## Supplementary Materials and Methods

*Mice*

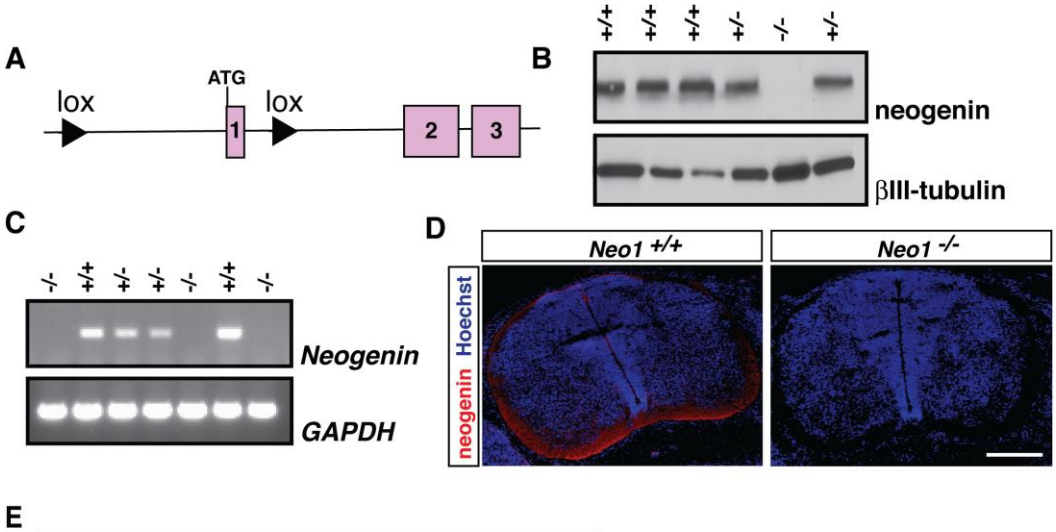
We generated *Neo1* mutant mice by standard homologous recombination methods to introduce two loxP sites flanking the first exon of the *Neo1* allele, which encodes the signal sequence. Following germline transmission, heterozygous mice (*Neo1*$^{+/lox}$) were crossed to FlpE mice to excise the Neomycin cassette used for G-418 selection of targeted ES cell clones. The *Neo1*$^{+/lox}$ mice were crossed with CMV-Cre mice to obtain germline deletion of the floxed *neogenin* allele (*Neo1*$^{+/-}$). Those heterozygous mice were bred together to obtain *neogenin* mutant mice.

We generated *Rgm-b* mutant mice by standard homologous recombination methods to introduce a tau-GFP cassette, 3' UTR and polyA signal into the first exon of the *Rgm*-b gene, in frame with the ATG start site but deleting the endogenous amino acid signal sequence of *Rgm-b*. In this way, tau-GFP is expressed under the control of endogenous *Rgm-b* regulatory sequences, but Rgm-b cannot follow the secretory pathway to reach the cell surface. Heterozygous mice (*Rgm-b*$^{+/-}$) mice were bred with FlpE mice to remove the neomycin cassette.

*Reverse transcription polymerase chain reaction (RT-PCR)*

Brains were isolated from E16.5 *Neo1*$^{+/+}$, *Neo1*$^{+/-}$, and *Neo1*$^{-/-}$ embryos. RNA was purified using the Qiazol reagent (Qiagen) followed by the RNeasy Lipid Tissue Mini Kit (Qiagen). DNase treatment was performed using DNase I (Invitrogen). cDNA was generated using the SuperScript II reverse transcriptase system (Invitrogen). PCR oligonucleotides for *Gapdh*, 5′-GCATCCTGCACCACCAACTG-3′ and 5′-CGGCCGCCTGCTTCACCACCTTCT-3′; *Neogenin Primers A, 5'-*

GAGCCCTCTCCAAACATTGA- 3, and 5'- TCAACAATGCAGCGGTAGAG- 3'; *Neogenin Primers C*, 5′- CCTCTGAAGAGCTTCGCTGT -3′ and 5′- TCCTGTACTTCAGGGGCACT -3′. PCR reactions were conducted for 30 cycles using the GoTaq system (Promega).




