

HALF-LIFE EXTENDING MOIETIES AND METHODS OF USING THE SAME

[001] The disclosure relates generally to biology and medicine, and more particularly it relates to single domain antibodies known as variable domains of heavy chain-only antibodies (VHH) that are engineered/modified to act as half-life ($t_{1/2}$)-extending moieties for use with therapeutic agents, especially for improving $t_{1/2}$ of biological-based therapeutic agents (*i.e.*, biotherapeutics or biologics). The disclosure further relates to fusions and conjugates that include one or more of the VHH-based $t_{1/2}$ -extending moieties and a therapeutic agent, as well as pharmaceutical compositions including the same and their use in treating various conditions, diseases or disorders.

[002] Biotherapeutics are native or modified components of physiological pathways and tend to be highly selective, efficacious and safe. However, they come with some limitations. One limitation, and with few exceptions, is that biotherapeutics cannot be orally administered. Another limitation is that many biotherapeutics have a relatively short $t_{1/2}$ when used in a clinical setting.

[003] Several strategies exist for extending the $t_{1/2}$ of biotherapeutics, which can improve their pharmacokinetic (PK) and/or pharmacodynamic (PD) profiles. Such strategies typically use bulking moieties or neonatal Fc receptor (FcRn)-mediated recycling. In this manner, antibodies (Abs) or fragments thereof (*e.g.*, Fab, Fc, *etc.*); polymers such as polyethylene glycol (PEG), polysialic acid (PSA), hyaluronic acid (HA) and hydroxy-ethyl-starch (HES); fatty acids and other lipids; N- or O-glycosylation; and serum albumin or other plasma proteins (such as transferrin), can be covalently and/or non-covalently bound to a given biotherapeutic to extend its $t_{1/2}$. *See, e.g.*, Hamers-Casterman *et al.* (1993) *Nature* 363:446-448; Harmsen & Haard (2007) *Appl. Microbiol. Biotechnol.* 77:13-22; Kontermann (2016) *Expert Opin. Biol. Ther.* 16:903-915; Müller *et al.* (2012) *MAbs* 4:673-685; Podust *et al.* (2013) *Protein Eng. Des. Sel.* 26:743-753; Strohl (2015) *BioDrugs* 29:215-239; and Werle U Bernkop-Schnürch (2006) *Amino Acids* 30:351-367.

[004] Despite the vast number of $t_{1/2}$ -extending strategies, there is a need for additional structures for extending or improving PK properties, such as the $t_{1/2}$, of biotherapeutics.

[005] To address this need, the disclosure first describes compounds that can be used as $t_{1/2}$ -extending moieties for biotherapeutics. In one instance, a compound is provided that

includes an amino acid sequence of selected from any one of SEQ ID NOS:1 to 37 and 124 to 126.

[006] In another instance, a compound is provided that includes an amino acid sequence of:

EVQLLES GGGLVQPGGSLRLSCAASGRYIDETAVAWFRQAPGKEREFVAGIGGG
VDITYYADSVKGRFTISRDN SKNTLYLQMNSLRPEDTAVYYCAARPGRPLITSKV
ADLYPYWGQGT LVT VSS (SEQ ID NO:1). Alternatively, the compound can have an amino acid sequence having at least about 90% to about 99% sequence similarity to SEQ ID NO:1.

[007] In another instance, a compound is provided that includes an amino acid sequence of:

EVQLLES GGGLVQPGGSLRLSCAASGRYIDETAVAWFRQAPGK GREFVAGIGGG
VDITYYADSVKGRFTISRDN SKNTLYLQMNSLRPEDTAVYYCAARPGRPLITSKV
ADLYPYWGQGT LVT VSS (SEQ ID NO:2). Alternatively, the compound can have an amino acid sequence having at least about 90% to about 99% sequence similarity to SEQ ID NO:2.

[008] In another instance, a compound is provided that includes an amino acid sequence of:

EVQLLES GGGLVQPGGSLRLSCAASGRYIDETAVAWFRQAPGKEREFVAGIGGG
VDITYYADSVKGRFTISRDN SKNTLYLQMNSLRPEDTAVYYCAARPGRPLITSKV
ADLYPYWGQGT LVT VSSPP (SEQ ID NO:3). Alternatively, the compound can have an amino acid sequence having at least about 90% to about 99% sequence similarity to SEQ ID NO:3.

[009] In another instance, a compound is provided that includes an amino acid sequence of:

EVQLLES GGGLVQPGGSLRLSCAASGRYIDETAVAWFRQAPGK GREFVAGIGGG
VDITYYADSVKGRFTISRDN SKNTLYLQMNSLRPEDTAVYYCAARPGRPLITSKV
ADLYPYWGQGT LVT VSSPP (SEQ ID NO:4). Alternatively, the compound can have an amino acid sequence having at least about 90% to about 99% sequence similarity to SEQ ID NO:4.

[0010] In another instance, a compound is provided that includes an amino acid sequence of:

EVQLVESGGGLVQAGGSLRLSCAASGRTVSSTAVAWFRQAPGKEREFVAGIGGS
VDITYYLDVSVKGRFTISKDNTKNTVYLQMNSLPEDTAVYYCAVRPGRPLITSRD
ANLYDYWGQGTQVTVSS (SEQ ID NO:5). Alternatively, the compound can have an
amino acid sequence having at least about 90% to about 99% sequence similarity to SEQ
ID NO:5.

[0011] In another instance, a compound is provided that includes an amino acid sequence
of:

[0012] EVQLLES GGGLVQPGGSLRLSCAASGRTVSSTAVAWFRQAPGKEREFV
AGIGGSVDITYYADSVKGRFTISRDN SKNTLYLQMNSLRPEDTAVYYCAVRPGRP
LITSRDANLYDYWGQGT LVT VSS (SEQ ID NO:6). Alternatively, the compound can
have an amino acid sequence having at least about 90% to about 99% sequence similarity
to SEQ ID NO:6.

[0013] In another instance, a compound is provided that includes an amino acid sequence
of:

EVQLLES GGGLVQPGGSLRLSCAASGRYIDSTAVAWFRQAPGKEREFVAGIGGG
VDITYYADSVKGRFTISRDN SKNTLYLQMNSLRPEDTAVYYCAARPGRPLITSRV
ANLYPYWGQGT LVT VSS (SEQ ID NO:7). Alternatively, the compound can have an
amino acid sequence having at least about 90% to about 99% sequence similarity to SEQ
ID NO:7.

[0014] In another instance, a compound is provided that includes an amino acid sequence
of:

EVQLLES GGGLVQPGGSLRLSCAASYRIDETAVAWFRQAPGKEREFVAGIGGG
VDITYYADSVKGRFTISRDN SKNTLYLQMNSLRPEDTAVYYCAARPGRPLITSKV
ADLYPYWGQGT LVT VSS (SEQ ID NO:8). Alternatively, the compound can have an
amino acid sequence having at least about 90% to about 99% sequence similarity to SEQ
ID NO:8.

[0015] In another instance, a compound is provided that includes an amino acid sequence
of:

EVQLLES GGGLVQPGGSLRLSCAASGAYIDETAVAWFRQAPGKEREFVAGIGGG
VDITYYADSVKGRFTISRDN SKNTLYLQMNSLRPEDTAVYYCAARPGRPLITSKV
ADLYPYWGQGT LVT VSS (SEQ ID NO:9). Alternatively, the compound can have an

amino acid sequence having at least about 90% to about 99% sequence similarity to SEQ ID NO:9.

[0016] In another instance, a compound is provided that includes an amino acid sequence of:

EVQLLES GGGLVQPGGSLRLSCAASGRYIDETAVAWFRQAPGKEREFVAGIGGG
VDETY YADSVKGRFTISRDN SKNTLYLQMNSLRPEDTAVYYCAARPGRPLITSKV
ADLYPYWGQGTLVTVSS (SEQ ID NO:10). Alternatively, the compound can have an amino acid sequence having at least about 90% to about 99% sequence similarity to SEQ ID NO:10.

[0017] In another instance, a compound is provided that includes an amino acid sequence of:

EVQLLES GGGLVQPGGSLRLSCAASGRYIDETAVAWFRQAPGKEREFVAGIGGG
VDQTY YADSVKGRFTISRDN SKNTLYLQMNSLRPEDTAVYYCAARPGRPLITSK
VADLYPYWGQGTLVTVSS (SEQ ID NO:11). Alternatively, the compound can have an amino acid sequence having at least about 90% to about 99% sequence similarity to SEQ ID NO:11.

[0018] In another instance, a compound is provided that includes an amino acid sequence of:

EVQLLES GGGLVQPGGSLRLSCAASGRYIDETAVAWFRQAPGKEREFVAGIGGG
VDITAYADSVKGRFTISRDN SKNTLYLQMNSLRPEDTAVYYCAARPGRPLITSKV
ADLYPYWGQGTLVTVSS (SEQ ID NO:12). Alternatively, the compound can have an amino acid sequence having at least about 90% to about 99% sequence similarity to SEQ ID NO:12.

[0019] In another instance, a compound is provided that includes an amino acid sequence of:

EVQLLES GGGLVQPGGSLRLSCAASGRYIDETAVAWFRQAPGKEREFVAGIGGG
VDITEYADSVKGRFTISRDN SKNTLYLQMNSLRPEDTAVYYCAARPGRPLITSKV
ADLYPYWGQGTLVTVSS (SEQ ID NO:13). Alternatively, the compound can have an amino acid sequence having at least about 90% to about 99% sequence similarity to SEQ ID NO:13.

[0020] In another instance, a compound is provided that includes an amino acid sequence of:

EVQLLES GGGLVQPGGSLRLSCAASGRYIDETAVAWFRQAPGKERE FVAGIGGG
VDITQYADSVKGRFTISRDN SKNTLYLQMNSLRPEDTAVYYCAARPGRPLITSKV
ADLYPYWGQGTLVTVSS (SEQ ID NO:14). Alternatively, the compound can have an amino acid sequence having at least about 90% to about 99% sequence similarity to SEQ ID NO:14.

[0021] In another instance, a compound is provided that includes an amino acid sequence of:

EVQLLES GGGLVQPGGSLRLSCAASGRYIDETAVAWFRQAPGKERE FVAGIGGG
VDITSYADSVKGRFTISRDN SKNTLYLQMNSLRPEDTAVYYCAARPGRPLITSKV
ADLYPYWGQGTLVTVSS (SEQ ID NO:15). Alternatively, the compound can have an amino acid sequence having at least about 90% to about 99% sequence similarity to SEQ ID NO:15.

[0022] In another instance, a compound is provided that includes an amino acid sequence of:

EVQLLES GGGLVQPGGSLRLSCAASGRYIDETAVAWFRQAPGKERE FVAGIGGG
VDITTYADSVKGRFTISRDN SKNTLYLQMNSLRPEDTAVYYCAARPGRPLITSKV
ADLYPYWGQGTLVTVSS (SEQ ID NO:16). Alternatively, the compound can have an amino acid sequence having at least about 90% to about 99% sequence similarity to SEQ ID NO:16.

[0023] In another instance, a compound is provided that includes an amino acid sequence of:

EVQLLES GGGLVQPGGSLRLSCAASGRYIDETAVAWFRQAPGKERE FVAGIGGG
VDITYYADSVKGRFTISRDN SKNTLYLQMNSLRPEDTAVYYCAARPGKPLITSKV
ADLYPYWGQGTLVTVSS (SEQ ID NO:17). Alternatively, the compound can have an amino acid sequence having at least about 90% to about 99% sequence similarity to SEQ ID NO:17.

[0024] In another instance, a compound is provided that includes an amino acid sequence of:

EVQLLES GGGLVQPGGSLRLSCAASGRYIDETAVAWFRQAPGKERE FVAGIGGG
VDITYYADSVKGRFTISRDN SKNTLYLQMNSLRPEDTAVYYCAARPGQPLITSKV
ADLYPYWGQGTLVTVSS (SEQ ID NO:18). Alternatively, the compound can have an

amino acid sequence having at least about 90% to about 99% sequence similarity to SEQ ID NO:18.

[0025] In another instance, a compound is provided that includes an amino acid sequence of:

EVQLLESGGGLVQPGGSLRLSCAASGRYIDETAVAWFRQAPGKEREFVAGIGGG
VDITYYADSVKGRFTISRDN SKNTLYLQMNSLRPEDTAVYYCAARPGSPLITSKV
ADLYPYWGQGTLVTVSS (SEQ ID NO:19). Alternatively, the compound can have an amino acid sequence having at least about 90% to about 99% sequence similarity to SEQ ID NO:19.

[0026] In another instance, a compound is provided that includes an amino acid sequence of:

EVQLLESGGGLVQPGGSLRLSCAASGRYIDETAVAWFRQAPGKEREFVAGIGGG
VDITYYADSVKGRFTISRDN SKNTLYLQMNSLRPEDTAVYYCAARPGRELITSKV
ADLYPYWGQGTLVTVSS (SEQ ID NO:20). Alternatively, the compound can have an amino acid sequence having at least about 90% to about 99% sequence similarity to SEQ ID NO:20.

[0027] In another instance, a compound is provided that includes an amino acid sequence of:

EVQLLESGGGLVQPGGSLRLSCAASGRYIDETAVAWFRQAPGKEREFVAGIGGG
VDITYYADSVKGRFTISRDN SKNTLYLQMNSLRPEDTAVYYCAARPGRQLITSKV
ADLYPYWGQGTLVTVSS (SEQ ID NO:21). Alternatively, the compound can have an amino acid sequence having at least about 90% to about 99% sequence similarity to SEQ ID NO:21.

[0028] In another instance, a compound is provided that includes an amino acid sequence of:

EVQLLESGGGLVQPGGSLRLSCAASGRYIDETAVAWFRQAPGKEREFVAGIGGG
VDITYYADSVKGRFTISRDN SKNTLYLQMNSLRPEDTAVYYCAARPGRSLITSKV
ADLYPYWGQGTLVTVSS (SEQ ID NO:22). Alternatively, the compound can have an amino acid sequence having at least about 90% to about 99% sequence similarity to SEQ ID NO:22.

[0029] In another instance, a compound is provided that includes an amino acid sequence of:

EVQLLES GGGLVQPGGSLRLSCAASGRYIDETAVAWFRQAPGKERE FVAGIGGG
VDITYYADSVKGRFTISRDN SKNTLYLQMNSLRPEDTAVYYCAARPGRPEITSKV
ADLYPYWGQGTLVTVSS (SEQ ID NO:23). Alternatively, the compound can have an
amino acid sequence having at least about 90% to about 99% sequence similarity to SEQ
ID NO:23.

[0030] In another instance, a compound is provided that includes an amino acid sequence
of:

EVQLLES GGGLVQPGGSLRLSCAASGRYIDETAVAWFRQAPGKERE FVAGIGGG
VDITYYADSVKGRFTISRDN SKNTLYLQMNSLRPEDTAVYYCAARPGRPGITSKV
ADLYPYWGQGTLVTVSS (SEQ ID NO:24). Alternatively, the compound can have an
amino acid sequence having at least about 90% to about 99% sequence similarity to SEQ
ID NO:24.

[0031] In another instance, a compound is provided that includes an amino acid sequence
of:

EVQLLES GGGLVQPGGSLRLSCAASGRYIDETAVAWFRQAPGKERE FVAGIGGG
VDITYYADSVKGRFTISRDN SKNTLYLQMNSLRPEDTAVYYCAARPGRPQITSKV
ADLYPYWGQGTLVTVSS (SEQ ID NO:25). Alternatively, the compound can have an
amino acid sequence having at least about 90% to about 99% sequence similarity to SEQ
ID NO:25.

[0032] In another instance, a compound is provided that includes an amino acid sequence
of:

EVQLLES GGGLVQPGGSLRLSCAASGRYIDETAVAWFRQAPGKERE FVAGIGGG
VDITYYADSVKGRFTISRDN SKNTLYLQMNSLRPEDTAVYYCAARPGRPTITSKV
ADLYPYWGQGTLVTVSS (SEQ ID NO:26). Alternatively, the compound can have an
amino acid sequence having at least about 90% to about 99% sequence similarity to SEQ
ID NO:26.

[0033] In another instance, a compound is provided that includes an amino acid sequence
of:

EVQLLES GGGLVQPGGSLRLSCAASGRYIDETAVAWFRQAPGKERE FVAGIGGG
VDITYYADSVKGRFTISRDN SKNTLYLQMNSLRPEDTAVYYCAARPGRPLITEKV
ADLYPYWGQGTLVTVSS (SEQ ID NO:27). Alternatively, the compound can have an

amino acid sequence having at least about 90% to about 99% sequence similarity to SEQ ID NO:27.

[0034] In another instance, a compound is provided that includes an amino acid sequence of:

EVQLLESGGGLVQPGGSLRLSCAASGRYIDETAVAWFRQAPGKGREFVAGIGGG
VDITYYADSVKGRFTISRDN SKNTLYLQMNSLRPEDTAVYYCATRPGRPLITSKV
ADLYPYWGQGTLVTVSS (SEQ ID NO:28). Alternatively, the compound can have an amino acid sequence having at least about 90% to about 99% sequence similarity to SEQ ID NO:28.

[0035] In another instance, a compound is provided that includes an amino acid sequence of:

EVQLLESGGGLVQPGGSLRLSCAASGRYIDETAVAWFRQAPGKGREFVAGIGGG
VDITYYADSVKGRFTISRDN SKNTLYLQMNSLRPEDTAVYYCAARQGRPLITSKV
ADLYPYWGQGTLVTVSS (SEQ ID NO:29). Alternatively, the compound can have an amino acid sequence having at least about 90% to about 99% sequence similarity to SEQ ID NO:29.

[0036] In another instance, a compound is provided that includes an amino acid sequence of:

EVQLLESGGGLVQPGGSLRLSCAASGRYIDETAVAWFRQAPGKGREFVAGIGGG
VDITYYADSVKGRFTISRDN SKNTLYLQMNSLRPEDTAVYYCATRQGRPLITSKV
ADLYPYWGQGTLVTVSS (SEQ ID NO:30). Alternatively, the compound can have an amino acid sequence having at least about 90% to about 99% sequence similarity to SEQ ID NO:30.

[0037] In another instance, a compound is provided that includes an amino acid sequence of:

EVQLLESGGGLVQPGGSLRLSCAASGRYIDETAVAWFRQAPGKGREFVAGIGGG
VDITYYADSVKGRFTISRDN SKNTLYLQMNSLRPEDTAVYYCAARPGRPLITSQV
ADLYPYWGQGTLVTVSS (SEQ ID NO:31). Alternatively, the compound can have an amino acid sequence having at least about 90% to about 99% sequence similarity to SEQ ID NO:31.

[0038] In another instance, a compound is provided that includes an amino acid sequence of:

EVQLLES GGGLVQPGGSLRLSCAASGRYIDETAVAWFRQAPGKGREFVAGIGGG
VDITYYADSVKGRFTISRDN SKNTLYLQMNSLRPEDTAVYYCAARPGRPLITSKQ
ADLYPYWGQGTLVTVSS (SEQ ID NO:32). Alternatively, the compound can have an
amino acid sequence having at least about 90% to about 99% sequence similarity to SEQ
ID NO:32.

[0039] In another instance, a compound is provided that includes an amino acid sequence
of:

EVQLLES GGGLVQPGGSLRLSCAASGRYIDETAVAWFRQAPGKGREFVAGIGGG
VDITYYADSVKGRFTISRDN SKNTLYLQMNSLRPEDTAVYYCAARPGRPLITSKV
AELYPYWGQGTLVTVSS (SEQ ID NO:33). Alternatively, the compound can have an
amino acid sequence having at least about 90% to about 99% sequence similarity to SEQ
ID NO:33.

[0040] In another instance, a compound is provided that includes an amino acid sequence
of:

EVQLLES GGGLVQPGGSLRLSCAASGRYIDETAVAWFRQAPGKGREFVAGIGGG
VDITYYADSVKGRFTISRDN SKNTLYLQMNSLRPEDTAVYYCAARPGRPLITSKV
ASLYPYWGQGTLVTVSS (SEQ ID NO:34). Alternatively, the compound can have an
amino acid sequence having at least about 90% to about 99% sequence similarity to SEQ
ID NO:34.

[0041] In another instance, a compound is provided that includes an amino acid sequence
of:

EVQLLES GGGLVQPGGSLRLSCAASGRYIDETAVAWFRQAPGKGREFVAGIGGG
VDITYYADSVKGRFTISRDN SKNTLYLQMNSLRPEDTAVYYCAARPGRPLITSKQ
AELYPYWGQGTLVTVSS (SEQ ID NO:35). Alternatively, the compound can have an
amino acid sequence having at least about 90% to about 99% sequence similarity to SEQ
ID NO:35.

[0042] In another instance, a compound is provided that includes an amino acid sequence
of:

EVQLLES GGGLVQPGGSLRLSCAASGRYIDETAVAWFRQAPGKGREFVAGIGGG
VDITYYADSVKGRFTISRDN SKNTLYLQMNSLRPEDTAVYYCAARPGRPLITSKQ
ASLYPYWGQGTLVTVSS (SEQ ID NO:36). Alternatively, the compound can have an

amino acid sequence having at least about 90% to about 99% sequence similarity to SEQ ID NO:36.

[0043] In another instance, a compound is provided that includes an amino acid sequence of:

EVQLLESGGGLVQPGGSLRLSCAASGRYIDETAVAWFRQAPGKGREFVAGIGGG
VDITYYADSVKGRFTISRDN SKNTLYLQMNSLRPEDTAVYYCAARPGRPLITSKV
ADLYPYWGQGTLVTVSSC (SEQ ID NO:37). Alternatively, the compound can have an amino acid sequence having at least about 90% to about 99% sequence similarity to SEQ ID NO:37.

[0044] In another instance, a compound is provided that includes an amino acid sequence of:

EVQLLESGGGLVQPGGSLRLSCAASGRYIDETAVAWFRQAPGKGREFVAGIGGG
VDITYYADSVKGRFTISRDN SKNTLYLQMNSLRPEDTAVYYCGARPGRPLITSKV
ADLYPYWGQGTLVTVSSC (SEQ ID NO:124). Alternatively, the compound can have an amino acid sequence having at least about 90% to about 99% sequence similarity to SEQ ID NO:124.

[0045] In another instance, a compound is provided that includes an amino acid sequence of:

EVQLLESGGGLVQPGGSLRLSCAASGRYIDETAVAWFRQAPGKGREFVAGIGGG
VDITYYADSVKGRFTISRDN SKNTLYLQMNSLRPEDTAVYYCGARPGRPLITSKV
ADLYPYWGQGTLVTVSSPP (SEQ ID NO:125). Alternatively, the compound can have an amino acid sequence having at least about 90% to about 99% sequence similarity to SEQ ID NO:125.

[0046] In another instance, a compound is provided that includes an amino acid sequence of:

EVQLLESGGGLVQPGGSLRLSCAASGRYIDETAVAWFRQAPGKGREFVAGIGGG
VDITYYADSVKGRFTISRDN SKNTLYLQMNSLRPEDTAVYYCGTRPGRPLITSKV
ADLYPYWGQGTLVTVSSPP (SEQ ID NO:126). Alternatively, the compound can have an amino acid sequence having at least about 90% to about 99% sequence similarity to SEQ ID NO:126.

[0047] Second, the disclosure describes compounds that include at least one of a VHH-based $t_{1/2}$ -extending moiety as described herein and a biotherapeutic. In some instances,

the compounds can include a structure from an amino-terminus (N-terminus) to a carboxy-terminus (C-terminus) of:

M-X₁,
 X₁-M,
 M-X₂,
 X₂-M,
 M-L₁-X₁,
 M-L₂-X₁,
 M-L₁-X₂,
 M-L₂-X₂,
 X₁-L₁-M,
 X₁-L₂-M,
 X₂-L₁-M,
 X₂-L₂-M,
 X₁-M-X₂,
 X₂-M-X₁,
 X₁-L₁-M-X₂,
 X₂-L₁-M-X₁,
 X₁-M-L₁-X₂,
 X₂-M-L₁-X₁,
 X₁-L₁-M-L₂-X₂,
 X₂-L₁-M-L₂-X₁,
 M-L₁-X₁-L₂-X₂,
 M-L₁-X₂-L₂-X₁,
 X₁-L₂-X₂-L₁-M,
 X₂-L₂-X₁-L₁-M, or

M-L₁-X₁ combined with M-L₂-X₂ (*i.e.*, non-covalently associated),

[0048] where M is a compound acting as a t_{1/2}-extending moiety and having an amino acid sequence selected from SEQ ID NOS:1 to 37 and 124 to 126 or having an amino acid sequence having at least about 90% to about 99% sequence similarity thereto, where L₁ (if present) is a first linker, where L₂ (if present) is a second linker, where X₁ is a biotherapeutic protein, peptide or oligomer, and where X₂ is a biotherapeutic protein, peptide or oligomer

that can be the same as or distinct from X_1 (such as when the biotherapeutic is a homodimer, or when the biotherapeutic is a heterodimer X_1 can be one chain (α -chain) and X_2 can be another chain (β -chain) thereof). X_1 and X_2 can also be fully distinct from one another. In some instances, L_1 can have an amino acid sequence of $(GGGGQ)_n$ (SEQ ID NO:38), $(GGGQ)_n$ (SEQ ID NO:39), $(GGGGS)_n$ (SEQ ID NO:40), $(PGPQ)_n$ (SEQ ID NO:41), $(PGPA)_n$ (SEQ ID NO:42), $GGGG(AP)_nGGGG$ (SEQ ID NO:43), $(GGE)_n$ (SEQ ID NO:44), $(GGGGE)_n$ (SEQ ID NO:45), $(GGK)_n$ (SEQ ID NO:46), $(GGGGK)_n$ (SEQ ID NO:47), $GGGG(EP)_nGGGG$ (SEQ ID NO:48), $GGGG(KP)_nGGGG$, (SEQ ID NO:49), $(PGPE)_n$ (SEQ ID NO:50) or $(PGPK)_n$ (SEQ ID NO:51), where n can be from 1 to 15, especially from about 5 to about 10. In other instances, L_1 can have an amino acid sequence selected from SEQ ID NOS:52 to 63. In still other instances, L_1 can have one or more additions, deletions, insertions or substitutions such that L_1 has an amino acid sequence having at least about 90% to about 99% sequence similarity to any one of SEQ ID NOS:52 to 63.

[0049] In some instances, L_2 can have an amino acid sequence selected from SEQ ID NO:64 to 65. In other instances, L_2 can have one or more additions, deletions, insertions or substitutions such that L_2 has an amino acid sequence having at least about 90% to about 99% sequence similarity to any one of SEQ ID NOS:64 to 65.

[0050] In still other instances, L_1 or L_2 can be a polymer such as a polyethylene glycol (PEG), especially $(PEG)_n$, where n can be from 1 to 20.

[0051] In some instances, X_1 , X_2 or X_1/X_2 is a peptide or protein (and even an oligomer, *e.g.*, a homodimer or heterodimer that may or may not be covalently linked). Examples of such peptides or proteins include, but are not limited to, an antibody (Ab), an antibody fragment (*e.g.*, Fab, scFv, Fab-Fab, VH, VL or VHH of different specificity), a ciliary neurotrophic factor (CNTF), growth/differentiation factor 15 (GDF15), an incretin (INC), an interleukin (IL), a neuregulin (NRG), or a hormone. In certain instances, the INC can be insulin (INS), glucose-dependent insulintropic peptide (GIP), glucagon-like peptide-1 (GLP-1), GIP/GLP-1, or even an INC having triple receptor activity (*i.e.*, glucagon-GIP-GLP-1 receptor activity). In certain instances, the IL is interleukin-2 (IL-2). In certain instances, the NRG is neuregulin 1 (NRG1) or neuregulin 4 (NRG4). In certain instances, the hormone is adrenocorticotrophic hormone (ACTH) or relaxin-2 (RLN-2). In certain

instances, the Fab binds to GITR; and in some instances the Fab binds to GITR and is a GITR antagonist.

[0052] In particular instances, the compounds can have an amino acid sequence of any one of SEQ ID NO:100 to 118. Alternatively, the compounds can have at least about 90% to about 99% sequence similarity to an amino acid sequence of any one of SEQ ID NOS: 100 to 118.

[0053] Third, the disclosure describes pharmaceutical compositions that include at least one compound herein and a pharmaceutically acceptable carrier.

[0054] Fourth, the disclosure describes methods of using the compounds and pharmaceutical compositions for medicaments and for extending the $t_{1/2}$ of biotherapeutics.

[0055] Fifth, the disclosure describes uses of the compounds herein in the manufacture of medicaments and in extending the $t_{1/2}$ of biotherapeutics.

[0056] An advantage of the $t_{1/2}$ -extending moieties and compounds including the same is that they can be chemically or recombinantly synthesized as a single-chain polypeptide (*i.e.*, monomeric) and thus do not require endoproteolytic processing for biological activity. It is contemplated, however, that in some instances, the compounds acting as $t_{1/2}$ -extending moieties can be fused not only to single-chain peptides and proteins but also to peptides with more than one chain, for example, two-chain peptides, multi-chain peptides and proteins as well. On the compounds acting as $t_{1/2}$ -extending moieties, one can chemically conjugate not only to the N- and C-terminus but also to any surface-exposed amino acid of the $t_{1/2}$ -extending moieties (with the proviso that such conjugation does not entirely abrogate albumin binding).

[0057] An advantage of the compounds acting as $t_{1/2}$ -extending moieties and compounds including the same is that the $t_{1/2}$ -extending moieties provide an extended duration of action in mammals such as humans and can have a $t_{1/2}$ of about 20 days to about 30 days, thereby allowing for at least weekly or biweekly administration when compared to native peptides and proteins, which can improve compliance and can improve quality of life, especially in cases of chronic diseases requiring life-long therapy.

[0058] An advantage of the compounds acting as $t_{1/2}$ -extending moieties herein is that they have tunable pharmacokinetics achieved by changing albumin affinity of the $t_{1/2}$ -extending moieties.

