**Supp. Figure S1** Summary of current amplitude evoked by maximally effective concentrations of agonists (100 mM glutamate and 100 mM glycine) determined by TEVC recordings from *Xenopus* oocytes expressing tri-heteromeric receptors (Swanger et al., 2016; Hansen, Ogden, Yuan, & Traynelis, 2014; Yi, Zachariassen, Dorsett, & Hansen, 2018; Yuan et al., 2014). The R518K/T690I double mutations are located in the agonist-binding pocket for glutamate and glycine and abolish glutamate/glycine binding, thereby rendering NMDA receptors with either the GluN1 subunit, GluN2A subunit or GluN2B subunit non-functional. Any current responses observed for oocytes expressing RK,TI mutant are mediated by receptors that have escaped ER retention. GluN1/GluN1/2A (858 ± 170 nA, n = 14), GluN1-RKTI/GluN1/2A (58 ± 18 nA, n = 14); 2A/2A (385 ± 58 nA, n = 16), 2A-RKTI/2A (29 ± 6.0 nA, n = 36);   and 2B/2B (704 ± 171 nA, n = 14), 2B-RKTI/2B (21 ± 6.2 nA, n = 40).
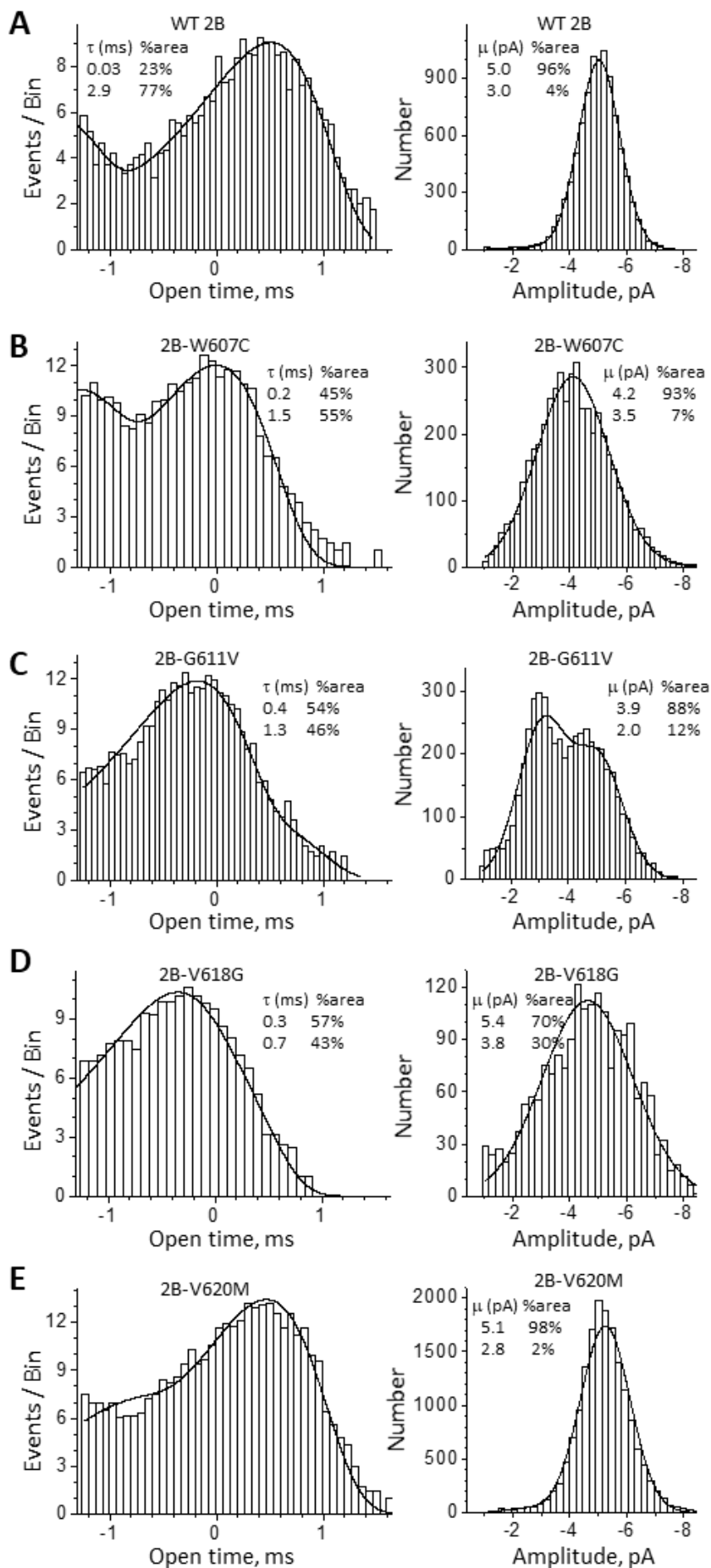
Supp. Figure S2 The pooled open time duration histograms (*left panels*) and amplitude histograms (*right panels*) are shown for WT and the GluN2B M2 variants. Fitted and statistical parameters are given in Table 5 and Supp. Table S4.

