

CRISPR-mediated KO/KI mutations at rat *Tyr* loci

Wistar	WT		CATGGTTTCCAGGATTATGTAATAGTGGTCCCT
#1	KI/KO*	T>C	CATGGTTTCCAGGATTACGTAATAGTGGTCCCT
		+1	CATGGTTTCCAGGATTATGTAAATAGTGGTCCCT
		-4	CATGGTTTCCAGGATTATGTAA----GGTCCCT
#2	KI/WT	T>C	CATGGTTTCCAGGATTACGTAATAGTGGTCCCT
#3	KI/KO	T>C	CATGGTTTCCAGGATTACGTAATAGTGGTCCCT
		+1	CATGGTTTCCAGGATTATGTAAATAGTGGTCCCT
#4	KI/KO/WT	T>C	CATGGTTTCCAGGATTACGTAATAGTGGTCCCT
		+1	CATGGTTTCCAGGATTATGTAAATAGTGGTCCCT
#5	KI/WT	T>C	CATGGTTTCCAGGATTACGTAATAGTGGTCCCT
#6	KI/WT	T>C	CATGGTTTCCAGGATTACGTAATAGTGGTCCCT
#7	KI/KO	T>C	CATGGTTTCCAGGATTACGTAATAGTGGTCCCT
		+1	CATGGTTTCCAGGATTATGTAAATAGTGGTCCCT
#8	KI/WT	T>C	CATGGTTTCCAGGATTACGTAATAGTGGTCCCT
#9	KI/KO	T>C	CATGGTTTCCAGGATTACGTAATAGTGGTCCCT
		-7	CATGGTTTCCAGGATTATGTAAT-----GTGGTCCCT
#10	KI/WT	T>C	CATGGTTTCCAGGATTACGTAATAGTGGTCCCT
#11	KI/KO/WT*	T>C	CATGGTTTCCAGGATTACGTAATAGTGGTCCCT
		+1	CATGGTTTCCAGGATTATGTAAATAGTGGTCCCT
#12	KI/WT	T>C	CATGGTTTCCAGGATTACGTAATAGTGGTCCCT
#14	KO	+1	CATGGTTTCCAGGATTATGTAAATAGTGGTCCCT
#15	KO/WT	+1	CATGGTTTCCAGGATTATGTAAATAGTGGTCCCT
#17	KI/KO/WT*	T>C	CATGGTTTCCAGGATTACGTAATAGTGGTCCCT
		-3	CATGGTTTCCAGGATTATGTAAAT---GGTCCCT
#18	KO	-1	CATGGTTTCCAGGATTATGTAATAGTGGTCCCT
		-4	CATGGTTTCCAGGATTATGT-----GTGGTCCCT
#19	KO/WT	+1	CATGGTTTCCAGGATTATGTAAATAGTGGTCCCT
#21	KO/WT	+1	CATGGTTTCCAGGATTATGTAAATAGTGGTCCCT
#22	KO/WT	+1	CATGGTTTCCAGGATTATGTAAATAGTGGTCCCT
#24	KO	-3	CATGGTTTCCAGGATTATGT---TAGTGGTCCCT
#26	KO/WT	+2	CATGGTTTCCAGGATTATGTAAATAGTGGTCCCT
#27	KO*	+1	CATGGTTTCCAGGATTATGTAAATAGTGGTCCCT
		-3	CATGGTTTCCAGGATTATGTAAAT---GGTCCCT
		-6	CATGGTTTCCAGGATTATGT-----GGTCCCT
#28	KO/WT	-3	CATGGTTTCCAGGATTATGTAAAT---GGTCCCT
#29	KO	+1	CATGGTTTCCAGGATTATGTAAATAGTGGTCCCT
#30	KO/WT	+1	CATGGTTTCCAGGATTATGTAAATAGTGGTCCCT
#31	KO/WT	+1	CATGGTTTCCAGGATTATGTAAATAGTGGTCCCT

Supplementary Figure 1. CRISPR-mediated KO/KI mutation using Cas9-poly(A) plasmids. Cas9-poly(A) mRNA, gRNA targeting of the *Tyr^c* allele, and ssODN for recovering the albino phenotype, were microinjected into the male pronucleus of fertilized Wistar rat embryos. Sequence analysis of the 31 pups delivered showed 13 KI alleles, indicated by an orange letter (KI), as well as a variety of indel mutations at the targeted *Tyr* locus, indicated by red letters (KO). Asterisks indicate pups carrying mosaic mutations.

Rat Thy1-ATG EGFP ssODN (+)

TATCACGGGGCTGACATGCAGCTTTCCCCACTGCAGACCCAGGACGGAGCTATTGGCACC**ATGG**
TGAGCAAGGGCGAGGAGCTGTTACCGGGGTGGTGCCCATCCTGGTCGAGCTGGACGGCGACGT
AAACGGCCACAAGTTCAGCGTGTCCGGCGAGGGCGAGGGCGATGCCACCTACGGCAAGCTGACC
CTGAAGTTCATCTGCACCACCGCAAGCTGCCCCTGCCCTGGCCCACCCTCGTGACCACCCTGA

EGFP Small F
CCTACGGCGTGCACTGCTTCAGCCG**CTACCCCGACCACATGAAG**CAGCACGACTTCTTCAAGTC
CGCCATGCCCCGAAGGCTACGTCCAGGAGCGCACCATCTTCTTCAAGGACGACGGCAACTACAAG
ACCCGCGCCGAGGTGAAGTTCGAGGGCGACACCCTGGTGAACCGCATCGAGCTGAAGGGCATCG
ACTTCAAGGAGGACGGC**AACATCCTGGGGCACAAG**CTGGAGTACAACCTACAACAGCCACAACGT
EGFP_Small_R

CTATATCATGGCCGACAAGCAGAAGAACGGCATCAAGGTGAACTTCAAGATCCGCCACAACATC
GAGGACGGCAGCGTGCACTCGCCGACCACTACCAGCAGAACACCCCATCGGGCGACGGCCCCG
TGCTGCTGCCCCGACAACCACTACCTGAGCACCCAGTCCGCCCTGAGCAAAGACCCCAACGAGAA
GCGCGATCACATGGTCCTGCTGGAGTTCGTGACCGCCGCCGGGATCACTCTCGGCATGGACGAG
CTGTACAAGAACCCAGTCATCAGCATCACTCTCCTGCTTTTCAAGGTACTGGGCAAGGGTCAGGGC
TGGCA

Supplementary Figure 2. Long ssODN (lsODN) used for GFP-KI at the start codon (ATG) of the rat thymus cell antigen 1 (*Thy1*) locus. The lsODN consists of GFP-coding sequences (green) and two 60-bp homology sequences (black) at either end of the GFP. A set of primers used for the PCR analysis is shown in boxes (see also **Supplementary Figure 3**).

rThy1 ATG Small F

GCTGACATGCAGCTTTCCCCACTGCAGACCCAGGACGGAGCTATTGGCACCCTGAACCCAGTCATCAGCATC

rThy1 ATG Small R

GATCACCTGCTGGTGGTGGTCTTTGACATATTGGAAATTCCTACTGGATCTAAGAACTCCTCTTGCTGGGT
GGTGGTGGTGGTGCACACCTTTAATCCCAGCACTTAGGAGGCAGAGTCAGGTGGGTCTGTAAGTTTGAGGCC
AACCTGGTCTACAGAGTGAGTTCCAGGATATCCAGAACTGTTTGCAAGACAAAGAAACCCTTTCTTGAAAA
CCATTTAAACACAAACACACACACACACACACACACACACACATTCTCTCTCTCTCTCTCTCTCTCTCTC
TC
TTGGGGGCCCAGAACTTCAGTAGATTAGATGGAAATTGAAGGTGGTAGAACATCCCACCAATACCCCAGGGAT
GAGTGCAAAGCTTGAATCCTCCCAGGTCAAGTTTACTTTCTCTGCAGGTGGGAGGCCCTAGTCTGTCTCTCC
CCCAATTACAGAGAAGACACTGCTGTGCCAGTCTTGCCAGATGTCCCGAGGACAGAGGGTGATCAGCCTGACAG
CCTGCCTGGTGAACCAGAACCTTCGACTGGACTGCCGTCATGAGAATAACACCAACTTGCCCATCCAGCATG
AGTTCAGCCTGACCCGAGAGAAGAAGAAGCACGTGCTGTGTCAGGCACCCTGGGGGTTCCCGAGCACACTTACC
GCTCCCGCGTCAACCTTTTCAGTGACCGCTTTATCAAGGTCCTTACTCTAGCCAACTTCACCACCAAGGATG
AGGGCGACTACATGTGTGAACTTCGAGTCTCGGGCCAGAATCCCACAAGCTCCAATAAACTATCAATGTGA
TCAGAGGTGAGACTGGTCCTCAGAAAAATGGAAGGTCCAGGTTAGCCAGTCCGGGGTAGCCAATGGGCCTGT
AAAACAACAGGCAGCTCCCGATAGCCAGGCCTGAGGGTAGAGGAATGCTCTAGCCATAACTGCTGGTCCCCCT
GGGGAACCGCTGCCCTCTGTGTGAGCGGGTGGGGTGGGGGGGATCAAGGGCCAGATAGAGAGAGCTTGCAGT
AAAAAATACAGCTGAGGACACTGCAAATGGGAAAGGTGGTAGCATGGCGGCTCTTGCAGTGTAGAGGTTCTG
TTACACCATCAGGTGGAGCTGCTAAGACATCAAAAAGCACCAGCTCTCTCCCTTGAACAGTCTTTGCCAGCC

rThy1 TGA Small F
Exon 4

TGAGTCTGATCTCCCTGCCTCCCGGTTGGTACCCCTTCTCTCCACAGACAAGCTGGTCAAGTGTGGTGGCAT

AAGCCTGCTGGTTCAAAACACTTCCTGGCTGCTGCTGCTCCTGCTTTCCCTCTCCTTCCTCCAAGCCACGGA

rThy1 ATG target PAM

CTTCATTTCCTCTCTGACTGGTTGGGCCCAAGGAGAAACAGGAAACCTCAAGGTCTGCTGAAGAGGTCTTGCT
TCTCCCGGTCTAGCTGACTCCCTCCCCAAGACCTTCAAATATCTCAAAACGCGGGGAGAAATGGGGACCCTGT
CCCTCCTAGGGAACCCAGTGCTGCATGCCATCACCCCCACCCTCACCCCCGCCACTTCACCCTCAGTGCAC
ACCACGAGCTGCCATTTTGTACTCTGTATTCCGGGGCTGCTTCTGATTAGTTTGTCTTGGAGACCCGATGGA
ACACCAGGGTGTATGATGGGCAGTGAAGAGGCAGGATATCACCCCCCGGGTGAGTTCCTCTTTGCCTTCTA
AGCCAGATGCCTGAAAGAGATATGGATGAGGGAAGTTGGAAGTGTGTCTGTGCCTGGTATAGTGATACTCTGT
TGAAAGTATCGCCCAGTAGGCA

rThy1 TGA Small R

Supplementary Figure 3. gRNAs designed to target the start codon (ATG) and the stop codon (TGA) of the rat *Thy1* locus. The gRNA-binding sequences (blue) and the PAM sequences (green) are indicated at the *Thy1* ATG and TGA sequences (red boxes), respectively. Exons are shown as orange boxes. The ssODN sequences are underlined (pink and yellow). The primer sets (small) used for the PCR analysis are shown in boxes (see **Supplementary Table 6**).

CRISPR-mediated KO/KI mutations at rat *Thy1* (ATG) locus

Wistar	WT		AGAGTGATGCTGATGACTGGGTTCATGGTGCCA
#1	KO/WT	+1	AGAGTGATGCTGATGACTGGGTTTCATGGTGCCA
#2	KO/WT*	-7	AGAGTGATGCTGATGACTG-----GGTGCCA
		-8	AGAGTGATGCTGATGACT-----GGTGCCA
#4	KO/WT*	-7	AGAGTGATGCTGATGACTG-----GGTGCCA
		-15	AGAGTGATG-----ATGGTGCCA
#5	KI/KO/WT	KI-150	AGAGTGATGCTGATGACTGGGTTCCTT (GFP) --CCA
		-7	AGAGTGATGCTGATGACTG-----GGTGCCA
#7	KO/WT	-7	AGAGTGATGCTGATGACTG-----GGTGCCA
#8	KO/WT	+1	AGAGTGATGCTGATGACTGGGTTTCATGGTGCCA
#9	KI/WT	KI-171	AGAGTGATGCTGATGACTGGGTTCCTT (GFP) ---AAG
#10	KO/WT	-7	AGAGTGATGCTGATGACTG-----GGTGCCA
#11	KO/WT	+2	AGAGTGATGCTGATGACTGGGTGTTTCATGGTGCCA
#12	KO/WT*	-7	AGAGTGATGCTGATGACTG-----GGTGCCA
		-8	AGAGTGATGCTGATGACT-----GGTGCCA
#14	KO/WT	-7	AGAGTGATGCTGATGACTG-----GGTGCCA
#15	KO	-8	AGAGTGATGCTGATGACT-----GGTGCCA
		-48	TGACC----- (-48bp) -----CCAAT
#16		LD	Large Deletion
#17	KO/WT*	+1	AGAGTGATGCTGATGACTGGGTTTCATGGTGCCA
	KO	-1	AGAGTGATGCTGATGACTGGG-TCATGGTGCCA
#18	KO/WT	-7	AGAGTGATGCTGATGACTG-----GGTGCCA
#19	KO/WT	LD	Large Deletion
#20	KO/WT	+1	AGAGTGATGCTGATGACTGGGTTTCATGGTGCCA
#21	KO/WT*	-9	AGAGTGATGCTGATGAC-----GGTGCCA
		+1	AGAGTGATGCTGATGACTGGGTTTCATGGTGCCA
#22	KO/WT	-11	AGAGTGAT-----ACACCATGGTGCCA
#23	KO/WT	-7	AGAGTGATGCTGATGACTG-----GGTGCCA
#24	KO/WT	+1	AGAGTGATGCTGATGACTGGGTTTCATGGTGCCA
#25	KO/WT	-7	AGAGTGATGCTGATGACTG-----GGTGCCA
#26	KO/WT	+1	AGAGTGATGCTGATGACTGGGTTTCATGGTGCCA
#27	KO/WT*	-7	AGAGTGATGCTGATGACTG-----GGTGCCA
		-8	AGAGTGATGCTGATGACT-----GGTGCCA
#28	KO/WT*	+1	AGAGTGATGCTGATGACTGGGTTTCATGGTGCCA
		-7	AGAGTGATGCTGATGACTG-----GGTGCCA
#30	KO/WT	-5	AGAGTGATGCTGATGA-----TTCATGGTGCCA
#31	KO/WT	+1	AGAGTGATGCTGATGACTGGGTTTCATGGTGCCA
#33	KO/WT	-7	AGAGTGATGCTGATGACTG-----GGTGCCA

Supplementary Figure 4. NHEJ-mediated KO mutations at the start codon of the rat *Thy1* locus.

Cas9-poly(A) mRNA, gRNA targeting the start codon (ATG) of the rat *Thy1* allele, and an lsoDN were microinjected into the male pronucleus of fertilized Wistar rat embryos. Sequence analysis of the 33 representative pups delivered showed a variety of indel mutations at the targeted *Thy1* (ATG) locus, as shown by red letters (KO). Asterisks indicate pups carrying mosaic mutations.

