Supplemental Table 1 PCR Primers

Gene	Forward Primer	Reverse Primer
AQP4	TTCTCTTCGGTGCTAGGAAAC	AGGAAGCTTATGTCTCTGGTG
CSPG4	CCAGGTACTGTTCAGCGTGAG	CATCAGCTGGTCAGAGGTGTC
GAPDH	AATGCATCCTGCACCACCAAC	TGGATGCAGGGATGATGTTCTG
GFAP	ACATCGAGATCGCCACCTAC	TGCTTCGACTCCTTAATGACC
GLAST	ACTTGATGAGCAATTATCAGTTACC	TGGATGAGACAAGGCTCACTC
GLT-1	TTGACTCCCAACACCGAATGC	AGGAATGGGAAAGGTACCTTGC
KCNJ10 (Kir4.1)	TGCTGGAGCCCTTCCTTTTCC	TCCATCCAGTCACATGGTCCTC
LIF	GTCAACTGGCTCAACTCAACG	TACGCGACCATCCGATACAGC
PDGFRα	GAGGACGAGACCATTGAGGAC	TGTCTCCACATCACCCAAGTC
S100ß	CTCAAGTCTCTTCTTCCACAGTG	TTGATTCTCGGTCGTGAGTTAG
VIM	AACACTCCTGATTAAGACGGTTG	TGCAGTAAAGGCACTTGAAAGC
LIF (genotyping)	CGCCTAACATGACAGACTTCCCAT	AGGCCCCTCATGACGTCTATAGTA
NEO (genotyping)	CAAGCTCTTCAGCAATATCACGGG	CCTGTCCGGTGCCCTGAATGAACT

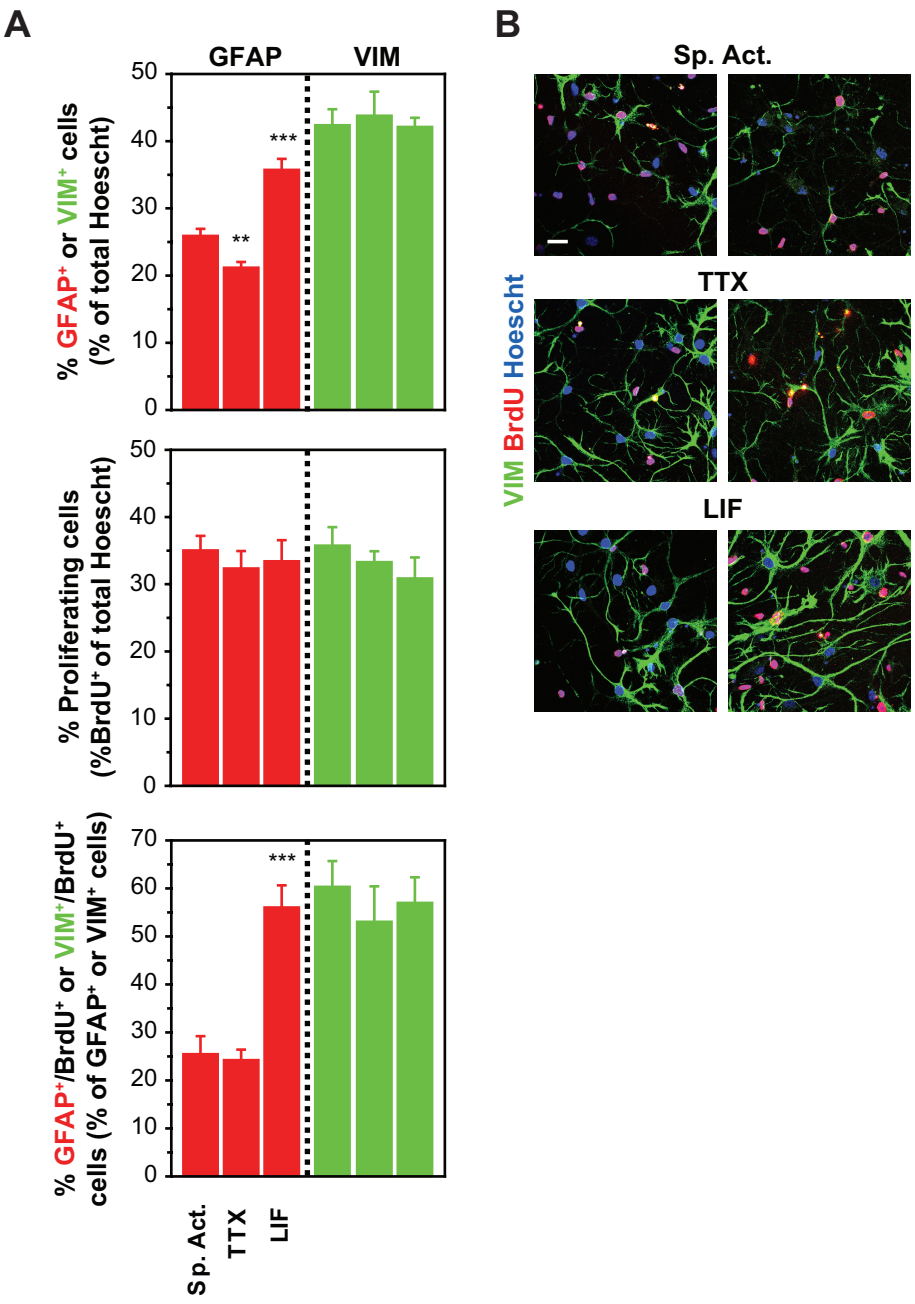
For semi-quantitative PCR, 2 µl of cDNA diluted 1:10 was amplified for 40 cycles under the following conditions: 95ºC for 5 sec, 58ºC for 5 sec, 72ºC for 10 sec. This was followed by a 10 min terminal extension and melting curve analysis.

