# Supporting Information

## Molecular Mechanism of Disease-Associated Mutations in the Pre-M1 Helix of NMDA Receptors and Potential Rescue Pharmacology

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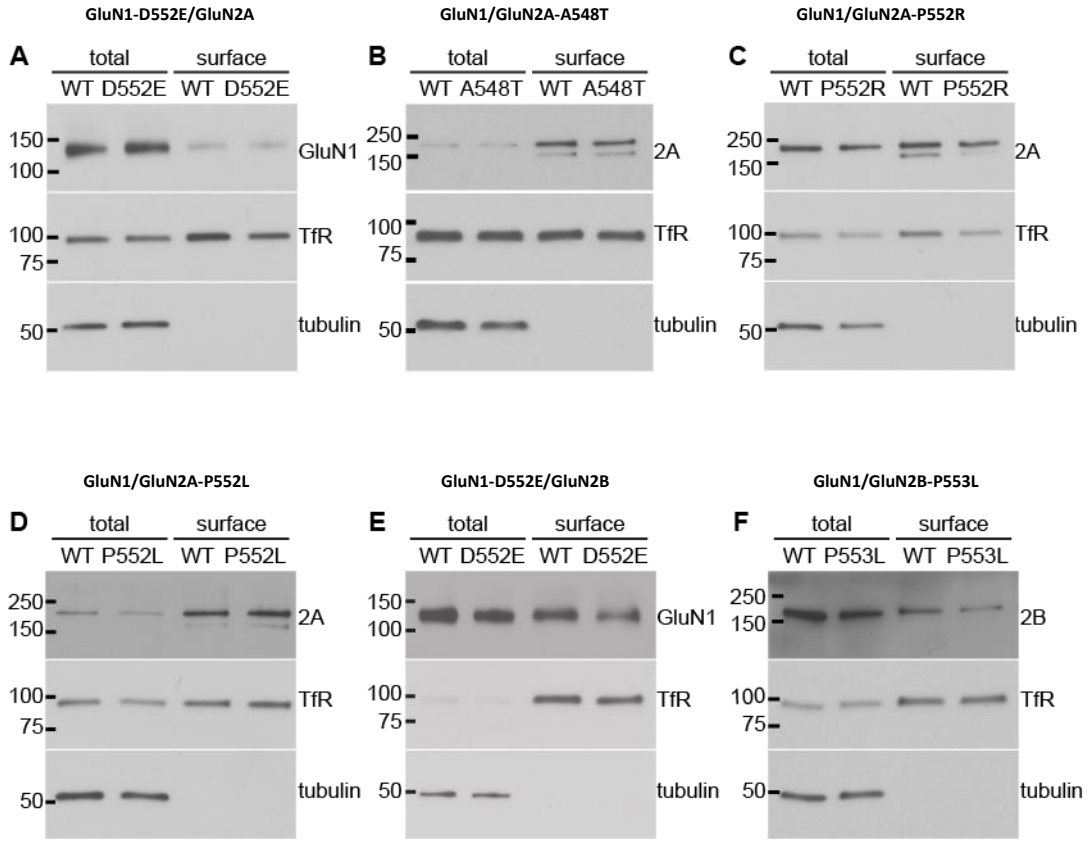
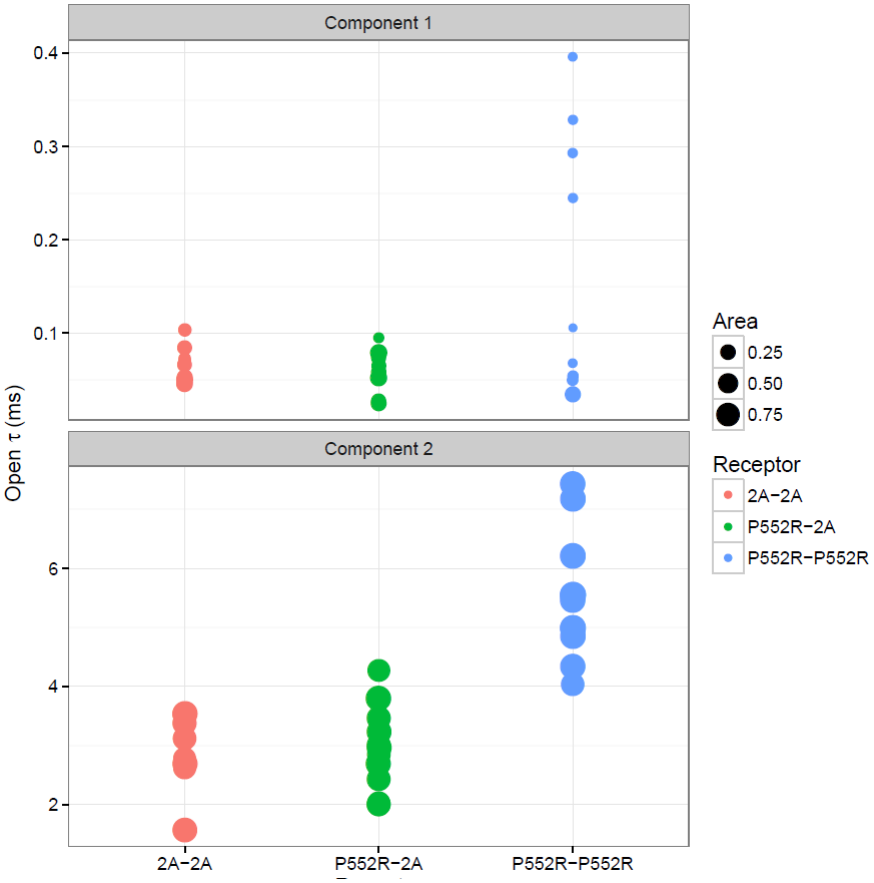
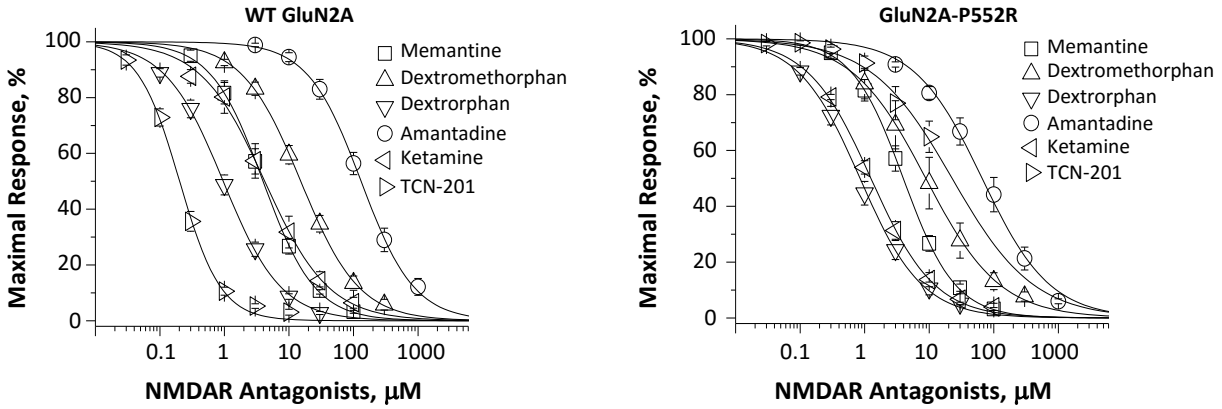
Supporting Information



