Supporting Information

Table S1. Primers used in this work.

Primer Name	Primer Sequence	Usage
P1	GCTTCCTTTCACATTGACAGAACACTCC	Detecting Site II in ***17DI-GFP***
	AGGAAGTAGGAATGCCCCTTTGAG	
P2	CACGCTCGTCGTTTGGTATGG	Detecting Site I in ***17DI-GFP*** & ***17M-GFP***
	GCACGAGGAAGCGGTCA	
P3	CCTGGACAGCAAGACTTTCCTG	Detecting Epas-1 SNP site within ***17DI-GFP***
	GGTAAGAACAGCAGCCTACACTGAC	
P4	GCACGAGGAAGCGGTCA	Detecting Site IV in ***17PI***& ***17M-GFP***
	CCTCCAGATGGTTCCTTCCG	
P5	CAAAGTCACCCACTGCCAAGTC	Detecting Site III in ***17PI***
	ACCCACACCTTGCCGATGTC	
P6	CAAAGTCACCCACTGCCAAGTC	Detecting Site III in ***17M-GFP***
	GCTTCCTTTCACATTGACAGAACACTCC	
P7	CTTTCATTCCCCCCTTTTTCTG	Detecting Site II in ***17M-GFP***
	GCGTGGATAGCGGTTTGACTC	
SRY	CTGCTGTGAACAGACACTAC	Detecting genders of de novo derived ES cells
	GACTCCTCTGACTTCACTTG	

Figure S1. Knock-in constructs for the four sites.

Genomic structure of wild-type (WT) locus and knock-in constructs for the four sites of inversions. Red triangles: *loxP* sites. Neo: the coding region of neomycin resistance gene; Puro: the coding region of puromycin resistance gene; PGK: phosphoglycerate kinase 1 promoter; CMV: Cytomegalovirus immediate-early enhancer and promoter; VMC: CMV promoter in inverted orientation; PGK-DTa: Diphtheria Toxin transgene driven by PGK promoter; K14-Agouti: Agouti transgene driven by K14 promoter. Empty boxes indicate inactive elements while solid ones indicate active components in the shuttling cassettes.

Figure S2. Scheme of the proximal inversion.

The CASS strategy-based process used for engineering the proximal inversion in ES cells. The labels are as in the Fig. S1.

Figure S3. Selection for internal inversion and alternative recombination outcomes.

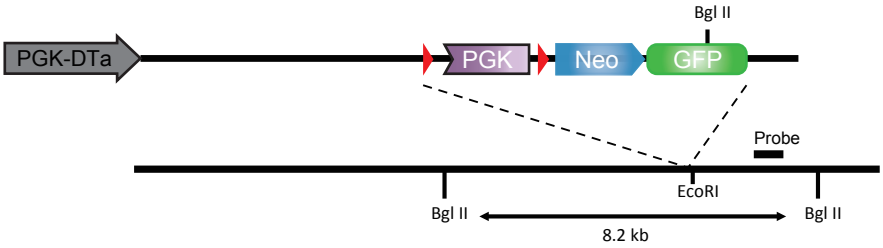
Recombination between different *loxP* sequences at the four inversion sites can cause chromosome deletions and inversions including the correct internal inversion. Possible recombination outcomes are illustrated. The Neo^+, $Puro^+$ double positive markers selects for the correct triple inversion chromosome 17, *17M-GFP*.

Figure S4. Breeding strategy for examining suppression of recombination by the inversions.

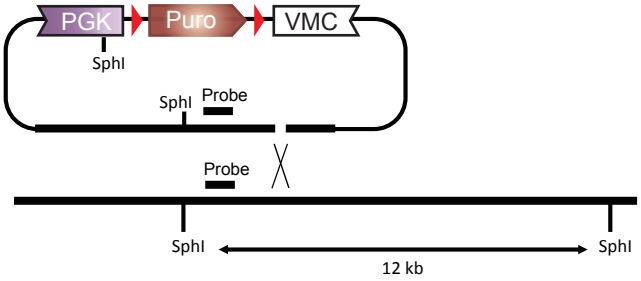
Mice carrying inversions, *17DI-GFP*, *17PI* or *17M-GFP*, were crossed to various test strains. The heterozygotes were then back crossed to these strains. The F2 offspring were examined for recombination by either PCR products across the inversion sites or SNP (see also methods).

