iScience, Volume *26*

Supplemental information

Pcdh19 mediates olfactory sensory neuron coalescence during postnatal stages and regeneration

Andrew P. Martinez, Alexander C. Chung, Suihong Huang, Adam J. Bisogni, Yingxin Lin, Yue Cao, Eric O. Williams, Jin Y. Kim, Jean Y.H. Yang, and David M. Lin

Supplemental Titles and Legends

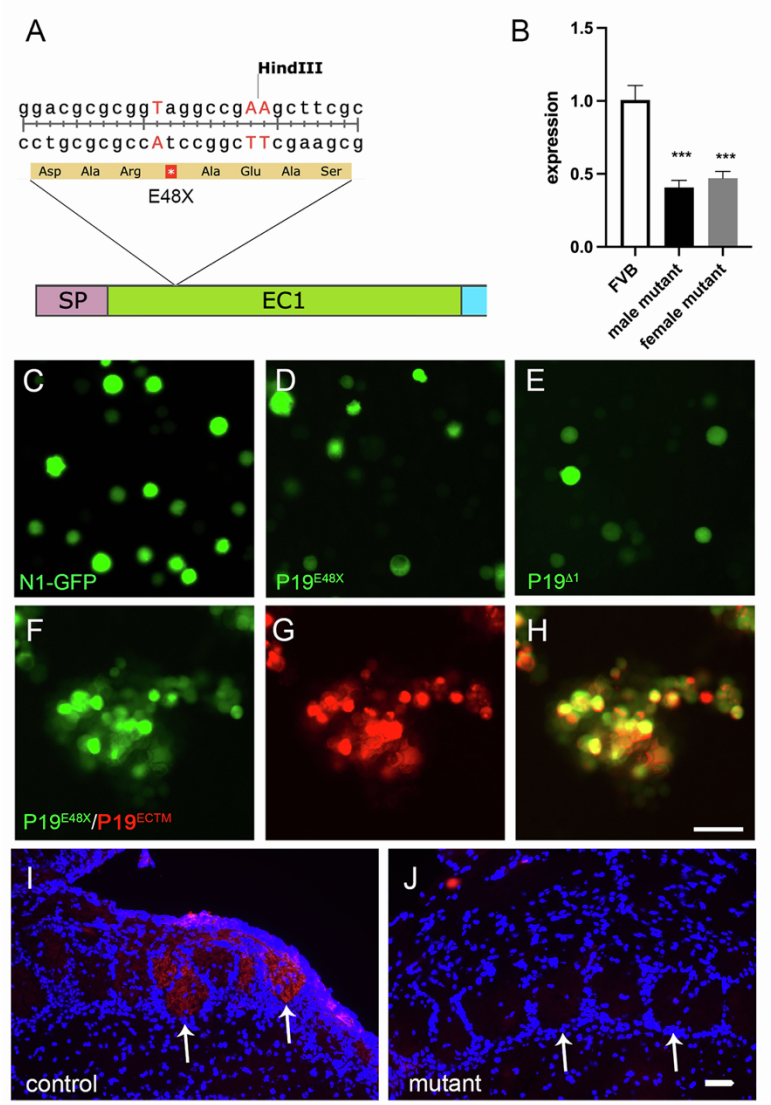

Figure S1. Generation of *Pcdh19*^{E48X} mutant mice, related to Figures 2-5. (A) CRISPR was used to introduce a known stop mutation (*Pcdh19*^{E48X}) in humans[24] into the corresponding position in mouse. A HindIII site was introduced downstream of the nonsense mutation for genotyping purposes. (B) Quantitative reverse transcriptase PCR was used to show reduced expression in both male hemizygous and female homozygous mice relative to controls, consistent with nonsense mediated decay. (C-H) K562 cells were transfected with constructs bearing a control N1-GFP plasmid (C), or *Pcdh19*^{E48X}-GFP (D). Both did not form aggregates. (E) Transfection of a construct that deleted the first extracellular domain (*Pcdh19*$^{\Delta 1}$-GFP) also showed no aggregation. Co-transfection of cells with *Pcdh19*^{E48X}-GFP and a construct containing the *Pcdh19* extracellular and transmembrane domains fused to RFP showed cells were able to aggregate (F-H). (I,J) PCDH19 antibody on bulbar tissue sections from control (I) and *Pcdh19*^{E48X} mutant mice (J) showed no detectable expression in mutant mice (J) as compared to control samples (arrows in (I)). Scale bar: 50 μm in all images. One way ANOVA, $F(2,6)=22.96$, Dunnett's test. ***$p<0.005$.

