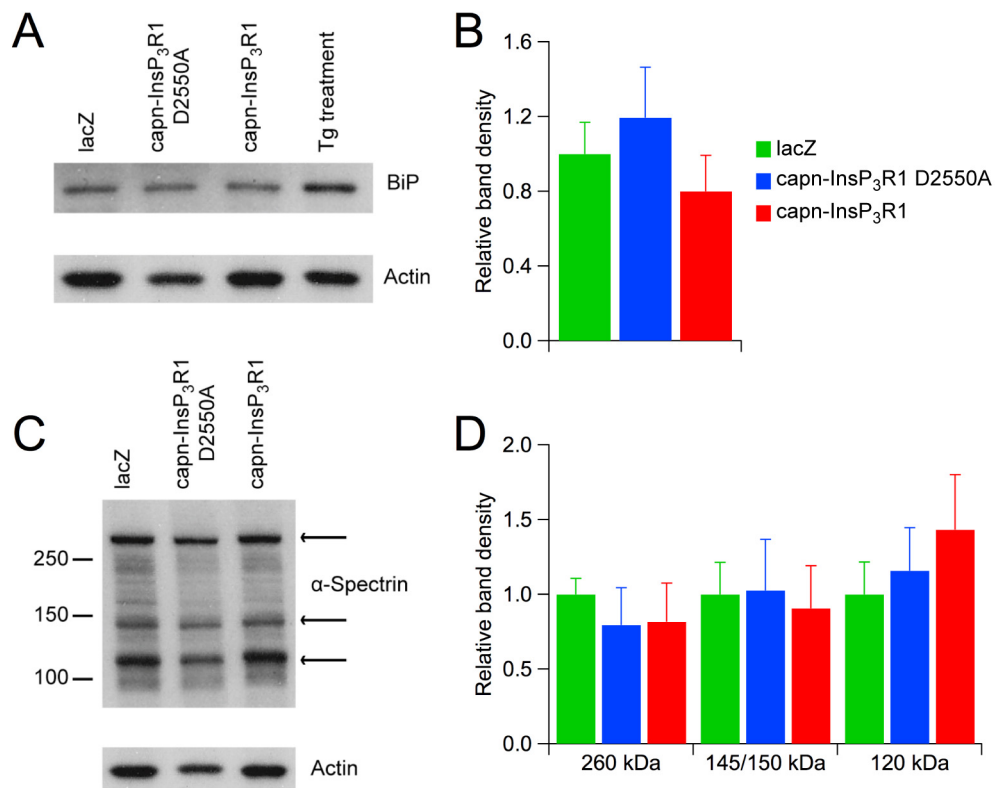
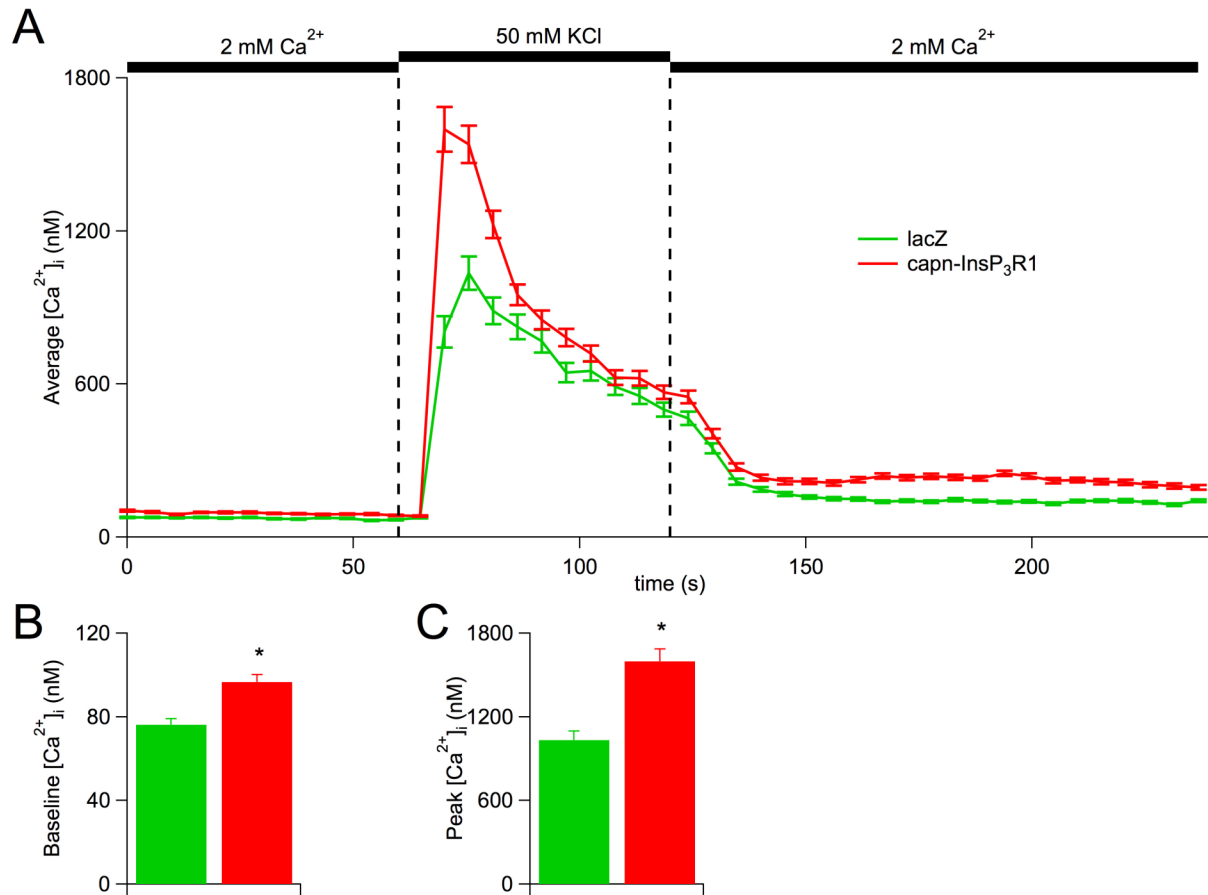


