Supplementary Information for

Super-Mendelian inheritance mediated by CRISPR/Cas9 in the female mouse germline

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Supplementary Methods

Cloning of the *Tyrosinase*^{CopyCat} transgene

All primers for cloning are listed in Supplementary Table 1. Using primers v851 and v852, we amplified a backbone for bacterial propagation that also contained a Human U6 promoter and gRNA scaffold. We amplified a second fragment of DNA that contained the CMV enhancer and promoter driving expression of the mCherry fluorophore from plasmid #548 (provided by Dr. Mark Tuszynski), using the primers v853 and v854. The two fragments were joined using the Gibson Assembly technique with reagents from New England Biolabs (NEB) (Cat.# E5520S) to obtain the plasmid pVG211, which carried all the components of the CopyCat except for the gRNA target sequence. To obtain the final transgene sequence, the *Tyrosinase* Exon 4 gRNA target (Tyr4a-gRNA) sequence was inserted by performing a plasmid primer mutagenesis using the primers v878 and v875 and the NEB Q5 Site-Directed Mutagenesis Kit (Cat.# E0554S) to obtain the pVG242 plasmid.

The Jackson Laboratory modified this plasmid to include homology arms for homologous recombination into the *Tyrosinase* locus (*Tyr*), precisely at the Tyr4a-gRNA target cut site. This donor plasmid was then used to introduce the targeting vector into the *Tyr* locus by pronuclear injection into zygotes of the C57BL/6J strain. Briefly, the Jackson Laboratory purchased capped Cas9 mRNA from Trilink for co-injection at 60 ng/ul together with 25-50 ng/ul of guide RNA (Tyr4a-gRNA target sequence: 5'-GTTATGGCCGATAGGTGCAT-3') and 10-20 ng/ul of the donor plasmid. The resulting founders were backcrossed, and offspring were screened for germline transmission. The inserted transgene is represented below (Supplementary Fig. 1, 2).

Tyrosinase^{CopyCat} transgene sequence

Below is displayed the sequence of the transgene in blue, inserted into the mouse genome (underlined sequences).

>Tyrosinase CopyCat Exon 4

TATTTTTGAACAATGGCTGCGAAGGCACCGCCCTCTTTTGGAAGTTTACCCAGAAGCCAATGCCATAG
AGCCCACCGCATCCCCAGCATGCCTGCTATTGTCTTCCCAATCCTCCCCCTTGCTGTCCTGCCCCACCCC
ACCCCCCAGAATAGAATGACACCTACTCAGACAATGCGATGCAATTTCCTCATTTTATTAGGAAAGGA
CAGTGGGAGTGGCACCTTCCAGGGTCAAGGAAGGCACGGGGGAGGGGCAAACAACAGATGGCTGGC
AACTAGAAGGCACAGTCGAGGCTGATCAGCGAGCTCTAGCATTTAGGTGACACTATAGAATAGGGCC
CTCTAGATGCATGCTCGAGCGGCCGCCAGTGTGATGGATATCTGCAGAATTCTTACTTGTACAGCTCGT
CCATGCCGCCGGTGGAGTGGCGGCCCTCGGCGCGTTCGTACTGTTCCACGATGGTGTAGTCCTCGTTGT
GGGAGGTGATGTCCAACTTGATGTTGACGTTGTAGGCGCCGGGCAGCTGCACGGGCTTCTTGGCCTTG
TAGGTGGTCTTGACCTCAGCGTCGTAGTGGCCGCCGTCCTTCAGCTTCAGCCTCTGCTTGATCTCGCCC
TTCAGGGCGCCGTCCTCGGGGTACATCCGCTCGGAGGAGGCCTCCCAGCCCATGGTCTTCTTCTGCATT
ACGGGGCCGTCGGAGGGGAAGTTGGTGCCGCGCAGCTTCACCTTGTAGATGAACTCGCCGTCCTGCAG
GGAGGAGTCCTGGGTCACGGTCACCACGCCGCCGTCCTCGAAGTTCATCACGCGCTCCCACTTGAAGC
CCTCGGGGAAGGACAGCTTCAAGTAGTCGGGGATGTCGGCGGGGTGCTTCACGTAGGCCTTGGAGCCG
TACATGAACTGAGGGGACAGGATGTCCCAGGCGAAGGGCAGGGGGCCACCCTTGGTCACCTTCAGCTT
GGCGGTCTGGGTGCCCTCGTAGGGGCGGCCCTCGCCCTCGCCCTCGATCTCGAACTCGTGGCCGTTCAC
GGAGCCCTCCATGTGCACCTTGAAGCGCATGAACTCCTTGATGATGGCCATGTTATCCTCCTCGCCCTT
GCTCACCATGGTGGCGGGATCCGAGCTCGGTACCAAGCTTGGGTCTCCCTATAGTGAGTCGTATTAATT
TCGATAAGCCAGTAAGCAGTGGGTTCTCTAGTTAGCCAGAGAGCTCTGCTTATATAGACCTCCCACCG
TACACGCCTACCGCCCATTTGCGTCAATGGGGCGGAGTTGTTACGACATTTTGGAAAGTCCCGTTGATT
TTGGTGCCAAAACAAACTCCCATTGACGTCAATGGGGTGGAGACTTGGAAATCCCCGTGAGTCAAACC
GCTATCCACGCCCATTGATGTACTGCCAAAACCGCATCACCATGGTAATAGCGATGACTAATACGTAG
ATGTACTGCCAAGTAGGAAAGTCCCATAAGGTCATGTACTGGGCATAATGCCAGGCGGGCCATTTACC