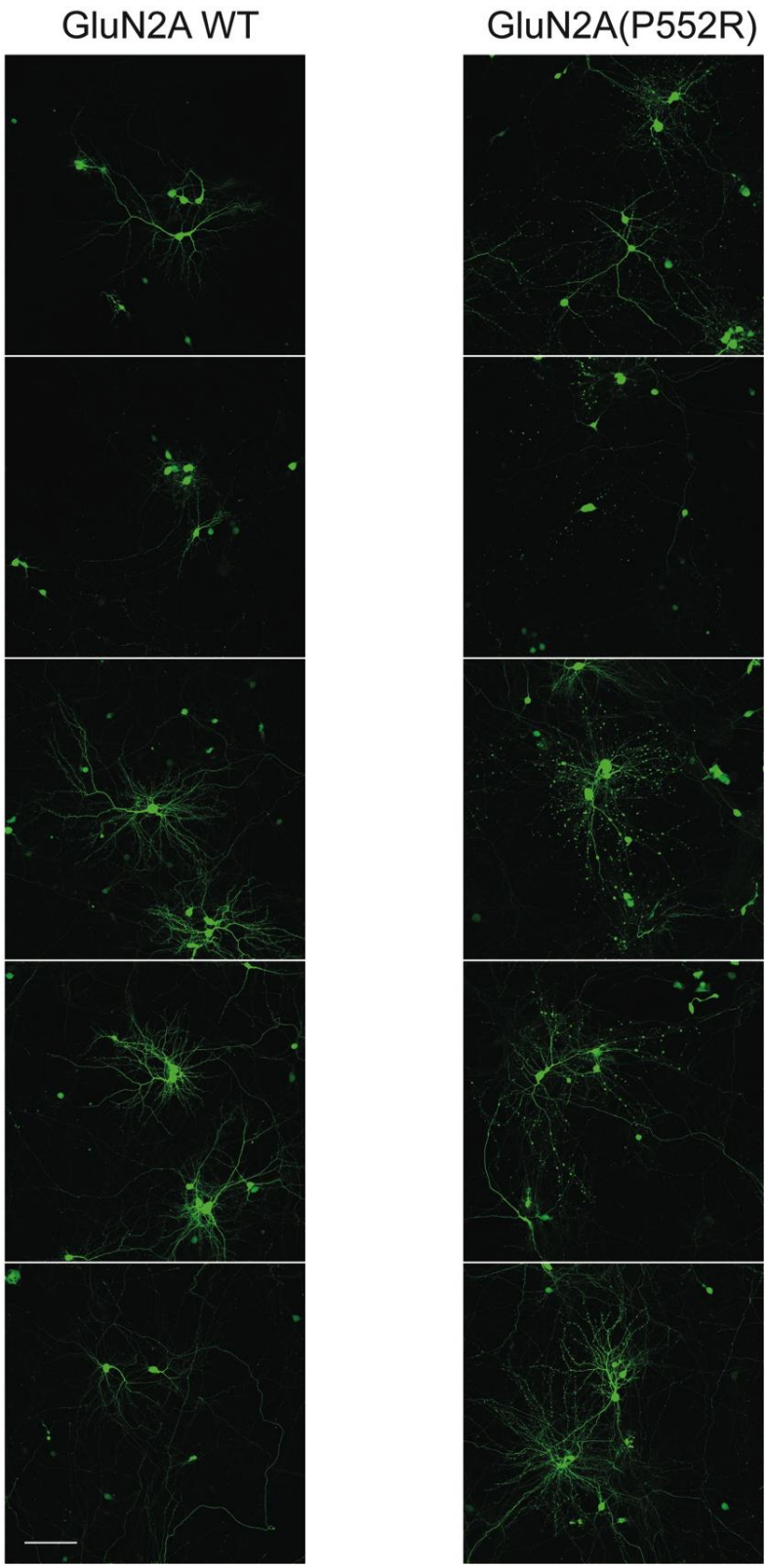

**S1 Figure. Surface expression of pre-M1 mutations (related to Figure-3)** The surface proteins of HEK293 cells transiently expressing wild type or mutated human NMDA receptors were labeled with biotin and pulled down with avidin-conjugated beads. The total and surface protein fractions were run on SDS-PAGE gels and immunoblotted for GluN1, GluN2A or GluN2B, transferrin receptor (TfR), and tubulin. Representative western blots are shown for HEK cells expressing GluN1/GluN2A and GluN1-D552E/GluN2A (**A**), GluN1/GluN2A and GluN1/GluN2A-A548T (**B**), GluN1/GluN2A and GluN1/GluN2A-P552R (**C**), GluN1/GluN2A and GluN1/GluN2A-P552L (**D**), GluN1/GluN2B and GluN1-D552E/GluN2B (**E**), and GluN1/GluN2B and GluN1/GluN2B-P553L (**F**)**.**



