCGGTGGTGGC GGATCCGGTGGAGGCGGATCGGGTGGAGGGGGATCCCGGA ACCTCCCGGTGGCCACTCCTGACCCTGGAATGTTCCCGTGC CTGCACCATAGCCAGAATCTGCTGAGGGCCGTGTCGAATA TGTTACAGAAAGCTCGGCAGACCCTCGAGTTTACCCCTGC ACCTCGGAAGAGATCGATCACGAGGACATCACTAAGGACA AGACCTCCACAGTGGAAGCGTGCCTGCCACTCGAACTGAC CAAGAACGAATCCTGCCTGAACTCCCGGGAAACCTCGTTC ATCACTAACGGCTCTTGCTTGGCGTCGCGCAAGACCTCATT

		CATGATGGCCCTTTGCCTCTCATCCATCTACGAAGATCTGA AAATGTACCAGGTTCGAGTTCAAGACCATGAACGCTAAGTT GCTGATGGATCCAAAGCGCCAGATTTTCTGGACCAGAAC ATGCTGGCCGTGATCGACGAGCTCATGCAGGCCTTGAACCT TAACAGCGAAACCGTGCCGCAGAAGAGTAGCCTCGAGGAA CCCGACTTCTACAAGACTAAGATTAAGCTGTGCATTCTCCT GCACGCCTTCCGGATTAGGGCCGTCACCATCGACCGCGTCA TGTCATACTGAACGCATCCGGGGGAGGAGGATCGGGAGG TGGTGGCAGCGAAGAGGGCGGAGGGGGTTCAGAGGAAGG CGGTGGAGGGTCCGAAGAGGGTGGTGGTGGGTCCGAGGAG GGTGGCGGTGGAAGCCACCATCACCACCACCAT
74	hIL12- ABP20-G4- 8x-GE (mature polypeptide)	ATCTGGGAACTGAAGAAGGACGTCTACGTGGTGGAACTGG ATTGGTACCCAGACGCCCCCTGGAGAAATGGTGGTGTCTAC CTGTGATACCCCCGAAGAGGACGGGATTACTTGGACCCTG GATCAGTCATCCGAAGTGCTGGGATCCGGAAAGACCCTGA CGATCCAGGTCAAGGAATTCGGGGACGCGGGACAGTACAC TTGCCATAAGGGCGGCGAAGTGCTGTCCACTCCTTGCTCC TGCTCCACAAGAAAGAAGATGGAATTTGGAGCACCGACAT CCTGAAGGACCAGAAAGAGCCGAAGAACAAGACCTTCCTG CGCTGCGAGGCCAAGAAGTACAGCGGACGCTTTACCTGTT GGTGGCTGACCACTATCTCCACCGACCTGACCTTCTCGGTC AAGTCCTCGCGCGGGAGCAGCGATCCTCAGGGAGTGACTT GCGGTGCCGCGACTCTGTGCGCCGAAAGGGTCCGGGGCGA CAACAAGGAGTACGAGTACTCAGTCGAGTGCCAGGAGGAC AGCGCATGTCCGGCCGCTGAGGAATCCCTGCCTATTGAAGT CATGGTGGACGCCGTCCACAAGCTGAAATACGAGAACTAC ACCTCCTCTTTCTTTATCCGGGATATCATCAAGCCCGACCC GCCTAAGAACCTTCAACTGAAGCCACTGAAGAACAGCAGA CAAGTGGAGGTGTCCTGGGAGTACCCGGATACTTGGTCCA CTCCGCACTCCTATTTCTCGCTCACCTTCTGCGTCCAAGTGC AGGGAAAGTCGAAACGGGAGAAGAAGGACAGAGTGTTCA CTGACAAGACCAGCGCAACTGTGATCTGTGCGGAAGAACGC CTCCATTTCCGTGCGCGCCAGGACCGGTACTATTCTCAT CCTGCTCGGAGTGGGCCTCAGTGCCCTGCAGCGGCGGGGG CGGTTCCGGTGGTGGCGGCAGCGGGGGCGGAGGGTCCCGG AATCTGCCGGTGGCGACGCCCCGACCCCGGAATGTTCCCGT GCCTGCACCATAGCCAGAACTTGCTCCGCGCGGTGTCTAAC ATGCTGCAGAAGGCCCGGCAGACCCTGGAATTCTACCCCT GCACTAGTGAAGAGATCGACCATGAAGATATTACCAAAGA CAAGACTTCCACCGTGGAAGCGTGCCTCCCCTGGAAGTGC ACCAAGAATGAGTCCTGCCTGAATTCAAGGGAACTTCGT TCATTACCAACGGTTCTGCTGCTGCTGCTGCTGCTGCTGCT TTCATGATGGCTCTCTGCTCTCCTCAATCTACGAAGATCT CAAAATGTACCAAGTGGAGTTCAAGACCATGAACGCTAAG CTGCTGATGGACCCGAAGCGGCAGATTTTCTCGACCAAA ACATGTTGGCCGTGATCGACGAGTTGATGCAGGCCCTTAAC

		TTCAACTCCGAAACAGTGCCTCAGAAGTCGAGCCTCGAGG AACCCGACTTCTACAAGACTAAGATCAAGCTGTGCATCCTG CTGCACGCATTTTGAATTTCGGGCCGTGACCATCGACCGCGT GATGAGCTACCTGAACGCATCCGGGGGCGGCGGGGAAGGA GGAGGCGGCTCAGAGGAGGGAGGAGGAGGCAGCGAGGAA GGAGGCGGAGGAAGCGAAGAAGGAGGGGGAGGCTCAGAA GAGGGAGGGGGTGGATCCGAGGAAGGCGGAGGAGGATCC GAAGAGGGCGGTGGTGGATCGGAGGAAGGGGGAGGCGGT TCTGAGGAGGGTGGAGGAGGCTCC
75	Signal polypeptide	ATGTATAGAATGCAGCTTCTGTCTGTATCGCCCTCTCCCT CGCCCTTGTGACCAACTCC
76	hIL12- ABP20-G4- 8x-GE w/ signal polypeptide	ATGTATAGAATGCAGCTTCTGTCTGTATCGCCCTCTCCCT CGCCCTTGTGACCAACTCCATCTGGGAAGTGAAGAAGGAC GTCTACGTGGTGGAACTGGATTGGTACCCAGACGCCCTG GAGAAATGGTGGTGCTCACCTGTGATACCCCGAAGAGGA CGGGATTACTTGGACCCTGGATCAGTCATCCGAAGTGCTGG GATCCGGAAGACCCTGACGATCCAGGTCAAGGAATTCGG GGACGCGGGACAGTACACTTGCCATAAGGGCGGCGAAGTG CTGTCCCCTCCTTGCTCCTGCTCCACAAGAAAGAAGATGG AATTTGGAGCACCGACATCCTGAAGGACCAGAAAGAGCCG AAGAACAAGACCTTCCTGCGCTGCGAGGGCCAAGAAGTACA GCGGACGCTTTACCTGTTGGTGGCTGACCACTATCTCCACC GACCTGACCTTCTCGGTCAAGTCCTCGCGCGGGAGCAGCG ATCCTCAGGGAGTGACTTGCGGTGCCGCGACTCTGTGCGGCC GAAAGGGTCCGGGGCGACAACAAGGAGTACGAGTACTCA GTCGAGTGCCAGGAGGACAGCGCATGTCCGGCCGCTGAGG AATCCCTGCCTATTGAAGTCATGGTGGACGCCGTCCACAAG CTGAAATACGAGAACTACACCTCCTCTTTCTTTATCCGGGA TATCATCAAGCCCGACCCGCCTAAGAACCTTCAACTGAAG CCACTGAAGAACAGCAGACAAGTGGAGGTGTCTTGGGAGT ACCCGGATACTTGGTCCACTCCGCACTCCTATTTCTCGCTC ACCTTCTGCGTCCAAGTGCAGGGAAAGTCGAAACGGGAGA AGAAGGACAGAGTGTTCACTGACAAGACCAGCGCAACTGT GATCTGTGGAAGAACGCCTCCATTTCCGTGCGCGCCCAGG ACCGGTACTATTCTCATCCTGGTCGGAGTGGGCCTCAGTG CCCTGCAGCGGCGGGGGCGGTTCCGGGTGGTGGCGGCAGCG GGGGCGGAGGGTCCCGGAATCTGCCGGTGGCGACGCCCGA CCCCGGAATGTTCCCGTGCCTGCACCATAGCCAGAAGTTCG TCCGCGCGGTGTCTAACATGCTGCAGAAGGCCCGGCAGAC CCTGGAATTCTACCCCTGCACTAGTGAAGAGATCGACCATG AAGATATTACCAAAGACAAGACTTCCACCGTGGAAAGCGTG CCTCCCCTGGAAGTACCAAGAATGAGTCCTGCCTGAATT CAAGGGAACTTCGTTTATTACCAACGGTTCCTGCCTGGCC TCGAGAAAGACCTCCTTCATGATGGCTCTCTGCCTCTCCTC AATCTACGAAGATCTCAAAATGTACCAAGTGGAGTTCAAG

		ACCATGAACGCTAAGCTGCTGATGGACCCGAAGCGGCAGA TTTTCCTCGACCAAAACATGTTGGCCGTGATCGACGAGTTG ATGCAGGCCCTTAACCTCAACTCCGAAACAGTGCCTCAGA AGTCGAGCCTCGAGGAACCCGACTTCTACAAGACTAAGAT CAAGCTGTGCATCCTGCTGCACGCATTTCTGAATTCGGGCCG TGACCATCGACCGCGTGATGAGCTACCTGAACGCATCCGG GGGCGGCGGGGAAGGAGGAGGCGGCTCAGAGGAGGGAGG AGGAGGCAGCGAGGAAGGAGGCGGAGGAAGCGAAGAAGG AGGGGGAGGCTCAGAAGAGGGAGGGGGTGGATCCGAGGA AGGCGGAGGAGGATCCGAAGAGGGCGGTGGTGGATCGGA GGAAGGGGGAGGCGGTTCTGAGGAGGGTGGAGGAGGCTC C
77	hIL12- ABP10 (mature polypeptide)	ATCTGGGAACCTGAAGAAGGACGTCTACGTGGTGGAACTGG ATTGGTACCCAGACGCCCCCTGGAGAAATGGTGGTGCTCAC CTGTGATACCCCCGAAGAGGACGGGATTACTTGGACCCTG GATCAGTCATCCGAAGTGCTGGGATCCGGAAAGACCCTGA CGATCCAGGTCAAGGAATTCGGGGACGCGGGACAGTACAC TTGCCATAAGGGCGGCGAAGTGCTGTCCACTCCTTGCTCC TGCTCCACAAGAAAGAAGATGGAATTTGGAGCACCGACAT CCTGAAGGACCAGAAAGAGCCGAAGAACAAGACCTTCCTG CGCTGCGAGGCCAAGAAGTACAGCGGACGCTTTACCTGTT GGTGGCTGACCACTATCTCCACCGACCTGACCTTCTCGGTC AAGTCCTCGCGCGGGAGCAGCGATCCTCAGGGAGTGACTT GCGGTGCCGCGACTCTGTGCGCCGAAAGGGTCCGGGGCGA CAACAAGGAGTACGAGTACTCAGTCGAGTGCCAGGAGGAC AGCGCATGTCCGGCCGCTGAGGAATCCCTGCCTATTGAAGT CATGGTGGACGCCGTCCACAAGCTGAAATACGAGAACTAC ACCTCCTCTTTCTTTATCCGGGATATCATCAAGCCCGACCC GCCTAAGAACCTTCAACTGAAGCCACTGAAGAACAGCAGA CAAGTGGAGGTGTCCTGGGAGTACCCGGATACTTGGTCCA CTCCGCACTCCTATTTCTCGCTCACCTTCTGCGTCCAAGTGC AGGGAAAGTCGAAACGGGAGAAGAAGGACAGAGTGTTCA CTGACAAGACCAGCGCAACTGTGATCTGTGCGGAAGAACGC CTCCATTTCCGTGCGCGCCAGGACCGGTACTATTCTCAT CCTGGTCGGAGTGGGCCTCAGTGCCCTGCAGCGGCGGGGG CGGTTCCGGTGGTGGCGGCAGCGGGGGCGGAGGGTCCCGG AATCTGCCGGTGGCGACGCCCCGACCCCGGAATGTTCCCGT GCCTGCACCATAGCCAGAACTTGCTCCGCGCGGTGTCTAAC ATGCTGCAGAAGGCCCGGCAGACCCTGGAATTCTACCCCT GCACTAGTGAAGAGATCGACCATGAAGATATTACCAAAGA CAAGACTTCCACCGTGGAAGCGTGCCTCCCCTGGAAGTGC ACCAAGAATGAGTCCTGCCTGAATTCAAGGGAACTTCGT TCATTACCAACGGTTCTGCTGCCTCGAGAAAGACCTCC TTCATGATGGCTCTCTGCCTCTCCTCAATCTACGAAGATCT CAAAATGTACCAAGTGGAGTTCAAGACCATGAACGCTAAG CTGCTGATGGACCCGAAGCGGCAGATTTTCCTCGACCAAA

		ACATGTTGGCCGTGATCGACGAGTTGATGCAGGCCCTTAAC TTCAACTCCGAAACAGTGCCTCAGAAGTCGAGCCTCGAGG AACCCGACTTCTACAAGACTAAGATCAAGCTGTGCATCCTG CTGCACGCATTTTGAATTTCGGGGCCGTGACCATCGACCGCGT GATGAGCTACCTGAACGCATCCGGGGGCGGCGGGAGCTTC CAGTCCGAGGAACAACAGGGAGGGGGCTCCGGCGGATCA GAGGAGGGAGGCATGGAATCAGAAGAGAGCAACGGCGGC GGTTCCGGAGGATCGGAAGAGGGCGGCGGCGGATCG
78	hIL12- ABP10 w/ signal polypeptide	ATGTATAGAATGCAGCTTCTGTCTGTATCGCCCTCTCCCT CGCCCTTGTGACCAACTCCATCTGGGAAGTGAAGAAGGAC GTCTACGTGGTGGAACTGGATTGGTACCCAGACGCCCTG GAGAAATGGTGGTGCTCACCTGTGATACCCCGAAGAGGA CGGGATTACTTGGACCCTGGATCAGTCATCCGAAGTGCTGG GATCCGGAAGAGACCCTGACGATCCAGGTCAAGGAATTCGG GGACGCGGGACAGTACACTTGCCATAAGGGCGGCGAAGTG CTGTCCCCTCCTTGCTCCTGCTCCACAAGAAAGAAGATGG AATTTGGAGCACCGACATCCTGAAGGACCAGAAAGAGCCG AAGAACAAGACCTTCCTGCGCTGCGAGGCCAAGAAGTACA GCGGACGCTTTACCTGTTGGTGGCTGACCACTATCTCCACC GACCTGACCTTCTCGGTCAAGTCCTCGCGCGGGAGCAGCG ATCCTCAGGGAGTGACTTGCGGTGCCGCGACTCTGTGCGGCC GAAAGGGTCCGGGGCGACAACAAGGAGTACGAGTACTCA GTCGAGTGCCAGGAGGACAGCGCATGTCCGGCCGCTGAGG AATCCCTGCCTATTGAAGTCATGGTGGACGCCGTCCACAAG CTGAAATACGAGAAGTACACCTCCTCTTTCTTTATCCGGGA TATCATCAAGCCCGACCCGCCTAAGAACCTTCAACTGAAG CCACTGAAGAACAGCAGACAAGTGGAGGTGTCTTGGGAGT ACCCGGATACTTGGTCCACTCCGCACTCCTATTTCTCGCTC ACCTTCTGCGTCCAAGTGCAGGGAAAGTCGAAACGGGAGA AGAAGGACAGAGTGTTCACTGACAAGACCAGCGCAACTGT GATCTGTGCGAAGAACGCCTCCATTTCCGTGCGCGCCCAGG ACCGGTACTATTCCTCATCCTGGTCGGAGTGGGCCTCAGTG CCCTGCAGCGGCGGGGGCGGTTCCGGGTGGCGGACGCCCGA CCCCGGAATGTTCCCGTGCTGCACCATAGCCAGAAGTTGC TCCGCGCGGTGTCTAACATGCTGCAGAAGGCCCGGCAGAC CCTGGAATTCTACCCCTGCACTAGTGAAGAGATCGACCATG AAGATATTACCAAAGACAAGACTTCCACCGTGGAAGCGTG CCTCCCCTGGAAGTGAACCAAGAATGAGTCCCTGCCTGAATT CAAGGGAAACTTCGTTTCAATACCAACGGTTCCTGCCTGGCC TCGAGAAAGACCTCCTTCATGATGGCTCTCTGCCTCTCCTC AATCTACGAAGATCTCAAAATGTACCAAGTGGAGTTCAAG ACCATGAACGCTAAGCTGCTGATGGACCCGAAGCGGCAGA TTTTCTCGACCAAAAACATGTTGGCCGTGATCGACGAGTTG ATGCAGGCCCTTAAGTTCAACTCCGAAACAGTGCCTCAGA AGTCGAGCCTCGAGGAACCCGACTTCTACAAGACTAAGAT

		CAAGCTGTGCATCCTGCTGCACGCATTTTGAATTCGGGGCCG TGACCATCGACCGCGTGATGAGCTACCTGAACGCATCCGG GGGCGGCGGGAGCTTCCAGTCCGAGGAACAACAGGGAGG GGGCTCCGGCGGATCAGAGGAGGGAGGCATGGAATCAGA AGAGAGCAACGGCGGCGGTTCCGGAGGATCGGAAGAGGG CGGCGGCGGATCG
118	His-tagged canine IL12- ABP expressed and tested (mature sequence)	ATCTGGGAACCTGGAGAAGGATGTGTACGTGGTTCGAGCTCG ATTGGCATCCTGATGCGCCTGGCGAAATGGTGGTGTGCTGACT TGCCACACTCCCGAGGAGGACGACATTACCTGGACCTCGG CCCAGTCCTCCGAAGTCCTGGGAAGCGGGAAAACCTCTGAC AATTCAGGTCAAAGAGTTCGGCGACGCAGGACAGTACACC TGTCACAAGGGTGGAAAGGTCCTGAGCAGATCGCTGTTGC TGATCCACAAGAAGGAAGATGGCATCTGGTCCACGGACAT CCTGAAGGAGCAGAAGGAATCCAAGAACAAGATCTTTCTT AAGTGCGAAGCCAAGAATACTCGGGCCGGTTCACCTGTT GGTGGCTTACTGCCATATCAACCGACCTGAAGTTTTCCGTG AAATCGAGCCGCGGCTTCTCGGACCCTCAAGGAGTGACTT GCGGAGCCGTGACCCTCTCCGCCGAGAGAGTGCGGGTTCGA CAACCGGGACTACAAGAAGTACACCGTGGAGTGCCAGGAA GGATCCGCCTGTCCAAGCGCCGAGGAAAGCCTGCCGATTG AGGTGGTCGTCGATGCCATCCACAAGCTGAAATACGAGAA TTACACTTCGTCCTTCTTCATCCGGGACATCATCAAGCCCG ATCCGCCAACCACCTTGCAACTCAAGCCGCTGAAGAATTC GCGCCACGTGGAGGTGTCCTGGGAGTACCCCGATACGTGG TCCACCCCGCATAGCTATTTCTCCCTCACTTTCTGCGTGCAA GCCCAGGGAAAGAACAACCGCGAGAAGAAGGACCGGCTG TGCGTGGATAAGACCAGCGCGAAAGTGGTCTGCCACAAGG ACGCGAAGATCAGAGTGCAGGCTCGGGACAGATATTACAG CAGCTCCTGGTCCGACTGGGCCTCCGTGTCCTGTTCCGGCG GAGGAGGATCCGGAGGAGGGGGTTCCGGTGGAGGCGGTTCC GAGGTCCCTGCCTACTGCTAGCCCTTCCCCCGGGATTTTCC AGTGCCTGAACCACTCCCAAGAACTTGCTGAGGGCCGTGTCC AACACCCTTCAAAAGGCTCGGCAGACCCTGGAACCTGTACT CCTGCACTTCCGAGGAAATTGACCACGAAGATATCACCAA GGACAAGACTTCCACCGTGGAGGCCTGTCTGCCCTGGAA CTTACTATGAACGAGTCATGCCTCGCAAGCCGCGAGATTA GCCTGATTACCAACGGCTCCTGCCTGGCTTCGGGGAAGGC GTCGTTTCATGACCGTGCTCTGCCTGTCAAGCATCTACGAGG ACCTGAAAATGTACCAGATGGAATTCAAGGCCATGAACGC CAAGCTCCTGATGGACCCGAAGAGGCAGATCTTCCTGGAC CAAAACATGCTGACTGCCATTGACGAACTGCTTCAGGCACT CAACTTCAACTCTGTGACCGTGCCGAGAAAGTCATCGTTGG AGGAACCAGATTTCTACAAGACCAAGATTAAGCTTTGCATT CTCCTCCATGCGTTTTCGCATCCGCGCCGTGACCATCGACAG GATGATGTCCTACCTGAATTCAAGCGGAGGAGGCGGAGAA GGAGGCGGTGGAAGCGAAGAGGGCGGTGGAGGCAGTGAA

		GAAGGAGGGGGCGGATCTGAAGAAGGGGGCGGTGGATCA GAAGAGGGAGGGGGAGGCTCAGAGGAAGGAGGTGGCGGT TCCGAGGAAGGGGGTGGCGGATCAGAGGAGGGAGGCGGA GGATCGGAGGAAGGCGGAGGTGGACACCACCACCATCATC AC
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Fusion polypeptides

Immunomodulatory polypeptide

[0130] Fusion polypeptides of the present disclosure comprise at least one immunomodulatory polypeptide.

[0131] In some embodiments, an immunomodulatory polypeptide is or comprises at least one immune agonist moiety. In some embodiments, an immune agonist moiety is or comprises a functional fragment of a parent (*e.g.*, a wild type) polypeptide; for example, in some embodiments, an immunomodulatory polypeptide is or comprises a functional fragment that is a signaling competent fragment. In some embodiments, an immunomodulatory polypeptide comprises one, two, three, four, five, or six immune agonist moieties.

[0132] In some embodiments, a fusion polypeptide comprises two or more immunomodulatory polypeptides (*e.g.*, two or more immune agonist moieties). In some such embodiments, a fusion polypeptide comprises two or more immunomodulatory polypeptides that are the same; in some such embodiments, all immunomodulatory polypeptides in a fusion polypeptide in accordance with the present disclosure are the same. In some such embodiments, a fusion polypeptide comprises two or more immunomodulatory polypeptides that are different from one another.

[0133] Thus, in some embodiments, an immunomodulatory polypeptide may include more than one immune agonist moiety which, in various embodiments, may be the same or different. In some such embodiments, two or more such immune agonist moieties are the same; in some embodiments, all such immune agonist moieties are the same. In some embodiments, an immunomodulatory polypeptide includes two or more immune agonist moieties that differ from one another; in some embodiments, no two such immune agonist moieties are the same.

[0134] In some embodiments, a fusion polypeptide comprises two or more immunomodulatory polypeptides (*e.g.*, two or more immune agonist moieties) that include at least two subtypes of immunomodulatory polypeptides (*e.g.*, immune agonist moieties) – for example so that the fusion polypeptide includes at least two of a first subtype and at least two of a second subtype. In some such embodiments, a subset of the same immunomodulatory polypeptides (*e.g.*, immune agonist moieties) equals 1 immunomodulatory polypeptide out of 2 total moieties in the fusion polypeptide, 2 immunomodulatory polypeptides out of 2 total, 1 immunomodulatory polypeptides out of 3, 2 immunomodulatory polypeptides out of 3 total, or 3 immunomodulatory polypeptides out of 3. In some such embodiments, a subset of different immunomodulatory polypeptides equals 1 immune immunomodulatory polypeptides out of 2 total, 2 immunomodulatory polypeptides out of 2 total, 1 immunomodulatory polypeptides out of 3 total, 2 immunomodulatory polypeptides out of 3 total, or 3 immunomodulatory polypeptides out of 3 total.

[0135] In some embodiments, an immunomodulatory polypeptide (*e.g.*, an immune agonist moiety) activates or inhibits activity of a cell of the immune system (*e.g.*, is signaling competent). In some embodiments, an immunomodulatory polypeptide (*e.g.*, an immune agonist moiety) is assessed, for example as part of a fusion polypeptide, *e.g.*, as described herein.

[0136] For example, in some embodiments, signal competency is characterized in that, when assessed for binding to a particular binding partner, an immune agonist moiety or moieties or functional fragments thereof displays binding comparable to that of a reference standard (*e.g.*, a wild-type polypeptide). For example, in some embodiments, signal competency is characterized in that, when assessed for a biological effect, *e.g.*, *in vitro* or *in vivo*, an immune agonist moiety or moieties or functional fragments thereof displays said biological effect comparable to that of a reference standard (*e.g.*, a wild-type polypeptide).

[0137] For example, in some embodiments, an immunomodulatory polypeptide (*e.g.*, an immune agonist moiety) is an immune response stimulatory moiety. In some embodiments, a response stimulatory moiety is, for example, but without limitation, a

cytokine, a chemokine, an agonistic antibody, an immune checkpoint inhibitor, or a combination thereof.

IL-12

[0138] In some embodiments, an immunomodulatory polypeptide comprises an interleukin-12 (IL-12) immunomodulatory polypeptide (*e.g.*, an IL-12 immune agonist moiety).

[0139] IL-12 is a pro-inflammatory cytokine that plays an important role in innate and adaptive immunity. Wild type IL-12 is a heterodimeric protein comprising two subunits, p35 (IL-12A; GenBank GeneID: 3592) and p40 (IL-12B; GenBank GeneID: 3593), connected by disulfide bonds. Binding of IL-12 to the IL-12 receptor complex (IL-12R β 1 / IL-12R β 2) on T cells and Natural Killer (NK) cells leads to signaling via signal transducer and activator of transcription 4 (STAT4) and subsequent interferon γ (IFN- γ) production and secretion.

[0140] IL-12 subunits, IL-12A and IL-12B, can also form heterodimers with other IL-12 family members. For example, IL-12A may also dimerize with Epstein-Barr virus induced gene 3 (EBI3) to form IL-12 family member, IL-35 and IL-12B may dimerize with a p19 monomer, to form IL-12 family member, IL23.

[0141] IL-12 plays important roles in the innate and adaptive immune response, and dysregulation of IL-12 has been implicated in a number of disease states. Exemplary such disease states include, but are not limited to, inflammatory bowel disease, psoriasis, diabetes mellitus, multiple sclerosis, rheumatoid arthritis, cancer, lupus erythematosus, primarily biliary cholangitis and Sjögren's syndrome (Ulrich et al. *EXCLI journal* vol. 19 1563-1589, 11 Dec. 2020). Use of IL-12 as a therapeutic modality has been studied extensively, including for treatment of tumors (Nastala CL et al. *J Immunol.* 1994 Aug 15; Lasek et al. *Cancer immunology, immunotherapy: CII* vol. 63,5 (2014): 419-35).

[0142] In some embodiments, an immunomodulatory polypeptide disclosed herein is or comprises an IL-12 immune agonist moiety. In some embodiments, an immunomodulatory polypeptide disclosed herein comprises a plurality of IL-12 immune agonist moieties. In some embodiments, an immunomodulatory polypeptide disclosed herein

comprises exactly two IL-12 immune agonist moieties. In some embodiments, two or more IL-12 moieties of a plurality of (*e.g.*, two) IL-12 moieties are the same moiety. In some such embodiments, a plurality (*e.g.*, two) IL-12 moieties are different moieties. In some such embodiments, an IL-12 moiety comprises an IL-12A polypeptide or functional fragment thereof. In some embodiments, an IL-12 moiety comprises an IL-12B polypeptide or functional fragment thereof.

[0143] In some embodiments, an IL-12B immune agonist moiety is located N-terminal to an IL-12A immune agonist moiety in an immunomodulatory polypeptide. In some embodiments, an IL-12A immune agonist moiety is located N-terminal to an IL-12B immune agonist moiety in an immunomodulatory polypeptide.

[0144] In some embodiments, an immunomodulatory polypeptide comprising a plurality (*e.g.*, two) IL-12 moieties (*e.g.*, IL-12A and/or IL-12B) are linked directly. In some embodiments, an immunomodulatory polypeptide comprising a plurality (*e.g.*, two) IL-12 moieties (*e.g.*, IL-12A and/or IL-12B) are linked via a first linker. Non-limiting examples of linkers are discussed elsewhere herein.

[0145] In some embodiments, an immunomodulatory polypeptide disclosed herein comprises an IL-12A and/or IL-12B immune agonist moiety comprising a variant. In some embodiments, an IL-12A and/or IL-12B immune agonist moiety variant comprises a substitution, deletion, addition, and/or insertion of relative to a wild-type IL-12A or IL-12B polynucleotide or amino acid sequence. In some embodiments, an IL-12A and/or IL-12B immune agonist moiety comprises a plurality of variants. In some embodiments, a plurality of variants comprises one or more of a substitution, deletion, addition, and/or insertion relative to a wild-type IL-12A or IL-12B. In some embodiments, a variant comprises a substitute that does not change the amino acid sequence relative to a wild-type IL-12A or IL-12B.

[0146] In some embodiments, an IL12 variant comprises a S43X mutation relative to SEQ ID NO: 25, wherein X is any amino acid other than S. In some embodiments, an IL12 variant comprises a S154X mutation relative to SEQ ID NO: 25, wherein X is any amino acid other than S. In some embodiments, an IL12 variant comprises a S168X mutation relative to SEQ ID NO: 25, wherein X is any amino acid other than S. In some

embodiments, an IL12 variant comprises a S227X mutation relative to SEQ ID NO: 25, wherein X is any amino acid other than S. In some embodiments, an IL12 variant comprises a S233X mutation relative to SEQ ID NO: 25, wherein X is any amino acid other than S. In some embodiments, an IL12 variant comprises a T364X mutation relative to SEQ ID NO: 25, wherein X is any amino acid other than T. In some embodiments, an IL12 variant comprises a S365X mutation relative to SEQ ID NO: 25, wherein X is any amino acid other than S. In some embodiments, an IL12 variant comprises a S398X mutation relative to SEQ ID NO: 25, wherein X is any amino acid other than S. In some embodiments, an IL12 variant comprises a S365X mutation relative to SEQ ID NO: 25, wherein X is any amino acid other than S. In some embodiments, an IL12 variant comprises a S480X mutation relative to SEQ ID NO: 25, wherein X is any amino acid other than S. In some embodiments, an IL12 variant comprises a S481X mutation relative to SEQ ID NO: 25, wherein X is any amino acid other than S. In some embodiments, an IL12 variant comprises a S365X and S481X mutation relative to SEQ ID NO: 25, wherein X is any amino acid other than S. In some embodiments, an IL12 variant comprises a S365X, S398X, and S481X mutation relative to SEQ ID NO: 25, wherein X is any amino acid other than S. In some embodiments, an IL12 variant comprises any combination of variants, including, for example, those disclosed herein.

[0147] In some embodiments, an immunomodulatory polypeptide disclosed herein comprises an IL-12A and/or IL-12B immune agonist moiety that is a functional fragment thereof (*e.g.*, a signaling competent fragment). In some embodiments, an immunomodulatory polypeptide comprises a functional IL-12A fragment. In some embodiments, an immunomodulatory polypeptide comprises a functional IL-12B fragment. In some embodiments, an immunomodulatory polypeptide comprises a full length IL-12A and a functional IL-12B fragment. In some embodiments, an immunomodulatory polypeptide comprises a full length IL-12B and a functional IL-12A fragment.

[0148] In some embodiments, a IL-12A or IL-12B fragment comprises or consists of at least 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 99% or more of the monomeric units (*e.g.*, residues) as found in wild-type IL-12A or IL-12B.

Metal-hydroxide binding polypeptide

[0149] The Fusion Protein Filing has taught that hydroxyl replacement (*e.g.*, with phosphate groups) can increase a polypeptide's adsorption via ligand exchange with a metal hydroxide (*e.g.*, aluminum hydroxide), and furthermore can improve tumor retention and anti-tumor efficacy of such polypeptide (*e.g.*, specifically of a fusion polypeptide comprising an immunomodulatory polypeptide and a metal-hydroxide-binding polypeptide in which such hydroxyl replacement has occurred).

[0150] As discussed above, metal-hydroxide- (*e.g.*, alum-) binding polypeptides were developed that could be fused to an immunomodulatory polypeptide to allow strong binding to aluminum hydroxide. Various immunomodulatory polypeptides fused to ABPs were assessed. It was determined that the ABPs adsorbed to alum in serum and could be used to retain proteins and peptides in tumors. Additionally, polypeptides with greater phosphorylation tended to be retained on alum for much longer in serum conditions. Of the ABPs analyzed, the polypeptide,

GGGGSFQSEEQQGGSGGSEEGGMESEESNGGGSGGSEEGGGGSHHHHHH,
referred to as ABP10, demonstrated the highest phosphorylation with an increase of phosphorylation of 4-6-fold when the protein was expressed with a wild-type (WT) kinase (*e.g.*, WT Fam20C kinase), compared to when the protein was expressed with a mutant kinase (*e.g.*, mutant Fam20C kinase). ABP10 consists of four SXE motifs, a prevalent phosphorylation site motif, separated by short spacer sequences. It was demonstrated immunomodulatory polypeptides (*e.g.*, interleukin-2 and interleukin-12) linked to ABP10 showed improved survival in a mouse model of melanoma compared to the immunomodulatory polypeptides without ABP10.

