Differential functional consequences of *GRIN2A* mutations associated with schizophrenia and neurodevelopmental disorders

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Figure S1

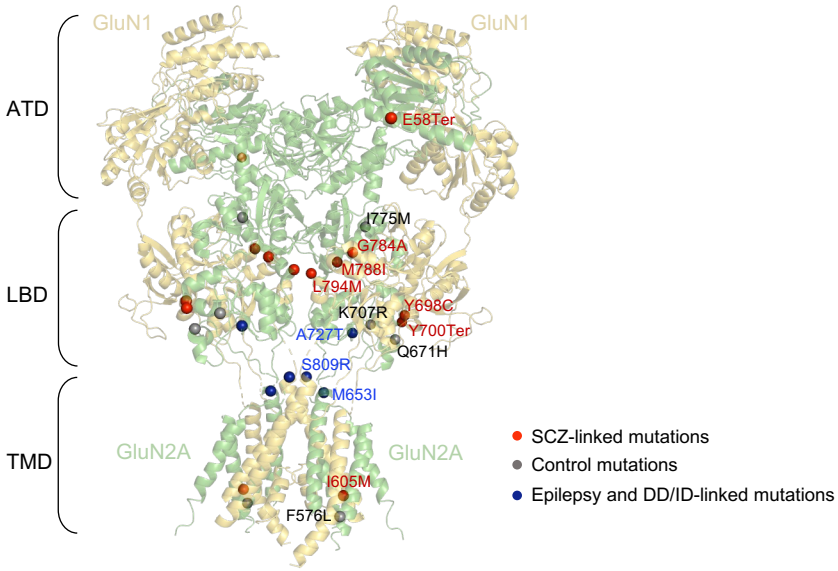
GluN1
GluN1
ATD
LBD
TMD
E58Ter
I775M
G784A
M788I
L794M
K707R
Y698C
Y700Ter
A727T
Q671H
S809R
M653I
GluN2A
GluN2A
I605M
F576L
SCZ-linked mutations
Control mutations
Epilepsy and DD/ID-linked mutations

Figure S1. *GRIN2A* mutations mapped on human NMDAR structure. Protein structure of human NMDAR (PDB ID 6IRH) with two GluN1 (light yellow) and two GluN2A (light green) subunits. *GRIN2A* mutations associated with SCZ (red) or epilepsy/DD/ID (blue) as well as control mutations (dark gray) are mapped on the two GluN2A subunits of human NMDAR. Two SCZ-associated missense mutations (Q811P, G591R), one control mutation (R586K), one DD/ID-linked mutation (L812M) as well as mutations within the CTD were not mapped due to no structure coverage.

