



Supporting Online Material for

Knockout Rats via Embryo Microinjection of Zinc-Finger Nucleases

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Materials and Methods

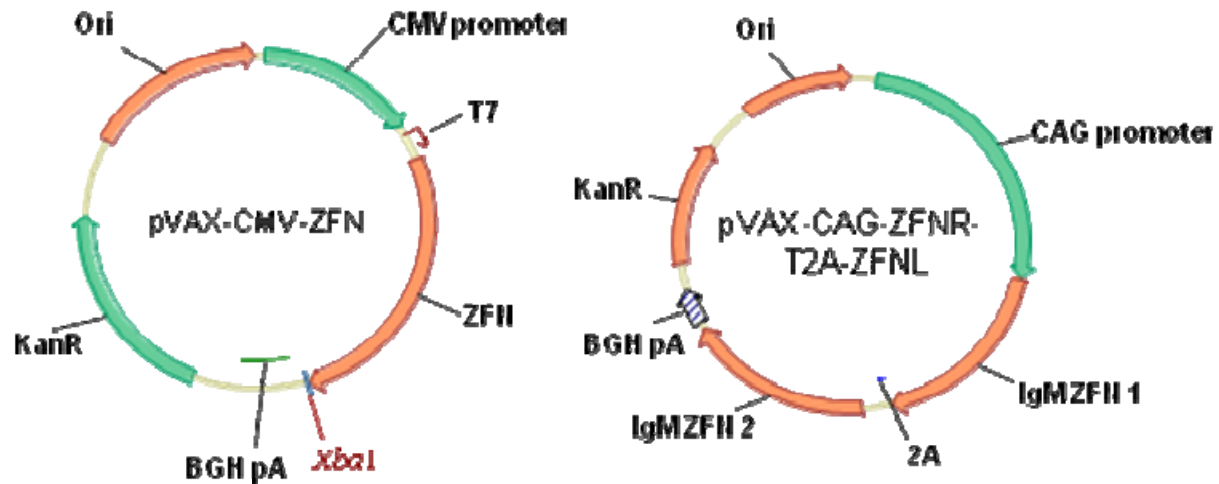
Design, assembly, in vitro and in vivo validation of zinc-finger nucleases

The ZFNs were designed, assembled, and validated using strategies and procedures described elsewhere (*S1, S2*); minor modifications to the procedures are detailed below.

ZFN design made use of an archive of pre-validated 2-finger and 1-finger modules (*S1, S3-5*). The target region of eGFP, *IgM*, and *Rab38* were scanned for positions where modules exist in the archive that allow the fusion of 2 or 3 such modules to generate a 4, 5 or 6 finger protein to recognize a 12-18 bp site on the top (“Watson”) strand, and the fusion of 2-3 different modules to recognize on the bottom (“Crick”) strand of a 12-18 bp site that lies 5 or 6 bp away.

The ZFNs were assembled using a PCR-based procedure and cloned into yeast expression vectors as described elsewhere (*S1*). Screening of ZFNs in the yeast-based proxy system (Fig. S5A) was done as described (*S1*), with one modification: the target regions of all ZFN pairs from a given gene were extracted and concatenated to yield a composite DNA stretch that was then used to generate a disrupted reporter construct. Final candidate ZFN pairs were subcloned into a CMV expression plasmid for testing in cultured rat cells (below, left).

Screening of ZFNs for gene disruption activity at the eGFP transgene, and at the *IgM* and *Rab38* loci was done by transfection of ZFN expression constructs into rat C6 cells (ATCC) using an Amaxa nucleofector according to the manufacturer’s instructions. Measurements of ZFN gene disruption activity were performed using the Surveyor endonuclease (Cel-1) assay exactly as described previously (*S6*) and below (Fig. S5B). The DNA recognition helices for the most active ZFN pairs that were used in all subsequent rat experiments are given below. The *IgM* ZFNs were subcloned into an expression plasmid as two open reading frames linked by the T2A peptide sequence as previously described (*S1*) (below, right) for the rat embryo plasmid DNA injections described below.



ZFN constructs

The GFP ZFNs used wild-type *Fok* I endonuclease domains, while the *IgM* and *Rab38* ZFNs utilized the obligate-heterodimer forms (S6). The DNA recognition helices for the GFP gene ZFNs are as follows: TSGSLSR QSGSLTR TSGSLSR QSSDLRR RSDALSR TSGSLTR from the N- to C-terminus of the left-hand ZFN; RSANLSV DRANLSR DRSDLSR RSDLSV DSSARKK from the N- to C-terminus of the right-hand ZFN. The DNA recognition helices for the *IgM* gene ZFNs are as follows: NKVGLIE TSSDLR RSDHLR RSDNLSE QNAHRKT from the N- to C-terminus of the left-hand ZFN; DRSHLTR RSDALTQ DRSDLSR RSDALAR RSDLSA TSSNRKT from the N- to C-terminus of the right-hand ZFN. The DNA recognition helices for the *Rab38* gene ZFNs are as follows: DRSNLSS RSHLLR RSDLSA TSGSLTR QSGNLAR QSGHLR from the N- to C-terminus of the left-hand ZFN; TSGHLR HKWQRNK DRSVLRR DSSTRKK RSDHLSE DKSNRKK from the N- to C-terminus of the right-hand ZFN.

Pronuclear injection

For the GFP and *Rab38* gene targeting, ZFN-encoding expression plasmids (above left) were linearized with *Xba*I, extracted with phenol-chloroform and precipitated with isopropanol. Messenger RNA was *in vitro* transcribed and polyadenylated using the MessageMax™ T7 ARCA-Capped Message Transcription Kit and A-plus polyadenosine tailing kits (Epicentre Biotechnologies, Madison, WI). The resulting mRNA was purified using the MegaClear Kit™ (Ambion, Austin, TX) before resuspension in RNase-free 0.1X TE (1mM Tris-Cl pH 8.0, 0.1mM EDTA), quantitated using a NanoDrop-1000 (Thermo Scientific) and stored at -80°C until use. Messenger RNAs encoding GFP and *Rab38* ZFNs were mixed to a final total concentration of 1.5-2.0 ng/μL in 1 mM Tris-Cl, 0.1 mM EDTA, pH 7.5. Plasmid DNA encoding *IgM* ZFNs was prepared using the GenElute HP midiprep kit (Sigma-Aldrich) and

diluted for microinjection to either 10 ng/ μ L, 2 ng/ μ L, or 0.4 ng/ μ L using 10 mM Tris-Cl, 0.1 mM EDTA, pH 7.5. Messenger RNAs encoding IgM ZFNs was prepared using the Ambion mMessage mMachine kit (Ambion, Austin, TX) following the manufacturer protocol and diluted at concentrations 0.4 ng/ μ L, 2 ng/ μ L, or 10 ng/ μ L with RNaseFree Water (Ambion) and stored at -80°C until use. GFP and IgM ZFN mRNAs were kept on ice during all microinjection procedures.

