KEY AMINO ACID RESIDUES WITHIN THE THIRD MEMBRANE DOMAINS OF NR1 AND NR2 SUBUNITS CONTRIBUTE TO THE REGULATION OF THE SURFACE DELIVERY OF NMDA RECEPTORS

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SUPPLEMENTARY FIGURE LEGENDS

Fig. S1. ***Immunofluorescence microscopy showed that key amino acid residues within the NR2B M3 domain contribute to the regulation of the surface targeting of the full-length NR1-1a/NR2B receptors.*** COS-7 cells transfected with indicated cDNAs were surface stained with rabbit anti-GFP antibodies. *A,* Representative images of total (left panel) and surface (right panel) signals are shown. *B,* data represent mean ± S.E. of the ratios of the fluorescence intensities per unit area obtained for surface and total GFP expression for each studied cell. More than 20 GFP-expressing cells for each combination of cDNAs were analyzed; **, $p < 0.001$, *t* test. The method was described previously (Horak et al., Journal of Neuroscience 28(13):3500-9, 2008). Images from stained cells were taken on an Olympus cell^R fluorescence microscope and analyzed using ImageJ software (NIH).

Fig. S2. ***Specific mutated NMDA receptors expressed in COS-7 cells exhibit functional properties similar to those of receptors expressed in HEK293 cells.*** Electrophysiological recordings were performed from COS-7 cells expressing the indicated NR1/NR2 receptors. Responses were elicited with 5 s applications of 1 mM glutamate (indicated by filled bar). Representative traces are shown. Please note that both mutated NMDA receptor subtypes exhibit functional properties similar to those obtained from the HEK293 cells (Fig. 2 and 3).

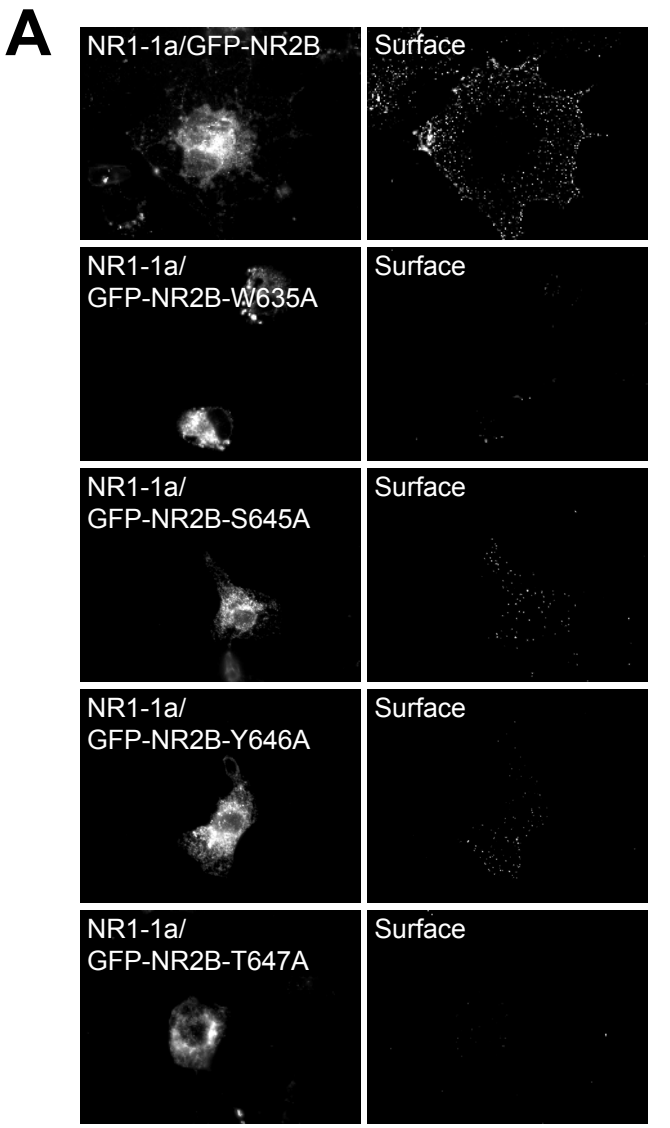
Fig. S3. ***Immunofluorescence microscopy showed that key amino acid residues within the NR1 M3 domain contribute to the regulation of the surface targeting of the full-length NMDA receptors.*** COS-7 cells transfected with indicated cDNAs were surface stained with rabbit anti-GFP antibodies. *A,* Representative images of total (left panel) and surface (right panel) signals are shown. *B,* data represent mean ± S.E. of the ratios of the fluorescence intensities per unit area obtained for surface and total YFP expression for each studied cell. More than 20 YFP-expressing cells for each combination of cDNAs were analyzed; **, $p < 0.001$, *t* test. The method was described previously (Horak et al., Journal of Neuroscience 28(13):3500-9, 2008). Images from stained cells were taken on an Olympus cell^R fluorescence microscope and analyzed using ImageJ software (NIH). Interestingly, although the YFP-NR1-1a-T648A/NR2B receptors exhibited no obvious surface expression in the vast majority of cells, there was a minor population of cells with positive surface signal (data not shown).

