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Review

The N terminus-only function of adhesion GPCRs: emerging concepts

Laura Lehmann[1,2], Victoria Elisabeth Groß[1,2], Rene Behlendorf[1,2], and Simone Prömel[1,*]

Adhesion G-protein-coupled receptors (aGPCRs) play key roles in health and disease. They are unique in that they not only activate G-protein pathways but also have distinct functions that rely solely on their N termini, making them complex drug targets. To date there have been only descriptive observations about these enigmatic N terminus-only functions. Emerging evidence from several aGPCRs now indicates that these are a defining characteristic of these receptors that allows them to operate bidirectionally across environments. Recent advances in characterizing aGPCR splice variants and receptor structure have revealed the G protein-independent mechanisms that underlie their N terminus-only functions. This review consolidates current findings, explores how the N termini integrate functions, and identifies common principles across aGPCRs. We consider the therapeutic implications and discuss how specifically targeting N terminus functions provides a novel perspective on the pharmacological potential of aGPCRs.

Highlights

Adhesion G protein-coupled receptors (GPCRs) mediate dual functions: one classical via G-protein pathways and a second solely by their extracellular N termini (N terminus-only/*trans*/seven transmembrane domain-independent).

The N terminus-only function can occur separately or together with G protein-mediated signaling.

The N terminus has large flexibility. In some cases it is released from the receptor whereas in others it remains attached to the rest of the receptor, resulting in different physiological outcomes. Differential splicing also produces variants containing only the aGPCR N terminus and provides several alternatives for how the N terminus-only function is realized.

The N terminus can serve either as a ligand for receptors on neighboring cells or as a modulator of other signaling cascades, thereby mediating signals by itself.

The N terminus-only function is implicated in several diseases, and targeting the N terminus has substantial pharmacological potential.

Adhesion GPCRs realize molecular functions beyond G-protein signals

aGPCRs (see Glossary) play crucial roles in diverse biological processes such as angiogenesis, metabolism, immunity, neurobiology, development, and cell polarity [1]. Genetic variants and mutations in aGPCR genes have been linked to severe diseases including neurodevelopmental disorders [1] and cancer [2]. However, the pharmacological potential of aGPCRs remains largely untapped, primarily because of the interplay of their complex functional mechanisms (Figure 1). Unlike classical GPCRs that mainly signal through G-proteins, aGPCRs additionally exhibit unique N terminus-dependent functions that are independent of G-protein pathways. These N-terminal roles enable aGPCRs to mediate diverse, context-specific cellular processes [3–5], but the precise mechanisms by which their N termini integrate the different functions remain poorly understood. This knowledge gap limits our ability to effectively target aGPCRs for therapeutic purposes.

Recent advances have provided valuable insights into these elusive **N terminus-only functions** of aGPCRs. Structural studies and new data on splice variants have revealed the dynamic nature of the N termini [3,4,6–12]. Furthermore, growing evidence directly links N terminus-driven mechanisms to disease processes, particularly in cancer [13–15], emphasizing the need for further investigation. These advances not only challenge the traditional view of GPCR signaling but also open new avenues for therapeutic intervention.

In this review we explore the N terminus-only functions of aGPCRs. By leveraging the recently solved structures and the extensive repertoire of splice variants associated with aGPCRs, we analyze the dynamics of the N terminus and how the N terminus-only functions are mediated. We outline the established mechanisms and identify common principles governing these unique functions. In addition, we examine the involvement of N terminus-only functions in various diseases and put forward a perspective on their potential in therapeutic applications.

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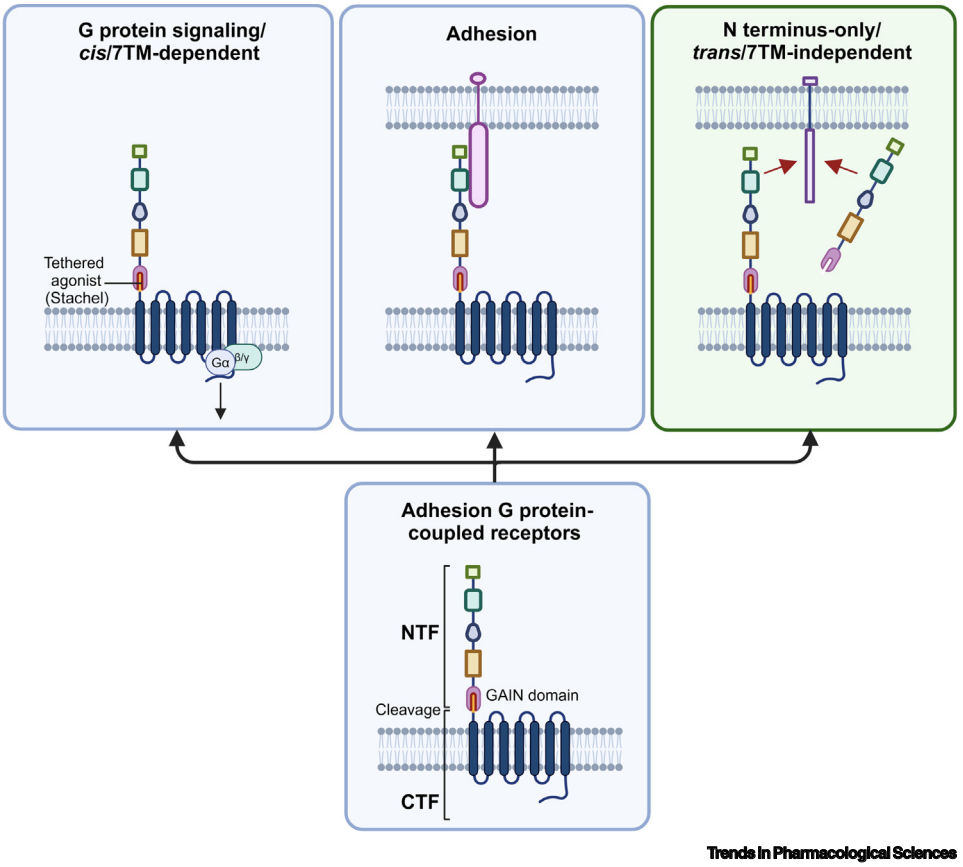

Figure 1. Schematic depiction of adhesion GPCR (aGPCR) signals and functions. aGPCRs have unusually large extracellular N termini that contain multiple domains, many of which are involved in adhesion. One domain common to all but one aGPCRs is the GPCR autoproteolysis-inducing (GAIN) domain that harbors the aGPCR proteolytic site (GPS) and the tethered agonist. Autoproteolytic cleavage at the GPS generates an N-terminal (NTF) and a C-terminal (CTF) fragment. The two fragments can remain noncovalently attached to each other but can also dissociate. Like other GPCRs, aGPCRs can signal via G-protein pathways [*cis*/seven transmembrane domain (7TM)-dependent; not all the mechanistic details are shown for simplicity] but, in addition to this classical mode of signaling, aGPCRs can also promote cell–cell and cell–matrix adhesion, as well as mediating functions that are dependent solely on their N termini (*trans*/7TM-independent). Their interaction partners can be present not only neighboring cells (as shown here) but also on the same cell. Figure created with BioRender.

aGPCRs integrate numerous functions via their N termini

The N termini of aGPCRs integrate different molecular functions. Like other GPCRs, they mediate classical signals into cells via G-proteins. Activation by an extracellular trigger exposes an internal tethered agonist, termed the Stachel sequence [16,17], which is buried within the **GPCR autoproteolysis-inducing (GAIN) domain** (Figure 1). The GAIN domain also harbors the **GPCR proteolytic site (GPS)** [18,19] at which several aGPCRs are autoproteolytically cleaved [1], potentially liberating the largest part of the N terminus [**N-terminal fragment (NTF)**] [9–12].

Unlike typical GPCRs, aGPCRs not only transduce classical G-protein signals [seven transmembrane (7TM) domain-dependent/*cis* functions] but also perform unique N terminus-only functions independently of G-proteins. These include adhesion to other cells or the extracellular matrix [1], as well as unique N terminus-dependent actions referred to as 7TM-independent, *trans*, or N terminus-only functions [4,5,20]. Exclusive to aGPCRs, these roles offer a plethora of context-specific functional pathways. Although underexplored, recent studies (e.g., [3,4,6,7,21]) reveal

Glossary

Adhesion GPCRs (aGPCRs): cell-surface receptors that constitute the second largest class of GPCRs. There are 33 members in human that are divided into nine subgroups. They contain extraordinarily large N termini with a complex domain structure that are the linchpins of different modes of function.

Bidirectional signaling: signaling in two directions, one affecting the cell on which the receptor is located (usually involving G-proteins) and one that only depends on the N terminus and has an influence on neighboring cells.

Cancer: a diverse set of diseases in which cells grow uncontrollably and spread throughout the body, disrupting normal function. Abnormal cell division avoids natural cell death and repair mechanisms; if left unchecked, cancerous cells invade nearby tissues and can metastasize to distant organs.

C-terminal fragment (CTF): the fragment of an aGPCR containing the seven transmembrane (7TM) domain and the C terminus that is produced by proteolytic cleavage of the aGPCR at the GPS.

GPCR autoproteolysis-inducing (GAIN) domain: a hallmark feature of all but one aGPCR. It contains the GPS and the tethered agonist.

GPCR proteolytic site (GPS): a motif at the very C-terminal end of the GAIN domain at which many aGPCRs undergo autocatalytic cleavage. This cleavage occurs during trafficking of the aGPCR through the endoplasmic reticulum.

N terminus-only function: also termed the *trans* or 7TM-independent function, this aGPCR function relies solely on the N terminus or parts of it. It is therefore independent of G-protein signaling and affects neighboring cells.

N-terminal fragment (NTF): the extracellularly located fragment of an aGPCR that results from cleavage at the GPS. It normally stays covalently attached to the CTF but can also be released under some conditions.

that many aGPCRs leverage N terminus-only functions, prompting investigation into the underlying mechanisms and shared features. This is particularly important because these functions are increasingly linked to diseases including cancer [13–15].

The N terminus-only function in different aGPCRs

To date, N terminus-only functions have been identified in seven of the 33 members of the mammalian aGPCR class, as well as in three aGPCRs from invertebrates, underscoring this mode of action as a prevalent and defining characteristic of this receptor class. In this section we outline the N terminus-only functions of the distinct aGPCRs, highlight the shared features that underpin them and highlight the distinct mechanisms that differentiate these functions.

