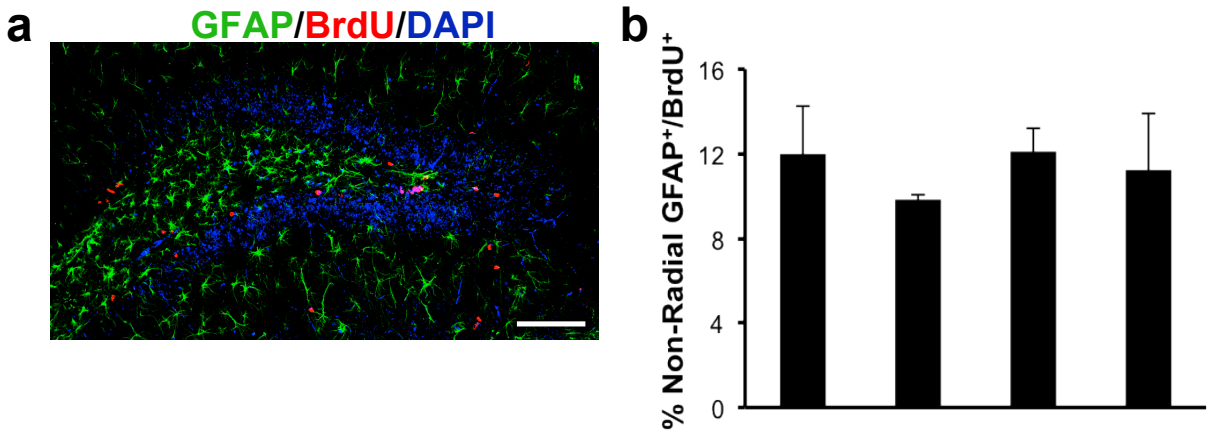
Supplementary Information

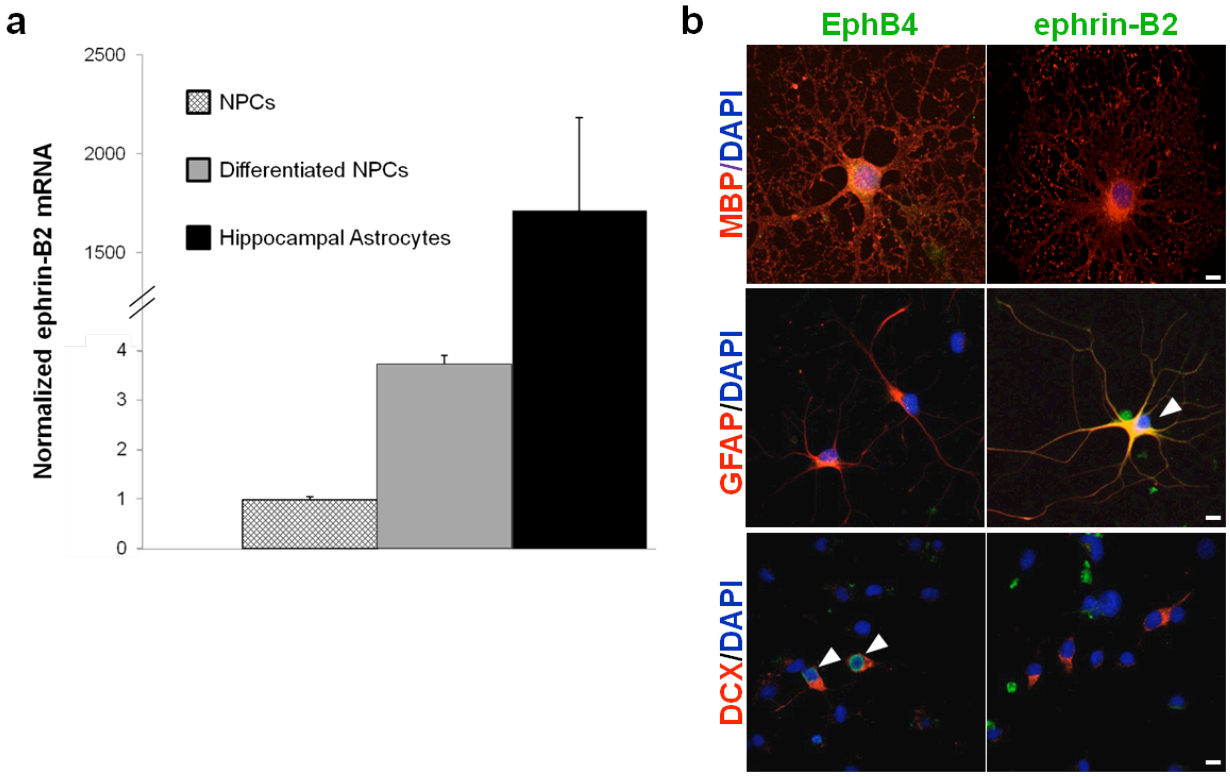
Astrocytes regulate adult hippocampal neurogenesis through ephrin-B signaling

Randolph S. Ashton, Anthony Conway, Chinmay Pangarkar, Jamie Bergen, Kwang-Il Lim, Priya Shah, Mina Bissell, and David V. Schaffer

