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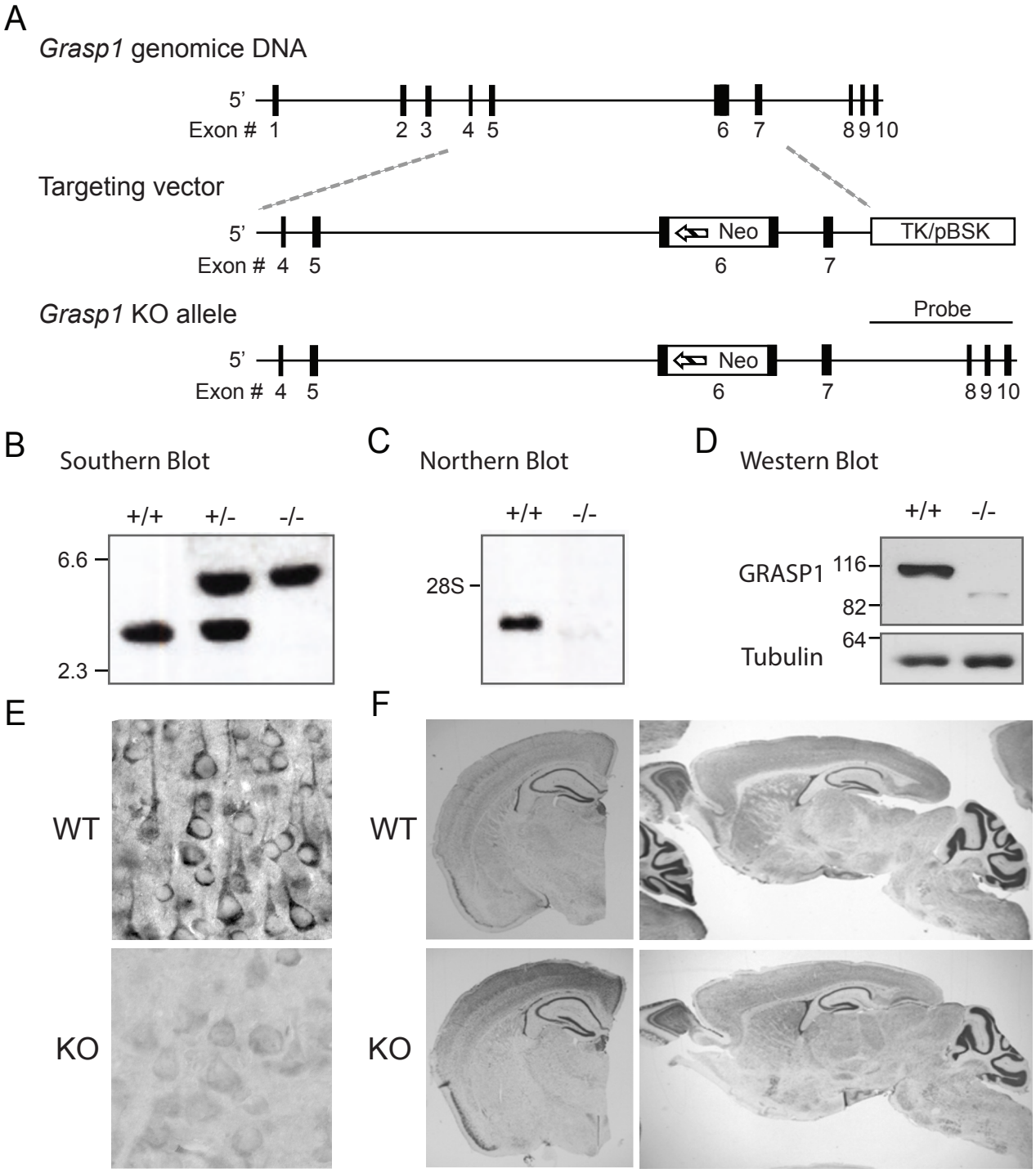

Figure S1 (related to Figures 1-3) Generation and evaluation of *Grasp1* KO mice
(A) Strategy of *Grasp1* KO mice generation in which exon 6 is disrupted with a neomycin-resistant gene cassette creating an early termination site.
(B) Southern blot analysis of the genomic DNA with the probe shown in (A) showed successful recombinant targeting.
(C) Northern blot analysis showed an absence of GRASP1 RNA products in homozygous KO mice.
(D) Western Blot analysis of the brain homogenates showed a completely loss of intact GRASP1 protein in homozygous KO mice. A light intensity band migrating around 85KD was observed in the KO mice suggesting a trace amount of truncated GRASP1 protein is expressed at a low level in the KO mice.
(E) Immunohistochemistry of the brain slices showed significant loss of GRASP1 protein in neurons.
(F) Nissl staining on 5-week old *Grasp1* mice showed comparable gross brain morphology.

