GDF11 expressed in the adult brain negatively regulates hippocampal neurogenesis

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Research

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Abstract

Growth differentiation factor 11 (GDF11) is a transforming factor-β superfamily member that functions as a negative regulator of neurogenesis during embryonic development. However, when recombinant GDF11 (rGDF11) is administered systemically in aged mice, it promotes neurogenesis, the opposite of its role during development. The goal of the present study was to reconcile this apparent discrepancy by performing the first detailed investigation into the expression of endogenous GDF11 in the adult brain and its effects on neurogenesis. Using quantitative histological analysis, we observed that Gdf11 is highly expressed in adult neurogenic niches and non-neurogenic regions within the hippocampus, choroid plexus, thalamus, habenula, and cerebellum. To investigate the role of endogenous GDF11 during adult hippocampal neurogenesis, we generated a tamoxifen inducible mouse that allowed us to reduce GDF11 levels. Depletion of Gdf11 during adulthood increased proliferation of neural progenitors and decreased the number of newborn neurons in the hippocampus, suggesting that endogenous GDF11 remains a negative regulator of hippocampal neurogenesis in adult mice. These findings further support the idea that circulating systemic GDF11 and endogenously expressed GDF11 in the adult brain have different target cells or mechanisms of action. Our data describe a role for GDF11-dependent signaling in adult neurogenesis that has implications for how GDF11 may be used to treat CNS disease.

Full Text

Due to technical limitations, full-text HTML conversion of this manuscript could not be completed. However, the manuscript can be downloaded and accessed as a PDF.

