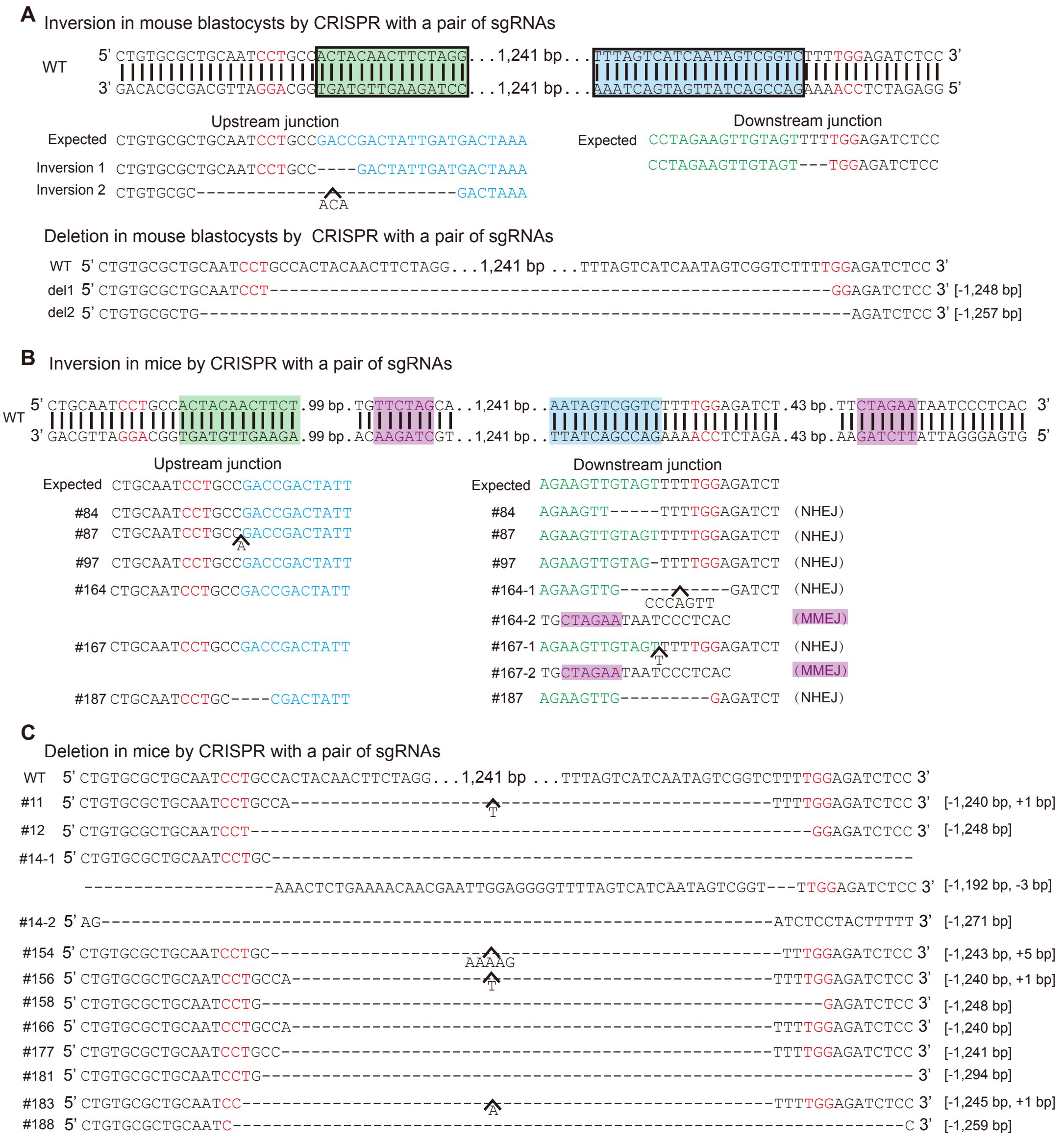
Efficient Inversions and Duplications of Mammalian Regulatory DNA Elements and Gene Clusters by CRISPR/Cas9

Online Supplementary Materials:

Supplementary Figures S1-S14

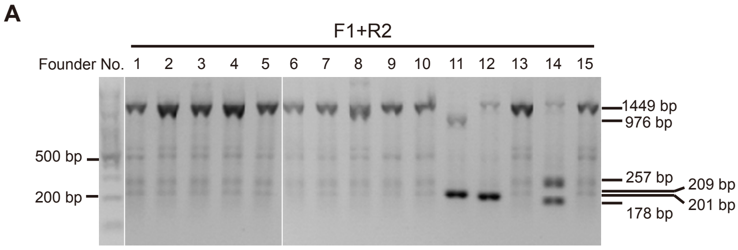
Supplementary Tables S1-S6

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.

A



B

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WT    5' GTGCGCTGCAATCCTGCCACTACAACTTCTAGGCTTGCT......AATAGTCGGTCTTTTGGAGATCTCCTACTTTTT 3'

#1    5' GTGCGCTGCAATCCTGCCACTACAACTTCTAGGCTTGCT......AATAGTCGGTCTTTTGGAGATCTCCTACTTTTT 3'
                                                                 ^
                                                                 C
#2    5' GTGCGCTGCAATCCTGCCACTACAACTTCTAGGCTTGCT......AATAGTCGGTCTTTTGGAGATCTCCTACTTTTT 3'

#3-1  5' GTGCGCTGCAATCCTGCCACTACAACTTCTAGGCTTGCT......------33 bp del----ATCTCCTACTTTTT 3'
#3-2  5' GTGC--------------ACTACAACTTCTAGGCTTGCT......------33 bp del----ATCTCCTACTTTTT 3'
#3-3  5' GTGCGCTGCAATCCTGCCACTACAACTTCTAGGCTTGCT......AATAGT-------62 bp del----------- 3'

#4-1  5' GTGCGCTGCAATCCTGCCACTACAACTTCTAGGCTTGCT......AATAGTCGGTC-------------CTACTTTTT 3'
#4-2  5' GTGCGCTGCAATCCTGCCACTACAACTTCTAGGCTTGCT......AATAGTCGGTCTTTTGGAGATCTCCTACTTTTT 3'
                                                                 ^
                                                                TTTT
#4-3  5' GTGCGCTGCAATCCTGCCACTACAACTTCTAGGCTTGCT......AATAGTCGGTCTTTTGGAGATCTCCTACTTTTT 3'
                                                                 ^
                                                                GT
#5    5' GTGCGCTGCAATCCTGCCACTACAACTTCTAGGCTTGCT......AATAGTCGGTCTTTTGGAGATCTCCTACTTTTT 3'

#6-1  5' GTGCGCTGCAATCCTGC------AACTTCTAGGCTTGCT......AATAGTCGGTCTTTTGGAGATCTCCTACTTTTT 3'
                                                                  ^
                                                                  T
#6-2  5' GTGCGCTGCAATCCTGC------AACTTCTAGGCTTGCT......AATAGTCGGT--TTTGGAGATCTCCTACTTTTT 3'
#6-3  5' GTGCGCTGCAATCCTGCCACTACAACTTCTAGGCTTGCT......AATAGTCGGT--TTTGGAGATCTCCTACTTTTT 3'
                          ^
                          A
#7-1  5' GTGCGCTGCAATCCTGCCACTACAACTTCTAGGCTTGCT......AATAGTCGGTCTTTTGGAGATCTCCTACTTTTT 3'
                                                                 ^
                                                                 T
#7-2  5' GTGCGCTGCAATCCTGCCACTACAACTTCTAGGCTTGCT......AATAGTCGGTCTTT-GGAGATCTCCTACTTTTT 3'
                                                                     ^
                                                          AAGTCTTAAAGTCAAAAGTC
#8-1  5' GTGCGCTGCAATCCTG-------226 bp del-----......AATAGTCGGTCTTTTGGAGATCTCCTACTTTTT 3'
                                                                 ^
                                                                 T
#8-2  5' GTGCGCTGCAATCCTG-------226 bp del-----......AATAGTCGGTCTTTTGGAGATCTCCTACTTTTT 3'
                                                                 ^
                                                                 TT
#8-3  5' GTGCGCTGCAATCCTG-------226 bp del-----......AATAGTCGGTCT-----------CCTACTTTTT 3'

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

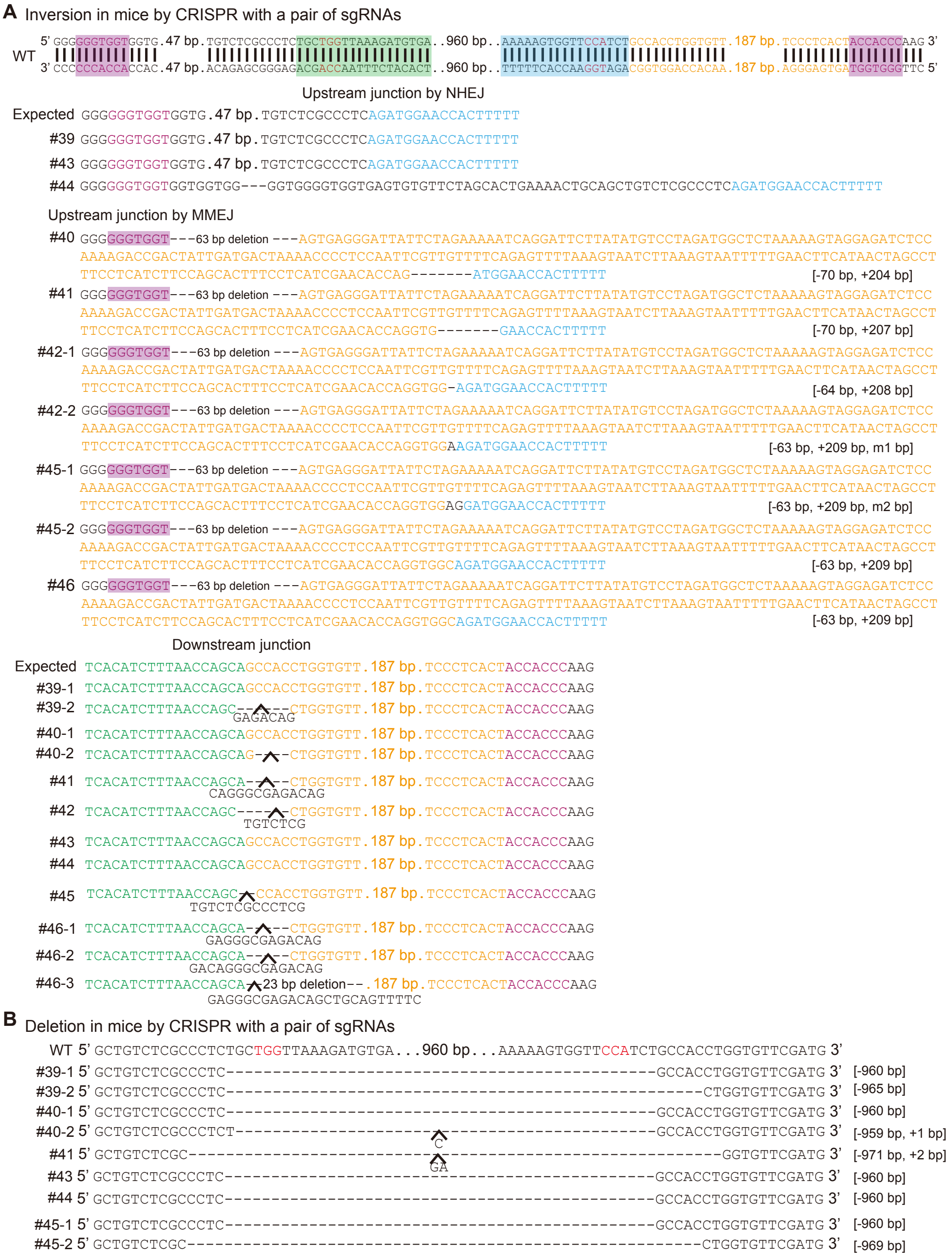
#10-1 5' GTGCGCTGCAATCCTGCCACTACAACTTCTAGGCTTGCT......AATAGTCGGT--TTTGGAGATCTCCTACTTTTT 3'
#10-2 5' GTGCGCTGCAATCCTGCCACTACAACTTCTAGGCTTGCT......AATAGTCGG--------AGATCTCCTACTTTTT 3'

