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	Luminescence readings were collected using BioTek Gen5 Software v3.11 from the Synergy Neo2 microplate reader (see https://www.biotek.com/products/software-robotics-software/gen5-microplate-reader-and-imager-software/overview/). Human Protein Atlas (HPA) RNA consensus tissue gene data (version 21.0 and Ensembl version 103.38.) was downloaded at https://www.proteinatlas.org/about/download
Data analysis	All data was analyzed using GraphPad Prism version 9.3.0

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

All data generated or analysed during this study are included in this published article (and its Supplementary Information files).

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 calculations were performed. Indicated sample sizes (n=3-8) were chosen based on the previous published work of experienced researchers in the field with cell-based experiments of similar design.
Data exclusions	Certain outlier values, which arose due to a couple of wells exhibiting significant cell detachment or cell aggregation/clumping, OR due to luminescence signal bleed-through between adjacent wells (when using white 384-well plates), were detected by checking microplates under the microscope prior to adding luciferase detection reagent. These outlier wells were noted and subsequently excluded during data processing.
Replication	Replication was ensured through a minimum of 3 independent measurements, and in certain cases, repeating the experiment multiple times (e.g., GPCR-ome screens, as indicated), especially for experiments producing novel results and/or results that contradict existing published findings (e.g., Fig 1J, ADORA3 result)
Randomization	No human and/or animal subjects were involved (only human immortalized cell lines), thus randomization was not relevant to our study
Blinding	No participant groups were involved, thus blinding was not relevant to our study

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
☒	☐ Human research participants
☒	☐ Clinical data
☒	☐ Dual use research of concern

Methods

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

Antibodies

Antibodies used	Monoclonal ANTI-FLAG® M2-Peroxidase (HRP) antibody, MilliporeSigma, Cat. #A8592, Clone M2
Validation	The antibody used was chosen based on the published methods of the original PRESTO-Tango (see PMID: 25895059, under Methods, subsection " Immunofluorescence")

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	HEK293T cells: ATCC, Cat# CRL-3216; HTL and HTLA cells : A gift from Dr. Richard Axel, Columbia University; Tango Trio cells (HTTL, HTTL-B1, HTTL-B2, HTTL-F): generated herein
Authentication	Cells were not authenticated
Mycoplasma contamination	Mycoplasma contamination was tested by PCR; if tested positive, cells were treated with 2-week treatment with Plasmocure™ (InvivoGen) and re-tested by PCR. All cell lines tested negative for mycoplasma before experiments.

Commonly misidentified lines (See ICLAC register)	No misidentified cell lines were used in this study based on the current ICLAC register