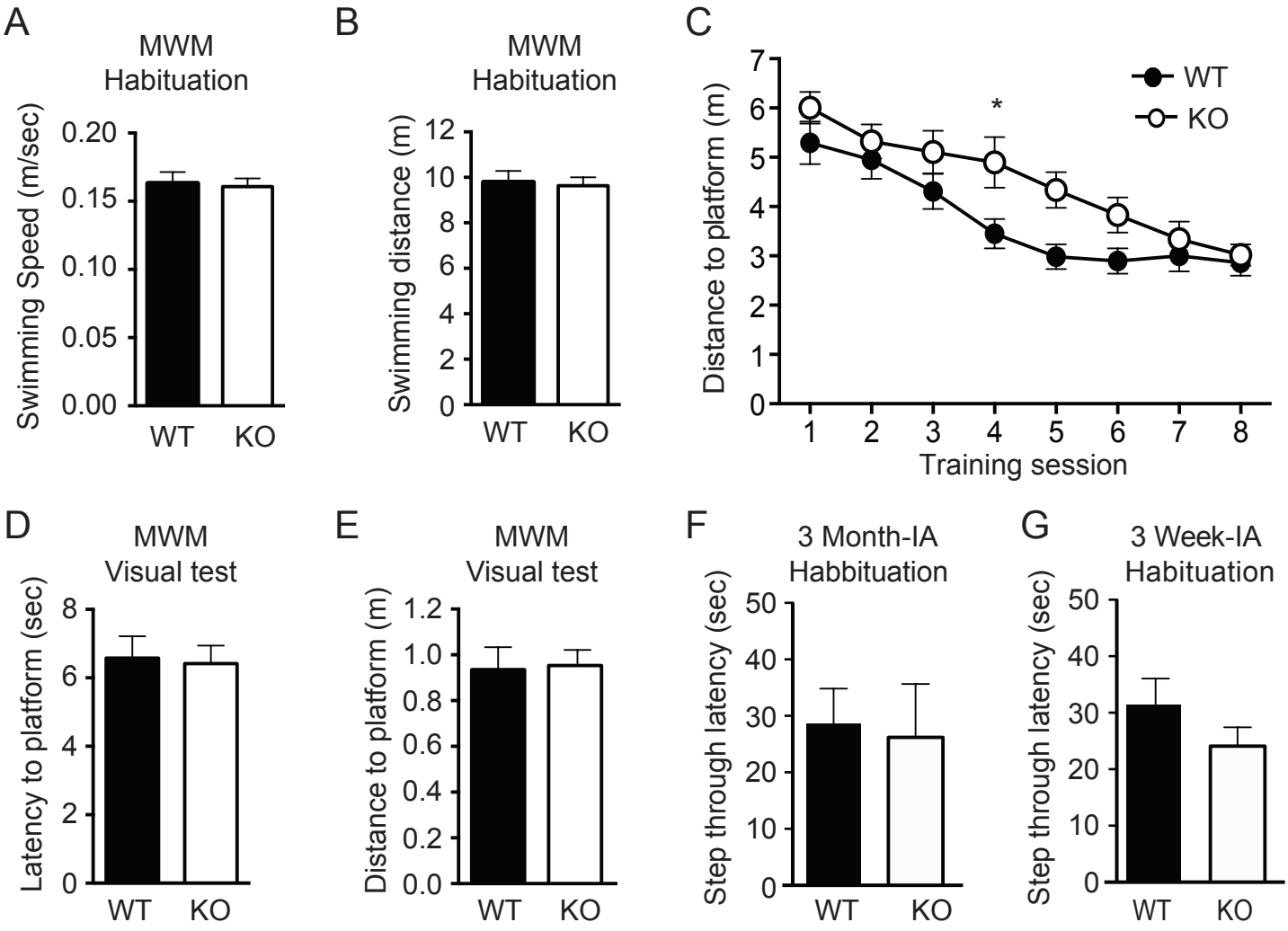

Figure S2 (related to Figure 3) Basal behavioral properties and swim distance to platform during MWM training

(A) *Grasp1* KO mice have comparable swim speed to their WT littermates during MWM habituation (WT= 0.16 ± 0.01 and KO= 0.16 ± 0.01 meter/sec, n= 20 mice/genotype; p= 0.83; unpaired t-test).

