

***i*-GONAD (improved genome-editing via oviductal nucleic acids delivery),
a convenient *in vivo* tool to produce genome-edited rats**

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Supplementary Table S1. Exp-1: *i*-GONAD-mediated induction of indels in fetuses of BSF1 (F1 hybrid between BN female and SD male).

Supplementary Table S2. Exp-2: *i*-GONAD-mediated induction of indels in fetuses of SBF1 (F1 hybrid between SD female and BN male).

Supplementary Table S3. Exp-3: *i*-GONAD-mediated KI into the *Tyr* locus in fetuses/newborn of LEW rats.

Supplementary Table S4. Exp-4: *i*-GONAD-mediated KI into the *Tyr* locus in fetuses/newborn of SD rats.

Supplementary Table S5. Exp-5: KI into the *Tyr* locus in SD fetuses/newborn offspring obtained after *i*-GONAD in the presence of 1 μ M SCR7.

Supplementary Table S6. Exp-5: KI into the *Tyr* locus in SD fetuses/newborn offspring obtained after *i*-GONAD in the presence of 10 μ M SCR7.

Supplementary Table S7. Mutations at putative off-target loci containing up to 3 bp mismatches for target gene are analyzed by sequencing.

Supplementary Table S8. Nucleotide sequences of oligonucleotides (oligo) used in this study.

Supplemental Fig. S1. Sequencing of the junction between endogenous *Tyr* and the ssODN using KI rats (LEW-#16 and SD-#55).

Supplemental Fig. S2. Genotyping of the unedited tails [isolated from SD, BN and offspring (C1-C3) obtained after mating between SD and BN) or fetal samples (Pax6-#4 and #5 shown in Fig. 4B-D)]. The PCR condition is the same shown in Fig. 4D. Note that there is no additional band throughout these samples, except for the presence of a single band at 1,489 bp.

Supplemental Fig. S3. Sequencing of the PCR products (derived from abnormal fetuses carrying indels in the target *Pax6* locus; shown in Fig. 4D) cloned into a TA cloning vector. Based on these sequence data, the deduced amino acid sequences are aligned and shown in Fig. 4F.

Supplemental Fig. S4. Sequencing results of off-target candidate loci in fetal/newborn offspring after *i*-GONAD. The 20-bp target sequences and PAM (underlined) are shown.

Supplementary Table S1. Exp-1: *i*-GONAD-mediated induction of indels in fetuses of BSF1 (F1 hybrid between BN female and SD male).

Name of pregnant females	Name of fetuses	Eye color	Genotype	Note for the target Tyr sequence
#1	BSF1-#1	Black	Normal (G/A)	-
#1	BSF1-#2	Black	Normal (G/A)	-
#1	BSF1-#3	White	Homozygous bi-allelic (A/A)	Transition of G to A in allele-G
#1	BSF1-#4	White	Heterozygous mono-allelic (del)	Deletion of (TTACGTA) in allele-G
#1	BSF1-#5	White	Heterozygous mono-allelic (del)	Deletion of (TTA) in allele-G
#1	BSF1-#6	White	Heterozygous mono-allelic (indels)	Deletion of 146-bp sequence (CATGTATGTAATTTTCATTCATATGTAAGTCCCTTGTCAGAGAT AACTCATTTTGCATAAAATTGGTTTTCACAGATCAITTCGAGCAGA TCAGAAGAGTATAATAGCCATCAGGTTTATGTGATGGAACACCT GAGGGACCAC) and insertion the sequence (AAACATGAAAA) is newly added in allele-G
#1	BSF1-#7	White	Homozygous bi-allelic (in)	Insertion of (TTT) in both alleles, as shown by CTA(TTT)TTA
#2	BSF1-#8	Black	Normal (G/A)	-
#2	BSF1-#9	White	Heterozygous mono-allelic (in)	Insertion of T in allele-G, as shown by TATT(T)ACG
#2	BSF1-#10	White	Heterozygous mono-allelic (del)	Deletion of (TATTAC) in allele-G (see Fig. 1G)
#3	BSF1-#11	Black	Normal (G/A)	-
#3	BSF1-#12	Black	Normal (G/A)	-
#3	BSF1-#13	White	Heterozygous mono-allelic (del)	Deletion of (CCACTA) in allele-G.

Abbreviations: in, insertion mutation; del, deletion mutation; indels, insertion and deletion mutations.

Supplementary Table S2. Exp-2: *i*-GONAD-mediated induction of indels in fetuses of SBF1 (F1 hybrid between SD female and BN male).