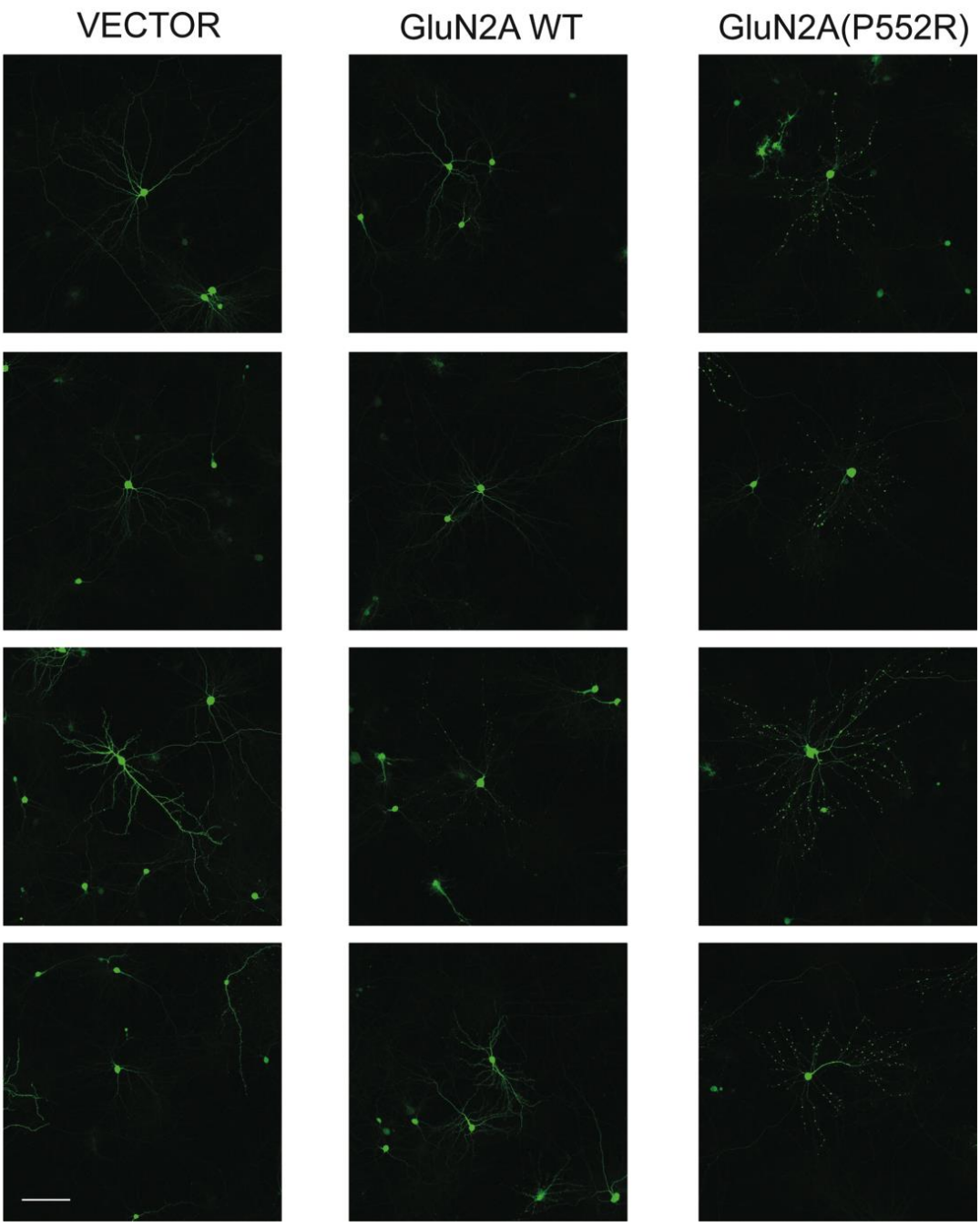

**S2 Figure. Fitted time constants for GluN2A-P552R open time histograms (related to Figure-4)** The open times for each patch were modelled as a mixture of two exponential components. The maximum likelihood estimates for the means of the two exponential components and their corresponding weights were determined for each patch. The top panel shows the estimated mean, tau, of the first exponential component and the bottom panel shows the estimated mean of the second component. The size of each point corresponds to the estimated area of that component, and points are colored by the receptor type.



