

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  |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> The exact sample size ( <i>n</i> ) for each experimental group/condition, given as a discrete number and unit of measurement   |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly  |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> The statistical test(s) used AND whether they are one- or two-sided<br><i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i>   |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> A description of all covariates tested   |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons  |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> 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) |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> For null hypothesis testing, the test statistic (e.g. <i>F</i> , <i>t</i> , <i>r</i> ) with confidence intervals, effect sizes, degrees of freedom and <i>P</i> value noted<br><i>Give P values as exact values whenever suitable.</i>                     |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings  |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes  |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> Estimates of effect sizes (e.g. Cohen's <i>d</i> , Pearson's <i>r</i> ), 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	Electrophysiological data were collected using pCLAMP10 (Molecular Devices, Sunnyvale, CA, USA). Molecular dynamics simulations were performed on structures modeled using AphaFold 3 ( <a href="https://alphafoldserver.com/">https://alphafoldserver.com/</a> ).
Data analysis	Electrophysiological results were analyzed using pCLAMP10 (Molecular Devices, Sunnyvale, CA, USA). Autoradiograms and Western blot images were analyzed using ImageQuant 5.2 (GE Healthcare) and ImageJ/Fuji ( <a href="https://imagej.net/software/fiji/">https://imagej.net/software/fiji/</a> ). Confocal images were analyzed using Zeiss LSM Image Browser (Carl Zeiss Jena GmbH), ImageJ/Fiji or LAS AF (Lecia Microsystems CMC GmbH) Image software. For molecular dynamics, coarse-grained constructs were modeled with CHARMM-GUI84 <a href="https://www.charmm-gui.org">https://www.charmm-gui.org</a> (accessed July 2024), simulations were conducted with Gromacs 2022.3 <a href="https://www.gromacs.org/">https://www.gromacs.org/</a> , contacts were analyzed using MDAnalysis91 <a href="https://www.mdanalysis.org/">https://www.mdanalysis.org/</a> . Analysis also used Python 3.7.4 routines MDAnalysis 0.20.1, NumPy 1.21.6, pandas 1.3.5, matplotlib 3.1.3, seaborn 0.11.1. For kinetic simulations we used Berkley Madonna (Berkeley Madonna, Inc., Albany, CA).

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 are presented in figures and tables in the main paper and in Supplementary Material. MD simulation data has been stored on Zenodo: doi: 10.5281/zenodo.15075373.

## 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

*Use the terms sex (biological attribute) and gender (shaped by social and cultural circumstances) carefully in order to avoid confusing both terms. Indicate if findings apply to only one sex or gender; describe whether sex and gender were considered in study design; whether sex and/or gender was determined based on self-reporting or assigned and methods used. Provide in the source data disaggregated sex and gender data, where this information has been collected, and if consent has been obtained for sharing of individual-level data; provide overall numbers in this Reporting Summary. Please state if this information has not been collected. Report sex- and gender-based analyses where performed, justify reasons for lack of sex- and gender-based analysis.*

### Reporting on race, ethnicity, or other socially relevant groupings

*Please specify the socially constructed or socially relevant categorization variable(s) used in your manuscript and explain why they were used. Please note that such variables should not be used as proxies for other socially constructed/relevant variables (for example, race or ethnicity should not be used as a proxy for socioeconomic status). Provide clear definitions of the relevant terms used, how they were provided (by the participants/respondents, the researchers, or third parties), and the method(s) used to classify people into the different categories (e.g. self-report, census or administrative data, social media data, etc.) Please provide details about how you controlled for confounding variables in your analyses.*

### Population characteristics

*Describe the covariate-relevant population characteristics of the human research participants (e.g. age, genotypic information, past and current diagnosis and treatment categories). If you filled out the behavioural & social sciences study design questions and have nothing to add here, write "See above."*

### Recruitment

*Describe how participants were recruited. Outline any potential self-selection bias or other biases that may be present and how these are likely to impact results.*

### Ethics oversight

*Identify the organization(s) that approved the study protocol.*

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](https://www.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

Sample size was determined based on previous experience with similar projects and on recommendations of the British Journal of Pharmacology <https://doi.org/10.1111/bph.15868>. No statistical analysis was performed on samples with less than 3 repeated independent measurements. The

### Data exclusions

No data were excluded from electrophysiological and biochemical experiments. In confocal imaging of whole oocytes and giant membrane patches, the predetermined exclusion criterion was signal-to-noise (background) ratio >2. The whole experiment was excluded if this condition was not fulfilled.

