

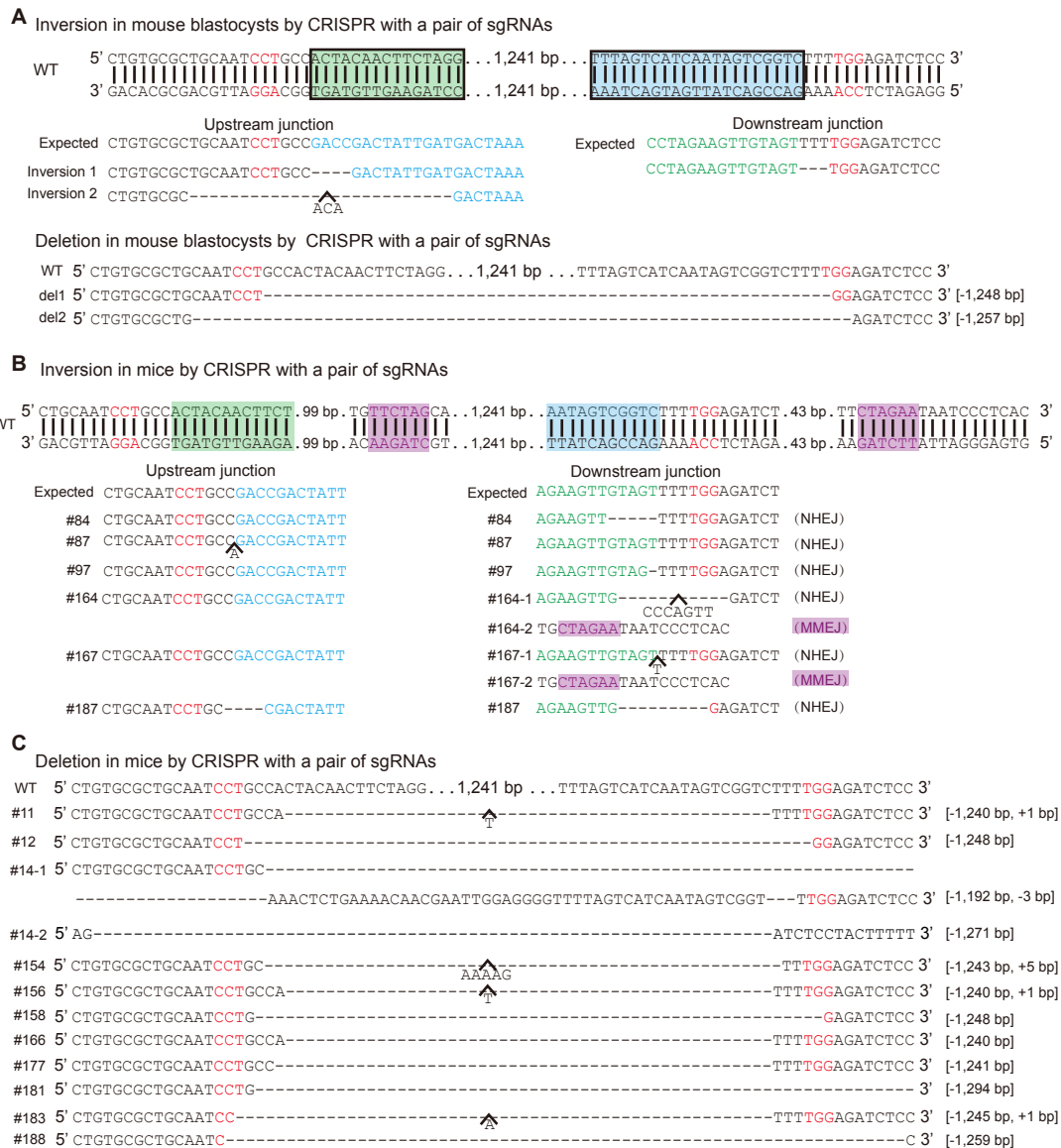
**Efficient Inversions and Duplications of Mammalian Regulatory
DNA Elements and Gene Clusters by CRISPR/Cas9**

Online Supplementary Materials:

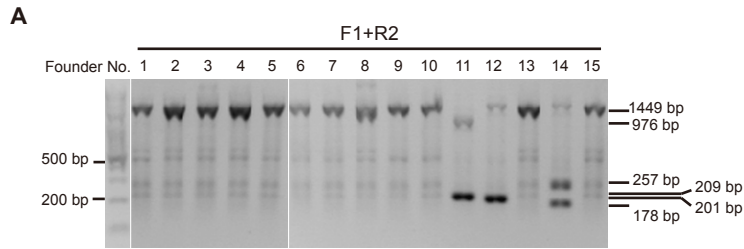
Supplementary Figures S1-S14

Supplementary Tables S1-S6

Supplementary Figures



Supplementary Figure S1. Inversion and deletion of a 1,241-bp DNA fragment by CRISPR with a pair of sgRNAs in mice. Shown are sequences of the upstream and downstream inversion junctions as well as deletion junctions in mouse blastocysts (A). Sequences of the upstream and downstream inversion junctions (B) and deletion junction (C) in the F0 founder mice are also shown. The nucleotides highlighted in purple in (B) indicate the inverted repeats used by MMEJ. Repair by the MMEJ pathway results in the inversion of the DNA fragment and simultaneous deletions of the sequences between the repeat and the DSB near both the upstream and downstream breaks.



B

WT 5' GTGCGCTGCAATCCTGCCACTACAACCTTAGGCTTGCT.AATAGTCGGTCTTTTGGAGATCTCCTACTTTTT 3'

#1 5' GTGCGCTGCAATCCTGCCACTACAACCTTAGGCTTGCT.AATAGTCGGTCTTTTGGAGATCTCCTACTTTTT 3'

#2 5' GTGCGCTGCAATCCTGCCACTACAACCTTAGGCTTGCT.AATAGTCGGTCTTTTGGAGATCTCCTACTTTTT 3'

#3-1 5' GTGCGCTGCAATCCTGCCACTACAACCTTAGGCTTGCT.-----33 bp del----ATCTCCTACTTTTT 3'

#3-2 5' GTGC-----ACTACAACCTTAGGCTTGCT.-----33 bp del----ATCTCCTACTTTTT 3'

#3-3 5' GTGCGCTGCAATCCTGCCACTACAACCTTAGGCTTGCT.AATAGT-----62 bp del----- 3'

#4-1 5' GTGCGCTGCAATCCTGCCACTACAACCTTAGGCTTGCT.AATAGTCGGTC-----CTACTTTTT 3'

#4-2 5' GTGCGCTGCAATCCTGCCACTACAACCTTAGGCTTGCT.AATAGTCGGTCTTTTGGAGATCTCCTACTTTTT 3'

#4-3 5' GTGCGCTGCAATCCTGCCACTACAACCTTAGGCTTGCT.AATAGTCGGTCTTTTGGAGATCTCCTACTTTTT 3'

#5 5' GTGCGCTGCAATCCTGCCACTACAACCTTAGGCTTGCT.AATAGTCGGTCTTTTGGAGATCTCCTACTTTTT 3'

#6-1 5' GTGCGCTGCAATCCTGC-----AACTTCTAGGCTTGCT.AATAGTCGGTCTTTTGGAGATCTCCTACTTTTT 3'

#6-2 5' GTGCGCTGCAATCCTGC-----AACTTCTAGGCTTGCT.AATAGTCGGT--TTGGAGATCTCCTACTTTTT 3'

#6-3 5' GTGCGCTGCAATCCTGCCACTACAACCTTAGGCTTGCT.AATAGTCGGT--TTGGAGATCTCCTACTTTTT 3'

#7-1 5' GTGCGCTGCAATCCTGCCACTACAACCTTAGGCTTGCT.AATAGTCGGTCTTTTGGAGATCTCCTACTTTTT 3'

#7-2 5' GTGCGCTGCAATCCTGCCACTACAACCTTAGGCTTGCT.AATAGTCGGTCTTTTGGAGATCTCCTACTTTTT 3'

#8-1 5' GTGCGCTGCAATCCTG-----226 bp del-----AATAGTCGGTCTTTTGGAGATCTCCTACTTTTT 3'

#8-2 5' GTGCGCTGCAATCCTG-----226 bp del-----AATAGTCGGTCTTTTGGAGATCTCCTACTTTTT 3'

#8-3 5' GTGCGCTGCAATCCTG-----226 bp del-----AATAGTCGGTCT-----CCTACTTTTT 3'

#9 5' GTGCGCTGCAATCCTGCCA----ACTTCTAGGCTTGCT.AAT-----TTTGGAGATCTCCTACTTTTT 3'

#10-1 5' GTGCGCTGCAATCCTGCCACTACAACCTTAGGCTTGCT.AATAGTCGGT--TTTGGAGATCTCCTACTTTTT 3'

#10-2 5' GTGCGCTGCAATCCTGCCACTACAACCTTAGGCTTGCT.AATAGTCGG-----AGATCTCCTACTTTTT 3'

#11 5' GTGCGCTGCAATCCTGCCACTACAACCTTAGGCTTGCT.-----473 bp del----- 3'

#12 5' GTGCGCTGCAATCCTGCCACTACAACCTTAGGCTTGCT.AATAGTCGGTCTTTTGGAGATCTCCTACTTTTT 3'

#13-1 5' GTGCGCTGCAATCCTGCCACTACAACCTTAGGCTTGCT.AATAGTCGG-----AGATCTCCTACTTTTT 3'

#13-2 5' GTGCGCTGCAATCCTGCCACTACAACCTTAGGCTTGCT.AATAGTCGGT--TTGGAGATCTCCTACTTTTT 3'

#14 5' GTGCGCTGCAATCCTGCCACTACAACCTTAGGCTTGCT.AATAGTCGGTCTTTTGGAGATCTCCTACTTTTT 3'

#15-1 5' GTGCGCTGCAATCCTGCCACTACAACCTTAGGCTTGCT.AATAGTCGG--TTTGGAGATCTCCTACTTTTT 3'

#15-2 5' GTGCGCTGCAATCCTGCCACTACAACCTTAGGCTTGCT.AATAGTCGG--TTTGGAGATCTCCTACTTTTT 3'

Supplementary Figure S2. Sequences of indels at the two targeted sites by CRISPR with a pair of sgRNAs in the F0 founder mice. (A) Genotyping of the 15 F0 founder mice. (B) Sequences of alleles of indels at the two targeted sites of the 15 F0 founder mice.

A Inversion in mice by CRISPR with a pair of sgRNAs

WT 5' GGGGGGGTGGG. 47 bp. TGCTCGCCCTCGTGGTTAAAGATGGA... 960 bp. . AAAAAGTGGTTCCTGCCACCTGGTGT. 187 bp. TCCCTCACTACCACCAAG 3'
 3' CCCCCACCAAC. 47 bp. ACAGAGCGGGAGACGACCAATTTCTACACT... 960 bp. . TTTTTCACCAAGGTAGACGGTGGACCACAA. 187 bp. AGGGAGTGAATGGGCTTC 5'

Upstream junction by NHEJ

Expected GGGGGTGGTGGT. 47 bp. TGCTCGCCCTCAGATGGAACCACCTTTT

#39 GGGGGTGGTGGT. 47 bp. TGCTCGCCCTCAGATGGAACCACCTTTT

#43 GGGGGTGGTGGT. 47 bp. TGCTCGCCCTCAGATGGAACCACCTTTT

#44 GGGGGTGGTGGTGG---GGTGGGGTGGTGGTGGTTCTAGCACTGAAAACGCAGCTGTCTCGCCCTCAGATGGAACCACCTTTT

Upstream junction by MMEJ

#40 GGGGGTGGT---63 bp deletion---AGTGAGGGATTATTTCTAGAAAAATCAGGATTCCTATATGCTCCTAGATGGCTCTAAAAAGTAGGAGATCTCC
 AAAAGACCGACTATTGATGACTAAAACCCCTCCAATTCGTTGTTTCAGAGTTTAAAGTAATCTTAAAGTAATTTTGAACCTTCATAACTAGCCT
 TTCCCTCATCTCCAGCACTTTCCCTCATCGAACACCAG-----ATGGAACCACCTTTT [-70 bp, +204 bp]

#41 GGGGGTGGT---63 bp deletion---AGTGAGGGATTATTTCTAGAAAAATCAGGATTCCTATATGCTCCTAGATGGCTCTAAAAAGTAGGAGATCTCC
 AAAAGACCGACTATTGATGACTAAAACCCCTCCAATTCGTTGTTTCAGAGTTTAAAGTAATCTTAAAGTAATTTTGAACCTTCATAACTAGCCT
 TTCCCTCATCTCCAGCACTTTCCCTCATCGAACACCAGGTG-----GAACCACCTTTT [-70 bp, +207 bp]

