SUPPLEMENTARY INFORMATION

An efficient *i*-GONAD method for creating and maintaining lethal mutant mice using inversion balancer identified from C3H/HeJJcl strain

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Supplementary Figures:
Figure S1; PCR amplification of the inversion *In(6)1J* break points in (*Tprkb*^{em1Cu}/*In(6)1J* × *Tprkb*^{em1Cu}/*In(6)1J*) F2 mice.
Figure S2; Schematic diagram depicting the B6.C3H-*In(6)1J Mitf*^{em1Cu} mutagenesis screen.

Supplementary Tables:
Table S1; List of all primers used in the present study.
Table S2; List of gRNAs used in the present study.

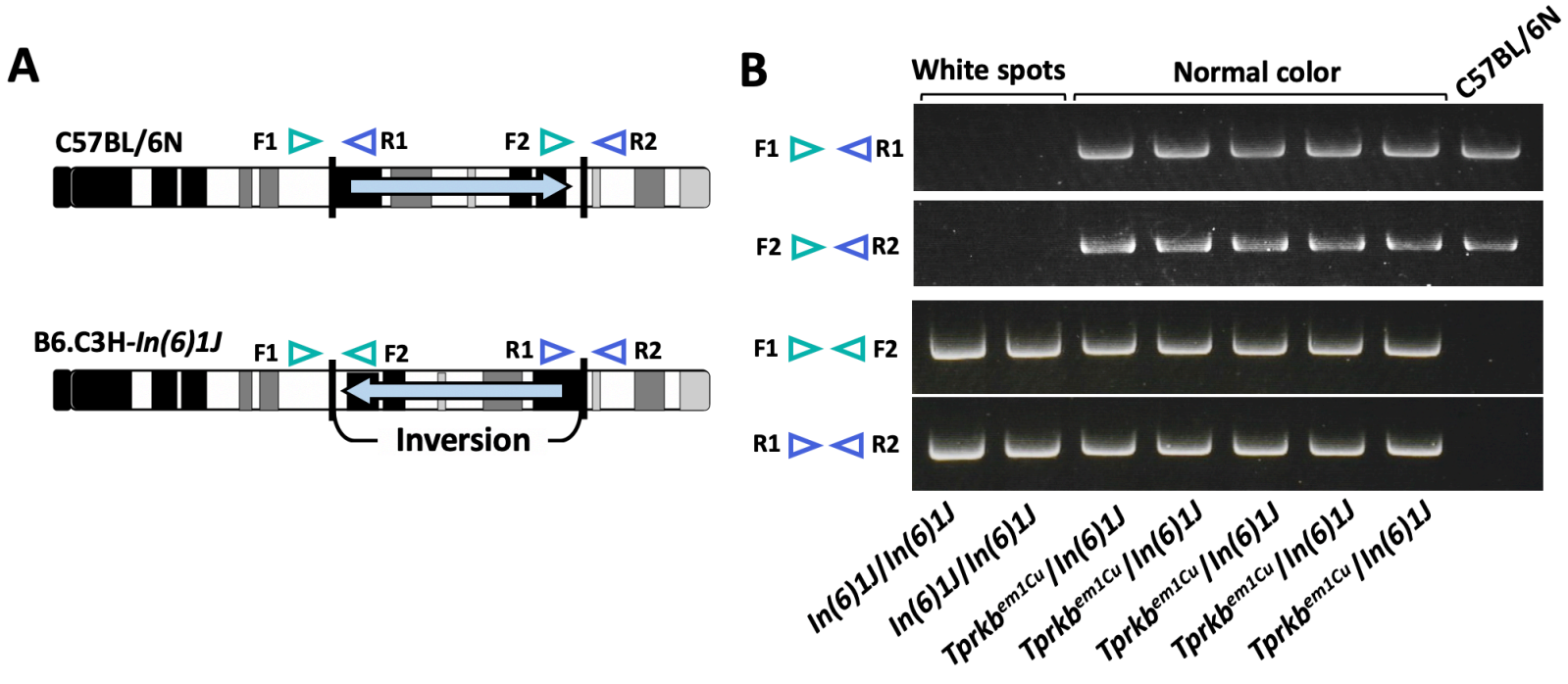

Figure S1

PCR amplification of the inversion *In(6)1J* break points in (*Tprkb*^{em1Cu}/*In(6)1J* × *Tprkb*^{em1Cu}/*In(6)1J*) F2 mice. (A) Schematic representation of the PCR primer positions for detecting the inversion *In(6)1J*. (B) Genotyping of F2 mice was performed to confirm *In(6)1J* genotypes.

