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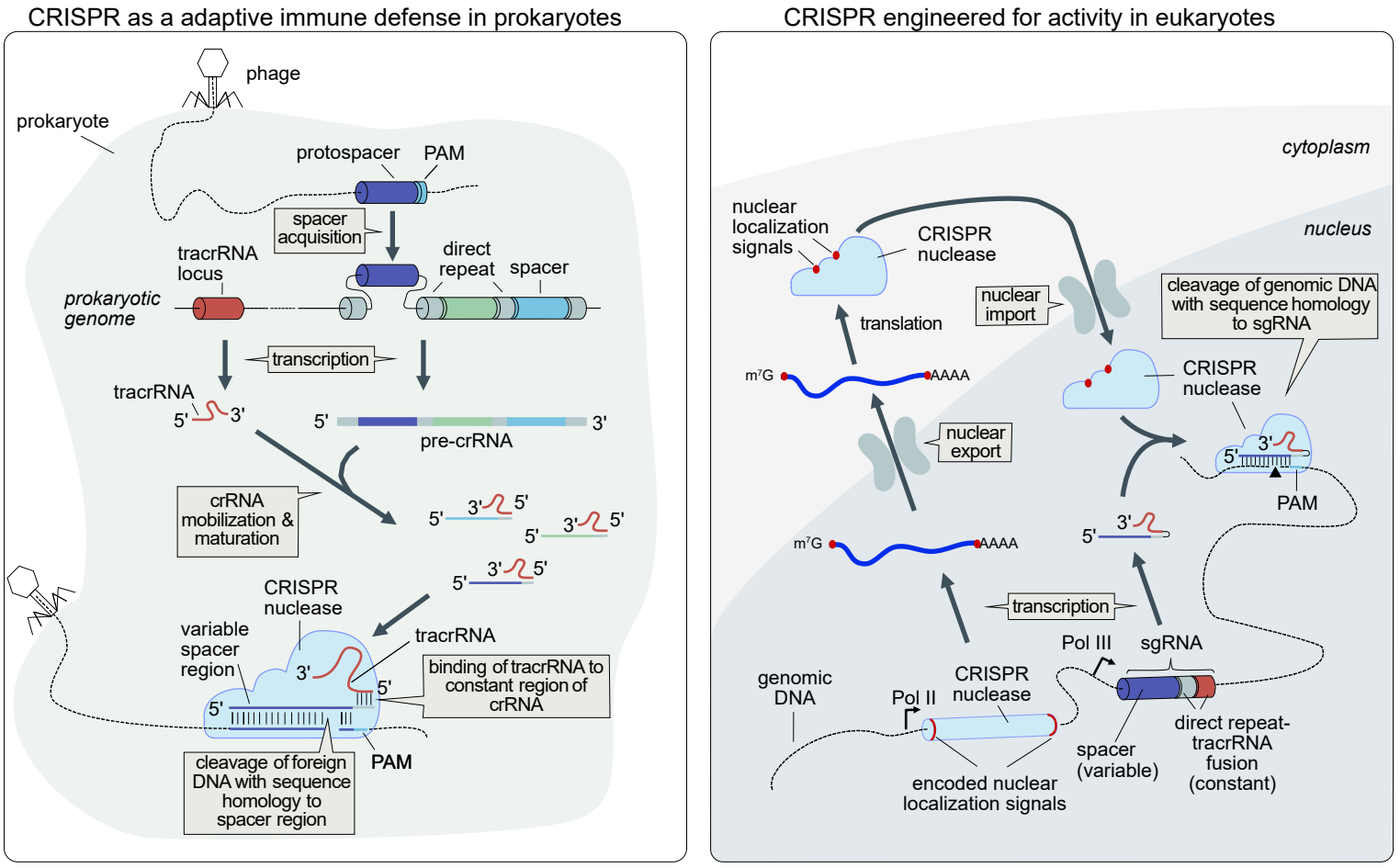
Supplementary information

