Guo et al, 2011

Supplemental Information

The Supplemental Information file includes Supplemental Data, Supplemental Experimental Procedures, and Supplemental References.

Supplemental Data includes 8 supplemental figures.

Figure S1 is related to Figure 1. It shows that FXR2 is expressed in the hippocampus of *WT* mice, and there is no compensatory increase in FMRP levels in *Fxr2 KO* mice. It also shows that FXR2 is expressed in DCX+ young neurons, but not in NG2+ or O1+ oligodendrocyte precursors or S100β+ astrocytes in the adult DG.

Figure S2 is related to both Figures 3 and 4. This figure indicates that FXR2 is expressed in proliferating DG-NPCs. This figure shows FXR2 deficiency does not affect either proliferation or self-renewal analyses of SVZ-NPCs. This figure also shows that reduced GFAP+ astrocytes in differentiated DG-NPCs is not due to increased death of GFAP+ cells.

Figures S3 is related to Figures 2, 3, and 4. This figure shows that acute knockdown of FXR2 in either cultured DG-NPCs or in the NPCs of adult DG leads to altered proliferation and differentiation of NPCs.

Figure S4 is related to Figure 5. This figure shows the differential gene expression in *Fxr2 KO* DG-NPCs based on mouse Neural Stem Cell Pathway Array analysis. It shows the results for RNA-IP validation of the top candidates related to NPC functions

Figure S5 is related to Figures 5 and 6. It shows that FXR2 and Noggin are co-expressed in cultured DG-NPCs and neurons in the adult DG. Noggin levels were increased and p-Smad levels were decreased in *Fxr2 KO* DG tissue. This figure also shows that conditioned medium derived from *Fxr2 KO* DG-NPCs could promote proliferation of WT DG-NPCs.

Figure S6 is related to Figure 7. It illustrates the structure of the lentiviral vector expressing shRNA, validation of Noggin-shRNA, and low magnification images of mouse DG grafted with lentivirus expressing either Noggin-shRNA or control shRNA.

Figure S7 is related to Figure 8. It provides support for the models shown in Figure 8F and 8G. Basically, SVZ-NPCs possess intact intrinsic BMP signaling.

Figure S8 is related to Figure 8. It shows that Noggin expression as detected by an anti-Noggin antibody is restricted to ependymal cells in the SVZ, further confirming the results by using $Noggin^{LacZ}$ mice as shown in Figure 8.

Supplemental Experimental Procedures include detailed materials and methods.
Supplemental References list the references used in the Supplemental Information.

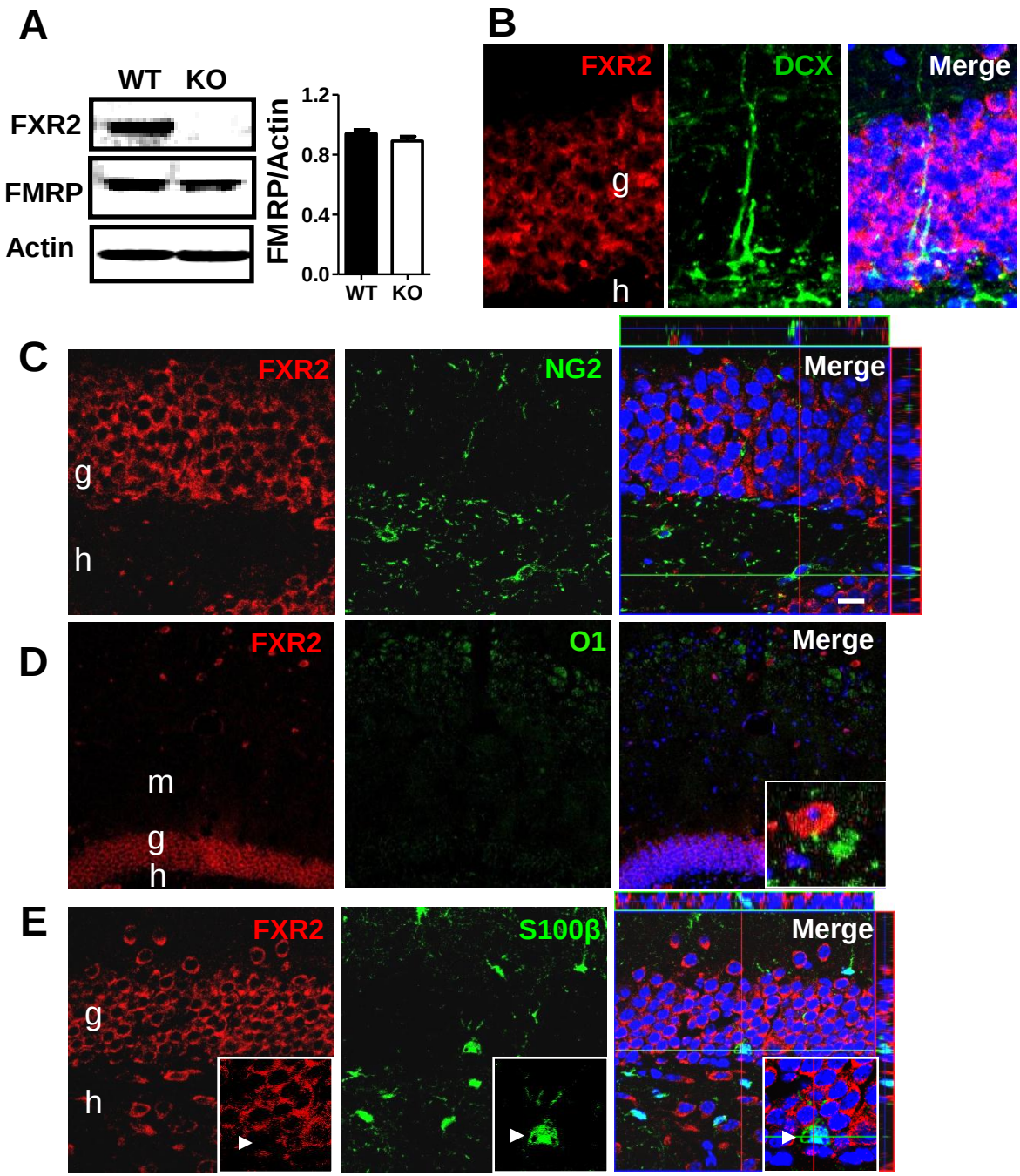

Figure S1 (related to Figure 1). FXR2 is expressed in neurons but not in glia of the adult hippocampus.

(A) The expression levels of FMRP does not change in the *Fxr2 KO* hippocampus. Left panel, representative western blot; right panel, quantification of 3 different western blots. Data are presented as mean ± SEM; *, $p < 0.05$, Student t-test. **(B-E)** In the DG of adult hippocampus, FXR2 (red) is expressed in DCX+ (green) young neurons (B) but not in either NG2+ (green) O1+(green) oligodendrocytes (C and D) or S100β+ (green) astrocytes (E). g, granule cell layer. h, hilar region. m, molecular cell layer. Scale bars = 20 μm.