Fig. S4. ***Key amino acid residues within the NR1 and NR2B M3 domains contribute to the regulation of the surface targeting of NR1/NR2B receptors expressed in HEK293 cells.*** HEK293 cells transfected with the indicated NMDA receptor subunits were labeled with anti-GFP antibodies using a quantitative colorimetric assay. Quantification of surface (black) and total (white) expression using quantitative colorimetric assay is shown. Plotted data represent mean ± S.E.; n = 6 in two experiments. **, $p<0.001$ relative to control (YFP-NR1-1a/GFP-NR2B), *t* test.

Fig. S5. ***The distribution of indicated mutated NMDA receptors closely matches the distribution of an ER marker but not a GA marker.*** Images were taken on fixed COS-7 cells expressing indicated NMDA receptors using a confocal microscope. The labeling method is described in the Experimental Procedures.

Fig. S6. ***The expression of mutated NMDA receptors.*** *A-B,* COS-7 cells co-transfected with indicated NR1/NR2B subunits were solubilized with 1% DOC and probed with rabbit anti-GFP antibody. Please note that similar protein expression levels were observed for the wild type and mutated receptors (n=3).

Figure S1


A
NR1-1a/GFP-NR2B
Surface
NR1-1a/
GFP-NR2B-W635A
Surface
NR1-1a/
GFP-NR2B-S645A
Surface
NR1-1a/
GFP-NR2B-Y646A
Surface
NR1-1a/
GFP-NR2B-T647A
Surface

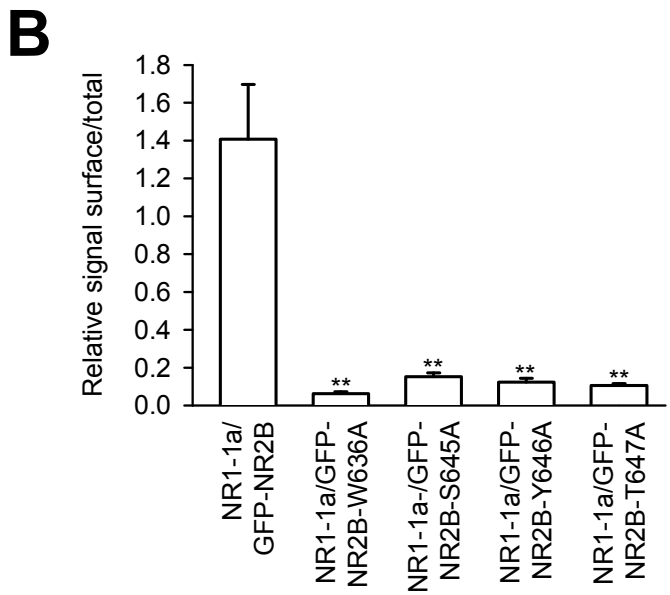

B
Relative signal surface/total
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
**
**
**
**
NR1-1a/
GFP-NR2B
NR1-1a/GFP-
NR2B-W636A
NR1-1a-/GFP-
NR2B-S645A
NR1-1a/GFP-
NR2B-Y646A
NR1-1a/GFP-
NR2B-T647A

