Supplementary Information

Simple knockout by electroporation of engineered endonucleases into intact rat embryos

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Supplementary Table S1. Germ-line transmission in offspring that were derived from embryos with *Il2rg*-targeted ZFN mRNA.

		Offspring		Offspring with an edited target gene	
Fathers	Mothers	Males	Females	Males	Females
G0 Δ13/Y	+/+	8	5	0	5
G0 Δ7/Y	+/+	7	5	0	5
G0 Δ5/Y	+/+	6	4	0	2
+/Y	F1 Δ13/+	5	6	3	3
+/Y	F1 Δ13/+	9	1	5	1

Supplementary Figure Legends

Supplementary Figure S1. ZFN and TALEN pairs and gRNA designed to target the *Il2rg* locus and primer sequences used for the PCR analysis of the *Il2rg* gene. Each exon is underlined. The start codon is indicated by a red box. The two primer sets (small and large) for the PCR analysis of *Il2rg* are shown by boxes.

Supplementary Figure S2. Validation of ZFN and TALEN activity in rat fibroblasts (Rat-1). (a) ZFN or TALEN expression vectors, and the GFP expression vector as a negative control, were electroporated into Rat-1 fibroblast cells using the Super Electroporator NEPA 21. The electroporation conditions are as follows: (A) pulse voltage, 275 V; pulse interval, 50 ms; pulse width, 2.5 ms; and pulse number, 2; or (B) pulse voltage, 275 V; pulse interval, 50 ms; pulse width, 1.0 ms; and pulse number, 3. The Surveyor (Cel-I) nuclease assay was performed to detect mutations in the *Il2rg* locus. (b) The Surveyor assay indicated the activity of ZFN and TALEN in Rat-1 cells. Data are expressed as means ± SEM (n = 4).

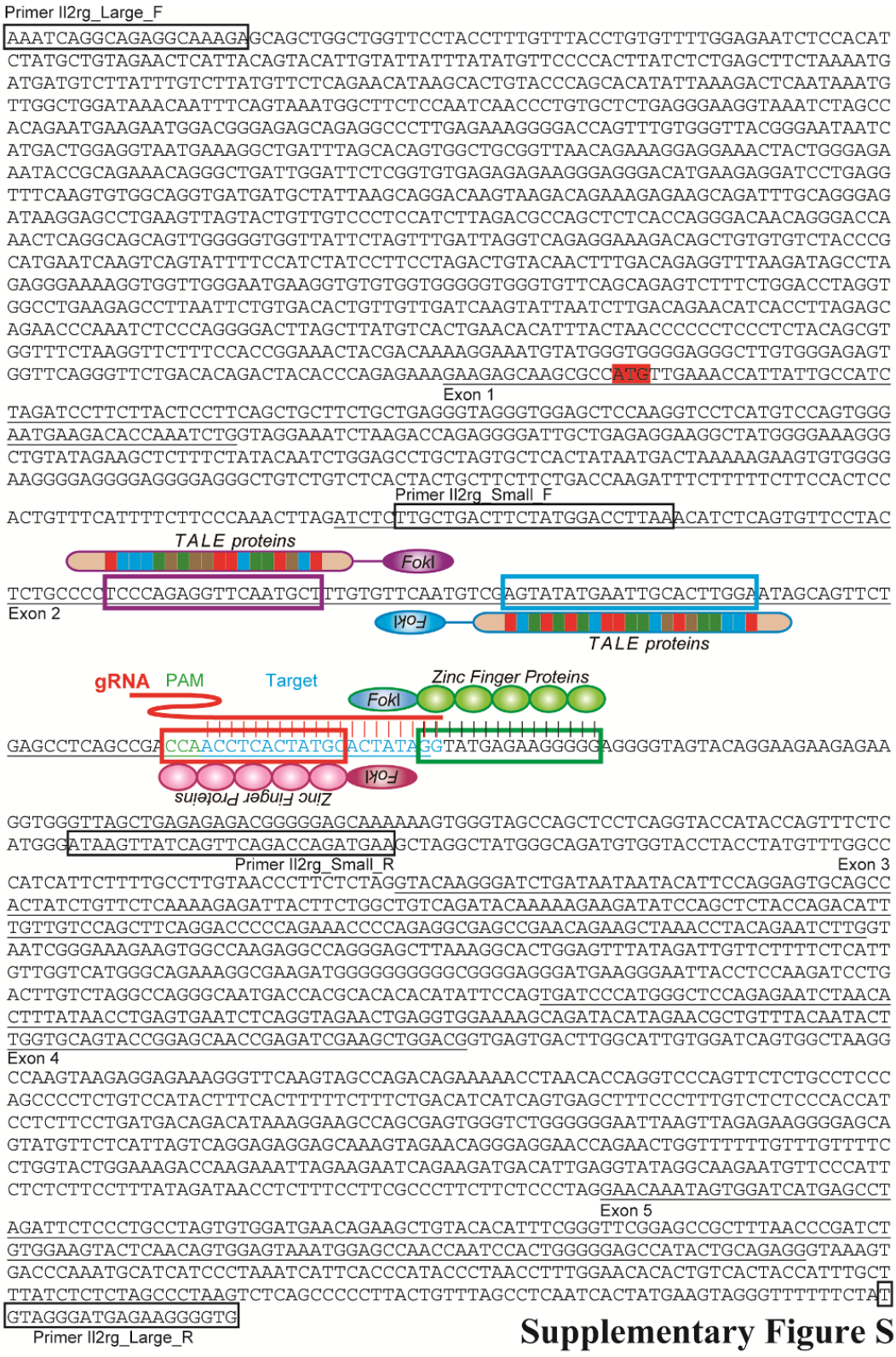
Supplementary Figure S3. Sequencing assay for ZFN- and TALEN-induced mutations in the *Il2rg*-targeted locus in rat fibroblasts (Rat-1). Multiple deletions or insertions, which are depicted using yellow dashes or letters, respectively, are aligned along the wildtype sequence