Rat *Thy1*-TGA 2A-EGFP-HA ssODN (-)

CACTGCCCATCATACACCCTGGTGTTCATCGGGTCTCCAGGACAACTAATCAGAAGCAGCCC
CGGAATACAGAGTACAAAATGGCAGCTCGTGGTGTGCACTGAGGGTGAAGTGGCGGGGGTGAGG
GTGGGGGTGATGGCATGCAGCACTGGGGTTCCCTAGGAGGGACAGGGTCCCCATTTCTCCCCGC
GTTTTGAGATATTTGAAGGTCTTGGGGAGGGAGTCAGCTGACCGGGAGAAGCAAGACCTCTTCA
GCAGACCTTGAGGTTTCCTGTTTCTCCTTGGGCCCAACCAGTCAggtggggtgggcgaagaact
ccagcatgagatccccgcgctgcagTTACTTGTACAGCTCGTCCATGCCGAGAGTGATCCCGGC
GGCGGTACGAACTCCAGCAGGACCATGTGATCGCGCTTCTCGTTGGGGTCTTTGCTCAGGGCG
GACTGGGTGCTCAGGTAGTGGTTGTCGGGCAGCAGCACGGGGCCGTCGCCGATGGGGGTGTTCT
GCTGGTAGTGGTCGGCGAGCTGCACGCTGCCGTCTCGATGTTGTGGCGGATCTTGAAGTTCAC
CTTGATGCCGTTCTTCTGCTTGTCTCGGCCATGATATAGACGTTGTGGCTGTTGTAGTTGTACTCC

EGFP_Small_R

AGCTTGTGCCCCAGGATGTTGCCGTCCTCCTTGAAGTCGATGCCCTTCAGCTCGATGCGGTTCA
CCAGGGTGTGCCCCTCGAACTTCACCTCGGCGCGGGTCTTGTAGTTGCCGTCGTCTTGAAGAA
GATGGTGCCTCCTGGACGTAGCCTTCGGGCATGGCGGACTTGAAGAAGTCGTGCTGCTTCATG
TGGTCGGGGTAGCGGCTGAAGCACTGCACGCCGTAGGTCAGGGTGGTC

EGFP_Small_F

ACGAGGGTGGGCCAGGGCACGGGCAGCTTGCCGGTGGTGCAGATGAACTTCAGGGTCAGCTTGC
CGTAGGTGGCATCGCCCTCGCCCTCGCCGGACACGCTGAACTTGTGGCCGTTTACGTCGCCGTC
CAGCTCGACCAGGATGGGCACCAACCCGGTGAACAGCTCCTCGCCCTTGCTCACATGGGCCG
GGATTCTCCTCCACGTCACCGCATGTTAGAAGACTTCTCTGCCCTCTCCGCCGCCGGACCTCA
GAGAAATGAAGTCCGTGGCTTGGAGGAAGGAGAGGGAAAGCAGGAGCAGCAGCAGCCA

Supplementary Figure 5. Long ssODN (lsODN) used for GFP-KI at the stop codon (TGA) of the rat *Thy1* locus. The lsODN consists of GFP-coding sequences (green), 2A peptide sequences (blue), extra 3'-UTR sequences (small), and a 300-bp homology sequence (large) at the 5' end of the lsODN that can be deleted, and a 60-bp homology sequences (black) at the 3' end of the lsODN. A set of primers used for PCR analysis is shown in boxes (see also **Supplementary Figure 3**).

CRISPR-mediated KO/KI mutations at rat *Thy1* (TGA) locus

Wistar	WT		GCCACGGACTTCATTTCTCTGTGACTGGTTGGG
#1	KO	+1	GCCACGGACTTCATTTCTCTGT T GACTGGTTGGG
#2	KO	+1	GCCACGGACTTCATTTCTCTGT T GACTGGTTGGG
#3	KO	+1	GCCACGGACTTCATTTCTCTGT T GACTGGTTGGG
#4	KO	-6	GCCACGGACTTCATTTCTCTG-----GTTGGG
		+1	GCCACGGACTTCATTTCTCTGT T GACTGGTTGGG
#5	KO	+1	GCCACGGACTTCATTTCTCTGT T GACTGGTTGGG
#6	KO	+1	GCCACGGACTTCATTTCTCTGT T GACTGGTTGGG
#7	KI/KO	KI	GCCACGGACTTCATTTCTCTG (2A, GFP, 3' UTR) T GACT
		+1	GCCACGGACTTCATTTCTCTGT T GACTGGTTGGG
#8	KO	TG>AT	GCCACGGACTTCATTTCTCTG A TACTGGTTGGG
		+1	GCCACGGACTTCATTTCTCTGT T GACTGGTTGGG
#9	KI/KO	KI	GCCACGGACTTCATTTCTCTG (2A, GFP, 3' UTR) T GACT
		+1	GCCACGGACTTCATTTCTCTGT T GACTGGTTGGG
#10	KI	KI	GCCACGGACTTCATTTCTCTG (2A, GFP, 3' UTR) T GACT
#11	KI/KO	KI	GCCACGGACTTCATTTCTCTG (2A, GFP-----
		+1	GCCACGGACTTCATTTCTCTGT T GACTGGTTGGG
#12	KO	-7	GCCACGGACTTCATTTCT-----TGGTTGGG
		+1	GCCACGGACTTCATTTCTCTGT T GACTGGTTGGG
#13	KO	+1	GCCACGGACTTCATTTCTCTGT T GACTGGTTGGG
#14	KO	+1	GCCACGGACTTCATTTCTCTGT T GACTGGTTGGG
#15	KO*	-8	GCCACGGACTTCAT-----GACTGGTTGGG
		+1	GCCACGGACTTCATTTCTCTGT T GACTGGTTGGG
#16	KO	+1	GCCACGGACTTCATTTCTCTGT T GACTGGTTGGG
#17	KO	-1	GCCACGGACTTCATTTCT-GAGAACTGGTTGGG
		+1	GCCACGGACTTCATTTCTCTGT T GACTGGTTGGG
#18	KI/KO*	KI	-----GFP, 3' UTR) T GACT
		-349	GCCACGGACTTCATTTCTCTG--- (-349bp) ---CCAGA
		-25	GCCA-----TGGG
		+1	GCCACGGACTTCATTTCTCTGT T GACTGGTTGGG
#19	KO	+1	GCCACGGACTTCATTTCTCTGT T GACTGGTTGGG
#20	KO	-10	GCCACGGACTT-----TGACTGGTTGGG
#21	KO	-15	GCCACGGACT-----GGAATTGGG
		+1	GCCACGGACTTCATTTCTCTGT T GACTGGTTGGG
#22	KO	-6	GCCACGGACTTCATTTCTCTG-----GTTGGG
#23	KO	-1	GCCACGGACTTCATTTCTCTG-AGCTGGTTGGG
		+1	GCCACGGACTTCATTTCTCTGT T GACTGGTTGGG
#24	KI	KI	GCCACGGACTTCATTTCTCTG (2A, GFP, 3' UTR) T GACT
#25	KO	+1	GCCACGGACTTCATTTCTCTGT T GACTGGTTGGG
#26	KO	+1	GCCACGGACTTCATTTCTCTGT T GACTGGTTGGG
#27	KO	+1	GCCACGGACTTCATTTCTCTGT T GACTGGTTGGG
#28	KO	+1	GCCACGGACTTCATTTCTCTGT T GACTGGTTGGG
#30	KO	-6	GCCACGGACTTCATTTCTCTG-----GTTGGG
		+1	GCCACGGACTTCATTTCTCTGT T GACTGGTTGGG
#31	KO	+1	GCCACGGACTTCATTTCTCTGT T GACTGGTTGGG
#32	KO/WT	+1	GCCACGGACTTCATTTCTCTGT T GACTGGTTGGG
#33	KO	+1	GCCACGGACTTCATTTCTCTGT T GACTGGTTGGG
#34	KO/WT	+1	GCCACGGACTTCATTTCTCTGT T GACTGGTTGGG
#35	KO	+1	GCCACGGACTTCATTTCTCTGT T GACTGGTTGGG
#36	KO/WT	+1	GCCACGGACTTCATTTCTCTGT T GACTGGTTGGG

Supplementary Figure 6. NHEJ-mediated KO mutations at the stop codon of the rat *Thy1* locus.

Cas9-poly(A) mRNA, gRNA targeting the stop codon (TGA) of the rat *Thy1* allele, and an lsODN were microinjected into the male pronucleus of fertilized Wistar rat embryos. Sequence analysis of the 36 pups delivered showed a variety of indel mutations at the targeted *Thy1* (TGA) locus, as shown by red letters (KO). Asterisks indicate pups carrying mosaic mutations.

pCAG-GFP vector (5497bp)

pCAGGS Large F

TCAAGGCGAGTTACATGATCCCCCATGTTGTGCAAAAAAGCGGTTAGCTCCTTCGGTCCTCCGA
TCGTTGTCAGAAGTAAGTTGGCCGAGTGTTATCACTCATGGTTATGGCAGCACTGCATAATTC
TCTTACTGTCATGCCATCCGTAAGATGCTTTTCTGTGACTGGTGAGTACTCAACCAAGTCATTC
TGAGAATAGTGTATGCGGCGACCGAGTTGCTCTTGCCCGGCGTCAATACGGGATAATACCGCGC
CACATAGCAGAACTTTAAAAGTGCTCATCATTGGAAAACGTTCTTCGGGGCGAAAACCTCTCAAG
GATCTTACCCTGTTGAGATCCAGTTCGATGTAACCCACTCGTGCACCCAACTGATCTTCAGCA

pCAGGS Small F

TCTTTTACTTTCACCAGCGTTTCTGGGTGAGCAAAAACAGGAAGGCAAAATGCCGCAAAAAGG
GAATAAGGGCGACACGGAAATGTTGAATACTCATACTCTTCCTTTTCAATATTATTGAAGCAT

pCAGGS_target PAM

TTATCAGGGTTATTGTCTCATGAGCGGATACATATTTGAATGTATTTAGAAAAATAAACAAATA

pCAGGS Small R

GGGGTTCGCGCACATTTCCCCGAAAAGTGCCACCTGGGTCGACATTGATTATTGACTAGTTAT
TAATAGTAATCAATTACGGGGTCATTAGTTCATAGCCCATATATGGAGTTCGCGGTTACATAAC
TTACGGTAAATGGCCCGCCTGGCTGACCGCCCAACGACCCCGCCCATTGACGTCAATAATGAC
GTATGTTCCCATAGTAACGCCAATAGGGACTTTCCATTGACGTCAATGGGTGGAGTATTTACGG
TAACTGCCCCTTGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCCCTATTGACGTCA

pCAGGS Large R

ATGACGGTAAATGGCCCGCCTGGCATTATGCCCAGTACATGACCTTATGGGACTTTCCTACTTG
GCAGTACATCTACGTATTAGTCATCGCTATTACCATGGTCGAGGTGAGCCCCACGTTCTGCTTC

CAG promoter + 1200bp

TTCTGGCGTGAGACCGGCGGCTTCTAGAGCCTCTGCTAACCATGTTTCATGCCTTCTTCTTTTCC
TACAGTCTCTGGGCAACGTGCTGGTTATTGTGCTGTTAACATGGTGAGCAAGGGCGAGGAGCTG
TTCACCGGGGTGGTGCCCATCCTGGTCGAGCTGGACGGCGACGTAAACGGCCACAAGTTCAGCG
TGTCCGGCGAGGGCGAGGGCGATGCCACCTACGGCAAGCTGACCCTGAAGTTCATCTGCACCAC
CGGCAAGCTGCCCCTGCCCTGGCCACCCCTCGTGACCACCTTCACCTACGGCGTGACGTGCTTC

GFP Small F

GFP protein (726bp)

GCCCCCTACCCCGACCACATGAAGCAGCACGACTTCTTCAAGTCCGCCATGCCCGAAGGCTACG
TCCAGGAGCGCACCATCTTCTTCAAGGACGACGGCAACTACAAGACCCGCGCCGAGGTGAAGTT
CGAGGGCGACACCCTGGTGAACCGCATCGAGCTGAAGGGCATCGACTTCAAGGAGGACGGCAAC

GFP Small R

ATCCTGGGGCACAAGCTGGAGTACAACACAAGGCTCTATATCACCGCCGACAAGC
AGAAGAACGGCATCAAGGTGAACCTCAAGACCCGCCACAACATCGAGGACGGCAGCGTGACGCT
CGCCGACCACTACCAGCAGAACACCCCATCGGCGACGGCCCGTGCTGCTGCCGACAACCAC
TACCTGAGCACCCAGTCCGCCCTGAGCAAAGACCCCAACGAGAAGCGCGATCACATGGTCTGCTG
TGGAGTTCGTGACCGCCGCGGGATCACTCTCGGCATGGACGAGCTGTACAAGTAATGATAAAC
TCCTCAGGTGCAGGCTGCCTATCAGAAGGTGGTGGCTGGTGTGGCCAATGCCCTGGCTCACAAA
TACCACTGAGATCTTTTTTCCCTCTGCCAAAAATTATGGGGACATCATGAAGCCCCTTG

beta-globin poly(A) + 400bp

Supplementary Figure 7. gRNAs designed to target CAGGS sequences in the CAG-GFP plasmids. The gRNA-binding sequences (blue) and the PAM sequences (green) are shown in the CAGGS sequences. The ssODN sequences are underlined (pink and yellow). The primer sets (small and large) used for the PCR analysis are shown in boxes (see **Supplementary Table 6**).

