

Supplemental figure legends

Supplemental Fig. 1 BCAS2 cKO mice show reduced dentate gyrus (DG) volume and low NeuN⁺ cells in DG versus WT mice. **A** The DG volume was measured by stereological analysis with ImageJ (NIH), and presented by a bar histogram at age 3 and 6 weeks. The 4- μ m paraffin sections of WT and cKO underwent immunohistochemistry assay with anti-NeuN antibody. NeuN⁺ cell density in the DG was quantified by 50 randomly chosen counting frames (N = 3, counting frame: 25 x 25 μ m). Estimated NeuN⁺ cells in DG were calculated by multiplying the granule cell density with DG volume as described [5]. Data are mean \pm SEM by Student's t test, **P* < 0.05, ***P* < 0.01. **B** Newborn neuron measurement (NeuN⁺). The scheme of newborn neuron maturation measurement. Daily BrdU injection for 4 constitutive days at age 6 weeks, followed by IFA of newborn neuron maturation with anti-NeuN antibody on day 28. **C** Representative images of NeuN⁺BrdU⁺ by confocal microscopy. Scale bar: 200 μ m. Quantification of **Ca** number of NeuN⁺BrdU⁺ cells in DG and **Cb** proportion of NeuN⁺BrdU⁺ to total BrdU⁺. Data are mean \pm SEM by Student's t test. **P* < 0.05

Supplemental Fig. 2 CaMKII α -Cre expression pattern in Sox2⁺ NSCs in SGZ of DG. **A** Series of every 12th 40- μ m vibratome sections underwent IFA with anti-Sox2 and anti-Cre antibodies. Images were taken by confocal microscopy. Sox2⁺ cells are indicated by arrowhead and Cre⁺ Sox2⁺ cells by arrows. Scale bar: 100 μ m. **B** More than 1000 Sox2⁺ cells were analyzed and Cre was expressed in NSCs by measuring the percentage of Cre⁺Sox2⁺ cells to Sox2 single-positive cells in the SGZ in WT mice, N = 3.

Supplemental Fig. 3 Reduced number of Ki67⁺ proliferative cells in SGZ of BCAS2-knockdown mice. Representative images of Ki67 expression in DG in shBCAS2- and mock-treated mice. The 40- μ m vibratome coronal brain sections underwent IFA staining for Ki67. Scale bar: 100 μ m.

Supplemental Fig. 4 Reduced number of DCX⁺ immature neurons in hippocampus of BCAS2-knockdown mice. Representative images of DCX expression in hippocampal DG. Expression of DCX and dendrite formation (arrow) after BCAS2 knockdown in 40- μ m vibratome sections after IFA staining. Scale bar: 100 μ m.

