Supplemental Data

Rationally Designed Anti-CRISPR Nucleic Acid Inhibitors of CRISPR-Cas9

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Table S1: Sequence of RNA and DNA Oligonucleotides

Guide RNAs and Oligonucleotides	Sequence (lowercase = RNA, uppercase = DNA)
crE2	uaccagcaaaacacuccgauguuuuagagcuaugcuguuuug
crTR	gcagagccagccuucuuauuguuuuagagcuaugcuguuuug
crEGlP	gggcgaggagcuguucaccgguuuuagagcuaugcuguuuug
tracrRNA	aaacagcauagcaaguuaaaauaaggcuaguccguuaucaacuugaaaaaguggcaccgagucggugcuu
sgEGlP	gggcgaggagcuguucaccgguuuuagagcuagaaauagcaaguuaaaauaaggcuaguccguuaucaacuugaaaaaguggcaccgagucggugcu
T7 promoter oligo	TAATACGACTCACTATA
T7_tracrRNA_as	AAGCACCGACTCGGTGCCACTTTTTCAAGTTGATAACGGACTAGCCTTATTTTAACTTGCTATGCTGTTTCCCTATAGTGAGTCGTATTA
T7_sgEGlP_as	AAGCACCGACTCGGTGCCACTTTTTCAAGTTTGATAACGGACTAGCCTTATTTTAACTTGCTATTTCTAGCTCTAAAACCGGTGAACAGCTCCTCGCCCTATAGTGAGTCGTATTA
pEGlP_vitro targ_F	ATGCGATGGAGTTTCCCCA
pEGlP_vitro targ_R	CCGCTTTACTTGTACAGCTCG

