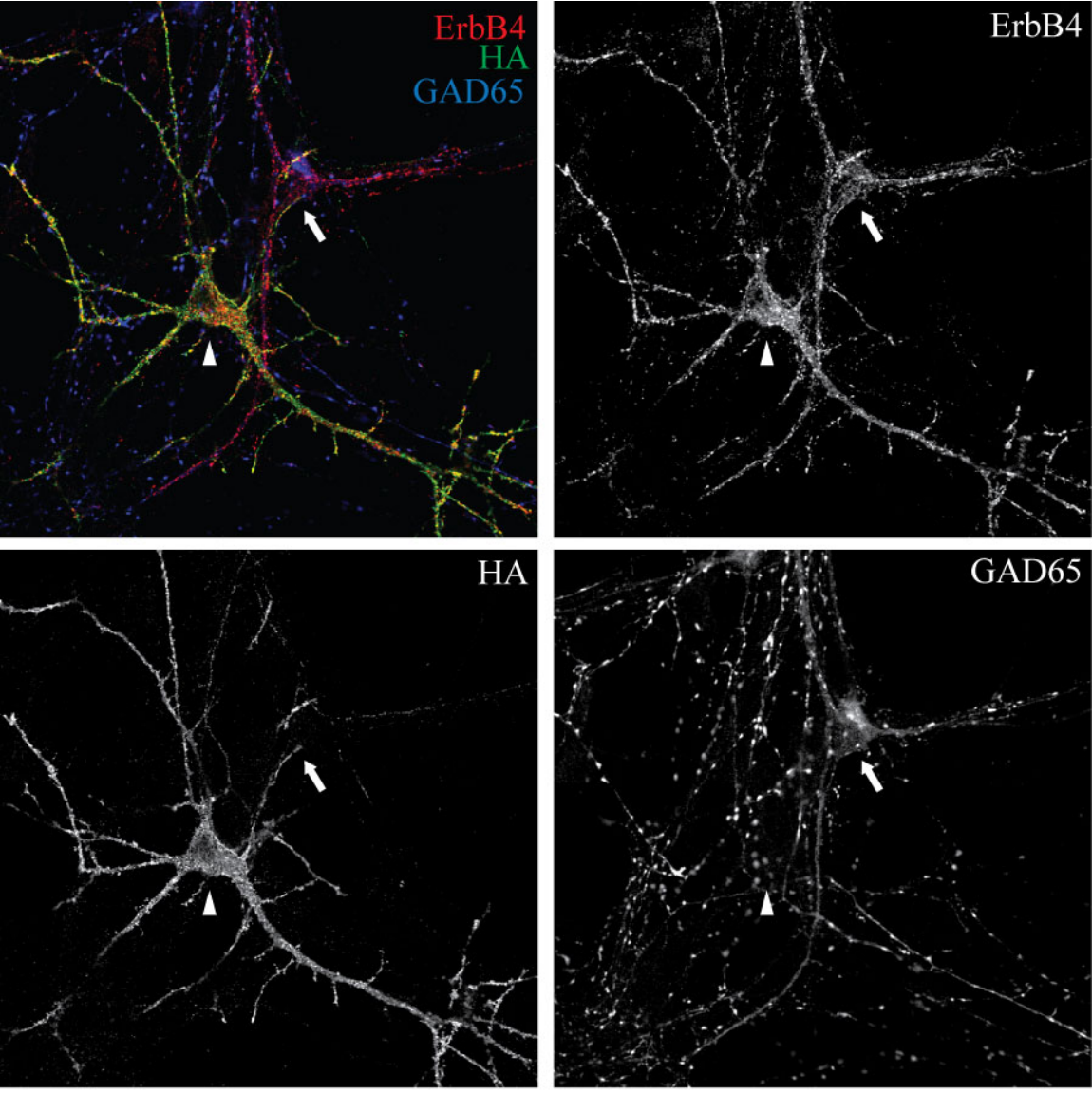
