Supplemental information

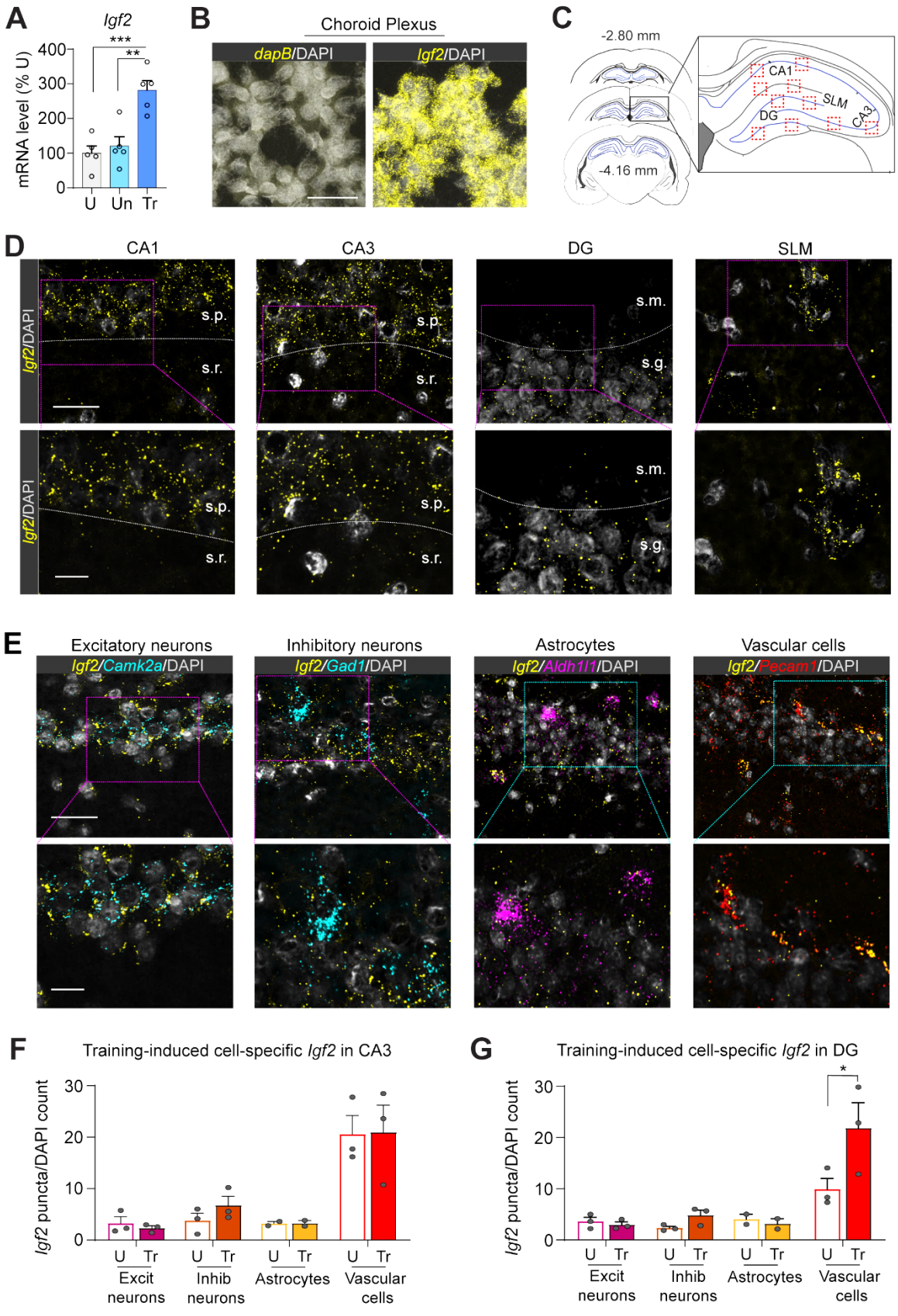


Figure S1: IGF2 is expressed in the choroid plexus, and vascular cells of dHC subregions, related to Figure 1. **(A)** Bargraphs showing *Igf2* mRNA levels in the RNA extracts from dHC of rats that were trained on IA (Tr) or received unpaired training (Un) and euthanized 20 hours later. The untrained rats (U) were left in their homecages and euthanized at matched timepoint. Data are expressed as percent of the *Igf2* mRNA levels in the untrained control rats (U = 100%). n = 5 rats/group, 2 independent experiments. One-way ANOVA followed by Tukey's post-hoc test. **(B)** Representative confocal images (scale bar 25 μm) showing RNAscope hybridization in the choroid plexus: left, *dapB* (negative control); right, *Igf2*. **(C)** Schema showing bregma sections and the areas imaged and analyzed for each dHC subregion (adapted from Paxinos & Watson, 1998). Representative confocal images (scale bar 25 μm) and higher magnification images (scale bar 15 μm) showing **(D)** *Igf2* RNAscope signal in CA1, CA3, DG and SLM subregions, and **(E)** *Igf2* RNAscope colocalized with cell-specific markers for excitatory (*Camk2a*) and inhibitory (*Gad1*) neurons, astrocytes (*Aldh1l1*), and vascular cells (*Pecam1*) in rats. High magnification confocal images from the boxed regions are shown in Figures 1A and 1B. Quantification of *Igf2* puncta by cell-type in **(F)** CA3 and **(G)** DG. n = 3 rats/group. 42-45 images/group. Dots on graphs represent the number of *Igf2* puncta normalized to the total DAPI count for each rat. Mixed-effects analyses followed by Bonferroni's multiple comparison tests. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Numeric values and detailed statistical analyses are reported in Table S1.

