Supplemental Figure S1

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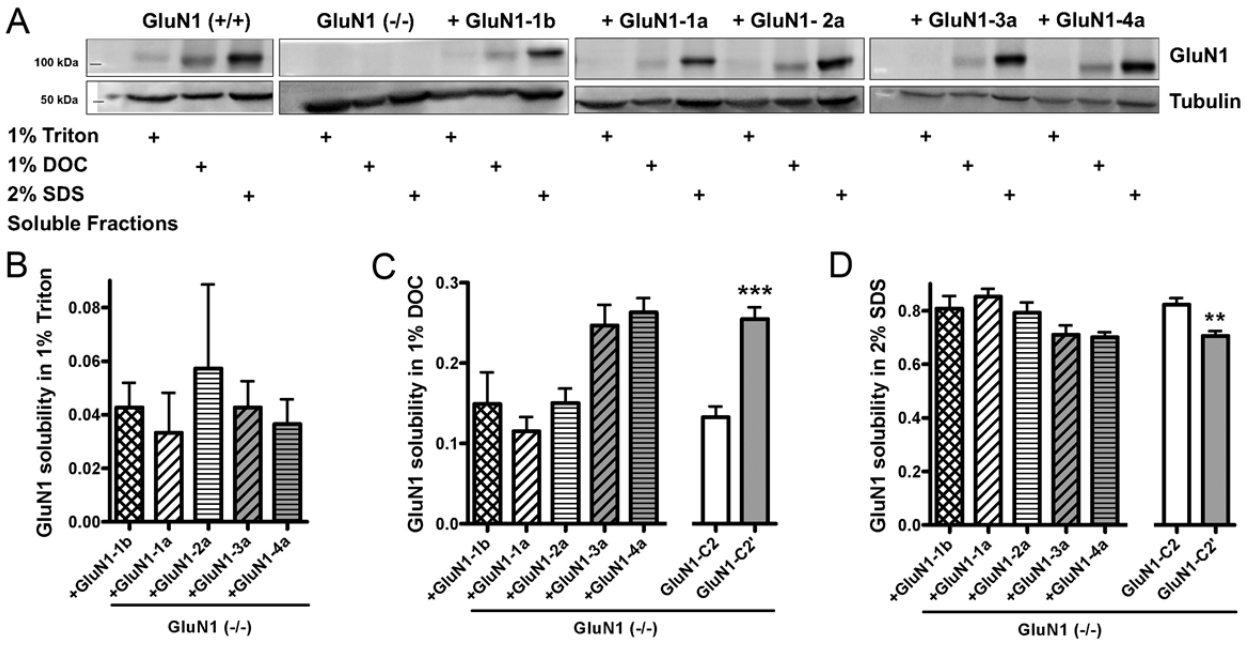
Activity and Protein Kinase C Regulate Synaptic Accumulation of NMDA Receptors Independently of GluN1 Splice Variant



Supplemental Fig. S1. The GluN1 C2 and C2' cassettes regulate detergent solubility of GluN1 splice variants.
(A) High density cortical cultures from GluN1 (-/-) mice were transduced at 3 DIV with lentivirus expressing GluN1 splice variants GluN1-1b, GluN1-1a, GluN1-2a, GluN1-3a or GluN1-4a. Proteins were sequentially extracted in 1% Triton, 1% DOC and 2% SDS. GluN1 protein levels were assessed by Western blot using an antibody against the GluN1 N-terminal region. Tubulin was used as a loading control. (B-D) The amount of GluN1 solubilized in 1% Triton X-100 (B), 1% DOC (C) and 2% SDS (D) was quantified and normalized to the total expression of GluN1, corresponding to the sum of all soluble fractions. Data are presented as mean ± SEM of four independent experiments, and analysed by ANOVA (B, $p>0.1$; C, $p<0.005$; D, $p<0.05$). To determine the role of specific splice cassettes, data were then analyzed by grouping GluN1 variants according to cassette usage. Only groups showing a significant difference are graphed, specifically splice variants containing the C2' cassette (GluN1-3a + GluN1-4a) show higher solubility in DOC (*** $p<0.001$, t-test), and lower solubility in SDS (** $p<0.01$, t-test), than those containing the C2 cassette (GluN1-1a + GluN1-2a). Tubulin levels in the different detergent soluble fractions show no significant differences between splice variants (data not shown).