Asymmetric parental genome engineering by Cas9 during mouse meiotic exit

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Supplementary Figure legends

Supplementary Figure 1 | Disruption of green fluorescence in blastocysts following mII editing. (A) Paired Hoffman modulation (upper) and eGFP expression (eGFP) images of E4.0 blastocysts produced by 1-step injection of wild-type (wt) mII oocytes with Nanog-eGFP sperm from hemizygotes with concentrations of injected *Cas9* cRNA and *eGFP* gRNA indicated beneath. (B) Paired Hoffman modulation (upper) and eGFP expression (eGFP) images as per (A), except that oocytes were used from *Nanog-eGFP* knock-in hemizygotes, with wt sperm. Red highlights accompanying histograms provide an at-a-glance indication of RNA concentrations. Bars, 100 μm.

Supplementary Figure 2 | Pedigree analysis of offspring produced after mII editing. (A) Pedigree analyses of founder lines produced by the 1-step method of mII editing (Fig. 1A). Inset tables indicate injected concentrations (ng/μl) of *Cas9* cRNA (c) and gRNA (g) against tyrosinase (*Tyr*) and *Foxn1*, the number of 2-cell embryos transferred and term offspring, indicating perinatal mortality and survivors (number and percentage) possessing a mutant phenotype. Symbols are: box, male; circle, female; black coat, filled; white coat, open; mosaic (white and black coat), half-filled; red, genomic sequencing revealed one or more mutations; br, two F1 females with brown coats; wt, wild-type *Tyr* sequence; i, eye phenotype; CD1, the outbred strain, CD1 (ICR). (B) Pedigree analysis of founder lines as per (A), except that offspring were produced by the sequential method of mII editing (Fig. 1A).

Supplementary Figure 3 | Genome sequence analysis of offspring produced by native gene mII editing. (A) Genomic sequences of offspring produced by the 1-step method of mII editing at the tyrosinase (*Tyr*) locus by injecting 30 ng/ml *Cas9* cRNA and *Tyr* gRNA. The gRNA-corresponding sequence (green) plus adjacent 5' sequence is displayed on the top row and mutants beneath, with the proto-spacer adjacent motif (PAM) highlighted in green. Mutations are indicated in red type-face. 5' +, mutations detected 5' (but not 3') of the displayed sequence; 3' +, mutations detected 3' of the displayed sequence. Yellow highlighting indicates ambiguous calls presumptively produced by multiple targeting events. The inset table indicates the corresponding phenotypic change (if any) exhibited by offspring. (B) Founder (F0, top) and F1 offspring produced by Cas9-mediated mII editing of the *Tyr* locus by injecting 30 ng/ml *Cas9* cRNA and *Tyr* gRNA as indicated. Editing to produce founders was performed in C57BL/6. White arrowheads, mosaic; red arrowheads, apparently non-mosaic mutants. The F1 litter was produced by crossing a black coat-colour female founder (indicated) with a CD1 male; the founder carried a germline *Tyr* mutation confirmed by sequencing, to produce white coat-colour pups (indicated). Non-white coat colour mutations associated with Tyr are

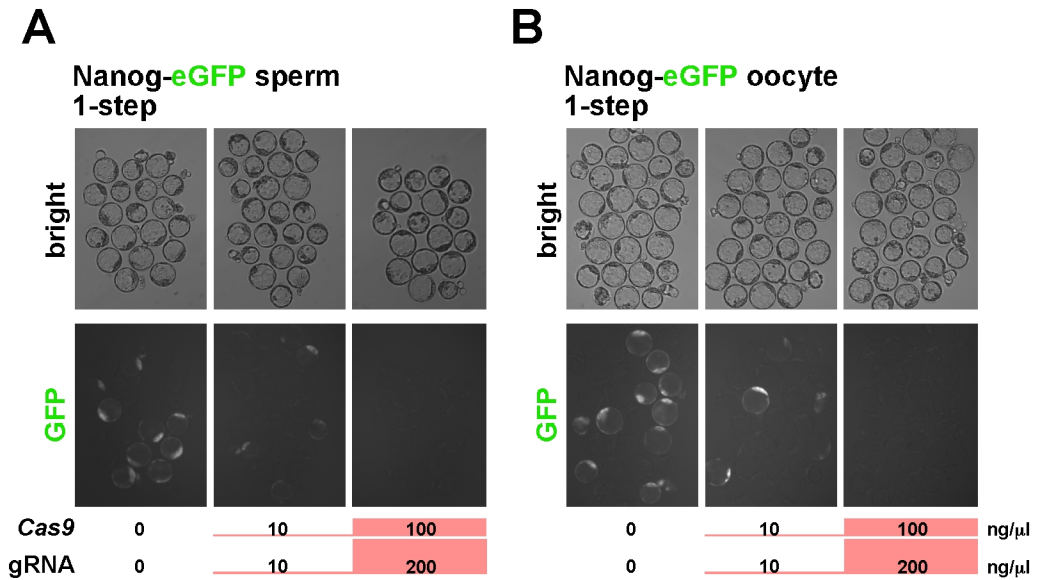
presumptively responsible for the brown coat colour F1 phenotype[37].

Supplementary reference

37. Lavado, A., Olivares, C., García-Borrón, J.C. & Montoliu, L. (2005). Molecular basis of the extreme dilution mottled mouse mutation: a combination of coding and noncoding genomic alterations. *J. Biol. Chem.* **280**, 4817-4824.