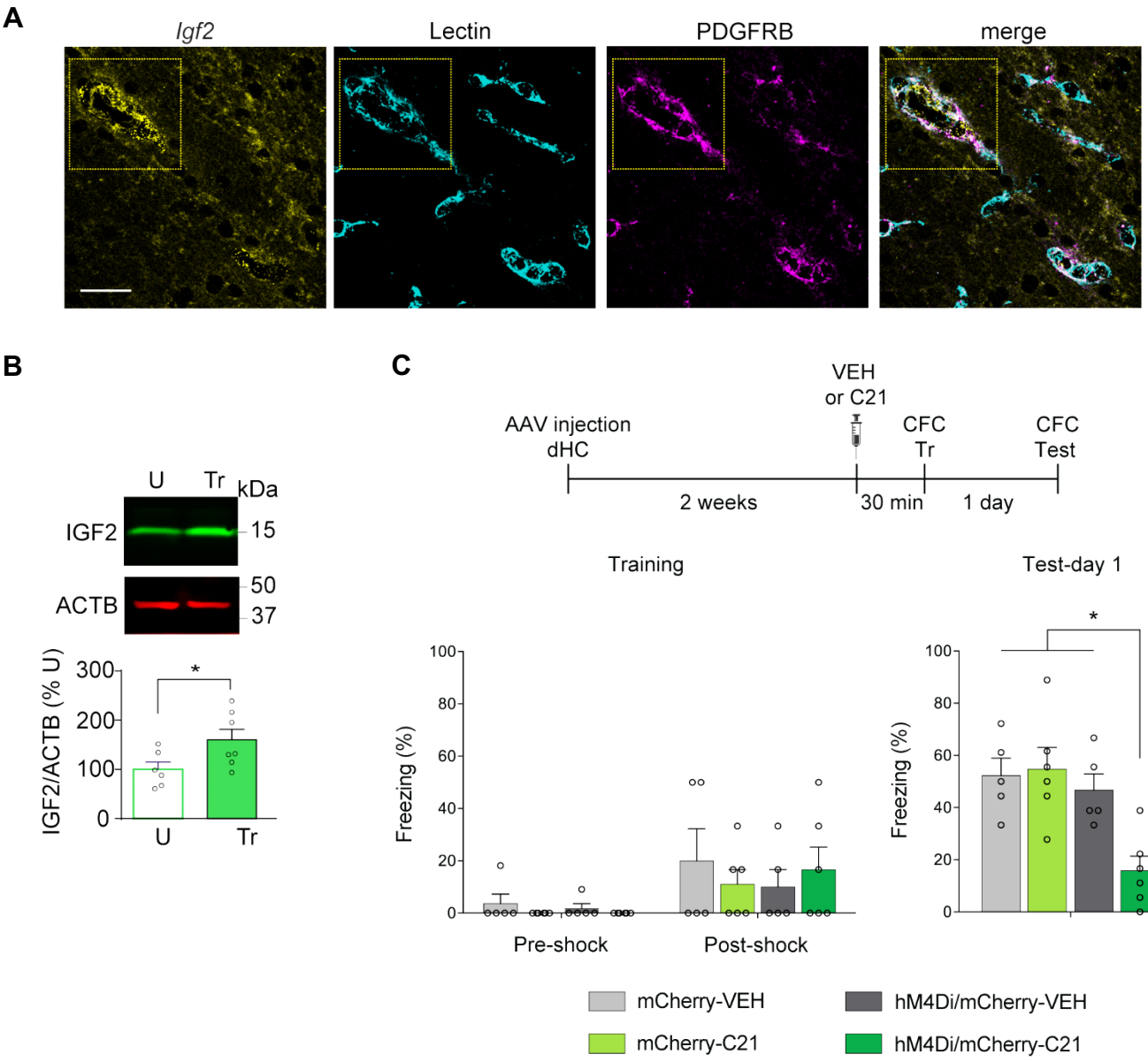
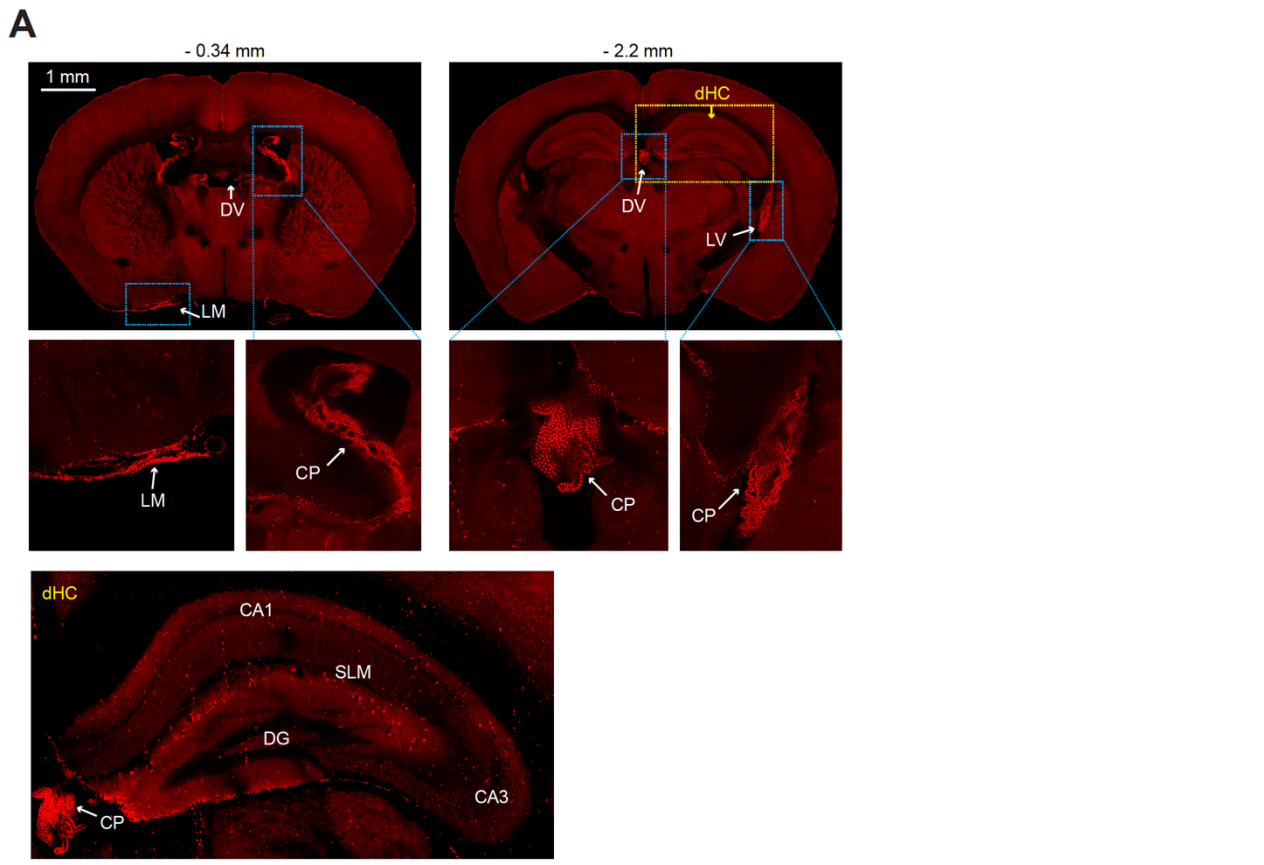
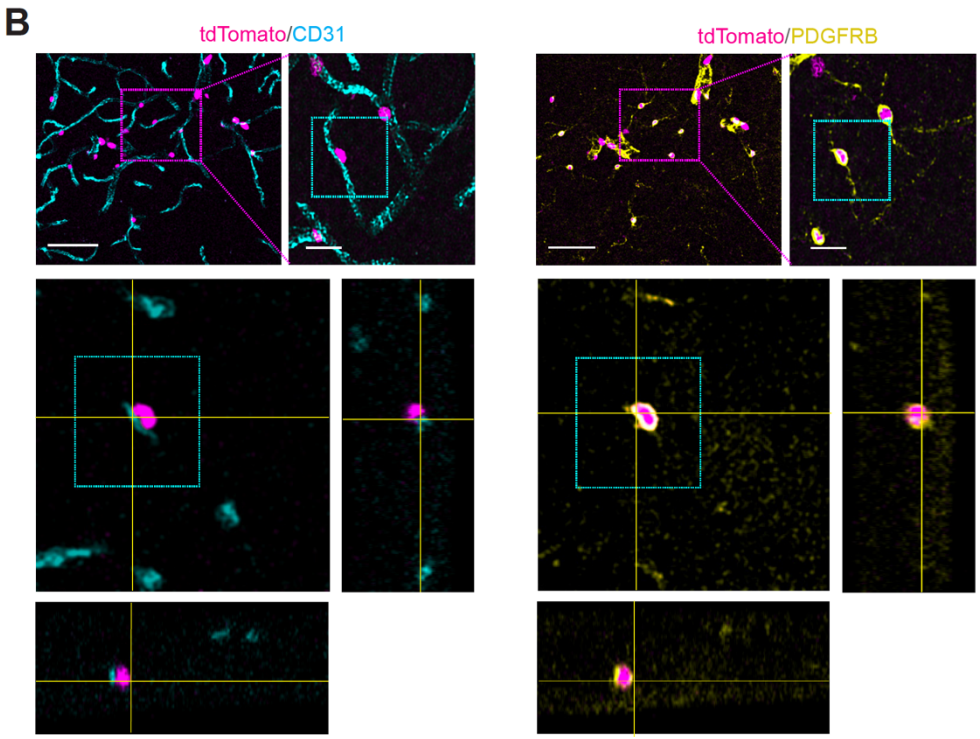