Rat Rosa26 locus

Exon 1

TCAGAGAGCCTCGGCTAGGTAGGGGATCGGGACTCTGGCGGGAGGGTGGCTTGGCGCGTTTGCG
GGGGCGGGCGGCCGCGGTAGGCCCTCCAAGGACGGTGGAGCCGCTTTGTGGGACAGCTGGGTTC
GATTCGTTAACCTTGAAGGGGCAAGCGGGTGGTAGTCAGGAATCCGGCCGCCCTGCAGCAACC
GGAGGGGGAGGGAGAAGGGAGCGGAAAAGTCTCCACCGGACGCGGCCATGGCTCCCACGGGGGG
CGGAGAAGCGCTTCCGGTCGATGTCTCATCGCTGATTGGCTGCTTTTCTCCCGCCGCGTGTGA
AAACACAAATGGCGTGTTTTGGTTGGAGTGAGGCGCCTGTCAATTAACGGCTGCCGGAGTGCGC
AGCCGCTGACTGCCTCGCTGTGCCACTGGGTGGGGCGGGAGGTAGGTGGGGTGAGGCGAGCTG
GACGTGCGGGCGCGGTTCGGCCTCTGGCGGGGCGGGGAGGGGAGGGTCAGCGAAAGTGGCTGGC
GCGTGAGCGGCCTCCACCCCTCCCCTTCTCTGGGGGAGTCGTTTTACCCGCCGCGGCCTGGC

rRosa26 Large F

CTCGTCATCTGATTGGCTCTCGGGGCTCAGAAAAGTGGCCTTTGCAATTGGCCCGCGTTCATGC
AAGTTCAGTCCCTAAGCTGGCTGGCGGGGGCGGCAGGGAGGCGCTCACAGGTTCGGGCCCTCCC
CCCAGGCCCCCGCGCCGAGAGTCTGGCCCCGCGCCCCTGCGCAACGTGGCAGGAAGCGCGCGCT
GGGGGCGGGGACGGGCGGTTCGGTCTGAGCGGCGGGCGGGTGCAAACGGGATTCCTCCTTGAGTT
GTGGCACTGAGGAACGTGCTGAACAAGACCTACATTGCACTCCAGGGAGTGGATGAAGGAGTTG
GGGCTCAGTCGGGTTGTATTGGAGACAAGAAGCACTTGCTCTCCAAAAGTCGGTTTGAGTTATC

rRosa26 Small F

ATTAAAGGGAGCTGCAGTGGAGTAGGCGGAGAAAAGGCCGCACCCTTCTCAGGACGGGGGAGGGG
AGTGTTGCAATACCTTTCTGGGAGTTCTCTGCTGCCTCCTGTCTTCTGAGGACCGCCCTGGGCC

PAM

rRosa26_target

TGGAAGATTCCCTTCCCCCTTCTTCCTCGTGGATCTGCAACTGGAGTCTTTCTGGAAGATAGGC
GGGAGTCTTCTGGGCAGGCTTAAAGGCTAACCTGGTGCGTGGGGCGTTGTCCTGCAGAGGAATT
GAACAGGTGTAAATTTGGAGGGGCAAGACTTCCACAGATTTTCGATTGTGTTGTTAAGTATTG

rRosa26 Small R

TAATAGGGGCAAATAAGGGAAATAGACTAGGCACCTACCTGGGGTTTTATGCAGCAAACTACA
GGTTATTATTGCTTGTGATCCGCCCTGGAGAATTTTTCACCGAGGTAGATTGAAGACATGCCCA
CCCAAATTTTAATATTCTTCCACTTGCATCCTTGCTACAGTATGAAATTACAGTATCGTGAAT
TAGAATATATAAGCAGAATTTTAAGCATTTTAAAGAGCCCAGTACTTCATGTCTGTCTCTCCC
ACTTCTGCAGCCCTATCAAAGGGTATTTTAGCACACTCATTTTAGTCCCATTTTCATTTGTTGT
ACTGGCTTATCCAATCCCTAGACAGAGCACTGGCATTCCCTCTCTCCT

rRosa26 Large_R

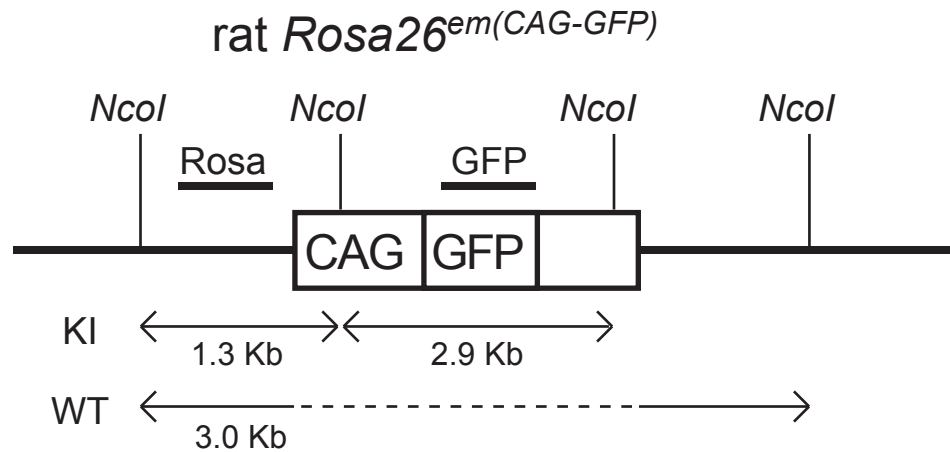
Supplementary Figure 8. gRNAs designed to target the rat *Rosa26* locus. The gRNA-binding sequences (blue) and the PAM sequences (green) are shown in intron 1 of the rat *Rosa26* sequence. Exon 1 is shown by an orange box. The ssODN sequences are underlined (pink and yellow). The primer sets (small and large) used for the PCR analysis are shown in boxes (see **Supplementary Table 6**).

CRISPR-mediated KO/KI mutations at rat *Rosa26* locus

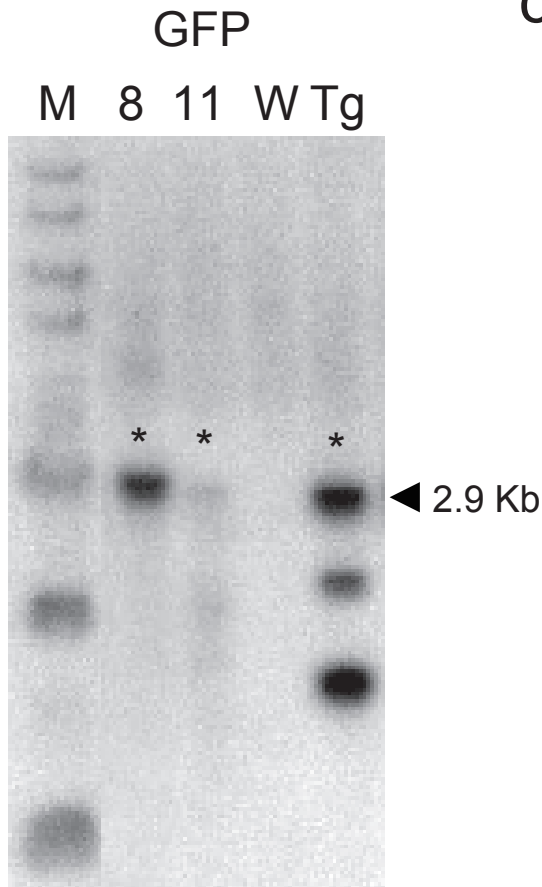
Wistar	WT		AGAAAGACTCCAGTTGCAGATCACGAGGGAAGA
#1	KO	-1	AGAAAGACTCCAGTTGCAGATC G -CGAGGGAAGA
#2	KO/WT*	-2	AGAAAGACTCCAGTTGCAGA--ACGAGGGAAGA
#3	KO	-6	AGAAAGACTCCAGTTGCA-----GAGGGAAGA
		+35	AGAAAGACTCCAGTTGCAGATCATGAG (+35bp) TAATAACGAGGGAAGA
#5	KO/WT*	-2	AGAAAGACTCCAGTTGCAGA--ACGAGGGAAGA
#6	KI/KO/WT*	-6	AGAAAGACTCCAGTTGCAGATCATGAG (pCAG-GFP) CGC-----GGGAAGA
		+1	AGAAAGACTCCAGTTGCAGATC G ACGAGGGAAGA
#7	KI	+27 -256	AGAAAGACTCCAGTTGCAGAGTA (+30bp) GACTGAG (pCAG-GFP) CGCTC (-256bp) CCGCC
#8	KO/Tg?	-506	TACAA----- (-506bp) -----CAGGC
#9	KO	+1	AGAAAGACTCCAGTTGCAGATC A ACGAGGGAAGA
		+42	AGAAAGACTCCAGTTGCAGATCATGAG (+46bp) CAATCACGAGGGAAGA
#10	KO/WT*	-106	AGAAAGACTCC----- (-106bp) -----CCCCT
#11	KI/KO	KI	AGAAAGACTCCAGTTGCAGATCATGAG (pCAG-GFP) CGCTCACGAGGGAAGA
		+54	AGAAAGACTCCAGTTGCAGATCATGAG (+54bp) CGCTCACGAGGGAAGA
#12	KO	+11	AGAAAGACTCCAGTTGCAGATCATGAGACAATAACGAGGGAAGA
		+46	AGAAAGACTCCAGTTGCAGATCATGAG (+46bp) AAATAACGAGGGAAGA
#14	KO/WT	+4	AGAAAGACTCCAGTTGCAGATCTGCTACTAGGGAAGA
#15	KO/WT	+5	AGAAAGACTCCAGTTGCAGATCATGAGATGAGGGAAGA
#16	KO	+1	AGAAAGACTCCAGTTGCAGATC A CCGAGGGAAGA
#17	KO	-2	AGAAAGACTCCAGTTGCAGA--ACGAGGGAAGA
		+2	AGAAAGACTCCAGTTGCAGATC A CACGAGGGAAGA

Supplementary Figure 9. CRISPR-mediated KO/KI mutation at rat *Rosa26* locus. A mix of the Cas9-poly(A), two gRNAs, two ssODNs, and the CAG-GFP plasmids were microinjected into the male pronucleus of fertilized Wistar rat embryos. Sequence analysis of the 17 pups delivered showed three GFP-KI alleles, as indicated by orange letters (KI), as well as a variety of indel mutations at the targeted *Tyr* locus, as shown by red letters (KO). Asterisks indicate pups carrying mosaic mutations.

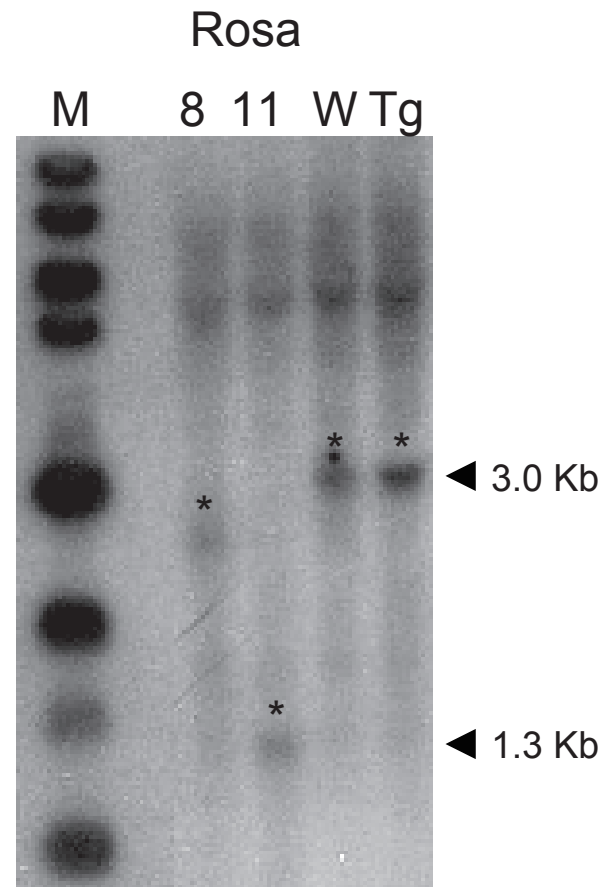
a



b

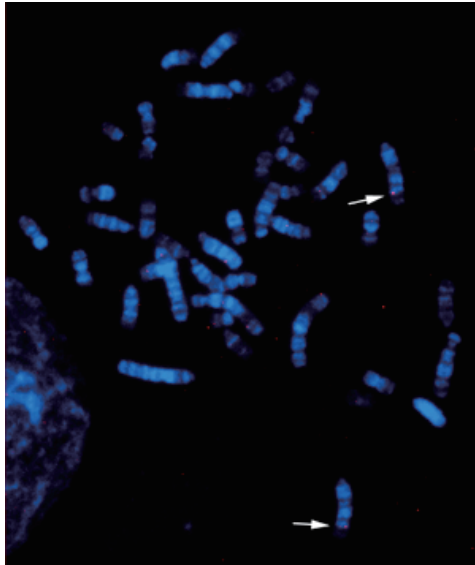


c

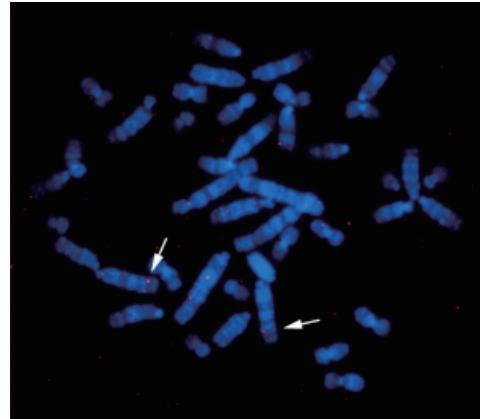


Supplementary Figure 10. Southern blot analysis of the rat *Rosa26* locus in CRISPR-mediated GFP-KI rats. (a) Schematic representation of the rat *Rosa26* and CAG-GFP knock-in loci. *NcoI* restriction sites are indicated. Radioisotope-labeled probes for GFP and *Rosa26* were used. Estimated size of each allele, knock-in (KI) or wild-type (WT), is indicated below. (b) The GFP-hybridizing band indicates that rat no. 8 carries three copies of the GFP transgene and no. 11 has one copy. W: Wistar rat as a negative control. Tg: GFP transgenic rat (NBRP-Rat No.0273: W-Tg(CAG-GFP)184Ys) as a positive control. (c) A 1.3-kb short band was detected by *Rosa26* probes in no. 11. A 2.5-kb band corresponding to a 506-bp deletion KO allele was detected in no. 8. The absence of another band detected in no. 8 means that the GFP transgene was randomly inserted at another chromosomal site. Wild-type 3.0-kb bands were detected in the Wistar and GFP transgenic rats.