[0151] We have surprisingly found that improved metal-hydroxide binding polypeptides can be developed. Among other things, we have developed metal-hydroxide binding polypeptides characterized by enhanced metal hydroxide (*e.g.*, alum) retention relative to an appropriate reference (*e.g.*, to ABP10). Alternatively or additionally, provided metal-hydroxide binding polypeptides are characterized by improved efficacy, as compared to an appropriate reference (*e.g.*, to ABP10), when administered to a subject with a tumor.

[0152] Thus, among other things, the present disclosure identifies the source of a problem with certain metal-hydroxide binding polypeptides and/or fusion polypeptides that include them. For example, the present disclosure appreciates that manufacturing challenges can be associated with certain such polypeptides and/or fusions. Without wishing to be bound by any particular theory, the present disclosure notes that secondary phosphorylation on the polypeptide may contribute to and/or be responsible for certain such manufacturing challenges. Among other things, the present disclosure provides metal-hydroxide-binding polypeptides, and fusion polypeptides that include them, which demonstrate high levels of adsorption to metal hydroxides and also desirable manufacturing characteristics (*e.g.*, one or more of reproducibility, consistency, production of a homogeneously-phosphorylated preparation, etc.).

[0153] In some embodiments, a metal-hydroxide binding polypeptide comprises an amino acid sequence that includes a plurality of phosphorylation sites, so that it can adopt phosphorylated and unphosphorylated forms. In some embodiments, a metal-hydroxide binding polypeptide comprises at least one kinase target motif. A target kinase motif comprises an amino acid that is phosphorylated by a kinase. Amino acids that are typically phosphorylated include a hydroxyl, such as serine (Ser, S), threonine (Thr, T), and tyrosine (Tyr, Y) residues. A kinase motif refers to the amino acid sequence immediately N- and/or C-terminal to the amino acid residue capable of being phosphorylated. Without wishing to be bound by any one theory, many kinases comprise structural features that confer specificity such that the kinase phosphorylates a particular amino acid (*e.g.*, serine, threonine, or tyrosine) of a particular kinase target motif.

[0154] Kinase target motifs recognized are highly diverse depending on the particular type of kinase. In some embodiments, the present disclosure provides metal-hydroxide binding polypeptides comprising one or more kinase target motifs of a secretory pathway kinase. The secretory pathway, which is the pathway by which a cell secretes proteins and/or other biomolecules into the extracellular space, refers to the endoplasmic reticulum (ER), Golgi apparatus (Golgi), cell membrane, and lysosomal storage compartments as well as the vesicles that travel between them. Secretory pathway kinases are localized throughout the secretory pathway (*e.g.*, at the ER, Golgi, etc.) and function to

phosphorylate proteins destined for secretion (Sreelatha et al. *Biochimica et biophysica acta* vol. 1854,10 Pt B (2015): 1687-93).

[0155] In some embodiments, a relevant kinase is a naturally occurring secretory pathway kinase (*e.g.*, is endogenously targeted to the secretory pathway to function). In some embodiments, a secretory pathway kinase comprises a signal sequence that targets the kinase to the secretory pathway. Naturally-occurring human secretory pathway kinases include, for example, four-jointed box kinase 1, Fam20A, Fam20B, Fam20C, vertebrate lonesome kinase (VLK), SGK196, and Fam69A, Fam69B, and Fam69C.

[0156] In some embodiments, a relevant kinase is a non-naturally occurring secretory pathway kinase. In some embodiments, a non-naturally occurring kinase is produced by linking a secretory signal peptide to a kinase endogenously localized to a non-secretory pathway cellular compartment.

[0157] In some embodiments, a kinase target motif is a target kinase motif of a secretory pathway kinase. In some embodiments, a secretory pathway kinase target kinase motif comprises an S-X-E motif. For example, Fam20C phosphorylates serine and has been shown to phosphorylate kinase targets motif comprising the amino acid sequence Ser-X-Glu (*e.g.*, S-X-E), Ser-X-pSer (*e.g.*, S-X-pS), and Ser-X-Gln-X-X-Asp-Glu-Glu (S-X-Q-X-X-D-F-E) wherein X is any amino acid, and pS is phosphorylated serine (Mercier, et al (1981) *Biochimie*, 63: 1-17; Mercier et al (1971) *Eur J. Biochem.* 23:41-51; Lasa-Benito (1996) *FEES Lett.* 382:149; Brunati, et al (2000) 3:765, Tagliabracci, et al (2015) *Cell* 161:1619-1632; Tagliabracci, et al (2012) *Science* 336:1150-1153). In some embodiments, a target kinase motif comprises the amino acid sequence SEEE. In some embodiments, a target kinase motif comprises the amino acid sequence SEEA. In some embodiments, a target kinase motif comprises the amino acid sequence SEEQ. In some embodiments, a target kinase motif comprises the amino acid sequence SEE.

[0158] In some embodiments, a metal-hydroxide binding polypeptide comprises at least one, two, three, four, five, six, seven, eight, nine, ten, eleven, or twelve target kinase motifs. In some embodiments, a metal-hydroxide binding polypeptide comprises at least one, two, three, four, five, six, seven, eight, nine, ten, eleven, or twelve S-X-E motifs. In some embodiments, a metal-hydroxide binding polypeptide comprises more than four S-X-E

motifs. In some embodiments, a metal-hydroxide binding polypeptide comprises eight S-X-E motifs. In some embodiments, the number of target kinase motifs (*e.g.*, S-X-E motifs) contributes to the number of phosphorylated residues on a metal-hydroxide binding polypeptide.

[0159] In some embodiments, a metal-hydroxide binding polypeptide is a metal-hydroxide binding polypeptide whose amino acid sequence includes a plurality of phosphorylation sites. In some embodiments, a plurality of phosphorylation sites comprises at least one, two, three, four, five, six, seven, eight, nine, ten, eleven, or twelve target kinase motifs. In some embodiments, a plurality of phosphorylation sites comprises at least one, two, three, four, five, six, seven, eight, nine, ten, eleven, or twelve S-X-E motifs. In some embodiments, a plurality of phosphorylation sites comprises more than four S-X-E motifs. In some embodiments, a plurality of phosphorylation sites comprises more than eight S-X-E motifs. In some embodiments, the number of target kinase motifs (*e.g.*, S-X-E motifs) contributes to the number of phosphorylated residues on a metal-hydroxide binding polypeptide.

[0160] In some embodiments, the at least one, two, three, four, five, six, seven, eight, nine, ten, eleven, or twelve target kinase motifs (*e.g.*, S-X-E motifs) are directly adjacent (*e.g.*, linked) to the next target kinase (*e.g.*, S-X-E motif). In some embodiments, the at least one, two, three, four, five, six, seven, eight, nine, ten, eleven, or twelve target kinase motifs (*e.g.*, S-X-E motifs) are separated (*e.g.*, linked) to the next target kinase motif (*e.g.*, S-X-E motif) by a spacer. In some embodiments, the spacer comprises at least one glycine residue. In some embodiments, the spacer comprises a plurality of glycine residues. In some embodiments, the spacer comprises three glycine residues. In some embodiments, the spacer comprises at least four glycine residues. In some embodiments, the spacer has a sequence that comprises four glycine residues. In some embodiments, the spacer has an amino acid sequence comprising GGGSGGGG. In some embodiments, the spacer has an amino acid sequence comprising GGGEGGGG.

[0161] In some embodiments, a metal-hydroxide binding polypeptide comprises four S-X-E motifs and three spacers comprising four glycine residues. In some embodiments, a metal-hydroxide binding polypeptide comprises six S-X-E motifs and five spacers

comprising four glycine residues. In some embodiments, a metal-hydroxide binding polypeptide comprises eight S-X-E motifs and seven spacers comprising four glycine residues. In some embodiments, a metal-hydroxide binding polypeptide comprises four motifs with the amino acid sequence, SEEE, and three spacers comprising three glycine residues. In some embodiments, a metal-hydroxide binding polypeptide comprises four motifs with the amino acid sequence, SEEA, and three spacers comprising three glycine residues. In some embodiments, a metal-hydroxide binding polypeptide comprises four motifs with the amino acid sequence, SEEQ, and three spacers comprising three glycine residues. In some embodiments, a metal-hydroxide binding polypeptide comprises four motifs with the amino acid sequence, SEE, and three spacers comprising the amino acid sequence, GGGS GGGG. In some embodiments, a metal-hydroxide binding polypeptide comprises four motifs with the amino acid sequence, SEE, and three spacers comprising the amino acid sequence, GGGE GGGG.

[0162] In some embodiments, a metal-hydroxide binding polypeptide comprises six S-X-E motifs, wherein each S-X-E motif is directly adjacent to the next S-X-E motif. In some embodiments, a metal-hydroxide binding polypeptide comprises eight S-X-E motifs, wherein each S-X-E motif is directly adjacent to the next S-X-E motif.

[0163] In some embodiments, a metal-hydroxide binding polypeptide comprise an ending sequence (*e.g.*, an amino acid sequence at the c-terminus of the fusion polypeptide. In some embodiments, an ending sequence comprises a plurality of amino acid residues. In some embodiments, a plurality of amino acid residues comprises GGGG. In some such embodiments, an ending sequence comprises the amino acid sequence GGGS. In some such embodiments, an ending sequence comprises the amino acid sequence GGGGHHHHHH. In some such embodiments, an ending sequence comprises the amino acid sequence GGGGSHHHHHH. In some embodiments, an ending sequence comprises an optional tag, as discussed elsewhere herein.

[0164] Among other things, the present disclosure identifies the source of a problem with certain metal-hydroxide binding polypeptides and/or fusion polypeptides that include them. Among other things, the present disclosure provides metal-hydroxide-binding polypeptides, and fusion polypeptides that include them, which demonstrate high levels of

adsorption to metal hydroxides and also desirable manufacturing characteristics (e.g., one or more of reproducibility, consistency, production of a homogeneously-phosphorylated fusion polypeptide, etc.). For example, in some embodiments, an optimal number of kinase target motifs (e.g., phosphorylation sites) are utilized. In some embodiments, for example, spacing of kinase target motifs (e.g., phosphorylation sites) is or has been optimized.

[0165] In some embodiments, a desired (e.g., optimal) number of kinase target motifs and/or spacing of kinase motifs may be determined based on one or more of, for example, desired phosphate content to achieve strong metal-hydroxide retention and/or avoidance of one or more manufacturing challenges (e.g., which the present disclosure appreciates may be associated with highly phosphorylated elements). In some embodiments, a desired (e.g., optimal) number and/or spacing of kinase motifs results in an exposure of the polypeptide to the kinase to achieve a desired level of fusion polypeptide phosphorylation. In some embodiments, improved fusion polypeptides as described herein, results in one or more of improved reproducibility, consistency, and/or production of a homogeneously-phosphorylated fusion polypeptide. For example, in some embodiments, provided technologies achieve reproducible manufacturing of comparable preparations (e.g., preparations that are consistently within established parameters) of fusion polypeptides (e.g., phosphorylated fusion polypeptides) and/or complexes as described herein. For example, in some embodiments, provided technologies achieve reduced immunogenicity compared to an appropriate reference standard (e.g., fusion polypeptides comprising ABP-10). Without wishing to be bound by any one theory, reduced immunogenicity is achieved by removing hydrophobic amino acids, thus, reducing binding to major histocompatibility complex.

Linkers

[0166] In some embodiments, fusion polypeptides as described herein may include one or more linkers or spacers.

[0167] For example, in some embodiments, fusion polypeptides comprise an immunomodulatory polypeptide comprising a first and a second immune agonist moiety. In some embodiments, a first and a second immune agonist moiety are linked via a first linker.

[0168] In some embodiments, fusion polypeptides of the present disclosure comprise an immunomodulatory polypeptide and a metal-hydroxide binding polypeptide. In some embodiments, an immunomodulatory polypeptide and a metal-hydroxide binding polypeptide are linked via a second linker.

[0169] In some embodiments, a first linker and/or a second linker is a polypeptide linker. In some embodiments, a polypeptide linker is synthetic. For example, a synthetic polypeptide linker may comprise non-naturally occurring polypeptides which are modified forms of naturally occurring polypeptides.

[0170] In some embodiments, polypeptide linkers of the present disclosure are at least one amino acid in length and can be any suitable number of amino acids. In some embodiments, a polypeptide linker is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, or 50 amino acids in length.

[0171] In some embodiments, a first linker comprises a polypeptide linker. In some embodiments, a first linker comprises or consists of a Glycine-Serine (Gly-Ser or G-S linker). A Gly-Ser linker is a polypeptide linker that consists of glycine and serine residues. In some embodiments, a Gly-Ser linker comprises an amino acid sequence of (Gly₄Ser)_n, wherein n is a positive integer (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10). In some embodiments, a Gly-Ser linker is (Gly₄Ser)₁. In some embodiments, a Gly-Ser linker is (Gly₄Ser)₂. In some embodiments, a Gly-Ser linker is (Gly₄Ser)₃. In some embodiments, a Gly-Ser linker is (Gly₄Ser)₄. In some embodiments, a Gly-Ser linker is (Gly₄Ser)₅. In some embodiments, a Gly-Ser linker is (Gly₄Ser)₆. In some embodiments, a Gly-Ser linker is (Gly₄Ser)₇. In some embodiments, a Gly-Ser linker is (Gly₄Ser)₈. In some embodiments, a Gly-Ser linker is (Gly₄Ser)₉. In some embodiments, a Gly-Ser linker is (Gly₄Ser)₁₀.

[0172] In some embodiments, a second linker comprises a polypeptide linker. In some embodiments, a second linker comprises a plurality of glycine residues. In some embodiments, a second linker comprises a polypeptide linker with the amino acid sequence, GGGGSGGGG. In some embodiments, a second linker comprises a polypeptide linker with the amino acid sequence, GGGGEGGGG.

Variants

[0173] In some embodiments, an immunomodulatory polypeptide or a metal-hydroxide-binding polypeptide utilized in accordance with the present disclosure is a variant of a relevant reference polypeptide (*e.g.*, a wild type polypeptide or functional portion thereof).

[0174] In some embodiments, a variant shows at least 70% identity to its reference polypeptide. In some such embodiments, a variant shows at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or higher identity to its references polypeptide

[0175] In some embodiments, a variant comprises one or more conservative or otherwise non-disruptive modifications (*e.g.*, substitutions, deletions or additions) relative to its reference. In some embodiments, a variant is free of any disruptive modifications (*e.g.*, substitutions, deletions or additions) so that an immunomodulatory polypeptide maintains one or more functional characteristics of the reference. In some embodiments, maintains means an immunomodulatory polypeptide display comparable activity (*e.g.*, signaling competency or binding) compared to an appropriate reference standard (*e.g.*, a wild-type immunomodulatory polypeptide). For example, in some such embodiments, an immunomodulatory polypeptide maintains at least about 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or higher activity compared to an appropriate reference standard (*e.g.*, a wild-type immunomodulatory polypeptide).

Metal hydroxides

[0176] In some embodiments, the present disclosure provides fusion polypeptides comprising an immunomodulatory polypeptide and a metal-hydroxide binding polypeptides, wherein the fusion polypeptide, when exposed to a metal-hydroxide forms a complex therewith. A complex is formed via adsorption of the fusion polypeptide to a metal-hydroxide. Without wishing to be bound by any one theory, it is hypothesized adsorption of a fusion polypeptide to a metal hydroxide occurs by ligand exchange. Ligand exchange, for example, is a substitution or exchange of a surface hydroxyl by another ligand. In some embodiments, substitution or exchange of a surface hydroxyl occurs by a hydroxyl-replacement group (*e.g.*, a phosphate group).

[0177] In some embodiments, a metal-hydroxide is a substance that includes at least one hydroxyl group bound to a metal. In accordance with the present disclosure, in some embodiments, a metal-hydroxide can adsorb fusion polypeptides comprising a hydroxyl-replacement moiety. In some embodiments, a hydroxyl-replacement moiety is a phosphate group.

[0178] In some embodiments, a metal-hydroxide is selected based on its inherent qualities or characteristics. In some embodiments, a metal-hydroxide is selected due to its biocompatibility for use in a subject (*e.g.*, a mammal, *e.g.*, a human). In some embodiments, a metal-hydroxide is aluminum hydroxide (*e.g.*, alum). In some embodiments, a metal-hydroxide is iron-hydroxide. A skilled artisan will recognize any number of metal-hydroxide may be successfully utilized in accordance with the present disclosure.

Tags

[0179] In some embodiments, a fusion polypeptide of the present disclosure comprises an immunomodulatory polypeptide that comprises an immune agonist moiety and a metal-hydroxide binding polypeptide whose amino acid sequence includes a plurality of phosphorylation sites, so that it can adopt phosphorylated and unphosphorylated forms. In some embodiments, a fusion polypeptide of the present disclosure comprises an ending sequence. In some embodiments, an ending sequence comprises a tag. A tag, as used herein, is an amino acid sequence that, when detected and/or measured in a particular sample, indicates that the protein to which it was linked is present in the sample and can provide a quantitative measurement thereof. A variety of tags known in the art may be used in accordance with the present disclosure. For example, in some embodiments, a tag comprises a FLAG tag, a polyhistidine tag, a V5 tag, a MBP tag.

[0180] In some embodiments, a tag is inserted at the N- or C-terminus of the fusion polypeptide to minimize interference with fusion polypeptide function. In some embodiments, the tags are placed internally in-frame within the fusion polypeptide sequence without affecting functionality. Preferred locations for the tag may be determined in part based on available empirical data (*e.g.*, from affinity tag or other fusion polypeptide experiments such as polyhistidine, FLAG tag, MBP tag, etc.), three dimensional structures

of the fusion polypeptide or similar polypeptides and/or *in vivo* or *in vitro* expression experiments.

[0181] In some embodiments, a tag can also contain a short sequence motif, such as an affinity tag or chromatography tag to allow for partial or complete purification from a complex mixture or preparation. In some embodiments, the peptide tags can be designed to allow inclusion of a fixed number (e.g., one, two, three, or more) of a set of preselected (e.g., one, two, three, or more) amino acids.

Production of fusion polypeptide metal-hydroxide complexes

[0182] In one aspect of the present disclosure, fusion polypeptide-metal hydroxide complexes described herein are produced by (1) making a fusion polypeptide; (2) phosphorylating a fusion polypeptide; and (3) contacting a fusion polypeptide with metal hydroxide.

Production of fusion polypeptides

[0183] In some embodiments, fusion polypeptides described herein are made in host cells using techniques for exogenous expression. Methods of exogenously expressing polypeptides are well known in the art and the skilled artisan would recognize a variety of techniques could be successfully utilized.

[0184] Recombinant polynucleotides (*e.g.*, DNA or RNA) encoding for fusion polypeptides of the present disclosure may be prepared by a variety of methods available. For example, sequences encoding for fusion polypeptides may be excised from DNA using restriction enzymes, may be amplified from plasmids or genomic polynucleotide sequences using, for example, polymerase chain reaction, or may be synthesized using chemical synthesis techniques. In some embodiments, a combination of known methods is utilized to prepare a recombinant polynucleotide encoding for fusion polypeptides of the present disclosure.

[0185] Recombinant polynucleotides encoding fusion polypeptides of the present disclosure may be cloned into a vector capable of expressing a fusion polypeptide. Cloning

may be carried out according to a variety of methods available (*e.g.*, Gibson assembly, restriction digest and ligation, etc.). In some embodiments, a vector is a viral vector. In some embodiments, a vector is a non-viral vector. In some embodiments, a vector is a plasmid.

[0186] In some embodiments, a vector capable of expression comprises a recombinant polynucleotide that encodes a fusion polypeptide of the present disclosure operatively linked to a sequence or sequences that control expression (*e.g.*, promoters, start signals, stop signals, polyadenylation signals, activators, repressors, etc.). In some embodiments, a sequence or sequences that control expression are selected to achieve a desired level of expression. In some embodiments, more than one sequence that controls expression (*e.g.*, promoters) are utilized. In some embodiments, more than one sequence that controls expression (*e.g.*, promoters) are utilized to achieve a desired level of expression of a plurality of recombinant polynucleotides that encode a plurality polypeptides. In some embodiments, a plurality of recombinant polypeptides are expressed from the same vector (*e.g.*, a bi-cistronic vector, a tri-cistronic vector, multi-cistronic.). In some embodiments, a plurality of recombinant polypeptides are expressed, each of which is expressed from a separate vector.

[0187] In some embodiments, a vector capable of expression comprising a recombinant polynucleotide encoding a fusion polypeptide of the present disclosure is used to express a fusion polypeptide in a host cell. A host cell may be selected from a variety of the available and known host cells (*e.g.*, Human Embryonic Kidney (HEK) cells, suspension HEK293 cells, Chinese Hamster Ovary cells) suitable expressing fusion polypeptides disclosed herein.

[0188] A variety of methods are available to introduce a vector into host cells. In some embodiments, a vector may be introduced into host cells using transfection. In some embodiments, transfection is completed, for example, using calcium phosphate transfection, lipofection, or polyethylenimine-mediated transfection. In some embodiments, a vector may be introduced into a host cell using transduction.

[0189] In some embodiments, a transformed host cells are cultured following introduction of a vector into a host cell to allow for expression of said recombinant polynucleotides. In some embodiments, a transformed host cells are cultured for at least 12

hours, 16 hours, 20 hours, 24 hours, 28 hours, 32 hours, 36 hours 40 hours, 44 hours, 48 hours, 52 hours, 56 hours, 60 hours, 64 hours, 68 hours, 72 hours or longer. Transformed host cells are cultured in growth conditions (*e.g.*, temperature, carbon-dioxide levels, growth medium) in accordance with the requirements of a host cell selected. A skilled artisan would recognize culture conditions for host cells selected are well known in the art.

Phosphorylating fusion polypeptides

[0190] In some embodiments, fusion polypeptides described herein are phosphorylated by contacting a fusion polypeptide with a kinase. In some embodiments, a fusion polypeptide is contacted with a kinase by co-expressing a fusion polypeptide in a host cell with a kinase. In some embodiments, co-expression is achieved by introducing two vectors, one comprising a recombinant polynucleotide encoding a fusion polypeptide and one comprising a recombinant polynucleotide encoding a kinase, into a host cell. In some embodiments, co-expression is achieved by introducing a single, multi-cistronic (*e.g.*, bi-cistronic) vector that comprises a plurality of recombinant polynucleotides. In some embodiments, a recombinant polynucleotides encode a fusion polypeptide and a recombinant polynucleotide encoding a kinase. In some embodiments, a transformed host cell is cultured following introduction of a vector into a host cell. Without wishing to be bound by any one theory, upon co-expression of a fusion polypeptide and a kinase in a host cell, a kinase can contact a fusion polypeptide, phosphorylating it.

[0191] In some embodiments, co-expression is achieved by introducing two vectors, one comprising a recombinant polynucleotide encoding a fusion polypeptide and one comprising a recombinant polynucleotide encoding a kinase, into a host cell. In some embodiments, two vectors are introduced at a ratio of vector encoding fusion polypeptide to vector encoding a kinase introduced into a host cell optimized to achieve a desired, relative level of expression of fusion polypeptide to kinase. In some embodiments, a ratio of vector encoding fusion polypeptide to vector encoding a kinase is 1:1, 2:1, 3:1, 4:1, 5:1, 6:1, 7:1, 8:1, 9:1, 10:1, 11:1, 12:1, 13:1, 14:1, 15:1, 16:1, 17:1, 18:1, 19:1, 20:1, 30: 1, 40:1, 50:1, 60:1, 70:1, 80:1, 90:1, or 100:1.

[0192] In some embodiments, co-expression is achieved by introducing one vector comprising both a recombinant polynucleotide encoding a fusion polypeptide and a

recombinant polypeptide encoding a kinase (*e.g.*, a bi-cistronic vector) into a host cell. In some embodiments, a recombinant polynucleotide encoding a fusion polypeptide and a recombinant polynucleotide encoding a kinase are operatively linked to a sequence or sequences that control expression (*e.g.*, promoters, start signals, stop signals, polyadenylation signals, activators, repressors, etc.). In some embodiments, a sequence or sequences that control expression are selected to achieve a desired level of expression. In some embodiments, multiple sequences that control expression (*e.g.*, promoters) are utilized to achieve a desired ratio of expression of fusion polypeptide to kinase. In some embodiments, a ratio is 1:1, 2:1, 3:1, 4:1, 5:1, 6:1, 7:1, 8:1, 9:1, 10:1, 11:1, 12:1, 13:1, 14:1, 15:1, 16:1, 17:1, 18:1, 19:1, 20:1, 30:1, 40:1, 50:1, 60:1, 70:1, 80:1, 90:1, or 100:1.

[0193] Exemplary nucleotide and amino acid sequences of Fam20C kinases include those described in **Table 3**.

Table 3: Exemplary nucleotide and amino acid sequences of Fam20C kinases

SEQ ID NO:	Fam20C	Sequence
99	hFam20C (w/ signal polypeptide)	MKMMMLVRRFRVLILMVFLVACALHIALDLLPRLERRGARPSG EPGCSCAQPAAEVAAPGWAQVRGRPGEPAAASSAAGDAGWP NKHTLRILQDFSSDPSSNLSSHLEKLPPAAEPAERALRGRDPG ALRPHDPAHRPLLRLDPGPRRSESPPGGGDASLLARLFEHPLY RVAVPPLTEEDVLFNVNSDTRLSPKAAENPDWPHAGAEGAEF LSPGEAAVDSYPNWLKFHIGINRYELYSRHNPAIEALLHDLSS QRITSVAMKSGGTQLKLIMTFQNYGQALFKPMKQTREQETPP DFFYFSDYERHNAEIAAFHLDRILDFRRVPPVAGRMVNMTKEI RDVTRDKKLWRTFFISPANNICFYGECSYYCSTEHALCGKPD QIEGSLAAFLPDLSLAKRKTWRNPWRRSYHKRKKAEWEVDP DYCEEVKQTPPYDSSHRILDVMDMTIFDFLMGNMDRHHYET FEKFGNETFIIHLDNNGRGFGKYSHDELSILVPLQQCCRIKSTY LRLQLLAKEEYKLSLLMAESLRGDQVAPVLYQPHLEALDRRL RVVLKAVRDCVERNGLHSVDDDLDEHRAASAR
79	hFam20C-KDEL (w/ signal polypeptide)	MKMMMLVRRFRVLILMVFLVACALHIALDLLPRLERRGARPSG EPGCSCAQPAAEVAAPGWAQVRGRPGEPAAASSAAGDAGWP NKHTLRILQDFSSDPSSNLSSHLEKLPPAAEPAERALRGRDPG ALRPHDPAHRPLLRLDPGPRRSESPPGGGDASLLARLFEHPLY RVAVPPLTEEDVLFNVNSDTRLSPKAAENPDWPIIAGAEGAEF LSPGEAAVDSYPNWLKFHIGINRYELYSRHNPAIEALLHDLSS QRITSVAMKSGGTQLKLIMTFQNYGQALFKPMKQTREQETPP DFFYFSDYERHNAEIAAFHLDRILDFRRVPPVAGRMVNMTKEI RDVTRDKKLWRTFFISPANNICFYGECSYYCSTEHALCGKPD

		QIEGSLAAFLPDLSLAKRKTWRNPWRRSYHKRKKAEWEVDP DYCEEVKQTPPYDSSHRILDVMDMTIFDFLMGNMDRHHYET FEKFGNETFIIHLDNNGRGFGKYSHDELSILVPLQQCCRIRKSTY LRLQLLAKEEYKLSLLMAESLRGDQVAPVLYQPHLEALDRRL RVVLKAVRDCVERNGLHSVVDLDDTEHRAASARGGGSKD EL
80	hFam20C (w/ signal polypeptide)	ATGAAGATGATGCTTGTTTCGAAGATTCCGGGTCCTGATTCT GATGGTATTCCTCGTCGCATGCGCCCTGCACATCGCCTTGG ACCTCCTCCCTCGGCTGGAACGCAGAGGAGCACGGCCTTC CGGAGAACC GGCTGCTCGTGTGCACAACCAGCCGCCGAA GTCGCTGCCCCCTGGATGGGCTCAAGTCAGAGGACGGCCTG GAGAACCCCGGCAGCTTCCTCCGCGGCTGGAGATGCGGG ATGGCCCAACAAGCACACCCTCCGGATCCTGCAGGACTTCT CCTCCGACCCCTCCTCGAATCTGAGCTCCCACTCCCTCGAA AAGCTGCCTCCTGCTGCCGAGCCAGCTGAACGGGCCCCTGA GGGGTAGAGATCCCGGTGCACTCAGGCCACATGACCCTGC GCACCGACCTCTGTTGAGAGATCCGGGTCTCGGAGGTCTG AATCACCGCCAGGTCTTGGTGGCGATGCGTCCCTTCTTGCG CGCTTGTTTCGAGCACCCGCTGTACCGGGTGGCAGTGCCTCC CCTCACCGAGGAAGATGTGCTGTTCAACGTGAACAGCGAT ACCCGCCTGAGCCCGAAGGCCGCGAGAATCCCGACTGGC CTCATGCCGGAGCCGAAGGAGCCGAGTTCCTGAGCCCTGG AGAAGCGGCCGTGGATTTCGTACCCGAAGTGGCTAAAGTTC CATATCGGCATCAACCGCTACGAGCTCTACTCGAGGCATA ACCCGGCCATTGAGGCCCTGCTGCACGACCTCAGCAGCCA ACGGATCACTAGCGTGGCCATGAAGTCCGGGGGCACTCAG CTGAAGTTGATCATGACCTTTCAGAACTACGGCCAGGCCCT GTTTAAGCCCATGAAGCAGACCCGGGAGCAGGAAACACCG CCGGACTTTTTCTACTTCTCGGACTACGAACGCCACAACGC CGAGATTGCCGCCTTCCACCTGGATCGCATCCTGGATTTC GACGGGTGCCACCTGTGGCGGGACGCATGGTCAATATGAC CAAAGAAATCCGCGACGTCACTCGCGACAAGAAGCTCTGG CGGACGTTCTTCATTTCCCGGCCAACACATCTGCTTCTA CGGGGAGTGCTCATACTACTGCTCCACCGAGCATGCCCTTT GCGGAAAGCCCGACCAGATCGAAGGGTCCCTGGCAGCCTT CCTTCCCGACCTGAGCCTGGCGAAGCGCAAGACTTGGAGA AACCCATGGCGCCGCTCATACCACAAGCGGAAGAAGGCGG AGTGGGAGGTGGACCCCGACTACTGCGAGGAAGTCAAGCA GACCCCCCCTACGACTCCTCCACCGGATTCTGGACGTGA TGGACATGACTATTTTCGATTTCCTCATGGGCAACATGGAC CGGCACCACTATGAAACCTTCGAAAAGTTCGGGAACGAGA CTTTCATTATTCACCTGGACAACGGCCGGGGATTTCGGAAAG TACAGCCACGACGAAGTCTCCATCCTGGTGCCGCTGCAGCA GTGTTGCCGGATCCGCAAGTCCACGTACCTGAGACTGCAG CTGTTGGCCAAAGAGGAGTATAAGCTGTGCTCCTGATGG CCGAATCCCTCCGCGGCGATCAAGTCGCCCCGGTGCTGTAC

		CAGCCGCATCTCGAGGCCCTGGACAGGAGACTGCGCGTGG TGCTCAAAGCTGTGCGGGATTGTGTGGAACGGAACGGCCT GCATTCTGTGGTGGACGACGACCTGGACACCGAACACAGA GCCGCATCCGCTAGG
81	hFam20C- KDEL (w/ signal polypeptide)	ATGAAGATGATGCTTGTTCTGAAGATTCCGGGTCCTGATTCT GATGGTATTCCTCGTCGCATGCGCCCTGCACATCGCCTTGG ACCTCCTCCCTCGGCTGGAACGCAGAGGAGCACGGCCTTC CGGAGAACCGGGCTGCTCGTGTGCACAACCAGCCGCCGAA GTCGCTGCCCCCTGGATGGGCTCAAGTCAGAGGACGGCCTG GAGAACCCCCGGCAGCTTCCTCCGCGGCTGGAGATGCGGG ATGGCCCAACAAGCACACCCTCCGGATCCTGCAGGACTTCT CCTCCGACCCCTCCTCGAATCTGAGCTCCCACTCCCTCGAA AAGCTGCCTCCTGCTGCCGAGCCAGCTGAACGGGCCCTGA GGGGTAGAGATCCCGGTGCACTCAGGCCACATGACCCTGC GCACCGACCTCTGTTGAGAGATCCGGGTCTCTCGGAGGTCTG AATCACCGCCAGGTCCTGGTGGCGATGCGTCCCTTCTTGCG CGCTTGTTGAGCACCCGCTGTACCGGGTGGCAGTGCCTCC CCTCACCGAGGAAGATGTGCTGTTCAACGTGAACAGCGAT ACCCGCCTGAGCCCGAAAGCCGCGGAGAATCCCGACTGGC CTCATGCCGGAGCCGAAGGAGCCGAGTTCCTGAGCCCTGG AGAAGCGGCCGTGGATTTCGTACCCGAACTGGCTAAAGTTC CATATCGGCATCAACCGCTACGAGCTCTACTCGAGGCATA ACCCGGCCATTGAGGCCCTGCTGCACGACCTCAGCAGCCA ACGGATCACTAGCGTGGCCATGAAGTCCGGGGGCACTCAG CTGAAGTTGATCATGACCTTTCAGAACTACGGCCAGGCCCT GTTTAAGCCCATGAAGCAGACCCGGGAGCAGGAAACACCG CCGGACTTTTTCTACTTCTCGGACTACGAACGCCACAACGC CGAGATTGCCGCCTTCCACCTGGATCGCATCCTGGATTTC GACGGGTGCCACCTGTGGCGGGACGCATGGTCAATATGAC CAAAGAAATCCGCGACGTCACTCGCGACAAGAAGCTCTGG CGGACGTTCTTCATTTCCCCGGCCAACAACATCTGCTTCTA CGGGGAGTGCTCATACTACTGCTCCACCGAGCATGCCCTTT GCGGAAAGCCCGACCAGATCGAAGGGTCCCTGGCAGCCTT CCTTCCCGACCTGAGCCTGGCGAAGCGCAAGACTTGGAGA AACCCATGGCGCCGCTCATACCACAAGCGGAAGAAGGCGG AGTGGGAGGTGGACCCCGACTACTGCGAGGAAGTCAAGCA GACCCCCCCTACGACTCCTCCACCGGATTCTGGACGTGA TGGACATGACTATTTTCGATTTCCTCATGGGCAACATGGAC CGGCACCACTATGAAACCTTCGAAAAGTTCGGGAACGAGA CTTTCATTATTCACCTGGACAACGGCCGGGGATTTCGGAAAG TACAGCCACGACGAACCTCTCCATCCTGGTGCCGCTGCAGCA GTGTTGCCGGATCCGCAAGTCCACGTACCTGAGACTGCAG CTGTTGGCCAAAGAGGAGTATAAGCTGTGCTCCTGATGG CCGAATCCCTCCGCGGCGATCAAGTCGCCCCGGTGCTGTAC CAGCCGCATCTCGAGGCCCTGGACAGGAGACTGCGCGTGG TGCTCAAAGCTGTGCGGGATTGTGTGGAACGGAACGGCCT

		GCATTCTGTGGTGGACGACGACCTGGACACCGAACACAGA GCCGCATCCGCTAGGGGTGGAGGGTCCAAAGATGAGCTG
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Complex formation

[0194] In some embodiments, a fusion polypeptide described herein, when exposed to a metal-hydroxide (*e.g.*, aluminum hydroxide) forms a complex therewith. In some embodiments, fusion polypeptides comprise hydroxyl replacement groups (*e.g.*, phosphate groups) for adsorption via ligand exchange with a metal hydroxide. In some embodiments, a fusion polypeptide can, via electrostatic interactions, form a complex with a metal hydroxide.