A
Igf2
Lectin
PDGFRB
merge
B
U
Tr
kDa
IGF2
15
ACTB
50
37
IGF2/ACTB (% U)
300
200
100
0
*
U
Tr
C
VEH
or C21
AAV injection
dHC
CFC
Tr
CFC
Test
2 weeks
30 min
1 day
Training
Test-day 1
Freezing (%)
100
80
60
40
20
0
Pre-shock
Post-shock
*
mCherry-VEH
hM4Di/mCherry-VEH
mCherry-C21
hM4Di/mCherry-C21
D
Pdgfrb
DREADD + Veh
DREADD + C21
fold total U
40
30
20
10
0
U Tr
total
U Tr
CD13+
U Tr
total
U Tr
CD13+

Figure S2: IGF2 is highly expressed in the PDGFRB+ cells and not in the Lectin+ cells, and upregulated in the dHC following CFC training; chemogenetic inactivation of the dHC impairs CFC memory and does not change *Pdgrfb* level, related to Figures 1, 2. (A) Representative confocal images (scale bar 25 μm) showing *Igf2* mRNA puncta (yellow) with staining for endothelial cells (tomato lectin; cyan) and immunohistochemical labeling for pericytes (PDGFRB; magenta). High magnification confocal images from the boxed regions are shown in Figure 1G. (related to Figure 1). **(B)** Representative western blots and bargraphs showing the increase in IGF2 levels in the dHC of mice 20 hours after CFC training (Tr). Untrained (U, homecage) control mice were euthanized at matched time point. ACTB was used as a loading control. Data are expressed as mean percent ± s.e.m. of untrained mice (U = 100%). Dots on graphs represent individual values for each mouse. n = 6-7 mice/group. Unpaired t-test. * $p < 0.05$. (related to Figure 2). **(C)** Top: Experimental schedule. Wild-type C57/BL6 mice were injected in the dHC with a AAV8-hSyn-hM4Di-mCherry or a AAV8-hSyn-mCherry then trained in CFC (CFC Tr) and tested one day later (CFC Test). Thirty minutes before CFC training, the mice received an intra-peritoneal injection of vehicle (VEH) or Compound 21 (C21). Bottom: CFC training (left panel) and memory tested (right panel) at 1 day after training. CFC memory is expressed as mean percent freezing ± s.e.m. n = 5–6 mice/group, 2 independent experiments. Dots on graphs represent the value for each mouse. One-way ANOVA followed by Tukey's post-hoc test. * $p < 0.05$. (related to Figure 2). **(D)** Mice were bilaterally injected in the dHC with AAV8/hSyn-hM4D(Gi)-mCherry (DREADD), 2 weeks following which they were injected intraperitoneally with C21 compound (C21) or vehicle (Veh), trained in CFC 30 min later, and euthanized at 20 hours after training. Control untrained rats (U) were left in their homecages and euthanized at matched time points. Bargraph showing the *Pdgfrb* mRNA levels in the RNA extracts from dHC (total) and FACS-

