***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/" \t "_blank)), or the [ARRIVE guidelines](http://www.plosbiology.org/article/info:doi/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.

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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:

For Fig. 1G, we used 5-6 dummy spacers for each 10%-GC increment (10-90%) because we hypothesized that this amount would sufficiently capture the variability of array performance. This information is included in the Materials and Methods section (“Design of short CRISPR arrays (2 gRNAs) for testing effect of GC content of dummy spacer”).

For Figs 2 and Figure 2-figure supplement1, we used all Cas12a/Cas13d variants we could find using the method outlined in the Materials and Methods section (“Multiple sequence alignment of naturally occurring CRISPR sequences”)

**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:

For Fig. 1G, we designed 5-6 dummy spacers for each 10%-GC increment (10-90%) for a total of 51 dummy spacer variants. This information is included in the Materials and Methods section (“Design of short CRISPR arrays (2 gRNAs) for testing effect of GC content of dummy spacer”). Three replicate transfections were analyzed for each array variant. This information is found in the Figure 1 legend.

For Figs 3 and Figure 3-figure supplement 1, we performed three replicate transfections for each experimental condition, as can be seen from the data points in the graphs, where each data point represents one replicate transfection.

For Fig. 4E, three replicates were analyzed for each time point. For the array with a 30%-GC dummy spacer at the 60-minute time point, five replicates were performed. This information is contained in the Fig. 4 legend.

For Fig. 5B, two biological replicates and three technical replicates were used.

**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:

All statistical analysis methods are reported in the Materials and Methods section (e.g., “Computation of GC content in sliding window”, “Calculation of the predictive power of spacer GC content”, “Multiple sequence alignment of naturally occurring CRISPR sequences”, “Cas12a cleavage assay”)

(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

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Sample allocation into experimental groups was not performed 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:

Source data for figures 1G, 1L, 1M, 2A, 3D, 3F, 4A, 5B and Figure 1-figure supplement 1C are included in the separate source data files.