that is shown on the top line.

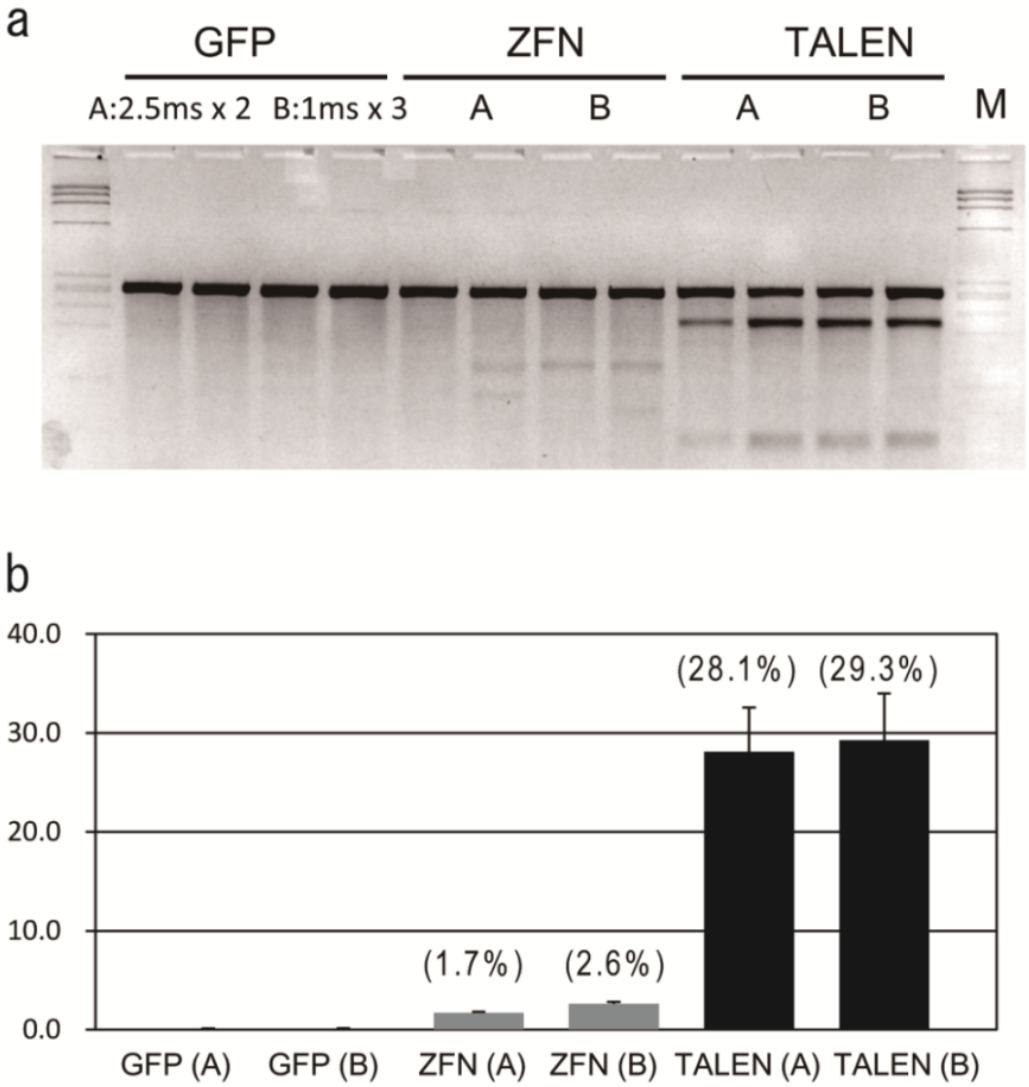
Supplementary Figure S4. Sequencing assay for ZFN-induced mutations in the *Il2rg*-targeted locus in the edited offspring. Multiple deletions or insertions, which are depicted using yellow dashes or letters, respectively, are aligned along the wildtype sequence that is shown on the top line. MI, microinjection; EP, electroporation.