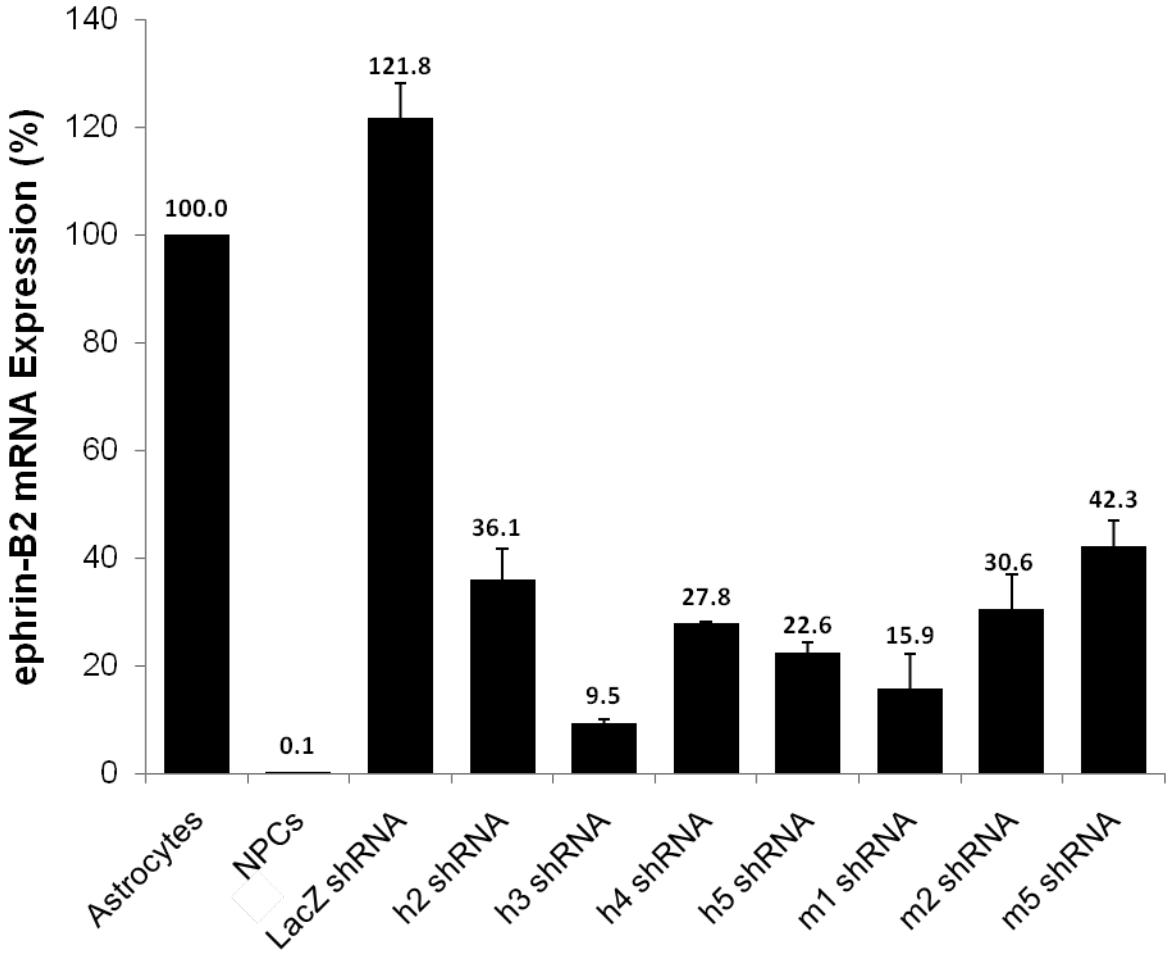
Inventory of Supplementary Information



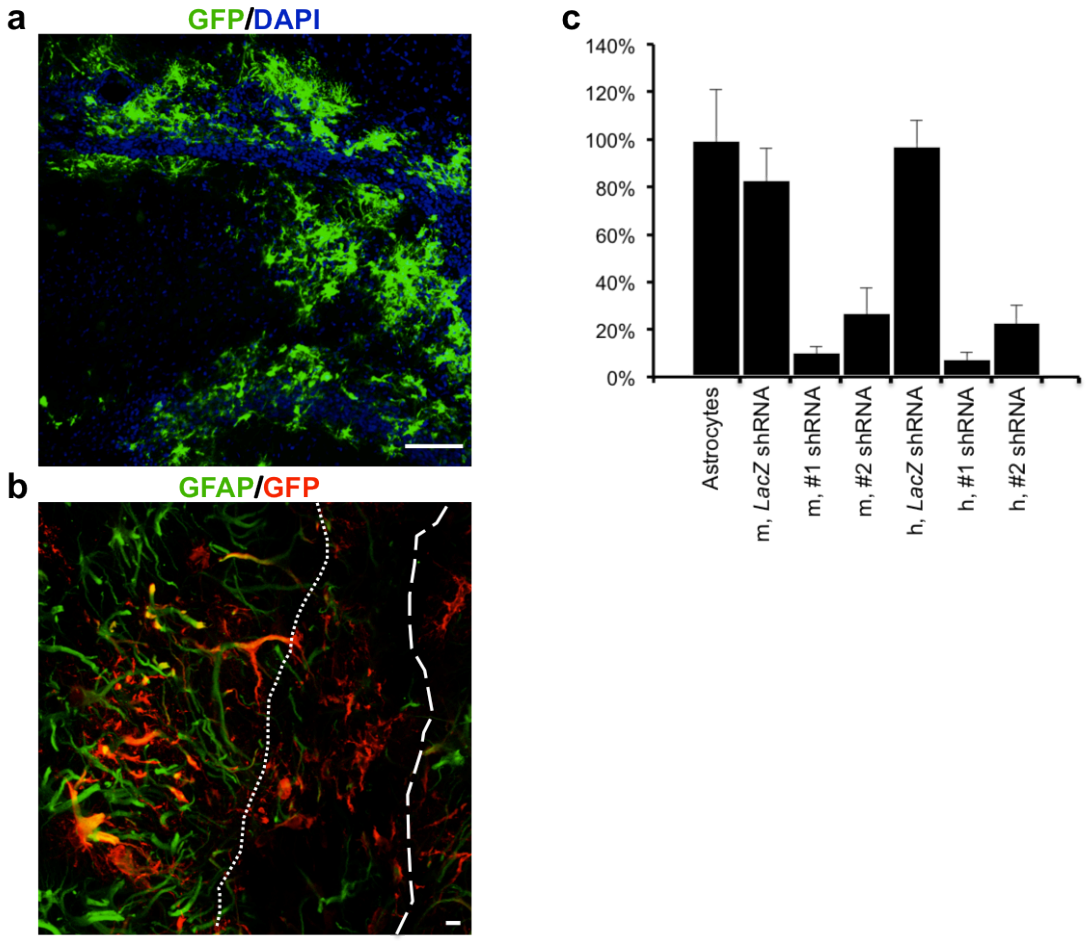
Supplementary Figure 1, related to **Figure 3**. Quantification of hippocampal gliogenesis. Fc-ephrin-B2 does not affect gliogenesis in vivo. (**a**) In general, only a small fraction of $BrdU^+$ cells co-labeled as astrocytes ($GFAP^+$). (**b**) The percentage of non-radial $GFAP^+/BrdU^+$ astrocytes was not affected by Fc-ephrin-B2 induced signaling (*n* = 4 experimental replicates ± s.d.).



