Supporting Information

Ohsawa et al. 10.1073/pnas.0910488107

SI Materials and Methods

Construction. pEX-Sema7A-Fc was kindly provided by A. L. Kolodkin (The Johns Hopkins University School of Medicine, Baltimore) (1). Caspase-cleaved N-terminal fragment of Sema7A (1–236aa) were subcloned into pEX.

Cells, Transfection, and Immunoblotting. Neuro2a cells were cultured in DMEM containing 10% FBS and 2 mM L-glutamine. Cells were transfected using Lipofectamine 2000 reagent (Invitrogen) following the manufacturer's instructions. Methods used for immunoblotting have been described previously (2). The following antibodies were used at the indicated dilutions: rabbit monoclonal anti-p44/42 MAPK (Erk1/2) (137F5) (Cell Signaling; 1:2,000), rabbit monoclonal anti–phospho-p44/42 MAPK (Erk1/2)(Thr202/Tyr204) (D13.4.4E)XP (Cell Signaling; 1:2,000), goat polyclonal anti–human IgG Fc (1:5,000; Jackson Immunoresearch Laboratories), and mouse monoclonal anti–β-tubulin (Millipore Bioscience Research Reagents; 1:1,000).

1. Pasterkamp RJ, Peschon JJ, Spriggs MK, Kolodkin AL (2003) Semaphorin 7A promotes axon outgrowth through integrins and MAPKs. *Nature* 424:398–405.

2. Ohsawa S, Hamada S, Yoshida H, Miura M (2008) Caspase-mediated changes in histone H1 in early apoptosis: Prolonged caspase activation in developing olfactory sensory neurons. *Cell Death Differ* 15:1429–1439.

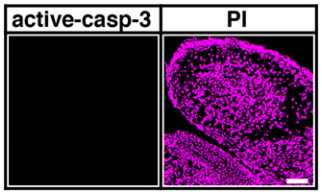

Fig. S1. Apaf-1–mediated caspase-3 activation in the olfactory system. An E16.5 *apaf-1*–deficient OB coronal section was stained with the anti–active-caspase-3 antibody (green). Cell nuclei were visualized by propidium iodide (PI; magenta) staining. (Scale bar, 50 μm.)

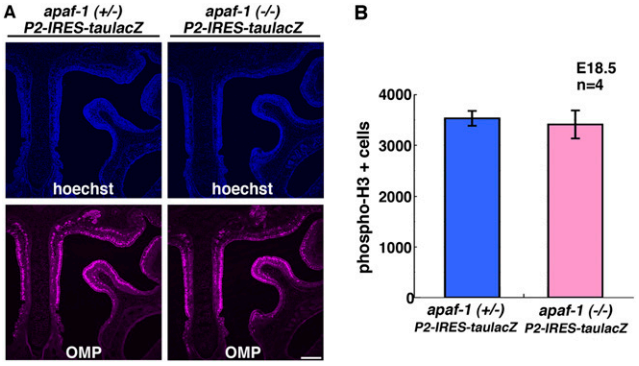

Fig. S2. Neither distribution of OMP-positive mature OSNs nor the number of mitotic cells in the OE were affected in *apaf-1*$^{-/-}$ mice. (*A*) Coronal sections (5-μm thick) of E18.5 *apaf-1 P2-IRES-taulacZ*$^{+/-}$ and $^{-/-}$ mice were stained with anti-OMP antibody (magenta). Cell nuclei were visualized by Hoechst 33342 staining (blue). (*B*) Serial coronal sections (5-μm thick) were prepared from entire OE of E18.5 *apaf-1*$^{+/-}$ and *apaf-1*$^{-/-}$ *P2-IRES-taulacZ* mice and stained with anti-phospho-H3 antibody. Phospho-H3–positive cells in every 10th section were counted. Values are mean ± SEM (n = 4; t test, $P < 0.01$). (Scale bar, 200 μm.)

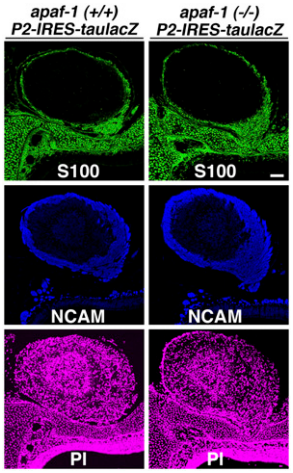

Fig. S3. S100-positive olfactory ensheathing cells were not affected in the OE of *apaf-1*$^{-/-}$ mice. Coronal sections (5 μm thick) of E18.5 *apaf-1*$^{+/+}$ and *apaf-1*$^{-/-}$ mice were stained with anti-S100 (green) and anti-NCAM (blue) antibody. Cell nuclei were visualized by PI staining (magenta). (Scale bar, 100 μm.)

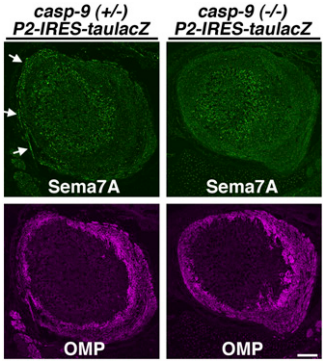

Fig. S4. Caspase-9–dependent cleavage of Sema7A in the developing olfactory system. Coronal sections (5 μm thick) of E17.5 *caspase-9*$^{+/-}$ or *caspase-9*$^{-/-}$ *P2-IRES-taulacZ* OB were stained with anti-Sema7A (green) and anti-OMP (magenta) antibodies. Arrows show Sema7A-positive axons. (Scale bar, 100 μm.)

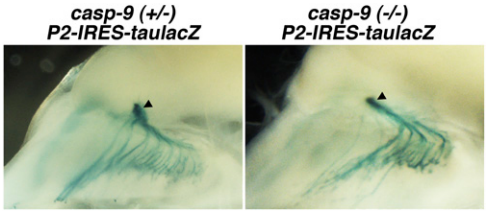

Fig. S5. Impaired development of P2 glomeruli in *caspase-9*$^{-/-}$ mice. View of nasal septum and medial aspect of the OB of E18.5 *caspase-9*$^{+/-}$ (*A*) and *caspase-9*$^{-/-}$ *P2-IRES-taulacZ* (*B*) mice. Arrowheads show P2 glomeruli in their mice.

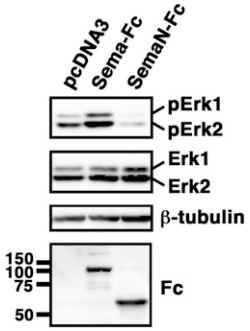

Fig. S6. Caspase-cleaved fragment of Sema7A fails to induce Erk1/2 phosphorylation in Neuro2a cells. Neuro2a cells were transfected with either pcDNA3, Fc-tagged full-length Sema7A-pEX (Sema-Fc), Fc-tagged caspase-cleaved N-terminal fragment of Sema7A-pEX (SemaN-Fc). Whole-cell extracts were analyzed by immunoblotting using anti–phospho-Erk1/2 (Thr202/Thr204), anti-Erk1/2, anti–β-tubulin, and anti-Fc antibodies.