Staphyloccocus aureus Cas9:

an alternative Cas9 for genome editing applications

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Summary

S. aureus Cas9:

- Is encoded by 3159 nucleotides, compared to 4104 nucleotides for *S. pyogenes* Cas9.
- Recognizes an NNGRRT PAM.
- Cleaves dsDNA with efficiency comparable to *S. pyogenes* Cas9.
- Cleaves dsDNA with gRNAs of different spacer lengths, with shorter guides generally less tolerant of sequence mismatches.

Cleavage efficiency depends on gRNA spacer length

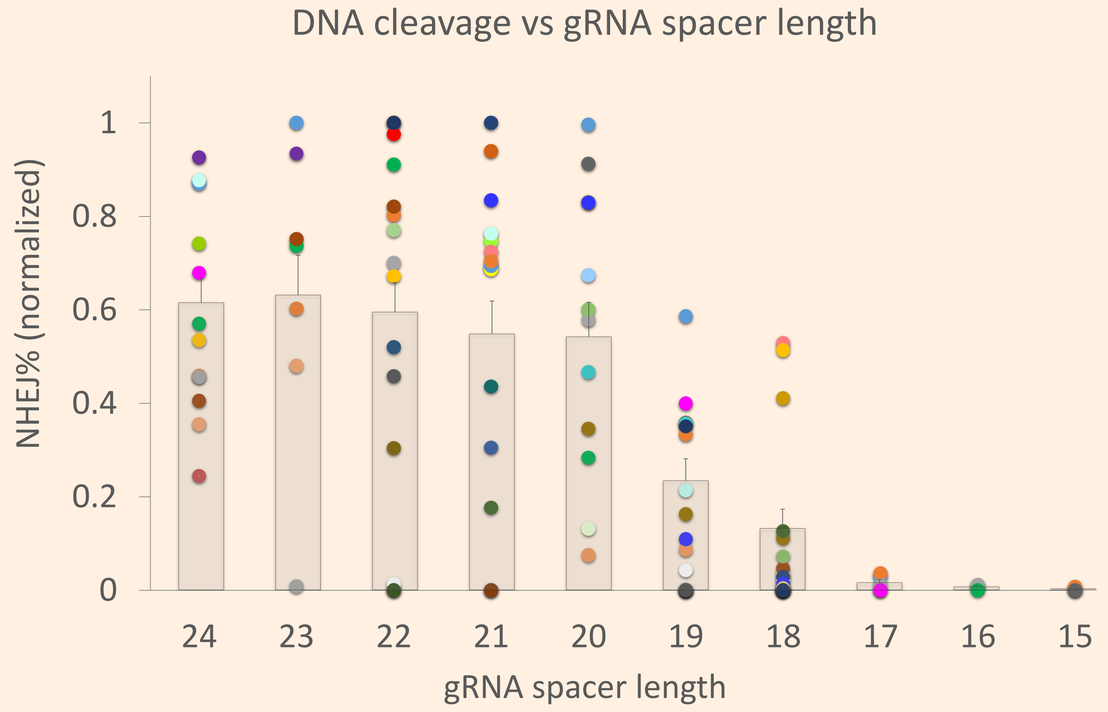

Figure 2. *S. aureus* Cas9 cleavage of target DNA with "sibling" gRNAs, which have spacers of different lengths but target the same precise locus. Same-colored dots represent gRNA siblings, all of which initiate with a G for optimal U6 promoter expression. Data points come from multiple experiments, with maximum cleavage in each experiment set to 1 and all other data points normalized. Grey bars represent mean cleavage for gRNAs of that length.