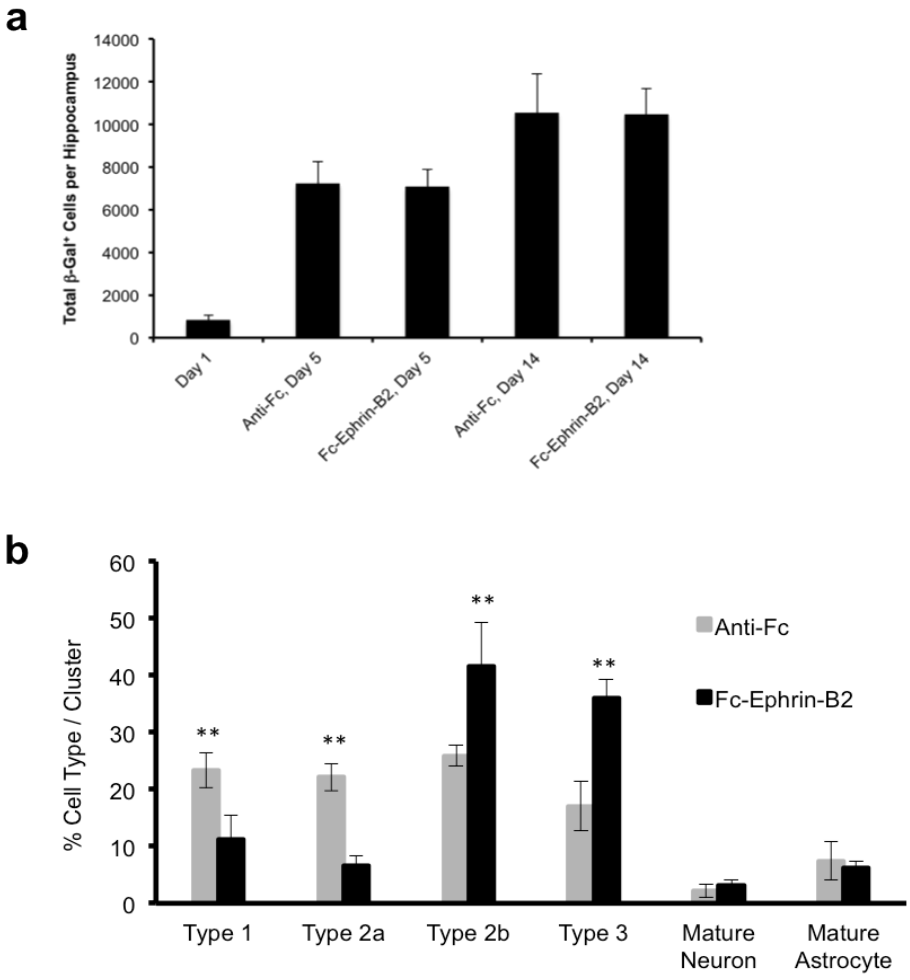
Supplementary Figure 2, related to **Figure 2** and **Figure 4**. Ephrin-B2 expression upon astrocytic differentiation. In vitro analysis of ephrin-B2 expression in hippocampus-derived astrocytes and differentiated NSCs. (**a**) Using QPCR, we compared *efnb2* expression levels in cultures of NSCs, differentiated NSCs, and hippocampus-derived astrocytes. Hippocampus-derived astrocytes express *efnb2* at levels three order-of-magnitude higher than NSCs, and it appears as though *efnb2* expression increased as the fraction and maturity of astrocytes in the cell population also increased. Data is normalized to NSC *efnb2* expression level (*n* = 3, technical replicates ± s.d.). (**b**) NSCs were differentiated into oligodendrocytes (MBP^+), astrocytes ($GFAP^+$), and immature neurons (DCX^+) and stained for expression of ephrin-B2 and EphB4. NSCs differentiated into astrocytes down-regulated EphB4 and up-regulated ephrin-B2 expression, while NSCs differentiated into neurons still expressed EphB4 on the cell soma. NSCs differentiated into oligodendrocytes did not express high levels of either ephrin-B2 or EphB4. The scale bar represents 10 μm.