[0059] An advantage of the compounds acting as $t_{1/2}$ -extending moieties herein is that they may enable recombinant expression in standard manufacturing organisms such as yeast, mammalian or prokaryotes.

[0060] Moreover, and an advantage of the compounds acting as $t_{1/2}$ -extending moieties herein is that they have similar binding not only to human serum albumin but also to monkey, mouse, rat, dog and pig serum albumin, which allows for pharmacodynamic, pharmacokinetic and toxicology studies to more readily translate from these species to humans. As such, the $t_{1/2}$ -extending moieties herein can be used not only for treating humans but also for treating animals.

DETAILED DESCRIPTION

[0061] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of skill in the art to which the disclosure pertains. Although any methods and materials similar to or equivalent to those described herein can be used in the practice or testing of the analogs, pharmaceutical compositions and methods, the preferred methods and materials are described herein.

[0062] Moreover, reference to an element by the indefinite article “a” or “an” does not exclude the possibility that more than one element is present, unless the context clearly requires that there be one and only one element. The indefinite article “a” or “an” thus usually means “at least one.”

[0063] *Definitions*

[0064] As used herein, “about” means within a statistically meaningful range of a value or values such as, for example, a stated concentration, length, molecular weight, pH, sequence similarity, time frame, temperature, volume, *etc.* Such a value or range can be within an order of magnitude typically within 20%, more typically within 10%, and even more typically within 5% of a given value or range. The allowable variation encompassed by “about” will depend upon the particular system under study, and can be readily appreciated by one of skill in the art.

[0065] As used herein, and in reference to one or more of receptors, “activity,” “activate,” “activating” and the like means a capacity of a compound, such as a fusion herein, to bind

to and induce a response at the receptor(s), as measured using assays known in the art, such as the *in vitro* assays described below.

[0066] As used herein, “adrenocorticotrophic hormone” or “ACTH” means an ACTH obtained or derived from any species, such as a mammalian species, especially a human. ACTH includes both native ACTH (*i.e.*, full-length) and variations thereof (*i.e.*, additions, deletions, insertions and/or substitutions of native ACTH). One sequence for ACTH is set forth in SEQ ID NO:95 (UniProt/SwissProt Database Accession No. P01189). In humans, ACTH binds to the ACTH receptor (ACTHR, also known as the melanocortin receptor 2 or MC2R), and one sequence for ACTHR is set forth in SEQ ID NO:96 (UniProt/SwissProt Database Accession No. Q01718). ACTHR is a G protein-coupled receptor located on the external cell plasma membrane and is coupled to G α s and upregulates levels of cAMP by activating adenylyl cyclase.

[0067] As used herein, “amino acid” means a molecule that, from a chemical standpoint, is characterized by a presence of one or more amine groups and one or more carboxylic acid groups, and may contain other functional groups. As is known in the art, there is a set of twenty amino acids that are designated as standard amino acids and that can be used as building blocks for most of the peptides/proteins produced by any living being. The amino acid sequences in the disclosure contain the standard single letter or three letter codes for the twenty naturally occurring amino acids.

[0068] As used herein, “analog” means a compound, such as a synthetic peptide or polypeptide, that activates a target receptor and that elicits at least one *in vivo* or *in vitro* effect elicited by a native agonist for that receptor.

[0069] As used herein, “biotherapeutic” and the like means an amino acid- or nucleic acid-based compounds such as antibodies, coagulation factors, clotting factors, cytokines, enzymes, growth factors, hormones, and fragments thereof, having at least one therapeutic activity/applicability, as well as therapeutic DNA and/or RNA molecules.

[0070] As used herein, “CNTF” or “ciliary neurotrophic factor” means a CNTF obtained or derived from any species, such as a mammalian species, especially a human. CNTF includes both native CNTF (*i.e.*, full-length) and variations thereof (*i.e.*, additions, deletions, insertions and/or substitutions of native CNTF). One sequence for CNTF is set forth in SEQ ID NO:97 (UniProt/SwissProt Database Accession No. P26441). In humans, CNTF binds to the CNTF α -receptor (CNTFR α), and one sequence for CNTFR α is set forth

in SEQ ID NO:98 (UniProt/SwissProt Database Accession No. P26992). CNTFR α also uses two signal-transducing transmembrane subunits, LIFR β and gp130, which together activate the Jak-STAT signaling pathway. *See, Stahl et al. (1994) Science 263:92-95 and Stahl & Yancopoulos (1994) J. Neurobiol. 25:1454-1466.*

[0071] As used herein, “conservative substitution” means a variant of a reference peptide or polypeptide that is identical to the reference molecule, except for having one or more conservative amino acid substitutions in its amino acid sequence. In general, a conservatively modified variant includes an amino acid sequence that is at least about 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to a reference amino acid sequence. More specifically, a conservative substitution refers to substitution of an amino acid with an amino acid having similar characteristics (*e.g.*, charge, side-chain size, hydrophobicity/hydrophilicity, backbone conformation and rigidity, *etc.*) and having minimal impact on the biological activity of the resulting substituted peptide or polypeptide. Conservative substitutions of functionally similar amino acids are well known in the art and thus need not be exhaustively described herein.

[0072] As used herein, “effective amount” means an amount or dose of one or more of the compounds herein, or a pharmaceutically acceptable salt thereof that, upon single or multiple dose administration to an individual in need thereof, provides a desired effect in such an individual under diagnosis or treatment (*i.e.*, may produce a clinically measurable difference in a condition of the individual such as, for example, increased angiogenesis, increased vascular compliance, increased cardiac blood flow, increased hepatic blood flow, increased pulmonary blood flow, increased renal blood flow, increased glomerular filtration rate, decreased blood pressure, decreased (or prevented) inflammation and/or reduced (or prevented) fibrosis in the heart, kidney, liver or lung). An effective amount can be readily determined by one of skill in the art by using known techniques and by observing results obtained under analogous circumstances. In determining the effective amount for an individual, a number of factors are considered, including, but not limited to, the species of mammal, its size, age and general health, the specific disease or disorder involved, the degree of or involvement or the severity of the disease or disorder, the response of the individual, the particular compound administered, the mode of administration, the bioavailability characteristics of the preparation administered, the dose regimen selected, the use of concomitant medication, and other relevant circumstances.

[0073] As used herein, “extended duration of action” means that binding affinity and activity for a fusion including at least one compound herein and a biotherapeutic herein continues for a period of time greater than a native biotherapeutic, allowing for dosing at least as infrequently as once daily or even thrice-weekly, twice-weekly or once-weekly. The time action profile may be measured using known pharmacokinetic test methods such as those utilized in the Examples below.

[0074] As used herein, “glucocorticoid-induced TNFR-related protein” or “GITR”, also known as tumor necrosis factor receptor superfamily member 18 (TNFRSF18), means a GITR obtained or derived from any species, such as a mammalian species, especially a human. GITR includes both native GITR (*i.e.*, full-length) and variations thereof (*i.e.*, additions, deletions, insertions and/or substitutions of native GITR). One sequence for human GITR full-length (but without the signal peptide) is set forth in SEQ ID NO:122 (*see also*, UniProt/SwissProt Database Accession No. Q9Y5U5). One sequence for human GITR ECD (but without the signal peptide) is set forth in SEQ ID NO:123.

[0075] As used herein, “glucose-dependent insulintropic peptide(s),” “gastric inhibitory peptide(s)” or “GIP(s)” means a GIP obtained or derived from any species, such as a mammalian species, especially a human. GIP includes both native GIP (*i.e.*, full-length) and variations thereof (*i.e.*, additions, deletions, insertions and/or substitutions of native GIP). GIP is processed from a precursor, proGIP. One sequence for proGIP is set forth in SEQ ID NO:67 (*see also*, UniProt/SwissProt Database Accession No. P09681) and one sequence for GIP is set forth in SEQ ID NO:68. An alternative GIP is GIP₁₋₃₀ (*see*, Hansen *et al.* (2016) *Br. J. Pharmacol.* 173:826-838). In humans, there is one GIP receptor (GIPR; SEQ ID NO:69; *see also*, UniProt/SwissProt Database Accession No. P48546), which acts as G protein-coupled receptor. *See*, Yaqub *et al.* (2010) *Mol. Pharmacol.* 77:547-558.

[0076] As used herein, “glucagon-like peptide-1” or “GLP-1” means a GLP-1 obtained or derived from any species, such as a mammalian species, especially a human. GLP-1 includes both native GLP-1 (*i.e.*, full-length) and variations thereof (*i.e.*, additions, deletions, insertions and/or substitutions of native GLP-1). GLP-1 is processed from a precursor, proglucagon (proGCG). One sequence for proGCG is set forth in SEQ ID NO:70 (*see also*, UniProt/SwissProt Database Accession No. P01275), and one sequence for GLP-1 is set forth in SEQ ID NO:71. Two active, physiological variants are known, which are set forth in SEQ ID NOS:72 and 73. In humans, there is one GLP-1 receptor (GLP-1R;

SEQ ID NO:74; *see also*, UniProt/SwissProt Database Accession No. P43220), which acts as G protein-coupled receptor. *See*, Dillon *et al.* (1993) *Endocrinol.* 133:1907-1910; Graziano *et al.* (1993) *Biochem. Biophys. Res. Commun.* 196:141-146; and Thorens *et al.* (1993) *Diabetes* 42:1678-1682.

[0077] As used herein, “growth/differentiation factor 15” or “GDF15” means a GDF15 obtained or derived from any species, such as a mammalian species, especially a human. GDF15 includes both native GDF15 (*i.e.*, full-length) and variations thereof (*i.e.*, additions, deletions, insertions and/or substitutions of native GDF15). GDF15 is a homodimeric peptide that is processed from a precursor, proGDF15. One sequence for the precursor is set forth in SEQ ID NO:75, and one sequence for GDF15 is set forth in SEQ ID NO:76 (*see also*, UniProt/SwissProt Database Accession No. Q99988). In humans, there is one GDF15 receptor (GFRAL; SEQ ID NO:77; *see also*, UniProt/SwissProt Database Accession No. Q6UXV0), which acts as a RET-receptor tyrosine kinase. *See*, Emmerson *et al.* (2017) *Nat Med.* 23:1215-1219; Yang *et al.* (2017) *Nat. Med.* 23:1158-1166; and Baek & Eling (2019) *Pharmacol. Ther.* 198:46-58.

[0078] As used herein, “half-life” or “ $t_{1/2}$ ” means a time it takes for one-half of a quantity of a compound, such as a fusion described herein, to be removed from a fluid or other physiological space such as serum or plasma of an individual by biological processes. Alternatively, $t_{1/2}$ also can mean a time it takes for a quantity of such a fusion to lose one-half of its pharmacological, physiological or radiological activity.

[0079] As used herein, “half-maximal effective concentration” or “ EC_{50} ” means a concentration of compound that results in 50% activation/stimulation of an assay endpoint, such as a dose-response curve (*e.g.*, CNTF: Jak, STAT, Ras, PI3K/Akt and MAPK/ERK; NRG: PI3K/Akt, Jak, STAT, Ras and PLC γ ; GDF15: PI3K/AKT and MAPK/ERK and Smad; IL-2: JAK-STAT, PI3K/Akt and MAPK/ERK; GLP1: cAMP, PI3K, MAPK/ERK, PKC δ ; TNF: TRAF, MKK, IKK and NF κ B; ACTH: cAMP and PKA).

[0080] As used herein, “in combination with” means administering at least one of the fusions herein either simultaneously, sequentially or in a single combined formulation with one or more additional therapeutic agents.

[0081] As used herein, “individual in need thereof” means a mammal, such as a human, with a condition, disease, disorder or symptom requiring treatment or therapy, including

for example, those listed herein. In particular, the preferred individual to be treated is a human.

[0082] As used herein, “incretin(s)” or “INC(s)” means a peptide secreted from enteroendocrine cells that can increase insulin secretion following feeding. INC can be an incretin obtained or derived from any species, such as a mammalian species, especially a human. In humans, INCs include INS, GIP and GLP-1, which are discussed above.

[0083] As used herein, “insulin” or “INS” means an insulin obtained or derived from any species, such as a mammalian species, especially a human, where the native form is a heterodimeric peptide having two peptide chains (*e.g.*, an A chain and a B chain) connected via two disulfide bonds, and with the A chain further having a single intramolecular disulfide bond. In humans, INS processing begins with preproinsulin (*see*, UniProt/SwissProt Database Accession No. P01308), which is processed to proinsulin (includes A chain, B chain and C peptide; native INS has a structure of B-C-A; *see*, SEQ ID NO:78; *see also*, UniProt/Swiss Prot Database Accession No. P01308). Proinsulin undergoes further processing in which the C peptide is cleaved to arrive at INS (*see*, SEQ ID NO:79 for A chain of native, human INS and SEQ ID NO:80 for B chain of native, human INS; *see also*, UniProt/SwissProt Database Accession No. P01308).

[0084] As used herein, “interleukin(s)” or “IL(s)” means an interleukin obtained or derived from any species, such as a mammalian species, especially a human. IL includes both native IL (*i.e.*, full-length) and variations thereof (*i.e.*, additions, deletions, insertions and/or substitutions of native IL). In humans, there are a number of native IL isoforms; however, of interest herein is IL-2. IL-2 is a cytokine that can transduce signals via three different signaling pathways, which include JAK-STAT, PI3K/Akt/mTOR and MAPK/ERK pathways. One sequence for human IL-2 is set forth in SEQ ID NO:83 (*see also*, UniProt/SwissProt Database Accession No. P60568). In humans, there is one IL-2 receptor that includes α -, β - and γ -subunits (IL-2R; SEQ ID NOS:84 to 86; *see also*, UniProt/SwissProt Database Accession No. P01589, P14784 and P31785). *See*, Liao *et al.* (2011) *Curr. Opin. Immunol.* 23:598-604; and Malek & Castro (2010) *Immunity* 33:153-165.

[0085] As used herein, “long-acting” means that binding affinity and activity of a composition herein continues for a period of time greater than native peptide or protein, allowing for dosing at least as infrequently as once daily or even thrice-weekly, twice-

weekly, once-weekly or monthly. The time action profile of the compounds herein may be measured using known pharmacokinetic test methods such as those described in the Examples below.

[0086] As used herein, “neuregulin(s)” or “NRG(s)” means a neuregulin obtained or derived from any species, such as a mammalian species, especially a human. NRG includes both native NRG (*i.e.*, full-length) and variations thereof (*i.e.*, additions, deletions, insertions and/or substitutions of native NRG). In humans, there are a number of native NRG family members; however, of interest herein is NRG1. Like all NRGs, NRG1 is processed from a larger precursor. One sequence for the precursor is set forth in SEQ ID NO:87, and one sequence for NRG1 is set forth in SEQ ID NO:88 (*see also*, UniProt/SwissProt Database Accession Nos. Q02297). In humans, there are two NRG1 receptors, ErbB3 (SEQ ID NO:89; *see also*, UniProt/SwissProt Database Accession No. P21860) and ErbB4 (SEQ ID NO:90; *see also*, UniProt/SwissProt Database Accession No. Q15303). *See*, Mei & Xiong (2008).

[0087] As used herein, “non-standard amino acid” means an amino acid that may occur naturally in cells but does not participate in peptide synthesis. Non-standard amino acids can be constituents of a peptide and oftentimes are generated by modification of standard amino acids in the peptide (*i.e.*, via post-translational modification). Non-standard amino acids can include D-amino acids, which have an opposite absolute chirality of the standard amino acids above.

[0088] As used herein, “oligomer” means a molecule having a few similar or identical repeating units that could be derived from copies of a smaller molecule, its monomer. These monomers can be joined by bonds that are either strong or weak, covalent or non-covalent (*e.g.*, intramolecular).

[0089] As used herein, “patient,” “subject” and “individual,” are used interchangeably herein, and mean a mammal, especially a human. In certain instances, the individual is further characterized with a condition, disease, disorder or symptom that would benefit from administering a compound or composition herein.

[0090] As used herein, “pharmaceutically acceptable buffer” means any of the standard pharmaceutical buffers known to one of skill in the art.

[0091] As used herein, “relaxin-2” or “RLN-2” means a relaxin-2 obtained or derived from any species, such as a mammalian species, especially a human, where the native form

is a heterodimeric peptide having two peptide chains (*e.g.*, an A chain and a B chain) connected via two disulfide bonds, and with the A chain further having a single intramolecular disulfide bond. In humans, RLN-2 processing begins with preprorelaxin (*see*, UniProt/SwissProt Database Accession No. P04090), which is processed to prorelaxin (includes A chain, B chain and C peptide; native RLN has a structure of B-C-A; *see*, SEQ ID NO:91; *see also*, UniProt/SwissProt Database Accession No. P04090). Prorelaxin undergoes further processing in which the C peptide is cleaved to arrive at RLN-2 (*see*, SEQ ID NO:92 for the A chain of RLN-2 and SEQ ID NO:93 for the B chain of RLN-2; *see also*, UniProt/SwissProt Database Accession No. P04090).

[0092] As used herein, “sequence similarity” means a quantitative property of two or more nucleic acid sequences or amino acid sequences of biological compounds such as, for example, a correspondence over an entire length or a comparison window of the two or more sequences. Sequence similarity can be measured by (1) percent identity or (2) percent similarity. Percent identity measures a percentage of residues identical between two biological compounds divided by the length of the shortest sequence, whereas percent similarity measures identities and, in addition, includes sequence gaps and residue similarity in the evaluation. Methods of and algorithms for determining sequence similarity are well known in the art and thus need not be exhaustively described herein. A specified percentage of identical nucleotide or amino acid positions is at least about 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or higher.

[0093] As used herein, “treating” or “to treat” means managing and caring for an individual having a condition, disease, disorder or symptom for which administration of a compound herein is indicated for the purpose of attenuating, restraining, reversing, slowing or stopping progression or severity of the condition, disease, disorder or symptom. Treating includes administering a compound herein or composition containing a compound herein to the individual to prevent the onset of symptoms or complications, alleviating the symptoms or complications, or eliminating the condition, disease, disorder or symptom. Treating includes administering a compound herein or composition containing a compound herein to the individual to result in such as, for example, increased angiogenesis, increased vascular compliance, increased cardiac blood flow, increased hepatic blood flow, increased pulmonary blood flow, increased renal blood flow, increased glomerular filtration rate, decreased blood pressure, decreased (or prevented) inflammation and/or reduced (or

prevented) fibrosis in the heart, kidney, liver or lung. The individual to be treated is a mammal, especially a human.

[0094] As used herein, “VHH” or “VHH moiety” means a form of single-domain antibody, especially an antibody fragment comprising a single, monomeric variable region of a heavy chain only antibody (HcAb), which may have a size of about 15 kDa. It has been found herein that engineered/modified VHH-based compounds can be used as a pharmacokinetic enhancer to extend the duration of action of and/or to improve the $t_{1/2}$ of biotherapeutics. The VHH-based compounds bind serum albumin; however, the VHH-based compounds can be used to bind IgG (including Fc domain), neonatal Fc receptor (FcRn) or other long-lasting serum proteins. The VHH-based compound therefore can be used to improve the $t_{1/2}$ of a compound such as a peptide or protein or even other molecules such as, for example, small molecules.

[0095] Certain abbreviations are defined as follows: “ACR” refers to urine albumin/urine creatinine ratio; “AUC” refers to area under the curve; “cAMP” refers to cyclic adenosine monophosphate; “CMV” refers to cytomegalovirus; “DNA” refers to deoxyribonucleic acid; “ECD” refers to extracellular domain; “EDC” refers to 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride; “ETA” refers to ethanolamine; “GS” refers to glutamine synthetase; “HC” refers to heavy chain; “HIC” refers to hydrophobic interaction chromatography; “hr” refers to hour or hours; “IV” refers to intravenous; “kDa” refers to kilodaltons; “LC” refers to light chain; “LC-MS” refers to liquid chromatography-mass spectrometry; “min” refers to minute or minutes; “MS” refers to mass spectrometry; “MSX” refers to methionine sulfoximine; “NHS” refers to N-hydroxysuccinimide; “OtBu” refers to O-tert-butyl; “PEI” refers to polyethylenimine; “RP-HPLC” refers to reversed-phase high performance liquid chromatography; “sec” refers to second or seconds; “NaOAc” refers to sodium acetate; “rcf” means relative centrifugal force; “RT” means room temperature; “RU” means resonance units; “SQ” refers to subcutaneous; “SEC” refers to size-exclusion chromatography; “SEM” refers to standard error of the mean; “SPR” means surface plasmon resonance; “TFA” refers to trifluoroacetic acid; and “Trt” refers to trityl.

[0096] *VHH-Based Compounds Acting as Half-Life Extenders and Fusion or Conjugated Compounds Including the Same*

[0097] Briefly, the compounds herein can include an amino acid sequence from the N-terminus to the C-terminus having one of the following structures:

M-X₁,
X₁-M,
M-X₂,
X₂-M,
M-L₁-X₁,
M-L₂-X₁,
M-L₁-X₂,
M-L₂-X₂,
X₁-L₁-M,
X₁-L₂-M,
X₂-L₁-M,
X₂-L₂-M,
X₁-M-X₂,
X₂-M-X₁,
X₁-L₁-M-X₂,
X₂-L₁-M-X₁,
X₁-M-L₁-X₂,
X₂-M-L₁-X₁,
X₁-L₁-M-L₂-X₂,
X₂-L₁-M-L₂-X₁,
M-L₁-X₁-L₂-X₂,
M-L₁-X₂-L₂-X₁,
X₁-L₂-X₂-L₁-M,
X₂-L₂-X₁-L₁-M, or

M-L₁-X₁ combined with M-L₂-X₂ (*i.e.*, non-covalently associated),

[0098] where M is a VHH-based compound acting as a t_{1/2}-extending moiety and having an amino acid sequence selected from SEQ ID NOS:1 to 37 and 124 to 126 or having an amino acid sequence having at least about 90% to about 99% sequence similarity thereto, where L₁ (if present) is a first linker, where L₂ (if present) is a second linker, where X₁ (if present) is a biotherapeutic protein, peptide or oligomer, and where X₂ (if present) likewise

is a biotherapeutic protein, peptide, or oligomer that can be the same as or distinct from X₁ (such as when the biotherapeutic is a homodimer, or when the biotherapeutic is a heterodimer X₁ can be one chain (α -chain) and X₂ can be another chain (β -chain) thereof). X₁ and X₂ can also be fully distinct from one another.

[0099] In some instances, L₁ can have an amino acid sequence of (GGGGQ)_n (SEQ ID NO:38), (GGGQ)_n (SEQ ID NO:39), (GGGGS)_n (SEQ ID NO:40), (PGPQ)_n (SEQ ID NO:40), (PGPA)_n (SEQ ID NO:42), GGGG(AP)_nGGGG (SEQ ID NO:43), (GGE)_n (SEQ ID NO:44), (GGGGE)_n (SEQ ID NO:45), (GGK)_n (SEQ ID NO:46), (GGGGK)_n (SEQ ID NO:47), GGGG(EP)_nGGGG (SEQ ID NO:48), GGGG(KP)_nGGGG (SEQ ID NO:49), (PGPE)_n (SEQ ID NO:50), or (PGPK)_n (SEQ ID NO:51), where n can be from 1 to 15, especially from about 5 to about 10. In other instances, L₁ can have an amino acid sequence selected from any one of SEQ ID NOS:52 to 63. In still other instances, L₁ can have one or more additions, deletions, insertions or substitutions such that L₁ has an amino acid sequence having at least about 90% to about 99% sequence similarity to any one of SEQ ID NOS:52 to 63.

[00100] In some instances, L₂ can have an amino acid sequence selected from any one of SEQ ID NOS:64 to 65. In other instances, L₂ can have one or more additions, deletions, insertions or substitutions such that L₂ has an amino acid sequence having at least about 90% to about 99% sequence similarity to any one of SEQ ID NOS:64 to 65.

[00101] In still other instances, L₁ or L₂ can be a polymer such as a polyethylene glycol (PEG), especially maleimide-(PEG)₁₂.

[00102] Taken together, exemplary fusions are as follows:

[00103] Compound 1, which is a VHH-CNTF fusion that includes CNTF, a (G₄Q)₅ linker (*italicized*) and a VHH-based compound acting as a t_{1/2}-extending moiety (underlined), has the following amino acid sequence:

MAFTEHSPLTPHRRDLASRSIWLARKIRSDLTALTESYVKHQGLNKNINLDSADG
MPVASTDRWSELTEAERLQENLQAYRTFHVLLARLLEDQQVHFTPTEGDFHQAI
HTLLLQVA AFAYQIEELMILLEYKIPRNEADGMPINVG DGGLFEKKLWGLKVLQE
LSQWTVRSIHDLRFISSHQTGGGGGQGGGGQGGGGQGGGGQGGGGQEVQLLES
GGGLVQPGGSLRLSCAASGRYIDETAVAWFRQAPGKEREFVAGIGGGVDITYYA
DSVKGRFTISRDN SKNTLYLQMNSLRPEDTAVYYCAARPGRPLITSKVADLYPY
WGQGT LVT VSSPP (SEQ ID NO:100).

[00104] Compound 2, which is a VHH-CNTF fusion that includes a VHH-based compound acting as a $t^{1/2}$ -extending moiety (underlined), a (G₄Q)₅ linker (*italicized*) and CNTF, has the following amino acid sequence:

EVQLLES*GGGLVQPGGSLRLSCAASGRYIDETAVAWFRQAPGKEREFVAGIGGG*
VDITYYADSVKGRFTISRDN*SKNTLYLQMNSLRPEDTAVYYCAARPGRPLITSKV*
ADLYPYWGQGT*LVTVSSGGGGQGGGGQGGGGQGGGGQGGGGQ*MAFTEHSPLT
 PHRRDLASRSIWLARKIRSDLTALTESYVKHQGLNKNINLDSADGMPVASTDRW
 SELTEAERLQENLQAYRTFHVLLARLLEDQQVHFTPTGDFHQAIHTLLLQVAAF
 AYQIEELMILLEYKIPRNEADGMPINVGDGGLFEKKLWGLKVLQELSQWTVRSIH
 DLRFISSHQTG (SEQ ID NO:101).

[00105] Compound 3, which is a VHH-NRG1 fusion that includes NRG1, a (G₄Q)₅ linker (*italicized*) and a VHH-based compound acting as a $t^{1/2}$ -extending moiety (underlined), has the following amino acid sequence:

SHLVKCAEKEKTFVCVNGGECFMVKDLSNPSRYLCKCPNEFTGDRCQNYVMASF
 YKHLGIEFMEAEELYQGGGGQGGGGQGGGGQGGGGQGGGGQEVQLLES

GGGLVQPGGSLRLSCAASGRYIDETAVAWFRQAPGKEREFVAGIGGGVDITYYADSVK
GRFTISRDN*SKNTLYLQMNSLRPEDTAVYYCAARPGRPLITSKVADLYPYWGQGT*
LVTVSSPP (SEQ ID NO:102).

[00106] Compound 4, which is a VHH-NRG1 fusion that includes a VHH-based compound acting as a $t^{1/2}$ -extending moiety (underlined), a (G₄Q)₅ linker (*italicized*) and NRG1, has the following amino acid sequence:

EVQLLES*GGGLVQPGGSLRLSCAASGRYIDETAVAWFRQAPGKEREFVAGIGGG*
VDITYYADSVKGRFTISRDN*SKNTLYLQMNSLRPEDTAVYYCAARPGRPLITSKV*
ADLYPYWGQGT*LVTVSSGGGGQGGGGQGGGGQGGGGQGGGGQ*SHLVKCAEK
 EKTFCVNGGECFMVKDLSNPSRYLCKCPNEFTGDRCQNYVMASFYKHLGIEFME
 AEELYQ (SEQ ID NO:103).

[00107] Compound 5, which is a VHH-GDF15 fusion that includes a VHH-based compound acting as a $t^{1/2}$ -extending moiety (underlined), a (G₄Q)₅ linker (*italicized*) and GDF15, has the following amino acid sequence:

EVQLLES*GGGLVQPGGSLRLSCAASGRYIDETAVAWFRQAPGKEREFVAGIGGG*
VDITYYADSVKGRFTISRDN*SKNTLYLQMNSLRPEDTAVYYCAARPGRPLITSKV*
ADLYPYWGQGT*LVTVSSGGGGQGGGGQGGGGQGGGGQGGGGQ*GDHCPPLPGP

EVQLVESGGGLVQPGRSLRLSCAASGFTFDDYAMHWVRQAPGKGLEWVSAITW
NSGHIDYADSVGRFTISRDNANKNSLYLQMNSLRAEDTAVYYCAKVSYLSTASSL
DYWGQGTLLTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSW
NSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDK
KVEPKSCDKTHTGGGGQGGGGQGGGGQGGGGQGGGGQEVQLLES GGGLVQPG
GSLRLSCAASGRYIDETAVAWFRQAPGKEREFVAGIGGGVDITYYADSVKGRFTI
SRDNSKNTLYLQMNSLRPEDTAVYYCAARPGRPLITSKVADLYPYWGQGTLLTV
SSPP (SEQ ID NO:108).

[00112] Compound 9 further includes a LC having the following amino acid sequence:
DIQMTQSPSSLSASVGDRVTITCRASQGIRNYLAWYQQKPGKAPKLLIYAASLTQ
SGVPSRFSGSGSGTDFTLTISLQPEDVATYYCQRYNRAPYTFGGGTKVEIKRTVA
APSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQ
DSKDSTYLSSTLTLSKADYEKHKVYACEVTHQGLSPVTKSFNRGEC (SEQ ID
NO:109).

