Journal: Molecular Neurobiology

NMDA receptors regulate Neuregulin 2 binding to ER-PM junctions and ectodomain release

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Supplementary Material

Supplementary Figures 1-3

Captions for Online Resources 1

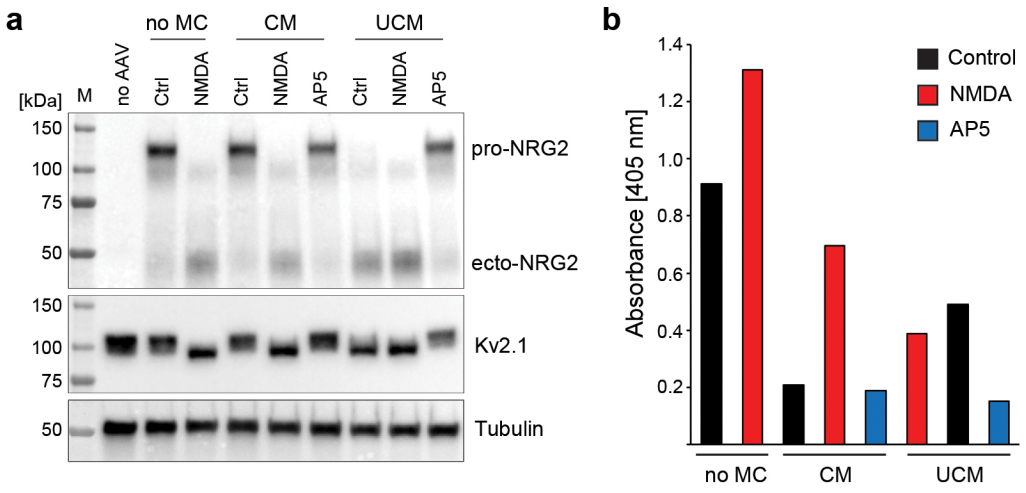

Fig. S1 **Effect of medium change on pro-NRG2 processing.** AAV-transduced hippocampal neurons (DIV 31) were subjected to medium change (2 brief washes) using conditioned supernatants collected from untransduced hippocampal neurons (*CM*) or unconditioned, freshly prepared Neurobasal/B27 medium (*UCM*), returned to the incubator for 10 min and then processed for Western blotting of whole-cell lysates and ELISA measurements of ecto-NRG2 in the corresponding culture supernatants. (**a**) Western blot analysis shows that pro-NRG2 levels after medium change using conditioned medium (CM/Ctrl; lane 5) were similar to untreated controls (no MC/Ctrl; lane 3), whereas unconditioned medium (UCM/Ctrl; lane 8) triggered pro-NRG2 processing to a similar extent than treatment with 50 µM NMDA (lanes 4,6,9). Importantly, the effects of unconditioned medium on pro-NRG2 processing were fully blocked by the NMDAR inhibitor AP5 (100 µM; lane 10). A sample from untransduced neurons is also shown (lane 2) to demonstrate specificity of the detected bands. The Western blot was additionally probed for Kv2.1 to show that medium change similarly affected electrophoretic mobility of the Kv2.1 protein. Tubulin was included as a loading control. (**b**) ELISA measurements of ecto-NRG2 collected from the same experiment. Note the reciprocal relationship between pro-NRG2 signal levels in the Western blotting data shown in (a) and the corresponding ecto-NRG2 signals in (b). Elevated ecto-NRG2 levels in control and NMDA-treated samples that were not subject to medium change reflect the accumulation of ecto-NRG2 prior to treatments. Data from this experiment are representative of two independent experiments performed.

