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Supplemental Information

sgRNA Sequence Motifs Blocking Efficient CRISPR/Cas9-Mediated Gene Editing

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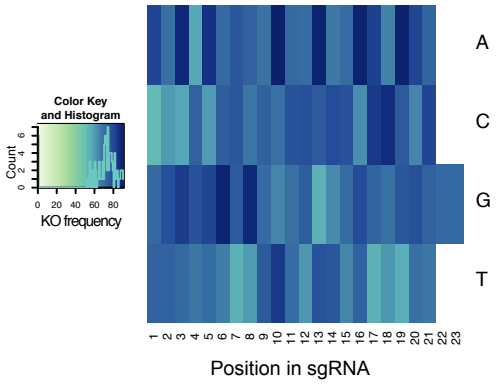
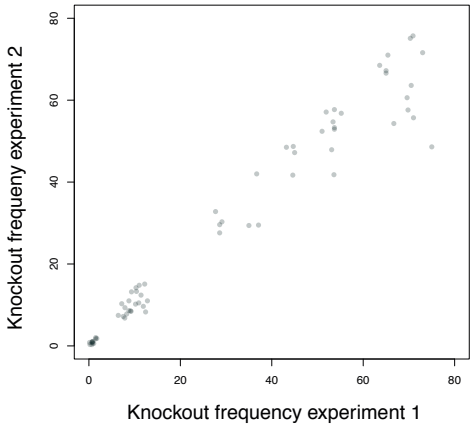
Supplementary Figures

A



B



C

sgRNA sequence (with PAM)	Percent GFP knockout					
	Cl1ex1	Cl2ex1	Cl3ex1	Cl1ex2	Cl2ex2	Cl3ex2
GAGGCGTCATCGATGACCGA TGG	◔ 53.7	◔ 66.7	◔ 55.2	57.7	54.3	56.8
GAGGCGTCATCGATGACCGT TGG	◔ 45.0	◔ 53.6	● 43.2	47.2	41.8	48.5
GAGGCGTCATCGATGACCGG TGG	◔ 70.3	◔ 75.0	● 69.8	75.1	48.6	57.6
GAGGCGTCATCGATGACCGC TGG	◔ 37.1	◔ 35.0	● 28.6	29.5	29.4	27.6
GAGGCGTCATCGATGATTGG TGG	◔ 65.4	◔ 69.6	● 65.0	71.0	60.6	66.6
GAGGCGTCATCGATGATGTG TGG	◔ 70.9	◔ 73.0	◔ 71.0	75.7	71.6	55.7
GAGGCGTCATCGATGAGGTG TGG	◔ 65.0	◔ 70.5	◔ 63.6	67.2	63.6	68.5
GAGGCGTCATCGATGACTTA TGG	◔ 7.8	◔ 6.4	● 7.5	6.8	7.5	7.2
GAGGCGTCATCGATGACCTT TGG	◔ 12.8	◔ 12.2	● 11.0	11.0	15.1	14.8
GAGGCGTCATCGATGATTTT TGG	◔ 0.2	◔ 0.7	◔ 0.1	0.3	0.3	0.8
GAGGCGTCATCGATGATTTG TGG	◔ 10.4	◔ 10.9	● 10.2	13.3	10.5	10.2
GAGGCGTCATCGATGATTTC TGG	◔ 0.8	◔ 0.7	● 1.1	0.7	0.8	0.6
GAGGCGTCATCGATGATTTA TGG	◔ 1.5	◔ 1.5	● 1.7	1.7	2.0	1.8
GAGGCGTCATCGATGACTTT TGG	◔ 0.7	◔ 0.9	● 0.7	1.1	1.1	0.9
GAGGCGTCATCGATGATCTC TGG	◔ 7.9	◔ 7.2	◔ 8.8	9.3	10.3	8.6
GAGGCGTCATCGATGAGCCA TGG	◔ 53.7	◔ 51.9	● 53.1	52.9	57.1	47.9
GAGGCGTCATCGATGAGCCG TGG	◔ 44.6	◔ 44.7	◔ 36.7	41.7	48.7	42.0
GAGGCGTCATCGATGAGCCC TGG	◔ 51.0	◔ 53.4	◔ 53.7	52.4	54.7	53.3
GAGGCGTCATCGATGAGCCT TGG	◔ 8.3	◔ 8.8	● 9.2	7.8	11.0	8.4
GAGGCGTCATCGATGAGGCC TGG	◔ 11.4	◔ 11.9	● 12.4	12.4	9.7	8.3
GAGGCGTCATCGATGATGCC TGG	◔ 9.1	◔ 9.3	● 10.3	8.5	13.2	14.2
GAGGCGTCATCGATGAGTCC TGG	◔ 28.6	◔ 27.7	● 29.1	29.6	32.8	30.3

◔ Heterozygous knock-in ● Homozygous knock-in

D

Figure S1. Related to Figure 1, sgRNA sequence motifs blocking efficient gene editing.

(A) Heatmap of the average knockout (KO) frequencies per nucleotide per position as detected in the surface marker knockout screen reported in Chu *et al.* (Chu et al., 2016). Positions 21-23 correspond to the Protospacer Adjacent Motif (PAM). (B) Dotplot of GFP knockout frequencies measured by flow cytometry in two independent experiments. Each dot corresponds to the knockout frequency measured in one sub-cloned cell line eight days post transduction with the matching sgRNA. (C) GFP KO frequencies (in %) eight days post transduction in three sub-clones (Cl) with the indicated sgRNAs in experiment (ex) 1 and experiment 2. Homozygous and heterozygous clones are annotated with filled and semi-filled circles,

respectively. TT- and GCC-motifs are highlighted in orange. (D) Mutation analysis of heterozygous clones with the indicated target sequences eight days post transduction with the respective sgRNAs. The indicated cell populations were sorted and the loci were analyzed using PCR-fragment sub-cloning and Sanger sequencing. Big deletions (≥ 100 nts) were grouped with out-of-frame indels.