ADGRA2/GPR124/TEM5

An N terminus-only function for the aGPCR ADGRA2/GPR124/TEM5 (tumor endothelial marker 5) has been clearly identified, while no evidence of G protein-mediated signaling has been reported thus far. ADGRA2 plays a crucial role in central nervous system (CNS) angiogenesis, as well as in the formation and maintenance of the blood–brain barrier, particularly under pathological conditions such as stroke [22]. Here, the aGPCR interacts with the Wnt pathway, one of the modulators of brain angiogenesis and blood–brain barrier formation. Wnt7 activates the receptors Fz (frizzled) and Lrp5/6 (low-density lipoprotein-related receptors 5/6), leading to canonical β-catenin signaling via the intracellular protein Dvl (disheveled) [23]. This interaction can be inhibited when Wnt7 is bound by the glycosylphosphatidylinositol (GPI)-anchored protein Reck (reversion-inducing cysteine-rich protein with kazal motifs), rendering Wnt7 unavailable to Fz. When ADGRA2 interacts with the Reck–Wnt7 complex, it transforms the Wnt signaling output from inhibition to robust activation by facilitating the assembly of ADGRA2/Reck/Fz/Lrp5/6 complexes [24] (Figure 2A). Thus, it enhances canonical β-catenin signaling in both brain endothelial [23,25,26] and cancer cells [14]. Notably, in mammals, the N terminus of ADGRA2 in sufficient to initiate [6] and enhance [7] this Wnt7 signaling. However, in other species such as zebrafish the C terminus of ADGRA2 is additionally required to stabilize the ADGRA2/Reck/Fz/Lrp5/6 complex. This stabilization occurs, for example, through binding to Dvl [27], which also interacts with Fz, thereby creating an indirect intracellular link to Fz [6].

In addition to this function, which is mediated by the ADGRA2 N terminus attached to the rest of the receptor, a soluble ADGRA2 fragment (sTEM5) also plays a role in angiogenesis [28]. sTEM5 comprises the aGPCR NTF, is released by endothelial cells upon growth factor stimulation during angiogenesis, and binds to glycosaminoglycans in the extracellular matrix (Figure 2B). Further cleavage of sTEM5 by MMP-9 (matrix metalloprotease 9) and more efficiently by the thrombin [28] yields a shorter fragment termed proteolytic processed sTEM5 (ppsTEM5). This cleavage unmasks an additional $\alpha_v\beta_3$ -integrin binding site that contains an RGD (arginine-glycine-aspartate) motif. The interaction of ppsTEM5 with integrins potentially triggers integrin signaling, ultimately leading to inhibition of the proteolytic activation of procaspase-3 [29]. By this pathway, ppsTEM5 promotes the survival of endothelial cells [29]. Thus, the N terminus-only function of ADGRA2 controls angiogenesis via distinct mechanisms.

ADGRB1/BAI1

ADGRB1/BAI1 (brain-specific angiogenesis inhibitor 1) was one of the first identified aGPCRs with an N terminus-only function and the function in ADGRB1 is among the most extensively characterized ones within the receptor class. While its 7TM signaling mediated through $G_{12/13}$ [30] proteins is essential for processes such as bacterial engulfment [31], apoptotic cell clearance [32], and myoblast fusion [33], its N terminus plays a crucial role in angiogenesis. The receptor is expressed in the brain where it inhibits endothelial cell migration and proliferation *in vitro* [34], and

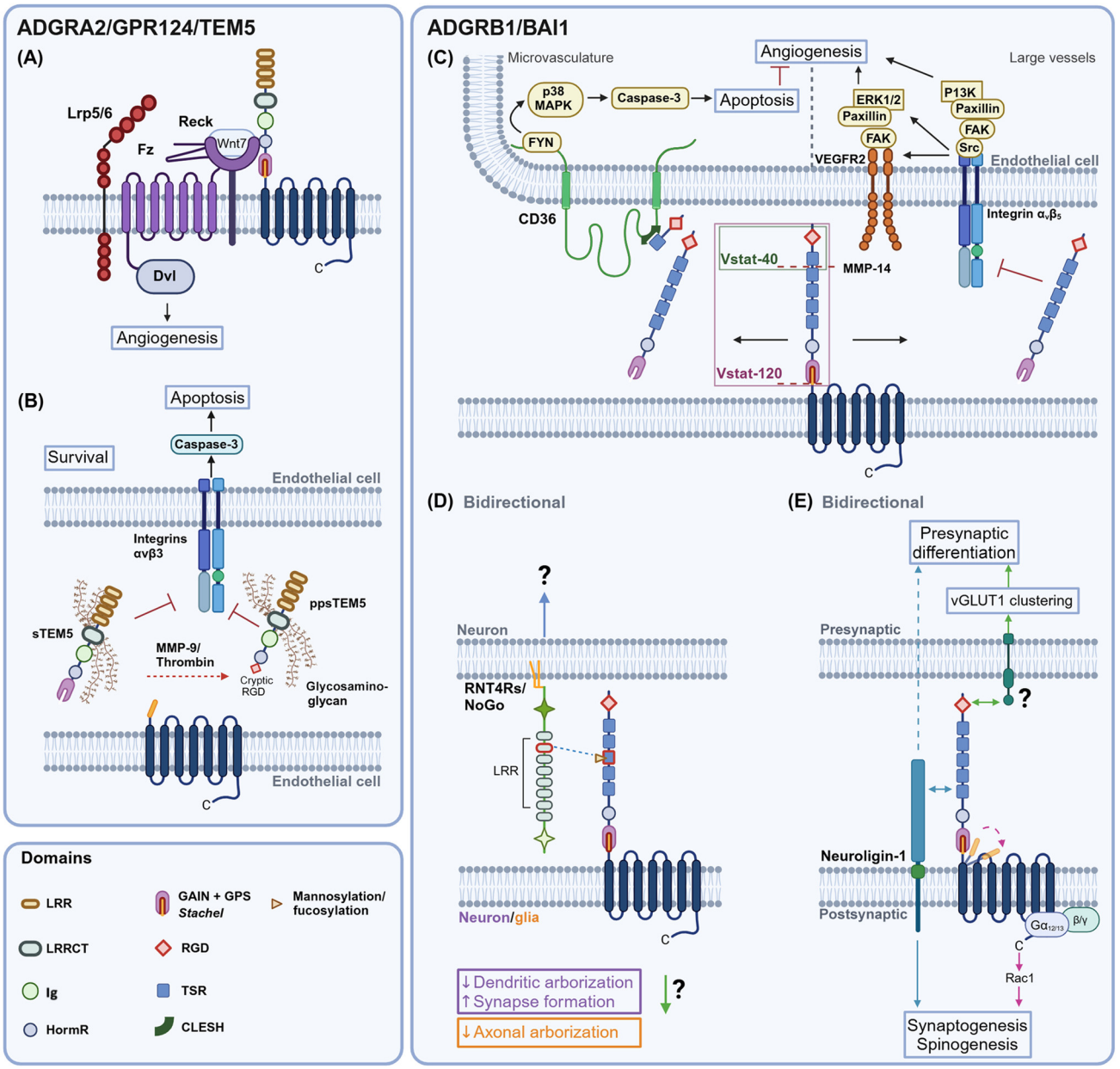

Figure 2. N terminus-only functions of ADGRA2 and ADGRB1. (A,B) ADGRA2 has two different roles both mediated by the sole N terminus. (A) The receptor interacts with Reck to regulate Wnt7 signaling. When bound to Wnt7–Reck, the aGPCR N terminus enables Wnt7 to activate Fz with the help of Lrp5/6. It is unknown whether the N terminus is released or different splice variants are at play. (B) The soluble ADGRA2 N terminus is released through autocatalytic cleavage at the GPS (sTEM5) or through processing by MMP-9 or thrombin (ppsTEM5). ppsTEM5 cleavage exposes a cryptic RGD motif within the aGPCR. Both interact with glycosaminoglycans and integrins. This interaction blocks integrin function, thereby reducing caspase-3 activation. ppsTEM5 is more efficient than sTEM5. (C–E) The N terminus-only functions of ADGRB1. (C) The ADGRB1 N termini Vstat-120 and Vstat-40 bind to CD36, which mediates apoptosis of endothelial cells, thus suppressing endothelial cell growth and migration. This process is initiated by the binding of Vstat-120 and Vstat-40 TSRs to the CLESH domain of CD36. Vstat-120 is produced by autoproteolytic cleavage at the GPS, whereas Vstat-40 is released by MMP-14-mediated and furin-facilitated cleavage of the NTF. In addition, Vstats can bind to integrins and block their function, resulting in reduced apoptosis. Note that the molecules and pathways depicted in yellow act in the context of CD36 and integrins but have not been shown to act in the context of Vstat. However, it can be speculated that they also act downstream of Vstat. These pathways are inferred based on signals involving integrin/CD36 in other contexts. (D) The N terminus interacts with the RNT4Rs (NoGo receptors) through one of its TSRs. (E) ADGRB1 is involved in

(Figure legend continued at the bottom of the next page.)

thus has significant antiangiogenic effects. In brain tumor contexts such as glioblastoma, expression is reduced and correlates with phenomena such as increased neovascularization [34–39].

The antiangiogenic function of ADGRB1 is controlled by two different soluble NTFs: the 120 kDa Vstat-120 (vasculostatin-120) [34,37] and the 40 kDa Vstat-40 [39] (Figure 2C). Vstat-120 is generated via proteolytic cleavage of the receptor at the GPS [34,37]. Vstat-40, by contrast, is produced from the ADGRB1 N terminus through cleavage between S^{326} and L^{327} by MMP-14 (matrix metalloproteinase 14) [34,37]. It consists of an RGD motif and the first thrombospondin type 1 repeat (TSR) [39], and is abundant in the brain. Release of Vstat-40 requires the presence of the receptor 7TM domain and association with the cell membrane [39].

Notably, the production of Vstat-40 appears to be independent of Vstat-120 because the latter is not a prerequisite for the formation of Vstat-40. Both Vstat-120 and Vstat-40 are soluble proteins and fulfill their functions by interacting through their TSR (thrombospondin type 1 repeat) domain containing five TSRs with the CLESH (CD36/LIMP-2/Emp sequence homology) domain of CD36 (cluster of differentiation 36) on endothelial cells. This interaction triggers antiangiogenic effects [37,39]. The exact mechanisms linking the interaction to the observed effects remain unclear. However, binding of TSRs in the context of type 1 thrombospondin to CD36 initiates a signaling cascade involving the $p59^{fyn}$ Src family, caspase 3-like proteases, p38 MAPK (mitogen-activated protein kinase), JNK-1 (c-Jun N-terminal kinase), and Fas/Fas ligand. This pathway triggers apoptosis and inhibition of angiogenesis [40,41]. Because Vstat-120 and Vstat-40 appear to induce similar signaling cascades, it remains unclear why both are produced. An earlier study might provide an explanation. It showed that extracellular ADGRB1 fragments corresponding roughly to Vstat-120 bind to and block $\alpha_v\beta_5$-integrins via the TSRs [42]. $\alpha_v\beta_5$ -integrins normally stimulate VEGF (vascular endothelial growth factor)-induced angiogenesis (Figure 2C). Thus, the ADGRB1 fragments inhibit angiogenesis by inducing apoptosis. Because $\alpha_v\beta_5$ -integrins are present on large vessels whereas CD36 is in the microvasculature, it can be speculated that Vstats exert their antiangiogenic effects tissue-specifically by two different mechanisms. Supporting this hypothesis, the generation of Vstat-120 inhibits the formation of Vstat-40, suggesting that the ratio of these fragments may influence the context-dependent outcome [39].

