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Supplemental Information

Insulin-like Growth Factor II: An Essential Adult Stem Cell Niche Constituent in Brain and Intestine

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Supplemental Figures

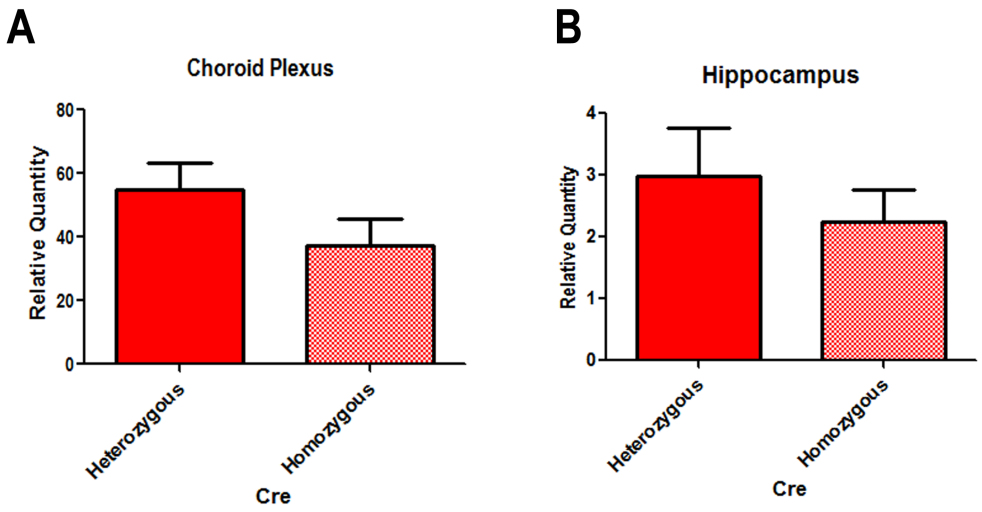

Figure S1. Comparison of *Igf2* RNA in mice heterozygous and homozygous for Cre. Related to Figure 1. Choroid plexus (**A**) and hippocampus (**B**) *Igf2* expression was compared after recombination induced by tamoxifen administration to mice heterozygous or homozygous for Cre and homozygous for floxed *Igf2*. No significant difference was found. n=5 WT and n=12 iKO mice. Error bars represent SEM.