#42-1 GGGGGTGGT---63 bp deletion---AGTGAGGGATTATTTCTAGAAAAATCAGGATTCCTATATGCTCCTAGATGGCTCTAAAAAGTAGGAGATCTCC
 AAAAGACCGACTATTGATGACTAAAACCCCTCCAATTCGTTGTTTCAGAGTTTAAAGTAATCTTAAAGTAATTTTGAACCTTCATAACTAGCCT
 TTCCCTCATCTCCAGCACTTTCCCTCATCGAACACCAGGTG---AGATGGAACCACCTTTT [-64 bp, +208 bp]

#42-2 GGGGGTGGT---63 bp deletion---AGTGAGGGATTATTTCTAGAAAAATCAGGATTCCTATATGCTCCTAGATGGCTCTAAAAAGTAGGAGATCTCC
 AAAAGACCGACTATTGATGACTAAAACCCCTCCAATTCGTTGTTTCAGAGTTTAAAGTAATCTTAAAGTAATTTTGAACCTTCATAACTAGCCT
 TTCCCTCATCTCCAGCACTTTCCCTCATCGAACACCAGGTGAGATGGAACCACCTTTT [-63 bp, +209 bp, m1 bp]

#45-1 GGGGGTGGT---63 bp deletion---AGTGAGGGATTATTTCTAGAAAAATCAGGATTCCTATATGCTCCTAGATGGCTCTAAAAAGTAGGAGATCTCC
 AAAAGACCGACTATTGATGACTAAAACCCCTCCAATTCGTTGTTTCAGAGTTTAAAGTAATCTTAAAGTAATTTTGAACCTTCATAACTAGCCT
 TTCCCTCATCTCCAGCACTTTCCCTCATCGAACACCAGGTGAGATGGAACCACCTTTT [-63 bp, +209 bp, m2 bp]

#45-2 GGGGGTGGT---63 bp deletion---AGTGAGGGATTATTTCTAGAAAAATCAGGATTCCTATATGCTCCTAGATGGCTCTAAAAAGTAGGAGATCTCC
 AAAAGACCGACTATTGATGACTAAAACCCCTCCAATTCGTTGTTTCAGAGTTTAAAGTAATCTTAAAGTAATTTTGAACCTTCATAACTAGCCT
 TTCCCTCATCTCCAGCACTTTCCCTCATCGAACACCAGGTGGCAGATGGAACCACCTTTT [-63 bp, +209 bp]

#46 GGGGGTGGT---63 bp deletion---AGTGAGGGATTATTTCTAGAAAAATCAGGATTCCTATATGCTCCTAGATGGCTCTAAAAAGTAGGAGATCTCC
 AAAAGACCGACTATTGATGACTAAAACCCCTCCAATTCGTTGTTTCAGAGTTTAAAGTAATCTTAAAGTAATTTTGAACCTTCATAACTAGCCT
 TTCCCTCATCTCCAGCACTTTCCCTCATCGAACACCAGGTGGCAGATGGAACCACCTTTT [-63 bp, +209 bp]

Downstream junction

Expected TCACATCTTTAACCAGCAGCCACCTGGTGT. 187 bp. TCCCTCACTACCACCAAG

#39-1 TCACATCTTTAACCAGCAGCCACCTGGTGT. 187 bp. TCCCTCACTACCACCAAG

#39-2 TCACATCTTTAACCAGCAGCCACCTGGTGT. 187 bp. TCCCTCACTACCACCAAG

#40-1 TCACATCTTTAACCAGCAGCCACCTGGTGT. 187 bp. TCCCTCACTACCACCAAG

#40-2 TCACATCTTTAACCAGCAGCCACCTGGTGT. 187 bp. TCCCTCACTACCACCAAG

#41 TCACATCTTTAACCAGCAGCCACCTGGTGT. 187 bp. TCCCTCACTACCACCAAG

#42 TCACATCTTTAACCAGCAGCCACCTGGTGT. 187 bp. TCCCTCACTACCACCAAG

#43 TCACATCTTTAACCAGCAGCCACCTGGTGT. 187 bp. TCCCTCACTACCACCAAG

#44 TCACATCTTTAACCAGCAGCCACCTGGTGT. 187 bp. TCCCTCACTACCACCAAG

#45 TCACATCTTTAACCAGCAGCCACCTGGTGT. 187 bp. TCCCTCACTACCACCAAG

#46-1 TCACATCTTTAACCAGCAGCCACCTGGTGT. 187 bp. TCCCTCACTACCACCAAG

#46-2 TCACATCTTTAACCAGCAGCCACCTGGTGT. 187 bp. TCCCTCACTACCACCAAG

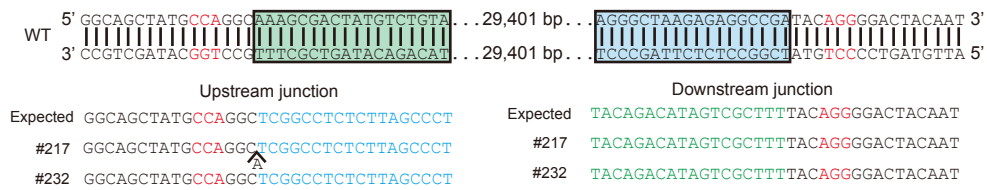
#46-3 TCACATCTTTAACCAGCAGCCACCTGGTGT. 187 bp. TCCCTCACTACCACCAAG

B Deletion in mice by CRISPR with a pair of sgRNAs

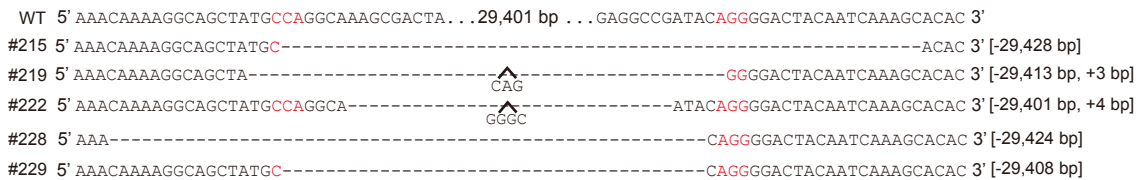
WT 5' GCTGTCTCGCCCTCTGCTGGTTAAAGATGGA... 960 bp. . AAAAAGTGGTTCCTGCCACCTGGTGTTCGATG 3'
 #39-1 5' GCTGTCTCGCCCTC-----GCCACCTGGTGTTCGATG 3' [-960 bp]
 #39-2 5' GCTGTCTCGCCCTC-----CTGGTGTTCGATG 3' [-965 bp]
 #40-1 5' GCTGTCTCGCCCTC-----GCCACCTGGTGTTCGATG 3' [-960 bp]
 #40-2 5' GCTGTCTCGCCCTC-----GCCACCTGGTGTTCGATG 3' [-959 bp, +1 bp]
 #41 5' GCTGTCTCGC-----GGTGTTCGATG 3' [-971 bp, +2 bp]
 #43 5' GCTGTCTCGCCCTC-----GCCACCTGGTGTTCGATG 3' [-960 bp]
 #44 5' GCTGTCTCGCCCTC-----GCCACCTGGTGTTCGATG 3' [-960 bp]
 #45-1 5' GCTGTCTCGCCCTC-----GCCACCTGGTGTTCGATG 3' [-960 bp]
 #45-2 5' GCTGTCTCGC-----CTGGTGTTCGATG 3' [-969 bp]

Supplementary Figure S3. Inversion and deletion of a 960-bp DNA fragment by CRISPR with a pair of sgRNAs in the F0 founder mice. (A) Sequences of the upstream and downstream inversion junctions in F0 founder mice. The nucleotides highlighted in purple indicate the inverted repeats used by MMEJ. Repair by the MMEJ pathway results in the inversion of the DNA fragment between the upstream breakpoint and downstream microhomologous repeat as well as the simultaneous deletion of the sequences between the upstream microhomologous repeat and upstream breakpoint. (B) Sequences of the deletion junctions in F0 founder mice.

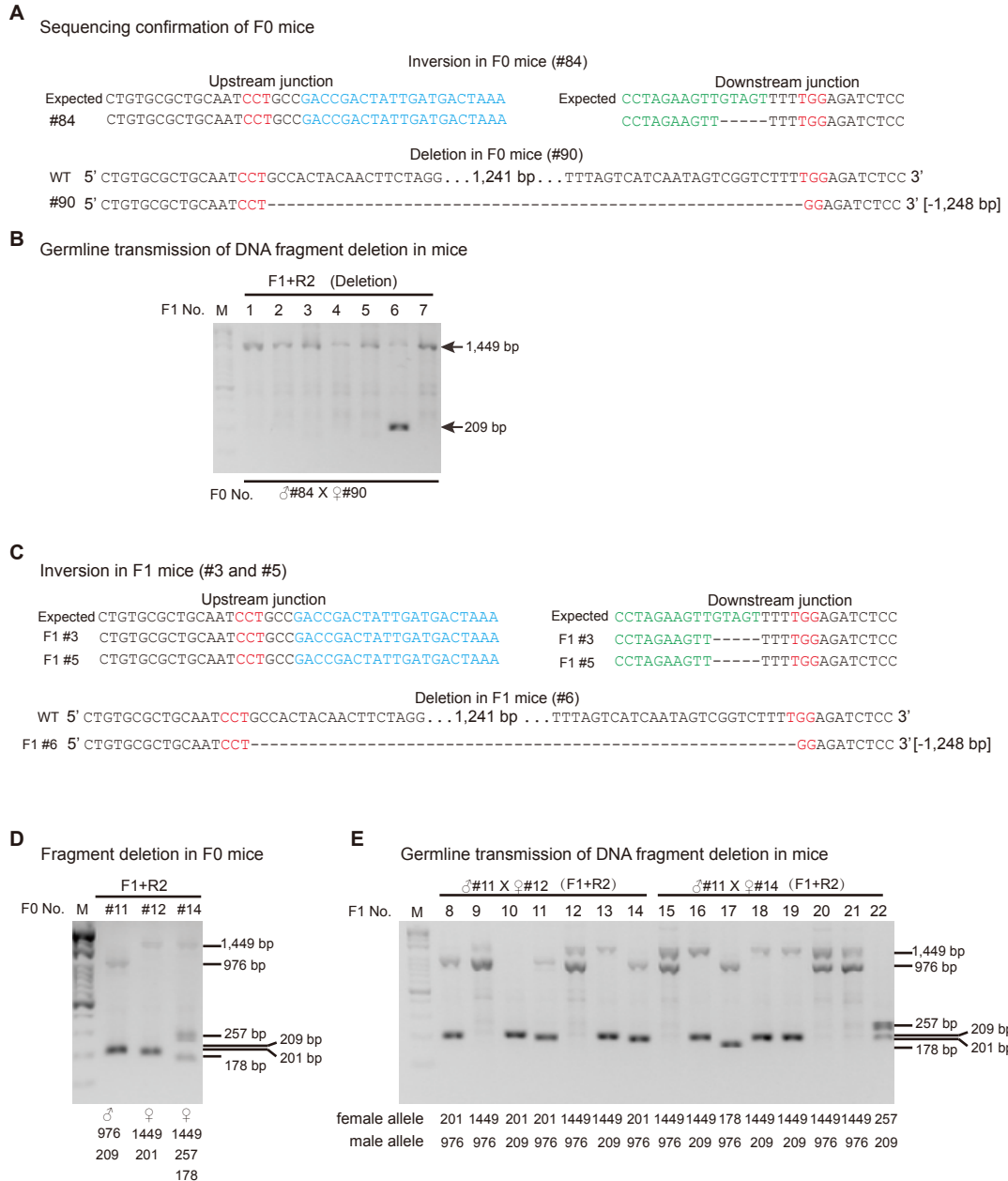
A Inversion in mice by CRISPR with a pair of sgRNAs



