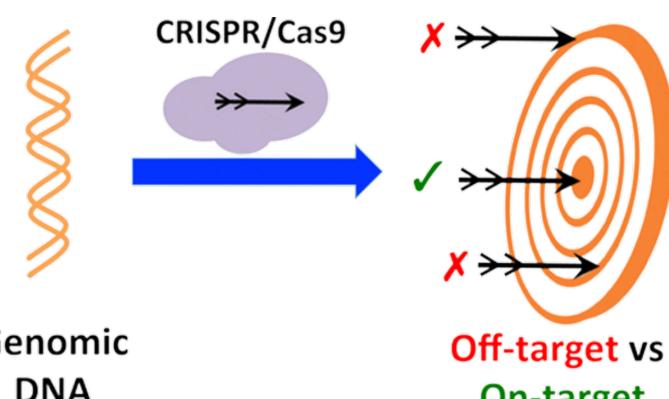


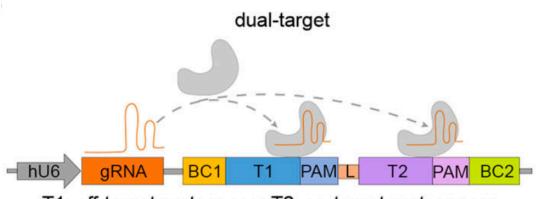
From beginner to expert



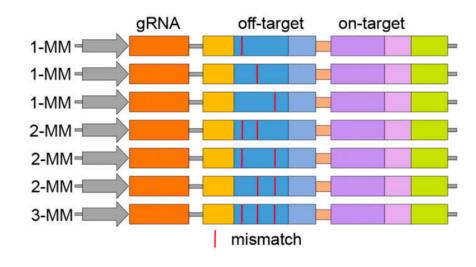
Genomic DNA

On-target Editing

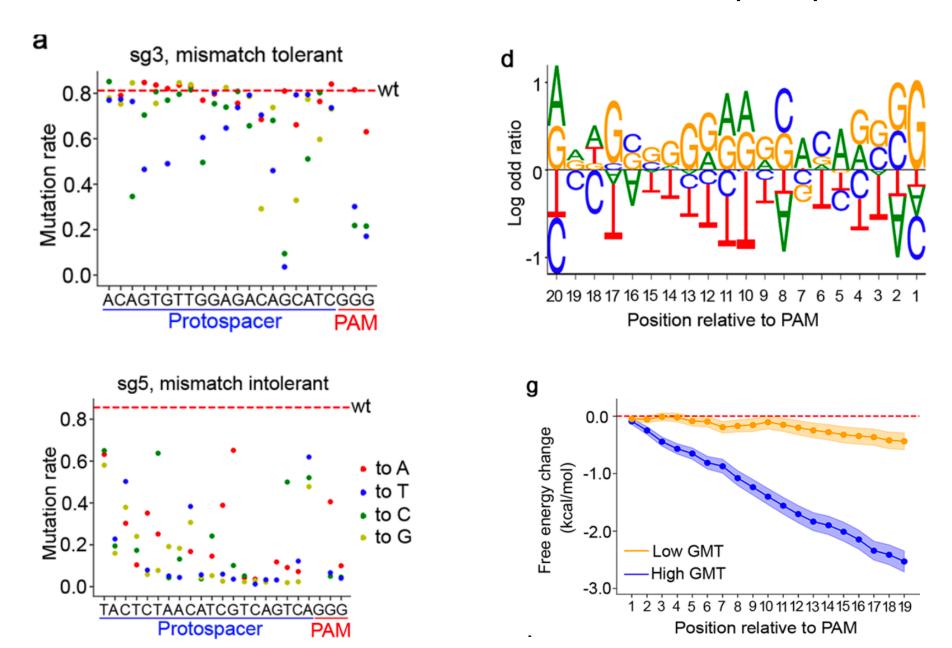
Experimental design for CRISPR off-target study



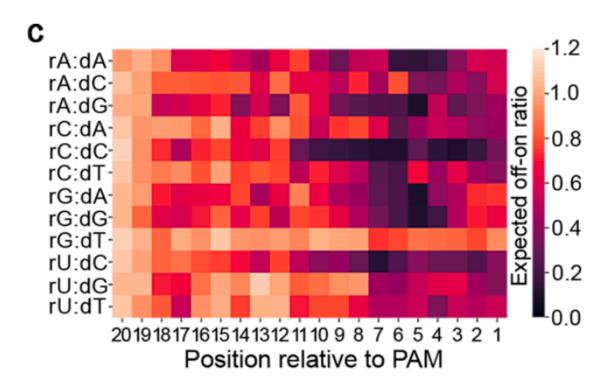
T1: off-target protospacer T2: on-target protospacer BC: barcode L: linker



