Supplementary data

Structural features in the glycine-binding sites of the GluN1 and GluN3A subunits regulate the surface delivery of NMDA receptors

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Supplementary Figure S1

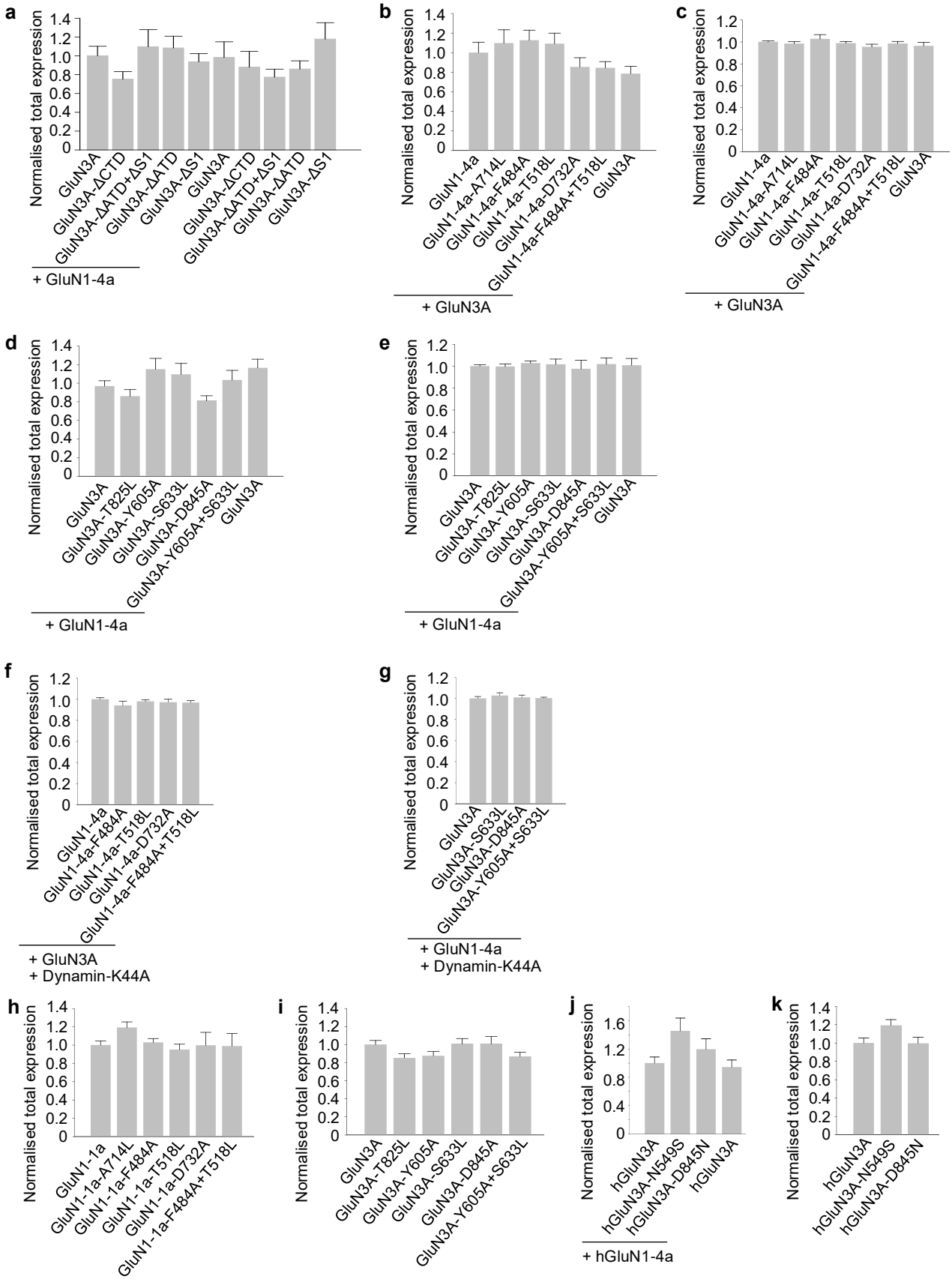

Figure S1. The total expression levels were not significantly different among the studied GluN subunit combinations. **(a)** corresponds to Fig. 1c; **(b)** corresponds to Fig. 2c; **(c)** corresponds to Fig. 2d; **(d)** corresponds to Fig. 3c; **(e)** corresponds to Fig. 3d; **(f)** corresponds to Fig. 4a; **(g)** corresponds to Fig. 4b; **(h)** corresponds to Fig. 5b; **(i)** corresponds to Fig. 5d; **(j)** corresponds to Fig. 6d; **(k)** corresponds to Fig. 6j. (p>0.05; ANOVA).