sorted CD13+ (pericytes) of mice expressing DREADD and injected with either Veh or C21. Data are shown as fold change relative to the mRNA levels present in the dHC extract (total) of untrained mice expressing DREADD and injected with Veh (U, DREADD + Veh). n = 2-3 mice/group per experiment, 3-4 independent experiments. Dots on graphs represent the individual value for each experiment. (related to Figure 2). Numerical values and detailed statistical analyses are reported in Table S1.


A
- 0.34 mm
- 2.2 mm
1 mm
DV
LM
dHC
DV
LV
LM
CP
CP
CP
dHC
CA1
SLM
DG
CA3
CP


B
tdTomato/CD31
tdTomato/PDGFRB

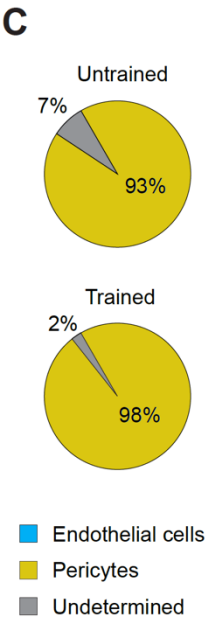

C
Untrained
7%
93%
Trained
2%
98%
Endothelial cells
Pericytes
Undetermined

Figure S3: *Igf2* promoter-driven tdTomato expression is high in the choroid plexus and leptomeninges; tdTomato labels nuclei of pericytes but not of endothelial cells in the dHC, related to Figure 3. (A) Images of coronal brain sections (scale bar 1 mm) at -0.34 mm (left) and -2.2 mm (right) from the bregma and zoomed in images showing tdTomato expression under *Igf2* promoter in the choroid plexus (CP) lining the dorsal (DV) and lateral ventricles (LV), the leptomeninges (LM), and dorsal hippocampus with the indicated subregions. **(B)** Representative confocal images (scale bar 25 μm), higher magnification images (scale bar 15 μm) and orthogonal projections showing tdTomato-labeled nuclei (magenta) and CD31-immunostained endothelial cells (cyan, left) and PDGFRB-immunostained pericytes, (yellow, right) from SLM. High magnification confocal images from the boxed regions are shown in Figures 3A and 3B. (**C**) Pie chart showing percentage of tdTomato-labeled nuclei co-localized with markers of pericytes, endothelial cells, or neither (undetermined cell type). n = 2 mice/group, 2 images/brain section, 2 brain sections/mouse. Numeric values and detailed statistical analyses are reported in Table S1.