(B) *Grasp1* KO mice swam similar distance as their WT littermates during MWM habituation (WT= 9.79 ± 0.50 and KO= 9.64 ± 0.37 meter; p= 0.82; unpaired t-test).

(C) Swim distances to hidden platform during MWM training (session 4: WT= 3.45 ± 0.30 and KO= 4.90 ± 0.52 meter, p= 0.03; two-way ANOVA; see Table S1 for values of other sessions).

(D) *Grasp1* WT and KO mice have comparable latency to find the visible platform (WT= 6.58 ± 0.64 and KO= 6.42 ± 0.53, n= 10 mice/genotype; p= 0.85, unpaired t-test).

(E) *Grasp1* WT and KO mice have comparable swim distance to the visible platform (WT= 0.94 ± 0.10 and KO= 0.95 ± 0.07 meter; p= 0.30; unpaired t-test).

(F) Adult *Grasp1* WT and KO mice have comparable step-through latency (WT= 28.65 ± 6.18 and KO= 26.19 ± 9.46 meter; p= 0.82; n=10-13 mice/genotype, unpaired t-test).

(G) Juvenile *Grasp1* WT and KO mice have comparable step-through latency (WT= 31.46 ± 4.61 and KO= 24.09 ± 3.32 meter; p= 0.20; n=16 mice/genotype, unpaired t-test).

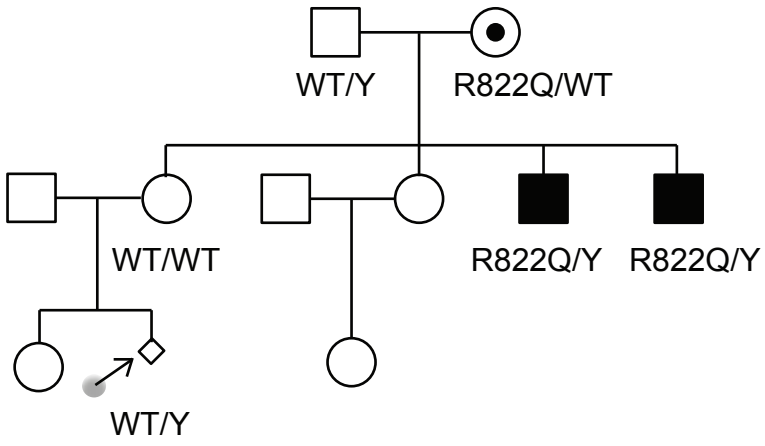

Figure S3 (related to Figure 5) Genetic pedigrees of XL-ID patients carrying R822Q mutation
Two affected brothers were found to be the carriers inherited from their un-affected mother. Genotypes are included wherever is available. A diamond symbol indicates miscarriage. In addition to severe nonsyndromic ID, shared phenotypes of the affected brothers include normal head circumference (60-70th%), short stature (height <3rd%), and increased deep tendon reflexes of lower extremities.