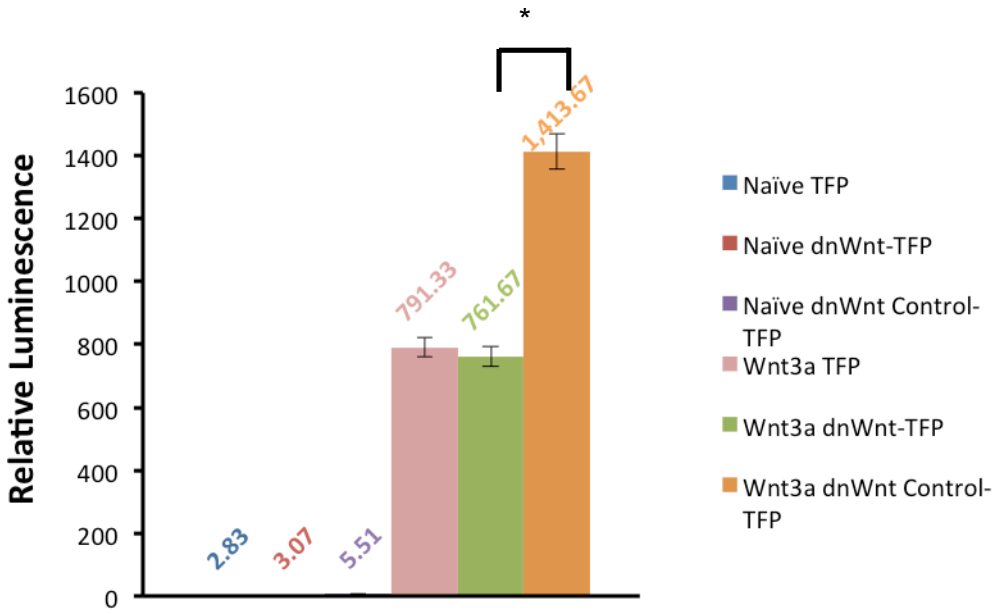
Supplementary Figure 3, related to **Figure 4**. Ephrin-B2 shRNA screen. Screen for effective shRNA targeting *efnb2.* QPCR analysis of hippocampus-derived astrocytes expressing shRNA sequences designed to knockdown expression of *efnb2*. Five shRNA sequences were tested using human or mouse U6 promoters. Naïve astrocytes, NSCs, and astrocytes expressing a shRNA sequence targeting *LacZ* mRNA were used as controls, and data was normalized to *efnb2* expression of non-infected astrocytes (*n* = 3, technical replicates ± s.d.).



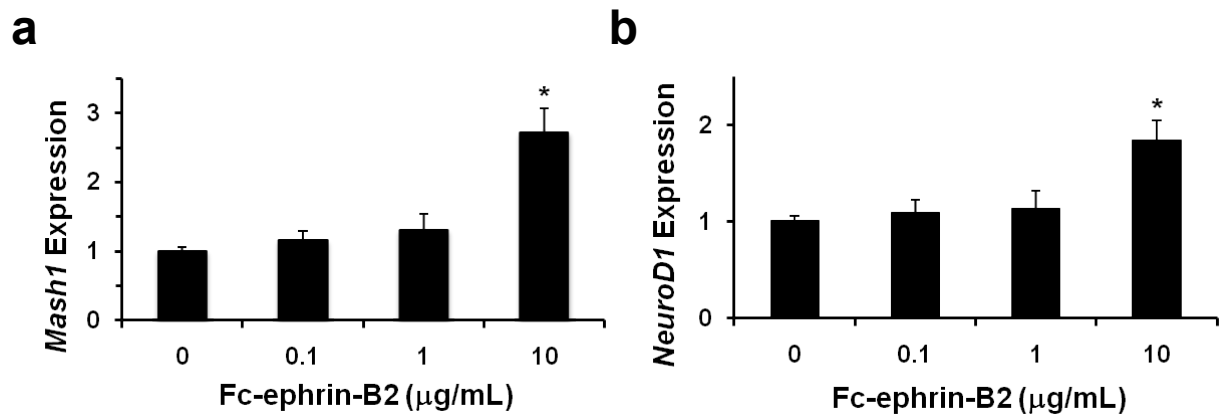
Supplementary Figure 4, related to **Figure 5**. *In vivo* validation of astrocytic shRNA expression. We hypothesized that selective expression of GFP in hippocampal neurons (see **Figure 5**) was due to low activity of the ubiquitin-C promoter in astrocytes, not inactivity of the U6 promoter-shRNA cassette or a neuronal tropism of the lentiviral vector. (**a,b**) The ubiquitin-C promoter was replaced by a mouse (m) and a human (h) GFAP promoter, and as evidenced by histology, the new shRNA vectors were expressed by $GFAP^+$ astrocytes proving that prior localization of GFP expression