Supporting Information inventory

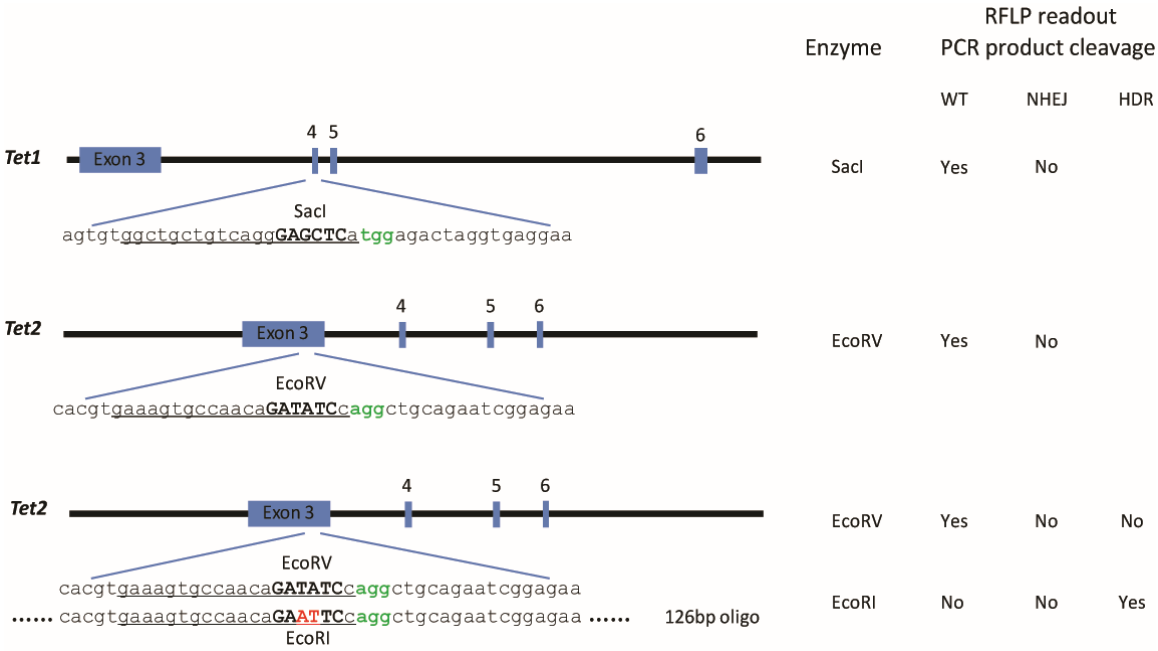

Figure S1 Schematic of the Cas9/sgRNA-targeting sites at the *Tet1* and *Tet2* loci, and RFLP genotyping strategy. The sgRNA-targeting sequence is underlined, and the protospacer-adjacent motif (PAM) sequence colored in green. Restriction sites at the target regions are bold and capitalized. Restriction enzymes used for RFLP analysis and the expected results from different alleles are shown in the right panel. Oligonucleotide directed 2bp changes are colored in red.

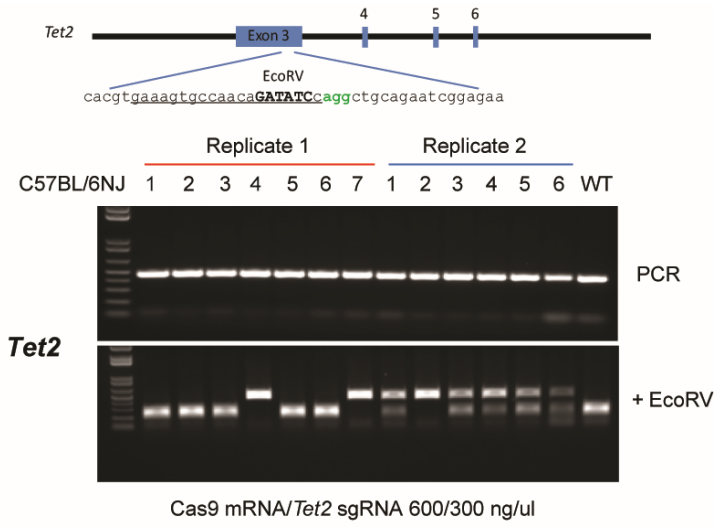

Figure S2 CRISPR/Cas9 mediated gene editing delivered by ZEN in C57BL/6NJ strain. The sgRNA-targeting sequence is underlined, and the PAM sequence colored in green. The EcoRV restriction site at the target region is bold and capitalized. RFLP analysis results using EcoRV are shown for mice derived from two replicate experiments.

Table S1 Effect of voltage and pulse interval on embryo survival

Voltage (v)	Pulse Interval (ms)	Zygotes Electroporated	Blastocysts Developed	Embryo Survival Rate
30	100	13	12	92%
30	500	13	9	69%
30	1000	10	8	80%
100	100	10	2	20%
100	500	11	3	27%
100	1000	11	2	18%
300	100	14	0	0
300	500	12	0	0
300	1000	14	0	0

All zygotes were derived from B6D2F2/J and treated with acidic Tyrode's solution for 10 seconds before electroporation. The embryos used in this experiment had not been exposed to CRISPR/Cas9 reagents.

Table S2 Composition of electroporation solution on embryo survival

TE (ul)	Opti-MEM (ul)	Total Volume (ul)	Zygotes Electroporated	Blastocysts Developed	Embryo Survival Rate
20	0	20	19	7	37%
20	0	20	19	9	47%
15	5	20	17	13	76%
15	5	20	21	16	76%
10	10	20	14	13	93%
10	10	20	18	17	94%
0	20	20	17	17	100%
0	20	20	20	19	95%

All zygotes were derived from B6D2F2/J and treated with acidic Tyrode's solution for 10 seconds before electroporation. Embryos used in this experiment had not been exposed to CRISPR/Cas reagents.