One goal of this study was to test the ability of ZFNs to induce mutations in a variety of inbred and outbred rat strains. The GFP rat was previously produced on the SS (Dahl S) strain background (S7), the SD (Sprague Dawley) rat was chosen to build the humanized monoclonal antibody platform due to its robust breeding characteristics, and the FHH (Fawn Hooded Hypertensive) rat is a model of hypertension where the *Rab38* gene is thought to play a role in end-stage renal disease (S8).

Inbred SS and FHH and outbred SD embryo pronuclear injections were performed at different institutions. At the Medical College of Wisconsin, inbred strain animals are housed in standard cages under approved animal care protocols in an American Association of Laboratory Animal Care-approved facility. The rats are maintained on a 12 h light/dark cycle with *ad libitum* access to food and water. Five to eight week old female SS female rats were injected with 20 IU pregnant mare serum gonadotropin (PMSG) (Sigma-Aldrich or National Hormone and Peptide Program) followed 48 hours later with 25 IU human chorionic gonadotropin (hCG) (Sigma-Aldrich). Wild-type SS females were bred to homozygous SS-Tg(CAG-eGFP)1Mcwi males (7) to generate hemizygous GFP embryos for microinjection. Wild-type FHH females, harboring the natural knockout allele of *Rab38* were superovulated for breeding to FHH.BN-*Rab38* congenic males harboring a wild-type copy of the *Rab38* gene from the BN (Brown Norway) strain which has been introgressed into the FHH inbred background (S8). The *Rab38* ZFNs can target both alleles equally. Embryos were microinjected with ZFNs into the pronucleus or cytoplasm using an Eppendorf Microinjection system under standard conditions. Manipulated embryos were transferred to pseudopregnant SD/Hsd female rats (Harlan Laboratories, Inc.) to be carried to parturition.

At the Caliper Life Sciences (Xenogen Biosciences) facility, outbred SD/NTac strain animals (Taconic) were housed in standard microisolator cages under approved animal care protocols in animal facility that is accredited by the Association for the Assessment and

Accreditation for Laboratory Animal Care (AAALAC). The rats were maintained on a 14-10 h light/dark cycle with *ad libitum* access to food and water. Four to five week old SD/Hsd female rats were injected with 20-25 IU PMSG (Sigma-Aldrich, St. Louis, MO) followed 48 hours later with 20-25 IU hCG (Sigma-Aldrich, St. Louis, MO) before breeding to outbred SD/Hsd males. Fertilized 1-cell stage embryos were collected for subsequent IgM plasmid DNA microinjection. Manipulated embryos were transferred to pseudopregnant SD/NTac female rats to be carried to parturition.

At the INSERM UMR 643 Transgenic Rat Facility (<http://www.ifr26.nantes.inserm.fr/ITER/Transgenese-rat/>), Sprague-Dawley (SD/Crl) outbred strain animals (Charles River France, L'Arbresle, France) were housed in standard cages and protocols were conducted in accordance with the guidelines for animal experiments of the French Veterinary Services and were performed by officially authorized personnel in a certified animal facility. The rats are maintained on a 12 h light/dark cycle with *ad libitum* access to food and water. Females, 26-30 days old SD/Crl were injected with 30 IU pregnant mare serum gonadotropin (PMSG, Intervet, France) and followed 48 hours later with 20 IU human chorionic gonadotropin (hCG) (Intervet, France) before breeding. Fertilized 1-cell stage embryos were collected for subsequent microinjection using previously published procedures (S9). The male pronucleus or cytoplasm of one-cell stage embryos were microinjected with ZFN mRNA and surviving embryos were implanted on the same day in the oviduct of pseudo-pregnant SD/Crl females and allowed to develop to full term following described procedures (S9). The microinjection pipettes for mRNA IgM ZFNs of pronuclear or cytoplasmic injections were changed after injections of 20-30 embryos.

Interestingly, as is shown in Table 1, lower birth rates were observed after microinjection of the highest doses of both plasmid DNA or mRNA encoding *IgM* ZFNs at 10 ng/μL. While this is suggestive of toxicity due to ZFN activity in the genome, this dosage of nucleic acid delivered to an embryo is high by some laboratory standards (S9, S10), and could be due to cytotoxicity of the nucleic acid itself and therefore unrelated to ZFN activity.

Analysis of genome editing at ZFN target sites

For genome editing of the GFP locus, tail biopsy DNA was amplified in a ³²P-body-labeled reaction using the primers eGF_out_F1 (5'-GTTGTGCTGTCTCATCATTTTGG-3') and

eGFP_out_R4 (5' ACATAGCGTAAAAGGAGCAACAT-3') as previously described (*S11*). Genotyping of F1 offspring from founder GFPm2 was performed on Proteinase K-extracted DNA under standard PCR conditions using the primers GFP-F3 (5' - CAGTGCTTCAGCCGCTACC-3') and GFP-R5 (5' -TTGGGGTCTTTGCTCAGGGC-3'). For *IgM* targeted genome editing on founder generation animals and F1 genotyping, tail biopsy DNA from liveborn neonates was extracted following treatment with Proteinase K, and the extracted DNA was PCR amplified using Accuprime DNA polymerase (Invitrogen, Carlsbad, CA) and the primers GJC 153F (5' -GGAGGCAAGAAGATGGATTTC-3') and GJC 154R (5' -GAATCGGCACATGCAGATCT-3'). For *Rab38* genomic DNA was extracted amplified and amplified in a similar manner using the primers Rab38-F4 (5' - GTAATCGGCGACCTAGGTG-3') and Rab38-R4 (5' -TCCATTCCCGGAACCTTCAC-3'). The amplified DNA for both genomic targets was assayed for mutations using Surveyor nuclease (Transgenomic) as described below (*6*). GFP, *Rab38*, and *IgM* PCR products were directly subcloned into the pCR4-TOPO vector (Invitrogen, Carlsbad, CA), and plasmid DNA was prepared and sequenced using standard methods.