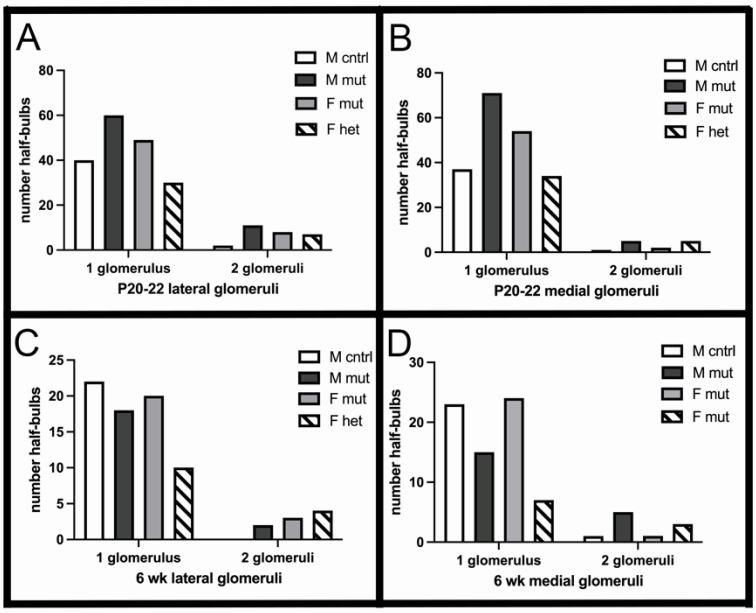

Figure S2, Related to Figure 2. No significant effects on MOR28 glomeruli are seen in *Pcdh19*^{E48X} mice at P20-22 or 6 weeks of age. (A,B) No statistically significant differences are observed at P20-22. (C,D) No statistically significant differences were observed at 6 weeks of age. Fisher's exact test with Bonferroni correction. n=20 (M cntrl), 40 (M mut), 30 (F mut) and 20 (F het) for P20-22 mice, and n=12 (M cntrl), 10 (M mut), 13 (F mut), and 7 (F het) for 6 wk mice.

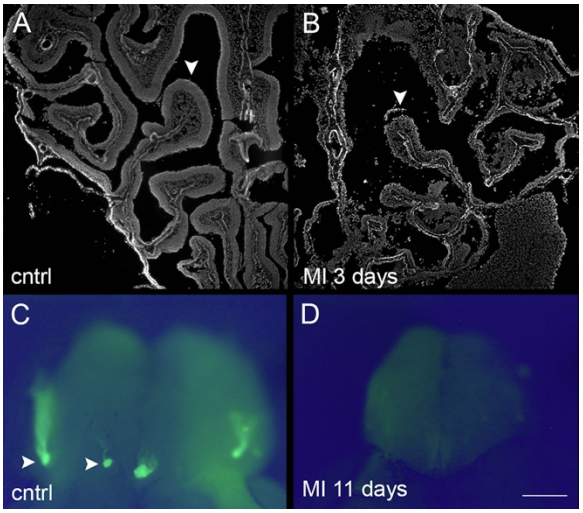

Figure S3. Methimazole treatment severely disrupts epithelial structure and coalescence, related to Figures 3-5. Epithelia from (A) saline and (B) methimazole treated mice isolated three days after injection results in severe disruption of the epithelium (compare arrowheads; DAPI stained sections). (C,D) Glomeruli (arrowheads) are not clearly observed eleven days after treatment. Scale bar: 800 μm (A,B) and 1 mm (C,D).

