

## 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.**

Fathers	Mothers	Offspring		Offspring with an edited target gene	
		Males	Females	Males	Females
G0 $\Delta 13/Y$	+/+	8	5	0	5
G0 $\Delta 7/Y$	+/+	7	5	0	5
G0 $\Delta 5/Y$	+/+	6	4	0	2
+/Y	F1 $\Delta 13/+$	5	6	3	3
+/Y	F1 $\Delta 13/+$	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  $\pm$  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.

Primer II2rg\_Large\_F

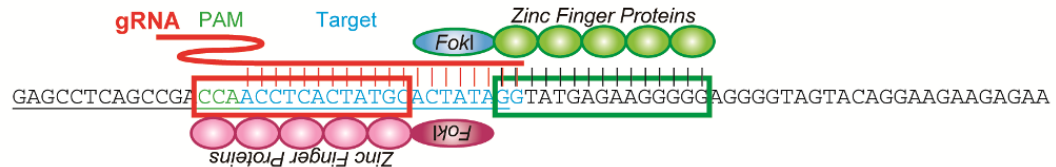
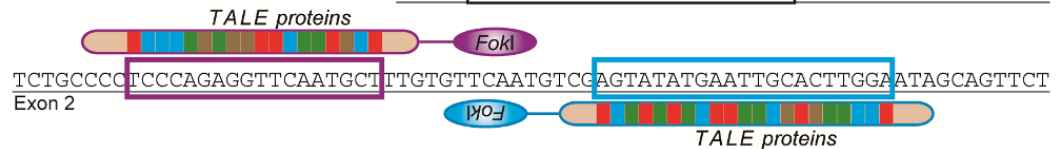
AAATCAGGCAGAGGCAAAGA GCAGCTGGCTGGTTCTACCTTTGTTTACCTGTGTTTTGGAGAATCTCCACAT  
CTATGCTGTAGAACTCATTACAGTACATTGTATTATTTATATGTTCCCCACTTATCTCTGAGCTTCTAAAATG  
ATGATGTCTTATTTGTCTTATGTTCTCAGAACATAAGCACTGTACCCAGCACATATTAAGACTCAATAAATG  
TTGGCTGGATAAAACAATTTTCAGTAAATGGCTTCTCCAATCAACCCTGTGCTCTGAGGGGAAAGTAAATCTAGCC  
ACAGAATGAAGAAATGGACGGGAGAGCAGAGGCCCTTGAGAAAGGGGACCAGTTTGTGGGTTACGGGAATAATC  
ATGACTGGAGGTAATGAAAGGCTGATTTAGCACAGTGGCTGCGGTTAACAGAAAGGAGGAAACTACTGGGAGA  
AATACCGCAGAAAACAGGGCTGATTGGATTCTCGGTGTGAGAGAGAAGGGAGGGACATGAAGAGGATCCTGAGG  
TTTCAAGTGTGGCAGGTGATGATGCTATTAAGCAGGACAAGTAAGACAGAAAGAGAAGCAGATTTGCAGGGAG  
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AACTCAGGCAGCAGTTGGGGTGGTTATTCTAGTTTGGATTAGGTGAGAGGAAAGACAGCTGTGTGCTTACCCG  
CATGAATCAAGTCAGTATTTTCCATCTATCCTTCTAGACTGTACAACCTTTGACAGAGGTTTAAAGATAGCCTA  
GAGGGAAAAGTGGTTGGGAATGAAGGTGTGTTGGTGGGGTGGGTGTTTCTGACAGAGTCTTTCTGGACCTAGGT  
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AGAAACCAAATCTCCAGGGGACTTAGCTTATGTCACTGAACACATTTACTAACCCTTCCCTCTACAGCGT  
GGTTTCTAAGGTTCTTTCCACCGGAAACTACGACAAAAGGAAATGTATGGGTGGGGAGGGCTTGTGGGAGAGT  
GGTTCAGGGTTCTGACACAGACTACACCCAGAGAAAGAGCAAGCGCCATGTTGAAACCATTATTGCCATC

Exon 1

TAGATCCTTCTTACTCCTTCAGCTGCTTCTGCTGAGGGTAGGGTGGAGCTCCAAGGTCCTCATGTCCAGTGGG  
AATGAAGACACCAAATCTGGTAGGAAATCTAAGACCAGAGGGGATTGCTGAGAGGAAGGCTATGGGGAAAGGG  
CTGTATAGAAGCTCTTTCTATAACAATCTGGAGCCTGCTAGTGTCTCACTATAATGACTAAAAGAAGTGTGGGG  
AAGGGGAGGGGAGGGGAGGGTGTCTGTCTCACTACTGCTTCTTCTGACCAAGATTTCTTTTCTTCCACTCC