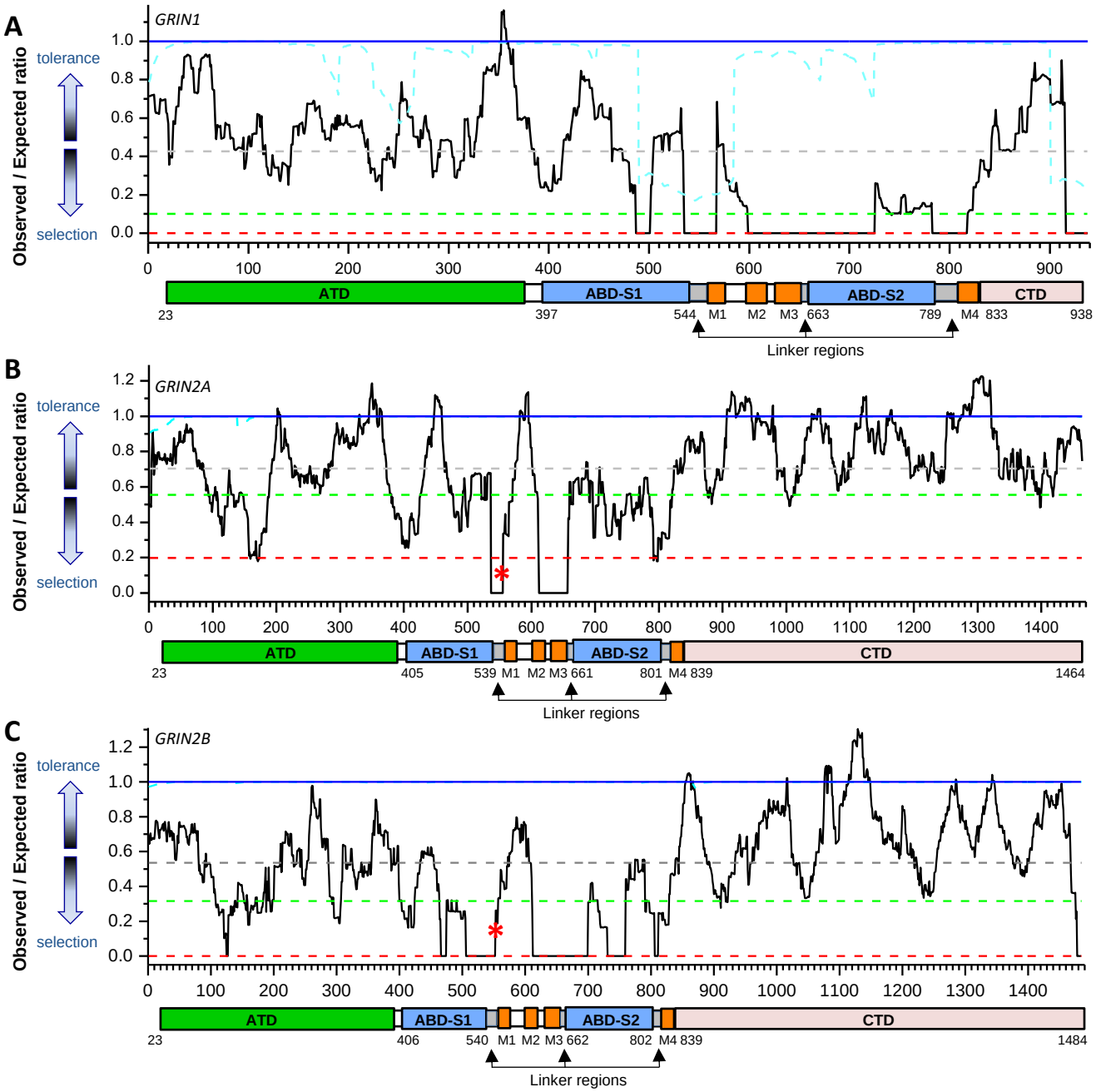

**S3 Figure. (related to Figure-8 and RESULTS).** Rescue pharmacology to evaluate the ability of NMDAR antagonists including FDA-approved drugs on inhibition of human NMDAR function by using two-electrode voltage clamp current recordings (holding at -40 mV) on *Xenopus* oocytes. The data are expressed as $IC_{50}$ value ± SEM (n, maximal inhibition % at 100 μM for memantine, 300 μM for dextromenthorphan, 30 μM for dextrorphan, 1000 μM for amantadine, 100 μM for ketamine, 10 μM for TCN-201).



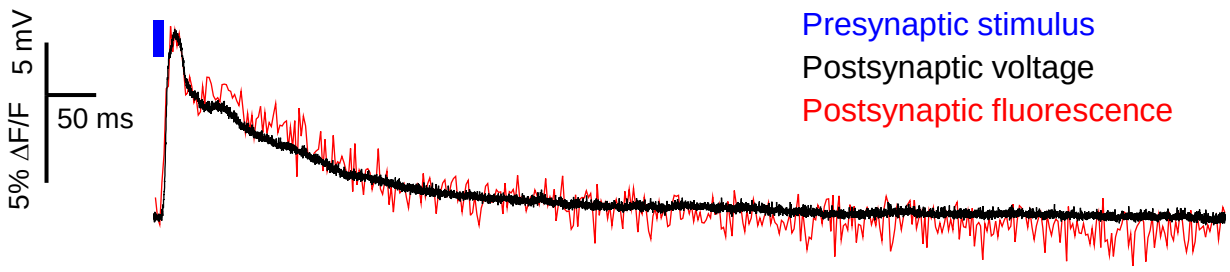

**S4 Figure. Comparison of blebbing produced by transfection of neurons with GluN2A-P552R cDNA** (related to **Figure-8, S5 Fig,** and **RESULTS**)**.** Morphological features of rat cortical neurons in culture (DIV 18-19) expressing GFP and either GluN2A WT (0.6 μ g; see **Methods** and **Fig. 8**), or GluN2A-P552R (0.6 μ g) for 24 hours. Blebs are a telltale and nearly ubiquitous sign of neuronal expression of GluN2A-P552R, but not GluN2A WT. Panels are representative of 5 independent transfection experiments for each vector, not necessarily paired across rows. Scale bar = 100 μm.



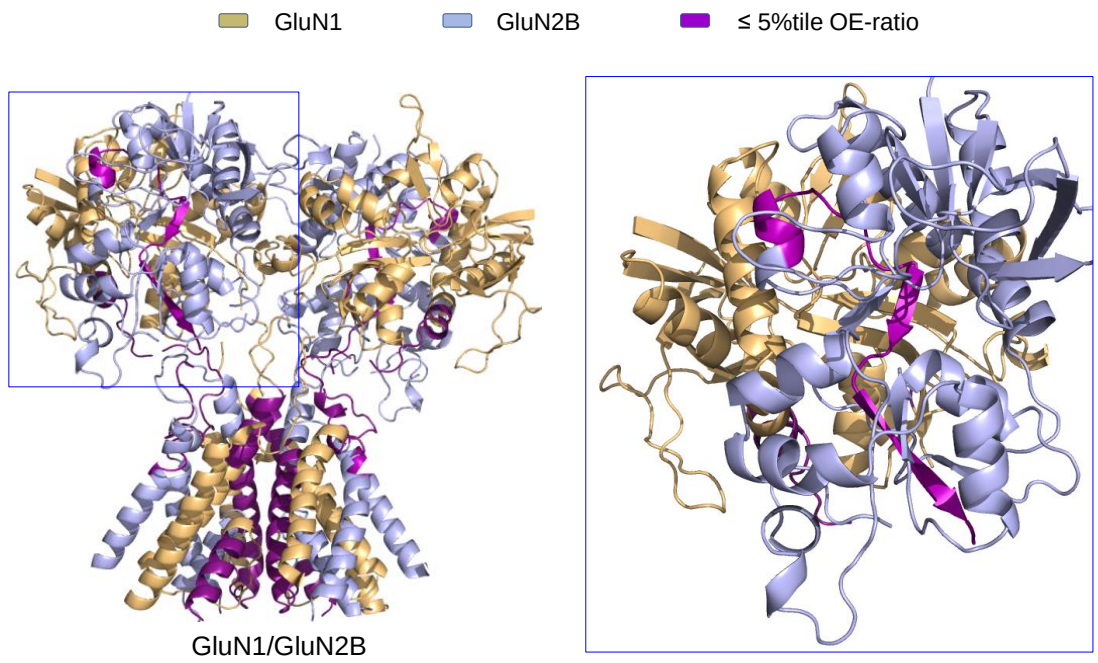

**S5 Figure. Quantification of GluN2A-P552R induced blebbing in transfected neurons** (related to **Figure-8, S4 Fig,** and **RESULTS**). Morphological features of rat cortical neurons in culture (DIV 18-19) expressing GFP and either empty vector, GluN2A WT (0.3 μ g; see Methods and Fig. 8), or GluN2A-P552R (0.3 μ g) for 24 hours. Although very rarely some dendritic blebs are observed in WT GluN2A-expressing neurons (e.g. see third panel from the top, middle row), blebs are a telltale and nearly ubiquitous sign of neuronal expression of GluN2A-P552R. Panels are representative of 11 different fields obtained from three separate coverslips for each condition for one representative experiment. We utilized an unbiased object count program (NIS elements, Nikon) to obtain the total number of blebs per field. The mean intensity for each field was utilized to set the threshold intensity and all objects from 2-100 μm were counted (cell bodies were excluded). Circularity was set to 0.25, with 1 being a perfect circle. Although these parameters detected objects in vector-expressing cells, these were attributed to spines or intrinsic dendritic tortuosity. Vector: 29.8 ± 6.2 objects/field; GluN2A WT: 59.2 ± 11.8; GluN2A(P552R): 115.4 ± 12.5. No statistical difference was observed between vector and WT; significant differences were observed between vector and mutant, and WT and mutant ($p<0.001$, 0.01, respectively; ANOVA/Tukey). Please note that we used a different pinhole in the confocal microscope (1.7) than the one used in S4 Figure (1.2) to increase the signal-to-noise ratio, which aided in the quantification procedure. Scale bar = 100 μm.