Name of pregnant females	Name of fetuses	Eye color	Genotype	Note for the target <i>Tyr</i> sequence
#1	SBF1-#14	Black	Normal (G/A)	-
#1	SBF1-#15	Black	Normal (G/A)	- (see Fig. 1G)
#1	SBF1-#16	White	Heterozygous mono-allelic (del)	Deletion of 89-bp sequence (AAATTGGTTTTTCACAGATCATTGTCAGCAGATCAGAAGAGTATAATAGCCATCAGGTTTTATGTGATGGAACACCTGAGGGACCAC TAT) in allele-G
#2	SBF1-#17	Black	Normal (G/A)	-
#3	SBF1-#18	Black	Normal (G/A)	-
#3	SBF1-#19	Black	Normal (G/A)	-
#3	SBF1-#20	Black	Normal (G/A)	-
#3	SBF1-#21	Black	Normal (G/A)	-
#3	SBF1-#22	White	Heterozygous mono-allelic (del)	Deletion of 51-bp sequence (TGAGGGACCACTATTACGTAATCCTGGAAACCATGACAAAGC CAAAACCCC) in allele-G
#3	SBF1-#23	White	Heterozygous mono-allelic (del)	Deletion of 214-bp sequence (TCCTCCCTTCACTTTCATCTAACTGCTAGGAAGCTGAATGATA ATAATGATATGTAATTTTCATTGTATGTAATTTTCATTCATATGTAAG TCCCTTGCTCAGAGATAACTCAITTTGCATAAATTGGTTTCA CAGATCATTGTCAGCAGATCAGAAGAGTATAATAGCCATCAGG TTTTATGTGATGGAACACCTGAGGGACCACTATTACGT) in allele-G
#3	SBF1-#24	White	Heterozygous mono-allelic (in)	Insertion of (AA) in allele-G, as shown by CTA(AA)TTACG
#3	SBF1-#25	White	Compound heterozygous bi-allelic (indels)	Deletion of (TTACGTA) in allele-G, and insertion of (CA) in the allele-A, as shown by CTA(CA)TTACA
#3	SBF1-#26	White	Heterozygous mono-allelic (in)	Insertion of (T) in allele-G, as shown by TATT (T)ACG
#3	SBF1-#27	White	Mosaic (in)	Allele-A is normal, but there are deletion of 15-bp (TTACGTAATCCTGGA) sequence in allele-G, and deletion of 3-bp (TTA) sequence in the other allele-G
#3	SBF1-#28	White	Mosaic (indels)	Allele-A is normal, but there are insertion of (T) in allele-G, as shown by TATT(T)ACG, and deletion of 7-bp (TTACGTA) sequence in the other allele-G
#3	SBF1-#29	White	Mosaic (indels)	One allele is allele-A, but there are insertion of (TA) in the other allele-A, as shown by TATTA(TA)CAT, and deletion of 22-bp sequence (ACTATTACGTAATCCTGGAAAC) in the other allele-G

Abbreviations: in, insertion mutation; del, deletion mutation; indels, insertion and deletion mutations.

Supplementary Table S3. Exp-3: *i*-GONAD-mediated KI into the *Tyr* locus in fetuses/newborn of LEW rats.

Name of pregnant females	Name of fetuses	Eye color	Genotype	Note for the target <i>Tyr</i> sequence
#1	LEW-#1	White	Normal (A/A)	-
#1	LEW-#2	White	Normal (A/A)	-
#2	LEW-#3	White	Compound heterozygous bi-allelic (indels)	Insertion of GA in one allele, as shown by ACCACTA(GA)TTACAT, and deletion of 103-bp sequence (ATAATCCTGGAAACCATGACAAAGCCAAAACCCCAGGCT CCCATCTTCAGCAGACGTGGAATTTGTCTGAGTTTGACCC AGTATGAATCTGGATCAATGGA) in the other allele
#2	LEW-#4	White	Heterozygous mono-allelic (in)	Insertion of (TT) in one allele, as shown by ACCACTATT(TT)ACATA
#2	LEW-#5	White	Normal (A/A)	-
#2	LEW-#6	White	Heterozygous mono-allelic (in)	Insertion of (A) in one allele, as shown by ACCACTA(A)TTACAT (see Fig. 2D)
#3	LEW-#7	White	Normal (A/A)	-
#3	LEW-#8	White	Normal (A/A)	-
#3	LEW-#9	White	Normal (A/A)	-
#3	LEW-#10	White	Normal (A/A)	-
#3	LEW-#11	White	Normal (A/A)	-
#4	LEW-#12	White	Compound heterozygous bi-allelic (indels)	Deletion of (TTA) in one allele, and deletion of (TTAC) and insertion of (A) in the other allele, as shown by ACCACTATTAC(A)ATAAT
#4	LEW-#13	White	Normal (A/A)	-
#4	LEW-#14	White	Heterozygous mono-allelic (replacement)	Replacement of CCCAACTAAACCTTGAGC by TAATAGCCATCAGGTTT in one allele
#4	LEW-#15	White	Heterozygous mono-allelic (in)	Insertion of (TT) in one allele, as shown by ACCACTATT(TT)ACA
#5	LEW-#16	Black	KI (G)/ mosaic (in)	Successful KI of ssODN in one allele (allele-G) (see Fig. 2D and Supplemental Fig. S1), insertion of A in the other allele (allele-A), as shown by ACCACTA(A)TTACA, and insertion of CA in one more another allele (allele-A'), as shown by ACCACTA(CA)TTACA
#5	LEW-#17	White	Normal (A/A)	-
#5	LEW-#18	White	Normal (A/A)	-
#5	LEW-#19	White	Normal (A/A)	-
#5	LEW-#20	White	Mosaic (indels)	One allele is allele-A, but there are insertion of (TT) in one allele, as shown by ACCACTATT(TT)ACA, and deletion of 23-bp sequence (CTATTACATAATCCTGGAAACCA) in the other allele
#5	LEW-#21	White	Compound heterozygous bi-allelic (indels)	Insertion of (T) in one allele, as shown by ACCACTATT(T)ACA and deletion of 21-bp sequence (CCACTATTACATAATCCTGGA) in the other allele
#5	LEW-#22	White	Normal (A/A)	-

Abbreviations: in, insertion mutation; del, deletion mutation; indels, insertion and deletion mutations.

Supplementary Table S4. Exp-4: *i*-GONAD-mediated KI into the *Tyr* locus in fetuses/newborn of SD rats.

