***eLife’s* transparent reporting form**

We encourage authors to provide detailed information *within their submission* to facilitate the interpretation and replication of experiments. Authors can upload supporting documentation to indicate the use of appropriate reporting guidelines for health-related research (see [EQUATOR Network](http://www.equator-network.org/%20)), life science research (see the [BioSharing Information Resource](https://biosharing.org/%22%20%5Ct%20%22_blank)), or the [ARRIVE guidelines](http://www.plosbiology.org/article/info%3Adoi/10.1371/journal.pbio.1000412) for reporting work involving animal research. Where applicable, authors should refer to any relevant reporting standards documents in this form.

If you have any questions, please consult our Journal Policies and/or contact us: editorial@elifesciences.org.

**Sample-size estimation**

* You should state whether an appropriate sample size was computed when the study was being designed
* You should state the statistical method of sample size computation and any required assumptions
* If no explicit power analysis was used, you should describe how you decided what sample (replicate) size (number) to use

Please outline where this information can be found within the submission (e.g., sections or figure legends), or explain why this information doesn’t apply to your submission:

No statistical analysis other than standard error of measurement was used to show variance between replicates, so no sample size pre-determination was required. At least n=3 independent experiments were performed for each mutant, which is standard for biochemical assays and sufficient for determining a mean and SEM.

**Replicates**

* You should report how often each experiment was performed
* You should include a definition of biological versus technical replication
* The data obtained should be provided and sufficient information should be provided to indicate the number of independent biological and/or technical replicates
* If you encountered any outliers, you should describe how these were handled
* Criteria for exclusion/inclusion of data should be clearly stated
* High-throughput sequence data should be uploaded before submission, with a private link for reviewers provided (these are available from both GEO and ArrayExpress)

Please outline where this information can be found within the submission (e.g., sections or figure legends), or explain why this information doesn’t apply to your submission:

Data were reproduced in multiple, independent experiments. Each construct was prepared from at least two independent protein preparations. No prep-to-prep variation was observed, which can be visualized in Figures 1d, 2f, 3c, 5-Table S1, 2-S1, and 4-S3. Macroscopic bilayer data was collected from 3-6 independent bilayers, single channel data from at least 9 independent channel fusions, and liposome efflux data from at least 3 independent experiments derived from 2 protein preps. All attempts at replication were successful.

We strove to get at least 3 replicates for each datapoint. Because bilayers sometimes break midway through a titration series, in some cases we repeated titrations and collected more than 3 replicates for a single datapoint.

No data was excluded. We did not remove any outliers.

Information on the number of replicates for each experiment can be found in the figure legends and, for flux assays, Table 5

**Statistical reporting**

* Statistical analysis methods should be described and justified
* Raw data should be presented in figures whenever informative to do so (typically when N per group is less than 10)
* For each experiment, you should identify the statistical tests used, exact values of N, definitions of center, methods of multiple test correction, and dispersion and precision measures (e.g., mean, median, SD, SEM, confidence intervals; and, for the major substantive results, a measure of effect size (e.g., Pearson's r, Cohen's d)
* Report exact p-values wherever possible alongside the summary statistics and 95% confidence intervals. These should be reported for all key questions and not only when the p-value is less than 0.05.

Please outline where this information can be found within the submission (e.g., sections or figure legends), or explain why this information doesn’t apply to your submission:

Data is reported as the mean and SEM of independent measurements. This information can be found in the associated figure legends.

(For large datasets, or papers with a very large number of statistical tests, you may upload a single table file with tests, Ns, etc., with reference to sections in the manuscript.)

**Group allocation**

* Indicate how samples were allocated into experimental groups (in the case of clinical studies, please specify allocation to treatment method); if randomization was used, please also state if restricted randomization was applied
* Indicate if masking was used during group allocation, data collection and/or data analysis

Please outline where this information can be found within the submission (e.g., sections or figure legends), or explain why this information doesn’t apply to your submission:

Samples were not allocated into groups for data collection or analysis since standard error of measurement was the only statistical test used to show variance between replicates

**Additional data files (“source data”)**

* We encourage you to upload relevant additional data files, such as numerical data that are represented as a graph in a figure, or as a summary table
* Where provided, these should be in the most useful format, and they can be uploaded as “Source data” files linked to a main figure or table
* Include model definition files including the full list of parameters used
* Include code used for data analysis (e.g., R, MatLab)
* Avoid stating that data files are “available upon request”

Please indicate the figures or tables for which source data files have been provided:

Source data for Figures 1d, 2a, 2b, 2e, 3c, 3d, 4a, 4c, 4d, 4e, 4f,1-s2, 2-s1, 2-s2, 3-s1, 3-s2, 3-s3, 4-s1,4-s4, 4-s6 are provided in Excel files. Pdb and mtz files have been provided for all structures.