**S6 Figure. OE-ratio calculated from ExAC** (related to **METHODS**, **Figure-1,** and **RESULTS**). **A**, GluN1, **B**, GluN2A and **C**, GluN2B sliding window OE-ratio estimates (black full line), neutrality expected OE-ratio estimates derived from ExAC server accessed April, 2016 (blue full line), median OE-ratio for the gene (dark grey dashed line), 25th percentile of OE-ratio (green dashed line), 5th percentile of OE-ratio (red dashed line). The proportion of the 60,706 ExAC samples that had at least 10-fold coverage to be able to call a variant at the residue (cyan dashed line). The asterisk shows the Pro552 in GluN2A and Pro 553 in GluN2B.




**S7 Figure.** Simultaneous electrical and optical voltage recording form neurons (related to **Figure-7** and **RESULTS**). Simultaneous fluorescence and manual patch clamp measurements of an excitatory post-synaptic potential in a neuron expressing a QuasAr voltage indicator. Presynaptic CheRiff-expressing neurons were stimulated with a single flash of blue light at the start of the measurement. See Lou et al (2016) for current recording methods.




**S8 Figure. Regional purifying selection of GluN1/GluN2B** (related to **Figure-9** and **Discussion**). Left panel shows a ribbon structure of the GluN1/GluN2B receptors without the amino terminal domain. GluN1 is tan and GluN2B is light blue; regions with purple color reflect an OE-ratio below the 5$^{th}$ percentile, indicating the regions under the strongest purifying selection. Side view of agonist binding domain (ABD) in an expanded panel (*right*) shows a strong purifying selection (in purple) in regions of GluN1 S2 connecting with pre-M4 helix and GluN2B S1 connecting with pre-M1 helix.

## S1 Table. Patient ascertained de novo mutations (related to **Table-1**)

| HGNC | GRCh37/hg19 | HGVSc | HGVSp | Database Phenotype | PUBMED |
|---|---|---|---|---|---|
| GRIN1 | chr9:140057162G>A | NM_007327.3:c.1984G>A | NP_015566.1:p.Glu662Lys | Mental retardation, autosomal dominant 8 | 21376300 |
| GRIN1 | chr9:140058120G>A | NM_007327.3:c.2443G>A | NP_015566.1:p.Gly815Arg | Musculoskeletal/Structural (child onset); Seizures | 25356970 |
| GRIN1 | chr9:140056647C>G | NM_007327.3:c.1656C>G | NP_015566.1:p.Asp552Glu | Epileptic encephalopathy early onset with involuntary movements developmental delay & intellectual disability | 25864721 |
| GRIN1 | chr9:140056647C>A | NM_007327.3:c.1656C>A | NP_015566.1:p.Asp552Glu | Epileptic encephalopathy nonsyndromic | 26482601 |
| GRIN1 | chr9:140056661C>G | NM_007327.3:c.1670C>G | NP_015566.1:p.Pro557Arg | Intellectual disability | 25167861 |
| GRIN1 | chr9:140057101G>A | NM_007327.3:c.1923G>A | NP_015566.1:p.Met641Ile | Epileptic encephalopathy early onset with involuntary movements developmental delay & intellectual disability | 25864721 |
| GRIN1 | chr9:140057118A>C | NM_007327.3:c.1940A>C | NP_015566.1:p.Tyr647Ser | Infantile spasms | 23934111 |
| GRIN1 | chr9:140057128C>G | NM_007327.3:c.1950C>G | NP_015566.1:p.Asn650Lys | Epileptic encephalopathy early onset with involuntary movements developmental delay & intellectual disability | 25864721 |
| GRIN1 | chr9:140058120G>C | NM_007327.3:c.2443G>C | NP_015566.1:p.Gly815Arg | Epileptic encephalopathy early onset with involuntary movements developmental delay & intellectual disability | 25864721 |
| GRIN1 | chr9:140058090C>T | NM_007327.3:c.2413C>T | NP_015566.1:p.Pro805Ser | Developmental Delay | DDD - biorxiv |
| GRIN1 | chr9:140057361T>C | NM_007327.3:c.2077T>C | NP_015566.1:p.Phe693Leu | Developmental Delay | DDD - biorxiv |
| GRIN1 | chr9:140057658G>A | NM_007327.3:c.2209G>A | NP_015566.1:p.Glu737Lys | Intellectual disability | 27479843 |
| GRIN2A | chr16:9943635A>G | NM_000833.4:c.1306T>C | NP_000824.1:p.Cys436Arg | Partial epilepsy atypical benign | 23933819 |
| GRIN2A | chr16:9934513C>T | NM_000833.4:c.1642G>A | NP_000824.1:p.Ala548Thr | Landau-Kleffner syndrome | 23933820 |
| GRIN2A | chr16:9928084G>C | NM_000833.4:c.1655C>G | NP_000824.1:p.Pro552Arg | Focal epilepsy with speech disorder with or without mental retardation | 23033978 |
| GRIN2A | chr16:9923442G>T | NM_000833.4:c.1845C>A | NP_000824.1:p.Asn615Lys | Focal epilepsy with speech disorder with or without mental retardation | 20890276 |
| GRIN2A | chr16:9923342G>C | NM_000833.4:c.1945C>G | NP_000824.1:p.Leu649Val | Focal epilepsy with speech disorder with or without mental retardation | 23033978 |
| GRIN2A | chr16:9923333A>C | NM_000833.4:c.1954T>G | NP_000824.1:p.Phe652Val | Focal epilepsy with speech disorder with or without mental retardation | 23933820 |
| GRIN2A | chr16:9923330T>C | NM_000833.4:c.1957A>G | NP_000824.1:p.Met653Val | Developmental Delay | DDD - biorxiv |
| GRIN2A | chr16:9923328C>T | NM_000833.4:c.1959G>A | NP_000824.1:p.Met653Ile | Intellectual disability | 27479843 |
| GRIN2A | chr16:9916208A>G | NM_000833.4:c.2081T>C | NP_000824.1:p.Ile694Thr | Landau-Kleffner syndrome | 23933820 |
| GRIN2A | chr16:9916194G>A | NM_000833.4:c.2095C>T | NP_000824.1:p.Pro699Ser | Benign epilepsy with centrotemporal spikes | 23933819 |
| GRIN2A | chr16:9862869G>T | NM_000833.4:c.2434C>A | NP_000824.1:p.Leu812Met | Epileptic encephalopathy | 24504326 |
| GRIN2A | chr16:9862854T>C | NM_000833.4:c.2449A>G | NP_000824.1:p.Met817Val | Global developmental delay & epilepsy | 24903190 |
| GRIN2A | chr16:9862853A>G | NM_000833.4:c.2450T>C | NP_000824.1:p.Met817Thr | Intellectual disability | 27479843 |
| GRIN2B | chr12:13769479T>C | NM_000834.3:c.1238A>G | NP_000825.2:p.Glu413Gly | Mental retardation, autosomal dominant 6 | ClinVar Submission |
| GRIN2B | chr12:13768560C>T | NM_000834.3:c.1367G>A | NP_000825.2:p.Cys456Tyr | Mental retardation, autosomal dominant 6 | 23160955 |
| GRIN2B | chr12:13768545C>A | NM_000834.3:c.1382G>T | NP_000825.2:p.Cys461Phe | Lennox-Gastaut syndrome | 23934111 |
| GRIN2B | chr12:13768132C>T | NM_000834.3:c.1570G>A | NP_000825.2:p.Asp524Asn | Intellectual disability | 27479843 |
| GRIN2B | chr12:13768083C>T | NM_000834.3:c.1619G>A | NP_000825.2:p.Arg540His | Epileptic encephalopathy, early infantile, 27 | 24272827 |
| GRIN2B | chr12:13764781G>A | NM_000834.3:c.1658C>T | NP_000825.2:p.Pro553Leu | Mental retardation, autosomal dominant 6 | 23033978 |
| GRIN2B | chr12:13764767C>T | NM_000834.3:c.1672G>A | NP_000825.2:p.Val558Ile | Intellectual disability | 27479843 |
| GRIN2B | chr12:13761703T>A | NM_000834.3:c.1844A>T | NP_000825.2:p.Asn615Ile | Epileptic encephalopathy, early infantile, 27 | 24272827 |
| GRIN2B | chr12:13761702G>C | NM_000834.3:c.1845C>G | NP_000825.2:p.Asn615Lys | Developmental Delay | DDD - biorxiv |
| GRIN2B | chr12:13761694A>C | NM_000834.3:c.1853T>G | NP_000825.2:p.Val618Gly | Epileptic encephalopathy, early infantile, 27 | 24272827 |
| GRIN2B | chr12:13761664G>A | NM_000834.3:c.1883C>T | NP_000825.2:p.Ser628Phe | Developmental Delay | DDD - biorxiv |
| GRIN2B | chr12:13761641C>G | NM_000834.3:c.1906G>C | NP_000825.2:p.Ala636Pro | Intellectual disability | 23718928 |
| GRIN2B | chr12:13761562T>G | NM_000834.3:c.1985A>C | NP_000825.2:p.Gln662Pro | Partial seizures & infantile spasms with intellectual / developmental disabilities | 26544041 |
| GRIN2B | chr12:13724865G>A | NM_000834.3:c.2044C>T | NP_000825.2:p.Arg682Cys | Mental retardation, autosomal dominant 6 | 20890276 |
| GRIN2B | chr12:13724856T>G | NM_000834.3:c.2053A>C | NP_000825.2:p.Thr685Pro | Epileptic encephalopathy, early infantile, 27 | ClinVar Submission |
| GRIN2B | chr12:13724849G>C | NM_000834.3:c.2060C>G | NP_000825.2:p.Pro687Arg | Developmental Delay | DDD - biorxiv |
| GRIN2B | chr12:13724844C>T | NM_000834.3:c.2065G>A | NP_000825.2:p.Gly689Ser | Developmental Delay | DDD - biorxiv |
| GRIN2B | chr12:13724793T>C | NM_000834.3:c.2116A>G | NP_000825.2:p.Met706Val | Intellectual disability | 27479843 |
| GRIN2B | chr12:13720138C>T | NM_000834.3:c.2419G>A | NP_000825.2:p.Glu807Lys | Developmental Delay | DDD - biorxiv |
| GRIN2B | chr12:13720098C>T | NM_000834.3:c.2459G>A | NP_000825.2:p.Gly820Glu | Intellectual disability | 25356899 |
| GRIN2B | chr12:13720098C>G | NM_000834.3:c.2459G>C | NP_000825.2:p.Gly820Ala | Developmental Delay | DDD - biorxiv |