Off-target site prediction

The prediction of off-target sites of ZFN cleavage is described in detail (*S1*, *S2*). Briefly, an in vitro SELEX strategy involving binding of the zinc-finger domain to a randomized pool of target sequences is used to identify a consensus binding site. Bioinformatics tools were used to scan the most current assembly of the rat genome (RGSCv3.4) to identify putative off target sequences based on this consensus. PCR primers were designed flanking the most likely off target sites based on the number of nucleotide differences and these regions were amplified in the founder animals and tested for ZFN cleavage using the Surveyor nuclease assay as described below.

Surveyor nuclease assay

The Surveyor nuclease assay for detection of ZFN mediated genome editing is described (*S6*). Briefly, 150-300 ng PCR product (5-15 μ L) is transferred to a fresh tube, denatured and re-annealed according to the following thermocycler program: (95° for 2 min, 95° to 85° -2°C per second, 85° to 25° -0.1° per second, 4° indefinitely). 0.5 μ L of the Surveyor nuclease was added

and incubated at 42°C for 20 minutes. After immediately placing the reactions on ice, 6X Surveyor nuclease stop buffer and 0.25% Orange G (Sigma) is added and the sample is electrophoresed on a 10% polyacrylamide gel (BioRad, Hercules, CA) at 10-15 V/cm. The percentage of cleavage was estimated as previously described (56). While Surveyor nuclease digestion occasionally resulted in partial digestion, all Surveyor digestion reactions that were quantitated proceeded to completion.

Fig. S1 – Surveyor nuclease (CEL-I) assay detection of targeted mutations in IgM and Rab38

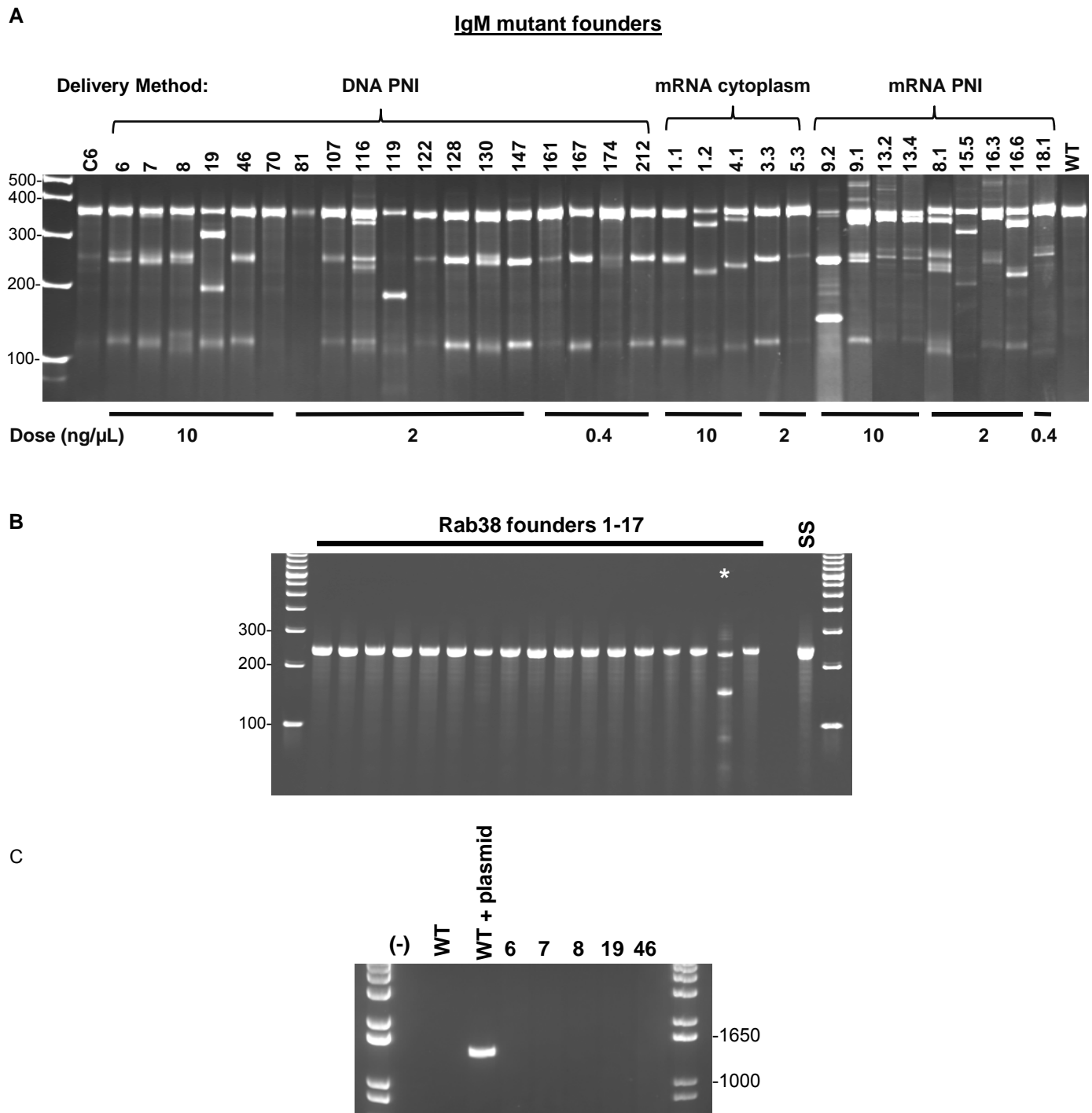


Fig. S1. Surveyor nuclease (Cel-1) analysis of mutant founders for *IgM* and *Rab38* ZFN injections. **(A)** A total of 295 founder animals were screened to identify 32 mutant founder animals. Animals are shown by delivery method (above gel) and dose (below). A positive control from transfected C6 cells and negative control from unmodified wild-type DNA (WT) are shown at either end. **(B)** Seventeen founders born after *Rab38* ZFN mRNA injections were screened using the Surveyor nuclease assay to reveal a putative mutation in founder 16 (asterisk). SS – control parental SS strain. **(C)** Of note, disruption of *IgM* after injection of ZFN-encoding plasmid is due to transient expression as no evidence for integration of the ZFN plasmid was detected by PCR for plasmid sequences among five mutant founders injected with the highest dose of plasmid (10ng/μL) (see Table 1). WT control DNA spiked with 1 copy per genome of the injected plasmid was used as a positive control. (-) – no template.

