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Supplemental Information

YAP signaling in horizontal basal cells promotes the regeneration of olfactory epithelium after injury

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Supplemental Information

Supplemental Figures and legends

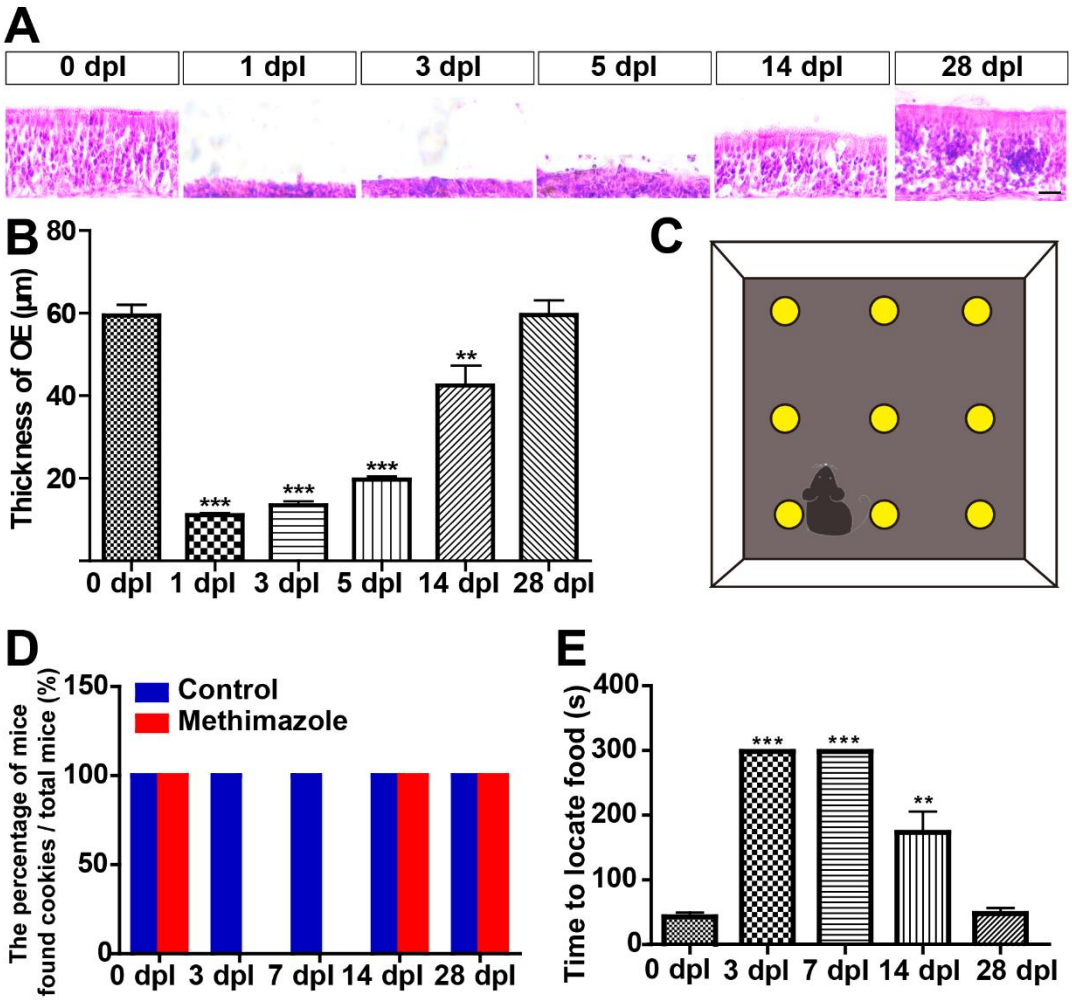

Figure S1. Establishment of methimazole-induced OE injury model.