[0195] In some embodiments, a fusion polypeptide metal-hydroxide complex of the present disclosure is formed by mixing. In some embodiments, mixing occurs in a buffer (*e.g.*, a tris-buffered saline buffer). In some such embodiments, a buffer does not contain phosphate. In some such embodiments, a buffer does not contain a substance or substances that solubilize a metal-hydroxide (*e.g.*, citric acid, malic acid, or lactic acid). Without wishing to be bound by any one theory, buffers that contain phosphate compete with, and can hinder, complex formation. In some embodiments, mixing occurs for a duration of time at a particular temperature. In some such embodiments, a duration of time is 5 minutes, 10 minutes, 15 minutes, 20 minutes, 25 minutes, 30 minutes, 35 minutes, 40 minutes, or 45 minutes. In some such embodiments, a particular temperature is approximately 15°C, 16°C, 17°C, 18°C, 19°C, 20°C, 21°C, 22°C, 23°C, 24°C, 25°C, 26°C, 27°C, 28°C, 29°C, 30°C, 31°C, 32°C, 33°C, 34°C, or 35°C.

Purification

[0196] Commonly aberrant products (*e.g.*, residual protein, host cell contaminants, etc.) are removed from fusion polypeptide preparations. A variety of purification technologies are available and known to the skilled artisan.

[0197] In some embodiments, a fusion polypeptide can be purified by a chromatography method. In some embodiments, such a chromatography purification method can be performed with a chromatography method known in the art, including, but not

limited to, high-performance liquid chromatography, size exclusion chromatography, ion exchange chromatography, wherein components of a mixture travel through a stationary phase at different speeds, resulting in separation from one another. It will be apparent to a skilled artisan a variety of solid substrates may be used (*e.g.*, beads, particles, microspheres, resins, etc.). For example, in some embodiments, a solid substrate has properties such that, in accordance with the present disclosure, permits a different retention time for a fusion polypeptide relative to any other undesirable components in the preparation of fusion polypeptide.

[0198] In some embodiments, a fusion polypeptide can be purified by an affinity-based purification method. In some embodiments, such an affinity-based purification method may be performed with a solid substrate known in the art. A wide variety of substrates could be utilized, such as, for example, membranes, polymeric beads, magnetic beads, or composites. For example, in some embodiments, a solid substrate (*e.g.*, polymeric beads or particles) coated or pre-charged with a substance or composition (*e.g.*, nickel ions) that has a high binding affinity for a fusion polypeptides can be useful in accordance with the present disclosure such that a fusion polypeptide will bind to a solid substrate, while any other undesirable components present in a preparation will remain in solution. In some embodiments, a fusion polypeptide may be eluted from a solid substrate. In some embodiments, elution may be carried out using specific elution. For example, in some embodiments specific elution is completed by challenging a polypeptide-substrate complex by an agent or agents that will compete for complexation with either a substrate or a polypeptide, releasing a polypeptide into solution. In some embodiments, elution may be carried out using non-specific elution. For example, in some embodiments, non-specific elution is completed by manipulating solvent or buffer conditions (*e.g.*, increasing concentration of a buffer, *e.g.*, an imidazole buffer) to reduce the associate rate constant, resulting in dissociation of the polypeptide from the substrate.

Characterization

[0199] Among other things, in some embodiments, the present disclosure provides technologies for characterizing fusion polypeptides (*e.g.*, phosphorylated or unphosphorylated preparations thereof) and/or of complexes comprising such fusion

polypeptides and metal hydroxides. In characterization is performed during and/or following production process. In some embodiments, a particular preparation process may be modified or terminated in light of a characterization (*e.g.*, if a particular preparation fails to meet one or more specifications). In some embodiments, such characterization may involve assessment of one or more of metal-hydroxide retention, degree of phosphorylation, heterogeneity of phosphorylation, signaling activity, and/or efficacy.

Exemplary characterization of phosphate content

[0200] In some embodiments, degree of phosphorylation (*e.g.*, of fusion polypeptides of the present disclosure and/or preparations thereof) is characterized. A variety of methods are available for measurement of degree of phosphorylation (*e.g.*, the average number of phosphate molecules per polypeptide). For example, in some embodiments, degree of phosphorylation can be determined by a colorimetric method. In some embodiments, a colorimetric method is or comprises a malachite green assay. Without wishing to be bound by any one theory, a malachite green assay is based on quantification of a green complex formed between Malachite green, molybdate, and free orthophosphate which can be measured (*e.g.*, using a spectrophotometer or plate reader).

[0201] In some embodiments, degree of phosphorylation (*e.g.*, the average number of phosphate molecules per polypeptide) is 0.5-7, 1-7, 2-7, 3-7, 4-7, 5-7, 6-7, 0.5-6, 0.5-5, 0.5-4, 1-6, 2-6, 3-6, 4-6, 5-6, 0.5-10, 0.5-9, 0.5-8, 1-10, 1-9, 1-8, 2-10, 2-9, 2-8, 3-10, 3-9, 3-8, 4-10, 4-9, 4-8, 5-10, 5-9, 5-8, 6-10, 6-9, or 6-8. In some embodiments, degree of phosphorylation (*e.g.*, the average number of phosphate molecules per polypeptide) is 3, 3.1, 3.2, 3.3, 3.4, 3.5, 3.6, 3.7, 3.8, 3.9, 4.0, 4.1, 4.2, 4.3, 4.4, 4.5, 4.6, 4.7, 4.8, 4.9, 5.0, 5.1, 5.2, 5.3, 5.4, 5.5, 5.6, 5.7, 5.8, 5.9, 6.0, 6.1, 6.2, 6.3, 6.4, 6.5, 6.6, 6.7, 6.8, 6.9, 7.0, 7.1, 7.2, 7.3, 7.4, 7.5, 7.6, 7.7, 7.8, 7.9, 7.10, 8.0, 8.1, 8.2, 8.3, 8.4, 8.5, 8.6, 8.7, 8.8, 8.9, 9.0, 9.1, 9.2, 9.3, 9.4, 9.5, 9.6, 9.7, 9.8, 9.9 or 10.

[0202] In some embodiments, heterogeneity of phosphorylation of fusion polypeptides of the present disclosure and/or preparations thereof are characterized. In some embodiments, heterogeneity of phosphorylation is a measurement of the degree of phosphorylation within a given preparation of fusion polypeptide. In some embodiments, heterogeneity of phosphorylation is a measurement of the degree of phosphorylation across

a plurality of preparations of fusion polypeptide. In some embodiments, heterogeneity of phosphorylation is a measurement of location of particular phosphate groups on a polypeptide within a given preparation of fusion polypeptide. In some embodiments, heterogeneity of phosphorylation is a measurement of location of particular phosphate groups on a polypeptide across a plurality of preparations of fusion polypeptide.

[0203] A variety of technologies is available for measurement of heterogeneity of phosphorylation. For example, in some embodiments, degree of phosphorylation can be determined by a chromatography method. In some embodiments, a chromatography method comprises ion-exchange chromatography. In some embodiments, for example, a chromatography method comprises analytical anion-exchange chromatography. Anion-exchange chromatography is a form of ion exchange where a negatively charged biomolecule (*e.g.*, a phosphorylated form of a fusion polypeptide disclosed herein) binds to a positively charged resin. In some embodiments, anion-exchange chromatography can be used to resolve polypeptides with different numbers of phosphorylated amino acid residues (*e.g.*, differentially phosphorylated polypeptides). Without wishing to be bound by any one theory, polypeptide phosphorylation confers variability in a polypeptide's charge, permitting separation of differentially phosphorylated polypeptides using ion-exchange chromatography (*e.g.*, anion-exchange chromatography). Use of a gradient elution buffer (*e.g.*, a buffer with increasing salt concentrations) to elute from the ion exchange (*e.g.*, anion exchange) column permits separation of differentially phosphorylated polypeptides. In some embodiments, a buffer is, for example, a Tris buffer. In some embodiments, a linear gradient of Tris buffer is utilized. In some embodiments, a linear gradient of Tris buffer comprises over a linear gradient from 20 mM Tris, pH 7.1 to 20 mM Tris, 525 mM NaCl, pH 7.1 over a pre-defined period of time. In some embodiments, a linear gradient is conducted over a period of 1 minute, 5 minutes, 10 minutes, 15 minutes, 20 minutes, 22 minutes, 24 minutes, 26 minutes, 28 minutes, 30 minutes, 32 minutes, 34 minutes, 36 minutes, 38 minutes, 40 minutes, or longer.

[0204] In some embodiments, differentially phosphorylated polypeptides are dephosphorylated. In some embodiments, dephosphorylation comprises use of a phosphatase (*e.g.*, a lambda phosphatase). In some embodiments, a fusion polypeptide is incubated with a

phosphatase for a period of time and at a temperature that permits activity of said phosphatase and dephosphorylation of said fusion polypeptide. In some embodiments, dephosphorylation occurs at an incubation temperature of approximately 25°C, 26°C, 27°C, 28°C, 29°C, 30°C, 31°C, 32°C, 33°C, 34°C, 35°C, or higher. In some embodiments, dephosphorylation occurs for an incubation time of 25 minutes, 30 minutes, 35 minutes, 40 minutes, 45 minutes, 50 minutes, 55 minutes, 60 minutes, 65 minutes, or longer. In some embodiments, dephosphorylation occurs for an incubation time of 25-65 minutes, 30-60 minutes, 35-55 minutes, 40-50 minutes, 30-65 minutes, 35-65 minutes, 40-65 minutes, 45-65 minutes, 50-65 minutes, or 55-65 minutes.

[0205] In some embodiments, differentially phosphorylated polypeptides are dephosphorylated prior to separation. In some embodiments, differentially phosphorylated polypeptides of the present disclosure are assessed relative to an appropriate reference standard (*e.g.*, a dephosphorylated and/or non-phosphorylated form of a fusion polypeptide).

[0206] In some embodiments, after separation of differentially phosphorylated polypeptides (*e.g.*, by ion-exchange chromatography), the amount of each differentially phosphorylated polypeptide is measured. In some embodiments, the amount of each differentially phosphorylated polypeptide is measured according to a variety of methods available in the art. In some embodiments, for example and without limitation, differentially phosphorylated polypeptides are measured using a malachite green assay, analytical ion exchange, spectrophotometer, colorimetric assays, and/or western blot.

Exemplary characterization of metal-hydroxide retention

[0207] In some embodiments, fusion polypeptides of the present disclosure, when exposed to a metal-hydroxide (*e.g.*, aluminum hydroxide) forms a complex therewith. In some embodiments, retention of a fusion polypeptide of the present disclosure on a metal-hydroxide (*e.g.*, metal-hydroxide retention) is characterized. A variety of methods are available to measure metal-hydroxide retention. In some embodiments, for example and without limitation, metal-hydroxide retention can be measured by ellipsometry, surface plasmon resonance, optical waveguide lightmode spectroscopy, attenuated total internal reflectance-infrared spectroscopy, circular dichroism spectroscopy (CD), total internal reflectance-infrared spectroscopy (TIRF), and other high resolution microscopy techniques.

[0208] In some embodiments, metal-hydroxide retention is characterized using an *in vitro* assay. For example, fusion polypeptides at a known concentration are mixed with an excess of metal-hydroxide. The concentration of free, non-complexed fusion polypeptides is quantified and compared to a standard curve to determine metal-hydroxide retention. The concentration of free, non-complexed fusion polypeptide can be assessed according to a variety of method known to those of skill in the art. For example, and without limitation, in some embodiments, free, non-complexed, fusion polypeptides are quantified by enzyme-linked immunosorbent assay (ELISA), western blot, bicinchoninic acid assay, or Bradford assay.

[0209] In some embodiments, at least 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 99%, or 100% of fusion-polypeptide, when mixed with a metal-hydroxide, forms a complex therewith (*e.g.*, is retained).

Exemplary characterization of signaling activity

[0210] In some embodiments, fusion polypeptides (and/or complexes thereof) as described herein are characterized for activity (*e.g.*, signaling activity). In some embodiments, activity is characterized by assessing signaling activity (*e.g.*, signaling competency) compared to an appropriate reference standard. An appropriate reference standard can be, for example, a wild-type polypeptide and/or a fusion polypeptide lacking a metal-hydroxide binding polypeptide.

[0211] A variety of methods are available to assess signaling competency. In some embodiments, for example, signaling competency is assessed using an *in vitro*- or *in vivo*-based activity assay.

[0212] In some embodiments, signaling activity is assessed with an *in vitro* activity assay. In some embodiments, an *in vitro* activity assay comprises measuring activation or inhibition of downstream signaling of a fusion polypeptide. In some embodiments, measuring activation or inhibition of downstream activity comprises use of a reporter (*e.g.*, a reporter assay). In some embodiments, a reporter assay measures activity using a detectable molecule (*e.g.*, a reporter) that correlates with fusion polypeptide activity.

[0213] In some embodiments, a reporter comprises a fluorescent, bioluminescent, and/or other detectable probe known to those of skill in the art. In some embodiments, a reporter comprises use of a gene reporter. A gene reporter, for example, can be activated upon signaling elicited from a polypeptide. For example, upon activation of gene reporter transcription, a detectable product or enzyme that can be activated upon addition of substrate, generating a detectable product and/or by-product, can be utilized. In some embodiments, an enzyme useful in accordance with a reporter assay is, for example, luciferase or an alkaline phosphatase (*e.g.*, secreted alkaline phosphatase, SEAP). In some such embodiments, a HEK-blue-IL12 reporter assay is utilized.

[0214] In some embodiments, signaling activity is assessed with an *in vivo* activity assay. In some embodiments, a fusion polypeptide is administered to a subject (*e.g.*, a mouse, non-human primate, human, etc.) and activity is assessed. In some embodiments, activity is assessed, for example, by measuring activation or inhibition of downstream signaling of a fusion polypeptide as compared to an appropriate reference standard (*e.g.*, activity of a wild-type polypeptide). A variety of methods are available to measure activation or inhibition of downstream signaling of a fusion polypeptide. For example, and without limitation, differential gene expression, protein expression, and/or alterations in post-translational modifications induced by a fusion polypeptide can be measured.

Exemplary efficacy characterization

[0215] In some embodiments, efficacy can be characterized according to a variety of methods that are available. In some embodiments, for example, a fusion polypeptide (or complex thereof) as described herein is administered (*e.g.*, by intratumoral or peritumoral injection) to a subject (*e.g.*, mouse, non-human primate, human, etc.) and efficacy is determined in comparison to an appropriate reference standard. An appropriate reference standard can be, for example, a wild-type polypeptide and/or a polypeptide lacking a metal-hydroxide binding polypeptide, or having a metal-hydroxide binding polypeptide in a non-binding (*e.g.*, non-phosphorylated) state.

[0216] In some embodiments, efficacy is determined pre-clinically in an animal model (*e.g.*, in mice, rats, non-human primates, *etc.*). In some embodiments, a fusion polypeptide is administered (*e.g.*, by intratumoral or peritumoral injection) to an animal

model. For example, in some embodiments, an animal model is an animal model with a tumor (*e.g.*, an animal model of cancer). In some embodiments, a cancer animal model is generated by inoculating said animal model with tumor cells. In some embodiments, an animal model is inoculated with tumor cells at the flank region. In some embodiments, an animal model is inoculated with tumor cells in a clinically relevant region (*e.g.*, a mammary fat pad).

[0217] In some embodiments, an animal model of cancer is administered a fusion polypeptide of the present disclosure. In some embodiments, an animal model of cancer is administered a reference standard (*e.g.*, a wild-type polypeptide and/or a polypeptide lacking a metal-hydroxide binding polypeptide). In some embodiment, a variety of available, pre-determined measurements for efficacy known in the art, such as, for example, tumor volume and/or percent survival are assessed over time relative to an appropriate reference standard (*e.g.*, a wild-type polypeptide and/or a polypeptide lacking a metal-hydroxide binding polypeptide).

[0218] In some embodiments, efficacy of a fusion polypeptide is determined clinically. In some embodiments, a fusion polypeptide is administered (*e.g.*, by intratumoral, peritumoral injection, or into a tumor-draining lymph node) to a subject with a tumor. In some embodiments, a variety of available, pre-determined measurements for efficacy known in the art, such as, for example, tumor volume and/or percent survival are assessed over time relative to a subject with a tumor administered reference standard (*e.g.*, a treatment in the art of known efficacy and/or placebo).

Compositions

Preparations of fusion polypeptides

[0219] In some embodiments, the present disclosure, among other things, provides fusion polypeptide preparations. Fusion polypeptide preparations are preparations comprising a fusion polypeptide that, in some embodiments, is purified from a cell culture production of said fusion polypeptide described herein. In some embodiments, a fusion polypeptide preparation comprises an unphosphorylated form of a fusion polypeptide. In some embodiments, a fusion polypeptide preparation comprises a phosphorylated form of

the fusion polypeptide. In some embodiments, a fusion polypeptide preparation comprises a mixture of both unphosphorylated and phosphorylated forms of a fusion polypeptide.

[0220] In some embodiments, a fusion polypeptide preparation is a preparation comprising pharmaceutical-grade fusion polypeptide. In some embodiments, a fusion polypeptide preparation is a preparation comprising fusion polypeptide which its one or more characterization attributes are assessed and determined to meet a release and/or acceptance criteria (*e.g.*, as described herein). Examples of such product quality attributes include, but are not limited to, degree of phosphorylation and/or heterogeneity of phosphorylation.

[0221] In some embodiments, a fusion polypeptide preparation comprises a phosphorylated form of a fusion polypeptide and a kinase used to phosphorylate a fusion polypeptide (*e.g.*, as described herein). In some embodiments, a kinase is removed from a fusion polypeptide preparation.

[0222] In some embodiments, a phosphorylated fusion polypeptide preparation comprises fusion polypeptides with varying degrees of phosphorylation. In some embodiments, the degree of phosphorylation (*e.g.*, the average number of phosphate molecules per polypeptide) of a preparation of fusion polypeptide is 0.5-7, 1-7, 2-7, 3-7, 4-7, 5-7, 6-7, 0.5-6, 0.5-5, 0.5-4, 1-6, 2-6, 3-6, 4-6, 5-6, 0.5-10, 0.5-9, 0.5-8, 1-10, 1-9, 1-8, 2-10, 2-9, 2-8, 3-10, 3-9, 3-8, 4-10, 4-9, 4-8, 5-10, 5-9, 5-8, 6-10, 6-9, or 6-8. In some embodiments, the degree of phosphorylation (*e.g.*, the average number of phosphate molecules per polypeptide) of a preparation of fusion polypeptide is 3, 3.1, 3.2, 3.3, 3.4, 3.5, 3.6, 3.7, 3.8, 3.9, 4.0, 4.1, 4.2, 4.3, 4.4, 4.5, 4.6, 4.7, 4.8, 4.9, 5.0, 5.1, 5.2, 5.3, 5.4, 5.5, 5.6, 5.7, 5.8, 5.9, 6.0, 6.1, 6.2, 6.3, 6.4, 6.5, 6.6, 6.7, 6.8, 6.9, 7.0, 7.1, 7.2, 7.3, 7.4, 7.5, 7.6, 7.7, 7.8, 7.9, 7.10, 8.0, 8.1, 8.2, 8.3, 8.4, 8.5, 8.6, 8.7, 8.8, 8.9, 9.0, 9.1, 9.2, 9.3, 9.4, 9.5, 9.6, 9.7, 9.8, 9.9 or 10.

[0223] In some embodiments, the present disclosure, among other things, provides a fusion polypeptide preparation comprising a fusion polypeptide-metal hydroxide complex. In some embodiments, a preparation comprising a fusion polypeptide-metal hydroxide complex comprises any of a variety of suitable metal-hydroxide known in the art. For example, and without limitation, a metal-hydroxide may be an aluminum hydroxide or an

iron hydroxide. In some embodiments, a fusion polypeptide-metal hydroxide complex comprises a mass ratio of fusion polypeptide to metal hydroxide (*e.g.*, aluminum hydroxide), for example as defined by metal (*e.g.*, aluminum) mass. In some embodiments, the ratio, is 1:1, 1:2, 1:3, 1:4, 1:5, 1:6, 1:7, 1:8, 1:9, 1:10, 1:11, 1:12, 1:13, 1:14, 1:15, 1:16, 1:17, 1:18, 1:19, or 1:20.

[0224] In some embodiments, the present disclosure, among other things, provides a pharmaceutical composition comprising a fusion polypeptide disclosed herein. In some embodiments, the pharmaceutical composition is formulated as a fusion polypeptide-metal hydroxide complex. In some embodiments, a pharmaceutical composition comprises a pharmaceutically acceptable diluent, carrier, solubilizer, emulsifier, preservative, and/or adjuvant.

[0225] In some embodiments, acceptable pharmaceutical composition formulation materials preferably are nontoxic to recipients at the dosages and concentrations employed. In some embodiments, formulation material(s) are for subcutaneous and/or intravenous administration. In some embodiments, formulation material(s) are for local administration (*e.g.*, intratumoral or peritumoral administration). In some embodiments, a pharmaceutical composition can contain formulation materials for modifying, maintaining or preserving, for example, the pH, osmolality, viscosity, clarity, color, isotonicity, odor, sterility, stability, rate of dissolution or release, adsorption or penetration of fusion polypeptides. In some embodiments, suitable formulation materials include, but are not limited to, amino acids (such as glycine, glutamine, asparagine, arginine or lysine); antimicrobials; antioxidants (such as ascorbic acid, sodium sulfite or sodium hydrogen- sulfite); buffers (such as borate, bicarbonate, Tris-HCl, citrates, phosphates or other organic acids); bulking agents (such as mannitol or glycine); chelating agents (such as ethylenediamine tetraacetic acid (EDTA)); complexing agents (such as caffeine, polyvinylpyrrolidone, beta-cyclodextrin or hydroxypropyl-beta- cyclodextrin); fillers; monosaccharides; disaccharides; and other carbohydrates (such as glucose, mannose or dextrans); proteins (such as serum albumin, gelatin or immunoglobulins); coloring, flavoring and diluting agents; emulsifying agents; hydrophilic polymers (such as polyvinylpyrrolidone); low molecular weight polypeptides; salt-forming counterions (such as sodium); preservatives (such as benzalkonium chloride,

benzoic acid, salicylic acid, thimerosal, phenethyl alcohol, methylparaben, propylparaben, chlorhexidine, sorbic acid or hydrogen peroxide); solvents (such as glycerin, propylene glycol or polyethylene glycol); sugar alcohols (such as mannitol or sorbitol); suspending agents; surfactants or wetting agents (such as pluronics, PEG, sorbitan esters, polysorbates such as polysorbate 20, polysorbate 80, triton, tromethamine, lecithin, cholesterol, tyloxapal); stability enhancing agents (such as sucrose or sorbitol); tonicity enhancing agents (such as alkali metal halides, preferably sodium or potassium chloride, mannitol sorbitol); delivery vehicles; diluents; excipients and/or pharmaceutical adjuvants. (Remington's Pharmaceutical Sciences, 18th Edition, A. R. Gennaro, ed., Mack Publishing Company (1995). In some embodiments, a pharmaceutical composition comprises PBS; 20 mM NaOAc, pH 5.2, 50 mM NaCl; and/or 10 mM NaOAc, pH 5.2, 9% Sucrose. In some embodiments, an optimal pharmaceutical composition will be determined by one skilled in the art depending upon, for example, the intended route of administration, delivery format and desired dosage. See, for example, Remington's Pharmaceutical Sciences, *supra*. In some embodiments, such compositions may influence the physical state, stability, rate of in vivo release and rate of in vivo clearance of a fusion polypeptide-metal hydroxide complex.

[0226] In some embodiments, a primary vehicle or carrier in a pharmaceutical composition can be either aqueous or non-aqueous in nature. For example, in some embodiments, a suitable vehicle or carrier can be water for injection, physiological saline solution or artificial cerebrospinal fluid, possibly supplemented with other materials common in compositions for parenteral administration. In some embodiments, the saline comprises isotonic phosphate-buffered saline. In some embodiments, neutral buffered saline or saline mixed with serum albumin are further exemplary vehicles. In some embodiments, pharmaceutical compositions comprise Tris buffer of about pH 7.0-8.5, or acetate buffer of about pH 4.0-5.5, which can further include sorbitol or a suitable substitute therefore. In some embodiments, a composition comprising a fusion polypeptide metal hydroxide complex or a fusion polypeptide is prepared for storage by mixing the selected composition having the desired degree of purity with optional formulation agents (Remington's Pharmaceutical Sciences, *supra*) in the form of a lyophilized cake or an aqueous solution. Further, in some embodiments, a composition comprising a fusion polypeptide-metal

hydroxide complex or a fusion polypeptide is formulated as a lyophilizate using appropriate excipients such as sucrose.

Use

Methods of treatment

[0227] In one aspect, the present disclosure relates to methods of treating a subject with a medical condition. In some embodiments, the present disclosure relates to methods of treating a subject with a tumor (*e.g.*, a subject with cancer). In general, methods of treatment are aimed at reducing tumor volume, reducing and/or preventing metastases, prolonging survival, and/or curing the condition. Appropriate subjects or individuals receiving a fusion polypeptide or fusion polypeptide metal hydroxide complex of the present disclosure include, for example, humans or other mammals (*e.g.*, mice, rats, rabbits, dogs, horses, cats, pigs, or non-human primates) that have a tumor (*e.g.*, cancer).

[0228] In some embodiments, a method of treating a subject with a tumor comprises a step of: treating a subject with a complex comprising: a fusion polypeptide comprising an immunomodulatory polypeptide that comprises an immune agonist moiety and a metal-hydroxide binding polypeptide and a metal hydroxide. In some embodiments, a method of treating a subject with a tumor comprises administering a fusion polypeptide comprising: an immunomodulatory polypeptide that comprises an immune agonist moiety and a metal-hydroxide binding polypeptide, wherein a fusion polypeptide is formulated with a metal hydroxide.

[0229] In some embodiments, a complex as described herein is administered as a monotherapy. In some embodiments, a complex as described herein is administered in combination with a second therapeutic. In some embodiments, a complex as described herein is administered to a subject wherein a subject has received or is receiving therapy with at least one additional therapeutic.

[0230] Fusion polypeptides and/or preparations and/or complexes thereof of the present disclosure, among other things, are useful for treating a subject with a tumor. Non-limiting examples of diseases associated with a tumor include cancer (*e.g.*, carcinoma,

sarcoma, metastatic diseases or hematopoietic neoplastic disorders). A tumor, including a metastatic tumor, can arise from a plurality of primary tumor types. For example, and without limitation, in some embodiments, a tumor or metastatic tumor can arise from a primary tumor of the kidney (*e.g.*, renal cell carcinoma), head and neck (*e.g.*, head and neck squamous cell carcinoma), prostate, breast (*e.g.*, triple-negative), colon, skin (*e.g.*, melanoma, merkel cell carcinoma, cutaneous T-cell lymphoma, cutaneous squamous cell carcinoma, basal cell carcinoma), lung (*e.g.*, non-small cell lung cancer), and pancreas. Accordingly, fusion polypeptides and preparations thereof disclosed herein, including fusion polypeptide metal-hydroxide complexes and preparations thereof, can be administered to subject who has cancer.

[0231] It will be appreciated by those skilled in the art that amounts of a fusion polypeptide-metal hydroxide complex, fusion polypeptide or a preparation thereof sufficient to reduce tumor growth and size, or a therapeutically effective amount, will vary not only on the particular compounds or preparations selected, but also with the route of administration, the nature of the condition being treated, and the age and condition of the patient, and will ultimately be at the discretion of the patient's physician or pharmacist and/or based upon clinical guidelines. The length of time during which the compounds used in the instant method will be given varies on an individual basis and/or be based upon clinical guidelines.

[0232] In some embodiments, a method of treating a subject with a tumor (*e.g.*, cancer) comprises a step of treating the subject with a complex comprising a fusion polypeptide comprising an immunomodulatory polypeptide that comprises an immune agonist moiety and a metal-hydroxide binding polypeptide and a metal hydroxide. In some embodiments, a fusion polypeptide and a metal-hydroxide are formulated together. Formulated together, for example, comprises a pre-formed complex of fusion polypeptide and metal-hydroxide. In some embodiments, a fusion polypeptide and metal-hydroxide are mixed immediately prior to administration.

[0233] In some embodiments, a method of treating a subject with a tumor (*e.g.*, cancer) comprises treating a subject with a complex wherein a complex is administered by intratumoral injection. In some embodiments, a method of treating a subject with a tumor (*e.g.*, cancer) comprises treating a subject with a complex wherein a complex is

administered by peritumoral injection. In some embodiments, a method of treating a subject with a tumor (*e.g.*, cancer) comprises treating a subject with a complex wherein a complex is administered to a tumor-draining lymph node or lymph nodes.

[0234] Methods of the present invention often involve administration of a therapeutically effective amount of a particular agent. A therapeutically effective amount is an amount sufficient to achieve (in principle, for a subject of comparable characteristics, such as species, body type, size, extent of disease or disorder, degree or type of symptoms, history of responsiveness, and/or overall health) an intended biological or medical response or therapeutic benefit in a tissue, system or subject. For example, a desirable response may include one or more of: delaying or preventing the onset of a medical condition, disease or disorder, slowing down or stopping the progression, aggravation, or deterioration of the symptoms of the condition, bringing about ameliorations of the symptoms of the condition, and curing the condition.

[0235] When combinations of therapeutic agents are administered, the amount of any individual agent required in the combination may be different from the amount required of that same agent to achieve its therapeutic effect alone. In some cases, synergies between or among therapeutic agents used in a combination may reduce amounts required; in other cases, inhibitory interactions may increase amounts required. Thus, in general, therapeutically effective amounts of a combination of agents may utilize different absolute amounts of the agents than constitute therapeutically effective amounts of the agents individually.

Combination therapies

[0236] In some embodiments, fusion polypeptide metal-hydroxide complexes or preparations thereof as disclosed herein are administered in combination with a second therapeutic agent. A second therapeutic agent may be selected from a variety of available anti-tumor agents known in the art. In some embodiments, a second therapeutic agent is administered prior to administration of a fusion polypeptide metal-hydroxide complex. In some embodiments, a second therapeutic agent is administered concurrently with a fusion polypeptide metal-hydroxide complex. In some embodiments, a second therapeutic agent is administered after administration with a fusion polypeptide metal-hydroxide complex.

[0237] For example, in some embodiments, a second therapeutic is radiation (*e.g.*, ionizing radiation). In some embodiments, an amount of ionizing radiation administered is between about 1 Gy and about 1,000 Gy, about 5 Gy and about 900 Gy, about 10 Gy to about 800 Gy, about 10 Gy to about 700 Gy, about 10 Gy to about 600 Gy, about 10 Gy to about 500 Gy, about 10 Gy to about 400 Gy, about 10 Gy to about 300 Gy, about 10 Gy to about 200 Gy, about 10 Gy to about 100 Gy, about 5 Gy and about 15 Gy, between about 7.5 Gy and about 12 Gy, or between about 10 Gy and about 12 Gy. In some embodiments, an amount of ionizing radiation administered is about 12 Gy. In some embodiments, an amount of ionizing radiation is greater than about 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 200, 300, 400, 500, or 1,000 Gy. In some embodiments, an amount of ionizing radiation is less than about 1,000, 900, 800, 700, 600, 500, 400, 300, 200, 100, 90, 80, 70, 60, or 50 Gy.

[0238] For example, in some embodiments, a second therapeutic agent is a chemotherapeutic agent. In some embodiments, a chemotherapeutic agent may be a targeted therapy (*e.g.*, BRAF inhibitor, MEK inhibitor, etc.). In some embodiments, a chemotherapeutic agent may be any approved chemotherapeutic agent. For example, and without limitation, a chemotherapeutic agent can be one or more of adriamycin, anastrozole, cyclophosphamide, docetaxel, doxifluridine, doxorubicin, erlotinib, fluorouracil, gemcitabine, imatinib, iressa, letrozole, methotrexate, paclitaxel, tarceva, and trastuzumab. A chemotherapeutic agent may be administered according to any approved and/or known regimen in the art.