In addition to ADGRB1, two homologs, ADGRB2 and ADGRB3, are also expressed in the brain, prompting the question of whether they exhibit N terminus-only functions similar to the Vstats. Despite their structural similarity to ADGRB1, no evidence has been found to suggest that ADGRB2 or ADGRB3 exert N terminus-only functions. One reason for this might be that they contain only four TSRs and lack RGD motifs.

Like ADGRA2, ADGRB1 exhibits both an N terminus-only function via a released NTF and a function in which the NTF remains attached to the receptor, highlighting the versatility of N terminus-only aGPCR functions. The N terminus of ADGRB1 interacts with the three RTN4Rs (reticulon 4 receptors), also termed NoGo proteins (neurite outgrowth inhibitors). They are receptors for ADGRB1, and the interaction interface has been identified as mannosylated tryptophan and fucosylated threonine residues in the third TSR [43] (Figure 2D). This interaction occurs on neurons or on glial cells and has distinct developmental effects: neuronal RTN4Rs initially suppress

presynaptic and postsynaptic development via bidirectional *trans*-synaptic signaling. Presynaptic ADGRB1 NTF interacts with a so far unidentified interaction partner to initiate vGLUT1 clustering and thus presynaptic differentiation. Furthermore, it is likely that synaptogenesis and spinogenesis are promoted by Stachel as a tethered agonist via $G\alpha_{12/13}$. The receptor also interacts with neuroligin 1 on the same cell via the TSR of the NTF. Abbreviations: aGPCR, adhesion GPCR; CLESH, CD36/LIMP-2/Emp sequence homology; GAIN, GPCR autoproteolysis-inducing; GPS, GPCR proteolytic site; HormR, hormone-responsive domain; IG, immunoglobulin-like domain; LRR, leucine-rich repeat; LRRCT, LRR C-terminal; NTF, N-terminal fragment; RGD, arginine-glycine-aspartate motif; TSR, thrombospondin type 1 repeat. Figure created with BioRender.

dendritic arborization by binding to ADGRB1 on neurons, and then inhibit axonal arborization via an interaction with ADGRB1 on glial cells, and finally promote synapse formation through binding to ADGRB1 on neurons [21].

In addition to this N terminus-only role that employs the NTF anchored to the full-length receptor, a very recent study showed that ADGRB1 is also capable of mediating N terminus-only functions (*trans*) and G protein-mediated signaling (*cis*) simultaneously. Thereby, it engages in so-called *trans*-synaptic **bi-directional signaling** that affects both pre- and postsynaptic development [5,21] (Figure 2E). In hippocampal neurons lacking ADGRB1, reduced spine density, longer spines, and smaller spine heads were observed. Full-length ADGRB1, but not the N terminus alone, restored normal features [5]. Postsynaptic ADGRB1 interacts via its TSRs with NRLN1 (neuroligin 1) on the same cell to influence synaptic localization. This binding is crucial for NRLN1-induced synaptogenesis and enhances presynaptic development [5,44]. In addition, postsynaptic ADGRB1 interacts with so far unidentified partners across synaptic clefts, leading to clustering of vGluT1 (glutamate transporter 1) in presynaptic axons. This interaction aids cell differentiation [5].

ADGRC/CELSR/Flamingo

The aGPCR group ADGRC/CELSR (cadherin EGF LAG seven-pass G-type receptor) includes three mammalian homologs (ADGRC1–3/CELSR1–3) and their invertebrate counterparts. Especially for the latter, a clear N terminus-only function has been shown, illustrating its ancient origin. ADGRC are key molecules in the planar cell polarity (PCP) pathway which organizes cells within a tissue plane in various contexts [45]. Studies, primarily in the invertebrate *Drosophila melanogaster*, have revealed that the ADGRC homolog Fmi localizes in a polarized manner at cell junctions, such as on wing cells. Fmi interacts homophilically with another Fmi molecule on adjacent cells and associates with two other PCP core proteins: the seven-pass transmembrane protein Fz and the four-pass transmembrane protein Vang (Van Gogh) [46] (Figure 3). Together with intracellular binding partners, these molecules form complexes that mediate signals into the cells, thereby polarizing them. Although G-protein coupling and thus intracellular signaling have recently been shown for ADGRC members [47], an N terminus-only function is plausible through Fz or Vang. For instance, Fz has been shown to mediate signals into the cell via Dvl [45]. These findings might even suggest a simultaneous G protein-mediated/N terminus-only role of ADGRC similar to that observed for ADGRB1.

The cadherin domains of mammalian ADGRC support *trans*-adhesive and *cis*-clustering interactions [48,49], suggesting further N terminus-only functions. In *Caenorhabditis elegans* the ADGRC homolog employs these functions beyond PCP. It controls axonal pathfinding [50], as well as collagen production and body size [51]. For the latter, a potential interaction with the Vang homolog was postulated, although there may also be binding partners other than PCP components [52].

Although similarly to ADGRB1 dual simultaneous G protein-mediated/N terminus-only roles for ADGRC are conceivable, it should be noted that, unlike ADGRA2 and ADGRB1, all ADGRC N terminus-only functions rely on a receptor-attached NTF. Direct effects of soluble ADGRC have not been reported so far.

ADGRE5/CD97

ADGRE5 offers insights into how N terminus-only functions can be separated from other receptor roles and how different N terminus-only functions are realized. One mechanism is based on the modular composition of the N terminus, which includes 3–5 EGF (endothelial growth factor)-like domains depending on alternative splicing: domains 1–5; 1, 2, 3, 5; and 1, 2, 5 [53]. The number of these domains affects ligand binding by the receptor.

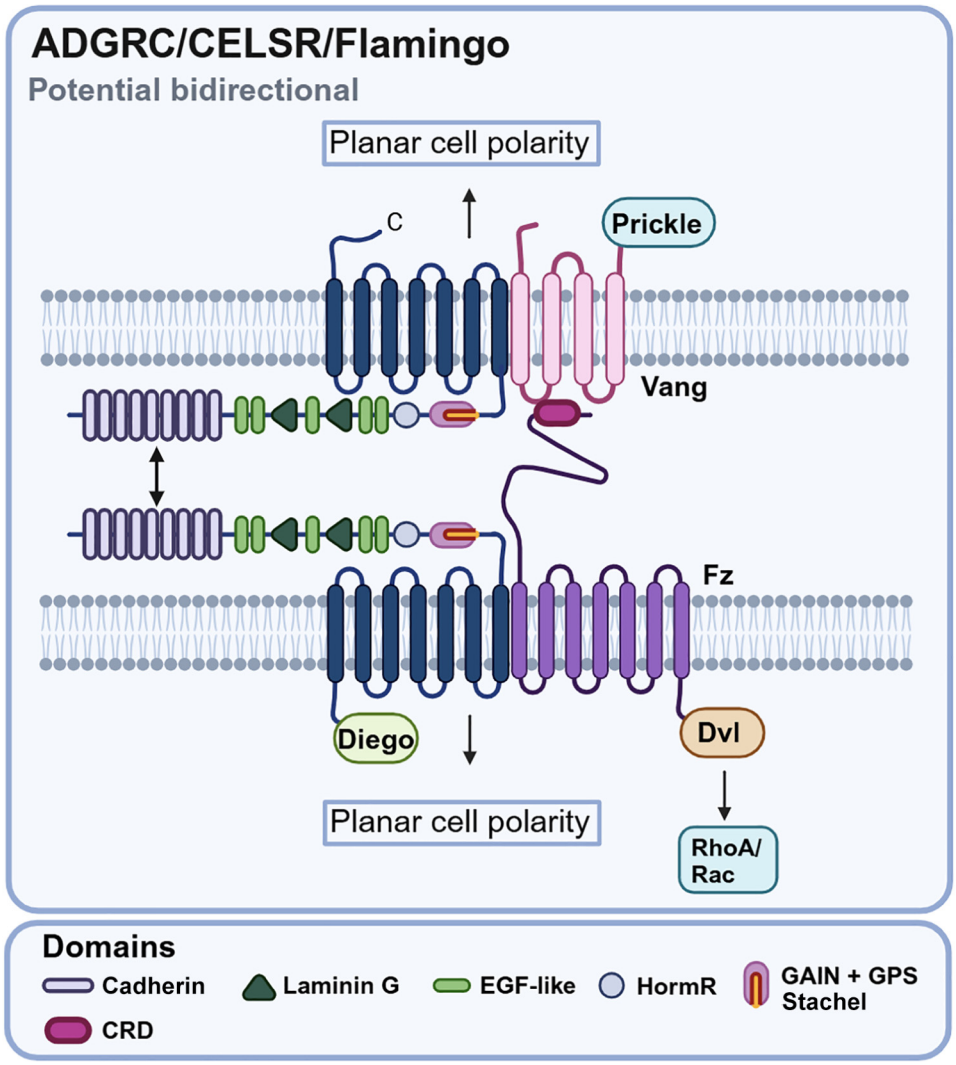

Figure 3. Interactions of ADGRC/CELSR/Flamingo in the context of planar cell polarity (PCP). An N terminus-only function of ADGRC in the PCP pathway can be assumed. The PCP pathway molecules are distributed asymmetrically in the cell. Frizzled (Fz) and Van Gogh (Vang) are located on opposing cells, whereas the adhesion GPCR (aGPCR) is localized on both cells. The N terminus of ADGRC forms homophilic interactions. Furthermore, Flamingo (Fmi) interacts with Fz and Vang on the same cell. Signals have been described to be mediated for instance by Fz. Thus, an N terminus-only function of ADGRC is conceivable through binding of ADGRC to Fz or Vang. Intracellularly, Prickle (Pk) binds to Vang on the opposing cell. Disheveled (Dvl) forms a complex with Fz. Diego bound to ADGRC is important for forming the aGPCR–Fz complex. Abbreviations: CRD, cysteine-rich domain; EGF-like, epidermal growth factor-like; GAIN, GPCR autoproteolysis-inducing; GPS, GPCR proteolytic site; HormR, hormone-responsive domain. Figure created with BioRender.

The variant with five EGF-like domains binds the GPI-anchored membrane protein THY1/CD90 via the GAIN domain [54,55] or the glycosaminoglycan chondroitin sulfate B through the EGF-like domain 4 [56] (Figure 4A). Variants with EGF-like domains 1, 2, and 5 interact with CD55, and binding is influenced by glycosylation [57] (Figure 4A). Integrins $\alpha_5\beta_1$ or $\alpha_v\beta_3$ bind to the full-length ADGRE5 via three EGF-like domains (1, 2, 5; or 3, 4, 5) and an RGD motif [58,59] (Figure 4B–D).

