

COMPOSITIONS COMPRISING GPCR BASED CHIMERIC ANTIGEN RECEPTOR AND METHODS OF USE

Field

- 5 The invention is in the field of therapeutics, specifically in the field of targeted drug delivery.

Background

- 10 Chimeric antigen receptors (CARs) are synthetic receptors that combine the high affinity binding ability of antibodies with internal signalling ITAM domains of a T cell receptor. They have the ability to redirect the specificity of immune cells, typically T cells, towards targets of interest, which are typically found on cancer cells. CARs are designed to enhance the ability of the immune system to recognize and eliminate
15 cancer cells.

- ITAM domains are short sequences found in the cytoplasmic tails of various immune receptors, including the T cell receptor and B cell receptor. In CARs, ITAM domains are incorporated into the intracellular signalling domain to initiate T cell activation upon
20 antigen recognition. When the CAR binds to the target antigen on cancer cells, the ITAM domains become phosphorylated, triggering a cascade of signalling events that activate the T cell. This activation ultimately leads to the killing of cancer cells by the T cell.

- 25 The incorporation of ITAM domains into CARs allows for the bypassing of several steps involved in the normal immune response against cancer cells. CAR T cells can recognize and kill cancer cells independently of major histocompatibility complex (MHC) presentation, which is the usual mode of antigen recognition by T cells. This feature makes CAR T cell therapy applicable to a wide range of cancer types.

- 30 CAR T cell therapy has shown remarkable success in the treatment of certain hematological malignancies, such as acute lymphoblastic leukemia and certain types of lymphoma. Ongoing research is focused on improving the efficacy and safety of CAR T cell therapy and extending its application to solid tumors.

- 35 Other examples of chimeric receptors incorporating ITAM signalling domains are described in WO 2022/263824, which presents a range of chimeric ITAM-based receptors which can be used to redirect the natural degranulation response of platelets.

Degranulation typically occurs in response to binding of receptors to markers of physical damage, such as collagen and to trigger thrombogenesis. WO 2022/263824 describes the use of the Chimeric Platelet Receptors to redirect this response to instead to deliver therapeutic cargo to targets of interest – for example an anti-cancer agent to a tumour. WO 2022/263824 also describes how the platelets can be engineered to express a cargo of interest from within the precursor megakaryocyte, or that exogenous cargo can be directly loaded into a precursor megakaryocyte or platelet. WO 2022/263824 also describes platelets that have been engineered to remove or reduce thrombogenicity – i.e. to prevent the platelet from degranulating in response to the natural signals that will be found in a subject such as a patient in need of treatment. In this way WO 2022/263824 describes a complete system that can comprise non-thrombogenic platelets (or Synlets) and that which also comprise the chimeric receptor, and that can be used to deliver various types of cargo to target sites.

There are however potential disadvantages with the ITAM based approach of these systems. ITAM receptors give an on-off response with little nuance in the responses that can be obtained relative to the strength of the target signal. In addition ITAM receptors tend to initiate signalling via the activation of a single kinase, such as Syk or Zap70, giving little variation in the downstream effects that target binding can achieve. Finally, stochastic ITAM signalling can occur in the absence of target binding by virtue of receptor aggregation on the cell surface for example via overexpression of the receptor, leading to a lack of sensitivity in signalling, i.e. a high background rate of signalling.

The present invention provides receptors that address these issues with known chimeric receptors.

Summary of invention

G protein-coupled receptors (GPCRs) are a large family of transmembrane receptors that play a crucial role in intracellular signaling pathways. Upon activation by their ligands (such as neurotransmitters, hormones, or other signaling molecules), GPCRs undergo a conformational change that leads to the activation of intracellular signaling pathways. Upon activation of the GPCR, the G protein undergoes a conformational change (i.e. from an inactive conformation to an active conformation) that allows it to exchange GDP for GTP, leading to the activation of downstream effectors. Accordingly, by “activation of an aGPCR” herein we include the meaning of initiation of downstream signalling via the corresponding G protein(s).

Adhesion GPCRs (aGPCRs) are a particular class of GPCR that, in addition to the standard seven transmembrane domain (7TM) common to all GPCRs, have an extracellular domain that comprises a domain (the GPCR Autoproteolysis Inducing (GAIN) domain) that in most cases causes the constitutive autoproteolytic cleavage of a site called the GPS site which is located proximal to the first transmembrane helix. The GAIN domain typically ends 7-18 residues before the start of the first transmembrane span of the 7TM bundle. Cleavage of the site results in the production of a N terminal fragment (NTF) and a C terminal fragment (CTF – comprising the 7TM).
A dense network of hydrogen bonds within the GAIN domain allows the NTF and CTF to remain noncovalently bound after self-proteolysis, which is considered to occur early during receptor biosynthesis in an intracellular compartment. The two-fragment holoreceptor is trafficked to the plasma membrane where it resides, poised for signaling.

The GAIN domain also harbours an agonistic peptide, called the “tethered agonist” or “Stachel sequence”. Autoproteolytic cleavage occurs N-terminal of this sequences, so the tethered agonist remains associated with the CTF. Following cleavage, the tethered agonist is still hidden due to the non-covalent association between the NTF and the CTF. However, mechanical forces exerted on this interaction due to ligand binding physically pulls the NTF from the CTF, exposing the tethered agonist peptide. The tethered agonist peptide then interacts with the 7TM domain, activating the aGPCR.

Some aGPCRs are unable to undergo proteolytic cleavage, but are still activated by binding to the respective target.

Despite the complex structure and inter-molecular interactions of the aGPCR receptors, the inventors have surprisingly produced and demonstrated functionality of chimeric aGPCRs in which the extracellular ligand binding domain has been modified or substituted to target a new ligand, but which still triggers appropriate intracellular signalling upon ligand binding. In this way the sensing repertoire of the chimeric aGPCR is expanded.

One potential advantage of chimeric aGPCR signalling over ITAM based chimeric receptor signalling is that it allows for a more sensitive and dynamic response to extracellular stimuli since GPCRs can activate multiple downstream effectors, including G proteins, β -arrestins, and various kinases. For example, GPCRs that interact with a $G_{\alpha q}$ subunit (also called the called $G_{\alpha q/11}$ or $G_{\alpha q/11/14/15}$ subunit) activates beta-type

phospholipase C (PLC β); a GPCR that interacts with a G $_{\alpha s}$ subunit stimulates the cAMP-dependent pathway by activating adenylyl cyclase and increasing intracellular cAMP levels; and a GPCR that interacts with a G $_{\alpha i/o}$ subunit inhibits adenylyl cyclase and decreases intracellular cAMP.

5

In contrast, ITAM signalling typically involves the activation of a single kinase, such as Syk or Zap70, which leads to a more limited set of downstream effects. Complex signalling pathways with multiple layers of regulation can be designed using GPCRs, or a combination of GPCRs and ITAMs.

10

GPCRs can be regulated by a variety of mechanisms, including receptor desensitization, internalization, and degradation, as well as by feedback mechanisms involving downstream effectors. This allows cells to modulate the strength and duration of GPCR signalling in response to changing conditions.

15

In contrast, the strength of the response of ITAM based signalling depends primarily on the number of activated receptors, and acts more in a "on or off" manner.

Since aGPCRs are primarily activated by mechanical forces, and since cells such as cells found in the circulatory system (e.g. platelets and engineered platelets) are typically in constant motion, they are able to exert mechanical forces on any target to which they bind, expanding the repertoire of ligands which can activate aGPCRs. Expressing these chimeric aGPCRs in cells, platelets and engineered platelets provides an opportunity for increased control and sophistication in the activation of intracellular (or intraplatelet/intra-engineered platelet) signalling and downstream effects, such as in some cases release of a therapeutic cargo. Nonlimiting examples include T cells, B cells, NK cells, red blood cells, macrophages, megakaryocyte, pluripotent cells or stem cells.

20
25
30 The invention provides chimeric aGPCR receptors, compositions and methods that use the chimeric aGPCRs.

The chimeric aGPCRs described herein can be used instead of, or in addition, to the chimeric ITAM based receptors described in WO 2022/263824 in the context of a platelet, or a non-thrombogenic platelet as described therein. The chimeric aGPCRs described herein may also be used in a different context, for example in a different cellular context. For example the chimeric aGPCRs might be expressed from within a T cell and used in a similar way to current CAR-T therapies.

By chimeric we include the meaning that various portions or domains of the aGPCR are different to those that would be found together in the same aGPCR in nature. In some embodiments the chimeric nature comes from one or more substitutions, deletions or insertions into the amino acid sequence of the aGPCR, relative to the wild type receptor. In some embodiments the chimeric nature of the aGPCR comes from the substitution of a whole domain of the aGPCR with a different domain, for example a different wildtype domain that is found in another aGPCR, or a different wildtype domain that comprises one or more mutations corresponding to that wildtype domain, or is substituted with a domain that is not from any aGPCR. For example the chimeric aGPCR may comprise a target binding domain that is not the same as any natural aGPCR target binding domain. For example the target binding domain may be an antibody or antibody fragment thereof, or ligand capable of binding to a particular receptor.

The chimeric aGPCRs described herein may also be alternatively termed redirected aGPCRs, since the response elicited by the aGPCR upon target binding is redirected to a new target by virtue of substitution or mutation of the target binding domain of the aGPCR.

The chimeric aGPCRs may also be considered to be Synthetically Agonised G-protein Adhesion Receptors (or abbreviated to SAGA receptors) since in preferred embodiments the aGPCRs have been designed to be activated by the non-native target and so can be considered to be synthetically agonised.

The skilled person will appreciate that aGPCRs, like standard GPCRs, comprise a 7 transmembrane domain (7TM) and an intracellular tail domain. The intracellular tail domain is typically at the C-terminal end of the protein and so in preferred instances may be termed a C-terminal tail. Intracellular signalling is transduced from binding of the target to the aGPCR through the intracellular interaction of different G-proteins with the intracellular loops (ICL), of which there are 3 in a 7TM, and/or the intracellular tail domain (or the C-terminal tail domain). G-proteins (such as beta-arrestin 1/2) can interact with **just** the C-terminal tail domain alone (i.e. rather than also interacting with the three ICLs) to effect signalling, or can interact with one, two or all three of the intracellular loops of the 7TM. See Figure 10 which depicts the 7TM and intracellular loops of a GPCR (note the extracellular domains of the depicted protein are those of a GPCR, not an aGPCR). Accordingly, the C-terminal tail and the three

ICLS can collectively be termed the "intracellular signalling domain", even though the residues that make up that domain are not contiguous in sequence.

Accordingly, in a first aspect the invention provides a chimeric adhesion G-Protein

5 Coupled Receptor (chimeric aGPCR) comprising:

- a) an intracellular tail domain, optionally a C-terminal tail;
- b) a seven-transmembrane domain (7TM) that comprise three intracellular loops (ICLs); and
- 10 c) an extracellular domain comprising:
 - (i) a target binding domain that is heterologous to the intracellular tail domain;
 - (ii) a GPCR autoproteolysis-inducing domain (GAIN domain) that
 - 15 optionally comprises a tethered agonist peptide.

The chimeric aGPCR may also further comprise:

- (iii) a linker, optionally wherein the linker is in the extracellular domain, for
- 20 example is between the GAIN domain and the target binding domain.

As mentioned above, some aGPCRs do not require cleavage of the GPCR-proteolytic site (GPS) site for activation. Accordingly in some embodiments of the chimeric aGPCR the GAIN domain does not comprise a GPS. In some embodiments the GAIN domain

25 comprises a GPS but the GPS is an uncleavable GPS, for example the GPS is not capable of autoproteolysis. In other, more usual embodiments the GPS is cleavable. The GPS is in preferred instances cleavable by the GAIN domain. In some embodiments the GPS is constitutively cleaved by the GAIN domain. In some embodiments the GPS is cleaved *in vitro*. In some embodiments the GPS is cleaved when expressed by a cell,

30 for example when expressed by eukaryotic cell such as a mammalian cell such as a human cell; or a prokaryotic cell such as a bacterial cell. In some embodiments the cleavage is constitutive cleavage.

In some embodiments the extracellular domain comprises a GAIN domain that is not

35 cleavable by autoproteolysis but also comprises an additional protease site that has been introduced or engineered into the protein. The protease site is arranged so that following proteolysis, the aGPCR becomes activated. In some embodiments the chimeric aGPCR becomes activated upon cleavage of the protease site in the absence

of binding of the target binding domain to the target. In some embodiments, the chimeric aGPCR becomes activated upon cleavage of the protease site only and binding of the target binding domain to the target. Such embodiments where the chimeric aGPCR comprises an additional protease site that has been introduced or engineered into the protein have particular uses in sensing and detection of particular proteases. In particular embodiments the protease site is a site that can be cleaved by, for example, a protease that is expressed in the tumour microenvironment. In this way, the chimeric aGPCR would only become activated in the presence of the corresponding protease. This can be used to a) determine whether a particular protease is present in a particular environment such as the tumour microenvironment; and b) can help to reduce off target effects in embodiments where the chimeric aGPCR is activated only when the protease site is cleaved by the appropriate protease and where there is simultaneous binding of the target binding domain to the target. Tumours are known to express many different types of proteases, for example the cysteine proteases that include cathepsins, caspases and calpains; the metallo proteases, aspartate proteases, serine proteases and threonine proteases. Any one or more of the corresponding proteolytic cleavage sites may be present in the extracellular domain of some embodiments of the chimeric aGPCR.

The GPS site is typically positioned within the GAIN domain N-terminal of the tethered peptide agonist, such that following cleavage the tethered peptide agonist remains with the C-terminal fragment.

As described above, for most aGPCRs, following cleavage of the GPS non-covalent interactions between the C-terminal fragment (CTF) and the N-terminal fragment (NTF) keep the two fragments in close association, and prevent receptor activation in the absence of external forces which may generated when the receptor binds the target and which may act to perturb the non-covalent interactions between the CTF and NTF.

Accordingly in some embodiments of the chimeric aGPCR cleavage of the GPS motif produces an N-terminal fragment (NTF) and a C-terminal fragment (CTF), and the NTF and the CTF remain associated with one another via non-covalent interactions. In some embodiments the CTF and NTF remain associated via non-covalent interactions when present in a lipid membrane, for example when the 7TM is present in a lipid membrane, such as the lipid membrane of a cell, platelet, or engineered platelet. To be clear, the target binding domain that is heterologous to the intracellular tail domain is part of the NTF.

In some embodiments association of the NTF and the CTF prevents the tethered peptide agonist from interacting with the 7TM domain and so prevents activation of intracellular signalling.

- 5 In all embodiments the chimeric aGPCR is inactive in the absence of binding to the target. By inactive we include the meaning of not initiating the corresponding intracellular signalling cascade relevant to the particular intracellular signalling domain (which, as mentioned elsewhere, is comprised of the 3 ICLs of the 7TM and/or the intracellular tail domain, e.g. the C-terminal tail). By inactive we also include the
- 10 meaning of a basal level of activation. GPCRs exhibit different basal activity levels that depend on the individual characteristics of each receptor. See for example Vizurraga et al 2020 J Biol Chem 295: 14065-14083. Basal activity is one state that GPCRs occupy within a dynamic energy landscape of active and inactive conformations. The skilled person will appreciate that there is often some "bleed through" in any biological
- 15 system, and that even in the absence of the relevant target, there may be some low level of receptor activation and subsequent intracellular signalling due to the low level of switching between inactive and active conformations in the absence of the target. However the skilled person will also recognise a difference in the level of signalling from an unactivated (but basal) level of signalling versus the level of signalling obtained
- 20 in the presence of the relevant target.

By inactive we also include the meaning that the chimeric aGPCR is in the inactive conformation. Accordingly the chimeric aGPCR shows an increased level of intracellular signalling when bound to the target versus when not bound to the target. Accordingly

25 in some embodiments, the chimeric aGPCR:

- a) is inactive in the absence of binding of the target binding domain to the target;
- b) predominantly occupies the inactive conformation in the absence of binding of the target binding domain to the target; and/or
- 30 c) remains in a basal activity state in the absence of binding of the target binding domain to the target.

The skilled person can readily test for this. For example a receptor which is constitutively active will show a high level of intracellular signalling whether the target

35 is present or not; and a receptor that is incapable of being activated by target binding will show the same absent or basal level of intracellular signalling whether the target is present or not.

Suitable methods to determine the activity of the receptor are described in the examples, for example the functional assay of Example 2 and the agitation method in Example 3.

- 5 In instances where the chimeric aGPCR is a receptor that comprises a cleavable GPS site and cleavage of the GPS produces a NTF and a CTF that remain associated via non-covalent interactions, the aGPCR may be activated by target binding resulting in either the physical removal the NTF from the CTF allowing activation of the intracellular domain (the "rupture" mechanism); or modulation of the non-covalent interactions
10 between the NTF and CTF allowing the CTF to be activated – i.e. the NTF and CTF remain associated in this context but conformational changes due to the application of the force allows activation of the receptor (the "tuneable" mechanism).

In some embodiments binding of the target binding domain to the target causes
15 mechanical forces that are exerted on the NTF/CTF non-covalent interaction.

In some embodiments the mechanical forces are able to:

- a) modulate the non-covalent interactions between the NTF and CTF and
20 activate intracellular signalling, for example by allowing the tethered peptide agonist to interact with the 7TM and activate intracellular signalling; or
b) rupture the non-covalent interactions between the NTF and CTF and activate intracellular signalling, for example by exposing the tethered peptide agonist allowing it to interact with the 7TM and activate intracellular signalling.

25 In some embodiments where the chimeric aGPCR is a receptor that does not comprise a cleavable GPS site and cleavage of the GPS does not occur, binding of the target binding domain to the target causes perturbation of the conformation of the receptor activating intracellular signalling.

30 As discussed elsewhere herein, in some embodiments it is important that mechanical forces are exerted on the receptor by target binding. For example in some embodiments where the GPS has been cleaved, target binding should exert sufficient mechanical forces so as to rupture the non-covalent interactions between the NTF and
35 CTF; or so as to modulate those interactions sufficiently so as to allow activation of intracellular signalling. These forces may be triggered by, in some instances, the target being a "fixed" target. By a "fixed" target in some embodiments we include the meaning of a target that is fixed to a physical structure for example within the body,

for example is present on a blood vessel, epithelial layer, or a tumour for example. In the same or different embodiments we include the meaning that the target is fixed relative to the dynamics of the chimeric aGPCR, for example there may be some relative difference in motion between the aGPCR (which will typically be present in the plasma membrane of a platelet, engineered platelet or cell) and the target, such that when target binding occurs there is some force exerted on the receptor, for example a force that can rupture the NTF/CTF interaction or modulate the NTF/CTF interaction to trigger intracellular signalling; or where the GPS is not present or is not cleaved, a force sufficient to trigger intracellular signalling.

However, it is also considered that binding to any target will exert sufficient forces so as to result in intracellular signalling. For example the random Brownian motion of the receptor present in the plasma membrane relative to the target is expected to exert sufficient forces to trigger receptor activation and intracellular signalling.

The force required to rupture or perturb the non-covalent interactions between the CTF and NTF can be designed by using a particular GAIN domain, and/or by engineering the sequence of the GAIN domain – see for example Dumas (Uncovering and engineering the mechanical properties of the adhesion GPCR ADGRG1 GAIN domain doi: <https://doi.org/10.1101/2023.04.05.535724>), and discussion elsewhere herein.

As will be apparent from the disclosure herein, in some contexts the chimeric aGPCR of the invention has a use in the context of platelets or engineered platelets as drug delivery vehicles. Upon target binding the platelet or engineered platelet, which may be loaded with or expressing a cargo such as a therapeutic cargo, degranulates, releasing the cargo in the vicinity of the target. Clearly then the chimeric aGPCRs described herein have a particular utility in the treatment of cancer, and in some embodiments the target binding domain is able to bind to, and elicit a response to, a target associated with cancer.

However, the chimeric aGPCRs described herein are considered to have a wider use beyond the context of platelets and drug delivery and cancer. For example in some embodiments the chimeric aGPCRs are considered to have use in the redirected immunotherapy field, in a similar way to chimeric antigen receptors are currently used in the context of T cells.

Accordingly, the target may be any target. For example the target may be any target towards which a specific binding activity can be arranged, for example an antigenic

target towards which an antibody or antigen binding fragment thereof can be generated, or a receptor/ligand arrangement. It will be apparent to the skilled person, and as discussed elsewhere herein, the target binding domain may be an antibody or antigen binding fragment thereof.

5

In some embodiments the target is a target that causes, upon binding to the target binding domain of the aGPCR:

a) modulation of the non-covalent interactions between the NTF and CTF and activation of intracellular signalling, for example by allowing the tethered peptide agonist to interact with the 7TM and activate intracellular signalling; or

10

b) rupture of the non-covalent interactions between the NTF and CTF and activation of intracellular signalling, for example by exposing the tethered peptide agonist allowing it to interact with the 7TM and activate intracellular signalling.

15

In some embodiments where the chimeric aGPCR is a receptor that does not comprise a cleavable GPS site and cleavage of the GPS does not occur, the target is a target that causes, upon binding to the target binding domain, perturbation of the conformation of the receptor activating intracellular signalling.

20

The target may be a protein or peptide, an antigenic protein or peptide, a carbohydrate or a lipid. The target may be any type of target towards which specific antibodies or antigen binding fragments can be made.

25

In some embodiments the target binding domain is able to bind to a target that is an endogenous target that is found on a tissue or subset of a tissue in the body of a subject or on a cell or in a particular location of a subject, for example cancer tissue or a cancer cell; and/or present on a cell surface, present on a physical structure, present on the inside of a blood vessel, present on an organ, present on a solid tumour, an anchored target, a target that has an opposite relative movement to the chimeric aGPCR when the chimeric aGPCR is present in the plasma membrane of a cell, platelet or engineered platelet, present on a cell surface or a tissue surface, a cellular matrix component, connective tissue, a carbohydrate, collagen, and/or immobilised on a solid substrate.

30

35

For example in some embodiments the target is an endogenous target that is found on a tissue or subset of a tissue in the body of a subject or on a cell or in a particular location of a subject, for example cancer tissue or a cancer cell; and/or is present on a cell surface, present on a physical structure, present on the inside of a blood vessel,

present on an organ, present on a solid tumour, an anchored target, a target that has an opposite relative movement to the chimeric aGPCR when the chimeric aGPCR is present in the plasma membrane of a cell, platelet or engineered platelet, present on a cell surface or a tissue surface, a cellular matrix component, connective tissue, a carbohydrate, collagen, and/or immobilised on a solid substrate.

In some embodiments the target is only presented during one or more disease states, for example in some embodiments the target is a neoantigen that arises in a tumour cell; and/or is only present in significant amounts for example abnormal levels on a tissue or cell that does not normally express the target; and/or is only present in a localised manner during one or more disease states; and/or is an antigen associated with a disease, disorder or condition, for example a tumour neoantigen or a tumour specific antigen.

In some embodiments the target binding domain comprises a peptide associated with autoimmunity, for example:

a peptide or portion of any one or more of the following proteins: MOG, GAD65, MAG, PMP22, TPO, VGKC, PLP, AChR, TRIB2, NMDA, GluR, GAD2, ARMC9, CYP21A2, CASR, NASP, insulin, TSHR, thyroperoxidase, asialoglycoprotein receptor, CYP2D6, LF, TTG, H/K ATP-ase, Factor XIII, Beta2-GPI, ITGB2, G-CSF, GP IIb/IIIa, COLII, FBG beta alpha, MPO, CYO, PRTN3, TGM, COLVII, COIL, DSG1, DSG3, SOX10, 70SNRNP70, SAG and $\alpha 3(\text{IV})\text{NC1}$ collagen; or

a peptide or portion that has at least 75%, 80%, 85%, 90%, 92%, 94%, 96%, 98% or 100% sequence identity to any one or more of the following proteins: MOG, GAD65, MAG, PMP22, TPO, VGKC, PLP, AChR, TRIB2, NMDA, GluR, GAD2, ARMC9, CYP21A2, CASR, NASP, insulin, TSHR, thyroperoxidase, asialoglycoprotein receptor, CYP2D6, LF, TTG, H/K ATP-ase, Factor XIII, Beta2-GPI, ITGB2, G-CSF, GP IIb/IIIa, COLII, FBG beta alpha, MPO, CYO, PRTN3, TGM, COLVII, COIL, DSG1, DSG3, SOX10, 70SNRNP70, SAG and $\alpha 3(\text{IV})\text{NC1}$ collagen.

The present invention is considered to be particularly useful in the context of an engineered platelet, such as those described in WO 2022/263824. In some embodiments the platelet has been engineered to have reduced thrombogenicity or to be non-thrombogenic. In these instances, where the chimeric aGPCR is present in the membrane of a platelet or an engineered platelet, binding of the target binding domain to the target:

- a) results in degranulation of the platelet or engineered platelet;
- b) results in the release of contents from the platelet or engineered platelet;
- c) results in the presence of intraplatelet contents on the plasma membrane of the platelet or engineered platelet;
- d) results in the release of extracellular vesicles via blebbing from the plasma membrane; and/or
- e) results in a change of shape of the platelet or engineered platelet from a biconcave disk to fully spread cell fragments.

10

Some exemplary targets are described below – for example in some embodiments, the target binding domain binds to a target that is:

- an endogenous target;
- an endogenous target that is found on a tissue or subset of a tissue in the body of a subject or on a cell or in a particular location of a subject, for example cancer tissue or a cancer cell;
- present on a cell surface;
- present on a physical structure for example inside the body or coated on to the walls of a well of a plate;
- present on the inside of a blood vessel;
- present in plasma or blood of a subject;
- present on an organ;
- present on a solid tumour
- an anchored target;
- target that has an opposite relative movement to the chimeric aGPCR when the chimeric aGPCR is present in the plasma membrane of a cell, platelet or engineered platelet;
- a cellular matrix component;
- is or is present on connective tissue
- a carbohydrate;
- collagen; and/or
- immobilised on a solid substrate
- present on a cancer tissue or a cancer cell;
- only presented during one or more disease states, for example in some embodiments the target is a neoantigen that arises in a tumour cell;

only present in significant amounts for example abnormal levels on a tissue or cell that does not normally express the target and/or is only present in a localised manner during or more disease states;
 an antigen associated with a disease, disorder or condition, for example a tumour neoantigen or a tumour specific antigen;
 an artificial or exogenous target;
 CD19;
 CD276;
 IL2;
 10 KLK;
 Amyloid;
 a Notch receptor;
 OLR1;
 MadCAM1;
 15 a cytokine receptor;
 collagen;
 not collagen;
 a designer drug;
 a drug that has been designed using DREADD;
 20 a protein selected from Table 2 on pages 23-31 of PCT/GB2020/053247 which is hereby incorporated by reference; and/or
 an autoimmune B cell.

D276 is an identified immunoregulatory protein which is overexpressed in tumor tissues (see Zhao et al 2022 Journal of Hematology and Oncology 15: article 153).
 25 <https://jhoonline.biomedcentral.com/articles/10.1186/s13045-022-01364-7>)

Mucosal vascular addressin cell adhesion molecule 1 (MAdCAM-1) proteins are cell adhesion molecules expressed on the endothelium in mucosa. The protein guides the specific homing of lymphocytes into mucosal tissues. Antibody targeting MAdCAM1 has
 30 been developed for the treatment of ulcerative colitis and Crohn's disease (see Wang et al 2020 J Clin Pharmacol 60: 903-914).

The target binding domain preferably binds to an endogenous target as described herein. In some embodiments the target binding domain binds to an artificial or
 35 exogenous target.

In some embodiments the target is not a soluble target.

Although typically the chimeric aGPCR will be designed so as to have a target binding domain that binds directly to the ultimate target, or the desired target, i.e. the target towards which a particular response is to be directed, such as to a neoantigen present on a cancer cell, in some embodiments the aGPCR may be considered to be a universal aGPCR which is designed so as to bind to an intermediate adaptor protein or peptide, for example that itself comprises a peptide tag and a target binding domain that can bind to the ultimate target or the desired target i.e. any target towards which the aGPCR is intended to be targeted to. The premise is that the subject is administered an intermediate adaptor protein or peptide that itself binds to the targets as described herein (herein termed the ultimate target binding domain, in the context of the intermediate adaptor protein or peptide), such as a cancer antigen, but which also comprises a portion, such as a peptide tag, that can be bound by the target binding domain of the chimeric aGPCR. In this way the target is essentially coated with the intermediate adaptor protein or peptide allowing the chimeric aGPCR described herein to bind to the cell or target (i.e. the ultimate target) via the intermediate adaptor protein or peptide. Alternatively, a complex is pre-formed prior to administration between the chimeric aGPCR that has a target binding domain that can bind to a tag (i.e. a tag binding domain), and the intermediate adaptor protein or peptide that comprises the ultimate target binding domain, so that the pre-formed complex is administered to a patient. An advantage of this system is that the aGPCR does not necessarily have to be redesigned for each new target, and instead a new intermediate target protein or polypeptide can be designed to the relevant target and incorporating the corresponding tag peptide. Such systems in the context of ITAM containing Chimeric Antigen Receptors have been proposed by Janh Hwan Cho *et al* in 2018 Cell 173:1426-1438.

In these instances the target binding domain of the chimeric aGPCR could be said to be an adaptor protein or peptide binding domain. Said adaptor protein or peptide comprises a portion that is able to bind to the target binding domain of the chimeric aGPCR, and a portion that is a target binding domain. Preferences for the target binding domain of the adaptor protein or peptide are as described elsewhere herein in relation to the target binding domain of the chimeric aGPCR.

Preferably the intermediate adaptor protein or peptide comprises a portion that is a peptide tag, and the target binding domain of the chimeric aGPCR binds to the peptide tag

It will be appreciated that the invention also provides a complex comprising:

a) a chassis of the invention that expresses one or more chimeric aGPCR of the invention; and

b) an intermediate adaptor protein or peptide,

5

wherein the intermediate adaptor protein or peptide comprises an ultimate target binding domain and a tag, optionally a peptide tag,

and where the target binding domain of the chimeric aGPCR of the invention is a tag binding domain that can bind to the tag of the intermediate adaptor protein or peptide

10 and wherein the ultimate target binding domain of the intermediate adaptor polypeptide or protein is able to simultaneously bind to the ultimate target and to the chimeric aGPCR of the invention.

Preferences for the ultimate target binding domain are as described herein for the
15 target binding domain of the chimeric aGPCR.

The skilled person will appreciate that when any of the chassis as described herein are described as expressing one or more chimeric aGPCRs, it is intended to include the meaning that the chassis expresses the one or more chimeric aGPCRs on the cell
20 surface i.e. in the plasma membrane as is usual for an aGPCR.

Accordingly, the contact between the chassis that expresses the chimeric aGPCR and the intermediate adaptor protein occurs at the cell surface or platelet surface or engineered platelet surface, between the chimeric aGPCR present in the cell or platelet
25 surface, and the intermediate adaptor protein or peptide.

Accordingly in some embodiments the target to which the target binding domain of the chimeric aGPCR is a peptide tag. In preferred embodiments the peptide tag is expressed as part of the larger targeting peptide and is an integral part of the larger
30 targeting peptide i.e. in such embodiments the tagged intermediate adaptor protein or peptide is a single protein or peptide that comprises both the tag and the ultimate target binding domain.

In other embodiments the target binding domain of the chimeric aGPCR does not bind
35 to a peptide tag.

Peptide tags as described herein are typically short peptide sequences (i.e. sequences of amino acids) well known in the molecular biology field, where it is routine to express

a peptide or polypeptide sequence of interest wherein the sequence has been extended to include a relatively short additional sequence, encoding the tag. Examples of suitable peptide tags include the FLAG-tag, V5-tag, Myc-tag, HA-tag, Spot-tag, T7-tag, NE-tag and a leucine-zipper (Hwan et al 2018 Cell 173: 1426-1438).

5

By peptide tag we also include the meaning of protein domains that are known to have interactions with the same or heterologous protein domains. For example in some embodiments the target binding domain of the aGPCR may comprise a leucine zipper domain. In this embodiment the intermediate adaptor protein or peptide may comprise an ultimate target binding domain, such as an scFv fused to a leucine zipper domain, allowing association between the chimeric aGPCR of the invention and the leucine zipper of the intermediate adaptor protein or peptide. Similarly, coiled-coil domains may be employed that form associations with one another (forming either homodimers or heterodimers) to allow association of the chimeric aGPCR and the ultimate target binding domain of the intermediate adaptor protein or peptide.