(A) HE staining in the OE of 2 months old C57BL/6 mice at 0, 1, 3, 5, 14 and 28 dpl. (B) Quantitative analysis of the thickness of 2 months old OE as shown in (A) (n = 10 sections from 5 mice per group). (C) The schematic diagram of buried pellet test. (D) The percentage of control- or methimazole-treated 2 months old C57BL/6 mice that found cookies over total observed mice at 0, 3, 7, 14 and 28 dpl (n = 13 mice per group, two-way ANOVA with Bonferroni's post-tests, compared with control group). (E) Quantitative analysis of the time to locate food in the buried pellet test at 0, 3, 7, 14 and 28 dpl (n = 13 mice per group). Data were mean ± SEM, one-way ANOVA with Bonferroni's post-tests unless otherwise indicated, compared with 0 dpl, $^{**}P < 0.01$, $^{***}P < 0.001$. Scale bars, 20 μm.

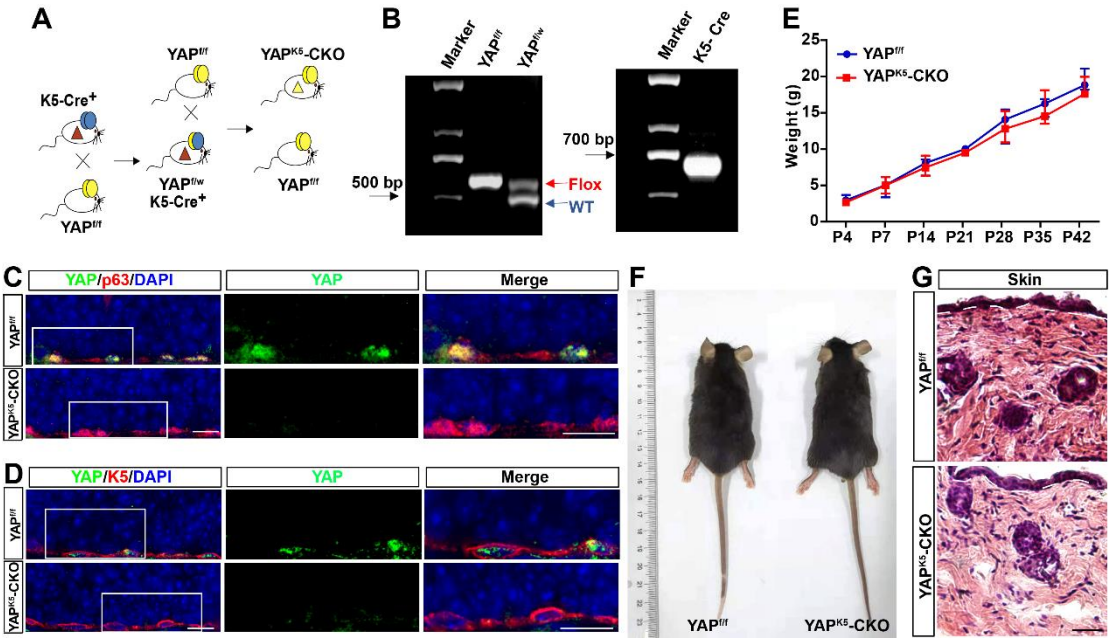

Figure S2. Identification of YAP^{K5}-CKO mice and normal development of skin and hair in YAP^{K5}-CKO mice, related to Figures 2, 3, 4.

(A) The flow chart showed the process of obtaining YAP^{K5}-CKO mice and their littermate control. (B) Genotyping was conducted by agarose gel electrophoresis (YAP$^{f/f}$ and YAP$^{f/w}$ mice were shown in the left panel, and K5-Cre mice were shown in the right panel). (C-D) Double immunostaining of YAP (green) and p63 (red) (C), and YAP (green) and K5 (red) (D) in OE of 2 months old YAP$^{f/f}$ and YAP^{K5}-CKO mice. (E) Body weight of YAP$^{f/f}$ and YAP^{K5}-CKO mice at different developmental stages (n = 10 mice per group). (F) The gross morphologies of skin and hair of 2 months old YAP$^{f/f}$ and YAP^{K5}-CKO mice. (G) HE staining of the skin of 2 months old YAP$^{f/f}$ and YAP^{K5}-CKO mice. Images of selected regions were shown at higher magnification. Data were mean ± SEM, two-way ANOVA with Bonferroni's post-tests, compared with YAP$^{f/f}$ group. Scale bars, 20 μm.