E

| Age(Total number of embryos) | *Neo1* $^{+/+}$ | *Neo1* $^{+/-}$ | *Neo1* $^{-/-}$ |
|---|---|---|---|
| E11 (22 from 3 litters) | 6 (27%) | 12 (55%) | 4 (18%) |
| E14 (124 from 17 litters) | 29 (24%) | 62 (50%) | 33 (26%) |
| E16 (181 from 26 litters) | 39 (22%) | 99 (55%) | 43 (23%) |
| P0 (30 from 4 litters) | 8 (27%) | 20 (67%) | 2 (7%) |

**Fig. S1: Generation and characterization of a floxed *Neogenin* mutant.**

(A) Diagram of the *Neo1* targeted allele containing two LoxP sites inserted 5' and 3' of exon 1. (B) Immunoblots on brain lysates collected from littermates of a cross between two *Neo1*$^{+/-}$ mice. Neogenin protein expression is abolished in *Neo1*$^{-/-}$ embryos. (C) RT-PCR analysis on brain mRNA samples isolated from control and *Neo1*$^{-/-}$ embryos. *Neogenin* mRNA expression is abolished in *Neo1*$^{-/-}$ embryos. (D) Immunolabeling of spinal cord sections from E16.5 embryos with a neogenin antibody. Neogenin signal is absent in *Neo1*$^{-/-}$ embryos. (E) Table indicating the number of *Neo1*$^{+/+}$, *Neo1*$^{+/-}$, and *Neo1*$^{-/-}$ embryos obtained in litters at different time points in embryonic development. None of the *neo1*$^{-/-}$ mice survive past P0. Scale bar: 200 μm.

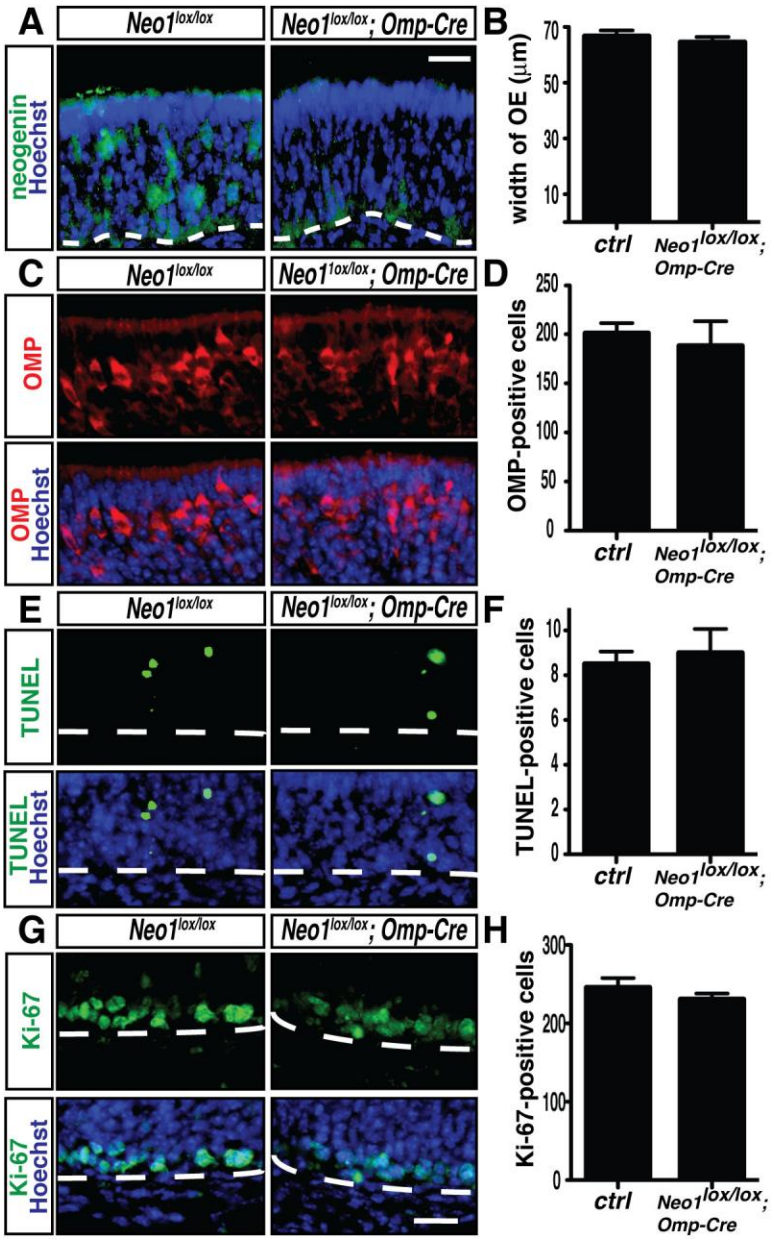



**Fig. S2: Deletion of Neogenin from OMP expressing cells does not alter ORN generation and survival.**

(A) Immunolabeling of OE sections from E16.5 embryos with a neogenin antibody. OMP-Cre mediated deletion of neogenin in the OE. The expression of neogenin is

abolished in mature ORNs but not in basal cells of the OE in E16.5 *Neo1*$^{lox/lox}$*;Omp-Cre* embyos. (B) Quantification of the width of OE shows comparable OE width between control and mutant littermates. (C-H) Immunolabeling of OE sections from E16.5 embryos reveal that the number of OMP- (C,D), TUNEL- (E,F) and Ki-67 (G,H)-positive cells is unchanged in *Neo1*$^{lox/lox}$*;Omp-Cre* embryos. All cell counts are represented as cells per 2.5 mm of OE. Students unpaired t-test was performed to compare values between groups. Scale bar: 100 μm.