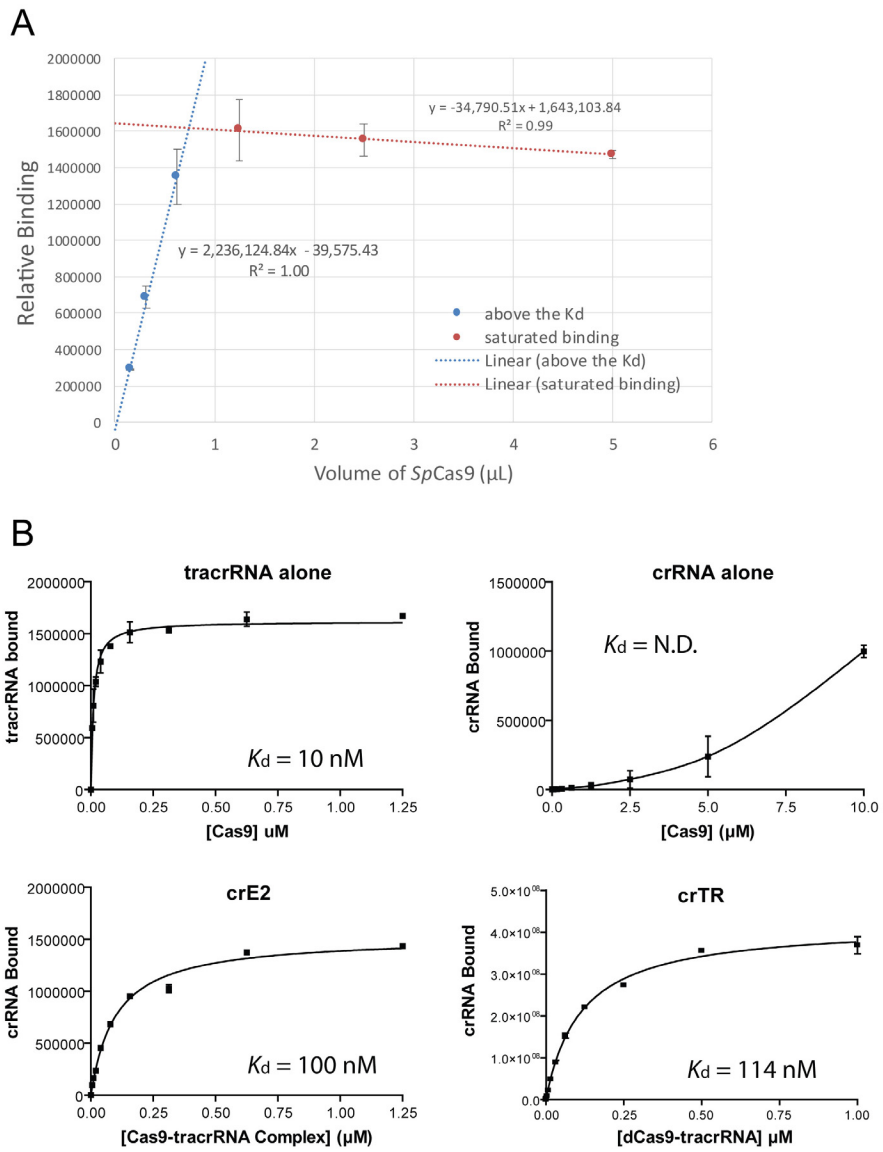

Figure S1. Determining the active concentration of Cas9 and its binding affinity to native dual-RNA guides. (**A**) Cas9 enzyme stocks were titrated with a fixed concentration of tracrRNA-crRNA complex (0.5 µM). Active concentration of Cas9 was determined by solving for x when the equations of the two lines were set equal to each other, as described in Methods. (**B**) Binding of Cas9 to tracrRNA or Cas9-tracrRNA complex to crRNA. Binding affinities were calculated by non-linear regression and fitting to a one-site binding curve. Error bars are standard error of the mean (s.e.m.).

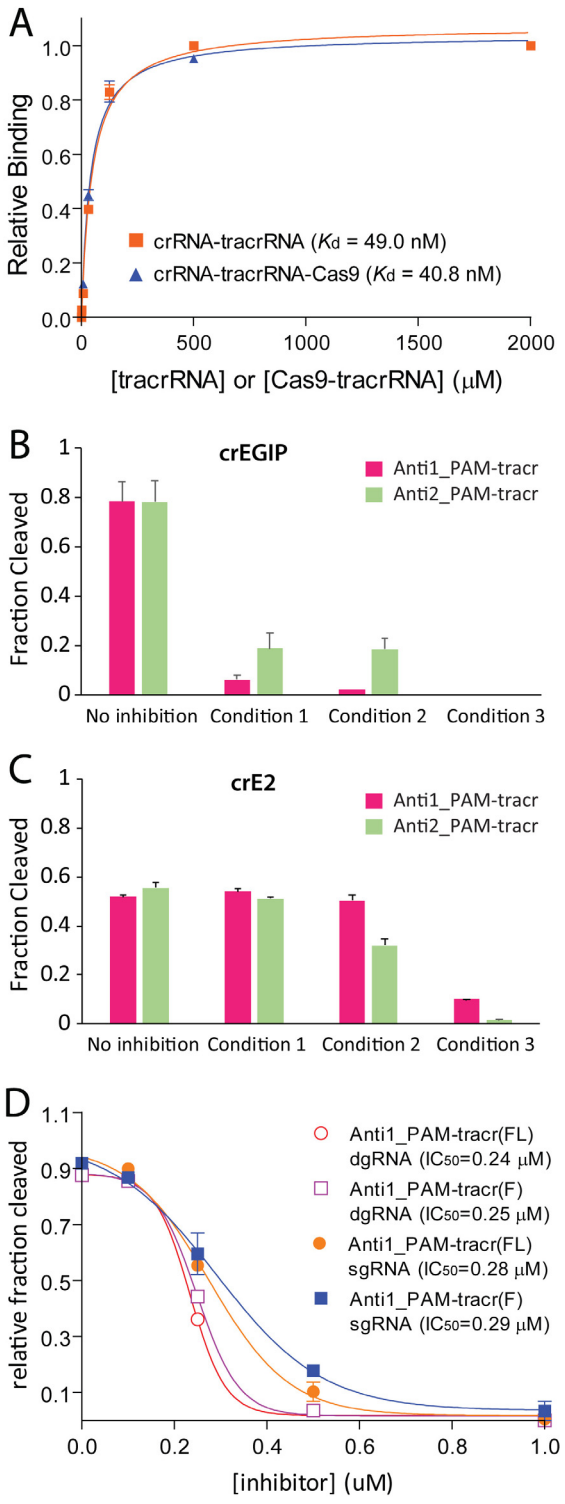

Figure S2. (**A**) Quantification of gel shift analyses comparing crRNA binding and affinity to Cas9-tracrRNA complex or tracrRNA alone. (**B-C**) Testing of different conditions for inhibitor testing in in vitro cleavage assays. Condition 1: Pre-assemble RNP complex, then mix with target and inhibitor, Condition 2: Pre-assemble RNP, incubate with inhibitor, then mix with target, Condition 3: Pre-incubate inhibitor with Cas9-tracrRNA complex, then mix with crRNA and target. (**D**) Quantification of dgRNA and sgRNA Cas9 inhibition. IC_{50} values were calculated by fitting to a variable slope dose-response inhibition curve. All error bars are standard error of the mean (s.e.m.).