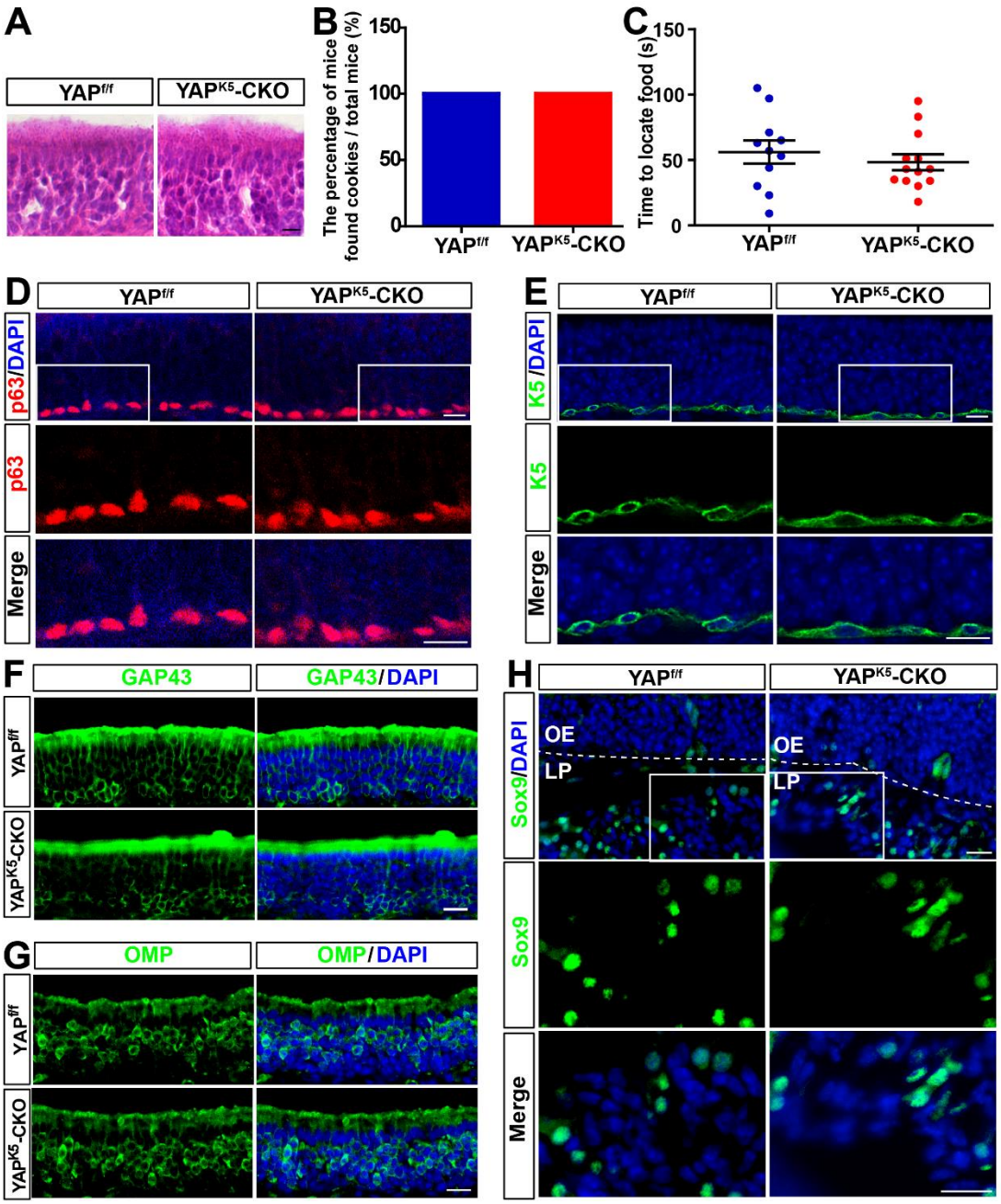

Figure S3. Normal OE development and olfactory functions of YAP^{K5}-CKO mice, related to Figures 2, 3, 4.