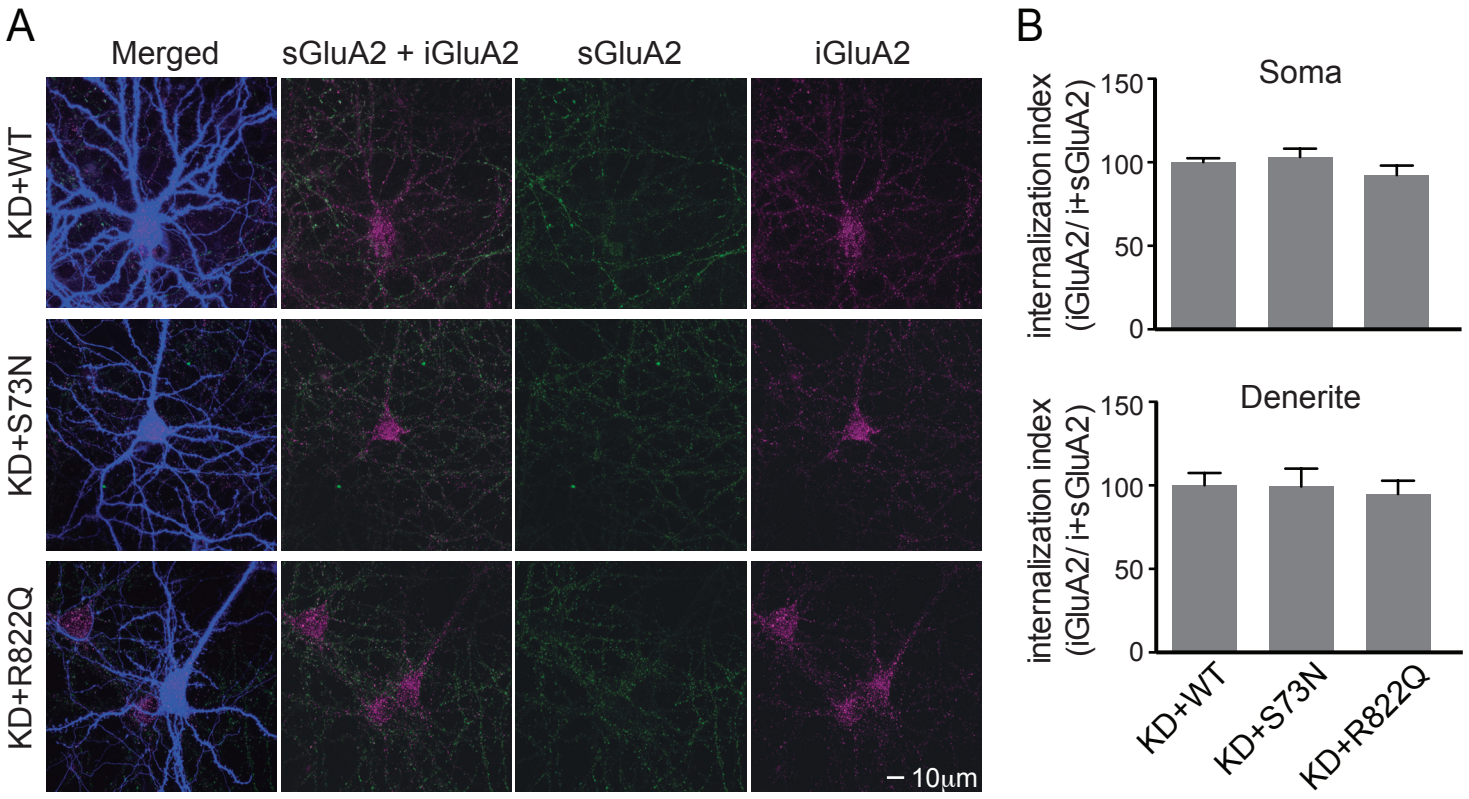

Figure S4 (related to Figures 5&6) GRASP1 ID mutants do not affect AMPAR internalization.
(A) Representative images of hippocampal neurons stained for surface GluA2 (sGluA2) and internalized GluA2 (iGluA2) at the peak endocytosis. Transfected hippocampal neurons were live labeled with GluA2 antibodies, AMPA stimulated and stained for sGluA2 (green) and iGluA2 (magenta) at 8 minutes following the AMPA treatment.
(B) Quantification of the internalized AMPARs measured as the ratio of internalized/ total labeled (surface and internalized) GluA2 in (A). (% KD+WT; soma: WT= 100.0 ± 2.4%, S73N= 103.2 ± 5.0%, R822Q= 92.4 ± 5.7%; dendrite: WT= 100.0 ± 7.5%, S73N= 99.4 ± 10.8%, R822Q= 94.9 ± 8.0%; p> 0.05; n= 21 neurons/group, one-way ANOVA).