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

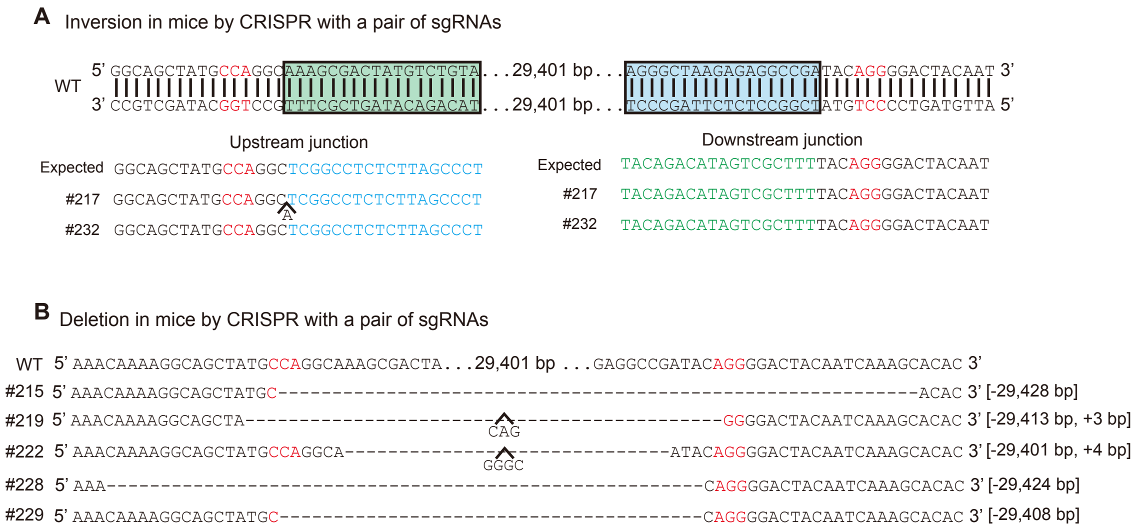
#12   5' GTGCGCTGCAATCCTGCCACTACAACTTCTAGGCTTGCT......AATAGTCGGTCTTTTGGAGATCTCCTACTTTTT 3'
                                                                 ^
                                                                 T
#13-1 5' GTGCGCTGCAATCCTGCCACTACAACTTCTAGGCTTGCT......AATAGTCGG--------AGATCTCCTACTTTTT 3'
#13-2 5' GTGCGCTGCAATCCTGCCACTACAACTTCTAGGCTTGCT......AATAGTCGGT---TTGGAGATCTCCTACTTTTT 3'