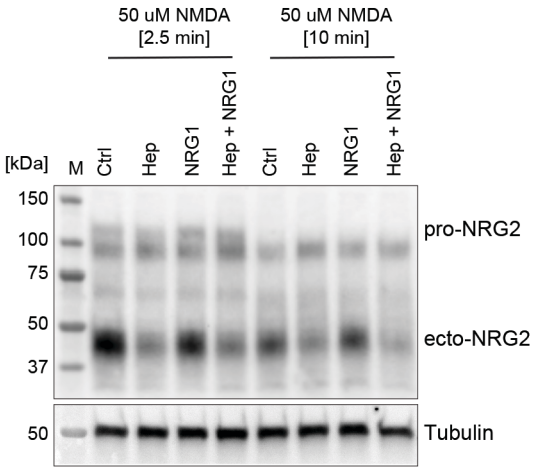

Fig. S2 **Heparinase treatment reduces ecto-NRG2 levels in whole-cell lysates.** AAV-transduced hippocampal neurons were pre-incubated for 3 h at 37°C with a cocktail of Heparinase I-III (0.5 U/ml each) to digest heparan sulfate proteoglycans in the ECM (*Hep*) or with 60 nM recombinant human NRG1 (EGF-like domain) to block ErbB receptors (*NRG1*), or both. Neurons were then treated with 50 µM NMDA for 2.5 or 10 min and processed for Western blotting. The results show how Heparinase treatment dramatically reduces ecto-NRG2 levels in the cell lysates at 2.5 min compared to controls that were not pre-treated (*Ctrl*), while ErbB receptor blockade has little effect. Furthermore, ecto-NRG2 levels associated with the cellular fraction in control samples were noticeably lower after 10 min, consistent data shown in **Fig. 1**, indicating the transient nature of the observed interactions with the ECM. Tubulin was included as loading control.

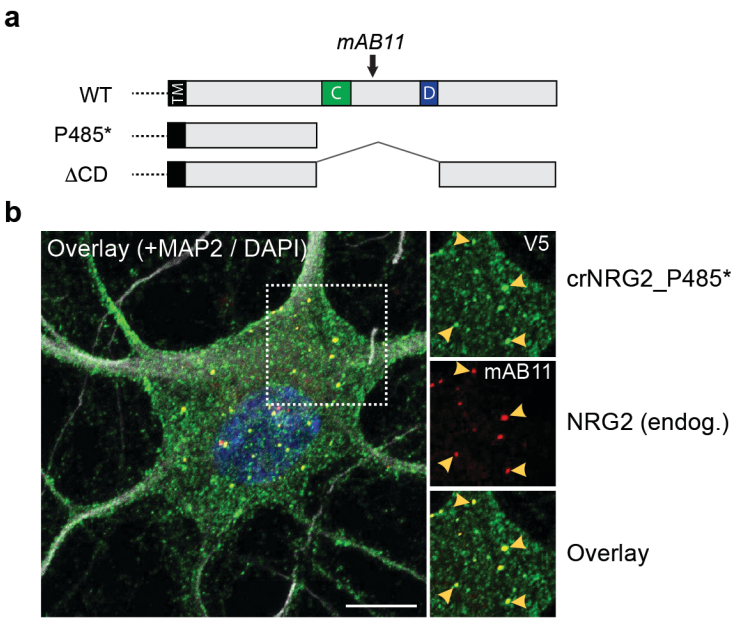

Fig. S3 Limited colocalization of mis-targeted crNRG2_P485* protein with endogenous NRG2. (**a**) Rabbit monoclonal NRG2 antibody mAB11 binds to an epitope located in the ICD that is deleted in crNRG2_P485* and NRG2_ΔCD (see also (Vullhorst et al., 2015)). (**b**) Representative Z-projected confocal image of a transduced hippocampal neuron, surface-labeled for crNRG2_P485* using anti-V5 and for endogenous NRG2 using mAB11 following permeabilization. MAP-2 and DAPI are included in the overlay image. The area outlined by the bounding box is magnified on the right and shows colocalization of crNRG2_P485* and endogenous NRG2 puncta (arrowheads). Scale bar = 10 µm.

Online Resource 1 **NRG2_ΔCD protein is expressed on the plasma membrane but also accumulates intracellularly.** The movie shows an animated Z-stack of a representative hippocampal neuron transduced with an AAV expressing NRG2_ΔCD and incubated with anti-V5 under non-permeabilizing conditions to label surface protein, and then with anti-NRG2 antibody 8D11 [28] following permeabilization with 0.1% TX-100. Channels for pre- and post-permeabilization images are shown separately. Image stack was acquired using an Zeiss LSM 880 Airyscan confocal microscope.

Online Resource 2 **Distribution of endogenous NRG2 puncta.** The movie shows an animated Z-stack of a representative untransduced hippocampal neuron incubated with anti-NRG2 antibody 8D11 under permeabilizing conditions. Image stack was acquired as described for Online Resource 1.