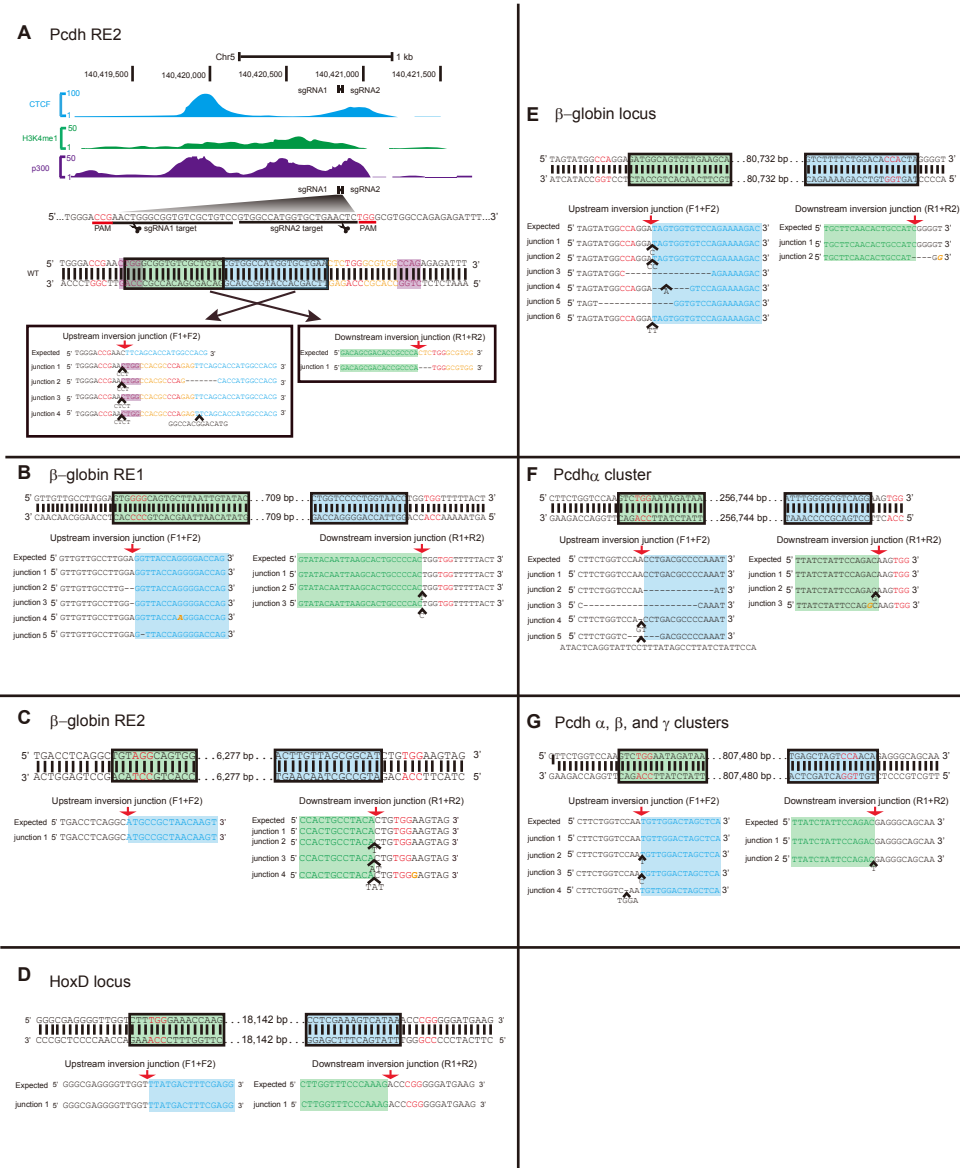
B Deletion in mice by CRISPR with a pair of sgRNAs



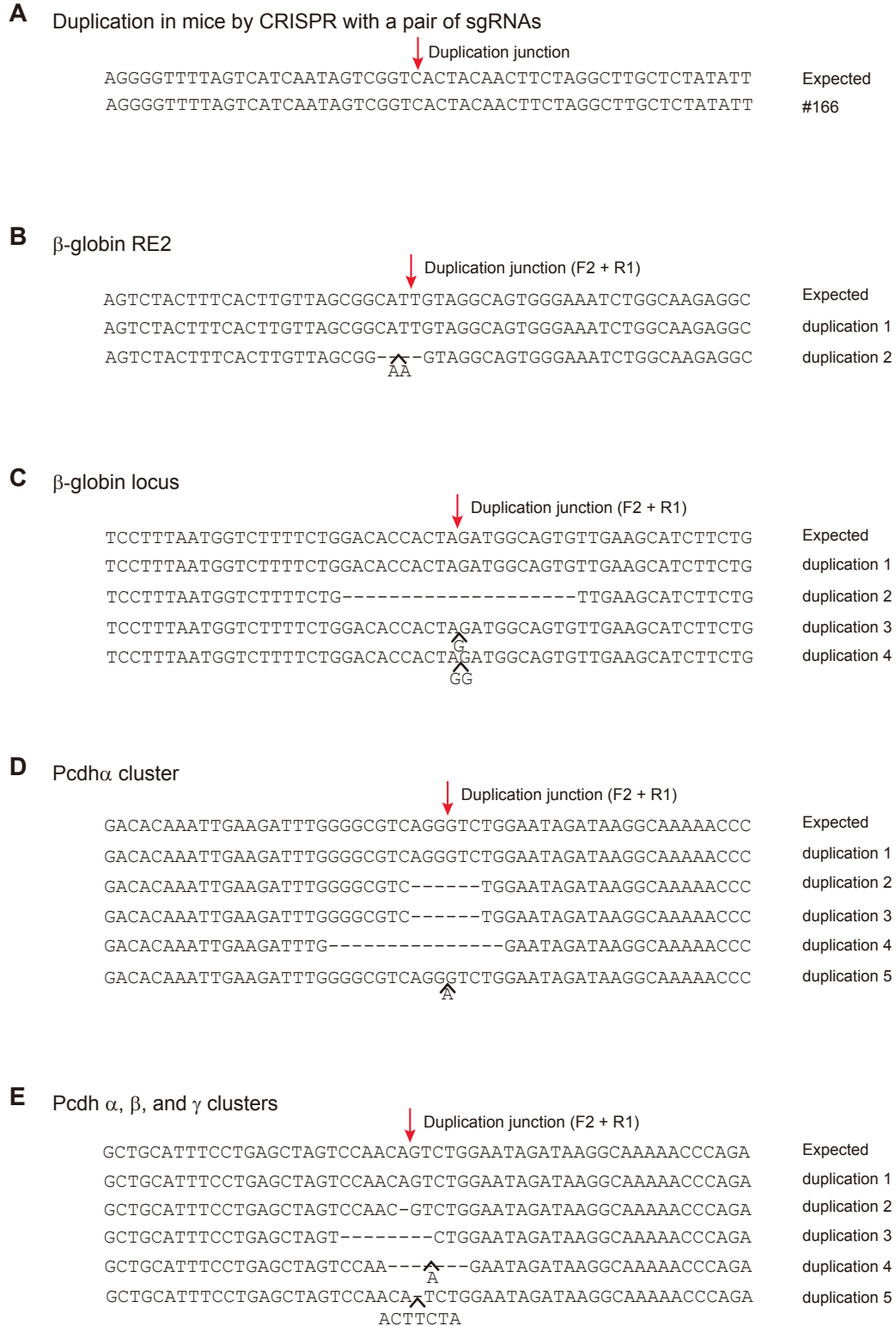
Supplementary Figure S4. Inversion and deletion of a 29,401-bp DNA fragment by CRISPR with a pair of sgRNAs in F0 founder mice. (A) Sequences of the upstream and downstream inversion junctions in F0 founder mice. (B) Sequences of the deletion junctions in F0 founder mice.



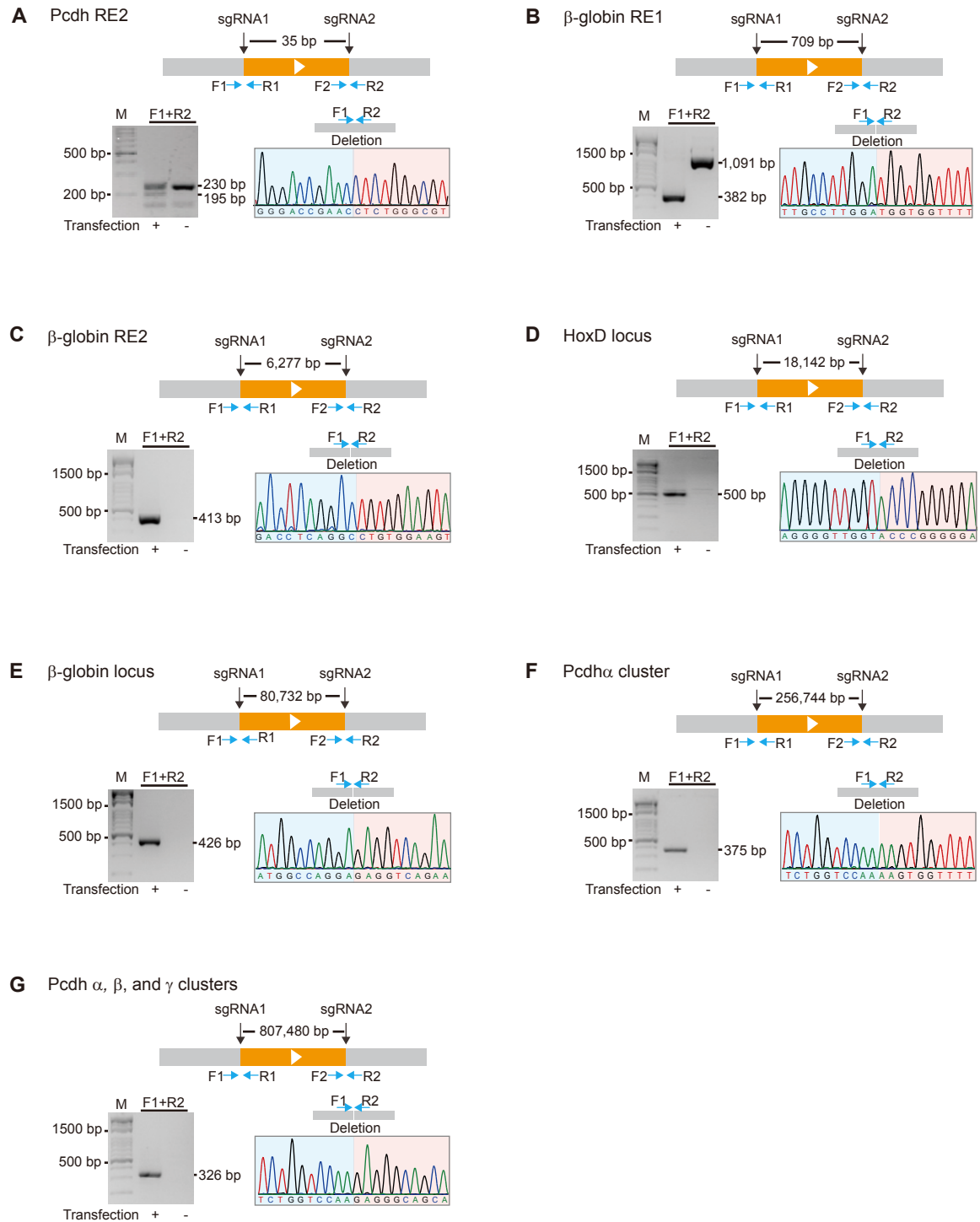
Supplementary Figure S5. Germline transmission in mice of the DNA-fragment inversion and deletion induced by CRISPR with a pair of sgRNAs. (A) Sequencing of alleles of inversion and deletion junctions in F0 mice. (B) Genotyping of DNA fragment deletion in F1 mice from crossing of two F0 founder mice. (C) Sequencing of alleles of inversion and deletion junctions in F1 mice. (D) Genotyping of DNA fragment deletion in F0 founder mice. (E) F1 genotyping demonstrates the germline transmission of the deletion alleles.



Supplementary Figure S6. Inversions of a set of DNA fragments of different sizes in human cell. (A) Inversion of a 35-bp DNA fragment of the *Pcdh* regulatory element 2 (RE2) by CRISPR with a pair of sgRNAs. Shown are diagram of CRISPR with a pair of sgRNAs. The sgRNAs targeting sequences are underlined. The PAM sequences are highlighted in red. The ChIP-seq signals of CTCF, H3K4me1, and p300 are obtained from the Human ENCODE Project. The sequences of the upstream and downstream inversion junctions are also shown. The nucleotides highlighted in purple indicate the inverted repeats used by the microhomology-mediated end joining (MMEJ) repair pathway. Repair by the MMEJ pathway results in the inversion of the DNA fragment between the upstream breakpoint and downstream microhomologous repeat. Shown are also sequences of the upstream and downstream inversion junction of 709-bp (B) and 6,277-bp (C) DNA fragments at the *β-globin* locus, of an 18,142-bp DNA fragment at the *HoxD* locus (D), of an 80,732-bp DNA fragment at the *β-globin* locus (E), of a 256,744-bp DNA fragment covering the *Pcdhα* gene cluster (F), and of an 807,480-bp DNA fragment covering the *Pcdh α, β, and γ* gene clusters (G).



Supplementary Figure S7. Sequences of duplication junctions by CRISPR through *trans*-allelic recombination in mice and human cells. Shown are sequences of duplication junctions of 1,241-bp DNA fragment (A) in mouse, of 6,277-bp (B) and 80,732-bp (C) DNA fragments at the β -globin locus, of a 256,744-bp DNA fragment covering the $Pcdh\alpha$ gene cluster (D), and of an 807,480-bp DNA fragment covering the $Pcdh$ α , β , and γ gene clusters (E) in human cells.



