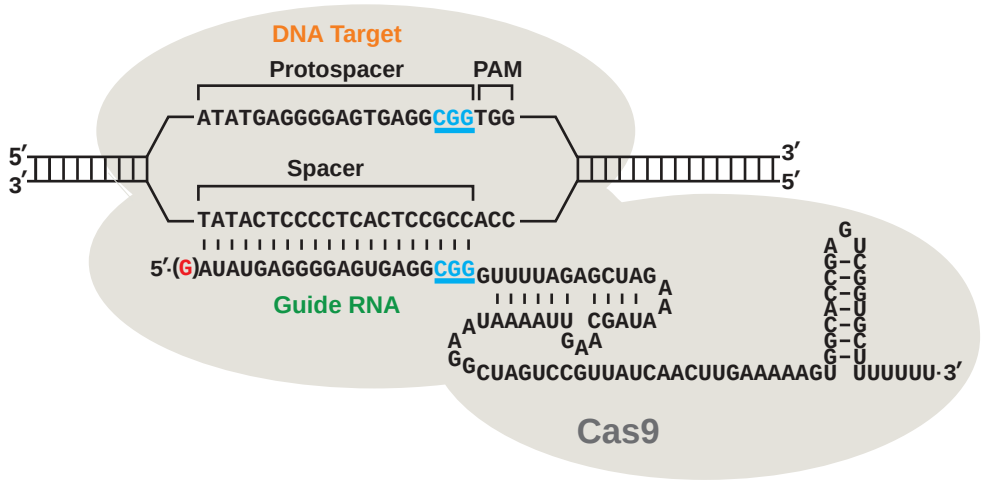
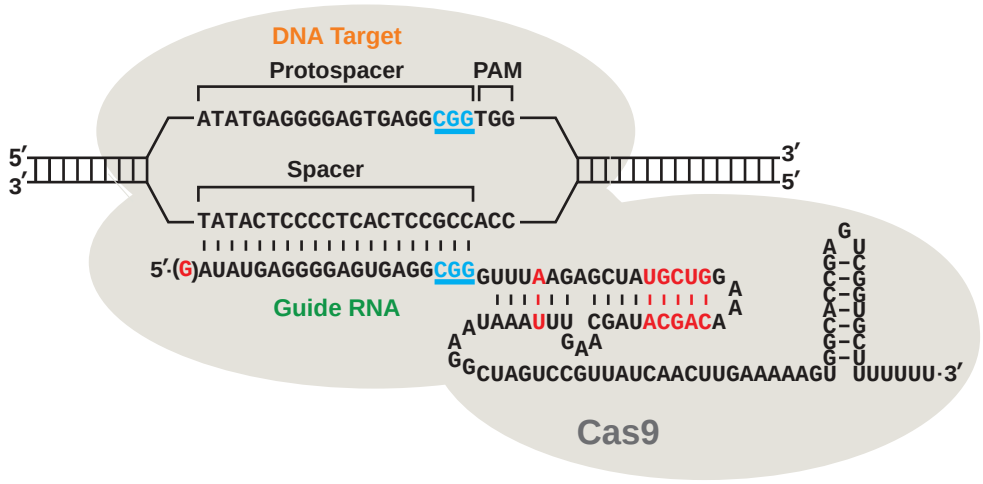
A



B



C

fox-1 guides	F_1s with transformation markers	Heterozygous F_1 mutants	Mutagenesis rate (%)
Guide (1)	38	11	29
Modified Guide (1)	94	23	25
Guide (2)	62	8	23
Modified Guide (2)	57	8	14
Guide (3)	94	20	21
Modified Guide (3)	76	18	23

Figure S1 Alternate guide RNA design with more extensive stem-loop structures does not enhance the Cas9 mutagenesis frequency achieved by 3' GG guide RNAs. (A-B) Shown are diagrams of two related guide RNAs bound in a complex with Cas9 to the same DNA target site. The 3' GG guide RNAs target Cas9 to sites with an NGG motif at the 3' end of the protospacer. The modified guide has the same target specificity as the 3' GG guide, but has a longer stem-loop structure, and an A-U flip that improved its efficacy in mammalian cell Cas9 experiments (Chen *et al.* 2013). (C) The addition of the longer stem-loop structure to three different 3' GG guide RNAs corresponding to three different targets in *fox-1* did not improve the frequency of mutagenesis directed by Cas9. Experiments were conducted as those presented in Figure 2.