Figure S2

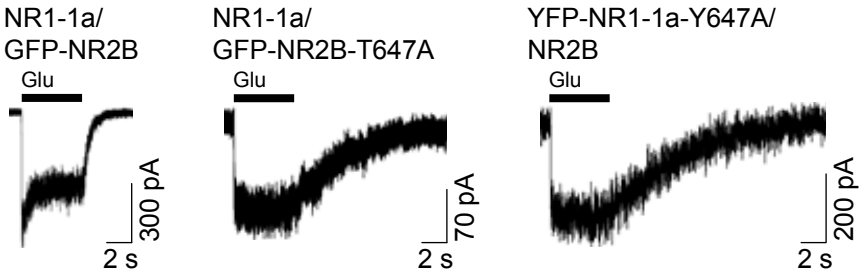
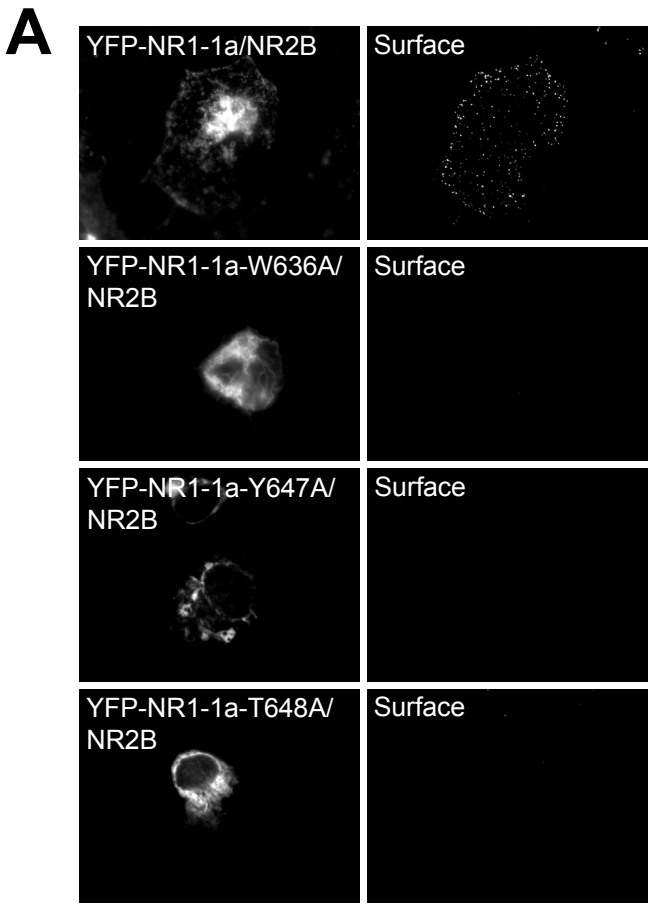

NR1-1a/
GFP-NR2B
Glu
300 pA
2 s
NR1-1a/
GFP-NR2B-T647A
Glu
70 pA
2 s
YFP-NR1-1a-Y647A/
NR2B
Glu
200 pA
2 s

Figure S3


A
YFP-NR1-1a/NR2B
Surface
YFP-NR1-1a-W636A/
NR2B
Surface
YFP-NR1-1a-Y647A/
NR2B
Surface
YFP-NR1-1a-T648A/
NR2B
Surface

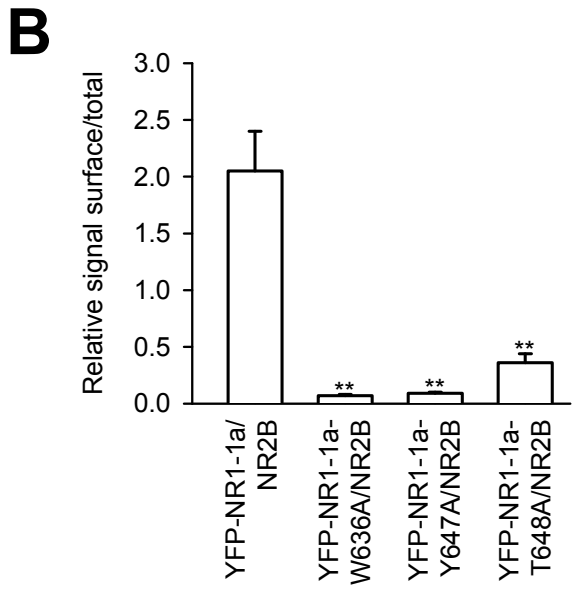

B
Relative signal surface/total
3.0
2.5
2.0
1.5
1.0
0.5
0.0
**
**
**
YFP-NR1-1a/
NR2B
YFP-NR1-1a-
W636A/NR2B
YFP-NR1-1a-
Y647A/NR2B
YFP-NR1-1a-
T648A/NR2B

