Supporting Information

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SI Materials and Methods

Use of Distribution Profiles to Predict the Colocalization of Two Markers. We used the distribution profiles of each marker (GAP-43, OMP, AC3, or ORs) and those obtained for BrdU at different DPI to analyze the theoretical colocalization expected at each DPI and to predict the appearance of double labeling for any two markers. From the overlapping area (shadowed in gray) between the distribution profile of each marker (GAP-43, OMP, AC3, or ORs) and those observed for BrdU at different DPI (Fig. S1 *B*, *D*, *F*, and *H*) we determined the potential maximal frequency of double labeling at each DPI, which is represented by the peak of the gray area in each graph. Although the distribution of newly born cells at different DPIs (represented in Fig. S1*A*) is independent of the labeling efficiency, the number of BrdU-labeled cells does depend on the labeling efficiency and the detection method. Therefore, the frequency of double-labeled cells is going to be less than the maximum predicted. We determined that at 1 DPI the percentage of cells labeled with BrdU in the horizontal and globose basal cells niche is almost 40%. We therefore multiplied the maximal frequency of double labeling by 0.40 to obtain the theoretical maximal frequency of double labeling for each marker and BrdU at each DPI (Fig. S1 *C*, *E*, *G*, and *I*) and considered an arbitrary threshold for experimental detection of 5%. This means that if, for example, we consider the distribution profiles for OMP and BrdU at 4 DPI (Fig. S1*B*, *Bottom Left*): (*i*) Of all of the OMP cells, fewer than 17% are found positioned in the OE below 0.55 (right vertical dotted line), which is the area of overlap with BrdU at 4 DPI, but this does not imply that both markers are expressed in the same cell. (*ii*) Considering that the maximum probability of having OSNs double-labeled is 0.03 (Fig. S1*C*), it would be possible to observe at the most three double-labeled cells for every 100 OMP^+ cells that are in the overlapping zone.

Our arbitrary threshold of 5% implies that we should be able to detect in our experiments double-labeled OMP/BrdU cells at 4 DPI after counting over 1,000 OMP^+ OSNs (which is approximately the number of cells that we counted; see main text).

Following the previous reasoning we can predict a significant experimental detection of double-labeled GAP-43 and BrdU cells at 1 DPI because the theoretical value is higher than our threshold (Fig. S1 *D* and *E*), double-labeled OMP and BrdU at 6 DPI (Fig. S1 *B* and *C*), double-labeled AC3 and BrdU at 5–6 DPI (Fig. S1 *F* and *G*), and double-labeled ORs and BrdU at 3–4 DPI (Fig. S1 *H* and *I*).

In situ hybridization data supported our predictions (see main text), demonstrating that distribution profiles are a useful tool to determine expected double-labeled frequencies for two OSN markers. This analysis technique will be beneficial in the future to compare profiles between different, noncompatible techniques or even to compare results between different studies.

Immunohistochemistry. Sections were stained with antibodies as previously described (1). Briefly, tissue was thawed, air dried, and then incubated in blocking buffer [2% (wt/vol) BSA (Sigma) in PBS-T (PBS with 0.3% Triton X-100, Sigma)] for 30 min to block nonspecific binding sites. Incubation with primary antibodies diluted in blocking buffer was performed at 4 °C for 48 h. The primary antibodies used were the following: goat anti-OMP (1:1,000, Wako, catalog # 544–10001), rabbit anti–GAP-43 (1:1,000, Novus Biological, catalog # NB300-143), rat anti-BrdU [1:250, Accurate Chemical & Scientific, clone BU1/75 (ICR1)], rabbit anti-ZsGreen (1:300, Origene, catalog # TA180002), rabbit anti–MOR244-1 (kind gift from Hitoshi Sakano, University of Fukui, Fukui, Japan), and chicken anti-GFP (1:1,000, AbCam, Accurate Chemical & Scientific catalog # ab13970). Sections were then washed three times in PBS-T for 5 min and incubated in secondary antibodies conjugated to Alexa Fluor (1:1,000, Molecular Probes) in blocking buffer for 1 h at room temperature with DRAQ5 (1:1,000, Biostatus) for nuclear staining and streptavidin-555 (Molecular Probes) to enhance dextran labeling. Coverslips were applied with Gel/Mount mounting medium (Biomeda).