Name of pregnant females	Name of fetuses	Eye color	Genotype	Note for the target <i>Tyr</i> sequence
#1	SD-#1	White	Normal (A/A)	-
#1	SD-#2	White	Normal (A/A)	-
#1	SD-#3	White	Normal (A/A)	-
#1	SD-#4	White	Normal (A/A)	-
#1	SD-#5	White	Heterozygous mono-allelic (in)	Insertion of (A) in one allele, as shown by ACCACTA(A)TTACA
#1	SD-#6	White	Normal (A/A)	-
#1	SD-#7	White	Mosaic (in)	One allele has albino-specific allele A (allele-A). Insertion of A in another allele, as shown by ACCACTA(A)TTACA (allele-A'), and insertion of (AT) in the other allele, as shown by ACCACTA(AT)TTACA (allele-A'')
#1	SD-#8	White	Heterozygous mono-allelic (KI and in)	Successful KI of ssODN and insertion of (ATTACTAA) in allele-G, as shown by CCACTA(ATTACTAA)TTACG
#1	SD-#9	White	Heterozygous mono-allelic (in)	Insertion of (AATA) in one allele, as shown by ACCACTA(AATA)TTACA
#1	SD-#10	White	Normal (A/A)	-
#1	SD-#11	White	Compound heterozygous bi-allelic (in)	Insertion of (A) in one allele, as shown by ACCACTA(A)TTACA, and insertion of (TA) in the other allele, as shown by ACCACTA(TA)TTACA
#2	SD-#12	White	Normal (A/A)	-
#2	SD-#13	White	Normal (A/A)	-
#2	SD-#14	White	Normal (A/A)	-
#2	SD-#15	White	Mosaic (indels)	One allele has albino-specific allele A (allele-A). Insertion of (TT) in another allele (allele-A'), as shown by ACCACTATT(TT)ACA, deletion of 41-bp sequence (ACTATTACATAATCCTGGAAACCATGACAAAGCCAAAACCC) in the other allele (allele-A''), and deletion of 31-bp sequence (GACCACTATTACATAATCCTGGAAACCATGA) in one more another allele (allele-A''')
#2	SD-#16	White	Mosaic (in)	One allele has albino-specific allele A (allele-A). Insertion of (AA) in another allele (allele-A'), as shown by ACCACTA(AA)TTACA, and insertion of (T) in the other allele (alleles A''), as shown by ACCACTATT(T)ACA
#2	SD-#17	White	Normal (A/A)	-
#2	SD-#18	White	Homozygous bi-allelic (KI and in)	Successful KI of ssODN and three insertions in both alleles, as shown by GTGAT(T)GGAACACCT(G)AGGACCACTATTACGTAATC(C)TGGAAA
#2	SD-#19	White	Heterozygous mono-allelic (del)	Deletion of 6-bp sequence (CCACTAT) in one allele
#2	SD-#20	White	Mosaic (indels)	Insertion of A in one allele (allele-A), as shown by ACCACTA(A)TTACA, insertion of TT in another allele (allele-A'), as shown by ACCACTATT(TT)ACA, and deletion of 7-bp sequence (ACATAAT) in one more another allele (allele-A'')
#2	SD-#21	White	Normal (A/A)	-
#2	SD-#22	White	Normal (A/A)	-
#2	SD-#23	White	Heterozygous mono-allelic (in)	Insertion of (A) in one allele, as shown by ACCACTA(A)TTACA
#3	SD-#24	White	Normal (A/A)	-
#3	SD-#25	White	Normal (A/A)	-
#3	SD-#26	White	Compound heterozygous bi-allelic (del)	Deletion of 6-bp sequence (TATTAC) in one allele, and deletion of 7-bp sequence (TATTACA) in the other allele
#4	SD-#54	Black	Heterozygous KI (G/A)	Successful KI of ssODN in one allele (see Fig. 2F)

#4	SD-#55	Black	KI (G)/ heterozygous mono-allelic (indels)	Successful KI of ssODN in one allele (allele-G; see Supplemental Fig. S1) and deletion of (T)/insertion of (A) in the other allele, as shown by TATGTGATGGAACACCTGAGGGACC(A) (allele-A)
#4	SD-#56	White	Heterozygous mono-allelic (in)	Insertion of (T) in one allele, as shown by ACCACTATT(T)ACA.
#4	SD-#57	White	Normal (A/A)	-
#4	SD-#58	White	Mosaic (indels)	One allele has albino-specific allele A (allele-A). There are insertion of (AA) in the other allele (allele-A'), as shown by ACCACTA(AA)TTACA, and deletion of 13-bp sequence (ATTACATAATCCT) in one more another allele (allele-A'')
#4	SD-#59	White	Normal (A/A)	-
#4	SD-#60	White	Normal (A/A)	-
#4	SD-#61	White	Normal (A/A)	-
#4	SD-#62	White	Normal (A/A)	-
#4	SD-#63	White	Normal (A/A)	-
#4	SD-#64	White	Normal (A/A)	-
#4	SD-#65	White	Normal (A/A)	-
#4	SD-#66	White	Normal (A/A)	-
#4	SD-#67	White	Heterozygous mono-allelic (in)	Insertion of (AA) in one allele, as shown by ACCACTA(AA)TTACA

Abbreviations: in, insertion mutation; del, deletion mutation; indels, insertion and deletion mutations.

Supplementary Table S5. Exp-5: KI into the *Tyr* locus in SD fetuses/newborn offspring obtained after *i*-GONAD in the presence of 1 μ M SCR7.