Figure S4

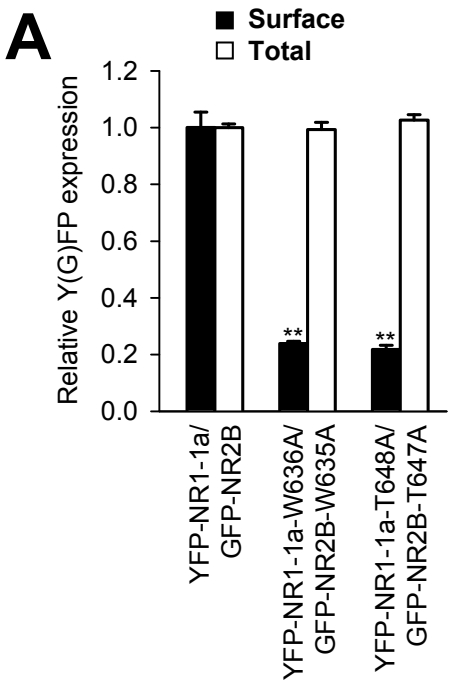

A
Surface
Total
Relative Y(G)FP expression
1.2
1.0
0.8
0.6
0.4
0.2
0.0
**
**
YFP-NR1-1a/
GFP-NR2B
YFP-NR1-1a-W636A/
GFP-NR2B-W635A
YFP-NR1-1a-T648A/
GFP-NR2B-T647A