Dextran Tracings. PND 7 mice were anesthetized with an i.p. injection of ketamine (100 mg/kg) and xylazine (10 mg/kg). Approximately 1 μL of dextran (3 kDa, tetramethylrhodamine and biotin; D-7162: Invitrogen), dissolved in sterile dimethyl sulfoxide at 12 mM, was injected in the most rostral part of one olfactory bulb. Following the injection, pups recovered on a warm blanket before returning to their home cage. After 24 h the pups were anesthetized and perfused as described above.

Countings and Analysis. Cell counts were done manually on images acquired using a Leica TCS SL laser-scanning confocal microscope (Leica Microsystems) or an Olympus BX51 epifluorescent microscope. Graphs were done using the Prism 4.03 package (GraphPad Software). One-way ANOVA was used to analyze differences on $BrdU^+$ cell position in the OE, with Bonferroni's multiple comparison test. Nonparametric one-way ANOVA was used to analyze differences in double-labeled BrdU/ZsGreen OSNs.

1. Rodriguez-Gil DJ, Greer CA (2008) Wnt/Frizzled family members mediate olfactory sensory neuron axon extension. *J Comp Neurol* 511(3):301–317.

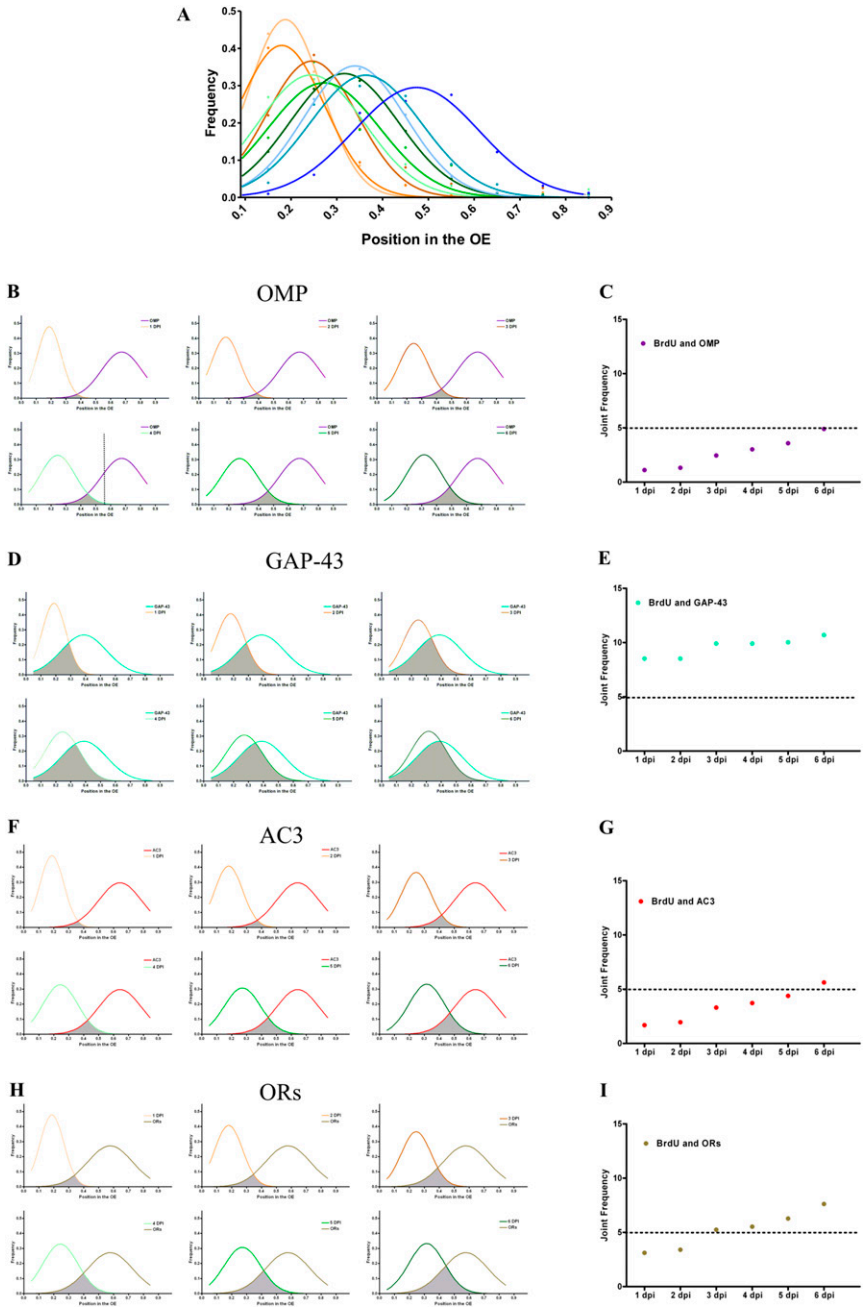

Fig. S1. Distribution profiles are good predictors of double labeling. Normal distributions associated with the histograms shown in Fig. 1, showing the progress of BrdU-labeled cells across the OE (*A*). The distribution for OMP (purple in *B*), GAP-43 (cyan in *D*), AC3 (red in *F*), and ORs (light green in *H*) and BrdU at 1–6 DPIs are shown. The overlapping area under the curves is shaded (gray). Theoretical maximal frequency of double labeling (joint frequency) for BrdU and OMP (*C*), GAP-43 (*E*), AC3 (*G*), and ORs (*I*) at each studied DPI. Dashed line: arbitrary threshold of detection defined at 5%.