Supplementary Figure S8. Targeted deletions of seven additional DNA fragments of different sizes. (A) Deletion of the *Pcdh* RE2. The deletion junctions are identified by PCR with specific primer pairs. The PCR product is cloned and sequenced. An example of the sequence chromatograms at the deletion junctions is shown. Deletion of 709-bp (B) and 6,277-bp (C) DNA fragments at the *β-globin* locus. (D) Deletion of an 18,142-bp DNA fragment at the *HoxD* locus. (E) Deletion of an 80,732-bp DNA fragment at the *β-globin* locus. (F) Deletion of a 256,744-bp DNA fragment covering the *Pcdhα* gene cluster. (G) Deletion of an 807,480-bp DNA fragment covering the *Pcdh α, β, and γ* gene clusters.

A Pcdh RE2

5' TCTGCAGCGAGTCATGGGACCGAACTGGGCGGTGTCGCTGTCCTGGCCATGGTGTGAACCTGGGCGTGGCCAGAGAGAT 3' WT
 5' TCTGCAGCGAGTCATGGGACCGAACTGGGCGGTG-----CTCTGGGCGTGGCCAGAGAGAT 3' [-26 bp]
 5' TCTGCAGCGAGTCATGGGACCGAACTGGGCGGTG-----TCTGGGCGTGGCCAGAGAGAT 3' [-27 bp]
 5' TCTGCAGCGAGTCATGGGACCGAACT-----CTCTGGGCGTGGCCAGAGAGAT 3' [-34 bp]
 5' TCTGCAGCGAGTCATGGGACCGAAC-----CTCTGGGCGTGGCCAGAGAGAT 3' [-35 bp]
 5' TCTGCAGCGAGTC-----CTCTGGGCGTGGCCAGAGAGAT 3' [-47 bp, +1 bp]

B β -globin RE1

5' GTTGTGTCCTGGAGTGGGGCAGTGTCTAATTGTATAC...668 bp...CTGGTCCCCTGGTAACCTGGTGGTTTTTTACT 3' WT
 5' GTTGTGTCCTTGG-----TGGTGGTTTTTTACT 3' [-709 bp]
 5' GTTGTGTCCTTGG-----GGTGGTTTTTTACT 3' [-711 bp]

C β -globin RE2

5' GCAGACCCAATGACCTCAGGCTGTAGGCAGTGG...6,242 bp...CTACTTTCACTTGTAGCGGCATCTGTGGGAAGTAG 3' WT
 5' GCAGACCCAATGACCTCAGGC-----CTGTGGGAAGTAG 3' [-6,277 bp]
 5' GC-----TAG 3' [-6,305 bp]
 5' GCAGACCCAATGACCTCAGGCT-----CTGTGGGAAGTAG 3' [-6,276 bp]
 5' GCAG-----TAG 3' [-6,303 bp]

D HoxD locus

5' GAGGGGTGGTCTTTGGGAA...18,121 bp...GAAAGTCATAAAACCCGGGGGATGAAGTGGGAAGACTGTCCAAGGAG 3' WT
 5' GAGGGGTGGT-----ACCCGGGGGATGAAGTGGGAAGACTGTCCAAGGAG 3' [-18,142 bp]
 5' GA-----GGGGGATGAAGTGGGAAGACTGTCCAAGGAG 3' [-18,155 bp]
 5' GAGG-----AG 3' [-18,182 bp]
 5' GAGGGGTGGT-----ATGAAGTGGGAAGACTGTCCAAGGAG 3' [-18,151 bp]

E β -globin locus

5' TAGTATGGCCAGGAGATGGCAGTGTGA...80,695 bp...TCTGGACACCACTAGGGGTGAGAAAGTAGTTCATCAAAAC 3' WT
 5' TAGTATGGCCAGGA-----GAGGTCAGAAGTAGTTCATCAAAAC 3' [-80,723 bp]
 5' TAGTATGG-----TCAGAAGTAGTTCATCAAAAC 3' [-80,733 bp]
 5' TAGTATGGCC-----GT-----GTCAGAAGTAGTTCATCAAAAC 3' [-80,730 bp, +2 bp]
 5' TAGTATGGCCAGGA-----AGTAGTTCATCAAAAC 3' [-80,732 bp]
 5' TAGTATGGCCA-----TCATACTAGTTCATCAAAAC-----TAGTTCATCAAAAC 3' [-80,737 bp, +18 bp]

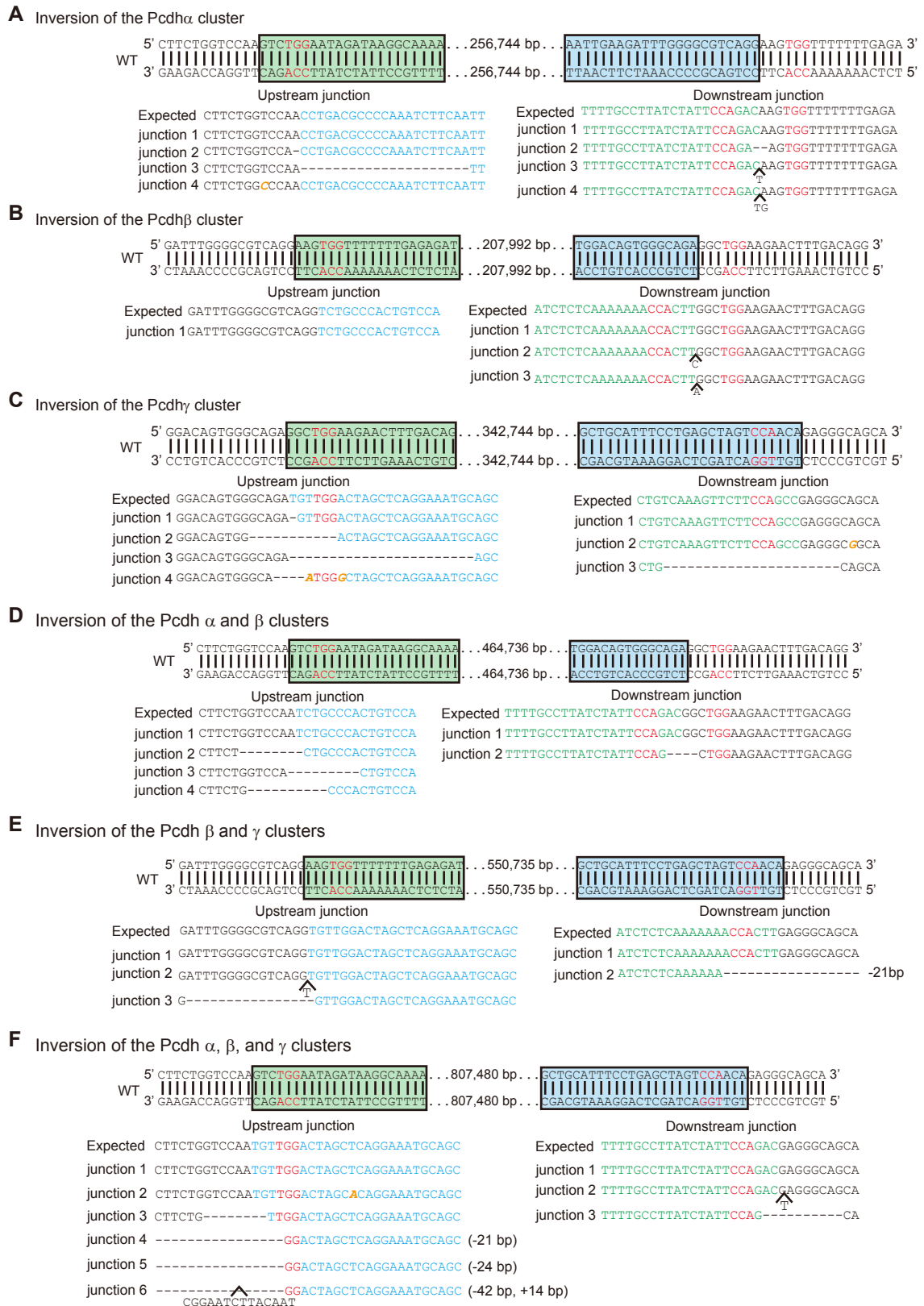
F Pcdh α cluster

5' TCTGGTCCAAGTCTGGAAATAGATAAGGCCAAAACCC...256,702 bp...AGATTGGGGCGTCAGGAAGTGGTTTTTT 3' WT
 5' TCTGGTCCAA-----AAGTGGTTTTTT 3' [-256,744 bp]
 5' TCTGGTCCAA-----AA-----AAGTGGTTTTTT 3' [-256,744 bp, +2 bp]
 5' TCTGGTCC-----AGTGGTTTTTT 3' [-256,747 bp]
 5' TCTGGT-----T 3' [-256,759 bp]

G Pcdh α , β , and γ clusters

5' TCTGGTCCAAGTCTGGAAATAGATAAGGCCAAAACCC...807,429 bp...TGAGCTAGTCCACAGAGGGCAGCAACAA 3' WT
 5' TCTGGTCCAA-----GAGGGCAGCAACAA 3' [-807,480 bp]
 5' TCTGGTCCAA-----AA 3' [-807,492 bp]
 5' TCTGGTCCAA-----CACTA-----GGCAGCAACAA 3' [-807,483 bp, +5 bp]
 5' TCTGGTCC-----GATGGAAC-----CAGCAACAA 3' [-807,486 bp, +8 bp]

Supplementary Figure S9. Sequences of deletion junctions of the seven additional DNA fragments. Shown are sequences of deletion junction of a 35-bp DNA fragment of the *Pcdh* RE2 (A), of 709-bp (B) and 6,277-bp (C) DNA fragments at the β -globin locus, of an 18,142-bp DNA fragment at the *HoxD* locus (D), of an 80,732-bp DNA fragment at the β -globin locus (E), of a 256,744-bp DNA fragment covering the *Pcdh* α gene cluster (F), and of an 807,480-bp DNA fragment covering the *Pcdh* α , β , and γ gene clusters (G).



Supplementary Figure S10. Sequences of the upstream and downstream junctions of combinatorial inversions of the *Pcdh* α , β , and γ gene clusters induced by CRISPR with four sgRNAs. Shown are the sequences of the upstream and downstream inversion junctions of the *Pcdh* α (A), β (B), γ (C), α/β (D), β/γ (E), and $\alpha/\beta/\gamma$ (F) gene clusters.

A Duplication of the *Pcdh α* cluster

↓ Duplication junction

```
CACAAATTGAAGATTTGGGGCGTCAGGGTCTGGAAATAGATAAGGCCAAAAACCCAGAGATTTCCACC Expected duplication
CACAAATTGAAGATTTGGGGCGTCAGGGTCTGGAAATAGATAAGGCCAAAAACCCAGAGATTTCCACC junction 1
CACAAATTGAAGAT-----CTGGAAATAGATAAGGCCAAAAACCCAGAGATTTCCACC junction 2
CACAAATTGAAGATTTGGGGCGTCAG-----AGATTTCCACC junction 3
CACAAATTGAAGATTTGGGGCGTCAGGGTCTGGAAATAGATAAGGCCAAAAACCCAGAGATTTCCACC junction 4
      A
```

B Duplication of the *Pcdh β* cluster

↓ Duplication junction

```
TGTGCAGATGGCTTCTGAATTGGACAGTGGGCAGAAAAGTGGTTTTTTTTGAGAGATCTGCCTATTT Expected duplication
TGTGCAGATGGCTTCTGAATTGGACAGTGGGCAGAAAAGTGGTTTTTTTTGAGAGATCTGCCTATTT junction 1
TGTGCAGATGGCTTCTGAATTGGACAGTGGGCAGAAAAGTGGTTTTTTTTGAGAGATCTGCCTATTT junction 2
TGTGCAGATGGCTTCTGAATTGGACAGTGGGCAGAAAAGTGGTTTTTTTTGAGAGATCTGCCTATTT junction 3
      AG
TGTGCAGATGGCTTCTGAATTGGACAGTGGGCAGAAAAGTGGTTTTTTTTGAGAGATCTGCCTATTT junction 4
      ATTGGAC
```

C Duplication of the *Pcdh γ* cluster

↓ Duplication junction

```
TTTCCTGAGCTAGTCCAACAGGCTGGAAAGAACTTTGACAGGCATGACAGAAAAAGCCTCGCTTGC Expected duplication
TTTCCTGAGCTAGTCCAACAGGCTGGAAAGAACTTTGACAGGCATGACAGAAAAAGCCTCGCTTGC junction 1
TTTCCTGAGCTAGTCCAACAGGC-----ATGACAGAAAAAGCCTCGCTTGC junction 2
TTTCCTGAGCTAGTCCAACA-----AGCCTCGCTTGC junction 3
```