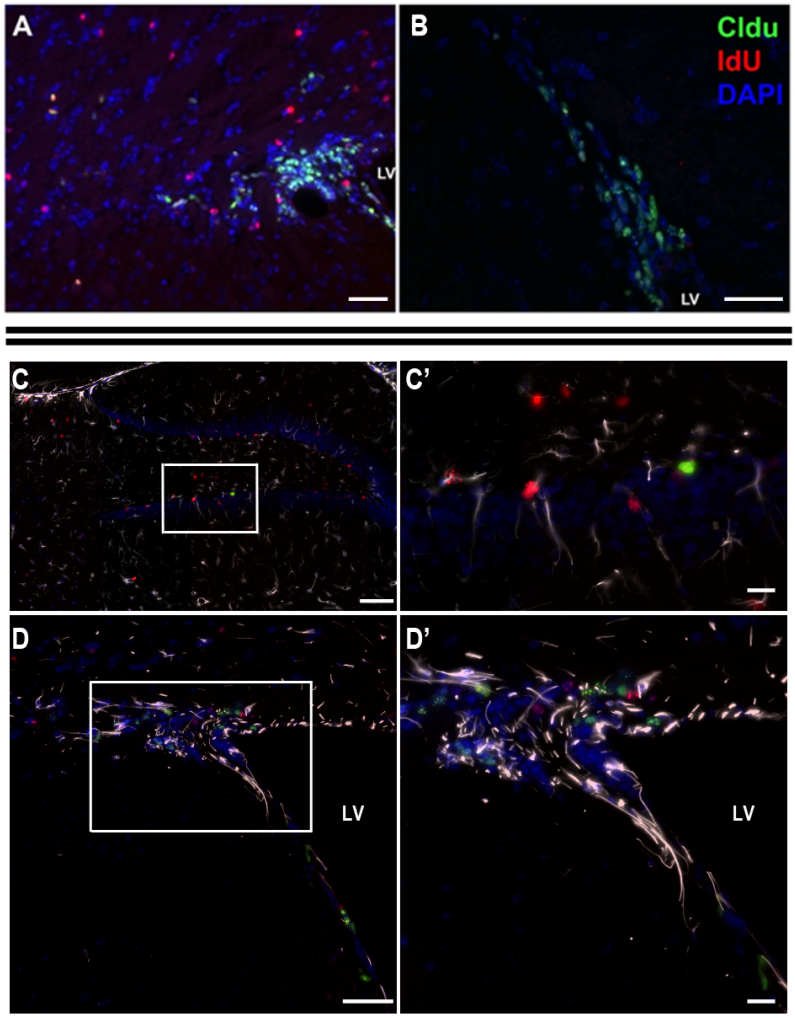

Figure S2. Dual nucleotide staining validity test. Related to Figures 2 and 3.
IdU antibodies have been reported to cross-react and recognize CldU analog, therefore, studies were performed to establish that the IdU antibody that we used did not cross react with CldU. Tissue from a mouse that received IdU and CldU analogs (**A**) was stained for CldU (green) and IdU (red) GFAP (white) panels (**C, D**) and counterstained with DAPI (blue). (**B**), Tissue from a mouse that received only the CldU analog was stained similarly. In our hands there was no cross-reactivity between IdU antibody and CldU analog (**A, B**). IdU was administered for 3 weeks followed by a 2-week washout, and then a single injection of CldU was given 4 hours prior to tissue collection (**C, D**). Very few CldU cells (green) were detected in the hippocampus (**C**) and inset (**C'**). By contrast, the SVZ contained many CldU positive (green) cells (**D**) and inset (**D'**). Double positive cells (yellow) were not detected in hippocampus or SVZ. LV = lateral ventricle. Scale bars = 100 µM (A, B, C, and D) and 20 µM (C' and D').

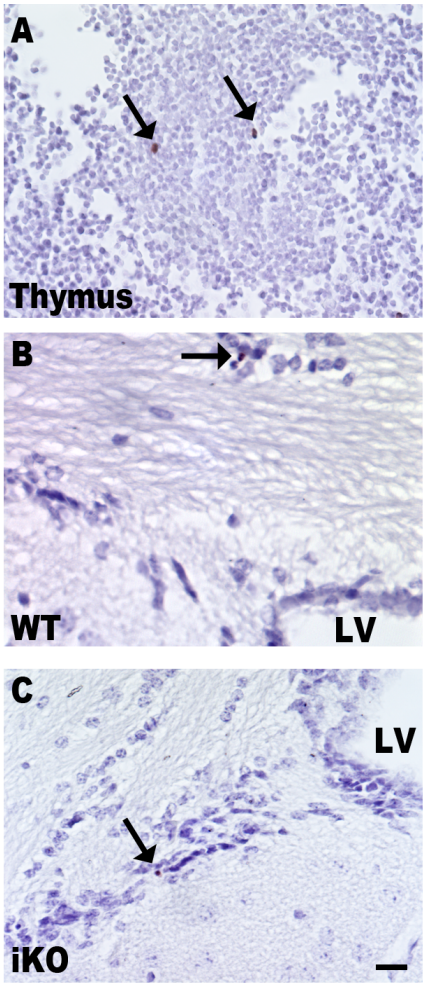

Figure S3. *In-situ* end labeling reveals no increase in cell death within the SVZ or the SGZ. Related to Figures 2, 3 and Table 1.

In-situ end labeling (ISEL) was performed on mouse thymus (A) (positive control tissue) and on WT (**B**) and iKO (**C**) mouse forebrains; lateral ventricle (LV). $ISEL^{+}$ cells, which have condensed reddish-brown nuclei, were rare in the SVZ and no positive cells were observed in the hippocampus. Sections were counterstained with hematoxylin. Scale bar = 50 μM.