#14   5' GTGCGCTGCAATCCTGCCACTACAACTTCTAGGCTTGCT......AATAGTCGGTCTTTTGGAGATCTCCTACTTTTT 3'
                                                                       ^
                                                                      TT
#15-1 5' GTGCGCTGCAATCCTGCCACTACAACTTCTAGGCTTGCT......AATAGTCGG--TTTGGAGATCTCCTACTTTTT 3'
                                                                ^
                                                                C
#15-2 5' GTGCGCTGCAATCCTGCCACTACAACTTCTAGGCTTGCT......AATAGTCGG--TTTTGGAGATCTCCTACTTTTT 3'
```

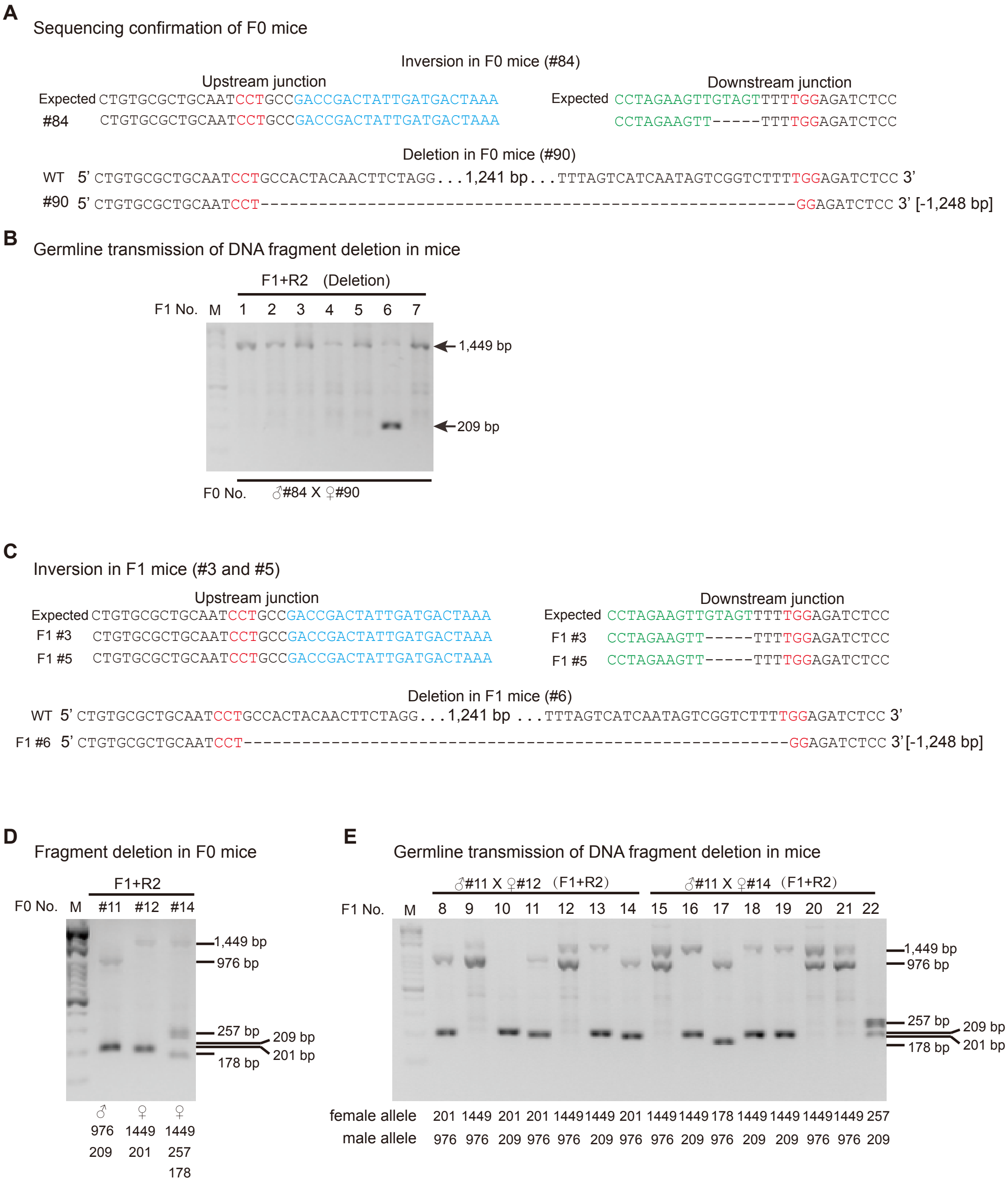
Supplementary Figure S2. Sequences of indels at the two targeted sites by CRISPR with a pair of sgRNAs in the F0 founder mice. (*A*) Genotyping of the 15 F0 founder mice. (*B*) Sequences of alleles of indels at the two targeted sites of the 15 F0 founder mice.



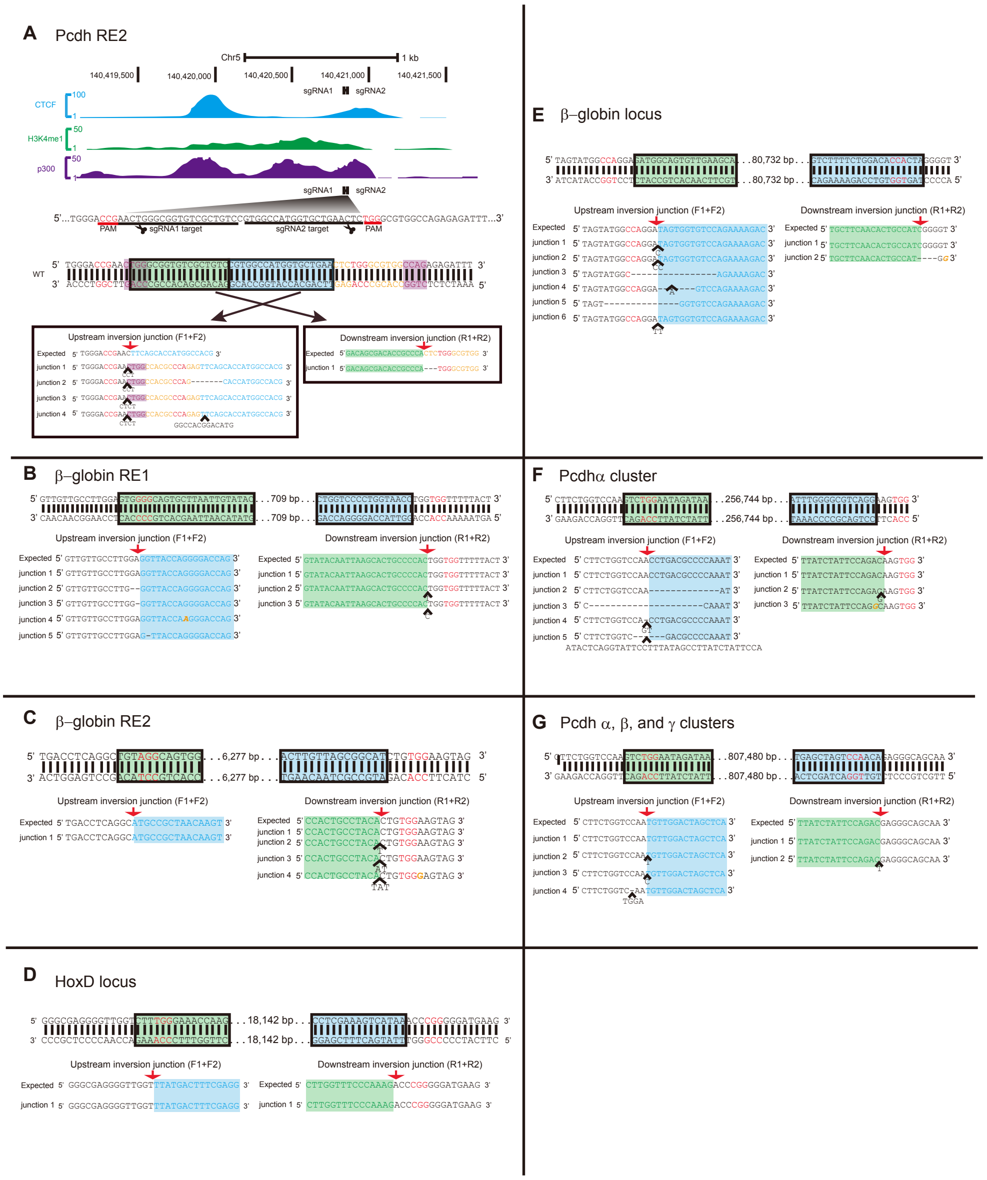
Supplementary Figure S3. Inversion and deletion of a 960-bp DNA fragment by CRISPR with a pair of sgRNAs in the F0 founder mice. (*A*) Sequences of the upstream and downstream inversion junctions in F0 founder mice. The nucleotides highlighted in purple indicate the inverted repeats used by MMEJ. Repair 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.



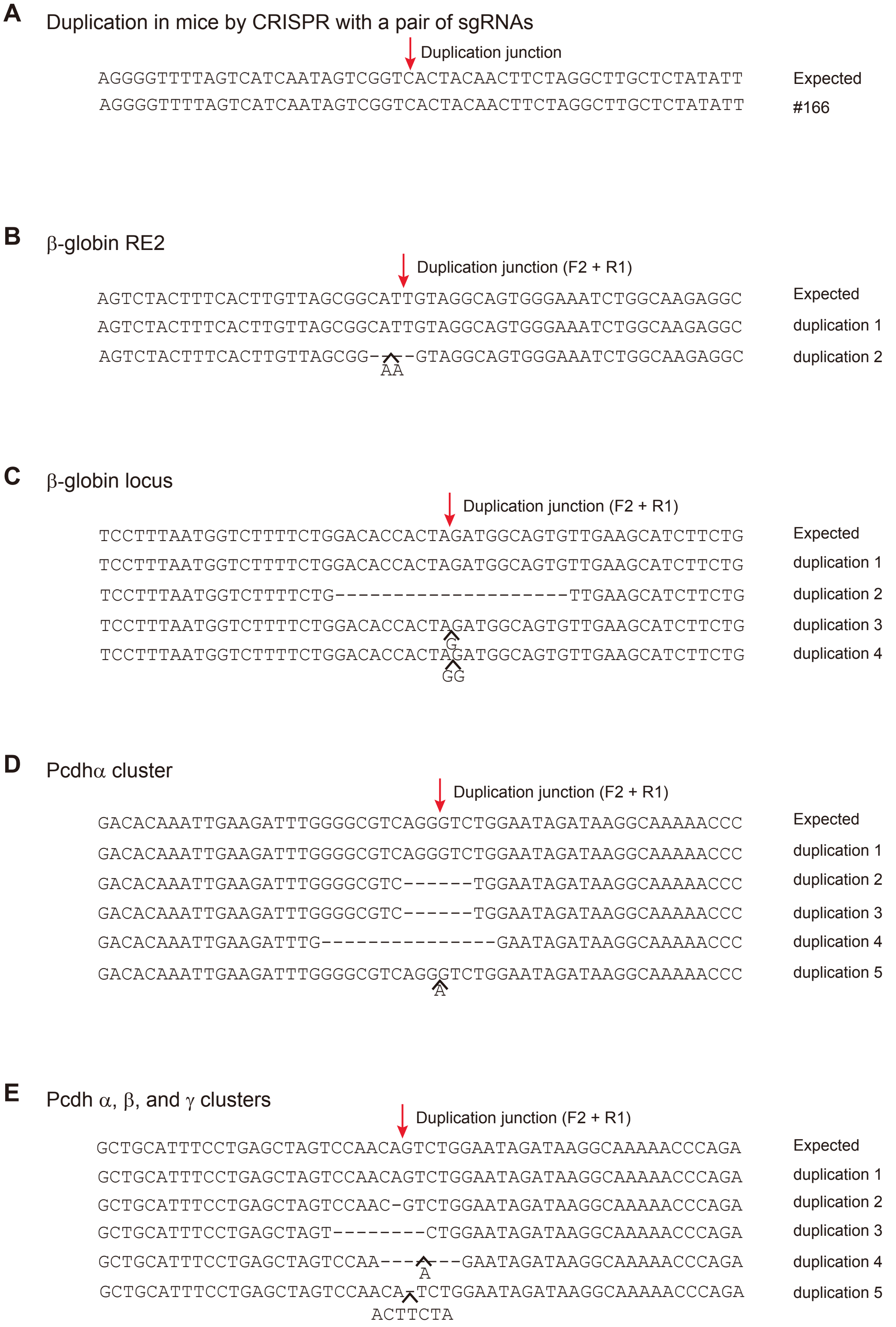
Supplementary Figure S4. Inversion and deletion of a 29,401-bp DNA fragment by CRISPR with a pair of sgRNAs in F0 founder mice. (*A*) Sequences of the upstream and downstream inversion junctions in F0 founder mice. (*B*) Sequences of the deletion junctions in F0 founder mice.



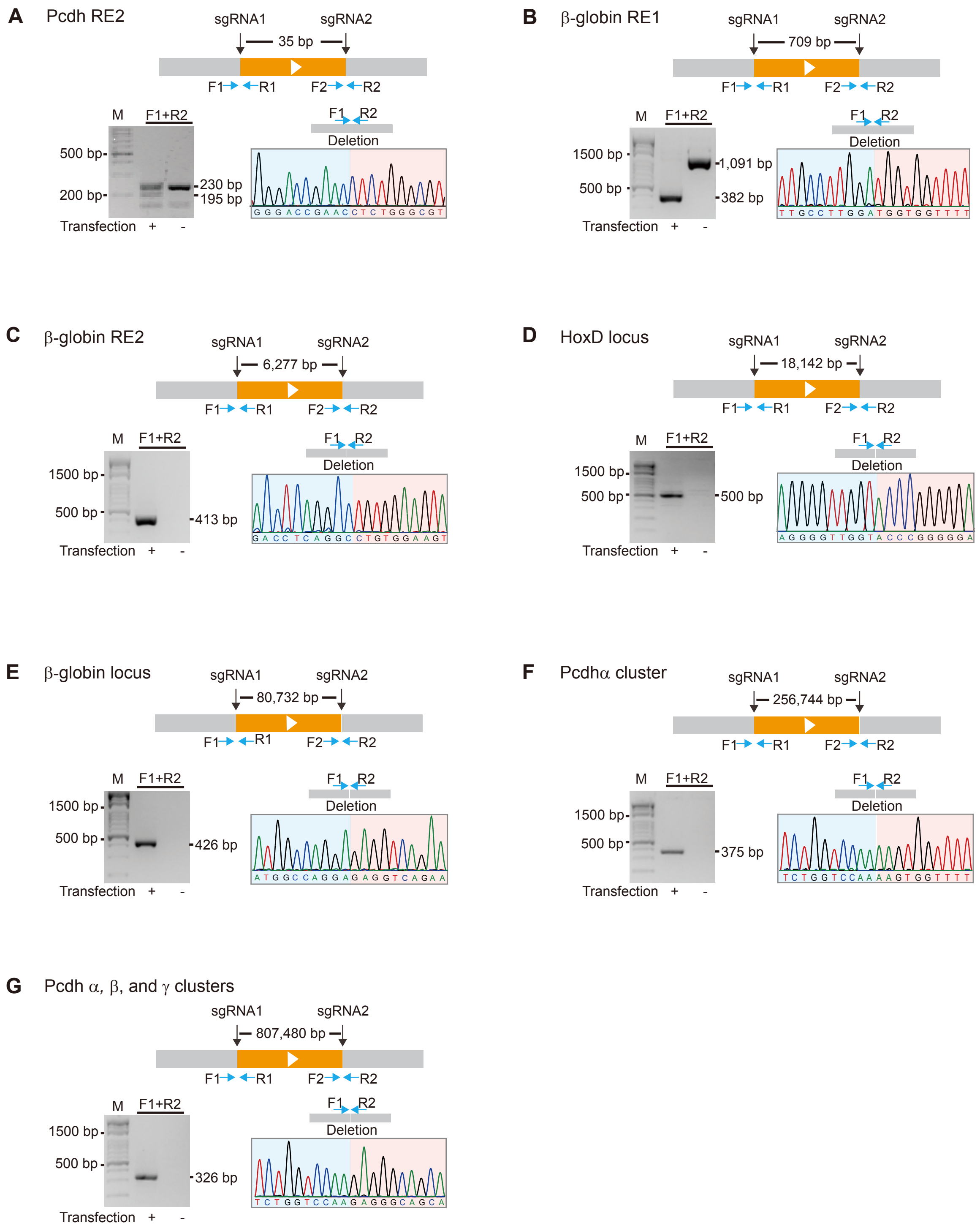
Supplementary Figure S5. Germline transmission in mice of the DNA-fragment inversion and deletion induced by CRISPR with a pair of sgRNAs. (*A*) Sequencing of alleles of inversion and deletion junctions in F0 mice. (*B*) Genotyping of DNA fragment deletion in F1 mice from crossing of two F0 founder mice.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



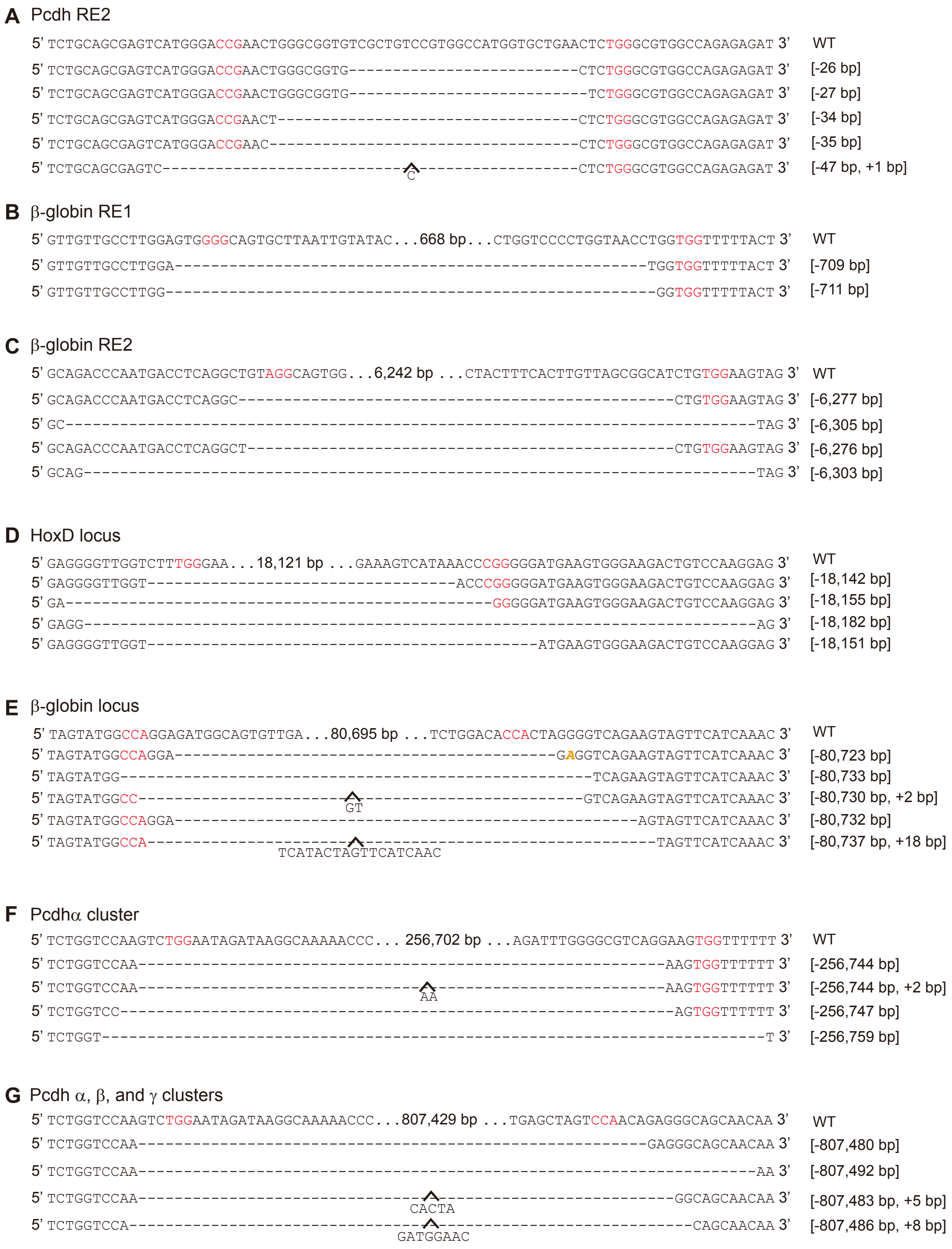
Supplementary Figure S6. Inversions of a set of DNA fragments of different sizes in human cell. (*A*) Inversion of a 35-bp DNA fragment of the *Pcdh* regulatory element 2 (RE2) by CRISPR with a pair of sgRNAs. Shown are diagram of CRISPR with a pair of sgRNAs. The sgRNAs targeting sequences are underlined. 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*).



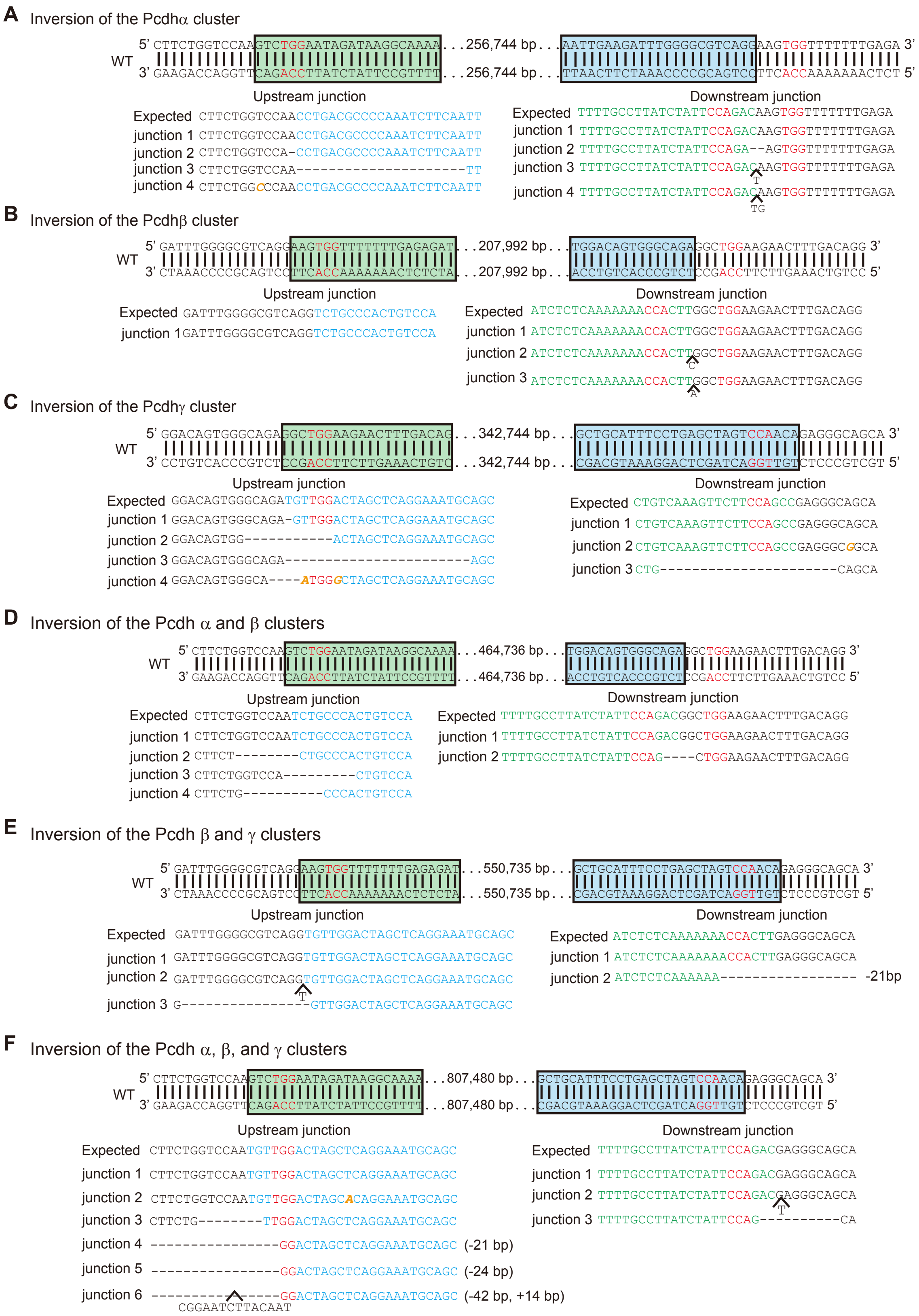
Supplementary Figure S7. Sequences of duplication junctions by CRISPR through *trans*-allelic recombination in mice and human cells. Shown are sequences of duplication junctions of 1,241-bp DNA fragment (*A*) in mouse, of 6,277-bp (*B*) and 80,732-bp (*C*) DNA fragments at the *β-globin* locus, of a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



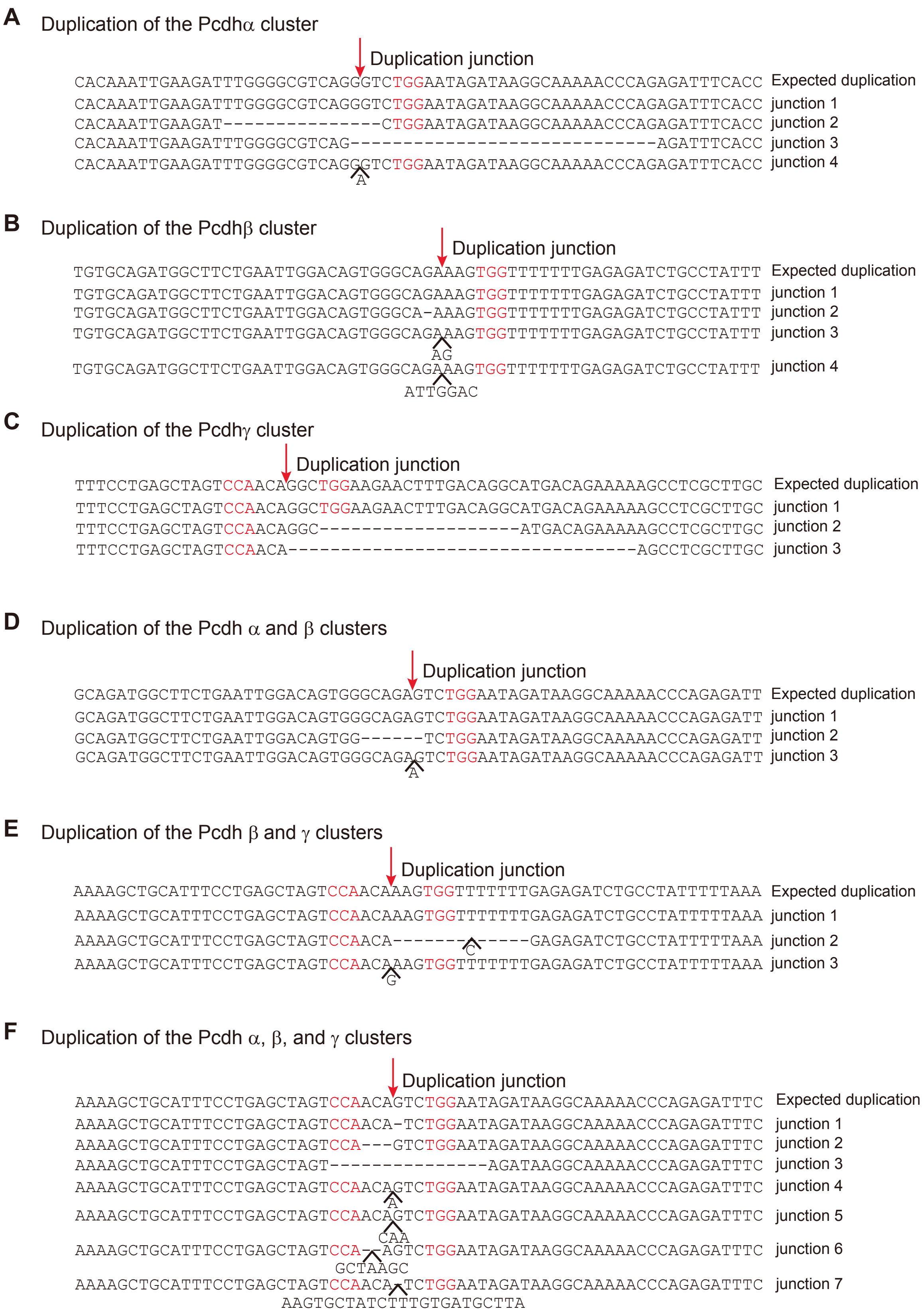
Supplementary Figure S8. Targeted deletions of seven additional DNA fragments of different sizes. (*A*) Deletion of the *Pcdh* RE2. The deletion junctions are identified by PCR with specific primer pairs. The PCR product is cloned and sequenced. An example of the sequence chromatograms at the deletion 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.



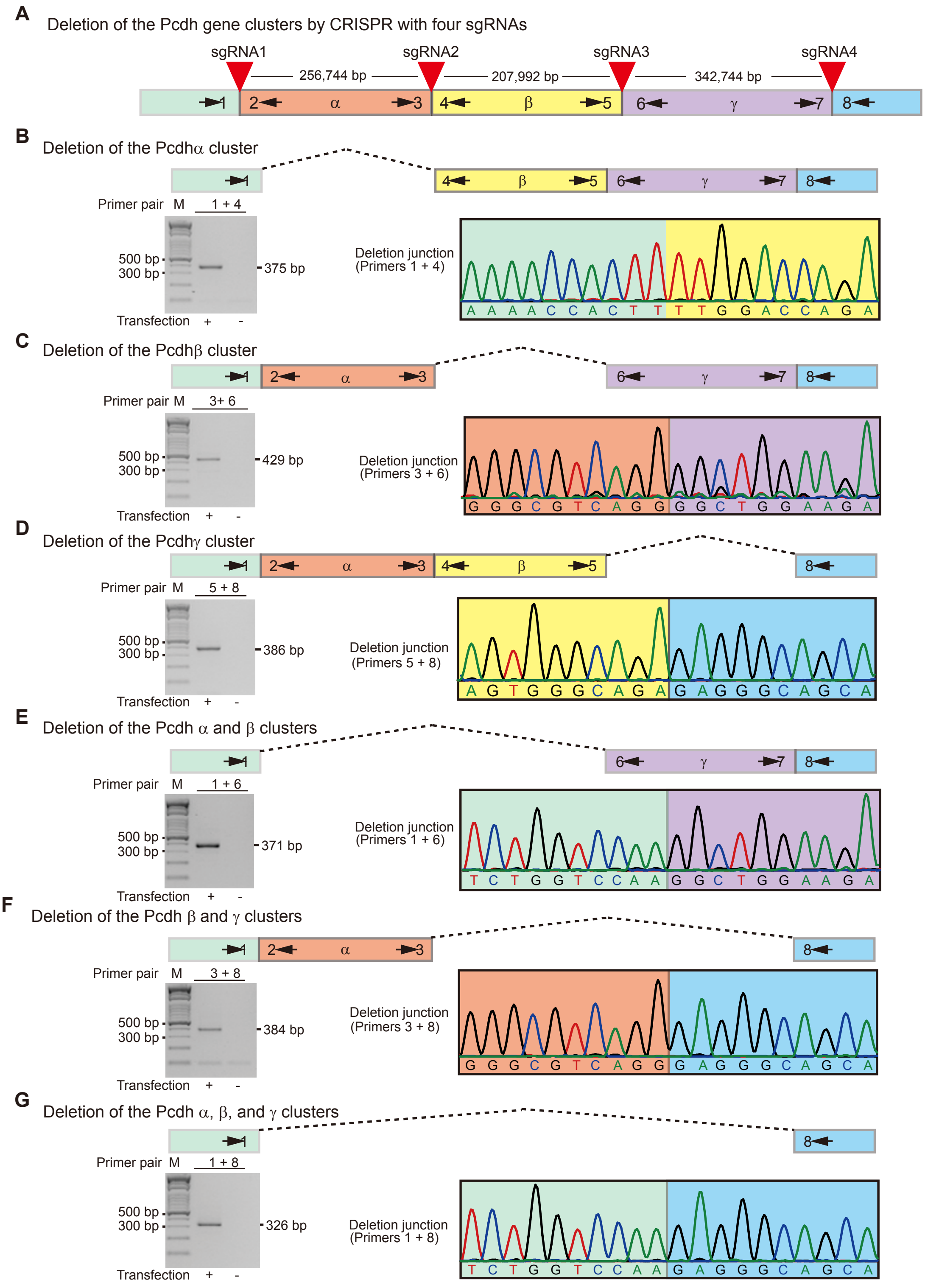
Supplementary Figure S9. Sequences of deletion junctions of the seven additional DNA fragments. Shown are sequences of deletion junction of a 35-bp DNA fragment of the *Pcdh* RE2 (*A*), of 709-bp (*B*) and 6,277-bp (*C*) DNA fragments at the *β-globin* locus, of an 18,142-bp DNA fragment at the *HoxD*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