Supp. Table S2 Statistical analysis for data in Table 3

			Glutamate EC_{50}	Glycine EC_{50}	%, pH6.8/pH7.6
GluN1/GluN2A	ANOVA	F statistic	$F_{(5, 85)} = 18.75$	$F_{(5, 78)} = 18.88$	$F_{(5, 80)} = 27.06$
		P value	P<0.0001	P<0.0001	P<0.0001
	P-value	1-G618R/2A	0.9996	0.0013	0.13
		1-G620R/2A	0.1459	0.4463	<0.0001
		2A-L611Q	<0.0001	0.0049	<0.0001
		2A-N614S	<0.0001	<0.0001	0.0002
		2A-N615K	0.0004	0.0005	>0.9999
GluN1/GluN2B	ANOVA	F statistic	$F_{(9, 114)} = 6.206$	$F_{(8, 124)} = 11.29$	$F_{(9, 117)} = 9.542$
		P value	P<0.0001	P<0.0001	P<0.0001
	P-value	1-G618R/2B	0.9875	n.d.	0.0393
		1-G620R/2B	0.1505	0.986	0.9993
		2B-W607C	0.0004	0.9935	0.0048
		2B-G611V	0.9875	0.0942	0.087
		2B-N615I	>0.9999	0.0286	0.0769
		2B-N615K	0.5725	<0.0001	0.9993
		2B-N616K	0.0908	<0.0001	>0.9999
		2B-V618G	0.9208	0.5699	0.0042
		2B-V620M	0.0936	0.1983	0.0006

Supp. Table S3 Statistical analysis for data in Table 4

			IC$_{50}$, μM	% at 1 mM	% at -60mV
di-heteromeric receptors	GluN1/GluN2A	ANOVA	**F statistic**	F (3, 65) = 194.3	F (5, 98) = 140.8	F (5, 88) = 76
			P value	P<0.0001	P<0.0001	P<0.0001
		P-value	**1-G618R/2A**	n.d.	<0.0001	<0.0001
			1-G620R/2A	<0.0001	<0.0001	0.0031
			2A-L611Q	<0.0001	<0.0001	<0.0001
			2A-N614S	<0.0001	0.0014	0.9025
			2A-N615K	n.d.	<0.0001	<0.0001
	GluN1/GluN2B	ANOVA	**F statistic**	F (2, 67) = 101	F (9, 135) = 193.3	F (8, 118) = 90.86
			P value	P<0.0001	P<0.0001	P<0.0001
		P-value	**1-G618R/2B**	<0.0001	<0.0001	<0.0001
			1-G620R/2B	n.d.	<0.0001	<0.0001
			2B-W607C	n.d.	<0.0001	0.0025
			2B-G611V	n.d.	<0.0001	<0.0001
			2B-N615I	n.d.	<0.0001	<0.0001
			2B-N615K	n.d.	<0.0001	<0.0001
			2B-N616K	n.d.	<0.0001	<0.0001
			2B-V618G	n.d.	<0.0001	0.0012
			2B-V620M	<0.0001	0.0726	n.d.
di-heteromeric receptors	GluN1/GluN2A	ANOVA	**F statistic**	F (2, 33) = 31.73	F (2, 33) = 34.34	F (2, 39) = 62.56
			P value	P<0.0001	P<0.0001	P<0.0001
		P-value	**1-G620R/N1/2A**	0.0001	0.0004	0.0738
			1-G620R/1-G620R/2A	<0.0001	<0.0001	<0.0001
	GluN1/GluN2A	ANOVA	**F statistic**	F (4, 87) = 98.44	F (6, 105) = 151.6	F (6, 102) = 174.8
			P value	P<0.0001	P<0.0001	P<0.0001
		P-value	**N1/2A-L611Q/2A**	<0.0001	<0.0001	<0.0001
			N1/2A-L611Q/2A-L611Q	<0.0001	<0.0001	<0.0001
			N1/2A-N614S/2A	<0.0001	0.6927	0.9825
			N1/2A-N614S/2A-N614S	<0.0001	0.0807	0.2994
			N1/2A-N615K/2A	n.d.	<0.0001	<0.0001
			N1/2A-N615K/2A-N615K	n.d.	<0.0001	<0.0001
	GluN1/GluN2B	ANOVA	**F statistic**	F (6, 120) = 28.45	F (14, 199) = 261.5	F (14, 266) = 66.1
			P value	P<0.0001	P<0.0001	P<0.0001
		P-value	**N1/2B-W607C/2B**	0.0002	0.5183	0.9998
			N1/2B-W607C/2B-W607C	<0.0001	0.0002	0.8144
			N1/2B-G611V/2B	<0.0001	<0.0001	0.181
			N1/2B-G611V/2B-G611V	n.d.	<0.0001	0.0002
			N1/2B-N615I/2B	<0.0001	0.5793	0.2273
			N1/2B-N615I/2B-N615I	n.d.	<0.0001	<0.0001
			N1/2B-N615K/2B	n.d.	<0.0001	<0.0001
			N1/2B-N615K/2B-N615K	n.d.	<0.0001	<0.0001
			N1/2B-N616K/2B	n.d.	<0.0001	<0.0001
			N1/2B-N616K/2B-N616K	n.d.	<0.0001	<0.0001
			N1/2B-V618G/2B	n.d.	<0.0001	0.0036
			N1/2B-V618G/2B-V618G	n.d.	<0.0001	<0.0001
			N1/2B-V620M/2B	<0.0001	0.1675	0.9682
			N1/2B-V620M/2B-V620M	<0.0001	0.0002	0.8456