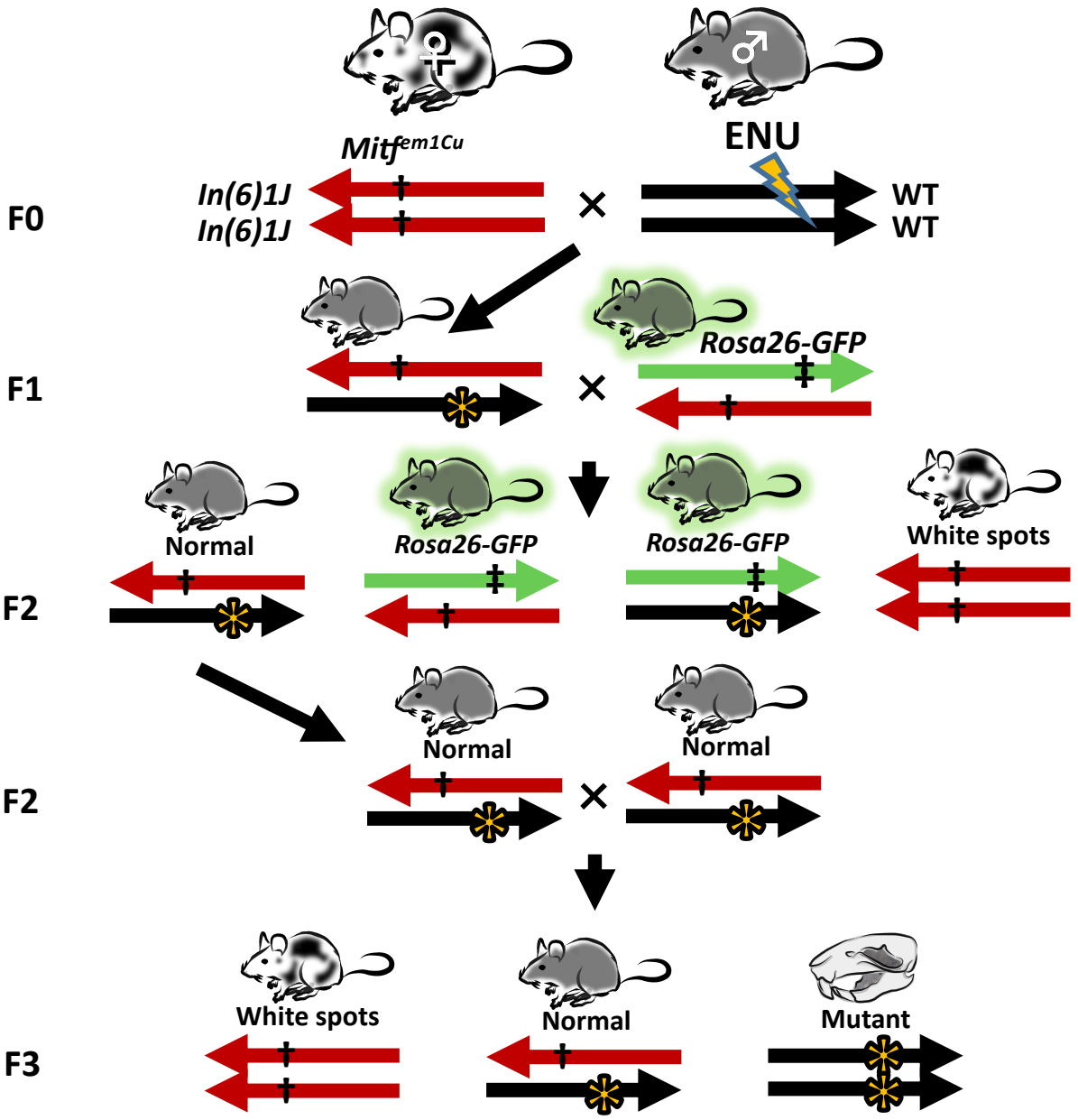

Figure S2

Schematic diagram depicting the B6.C3H-*In(6)1J Mitf*^{em1Cu} mutagenesis screen.