### Replication

Experiments presented in the paper were repeated at least twice with oocytes from separate donor frogs, except for Gbg dose-response relationships shown in Supplementary Figures 5 and 6 where one experiment with a large number of oocytes was conducted, as reported in main text and in Figure legends. In each experiment 3 to 18 oocytes were tested in each experimental group. Biochemical experiments such as pulldowns and Western blots were repeated at least 3 times with independent samples (e.g. oocytes from different donors), except Supplementary Fig.11 which shows one of the two replicated experiments.

Randomization	Randomization is not applicable to the kind of experiments performed here.
Blinding	Most experiments were performed by the same person, from sample preparation to analysis, therefore blinding was not applicable. For results of randomly selected experiments, the PI (ND) repeated the analyses of raw data without referring to the results reported by the experimenter. In the vast majority of cases the results were in very good agreement.

## 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
<input type="checkbox"/>	<input checked="" type="checkbox"/> Antibodies
<input checked="" type="checkbox"/>	<input type="checkbox"/> Eukaryotic cell lines
<input checked="" type="checkbox"/>	<input type="checkbox"/> Palaeontology and archaeology
<input type="checkbox"/>	<input checked="" type="checkbox"/> Animals and other organisms
<input checked="" type="checkbox"/>	<input type="checkbox"/> Clinical data
<input checked="" type="checkbox"/>	<input type="checkbox"/> Dual use research of concern
<input checked="" type="checkbox"/>	<input type="checkbox"/> Plants

### Methods

n/a	Involved in the study
<input checked="" type="checkbox"/>	<input type="checkbox"/> ChIP-seq
<input checked="" type="checkbox"/>	<input type="checkbox"/> Flow cytometry
<input checked="" type="checkbox"/>	<input type="checkbox"/> MRI-based neuroimaging

## Antibodies

Antibodies used	All antibodies, with all the required details (manufacturer, catalog #, lot where available, dilution) are reported in the Methods and summarized in Supplementary Table 10.
Validation	All antibodies are commercially available and validated by vendors. In each experiment involving detection of a protein in a sample derived from living cells, the specificity was validated by the absence of signal in cells not expressing the protein under study, and by comparison with purified recombinant proteins such as G-beta.

## Animals and other research organisms

Policy information about [studies involving animals; ARRIVE guidelines](#) recommended for reporting animal research, and [Sex and Gender in Research](#)

Laboratory animals	Female <i>Xenopus laevis</i> frogs, aged 1.5-5 years
Wild animals	None
Reporting on sex	N/A
Field-collected samples	N/A
Ethics oversight	Experiments have been approved by Tel Aviv University Institutional Animal Care and Use Committee (permits #01-20-083 and TAU-MD-IL-2411-174-3).

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

## Plants

Seed stocks	Report on the source of all seed stocks or other plant material used. If applicable, state the seed stock centre and catalogue number. If plant specimens were collected from the field, describe the collection location, date and sampling procedures.
Novel plant genotypes	Describe the methods by which all novel plant genotypes were produced. This includes those generated by transgenic approaches, gene editing, chemical/radiation-based mutagenesis and hybridization. For transgenic lines, describe the transformation method, the number of independent lines analyzed and the generation upon which experiments were performed. For gene-edited lines, describe the editor used, the endogenous sequence targeted for editing, the targeting guide RNA sequence (if applicable) and how the editor was applied.
Authentication	Describe any authentication procedures for each seed stock used or novel genotype generated. Describe any experiments used to assess the effect of a mutation and, where applicable, how potential secondary effects (e.g. second site T-DNA insertions, mosaicism, off-target gene editing) were examined.