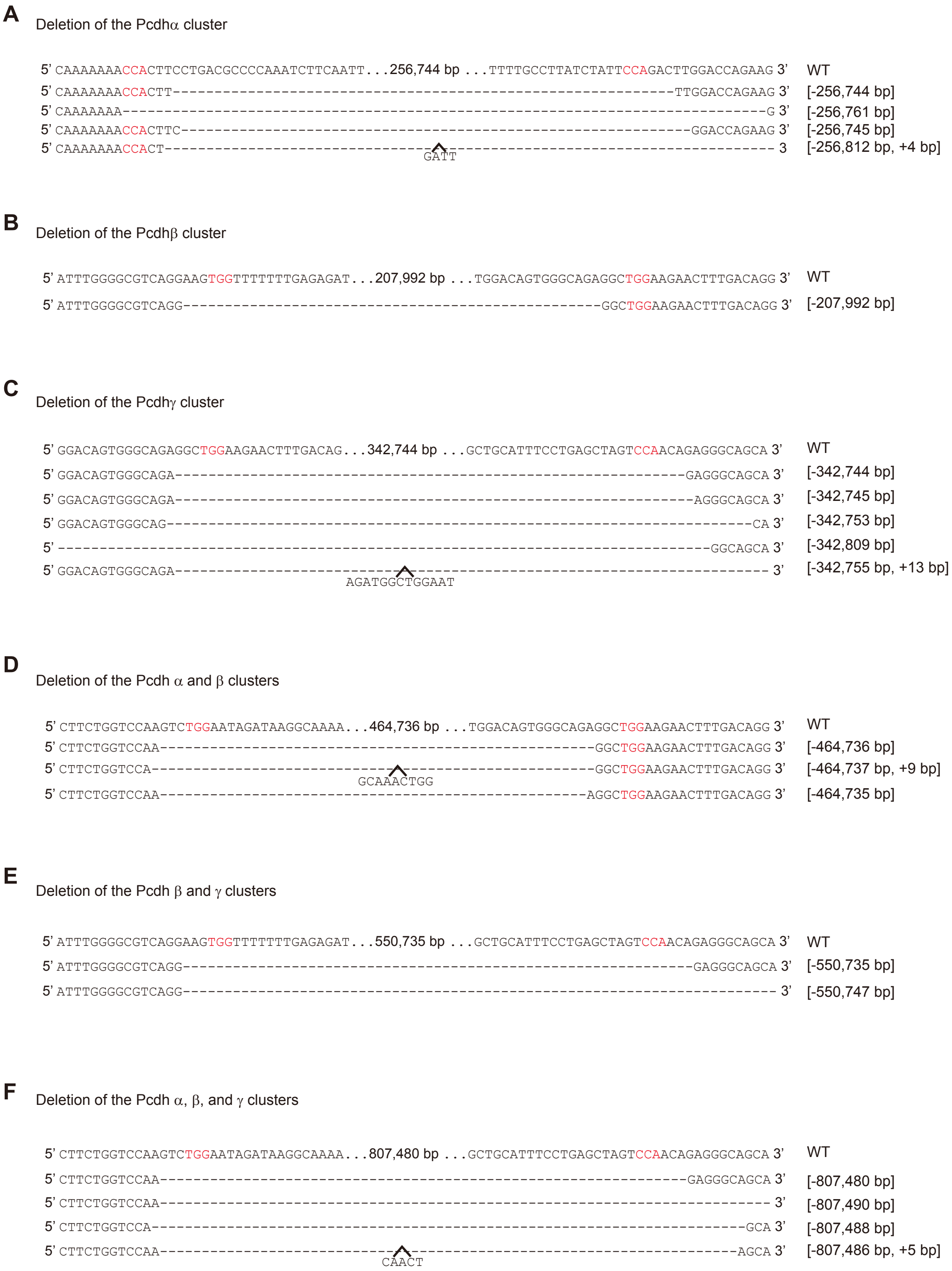
Supplementary Figure S10. Sequences of the upstream and downstream junctions of combinatorial inversions of the *Pcdh α*, *β*, and *γ* gene clusters induced by CRISPR with four sgRNAs. Shown are the sequences of the upstream and downstream inversion junctions of the *Pcdh α* (*A*), *β* (*B*), *γ* (*C*),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



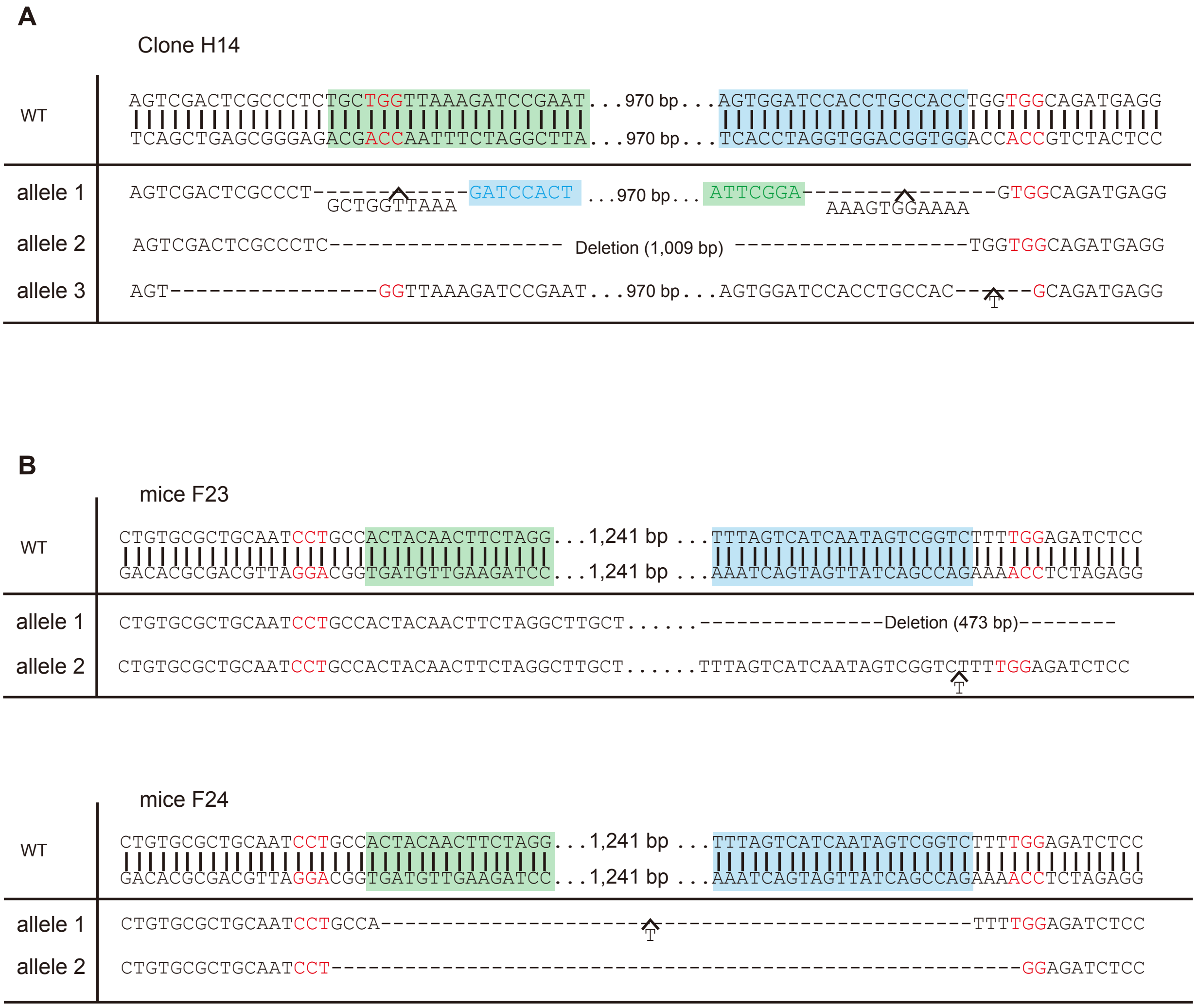
Supplementary Figure S11. Sequences of duplication junctions of combinatorial duplications of the *Pcdh α*, *β*, and *γ* gene clusters induced by CRISPR with four sgRNAs. Shown are the sequences of the duplication junctions of the *Pcdh α* (*A*), *β* (*B*), *γ* (*C*), *α*/*β* (*D*), *β*/*γ* (*E*), and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



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.



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
Pcdh RE1	sgRNA1	F	ACCGCCACACATCCAAGGCTGAC
		R	AAACGTCAGCCTTGGATGTGTGG
	sgRNA2	F	ACCGAGATTTGGGGCGTCAGGAAG
		R	AAACCTTCCTGACGCCCCAAATCT
Pcdh RE2	sgRNA1	F	ACCGGACAGCGACACCGCCCAGTT
		R	AAACAACTGGGCGGTGTCGCTGTC
	sgRNA2	F	ACCGGTGGCCATGGTGCTGAACTC
		R	AAACGAGTTCAGCACCATGGCCAC
β-globin RE1	sgRNA1	F	ACCGATTGTTGTTGCCTTGGAGTG
		R	AAACCACTCCAAGGCAACAACAAT
	sgRNA2	F	ACCGCTGGTCCCCTGGTAACCTGG
		R	AAACCCAGGTTACCAGGGGACCAG
β-globin RE2	sgRNA1	F	ACCGACCCAATGACCTCAGGCTGT
		R	AAACACAGCCTGAGGTCATTGGGT
	sgRNA2	F	ACCGTCACTTGTTAGCGGCATCTG
		R	AAACCAGATGCCGCTAACAAGTGA
HoxD locus	sgRNA1	F	ACCGGGGGCGAGGGGTTGGTCTT
		R	AAACAAGACCAACCCCTCGCCCC
	sgRNA2	F	ACCGACCTCGAAAGTCATAAACC
		R	AAACGGTTTATGACTTTCGAGGT
β-globin locus	sgRNA1	F	GCTTCAACACTGCCATCTCCGTTTTAGAGCTAGAAATAG
		R	GGAGATGGCAGTGTTGAAGCGGTGTTTCGTCCTTTCCAC
	sgRNA2	F	GAACTACTTCTGACCCCTAGGTTTTAGAGCTAGAAATAG
		R	CTAGGGGTCAGAAGTAGTTCGGTGTTTCGTCCTTTCCAC
Pcdhα cluster	sgRNA1	F	ACCGGCTTCTTCTGGTCCAAGTC
		R	AAACGACTTGGACCAGAAGAAGC
	sgRNA2	F	ACCGAGATTTGGGGCGTCAGGAAG
		R	AAACCTTCCTGACGCCCCAAATCT
Pcdhα, *β*, and *γ* clusters	sgRNA1	F	ACCGGCTTCTTCTGGTCCAAGTC
		R	AAACGACTTGGACCAGAAGAAGC
	sgRNA2	F	GTGGTTGTTGCTGCCCTCTGTGTTTTAGAGCTAGAAATAG
		R	ACAGAGGGCAGCAACAACCACGGTGTTTCGTCCTTTCCAC
Pcdh clusters with four sgRNAs	sgRNA1	F	ACCGGCTTCTTCTGGTCCAAGTC
		R	AAACGACTTGGACCAGAAGAAGC
	sgRNA2	F	ACCGAGATTTGGGGCGTCAGGAAG
		R	AAACCTTCCTGACGCCCCAAATCT
	sgRNA3	F	ACCGATTGGACAGTGGGCAGAGGC
		R	AAACGCCTCTGCCCACTGTCCAAT
	sgRNA4	F	GTGGTTGTTGCTGCCCTCTGTGTTTTAGAGCTAGAAATAG

		R	ACAGAGGGCAGCAACAACCACGGTGTTTCGTCCTTTCCAC
m*Pcdh* locus 1	sgRNA1	F	TAATACGACTCACTATAGGGCAGCTGTCTCGCCCTCTGCGTTTTAGAGCTAGAAATAG
		R	AAAAGCACCGACTCGGTGCC
	sgRNA2	F	TAATACGACTCACTATAGGATCGAACACCAGGTGGCAGAGTTTTAGAGCTAGAAATAG
		R	AAAAGCACCGACTCGGTGCC
m*Pcdh* locus 2	sgRNA1	F	TAATACGACTCACTATAGGAGCCTAGAAGTTGTAGTGGCGTTTTAGAGCTAGAAATAG
		R	AAAAGCACCGACTCGGTGCC
	sgRNA2	F	TAATACGACTCACTATAGGGTCATCAATAGTCGGTCTTTGTTTTAGAGCTAGAAATAG
		R	AAAAGCACCGACTCGGTGCC
m*Pcdh* locus 3	sgRNA1	F	TAATACGACTCACTATAGGGACAGACATAGTCGCTTTGCCGTTTTAGAGCTAGAAATAG
		R	AAAAGCACCGACTCGGTGCC
	sgRNA2	F	TAATACGACTCACTATAGGGCTAAGAGAGGCCGATACGTTTTAGAGCTAGAAATAG
		R	AAAAGCACCGACTCGGTGCC
Pcdh enhancer	sgRNA1	F	GCAGTCGACTCGCCCTCTGCGTTTTAGAGCTAGAAATAG
		R	GCAGAGGGCGAGTCGACTGCGGTGTTTCGTCCTTTCCAC
	sgRNA2	F	GTGGATCCACCTGCCACCTGGGTTTTAGAGCTAGAAATAG
		R	CCAGGTGGCAGGTGGATCCACGGTGTTTCGTCCTTTCCAC

Primers used for identifying inversions, duplications, and deletions

DNA fragment		Direction	Sequence
Pcdh RE1	Del	F1	TTCATCCCCGCTTCCTACTG
		R2	CACTCTGATAGTTTATGTATTAGGCTTG
	InvF	F1	TTCATCCCCGCTTCCTACTG
		F2	CTTGGAACCAGTTGGGATTG
	InvR	R1	TTCCGTACCATATGGATTGCTT