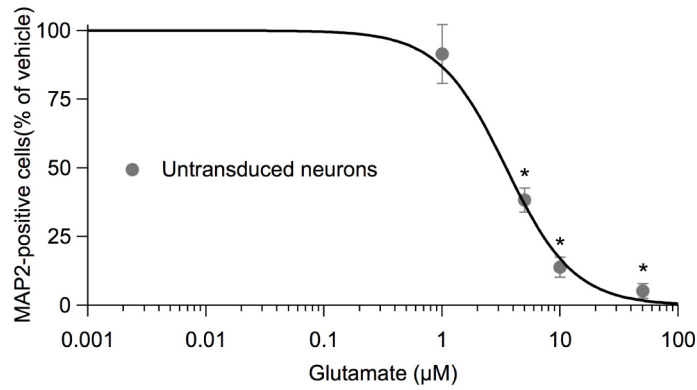
## SUPPLEMENTAL DATA



**Supplemental Figure 1      Activation of ER stress markers and proteases in transduced primary cortical neurons.** Western blot analysis of whole cell lysates from primary cortical cultures transduced with AAV 2/1 expressing lacZ, capn-InsP<sub>3</sub>R1 D2550A, or capn-InsP<sub>3</sub>R1 at 1 week post-transduction (14 DIV). (A) Antibody against BiP was used to detect activation of ER stress response. Untransduced cultures were treated with thapsigargin (*Tg*; 1 μM) as a positive control for ER stress and increased BiP. Antibody against actin was used as a loading control. (B) BiP band densities were quantified and normalized to actin. Changes in protein expression are expressed relative to lacZ controls. (C) Antibody against α-spectrin was used to detect calpain- and caspase-mediated proteolysis. Calpain-mediated cleavage of α-spectrin (260 kDa) generates 150 and 145 kDa polypeptides while caspase-mediated cleavage generates 150 and 120 kDa polypeptides. (D) Full-length (260 kDa) and proteolyzed (145/150 kDa and 120 kDa) α-spectrin band densities were quantified and normalized to actin. Changes in intact α-spectrin and α-spectrin fragment densities are expressed relative to lacZ controls. There are no statistically significant differences between BiP or α-spectrin band densities in lacZ-, capn-InsP<sub>3</sub>R1 D2550- and capn-InsP<sub>3</sub>R1-transduced cultures.



**Supplemental Figure 2**      **Neurons expressing capn-InsP<sub>3</sub>R1 have increased rises in [Ca<sup>2+</sup>]<sub>i</sub> in response to depolarization.** (A) Averaged single-cell [Ca<sup>2+</sup>]<sub>i</sub> responses to depolarization with 50 mM KCl in Fura-2 loaded primary cortical neurons (14 DIV) transduced with lacZ or capn-InsP<sub>3</sub>R1. Total number of single-cell Ca<sup>2+</sup> responses analyzed in these experiments was 94 and 95 for lacZ and capn-InsP<sub>3</sub>R1 transduced cultures, respectively. (B) Summary of average resting [Ca<sup>2+</sup>]<sub>i</sub> in neurons from cultures used for KCl Ca<sup>2+</sup> imaging experiments. Consistent with data presented in Figures 3B and 4B, cultures expressing capn-InsP<sub>3</sub>R1 demonstrate increased baseline [Ca<sup>2+</sup>]<sub>i</sub> (capn-InsP<sub>3</sub>R1=96.7 ± 3.5 nM, lacZ=76.2 ± 2.9 nM; unpaired *t*-tests with unequal variance; \*, *p*<0.001). (C) Summary of average peak [Ca<sup>2+</sup>]<sub>i</sub> responses elicited by KCl shows an increased maximum [Ca<sup>2+</sup>]<sub>i</sub> achieved in capn-InsP<sub>3</sub>R1 expressing cells (capn-InsP<sub>3</sub>R1=1540 ± 73 nM, lacZ=1035 ± 65 nM; unpaired *t*-tests with unequal variance; \*, *p*<0.001).



**Supplemental Figure 3 Characterization of glutamate injury model.** Untransduced rat primary cortical cultures (14 DIV) were exposed to HBS vehicle or 1, 5, 10 or 50 µM glutamate. Twenty-four hours later, cultures were stained for MAP2 and percent neuronal survival quantified relative to vehicle treated controls. The percentage of surviving cells was significantly reduced in cultures treated with glutamate concentrations of 5 µM and higher (1 µM=91.5 ± 10.6 %, 5 µM=38.3 ± 4.4 %, 10µM=13.9 ± 3.7 %, 50µM=4.3 ± 2.6 %; one-way analysis of variance; \*,  $p < 0.01$ ). LD<sub>50</sub> for glutamate in these cultures is 3.49 ± 0.48 µM.