[00113] Compound 10, which is a VHH-Fab fusion that includes a LC, a (G₄Q)₅ linker
(italicized) and a VHH-based compound acting as a t_{1/2}-extending moiety (underlined), has
the following amino acid sequence:

DIQMTQSPSSLSASVGDRVTITCRASQGIRNYLAWYQQKPGKAPKLLIYAASLTQ
SGVPSRFSGSGSGTDFTLTISLQPEDVATYYCQRYNRAPYTFGGGTKVEIKRTVA
APSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQ
DSKDSTYLSSTLTLSKADYEKHKVYACEVTHQGLSPVTKSFNRGECGGGGQG
GGGQGGGGQGGGGQGGGGQGGGGQEVQLLES GGGLVQPGGSLRLSCAASGRYIDETAV
AWFRQAPGKEREFVAGIGGGVDITYYADSVKGRFTISRDNKNTLYLQMNSLRP
EDTAVYYCAARPGRPLITSKVADLYPYWGQGTLLTVSSPP (SEQ ID NO:111).

[00114] Compound 10 further includes a HC having the following amino acid sequence:
EVQLVESGGGLVQPGRSLRLSCAASGFTFDDYAMHWVRQAPGKGLEWVSAITW
NSGHIDYADSVGRFTISRDNANKNSLYLQMNSLRAEDTAVYYCAKVSYLSTASSL
DYWGQGTLLTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSW
NSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDK
KVEPKSCD (SEQ ID NO:110).

[00115] Compound 11, which is a VHH-Fab fusion that includes a VHH-based compound acting as a $t\frac{1}{2}$ -extending moiety (underlined), a (G₄Q)₅ linker (*italicized*) and a HC, has the following amino acid sequence:

EVQLLESGGGLVQPGGSLRLSCAASGRYIDETAVAWFRQAPGKEREFVAGIGGG
VDITYYADSVKGRFTISRDNSKNTLYLQMNSLRPEDTAVYYCAARPGRPLITSKV
ADLYPYWGQGTLVTVSS*GGGGQGGGGQGGGGQGGGGQGGGGQ*EVQLVESGG
GLVQPGSLRLSCAASGFTFDDYAMHWVRQAPGKGLEWVSAITWNSGHIDYAD
SVEGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCAKVSYLSTASSLDYWGQGT
LVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTSVWNSGALTSGV
HTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCD
 (SEQ ID NO:112).

[00116] Compound 11 further includes a LC having the following amino acid sequence:
 DIQMTQSPSSLSASVGDRVTITCRASQGIRNYLAWYQQKPGKAPKLLIYAASLTQ
 SGVPSRFGSGSGTDFTLTISSLQPEDVATYYCQRYNRAPYTFGQGTKVEIKRTVA
 APSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQ
 DSKDSTYLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID
 NO:113).

[00117] Compound 12, which is a VHH-Fab fusion that includes a VHH-based compound acting as a $t\frac{1}{2}$ -extending moiety (underlined), a (G₄Q)₅ linker (*italicized*) and a LC, has the following amino acid sequence:

EVQLLESGGGLVQPGGSLRLSCAASGRYIDETAVAWFRQAPGKEREFVAGIGGG
VDITYYADSVKGRFTISRDNSKNTLYLQMNSLRPEDTAVYYCAARPGRPLITSKV
ADLYPYWGQGTLVTVSS*GGGGQGGGGQGGGGQGGGGQGGGGQ*DIQMTQSPSS
LSASVGDRVTITCRASQGIRNYLAWYQQKPGKAPKLLIYAASLTQSGVPSRFGS
GSGTDFTLTISSLQPEDVATYYCQRYNRAPYTFGQGTKVEIKRTVAAPSVFIFPPS
DEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSL
SSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO:115).

[00118] Compound 12 further includes a HC having the following amino acid sequence:
 EVQLVESGGGLVQPGSLRLSCAASGFTFDDYAMHWVRQAPGKGLEWVS
 AITWNSGHIDYADSVEGRFTISRDN
 AKNSLYLQMNSLRAEDTAVYYCAKVS
 YLSTASSLDYWGQGT
 LVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVT
 VSW

[00122] *Pharmaceutical Compositions and Kits*

[00123] The compounds (*i.e.*, VHH-based fusions or VHH-based conjugates such as, for example, Compounds 1 to 15 above) herein can be formulated as pharmaceutical compositions, which can be administered by parenteral routes (*e.g.*, intravenous, intraperitoneal, intramuscular, subcutaneous or transdermal). Such pharmaceutical compositions and techniques for preparing the same are well known in the art. *See, e.g.*, Remington, “The Science and Practice of Pharmacy” (D.B. Troy ed., 21st Ed., Lippincott, Williams & Wilkins, 2006). In particular instances, the compositions are administered SQ or IV. Alternatively, however, the compositions can be formulated in forms for other pharmaceutically acceptable routes such as, for example, tablets or other solids for oral administration; time release capsules, and any other form currently used, including creams, lotions, inhalants and the like.

[00124] As noted above, and to improve their *in vivo* compatibility and effectiveness, the VHH-based fusions or VHH-based conjugates herein may be reacted with any number of inorganic and organic acids/bases to form pharmaceutically acceptable acid/base addition salts. Pharmaceutically acceptable salts and common techniques for preparing them are well known in the art (*see, e.g.*, Stahl *et al.*, “Handbook of Pharmaceutical Salts: Properties, Selection and Use” (2nd Revised Ed. Wiley-VCH, 2011)). Pharmaceutically acceptable salts for use herein include sodium, trifluoroacetate, hydrochloride and acetate salts.

[00125] The compounds herein may be administered by a physician or self-administered using an injection. It is understood the gauge size and amount of injection volume can be readily determined by one of skill in the art. However, the amount of injection volume can be $\leq$ about 2 mL or even $\leq$ about 1 mL, and the needle gauge can be $\geq$ about 27 G or even $\geq$ about 29 G.

[00126] The disclosure also provides and therefore encompasses novel intermediates and methods useful for synthesizing the compounds herein, or a pharmaceutically acceptable salt thereof. The intermediates and compounds can be prepared by a variety of techniques that are well known in the art. For example, a method using recombinant synthesis is illustrated in the Examples below. The specific steps for each of the techniques described may be combined in different ways to prepare the compounds. The reagents and starting materials are readily available to one of skill in the art.

[00127] The compounds herein are generally effective over a wide dosage range. Exemplary doses of the compounds or of pharmaceutical compositions including the same can be milligram (mg) or microgram (μg), nanogram (ng), or picogram (pg) amounts per kilogram (kg) of an individual. In this manner, a daily dose can be from about 1 μg to about 100 mg.

[00128] Here, the effective amount of the compound in a pharmaceutical composition can be a dose of about 0.25 mg to about 5.0 mg. One of skill in the art, however, understands that in some instances the effective amount (*i.e.*, dose/dosage) may be below the lower limit of the aforesaid range and be more than adequate, while in other cases the effective amount may be a larger dose and may be employed with acceptable side effects.

[00129] In addition to a compound herein, the pharmaceutical composition also can include at least one additional therapeutic agent such as, for example, a therapeutic agent typically used as the standard of care in of a particular condition, disease and disorder (*e.g.*, a cardiovascular, neurological, immunological, metabolic, oncological, psychological, pulmonary and/or renal condition, disease or disorder).

[00130] In this manner, a pharmaceutical composition can include an effective amount of one or more compounds herein, a pharmaceutically acceptable carrier and optionally at least one additional therapeutic agent. For example, the pharmaceutical composition can include an effective amount of a compound of SEQ ID NO:100 and a pharmaceutically acceptable carrier, an effective amount of a compound of SEQ ID NO:101 and a pharmaceutically acceptable carrier, an effective amount of a compound of SEQ ID NO:102 and a pharmaceutically acceptable carrier, an effective amount of a compound of SEQ ID NO:103 and a pharmaceutically acceptable carrier, an effective amount of a compound of SEQ ID NO:104 and a pharmaceutically acceptable carrier, an effective amount of a compound of SEQ ID NO:105 and a pharmaceutically acceptable carrier, an effective amount of a compound of SEQ ID NO:106 and a pharmaceutically acceptable carrier, an effective amount of a compound of SEQ ID NO:107 and a pharmaceutically acceptable carrier, an effective amount of a compound of SEQ ID NOS:108 and 109 and a pharmaceutically acceptable carrier, an effective amount of a compound of SEQ ID NOS:110 and 111 and a pharmaceutically acceptable carrier, an effective amount of a compound of SEQ ID NOS:112 and 113 and a pharmaceutically acceptable carrier, an effective amount of a compound of SEQ ID NOS:114 and 115 and a pharmaceutically

acceptable carrier, an effective amount of a compound of SEQ ID NO:116 and a pharmaceutically acceptable carrier, an effective amount of a compound of SEQ ID NO:117 and a pharmaceutically acceptable carrier, and an effective amount of a compound of SEQ ID NO:118 and a pharmaceutically acceptable carrier.

[00131] Alternatively, the compounds herein can be provided as part of a kit. In some instances, the kit includes a device for administering at least one compound (and optionally at least one additional therapeutic agent) to an individual. In certain instances, the kit includes a syringe and needle for administering the at least one compound (and optionally at least one additional therapeutic agent). In particular instances, the compound (and optionally at least one additional therapeutic agent) is pre-formulated in aqueous solution within the syringe.

[00132] *Methods of Making and Using VHH-Based Compounds Acting as Half-Life Extenders or Fusions and Conjugates Thereof*

[00133] The compounds herein can be made via any number of standard recombinant DNA methods or standard chemical peptide synthesis methods known in the art. With regard to recombinant DNA methods, one can use standard recombinant techniques to construct a polynucleotide having a nucleic acid sequence that encodes an amino acid sequence for a compound (*i.e.*, fusion peptide or fusion protein or fusion conjugate), incorporate that polynucleotide into recombinant expression vectors, and introduce the vectors into host cells, such as bacteria, yeast and mammalian cells, to produce the compound. *See, e.g.*, Green & Sambrook, "Molecular Cloning: A Laboratory Manual" (Cold Spring Harbor Laboratory Press, 4th ed. 2012).

[00134] With regard to recombinant DNA methods, the compounds herein can be prepared by producing a protein or precursor protein molecule using recombinant DNA techniques. DNA, including cDNA and synthetic DNA, may be double-stranded or single-stranded, and the coding sequences therein encoding a compound herein may vary as a result of the redundancy or degeneracy of the genetic code. Briefly, the DNA sequences encoding the compounds herein are introduced into a host cell to produce the compound or precursor thereof. The host cells can be bacterial cells such as K12 or B strains of *Escherichia coli*, fungal cells such as yeast cells, or mammalian cells such as Chinese hamster ovary (CHO) cells.

[00135] An appropriate host cell is transiently or stably transfected or transformed with an expression system, such as expression vectors, for producing a compound herein or a precursor thereof. Expression vectors typically are replicable in the host organisms either as episomes or as an integral part of the host chromosomal DNA. Commonly, expression vectors will contain selection markers such as, for example, tetracycline, neomycin, G418 and dihydrofolate reductase, to permit selection of those cells transformed with the desired DNA sequences.

[00136] The specific biosynthetic or synthetic steps for each of the steps described herein may be used, not used or combined in different ways to prepare the compounds herein.

[00137] With regard to chemical peptide synthesis methods, one can use standard manual or automated solid-phase synthesis procedures. For example, automated peptide synthesizers are commercially available from, for example, Applied Biosystems (Foster City, CA) and Protein Technologies Inc. (Tucson, AZ). Reagents for solid-phase synthesis are readily available from commercial sources. Solid-phase synthesizers can be used according to the manufacturer's instructions for blocking interfering groups, protecting amino acids during reaction, coupling, deprotecting and capping of unreacted amino acids.

[00138] One use for the compounds herein is in treating cardiovascular, neurological, immunological, metabolic, oncological, psychological, pulmonary and/or renal condition, disease or disorder.

[00139] The methods can include the steps described herein, and these may be, but not necessarily, carried out in the sequence as described. Other sequences, however, also are conceivable. Moreover, individual or multiple steps may be carried out either in parallel and/or overlapping in time and/or individually or in multiply repeated steps. Furthermore, the methods may include additional, unspecified steps.

[00140] Such methods therefore can include selecting an individual having, for example, a neurological condition, disease or disorder, or who is predisposed to the same. Alternatively, the methods can include selecting an individual having a metabolic condition, disease or disorder, or who is predisposed to the same. Alternatively, the methods can include selecting an individual having a cardiovascular condition, disease or disorder, or who is predisposed to the same. Alternatively, the methods can include selecting an individual having an oncology condition, disease or disorder, or who is predisposed to the same. Alternatively, the methods can include selecting an individual

having a psychological condition, disease or disorder, or who is predisposed to the same. Alternatively, the methods can include selecting an individual having a pulmonary condition, disease or disorder, or who is predisposed to the same. Alternatively, the methods can include selecting an individual having a renal condition, disease or disorder, or who is predisposed to the same. Alternatively, the methods can include selecting an individual having an autoimmune condition, disease or disorder, or who is predisposed to the same.

[00141] The methods also can include administering to the individual an effective amount of at least one compound herein, which may be in the form of a pharmaceutical composition as also described herein. In some instances, the compound/pharmaceutical composition can include an additional therapeutic agent.

[00142] The concentration/dose/dosage of the compound and optional additional therapeutic agent are discussed elsewhere herein.

[00143] With regard to a route of administration, the compound or pharmaceutical composition including the same can be administered in accord with known methods such as, for example, orally; by injection (*i.e.*, intra-arterially, intravenously, intraperitoneally, intracerebrally, intracerebroventricularly, intramuscularly, intraocularly, intraportally or intralesionally); by sustained release systems, or by implantation devices. In certain instances, the compound or pharmaceutical composition including the same can be administered SQ by bolus injection or continuously.

[00144] With regard to a dosing frequency, the compound or pharmaceutical composition including the same can be administered daily, every other day, three times a week, two times a week, one time a week (*i.e.*, weekly), biweekly (*i.e.*, every other week), or monthly. In certain instances, the compound or pharmaceutical composition including the same is administered SQ every other day, SQ three times a week, SQ two times a week, SQ one time a week, SQ every other week or SQ monthly. In particular instances, the compound or pharmaceutical composition including the same is administered SQ one time a week (QW).

[00145] Alternatively, and if administered IV, the compound or pharmaceutical composition including the same is administered IV every other day, IV three times a week, IV two times a week, IV one time a week, IV every other week or IV monthly. In particular

instances, the compound or pharmaceutical composition including the same is administered IV one time a week (IW).

[00146] With regard to those instances in which the compound or pharmaceutical composition including the same is administered in combination with an effective amount of at least one additional therapeutic agent, the additional therapeutic agent can be administered simultaneously, separately or sequentially with the compound or pharmaceutical composition including the same.

[00147] Moreover, the additional therapeutic agent can be administered with a frequency same as the compound or pharmaceutical composition including the same (*i.e.*, every other day, twice a week, or even weekly). Alternatively, the additional therapeutic agent can be administered with a frequency distinct from the compound or pharmaceutical composition including the same. In other instances, the additional therapeutic agent can be administered SQ. In other instances, the additional therapeutic agent can be administered IV. In still other instances, the additional therapeutic agent can be administered orally.

[00148] It is further contemplated that the methods may be combined with diet and exercise and/or may be combined with additional therapeutic agents other than those discussed above.

EXAMPLES

[00149] The following non-limiting examples are offered for purposes of illustration, not limitation.

[00150] POLYPEPTIDE EXPRESSION, PURIFICATION AND CONJUGATION

[00151] Example 1: Recombinant Expression and Purification of VHH-Based Fusion 1

[00152] Example 1 is a CNTF-VHH fusion having an amino acid sequence of:

MAFTEHSPLTPHRRDLASRSIWLARKIRSDLTALTESYVKHQGLNKNINLDSADG
MPVASTDRWSELTEAERLQENLQAYRTFHVLLARLLEDQQVHFTPTEGDFHQAI
HTLLLQVA AFAYQIEELMILLEYKIPRNEADGMPINVG DGGLFEKKLWGLKVLQE
LSQWTVRSIHDLRFISSHQTGGGGGQGGGGQGGGGQGGGGQGGGGQEVQLLES
GGGLVQPGGSLRLSCAASGRYIDETAVAWFRQAPGKEREFVAGIGGGVDITYYA
DSVKGRFTISRDN SKNTLYLQMNSLRPEDTAVYYCAARPGRPLITSKVADLYPY
WGQGTLVTVSSPP (SEQ ID NO:100).

[00153] Here, the VHH fusion of SEQ ID NO:100 is generated in a mammalian cell expression system using CHOK1 cell derivatives. A cDNA sequence encoding SEQ ID NO:100 is sub-cloned into GS-containing expression plasmid backbone (pEE12.4-based plasmids). The cDNA sequence is fused in frame with the coding sequence of a signal peptide sequence, METDTLLLWVLLLWVPGSTG (SEQ ID NO:66) to enhance secretion of the VHH fusion analog into the tissue culture medium. The expression is driven by the viral CMV promoter.

[00154] For generating the VHH fusion via transient transfection, CHOK1 cells are transfected with the recombinant expression plasmid using a PEI-based method. Briefly, the appropriate volume of CHOK1 suspension cells at a density of 4×10^6 cells/mL is transferred in shake flasks, and both PEI and recombinant plasmid DNA are added to the cells. Cells are incubated in a suspension culture at 32°C for 6 days. At the end of the incubation period, cells are removed by low-speed centrifugation and the VHH fusion is purified from the conditioned medium.

[00155] Alternatively, and for generating the VHH fusion via stable transfections, CHOK1 cells are stably transfected using electroporation and an appropriate amount of recombinant expression plasmid, and the transfected cells are maintained in suspension culture at an adequate cell density. Selection of the transfected cells is accomplished by growth in 25 μ M MSX-containing serum-free medium and incubated at about 37°C and about 6% CO₂.

[00156] The VHH fusion secreted into the media from the CHO cells is purified by Protein A affinity chromatography followed by ion exchange, hydrophobic interaction, or size-exclusion chromatography. Specifically, the VHH fusion from harvested media is captured onto Mab Select Protein A resin (GE Healthcare). The resin then is briefly washed with a running buffer, such as a phosphate-buffered saline (PBS; pH 7.4) or a running buffer containing Tris, to remove non-specifically bound material. The VHH fusion is eluted from the resin with a low pH solution, such as 10 mM citric acid pH 3. Fractions containing the VHH fusion are pooled and may be held at a low pH to inactivate potential viruses. The pH may be neutralized by adding a base such as 1M Tris pH 8.0. The VHH fusion may be further purified by ion exchange chromatography using resins such as Poros 50 HS (ThermoFisher). The VHH fusion is eluted from the ion exchange column using a 0 to 500 mM NaCl gradient in 20 mM NaOAc, pH 5.0 over 15 column volumes.

[00157] The VHH fusion may be further purified by hydrophobic interaction chromatography by using a Capto Phenyl ImpRes HIC Column (GE Healthcare). The purification is performed by adjusting the column charge solution to around 0.5 M sodium sulfate and eluting using a 10 Column Volume (CV) gradient going from 0.5 M to 0 M sodium sulfate in a 20 mM Tris pH 8 solution. After HIC, the VHH fusion may be even further purified by SEC by loading the concentrated Capto Phenyl ImpRes pool on a Superdex200 or Superdex75 (GE Healthcare) with isocratic elution in PBS pH 7.4 or in 20mM histidine, 50mM NaCl pH6.0.

[00158] Purified VHH fusion may be passed through a viral retention filter such as Planova 20N (Asahi Kasei Medical) followed by concentration/diafiltration into 20 mM histidine, 20 mM NaCl pH 6 using tangential flow ultrafiltration on a regenerated cellulose membrane (Millipore).

[00159] The VHH fusion therefore is prepared in this manner or in a similar manner that would be readily determined by one of skill in the art.

[00160] Example 2: Recombinant Expression and Purification of VHH-Based Fusion 2

[00161] Example 2 is a VHH-CNTF fusion having an amino acid sequence of:

EVQLLES GGGLVQPGGSLRLSCAASGRYIDETAVAWFRQAPGKEREFVAGIGGG
VDITYYADSVKGRFTISRDN SKNTLYLQMNSLRPEDTAVYYCAARPGRPLITSKV
ADLYPYWGQGT LVTVSSGGGGQGGGGQGGGGQGGGGQGGGGQMAFTEHSPLT
PHRRDLASRSIWLARKIRSDLTALTESYVKHQGLNKNINLDSADGMPVASTDRW
SELTEAERLQENLQAYRTFHVLLARLLEDQQVHFTPTEGDFHQAIHTLLLQVAAF
AYQIEELMILLEYKIPRNEADGMPINVG DGGLFEKKLWGLKVLQELSQWTVRSIH
DLRFISSHQTG (SEQ ID NO:101).

[00162] Here, the VHH fusion of SEQ ID NO:101 is generated essentially as described for Example 1 except that a cDNA sequence encoding SEQ ID NO:101 is used in the expression plasmid.

[00163] Example 3: Recombinant Expression and Purification of VHH-Based Fusion 3

[00164] Example 3 is a NRG1-VHH fusion having an amino acid sequence of:

SHLVKCAEKEKTF CVNGGECFMVKDL SNPSRYLCKCPNEFTGDRCQNYVMASF
YKHLGIEFMEAEELYQGGGGQGGGGQGGGGQGGGGQGGGGQGGGGQEVQLLES GGGL

VQPGGSLRLSCAASGRYIDETAVAWFRQAPGKEREVAGIGGGVDITYYADSVK
GRFTISRDN SKNTLYLQMNSLRPEDTAVYYCAARPGRPLITSKVADLYPYWGQG
TLVTVSSPP (SEQ ID NO:102).

[00165] Here, the VHH fusion of SEQ ID NO:102 is generated essentially as described for Example 1 except that a cDNA sequence encoding SEQ ID NO:102 is used in the expression plasmid.

[00166] Example 4: Recombinant Expression and Purification of VHH-Based Fusion 4

[00167] Example 4 is a VHH-NRG1 fusion having an amino acid sequence of:

EVQLLESGGGLVQPGGSLRLSCAASGRYIDETAVAWFRQAPGKEREVAGIGGG
VDITYYADSVKGRFTISRDN SKNTLYLQMNSLRPEDTAVYYCAARPGRPLITSKV
ADLYPYWGQGTTLVTVSSGGGGQGGGGQGGGGQGGGGQGGGGQSHLVKCAEK
EKTFCVNGGECFMVKDLSNPSRYLCKCPNEFTGDRCQNYVMASFYKHLGIEFME
AEELYQ (SEQ ID NO:103).

[00168] Here, the VHH fusion of SEQ ID NO:103 is generated essentially as described for Example 1 except that a cDNA sequence encoding SEQ ID NO:103 is used in the expression plasmid.

[00169] Example 5: Recombinant Expression and Purification of VHH-Based Fusion 5

[00170] Example 5 is a VHH-GDF15 fusion having an amino acid sequence of:

EVQLLESGGGLVQPGGSLRLSCAASGRYIDETAVAWFRQAPGKEREVAGIGGG
VDITYYADSVKGRFTISRDN SKNTLYLQMNSLRPEDTAVYYCAARPGRPLITSKV
ADLYPYWGQGTTLVTVSSGGGGQGGGGQGGGGQGGGGQGGGGQGDHCPLGPG
RCCRLHTVRASLEDLGWADWVLSPREVQVTMCIGACPSQFRAANMHAQIKTSL
HRLKPDTVPAPCCVPASYNPMVLIQKTD TGVS LQTYDDLAKDCHCI (SEQ ID
NO:104).

[00171] Here, the VHH fusion of SEQ ID NO:104 is generated essentially as described for Example 1 except that a cDNA sequence encoding SEQ ID NO:104 is used in the expression plasmid.

[00172] Example 6: Recombinant Expression and Purification of VHH-Based Fusion 6

[00173] Example 6 is an IL-2-VHH fusion having an amino acid sequence of:

[00181] Example 9: Recombinant Expression and Purification of VHH-Based Fusion 9

[00182] Example 9 is a Fab-VHH fusion comprising a HC-VHH and a LC, with the HC-VHH amino acid sequence of:

EVQLVESGGGLVQPGRSLRLSCAASGFTFDDYAMHWVRQAPGKGLEWVSAITW
NSGHIDYADSVEGRFTISRDNANKNSLYLQMNSLRAEDTAVYYCAKVSYLSTASSL
DYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSW
NSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDK
KVEPKSCDKTHTGGGGQGGGGQGGGGQGGGGQGGGGQEVQLLES GGGLVQPG
GSLRLSCAASGRYIDETAVAWFRQAPGKEREFVAGIGGGVDITYYADSVKGRFTI
SRDNSKNTLYLQMNSLRPEDTAVYYCAARPGRPLITSKVADLYPYWGQGTLVTV
SSPP (SEQ ID NO:108).

[00183] and the LC amino acid sequence of:

DIQMTQSPSSLSASVGDRVTITCRASQGIRNYLAWYQQKPGKAPKLLIYAASLTQ
SGVPSRFSGSGSGTDFTLTISLQPEDVATYYCQRYNRAPYTFGGGTKVEIKRTVA
APSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQ
DSKDYSTYLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID
NO:109).

[00184] Here, the VHH fusion of SEQ ID NOS:108 and 109 is generated essentially as described for Example 1 except that cDNA sequence encoding SEQ ID NOS:108 and 109 are cloned into two expression plasmids and then a 1:1 mix of both plasmids is used for the transfection.

[00185] Example 10: Recombinant Expression and Purification of VHH-Based Fusion 10

[00186] Example 10 is a Fab-VHH fusion comprising a HC and a LC-VHH, with the HC amino acid sequence of:

EVQLVESGGGLVQPGRSLRLSCAASGFTFDDYAMHWVRQAPGKGLEWVSAITW
NSGHIDYADSVEGRFTISRDNANKNSLYLQMNSLRAEDTAVYYCAKVSYLSTASSL
DYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSW
NSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDK
KVEPKSCD (SEQ ID NO:110).

[00187] and the LC-VHH amino acid sequence of:

DIQMTQSPSSLSASVGDRVTITCRASQGIRNYLAWYQQKPGKAPKLLIYAASLTQ
 SGVPSRFSGSGSGTDFTLTISLQPEDVATYYCQRYNRAPYTFGQGTKVEIKRTVA
 APSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQ
 DSKDSTYLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGECGGGGQG
 GGGQGGGGQGGGGQGGGGQEVQLLESGGGLVQPGGSLRLSCAASGRYIDETAV
 AWRQAPGKEREFEVAGIGGGVDITYYADSVKGRFTISRDN SKNTLYLQMNSLRP
 EDTAVYYCAARPGRPLITSKVADLYPYWGQGTLLTVSSPP (SEQ ID NO:111).

[00188] Here, the VHH fusion analog of SEQ ID NOS:110 and 111 is generated essentially as described for Example 1 except that cDNA sequence encoding SEQ ID NOS:110 and 111 are cloned into two expression plasmids and then a 1:1 mix of both plasmids is used for the transfection.

[00189] Example 11: Recombinant Expression and Purification of VHH-Based Fusion 11

[00190] Example 11 is a Fab-VHH fusion comprising a VHH-HC and a LC, with the VHH-HC amino acid sequence of:

EVQLLESGGGLVQPGGSLRLSCAASGRYIDETAVAWFRQAPGKEREFEVAGIGGG
 VDITYYADSVKGRFTISRDN SKNTLYLQMNSLRPEDTAVYYCAARPGRPLITSKV
 ADLYPYWGQGTLLTVSSGGGGQGGGGQGGGGQGGGGQGGGGQEVQLVESGG
 GLVQPGSLRLSCAASGFTFDDYAMHWVRQAPGKGLEWVSAITWNSGHIDYAD
 SVEGRFTISRDN AKNSLYLQMNSLRAEDTAVYYCAKVSYLSTASSLDYWGQGTLL
 TVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTWNSGALTSGV
 HTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCD
 (SEQ ID NO:112).

[00191] and the LC amino acid sequence of:

DIQMTQSPSSLSASVGDRVTITCRASQGIRNYLAWYQQKPGKAPKLLIYAASLTQ
 SGVPSRFSGSGSGTDFTLTISLQPEDVATYYCQRYNRAPYTFGQGTKVEIKRTVA
 APSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQ
 DSKDSTYLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID
 NO:113).

[00192] Here, the VHH fusion of SEQ ID NOS:112 and 113 is generated essentially as described for Example 1 except that cDNA sequence encoding SEQ ID NOS:112 and 113

are cloned into two expression plasmids and then a 1:1 mix of both plasmids is used for the transfection.

[00193] Example 12: Recombinant Expression and Purification of VHH-Based Fusion 12

[00194] Example 12 is a VHH-Fab comprising a HC and a VHH-LC, with the HC amino acid sequence of:

EVQLVESGGGLVQPGRSLRLSCAASGFTFDDYAMHWVRQAPGKGLEWVSAITW
NSGHIDYADSVETRFTISRDNKNSLYLQMNSLRAEDTAVYYCAKVSYLSTASSL
DYWGQGTLLTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSW
NSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDK
KVEPKSCD (SEQ ID NO:114).

[00195] and the VHH-LC amino acid sequence of:

EVQLLES GGGLVQPGGSLRLSCAASGRYIDETAVAWFRQAPGKEREFVAGIGGG
VDITYYADSVKGRFTISRDN SKNTLYLQMNSLRPEDTAVYYCAARPGRPLITSKV
ADLYPYWGQGTLLTVSSGGGGGQGGGGGQGGGGGQGGGGQGGGGQDIQMTQSPSS
LSASVGDRVTITCRASQGIRNYLAWYQQKPGKAPKLLIYAASLTQSGVPSRFSGS
GSGTDFTLTISSLQPEDVATYYCQRYNRAPYTFGGGTKVEIKRTVAAPSVFIFPPS
DEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSSTYSLS
SSTLTLSKADYEKHKVYACEVTHQGLSPVTKSFNRGEC (SEQ ID NO:115).

[00196] Here, the VHH fusion analog of SEQ ID NOS:114 and 115 is generated essentially as described for Example 1 except that cDNA sequence encoding SEQ ID NOS:114 and 115 are cloned into two expression plasmids and then a 1:1 mix of both plasmids is used for the transfection.