		R2	CACTCTGATAGTTTATGTATTAGGCTTG
	Dup	F2	CGAGTCATGGGACCGAACTG
		R1	TAACCAGCAGAGGGCGAGTC
Pcdh RE2	Del	F1	CTTGGAACCAGTTGGGATTG
		R2	TTATCAATAGCATTTTCCTCATCTG
	InvF	F1	CAAATGTAGTCCGTCCCAGTG
		F2	GTCGCTGTCCGTGGCCAT
	InvR	R1	CATGGCCACGGACAGCGAC
		R2	TTATCAATAGCATTTTCCTCATCTG
β-globin RE1	Del	F1	GTTCCTCCTTGTATTAACAGACCTG
		R2	CAGCTCTGCCTGAAAGGAGTC
	InvF	F1	GTTCCTCCTTGTATTAACAGACCTG

		F2	AGATTGAGTTCTGTTTGTTTCATCTAC
	InvR	R1	TTTTATGAAAGCAGCCTCTTGAG
		R2	CAGCTCTGCCTGAAAGGAGTC
β-globin RE2	Del	F1	AGGTTGAATGAATGCGTGACTG
		R2	GCACAGCCCTGCTCTATTACG
	InvF	F1	CTGCCTCTTTATGGGTCTAATGTAC
		F2	CTGGTCCTGATTCTTCCTCACTC
	InvR	R1	AGAGCCACCAGTCCACAGATC
		R2	GCACAGCCCTGCTCTATTACG
	Dup	F2	ATAGCAATGAAATCTTGAAGGAGTG
		R1	ACGCAGGAGCCGTATCATG
HoxD locus	Del	F1	GTTGTGGTTCTCCGTTGCTTC
		R2	CCCAGGCATAGAGACTCACTCG
	InvF	F1	GTTGTGGTTCTCCGTTGCTTC
		F2	ACCGCCCTTCACTGTTGATC
	InvR	R1	TCCGCTCACCAGATAACTACG
		R2	CATCCTATTTTCGAGGAGCTGC
β-globin locus	Del	F1	GATGTACTGTT TCACCTCCACTTG
		R2	TGTTCTTACCTCTTCAGCCATC
	InvF	F1	TAAAGCCAGCCATTTCTAAGG
		F2	ATTCCCTGTGTGATTACTTGCTTAC
	InvR	R1	CAAATAAGTGAGAACGAGATAAACC
		R2	TGTTCTTACCTCTTCAGCCATC
	Dup	F2	CAGTGGAGTTTTTGCTGTTCTTAG
		R1	CAAATAAGTGAGAACGAGATAAACC
Pcdhα cluster	Del	F1	TGCTAGGCTGCCTATTTCCTG
		R2	TTTTTGGCTAACAACATAGTGCTTC
	InvF	F1	TGCTAGGCTGCCTATTTCCTG
		F2	CTTGGAACCAGTTGGGATTG
	InvR	R1	GAGAACATTGTCAACATTTGGAAGT
		R2	TTTTTGGCTAACAACATAGTGCTTC
	Dup	F2	CTTGGAACCAGTTGGGATTG
		R1	GAGAACATTGTCAACATTTGGAAGT
Pcdh α, *β*, and *γ* clusters	Del	F1	GGATTCCTCATTAACAGCTTTGC
		R2	CTGTTTGAGTGTTGTATTGGATTC
	InvF	F1	GGATTCCTCATTAACAGCTTTGC
		F2	CCCATGTTTTAGTGTTCAATTTGG
	InvR	R1	GAAAGCCTAAATCCTTGTTTACTTG
		R2	CTGTTTGAGTGTTGTATTGGATTC
	Dup	F2	CCCATGTTTTAGTGTTCAATTTGG
		R1	GAAAGCCTAAATCCTTGTTTACTTG
m*Pcdh* locus 1	Del	F1	CAATGGAGGCAATCCCTTCTG
		R2	GGGTGGTAGTGAGGGATTATTCTAG

	InvF (blastocysts)	F1	AGGTACTGAGCAGCCCTGTATCC
		F2	GTTCCTTTGTCAGGTGAAAATCTC
	InvR (blastocysts)	R1	AAACGAATTGCCTCCTCTGC
		R2	GGGTGGTAGTGAGGGATTATTCTAG
	InvF (mice)	F1	CAATGGAGGCAATCCCTTCTG
		F2	GTTCCTTTGTCAGGTGAAAATCTC
	InvR (mice)	R1	AGGTAGAGCTAAAGACTCGTGGTG
		R2	GGGTGGTAGTGAGGGATTATTCTAG
	Dup (mice)	F2	GTTCCTTTGTCAGGTGAAAATCTC
		R1	AGGTAGAGCTAAAGACTCGTGGTG
		F1	AGGTACTGAGCAGCCCTGTATCC
		R2	TGACCATCAAGATTGCTTTCACA
m*Pcdh* locus 2	Del	F1	CAATGGAGGCAATCCCTTCTG
		R2	GGGTGGTAGTGAGGGATTATTCTAG
	InvF	F1	AGGTACTGAGCAGCCCTGTATCC
		F2	GTTCCTTTGTCAGGTGAAAATCTC
	InvR	R1	AGGTAGAGCTAAAGACTCGTGGTG
		R2	GGGTGGTAGTGAGGGATTATTCTAG
m*Pcdh* locus 3	Del	F1	TATTCTTCAGCCCTGCAACC
		R2	ACAGGACACTACTCCGTGGT
	InvF	F1	AGCCAGACCAGCATAGCAAAT
		F2	ACAGGATAATGGGTTCTGGAGC
	InvR	R1	CCAAGCCCACAGTCGTTGAT
		R2	TCCAGATTACGAGCTGAGCG

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

Pcdh clusters with four sgRNAs	*Pcdhα* cluster	Del	1	TGCTAGGCTGCCTATTTCCTG
			4	TTTTTGGCTAACAACATAGTGCTTC
		InvF	1	TGCTAGGCTGCCTATTTCCTG
			3	CTTGGAACCAGTTGGGATTG
		InvR	2	GAGAACATTGTCAACATTTGGAAGT
			4	TTTTTGGCTAACAACATAGTGCTTC
		Dup	3	CTTGGAACCAGTTGGGATTG
			2	GAGAACATTGTCAACATTTGGAAGT
	Pcdhβ cluster	Del	3	CTTGGAACCAGTTGGGATTG
			6	GTTTCTCCAACATGCTCCTCAC
		InvF	3	CTTGGAACCAGTTGGGATTG
			5	GACTTGTCCAGCCACGTTTCA
		InvR	4	TTTTTGGCTAACAACATAGTGCTTC
			6	GTTTCTCCAACATGCTCCTCAC
		Dup	5	GACTTGTCCAGCCACGTTTCA
			4	TTTTTGGCTAACAACATAGTGCTTC
	Pcdhγ cluster	Del	5	AAGCAGAGTTGAGTCTTCCGTG

			8	CTGTTTGAGTGTTGTATTGGATTC
		InvF	5	AAGCAGAGTTGAGTCTTCCGTG
			7	CCCATGTTTTAGTGTTCAATTTGG
		InvR	6	TCCTCATTTCTGCACCCTCAC
			8	CTGTTTGAGTGTTGTATTGGATTC
		Dup	7	CCCATGTTTTAGTGTTCAATTTGG
			6	GTTTCTCCAACATGCTCCTCAC
	Pcdh α and β clusters	Del	1	GGATTCCTCATTAACAGCTTTGC
			6	GTTTCTCCAACATGCTCCTCAC
		InvF	1	TGCTAGGCTGCCTATTTCCTG