10
15

Although the tag is typically a peptide tag, in some embodiments the tag may be any moiety that can act as a binding partner for the chimeric aGPCR. Thus, a non-peptide tag can be any chemical entity to which the tag binding domain has affinity. The tag can be selected from, for example, any organic molecule, a small molecule, or a hapten. Tags can for example take the form of nucleic acids, for example aptamers.

20

Accordingly, it will be clear to the skilled person that the invention also provides a complex comprising a chimeric aGPCR of the invention that is bound to or interacts with an intermediate adaptor protein or peptide that comprises an ultimate target binding domain and a tag such as a peptide tag. In any embodiment of the invention, the target binding domain (or ultimate target binding domain on in the context of the adaptor intermediate protein or peptide) may be any domain with the ability to the bind to the required target.

25
30

Typically the target binding domain is a proteinaceous domain, such as a target-binding ligand or fragment thereof, or antibody or antigen binding fragment thereof that binds specifically to said target.

35

The target binding domain may be the same as or a variant of an endogenous target binding domain. By endogenous we include the meaning of being endogenous to a particular cell, endogenous to a particular tissue, or endogenous to a particular host organism such as a mammal such as a human. For example in some embodiments

the target binding domain is a human target binding domain with an amino acid sequence that has at least 75%, 80%, 85%, 90%, 92%, 94%, 96%, 98% or 100% sequence identity to a human target binding domain sequence. However, although the target binding domain may be endogenous to a specific host or species such as a human, it is still heterologous to the intracellular tail domain, for example the C-terminal tail domain of the chimeric aGPCR.

The target binding domain may comprise a non-human target binding domain sequence, for example a humanised sequence or a sequence from a mouse.

10

In some preferred embodiments the target binding domain comprises:

- an antibody or antibody fragment that binds specifically to said target;
- a variable heavy chain domain and/or a variable light chain domain;
- 15 an scFv;
- a nanobody;
- a Fab;
- a kappa light chain or a fragment thereof targeting;
- a synthetic binding scaffold such as a monobody, affibody, DARPin or knottin;
- 20 anti-CD19 scFv domains, such as FMC63 scFv domains see for example SEQ ID NO: 15;
- anti-CD276 scFv domains, such as Enoblituzumab scFv domains see for example SEQ ID NO: 16 or Omburtamab see for example SEQ ID NO:17; and/or
- anti- MAdCAM1 scFv domains, such as Ontamalimab scFv domains see for
- 25 example SEQ ID NO: 18.

The target binding domain may be any antibody or antibody derived fragment, including those described above. Antigen binding fragments of antibodies are available in many formats, and all antigen-binding fragments and derivatives, natural or engineered/synthetic, are contemplated by this invention.

30

The skilled person will appreciate that an antibody or antigen binding fragment thereof typically comprises of a heavy chain and a light chain polypeptide, each contributing to the variable epitope-binding region via their three CDR regions supported by a framework region. Single chain antibodies that can bind specifically to a target are known, which require only three CDR regions.

35

Accordingly in some embodiments the target binding domain comprises one, two, three, four, five, or six CDR regions, for example comprises one, two, three, four, five,

or six CDR regions from a light chain or a heavy chain of an antibody. In some embodiments the target binding domain comprises one, two or three CDR regions from a light chain. In other embodiments the target binding domain comprises one, two or three CDRs from a heavy chain. In other embodiments the target binding domain
5 comprises one, two or three CDRs from a light chain and one, two or three CDRs from a heavy chain. In specific embodiments the target binding domain comprises three CDRs from a light chain and three CDRs from a heavy chain.

As set out above, the premise of the chimeric aGPCR of the invention is to direct a
10 particular intracellular signalling response to a particular target. For example the chimeric aGPCR may comprise a target binding domain that binds to a desired target, and the appropriate intracellular tail domain for example the C-terminal tail and/or the ICLs of the 7TM that associate with a particular G protein to elicit the desired intracellular signalling response.

15 In this way the aGPCR may be considered to comprise of a number of modular units or domains that may be interchanged with other known units, or interchanged with rationally designed or synthetic units. All combinations of domains are considered to be appropriate in the context of the chimeric aGPCR.

20 In some embodiments some of the domains of the chimeric aGPCR are found naturally together in natural or wildtype aGPCRs. For example in some embodiments the intracellular tail domain for example the C-terminal tail is autologous to the 7TM domain i.e. the intracellular tail domain for example the C-terminal tail and 7TM are
25 found together in nature and it is only the N-terminal domain that is interchanged. In some embodiments the intracellular tail domain for example the C-terminal tail is autologous to the GAIN domain; and/or

the intracellular tail domain for example the C-terminal tail is autologous to the GPS motif.

30

In some embodiments the:

- a) the intracellular tail domain for example the C-terminal tail;
- b) seven-transmembrane domain (7TM); and
- 35 c) GPCR autoproteolysis-inducing domain (GAIN domain) that comprises a tethered agonist peptide and that constitutively cleaves a GPS motif; and
- d) GPS motif that is cleaved by the GAIN domain

are autologous to one another.

In some embodiments the:

- 5 GAIN domain is heterologous to:
the 7TM domain;
the intracellular tail domain for example the C-terminal tail; and/or
the target binding domain.

10 In some embodiments the:

- a) intracellular tail domain for example the C-terminal tail;
b) seven-transmembrane domain (7TM); and
c) GPCR autoproteolysis-inducing domain (GAIN domain) that comprises a
15 tethered agonist peptide and that constitutively cleaves a GPS motif; and
d) GPS motif that is cleaved by the GAIN domain

are from the same naturally occurring aGPCR.

20 In some embodiments the:

- a) intracellular tail domain for example the C-terminal tail;
b) seven-transmembrane domain (7TM); and
c) GPCR autoproteolysis-inducing domain (GAIN domain) that comprises a
tethered agonist peptide and that constitutively cleaves a GPS motif; and/or
25 d) GPS motif that is cleaved by the GAIN domain

are from the same naturally occurring aGPCR, and wherein any one or more of the:

- a) intracellular tail domain for example the C-terminal tail;
30 b) seven-transmembrane domain (7TM); and
c) GPCR autoproteolysis-inducing domain (GAIN domain) that comprises a
tethered agonist peptide and that constitutively cleaves a GPS motif; and/or
d) GPS motif that is cleaved by the GAIN domain

35 comprises an amino acid sequence that has at least 75%, 80%, 85%, 90%, 92%,
94%, 96%, 98% or 100% sequence identity to the corresponding domain of the
naturally occurring aGPCR.

In any of the embodiments, any one or more of the three ICLs of the 7TM may comprise one or more mutations, substitutions or insertions or deletions. For example such mutations, substitutions, insertions or deletions may be introduced to alter the interaction between the 7TM and particular G proteins, for example may be to allow interaction of one of more of the ICLs with a different G protein to the G protein the one or more ICLs would interact with absent those mutations, substitutions, insertions or deletions.

As mentioned above, the chimeric aGPCR of the invention can be fully modularised, and all combinations of domains, naturally occurring, synthetic or engineered are contemplated. In some embodiments the chimeric aGPCR comprises the following combination of naturally occurring and engineered and/or synthetic domains:

Naturally occurring	Naturally occurring	Naturally occurring	Engineered and/or synthetic domain
(a)	(b)	(c)	(d)
(a)	(b)	(d)	(c)
(a)	(c)	(b)	(d)
(a)	(c)	(d)	(b)
(a)	(d)	(b)	(c)
(a)	(d)	(c)	(b)
(b)	(a)	(c)	(d)
(b)	(a)	(d)	(c)
(b)	(c)	(a)	(d)
(b)	(c)	(d)	(a)
(b)	(d)	(a)	(c)
(b)	(d)	(c)	(a)
(c)	(a)	(b)	(d)
(c)	(a)	(d)	(b)
(c)	(b)	(a)	(d)
(c)	(b)	(d)	(a)
(c)	(d)	(a)	(b)
(c)	(d)	(b)	(a)

(d)	(a)	(b)	(c)
(d)	(a)	(c)	(b)
(d)	(b)	(a)	(c)
(d)	(b)	(c)	(a)
(d)	(c)	(a)	(b)
(d)	(c)	(b)	(a)
(d)	(c)	(b)	(a)

Naturally occurring	Naturally occurring	Engineered and/or synthetic domain	Engineered and/or synthetic domain
(a)	(b)	(c)	(d)
(a)	(b)	(d)	(c)
(a)	(c)	(b)	(d)
(a)	(c)	(d)	(b)
(a)	(d)	(b)	(c)
(a)	(d)	(c)	(b)
(b)	(a)	(c)	(d)
(b)	(a)	(d)	(c)
(b)	(c)	(a)	(d)
(b)	(c)	(d)	(a)
(b)	(d)	(a)	(c)
(b)	(d)	(c)	(a)
(c)	(a)	(b)	(d)
(c)	(a)	(d)	(b)
(c)	(b)	(a)	(d)
(c)	(b)	(d)	(a)
(c)	(d)	(a)	(b)
(c)	(d)	(b)	(a)
(d)	(a)	(b)	(c)
(d)	(a)	(c)	(b)
(d)	(b)	(a)	(c)
(d)	(b)	(c)	(a)

(d)	(c)	(a)	(b)
(d)	(c)	(b)	(a)
(d)	(c)	(b)	(a)

Naturally occurring	Engineered and/or synthetic domain	Engineered and/or synthetic domain	Engineered and/or synthetic domain
(a)	(b)	(c)	(d)
(a)	(b)	(d)	(c)
(a)	(c)	(b)	(d)
(a)	(c)	(d)	(b)
(a)	(d)	(b)	(c)
(a)	(d)	(c)	(b)
(b)	(a)	(c)	(d)
(b)	(a)	(d)	(c)
(b)	(c)	(a)	(d)
(b)	(c)	(d)	(a)
(b)	(d)	(a)	(c)
(b)	(d)	(c)	(a)
(c)	(a)	(b)	(d)
(c)	(a)	(d)	(b)
(c)	(b)	(a)	(d)
(c)	(b)	(d)	(a)
(c)	(d)	(a)	(b)
(c)	(d)	(b)	(a)
(d)	(a)	(b)	(c)
(d)	(a)	(c)	(b)
(d)	(b)	(a)	(c)
(d)	(b)	(c)	(a)
(d)	(c)	(a)	(b)
(d)	(c)	(b)	(a)
(d)	(c)	(b)	(a)

Engineered and/or synthetic domain	Engineered and/or synthetic domain	Engineered and/or synthetic domain	Engineered and/or synthetic domain
(a)	(b)	(c)	(d)

wherein (a) is the intracellular tail domain for example the C-terminal tail domain; (b) is the seven-transmembrane domain (7TM) that comprises three ICLs; (c) is the GPCR autoprolysis-inducing domain (GAIN domain) that optionally comprises a tethered agonist ; and (d) is the GPS motif that is optionally present and which in some embodiments is cleaved by the GAIN domain;

- and where an engineered domain is:
- a) a domain that comprises an amino acid sequence that has at least one substitution, insertion or deletion relative to the native or naturally occurring domain;
 - b) a domain that comprises an amino acid sequence that has at least 75%, 80%, 85%, 90%, 92%, 94%, 96%, 98% or 100% sequence identity to the naturally occurring domain; and/or
 - c) is a rationally designed or synthetic domain.

By rationally designed or synthetic domain we include the meaning that the domain has been designed de novo, rather than relatively minor engineering of known domains. We also include the meaning that the domain has an amino acid sequence with less than 75% sequence identity to known corresponding naturally occurring domains. For example it is known that it is possible to have functionally and conformationally equivalent domains which show very little sequence identity.

The inventors have found that different GAIN domains have different sequence and/or structural complexities, whilst retaining similar functions, and there are some advantages in using a relatively simple GAIN domain in the context of the chimeric aGPCRs. For example in some embodiments it is considered that the GAIN domain of the ADGRG1 aGPCR is relatively simple compared to the GAIN domain from ADGRG5. Accordingly in some embodiments of the chimeric aGPCR the GAIN domain (domain (c) in the above table) is the GAIN domain from ADGRG1 [SEQ ID NO: 35], or is a GAIN domain with an amino acid sequence that has at least 75%, 80%, 85%, 90%, 92%, 94%, 96%, 98% or 100% sequence identity to SEQ ID NO: 35. For example the GAIN domain may be from, or may be derived from (i.e. have some sequence

divergence from) the GAIN domain of ADGRG1, but the target binding domain, intracellular tail for example the C-terminal tail and 7TM domain may all be from proteins other than ADGRG1, or indeed one or more of them may be rationally designed, engineered or synthetic.

5

For example in some embodiments of the chimeric aGPCR, the GAIN domain is a GAIN domain with an amino acid sequence that has at least 75%, 80%, 85%, 90%, 92%, 94%, 96%, 98% or 100% sequence identity to SEQ ID NO: 35, and wherein:

- the intracellular tail is not from ADGRG1;
- 10 the target binding domain is not from ADGRG1; and/or
- the 7TM domain is not from ADGRG1.

In some embodiments the GAIN domain is:

- The GAIN domain from ADGRL1 and has a sequence of SEQ ID NO: 27;
- 15 The GAIN domain from ADGRL3 and has a sequence of SEQ ID NO: 28;
- The GAIN domain from ADGRE2 and has a sequence of SEQ ID NO: 29;
- The GAIN domain from ADGRG2 and has a sequence of SEQ ID NO: 30;

- or is a GAIN domain with an amino acid sequence that has at least 75%, 80%, 85%, 90%, 92%, 94%, 96%, 98% or 100% sequence identity to any of SEQ ID NO: 27, 28, 29 or 30.
- 20

- For example in some embodiments the GAIN domain is from ADGRL1 and has a sequence of SEQ ID NO: 27 or has at least 75%, 80%, 85%, 90%, 92%, 94%, 96%, 98% or 100% sequence identity to SEQ ID NO: 27 but the target binding domain, intracellular tail for example the C-terminal tail, and the 7TM domain may all be from proteins other than ADGRL1, or indeed one or more of them may be rationally designed, engineered or synthetic.
- 25

- For example in some embodiments the GAIN domain is from ADGRL3 and has a sequence of SEQ ID NO: 28 or has at least 75%, 80%, 85%, 90%, 92%, 94%, 96%, 98% or 100% sequence identity to SEQ ID NO: 28 but the target binding domain, intracellular tail for example the C-terminal tail, and the 7TM domain may all be from proteins other than ADGRL3, or indeed one or more of them may be rationally designed, engineered or synthetic.
- 30
- 35

For example in some embodiments the GAIN domain is from ADGRE2 and has a sequence of SEQ ID NO: 29 or has at least 75%, 80%, 85%, 90%, 92%, 94%, 96%,

98% or 100% sequence identity to SEQ ID NO: 29 but the target binding domain, intracellular tail for example the C-terminal tail, and the 7TM domain may all be from proteins other than ADGRE2, or indeed one or more of them may be rationally designed, engineered or synthetic.

5

For example in some embodiments the GAIN domain is from ADGRG2 and has a sequence of SEQ ID NO: 30 or has at least 75%, 80%, 85%, 90%, 92%, 94%, 96%, 98% or 100% sequence identity to SEQ ID NO: 30 but the target binding domain, intracellular tail for example the C-terminal tail, and the 7TM domain may all be from proteins other than ADGRG2, or indeed one or more of them may be rationally designed, engineered or synthetic.

10

The GAIN domain may be an engineered GAIN domain. For instance Dumas *et al* (Uncovering and engineering the mechanical properties of the adhesion GPCR ADGRG1 GAIN domain doi: <https://doi.org/10.1101/2023.04.05.535724>) have shown that computational designed of GAIN variants to lock the alpha and beta subdomains and rewire mechanically-induced structural deformations modulates the GPS-Stachel rupture forces. Accordingly in some embodiments the GAIN domain is an engineered GAIN domain, having at least 75%, 80%, 85%, 90%, 92%, 94%, 96%, 98% or 100% sequence identity to naturally occurring GAIN domains. In some embodiments the GAIN domain is a rationally designed or synthetic GAIN domain which has less than 75% sequence identity to naturally occurring GAIN domains. In some embodiments the GAIN domain has been engineered to increase the force required to rupture the association between the GPS and tethered agonist, or between the NTF and the CTF. In other embodiments the GAIN domain has been engineered to decrease the force required to rupture the association between the GPS and tethered agonist, or between the NTF and the CTF

20

The chimeric aGPCRs of the invention are considered to be useful in triggering intracellular signalling in response to an appropriate target. In preferred embodiments then the chimeric aGPCR is capable of activating intracellular signalling. For example is capable of activating intracellular signalling when the chimeric aGPCR is localised to the plasma membrane of a cell, a platelet or an engineered platelet. The skilled person can readily test the ability of a particular aGPCR to activate intracellular signalling. For example in some embodiments the cell, platelet or engineered platelet that expresses the aGPCR is incubated and agitated in the presence of the target and a determination of the activation of intracellular signalling is made. However, as described elsewhere

30

herein, it is considered that the general molecular motion of the target-bound aGPCR relative to the cell and the target is sufficient to trigger activation of the chimeric aGPCR and intracellular signalling.

- 5 Where agitation is employed in an assay to determine chimeric aGPCR activation, the agitation may be performed by shaking at a speed of:

At least 10 rpm, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190 or at least 200 rpm;

- 10 Less than 200 rpm, 190, 180, 170, 160, 150, 140, 130, 120, 110, 100, 90, 80, 70, 60, 50, 40, 30, 20 or less than 10 rpm; and/or

Between 10 rpm and 200 rpm, 20 rpm and 190 rpm, 30 rpm and 180 rpm, 40 rpm and 170 rpm, 50 rpm and 160 rpm, 60 rpm and 150 rpm, 70 rpm and 140 rpm, 80 rpm and 130 rpm.

15

The skilled person will appreciate the various methods by which activation of the chimeric aGPCR and subsequent intracellular signalling can be determined. For example in some instances the cell, platelet or engineered platelet that expresses the chimeric aGPCR also comprises a reporter system that comprises a reporter protein that is expressed when intracellular signalling is activated. For example the reporter protein may be expressed from a promoter that comprises a nuclear factor of activated T cells (NFAT) response element, for example the reporter protein may be a luciferase enzyme. Similar assays can be performed to detect the increase in intracellular calcium using calcium indicator dyes such as Fluo-4. Other methods are available to detect the different intracellular signalling responses elicited by interaction of the C-terminal signalling domain (that as described elsewhere may comprise the intracellular tail for example the C-terminal tail and/or one or more of the ICLs of the 7TM) with different G proteins.

20

25

30

Determination of whether a particular chimeric aGPCR has been activated or not can be made by detecting the expression or activity of the reporter protein, for example detection of the expression or activity of luciferase.

35

As well as engineering aGPCRs to redirect the intracellular signalling response to new targets, the intracellular signalling domain, i.e. the intracellular tail and/or any one, two or three of the ICLs of the 7TM, itself may be engineered to alter the intracellular signalling response.

As set out elsewhere herein, the skilled person will appreciate that the C-terminal intracellular signalling domain encompasses one, two or three of the 7TM intracellular loops (ICLs) and/or the C-terminal tail. G proteins, in particular $G\alpha$, interact with GPCRs through the ICLs of the 7TM region. G proteins also interact with C-terminal tail. In this way the intracellular signalling domain is actually composed of four discontinuous regions – the three ICLs and the C-terminal tail. The C-terminal tail mainly interacts with signaling proteins such as arrestin, which prevent GPCR activation and recruits other signaling molecules such as ERK/JNK.

10

For example in some embodiments the intracellular tail domain, for example the C-terminal tail domain, comprises one or more substitutions, insertions or deletions as compared to the autologous intracellular tail for example the autologous C-terminal tail or to a wild-type intracellular tail for example the autologous C-terminal tail. Similarly, and as mentioned above, in some embodiments the 7TM domain comprises one or more substitutions, insertions or deletions as compared to the autologous 7TM or to a wild-type 7TM,, for example in some embodiments:

15

the one or more substitutions, insertions or deletions in the intracellular tail for example the C-terminal tail, and/or the 7TM increases the intracellular signalling response as compared to the autologous intracellular tail for example the C-terminal tail, and/or the 7TM or to a wild-type intracellular tail for example the C-terminal tail, and/or the 7TM, that does not comprise the same one or more substitutions, insertions or deletions; or

20

the one or more substitutions, insertions or deletions in the intracellular tail for example the C-terminal tail, and/or the 7TM decreases the intracellular signalling response as compared to the autologous intracellular tail for example the C-terminal tail, and/or the 7TM i or to a wild-type intracellular tail for example the C-terminal tail, and/or the 7TM that does not comprise the same one or more substitutions, insertions or deletions;

25

and/or

the one or more substitutions, insertions or deletions in the intracellular tail for example the C-terminal tail, and/or the 7TM alters the specificity of the intracellular signalling response as compared to the autologous intracellular tail for example the C-terminal tail, and/or the 7TM or to a wild-type intracellular tail for example the C-terminal tail, and/or the 7TM i that does not comprise the same one or more substitutions, insertions or deletions resulting in altered signalling pathway activation.

30

35

By alters the specificity of the intracellular signalling response we include the meaning that the intracellular tail for example the C-terminal tail, and/or the 7TM is modified so as to allow interactions with different or additional G proteins. For example, the C-terminal tail, and/or the 7TM ICLs that would normally interact with G protein $G_{\alpha q}$ / $G_{\alpha s}$ is engineered by replacing the original $G_{\alpha q}$ C-terminal GPCR binding sequence with the sequence from $G_{\alpha s}$. The chimeric G protein $G_{\alpha q}$ / $G_{\alpha s}$ enables the $G_{\alpha s}$ dependent aGPCR (rather than original $G_{\alpha q}$ dependent aGPCR) to activate downstream $G_{\alpha q}$ signalling pathway upon activation.

In some embodiments any one or more of the 3 cytoplasmic loops of the 7TM, or the C-terminal tail may be modified, for example may comprise one or more substitutions or insertions or deletions, or otherwise engineered or designed sequences, so as to effect the desired intracellular signalling.

The sensitivity of the response of a cell or platelet or engineered platelet for example to a particular signal is considered to reside, at least in part, by the number of relevant chimeric aGPCR molecules present on the cell or platelet or engineered platelet surface. One means of affecting the level or amount of receptors present on the cell surface is to use an appropriate signal peptide. Signal peptides are short peptide sequences that are read internally by the cell as a signal to transport the protein to the cell surface.

In some embodiments then the chimeric aGPCR of the invention comprises a signal peptide. In some embodiments the signal peptide has been selected to achieve a desired level of chimeric aGPCR at the cell, platelet, or engineered platelet surface. The skilled person will understand how to select the appropriate signal peptide for the given situation. See for example O'Neill et al 2023 Protein-specific signal peptides for mammalian vector engineering <https://www.biorxiv.org/content/10.1101/2023.03.14.532380v1>

Exemplary signal peptides include the following:

ADGRG1 signal peptide	MTPQSLLQTTLFLLSLLFLVQGAHG [SEQ ID NO: 1]
ADGRF5 signal peptide	MKSPRRTLCLMFIVYSSKA [SEQ ID NO: 2]
FCERG signal peptide	MIPAVVLLLLLLVEQAAA [SEQ ID NO: 3]
CD28 signal peptide	MLRLLLALNLFPSIQVTG [SEQ ID NO: 4]
Platelet signal peptide – GPIIb (aIIb)	

- MARALCPLQALWLEWVLLLLGPCAAPPWA [SEQ ID NO: 5]
- Platelet signal peptide – GPIIIa ($\beta 3$)
- MRARPRRPLWATVLALGALAGVG [SEQ ID NO: 6]
- 5 Platelet signal peptide - GPIBa MPLLLLLLLPSPLHP [SEQ ID NO: 7]
- Platelet signal peptide - GPIX MPAWGALFLLWATAEA [SEQ ID NO: 8]
- Platelet signal peptide - GPV MLRGTLLCAVLGLLRA [SEQ ID NO: 9]
- 10 MAPFASLASGILLLSLITSSKA [SEQ ID NO: 36]
 MLLGPGHTLSAPALALAVTLTLVRSASP [SEQ ID NO: 37]
 MLLSVPLLGLLGLAAA [SEQ ID NO: 38]
 MQELRGILLCLLLAAVPTTP [SEQ ID NO: 39]
 MRYVASYLLAALGGNS [SEQ ID NO: 40]
- 15 MGKSPEAWCIVLFSVLASFSA [SEQ ID NO: 41] MASSGSVQQPRLVLLMLVLAGAARA [SEQ ID NO: 42]
 MRWKIIQLQYCFLLVPCMLTALEA [SEQ ID NO: 43]
 MLRSRLCLALAWVARVGA [SEQ ID NO: 44]
 MRFSCALLPGVALLASARLAAA [SEQ ID NO: 45]
 MRVLWVLGLCCVLLTFGFVRA [SEQ ID NO: 46]
- 20 MKFPMVAAALLLCAVRA [SEQ ID NO: 47]
 MRSLLASFCLLAVALA [SEQ ID NO: 48]
 MKILLLCVGLLLTWDNGMVLG [SEQ ID NO: 49]
 MLRISGRNMKVLFAAALIVGSVVFLLPGPSVA [SEQ ID NO: 50]
 MAATVRRQRPRRLCWTAVLLADLLALS [SEQ ID NO: 51]
- 25 MKMGVRLAARAWPLCGLLAALGGVCA [SEQ ID NO: 52]
 MWWRLWVLLLLLLWLALAAA [SEQ ID NO: 53] MGWSLILLFLVAVATRVLS [SEQ ID NO: 54]
 MDFQVQIISFLLISASVMSRG [SEQ ID NO: 55]
 MEFGLSWVFLVALFRGVQC [SEQ ID NO: 56]
- 30 MKWVTFISLLFLFSSAYS [SEQ ID NO: 57]
 MKLPVRLVLMFWIPAASA [SEQ ID NO: 58]
 MNLLLLITFVAAAVA [SEQ ID NO: 59]
 MGSAALLWVLLWVPSSRA [SEQ ID NO: 60]
 MTRLTVLALLAGLLASSRA [SEQ ID NO: 61]
- 35 MWWRLWVLLLLLLWPMVWA/AA [SEQ ID NO: 62]
 MKLPVRLVLMFWIPASSS [SEQ ID NO: 63]
 MDMRVPAQLLGLLLWLSGARC [SEQ ID NO: 64]
 MKYLLPTAAAGLLLAAPAMA [SEQ ID NO: 65]
 MGVKVLFALICIAVAEA [SEQ ID NO: 66]
 MPLLLLLPLWAGALA [SEQ ID NO: 67]
- 40 MRARALLAVLLLLLVGIAAAA Synthetically designed [SEQ ID NO: 68]
 MATATLLAVLLLLLVGSAGGA Synthetically designed [SEQ ID NO: 69]
 MRARALLVVLVLLGVASSA Synthetically designed [SEQ ID NO: 70]
 MPGPGAALLLLLLVLLGLGSAA Synthetically designed [SEQ ID NO: 71]
- 45 MTTTTLVLLLLLVVLAGLTSGA Synthetically designed [SEQ ID NO: 72]

The signal peptide may have at least 75%, 80%, 85%, 90%, 92%, 94%, 96%, 98% or 100% sequence identity to the above sequences.

50

In some embodiments the signal peptide may be a naturally occurring signal peptide.

In some embodiments the signal peptide is not a naturally occurring signal peptide.
In some embodiments the signal is an engineered signal peptide. For example in some
embodiment the signal peptide comprises a sequence that has at least 75%, 80%,
85%, 90%, 92%, 94%, 96%, 98% or 100% sequence identity to a naturally occurring
5 signal peptide.

In some embodiments the signal peptide is a rationally designed signal peptide and in
some further embodiments has less than 75% sequence identify to a naturally
occurring signal peptide. For example the signal peptide may have less than 75%
10 sequence identity to any of SEQ ID NO: 1-9, 36-72.

As mentioned elsewhere herein, the intracellular tail domain, for example the C-
terminal tail is heterologous to the target binding domain, but the intracellular tail
domain, for example the C-terminal tail may be autologous to the GAIN domain and/or
15 the 7TM domain.

Various intracellular signalling domains (i.e. combinations of one, two or three ICLs
from the 7TM and/or the C-terminal tail) from GPCRs are known, and they interact
with different G proteins, triggering different intracellular signalling response. In some
20 embodiments the C-terminal signalling domain (i.e. combinations of one, two or three
ICLs from the 7TM and/or the C-terminal tail) is selected so as to provide an
appropriate or desired intracellular signalling response upon target binding to the
target binding domain.

25 For example, when the target binding domain binds to the target, an:
intracellular domain that comprises a domain that interacts with a Gq subunit
activates beta-type phospholipase C (PLC β);
intracellular domain that comprises a domain that interacts with a Gs subunit
stimulates the cAMP-dependent pathway by activating adenylyl cyclase and increasing
30 intracellular cAMP levels;
intracellular domain that comprises a domain that interacts with a Gai/o subunit
inhibits adenylyl cyclase and decreases intracellular cAMP;
Intracellular domain that comprises a domain that interacts with a G α 12/13
subunit activates the RhoA pathway; or
35 Intracellular domain that comprises a domain that interacts with a G α 16 subunit
activates PLC- β /PI3K/Akt/MAPK/NF- κ B pathways.

In the context of PLC- β , once PLC- β is activated PLC- β hydrolyzes phosphatidylinositol 4,5-bisphosphate (PIP₂) to diacyl glycerol (DAG) and inositol trisphosphate (IP₃). IP₃ acts as a second messenger to release stored calcium into the cytoplasm, while DAG acts as a second messenger that activates protein kinase C (PKC).

5

For example, in some embodiments the intracellular tail, for example the C-terminal tail:

Comprises a domain that interacts with a G α q subunit and when the aGPCR is activated activates beta-type phospholipase C (PLC β);

10

Comprises a domain that interacts with a G α s subunit and when the aGPCR is activated stimulates the CAMP-dependent pathway by activating adenylyl cyclase and increasing intracellular cAMP levels;

Comprises a domain that interacts with a G α i/o subunit and when the aGPCR is activated inhibits adenylyl cyclase and decreases intracellular cAMP;

15

Comprises a G α 12/13 subunit and when the chimeric aGPCR is activated activates the RhoA pathway; and/or

Comprises a domain that interacts with a G α 16 subunit and when the chimeric aGPCR is activated activates PLC- β /PI3K/Akt/MAPK/NF- κ B pathways.

20

In platelets, intracellular calcium plays a central role in the regulation of degranulation. Inositol triphosphate (IP₃), which is produced by the activation of G α -coupled receptors on the platelet surface, binds to IP₃ receptors on the endoplasmic reticulum, leading to the release of Ca²⁺ into the cytosol. This increase in intracellular Ca²⁺ concentration activates several downstream effectors, including protein kinase C (PKC) and calmodulin-dependent protein kinase II (CaMKII).

25

PKC and CaMKII both contribute to the regulation of platelet degranulation. PKC phosphorylates a number of targets involved in granule release, including the cytoskeletal protein myosin light chain and the vesicular trafficking protein syntaxin-4. These phosphorylation events promote the interaction between the platelet membrane and the granule membrane, leading to the fusion and release of the granules.

30

CaMKII also plays a role in platelet degranulation by promoting the fusion of the granules with the platelet membrane. CaMKII phosphorylates the vesicular trafficking protein synaptotagmin, which is involved in the docking and fusion of the granules with the platelet membrane.

35

Since the intracellular signalling response that triggers platelet degranulation is an increase in Ca^{2+} , in preferred embodiments, where the chimeric aGPCR is to be used in the context of a platelet or engineered platelet such as those described in WO 2022/263824, the intracellular signalling domain is a domain that can trigger an increase in the amount of Ca^{2+} in the cell, leading to degranulation of the platelet or engineered platelet. For example in these embodiments the intracellular signalling domain may comprise a Gq subunit.