Table S3 CRISPR/Cas-mediated HDR at the *Tet2* Locus in Mouse Embryos Delivered by Electroporation

Group	Cas9/sgRNA/donor oligo Conc. (ng/ul)	Embryos Electroporated	Blastocysts Developed	NHEJ+ HDR	Efficiency	HDR	Efficiency
1	400/200/1000	15	12	9	75%	4	33%
2	600/300/1000	15	13	10	77%	4	31%

Zygotes were derived from B6D2F2/J and treated with acidic Tyrode's solution for 10 seconds before electroporation. A total of 15 embryos for each concentration were electroporated in a cuvette of 1 mm gap size and in 20 ul of TE/Opti-MEM at 1:1 volume ratio. Following electroporation, the embryos were transferred to 100 ul of M2 media and cultured in vitro for 3.5 days and blastocysts analyzed.

Table S4 Off target analysis for four mice carrying targeted *Tet2* mutation

Site	Coordinate*	Sequence	Mismatches	Mutation
On Target	chr3:133148617-133148639	GAAAGTGCCAACAGATATCCAGG	none	yes
Off Target 1	chr11:31327722-31327744	aAAtGTGCaAACAGATATCCTGG	1,4,9	No
Off Target 2	chr8:61482458-61482480	GAAAagGgCAACAGATATCCTGG	5,6,8	No
Off Target 3	chr5:134923409-134923431	GAAcGTGgCAcCAGATATCCTGG	4,8,11	No
Off Target 4	chr1:73824333-73824355	GAcAGTGCCAAaAcATATCCTGG	3,12,14	No
Off Target 5	chr2:150088681-150088703	cAAAGaGCCAACAGAaATCCAGG	1,6,16	No
Off Target 6	chr17:36494664-36494686	GAAgGTGgCAACAGAcATCCTGG	4,8,16	No
Off Target 7	chr17:36923721-36923743	GAAgGTGgCAACAGAcATCCTGG	4,8,16	No
Off Target 8	chr17:36961245-36961267	GAAgGTGgCAACAGAcATCCTGG	4,8,16	No
Off Target 9	chr1:89767331-89767353	GgAAtTGCCAACAGATcTCCTGG	2,5,17	No
Off Target 10	chr12:4599503-4599525	GAAAGgGCCAAgAGATgTCCAGG	6,12,17	No
Off Target 11	chr5:148012894-148012916	GAAAaTGgCAACAGATAcCCTGG	5,8,18	No
Off Target 12	chr7:121431500-121431522	GtAAGTtCCAACAGATATgCTGG	2,7,19	No
Off Target 13	chr14:26417940-26417962	GAAgGTGCCAgCAGATATCaAGG	4,11,20	No
Off Target 14	chr7:115756427-115756449	GAcAGTGCCAACAGATATagTGG	3,19,20	No

*chromosome coordinate is based on July 2007, NCBI37/mm9.

PCR products were amplified from samples 86EP1, 2, 3, and 4 encompassing the 14 potential off target sites and analyzed by Sanger sequencing. All were found to be wild type at the loci.

Table S5 Oligonucleotides used in this study.

Oligonucleotides used in a PCR reaction to generate DNA template for in vitro transcription of the Cas9 mRNA

Gene	Direction	Sequence (5' to 3')	Template
Cas9	F	TAATACGACTCACTATAGGGAGA CCACC ATGGACTATAAGGACCACGAC	Px330 (Cong *et al*. 2013)
	R	GCGAGCTCTAGgaattcttaC	

Oligonucleotides used in a PCR reaction to generate DNA template for in vitro transcription of the Tet1 and Tet2 single guide RNA

sgRNA	Direction	Sequence (5' to 3')
sgRNA	R	AAAAGCACCGACTCGGTGCCACTTTTTCAAGTTGATAACGG ACTAGCCTTATTTTAACTTGCTATTTCTAGCTCTAAAAC
Tet1	F	TAATACGACTCACTATAG ggctgctgtcagggagctca gtttAagagctaTGCTGGAAAC
Tet2	F	TAATACGACTCACTATAG gaaagtgccaacagatatcc gtttAagagctaTGCTGGAAAC

Oligonucleotide used as donor for HDR-mediated repair at the *Tet2* locus

Gene target	Sequence (5' to 3')
Tet2 EcoRV to EcoRI	tcactctgtgactataaggctctgactctcaagtcacagaaacacgtgaaagtgccaacaGAatTCcaggc tgcagaatcggagaaccacgcccgagctgcagagcctcaagcaaccaaaagcaca

Forward and reverse primers used to produce DNA template for in vitro transcription of sgRNA and guide sequences for the 10 genes from the study

Name	Sequence (5' to 3')
sgRNA_F	gaaattaatacgactcactatagg(N20)gttttagagctagaaatagc
sgRNA_R	aaaagcaccgactcggtgccacttttcaagttgataacggactagccttattttaacttgc tatttctagctctaaaac
Cd69	TTCTGAAAACTGTTCTATAA
Cd226	AAGTCCTGAGTCAGCGGCCA
Clec16a	GAGATGGTGATCATGAAGCT
Cyp27b1	CAACCAGTTGGGCATCGCCA
Fut2	ATGTAGCATATTCGCCCATC
Ormdl3	ACACGGGTGATGAACAGTCG
Rgs1	CGGCAGCCATCTCCATGCCA
Tlr7	ATTTACAGGTGTTTTCGATG

Tlr8	CGTCAGAATCCATGACTGAG
Tnfsf9	GCACTGACCGACCGTGGTAA