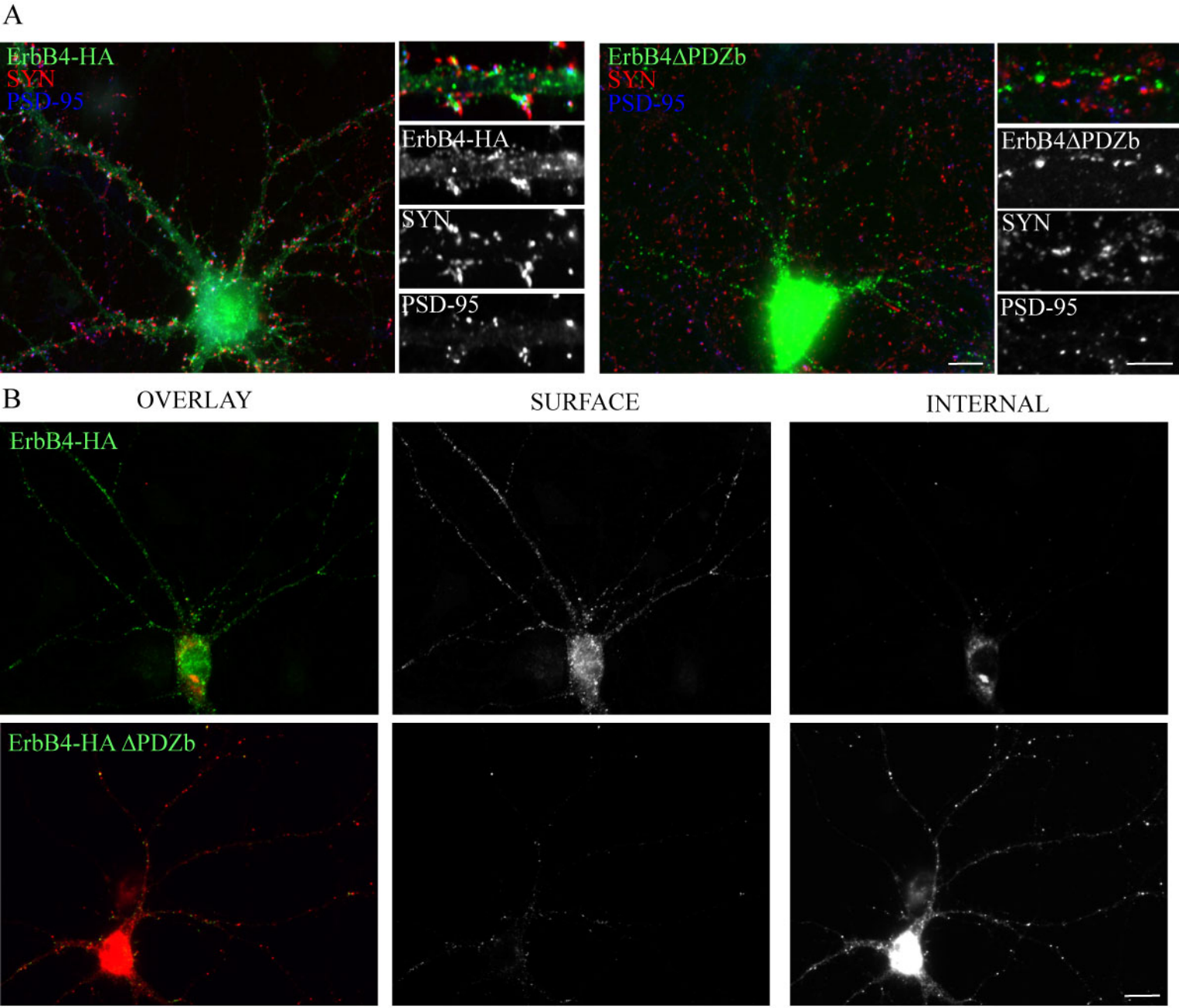