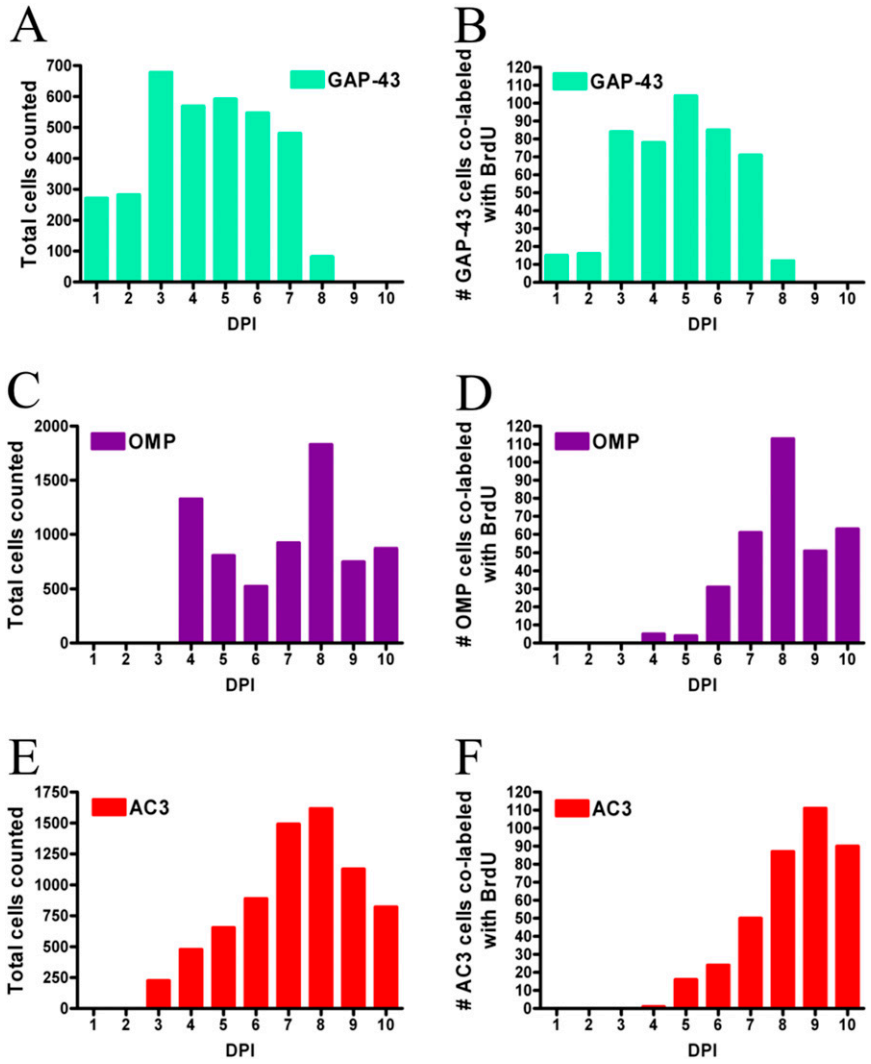

Fig. S2. Absolute cell counts after in situ hybridization. Total number of cells counted (*A*, *C*, and *E*) and total number of cells double-labeled with BrdU (*B*, *D*, and *F*) for GAP-43 (*A* and *B*), OMP (*C* and *D*), AC3 (*E* and *F*).

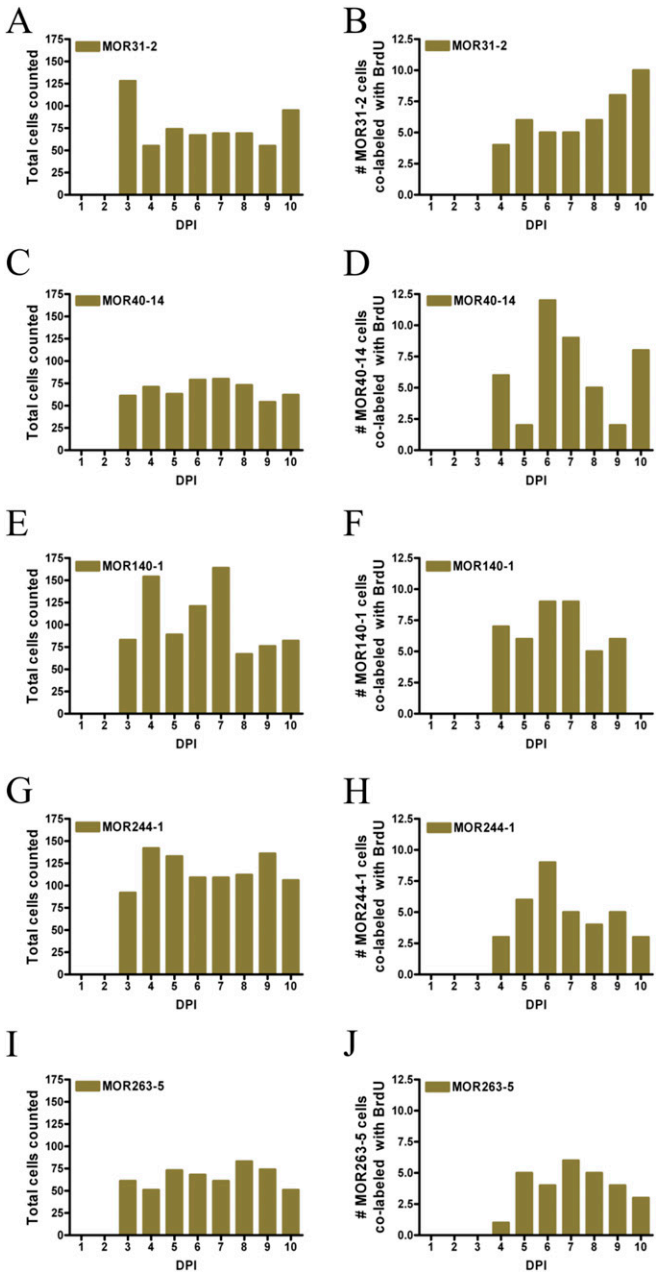

Fig. S3. Absolute cell counts after OR in situ hybridization. Total number of cells counted (*A*, *C*, *E*, *G*, and *I*) and total number of cells double-labeled with BrdU (*B*, *D*, *F*, *H*, and *J*) for MOR31-2 (*A* and *B*), MOR40-14 (*C* and *D*), MOR140-1 (*E* and *F*), MOR244-1 (*G* and *H*), and MOR 263–5 (*I* and *J*).