Figures

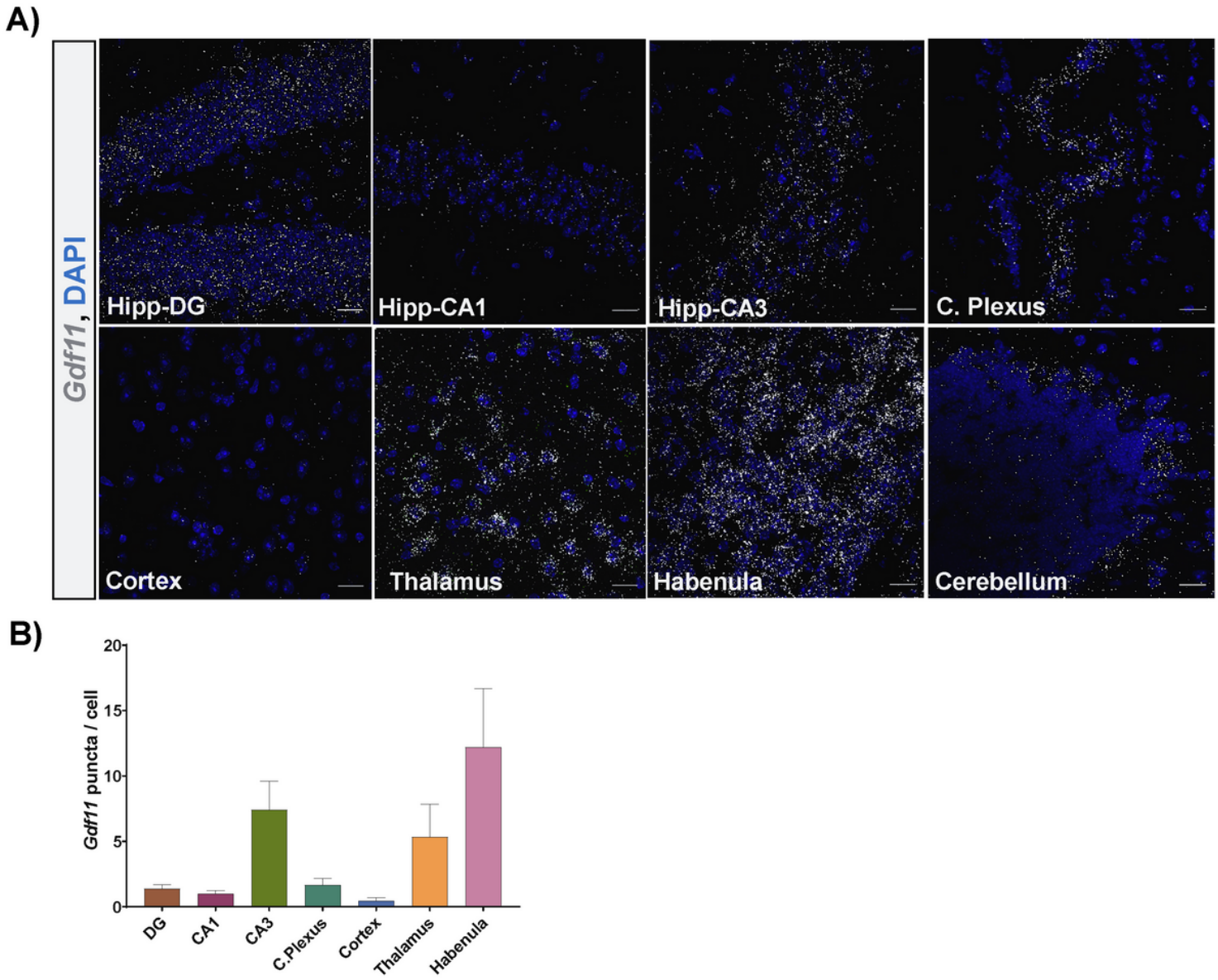

Figure 1

Gdf11 is expressed throughout the young adult brain. A) Representative RNAscope micrographs from a 3-month-old male C57BL/6 mouse probed for Gdf11 (white puncta) and stained with DAPI (blue) for the following brain regions: hippocampus dentate gyrus (DG), CA1, CA3, cortex, thalamus, habenula, and cerebellum. Scale bar = 20μm. B) Bar graph depicting the quantification of RNAscope data (mean of 3-4 mice per brain region, error bars represent SEM).