DDD – biorxiv: http://biorxiv.org/content/biorxiv/early/2016/04/22/049056.full.pdf
The rows highlighted in orange are the S1-M1 mutations associated with diseases

**S2 Table. Deactivation time course after glycine removal for GluN1-P557R and GluN2A-P552R (**related to **Figure-4)**

| | Di-heteromeric Receptors | | | Tri-heteromeric Receptors | | |
|---|---|---|---|---|---|---|
| | **WT N1/N2A** | **N1/N2A-P552R** | **N1-P557R/N2A** | **N2A/N2A** | **N2A-P552R/N2A** | **N2A-P552R/N2A-P552R** |
| **Amplitude (peak, pA/pF)** | 67 ± 12 | 18 ± 5.1$^{\#}$ | 4.5 ± 2.0$^{\#}$ | 57 ± 15 | 29 ± 4.3 | 11 ± 2.7$^{\#\$}$ |
| **Amplitude (SS, pA/pF)** | 44 ± 8.5 | --- | 3.9 ± 1.7* | 49 ± 11 | 24 ± 3.4 | --- |
| **$I_{SS}/I_{PEAK}$%** | 70 ± 4.5 % | --- | 88 ± 1.2 %* | 88 ± 2.8 % | 85 ± 4.9 % | --- |
| **Rise time (ms)** | 13 ± 0.75 | 874 ± 75$^{\#}$ | 8.6 ± 1.4 | 15 ± 1.1 | 14 ± 1.3 | 1176 ± 22$^{\#\$}$ |
| **$\tau_{FAST}$ (ms)** | 91 ± 5.2 | 1093 ± 96$^{\#}$ | 415 ± 148$^{\#}$ | 127 ± 9.6 | 387 ± 62 | 1690 ± 90$^{\#\$}$ |
| **$\tau_{SLOW}$ (ms)** | 594 ± 87 | 3034 ± 1263$^{\#}$ | 953 ± 99 | 1346 ± 242 | 1800 ± 254 | 2368 ± 304$^{\#}$ |
| **%$\tau_{FAST}$** | 92 ± 1.9 % | 89 ± 6.5 % | 57 ± 14.5 %$^{\#}$ | 95 ± 1.5 % | 81 ± 3.0 % | 91 ± 3.8 % |
| **$\tau_W$(ms)** | 125 ± 7.6 | 1243 ± 84$^{\#}$ | 801 ± 103$^{\#}$ | 177 ± 9.5 | 621 ± 75$^{\#}$ | 1915 ± 85$^{\#\$}$ |
| **n** | 17 | 12 | 7 | 8 | 7 | 17 |

Data are from human NMDARs. All parameters describing the time course of the macroscopic current were from responses to 1.5 sec glutamate application.