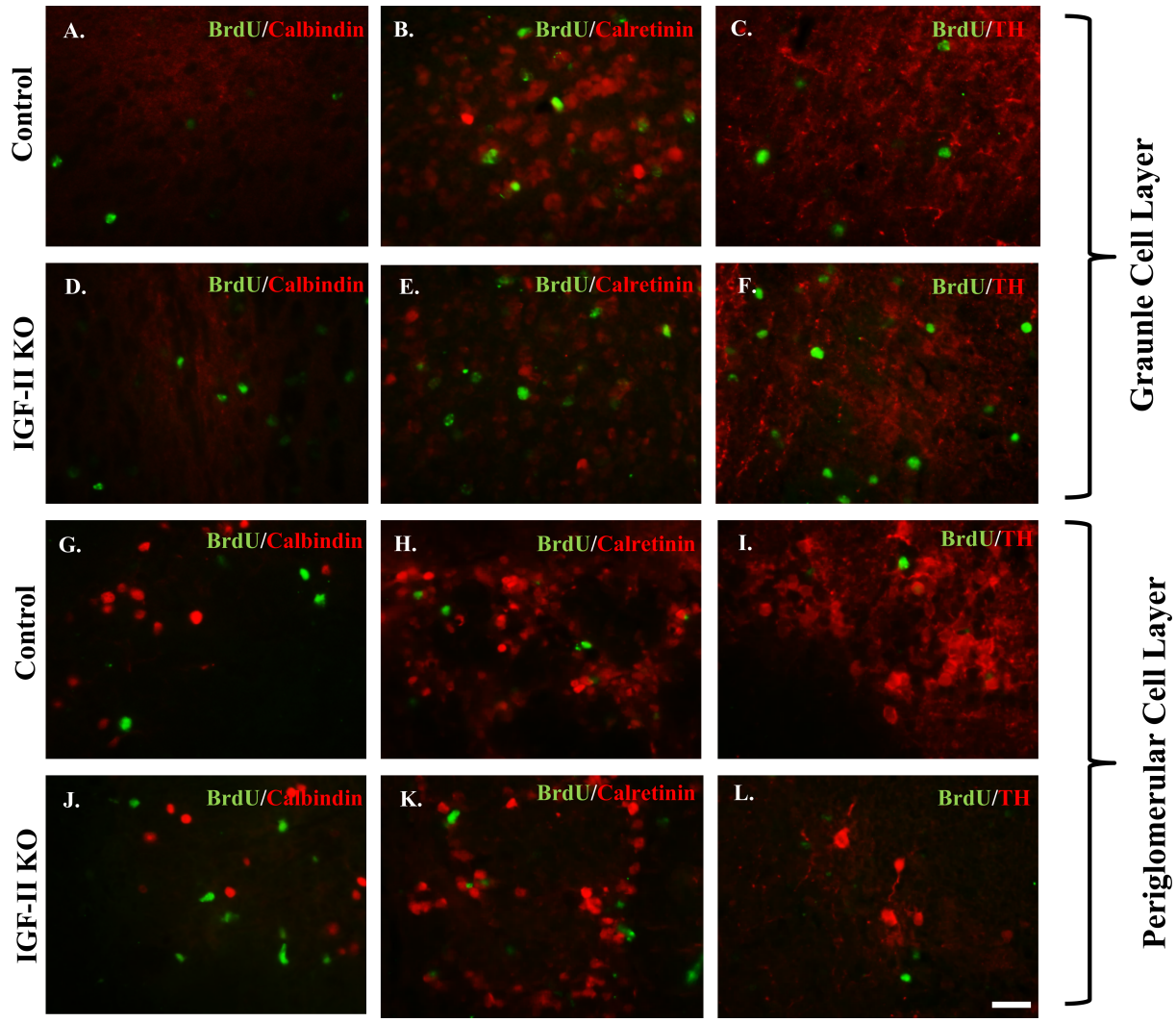

Figure S4: Representative images of BrdU and calbindin, calretinin and tyrosine hydroxylase immunofluorescence in the olfactory bulbs of WT and iKO mice. Related to Figure 3.
WT (**A-C, G-I**) and iKO (**D-F, J-L**) olfactory bulb granule cell layer (**A-F**) and periglomerular layer (**G-L**) stained for BrdU, calbindin (**A, D, G, J**), calretinin (**B, E, H, K**) and tyrosine hydroxylase (**C, F, I, L**), 5 weeks after BrdU administration. Scale bar = 100 μm.