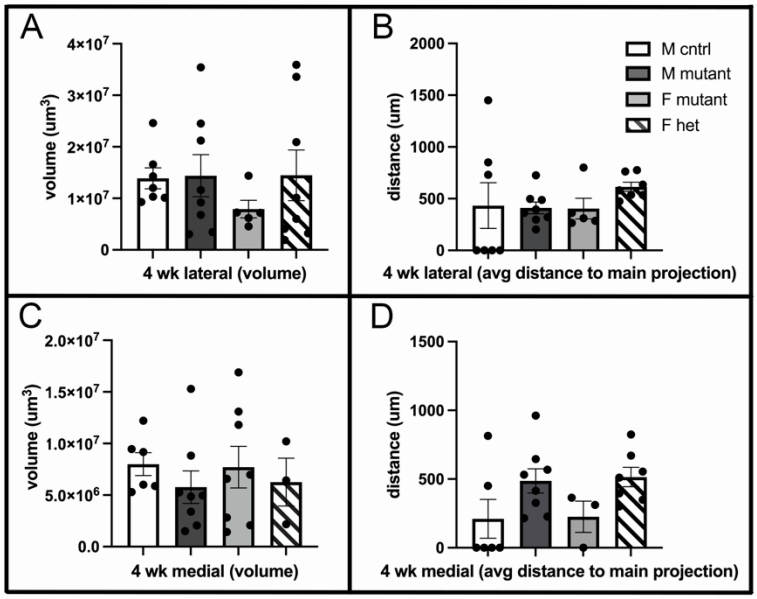

Figure S4, Related to Figures 3 and 4. Volume and spread of MOR28 projection patterns in *Pcdh19*^{E48X} mice are similar to control. No significant differences in the overall volume of the initial projection region (A,C) or spread of signal (B,D) within the region are seen in lateral (A,B) or medial (C,D) glomeruli at four weeks post treatment.