Table S1 (related to Figure 3A)
Swim latency and distance to the hidden platform during MWM training

Training	Latency (second)			Distance (meter)		
Sessions	WT	KO	P-value	WT	KO	P-value
1	39.65 ± 2.72	40.54 ± 2.30	> 0.999	5.30 ± 0.44	6.00 ± 0.32	> 0.999
2	30.82 ± 2.39	33.43 ± 2.35	> 0.999	4.95 ± 0.38	5.32 ± 0.35	> 0.999
3	25.57 ± 1.94	32.27 ± 2.78	> 0.241	4.31 ± 0.36	5.11 ± 0.43	0.861
4	18.73 ± 1.58	29.75 ± 3.36	0.003**	3.45 ± 0.30	4.90 ± 0.52	0.030*
5	16.02 ± 1.09	26.10 ± 2.22	0.009**	2.97 ± 0.25	4.33 ± 0.36	0.055
6	15.87 ± 1.35	22.60 ± 2.89	> 0.237	2.90 ± 0.26	3.83 ± 0.36	0.474
7	15.94 ± 1.59	19.20 ± 2.17	> 0.999	3.00 ± 0.32	3.34 ± 0.36	> 0.999
8	15.13 ± 1.24	17.50 ± 1.20	> 0.999	2.86 ± 0.26	3.01 ± 0.22	> 0.999

Two-way ANOVA followed by Bonferroni post-hoc test

Table S2 (related to Figure 3B)
Percentage of time spent in each quadrant in probe trials

	WT		KO	
Quadrant	% time	P-value	% time	P-value
2 (target)	33.58 ± 2.02		25.79 ± 1.77	
1	25.73 ± 1.73	0.015 *	26.23 ± 1.35	> 0.999
3	21.46 ± 1.36	< 0.001***	24.66 ± 1.87	> 0.999
4	19.25 ± 1.45	< 0.001***	23.33 ± 1.75	> 0.999

Two-way ANOVA followed by Bonferroni post-hoc test

Table S3 (related to Figure 5)
PCR amplicons and primer sequences used for human GRASP1 mutation analysis

GRASP1 (NM020137)	Forward Primers	Reverse Primers	Amplicon Size (bp)
Exon 1	5'-GGGAACAAGACCAGAGCGAAGC-3'	5'-CCTTCTCAGAGCTGATAGCACGACC-3'	524
Exons 2-3	5'-GAGTAGCTACAGAGGCACATGGAGG-3'	5'-CATTAGGTCTGGTTCACTGTAGGTGC-3'	492
Exons 4-5	5'-TGTCATCCTTGTCCTCCCAAAACG-3'	5'-GGCCAGACCCTGTCCAATCACTC-3'	1134
Exon 6	5'-CTCTTGGTATTCTCTGACTCTGCCAC-3'	5'-ACCCTATTTCTGAAGCCCTTCTCC-3'	478
Exons 7-8	5'-GGCCAGTTCTCCCTCTATTCGAG-3'	5'-CCTAGGATTGGGAATGGAATGG-3'	583
Exons 9-11	5'-GTCAGGGCTATTTTGAGGATTCCAC-3'	5'-CTCCATGGACCTCAGACCTCTCC-3'	619
Exons 12-13	5'-GCTGCTGGGTGGTCAAACAGAG-3'	5'-CCCTGATGTGGACCAGAAGCAG-3'	579
Exon 14	5'-CTCCCTGTTTCCCTGGAACTAGG-3'	5'-CCTGACTCCAGTCTTCCCACTCC-3'	322
Exons 15-17	5'-CAGGGAGAAGGGAGATGGTCTACC-3'	5'-CAGAAAATCTCGGGAAGTTCCAGG-3'	1090
Exons 18-20	5'-TGTGACACTTCAGCATATTGCCTAAGG-3'	5'-CAGAAGCAGGAAAGCACAGCTGAG-3'	923
Exon 21	5'-CATGCCCAGGTGTCTCTCTTTCC-3'	5'-CATGTCGCAATCTGAATCCAAGTTG-3'	417
Exons 22-23	5'-ACCCACCTCTGGCCTGGTTCTC-3'	5'-AGTAGCTTCGCTGAGTTCCGAGAGG-3'	556
Exon 24	5'-GGGCCATAGAGCTAGTGAGTGACAG-3'	5'-GGGTCAATGGAAGGAATGAATGG-3'	543
Exon 25	5'-AGATGATACCCTACGTTTGGCAAGG-3'	5'-CCAGGACCAAGAAGAAGGAGAAATC-3'	401