Primer II2rg\_Small F

ACTGTTTCATTTTCTTCCAACTTAGATCTCTTGTGCTGACTTCTATGGACCTTAAACATCTCAGTGTTCCTAC



GGTGGGTTAGCTGAGAGAGACGGGGAGCAAAAAGTGGGTAGCCAGCTCCTCAGGTACCATAACCAGTTTCTC  
ATGGGATAAGTTATCAGTTCAGACCAGATGAAAGCTAGGCTATGGGCAGATGTGGTACCTACCTATGTTTGGCC

Primer II2rg\_Small R

Exon 3

CATCATTCTTTTGCCTTGTAACTTCTCTAGGTACAAGGATCTGATAATAATACATTCCAGGAGTGCAGCC  
ACTATCTGTTCTCAAAAAGAGATTACTTCTGGCTGTGATACAAAAGAGATATCCAGTCTTACCAGACATT  
TGTTGTCCAGCTTTCAGGACCCCAAGAAACCCAGAGCGAGCCGAACAGAAAGCTAAACCTACAGAATCTTGGT  
AATCGGAAAGAAAGTGGCCAAGAGGCCAGGGAGCTTAAAGGCACTGGAGTTTATAGATTGTTCTTTTCTCATT  
GTTGGTTCATGGGCAGAAAGGCGAAGATGGGGGGGGGGCGGGGAGGGATGAAGGGAATTACCTCCAAGATCCTG  
ACTTGTCTAGGCCAGGGCAATGACCACGCACACATATTCAGTGTATCCCATGGGCTCCAGAGAATCTAACA  
CTTTATAACCTGAGTGAATCTCAGGTAGAAGTGAAGTGGAAAAGCAGATACATAGAACGCTGTTTACAATACT  
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Exon 4

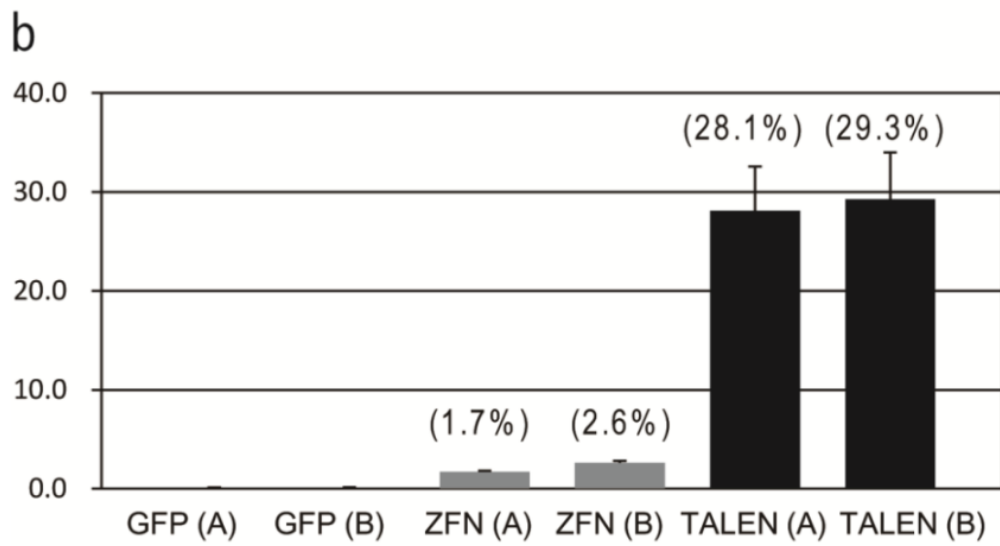
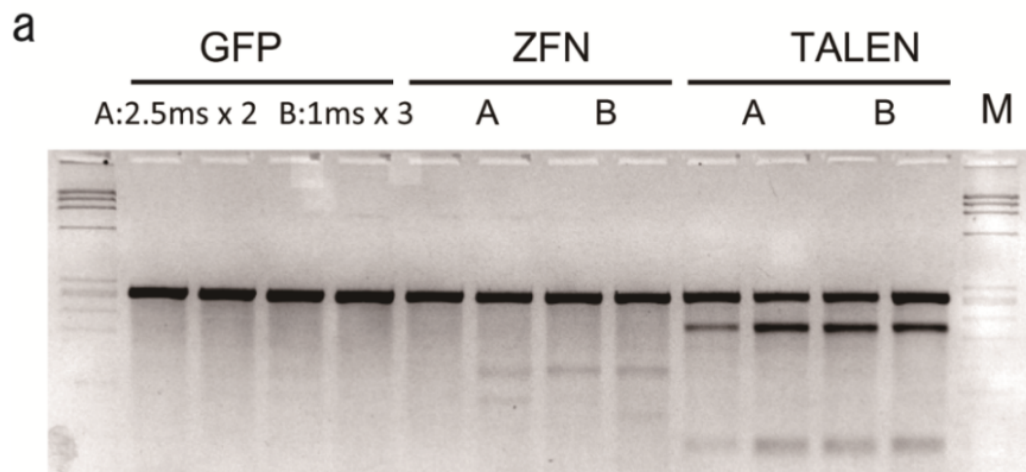
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CCTCTTCTGATGACAGACATAAAGGAAGCCAGCGAGTGGTCTGGGGGAATTAAGTTAGAGAAGGGGAGCA  
GTATGTTCTCATTAGTCCAGGAGAGGAGCAAAGTAGAACAGGGAGGAACCAGAACTGGTTTTTTGTTTGTTC  
CTGGTACTGGAAAGACCAAGAAATTAGAAGAAATCAGAAGATGACATTGAGGTATAGGCAAGAATGTTCCATT  
CTCTCTTCTTTATAGATAACCTCTTTCTTCCGCTTCTTCTCCCTAGGAAACAAATAGTGGATCATGAGCCT