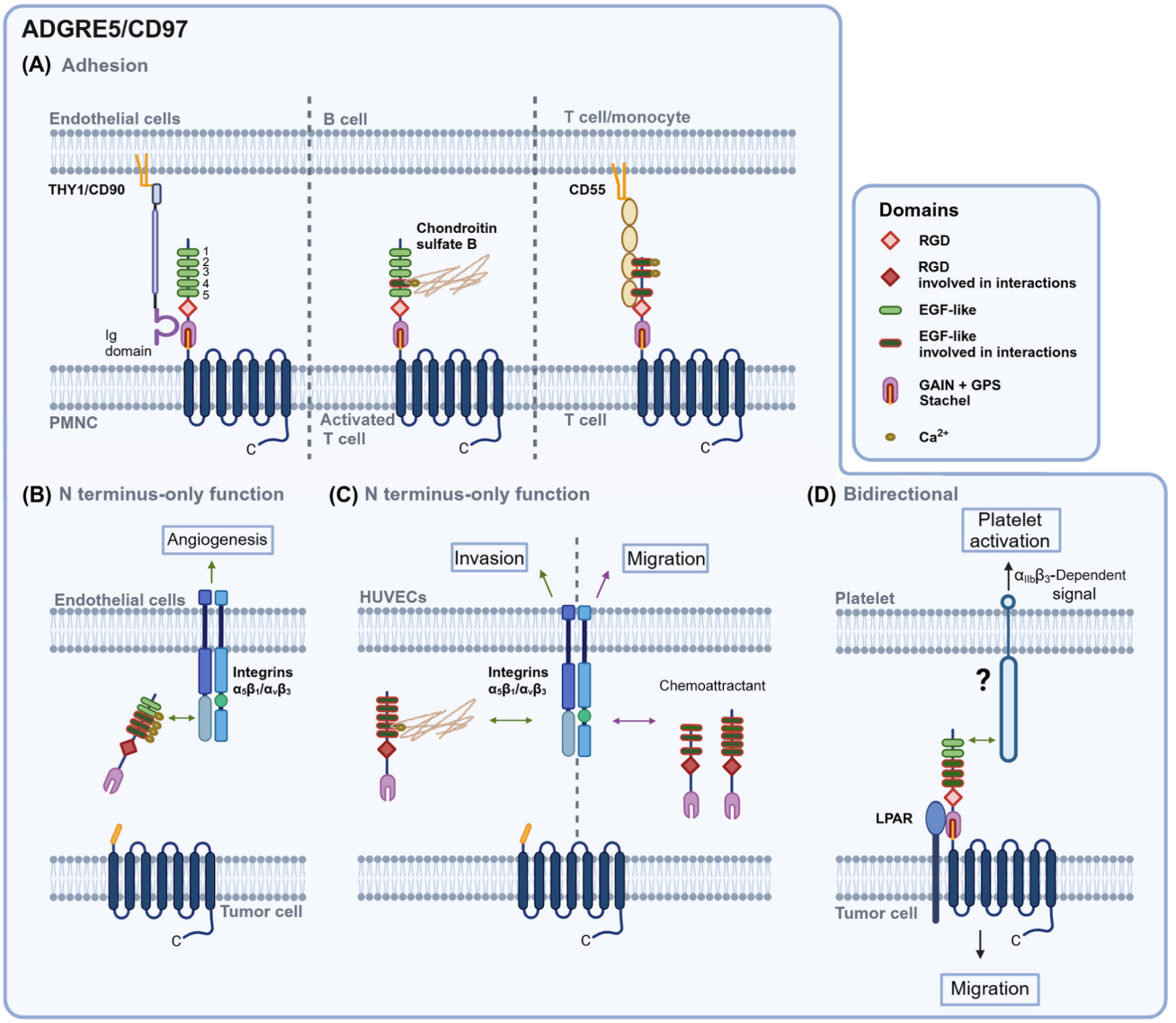

Figure 4. Interactions of ADGRE5 are dependent of the EGF-like domain architecture. (A) Interactions of ADGRE5 potentially involving adhesion. ADGRE5 variants containing all five EGF-like domains can interact with THY1/CD90 via the GAIN domain. Chondroitin sulfate B interacts with the fourth EGF-like domain and CD55 with a variant that only contains three EGF-like domains. (B,C) Interactions that mediate the N terminus-only function of the receptor. Binding to integrins requires the variant containing all EGF-like domains, and the different domains play distinct roles in these interactions. This interaction induces angiogenesis in endothelial cells (B) and migration and invasion in HUVECs (C) where two different mechanisms mediate migration versus invasion. Although the NTF acts as a chemoattractant in both migration/invasion, different domains are essential for the interaction. (D) Bidirectional signaling by ADGRE5. The N terminus-only function activates platelets by a so far unknown mechanism which is integrin-dependent, whereas on tumor cells the receptor interacts with the LPA receptor (LPAR) to stimulate transendothelial migration. Abbreviations: EGF-like, epidermal growth factor-like; GAIN, GPCR autoproteolysis-inducing; GPS, GPCR proteolytic site; HUVEC, human umbilical vein endothelial cells; Ig domain, immunoglobulin domain; LPA, lysophosphatidic acid; LPAR, LPA receptor; NTF, N-terminal fragment; RGD, arginine-glycine-aspartate; Figure created with BioRender.

Notably, none of the reported interaction partners have been shown to directly activate G-protein signaling in ADGRE5 thus far, although the receptor can activate $G_{12/13}$ pathways [60–62]. Interactions with GPI-anchored proteins and chondroitin sulfate B are linked to adhesive functions, where the latter acts as an extracellular matrix anchor. However, because CD55 and THY1/

CD90 can mediate intracellular signaling [59,60], their interaction with ADGRE5 may involve its N terminus-only function.

A clear N terminus-only role is evident in the ADGRE5 interaction with integrins that is similar to those of ADGRA2/ADGRB1 where the NTF alone suffices [58]. *In vivo*, this interaction on endothelial cells promotes angiogenesis, including in tumor cells (Figure 4B). The NTF of this variant also stimulates cell migration and invasion of human umbilical vein endothelial cells (HUVECs) [58]. Invasion requires all five EGF-like domains and is facilitated by the RGD motif that interacts with integrins $\alpha_5\beta_1/\alpha_v\beta_3$, alongside a proteoglycan containing chondroitin sulfate B. Migration is mediated by the full-length variant or the variant with EGF-like domains 1, 2, and 5, and both act as potent chemoattractants (Figure 4C) [58].

Interestingly, soluble N terminus-only versions of ADGRE5 are found in the body fluids of patients with breast, colorectal, or pancreatic cancer [63], as well as in individuals with rheumatoid arthritis [64,65]. Although it can be speculated that these ADGRE5 molecules comprise the liberated NTF, it has not formally been proven which domains/parts of the receptor they contain. The role of this soluble ADGRE5 in these pathological contexts remains elusive, but it is conceivable that increased NTF amounts favors tumor progression, for example, given the known effects of these molecules.

As in ADGRB1/ADGRG6, ADGRE5 in which the NTF remains attached to the **C-terminal fragment (CTF) can also mediate bidirectional signals**. In tumor cell-mediated activation of platelets, the N terminus supports $\alpha_{IIb}\beta_3$-dependent platelet activation (Figure 4D), although a direct integrin interaction has not been demonstrated. The receptor binds simultaneously to the lysophosphatidic acid (LPA) receptor on tumor cells to coordinate invasion and endothelial retraction. Platelet activation is not absolutely dependent on the RGD motif, but the EGF-like domains 3, 4, and 5 are essential [66].

In summary, like other aGPCRs with N terminus-only functions, ADGRE5 could act both as a cell-bound receptor on endothelial cells and as a soluble ligand in a chemotactic gradient by signaling through integrins. It is possible that NTFs from different ADGRE5 variants interact with specific, potentially unknown partners, leading to varied (patho)physiological effects.

ADGRG6/GPR126

The N terminus-only function of ADGRG6 was identified through studies on mouse and zebrafish homologs. Receptor-mediated G-protein signals are crucial for Schwann cell [67] and placental [68] development, as well as for peripheral nerve myelination/regeneration [67,69], and the N terminus-only function also plays roles in cardiac [70] and Schwann cell [67] development. Mice lacking ADGRG6 exhibit high embryonic lethality [69,71], possibly due to heart defects, including hypotrabeculation and myocardial wall thinning [65,67,68,70]. By contrast, zebrafish with a premature stop codon in the *Adgrg6* GPS (*gpr126st49*) show myelination defects but no cardiac issues or increased lethality [69,70]. This is because a functional NTF is still expressed without the CTF in these animals. Indeed, complete depletion of ADGRG6 caused trabeculation defects, whereas ectopic NTF overexpression restored cardiac function but not myelination [70]. These findings confirm the tissue-specific role of the ADGRG6 N terminus-only function in cardiac development.

The molecular mechanisms and binding partners underlying this function remain largely unclear. It is known that the NTF is secreted from endocardial cells and binds to cardiomyocytes and pericardial cells [70]. Possible scenarios include the NTF acting as a ligand or coreceptor to activate unknown receptors or membrane components on cardiomyocytes. This triggers signaling in endocardial cells and a yet unknown signaling pathway in cardiomyocytes. Alternatively, NTF cleavage and dissociation from endocardial cells may induce a so far unknown signal in

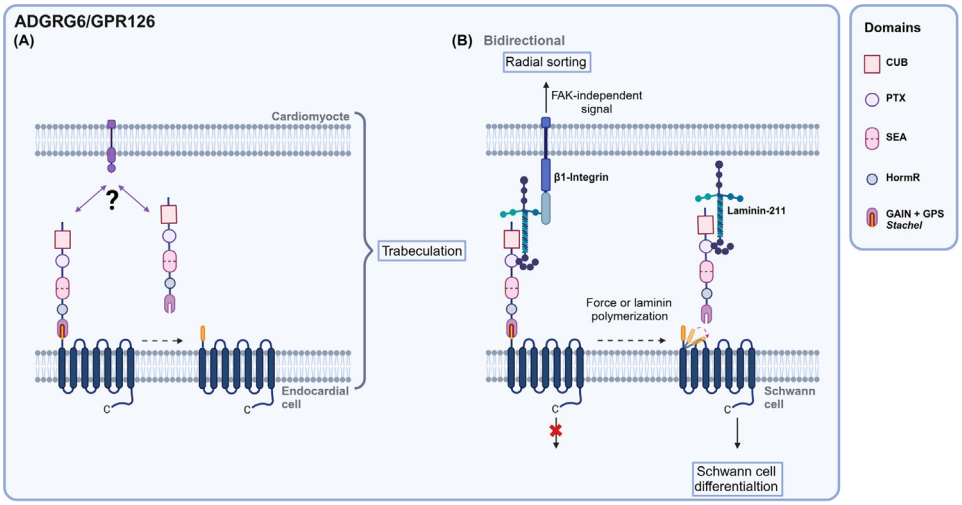

Figure 5. The N terminus-only function of ADGRG6 controls heart trabeculation and radial sorting by Schwann cells. (A) The N terminus of ADGRG6 on endocardial cells interacts with a so far unknown molecule on cardiomyocytes to regulate trabeculation. (B) Bidirectional signaling of ADGRG6 in Schwann cell development. The N terminus-only function is realized via laminin-211, which binds to the N-terminal fragment (NTF) and to β_1-integrins, and subsequently signals in a FAK-independent manner. In this constellation, the receptor remains inactive and no G protein-mediated signals are transduced. Upon mechanical force or laminin polymerization, the NTF is removed and the C-terminal fragment (CTF) is activated, thereby initiating Schwann cell differentiation. Abbreviations: CUB, complement C1r/C1s, Uegf, Bmp1 domain; GAIN, GPCR autoproteolysis-inducing; GPS, GPCR proteolytic site; HormR, hormone-responsive domain; PTX, pentraxin; SEA, sperm protein, enterokinase, and Agrin. Figure created with BioRender.

cardiomyocytes [72] (Figure 5A). The high binding affinity and specificity of collagen IV to the CUB (complement C1r/C1s, Uegf, and Bmp1) and PTX (pentraxin) domains of the NTF suggests that it may interact with this extracellular matrix protein [73].