(A) HE staining images of OE from 2 months old YAP$^{f/f}$ and YAP^{K5}-CKO mice. (B) The percentage of mice that found cookies over total observed 2 months old YAP$^{f/f}$ (n = 11 mice) or YAP^{K5}-CKO mice (n = 13 mice) in the buried pellet test. (C) Quantitative analysis of the time to locate food in the buried pellet test of 2 months old YAP$^{f/f}$ (n = 11 mice) or YAP^{K5}-CKO mice (n = 13 mice). (D-H) Immunostaining of p63 (red) (D), K5 (green) (E), GAP43 (green) (F), or OMP (green) (G), or Sox9 (green) (H) in OE of 2 months old YAP$^{f/f}$ and YAP^{K5}-CKO mice. Images of selected regions were shown at higher magnification. Data were mean ± SEM, Student's t-test, compared with YAP$^{f/f}$ group. Scale bars, 20 μm.

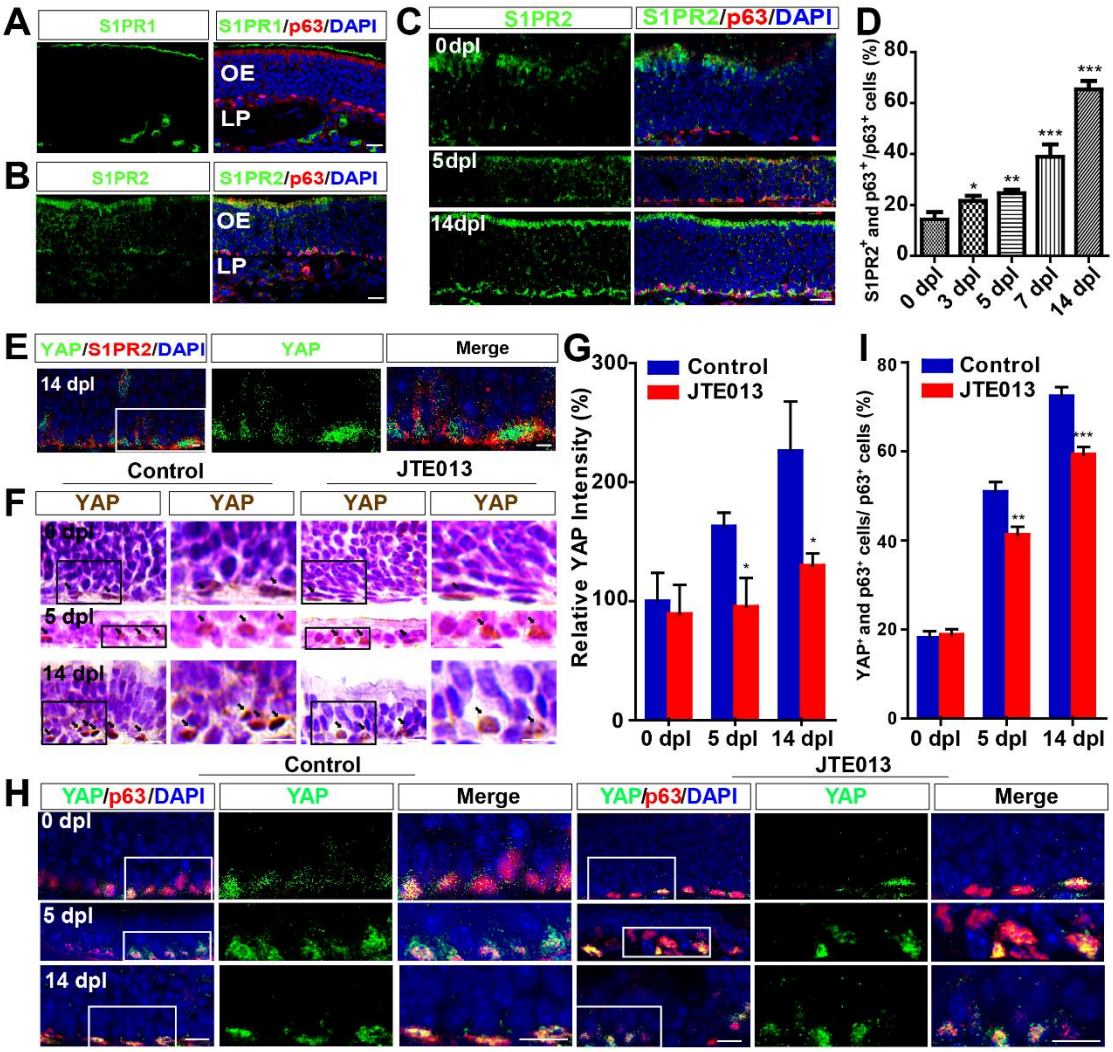

Figure S4. S1PR2/YAP signaling in HBCs is required for OE regeneration after injury, related to Figures 5, 6.

(A-B) Double immunostaining of S1PR1 (green) and p63 (red) (A), or S1PR2 (green) and p63 (red) (B) in the OE of 2 months old C57BL/6 mice. (C) Double immunostaining of S1PR2 (green) and p63 (red) in the OE of 2 months old C57BL/6 mice at 0, 5 and 14 dpl. (D) Quantitative analysis of the percentage of cells that showed both S1RP2^{+} and p63^{+} cells over total p63^{+} HBCs at 0, 3, 5, 7 and 14 dpl (n = 8 sections from 4 mice per group) (one-way ANOVA with Bonferroni's post-tests, compared with 0 dpl group). (E) Double immunostaining of YAP (green) and S1PR2 (red) in the OE of 2 months old C57BL/6 mice at 14 dpl. (F) Immunohistochemistry detected the expression of YAP in the OE of control- and JTE013-treated 2 months old C57BL/6 mice at 0, 5 and 14 dpl. (G) Quantitative analysis of the relative YAP density as shown in (F) (n = 6 sections from 3 mice per group). (H) Double immunostaining of YAP (green) and p63 (red) in the OE of control- and JTE013-treated 2 months old C57BL/6 mice at 0, 5 and 14 dpl. (I) Quantitative analysis of the percentage of both YAP^{+} and p63^{+} cells over p63^{+} cells as shown in (H) (n = 5 sections from 3 mice per group). Images of selected regions were shown at higher magnification. Data were mean ± SEM, two-way ANOVA with Bonferroni's post-tests unless otherwise indicated, compared with control group. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. Scale bars, 20 μm.