			5	GACTTGTCCAGCCACGTTTCA
		InvR	2	GAGAACATTGTCAACATTTGGAAGT
			6	GTTTCTCCAACATGCTCCTCAC
		Dup	5	GACTTGTCCAGCCACGTTTCA
			2	GAAAGCCTAAATCCTTGTTTACTTG
	Pcdh β and γ clusters	Del	3	CTTGGAACCAGTTGGGATTG
			8	CTGTTTGAGTGTTGTATTGGATTC
		InvF	3	CTTGGAACCAGTTGGGATTG
			7	CCCATGTTTTAGTGTTCAATTTGG
		InvR	4	TTTTTGGCTAACAACATAGTGCTTC
			8	CTGTTTGAGTGTTGTATTGGATTC
		Dup	7	CCCATGTTTTAGTGTTCAATTTGG
			4	TTTTTGGCTAACAACATAGTGCTTC
	Pcdh α, β, and γ clusters	Del	1	GGATTCCTCATTAACAGCTTTGC
			8	CTGTTTGAGTGTTGTATTGGATTC
		InvF	1	GGATTCCTCATTAACAGCTTTGC
			7	CCCATGTTTTAGTGTTCAATTTGG
		InvR	2	GAAAGCCTAAATCCTTGTTTACTTG
			8	CTGTTTGAGTGTTGTATTGGATTC
		Dup	7	CCCATGTTTTAGTGTTCAATTTGG
			2	GAAAGCCTAAATCCTTGTTTACTTG

Primers used for quantitative PCR for measuring efficiency

Targeted locus		Direction	Sequence
β-*globin* RE1	Del	F1	GTTCCTCCTTGTATTAACAGACCTG
		R2	CAGCTCTGCCTGAAAGGAGTC
	Inv	F1	GTTCCTCCTTGTATTAACAGACCTG
		F2	AGATTGAGTTCTGTTTGTTTCATCTAC
	WT	F2	AGATTGAGTTCTGTTTGTTTCATCTAC
		R2	CAGCTCTGCCTGAAAGGAGTC
Pcdh RE1	Del	F1	TTCATCCCCGCTTCCTACTG
		R2	TTTTTGGCTAACAACATAGTGCTTC
	Inv	R1	TTCCGTACCATATGGATTGCTT

		R2	TTTTTGGCTAACAACATAGTGCTTC
	Dup	F2	CGAGTCATGGGACCGAACTG
		R1	TAACCAGCAGAGGGCGAGTC
	WT	F1	TTCATCCCCGCTTCCTACTG
		R1	TTCCGTACCATATGGATTGCTT
β-globin RE2	Del	F1	AGGTTGAATGAATGCGTGACTG
		R2	GCACAGCCCTGCTCTATTACG
	Inv	F1	CTGCCTCTTTATGGGTCTAATGTAC
		F2	CTGGTCCTGATTCTTCCTCACTC
	Dup	F2	ATAGCAATGAAATCTTGAAGGAGTG
		R1	ACGCAGGAGCCGTATCATG
	WT	F2	CTGGTCCTGATTCTTCCTCACTC
		R2	GCACAGCCCTGCTCTATTACG
HoxD locus	Del	F1	GTTGTGGTTCTCCGTTGCTTC
		R2	CCCAGGCATAGAGACTCACTCG
	Inv	F1	GTTGTGGTTCTCCGTTGCTTC
		F2	ACCGCCCTTCACTGTTGATC
	WT	F1	TGAAAGAACGTTTGCCACCTC
		R1	GAAGGGAACCTGCTCCGAA
β-globin locus	Del	F1	GATGTACTGTTTCACCTCCACTTG
		R2	TGTTCTTACCTCTTCAGCCATC
	Inv	F1	TAAAGCCAGCCATTTCTAAGG
		F2	CAGTGGAGTTTTTGCTGTTCTTAG
	Dup	F2	CAGTGGAGTTTTTGCTGTTCTTAG
		R1	CAAATAAGTGAGAACGAGATAAACC
	WT	F1	GATGTACTGTTTCACCTCCACTTG
		R1	CAAATAAGTGAGAACGAGATAAACC
Pcdhα cluster	Del	F1	GGATTCCTCATTAACAGCTTTGC
		R2	TTTTTGGCTAACAACATAGTGCTTC
	Inv	R1	GAGAACATTGTCAACATTTGGAAGT
		R2	TTTTTGGCTAACAACATAGTGCTTC
	Dup	F2	CTTGGAACCAGTTGGGATTG
		R1	GAGAACATTGTCAACATTTGGAAGT
	WT	F1	GGATTCCTCATTAACAGCTTTGC
		R1	GAGAACATTGTCAACATTTGGAAGT
Pcdh α, *β*, and *γ* clusters	Del	F1	GGATTCCTCATTAACAGCTTTGC
		R2	CTGTTTGAGTGTTGTATTGGATTC
	Inv	F1	GGATTCCTCATTAACAGCTTTGC
		F2	CCCATGTTTTAGTGTTCAATTTGG
	Dup	F2	CCCATGTTTTAGTGTTCAATTTGG
		R1	GAAAGCCTAAATCCTTGTTTACTTG
	WT	F2	CCCATGTTTTAGTGTTCAATTTGG
		R2	CTTGTCATGATCTAGGAACTTATTC

Primers used for quantitative PCR for measuring efficiency with four sgRNAs

Pcdh clusters with four sgRNAs	*Pcdh*α cluster	Del	F1	GGATTCCTCATTAACAGCTTTGC
			R2	TTTTTGGCTAACAACATAGTGCTTC
		Inv	R1	GAGAACATTGTCAACATTTGGAAGT
			R2	TTTTTGGCTAACAACATAGTGCTTC
		Dup	F2	CTTGGAACCAGTTGGGATTG
			R1	GAGAACATTGTCAACATTTGGAAGT
		WT	F1	GGATTCCTCATTAACAGCTTTGC
			R1	GAGAACATTGTCAACATTTGGAAGT
	Pcdhβ cluster	Del	F1	CGAGTCATGGGACCGAACTG
			R2	GTTTCTCCAACATGCTCCTCAC
		Inv	F1	CTTGGAACCAGTTGGGATTG
			F2	GACTTGTCCAGCCACGTTTCA
		Dup	F2	GACTTGTCCAGCCACGTTTCA
			R1	TTTTTGGCTAACAACATAGTGCTTC
		WT	F2	GACTTGTCCAGCCACGTTTCA
			R2	GTTTCTCCAACATGCTCCTCAC
	Pcdhγ cluster	Del	F1	GACTTGTCCAGCCACGTTTCA
			R2	CTTGTCATGATCTAGGAACTTATTC
		Inv	R1	TCCTCATTTCTGCACCCTCAC
			R2	CTGTTTGAGTGTTGTATTGGATTC
		Dup	F2	CCCATGTTTTAGTGTTCAATTTGG
			R1	GTTTCTCCAACATGCTCCTCAC
		WT	F1	GACTTGTCCAGCCACGTTTCA
			R1	GTTTCTCCAACATGCTCCTCAC
	Pcdh α and β clusters	Del	F1	AAGTATTGGGATTCCTCATTAACAG
			R2	TCCTCATTTCTGCACCCTCAC
		Inv	F1	TGCTAGGCTGCCTATTTCCTG
			F2	GACTTGTCCAGCCACGTTTCA
		Dup	F2	GACTTGTCCAGCCACGTTTCA
			R1	GAGAACATTGTCAACATTTGGAAGT