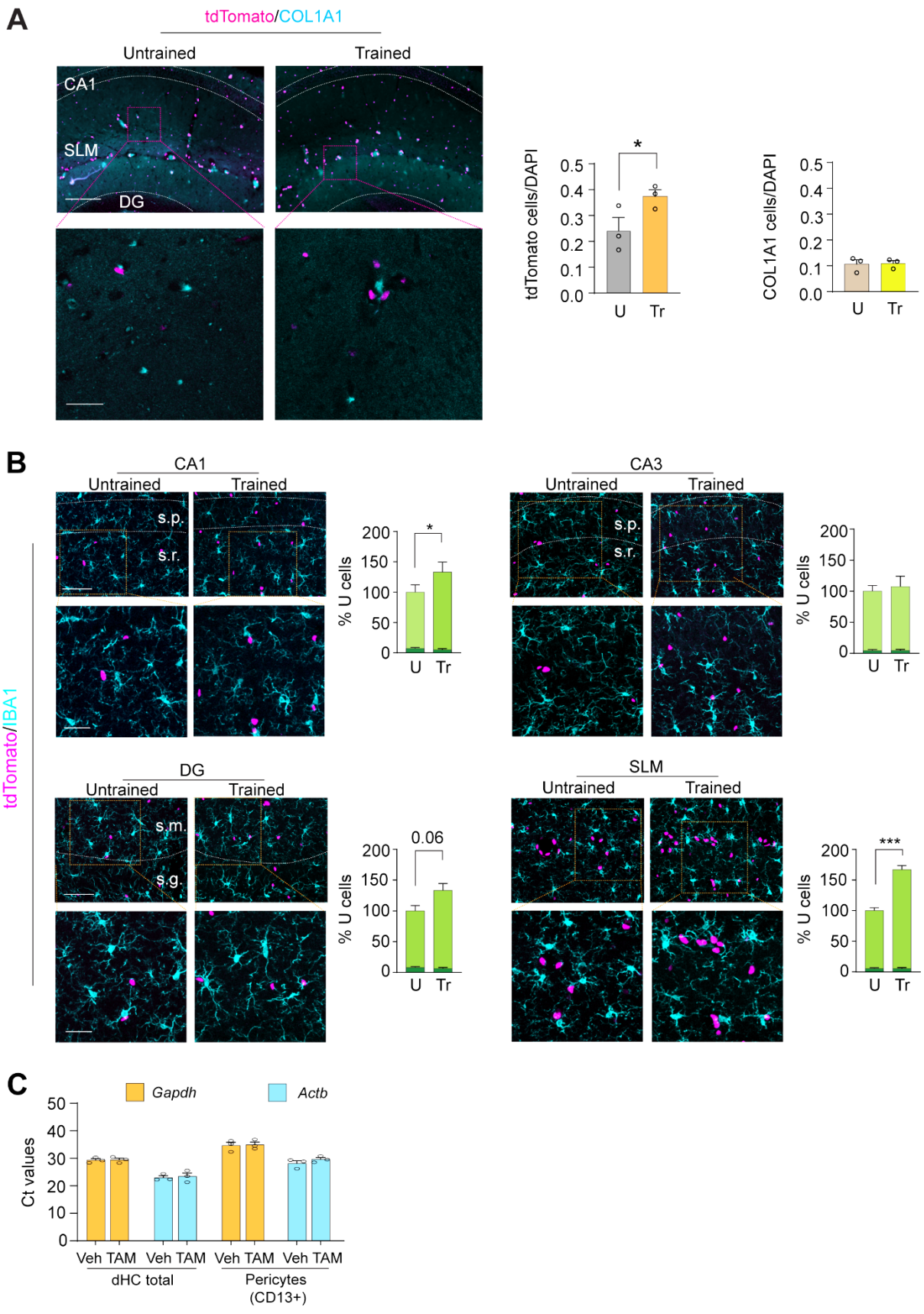

A
tdTomato/COL1A1
Untrained
Trained
CA1
SLM
DG
tdTomato cells/DAPI
COL1A1 cells/DAPI
U
Tr
B
tdTomato/IBA1
CA1
CA3
DG
SLM
s.p.
s.r.
s.m.
s.g.
% U cells
0.06
C
Gapdh
Actb
Ct values
Veh TAM Veh TAM Veh TAM Veh TAM
dHC total
Pericytes
(CD13+)

Figure S4: tdTomato does not label nuclei of perivascular fibroblasts in SLM, and tdTomato expression in the dHC does not colocalize with IBA1, related to Figures 3 and 4. As in rats SLM had the highest level of *Igf2*, we quantified the *Igf2* promoter-driven reporter tdTomato in SLM cells expressing markers of fibroblasts, and microglia. **(A)** Representative confocal images of SLM areas (scale bar: 50 μm), higher magnification images (scale bar: 20 μm), and quantifications showing tdTomato-labeled nuclei (magenta) and COL1A1-immunostained cells (cyan) from untrained (U) and CFC trained (Tr) IGF2-tdTomato-H2B mice. Data are expressed as mean percent ± s.e.m. Dots on graphs represent the summed values for all brain sections from each mouse. (related to Figure 3). **(B)** Representative confocal images (scale bar: 50 μm), higher magnification images (scale bar: 20 μm) of CA1, CA3, DG and SLM subregions, and quantifications showing tdTomato-labeled nuclei (magenta) and IBA1-immunostained microglia (cyan) from untrained (U) and CFC trained (Tr) IGF2-tdTomato-H2B mice. Data are expressed as mean percent ± s.e.m. Dots on graphs represent the summed values for all brain sections from each mouse. n = 3 mice/group, 3 independent experiments. Unpaired t-test. * $p < 0.05$, *** $p < 0.001$. (related to Figure 3). **(C)** Cycle threshold (Ct) values of *Gapdh* and *Actb* in total extracts and FACS-sorted CD13+ cells (pericytes) from the dHC of tamoxifen (TAM) or vehicle (Veh)-injected PDGFRB-Cre *Igf2*-floxed mice. n = 3 mice/group per experiment, 3 independent experiments. Dots on graphs represent individual values for each experiment. (related to Figure 4). Numerical values and detailed statistical analyses are reported in Table S1.