Fig. S2 - GFP ZFN off target analysis

| Site # (ranked) | Chromosome | Location | Sequence | Number of Mismatches | Homodimer (+)/ Heterodimer((-) | Gene ? |
|--------------------|------------|-----------|--|-------------------------|-----------------------------------|------------|
| 1 | chr6 | 74194373 | GAAtgtCatCgGCCACACCATCGGCcTTGacGtGGTTGTAGTTT | 9 | + | |
| 2 | chr4 | 182366143 | CcACcACAACCaCCACCACAACGAGGTGtGGTaGaAGTgT | 8 | + | |
| 3 | chr2 | 141169434 | CcACcACcACCaCCACCACcAGTAAGCTaTGGgAGaTGTcGTcA | 9 | + | |
| 4 | chr4 | 118569486 | CtcCgcCggCCGCCACAGCCTTAATGGTGTGaCGGTTGTAcTcA | 9 | + | |
| 5 | chr9 | 102238166 | AgACTAtAACTtCACCACTGCCAGTTGTGGaGGacGggGTTc | 9 | + | |
| 6 | chr15 | 67855112 | TagTtTCaTCCATcTTATCTAGTtRTGGtGGTTGTAtaTA | 9 | - | |
| 7 | chr2 | 125060854 | AAACTgCAACCTtCagAAgCCCGGtCTGTGGaGGcGTAGaTG | 9 | + | |
| 8 | chr2 | 181418141 | TAACTgtAtCTICCAcCGCTGGAAGGGGaGGTGGTgGTgGTgG | 9 | + | |
| 9 | chr6 | 112904920 | TcAtTtCcACTGCCAtACCTGATCAGGTtGagAGTTGgAGTTT | 9 | + | Nrxn3 |
| 10 | chr19 | 20556187 | TcACcACcACCaCCACcATCATcCGTGGCAGTgGcAGTgT | 9 | + | Zfp423 |
| 11 | chr4 | 146549672 | AAAtaTcCcACCaCCACATCCATCTtTTGTGctGGTTGTtGTTG | 9 | + | Gm7 |
| 12 | chr17 | 36614825 | TAgCcACAgCCGCCtCCTCCCTGGCcTTGTGaCGGTcTgGTTG | 9 | + | RGD1311307 |

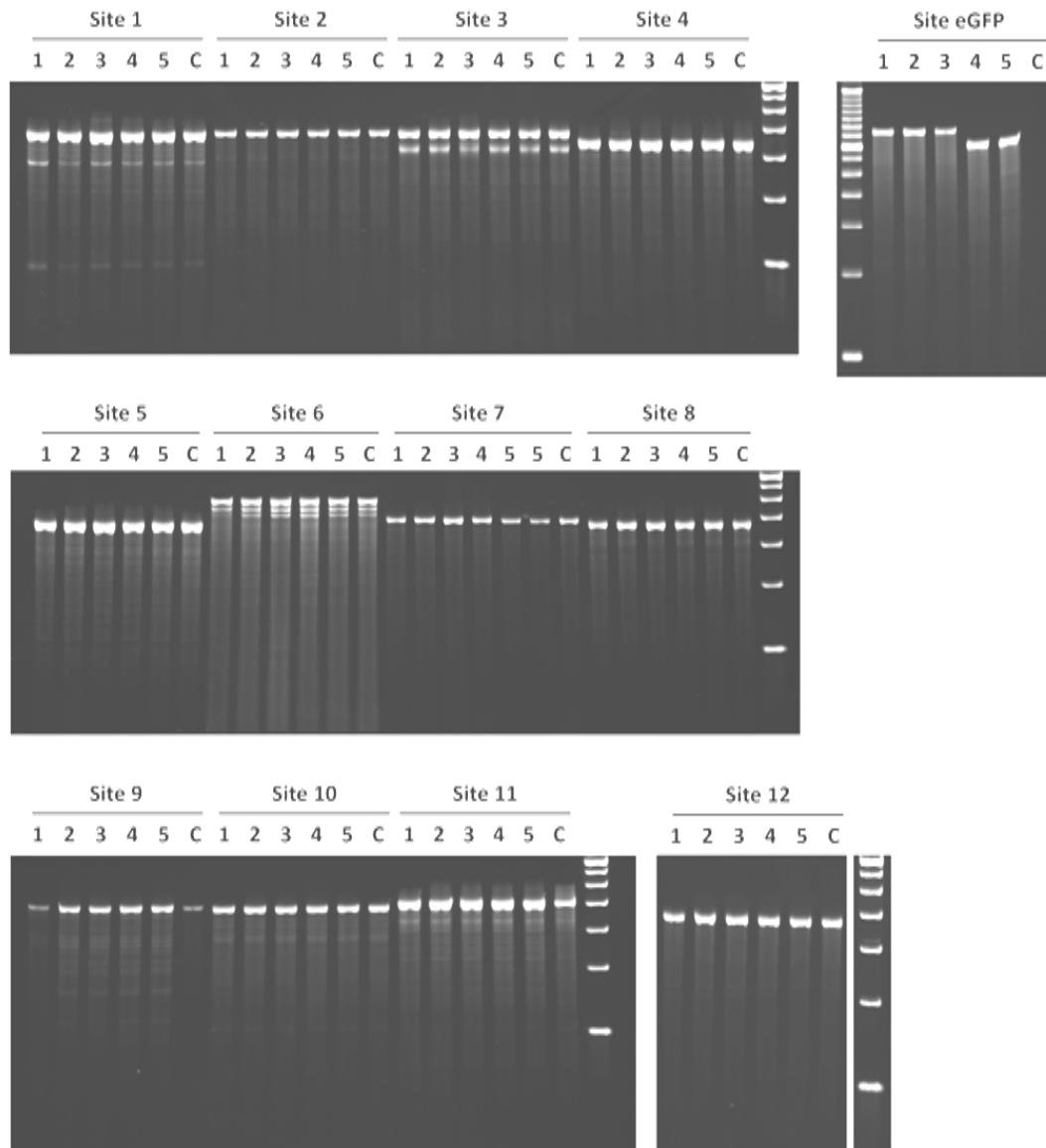


Fig. S2. Twelve off-target sites were identified as described in the materials and methods (Table). Bases differing from the consensus target sequence are shown as lowercase. The Surveyor nuclease detection method was used to screen these predicted off-target sites (see methods) in the five pups shown in Fig. 1B. In all cases we could not detect any evidence of new bands of the predicted sizes appearing in the test samples compared to wild-type SS parental strain control (C). All samples were treated with the Surveyor nuclease. Lanes 1-3 are GFP-expressing founders and lanes 4 and 5 correspond to GFP-null mutant founders GFPm1 and GFPm2, respectively. Primer sequences are found in Table S2.

