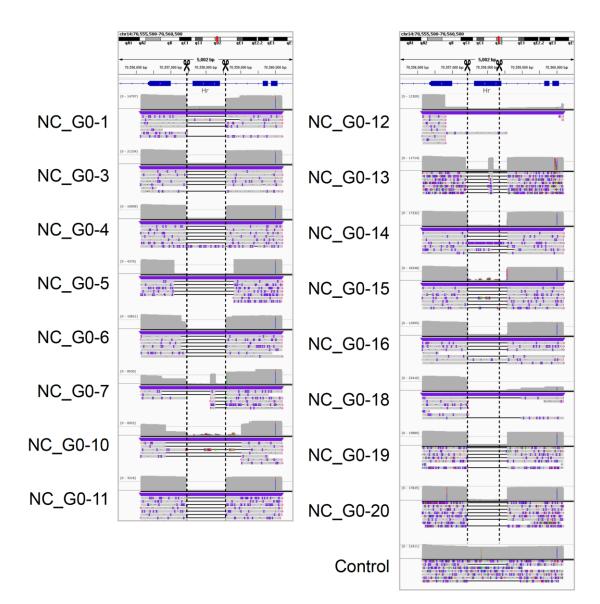


## Fig. S1. Electrophoresis results of long-PCR in G0 mice

Numbers indicate mouse IDs, and black boxes indicate mice subjected to long-read analysis owing to the intended loss of target exons. WT represents wild-type mice of each inbred strain.

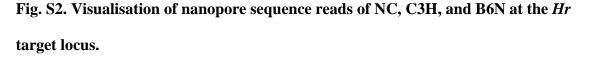


NC

C3H

C3H\_G0-1 B6N\_G0-1 C3H\_G0-2 B6N\_G0-2 C3H\_G0-3 B6N G0-3 C3H\_G0-4 ч**п** 1 C3H\_G0-8 B6N\_G0-4 C3H\_G0-9 B6N\_G0-5 C3H\_G0-10 B6N G0-6 C3H\_G0-11 B6N\_G0-7 C3H G0-12 B6N\_G0-9 C3H\_G0-13 C3H\_G0-14 Control Control

B6N



Visualisation of nanopore sequence reads at the Hr target locus in GRCm38.p6.

Scissors and dotted lines represent cleavage sites predicted by Cas9.

# Table S1. Survival and re-derivation rate of electroporated B6N zygotes in parallelexperiments.

Strain	# of examined zygotes <sup>A</sup>	# of survived zygotes <sup>B</sup> (B/A)	# of embryos developed to two-cell stage <sup>C</sup> (C/B)	# of transferred two-cell embryos <sup>D</sup>	# of recipients <sup>E</sup>	# of pregnancy <sup>F</sup> (F/E)	# of newborns <sup>G</sup> (G/D)
B6N	186	186 (100%)	181 (97.3%)	174	6	6 (100.0%)	61 (35.1%)

#, number.

#### Table S2. Details of the alleles in G1 mice.

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#### Table S3. Age of female mice for super-ovulation and results of retired breeders.

Strain	Age of	# of examined females <sup>A</sup>		# of	# of zygotes <sup>D</sup> (D/C)
BALB/c	11wks	12	12 (100.0%)	204	195 (95.6%)
	Retired breeders	15	12 (80.0%)	117	115 (98.3%)
NC	9-10 wks				
СВА	9-11 wks				
СЗН	11 wks				
SJL	9 wks				
	11wks	12	12 (100.0%)	268	186 (69.4%)
DBA1	Retired breeders	12	11 (91.7%)	242	242 (100.0%)
DBA2	9-10 wks				
B6N	9-11 wks				

Age of retired females ranged from 8-weeks- to approximately 12-months-old. #, number.

#### Table S4. Genomic DNA extraction methods in G0 mice.

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### Table S5. Primer sequences used for long-read and short-read sequencing.

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