For example in some embodiments where the chimeric aGPCR is present in the membrane of a platelet or engineered platelet, and once activated the chimeric aGPCR:

- a) results in degranulation of the platelet or engineered platelet;
- b) results in the release of contents from the platelet or engineered platelet;
- c) results in the presence of intraplatelet contents on the plasma membrane of the platelet or engineered platelet;
- d) results in the release of extracellular vesicles via blebbing from the plasma membrane; and/or
- e) results in a change of shape of the platelet or engineered platelet from a biconcave disk to fully spread cell fragments;

wherein in some embodiments one, two or three of the ICLs of the 7TM and/or the C-terminal tail comprises a Gq subunit.

In the context of platelet degranulation, activation of the G_s pathway increases cAMP and inhibits platelet activation. There are some instances where inhibition of platelet activation is desired. In some embodiments where the chimeric aGPCR is present in the membrane of a platelet or engineered platelet, and once activated the chimeric aGPCR:

- a) prevents degranulation of the platelet or engineered platelet;
- b) prevents the release of contents from the platelet or engineered platelet;
- c) prevents the presence of intraplatelet contents on the plasma membrane of the platelet or engineered platelet;
- d) prevents the release of extracellular vesicles via blebbing from the plasma membrane; and/or
- e) prevents a change of shape of the platelet or engineered platelet from a biconcave disk to fully spread cell fragments;

wherein in some embodiments one, two or three of the ICLs of the 7TM and/or the C-terminal tail comprises a Gas subunit.

It is generally considered that for protein domains to function correctly, there must be no steric hindrance between the functional domains. To achieve this linkers such as peptide linkers are typically used that provide a short stretch of amino acids sufficient to separate the two domains. In the present case it is considered necessary in some embodiments to include a linker, for example some linker amino acid residues between the GAIN domain and the target binding domain. In some embodiments the chimeric aGPCR comprises a linker. In some preferred embodiments the chimeric aGPCR comprises a linker between the GAIN domain and the target binding domain. In some embodiments the chimeric aGPCR comprises a peptide linker between the GAIN domain and the target binding domain. For example where the target binding domain comprises an antibody or antigen binding fragment thereof as described herein, in preferred embodiments the chimeric a GPCR comprises a linker between the GAIN domain and the target binding domain. Exemplary linkers include SPHTAAHNA [SEQ ID NO: 10].

Where the linker is a peptide linker, the linker may be any number of amino acids in length. Preferably the linker is at least 5 amino acids in length, for example at least 6, 7, 8, 9, or at least 10 amino acids in length.

As mentioned elsewhere herein, the chimeric aGPCR of the invention may be considered to be a universal chimeric aGPCR and may bind to an intermediate adaptor protein or peptide that itself binds to the final target – for example may bind to a tag present on the intermediate adaptor protein or peptide.

Accordingly the invention also provides a system comprising:

a chimeric aGPCR of the invention; and
an intermediate adaptor polypeptide or protein, for example that comprises a tag for example a peptide tag, and that comprises an ultimate target binding domain (i.e. a binding domain that can bind to a target as described herein)

wherein the target binding domain of the aGPCR is able to bind to the intermediate adaptor protein or peptide, for example bind to a tag present on the intermediate adaptor polypeptide or peptide, and the ultimate target binding domain of the adaptor polypeptide or protein is able to simultaneously bind to a the desired

target – i.e. the target towards which the chimeric aGPCR is to be directed. Preferences for all features of this aspect are as described elsewhere herein, for example preferences for the ultimate target are as described elsewhere herein for the target to which the chimeric aGPCR binds, for example the target may be present on a tumour or cancer cell. Preferences for the tag are also as described elsewhere.

It will be clear that binding of the intermediate adaptor protein or peptide itself to the target binding domain of the chimeric aGPCR, in the absence of simultaneous target binding of the intermediate adaptor protein to the ultimate target, should not be sufficient to trigger receptor activation and intracellular signalling. For example, in instances where the chimeric aGPCR is present in a platelet plasma membrane or in an engineered platelet plasma membrane, binding of the intermediate adaptor polypeptide or peptide to the target binding domain of the chimeric aGPCR in the absence of simultaneous binding of the target binding domain of the intermediate adaptor polypeptide or protein to the target not sufficient to activate degranulation of the platelet or engineered platelet.

On the other hand, simultaneous binding of the ultimate target binding domain of the intermediate adaptor protein or peptide to the ultimate target and binding of the target binding domain of the chimeric aGPCR to the intermediate adaptor protein or peptide for example binding to a tag (for example a peptide tag) on the intermediate adaptor protein or peptide, triggers activation of the chimeric aGPCR and intracellular signalling.

The invention also provides one or more polynucleotides encoding any one or more of the chimeric aGPCRs described herein. The invention also provides one or more nucleic acids encoding the intermediate adaptor protein or peptide described herein.

In some embodiments the polynucleotide is DNA.

30

In some embodiments the polynucleotide is RNA.

The skilled person understands the concepts of promoters and appreciates that for transcription to be initiated from DNA a promoter must be operably linked to the appropriate open reading frame. Accordingly in some embodiments the polynucleotide is operatively linked to a promoter. In some embodiments the promoter is a heterologous promoter. The nucleic acid may also comprise an appropriate enhancer.

Depending on the context on which the chimeric aGPCR is to be used, the promoter may be cell-specific promoter. For example in some embodiments the cell-specific promoter is a megakaryocyte-specific promoter, or a pluripotent cell-specific promoter or stem cell-specific promoter, optionally an induced pluripotent stem cell (iPSC) cell-specific promoter, or a T cell-specific promoter, or a NK cell-specific promoter, or a B cell-specific promoter.

The promoter may be an inducible promoter, for example inducible in a particular cellular context or intended subject. The promoter may be constitutive, for example constitutive in a particular cellular context or intended subject.

The invention also provides a vector comprising the polynucleotide of the invention, for example comprises the polynucleotide of the invention that encodes a chimeric aGPCR as described herein operably linked to a promoter. The vector may be a plasmid or circular nucleic acid. The vector may also comprise an appropriate enhancer.

The invention also provides a viral vector or viral particle comprising the polynucleotide of the invention or the vector of the invention, such as an AAV or lentivirus.

The invention also provides a cell or a chassis comprising

- a) one or more chimeric aGPCRs of the invention;
- b) one or more polynucleotides of the invention;
- c) one or more vectors of the invention;
- d) one or more of the viral vectors of the invention
- e) a system of the invention.

In preferred embodiments the cell or the chassis expresses a chimeric aGPCR of the invention.

The skilled person will recognise the term chassis, and understand that it refers to an entity, for example a cell such as a mammalian cell or a microbial cell, or an entity such as a liposome or the like, that houses genetic or protein compartments. In the present case a chassis is an entity that is capable of expressing or carrying one or more chimeric aGPCRs of the invention. In some embodiment the chassis is a cell, a platelet or an engineered platelet. In some embodiments the cell is a T cell, B cell, NK cell, or macrophage, or iPSC cell.

In some embodiments the chassis has been engineered. In some embodiments the chassis is an engineered platelet such as those described in WO 2022/263824 which is specifically incorporated herein in its entirety, for example the various chassis described on pages 43-122 of WO 2022/263824, said pages of which are specifically
5 incorporated herein by reference. In some embodiments the platelet has been engineered:

10 to disrupt the thrombogenic pathway and/or engineered to disrupt a platelet inflammatory signaling pathway and/or engineered to make the engineered platelet less immunogenic; and/or

to enhance or disrupt one or more base functions of the chassis, optionally wherein the one or more base functions are involved in the innate and/or adaptive immune response, inflammation, angiogenesis, atherosclerosis,
15 lymphatic development and tumour growth.

In some embodiments the chassis is:

20 a) a progenitor-chassis, for example is a myeloid stem cell; an iPSC; adipocyte; adipose-derived mesenchymal stromal/stem cell line (ASCL); or cancer cell-line that is capable of producing a producer-chassis; or other immortal cell that is capable of producing a producer-chassis;

b) a producer-chassis, for example is a megakaryoblast; a megakaryocyte; a megakaryocyte-like cell; a cancer cell line that is capable of forming a platelet, a
25 platelet-like membrane-bound cell fragment or an anucleate cell fragment for example a MEG01 or DAMI cancer cell line; or other immortal cell that is capable of forming a platelet, a platelet-like membrane-bound cell fragment or an anucleate cell fragment;
or

c) an effector-chassis, for example is a platelet, a platelet-like membrane-bound
30 cell fragment or anucleate cell fragment.

Platelets that have been engineered to have reduced thrombogenicity may be termed a "Synlet", and the term Synlet as used herein, for example in the Examples, refers to an engineered platelet that has a disruption or deletion of genes that are involved in
35 thrombogenicity, resulting a platelet with reduced thrombogenicity. Such engineered platelets, or Synlets, are described in WO 2022/263824 and are contemplated by the present invention.

In some embodiments the chassis has been modified so as to drive differentiation to a producer-chassis, for example drive differentiation to a megakaryocyte or a megakaryocyte-like cell, for example has been forward programmed to differentiate into a megakaryocyte or a megakaryocyte-like cell.

5

In some embodiments the chassis has been engineered so as to have inhibited expression from the beta 2 microglobulin gene, for example wherein the beta 2 microglobulin gene has been knocked out or deleted.

- 10 The chassis may be a mammalian chassis, for example a human chassis, bovine chassis, equine chassis or murine chassis.

- In some preferred embodiments, the chassis has been engineered to disrupt a platelet thrombogenic pathway. For example the chassis may have been engineered so as to have reduced thrombogenicity relative to a chassis that has not been engineered so as to have reduced thrombogenic potential, for example wherein the engineered chassis has no thrombogenic potential.

- 20 In some instances the chassis has been engineered to disrupt or delete at least two, three, four, five, six, seven, eight, nine, or at least ten genes involved in the thrombogenic pathway, for example wherein the genes are selected from the group of genes encoding:

- 25 a protein involved in recognition of primary stimuli of thrombus formation;
a protein involved in recognition of secondary mediators of thrombus formation;
and/or
a protein involved in the release of secondary mediators of thrombus formation.

- 30 The chassis may comprise a disruption or deletion of at least:
one gene that encodes a protein involved in recognition of primary stimuli of thrombus formation;
one gene that encodes a protein involved in recognition of secondary mediators of thrombus formation; and
35 one gene that encodes a protein involved in the release of secondary mediators of thrombus formation;

for example comprises a disruption of at least:

two genes that encode a protein involved in recognition of primary stimuli of thrombus formation;

two genes that encode a protein involved in recognition of secondary mediators of thrombus formation; and

5 two genes that encode a protein involved in the release of secondary mediators of thrombus formation;

for example comprises a disruption of at least:

10 three genes that encode a protein involved in recognition of primary stimuli of thrombus formation;

three genes that encode a protein involved in recognition of secondary mediators of thrombus formation; and

three genes that encode a protein involved in the release of secondary mediators of thrombus formation.

15

In some exemplary embodiments:

20 the at least one, two or three genes that encode a protein involved in recognition of primary stimuli of thrombus formation are selected from the group consisting of: GPIb/V/IX and GPVI (GP6), ITGA2B, CLEC2, integrins $\alpha_{IIb}\beta_3$, $\alpha_2\beta_1$, $\alpha_5\beta_1$ and $\alpha_6\beta_1$, or from the group consisting of GPVI and ITGA2B;

25 the at least one, two or three that encode a protein involved in recognition of secondary mediators of thrombus formation are selected from the group consisting of Par1, Par4, P2Y12, GPIb/V/IX, the Thromboxane receptor (TBXA2R), P2Y1, P2X1 and integrin $\alpha_{IIb}\beta_3$ or from the group consisting of Par1, Par4 and P2Y12; and/or

30 the at least one, two or three genes that encode a protein involved in the release of secondary mediators of thrombus formation are selected from the group consisting of Cox1, HPS and thromboxane-A synthase (TBXAS1) or from the group consisting of Cox1 and HPS.

In some embodiments of the chassis, each of the following genes is disrupted or deleted:

35 ITGA2B, Par1 and HPS;
ITGA2B, P2Y12 and HPS; or
GPVI, ITGA2B, Par1, Par4, P2Y12, Cox1 and HPS.

In some embodiments, instead of, or in addition to, the genes that are disrupted above, the chassis may comprise a disruption in any one or more of the following genes:

TBXAS1, ITGB1, TMEM16F and/or B2m.

5

By disrupt we include the meaning of entirely knocking out the protein expression of the gene. The gene itself may be deleted, or may be disrupted by other modifications that can reduce or knockout the expression from a gene.

10 In some embodiments the chassis is that:

- a) does not respond to endogenous stimuli that usual results in clot formation;
- b) is not recruited by other activated platelets; and/or
- c) on activation, is not able to recruit and activate endogenous platelets in a

15

patient.

In some embodiments the chassis has been engineered to have reduced immunogenicity relative to a non-engineered chassis.

20 In some embodiments of the chassis :

a) the function of endogenous MHC Class 1 and/or MHC Class 2 has been disrupted; and/or

25 b) expression from the $\beta 2$ microglobulin gene has been disrupted, for example has been knocked out.

In some embodiments the chassis has been engineered to have disrupted expression from one or more HLA genes, for example has been engineered to have disrupted expression from any one or more of HLA-A, HLA-B and/or HLA-C, optionally wherein expression of HLA-A and HLA-B has been entirely disrupted but wherein expression of

30 HLA-C has been partially disrupted, optionally wherein expression from both alleles of HLA-A and HLA-B have been disrupted but wherein expression from only one allele of HLA-C has been disrupted.

In some embodiments the chassis has been engineered to overexpress anyone or more

35 of the HLA class Ib genes, optionally any one or more of HLA-G, HLA-E, CD47 and PD-L1, and may also for example may been engineered to have inhibited expression from the beta 2 microglobulin gene.

In some embodiments the chassis has been engineered to overexpress one or more immunomodulatory genes, optionally wherein the one or more immunomodulatory genes is selected from the group comprising CD47 and PD-L1.

- 5 In some embodiments the chassis has been engineered to eliminate one or more genes of which the product(s) could negatively affect the potency of a cargo.

In some embodiments the chassis has been engineered to tune up or down the innate/adaptive response.

10

In some embodiments the chassis has been engineered to reduce inflammation, angiogenesis, atherosclerosis, lymphatic development and tumour growth.

- 15 In some embodiments the chassis has been engineered to disrupt one or more genes encoding adhesive proteins and/or cargo entities which are likely to indirectly counter the biological action of an engineered cargo.

In some embodiments the chassis has been engineered to downregulate or inhibit expression of TGF β and/or GARP and/or CD40L.

20

- In some embodiments the chassis has been engineered to downregulate or inhibit expression of any one or more of CD36, NOD2, SRB1, TLR1, TLR2, TLR3, TLR4, TLR6, TLR9, CD40L, CD93 (C1qRp), C3aR, CD88 (C5aR), CD89 (Fc α R1), CD23 (Fc ϵ R1), CD32 (Fc γ RIIa), MHC class1, CD191 (CCR1), CD193 (CCR3), CD194 (CCR4), CD184 (CXCR4), CX3CR1, CD102 (ICAM-2), JAM-C/JAM-3, CD62P (P-selectin), CD31 (PECAM-1), CD150 (SLAMF1), CCL2, CCL3, CCL5, CXCL1, CXCL12, CXCL4/PF4, CXCL5, CXCL8, NAP2 (CXCL7), IL-1 β .

25

- 30 In some embodiments the chassis has been engineered to disrupt or inhibit expression of TGF β and/or GARP2.

In some embodiments the chassis has been engineered to disrupt or inhibit expression of any one or more of Siglec-7, Siglec-9, Siglec-11 and TGF β .

- 35 In some embodiments the chassis has been engineered to express one or more additional ITAM receptors to enhance T cell signaling and stimulate an immune response.

In some embodiments the chassis has been engineered to have reduced immunogenicity relative to a non-engineered chassis, wherein the chassis has been engineered to:

- 5 a) have disrupted function of MHC Class 1 genes or proteins;
- b) have disrupted expression from the $\beta 2$ microglobulin gene, optionally to knock out the $\beta 2$ microglobulin gene;
- c) have disrupted expression from one or more HLA genes;
- d) have disrupted expression from any one or more of HLA-A, HLA-B and/or
- 10 HLA-C, optionally wherein expression of HLA-A and HLA-B has been entirely disrupted but wherein expression of HLA-C has been partially disrupted, optionally wherein expression from both alleles of HLA-A and HLA-B have been disrupted but wherein expression from only one allele of HLA-C has been disrupted;
- e) overexpress anyone or more of the HLA class Ib genes, optionally any one or more of HLA-G, HLA-E, CD47 and PD-L1;
- f) been engineered to overexpress any one or more of HLA-G, HLA-E, CD47 and PD-L1 and optionally has been engineered to have disrupted expression from the beta 2 microglobulin; and/or
- 20 g) overexpress one or more immunomodulatory genes, optionally wherein the one or more immunomodulatory genes is selected from the group comprising CD47 and PD-L1.

In some embodiments the chassis has been engineered to:

- 25 a) have disrupted function of MHC Class 1 genes or proteins;
- b) have disrupted expression from the $\beta 2$ microglobulin gene, optionally to knock out the $\beta 2$ microglobulin gene;
- c) have disrupted expression from one or more HLA genes;
- d) have disrupted expression from any one or more of HLA-A, HLA-B and/or HLA-C,
- 30 optionally wherein expression of HLA-A and HLA-B has been entirely disrupted but wherein expression of HLA-C has been partially disrupted, optionally wherein expression from both alleles of HLA-A and HLA-B have been disrupted but wherein expression from only one allele of HLA-C has been disrupted;
- e) overexpress anyone or more of the HLA class Ib genes, optionally any one or more of HLA-G, HLA-E, CD47 and PD-L1;
- 35

- f) overexpress any one or more of HLA-G, HLA-E, CD47 and PD-L1 and optionally has been engineered to have disrupted expression from the beta 2 microglobulin gene; and/or
- g) overexpress one or more immunomodulatory genes, optionally wherein the one or more immunomodulatory genes is selected from the group comprising CD47 and PD-L1;
- h) eliminate one or more genes or gene products for which the product(s) could negatively affect the potency of a cargo;
- i) tune up or down the innate/adaptive response;
- 10 j) reduce inflammation, angiogenesis, atherosclerosis, lymphatic development and tumour growth;
- k) have disrupted expression of one or more genes encoding adhesive proteins and/or cargo entities which are likely to indirectly counter the biological action of the engineered cargo, potentially leading to a greater net therapeutic effect;
- 15 l) downregulate or inhibit expression of TGF β and/or GARP and/or CD40L;
- n) downregulate or inhibit expression of any one or more of CD36, NOD2, SRB1, TLR1, TLR2, TLR3, TLR4, TLR6, TLR9, CD40L, CD93 (C1qRp), C3aR, CD88 (C5aR), CD89 (Fc α R1), CD23 (Fc ϵ R1), CD32 (Fc γ RIIa), MHC class1, CD191 (CCR1), CD193 (CCR3), CD194 (CCR4), CD184 (CXCR4), CX3CR1, CD102 (ICAM-2), JAM-C/JAM-3, CD62P (P-selectin), CD31 (PECAM-1), CD150 (SLAMF1), CCL2, CCL3, CCL5, CXCL1, CXCL12, CXCL4/PF4, CXCL5, CXCL8, NAP2 (CXCL7), IL-1 β
- 20 o) disrupt or inhibit expression of TGF β and/or GARP;
- q) disrupt or inhibit expression of any one or more of Siglec-7, Siglec-9, Siglec-11 or TGF β
- 25 s) disrupt or inhibit expression of any one or more of GPIb/V/IX and GPVI (GP6), ITGA2B, CLEC2, integrins α IIBb3, α 2b1, α 5b1 and α 6b1, GPVI and ITGA2B;
- t) disrupt or inhibit expression of anyone or more of Par1, Par4, P2Y12, GPIb/V/IX, the Thromboxane receptor (TBXA2R), P2Y1, P2X1 and integrin α IIBb3 or from the group consisting of Par1, Par4 and P2Y12;
- 30 u) disrupt or inhibit expression of anyone or more of Cox1, Cox2, HPS, prothrombin, PDGF, EGF, von Willebrand Factor and thromboxane-A synthase (TBXAS1);
- v) to synthesise a protein or RNA of interest in response to activation of the platelet or platelet-like membrane-bound cell fragment, optionally wherein the protein or RNA of interest is expressed from the BCL-3 mRNA untranslated regions, optionally 5'UTR;
- 35 z) to express one or more cargo proteins or cargo RNAs, optionally wherein the cargo protein or cargo RNA comprises an alpha-granule targeting signal, optionally comprises a platelet factor 4 (PF4) or von Willebrand factor (vWf);

- aa) express at least two chimeric aGPCRs, optionally express at least 3, 4, 5, 6, 7, 8, 9 or at least 10 different aGPCRs, as described herein;
- bb) express at least two chimeric aGPCRs and wherein the target binding domain of the at least two chimeric aGPCRs are directed towards different targets;
- 5 cc) express at least two chimeric aGPCRs that operate together to form a logic circuit;
- Dd) express at least one chimeric aGPCR of the present invention and at least one chimeric platelet receptor as defined in WO2022263824, i.e. a chimeric platelet receptor that comprises a) an intracellular domain that is a platelet stimulation domain and comprises domains from an immunoreceptor tyrosine-based activation motif (ITAM) receptor; and b) a heterologous targeting domain that recognizes and binds a target;
- 10 ee) express one or more cargo, optionally wherein the cargo is selected from the group comprising:
- a) a protein or peptide – optionally wherein the protein or peptide is:
 - i) an antibody or antigen binding fragment thereof, for example an antibody or antigen binding fragment thereof binds to a tumor antigen or a neoantigen;
 - 20 ii) an enzyme, such as a nuclease for example a TALEN;
 - iii) a cytokine for example IL-10; or
 - iv) a CRISPR associated protein, for example Cas9;
 - v) a bispecific protein, for example a bispecific antibody or optionally a T-cell engager (BiTE);
 - 25 vi) a fusion protein comprising an exosome targeting domain, optionally wherein the fusion protein comprises:
 - a) the cargo protein or peptide; and
 - 30 b) an exosome targeting domain, optionally wherein the exosome targeting domain is selected from the group comprising or consisting of:
 - i) an exosome specific membrane protein or exosome membrane targeting portion thereof, for example:
 - 35 a tetraspanin, for example CD63; or

a non-tetraspanin such as PTGFRN or BASP1

5 ii) an exosome targeting sequence from a soluble protein, optionally the WW domain of Nedd4 ubiquitin ligases;

iii) a ubiquitin tag; and/or

iv) a tag binding domain, optionally a nanobody directed against a tag, optionally a nanobody directed against GFP.

15 b) a nucleic acid, optionally wherein the nucleic acid is:
 i) an RNA, for example selected from mRNA, a miRNA, shRNA, and a
 clustered regularly interspaced short palindromic repeats (CRISPR)
 sequence; and/or

20 ii) an RNA that comprises an exosome targeting domain, optionally wherein the exosome targeting domain is selected from the group comprising or consisting of:

- a) an exosome targeting hairpin or linear motif;
- b) a viral exosome targeting RNA or exosome targeting fragment thereof;

25 iii) an RNA that comprises an aptamer domain, optionally wherein the aptamer domain is selected from:

30 a) a MS2 binding stem-loop;
b) a C/D box; and/or
c) an AU rich element, optionally wherein the RNA is an mRNA that encodes Cas9;

ff) express a fusion protein wherein the fusion protein comprises:

35 i) the bacteriophage coat protein MS2 fused to an exosome membrane protein, optionally wherein the exosome membrane protein is selected from the group comprising or consisting of Lamp2b, VSVG, CD63; and/or

ii) the archaeal ribosomal protein L7Ae fused to an exosome membrane protein, optionally wherein the exosome membrane protein is selected from the group comprising or consisting of Lamp2b, VSVG, CD63; and/or

iii) a CD9-HuR fusion protein;

optionally wherein the fusion protein further comprises a light activated dimerization protein;

5

gg) to express one or more cargo only upon binding of one or more CPRs, universal CPRs, complexes of universal CPRs and tagged targeting peptides, SAPRs or ePARs to the target, optionally wherein the cargo is selected from the group comprising:

a) a protein or peptide, optionally:

10 i) an antibody or antigen binding fragment thereof, for example an antibody or antigen binding fragment thereof binds to a tumor antigen or a neoantigen;

ii) an enzyme, such as a nuclease for example a TALEN;

iii) a cytokine for example IL-10; or

15 iv) a CRISPR associated protein, for example Cas9;

v) a bispecific protein, for example a bispecific antibody or optionally a T-cell engager (BiTE)

b) a nucleic acid – in some embodiments the nucleic acid is:

20 i) an RNA, for example selected from mRNA, a miRNA, shRNA, and a clustered regularly interspaced short palindromic repeats (CRISPR) sequence,

optionally wherein the cargo is expressed from the Bcl-3 mRNA untranslated regions, optionally 5'UTR.

25 Where the chassis is a platelet or an engineered platelet, and as described in WO 2022/263824, the platelet or engineered platelet can express a cargo or be loaded with a cargo. Upon binding of the chimeric GCPR to the target, the platelet or engineered platelet degranulates, releasing the cargo in the vicinity of the target and so can be used for, amongst other things, targeted drug delivery.

30

The cargo may be any cargo, for example may be:

a therapeutic agent;

an imaging agent,

a non-therapeutic agent; and/or

35 a cosmetic-agent.

Accordingly in some embodiments the chassis of the invention comprises one or more cargo. For example the chassis have been:

- a) loaded with one or more cargo; and/or
- b) engineered so as to express one or more cargo.

5 In some embodiments the cargo may be selected from any one or more of:

- a) a protein or peptide – in some embodiments the protein or peptide is:
 - i) an antibody or antigen binding fragment thereof, for example an antibody or antigen binding fragment thereof binds to a tumor antigen or a neoantigen;
 - 10 ii) an enzyme, such as a nuclease for example a TALEN;
 - iii) a cytokine for example IL-10; or
 - iv) a CRISPR associated protein, for example Cas9;
 - v) a bispecific protein, for example a bispecific antibody or optionally a T-cell engager (BiTE)
 - 15
- b) a nucleic acid – in some embodiments the nucleic acid is:
 - i) an RNA, for example selected from mRNA, a miRNA, shRNA, and a clustered regularly interspaced short palindromic repeats (CRISPR) sequence; or
 - 20 ii) a DNA vector;
- c) a toxin;
- d) a small molecule drug, imaging agent, radionucleotide drug, radionucleotide tagged antibody, or any conjugate thereof;
- e) a viral vector such as AAV;
- 25 f) a virus such as oncolytic virus;
- g) agents for performing CRISPR mediated gene editing;
- h) an exosome, for example an exosome pre-loaded with a second cargo;
 - i) or a nanoparticle or nanoparticles;
 - and/or
- 30 j) a lipid nanoparticle (LNP) comprising an RNA or an mRNA; or any combination thereof.

In some instances the cargo is an endogenously expressed cargo.

For example the endogenously expressed cargo may be any one or more of:

35

- a) a protein or peptide – in some embodiments the protein or peptide is:

- i) an antibody or antigen binding fragment thereof, for example an antibody or antigen binding fragment thereof binds to a tumor antigen or a neoantigen;
- ii) an enzyme, such as a nuclease for example a TALEN;
- 5 iii) a cytokine for example IL-10; or
- iv) a CRISPR associated protein, for example Cas9;
- v) a bispecific protein, for example a bispecific antibody or optionally a T-cell engager (BiTE)
- b) a nucleic acid – in some embodiments the nucleic acid is:
 - 10 i) an RNA, for example selected from mRNA, a miRNA, shRNA, and a clustered regularly interspaced short palindromic repeats (CRISPR) sequence.

In other instances, the cargo is exogenously loaded into the chassis,
For example the exogenously loaded cargo may be any one or more of:

- 15 a) a protein or peptide – in some embodiments the protein or peptide is:
 - i) an antibody or antigen binding fragment thereof, for example an antibody or antigen binding fragment thereof binds to a tumor antigen or a neoantigen;
 - 20 ii) an enzyme, such as a nuclease for example a TALEN;
 - iii) a cytokine for example IL-10; or
 - iv) a CRISPR associated protein, for example Cas9;
 - v) a bispecific protein, for example a bispecific antibody or optionally a T-cell engager (BiTE)
- 25 b) a nucleic acid – in some embodiments the nucleic acid is
 - an RNA, for example selected from mRNA, a miRNA, shRNA, and a clustered regularly interspaced short palindromic repeats (CRISPR) sequence;
 - c) a lipid nanoparticle comprising an RNA nucleic acid such as a mRNA, a miRNA, or shRNA.

30

The skilled person will understand what is meant by the term lipid nanoparticle in the context of RNA delivery. See for example Hou et al 2021 Nature Reviews Materials 6: 1078-1094.

- 35 In some instances the cargo comprises an exosome targeting domain.
In some instances the cargo is a fusion protein comprising:

- a) the cargo protein or peptide; and

b) an exosome targeting domain, optionally wherein the exosome targeting domain is selected from the group comprising or consisting of:

- 5 i) an exosome specific membrane protein or exosome membrane targeting portion thereof, for example:
a tetraspanin, for example CD63; or
a non-tetraspanin such as PTGFRN or BASP1
- 10 ii) an exosome targeting sequence from a soluble protein, optionally the WW domain of Nedd4 ubiquitin ligases;
- iii) a ubiquitin tag; and/or
- iv) a tag binding domain, optionally a nanobody directed against a tag,
15 optionally a nanobody directed against GFP; and/or
- v) a protein selected from the proteins listed in Table A.

Where the cargo is an RNA, and the exosome targeting domain may be is:

- 20 a) an exosome targeting hairpin or linear motif;
- b) a viral exosome targeting RNA or exosome targeting fragment thereof;
- c) an aptamer, optionally:
- 25 i) a MS2 binding stem-loop;
- ii) a C/D box; and/or
- iii) an AU rich element, optionally wherein the RNA is an mRNA that encodes Cas9.

30

Where the chassis has been engineered to express a fusion protein, the fusion protein may comprise:

- a) the bacteriophage coat protein MS2 fused to an exosome membrane protein,
35 optionally wherein the exosome membrane protein is selected from the group comprising or consisting of Lamp2b, VSVG, CD63 or any of the proteins of Table A; and/or
- b) a fusion protein comprising the archaeal ribosomal protein L7Ae fused to an exosome membrane protein, optionally wherein the exosome membrane protein is

selected from the group comprising or consisting of Lamp2b, VSVG, CD63 or any of the proteins of Table A; and/or

- 5 c) an aptamer binding protein fused to an exosome membrane protein, optionally wherein the exosome membrane protein is selected from the group comprising or consisting of Lamp2b, VSVG, CD63 or any of the proteins of Table A of WO 2022/263824 which is specifically herein incorporated by reference.

The fusion protein may further comprise a light activated dimerization protein.

- 10 In some instances, the chassis comprises a cargo that is an RNA that comprises an exosome targeting domain that is an MS2 binding stem-loop, the chassis has been engineered to express a fusion protein, wherein the fusion protein comprises the bacteriophage coat protein MS2 fused to an exosome membrane protein, optionally wherein the exosome membrane protein is selected from the group comprising or
15 consisting of Lamp2b, VSVG, CD63 or any of the proteins of Table A; WO 2022/263824 which is specifically herein incorporated by reference.

- In some instances the chassis comprises a cargo that is an RNA that comprises an exosome targeting domain that is a C/D box, the chassis has been engineered to
20 express a fusion protein, wherein the fusion protein comprises the archaeal ribosomal protein L7Ae fused to an exosome membrane protein, optionally wherein the exosome membrane protein is selected from the group comprising or consisting of Lamp2b, VSVG, CD63 or any of the proteins of Table A WO 2022/263824 which is specifically herein incorporated by reference.

- 25 In some instances the chassis comprises a cargo that is an RNA that comprises an aptamer, the chassis has been engineered to express a fusion protein, wherein the fusion protein comprises a protein or fragment thereof capable of being bound by the aptamer fused to an exosome membrane protein, optionally wherein the exosome
30 membrane protein is selected from the group comprising or consisting of Lamp2b, VSVG, CD63 or any of the proteins of Table A WO 2022/263824 which is specifically herein incorporated by reference.