Supplemental Table 2 Antibodies used in this study

Species	Clone	Antigen	Dilution	Provider
mouse	3CB2	Radial glial marker	1:10	DSHB, Iowa City, IA
mouse	40E-C	vimentin	1:10	DSHB, Iowa City, IA
mouse	74.5A5	Nkx2.2	1:10	DSHB, Iowa City, IA
mouse	G3G4	BrdU	1:50	DSHB, Iowa City, IA
mouse	OX42	CD11b	1:100	BD Biosciences, San Jose, CA
rabbit	-	GFAP	1:500	Zymed
mouse	GA-5	GFAP	1:1000	Chemicon, Billerica, MA
rabbit	M-179	LIF	1:100	Santa Cruz Biotech, Santa Cruz, CA
rabbit	C-19	LIF-R	1:500	Santa Cruz Biotech, Santa Cruz, CA
chicken	-	MAP2	1:2500	Chemicon, Billerica, MA
mouse	-	NG2	1:500	Chemicon, Billerica, MA
rabbit	-	NG2	1:500	Chemicon, Billerica, MA
mouse	Rat 401	Nestin	1:500	BD Biosciences, San Jose, CA
mouse	O4	O4	1:10	kindly provided by Ben Barres
mouse	SH-B1	S100ß	1:500	Sigma, St. Louis, MO