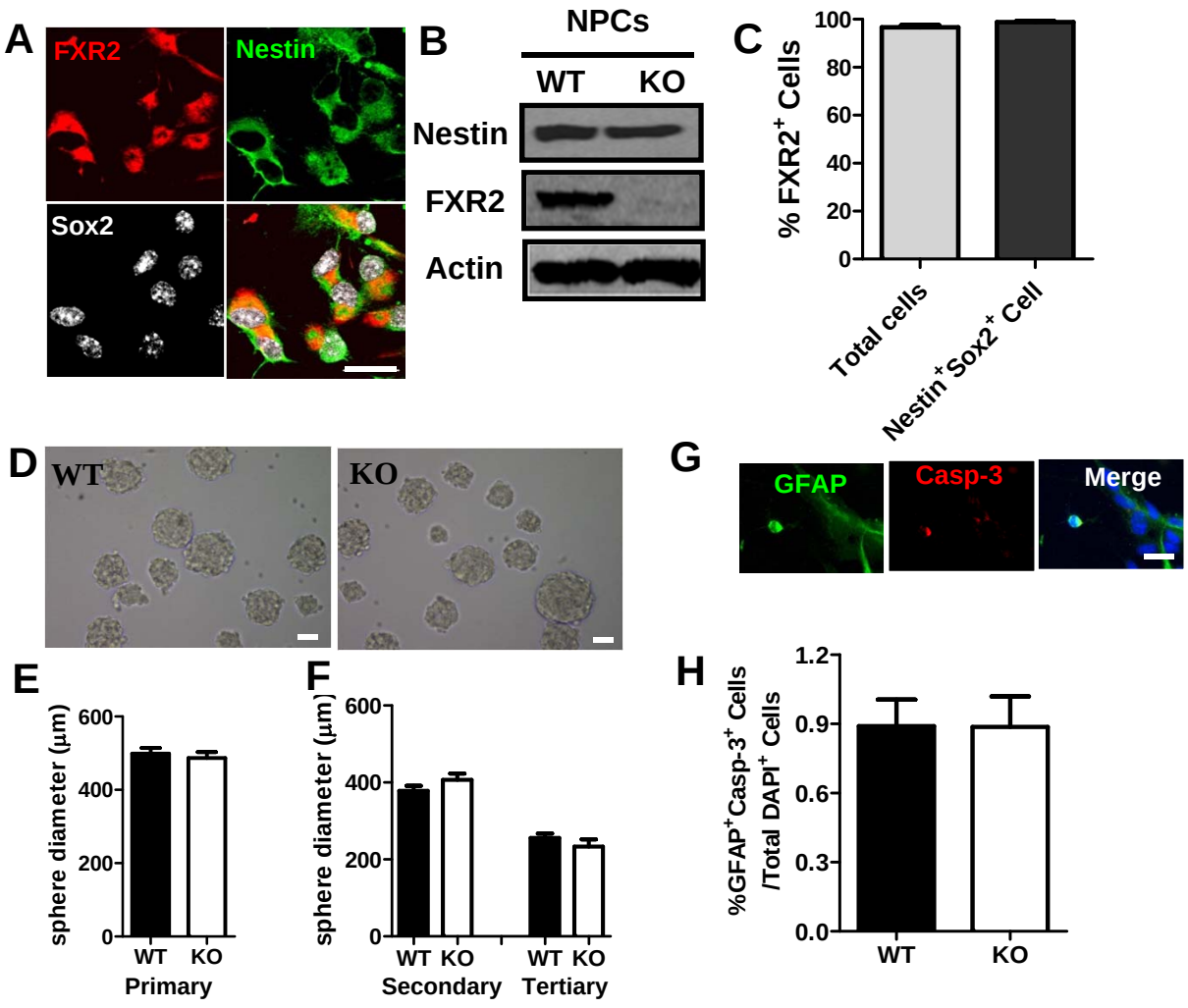

Figure S2 (related to Figure 3 and Figure 4). Astrocytes differentiated from *Fxr2 KO* adult DG-NPCs did not show increased cell death and Loss of FXR2 did not affect the self-renewal capability of SVZ-NPCs.

(A) DG-NPCs cultured under proliferating conditions expressed the neural progenitor markers Nestin (green) and Sox2 (red in the left panel), and also expressed FXR2 (red in the right panel). Scale bar = 20 μm. **(B)** Western blot showing that WT DG-NPCs expressed FXR2. (**C)** Quantification analyses showing percentage of cell expressed FXR2. **(D)** Sample images of WT and *Fxr2 KO* SVZ neurospheres. **(E)** The size (diameter) of primary neurospheres generated from WT and KO adult SVZ showed no difference. Scale bar = 50 μm. **(F)** The size (diameter) of secondary and tertiary neurospheres generated from WT and KO SVZ showed no difference. (n=3), Data is presented as mean ± SEM. **(G)** Examples of differentiated DG-NPCs stained with antibodies against GFAP for astrocyte lineage and active-Caspase-3 for apoptotic cells. Scale bar = 20 μm. **(H)** Quantification analyses showing that decreased astrocyte differentiation of *Fxr2 KO* DG-NPCs was not caused by increased astrocyte death. Data are presented as mean ± SEM.

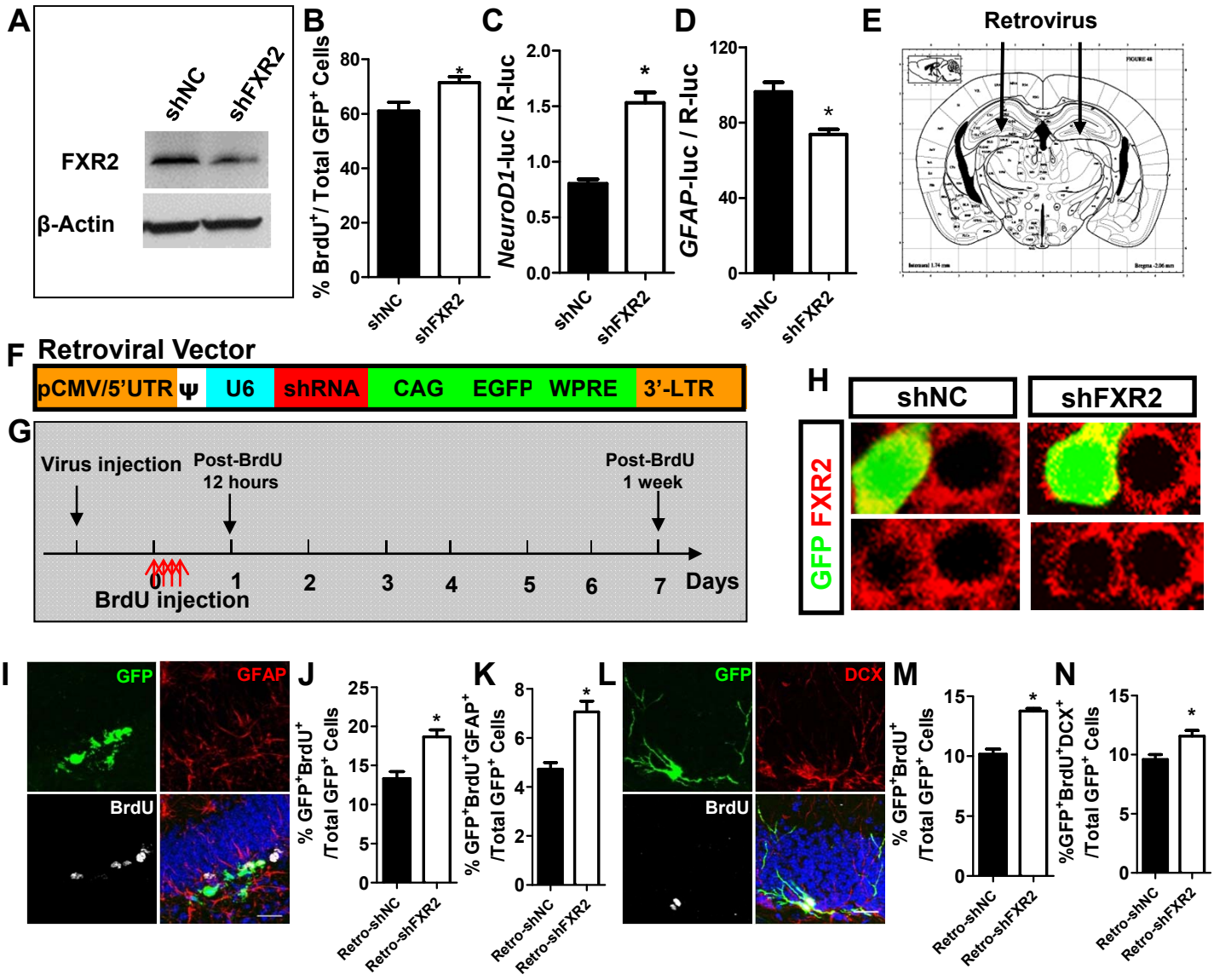

Figure S3 (relative to Figure 2,3,4). Acute knockdown of Fxr2 Affects Stem Cell Proliferation and Differentiation in vivo and in vitro.

(A) Western blot showing that the Fxr2-shRNA transfected into NPCs could efficiently reduce the endogenous Fxr2 protein levels. shNog, Noggin-shRNA. shNC, non-silencing control shRNA. **(B)** Quantitative analysis showing that acute knockdown of Fxr2 in DG-NPCs incorporated more BrdU compared with NC controls (N = 3, P<0.05). **(C, D)** Acute knockdown of Fxr2 in DG-NPCs increased neuronal differentiation (C) but reduced astrocyte differentiation (D) in vitro as demonstrated by NeuroD1 promoter activity and GFAP promoter activity assay respectively. A cotransfected Renilla luciferase (R-Luc) plasmid was used as a transfection control (n = 3, p<0.05). **(E)** Retroviral vector expressing shRNA also expressed eGFP. **(F)** Experimental scheme for assessing neural stem cell proliferation and early stage differentiation in adult mice after retrovirus graft. **(G)** retrovirus grafting scheme in adult DG. **(H)** Examples of retroviral infected cells showing that shFXR2-virus-infected cells had reduced FXR2 protein expression compared to shNC virus-infected cells. shRNA (green), FXR2 (red). Lower panel shows the same cells as the upper panel without the green channel. **(I)** Examples of brain sections (12 hours post-BrdU injection) stained with antibodies against GFP (green), GFAP (red), and BrdU (white). DAPI, blue. Scale bar = 20 μm. **(J, K)** Acute knockdown of Fxr2 showed increased BrdU incorporation (J, GFP+BrdU+) and promoted proliferation of stem cells (K, GFP+BrdU+GFAP+) in adult DG analyzed at 12 hours after the last BrdU injection (n = 3, p < 0.05). **(L)** Examples of brain sections stained with antibodies against immature neuronal marker DCX (red) and BrdU (white) at one week post-BrdU injection to assess early stage neuronal differentiation. Scale bar = 20 μm. **(M, N)** Acute knockdown of Fxr2 showed higher percentage of BrdU+ cells (M) and BrdU+DCX+ (N) in GFP+ cells suggesting increased early neuronal differentiation (n = 3, p < 0.01).

