

## Supplementary Tables

**Table S1.** Sequence of target strand of DNA duplexes used as substrates for Cas9/sgRNA or crRNA:tracrRNA cleavage.

Name	Structure (5'-3')
DNA_target strand (531nt)	TTGTGTCTTCGTCAGTCCCGAGTCCCTTCATGGCTTTCCGC AGCGCTTTGGCATCTGCGTCAGGGTTGAAGTCATTGGCTG GGCGCACAGTTCCCTTCAGCTCTACTCGGGCCACTGCACT AAGTTCCCACATCTGATAGGCCACCTGCGCTGCCTCCGGG AAGAACTGGCCAGCAGCATCATCTCCCCCAGACAGCT TCAGCAGAGTCTTCTTGTACTCGCCAGAGGTGTCATTCTT GATCATGCTGTAGAGGGACTTCTCATACTTGGTCCGGAAG ATCT <b>CCC</b> <u>GAA</u> TGTCGAGCATGTCCA <u>ACT</u> CACTACGGGAG ACCATGATGCGGATCAGGGTGTGTCCCGAGTCCCCAGGC CCTTCATAGCCTTGAAGAGCCTTTCAGCAAAATATTCCGG GGTGCTCCGGATACACTTCACTACGGCCAGCATTAGCTTC TCAAAGTCCCCAGACAGCTCCCCTCGGATGCTGGCTTCAA TCGGCTTCCCTGTGGTCTTCAGATACTCATCGAACACCAA CCGAAGATGC

The spacer in the DNA duplex is underlined and the PAM is in red and bold.

**Table S2.** Amplification primers used to generate the fluorescently labeled DNA duplexes.

Name	Structure (5'-3')
F_ANX	gcatcttcggttggtgttcg
R-FAM_ANX_target strand	FAM-ttgtgtcttcgtcagtcceg
F_ANX_mismatch	aatattttgctgaaaggctcttcaa

**Table S3.** Calculated P-values for the specificity experiment (for Figure 3A, B).

Name of sample	P-value
sgRNA_m1Ψ_WT	0,00058
sgRNA_m1Ψ_S1	0,00014
sgRNA_m1Ψ_S2	0,00053
sgRNA_m1Ψ_S3	0,00003
sgRNA_m1Ψ_S4	0,00005
sgRNA_m1Ψ_S5	0,00042
sgRNA_m1Ψ_S6	0,00000
sgRNA_m1Ψ_S7	0,00056
sgRNA_m1Ψ_S8	0,00004
sgRNA_m1Ψ_S9	0,00036
sgRNA_m1Ψ_S10	0,00001

sgRNA_m1Ψ_D9,10	0,00011
sgRNA_m1Ψ_D11,12	0,00007
sgRNA_m1Ψ_D13,14	0,00146
sgRNA_m1Ψ_D15,16	0,00055
sgRNA_m1Ψ_D17,18	0,00063
sgRNA_m1Ψ_D19,20	0,00020

**Table S4.** Sequences of mutant DNA substrates used for the experiments determining specificity of the CRISPR-Cas9 system.

Name; S- single-point mismatch; D- double-point mismatches	Sequence, spacer region (5'-3')
WT	GAATGTCGAGCATGTCCAAC
S1	<u>C</u> AATGTCGAGCATGTCCAAC
S2	G <u>T</u> ATGTCGAGCATGTCCAAC
S3	GAT <u>T</u> GTCGAGCATGTCCAAC
S4	GAA <u>A</u> GTCGAGCATGTCCAAC
S5	GAAT <u>C</u> TCGAGCATGTCCAAC
S6	GAATG <u>A</u> CGAGCATGTCCAAC
S7	GAATGT <u>G</u> GAGCATGTCCAAC
S8	GAATGTCC <u>A</u> GAGCATGTCCAAC
S9	GAATGTCG <u>T</u> GAGCATGTCCAAC
S10	GAATGTCGAC <u>C</u> ATGTCCAAC
D9,10	GAATGTCG <u>TCC</u> ATGTCCAAC
D11,12	GAATGTCGAG <u>GTT</u> GTCCAAC
D13,14	GAATGTCGAGCA <u>ACT</u> CCAAC
D15,16	GAATGTCGAGCATG <u>AG</u> CAAC
D17,18	GAATGTCGAGCATGTC <u>GT</u> AC
D19,20	GAATGTCGAGCATGTCCAT <u>G</u>

Mismatches in DNA duplexes are underlined.