[00197] Example 13: Recombinant Expression and Purification of Control Fab

[00198] Example 13 is a Fab comprising a HC and a LC, with the HC amino acid sequence of:

EVQLVESGGGLVQPGRSLRLSCAASGFTFDDYAMHWVRQAPGKGLEWVSAITW
NSGHIDYADSVETRFTISRDNKNSLYLQMNSLRAEDTAVYYCAKVSYLSTASSL
DYWGQGTLLTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSW
NSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDK
KVEPKSCD (SEQ ID NO:120).

are added and incubated for 4 hours at RT. Successful reduction to homogenous monomers is confirmed via mass spectrometry.

[00206] The reaction mixture is desalted using a 2 mL 7K MW Cutoff ZEBRA desalting column. Column storage buffer is removed by spinning at 1000 rcf for 1 min. The column is washed by adding 2 mL of PBS pH 7.2 and spinning at 1000 rcf for 1 min. The reaction mixture is loaded onto a ZEBRA column and desalted by spinning for 1 min at 1000 rcf into a clean 15 mL conical vial. The desalted sample is concentrated to ~700 μ M (~100 μ L) using a 3K MW cutoff Amicon Ultra spin column.

[00207] ACTH-(PEG)₁₂-maleimide-NH₂ (SEQ ID NO:119) is prepared at 20 mg/mL (5.2 mM) in PBS pH 7.2. 20 μ L ACTH-(PEG)₁₂-maleimide-NH₂ corresponding to 2 stoichiometric equivalents is added to the desalted and concentrated solution of VHH fusion of Example 14 (SEQ ID NO:116). A white precipitate forms immediately. The reaction is allowed to continue for 10 min., and the solution is solubilized by lowering the pH to 5.5 by slowly adding 0.1 M HCl until solution is clear. Successful conjugation is confirmed via mass spectrometry.

[00208] The desired product is purified on an AKTA purification system using a 5 mL MabSelect Protein A resin (GE Healthcare) column with a loading buffer of PBS, pH 7.2, and an elution buffer of 20 mM citrate, pH 3. The sample is loaded onto the column, and loading buffer phase is held until a peak for unreacted ACTH-(PEG)₁₂-maleimide-NH₂ passes through the column. When the signal returns to baseline, the buffer is switched to 20 mM citrate, pH 3, to elute the desired product from the column. 1 mL fractions are collected upon elution, pooled and the pH is adjusted to 7 using tris HCl, pH 8.

[00209] To open the maleimide ring, the pH is adjusted to 8 by adding 0.1 M NaOH and left overnight at RT. To drive partial ring opening to completion, the pH is adjusted to 8.5 and incubated for another 24 hr at RT. Completion of ring opening is confirmed by mass spectrometry, and a final pH adjustment back to about 7.2 is performed by adding 0.1 N HCl. The VHH conjugate of Example 15 is then stored at 4°C.

[00210] Example 16: Production of VHH-Based Conjugate 2

[00211] Example 16 is an ACTH-VHH conjugate having a chemical structure of:

[00212] EVQLLESGGGLVQPGGSLRLSCAASGRYIDETAVAWFRQAPGKGREFV
AGIGGGVDITYYADSVKGRFTISRDNKNTLYLQMNSLRPEDTAVYYCAARPGR

PLITSKVADLYPYWGQGLTVTVSSC-maleimide-(PEG)₁₂-(ACTH) (in a C-terminal to N-terminal orientation; SEQ ID NO:118).

[00213] The VHH conjugate of SEQ ID NO:118 is generated as follows. 1 mg of a control VHH (SEQ ID NO:37) is prepared at 1 mg/mL (about 70 μ M) by diluting a 10 mg/mg stock solution 10-fold with PBS, pH 7.2. Four equivalents of TCEP in PBS, pH 7.2, are added and incubated for 4 hr at RT. Successful reduction to homogenous monomers is confirmed via mass spectrometry.

[00214] The reaction mixture is desalted using a 2 mL 7K MW Cutoff ZEBRA desalting column. Column storage buffer is removed by spinning at 1000 rcf for 1 min. The column is washed by adding 2 mL of PBS, pH 7.2, and spinning at 1000 rcf for 1 min. The reaction mixture is loaded onto the ZEBRA column and desalted by spinning for 1 min at 1000 rcf into a clean 15 mL conical vial. The desalted sample is concentrated to about 700 μ M (~100 μ L) using a 3K MW cutoff Amicon Ultra spin column.

[00215] ACTH-(PEG)₁₂-maleimide-NH₂ (SEQ ID NO:119) is prepared and conjugated to the VHH as described in Example 15.

[00216] *IN VITRO* FUNCTION – ALBUMIN BINDING

[00217] Example 17: Albumin-Binding Studies via SPR of VHH Moieties

[00218] *In vitro* binding of various VHH moieties to human, cynomolgus monkey, mouse, rat, pig, dog and cow serum albumin (SA) is determined by SPR. In particular, the binding of the VHH moieties herein to the SA of these species is summarized below in Table 1. Binding of the VHH moieties of SEQ ID NOS:3 and 8 to 28 to various SAs is carried out on Biacore 8K instrument.

[00219] Immobilization of SA to a Series S Sensor Chip CM5 surface is performed according to the manufacturer's instructions (amine coupling kit BR-1000-50). Briefly, carboxyl groups on the sensor chip surfaces (flow cell 1 and 2) are activated by injecting 70 μ L of a mixture containing 75 mg/mL 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) and 11.5 mg/mL N-hydroxysuccinimide (NHS) at 10 μ L/min. Human, cynomolgus monkey, mouse, rat, pig, dog and cow SA are diluted in 10 mM sodium acetate, pH 4.0, (BR-1003-49) at 1, 1, 3, 1, 1, 1 and 1 μ g/mL and then injected over the activated chip surfaces (flow cell 2, channel 1 to 8) at 10 μ L/min for 90 sec. Human SA is obtained from Sigma Aldrich (St. Louis, MO; Cat. No. A8763). Cynomolgus

monkey SA is obtained from Athens R&T (Athens, GA; Cat. No. 16-16-011202-CM). Mouse SA is obtained from Sigma Aldrich (Cat. No. A3559). Rat SA is obtained from Sigma Aldrich (Cat. No. A4538). Pig SA is obtained from Sigma Aldrich (Cat. No. A4414). Dog SA is obtained from Molecular Innovations (Novi, MI; Cat. No. DSA-1213 NC0739153). Cow SA is obtained from Sigma Aldrich (Cat. No. A7030). The various SAs are covalently immobilized through free amines onto a carboxymethyl dextran-coated sensor chip CM5 targeting a surface density of average approximately 77 (58-98) RU. Excess reactive groups on the surfaces (flow cell 1 and 2) are deactivated by injecting 70 μ L of 1 M ethanolamine hydrochloride-NaOH, pH 8.5, at 10 μ L/min.

[00220] VHH moieties are diluted in HBS-EP + buffer (10 mM HEPES, pH 7.6, 150 mM NaCl, 3 mM EDTA, and 0.05% Polysorbate 20) at concentrations of 300 nM. 150 μ L of sample is individually injected sequentially across the immobilized SAs surface and is dissociated for 600 sec at a flow rate of 50 μ L/min at 25 C. The surface is regenerated by injecting 10 mM glycine-HCl, pH 1.5, (BR-1003-54) at 50 μ L/min for 100 sec. The resulting sensorgrams are analyzed using Biacore 8K Insight Evaluation Software (version 2.0.15.12933) to calculate the dissociation rate (kd).

[00221] Table 1: Binding of Exemplary VHH Moieties to Human, Cynomolgus Monkey, Mouse, Rat, Pig, Dog, and Cow SA at 25°C.

VHH Moieity	Human SA	Cyno SA	Mouse SA	Rat SA	Pig SA	Dog SA	Cow SA
SEQ ID NO:	kd (1/s)	kd (1/s)	kd (1/s)	kd (1/s)	kd (1/s)	kd (1/s)	kd (1/s)
3	8.0E-04	2.0E-03	4.3E-03	3.5E-03	5.2E-03	2.2E-03	1.5E-02
3	7.8E-04	1.9E-03	4.3E-03	3.4E-03	5.2E-03	2.3E-03	1.4E-02
8	1.0E-03	3.0E-03	3.2E-03	7.5E-03	9.2E-03	2.2E-03	1.5E-02
9	1.2E-03	3.2E-03	1.3E-02	1.1E-02	1.2E-02	1.1E-02	very weak
10	weak	very weak	very weak	very weak	very weak	very weak	no binding
11	7.4E-03	very weak	no binding	no binding	no binding	no binding	no binding
12	1.1E-02	very weak	no binding	no binding	no binding	no binding	no binding
13	very weak	no binding	no binding	no binding	no binding	no binding	no binding
14	1.3E-02	no binding	no binding	no binding	no binding	no binding	no binding
15	5.8E-03	very weak	no binding	no binding	no binding	no binding	no binding
16	3.9E-03	1.5E-02	no binding	no binding	no binding	very weak	no binding
17	1.5E-03	2.2E-03	1.0E-02	8.1E-03	1.3E-02	8.2E-03	1.5E-02
3	7.7E-04	1.7E-03	4.2E-03	3.1E-03	5.1E-03	2.0E-03	1.5E-02
18	7.6E-03	7.2E-03	very weak	1.4E-02	very weak	1.5E-02	no binding
19	4.5E-03	7.3E-03	1.5E-02	1.5E-02	1.4E-02	1.4E-02	no binding
20	9.2E-03	no binding	no binding	no binding	no binding	very weak	no binding
21	2.5E-03	1.4E-02	1.3E-02	1.1E-02	1.0E-02	8.1E-03	very weak
22	2.8E-03	1.4E-02	1.0E-02	1.0E-02	1.1E-02	7.4E-03	very weak
23	no binding	no binding	no binding	no binding	no binding	no binding	no binding
24	no binding	no binding	no binding	no binding	no binding	no binding	no binding
25	very weak	no binding	very weak	very weak	very weak	very weak	no binding

26	very weak	no binding	no binding	no binding	no binding	no binding	no binding
27	1.4E-02	no binding	no binding	no binding	no binding	no binding	no binding
3	8.6E-04	1.5E-03	4.2E-03	3.0E-03	5.0E-03	1.9E-03	1.5E-02
4	2.1E-04	5.3E-04	4.3E-03	3.3E-03	5.7E-03	1.7E-03	n/d
125	9.4E-05	6.0E-04	5.6E-03	3.8E-03	6.5E-03	2.1E-03	n/d
126	1.4E-04	7.2E-04	5.4E-03	5.3E-03	5.8E-03	2.1E-03	n/d

[00222] Example 18: VHH-Based Fusions Albumin-Binding Studies via SPR

[00223] *In vitro* binding of VHH-based fusions to human, cynomolgus monkey, mouse, rat, pig, dog, cow and rabbit SA is determined by SPR at 25°C. In particular, the affinity of the VHH-fusions of Examples 1 to 9 to SA of these species is summarized below in Tables 2 to 10.

[00224] Binding of the VHH-based fusions of Examples 1 to 9 to various SAs is carried out on a Biacore 8K instrument. The immobilization of various SA orthologs to a Series S Sensor Chip CM5 (BR-1006-68) surface are performed according to manufacturer's instructions (amine coupling kit BR-1000-50). Briefly, carboxyl groups on the sensor chip surfaces (flow cell 1 and 2) are activated by injecting 70 µl of mixture containing 75 mg/ml 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC), and 11.5 mg/ml N-Hydroxysuccinimide (NHS), at 10 µl/min. Human, cynomolgus monkey, rat, mouse, dog, pig, cow, and rabbit SA are diluted in 10 mM sodium acetate pH 4.0 (BR-1003-49) at 1 and 0.8 µg/ml, 1 and 0.8 µg/ml, 1.5 and 0.8 µg/ml, 4 and 2.5 µg/ml, 1 and 0.8 µg/ml, 1 and 1 µg/ml, 1 and 1 µg/ml, and 1 and 1.5 µg/ml, and then are injected over the activated chip surfaces (flow cell 2, channel 1 to 8) at 10 µl/min for 100 seconds. Human SA is obtained from Sigma Aldrich (Cat. No. A8763). Cynomolgus monkey SA is obtained from Athens R&T (Cat. No. 16-16-011202-CM). Mouse SA is obtained from Sigma Aldrich (Cat. No. A3139). Rat SA is obtained from Sigma Aldrich (Cat. No. A4538). Pig SA is obtained from Sigma Aldrich (Cat. No. A4414). Dog SA is obtained from Molecular Innovations (Novi, MI; Cat. No. DSA-1213 NC0739153). Cow SA is obtained from Sigma Aldrich (Cat. No. A7030). Rabbit SA is obtained from Fitzgerald Industries International (Acton, MA; Cat. No. 30R-3303). The SAs are covalently immobilized through free amines onto a carboxymethyl dextran-coated sensor chip CM5 at surface densities of 25-78 resonance units (RU) for human, cynomolgus monkey, rat, mouse, dog, pig, and cow SA as well as 118-372 resonance units (RU) for rabbit SA. Excess reactive groups on the surfaces (flow cell 1 and 2) are deactivated by injecting 70 µl of 1 M Ethanolamine hydrochloride-NaOH pH 8.5.

[00225] VHH-fusions are diluted in HBS-EP+ buffer (10 mM HEPES pH 7.6, 150 mM NaCl, 3 mM EDTA, 0.05% Polysorbate 20) at concentrations of 1000, 333.3, 111.1, 37.04,

12.35, 4.12, 1.37, 0.457, 0.152, 0.051 and 0.017 nM. 180 μ l of sample are individually injected sequentially across the immobilized SAs on the chip's surface and dissociated for 600 sec at 60 μ l/min flow rate at 25°C. The surface is regenerated by injecting 10 mM glycine-HCl pH 1.5 (BR-1003-54) at 60 μ l/min for 100 sec. The resulting sensorgrams are analyzed using Biacore 8K Insight Evaluation Software (version 3.0.11.15423) 1:1 binding kinetics model fitting to calculate the binding kinetic parameters: association rate (k_a), dissociation rate (k_d), and equilibrium dissociation constant (K_D).

[00226] Table 2: Binding Kinetics of VHH-Based Fusion of Example 1 to Human, Cynomolgus Monkey, Mouse, Rat, Pig, Dog, Cow and Rabbit SA at 25°C.

Binding to Immobilized SAs	k_a (1/Ms)	k_d (1/s)	K_D (M)
Human SA	7.6E+04	2.4E-04	3.2E-09
Cyno SA	6.8E+04	8.6E-04	1.3E-08
Mouse SA	8.1E+04	8.2E-03	1.0E-07
Rat SA	7.5E+04	6.3E-03	8.3E-08
Pig SA	5.8E+04	1.1E-02	1.9E-07
Dog SA	7.9E+04	3.2E-03	4.0E-08
Cow SA	9.1E+04	7.4E-02	8.2E-07
Rabbit SA	No binding		

[00227] K_D is determined as 3.2, 13, 100, 83, 190, 40 and 820 nM for human, cynomolgus monkey, mouse, rat, pig, dog and cow SA binding, respectively, with the VHH-based fusion of Example 1.

[00228] Table 3: Binding Kinetics of VHH-Based Fusion of Example 2 to Human, Cynomolgus Monkey, Mouse, Rat, Pig, Dog, Cow and Rabbit SA at 25°C.

Binding to Immobilized SAs	k_a (1/Ms)	k_d (1/s)	K_D (M)
Human SA	4.3E+05	2.5E-04	5.8E-10
Cyno SA	5.0E+05	6.0E-04	1.2E-09
Mouse SA	4.8E+05	4.2E-03	8.8E-09
Rat SA	4.9E+05	3.0E-03	6.1E-09
Pig SA	3.0E+05	5.7E-03	1.9E-08
Dog SA	4.6E+05	1.7E-03	3.7E-09
Cow SA	4.6E+05	5.2E-02	1.1E-07
Rabbit SA	No binding		

[00229] K_D is determined as 0.58, 1.2, 8.8, 6.1, 19, 3.7 and 110 nM for human, cynomolgus monkey, mouse, rat, pig, dog and cow SA binding, respectively, with the VHH-based fusion of Example 2.

[00230] Table 4: Binding Kinetics of VHH-Based Fusion of Example 3 to Human, Cynomolgus Monkey, Mouse, Rat, Pig, Dog, Cow and Rabbit SA at 25°C.

Binding to Immobilized SAs	k_a (1/Ms)	k_d (1/s)	K_D (M)
Human SA	1.2E+05	2.3E-04	1.9E-09
Cyno SA	1.4E+05	8.7E-04	6.1E-09
Mouse SA	1.4E+05	8.8E-03	6.2E-08
Rat SA	1.2E+05	6.0E-03	5.2E-08
Pig SA	9.3E+04	1.2E-02	1.3E-07
Dog SA	1.7E+05	3.2E-03	1.9E-08
Cow SA	1.6E+05	7.2E-02	4.7E-07
Rabbit SA	No binding		

[00231] K_D is determined as 1.9, 6.1, 62, 52, 130, 19 and 470 nM for human, cynomolgus monkey, mouse, rat, pig, dog and cow SA binding, respectively, with the VHH-based fusion of Example 3.

[00232] Table 5: Binding Kinetics of VHH-Based Fusion of Example 4 to Human, Cynomolgus Monkey, Mouse, Rat, Pig, Dog, Cow and Rabbit SA at 25°C.

Binding to Immobilized SAs	k_a (1/Ms)	k_d (1/s)	K_D (M)
Human SA	1.0E+06	1.9E-04	1.9E-10
Cyno SA	6.8E+05	6.3E-04	9.4E-10
Mouse SA	6.6E+05	4.2E-03	6.4E-09
Rat SA	9.7E+05	3.0E-03	3.1E-09
Pig SA	4.0E+05	6.0E-03	1.5E-08
Dog SA	7.8E+05	1.7E-03	2.2E-09
Cow SA	5.7E+05	5.8E-02	1.0E-07
Rabbit SA	No binding		

[00233] K_D is determined as 0.19, 0.94, 6.4, 3.1, 15, 2.2 and 100 nM for human, cynomolgus monkey, mouse, rat, pig, dog and cow SA binding, respectively, with the VHH-based fusion of Example 4.

[00234] Table 6: Binding Kinetics of VHH-Based Fusion of Example 5 to Human, Cynomolgus Monkey, Mouse, Rat, Pig, Dog, Cow and Rabbit SA at 25°C.

Binding to Immobilized SAs	k_a (1/Ms)	k_d (1/s)	K_D (M)
Human SA	6.7E+05	1.4E-04	2.0E-10
Cyno SA	7.4E+05	3.4E-04	4.5E-10
Mouse SA	1.4E+06	1.2E-03	8.6E-10
Rat SA	1.0E+06	7.7E-04	7.4E-10
Pig SA	8.5E+05	2.8E-03	3.3E-09
Dog SA	1.1E+06	6.8E-04	6.0E-10
Cow SA	*not applicable	*not applicable	2.8E-08
Rabbit SA	No binding		

[00235] K_D is determined as 0.2, 0.45, 0.86, 0.74, 3.3, 0.6 and 28 nM for human, cynomolgus monkey, mouse, rat, pig, dog and cow SA binding, respectively, with the VHH-based fusion of Example 5.

[00236] Table 7: Binding Kinetics of VHH-Based Fusion of Example 6 to Human, Cynomolgus Monkey, Mouse, Rat, Pig, Dog, Cow and Rabbit SA at 25°C.

Binding to Immobilized SAs	k_a (1/Ms)	k_d (1/s)	K_D (M)
Human SA	1.8E+05	2.6E-04	1.5E-09
Cyno SA	1.8E+05	8.8E-04	5.0E-09
Mouse SA	1.7E+05	8.7E-03	5.0E-08
Rat SA	1.9E+05	6.6E-03	3.4E-08
Pig SA	1.2E+05	1.2E-02	1.0E-07
Dog SA	1.7E+05	3.4E-03	1.9E-08
Cow SA	1.9E+05	7.1E-02	3.8E-07
Rabbit SA	No binding		

[00237] K_D is determined as 1.5, 5.0, 50, 34, 100, 19 and 380 nM for human, cynomolgus monkey, mouse, rat, pig, dog and cow SA binding, respectively, with the VHH-based fusion of Example 6.

[00238] Table 8: Binding Kinetics of VHH-Based Fusion of Example 7 to Human, Cynomolgus Monkey, Mouse, Rat, Pig, Dog, Cow and Rabbit SA at 25°C.

Binding to Immobilized SAs	k_a (1/Ms)	k_d (1/s)	K_D (M)
Human SA	9.1E+05	2.1E-04	2.3E-10
Cyno SA	1.4E+06	5.6E-04	3.9E-10
Mouse SA	9.7E+05	3.8E-03	3.9E-09
Rat SA	9.7E+05	2.9E-03	3.0E-09
Pig SA	4.5E+05	5.8E-03	1.3E-08
Dog SA	9.2E+05	1.6E-03	1.8E-09
Cow SA	6.7E+05	5.2E-02	7.8E-08
Rabbit SA	No binding		

[00239] K_D is determined as 0.23, 0.39, 3.9, 3.0, 13, 1.8 and 78 nM for human, cynomolgus monkey, mouse, rat, pig, dog and cow SA binding, respectively, with the VHH-based fusion of Example 7.

[00240] Table 9: Binding Kinetics of VHH-Based Fusion of Example 8 to Human, Cynomolgus Monkey, Mouse, Rat, Pig, Dog, Cow and Rabbit SA at 25°C.

Binding to Immobilized SAs	k_a (1/Ms)	k_d (1/s)	K_D (M)
Human SA	7.2E+05	1.1E-04	1.5E-10
Cyno SA	9.1E+05	8.7E-04	9.6E-10
Mouse SA	5.6E+05	1.0E-02	1.8E-08
Rat SA	6.6E+05	7.1E-03	1.1E-08
Pig SA	4.9E+05	1.3E-02	2.7E-08
Dog SA	7.1E+05	3.8E-03	5.4E-09
Cow SA	8.0E+05	9.3E-02	1.2E-07
Rabbit SA	No binding		

[00241] K_D is determined as 0.15, 0.96, 18, 11, 27, 5.4 and 120 nM for human, cynomolgus monkey, mouse, rat, pig, dog and cow SA binding, respectively, with the VHH-based fusion of Example 8.

[00242] Table 10: Binding Kinetics of VHH-Based Fusion of Example 9 to Human, Cynomolgus Monkey, Mouse, Rat, Pig, Dog, Cow and Rabbit SA at 25°C.

Binding to Immobilized SAs	k_a (1/Ms)	k_d (1/s)	K_D (M)
Human SA	2.1E+05	2.8E-04	1.3E-09
Cyno SA	2.2E+05	9.4E-04	4.3E-09
Mouse SA	2.3E+05	1.0E-02	4.3E-08
Rat SA	2.1E+05	7.1E-03	3.3E-08
Pig SA	1.5E+05	1.3E-02	8.3E-08
Dog SA	2.4E+05	3.7E-03	1.5E-08
Cow SA	2.5E+05	8.5E-02	3.4E-07
Rabbit SA	No binding		

[00243] K_D is determined as 1.3, 4.3, 43, 33, 83, 15, and 340 nM for human, cynomolgus monkey, mouse, rat, pig, dog and cow SA binding, respectively, with the VHH-based fusion of Example 9.

[00244] *IN VITRO* FUNCTION – SPECIFIC ACTIVITY OF PAYLOADS

[00245] Example 19: *In Vitro* Potency of VHH-Based Fusions of Examples 1 and 2

[00246] Human CNTFR pSTAT3 assay: IMR-32 cells (ATCC, Cat# CCL-127), which endogenously express human CNTFR, LIFR and gp130 are cultured at 37°C, 5% CO₂, 90% humidity in RPMI-1640 (ATCC, Cat# 30-2001) media supplemented with final 10% FBS, 1 mM sodium pyruvate and 1X Antibiotic-Antimycotic (Thermo Fisher Scientific, 15240062). On Day -1 (the day before the pSTAT3 assay), cells are washed once with 1X PBS, lifted from flasks with cell dissociation buffer (Gibco, 13151) and resuspended in above mentioned culture media. Cells are plated in 96-well poly D lysine coated plates (Corning; Cat# 354640) at 150,000 cells/0.1 mL/well. Cells are cultured at 37°C, 5% CO₂, 90% humidity overnight. On Day 1 (the day of the pSTAT3 assay), medium is aspirated off and is replaced with 100 µL EMEM (ATCC, Cat# 30-2003). Cells are serum starved at 37°C, 5% CO₂, 90% humidity for 4 hr, then 50 µL of 3x serially diluted human CNTF (R&D Systems; Cat# 257-NT-010), Example 1 or Example 2 in 0.3% IgG free BSA (Jackson ImmunoResearch Laboratories, Inc.; Cat# 001-000-162) are added. EMEM is added for a final 1X concentration. Cells are incubated with these serially diluted proteins for an additional 10 min at 37°C, 5% CO₂, 90% humidity. After the incubation period is complete, contents are removed from the plate. Cells are lysed, and pSTAT3 is detected using an AlphaLISA Surefire Ultra p-STAT3 (Tyr705) Assay Kit (Perkin Elmer; ALSU-PST3) and its two-plate assay protocol for adherent cells. Plates are read on an Envision 2102 instrument (Perkin Elmer).

[00247] Statistical analysis of data: Data is imported from an Envision 2102 instrument reader into Excel® (Microsoft). % of maximum (1 nM) human CNTF stimulated pSTAT3 signal is calculated for each concentration of protein tested. EC₅₀ values are generated from these calculations by a variable slope-four parameter dose response curve analysis using GraphPad Prism® software (GraphPad Software, LLC; La Jolla, CA; version 8.4.3). The results of the assay are presented below in Table 11.

[00248] Table 11: *In Vitro* Potency of VHH-Based Fusions to Human CNTF Receptor.

Compound	hCNTFR EC ₅₀ pM GeoMean	SEM	N
human CNTF	3.50	0.45	2
Example 1	7.09	0.35	2
Example 2	8.39	0.65	2

[00249] Example 20: *In Vitro* Potency of VHH-Based Fusions of Examples 3 and 4

[00250] A cell-based dimerization bioassay from DiscoverX/eurofins (PathHunter eXpress ErbB4/ErbB4 Dimerization Assay Cat# 93-0961E3) is performed to test potency of the VHH fusions of Examples 3 and 4 at the human Eb4 receptor (NCBI Ref. Seq. No. NP_001036064.1). The assay detects ligand induced dimerization of two subunits of a receptor-dimer pair and is designed to assess potency. Testing is performed according to the protocol provided by the manufacturer. Briefly, cells are thawed and are plated in the provided 96-well plate at 100 μ L per well. After 24 hr incubation at 37°C in 5% CO₂, 10 μ L 11X of Example 3, Example 4 and a control agonist, rhNRG-1 (eurofins cat# 92-1091), are added in an 11-point serial dilution curve (1:3) to appropriate wells in duplicate. The plate is incubated at 37°C for 6 hr. Following the 6-hour incubation, 110 μ L detection reagents are added to each well. The plate is incubated at RT in the dark for an additional hr. The chemiluminescent signal is read on plate reader using a 0.5 sec integration time (SpectraMax i3x plate reader by Molecular Devices). Statistical analysis of raw data is performed with GraphPad PRISM version 9.0. The average background reading is subtracted from all values, raw data are transformed and a non-linear regression analysis (log(agonist) vs. response - variable slope (four parameters)) is run to obtain EC₅₀ values and to determine goodness of fit (n=2 for all data).

[00251] Table 12: Potency of VHH-Based Fusions of Examples 3 and 4 in the DiscoverX PathHunter ErbB4 Bioassay.

	rhNRG-1	Example 3	Example 4
EC ₅₀ (ng/mL)	16.51	33.55	112.2
R ²	0.9884	0.9863	0.9986

[00252] Example 21: *In Vitro* Potency of VHH-Based Fusion of Example 5

[00253] Generating HEK293 human GFRAL- and human RET-expressing cell line: HEK293 cells (ATCC) are cultured in DMEM with 10% FBS and 25 mM HEPES, 1x antibiotics and split 1:16 every 3-4 days with TrypLE™ Express (Gibco). Cells are transfected with plasmid DNA of human GFRAL (GDNF Receptor Alpha Like; NCBI Reference Sequence No. NP_997293.2), human RET (Proto-oncogene tyrosine-protein kinase receptor RET; NCBI Reference Sequence No. NP_066124.1), and Fugene 6 (Promega) according to the manufacturer's instructions. Transfected cells are selected with geneticin (Gibco, 1 mg/ml) and puromycin (Gibco, 0.1 mg/ml) for 3-4 weeks. Clonal lines are obtained by limited dilution cloning into 96-well plates and are confirmed with a GDF15 response by AlphaLISA SureFire Ultra p-ERK1/2 (Thr202/Tyr204) Assay Kit (PerkinElmer). Clones are expanded, harvested, resuspended in freezing media, aliquoted into cryovials, and kept in liquid nitrogen for long-term storage. The top responder is selected with the best GDF15 response (signal to background ratio), clonal line #7.

[00254] Human GFRAL and RET receptor AlphaLISA SureFire Ultra p-ERK1/2 (Thr202/Tyr204) assay: HEK293 cell lines expressing the human GFRAL and the human RET are cultured with selection medium (DMEM with 10% FBS with 25 mM HEPES, 1x antibiotics, 1 µg/mL puromycin, 1 mg/mL Geneticin). On Day -3 (the day of cell plating), cells are washed once with PBS, lifted from flasks with TrypLE™ Express, and resuspended in plating medium (DMEM with 25 mM HEPES, 1x antibiotics, 10% FBS). Cells are plated in a 96-well plate (Corning Cat# 356461) at 20,000 cells/0.1 mL/well. Cells are cultured at 37°C 5% CO₂ for 72 hr. On Day 1 (the day of assay), medium is removed and replaced with 50 µL serum-free medium (DMEM with 25 mM HEPES, 1x antibiotics). Plates are incubated at 37°C for 4 hr, then 50 µL of 2x ligand is added (GDF15, final 1x). Plates are incubated for an additional 10 min at 37°C. After the incubation period is complete, medium is removed from plates by decanting and blotting on white utility wipes. Then, 50 µL of 1x AlphaLISA SureFire Ultra Lysis Buffer is added to each well, and the plates are incubated on a plate shaker at 350 rpm at RT for 10 min. Subsequently, according to the manufacturer's

instructions (AlphaLISA SureFire Ultra p-ERK (Thr202/Tyr204) Assay Kit, PerkinElmer Cat# ALSU-pERK-A10K) for the 2-Plate/1-Incubation protocol for adherent cells, 10 μ L cell lysate, 5 μ L Acceptor Mix, and 5 μ L Donor Mix are added to an OptiPlate™-384 plate (PerkinElmer cat# 6007290). The plate is sealed, wrapped in foil, incubated for 1 min on a plate shaker at 250 rpm at RT, incubated at RT in the dark for 8 hr, and then read on an EnVision 2102 Multilabel Reader with EnVision Manager software (PerkinElmer).