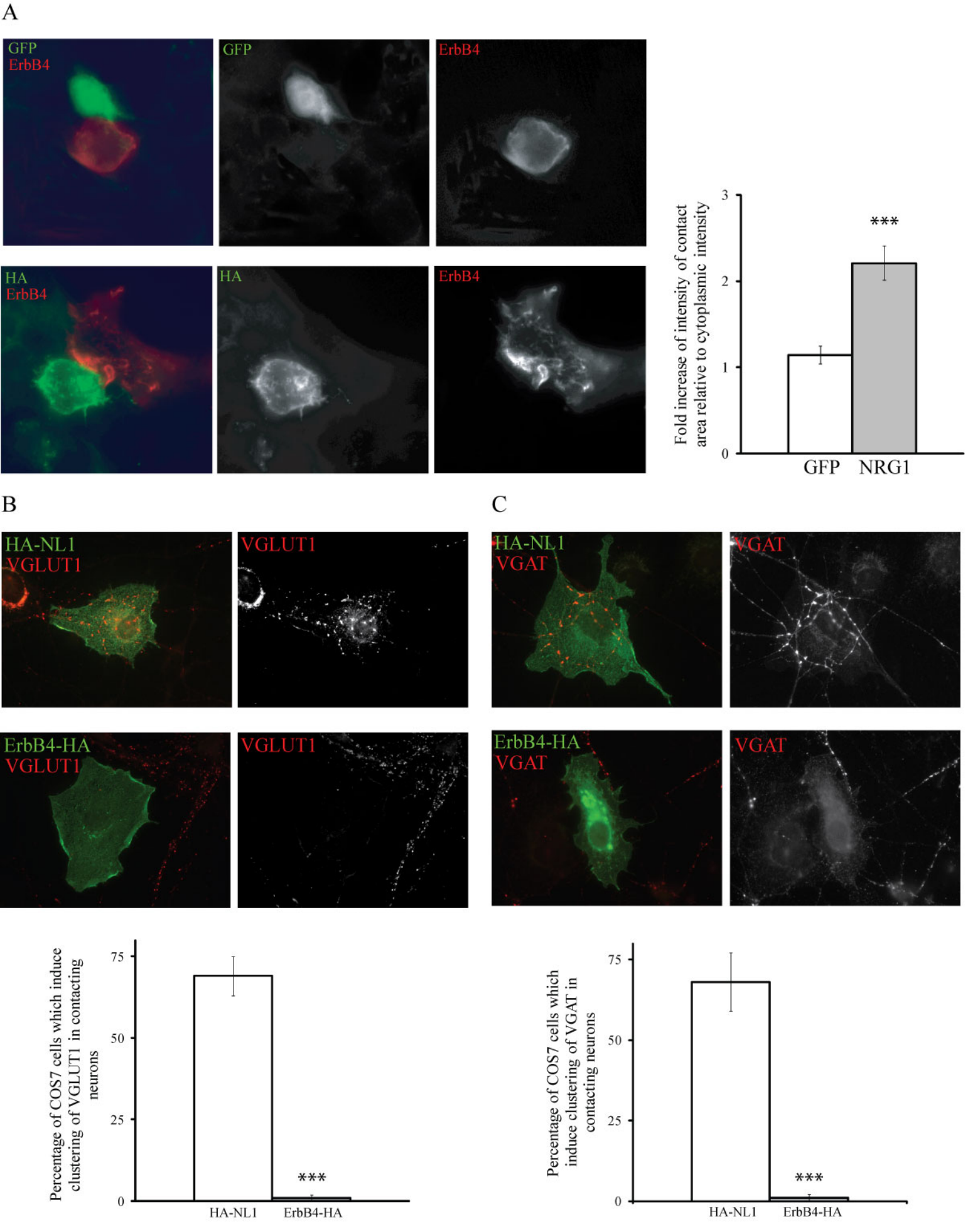
Suppl. Figure 1

Supplementary Figure 1. Overexpression levels of ErbB4-HA are comparable to endogenous ErbB4 levels. Hippocampal neurons were transfected with ErbB4-HA and immunostained for the HA epitope, ErbB4, and GAD65. Expression levels of ErbB4 in transfected cells, as assessed by HA staining (arrowheads), were directly compared to endogenous levels of ErbB4 in GABAergic neurons (arrows). It was determined that ErbB4-HA expression levels were similar to those of endogenous ErbB4.