Tutorial: design and execution of CRISPR in vivo screens

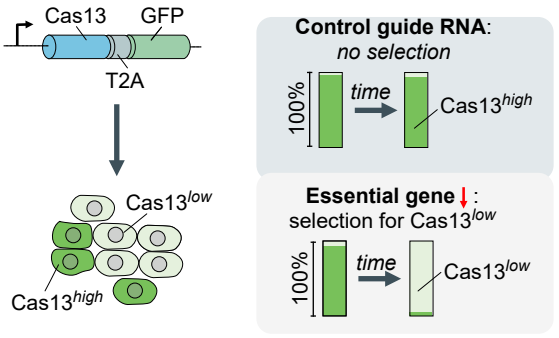
In the format provided by the authors and unedited

Supplementary Figure 1



Supplementary Figure 1: Repurposing prokaryotic CRISPR systems for *in vivo* genome editing in eukaryotes. *Left panel:* In prokaryotes, CRISPR components function as an adaptive immune response mechanism: Protospacers are cleaved out from invading DNA molecules by specific nucleases and become part of a CRISPR array to form a lasting immunological memory. They can later be re-mobilized and contribute to adaptive immune defence. In *S. pyogenes*, after transcription individual crRNAs are mobilized from a pre-crRNA molecule, bind a tracrRNA and form a complex with Cas9. This complex can then bind and cleave foreign DNA molecules with a protospacer adjacent motif (PAM) and sequence homology to the spacer region. *Right panel:* The repurposing of CRISPR for genome editing in eukaryotes requires the expression of a CRISPR nuclease and an sgRNA in a eukaryotic cell. SgRNA-nuclease complexes bind and cleave DNA sequences with a protospacer adjacent motif (PAM) and sequence homology to the sgRNA.

Supplementary Figure 2



Supplementary Figure 2: High nuclease expression boosts functional phenotypes. The probing of functional phenotypes with CRISPR typically requires a high nuclease expression level in eukaryotic cells. Here, the CRISPR nuclease Cas13 tagged with the fluorophore GFP was delivered to cells and is found to show heterogeneous levels of expression across cells. The amount of fluorescence acts as a surrogate marker for Cas13 expression levels. When a crRNA, which elicits a Cas13-mediated knockdown of an essential gene (red arrow), is delivered to this mixed population, cells with a higher expression level of Cas13 ($Cas13^{high}$) exhibit a bigger fitness defect than those with lower Cas13 expression levels ($Cas13^{low}$). If this experiment is repeated with a non-targeting crRNA control, Cas13 expression levels have no influence on cellular fitness. This experiment highlights two important prerequisites for screens: (i) The extent of a phenotype depends on nuclease expression levels, therefore an even nuclease expression across cells reduces unwanted selection effects, (ii) The overall nuclease expression level should be high for strong phenotypes.

Suppl. Table 1 | Comparison of the three Class 2 nuclease families Cas9, Cas12 and Cas13

Quality	Cas9	Cas12	Cas13
Separate tracrRNA required?	Yes	Subtype-specific (not for Cas12a)	No
Artificial RNA to circumvent tracrRNA requirement	SgRNA	Not necessary / sgRNA	Not required
Nucleic acid substrate	DNA	DNA	RNA
PAM	Subtype-specific (5'-NGG for SpCas9)	Subtype-specific (5'-TTTV, with V = A, C or G for Cas12a)	No PAM, but PFS for some subtypes (none for Cas13d)
Size of nuclease [AAs]	1300s (SpCas9)	1300s (Cas12a)	900s (Cas13d)
Type of induced double strand break	Blunt end	5 nt 5' overhang	No double-strand breaks induced
Spacer length	~20 nt	~20-23 nt	~20-23 nt
Total guide length (artificial sgRNA, when tracrRNA required)	~100 nt	40-50 nt	50-60 nt
Intrinsic multiplexing capability (nuclease can mobilize individual crRNAs from multiplexed precrRNA)?	No	Yes	Yes
DNA editing	All 12 conversions possible: CBEs (C→T) ABEs (A→G) Prime editing C-to-G base editors	Only 4 transitions possible so far: CBEs (C→T) ABEs (A→G)	No
Epigenome editing	Yes	Yes	No
RNA editing	Adenosine deamination: A→I (functionally: A → G)	No	Adenosine deamination: A→I (functionally: A → G) Cytosine deamination: C→U
Epitranscriptome editing	m6A methylation m6A demethylation	No	m6A methylation m6A demethylation