[00255] Statistical analysis of data: Data is imported from the EnVision 2102 Multilabel Reader into GraphPad Prism® software (GraphPad Software, LLC; La Jolla, CA; version 8). EC₅₀ values are generated by a variable slope-four parameter dose response curve analysis.

[00256] Table 13: Stimulation of ERK1/2 Phosphorylation in HEK293 Cells Expressing Human GFRAL & RET51 by VHH-Based Fusion of Example 5.

Compound	hGFRAL/RET EC ₅₀ (pM)	SEM	N
Human GDF15	2.85	1.08	2
Example 5	101	19.5	2

[00257] Example 22: *In Vitro* Potency of VHH-Based Fusions of Examples 6 and 7

[00258] A cell-based dimerization bioassay from DiscoverX, PathHunter IL-2 BioAssay Kit (eurofins/DiscoverX part # 93-1003Y3-00091) is used to evaluate the potency of the VHH-based fusions of Examples 6 and 7. The assay detects IL-2 induced dimerization of two subunits of an IL-2 receptor-dimer pair and is designed to assess IL-2 potency. The assay is performed according to the manufacturer's protocol. Briefly, cells are thawed and plated in the provided 96-well plate at 80 μ L per well. After 20 hr incubation at 37°C in 5% CO₂, 20 μ L of a 5X stock concentration of Examples 6 and 7 and the provided IL-2 reference standard is added in an 11-point serial dilution curve (1:3) to appropriate wells in duplicate. The plate is incubated at 37°C for 6 hr. Following the 6-hr incubation with the Examples 6 and 7 and the reference standard, 10 μ L detection reagent 1 are added to each well. Mixing is achieved on a plate shaker at 350 rpm for 1 min, and the plate is incubated at RT in the dark for 15 min. Then 40 μ L of detection reagent 2 are added to each well and incubated at RT in the dark for 1 additional hr. Chemiluminescent signal is read on plate reader using a 1-sec integration time (SpectraMax i3x plate reader by Molecular Devices). Statistical analysis of raw data is

performed with GraphPad PRISM version 8.4.3. Raw data are transformed and a non-linear regression analysis (log(agonist) vs. response - variable slope (four parameters)) is run to obtain EC₅₀ values and to determine goodness of fit. (n=2 for all data).

[00259] Table 14: Potency of VHH-Based Fusions of Examples 6 and 7 in the DiscoverX PathHunter IL-2 BioAssay.

	EC ₅₀ (nM)	R ²	N
Human IL-2	0.13	0.9983	2
Example 6	6.7	0.9978	2
Example 7	0.49	0.9980	2

[00260] Example 23: *In Vitro* Potency of VHH-Based Fusions of Examples 9 and 13

[00261] A L929 cell-based cytotoxicity assay is used to assess in vitro neutralization potency of Examples 9 and 13 of soluble human TNF α , soluble cyno TNF α , or membrane-bound human TNF α .

[00262] L929 cells (ATCC) endogenously expressing the human TNF α receptor are cultured in DMEM High Glucose media with 10% heat-inactivated FBS, 2 mM L-glutamine, 1x non-essential amino acids, 1x sodium pyruvate, 1x antibiotics and split 1:20 every 3-4 days with TrypLE™ Express (Gibco).

[00263] Soluble human TNF α protein is obtained from custom synthesis by Syngene (Bangalore, India). Soluble cyno TNF α protein is obtained from R&D Systems (Minneapolis, MN; Cat. #1070-RM/CF).

[00264] A stable, MT104 H2 CHO-membrane human TNF α -expressing cell line is generated at Eli Lilly and Company (Indianapolis, IN). The cells are cultured in Lilly media LM7300 with 8 mM L-glutamine and selection agent G418 (500 μ g/mL) split 1:10 every 3-4 days.

[00265] L929 cytotoxicity assay: On Day 1, L929 cells are trypsinized with 5 mL TrypLE™ (Gibco# 12605036), complete media is added (1:3 volume) and centrifuged at 1000 rpm for 5 min at RT. The supernatant is gently aspirated, and the cells are re-suspended in 15 mL of complete media (DMEM High Glucose Media with 10% heat-inactivated FBS, 2 mM L-glutamine, 1x non-essential amino acids, 1x sodium pyruvate, 1x antibiotics), and an aliquot of cells is counted using a Viacell counter. Cells are plated in 96-well Poly-D Lysine-coated

plates (Corning#354496) at 10,000 cells/0.1 mL/well and cultured at 37°C 5% CO₂ overnight. On Day 2, Examples 9 and 13 are titrated with fixed amounts of antigens: soluble human TNF α (200 pg/mL) or soluble cyno TNF α (750 pg/mL) or membrane human TNF α expressing cells (5000 cells/mL) in DMEM + FBS with Actinomycin D (6.25 μ g/mL). Starting concentrations are 1.5 μ g/mL, 3 μ g/mL and 10 μ g/mL for soluble human TNF, soluble cyno TNF and membrane human TNF neutralization, respectively and are titrated by 3-fold serial dilution 8 points down. Titrated compound-antigen complex is added to 96-well plates containing L929 cells after removing media, and the plates are incubated at 37°C 5% CO₂ overnight. Stimulated negative control wells contain L929 cells + Actinomycin D + TNF α antigen, whereas unstimulated control wells include only L929 cells + Actinomycin D. [00266] On Day 3, media is removed from 96-well plates, and 120 μ L of Cell Titer AQueous ONE substrate solution (1:6 dilution in DMEM media +FBS) is added to each well on all the plates. Plates are read at an OD 490 nm after 2 hr on a Spectra max plate reader using Softmax 4.7 software.

[00267] Data Analysis: OD readings versus concentration is graphed in Excel® (Microsoft; Redmond, WA); EC₅₀ calculation and statistical analysis are done using Eli Lilly statistical software tools (Global Stats Discovery Team, Lilly).

[00268] Table 15: *In Vitro* Potency of VHH-Based Fusions of Examples 9 and 13 Neutralizing Soluble Human TNF α .

Compound	EC ₅₀ Geo Mean (μ g/mL)	Error	N	Log Mean	Sd
Example 9	0.0562	0.0115	3	-1.2506	0.0153
Example 13	0.0333	0.0004	3	-1.4781	0.0089
Adalimumab	0.0392	0.0009	3	-1.407	0.01678

[00269] Table 16: *In Vitro* Potency of VHH-Based Fusions of Examples 9 and 13 Neutralizing Soluble Cyno TNF α .

Compound	EC ₅₀ Geo Mean (μ g/mL)	Error	N	Log Mean	Sd
Example 9	0.1748	0.0040	3	-0.7574	0.0170
Example 13	0.1025	0.0045	3	-0.9893	0.0333
Adalimumab	0.0944	0.0021	3	-0.01633	0.0163

[00270] Table 17: *In Vitro* Potency of VHH-Based Fusions of Examples 9 and 13 Neutralizing Membrane Human TNF α .

Compound	EC ₅₀ Geo Mean (μ g/mL)	Error	N	Log Mean	Sd
Example 9	0.2943	0.0040	3	-0.5312	0.0102
Example 13	0.2005	0.0196	3	-0.6979	0.0736
Adalimumab	0.2165	0.0068	3	-0.6645	0.0238

[00271] Table 18: *In Vitro* Potency of VHH-Based Fusions of Examples 9 and 13 Neutralizing Various Forms of Human and Cyno TNF α .

Compound	EC ₅₀ (nM)-Geo Mean		
	Soluble Human TNF α Inhibition	Soluble Cyno TNF α Inhibition	Membrane Human TNF α Inhibition
Example 9	0.886	2.7565	4.641
Example 13	0.703	2.1668	4.238
Adalimumab	0.2613	0.6292	1.443

[00272] Example 24: *In Vitro* Activity of Multi-Specific VHH-Based Fusion and Conjugate of Examples 15 and 16

[00273] Cell-based cyclic AMP assay kits purchased from DiscoverX (eurofins) are used to evaluate activity of the VHH-based conjugates of Examples 15 and 16 at GLP1R and MC3R. For the cAMP, Hunter eXpress GLP1R CHO-K1 GPCR assay (cat# 95-0062E2) is used, and an internal GLP-1 standard (SEQ ID NO:99) is used as a positive control. For the cAMP Hunter eXpress MC3R CHO-K1 GPCR assay (cat # 95-0045E2) is used, and α -MSH is used as a positive control. Assays are performed according to the GPCR assay protocol provided by

the manufacturer. Briefly, cells are thawed and are plated in a 96-well plate at 100 μ L per well. After a 24-hr incubation at 37°C in 5% CO₂, 15 μ L 3X agonist (VHH conjugate/fusion and controls) and cAMP standard are added to appropriate wells in duplicate. The plate is incubated at 37°C for 30 minutes. cAMP working detection solution is made up and kept at RT protected from light. Following a 30-min incubation with a VHH fusion or conjugate, 15 μ L cAMP antibody reagent is added to each well. Immediately after adding antibody reagent, 60 μ L of a cAMP working detection solution is added, which is made up during the incubation period. The plate is incubated at RT in the dark for 1 hr, then 60 μ L of cAMP solution A are added to each well, and the plate is incubated at RT in the dark for 3-18 hr. Chemiluminescent signal is read on a plate reader (SpectraMax i3x plate reader by Molecular Devices). Statistical analysis of raw data is performed with GraphPad PRISM version 8. Raw data is transformed, and a non-linear regression analysis is run (log(agonist) vs. response - variable slope (4 parameters)) to obtain EC₅₀ values and to determine goodness of fit (n=2 for all data).

[00274] Table 19: *In Vitro* Potency of VHH-Based Conjugates of Examples 15 and 16 at MC3R.

	Example 15	Example 16	cAMP standard
EC ₅₀	54.2	46.17	36.25
R squared	0.9941	0.9888	0.9932

[00275] Table 20: *In Vitro* Potency of VHH-Based Conjugate of Example 15 at GLP1R.

	Example 15	GLP-1 positive control	cAMP standard
EC ₅₀	1.807	0.5044	39.89
R squared	0.9925	0.9832	0.9971

[00276] *IN VIVO* FUNCTION – LONG-ACTING SPECIFIC PD FOR EACH PAYLOAD

[00277] Example 25: *In vivo* activity (Body Weight Loss in Healthy Mice) of VHH-Based Fusions of Examples 1 and 2

The study is performed with 48 male DIO C57BL6 mice sourced from Taconic. Mice are 15 weeks of age at study start. Animals are single housed in micro-isolator cages with ad-libitum access to automated house water (2-5 ppm chlorine) and high-fat chow (TD95217). Mice are

randomized into 8 groups (n = 6) based on body weight using the block randomized allocation tool developed by Lilly statisticians for in vivo group randomization. To reduce potential stress-related study-effects, all animals are acclimated to daily handling for at least 3 days prior to the start of the study. Body weights and food weights are recorded for 2 days prior to study start, then daily throughout the study. Treatments are administered subcutaneously in the interscapular region over the course of the study (QD dosing schedule) using a 0.5 mL 28G insulin syringe (BD cat# 329461). Native CNTF (R&D systems, recombinant human CNTF cat# 257-NT/CF, lot # GL402101A), Example 1, and Example 2 are diluted to appropriate concentrations in sterile PBS pH 7.2 within 30 minutes of administration. Native CNTF is dosed at 0.25 mpk. Example 1 and Example 2 are dosed at either 0.25 mpk or 0.1 mpk. Dilutions are calculated for each treatment daily based on average group body weights from the previous day. 200 µL of PBS are administer to animals in the vehicle control group. Body weight changes are analyzed using an internal statistical tool with a rigorous model-based approach developed by Lilly statisticians for in vivo PD studies. Animals are removed from study regardless of dosing date upon reaching 20% body weight loss.

[00278] Table 21: Effect of Varied Doses of VHH-based Fusions of Examples 1 and 2 on Body Weight in Healthy Mice at Various Timepoints.

Compound	Dose	N	% change in body weight by study day						
			0	1	2	3	4	5	6
Vehicle	n/a	6	102.47	102.11	101.01	99.98	99.50	98.61	99.32
Example 1	0.1 mpk	6	101.09	100.51	97.61	95.38	94.24	92.73	91.44
Example 1	0.25 mpk	6	100.81	97.18	91.21	85.31	81.18	79.19	82.31
Example 2	0.1 mpk	6	102.13	100.70	98.04	95.39	94.87	93.02	91.91
Example 2	0.25 mpk	6	102.77	99.81	95.46	90.42	85.29	82.23	82.91
rhCNTF	0.25 mpk	6	100.51	98.95	96.96	94.76	94.03	91.74	90.62

[00279] Table 22: Statistical Analysis of Effect of Varied Doses of VHH-based Fusions of Examples 1 and 2 on Body Weight in Healthy Mice at Various Timepoints.

Compound	Dose		Study day						
			0	1	2	3	4	5	6
Example 1	0.1 mpk	effect size	0.580	1.566	0.650	0.626	0.208	0.989	0.823
		p-val	0.997	0.751	0.995	0.996	1.000	0.958	0.983
Example 1	0.25 mpk	effect size	0.292	-1.764	-5.750	-9.442	-12.853	-12.552	-8.309
		p-val	1.000	0.650	0.00026	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Example 2	0.1 mpk	effect size	1.617	1.754	1.077	0.638	0.837	1.281	1.289
		p-val	0.726	0.655	0.939	0.995	0.981	0.876	0.873
Example 2	0.25 mpk	effect size	2.252	0.867	-1.501	-4.332	-8.743	-9.516	-7.715
		p-val	0.403	0.977	0.783	0.012	< 0.0001	< 0.0001	< 0.0001

P-values are in comparison to the native CNTF 0.25 mpk treatment group

[00280] As shown in Table 21 and 22, all treatment groups demonstrate significant body weight loss when compared to the vehicle group by study day 3. Example 1 and Example 2 dosing at 0.1 mpk both show body weight loss equivalent to rhCNTF dosed at 0.25 mpk. Animals receiving Example 1 at 0.25 mpk display significantly more body weight loss by study day 2 when compared to rhCNTF at 0.25 mpk. Animals receiving Example 1 at 0.25 mpk display significantly more body weight loss by study day 3 when compared to rhCNTF at 0.25 mpk. Animals in the groups receiving 0.25 mpk dosage of both Example 1 and 2 are not dosed on days 5 or 6 due to clinical signs of dehydration and/or achievement of 20% BWL.

[00281] Example 26: In vivo Activity (Blood Chemistry Analysis of Healthy Mice) of VHH-Based Fusions of Examples 3 and 4

The study is performed with 48 female C57BL6 mice sourced from Envigo at 6 weeks of age. Animals are pair housed in micro-isolator cages with ad-libitum access to automated house water (2-5 ppm chlorine) and standard chow (Teklad 2014 rodent maintenance diet). Mice are randomized into 6 groups (n = 8) based on body weight using the block randomized allocation tool developed by Lilly statisticians for in vivo group randomization. To reduce potential stress-related study-effects, all animals are acclimated to daily handling for at least 3 days prior to study start. Body weights and food weights are recorded for 2 days prior to study start, then monitored and recorded daily throughout the study. Animals are 8 weeks of age at the time of the first dose. Treatments are administered subcutaneously in the interscapular region every other day for 7 days for a total of 3 doses (Q2D dosing schedule) using a 0.5 mL 28G insulin syringe (BD cat# 329461). Native NRG-1 (R&D systems, recombinant human NRG1-beta 1 EGF Domain, CF, cat# 396-HB/CF, batch# ACD182101A), Example 3 and Example 4 are diluted to appropriate concentrations in sterile PBS pH 7.2 within 30 minutes of administration. Native NRG-1 is dosed at 1 mpk. Example 3 and Example 4 are dosed at 1 and 0.3 mpk. Dilutions for each treatment are calculated daily based on average group body weights from the previous day. Animals in the vehicle control group receive 200 μ L of PBS SC. Treatments are administered in the morning on study days 1, 3, & 5. All animals are anesthetized with isofluorane (4%) to a surgical plane and exsanguinated via retro-orbital bleed in accordance with animal care and use protocols on study day 7. Blood is collected into serum separator tubes (BD cat # 365967) and kept at room temperature for up to 90 minutes. Blood is spun down for 10 minutes at 10,000 RPM. Serum is collected from each tube and aliquoted for Chem 18 analysis. Serum is analyzed on the Roche Cobas 8000 Modular chemistry analyzer. All reagents for the Chem 18 analysis are sourced from Roche except for the triglyceride test which uses Fujifilm WAKO reagent. JMP software oneway analysis of CI by group using Dunnett's Method is used for statistical analysis of blood chemistry values.

Table 23: Statistical Analysis of Effect of Varied Doses of VHH-based Fusions of Examples 3 and 4 on Blood Chemistry Values of Healthy Mice.

Compound	Dose	N		BUN mg/dL	creatinine mg/dL	cholesterol mg/dL	ALP IU/L
Vehicle	n/a	8	mean	17.51	0.1025	79.125	173
			p val	1	1	1	1
Example 3	1 mpk	8	mean	16.63	0.08	98.375	128.75
			p val	0.99	0.0462	0.0004*	< 0.0001*
Example 3	0.3 mpk	8	mean	17.22	0.08	85	156.625
			p val	0.8	0.0462	0.5397	0.1347
Example 4	1 mpk	8	mean	17.39	0.0675	173	81.25
			p val	1	0.0008*	< 0.0001*	< 0.0001*
Example 4	0.3 mpk	8	mean	14.7	0.0787	99.75	138.25
			p val	0.015*	0.0324*	0.0001*	0.0002*
rhNRG-1 (native control)	1 mpk	8	mean	14.55	0.0775	79.375	157.5
			p val	0.01*	0.0224*	1	0.1695

* Indicative of statistically significant difference from vehicle treatment group

[00282] As shown in Table 23, the VHH fusions of Examples 3 & 4 demonstrate significant effects on blood chemistry as compared to the vehicle control. A reduction in serum creatinine is observed across all treatment groups with both dose levels of Example 4 and the native rhNRG1 control producing statistically significant drops. A significant decrease in ALP from baseline occurs for animals dosed with the native rhNRG1 control and 1mpk dosing groups for both Example 3 and 4. For both dose levels of Example 3, at the 1mpk dose of Example 4, and in the native rhNRG1 control group cholesterol levels are increased. The native rhNRG1 control and the 0.3 mpk dose group of Example 4 also significantly decrease BUN.

[00283] Example 27: In vivo Activity (Body Weight Loss in Healthy Mice) of VHH-Based Fusion of Example 5.

[00284] 24 male DIO C57BL6 mice are sourced from Taconic at 12 weeks of age. Animals are single housed in micro-isolator cages with ad-libitum access to automated house water (2-5 ppm chlorine) and high-fat chow (TD95217). Randomize mice into 4 groups (n = 6) based on body weight using the block randomized allocation tool developed by Lilly statisticians for in vivo group randomization. To reduce potential stress-related study-effects, all animals

are acclimated to daily handling for at least 3 days prior to the start of the study. Body weights and food weights are recorded for 2 days prior to study start, then monitored and recorded daily throughout the study. 5 doses of each treatment are administered subcutaneously in the interscapular region over the course of the study (Q3D dosing schedule) using a 0.5 mL 28G insulin syringe (BD cat# 329461). Native GDF-15 (R&D systems, recombinant, cat #957-GD/CF; batch EHF232101A) and VHH fusion protein of Example 5 are diluted to appropriate concentrations in sterile PBS pH 7.2 within 30 minutes of administration. Native GDF-15 is dosed at 0.1 mpk and VHH fusion protein of Example 5 is dosed at either 1 mpk or 0.1 mpk based on average group body weights from the previous day. Animals in the vehicle control group receive 200 μ L of PBS. Body weight changes are analyzed using an internal statistical tool with a rigorous model-based approach developed by Lilly statisticians for in vivo PD studies.

[00285] Table 24: Effect of Varied Doses of VHH-based Fusion of Example 5 on Body Weight in Healthy Mice at Various Timepoints.

Compound	Dose	N	% change in body weight by study day				
			0	3	6	9	12
Vehicle	n/a	6	100.3	101.9	100.2	102.3	104.4
Example 5	0.1	6	100.9	95.8	90.7	89.2	86.2
Example 5	1	6	101.9	95.6	91.3	87.7	85.5
Native GDF-15	0.1	6	99.7	99.2	97.1	95.7	94.8

[00286] Table 25: Statistical Analysis of Effect of Varied Doses of VHH-based Fusion of Example 5 on Body Weight in Healthy Mice at Various Timepoints.

Compound	Dose		Study Day				
			0	3	6	9	12
Example 5	0.1	Effect size	0.583	-6.02	-9.48	-13.08	-18.15
		P-val	0.971	6.88E-04	< 0.001	< 0.001	< 0.001
Example 5	1	Effect size	1.657	-6.247	-8.862	-14.644	-18.825
		P-val	0.616	4.53E-04	< 0.001	< 0.001	< 0.001
Native GDF-15	0.1	Effect size	-0.631	-2.673	-3.030	-6.611	-9.589
		P-val	0.963	0.242	0.158	1.85E-04	< 0.001

P-values are in comparison to the vehicle treated control group

[00287] As shown in Tables 24 and 25, significant body weight loss occurs in both groups treated with VHH fusion protein of Example 5 at either 0.1 mpk or 1 mpk by day 3 compared to vehicle control. Body weight loss in the native GDF-15 treated group reaches significance by day 9. There is no difference in body weight loss at any time point between the 1 and 0.1 mpk dose groups of VHH fusion protein of Example 5. Animals receiving 1 mpk or 0.1 mpk VHH fusion protein of Example 5 lost significantly more body weight by day 6 on study as compared to those treated with native GDF15. This significance persists for the remainder of the study.

[00288]

[00289] Example 28: In vivo Activity (Corticosterone Induction and Body Weight Loss in Healthy Mice) of VHH-Based Conjugate of Example 15

[00290] Healthy 6-week-old female C57BL6 mice are purchased from Envigo, group housed (3 mice per cage) in micro-isolator cages with house filtered water in bottles and fed a standard chow diet (2014 Teklad global rodent maintenance diet). Mice are allowed to acclimate after receipt for at least 72 hr. Mice are weighed 1 day prior to dosing. A single subcutaneous dose of the VHH fusion conjugate of Example 15 at 0.1, 0.3, 1 or 3 nmol/kg, or vehicle (DPBS) is administered on day 1; n = 3 per group. A tail stick for collection of dried blood spots (DBS) samples for corticosterone level analysis is performed just prior to dosing (T0) and at 2, 6, 24, 30, 48 and 72 hr post dose. About 20-30 μ L of blood from the tail of each animal is collected on Whatman DMPK cards (WB129243). DBS cards are provided to in-house LC-MS specialists for corticosterone level analysis. Animal body weight is also recorded on a daily basis throughout the study. All *in vivo* experimental procedures are conducted in compliance with IACUC standards and according to an approved animal use protocol (19-033). Data are reported in Tables 26 and 27 as mean corticosterone levels (ng/mL) or mean percent change from body weight recorded on day 0, respectively.

[00291] Table 26: Effect of Varied Doses of VHH-Based Conjugate of Example 15 on Corticosterone Levels in Healthy Mice at Various Timepoints.

			Hours Post Dose:						
Dose	N		0	2	6	24	30	48	72
0.1 nmol/kg	3	Mean	8.53	331.44	542.62	52.60	32.77	16.36	7.54
		SEM	0.65	48.23	43.88	33.65	1.43	4.00	2.73
		P-val	0.17	0.018	0.007	0.27	0.032	0.2	0.002
0.3 nmol/kg	3	Mean	44.71	788.87	1278.7	132.09	53.99	5.86	18.96
		SEM	11.96	125.20	120.20	96.47	11.99	0.18	1.83
		P-val	0.49	0.006	0.0013	0.974	0.053	0.078	0.015
1 nmol/kg	3	Mean	25.16	1027.8	1315.4	1003.8	275.68	8.02	8.28
		SEM	15.44	52.55	109.56	297.4	151.60	3.08	0.16
		P-val	0.3	< .0001	0.0008	0.045	0.63	0.098	0.0006
3 nmol/kg	3	Mean	52.85	1071.9	1315.5	1746.4	1748.0	260.69	10.24
		SEM	19.44	63.01	15.20	82.73	92.80	187.38	2.95
		P-val	0.62	0.0001	< .0001	< .0001	0.0001	0.302	0.005
Vehicle	3	Mean	77.11	126.99	321.41	135.83	192.73	38.37	30.81
		SEM	41.11	21.57	2.44	55.38	49.68	13.82	2.30

P-values are calculated to determine significance using an unpaired T-test of treatment groups vs Vehicle by individual time point in GraphPad PRISM software.

[00292] As shown in Table 26, treatment with VHH-based conjugate of Example 15 leads to significant induction of corticosterone levels as compared to vehicle control at 2 and 6 hours post dose for all dose levels. For animals in the 3mpk dosing groups, the increase in corticosterone levels remains significantly higher than the vehicle control group out to 30 hours post dose.

[00293] Table 27: Effect of Varied Doses of VHH-Based Conjugate of Example 15 on Body Weight in Healthy Mice 72 hr Post-Injection.

Treatment Group	Dose (nmol/kg)	N	% BW Mean	SEM	P-value
Vehicle	n/a	3	106.7	1.125	1
Example 15	0.1	3	105.1	0.563	0.2846
Example 15	0.3	3	102.1	2.183	0.1346
Example 15	1	3	97.9	0.671	0.0026
Example 15	3	3	100.1	1.385	0.0211

[00294] As shown in Table 27, treatment with VHH-based conjugate of Example 15 leads to prevention of body weight gain in the 1nmol/kg and 3nmol/kg treatment groups over the course of the study. The difference in percent body weight gain is significant for these two treatment groups by day 3 when compared to vehicle control. P-values are calculated using an unpaired T-test of treatment groups vs Vehicle in GraphPad PRISM software.

[00295] *IN VIVO* FUNCTION – PK

[00296] Example 29: PK of VHH-Based Fusions of Example 4 in Male Sprague Dawley Rats

[00297] Male Sprague Dawley rats are administered a single intravenous (IV) or subcutaneous (SC) dose of 106.8 nmol/kg for Example 4, formulated in PBS buffer (pH 7.4) at a dose volume of 2.6 mL/kg. Blood is collected for PK characterization at 1, 6, 12, 24, 48, 96, 144, 168 and 240 hr post-dose for IV the route; and at 6, 12, 24, 48, 96, 144, 168 and 240 hr post-dose for the SC route.

[00298] Plasma concentrations of the VHH-based fusions of Example 4 are determined by a qualified LC/MS assay at Altascience Company (Laval, Quebec, Canada) using a Q/Exactive Plus mass spectrometer (Thermo Scientific, San Jose, CA). The VHH fusions of Example 4 and an internal standard are isolated from K3EDTA rat plasma via immunoprecipitation with an anti-VHH-antibody-biotin conjugate and streptavidin-coated magnetic beads. Following wash steps to remove interfering endogenous proteins, the isolated VHH fusions of Example 4 are reduced, alkylated and digested with trypsin, and the tryptic peptides are analyzed by LC/MS as a surrogate measure of the intact fusions. The plasma concentrations of the VHH fusions of Example 4 are used to calculate the PK parameters shown in Table 28.

[00299] Table 28: Mean Plasma Pharmacokinetic Parameters for VHH-Based Fusions of Example 4 Following Single IV or SC Dose Administered to Male Sprague Dawley Rats.

Compound	Route	Dose (nmol/kg)	T _{max} (hr)	C ₀ (IV) C _{max} (SC) (nmol/L)	AUC _{0-inf} (hr*nmol/L)	CL (IV) CL/F (SC) (mL/hr/kg)	t _{1/2} (hr)
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Example 4	IV	106.8	NA	2310 (461)	4.40E4 (5.27E3)	2.45 (0.274)	30 (1.3)
	SC	106.8	24 (0)	127 (27)	6.83E3 (1.51E3)	16.2 (3.50)	38 (3.2)

NOTE: Abbreviations: $t_{1/2}$ = half-life, T_{max} = time to maximum concentration, C_{max} = maximum observed plasma concentration, C_0 = plasma concentration extrapolated to time zero, AUC_{0-inf} = area under the curve from time 0 hours to infinity, CL = clearance, CL/F = clearance/bioavailability. Standard deviation values are included in the table below the mean PK parameters, in parentheses. (N = 3/group)

[00300] As shown in Table 28, the VHH fusion of Example 4 demonstrates an extended PK profile in Sprague Dawley rats relative to the non-fused proteins (data not shown).

[00301] Example 30: PK of VHH-Based Fusions of Examples 5, 6 and 7 in Male Sprague Dawley Rats

[00302] Male Sprague Dawley rats are administered a single intravenous (IV) or subcutaneous (SQ) dose of 50 nmol/kg for Example 5, 25 nmol/kg for Example 6 or 7, formulated in PBS buffer (pH 7.4) at a dose volume of 4 mL/kg. Blood is collected for PK characterization at 1, 6, 12, 24, 48, 96, 144, 168 and 240 hr post-dose for IV routes; and at 6, 12, 24, 48, 96, 144, 168 and 240 hr post-dose for SQ routes.

