

Legends for Supplemental Figures

Figure S1. The sequence of donor DNAs used for generating conditional mouse allele of the *Prune1*. The sequence of homologous arms and *loxP* site are indicated. The red front represents the sequence used for gRNA synthesis, and the green front indicates the protospacer adjacent motif (PAM).

Figure S2. Genotyping of targeted alleles through zygote electroporation. Zygote electroporation of 100 ng/ μ L of gRNA, 200 ng/ μ L of donor DNA and 50 ng/ μ L Cas9 in a 10 μ L volume of Opti-MEM® was used for both targeting. The ratio of electroporated zygotes developed to the blastocytes in both targeting was around 50%. gRNA/donor DNA used for targeting 5' *loxP* showed significantly higher efficiency on generating homozygous knock-in (indicated by red arrows) than the one used for targeting 3' *loxP*.

Figure S3. The DNA sequencing data to confirm the corrected insertion of *loxP* sites into the *Prune1* locus in the *Prune1*^{F/F} allele.

Figure S4. Generation of the *Prune1* ^{Δ exon6} allele. (a) The *Prune1*^{F/F} allele was bred with *EIIa-Cre* mice for the excision of exon 6, resulting in the *Prune1* ^{Δ exon6} allele. (b) PCR genotyping for the detection of the excision of exon 6 in the *Prune1* ^{Δ exon6} allele. Lane 1, the wild-type with a ~1.4 kb PCR product; Lane 2, the *Prune1* ^{Δ exon6/ Δ exon6} homozygote in which a single 329 bp PCR product was detected.



5' donor DNA

cacacccagtaaagctgctcttctttaccctcc**tacaaagtcctcaatc**ATAACTTCGTATAGCATACATTATACGAAGTTAT

Homologous arm

loxP

tacaggcagccatttaactattgtgacctgattgtagcatttagagtttctttat

Homologous arm

3' donor DNA

tgctcatggcacaaggggattggtttgtggtttgt**tagtagtcgtgctcaa**ATAACTTCGTATAGCATACATTATACGAAGTTAT

Homologous arm

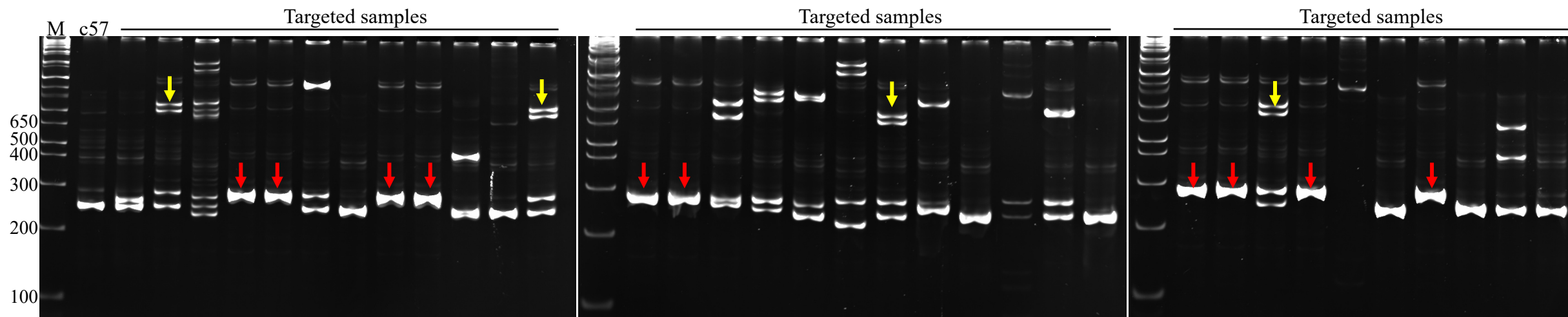
loxP

agaaggaagaaattagattcagaaatctctctctctcccttctcccctctccttct

Homologous arm

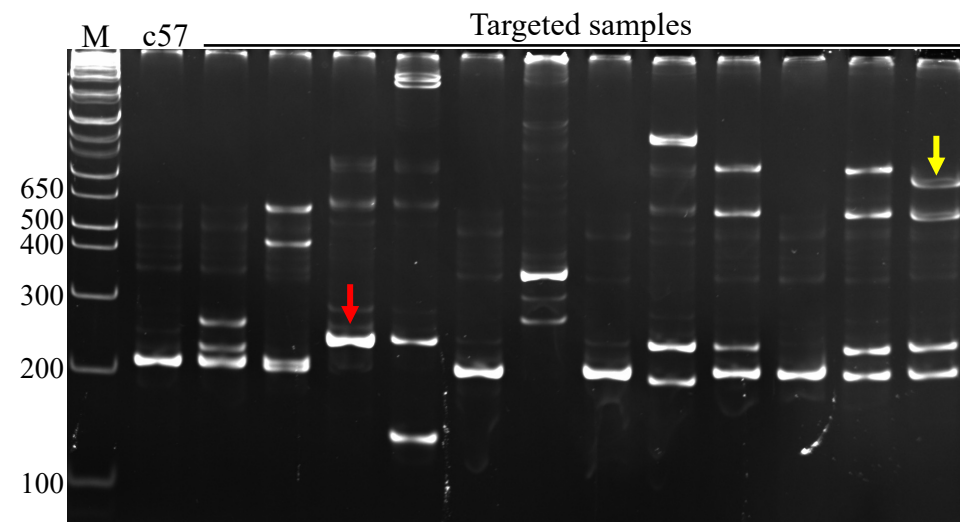
Figure S1

(a) Zygote electroporation with gRNA/Donor for 5' *loxP* site



10/34 samples showed homozygous knock-in (indicated by red arrows)
4/34 samples showed heterozygous knock-in (yellow arrows)
20/34 samples contained Indel or other mutations

