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Supplemental information

EphrinB2 and GRIP1 stabilize mushroom spines during denervation-induced homeostatic plasticity

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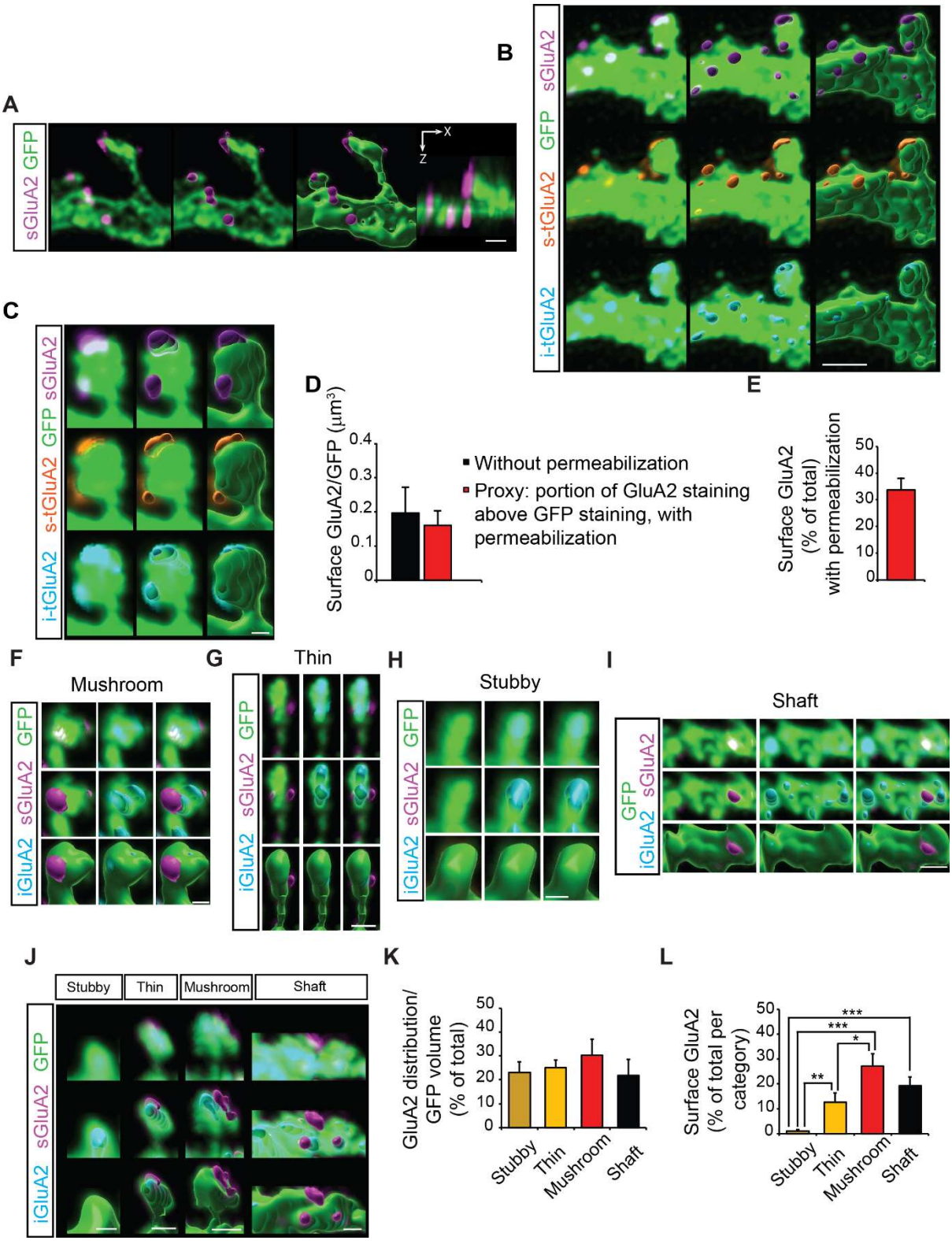
A
sGluA2 GFP
X
Z
B
i-tGluA2
s-tGluA2
GFP
sGluA2
C
i-tGluA2
s-tGluA2
GFP
sGluA2
D
Surface GluA2/GFP (μm³)
0.4
0.3
0.2
0.1
0
Without permeabilization
Proxy: portion of GluA2 staining above GFP staining, with permeabilization
E
Surface GluA2 (% of total) with permeabilization
50
40
30
20
10
0
F
Mushroom
iGluA2
sGluA2
GFP
G
Thin
iGluA2
sGluA2
GFP
H
Stubby
iGluA2
sGluA2
GFP
I
Shaft
GFP
iGluA2
sGluA2
J
Stubby
Thin
Mushroom
Shaft
iGluA2
sGluA2
GFP
K
GluA2 distribution/ GFP volume (% of total)
50
40
30
20
10
0
Stubby
Thin
Mushroom
Shaft
L
Surface GluA2 (% of total per category)
50
40
30
20
10
0
***
***
*
**
Stubby
Thin
Mushroom
Shaft

