Expanded View Figures

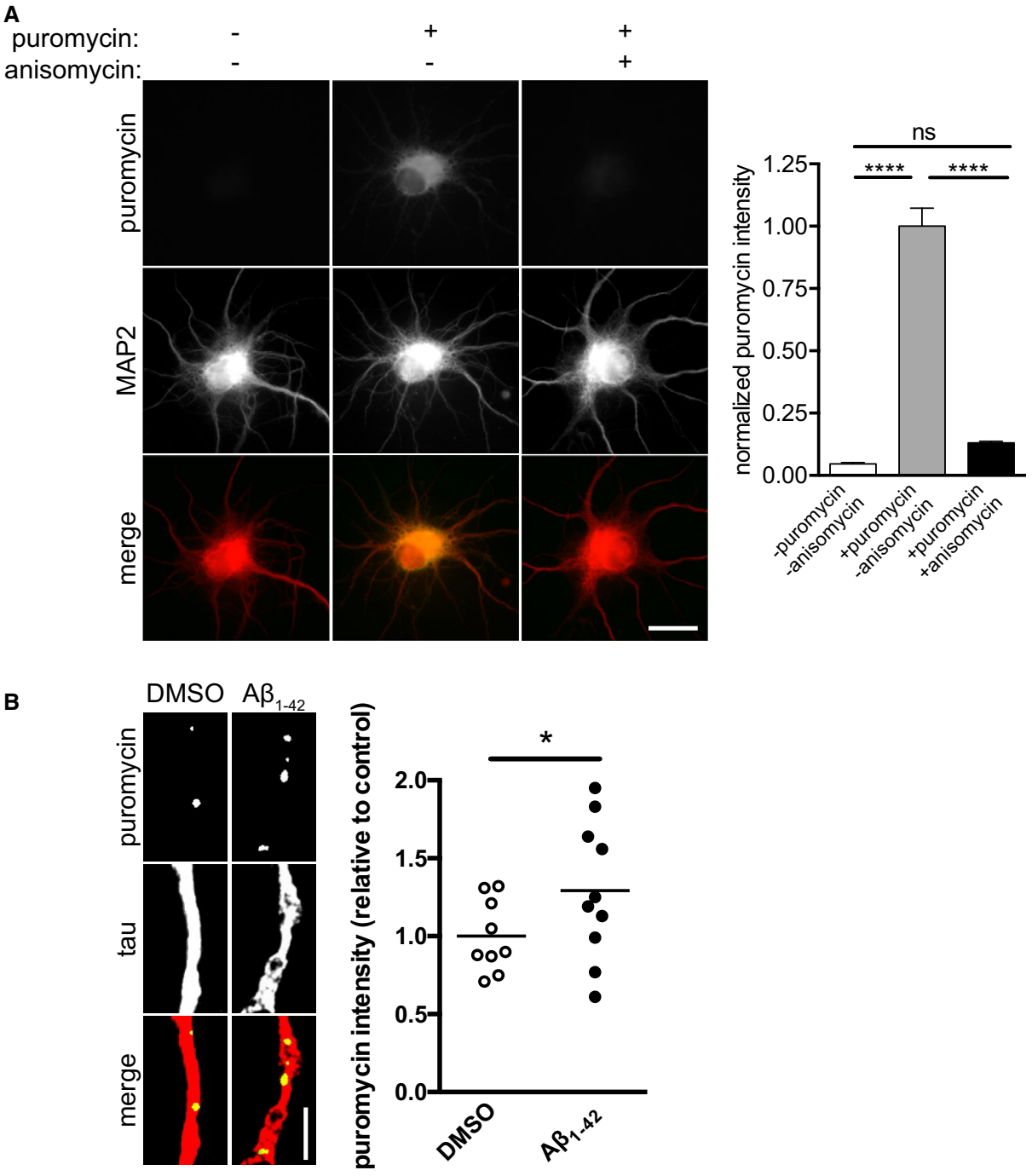

Figure EV1. Validation of puromycylation assay.