Name of pregnant females	Name of fetuses	Eye color	Genotype	Note for the target <i>Tyr</i> sequence
#1	SD-#27	Black	KI (G)/ mosaic (indels)	Successful KI of ssODN in one allele (allele-G). There are deletion of 7-bp sequence (ACATAAT) in another allele (allele-A'), and insertion of (A) in one more another allele (allele-A''), as shown by ACCACTA(A)TTACA
#1	SD-#28	White	Normal (A/A)	-
#1	SD-#29	White	Heterozygous mono-allelic (del)	Deletion of 2-bp sequence (TA) in one allele.
#2	SD-#30	White	Normal (A/A)	-
#2	SD-#31	White	Normal (A/A)	-
#2	SD-#32	White	Normal (A/A)	-
#2	SD-#33	White	Normal (A/A)	-
#2	SD-#34	White	Normal (A/A)	-
#2	SD-#35	White	Normal (A/A)	-
#3	SD-#36	White	Normal (A/A)	-
#3	SD-#37	White	Normal (A/A)	-
#3	SD-#38	White	Normal (A/A)	-
#4	SD-#39	White	Compound heterozygous bi-allelic (indels)	Deletion of (T) in one allele, as shown by ACCACTAATTACA and deletion of (TTAC)/insertion of (A) in the other allele, as shown by ACCACTATTAG(A)ATAA
#5	SD-#40	White	Heterozygous mono-allelic (in)	Insertion of (TT) in one allele, as shown by ACCACTATT(TT)ACA
#5	SD-#41	White	Heterozygous mono-allelic (in)	Insertion of (TT) in one allele, as shown by ACCACTATT(TT)ACA
#5	SD-#42	White	Normal (A/A)	-
#5	SD-#43	White	Normal (A/A)	-
#5	SD-#44	White	Mosaic (indels)	One allele has albino-specific allele A (allele-A). There are insertion of (A) in another allele (allele-A'), as shown by ACCACTA(A)TTACA, and deletion of 23-bp sequence (TATTACATAATCCTGGAAACCAT) in one more another allele (allele-A'')
#5	SD-#45	White	Mosaic (indels)	One allele has albino-specific allele A (allele-A). There are insertion of (A) in another allele (allele-A'), as shown by ACCACTA(A)TTACA, and deletion of (CTATTACA) in one more another allele (allele-A'')
#6	SD-#46	Black	KI (G)/ mosaic (in)	Successful KI of ssODN in one allele (allele-G). There are insertion of (TT) in another allele (allele-A'), as shown by ACCACTATT(TT)ACA, and insertion of (A) in one more another allele (allele-A''), as shown by ACCACTA(A)TTACA
#6	SD-#47	White	Homozygous bi-allelic (del)	Deletion of 96-bp sequence (CATAAATTGGTTTTTCACAGATCATTTGCAGCAGATCA GAAGAGTATAATAGCCATCAGGTTTTATGTGATGGAAC ACCTGAGGGACCACTATTACA) in both alleles
#6	SD-#48	White	Normal (A/A)	-
#6	SD-#49	White	Normal (A/A)	-
#6	SD-#50	White	Normal (A/A)	-
#6	SD-#51	White	Normal (A/A)	-
#6	SD-#52	White	Mosaic (indels)	One allele has albino-specific allele A (allele-A). There are insertion of (T) in another allele (allele-A'), as shown by ACCACTATT(T)ACA, and deletion of 7-bp sequence (TTACATA) in one more another allele (allele-A'')

#6	SD-#53	White	Mosaic (indels)	One allele has albino-specific allele A (allele-A). There are insertion of (TT) in another allele (allele-A'), as shown by ACCACTATT(TT)ACA, and deletion of 115-bp sequence (ATTACATAATCCTGGAAACCATGACAAAGCCAAAACCCAGGCTCCCATCTTCAGCAGACGTGGAATTTGTCTGAGTTTGACCCAGTATGAATCTGGATCAATGGATAGAACT) in one more another allele (allele-A'')
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Abbreviations: in, insertion mutation; del, deletion mutation; indels, insertion and deletion mutations.

Supplementary Table S6. Exp-5: KI into the *Tyr* locus in SD fetuses/newborn offspring obtained after *i*-GONAD in the presence of 10 μ M SCR7.