GTCATTGACGTCAATAGGGGGCGTACTTGGCATATGATACACTTGATGTACTGCCAAGTGGGCAGTTT
ACCGTAAATACTCCACCCATTGACGTCAATGGAAAGTCCCTATTGGCGTTACTATGGGAACATACGTC
ATTATTGACGTCAATGGGCGGGGGTCGTTGGGCGGTCAGCCAGGCGGGCCATTTACCGTAAGTTATGT
AACGCGGAACTCCATATATGGGCTATGAACTAATGACCCCGTAATTGATTACTATTAATAACTAGTCA
ATAATCAATGTCCCAGCTAGCAGAGGGCCTATTTCCCATGATTCCTTCATATTTGCATATACGATACAA
GGCTGTTAGAGAGATAATTGGAATTAATTTGACTGTAAACACAAAGATATTAGTACAAAATACGTGAC
GTAGAAAGTAATAATTTCTTGGGTAGTTTGCAGTTTTAAAATTATGTTTTAAAATGGACTATCATATGC
TTACCGTAACTTGAAAGTATTTCGATTTCTTGGCTTTATATATCTTGTGGAAAGGACGAAACACCGTTA
TGGCCGATAGGTGCATGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTT
GAAAAAGTGGCACCGAGTCGGTGCTTTTTACATCAGGTTGTTTTTCTGTTTTTACATCAGGTTGTTTTTC
TGTTTGGTTTTTTTTTTACACCACGTTTATACGCCGGTGCACGGTTTACCACTGAAAACACCTATCGGCC
ATAACAGAGACTCTTACATGGTTCCTTTCATACCGCTCTATAGAAATGGTGATTTCTTCATAACATCCA
AGGATCTGGGATATGACTACAGCTACCTCCAAGAGTCAG

Mouse care

Mouse stocks used in this study are listed in Supplementary Table 2. All mice were housed in accordance with UCSD Institutional Animal Care and Use Committee protocols and fed on a standard breeders diet. Adult males and females were used for breeding.

DNA extraction

We obtained <5 mm of tail tissue from each mouse between birth and postnatal day 21 for genotyping. We sealed tail wounds with KwikStop Stypic Powder. We then screened tails for expression of mCherry using a fluorescent dissecting microscope. We submerged tails in 500 uL of TNES buffer (10mM Tris, pH 7.5; 400mM NaCl; 100 mM EDTA; 0.6% SDS) with 3 uL of 10 mg/mL Proteinase K and digested overnight (8-20 hr) in a 56°C water bath. We then added 139 uL of 6 M NaCl to each sample, vortexed, and centrifuged for 10 minutes at 14,000g at room temperature. We transferred supernatant to a clean tube and precipitated DNA by adding 700 uL ice-cold 95% EtOH and placing samples overnight at -20°C. We pelleted the precipitated DNA by centrifugation at 14,000g for 10 minutes at 4°C. We washed the pelleted DNA with ice-cold 70% EtOH and allowed it to air-dry before resuspension in TE.