[00303] Plasma concentrations of the VHH-based fusions of Examples 5-7 are determined by a qualified LC/MS assay at Covance Laboratories (Greenfield, IN) using a Q/Exactive Plus mass spectrometer (Thermo Scientific, San Jose, CA). The VHH fusions of Examples 5-7 and an internal standard are isolated from K3EDTA rat plasma via immunoprecipitation with an anti-VHH-antibody-biotin conjugate and streptavidin-coated magnetic beads. Following wash steps to remove interfering endogenous proteins, the isolated VHH fusions of Examples 5-7 are reduced, alkylated and digested with trypsin, and the tryptic peptides are analyzed by LC/MS as a surrogate measure of the intact fusions. The plasma concentrations of the VHH fusions of Examples 5-7 are used to calculate the PK parameters shown in Table 29.

[00304] Table 29: Mean Plasma Pharmacokinetic Parameters for VHH-Based Fusions of Examples 5, 6 and 7 Following Single IV or SQ Dose Administered to Male Sprague Dawley Rats.

Compound	Route	Dose (nmol/kg)	T _{max} (hr)	C ₀ (IV) C _{max} (SQ) (nmol/L)	AUC _{0-inf} (hr*nmol/L)	CL (IV) CL/F (SQ) (mL/hr/kg)	t _{1/2} (hr)
Example 5	IV	50	NA	1480 (231)	1.48E5* (7.08E3)	0.438* (0.0290)	106* (12.9)
	SQ	50	64 (28)	269 (56)	8.77E4* (1.89E4)	1.14* (0.160)	206* (102)
Example 6	IV	25	NA	383 (42.4)	8390 (892)	3.00 (0.317)	15.7 (2.35)
	SQ	25	32 (14)	57.8 (12.0)	3240 (746)	7.96 (1.62)	12.6 (0.968)
Example 7	IV	25	NA	309 (29.2)	5830 (180)	4.30 (0.134)	13.9 (4.78)
	SQ	25	24 (0)	31.6 (16.9)	1520 (755)	20.2 (11.6)	13.2 (3.19)

COMMENTS: *The study duration is too short to fully characterize the elimination phase of the PK profile. The data are mean values (standard deviation in parentheses): Example 5 values are N = 3 (IV and SC from 1-240 hours); Example 6 IV values are N = 3 (1-168 hours) and N = 1 (240 hours), and SC values are N = 3 (6-144 hours); Example 7 IV values are N = 3 (1-96 hours), N = 2 (144 hours), and N = 1 (168, 240 hours); and SC values are N = 3 (6 to 96 hours), N = 2 (144 hours), and N = 1 (168 hours).

NOTE: Abbreviations: t_{1/2} = half-life, T_{max} = time to maximum concentration, C_{max} = maximum observed plasma concentration, C₀ = plasma concentration extrapolated to time zero, AUC_{0-inf} = area under the curve from time 0 hours to infinity, CL = clearance, CL/F = clearance/bioavailability.

[00305] As shown in Table 29, the VHH fusions of Examples 5, 6 and 7 demonstrate an extended PK profile in Sprague Dawley rats relative to the non-fused proteins (data not shown).

[00306] Example 31: PK of Fab-VHH Fusion of Example 9 and the corresponding non-fused Fab (Example 13) in Male Sprague Dawley Rats

[00307] Male Sprague Dawley rats are administered a single intravenous (IV) or subcutaneous (SC) dose of 63.4 nmol/kg for Example 9, 78.9 nmol/kg for Example 13, formulated in PBS buffer (pH 7.4) at a dose volume of 4 mL/kg. Blood is collected for PK characterization at 1, 6, 12, 24, 48, 96, 144, 168 and 240 hr post-dose for IV routes; and at 6, 12, 24, 48, 96, 144, 168 and 240 hr post-dose for SC routes.

[00308] Plasma concentrations of the Fab-VHH fusion of Example 9 and the corresponding non-fused Fab of Example 13 are determined by a qualified LC/MS assay at Altascience Company (Laval, Quebec, Canada) using a Q/Exactive Plus mass spectrometer (Thermo Scientific, San Jose, CA). The Fab-VHH fusion of Examples 9 and the Fab of Example 13 and an internal standard are isolated from K3EDTA rat plasma via immunoprecipitation with an anti-human kappa light chain-biotin conjugate and streptavidin-coated magnetic beads, or an anti-VHH-antibody-biotin conjugate and streptavidin-coated magnetic beads. Following wash steps to remove interfering endogenous proteins, the isolated proteins of Examples 9 and 13 are reduced, alkylated and digested with trypsin, and the tryptic peptides are analyzed by LC/MS as a surrogate measure of the intact fusions. The plasma concentrations (Table 30) of Fab-VHH of Example 9 are used to calculate the PK parameters shown in Table 31. The plasma concentrations of the non-fused Fab of Example 13 are too low to calculate PK parameters.

[00309] Table 30: Mean Plasma Concentration (reported in nmol/L) for Fab-VHH Fusion of Example 9 and the Corresponding Non-Fused Fab (Example 13) Following Single IV or SC Dose Administered to Male Sprague Dawley Rats.

Compound	Route	Time (hour)								
		1	6	12	24	48	96	144	168	240
Example 9	IV	1909.8 (35.4)	1378.2 (93.2)	999.7 (19.7)	797.8 (40.3)	515.3 (44.0)	257.4 (10.6)	133.1 (17.4)	96.9 (14.2)	34.6 (5.0)
	SC	NA	28.1 (7.1)	79.2 (26.5)	159.6 (30.0)	229.8 (16.2)	183.1 (16.4)	103.0 (13.4)	85.0 (8.3)	46.0 (20.9)
Example 13	IV	75.7 (7.1)	18.8 (11.3)	6.9 (4.2)	2.7 (NC)	BQL	BQL	BQL	BQL	BQL
	SC	NA	12.1	5.6	BQL	BQL	BQL	BQL	BQL	BQL

SEQUENCES

[00313] The following nucleic and/or amino acid sequences are referred to in the disclosure and are provided below for reference.

[00314] SEQ ID NO:1 – VHH moiety 1 (MC6.1C22)

EVQLLESGGGLVQPGGSLRLSCAASGRYIDETAVAWFRQAPGKREFVAGIGGGV
DITYYADSVKGRFTISRDN SKNTLYLQMNSLRPEDTAVYYCAARPGRPLITSKVAD
LPHYWGQGTLVTVSS

[00315] SEQ ID NO:2 – VHH moiety 2 (MC6.1C80)

EVQLLESGGGLVQPGGSLRLSCAASGRYIDETAVAWFRQAPGKGREFVAGIGGGV
DITYYADSVKGRFTISRDN SKNTLYLQMNSLRPEDTAVYYCAARPGRPLITSKVAD
LPHYWGQGTLVTVSS

[00316] SEQ ID NO:3 – VHH moiety 3 (MC6.1C22.43)

EVQLLESGGGLVQPGGSLRLSCAASGRYIDETAVAWFRQAPGKREFVAGIGGGV
DITYYADSVKGRFTISRDN SKNTLYLQMNSLRPEDTAVYYCAARPGRPLITSKVAD
LPHYWGQGTLVTVSSPP

[00317] SEQ ID NO:4 – VHH moiety 4 (MC6.1C80.43)

EVQLLESGGGLVQPGGSLRLSCAASGRYIDETAVAWFRQAPGKGREFVAGIGGGV
DITYYADSVKGRFTISRDN SKNTLYLQMNSLRPEDTAVYYCAARPGRPLITSKVAD
LPHYWGQGTLVTVSSPP

[00318] SEQ ID NO:5 – VHH moiety 5 (MC6)

EVQLVESGGGLVQAGGSLRLSCAASGRTVSSTAVAWFRQAPGKEREF TAGIGGSV
DITYYLD SVKGRFTISKDN TKNTVYLQMNSLKPEDTAVYYCAVRPGRPLITSRDAN
LYDYWGQGTQVTVSS

[00319] SEQ ID NO:6 – VHH moiety 6 (MC6.1)

EVQLLESGGGLVQPGGSLRLSCAASGRTVSSTAVAWFRQAPGKEREFVAGIGGSVD
ITYYADSVKGRFTISRDN SKNTLYLQMNSLRPEDTAVYYCAVRPGRPLITSRDANL
YDYWGQGTLVTVSS

[00320] SEQ ID NO:7 – VHH moiety 7 (MC6.1C6)

EVQLLESGGGLVQPGGSLRLSCAASGRYIDSTAVAWFRQAPGKEREFVAGIGGGVD
ITYYADSVKGRFTISRDN SKNTLYLQMNSLRPEDTAVYYCAARPGRPLITSRVANL
YPYWGQGTLVTVSS

[00321] SEQ ID NO:8 – VHH moiety 8 (MC6.1C22-G26Y)

EVQLLESGGGLVQPGGSLRLSCAASYRYIDETAVAWFRQAPGKEREFVAGIGGGV
DITYYADSVKGRFTISRDN SKNTLYLQMNSLRPEDTAVYYCAARPGRPLITSKVAD
LYPYWGQGTLVTVSS

[00322] SEQ ID NO:9 – VHH moiety 9 (MC6.1C22-R27A)

EVQLLESGGGLVQPGGSLRLSCAASGAYIDETAVAWFRQAPGKEREFVAGIGGGV
DITYYADSVKGRFTISRDN SKNTLYLQMNSLRPEDTAVYYCAARPGRPLITSKVAD
LYPYWGQGTLVTVSS

[00323] SEQ ID NO:10 – VHH moiety 10 (MC6.1C22-I57E)

EVQLLESGGGLVQPGGSLRLSCAASGRYIDETAVAWFRQAPGKEREFVAGIGGGV
DETYYADSVKGRFTISRDN SKNTLYLQMNSLRPEDTAVYYCAARPGRPLITSKVAD
LYPYWGQGTLVTVSS

[00324] SEQ ID NO:11 – VHH moiety 11 (MC6.1C22-I57Q)

EVQLLESGGGLVQPGGSLRLSCAASGRYIDETAVAWFRQAPGKEREFVAGIGGGV
DQTYYADSVKGRFTISRDN SKNTLYLQMNSLRPEDTAVYYCAARPGRPLITSKVAD
LYPYWGQGTLVTVSS

[00325] SEQ ID NO:12 – VHH moiety 12 (MC6.1C22-Y59A)

EVQLLESGGGLVQPGGSLRLSCAASGRYIDETAVAWFRQAPGKEREVAVAGIGGGV
DITAYADSVKGRFTISRDN SKNTLYLQMNSLRPEDTAVYYCAARPGRPLITSKVAD
LTPYWGQGTLVTVSS

[00326] SEQ ID NO:13 – VHH moiety 13 (MC6.1C22-Y59E)

EVQLLESGGGLVQPGGSLRLSCAASGRYIDETAVAWFRQAPGKEREVAVAGIGGGV
DITEYADSVKGRFTISRDN SKNTLYLQMNSLRPEDTAVYYCAARPGRPLITSKVADL
YPYWGQGTLVTVSS

[00327] SEQ ID NO:14 – VHH moiety 14 (MC6.1C22-Y59Q)

EVQLLESGGGLVQPGGSLRLSCAASGRYIDETAVAWFRQAPGKEREVAVAGIGGGV
DITQYADSVKGRFTISRDN SKNTLYLQMNSLRPEDTAVYYCAARPGRPLITSKVAD
LTPYWGQGTLVTVSS

[00328] SEQ ID NO:15 – VHH moiety 15 (MC6.1C22-Y59S)

EVQLLESGGGLVQPGGSLRLSCAASGRYIDETAVAWFRQAPGKEREVAVAGIGGGV
DITSYADSVKGRFTISRDN SKNTLYLQMNSLRPEDTAVYYCAARPGRPLITSKVADL
YPYWGQGTLVTVSS

[00329] SEQ ID NO:16 – VHH moiety 16 (MC6.1C22-Y59T)

EVQLLESGGGLVQPGGSLRLSCAASGRYIDETAVAWFRQAPGKEREVAVAGIGGGV
DITTYADSVKGRFTISRDN SKNTLYLQMNSLRPEDTAVYYCAARPGRPLITSKVADL
YPYWGQGTLVTVSS

[00330] SEQ ID NO:17 – VHH moiety 17 (MC6.1C22-R102K)

EVQLLESGGGLVQPGGSLRLSCAASGRYIDETAVAWFRQAPGKEREVAVAGIGGGV
DITYYADSVKGRFTISRDN SKNTLYLQMNSLRPEDTAVYYCAARPGKPLITSKVAD
LTPYWGQGTLVTVSS

[00331] SEQ ID NO:18 – VHH moiety 18 (MC6.1C22-R102Q)

EVQLLESGGGLVQPGGSLRLSCAASGRYIDETAVAWFRQAPGKEREVAGIGGGV
DITYYADSVKGRFTISRDN SKNTLYLQMNSLRPEDTAVYYCAARPGQLITSKVAD
LPHYWGQGTLVTVSS

[00332] SEQ ID NO:19 – VHH moiety 19 (MC6.1C22-R102S)

EVQLLESGGGLVQPGGSLRLSCAASGRYIDETAVAWFRQAPGKEREVAGIGGGV
DITYYADSVKGRFTISRDN SKNTLYLQMNSLRPEDTAVYYCAARPGSPLITSKVADL
YPYWGQGTLVTVSS

[00333] SEQ ID NO:20 – VHH moiety 20 (MC6.1C22-P103)

EVQLLESGGGLVQPGGSLRLSCAASGRYIDETAVAWFRQAPGKEREVAGIGGGV
DITYYADSVKGRFTISRDN SKNTLYLQMNSLRPEDTAVYYCAARPGRELITSKVAD
LPHYWGQGTLVTVSS

[00334] SEQ ID NO:21 – VHH moiety 21 (MC6.1C22-P103Q)

EVQLLESGGGLVQPGGSLRLSCAASGRYIDETAVAWFRQAPGKEREVAGIGGGV
DITYYADSVKGRFTISRDN SKNTLYLQMNSLRPEDTAVYYCAARPGRQLITSKVAD
LPHYWGQGTLVTVSS

[00335] SEQ ID NO:22 – VHH moiety 22 (MC6.1C22-P103S)

EVQLLESGGGLVQPGGSLRLSCAASGRYIDETAVAWFRQAPGKEREVAGIGGGV
DITYYADSVKGRFTISRDN SKNTLYLQMNSLRPEDTAVYYCAARPGRSLITSKVAD
LPHYWGQGTLVTVSS

[00336] SEQ ID NO:23 – VHH moiety 23 (MC6.1C22-L104E)

EVQLLESGGGLVQPGGSLRLSCAASGRYIDETAVAWFRQAPGKEREVAGIGGGV
DITYYADSVKGRFTISRDN SKNTLYLQMNSLRPEDTAVYYCAARPGRPEITSKVAD
LPHYWGQGTLVTVSS

[00337] SEQ ID NO:24 – VHH moiety 24 (MC6.1C22-L104G)

EVQLLESGGGLVQPGGSLRLSCAASGRYIDETAVAWFRQAPGKEREFVAGIGGGV
DITYYADSVKGRFTISRDN SKNTLYLQMNSLRPEDTAVYYCAARPGRP GITSKVAD
LTPYWGQGTLVTVSS

[00338] SEQ ID NO:25 – VHH moiety 25 (MC6.1C22-L104Q)

EVQLLESGGGLVQPGGSLRLSCAASGRYIDETAVAWFRQAPGKEREFVAGIGGGV
DITYYADSVKGRFTISRDN SKNTLYLQMNSLRPEDTAVYYCAARPGRP QITSKVAD
LTPYWGQGTLVTVSS

[00339] SEQ ID NO:26 – VHH moiety 26 (MC6.1C22-L104T)

EVQLLESGGGLVQPGGSLRLSCAASGRYIDETAVAWFRQAPGKEREFVAGIGGGV
DITYYADSVKGRFTISRDN SKNTLYLQMNSLRPEDTAVYYCAARPGRP TITSKVAD
LTPYWGQGTLVTVSS

[00340] SEQ ID NO:27 – VHH moiety 27 (MC6.1C22-S107E)

EVQLLESGGGLVQPGGSLRLSCAASGRYIDETAVAWFRQAPGKEREFVAGIGGGV
DITYYADSVKGRFTISRDN SKNTLYLQMNSLRPEDTAVYYCAARPGRP LITEKVAD
LTPYWGQGTLVTVSS

[00341] SEQ ID NO:28 – VHH moiety 28 (MC6.1C80-A98T)

EVQLLESGGGLVQPGGSLRLSCAASGRYIDETAVAWFRQAPGK GREFVAGIGGGV
DITYYADSVKGRFTISRDN SKNTLYLQMNSLRPEDTAVYYCATRPRPLITSKVADL
YTPYWGQGTLVTVSS

[00342] SEQ ID NO:29 – VHH moiety 29 (MC6.1C80-P100Q)

EVQLLESGGGLVQPGGSLRLSCAASGRYIDETAVAWFRQAPGK GREFVAGIGGGV
DITYYADSVKGRFTISRDN SKNTLYLQMNSLRPEDTAVYYCAARQGRPLITSKVAD
LTPYWGQGTLVTVSS

[00343] SEQ ID NO:30 – VHH moiety 30 (MC6.1C80-A98T, P100Q)

EVQLLESGGGLVQPGGSLRLSCAASGRYIDETAVAWFRQAPGKGREFVAGIGGGV
DITYYADSVKGRFTISRDN SKNTLYLQMNSLRPEDTAVYYCATRQGRPLITSKVAD
LPHYWGQGTLVTVSS

[00344] SEQ ID NO:31 – VHH moiety 31 (MC6.1C80-K108Q)

EVQLLESGGGLVQPGGSLRLSCAASGRYIDETAVAWFRQAPGKGREFVAGIGGGV
DITYYADSVKGRFTISRDN SKNTLYLQMNSLRPEDTAVYYCAARPGRPLITSQVAD
LPHYWGQGTLVTVSS

[00345] SEQ ID NO:32 – VHH moiety 32 (MC6.1C80-V109Q)

EVQLLESGGGLVQPGGSLRLSCAASGRYIDETAVAWFRQAPGKGREFVAGIGGGV
DITYYADSVKGRFTISRDN SKNTLYLQMNSLRPEDTAVYYCAARPGRPLITSQVAD
LPHYWGQGTLVTVSS

[00346] SEQ ID NO:33 – VHH moiety 33 (MC6.1C80-D111E)

EVQLLESGGGLVQPGGSLRLSCAASGRYIDETAVAWFRQAPGKGREFVAGIGGGV
DITYYADSVKGRFTISRDN SKNTLYLQMNSLRPEDTAVYYCAARPGRPLITSKVAEL
YPYWGQGTLVTVSS

[00347] SEQ ID NO:34 – VHH moiety 34 (MC6.1C80-D111S)

EVQLLESGGGLVQPGGSLRLSCAASGRYIDETAVAWFRQAPGKGREFVAGIGGGV
DITYYADSVKGRFTISRDN SKNTLYLQMNSLRPEDTAVYYCAARPGRPLITSKVASL
YPYWGQGTLVTVSS

[00348] SEQ ID NO:35 – VHH moiety 35 (MC6.1C80-V109Q, D111E)

EVQLLESGGGLVQPGGSLRLSCAASGRYIDETAVAWFRQAPGKGREFVAGIGGGV
DITYYADSVKGRFTISRDN SKNTLYLQMNSLRPEDTAVYYCAARPGRPLITSKQVAD
YPYWGQGTLVTVSS

[00349] SEQ ID NO:36 – VHH moiety 36 (MC6.1C80-V109Q, D111S)
EVQLLES GGGLVQP GGS LRLSCAASGRYIDETAVAWFRQAPGKGREFVAGIGGGV
DITYYADSVKGRFTISRDN SKNTLYLQMNSLRPEDTAVYYCAARPGRPLITSKQASL
YPYWGQGTLVTVSS

[00350] SEQ ID NO:37 – VHH moiety 37 (MC6.1C80Cys)
EVQLLES GGGLVQP GGS LRLSCAASGRYIDETAVAWFRQAPGKGREFVAGIGGGV
DITYYADSVKGRFTISRDN SKNTLYLQMNSLRPEDTAVYYCAARPGRPLITSKVAD
LYPYWGQGTLVTVSSC

[00351] SEQ ID NO:38 – L₁ (basic sequence of (GGGGQ)_n)
GGGGQ

[00352] SEQ ID NO:39 – L₁ (basic sequence of (GGGQ)_n)
GGGQ

[00353] SEQ ID NO:40 – L₁ (basic sequence of (GGGGS)_n)
GGGGS

[00354] SEQ ID NO:41 – L₁ (basic sequence of (PGPQ)_n)
PGPQ

[00355] SEQ ID NO:42 – L₁ (basic sequence of (PGPA)_n)
PGPA

[00356] SEQ ID NO:43 – L₁ (basic sequence of GGGG(AP)_nGGGG)
GGGG-AP-GGGG

[00357] SEQ ID NO:44 – L₁ (basic sequence of (GGE)_n)

GGE

[00358] SEQ ID NO:45 – L₁ (basic sequence of (GGGGE)_n)

GGGGE

[00359] SEQ ID NO:46 – L₁ (basic sequence of (GGK)_n)

GGK

[00360] SEQ ID NO:47 – L₁ (basic sequence of (GGGGK)_n)

GGGGK

[00361] SEQ ID NO:48 – L₁ (basic sequence of GGGG(EP)_nGGGG)

GGGG-EP-GGGG

[00362] SEQ ID NO:49 – L₁ (basic sequence of GGGG(KP)_nGGGG)

GGGG-KP-GGGG

[00363] SEQ ID NO:50 – L₁ (basic sequence of (PGPE)_n)

PGPE

[00364] SEQ ID NO:51 – L₁ (basic sequence of (PGPK)_n)

PGPK

[00365] SEQ ID NO:52 – L₁ 1 (GGGGQ)₅

GGGGQGGGGQGGGGQGGGGQGGGGQ

[00366] SEQ ID NO:53 – L₁ 2 (PGPQ)₈

PGPQPGPQPGPQPGPQPGPQPGPQPGPQPGPQ

[00367] SEQ ID NO:54 – L₁ 3 (PGPA)₈
PGPAPGPAPGPAPGPAPGPAPGPAPGPAPGPA

[00368] SEQ ID NO:55 – L₁ 4 ((GGE)₈)
GGEGGEGGEGGEGGEGGEGGEGGEGGE

[00369] SEQ ID NO:56 – L₁ 5 ((GGGGE)₅)
GGGGEGGGGEGGGGEGGGGEGGGGE

[00370] SEQ ID NO:57 – L₁ 6 ((GGK)₈)
GGKGGKGGKGGKGGKGGKGGKGGK

[00371] SEQ ID NO:58 – L₁ 7 ((GGGGK)₅)
GGGGKGGGGKGGGGKGGGGKGGGGK

[00372] SEQ ID NO:59 – L₁ 8 ((GGGG(AP)₁₀GGGG))
GGGGAPAPAPAPAPAPAPAPAPAPGPPGGG

[00373] SEQ ID NO:60 – L₁ 9 ((GGGG(EP)₁₀GGGG))
GGGGEPEPEPEPEPEPEPEPEPEPGGGG

[00374] SEQ ID NO:61 – L₁ 10 ((GGGG(KP)₁₀GGGG))
GGGGKPKPKPKPKPKPKPKPKPKPGGGG

[00375] SEQ ID NO:62 – L₁ 11 ((PGPE)₈)
PGPEPGPEPGPEPGPEPGPEPGPEPGPE

[00376] SEQ ID NO:63 – L₁ 12 ((PGPK)₈)
PGPKPGPKPGPKPGPKPGPKPGPKPGPK

[00377] SEQ ID NO:64 – L₂ 1

GGGSGGSGGG

[00378] SEQ ID NO:65 – L₂ 2

GGGSGGSGGSGGG

[00379] SEQ ID NO:66 – signal peptide

METDTLLLWVLLLWVPGSTG

[00380] SEQ ID NO:67 – human proGIP

MVATKTFALLLLSLFLAVGLGEKKEGHFSALPSLPVGSNAKVSSPQPRGPRYAEGT
FISDYSIAMDKIHQQDFVNWLLAQKGKKNDWKHNITQREARALELASQANRKEEE
AVEPQSSPA KNPSDEDLLR DLLIQELLACLLDQTNLCRLRSR

[00381] SEQ ID NO:68 – human GIP

YAEGTFISDYSIAMDKIHQQDFVNWLLAQKGKKNDWKHNITQ

[00382] SEQ ID NO:69 – human GIP receptor

RAETGSKGQTAGELYQRWERYRRECQETLAAAEPSPGLACNGSFDMYVCWDYAA
PNATARASCPWYLPWHHHVAAGFVLRQCGSDGQWGLWRDHTQCENPEKNEAFL
DQRLILERLQVMYTVGYSLSLATLLLALLILSLFRRLHCTRNYIHINLFTSFMLRAAA
ILSRDRLLPRPGPYLGDQALALWNQALAAACRTAQIVTQYCVGANYTWLLVEGVYL
HSLVLVGGSEEGHFRYYLLLGWGAPALFVIPWVIVRYLYENTQCWERNEVKAIW
WIIRTPILMTILINFLIFIRILGILLSKLRTRQMRCRDYRLRLARSTLTLVPLLGVHEVV
FAPVTEEQARGALRFAKLGFEIFLSSFQGFVSVLYCFINKEVQSEIRRGWHHCRLR
RSLGEEQRQLPERAFRALPSGSGPGEVPTSRGLSSGTLPGPGNEASRELESYC

[00383] SEQ ID NO:70 – human proglucagon

MKSIYFVAGLFVMLVQGSWQRSLQDTEEKSRFSASQADPLSDPDQMNE DKRHSQ
GTFTSDYSKYLDSSRAQDFVQWLMNTKRNRNNIAKRHDEFERHAEGTFTSDVSSY
LEGQAAKEFIAWLVKGRGRRDFPEEVAIVEELGRRHADGSFSDEMNTILDNLAARD
FINWLIQTKITDRK

[00384] SEQ ID NO:71 – human GLP-1

HDEFERHAEGTFTSDVSSYLEGQAAKEFIAWLVKGRG

[00385] SEQ ID NO:72 – human GLP-1₇₋₃₇

HAEGTFTSDVSSYLEGQAAKEFIAWLVKGRG

[00386] SEQ ID NO:73 – human GLP-1₇₋₃₆

HAEGTFTSDVSSYLEGQAAKEFIAWLVKGR

[00387] SEQ ID NO:74 – human GLP-1 receptor

RPQGATVSLWETVQKWREYRRQCQRSLTEDPPPATDLFCNRTFDEYACWPDGEPG
SFVNVSCPWYLPWASSVPQGHVYRFCTAEGLWLQKDNSSLPWRDLSECEESKRGE
RSSPEEQLLFLYIIYTVGYALSFSALVIASAILLGFRHLHCTRNYIHLNLFASFILRALS
VFIKDAALKWMYSTAAQQHQWDGLLSYQDSLSCRLVFLLMQYCVAANYYWLLV
EGVYLYTLLAFSVLSEQWIFRLYVSIGWGVPLLFVVPWGIVKYL YEDEGCWTRNSN
MNYWLIIRLPILFAIGVNFLIFVRVICIVVSKLKANLMCKTDIKCRLAKSTLTLIPLLG
THEVIFAFVMDEHARGTLRFIKLFTELSFTSFQGLMVAILYCFVNNEVQLEFRKSWE
RWRLEHLHIQRDSSMKPLKCPTSSLSSGATAGSSMYTATCQASCS

[00388] SEQ ID NO:75 – human proGDF15

MPGQELRTVNGSQMLLVLLVLSWLPHGGLSLAEASRASFPGPSELHSEDSRFREL
RKRYEDLLTRLRANQSWEDSNTDLVPAPAVRILTPEVRLGSGGHLHLRISRAALPE
GLPEASRLHRALEFRLSPTASRSWDVTRPLRRQLSLARPQAPALHLRLSPPPSQSDQL
LAESSSARPQLELHLRPQAARGRRRARARNGDHCPLGPGRCCRLHTVRLASLEDLG
WADWVLSPREVQVTMCIGACPSQFRAANMHAQIKTSLHRLKPDTVPAPCCVPASY
NPMVLIQKTDGTGVSLQTYDDLLAKDCHCI

[00389] SEQ ID NO:76 – human GDF15

ARNGDHCPLGPGRCCRLHTVRLASLEDLGWADWVLSPREVQVTMCIGACPSQFRAA
NMHAQIKTSLHRLKPDTVPAPCCVPASYNPMVLIQKTDGTGVSLQTYDDLLAKDCH
CI

[00390] SEQ ID NO:77 – human GDF15 (GFRAL) receptor

MIVFIFLAMGLSLENEYTSQTNNCTYLREQCLRDANGCKHAWRVMEDACNDSDPG
DPCKMRNSSYCNLSIQYLVESNFQFKECLCTDDFYCTVNKLLGKKCINKSDNVKED
KFKWNLTTTRSHHGFKGMWSCLEVAEACVGDVVCNAQLASYLKACSANGNPCDL
KQCQAAIRFFYQNIPFNIAQMLAFCDCAQSDIPCQQSKEALHSKTCAVNMVPPPTCL
SVIRSCQNDELCCRHYRTFQSKCWQRVTRKCHEDENCISTLSKQDLTCSGSDDCKA
AYIDILGTVLQVQCTCRTITQSEESLCKIFQHMLHRKSCFNYPITLSNVKGMALYTRK
HANKITLTGFHSPFNGEVIYAAMCMTVTCGILLVMVKLRISRISSKARDPSSIQIPG
EL