S. aureus Cas9 cleavage efficiency is comparable to *S. pyogenes* Cas9

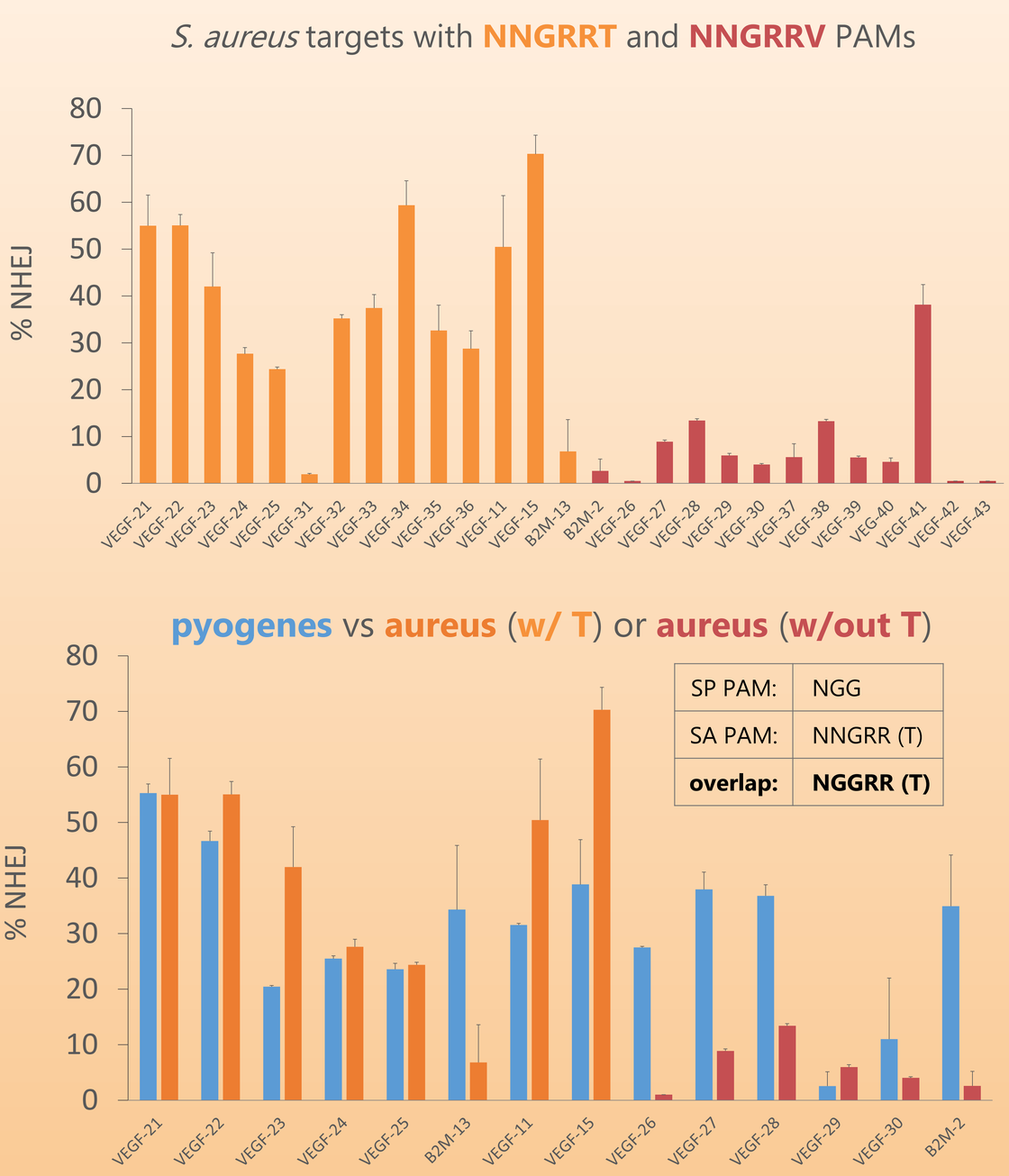

Figure 1. *(Above) S. aureus* Cas9 with gRNAs targeting loci with different PAMs. NHEJ % on the Y axis represents on-target cleavage rates as measured by T7E1 assay. (*Below*) Comparison of *S. aureus* Cas9 and *S. pyogenes* Cas9 DNA cleavage at identical target sites, with dual-compatible NNGRR(T) PAMs.