Figure S1. Expansion microscopy provides sufficient spatial resolution to discriminate between surface and internal GluA2-containing AMPARs, and AMPAR distribution is similar in CA1 pyramidal cells compared to dentate granule cells. Related to Figure 1.

(A) Representative images from GFP (green) - transfected hippocampal cultures stained for surface GluA2 (sGluA2, magenta) without permeabilization and submitted to expansion microscopy. From left to right: GluA2 and GFP stainings; GluA2 reconstruction and GFP staining; GluA2 and GFP reconstructions. The fourth panel displays GluA2 and GFP staining of the same spine as visible along the Y axis. Scale bar, 1µm.

(B-C) Representative images from GFP (green) - transfected hippocampal cultures stained for surface GluA2 without permeabilization (sGluA2, magenta, top row) and for total GluA2 after permeabilization (tGluA2) using the same antibody; based on the reconstruction of the GFP staining, the latter has been divided between surface GluA2 (s-tGluA2, orange, middle row) and internal GluA2 (i-tGluA2, cyan, bottom row). The left panels show GluA2 and GFP stainings; the middle panels show GluA2 reconstruction and GFP staining; the right panels show both reconstructions. (C) Higher magnification of the mushroom spine in (B). Scale bar, 1µm (B), 0.5µm (C).

(D) Quantification of the amount of GluA2 signal at the surface of mushroom spines, corresponding to (C). For surface GluA2 without permeabilization, the volume of each GluA2 reconstruction was reported to the GFP volume of the corresponding mushroom spine, all volumes were summed per spine, and the average was calculated across all mushroom spines. For total GluA2 after permeabilization, only GluA2 reconstructions at the surface of the GFP volume were considered. Of note, the baseline proportion of surface GluA2 in mushroom spines in hippocampal cultures is statistically similar to what is observed in OTCs under constitutive conditions. Data are shown as mean +/- SEM.

(E) Quantification of surface GluA2 with permeabilization, shown as percentage of total GluA2 (+/- SEM) corresponding to (C).

(F-I) Representative surface reconstructions of the mushroom (F), thin (G), stubby (H) spines and the shaft (I) shown in Fig. 1C. Surface GluA2 (magenta, left column) and internal GluA2 (cyan, middle column) are shown separately and together (right column; as seen in Fig. 1C). From top to bottom: GluA2 and GFP staining; GluA2 reconstruction and GFP staining; GluA2 and GFP reconstruction. Scale bars, 1µm.

(J) Representative surface reconstructions of stubby, thin and mushroom spines and shafts from secondary stretches in the *stratum radiatum* of CA1 pyramidal cells. The surface reconstruction of the GFP staining is not visible in the bottom row to enable visualization of internal GluA2. Scale bars, 1µm.

(K) Quantification of GluA2 distribution normalized to GFP volume, shown as percentage of total GluA2/GFP (+/- SEM) corresponding to (E). n = 5 neurons, 2 experiments.

(L) Quantification of surface GluA2, shown as percentage of total GluA2 per compartment (+/- SEM) corresponding to (E). n = 5 neurons, 2 experiments. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

Exact p-values in Table S1.