PCR reactions

We performed PCR using either Bioline Red MyTaq MasterMix or NEB Q5 2X MasterMix (if the product was to be submitted for Sanger sequencing) with the following recipes and cycling parameters.

Bioline Red MyTaq: 1X MasterMix, 0.5 uM primers, 1 uL DNA (between 10-200 ng DNA) in 20 uL with the following cycle parameters. “n” represents the annealing temperature, and “q” represents the elongation time; each is designated in Supplementary Table 3.
95°C for 3’
30 repeats of 95°C for 15”, n°C for 15”, 72°C for q”
72°C for 5’
10°C for ∞

NEB Q5: 1X MasterMix, 0.5 uM primers, 1 uL DNA (between 10-200 ng DNA) in 50 uL
98°C for 30”
35 repeats of 98°C for 30”, 64°C for 30”, 72°C for 3’
72°C for 5’
10°C for ∞

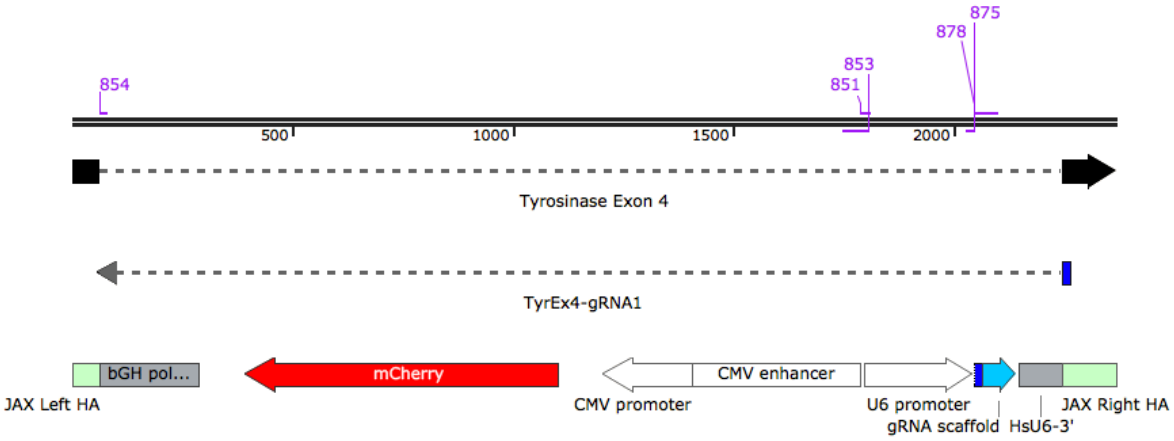
Gel purification

We ran samples on 1-2% agarose gels to separate bands. Representatives of each genotyping reaction are shown in Supplementary Fig. 3. Non-homologous end joining (NHEJ) amplicons or *Tyr*^{ch} amplicons were gel extracted using a QiaQuick Gel Extraction Kit as instructed. We submitted purified DNA for Sanger sequencing using the amplification primers noted in Supplementary Table 3.

Blinded genotyping of F3 constitutive and F4 germline conditional offspring

The researcher did not have information about the status of mCherry fluorescence or coast color when exon 4 and exon 5 were independently amplified from each tail tip DNA sample. Presence or absence of the *Tyr*^{CopyCat} allele (higher molecular weight band) and presence or absence of the *Tyr*^{Chinchilla} SNP (determined from Sanger sequence trace) was documented separately for each individual and then merged to annotate the genotype at both sites. Randomization was not appropriate for this work.