Fig. S3 – IgM ZFN off target analysis

| Site# (ranked) | Chromosome | Location | Sequence | Mismatches | Homodimer (+)/ Heterodimer (-) | Gene? |
|-------------------|------------|-----------|---|------------|-----------------------------------|-----------|
| 1 | chr8 | 92436990 | AGtcAGCttCCTGTCTAGAAGAGAAcTgGGTGtCtATGGGGCC | 8 | - | |
| 2 | chr1 | 170756822 | CaAatGCCaCCTGTCTGAATGGtTaTgCtGGCaATGGGCT | 9 | - | |
| 3 | chr11 | 36783404 | GGtGAGaCCCCtGTCTTAACAAAgaTGgGgGggtTGGGaA | 9 | - | |
| 4 | chr11 | 66707758 | GatCCaAGGCCACCAAcTgGAGTTTAAGACAaaGGGCTCTgC | 8 | - | |
| 5 | chr2 | 40914750 | TGtCCATGGCCtCCtcTcTTTGCTAGAgcGGtGGCTCTCA | 9 | - | Pde4d |
| 6 | chr3 | 141510596 | GGAttGCCCCCTGTcAGTCACAGcATaTGGTGGCCATaGatG | 8 | - | LOC499913 |
| 7 | chr4 | 35317164 | GGAGAagCCCaTGtTACTCTTtAgTTGGTGGCtcTGGGaG | 9 | - | |
| 8 | chr6 | 103216965 | GcCCataGGCCAaCAAcTcTCAGGCTAGACAacGGGCTCTCA | 9 | - | Actn1 |

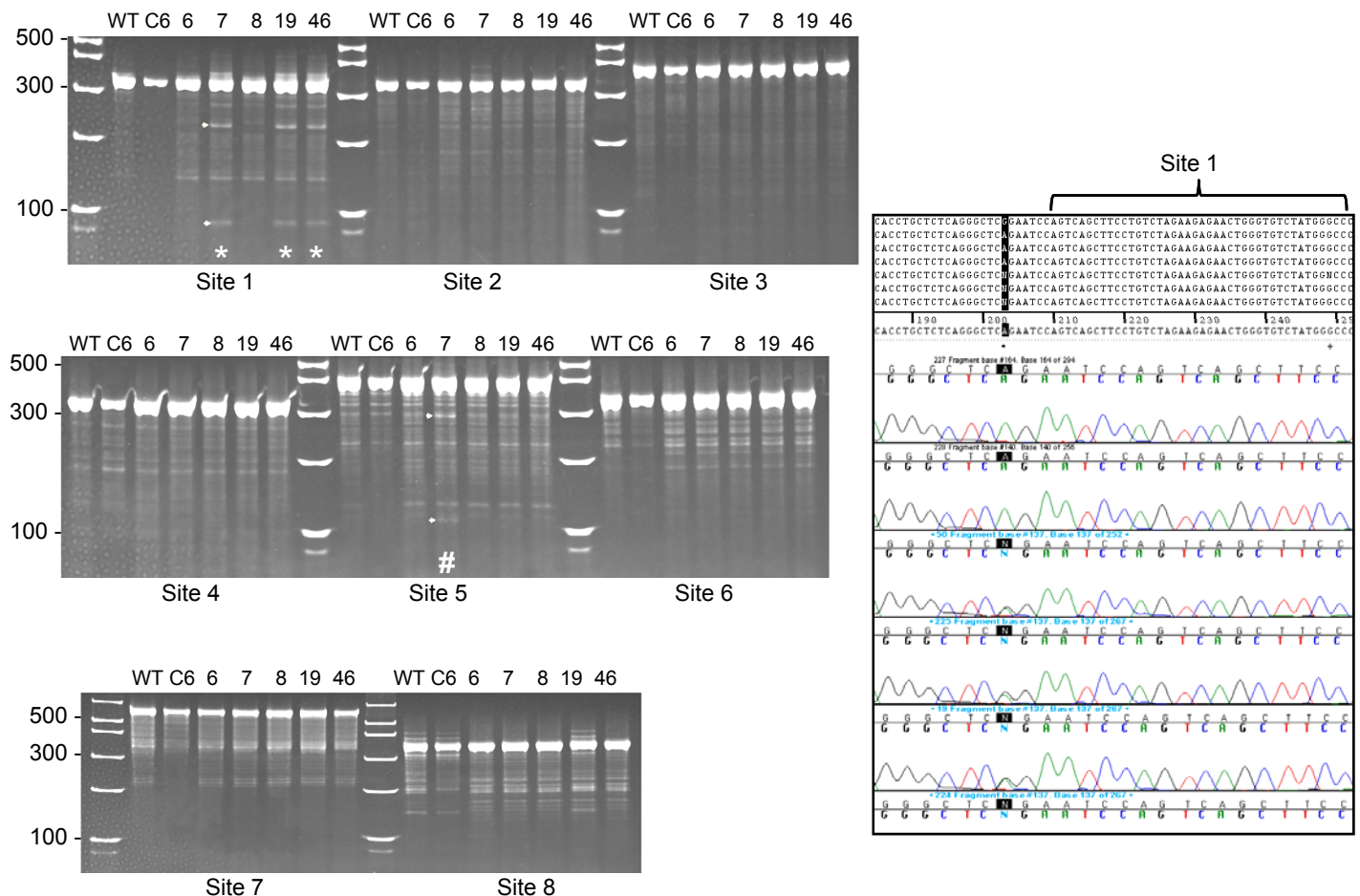


Fig. S3. Eight off-target sites were identified as described in the materials and methods (Table). Bases differing from the consensus target sequence are shown as lowercase. The Surveyor nuclease detection method was used to screen these predicted off-target sites (see methods) in five mutant founders. Site 1, which shared the most sequence similarity to the consensus target site, demonstrated cleavage at approximately 3% of chromosomes in 3 out of 5 founders (*). This cleavage was due to an A→G single nucleotide polymorphism (SNP) found in the heterogeneous SD rat strain seven base pairs upstream of Site 1 in these 3 founders by sequencing this region in all 5 animals (right panel). Since this SNP would cause heteroduplex formation, the observed cleavage is not due to off target ZFN activity, further supported by the sequence traces which show no mutation in Site 1 in any of these founders. Site 5 in founder 7 demonstrated Cel-1 cleavage, but the resulting fragments were not of the correct size to be the result of ZFN activity (#) and is likely due to a SNP. In all samples, in all other cases we could not detect any evidence of new bands of the predicted sizes appearing in the test samples, including transfected cells (C6), compared to wild-type (WT) control DNA. Primer sequences are found in Table S2.

