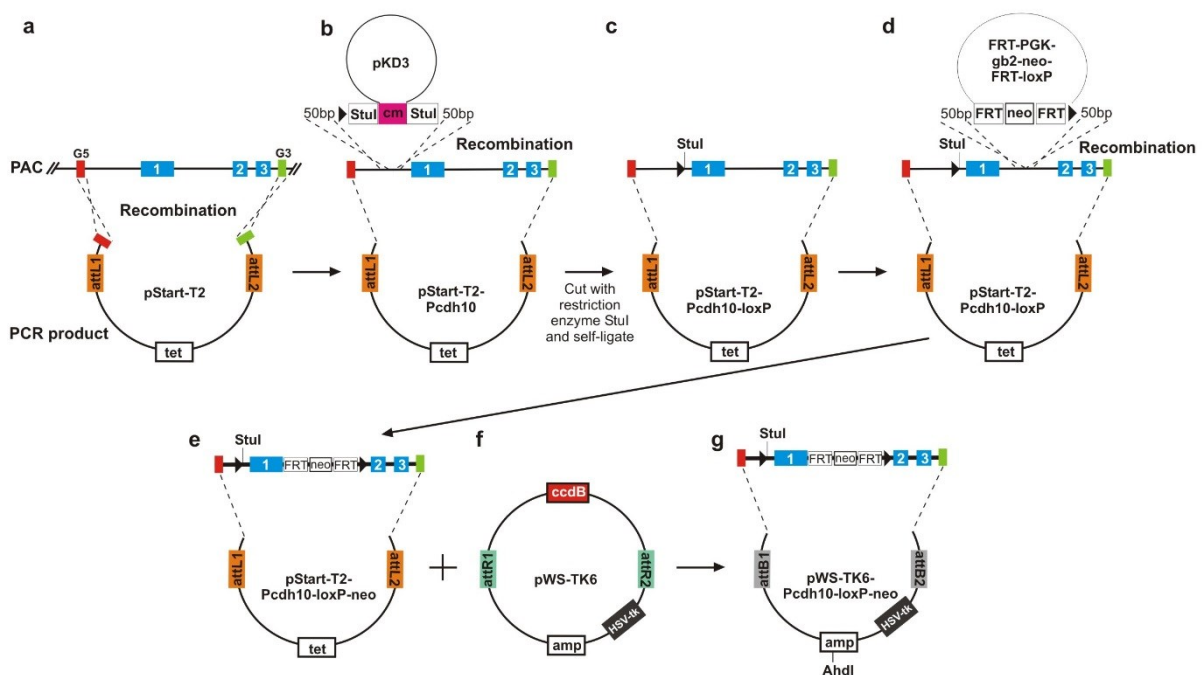


Strategy for conditional knockout of all isoforms of the *Pcdh10* allele

Cloning of the targeting vector was by recombineering on the basis of the Red/ET recombination system (1, 2). PAC clone RP21-402C3 (MRC Geneservice, UK) served as genomic start material. A fragment comprising exons 1, 2 and 3 of the *Pcdh10* gene was subcloned into the Gateway-compatible vector pStart-T2 (see below: Additional Fig. S1 and Table S1). Thereafter, a first loxP site was inserted upstream of exon 1, followed by insertion into intron 1 of a neo-resistance cassette, flanked by FRT sites and followed by the second loxP site. Finally, by Gateway recombination, the negative selection cassette HSV-tk was introduced downstream of the genomic insert. The final targeting vector (Additional Fig. S1g) was linearized with *AhdI* before electroporation of ES cells. All vectors used and generated were quality verified by restriction analysis and DNA sequencing.



Additional Fig. S1 Schematic representation of the recombineering strategy of the *Pcdh10* targeting construct. Numbered blue blocks represent mouse *Pcdh10* exons. All PCR primers used are listed in Additional Table S1. **(a)** To subclone a fragment from PAC clone RP21-402C3 into pStart-T2, two oligonucleotides were designed, which have 50 bp homology to the PAC fragment and 20 bp specific for the pStart-T2 plasmid. These oligos were used as PCR primer. **(b)** To insert the first loxP site, two oligonucleotides were designed for PCR amplification of a chloramphenicol resistance gene (cm) flanked by two unique restriction enzyme sites (*StuI*) and a loxP site (black triangle). The unique restriction site was designed for use in Southern screening of ES cells, ensuring that the 5' loxP site was inserted. **(c)** The plasmid was cut with the restriction enzyme *StuI* and self-ligated to remove the cm resistance gene and to obtain a single loxP site and a unique restriction enzyme site upstream of exon 1. **(d)** To insert the second loxP site and a neo-resistance cassette, two oligo-nucleotides were designed for PCR amplification of a neo-resistance cassette flanked by two FRT sites and a loxP site. **(e)** With the resulting plasmid, a Gateway recombination step is performed with the pWS-TK6 plasmid **(f)** to add a negative selection cassette (HSV-tk). **(g)** The final targeting vector contains a unique restriction enzyme site (*AhdI*) for plasmid linearization.

Additional Table S1 Recombineering primers used for generation of the *Pcdh10*all targeting construct.

Genomic subcloning	forward	5'CTTTTAGTAAGTGATGTTTTGCCACCCATATTAATGTTTTGTAGTATAAA gccgcactcgagatatctagacca-3'
	reverse	5'TATCTGAAATTTATTTGAATTTGAGGTATGGTCTCACTATGTTGTCCA cgactgaattggtcctttaagc-3'
First loxP insertion	forward	5'TTTAGTAACCAAGCATTGTGGGTAGACATTAGATGGGTAGACGTTCA AataacttcgtatagcatattatacgaagttatAGGCCT agcattacagctctgagcgattgt-3'
	reverse	5'CAATCAAAGCTAGAGGGTCAGAAGGGGAGGTGTGGGGAGGATAGACC AGG AGGCCT cacttaacggctgacatgggaatta-3'
FRT-neo-FRT-loxP insertion	forward	5'CAGACTATGATTAGAGTAATTTATGGTAGACGCAACAGTTTATTAAAC Ctgtcctactcaggagagcg-3'
	reverse	5'TTGCTGCCCCAGAGAAAATGTGTCTTATTTAAAAGGAGAATGCAAAAA AAacccggtagaattcgtcgac-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 *StuI* (AGGCCT) consensus sequence. The bold lowercase sequence is the loxP site.

References

1. Wu S, Ying G, Wu Q, Capecchi MR. A protocol for constructing gene targeting vectors: generating knockout mice for the cadherin family and beyond. *Nat Protoc.* 2008;3:1056-76.
2. Fu J, Bian X, Hu S, Wang H, Huang F, Seibert PM, et al. Full-length RecE enhances linear-linear homologous recombination and facilitates direct cloning for bioprospecting. *Nat Biotechnol.* 2012;30:440-6.