A
ErbB4-HA
SYN
PSD-95
ErbB4-HA
SYN
PSD-95
ErbB4ΔPDZb
SYN
PSD-95
ErbB4ΔPDZb
SYN
PSD-95
B
OVERLAY
SURFACE
INTERNAL
ErbB4-HA
ErbB4-HA ΔPDZb

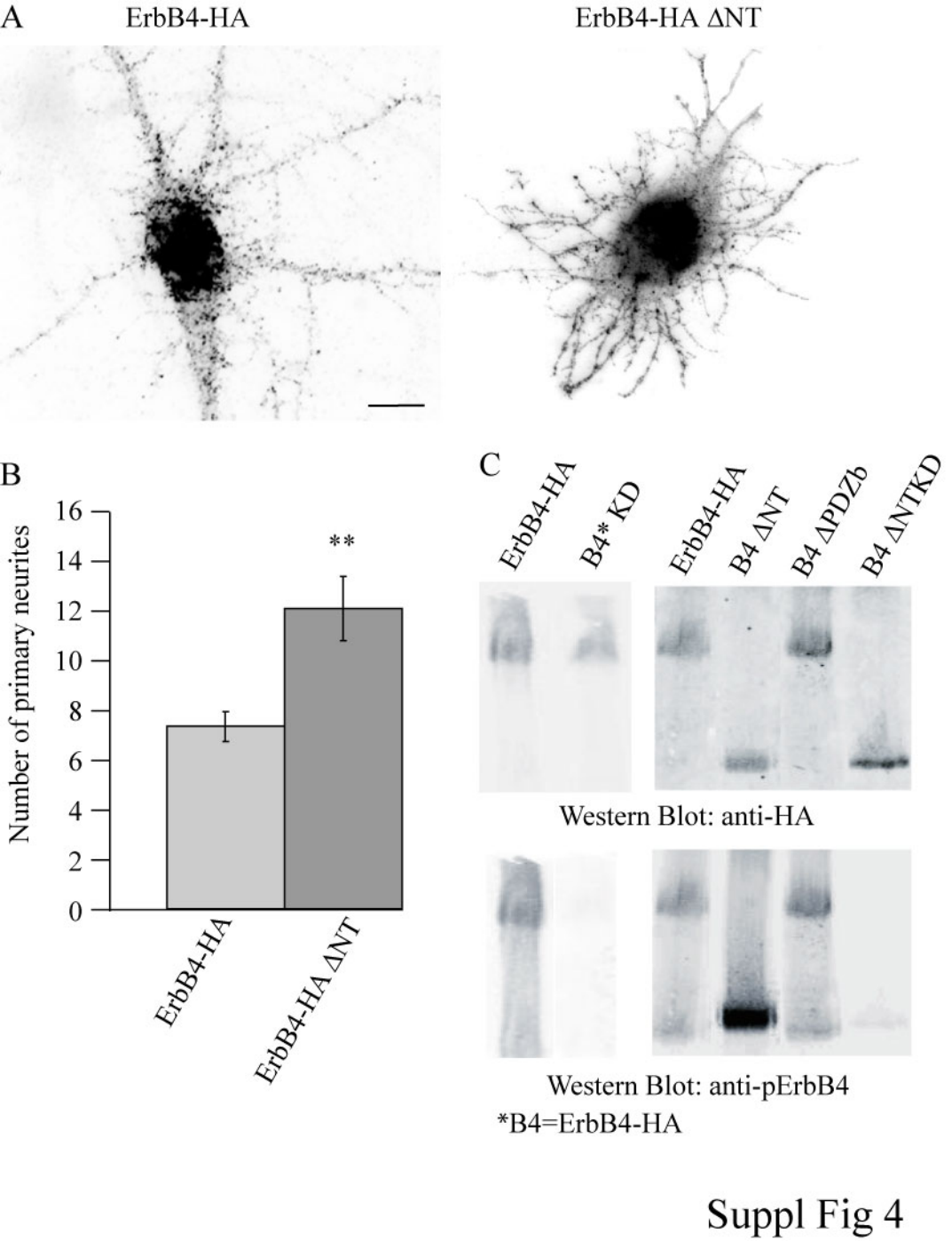
Suppl Fig. 2

Supplementary Figure 2. PDZ-mediated interactions regulate ErbB4 surface expression and trafficking to the synapse. (A) Hippocampal neurons were transfected with either full-length ErbB4-HA (left panel) or ErbB4-HA ΔPDZb (right panel), fixed at DIV 14 and immunostained for SYN and the postsynaptic scaffolding protein, PSD-95. Full-length ErbB4 was highly clustered at synaptic sites containing both SYN and PSD-95. In contrast, deletion of the PDZ-binding motif dramatically reduced ErbB4 presence at synapses (Scale bars: 10 μm, inset: 5 μm). **(B)** Hippocampal neurons were transfected with ErbB4-HA (top panel) or ErbB4-HA ΔPDZb (bottom panel). Cells were then fixed and immunostained under non-permeabilizing conditions with rabbit anti-HA antibodies to visualize surface protein (green). Cells were then permeabilized with Triton-X 100, and the internal pool of protein was visualized with mouse anti-HA antibodies (red). Reduced surface labeling and intracellular cluster formation was observed in neurons expressing ErbB4-HA ΔPDZ (Scale bars: 10 μm).