Figure S2

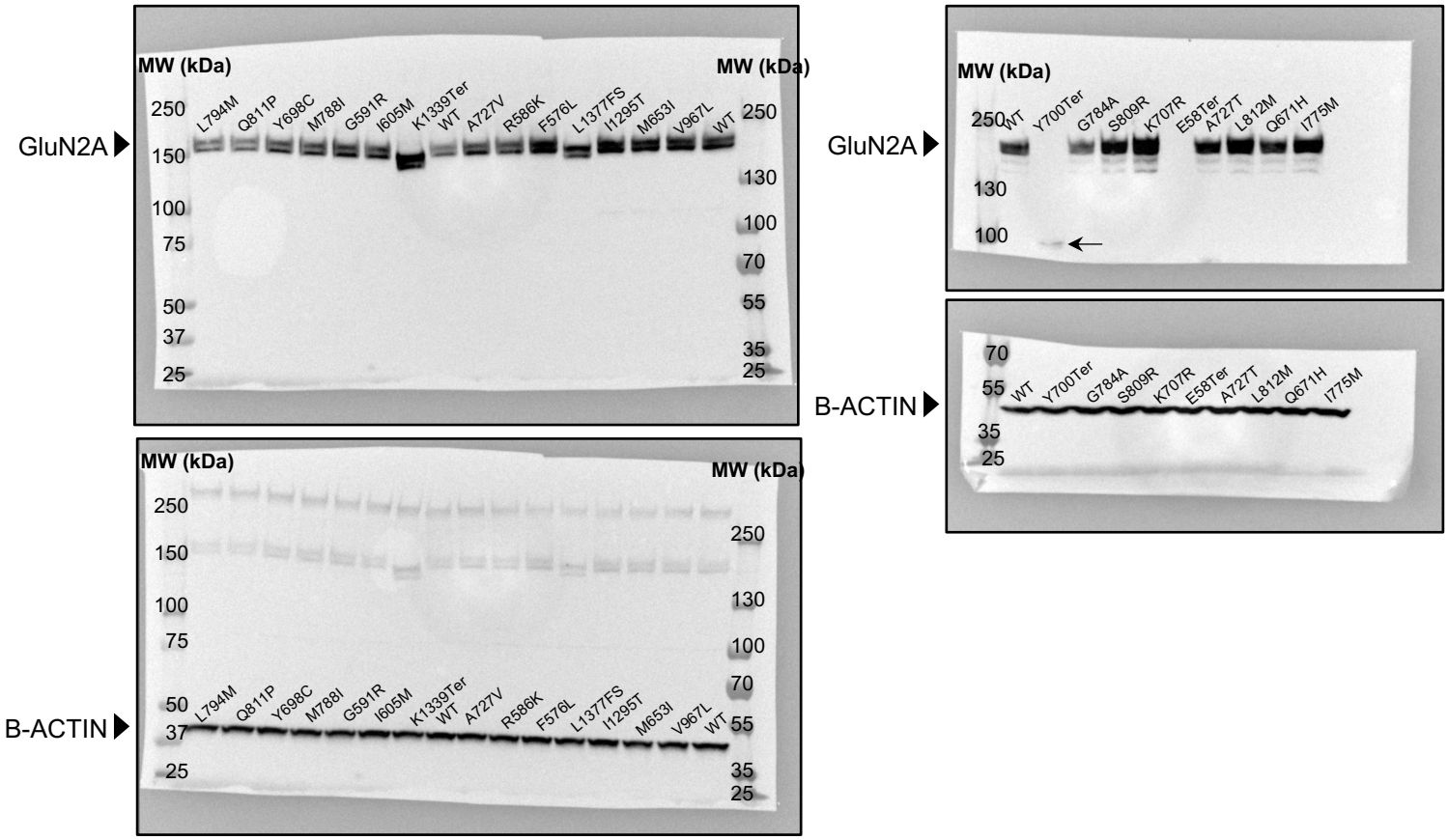
GluN2A
MW (kDa)
MW (kDa)
250
150
100
75
50
37
25
L794M
Q811P
Y698C
M788I
G591R
I605M
K1339Ter
WT
A727V
R586K
F576L
L1377FS
I1295T
M653I
V967L
WT
250
130
100
70
55
35
25
B-ACTIN
MW (kDa)
MW (kDa)
250
150
100
75
50
37
25
L794M
Q811P
Y698C
M788I
G591R
I605M
K1339Ter
WT
A727V
R586K
F576L
L1377FS
I1295T
M653I
V967L
WT
250
130
100
70
55
35
25
GluN2A
MW (kDa)
250
WT
Y700Ter
G784A
S809R
K707R
E58Ter
A727T
L812M
Q671H
I775M
130
100
B-ACTIN
70
55
35
25
WT
Y700Ter
G784A
S809R
K707R
E58Ter
A727T
L812M
Q671H
I775M

Figure S2. Uncropped western blot images of GluN2A variants. Western blots probing for GluN2A and β-ACTIN in lysates of HEK cells transiently transfected with GRIN1-GRIN2A constructs to express wild-type or mutant NMDARs. The arrow in Y700Ter lane indicates a faint band near the expected size for the Y700Ter fragment (~ 80 kDa).