[0239] For example, in some embodiments, a second therapeutic agent is an anti-tumor antibody. In some embodiments, an anti-tumor antibody is an immune modulator. In some embodiments, an immune modulator is a checkpoint inhibitor. In some embodiments, a checkpoint inhibitor is an antibody or a functional fragment thereof. In some embodiments, an antibody targets one or more of PD-1, PD-L1, CTLA-4, TIM3, TIGIT, and/or LAG3. In some embodiments, an antibody targets PD-1 (*e.g.*, pembrolizumab). An anti-tumor antibody may be administered according to any approved and/or known regimen in the art.

[0240] For example, in some embodiments, a second therapeutic agent is a surgical tumor resection. In some embodiments, a fusion polypeptide metal-hydroxide complex is

administered prior to surgical tumor resection. In some embodiments, a fusion polypeptide metal-hydroxide complex is administered to tissue after tumor resection, which tissue may include, for example, remaining tumor (*e.g.*, tumor cells). In some embodiments, a fusion polypeptide metal-hydroxide complex is administered to tissue which cannot be removed by surgical tumor resection, or tissue proximal to the resection, during said resection.

[0241] For example, in some embodiments, a second therapeutic agent is or comprises cell therapy. In some embodiments, a cell therapy is or comprises natural killer (NK) cells. In some embodiments, a cell therapy is or comprises tumor infiltrating lymphocytes (TILs). In some embodiments, a cell therapy is or comprises macrophages or other myeloid cells. In some embodiments, a cell therapy is or comprises cells that have been expanded *ex vivo*. In some embodiments, a cell therapy is or comprises Chimeric Antigen Receptor (CAR) effector cell therapy (*e.g.*, CAR T cells). CARs are genetically-engineered, artificial transmembrane receptors, which confer a selected specificity for a ligand of choice onto an immune effector cell (*e.g.* a T cell, natural killer cell or other immune cell) and which results in activation of the effector cell upon recognition and binding to the ligand. Often, such ligand specificity is achieved by engineering the antigen specificity of a monoclonal antibody into the CAR, thereby targeting the CAR T cell to the antigen recognized by the antibody.

[0242] In some embodiments, chimeric antigen receptor-expressing effector cells (*e.g.*, CAR-T cells) are cells that are derived (*e.g.*, isolated) from a patient with a disease or condition and genetically modified *in vitro* to express at least one CAR with an arbitrary specificity to a ligand. The cells perform at least one effector function (*e.g.* induction of cytokines) that is stimulated or induced by the specific binding of the ligand to the CAR and that is useful for treatment of the same patient's disease or condition. The effector cells may be T cells (*e.g.* cytotoxic T cells or helper T cells). One skilled in the art, reading the present disclosure, will appreciate that, in some embodiments, cells other than T cells (*e.g.*, natural killer cells, stem cells, *etc*) may be engineered to express CARs, so that a chimeric antigen receptor effector cell may comprise an effector cell other than a T cell. In some embodiments, a CAR effector cell is a T cell (*e.g.* a cytotoxic T cell); in some embodiments, such CAR-T cell exerts its effector function (*e.g.* a cytotoxic T cell response) on a target cell

when brought in contact or in proximity to the target or target cell (e.g. a cancer cell) (see e.g., Chang and Chen (2017) Trends Mol Med 23(5):430-450). In some embodiments, a cell therapy (e.g., a CAR effector cell therapy) utilizes Tumor Infiltrating Lymphocytes (TILs). TILs target cancer cells. In some embodiments, TILs are isolated from a subject with cancer and expanded *ex vivo*. In some such embodiments, TILs are isolated and expanded *ex vivo* after surgical resection of the tumor. In some embodiments, before administration of TILs, a subject is treated with a lymphodepleting conditioning regimen (Rohaan, Maartje W et al. "Adoptive cellular therapies: the current landscape." *Virchows Archiv: an international journal of pathology* vol. 474,4 (2019): 449-461).

[0243] In some embodiments, a cell therapy (e.g., a CAR effector cell therapy) utilizes Natural Killer (NK) cells. Natural killer (NK) cells are an essential part of tumor immunosurveillance, evidenced by higher cancer susceptibility and metastasis in association with diminished NK activity in mouse models and clinical studies. In some embodiments, for example, using an array of germline-encoded surface receptors, NK cells are able to recognize and rapidly act against malignant cells without prior sensitization (Gu, S., Galat, V., Galat, Y. et al. NK cell-based cancer immunotherapy: from basic biology to clinical development. *J Hematol Oncol* 14, 7 (2021)).

[0244] In some embodiments, fusion polypeptide metal-hydroxide complexes or preparations thereof as disclosed herein are administered to a subject who has received or is receiving a therapy with at least one additional therapeutic. An additional therapeutic agent may be selected from a variety of anti-tumor agents known in the art. In some embodiments, an additional therapeutic agent is administered prior to administration of a fusion polypeptide metal-hydroxide complex. In some embodiments, an additional therapeutic agent is administered concurrently with a fusion polypeptide metal-hydroxide complex. In some embodiments, an additional therapeutic agent is administered after administration with a fusion polypeptide metal-hydroxide complex.

[0245] For example, in some embodiments, an additional therapeutic is radiation (e.g., ionizing radiation). In some embodiments, an amount of ionizing radiation administered is between about 1 Gy and about 1,000 Gy, about 5 Gy and about 900 Gy, about 10 Gy to about 800 Gy, about 10 Gy to about 700 Gy, about 10 Gy to about 600 Gy,

about 10 Gy to about 500 Gy, about 10 Gy to about 400 Gy, about 10 Gy to about 300 Gy, about 10 Gy to about 200 Gy, about 10 Gy to about 100 Gy, about 5 Gy and about 15 Gy, between about 7.5 Gy and about 12 Gy, or between about 10 Gy and about 12 Gy. In some embodiments, an amount of ionizing radiation administered is about 12 Gy. In some embodiments, an amount of ionizing radiation is greater than about 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 200, 300, 400, 500, or 1,000 Gy. In some embodiments, an amount of ionizing radiation is less than about 1,000, 900, 800, 700, 600, 500, 400, 300, 200, 100, 90, 80, 70, 60, or 50 Gy.

[0246] For example, in some embodiments, an additional therapeutic agent is a chemotherapeutic agent. In some embodiments, an additional therapeutic agent is or comprises a targeted therapy (e.g., BRAF inhibitor, MEK inhibitor, etc.). In some embodiments, a chemotherapeutic agent may be any approved chemotherapeutic agent. For example, and without limitation, a chemotherapeutic agent can be one or more of adriamycin, anastrozole, cyclophosphamide, docetaxel, doxorubicin, erlotinib, fluorouracil, gemcitabine, imatinib, iressa, letrozole, methotrexate, paclitaxel, tarceva, and trastuzumab. A chemotherapeutic agent may be administered according to any approved and/or known regimen in the art. In some embodiments, an additional therapeutic agent is an anti-tumor antibody. In some embodiments, an anti-tumor antibody is an immune modulator. In some embodiments, an immune modulator is a checkpoint inhibitor. In some embodiments, a checkpoint inhibitor is an antibody or a functional fragment thereof. In some embodiments, an antibody targets one or more of PD-1, PD-L1, CTLA-4, TIM3, TIGIT, and/or LAG3. In some embodiments, an antibody targets PD-1 (e.g., pembrolizumab). An anti-tumor antibody may be administered according to any approved and/or known regimen in the art. For example, in some embodiments an additional therapeutic agent is or comprises cell therapy. In some embodiments, a cell therapy is or comprises Chimeric Antigen Receptor (CAR) effector cell therapy (e.g., CAR T cells). CARs are genetically-engineered, artificial transmembrane receptors, which confer a selected specificity for a ligand of choice onto an immune effector cell (e.g. a T cell, natural killer cell or other immune cell) and which results in activation of the effector cell upon recognition and binding to the ligand. Often, such ligand specificity is achieved by

engineering the antigen specificity of a monoclonal antibody into the CAR, thereby targeting the CAR T cell to the antigen recognized by the antibody.

[0247] In some embodiments, chimeric antigen receptor-expressing effector cells (*e.g.*, CAR-T cells) are cells that are derived (*e.g.*, isolated) from a patient with a disease or condition and genetically modified *in vitro* to express at least one CAR with an arbitrary specificity to a ligand. The cells perform at least one effector function (*e.g.*, induction of cytokines) that is stimulated or induced by the specific binding of the ligand to the CAR and that is useful for treatment of the same patient's disease or condition. The effector cells may be T cells (*e.g.*, cytotoxic T cells or helper T cells). One skilled in the art, reading the present disclosure, will appreciate that, in some embodiments, cells other than T cells (*e.g.*, natural killer cells, stem cells, *etc*) may be engineered to express CARs, so that a chimeric antigen receptor effector cell may comprise an effector cell other than a T cell. In some embodiments, a CAR effector cell is a T cell (*e.g.*, a cytotoxic T cell); in some embodiments, such CAR-T cell exerts its effector function (*e.g.*, a cytotoxic T cell response) on a target cell when brought in contact or in proximity to the target or target cell (*e.g.*, a cancer cell) (see *e.g.*, Chang and Chen (2017) Trends Mol Med 23(5):430-450). In some embodiments, a cell therapy (*e.g.*, a CAR effector cell therapy) utilizes Tumor Infiltrating Lymphocytes (TILs). TILs target cancer cells. In some embodiments, TILs are isolated from a subject with cancer and expanded *ex vivo*. In some such embodiments, TILs are isolated and expanded *ex vivo* after surgical resection of the tumor. In some embodiments, before administration of TILs, a subject is treated with a lymphodepleting conditioning regimen (Rohaan, Maartje W et al. "Adoptive cellular therapies: the current landscape." *Virchows Archiv: an international journal of pathology* vol. 474,4 (2019): 449-461).

[0248] In some embodiments, a cell therapy (*e.g.*, a CAR effector cell therapy) utilizes Natural Killer (NK) cells. Natural killer (NK) cells are an essential part of tumor immunosurveillance, evidenced by higher cancer susceptibility and metastasis in association with diminished NK activity in mouse models and clinical studies. In some embodiments, for example, using an array of germline-encoded surface receptors, NK cells are able to recognize and rapidly act against malignant cells without prior sensitization (Iu, S., Galat,

V., Galat4, Y. *et al.* NK cell-based cancer immunotherapy: from basic biology to clinical development. *J Hematol Oncol* **14**, 7 (2021)).

[0249] In some embodiments, a cell therapy (e.g., a CAR effector cell therapy) comprises myeloid cells. In some embodiments, myeloid cells are or comprise macrophages. Macrophages have been shown to take up alum.

Exemplification

Example 1: Optimization of exemplary metal-hydroxide binding polypeptides (e.g., alum binding polypeptide (ABP)) sequence for increased phosphorylation and aluminum hydroxide binding

Methods

[0250] *Gene synthesis* - Genes were synthesized at ATUM Bio (Newark, CA) and cloned into the pD2610-v10 vector. Human Fam20C (Uniprot Q8IXL6) was cloned with its native signal peptide and a linker-KDEL sequence (GGGSKDEL) for intracellular retention fused at the c-terminus. Single-chain human IL12 constructs were cloned with a human IL2 signal peptide and mature human IL12B/p40 (Uniprot P29460) fused to mature human IL12A/p35 (Uniprot P29459) through a (G₄S)₃ linker. Single-chain mouse IL12 constructs were cloned with a human IL2 signal peptide and mature mouse IL12B/p40 (Uniprot P43432) fused to mouse IL12A/p35 (Uniprot P43431) through a (G₄S)₃ linker. Various alum binding polypeptide (ABP) sequences were genetically fused to the c-terminus of mouse and human IL12 followed by a His₆ tag for affinity purification.

[0251] *Polypeptide expression and purification* - Plasmids encoding human or mouse IL12 constructs were transiently transfected in suspension HEK-293 cells either with or without co-transfection with the human Fam20C-KDEL plasmid. In most co-transfections, a 4:1 mass ratio of IL12 plasmid to Fam20C-KDEL plasmid was used. Supernatants were harvested and IL12 fusion polypeptides purified by affinity chromatography on NiSepharose Excel (Cytiva 17-3712-02). Eluted fusion polypeptides were formulated in Tris-buffered saline (TBS), pH 7.4 and purity determined on a Perkin Elmer GXII capillary electrophoresis system. Polypeptide aggregation was assessed by HPLC-SEC with a 300Å pore size. Where needed, proteins were further purified by FPLC-SEC on a HiLoad 16/600 or 26/600 Superdex 200 pg column (Cytiva 28-9893-36) and monomeric peak fractions were pooled.

[0252] In some cases, fusion polypeptides were further polished by anion exchange chromatography to enrich for highly phosphorylated species. Fusion polypeptides in TBS were diluted 3-fold in WFI and loaded on a HiTrap Q Sepharose column. Samples were washed with 1% Tx-13 in 0.33x TBS followed by 15 column volumes of 20 mM Tris, pH

8.1. Samples were eluted with a linear gradient from 20 mM Tris, pH 8.1 to 20 mM Tris, pH 8.1, 600 mM NaCl over 20 column volumes. Selected fractions were pooled and buffer exchanged into TBS.

[0253] *Malachite green assay* - The average number of phosphate molecules per polypeptide was determined using the Pierce Phosphoprotein Estimation Assay Kit (23270) according to manufacturer's instructions. The phosphorylated protein standard Phosvitin was resuspended in TBS and diluted from 100 to 2.5 $\mu\text{g/mL}$ to generate a standard curve. Each test polypeptide was diluted in TBS to 300 $\mu\text{g/mL}$ and 100 $\mu\text{g/mL}$. 50 μL of each standard or test agent was mixed with 50 μL of 2.0N NaOH in a flat bottom, 96 well plate (Griener 655101) for alkaline hydrolysis of phosphate from seryl and threonyl residues. Samples were incubated at either 65°C for 30 minutes or 37°C for 1 hour, then neutralized by adding 50 μL of 4.7N HCL to each well and mixing for 30 seconds on a shaker. 50 μL of phosphate reagent comprised of one volume ammonium molybdate solution and 3 volumes of malachite green solution was then added to each well and mixed for 30 seconds. Samples were incubated at room temperature for 30 minutes, then absorbance measured at 650 nm. The number of phosphate molecules per test agent was derived based on the known phosphate content in the Phosvitin standard curve.

[0254] *Aluminum hydroxide (alum) retention assay* - Binding and retention of fusion polypeptides to aluminum hydroxide was tested *in vitro*. Test polypeptides at a final concentration of 100-250 $\mu\text{g/mL}$ in TBS were mixed with a 10-fold mass excess of aluminum hydroxide as defined by metal mass (Invivogen Cat# alu-vac-250) or TBS only as a control to a final volume of 50 μL . Fusion polypeptide/alum mixtures were resuspended thoroughly by pipetting and incubated at room temperature for 30 minutes. Halfway through the incubation, the mixtures were resuspended again by pipetting. The fusion polypeptide/alum mixtures or polypeptide only controls were then diluted 20x in elution buffer containing a final concentration of 1 mM phosphate, 20% mouse serum to a final volume of 1 mL. Diluted samples were incubated at 37°C with gentle rotating for 24-48 hours. At each timepoint, 50 μL of sample was removed and centrifuged at 18,000xg for 10 minutes to pellet the aluminum hydroxide. Cleared supernatant was transferred to a new tube and stored at 4°C until ready for analysis. The concentration of free polypeptide in

each supernatant sample was quantified using a mouse IL12p70 ELISA kit (R&D Systems m1270). All dilutions were made in TBS + 1% BSA + 0.1% Tween-20. Test agents were used for standard curves with a top concentration of 1 ng/mL and 2x dilutions and supernatant samples were diluted to a theoretical concentration of 1 ng/mL and 0.5 ng/mL if all polypeptide was released.

[0255] *SAX-10 HPLC assay* - Fusion polypeptide phosphorylation was also assessed by analytical anion-exchange chromatography on a Thermo ProPac SAX-10 column (4 x 250 mM, 10 μ m). Samples were diluted 4-fold in 20 mM Tris, pH 7.1 and loaded on the column at a 1.0 mL/min flow rate. Samples were eluted over a linear gradient from 20 mM Tris, pH 7.1 to 20 mM Tris, 525 mM NaCl, pH 7.1 over 28 minutes. In some cases, fusion polypeptides were dephosphorylated with lambda protein phosphatase (New England Biolabs, P0753L) prior to running on the column. 50 μ g of fusion polypeptide was incubated with 10x PMP buffer, 10 mM $MnCl_2$, and 2-4 μ L phosphatase at 30°C for 30-60 minutes.

[0256] *Syngeneic tumor models* - B16F10 cells were grown in DMEM + 10% FBS at 37°C, 5% CO_2 . C57BL/6 mice aged 7-9 weeks were inoculated with 1×10^6 B16F10 cells in 0.1 mL of PBS solution at the right flank region. When tumors reached ~ 75 mm³, mice were randomized and injected intratumorally with 20 μ L vehicle or 5-7.7 μ g mIL12-ABP that had been mixed and pre-incubated with 25-50 μ g aluminum hydroxide as defined by metal mass for 30 minutes at room temperature. In some groups, 200 μ g anti-PD1 (Bioxcell BP0273) in a 50 μ L volume was injected intraperitoneally on days 0, 3, 6, and 9. Tumor volume and body weight were measured 3x weekly for the duration of the study and mice were euthanized when their tumor volume reach 2000 mm³. In some studies, two tumors were inoculated in each animal with 1×10^6 B16F10 cells injected in the right flank and 1×10^5 cells injected in the left flank. In these dual flank studies, the mIL12-ABP/alum complex or controls were injected in the larger right flank tumor only.

Results

[0257] Single-chain human and mouse IL12 constructs were generated containing the mature IL12B and IL12A sequences linked by a (G₄S)₃ linker with a c-terminal His6 tag for affinity purification. Alum binding polypeptide containing variants (IL12-ABP) were

cloned by inserting nucleotide sequences encoding various ABP sequences c-terminal to IL12 and before the His tag. A first set of human and mouse IL12-ABP fusion polypeptides were transiently expressed in 100 mL HEK cultures either with or without co-transfection with a plasmid encoding human Fam20C kinase with a c-terminal KDEL sequence for intracellular retention. Fusion polypeptides were purified by Ni-NTA chromatography and average phosphorylation levels per fusion polypeptide assessed by malachite green assay (**Figure 3A**). hIL12-ABP and mL12-ABP fusion polypeptides co-expressed with Fam20C (denoted as “-K”) had significantly higher phosphate levels compared to fusion polypeptides without Fam20C co-expression or wild-type hIL12 or mL12 lacking the ABP co-expressed with Fam20C. While all ABP sequences were phosphorylated, fusion polypeptides with the ABP10 sequence had reproducibly higher phosphorylation levels than the other ABP variants tested.

[0258] Mouse IL12-ABP variants were expressed at 1 L scale in transient HEK with Fam20C co-expression and purified by sequential Ni-NTA and SEC chromatography. In the malachite green assay, mL12-ABP10 again had the highest phosphorylation followed in order by ABP20-G4-GS20, ABP20-G4, and ABP20 (**Figure 3B**). Mouse IL12-ABP fusion polypeptides were then tested in a dual flank B16F10 syngeneic tumor model in combination with systemic PD-1 blockade. 5 µg mL12 or mL12-ABP was mixed with 25 µg aluminum hydroxide as defined by metal mass and incubated for 30 minutes prior to injection into the right flank tumor on Day 6 post tumor implantation. All mL12-ABP / alum complexes delayed tumor growth compared to PD-1 alone or mL12 + PD-1, but the greatest delays were observed with mL12-ABP10 suggesting that increased phosphorylation can improve *in vivo* efficacy after a single injection when complexed with alum (**Figures 4A-4G**).

[0259] To further enhance ABP phosphorylation, alum retention, and *in vivo* efficacy, a larger panel of mL12-ABP constructs was generated with ABP sequences listed in **Figure 5** differing in number of SXE sites, spacing of SXE sites, flanking residues, and linker sequences. Polypeptides were co-transfected with Fam20C in HEK cells and purified by Ni-NTA chromatography. Phosphorylation levels of the purified polypeptides were characterized in the malachite green assay (**Figure 6A** and **Figure 6B**). While all ABP's

had significantly higher phosphorylation compared to negative controls, the highest phosphorylation levels were detected in mIL12 fusion polypeptides comprising ABP10, ABP20-G8-GS, ABP20-G4-6x, and ABP20-G4-8x.

[0260] To reduce the chance of off-target phosphorylation of additional serines in the ABP sequence, the linkers for ABP20-G4-6x and ABP20-G4-8x were changed from GGGGSGGGG to GGGGEGGGG and the serine immediately upstream of the His-tag was removed. These new sequences were referred to as ABP20-G4-6x-GE and ABP20-G4-8x-GE (**Figure 5**). mIL12 fused to ABP10, ABP20-G4-6x-GE, or ABP20-G4-8x-GE was co-expressed with Fam20C at 1 L scale in transient HEK. Fusion polypeptides were purified by sequential Ni-NTA affinity and size exclusion chromatography. All fusion polypeptides ran as a single peak on SDS-PAGE and SEC demonstrating that there was no significant degradation or aggregation (**Figures 7A-7C**). The three exemplary fusion polypeptides were tested in an alum retention assay in which fusion polypeptides were incubated with a 10-fold mass excess of aluminum hydroxide as defined by metal mass for 30 minutes in TBS then diluted in elution buffer containing 5 mM phosphate and 20% mouse serum for 24 hours. While all 3 fusion polypeptides had significantly greater retention on alum compared to unmodified IL12, mIL12-ABP20-G4-6x-GE was eluted more quickly than both mIL12-ABP10 and mIL12-ABP20-G4-8x-GE (**Figure 8**).

[0261] Following transient co-transfection with Fam20C in HEK cells, there is potential for heterogeneity in phosphate levels between IL12-ABP molecules within a given fusion polypeptide sample. In order to generate more homogeneously phosphorylated material, mIL12-ABP10 and mIL12-ABP20-G4-8x-GE were further purified by anion exchange chromatography which is able to separate different phospho-species on the basis of the added negative charge with more heavily phosphorylated fusion polypeptides binding tighter to the column and eluting later. Samples were eluted with a linear salt gradient and the second half of each elution peak was collected and pooled to eliminate early eluting fractions containing lower levels of phosphorylation (**Figures 9A-9B**). The enriched pools of mIL12-ABP10 and mIL12-ABP20-G4-8x-GE after preparative anion exchange chromatography were compared to the original material by analytical ion exchange chromatography (**Figures 10A-10C**). An additional sample of each fusion polypeptide was

enzymatically dephosphorylated with phosphatase and run as a control. Prior to anion exchange polishing, both samples have an early eluting fraction that partially overlaps with the dephosphorylated trace. However, after ion exchange polishing, the samples have a more homogeneous profile that elutes later than the dephosphorylated fusion polypeptide suggesting enrichment for fusion polypeptides with higher phosphorylation.

[0262] Ion-exchange enriched mIL12-ABP10 and mIL12-ABP20-G4-8x-GE were tested in the alum retention assay along with a sample of mIL12-ABP20-G4-8x-GE prior to ion-exchange polishing (**Figure 11A**). Polypeptides were complexed with a 10-fold mass excess of aluminum hydroxide as defined by metal mass then diluted in elution buffer containing 5 mM phosphate and 20% mouse serum for 24 hours before quantifying free polypeptide in the supernatant. The ion exchange purified mIL12-ABP20-G4-8x-GE had the highest retention with 89% bound to alum at 24 hours compared to 86% for mIL12-ABP10 and 78% for the non-ion exchanged material demonstrating that the low phosphate fractions elute off the alum faster. In a second alum retention assay, samples were bound to a 10-fold mass excess of aluminum hydroxide as defined by metal mass then eluted with 5 mM phosphate and 20% mouse serum for up to 48 hours (**Figure 11B**). The ion exchange purified mIL12-ABP20-G4-8x-GE again had the highest retention with 82% bound to alum at 48 hours compared to 78% for mIL12-ABP10 and 17% for unmodified mIL12.

Example 2: Cellular activity of exemplary fusion-polypeptide-metal hydroxide complex

Methods

[0263] *HEK-Blue-IL12 potency assay* - *In vitro* IL12 signaling activity was assessed using the HEK-Blue-IL12 reporter assay (Invivogen hkb-il12) according to manufacturer's instructions. This cell line is derived from HEK293 cells stably transfected with human IL12R β 1 and hIL12R β 2 and a secreted alkaline phosphatase (SEAP) reporter under the control of a STAT4 inducible promoter. Since mouse IL12 cross-reacts with the human IL12 receptors, this cell line can be used to assess potency of both human and mouse IL12 derived constructs. HEK-Blue-IL12 cells were cultured in DMEM + 4.5 g/l glucose, 2 mM L-glutamine, 10% heat inactivated FBS, Pen-Strep (100 U/mL) and 100 ug/mL

Normocin and passaged at 70-80% confluency. For potency testing, the same media was used without Normocin.

[0264] Test agents or IL12 controls were diluted in assay media to generate a titration series with a top concentration of 10 µg/mL and 3x dilutions. For samples mixed with alum, fusion polypeptides at a final concentration of 50 µg/mL were mixed with a 10x mass excess of aluminum hydroxide as defined by metal mass in TBS and incubated at room temperature for 30 minutes with shaking before diluting in assay media as above. 20 µL of each sample in the titration series was transferred to a 96 well plate and mixed with 180 µL of HEK-Blue-IL12 cell suspension (280,000 cells/mL) for a final top fusion polypeptide concentration of 1 µg/mL and 50,000 cells/well. Plates were then incubated overnight at 37°C in 5% CO₂. The next day, 20 µL of supernatant from each well was transferred to a new plate and mixed with 180 µL of QUANTI-Blue solution (Invivogen rep-qbs), a colorimetric reagent that turns blue in the presence of secreted alkaline phosphatase. Plates were incubated for at 37°C for 3 hours, then absorbance measured at 620-655 nm.

[0265] In some experiments, test agents in TBS were mixed with aluminum hydroxide to a final concentration of 200 µg/mL fusion polypeptide and 2 mg/mL aluminum hydroxide as defined by metal mass, then incubated at RT for 30 minutes. Mixtures were then diluted 5x in elution buffer to a final concentration of 40 µg/mL fusion polypeptide with 1 mM phosphate and 20% mouse serum and incubated at 37°C with rotating for 24 hours. Samples were centrifuged at 18,000xg at 4°C for 10 minutes to pellet alum and the supernatant carefully removed and saved. Pellets were resuspended in an equal volume of elution buffer. Supernatant and resuspended alum pellets were then diluted in assay media and tested for activity in the HEK-Blue-IL12 assay as described above.

Results

[0266] mIL12-ABP10 and mIL12-ABP20-G4-8x-GE were tested in a HEK-Blue-IL12 reporter assay that expresses SEAP in response to IL12 induced signaling. The IL12-ABP fusion polypeptides were titrated either alone or after mixing with a 10-fold mass excess of aluminum hydroxide as defined by metal mass in TBS and incubating for 30 minutes. Both fusion polypeptides were active in the assay with EC₅₀ values of 3.6 and 5.4 ng/mL for mIL12-ABP10 and mIL12-ABP20-G4-8x-GE, respectively, compared to 3.5-5.4

ng/mL for unmodified mIL12 (**Figures 12A-12B**). When bound to alum the EC50 values shifted ~2-4 fold with EC50 values of 8 ng/mL for mIL12-ABP10 and 22 ng/mL for mIL12-ABP20-G4-8x. The potency shift is likely a product of steric hindrance in the way the IL12 is presented while complexed with aluminum hydroxide.

[0267] To assess activity of the alum bound and eluted fractions, mIL12-ABP20-G4-8x-GE was complexed with a 10-fold mass excess of aluminum hydroxide as defined by metal mass then incubated in elution buffer containing 1 mM phosphate and 20% mouse serum for 24 hours. The alum was then pelleted by centrifugation, supernatant removed, and the pellet resuspended in an equal volume of elution buffer. The supernatant fraction, resuspended alum pellet, and a control sample of mIL12-ABP20-G4-8x-GE incubated overnight in elution buffer without alum were tested in the HEK-Blue-IL12 assay. While the control sample without alum had a similar EC50 to previous measurements at 7 ng/mL, the supernatant fraction had an EC50 >1000 ng/mL demonstrating that minimal IL12 eluted off the alum during the extended incubation. In contrast, the alum pellet was active in the assay with a potency shift of ~5x demonstrating that IL12 remains active while retained on alum for extended time (**Figure 13**).

Example 3: Use of fusion-polypeptide-metal hydroxide complex as monotherapy and in combination therapy

Methods

[0268] **Syngeneic tumor models** - CT26 cells were cultured in RPMI1640 + 10% FBS. BALB/c mice aged 7-9 weeks were inoculated subcutaneously with 5×10^5 CT26 cells in 0.1 mL PBS at the right flank region. When tumors reached ~75 mm³, mice were randomized and injected intratumorally with 20 μ L vehicle or 5 μ g mIL12-ABP that had been mixed and pre-incubated with 50 μ g aluminum hydroxide as defined by metal mass for 30 minutes at room temperature. Tumor volume and body weight were measured 3x weekly for the duration of the study and mice were euthanized when their tumor volume reach 2000 mm³. Tumor volumes were measured in two dimensions using a caliper, and the volume was calculated using the formula: $V = (L \times W \times W)/2$, where V is tumor volume, L is tumor

length (the longest tumor dimension) and W is tumor width (the longest tumor dimension perpendicular to L).

[0269] B16F10 cells were grown in DMEM + 10% FBS at 37°C, 5% CO₂. C57BL/6 mice aged 7-9 weeks were inoculated with 1x10⁶ B16F10 cells in 0.1 mL of PBS solution at the right flank region. When tumors reached ~75 mm³, mice were randomized and injected intratumorally with 20 µL vehicle or 7.7 µg mIL12-ABP that had been mixed and pre-incubated with 50 µg aluminum hydroxide as defined by metal mass for 30 minutes at room temperature. In some groups, 200 µg anti-PD1 (Bioxcell BP0273) in a 50 µL volume was injected intraperitoneally on days 0, 3, 6, and 9. Tumor volume and body weight were measured 3x weekly for the duration of the study and mice were euthanized when their tumor volume reach 2000 mm³.

[0270] 4T1 cells were grown in RPMI1640 + 10% FBS at 37°C, 5% CO₂. BALB/c mice aged 7-9 weeks were inoculated in the right mammary fat pad with 3x10⁵ 4T1 cells in 0.1 mL PBS. When tumors reached ~75 mm³, mice were randomized and injected intratumorally with 20 µL vehicle or 5 µg mIL12-ABP that had been mixed and pre-incubated with 50 µg aluminum hydroxide as defined by metal mass for 30 minutes at room temperature. Tumor volume and body weight were measured 3x weekly. On day 28 post-tumor inoculation, mice were euthanized and metastases counted in the lung.

Results

[0271] mIL12-ABP10 and mIL12-ABP20-G4-8x-GE were compared in multiple syngeneic tumor models. Fusion polypeptides were mixed with a 10-fold mass excess of aluminum hydroxide as defined by metal mass in TBS and incubated at RT for 30 minutes to form a fusion polypeptide-aluminum hydroxide complex prior to injecting in the animals. In the CT26 colorectal cancer model, a single intratumoral injection of 5 µg of either mIL12-ABP or mIL12-ABP20-G4-8x-GE fusion polypeptides complexed with 50 µg alum as defined by metal mass on Day 6 post tumor inoculation led to significant tumor delays and regressions compared to vehicle treated mice. Complete responses with no measurable tumor were observed in 4/10 mice treated with mIL12-ABP10 + alum and 5/10 mice treated with mIL12-ABP20-G4-8x-GE + alum (**Figures 14A-14C**).

[0272] In a second study in the refractory B16F10 tumor model, 7.7 µg of either mIL12-ABP10 or mIL12-ABP20-G4-8x-GE complexed with 50 µg alum as defined by metal mass was injected intratumorally on Day 6 and 13 following tumor inoculation. In some animals, anti-PD1 antibody was also administered intraperitoneally on Day 0, 3, 6, & 9. Both mIL12-ABP agents complexed with alum significantly delayed tumor growth compared to vehicle. The tumor delay was further extended in groups receiving the combination treatment with systemic PD-1 blockade. In both the monotherapy and PD-1 combination groups, the median survival was longer in mice treated with mIL12-ABP20-G4-8x-GE compared to mIL12-ABP10 (**Figures 15A-15E**).

[0273] mIL12-ABP20-G4-8x-GE was also tested in an orthotopic 4T1 model that forms spontaneous lung metastases (**Figures 16A-16B**). Tumor-bearing mice were treated with a single intratumoral injection of 5 µg of mIL12-ABP fusion polypeptide complexed with 50 µg alum as defined by metal mass on Day 7 post tumor inoculation. mIL12-ABP20-G4-8x-GE /alum treatment led to growth delay of the primary tumor compared to IT injection of vehicle (**Figure 16A**). On Day 28, animals were sacrificed and metastases counted in the lung. While 10/10 vehicle treated mice had at least one lung metastasis, 6/10 of the mice in the mIL12-ABP20-G4-8x-GE /alum treated mice were metastases-free demonstrating that local injection of the mIL12-ABP/alum complex had also induced anti-tumor effects against distal, non-injected lesions (**Figure 16B**).