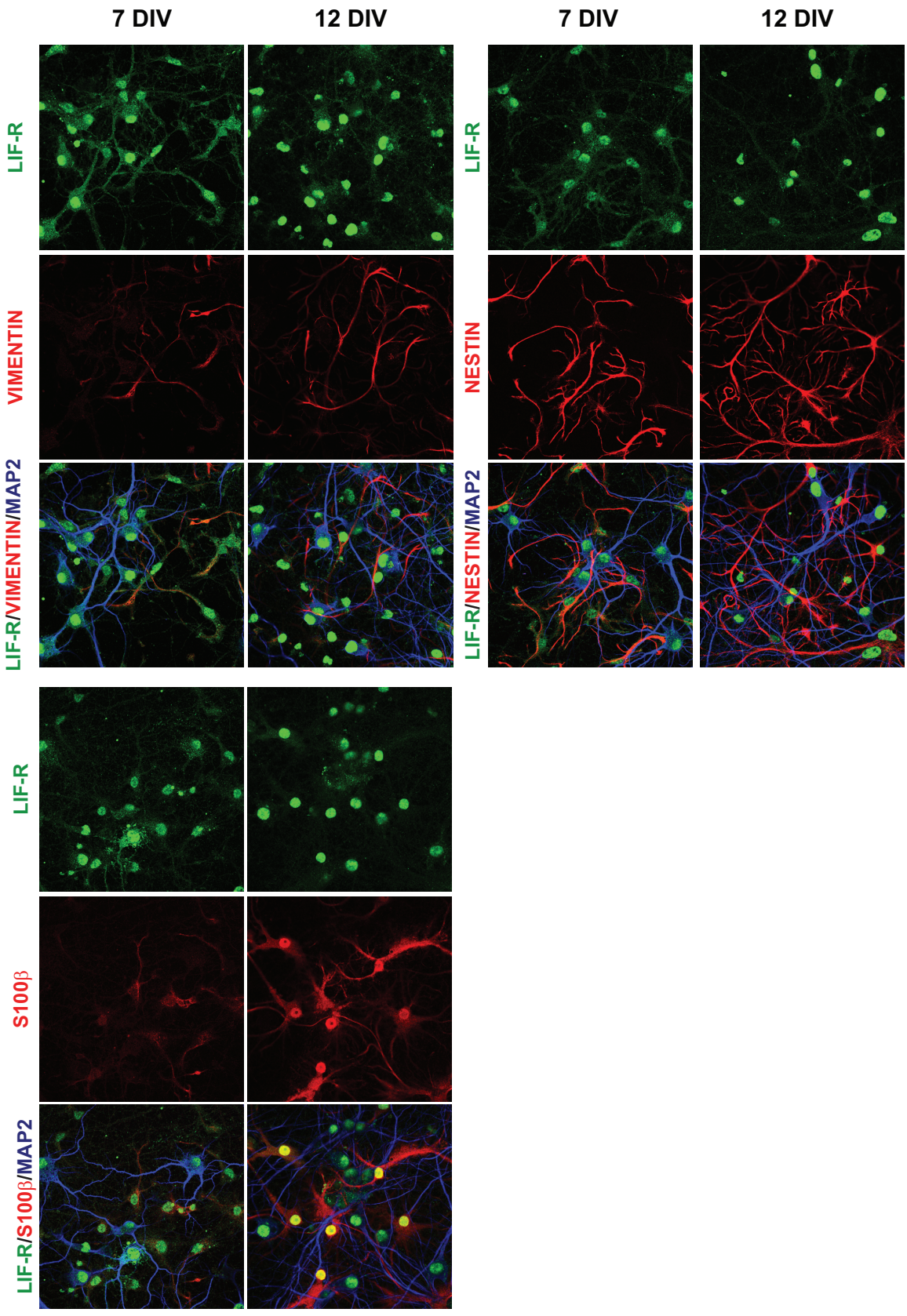
Supplemental Figure 1. Proliferation of glial cells in mixed hippocampal cultures

Rat hippocampal cultures were treated with 1 μM TTX or 1 ng/ml LIF at 7 DIV. At 10 DIV, 10 μM BrdU was added to cultures and cells were fixed 48 hrs later at 12 DIV. Cultures were immunostained for GFAP, MAP2, and BrdU or Vimentin, MAP2, and BrdU and % proliferation was calculated. Blocking spontaneous activity did not significantly decrease either the percentage of $BrdU^+$-proliferating cells or the percentage of GFAP+/$BrdU^+$ proliferating cells whereas the number of $GFAP^+$ cells was significantly decreased. In contrast, 1 ng/ml LIF significantly increased both the percentage of $GFAP^+$ astrocytes and $GFAP^+/BrdU^+$ proliferating astrocytes. Neither blocking spontaneous activity or treatment with LIF affected the percentage of VIM^+-precursors or proliferating $VIM^+/BrdU^+$-precursors. However, expression of vimentin was increased in both TTTX-treated and LIF-treated cultures (B). Scale bar = 25 μm **p <.01 ***p < 0.005


A
GFAP
VIM
% GFAP+ or VIM+ cells (% of total Hoescht)
50
40
30
20
10
0
**
***
% Proliferating cells (%BrdU+ of total Hoescht)
50
40
30
20
10
0
% GFAP+/BrdU+ or VIM+/BrdU+ cells (% of GFAP+ or VIM+ cells)
70
60
50
40
30
20
10
0
***
Sp. Act.
TTX
LIF
B
Sp. Act.
TTX
LIF
VIM BrdU Hoescht

Supplemental Figure 2. LIF receptor is expressed in GPCs and mature astrocytes

Rat hippocampal cultures were immunostained for LIF receptor and markers of GPCs (vimentin and nestin) and mature astrocytes (S100β). Immunoreactivity for LIF-R (Alexa 488) was present in vimentin^{+}-, nestin^{+}-, and S100β^{+}-cells (Alexa 568). Nestin and S100β immunoreactivity increased from 7 DIV to 12 DIV. LIF-R staining in glial processes was more prominent at 7 DIV, whereas strong nuclear staining was present in all cell types and at both time points. Also shown is MAP2 staining (Alexa 633). Scale bar = 25 μm


7 DIV
12 DIV
7 DIV
12 DIV
LIF-R
VIMENTIN
LIF-R/VIMENTIN/MAP2
LIF-R
NESTIN
LIF-R/NESTIN/MAP2
LIF-R
S100β
LIF-R/S100β/MAP2