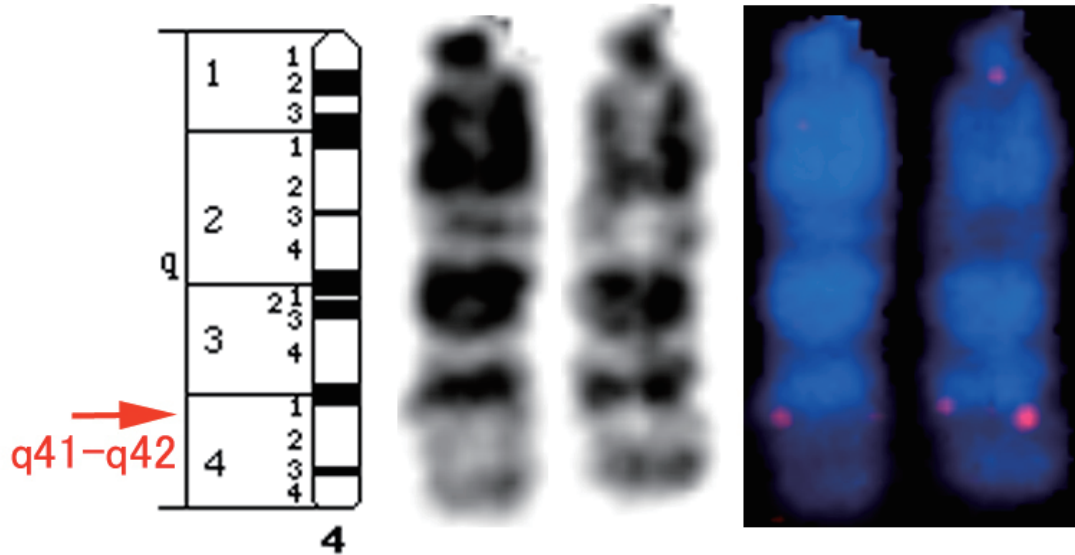
a



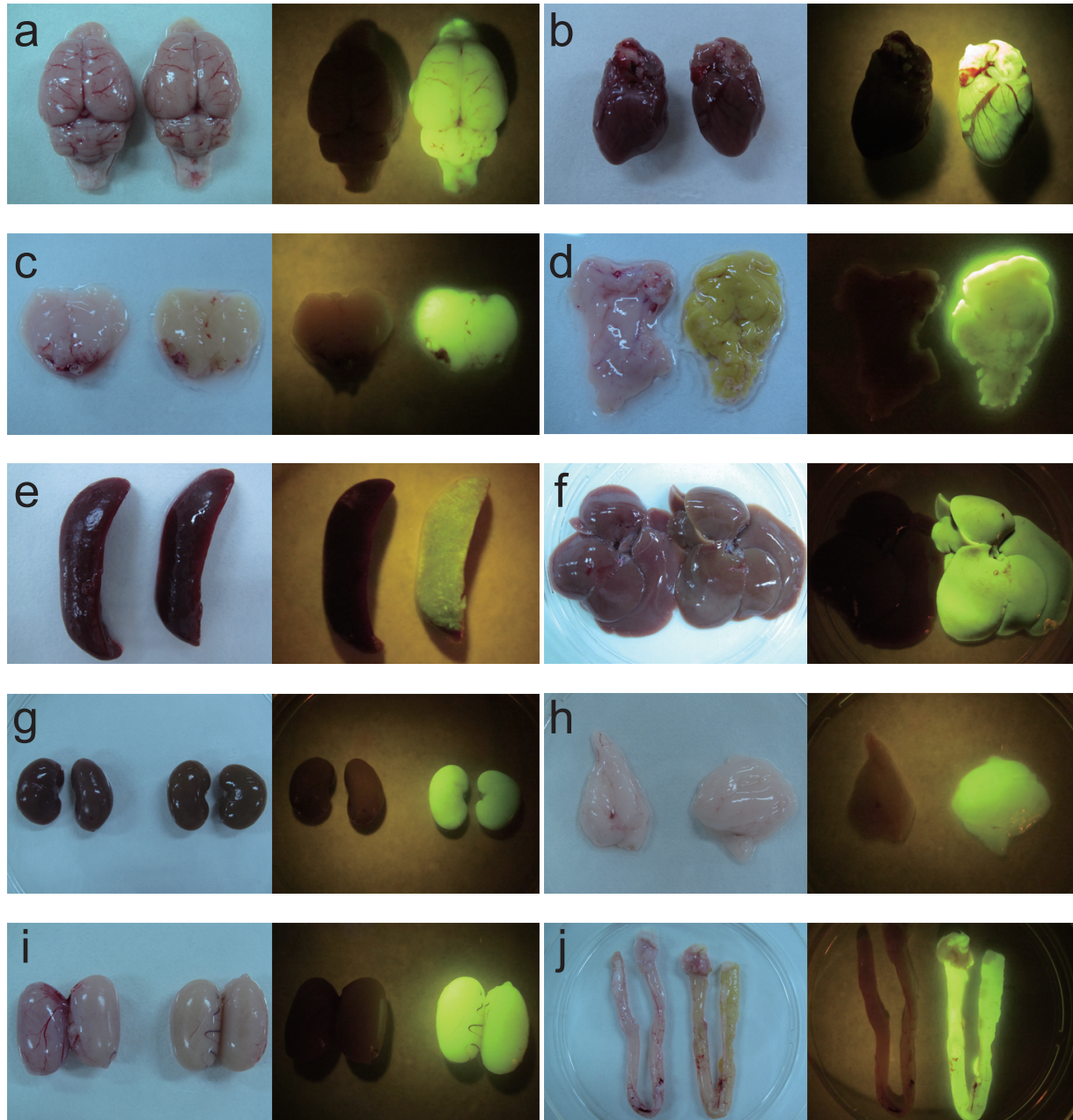
b



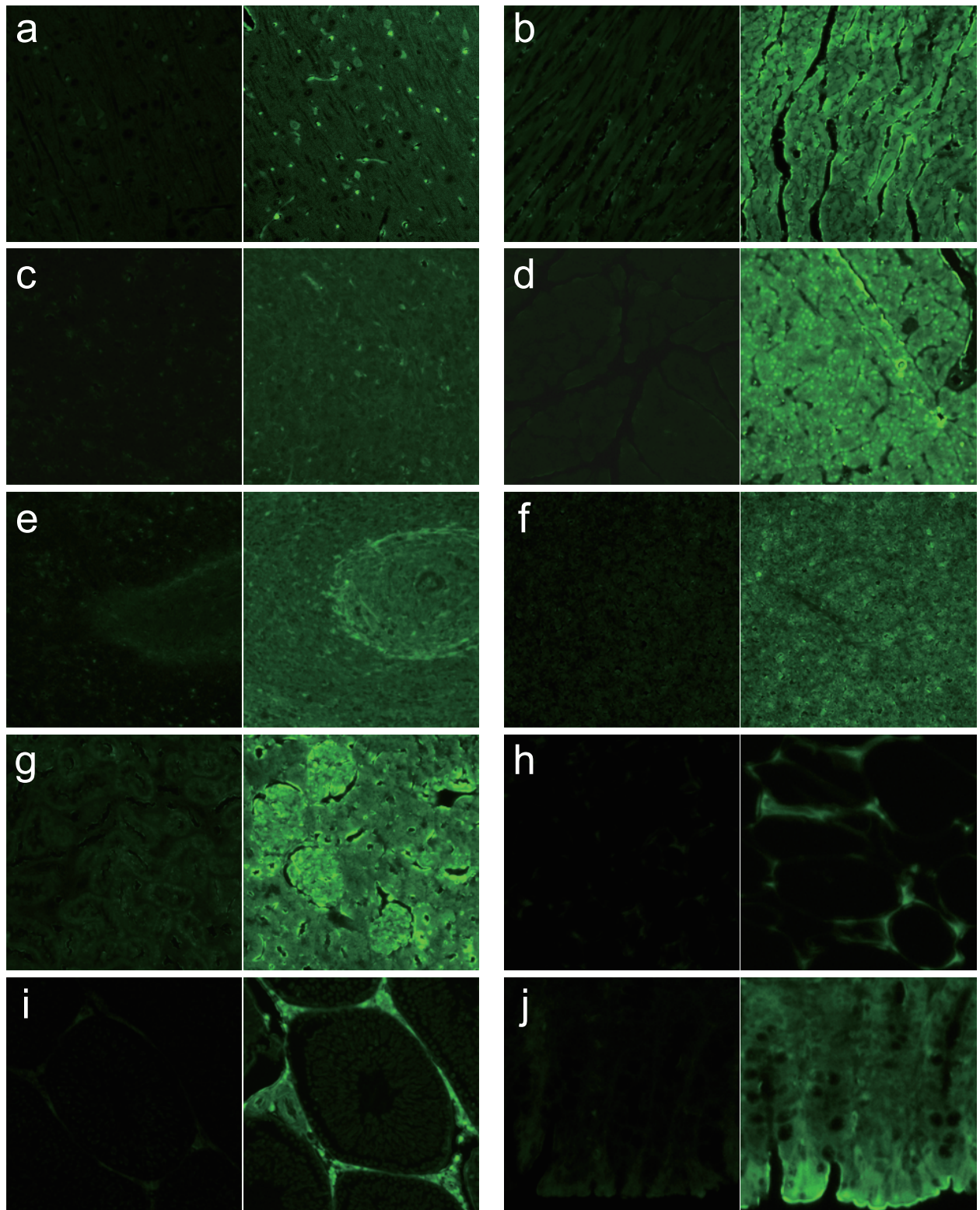
c



Supplementary Figure 11. Fluorescent in situ hybridization (FISH) analysis of the homozygous GFP-KI rats. The GFP-KI allele hybridized with the fluorescent labeled probes (arrows) (a, b). Homozygous KI alleles were confirmed on rat chromosome 4q41-q42 (c).



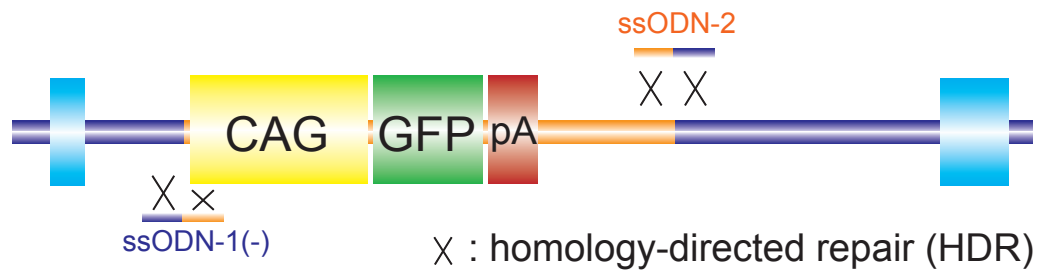
Supplementary Figure 12. Fluorescence photos for GFP-KI rat organs. Each sample was taken from the heterozygous W-*Rosa26^{em1}(CAG-GFP)^{Kyo}* rats (NBRP-Rat: No.0273) (right) and the control Wistar rat (left). (a) brain, (b) heart, (c) thymus, (d) pancreas, (e) spleen, (f) liver, (g) kidney, (h) adipose tissue, (i) testis, (j) large intestine.



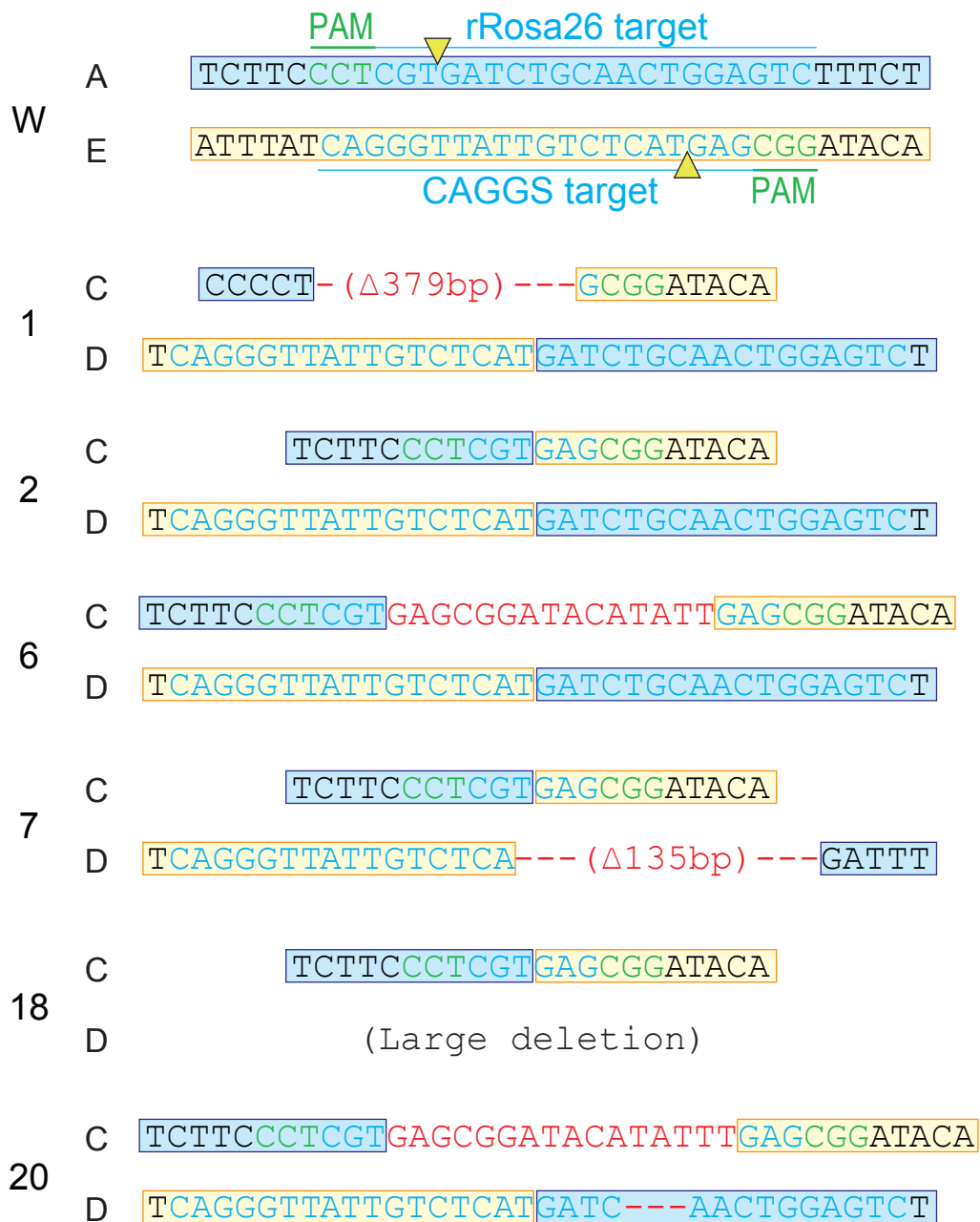
Supplementary Figure 13. Fluorescent tissue sections of GFP-KI rat organs. Each sample was taken from the heterozygous *W-Rosa26^{em1}(CAG-GFP)^{Kyo}* rats (NBRP-Rat: No.0273) (right) and the control Wistar rat (left). (a) brain, (b) heart, (c) thymus, (d) pancreas, (e) spleen, (f) liver, (g) kidney, (h) adipose tissue, (i) testis, (j) large intestine.

a

rat *Rosa26*^{em(CAG-GFP)}



b



Supplementary Figure 14. CAG-GFP knock-in rats generated by 2H2OP with complementary ssODN (-). (a) Schematic representation of the 2H2OP method with a complementary ssODN-1(-) upstream of the rat *Rosa26* cut site. (b) Sequence analysis of the delivered pups indicated six pups carried GFP-KI alleles.