Fig. S1

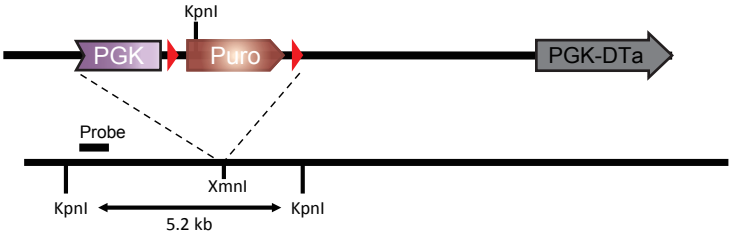
Site I



Site II



Site III



Site IV

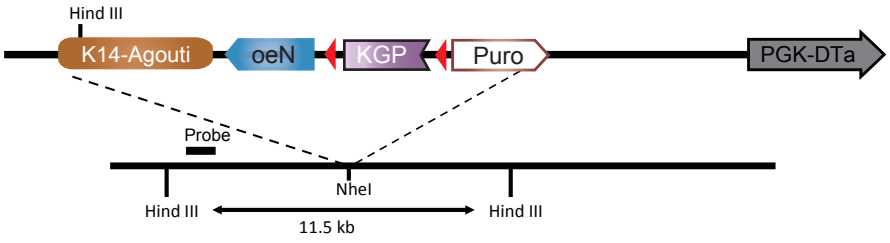


Fig. S2

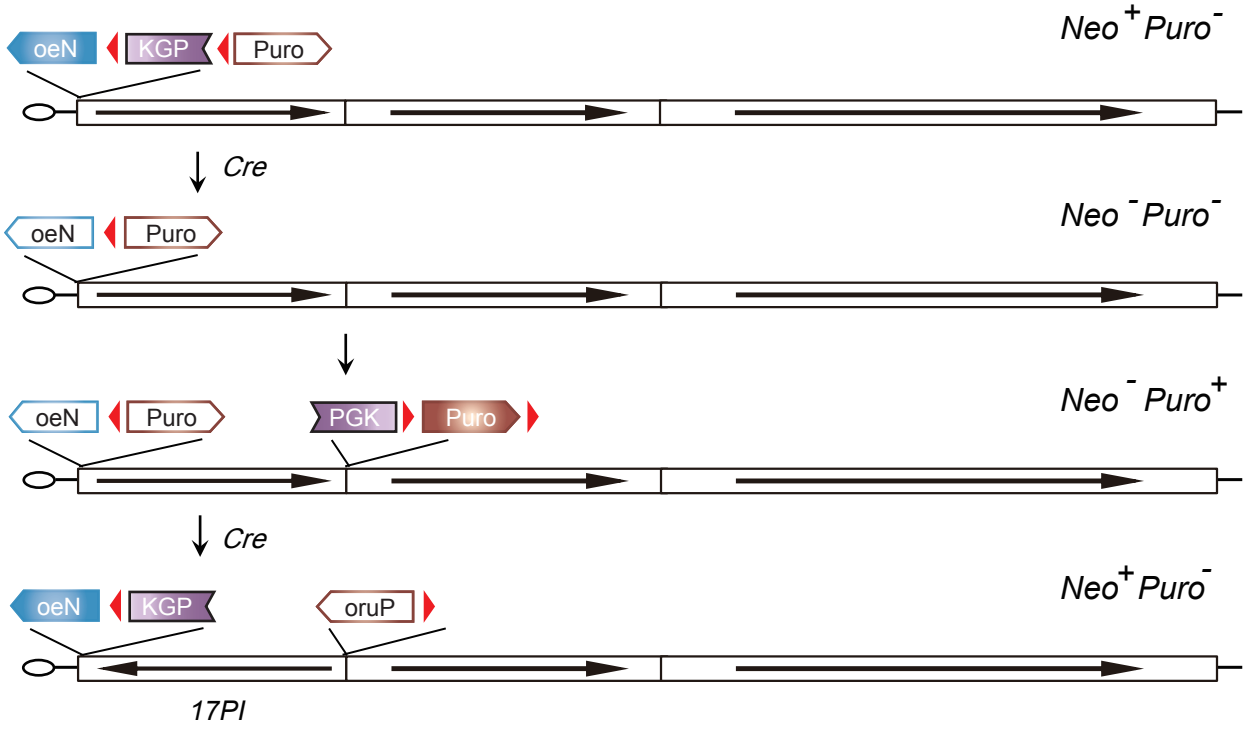

oeN
KGP
Puro
Neo+Puro-
Cre
oeN
Puro
Neo-Puro-
oeN
Puro
PGK
Puro
Neo-Puro+
Cre
oeN
KGP
oruP
Neo+Puro-
17PI