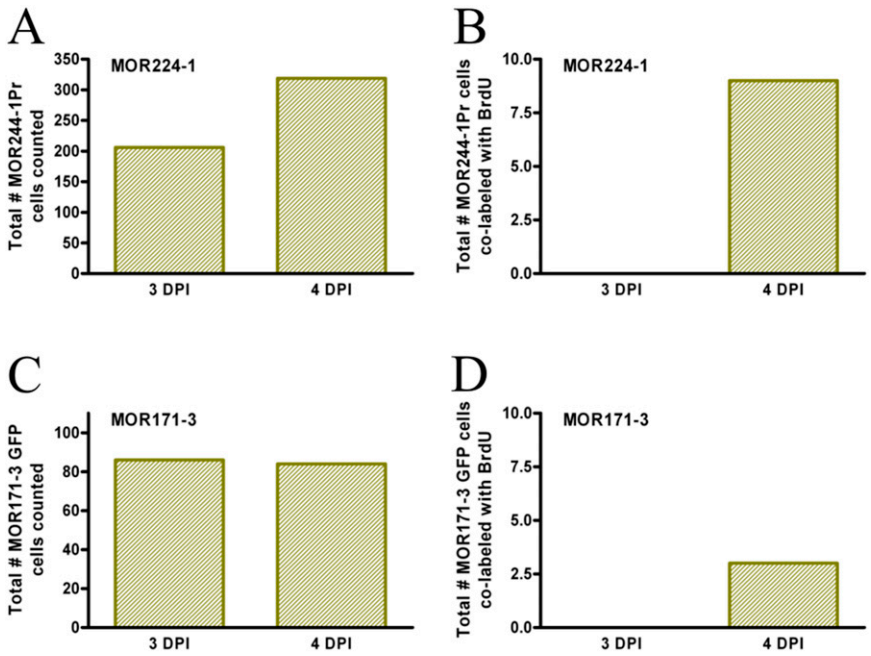

Fig. S4. Absolute cell counts after immunohistochemistry. Total number of cells counted (*A* and *C*) and total number of cells double-labeled with BrdU (*B* and *D*) for 244–1 protein (*A* and *B*) and MOR171-3 GFP (*C* and *D*).