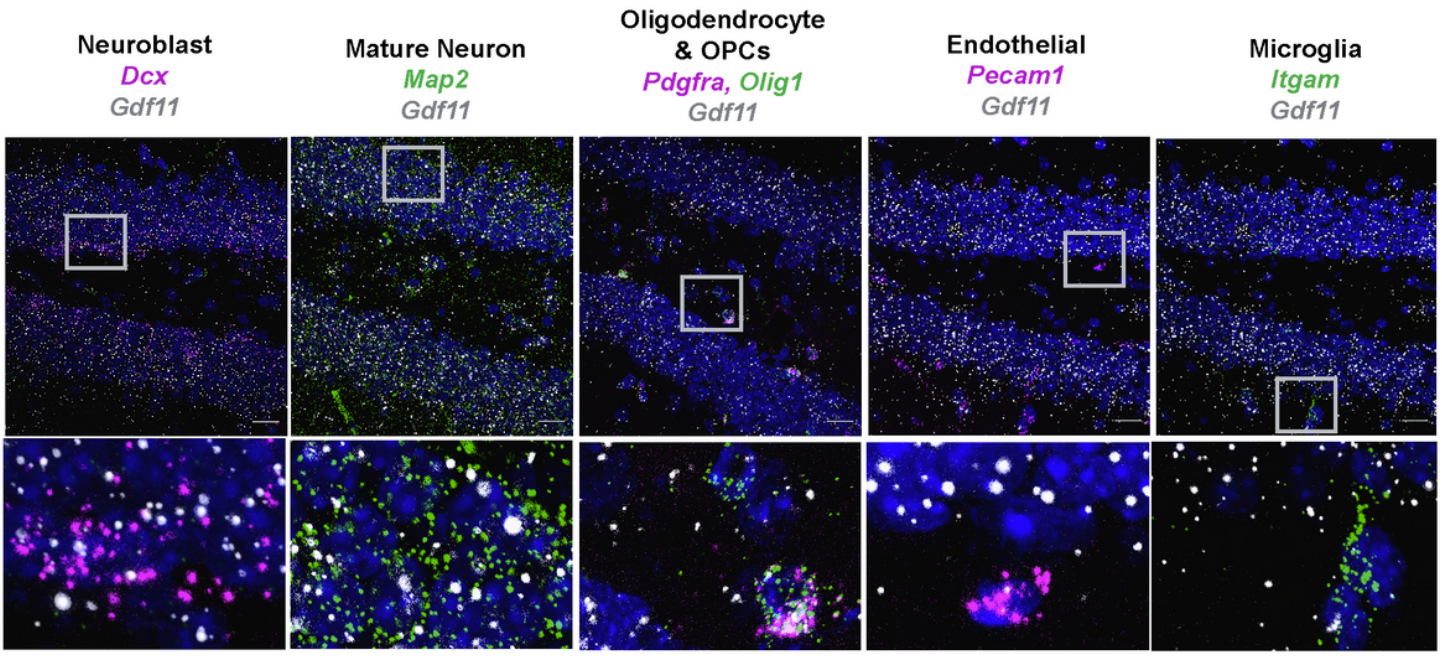

Figure 2

Gdf11 is expressed by neuronal lineage and oligodendrocyte progenitors in the adult dentate gyrus. Representative RNAscope micrographs from a 3-month-old male C57BL/6 mouse probed for Gdf11 (white puncta), the specified cell type specific marker (green or pink) and stained with DAPI (blue). Gray boxes mark the regions chosen for the inserts below each micrograph shown. Scale bar = 20μm. Cell types studied for colocalization with Gdf11 include mature neurons (Map2), neuroblasts (Dcx), oligodendrocytes (Olig1+, Pdgfra-), oligodendrocyte precursor cells, OPCS (Pdgfra), microglia (Itgam), and endothelial cells (Pecam1). hippocampus, cerebellum, kidney, heart, spleen, lung, muscle (tibialis anterior), and liver tissue isolated from Gdf11flox/flox and Gdf11cKO mice. Hprt was used as a housekeeping gene. Relative mRNA levels for all samples are normalized to Gdf11flox/flox mice and error bars depict SEM. D) Concentration of GDF11 protein in serum of Gdf11flox/flox and Gdf11cKO mice at 3 months old, as detected by mass spectrometry (n=9 mice per genotype, males and females pooled.) Individual data points are overlaid with mean +/- SEM. E) RNAscope in situ hybridization micrographs of mouse hippocampi (dentate gyrus) probed for Gdf11 (white puncta) from representative Gdf11flox/flox and Gdf11cKO mice. Scale bar = 20μm. F) Scatterplot showing the quantification of RNAscope data (data includes mean of 4 control and 4 Gdf11cKO brains). G) Quantitative PCR analysis in isolated hippocampi from Gdf11flox/flox and Gdf11cKO mice. Analysis of TGFβ family members Mstn, Tgfβ1, Tgfβ2, Tgfβ3 expression and GDF11 receptors Alk4 and Alk5 expression. Relative mRNA levels for all samples were normalized to the Gdf11flox/flox group and error bars depict SEM. No statistically significant difference between groups was observed for any GDF11 family member or receptor studied. H) Body weight (grams) measured for male and female Gdf11flox/flox or Gdf11cKO mice over varying lengths of time post 3 consecutive days of single tamoxifen IP injections (0.2mg/gram b.w.). All statistics were calculated using two-tailed t- test. * $p<0.05$, ** $p<0.01$.