Supplementary Table S1. Primers used in this work

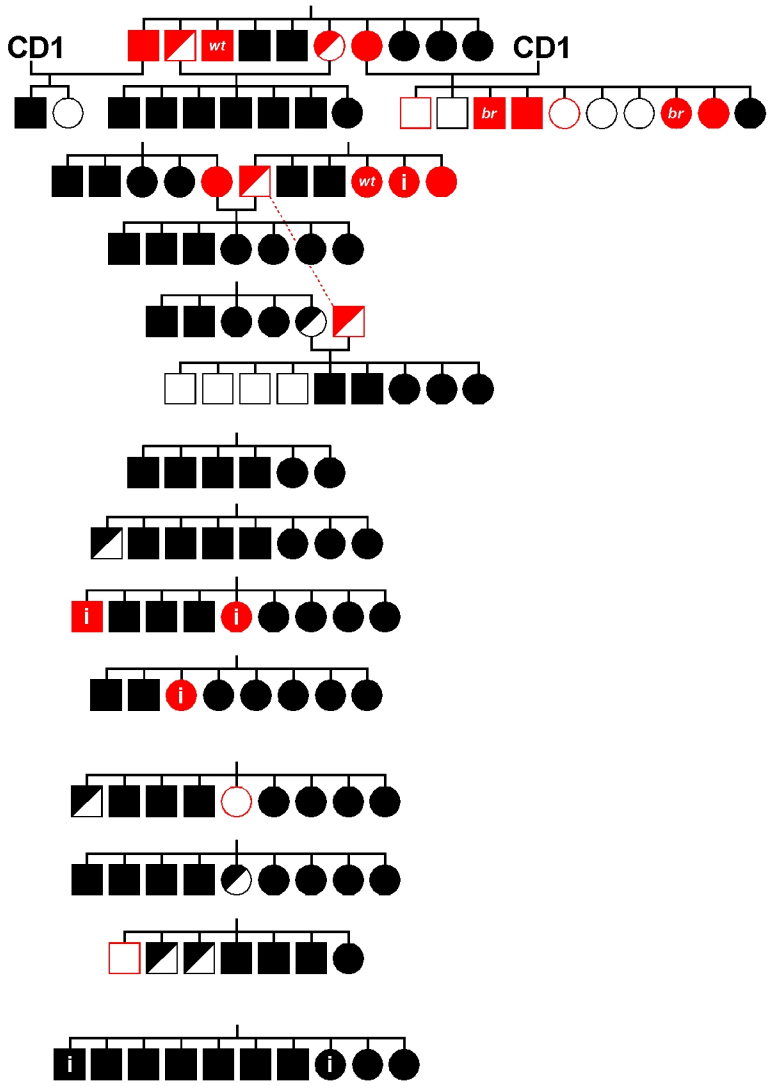
primer	target	sequence (5'→3')
GFP-Venus-S	*eGFP*	GACGTAAACGGCCACAAGTT
GFP-Venus-AS2	*eGFP*	GTCCTCCTTGAAGTCGATGC
Tyr 55F	*Tyr*	GGAGAAAATGTTCTTGGCTG
Tyr 651R	*Tyr*	TCCCTCCATATTTCAGAGCCC
β-actin-F	*Actb*	CCACCACAGCTGAGAGGGAA
β-actin-R	*Actb*	AGCCACCGATCCACACAGAG



Supplementary Figure S1

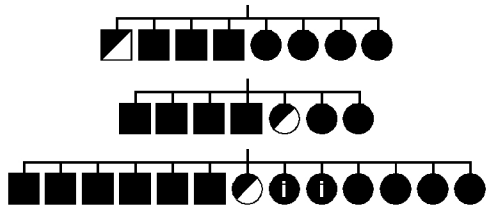
A

Tyr	c30g30
birth/emb tf	60/106
perinatal death	3
phenotypic change	9/57 (15.8%)

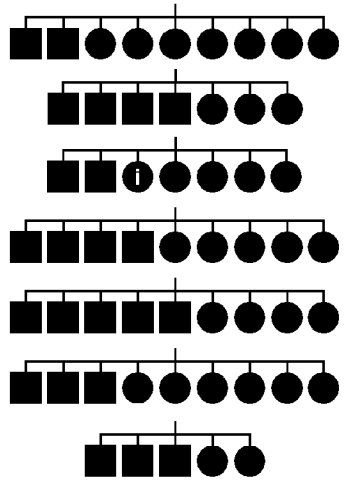


Tyr	c100g200
birth/emb tf	28/48
perinatal death	3
phenotypic change	6/25 (24.0%)

Tyr + Foxn1	c30g30
birth/emb tf	11/60
perinatal death	1
phenotypic change	2/10 (20.0%)

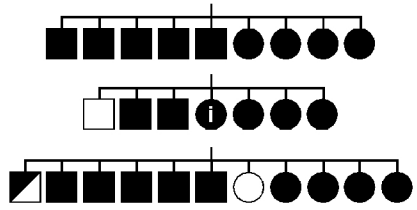


Tyr + Foxn1	c100g200
birth/emb tf	31/48
perinatal death	3
phenotypic change	5/28 (17.9%)



Foxn1	c30g30
birth/emb tf	59/108
perinatal death	4
phenotypic change	1/55 (1.8%)

B



Tyr + Foxn1	c30g30
birth/emb tf	30/60
perinatal death	3
phenotypic change	4/27 (14.8%)

Supplementary Figure S2

Supplementary Figure S3