Fig. S4 – Germline transmission of GFP and IgM mutant alleles

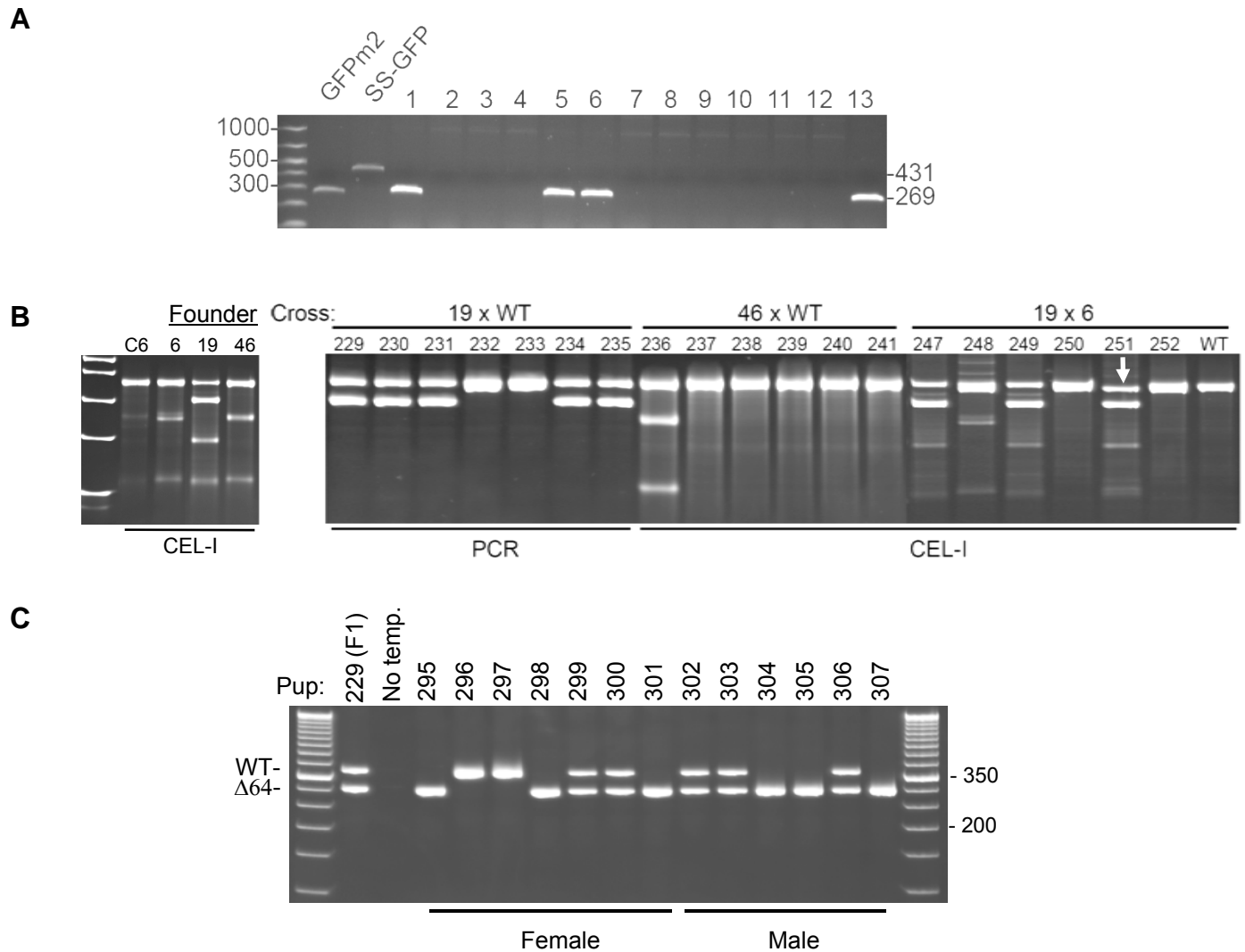


Fig. S4. Germline transmission of ZFN induced mutations. **(A)** Founder GFPm2 was bred to a wild-type SS female and 4 of 13 offspring inherited the mutant GFP sequence, but none inherited the wild-type GFP sequence as detected by genotyping PCR (see methods). SS-GFP – positive control from SS/Tg(CAG-eGFP)1Mcwi strain. **(B)** Three founders transmitted mutant IgM alleles to their offspring after backcross to wild-type (WT) or intercross as determined by PCR (founder #19) or CEL-I (founders #6, #46) (see methods). F1 animal #251 inherited the mutant allele from both founders 6 and 19. The inheritance of both alleles causes a subtle, but detectable mobility shift in the upper band (arrow) due to the 9-bp deletion transmitted by founder #6 (All genotypes were confirmed by Sanger sequencing). The lower migrating minor bands are cleavage products from the Surveyor nuclease. C6 – positive control from transfected C6 cells; WT – control wild-type DNA from the SD parental strain. **(C)** PCR genotyping 13 offspring from an intercross of F1 heterozygotes descendent of founder #19 reveals six male and female homozygous offspring carrying only the $\Delta 64$ allele, two wild-type (WT) and five heterozygotes.