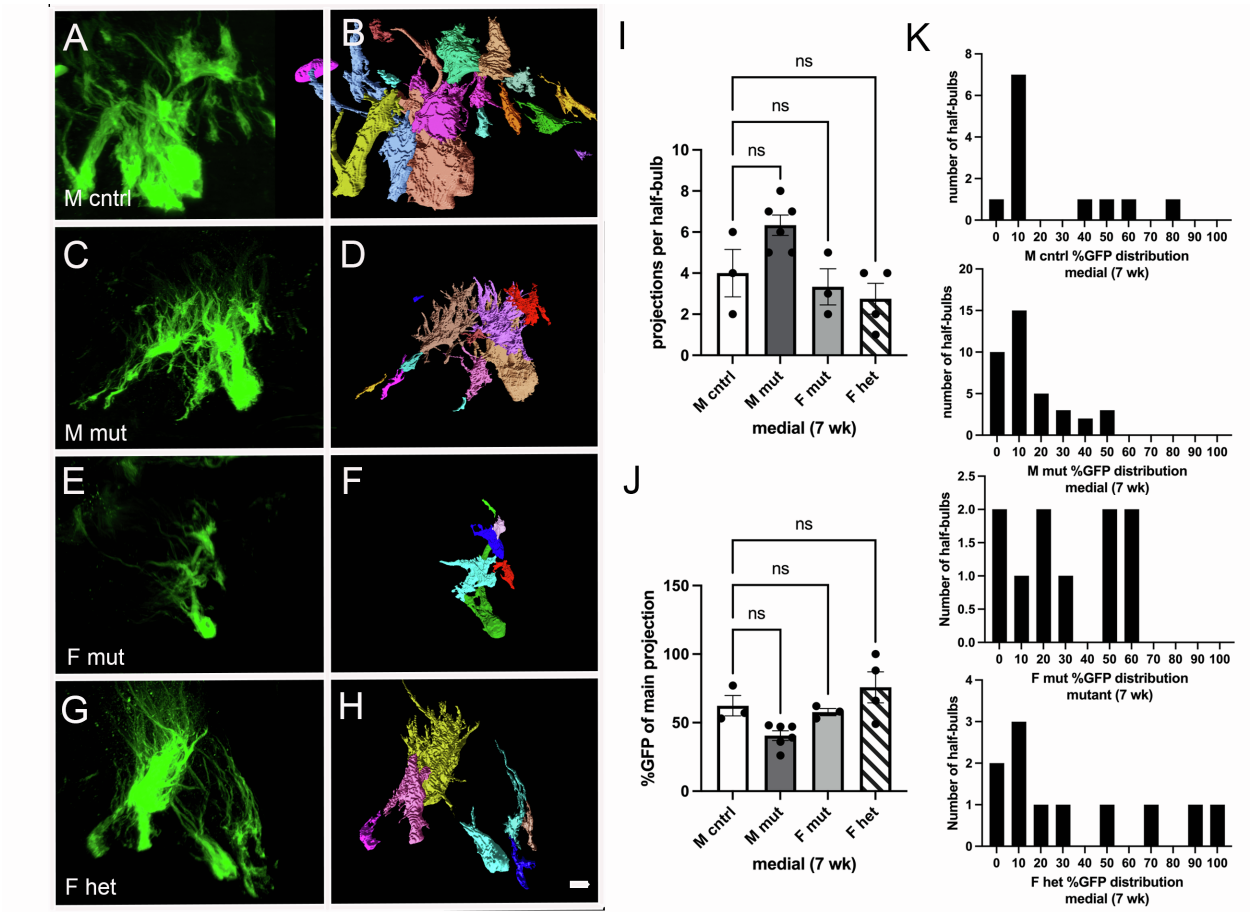

Figure S5, Related to Figure 5. The *Pcdh19*^{E48X} mutation does not affect medial MOR28 coalescence at 7 weeks. A-H) Projection of image stack obtained by light sheet microscopy (A,C,E,G) and segmented to define projection patterns on the medial surface (B,D,F,H). I) The number of lateral projections per half-bulb. J) The percent GFP present in the main projection. K) Histogram of distribution of GFP expression within each projection (greater than 3%) in the various genotypes. Scale bar: 100 μm. n=3 (M cntrl), 6 (M mut), 3 (F mut) and 4 (F het).

Supplemental Table 1: qPCR primers used to validate single cell data, related to Figures 6 and 8 and STAR Methods

gene	5' flanking primer	3' flanking primer	5' nested primer	3' nested primer
pcdh1	ggcaacccttatgggctct	gacgtaaaggtggactaaggctg	gatcacattcgggagccatcac	ctgaccttcaccacgaggcg
pcdh8	gcgggtcagctgtgcc	ggtggcgatggcgatg	ccacccggctctcgc	cagctcccggctaacacg
pcdh9	tatttctttatgtgaacgacactgctgg	catggccccagcaacgat	ttgatccgtaggactatggagac	atggtgagatagtcctcgttctga
pcdh17	gacgagtacaatgtgaccattgtgg	ctagcactgacccgaggtattct	tccacttaactccaccaagtcctt	tggagcacgtaaagtcctttgg
pcdh19	accacccacacttctccaag	aggtgaagactggcatgtctcg	ggtgcctatctgctctcagtg	gtgatggcacaatttgataggagac
pcdhga7	cctctacctggtggtggcagtg	ccgtccaaacctacaaagtgtgac	ttattggctctcaggctgtgg	ggcacatcttccaggccac
dsg1a	agaggacctgcacgggg	gatcctgtgacgcgttgt	cccttcagaggacctgcac	ctgccaccactttctgaaccag
pcdhga6	tccaggaagagccatctgatctt	gccagtcagtgttgggcg	gtcaggagggctgtgagaaaa	tgctgatcaagagagtcttcctcttt
pcdhga11	cgaagtttcgctcactgcagac	cggggcttgctgaatatctgt	cttcccgcagcccaacta	gagtcttcagttatcaggagaggct