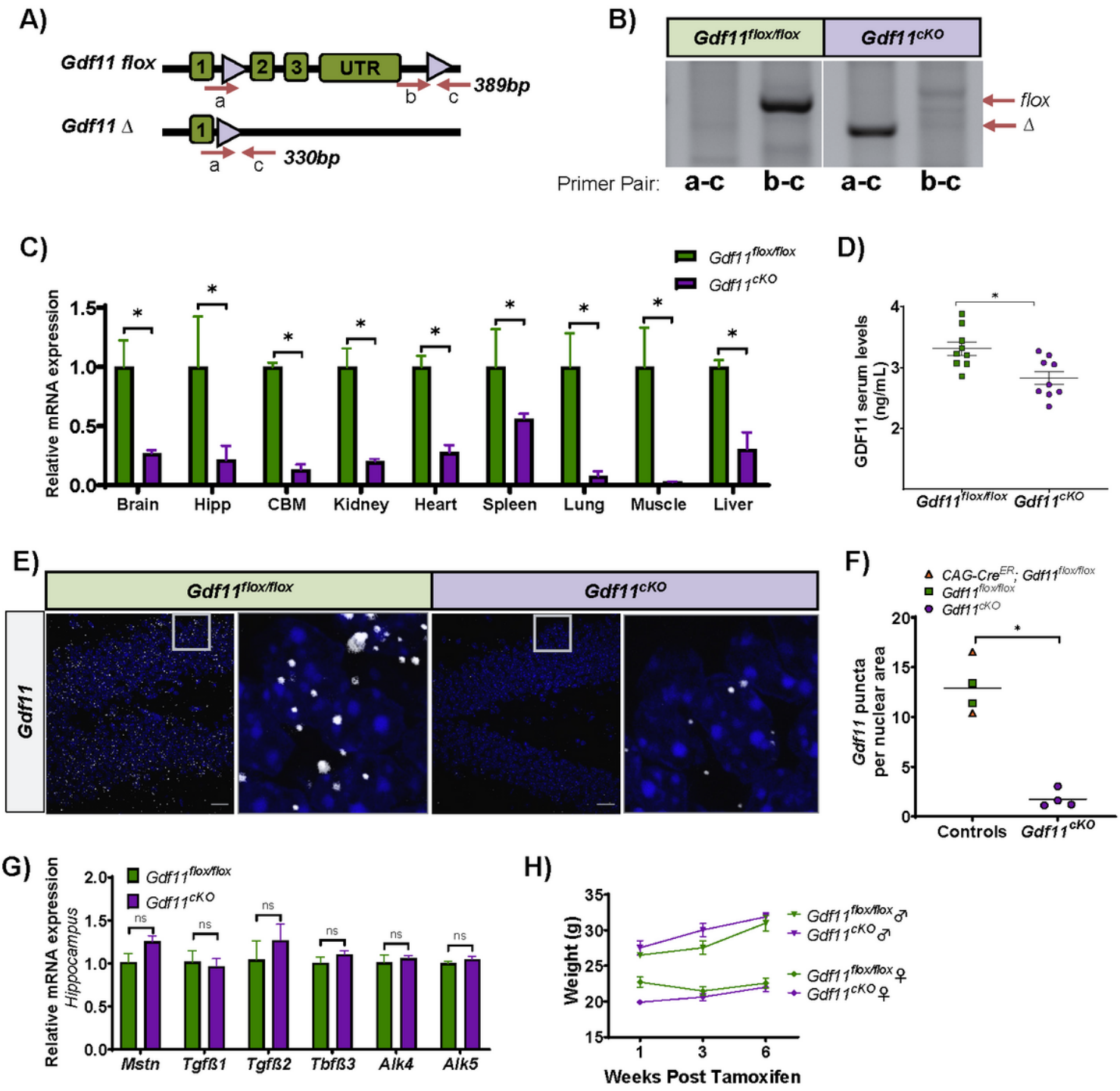

Figure 3

Construction and characterization of Gdf11cKO mice A) Schematic of Gdf11 flox (top), and Gdf11 Δ (bottom) alleles. Primer binding sites are labeled by a, b, and c alongside their amplicon sizes. B) Genomic DNA PCR for Gdf11 flox (b-c amplicon: bp) and Gdf11 Δ (a-c amplicon: 330bp) amplicons from representative Gdf11flox/flox and Gdf11cKO mice. These data confirm recombination of the Gdf11 floxed allele in the Gdf11cKO, but not Gdf11flox/flox, mice. C) Quantitative PCR analysis of Gdf11

expression in whole brain, hippocampus, cerebellum, kidney, heart, spleen, lung, muscle (tibialis anterior), and liver tissue isolated from Gdf11flox/flox and Gdf11cKO mice. Hprt was used as a housekeeping gene. Relative mRNA levels for all samples are normalized to Gdf11flox/flox mice and error bars depict SEM. D) Concentration of GDF11 protein in serum of Gdf11flox/flox and Gdf11cKO mice at 3 months old, as detected by mass spectrometry (n=9 mice per genotype, males and females pooled.) Individual data points are overlaid with mean +/- SEM. E) RNAscope in situ hybridization micrographs of mouse hippocampi (dentate gyrus) probed for Gdf11 (white puncta) from representative