Exon 5

AGATTCTCCCTGCCTAGTGTGGATGAACAGAAGCTGTACACATTTCCGGTTCCGGAGCCGCTTTAACCCGATCT  
GTGGAAGTACTCAACAGTGGAGTAAATGGAGCCAACCAATCCACTGGGGGAGCCATACTGCAGAGGGTAAAGT  
GACCCAAATGCATCATCCCTAAATCATTACCACATACCCTAACCTTTGGACACACTGTCACTACCATTGCT  
TTATCTCTTAGCCCTAAGTCTCAGCCCCCTTACTGTTTGTAGCTCAATCACTATGAAGTAGGGTTTTTTCTA  
GTAGGGATGAGAGGGGTG

Primer II2rg\_Large\_R

Supplementary Figure S1



**Supplementary Figure S2**

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

Wild-type GCCGACCAACCTCACTATGCACTATAGGTATGAGAAGGGGGAGGGG  
#6 GCCGACCAACCTCACTATGCACTATATAGGTATGAGAAGGGGGAGGGG  
#10 GCCGACCAACCTCACTATGCACTATCTATAGGTATGAGAAGGGGGAGGGG  
#60 CAGTT----- ( $\Delta 83$ bp) -----AGCTG  
#24 GCCGACCAACCTCACTATGCACTATGGTATGAGAAGGGGGAGGGG

TALEN-induced mutations: 17/86 colony (19.8%)

Wild-type GCCCCTCCAGAGGTTCAATGCTTTGTGTTCAATGTCGAGTATATGAATTGCACTTGGAAATAGC  
#3 GCCCCTCCAGAGGTTCAATGCTTTGTGTTCAATGTCGAGTAG----- ( $\Delta 163$ bp) -----ACCAG  
#5 GCCCCTCCAGAGGTTCAATGCTTTGTGTTCA---TCGAGTATATGAATTGCACTTGGAAATAGC  
#9 GCCCCTCCAGAGGTTCAATGCTTTGTGTT-----GTATATGAATTGCACTTGGAAATAGC  
#12 GCCCCTCCAGAGGTTCAATGCTTTGTGT-----CGAGTATATGAATTGCACTTGGAAATAGC  
#17 GCCCCTCCAGAGGTTCAATGCTTT-----TGTCGAGTATATGAATTGCACTTGGAAATAGC  
#52 GCCCCTCCAGAGGTTCAATGCTTTGTGTTCAATGTTGAGTATATGAATTGCACTTGGAAATAGC

Supplementary Figure S3

F344/Stm GCCGACCAACCTCACTATGCACTATAGGTATGAGAAGGGGGAGGGG

MI GCCGACCAACCTCAC--- ( $\Delta 13$ bp) ---TATGAGAAGGGGGAGGGG  
GCCGACCAACCTCACTATGCA---TA---GTATGAGAAGGGGGAGGGG

EP  
(0.5ms) GCCGACCAACCTCAC--- ( $\Delta 13$ bp) ---TATGAGAAGGGGGAGGGG  
GCCGACCAACCTCACTATGC-----AGGTATGAGAAGGGGGAGGGG  
GCCGACCAACCTCACTATGCA-----TATGAGAAGGGGGAGGGG  
GGAGG----- ( $\Delta 262$ bp) -----AAGAA  
GCCGACCAACCTCACTATGCA---TAGGTATGAGAAGGGGGAGGGG  
GCCGACCAACCTCACTATG-----AGGTATGAGAAGGGGGAGGGG  
GCCGACCAACCTCACTATGCATGAGAGGTATGAGAAGGGGGAGGGG