Supplementary Figures

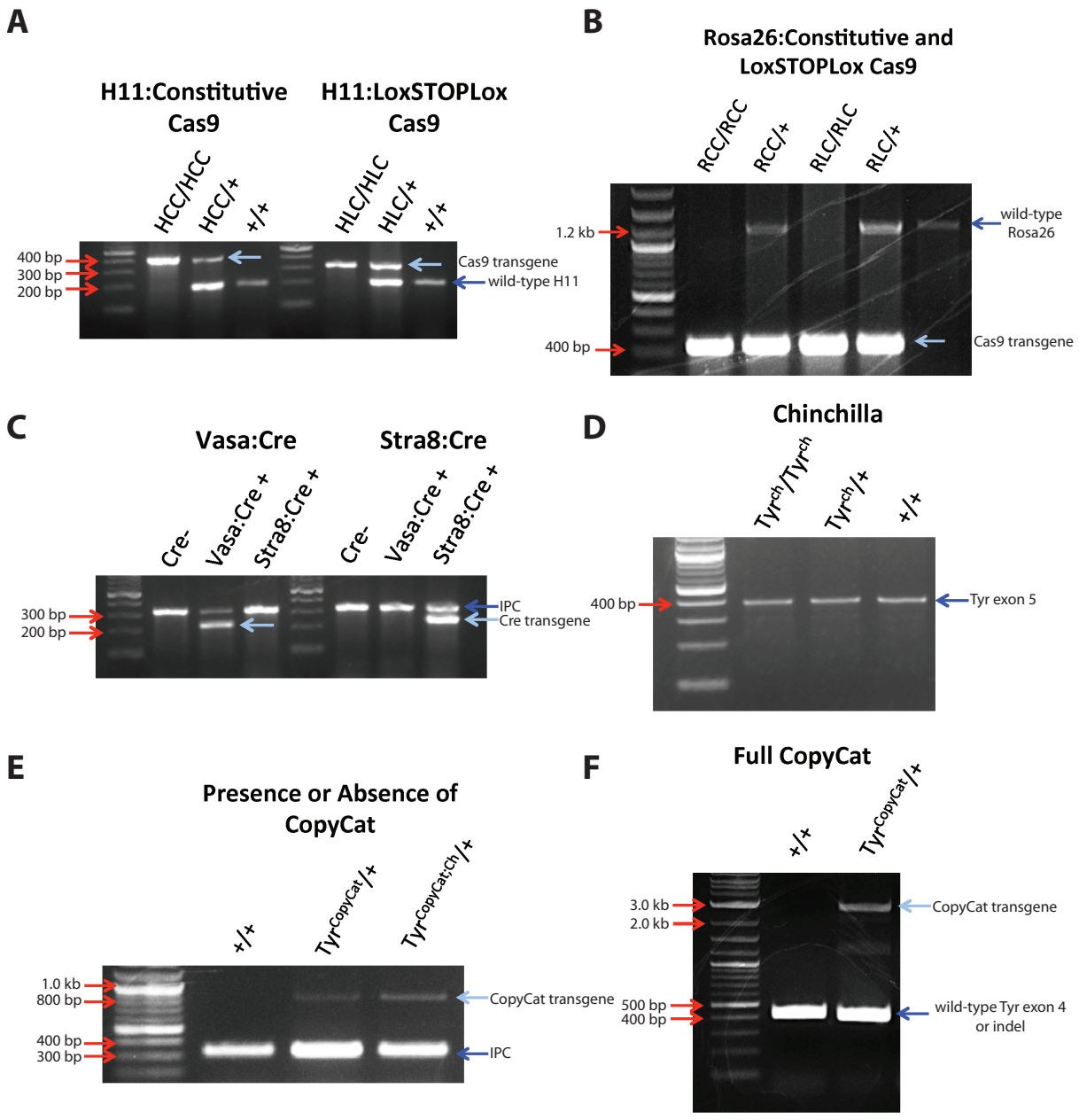


Supplementary Fig. 1.

The *Tyrosinase* Exon 4 "CopyCat" transgene that was inserted into the mouse genome by homologous recombination. As a visible marker of inheritance, the knock in allele carries a CMV Enhancer and Promoter-driven *mCherry* transgene with a bovine growth hormone polyadenylation signal (bGH poly[A]). A Human U6 Promoter (and downstream sequence) controls the transcription of the gRNA (Tyr4a-gRNA) in reverse orientation.

Supplementary Fig. 2.

Sequence annotation of the *Tyr*^{CopyCat} transgene after insertion into *Tyr* Exon 4 in the mouse genome. The flanking sequence of Exon 4 is represented as shaded in grey. Each of the primers used for the cloning this transgene is shown in purple. Dark blue annotations in the right and left homology arms (HA) show the disrupted target site for the Tyr4a-gRNA that is encoded in this transgene.



