Figure S5

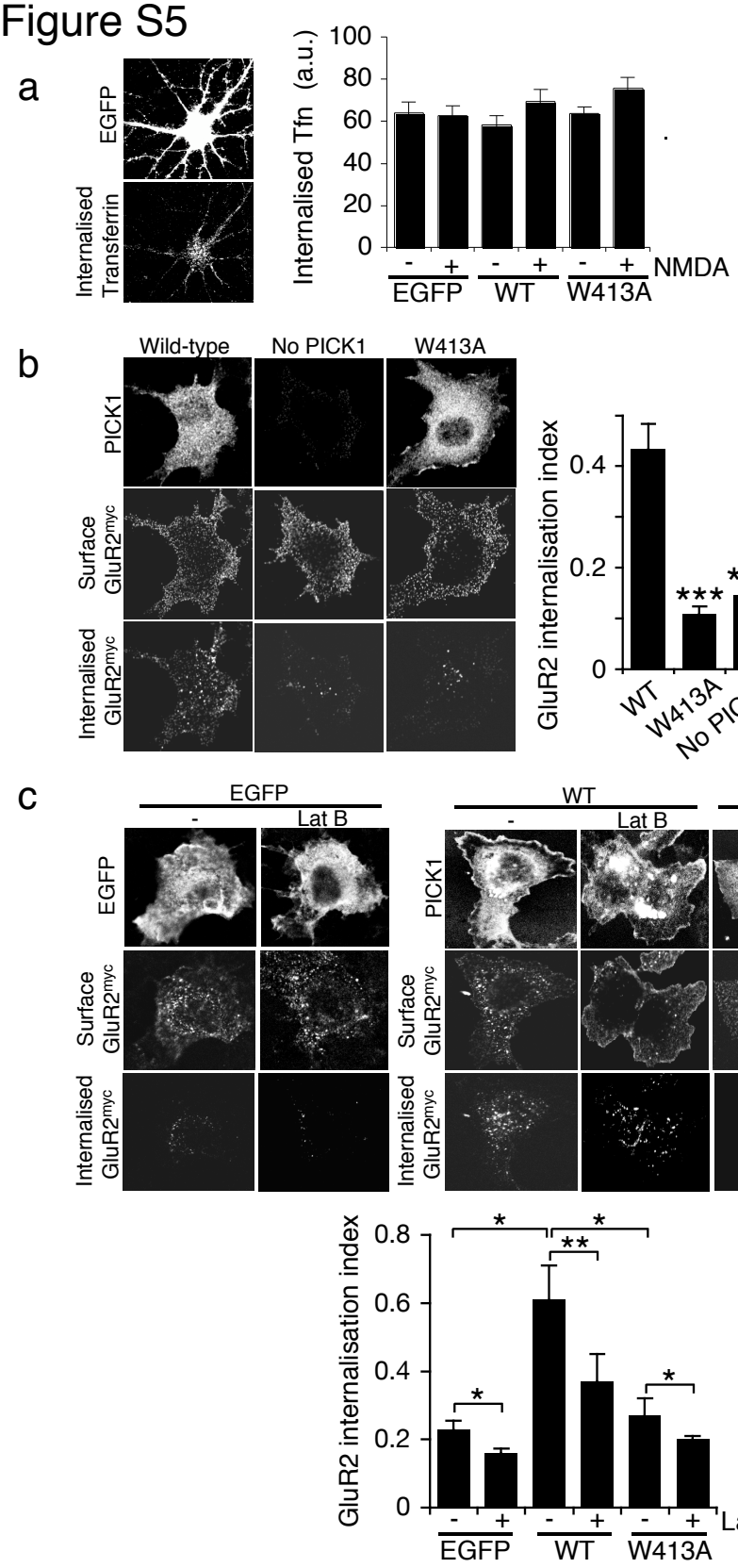
a
EGFP
Internalised Transferrin
Internalised Tfn (a.u.)
100
80
60
40
20
0
- + - + - +
NMDA
EGFP
WT
W413A
b
Wild-type
No PICK1
W413A
PICK1
Surface GluR2myc
Internalised GluR2myc
GluR2 internalisation index
0.4
0.2
0
***
***
WT
W413A
No PICK1
c
EGFP
WT
W413A
- Lat B
- Lat B
- Lat B
EGFP
PICK1
Surface GluR2myc
Internalised GluR2myc
GluR2 internalisation index
0.8
0.6
0.4
0.2
0
*
*
**
*
*
- + - + - +
Lat B
EGFP
WT
W413A

Figure S5 Supplementary trafficking data.

a) W413A-PICK1 does not affect transferrin receptor endocytosis. Hippocampal cultures overexpressing WT-PICK1 or W413A-PICK1 were subjected to NMDA-treatment and analysed using a fluorescent-conjugated transferrin endocytosis assay. Representative images are shown for EGFP-transduced cultures in the absence of NMDA. n=15 cells. Data are presented as mean +/- SEM.

b) Arp2/3 binding is required for PICK1-mediated GluR2 endocytosis in heterologous cells. COS7 cells were transfected with myc-GluR2 and either WT-PICK1 or W413A-PICK1. Myc-GluR2 internalised in response to increased extracellular Ca^{2+} was analysed by antibody-feeding using anti-myc. Cells were fixed and subsequently stained for surface (red channel) and internalised (blue channel) myc-GluR2 using appropriate secondary antibodies. Total PICK1 was visualised with anti-PICK1 antibody. Representative images are shown for each condition, and graph shows pooled data for GluR2 internalisation index. n=15 cells, *p<0.001. Data are presented as mean +/- SEM.

c) Latrunculin B does not reverse the blockade of GluR2 endocytosis by the W413A mutation in heterologous cells. COS7 cells co-expressing WT- or W413A- PICK1 and myc-GluR2 were treated with 2.5μM Latrunculin B or vehicle control for 1 hour prior to antibody-feeding using anti-myc, as in (a), above. Total PICK1 and GFP expression were visualised using anti-PICK1 antibody or inherent GFP fluorescence. Representative images are shown for each condition and graph shows pooled data for GluR2 internalisation index (internalized/surface + internalized). n=15 cells. *p<0.05, **p<0.01. Data are presented as mean ± SEM.