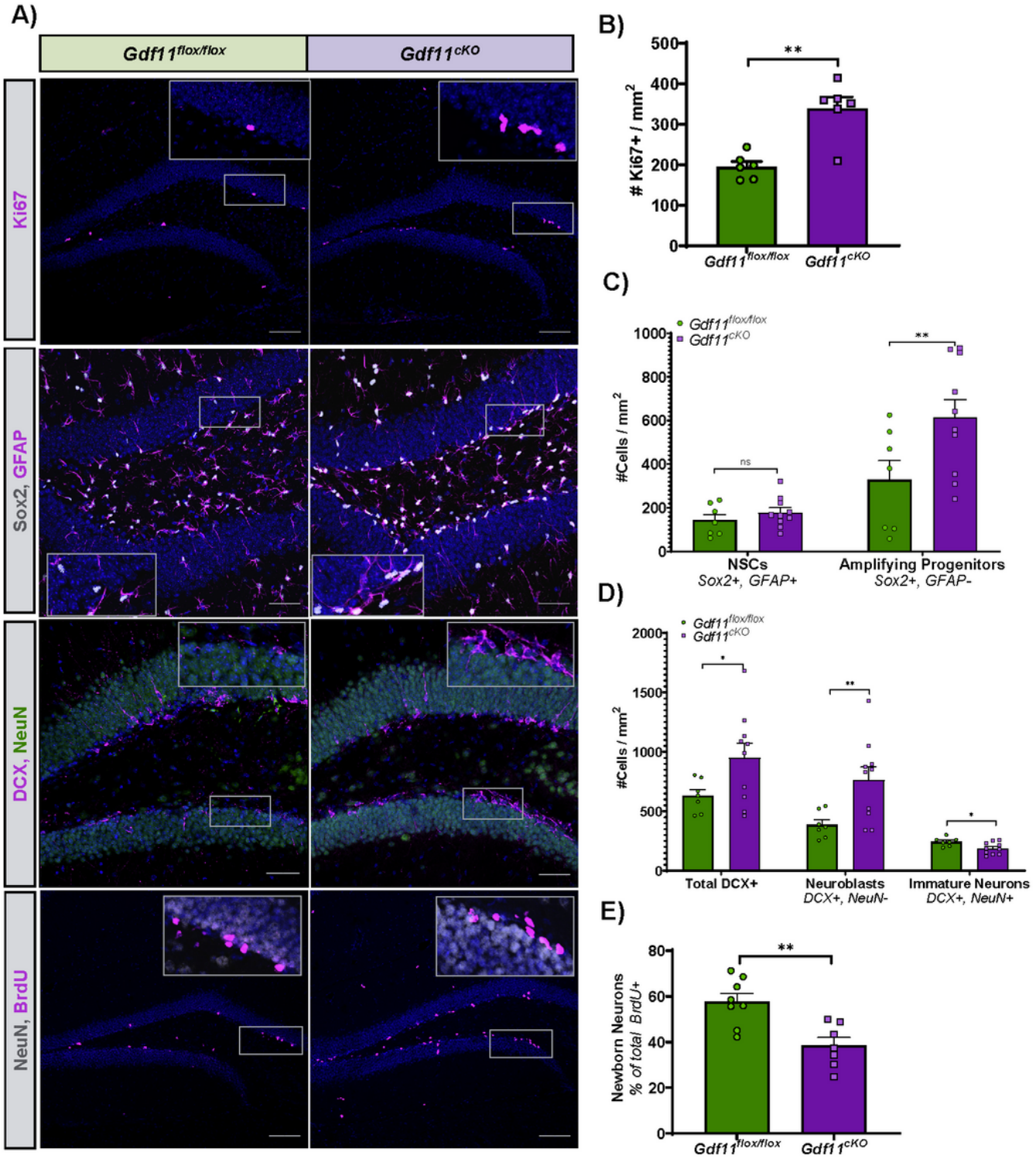

Figure 4

Gdf11 depletion increases overall proliferation, increases the number of neural 325 progenitors, and decreases the number of newborn neurons in the adult hippocampus A) Representative immunohistochemical micrographs comparing the expression of Ki67, Sox2/GFAP, DCX/NeuN, and NeuN/BrdU in the DG of Gdf11flox/flox and Gdf11cKO mice. Scale bar = 20μm. B) Quantification of overall proliferation as measured by the number of Ki67+ cells per DG mm2 (74% increase in Gdf11cKO mice, n=6 mice per group). C) Quantification of the number of neural stem cells (25% nonsignificant increase in Gdf11cKO mice, n=7-10 mice per group) and the number of amplifying progenitors (87% increase, n=7-10 per group). D) Quantification of neuroblasts (DCX+, NeuN-) and immature neurons (DCX+, NeuN+), as measured by the proportion of DCX+ cells that colabeled with or without NeuN+ cells per DG mm2 (% decrease