In addition to its N terminus-only function in heart development, evidence has surfaced that the NTF is also involved in the nervous system where it guides radial axon sorting by Schwann cells (before myelinization) independently of G-protein signals (Figure 5B), whereas the CTF is necessary for axonal wrapping and myelin production via G-protein signaling and cAMP production [67]. It is hypothesized that the NTF interacts with laminin-211 to stabilize an inactive CTF and enable radial sorting. Mechanical forces, such as through laminin polymerization, lead to removal of the NTF, thereby activating the CTF and triggering Schwann cell differentiation. Although the exact mechanism remains unclear, it is speculated that the N terminus-only function involves NTF–laminin-211 complex signaling via β_1-integrins. Laminin-211 signaling through β_1-integrins is known to activate intracellular FAK (focal adhesion kinase) [74]. However, the data suggest that ADGRG6 signaling is independent of FAK. This dual role of ADGRG6 in Schwann cell development is another example where N terminus-only and G-protein signaling by an aGPCR act together in a single biological process.

Recent studies indicate additional roles of the N terminus-only function of ADGRG6 that are of clinical relevance, particularly in cartilage development [75,76]. In adolescent idiopathic scoliosis (AIS) patients, two *ADGRG6* splice variants, one with exon 6 and a second without, are asymmetrically expressed in the spine, and with the exon 6-containing transcript higher on the convex side. Overexpression of the exon 6-containing NTF delayed ossification of human mesenchymal stem cells, suggesting a role in bone formation. In AIS, increased expression of this variant may result in a spinal asymmetry by slowing osteogenic differentiation [76]. Thus, ADGRG6 is another key example demonstrating that the N terminus-only function of aGPCRs is utilized in various contexts.

ADGRL1–3/LPHN1–3/latrophilins

Many recent insights in the N terminus-only function of aGPCRs stem from research on latrophilins, mostly in the invertebrates *C. elegans* [3,4] and *D. melanogaster* [77], but also in mammals [21]. Latrophilins are among the oldest and most evolutionarily conserved aGPCR groups [1]. Mammalian latrophilins are crucial for processes in the nervous, cardiovascular, and endocrine systems [78], and are implicated in severe neurodevelopmental [79] and mental [80] disorders. Consistently, neuronal interaction partners include FLRT (fibronectin leucine-rich transmembrane) proteins [81–84] and teneurins [85,86] that interact with ADGRL1–3, as well as neurexins [87] and contactins [88] that bind to ADGRL1.

Although classical 7TM-dependent signaling has been well established [89], recent studies also suggest N terminus-only functions [21]. For instance, ADGRL3 modulates cone synaptic transmission from lateral inhibitory neurons through teneurins and FLRTs [21]. This involves calcium channel blockade (Figure 6A). Further studies suggest that there may be even more N terminus-only functions. Structural analyses have revealed that ADGRL3 forms supercomplexes with FLRT2 and the receptor Unc5D (uncoordinated-5D) that mediate cell repulsion across synapses (Figure 6B) [90]. Although the signaling mechanism of this complex remains unclear, an N terminus-only function – potentially in the context of bidirectional signals – is plausible, and possibly modulates Unc5D signaling.

Although knowledge on the N terminus-only functions of mammalian latrophilins is only now emerging, invertebrate aGPCRs clearly exert numerous functions that are completely independent of the 7TM region and the C terminus. In the fruit fly *D. melanogaster*, the only ADGRL homolog, dCIRL (calcium-independent receptor of α-latrotoxin), interacts with the receptor Toll-8 (Tollo) via its N terminus [77,91] (Figure 6C). This interaction, occurring between dCIRL on cortical glia cells and Tollo on neighboring neuroblasts in the larval brain, triggers NTF release. This so-called *trans* activation regulates neuroblast numbers. Interestingly, Tollo expression on the same cells as dCIRL inhibits NTF release. The interacting domains and Tollo downstream signals remain unclear and the possibility of simultaneous G-protein signaling by dCIRL in cortical glia warrants further investigation.

In the roundworm *C. elegans*, the N terminus-only function of the latrophilin homolog LAT-1 regulates reproduction, mating [3,4], and neuronal morphogenesis [4]. All are controlled by the membrane-anchored LAT-1 N terminus [3,4] (Figure 6D). Notably, they do not require GPS cleavage, the NTF and CTF remain attached, and both the RBL (rhamnose-binding lectin) and GAIN domains are essential, suggesting interactions with various partners [92]. Although ligands for mammalian latrophilins are conserved in *C. elegans*, most are unlikely to bind to LAT-1 because of the absence of the OLF (olfactomedin) domain. In addition, no evidence currently supports binding to the teneurin homolog, which in mammals interacts via the RBL domain.

Similarly to ADGRE5, studies suggest that, in addition to cleavage, splice variants help to realize and/or fine-tune the N terminus-only function of latrophilins. In mammals, latrophilin splice variants have different ligand-binding properties [86] and distinct G-protein coupling [8,93]. In *C. elegans*, RNA sequencing has identified *lat-1* transcripts containing only the N terminus [3], raising the possibility that these variants indeed play a key role in latrophilin N terminus-only function.

ADGRL4/ELTD1

ADGRL4/ELTD1 (epidermal growth factor, latrophilin, and seven transmembrane domain-containing 1) also belongs to the ADGRL group of aGPCRs [1] but, in contrast to the other group members, its N terminus in humans harbors, in addition to the GAIN domain, one EGF- and one Ca^{2+}-binding EGF-like domain [94] (Figure 6E). Although its 7TM-dependent

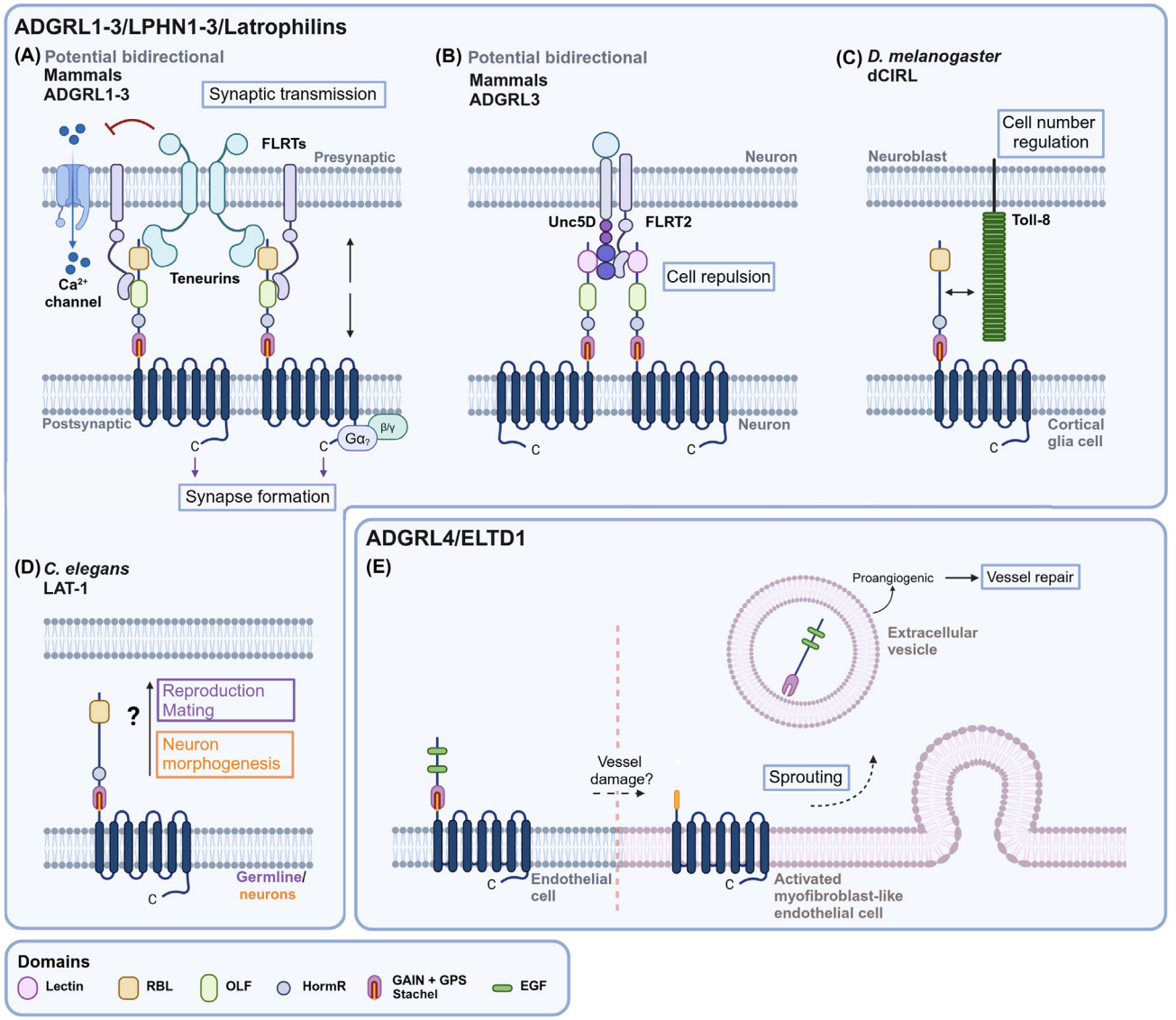

Figure 6. N terminus-only functions of members of the ADGRL group. (A) Mammalian ADGRL molecules can form a complex with teneurins and FLRTs. This complex has recently been shown to involve the N terminus-only function of adhesion GPCRs (aGPCRs). It regulates a calcium channel on the opposing presynaptic cell. (B) A supercomplex is formed between ADGRL, FLRT2, and the receptor Unc5D that modulates cell repulsion. It can be speculated that this can result in an N terminus-only function. (C) In the fruit fly *Drosophila melanogaster*, the N terminus-only function acts on the Toll-like receptor Tollo (Toll-8) and this interaction controls the number of neuroblasts. Bidirectional signaling with an effect on dCIRL-carrying cortical glial cells is also possible. (D) In the nematode *Caenorhabditis elegans* the N terminus-only function is involved in several contexts. (E) For ELTD1, the N-terminal fragment (NTF) is thought to be released during vessel damage, leading to the production of extracellular vesicles (EVs) and promoting angiogenesis and vessel repair. Abbreviations: dCIRL, calcium-independent receptor of α-latrotoxin; EGF, epidermal growth factor-like; FLRT, family of leucine-rich repeat transmembrane proteins; GAIN, GPCR autoproteolysis-inducing; GPS, GPCR proteolytic site; HormR, hormone-responsive domain; OLF, olfactomedin; RBL, rhamnose binding lectin. Figure created with BioRender.

signaling is not well understood, recent studies provide evidence for an N terminus-only function in endothelial cells that contributes to angiogenesis [95], wound repair, and inflammation [96,97].