		WT	F2	GACTTGTCCAGCCACGTTTCA
			R2	GTTTCTCCAACATGCTCCTCAC
	Pcdh β and γ clusters	Del	F1	CGAGTCATGGGACCGAACTG
			R2	CTGTTTGAGTGTTGTATTGGATTC
		Inv	F1	CTTGGAACCAGTTGGGATTG
			F2	CCCATGTTTTAGTGTTCAATTTGG
		Dup	F2	CCCATGTTTTAGTGTTCAATTTGG
			R1	TTTTTGGCTAACAACATAGTGCTTC
		WT	F2	CCCATGTTTTAGTGTTCAATTTGG
			R2	CAAACAAGGAGAATGATGAAAGTGG
	Pcdh α, β, and γ	Del	F1	GGATTCCTCATTAACAGCTTTGC

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

Primers used for off-target analyses

DNA fragment	sgRNAs	Direction	Sequence
Pcdh RE1	sgRNA1	OTF	TCCTGGAACACGGATTTATGC
		OTR	TGTTCCTCCTTTCCTTTGTGAG
β-globin RE1	sgRNA1	OTF	CTCAACCTCCCAAGTTTGGATC
		OTR	GAGAGGTGATGTGGAGGTGAGTG
	sgRNA2	OTF	GCCTACTGTGACTGTGGCAATG
		OTR	CCACGCTACATTCACCCATC
β-globin RE2	sgRNA1	OTF	GGAGGGCTGGGAAGTGCA
		OTR	GGGCATCCATTGGTTGTCTG
	sgRNA2	OTF	CATTCAAGGGTAATAACTCACTGG
		OTR	CACCCTCAAACATTTCCACAAG
HoxD locus	sgRNA1	OTF	CAGAGCCTCCAGCAGTCCTAC
		OTR	GAGGCACCCATACCAGAAGC
	sgRNA2	OTF	AGAAGCGCCCATTGAAAGAG
		OTR	TTTCAGTTCCAGAATCCCGTC
β-globin locus	sgRNA1	OTF	CCATCTGGGGATAGGTTGTTC
		OTR	GCTTACGGTGTTTCCTCCATC
	sgRNA2	OTF	CATCCCATTCCTATCAACCAGG
		OTR	AAAATAGGCTGGAGCAAGTCC

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

Gene name	Direction	Sequence
hPcdhα6	F	GATGGGTAAGGCGGAGAATC
	R	AGGTCCAGCTGTTGCTGTTGAC
hPcdhα12	F	CAAGCCTTCAGCTGTCTCGA
	R	AGGTCCAGCTGTTGCTGTTGAC
hPcdhαc1	F	CTTGCCACTGGGGTAGGACTG
	R	AGGTCCAGCTGTTGCTGTTGAC
hPcdhαc2	F	AGGCCAAAGTGGTCAGAATG
	R	AGGTCCAGCTGTTGCTGTTGAC
hPcdhβ3	F	TGTCTTCAGTAAACAGCCCTATTC
	R	TCACTCTTCCGTTGTCTCCAG
hPcdhβ9	F	ATTCCAATGACAATCCTCCTG
	R	TAAAAACAGCCAATACTATCCCAG

hPcdhβ15	F	GCTCTGTCTCTGTTAAGGTGCTG
	R	GAGTCTCGGTCTCTAATCCTAAAC
hPcdhγb5	F	CGACTTCCCATCCTGAGTTG
	R	CGACTTCTTCTTGTTGCCATTG
hPcdhγa10	F	CCTTTGTCTTTGTTAGATGATTCG
	R	CGACTTCTTCTTGTTGCCATTG
hGAPDH	F	GGAGTCCACTGGCGTCTTCAC
	R	GCAGGAGGCATTGCTGATGAT

Supplementary Table S2. List of sgRNA targeting sequences

DNA fragment	sgRNA	Species	Chr	Genomic coordinates (hg19 or mm9)	Sequence
Pcdh RE1	sgRNA1	Human	5	140419774-140419793	GCCACACATCCAAGGCTGAC
	sgRNA2	Human	5	140421046-140421065	AGATTTGGGGCGTCAGGAAG
Pcdh RE2	sgRNA1	Human	5	140420832-140420851	GACAGCGACACCGCCCAGTT
	sgRNA2	Human	5	140420853-140420872	GTGGCCATGGTGCTGAACTC
β-globin RE1	sgRNA1	Human	11	4093748-4093767	ATTGTTGTTGCCTTGGAGTG
	sgRNA2	Human	11	4094457-4094476	CTGGTCCCCTGGTAACCTGG
β-globin RE2	sgRNA1	Human	11	4203109-4203128	ACCCAATGACCTCAGGCTGT
	sgRNA2	Human	11	4209386-4209405	TCACTTGTTAGCGGCATCTG
HoxD locus	sgRNA1	Human	2	176944510-176944529	GGGGGCGAGGGGTTGGTCTT
	sgRNA2	Human	2	176962653-176962672	GACCTCGAAAGTCATAAACC
β-globin locus	sgRNA1	Human	11	5145473-5145492	GCTTCAACACTGCCATCTCC
	sgRNA2	Human	11	5226196-5226215	GAACTACTTCTGACCCCTAG
Pcdh 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
m*Pcdh* locus 1	sgRNA1	Mouse	18	37217457-37217476	GCAGCTGTCTCGCCCTCTGC
	sgRNA2	Mouse	18	37218428-37218447	TCTGCCACCTGGTGTTCGAT
m*Pcdh* locus 2	sgRNA1	Mouse	18	37217321-37217340	GCCACTACAACTTCTAGGCT
	sgRNA2	Mouse	18	37218551-37218570	GTCATCAATAGTCGGTCTTT
m*Pcdh* locus 3	sgRNA1	Mouse	18	38011468-38011487	GGCAAAGCGACTATGTCTGT
	sgRNA2	Mouse	18	38040855-38040874	GGGCTAAGAGAGGCCGATAC
Pcdh 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
Pcdh RE1	sgRNA1	GCCACACATCCAAGGCTGACAGG	chr5:140419774-140419796
		G**TAGGC**CATCCAAGGCTGACAGG	chr5:73541775-73541797
		AGGA**G**A**A**ATCCAAGGCTGACAGG	chr16:29283392-29283414
		AGGA**G**A**A**ATCCAAGGCTGACAGG	chr16:88279138-88279160
	sgRNA2	AGATTTGGGGCGTCAGGAAGTGG	chr5:140421046-140421068
		not found	
β-globin RE1	sgRNA1	ATTGTTGTTGCCTTGGAGTGGGG	chr11:4093748-4093770