# $p < 0.05$ compared to corresponding WT receptors; one way ANOVA, Tukey post hoc

$ $p < 0.05$ compared to N2A-P552R/N2A; one way ANOVA, Tukey post hoc

* $p < 0.05$ compared to corresponding WT receptors, unpaired t-test

See **S6 Table** for F statistics.

**S3 Table. Sensitivity of GluN2A-P552R to endogenous negative modulators (**related to **RESULTS)**

| | WT N1/N2A | N1/N2A-P552R | P-value[#] |
|---|---|---|---|
| **$Mg^{2+}$, $IC_{50}$, μM (n)** | 20 ± 3.8 (6) | 13 ± 2.2 (8) | 0.062 |
| **Proton, $I_{pH6.8}/I_{pH7.6}$% (n)** | 44 ± 1.0% (12) | 47 ± 1.6% (11) | 0.108 |
| **$Zn^{2+}$, $IC_{50}$, nM (n)** | 27 ± 5.7 (24) | 8.9 ± 0.4 (24) | < 0.001 |
| **%inhibition by saturating $Zn^{2+}$** | 60 ± 2.7% (23) | 91 ± 1.7% (22) | < 0.001 |

Data are from human NMDARs.

[#] p-values for unpaired t-tests comparing WT N1/N2A to N1/N2A-P552R

## S4 Table. Statistical analysis for data in Table-3.

| | GluN1/GluN2A | | | | | GluN1/GluN2B | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | ANOVA | | Post hoc Tukey's P-value | | | ANOVA | | Post hoc Tukey's P-value | | |
| | F statistic | P value | GluN1-D552E/N2A | GluN1-P557R/N2A | GluN1/N2A-A548T | F statistic | P value | GluN1-D552E/N2B | GluN1-P557R/N2B | GluN1/N2B-P553L |
| **Amplitude (peak)** | F (3,32) = 6.884 | 0.001 | 0.0082 | 0.0074 | 0.0028 | F (3,27) = 10.33 | 0.0001 | 0.0024 | 0.001 | 0.0009 |
| **Glutamate, $EC_{50}$** | F (3,50) = 307.8 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | F (2,21) = 256.7 | <0.0001 | 0.0001 | <0.0001 | - |
| **Glycine, $EC_{50}$** | F (3,38) = 146.6 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | F (2,21) = 86.34 | <0.0001 | 0.0048 | <0.0001 | - |

**S5 Table. Statistical analysis for data in Table-4.**

| | Di-heteromeric NMDA Receptors | Tri-heteromeric NMDA Receptors | | | | |
|---|---|---|---|---|---|---|
| | GluN2A vs GluN2A-P552R | ANOVA | | Post hoc Tukey's P-value | | |
| | unpaired t-test p value | F statistic | P value | N2A/N2A vs N2A-P552R/N2A | N2A/N2A vs N2A-P552R/N2A-P552R | N2A/N2A-P552R vs N2A-P552R/N2A-P552R |
| **Glutamate, $EC_{50}$** | < 0.0001 | F (2,77) = 136.9 | <0.0001 | <0.0001 | <0.0001 | <0.0001 |
| **Glycine, $EC_{50}$,** | < 0.0001 | F (2,71) = 331 | <0.0001 | <0.0001 | <0.0001 | <0.0001 |
| **Amplitude (peak, pA/pF)** | 0.9289 | F (2,22) = 0.011 | 0.9891 | --- | --- | --- |
| **Amplitude (SS, pA/pF)*** | --- | --- | --- | 0.6242 | --- | --- |
| **$I_{SS}/I_{PEAK}$%*** | --- | --- | --- | 0.1478 | --- | --- |
| **Rise time (ms)** | < 0.0001 | F (2,23) = 1662 | <0.0001 | 0.9958 | <0.0001 | <0.0001 |
| **$t_{FAST}$ (ms)** | < 0.0001 | F (2,23) = 110.7 | <0.0001 | 0.4742 | <0.0001 | <0.0001 |
| **$t_{SLOW}$ (ms)** | --- | F (2,14) = 20.7 | <0.0001 | 0.7029 | 0.0001 | 0.0002 |
| **%$t_{FAST}$** | 0.0547 | F (2,23) = 11.12 | 0.0004 | 0.001 | 0.9869 | 0.0014 |
| **$t_W$(ms)** | < 0.0001 | F (2,23) = 152.7 | <0.0001 | 0.0672 | <0.0001 | <0.0001 |
| **Charge transfer pA ms/pF** | 0.00061 | F (2,20) = 3.707 | 0.0427 | 0.5520 | <0.05 | 0.1938 |
| **Mean Open Time (ms)** | --- | F (2,28) = 39.18 | <0.0001 | 0.9996 | <0.0001 | <0.0001 |
| **Current Amplitude (pA)** | --- | F(2,28) = 32.01 | <0.0001 | 0.4623 | <0.0001 | <0.0001 |
| **Open t1 (ms)** | --- | F (2,28) = 3.816 | 0.0342 | 0.9861 | 0.1011 | 0.0424 |
| **Open t2 (ms)** | --- | F (2,28) = 33.33 | <0.0001 | 0.9999 | <0.0001 | <0.0001 |
| **Open Area 1 (%)** | --- | F (2,28) = 11.21 | 0.0003 | 0.9915 | 0.0016 | 0.0007 |
| **Open probability** | 0.2184 | --- | --- | --- | --- | --- |