D Duplication of the *Pcdh α and β* clusters

↓ Duplication junction

```
GCAGATGGCTTCTGAATTGGACAGTGGGCAGAGTCTGGAAATAGATAAGGCCAAAAACCCAGAGATT Expected duplication
GCAGATGGCTTCTGAATTGGACAGTGGGCAGAGTCTGGAAATAGATAAGGCCAAAAACCCAGAGATT junction 1
GCAGATGGCTTCTGAATTGGACAGTGG-----TCTGGAAATAGATAAGGCCAAAAACCCAGAGATT junction 2
GCAGATGGCTTCTGAATTGGACAGTGGGCAGAGTCTGGAAATAGATAAGGCCAAAAACCCAGAGATT junction 3
      A
```

E Duplication of the *Pcdh β and γ* clusters

↓ Duplication junction

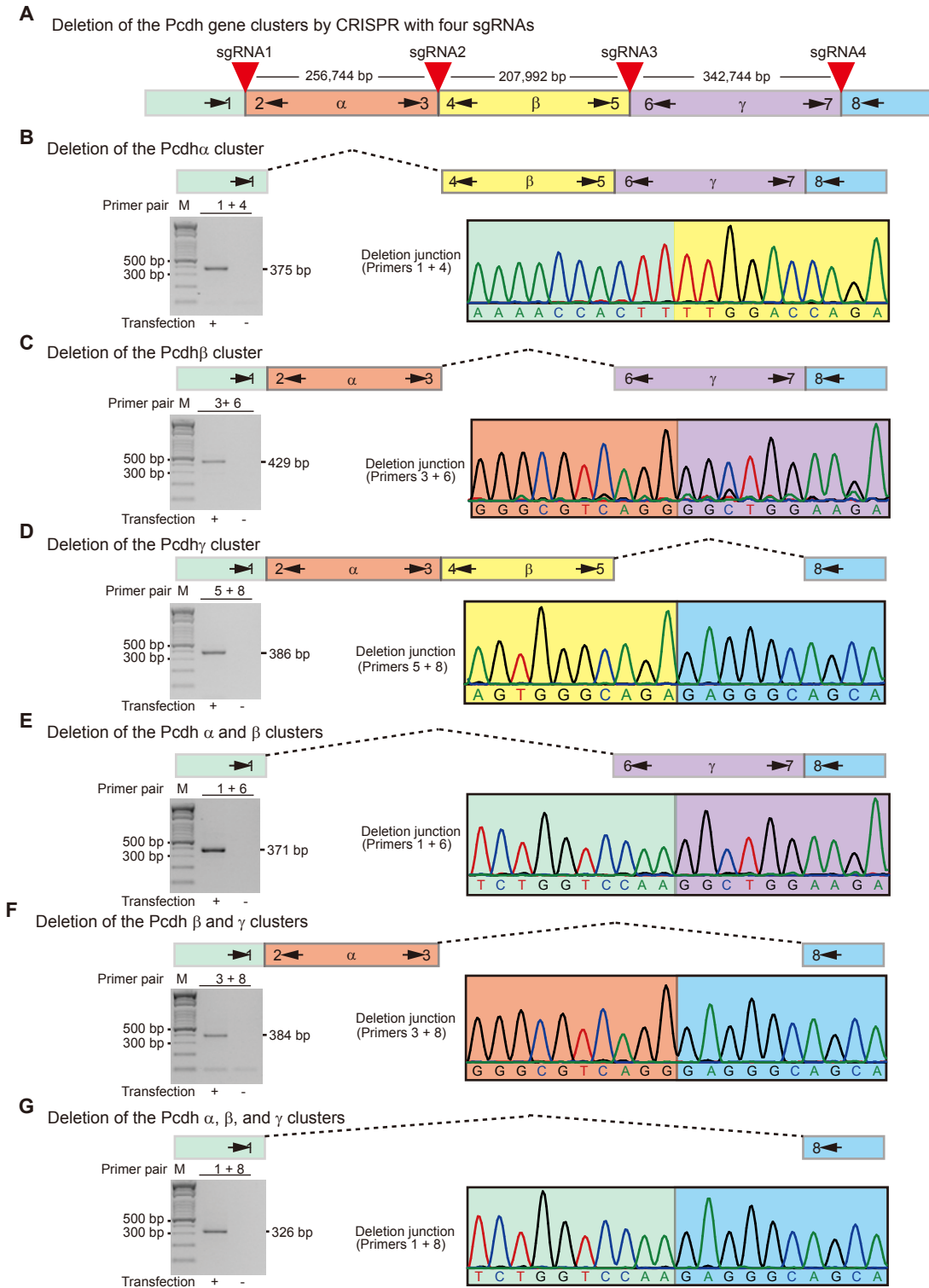
```
AAAAGCTGCATTTCTGAGCTAGTCCAACAAGTGGTTTTTTTTGAGAGATCTGCCTATTTTTTAAA Expected duplication
AAAAGCTGCATTTCTGAGCTAGTCCAACAAGTGGTTTTTTTTGAGAGATCTGCCTATTTTTTAAA junction 1
AAAAGCTGCATTTCTGAGCTAGTCCAACA-----GAGAGATCTGCCTATTTTTTAAA junction 2
AAAAGCTGCATTTCTGAGCTAGTCCAACAAGTGGTTTTTTTTGAGAGATCTGCCTATTTTTTAAA junction 3
      G
```

F Duplication of the *Pcdh α , β , and γ* clusters

↓ Duplication junction

```
AAAAGCTGCATTTCTGAGCTAGTCCAACAGTCTGGAAATAGATAAGGCCAAAAACCCAGAGATTTTC Expected duplication
AAAAGCTGCATTTCTGAGCTAGTCCAACA-TCGGAAATAGATAAGGCCAAAAACCCAGAGATTTTC junction 1
AAAAGCTGCATTTCTGAGCTAGTCCA----GTCGGAAATAGATAAGGCCAAAAACCCAGAGATTTTC junction 2
AAAAGCTGCATTTCTGAGCTAGT-----AGATAAGGCCAAAAACCCAGAGATTTTC junction 3
AAAAGCTGCATTTCTGAGCTAGTCCAACAGTCTGGAAATAGATAAGGCCAAAAACCCAGAGATTTTC junction 4
      A
AAAAGCTGCATTTCTGAGCTAGTCCAACAGTCTGGAAATAGATAAGGCCAAAAACCCAGAGATTTTC junction 5
      CAA
AAAAGCTGCATTTCTGAGCTAGTCCA-AGTCTGGAAATAGATAAGGCCAAAAACCCAGAGATTTTC junction 6
      GCTAAGC
AAAAGCTGCATTTCTGAGCTAGTCCAACA-TCGGAAATAGATAAGGCCAAAAACCCAGAGATTTTC junction 7
      AAGTGCTATCTTTGTGATGCTTA
```

Supplementary Figure S11. Sequences of duplication junctions of combinatorial duplications of the *Pcdh α , β , and γ* gene clusters induced by CRISPR with four sgRNAs. Shown are the sequences of the duplication junctions of the *Pcdh α* (A), *β* (B), *γ* (C), *α/β* (D), *β/γ* (E), and *$\alpha/\beta/\gamma$* (F) gene clusters.



Supplementary Figure S12. Combinatorial genomic deletions by CRISPR targeted with four sgRNAs. (A) Diagram of CRISPR with four sgRNAs targeted at the *Pcdh* α , β , and γ gene clusters. The examples of PCR gels and sequence chromatograms of the deletion junctions of the *Pcdh* α (B), β (C), γ (D), α/β (E), β/γ (F), and $\alpha/\beta/\gamma$ (G) gene clusters are shown. Deletion junctions are amplified by PCR with specific primer pairs and confirmed by Sanger sequencing.

A Deletion of the *Pcdh α* cluster

```

5' CAAAAAAACCACTTCTCTGACGCCCAATCTTCAATT...256,744 bp...TTTGCCTTATCTATTCCAGACTTGGACCAGAAG 3' WT
5' CAAAAAAACCACTT-----TTGACCAGAAG 3' [-256,744 bp]
5' CAAAAAA-----G 3' [-256,761 bp]
5' CAAAAAAACCACTTC-----GGACCAGAAG 3' [-256,745 bp]
5' CAAAAAAACCACT-----GATT-----3 [-256,812 bp, +4 bp]

```

B Deletion of the *Pcdh β* cluster

```

5' ATTTGGGCGTCAGGAAGTGGTTTTTTTGAGAGAT...207,992 bp...TGGACAGTGGGCAGAGGCTGGAAGAAGTTTGACAGG 3' WT
5' ATTTGGGCGTCAGG-----GGCTGGAAGAAGTTTGACAGG 3' [-207,992 bp]

```

C Deletion of the *Pcdh γ* cluster

```

5' GGACAGTGGGCAGAGGCTGGAAGAAGTTTGACAG...342,744 bp...GCTGCATTTCTGAGCTAGTCCAACAGAGGGCAGCA 3' WT
5' GGACAGTGGGCAG-----GAGGGCAGCA 3' [-342,744 bp]
5' GGACAGTGGGCAG-----AGGGCAGCA 3' [-342,745 bp]
5' GGACAGTGGGCAG-----CA 3' [-342,753 bp]
5' -----GGCAGCA 3' [-342,809 bp]
5' GGACAGTGGGCAG-----AGATGGCTGGAAT-----3 [-342,755 bp, +13 bp]

```

D Deletion of the *Pcdh α* and *β* clusters

```

5' CTTCGGTCCAAGTCTGGAATAGATAAGGCCAAAA...464,736 bp...TGGACAGTGGGCAGAGGCTGGAAGAAGTTTGACAGG 3' WT
5' CTTCGGTCCAA-----GGCTGGAAGAAGTTTGACAGG 3' [-464,736 bp]
5' CTTCGGTCCA-----GCAAACTGG-----GGCTGGAAGAAGTTTGACAGG 3' [-464,737 bp, +9 bp]
5' CTTCGGTCCAA-----AGGCTGGAAGAAGTTTGACAGG 3' [-464,735 bp]

```

E Deletion of the *Pcdh β* and *γ* clusters

```

5' ATTTGGGCGTCAGGAAGTGGTTTTTTTGAGAGAT...550,735 bp...GCTGCATTTCTGAGCTAGTCCAACAGAGGGCAGCA 3' WT
5' ATTTGGGCGTCAGG-----GAGGGCAGCA 3' [-550,735 bp]
5' ATTTGGGCGTCAGG-----3 [-550,747 bp]

```

F Deletion of the *Pcdh α* , *β* , and *γ* clusters

```

5' CTTCGGTCCAAGTCTGGAATAGATAAGGCCAAAA...807,480 bp...GCTGCATTTCTGAGCTAGTCCAACAGAGGGCAGCA 3' WT
5' CTTCGGTCCAA-----GAGGGCAGCA 3' [-807,480 bp]
5' CTTCGGTCCAA-----3 [-807,490 bp]
5' CTTCGGTCCA-----GCA 3' [-807,488 bp]
5' CTTCGGTCCAA-----CAACT-----AGCA 3' [-807,486 bp, +5 bp]

```

Supplementary Figure S13. Sequences of deletion junctions of combinatorial genomic deletions of the *Pcdh α* , *β* , and *γ* gene clusters induced by CRISPR with four sgRNAs. Shown are the sequences of the deletion junctions of the *Pcdh α* (A), *β* (B), *γ* (C), *α/β* (D), *β/γ* (E), and *$\alpha/\beta/\gamma$* (F) gene clusters. Both precise and indel junctions are detected.

