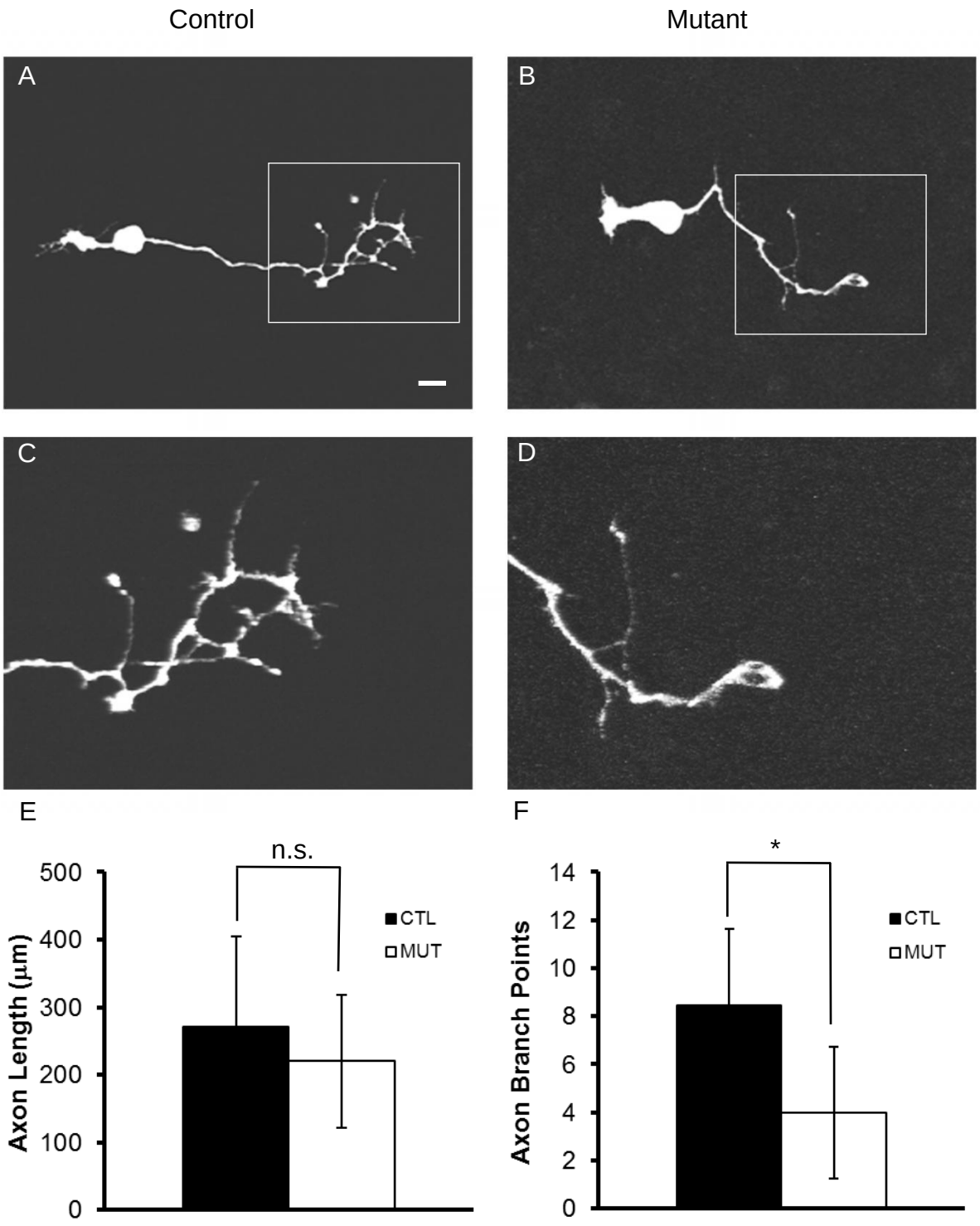
Supplemental Material:

Supplemental Figure 1.