to neurons was an artifact of the ubiquitin-C promoter. Dotted line marks SGZ/Hilus boundary and dashed line marks GCL/MCL boundary. (**c**) QPCR analysis of shRNA vector-expressing astrocytes in culture demonstrated sustained effectiveness of the *efnb2* shRNA #1 and #2 vectors (n = 3, technical replicates ± s.d.).



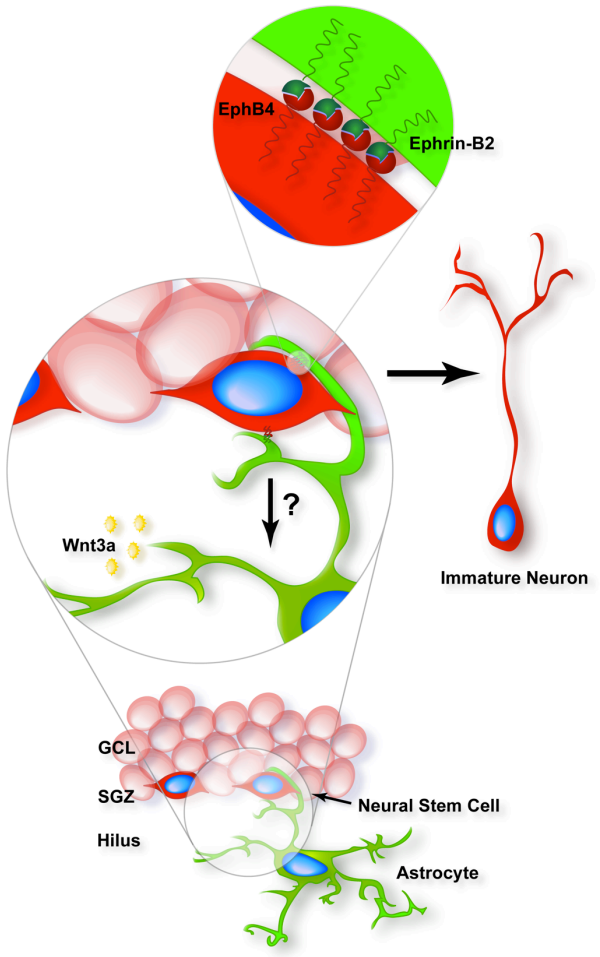
Supplementary Figure 5, related to **Figure 6**. Effect of Fc-ephrin-B2 on Nestin^{+} (β-gal^{+}) NSCs *in vivo*. (**a**) In fate mapping experiments using *Nestin-CreER^{T2}; R26-stop$^{fl/fl}$-lacZ* mice, the proliferative rate of recombined cells was consistent between Anti-Fc and Fc-ephrin-B2 experimental groups indicating that the increase in BrdU^{+} cells observed in **Figure 3c** was not due to ephrin-B2 induced proliferation of Nestin^{+} NSCs (*n* = 4 experimental replicates ± s.d.). (**b**) Analysis of cell phenotype amongst clonal β-gal^{+} cell clusters at Day 5 showed a similar distribution as observed at the population level in **Figure 6d-h** and no significant change in levels of gliogenesis between Anti-Fc and Fc-ephrin-B2 treated groups. ** indicates *P* <0.05; *n* = 4 brains, analyzed 8 hippocampal sections per brain.