A

Clone H14

WT	AGTCGACTCGCCCTCTGCTGGTTAAAGATCCGAAT...970 bp...AGTGGATCCACCTGCCACCTGGTGGCAGATGAGG TCAGCTGAGCGGGAGACGACCAATTTCTAGGCTTA...970 bp...TCACCTAGGTGGACGGTGGACCACCGTCTACTCC
allele 1	AGTCGACTCGCCCT-----GCTGGTTAAA [^] -----GATCCACT...970 bp...ATTTCGGA-----AAAGTGGAAAA [^] -----GTGGCAGATGAGG
allele 2	AGTCGACTCGCCCTC-----Deletion (1,009 bp)-----TGGTGGCAGATGAGG
allele 3	AGT-----GGTTAAAGATCCGAAT...970 bp...AGTGGATCCACCTGCCAC-- [^] -----GCAGATGAGG

B

mice F23

WT	CTGTGCGCTGCAATCCTGCCACTACAACCTTCTAGG...1,241 bp...TTTAGTCATCAATAGTCGGTCTTTTGGAGATCTCC GACACGCGACGTTAGGACGGTGATGTTGAAGATCC...1,241 bp...AAATCAGTAGTTATCAGCCAGAAAACCTCTAGAGG
allele 1	CTGTGCGCTGCAATCCTGCCACTACAACCTTCTAGGCTTGCT.....-----Deletion (473 bp)-----
allele 2	CTGTGCGCTGCAATCCTGCCACTACAACCTTCTAGGCTTGCT.....TTTAGTCATCAATAGTCGGTCTTTTGGAGATCTCC [^]

mice F24

WT	CTGTGCGCTGCAATCCTGCCACTACAACCTTCTAGG...1,241 bp...TTTAGTCATCAATAGTCGGTCTTTTGGAGATCTCC GACACGCGACGTTAGGACGGTGATGTTGAAGATCC...1,241 bp...AAATCAGTAGTTATCAGCCAGAAAACCTCTAGAGG
allele 1	CTGTGCGCTGCAATCCTGCCA----- [^] -----TTTGGAGATCTCC
allele 2	CTGTGCGCTGCAATCCT-----GGAGATCTCC

Supplementary Figure S14. Cell clone and F1 mice by CRISPR. (A) Hec-1-B single-cell subclone of H14 screened by CRISPR with a pair of sgRNAs. Shown are sequences of the three alleles of the H14 CRISPR cell line. (B) F1 mice with deletion. Shown are sequences of the two alleles of F23 and F24 mice.

Supplementary Tables

Supplementary Table S1. Sequences of oligos used.

Oligos used for constructing sgRNAs

DNA fragment	sgRNAs	Direction	Sequence
<i>Pcdh</i> RE1	sgRNA1	F	ACCGCCACACATCCAAGGCTGAC
		R	AAACGTCAGCCTTGGATGTGTGG
	sgRNA2	F	ACCGAGATTTGGGGCGTCAGGAAG
		R	AAACCTTCCTGACGCCCCAAATCT
<i>Pcdh</i> RE2	sgRNA1	F	ACCGGACAGCGACACCGCCAGTT
		R	AAACAAC TGGGCGGTGTCGCTGTC
	sgRNA2	F	ACCGGTGGCCATGGTGTGCTGAACTC
		R	AAACGAGTTCAGCACCATGGCCAC
β -globin RE1	sgRNA1	F	ACCGATTTGTTGCTGCCTTGGAGTG
		R	AAACCACTCCAAGGCAACAACAAT
	sgRNA2	F	ACCGCTGGTCCCCTGGTAACCTGG
		R	AAACCCAGGTTACCAGGGGACCAG
β -globin RE2	sgRNA1	F	ACCGACCAATGACCTCAGGCTGT
		R	AAACACAGCCTGAGGTGATTGGGT
	sgRNA2	F	ACCGTCACTTGTAGCGGCATCTG
		R	AAACCAGATGCCGCTAACAAGTGA
<i>HoxD</i> locus	sgRNA1	F	ACCGGGGCGAGGGGTTGGTCTT
		R	AAACAAGACCAACCCCTCGCCCC
	sgRNA2	F	ACCGACCTCGAAAGTCATAAACC
		R	AAACGGTTTATGACTTTCGAGGT
β -globin locus	sgRNA1	F	GCTTCAACACTGCCATCTCCGTTTTAGAGCTAGAAATAG
		R	GGAGATGGCAGTGTGAAGCGGTGTTTCGTCCTTTCCAC
	sgRNA2	F	GAACTACTTCTGACCCCTAGGTTTTAGAGCTAGAAATAG
		R	CTAGGGGTCAGAAGTAGTTCGGTGTTCGTCCTTTCCAC
<i>Pcdha</i> cluster	sgRNA1	F	ACCGGCTTCTTCTGGTCCAAGTC
		R	AAACGACTTGGACCAGAAGAAGC
	sgRNA2	F	ACCGAGATTTGGGGCGTCAGGAAG
		R	AAACCTTCCTGACGCCCCAAATCT
<i>Pcdha</i> , β , and γ clusters	sgRNA1	F	ACCGGCTTCTTCTGGTCCAAGTC
		R	AAACGACTTGGACCAGAAGAAGC
	sgRNA2	F	GTGGTTGTGCTGCCCTCTGTGTTTTAGAGCTAGAAATAG
		R	ACAGAGGGCAGCAACAACCACGGTGTTCGTCCTTTCCAC
<i>Pcdh</i> clusters with four sgRNAs	sgRNA1	F	ACCGGCTTCTTCTGGTCCAAGTC
		R	AAACGACTTGGACCAGAAGAAGC
	sgRNA2	F	ACCGAGATTTGGGGCGTCAGGAAG
		R	AAACCTTCCTGACGCCCCAAATCT
	sgRNA3	F	ACCGATGGACAGTGGGCAGAGGC
		R	AAACGCCTCTGCCCACTGTCCAAT
sgRNA4	F	GTGGTTGTGCTGCCCTCTGTGTTTTAGAGCTAGAAATAG	

		R	ACAGAGGGCAGCAACAACCACGGTGTTCGTCCTTTCCAC
m <i>Pcdh</i> locus 1	sgRNA1	F	TAATACGACTCACTATAGGGCAGCTGTCTCGCCCTCTGCGTT TTAGAGCTAGAAATAG
		R	AAAAGCACCGACTCGGTGCC
	sgRNA2	F	TAATACGACTCACTATAGGATCGAACACCAGGTGGCAGAGTT TTAGAGCTAGAAATAG
		R	AAAAGCACCGACTCGGTGCC
m <i>Pcdh</i> locus 2	sgRNA1	F	TAATACGACTCACTATAGGAGCCTAGAAGTTGTAGTGGCGTT TTAGAGCTAGAAATAG
		R	AAAAGCACCGACTCGGTGCC
	sgRNA2	F	TAATACGACTCACTATAGGGTCATCAATAGTCGGTCTTTGTT TTAGAGCTAGAAATAG
		R	AAAAGCACCGACTCGGTGCC
m <i>Pcdh</i> locus 3	sgRNA1	F	TAATACGACTCACTATAGGGACAGACATAGTCGCTTTGCCGT TTAGAGCTAGAAATAG
		R	AAAAGCACCGACTCGGTGCC
	sgRNA2	F	TAATACGACTCACTATAGGGCTAAGAGAGGCCGATACGTTTT AGAGCTAGAAATAG
		R	AAAAGCACCGACTCGGTGCC
<i>Pcdh</i> enhancer	sgRNA1	F	GCAGTCGACTCGCCCTCTGCGTTTTAGAGCTAGAAATAG
		R	GCAGAGGGCAGTCGACTGCGGTGTTCGTCCTTTCCAC
	sgRNA2	F	GTGGATCCACCTGCCACCTGGGTTTTAGAGCTAGAAATAG
		R	CCAGGTGGCAGGTGGATCCACGGTGTTCGTCCTTTCCAC

Primers used for identifying inversions, duplications, and deletions

DNA fragment		Direction	Sequence
<i>Pcdh</i> RE1	Del	F1	TTCATCCCCGCTTCCTACTG
		R2	CACTCTGATAGTTTATGTATTAGGCTTG
	InvF	F1	TTCATCCCCGCTTCCTACTG
		F2	CTTGAACCAGTTGGGATTG
	InvR	R1	TTCCGTACCATATGGATTGCTT
		R2	CACTCTGATAGTTTATGTATTAGGCTTG
Dup	F2	CGAGTCATGGGACCGAACTG	
	R1	TAACCAGCAGAGGGCGAGTC	
<i>Pcdh</i> RE2	Del	F1	CTTGAACCAGTTGGGATTG
		R2	TTATCAATAGCATTTTCCTCATCTG
	InvF	F1	CAAATGTAGTCCGTCCAGTG
		F2	GTCGCTGTCCGTGGCCAT
	InvR	R1	CATGGCCACGGACAGCGAC
		R2	TTATCAATAGCATTTTCCTCATCTG
β -globin RE1	Del	F1	GTTCTCCTTGTATTAACAGACCTG
		R2	CAGCTCTGCCTGAAAGGAGTC
	InvF	F1	GTTCTCCTTGTATTAACAGACCTG

		F2	AGATTGAGTTCTGTTTGTTCATCTAC
	InvR	R1	TTTTATGAAAGCAGCCTCTTGAG
		R2	CAGCTCTGCCTGAAAGGAGTC
<i>β-globin</i> RE2	Del	F1	AGGTGAATGAATGCGTGACTG
		R2	GCACAGCCCTGCTCTATTACG
	InvF	F1	CTGCCTCTTATGGGTCTAATGTAC
		F2	CTGGTCCTGATTCTTCCTCACTC
	InvR	R1	AGAGCCACCAGTCCACAGATC
		R2	GCACAGCCCTGCTCTATTACG
Dup	F2	ATAGCAATGAAATCTTGAGGAGTG	
	R1	ACGCAGGAGCCGTATCATG	
<i>HoxD</i> locus	Del	F1	GTTGTGGTTCTCCGTTGCCTC
		R2	CCCAGGCATAGAGACTCACTCG
	InvF	F1	GTTGTGGTTCTCCGTTGCCTC
		F2	ACCGCCCTCACTGTTGATC
	InvR	R1	TCCGCTCACCAGATAACTACG
		R2	CATCCTATTTTCGAGGAGCTGC
<i>β-globin</i> locus	Del	F1	GATGTACTGTT TCACCTCCACTTG
		R2	TGTTCTTACCTCTTCAGCCATC
	InvF	F1	TAAAGCCAGCCATTTCTAAGG
		F2	ATTCCCTGTGTGATTACTTGCTTAC
	InvR	R1	CAAATAAGTGAGAACGAGATAAACC
		R2	TGTTCTTACCTCTTCAGCCATC
Dup	F2	CAGTGGAGTTTTTGCTGTTCTTAG	
	R1	CAAATAAGTGAGAACGAGATAAACC	
<i>Pcdhα</i> cluster	Del	F1	TGCTAGGCTGCCTATTTCCCTG
		R2	TTTTTGGCTAACAACATAGTGCTTC
	InvF	F1	TGCTAGGCTGCCTATTTCCCTG
		F2	CTTGGAACCCAGTTGGGATTG
	InvR	R1	GAGAACATTGTCAACATTTGGAAGT
		R2	TTTTTGGCTAACAACATAGTGCTTC
Dup	F2	CTTGGAACCCAGTTGGGATTG	
	R1	GAGAACATTGTCAACATTTGGAAGT	
<i>Pcdh α, β, and γ</i> clusters	Del	F1	GGATTCCCTATTAACAGCTTTGC
		R2	CTGTTTGAGTGTGTATTGGATTG
	InvF	F1	GGATTCCCTATTAACAGCTTTGC
		F2	CCCATGTTTTAGTGTTC AATTTGG
	InvR	R1	GAAAGCCTAAATCCTTGT TACTTG
		R2	CTGTTTGAGTGTGTATTGGATTG
Dup	F2	CCCATGTTTTAGTGTTC AATTTGG	
	R1	GAAAGCCTAAATCCTTGT TACTTG	
<i>mPcdh</i> locus 1	Del	F1	CAATGGAGGCAATCCCTTCTG
		R2	GGTGGTAGTGAGGGATTATTCTAG

	InvF (blastocysts)	F1	AGGTAAGTGCAGCCCTGTATCC
		F2	GTTCCCTTTGTCAGGTGAAAATCTC
	InvR (blastocysts)	R1	AAACGAATGCCTCCTCTGC
		R2	GGGTGGTAGTGAGGGATTATTCTAG
	InvF (mice)	F1	CAATGGAGGCAATCCCTTCTG
		F2	GTTCCCTTTGTCAGGTGAAAATCTC
	InvR (mice)	R1	AGGTAGAGCTAAAGACTCGTGGTG
		R2	GGGTGGTAGTGAGGGATTATTCTAG
	Dup (mice)	F2	GTTCCCTTTGTCAGGTGAAAATCTC
		R1	AGGTAGAGCTAAAGACTCGTGGTG
F1		AGGTAAGTGCAGCCCTGTATCC	
R2		TGACCATCAAGATTGCTTTCACA	
m <i>Pcdh</i> locus 2	Del	F1	CAATGGAGGCAATCCCTTCTG
		R2	GGGTGGTAGTGAGGGATTATTCTAG
	InvF	F1	AGGTAAGTGCAGCCCTGTATCC
		F2	GTTCCCTTTGTCAGGTGAAAATCTC
	InvR	R1	AGGTAGAGCTAAAGACTCGTGGTG
		R2	GGGTGGTAGTGAGGGATTATTCTAG
m <i>Pcdh</i> locus 3	Del	F1	TATTCTTCAGCCCTGCAACC
		R2	ACAGGACACTACTCCGTGGT
	InvF	F1	AGCCAGACCAGCATAGCAAAT
		F2	ACAGGATAATGGGTCTGGAGC
	InvR	R1	CCAAGCCCACAGTCGTTGAT
		R2	TCCAGATTACGAGCTGAGCG