[00391] SEQ ID NO:78 – human proINS

FVNQHLCGSHLVEALYLVCGERGFFYTPKTRREAEDLQVGQVELGGGPGAGSLQP
LALEGLSLQKRGIVEQCCTSICSLYQLENYCN

[00392] SEQ ID NO:79 – human INS A chain

GIVEQCCTSICSLYQLENYCN

[00393] SEQ ID NO:80 – human INS B chain
FVNQHLCGSHLVEALYLVCGERGFFYTPKT

[00394] SEQ ID NO:81 – human INS receptor α -subunit

HLYPGEVCPGMDIRNNLTRLHELENCVIEGHLQILLMFKTRPEDFRDLSFPKLIMIT
DYLLLFRVYGLESLKDLFPNLTVIRGSRLFFNYALVIFEMVHLKELGLYNLMNITRG
SVRIEKNNELCYLATIDWSRILDSVEDNYIVLNKDDNEECGDICPGTAKGKTNCPAT
VINGQFVERCWTHSHCQKVCPTICKSHGCTAEGLCCHSECLGNCSQPDDPTKCVAC
RNFYLDGRCVETCPPPYHFQDWRCVNFSCQDLHHKCKNSRRQGCHQYVIHNNK
CIPECPSGYTMNSSNLLCTPCLGPCPKVCHLLEGEKTIDSVTSAQELRGCTVINGSLII
NIRGGNNLAAELEANLGLIEEISGYLKIRRSYALVSLSFFRKLRLIRGETLEIGNYSFY
ALDNQNLRLQLWDWSKHNLTITQGKLFHYNPKLCLSEIHKMEEVSGTKGRQERND
IALKTNGDQASCENELLKFSYIRTSFDKILLRWEPYWPPDFRDLLGFMLFYKEAPYQ
NVTEFDGQDACGSNSWTVVDIDPPLRSNDPKSQNHPGWLMRGLKPWTQYAIFVKT
LVTFSDERRTYGAKSDIIVQTDATNPSPVLPDISVSNSSSQILKWKPPSDPNGNITH
YLVFWEQAEDESELFELDYCLKGLKLPSRTWSPPFESEDSQKHNQSEYEDSAGECC
SCPKTDSQILKELEESSFRKTFEDYLHNVVVFVPRKTSSTGTAEDPRPS

[00395] SEQ ID NO:82 – human INS receptor β -subunit

SLGDVGNVTVAVPTVAAFPNTSSTSVPSPPEHRPFKVVNKESLVISGLRHFTGYRI
ELQACNQDTPPEERCSVAAYVSARTMPEAKADDIVGPVTHEIFENNVVHLMWQEPK
EPNGLIVLYEVSYYRYGDEELHLCVSRKHFALERGCRLRGLSPGNYSVRIRATSLAG
NGSWTEPTYFYVTDYLDVPSNIAKIIIGPLIFVFLFSVVIGSIYFLRKRQPDGPLGPLY
ASSNPEYLSASDVFPSCSVYVPDEWEVSRKITLLRELQGGSFGMVYEGNARDIHKGE
AETRVAVKTVNESASLRERIEFLNEASVMKGFTCHHVVRLLGVVSKGQPTLVVME
LMAHGDLKSYLRSLRPEAENNPGRPPPTLQEMIQMAAEIADGMAYLNKKFVHRD
LAARNCMVAHDFTVKIGDFGMTRDIYETDYRKGKGKLLPVRWMAPESLKDGVF
TTSSDMWSFGVVLWEITSLAEOPYQGLSNEQVLKFVMDGGGYLDQPDNCPERVTDL

MRMCWQFNPKMRPTFLEIVNLLKDDLHPSFPEVSFFHSEENKAPESSELEMEFEDM
ENVPLDRSSHQCREEAGGRDGGSSLGFKRSYEEHIPYTHMNGGKKNRILTLPRSN
PS

[00396] SEQ ID NO:83 – human IL-2

APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTFKFYMPKKATELKHL
QCLEEELKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATI
VEFLNRWITFCQSIISTLT

[00397] SEQ ID NO:84 – human IL-2 receptor α -subunit

ELCDDDPPEIPHATFKAMAYKEGTMLNCECKRGFRRIKSGSLYMLCTGNSSHSSWD
NQCQCTSSATRNTTKQVTPQPEEQKERKTTEMQSPMQPVDQASLPGHCREPPPWE
NEATERIYHFVVGQMVYYQCVQGYRALHRGPAESVCKMTHGKTRWTQPQLICTG
EMETSQFPGEEKPQASPEGRPESETSCLVTTTDFQIQTEMAATMETSIFTTEYQVAV
AGCVFLLISVLLLSGLTWQRRQRKSRRTI

[00398] SEQ ID NO:85 – human IL-2 receptor β -subunit

AVNGTSQFTCFYNSRANISCVWSQDQALQDTSCQVHAWPDRRRWNQTCELLPVSQ
ASWACNLILGAPDSQKLTTVDIVTLRVLCREGVRWRVMAIQDFKPFENLRMAPIS
LQVVHVETHRCNISWEISQASHYFERHLEFEARTLSPGHTWEEAPLLTLKQKQEWI
CLETLPDTQYEFQVRVKPLQGEFTTWSPWSQPLAFRTKPAALGKDTIPWLGHLLV
GLSGAFGFILVYLLINCRNTGPWLKKVLKCNTPDPSKFFSQLSSEHGGDVQKWSS
PFPSSSFSPGGLAPEISPLEVLERDKVTQLLLQQDKVPEPASLSSNHSLTSCFTNQGYF
FFHLPDALEIEACQVYFTYDPYSEEDPDEGVAGAPTGSSPQPLQPLSGEDDAYCTFP
SRDDLLLFSPSLLGGPSPPSTAPGGSGAGEERMPPSLQERVPRDWDPPQLGPPTPGV
PDLVDFQPPPELVLEAGEEVPDAGPREGVSFPWSRPPGQGEFRALNARLPLNTDA
YLSLQELQGQDPTHLV

[00399] SEQ ID NO:86 – human IL-2 receptor γ -subunit

LNTTILTPNGNEDTTADFFLTMTPTDSLVSSTLPLPEVQCFVFNVEYMNCTWNSSSE
PQPTNLTLYHYWYKNSDNDKVQKCSHYLFSEEITSGCQLQKKEIHL YQTFVVQLQDP
REPRRQATQMLKLQNLVIPWAPENLT LHKLSSESQLELNWNNRFLNHCLEHLVQYR
TDWDHSWTEQSVDYRHKFSLPSVDGQKRYTFRVRSRFNPLCGSAQHWSEWSHPIH
WGSNTSKENPFLFALEAVVISVGSMLIISLLCVYFWLERTMPRIPTLKNLEDLVTE
YHGNFSAWSGVSKGLAESLQPDYSERLCLVSEIPPKGGALGEGPGASPCNQHSPIW
APPCYTLKPET

[00400] SEQ ID NO:87 – human proNRG1

MSEKKEGRGKGKGKKKERGSGKKPESAAGSQSPALPPRLKEMKSQESAAGSKLV
RCETSSEYSSLRFKWFKNGNELNRKNKPQNIQKKPGKSEL RINKASLADSGEYM
CKVISKLGNDASANITIVESNEIITGMPASTEGAYVSSESPIRISVSTEGANTSSSTST
STTGTSHLVKCAEKEKTF CVNGGECFMVKDLSNPSRYLCKCQPGFTGARCTENV
MKVQNQEKAEELYQKRVLTITGICIALLVGIMCVVAYCKTKKQRKKLHDLRQ
LRSERNNMMNIANGPHHPNPPENVQLVNQYVSKNVISSEHIVEREAETSFSTSHYT
STAHHSTTVTQTPSHWSNGHTESILSEHSVIVMSSVENSRRHSSPTGGPRGRLNGT
GGPRECNSFLRHARETPDSYRDSPHSERYVSAMTTPARMSPVDFHTPSSPKSPPSEM
SPPVSSMTVSMPSMAVSPFMEEERPLLVTPPRLREKKFDHHPQQFSSFHHPAHDS
NSLPASPLRIVEDEEYETTQEYEPAQEPVKKLANSRRAKRTKPNGHIANRLEVDST
SSQSSNSESETEDERVGEDTPFLGIQNPLAASLEATPAFRLADSRTNPAGRFSTQEEI
QARLSSVIANQDPIAV

[00401] SEQ ID NO:88 – human NRG1

SGKKPESAAGSQSPALPPRLKEMKSQESAAGSKLVRCETSSEYSSLRFKWFKN
ELNRKNKPQNIQKKPGKSEL RINKASLADSGEYMCKVISKLGNDASANITIVES
NEIITGMPASTEGAYVSSESPIRISVSTEGANTSSSTSTTGTSHLVKCAEKEKTF
CVNGGECFMVKDLSNPSRYLCKCQPGFTGARCTENVPMKVQNQEKAEELYQK

[00402] SEQ ID NO:89 – human NRG1 receptor ErbB3

MRANDALQVLGLLFSLARGSEVGNSQAVCPGTLNGLSVTGD AENQYQTL YKLYER
CEVVMGNLEIVLTGHNADLSFLQWIREVTGYVLVAMNEFSTLPLPNLRVVRGTQV
YDGKFAIFVMLNYNTNSSHALRQLRLTQLTEILSGGVYIEKNDKLCHMDTIDWRDI
VRDRDAEIVVKDNGRSCPPCHEVCKGRCWGPGESEDCQTLTKTICAPQCNGHCFGP
NPNQCCHDECAGGCSGPQDTCFACRHFND SGACVPRCPQPLVYNKLT FQLEPNP
HTKYQYGGVCVASCPHNFVVDQTSV RACPPDKMEVDKNGLKMCEPCGGLCPKA
CEGTGSGSRFQTV DSSNIDGFVNCTKILGNLDFLITGLNGDPWHKIPALDPEKLN VF
RTVREITGYLNIQSWPPHMHNF SVFSNLTTIGGRSLYNRGFSLLIMKNLNV TSLGFRS
LKEISAGRIYISANRQLCYHHSLN WTKVLRGPTEERLDIKHNRPRRDCVAEGKVCD
PLCSSGGCWGPGPGQCLSCRNY SRGGVCVTHCNFLNGEPREFAHEAECFSCHPECQ
PMEGTATCNGSGSDTCAQCAHFRDGP HC VSSCPHGV LGAKGPIYKYPDVQNECRP
CHENCTQGCKGPELQDCLGQTLVLIGKTHLTMALTVIAGLVVIFMMLGGTFLYWR
GRIQNKRAMRRYLERGESIEPLDPSEKANKVLARIFKETELRKLKVLGSGVFGTVH
KGVWIP EGESIKIPVCIKVIEDKSGRQSFQAVTDHMLAIGSLDHAHIVRL LGLCPGSS
LQLVTQYLPLGSLLDHVRQHRGALGPQLLLNWGVQIAKGMYYLEEHGMVHRNLA
ARNVLLKSPSQVQVADFGVADLLPPDDKQLLYSEAKTPIKWMALESIHF GK YTHQS
DVWSYGVTVWELMTFGAEPYAGLRLAEVPDLLEKGERLAQPQICTIDVYMVMVK
CWMIDENIRPTFKELANEFTRMARDPPRYLVIKRESGPGIAPGPEPHGLTNKKLEEV
ELEPELDLDLDLEAEEDNLATTTLG SALSLPVGTLNRPRGSQSLLSPSSGYMPMNQ G
NLGESQESAVSGSSERCPRPVSLHPMPRGCLASESSEGHVTGSEAELQEKVSMCRS
RSRSRSPRPRGDSAYHSQRHSL LTPVTPLSPPGLEEEDVNGYVMPDTHLKGTPSSRE
GTLSSVGLSSVLGTEEEDEDEEY EYMNRRRRHSPPHPPRPSLEELGYEYMDVGSD
LSASLGSTQSCPLHPVPIMPTAGTTPDEDYEYMNQRDGGGPGGDYAAMGACPAS
EQGYEEMRAFQGP GHQAPHVHYARLKTLSLEATDSAFDNP DYWHSRLFPKANA
QRT

[00403] SEQ ID NO:90 – human NRG1 receptor ErbB4

MKPATGLWVWVSLVAAGTVQPSDSQSV CAGTENKLSSLS DLEQQYRALRKYYE
NCEVVMGNLEITSIEHNRDLSFLRSVREVTGYVLVALNQFRYLPLENLRIIRGTKLY

EDRYALAIFLNYRKDGNFGLQELGLKNLTEILNGGVYVDQNKFLCYADTIHWQDIV
RNPWPSNLTLVSTNGSSGCGRCHKSGCTGRCWGPTENHCQTLTRTVCAEQCDGRCY
GPYVSDCCHRECAGGCSGPKDTCDFACMNFND SGACVTQCPQTFVYNPTTFQLEH
NFN AKYTYGAF CVKKCPHNFVVDSSSCVRACPSSKMEVEENGIKMCKPCTDICPKA
CDGIGTGSLMSAQTV DSSNIDKFINCTKINGNLIFLVTGIHGDPYNAIEAIDPEKLN VF
RTVREITGFLNIQSWPPNMTDFS VFSNLVTIGGRVLYSGLSLLILKQQGITS LQFQSL
KEISAGNIYITDNSNLCYYHTINWTTLFSTINQRIVIRDNRKAENCTAEGMVCNHLCS
SDGCWGP GPDQCLSCRRFSRGRICIESCNLYDGEFREFENG SICVECDPQCEK MEDG
LLTCHGPGPDNCTKCSHF KDGPNCVEKCPDGLQGANSFIFKYADPDRECHPCHPNC
TQGCNGPTSHDCIYYPW TGHSTLPQHARTPLIAAGVIGGLFILVIVGLTF AVYVRRK
SIKKKRALRRFLETELVEPLTPSGTAPNQAQLRILKETELKRVKVLGSGAFGT VYKG
I WVPEGETVKIPVAIKILNETTGPKANVEFMDEALIMASMDPHLVRL LGVCLSPTI
QLVTQLMPHGCLLEYVHEHKDNIGS QLLL NWCVQIAKGMMYLEERRLVHRDLAA
RNVLVKSPNHVKITDFGLARLLEGDEKEYNADGGKMPIKWMALECIHYRKFT HQS
DVWSYGV TIWELMTFGGKPYDGIPTREIPDLLEKGERLPQPPICTIDVYVMVMVKCW
MIDADSRPKFKELAAEF SRMARDPQRYLVIQGDDRMKLPSPNDSKFFQNL LDEEDL
EDMMDAEEYLVPQAFNIPPPIYTSRARIDS NRSEIGHSPPPAYTPMSGNQFVYRDGG
FAAEQGVSVPYRAP TSTIPEAPVAQGATAEIFDDSCCNGTLRKPVAPHVQEDSSTQR
YSADPTVFAPERSPRGELDEEGYMT PMRDKPKQEYLN PVEENPFVSRRKNGDLQA
LDNPEYHNASNGPPKA EDEYVNEPLYLNTFANTLGKAEYLKNNILSMPEKAKKAF
DNP DYWNHSLPPRSTLQHPDYLQEYSTKYFYKQNGRIRPIVAENPEYLSEFSLKPGT
VLPPPPYRHRNTVV

[00404] SEQ ID NO:91 – human proRLN2

MPRLFFFHLLGVCLLLNQFSRAVADSWMEEVIKLCGRELVRAQIAICGMSTWSKRS
LSQEDAPQTPRPVAEIVPSFINKDTETINMMSEFVANLPQELKLT LSEMQPALPQLQ
QHVPVLKDSSLLFEEFKKLIRNRQSEAADSSPSELKYLGLDTHSRKKRQLYSALANK
CCHVGCTKRSLARFC

[00405] SEQ ID NO:92 – human RLN2 A chain
QLYSALANKCCHVGCTKRSLARFC

[00406] SEQ ID NO:93 – human RLN2 B chain
DSWMEEVIKLCGRELVRAQIAICGMSTWS

[00407] SEQ ID NO:94 – human RXFP1 receptor
MTSGSVFFYILIFGKYFSHGGGQDVKCSLGYPFCGNITKCLPQLLHCNGVDDCGNQ
ADEDNCGDNNGWSLQFDKYFASYKMTSQYPFEAETPECLVGSVPVQCLCQGLEL
DCDETNLRAVPSVSSNVTAMSLQWNLIRKLPPDCFKNYHDLQKLYLQNNKITSISIIY
AFRGLNSLTKLYLSHNRITFLKPGVFEDLHRLEWLIIEDNHLSRISPPTFYGLNSLILL
VLMNNVLTRLDPKPLCQHMPRLHWLDLEGNIHNLRLNLTFIGSCSNLTVLVMRKNKI
NHLNENTFAPLQKLDELDLGSNKIENLPPLIFKDLKELSQLNLSYNPIQKIQANQFDY
LVKLKSLSLEGIEISNIQORMFRPLMNLSHIYFKKFQYCGYAPHVRSCKPNTDGISSL
ENLLASIIQRFVWVVS AVTCFGNIFVICMRPYIRSENKLYAMSIISLCCADCLMGIY
LFVIGGFDLKFRGEYNKHAQLWMESTHCQLVGSLAILSTEVSVLLL TFLTLEKYICI
VYPFR CVRPGK CRTITVLILIWITGFIVAFIPLSNKEFFKNYYGTNGVCFPLHSEDTEI
GAQIYSVAIFLGINLAAFIIVFSYGSMFYSVHQSAITATEIRNQVKKEMILAKRFFFIV
FTDALCWIPFVVKFLLQVEIPGTITSWVVIFILPINSALNPILYTLTTRPFKEMIHRF
WYNYRQRKSMDSKGQKTYAPSWVEMWPLQEMPELMKPDLTYPCEMSLISQS
TRLNSYS

[00408] SEQ ID NO:95 – human ACTH
SYSMEHFRWGKPVGKKRRPVKVYPNGAEDESAEAFPLEF

[00409] SEQ ID NO:96 – human ACTH receptor (ACTHR, MC2R)
MKHIINSYENINNTARNNSDCPRVVLPEEIFFTISIVGVLENLIVLLAVFKNKNLQAP
MYFFICSLAISDMLGSLYKILENIIILRNMGYLYKPRGSFETTADDIIDSFLVLSLLGSI
FSLSVIAADRYITIFHALRYHSIVTMRRTVVVLTVIWTFTCTGTGITMVIFSHHVPTVIT

FTSLFPLMLVFILCLYVHMFLARSHTRKISTLPRANMKGAITLTILLGVFIFCWAPF
VLHVLLMTFCPSNPYCACYMSLFQVNGMLIMCNAVIDPFIYAFRSPELRDAFKKMI
FCSRYW

[00410] SEQ ID NO:97 – human CNTF

MAFTEHSPLTPHRRDLCSRSIWLARKIRSDLTALTESYVKHQGLNKNINLDSADGM
PVASTDQWSELTEAERLQENLQAYRTFHVLLARLLEDQQVHFTPTEGDFHQAIHTL
LLQVAAFAYQIEELMILLEYKIPRNEADGMPINVGDGGLFEKKLWGLKVLQELSQW
TVRSIHDLRFISSHQTGIPARGSHYIANNKKM

[00411] SEQ ID NO:98 – human CNTF receptor alpha

QRHSPQEAPHVQYERLGSDVTLPCGTANWDAAVTWRVNGTDLAPDLLNGSQLVL
HGLELGHSGLYACFHRDSWHLRHQVLLHVGLPPREPVLSRCSNTYPKGFYCSWHL
PTPTYIPNTFNVTVLHGSKIMVCEKDPALKNRCHIRYMHLFSTIKYKVSISVSNALG
HNATAITFDEFTIVKPDPPENVVARPVPSNPRRLEVTWQTPSTWPDPEFPLKFFFLRY
RPLILDQWQHVELSDGTAHTITDAYAGKEYIIQVAAKDNEIGTWSDWSVAAHATP
WTEEPRHLTTEAQA AETTTSTTSSLAPPPTTKICDPGELGS

[00412] SEQ ID NO:99 – GLP1-standard

HGEGTFTSDVSSYLEEQAAKEFIAWLVKGRGGGGGSGGGGSGGGGSESKYGPPCPP
CPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEV
HNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAK
GQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPP
VLDSGDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLGLG

[00413] SEQ ID NO:100 – VHH fusion 1 (CNTF-(G₄Q)₅-MC6.1C22.43)

MAFTEHSPLTPHRRDLASRSIWLARKIRSDLTALTESYVKHQGLNKNINLDSADGM
PVASTDWRSELTEAERLQENLQAYRTFHVLLARLLEDQQVHFTPTEGDFHQAIHTL
LLQVAAFAYQIEELMILLEYKIPRNEADGMPINVGDGGLFEKKLWGLKVLQELSQW
TVRSIHDLRFISSHQTGGGGGQGGGGQGGGGQGGGGQGGGGQEVQLLESGGGLV
QPGGSLRLSCAASGRYIDETAVAWFRQAPGKEREVAGIGGGVDITYYADSVKGRF
TISRDN SKNTLYLQMNSLRPEDTAVYYCAARPGRPLITSKVADLYPYWGQGTLVTV
SSPP

[00414] SEQ ID NO:101 – VHH fusion 2 (MC6.1C22-(G₄Q)₅-CNTF)

EVQLLES GGGLVQPGGSLRLSCAASGRYIDETAVAWFRQAPGKEREVAGIGGGV
DITYYADSVKGRFTISRDN SKNTLYLQMNSLRPEDTAVYYCAARPGRPLITSKVAD
LYPYWGQGTLVTVSSGGGGQGGGGQGGGGQGGGGQGGGGQMAFTEHSPLTPHR
RDLASRSIWLARKIRSDLTALTESYVKHQGLNKNINLDSADGMPVASTDRWSELTE
AERLQENLQAYRTFHVLLARLLEDQQVHFTPTEGDFHQAIHTLLQVAAFAYQIEE
LMILLEYKIPRNEADGMPINVG DGGLFEKKLWGLKVLQELSQWTVRSIHDLRFISSH
QTG

[00415] SEQ ID NO:102 – VHH fusion 3 (NRG1-(G₄Q)₅-MC6.1C22.43)

SHLVKCAEKEKTFCVNGGECFMVKDLSNPSRYLCKCPNEFTGDRCQNYVMASFYK
HLGIEFMEAEELYQGGGGQGGGGQGGGGQGGGGQGGGGQEVQLLES GGGLVQP
GGSLRLSCAASGRYIDETAVAWFRQAPGKEREVAGIGGGVDITYYADSVKGRFTI
SRDN SKNTLYLQMNSLRPEDTAVYYCAARPGRPLITSKVADLYPYWGQGTLVTVS
SPP

[00416] SEQ ID NO:103 – VHH fusion 4 (MC6.1C22-(G₄Q)₅-NRG1)

EVQLLES GGGLVQPGGSLRLSCAASGRYIDETAVAWFRQAPGKERE FVAGIGGGV
DITYYADSVKGRFTISRDN SKNTLYLQMNSLRPEDTAVYYCAARPGRPLITSKVAD
LYPYWGQGTLVTVSSGGGGQGGGGQGGGGQGGGGQGGGGQSHLVKCAEKEKTF
CVNGGECFMVKDL SNPSRYLCKCPNEFTGDRCQNYVMASFYKHLGIEFMEAEELY
Q

[00417] SEQ ID NO:104 – VHH fusion 5 (MC6.1C22-(G₄Q)₅-GDF15)

EVQLLES GGGLVQPGGSLRLSCAASGRYIDETAVAWFRQAPGKERE FVAGIGGGV
DITYYADSVKGRFTISRDN SKNTLYLQMNSLRPEDTAVYYCAARPGRPLITSKVAD
LYPYWGQGTLVTVSSGGGGQGGGGQGGGGQGGGGQGGGGQGDH CPLGPGRCCR
LHTVRASLEDLGWADWVLSPREVQVTMCIGACPSQFRAANMHAQIKTSLHRLKPD
TVPAPCCVPASYNPMVLIQKTD TGVS LQTYDDLLAKDCHCI

[00418] SEQ ID NO:105 – VHH fusion 6 (IL2-(G₄Q)₅-MC6.1C22.43)

APQSSSTQQTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTFKFYMPKKATELKHL
QCLEEELKPLEEVLNLAQSKNFHLRPRDLISRINVIVLELKGSETTFMCEYADETATI
VEFLNRWITFAQSIISTLTGGGGQGGGGQGGGGQGGGGQGGGGQGGGGQEVQLLES GGGL
VQPGGSLRLSCAASGRYIDETAVAWFRQAPGKERE FVAGIGGGVDITYYADSVKG
RFTISRDN SKNTLYLQMNSLRPEDTAVYYCAARPGRPLITSKVADLYPYWGQGTLV
TVSSPP

[00419] SEQ ID NO:106 – VHH fusion 7 (MC6.1C22-(G₄Q)₅-IL2)

EVQLLES GGGLVQPGGSLRLSCAASGRYIDETAVAWFRQAPGKERE FVAGIGGGV
DITYYADSVKGRFTISRDN SKNTLYLQMNSLRPEDTAVYYCAARPGRPLITSKVAD
LYPYWGQGTLVTVSSGGGGQGGGGQGGGGQGGGGQGGGGQAPQSSSTQQTQLQ
LEHLLLDLQMILNGINNYKNPKLTRMLTFKFYMPKKATELKHLQCLEEELKPLEEVLN
LAQSKNFHLRPRDLISRINVIVLELKGSETTFMCEYADETATIVEFLNRWITFAQSI
ISTLT

[00423] SEQ ID NO:110 – HC (AdaFab VHCH₁)

EVQLVESGGGLVQPGRSLRLSCAASGFTFDDYAMHWVRQAPGKGLEWVSAITWN
SGHIDYADSVEGRFTISRDNANKNSLYLQMNSLRAEDTAVYYCAKVSYLSTASSLDY
WGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGA
LTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKS
CD

[00424] SEQ ID NO:111 – LC-VHH (AdaFab VLCL-(G₄Q)₅-MC6.1C22.43)

DIQMTQSPSSLSASVGDRVTITCRASQGIRNYLAWYQQKPGKAPKLLIYAASLTQSG
VPSRFGSGSGTDFTLTISLQPEDVATYYCQRYNRAPYTFGGGTKVEIKRTVAAPS
VFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKD
STYLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGECGGGGQGGGGQG
GGGQGGGGQGGGGQEVQLLES GGGLVQPGGSLRLSCAASGRYIDETA VAWFRQA
PGKEREFVAGIGGGVDITYYADSVKGRFTISRDN SKNTLYLQMNSLRPEDTAVYYC
AARPGRPLITSKVADLYPYWGQGTLVTVSSPP

[00425] SEQ ID NO:112 – VHH-HC (MC6.1C22-(G₄Q)₅-AdaFab VHCH₁)

EVQLLES GGGLVQPGGSLRLSCAASGRYIDETA VAWFRQAPGKEREFVAGIGGGV
DITYYADSVKGRFTISRDN SKNTLYLQMNSLRPEDTAVYYCAARPGRPLITSKVAD
LYPYWGQGTLVTVSSGGGGQGGGGQGGGGQGGGGQGGGGQEVQLVESGGGLVQ
PGRSLRLSCAASGFTFDDYAMHWVRQAPGKGLEWVSAITWNSGHIDYADSVEGRF
TISRDNANKNSLYLQMNSLRAEDTAVYYCAKVSYLSTASSLDYWGQGTLVTVSSAS
TKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQS
SGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCD

[00426] SEQ ID NO:113 – LC (AdaFabVLCL)

DIQMTQSPSSLSASVGDRVITITCRASQGIRNYLAWYQQKPGKAPKLLIYAASLTQSG
VPSRFSGSGSGTDFTLTISLQPEDVATYYCQRYNRAPYTFGQGTKVEIKRTVAAPS
VFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKD
STYLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

[00427] SEQ ID NO:114 – HC (AdaFabVHCH₁)

EVQLVESGGGLVQPGRSLRLSCAASGFTFDDYAMHWVRQAPGKGLEWVSAITWN
SGHIDYADSVETRFTISRDNANKSLYLQMNSLR AEDTAVYYCAKVSYLSTASSLDY
WGQGTLLTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGA
LTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKS
CD

[00428] SEQ ID NO:115 – VHH-LC (MC6.1C22-(G₄Q)₅-AdaFabVLCL)

EVQLLES GGGLVQPGGSLRLSCAASGRYIDETAVAWFRQAPGKEREFVAGIGGGV
DITYYADSVKGRFTISRDN SKNTLYLQMNSLRPEDTAVYYCAARPGRPLITSKVAD
LYPYWGQGTLLTVSSGGGGQGGGGQGGGGQGGGGQGGGGQDIQMTQSPSSLSAS
VGDRVITITCRASQGIRNYLAWYQQKPGKAPKLLIYAASLTQSGVPSRFSGSGSGTD
FTLTISLQPEDVATYYCQRYNRAPYTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSG
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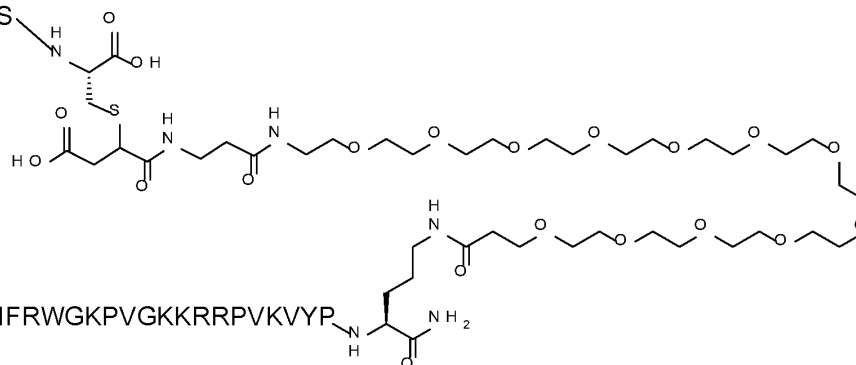
[00429] SEQ ID NO:116 – VHH Fusion 13 (GLP1-MC6.1C80Cys)

HGEGTFTSDVSSYLEEQAAKEFIAWLVKGGGGGGGQGGGGQGGGGQGGGGQGGG
GGQEVQLLESGGGLVQPGGSLRLSCAASGRYIDETAVAWFRQAPGKGREFVAGIG
GGVDITYYADSVKGRFTISRDN SKNTLYLQMNSLRPEDTAVYYCAARPGRPLITSK
VADLYPYWGQGTLVTVSSC