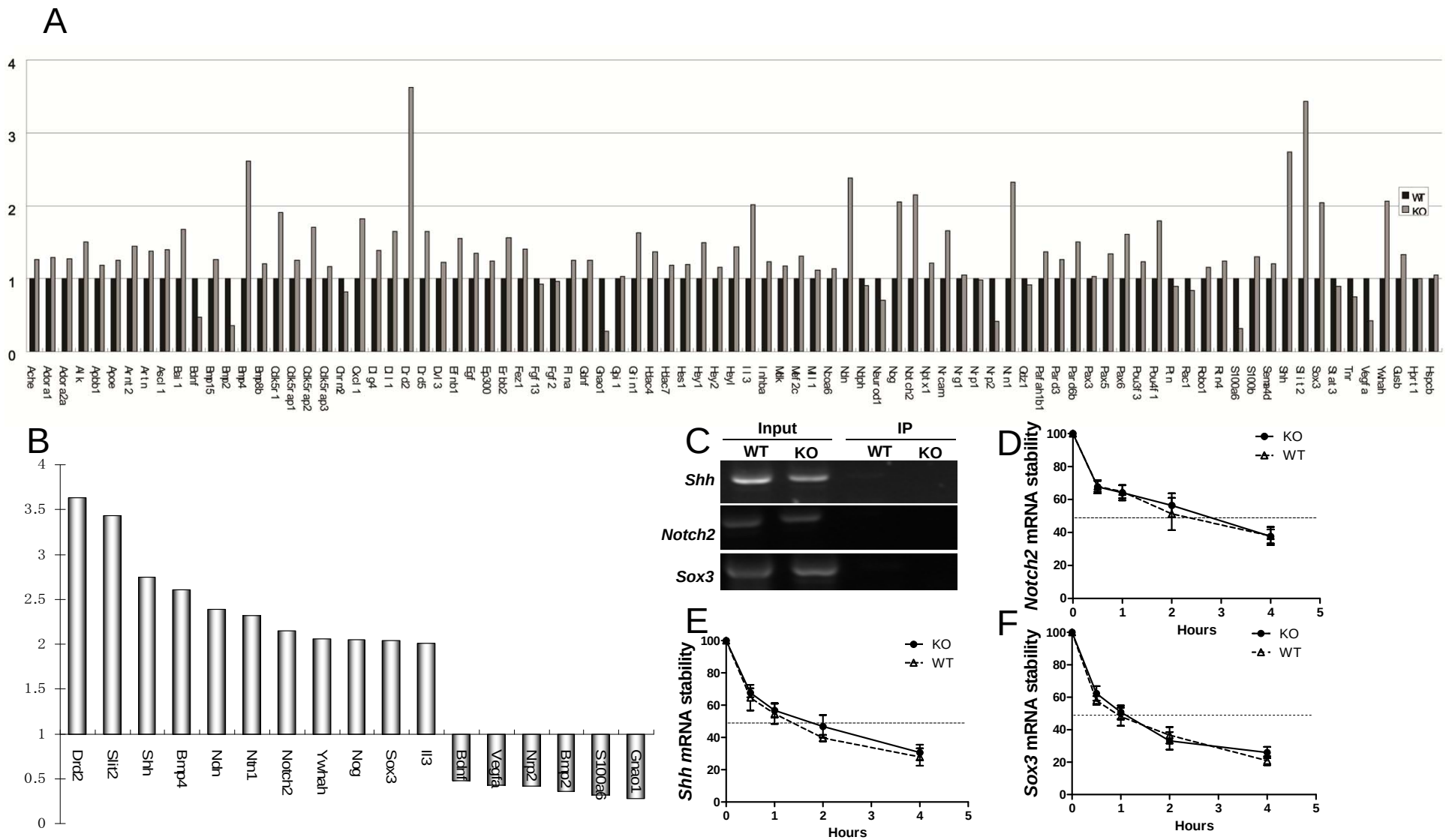

Figure S4 (related to Figure 5). Gene expression changes in *Fxr2 KO* DG-NPCs compared to WT cells

(A) Results of Neural stem cell pathway arrays showing multiple genes with differential expressed in *Fxr2 KO* DG-NPCs relative to WT DG-NPCs (WT is normalized to"1"; n=3). White bar, KO. Black bar, WT. **(B)** The genes with > 2 Fold changes were shown. **(C)** FXR2 RNA-IP followed by RT-PCR assays showing that FXR2 did not bind Shh, Notch2, and Sox3 mRNAs in DG-NPCs. **(D-F)** DG-NPCs were treated with Actinomycin D to inhibit gene transcription and the percentage of *Notch2* (D), *Shh* (E) and *Sox2* (F) mRNA in *Fxr2 KO* and WT cells was quantified using real-time PCR at the indicated time point (n = 3). *Fxr2 KO* cells did not change these mRNAs stability.

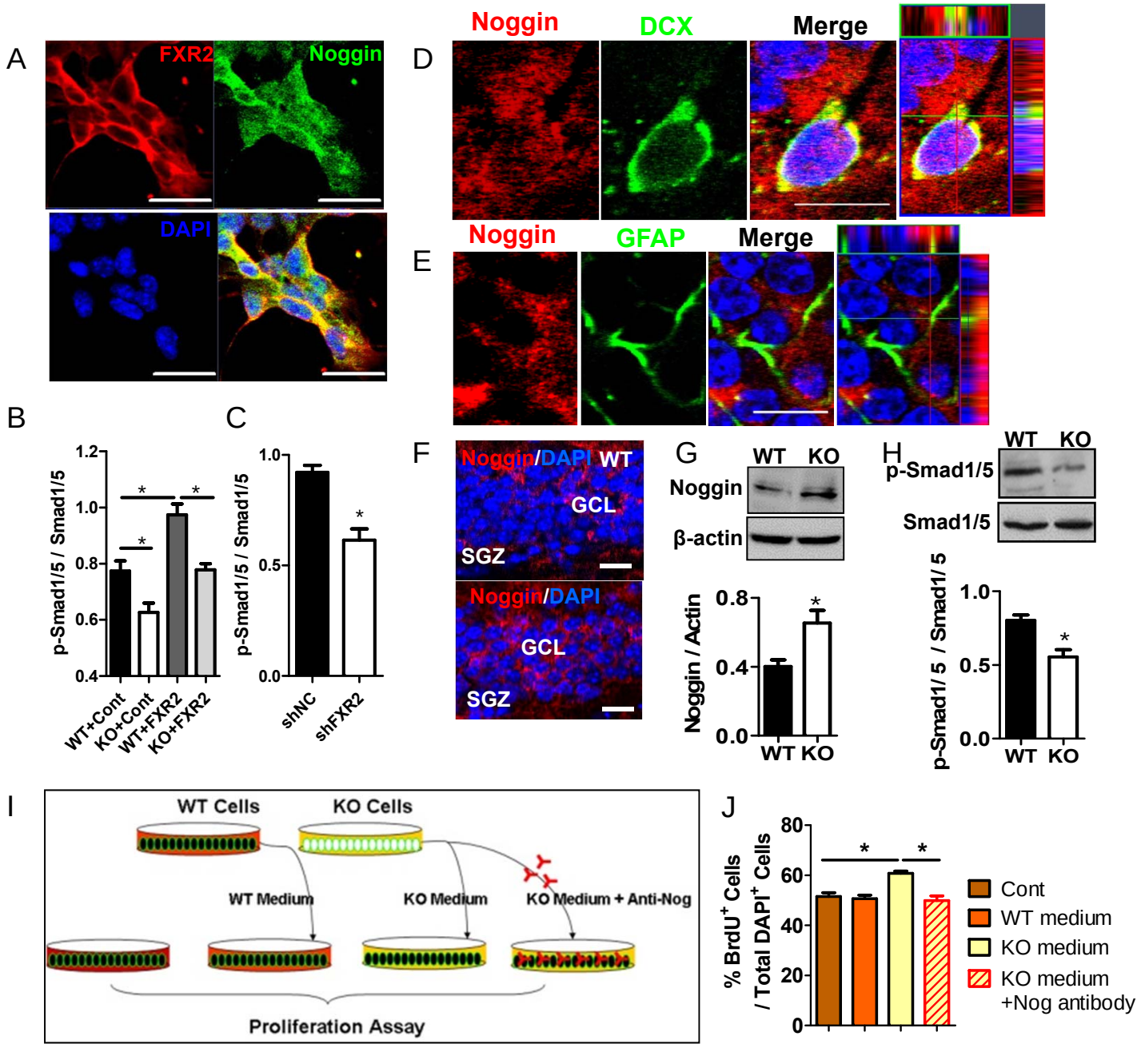

Figure S5 (relatived to Figure 5 and Figure 6) Noggin expressed in NPCs in vitro and neuronal lineage in vivo