ADGRL4 activity promotes epithelial–mesenchymal transition in myofibroblast-like cells with elevated angiogenic ability [96]. The NTF is present in endothelial extracellular vesicles (EVs), and expressing the NTF only in EVs enhances endothelial sprouting. ADGRL4-enriched EVs are mostly found in damaged endothelia and have proangiogenic activity *in vitro* and *in vivo* [98]. It is hypothesized that the NTF is only separated from the CTF upon damage to epithelia [98]. Subsequently, the receptor is active and mediates the epithelial–mesenchymal transition of myofibroblast-like cells involved in vessel repair via its CTF. The NTF is packaged into EVs and contributes to their proangiogenic properties, thereby aiding the repair process (Figure 6B).

Current knowledge therefore suggests that the ADGRL4 N terminus-only function requires NTF release for activation. Although the molecular details such as binding partners remain unknown, they are crucial for future studies given the role of the receptor in wound healing where new therapeutic interventions are needed.

Common concepts and different signals of the N terminus-only function

Knowledge from the past years combined with the increasing body of recent findings is beginning to yield a picture of the N terminus-only function of aGPCRs. This understanding is especially important given the involvement of these receptor functions in the onset and/or progression of various diseases (ADGRB1, ADGRE5, and ADGRL), or inflammation and wound repair (ADGRL4).

The ability of aGPCRs to perform functions solely through their N termini is a remarkable and evolutionarily ancient trait that underscores their significance. Invertebrate aGPCRs, such as ADGRC/CELSR/Flamingo and ADGRL/LPHN/latrophilin homologs in *C. elegans*, and ADGRL/LPHN/latrophilin in *D. melanogaster*, are known to exhibit N terminus-only functions across various contexts [11,13,44,45,97]. It is likely that more aGPCRs with similar properties will be identified in the future.

It is increasingly clear that each N terminus-only function of aGPCRs is specifically tailored to the contextual requirements, but common concepts are emerging. First, the N termini of the aGPCRs are highly dynamic – some require release to act as soluble entities (e.g., ADGRB1, ADGRE5, and ADGRL4) whereas others depend on membrane-tethering (e.g., LAT-1). N terminus release can occur through autoproteolysis at the GPS, as confirmed by recently solved structures for several aGPCRs [9–12]. This process can expose the tethered agonist, potentially activating G-protein signaling. This raises the question of whether aGPCRs relying on liberated NTFs also signal via G-proteins in parallel (as suggested for ADGRL4) and how these receptors coordinate the two functions.

As noted, many aGPCRs function bidirectionally, but apparently do not require a liberated NTF for their N terminus-only function (e.g., dCIRL and ADGRE5 in platelet activation). Some, such as LAT-1, do not even require NTF cleavage for functionality. It can be speculated that these aGPCRs perform N terminus-only and G protein-mediated functions in different contexts rather than simultaneously. However, the advent of the numerous structures in the past years can help to understand this matter and suggest that parallel functions might still be possible. For instance, the ADGRL3 structure shows highly flexible extracellular regions [9] that would potentially allow the NTF to move and support simultaneous N terminus-only functions and G-protein activation via Stachel activation. Structural analyses reveal that even uncleaved receptors can be activated by Stachel [11]. In these cases, enzymatic cleavage at sites within the N terminus other than the GPS could produce N-terminal fragments of varying size, thereby enabling N terminus-only function based on a soluble N terminus with an inactive 7TM-dependent function. In some instances, enzymatic cleavage may be essential for functional fine-tuning, especially

because several aGPCRs contain enzyme recognition sites. For example, in ADGRB1, Vstat-120 and Vstat-40 likely have distinct roles in different contexts and may even regulate each other's presence.

Another mechanism for generating soluble N termini is through splice variants. A diverse array of aGPCR splice variants are known, including many N-terminal variants [99,100]. It is conceivable that variants comprising only N-terminal fragments are employed for N terminus-only functions. For instance, N-terminal variants of LAT-1 are found in cells where the receptor fulfills an N terminus-only function.

Interestingly, four of the aGPCRs with N terminus-only functions are directly involved in angiogenesis: ADGRA2, ADGRB1, ADGRE5, and ADGRL4. Although ADGRA2, ADGRE5, and ADGRL4 promote angiogenesis, ADGRB1 inhibits it. All rely on a liberated N terminus and predominantly act through integrin interactions. Notably, both ADGRA2 (sTEM/ppsTEM) and ADGRB1 (Vstat) bind to $\alpha_v\beta_3$-integrins, but with opposing effects on angiogenesis. Thus, it is tempting to speculate that they crosstalk or interfere with each other. Although there is no evidence for such a scenario, the intricate interplay of N terminus-only functions in the same context generally emphasizes the nuanced roles of aGPCRs in vascular biology, where these mechanisms crucially influence endothelial cell behavior and angiogenic processes. Although these aGPCRs all feature an RGD motif, their interactions also depend on additional motifs/domains such as TSRs (ADGRB1) and EGF-like domains (ADGRE5). The N terminus-only functions of ADGRG6 and ADGRL4 also involve integrins, but no direct interactions have been confirmed. These findings suggest that other angiogenesis-related aGPCRs, such as ADGRF5/GPR116 [101], might regulate this process through N terminus-only functions.

A common role of all N terminus-only functions is in acting as ligands or modulators of nearby cells through other signaling cascades. However, the term '*trans* functions' should be used with caution because N terminus effects typically extend in *trans* but do not always involve partners on separate cells. Therefore, 'N terminus-only' or '7TM-independent' function is more precise than '*trans* function'.

The clinical and therapeutic potential of the N terminus of aGPCRs

GPCRs account for ~30% of all drug targets [102], and aGPCRs hold vast pharmacological potential. However, in contrast to other GPCRs, their druggability is more challenging because of their N terminus-only functions. Targeting aGPCRs risks unintentionally affecting the non-canonical mechanisms mediated by their large N termini. Conversely, specifically targeting the N termini is crucial because of their roles in diseases such as cancer development and progression (ADGRA2, ADGRB1, and ADGRE5). A detailed understanding of N terminus-only functions will be essential to develop drugs that selectively modulate G protein-dependent signals, N terminus-only functions, or both in specific tissues. Addressing this remains a key research challenge. Promising approaches include: (i) specifically targeting N terminus-only functions with molecules or antibodies, (ii) using the NTF itself as a drug, and (iii) employing the NTF as a disease biomarker.

Molecules and antibodies against the N terminus

Given their extraordinarily size and complex architecture, the N termini of aGPCRs offer multiple topographically distinct regions for pharmacological targeting, allowing discrimination between different receptor functions. In addition to classical small-molecule approaches, aGPCRs have been successfully targeted with modulating anti-, mono-, or nanobodies. For example, a neutralizing antibody for ADGRE5 has shown effectiveness in treating rheumatoid arthritis

[103,104]. Such neutralizing antibodies hold significant potential for targeting the N terminus-only functions of aGPCRs. Increasing structural knowledge of the NTF will help in identifying targetable regions crucial for these functions.

The NTF as a drug

Although the N terminus-only functions make selective aGPCR targeting more cumbersome, they also open new avenues for therapeutic intervention by using the N termini themselves as drugs. This approach could precisely modulate N terminus-only activity, thus offering selective therapeutic effects. At the forefront of this approach is Vstat-120, where ADGRB1 inhibits angiogenesis in various tumor contexts [34–39] whereas reduced receptor expression favors tumor growth [34]. Thus, the N terminus (or parts of it) could be employed in antitumor therapy. Indeed, preclinical studies involving rapid antiangiogenesis mediated by an oncolytic virus (RAMBO), which expresses Vstat-120 under herpes simplex virus control, demonstrated antiangiogenesis effects *in vitro* as well as in mouse models of glioma, head and neck squamous cell carcinoma, and ovarian cancer [105–109].

When combined with the monoclonal antibody bevacizumab, which targets vascular endothelia growth factor VEGF, RAMBO significantly decreases glioma cell migration and invasion, potentially by downregulating CCN1 (cellular communication network factor 1) and AKT signaling pathways [107]. Furthermore, RAMBO together with the integrin inhibitor cilengitide showed a synergistic effect in enhancing tumor cell killing and inhibiting tumor growth in glioma xenograft mouse models [110]. This suggests promising potential for clinical studies. This approach could also be feasible for other aGPCR N termini, and offers an exciting therapeutic strategy for aGPCR-related diseases. ADGRL4 in particular, given its proangiogenic role in vessel repair and wound healing, appears to be a strong candidate. However, this strategy may not be suitable for ADGRB1 and ADGRE5 because they activate rather than inhibit integrins.

The NTF as a biomarker

In addition to their therapeutic potential, the N termini of aGPCRs are increasingly recognized as valuable biomarkers, and ADGRE5 serves as a prime example. Soluble ADGRE5, likely representing the NTF, is present in fluids from inflammatory sites, such as synovial fluid in rheumatoid arthritic joints [64,65], as well as in patients with breast, colorectal, pancreatic, or bile duct cancers [63,111]. Thus, soluble ADGRE5 levels could serve as reliable biomarker for metastasis and disease progression [111], thereby aiding cancer diagnosis, tumor staging, and treatment evaluation. This is of particular interest given the role of ADGRE5 in other cancers such as glioblastoma [15] and prostate cancer [13].

Concluding remarks and future perspectives

aGPCRs are intriguing receptors with significant pharmacological potential. However, their complex multifaceted functions, particularly N terminus-only signaling, pose challenges for effective evaluation and drug targeting. Recent years have seen substantial progress in understanding these functions, but unresolved issues remain (see Outstanding questions). Future research needs to focus on elucidating the mechanistic details of N terminus-only signaling. Identifying specific binding partners will be essential to understand how the receptors distinguish between N terminus-only and G protein-mediated signals. This knowledge is vital for understanding their roles in diseases such as cancer, neurodevelopmental disorders, and immune dysfunction. Advances in structural biology, including cryo-electron microscopy (EM) and advanced imaging, provide valuable insights into their structure–function relationships and hold promise for the development of targeted therapeutics. A major challenge is to selectively target either G protein-mediated or N terminus-only functions. Exploring splice variants and their expression and

Outstanding questions

Do different binding partners mediate the N terminus-only functions versus the G-protein functions of aGPCRs?

What is the impact of different splice variants containing the N termini of aGPCRs on their N terminus-only functions?