*unpaired t-test

**S6 Table. Statistical analyses for S2 Table.**

| | Di-heteromeric Receptors | | | | Tri-heteromeric Receptors | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | ANOVA | | p value | | ANOVA | | p value | | |
| | F statistic | P value | N1/N2A vs N1/N2A-P552R | N1/N2A vs N1-P557R/N2A | F statistic | P value | N2A/N2A vs N2A-P552R/N2A | N2A/N2A vs N2A-P552R/N2A-P552R | N2A-P552R/N2A vs N2A-P552R/N2A-P552R |
| **Amplitude (peak, pA/pF)** | F (2,33) = 9.869 | 0.0004 | 0.0037 | 0.0018 | F (2,29) = 11.37 | 0.0002 | 0.0542 | 0.0001 | 0.2076 |
| **Amplitude (SS, pA/pF)*** | --- | --- | --- | 0.0073 | --- | --- | 0.0761 | --- | --- |
| **$I_{SS}/I_{PEAK}$%*** | --- | --- | --- | 0.0152 | --- | --- | 0.5758 | --- | --- |
| **Rise time (ms)** | F (2,35) = 143.9 | <0.0001 | <0.0001 | 0.9975 | F (2,39) = 1369 | <0.0001 | 0.9987 | <0.0001 | <0.0001 |
| **$t_{FAST}$ (ms)** | F (2,35) = 44.7 | <0.0001 | <0.0001 | 0.0224 | F (2,39) = 118.5 | <0.0001 | 0.1479 | <0.0001 | <0.0001 |
| **$t_{SLOW}$ (ms)** | F (2,19) = 6.178 | 0.0086 | 0.0068 | 0.8666 | F (2,22) = 8.969 | 0.0014 | 0.7856 | 0.0012 | 0.0061 |
| **%$t_{FAST}$** | F (2,35) = 6.432 | 0.0042 | 0.9517 | 0.0044 | F (2,39) = 2.988 | 0.062 | --- | --- | --- |
| **$t_W$(ms)** | F (2,35) = 93.03 | <0.0001 | <0.0001 | <0.0001 | F (2,39) = 147.9 | <0.0001 | 0.0045 | <0.0001 | <0.0001 |

* unpaired t-tests

## S7 Table. Statistical analysis for Table-6.

| | unpaired t-test p value | |
|---|---|---|
| | **GluN1/GluN2A vs. GluN1-P557R/GluN2A** | **GluN1/GluN2B vs. GluN1/GluN2B-P553R** |
| **Amplitude (peak, pA/pF)** | 0.0443 | 0.5815 |
| **Amplitude (SS, pA/pF)** | 0.0321 | --- |
| **$I_{SS}/I_{PEAK}$%** | 0.0006 | --- |
| **Rise time (ms)** | 0.8589 | < 0.0001 |
| **$t_{FAST}$ (ms)** | < 0.0001 | < 0.0001 |
| **$t_{SLOW}$ (ms)** | 0.4272 | 0.0013 |
| **%$t_{FAST}$** | 0.0201 | 0.9024 |
| **$t_W$ (ms)** | < 0.0001 | < 0.0001 |
| **Charge transfer, pA·ms/pF** | 0.48208 | --- |

**S8 Table. Statistical analysis for Table-7.**

| | Q and K ANOVA | | Post hoc Tukey's P-value vs WT 2A | | G, A, I, and L ANOVA | | Post hoc Tukey's P-value vs WT 2A | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | **F statistic** | **P value** | **P552Q** | **P552K** | **F statistic** | **P value** | **P552G** | **P552A** | **P552I** | **P552L** |
| **Glutamate, $EC_{50}$** | F (6,36) = 124.8 | <0.0001 | <0.0001 | 0.9473 | <0.0001 | <0.0001 | <0.0001 | 0.9473 | <0.0001 | 0.0606 |
| **Glycine, $EC_{50}$** | F (6,79) = 83.39 | <0.0001 | <0.0001 | <0.0001 | 0.9999 | 0.0275 | <0.0001 | <0.0001 | 0.0003 | 0.3416 |
| **Amplitude (peak, pA/pF)** | F (2, 27) = 19.16 | <0.0001 | 0.0002 | <0.0001 | F (4, 58) = 1.517 | 0.2091 | --- | --- | --- | --- |
| **Amplitude (SS, pA/pF)** | --- | --- | 0.0002* | --- | F (4, 58) = 43.78 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 |
| **$I_{SS}/I_{PEAK}$** | --- | --- | <0.0001* | --- | F (4, 58) = 43.78 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 |
| **$t_W$ desensitization (ms)** | --- | --- | <0.0001 | --- | F (4, 59) = 29.62 | <0.0001 | <0.0001 | <0.0001 | 0.998 | <0.0001 |
| **Rise time (ms)** | F (2, 28) = 6131 | <0.0001 | 0.9786 | <0.0001 | F (4, 57) = 5.376 | 0.001 | 0.0022 | 0.9969 | 0.5998 | 0.9994 |
| **$t_{FAST}$ (ms)** | F (2, 28) = 153.2 | <0.0001 | 0.9945 | <0.0001 | F (4, 58) = 10.72 | <0.0001 | <0.0001 | 0.0806 | 0.0003 | 0.0009 |
| **$t_{SLOW}$ (ms)** | --- | --- | 0.6758* | --- | F (4, 44) = 3.181 | 0.0222 | 0.7861 | 0.9441 | 0.8359 | 0.0395 |
| **$\%t_{FAST}$** | F (2, 28) = 3.667 | 0.0385 | 0.0634 | 0.999 | F (4, 59) = 1.547 | 0.2004 | --- | --- | --- | --- |
| **$t_W$(ms)** | F (2, 28) = 219.9 | <0.0001 | 0.8239 | <0.0001 | F (4, 58) = 4.52 | 0.003 | 0.079 | 0.7566 | 0.0021 | 0.213 |

*unpaired t-test

## S9 Table. Statistical Data for Figure-8.

**A. Repeated Measures ANOVA/Bonferroni: Luciferase Assays (0.3 μg DNA/well)**

| | P value | F statistic | WT GluN2A (- mem) vs. WT GluN2A (+ mem) | WT GluN2A (- mem) vs. GluN2A-P552R (- mem) | GluN2A-P552R (- mem) vs. GluN2A-P552R (+ mem) |
|---|---|---|---|---|---|
| *Viability (% of control; n=7)* | < 0.0001 | F (3, 6, 18) = 64.07 | < 0.001 | < 0.05 | < 0.001 |

**B. Repeated Measures ANOVA/Bonferroni: Luciferase Assays (0.6 μg DNA/well)**

| | P value | F statistic | WT GluN2A (- mem) vs. WT GluN2A (+ mem) | WT GluN2A (- mem) vs. GluN2A-P552R (- mem) | GluN2A-P552R (- mem) vs. GluN2A-P552R (+ mem) |
|---|---|---|---|---|---|
| *Viability (% of control; n=8)* | < 0.0001 | F (3, 7, 21) = 23.11 | < 0.01 | ns | < 0.001 |

**C. Repeated Measures ANOVA/Bonferroni: Cell Counts (0.6 μg DNA/well)**

| | P value | F statistic | WT GluN2A (- mem) vs. WT GluN2A (+ mem) | WT GluN2A (- mem) vs. GluN2A-P552R (- mem) | GluN2A-P552R (- mem) vs. GluN2A-P552R (+ mem) |
|---|---|---|---|---|---|
| *Viability (% of control; n=6)* | 0.0079 | F (3, 5, 15) = 5.77 | ns | ns | < 0.01 |