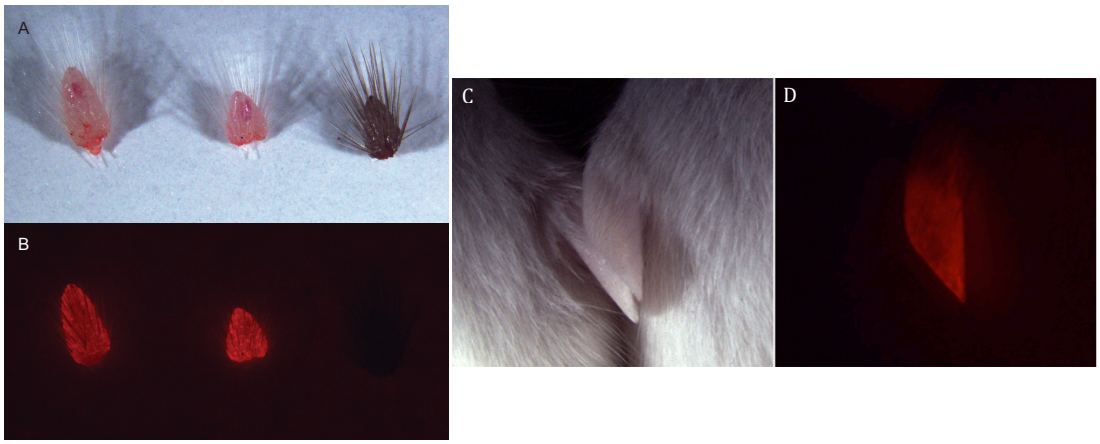
Supplementary Fig. 3.

Sample genotype results using primers as indicated in Supplementary Table 3. Each Cre and Cas9 PCR is representative of dozens of individuals with similar results. *Tyr*^{Chinchilla} and *TyrCopyCat* PCR results are representative of hundreds of individuals with similar results. All primary data for each of these *Tyr* alleles from F3 offspring of the constitutive crosses and F4 offspring of the germline crosses is accessible through a link provided in the "Data Accessibility" statement in the online version of the manuscript. For all: dark blue arrows indicate the wild type alleles or internal positive controls (IPC, amplifies *interleukin2* on chromosome 3), light blue arrows indicate transgenes. Red arrows denote relevant size markers in the DNA ladder for comparison.

(A) Genotyping for constitutive *H11-Cas9* (HCC) and *H11-LoxSTOPLox-Cas9* (HLC). Right- HCC: Band at 425 bp indicates the *Cas9* transgene. The band at 200 bp indicates the wild type (non-transgenic) *H11* allele. Left- HLC: The band at 350 bp indicates the *LoxSTOPLox-Cas9* transgene. The band at 200 bp indicates wild type (non-transgenic) *H11* allele.
(B) Genotyping for constitutive *Rosa26-Cas9* (RCC) or *Rosa26-LoxSTOPLox-Cas9* (RLC). The band at 1.2 kb indicates wild type (non-transgenic) *Rosa26* allele. The bands at 220 bp indicate each respective *Cas9* transgene.
(C) Genotyping for *Vasa-Cre* and *Stra8-Cre*. This genotyping strategy identifies presence or absence of the *Cre* transgene but not copy number. In both cases, an internal positive control at 324 bp (IPC) confirms successful amplification. Left: *Vasa-Cre* primers only amplify the *Vasa-Cre* transgene. Right: *Stra8-Cre* primers only amplify the *Stra8-Cre* transgene. Each primer pair contains a primer specific to the regulatory sequence and a primer within *Cre*.
(D) Genotyping for $Tyr^{chinchilla}$. The $Tyr^{chinchilla}$ primers flank the SNP and therefore amplify a 392 bp product regardless of $Tyr^{chinchilla}$ genotype. This amplicon was gel purified and Sanger sequenced to reveal the genotype as in Supplementary Figure 4.
(E) Genotyping to determine presence of $Tyr^{CopyCat}$ transgene. This strategy identifies presence or absence of the transgene but not copy number. An internal positive control (IPC) at 324 bp confirms successful amplification. The band at 838 bp indicates the presence of the $Tyr^{CopyCat}$ transgene in animals that inherit the original $Tyr^{CopyCat}$ chromosome and also in animals that copy the $Tyr^{CopyCat}$ allele to the $Tyr^{chinchilla}$ marked chromosome by homology directed repair (HDR).
(F) Genotyping to amplify *Tyr* exon 4 which can include the $Tyr^{CopyCat}$ transgene. The band at 2606 bp is an amplicon that includes the $Tyr^{CopyCat}$ transgene. The band at approximately 400 bp is the non-transgenic allele that was gel purified and Sanger sequenced to reveal NHEJ indels in individuals that inherited *Cas9* and did not repair double strand breaks (DSBs) by HDR.

