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**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:

Sample size calculations were not performed in this study.

**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)

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For Figure 2C, each measurement with the WT Were-1 was performed at least in triplicate (see Source Data 1 for tabulated values); Were-1 mutants were tested twice.

For Figure 2E, each kinetic run is performed twice.

For Figure 2F, the replicates are shown in Source Data 2.

For Figure 2H, each kinetic run is performed in triplicate.

For Figure 2I, each experiment is performed at least in triplicate; the replicates are shown in Source Data 2.

Figure 2 supplement 1B, each kinetic run is performed twice.

Figure 2 supplement 3, each experiment is performed twice.

Figure 2 supplement 4, each experiment is performed twice.

For Figure 2 supplement 5, experiment was performed in triplicate as biological replicates.

For Figure 3, experiments were performed at least in triplicate as biological replicates.

Figure 3 supplement 2, each experiment is performed twice.

For Figure 3 supplement 3, experiment was performed twice as biological replicates.

**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.

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(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
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No experimental groups were allocated in this study.

**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:

A table of all oligonucleotide, pool, Were-1, and luciferase sequences used in this study is provided as Supplementary Table 1.

Graphed values presented throughout the manuscript are tabulated in Source Data files 1–3.