

Supplementary Information

Furin inhibitor protects against neuronal cell death induced by activated NMDA receptors

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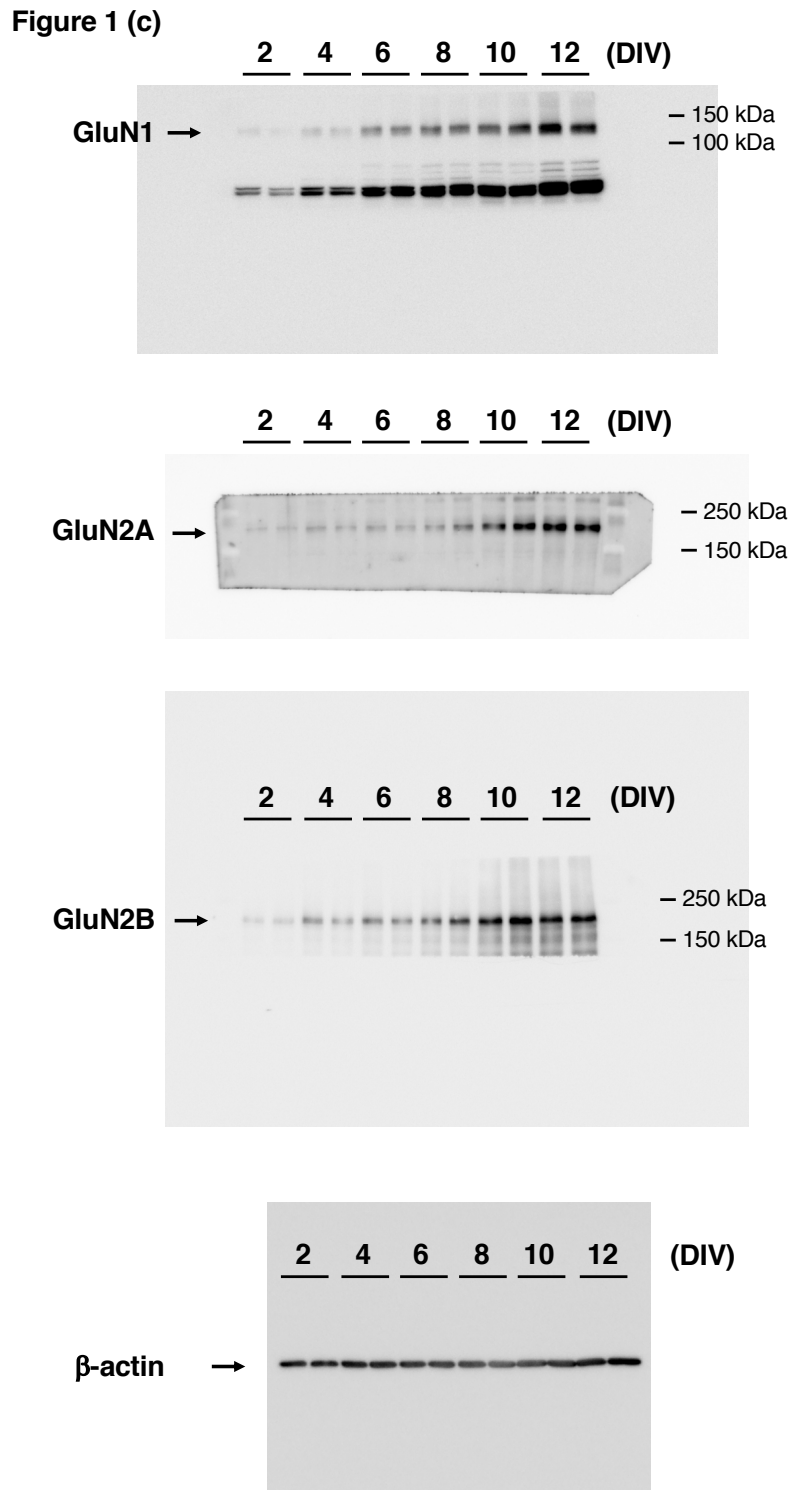
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Supplemental materials and methods

The direct effect of calpeptin and furin inhibitor on calpain activity.

To examine direct effect of furin inhibitor on calpain activity *in vitro*, calpain-1 (Cat. No. 208713) were procured from Millipore (Billerica, MA, USA). The effects of calpeptin (calpain; $K_i = 7$ nM) and furin inhibitor 1 (FI-1 : furin; $K_i = 1$ nM) on activity of recombinant calpain were measured by performing the Calpain-Glo™ protease assay according to the manufacturer's instructions. Briefly, a volume of Calpain-Glo reagent equal to that of the indicated concentration of calpeptin or furin inhibitor in recombinant calpain (10 nM) was added. Then, they were incubated for 10 min at 37 °C. Luminescence was measured with a plate-reading luminometer, ARVO X2 (PerkinElmer, Waltham, MA, USA). The doses (10, 30, and 100 nM) used in this experiment were based on the K_i values for calpeptin and furin inhibitor 1 (FI-1).