in Gdf11cKO mice, n=7-8 mice per group). E) Quantification of newborn neurons, as measured by the proportion of BrdU+ cells that colabeled with NeuN+ cells per DG mm2 (50% decrease in Gdf11cKO mice, n=7-8 mice per group). See methods for details on cell classification scheme used. All statistics were calculated using two-tailed t-test.* p<0.05 and ** p<0.01.

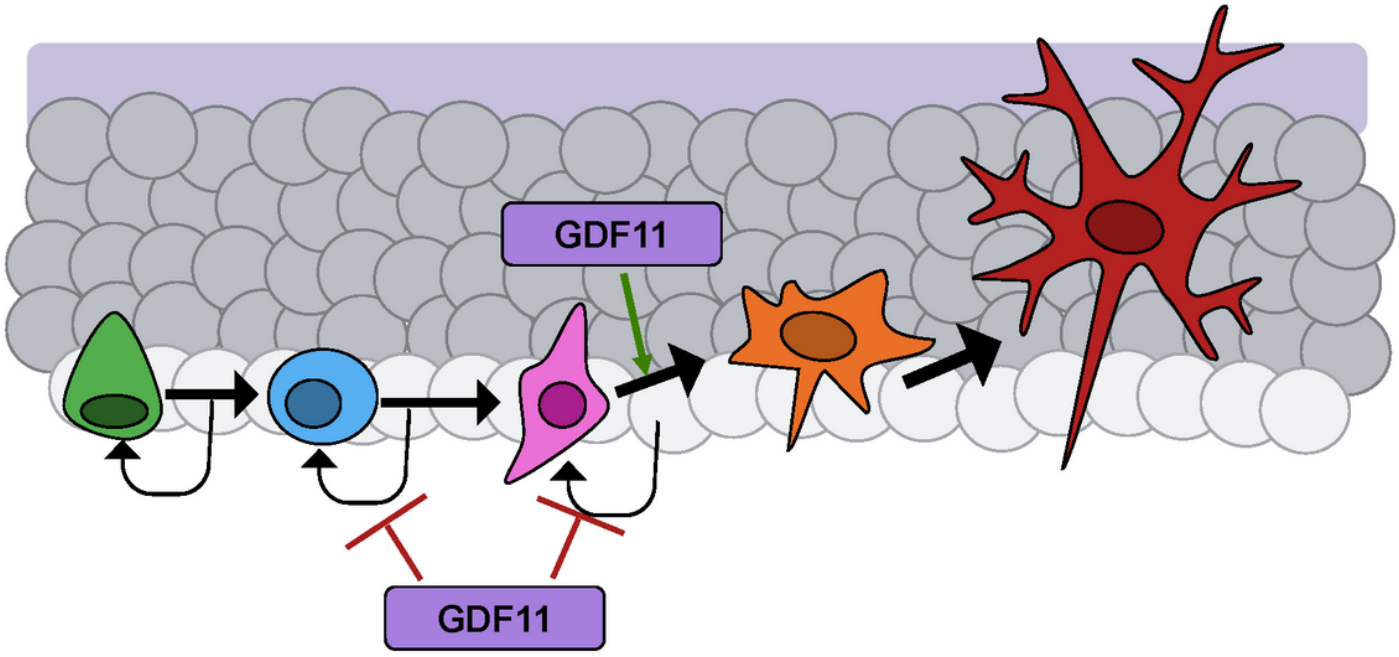

Figure 5

Model summarizing the role of GDF11 in adult hippocampal neurogenesis. GDF11 inhibits the proliferation of amplifying progenitors and neuroblasts under normal conditions. In Gdf11cKO mice there is an increased number of these neural precursor cells. GDF11 promotes the differentiation of neuroblasts into immature neurons and in Gdf11cKO mice there are fewer newborn neurons produced.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- GDF11ManuscriptSupplementalFigure1.pdf
- GDF11ManuscriptSupplementalFigure2.pdf