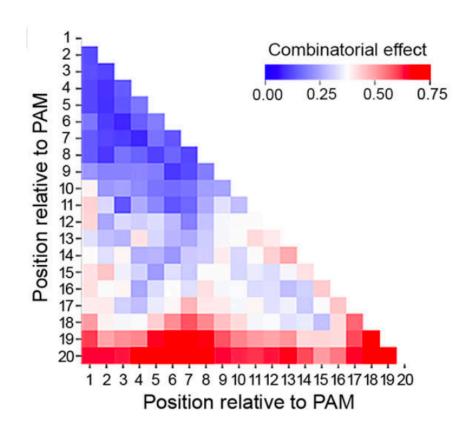
Guide-intrinsic mismatch tolerance (GMT)

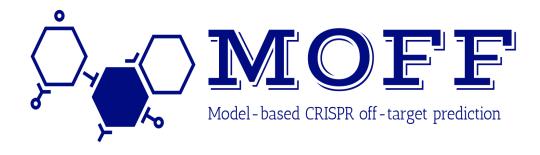


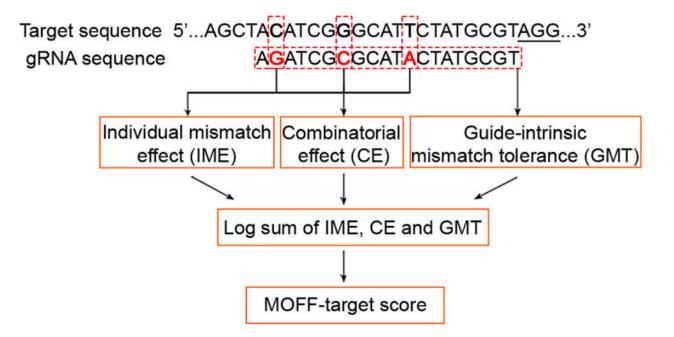
Single-mismatch effects

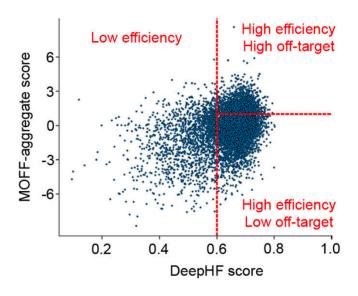


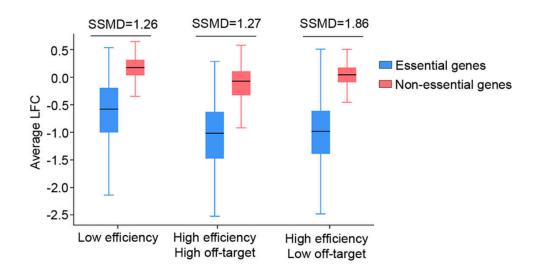
Combinatorial effects







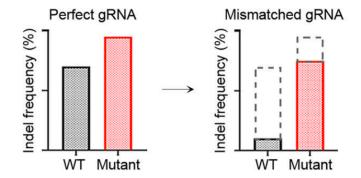


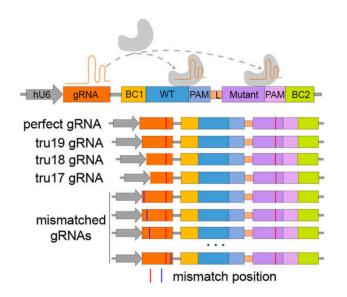


Allele-specific knockout using mismatched sgRNA

KRAS WT: GTAGTTGGAGCTGGTGGCGT<u>AGG</u>CAA KRAS G12D: GTAGTTGGAGCTG<mark>A</mark>TGGCGT<u>AGG</u>CAA

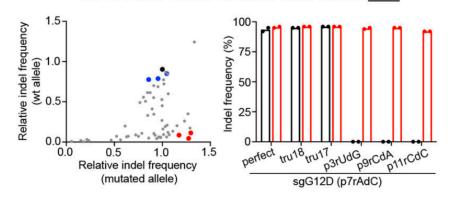
Perfect gRNA: GTAGTTGGAGCTGATGGCGT Mismatched gRNA: GTAGTTGGAGCCGATGGCGT







KRAS WT: GTAGTTGGAGCTGGTGGCGTAGG KRAS G12D: GTAGTTGGAGCTGATGGCGTAGG



- perfect gRNA
 truncated gRNA
- validated mismatched gRNAs
- other mismatched gRNAs
- ☐ WT allele ☐ Mutated allele

p53 WT: GCATGGGCGGCATGAACCGGAGG p53 R248W: GCATGGGCGGCATGAACTGGAGG