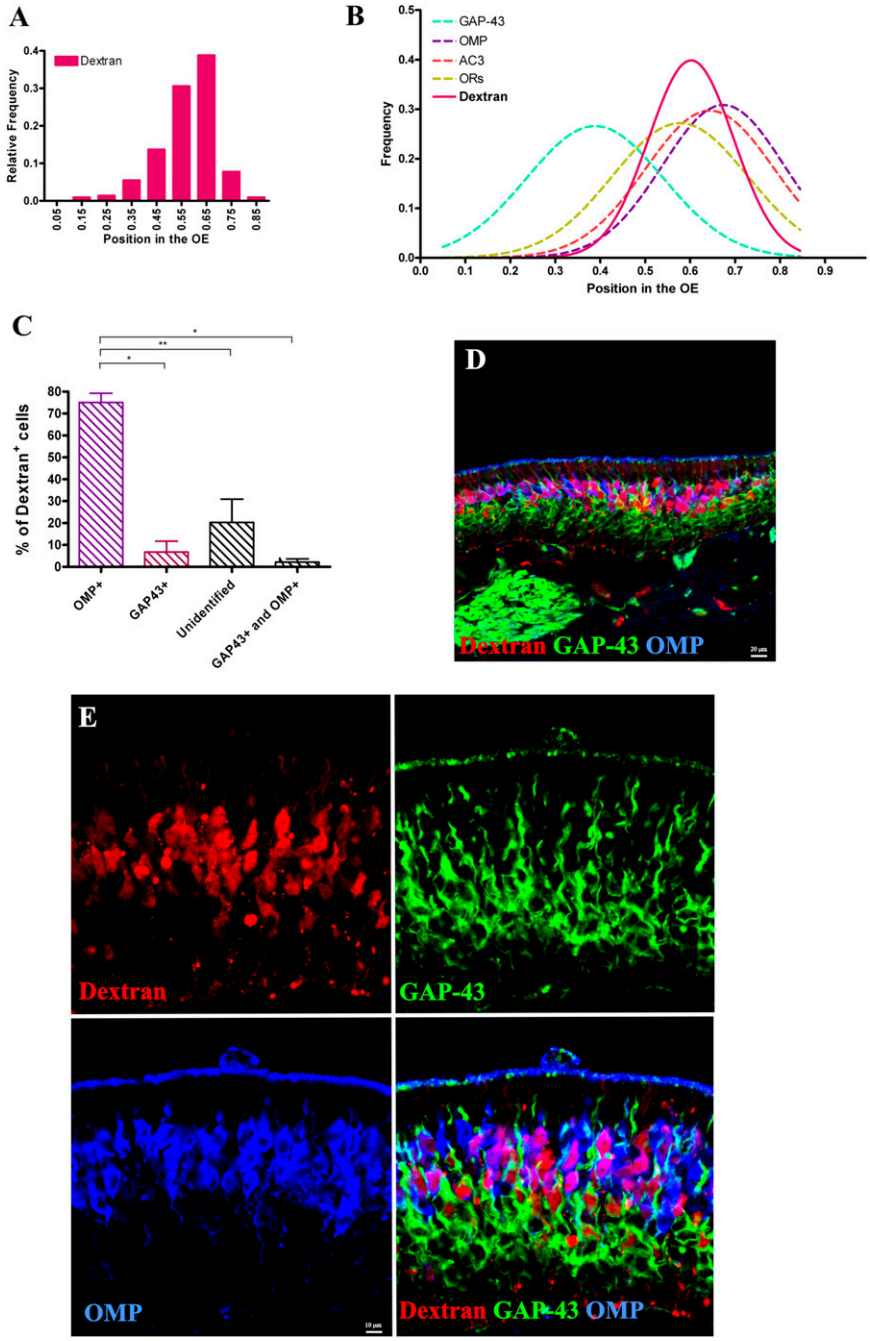

Fig. S5. Dextran in the OB predominantly labels OMP-expressing OSNs. (*A*) Histogram distribution showing the relative frequency of retrogradely labeled dextran$^+$ OSNs across the OE width. (*B*) Normal distribution of dextran-labeled cells (solid line) in relation to OMP, GAP-43, AC3, and ORs (dashed lines). (*C*) Quantification of retrogradely labeled dextran$^+$ cell bodies. Double-labeled OMP/dextran cells were significantly more abundant than any of the other categories analyzed. *$P < 0.01$; **$P < 0.05$. (*D* and *E*) Representative examples of sections stained for dextran (red), OMP (blue), and GAP-43 (green).