Fig. S5 – Pathway to ZFN validation in yeast and cultured rat C6 cells

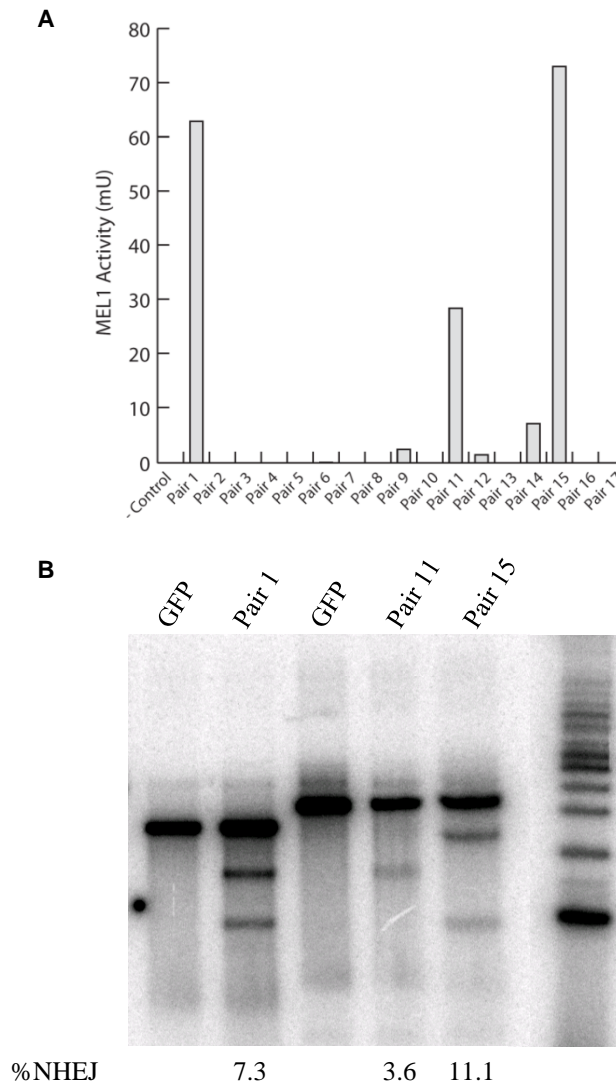


Fig. S5. Pathway to validation of GFP-targeted ZFNs. Methods are described and referenced in the supplemental methods. **(A)** Three out of seventeen pairs of designed ZFNs demonstrated significant activity in a yeast reporter assay compared to untransformed control. **(B)** These same pairs demonstrate activity in cultured rat C6 cells as determined using the Surveyor nuclease (CEL-I) assay as described in the methods. GFP mRNA was used as a negative control. Pair 15 was chosen for all subsequent rat experiments as described in the text and methods. ZFN reagents for the other genomic targets were validated in a similar fashion.

Table S1: Modified alleles from ZFN-injected rats.

| Gene | ZFN Source | Founder | Sequence* |
|--------------|------------|---------|--|
| <i>GFP</i> | | WT | AAGCTGGAGTACA <u>AACTACAACAGCCACAACG</u> TCTATATCATGGCCGACAAGCAGAAGAACGGCATCAAGGTGAACCTTCAAGATCCGCCA |
| <i>GFP</i> | mRNA | GFPm1 | GCGCACCATCTTCTTCAAGG---(156 bp deletion)---CCGACAAGCAGAAGAACGGCATCAAGGTGAACCTTCAAGATCCGCCA |
| <i>GFP</i> | mRNA | GFPm2 | AAGCTGGAGTACA <u>ACTACAACAGCCACAAC</u> ---(162 bp deletion)---CTGAGCACCAGTCCGCCCTGAGCAAAGACCCCAA |
| <i>IgM</i> | | WT | TCTCCTGCGAGAG <u>CCCCCTGTCT</u> GATGAGAATTTGGTGGCCATGGGCTGCCTGGCCCGGGACTTCCTGCCAGCTCCATTTCCTTCTCC |
| <i>IgM</i> | DNA | 6 | TCTCCTGCGAGAGCCCCCTGTCT-----TTGGTGGCCATGGGCTGCCTGGCCCGGGACTTCCTGCCAGCTCCATTTCCTTCTCC |
| <i>IgM</i> | DNA | 7 | TCTCCTGCGAGAGCCCCCTG-----GTGGCCATGGGCTGCCTGGCCCGGGACTTCCTGCCAGCTCCATTTCCTTCTCC |
| <i>IgM</i> | | 7 | TCTCCTGCGAGAGCCCCCTGT-----GGTGGCCATGGGCTGCCTGGCCCGGGACTTCCTGCCAGCTCCATTTCCTTCTCC |
| <i>IgM</i> | | 7 | TC-----ATGGGCTGCCTGGCCCGGGACTTCCTGCCAGCTCCATTTCCTTCTCC |
| <i>IgM</i> | | 7 | TCTCCTGCGAGAGCCCCCTGTC-----CATGGGCTGCCTGGCCCGGGACTTCCTGCCAGCTCCATTTCCTTCTCC |
| <i>IgM</i> | | 7 | TCTCCTGCGAGAGCCCCCTGTCTGA-----ATTTGGTGGCCATGGGCTGCCTGGCCCGGGACTTCCTGCCAGCTCCATTTCCTTCTCC |
| <i>IgM</i> | DNA | 8 | TCTCCTGCGAGAGCGGCC-----ATGGGCTGCCTGGCCCGGGACTTCCTGCCAGCTCCATTTCCTTCTCC |
| <i>IgM</i> | | 8 | TCTCCTGCGAGAGCCCCCT---ATCACAAATTTGGTGGCCATGGGCTGCCTGGCCCGGGACTTCCTGCCAGCTCCATTTCCTTCTCC |
| <i>IgM</i> | DNA | 19 | TCTCCTGCGAGAGCCCCCTGTCT-----CC |
| <i>IgM</i> | DNA | 46 | TCTCCTGCGAGAGCCCCCTGTCTGA-----ATTTGGTGGCCATGGGCTGCCTGGCCCGGGACTTCCTGCCAGCTCCATTTCCTTCTCC |
| <i>IgM</i> | DNA | 81 | TCTCCTGCGAGAGCCCCCT---ATCACAAATTTGGTGGCCATGGGCTGCCTGGCCCGGGACTTCCTGCCAGCTCCATTTCCTTCTCC |
| <i>IgM</i> | DNA | 107 | TCTCCTGCGAGAGCCCCCTGTCTGA-----ATTTGGTGGCCATGGGCTGCCTGGCCCGGGACTTCCTGCCAGCTCCATTTCCTTCTCC |
| <i>IgM</i> | DNA | 119 | TCTCCTGCGAGAGCCCCCTTG----- (187 bp deletion) -----AAGGTTCAGA |
| <i>IgM</i> | DNA | 122 | TCTCCTGCGAGAGCCCCCTGTCTG---GGAATTTGGTGGCCATGGGCTGCCTGGCCCGGGACTTCCTGCCAGCTCCATTTCCTTCTCC |
| <i>IgM</i> | | 122 | TCTCCTGCGAGAGCCCCCTGTCTG---GAATTTGGTGGCCATGGGCTGCCTGGCCCGGGACTTCCTGCCAGCTCCATTTCCTTCTCC |
| <i>IgM</i> | mRNA | 1.1 | TCTCCTGCGAGAGCCCCCTGTCT---GAATTTGGTGGCCATGGGCTGCCTGGCCCGGGACTTCCTGCCAGCTCCATTTCCTTCTCC |
| <i>IgM</i> | mRNA | 1.2 | TCTCCTGCGAGAGCCCC-----GGGACTTCCTGCCAGCTCCATTTCCTTCTCC |
| <i>IgM</i> | mRNA | 3.3 | TCTCCTGCGAGAGCCCCCTGTCTGATG-GAATTTGGTGGCCATGGGCTGCCTGGCCCGGGACTTCCTGCCAGCTCCATTTCCTTCTCC |
| <i>IgM</i> | mRNA | 4.1 | TCTCCTGCGAGAGCCCCCTG-----GCTGCCTGGCCCGGGACTTCCTGCCAGCTCCATTTCCTTCTCC |
| <i>IgM</i> | mRNA | 8.1 | TCTCCTGCGAGAGCCCCCTGTCTG-----GCCCCGGGACTTCCTGCCAGCTCCATTTCCTTCTCC |
| <i>IgM</i> | | 8.1 | TCTCCTGCGAGAGCC---ATGGTGGCCCGGAATTTGGTGGCCATGGGCTGCCTGGCCCGGGACTTCCTGCCAGCTCCATTTCCTTCTCC |
| <i>IgM</i> | mRNA | 9.2 | TCTCCTGCGAGAGCC----- (224 bp deletion) -----ACCATGGCAACAAAAACAAGATCTGCATGTGCCGATTCCAG |
| <i>IgM</i> | mRNA | 9.2 | TCCTCAGAGAGTGGT----- (123 bp deletion) -----CAGAACAACTGAA |
| <i>Rab38</i> | | WT | AGGCAAGACCAGCATCATCAAGCGCTACGTGCACCAAACTTCTCTCCACTACCGGGCCACCATTTGGTGTGGACTTCGCGCTGAAGG |
| <i>Rab38</i> | mRNA | R38-16 | AGGCAAGACCAGCATCATCAAGCGCTACGTGCACCAAACTT----- (96 bp deletion) -----CGCAGGT |
| <i>Rab38</i> | | R38-16 | AGGCAAGACCAGCATCATCAAGCGCTACGTGCACCAAACTTCTC-----CGGGCCACCATTTGGTGTGGACTTCGCGCTGAAGG |