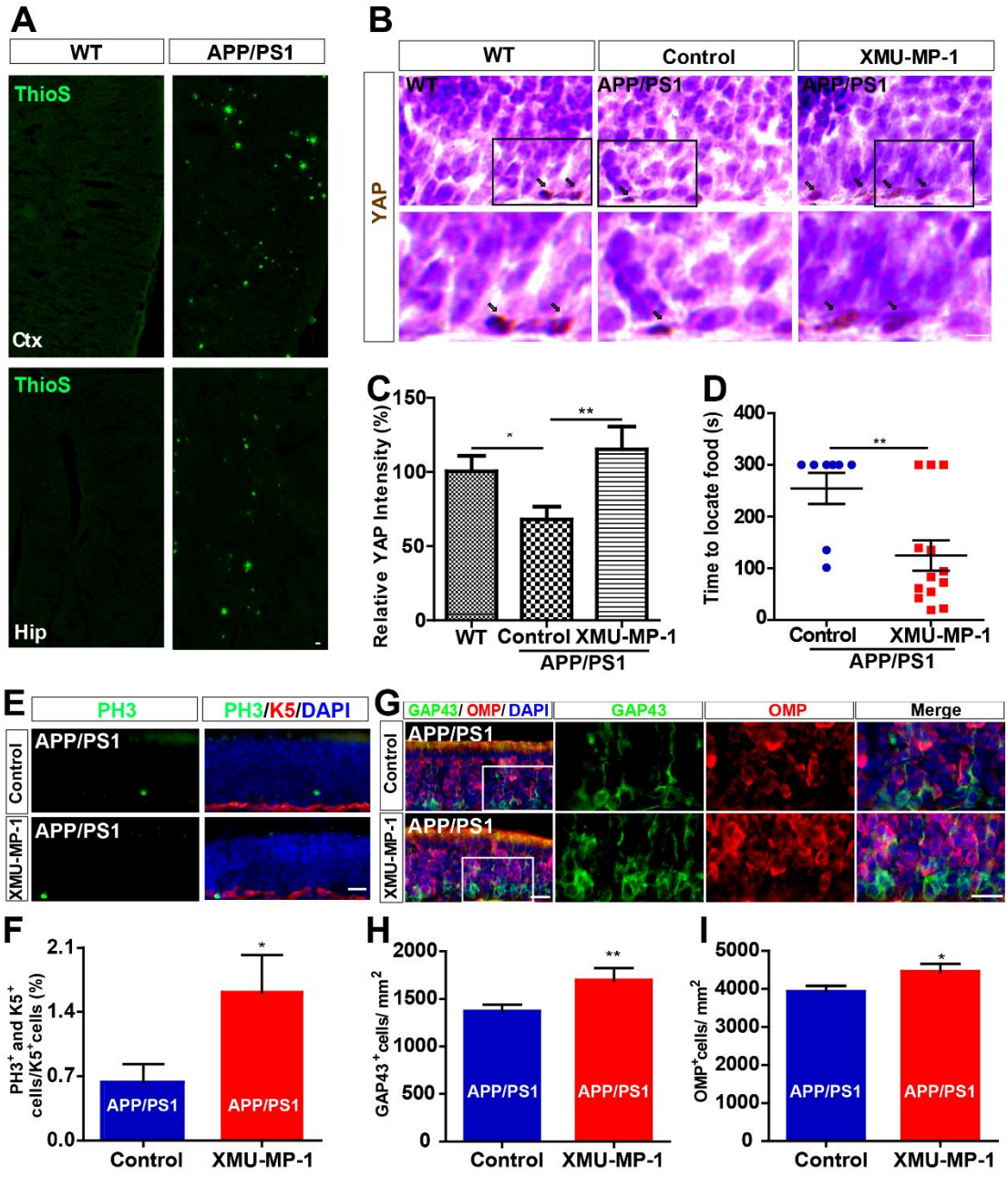

Figure S5. Activation of YAP signaling by XMU-MP-1 promotes the olfactory functional recovery of AD model mice, related to Figure 7.

(A) Cortex and hippocampus sections of 9 months old WT and AD model mice were stained with thioflavin-S staining to detect Aβ deposition. Ctx: cortex; Hip: hippocampus. (B) Immunohistochemistry detected the expression of YAP in OE of control-treated 9 months old WT mice, control- and XMU-MP-1-treated 9 months old AD model mice. (C) Quantitative analysis of the relative YAP density as shown in (B) (n = 10 sections from 5 mice per group) (one-way ANOVA with Bonferroni's post-tests, compared with WT group). (D) Quantitative analysis of the time to locate food in control- (n = 8 mice) and XMU-MP-1-treated 9 months old AD model mice (n = 13 mice) in the buried pellet assays. (E) Double immunostaining of PH3 (green) and K5 (red) in the OE of control- and XMU-MP-1-treated 9 months old AD model mice. (F) Quantitative analysis of the percentage of $PH3^+$ and $K5^+$ cells over total $K5^+$ HBCs as shown

