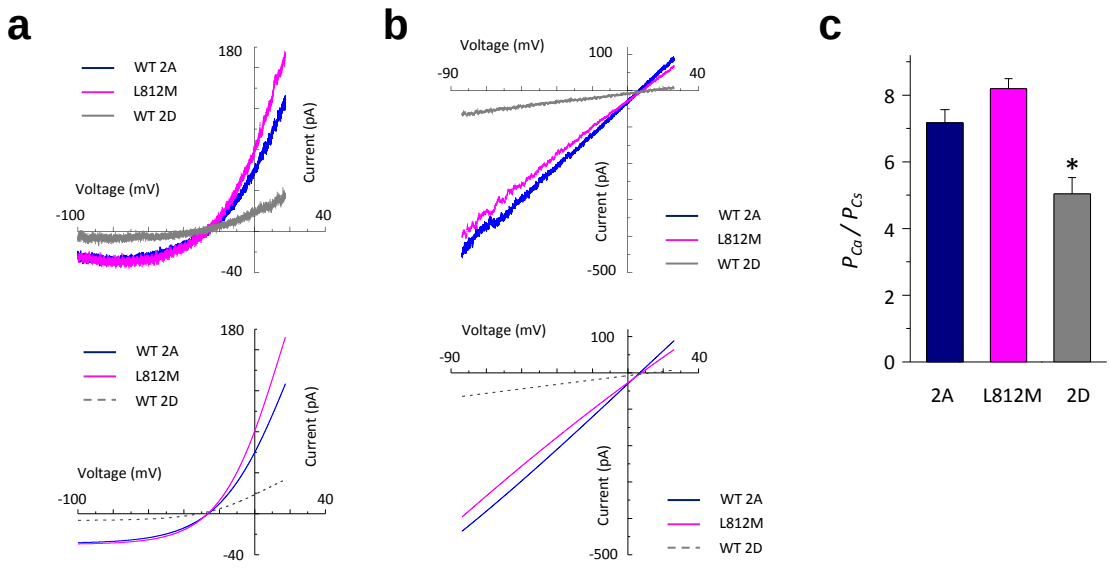


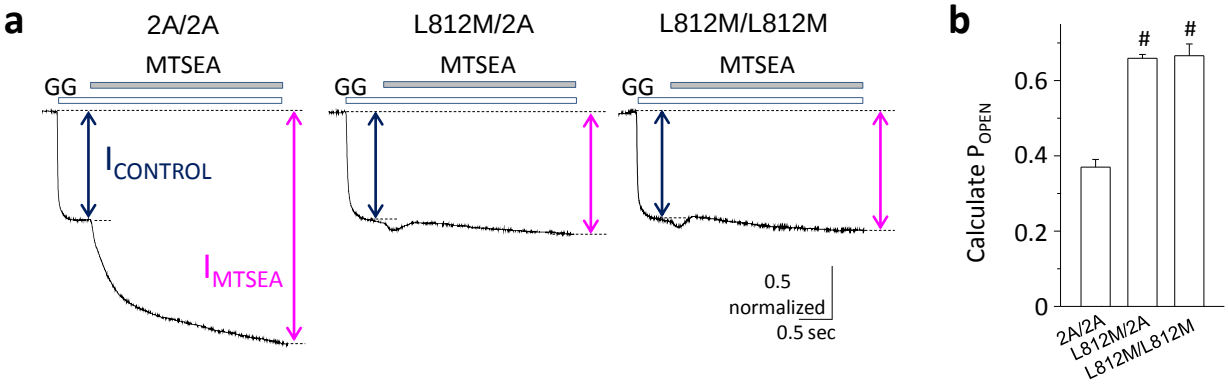
Supplementary Figure 1. L812M mutation does not significantly alter the relative calcium permeability.

(a) Representative current-voltage curves recorded in 1.8 mM extracellular Ca^{2+} are shown for WT GluN2A, GluN2A(L812M), and WT GluN2D (upper: representative traces; lower: fitted traces). The average values of $V_{rev\ (Ca)}$ for L812M (-25 ± 1.4 mV; n = 7) are not significantly different from those obtained for WT 2A (-27 ± 1.0 mV; n = 7) (p = 0.74, one way ANOVA, Tukey post hoc), whereas WT 2D has more negative $V_{rev\ (Ca)}$ (-35 ± 1.3 mV; n = 7; p < 0.001, one way ANOVA, Tukey post hoc).

(b) Representative current-voltage traces in 143 mM extracellular Cs^+ of WT GluN2A, GluN2A(L812M), and WT GluN2D (upper: original traces; lower: fitted traces). Average values of $V_{rev\ (Cs)}$ are 6.8 ± 1.6 mV (n = 7) for WT 2A, 5.5 ± 0.8 mV (n = 7) for L812M, and 6.0 ± 1.6 mV (n = 7) for WT 2D. There is no significant difference of average values of $V_{rev\ (Cs)}$ between the three receptors (p = 0.80, one way ANOVA, Tukey post hoc).