Mouse *Rosa26* locus

Exon 1

TCAGAGAGCCTCGGCTAGGTAGGGGATCGGGACTCTGGCGGGAGGGCGGCTTGGTGC GTTTGCG
GGGATGGGCGGCCGCGGCAGGCCCTCCGAGCGTGGTGGAGCCGTTCTGTGAGACAGCCGGGTAC
GAGTCGTGACGCTGGAAGGGGCAAGCGGGTGGTGGGCAGGAATGCGGTCCGCCCTGCAGCAACC
GGAGGGGGAGGGAGAAGGGAGCGGAAAAGTCTCCACCGGACGCGGCCATGGCTCGGGGGGGGGG
GGGCAGCGGAGGAGCGCTTCCGGCCGACGTCTCGTCTGCTGATTGGCTTCTTTTCCTCCCGCCGT
GTGTGAAAACACAAATGGCGTGTTTTGGTTGGCGTAAGGCGCCTGTCAGTTAACGGCAGCCGGA
GTGCGCAGCCGCCGGCAGCCTCGCTCTGCCCACTGGGTGGGGCGGGAGGTAGGTGGGGTGAGGC
GAGCTGGACGTGCGGGCGCGGTTCGGCCTCTGGCGGGGCGGGGAGGGGAGGGAGGGTCAGCGAA
AGTAGCTCGCGCGAGCGGCCGCCACCCTCCCCTTCTCTGGGGGAGTCGTTTTACCCGCCG

mRosa26 Large F

CCGGCCGGGCGCTCGTCTCTGATTGGCTCTCGGGGCCAGAAAAGTGGCCCTTGCCATTGGCTC
GTGTTCTGTGCAAGTTGAGTCCATCCGCCGGCCAGCGGGGGCGGCGAGGAGGCGCTCCCAGGTTT
CGGCCCTCCCCTCGGCCCGCGCCGAGAGTCTGGCCGCGCGCCCTGCGCAACGTGGCAGGAA
GCGCGCGCTGGGGGCGGGGACGGGCAGTAGGGCTGAGCGGCTGCGGGGCGGGTGCAAGCACGTT
TCCGACTTGAGTTGCCTCAAGAGGGGCGTGCTGAGCCAGACCTCCATCGCGCACTCCGGGGAGT
GGAGGGAAGGAGCGAGGGCTCAGTTGGGCTGTTTTGGAGGCAGGAAGCACTTGCTCTCCCAAAG

mRosa26 Small F

TCGCTCTGAGTTGTTATCAGTAAGGGAGCTGCAGTGAGTAAGCGGGGAGAAGGCCGCACCCTT
CTCCGGAGGGGGGAGGGGAGTGTTGCAATACCTTTCTGGGAGTTCTCTGCTGCCTCCTGGCTTC

PAM

mRosa26_target

TGAGGACC GCCCTGGGCCTGGGAGAATCCCTTCCCCCTCTTCCTCGT GATCTGCAACTCCAGT
CTTTCTAGAAGATGGGCGGGAGTCTTCTGGGCAGGCTTAAAGGCTAACCTGGTGTGTGGGCGTT

mRosa26 Small R

GTCCTGCAGGGGAATTGAACAGGTGTAAAATTGGAGGGACAA GACTTCCCACAGATTTTCGGTTT
TTGTCGGGAAGTTTTTTAATAGGGGCAAATAAGGAAAATGGGAGGATAGGTAGTCATCTGGGGT
TTTATGCAGCAAACTACAGGTTATTATTGCTTGTGATCCGCCTCGGAGTATTTTCCATCGAGG
TAGATTAAAGACATGCTCACCCGAGTTTTTATACTCTCCTGCTTGAGATCCTTACTACAGTATGA
AATTACAGTGTCGCGAGTTAGACTATGTAAGCAGAATTTTAATCATTTTTAAAGAGCCCAGTAC
TTCATATCCATTTCTCCCGCTCCTTCTGCAGCCTTATCAAAGGTATTTTGAACACTCATTTT
AGCCCCATTTTCATTTATTATACTGGCTTATCCAACCCCTAGACAGA GCATTGGCATTTTCCCT

TTCCT

mRosa26_Large_R

Supplementary Figure 15. gRNAs designed to target mouse *Rosa26* locus. The gRNA-binding sequences (blue) and the PAM sequences (green) are shown in intron 1 of the mouse *Rosa26* sequence. Exon 1 is shown in an orange box. The ssODN sequences are underlined (pink and yellow). The primer sets (small and large) used for the PCR analysis are shown in boxes (see **Supplementary Table 6**).

CRISPR-mediated KO/KI mutations at mouse *Rosa26* locus

C57BL/6	WT		TCTAGAAAGACTGGAGTTGCAGATCACGAGGGAAGAGGGG
#1-1	KI/KO	KI	AGACTGGAGTTGCAGATCATGAG (pCAG-GFP) CGCTCACGAGGGAAG +1 TCTAGAAAGACTGGAGTTGCAGATCAGCAGGGAAGAGGGG
#1-2	KO	+29	AGACTGGAGTTGCAGATCATGAG (+29bp) CTTCAACGAGGGAAG +10 TCTAGAAAGACTGGAGTTGCAGATCATGCGACATTGGAGGGAAG
#1-3	KO	+1	TCTAGAAAGACTGGAGTTGCAGATCACGAGGGAAGAGGGG -6 TCTAGAAAGACTGGAGTTGCA-----GAGGGAAGAGGGG
#1-4	KO	+1	TCTAGAAAGACTGGAGTTGCAGATCAGCAGGGAAGAGGGG
#1-5	KO	-18	TCTAGAAAGACT-----GGAAGAGGGG
#1-6	KO	-60	GTTAGAGG----- (-63bp) -----GGAAGAGGGG

Supplementary Figure 16. CRISPR-mediated KO/KI mutation at the mouse *Rosa26* locus. A mix of Cas9-poly(A), two gRNAs, two ssODNs, and the CAG-GFP plasmid were microinjected into the male pronucleus of fertilized C57BL/6J mouse embryos. Sequence analysis of the representative six pups delivered showed GFP-KI alleles (#1-1), as shown by orange letters, as well as a variety of indel mutations at the targeted *Rosa26* locus, as shown by red letters (KO).

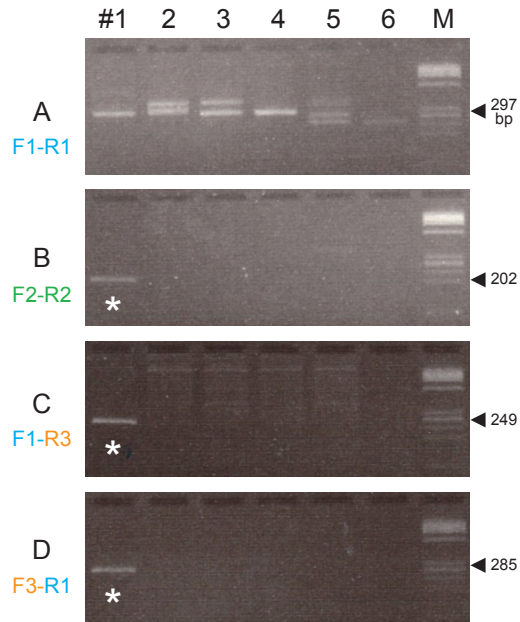
a

Embryos injected	Two-cell embryos (%)	Pups delivered (%)	Knockout (%)	Knock-in (%)
165	132 (80.0)	31 (23.5)	31/31 (100)	3/31 (9.7)

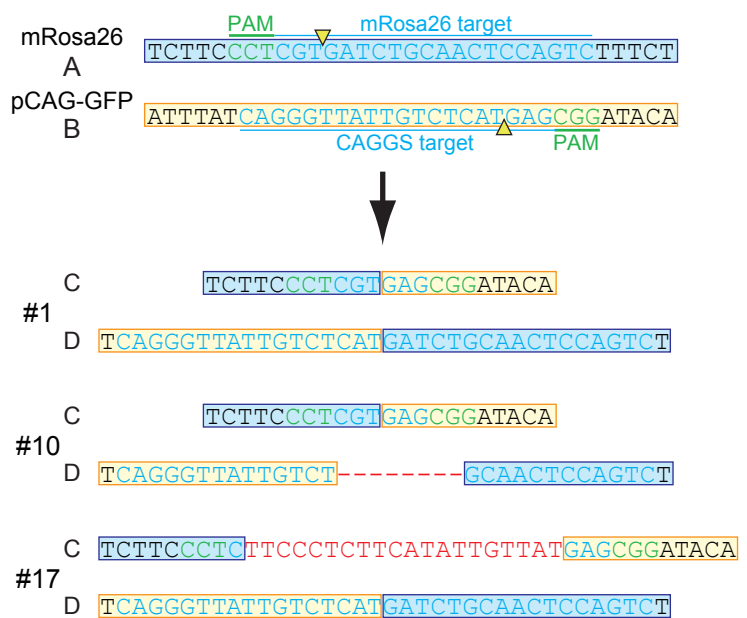
b



c



d



Supplementary Figure 17. CAG-GFP KI mice generated by two-hit gRNA and two-oligo with a targeting plasmid (2H2OP). (a) Cas9-poly(A) mRNA, two gRNAs, two ssODNs, and the CAG-GFP plasmids were co-injected into C57BL/6J mouse embryos. (b) Photos of pups delivered from C57BL/6J mouse embryos injected with a mix of Cas9 mRNA, two gRNAs, two ssODNs, and CAG-GFP plasmid (left). One of the pups expressing GFP at high levels in the body (asterisk) (right). (c) PCR analysis on the pups with primer sets, F1–3 and R1–3, as indicated in **Figure 3**. Three pups showed a GFP positive PCR band (asterisk). No. 1 was positive for the ligation either side of *Rosa26* and the CAG-GFP (C and D). (d) Sequence analysis of the pups indicated that No 1 carried the accurate conjunction at both cut ends.

Rat *Sirpa* Exon2

rSirpa Large F

TTCAGGCTCCCATTTTTGTTCAGGGTTTCTATTCTGCACAAAACATCATGACCAAGAAGTAA
GTTGGGGAGGGAAGGGTTTATTTCAGCTTACACTTCCACATTACTGTTTCATCACCAAAGGAAGTC
AGGACTGGAACCTCACACAGGGCAGGAAGCAGGAGCTGATGCAGAGGCCACGGAGGGTGCTGCTT
CCTGGATGGCTTCCCCTGGCTTGCTCAGCCTGCTCTCTTATAGAACCCAGTACTACCAGCCCAG
GGATGGCACCACCCATGATCAGTAATTGAGAAAATGCCTTACAGATGGGTCTCATGGAGGCCTT
TCCTCAAGGGAGGCGCCTTTCTCTGTATTAACCTACAGCTGTGTCAAGTTGATACACAAAACCAG
CCAGTACACATTTTCACTGACAAATTCAGATTTGAATTTTCAGAGCCCAGCTCAGATAGGACATT
TCCCCCAAAGGGCTTGGGCACTCTACTACACCAAACCATGTGACTTAGCCCTCTGTGCCCCAG
TTTTCTCACCACAGTCTTGATTTACAGGTTTCATGTGGGGGATAACACATGCCGAACATTGTGCT
GAACTCAATACCACTGTGTGTGATCAATCAGTGAAAACCATTGATAAACTTGAGCTTGTAGGAT

rSirpa Small F Exon 2

CCCTTAAGGTGAATCATGGCTGCCATCTTTCTCTCCAGGAGCCAGCGGGAAAGAACTGAAGGTG
ACTCAGGCTGACAAATCAGTGTCTGTTGCTGCTGGAGATTCGGCCACTCTGAACTGCACTGTGT

rSirpa target PAM

CCTCCCTGACGCCTGTGGGACCCATTAAGTGGTTCAAAGGAGAAGGGCAAATCGGAGCCCGAT
CTACAGTTTCATAGGAGGAGAACACTTTCCTCGAATTACAAATGTTTCAGATGCTACTAAGAGA

rSirpa Small R

AACAATATGGACTTTAGCATCTGTATCAGTAATGTCACCCCAGAAGATGCTGGCACCTACTACT
GTGTGAAGTTCCAGAAAGGAATAGTAGAGCCTGACACAGAAATTAAATCTGGAGGGGGAACAAC
GCTCTATGTACTCGGTAAGTAGGCTGTGCCTTCCTCATCCCCTCAGGTGTAGCATTAGGTCAGA
GAGTAGCATCCCGGTGAGTGTGGCTCACAAGGGCACAATCTGATCTCAACCTTCAGCAGGTCAG
GGACGTCAAGGCCAATCGTTAGCTCCAGGTTCCAGTGCTAGCAAGTAAGAGGGCATTAGCATCC
CTCATCTGCCCCGCCACGGCCCTCTCTTCCTTGACACAGGTTCAAGTACCATGAGTACCAGAA
TGCAGATCCCAGCAGGCTCCTTGCTGCCCAGGTATTAGGCATCCCCACAGGTGAGCCCAGGACC
TAGGAAGCACTGAACCTCTAGGAGATGCAGCCTCTCCACACATCACTTTCACAGAGATTCAGGA
AGCCCCTTCCATTCAGAACCCGAGGCCTGTCAGTGACAGAAAGCTCTCCACATGTCAGTCAGG
ACACAAAGGACCCCTCCATTATATAAATGGCTCCATTTTACTCTAGCATCTAGAAGTTCCACAA
TTTATTGTAGTCTGTTGGTAGATAATTCAGTTTTTCCATGCTGTGTATTTTTTTTAAATAAATGC

TGCATCGAATGCC

*rSirpa*_Large_R

Supplementary Figure 18. gRNAs designed to target the rat *Sirpa* locus. The gRNA-binding sequences (blue) and the PAM sequences (green) are shown in exon 2 of the rat *Sirpa* sequence (orange box). The ssODN sequences are underlined (navy). The primer sets (small and large) used for the PCR analysis are shown in boxes (see **Supplementary Table 6**).

pBACe3.6-PISecI site

PISecI Large F

TCCGGCCTTTATTACATTCTTTGCCCCGCTGATGAATGCTCATCCGGAGTTCCGTATGGCAATG
AAAGACGGTGAGCTGGTGATATGGGATAGTGTTACCCTTGTTACACCGTTTTTCCATGAGCAAA
CTGAAACGTTTTTCATCGCTCTGGAGTGAATACCACGACGATTTCCGGCAGTTTCTACACATATA
TTCGCAAGATGTGGCGTGTTACGGTGAAAACCTGGCCTATTTCCCTAAAGGGTTTATTGAGAAT
ATGTTTTTTCGTCTCAGCCAATCCCTGGGTGAGTTTCACCAGTTTTGATTTAAACGTGGCCAATA
TGGACAACCTTCTTCGCCCCCGTTTTTCACCATGGGCAAATATTATACGCAAGGCGACAAGGTGCT
GATGCCGCTGGCGATTCAGGTTTCATCATGCCGTTTGTGATGGCTTCCATGTCGGCAGAATGCTT
AATGAATTACAACAGTACTGCGATGAGTGGCAGGGCGGGCGTAATTTTTTTAAGGCAGTTATT

PISecI Small_F

GGTGCCCTTAAACGCCTGGTTGCTACGCCTGAATAAGTGATAATAAGCGGATGAATGGCAGAAA
TTCGATGATAAGCTGTCAAACATGAGAATTGGTCGACGGCGCGCCAAAGCTTGCATGCCTGCAG
CCGCGTAACCTGGCAAAAATCGGTTACGGTTGAGTAATAAATGGATGCCCTGCGTAAGCGGGGCA

PI-SceI PAM PISecI_target LoxP
CATTTCATTACCTCTTTCTCCGCACCCGACATAGATAATAACTTCGTATAGTATACATTATACG
AAGTTATCTAGTAGACTTAATTAAGGATCGATCCGGCGCGCCAATAGTCATGCCCCGCGCCAC
CGGAAGGAGCTGACTGGGTTGAAGGCTCTCAAGGGCATCGGTTCGAGCTTGACATTGTAGGACTA
PISecI_Small_R