EP  
(1.5ms) GCCGACCAACCTCA--- ( $\Delta 12$ bp) --GGTATGAGAAGGGGGAGGGG  
GCCGACCAACCTCACTATGCATTTACTAACATACAGGTGGTAG  
TAGGTATGAGAAGGGGGAGGGG  
GGAGGCCAACCTCACTATGCAC-----GTATGAGAAGGGGGAGGGG  
GCCAT----- ( $\Delta 494$ bp) ---GTATGAGAAGGGGGAGGGG  
GCCGACCAACCTCACTATG-- ( $\Delta 9$ bp) -TATGAGAAGGGGGAGGGG  
CAAGG----- ( $\Delta 827$ bp) -----AAAGA  
GCCGACCAACCTCACTATGCAC--TAGGTATGAGAAGGGGGAGGGG  
AGAAG----- ( $\Delta 261$ bp) -----GTATGAGAAGGGGGAGGGG  
CTGAG----- ( $\Delta 33$ bp) -----TATGAGAAGGGGGAGGGG  
GCCGACCAACCTCACTATGCA---TAGGTATGAGAAGGGGGAGGGG

EP  
(2.5ms) GCCGACCAACCTCACTATGCACTATTAGGTATGAGAAGGGGGAGGGG  
GCCGACCAACCTCACTATGCATACACAAAGCATA----GGGAGGGG  
GCCGACCAACCTCACTATGCA-----GGTATGAGAAGGGGGAGGGG  
GCCGACCAACCTCACTATGCATACACAAAGCATATGAGAAGGGGGAGGGG  
CTTTG----- ( $\Delta 650$ bp) -----ACCAC

## Supplementary Figure S4

F344/Stm GCCCC**TCCAGAGGTTCAATGCT**TTGTGTTCAATGTCGAGTATATGAATTGCACTTGAATAGC

MI GCCCC**TCCAGAGGTTCAATGCT**TTGT-----GTCGAGTATATGAATTGCACTTGAATAGC  
AGGAA-----( $\Delta 286$ bp)-----GAATTGCACTTGAATAGC  
GCCCC**TCCAGAGGTTCAATGCT**TTGT-----CGAGTATATGAATTGCACTTGAATAGC

EP  
(1.5ms) GCCCC**TCCAGAGGTTCAATGCT**TTGTGTTT-ATGTCGAGTATATGAATTGCACTTGAATAGC

EP  
(2.5ms) GCCCC**TCCAGAGGTTCAATGCT**TTGTGTTT-ATGTCGAGTATATGAATTGCACTTGAATAGC

## Supplementary Figure S5



F344/Stm AGCCTCAGCCGACCAACCTCACTATGCACTATAGGTATGAGAA

MI AGCCTCAGCCGACCAACCTTTTAAAAAGCTCTATGCACTATAGGTATGAGAA  
AGCCTCAGCCGACCAACCTTCACTATGCACTATAGGTATGAGAA  
AGCCTCAGCCGACCAACCATCACTATGCACTATAGGTATGAGAA  
AGCCTCAGCCGACCAACC---GTATGCACTATAGGTATGAGAA  
AGCCTCAGCCGACCAAC----CTATGCACTATAGGTATGAGAA  
AGCCTCAGCCGACCA-----ACTATGCACTATAGGTATGAGAA  
AGCCTCAGCCGACC-----TATGCACTATAGGTATGAGAA  
AGCCTCAGCCGACCA-----ATGCACTATAGGTATGAGAA  
AGCCTCAGCCGAC-----CACTATAGGTATGAGAA  
AGCCTCAG-----TATGCACTATAGGTATGAGAA  
AGCCTCAGCCGACCAACCT-----AGGT-TGAGAA  
GAATA---- ( $\Delta 29$ bp) ----ACTATGCACTATAGGTATGAGAA  
CTGAG----- ( $\Delta 85$ bp) -----AGAGA  
AGCCTCAGCCGACCAA----- ( $\Delta 87$ bp) -----AAAAG

EP AGCCTCAGCCGACCAACCTTTCACCTATGCACTATAGGTATGAGAA  
(2.5ms) AGCCTCAGCCGACCAACCT---TATGCACTATAGGTATGAGAA  
AGCCTCAGCCGACCA-----TACTCTATAGTTATGAGAA

## Supplementary Figure S6