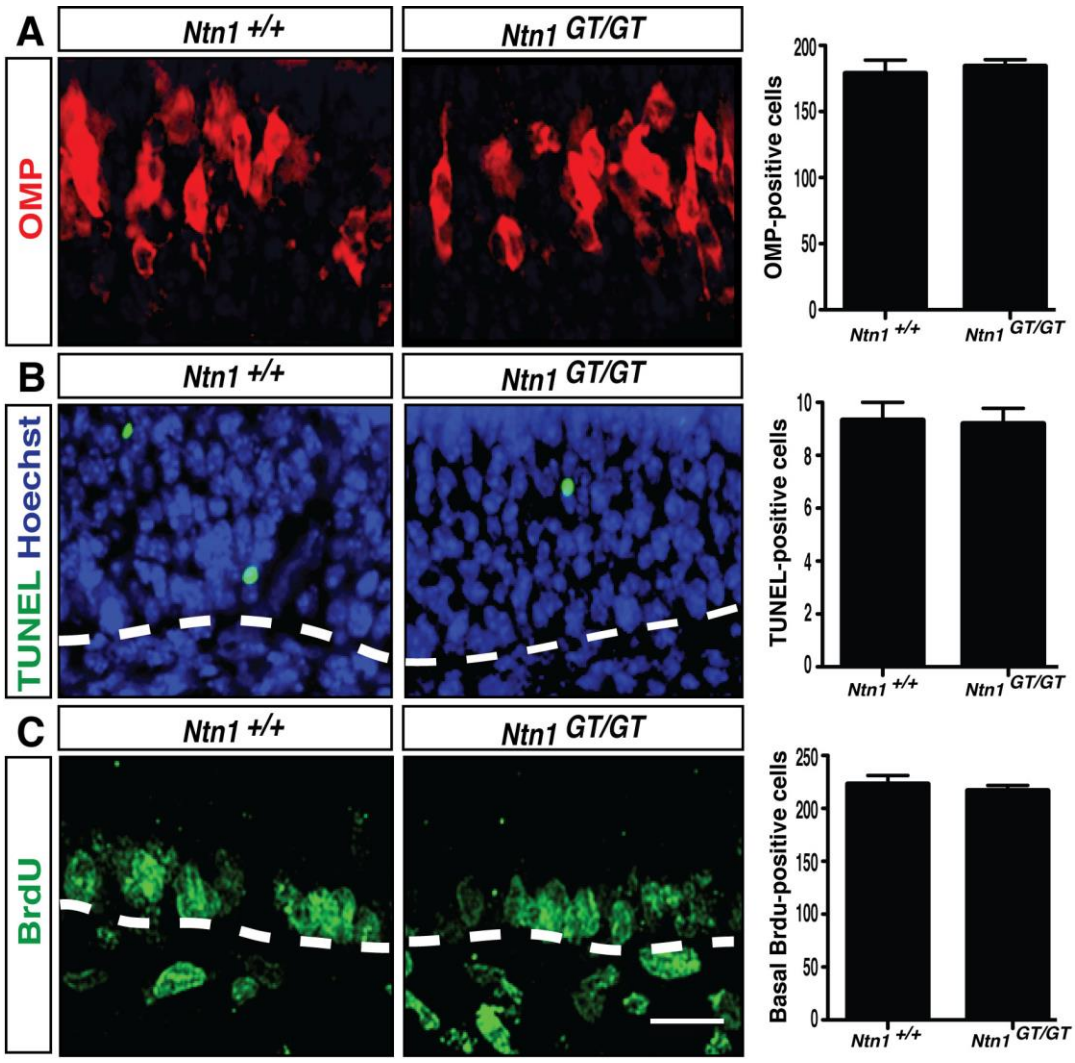



**Fig. S3: OE development is normal in *Ntn1*$^{GT/GT}$ mice.**

**(A-C)** OE sections from E16.5 embryos were processed for OMP (A), TUNEL (B), and BrdU (C) staining. The OE of *Ntn1*$^{GT/GT}$ embryos shows similar numbers of mature ORNs (A), cell death (B), and basal proliferating cells (C), as control embryos. All cell counts are represented as cells per 2.5 mm of OE. Students unpaired t-test was performed to compare values between groups. Scale bar: 100 μm.