A
GFP
ErbB4
GFP
ErbB4
HA
ErbB4
HA
ErbB4
Fold increase of intensity of contact area relative to cytoplasmic intensity
3
2
1
0
***
GFP
NRG1
B
HA-NL1
VGLUT1
VGLUT1
ErbB4-HA
VGLUT1
VGLUT1
Percentage of COS7 cells which induce clustering of VGLUT1 in contacting neurons
75
50
25
0
***
HA-NL1
ErbB4-HA
C
HA-NL1
VGAT
VGAT
ErbB4-HA
VGAT
VGAT
Percentage of COS7 cells which induce clustering of VGAT in contacting neurons
75
50
25
0
***
HA-NL1
ErbB4-HA

Suppl Fig. 3

Supplementary Figure 3. NRG1 induces intercellular clustering of ErbB4 in heterologous cells. (A) NRG1 expressed in heterologous cells induces accumulation of ErbB4 at contact sites. HEK cells were transfected with either GFP, untagged ErbB4, or HA-NRG1. One day post-transfection, cells expressing ErbB4 were replated together with cells expressing either GFP or HA-NRG1. Analysis revealed a 2-fold increase in intensity of ErbB4 at sites of contact with HA-NRG1-expressing cells, when compared to the intensity of the contact area with cells expressing GFP. **(B, C) Expression of ErbB4 in heterologous cells is not sufficient for induction of presynaptic differentiation.** COS7 cells were transfected with either HA-tagged neuroligin 1 (HA-NL1) or ErbB4-HA, co-cultured with hippocampal neurons for 24 h, and then fixed and immunostained for HA (green) and either VGLUT1 (B) or VGAT (C). Quantification of the number of COS7 cells that induce presynaptic maturation revealed that HA-NL1, but not ErbB4, induces clustering of VGLUT1 and VGAT (n= 117 and 138 respectively from 3 different cultures). ***, $p < 0.001$


A
ErbB4-HA
ErbB4-HA ΔNT
B
Number of primary neurites
16
14
12
10
8
6
4
2
0
**
ErbB4-HA
ErbB4-HA ΔNT
C
ErbB4-HA
B4* KD
ErbB4-HA
B4 ΔNT
B4 ΔPDZb
B4 ΔNTKD
Western Blot: anti-HA
Western Blot: anti-pErbB4
*B4=ErbB4-HA

Suppl Fig 4

Supplementary Figure 4. Deletion of the extracellular domain enhances ErbB4 kinase activity and neurite outgrowth. Cultured hippocampal neurons were transfected with full length ErbB4-HA or with a truncated form lacking the extracellular domain (ErbB4-HA ΔNT). Cells were then fixed and immunostained with HA antibody to visualize transfected cells. **(A)** ErbB4-HA-transfected neurons displayed normal morphology, while ErbB4-HA ΔNT-expressing cells showed enhanced primary neurite formation **(B)** Quantification of changes in the number of primary neurites in neurons expressing ErbB4-HA ΔNT, compared to the full length ErbB4-HA (12±1 versus 7.3±0.6, respectively). **(C)** Assessment of the basal phosphorylation level of ErbB4 constructs in heterologous cells**.** COS7 cells were transfected with wild type or various mutant forms of ErbB4, including ErbB4-HA, ErbB4-HA KD, ErbB4-HA ΔNT and ErbB4-HA ΔPDZb. Cell lysates were then subjected to western blot analysis using antibodies against HA (top panel) or phospho-ErbB4 specific antibodies (pErbB4) (bottom panel). The basal level of phosphorylation for full length ErbB4-HA is comparable to that for ErbB4-HA ΔPDZb. In contrast, ErbB4-HA ΔNT exhibits strong basal levels of tyrosine phosphorylation. **, $p<0.01$ (Scale bars: 10 μm)