A Hippocampal neurons were cultured for 10–11 DIV. Puromycin was added to dissociated hippocampal neurons in the presence of vehicle or anisomycin. The neurons were fixed and immunostained for puromycin and MAP2. Mean ± SEM of 25–30 optical fields per condition (n = 3 independently performed experiments per group). ****$P < 0.0001$; ns, not significant; one-way ANOVA with Bonferroni's multiple comparisons test. Scale bar, 10 μm.

B Hippocampal neurons were cultured for 10–11 DIV. $A\beta_{1-42}$ was added to the cultured for 30 min, and puromycin was added 10 min prior to fixation. Neurons were fixed and immunostained for puromycin and tau. Mean of 9–10 optical fields per condition (sampled from two coverslips per condition) from one primary culture. *$P < 0.05$; unpaired, one-tailed t-test. Scale bar, 5 μm.

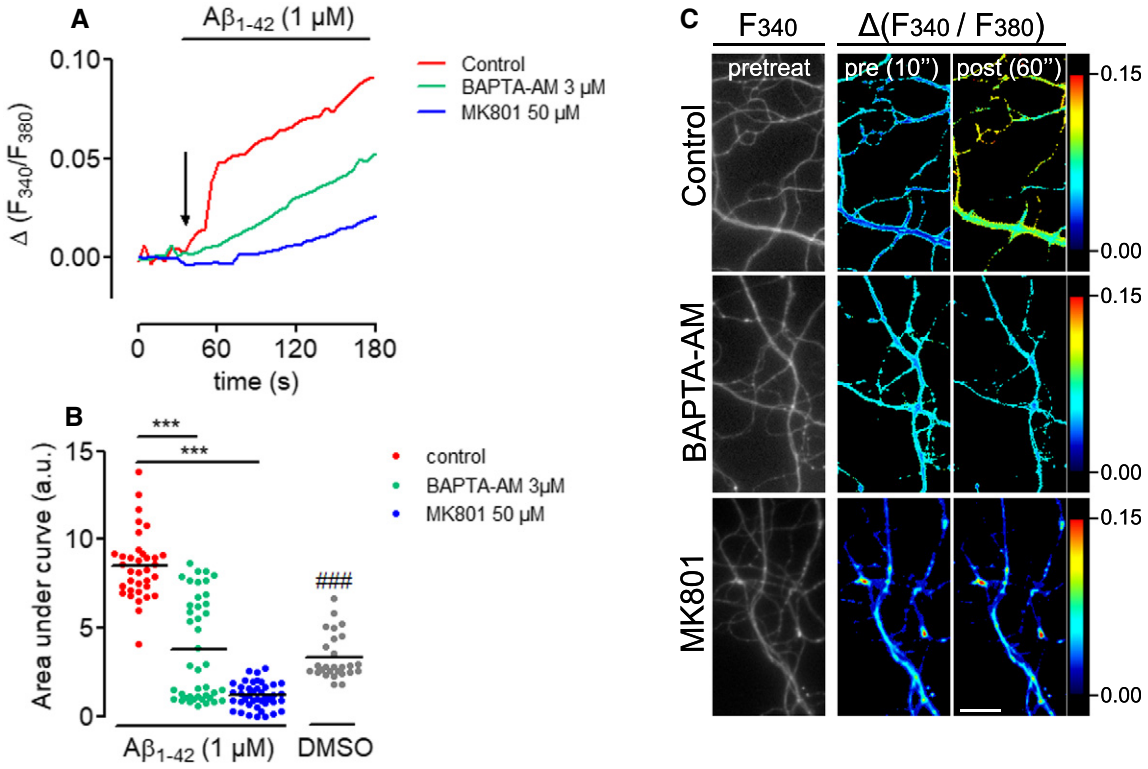

Figure EV2. Aβ1–42 oligomers increase Ca^{2+} levels in distal neurites in an NMDA-dependent manner.

A Hippocampal neurons were grown at low density (5,000 cells cm^{-2}) for > 10 DIV and loaded with Fura2-AM. Ca^{2+} levels were measured before and during perfusion with Aβ1–42 oligomers in the presence or absence of the NMDA receptor antagonist MK801 and the Ca^{2+}-chelator BAPTA-AM. Relative changes in Fura2-AM fluorescence intensity were plotted as means of 35–42 neurites imaged in two independent experiments.