(A) DG-NPCs cultured under proliferating conditions expressed Fxr2 (red) and Noggin (green). **(B)** Quantification of western blot for Figure 5K showing that exogenous FXR2 rescued the Smad1/5 phosphorylation levels in *Fxr2 KO* DG-NPCs. **(C)** Quantification of western blot for Figure 5M showing that acute knockdown of FXR2 in *WT* DG-NPCs led to decreased Smad1/5 phosphorylation. **(D)** Noggin (red) was expressed in DCX+ cells in adult DG. **(E)** Noggin (red) may be expressed in GFAP+ astrocyte outside of SGZ. **(F)** WT and *Fxr2 KO* brain sections containing the DG stained with an antibody against Noggin (Noggin, red; DAPI, blue). **(G, H)** Western blotting analysis demonstrates that Noggin expression was increase, and then p-Smad1/5 levels were decreased in the hippocampal tissue dissected from Fxr2 KO mice compared with that from WT mice (n = 3, $p < 0.05$) **(I)** Experimental scheme for assessing conditional medium (with secretive noggin) from *Fxr2* WT and KO DG-NPC on NPCs proliferation differentiation. **(J)** Quantification data showed conditional medium form *Fxr2 KO* DG-NPCs could promote WT DG-NPCs differentiation, but this effect can be blocked by adding anti-Nog antibody. Data are presented as mean ± SEM; *, $p < 0.05$. All scale bars = 20 μm

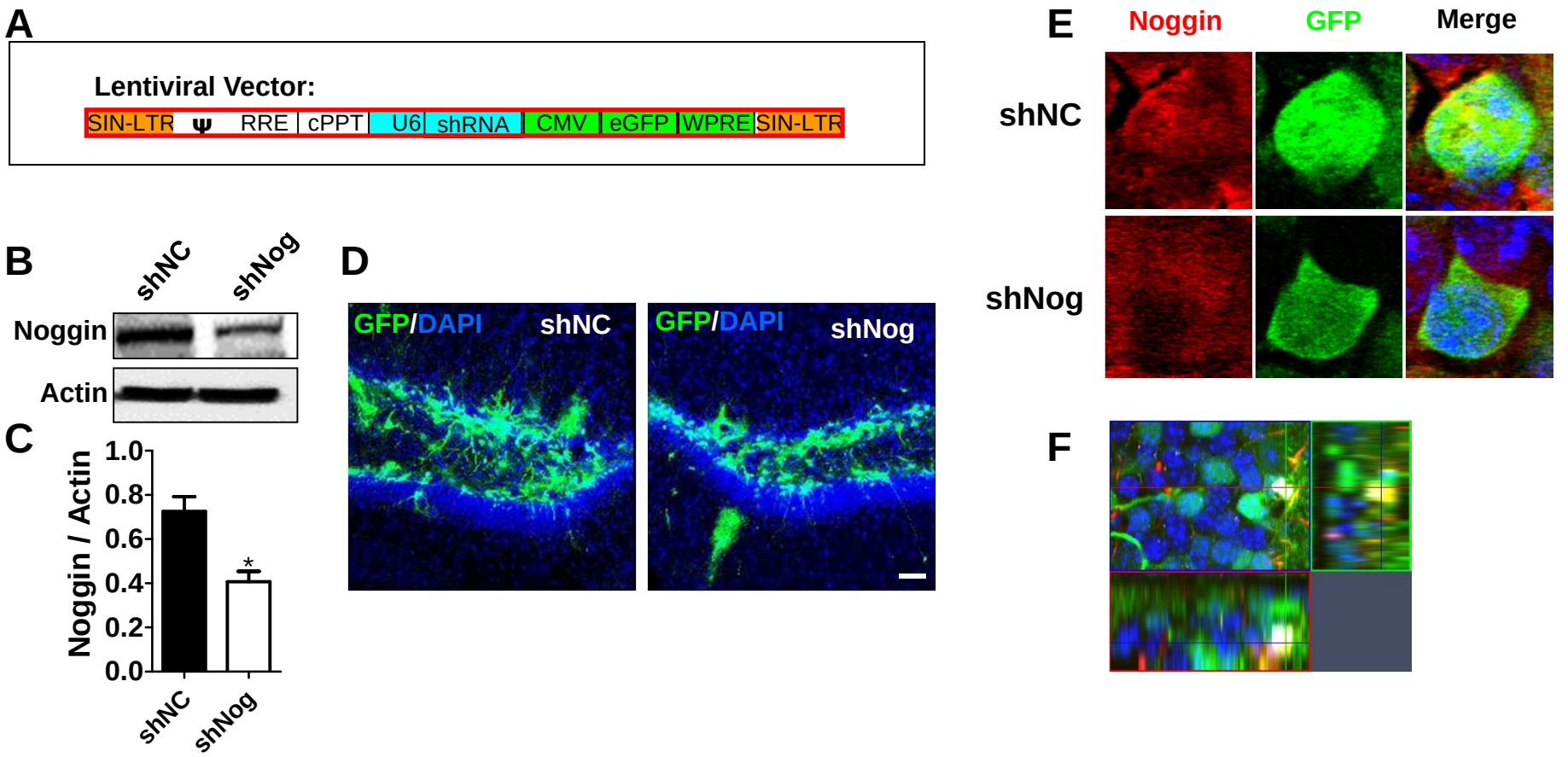

Figure S6 (related to Figure 7). Lentivirus expressing Noggin-shRNA

(A) Lentiviral vector expressing shRNA also expressed eGFP. **(B, C)** Western blot and quantfication showed that the Noggin-shRNA transfected into P19 embryonic carcinoma cells could efficiently reduce the endogenous Noggin protein levels. shNog, Noggin-shRNA. shNC, non-silencing control shRNA. **(D)** Sample images of mouse brain sections from animals with lentivirus grafted into adult mouse DG analyzed at 3-week post-viral grating (see Figure 7A). Based on a published criteria (Clelland, et al 2009), we only included the animal in the data analysis shown in Figure 7, if lentivirus-infected (GFP+) cells were found in > 50% of the DG area of adult hippocampus as shown in the figure,. Scale bar = 100 µm. **(E)** Example of a lentivirus-sh-Nog-infected cells (green) expressed reduced amount of Noggin protein compared to a lentivirus-sh-NC-infected cell, as assay by immunostaining with a anti-Noggin antibody (red**) (F)** Orthogonal image of the cell that is positive for GFP (green), GFAP (red), BrdU (white), and Dapi (blue) as shown in Figure 7F (arrow pointed).