Figure S3

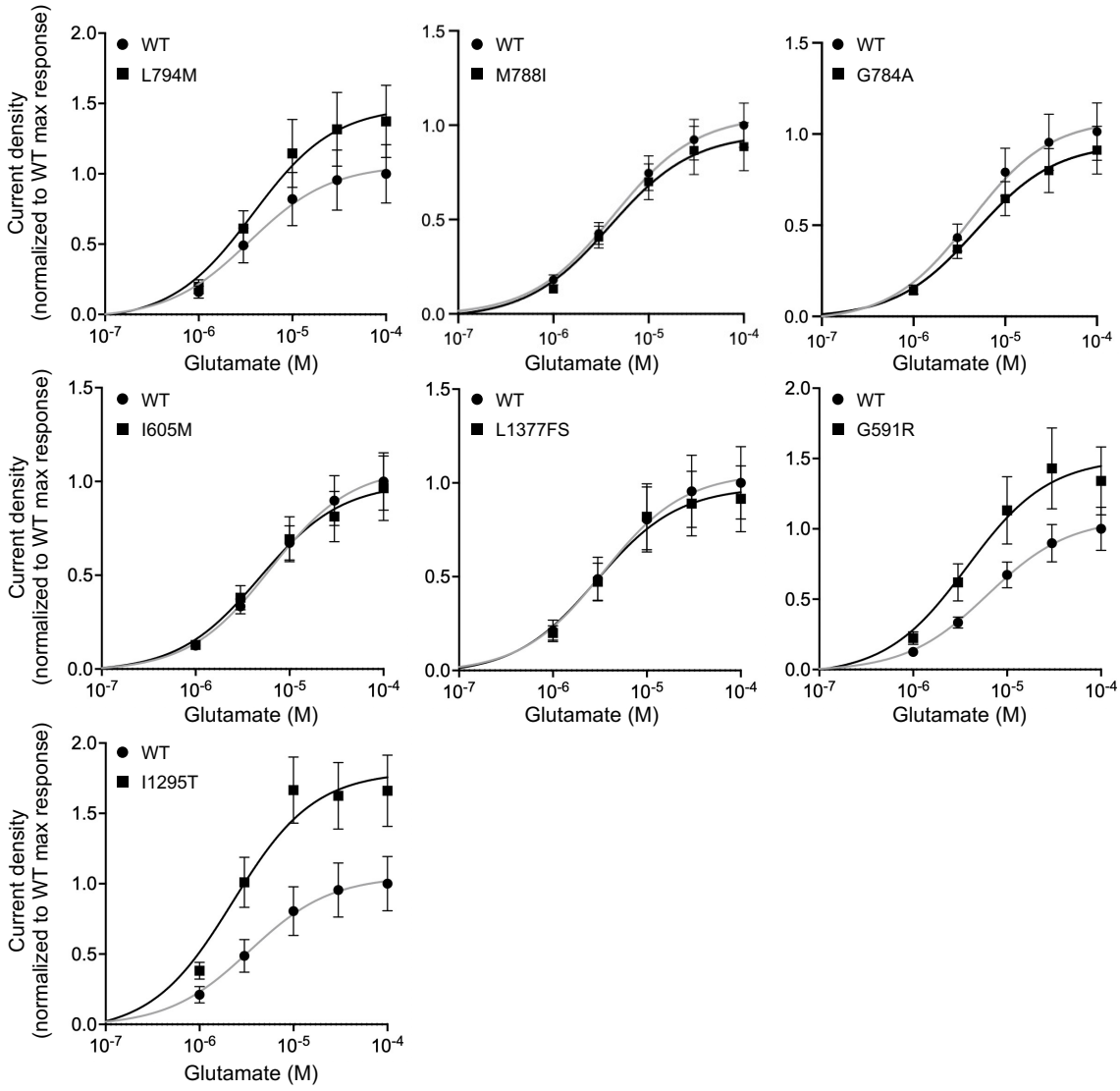
WT
L794M
M788I
G784A
I605M
L1377FS
G591R
I1295T
Current density
(normalized to WT max response)
Glutamate (M)

Figure S3. Effect of SCZ-associated *GRIN2A* variants on NMDAR function. Averaged current density in response to increasing concentrations of glutamate in the constant presence of 30 μM glycine, normalized to maximal response, for NMDARs containing the wild-type or SCZ-associated GluN2A variants. The lines indicate a nonlinear regression three-parameter fit to each dataset. Data are displayed as mean ± SEM, n = 31-81; see Table 1 for number of cells recorded per variant.