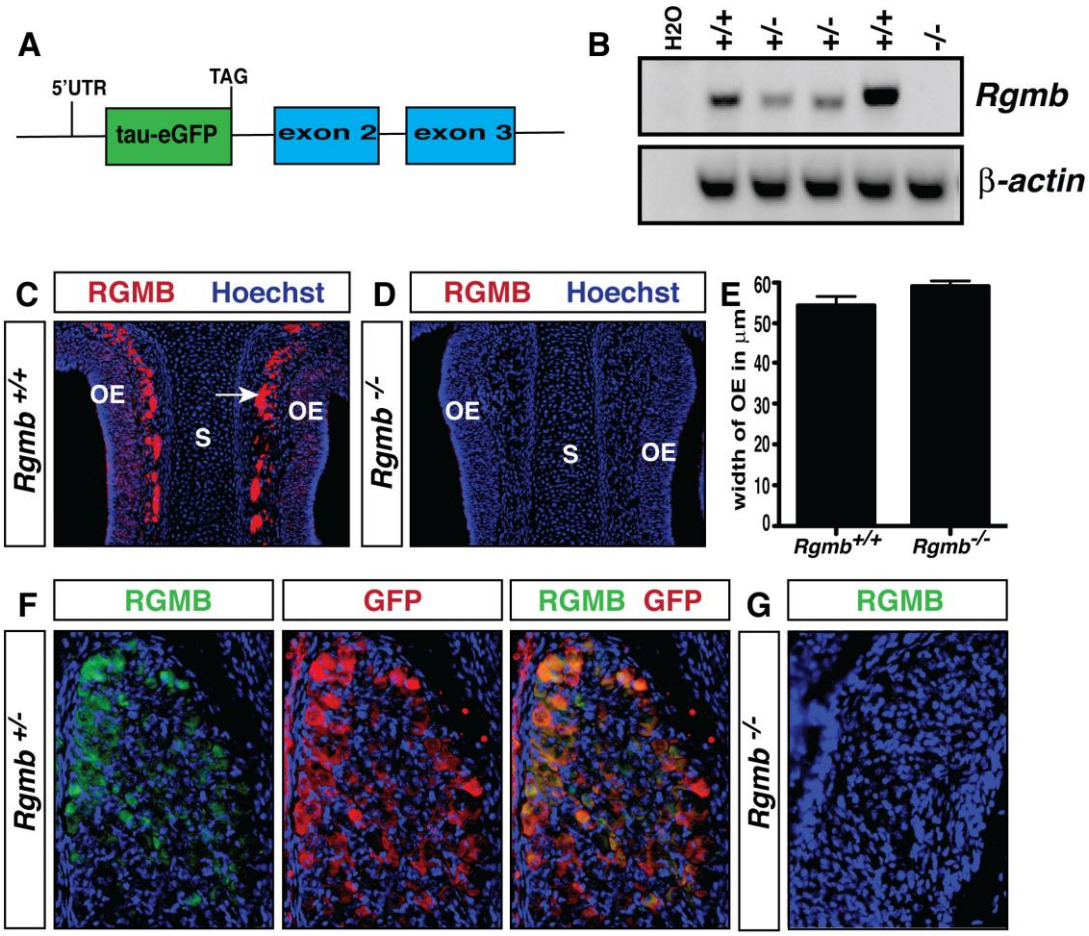



**Fig. S4: Generation and characterization of the *Rgmb* knock-in mice.**

(A) Diagram of the *Rgmb* targeted allele. An eGFP cassette was inserted into exon 1 of the *Rgmb* allele. (B) RT-PCR analysis on brain mRNA samples isolated from control and $Rgmb^{-/-}$ embryos. *Rgmb* mRNA expression is abolished in $Rgmb^{-/-}$ embryos. (C,D) RGMB immunolabeling of OE sections from E16.5 $Rgmb^{+/+}$ (C) and $Rgmb^{-/-}$ (D) embryos. RGMB protein expression is abolished in $Rgmb^{-/-}$ embryos. (E) Quantification of the width of OE (μm) in $Rgmb^{+/+}$ and $Rgmb^{-/-}$ embryos. The width of the OE is unchanged in $Rgmb^{-/-}$ embryos. (F-G) Immunolabeling of dorsal root ganglia from an

E16.5 *Rgmb*$^{+/-}$ embryo with GFP and RGMB antibodies. GFP is expressed in RGMB-positive cells and GFP expression recapitulates RGMB expression in the dorsal root ganglia (F). RGMB expression is abolished in the dorsal root ganglia of *Rgmb*$^{-/-}$ embryos (G). Students unpaired t-test was performed to compare values between groups. Scale bars: 100 μm.