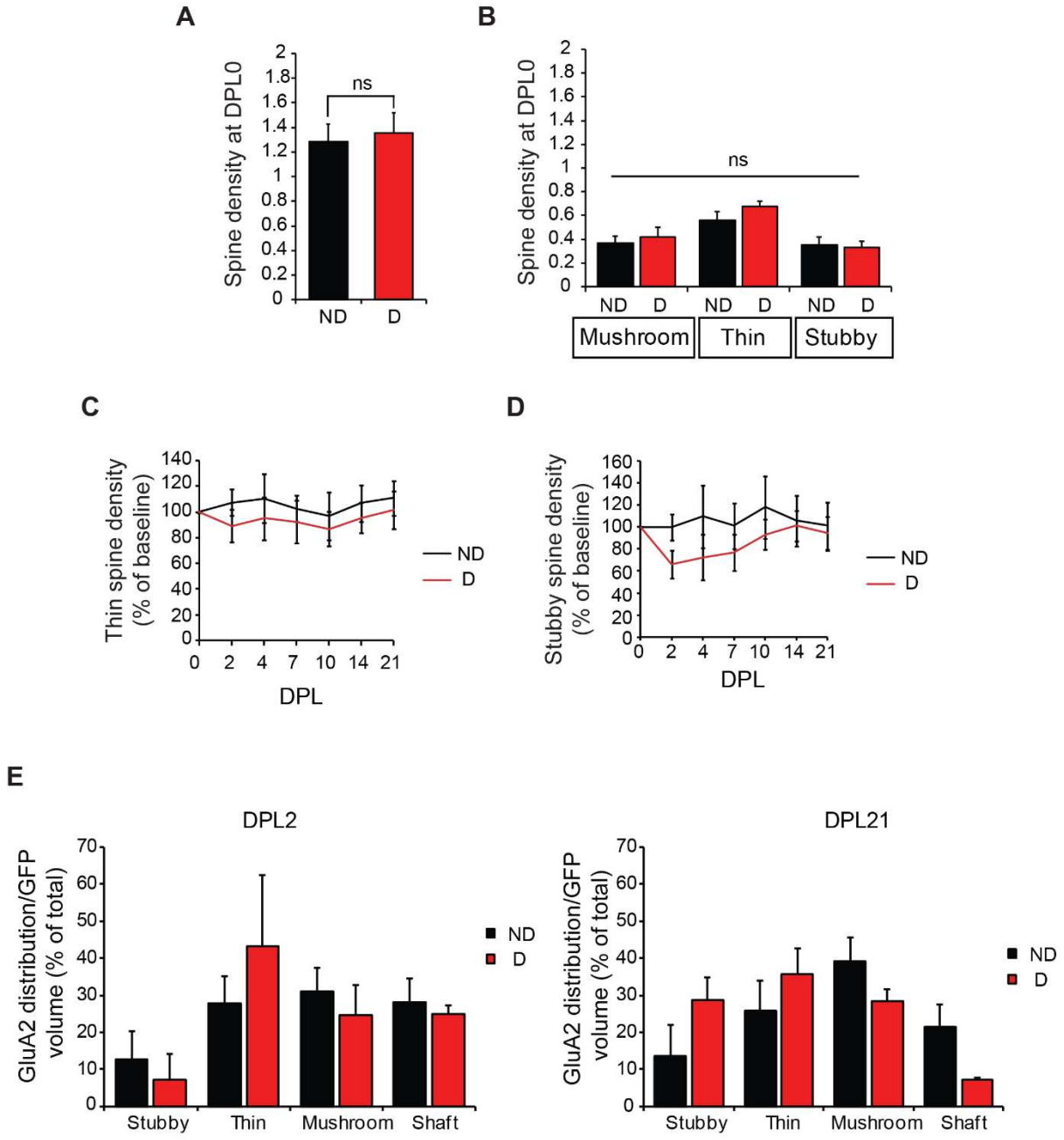

Figure S2. Denervation does not affect thin and stubby spine density nor total AMPAR content. Related to Figure 1.

(A-B) Total spine density (A) and mushroom, thin and stubby spine density (B) at baseline (day post lesion (DPL)0) (+/- SEM) are similar between stretches from OTCs subsequently selected for denervation (D) or as non-denervated controls (ND). ns, not significant.

(C-D) Quantification of thin (C) and stubby (D) spine density in non-denervated and denervated OTCs as relative ratios to baseline (DPL 0) (+/- SEM) corresponding Figure 1 (G). n = 5-7 neurons per condition, 4 experiments.

(E) Quantification of GluA2 distribution normalized to GFP volume at DPL2 and DPL21 in denervated (D) and non-denervated (ND) OTCs (+/- SEM) corresponding to Figure 1 (I-K). n = 5 neurons per condition, 2 experiments.

Exact p-values in Table S1.