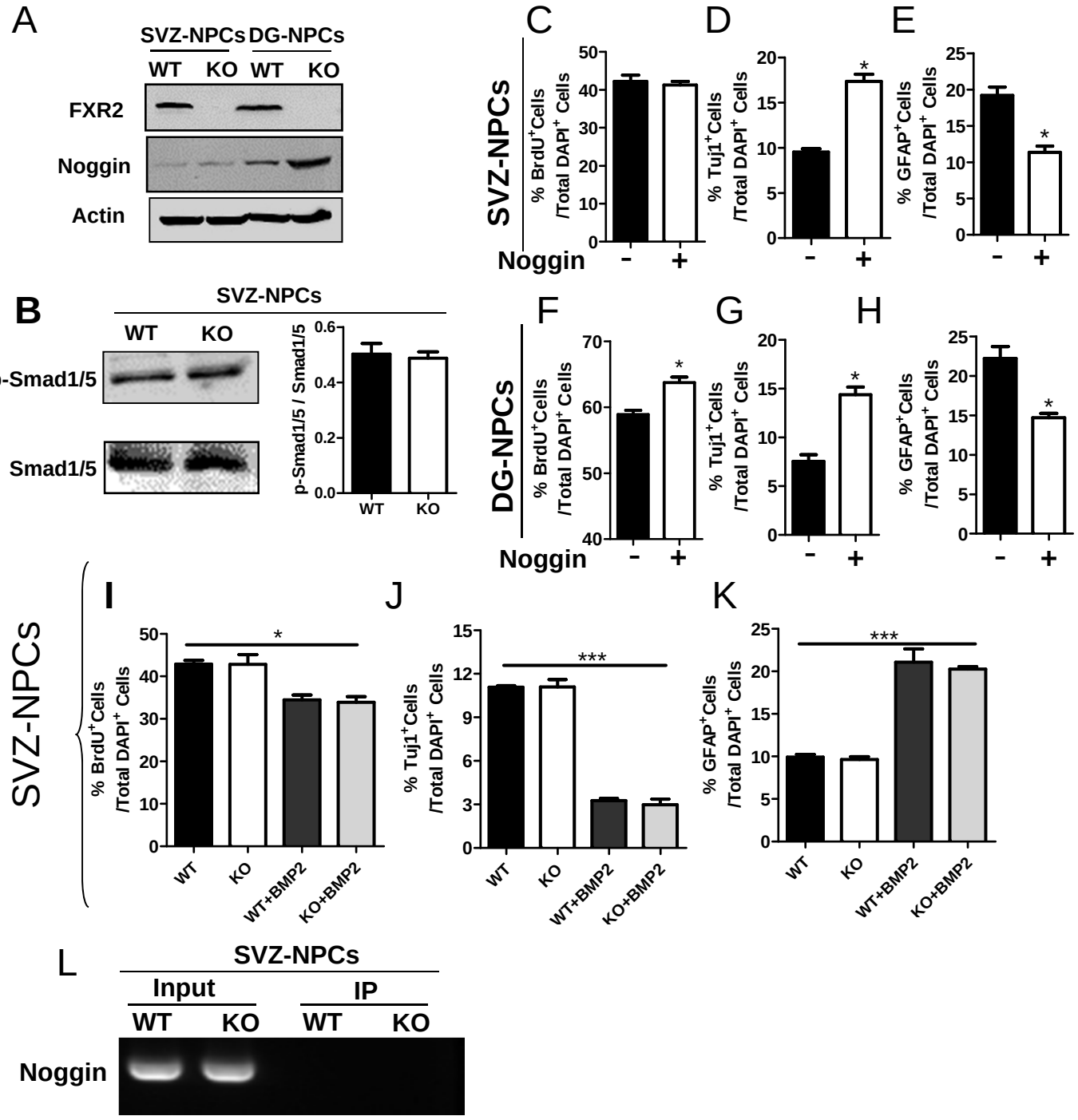

Figure S7 (related to Figure 8) The effect of exogenous Noggin and BMP2 on proliferation and differentiation of SVZ-NPCs and DG-NPCs.

(A) Western blot showing that very low level of Noggin was detected in cultured SVZ-NPCs. **(B)** The p-Smad1/5 levels were no different between WT and KO SVZ-NPCs, suggesting no alteration in BMP signaling in *KO* SVZ-NPCs. **(C-E)** In SVZ-NPCs, Noggin did not affect the proliferation (C), but promoted neuronal differentiation (D) and repressed astrocyte differentiation (E). **(F-H)** In DG-NPCs, Noggin increased the proliferation (F), incresed neuronal differentiation (G) but decrease astrocyte differentiation (H). **(I-K)** in both *WT* and *KO* SVZ-NPCs, exogenous BMP2 repressed proliferation (I), repressed neuronal differentiation (J), and enhanced astrocyte differentiation (K). **(L)** RNA-IP followed by RT-PCR assay indicate that FXR2 did not bind *Noggin* mRNA in SVZ-NPCs. Data are presented as mean ± SEM; *, $p < 0.05$, **, $p < 0.001$, ***, $p < 0.0001$ Student t-test.

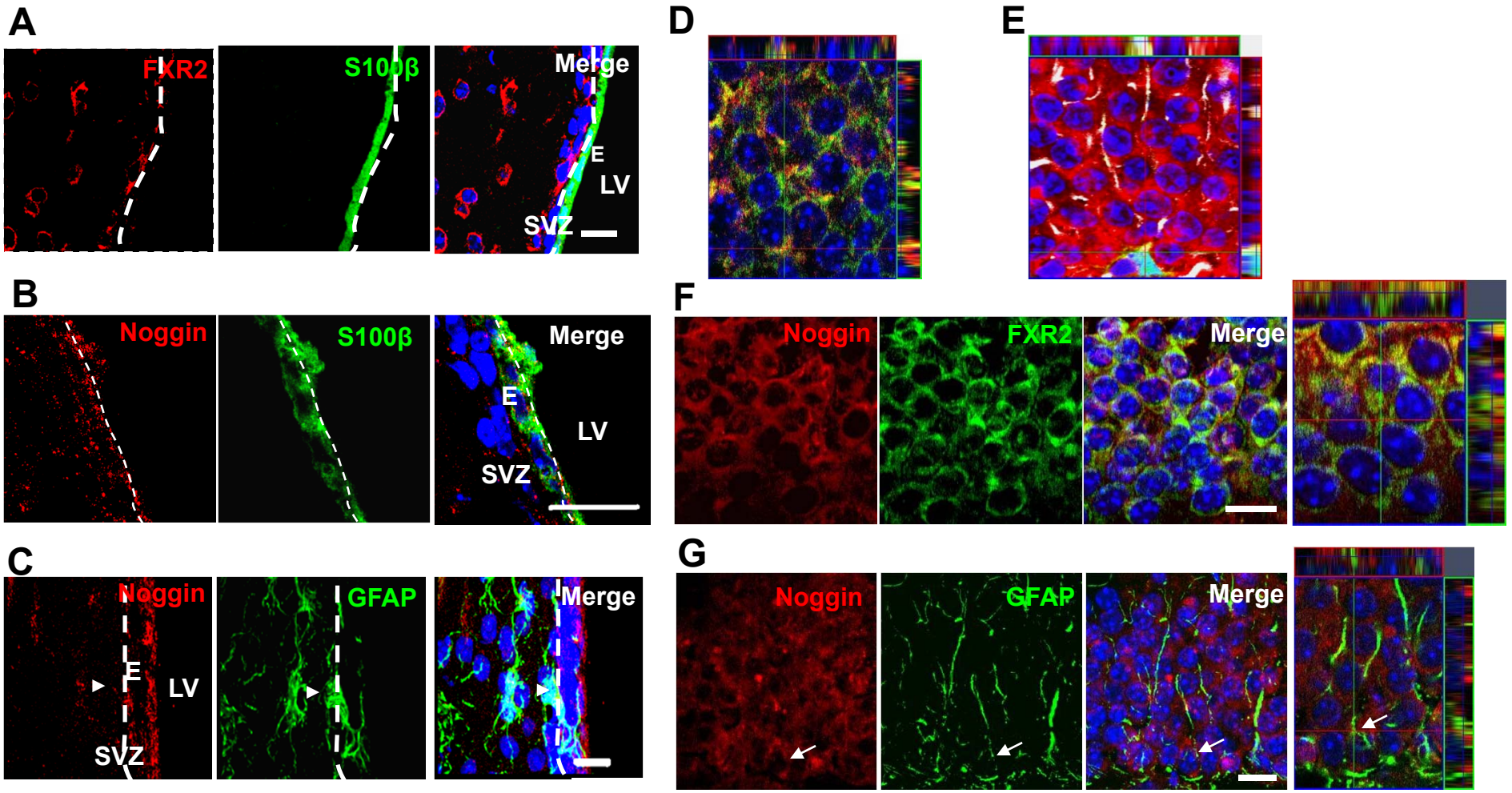

Figure S8 (related to Figure 8). Noggin and FXR2 are Colocalized in the DG but Not in the SVZ
(A) FXR2 (red) was not expressed in S100β+ (green) ependymal cells in the SVZ. Scale bars = 10 μm. **(B)** Noggin (red) was expressed in S100β+ (green) ependymal cell in the SVZ. Scale bars = 20 μm. **(C)** Noggin (red) was not expressed in GFAP+ (green) neural stem cells in SVZ. Scale bars = 5 μm. **(D)** Orthogonal image of a cell that is positive for FXR2 (green), Noggin (red), and Dapi (blue) as shown in Figure 8D. **(E)** Orthogonal image of the cell that is positive for Nestin (green), GFAP (white), Noggin (red), and Dapi (blue) as shown in Figure 8E. **(F)** FXR2 (green) and Noggin (red) were colocalized in neurons of the DG. Scale bars = 10 μm. **(G)** Noggin (red) was expressed in GFAP+ (green) neural stem cells in the DG (arrow). Scale bars = 10 μm.