Supplementary Figure S2

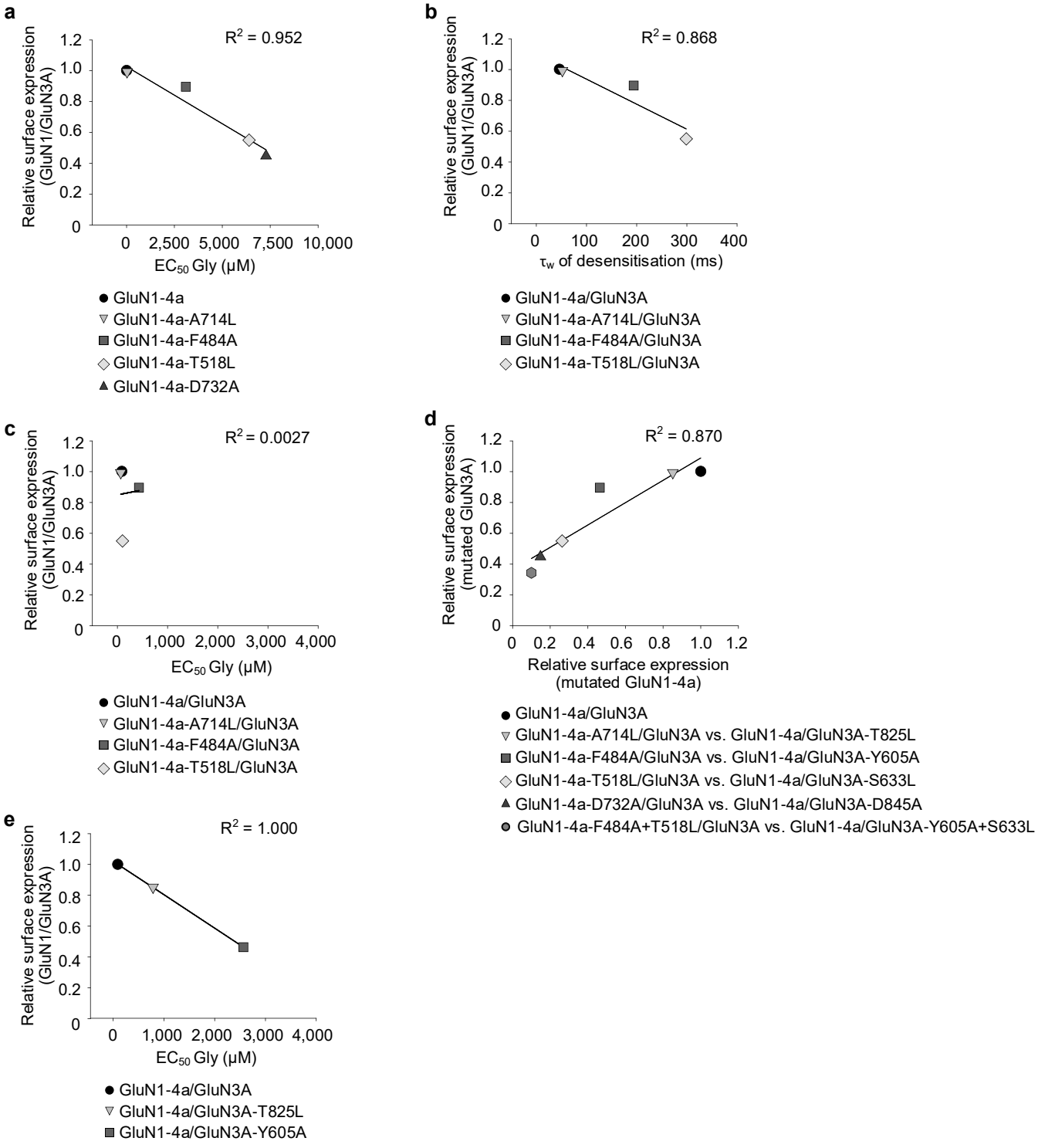

Figure S2. The correlation analysis of surface expression of the mutated GluN1/GluN3 receptors. (a) Correlation of relative surface expression shown in Fig. 2d with the EC_{50} values for glycine reported in the following studies: GluN1 (reported EC_{50} value at the GluN1/GluN2A receptors was 1.2 μM); GluN1-A714L mutation (reported EC_{50} value at the GluN1/GluN2A receptors was 27 μM); the GluN1-F484A mutation (reported EC_{50} value at the GluN1/GluN2A receptors was 3100 μM); the GluN1-T518L mutation (reported EC_{50} value at the GluN1/GluN2A receptors was 6400 μM) [1]; the GluN1-D732A mutation (reported EC_{50} value at the GluN1/GluN2B receptors was 7284 μM) [2]. **(b)** Correlation of relative surface expression from Fig. 2d with the τ_w of desensitisation (1000 μM glycine) shown in Fig. 2f. **(c)** Correlation of relative surface expression shown in Fig. 2d with the EC_{50} values shown in **Table 1**. **(d)** Correlation of relative surface expression shown in Fig. 2d with the relative surface expression shown in Fig. 3d. **(e)** Correlation of relative surface expression shown in Fig. 3d with the EC_{50} values shown in **Table 1**. The calculated coefficient of determination (R^2) is shown in each graph.