Figure S5

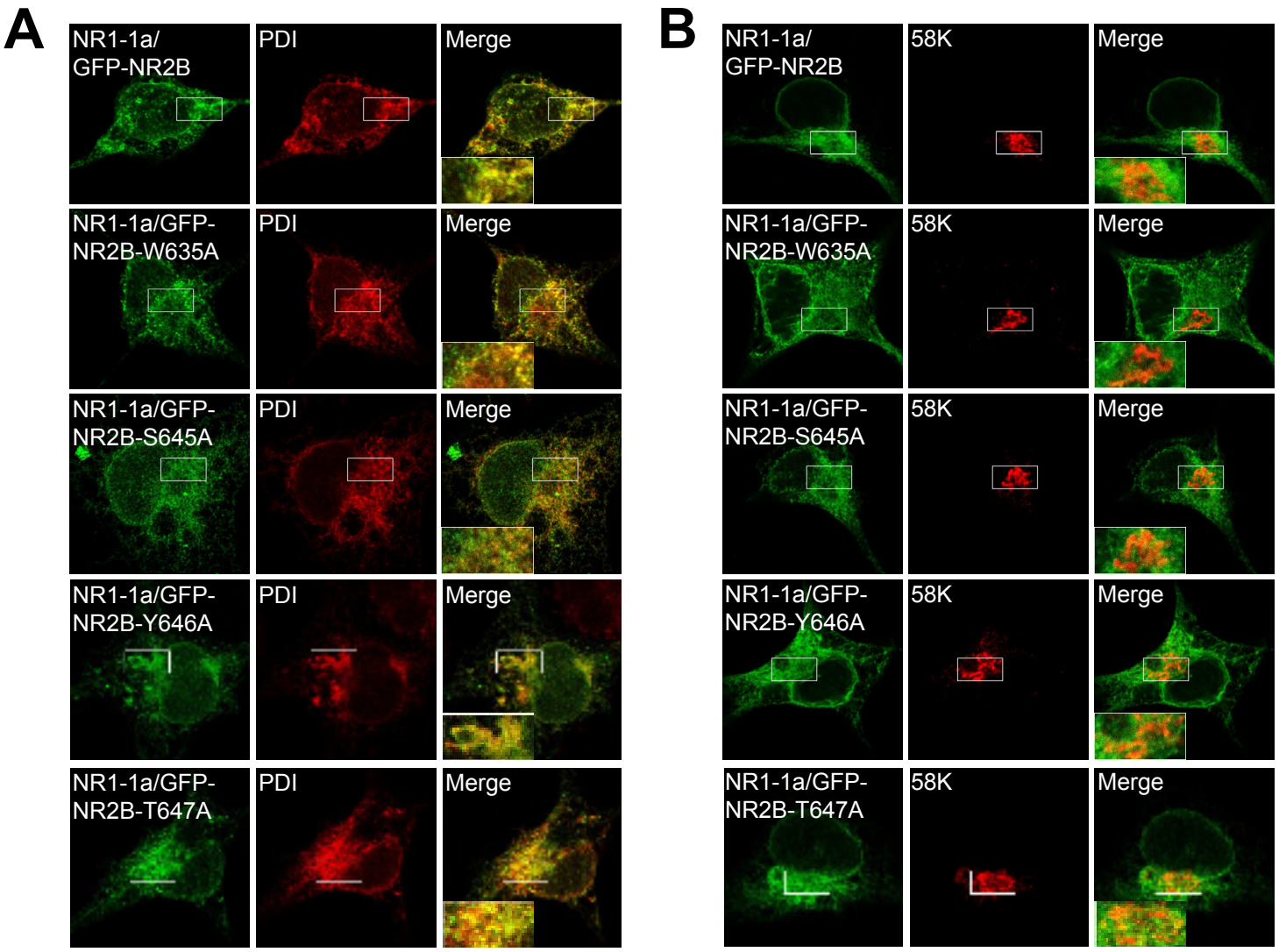
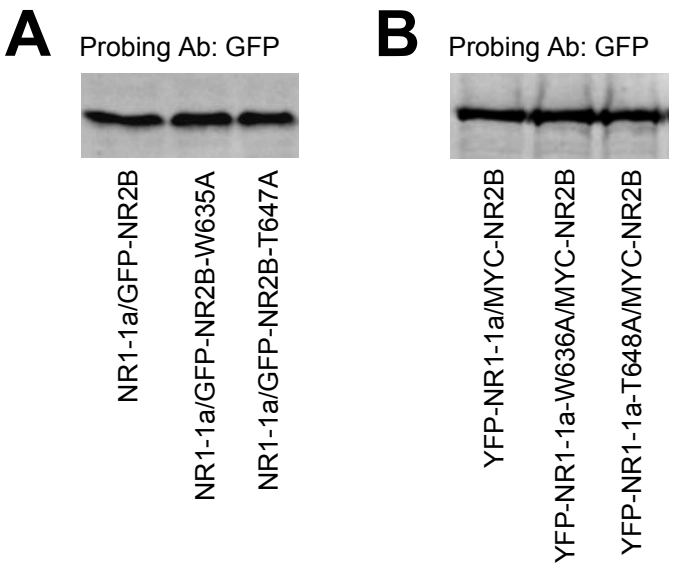

A
NR1-1a/
GFP-NR2B
PDI
Merge
NR1-1a/GFP-
NR2B-W635A
PDI
Merge
NR1-1a/GFP-
NR2B-S645A
PDI
Merge
NR1-1a/GFP-
NR2B-Y646A
PDI
Merge
NR1-1a/GFP-
NR2B-T647A
PDI
Merge
B
NR1-1a/
GFP-NR2B
58K
Merge
NR1-1a/GFP-
NR2B-W635A
58K
Merge
NR1-1a/GFP-
NR2B-S645A
58K
Merge
NR1-1a/GFP-
NR2B-Y646A
58K
Merge
NR1-1a/GFP-
NR2B-T647A
58K
Merge

Figure S6


A
Probing Ab: GFP
NR1-1a/GFP-NR2B
NR1-1a/GFP-NR2B-W635A
NR1-1a/GFP-NR2B-T647A
B
Probing Ab: GFP
YFP-NR1-1a/MYC-NR2B
YFP-NR1-1a-W636A/MYC-NR2B
YFP-NR1-1a-T648A/MYC-NR2B