T7

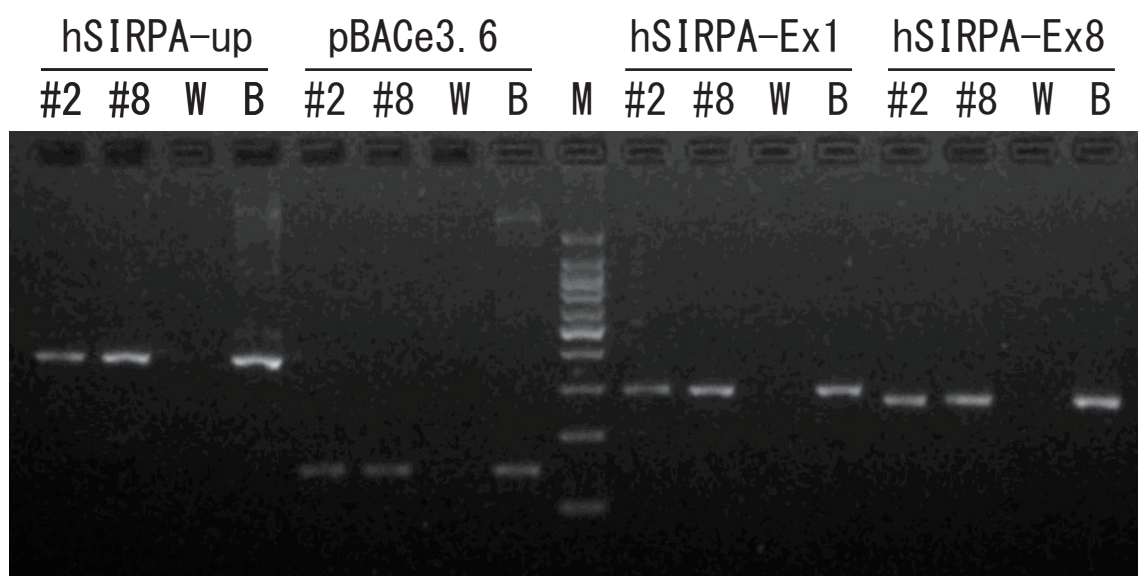
TATTGCTCTAATAAATTTGCGGCCGCTAATACGACTCACTATAGGGAGAGGATCCGCGGAATTC
GAGCTCACGCGTACTGATGCATGATCCGGGTTTAAACCCAGTACTCTAGATCCTCTAGAGTCGA
CCTGCAGGCATGCAAGCTTGGCGTAATCATGGTCATAGCTGTTTCCTGTGTGAAATTGTTATCC
GCTCACAATTCCACACAACATACGAGCCGGAAGCATAAAGTGTAAGCCTGGGGTGCCTAATGA
GTGAGCTAACTCACATTAATTGCGTTGCGCTCACTGCCCCTTTCCAGTCGGGAAACCTGTCGT
GCCAGCTGCATTAATGAATCGGCCAACGCGCGGGGAGAGGCGGTTTTCGTATTGGGCGCTCTTC
CGTTTCCTCGCTCACTGACTCGCTGCGCTCGGTTCGCTCGGCGAGCGGTATCAGCTCAC
TCAAAGGCGGTAATACGGTTATCCACAGAATCAGGGGATAACGCAGGAAAGAACATGTGAGCAA
AAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGCGTTGCTGGCGTTTTTCCATAGGCTCCG
CCCCCTGACGAGCATCACAAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTA
TAAAGATACCAGGCGTTTCCC
PISecI_Large_R

Supplementary Figure 19. gRNAs designed to target PI-SceI sequences in the human BAC plasmid. The gRNA-binding sequences (blue) and the PAM sequences (green) are shown in the PI-SceI sequence (yellow). The two ssODN sequences are underlined (pink and yellow). The primer sets (small and large) used for the PCR analysis are shown in boxes (see **Supplementary Table 6**).

CRISPR-mediated KO/KI mutations at rat *Sirpa* locus

Wistar	WT		GCACTGTGTCCTCCCTGACGCCGTGGGACCCA
#1	KO	+93	GCACTGTGTCCTCCCTGACGCCAAGAG (+93bp) GGAGATGTGGGACCCA
#2	KI/KO	KI	GCACTGTGTCCTCCCTGACGCCAAGAG (BAC-hSIRPA) GGAGATGTGGGACCCA
		+72	GCACTGTGTCCTCCCTGACGCCAAGAG (+72bp) GGAGATGTGGGACCCATTAA
#3	KO	+1	GCACTGTGTCCTCCCTGACGCCTTGTGGGACCCATTAA
#4	KO	+10	GCACTGTGTCCTCCCT-----ATGTC (+16bp) GGAGATGTGGGACCCATTAA
#5	KO	+88	GCACTGTGTCCTCCCTGACGCCAAGAG (+88bp) GGAGATGTGGGACCCATTAA
#6	KO	-83	GCACTGTGTCCTC----- (-83bp) -----ACTTT
#7	KO*	-91	GCACTGTGTCCTCCCTGACGC----- (-91bp) -----ACTAA
#8	KO/WT	-2	GCACTGTGTCCTCCCTGACG--TCAGGGACCCA
#10	KO	-11	GCACTGTGTCCTCCCTGAC-----CCATT
		-93	GCACTGTGTCCTCCCTGAC--- (-93bp) ---AAATG
#11	KO/WT*	-10	GCACTGTGTCCTCCCTGAC-----CCCAT
#12	KO	LD	Large Deletion
#14	KO	+1	GCACTGTGTCCTCCCTGACGCCGTGTGGGACCCA
#15	KO/WT*	+89	GCACTGTGTCCTCCCTGACGCCAAGAG (+89bp) GGAGATGTGGGACCCA

Supplementary Figure 20. NHEJ-mediated KO mutations at the rat *Sirpa* locus. Cas9-poly(A) mRNA and gRNA targeting exon 2 of the rat *Sirpa* allele were microinjected into the male pronucleus of fertilized Wistar rat embryos. Sequence analysis of the seven pups delivered showed a variety of indel mutations at the targeted *Sirpa* locus, as shown by red letters (KO). Asterisks indicate pups carrying mosaic mutations.



Supplementary Figure 21. PCR analysis for the SIRPA BAC knock-in. Primer sets amplifying the upstream region (hSIRPA-up), exon 1 (hSIRPA-Ex1), and exon 8 (hSIRPA-Ex8) of the human SIRPA gene, and the BAC vector sequences (pBACe3.6) were used (see **Supplementary Table 6**). BAC KI rat no. 2 (#2), BAC Tg rat no. 8 (#8), Wistar control rat (W), human BAC clone, RP11-933C19 (B).

rCyp2d1-5 (58Kbp)

rCyp2d-up Large F

GACCTCCCACACCTGTCTGGTACTTATCTCACAGGACTGAGCTGGCTAGGTTGTCCCCAGGCCTGCAAAGAGGC
TGGGGCCCATCTGGTTCTCAGGCTCTTGGGCATCCAGGCACCACTGGAACTGAAAGAGCTGAGAACAGAGTGA
GGCCCGCAGGCTGGATCTAGCCGGAGGCTGAGTGGGAAAGGGGTGCTCCAGCTGGTCCTGCAGGGGGAGTCCTTG
GCTCAATGGTAGCAGGTTTCACTTAGCTTGGTGGTGGCTGCCTCTGGGAGCAAAGTGAGGCCTTCTATGGCCTTC
TCTGTGGCTTTCTGTGGCAGATCCCAATGAAAAGAGACAGTCCATGATTTTAAGGTATTTATTCTCATGGTAGAA
AGTGGATGAGTAGAACTGTACTGCACTTCTGAGGGTGGGCCTCAGATTAAGTACTTTTGCAGGAATGAGTGTGTTG
GGAAGAAAGGTTATTGGCTCAGTCTCCAGGCTTTTAGGTACCTCATTATAATGGAGATCTGACTTGAAATGACCA
GGTCACCTCCTCTCCATGTGGAGGGGCTTGGGGCATTGCCCTTACATGACTGATGGCAACAAATGTATGGGGGGC
*rCyp2d-up*_Small_F

rCyp2d-up_target PAM

TGCGGCTAGTGACAGGGCCTGGTGCCAGGAGTCAGGCAAAACACCTACCGTCTCTTCAGGGTAACTGTGTTTT
AAGGCTTAGCTCAACTGGGAATCAGGGTGCCTTTGACAGTCCCACAGGGATTGACCTATCAAGAAGACAAGGATG
AATGAAATTAGATAGTGGTCTCCATGAGACAAGCACCTGGACTCCTAGCTGGTCTCCCCAGGACTCTTGACAGTT
*rCyp2d-up*_Small_R

GGTGACAACCTTTTCAGCTTTGTGAGGTGAGAGCTAAGAAGTCCCTAGGTTTTGAATTTGGCCATCCTTGAGACTT
GAACGACCTTCCACCTGTACTTGAAGCTTACAGGCTGGAGTTACCTTCTCACACTGCCTCTCCCTCACCCTCA
TCAGTACCCACACAAGCCACTTCAGTGGCCCTTGCTGCATACTGATAGTGGCCATGGTGTGAGTCAGACCCCAT
GTCTGACTAGCCAAGTGTCTTGAAGCCTCCAAAAGTTCTCTTCCAATCTGAGACGATGCCCTCAGGCCCCGT
GGATCTCTGCCCATAGGCAAAATCAGCAGCTTGCACACTGAGGAAGGGTTCATGTTATGTGCTTGTCTTGCTCAA
AATGTCCAGCCCAACATAACCACTATGCTGCTTTCTGTGGAGATTTCAATGCCACTCCCATGGACAAGCACCACC

*rCyp2d-up*_Large_R

GGAGAGAAGAAAGGGCAAGAACCTCTGATGTTTATTTTCCCTGGACTTTTCTGACACCCCTTCCCCATACAGGC
TCTGTTTGAACATTAACACAGCTACTTGGCTCAATTTAGAGAACAAAGTGCAGCTTTACCCCTTTTAAGGGACC
AGGTTCCCAGTCTGTTGTAAGAGGATCTTTCCTGATTCTAAGTGTCTGGACAAGTATTCCTTCAAAAGCCTGCAA
GCCGTGGGGCAGCCATGCGGGCTGCTGATTGGAGATGACCTCTGGGCTGTGGTCATATTCACAGCCATCTTCTTGC

rCyp2d1-5 55Kbp

⋮

ACTATGGCGTCTTTGGTGTCTGACCACCCCGCGCCCTACCAGCTCTGTGCTTCACCCCGCTAAGGGGAGGCAC
AGCATCTCACTCACTGTGCTTGTGTTGGGTCCTAGTGTGCAATAAAATGGTTTTACTCTGAACCGAATCATCCCTGT
GAGCTCTCCAGGCTGTAAGGGGCTGAGCAGCCTTCCCGTGGACATCCGCACCCCTACTTAATCTTCCTTGACCA
TGTGCCCCAATGGAAGGGCTGCTCTACTGACCTCCGAAATGGCAGCCATTCTTGCTTTCACCCCTGCCCCCTCTT

rCyp2d-down Large F

TTACCCCAAATTGATGATGTTTATTCATAGATGCCAACATCTGGAAGGAGGGCCAGAAAGGACTGCTGTGAAGGG
TCAGTGTAAGTCACACAGATGAGGGAAGGGGCGGTGGAGGTAATGGTGGGCAGAATTGTCCCTTTCCACTTGAG
ATGTTTCTCCAGACGCCCCCATTTACAGACCCACTACACAACCAAGGCTAACTCCTCAGCCAGCATCATCACAAC
TTCTTATATGACGTCGCAGAGATGTAGAGAAGTCGGGGAGGCTGGAAATGACATGCAGGTAAAGTGCCCAAGGTT
ACCTGTTGGGTACCACATGCTTCCCTAAACGGTTTTGTGGGGGTCCAGAAGCAGGTTGCCTCCTAAGCTTCTTTG
TCACCATTAATTCCATGACCCAGCAGGGATACTGGTGTCCAGGCCCATGCACAGTAAGAAAGTGACTCTAACCAG
GGATGGAAGGACCCGCAAGCTTAGTGTTGACACAGACTCCCAGACCTTAGCACAACTGACTCCATGGTAGAAGTA
CCATTGGGCCATAAACTTAGCACGTAGACAGCAGCTCCTCTCATAATGAAAACAAAGACCTAACCCATCAAAT

PAM

*rCyp2d-down*_Small_F

rCyp2d-down_target

TCTATCCTGGGAAGGTCTCTTGAAGCACTCCTCTTGGCTTCTTGGCTTCTGTAGTTCTCCTAGCTAACTGCTCTT
GCTAACTGAAGTATGTCAACCCAGGATATGGTTGTTGGTAAAAGCTCGCCCTGAGAACAGCTCAGGACGACATTG
AGGTGACCAGTGTAGTCACCAGCCAGCTAATAAAGACCTCCTTTTGGTTAAATCCATATCTGAGTAGTCTTCT
*rCyp2d-down*_Small_R

CTGGTGCATACCTCACACCATTTCTAAAGGTTGCAACAAGATCCCTAGAGACAGACCTTGAGGCACCATGGGTCT
CAGATCCCCATGGTGCAGAGAAGAGGAGTATGGTAGTCTAGGGGCTCCCAGGAAGTGTGCAACCAGAAGACTTTC
CAGGGCCTTAGGACTGCCTTTGATCATTTGCTGCCTAAAAGCTTTCTGACACTGCACCTCCCCCCCCAAAAGAAA
CAAACCTCAAGTGTGCTGGTCCGTTACCTCCAGAGGCTCTGTGTCCCTCTGTTAGGTAGGGCTGACCCAGTGTC
TGGGATCCAGGTGAGACATTACCAGACTCCCCTGGCCTGTCTGTATGAATGTATGGTGACCACCCCTGCTTGTCT
TTACGTGTGCCTTTCTATATGATTCTGTGCTAGTTCTATAGACTGGAGAAACATCGAAAGTAGAAAGGGAACAGT
*rCyp2d-down*_Large_R

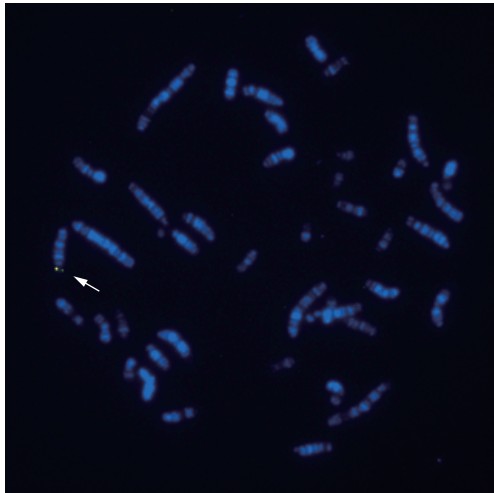
Supplementary Figure 22. gRNAs designed to target upstream of the rat *Cyp2d2* locus and downstream of the rat *Cyp2d4* locus. The gRNA-binding sequences (blue) and the PAM sequences (green) are shown on the rat *Cyp2d* cluster sequences (yellow and green boxes). The ssODN sequences are underlined (navy). The primer sets (small and large) used for the PCR analysis are shown in boxes (see Supplementary Table 6).