Supplemental Experimental Procedures

In vivo Cell Proliferation and Differentiation Analysis and Immunohistology

In vivo neurogenesis analyses were performed essentially as described in our publications (Guo et al., 2011; Luo et al., 2010; Smrt et al., 2007; Zhao et al., 2003). Mice were given 4 injections of BrdU (50mg/kg) within 12 hours to label all dividing cells in adult germinal zones within this time period and sacrificed at 4 hours post-last injection based on published paradigm (Hayes and Nowakowski, 2002). Mice were then euthanized either at 12 hours or 1 week after the last BrdU injection, by intraperitoneal injection of sodium pentobarbital followed by transcardially perfusion with saline followed by 4% paraformaldehyde (PFA). Brains were dissected out, post-fixed overnight in 4% PFA, and then equilibrated in 30% sucrose. Forty-μm brain sections were generated using a sliding microtone and stored in a -20℃ freezer as floating sections in 96-well plates filled with cryoprotectant solution (glycerol, ethylene glycol, and 0.1M phosphate buffer, pH 7.4, 1:1:2 by volume).

We performed immunohistological analysis on 1-in-6 serial floating brain sections (240 μm apart) based on the published method (Luo et al., 2010; Smrt et al., 2007; Zhao et al., 2003). The primary antibodies used were: rat-anti-BrdU (1:3000, Abcam, ab-6326), rabbit anti-S100β (1:500; Sigma), chicken anti-Nestin (1:1000, Aves, #mNES), rabbit anti-GFAP (1:1000, Dako, Z0334), Doublecortin (1:200, Santa Cruz), and mouse anti-FXR2 (1:500, Sigma), goat anti-Noggin (1:200, R&D), mouse anti-β-galactosidase (1:50, Developmental Studies Hybridoma Bank), chicken anti-β-galactosidase (ab9361,1:200, Abcam), mouse anti-Sox2 (MAB4343, 1:1000, Millipore), Rabbit anti-O1 (ab7474, 1:500, Abcam). Fluorescent secondary antibodies were used at 1:250 dilutions (donkey from Jackson ImmunoResearch

or goat from Invitrogen). After staining, sections were mounted, coverslipped, and maintained at 4ºC in the dark until analysis. BrdU-positive cells in the granule layer were counted using unbiased stereology (StereoInvestigator, MBF Biosciences, Inc) with a 5-μm guard zone, and the total number of BrdU+ cells in the DG was then calculated by StereoInvestigator, as described in our publications. The results were presented as number of BrdU+ cells in a cubic millimeter of dentate gyrus (DG). Phenotype analysis of BrdU+ cells was performed as described previously (Luo et al., 2010; Smrt et al., 2007; Zhao et al., 2003). Briefly, 50 BrdU+ cells in the DG were randomly selected, and their phenotypes (double labeling with either NeuN, S100β, GFAF, or Nestin) were determined using either a Zeiss LSM510 laser scanning confocal microscope or a Nikon TE2000 microscope equipped with a spin disc confocal microscope and MetaMorph quantification software. The data were analyzed using a Student's t-test (GraphPad software, www.graphpad.com).

Isolation and Culture of Adult NPCs

NPCs used in this study were isolated from the DG and SVZ of 8 to 10-week-old male *Fxr2 KO* mice and wild-type (*WT*) littermate controls based on published methods (Babu et al., 2007; Bull and Bartlett, 2005; Luo et al., 2010; Seaberg and van der Kooy, 2002). Briefly, SVZ region and DG region were dissected from 400 um coronal sections of forebrain under a microscope. After enzymatic digestion using MACS Neural Tissue Dissociation kit (Miltenyi Biotech, Germany), we added 5 ml of DMEM/F-12 containing 10% FBS (Sigma-Aldrich, #F 4135), 2 mM L-glutamine (GIBCO, #25030-081), and 1% Antibiotic-Antimycotic (GIBCO, #15240-062) into each sample to stop digestion. After filtering through a 70-μm cell strainer

(BD Falcon, #252350, CA) and washing with DMEM/F-12 (2 mM L-Glutamine, 1% Antibiotic-Antimycotic), the single-cell suspension was collected and cultured with DMEM/F-12 medium containing 20 ng/ml basic fibroblast growth factor (FGF-2, PeproTech, #K1606), 20 ng/ml epidermal growth factor (EGF, PeproTech, #A2306), 1% N2 supplement (GIBCO, #17502-048), 1% Antibiotic-Antimycotic, and 2 mM L-glutamine in a 5% CO2 incubator at 37°C. Half of the medium was replaced every two days.

Proliferation and Differentiation Analyses of Cultured NPCs

Proliferation and differentiation of aNPCs were analyzed using our published method (Barkho et al., 2008; Liu et al., 2010; Luo et al., 2010; Szulwach et al., 2010). We used only early passage cells (between passage 4 and 10) and only the same passage numbers of wild type (*WT*) and *Fxr2 KO* cells. For each experiment, triplicate wells of cells were analyzed, and results were averaged as one data point (n = 1). At least 3 independent experiments (n = 3) were performed and used for statistical analyses for each analysis. To study cell proliferation, we dissociated neural stem cells with trypsin and plated them on poly-L-ornithin/laminin-coated chamber slides (Nunc, #154526) at a density of 50,000 cells/well in proliferation medium (see above). At 20 h post-plating, 5 μM 5-bromo-2'-deoxyuridine (BrdU, Sigma-Aldrich) was added into the culture medium for 16 hours. NPCs were then washed with PBS and fixed with 4% paraformaldehyde for 30 min at room temperature, followed by immunohistochemical analysis. To detect BrdU incorporation, fixed cells were pretreated with 1M HCl for 30 min at 37°C, and then washed with borate buffer, pH 8.5, for 30 min. We then followed our standard immunohistochemistry protocol.

Neurosphere self-renewal assays were performed based on published methods (Liu et al., 2010; Seaberg and van der Kooy, 2002). Briefly, neurospheres from newly isolated primary culture (passage 0) were collected and dissociated to single cells using trypsin digestion. Cell viability was assessed using 0.4% trypan blue exclusion. Dissociated cells were plated at 1 viable cell per µl (1000 cells per 35 mm dish) in proliferation medium (see above) onto uncoated 35mm dishes. Lentivirus was added to the dish at one day post-plating. Fresh medium was added to the culture dishes every other day. The total number of spheres that formed in each dish was counted after a 7-day in vitro culture. Only phase bright live spheres containing at least 10 cells were counted.

For the differentiation assay, at 24 h post-plating, cells were changed into differentiation medium, DMEM/F12 (1:1), containing 5 µM forskolin (Fsk, Sigma-Aldrich, #F-6886), 1 µM retinoic acid (RA, Sigma-Aldrich, #R-2625), and sometimes with 0.5% fetal bovine serum (FBS, Sigma-Aldrich, #F-2442) for 4 days, followed by fixation with 4% paraformaldehyde for 30 min, then washing with Dulbecco's Phosphate-Buffered Saline, pH 7.4 (DPBS) for 30 min. Immunocytochemistry staining was carried out as described (Luo et al; 2010; Liu et al; 2010). Briefly, cells were pre-blocked using DPBS containing 5% normal goat serum (VECTOR, #S-1000) and 0.1% Triton X-100 for 30 min, followed by overnight incubation with primary antibodies: mouse neuron-specific type ß-III tubulin (Tuj1, 1:4000, Promega, #G712A), rabbit glial fibrillary acidic protein (GFAP, 1:1000, DAKO, #Z-0334), or rat anti-BrdU (1:3000, Abcam, ab-6326). After washing with DPBS, cells were incubated with secondary antibodies that included goat anti-mouse Alexa Fluor 568 (1:500, Invitrogen, #A11031), goat anti-rabbit Alexa Fluor 647 (1:500, Invitrogen, #A21245), or goat anti-rat Alexa Fluor 568 (1:500, Invitrogen,

#A11077), followed by counterstaining with the fluorescent nuclear dye 4',6-dimidino-2'-phenylindole dihydrochloride (DAPI, Sigma-Aldrich, #B2261). After the cells were mounted with VECTASHIELD (VECTOR, #H-1000), the numbers of Tuj1, GFAP, BrdU, or activated caspase-3-positive cells were quantified using an Olympus BX51 microscope equipped with a MicroFire digital camera (Optronics) and a motorized stage using a 20X objective lens. The quantification was carried out using an unbiased stereology method with assistance from StereoInvestigator software (MBF Biosciences). The percentage of differentiated cells was calculated as the number of Tuj1- or GFAP-labeled cells divided by the total number of cells stained with DAPI. The percentage of proliferating cells was defined as the number of BrdU-labeled cells divided by total DAPI-positive cells.