Example 4: Identification of an optimal level of phosphorylation on mIL12-ABP

[0274] mIL12-ABP20-G4-8x-GE was co-expressed with Fam20C-KDEL transiently in HEK as described above. Fusion polypeptide was purified by Ni-NTA chromatography and size exclusion chromatography, then run on a HiTrap Q Sepharose anion exchange column with a linear salt elution gradient. Individual elution fractions were collected and phosphate levels measured by malachite green assay. Average phosphate levels per fraction ranged from 2 to 7 with lower phosphorylated fusion polypeptide eluting earlier and highly phosphorylated fractions retained longer on the column (**Figures 17A-17B**). Individual fractions were then assessed in the alum retention assay. Fusion polypeptides were bound to a 10-fold mass excess of aluminum hydroxide as defined by metal mass then eluted in

solution containing 1 mM phosphate and 50% mouse serum. There was a clear trend where fractions eluting earlier off the anion exchange resin and containing lower phosphorylation levels had lower retention on alum compared to later eluting fractions with higher phosphorylation levels. Fraction 110 with 2.4 phosphates per fusion polypeptide had <50% fusion polypeptide retained on alum at 24 hours and fraction 111 with 3.7 phosphates per fusion polypeptide had ~80% fusion polypeptide retained on alum. Fractions 112 and 113 with 4.6 and 5.8 phosphates per fusion polypeptide respectively were highly retained with >95% bound to alum at 24 hours. Fractions 114-117 with >6 phosphates per fusion polypeptide had no measurable free fusion polypeptide eluting off alum in the assay (**Figures 18A-18B**).

[0275] Individual fractions were also tested in the HEK-Blue-IL12 reporter assay either alone or complexed with a 10-fold mass excess of aluminum hydroxide as defined by metal mass (**Figures 19A-19D**). Fractions 110 and 112 had similar peak activity levels with or without alum and 2-3 fold shifts in EC₅₀ when bound to alum. In contrast, fraction 114 and 117 had reduced peak activity when complexed with alum. This suggests that beyond a certain point, excess phosphorylation on the fusion polypeptide can decrease functional activity in the presence of alum

[0276] Together, these data, summarized in **Figure 20**, suggest that there may be an optimal levels of phosphorylation per murine fusion polypeptide at ~4-6 with less than that having insufficient alum retention and >6 losing activity in functional assays.

Example 5: Production and characterization of tagless human IL12-ABP20-G4-8x-GE

[0277] Genes were synthesized encoding single-chain human IL12-ABP20-G4-8x-GE comprising mature human IL12B/p40 (Uniprot P29460) fused to mature human IL12A/p35 (Uniprot P29459) through a (G₄S)₃ linker with the ABP20-G4-8x-GE peptide at the c-terminus. Unlike the mouse constructs, the fusion polypeptides were cloned without the c-terminal His tag, instead ending in a single serine residue. Human IL12-ABP20-G4-8x-GE constructs and Fam20C-KDEL genes were cloned into a single vector with two expression cassettes with different promoters selected to express the hIL12-ABP20-G4-8x-GE fusion polypeptide at an 8-fold higher level than Fam20C-KDEL. Fusion polypeptides

were stably transfected in CHO cells using the ATUM Leap-In-Transposase system and stable pools selected. After 14 day expression in a fed-batch culture, supernatants were harvested and purified by multiple chromatography steps including anion exchange chromatography capture and size exclusion chromatography polishing. After purification, the untagged hIL12-ABP20-G4-8x-GE was >97% pure as measured by SDS-PAGE and SEC.

[0278] Human IL12-ABP20-G4-8x-GE is active in the HEK-Blue-IL12 assay with an EC₅₀ of 3.6 ng/mL alone or 8.2 ng/mL when complexed with aluminum hydroxide (**Figure 21A**). Following incubation of the fusion polypeptide /alum complex in elution buffer containing 1 mM phosphate and 20% mouse serum, the supernatant EC₅₀ shifts >300-fold suggesting minimal fusion polypeptide elution after 24 hours, while the pellet maintained activity with a 5x EC₅₀ shift (**Figure 21B**). In a separate alum retention assay, hIL12 or hIL12-ABP was bound to a 10-fold mass excess of aluminum hydroxide as defined by metal mass then diluted in elution buffer containing 1 mM phosphate and 20% mouse serum with free fusion polypeptide detected by ELISA (**Figure 21C**). ~80% of the hIL12-ABP20-G4-8x-GE is retained on the alum at 24 hours compared to 0% for unmodified IL12.

[0279] A second lot of human IL12-ABP20-G4-8x-GE was prepared as described above and further purified by anion exchange chromatography and eluted with a linear salt gradient. Individual elution fractions were collected and phosphate levels were measured by malachite green assay. Average phosphate levels per fraction ranged from 5.8 to 8.8 with lower phosphorylated fusion polypeptide eluting earlier and highly phosphorylated fractions retained longer on the column (**Figure 22**).

[0280] Individual fractions were assessed in an alum retention assay in which fusion polypeptides were bound to a 10-fold mass excess of aluminum hydroxide as defined by metal mass then eluted in solution containing 1 mM phosphate and 40% serum (**Figure 23**). Similar to the murine IL12-ABP20-G4-8x-GE protein, fractions of human IL12-ABP20-G4-8x-GE eluting earlier off the anion exchange resin and containing lower phosphorylation levels had lower retention on alum compared to later eluting fractions with higher phosphorylation levels.

[0281] Individual fractions were also tested in the HEK-Blue-IL12 reporter assay either alone or complexed with a 10-fold mass excess of aluminum hydroxide as defined by metal mass (**Figure 24**). In the absence of aluminum hydroxide complexation, all tested fractions had similar potency in the assay with EC50 values between 3.4 – 3.6 ng/mL. Following aluminum hydroxide complexation, earlier eluting fractions with lower phosphorylation had lower EC50 values (5 and 6.4 ng/mL for fraction 645 and 650, respectively), while later eluting fractions with higher phosphorylation levels had more significant shifts in the EC50 (8.6 and 14 ng/mL for fractions 655 and 660, respectively). All tested fractions had similar maximal activity in the assay either with or without aluminum hydroxide complexation.

[0282] Together, fractions 650-660 were identified as having a particularly beneficial balance of alum retention and IL12 signaling activity. Thus, preparations characterized by about 5.8 phosphates or more (e.g., about 5.8 to more than about 8.4) per fusion polypeptide showed particularly desirable properties. Additional assessments may be performed to confirm such properties, including, for example, further *in vitro* and/or *in vivo* assessments. In some such studies, fractions 650-660 (or other reasonably comparable preparations – e.g., from another batch, or produced via a different process but achieving comparable phosphorylation characteristics, etc.) may be pooled or otherwise combined for assessment; alternatively or additionally, these fractions (or other reasonably corresponding preparations) may be separately assessed.

Example 6: Production and characterization of canine IL12-ABP20-G4-8x-GE-His

[0283] The present Example documents production and activity of a beneficially phosphorylated preparation of an IL-12 fusion polypeptide as described herein, and of metal hydroxide complexes thereof. Among other things, the present Example documents that preparations of this fusion polypeptide which are characterized by about 5.5 phosphates or more (e.g., about 5.5 to more than about 8.3) per fusion polypeptide showed a particularly desirable balance of alum-binding and IL-12 signaling activities.

[0284] An exemplary gene cassette encoding for single-chain canine-IL12-ABP20-G4-8x-GE was synthesized at ATUM Bio and cloned into the pD2610-v5 expression vector.

The exemplary canine IL12-ABP20-G4-8x-GE sequence comprises mature canine IL12B/p40 (Uniprot Q28268) fused to mature canine IL12A/p35 (Uniprot F1PPC0) through a (G4S)₃ linker with the ABP20-G4-8x-GE peptide and His tag at the C-terminus. The exemplary construct was transiently co-transfected in suspension HEK-293 cells with a human Fam20C-KDEL plasmid at a 4:1 mass ratio. Supernatants were harvested and IL12 fusion polypeptides purified by affinity chromatography on NiSepharose Excel resin (Cytiva 17-3712-02) followed by FPLC-SEC on a HiLoad 16/600 Superdex 200 pg column (Cytiva 28-9893-36) to remove aggregates. The exemplary canine IL12-ABP20-G4-8x-GE protein was further polished by anion exchange chromatography on a HiTrap Q Sepharose column to enrich for differentially phosphorylated species and eluted with a linear salt gradient. Selected fractions were assessed for numbers of phosphate per protein using a malachite green assay, IL12 signaling potency using a HEK-Blue-IL12 assay, and aluminum hydroxide binding using an alum retention assay with protocols described above (**Figure 25**).

[0285] Average phosphate levels in each fraction ranged from 0.4 to 11.3 PO₄/protein with lower phosphorylated proteins eluting earlier and highly phosphorylated proteins retained longer on the column. Individual fractions were then assessed in an alum retention assay in which polypeptides were bound to a 10-fold mass excess of aluminum hydroxide as defined by metal mass then eluted in solution containing 1 mM phosphate and 40% serum with free protein quantified over time using a canine IL12p40 ELISA kit (R&D Systems). A trend was observed where fractions eluting earlier off the anion exchange resin and containing lower phosphorylation levels had lower retention on alum compared to later eluting fractions with higher phosphorylation levels (**Figure 26**). Fractions 91 & 92 with 2.0 and 4.5 PO₄/protein respectively had the lowest retention with 28% of the IL12 polypeptide in fraction 91 and 65% in fraction 92 retained on the alum at 24 hours. Fractions 93 and 93.5 with 5.5 and 6.5 phosphates per fusion polypeptide respectively were highly retained with >95% bound to alum at 24 hours. Fractions 94-98 with >6.5 phosphates per fusion polypeptide had no measurable free fusion polypeptide eluting off alum in the assay.

[0286] Individual fractions were also tested in a HEK-Blue-IL12 reporter assay either alone or complexed with a 10-fold mass excess of aluminum hydroxide as defined by metal mass (**Figure 27**). Fractions had similar EC50 values when tested as free protein but different potency when complexed on alum with fractions containing high levels of PO₄ having larger increases in EC50 after alum complexation. Fractions 93-94.5 were identified as having a particularly beneficial balance of alum retention and IL12 signaling activity. Thus, preparations characterized by about 5.5 phosphates or more (e.g., about 5.5 to more than about 8.3) per fusion polypeptide showed particularly desirable properties. Additional assessments may be performed to confirm such properties, including, for example, further *in vitro* and/or *in vivo* assessments (e.g., in an appropriate animal system – e.g., in this case, in dogs). In some such studies, fractions 93-94.5 (or other reasonably comparable preparations – e.g., from another batch, or produced via a different process but achieving comparable phosphorylation characteristics, etc.) may be pooled or otherwise combined for assessment; alternatively or additionally, these fractions (or other reasonably corresponding preparations) may be separately assessed.

Example 7: Exemplary canine studies

[0287] The present Example documents further assessment of technologies of the present disclosure (e.g., IL-12 fusion polypeptides, fusion polypeptide preparations). For example, the present Example provides further confirmation and/or assessment of phosphate content, metal-hydroxide retention, signaling activity, efficacy, and/or potency, *etc.*. Among other things, the present Example documents assessment and/or further confirmation of use of IL-12 fusion polypeptides and fusion polypeptide preparations of the present disclosure in a subject, including in a non-human subject, including, for example, a rodent, a mouse, a rat, a rabbit, a monkey, a dog, a cat, a horse, a sheep, cattle, a primate, and/or a pig. Technologies of the present disclosure (e.g., IL-12 fusion polypeptides, fusion polypeptide preparations) are assessed using any suitable animal model known in the art (see, e.g., Paoloni M et al. Defining the Pharmacodynamic Profile and Therapeutic Index of NHS-IL12 Immunocytokine in Dogs with Malignant Melanoma. PLoS One. 2015;10(6):e0129954; Cutrera J et al. Safe and effective treatment of spontaneous neoplasms with interleukin 12

electro-chemo-gene therapy. J Cell Mol Med. 2015;19(3):664-675; Cutrera J et al. Safety and efficacy of tumor-targeted interleukin 12 gene therapy in treated and non-treated, metastatic lesions. Curr Gene Ther. 2015;15(1):44-54; Von Rueden SK et al. Cancer-Immunity Cycle and Therapeutic Interventions- Opportunities for Including Pet Dogs With Cancer. Front Oncol. 2021;11:773420. Published 2021 Nov 19).

Equivalents

[0289] Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. The scope of the present invention is not intended to be limited to the above Description, but rather is as set forth in the following claims:

Claims

We claim:

1. A fusion polypeptide comprising:
 - (a) an immunomodulatory polypeptide that comprises an immune agonist moiety; and
 - (b) a metal-hydroxide binding polypeptide whose amino acid sequence includes a plurality of phosphorylation sites, so that it can adopt phosphorylated and unphosphorylated forms.
2. The fusion polypeptide of claim 1, wherein the fusion polypeptide, when exposed to a metal-hydroxide forms a complex therewith.
3. The complex of claim 2, wherein the metal hydroxide is aluminum hydroxide.
4. The complex of claim 2, wherein the complex forms more readily when the metal-hydroxide-binding polypeptide is in a phosphorylated form than when it is in an unphosphorylated form.
5. The complex of claim 4, wherein one or more of the phosphorylation sites is targeted by a Fam20C kinase.
6. The complex of claim 5, wherein the phosphorylation site is or comprises an S-X-E motif.
7. The fusion polypeptide of claim 1, wherein the plurality of phosphorylation sites comprises more than 4 S-X-E motifs.
8. The fusion polypeptide of claim 7, wherein the plurality of phosphorylation sites comprises 8 S-X-E motifs.

9. The fusion polypeptide of claim 7, wherein at least two adjacent S-X-E motifs are separated by a spacer.
10. The fusion polypeptide of claim 9, wherein the spacer comprises at least one glycine residue.
11. The fusion polypeptide of claim 10, wherein the spacer comprises a plurality of glycine residues.
12. The fusion polypeptide of claim 11, wherein the spacer comprises at least four glycine residues.
13. The fusion polypeptide of claim 12, wherein the spacer has a sequence that comprises four glycine residues.
14. The fusion polypeptide of claim 7, wherein each SXE motif is separated from each adjacent S-X-E motif by a spacer.
15. A method of treating a subject with a tumor, the method comprising a step of:
treating the subject with a complex comprising:
 - (a) fusion polypeptide comprising:
 - (i) an immunomodulatory polypeptide that comprises an immune agonist moiety; and
 - (ii) a metal-hydroxide binding peptide; and
 - (b) a metal hydroxide.
16. The method of claim 15, wherein (a) and (b) are formulated together.
17. The method of claim 15, wherein (a) and (b) are mixed prior to administration.

18. The method of claim 15, wherein the complex is administered by intratumoral injection.
19. The method of claim 15, wherein the complex is administered by peritumoral injection.
20. The method of claim 15, wherein the complex is administered to a tumor-draining lymph node or lymph nodes.
21. The method of claim 15, wherein the complex is administered in combination with a second therapeutic.
22. The method of claim 21, wherein the second therapeutic is radiation.
23. The method of claim 21, wherein the second therapeutic is surgical tumor resection.
24. The method of claim 23, wherein the fusion polypeptide is administered prior to surgical tumor resection.
25. The method of claim 21, wherein the second therapeutic is a chemotherapy or targeted therapy.
26. The method of claim 21, wherein the second therapeutic is an anti-tumor antibody.
27. The method of claim 21, wherein the second therapeutic is an immune modulator.
28. The method of claim 27, wherein the immune modulator is a checkpoint inhibitor.
29. The method of claim 28, wherein the checkpoint inhibitor is an antibody or a functional fragment thereof.

30. The method of claim 29, wherein the antibody targets one or more of PD-1, PD-L1, CTLA-4, TIM3, TIGIT, and LAG3.
31. The method of claim 30, wherein the antibody targets PD-1.
32. The method of claim 26, wherein the antibody is a tumor-targeting CD3 bispecific antibody.
33. The method of claim 27, wherein the immune modulator is a cell therapy.
34. The method of claim 33, wherein the cell therapy is selected from the group consisting of: CAR-T cells, ex-vivo expanded TILs, and NK cells.
35. A method of treating a subject with a tumor comprises administering a fusion polypeptide comprising:
(a) an immunomodulatory polypeptide that comprises an immune agonist moiety; and
(b) a metal-hydroxide binding peptide,
wherein the fusion polypeptide is formulated with a metal hydroxide; and
wherein the subject has received or is receiving therapy with at least one additional therapeutic.
36. The method of claim 35, wherein the fusion polypeptide and metal-hydroxide are formulated together, forming a complex.
37. The method of claim 35, wherein the fusion polypeptide and metal-hydroxide are mixed prior to administration.

38. The method of claim 35, wherein the fusion polypeptide is administered by intratumoral injection.
39. The method of claim 35, wherein the fusion polypeptide is administered by peritumoral injection.
40. The method of claim 35, wherein the fusion polypeptide is administered to a tumor-draining lymph node or lymph nodes.
41. The method of claim 35, wherein the at least one additional therapeutic is radiation.
42. The method of claim 35, wherein the at least one additional therapeutic is surgical tumor resection.
43. The method of claim 42, wherein the fusion polypeptide is administered prior to surgical tumor resection.
44. The method of claim 35, wherein the at least one additional therapeutic is a chemotherapy or a targeted therapy.
45. The method of claim 35, wherein the at least one additional therapeutic is an anti-tumor antibody.
46. The method of claim 45, wherein the second therapeutic is an immune modulator.
47. The method of claim 46, wherein the immune modulator is a checkpoint inhibitor.
48. The method of claim 47, wherein the checkpoint inhibitor is an antibody or a functional fragment thereof.

49. The method of claim 48, wherein the antibody targets one or more of PD-1, PD-L1, CTLA-4, TIM3, TIGIT, and LAG3.
50. The method of claim 49, wherein the antibody targets PD-1.
51. The method of claim 45, wherein the antibody is a tumor-targeting CD3 bispecific antibody.
52. The method of claim 46, wherein the immune modulator is a cell therapy.
53. The method of claim 52, wherein the cell therapy is selected from the group consisting of: CAR-T cells, ex-vivo expanded TILs, NK cells, and macrophages.
54. The fusion polypeptide of claim 1, wherein the immune agonist moiety comprises a first moiety or a functional fragment thereof.
55. The fusion polypeptide of claim 54, wherein the functional fragment is signaling competent.
56. The fusion polypeptide of claim 54, wherein the first moiety comprises an IL12 moiety or a functional fragment thereof.
57. The fusion polypeptide of claim 56, wherein the IL12 moiety comprises IL12B or a functional fragment thereof.
58. The fusion polypeptide of claim 1, wherein the immune agonist moiety comprises a first and a second moiety or a functional fragment thereof.

59. The fusion polypeptide of claim 58, wherein the first moiety comprises an IL12 moiety or a functional fragment thereof.
60. The fusion polypeptide of claim 59, wherein the IL12 moiety comprises IL12B or a functional fragment thereof.
61. The fusion polypeptide of claim 58, wherein the second moiety comprises an IL12 moiety or a functional fragment thereof.
62. The fusion polypeptide of claim 61, wherein the second IL12 moiety comprises IL12A or a functional fragment thereof.
63. The fusion polypeptide of claim 58, wherein the first and second moieties or functional fragments thereof are linked via a first linker.
64. The fusion polypeptide of claim 63, wherein the first linker comprises a polypeptide.
65. The fusion polypeptide of claim 64, wherein the polypeptide comprises a (G₄S)₃ linker.
66. The fusion polypeptide of claim 1, wherein an immunomodulatory polypeptide and a metal-hydroxide binding polypeptide are linked via a second linker.
67. The fusion polypeptide of claim 66, wherein the second linker comprises a polypeptide.
68. The fusion polypeptide of claim 67, wherein the polypeptide comprises the amino acid sequence, GGGGEGGGG.
69. The fusion polypeptide of claim 67, wherein the polypeptide comprises the amino acid sequence, GGGGSGGGG.

70. The fusion polypeptide of claim 1, wherein the metal-hydroxide binding polypeptide is linked directly to the c-terminus of the immunomodulatory polypeptide.
71. The fusion polypeptide of claim 1, wherein the metal-hydroxide binding polypeptide is linked via a second linker to the c-terminus of the immunomodulatory polypeptide.
72. A method of manufacturing a phosphorylated form of the fusion polypeptide of claim 1, by contacting the fusion polypeptide with a kinase.
73. The method of claim 72, wherein the contacting comprises co-expressing the fusion polypeptide and a kinase.
74. The method of claim 73, wherein the fusion polypeptide and kinase are co-expressed at a ratio of 2:1 to 100:1.
75. The method of claim 74, wherein the ratio is 4:1.
76. The method of claim 75, wherein the 4:1 ratio is achieved using two separate plasmids to express the fusion polypeptide and the kinase.
77. The method of claim 76, wherein the two separate plasmids comprise promoters of differing strength to produce the ratio of 4:1.
78. The method of claim 74, wherein the ratio is 8:1.
79. The method of claim 78, wherein the 8:1 ratio is achieved using a single vector with two promoters to express the fusion polypeptide and the kinase.

80. The method of claim 72, wherein the kinase is Fam20C.
81. The method of claim 72, the method further comprising a step of purifying the phosphorylated form.
82. The method of claim 81, wherein the step of purifying comprises chromatography.
83. The method of claim 73, wherein the step of co-expressing comprises expressing from promoters established to direct expression at the ratio.
84. The method of claim 73, where the step of co-expressing comprises expressing from a bi-cistronic construct.
85. The method of claim 72, wherein the fusion polypeptide is exposed to a metal-hydroxide to form a complex therewith.
86. The method of claim 85, wherein the complex is prepared prior to administering to a subject.
87. The method of manufacturing a complex by contacting a phosphorylated form of a fusion polypeptide of claim 1 with a metal hydroxide.
88. A complex comprising the fusion polypeptide of claim 1 and a metal hydroxide.
89. The complex of claim 88, wherein the complex comprises an average of 2-8 phosphates per fusion polypeptide.
90. The complex of claim 88, wherein the complex is characterized as having greater than 95% metal hydroxide retention.

91. The complex of claim 88, wherein the complex comprises a ratio of 1:1 to 1:20 by mass of fusion polypeptide to metal hydroxide as defined by metal mass.
92. The complex of claim 91, wherein the ratio is 1:5 to 1:20 by mass of fusion polypeptide to metal hydroxide as defined by metal mass.
93. The complex of claim 91, wherein the ratio is 1:10 by mass of fusion polypeptide to metal hydroxide as defined by metal mass.
94. The complex of claim 91, wherein the ratio is 1:5 by mass of fusion polypeptide to metal hydroxide as defined by metal mass.
95. A pharmaceutical composition comprising the fusion polypeptide of claim 1.
96. The pharmaceutical composition of claim 95, formulated as a fusion polypeptide metal-hydroxide complex.
97. The method of characterizing a preparation of a fusion polypeptide of claim 1 by assessing degree of phosphorylation.
98. The method of claim 97, wherein the degree of phosphorylation is assessed by determining the number of phosphates per protein.
99. The method of claim 98, wherein the number of phosphates per protein is determined using a colorimetric assay.
100. The method of claim 99, wherein the colorimetric assay is a malachite green assay.

101. The method of characterizing a preparation of a fusion polypeptide of claim 1 by assessing heterogeneity of the preparation.
102. The method of claim 101, wherein heterogeneity of the preparation is assessed using analytical ion exchange.
103. The method of characterizing a preparation of a fusion polypeptide of claim 1 by assessing retention of the fusion polypeptide on the metal hydroxide.
104. The method of claim 103, wherein retention is assessed using an *in vitro* retention assay.
105. A method of characterizing a preparation of a fusion polypeptide of claim 1 by assessing signaling activity of the fusion polypeptide.
106. The method of claim 105, wherein the signaling activity is assessed *in vitro*.
107. The method of claim 106, wherein *in vitro* assessment utilizes a reporter assay.
108. The method of characterizing a preparation of a fusion polypeptide of claim 1 comprising assessing purity of the preparation.
109. The method of characterizing a preparation of a fusion polypeptide of claim 1 comprising assessing phosphate content.
110. The method of claim 109, wherein assessing phosphate content comprises use of a malachite green assay.
111. The method of claim 110, wherein assessing phosphate content comprises use of a high performance liquid chromatography assay.

112. The method of claim 111, wherein the high performance liquid chromatography assay utilizes a SAX-10 column.
113. The method of characterizing a preparation of a fusion polypeptide of claim 1 comprising assessing potency of the preparation.
114. The method of claim 113, wherein potency is characterized by assessing immune moiety signaling.
115. The method of claim 114, wherein immune moiety signaling is determined using a reporter assay.
116. The method of claim 114, wherein potency is characterized by assessing IL12 signaling.
117. The method of claim 116, wherein IL12 signaling is determined using a reporter assay.
118. A method of characterizing the complex of claim 88 comprising assessing retention of the fusion polypeptide to the metal hydroxide.
119. The method of claim 118, wherein assessing retention comprises use of a metal hydroxide retention assay.
120. A method of characterizing a pharmaceutical composition of claim 95 comprising assessing one or more of:
- (a) the purity of the preparation;
 - (b) phosphate content;
 - (c) potency of the pharmaceutical composition;
 - (d) retention of the fusion polypeptide to the metal hydroxide;

- (e) efficacy of treating a subject having a tumor; and
- (f) combination with a second therapeutic agent.

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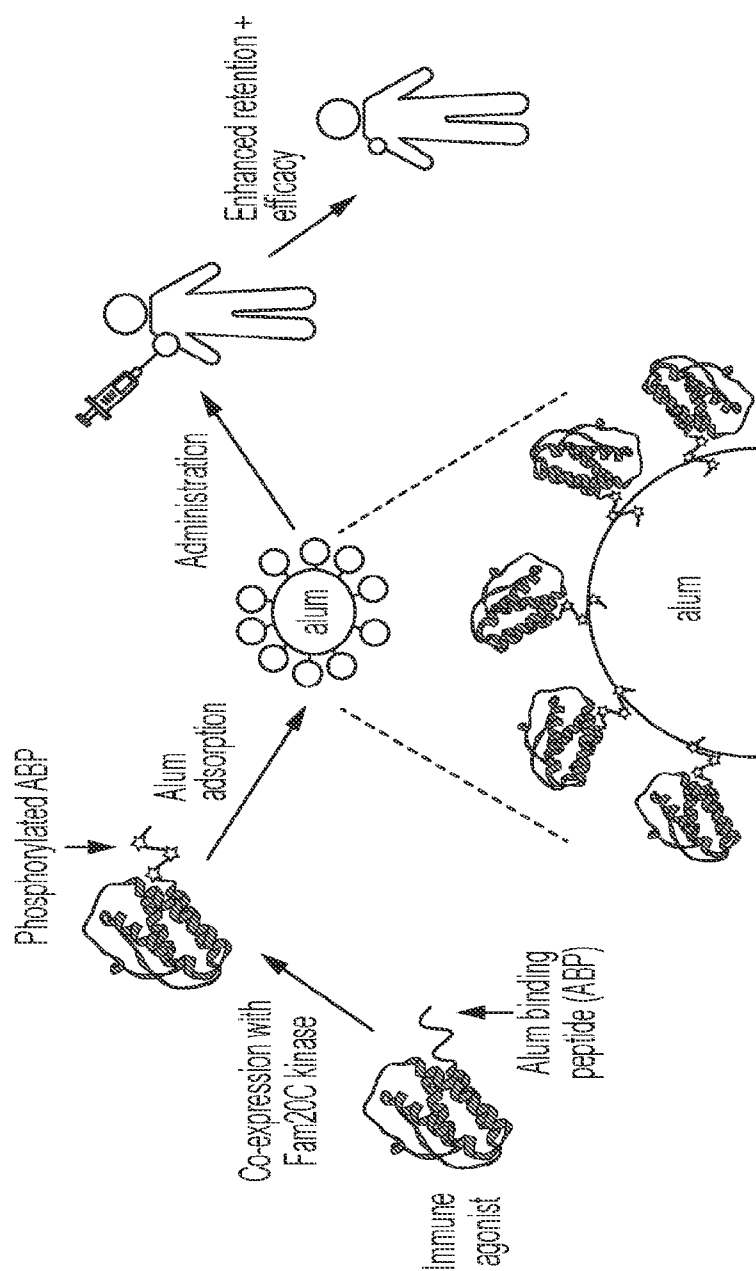


FIG. 1

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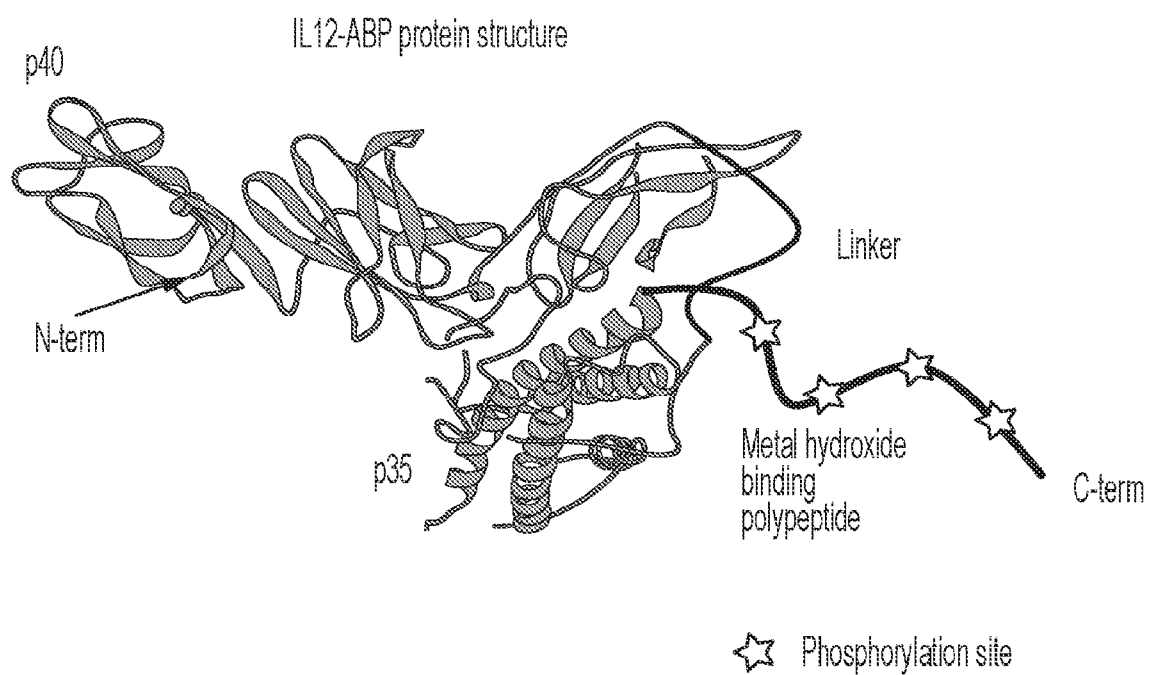


FIG. 2

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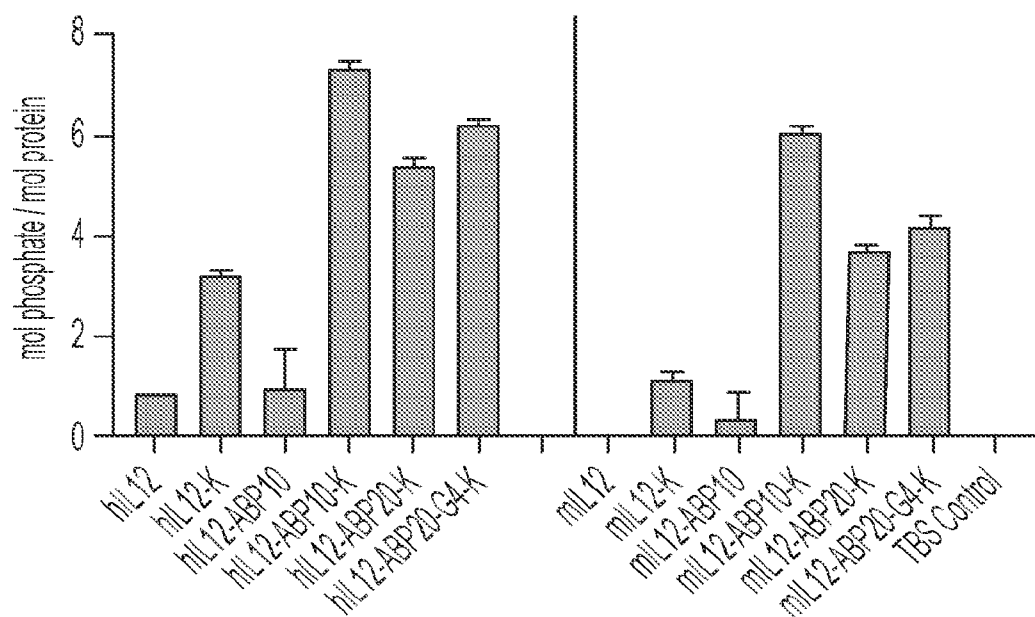


FIG. 3A

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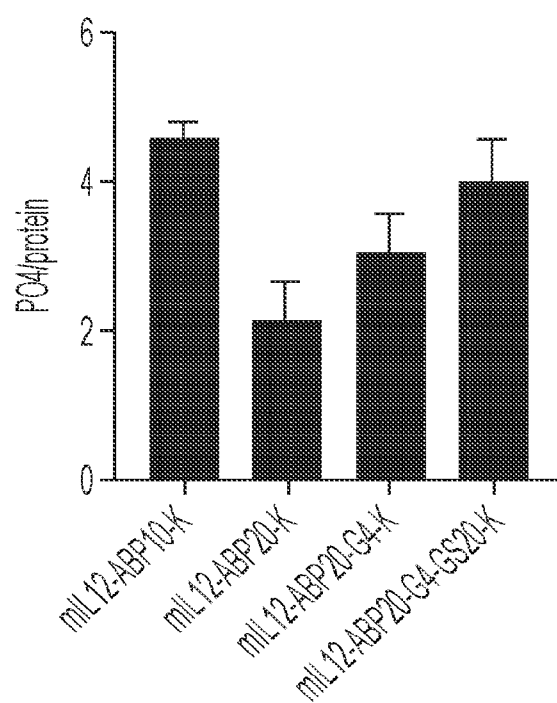