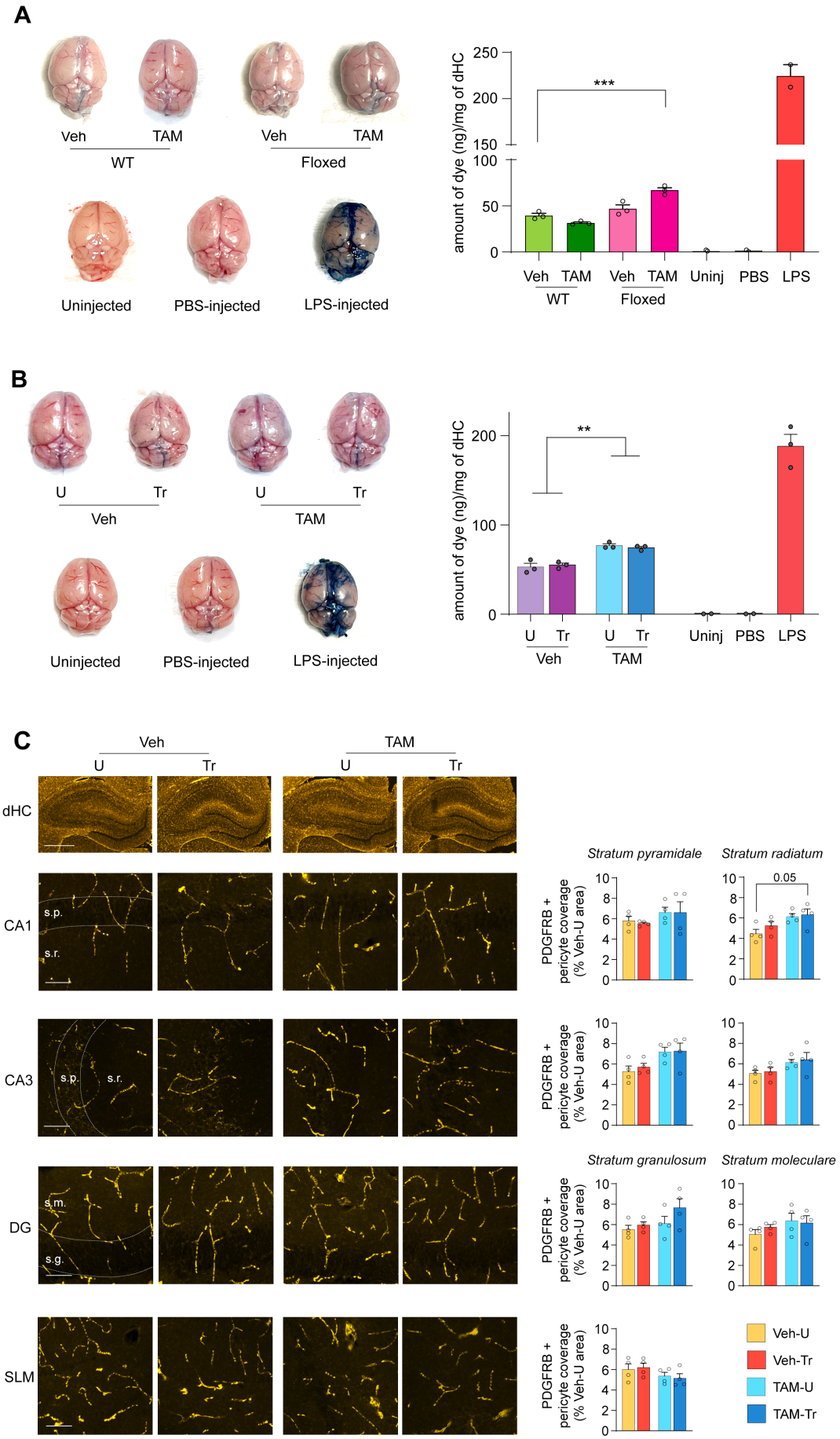
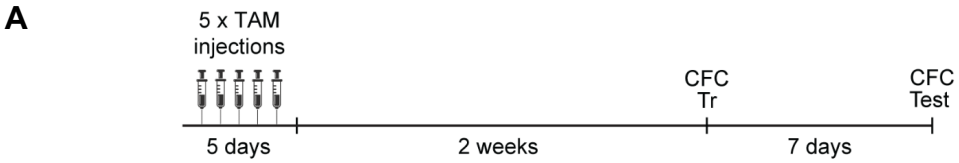
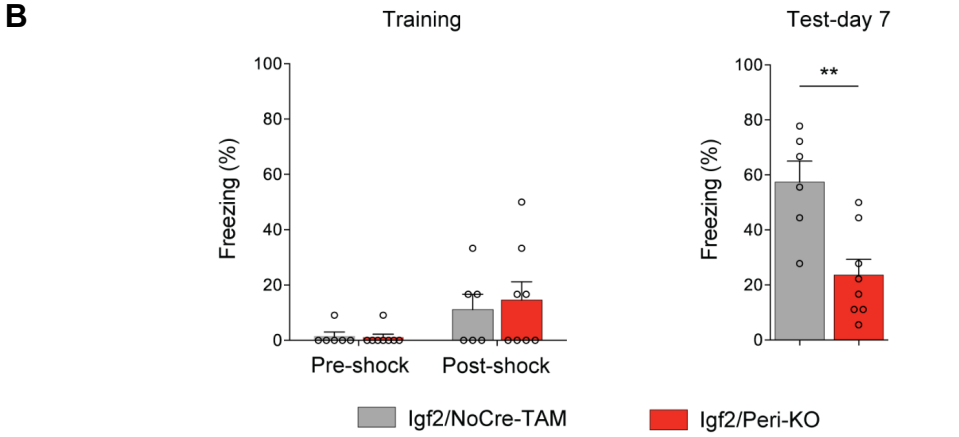
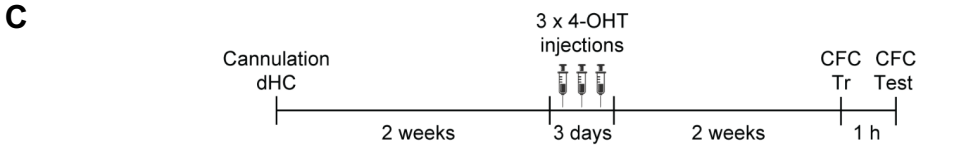
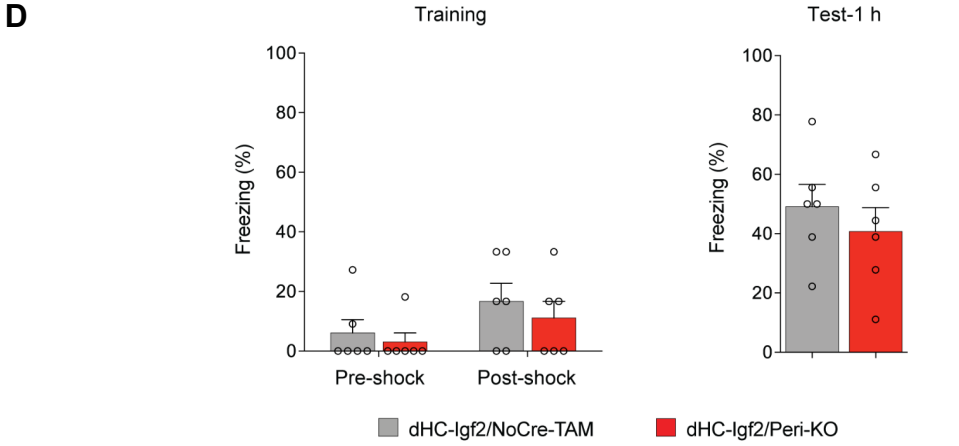