Name of pregnant females	Name of fetuses	Eye color	Genotype	Note for the target <i>Tyr</i> sequence
#1	SD-#68	White	Compound heterozygous bi-allelic (del)	Deletion of (TAT) in one allele, as shown by ACCACTAFTACA and deletion of (TATTAC) in the other allele, as shown by ACCACTATTAGATAA
#1	SD-#69	White	Normal (A/A)	-
#1	SD-#70	White	Normal (A/A)	-
#1	SD-#71	White	Mosaic (indels)	One allele has albino-specific allele A (allele-A). There are insertion of (AT) in another allele (allele-A'), as shown by ACCACTAT(AT)TACA, and deletion of (T) in one more another allele (allele-A''), as shown by ACCACTATFACA.
#1	SD-#72	White	Normal (A/A)	-
#1	SD-#73	White	Normal (A/A)	-
#1	SD-#74	White	Heterozygous mono-allelic (in)	Insertion of (CCTGAGGGACCAC) in one allele, as shown by ACCACTA(CCTGAGGGACCAC)TTACA
#1	SD-#75	Black	KI (G)/ mosaic (in)	Successful KI of ssODN in one allele (allele-G). One allele has albino-specific allele A (allele-A). Deletion of 5-bp sequence (TACAT) in one more another allele (allele-A''), as shown by ACCACTATFAGATAAT (see Fig. 3B).
#2	SD-#76	Black	KI (G)/ mosaic (in)	Successful KI of ssODN in one allele (allele-G). There are insertion of (C) in another allele (allele-A'), as shown by ACCACTA(C)TTACA, and deletion of 5-bp sequence (CTATTA) in one more another allele (allele-A''), as shown by ACCACTATFACATAAT.
#2	SD-#77	White	Compound heterozygous bi-allelic (indels)	Deletion of (TTACATAATCCTGG) in one allele, as shown by ACCACTATFACATAATCCTGGAAACCA and deletion of (ACTA)/insertion of (TGGAAA) in the other allele, as shown by ACCACTA(TGGAAA)TTACATAAT.
#2	SD-#78	White	Homozygous bi-allelic (del)	Deletion of (TACAT) in both alleles, as shown by ACCACTATFAGATAATCCTGG.
#2	SD-#79	White	Normal (A/A)	-
#2	SD-#80	White	Normal (A/A)	-
#2	SD-#81	White	Mosaic (indels)	Deletion of (TTACA) in one allele (allele-A), as shown by ACCACTATFACATAAT and insertion of (A) in the other allele (allele-A'), as shown by ACCACTA(A)TTACATAAT, and deletion of (CTATTACA) in one more another allele (allele-A''), as shown by ACCACTATFACATAAT.
#2	SD-#82	White	Mosaic (indels)	Insertion of A in one allele (allele-A), as shown by ACCACTA(A)TTACA, insertion of A in another allele (allele-A'), as shown by ACCACTA(A)TTACA, and deletion of (CTA) in one more another allele (allele-A''), as shown by ACCACTATTACA.
#2	SD-#83	White	Normal (A/A)	-
#2	SD-#84	White	Normal (A/A)	-
#2	SD-#85	White	Normal (A/A)	-
#3	SD-#86	White	Normal (A/A)	-
#3	SD-#87	White	Normal (A/A)	-
#3	SD-#88	White	Heterozygous mono-allelic (in)	Insertion of (AT) in one allele, as shown by ACCACTA(AT)TTACATAAT

Abbreviations: in, insertion mutation; del, deletion mutation; indels, insertion and deletion mutations.

Supplementary Table S7. Mutations at putative off-target loci containing up to 3 bp mismatches for target gene are analyzed by sequencing.

Name	Sequence (5'-3')	Locus	Gene name	Mutation	Primer-F	Primer-R
Tyr-wild-crRNA	TTTCCAGGATTACGTAAATAGTGG	chr1 : +151097594	Tyr	12/12	GCTCAAGGTTTAGTTGGGTACT	CTGGCTAGGTTTACTATCTCCTTG
W-offtarget 1	TTTCCAGGAAAGGTAATAGTGG	chr14 : +37687330	Fryl	0/12	ATCAGCACTAGACTGGTCAA	GAGAGTCTGTCACAAGCTTC
Name	Sequence (5'-3')	Locus	Gene name	Mutation	Primer-F	Primer-R
Tyr-mutant-crRNA	TTTCCAGGATTATGTAATAGTGG	chr1 : +151097594	Tyr	12/12	GCTCAAGGTTTAGTTGGGTACT	CTGGCTAGGTTTACTATCTCCTTG
M-offtarget 1	TTTCCAGAACTATGTAATAGTGG	chr11 : +30871276	-	0/12	GAGTGAGCAATGGCGGATAC	CACAGTIGCTCACACGCTTT
M-offtarget 2	TTTCCAGGATTGTGTCTAGTGG	chr11 : +36928561	-	0/12	TACTGGGGAGGTACAAGGAG	AGCATCTCCAAGGATGGGT
M-offtarget 3	TTTCCAGGAAAGGTAATAGTGG	chr14 : +37687330	Fryl	0/12	ATCAGCACTAGACTGGTCAA	GAGAGTCTGTCACAAGCTTC
M-offtarget 4	TTTCCACGAATATGTAAAGTGG	chr8 : +90874461	-	0/12	CAGCTCAAGCTTCTGGGATT	CGTGAGGATCTGTTCTGTIG
Name	Sequence (5'-3')	Locus	Gene name	Mutation	Primer-F	Primer-R
Pax6-crRNA1	GGAGGATCACCTGCAGAATTCGG	chr3 : -95715312	Pax6	4/4	CCTCCTTTCCTCAGGTACA	GGAGACCCATGCAAAATCAGG
P1-offtarget 1	GAAAGATCAGCTGCAGAATCGG	chr5 : +134499947	Cyp4a1	0/4	TCCTCTAACCTACCCAATCC	CAGGACACTCTGAACCTCCTC
Name	Sequence (5'-3')	Locus	Gene name	Mutation	Primer-F	Primer-R
Pax6-crRNA2	GGATGTGTGAGTAAAATTCTGGG	chr3 : +95716248	Pax6	4/4	CCTCCTTTCCTCAGGTACA	GGAGACCCATGCAAAATCAGG
P2-offtarget 1	GGATGTAGAGTCAAATTCTGGG	chr1 : -32441545	-	0/4	ATGTCCACAACAGCAGACAG	GTGGCTAAGACATACCACAACC
P2-offtarget 2	GGATGTGTGGTACATTTCTGGG	chr14 : +47851159	-	0/4	GTCTTTAGCCACTTGGACTT	GTGCAATAATGCTGGAGGATG
P2-offtarget 3	GCAATGTGTGAGAAATATTCTGGG	chr15 : +27165193	-	0/4	GTTGCAACTTTTGGGAAGTCTG	AGAAATGTTTCCCTCTCGATG
P2-offtarget 4	TGATATGTGACTAAAATTCTGGG	chr9 : +94093203	Dis3l2	0/4	CTCATGAGTGTGAGGTCAGTC	CTAAGTCTGCTCCACATC