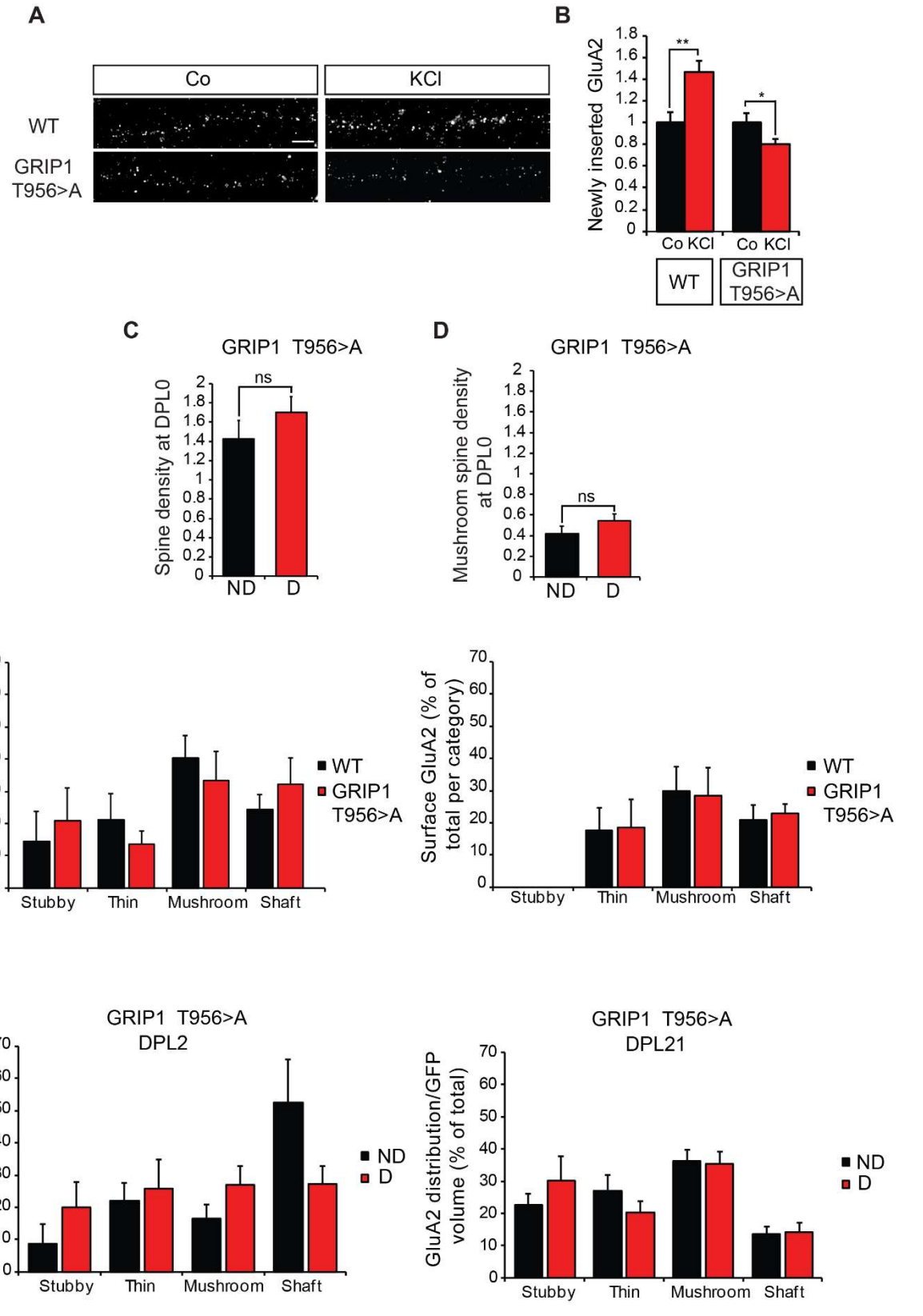
A
Co
KCl
WT
GRIP1
T956>A
B
Newly inserted GluA2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
**
*
Co KCl
Co KCl
WT
GRIP1
T956>A
C
GRIP1 T956>A
Spine density at DPL0
ns
ND
D
D
GRIP1 T956>A
Mushroom spine density
at DPL0
ns
ND
D
E
GluA2 distribution/GFP
volume (% of total)
70
60
50
40
30
20
10
0
Stubby
Thin
Mushroom
Shaft
WT
GRIP1
T956>A
Surface GluA2 (% of
total per category)
WT
GRIP1
T956>A
F
GRIP1 T956>A
DPL2
GluA2 distribution/GFP
volume (% of total)
ND
D
GRIP1 T956>A
DPL21
GluA2 distribution/GFP
volume (% of total)
ND
D

Figure S3. T956 phosphorylation is required for GRIP1 function during activity-induced *de novo* AMPA receptor insertion and stabilization, but not for baseline spine density and morphology, nor for AMPAR content and distribution. Related to Figure 2.