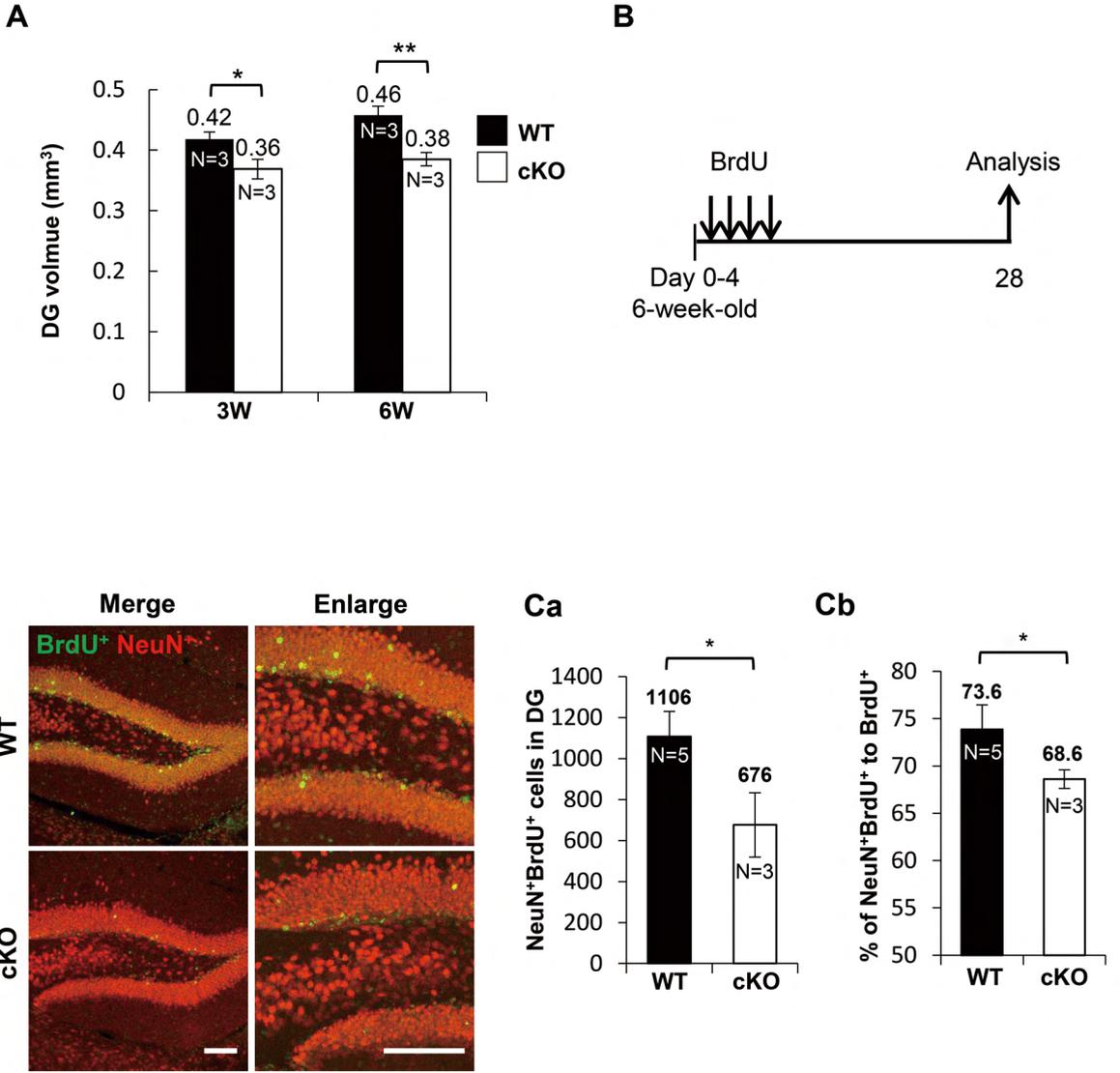
Supplemental Fig. 5 Co-expression of β -catenin and BCAS2 in the inner cell layer of hippocampal granular cell layer of WT mice. **A** Representative images of BCAS2 and β -catenin. The 10- μ m forebrain paraffin sections underwent IFA with mouse anti- β -catenin and rabbit anti-BCAS2 antibodies. WT: β -catenin predominantly expressed in the SGZ and inner granular cell layer of DG and co-stained with BCAS2, indicated by arrow. cKO: IFA analysis of β -catenin expression in Cre-positive cells of cKO. Arrow: low level Cre with high expression β -catenin. Arrowhead: high level Cre with low β -catenin. Scale bar: 20 μ m. **B** Western blot analysis of protein level of β -catenin in the hippocampus of 3-, 6- and 12-week-old cKO mice.

Supplemental Fig. 6 Intracranial hippocampal injection of AAV-BCAS2 can restore cell proliferation in 8-week-old mice. Representative images of Ki67 expression in dentate gyrus in cKO-BCAS2 and cKO-GFP mice. The 40- μ m vibratome brain sections underwent IFA staining for Ki67. Scale bar: 100 μ m.