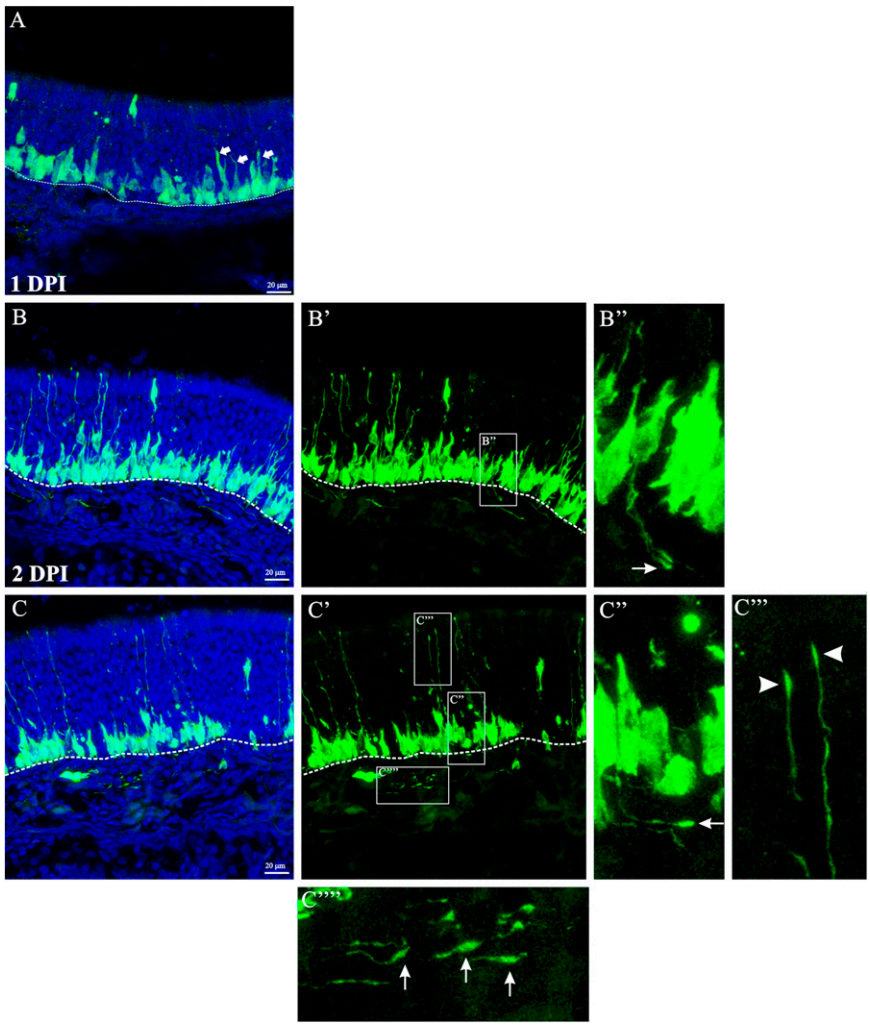

Fig. S6. Ascl1-CreERT2;R26R mice provide high resolution of dendritic and axonal growth cones. (*A*) At 1 DPI, Ascl1$^{CreERT2/+}$;R26R$^{ZsGreen/-}$ mice show that some cells already extend a process (arrows) toward the surface of the OE. (*B* and *C*) By 2 DPI, the extension of apical dendrites and axons in the underlying lamina propria is visible. (*B′* and *C′*) Same images as *B* and *C* without the nuclei staining. At this stage, enlarged terminals can be detected both in the dendrites (arrowheads in *C′′′*) and in the axons (presumably growth cones; arrows in *B′′*, *C′′*, and *C′′′′*). ZsGreen is shown in green. Nuclei were stained with Draq5 and are shown in blue.

