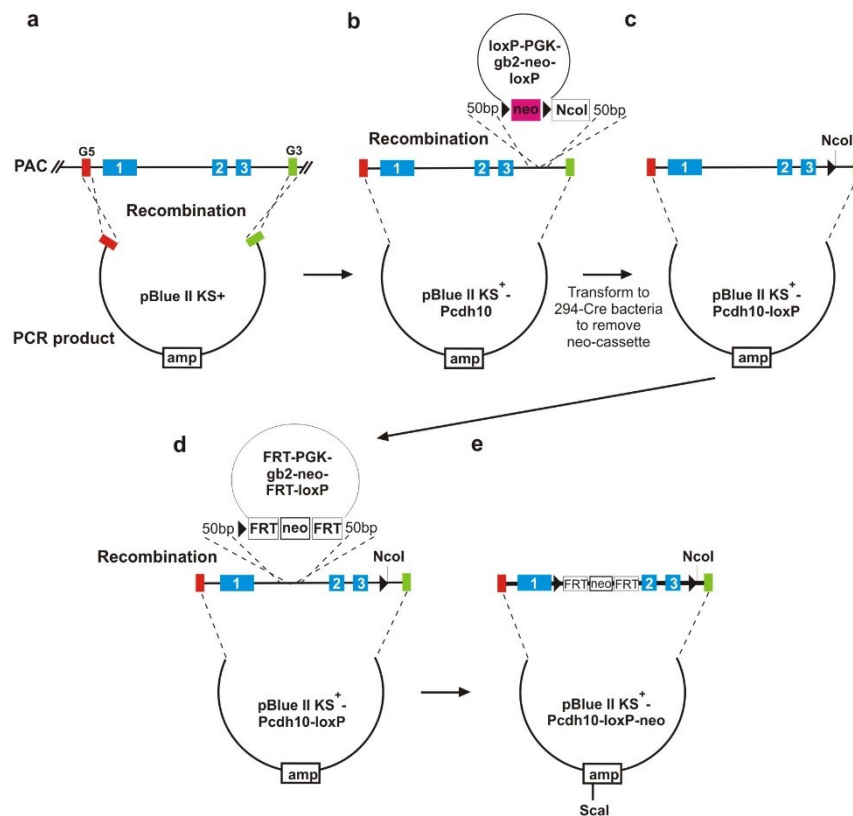


Strategy for conditional knockout of the long isoforms of the *Pcdh10* allele

PAC clone RP21-402C3 (MRC Geneservice, UK) served as genomic start material. The targeting vector was constructed by following the Red/ET recombination protocols K003 and K005 from Gene Bridges GmbH (Heidelberg, Germany). Briefly, a fragment of PAC clone RP21-402C3, comprising exons 1, 2 and 3 of the *Pcdh10* gene, was subcloned into the pBluescript II KS⁺ vector (see below: Additional Fig. S2 and Table S2). Then, a first loxP site was inserted downstream of exon 3, followed by insertion upstream of exon 2 of a neo-resistance cassette, flanked by FRT sites and preceded by a second loxP site. The final targeting vector (Additional Fig. S2e) was linearized with *ScaI* before electroporation of ES cells. All vectors used and generated were quality verified by restriction analysis and DNA sequencing.



Additional Fig. S2 Schematic representation of the recombineering strategy of the *Pcdh10* long targeting construct. Numbered blue blocks represent mouse *Pcdh10* exons. All PCR primers used are listed in Additional Table S2. **(a)** To subclone a fragment from a PAC into pBluescript II KS⁺, two oligonucleotides were designed, which have 50 bases homologous to the PAC fragment while 20 bases are used as PCR primer. **(b)** To insert the first loxP site, two oligonucleotides were designed for PCR amplification of a resistance gene (neo) flanked by two loxP sites and a unique restriction enzyme site (*NcoI*). The unique restriction site is designed for use in Southern screening of ES cells, ensuring that the 3' loxP site is inserted. **(c)** The plasmid was transformed to 294-Cre bacteria to remove the neo resistance gene and to obtain a single loxP site and a unique restriction enzyme site downstream of exon 3. **(d)** To insert the second loxP site and neo-cassette, two oligonucleotides were designed for PCR amplification of a neo resistance cassette flanked by two FRT sites and a loxP site. **(e)** The final targeting vector contains a unique restriction enzyme site (*ScaI*) for plasmid linearization.

Additional Table S2 Recombineering primers used for generation of the the *Pcdh10*long targeting construct.

Genomic subcloning	forward	5'TGAGATTTCCCTGTAAATAATCAGTGCATCCACTGTAACACTTGAGCCGCat agacggttttcgcccttgac-3'
	reverse	5'TTACTGATAAACTGGTGAAATACTCAGGCCTTATTCGAGAGACCTCACTc ccagctttgtcccttagtga-3'
First loxP insertion	forward	5'GCATACAAAGTATCACTGGAGAAGGGGATGATGTTAAAAGTCACAAATG Gtcccggcggattgtcctactcaggagagc-3'
	reverse	5'CTATTACTAATGTTATGAAGCCCACAGACACCTGGACTGCAAACTAAAC CCATGG tctgcaaaccctatgctactcgcgc-3'
loxP-FRT-neo-FRT insertion	forward	5'GGAATTGCCTACTGTTGGCTTCTTTCTTCAGCAAAGAATTTGGCTTGTCAcc cctaccggtagaattcgcgcac-3'
	reverse	5'ACCCTCCAACCTTCCACTATGTCTCTACCTTTAAAAAGAAATTGAAATTcc cggcggattgtcctactcaggagagc-3'

Uppercase sequences are 50 bases homologous to flanking genomic DNA of *Pcdh10* for Red-recombination. Lowercase sequences are primers homologous to the plasmid DNA to be amplified. Bold italic uppercase sequences correspond to the *Nco*I (CCATGG) consensus sequence.