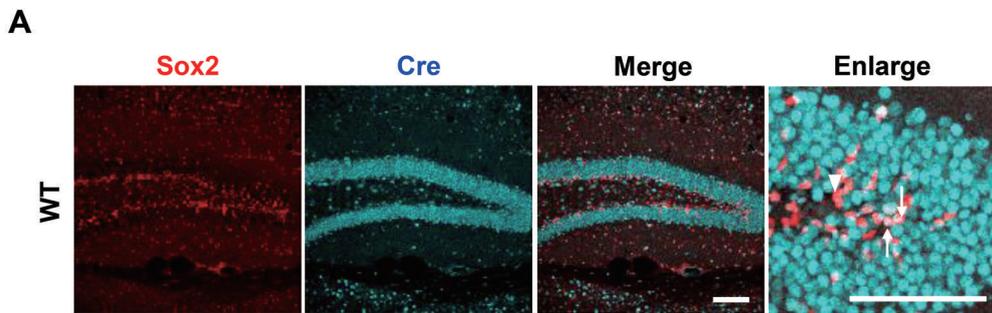
Supplemental Fig. 7 Representative images of DCX expression in hippocampal DG of cKO-BCAS2 mice. Increasing expression of DCX and dendrite formation in AAV-BCAS2-injected cKO mice. The 40- μ m vibratome sections underwent IFA staining for DCX. Scale bar: 100 μ m.

Supplemental Fig. 8 Proliferating cells increased in number in AAV- β -catenin-treated BCAS2 cKO mice. Representative images of Ki67 and Flag (β -catenin) expression in hippocampal DG of cKO- β -catenin mice. Increasing expression of Ki67⁺ proliferating cells in cKO- β -catenin mice. Scale bar: 100 μ m.