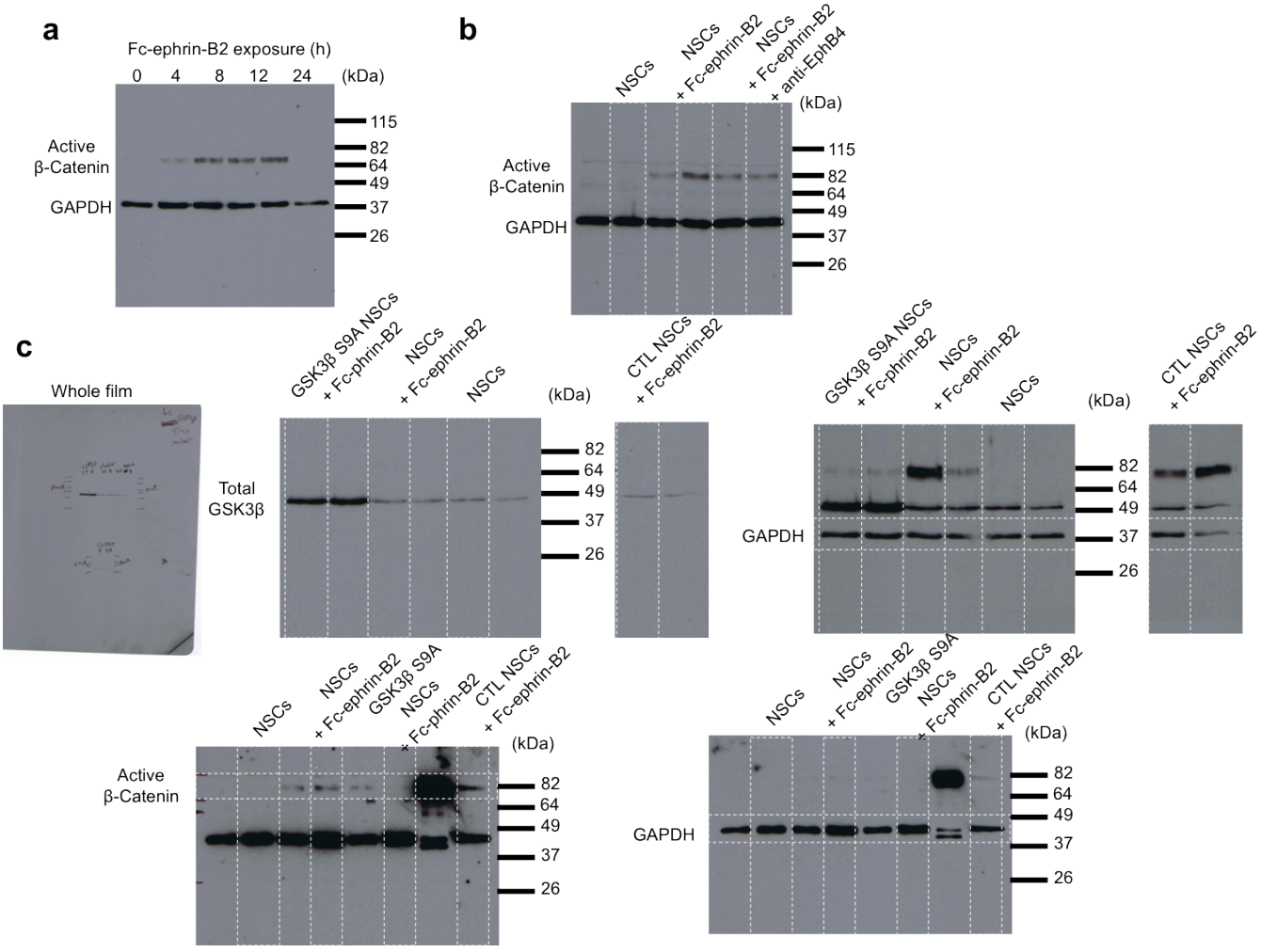
Supplementary Figure 6, related to **Figure 7**. In vitro validation of lentiviral vectors encoding Tcf-Luc reporter (TFP), dnWnt-IRES-GFP (dnWnt), and IRES-GFP (dnWnt Control) cassettes. Naïve NSCs, NSCs infected with TFP vector, and NSCs co-infected with TFP and either dnWnt or dnWnt control vectors were assayed for their ability to report β-catenin signaling, as evidenced by luciferase expression, in response to a 24-hour incubation in Wnt3a supplemented (200 ng/mL) or standard media. No Luc expression was observed in the absence of Wnt3a, and a significant decrease in Wnt3a-induced Luc reporting was observed in NSCs expressing dnWnt as compared to the dnWnt control construct, thus validating proper activity of the three constructs (n = 3 technical replicates ± s.d.). * indicates a P <0.01.