Supplementary Figure S3

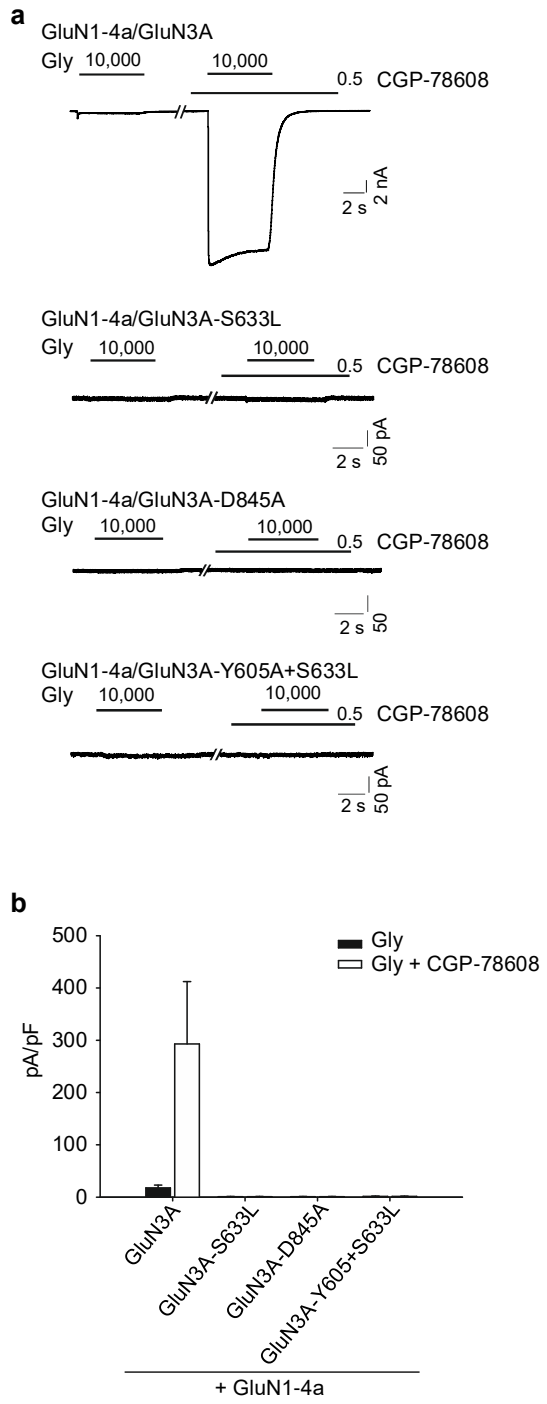

Figure S3. The pharmacological analysis with CGP-78608 at the GluN1/GluN3A receptors. (a) Representative whole-cell patch-clamp recordings from HEK293 cells transfected with the indicated wild-type or mutant GluN1-4a/GluN3A receptors at a membrane potential of -60 mV. Currents were elicited by applying 10,000 μM glycine; 0.5 μM CGP-78608 was applied as indicated. **(b)** Summary of current densities (pA/pF) obtained from the HEK293 cells expressing the indicated GluN1-4a/GluN3A receptors (n≥6 cells per group).

Supplementary Figure S4

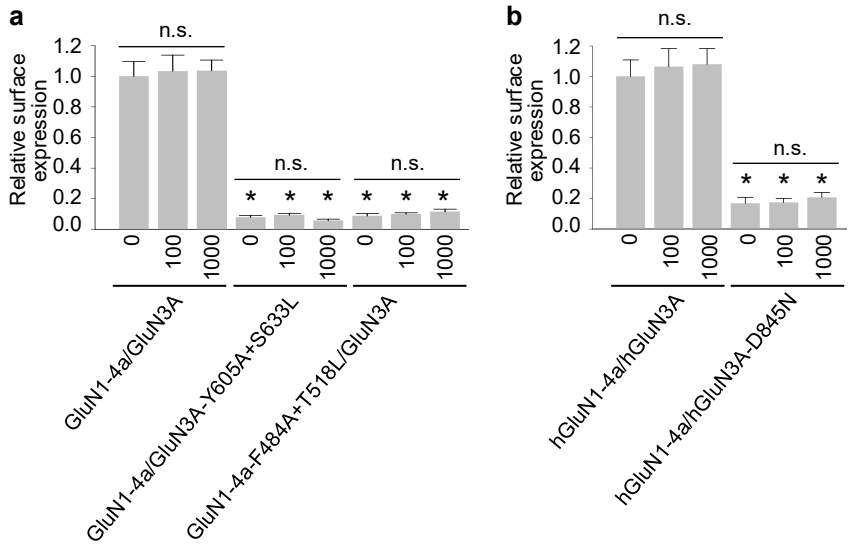

Figure S4. Chronic application of glycine does not affect the surface expression of wild-type or mutant GluN1/GluN3A receptors. COS-7 cells were transfected with the indicated rat (a) or human (b) GluN1 and GluN3A subunits. The cells were then incubated for 48 h in the absence or presence 100 μM or 1000 μM glycine, after which surface and total subunits were measured using fluorescence microscopy ($n \geq 32$ cells per group); *$p<0.05$ vs. the respective GluN1-4a/GluN3A (a) or hGluN1-4a/hGluN3A (b) receptor group (ANOVA).

Supplementary references

1 Kvist, T., Greenwood, J. R., Hansen, K. B., Traynelis, S. F. & Brauner-Osborne, H. Structure-based discovery of antagonists for GluN3-containing N-methyl-D-aspartate receptors. *Neuropharmacology* **75**, 324-336, doi:S0028-3908(13)00360-2 [pii]10.1016/j.neuropharm.2013.08.003 (2013).

2 Williams, K., Chao, J., Kashiwagi, K., Masuko, T. & Igarashi, K. Activation of N-methyl-D-aspartate receptors by glycine: role of an aspartate residue in the M3-M4 loop of the NR1 subunit. *Mol Pharmacol* **50**, 701-708 (1996).