Primers used for identifying inversions, duplications, and deletions with four sgRNAs

Pcdh clusters with four sgRNAs	<i>Pcdh</i> α cluster	Del	1	TGCTAGGCTGCCTATTTCTG
			4	TTTTTGGCTAACAACATAGTGCTTC
		InvF	1	TGCTAGGCTGCCTATTTCTG
			3	CTTGGAACCAGTTGGGATTG
		InvR	2	GAGAACATTGTCAACATTTGGAAGT
			4	TTTTTGGCTAACAACATAGTGCTTC
	Dup	3	CTTGGAACCAGTTGGGATTG	
		2	GAGAACATTGTCAACATTTGGAAGT	
	<i>Pcdh</i> β cluster	Del	3	CTTGGAACCAGTTGGGATTG
			6	GTTTCTCCAACATGCTCCTCAC
		InvF	3	CTTGGAACCAGTTGGGATTG
			5	GACTTGTCAGCCACGTTTCA
		InvR	4	TTTTTGGCTAACAACATAGTGCTTC
			6	GTTTCTCCAACATGCTCCTCAC
Dup	5	GACTTGTCAGCCACGTTTCA		
	4	TTTTTGGCTAACAACATAGTGCTTC		
<i>Pcdh</i> γ cluster	Del	5	AAGCAGAGTTGAGTCTTCCGTG	

		InvF	8	CTGTTTGAGTGTGTATTGGATTC
			5	AAGCAGAGTTGAGTCTTCCGTG
		InvR	7	CCCATGTTTGTAGTGTCAATTGG
			6	TCCTCATTTCTGCACCCTCAC
		Dup	8	CTGTTTGAGTGTGTATTGGATTC
			7	CCCATGTTTGTAGTGTCAATTGG
	<i>Pcdh</i> α and β clusters	Del	6	GTTTCTCCAACATGCTCCTCAC
			1	GGATTCCTCATTAACAGCTTTGC
		InvF	5	GACTTGCCAGCCACGTTTCA
			1	TGCTAGGCTGCCTATTTCTTG
		InvR	6	GTTTCTCCAACATGCTCCTCAC
			2	GAGAACATTGTCAACATTTGGAAGT
	Dup	5	GACTTGCCAGCCACGTTTCA	
		2	GAAAGCCTAAATCCTTGTACTTG	
	<i>Pcdh</i> β and γ clusters	Del	8	CTGTTTGAGTGTGTATTGGATTC
			3	CTTGAACCAGTTGGGATTG
		InvF	7	CCCATGTTTGTAGTGTCAATTGG
			3	CTTGAACCAGTTGGGATTG
		InvR	8	CTGTTTGAGTGTGTATTGGATTC
			4	TTTTTGGCTAACACATAGTGCTTC
	Dup	7	CCCATGTTTGTAGTGTCAATTGG	
		4	TTTTTGGCTAACACATAGTGCTTC	
	<i>Pcdh</i> α , β , and γ clusters	Del	8	CTGTTTGAGTGTGTATTGGATTC
			1	GGATTCCTCATTAACAGCTTTGC
		InvF	7	CCCATGTTTGTAGTGTCAATTGG
			1	GGATTCCTCATTAACAGCTTTGC
		InvR	8	CTGTTTGAGTGTGTATTGGATTC
			2	GAAAGCCTAAATCCTTGTACTTG
Dup	7	CCCATGTTTGTAGTGTCAATTGG		
	2	GAAAGCCTAAATCCTTGTACTTG		

Primers used for quantitative PCR for measuring efficiency

Targeted locus		Direction	Sequence
<i>β-globin</i> RE1	Del	F1	GTTCCCTCCTTGATTAAACAGACCTG
		R2	CAGCTCTGCCTGAAAGGAGTC
	Inv	F1	GTTCCCTCCTTGATTAAACAGACCTG
		F2	AGATTGAGTTCTGTTTGTTCATCTAC
	WT	F2	AGATTGAGTTCTGTTTGTTCATCTAC
		R2	CAGCTCTGCCTGAAAGGAGTC
<i>Pcdh</i> RE1	Del	F1	TTCATCCCCGCTTCCTACTG
		R2	TTTTTGGCTAACACATAGTGCTTC
	Inv	R1	TTCCGTACCATATGGATTGCTT

		R2	TTTTTGGCTAACAAACATAGTGCTTC
	Dup	F2	CGAGTCATGGGACCGAACTG
		R1	TAACCAGCAGAGGGCGAGTC
	WT	F1	TTCATCCCCGCTTCCTACTG
		R1	TTCCGTACCATATGGATTGCTT
<i>β-globin</i> RE2	Del	F1	AGGTTGAATGAATGCGTGACTG
		R2	GCACAGCCCTGCTCTATTACG
	Inv	F1	CTGCCTCTTTATGGGTCTAATGTAC
		F2	CTGGTCTGATTCTTCCTCACTC
	Dup	F2	ATAGCAATGAAATCTTGAAGGAGTG
		R1	ACGCAGGAGCCGTATCATG
WT	F2	CTGGTCTGATTCTTCCTCACTC	
	R2	GCACAGCCCTGCTCTATTACG	
<i>HoxD</i> locus	Del	F1	GTTGTGGTTCTCCGTTGCTTC
		R2	CCCAGGCATAGAGACTCACTCG
	Inv	F1	GTTGTGGTTCTCCGTTGCTTC
		F2	ACCGCCCTTCACTGTTGATC
	WT	F1	TGAAAGAACGTTGCCACCTC
		R1	GAAGGGAACCTGCTCCGAA
<i>β-globin</i> locus	Del	F1	GATGTACTGTTTCACCTCCACTTG
		R2	TGTTCTTACCTCTTCAGCCATC
	Inv	F1	TAAAGCCAGCCATTTCTAAGG
		F2	CAGTGGAGTTTTTGCTGTTCTTAG
	Dup	F2	CAGTGGAGTTTTTGCTGTTCTTAG
		R1	CAAATAAGTGAGAACGAGATAAACC
WT	F1	GATGTACTGTTTCACCTCCACTTG	
	R1	CAAATAAGTGAGAACGAGATAAACC	
<i>Pcdhα</i> cluster	Del	F1	GGATTCCCTCATTAACAGCTTTGC
		R2	TTTTTGGCTAACAAACATAGTGCTTC
	Inv	R1	GAGAACATTGTCAACATTTGGAAGT
		R2	TTTTTGGCTAACAAACATAGTGCTTC
	Dup	F2	CTTGGAAACCAGTTGGGATTG
		R1	GAGAACATTGTCAACATTTGGAAGT
WT	F1	GGATTCCCTCATTAACAGCTTTGC	
	R1	GAGAACATTGTCAACATTTGGAAGT	
<i>Pcdh α, β, and γ</i> clusters	Del	F1	GGATTCCCTCATTAACAGCTTTGC
		R2	CTGTTTGAGTGTGTATTGGATTC
	Inv	F1	GGATTCCCTCATTAACAGCTTTGC
		F2	CCCATGTTTTAGTGTTC AATTTGG
	Dup	F2	CCCATGTTTTAGTGTTC AATTTGG
		R1	GAAAGCCTAAATCCTTGT TACTTG
WT	F2	CCCATGTTTTAGTGTTC AATTTGG	
	R2	CTTGTCATGATCTAGGA ACTTATTC	

Primers used for quantitative PCR for measuring efficiency with four sgRNAs

Pcdh clusters with four sgRNAs	Pcdh α cluster	Del	F1	GGATTCCTCATTAACAGCTTTGC
			R2	TTTTTGGCTAACAAACATAGTGCTTC
		Inv	R1	GAGAACATTGTCAACATTTGGAAGT
			R2	TTTTTGGCTAACAAACATAGTGCTTC
		Dup	F2	CTTGAACCAGTTGGGATTG
			R1	GAGAACATTGTCAACATTTGGAAGT
	WT	F1	GGATTCCTCATTAACAGCTTTGC	
		R1	GAGAACATTGTCAACATTTGGAAGT	
	Pcdh β cluster	Del	F1	CGAGTCATGGGACCGAACTG
			R2	GTTTCTCCAACATGCTCCTCAC
		Inv	F1	CTTGAACCAGTTGGGATTG
			F2	GACTTGTCAGCCACGTTTCA
		Dup	F2	GACTTGTCAGCCACGTTTCA
			R1	TTTTTGGCTAACAAACATAGTGCTTC
	WT	F2	GACTTGTCAGCCACGTTTCA	
		R2	GTTTCTCCAACATGCTCCTCAC	
	Pcdh γ cluster	Del	F1	GACTTGTCAGCCACGTTTCA
			R2	CTTGTCATGATCTAGGAACCTTATTC
		Inv	R1	TCCTCATTCTGCACCCTCAC
			R2	CTGTTTGAGTGTGTATTGGATTTC
		Dup	F2	CCCATGTTTTAGTGTTCAATTTGG
			R1	GTTTCTCCAACATGCTCCTCAC
	WT	F1	GACTTGTCAGCCACGTTTCA	
		R1	GTTTCTCCAACATGCTCCTCAC	
	Pcdh α and β clusters	Del	F1	AAGTATTGGGATTCCTCATTAACAG
			R2	TCCTCATTCTGCACCCTCAC
		Inv	F1	TGCTAGGCTGCCTATTTCTCTG
			F2	GACTTGTCAGCCACGTTTCA
		Dup	F2	GACTTGTCAGCCACGTTTCA
			R1	GAGAACATTGTCAACATTTGGAAGT
WT	F2	GACTTGTCAGCCACGTTTCA		
	R2	GTTTCTCCAACATGCTCCTCAC		
Pcdh β and γ clusters	Del	F1	CGAGTCATGGGACCGAACTG	
		R2	CTGTTTGAGTGTGTATTGGATTTC	
	Inv	F1	CTTGAACCAGTTGGGATTG	
		F2	CCCATGTTTTAGTGTTCAATTTGG	
	Dup	F2	CCCATGTTTTAGTGTTCAATTTGG	
		R1	TTTTTGGCTAACAAACATAGTGCTTC	
WT	F2	CCCATGTTTTAGTGTTCAATTTGG		
	R2	CAAACAAGGAGAATGATGAAAGTGG		
Pcdh α , β , and γ	Del	F1	GGATTCCTCATTAACAGCTTTGC	

	clusters		R2	CTGTTTGAGTGTTGTATTGGATTC
		Inv	R1	GGATTCCTCATTAACAGCTTTGC
	Dup		R2	CCCATGTTTTAGTGTTCAATTTGG
		WT	F2	CCCATGTTTTAGTGTTCAATTTGG
	R1		GAAAGCCTAAATCCTTGTTTACTTG	
	R2	CTTGTCATGATCTAGGAACCTTATTC		

Primers used for off-target analyses

DNA fragment	sgRNAs	Direction	Sequence
<i>Pcdh</i> RE1	sgRNA1	OTF	TCCTGGAACACGGATTTATGC
		OTR	TGTTCTCCTTTCCCTTTGTGAG
β -globin RE1	sgRNA1	OTF	CTCAACCTCCCAAGTTGGATC
		OTR	GAGAGGTGATGTGGAGGTGAGTG
	sgRNA2	OTF	GCCTACTGTGACTGTGGCAATG
		OTR	CCACGCTACATTCACCCATC
β -globin RE2	sgRNA1	OTF	GGAGGGCTGGGAAGTGCA
		OTR	GGGCATCCATGGTTGTCTG
	sgRNA2	OTF	CATTCAAGGTAATAACTCACTGG
		OTR	CACCCTCAACATTTCCACAAG
<i>HoxD</i> locus	sgRNA1	OTF	CAGAGCCTCCAGCAGTCCCTAC
		OTR	GAGGCACCCATACCAGAAGC
	sgRNA2	OTF	AGAAGCGCCCATTGAAAGAG
		OTR	TTTCAGTTCAGAAATCCCGTC
β -globin locus	sgRNA1	OTF	CCATCTGGGGATAGGTTGTTC
		OTR	GCTTACGGTGTTCCTCCATC
	sgRNA2	OTF	CATCCCATTCTATCAACCAGG
		OTR	AAAAATAGGCTGGAGCAAGTCC

Primers used for quantitative RT-PCR of gene expression of the *Pcdh* gene clusters