in (E) (n = 11 sections from 5 mice per group). (G) Double immunostaining of GAP43 (green) and OMP (red) in the OE of control- and XMU-MP-1-treated 9 months old AD model mice. (H-I) Quantitative analysis of the density of $GAP43^+$ (H) or OMP^+ (I) cells as shown in (G) (n = 11 sections from 5 mice per group). Images of selected regions were shown at higher magnification. Data were mean ± SEM, Student's t-test unless otherwise indicated, compared with control group, $^*P < 0.05$, $^{**}P < 0.01$. Scale bars, 20 μm.

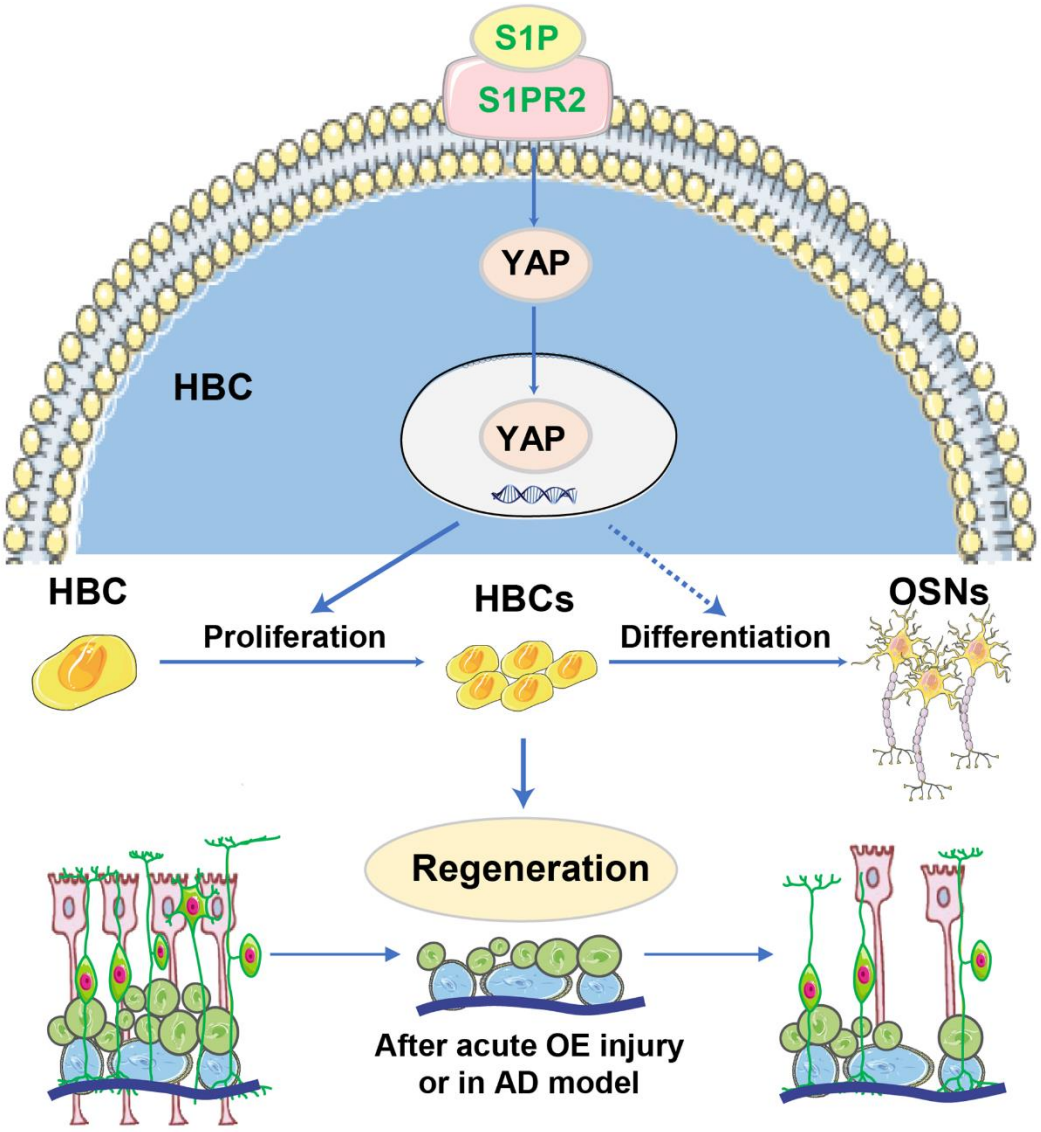

Figure S6. Working model of YAP's functions in OE regeneration through promoting HBCs proliferation after injury.

After OE injury, S1P binds to S1PR2 to activate YAP in HBCs, and may trigger OE regeneration through promotion of the proliferation and/ or differentiation of HBCs.

Supplemental Experimental Procedures

TUNEL staining

TUNEL staining of the olfactory tissue sections (20 μm in thickness) of 2 months old mice was conducted to evaluate apoptosis. Briefly, the sections were blocked in 5% BSA plus 0.3% Triton X-100 at room temperature for 1 h, after washing with PBS. The *in situ* detection kit was then used according to the manufacturer's protocol (MK500, TaKaRa Bio Inc, Shiga, Japan). The images were acquired through SLIDEVIEW™ VS200 microscope (Olympus, Germany) and analyzed by Image J or Adobe Photoshop CC 14.0 software.

Hematoxylin-Eosin (HE) staining

Following cardiac perfusion, the tissues were fixed with fresh 4% paraformaldehyde for 1 d, then they were decalcified using 10% EDTA in PBS at 4 °C for 5 d. All tissues were also dehydrated for 3 d using 30% sucrose in PBS until they sank. Thereafter, the olfactory tissues were embedded in optimal cutting temperature before being cut into 20 μm-thick sections, using a freezing microtome (Thermo, USA). The sections were then washed 3 times in double-distilled