CRISPR-mediated indel mutations at rat *Cyp2d-up* and *Cyp2d-down* loci

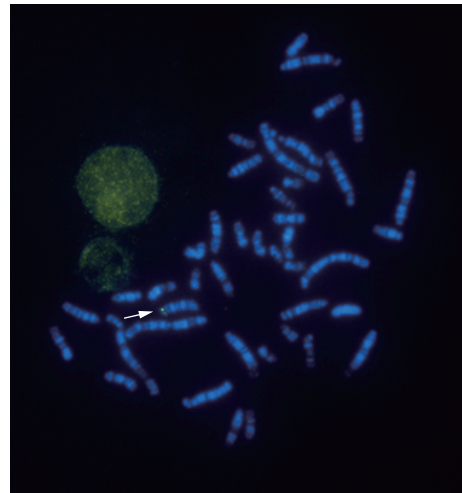
<i>rCyp2d-up</i>			<i>rCyp2d-down</i>		
Wistar	WT	ACCTACCGTCTCTTCAGGGTAACTGTGGTTTTA	WT	AGACCTAACCCATCAAATTCTATCCTGGGAAGG	
#1	KI/KO -9	ACCTACCGTCTCTTCAG-----GGTTTTA	KI/KO +1	AGACCTAACCCATCAAATTCTATTCCTGGGAAGG	
#2	KO -2	ACCTACCGTCTCTTCAGGGTAA--GTGGTTTTA	KO +1	AGACCTAACCCATCAAATTCTACTCCTGGGAAGG	
#3	KO/WT +1	ACCTACCGTCTCTTCAGGGTAAACTGTGGTTTTA	KO/WT -6	AGACCTAACCCATCAA-----TCCTGGGAAGG	
#4	KO -6	ACCTACCGTCTCTTCAGG-----GTGGTTTTA	KO/WT +4	AGACCTAACCCATCAAATTCTATTTTCCTGGGAA	
	-8	ACCTACCGTCTCTTCA-----GTGGTTTTA			
#5	KO -6	ACCTACCGTCTCTTCAGG-----GTGGTTTTA	KO/WT* +1	AGACCTAACCCATCAAATTCTATTCCTGGGAAGG	
			-5	AGACCTAACCCATCAAATT-----CTGGGAAGG	
#6	KO/WT -75	ACCTACCGTCTCTTCAGGGT--(-75bp)--AAGAA	KO/WT +1	AGACCTAACCCATCAAATTCTAATCCTGGGAAGG	
#7	KO -6	ACCTACCGTCTCTTCAGG-----GTGGTTTTA	KO +1	AGACCTAACCCATCAAATTCTACTCCTGGGAAGG	
	-8	ACCTACCGTCTCTTC-----TGTGGTTTTA	-4	AGACCTAACCCATCAAAT-----TCCTGGGAAGG	
#8	KO -8	ACCTACCGTCTCTTC-----TGTGGTTTTA	KO +1	AGACCTAACCCATCAAATTCTACTCCTGGGAAGG	
#9	KO +1	ACCTACCGTCTCTTCAGGGTAAACTGTGGTTTTA	KO/WT +1	AGACCTAACCCATCAAATTCTACTCCTGGGAAGG	
	+3	ACCTACCGTCTCTTCAGGGTAGTTTCTGTGGTTTTA			
#10	KO/WT -9	ACCTACCGTCTCTTCAG-----GGTTTTA	KO -8	AGACCTAACCCATCAAATTT-----GAAGG	
#11	KO/WT -6	ACCTACCGTCTCTTCAGG-----GTGGTTTTA	KO -3	AGACCTAACCCATCAAATTC--CCTGGGAAGG	
#12	KO -9	ACCTACCGTCTCTTCAG-----GGTTTTA	KO/WT +1	AGACCTAACCCATCAAATTCTACCCCTGGGAAGG	
	+2	ACCTACCGTCTCTTCAGGGTAAACTGTGGTTTTA			
#13	KO -8	ACCTACCGTCTCTTC-----TGTGGTTTTA	KO +1	AGACCTAACCCATCAAATTCTACTCCTGGGAAGG	
	+14	ACCTACCGTCTCTTCAGGGTAAAG(+14bp)TCCTGT	+2	AGACCTAACCCATCAAATTCTATTTTCCTGGGAAGG	
#14	KO/WT -8	ACCTACCGTCTCTTC-----TGTGGTTTTA	KO/WT* +1	AGACCTAACCCATCAAATTCTACTCCTGGGAAGG	
			-14	AGACCTAA-----TCCTGGGAAGG	
#15	KO/WT* -2	ACCTACCGTCTCTTCAGGGT--CTGTGGTTTTA	KO/WT* -1	AGACCTAACCCATCAAATTCT--TCCTGGGAAGG	
	-9	ACCTACCGTCTCTTCAG-----GGTTTTA	+2	AGACCTAACCCATCAAATTCTATTTTCCTGGGAAGG	
#16	KO -8	ACCTACCGTCTCTTCA-----GTGGTTTTA	WT	w	
#17	KO/WT -6	ACCTACCGTCTCTTCAGG-----GTGGTTTTA	KO/WT* -1	AGACCTAACCCATCAAATTCTAT--CTGGGAAGG	
			-4	AGACCTAACCCATCAAATTCTAT----GGAAGG	
#18	KO -1	ACCTACCGTCTCTTCAGGGTT--CTGTGGTTTTA	KO +1	AGACCTAACCCATCAAATTCTATTCCTGGGAAGG	
#20	KO/WT -9	ACCTACCGTCTCTTCAG-----GGTTTTA	KO/WT -7	AGACCTAACCCATCAAATTCTAG-----AGGTC	
#21	KO -9	ACCTACCGTCTCTTCAG-----GGTTTTA	KO/WT* +1	AGACCTAACCCATCAAATTCTACTCCTGGGAAGG	
			+4	AGACCTAACCCATCAAATTCTATTTTCCTGGGAA	
#22	KO -10	ACCTACCGTCTCTTCA-----GGTTTTA	KO +1	AGACCTAACCCATCAAATTCTATTCCTGGGAAGG	
			+19	AGACCTAACCCATCAAATTCTAAT(+19bp)ATTTCCT	
#23	KO -6	ACCTACCGTCTCTTCAGG-----GTGGTTTTA	KO -1	AGACCTAACCCATCAAATTCT--TCCTGGGAAGG	

Supplementary Figure 23. NHEJ-mediated KO mutations at rat *Cyp2d* cluster sites. Cas9 mRNA and three gRNAs; one targeting the upstream of rat *Cyp2d2*, the second targeting downstream of rat *Cyp2d4*, and the third on the plasmid DNA, together with two ssODNs and the human *CYP2D6* plasmid were microinjected into the male pronucleus of fertilized Wistar rat embryos. Sequence analysis of the 23 pups delivered showed a variety of indel mutations at the targeted *Cyp2d2* and *Cyp2d4* loci, as shown by red letters (KO). Asterisks indicate pups carrying mosaic mutations.

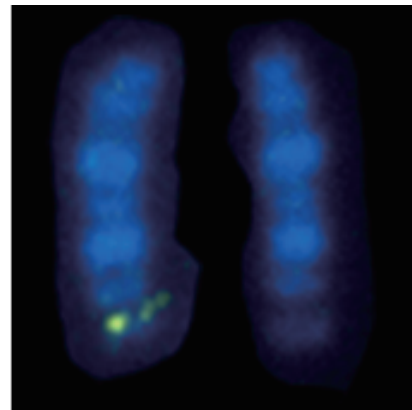
a



b

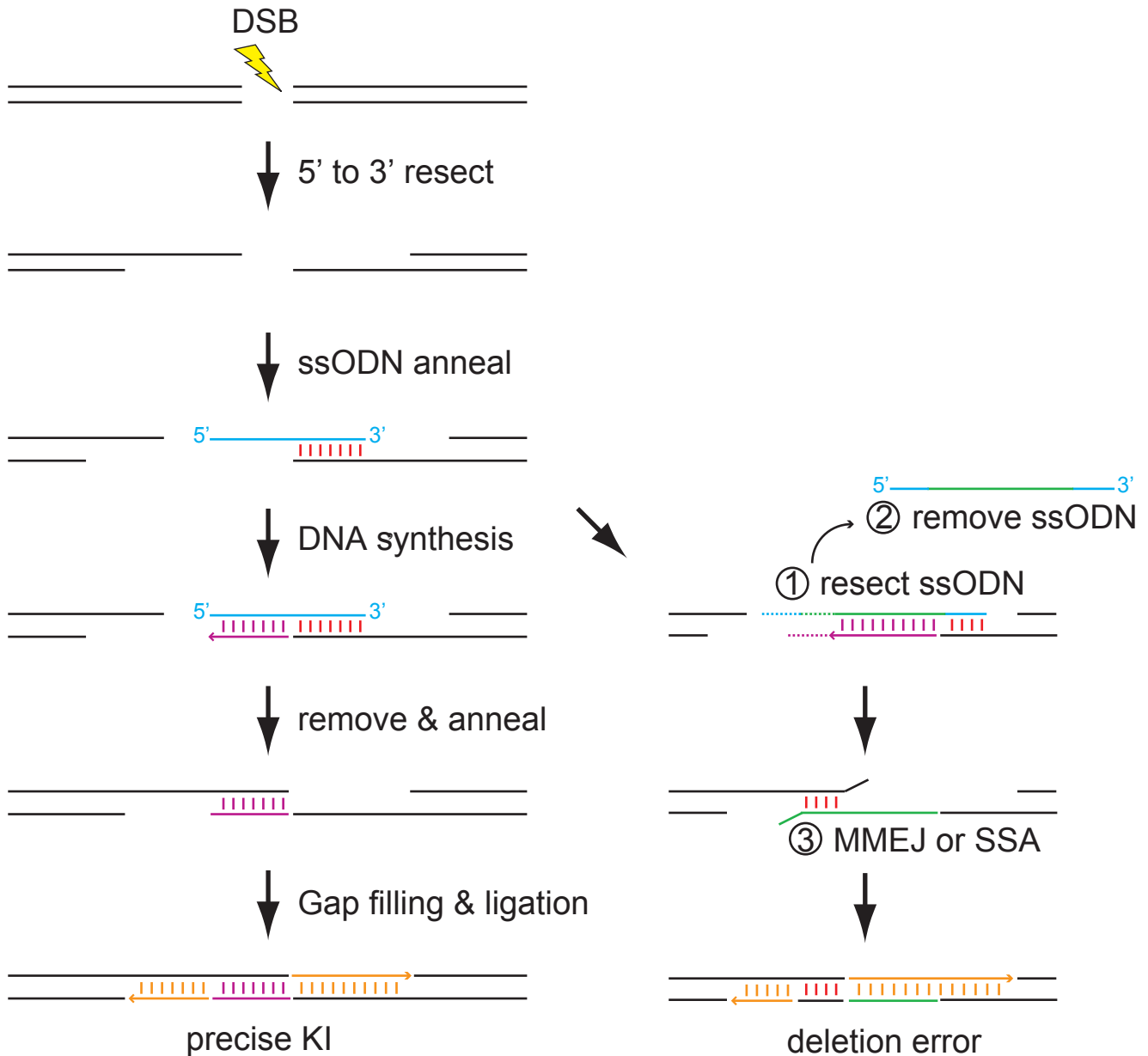


c



Supplementary Figure 24. Fluorescent in situ hybridization (FISH) analysis of the homozygous CYP2D6-KI rats. CYP2D6-KI allele hybridized with the fluorescent labeled probes (arrows) (a, b). The presence of the heterozygous KI allele was confirmed on rat chromosome 7q34 (c).

Synthesis-dependent strand annealing (SDSA)



Supplementary Figure 25. Hypothesis of the HDR mechanism. In the synthesis-dependent strand annealing (SDSA) pathway, the 3' end of an ssODN anneals to the homologous sequences on the 3' strand of the DSB after resection of the 5' strand of the DSB. DNA synthesis occurs from the 3' end of the DSB to copy the 5' remaining strand of the ssODN. A second annealing occurs with the other 3' end of the DSB after removal of the ssODN. Finally, gap filling and ligation complete the repair event. In the pathway, (1) resection of 5' ssODN by exonuclease activity, or (2) removal of the ssODN by helicase activity concurrently occurs with DNA synthesis, which causes incomplete repair, such as indel mutations. (3) Microhomology sequences between the 3' strand of the DSB and the 3' of the newly synthesized strand from the ssODN may also cause indel mutations via microhomology-mediated end joining (MMEJ) or single-strand annealing (SSA).

Supplementary Table 1. Comparison of genome editing efficiency between Cas9 and Cas9-polyA in rats using the 2-cell embryo assay.

Targeted locus	Injected RNA	Embryos injected	2-cell embryos (%)	PCR-amplified (%)	Knockout (%)
<i>Tyr</i>	Cas9-GFP	29	24 (82.8)	20 (83.3)	2 (10.0)
	Cas9-GFP-polyA	35	24 (68.6)	19 (79.2)	7 (36.8)*

Two-cell embryos were collected 24 h after injection of Cas9 mRNA/gRNA, and genomic DNA was amplified by PCR to detect NHEJ-mediated knockout mutations at the targeted locus. *Tyr*: tyrosinase, *P<0.05, Cas9 vs. Cas9-polyA by Fisher's exact test.

Supplementary Table 2. Potential off-target sites for CRISPR/Cas targeting in the rat genome.

Name	Sequence	Hit Score*	No. of Mismatches	Coordinate (rn5)	Mutation Frequency (Mutant/Total)	Primer Forward	Primer Reverse
gRNA:Tyr ^c	TTTCCAGGATTATGTAATA						
	GTGG						
Tyr ^c -OT1	CTCCCAGGGTTATGTAATAG AAG	1.6	3	chr19:+32965297	0/13	TTGAATCCTGTGATACAGTTCAG AC	CATATGCCGAGGTCACAACA
Tyr ^c -OT2	TATCCAGGCATATGTAATAG CAG	1.5	3	chr10:-32914681	0/13	TGGCCATACTCCAAATCTTCTT	CCCTGATCCTGTAGTTTGT GA
Tyr ^c -OT3	ATTCCAGGAGTATGTAATAC TAG	1.4	4	chr19:+24325208	0/13	GGACACCTGCAGTAAGGACA	TGAGATTGAGATCATTTTGG TCA
Tyr ^c -OT4	TTTCCAGAACTATGTAATAC TGG	1.1	3	chr11:+34484150	0/13	GAGTGAGCAATGGCGGATAC	CACAGTTGCTCACACGCTTT
Tyr ^c -OT5	TTTCCATGTGTATGTAATAG AAG	0.9	3	chr4:+22739080	0/13	TGTGGTATCAGCTGGAGCAT	CAGAGCAGATGGCACTCCTA
Tyr ^c -OT6	TTACTATTATTATGTAATAG TAG	0.9	4	chr10:-78685738	0/13	AGGCGAGGATGTATTGGTTG	CATGCATGTGAAGCAGGACT
Tyr ^c -OT7	TTGCTATGACTATGTAATAG GAG	0.9	4	chr13:-118286168	0/13	TCAACCACAGGTTTGGGAAT	CTCTTGTTATGGCCTCTCC
gRNA:Thy1-ATG	GATGCTGATGACTGGGGTTC						
	ATGG						
Thy1-ATG-OT1	GAAGCTGATGGCTGGGTTCA TGG	4.1	2	chr1:+95817250	0/8	ACCAGGTCACAGGGCTACTG	CAAGCTGGGCGAACTCTCTA
Thy1-ATG-OT2	GACTATGTTGACTGGGTTCA AAG	1.3	4	chr1:-203590002	0/8	GCCTAGTGAGCCCAGACAGT	AGCGACATGTACAACCACCA
Thy1-ATG-OT3	GATGCTAATGACTGGGTGCA CAG	1.2	2	chr1:+104389339	0/8	CTGACTGGGCATCACCATT	GACCAGTTTGGCCTTTAGGA
Thy1-ATG-OT4	TATGCTAATGTCTGGGTTCA AAG	1.1	3	chr5:-155608310	0/8	CAGAGTGGGAAGCCATT	ACATTACTGGCCCACTCC
Thy1-ATG-OT5	GAATATTATGACTGGGTTCA TAG	0.9	4	chr2:-99063815	0/8	CCCAGCCCCACGTATATTT	AAGAGCTCTGGGTCCCATTT
Thy1-ATG-OT6	TAAGCTTATCACTGGGTTCA CAG	0.9	4	chr19:-54038098	0/8	TGTCAATCTCCCTCGCTTCT	TTGCAATGTCCCAGGTTACA
Thy1-ATG-OT7	GTTGCTCCTCACTGGGTTCA TAG	0.9	4	chr13:-103736046	0/8	CCGGGTTACATGAGACGAAC	CTGACAACCCACTGATCCAC T
Thy1-ATG-OT8	GCTTCTCATCACTGGGTTCA TGG	0.9	4	chr15:-64251828	0/8	CCTTGAATTCTTCCAAAGCAG	CACAACCATGCACACATGAA
gRNA:Thy1-TGA	GGACTTCATTCTCTGTGA						
	CTGG						
Thy1-TGA-OT1	GAATTCTTTCTCTGTGAC AGG	2.5	3	chr3:-94266024	0/8	CACACACACACAGTGAACAA	TTGATGAGAGGTTGGTCTTT G
Thy1-ATG-OT2	GAAATTCATATCTCTGTGAC TGG	2.5	3	chr9:+72220986	0/8	GGTTTGAACTTTGGGCTTTG	TGTAGGGGGTGCTAGAGTGG
Thy1-ATG-OT3	GGACATTCTTTCTCTGTGAC TGG	1.6	3	chr2:-223297766	0/8	CCCACAGAAAATACACGCAAA	ACACAGAGGCTGCAGGACTT
Thy1-ATG-OT4	GCTCTTCAGTTCTCTGTGAC TGG	1.6	3	chr8:-75966086	0/8	CGGGAAAAAGCACTGGTAAA	TACCAAGTGGCTCACAACCA
Thy1-ATG-OT5	TATCATCATTTCTCTGTGACT GG	1.3	4	chr1:+159654698	0/8	TCCATTTCATGTGTTTGGT	TGTGAATGGAGCTGGCTTAG
Thy1-ATG-OT6	TGACTTCCTTTCTCTGGGAC CAG	1.3	3	chr14:-82502071	0/8	GTAGATGGCCAGGGATAGCA	TGCAGGATTCTGACAAGTGG
Thy1-ATG-OT7	GGATTTCATTTCTTTGTGAC CAG	1.3	2	chrX:+54020076	0/8	CGTTCTTGTTCCCTTTGTGT	TTTGTTTGCCTGTATGTTGGA
Thy1-ATG-OT8	TTACTTCATTTCTCTGTGGCA	1.1	3	chr1:-36741643	0/8	TCAACAGTCTCTGCCTCTGC	CCTTGGCTGCATAAATGTT