Supplementary Figure S5. Sequencing assay for TALEN-induced mutations in the *Il2rg*-targeted locus in the edited offspring. Multiple deletions, which are depicted using yellow dashes, are aligned along the wildtype sequence that is shown on the top line. MI, microinjection; EP, electroporation.

Supplementary Figure S6. Sequencing assay for CRISPR/Cas-induced mutations in the *Il2rg*-targeted locus in the edited offspring. Multiple mutations, which are depicted using yellow, are aligned along the wildtype sequence that is shown on the top line. MI, microinjection; EP, electroporation.



Supplementary Figure S1



Supplementary Figure S2

ZFN-induced mutations: 10/96 colony (10.4%)

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Wild-type  GCCGACCAACCTCACTATGCACTATAGGTATGAGAAGGGGGAGGGG
#6         GCCGACCAACCTCACTATGCACTATTATAGGTATGAGAAGGGGGAGGGG
#10        GCCGACCAACCTCACTATGCACTATCTATAGGTATGAGAAGGGGGAGGGG
#60        CAGTT----------(Δ83bp)----------AGCTG
#24        GCCGACCAACCTCACTATGCACTATGGGTATGAGAAGGGGGAGGGG
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TALEN-induced mutations: 17/86 colony (19.8%)

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Wild-type  GCCCCTCCCAGAGGTTCAATGCTTTGTGTTCAATGTCGAGTATATGAATTGCACTTGGAATAGC
#3         GCCCCTCCCAGAGGTTCAATGCTTTGTGTTCAATGTCGAGTAG----(Δ163bp)----ACCAG
#5         GCCCCTCCCAGAGGTTCAATGCTTTGTGTTCA---TCGAGTATATGAATTGCACTTGGAATAGC
#9         GCCCCTCCCAGAGGTTCAATGCTTTGTGTT---------GTATATGAATTGCACTTGGAATAGC
#12        GCCCCTCCCAGAGGTTCAATGCTTTGTGT-------CGAGTATATGAATTGCACTTGGAATAGC
#17        GCCCCTCCCAGAGGTTCAATGCTTT--------TGTCGAGTATATGAATTGCACTTGGAATAGC
#52        GCCCCTCCCAGAGGTTCAATGCTTTGTGTTCAATGTTGAGTATATGAATTGCACTTGGAATAGC
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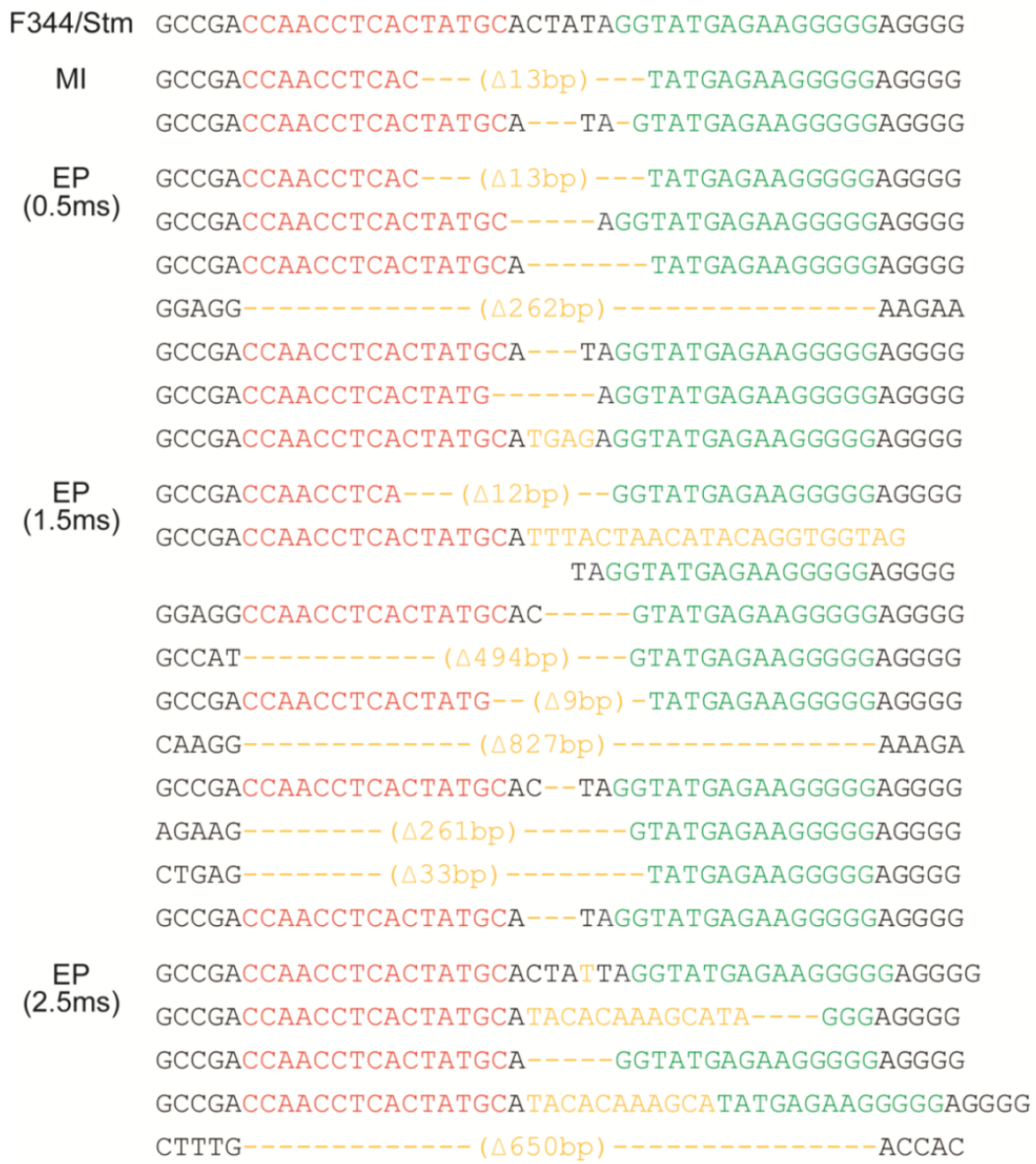
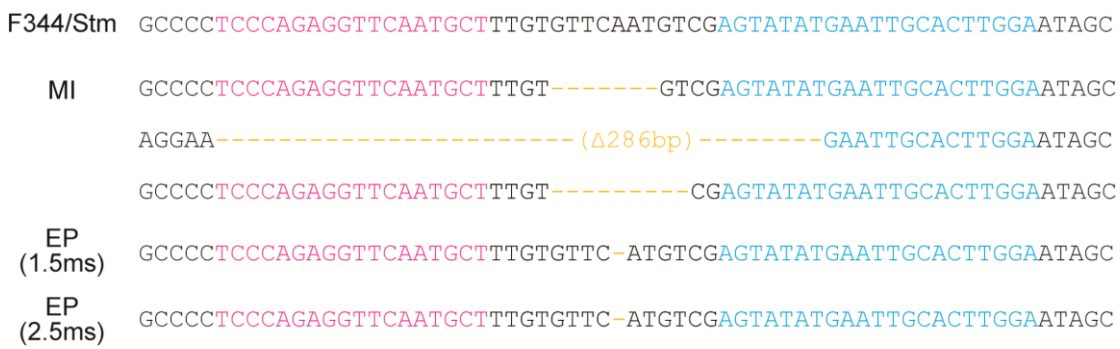
Supplementary Figure S3



Supplementary Figure S4



Supplementary Figure S5

Supplementary Figure S6