nature portfolio

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Reporting Summary

Nature Portfolio wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Portfolio policies, see our Editorial Policies and the Editorial Policy Checklist.

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a	Confirmed
☐	☒ The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
☐	☒ A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒ The statistical test(s) used AND whether they are one- or two-sided *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☒	☐ A description of all covariates tested
☒	☐ A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☐	☒ A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☐	☒ For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☒	☐ For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☒	☐ For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☒	☐ Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection	Titan Krios electron microscope (FEI) operating at 300 kV and equipped with a K3 direct electron detector (Gatan, Inc., cryo-EM); Tecnai G2 F30 microscope (nsEM); BioTek Gen5 (signaling data collection); Zeiss LSM800 confocal microscope (live cell images); Home-built TIRF microscope with smCamera (v1.0)
Data analysis	cryoSPARC 4.4 (cryo-EM data processing); Relion 4.0 (cryo-EM data processing); EMAN2 (nsEM data processing); PHENIX 1.19.2-4158 and COOT 0.9.6 (Model building and refinement); UCSF ChimeraX 1.5 (structure visualization), GraphPad Prism 10.0.2 (signaling data analysis), OriginPro 9.5.5.409 (smFRET data analysis), ImageJ 1.52P (smFRET movies analysis), vbFRET 2.5 (single molecule data analysis)

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our policy

The cryo-EM density map has been deposited in the Electron Microscopy Data Bank under accession code EMD-43523 [https://www.ebi.ac.uk/emdb/EMD-43523], and the coordinates for the model of HormR/GAIN domains of ADGRL3 in complex with sAB LK3 generated in this study have been deposited in the Protein Data Bank under accession code PDB 8vti [https://doi.org/10.2210/pdb8vti/pdb]. The available structure of apo-state 7TM region of ADGRL3 referenced in this work is available under the accession code 8jmt [https://doi.org/10.2210/pdb8jmt/pdb]. Sample single molecule image data of ADGRL3 WT and ADGRL3 S810L/E811Q has been deposited in the Harvard Dataverse repository at [https://doi.org/10.7910/DVN/W446VI]. All data supporting the findings of this study are available within the article and Supplementary Information/Source Data files. Source Data file with raw data underlying Figs. 4e, 5a-b, 5d, 5g-i, 6b-i, and Supplementary Figs. 1, 2, 9, 10, 13, 15 are provided with this paper as a Source Data file. Raw smFRET traces data sets can be provided upon a reasonable request to the corresponding author R.V.

Research involving human participants, their data, or biological material

Policy information about studies with human participants or human data. See also policy information about sex, gender (identity/presentation), and sexual orientation and race, ethnicity and racism.

Reporting on sex and gender	N/A
Reporting on race, ethnicity, or other socially relevant groupings	N/A
Population characteristics	N/A
Recruitment	N/A
Ethics oversight	N/A

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	No sample size calculation was performed. A sample size of n=3 is commonly used in biological studies using cell cultures. Sample size of more than 500 molecules per condition was acquired and was found to adequately sample the behavior of molecules. All experiments were performed in at least three independent preparations with at least three independent biologial replicates.
Data exclusions	No data were excluded.
Replication	All experiments were repeated in part or in whole at least two times to ensure reproducibility. Signaling assays and smFRET experiments were done in triplicates, and all attempts at replication were successful. Image analysis were done in three independent replicates with quantifications from randomly selected imaging fields per replicate. No biological was used in more than one set of experiments.
Randomization	Quantifications from randomly selected imaging fields per experimental condition replicate. Cells placed in different positions on 96-well signaling assay plate and randomly allocated into control and treatment groups.
Blinding	Blinding is not relevant to this study, all experiments were performed based on standardized protocols and readouts, and are not influenced by the investigator.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☐	☒ Antibodies
☐	☒ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☒	☐ Animals and other organisms
☒	☐ Clinical data
☒	☐ Dual use research of concern
☒	☐ Plants

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Antibodies

Antibodies used: HRP-conjugated Mouse Anti-M13 monoclonal antibody; 1:5000 dilution (GE Healthcare 27942101), anti-GFP antibody (Abcam, #ab6658)

Validation: Most commercially available antibodies (from various suppliers) that are being used during the course of our studies have been validated by our lab and others through comparison of obtained immunohistochemistry (IHC) and/or Western blot (WB) results between labs and, in many cases, confirmation of specificity using mutant mice lacking the proteins detected by the antibodies. We validate each new lot of antibodies by reproducing results obtained with previous batches of antibody.

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s): HEK293T cells (ATCC, CRL-3216);
HEK293 cells (ATCC CRL-1573);
High Five cells (Thermo Fisher, B85502);
Sf9 cells (Thermo Fisher, 11496015)

Authentication: HEK293T/HEK293 cell lines (from ATCC) have been validated by the supplier through STR analysis and cytogenetic studies. Sf9 and High Five were not authenticated. We also employ a protocol for sequencing of baculoviral stocks postamplification to confirm identities of proteins over-expressed in lepidopteran insect cells, especially for mutant variants that are hard to differentiate otherwise.

Mycoplasma contamination: Cell lines tested negative for mycoplasma contamination (ATCC)

Commonly misidentified lines (See ICLAC register): No commonly misidentified cell lines were used in this study

Plants

Seed stocks: n/a

Novel plant genotypes: n/a

Authentication: n/a