Cell Transfection and Luciferase Assay

Transfection of aNPCs was carried out using Stemfect kit (Stemgent, San Diego, CA) based on the manufacturer's protocol and our publication (Luo et al., 2010). Briefly, aNPCs were plated into 24-well P/L-coated plate for 24 hours. Then 3 µg DNA and 0.9 µl Stemgent reagent were mixed, incubated for 10 minute, and then added to the cells. Sixteen hours later, the transfected cells were then changed into differentiation medium for 48 hours. The cells were then collected and luciferase activity was detected using the Dual-Luciferase Reporter 1000 System (Promega, Cat# E1980) based on the manufacturer's protocol. Briefly, collected cells were lysed in 100 µl of 1X passive lysis buffer at room temperature for 15 min. Then 20 µl of the lysate was added to 100 µl of Luciferase Assay Buffer II and mixed briefly. Firefly luciferase (F-luc) activity was immediately read using a SpectraMax M2E plate reader

(Molecular Devices Corp.). Next, 100 µl of Stop & Glo Buffer with Stop & Glo substrate was added and mixed briefly. Renilla luciferase (R-luc) activity was immediately read. F-luc activity was normalized to R-luc activity to account for variation in transfection efficiencies. Each experiment was independently repeated 3 times. For each electroporation, 3 µg (NeuroD1- or GFAP-) luciferase DNA, 0.2 µg R-Luc.

RNA Immunoprecipitation (IP)

RNA-IP was carried out as described (Brown et al., 2003; Luo et al., 2010). Briefly, *WT* and *Fxr2 KO* NPCs (2 X 10^6) were harvested and homogenized in 2 ml of ice-cold lysis buffer (10 mM Hepes [pH 7.4], 200 mM NaCl, 30 mM EDTA, and 0.5% Triton X-100) with 2X complete protease inhibitors (Boehringer-Mannheim) and 400 U/ml rRNasin (Promega). Nuclei and debris were pelleted at 3,000 X g for 10 min; the supernatant was collected and raised to 300 mM NaCl, and clarified at 14,000 X g for 30 min. The resulting supernatant was pre-cleared for 1 h with 100 µl recombinant protein G agarose (Invitrogen) (washed with lysis buffer first). An aliquot of precleared input was saved for RNA extraction (200 µl) and protein analysis (100 µl). A monoclonal antibody against FXR2 (F1554, Sigma) was incubated with recombinant protein G agarose at 4ºC for 2 h and washed 3 times with lysis buffer. RNase Inhibitors (Invitrogen) will be added to the remaining lysates. The precleared lysates were immunoprecipitated with antibody-coated recombinant protein G agarose at 4ºC overnight. After third wash with the lyses buffer, 10% of immunoprecipitate was saved for protein analysis. The remaining was washed one more time and the immunoprecipitate was re-suspended into Trizol (Invitrogen) for RNA isolation.

RT-PCR, Real Time PCR and Neural Stem Cell Pathway Arrays

RT-PCR and real time PCR were performed using standard methods as described (Liu et al., 2010; Luo et al., 2010). The first-strand cDNA was generated by reverse transcription with oligo dT primer or random hexamers. Standard RT-PCR was performed using GoTaq DNA polymerase (Promega). To quantify the mRNA levels using real-time PCR, aliquots of first-stranded cDNA were amplified with gene-specific primers and Power SYBR Green PCR Master Mix (Applied Biosystems) using a 7300 Real-Time PCR System (Applied Biosystems). The PCR reactions contained 20-40 ng of cDNA (except the cDNA for the IP, for which 5% of the cDNA was used for each gene examined), Universal Master Mix (Applied Biosystems), and 300 nM of forward and reverse primers in a final reaction volume of 20 µl. The ratio of different samples was calculated by the data analysis software built in with the 7300 Real-Time PCR System.

To determine differential gene expression in *Fxr2 KO* DG-NPCs, cDNA were synthesized from the RNA of *WT* and *Fxr2 KO* DG-NPCs as described above. Forty ng cDNA was added into each well of a 96-well pre-manufactured mouse Neural Stem Cell Pathway Array (Qiagen/SABioscience). Each sample was applied to one array and independent duplicates were analyzed for *WT* and *Fxr2 KO.* Real time PCR and data analyses were performed according to manufacture's instruction (Qiagen/SABioscience). Briefly, the expression level of each gene was obtained by comparing to internal controls on the same array. The relative expression levels of genes in *Fxr2 KO* compared with *WT* samples were then calculated with *WT* samples set as "1" to create data shown in Figure S3.

The sequences of primers used for PCR reactions are as the following:

Noggin: sense 5’-CCAGACCCTATCTTTGACCC-3’, anti-sense 5’ GCACAGACTTGGATGGCTTA-3’.

Shh: sense 5'-CCCTTGTCCTGCGTTTCA-3', antisense 5'-TAAACGAAACATTGGCGGT-3'

Notch2: sense 5'-CAAGAAAGCCGTCAGCAC-3', antisense 5’-ACAGATACAGGACTCCGGG-3'

Sox3: sense: 5'-ACGGTGCCGCTGACCCACAT-3', antisense: 5'-ACTCCTCCTGGCTTTCAAACCG-3'

In vitro transcription of biotin-labeled Noggin mRNA and binding assay:

This procedure was performed based on published methods with modifications (Deschenes-Furry et al., 2007). The pCR4-TOPO vector containing mouse Noggin cDNA sequence was purchased from the Open Biosystems (MMM1013-99826952, Thermo scientific). The plasmid was linearized using SpeI and NotI restriction enzymes to generate sense and anti-sense templates, respectively. For in vitro transcription of biotin-labeled noggin mRNA, Noggin sense template was transcribed using AmpliScritbe™ T7-Flash™ biotin-RNA transcription Kit (ASB71110, Epicentre Biotechnologies). For in vitro transcription of biotin-labeled noggin anti-sense mRNA, Noggin anti-sense template was transcribed using T3 RNA polymerase (T4905K, Epicentre Biotechnologies). To determine whether synthetic biotinylated Noggin mRNA could bind endogenous FXR2 in NPCs, protein lysate was prepared by homogenizing NPCs using RIPA buffer (50mM Tris HCl pH 8, 150 mM NaCl, 1% NP-40, 0.5% sodium Deoxycholate, 0.1% SDS). Then, thirty micrograms of purified

biotinylated transcripts (the antisense transcript was used as control) were incubated with 500 µg of total protein in the binding buffer (10 mM HEPES, pH 7.4, 3 mM MgCl2, 5% glycerol, and 1 mM DTT) for 30 min at room temperature to allow the binding of biotinylated RNA and their binding proteins. Yeast transfer RNA (50 ng/ml) and heparin (5 mg/ml) were then added to the mixture to block non-specific binding and the binding reaction was allowed to continue for 10 more min. Dynabeads M-280 streptavidin (112-05D, Invitrogen) was then added to the mixture, and incubated with constant mixing overnight at 4°C. To collect the protein bound by biotinylated Noggin mRNA, the beads were washed three times with 1x binding buffer and resuspended in 1x binding buffer with SDS gel loading dye to dissolve protein bound to biotinylated Noggin RNA. The proteins in the pull-down material were separated by 4–20% SDS-PAGE gel, transferred to nitrocellulose membrane for Western blot analysis using an anti-FXR2 antibody.

Actinomycin D Treatment (Noggin mRNA Stability Assay)

DG-NPCs from *WT* and *Fxr2 KO* mice were grown in proliferating condition (see above). 10 µg/ml of Actinomycin D (Sigma-Aldrich) was added based on published protocol (Ghosh et al., 2009) and total RNA was isolated at various time intervals and the Noggin mRNA level normalized to GAPDH was measured by real time PCR.