Supp. Table S4 Statistical analysis for data in Table 5

			amplitude, pA/pF	deactivation tw, ms	P_{OPEN}, MTSEA	Mean open time, ms	main conductance, pS	Pca/PNa	surface/total ratio (b-lac)
GluN1/GluN2A	ANOVA	F statistic	F (5, 65) = 3.861	F (3, 53) = 15.82	F (3, 65) = 52.88	F (2, 6) = 6.909	F (2, 6) = 117.1	F (3, 22) = 40.61	F (3, 26) = 3.417
		P value	P=0.0040	P<0.0001	P<0.0001	P=0.0278	P<0.0001	P<0.0001	P=0.0321
	P-value	1-G618R/2A	0.0297	n.d.	<0.0001	n.d.	n.d.	n.d.	<0.0001
		1-G620R/2A	0.0202	0.2086	<0.0001	n.d.	n.d.	<0.0001	<0.0001
		2A-L611Q	0.932	<0.0001	0.0049	0.0969	0.0003	<0.0001	0.7047
		2A-N614S	0.0109	n.d.	<0.0001	n.d.	n.d.	n.d.	0.0102
		2A-N615K	0.796	0.8099	0.0006	0.3764	<0.0001	<0.0001	0.8663
GluN1/GluN2B	ANOVA	F statistic	F (9, 117) = 7.368	F (8, 111) = 5.037	F (6, 121) = 26.36	F (4, 12) = 5.222	F (4, 12) = 2.646	F (3, 19) = 34.74	F (7, 32) = 3.184
		P value	P<0.0001	P<0.0001	P<0.0001	P=0.0113	P=0.0858	P<0.0001	P=0.0113
	P-value	1-G618R/2B	0.0094	n.d.	n.d.	n.d.	n.d.	n.d.	<0.0001
		1-G620R/2B	<0.0001	0.3072	n.d.	n.d.	n.d.	n.d.	<0.0001
		2B-W607C	0.001	0.3926	<0.0001	0.0303	0.4346	n.d.	0.8521
		2B-G611V	0.0098	0.137	<0.0001	0.0187	0.1691	n.d.	0.6657
		2B-N615I	0.0096	0.9901	0.0007	n.d.	n.d.	<0.0001	0.866
		2B-N615K	0.0114	0.9651	<0.0001	n.d.	n.d.	n.d.	0.037
		2B-N616K	0.5422	0.8549	<0.0001	n.d.	n.d.	n.d.	0.9759
		2B-V618G	0.0004	0.2176	n.d.	0.0241	0.8648	0.0068	0.2808
		2B-V620M	0.7917	0.0126	<0.0001	0.9675	0.9983	0.001	0.0726