Fig. S3

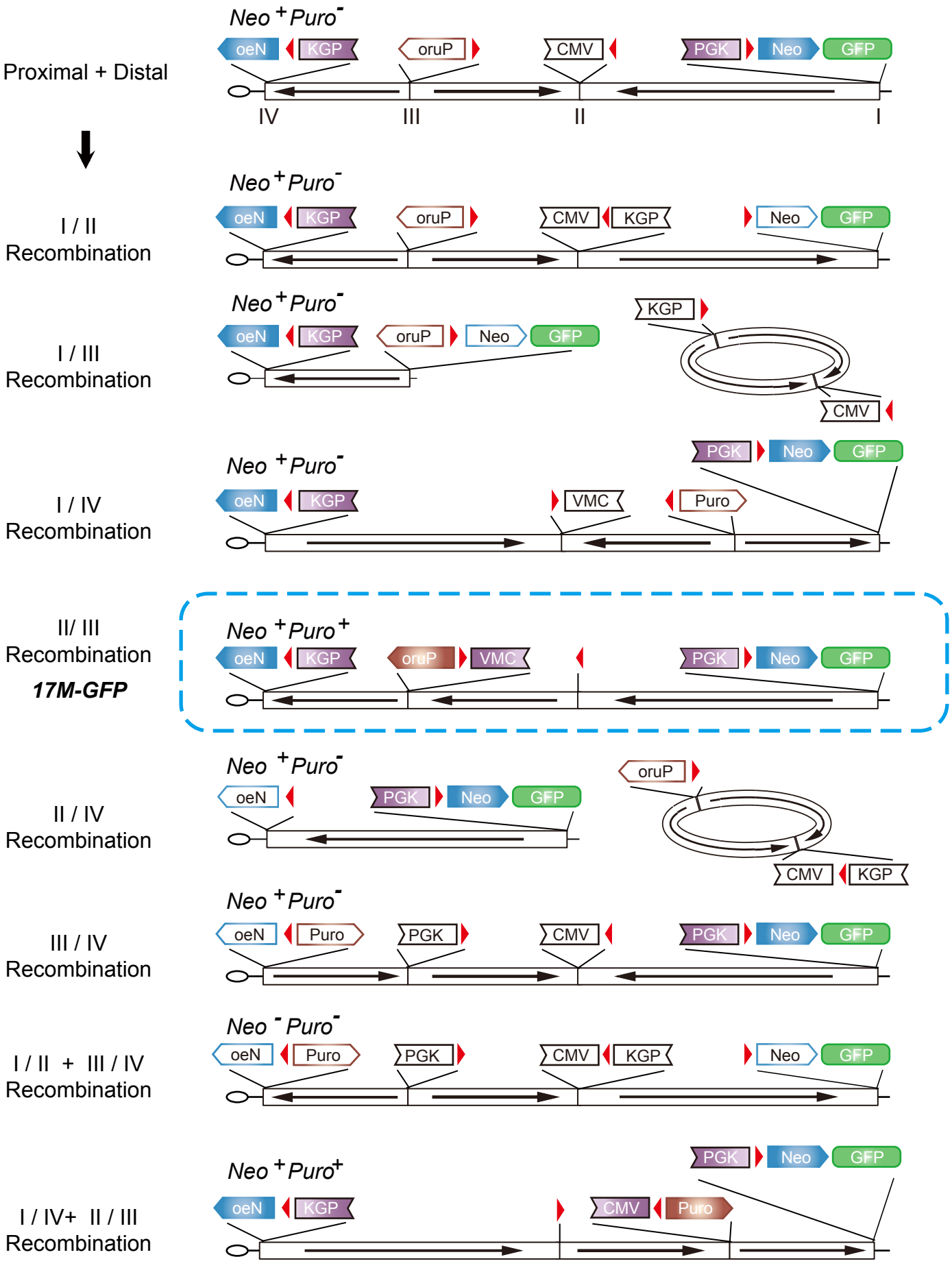
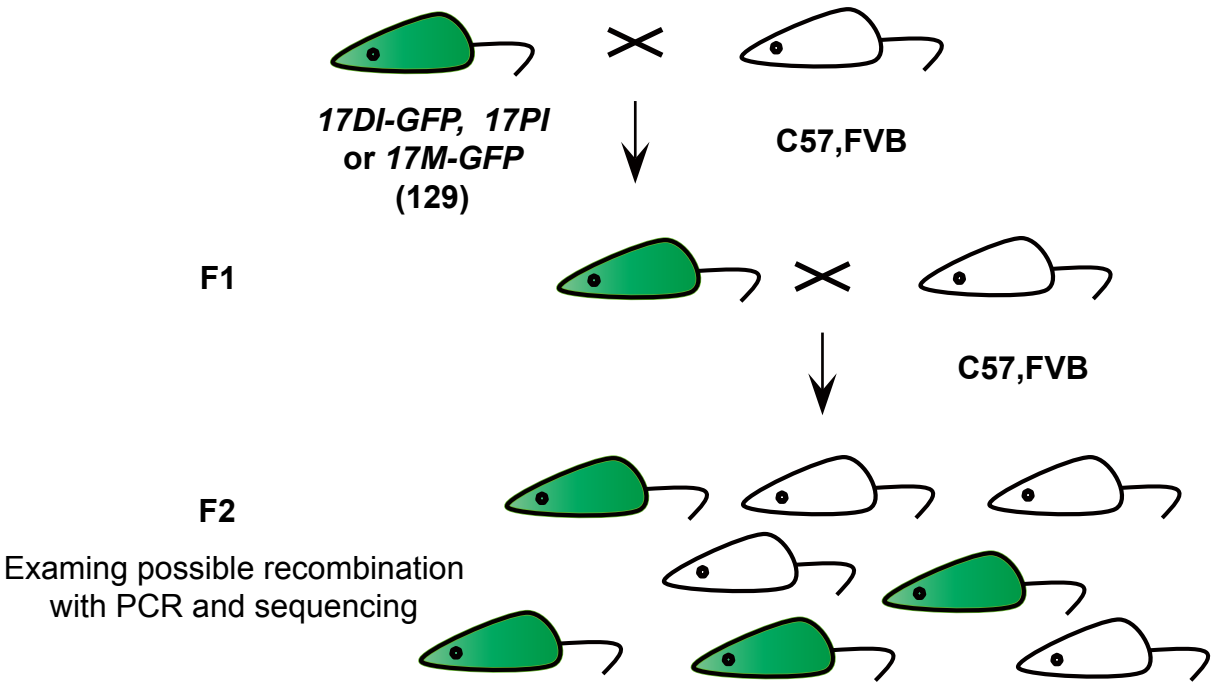

Proximal + Distal
Neo +Puro-
oeN
KGP
oruP
CMV
PGK
Neo
GFP
IV
III
II
I
I / II Recombination
I / III Recombination
I / IV Recombination
VMC
Puro
II/ III Recombination
17M-GFP
Neo +Puro+
II / IV Recombination
III / IV Recombination
I / II + III / IV Recombination
I / IV+ II / III Recombination

Fig. S4


17DI-GFP, 17PI
or 17M-GFP
(129)
C57,FVB
F1
C57,FVB
F2
Examing possible recombination
with PCR and sequencing