Supplemental Figure 1



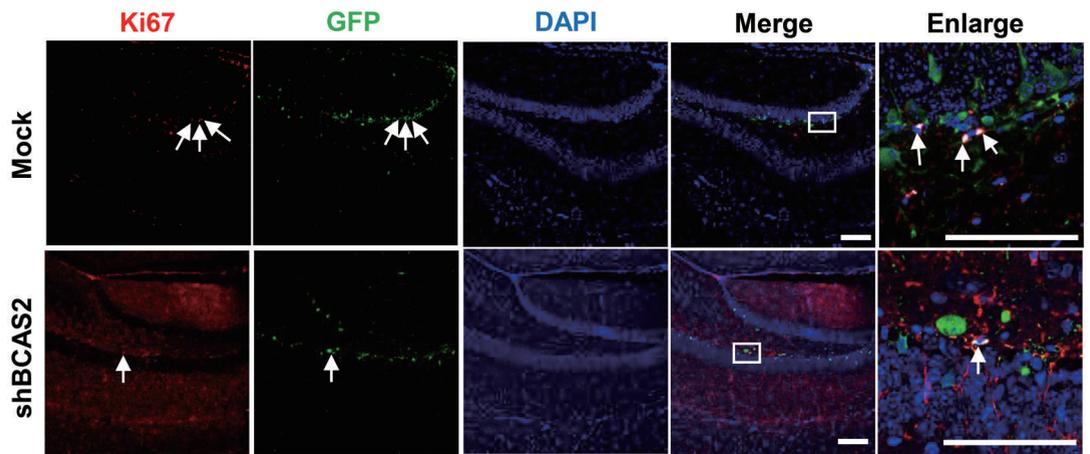
Supplemental Figure 2



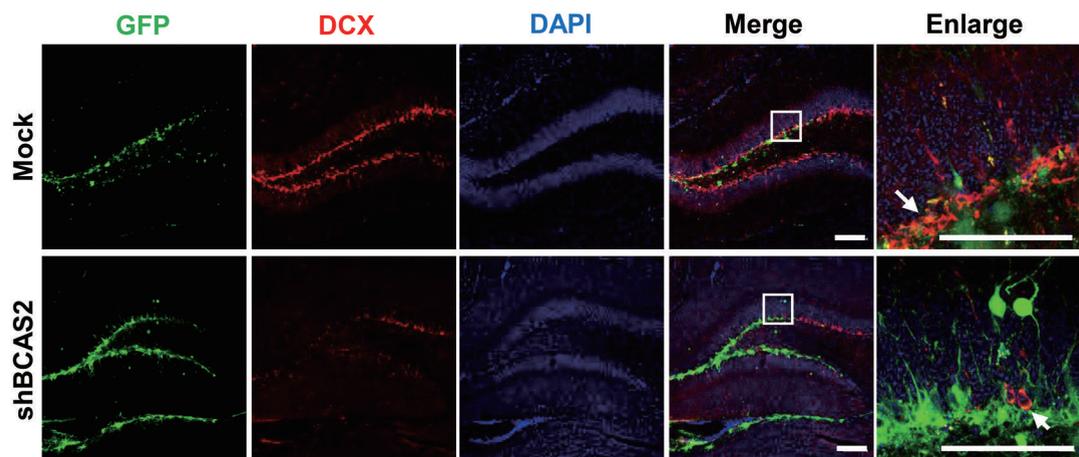
B

	WT Ctrl
Percentage of Cre⁺Sox2⁺/Sox2⁺ (\pmSEM)	3.3% \pm 0.25

Supplemental Figure 3

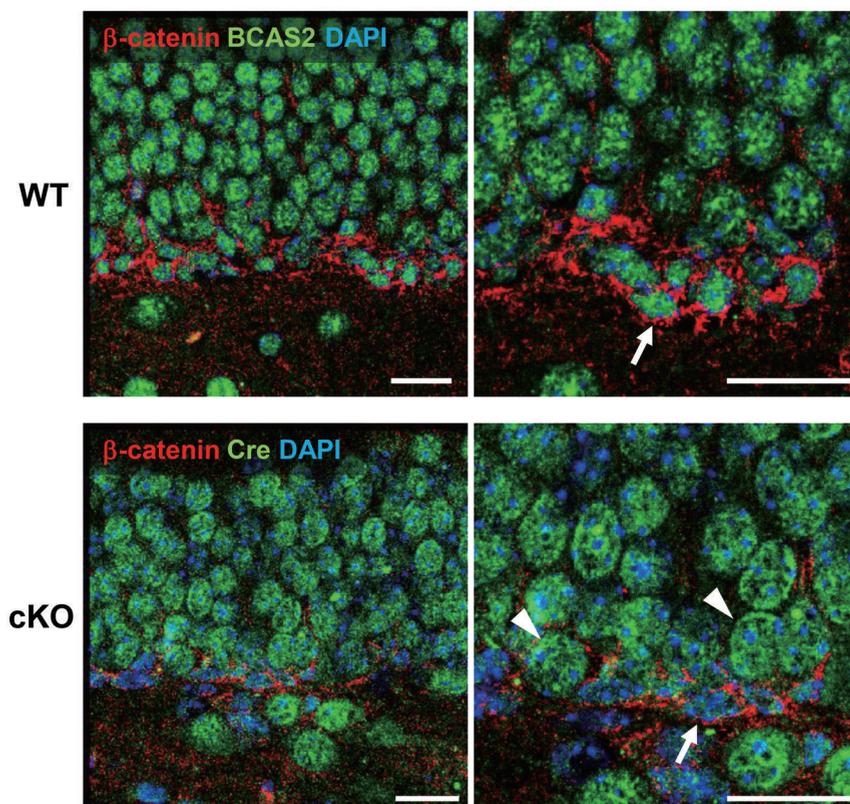


Supplemental Figure 4