(c) The relative Ca^{2+} permeability of GluN2A(L812M) (P_{Ca}/P_{Cs} 8.2 ± 0.5, n = 7) is not significant different from WT GluN2A (7.2 ± 0.4, n = 7, = 0.52, one way ANOVA, Tukey post hoc). We also determined the Ca^{2+} permeability for WT GluN2D, which has a significant lower Ca^{2+} permeability (5.0 ± 0.3, n = 7; p < 0.001, one way ANOVA, Tukey post hoc) compared with the WT GluN2A. Relative permeability values obtained for WT GluN2A and GluN2D are similar to those described by Siegler Retchless et al [67].

All data were expressed as Mean ± SEM. Error bars in figure are SEM.

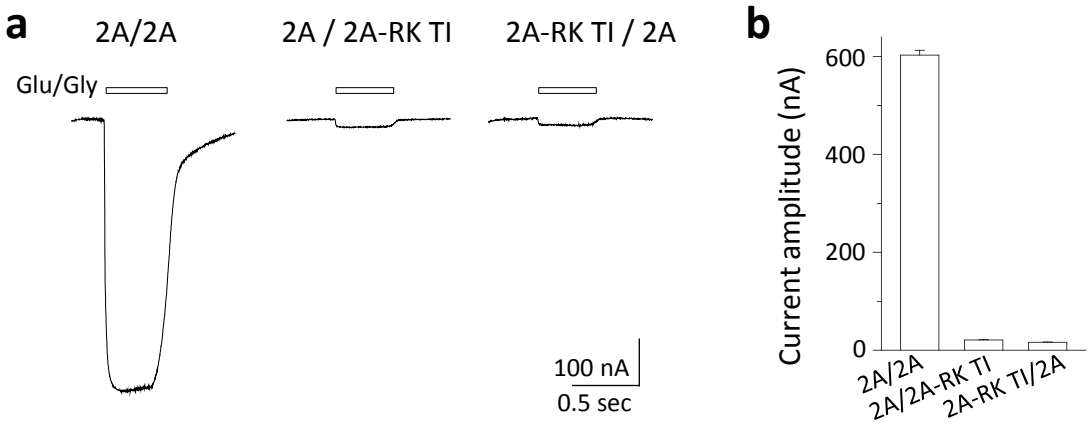


Supplementary Figure 2. A single copy of the mutant L812M receptor changes open probability measured by the degree of MTSEA (methanethiosulfonate ethylammonium) potentiation.

(a) Representative current traces evoked by agonists (100 μM glutamate and glycine) followed by 200 μM MTSEA were determined by TEVC recordings from *Xenopus* oocytes expressing tri-heteromeric GluN1-A652C/GluN2A/GluN2A (2A/2A) receptors, GluN1-A652C/GluN2A-L812M/GluN2A (L812M/2A) receptors, and GluN1-A652C/GluN2A-L812M/GluN2A–L812M (L812M/L812M) receptors.

(b) Summary of calculated P_{OPEN} of 2A/2A (0.37 ± 0.02, n = 11), L812M/2A (0.66 ± 0.01, n = 8), and L812M/L812M (0.67 ± 0.03, n = 8). We conclude that one copy of the mutant GluN2A subunit has a dominant effect on channel open probability.

All data were expressed as Mean ± SEM. Error bars in figure are SEM.
 # compared to 2A/2A; one way ANOVA, Tukey post hoc



Supplementary Figure 3. Control experiments evaluating the escape of non-tri-heteromeric receptors from ER retention.

(a) Representative current traces evoked by agonists (100 μM glutamate and 100 μM glycine) were determined by TEVC recordings from *Xenopus* oocytes expressing tri-heteromeric GluN1/GluN2A/GluN2A (2A/2A) receptors, GluN1/GluN2A/GluN2A-R518K,T690I (2A/2A-RK TI) receptors, and GluN1/GluN2A-R518K,T690I/GluN2A (2A-RK TI/2A) receptors. The R518K,T690I double mutations are located in the glutamate binding pocket and abolish glutamate binding, thereby rendering NMDA receptors with one or two mutated GluN2A subunits non-functional. Any current responses observed for oocytes expressing 2A-RK TI/2A or 2A/2A-RK TI are mediated by receptors that have escaped ER retention.

(b) Summary of current amplitude of 2A/2A (603 ± 8.8 nA, n = 18), 2A/2A-RK TI (20 ± 0.2 nA, n = 16), and 2A-RK TI/2A (16 ± 0.1 nA, n = 16). We conclude that less than 4% of the recorded currents arises from diheteromeric receptors that escape the engineered ER retention signal.

All data were expressed as Mean ± SEM. Error bars in figure are SEM.