- In some embodiments the chassis comprises a cargo that is an RNA that comprises an
35 exosome targeting domain that is an AU rich element, the producer or effector-chassis has been engineered to express a fusion protein, wherein the fusion protein is a CD9-HuR fusion protein.

In some embodiments the cargo is an RNA that encodes a Cas protein, optionally a Cas9 protein. In the same or different embodiments the chassis has been engineered to express one or more sgRNAs.

- 5 It will be apparent that the invention also provides various therapeutic uses of the chimeric aGPCRs, nucleic acids, systems and chassis described herein, along with targeted delivery systems.

10 The invention also provides a targeted delivery system comprising a chassis of the invention wherein the chassis expresses one or more chimeric aGPCRs of the invention, and comprises a cargo, for example wherein the targeted delivery system is a therapeutic targeted delivery system or a non-therapeutic delivery system. In preferred embodiments the chassis will comprise a cargo that is a therapeutic agent.

15 The invention also provides a chimeric aGPCR, nucleic acid, vector, system, complex or chassis according to the invention for use in medicine.

20 The invention also provides a chimeric aGPCR, nucleic acid, vector, system, complex or chassis according to the invention for use in delivering a therapeutic or imaging cargo; or treating or preventing cancer, an autoimmunity disease, genetic disease, cardiovascular disease and/or an infection.

25 The invention also provides a method of delivering a cargo comprising administering an effective amount of any one or more of a chimeric aGPCR, nucleic acid, vector, system, complex or chassis of the invention, where the chassis comprises or expresses a cargo.

30 The invention also provides a method of targeted cargo delivery to a target cell, tissue or site in the body wherein the method comprises administering an effective amount of any one or more of a chimeric aGPCR, nucleic acid, vector, system, complex or chassis of the invention, for example a chassis of the invention that comprises a cargo.

35 The invention also provides a non-therapeutic method of delivering cargo to a subject in need thereof comprising administering an effective amount of any one or more of a chimeric aGPCR, nucleic acid, vector, system, complex or chassis of the invention, for example a chassis of the invention that comprises a non-therapeutic cargo.

The invention also provides a method of treatment comprising administering an effective amount of any one or more of a chimeric aGPCR, nucleic acid, vector, system, complex or chassis of the invention, for example wherein the method is for the treatment or prevention of any one or more of cancer, an autoimmunity disease,
5 genetic disease, cardiovascular disease and/or an infection, for example a chassis of the invention that comprises a therapeutic cargo.

The invention also provides the use of any one or more of a chimeric aGPCR, nucleic acid, vector, system, complex or chassis of the invention in the manufacture of a medicament for the treatment or prevention of disease or infection, for example for
10 the treatment or prevention of any one or more of cancer, an autoimmunity disease, genetic disease, cardiovascular disease and/or an infection, for example wherein the chassis is an effector-chassis or engineered effector-chassis.

The invention also provides a method of using the chimeric aGPCR, nucleic acid, vector,
15 system, complex or chassis of the invention to deliver a cargo, for example a therapeutic agent, by administering the chimeric aGPCR, nucleic acid, vector, system, complex or chassis of the invention.

The invention also provides various kits and kits of parts comprising any one or more
20 of the agents described herein. For example the invention provides a kit comprising any two or more of the following:

- A chassis of the invention;
- A chimeric aGPCR of the invention;
- 25 A nucleic acid of the invention;
- A vector of the invention;
- A system of the invention;
- A complex of the invention.

30 The invention also provides a kit comprising a chimeric aGPCR of the invention and a corresponding intermediate adaptor protein or peptide, wherein the chimeric aGPCR can bind to the intermediate adaptor protein or peptide.

The invention also provides the following embodiments:
35

Embodiments

1. A chimeric adhesion G-Protein Coupled Receptor (aGPCR) comprising:
 - a) an intracellular tail domain, optionally a C-terminal tail;
 - b) a seven-transmembrane domain (7TM) that comprises three intracellular loops (ICLs); and
 - c) an extracellular domain comprising:
 - (i) a target binding domain that is heterologous to the intracellular tail domain;
 - (ii) a GPCR autoproteolysis-inducing domain (GAIN domain) that optionally comprises a tethered agonist peptide;
- 10 and optionally further comprising
 - (iii) a linker, optionally wherein the linker is in the extracellular domain, optionally between the GAIN domain and the target binding domain.
2. The chimeric aGPCR of embodiment 1 wherein the GAIN domain comprises a GPCR-proteolytic site (GPS) site optionally positioned N-terminal of the tethered peptide agonist.
- 15 3. The chimeric aGPCR of embodiment 2 wherein the GPS site is cleaved by the GAIN domain, optionally is constitutively cleaved by the GAIN domain.
- 20 4. The chimeric aGPCR of embodiment 2 wherein the GPS site is not cleaved, optionally not cleaved by the GAIN domain.
5. The chimeric aGPCR of embodiment 4 wherein the N-terminal domain further comprises a protease site, optionally comprises a protease site that is a cysteine protease site, a metallo protease site, an aspartate protease site, a serine protease site or a threonine protease site, optionally wherein the protease site is a site for a protease that is associated with the tumour microenvironment.
- 25 6. The chimeric aGPCR of embodiment 5 wherein the chimeric aGPCR becomes activated upon cleavage of the protease site in the absence of binding of the target binding domain to the target.
- 30 7. The chimeric aGPCR of embodiment 5 wherein the chimeric aGPCR becomes activated only upon cleavage of the protease site and binding of the target binding domain to the target.
- 35

8. The chimeric aGPCR of embodiments 2 or 3 wherein cleavage of the GPS motif produces an N-terminal fragment (NTF) and a C-terminal fragment (CTF), and wherein the NTF and the CTF remain associated with one another via non-covalent interactions, optionally remain associated via non-covalent interactions when present in a lipid membrane, optionally the lipid membrane of a cell, platelet, or engineered platelet.
9. The chimeric aGPCR of embodiment 8 where the association of the NTF and the CTF prevents the tethered peptide agonist from interacting with the 7TM domain and so prevents activation of intracellular signalling.
10. The chimeric aGPCR of any of embodiments 1-9 wherein the chimeric aGPCR is:
- a) is inactive in the absence of binding of the target binding domain to the target;
 - c)-predominantly occupies the inactive conformation in the absence of binding of the target binding domain to the target; and/or
 - c) remains in a basal activity state in the absence of binding of the target binding domain to the target.
11. The chimeric aGPCR of any of embodiments 1-3 or 5-10 wherein binding of the target binding domain to the target results in:
- a) modulation of the non-covalent interactions between the NTF and CTF and activation of the CTF, optionally by allowing the tethered peptide agonist to interact with the 7TM and activate intracellular signalling; or
 - b) rupture of the non-covalent interactions between the NTF and CTF and activation of the CTF, optionally by exposing the tethered peptide agonist allowing it to interact with the 7TM and activate intracellular signalling.
12. The chimeric aGPCR of any of embodiments 1-3 or 5-11 wherein binding of the target binding domain to the said target causes mechanical forces to be exerted on the NTF/CTF non-covalent interaction.
13. The chimeric aGPCR of any of embodiments 1-12 wherein the:
- intracellular tail domain, optionally the C-terminal tail is autologous to the 7TM domain;
 - intracellular tail domain, optionally the C-terminal tail is autologous to the GAIN domain; and/or

intracellular tail domain, optionally the C-terminal tail is autologous to the GPS motif.

14. The chimeric aGPCR of any of embodiments 1-13 wherein the chimeric aGPCR activates intracellular signalling when:

5

a) the chimeric aGPCR is localised to the plasma membrane of a chassis, optionally a cell, a platelet or an engineered platelet; and optionally when

b) the chassis, optionally the cell, platelet or engineered platelet is incubated and agitated in the presence of the target.

10

15. The chimeric aGPCR of embodiment 14 where the chassis, optionally the cell, platelet or engineered platelet comprises a reporter system

Optionally where the reporter system comprises a reporter protein that is expressed when intracellular signalling is activated, optionally wherein the reporter protein is expressed from a promoter that comprises a nuclear factor of activated T cells (NFAT) response element, optionally wherein the reporter protein is a luciferase enzyme.

15

16. The chimeric aGPCR of embodiment 15 where the activation of intracellular signalling is determined by the detection of the expression or activity of the reporter protein, optionally detection of the expression or activity of luciferase.

20

17. The chimeric aGPCR of embodiment 16 wherein the activation of intracellular signalling is detected by the use of a calcium indicator dye, optionally Fluo-4.

25

18. The chimeric aGPCR of any 14-17 where the agitation is performed by shaking at a speed of:

At least 10 rpm, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190 or at least 200 rpm;

30

Less than 200 rpm, 190, 180, 170, 160, 150, 140, 130, 120, 110, 100, 90, 80, 70, 60, 50, 40, 30, 20 or less than 10 rpm; and/or

Between 10 rpm and 200 rpm, 20 rpm and 190 rpm, 30 rpm and 180 rpm, 40 rpm and 170 rpm, 50 rpm and 160 rpm, 60 rpm and 150 rpm, 70 rpm and 140 rpm, 80 rpm and 130 rpm.

35

19. The chimeric aGPCR of any of embodiments 1-18 wherein the intracellular domain comprises one or more substitutions, insertions or deletions as compared to

the autologous intracellular domain or to a wild-type intracellular domain, optionally wherein

the one or more substitutions, insertions or deletions increases the intracellular signalling response as compared to the autologous intracellular domain or to a wild-type intracellular domain that does not comprise the same one or more substitutions, insertions or deletions; or

the one or more substitutions, insertions or deletions decreases the intracellular signalling response as compared to the autologous intracellular domain or to a wild-type intracellular domain that does not comprise the same one or more substitutions, insertions or deletions;
and/or

the one or more substitutions, insertions or deletions alters the specificity of the intracellular signalling response as compared to the autologous intracellular domain or to a wild-type intracellular domain that does not comprise the same one or more substitutions, insertions or deletions resulting in altered signalling pathway activation.

20. The chimeric aGPCR of any of embodiments 1-19 further comprising a signal peptide, optionally a signal peptide that has been selected to achieve a desired level of chimeric aGPCR at the cell, platelet, or engineered platelet surface.

21. The chimeric aGPCR of embodiment where the signal peptide is an engineered signal peptide.

22. The chimeric aGPCR of embodiment 20 or 21 where the signal peptide is selected from the group comprising or consisting of:

ADGRG1 signal peptide MTPQSLLQTTLFLLSLLFLVQGAHG [SEQ ID NO: 1]

ADGRF5 signal peptide MKSPRRTTLCLMFIVYSSKA [SEQ ID NO: 2]

FCERG signal peptide MIPAVVLLLLLLVEQAAA [SEQ ID NO: 3]

CD28 signal peptide MLRLLLALNLFPSIQVTG [SEQ ID NO: 4]

Platelet signal peptide – GPIIb (aIIb) MARALCPLQALWLEWVLLLLGPCAAPPAAWA [SEQ ID NO: 5]

Platelet signal peptide – GPIIIa (β 3)

- MRARPRPRPLWATVLALGALAGVGVG [SEQ ID NO: 6]
Platelet signal peptide - GPIBaMPLLLLLLLPSPLHP [SEQ ID NO: 7]
Platelet signal peptide - GPIX MPAWGALFLLWATAEA [SEQ ID NO: 8]
Platelet signal peptide - GPV MLRGTLCAVLGLLRA [SEQ ID NO: 9]
- 5
- MAPFASLASGILLLSLITSSKA [SEQ ID NO: 36]
MLLGPGHTLSAPALALAVTLTLLVRSASP [SEQ ID NO: 376]
MLLSVPLLLGLLGLAAA [SEQ ID NO: 386]
MQELRGILLCLLAAAAPTTP [SEQ ID NO: 369]
- 10
- MRYVASYLLAALGGNS [SEQ ID NO: 40]
MGKSPEAWCIVLFSVLASFSA [SEQ ID NO: 41]
MASSGSVQQPRLVLLMLVLAGAARA [SEQ ID NO: 42]
MRWKIIQLQYCFLLVPCMLTALEA [SEQ ID NO: 43]
MLSRSLCLALAWVARVGA [SEQ ID NO: 44]
- 15
- MRFSCLALLPGVALLASARLAAA [SEQ ID NO: 45]
MRVLWVLGLCCVLLTFGFVRA [SEQ ID NO: 46]
MKFPMVAAAALLLCAVRA [SEQ ID NO: 47]
MRSLLASFCLLAVALA [SEQ ID NO: 48]
MKILLLCVGLLLTWDNGMVLG [SEQ ID NO: 49]
- 20
- MLRISGRNMKVLFAAALIVGSVVFLLPGPSVA [SEQ ID NO: 50]
MAATVRRQRPRRLCWTLVAVLLADLLALS [SE [SEQ ID NO: 51]
MKMGVRLAARAWPLCGLLAALGGVCA [SEQ ID NO: 52]
MWWRLWWLLLLLLLWLALAAAA [SEQ ID NO: 53]
MGWSLILLFLVAVATRVLS [SEQ ID NO: 54]
- 25
- MDFQVQIISFLLISASVIMSRG [SEQ ID NO: 55]
MEFGLSWVFLVALFRGVQC [SEQ ID NO: 56]
MKWVTFISLLFLFSSAYS [SEQ ID NO: 57]
MKLPVRLVLMFWIPAASA [SEQ ID NO: 58]
MNLILLITFVAAAVA [SEQ ID NO: 59]
- 30
- MGSAALLLVVLLLVWPSSRA [SEQ ID NO: 60]
MTRLTVLALLAGLLASSRA [SEQ ID NO: 61]
MWWRLWWLLLLLLLWPMVWA/AA [SEQ ID NO: 62]
MKLPVRLVLMFWIPASSS [SEQ ID NO: 63]
MDMRVPAQLLGLLLWLSGARC [SEQ ID NO: 64]
- 35
- MKYLLPTAAAGLLLLAAQPAMA [SEQ ID NO: 65]
MGVKVLFALICIAVAEA [SEQ ID NO: 66]
MPLLLLLPLWAGALA [SEQ ID NO: 67]
MRARALLAVLLLLLLVGIAAAA [SEQ ID NO: 68]

MATATLLAVLLLLLLVGSAGGA [SEQ ID NO: 69]

MRARALLVVLVLVLLGVASSA [SEQ ID NO: 70]

MPGPAAALLLLLLVLLGLGSAA [SEQ ID NO: 71]

MTTTTVLLLLVLVLAGLTSGA [SEQ ID NO: 72]

5

or a signal peptide sequence with at least 75%, 80%, 85%, 90%, 92%, 94%, 96%, 98% or 100% sequence identity to the above sequences.

23. The chimeric aGPCR of any of embodiments 1-22 wherein the:

10

- a) intracellular tail;
- b) seven-transmembrane domain (7TM); and
- c) GPCR autoproteolysis-inducing domain (GAIN domain) that optionally comprises a tethered agonist peptide and that optionally cleaves a GPS motif; and
- 15 d) GPS motif that is optionally cleaved by the GAIN domain

are autologous to one another

20 24. The chimeric aGPCR of any of embodiments 1-23 wherein the:

GAIN domain is heterologous to:
the 7TM domain;
the intracellular tail; and/or
25 the target binding domain.

25. The chimeric aGPCR of any of embodiments 1-23 wherein the:

- a) intracellular tail;
- 30 b) seven-transmembrane domain (7TM); and
- c) GPCR autoproteolysis-inducing domain (GAIN domain); and
- d) GPS motif

are from the same naturally occurring aGPCR.

35

26. The chimeric aGPCR of any of embodiments 1-23 wherein the:

- a) intracellular tail;

- b) seven-transmembrane domain (7TM); and
- c) GPCR autoproteolysis-inducing domain (GAIN domain); and/or
- d) GPS motif

5 are from the same naturally occurring aGPCR, and wherein any one or more of the:

- a) intracellular tail;
- b) seven-transmembrane domain (7TM); and
- c) GPCR autoproteolysis-inducing domain (GAIN domain); and/or
- 10 d) GPS motif

comprises an amino acid sequence that has at least 75%, 80%, 85%, 90%, 92%, 94%, 96%, 98% or 100% sequence identity to the corresponding domain of the naturally occurring aGPCR.

15

27. The chimeric aGPCR of any of embodiments 1-26 wherein the GAIN domain is from:

ADGRG1 [SEQ ID NO: 35];

ADGRL1 and has an amino acid sequence of [SEQ ID NO: 27];

20 ADGRL3 and has an amino acid sequence of [SEQ ID NO: 28];

ADGRE2 and has an amino acid sequence of [SEQ ID NO: 29]; ADGRG2 and has an amino acid sequence of [SEQ ID NO: 30];

or is a GAIN domain with an amino acid sequence that has at least 75%, 80%, 85%, 90%, 92%, 94%, 96%, 98% or 100% sequence identity to SEQ ID NO: 35, 27, 28, 29

25 or 30.

28. The chimeric aGPCR of embodiment 27 wherein:

the GAIN domain has at least 75%, 80%, 85%, 90%, 92%, 94%, 96%, 98% or 100% sequence identity to SEQ ID NO: 35 and:

30

the intracellular tail and/or one, two or three ICLs are not from ADGRG1;

the target binding domain is not from ADGRG1; and/or

the 7TM domain is not from ADGRG1;

the GAIN domain has at least 75%, 80%, 85%, 90%, 92%, 94%, 96%, 98% or 100% sequence identity to SEQ ID NO: 27 and:

35

the intracellular tail and/or one, two or three ICLs are not from ADGRL1;

the target binding domain is not from ADGRL1; and/or

the 7TM domain is not from ADGRL1;

the GAIN domain has at least 75%, 80%, 85%, 90%, 92%, 94%, 96%, 98% or 100% sequence identity to SEQ ID NO: 28 and:

- 5 the intracellular tail and/or one, two or three ICLs are not from ADGRL3;
the target binding domain is not from ADGRL3; and/or
the 7TM domain is not from ADGRL3;

the GAIN domain has at least 75%, 80%, 85%, 90%, 92%, 94%, 96%, 98% or 100% sequence identity to SEQ ID NO: 29 and:

- 10 the intracellular tail and/or one, two or three ICLs are not from ADGRE2;
the target binding domain is not from ADGRE2; and/or
the 7TM domain is not from ADGRE2; or

the GAIN domain has at least 75%, 80%, 85%, 90%, 92%, 94%, 96%, 98% or 100% sequence identity to SEQ ID NO: 30 and:

- 15 the intracellular tail and/or one, two or three ICLs are not from ADGRG2;
the target binding domain is not from ADGRG2; and/or
the 7TM domain is not from ADGRG2.

20

29. The chimeric aGPCR of any of embodiments 1-28 wherein the chimeric aGPCR comprises the following combination of naturally occurring and engineered domains:

Naturally occurring	Naturally occurring	Naturally occurring	Engineered domain
(a)	(b)	(c)	(d)
(a)	(b)	(d)	(c)
(a)	(c)	(b)	(d)
(a)	(c)	(d)	(b)
(a)	(d)	(b)	(c)
(a)	(d)	(c)	(b)
(b)	(a)	(c)	(d)
(b)	(a)	(d)	(c)
(b)	(c)	(a)	(d)
(b)	(c)	(d)	(a)
(b)	(d)	(a)	(c)
(b)	(d)	(c)	(a)

(c)	(a)	(b)	(d)
(c)	(a)	(d)	(b)
(c)	(b)	(a)	(d)
(c)	(b)	(d)	(a)
(c)	(d)	(a)	(b)
(c)	(d)	(b)	(a)
(d)	(a)	(b)	(c)
(d)	(a)	(c)	(b)
(d)	(b)	(a)	(c)
(d)	(b)	(c)	(a)
(d)	(c)	(a)	(b)
(d)	(c)	(b)	(a)
(d)	(c)	(b)	(a)

Naturally occurring	Naturally occurring	Engineered domain	Engineered domain
(a)	(b)	(c)	(d)
(a)	(b)	(d)	(c)
(a)	(c)	(b)	(d)
(a)	(c)	(d)	(b)
(a)	(d)	(b)	(c)
(a)	(d)	(c)	(b)
(b)	(a)	(c)	(d)
(b)	(a)	(d)	(c)
(b)	(c)	(a)	(d)
(b)	(c)	(d)	(a)
(b)	(d)	(a)	(c)
(b)	(d)	(c)	(a)
(c)	(a)	(b)	(d)
(c)	(a)	(d)	(b)
(c)	(b)	(a)	(d)
(c)	(b)	(d)	(a)
(c)	(d)	(a)	(b)
(c)	(d)	(b)	(a)
(d)	(a)	(b)	(c)
(d)	(a)	(c)	(b)

(d)	(b)	(a)	(c)
(d)	(b)	(c)	(a)
(d)	(c)	(a)	(b)
(d)	(c)	(b)	(a)
(d)	(c)	(b)	(a)

Naturally occurring	Engineered domain	Engineered domain	Engineered domain
(a)	(b)	(c)	(d)
(a)	(b)	(d)	(c)
(a)	(c)	(b)	(d)
(a)	(c)	(d)	(b)
(a)	(d)	(b)	(c)
(a)	(d)	(c)	(b)
(b)	(a)	(c)	(d)
(b)	(a)	(d)	(c)
(b)	(c)	(a)	(d)
(b)	(c)	(d)	(a)
(b)	(d)	(a)	(c)
(b)	(d)	(c)	(a)
(c)	(a)	(b)	(d)
(c)	(a)	(d)	(b)
(c)	(b)	(a)	(d)
(c)	(b)	(d)	(a)
(c)	(d)	(a)	(b)
(c)	(d)	(b)	(a)
(d)	(a)	(b)	(c)
(d)	(a)	(c)	(b)
(d)	(b)	(a)	(c)
(d)	(b)	(c)	(a)
(d)	(c)	(a)	(b)
(d)	(c)	(b)	(a)
(d)	(c)	(b)	(a)

Engineered domain	Engineered domain	Engineered domain	Engineered domain
-------------------	-------------------	-------------------	-------------------

(a)	(b)	(c)	(d)
-----	-----	-----	-----

wherein (a) is the intracellular tail domain for example the C-terminal tail; (b) is the
5 seven-transmembrane domain (7TM); (c) is the GPCR autoproteolysis-inducing domain
(GAIN domain) that comprises a tethered agonist peptide and that constitutively
cleaves a GPS motif; and (d) is the GPS motif that is cleaved by the GAIN domain;

and where an engineered domain is:

- 10 a) a domain that comprises an amino acid sequence that has at least one
substitution, insertion or deletion relative to the native or naturally occurring domain;
 b) a domain that comprises an amino acid sequence that has at least 75%,
80%, 85%, 90%, 92%, 94%, 96%, 98% or 100% sequence identity to the naturally
occurring domain; and/or
15 c) is a rationally designed domain.

30. The chimeric aGPCR of any of embodiments 1-29 wherein the target binding
domain is able to bind to a target that is:
 fixed; and/or
20 able to exert a mechanical force on the chimeric aGPCR once bound to the
chimeric aGPCR by virtue of the relative movement of the target and the chimeric
aGPCR, optionally the chimeric aGPCR when present in the plasma membrane of a cell,
platelet or engineered platelet.

25 31. The chimeric aGPCR of any of the embodiments 1-30 wherein the target binding
domain is able to bind to a target that is:
Present on a cell surface;
Present on a physical structure;
Present on the inside of a blood vessel;
30 Present on an organ;
Present on a solid tumour;
An anchored target;
A target that has an opposite relative movement to the chimeric aGPCR when the
chimeric aGPCR is present in the plasma membrane of a cell, platelet or engineered
35 platelet; and/or
Immobilised on a solid substrate.

32. The chimeric aGPCR of any of embodiments 1-31 wherein the target binding domain is able to bind to a target that is a peptide tag, optionally a peptide tag present on an antibody or fragment thereof, optionally an scFv, nanobody or Fab.
- 5 33. The chimeric aGPCR according to any of the preceding embodiments wherein the target binding domain binds to a target that is endogenous to a subject, optionally wherein the target is a human target.
- 10 34. The chimeric aGPCR of any of the preceding embodiments wherein the target is present on a cell surface or a tissue surface.
35. The chimeric aGPCR according to any of the preceding embodiments wherein the target binding domain comprises a human target binding domain sequence or a sequence that has at least 75%, 80%, 85%, 90%, 92%, 94%, 96%, 98% or 100% sequence identity to a human target binding domain sequence.
- 15 36. The chimeric aGPCR according to any of the preceding embodiments wherein the target binding domain comprises a non-human target binding domain sequence, optionally:
20 a humanised sequence; or
a sequence from a mouse.
37. The chimeric aGPCR according to any of the preceding embodiments wherein said target binding domain comprises a target-binding ligand or fragment thereof that
25 binds specifically to said target.
38. The chimeric aGPCR according to any of the preceding embodiments wherein said target binding domain comprises:
30 an antibody or antibody fragment that binds specifically to said target;
a variable heavy chain domain and/or a variable light chain domain;
one, two or three CDRs of a heavy chain and/or one, two or three CDRs of a light chain;
an scFv;
a nanobody;
35 a Fab;
a kappa light chain or a fragment thereof targeting;
a synthetic binding scaffold such as a monobody, affibody, DARPin or knottin;

anti-CD19 scFV domains, such as FMC63 scFv domains optionally of example SEQ ID NO: 15;

anti-CD276 scFV domains, such as Enoblituzumab scFv domains optionally of SEQ ID NO: 16 or Omburtamab optionally of SEQ ID NO:17; and/or

5 anti- MAdCAM1 scFV domains, such as Ontamalimab scFv domains optionally of SEQ ID NO: 18.

39. The chimeric aGPCR according to any of the preceding paragraphs wherein the target is a tumor antigen, neoantigen or autoantigen.

10

40. The chimeric aGPCR according to any of the preceding embodiments wherein the target binding domain binds to a target that is:

an endogenous target that is found on a tissue or subset of a tissue in the body of a subject or on a cell or in a particular location of a subject, for example cancer tissue or

15

a cancer cell;

present in plasma or blood of a subject;

only presented during one or more disease states, for example in some embodiments the target is a neoantigen that arises in a tumour cell;

20

only present in significant amounts for example abnormal levels on a tissue or cell that does not normally express the target and/or is only present in a localised manner during one or more disease states;

an antigen associated with a disease, disorder or condition, for example a tumour neoantigen or a tumour specific antigen;

an artificial or exogenous target;

25

CD19;

CD276

IL2

KLK

Amyloid

30

a Notch receptor

OLR1

MadCAM1

a cytokine receptor

collagen

35

not collagen

a designer drug;

a drug that has been designed using DREADD;

a protein selected from Table 2 on pages 23-31 of PCT/GB2020/053247 which is hereby incorporated by reference; and/or
an autoimmune B cell.

- 5 41. The chimeric aGPCR according to any of the preceding embodiments wherein the target binding domain comprises a peptide associated with autoimmunity, optionally:

a peptide or portion of any one or more of the following proteins: MOG, GAD65,
10 MAG, PMP22, TPO, VGKC, PLP, AChR, TRIB2, NMDA, GluR, GAD2, ARMC9, CYP21A2, CASR, NASP, insulin, TSHR, thyroperoxidase, asioglycoprotein receptor, CYP2D6, LF, TTG, H/K ATP-ase, Factor XIII, Beta2-GPI, ITGB2, G-CSF, GP IIb/IIa, COLII, FBG beta alpha, MPO, CYO, PRTN3, TGM, COLVII, COIL, DSG1, DSG3, SOX10, 70SNRNP70, SAG and a3(IV)NC1 collagen; or

15 a peptide or portion that has at least 75%, 80%, 85%, 90%, 92%, 94%, 96%, 98% or 100% sequence identity to any one or more of the following proteins: MOG, GAD65, MAG, PMP22, TPO, VGKC, PLP, AChR, TRIB2, NMDA, GluR, GAD2, ARMC9, CYP21A2, CASR, NASP, insulin, TSHR, thyroperoxidase, asioglycoprotein receptor,
20 CYP2D6, LF, TTG, H/K ATP-ase, Factor XIII, Beta2-GPI, ITGB2, G-CSF, GP IIb/IIa, COLII, FBG beta alpha, MPO, CYO, PRTN3, TGM, COLVII, COIL, DSG1, DSG3, SOX10, 70SNRNP70, SAG and a3(IV)NC1 collagen.

42. The chimeric aGPCR of any of the preceding embodiments wherein when the
25 chimeric aGPCR is present in the membrane of a platelet or an engineered platelet, binding of the target binding domain to the target:

- a) results in degranulation of the platelet or engineered platelet;
b) results in the release of contents from the platelet or engineered platelet;
30 c) results in the presence of intraplatelet contents on the plasma membrane of the platelet or engineered platelet;
d) results in the release of extracellular vesicles via blebbing from the plasma membrane; and/or
e) results in a change of shape of the platelet or engineered platelet from a biconcave
35 disk to fully spread cell fragments.

43. The chimeric aGPCR of any of the preceding embodiments wherein the intracellular tail for example the C-terminal tail that:

Comprises a domain that interacts with a G_{oq} subunit and when the chimeric aGPCR is activated activates beta-type phospholipase C (PLC β);

5 Comprises a domain that interacts with a G_{os} subunit and when the chimeric aGPCR is activated stimulates the cAMP-dependent pathway by activating adenylyl cyclase and increasing intracellular cAMP levels; or

Comprises a domain that interacts with a $G_{oi/o}$ subunit and when the cGPCR is activated inhibits adenylyl cyclase and decreases intracellular cAMP;

10 Comprises a domain that interacts with a $G_{o12/13}$ subunit and when the chimeric aGPCR is activated activates the RhoA pathway; and/or

Comprises a domain that interacts with a G_{o16} subunit and when the chimeric aGPCR is activated activates PLC- β /PI3K/Akt/MAPK/NF- κ B pathways.

15 44. The chimeric aGPCR of any of the preceding embodiments wherein the intracellular tail for example the C-terminal tail that, once activated:

- a) results in degranulation of the platelet or engineered platelet;
- b) results in the release of contents from the platelet or engineered platelet;
- c) results in the presence of intraplatelet contents on the plasma membrane of the platelet or engineered platelet;
- 20 d) results in the release of extracellular vesicles via blebbing from the plasma membrane; and/or
- e) results in a change of shape of the platelet or engineered platelet from a biconcave disk to fully spread cell fragments,
- 25 optionally wherein the intracellular tail and/or one, two or three ICLs are comprises a G_{aq} subunit.

30 45. The chimeric aGPCR of any of the preceding embodiments wherein the intracellular tail for example the C-terminal tail that, once activated:

- a) prevents degranulation of the platelet or engineered platelet;
- b) prevents the release of contents from the platelet or engineered platelet;
- c) prevents the presence of intraplatelet contents on the plasma membrane of the platelet or engineered platelet;
- 35 d) prevents the release of extracellular vesicles via blebbing from the plasma membrane; and/or
- e) prevents a change of shape of the platelet or engineered platelet from a biconcave disk to fully spread cell fragments;

optionally wherein the intracellular tail and/or one, two or three ICLs are comprises a Gas subunit.

5 46. The chimeric aGPCR according to any of the preceding embodiments wherein the chimeric aGPCR comprises a linker between the GAIN domain and the target binding domain, optionally comprises a peptide linker between the GAIN domain and the target binding domain.

10 47. The chimeric aGPCR according to embodiment 46 wherein the linker comprise SPPHTAAHNA [SEQ ID NO: 10].

48. A system comprising:
a chimeric aGPCR as described in any of the preceding embodiments; and
15 an intermediate adaptor protein or peptide, optionally comprising a tag optionally a peptide tag, and an ultimate target binding domain;

wherein the target binding domain of the chimeric aGPCR is able to bind to the intermediate adaptor protein or peptide, optionally bind to the tag when present on the adaptor polypeptide or peptide, and the ultimate target binding domain of the
20 adaptor polypeptide or protein is able to bind to the ultimate target.