[00430] SEQ ID NO:117 – VHH fusion conjugate 1 (GLP1-C80Cys-ACTH conjugate)

HGEGTFTSDVSSYLEEQAAKEFIAWLVKGGGGGGGQGGGGQGGGGQGGGGQGGG
GGQEVQLLESGGGLVQPGGSLRLSCAASGRYIDETAVAWFRQAPGKGREFVAGIG
GGVDITYYADSVKGRFTISRDN SKNTLYLQMNSLRPEDTAVYYCAARPGRPLITSK
VADLYPYWGQGTLVTVSSC-MALEIMIDE-(PEG)₁₂-ACTH (in a C-terminal to N-
terminal orientation)

HGEGTFTSDVSSYLEEQAAKEFIAWLVKGGGGGGGQGGGGQGGGGQGGGGQGGGGQ
EVQLLESGGGLVQPGGSLRLSCAASGRYIDETAVAWFRQAPGKGREFVAGIGGGVDITY
YADSVKGRFTISRDN SKNTLYLQMNSLRPEDTAVYYCAARPGRPLITSKVADLYPYWG
QGTLVTVSS

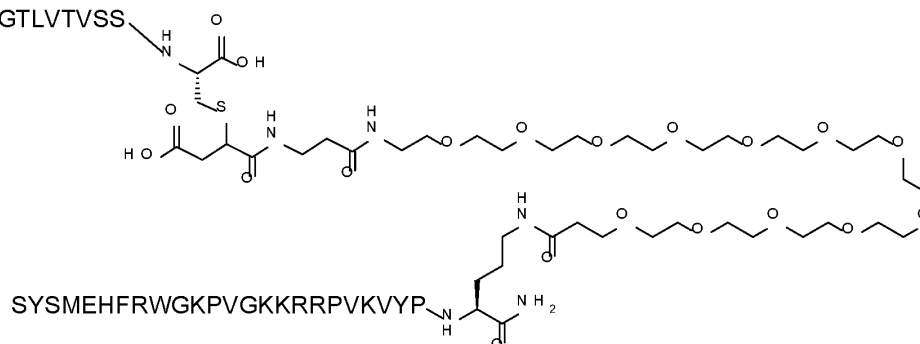


SYSMEHFRWGKPVGKKRRPVKVYP-NH₂

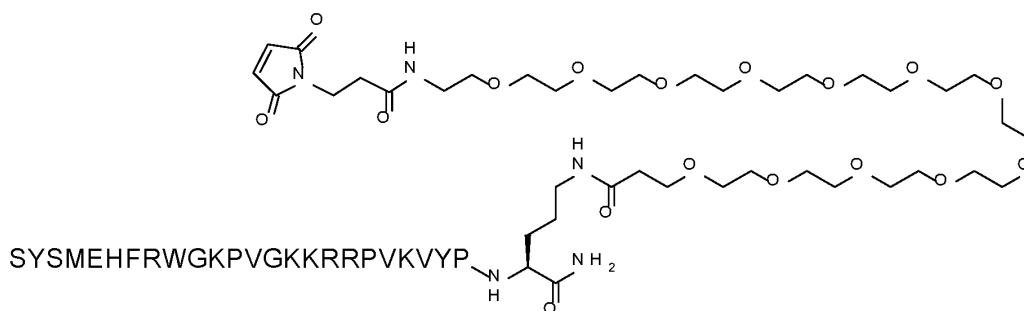
100

[00431] SEQ ID NO:118 – VHH fusion conjugate 2 (MC6.1C80Cys-ACTH conjugate)
 EVQLLESGGGLVQPGGSLRLSCAASGRYIDETAVAWFRQAPGKGREFVAGIGGGV
 DITYYADSVKGRFTISRDN SKNTLYLQMNSLRPEDTAVYYCAARPGRPLITSKVAD
 LYPYWGQGTLVTVSSC-maleimide-(PEG)₁₂-ACTH (in a C-terminal to N-terminal
 orientation)

EVQLLESGGGLVQPGGSLRLSCAASGRYIDETAVAWFRQAPGKGREFVAGIGGGVD
 ITYYADSVKGRFTISRDN SKNTLYLQMNSLRPEDTAVYYCAARPGRPLITSKVADLY
 PYWGQGTLVTVSS



[00432] SEQ ID NO:119 – Intermediate 1 (ACTH-(PEG)₁₂-maleimide conjugate)
 SYSMEHFRWGKPVGKKRRPVKVYPK-(PEG)₁₂-maleimide-NH₂



[00433] SEQ ID NO:120 – Fab HC (AdaFabVHCH₁)

EVQLVESGGGLVQPGRSLRLSCAASGFTFDDYAMHWVRQAPGKGLEWVSAITWN
SGHIDYADSVTEGRFTISRDNNAKNSLYLQMNSLRAEDTAVYYCAKVSYLSTASSLDY
WGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGA
LTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKS
CD

[00434] SEQ ID NO:121 – Fab LC (AdaFab VLCL)

DIQMTQSPSSLSASVGDRVTITCRASQGIRNYLAWYQQKPGKAPKLLIYAASSTLQSG
VPSRFGSGSGTDFTLTISSLQPEDVATYYCQRYNRAPYTFGQGTKVEIKRTVAAPS
VFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKD
STYLSSTLTLSKADYEKHKVYACEVTHQGLSPVTKSFNRGEC

[00435] SEQ ID NO:122 – Human GITR full-length (w/o signal peptide)

QRPTGGPGCGPGRLLLGTGTDARCCRVHTTRCCRDYPGEECCSEWDCMCVQPEFH
CGDPCCTTCRHHPCPPGQGVQSQGKFSFGFQCIDCASGTFSGGHEGHCKPWTDCQ
FGFLTVPFGNKTHNAVCPGSPPAEPLGWLTVVLLAVAACVLLLTSAQLGLHIWQL
RSQCMWPRETQLLLEVPPSTEDARSCQFPEEERGERSAEEKGRLGDLWV

[00436] SEQ ID NO:123 – Human GITR ECD (w/o signal peptide)

QRPTGGPGCGPGRLLLGTGTDARCCRVHTTRCCRDYPGEECCSEWDCMCVQPEFH
CGDPCCTTCRHHPCPPGQGVQSQGKFSFGFQCIDCASGTFSGGHEGHCKPWTDCQ
FGFLTVPFGNKTHNAVCPGSPPAE

[00437] SEQ ID NO:124 – VHH moiety 38 (MC6.1C90Cys)

EVQLLES GGGLVQPGGSLRLSCAASGRYIDETAVAWFRQAPGKGREFVAGIGGGV
DITYYADSVKGRFTISRDN SKNTLYLQMNSLRPEDTAVYYCGARPGRPLITSKVAD
LPYWGQGTLVTVSSC

[00438] SEQ ID NO:125 – VHH moiety 39 (MC6.1C90.43)

EVQLLESGGGLVQPGGSLRLSCAASGRYIDETAVAWFRQAPGKGREFVAGIGGGV
DITYYADSVKGRFTISRDN SKNTLYLQMNSLRPEDTAVYYCGARPGRPLITSKVAD
LTPYWGQGTLVTVSSPP

[00439] SEQ ID NO:126 – VHH moiety 40 (MC6.1C95.43)

EVQLLESGGGLVQPGGSLRLSCAASGRYIDETAVAWFRQAPGKGREFVAGIGGGV
DITYYADSVKGRFTISRDN SKNTLYLQMNSLRPEDTAVYYCGTRPGRPLITSKVADL
YPYWGQGTLVTVSSPP

CLAIMS

The invention claimed is:

1. A compound comprising an amino acid sequence selected from the group consisting of SEQ ID NOS:1-37 and 124-126, or at least about 90% sequence similarity thereto.
2. The compound of Claim 1, wherein the amino acid sequence is:
EVQLLES GGGLVQPGGSLRLSCAASGRYIDETAVAWFRQAPGKREFVAGIGGGV
DITYYADSVKGRFTISRDN SKNTLYLQMNSLRPEDTAVYYCAARPGRPLITSKVAD
LPHYWGQGTLVTVSS (SEQ ID NO:1) or at least about 90% sequence similarity thereto.
3. The compound of Claim 1, wherein the amino acid sequence is:
EVQLLES GGGLVQPGGSLRLSCAASGRYIDETAVAWFRQAPGKREFVAGIGGGV
DITYYADSVKGRFTISRDN SKNTLYLQMNSLRPEDTAVYYCAARPGRPLITSKVAD
LPHYWGQGTLVTVSS (SEQ ID NO:2) or at least about 90% sequence similarity thereto.
4. The compound of Claim 1, wherein the amino acid sequence is:
EVQLLES GGGLVQPGGSLRLSCAASGRYIDETAVAWFRQAPGKREFVAGIGGGV
DITYYADSVKGRFTISRDN SKNTLYLQMNSLRPEDTAVYYCAARPGRPLITSKVAD
LPHYWGQGTLVTVSSPP (SEQ ID NO:3) or at least about 90% sequence similarity thereto.
5. The compound of Claim 1, wherein the amino acid sequence is:
EVQLLES GGGLVQPGGSLRLSCAASGRYIDETAVAWFRQAPGKREFVAGIGGGV
DITYYADSVKGRFTISRDN SKNTLYLQMNSLRPEDTAVYYCAARPGRPLITSKVAD
LPHYWGQGTLVTVSSPP (SEQ ID NO:4) or at least about 90% sequence similarity thereto.

6. The compound of Claim 1, wherein the amino acid sequence is:
EVQLVESGGGLVQAGGSLRLSCAASGRTVSSTAVAWFRQAPGKEREFVAGIGGSV
DITYYLD SVKGRFTISKDNTKNTVYLQMNSLKPEDTAVYYCAVRPGRPLITSRDAN
LYDYWGQGTQVTVSS (SEQ ID NO:5) or at least about 90% sequence similarity thereto.
7. The compound of Claim 1, wherein the amino acid sequence is:
EVQLLES GGGLVQPGGSLRLSCAASGRTVSSTAVAWFRQAPGKEREFVAGIGGSVD
ITYYADSVKGRFTISRDN SKNTLYLQMNSLRPEDTAVYYCAVRPGRPLITSRDANL
YDYWGQGT LVTVSS (SEQ ID NO:6) or at least about 90% sequence similarity thereto.
8. The compound of Claim 1, wherein the amino acid sequence is:
EVQLLES GGGLVQPGGSLRLSCAASGRYIDSTAVAWFRQAPGKEREFVAGIGGGVD
ITYYADSVKGRFTISRDN SKNTLYLQMNSLRPEDTAVYYCAARPGRPLITSRVANL
YPYWGQGT LVTVSS (SEQ ID NO:7) or at least about 90% sequence similarity to thereto.
9. The compound of Claim 1, wherein the amino acid sequence is:
EVQLLES GGGLVQPGGSLRLSCAASYRYIDETAVAWFRQAPGKEREFVAGIGGGV
DITYYADSVKGRFTISRDN SKNTLYLQMNSLRPEDTAVYYCAARPGRPLITSKVAD
LPHYWGQGT LVTVSS (SEQ ID NO:8) or at least about 90% sequence similarity thereto.
10. The compound of Claim 1, wherein the amino acid sequence is:
EVQLLES GGGLVQPGGSLRLSCAASGAYIDETAVAWFRQAPGKEREFVAGIGGGV
DITYYADSVKGRFTISRDN SKNTLYLQMNSLRPEDTAVYYCAARPGRPLITSKVAD
LPHYWGQGT LVTVSS (SEQ ID NO:9) or at least about 90% sequence similarity thereto.
11. The compound of Claim 1, wherein the amino acid sequence is:
EVQLLES GGGLVQPGGSLRLSCAASGRYIDETAVAWFRQAPGKEREFVAGIGGGV
DEYYADSVKGRFTISRDN SKNTLYLQMNSLRPEDTAVYYCAARPGRPLITSKVAD
LPHYWGQGT LVTVSS (SEQ ID NO:10) or at least about 90% sequence similarity thereto.

12. The compound of Claim 1, wherein the amino acid sequence is:
EVQLLES G G G L V Q P G G S L R L S C A A S G R Y I D E T A V A W F R Q A P G K E R E F V A G I G G G V
D Q T Y Y A D S V K G R F T I S R D N S K N T L Y L Q M N S L R P E D T A V Y Y C A A R P G R P L I T S K V A D
L Y P Y W G Q G T L V T V S S (SEQ ID NO:11) or at least about 90% sequence similarity thereto.

13. The compound of Claim 1, wherein the amino acid sequence is:
EVQLLES G G G L V Q P G G S L R L S C A A S G R Y I D E T A V A W F R Q A P G K E R E F V A G I G G G V
D I T A Y A D S V K G R F T I S R D N S K N T L Y L Q M N S L R P E D T A V Y Y C A A R P G R P L I T S K V A D
L Y P Y W G Q G T L V T V S S (SEQ ID NO:12) or at least about 90% sequence similarity thereto.

14. The compound of Claim 1, wherein the amino acid sequence is:
EVQLLES G G G L V Q P G G S L R L S C A A S G R Y I D E T A V A W F R Q A P G K E R E F V A G I G G G V 3
D I T E Y A D S V K G R F T I S R D N S K N T L Y L Q M N S L R P E D T A V Y Y C A A R P G R P L I T S K V A D L
Y P Y W G Q G T L V T V S S (SEQ ID NO:13) or at least about 90% sequence similarity thereto.

15. The compound of Claim 1, wherein the amino acid sequence is:
EVQLLES G G G L V Q P G G S L R L S C A A S G R Y I D E T A V A W F R Q A P G K E R E F V A G I G G G V
D I T Q Y A D S V K G R F T I S R D N S K N T L Y L Q M N S L R P E D T A V Y Y C A A R P G R P L I T S K V A D
L Y P Y W G Q G T L V T V S S (SEQ ID NO:14) or at least about 90% sequence similarity thereto.

16. The compound of Claim 1, wherein the amino acid sequence is:
EVQLLES G G G L V Q P G G S L R L S C A A S G R Y I D E T A V A W F R Q A P G K E R E F V A G I G G G V
D I T S Y A D S V K G R F T I S R D N S K N T L Y L Q M N S L R P E D T A V Y Y C A A R P G R P L I T S K V A D L
Y P Y W G Q G T L V T V S S (SEQ ID NO:15) or at least about 90% sequence similarity thereto.

17. The compound of Claim 1, wherein the amino acid sequence is:
EVQLLES G G G L V Q P G G S L R L S C A A S G R Y I D E T A V A W F R Q A P G K E R E F V A G I G G G V
D I T T Y A D S V K G R F T I S R D N S K N T L Y L Q M N S L R P E D T A V Y Y C A A R P G R P L I T S K V A D L
Y P Y W G Q G T L V T V S S (SEQ ID NO:16) or at least about 90% sequence similarity thereto.

18. The compound of Claim 1, wherein the amino acid sequence is:
EVQLLES GGGLVQP GGSRLRLSCAASGRYIDETAVAWFRQAPGKEREFVAGIGGGV
DITYYADSVKGRFTISRDN SKNTLYLQMNSLRPEDTAVYYCAARPGKPLITSKVAD
LPHYWGQGTLVTVSS (SEQ ID NO:17) or at least about 90% sequence similarity thereto.

19. The compound of Claim 1, wherein the amino acid sequence is:
EVQLLES GGGLVQP GGSRLRLSCAASGRYIDETAVAWFRQAPGKEREFVAGIGGGV
DITYYADSVKGRFTISRDN SKNTLYLQMNSLRPEDTAVYYCAARPGQPLITSKVAD
LPHYWGQGTLVTVSS (SEQ ID NO:18) or at least about 90% sequence similarity thereto.

20. The compound of Claim 1, wherein the amino acid sequence is:
EVQLLES GGGLVQP GGSRLRLSCAASGRYIDETAVAWFRQAPGKEREFVAGIGGGV
DITYYADSVKGRFTISRDN SKNTLYLQMNSLRPEDTAVYYCAARPGSPLITSKVADL
PHYWGQGTLVTVSS (SEQ ID NO:19) or at least about 90% sequence similarity thereto.

21. The compound of Claim 1, wherein the amino acid sequence is:
EVQLLES GGGLVQP GGSRLRLSCAASGRYIDETAVAWFRQAPGKEREFVAGIGGGV
DITYYADSVKGRFTISRDN SKNTLYLQMNSLRPEDTAVYYCAARPGRELITSKVAD
LPHYWGQGTLVTVSS (SEQ ID NO:20) or at least about 90% sequence similarity thereto.

22. The compound of Claim 1, wherein the amino acid sequence is:
EVQLLES GGGLVQP GGSRLRLSCAASGRYIDETAVAWFRQAPGKEREFVAGIGGGV
DITYYADSVKGRFTISRDN SKNTLYLQMNSLRPEDTAVYYCAARPGRQLITSKVAD
LPHYWGQGTLVTVSS (SEQ ID NO:21) or at least about 90% sequence similarity thereto.

23. The compound of Claim 1, wherein the amino acid sequence is:
EVQLLES GGGLVQP GGSRLRLSCAASGRYIDETAVAWFRQAPGKEREFVAGIGGGV
DITYYADSVKGRFTISRDN SKNTLYLQMNSLRPEDTAVYYCAARPGRSLITSKVAD
LPHYWGQGTLVTVSS (SEQ ID NO:22) or at least about 90% sequence similarity thereto.

24. The compound of Claim 1, wherein the amino acid sequence is:
EVQLLES GGGLVQPGGSLRLSCAASGRYIDETAVAWFRQAPGKERE FVAGIGGGV
DITYYADSVKGRFTISRDN SKNTLYLQMNSLRPEDTAVYYCAARPGRPEITSKVAD
LPHYWGQGTLVTVSS (SEQ ID NO:23) or at least about 90% sequence similarity thereto.

25. The compound of Claim 1, wherein the amino acid sequence is:
EVQLLES GGGLVQPGGSLRLSCAASGRYIDETAVAWFRQAPGKERE FVAGIGGGV
DITYYADSVKGRFTISRDN SKNTLYLQMNSLRPEDTAVYYCAARPGRPGITSKVAD
LPHYWGQGTLVTVSS (SEQ ID NO:24) or at least about 90% sequence similarity thereto.

26. The compound of Claim 1, wherein the amino acid sequence is:
EVQLLES GGGLVQPGGSLRLSCAASGRYIDETAVAWFRQAPGKERE FVAGIGGGV
DITYYADSVKGRFTISRDN SKNTLYLQMNSLRPEDTAVYYCAARPGRPQITSKVAD
LPHYWGQGTLVTVSS (SEQ ID NO:25) or at least about 90% sequence similarity thereto.

27. The compound of Claim 1, wherein the amino acid sequence is:
EVQLLES GGGLVQPGGSLRLSCAASGRYIDETAVAWFRQAPGKERE FVAGIGGGV
DITYYADSVKGRFTISRDN SKNTLYLQMNSLRPEDTAVYYCAARPGRPTITSKVAD
LPHYWGQGTLVTVSS (SEQ ID NO:26) or at least about 90% sequence similarity thereto.

28. The compound of Claim 1, wherein the amino acid sequence is:
EVQLLES GGGLVQPGGSLRLSCAASGRYIDETAVAWFRQAPGKERE FVAGIGGGV
DITYYADSVKGRFTISRDN SKNTLYLQMNSLRPEDTAVYYCAARPGRPLITEKVAD
LPHYWGQGTLVTVSS (SEQ ID NO:27) or at least about 90% sequence similarity thereto.

29. The compound of Claim 1, wherein the amino acid sequence is:
EVQLLES GGGLVQPGGSLRLSCAASGRYIDETAVAWFRQAPGKGREFVAGIGGGV
DITYYADSVKGRFTISRDN SKNTLYLQMNSLRPEDTAVYYCATRPGRPLITSKVADL
PHYWGQGTLVTVSS (SEQ ID NO:28) or at least about 90% sequence similarity thereto.

30. The compound of Claim 1, wherein the amino acid sequence is:
EVQLLES G GGLVQP G GSLRLSCAASGRYIDETAVAWFRQAPGKGREFVAGIGGGV
DITYYADSVKGRFTISRDN SKNTLYLQMNSLRPEDTAVYYCAARQGRPLITSKVAD
LPHYWGQGTLVTVSS (SEQ ID NO:29) or at least about 90% sequence similarity thereto.

31. The compound of Claim 1, wherein the amino acid sequence is:
EVQLLES G GGLVQP G GSLRLSCAASGRYIDETAVAWFRQAPGKGREFVAGIGGGV
DITYYADSVKGRFTISRDN SKNTLYLQMNSLRPEDTAVYYCATRQGRPLITSKVAD
LPHYWGQGTLVTVSS (SEQ ID NO:30) or at least about 90% sequence similarity thereto.

32. The compound of Claim 1, wherein the amino acid sequence is:
EVQLLES G GGLVQP G GSLRLSCAASGRYIDETAVAWFRQAPGKGREFVAGIGGGV
DITYYADSVKGRFTISRDN SKNTLYLQMNSLRPEDTAVYYCAARPGRPLITSQVAD
LPHYWGQGTLVTVSS (SEQ ID NO:31) or at least about 90% sequence similarity thereto.

33. The compound of Claim 1, wherein the amino acid sequence is:
EVQLLES G GGLVQP G GSLRLSCAASGRYIDETAVAWFRQAPGKGREFVAGIGGGV
DITYYADSVKGRFTISRDN SKNTLYLQMNSLRPEDTAVYYCAARPGRPLITSKQAD
LPHYWGQGTLVTVSS (SEQ ID NO:32) or at least about 90% sequence similarity thereto.

34. The compound of Claim 1, wherein the amino acid sequence is:
EVQLLES G GGLVQP G GSLRLSCAASGRYIDETAVAWFRQAPGKGREFVAGIGGGV
DITYYADSVKGRFTISRDN SKNTLYLQMNSLRPEDTAVYYCAARPGRPLITSKVAEL
YPYWGQGTLVTVSS (SEQ ID NO:33) or at least about 90% sequence similarity thereto.

35. The compound of Claim 1, wherein the amino acid sequence is:
EVQLLES G GGLVQP G GSLRLSCAASGRYIDETAVAWFRQAPGKGREFVAGIGGGV
DITYYADSVKGRFTISRDN SKNTLYLQMNSLRPEDTAVYYCAARPGRPLITSKVASL
YPYWGQGTLVTVSS (SEQ ID NO:34) or at least about 90% sequence similarity thereto.

36. The compound of Claim 1, wherein the amino acid sequence is:
EVQLLES GGG LVQP GGS LRLSCAASGRYIDETAVAWFRQAPGKGREFVAGIGGGV
DITYYADSVKGRFTISRDN SKNTLYLQMNSLRPEDTAVYYCAARPGRPLITSKQ AEL
YPYWGQGTLVTVSS (SEQ ID NO:35) or at least about 90% sequence similarity thereto.

37. The compound of Claim 1, wherein the amino acid sequence is:
EVQLLES GGG LVQP GGS LRLSCAASGRYIDETAVAWFRQAPGKGREFVAGIGGGV
DITYYADSVKGRFTISRDN SKNTLYLQMNSLRPEDTAVYYCAARPGRPLITSKQASL
YPYWGQGTLVTVSS (SEQ ID NO:36) or at least about 90% sequence similarity thereto.

38. The compound of Claim 1, wherein the amino acid sequence is:
EVQLLES GGG LVQP GGS LRLSCAASGRYIDETAVAWFRQAPGKGREFVAGIGGGV
DITYYADSVKGRFTISRDN SKNTLYLQMNSLRPEDTAVYYCAARPGRPLITSKVAD
LYPYWGQGTLVTVSS (SEQ ID NO:37) or at least about 90% sequence similarity
thereto.

39. The compound of Claim 1, wherein the amino acid sequence is:
EVQLLES GGG LVQP GGS LRLSCAASGRYIDETAVAWFRQAPGKGREFVAGIGGGV
DITYYADSVKGRFTISRDN SKNTLYLQMNSLRPEDTAVYYCGARPGRPLITSKVAD
LYPYWGQGTLVTVSS (SEQ ID NO:124) or at least about 90% sequence similarity
thereto.

40. The compound of Claim 1, wherein the amino acid sequence is:
EVQLLES GGG LVQP GGS LRLSCAASGRYIDETAVAWFRQAPGKGREFVAGIGGGV
DITYYADSVKGRFTISRDN SKNTLYLQMNSLRPEDTAVYYCGARPGRPLITSKVAD
LYPYWGQGTLVTVSSPP (SEQ ID NO:125) or at least about 90% sequence similarity
thereto.

41. The compound of Claim 1, wherein the amino acid sequence is:

EVQLLESGGGLVQPGGSLRLSCAASGRYIDETAVAWFRQAPGKGREFVAGIGGGV
DITYYADSVKGRFTISRDN SKNTLYLQMNSLRPEDTAVYYCGTRPGRPLITSKVADL
YPYWGQGTLVTVSSPP (SEQ ID NO:126) or at least about 90% sequence similarity
thereto.

42. A compound comprising a structure of:

M-X₁,
X₁-M,
M-L₁-X₁,
X₁-L₁-M,
X₁-M-X₂,
X₂-M-X₁,
X₁-L₁-M-X₂,
X₂-L₁-M-X₁,
X₁-M-L₁-X₂,
X₂-M-L₁-X₁,
X₁-L₁-M-L₂-X₂,
X₂-L₁-M-L₂-X₁,
M-L₁-X₁-L₂-X₂,
M-L₁-X₂-L₂-X₁,
X₁-L₂-X₂-L₁-M, or
X₂-L₂-X₁-L₁-M,

wherein M is an amino acid sequence selected from the group consisting of SEQ ID
NOS:1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26,
27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 124, 125 and 126, or at least about 90% sequence
similarity thereto,

wherein X₁ is a first biotherapeutic,

wherein X₂ (if present) is a second biotherapeutic,

wherein L₁ (if present) is a first linker having an amino acid sequence selected from the group consisting of (GGGGQ)_n (SEQ ID NO:38), (GGGQ)_n (SEQ ID NO:39), (GGGS)_n (SEQ ID NO:40), (PGPQ)_n (SEQ ID NO:41), (PGPA)_n (SEQ ID NO:42), (GGGG(AP)_nGGGG) (SEQ ID NO:43), (GGE)_n (SEQ ID NO:44), (GGGGGE)_n (SEQ ID NO:45), (GGK)_n (SEQ ID NO:46), (GGGGK)_n (SEQ ID NO:47), (GGGG(EP)_nGGGG) (SEQ ID NO:48), (GGGG(KP)_nGGGG) (SEQ ID NO:49), (PGPE)_n (SEQ ID NO:50), and (PGPK)_n (SEQ ID NO:51), wherein n can be from 1 to 10, and

wherein L₂ (if present) is a second linker having an amino acid sequence selected from the group consisting of SEQ ID NOS:64 and 65, or at least about 90% sequence similarity thereto.

43. The compound of Claim 42, wherein the first biotherapeutic is a peptide, protein or oligomer.

44. The compound of Claim 42 or 43, wherein the second biotherapeutic is a peptide, protein or oligomer.

45. The compound of any one of Claims 42 to 44, wherein the first biotherapeutic and the second biotherapeutic are distinct from one another.

46. The compound of any one of Claims 42 to 45, wherein L₁ has an amino acid sequence selected from the group consisting of SEQ ID NOS:52 to 63, or at least about 90% sequence similarity thereto.
47. The compound of any one of Claims 42 to 46, wherein the first biotherapeutic and/or the second biotherapeutic is a peptide or protein such as an antibody (Ab) or fragment thereof, adrenocorticotrophic hormone (ACTH), growth/differentiation factor 15 (GDF15), incretin (INC), insulin (INS), interleukin (IL), ciliary neurotrophic factor (CNTF), neuregulin (NRG) or relaxin (RLN).
48. The compound of Claim 47, wherein the peptide or protein is an INC.
49. The compound of Claim 48, wherein the INC is selected from the group consisting of glucose-dependent insulintropic peptide (GIP), glucagon-like peptide-1 (GLP-1) and GIP/GLP-1.
50. The compound of Claim 47, wherein the peptide or protein is IL, and wherein the IL is IL-2.
51. The compound of Claim 47, wherein the peptide or protein is NRG, and wherein the NRG is neuregulin 1 (NRG1) or neuregulin 4 (NRG4).
52. The compound of Claim 47, wherein the peptide or protein is RLN, and wherein the RLN is relaxin 2 (RLN2).
53. A pharmaceutical composition comprising a compound of any one of Claims 42 to 52 and a pharmaceutically acceptable buffer.

54. The pharmaceutical composition of Claim 53 further comprising an additional therapeutic agent.
55. A method treating an individual, the method comprising the step of:
administering to the individual an effective amount of a compound of any one of Claims 42 to 52 or a pharmaceutical composition of any one of Claims 53 to 54.
56. A compound of any one of Claims 1 to 41 for use in extending the $t_{1/2}$ of a biotherapeutic.
57. A compound of any one of Claims 42 to 52 for use in a therapy.
58. A compound of any one of Claims 42 to 52 for use in treating cardiovascular, neurological, immunological, metabolic, oncological, psychological, pulmonary and/or renal conditions, diseases and/or disorders.
59. Use of a compound of any one of Claims 42 to 52 for manufacturing a medicament.
60. A compound comprising any one of SEQ ID NOS:100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117 or 118, or at least about 90% sequence similarity thereto.
61. A pharmaceutical composition comprising a compound of Claim 60 and a pharmaceutically acceptable buffer.
62. The pharmaceutical composition of Claim 61 further comprising an additional therapeutic agent.

63. A method treating an individual, the method comprising the step of:
administering to the individual an effective amount of a compound of Claim 60 or a pharmaceutical composition of any one of Claims 61 to 62.
64. A compound of Claim 60 for use in a therapy.
65. A compound of Claim 60 for use in treating cardiovascular, neurological, immunological, metabolic, oncological, psychological, pulmonary and/or renal conditions, diseases and/or disorders.
66. Use of a compound of Claim 60 for manufacturing a medicament.