Figure S4

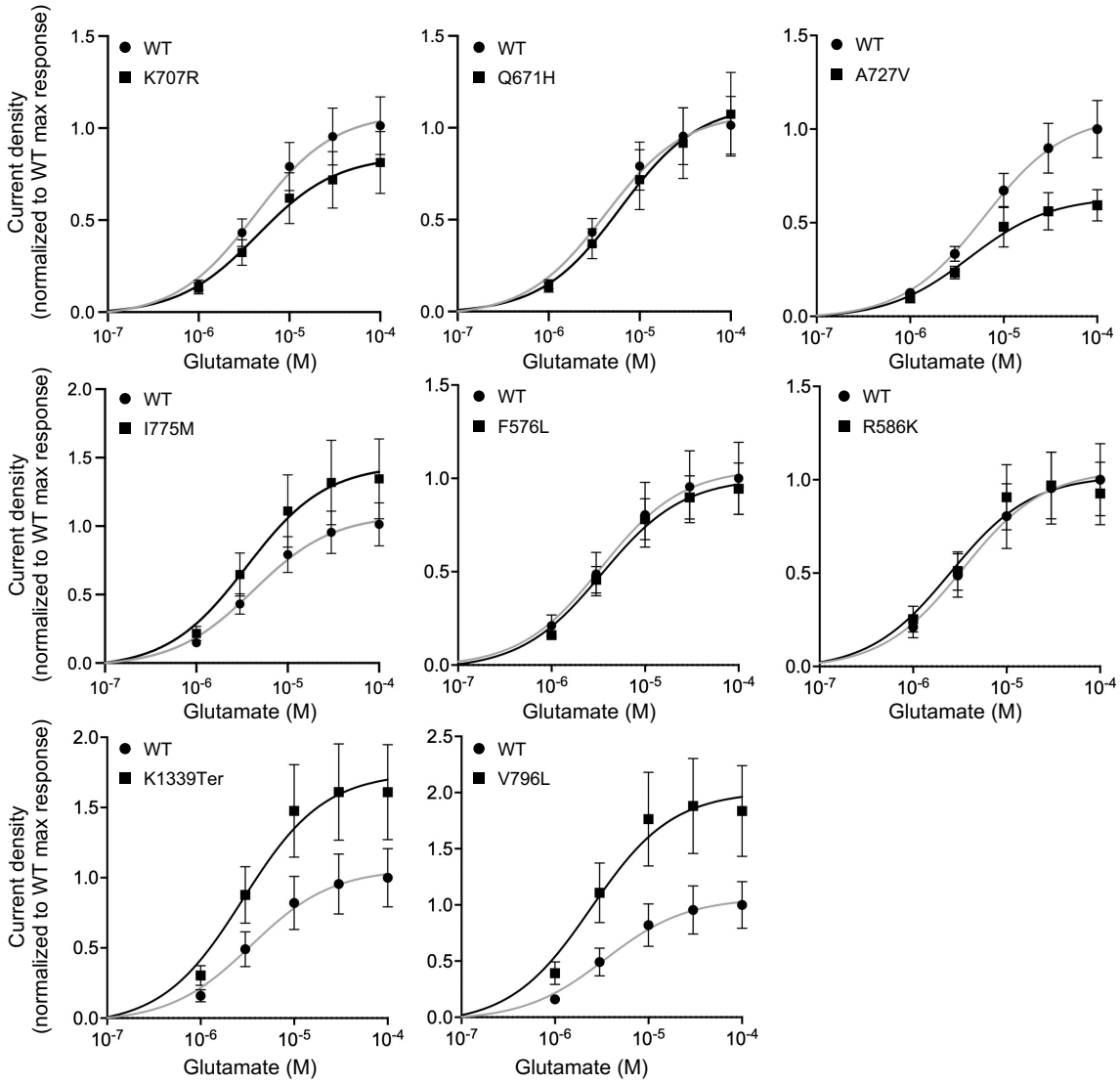
Current density
(normalized to WT max response)
WT
K707R
Q671H
A727V
I775M
F576L
R586K
K1339Ter
V796L
Glutamate (M)

Figure S4. Effect of non-pathogenic *GRIN2A* variants on NMDAR function. Averaged current density in response to increasing concentrations of glutamate in the constant presence of 30 μM glycine, normalized to maximal response, for NMDARs containing the wild-type or non-pathogenic GluN2A variants. The lines indicate a nonlinear regression three-parameter fit to each dataset. Data are displayed as mean ± SEM, n = 17-70; see Table 1 for number of cells recorded per variant.