49. The system according to embodiment 45 where the target is selected from the group comprising or consisting of:

25 an endogenous target that is found on a tissue or subset of a tissue in the body of a subject or on a cell or in a particular location of a subject, for example cancer tissue or a cancer cell;
present in plasma or blood of a subject;
only presented during one or more disease states, for example in some embodiments
30 the target is a neoantigen that arises in a tumour cell;
only present in significant amounts for example abnormal levels on a tissue or cell that does not normally express the target and/or is only present in a localised manner during or more disease states;
an antigen associated with a disease, disorder or condition, for example a tumour
35 neoantigen or a tumour specific antigen;
an artificial or exogenous target;
CD19;
CD276

- IL2
 KLK
 Amyloid
 a Notch receptor
- 5 OLR1
 MadCAM1
 a cytokine receptor
 collagen
 not collagen
- 10 a designer drug;
 a drug that has been designed using DREADD;
 a protein selected from Table 2 on pages 23-31 of PCT/GB2020/053247 which is hereby incorporated by reference; and/or
 an autoimmune B cell.
- 15 50. The system according to embodiment 48 or 49 wherein where the chimeric aGPCR is present in a platelet plasma membrane or in an engineered platelet plasma membrane, binding of the intermediate adaptor protein or peptide to the target binding domain of the chimeric aGPCR in the absence of simultaneous binding of the target binding domain of the intermediate adaptor polypeptide or protein to the target not
- 20 sufficient to activate degranulation of the platelet or engineered platelet.
51. The system according to any of embodiments 48-50 wherein where the chimeric aGPCR is present in a platelet plasma membrane or in an engineered platelet plasma membrane, binding of the intermediate adaptor protein or peptide to the target binding domain of the chimeric aGPCR with simultaneous binding of the target binding
- 25 domain of the intermediate adaptor polypeptide or protein to the target activates degranulation of the platelet or engineered platelet.
52. A complex comprising:
 a chimeric aGPCR as described in any of the preceding embodiments; and
- 30 an intermediate adaptor protein or peptide, that comprises an ultimate target binding domain and a tag optionally a peptide tag,
 wherein the target binding domain of the chimeric aGPCR binds to the tag of the intermediate adaptor protein. or peptide
- 35 53. The complex of embodiment 53 wherein the ultimate target binding domain of the intermediate adaptor polypeptide or protein is capable of binding to the ultimate target whilst the target binding domain of the chimeric aGPCR simultaneously binds to the tag, optionally peptide tag, of the intermediate adaptor protein or peptide.

- 5 54. A polynucleotide encoding any one or more of the chimeric aGPCRs of any of the preceding embodiments.
55. The polynucleotide of embodiment 54 wherein the polynucleotide is DNA.
- 10 56. The polynucleotide of embodiment 54 wherein the polynucleotide is RNA.
57. The polynucleotide according to embodiment 55 wherein the polynucleotide is operatively linked to a promoter, optionally a heterologous promoter.
- 15 58. The polynucleotide of any of embodiments 54-57 further comprising a cell-specific promoter, optionally a megakaryocyte-specific promoter, pluripotent cell - specific promoter or stem cell-specific promoter, optionally an induced pluripotent stem cell (iPSC) cell-specific promoter, T cell-specific promoter, NK cell-specific promoter, or B cell-specific promoter.
- 20 59. The polynucleotide according to any of paragraphs 57 or 58 wherein the promoter is an inducible promoter, optionally a promoter that is inducible in an intended subject.
- 25 60. The polynucleotide according to any of embodiments 57-59 wherein the promoter is a constitutive promoter, optionally a promoter that is constitutive in an intended subject.
61. A vector comprising a polynucleotide according to any of embodiments 54-61, 30 optionally wherein the vector is a plasmid or circular nucleic acid.
62. A viral vector or viral particle comprising a polynucleotide according to any of embodiments 54-60 or a vector according to embodiment 61, optionally where the viral vector is an AAV or lentivirus.
- 35 63. A chassis comprising:
- a) one or more chimeric aGPCRs of any of the preceding embodiments;

- b) one or more polynucleotides of any of the preceding embodiments;
- c) one or more vectors of any of the preceding embodiments;
- d) one or more of the viral vectors of any of the preceding embodiments;
- e) a system of any of the preceding embodiments.

5

64. The chassis of embodiment 63 wherein the chassis is a cell, platelet or engineered platelet, optionally wherein the cell is a T cell, NK cell, B cell, or macrophage, or stem cell optionally an iPSC cell.

10 65. The chassis of embodiment 63 or 64 wherein the chassis expresses a chimeric aGPCR according to any of the preceding embodiments.

66. The chassis of any of embodiments 63-65 wherein the chassis has been engineered:

15

to disrupt the thrombogenic pathway and/or engineered to disrupt a platelet inflammatory signaling pathway and/or engineered to make the engineered platelet less immunogenic; and/or

20

to enhance or disrupt one or more base functions of the chassis, optionally wherein the one or more base functions are involved in the innate and/or adaptive immune response, inflammation, angiogenesis, atherosclerosis, lymphatic development and tumour growth.

25

67. The chassis according to any embodiments 63-67 wherein the chassis is:

a) a progenitor-chassis, optionally is a myeloid stem cell; an iPSC; adipocyte; adipose-derived mesenchymal stromal/stem cell line (ASCL); or cancer cell-line that is capable of producing a producer-chassis; or other immortal cell that is capable of

30

producing a producer-chassis;

b) a producer-chassis, optionally is a megakaryoblast; a megakaryocyte; a megakaryocyte-like cell; a cancer cell line that is capable of forming a platelet, a platelet-like membrane-bound cell fragment or an anucleate cell fragment for example a MEG01 or DAMI cancer cell line; or other immortal cell that is capable of forming a

35

platelet, a platelet-like membrane-bound cell fragment or an anucleate cell fragment; or

c) an effector-chassis, optionally is a platelet, a platelet-like membrane-bound cell fragment or anucleate cell fragment.

68. The chassis according to any of embodiments 63-67 wherein the chassis is a mammalian chassis, optionally a human chassis, bovine chassis, equine chassis or murine chassis.
- 5
69. The chassis according to any of embodiments 63-68 wherein the chassis has been engineered to disrupt a platelet thrombogenic pathway.
70. The chassis according to any of embodiments 63-69 wherein the chassis has
- 10 been engineered so as to have reduced thrombogenicity relative to a chassis that has not been engineered so as to have reduced thrombogenic potential, optionally wherein the engineered chassis has no thrombogenic potential.
71. The chassis according to any of embodiments 63-70 wherein the chassis
- 15 comprises a disruption of or deletion of at least two, three, four, five, six, seven, eight, nine, or at least ten genes involved in the thrombogenic pathway, optionally wherein the genes are selected from the group of genes encoding:
- a protein involved in recognition of primary stimuli of thrombus formation;
 - 20 a protein involved in recognition of secondary mediators of thrombus formation;
 - and/or
 - a protein involved in the release of secondary mediators of thrombus formation.
72. The chassis according to any of embodiments 63-71 wherein the chassis
- 25 comprises a disruption or deletion of at least:
- one gene that encodes a protein involved in recognition of primary stimuli of thrombus formation;
 - one gene that encodes a protein involved in recognition of secondary mediators of thrombus formation; and
 - 30 one gene that encodes a protein involved in the release of secondary mediators of thrombus formation;
- optionally comprises a disruption of at least:
- two genes that encode a protein involved in recognition of primary stimuli of
 - 35 thrombus formation;
 - two genes that encode a protein involved in recognition of secondary mediators of thrombus formation; and

two genes that encode a protein involved in the release of secondary mediators of thrombus formation;

optionally comprises a disruption of at least:

5 three genes that encode a protein involved in recognition of primary stimuli of thrombus formation;

three genes that encode a protein involved in recognition of secondary mediators of thrombus formation; and

10 three genes that encode a protein involved in the release of secondary mediators of thrombus formation.

73. The engineered chassis according any of embodiments 63-72 wherein:

15 The at least one, two or three genes that encode a protein involved in recognition of primary stimuli of thrombus formation are selected from the group consisting of: GPIIb/IIIa and GPIIb/IIIa (GP6), ITGA2B, CLEC2, integrins $\alpha_{IIb}\beta_3$, $\alpha_2\beta_1$, $\alpha_5\beta_1$ and $\alpha_6\beta_1$, or from the group consisting of GPIIb/IIIa and ITGA2B;

20 The at least one, two or three that encode a protein involved in recognition of secondary mediators of thrombus formation are selected from the group consisting of Par1, Par4, P2Y12, GPIIb/IIIa, the Thromboxane receptor (TBXA2R), P2Y1, P2X1 and integrin $\alpha_{IIb}\beta_3$ or from the group consisting of Par1, Par4 and P2Y12; and/or

25 The at least one, two or three genes that encode a protein involved in the release of secondary mediators of thrombus formation are selected from the group consisting of Cox1, HPS and thromboxane-A synthase (TBXAS1) or from the group consisting of Cox1 and HPS.

74. The chassis according any of embodiments 63-73 wherein each of the following genes is disrupted or deleted:

30 ITGA2B, Par1 and HPS;
ITGA2B, P2Y12 and HPS; or
GPVI, ITGA2B, Par1, Par4, P2Y12, Cox1 and HPS.

75. The chassis according to any of claims 63-74 wherein the chassis comprises a disruption in any one or more of the following genes:

35 TBXAS1, ITGB1, TMEM16F and/or B2m.

76. The chassis according to any of the preceding embodiments wherein the chassis has been engineered to have reduced immunogenicity relative to a non-engineered chassis, wherein the chassis has been engineered to:

- 5 a) have disrupted function of MHC Class 1 genes or proteins;
- b) have disrupted expression from the $\beta 2$ microglobulin gene, optionally to knock out the $\beta 2$ microglobulin gene;
- c) have disrupted expression from one or more HLA genes;
- d) have disrupted expression from any one or more of HLA-A, HLA-B and/or
- 10 HLA-C, optionally wherein expression of HLA-A and HLA-B has been entirely disrupted but wherein expression of HLA-C has been partially disrupted, optionally wherein expression from both alleles of HLA-A and HLA-B have been disrupted but wherein expression from only one allele of HLA-C has been disrupted;
- e) overexpress anyone or more of the HLA class Ib genes, optionally any one
- 15 or more of HLA-G, HLA-E, CD47 and PD-L1;
- f) been engineered to overexpress any one or more of HLA-G, HLA-E, CD47 and PD-L1 and optionally has been engineered to have disrupted expression from the beta 2 microglobulin; and/or
- g) overexpress one or more immunomodulatory genes, optionally wherein the
- 20 one or more immunomodulatory genes is selected from the group comprising CD47 and PD-L1.

77. The chassis according to any of the preceding embodiments wherein the chassis comprises one or more cargo, optionally wherein the chassis has been:

- 25 a) loaded with one or more cargo; and/or
- b) engineered so as to express one or more cargo.

78. The chassis according to any of the preceding embodiments wherein the cargo

30 is selected from any one or more of:

- a) a protein or peptide – in some embodiments the protein or peptide is:
 - i) an antibody or antigen binding fragment thereof, for example an antibody or antigen binding fragment thereof binds to a tumor antigen or a neoantigen;
 - 35 ii) an enzyme, such as a nuclease for example a TALEN;
 - iii) a cytokine for example IL-10; or
 - iv) a CRISPR associated protein, for example Cas9;

- v) a bispecific protein, for example a bispecific antibody or optionally a T-cell engager (BiTE)
- b) a nucleic acid – in some embodiments the nucleic acid is:
 - i) an RNA, for example selected from mRNA, a miRNA, shRNA, and a clustered regularly interspaced short palindromic repeats (CRISPR) sequence; or
 - ii) a DNA vector;
 - c) a toxin;
 - d) a small molecule drug, imaging agent, radionucleotide drug, radionucleotide tagged antibody, or any conjugate thereof;
 - e) a viral vector such as AAV;
 - f) a virus such as oncolytic virus;
 - g) agents for performing CRISPR mediated gene editing;
 - h) an exosome, for example an exosome pre-loaded with a second cargo;
 - i) a nanoparticle or nanoparticles; and/or
 - j) a lipid nanoparticle (LNP) comprising an RNA or an mRNA;

or any combination thereof.

79. The chassis according to any of the preceding embodiment wherein the cargo is an endogenously expressed cargo, optionally wherein the endogenously expressed cargo is any one or more of:

- a) a protein or peptide – in some embodiments the protein or peptide is:
 - i) an antibody or antigen binding fragment thereof, for example an antibody or antigen binding fragment thereof binds to a tumor antigen or a neoantigen;
 - ii) an enzyme, such as a nuclease for example a TALEN;
 - iii) a cytokine for example IL-10; or
 - iv) a CRISPR associated protein, for example Cas9;
- v) a bispecific protein, for example a bispecific antibody or optionally a T-cell engager (BiTE)
- b) a nucleic acid – in some embodiments the nucleic acid is:
 - i) an RNA, for example selected from mRNA, a miRNA, shRNA, and a clustered regularly interspaced short palindromic repeats (CRISPR) sequence.

80. The chassis according to any of the preceding embodiments wherein a cargo is exogenously loaded into the chassis, optionally wherein exogenously loaded cargo is any one or more of:

- a) a protein or peptide – in some embodiments the protein or peptide is:
- i) an antibody or antigen binding fragment thereof, for example an antibody or antigen binding fragment thereof binds to a tumor antigen or a neoantigen;
 - 5 ii) an enzyme, such as a nuclease for example a TALEN;
 - iii) a cytokine for example IL-10; or
 - iv) a CRISPR associated protein, for example Cas9;
 - v) a bispecific protein, for example a bispecific antibody or optionally a T-cell engager (BiTE)
- 10 b) a nucleic acid – in some embodiments the nucleic acid is:
- i) an RNA, for example selected from mRNA, a miRNA, shRNA, and a clustered regularly interspaced short palindromic repeats (CRISPR) sequence;
 - j) a lipid nanoparticle (LNP) comprising an RNA or an mRNA;
 - or any combination thereof.

15

81. The chassis according to any of the preceding embodiments where the chassis comprises a cargo and wherein the cargo has been exogenously loaded into or onto the chassis, optionally into the cytoplasm, into the plasma membrane, or onto the

20 extracellular surface.

82. The chassis according to any of the preceding paragraphs wherein where the chassis comprises a cargo that is an RNA that comprises an exosome targeting domain that is an AU rich element, the producer or effector-chassis has been engineered to

25 express a fusion protein, wherein the fusion protein is a CD9-HuR fusion protein.

83. The chassis according to any of the preceding paragraphs wherein the cargo is an RNA that encodes a Cas protein, optionally a Cas9 protein.

30 84. The chassis according to any of the preceding paragraphs wherein the progenitor, producer or effector-chassis has been engineered to express one or more sgRNAs.

85. The chassis according to any of the preceding paragraphs where the chassis

35 comprises a cargo and the cargo is:

- a therapeutic agent;
- an imaging agent,
- a non-therapeutic agent; and/or

a cosmetic-agent.

86. A targeted delivery system comprising a chassis of any of the preceding
embodiments wherein the chassis expresses one or more chimeric aGPCRs as defined
5 in the preceding embodiments, optionally wherein the targeted delivery system is a
therapeutic targeted delivery system or a non-therapeutic delivery system.

87. A non-thrombogenic targeted delivery system comprising a chassis as defined
in any of the preceding embodiments wherein the chassis expresses one or more
10 chimeric aGPCRs as defined in any of the preceding embodiments and wherein the
chassis has been engineered to disrupt the thrombogenic pathway targeted delivery
system.

88. The targeted delivery system or the non-thrombogenic targeted delivery system
15 of the preceding embodiments wherein the system further comprises one or more
cargo, optionally wherein the cargo comprises one or more targeting domains,
optionally comprises an exosome targeting domain.

89. A chimeric aGPCR, nucleic acid, vector, system, complex or chassis according
20 to any of the preceding embodiments for use in medicine.

90. A chimeric aGPCR, nucleic acid, vector, system, complex or chassis according
to any of the preceding embodiments for use in delivering a therapeutic or imaging
cargo; or treating or preventing cancer, an autoimmunity disease, genetic disease,
25 cardiovascular disease and/or an infection, optionally wherein the chassis is an
effector-chassis or engineered effector-chassis.

91. A method of delivering a cargo comprising administering an effective amount
of any one or more of a chimeric aGPCR, nucleic acid, vector, system, complex or
30 chassis according to any of the preceding embodiments.

92. A method of targeted cargo delivery to a target cell, tissue or site in the body
wherein the method comprises administering an effective amount of any one or more
of a chimeric aGPCR, nucleic acid, vector, system, complex or chassis according to any
35 of the preceding embodiments.

93. A non-therapeutic method of delivering cargo to a subject in need thereof
comprising administering an effective amount of any one or more of a chimeric aGPCR,

nucleic acid, vector, system, complex or chassis according to any of the preceding embodiments, where the chassis comprises a non-therapeutic cargo

94. A method of treatment comprising administering an effective amount of any one or more of a chimeric aGPCR, nucleic acid, vector, system, complex or chassis according to any of the preceding embodiments, optionally wherein the method is for the treatment or prevention of any one or more of cancer, an autoimmunity disease, genetic disease, cardiovascular disease and/or an infection, optionally wherein the chassis is an effector-chassis or engineered effector-chassis.

10

95. Use of any one or more of a chimeric aGPCR, nucleic acid, vector, system, complex or chassis according to any of the preceding embodiments in the manufacture of a medicament for the treatment or prevention of disease or infection, optionally for the treatment or prevention of any one or more of cancer, an autoimmunity disease, genetic disease, cardiovascular disease and/or an infection, optionally wherein the chassis is an effector-chassis or engineered effector-chassis.

96. A method of using the chimeric aGPCR, nucleic acid, vector, system, complex or chassis according to any of the preceding embodiments to deliver a cargo, optionally a therapeutic agent, by administering the chimeric aGPCR, nucleic acid, vector, system, complex or chassis according to any of the preceding embodiments.

97. A kit comprising any two or more of the following:

- 25 A chassis according to any of the preceding embodiments;
- A chimeric aGPCR of any of the preceding embodiments;
- A nucleic acid of any of the preceding embodiments;
- A vector of any of the preceding embodiments;
- A system of any of the preceding embodiments;
- 30 A complex of any of the preceding embodiments.

98. A kit comprising a chimeric aGPCR of any of the preceding embodiments and a corresponding intermediate adaptor protein or peptide of any of the preceding embodiments, wherein the chimeric aGPCR can bind to the intermediate adaptor protein or peptide.

35

99. A complex comprising:

a) a chassis of any of the preceding embodiments that expresses one or more chimeric aGPCR of the invention; and

b) an intermediate adaptor protein or peptide,

5 wherein the intermediate adaptor protein or peptide comprises an ultimate target binding domain and a tag, optionally a peptide tag,
and where the target binding domain of the chimeric aGPCR of the invention is a tag binding domain that can bind to the tag of the intermediate adaptor protein or peptide
and wherein the ultimate target binding domain of the intermediate adaptor
10 polypeptide or protein is able to simultaneously bind to the ultimate target and to the chimeric aGPCR of the invention.

15 The listing or discussion of an apparently prior-published document in this specification should not necessarily be taken as an acknowledgement that the document is part of the state of the art or is common general knowledge.

Preferences and options for a given aspect, feature or parameter of the invention
20 should, unless the context indicates otherwise, be regarded as having been disclosed in combination with any and all preferences and options for all other aspects, features and parameters of the invention. For example, the invention provides:

A chimeric aGPCR that comprises:

25 an extracellular domain that comprises:
a target binding domain that is an antigen binding domain from
an antibody capable of binding to a cancer neoantigen; and
A GAIN domain that has been engineered to increase the force
required to rupture the association between the GPS and tethered
30 agonist, or between the NTF and the CTF;

And

An intracellular tail capable of triggering degranulation of a platelet or
engineered platelet when the target binding domain binds to the cancer
35 neoantigen and the association between the GPS and tethered peptide or
between the CTF and NTF is ruptured.

A non-thrombogenic platelet, or a platelet with reduced thrombogenicity comprising at least one chimeric aGPCR of the invention, wherein the aGPCR is able to trigger degranulation of the platelet non-thrombogenic platelet or platelet with reduced thrombogenicity upon binding of the target binding domain to the target, and wherein the non-thrombogenic platelet or platelet with reduced thrombogenicity comprises a therapeutic cargo that is released upon degranulation of the non-thrombogenic platelet or platelet with reduced thrombogenicity.

10

Table 1. Sequence information of chimeric aGPCRs

ID	Sequences	Note
ADGRG1 signal peptide	MTPQSLLQTTLFLLSLLFLVQGAHG [SEQ ID NO: 1]	
ADGRF5 signal peptide	MKSPRRTTLCLMFIVYSSKA [SEQ ID NO: 2]	
FCERG signal peptide	MIPAVVLLLLLLLVEQAAA [SEQ ID NO: 3]	
CD28 signal peptide	MLRLLLLALNLFPSIQVTG [SEQ ID NO: 4]	
Platelet signal peptide – GPIIb (aIIb)	MARALCPLQALWLEWVLLLLGPCAAPPAWA [SEQ ID NO: 5]	
Platelet signal peptide – GPIIIa (β3)	MRARPRRPLWATVLALGALAGVGVG [SEQ ID NO: 6]	
Platelet signal peptide - GPIBa	MPLLLLLLLLPSPLHP [SEQ ID NO: 7]	
Platelet signal peptide - GPIX	MPAWGALFLLWATAEA [SEQ ID NO: 8]	
Platelet signal peptide - GPV	MLRGTLCAVLGLLRA [SEQ ID NO: 9]	
Signal peptide	MAPFASLASGILLLLSLITSSKA [SEQ ID NO: 36]	
Signal peptide	MLLGPGHTLSAPALALAVTLTLLVRSASP [SEQ ID NO: 37]	
Signal peptide	MLLSVPLLLGLLGLAAA [SEQ ID NO: 38]	
Signal peptide	MQELRGILLCLLLAAVPTTP [SEQ ID NO: 39]	
Signal peptide	MRYVASYLLAALGGNS [SEQ ID NO: 40]	
Signal peptide	MGKSPEAWCIVLFSVLASFSA [SEQ ID NO: 41]	
Signal peptide	MASSGSVQQPRLVLLMLVLAGAARA [SEQ ID NO: 42]	

Signal peptide	MRWKIIQLQYCFLLVPCMLTALEA [SEQ ID NO: 43]	
Signal peptide	MLSRSLCLALAWVARVGA [SEQ ID NO: 44]	
Signal peptide	MRFSCLALLPGVALLLASARLAAA [SEQ ID NO: 45]	
Signal peptide	MRVLWVLGLCCVLLTFGFVRA [SEQ ID NO: 46]	
Signal peptide	MKFPMVAAAALLLCAVRA [SEQ ID NO: 47]	
Signal peptide	MRSLLASFCLLAVALA [SEQ ID NO: 48]	
Signal peptide	MKILLLCVGLLLTWDNGMVLG [SEQ ID NO: 49]	
Signal peptide	MLRISGRNMKVLFAAALIVGSVVFLLLPGPSVA [SEQ ID NO: 50]	
Signal peptide	MAATVRRQRPRLLCWTLVAVLLADLLALS [SEQ ID NO: 51]	
Signal peptide	MKMGVRLAARAWPLCGLLLAALGGVCA [SEQ ID NO: 52]	
Signal peptide	MWWRLWWLLLLLLLLWLALAAAA [SEQ ID NO: 53]	
Signal peptide	MGWSLILLFLVAVATRVLS [SEQ ID NO: 54]	
Signal peptide	MDFQVQIISFLLISASVIMSRG [SEQ ID NO: 55]	
Signal peptide	MEFGLSWVFLVALFRGVQC [SEQ ID NO: 56]	
Signal peptide	MKWVTFISLLFLFSSAYS [SEQ ID NO: 57]	
Signal peptide	MKLPVRLVLMTFWIPAASA [SEQ ID NO: 58]	
Signal peptide	MNLLLILTFVAAAVA [SEQ ID NO: 59]	
Signal peptide	MGSAALLWVLLLVWPSSRA [SEQ ID NO: 60]	
Signal peptide	MTRLTVLALLAGLLASSRA [SEQ ID NO: 61]	
Signal peptide	MWWRLWWLLLLLLLLWPMVWA/AA [SEQ ID NO: 62]	
Signal peptide	MKLPVRLVLMTFWIPASSS [SEQ ID NO: 63]	
Signal peptide	MDMRVPAQLLGLLLWLSGARC [SEQ ID NO: 64]	
Signal peptide	MKYLLPTAAAGLLLLAAQPAMA [SEQ ID NO: 65]	
Signal peptide	MGVKVLFALICIAVAEA [SEQ ID NO: 66]	
Signal peptide	MPLLLLPLWAGALA [SEQ ID NO: 67]	

Signal peptide	MRARALLAVLLLLLVGIAAAA Synthetically designed [SEQ ID NO: 68]	
Signal peptide	MATATLLAVLLLLLVGSAGGA Synthetically designed [SEQ ID NO: 69]	
Signal peptide	MRARALLVVLVVLVLLGVASSA Synthetically designed [SEQ ID NO: 70]	
Signal peptide	MPGPGAALLLLLLVLLGLGSAA Synthetically designed [SEQ ID NO: 71]	
Signal peptide	MTTTTVLLLLVVLVLAGLTSGA Synthetically designed [SEQ ID NO: 72]	
Target binding domain/GAIN domain linker	SPPHTAAHNA [SEQ ID NO: 10]	
Modularized ADGRG1.1 Without target binding domain but with linker at N-terminus that links GAIN domain, 7TM and C-terminal domain to the target binding domain.	SPPHTAAHNASVDMCELKRDQLLSQFLKH PQKASRRPSAAPASQQLQSLESKLTSVRFMG DMVSFEEDRINATVWKLQPTAGLQDLHIHSR QEEEQSEIMEYSVLLPRTLQRTKGRSGEAEK RLLLVDFSSQALFQDKNSSQVLGEKVLGIVVQ NTKVANLTPVVLTFQHQLQPKNVTLCVFW VEDPTLSSPGHWSSAGCETVRRETQTSCFCN HLTYFAVLMVSSVEVDVAVHKHYLSLLSYVGC VVSALACLVTIAAYLCSRVPPLCRRKPRDYTIK VHMNLLLAVFLDTSFLLSEPVALTGSEAGCRA SAIFLHFSLLTCLSWMGLEGYNLYRLVVEVFG TYVPGYLLKLSAMGWGFPIFLVTLVALVDVDN YGPIILAVHRTPEGVIYPSMCWIRDSLVSITN LGLFSLVFLFNMAMLATMVVQILRLRPHTQKW SHVLTLLGLSLVGLPWALIFFSFASGTFQLVV LYLSIITSFQGLFIWIWWSMRLQARGGPSPL KNSDSARLPISSGSTSSSRI [SEQ ID NO: 11]	SPPHTAAHNA is the extra-linker sequence comparing to Modularized ADGRG1.2 [SEQ ID NO: 10] TYFAVLM [SEQ ID NO: 73] is the tethered agonist peptide of ADGRG1 The GPS cleavage site is in front of peptide agonist. For the GPS site of ADGRG1 is HL[^]TYFAVLM
Modularized ADGRG1.2 Without target binding domain, and without linker that in ADGRG1.1 links the GAIN domain, 7TM and C-terminal domain to the target binding domain.	SVDMCELKRDQLLSQFLKH PQKASRRPSAA PASQQLQSLESKLTSVRFMGDMVSFEEDRIN ATVWKLQPTAGLQDLHIHSRQEEEQSEIMEY SVLLPRTLQRTKGRSGEAEKRLLLVDFSSQA LFQDKNSSQVLGEKVLGIVVQNTKVANLTPV VLTQFHQLQPKNVTLCVFWVEDPTLSSPGH WSSAGCETVRRETQTSCFCNHLTYFAVLMV SSVEVDVAVHKHYLSLLSYVGCVVSALACLVTI AAYLCSRVPPLCRRKPRDYTIKVHMNLLLAVFL DTSFLLSEPVALTGSEAGCRA SAIFLHFSLLT CLSWMGLEGYNLYRLVVEVFGTYVPGYLLKLS AMGWGFPIFLVTLVALVDVDNYGPIILAVHRT PEGVIYPSMCWIRDSLVSITNLGLFSLVFLFN MAMLATMVVQILRLRPHTQKWSHVLTLLGLSL VGLPWALIFFSFASGTFQLVVLYLSIITSFQG FLFIWIWWSMRLQARGGPSPLKNSDCARLPI SSGSTSSSRI [SEQ ID NO: 12]	TYFAVLM is the tethered agonist peptide of ADGRG1 The GPS cleavage site is in front of peptide agonist. For the GPS site of ADGRG1 is HL[^]TYFAVLM

Modularized ADGRF5.1	GENITCDDPVIGVGEPGKVIOKLCRFSNV PSSPESPIGGTITYKCVGSOWEEKRND SAPINSLQMAKALIKSPSQDEMLPTYLKDL SIDKAEHEISSSPGSLGAIINILDLLSTVPTQV NSEMMTHVLSTVNVILGKPVNTWKVLQQQ WTNQSSQLLHsverfsqalqsgdspplsfsq TNVQMSSMVIKSSHPETYQQRFFVFPYFDLWG NVVIDKSYLENLQSDSSIVTMAFPTLQAILAQ DIQENNFAESLVMTTTTVSHNTTTPFRISMTEK NNSPSGGETKCVFWNFRLANNTGGWDSSGC YVEEGDGDNVTCICDHLTSFSILMSPDSPDP SSLLGILLDIISYVGVGFSILSLAACLVVEAVV WKSVTKNRTSYMRHTCIVNIAASLLVANTWFI VVAIQDNRYILCKTACVAATFFIHFYLSVFF WMLTLGLMLFYRLVFIHETSRSTQKAIACFLG YGCPLAISVITLGATQPREVYTRKNVCWLNWE DTKALLAFAIPALIIVVNITITIVITKILRPSIG DKPCKQEKSSLFQISKSIGVLTPLLGLTWGFG LTTVPFGTNLVFHIIFAILNVFQGLFILLFGCLW DLKVQEALLNKFSLSRWSSQHSKSTSLGSST PVFSMSSPISRRFNNLFGKTGTYNVSTPEATS SSENSSSASSLLN [SEQ ID NO: 13]	1. GENITCDDPVIGVGEPGKVIOKLCRFSNVPSSPESPIGGTITYKCVGSOWEEKRND [SEQ ID NO: 80] is the HormR domain of ADGRF5 2. TSFSILMS [SEQ ID NO: 75] is the peptide agonist of ADGRF5 3. The GPS cleavage site is in front of peptide agonist. The GPS site for ADGRF5 is HL[^]TSFSILMS [SEQ ID NP: 76].
Modularized ADGRF5.2	ISAPINSLQMAKALIKSPSQDEMLPTYLKDL SIDKAEHEISSSPGSLGAIINILDLLSTVPTQV NSEMMTHVLSTVNVILGKPVNTWKVLQQQ WTNQSSQLLHsverfsqalqsgdspplsfsq TNVQMSSMVIKSSHPETYQQRFFVFPYFDLWG NVVIDKSYLENLQSDSSIVTMAFPTLQAILAQ DIQENNFAESLVMTTTTVSHNTTTPFRISMTEK NNSPSGGETKCVFWNFRLANNTGGWDSSGC YVEEGDGDNVTCICDHLTSFSILMSPDSPDP SSLLGILLDIISYVGVGFSILSLAACLVVEAVV WKSVTKNRTSYMRHTCIVNIAASLLVANTWFI VVAIQDNRYILCKTACVAATFFIHFYLSVFF WMLTLGLMLFYRLVFIHETSRSTQKAIACFLG YGCPLAISVITLGATQPREVYTRKNVCWLNWE DTKALLAFAIPALIIVVNITITIVITKILRPSIG DKPCKQEKSSLFQISKSIGVLTPLLGLTWGFG LTTVPFGTNLVFHIIFAILNVFQGLFILLFGCLW DLKVQEALLNKFSLSRWSSQHSKSTSLGSST PVFSMSSPISRRFNNLFGKTGTYNVSTPEATS SSENSSSASSLLN [SEQ ID NO: 14]	1. TSFSILMS is the peptide agonist of ADGRF5
CD19 binding domain – scFv FMC63	DIQMTOTTSSLSASLGDRVTISCRASQDI SKYLNWYQOKPDGTVKLLIYHTSRLHSGV PSRFSGSGSGTDYSLTISNLEQEDIATYF CQQGNTLPYTFGGGTKLEITGSTSGSGKPK GSGEGSTKGEVKLQESGPGLVAPSQSLSV	