ENU-treated C57BL/6N males (*/+) are mated to females carrying the balancer (Inv/Inv) to generate first generation (F1) animals. F1 animals are mated to mice carrying a balancer and Rosa26-Green fluorescent protein (GFP) (dominant visible marker, ‡/Inv), which marks the non-mutagenized chromosome. The F2 animals (Inv/*) can be visually identified their normal coat color, and ‡/Inv and Inv/Inv mice are not used further. The F2 animals (Inv/*) are mated to look for new mutations (*/*) in the F3 offspring. Sibling matings between the Inv/* mice generate three classes of F3 mice, Inv/*, Inv/Inv, and */*, which can be distinguished by the presence of *Mitf*^{em1Cu} on the balancer chromosome. Abbreviation: ENU, *N*-ethyl-*N*-nitrosourea.

Table S1: List of all primers used in the present study

Target loci	Genomic location (GRCm38/mm10)	Sequences (5'-3')
Genotyping primers		
In(6)1J#F1	chr6:63000428-63000449	TCCCCAAAACATCACAATACAA
In(6)1J#R1	chr6:63001001-63001022	GCAGCTAAAAGAGTCAGCTTCA
In(6)1J#F2	chr6:120826900-120826921	TCACTGCAGAAATTCTTGGAAA
In(6)1J#R2	chr6:120827428-120827449	TCGTAATAAGGGCATTTCACCT
rs387767483#F	chr6:38214496-38214515	GGAAGGCTCTTTGGGACTCC
rs387767483#R	chr6:38215034-38215053	GGAGCTGTCTCGGTCCTTAG
rs244130831#F	chr6:71403016-71403037	AAAACACTAAACACACCGTGGG
rs244130831#R	chr6:71403684-71403705	TGAAGGTTTCCCATTAGCACCT
rs238042460#F	chr6:92813194-92813213	ACTGGCCTTCCTACCATCCT
rs238042460#R	chr6:92813698-92813717	CGTGTTCGCCCTGAGATGAT
rs242839954#F	chr6:136950009-136950028	GGCTGTAGTTCTGACGTGCT
rs242839954#R	chr6:136950329-136950350	TTGGTCATTGGCTAGTGTCATT
Mitf#F1	chr6:98003838-98003859	AAACCTCTGCAGTCAGTCAACA
Mitf#R1	chr6:98004100-98004121	CACACTGGAAGAACACCCACTA
Tprkb (External) #F1	chr6:85920542-85920564	CTTCATGTGTCCAGAGTTCAGC
Tprkb (External) #R1	chr6:85925068-85925090	GCCTTGATAGAGGAGGTGTGTC
Tprkb (Internal) #F2	chr6:85924288-85924310	GAGAAACTGGGTGGAAAAGATG
Tprkb (Internal) #R2	chr6:85924539-85924561	TCTGCCTTGGTTAATTCATTCC
RT-PCR primers		
Mitf#F2	chr6: 97996436-97996455	CTTGATGGATCCGGCCTTGC
Mitf#R2	chr6: 98006169-98006190	GTGATTGTCCTTTTTCTGCCTC
Gapdh#F1	chr6: 125162689-125162708	ACCACAGTCCATGCCATCAC
Gapdh#R1	chr6: 125162063-125162082	TCCACCACCCTGTTGCTGTA

Table S2: List of gRNAs used in the present study

Target loci	Genomic location (GRCm38/mm10)	Target Sequences (PAM)	CHOPCHOP (https://chopchop.cbu.uib.no/) Number of mismatches 0	1	2	3	Efficiency
Mitf exon 6	chr6: 98003907	GATCGACCTCTACAGCAACC (AGG)	0	0	0	1	58.22
Tprkb intron 7	chr6: 85920611	AAGCGTAGTCATTTGCACAA (AGG)	0	0	0	4	65.52
Tprkb intron 10	chr6: 85924922	CGGGCGTAAGAGCTTACATC (TGG)	0	0	0	0	47.05