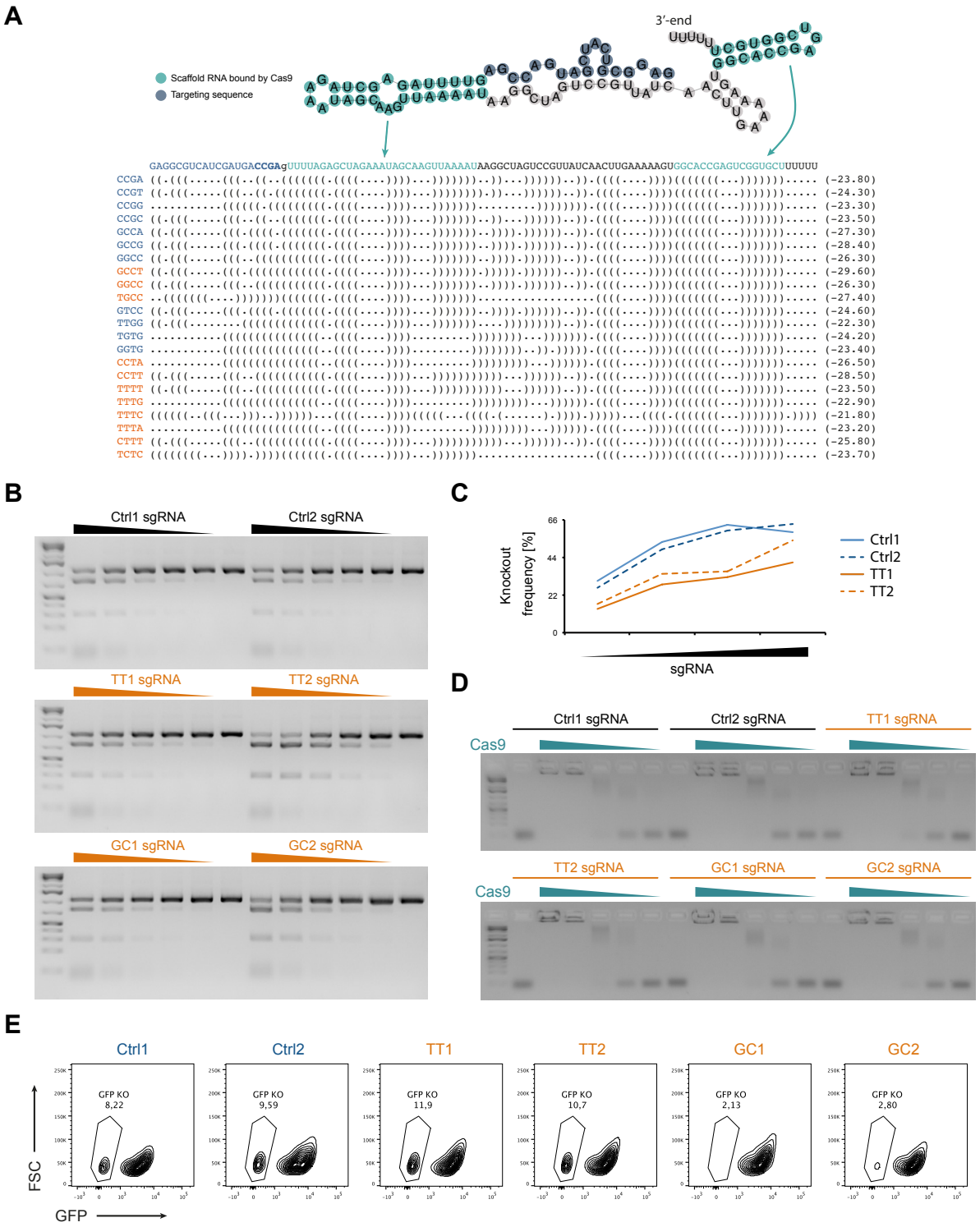


Figure S2. Related to Figure 2, mechanism of sequence motifs in sgRNAs blocking efficient gene editing.

(A) RNA secondary structures of the sgRNAs based on the RNAfold algorithm (Vienna package, Lorenz et al., 2011). The Paired nucleotides are shown as brackets. The key structures from the sgRNA are labeled in turquoise. (B) *In vitro* cleavage titration experiment. Decreasing amounts of RNPs with the indicated sgRNAs (1x, 0.5x, 0.25x, 0.125x, 0.063x, 0x) were incubated with the respective target DNA. The bands two and three (from top) correspond to the cleaved products. (C) Knockout efficiency based on increasing doses (1x, 2x, 4x, 6x) of the indicated synthetic sgRNAs eight days post electroporation. (D) Cas9 loading experiment. Decreasing amounts of Cas9 (0x, 4x, 2x, 1x, 0.5x, 0.25x) were incubated with the same amount of the indicated sgRNAs. The lower band corresponds to the sgRNA. (E) RNP-mediated knockout *in vivo*. The indicated RNPs were electroporated into the respective cell lines and the knockout frequency was assessed four days post electroporation. Data are representative for two independent experiments.