FIG. 3B

Name	Sequence	Tag
ABP10	GGGGSFQSEEQGGGSGGSEEGGMESEESNGGGSGGSEEGGGGS (SEQ ID NO: 90)	HHHHHH (SEQ ID NO: 98)
ABP20	GGGSGGGGSESESESESEEGGGGS (SEQ ID NO: 119)	HHHHHH (SEQ ID NO: 98)
ABP20-G4	GGGSGGGGSEEGGGSEEGGGSEEGGGSEEGGGGS (SEQ ID NO: 82)	HHHHHH (SEQ ID NO: 98)
ABP20-G4-GS20	GGGSGGGGSGGGSGGGSEEGGGSEEGGGSEEGGGSEEGGGGS (SEQ ID NO: 120)	HHHHHH (SEQ ID NO: 98)

FIG. 3C

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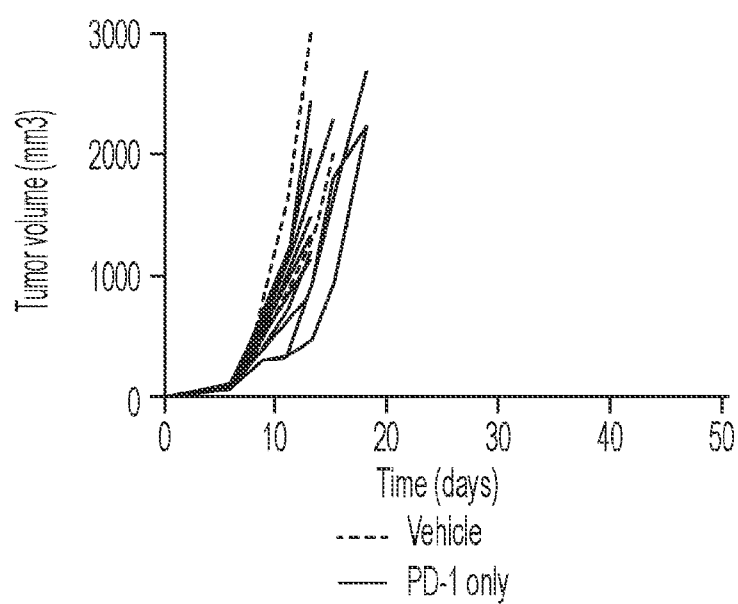


FIG. 4A

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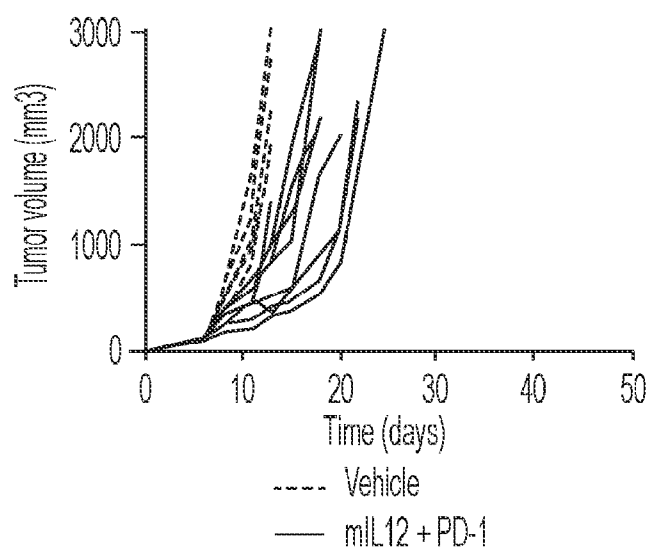


FIG. 4B

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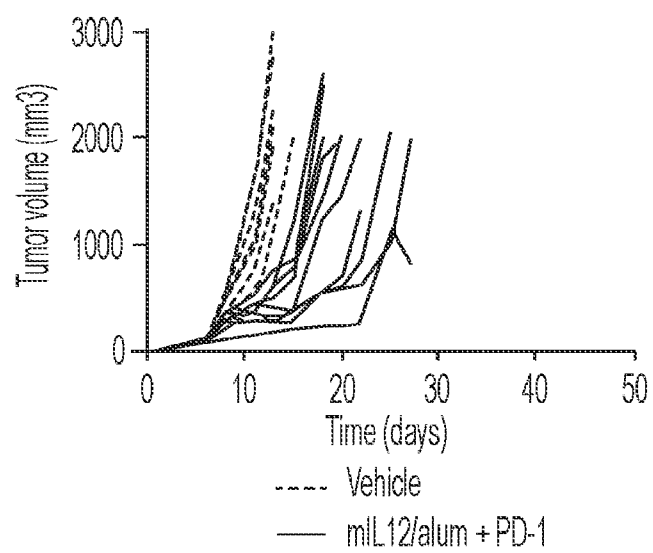


FIG. 4C

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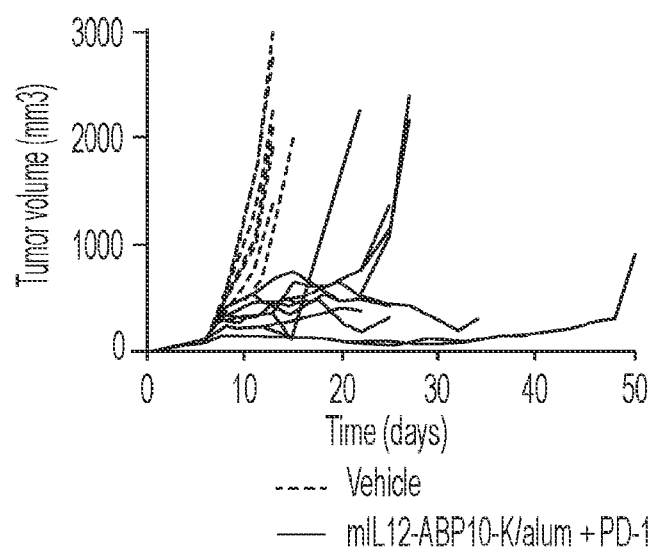


FIG. 4D

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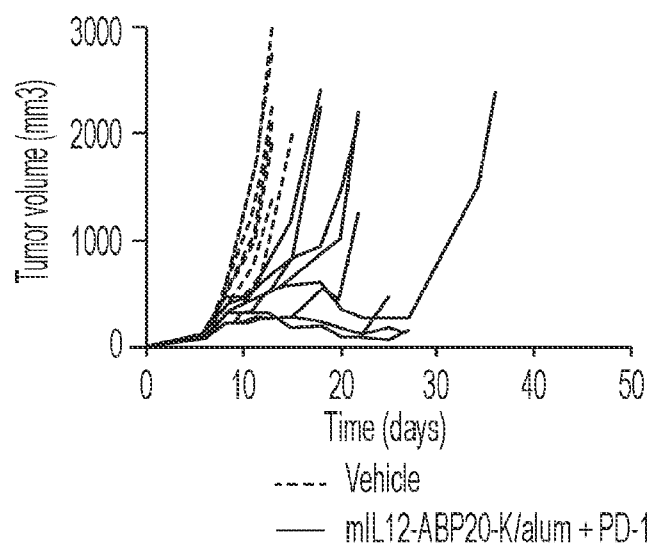


FIG. 4E

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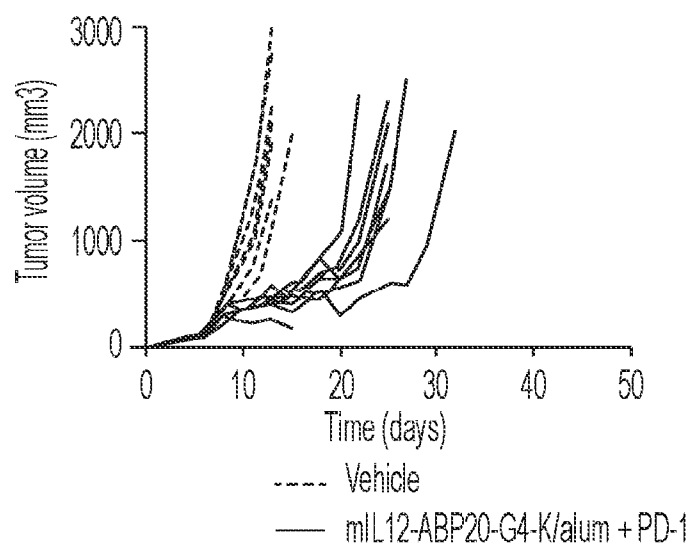


FIG. 4F

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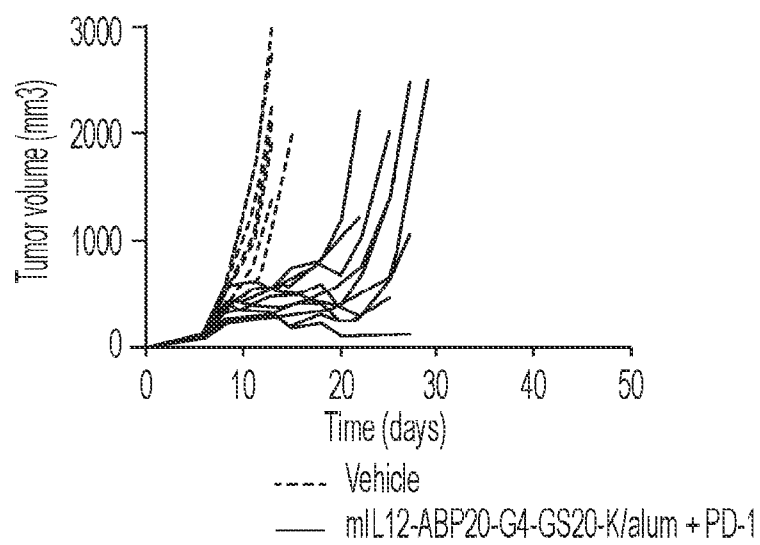


FIG. 4G

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Name	Sequence	Tag
ABP20-G4	GGGSGGGSEHEGGGSEHEGGGSEHEGGGGS (SEQ ID NO: 82)	HHHHHH (SEQ ID NO: 98)
ABP20-G4-6x	GGGSGGGSEHEGGGSEHEGGGSEHEGGGSEHEGGGGS (SEQ ID NO: 83)	HHHHHH (SEQ ID NO: 98)
ABP20-G4-8x	GGGSGGGSEHEGGGSEHEGGGSEHEGGGSEHEGGGSEHEGGGGS (SEQ ID NO: 84)	HHHHHH (SEQ ID NO: 98)
ABP20-6x	GGGSGGGSEHEHESEHESEHESEHEGGGGS (SEQ ID NO: 85)	HHHHHH (SEQ ID NO: 98)
ABP20-8x	GGGSGGGSEHESEHESEHESEHESEHESEHEGGGGS (SEQ ID NO: 86)	HHHHHH (SEQ ID NO: 98)
ABP20-G4-SEEE	GGGSGGGSEHEEGGSEHEEGGSEHEEGGSEHEEGGGS (SEQ ID NO: 87)	HHHHHH (SEQ ID NO: 98)
ABP20-G4-SEEA	GGGSGGGSEHEAGGSEHEAGGSEHEAGGSEHEAGGGS (SEQ ID NO: 88)	HHHHHH (SEQ ID NO: 98)
ABP20-G4-SEEQ	GGGSGGGSEHEQGGSEHEQGGSEHEQGGSEHEQGGGS (SEQ ID NO: 89)	HHHHHH (SEQ ID NO: 98)
ABP10	GGGSGTQSEHQGGSGGSEHEGGMESEHNGGSGGSEHEGGGS (SEQ ID NO: 90)	HHHHHH (SEQ ID NO: 98)
ABP10-GS	GGGSGGSEHEGGCGSGGSEHEGGCGSEHEGGCGSGGSEHEGGGS (SEQ ID NO: 91)	HHHHHH (SEQ ID NO: 98)
ABP10-GE	GGGCGGSEHEGGGGGSGGSEHEGGGSEHEGGGGGSGGSEHEGGGS (SEQ ID NO: 92)	HHHHHH (SEQ ID NO: 98)
ABP20-G8-GS	GGGCGGGSEHEGGSGGGSEHEGGSGGGSEHEGGSGGGSEHEGGGS (SEQ ID NO: 93)	HHHHHH (SEQ ID NO: 98)
ABP20-G8-GE	GGGCGGGSEHEGGCGGGSEHEGGCGGGSEHEGGCGGGSEHEGGGS (SEQ ID NO: 94)	HHHHHH (SEQ ID NO: 98)
ABP20-G4-GE	GGGCGGGSEHEGGGSGGSEHEGGGSEHEGGGSEHEGGGS (SEQ ID NO: 95)	HHHHHH (SEQ ID NO: 98)
ABP20-G4-6x-GE	GGGCGGGSEHEGGGSGGSEHEGGGSEHEGGGSEHEGGGSEHEGGGS (SEQ ID NO: 96)	HHHHHH (SEQ ID NO: 98)
ABP20-G4-8x-GE	GGGCGGGSEHEGGGSGGSEHEGGGSEHEGGGSEHEGGGSEHEGGGGS (SEQ ID NO: 97)	HHHHHH (SEQ ID NO: 98)

FIG. 5

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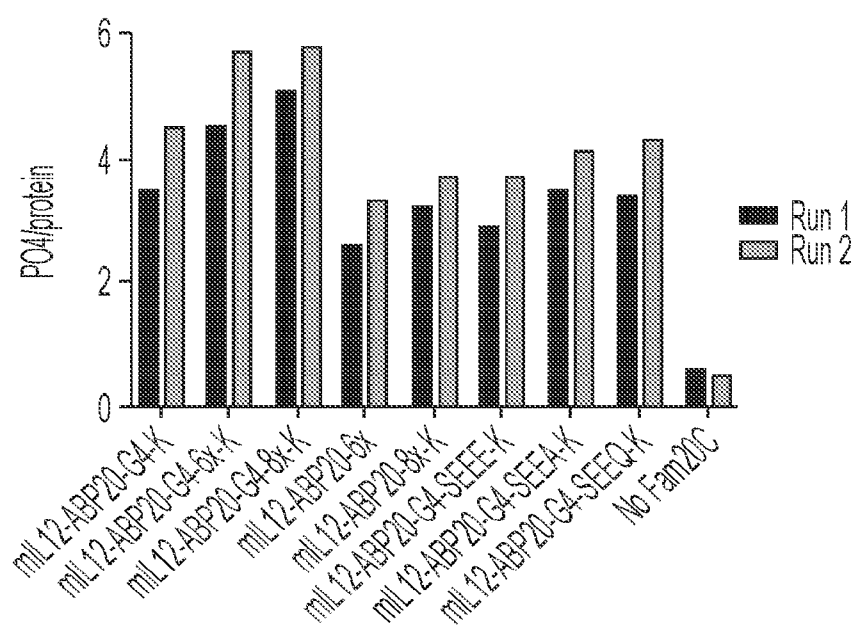


FIG. 6A

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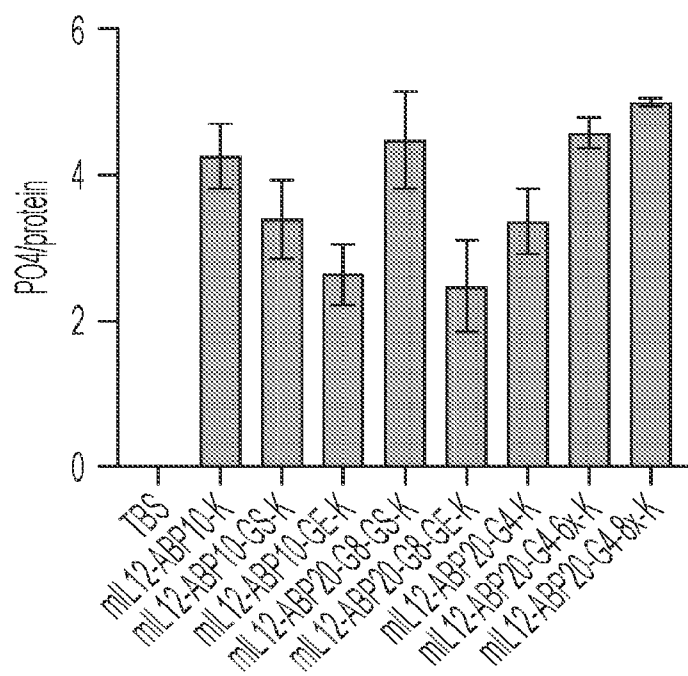


FIG. 6B

mIL12-ABP10

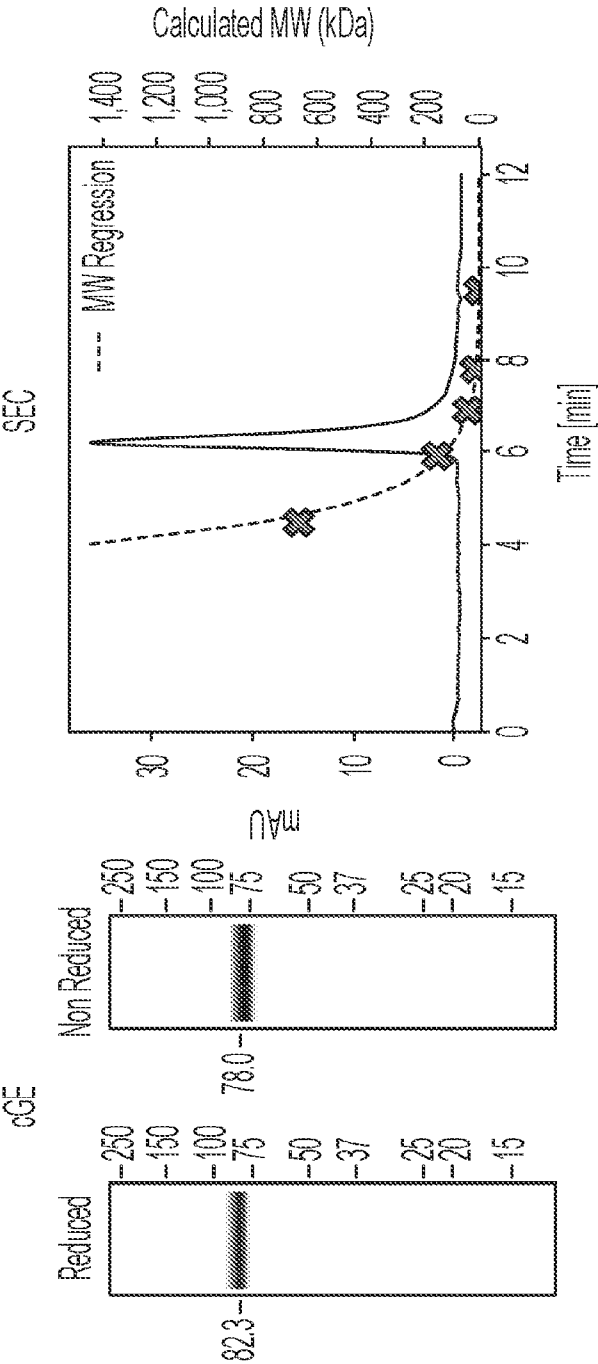


FIG. 7A

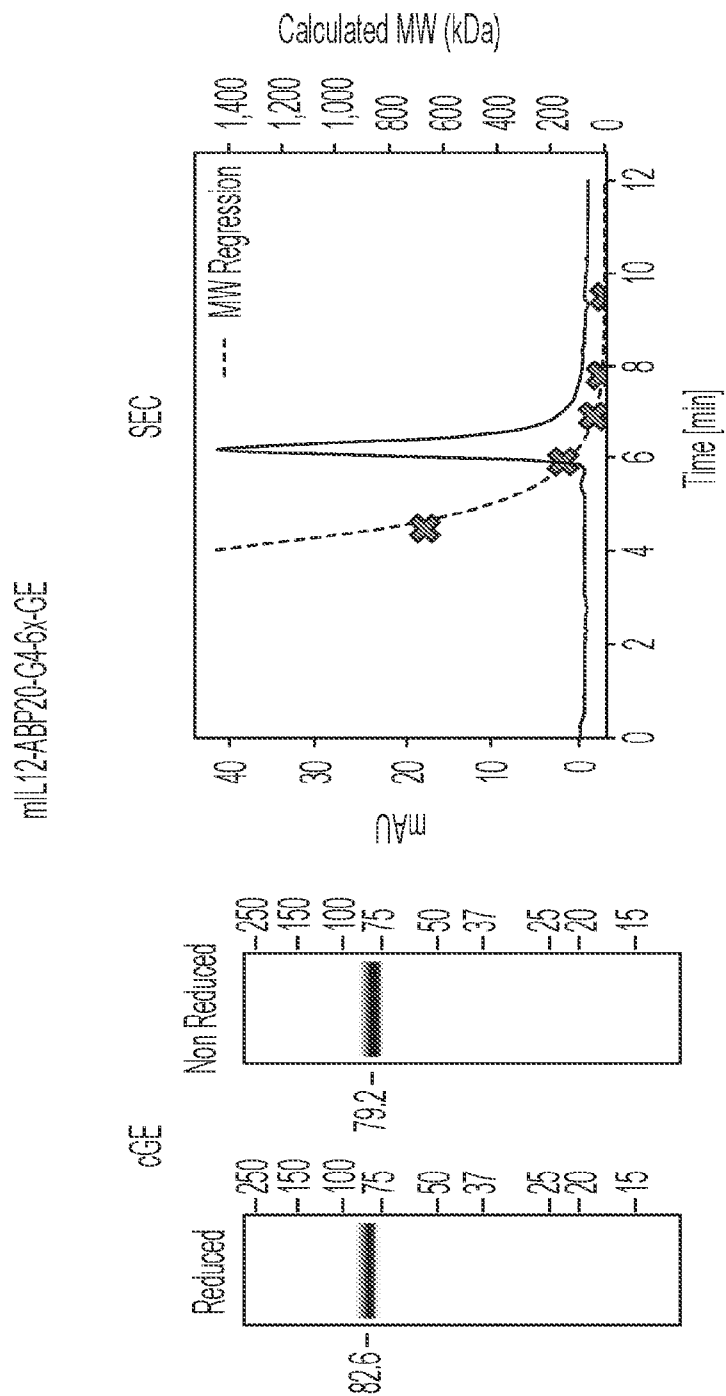


FIG. 7B

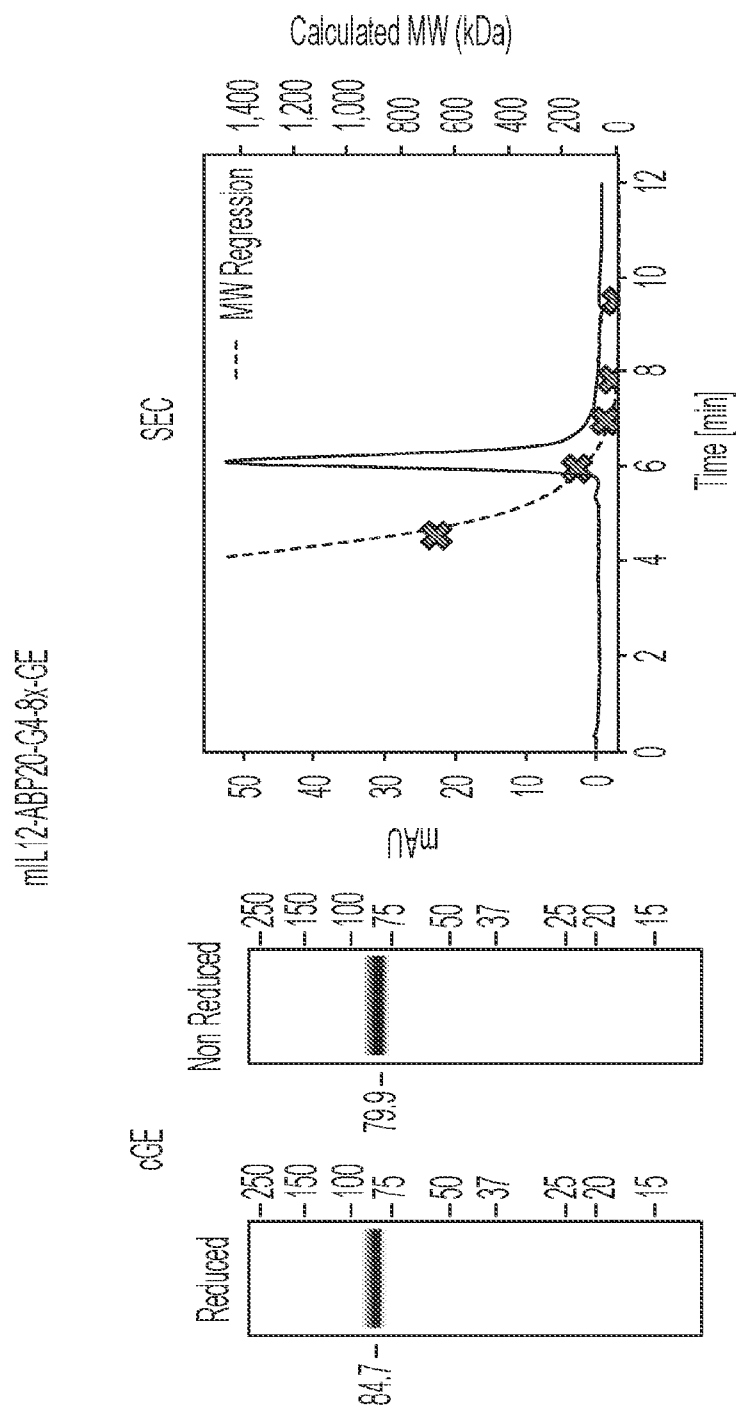


FIG. 7C

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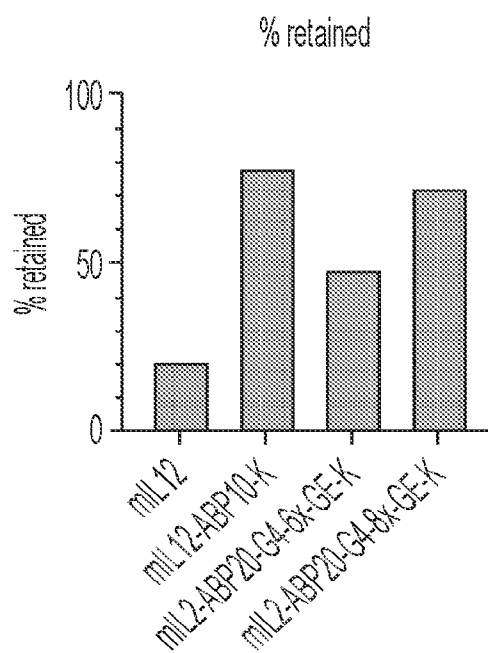