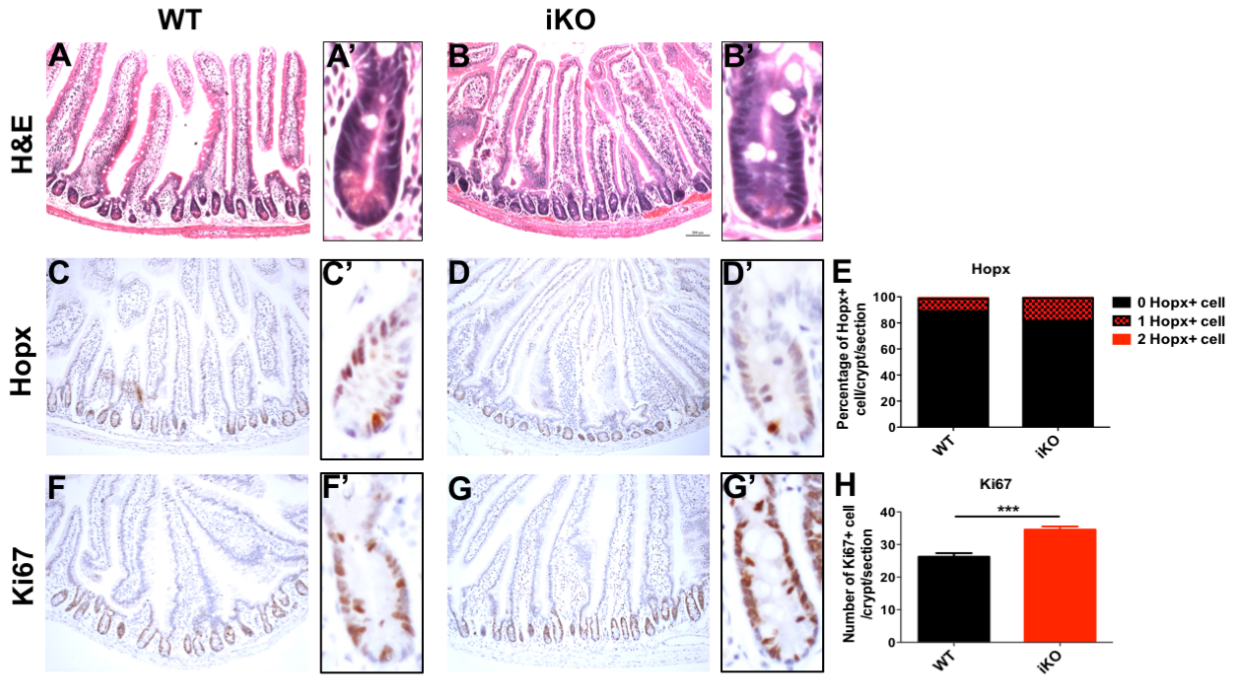

Figure S5: Long-term deletion of *Igf2* showed a compensatory phenotype in small intestine. Related to Figure 5.

Igf2 deletion was induced using 5 doses of tamoxifen administered every 3 days. Small intestine of WT and iKO mice were stained for H&E (**A, A', B, B'**), Hopx (**C, C', D, D', E**; for the quiescent stem cell), Ki67 (**F, F', G, G', H**; for proliferative progenitor cell) and Lysozyme (**I, I', J, J', K**; for Paneth cell) n=3 (WT) and 3 (iKO) mice. Scale bar = 200 μm. *, $p<0.05$; ***, $p<0.001$ by Student's T test. Error bars represent SEM.

Supplemental Experimental Procedures - Behavioral Tests

Buried Food test: A simple test for mouse olfaction was followed as described by Yang and Crawley (2009. Briefly mice were fasted for 12 hours and then placed into a clean test chamber where a piece of cereal was buried under the bedding. The mice were pre-exposed to the test chamber and the cereal prior to the test. All mice consumed the cereal prior to the test. The latency (seconds) to find the hidden cereal was recorded.

Morris Water Maze:
Hidden Platform: The water maze consisted of a pool with a 0.97 meter diameter and a platform (10 cm in diameter) placed in the target quadrant (TQ) submerged under water made opaque with white non-toxic paint (Art Minds Tempera Paint, Michael's Inc). Several visual cues were dispersed throughout the room. Mice were tested in the Morris Water Maze for 5 consecutive days. Day 1 consisted of 1 pre-training session. On days 2-5, mice received 2 training sessions per day, one in the morning and one in the afternoon. The latency to find the platform was measured for each trial. Each training session consisted of 4 trials with the mouse started in a different quadrant of the maze each time, omitting placement in the TQ. On day 6, mice received a probe trial where the platform was removed and the time spent searching the TQ was measured.

Visible Platform: Mice were placed in the maze containing a visible platform by extending a patterned cylinder beyond the surface of the water. Mice were given 60 seconds to swim to the platform. Each mouse received 3 sessions with 1 hour between each session of 4 trials each; each trial lasted 60 seconds or the time it took the mouse to reach to the platform. Mice were subjected to the visible platform task on day 7 of the experiment to ensure that visual and motor abilities were normal.

Open Field: Horizontal locomotor activity was measured using the open field photobeam activity system (PAS; SD Instruments). PAS recording software was programmed to collect data over 6 phases consisting of a 5 min interval per phase. The animals were placed in the center of the open field. Photobeam breaks were converted to total distance traveled in cm using the PAS reporter software (version 2). The resting time parameter in the software was set at 4 seconds. A center area was defined using the PAS software as an 8 by 8 inches square in the center of the arena from which Photobeam breaks were also assessed.