Supp. Table S5 Summary of patch clamp data with a brief application

	amplitude, pA/pF	deactivation τw, ms	charge transfer, pA·ms/pF
WT GluN1/WT 2A	154 ± 30 (18)	47 ± 3.4 (18)	7,600
1-G618R/2A	2.1 ± 0.84 (8)*	n.d.	n.d.
1-G620R/2A	24 ± 7.8 (12)*	20 ± 2.2 (12)*	533
2A-L611Q	128 ± 26 (15)	67 ± 8.8 (15)*	7,686
2A-N614S	n.d.	n.d.	n.d.
2A-N615K	126 ± 36 (10)	48 ± 4.4 (10)	6,031
WT GluN1/WT 2B	41 ± 9.4 (16)	548 ± 50 (18)	22,131
1-G618R/2B	n.d.	n.d.	n.d.
1-G620R/2B	2.1 ± 0.56 (10)*	328 ± 22 (10)	749
2B-W607C	4.3 ± 1.9 (10)*	414 ± 93 (9)	1,554
2B-G611V	11 ± 3.2 (9)	949 ± 87 (9)*	10,638
2B-N615I	15 ± 4.1 (15)	472 ± 30 (15)	7,108
2B-N615K	6.1 ± 2.2 (7)	639 ± 71 (7)	3,845
2B-N616K	28 ± 18 (6)	714 ± 89 (6)	17,069
2B-V618G	7.8 ± 3.9 (11)	749 ± 86 (11)	7,640
2B-V620M	67 ± 15 (16)	658 ± 63 (16)	39,071

Data were expressed as Mean ± SEM (n).

n.d. not determined due to too small current.

*$p < 0.05$ one way ANOVA, with Dunnett's multiple comparisons test, controlled FWER (family wise error rate) by using the Holm-Bonferroni correction.

Supp. Table S6 Statistical analysis for data in Supp. Table S5

			amplitude, pA/pF	deactivation tw, ms
GluN1/GluN2A	**ANOVA**	F statistic	F (4, 58) = 5.73	F (3, 51) = 13.51
		P value	P=0.0006	P<0.0001
	P-value	1-G618R/2A	0.002	n.d.
		1-G620R/2A	0.0026	0.0011
		2A-L611Q	0.8627	0.0118
		2A-N614S	n.d.	n.d.
		2A-N615K	0.8785	0.9994
GluN1/GluN2B	**ANOVA**	F statistic	F (8, 91) = 6.409	F (8, 92) = 7.383
		P value	P<0.0001	P<0.0001
	P-value	1-G618R/2B	n.d.	n.d.
		1-G620R/2B	0.019	0.0733
		2B-W607C	0.0306	0.5403
		2B-G611V	0.132	0.0002
		2B-N615I	0.142	0.8884
		2B-N615K	0.0938	0.9252
		2B-N616K	0.9532	0.468
		2B-V618G	0.0523	0.1027
		2B-V620M	0.1193	0.5825

Supp. Table S7 Summary of beta-lac total protein

	total (b-lac)
WT GluN1/WT 2A	1.0 ± 0.11 (10)
1-G618R/2A	0.68 ± 0.11 (4)*
1-G620R/2A	0.34 ± 0.14 (4)*
2A-L611Q	0.93 ± 0.15 (11)
2A-N614S	0.19 ± 0.02 (4)*
2A-N615K	0.89 ± 0.14 (4)
WT GluN1/WT 2B	1.0 ± 0.10 (10)
1-G618R/2B	1.1 ± 0.08 (4)*
1-G620R/2B	1.4 ± 0.2 (4)*
2B-W607C	1.1 ± 0.22 (4)
2B-G611V	1.3 ± 0.13 (3)
2B-N615I	0.91 ± 0.12 (7)
2B-N615K	0.62 ± 0.08 (4)
2B-N616K	0.94 ± 0.07 (3)
2B-V618G	0.89 ± 0.15 (5)
2B-V620M	0.57 ± 0.07 (4)*

*$p < 0.05$ one way ANOVA, with Dunnett's multiple comparisons test.

Supp. Table S8 Statistical analysis for beta-lac total protein

			total (b-lac)
GluN1/GluN2A	**ANOVA**	**F statistic**	F (2, 9) = 305988
		P value	P<0.0001
	P-value	**1-G618R/2A**	<0.0001
		1-G620R/2A	<0.0001
	ANOVA	**F statistic**	F (3, 22) = 5.168
		P value	P=0.0074
	P-value	**2A-L611Q**	0.9558
		2A-N614S	0.0041
		2A-N615K	0.9343
GluN1/GluN2B	**ANOVA**	**F statistic**	F (2, 9) = 216383
		P value	P<0.0001
	P-value	**1-G618R/2B**	<0.0001
		1-G620R/2B	<0.0001
	ANOVA	**F statistic**	F (7, 32) = 3.69
		P value	P=0.0049
	P-value	**2B-W607C**	0.917
		2B-G611V	0.2713
		2B-N615I	0.9637
		2B-N615K	0.0702
		2B-N616K	0.9994
		2B-V618G	0.9317
		2B-V620M	0.0299