Supplementary Figure S1. Full-length blots corresponding to crop blots presented in the main manuscript

Full-length blots for Figure 1C (GluN1, GluN2A, GluN2B and actin). Area displayed in the main manuscript delineated by red rectangle.

Figure 5 (a)

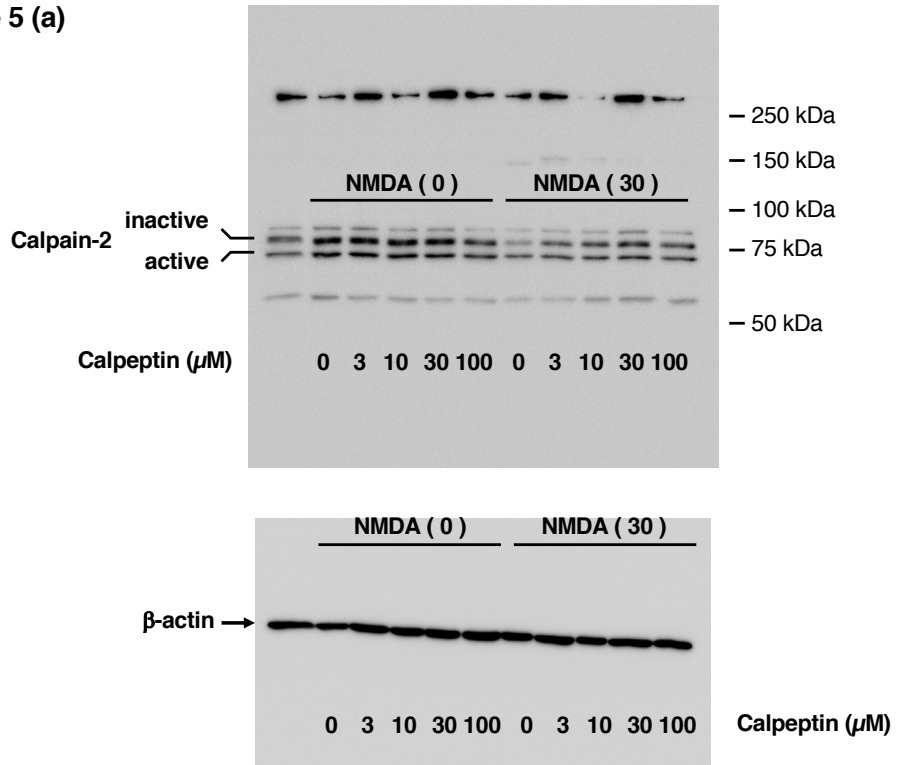
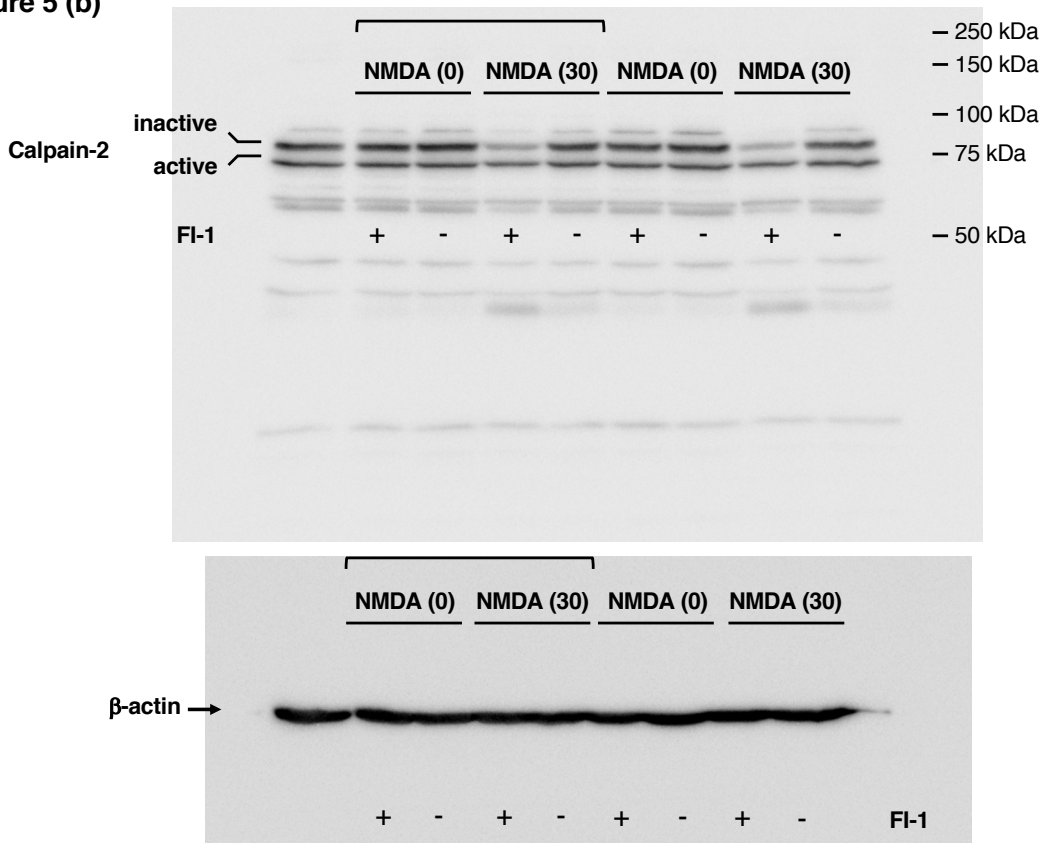
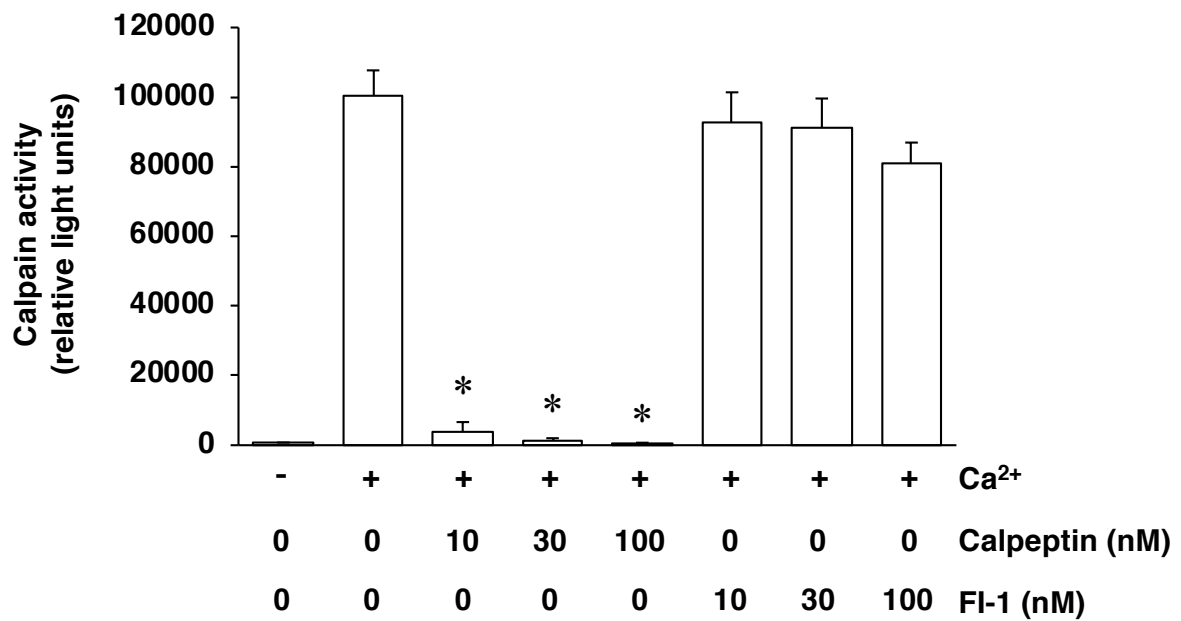


Figure 5 (b)



Supplementary Figure S2. Full-length blots corresponding to crop blots presented in the main manuscript.

Full-length blots for Figure 5A and 5B. Area displayed in the main manuscript delineated by red rectangle.



Supplementary Figure S3. The direct effect of calpeptin and FI-1 on the calpain activity. Direct effect of calpeptin and furin inhibitor 1 (FI1) on calpain activity in the presence of Ca²⁺. The activity of recombinant calpain treated with 0, 10, 30 and 100 nM calpeptin or furin inhibitor 1 is shown. Calpain activity was measured by performing the Calpain-Glo™ protease assay and expressed as the percentage of the inhibitor-untreated control group. Results are the means ± SE (n=3 independent experiments). * indicates a significant difference from the inhibitor-untreated group (p < 0.05).