FIG. 8

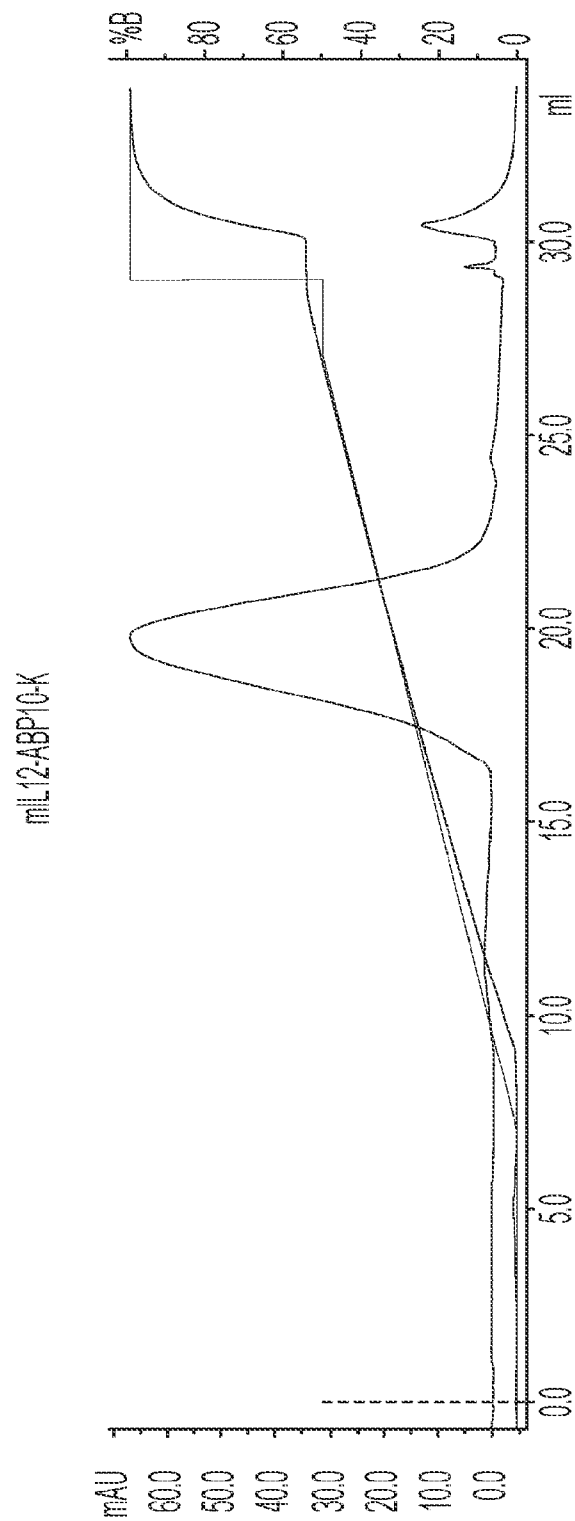


FIG. 9A

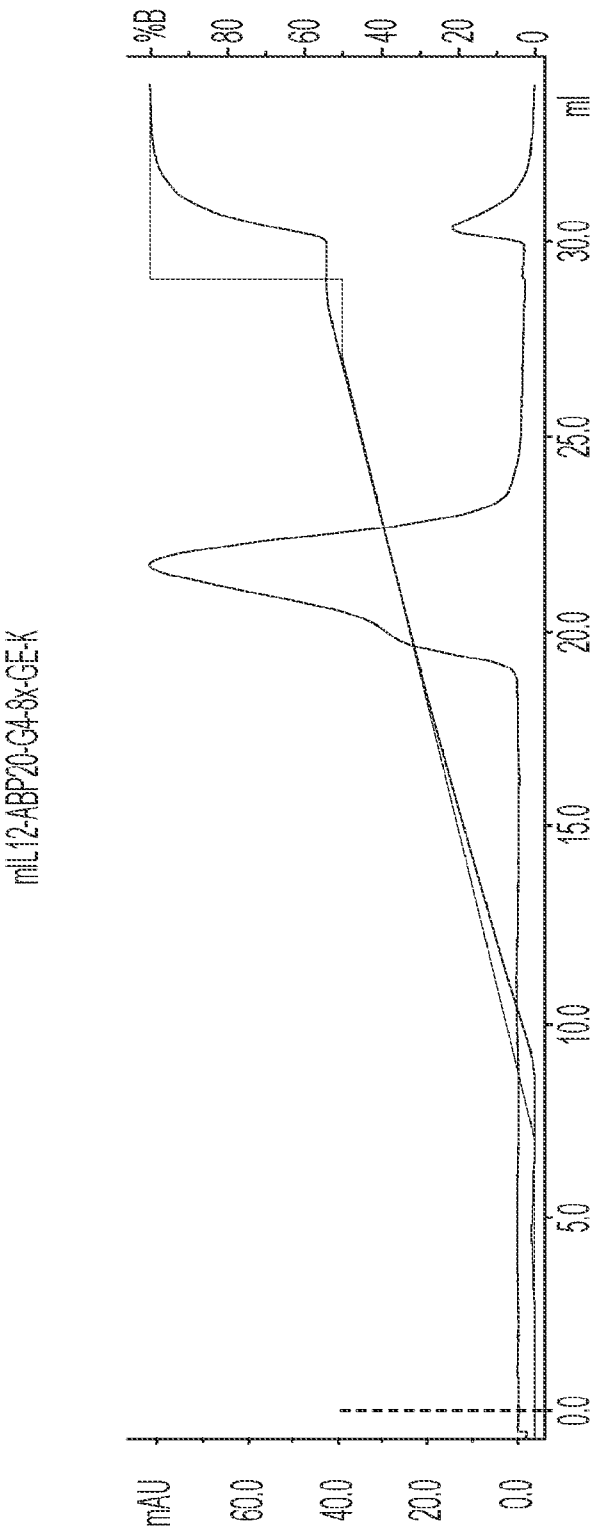


FIG. 9B

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mIL12-ABP10

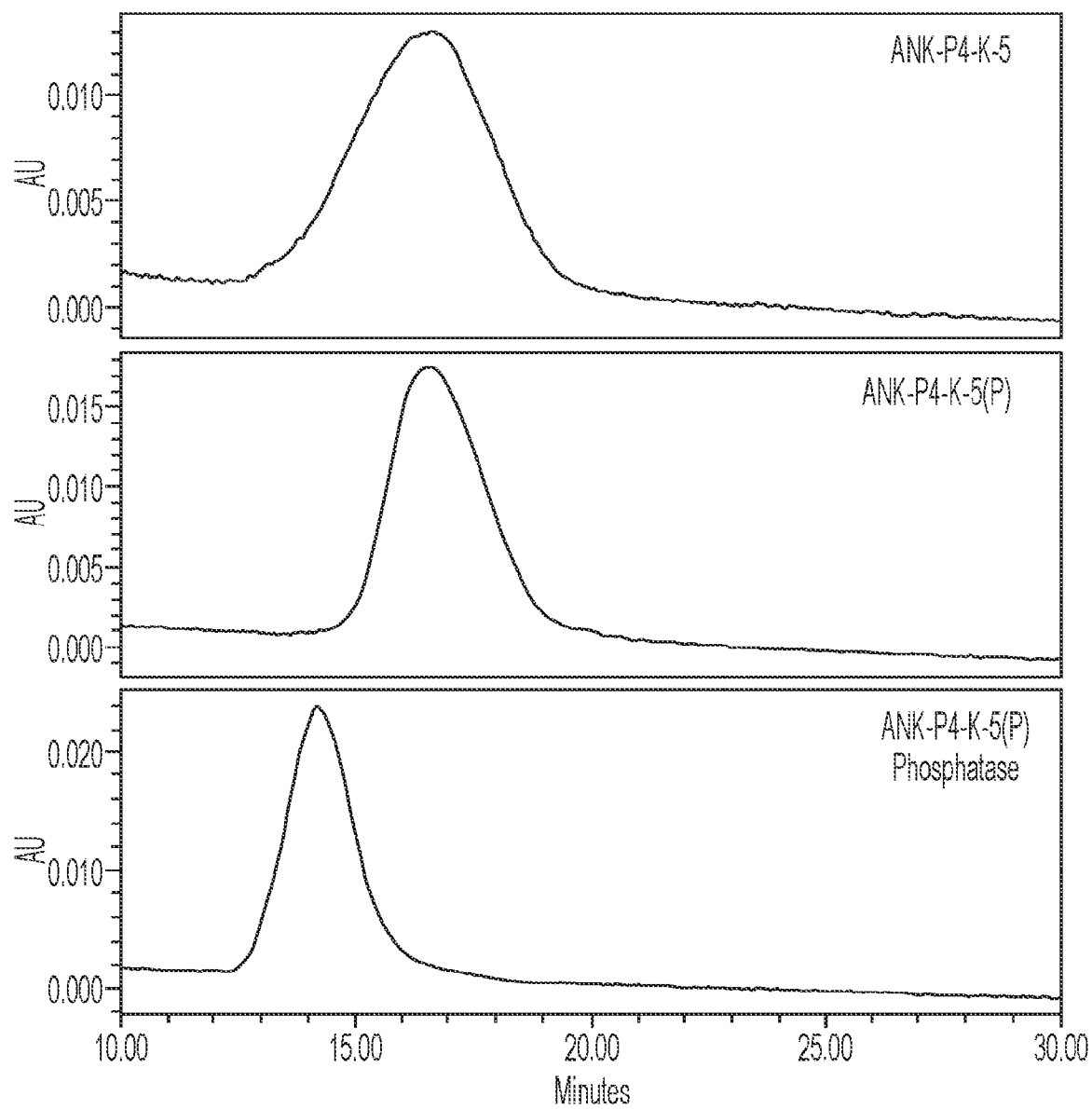


FIG. 10A

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mIL12-ABP20-G4-8x-GE

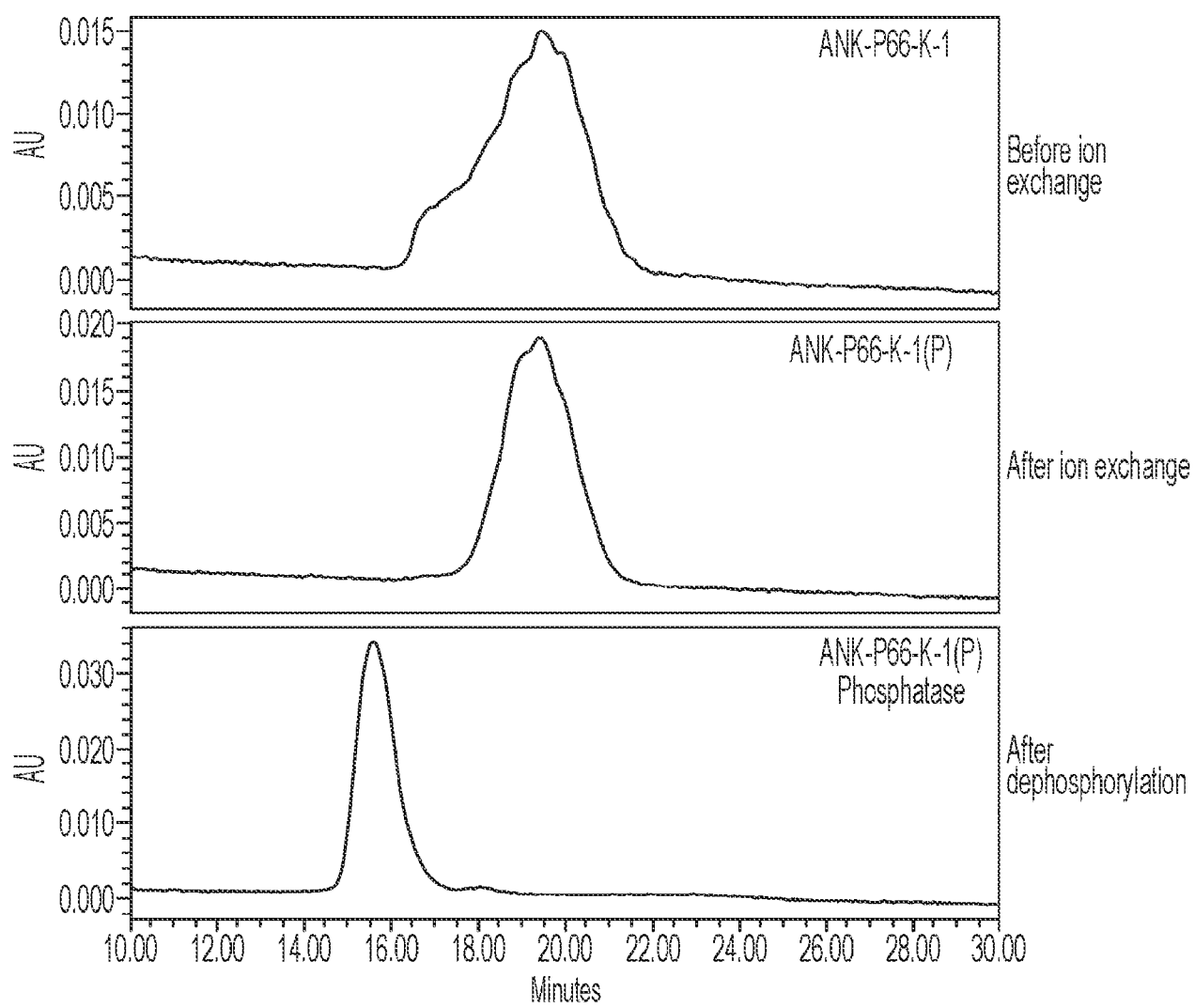


FIG. 10B

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Molecule	Peak elution time		
	Pre-AEX	Post-AEX	Post-AEX + phosphatase
mIL12-ABP10-K	16.7	16.6	14.2
mIL12-ABP20-G4-8x-GE-K	19.5	19.4	15.6

FIG. 10C

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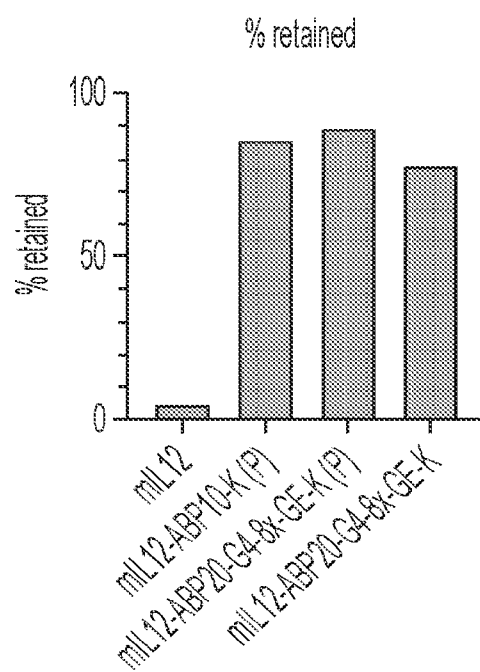


FIG. 11A

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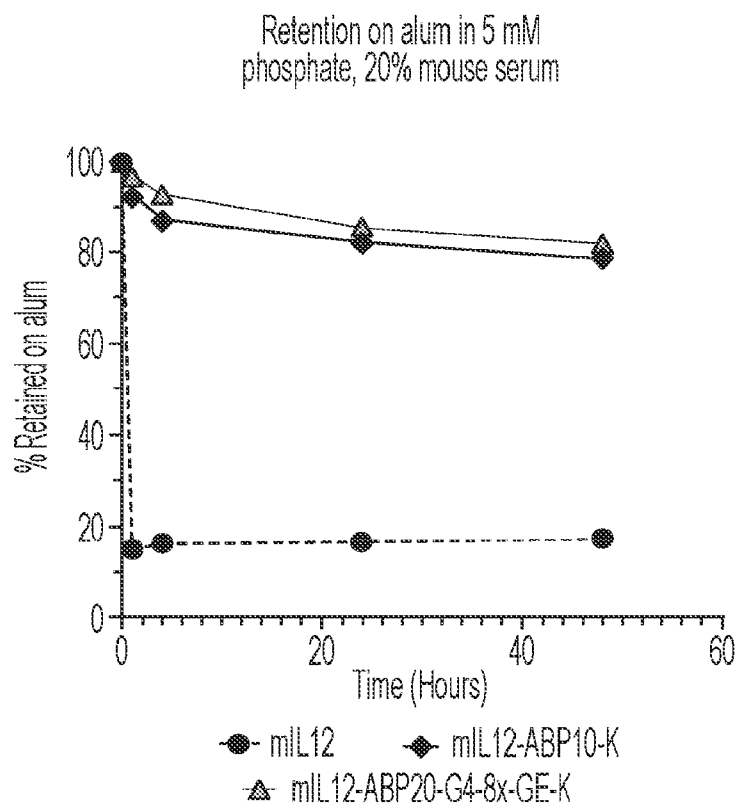


FIG. 11B

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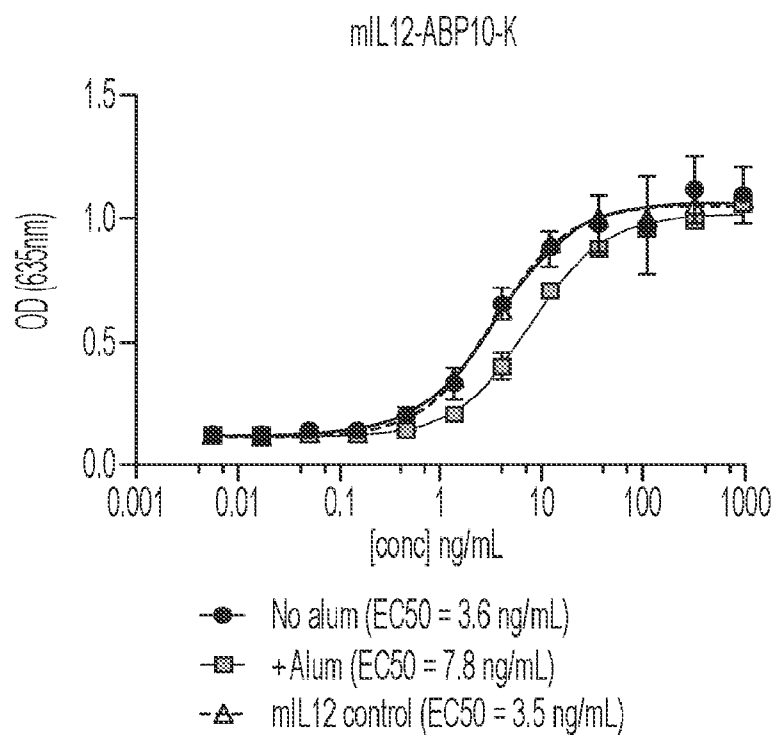


FIG. 12A

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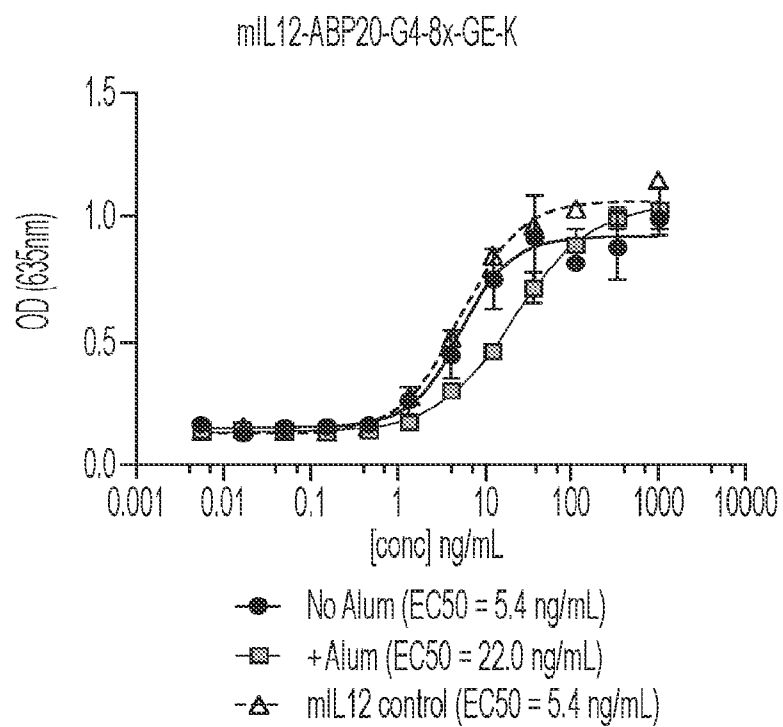


FIG. 12B

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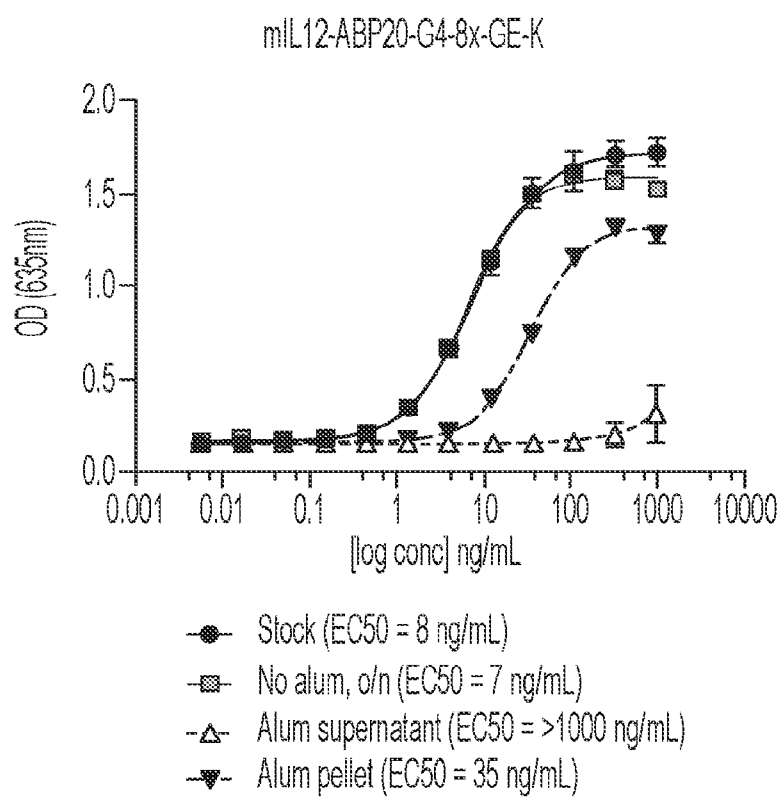


FIG. 13

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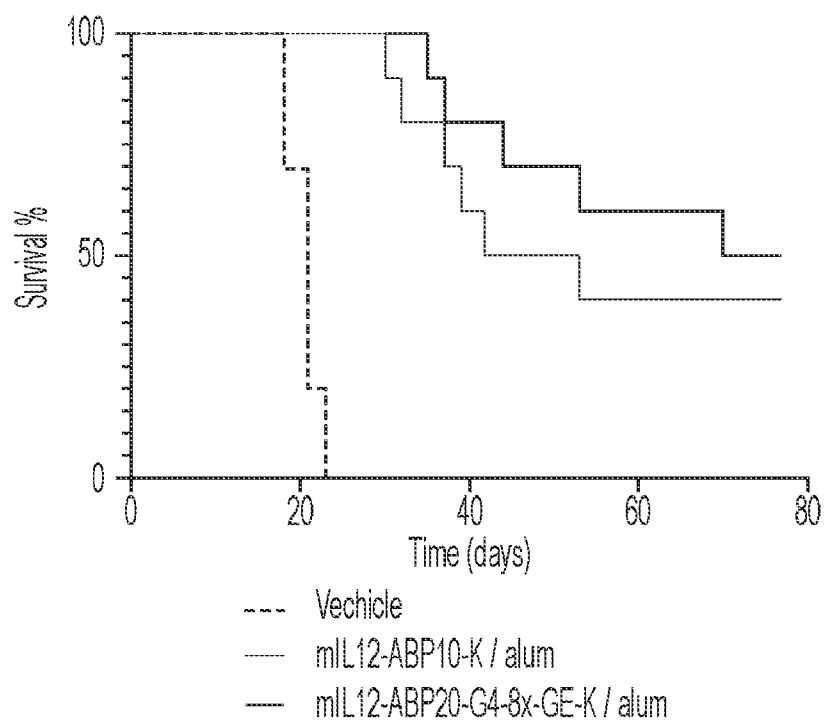


FIG. 14A

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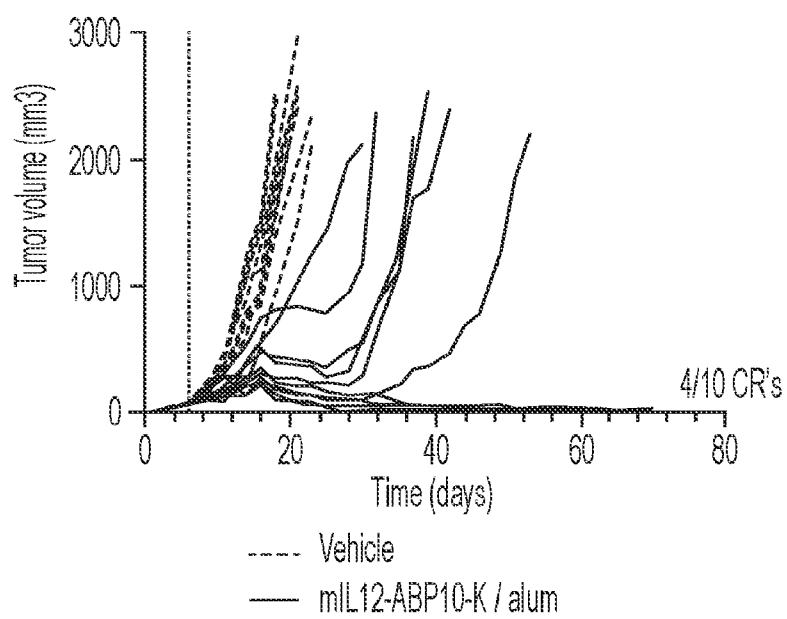


FIG. 14B

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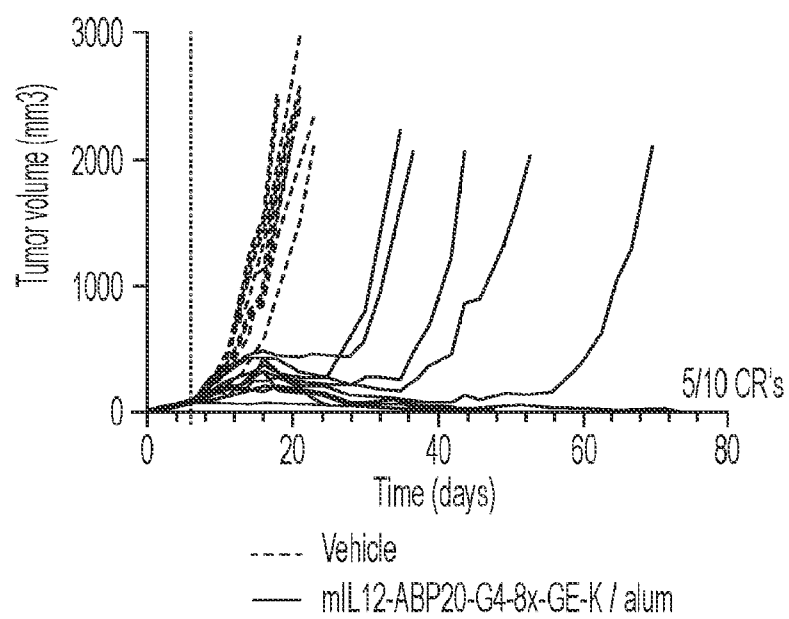


FIG. 14C

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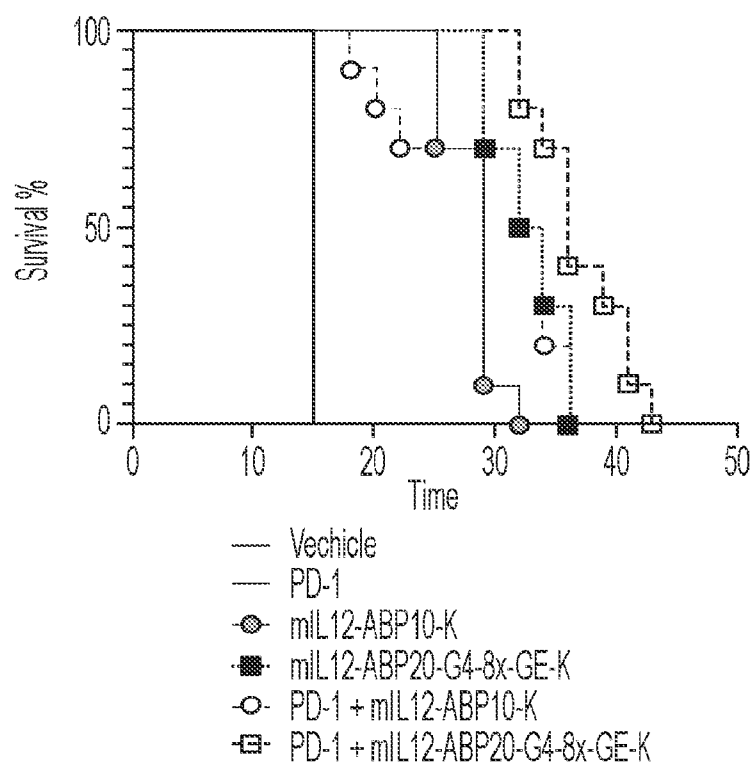


FIG. 15A

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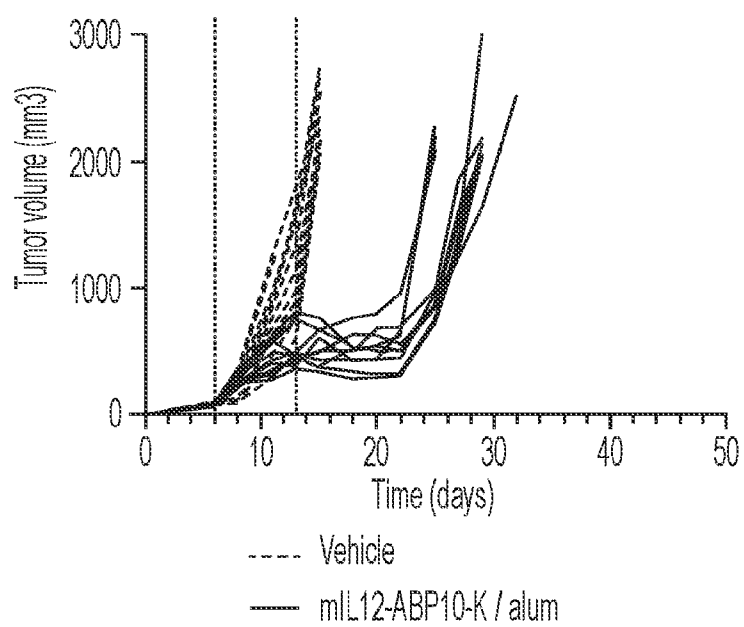


FIG. 15B

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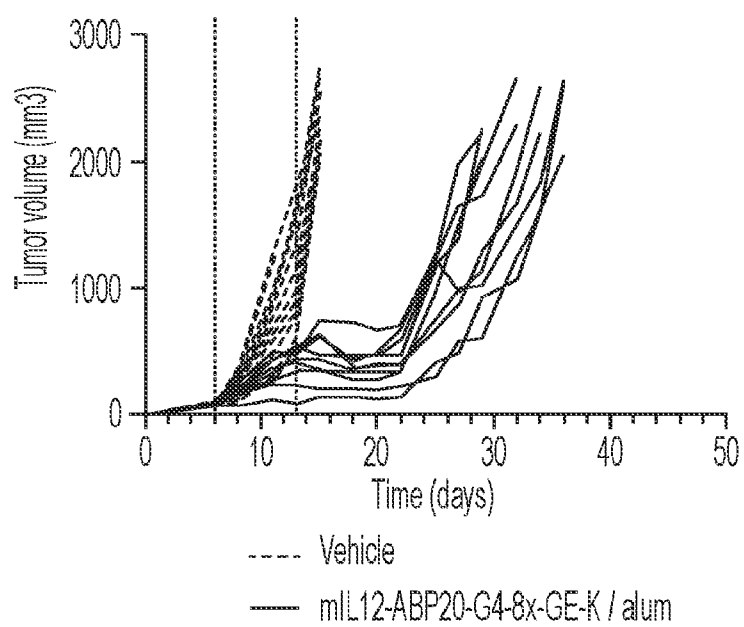


FIG. 15C

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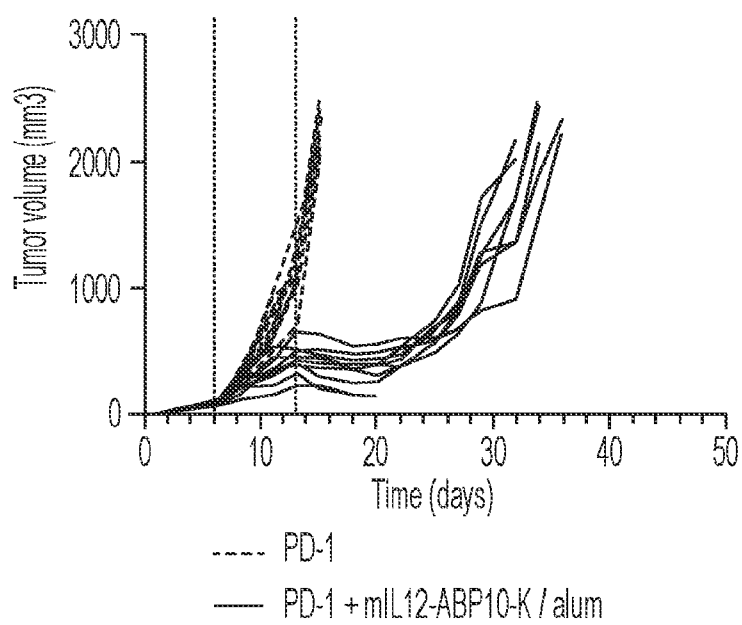


FIG. 15D

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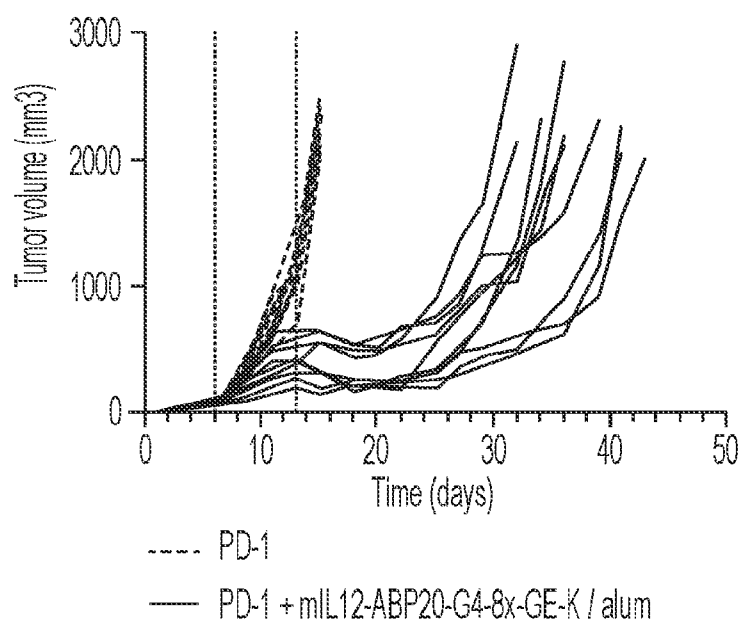


FIG. 15E

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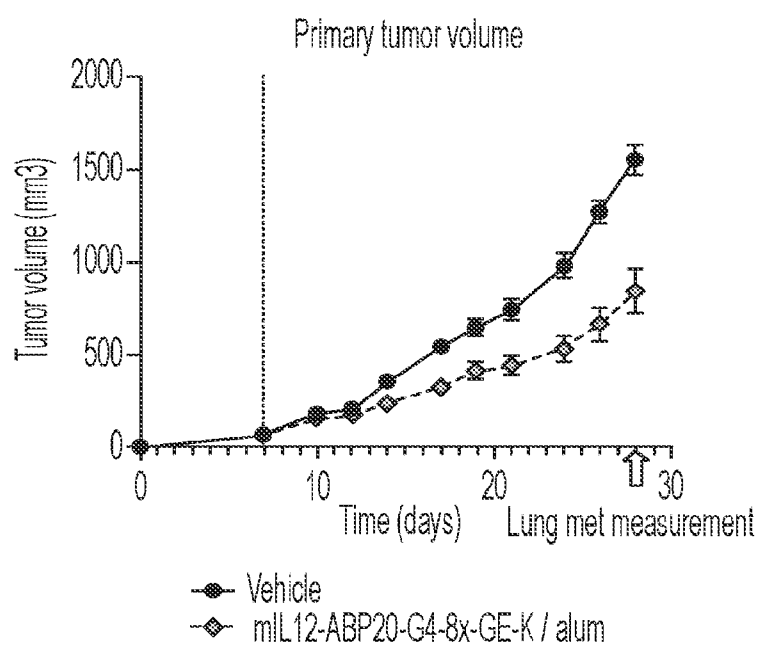


FIG. 16A

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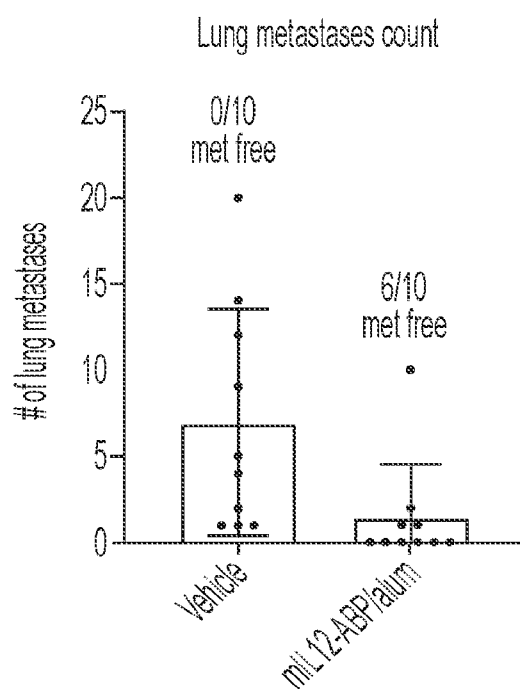