Supplemental Figure 1. ***In vitro*** **culture of dissociated OSNs from newborn mice.**

A, B) Confocal images show individual GFP labeled OSNs from control (Gγ8-tTA/TetO-GFP) and mutant (Gγ8-tTA/TetO-FAF1) mice, respectively, following 3 days in culture. **C, D)** Higher magnification view of boxed regions from A and B show branching of axon terminals in both control (C) and mutant (D) OSNs. **E, F)** Graphs illustrate that while the length of the axons from the mutant OSNs (220.0 ± 95.9; mean ± SD; n=22) show no significant difference (p=0.153; t=1.549) compared to control OSNs (271.5 ± 133.7; mean ± SD; n=21), the number of axonal branch points in mutant OSNs (4.0 ± 2.7; mean ± SD; n=22) is significantly reduced (*p<0.001; t=7.439) compared to controls (8.4 ± 3.2; mean ± SD; n=21).

Supplemental Materials and Methods

Mice

Gγ8-tTA/TetO-FAF1 mutant mice were generated as described in main text. Gγ8-tTA/TetO-GFP control mice were generated by crossing Gγ8-tTA driver mice with TetO-GFP reporter mice as previously described (Nguyen et al., 2007). In each case the TetO driven transgene is expressed specifically in immature OSNs.

Dissociated OSN Cultures

Primary OSN cultures were generated from P0 mice as previous published (Chen et al., 2008). Briefly, olfactory epithelial (OE) tissue was dissected from the nasal cavity of either mutant or control mice and incubated in 20μg/ml papain (Worthington, NJ), then washed and triturated in Waymouth's MB 752/1 medium with N2 supplement. Dissociated tissue was then filtered spun at 150 x g for 10 min, and resuspended in Waymouth's MB 752/1 medium with N2 supplement (Invitrogen, Carlsbad, CA, USA). Dissociated cells were then plated at a density of 1×10^4 cells/cm^2 on a confluent astrocyte feeder layer. Dissociated OSNs were maintained in Waymouth's MB 752/1 medium with N2 supplement for up to 3 days.

Cortical astrocytes feeder layer

Primary astrocytes were isolated from P0 ~ P2 mouse neocortex and cultured according to a published protocol (Weinstein, 2008). Briefly, P0 ~ P2 cortex was dissected from mice and digested with 1% trypsin /0.1% DNase for 15 minutes at 37^o C, then washed and triturated in 0.05% DNase, spun at 150 x g for 10 min and resuspended in 1 ml of a 1:1 mixture of DNase solution and PBS glucose /MgSO4. Dissociated cells were then added to a Percoll gradient, spun 150 x g for 10 min, and the astrocyte band was extracted with a Pasteur pipet. Extracted cells were again resuspended in PBS/glucose, spun at 150 x g for 10 min and resuspended in DMEM/10% FBS/ 2% glucose.

Cells were counted and plated at 2.5~5 x 10^4 cells/cm^2 on Poly –D –lysine and laminin coated coverslips (BD Biosciences, CA). Astrocytes culture were maintained in DMEM/10% FBS/ 2% glucose until used.

Images and Quantification

Fixed cultures were imaged for GFP fluorescence (excitation 488, emission 507) using a Zeiss LSM-510-Meta confocal (Carl Zeiss, Inc., NY) and 0.5mm optical sections. 3D-reconstructions of individual cells were traced to determine: axonal lengths, defined as longest continuous axonal segment starting at cell body and extending outward without crossing back on itself; and branch points defined as axonal splits producing branches >3μm. Measurement were compared using student T-test with statistical significance determined as $p<0.001$.

References

Nguyen MQ, Zhou Z, Marks CA, Ryba NJ, Belluscio L. Prominent roles for odorant receptor coding sequences in allelic exclusion. *Cell*. 2007 Nov 30;131(5):1009-17.

Chen H, Dadsetan S, Fomina AF, Gong Q. Expressing exogenous functional odorant receptors in cultured olfactory sensory neurons. *Neural Dev.* 2008 Sep 11;3:22.

Weinstein DE. Isolation and purification of primary rodent astrocytes. *Curr Protoc Neurosci.* 2001 May; Chapter 3:Unit 3.5.