* For each gene, the wild-type sequence is in the first row with the ZFN binding sites underlined and in italics. Multiple mutant alleles are detected in some founders, reflecting independent ZFN cleavage and NHEJ-mediated repair events. No wild-type sequences were recovered from GFP mutant pups (GFPm1, GFPm2). Wild-type IgM sequence was also recovered from all IgM ZFN-injected rats except rat 122 and no modified sequences were recovered for the weakly CEL-I positive rats 70 (0/12) and 5.3 (0/16).

Table S2: Primer sequences for off-target analysis.

| Name | SEQUENCE (5'-3') |
|-------------|--------------------------|
| eGFP-OT1_F | GCCCAAGTCTCCTTTACTGC |
| eGFP-OT1_R | TGGGTTTATTGTGTGCCCTA |
| eGFP-OT2_F | TGGTCCTAGGTCCTTTGGAA |
| eGFP-OT2_R | TCTGCATGCTGACCAGTCTC |
| eGFP-OT3_F | CCACTCATGCACATCGAAAG |
| eGFP-OT3_R | CAC TTGATATAAGCTGGGGTCAC |
| eGFP-OT4_F | TAAAATCCACGCAGCCAAAG |
| eGFP-OT4_R | GGTGATGATGCCATGAGATG |
| eGFP-OT5_F | CCAGCTAGGAGAAAGCAAGA |
| eGFP-OT5_R | AAGTCTGCTGCCCTACCTCTCC |
| eGFP-OT6_F | AACAAGGCCATCCTCTGCTA |
| eGFP-OT6_R | AACCAAAGTGCATAGGCTTCA |
| eGFP-OT7_F | ATGGTTCCGTGCAACAATTT |
| eGFP-OT7_R | TTTGCTCATTAGAGCCCTTCA |
| eGFP-OT8_F | GGTCACTTGGAAGCTCTTG |
| eGFP-OT8_R | TATTGAAGGCCTGGGTTGAC |
| eGFP-OT9_F | CCCTGATGACAGAGCAAAGG |
| eGFP-OT9_R | CTCTTGGGAAAATGCCCTTA |
| eGFP-OT10_F | GGTTAGCATTGTGGCCTGTT |
| eGFP-OT10_R | TGATCATTGGCTTTGAGCAG |
| eGFP-OT11_F | GCTCAGGAATTTGCCTCTCA |
| eGFP-OT11_R | CAAGCACAAAGACCTAAACTTGT |
| eGFP-OT12_F | TTTGCTGGAAAGCGTAGAGAC |
| eGFP-OT12_R | CCTCTCCAGTTTCCTCCACTC |
| eGFP-OTC_F | TCGTGACCACCCTGACCTAC |
| eGFP-OTC_R | GAACTCCAGCAGGACCATGT |
| IgM-OT#1 F | GGTCAATCAGTAGGAAGTTT |
| IgM-OT#1 R | GCTTCTCCAGCCTAAAAGCT |
| IgM-OT#2 F | GGTGTAGTTACATTGTTGTTGC |
| IgM-OT#2 R | TTAATAAACGCCAGGAACCA |
| IgM-OT#3 F | AGGGAGAAGAGTGTCCTG |
| IgM-OT#3 R | CTGGAGACTAGAAGTCCAAG |
| IgM-OT#4 F | TGAGAGTGTGTAGACTCACA |
| IgM-OT#4 R | CAAAGTTTTCTAGGGAAGTTCC |
| IgM-OT#5 F | CAGCATTCTCCTAATTTTCACA |
| IgM-OT#5 R | GCTACTCAGTCTGTGGGTTG |
| IgM-OT#6 F | GATAGTGAAGACACAGGTGAG |
| IgM-OT#6 R | GTTAGTTTTACATCATGCACCC |
| IgM-OT#7 F | CTCAACTCGTTCTTCTTATTCAG |
| IgM-OT#7 R | CATCTCTTATGTAGAGACACC |
| IgM-OT#8 F | GTCTCTTGGGATAAAAGACAC |
| IgM-OT#8 R | CATGCCGTCTTCTCTTGCTT |

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