	TCTVSGVSLFDYGVSWIROPPRKGLEWLG VIWGSETTYNSALKSRLTIKDNSKSQV FLKMNSLQTDITAIYYCAKHYYYGGSYAM DYWGQGTSTVTVSS [SEQ ID NO: 15]	
Exemplary target binding domains		
CD276 binding domain - Enoblituzumab scFv	EVQLVESGGGLVQPGGSLRLSCAASGFTF SSFGMHVVRQAPGKGLEWVAYISSDSSAI YYADTVKGRFTISRDNKNSLYLQMNSLR DEDTAVYYCGRGRENIIYGSRLDYWGQGT TVTVSSGSTSGSGKPGSGEGSTKGDILQLT QSPSELSASVGDRVTITCKASQNVDTNVA WYQQKPGQAPKALIYSASYRYSQVPSRFS GSGSGTDFTLTISSSLQPEDFATYYCQQYN NYPFTFGQGTKLEIKR [SEQ ID NO: 16]	
CD276 binding domain - Omburtamab scFv	QVKLQQSGAELVKPGASVKLSCKASGYTF TNYDINWVRQRPEQGLEWIGWIFPGDGST QYNEKFKGKATLTDTSSSTAYMQLSRLT SEDSAVYFCARQTTATWFAYWGQGTITVTV SSGSTSGSGKPGSGEGSTKGDIELTQSPT TLSTVTPGDRVSLSCRASQISDYLHWYQQ KSHESPRLLIKYASQISGIPSRFSGSGS GSDFTLSINSVEPEDVGVYYCQNGHSFPL TFGAGTKLELKR [SEQ ID NO: 17]	
MAdCAM1 binding domain - Ontamalimab	QVQLVQSGAEVKKPGASVKVSCASGYTF TSYGINWVRQAPGQGLEWMGWISVYSGNT NYAQKVQGRVTMTADTSTSTAYMDLRSLR SDDTAVYYCAREGSSSSGDIYYGMDVWGQ GTTVTVSSGSTSGSGKPGSGEGSTKGDIV MTQTPLSLSVTPGQPASISCKSSQSLHT DGTITYLYWYLQKPGQPPQLLIYEVSNRFS GVPDRFSGSGSGTDFTLKISRVEAEDVGI YYCMQNIQLPWTFGQGTKVEIKR [SEQ ID NO: 18]	
Modularized ADGRL1 (for Gαq (major), Gαi/o)	<u>QWPATQQGMLVERPCPKGTRGIASFQCLPALGLWN</u> <u>PRGPDLSNCTSPWVNQVAQKIKSGENAANIASELA</u> <u>RHTRGSIYAGDVSSSVKLMEQLLDILDAQLQALRP</u> <u>IERESAGKNYNKMHKRERTCKDYIKAVVETVDNLL</u> <u>RPEALESWKDMNATEQVHTATMLLDVLEEGAFLLA</u> <u>DNVREPARFLAAKENVVLEVTVLNTGQVQELVFP</u> <u>QEEYPRKNSIQLSAKTIKQNSRNGVVKVVFILYNN</u> <u>LGLFLSTENATVKLAGEAGPGGPGGASLVVNSQVI</u> <u>AASINKESSRVFLMDPVIFTVAHLEDKNHFNANCS</u> <u>FWNYERSMLGYWSTQGCRLVESNKTHHTTCACSHL</u> <u>TNFAVLM</u> MAHREIYQGRINELLSVITWVGIVI <u>SLVCLAICISTFCFLRGLQTDNRNTIHKNL</u> <u>CINLFLAELLFLVGIDKTQYEIACPIFAG</u> <u>LLHYFFLAAFSWLCLEGVHLYLLLVEVFE</u> <u>SEYSRTKYYYLGGYCFPALVVGIAAAIDY</u> <u>RSYGTEKACWLRVDNYFIWSFIGPVSEFVI</u> <u>VVNLVFLMVTLHKMIRSSSVLKPDSRLD</u>	TNFAVLM is the peptide agonist for ADGRL1 [SEQ ID NO: 77] Underline = GAIN domain Italics = 7TM Underline and italics = C-terminal signalling domain

	<u>NIKSWALGAIALLFLLGLTWAFGLLFINK</u> <u>ESVVMAYLFTTFNAFQGVFIFVFHICALQK</u> <u>KVHKEYSKCLRHSYCCIRSPPGGTHGSLK</u> <u>TSAMRSNTRYTGTQSRIRRMWNDTVRKQ</u> <u>TESSEFMAGDINSTPTLNRGTMGNHLLTNP</u> <u>VLQPRGGTSPYNTLIAESVGFNPSSPPVF</u> <u>NSPGSYREPKHPLGGREACGMDTLPLNGN</u> <u>FNNSYSLRSGDFPFGDGGPEPPRGRNLAD</u> <u>AAAFEKMIISELVHNNLRGSSSAAGKPPP</u> <u>PEPPVPPVPGGGGEEEAGGPGGADRAEIE</u> <u>LLYKALEEPLLLPRAQSVLYQSDLDESES</u> <u>CTAEDGATSRPLSSPPGRDSLYASGANLR</u> <u>DSPSYPDSSPEGPSEALPPPPPAPPGPPE</u> <u>IYYTSRPPALVARNPLQGYQVRRPSHEG</u> <u>YLAAPGLEGPDPDGDGQMQLVTSL</u> [SEQ ID NO: 19]	
Modularized ADGRL3 (for Gα 12/13 (main), Gαq (weak))	<u>PALEESCEAVEAREIMWFKTRQGQIAKQPCPAGTI</u> <u>GVSTYLCLAPDGIWDFQGPDLSCSSPWVNHITQK</u> <u>LKSGETAANIARELAEQTRNHLNAGDITYSVRAMD</u> <u>QLVGLLDVQLRNLTGGKDSAARSLNKAMVETVNN</u> <u>LLQPQALNAWRDLTTSQDLRAATMLLHTVEESAFV</u> <u>LADNLLKTDIVRENTDNIKLEVARLSTEGNLEDLK</u> <u>FPENMGHGSTIQLSANTLKQNGRNGEIRVAFVLYN</u> <u>NLGPYLSTENASMKLGTEALSTNHSVIVNSPVITA</u> <u>AINKEFSNKVYLADPVVETVKHIKQSEENFNPNCS</u> <u>FWSYSKRTMTGYWSTQGCRLLTNTKTHHTTCSNHL</u> <u>TNEAVLMA</u>HVEVKHSDAVHDLLLDVITWVGIL <u>LSLVCLLICIFTFCFFRGLQSDRNTIHK</u> <u>LCISLFLVAELLFLIGINRTDQPTACAVFA</u> <u>ALLHFFFLAAFTWMFLEGVQLYIMLVEVF</u> <u>ESEHSRRKYFYLVGYGMPALIVAVSAAVD</u> <u>YRSYGTDKVCWLRLDTYFIWSFIGPATLI</u> <u>IMLNVI FLGIALYKMFHHTAILKPESGCL</u> <u>DNIKSWVIGAIALLCLLGLTWAFGLMYIN</u> <u>ESTVIMAYLFTIFNSLQGMFIFIFHCVLQ</u> <u>KKVRKEYGKCLRTHCCSGKSTESSIGSGK</u> <u>TSGSRTPGRYSTGSQSRIRRMWNDTVRKQ</u> <u>SESSFITGDINSSASLNREGLLNARDTS</u> <u>VMDTLPLNGNHGNSYSIASGEYLSNCVQI</u> <u>IDRGYNHNETALEKKILKELTSNYIPSYL</u> <u>NNHERSSEQNRNLMNKLNVNNGSGREDDA</u> <u>IVLDDATSFNHEESLGLELIHEESDAPLL</u> <u>PPRVYSTENHQPHHYTRRRIPQDHSESFF</u> <u>PLLTNEHTEDLQSPHRDSLYTSMPTLAGV</u> <u>AATESVTTSTQTEPPPAKCGDAEDVYYKS</u> <u>MPNLGSRNHVHQLHTYYQLGRGSSDGFIV</u> <u>PPNKDGTPEEGSSKGAHLVTSL</u> [SEQ ID NO: 20]	<u>TNEAVLMA</u> is the peptide agonist of ADGRL3 [SEQ ID NO: 78] Underline = GAIN domain Italics = 7TM Underline and italics = C- terminal signalling domain
Modularized ADGRE2 (For Gα16/ PLC-β /PI3K/Akt/MAPK/NF- κB pathways)	<u>TWTPPPGIKSQRLSNFFERVQELHRDFKPALAQET</u> <u>IQDLIQEVDELLEIPGDLEALPHSEQHCVATNLLV</u> <u>GLEGVLFNVSQAMPNGPWTFNASAGTDLSLLEVQEE</u> <u>GYRNVTLSONLAKMMLKWDVVHKS GDSGSPSVVGLL</u> <u>STPGMGKLLAEAPLVLEPEKQAVLHGAPKGLLQGV</u> <u>SSVLLSDVISVFMSNKVTQKLSSPVTFIFSHHSAT</u>	SSFAVLMA is the peptide agonist of ADGRE2 [SEQ ID NO: 79] Underline = GAIN domain

	HEPKLVFCVFWEHSSQDECCHWSTRGCTVVDSDGT STTCQCTHLSSSFAVLMAHYDVQEEDLVLPVIT YVGLGLSLLCLLLAALTFLLCKAIQNTST SLHLQLLICLFLAHLLFLMAIDRTEIKVL CSIIAGALHYLYLASFTWMLLEGLHLFLT ARNLMVVNYSSVSMLMKKLMYPVGYGVPT LIVAISAASRSHLYGTRTRCWLNPEEFI WSFLGPVCTIFSVNLGFFLMTLWILKSKL SSLNSDVSTLQNTRMLTFKAIAQLFILGC TWCLGILQVGPAAHVMAYLFTIINSLOGV FIFLVYCLLSQOVREEYGKWFKGIRKTRA ESEKYTLSSRAMSDVNKPMMVN [SEQ ID NO: 21]	Italics = 7TM Italics and underlined = C-terminal signalling domain
Modularized ADGRG2 (For Gs, Gα 12/13 and Gαq pathway)	TSSISDLENQVLQMEKALSLSLEPNLAGEMINQV SRLHSPFDMLAPLAQRLKVVDDIGLQLNFSNTT ISLTSPSLALAVIRVNASSFNTTFFVAQDPANLQV SLETOAPENSIGTITLPSSLMNNLPAHDMELASRV QFNFFETPALFQDPSLENLSLISYVISSSVANLTV RNLTRNVTVTLKHINPSQDELTVRCVFWDLGRNGG RGGWSDNGCSVKDRRLNETICTCSHLTSFGVLLD LSRTSVLPAQMMALTFITYIGCGLSSIFLSVT LVTYIAFEKIRRDYPSKILIQLCAALLLL NLVFLLDSWIALYKMQGLCISVAVFLHYF LLVSFTWMGLEAFHMYLALVKVFNTYIRK YILKFCIVGWGVPVAVVTIILTISPDNYG LGSYGKFPNGSPDDFCWINNNAVFIITVV GYFCVIFLLNVSMFIVVLVQLCRIKKKKQ LGAQRKTSIQDLRSIAGLTFLLGITWGFA FFAWGPVNVTFMYLFAIFNTLQGFFIFIF YCVAKENVRKQWRRYLCCGKLRLAENSDW SKTATNGLKKQTVNQGVSSSSNSLQSSSN STNSTLLVNNDCSVHASGNGNASTERNG VSFSVQNGDVCLHDFTGKQHMFNEKEDSC NGKGRMALRRTSKRGSLHFIEQM [SEQ ID NO: 22]	TSFGVLL is the peptide agonist of ADGRG2 [SEQ ID NO: 74] Underline = GAIN domain Italics = 7TM Italics and underlined = C-terminal signalling domain
Exemplary intracellular signalling domains (inc the mechanisms by which they signal)		
Signalling via Gαq (major), Gαi/o (weak)	[SEQ ID NO: 23] (Based on ADGRL1) STFCFLRGLQTDRN/ CLEGVHLYLLLVEVFESEYSRTK/ VTLHKMIRSSSVLKPDSSRLDNIKSWA/ HCALQKKVHKEYSKCLRHSYCCIRSPPGG THGSLKTSAMRSNTRYYTGTQSRIRRMWN DTVRKQTESSFMAGDINSTPTLNRGTMGN HLLTNPVLQPRGGTSPYNTLIAESVGFNP SSPPVFNSPGSYREPKHPLGGREACGMDT LPLNGFNNSYSLSRSGDFPPGDGGPEPPR GRNLADAAAFEKMIISELVHNNLRGSSSA AKGPPPPPEPPVPPVPGGGGEEEAGGPGGA DRAEIELLYKALEEPLLLPRAQSVLYQSD	

	<u>LDESESC</u> <u>TAEDGATSRPLSSPPGRDSL</u> <u>YA</u> <u>SGANLRD</u> <u>SPSYPDSSPEGPSEALPPPP</u> <u>PA</u> <u>PPGPPEI</u> <u>YYTSRPPALVARNPLQGYYQVR</u> <u>RPSHEGYLAAPGLEGPDPDGDGQMQLVTS</u> <u>L</u>	
Signalling via Gα 12/13 (main), Gαq (weak))	[SEQ ID NO: 24] (Based on ADGRL3) <u>RGLQSDRNTIHK/</u> <u>VEVFESEHSRRK/</u> <u>YKMFHHTAILKPESGCLDN/</u> <u>KKVRKEYGKCLRTHCCSGKSTESSIGSGK</u> <u>TSGSRTPGRYSTGSQSRIRRMWNDTVRKQ</u> <u>SESSFITGDINSSASLNREGLLNARDTS</u> <u>VMDTLPLNGNHGNSYSIASGEYLSNCVQI</u> <u>IDRGYNHNETALEKKILKELTSNYIPSYL</u> <u>NNHERSSEQNRNLMNKLVNNLGSGREDDA</u> <u>IVLDDATSFNHEESLGLELIHEESDAPLL</u> <u>PPRVYSTENHQPHHYTRRRIPQDHSESEFF</u> <u>PLLTNEHTEDLQSPHRDSLYTSMPTLAGV</u> <u>AATESVTTSTQTEPPPAKCGDAEDVYYKS</u> <u>MPNLGSRNHVHQLHTYYQLGRGSSDGFIV</u> <u>PPNKDGTPEEGSSKGAHLVTSL</u>	
Signalling via Gα16/ PLC-β /PI3K/Akt/MAPK/NF-κB pathways	[SEQ ID NO: 25] (Based on ADGRE2) <u>ALTFLCKAIQNTST/</u> <u>LLEGLHLFLTARNLMVVNYSSVSMLMK/</u> <u>MTLWILKSKLSSLNSDVSTLQNTLRMLTFKAIQ/</u> <u>SQQVREYEGKWFKGIRKTRAESKEYTLSSRAMSDV</u> <u>NKPMNVN</u>	
Signalling via Gs, Gα 12/13 and Gαq pathway	[SEQ ID NO: 26] (Based on ADGRG2) <u>TYIAFEKIRRDYPSKILIQ/</u> <u>EAFHMYLALVKVFNTYIRKYILK/</u> <u>QLCRIKKKKQLGAQRKTSIQDLRS/</u> <u>YCVAKENVRKQWRRYLCCGKLRLAENS DWSKTATN</u> <u>GLKKQTVNQGVSSSSNSLQSSSNSTNSTLLVNND</u> <u>CSVHASGNGNASTERNGVSFVQNGDVCLHDFTGK</u> <u>QHMFNEKEDSCNGKGRMALRRTSKRGS LHFIEQM</u>	
Exemplary GAIN domains (inc any engineered domains as per Dumas et al)		
ADGRL1 GAIN domain	<u>QWPATQQGMLVERPCPKGTRGIASFQCLPALGLWN</u> <u>PRGPDLSNCTSPWVNQVAQKIKSGENAANIASELA</u> <u>RHTRGSIYAGDVSSSVKLMEQLLDILDAQLQALRP</u> <u>IERESAGKNYNKMHKRERTCKDYIKAVVETVDNLL</u> <u>RPEALESWKDMNATEQVHTATMLLDVLEEGAFLLA</u> <u>DNVREPARFLAAKENVVLEVTVLNTEGQVQELVFP</u> <u>QEEYPRKNSIQLSARTIKQNSRNGVVVKVFI LYNN</u> <u>LGLFLSTENATVKLAGEAGPGGPGGASLVVNSQVI</u> <u>AASINKESSRVFLMDPVI FTVAHLEDKNHFNANCS</u> <u>FWNYSERSMLGYWSTQGCRLVESNKTHTTACASHL</u> [SEQ ID NO: 27]	
ADGRL3 GAIN domain	<u>PALEESCEAVEAREIMWFKTRQGQIAKQPCPAGTI</u> <u>GVSTYLCLAPDGIWDEQGPDLNCSSPWVNHITQK</u> <u>LKSGETAANIARELAEQTRNHLNAGDITYSVRAMD</u> <u>QLVGLLDVQLRNLTGPKGKDSAARSLNKAMVETVNN</u> <u>LLQPQALNAWRDLTTS DQLRAATMLLHTVEESAFV</u>	

	LADNLLKTDIVRENTDNIKLEVARLSTEGNLEDLK FPENMGHGSTIQLSANTLKQNGRNGEIRVAFVLYN NLGPYLSTENASMKLGTEALSTNHSVIVNSPVITA AINKEFSNKVYLADPVVFTVKHIKQSEENFNPNCS FWSYSKRMTGTGYWSTQGCRLLTNKTHTTCSNHL [SEQ ID NO: 28]	
ADGRE2 GAIN domain	TWTPPPGIKSQRLSNFFERVQELHRDFKPALAQET IQDLIQEVDELLEIPGDLEALPHSEQHCVATNLLV GLEGVLRNVSQAMPNGPWTFNASAGTDLSLEVQEE GYRNVTLSONLAKMMLKWDVVHKSGDSDGSPVVGLL STPGMGKLLAEAPLVLEPEKQAVLHGAPKGLLQGV SSVLLSDVISVFMSNKVTQKLSSPVTFFSHHSAT HEPKLVFCVFEHWSQDECGHWSTRGCTVVDSDGT STTCQCTHL [SEQ ID NO: 29]	
ADGRG2 GAIN domain	TSSISDLENQVLQMEKALSLSLEPNLAGEMINQV SRLHSPDMLAPLAQRLLKVDDIGLQLNFSNTT ISLTSPSLALAVIRVNASSFNTTFVAQDPANLQV SLETQAPENSIGTITLPSLLMNNLPAHDMELASRV QFNFFETPALFQDPSLENLSLISYVISSSVANLTV RNLTRNVTVTLKHINPSQDELTVRCVFWDLGRNGG RGGWSDNGCSVKDRRLNETICTCSH [SEQ ID NO: 30]	
ADGRG1 GAIN domain	[SEQ ID NO: 35] SVDMECELKRDQLLSQFLKHPQKASRRPSAA PASQQLQSLESKLTSVRFMGDMVSFEEDRIN ATVWKLQPTAGLQDLHIHSRQEEQSEIMEY SVLLPRTLQRTKGRSGEAEKRLLLVDFSSQA LFQDKNSSQVLGEKVLGIVVQNTKVANLTPV VLTFFHQHQLQPKNVTLCVFWVEDPTLSSPGH WSSAGCETVRRETQTSCFCNHL	
Exemplary full length naturally occurring aGPCRs that can start as the base chimeric aGPCR		
ADGRL1	MARLA AVLWNLCVTAVLVTSATQGLSRAGLPF GLMRRELACEGYPIELRCPGSDVIMVEN ANYGRTDDKICDADPFQMENTVQCYLPDAFKIM SQRCNNRTQCVVAGSDAFDPDPCPGTYK YLEVQYDCVPYKVEQKVFCPGTLQKVLEPTST HESEHQSGAWCKDPLQAGDRIYVMPWI PYRTDTLTEYASWEDYVAARHTTTYRLPNRVDG TGFVVYDGA VFYNKERTRNIVKYDLRT RIKSGETVINTANYHDTSPYRWGGKTDIDLAVD ENGLWVIYATEGNNGRLVVSQNLNPLYTL RFEGTWETGYDKRSASNAFMVCGVLYVLRVY VDDDSEAAGNRVDYAFNTNANREEPVSL TFPNPYQFISSVDYNPRDNQLYVWNNYFVVRY LEFGPPDPSAGPATSPPLSTTTTARPT PLTSTASPAATPLRRAPLTTHPVGAINQLGPD PPATAPVPSTRRPPAPNLHVSPELFC EPREVRVQWPATQQGMLVERPCPKGTRGIAS FQCLPALGLWNPRGPDLSNCTSPWVNQV AQKIKSGENAANIASELARHTRGSIYAGDVSSSV KLMEQLLDILDAQLQALRPIERESAG KNYNKMHKRERTCKDYIKAVVETVDNLLRPEAL	>sp O94910 AGR L1_HUMAN Adhesion G protein-coupled receptor L1 OS=Homo sapiens OX=9606 GN=ADGRL1 PE=1 SV=1

	ESWKDMNATEQVHTATMLLDVLEEGAF LLADNVREPARFLAAKENVVLEVTNLNTEGQVQ ELVFPQEEYPRKNSIQLSAKTIKQNSR NGVVKVVFILYNNLGLFLSTENATVKLAGEAGP GGPGGASLVVNSQVIAASINKESSRVF LMDPVIFTVAHLEDKNHFNANCSFWNYSERSM LGYWSTQGCRLVESNKTHHTTCACSHLTN FAVLMAHREIYQGRINELLSVITWVGIVISLVCL AICISTFCFLRGLQTDNRNTHKNLC INLFLAELLFLVGIDKTQYEIACPIFAGLLHYFFLAA FSWLCLEGVHLYLLLVEVFESEY SRTKYYYLGGYCFPALVVGIAAAIDYRSYGTEKAC WLRVDNYFIWSFIGPVSFVIVVNLV FLMVTLHKMIRSSSVLKPSSRLDNIKSWALGAI ALLFLLGLTWAFGLLFINKESVVMAY LFTTFNAFQGVFIFVHFCALQKKVHKEYSKCLRH SYCCIRSPPGGTHGSLKTSAMRSNTR YYTGTQSRIRRMWNDTVRKQTESSFMAGDINS TPTLNRGTMGNHLLTNPVLQPRGGTSPY NTLIAESVGFNPSSPPVFNSPGSYREPKHPLGGR EACGMDTLPLNGNFNNSYSLRSGDFP PGDGGPEPPRGRNLADAAAFEKMIISELVHNNL RGSSSAAGPPPPPEPPVPPVPGGGGEE EAGGPGGADRAEIELLYKALEEPLLLPRAQSVLY QSDLDESECTAEDGATSRPLSSPPG RDSLYASGANLRDPSYPDSSPEGPSEALPPPPP APPGPPEIYYTSRPPALVARNPLQGY YQVRRPSHEGYLAAPGLEGPDPDGDGQMQLV TSL [SEQ ID NO: 31]	
ADGRL3	MWPSQLLIFMMLLAPIIHAFSRAPIPMAVVRRE LSCESYPIELRCPGTDVIMIESANYGR TDDKICDSDAQMENIRCYLPDAYKIMSQRCN NRTQCAVVAGPDVFPDPCPGTYKYLEVQ YECVPYKVEQKVFLCPGLLKGVYQSEHLFESDH QSGAWCKDPLQASDKIYYMPWTPYRTD TLTEYSSKDDFIAGRPTTTYKLPHRVDGTGFVVY DGALFFNKERTRNIVKFDLRTRIKSG EAIIANANYHDTSPYRWGGKSDIDLAVDENG WVIYATEQNNKGIVISQLNPYTLRIEGT WDTAYDKRSASNAFMICGILYVVKSVYEDDDNE ATGNKIDYIYNTDQSKDSLVDVPPFNS YQYIAAVDYNPRDNLLYVWNNYHVVKYSLDFG PLDSRSGQAHHGQVSYISPPIHLDSELE RPSVKDISTTGPLGMGSTTTTLRTTTLSPGRST TPSVSGRRNRSTSTPSPAVEVLDDM TTHLPSASSQIPALEESCEAVEAREIMWFKTRQG QIAKQPCPAGTIGVSTYLCCLAPDGIW	>sp Q9HAR2 AG RL3_HUMAN Adhesion G protein-coupled receptor L3 OS=Homo sapiens OX=9606 GN=ADGRL3 PE=1 SV=2

	DPQGPDLNCSSPWVNHITQKLKSGETAANIAR ELAEQTRNHLNAGDITYSVRAMDQLVG LLDVQLRNLTGGKDSAARSLNKAMVETVNNL LQPQALNAWRDLTSDQLRAATMLLHTV EESAFVLADNLLKTDIVRENTDNIKLEVARLSTEG NLEDLKFPENMGHGSTIQLSANTLK QNGRNGEIRVAFVLYNNLGPYLSTENASMKLGT EALSTNHSVIVNSPVITAAINKEFSNK VYLADPVVFTVKHIKQSEENFNPNCSEFWSYSKR TMTGYWSTQGCRLLTTNKTHHTTCSCNH LTNFAVLMAHVEVKHSDAVHDLDDVITWVGIL LSLVCLLICIFTFCFFRGLQSDRNTIH KNLCISLFVAELLFLIGINRTDQPIACAVFAALLHF FFLAAFTWMFLEGVQLYIMLVEVF ESEHSRRKYFYLVGYGMPALIVAVSAAVDYRSY GTDKVCWLRLDTYFIWSFIGPATLIIM LNVIFLGIALYKMFHHTAILKPESGCLDNIKSWVI GAIALLCLLGLTWAFGLMYINESTV IMAYLFTIFNSLQGMFIFIFHCVLQKKVRKEYGKC LRTHCCSGKSTESSIGSGKTSGSRT PGRYSTGSQSRIRRMWNDTVRKQSESSFITGDI NSSASLNREGLLNARDTSVMDTLPLN GNHGNYSIASGEYLSNCVQIIDRGYNHNETALE KKILKELTSNYIPSYLNNHERSSEQN RNL MNKLVNNLGS GREDDAIVLDDATSFNHEE SLGLELIHEESDAPLLPPRVYSTENHQP HHYTRRRIPQDHSESFPLLTNEHTEDLQSPHRD SLYTSMP TLAGVAATESVTTSTQTEP PPAKCGDAEDVYYKSMPNLGSRNHVHQLHTYY QLGRGSSDGFIVPPNKDGTPEGSSKGP AHLVTSL [SEQ ID NO: 32]	
ADGRE2	MRHGHPRLLPGLLMLLLLPLGAAAQKTSGCAR WCPPKSTCVNATTCRCSPGFSSLSGEIF SSPLESCDDIDECGPPPLVSCGRLADCQNTESGY HCMCSPGYALASGATTFMNESENTCR DVDECQLKPRVCKSRGICTNTKGSYTCCKPPGFE LNLGDLNLCTDVNECTSGQNPCHNST HCLNNIGGYECRCRPGWKVPVGPSPNGPKSTVC EDVDECSSGKHTCHYSTVCINTVGSYKC RCRRGWKPKPRFQDRQLNTTCEVPAEMSFT WTPPPGIKSQRLSNFFERVQELHRDFKPA LAQETIQDLIQEVDELLEIPGDLEALPHSEQHCV ATNLLVGLEGVLRNVSQAMPNGPWTF NASAGTDLSEVQEEGYRNVTLSQLAKMMLK WDVVHKSGDSGPSVVGLLSTPGMGKLLA EAPLVLEPEKQAVLHGAPKGLLQGVSSVLLSDVI SVFMSNKVTQKLSSPVTIFISHHSAT	>sp Q2Q421 AG RE2_CANLF Adhesion G protein-coupled receptor E2 OS=Canis lupus familiaris OX=9615 GN=ADGRE2 PE=2 SV=2

	HEPKLVFCVFEHSQDECGHWSTRGCTVVDS GDTSTTCQCTHLSSFAVLMAHYDVQEED LVLPVITYVGLGLSLLCLLLAALTFLCKAIQNTSTS LHLQLLICLFLAHLFLMAIDRT EIKVLCIIAGALHYLYLASFTWMLLEGLHLFLTA RNLMMVNYSSVSMLMKKLMYPVGYG VPTLIVAISAASRSHLYGTRTRCWLNPEERFIWSF LGPVCTIFSVNLGFFLMTLWILKSK LSSLNSDVSTLQNTRMLTFKAIQFLGCTWCL GILQVGPAAHVMAYLFTIINSLQGVF IFLVYCLLSQQVREEYGKWFKGIRKTRAESEKYTL SSRAMSDVNKPMMVN [SEQ ID NO: 33]	
ADGRG2	MVFSVRQCGHVGRTTEEVLLTFKIFLVIICLHVVLV TSLEEDTDNSSLSPPAKLSVVSFA PSSNGTPEVETTSLENDVTLSSLPSNETEKTITIVK TFNASGVKQQRNICNLSSICNDSA FFRGEIMFQYDKESTVPQNHITNGTLTGVLSSL ELKRSELNKTLQTLSETYFIMCATAE AQSTLNCTFTIKLNTMTNACAVIAALERVKIRP MEHCCCSVRIPCSSPEELEKLQCDLQ DPIVCLADHPRGPPFSSSQSIPVVPRAVLVSQVP KATSFAEPPDYSPVTHNVPSPIGEIQ PLSPQPSAPIASSPAIDMPPQSETISSPMPQTHV SGTPPPVKASFSSPTVSAPANVNTTS APPVQTDIVNTSSISDLENQVLQMEKALSLGSLE PNLAGEMINQVSRLHSPDMLAPLA QRLLKVDDIGLQLNFSNTTISLTSPSLAVIRV NASSFNTTTFVAQDPANLQVSLETQ APENSIGTITLPSSLMNNLPAHDMELASRVQFN FFETPALFQDPSLENLSLISYVISSSV ANLTVRNLTRNVTVLKHINPSQDELTVRCVFW DLGRNGGRGGWSDNGCSVKDRRLNETI CTCSHLTSFGVLLDSRTSVLPAQMMALTFITYI GCGLSSIFLSVTLVTYIAFEKIRRDY PSKILIQCAALLLNLFLLDSWIALYKMQGLCIS VAVFLHYFLLVSFTWMGLEAFHMY LALVKVFNTYIRKYILKFCIVGWGVPVAVVTIILT SPDNYGLGSYGKFPNGSPDDFCWI NNNAVFIYTVVGYFCVIFLLNVSMFIVVLVQLCRI KKKKQLGAQRKTSIQDLRSIAGLTF LLGITWGFAGFAWGPVNVTFMYLFAIFNTLQGF FIFIFYCAKENVRKQWRRYLCCGKLR LAENSDWSKTATNGLKKQTVNQGVSSSSNSLQ SSSNSTNSTLLVNDCSVHASGNGNAS TERNGVSVQNGDVCLHDFGKQHMFEKE DSCNGKGRMALRRTSKRGSLSHFIEQM [SEQ ID NO: 34]	>sp Q8IZP9 AGR G2_HUMAN Adhesion G- protein coupled receptor G2 OS=Homo sapiens OX=9606 GN=ADGRG2 PE=1 SV=2

Tethered peptide agonist of ADGRG1	<u>TYFAVLM [SEQ ID NO: 73]</u>	
Tethered peptide agonist of ADGRG2	TSFGVLL [SEQ ID NO: 74]	
Tethered peptide agonist of ADGRF5	<u>TSFSILMS [SEQ ID NO: 75]</u>	
GPS site for ADGRF5	HL^TSFSILMS [SEQ ID NO: 76]	
Tethered peptide agonist for ADGRL1	TNFAVLM [SEQ ID NO: 77]	
Tethered peptide agonist of ADGRL3	TNFAVLM A [SEQ ID NO: 78]	
Tethered peptide agonist of ADGRE2	SSFAVLMA [SEQ ID NO: 79]	
HormR domain of ADGRF5	<u>GENITCODPVIGVGEPGKVIOKLCRFSNV</u> <u>PSSPESPIGGTITYKCVGSOWEEKRND</u> <u>[SEQ ID NO: 80]</u>	

Sequence of the parts of the described aGPCRs that are involved in intracellular signalling i.e. the three intracellular loops and the C-terminal tail:

5

	ICL1	ICL2	ICL3	C-terminal tail
Based on ADGRL1 (Signalling via Gαq (major), Gα i/o (weak))	STFC FLRG LQTD RN <u>[SEQ ID NO: 81]</u>	CLEG VHLY LLL EVFE SEYS RTK <u>[SEQ ID NO: 82]</u>	VT LH KMIR SSSV LKPD SSRL DNIK SWA <u>[SEQ ID NO: 83]</u>	HCALQKKVHKEYSKCLRHS YCCIRSPPGGTHGSLKTS MRSNTRYTGTQSRIRRM WNDTVRKQTESSFMAGDIN STPTLNRGTMGNHLLTNPV LQPRGGTSPYNTLIAESVGF NPSSPPVFNSPGSYREPKH PLGGREACGMDTLPLNGNF NNSYSLRSGDFPPGDGGPE PPRGRNLADAAAFEKMIISE LVHNNLRGSSSAAGPPPP EPPVPPVPGGGGEEEAGG PGGADRAEIELLYKALEEPL LLPRAQSVLYQSDLDESES CTAEDGATSRPLSSPPGRD SLYASGANLRDSPSYDPSS PEGPSEALPPPPAPP EIIYYTSRPPALVARNPLQGY YQVRRPSHEGYLAAPGLEG PGPDGDGQMLVTSL <u>[SEQ ID NO: 84]</u>

Based on ADGRL3 (Signalling via G α 12/13 (main), G α q (weak)))	RGL QSD RNTI HK [SEQ ID NO: 85]	VEVF ESEH SRRK [SEQ ID NO: 86]	YKMF HHTA ILKPE SGCL DN [SEQ ID NO: 87]	KKVRKEYGKCLRTHCCSGK STESSIGSGKTSGSRTPGR YSTGSQSRIRRMWNDTVRK QSESSFITGDINSSASLNRE GLLNNARDTSVMDTLPLNG NHGNSYSIASGEYLSNCVQI IDRGYNHNETALEKKILKELT SNYIPSYLNNHERSSEQNR NLMNKL VNNLGSGREDDAI VLDDATSFNHEESLGLELIH EESDAPLLPPRVYSTENHQ PHHYTRRRIPQDHSESFFPL LTNEHTEDLQSPHRDSLTY SMPTLAGVAATESVTTSTQ TEPPPAKCGDAEDVYYKSM PNLGSRNHVHQLHTYYQLG RGSSDGFIVPPNKDGTPE GSSKGPAHLVTSL [SEQ ID NO: 88]
Based on ADGRE2 (Signalling via G α 16/ PLC- β /PI3K/Akt/MAPK/NF- κ B pathways)	ALTF LLCK AIQN TST [SEQ ID NO: 89]	LLEG LHLF LTAR NLMV VNYS SVSM LMK [SEQ ID NO: 90]	MTLW ILKSK LSSL NSDV STLQ NTRM LTFK AIAQ [SEQ ID NO: 91]	SQQVREEYGKWFKGIRKTR AESEKYTLSSRAMSDVNKP MMVN [SEQ ID NO: 92]
Based on ADGRG2 (Signalling via Gs, G α 12/13 and G α q pathway)	TYIA FEKI RRD YPSK ILIQL [SEQ ID NO: 93]	EAFH MYLA LVKV FNTYI RKYIL K [SEQ ID NO: 94]	QLCR IKKKK QLGA QRKT SIQD LRS/ [SEQ ID NO: 95]	YCVAKENVRKQWRRYLCC GKLRLAENSDWSKTATNGL KKQTVNQGVSSSSNSLQSS SNSTNSTLLVNNDCSVHA SGNGNASTERNGVSFSVQ NGDVCLHDFTGKQHMFNE KEDSCNGKGRMALRRTSK RGS LHFIEQM [SEQ ID NO: 96]

Sequences of the GPS cleavage site and CTF stalk of a range of aGPCRs.