ELISA Analysis of Secreted Noggin

To determine the amount of secreted Noggin protein in the cell medium, medium was collected after 24 hours of culture. Determination of secreted Noggin was performed using a

mouse Noggin ELISA kit (ABIN425343, antibodies-online.com, Atlanta, USA), according to the manufacture's instructions. Briefly, 200 ul medium of each independent culture medium (n=3) was placed in 96 well microtiter plates coated with monoclonal detective antibodies and incubated for 2 h at room temperature. After removing unbound material by washing with washing buffer (50 mM Tris, 200 mM NaCl, and 0.2% Tween 20), horseradish peroxidase conjugated streptavidin was added to bind to the antibodies. Horseradish peroxidase catalyzed the conversion of a chromogenic substrate (tetramethylbenzidine) to a colored solution, with color intensity proportional to the amount of protein present in the sample. The absorbance of each well was measured at 450 nm. Results are presented as total secreted noggin divided by total initial plated cells ($1X10^6$).

Treating NPCs with Exogenous Noggin and BMP2, anti-Noggin Blocking Antibody, and Conditioned Medium.

For growth factor and antibody treatment, the concentration of noggin was 250ng/ml (R&D Systems), BMP2 was 25ng/ml (R&D Systems), and noggin antibody was 2.5ng/ml (R&D Systems). Conditioned medium was collected from WT and KO DG-NPCs after 24 hour culture. To study cell proliferation, we dissociated neural stem cells with trypsin and plated them on poly-L-ornithin/laminin-coated slides at a density of 50,000 cells/well in proliferation medium (see above). At 20 h post-plating, the culture medium was changed to that with 5 μM BrdU and above treated concentration for 16 hours. For the differentiation assay, at 24 h post-plating, cells were changed into differentiation medium (see above) with treated concentration for 1 day, and then switched to differentiation medium for 3 days.

Western Blotting Analyses

Protein samples were separated on SDS-PAGE gels (Bio-Rad) and then transferred to PVDF membranes (Millipore). Membranes were processed following the ECL Western blotting protocol (GE Healthcare). FXR2 (1:1000. Sigma), FMRP (1:1000, Millipore), Noggin (1:500, R&D), p-Smad1/5 (1:1000, Cell signaling), total Smad1/5 (1:1000, Cell signaling), Nestin (1:1000, Millipore) were used as primary antibodies. HRP-labeled secondary antibodies were obtained from Sigma. For loading controls, membranes were stripped and reprobed with the mouse antibody against β-Actin (Sigma A5441).

Production of Lentivirus and Retrovirus Vectors

Lentivirus expressing control shRNA (shCon) was published previously (Barkho et al., 2008; Liu et al., 2010). Noggin-shRNA and FXR2-shRNA plasmids were purchased (Qiagen/SABioscience) and their efficiency on knocking down endogenous Noggin or FXR2 was tested by transfecting P19 cells followed by Western blotting analyses. The shRNA exhibiting highest efficacy was then cloned into the lentiviral vector inserting the Noggin-shRNA cassette or FXR2-shRNA cassette between the HpaI and ClaI sites as described (Barkho et al., 2008; Liu et al., 2010). For lentivirus expressing full length FXR2, pYX-Asc vector containing full length FXR2 was purchased from the Open Biosystems (MMM1013-9498022, Thermo scientific). The full length FXR2 was cloned into a pCDH Lentiviral vector (CD511B-1, System biosciences) using NotI and EcoRI sites. The expression of full length FXR2 from this viral vector was confirmed by western blot. Lentivirus

production was performed as described previously (Barkho et al., 2008; Liu et al., 2010). Briefly, lentiviral transfer vector DNA and packaging plasmid DNA were transfected into cultured 293T cells using calcium phosphate methods. The medium containing lentivirus was collected at 40, 64, and 88 hours post-transfection, pooled, filtered through a 0.2-µm filter, and concentrated using an ultracentrifuge at 19 k rpm for 2 hours at 20°C using a SW27 rotor (Beckman). The virus was washed once and then resuspended in 50 µl PBS. We routinely obtained $1X10^9$ infectious viral particles / ml.

Retroviral vector expressing both FXR2-shRNA and eGFP was engineered by deleting the original HpaI and ClaI sites in the CAG-EGFP vector (Zhao et al. 2006; Smrt et al. 2007) and inserting new HpaI and ClaI sites 5'-upstream from the CAG promoter. The U6-shRNA cassettes were then inserted between the HapI and ClaI sites. Retrovirus production was performed as described previously (Zhao et al. 2006; Smrt et al. 2007). Retrovirus production was performed as described previously (Barkho et al., 2008; Liu et al., 2010). Briefly, retroviral transfer vector DNA and packaging plasmid DNA were transfected into cultured 293T cells using calcium phosphate methods. The medium containing lentivirus was collected at 40, 64, and 88 hours post-transfection, pooled, filtered through a 0.2-µm filter, and concentrated using an ultracentrifuge at 19 k rpm for 2 hours at 20°C using a SW27 rotor (Beckman). The virus was washed once and then resuspended in 50 µl PBS. We routinely obtained $1X10^9$ infectious viral particles / ml.

In vivo and in vitro Acute Knockdown of FXR2 in NPCs using FXR2-shRNA

For in vivo acute knockdown of FXR2, retroviral grating was performed as described (Liu et

al., 2010; Smrt et al., 2007; Smrt et al., 2010; Szulwach et al., 2010). Briefly, 7- to 8-week-old C57B/L6 male mice were anesthetized with isofluorane, and virus (1.5 µl with titer greater than 5 × 105/µl) was injected stereotaxically into the dorsal ganglia (DG) using the following coordinates relative to bregma: anteroposterior, −(1/2)×d mm; lateral, +/−1.7 mm; ventral, −1.9 mm (from dura). At 1 day after viral grafting, mice were injected with BrdU at 50mg/Kg every 4 hours in 12 hours. One group of mice was perfused at 12 hours post-BrdU for cell proliferation analysis and another group of mice were perfused at one week after BrdU for cell differentiation analysis. Mice were deeply anesthetized with pentobarbital and perfused with saline followed by 4% PFA.

To analyze the phenotypes of GFP+ cells, we used 1 in 6 series of 40 µm brain sections starting at beginning of hippocampus (relative to bregma, −1.5 mm) to the end of hippocampus (relative to bregma, −3.5 mm). The data was calculated as percentage of GFP+BrdU+, GFP+GFAP+BrdU+ or GFP+DCX+BrdU+ cells divided by total GFP+ cells in counting.

To study the effects of in vitro acute knockdown of FXR2 by using FXR2-shRNA on DG-NPCs, ~60 µl Lentivirus was added to the NPCs cultured in proliferating condition on a 10 cm tissue culture plate. After a 1-2-day incubation, infected NPCs were trypsinized and plated into either coated coverslides, at a density of 1 X 10^5 cells /well, for differentiation or proliferation analysis.

In vitro and in vivo Functional Rescue Using Recombinant Lentivirus

In vivo lentiviral grating (with Noggin-shRNA) was performed as described(Liu et al., 2010;

Smrt et al., 2007; Smrt et al., 2010; Szulwach et al., 2010). Briefly, 7- to 8-week-old C57B/L6 male mice were anesthetized with isofluorane, and virus (1.5 µl with titer greater than 5 × 105/µl) was injected stereotaxically into the dorsal ganglia (DG) using the following coordinates relative to bregma: anteroposterior, −(1/2)×d mm; lateral, +/−1.7 mm; ventral, −1.9 mm (from dura). At two weeks after viral grafting, mice were injected BrdU at 50mg/Kg. One group of mice was perfused at 12 hours post-BrdU for cell proliferation analysis and another group of mice was perfused at one week after BrdU for cell differentiation analysis. Mice were deeply anesthetized with pentobarbital and perfused with saline followed by 4% PFA.

To analyze the phenotypes of BrdU+ cells, we used 1 in 12 series of 40 µm brain sections starting at beginning of hippocampus (relative to bregma, −1.5 mm) to the end of hippocampus (relative to bregma, −3.5 mm). Based on the published criteria (Clelland et al., 2009), if lentivirus-infected (GFP+) cells were found in > 50% of the DG area of adult hippocampus as shown in the figure, then the animal was included in the data analysis shown in Figure 7. The counting area in each section was defined by GFP+ cells and only BrdU+ or DCX+ cells within the areas counted. The counting areas were then used to calculate the volume of the DG containing GFP+. The data was calculated as number of either BrdU+ or DCX+ cells divided by the volume of the DG containing GFP+ cells.

To study the rescue effects of FXR2 on DG-NPCs, ~60 µl Lentivirus (contained full length FXR2) was added to the NPCs cultured in proliferating condition on a 10 cm tissue culture plate. After a 1-2-day incubation, infected WT and FXR2 KO NPCs were trypsinized and plated into either coated coverslides, at a density of 1 X 10^5 cells /well, for differentiation or

proliferation analysis.

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