A
Veh
TAM
Veh
TAM
WT
Floxed
Uninjected
PBS-injected
LPS-injected
amount of dye (ng)/mg of dHC
250
200
150
100
50
0
***
Veh
TAM
Veh
TAM
Uninj
PBS
LPS
WT
Floxed
B
U
Tr
U
Tr
Veh
TAM
Uninjected
PBS-injected
LPS-injected
amount of dye (ng)/mg of dHC
200
100
0
**
U
Tr
U
Tr
Uninj
PBS
LPS
Veh
TAM
C
Veh
TAM
U
Tr
U
Tr
dHC
CA1
s.p.
s.r.
CA3
s.p.
s.r.
DG
s.m.
s.g.
SLM
Stratum pyramidale
Stratum radiatum
Stratum granulosum
Stratum moleculare
PDGFRB + pericyte coverage (% Veh-U area)
0.05
Veh-U
Veh-Tr
TAM-U
TAM-Tr

Figure S5: Deletion of pericytic *Igf2* significantly increases vascular permeability in the dHC but does not affect PDGFRB level in the dHC, related to Figure 4. (A) Representative images of brains and quantifications of the amount of Evans Blue dye (ng) extravasated per mg of dHC of tamoxifen (TAM)- or vehicle (Veh)-injected wild type (WT) and PDGFRB-Cre *Igf2*-floxed mice. **(B)** Representative images of brains and quantifications of the amount of Evans Blue dye (ng) extravasated per mg of dHC of tamoxifen (TAM)- or vehicle (Veh)-injected WT mice trained (Tr) in CFC and euthanized 1 hour later. Control untrained mice (U) were left in their home cages and euthanized at matched timepoints. Mice injected with PBS- or lipopolysaccharides (LPS) were included as negative and positive controls, respectively. An uninjected (Uninj) control group of mice was also included. Data are expressed as mean amount of dye (ng) per mg of dHC ± s.e.m. n = 3 mice/group, 3 independent experiments. Dots on graphs represent values for each mouse. Two-way ANOVA followed by Tukey's post-hoc test. ** $p < 0.01$, *** $p < 0.001$. **(C)** Representative dHC images (scale bar: 500 μm) and zoomed confocal images of CA1, CA3, DG and SLM subregions (scale bar: 50 μm) and quantifications showing PDGFRB immunofluorescence intensities in *stratum pyramidale* (s.p.) and *stratum radiatum* (s.r.) layers of CA1 and CA3, *stratum granulosum* (s.g.) and *stratum moleculare* (s.m.) layers of DG, and in SLM of tamoxifen (TAM)- or vehicle (Veh)-injected PDGFRB-Cre *Igf2*-floxed mice trained (Tr) in CFC and euthanized 1 hour later. Control untrained mice (U) were left in their home cages and euthanized at matched timepoints. Data are expressed as mean percent area ± s.e.m. n = 4 mice/group. Two independent experiments of n = 2 each. Dots on graphs represent the mean values for each mouse. Two-way ANOVA followed by Tukey's post-hoc test. Numerical values and detailed statistical analyses are reported in Table S1.


A
5 x TAM injections
CFC Tr
CFC Test
5 days
2 weeks
7 days


B
Training
Test-day 7
Freezing (%)
Pre-shock
Post-shock
**
Igf2/NoCre-TAM
Igf2/Peri-KO


C
Cannulation dHC
3 x 4-OHT injections
CFC Tr
CFC Test
2 weeks
3 days
2 weeks
1 h


D
Training
Test-1 h
Freezing (%)
Pre-shock
Post-shock
dHC-Igf2/NoCre-TAM
dHC-Igf2/Peri-KO

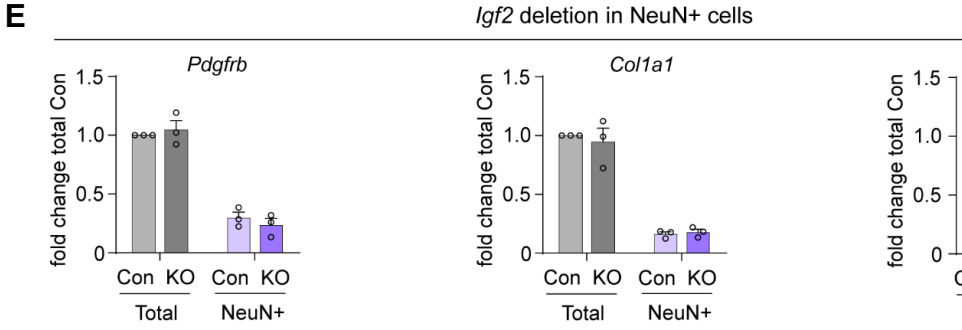

E
Igf2 deletion in NeuN+ cells
Pdgfrb
Col1a1
Igf2
fold change total Con
Con KO Con KO
Total
NeuN+
***

Figure S6: Pericyte-specific knockout (KO) of *Igf2* impairs long-term contextual fear memory; *Igf2* KO in dHC pericytes does not affect contextual fear learning and short-term memory, related to Figures 4, 5 and 6. (A) Experimental schedule of Igf2/NoCre-TAM and Igf2/Peri-KO. (related to Figure 4). **(B)** CFC training (left panel) and memory tested at 7 days (right panel) after training. CFC memory was expressed as mean percent freezing ± s.e.m. n = 6–8 mice/group. Two independent experiments. ** $p < 0.01$; Unpaired t-test. Dots on graphs represent the value for each mouse. (related to Figure 4). **(C)** Experimental schedule of dHC-Igf2/NoCre-TAM and dHC-Igf2/Peri-KO. (related to Figure 5). **(D)** CFC training (left panel) and memory tested at one hour (right panel) after training. CFC memory was expressed as mean percent freezing ± s.e.m. n = 6 mice/group. Two independent experiments. Dots on graphs represent the value for each mouse. (related to Figure 5). **(E)** Neuron-specific *Igf2* deletion was induced in Igf2 floxed mice by injecting into their dHC an adeno associated virus containing Cre-recombinase (dHC-Igf2/Neuro-KO or KO) or GFP (dHC-Igf2/Neuro-Control or Con) under Syn promoter. qPCR analyses of *Pdgfrb, Col1a1,* and *Igf2* mRNA in the RNA extracts from dHC (total) and FACS-sorted NeuN+ (neurons) of dHC-Igf2/Neuro-KO or dHC-Igf2/Neuro-Control mice. The graphs show the data shown in Figure 6D on a different scale. Data are shown as fold change relative to the mRNA levels in the dHC (total) extract of dHC-Igf2/Neuro-Control mice (Con, Total); n = 2-3 mice/group per experiment, 3 independent experiments. Dots on graphs represent the value for each experiment. Two-way ANOVA followed by Sidak's post-hoc test. *** $p < 0.001$. (related to Figure 6). Numeric values and detailed statistical analyses are reported in Table S1.

