

Fig. S1. A) qRT-PCR confirmation that Mcub-KO neurons do not express Mcub. **B)** Western blot to confirm that Mcub neurons do not have altered levels of Mcu. **C,D)** WT and Mcub-KO neurons (cultured from littermates) were transfected with mt-GCaMP2 and images taken before and after treatment with NMDA (20 μ M). Mitochondrial Ca²⁺ levels were calculated as a multiple of the Kd: (F-F_{min})/(F_{max}-F). Maximum (C) and average (D) Ca²⁺ levels were calculated before and after NMDA application; n=10 imaging experiments were performed for each genotype, within which 71 WT cells and 77 KO cells were measured. **E, F)** Example traces from individual experiments whose data contributed to 5C and 5D. In the WT example (E), the mean \pm s.e.m. of 10 cells within that single experiment is shown. In the KO example (F), the mean \pm s.e.m. of 6 cells within that single experiment is shown.