	GG						
Thy1-ATG-OT9	GAAGTGTATTCTCTGTGAC CAG	1	3	chr1:-275694272	0/8	GAGGACCTTGAGGCAGAGTG	CAGCTGGGGAGACAAAAGA G
Thy1-ATG-OT10	AGACTGCAGTTCTCTGTGAC CAG	1	3	chr16:+63188060	0/8	AGAACTCCATGGCAGATGGT	TGCTGTAAGGGGTGTGTGTG
Thy1-ATG-OT11	GAAGTACACTTCTCTGTGAC CAG	1	3	chr2:+143964172	0/8	AATGGTCAAAATGCCTGAGC	TCAACTGTTGCAAAAGGGAG T
gRNA:rRosa26	GACTCCAGTTGCAGATCAC GAGG						
rRosa26-OT1	GACCCAGTTGCAGATCACA GGG	6.4	2	chr6:-146497662	0/4	CACGACCCCTACCAITCATT	ATTGTGGCCTTACCTGGTTG
rRosa26-OT2	GACCCAGTTGCAGAGCAC GGAG	1.7	2	chr18:+55462095	0/4	CTGAAGGTAACCGTCCTCA	CTGTGCCCTCATCGTCCTAT
rRosa26-OT3	GCCTGCAGTTGCAGATCACA AGG	1.5	3	chr5:+172749987	0/4	GCTGCCACTACTGTGTCCAG	TCATTGTGGAGTCCCTGACC
rRosa26-OT4	GATTCCAGCAGCAGATCACG TGG	1.4	3	chr14:-78446863	0/4	GCAAGAGCCTGAAATCCTCA	TCTTCCTCGGTGACTGTCTT
rRosa26-OT5	ATCATCAGTTGCAGATCACG TGG	1.3	4	chr5:-30258553	0/4	TAAGGCCACTGGAAGACTGG	AGACCAACTCGGTTTGCATC
rRosa26-OT6	GGCTCCTGTGAAGATCACG AAG	0.9	3	chr3:+110807013	0/4	TCACATGGGAAAGTCCATGA	CCCTCTCCCTTCTTCCTTTC
rRosa26-OT7	GATTGCTGTAGCAGATCACG GGG	0.9	4	chr19:-71341543	0/4	TTGCTAAACTGGGCTTCAGG	CTCACTTGAGGGGCTTTGAG
rRosa26-OT8	ACCTCCATTCCAGATCACG GGG	0.8	4	chr3:+141310868	0/4	CGTCACGTCAGCAGTCAGTAA	ACAAGCACCCCTTACCTGTCTG
gRNA:CAGGS	CAGGGTTATTGTCTCATGA GCGG						
pCAG-OT1	CCTGATTATTGTCTCATGAG AAG	2.3	3	chr13:-43521965	0/4	TCTTAACATGCTGCCCTTC	TGAAAAAGCCATCAGTTGGT C
pCAG-OT2	TAGTGTAAATTGTCTCATGAG AAG	1.7	3	chr6:+3958889	0/4	TGCACTCTTGGCATAAGACG	CCAGGCTTCCATTGCTACTC
pCAG-OT3	GAGGATTATTATCTCATGAG GAG	1.6	3	chr4:+147313287	0/4	GGATTTCGATCCTTAGCACCA	TGACAAAGGGTTGTGTGCAT
pCAG-OT4	CATTGTATTTTCTCATGAG AAG	1.5	3	chr5:-77761908	0/4	GGGACAGACAGAAAGAGATACC A	GGGAAGCAGAGCAAAGAAT G
pCAG-OT5	TTGCGTTGTGTCTCATGAG TAG	1.4	4	chr13:+81796711	0/4	CATACCGTGGCACATGAACT	AAAACCCCAATGCTTTTTC
pCAG-OT6	TCTGATTATTGTCTCATGAG AAG	1.3	4	chr2:-142513379	0/4	TCGGACCTAGACACCTTTCC	TCCCTGCAGAGAAGAAGCAT
pCAG-OT7	GAAGGTTTTCTGTCTCATGAG TAG	1.3	4	chr7:+142228609	0/4	TGGCACCCATTCTTGAACAT	AGGGGACACCAGTGACTTTG
pCAG-OT8	TGCTGTTATTGTCTCATGAG TAG	1.3	4	chr1:-224044123	0/4	CTCCCTTCTCCCTCCAACT	ACTCTTGCCACTGTGCCTTT
pCAG-OT9	GAGAGTATTGTCTCATGAG AAG	0.9	4	chr11:-60248423	0/4	TTCAAGACCATGCACAGGAG	ATGCAATGTCCAAGCAATGA

*Off-target hit scores are calculated by CRISPR Design Tool (<http://crispr.mit.edu/>)

Supplementary Table 3. CRISPR/Cas-mediated knock-ins in rats.

Embryos injected	Two-cell embryos (%)	Pups delivered (%)	Knockouts /offspring (%)	Knock-ins /offspring (%)
145	88 (60.7)	48 (54.5)	37/48 (77.1)	5/48 (13.5)

Cas9-poly(A) mRNA, gRNA:Thy1-ATG, and lsODN-ATG(+) were injected into fertilized rat eggs. Two-cell embryos were transferred into pseudopregnant female rats and delivered offspring were genotyped by PCR and sequencing to detect knockout (KO) or knock-in (KI) alleles (See **Fig. 2c, d**).

Supplementary Table 4. Germline transmission from offspring generated by CRISPR/Cas.

KI rat	Founder No.	CRISPR-mediated mutation	G1 offspring	G1 mutants
CAG-GFP	8	Tg	16	8
CAG-GFP	11	KI	16	8
hCYP2D6	8	Tg	3	2
hCYP2D6	2	Large Del	9	3
hCYP2D6	18	KI	17	8

Supplementary Table 5. CRISPR/Cas-mediated knock-ins in rats.

Embryos injected	Two-cell embryos (%)	Pups delivered (%)	Knockouts /offspring (%)	Knock-ins /offspring (%)
100	56 (56.0)	21 (37.5)	20/21 (95.2)	6/21 (28.6)

Cas9-poly(A) mRNA, gRNA(s), ssODN-1(-) upstream and ssODN-2 (+) downstream of the rat *Rosa26* cut site, and a donor plasmid were injected into fertilized rat eggs. Two-cell embryos were transferred into pseudopregnant female rats and delivered offspring were genotyped by PCR and sequencing to detect knockout (KO) or knock-in (KI) alleles (See **Supplementary Fig. 14**).

Supplementary Table 6. Primers used for mutation screening.

Primer name	Forward	Reverse	PCR size (bp)
rTyr Small	TTGCATAAATTGGTTTTACAGA	ATTTAAACATGAAAATATTACCTTCCA	260
rThy1-ATG Small	GAGGTGTCCATTGTGTGATCC	CCCGAGTGTCTGAGACTATTGC	253
rThy1-TGA Small	GGTTGGTACCCCTTCTCTCC	GACAGGGTCCCCATTTCTC	266
EGFP Small	CTACCCCGACCACATGAAG	CTTGTGCCCCAGGATGTT	202
rRosa26 Small	AAGGGAGCTGCAGTGGAGTA	CCCAGGTGAGTGCCTAGTCT	360
rRosa26 Large	ATTGGCTCTCGGGGCTCAGAAAAC	AGGAGAGAGGGAATGCCAGTGC	1061
mRosa26 Small	AAGGGAGCTGCAGTGGAGTA	CCGAAAATCTGTGGGAAGTC	297
mRosa26 Large	ATTGGCTCTCGGGGCCAGAAAAC	AGGAAAGGGAAAATGCCAATGC	1072
CAGGS Small	ACTTTCACCAGCGTTTCTGG	AATCAATGTCGACCCAGGTG	237
CAGGS Large	TCAAGGCGAGTTACATGATCC	GCCAAGTAGGAAAGTCCATAA	962
rSirpa Exon2 Small	GCTGCCATCTTTCTCTCCAG	CAGATGCTAAAGTCCATATTGTTTC	261
rSirpa Exon2 Large	TTCAGGCTCCCATTTTGTGTC	GGCATTGATGCAGCATTAT	1613
PISceI Small	GGCAGTTATTGGTGCCCTTA	TTCAACCCAGTCAGCTCCTT	353
PISceI Large	TCCGGCCTTTATTCACATTC	GGGAAACGCCTGGTATCTTT	1557
hSIRPA up Small	AGGTCTTACTGGCCCCCTTA	TCTGTGCCTCAGCTTCTTCA	374
pBACe3.6 Small	CTTGTTGGGCGATAATCGTT	TACTGAGGACGCACTGGATG	165
hSIRPA Exon1 Small	GGGGGAGCCTTAGTCATTTT	AGGCGTCTCTCCCCCTATTA	287
hSIRPA Exon8 Small	CAGATGTCGCAAGCCCTATT	CTGTCCACATGGTCTGTTGG	263
rSirpa cDNA	AATGTCACCCCAGAAGATGC	TCCGTCCGTGATAAGTTTCC	563
hSIRPA cDNA	GCCGGCACCTACTACTGTGT	GGGCTGTTGAGTAACCTCCA	441
rCyp2d up Small	GGTCACCTCCTCTCCATGTG	GCTAGGAGTCCAGGTGCTTG	274
rCyp2d up Large	GACCTCCCACACCTGTCTGT	CTTTCTTCTCTCCGGTGGTG	1288
rCyp2d down Small	CCATTTGGGCCATAAACTT	GCTGGCTGGTGA CTACACTG	253
rCyp2d down Large	ATGCCAACATCTGGAAGGAG	GACTGTTCCCTTTCTACTTTTCG	1244
hCYP2D6 Exon1	TGGCATGAAGGACTGGATTT	AAGGCCTTTCCTTCTGGTGT	153

gRNA

Tyr	TAGGTTTCCAGGATTATGTAATAG	AAACCTATTACATAATCCTGGAAA
rThy1-ATG	TAGGGATGCTGATGACTGGGTCA	AAACTGAACCCAGTCATCAGCATC
rThy1-TGA	TAGGGGACTTCATTTCTCTGTGAC	AAACGTCACAGAGAAATGAAGTCC
rRosa26	TAGGGACTCCAGTTGCAGATCACG	AAACCGTGATCTGCAACTGGAGTC
mRosa26	TAGGGACTGGAGTTGCAGATCACG	AAACCGTGATCTGCAACTCCAGTC
pCAGGS	TAGGCAGGGTTATTGTCTCATGAG	AAACCTCATGAGACAATAACCCCTG
rSirpa	TAGGGTGTCCCTCCCTGACGCCTGT	AAACACAGGCGTCAGGGAGGACAC
PIScel	TAGGTATGTCGGGTGCGGAGAAAG	AAACCTTTCTCCGCACCCGACATA
rCyp2d up	TAGGCCGTCTCTTCAGGGTAACTG	AAACCAGTTACCCTGAAGAGACGG
rCyp2d down	TAGGTAACCCATCAAATTCTATCC	AAACGGATAGAATTTGATGGGTTA
ssODN		
Tyr ^C	AGGTTTTATGTGATGGAACACCTGAGGGACCACTATTACGTAATCCTGGAAACCATGACAAAGCCAAAACCCCCAGGCTC	
rRosa26-CAGGS	CCCTGGGCCTGGAAGATTCCCTTCCCCCTTCTTCCCTCGTGAGCGGATACATATTTGAATGTATTTAGAAAAATAAAACAA	
rRosa26-CAGGS (-)	TTGTTTATTTTTCTAAATACATTCAAATATGTATCCGCTCACGAGGGAAGAAGGGGGAAGGGAATCTTCCAGGCCCAGGG	
CAGGS-rRosa26	TTCAATATTATTGAAGCATTTATCAGGGTTATTGTCTCATGATCTGCAACTGGAGTCTTTCTGGAAGATAGGCGGGAGTC	
mRosa26-CAGGS	GCCCTGGGCCTGGGAGAATCCCTTCCCCCTTCTCCCTCGTGAGCGGATACATATTTGAATGTATTTAGAAAAATAAAACAA	
CAGGS-mRosa26	TTCAATATTATTGAAGCATTTATCAGGGTTATTGTCTCATGATCTGCAACTCCAGTCTTTCTAGAAGATGGGCGGGAGTC	
rSirpa-PIScel	AGTGTCTGTTGCTGCTGGAGATTCGGCCACTCTGAACTGCACTGTGTCTCCCTGACGCCAAGAGGTAATGAAATGTGCCCCGCTTACGCAGGGCATCCATTTATTAC TCAACCGTAACC	
PIScel-rSirpa	ACTAGATAACTTCGTATAATGTATACTATACGAAGTTATTATCTATGTCGGGTGCGGAGATGTGGGACCCATTAAGTGGTTCAAAGGAGAAGGGCAAAATCGGAGCC CGATCTACAGTTT	
rCyp2dUp-CAGGS	CTAGTGACAGGGCCTGGTGCCAGGAGTCAGGC AAAACACCTACCGTCTCTTCAGGGTAAGAGCGGATACATATTTGAATGTATTTAGAAAAATAAAACAAATAGGGG TTCCGCGCACATT	
CAGGS-rCyp2dDown	AATACTCATACTCTTCCTTTTTCAATATTATTGAAGCATTTATCAGGGTTATTGTCTCATTCCTGGGAAGGTCTCTTGAAGCACTCATCTTGGCTTCTTGGCTTCTGTAG TTCTCCTAGC	