TA refers to the tethered agonist.

5

[SEQ ID NO:	97	CACSHLTNFAVLMAHREIYQGRINELLSV
[SEQ ID NO:	98	CACSHLTNFAILMAHREIAYKDGVEHLLTV

[SEQ ID NO:	99	CSCNHLTNFAVLMAHVEVKHSDAVHDLLEDV
[SEQ ID NO:	100	CRCNHLTHFAILMSSGPSIGIKDYNILTRI
[SEQ ID NO:	101	CSCNQMANLAVIMASGELTMDFSL
[SEQ ID NO:	102	CRCTHLSSFAVLMAHYDVQEEDPVL
[SEQ ID NO:	103	CNCNHLSSFAVLMALTSQEEDPVL
[SEQ ID NO:	104	CKCFHLSSFAVLVALAPKEDPVL
[SEQ ID NO:	105	CQCCHLSSFAVLMALTSQEEDPVL
[SEQ ID NO:	106	LHCQHLGNVAVLMELSAFPREVGGAGAGLH
[SEQ ID NO:	107	IQCYLSNYAVLMDLTGSELYTQAASLLHP
[SEQ ID NO:	108	CQCSTASFAVLMDISRRENGEVLPLKI
[SEQ ID NO:	109	CQCNHMTSFAVLMDSRRENGEILPLKT
[SEQ ID NO:	110	CRCSTGTGVLMDASPRERLEGDLELL
[SEQ ID NO:	111	CRCTHLTNFAVLMQVPLELARGHQVALSS
[SEQ ID NO:	112	CFCNHSTSFALLQIYEVQRGPEEESLLR
[SEQ ID NO:	113	CQCCHLTSFSLMSPFVPSTIFPVVKWIT
[SEQ ID NO:	114	CKCRPSKLTSFSLMSPHILES
[SEQ ID NO:	115	CLCQHLTAFSVLMSPHTVPEEPALALLTQ
[SEQ ID NO:	116	CRCNYTSVMSFSLMSSKSMSTDK
[SEQ ID NO:	117	CICDHLTSFSLMSPDSPDPSSLLGILLD
[SEQ ID NO:	118	CLCDRLSTFAVLAQLSADANMEKATLP
[SEQ ID NO:	119	CQCQHLSTFAVLAQPPKDLTLELAGSPSVP
[SEQ ID NO:	120	CLCDRLSTFAVLAQPPREIMESSGTPSVT
[SEQ ID NO:	121	CFCNHLTYFAVLMVSSVEVDVHKHYLS
[SEQ ID NO:	122	CTCSHLTSFGVLLDLRSTSVLPAQMMAL
[SEQ ID NO:	123	CCCDHLTFFALLLRPTLDQSTVHILTR

[SEQ ID NO:	124	CQCDHLTHFGVLMDLRSSTVDSVNEQIL
[SEQ ID NO:	125	CRCNHLTYFAVLMQLSPALVPAELLAPL
[SEQ ID NO:	126	CLCNHFTHFGVLMDLPRSASQLDARNTKVL
[SEQ ID NO:	127	CRCNHTTNFAVLMTFKKDYQYPKSLD
[SEQ ID NO:	128	CACSHMSVYAVYARTDNLSSYNE
[SEQ ID NO:	129	CXCXHLTXFAVLMXXXXXXXXXXXXXXXXXX

Figure Legends

Figure 1 – Chimeric aGPCRs design and engineering. A) Schematic diagram of aGPCRs and their activation mechanism. The unique feature of aGPCR family members is the autoproteolysis within the highly conserved GAIN domain (GPCR Autoproteolysis-Inducing domain). It leads to the noncovalent association between the N-terminal extracellular domain (ECD) and the tethered agonist–7 transmembrane domain/cytoplasmic C-terminal fragment. After binding to target ligand, the mechanical forces will remove the N-terminal fragment, and then expose the tethered agonist peptide to activate the aGPCRs. B) Concept of chimeric aGPCR design. In order to expand the sensing repertoire of synthetic or chimeric aGPCRs, the original ligand binding domains of aGPCRs are replaced by diverse heterologous ~~synthetic~~ ligand binding scaffolds (e.g. scFv). The mechanical force caused by antibody – antigen interaction removes the GAIN domain and expose the tethered agonist peptide to activate GPCR signalling pathway. C) General strategy of chimeric aGPCR design and engineering. The chimeric aGPCR scaffold is composed of: 1. different aGPCR signal peptide for tuning receptor expression level; 2. diverse synthetic sensing scaffold against different target of interests; 3. linker sequences which are critical to receptor performance; 4. modularized aGPCR variants which are composed of the GAIN domain, corresponding tethered agonist, 7 transmembrane and cytoplasmic domain; 5. genetic circuit design for signal processing engineering; and 6. N-terminal c-Myc tag and C-terminal HiBIT tag for verifying receptor cell surface expression level and autoproteolysis status of chimeric aGPCRs.

Figure 2 – Verify expression level of chimeric aGPCRs on different cell surface. A) Express chimeric aGPCRs in different cell type. The chimeric aGPCR will be introduced into: 1) Jurkat NFAT-luciferase system (Promega) by mRNA nucleofection; 2) MK cells by mRNA nucleofection or lentivirus infection; and 3) iPSC cells by CRISPR

knock-in, and further transformed into MK cells and Synlets through directed differentiation and plate shaking. B) Analysis of chimeric aGPCR expression level by flow cytometry and western blot. C) Jurkat cell surface expression level of 4 different chimeric aGPCRs. 4 chimeric aGPCRs fused with FMC63 single chain variable fragment (scFv) which recognizes antigen CD19 were designed based on ADGRG1 and ADGRF5 (sequence and design detail see supplementary information). Their cell surface expression levels were analysed by anti FMC63-PE conjugated antibody and flow cytometry.

Figure 3 – Functional analysis of chimeric aGPCRs in Jurkat NFAT-luciferase system. A) Functional analysis of FMC63 chimeric aGPCRs in Jurkat NFAT-luciferase system (Promega). The Jurkat cells nucleofected with IVT mRNAs of different chimeric aGPCRs constructs were incubated in the 96 well streptavidin plates coated with biotinylated antigen CD19 at 37 degrees with gently shaking (120 rpm). The mechanical force generated through FMC63-CD19 interaction and plate shaking will separate the N-terminal scFv FMC63-GAIN domain fusion protein and the C-terminal aGPCR fragment, and then further activate the downstream luciferase reporter gene expression through NFAT signalling pathway. B) Functional analysis results of 4 different chimeric aGPCR constructs in Jurkat NFAT-luciferase system.

Figure 4 - Detect the release of N-terminal scFv FMC63-GAIN domain fusion protein. A) Schematic diagram of detecting the release of N-terminal scFv FMC63-GAIN domain fusion protein. The release of N-terminal scFv FMC63-GAIN domain fusion protein from Jurkat cell surface was analyzed under four different incubation conditions: i. without binding to antigen CD19 and without plate shaking; ii. without binding to antigen CD19 but with plate shaking; iii. with binding to antigen CD19 but without plate shaking; and iv. with binding to antigen CD19 and with plate shaking. B) Analyze surface remained scFv FMC63-GAIN domain fusion protein under four different incubation conditions by flow cytometry. C) Functional analysis of ADGRG1.2 under four different incubation conditions. The analysis was performed with three different cell amounts.

Figure. 5 – Different responses of engineered FMC63-ADGRG1.2wt and FMC63-ADGRG1.2_H381S for ligand binding and plate shaking. H381S mutation is in the GPCR proteolysis site (GPS) and inhibit the aGPCR autoproteolysis activity. The mechanical force generated by CD19-FMC63 scFv interaction and plate shaking should not be able to separate the GAIN domain of mutated ADGRG1.2_H381S from tether peptide agonist and unable to trigger the

aGPCR downstream signal response. 10.5 and 15 ug of ADGRG1.2wt and ADGRG1.2_H381S mRNA were nucleofected into Jurkat cells for functional assay. Comparing to ADGRG1.2_H381S, the ADGRG1.2wt has more significant response to ligand binding and plate shaking.

5

Figure 6- Functional analysis of chimeric aGPCRs in MK cells or Synlets. Since the activation of chimeric aGPCRs triggers downstream α -granule release through PLC β pathway, the function of chimeric aGPCRs in MK cells or synlets can be analysed through: the expose of P-selectin/CD62p on cell surface; the release of platelet factor 4 (PF4); and the release of pre-loaded therapeutic cargos. The other molecules involved in relative signalling pathways will also be applied for further engineering.

10

Figure 7 – *In vitro* and *in vivo* mouse models for functional analysis of chimeric aGPCRs. A) Establishment of endothelial cell line (e.g. MS1) with tuneable cell surface antigen (e.g. CD19/CD276/MadCam1) expression level. B) Multi-stage *in vitro* chimeric aGPCRs screening. In order to effectively select functional constructs, the chimeric aGPCR variants will be selected step-by-step through: I.) 2D culture cell surface binding assay; II.) Transwell therapeutic cargo release assay; and III) 3D microfluidic co-culture system for mimicking cancer micro-environments. C) Establishment of *in vivo* mouse model. MS1 cells expressed with target antigen forms hemangioma in NGS mice and will be used for evaluating the therapeutic effect of chimeric aGPCRs (Left). Co-injection of human cancer cell line and MS1 cells with or without expressing target antigen will form xenografted tumor vasculature as positive and negative control for functional analysis of chimeric aGPCRs (Right).

25

Figure.8 Rewiring chimeric aGPCR signalling through genetic circuit design. a) Rewiring aGPCR downstream signalling pathway through chimeric G protein (left) or modifying aGPCR G protein binding motif. The chimeric G protein can be engineered through alternating C-terminal GPCR binding sequences. For example, the chimeric G protein G α_q / G α_s is engineered by replacing the original G α_q C-terminal GPCR binding sequence with the sequence from G α_s . The chimeric G protein G α_q / G α_s enables the G α_s dependent aGPCR (rather than original G α_q dependent aGPCR) to activate downstream G α_q signalling pathway upon activation. B) Synthetic genetic circuit design based on close proximity induced by aGPCR activation. Nter: N-terminal. Cter: C-terminal.

30

35

Figure. 9 Sequences of the GPS cleavage site and CTF stalk of a range of aGPCRs.

TA refers to the tethered agonist.

Figure 10. Schematic of a GPCR protein depicting the position of the three intracellular loops involved in intracellular signalling with the C-terminal tail.

5

Figure 11. A – Schematic depicting the extracellular domain and intracellular tail of an embodiment of the chimeric aGPCR of the invention. B – Schematic depicting the arrangement of the intermediate adaptor protein or peptide, and the corresponding tag and ultimate target binding domains.

10

Examples

Outline

The aGPCRs sense and respond to environmental stimuli through the mechanical forces generated by the interaction between target ligands and their own ligand binding motifs (Fig. 1a). In order to expand the sensing repertoires of aGPCR, here we design and engineer chimeric aGPCRs through swapping their natural ligand binding motifs with synthetic (or the non-natural) sensing scaffolds (Fig. 1b). General strategies for engineering chimeric aGPCR include: a) fine tuning the receptor expression level through screening different aGPCR signal peptides; b) verifying receptor cell surface expression level by adding N-terminal c-myc tag for detection; c) swapping the natural sensing domain for different sensing domains, such as nanobodies, scFvs, or Fabs against diverse target of interest; d) tuning the signal threshold, signal output strength, and signal to noise ratio (S/N ratio) by modularizing diverse aGPCR GAIN domain, 7 transmembrane region and cytosolic domains; e) rewiring the downstream signal processing through genetic circuit design and engineering; and f) verifying auto-proteolysis status with C-terminal HiBiT tag labelling (Fig. 1c). Further details are described below.

Example 1. Express chimeric aGPCRs on cell surface

Based on the intended use and purpose, chimeric aGPCRs are expressed in different cell types through different methods, including: 1) The in vitro transcription (IVT) mRNA of different chimeric aGPCR constructs will be nucleofected into Jurkat NFAT-luciferase system for high throughput (HTP) screening of functional chimeric aGPCR variants; 2) The iPSC or human stem cells (hSCs) direct differentiated MK cells will be infected with lentivirus constructs of selected functional chimeric aGPCR variants for further verification; 3) The selected candidates of functional chimeric aGPCR constructs will be knocked-in into iPSC cell line and further directed differentiated into MK cells for functional analysis. The MK cells described above will be further used for producing Synlets through plate shaking (Fig. 2a). The surface expression level of chimeric

aGPCRs will be analysed through: 1) Fluorophore conjugated anti c-Myc tag antibody to detect the N-terminal c-Myc tag of chimeric aGPCRs by flow cytometry; 2) Fluorophore conjugated target ligand to detect the ligand-sensing scaffold interaction by flow cytometry; and 3) Fluorophore conjugated anti sensing scaffold antibody to detect the folding/expression of sensing scaffold. In addition, the auto proteolysis status of chimeric aGPCR constructs will be analysed through C-terminal HiBIT tag by western blot (**Fig. 2b**).

The expression level of 4 FMC63 chimeric aGPCRs were analysed on Jurkat cell surface. FMC63 is a CD19-specific IgG1 mouse monoclonal antibody. These chimeric aGPCRs were designed based on ADGRG1 and ADGRF5. Their ligand binding domains were swapped with the FMC63 scFv which recognizes antigen CD19 (design and sequence detail please see Table 1). Their cell surface expression levels were analysed by anti FMC63-PE conjugated antibody (Acrobiosystems) and flow cytometry (**Fig. 2c**). These four designed constructs were all able to express on Jurkat cell surface. The two constructs (ADGRG1.1 and ADGRG1.2) designed based on ADGRG1 have shown much higher expression level than the other two constructs designed based on ADGRF5 (ADGRF5.1 and ADGRF5.2). The differences between ADGRG1.1/ADGRG1.2 (with or without extra linker) or ADGRF5.1/ADGRF5.2 (with or without HormR domain) seems to have low or no influence on their cell surface expression level.

>In vitro transcription of mRNA

IVT mRNA production was followed the manual of HiScribe T7 mRNA Kit with CleanCap Reagent AG. In brief: T7 promoter, eIF4G aptamer, kozak sequence and 3'UTR were added into target gene sequence by PCR amplification. The purified DNA fragments were further in vitro transcribed into mRNA by CleanCap reaction which contains pseudo-UTP and other NTPs, reaction buffer, 5' Cap analog, and T7 RNA polymerase mix. After removing the DNA template by DNase I reaction, the poly A tail was added by *E. coli* Poly(A) Polymerase.

30

>Nucleofection of mRNA into Jurkat cells

After PBS wash to remove cell culture medium, 1×10^6 of Jurkat cells were resuspended in 20 μ L of SE buffer (Lonza). 1 – 6 μ g of IVT mRNA in 2 μ L of nuclease-free water was added into the Jurkat cells and transferred into nucleofection cuvette. The cells were nucleofected in the 4D nucleofector with program CM-120. After rescued by 100 μ L of Jurkat cell medium, the cells were further transferred into 2 mL of culture medium in 6 well culture plate and incubated in 37 degrees overnight for further analysis.

>Surface expression level assay

1 x 10⁵ of Jurkat cells nucleofected with the mRNA of chimeric aGPCRs were labelled with CD19-PE (Acrobiosystem, 1:100) or anti-FMC63 antibody-PE (Acrobiosystem, 1:100) for 20 – 30 mins in the dark. The cells were washed by MACSQuant running buffer to remove the unbound CD19-PE or antibody-PE. The pelleted cells were resuspended in MACSQuant running buffer with DAPI (1:200) and then further analysed by MACSQuant flow cytometry.

10 **Example 2. Functional assay of chimeric aGPCRs in Jurkat NFAT-Luciferase system**

The function of chimeric aGPCRs were tested in Jurkat NFAT-Luciferase system (Promega). The activation of aGPCR will increase cytoplasmic calcium concentration through phospholipase C (PLC) pathway, which will further activate downstream NFAT-Luciferase reporter gene expression.

The Jurkat NFAT-Luciferase reporter cells nucleofected with IVT mRNAs of different FMC63 chimeric aGPCRs constructs were incubated in the 96 well streptavidin plates coated with biotinylated antigen CD19. The plate was incubated at 37 degrees with gently shaking to generate the mechanical forces to activate the chimeric aGPCRs and downstream reporter gene expression (**Fig. 3a**). The functional assay of four FMC63 chimeric aGPCRs have shown that the two chimeric aGPCR designed based on ADGRG1 (ADGRG1.1 and ADGRG1.2) have response to target antigen CD19 (**Fig. 3b**). The different functional performance between ADGRG1.1 and ADGRG1.2 should result from the presence of an extra linker sequence between FMC63 scFv and the GAIN domain, which highlight the importance of linker sequences to the function of ADGRG1 based chimeric aGPCR design (sequence details please see the supplement information). The lack of function of the two ADGRF5 based FMC63 chimeric aGPCRs design may be due to the lower expression level comparing to ADGRG1 based design (**Fig. 3c**). Further tuning the surface expression level of ADGRF5 based chimeric design through signal peptide engineering, and verifying the folding and binding capability of surface expressed FMC63 scFv will be the main tasks to improve the ADGRF5 based chimeric aGPCR design.

35 **Example 3. Effects of agitation and cleavability of GPS site**

The release of N-terminal scFv FMC63 - GAIN domain fusion protein and the influence of plate shaking to the activation of aGPCR were verified with the chimeric aGPCR

construct ADGRG1.2. Jurkat NFAT-Luciferase reporter cells nucleofected with mRNA of ADGRG1.2 were incubated under 4 different conditions (**Fig. 4a**). Surface expression level of scFv FMC63-GAIN domain is significantly reduced due to ligand binding and plate shaking (**Fig. 4b**). The Jurkat NFAT-Luciferase reporter cells nucleofected with
 5 ADGRG1.2 can only be activated under ligand binding and plate shaking condition (**Fig. 4c**).

To verify if the exposure of tethered peptide agonist is essential for the activation of chimeric aGPCR, the ADGRG1 autoproteolysis resistant variant H381S were used as a
 10 negative control (**Fig.5**). The significant lower response of ADGRG1.2_H381S to ligand binding and plate shaking comparing to wild type ADGRG1.2 indicated that the proteolysis and exposure of tethered peptide agonis is important for aGPCR function.

15 >Functional assay of Jurkat NFAT-Luciferase system

The day before the Jurkat NFAT-Luciferase assay, the 96 white well streptavidin plate (Pierce) was coated with 0.4 µg CD19-Fc-Avitag (bio-technie) per well and store in 4 degrees overnight. 5 x 10⁴ of Jurkat cells nucleofected with the mRNA of chimeric aGPCRs were resuspend in Jurkat cell culture medium and added into each well. The
 20 plate was incubated at 37 degrees with gently shaking (120 rpm) for 6 hours. After incubation, 50 µL of Bio-Glo luciferase assay buffer (Promega) were added into each well. The luciferase signal was further analysed by FLUOstar Omega plate reader.

Example 4. Functional assay of chimeric aGPCRs in MK cells

25 The activation of chimeric aGPCR will lead to the activation of PLC and then further promote the α-granules to release cargos such as platelet factor 4 (PF4) or pre-loaded therapeutic cargos (e.g., scFvs or cytokines), or the expose of P-selectin/CD62p on platelet surface (**Fig. 6**). The exposure of P-selectin can be labelled by fluorophore conjugated anti-P-selectin antibodies and analysed by flow cytometry. The release of
 30 PF4 or pre-loaded therapeutic cargo can be also analysed through ELISA experiments. These assays provide us diverse methods to test the functional performance of chimeric aGPCRs in MK cells.

Example 5. Functional assay of chimeric aGPCRs in Synlets

35 Synlets are produced through gently shaking of MK cell culture plates overnight. The function of chimeric aGPCRs will be tested through: 1) the expose of P-selectin on Synlets surface by flow cytometry; 2) the release of PF4 or pre-loaded therapeutic cargo by ELISA.

Example 6. Expanding the sensing repertoire of chimeric aGPCRs

In order to expanding the sensing repertoire of chimeric aGPCRs, diverse sensing scaffolds including nanobodies, scFvs and Fabs will be used for further chimeric aGPCR designs. Different target of interest such as CD19, CD276, and MadCAM1 and their corresponding sensing scaffolds will be also introduced into chimeric aGPCR designs.

Example 7. *In vitro* screening of functional chimeric aGPCRs in cell models

The *in vitro* screening of functional chimeric aGPCRs in cell models will be established for further evaluating their capabilities to be activated by target antigen expressed on cell surface.

Immortalised mouse endothelial cell models (e.g. MS1 line) have been proven to be able to support the *in vitro* and *in vivo* targeting of endothelial cell markers. The expression level of target protein on endothelial cell models can also be fine-tuned by selecting different promoters or virus MOI (**Fig.7a**).

The functional chimeric aGPCRs will be selected through three *in vitro* models (**Fig.7b**): a) select the aGPCR design variants which are able to bind/interact with MS1 target presenting cells in 2D cell culture plate (**Fig.7b, left**); b) the selected variants will be further analysed in transwell assays to test their capability to release functional therapeutic cargo upon interacting with MS1 target presenting cells (**Fig.7b, middle**); c) the variants selected after two stage of screening will be applied to 3D microfluidic assay to mimic the environment in blood flow (**Fig.7b, right**).

Example 8. Functional assay of chimeric aGPCRs in MS1 mouse model.

MS1 cell lines expressing target antigen are able to form vascular hemangioma in NGS mice. It has been proved that such hemangioma can be removed by anti-target antigen CAR-T cells. Therefore, the NGS mice model with vascular hemangioma formed by target antigen expressing MS1 cells will be applied as the first step for evaluating the therapeutic effect of chimeric aGPCRs (**Fig.7c, left**).

In addition, it has been proved that the co-injection of human cancer cell line and MS1 cells in mice will form xenografted tumor vasculature derived from MS1 cells. The co-injection of human cancer cell line and MS1 cells with or without expressing target antigen will be applied as positive and negative control for evaluating the therapeutic effect of chimeric aGPCRs (**Fig.7c, right**) .

Example 9. Rewiring chimeric aGPCR signalling through genetic circuit design.

Modularization and adaptation of various aGPCRs into Synlet platform is the key step for optimizing the signalling performance of chimeric aGPCRs. To achieve this goal, rewiring diverse downstream signalling with $G_{\alpha q}$ protein pathway, which is the major pathway for the releasing of α -granule through GPCRs in platelet will be the first priority. Strategies such as introducing chimeric G protein or modify G protein interaction motif on GPCR based on structural information will be applied in this project (**Fig.8a**).

Implanting diverse Boolean logic gates has shown great potential in improving the precision of therapeutic cells. However, the possibility of synthetic ITAM and aGPCR receptor-based logic gate design is greatly limited by sharing the same downstream calcium signalling pathway. Therefore, there is an urgent need for orthogonal genetic circuit design for rewiring aGPCR downstream signalling. Close proximity induced by GPCR activation-based genetic circuit design will be recruited in this project (**Fig.7b**): the C-terminus of aGPCR is fused with a V2 tail sequence from arginine vasopressin receptor 2 (AVPR2) and a part of downstream split reporter (e.g. C-terminal fragment of split PLC β); and an adaptor protein, Beta-Arrestin-2 (ARRB2) is fused to the other part of downstream split reporter (e.g. N-terminal fragment of split PLC β). The interaction between V2 tail sequence and ARRB2 induced by aGPCR activation will bring the two split reporter fragments into close proximity and therefore restore PLC β function. The dimerized PLC β can be further activated through ITAM based signalling pathway for releasing therapeutic cargos in α -granule and form an "AND" Boolean logic gate for improve the precision of Synlets.

The invention also provides the following numbered embodiments

1. A chimeric adhesion G-Protein Coupled Receptor (aGPCR) comprising:
 - a) an intracellular tail domain, optionally a C-terminal tail;
 - b) a seven-transmembrane domain (7TM) that comprises three intracellular loops (ICLs); and
 - c) an extracellular domain comprising:
 - (i) a target binding domain that is heterologous to the intracellular tail domain;
 - (ii) a GPCR autoproteolysis-inducing domain (GAIN domain) that optionally comprises a tethered agonist peptide;

and optionally further comprising

(iii) a linker, optionally wherein the linker is in the extracellular domain, optionally between the GAIN domain and the target binding domain.

- 5 2. The chimeric aGPCR of embodiment 1 wherein the GAIN domain comprises a GPCR-proteolytic site (GPS) site optionally positioned N-terminal of the tethered peptide agonist.
3. The chimeric aGPCR of embodiment 2 wherein the GPS site is cleaved by the
- 10 GAIN domain.
4. The chimeric aGPCR of embodiment 2 wherein the GPS site is not cleaved, optionally not cleaved by the GAIN domain.
- 15 5. The chimeric aGPCR of embodiment 4 wherein the extracellular domain further comprises a protease site, optionally comprises a protease site that is a cysteine protease site, a metallo protease site, an aspartate protease site, a serine protease site or a threonine protease site; and/or
- 20 a protease site that is a protease that is associated with the tumour microenvironment, optionally wherein
 - i) the chimeric aGPCR becomes activated upon cleavage of the protease site in the absence of binding of the target binding domain to the target; or
 - 25 ii) the chimeric aGPCR becomes activated only upon cleavage of the protease site and binding of the target binding domain to the target.
6. The chimeric aGPCR of any of embodiments 1-5 wherein the:
 - 30 intracellular tail domain, optionally the C-terminal tail is autologous to the 7TM domain;
 - intracellular tail domain, optionally the C-terminal tail is autologous to the GAIN domain; and/or
 - 35 intracellular tail domain, optionally the C-terminal tail is autologous to the GPS motif.
7. The chimeric aGPCR of any of embodiments 1-6 wherein the chimeric aGPCR activates intracellular signalling when:

a) the chimeric aGPCR is localised to the plasma membrane of a chassis, optionally a cell, a platelet or an engineered platelet; and when

5 b) the chassis, optionally the cell, platelet or engineered platelet, is incubated and/or agitated in the presence of the target

optionally where the chassis, optionally the cell, platelet or engineered platelet comprises a reporter system

10 optionally where the reporter system comprises a reporter protein that is expressed when intracellular signalling is activated, optionally wherein the reporter protein is expressed from a promoter that comprises a nuclear factor of activated T cells (NFAT) response element, optionally wherein the reporter protein is a luciferase enzyme.

8. The chimeric aGPCR of any of embodiments 1-7 wherein the intracellular
15 domain comprises one or more substitutions, insertions or deletions as compared to the autologous intracellular domain or to a wild-type intracellular domain, optionally wherein

the one or more substitutions, insertions or deletions increases the intracellular
20 signalling response as compared to the autologous intracellular domain or to a wild-type intracellular domain that does not comprise the same one or more substitutions, insertions or deletions; or

the one or more substitutions, insertions or deletions decreases the intracellular
25 signalling response as compared to the autologous intracellular domain or to a wild-type intracellular domain that does not comprise the same one or more substitutions, insertions or deletions;
and/or

the one or more substitutions, insertions or deletions alters the specificity of
the intracellular signalling response as compared to the autologous intracellular domain
30 or to a wild-type intracellular domain that does not comprise the same one or more substitutions, insertions or deletions resulting in altered signalling pathway activation.

9. The chimeric aGPCR of any of embodiments 1-8 further comprising a signal
peptide, optionally a signal peptide that has been selected to achieve a desired level
35 of chimeric aGPCR at the cell, platelet, or engineered platelet surface.

10. The chimeric aGPCR of any of embodiments 1-9 wherein the:

- i)
 a) intracellular tail;
 b) seven-transmembrane domain (7TM); and
 c) GPCR autoproteolysis-inducing domain (GAIN domain) that optionally comprises a
 5 tethered agonist peptide and that optionally cleaves a GPS motif; and
 d) GPS motif that is optionally cleaved by the GAIN domain

are autologous to one another;

10 or

- ii) GAIN domain is heterologous to:
 the 7TM domain;
 the intracellular tail; and/or
 15 the target binding domain;

or

- iii)
 20 a) intracellular tail ;
 b) seven-transmembrane domain (7TM); and
 c) GPCR autoproteolysis-inducing domain (GAIN domain); and
 d) GPS motif

25 are from the same naturally occurring aGPCR;

or

- iv)
 a) intracellular tail;
 30 b) seven-transmembrane domain (7TM); and
 c) GPCR autoproteolysis-inducing domain (GAIN domain); and/or
 d) GPS motif

are from the same naturally occurring aGPCR, and wherein any one or more of the:

35

- a) intracellular tail;
 b) seven-transmembrane domain (7TM); and
 c) GPCR autoproteolysis-inducing domain (GAIN domain); and/or

d) GPS motif

comprises an amino acid sequence that has at least 75%, 80%, 85%, 90%, 92%, 94%, 96%, 98% or 100% sequence identity to the corresponding domain of the naturally occurring aGPCR.