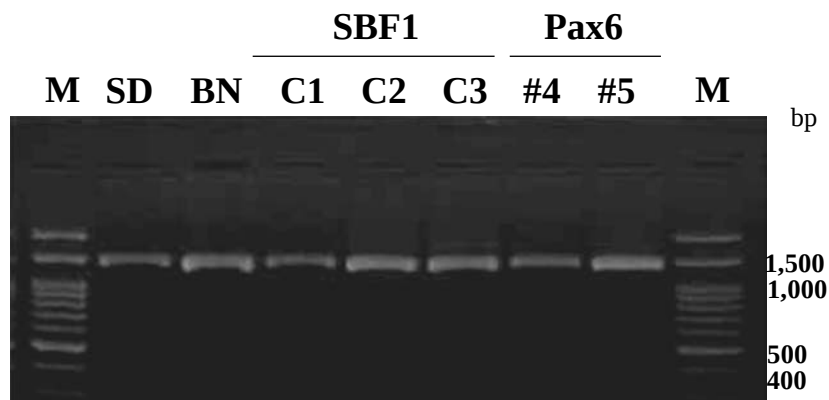
Mismatches compared to on-target sequence are shown in red; PAM sequences are labeled in blue.

Supplementary Table S8. Nucleotide sequences of oligonucleotides (oligo) used in this study.

Oligo-type	Sequence (5'-3')	Sequence (5'-3')
crRNA	Tyr-wild-crRNA	TTT CCA GGA TTA CGT AAT AG (TGG)
	Tyr-mutant-crRNA	TTT CCA GGA TTA TGT AAT AG (TGG)
	Pax6-crRNA1	GGA GGA TCA CCT GCA GAA TT(CGG)
	Pax6-crRNA2	GGA TGT GTG AGT AAA ATT CT(GGG)
ssODN	Tyr wild ssODN	AGA TCA GAA GAG TAT AAT AGC CAT CAG GTT TTA TGT GAT GGA ACA CCT GAG GGA CCA CTA TTA CGT AAT CCT GGA AAC CAT GAC AAA GCC AAA ACC CCC AGG CTC CCA TCT TCA GCA GAC GTG GAA TTT T
Primer	On-target primer	
	Rat Tyr-F	GCT CAA GGT TTA GTT GGG TAC T
	Rat Tyr-R	CTG GCT AGG TTT ACT ATC TCC TTG
	Rat Pax6-F	CCT CCT TTC CTC AGG TCA CA
	Rat Pax6-R	GGA GAC CCA TGC AAA TCA GG
	Off-target primer	
	W-offtarget 1 -F	ATC AGC ACT AGA CTG GTC AA
	W-offtarget 1 -R	GAG AGT CTG TCA CAA GCT TC
	M-offtarget 1 -F	GAG TGA GCA ATG GCG GAT AC
	M-offtarget 1 -R	CAC AGT TGC TCA CAC GCT TT
	M-offtarget 2 -F	TAC TGG GGA GGT ACA AGG AG
	M-offtarget 2 -R	AGC ATC TCC AAG GAT GGG T
	M-offtarget 3 -F	ATC AGC ACT AGA CTG GTC AA
	M-offtarget 3 -R	GAG AGT CTG TCA CAA GCT TC
	M-offtarget 4 -F	CAG CTC AAG CTT CTG GGA TT
	M-offtarget 4 -R	CGT GAG GAT CTG GTT CTG TTG
	P1-offtarget 1 -F	TCC TCT AAC CCT ACC CAA TCC
	P1-offtarget 1 -R	CAG GAC ACT CTG AAC TTC CTC
	P2-offtarget 1 -F	ATG TCC ACA ACA GCA GAC AG
	P2-offtarget 1 -R	GTG GCT AAG ACA TAC CAC AAC C

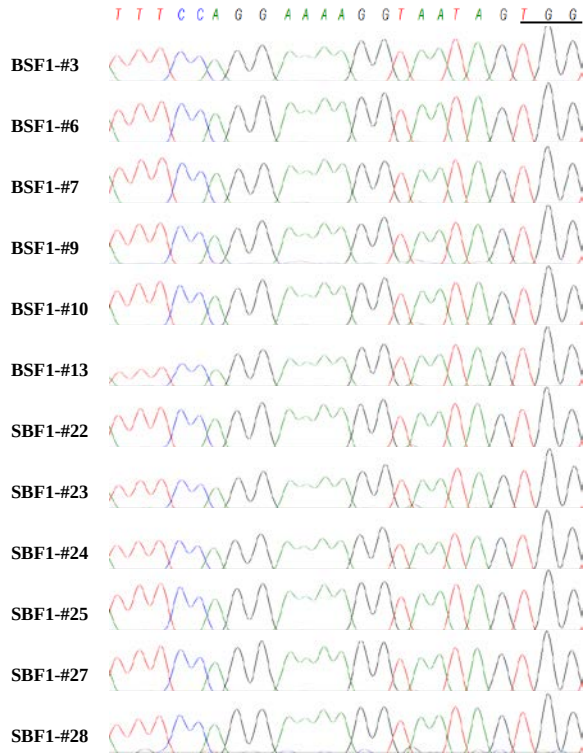
Primer	P2-offtarget 2 -F	GTC CTT TAG CCA CTT GGA CTT
	P2-offtarget 2 -R	GTG CAA TAA TGC TGG AGG ATG
	P2-offtarget 3 -F	GTG GCA ACT TTT GGA AGT CTG
	P2-offtarget 3 -R	AGA ATG TTT CCC CTC TCG ATG
	P2-offtarget 4 -F	CTC ATG AGT GTG AGG TCA GTC
	P2-offtarget 4 -R	CTA AGT CTG CTC CCA CAT C

Parentheses indicate PAM sequence in crRNA.