Supplementary Fig. 4.

Sanger sequencing traces of *Tyr* exon 5, which differentiate individuals that are wild type, heterozygous, and homozygous for the *Tyr*^{chinchilla} single nucleotide polymorphism. Sequence traces represent hundreds of F3 offspring of constitutive strategies and F4 offspring of germline crosses with similar results. All primary sequence traces for these individuals are available through a link provided in the "Data Accessibility" statement in the online version of the manuscript. The *Tyr*^{chinchilla} region was PCR amplified from tail tip genomic DNA using Chinchilla L3 and Chinchilla R1 primers. The gel pruified product was subsequently Sanger sequenced using the Chinchilla R1 primer.



Supplementary Fig. 5.

mCherry fluorescence marks *Tyr*^{CopyCat} tails and ears. (A and B) Two tail tips from F2 mice of the *Rosa26-Cas9* lineage with *Tyr*^{CopyCat} (left and middle) and one from a mouse that did not inherit the *Tyr*^{CopyCat} transgene (right, *Tyr*$^{WT/chinchilla}$). mCherry is visible only in tails with an allele of the *Tyr*^{CopyCat} transgene. (C and D) F3 offspring of the constitutive *Rosa26-Cas9* lineage. The right mouse inherited the original *Tyr*^{CopyCat} transgene with mCherry fluorescence in an outcross to CD-1 *Tyr*^{null}. The left mouse inherited the *Tyr*^{chinchilla}-marked recipient chromosome with an NHEJ mutation and no mCherry fluorescence. Fluorescent images are consistent with the *Tyr*^{CopyCat} genotype and representative of hundreds of animals in all constitutive and germline conditional strategies with similar results.

Supplementary Table 1.

Primers that were used for cloning the *Tyr*^{CopyCat} transgene.

V851	CCAGCTAGCAGAGGGCCTATTTCCC
V852	GAGCTCGAATTCACTGGCCGTC
V853	TAGGCCCTCTGCTAGCTGGGACATTGATTATTGACTAGTTATTAATAGTAATCAATTACG
V854	TGTAAAACGACGGCCAGTGAATTCGAGCTCCCATAGAGCCCACCGCAT
V875	GTTATGGCCGATAGGTGCATGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGG
V878	GGTGTTTCGTCCTTTCCACAAG

Supplementary Table 2.

Mouse stocks that were used in this study.

Jackson Labs Stock Number	Jackson Labs Stock Name	Notes
26175	B6J.129(B6N)-Gt(ROSA)26Sortm1(CAG-cas9*,-EGFP)Fezh/J	Rosa-loxSTOPlox-Cas9
26179	B6J.129(Cg)-Gt(ROSA)26Sortm1.1(CAG-cas9*,-EGFP)Fezh/J	Rosa constitutive Cas9
26816	B6;129-Igs2tm1(CAG-cas9*)Mmw/J	H11-loxSTOPlox-Cas9
27650	STOCK Igs2tm1.1(CAG-cas9*)Mmw/J	H11 constitutive Cas9
17490	B6.FVB-Tg(Stra8-icre)1Reb/LguJ	Stra8-Cre
6954	FVB-Tg(Ddx4-cre)1Dcas/J	Vasa-Cre
4828	FVB.129P2-Pde6b+ Tyrc-ch/AntJ	Tyrosinase^{chinchilla}

Supplementary Table 3.

PCR primers that were used for genotyping in this study.