B Cumulative Ca^{2+} levels represented by the area under the curves in (A). Mean of 27–42 neurites imaged in two independent experiments. $^{***}P < 0.001$; one-way ANOVA with Bonferroni's multiple comparisons test. $^{###}P < 0.001$, *t*-test control Aβ1–42 vs. DMSO.

C Micrographs of representative axonal segments quantified in (A and B). Fura2-AM levels before (two most left panels, grayscale and pseudocolor image) and after (right panel, pseudocolor image) Aβ1–42 perfusion at the indicated experimental conditions are shown. Scale bar, 25 μm.

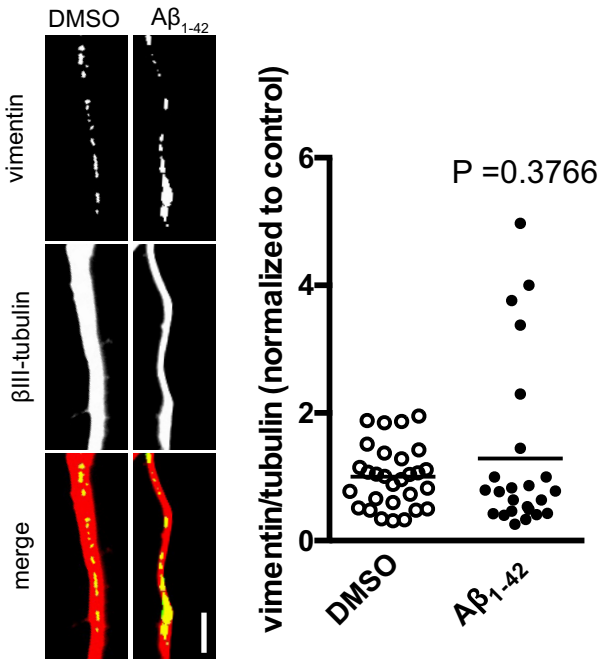

Figure EV3. Effect of 15-min treatment on axonal vimentin levels.

Hippocampal neurons were cultured in microfluidic chambers for 11–12 DIV, and axons were treated with vehicle or $A\beta_{1–42}$ for 15 min. Axons were immunostained for vimentin and βIII-tubulin, and the vimentin signal was normalized to the βIII-tubulin signal. Mean of 24–29 optical fields per condition (sampled from 5 to 6 coverslips per condition) from one primary culture. Unpaired *t*-test. Scale bar, 5 μm.

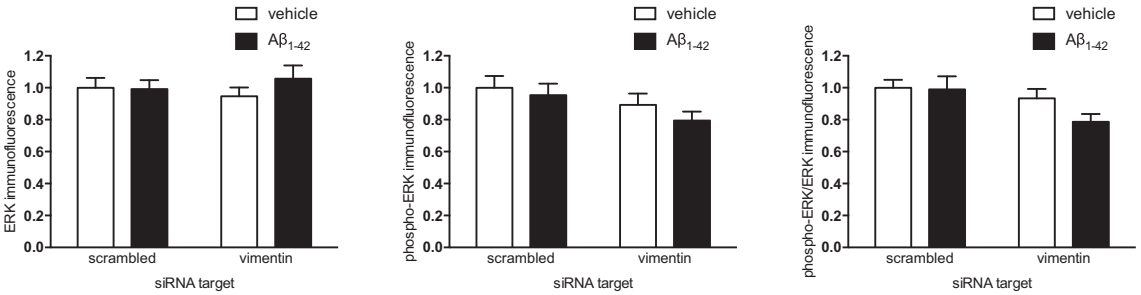

Figure EV4. ERK and phospho-ERK levels in the cell body are not affected by axonal $A\beta_{1–42}$ treatment.

Hippocampal neurons were cultured in microfluidic chambers for 10–11 DIV, and axons were transfected with scrambled or *Vim*-targeting siRNA. 24 h after transfection, axons were treated with vehicle or $A\beta_{1–42}$ for 1 h. ERK and phosphor-ERK levels were determined by quantitative immunocytochemistry. Mean ± SEM of 24–30 optical fields per condition ($n = 3$ independently performed experiments per group). No significant differences were detected between DMSO and $A\beta_{1–42}$ neurons; two-way ANOVA with Bonferroni's multiple comparisons test.