To what extent does enzymatic cleavage of the N terminus modulate the functionality of the N terminus-only function?

How are the tissue-specific activities of the different aGPCR functions realized?

function will help to tackle this obstacle and may enable innovative treatments that leverage the N-terminal functions of aGPCRs.

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Declaration of interests

The authors declare no competing interests.

References

1. Hamann, J. *et al.* (2015) International Union of Basic and Clinical Pharmacology. XCIV. Adhesion G protein-coupled receptors. *Pharmacol. Rev.* 67, 338–367
2. Scholz, N. (2018) Cancer cell mechanics: adhesion G protein-coupled receptors in action? *Front. Oncol.* 8, 59
3. Matus, D. *et al.* (2024) The N terminus-only (trans) function of the adhesion GPCR latrophilin-1 controls multiple processes in reproduction of *C. elegans*. *G3 (Bethesda)* 14, jkae206
4. Matus, D. *et al.* (2022) Latrophilin-1 drives neuron morphogenesis and shapes chemo- and mechanosensation-dependent behavior in *C. elegans* via a trans function. *Biochem. Biophys. Res. Commun.* 589, 152–158
5. Tu, Y.K. *et al.* (2018) The adhesion-GPCR BAI1 promotes excitatory synaptogenesis by coordinating bidirectional trans-synaptic signaling. *J. Neurosci.* 38, 8388–8406
6. America, M. *et al.* (2022) An integrated model for Gpr124 function in Wnt7a/b signaling among vertebrates. *Cell Rep.* 39, 110902
7. Yuki, K. *et al.* (2024) GPR124 regulates murine brain embryonic angiogenesis and BBB formation by an intracellular domain-independent mechanism. *Development* 151, dev202794
8. Wang, S. *et al.* (2024) Alternative splicing of latrophilin-3 controls synapse formation. *Nature* 626, 128–135
9. Barros-Alvarez, X. *et al.* (2022) The tethered peptide activation mechanism of adhesion GPCRs. *Nature* 604, 757–762
10. Ping, Y.Q. *et al.* (2022) Structural basis for the tethered peptide activation of adhesion GPCRs. *Nature* 604, 763–770
11. Qu, X. *et al.* (2022) Structural basis of tethered agonism of the adhesion GPCRs ADGRD1 and ADGRF1. *Nature* 604, 779–785
12. Xiao, P. *et al.* (2022) Tethered peptide activation mechanism of the adhesion GPCRs ADGRG2 and ADGRG4. *Nature* 604, 771–778
13. Lih, T.M. *et al.* (2022) Urinary marker panels for aggressive prostate cancer detection. *Sci. Rep.* 12, 14837
14. Yu, H. *et al.* (2024) RECK/GPR124-driven WNT signaling in pancreatic and gastric cancer cells. *Cancer Sci.* 115, 3013–3025
15. Langenhan, T. (2023) Adhesion GPCRs in glioblastoma revisited. *Cell Rep.* 42, 113474
16. Liebscher, I. *et al.* (2014) A tethered agonist within the ectodomain activates the adhesion G protein-coupled receptors GPR126 and GPR133. *Cell Rep.* 9, 2018–2026
17. Stoveken, H.M. *et al.* (2015) Adhesion G protein-coupled receptors are activated by exposure of a cryptic tethered agonist. *Proc. Natl. Acad. Sci. U. S. A.* 112, 6194–6199
18. Lin, H.H. *et al.* (2004) Autocatalytic cleavage of the EMR2 receptor occurs at a conserved G protein-coupled receptor proteolytic site motif. *J. Biol. Chem.* 279, 31823–31832
19. Arac, D. *et al.* (2012) A novel evolutionarily conserved domain of cell-adhesion GPCRs mediates autoproteolysis. *EMBO J.* 31, 1364–1378
20. Prömel, S. *et al.* (2016) Deciphering and modulating G protein signalling in *C. elegans* using the DREADD technology. *Sci. Rep.* 6, 28901
21. Wang, Y. *et al.* (2021) Adhesion GPCR latrophilin 3 regulates synaptic function of cone photoreceptors in a trans-synaptic manner. *Proc. Natl. Acad. Sci. U. S. A.* 118, e2106694118
22. Chang, J. *et al.* (2017) Gpr124 is essential for blood–brain barrier integrity in central nervous system disease. *Nat. Med.* 23, 450–460
23. Posokhova, E. *et al.* (2015) GPR124 functions as a WNT7-specific coactivator of canonical beta-catenin signaling. *Cell Rep.* 10, 123–130
24. Vallon, M. *et al.* (2018) A RECK–WNT7 receptor–ligand interaction enables isoform-specific regulation of Wnt bioavailability. *Cell Rep.* 25, 339–349
25. Zhou, Y. and Nathans, J. (2014) Gpr124 controls CNS angiogenesis and blood–brain barrier integrity by promoting ligand-specific canonical Wnt signaling. *Dev. Cell* 31, 248–256
26. Xu, Y. *et al.* (2024) GPR124 induces NLRP3 inflammasome-mediated pyroptosis in endothelial cells during ischemic injury. *Eur. J. Pharmacol.* 962, 176228
27. Eubelen, M. *et al.* (2018) A molecular mechanism for Wnt ligand-specific signaling. *Science* 361, eaat1178
28. Vallon, M. *et al.* (2012) Thrombin-induced shedding of tumour endothelial marker 5 and exposure of its RGD motif are regulated by cell-surface protein disulfide-isomerase. *Biochem. J.* 441, 937–944
29. Vallon, M. and Essler, M. (2006) Proteolytically processed soluble tumor endothelial marker (TEM) 5 mediates endothelial cell survival during angiogenesis by linking integrin alpha(v)beta3 to glycosaminoglycans. *J. Biol. Chem.* 281, 34179–34188
30. Stephenson, J.R. *et al.* (2013) Brain-specific angiogenesis inhibitor-1 signaling, regulation, and enrichment in the postsynaptic density. *J. Biol. Chem.* 288, 22248–22256
31. Das, G. *et al.* (2004) Diego interacts with Prickle and Strabismus/Van Gogh to localize planar cell polarity complexes. *Development* 131, 4467–4476
32. Park, D. *et al.* (2007) BAI1 is an engulfment receptor for apoptotic cells upstream of the ELMO/Dock180/Rac module. *Nature* 450, 430–434
33. Hochreiter-Hufford, A.E. *et al.* (2013) Phosphatidylserine receptor BAI1 and apoptotic cells as new promoters of myoblast fusion. *Nature* 497, 263–267
34. Kaur, B. *et al.* (2005) Vasculostatin, a proteolytic fragment of brain angiogenesis inhibitor 1, is an antiangiogenic and antitumorigenic factor. *Oncogene* 24, 3632–3642
35. Nishimori, H. *et al.* (1997) A novel brain-specific p53-target gene, BAI1, containing thrombospondin type 1 repeats inhibits experimental angiogenesis. *Oncogene* 15, 2145–2150
36. Duda, D.G. *et al.* (2002) Overexpression of the p53-inducible brain-specific angiogenesis inhibitor 1 suppresses efficiently tumour angiogenesis. *Br. J. Cancer* 86, 490–496
37. Kaur, B. *et al.* (2009) Vasculostatin inhibits intracranial glioma growth and negatively regulates in vivo angiogenesis through a CD36-dependent mechanism. *Cancer Res.* 69, 1212–1220
38. Izutsu, T. *et al.* (2011) Brain-specific angiogenesis inhibitor 1 is a putative factor for inhibition of neovascular formation in renal cell carcinoma. *J. Urol.* 185, 2353–2358
39. Cork, S.M. *et al.* (2012) A proprotein convertase/MMP-14 proteolytic cascade releases a novel 40 kDa vasculostatin from tumor suppressor BAI1. *Oncogene* 31, 5144–5152
40. Jimenez, B. *et al.* (2000) Signals leading to apoptosis-dependent inhibition of neovascularization by thrombospondin-1. *Nat. Med.* 6, 41–48