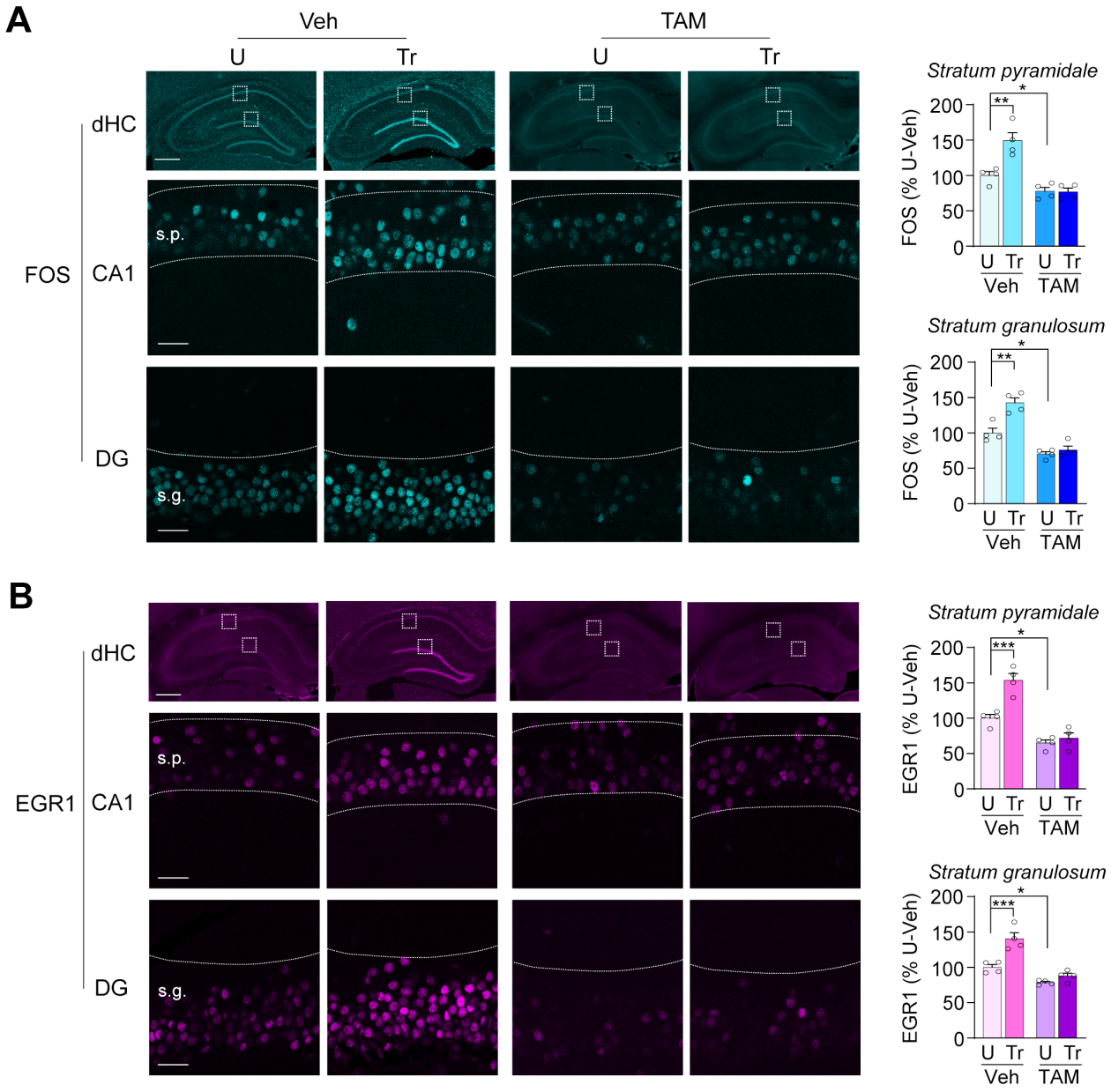


Figure S7: Ablation of pericytic IGF2 significantly decreases levels of immediate early genes FOS and EGR1, related to Figure 7. Representative dHC images (scale bar: 500 µm), zoomed confocal images (scale bar: 20 µm) and quantifications showing **(A)** FOS, and **(B)** EGR1 immunofluorescence intensities in the *stratum pyramidale* (s.p.) of CA1 and *stratum granulosum* (s.g.) of DG of tamoxifen (TAM)- or vehicle (Veh)-injected PDGFRB-Cre *Igf2*-floxed mice trained (Tr) in CFC and euthanized 1 hour later. Control untrained mice (U) were left in their home cages

and euthanized at matched timepoints. Data are expressed as mean percent ± s.e.m of Veh-injected untrained mice (U-Veh = 100%). n = 4 mice per group. Two independent experiments of n = 2 each. Dots on graphs represent the mean values for each mouse. Two-way ANOVA followed by Tukey's post-hoc test. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Numerical values and detailed statistical analyses are reported in Table S1.