Shorter gRNAs are generally less tolerant of mismatches

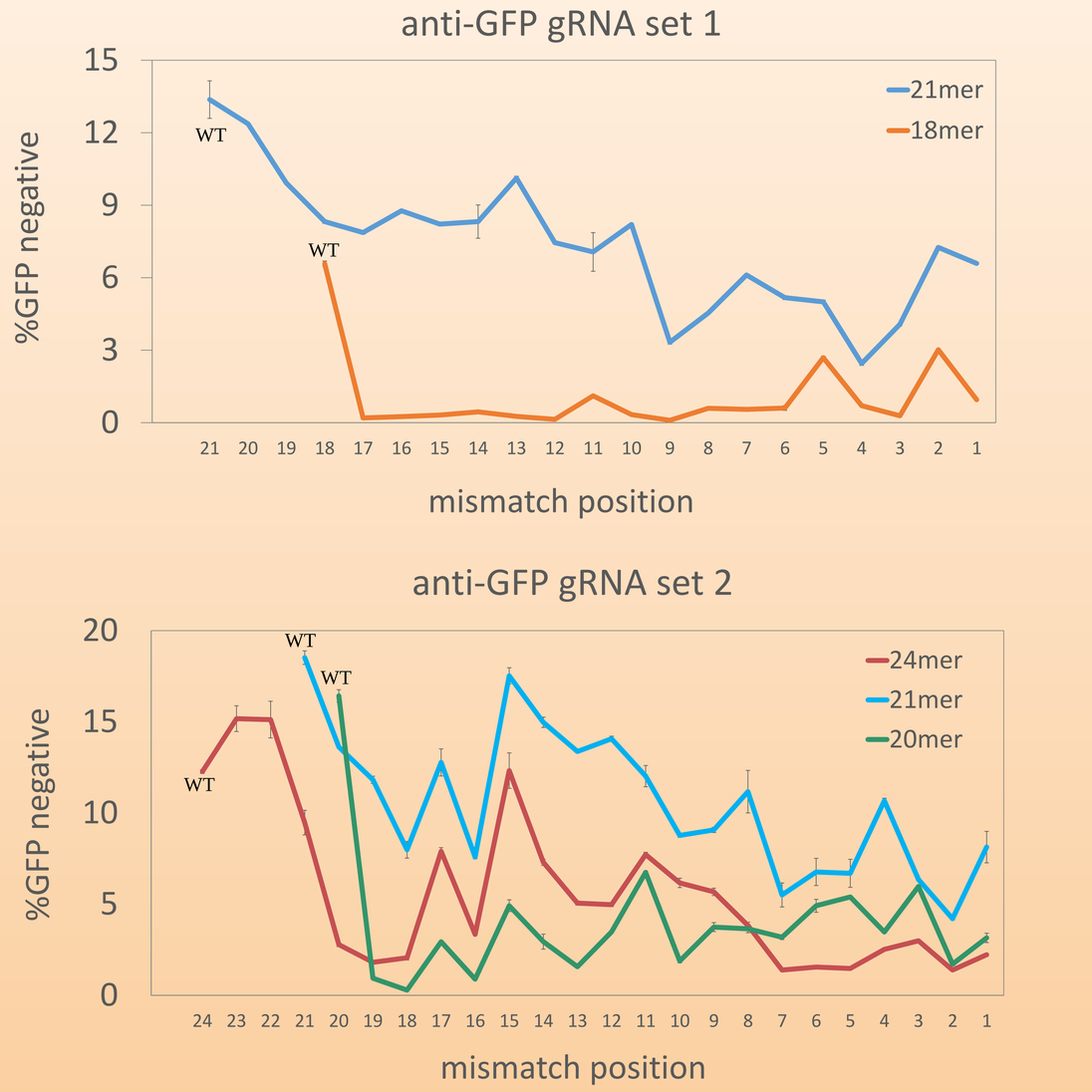

Figure 3. Knockdown of GFP with *S. aureus* Cas9 and gRNAs containing single mismatches. WT (wild type) gRNAs are perfect matches to the target sequence. *(Above)* gRNAs targeting GFP site 1 with spacer lengths of 21 and 18 bases. (Below) gRNAs targeting GFP site 2 with spacer lengths of 24, 21, and 20 bases.

Methods: All experiments were performed via transfection into HEK293T cells using Lipofectamine 3000 in 24-well format. Transfections consisted of 750 ng/well of Cas9 (driven by CMV promoter) plasmid and 250 ng/well of gRNA (driven by U6 promoter) construct. For most of these experiments, total DNA was harvested from cells 72 hours post transfection, and NHEJ % was determined by target locus PCR followed by T7E1 cleavage assay. Alternatively, for assays involving fluorescence, GFP knockdown was measured 3.5 days post transfection.