**Mapping the sequence determinants of basal activity in GPCRs**

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**Project Summary**

G protein-coupled receptors (GPCRs) are the largest human membrane receptor family and the target of roughly a third of approved drugs[1]. Almost everything we know about GPCRs is framed around what happens after ligand binding, but receptors are not always silent before that. GPCRs sit in an equilibrium between inactive and active states and often signal at some rate with no ligand present. This basal, or constitutive, activity is the baseline tone that a drug or endogenous stimulus acts on, and mutations that alter it are implicated in various endocrine and developmental diseases. How much of it a receptor has, and which residues constrain it, are basic properties of the family that cannot be read from sequence alone. We have a series of unpublished datasets that addresses this, functional testing a large-scale variant library and the entire GPCR family uncover hidden features determining basal receptor activity.

**What we will publish and why we generated it**

GPR161 is a clear case where basal signaling is crucial for its biological function. GPR161 sits in the primary cilium and holds the Hedgehog pathway off. Hedgehog patterns the neural tube, limbs and face in the embryo and remains active in adult stem compartments, and its resting state is not passive but actively repressed. Constitutive signaling by GPR161 maintains high ciliary cAMP, which keeps Hedgehog target genes silent[2]. The pathway is switched on not by giving GPR161 a ligand, but by removing the receptor from the cilium entirely[2,3]. GPR161's resting output is therefore the signal itself, and its magnitude sets the threshold for the whole Hedgehog pathway. Complete loss of GPR161 in mouse is embryonic lethal with fully penetrant exencephaly, and hypomorphic alleles give spina bifida and cataract[2,4]. In humans, germline GPR161 mutations cause a recognized tumor predisposition syndrome accounting for roughly 5% of infant Sonic Hedgehog medulloblastoma[5], and rare GPR161 variants are risk factors for spina bifida[6]. GPR161 is also an orphan, so basal activity is not merely the interesting readout for this receptor, it is the only one available.

Our principal dataset is a deep mutational scan of GPR161, generated to ask which residues govern receptor function. We screened a library covering every single amino acid substitution across three phenotypes: cAMP signaling, whole-cell protein abundance, and cell-surface expression (**Fig. 1**). Variants with the same apparent phenotype can arise by different mechanisms: a receptor can fail because it is never made, or because it is expressed but cannot be switched on. Using our data we can discern which variants are loss of function because of expression or trafficking defects from those that purely alter receptor signaling. Our scan therefore reports on variant phenotypes and the mechanisms behind them, including those that cause disease in humans. This scan began as a functional study alongside a structural study of GPR161 regulation[7], which ultimately investigated a different mechanistic question.

Alongside the GPR161 DMS, we will release a companion dataset that puts GPR161 basal activity in the context of the entire human GPCR family (**Fig. 1**). We developed a screening platform to measure basal cAMP signaling across all ~800 human GPCRs in a pooled format. Because basal activity is a relative quantity, pooling enables direct comparison between receptors without the plate, batch and normalization effects that confound arrayed screens. It is the less developed of the two datasets, and we present it as a resource and a starting point. As expected, GPR161 appears among the receptors with the highest basal cAMP. Others agree with reported literature, and still others are candidates we have not followed up on and would rather nominate to the field than leave in a drawer.

Together these data give a mechanistic account of variant effects in a receptor where basal signaling can drives development and disease. Across the GPCR family, they provide a common scale for a dimension of GPCR function that current screens cannot resolve.

**Why it has not been published:**

Both datasets sit squarely in the gap this program describes: comprehensive, machine-readable data that current publishing incentives leave unshared. The GPCRome cAMP signaling screen was performed as an internal benchmark, and we have not had the right opportunity to publish it in conjunction with another story. The GPR161 DMS dataset was generated to complement another story[7], but it was not included since the narrative moved in a different mechanistic direction. In both cases the data outlived the project that motivated it, and we prioritized work that was already headed somewhere publishable. Neither dataset has produced the single headline finding a conventional paper is built around, and neither is part of a paper we are preparing or holding them for. They are nonetheless complete and quality-controlled, they demonstrate the complexities of GPCR function and signaling, and they can serve as important resources similar to other GPCRome and GPCR-DMS papers[8–14]. Without this program we are unlikely to write either up, and we are fully committed to releasing everything and to a preprint-based model of publication for this work.

**How we will publish it:**

Following Astera's Open Science Policy, we will publish this information in several FAIR venues, depending on data type. First, a preprint describing the datasets and findings will be posted on bioRxiv (with affiliated supplemental data). Second, code used to analyze datasets will be posted on GitHub with an accompanying Zenodo deposition. Third, mutational scanning variant scores will be deposited in MAVEdb, a field-specific repository of DMS data. In each case, a permissive and open license (e.g., CC-BY/MIT, etc) will be used. The raw data, delivered as machine-readable measurements on shared GPCR residue coordinates, are immediately useful as benchmarks and training data for protein function models, as design constraints for receptor engineering, and as a prior for the many human GPCR variants where no assay data is available. Proposal is posted publicly here: https://matthewkhoward.com/

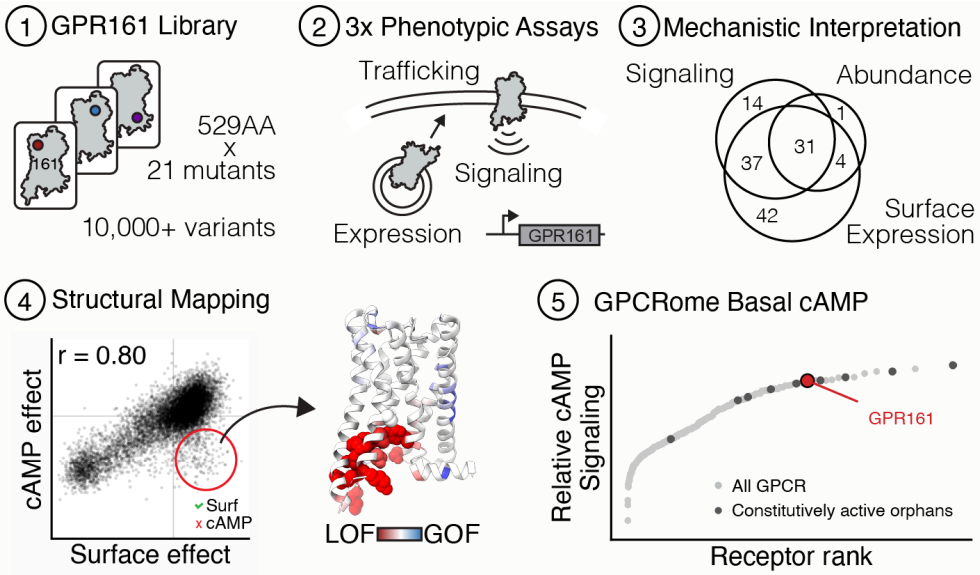


**Figure 1**
(1) A comprehensive missense mutational library was generates for GPR161
(2) Variant effects were assessed on surface expression, whole-cell abundance, and cAMP signaling
(3) Positions can alter multiple phenotypes
(4) Mapping variant effects to structure reveals functional hotspots
(5) Profiling basal cAMP signaling across the human GPCRome

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