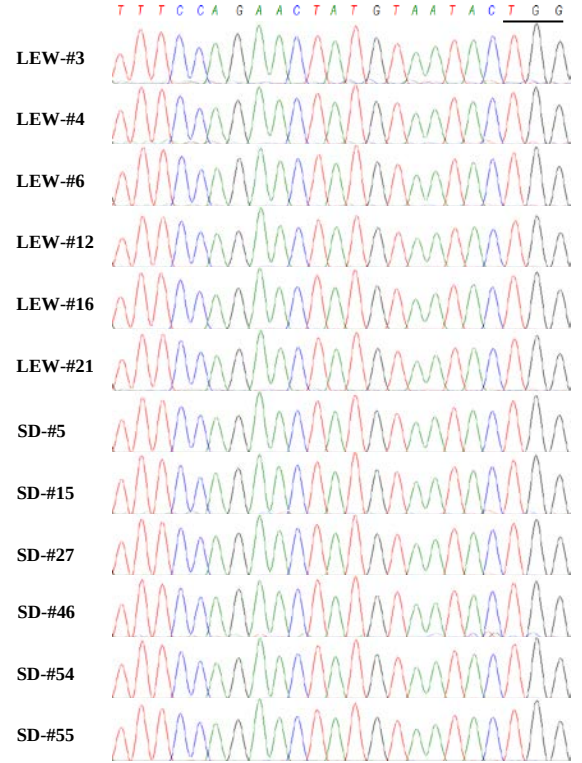


Supplemental Fig. S2. Genotyping of the unedited tails [isolated from SD, BN and offspring (C1-C3) obtained after mating between SD and BN) or fetal samples (Pax6-#4 and #5 shown in Fig. 4B-D)]. The PCR condition is the same shown in Fig. 4D. Note that there is no additional band throughout these samples, except for the presence of a single band at 1,489 bp.

