



Figure S4 *in vitro* DNA cleavage assays comparing the effectiveness of Cas9-crRNA: tracrRNA and Cas9-sgRNA complexes. Double-stranded DNA cleavage was tested in time course reactions using the molar ratio of Cas9 : guide RNA : target DNA as 0.5 μ M : 1 μ M : 0.5 μ M. Reactions were conducted at 37°C. The *in vitro* assays show that the dual RNA guides are more effective at promoting DNA cleavage than the sgRNAs.

(A) DNA cleavage assays for *gfp*. The target binding sequences in the *gfp* RNA guides are the following: crRNA7, 5' AAAGGGCAGAUUGUGUGGAC 3' and crRNA9, 5' GUGGACAGGUAAUGGUUGUC 3'. M, 100-bp markers.

(B) DNA cleavage assays for *rex-8*. The target binding sequences in the *rex-8* RNA guides are the following: crRNA5, 5' GGUGGAUAAAAUAAUUGAGC 3' and crRNA6, 5' UGGAUAAAAUAAUUGAGCGG 3'. The asterisk shows the location of Cas9-bound DNA in the gel.