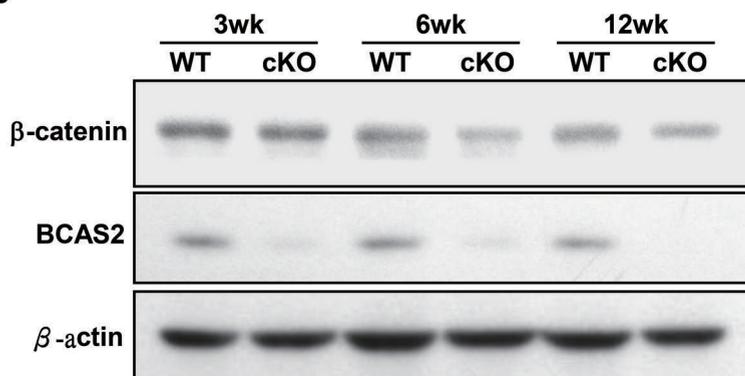


Supplemental Figure 5

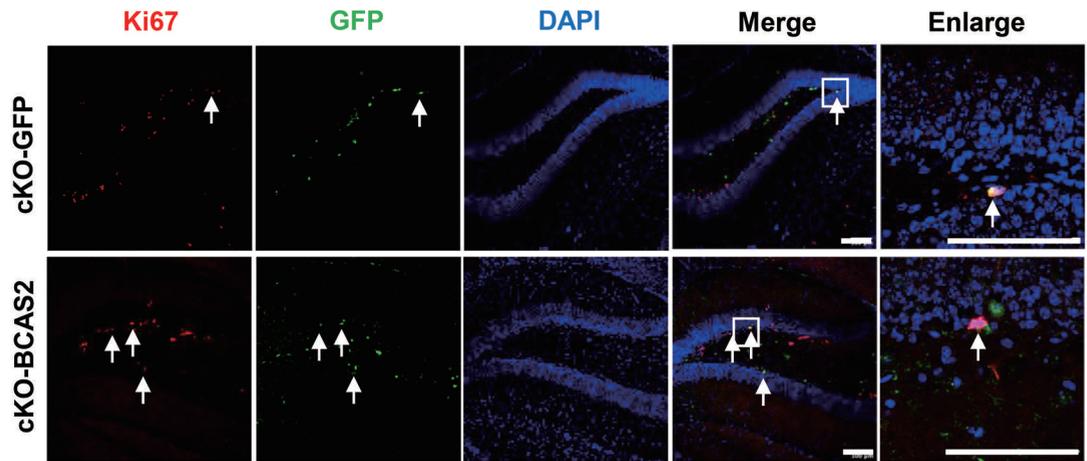
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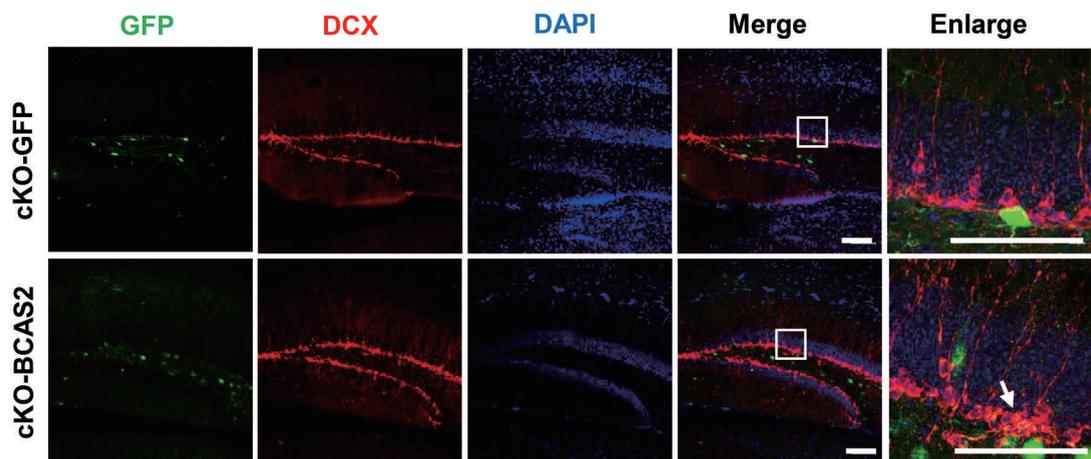
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Supplemental Figure 6



Supplemental Figure 7



Supplemental Figure 8