11. The chimeric aGPCR of any of embodiments 1-10 wherein the GAIN domain is:
 - from ADGRG1 and has an amino acid sequence of [SEQ ID NO: 35];
 - 10 from ADGRL1 and has an amino acid sequence of [SEQ ID NO: 27];
 - from ADGRL3 and has an amino acid sequence of [SEQ ID NO: 28];
 - from ADGRE2 and has an amino acid sequence of [SEQ ID NO: 29];
 - from ADGRG2 and has an amino acid sequence of [SEQ ID NO: 30];
- 15 or is a GAIN domain with an amino acid sequence that has at least 75%, 80%, 85%, 90%, 92%, 94%, 96%, 98% or 100% sequence identity to SEQ ID NO: 35, 27, 28, 29 or 30, optionally wherein:
 - the GAIN domain has at least 75%, 80%, 85%, 90%, 92%, 94%, 96%, 98%
 - 20 or 100% sequence identity to SEQ ID NO: 35 and:
 - the intracellular tail is not from ADGRG1;
 - the target binding domain is not from ADGRG1; and/or
 - the 7TM domain is not from ADGRG1;
- 25 the GAIN domain has at least 75%, 80%, 85%, 90%, 92%, 94%, 96%, 98% or 100% sequence identity to SEQ ID NO: 27 and:
 - the intracellular tail is not from ADGRL1;
 - the target binding domain is not from ADGRL1; and/or
 - the 7TM domain is not from ADGRL1;
- 30 the GAIN domain has at least 75%, 80%, 85%, 90%, 92%, 94%, 96%, 98% or 100% sequence identity to SEQ ID NO: 28 and:
 - the intracellular tail is not from ADGRL3;
 - the target binding domain is not from ADGRL3; and/or
 - 35 the 7TM domain is not from ADGRL3;
- the GAIN domain has at least 75%, 80%, 85%, 90%, 92%, 94%, 96%, 98% or 100% sequence identity to SEQ ID NO: 29 and:

the intracellular tail is not from ADGRE2;
the target binding domain is not from ADGRE2; and/or
the 7TM domain is not from ADGRE2; or

- 5 the GAIN domain has at least 75%, 80%, 85%, 90%, 92%, 94%, 96%, 98%
or 100% sequence identity to SEQ ID NO: 30 and:
the intracellular tail is not from ADGRG2;
the target binding domain is not from ADGRG2; and/or
the 7TM domain is not from ADGRG2.

10

12. The chimeric aGPCR of any of the embodiments 1-11 wherein the target binding
domain is able to bind to a target that is noncovalently bound or covalently bound or
15 is anchored in:
a cell surface;
a platelet or engineered platelet surface;
a physical structure;
the interior of a blood vessel;
20 an organ;
a solid tumour;
An anchored protein;
A target that has an opposite relative movement to the chimeric aGPCR when the
chimeric aGPCR is present in the plasma membrane of a cell, platelet or engineered
25 platelet; and/or
Immobilised on a solid substrate.

13. The chimeric aGPCR of any of embodiments 1-12 wherein the target is present
on a cell surface or a tissue surface.

30

14. The chimeric aGPCR according to any of embodiments 1-13 wherein the target
is a tumor antigen, neoantigen or autoantigen.

15. The chimeric aGPCR according to any of embodiments 1-14 wherein said target
35 binding domain comprises:

an antibody or antibody fragment that binds specifically to said target;
a variable heavy chain domain and/or a variable light chain domain;

- one, two or three CDRs of a heavy chain and/or one, two or three CDRs of a light chain;
 an scFv;
 a nanobody;
 a Fab;
- 5 a kappa light chain or a fragment thereof targeting;
 a synthetic binding scaffold such as a monobody, affibody, DARPin or knottin;
 anti-CD19 scFv domains, such as FMC63 scFv domains optionally of example SEQ ID
 NO: 15;.
 anti-CD276 scFv domains, such as Enoblituzumab scFv domains optionally of SEQ ID
 10 NO: 16 or Omburtamab optionally of SEQ ID NO:17; and/or
 anti- MAdCAM1 scFv domains, such as Ontamalimab scFv domains optionally of SEQ
 ID NO: 18;
 a target-binding ligand or fragment thereof that binds specifically to said target.
- 15 16. The chimeric aGPCR of any of embodiments 1-15 wherein when the chimeric
 aGPCR is present in the membrane of a platelet or an engineered platelet, binding of
 the target binding domain to the target:
- a) results in degranulation of the platelet or engineered platelet;
 20 b) results in the release of contents from the platelet or engineered platelet;
 c) results in the presence of intraplatelet contents on the plasma membrane of the
 platelet or engineered platelet;
 d) results in the release of extracellular vesicles via blebbing from the plasma
 membrane; and/or
 25 e) results in a change of shape of the platelet or engineered platelet from a biconcave
 disk to fully spread cell fragments.
17. The chimeric aGPCR of any of embodiments 1-16 wherein the intracellular tail
 and/or one, two or three ICLs:
- 30 Comprises a domain that binds to a $G_{\alpha q}$ subunit and when the chimeric aGPCR is
 activated activates beta-type phospholipase C ($PLC\beta$);
 Comprises a domain that binds to a $G_{\alpha s}$ subunit and when the chimeric aGPCR is
 activated stimulates the cAMP-dependent pathway by activating adenylyl cyclase and
 35 increasing intracellular cAMP levels; or
 Comprises a domain that binds to a $G_{\alpha i/o}$ subunit and when the cGPCR is activated
 inhibits adenylyl cyclase and decreases intracellular cAMP;

Comprises a domain that binds to a G α 12/13 subunit and when the chimeric aGPCR is activated activates the RhoA pathway; and/or

Comprises a G domain that binds to a α 16 subunit and when the chimeric aGPCR is activated activates PLC- β /PI3K/Akt/MAPK/NF- κ B pathways.

5

18. The chimeric aGPCR of any of embodiments 1-17 wherein, once activated:

a) results in degranulation of the platelet or engineered platelet;

b) results in the release of contents from the platelet or engineered platelet;

10 c) results in the presence of intraplatelet contents on the plasma membrane of the platelet or engineered platelet;

d) results in the release of extracellular vesicles via blebbing from the plasma membrane; and/or

e) results in a change of shape of the platelet or engineered platelet from a biconcave
15 disk to fully spread cell fragments,

optionally wherein the intracellular signalling domain comprises a G α q subunit.

19. The chimeric aGPCR of any of embodiments 1-17 wherein, once activated:

20 a) prevents degranulation of the platelet or engineered platelet;

b) prevents the release of contents from the platelet or engineered platelet;

c) prevents the presence of intraplatelet contents on the plasma membrane of the platelet or engineered platelet;

d) prevents the release of extracellular vesicles via blebbing from the plasma
25 membrane; and/or

e) prevents a change of shape of the platelet or engineered platelet from a biconcave disk to fully spread cell fragments;

optionally wherein the intracellular tail and/or one, two or three ICLs comprises a G α s
30 subunit.

20. The chimeric aGPCR according to any of embodiments 1-19 wherein the chimeric aGPCR comprises a linker between the GAIN domain and the target binding domain, optionally comprises a peptide linker between the GAIN domain and the target
35 binding domain, optionally wherein the linker comprises
SPPHTAAHNA [SEQ ID NO: 10].

21. A system comprising:

a chimeric aGPCR as described in any of embodiments 1-20; and
an intermediate adaptor protein or peptide that comprises an ultimate target binding domain and optionally a peptide tag

5 wherein the target binding domain of the chimeric aGPCR is able to bind to the intermediate adaptor protein or peptide, optionally bind to the tag when present on the adaptor polypeptide or peptide, and the ultimate target binding domain of the adaptor polypeptide or protein is able to bind to the ultimate target

10 optionally:

where the chimeric aGPCR is present in a platelet plasma membrane or in an engineered platelet plasma membrane, binding of the intermediate adaptor protein or peptide to the target binding domain of the chimeric aGPCR in the absence of
15 simultaneous binding of the ultimate target binding domain of the intermediate adaptor polypeptide or protein to the ultimate target is not sufficient to activate degranulation of the platelet or engineered platelet; and/or

where the chimeric aGPCR is present in a platelet plasma membrane or in an engineered platelet plasma membrane, binding of the intermediate adaptor protein or peptide to the target binding domain of the chimeric aGPCR with simultaneous binding
20 of the ultimate target binding domain of the intermediate adaptor polypeptide or protein to the ultimate target activates degranulation of the platelet or engineered platelet.

25

22. A polynucleotide encoding any one or more of the chimeric aGPCRs of any of the preceding embodiments.

23. A vector comprising the polynucleotide of embodiment 22 operably linked to a
30 promoter and/or enhancer, optionally where the vector is a plasmid.

24. A viral vector comprising the polynucleotide of embodiment 22, optionally where the viral vector is an AAV or lentivirus.

25. A cell or platelet or engineered platelet comprising any one or more of the chimeric aGPCRs of any of the preceding embodiments, optionally wherein the cell is a T cell, B cell, NK cell, red blood cell, macrophage, megakaryocyte, pluripotent cell or stem cell,
35 optionally an induced pluripotent stem cell (iPSC).

26. A chassis comprising:
- a) one or more chimeric aGPCRs of any of the preceding embodiments;
 - 5 b) one or more polynucleotides of any of the preceding embodiments;
 - c) a system of any of the preceding embodiments.
27. The chassis of embodiment 26 wherein the chassis is a cell, platelet or engineered platelet, optionally wherein the cell is a T cell, NK cell, macrophage, B cell,
- 10 red blood cell, megakaryocyte, pluripotent stem cell or stem cell, optionally an induced pluripotent stem cell (iPSC cell).
28. The chassis of any embodiments 26 or 27 wherein the chassis has been engineered:
- 15 to disrupt the thrombogenic pathway;
to disrupt a platelet inflammatory signaling pathway;
engineered to make the engineered platelet less immunogenic; and/or
to enhance or disrupt one or more base functions of the chassis,
- 20 optionally wherein the one or more or base functions are involved in the innate and/or adaptive immune response, inflammation, angiogenesis, atherosclerosis, lymphatic development and tumour growth.
- 25 29. The chassis according to any of embodiments 26-28 wherein the chassis has been engineered so as to have reduced thrombogenicity relative to a chassis that has not been engineered so as to have reduced thrombogenic potential, optionally wherein the engineered chassis has no thrombogenic potential.
- 30 30. The chassis according to any of the preceding embodiments wherein the chassis comprises one or more cargo, wherein the chassis has been:
- a) loaded with one or more cargo; and/or
 - b) engineered so as to endogenously express one or more cargo,
- 35 optionally wherein the cargo is selected from any one or more of:
- a) a protein or peptide – optionally that is:

- i) an antibody or antigen binding fragment thereof, for example an antibody or antigen binding fragment thereof binds to a tumor antigen or a neoantigen;
- ii) an enzyme, such as a nuclease for example a TALEN;
- iii) a cytokine for example IL-10; or
- 5 iv) a CRISPR associated protein, for example Cas9;
- v) a bispecific protein, for example a bispecific antibody or optionally a T-cell engager (BITE)
- b) a nucleic acid – optionally that is:
 - i) an RNA, for example selected from mRNA, a miRNA, shRNA, and a clustered regularly
 - 10 interspaced short palindromic repeats (CRISPR) sequence; or
 - ii) a DNA vector;
 - c) a toxin;
 - d) a small molecule drug, imaging agent, radionucleotide drug, radionucleotide tagged antibody, or any conjugate thereof;
 - 15 e) a viral vector such as AAV;
 - f) a virus such as oncolytic virus;
 - g) agents for performing CRISPR mediated gene editing;
 - h) an exosome, for example an exosome pre-loaded with a second cargo; and/or
 - i) or a nanoparticle or nanoparticles;
 - 20 and/or
 - j) a lipid nanoparticle (LNP) comprising an RNA or an mRNA.

or any combination thereof.

- 25 31. A complex comprising:
 - a) a chassis according to any of the preceding embodiments that expresses one or more chimeric aGPCR of any of the preceding embodiments; and
 - b) an intermediate adaptor protein or peptide,

- 30 wherein the intermediate adaptor protein or peptide comprises an ultimate target binding domain and optionally a peptide tag,
- and wherein the ultimate target binding domain of the intermediate adaptor polypeptide or protein is able to simultaneously bind to the ultimate target.

- 35 32. A targeted delivery system comprising a chassis of any of the preceding embodiments wherein the chassis expresses one or more chimeric aGPCRs as defined in the preceding embodiments, and wherein the chassis comprises one or more cargo.

33. A chassis, system or complex according to any of the preceding embodiments
for use in medicine,
optionally for use in
5 treating or preventing cancer, an autoimmunity disease, genetic disease,
cardiovascular disease and/or an infection; or
for delivering a therapeutic or imaging cargo.
34. A non-therapeutic method of delivering cargo to a subject in need thereof
10 comprising administering an effective amount of a chassis of any of the preceding
embodiments, wherein the chassis comprises one or more of a chimeric aGPCRs of any
of the preceding embodiments and one or more non-therapeutic cargo.
35. A kit comprising any two or more of the following:
15 A chassis according to any of the preceding embodiments;
A chimeric aGPCR of any of the preceding embodiments;
A nucleic acid of any of the preceding embodiments;
A vector of any of the preceding embodiments;
20 A system of any of the preceding embodiments;
A complex of any of the preceding embodiments.
36. A kit comprising a chimeric aGPCR of any of the preceding embodiments and a
corresponding intermediate adaptor protein or peptide of any of the preceding
25 embodiments, wherein the target binding domain of the chimeric aGPCR can bind to
the intermediate adaptor protein or peptide.

30

35

Claims

- 5
1. A chimeric adhesion G-Protein Coupled Receptor (aGPCR) comprising:
- a) an intracellular tail domain, optionally a C-terminal tail;
- b) a seven-transmembrane domain (7TM) that comprises three intracellular loops
- 10 (ICLs); and
- c) an extracellular domain comprising:
- (i) a target binding domain that is heterologous to the intracellular tail domain;
- (ii) a GPCR autoproteolysis-inducing domain (GAIN domain) that optionally comprises
- a tethered agonist peptide;
- 15 and
- (iii) a linker between the GAIN domain and the target binding domain.
2. The chimeric aGPCR of claim 1 wherein the GAIN domain comprises a GPCR-
- proteolytic site (GPS) site optionally positioned N-terminal of the tethered peptide
- 20 agonist.
3. The chimeric aGPCR of claim 2 wherein the GPS site is cleaved by the GAIN
- domain.
- 25 4. The chimeric aGPCR of claim 2 wherein the GPS site is not cleaved, optionally
- not cleaved by the GAIN domain.
5. The chimeric aGPCR of claim 4 wherein the extracellular domain further
- comprises a protease site,
- 30 optionally comprises
- a protease site that is a cysteine protease site, a metalloprotease site, an aspartate
- protease site, a serine protease site or a threonine protease site; and/or
- a protease site that is a protease that is associated with the tumour microenvironment,
- 35 optionally wherein
- i) the chimeric aGPCR becomes activated upon cleavage of the protease site in the
- absence of binding of the target binding domain to the target; or

ii) the chimeric aGPCR becomes activated only upon cleavage of the protease site and binding of the target binding domain to the target.

6. The chimeric aGPCR of any of claims 1-5 wherein the:

5

intracellular tail domain, optionally the C-terminal tail is autologous to the 7TM domain;

intracellular tail domain, optionally the C-terminal tail is autologous to the GAIN domain; and/or

10 intracellular tail domain, optionally the C-terminal tail is autologous to the GPS motif.

7. The chimeric aGPCR of any of claims 1-6 wherein the chimeric aGPCR activates intracellular signalling when:

15 a) the chimeric aGPCR is localised to the plasma membrane of a chassis, optionally a cell, a platelet or an engineered platelet; and when

b) the chassis, optionally the cell, platelet or engineered platelet, is incubated and/or agitated in the presence of the target

20 optionally where the chassis, optionally the cell, platelet or engineered platelet comprises a reporter system

optionally where the reporter system comprises a reporter protein that is expressed when intracellular signalling is activated, optionally wherein the reporter protein is expressed from a promoter that comprises a nuclear factor of activated T cells (NFAT)

25 response element, optionally wherein the reporter protein is a luciferase enzyme.

8. The chimeric aGPCR of any of claims 1-7 wherein the intracellular domain comprises one or more substitutions, insertions or deletions as compared to the autologous intracellular domain or to a wild-type intracellular domain, optionally

30 wherein

the one or more substitutions, insertions or deletions increases the intracellular signalling response as compared to the autologous intracellular domain or to a wild-type intracellular domain that does not comprise the same one or more substitutions,

35 insertions or deletions; or

the one or more substitutions, insertions or deletions decreases the intracellular signalling response as compared to the autologous intracellular domain or to a wild-

type intracellular domain that does not comprise the same one or more substitutions, insertions or deletions;

and/or

- 5 the one or more substitutions, insertions or deletions alters the specificity of the intracellular signalling response as compared to the autologous intracellular domain or to a wild-type intracellular domain that does not comprise the same one or more substitutions, insertions or deletions resulting in altered signalling pathway activation.

9. The chimeric aGPCR of any of claims 1-8 further comprising a signal peptide,
10 optionally a signal peptide that has been selected to achieve a desired level of chimeric aGPCR at the cell, platelet, or engineered platelet surface.

10. The chimeric aGPCR of any of claims 1-9 wherein the:

- 15 i)
a) intracellular tail;
b) seven-transmembrane domain (7TM); and
c) GPCR autoproteolysis-inducing domain (GAIN domain) that optionally comprises a
tethered agonist peptide and that optionally cleaves a GPS motif; and
20 d) GPS motif that is optionally cleaved by the GAIN domain

are autologous to one another;

or

- 25 ii) GAIN domain is heterologous to:
the 7TM domain;
the intracellular tail; and/or
the target binding domain;

30 or

- iii)
a) intracellular tail ;
35 b) seven-transmembrane domain (7TM); and
c) GPCR autoproteolysis-inducing domain (GAIN domain); and
d) GPS motif

are from the same naturally occurring aGPCR;

or

iv)

- 5 a) intracellular tail;
- b) seven-transmembrane domain (7TM); and
- c) GPCR autoproteolysis-inducing domain (GAIN domain); and/or
- d) GPS motif

- 10 are from the same naturally occurring aGPCR, and wherein any one or more of the:

- a) intracellular tail;
- b) seven-transmembrane domain (7TM); and
- c) GPCR autoproteolysis-inducing domain (GAIN domain); and/or
- 15 d) GPS motif

comprises an amino acid sequence that has at least 75%, 80%, 85%, 90%, 92%, 94%, 96%, 98% or 100% sequence identity to the corresponding domain of the naturally occurring aGPCR.

20

11. The chimeric aGPCR of any of claims 1-10 wherein the GAIN domain is:

- from ADGRG1 and has an amino acid sequence of [SEQ ID NO: 35];
- from ADGRL1 and has an amino acid sequence of [SEQ ID NO: 27];
- 25 from ADGRL3 and has an amino acid sequence of [SEQ ID NO: 28];
- from ADGRE2 and has an amino acid sequence of [SEQ ID NO: 29];
- from ADGRG2 and has an amino acid sequence of [SEQ ID NO: 30];

- or is a GAIN domain with an amino acid sequence that has at least 75%, 80%, 85%, 90%, 92%, 94%, 96%, 98% or 100% sequence identity to SEQ ID NO: 35, 27, 28, 29 or 30, optionally wherein:
- 30

the GAIN domain has at least 75%, 80%, 85%, 90%, 92%, 94%, 96%, 98% or 100% sequence identity to SEQ ID NO: 35 and:

- 35 the intracellular tail is not from ADGRG1;
- the target binding domain is not from ADGRG1; and/or
- the 7TM domain is not from ADGRG1;

the GAIN domain has at least 75%, 80%, 85%, 90%, 92%, 94%, 96%, 98% or 100% sequence identity to SEQ ID NO: 27 and:

the intracellular tail is not from ADGRL1;
the target binding domain is not from ADGRL1; and/or
5 the 7TM domain is not from ADGRL1;

the GAIN domain has at least 75%, 80%, 85%, 90%, 92%, 94%, 96%, 98% or 100% sequence identity to SEQ ID NO: 28 and:

the intracellular tail is not from ADGRL3;
10 the target binding domain is not from ADGRL3; and/or
the 7TM domain is not from ADGRL3;

the GAIN domain has at least 75%, 80%, 85%, 90%, 92%, 94%, 96%, 98% or 100% sequence identity to SEQ ID NO: 29 and:

15 the intracellular tail is not from ADGRE2;
the target binding domain is not from ADGRE2; and/or
the 7TM domain is not from ADGRE2; or

the GAIN domain has at least 75%, 80%, 85%, 90%, 92%, 94%, 96%, 98% or 100% sequence identity to SEQ ID NO: 30 and:

20 the intracellular tail is not from ADGRG2;
the target binding domain is not from ADGRG2; and/or
the 7TM domain is not from ADGRG2.

25 12. The chimeric aGPCR of any of the claims 1-11 wherein the target binding domain is able to bind to a target that is noncovalently bound or covalently bound or is anchored in:

a cell surface;
30 a platelet or engineered platelet surface;
a physical structure;
the interior of a blood vessel;
an organ;
a solid tumour;
35 An anchored protein;

A target that has an opposite relative movement to the chimeric aGPCR when the chimeric aGPCR is present in the plasma membrane of a cell, platelet or engineered platelet; and/or

Immobilised on a solid substrate.

13. The chimeric aGPCR of any of claims 1-12 wherein the target is present on a cell surface or a tissue surface.

5

14. The chimeric aGPCR according to any of claims 1-13 wherein the target is a tumor antigen, neoantigen or autoantigen.

15. The chimeric aGPCR according to any of claims 1-14 wherein said target binding domain comprises:

- an antibody or antibody fragment that binds specifically to said target;
a variable heavy chain domain and/or a variable light chain domain;
one, two or three CDRs of a heavy chain and/or one, two or three CDRs of a light chain;
15 an scFv;
a nanobody;
a Fab;
a kappa light chain or a fragment thereof targeting;
a synthetic binding scaffold such as a monobody, affibody, DARPin or knottin;
20 anti-CD19 scFv domains, such as FMC63 scFv domains optionally of example SEQ ID NO: 15;
anti-CD276 scFv domains, such as Enoblituzumab scFv domains optionally of SEQ ID NO: 16 or Omburtamab optionally of SEQ ID NO:17; and/or
anti- MADCAM1 scFv domains, such as Ontamalimab scFv domains optionally of SEQ
25 ID NO: 18;
a target-binding ligand or fragment thereof that binds specifically to said target.

16. The chimeric aGPCR of any of claims 1-15 wherein when the chimeric aGPCR is present in the membrane of a platelet or an engineered platelet, binding of the target binding domain to the target:

- a) results in degranulation of the platelet or engineered platelet;
b) results in the release of contents from the platelet or engineered platelet;
c) results in the presence of intraplatelet contents on the plasma membrane of the
35 platelet or engineered platelet;
d) results in the release of extracellular vesicles via blebbing from the plasma membrane; and/or

e) results in a change of shape of the platelet or engineered platelet from a biconcave disk to fully spread cell fragments.

17. The chimeric aGPCR of any of claims 1-16 wherein the intracellular tail and/or
5 one, two or three ICLs:

Comprises a domain that binds to a G_{oq} subunit and when the chimeric aGPCR is activated activates beta-type phospholipase C (PLC β);

10 Comprises a domain that binds to a G_{os} subunit and when the chimeric aGPCR is activated stimulates the cAMP-dependent pathway by activating adenylyl cyclase and increasing intracellular cAMP levels; or

Comprises a domain that binds to a $G_{ai/o}$ subunit and when the cGPCR is activated inhibits adenylyl cyclase and decreases intracellular cAMP;

15 Comprises a domain that binds to a $G_{12/13}$ subunit and when the chimeric aGPCR is activated activates the RhoA pathway; and/or

Comprises a G domain that binds to a $\alpha 16$ subunit and when the chimeric aGPCR is activated activates PLC- β /PI3K/Akt/MAPK/NF- κ B pathways.

18. The chimeric aGPCR of any of claims 1-17 wherein, once activated:

20

a) results in degranulation of the platelet or engineered platelet;

b) results in the release of contents from the platelet or engineered platelet;

c) results in the presence of intraplatelet contents on the plasma membrane of the platelet or engineered platelet;

25 d) results in the release of extracellular vesicles via blebbing from the plasma membrane; and/or

e) results in a change of shape of the platelet or engineered platelet from a biconcave disk to fully spread cell fragments,

optionally wherein the intracellular signalling domain comprises a G_{oq} subunit.

30

19. The chimeric aGPCR of any of claims 1-17 wherein, once activated:

a) prevents degranulation of the platelet or engineered platelet;

b) prevents the release of contents from the platelet or engineered platelet;

35 c) prevents the presence of intraplatelet contents on the plasma membrane of the platelet or engineered platelet;

d) prevents the release of extracellular vesicles via blebbing from the plasma membrane; and/or

e) prevents a change of shape of the platelet or engineered platelet from a biconcave disk to fully spread cell fragments;

5 optionally wherein the intracellular tail and/or one, two or three ICLs comprises a Gas subunit.

20. The chimeric aGPCR according to any of claims 1-19 wherein the linker between the GAIN domain and the target binding domain is a peptide linker between the GAIN domain and the target binding domain, optionally wherein the linker comprises
10 SPPHTAAHNA [SEQ ID NO: 10].

21. A system comprising:
a chimeric aGPCR as described in any of claims 1-20; and
an intermediate adaptor protein or peptide that comprises an ultimate target binding
15 domain and optionally a peptide tag

wherein the target binding domain of the chimeric aGPCR is able to bind to the intermediate adaptor protein or peptide, optionally bind to the tag when present on the adaptor polypeptide or peptide, and the ultimate target binding domain of the
20 adaptor polypeptide or protein is able to bind to the ultimate target

optionally:

where the chimeric aGPCR is present in a platelet plasma membrane or in an
25 engineered platelet plasma membrane, binding of the intermediate adaptor protein or peptide to the target binding domain of the chimeric aGPCR in the absence of simultaneous binding of the ultimate target binding domain of the intermediate adaptor polypeptide or protein to the ultimate target is not sufficient to activate degranulation of the platelet or engineered platelet; and/or

30

where the chimeric aGPCR is present in a platelet plasma membrane or in an engineered platelet plasma membrane, binding of the intermediate adaptor protein or peptide to the target binding domain of the chimeric aGPCR with simultaneous binding of the ultimate target binding domain of the intermediate adaptor polypeptide or
35 protein to the ultimate target activates degranulation of the platelet or engineered platelet.

22. A polynucleotide encoding any one or more of the chimeric aGPCRs of any of the preceding claims.
23. A vector comprising the polynucleotide of claim 22 operably linked to a promoter and/or enhancer, optionally where the vector is a plasmid.
24. A viral vector comprising the polynucleotide of claim 22, optionally where the viral vector is an AAV or lentivirus.
25. A cell or platelet or engineered platelet comprising any one or more of the chimeric aGPCRs of any of the preceding claims, optionally wherein the cell is a T cell, B cell, NK cell, red blood cell, macrophage, megakaryocyte, pluripotent cell or stem cell, optionally an induced pluripotent stem cell (iPSC).
26. A chassis comprising:
- a) one or more chimeric aGPCRs of any of the preceding claims;
 - b) one or more polynucleotides of any of the preceding claims;
 - c) a system of any of the preceding claims.
27. The chassis of claim 26 wherein the chassis is a cell, platelet or engineered platelet, optionally wherein the cell is a T cell, NK cell, macrophage, B cell, red blood cell, megakaryocyte, pluripotent stem cell or stem cell, optionally an induced pluripotent stem cell (iPSC cell).
28. The chassis of any claims 26 or 27 wherein the chassis has been engineered:
- to disrupt the thrombogenic pathway;
 - to disrupt a platelet inflammatory signaling pathway;
 - engineered to make the engineered platelet less immunogenic; and/or
 - to enhance or disrupt one or more base functions of the chassis,
- optionally wherein the one or more or base functions are involved in the innate and/or adaptive immune response, inflammation, angiogenesis, atherosclerosis, lymphatic development and tumour growth.
29. The chassis according to any of claims 26-28 wherein the chassis has been engineered so as to have reduced thrombogenicity relative to a chassis that has not

been engineered so as to have reduced thrombogenic potential, optionally wherein the engineered chassis has no thrombogenic potential.

30. The chassis according to any of the preceding embodiments wherein the chassis
5 comprises one or more cargo, wherein the chassis has been:

- a) loaded with one or more cargo; and/or
- b) engineered so as to endogenously express one or more cargo,

10 optionally wherein the cargo is selected from any one or more of:

- a) a protein or peptide – optionally that is:
 - i) an antibody or antigen binding fragment thereof, for example an antibody or antigen binding fragment thereof binds to a tumor antigen or a neoantigen;
 - 15 ii) an enzyme, such as a nuclease for example a TALEN;
 - iii) a cytokine for example IL-10; or
 - iv) a CRISPR associated protein, for example Cas9;
 - v) a bispecific protein, for example a bispecific antibody or optionally a T-cell engager (BITE)
- 20 b) a nucleic acid – optionally that is:
 - i) an RNA, for example selected from mRNA, a miRNA, shRNA, and a clustered regularly interspaced short palindromic repeats (CRISPR) sequence; or
 - ii) a DNA vector;
 - c) a toxin;
 - 25 d) a small molecule drug, imaging agent, radionucleotide drug, radionucleotide tagged antibody, or any conjugate thereof;
 - e) a viral vector such as AAV;
 - f) a virus such as oncolytic virus;
 - g) agents for performing CRISPR mediated gene editing;
 - 30 h) an exosome, for example an exosome pre-loaded with a second cargo; and/or
 - i) or a nanoparticle or nanoparticles;
 - and/or
 - j) a lipid nanoparticle (LNP) comprising an RNA or an mRNA.

35 or any combination thereof.

31. A complex comprising:

- a) a chassis according to any of the preceding claims that expresses one or more chimeric aGPCR of any of the preceding claims; and
- b) an intermediate adaptor protein or peptide,

5 wherein the intermediate adaptor protein or peptide comprises an ultimate target binding domain and optionally a peptide tag,
and wherein the ultimate target binding domain of the intermediate adaptor polypeptide or protein is able to simultaneously bind to the ultimate target.

10

32. A targeted delivery system comprising a chassis of any of the preceding claims wherein the chassis expresses one or more chimeric aGPCRs as defined in the preceding claims, and wherein the chassis comprises one or more cargo.

15

33. A chassis, system or complex according to any of the preceding claims for use in medicine,

optionally for use in

treating or preventing cancer, an autoimmunity disease, genetic disease, cardiovascular disease and/or an infection; or

20

for delivering a therapeutic or imaging cargo.

34. A non-therapeutic method of delivering cargo to a subject in need thereof comprising administering an effective amount of a chassis of any of the preceding claims, wherein the chassis comprises one or more of a chimeric aGPCRs of any of the preceding claims and one or more non-therapeutic cargo.

25

35. A kit comprising any two or more of the following:

A chassis according to any of the preceding claims;

30

A chimeric aGPCR of any of the preceding claims;

A nucleic acid of any of the preceding claims;

A vector of any of the preceding claims;

A system of any of the preceding claims;

A complex of any of the preceding claims.

35

36. A kit comprising a chimeric aGPCR of any of the preceding claims and a corresponding intermediate adaptor protein or peptide of any of the preceding claims,

wherein the target binding domain of the chimeric aGPCR can bind to the intermediate adaptor protein or peptide.

5

10

15

20

25

30

35