FIG. 16B

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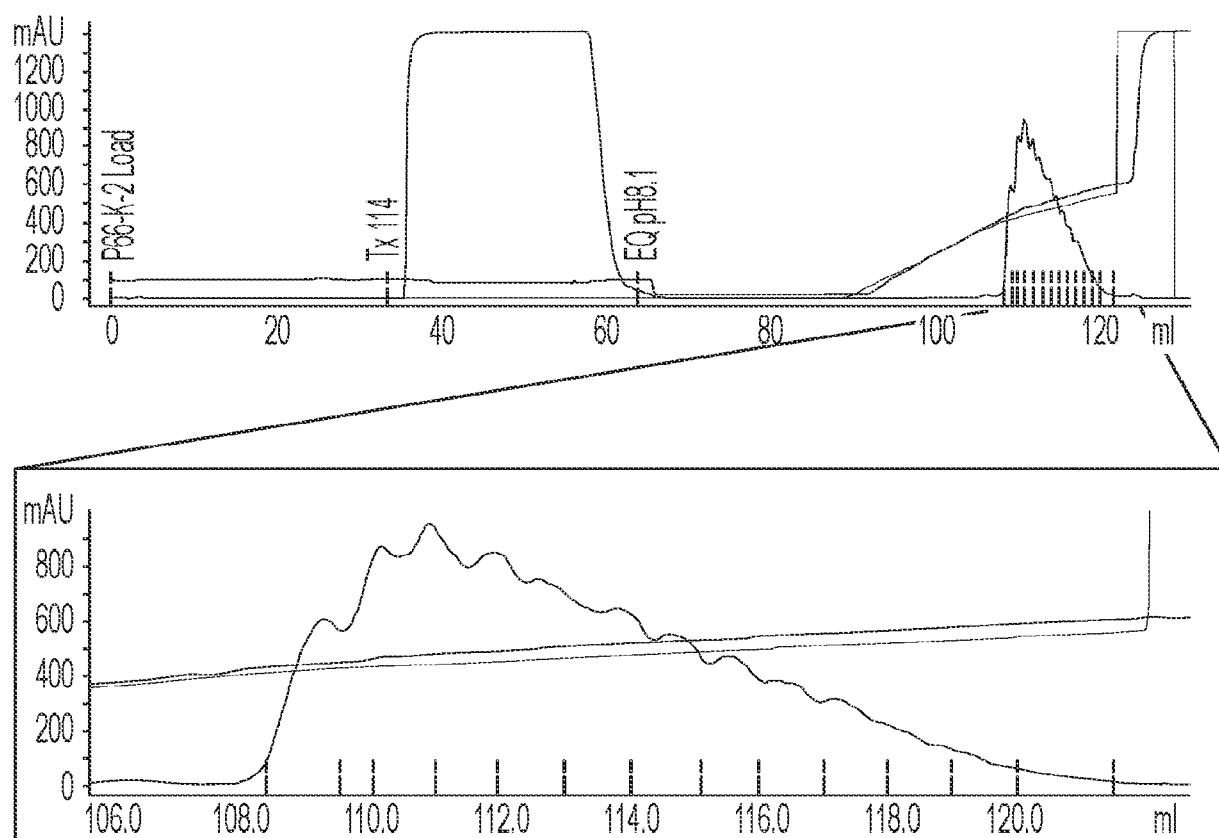
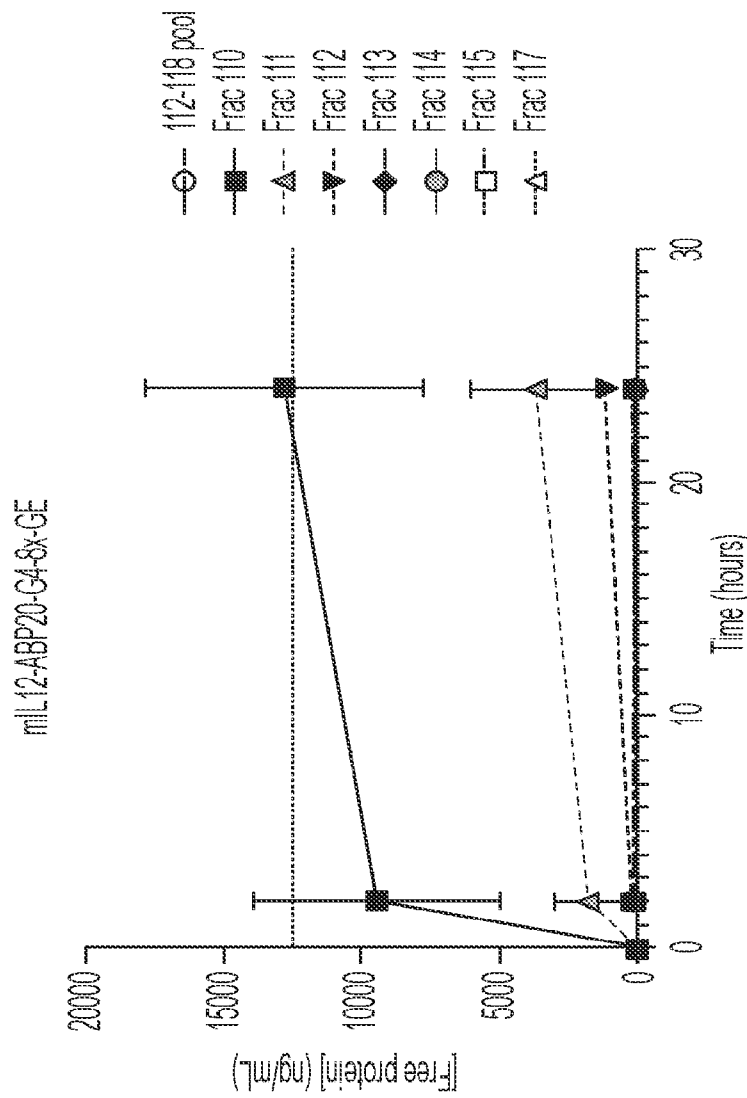


FIG. 17A

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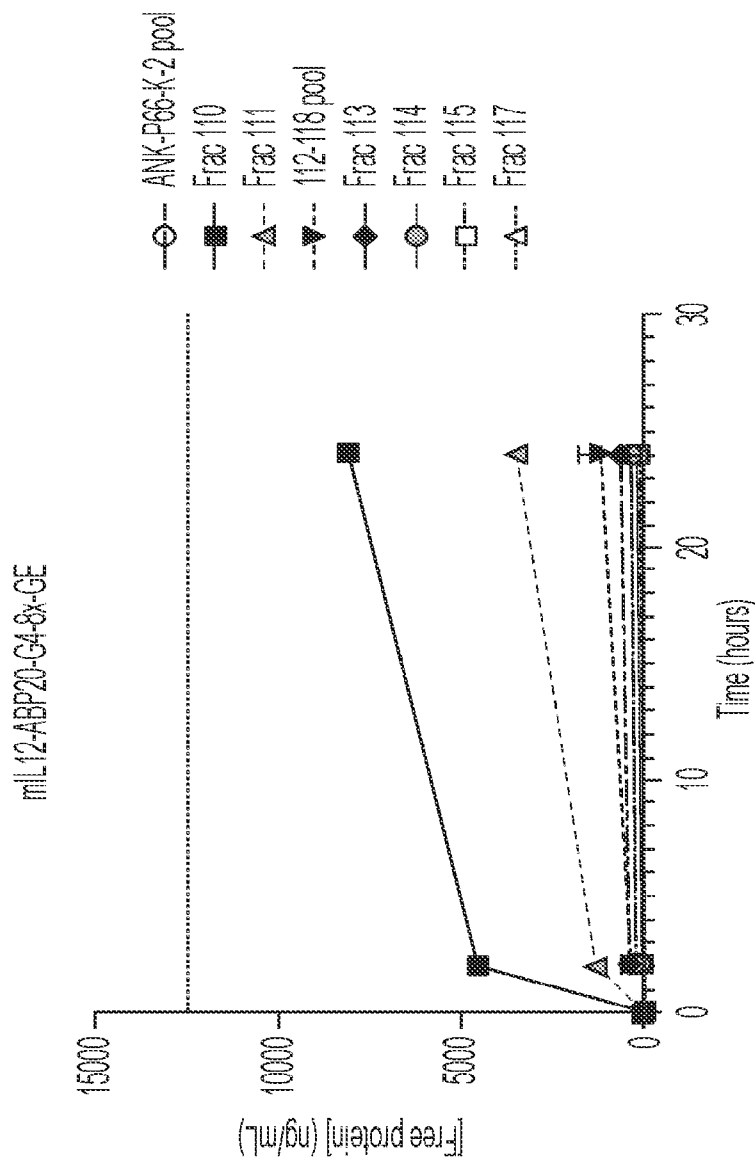
Fraction	% of pool	PO4/protein
No Fam20C control		0.6
110		2.4
111		3.7
112	24	4.6
113	20	5.8
114	17	6.2
115	14	6.3
116	11	6.4
117	9	6.9
POOL (112-118)		5.7

FIG. 17B



Dashed line represents 12,500 ng/mL theoretical concentration of free protein if all protein elutes off alum

FIG. 18A



Dashed line represents 12,500 ng/mL theoretical concentration of free protein if all protein elutes off alum

FIG. 18B

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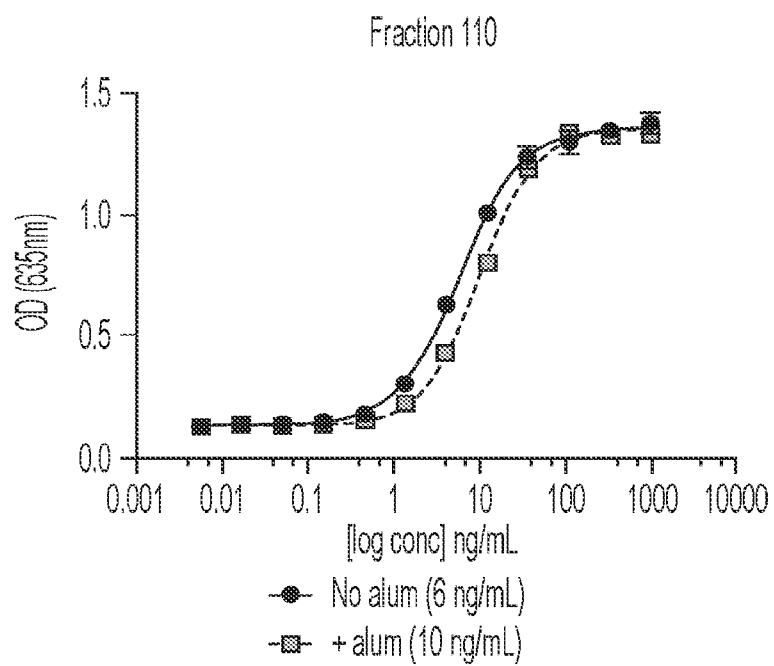


FIG. 19A

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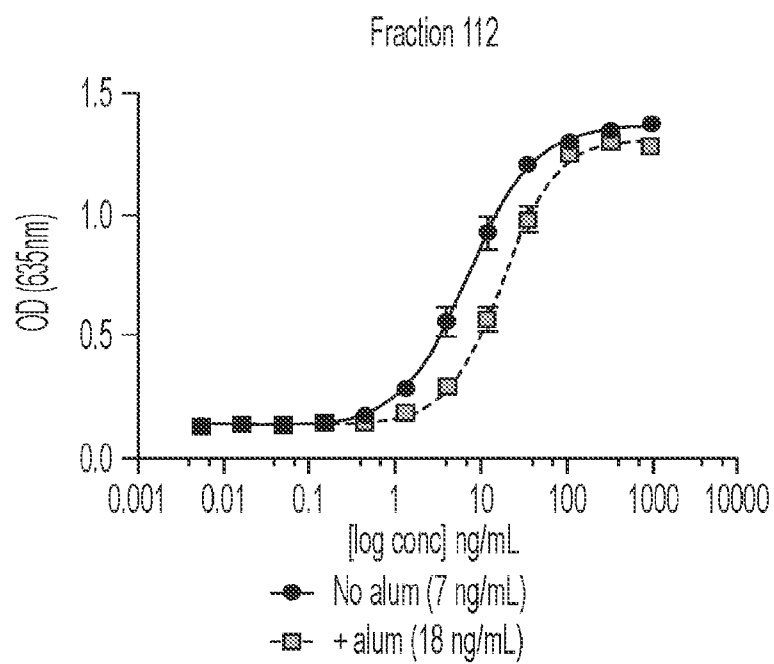


FIG. 19B

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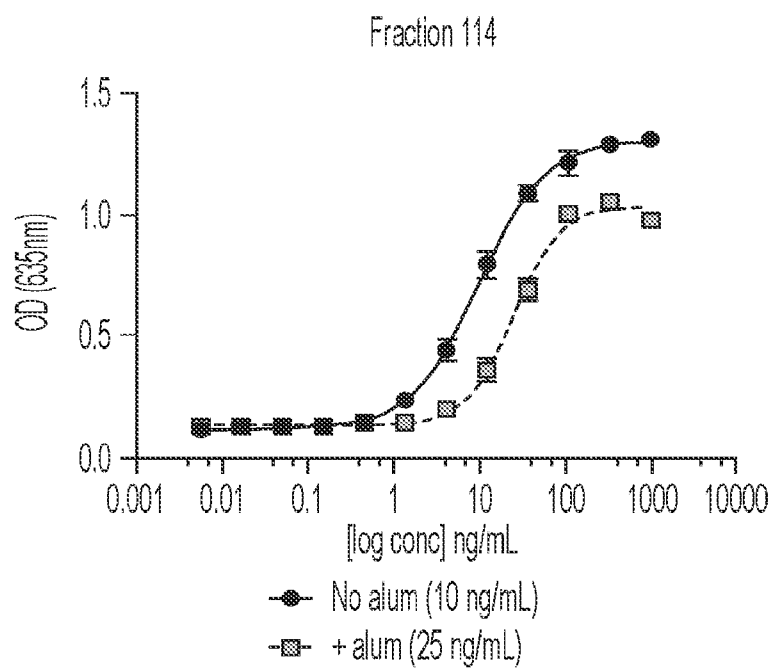


FIG. 19C

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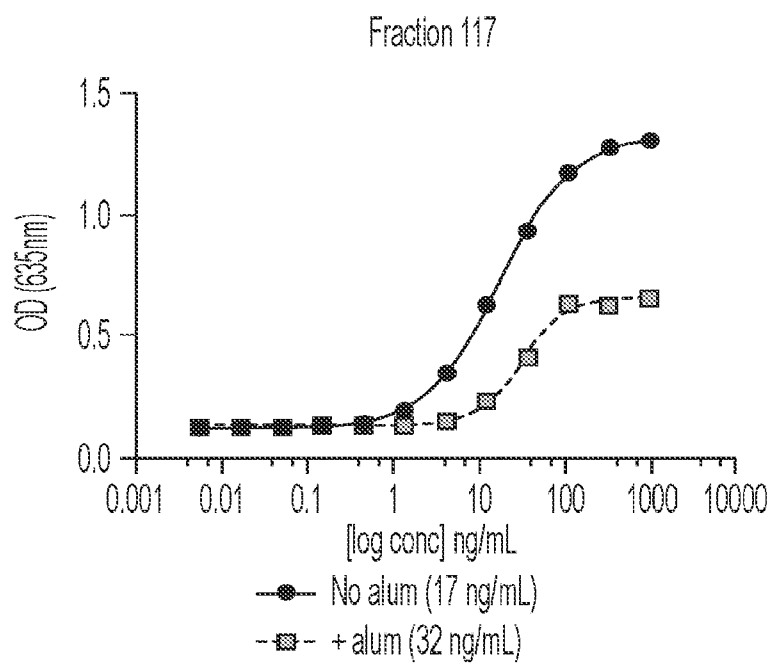


FIG. 19D

Fraction	% of pool	PO4/protein (mean)	Alum retention		HEK-Blue-L12		
			% Retained - Run 1	% Retained - Run 2	EC50 - No alum	EC50 w/ alum	% max signal
110		2.4	11	50	6	10	100
111		3.7	78	81			
112	24	4.6	95	95	7	18	100
113	20	5.8	99	96			
114	17	6.2	100	99	10	25	79
115	14	6.3	100	99			
116	11	6.4					
117	9	6.9	100	100	17	32	50

FIG. 20

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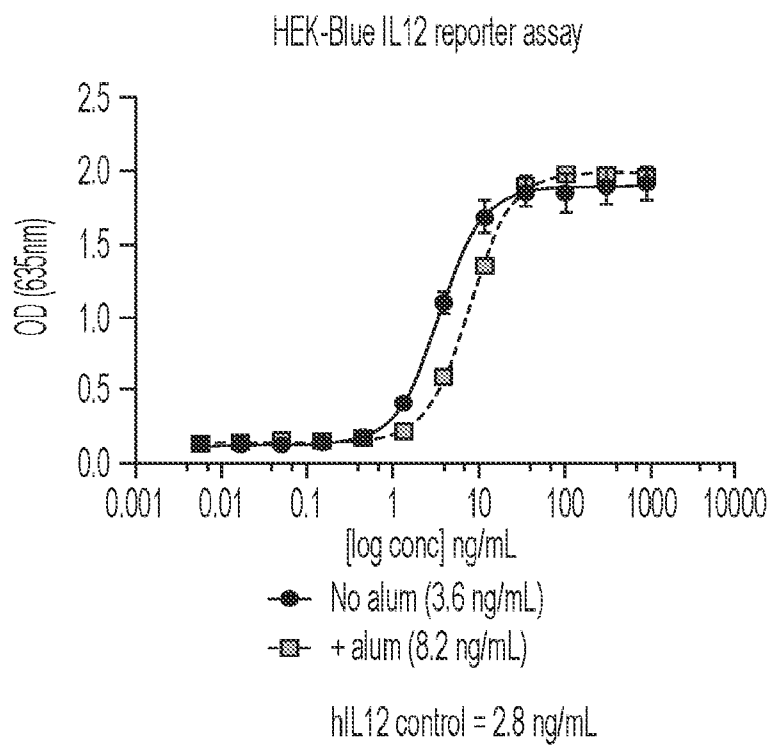


FIG. 21A

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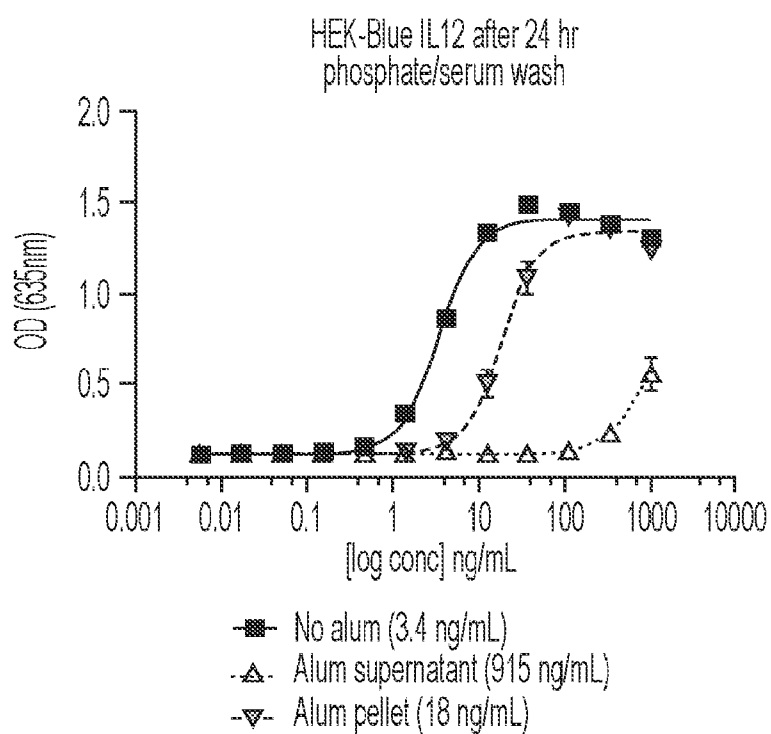


FIG. 21B

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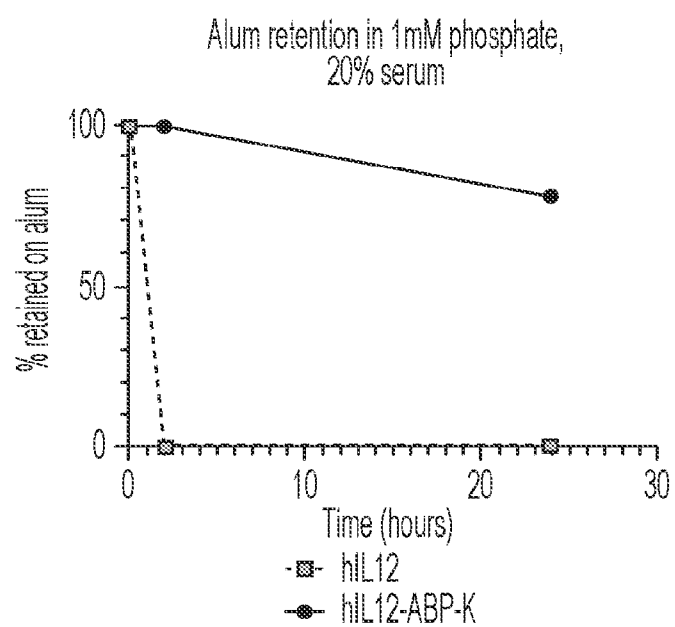


FIG. 21C

Fraction	Conc (mg/mL)	PO4/protein
645	7.4	5.8
650	13.9	5.8
655	12.7	6.3
660	10	8.4
665	4.8	8.8

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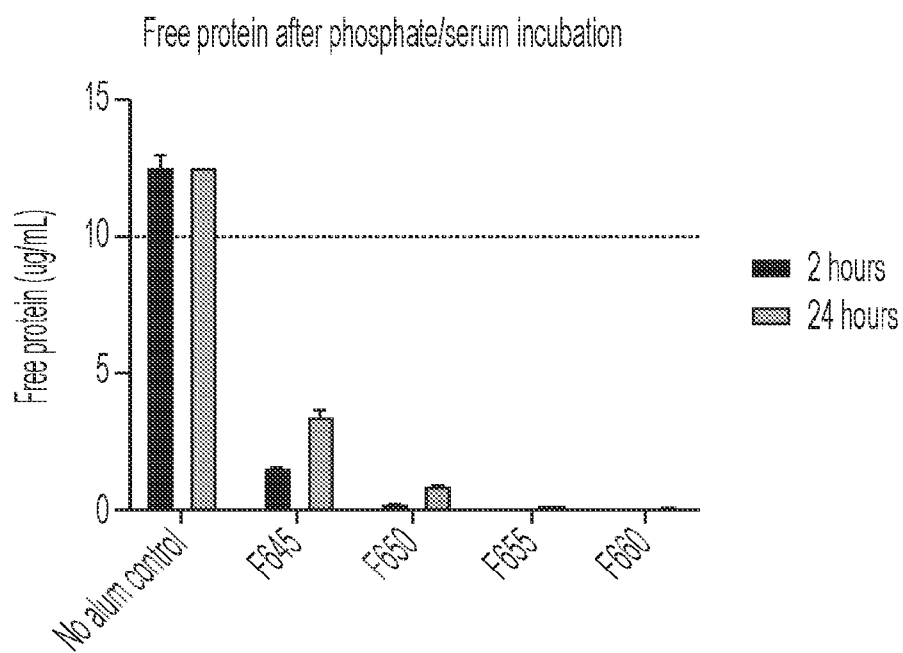
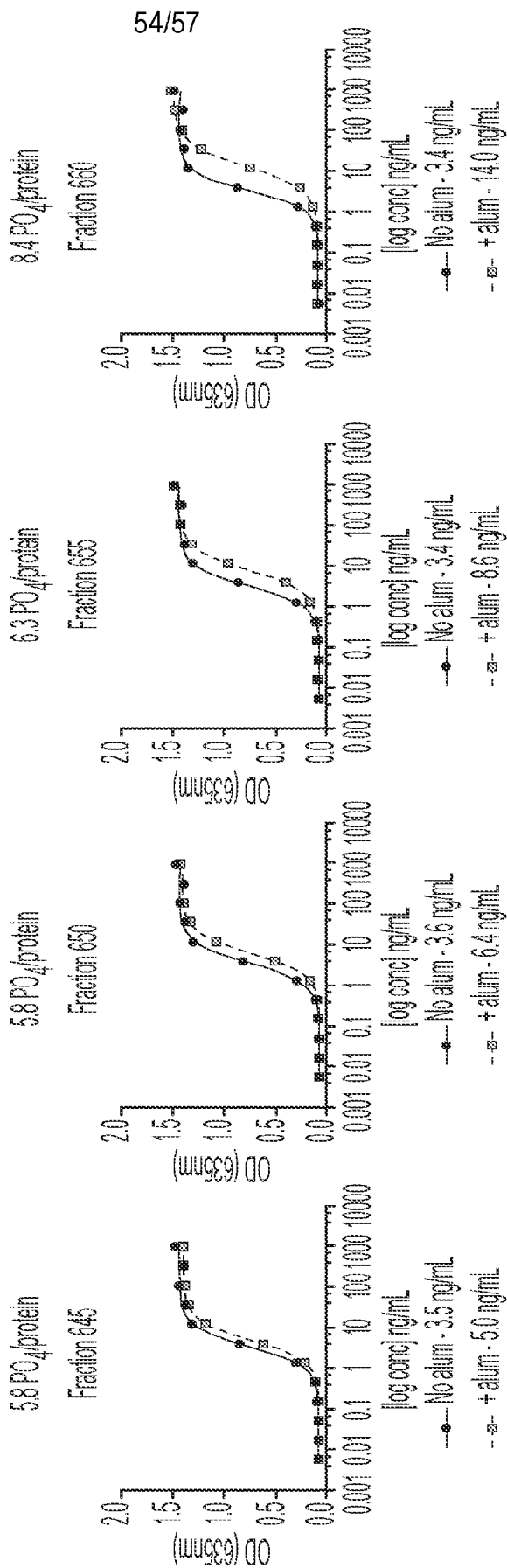


FIG. 23



* EC50 value reported in legend

FIG. 24

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Q-HP elution fraction	PO4/protein (malachite green)	% retained on alum at 24 hours	HEK-Blue-IL12 EC50 (ng/mL)	
			No alum	+ alum
f90	0.4			
f91	2.0	28		
f92	4.5	65		
f93	5.5	97	2.5	6.5
f93.5	6.5	99		
f94	6.8	100		9.4
f94.5	8.3	100		9.0
f95	9.4	100	3.0	14.4
f96	10.3	100		16.5
f97	10.8	100	3.9	30.1
f98	12.2			
f99	11.3			

FIG. 25

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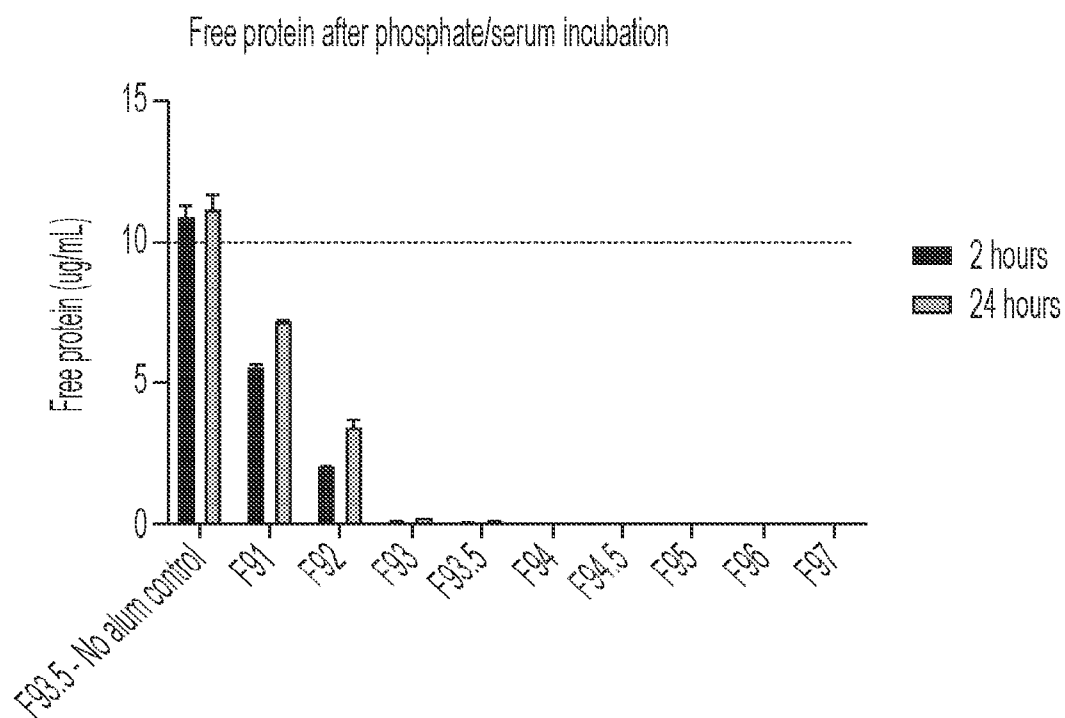


FIG. 26

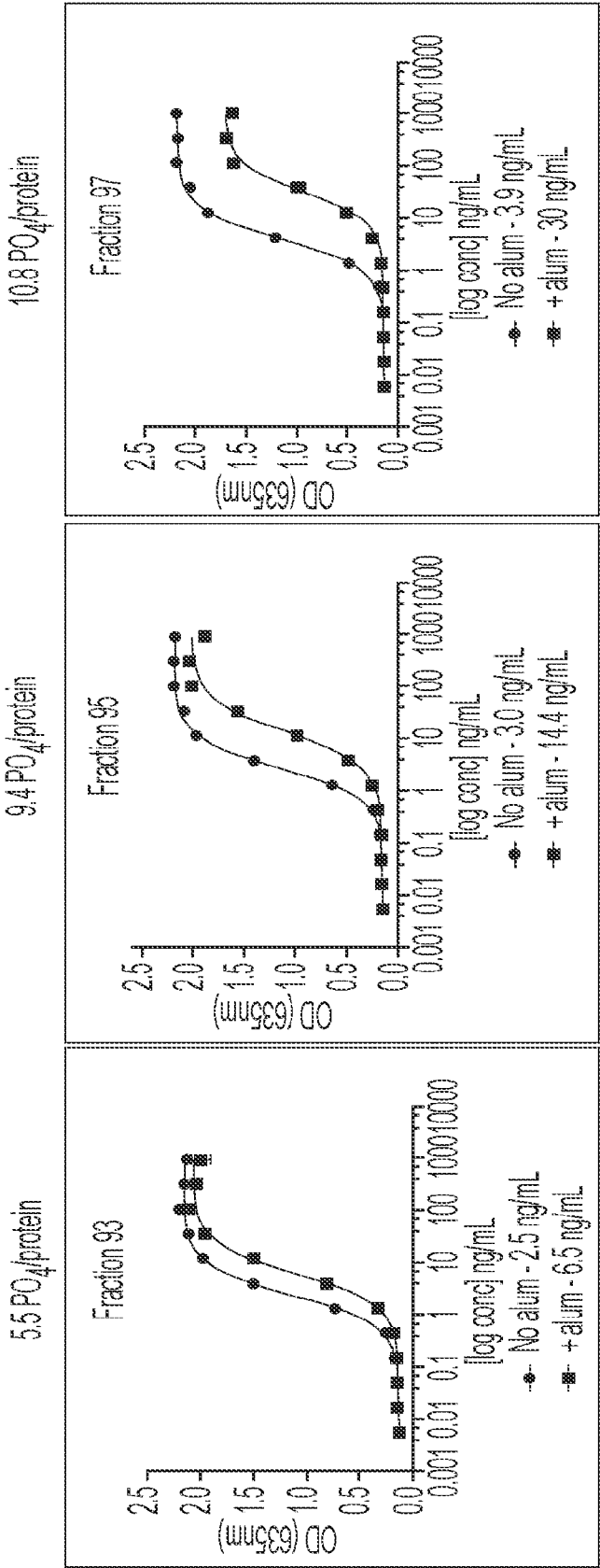


FIG. 27

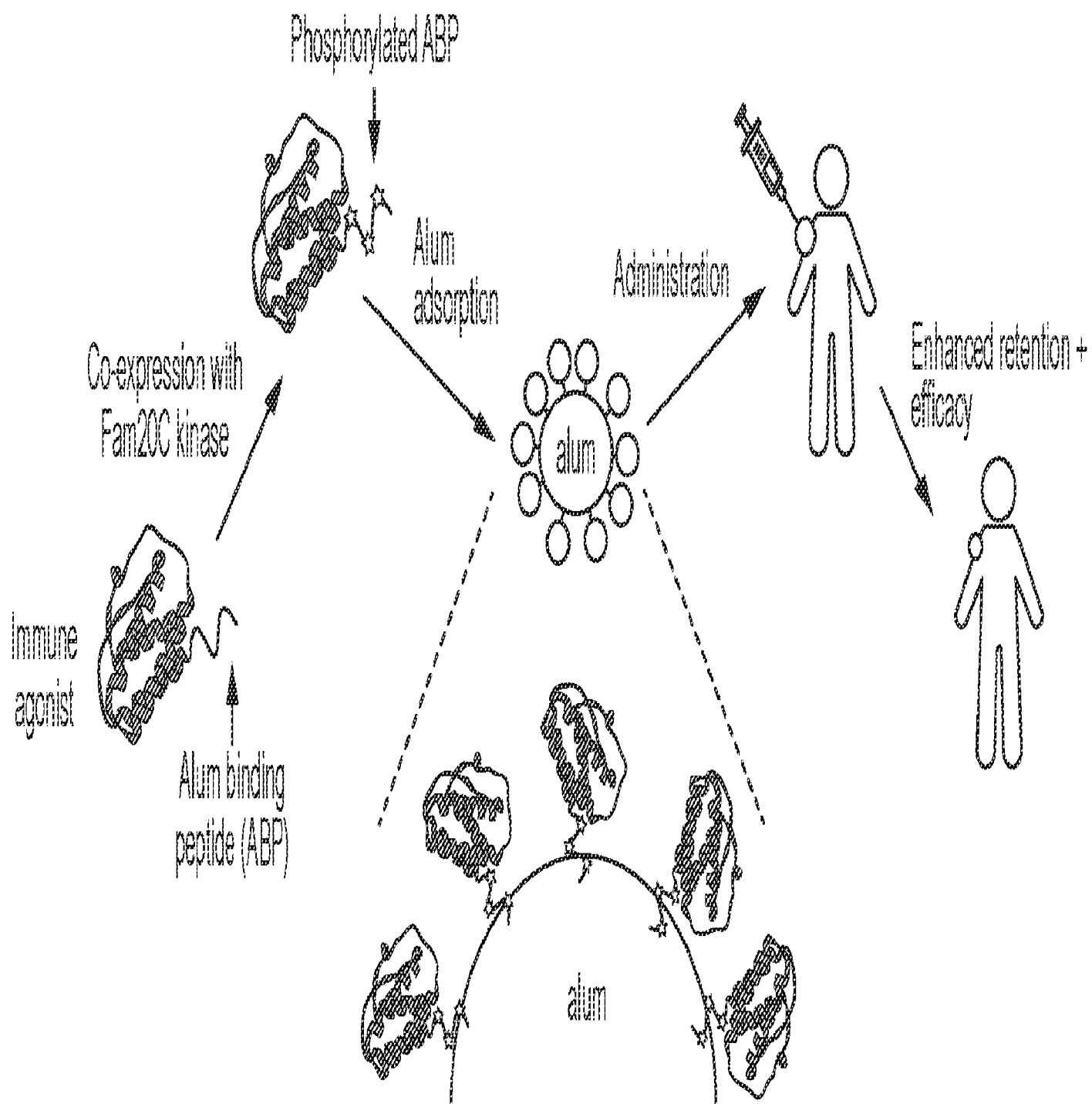


FIG. 1