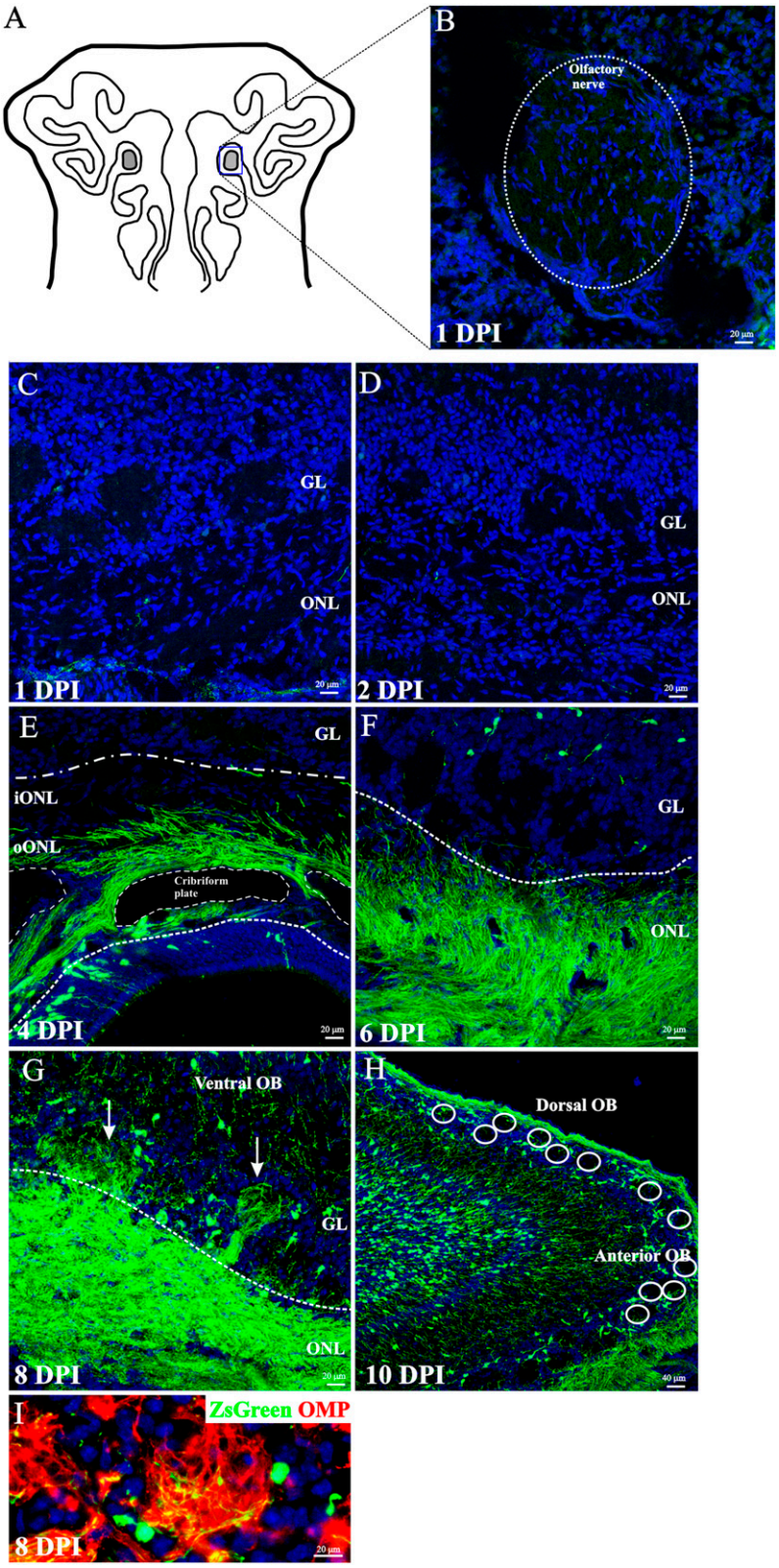

Fig. S7. Detailed tracking of OSN axon outgrowth in Ascl1-CreERT2;R26R mice. (*A* and *B*) Diagram of a coronal section through the nasal cavity of a rodent showing position of the olfactory nerve section in *B* at 1 DPI. (*C*–*G*) OB sections showing the absence of ZsGreen-expressing axons in the ONL and in the GL at 1 DPI (*C*) and 2 DPI (*D*) . At 4 DPI (*E*), the OSN axons are found almost exclusively in the oONL, and at only 6 DPI following 4OH-Tx injection (*F*) do axons invade the iONL. By 8 DPI, some glomeruli located in the most ventral part of the OB exhibit evidence of ZsGreen-labeled innervation (*G*). Nonetheless, 2 d later, no significant ZsGreen innervation was detected in the glomeruli located in the dorsal part of the OB (*H*). (*I*) At 8 DPI, ZsGreen-labeled axons entering the glomeruli also express olfactory marker protein (OMP, red). ZsGreen is shown in green. Nuclei were stained with Draq5 and are shown in blue.