		TTT**TAC**GTTGCCTTGGAGTG**A**GG	chr3:194529587-194529609
		A**GCCCAT**TTGCCTTGGAGTG**T**GG	chr10:61769557-61769579
		CACAGCCTTGCCTTGGAGTG**A**GG	chr12:105794596-105794618
	sgRNA2	CTGGTCCCCTGGTAACCTGGTGG	chr11:4094457-4094479
		AGACATTCCTGGTAACCTGG**C**GG	chr4:10135619-10135641
β-globin RE2	sgRNA1	ACCCAATGACCTCAGGCTGTAGG	chr11:4203109-4203131
		TCC**A**A**GG**GACCTCAGGCTGT**G**GG	chr1:35225451-35225473
		A**GG**C**CGG**GACCTCAGGCTGT**G**GG	chr7:155175311-155175333
		TC**TTGG**TGACCTCAGGCTGT**G**GG	chr11:72538049-72538071
		TGTC**TC**TGACCTCAGGCTGTAGG	chr22:29823268-29823290
	sgRNA2	TCACTTGTTAGCGGCATCTGTGG	chr11:4209386-4209408
		T**AGGA**T**T**TTAGCGGCATCTGTGG	chr1:200714321-200714343
		AGGAT**G**GTTAGCGGCATCTGTGG	chr16:5293528-5293550
		CAA**AAA**GTTAGCGGCATCTGTGG	chr17:47146780-47146802
HoxD locus	sgRNA1	GGGGGCGAGGGGTTGGTCTTTGG	chr2:176944510-176944532
		ATCTG**GA**AGGGGTTGGTCTT**A**GG	chr1:227824834-227824856
		G**CCCA**C**A**AGGGGTTGGTCTT**G**GG	chr3:119470371-119470393
		CAAG**TG**GAGGGGTTGGTCTT**G**GG	chr15:90131233-90131255
		AG**ACC**C**C**AGGGGTTGGTCTT**G**GG	chr16:5612529-5612551
	sgRNA2	GACCTCGAAAGTCATAAACCCGG	chr2:176962653-176962675
		CA**GAGTC**AAAGTCATAAACC**T**GG	chr4:165699237-165699259
		AA**AGC**CGAAAGTCATAAACC**A**GG	chr18:103855897-103855919
		AAC**A**TC**A**AAAGTCATAAACC**A**GG	chrX:89642974-89642996
β-globin locus	sgRNA1	CCAGGAGATGGCAGTGTTGAAGC	chr11:5145473-5145495
		CC**G**GGAGATGGCAGTG**GCTGG**G**A**	chr1:15136268-15136290
		CCAGGAGATGGCAGTGTTG**TGCA**	chr1:64140255-64140277
		CCAGGAGATGGCAGTGTTG**TCCT**	chr1:175121340-175121362
		CC**C**GGAGATGGCAGTG**AGCCCTG**	chr1:227603760-227603782
		CC**T**GGAGATGGCAGTG**GACTTAT**	chr4:123855569-123855591
		CC**T**GGAGATGGCAGTGT**GTGT**G**T**	chr4:183176777-183176799
		CCAGGAGATGGCAGTG**GGA**AAG**T**	chr5:73832096-73832118
		CCAGGAGATGGCAGTG**GG**GAA**AA**	chr7:6546692-6546714
		CCAGGAGATGGCAGTGTT**TCT**G**A**	chr7:12428914-12428936

		CC**T**GGAGATGGCAGTG**GATCCCA**	chr10:20603816-20603838
		CCAGGAGATGGCAGTGTTGA**GA**C	chr12:70273334-70273356
		CCAGGAGATGGCAGTG**GCAGCCA**	chr16:88882589-88882611
		CC**T**GGAGATGGCAGTGTT**CG**AG**A**	chr17:71503217-71503239
	sgRNA2	CCACTAGGGGTCAGAAGTAGTTC	chr11:5226196-5226218
		CC**T**CTAGGGGTCAGAA**AGCC**TT**T**	chr3:73189890-73189912
		CCACTAGGGGTCAGAA**T**TA**CACA**	chr18:41710505-41710527
Pcdhα cluster	sgRNA1	GGCTTCTTCTGGTCCAAGTCTGG	chr5:140164302-140164324
		ATGGAGTTCTGGTCCAAGTCTGG	chr1:84516811-84516833
		GG**TAATA**TCTGGTCCAAGTCTGG	chr3:197017861-197017883
		CCATT**AA**TCTGGTCCAAGTC**A**GG	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
Pcdh RE1	sgRNA1	GTAGGCCATCCAAGGCTGACAGG	chr5:73541775-73541797	Not found
β-globin RE1	sgRNA1	TTTTACGTTGCCTTGGAGTGAGG	chr3:194529587-194529609	Not found
	sgRNA2	AGACATTCCTGGTAACCTGGCGG	chr4:10135619-10135641	Not found
β-globin RE2	sgRNA1	TGTCTCTGACCTCAGGCTGTAGG	chr22:29823268-29823290	Not found
	sgRNA2	AGGATGGTTAGCGGCATCTGTGG	chr16:5293528-5293550	Not found
HoxD locus	sgRNA1	AGACCCCAGGGGTTGGTCTTGGG	chr16:5612529-5612551	Not found
	sgRNA2	AACATCAAAAGTCATAAACCAGG	chrX:89642974-89642996	Not found
β-globin locus	sgRNA1	CCAGGAGATGGCAGTGTTGAGAC	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 (%)	*Pcdhα* cluster	*Pcdhβ* cluster	*Pcdhγ* cluster	*Pcdh α* and *β* clusters	*Pcdh β* and *γ* clusters	*Pcdh α, β,* and *γ* 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 (%)
β-globin RE1	709 bp	78	38	48.72	0	0	46	58.97
Pcdh enhancer	1,272 bp	32	4	12.50	ND	/	12	37.50
β-globin RE2	6,277 bp	62	31	50.00	1	1.61	45	72.58
HoxD locus	18,142 bp	112	1	0.89	ND	/	4	3.57

ND: not determined.