(A) Representative pictures of newly inserted GluA2 in dendritic stretches of hippocampal neurons. Scale bar, 5µm.

(B) Quantification of the relative fluorescence intensities (+/- SEM) of the newly inserted GluA2, corresponding to (A). Stimulation is normalized to the control condition. n = 30-60 neurons per condition from 3 experiments. *$p < 0.05$, **$p < 0.01$.

(C-D) Total (C) and mushroom (D) spine density at baseline (DPL0) (+/- SEM) are similar between stretches from *Grip1*-T956>A OTCs subsequently selected for denervation (D) or as non-denervated OTCs (ND). ns, not significant.

(E) Quantification of total GluA2/GFP (left graph) and surface GluA2 (right graph) in non-denervated *Grip1*-T956>A OTCs compared to control (Ctrl) OTCs, (+/- SEM). n = 5 neurons per condition, 2 experiments.

(F) Quantification of total GluA2/GFP distribution at DPL2 and DPL21 in denervated (D) and non-denervated (ND) *Grip1*-T956>A OTCs (+/- SEM). n = 5 neurons per condition, 2 experiments. DPL, Day post lesion.

Exact p-values in Table S1.

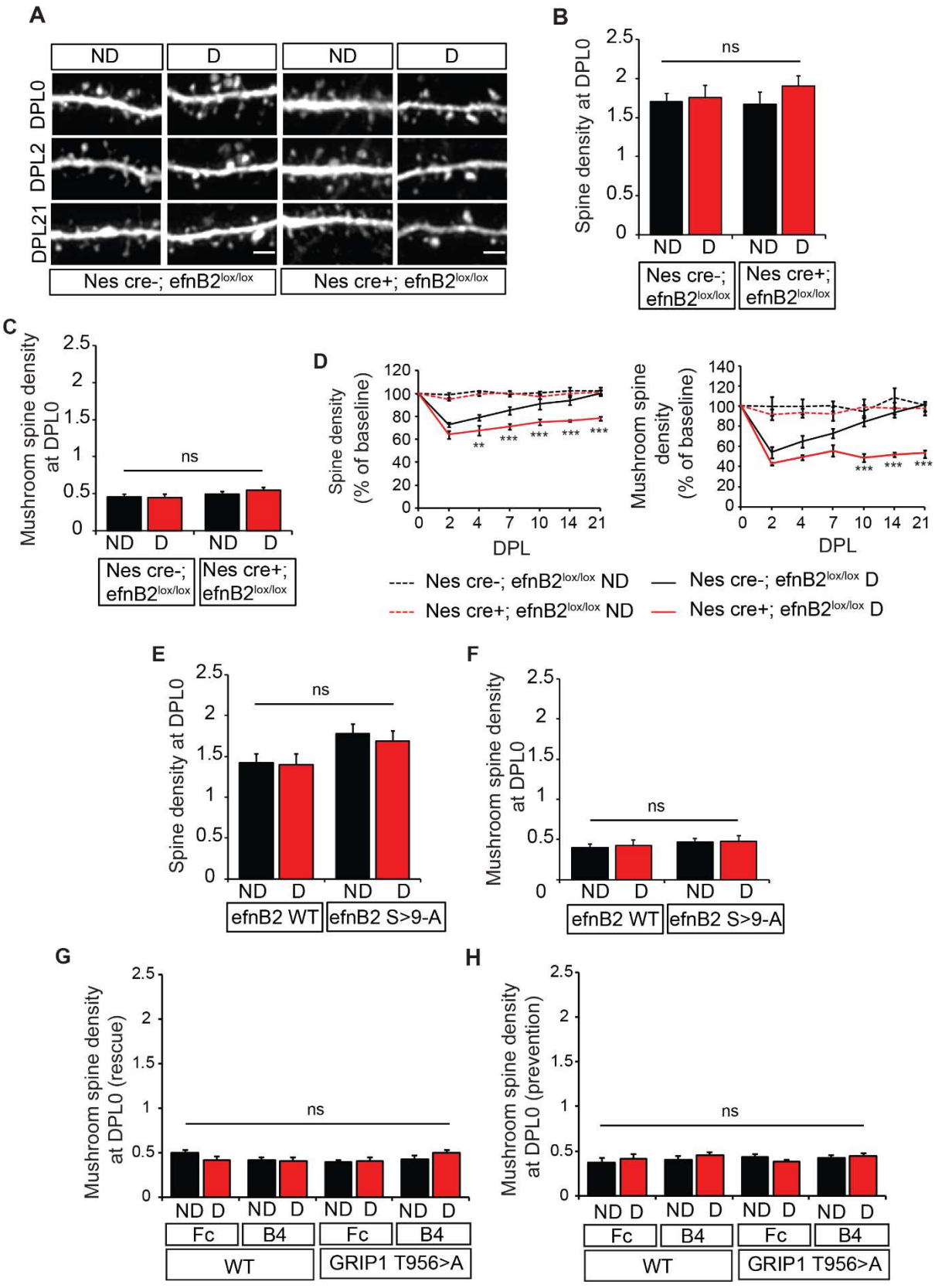
A
ND
D
ND
D
DPL0
DPL2
DPL21
Nes cre-; efnB2lox/lox
Nes cre+; efnB2lox/lox
B
Spine density at DPL0
ns
ND D ND D
Nes cre-; efnB2lox/lox
Nes cre+; efnB2lox/lox
C
Mushroom spine density at DPL0
ns
ND D ND D
Nes cre-; efnB2lox/lox
Nes cre+; efnB2lox/lox
D
Spine density (% of baseline)
DPL
Mushroom spine density (% of baseline)
DPL
---- Nes cre-; efnB2lox/lox ND
— Nes cre-; efnB2lox/lox D
---- Nes cre+; efnB2lox/lox ND
— Nes cre+; efnB2lox/lox D
E
Spine density at DPL0
ns
ND D ND D
efnB2 WT
efnB2 S>9-A
F
Mushroom spine density at DPL0
ns
ND D ND D
efnB2 WT
efnB2 S>9-A
G
Mushroom spine density at DPL0 (rescue)
ns
ND D ND D ND D ND D
Fc
B4
Fc
B4
WT
GRIP1 T956>A
H
Mushroom spine density at DPL0 (prevention)
ns
ND D ND D ND D ND D
Fc
B4
Fc
B4
WT
GRIP1 T956>A