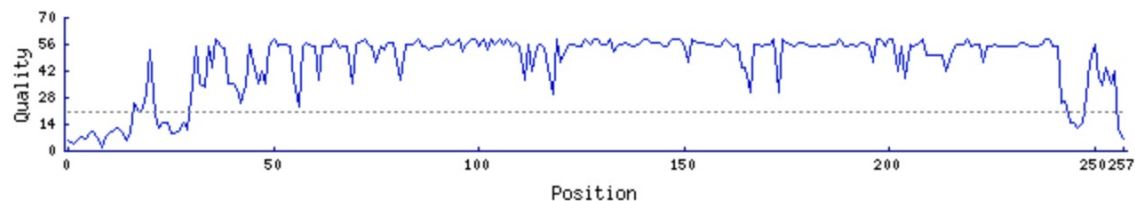
(b) Zygote electroporation with gRNA/Donor for 3' *loxP* site



1/12 samples showed homozygous knock-in (indicated by red arrow)
1/12 showed heterozygous knock-in (yellow arrow)

Figure S2

▼ Quality Graph



Read Length	258 b	Trimmed Length	243 b	Pos.	16-258
Q20	227 b	Q20/len	0.88		
Q40	197 b	Q40/len	0.76		

▼ Sequence

Previous Revision	n/a	Current Revision	1	Next Revision	n/a	Latest Revision	<u>1</u>
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Links: [View Revision History](#)

>5P_1RR_WPG1372-user_added CHROMAT_ID=1601340

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geatgggkateleaactcctgagctcaccagcctcttcctgcagagcactgggctcaagg      60
gtgtgcaccacccccacaccagtaaaagctgctcttcttttaccctcctacaaagtcct      120
caatcataaacttcgtatagcatatacgaagttattacaggcagccatttaactat      180
tgtgacctgattgttagcatttttagagttttctttattattggatatgtgtgggtgttt      240
tgctgatgtatgtcaaa
  
```

Targeted 5' loxP

▼ Quality Graph



Read Length	215 b	Trimmed Length	206 b	Pos.	10-215
Q20	194 b	Q20/len	0.90		
Q40	155 b	Q40/len	0.72		

▼ Sequence

Previous Revision	n/a	Current Revision	1	Next Revision	n/a	Latest Revision	<u>1</u>
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Links: [View Revision History](#)

>3P_1RR_WPG1374-user_added CHROMAT_ID=1601335

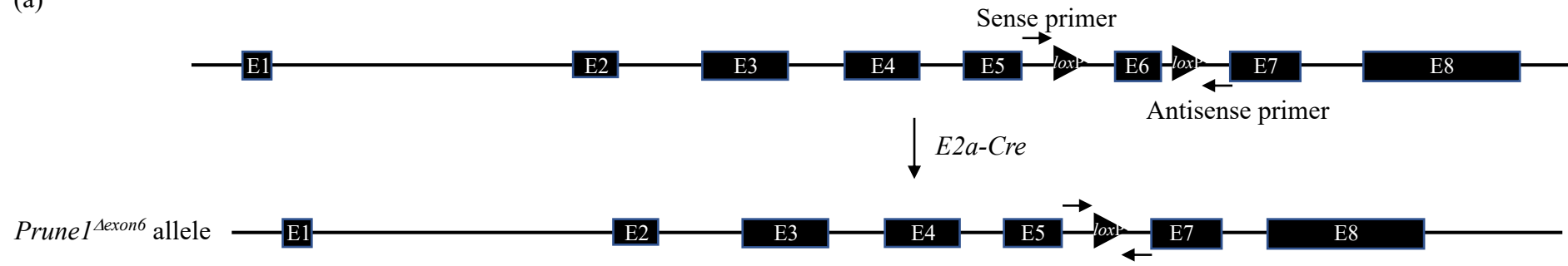
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gtcaattgatgggttggtttgtagtagtcgtgctcaataaacttcgtatagcatatacatt      60
atacgaagttatgaaggaagaaattagattcagaaatctctctcttcccttctccct      120
ctccttctttctctctatctattgatacagggtaaaattggaaggtgggaataaagga      180
tgggaaaaagaagaaccactacaaagtggcaaaga
  
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Targeted 3' loxP

Figure S3

(a)



(b)

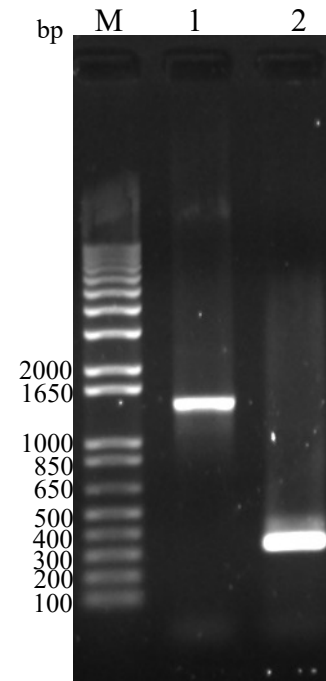


Figure S4