water after staining with hematoxylin for 4 min. Subsequently, the sections were incubated with acidic liquid alcohol differentiation for 30 s and stained with eosin for 40 s. This was then followed by 95% ethanol, 100% ethanol and a final clearance in xylene before mounting. Images were acquired by SLIDEVIEW™ VS200 microscope (Olympus, Germany) and analyzed by Image J software.

Thioflavin-S staining

Brain slices (20 μm in thickness) were incubated with 0.1% thioflavin-S (T1892, Sigma, USA) for 8 min at room temperature. The images were acquired through SLIDEVIEW™ VS200 microscope (Olympus, Germany) and analyzed by Image J or Adobe Photoshop CC 14.0 software.

Immunohistochemistry

Immunohistochemistry of the olfactory tissue sections (20 μm in thickness) of 2 months old mice or 9 months old AD model mice was performed using a kit (SP-9000, Zhong Shan Jin Qiao, China), according to the manufacturer's protocol. Antigen retrieval was accomplished using the sodium citrate antigen retrieval solution at 90°C for 30 min. Thereafter, the endogenous peroxidase activity of the tissue section was blocked with 3% H_2O_2 for 0.5 h followed by 5% BSA plus 0.3% Triton X-100 at room temperature for 1 h. After blocking, OE sections were incubated overnight with anti-YAP primary antibody (1:200, ab205270, Abcam) at 4 °C, then washed 3 times with PBS and incubated with a biotin-labeled goat anti-rabbit IgG secondary antibody at room temperature for 20 min. Streptavidin-peroxidase was applied for 20 min at room temperature followed by development with the diaminobenzidine substrate, and then the sections were counterstained with Mayer's Hematoxylin for 2 min. Subsequently, the sections were dehydrated and sealed with neutral gum. The sections were observed under SLIDEVIEW™ VS200 microscope (Olympus, Germany) and brown yellow particles in the nucleus were considered to be YAP positive (YAP^+) cells.