41. Jimenez, B. *et al.* (2001) c-Jun N-terminal kinase activation is required for the inhibition of neovascularization by thrombospondin-1. *Oncogene* 20, 3443–3448
42. Koh, J.T. *et al.* (2004) Extracellular fragment of brain-specific angiogenesis inhibitor 1 suppresses endothelial cell proliferation by blocking alphavbeta5 integrin. *Exp. Cell Res.* 294, 172–184
43. Wang, J. *et al.* (2021) RTN4/NoGo-receptor binding to BAI adhesion-GPCRs regulates neuronal development. *Cell* 184, 5869–5885
44. Scheiffele, P. *et al.* (2000) Neuroligin expressed in nonneuronal cells triggers presynaptic development in contacting axons. *Cell* 101, 657–669
45. Strutt, D. *et al.* (2016) Adhesion GPCRs govern polarity of epithelia and cell migration. *Handb. Exp. Pharmacol.* 234, 249–274
46. Strutt, H. and Strutt, D. (2008) Differential stability of flamingo protein complexes underlies the establishment of planar polarity. *Curr. Biol.* 18, 1555–1564
47. Bui, D.L.H. *et al.* (2023) The adhesion GPCRs CELSR1-3 and LPHN3 engage G proteins via distinct activation mechanisms. *Cell Rep.* 42, 112552
48. Basta, L.P. *et al.* (2022) Celsr1 and Celsr2 exhibit distinct adhesive interactions and contributions to planar cell polarity. *Front. Cell Dev. Biol.* 10, 1064907
49. Stahley, S.N. *et al.* (2021) Celsr1 adhesive interactions mediate the asymmetric organization of planar polarity complexes. *eLife* 10, e62097
50. Steimel, A. *et al.* (2010) The Flamingo ortholog FMI-1 controls pioneer-dependent navigation of follower axons in *C. elegans*. *Development* 137, 3663–3673
51. Schön, J.L. *et al.* (2024) The adhesion GPCR and PCP component flamingo (FMI-1) alters body size and regulates the composition of the extracellular matrix. *Matrix Biol.* 128, 1–10
52. Sakai, N. *et al.* (2023) Function of cell adhesion molecules in differentiation of ray sensory neurons in *C. elegans*. *G3 (Bethesda)* 13, jkac338
53. Hsiao, C.C. *et al.* (2011) GPS autoproteolysis is required for CD97 to up-regulate the expression of N-cadherin that promotes homotypic cell–cell aggregation. *FEBS Lett.* 585, 313–318
54. Ravn-Boess, N. *et al.* (2023) The expression profile and tumorigenic mechanisms of CD97 (ADGRE5) in glioblastoma render it a targetable vulnerability. *Cell Rep.* 42, 113374
55. Wandel, E. *et al.* (2012) Thy-1 (CD90) is an interacting partner for CD97 on activated endothelial cells. *J. Immunol.* 188, 1442–1450
56. Kwakkenbos, M.J. *et al.* (2005) Expression of the largest CD97 and EMR2 isoforms on leukocytes facilitates a specific interaction with chondroitin sulfate on B cells. *J. Leukoc. Biol.* 77, 112–119
57. Wobus, M. *et al.* (2004) N-glycosylation of CD97 within the EGF domains is crucial for epitope accessibility in normal and malignant cells as well as CD55 ligand binding. *Int. J. Cancer* 112, 815–822
58. Wang, T. *et al.* (2005) CD97, an adhesion receptor on inflammatory cells, stimulates angiogenesis through binding integrin counterreceptors on endothelial cells. *Blood* 105, 2836–2844
59. Tjong, W.Y. and Lin, H.H. (2019) The role of the RGD motif in CD97/ADGRE5- and EMR2/ADGRE2-modulated tumor angiogenesis. *Biochem. Biophys. Res. Commun.* 520, 243–249
60. Hilbig, D. *et al.* (2018) Mechano-dependent phosphorylation of the PDZ-binding motif of CD97/ADGRE5 modulates cellular detachment. *Cell Rep.* 24, 1986–1995
61. Ward, Y. *et al.* (2011) LPA receptor heterodimerizes with CD97 to amplify LPA-initiated RHO-dependent signaling and invasion in prostate cancer cells. *Cancer Res.* 71, 7301–7311
62. Slepak, T.I. *et al.* (2023) Dual role of the adhesion G-protein coupled receptor ADRGE5/CD97 in glioblastoma invasion and proliferation. *J. Biol. Chem.* 299, 105105
63. Vogl, U.M. *et al.* (2017) Evaluation of prognostic immune signatures in patients with breast, colorectal and pancreatic cancer receiving chemotherapy. *Anticancer Res.* 37, 1947–1955
64. Hamann, J. *et al.* (1999) Expression of the activation antigen CD97 and its ligand CD55 in rheumatoid synovial tissue. *Arthritis Rheum.* 42, 650–658
65. Gray, J.X. *et al.* (1996) CD97 is a processed, seven-transmembrane, heterodimeric receptor associated with inflammation. *J. Immunol.* 157, 5438–5447
66. Ward, Y. *et al.* (2018) Platelets promote metastasis via binding tumor CD97 leading to bidirectional signaling that coordinates transendothelial migration. *Cell Rep.* 23, 808–822
67. Petersen, S.C. *et al.* (2015) The adhesion GPCR GPR126 has distinct, domain-dependent functions in Schwann cell development mediated by interaction with laminin-211. *Neuron* 85, 755–769
68. Torregrosa-Carrion, R. *et al.* (2021) Adhesion G protein-coupled receptor Gpr126/Adgrg6 is essential for placental development. *Sci. Adv.* 7, eabj5445
69. Monk, K.R. *et al.* (2009) A G protein-coupled receptor is essential for Schwann cells to initiate myelination. *Science* 325, 1402–1405
70. Patra, C. *et al.* (2013) Organ-specific function of adhesion G protein-coupled receptor GPR126 is domain-dependent. *Proc. Natl. Acad. Sci. U. S. A.* 110, 16898–16903
71. Waller-Evans, H. *et al.* (2010) The orphan adhesion-GPCR GPR126 is required for embryonic development in the mouse. *PLoS One* 5, e14047
72. Liebscher, I. *et al.* (2014) New functions and signaling mechanisms for the class of adhesion G protein-coupled receptors. *Ann. N. Y. Acad. Sci.* 1333, 43–64
73. Paavola, K.J. *et al.* (2014) Type IV collagen is an activating ligand for the adhesion G protein-coupled receptor GPR126. *Sci. Signal.* 7, ra76
74. Feltri, M.L. *et al.* (2002) Conditional disruption of beta 1 integrin in Schwann cells impedes interactions with axons. *J. Cell Biol.* 156, 199–209
75. Karner, C.M. *et al.* (2015) Gpr126/Adgrg6 deletion in cartilage models idiopathic scoliosis and pectus excavatum in mice. *Hum. Mol. Genet.* 24, 4365–4373
76. Xu, E. *et al.* (2019) Asymmetric expression of GPR126 in the convex/concave side of the spine is associated with spinal skeletal malformation in adolescent idiopathic scoliosis population. *Eur. Spine J.* 28, 1977–1986
77. Scholz, N. *et al.* (2023) Molecular sensing of mechano- and ligand-dependent adhesion GPCR dissociation. *Nature* 615, 945–953
78. Liebscher, I. *et al.* (2022) A guide to adhesion GPCR research. *FEBS J.* 289, 7610–7630
79. Vitobello, A. *et al.* (2022) ADGRL1 haploinsufficiency causes a variable spectrum of neurodevelopmental disorders in humans and alters synaptic activity and behavior in a mouse model. *Am. J. Hum. Genet.* 109, 1436–1457
80. Arcos-Burgos, M. *et al.* (2010) A common variant of the latrophilin 3 gene, LPHN3, confers susceptibility to ADHD and predicts effectiveness of stimulant medication. *Mol. Psychiatry* 15, 1053–1066
81. O'Sullivan, M.L. *et al.* (2012) FLRT proteins are endogenous latrophilin ligands and regulate excitatory synapse development. *Neuron* 73, 903–910
82. O'Sullivan, M.L. *et al.* (2014) LPHN3, a presynaptic adhesion-GPCR implicated in ADHD, regulates the strength of neocortical layer 2/3 synaptic input to layer 5. *Neural Dev.* 9, 7
83. Jackson, V.A. *et al.* (2015) Structural basis of latrophilin–FLRT interaction. *Structure* 23, 774–781
84. Lu, Y.C. *et al.* (2015) Structural basis of latrophilin–FLRT–UNC5 interaction in cell adhesion. *Structure* 23, 1678–1691
85. Silva, J.P. *et al.* (2011) Latrophilin 1 and its endogenous ligand Lasso/teneurin-2 form a high-affinity transsynaptic receptor pair with signaling capabilities. *Proc. Natl. Acad. Sci. U. S. A.* 108, 12113–12118
86. Boucard, A.A. *et al.* (2014) Latrophilins function as heterophilic cell-adhesion molecules by binding to teneurins: regulation by alternative splicing. *J. Biol. Chem.* 289, 387–402
87. Boucard, A.A. *et al.* (2012) High-affinity neurexin binding to the cell-adhesion G-protein coupled receptor CIRL1/latrophilin-1 produces an intercellular adhesion complex. *J. Biol. Chem.*,
88. Zuko, A. *et al.* (2016) Association of cell adhesion molecules contactin-6 and latrophilin-1 regulates neuronal apoptosis. *Front. Mol. Neurosci.* 9, 143

89. Moreno-Salinas, A.L. *et al.* (2019) Latrophilins: a neuro-centric view of an evolutionary conserved adhesion G protein-coupled receptor subfamily. *Front. Neurosci.* 13, 700
90. Jackson, V.A. *et al.* (2016) Super-complexes of adhesion GPCRs and neural guidance receptors. *Nat. Commun.* 7, 11184
91. Lavalou, J. *et al.* (2021) Formation of polarized contractile interfaces by self-organized Toll-8/Cirl GPCR asymmetry. *Dev. Cell* 56, 1574–1588
92. Prömel, S. *et al.* (2013) Matching structure with function: the GAIN domain of adhesion-GPCR and PKD1-like proteins. *Trends Pharmacol. Sci.* 34, 470–478
93. Röthe, J. *et al.* (2019) Involvement of the adhesion GPCRs latrophilins in the regulation of insulin release. *Cell Rep.* 26, 1573–1584
94. Favara, D.M. *et al.* (2019) ADGRL4/ELTD1 is a highly conserved angiogenesis-associated orphan adhesion GPCR that emerged with the first vertebrates and comprises 3 evolutionary variants. *BMC Evol. Biol.* 19, 143
95. Masiero, M. *et al.* (2013) A core human primary tumor angiogenesis signature identifies the endothelial orphan receptor ELTD1 as a key regulator of angiogenesis. *Cancer Cell* 24, 229–241
96. Sheldon, H. *et al.* (2021) ELTD1 activation induces an endothelial EMT transition to a myofibroblast phenotype. *Int. J. Mol. Sci.* 22, 11293
97. Sheldon, H. *et al.* (2021) ADGRL4/ELTD1 expression in breast cancer cells induces vascular normalization and immune suppression. *Mol. Cancer Res.* 19, 1957–1969
98. Sheldon, H. *et al.* (2022) ELTD1 is present in extracellular vesicles derived from endothelial cells as a cleaved extracellular domain which induces in vivo angiogenesis. *J. Extracell. Biol.* 1, e52
99. Knierim, A.B. *et al.* (2019) Genetic basis of functional variability in adhesion G protein-coupled receptors. *Sci. Rep.* 9, 11036
100. Kuhn, C.K. *et al.* (2024) The repertoire and structure of adhesion GPCR transcript variants assembled from publicly available deep-sequenced human samples. *Nucleic Acids Res.* 52, 3823–3836
101. Niaudet, C. *et al.* (2015) Gpr116 receptor regulates distinctive functions in pneumocytes and vascular endothelium. *PLoS One* 10, e0137949
102. Hauser, A.S. *et al.* (2017) Trends in GPCR drug discovery: new agents, targets and indications. *Nat. Rev. Drug Discov.* 16, 829–842
103. de Groot, D.M. *et al.* (2009) Therapeutic antibody targeting of CD97 in experimental arthritis: the role of antigen expression, shedding, and internalization on the pharmacokinetics of anti-CD97 monoclonal antibody 1B2. *J. Immunol.* 183, 4127–4134
104. Kop, E.N. *et al.* (2006) CD97 neutralisation increases resistance to collagen-induced arthritis in mice. *Arthritis Res. Ther.* 8, R155
105. Hardcastle, J. *et al.* (2010) Enhanced antitumor efficacy of vasculostatin (Vstat120) expressing oncolytic HSV-1. *Mol. Ther.* 18, 285–294
106. Fujii, K. *et al.* (2013) The integrin inhibitor cilengitide enhances the anti-glioma efficacy of vasculostatin-expressing oncolytic virus. *Cancer Gene Ther.* 20, 437–444
107. Tomita, Y. *et al.* (2019) Oncolytic herpes virus armed with vasculostatin in combination with bevacizumab abrogates glioma invasion via the CCN1 and AKT signaling pathways. *Mol. Cancer Ther.* 18, 1418–1429
108. Hong, B. *et al.* (2021) Oncolytic HSV therapy modulates vesicular trafficking inducing cisplatin sensitivity and antitumor immunity. *Clin. Cancer Res.* 27, 542–553
109. Nair, M. *et al.* (2020) Enhancing antitumor efficacy of heavily vascularized tumors by RAMBO virus through decreased tumor endothelial cell activation. *Cancers (Basel)* 12, 1040
110. Rivera-Caraballo, K.A. *et al.* (2022) The complex relationship between integrins and oncolytic herpes simplex virus 1 in high-grade glioma therapeutics. *Mol. Ther. Oncolytics* 26, 63–75
111. Meng, Z.W. *et al.* (2017) Expression and prognostic value of soluble CD97 and its ligand CD55 in intrahepatic cholangiocarcinoma. *Tumour Biol.* 39, 1010428317694319