Supplementary Figure 7, related to **Figure 7**. Fc-ephrin-B2 induces expression of *Mash1* and *NeuroD1* in NSCs *in vitro*. Similar to experiments in **Figure 1**, Fc-ephrin-B2 stimulation also induced a dose-dependent increase in the expression of two proneural transcription factors, *Mash1* and *NeuroD1*, known to play important roles in adult hippocampal neurogenesis (n = 3, technical replicates ± s.d.). * indicates $P < 0.01$.



Supplementray Figure 8. Proposed model of ephrin-B2 signaling in regulating adult neurogenesis. In the SGZ, ephrin-B2^{+} hippocampal astrocytes induce neuronal differentiation of Sox2^{+}/EphB4^{+} NSCs through juxtacrine ephrin-B2/EphB4 forward signaling via a β-catenin dependent mechanism. However, the effect EphB4/ephrin-B2 reverse-signaling on hippocampal astrocytes remains unknown.



Supplementary Figure 9. Full-length pictures of the blots presented in **Fig. 7**. Each experimental group was assayed at both 8 and 24 h post-treatment. The bands for the 24-hour time point are included in **Fig.7**.

Supplementary Table 1. shRNA primers for RNAi.

***Efnb2* shRNA**	**Primer Sequence**
h2	5'-AAAATTAATTAAAAAGCCAAATTTCTACCCGGACATCTCTTGAATGTCCGGGTAGAAATTTGGCGGTGTTTCGTCCTTTCCACAAGATATATAAAGCC-3'
h3 (#1)	5'-AAAATTAATTAAAAAGCCGCAGGAGACACCGCAAATCTCTTGAATTTGCGGTGTCTCCTGCGGCGGTGTTTCGTCCTTTCCACAAGATATATAAAGCC-3'
h4	5'-AAAATTAATTAAAAAGAGCCGACAGATGCACTATTTCTCTTGAAAATAGTGCATCTGTCGGCTCGGTGTTTCGTCCTTTCCACAAGATATATAAAGCC-3'
h5	5'-AAAATTAATTAAAAAGCAGACAAGAGCCATGAAGATCTCTTGAATCTTCATGGCTCTTGTCTGCGGTGTTTCGTCCTTTCCACAAGATATATAAAGCC-3'
m1 (#2)	**5'-**AAAATTAATTAAAAAGGCTAGAAGCTGGTACGAATTCTCTTGAAATTCGTACCAGCTTCTAGCCAAACAAGGCTTTTCTCCAAGGGATATTTATAGTCTC-3'
m2	5'-AAAATTAATTAAAAAGCCAAATTTCTACCCGGACATCTCTTGAATGTCCGGGTAGAAATTTGGCAAACAAGGCTTTTCTCCAAGGGATATTTATAGTCTC-3'
m5	5'-AAAATTAATTAAAAAGCAGACAAGAGCCATGAAGATCTCTTGAATCTTCATGGCTCTTGTCTGCAAACAAGGCTTTTCTCCAAGGGATATTTATAGTCTC-3'
***LacZ* sense**	5'-GGGGTTAATTAAAAGGTCGGGCAGGAAGAGGGC-3'
***LacZ* anti-sense**	5'-GGGGTTAATTAAAAAAAGTGACCAGCGAATACCTGTTCTC-3'