Primer Name	Primer Sequence	Amplicon	Amplicon Length	Polymerase	Annealing Temp	Elongation Time	Sequencing Primer	Notes
Chinchilla L3	GGGAGGAAAGGGTGCTTGAG	Chinchilla SNP	392bp	MyTaq	60	1'	Chinchilla R1	SNP G>A at position 252
Chinchilla R1	CAGCAAGCTGTGGTAGTCGT							
EGFP L1	ACATGAAGCAGCACGACTTCT	EGFP	220bp	MyTaq	60	45"		Indicates presence of Rosa26-Cas9 and Rosa26-loxSTOPloxCas9
EGFP R1	ACGTTGTGGCTGTTGTAGTTGT							
RLC-WT F*	GTTCGTGCAAGTTGAGTCCATC	RLC-WT	~1200bp	MyTaq	60	45"		Indicates presence of Rosa26 wild type allele
RLC-WT R*	GGACTGAGAATAGGCCCAAATG							
HCC-Tg-F*	GGGCAACGTGCTGGTTATTG	HCC-Tg	425bp	MyTaq	60	10"		Indicates presence of H11-Cas9 allele
HCC-Tg-R*	CCAGGCCGATGCTGTACTTC							
HC-WT-F*	GGGGCCTCCAAGTCTTGACAGTAGAT	HLC-WT	200bp	MyTaq	60	10"		Indicates presence of H11 wild type allele
HC-Common-R*	CTGACCAGTGGGACTGCTTTTTCCAG							
HLC-Cas9-F*	CGGCCGCCACTCGACGATGTA	HLC-Tg	350bp	MyTaq	60	10"		Indicates presence of H11-loxSTOPloxCas9 allele
HC-Common-R*	CTGACCAGTGGGACTGCTTTTTCCAG							
HC-WT-F*	GGGGCCTCCAAGTCTTGACAGTAGAT	HLC-WT	200bp	MyTaq	60	10"		Indicates presence of H11 wild type allele
HLC-Common-R*	CTGACCAGTGGGACTGCTTTTTCCAG							
Stra8:Cre F*	AGATGCCAGGACATCAGGAACCTG	Stra8:Cre	236bp	MyTaq	60	10"		Indicates presence of Stra8:Cre
Stra8:Cre R*	ATCAGCCACACCAGACACAGAGATC							
IPC F*	CTAGGCCACAGAATTGAAAGATCT	Internal Positive Control	324bp	MyTaq	60	10"		Internal Positive Control confirms PCR success
IPC R*	GTAGGTGGAAATTCTAGCATCATCC							
Vasa:Cre F*	CACGTGCAGCCGTTTAAGCCGGGT	Vasa:Cre	240bp	MyTaq	59	10"		Indicates presence of Vasa:Cre
Vasa:Cre R*	TTCCCATTCTAAACAACACCCTGAA							
IPC F*	CTAGGCCACAGAATTGAAAGATCT	Internal Positive Control	324bp	MyTaq	59	10"		Internal Positive Control confirms PCR success
IPC R*	GTAGGTGGAAATTCTAGCATCATCC							
Tyr HAL F2	AATGGCTGCGAAGGCAC	CopyCat/NHEJ	CopyCat: 2606bp NHEJ/WT: ~400bp	Q5	64	3'	Tyr HAR R2	CopyCat band indicates presence of CopyCat. NHEJ/WT band can be sequenced to identify NHEJ
Tyr HAR R2	GGTTCAAAAGCTTCCCAATCCT							
cc F1	TCAATGTCCCAGCTAGCAGAGGG	CopyCat Internal Positive Control	838bp	MyTaq	60	1'		Indicates presence of CopyCat allele
Tyr HAR R2	GGTTCAAAAGCTTCCCAATCCT							
IPC F*	CTAGGCCACAGAATTGAAAGATCT		324bp	MyTaq	60	1'		Internal Positive Control confirms PCR success
IPC R*	GTAGGTGGAAATTCTAGCATCATCC							

*indicates primers that were designed at the Jackson Laboratory or in citations referenced in the manuscript.
IPC- internal positive control, amplifies *interleukin2* from chromosome 3.

Supplementary Table 4.

Coat color of *Tyr*$^{CopyCat/chinchilla}$ F3 individuals that inherited a germline *Cre* transgene and a *loxSTOPlox-Cas9* conditional allele.

	Vasa>Cre				Stra8>Cre	
	Rosa26>LSLCas9		H11>LSLCas9		Rosa26>LSLCas9	H11>LSLCas9
	Female	Male	Female	Male	Male	Male
White	0	0	0	0	0	0
Grey	3	4	5	4	2	1
Mosaic	2	1	0	1	0	0