Figure S4. EphrinB2 is crucial for mushroom spine formation and stabilization after lesion-induced loss, but not necessary for baseline spine density and morphology. Related to Figures 3 and 4.

(A) Representative pictures of secondary dendritic stretches of non-denervated and denervated *efnB2* knockout OTCs (*Nes cre+; efnB2*$^{lox/lox}$) and their control littermates (*Nes cre-; efnB2*$^{lox/lox}$) at DPL0, DPL2 and DPL21. DPL, Day post lesion; ND, not denervated; D, denervated. Scale bars, 2µm.

(B-C) Total (B) and mushroom (C) spine density at baseline (DPL0) (+/- SEM) are similar between stretches from *efnB2* knockout or control OTCs subsequently selected for denervation (D) or as non-denervated controls (ND). ns, not significant.

(D) Quantification of total spine (left graph) and mushroom spine (right graph) density at each time point as relative ratios to DPL0 (+/- SEM). Significance levels shown between *Nes cre+; efnB2*$^{lox/lox}$ D and *Nes cre-; efnB2*$^{lox/lox}$ D. n = 6-7 neurons per condition from 3 experiments. **p < 0.01, ***p< 0.001.

(E-F) Total (E) and mushroom (F) spine density at baseline (DPL0) (+/- SEM) are similar between stretches from *efnB2 S-9>A* or control (Ctrl) OTCs subsequently selected for denervation (D) or as non-denervated controls (ND). ns, not significant.

(G-H) Mushroom spine density at baseline (DPL0) (+/- SEM) is similar between stretches from *Grip1*-T956>A or control (Ctrl) OTCs subsequently selected for denervation (D), non-denervation (ND), EphB4-Fc stimulation (B4) or Fc-treatment (Fc) according to the rescue (G) or prevention (H) paradigm. ns, not significant.

Exact p-values in Table S1.