Figure S5

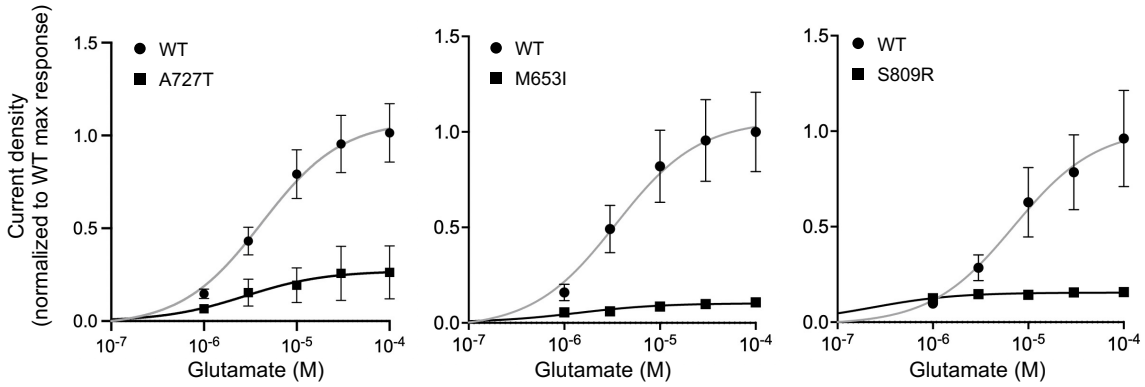
Current density
(normalized to WT max response)
WT
A727T
M653I
S809R
1.5
1.0
0.5
0.0
10^{-7}
10^{-6}
10^{-5}
10^{-4}
Glutamate (M)

Figure S5. Effect of Epilepsy and DD/ID-associated *GRIN2A* variants on NMDAR function. Averaged current density in response to increasing concentrations of glutamate in the constant presence of 30 μM glycine, normalized to maximal response, for NMDARs containing the wild-type or Epilepsy and DD/ID-linked GluN2A variants. The lines indicate a nonlinear regression three-parameter fit to each dataset. Data are displayed as mean ± SEM, n = 10-77; see Table 1 for number of cells recorded per variant.

Figure S6

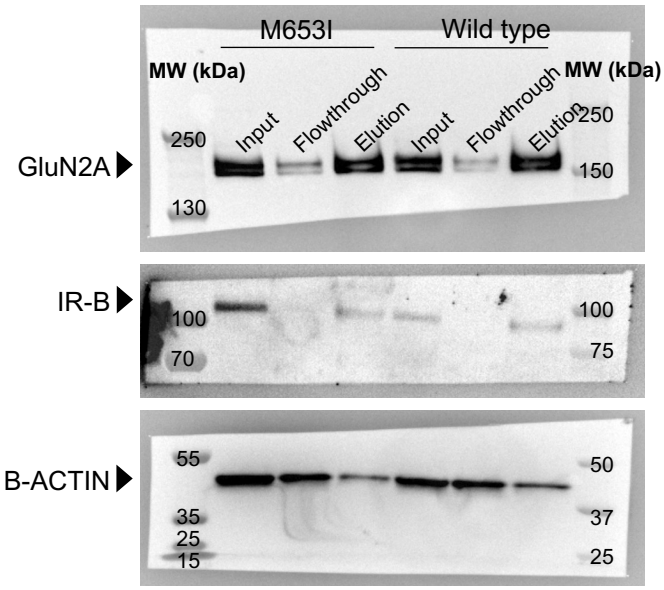
M653I
Wild type
MW (kDa)
Input
Flowthrough
Elution
Input
Flowthrough
Elution
MW (kDa)
250
130
250
150
GluN2A
100
70
IR-B
100
75
55
35
25
15
B-ACTIN
50
37
25

Figure S6. Original western blot images of the surface biotinylation experiment. Western blot probing for GluN2A, insulin receptor beta, and β-ACTIN in the input, flowthrough, and elution samples of surface biotinylation experiment done on HEK cells transiently transfected with *GRIN1-GRIN2A* constructs to express wild-type or M653I mutant NMDARs. Input, flowthrough, and elution represent total, internal, and surface expression respectively. IR-B: insulin receptor beta.