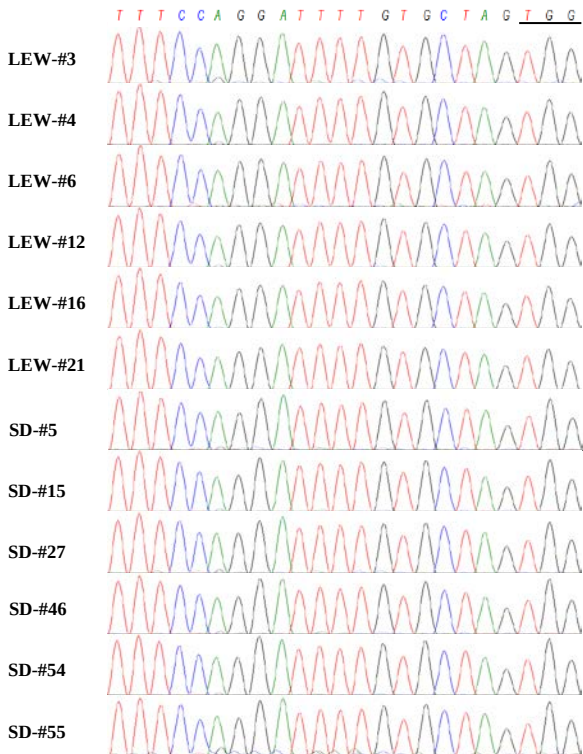
W-off target 1



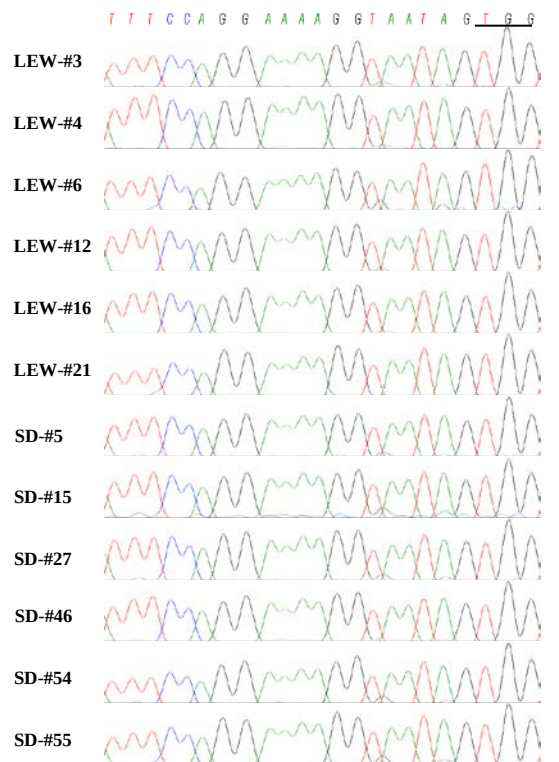
M-off target 1

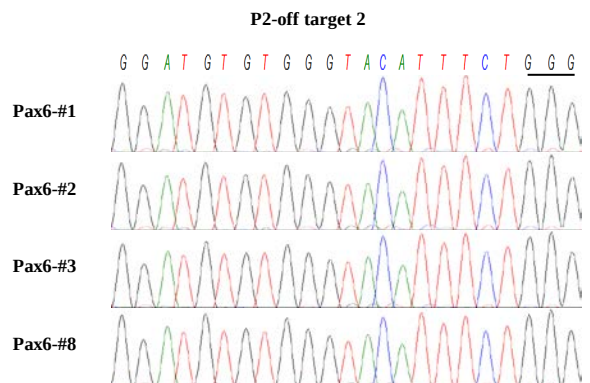
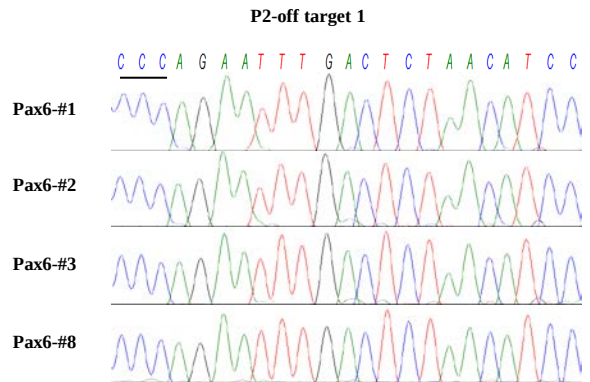
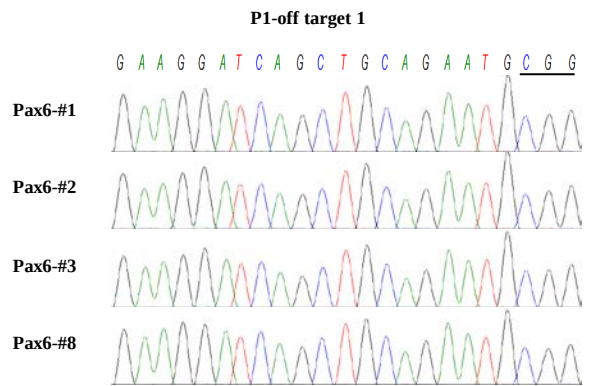
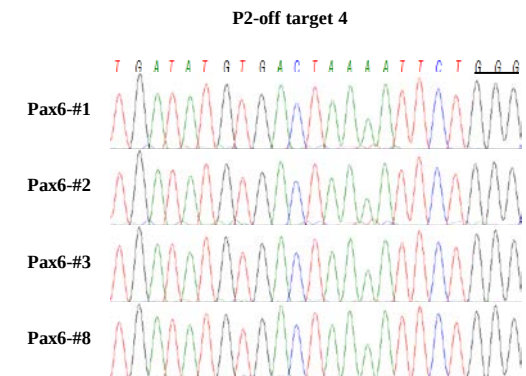
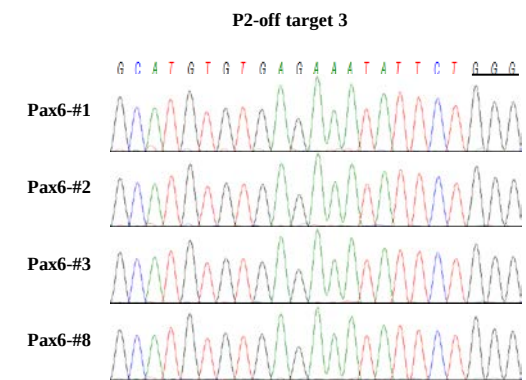
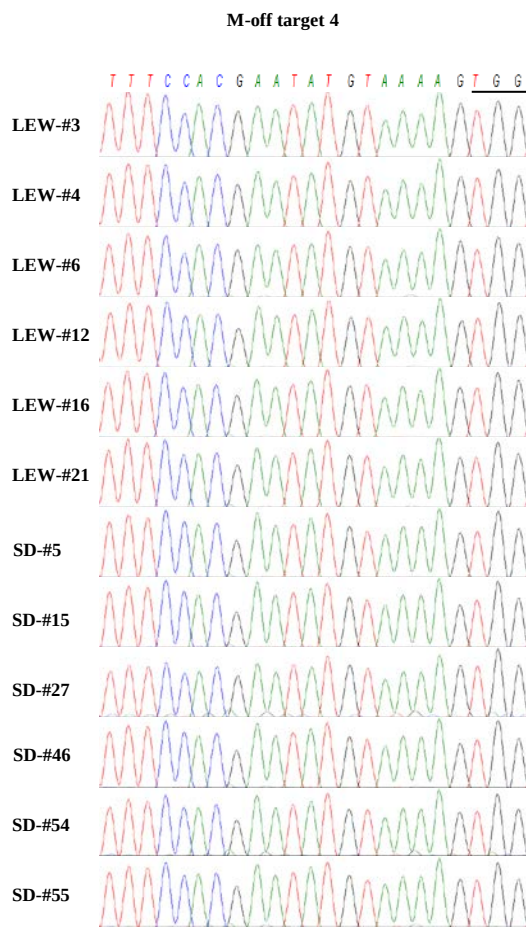


M-off target 2



M-off target 3





Supplemental Fig. S4. Sequencing results of off-target candidate loci in fetal/newborn offspring after *i*-GONAD. The 20-bp target sequences and PAM (underlined) are shown.