Gene name	Direction	Sequence
<i>hPcdhα6</i>	F	GATGGGTAAGGCGGAGAATC
	R	AGGTCCAGCTGTTGCTGTTGAC
<i>hPcdhα12</i>	F	CAAGCCTTCAGCTGTCTCGA
	R	AGGTCCAGCTGTTGCTGTTGAC
<i>hPcdhα1</i>	F	CTTGCCACTGGGGTAGGACTG
	R	AGGTCCAGCTGTTGCTGTTGAC
<i>hPcdhα2</i>	F	AGGCCAAAGTGGTCAGAATG
	R	AGGTCCAGCTGTTGCTGTTGAC
<i>hPcdhβ3</i>	F	TGTCTTCAGTAAACAGCCCTATTC
	R	TCACTCTTCCGTTGTCTCCAG
<i>hPcdhβ9</i>	F	ATTCCAATGACAATCCTCCTG
	R	TAAAAACAGCCAATACTATCCCAG

<i>hPcdhβ15</i>	F	GCTCTGTCTCTGTTAAGGTGCTG
	R	GAGTCTCGGTCTCTAATCCTAAAC
<i>hPcdhγb5</i>	F	CGACTTCCCATCCTGAGTTG
	R	CGACTTCTTCTGTGGCCATTG
<i>hPcdhγa10</i>	F	CCTTTGTCTTTGTTAGATGATTCG
	R	CGACTTCTTCTGTGGCCATTG
<i>hGAPDH</i>	F	GGAGTCCACTGGCGTCTTCAC
	R	GCAGGAGGCATTGCTGATGAT

Supplementary Table S2. List of sgRNA targeting sequences

DNA fragment	sgRNA	Species	Chr	Genomic coordinates (hg19 or mm9)	Sequence
<i>Pcdh</i> RE1	sgRNA1	Human	5	140419774-140419793	GCCACACATCCAAGGCTGAC
	sgRNA2	Human	5	140421046-140421065	AGATTTGGGGCGTCAGGAAG
<i>Pcdh</i> RE2	sgRNA1	Human	5	140420832-140420851	GACAGCGACACCGCCCAGTT
	sgRNA2	Human	5	140420853-140420872	GTGGCCATGGTCTGAACTC
<i>β-globin</i> RE1	sgRNA1	Human	11	4093748-4093767	ATTGTTGTTGCCTGGAGTG
	sgRNA2	Human	11	4094457-4094476	CTGGTCCCCTGGTAACCTGG
<i>β-globin</i> RE2	sgRNA1	Human	11	4203109-4203128	ACCCAATGACCTCAGGCTGT
	sgRNA2	Human	11	4209386-4209405	TCACTTGTAGCGGCATCTG
<i>HoxD</i> locus	sgRNA1	Human	2	176944510-176944529	GGGGCGAGGGGTTGGTCTT
	sgRNA2	Human	2	176962653-176962672	GACCTCGAAAGTCATAAACC
<i>β-globin</i> locus	sgRNA1	Human	11	5145473-5145492	GCTTCAACACTGCCATCTCC
	sgRNA2	Human	11	5226196-5226215	GAACTACTTCTGACCCCTAG
<i>Pcdh</i> cluster	sgRNA1	Human	5	140164302-140164321	GGCTTCTTCTGGTCCAAGTC
	sgRNA2	Human	5	140421046-140421065	AGATTTGGGGCGTCAGGAAG
	sgRNA3	Human	5	140629038-140629057	ATTGGACAGTGGGCAGAGGC
	sgRNA4	Human	5	140971774-140971793	TGGTTGTTGCTGCCCTCTGT
<i>mPcdh</i> locus 1	sgRNA1	Mouse	18	37217457-37217476	GCAGCTGTCTCGCCCTCTGC
	sgRNA2	Mouse	18	37218428-37218447	TCTGCCACCTGGTGTTCGAT
<i>mPcdh</i> locus 2	sgRNA1	Mouse	18	37217321-37217340	GCCACTACAACCTCTAGGCT
	sgRNA2	Mouse	18	37218551-37218570	GTCATCAATAGTCGGTCTTT
<i>mPcdh</i> locus 3	sgRNA1	Mouse	18	38011468-38011487	GGCAAAGCGACTATGTCTGT
	sgRNA2	Mouse	18	38040855-38040874	GGGCTAAGAGAGGCCGATAC
<i>Pcdh</i> enhancer	sgRNA1	Human	5	140419937-140419956	GCAGTCGACTCGCCCTCTGC
	sgRNA2	Human	5	140420945-140420965	GTGGATCCACCTGCCACCTGG

Supplementary Table S3. Potential off-target sites for sgRNAs.

DNA fragment	sgRNAs	Sequence	Coordinate
<i>Pcdh</i> RE1	sgRNA1	GCCACACATCCAAGGCTGACAGG	chr5:140419774-140419796
		<u>G</u> TAGGCATCCAAGGCTGACAGG	chr5:73541775-73541797
		<u>AGGAGAA</u> ATCCAAGGCTGACAGG	chr16:29283392-29283414
		<u>AGGAGAA</u> ATCCAAGGCTGACAGG	chr16:88279138-88279160
	sgRNA2	AGATTTGGGGCGTCAGGAAGTGG	chr5:140421046-140421068
		not found	
<i>β-globin</i> RE1	sgRNA1	ATTGTTGTTGCCTTGGAGTGGGG	chr11:4093748-4093770
		<u>T</u> TT <u>TAC</u> GTTCCTTGGAGT <u>G</u> AGG	chr3:194529587-194529609
		<u>AGCCCAT</u> TTGCCTTGGAGT <u>T</u> G	chr10:61769557-61769579
		<u>CACAGCC</u> TTGCCTTGGAGT <u>A</u> GG	chr12:105794596-105794618
	sgRNA2	CTGGTCCCCTGGTAACCTGGTGG	chr11:4094457-4094479
	<u>AGACATT</u> CCTGGTAACCTGG <u>C</u> GG	chr4:10135619-10135641	
<i>β-globin</i> RE2	sgRNA1	ACCCAATGACCTCAGGCTGTAGG	chr11:4203109-4203131
		<u>T</u> CC <u>AAGG</u> GACCTCAGGCTGT <u>G</u> GG	chr1:35225451-35225473
		<u>AGGCCGG</u> GACCTCAGGCTGT <u>G</u> GG	chr7:155175311-155175333
		<u>TCTTGG</u> TGACCTCAGGCTGT <u>G</u> GG	chr11:72538049-72538071
		<u>TGTCTC</u> TGACCTCAGGCTGTAGG	chr22:29823268-29823290
	sgRNA2	TCACTTGTAGCGGCATCTGTGG	chr11:4209386-4209408
		<u>TAGGATT</u> TTAGCGGCATCTGTGG	chr1:200714321-200714343
		<u>AGGATG</u> TTAGCGGCATCTGTGG	chr16:5293528-5293550
<u>CAAAA</u> ATTAGCGGCATCTGTGG		chr17:47146780-47146802	
<i>HoxD</i> locus	sgRNA1	GGGGCGAGGGGTTGGTCTTTGG	chr2:176944510-176944532
		<u>ATCTGGA</u> AGGGGTTGGTCTT <u>A</u> GG	chr1:227824834-227824856
		<u>GCCCA</u> AGGGGTTGGTCTT <u>G</u> GG	chr3:119470371-119470393
		<u>CAAGTG</u> GAGGGGTTGGTCTT <u>G</u> GG	chr15:90131233-90131255
		<u>AGACCC</u> CAGGGGTTGGTCTT <u>G</u> GG	chr16:5612529-5612551
	sgRNA2	GACCTCGAAAGTCATAAACCCGG	chr2:176962653-176962675
		<u>CAGAGT</u> CAAAGTCATAAACCT <u>T</u> GG	chr4:165699237-165699259
		<u>AAAGC</u> CGAAAGTCATAAAC <u>C</u> AGG	chr18:103855897-103855919
<u>AACATC</u> AAAAGTCATAAAC <u>C</u> AGG		chrX:89642974-89642996	
<i>β-globin</i> locus	sgRNA1	CCAGGAGATGGCAGTGTGAAGC	chr11:5145473-5145495
		CCGGGAGATGGCAGT <u>GCTGGGA</u>	chr1:15136268-15136290
		CCAGGAGATGGCAGTGT <u>TGCA</u>	chr1:64140255-64140277
		CCAGGAGATGGCAGTGT <u>TCCT</u>	chr1:175121340-175121362
		CCCGGAGATGGCAGT <u>AGCCTG</u>	chr1:227603760-227603782
		C <u>T</u> GGAGATGGCAGT <u>GA</u> CTTAT	chr4:123855569-123855591
		C <u>T</u> GGAGATGGCAGTGT <u>GTGT</u>	chr4:183176777-183176799
		CCAGGAGATGGCAGT <u>GGA</u> AAGT	chr5:73832096-73832118
		CCAGGAGATGGCAGT <u>GGG</u> AAA	chr7:6546692-6546714
CCAGGAGATGGCAGTGT <u>TCTGA</u>	chr7:12428914-12428936		

		CCTGGAGATGGCAGTGGATCCCA	chr10:20603816-20603838
		CCAGGAGATGGCAGTGTGAGAC	chr12:70273334-70273356
		CCAGGAGATGGCAGTGGCAGCCA	chr16:88882589-88882611
		CCTGGAGATGGCAGTGTTCGAGA	chr17:71503217-71503239
	sgRNA2	CCACTAGGGGTCAGAAGTAGTTC	chr11:5226196-5226218
		CCCTAGGGGTCAGAAAAGCCTTT	chr3:73189890-73189912
<i>Pcdhα</i> cluster		CCACTAGGGGTCAGAAATTACACA	chr18:41710505-41710527
	sgRNA1	GGCTTCTTCTGGTCCAAGTCTGG	chr5:140164302-140164324
		ATGGAGTTCTGGTCCAAGTCTGG	chr1:84516811-84516833
		GGTAATACTGGTCCAAGTCTGG	chr3:197017861-197017883
		CCATTAACTGGTCCAAGTCAAGG	chr15:78053927-78053949
	sgRNA2	AGATTTGGGGCGTCAGGAAGTGG	chr5:140421046-140421068
	not found		

Supplementary Table S4. Off-target analyses by PCR and sequencing.

DNA fragment	sgRNAs	Sequence	Coordinate	Indel mutation
<i>Pcdh</i> RE1	sgRNA1	GTAGGCCATCCAAGGCTGACAGG	chr5:73541775-73541797	Not found
<i>β-globin</i> RE1	sgRNA1	TTTACGTTCCTTGGAGTGAGG	chr3:194529587-194529609	Not found
	sgRNA2	AGACATTCCTGGTAACCTGGCGG	chr4:10135619-10135641	Not found
<i>β-globin</i> RE2	sgRNA1	TGTCCTGACCTCAGGCTGTAGG	chr22:29823268-29823290	Not found
	sgRNA2	AGGATGGTTAGCGCATCTGTGG	chr16:5293528-5293550	Not found
<i>HoxD</i> locus	sgRNA1	AGACCCCAGGGGTTGGTCTTGGG	chr16:5612529-5612551	Not found
	sgRNA2	AACATCAAAAGTCATAAACCAGG	chrX:89642974-89642996	Not found
<i>β-globin</i> locus	sgRNA1	CCAGGAGATGGCAGTGTGAGAC	chr12:70273334-70273356	Not found
	sgRNA2	CCACTAGGGGTCAGAATTACACA	chr18:41710505-41710527	Not found

Supplementary Table S5. The efficiency of inversions, duplications, and deletions by CRISPR with four sgRNAs

Efficiency (%)	<i>Pcdhα</i> cluster	<i>Pcdhβ</i> cluster	<i>Pcdhγ</i> cluster	<i>Pcdh α and β</i> clusters	<i>Pcdh β and γ</i> clusters	<i>Pcdh α, β, and γ</i> clusters
Inversion	3.474	8.178	5.488	8.574	4.597	0.550
Duplication	2.381	4.180	2.250	0.834	6.347	0.228
Deletion	4.048	11.904	6.154	20.989	5.871	0.318

Supplementary Table S6. The efficiency of inversions, duplications, and deletions by CRISPR for screening single-cell clones

DNA fragment	Length	Total clones	Inversion clones	Inversion efficiency (%)	Duplication clones	Duplication efficiency (%)	Deletion clones	Deletion efficiency (%)
<i>β-globin</i> RE1	709 bp	78	38	48.72	0	0	46	58.97
<i>Pcdh</i> enhancer	1,272 bp	32	4	12.50	ND	/	12	37.50
<i>β-globin</i> RE2	6,277 bp	62	31	50.00	1	1.61	45	72.58
<i>HoxD</i> locus	18,142 bp	112	1	0.89	ND	/	4	3.57

ND: not determined.