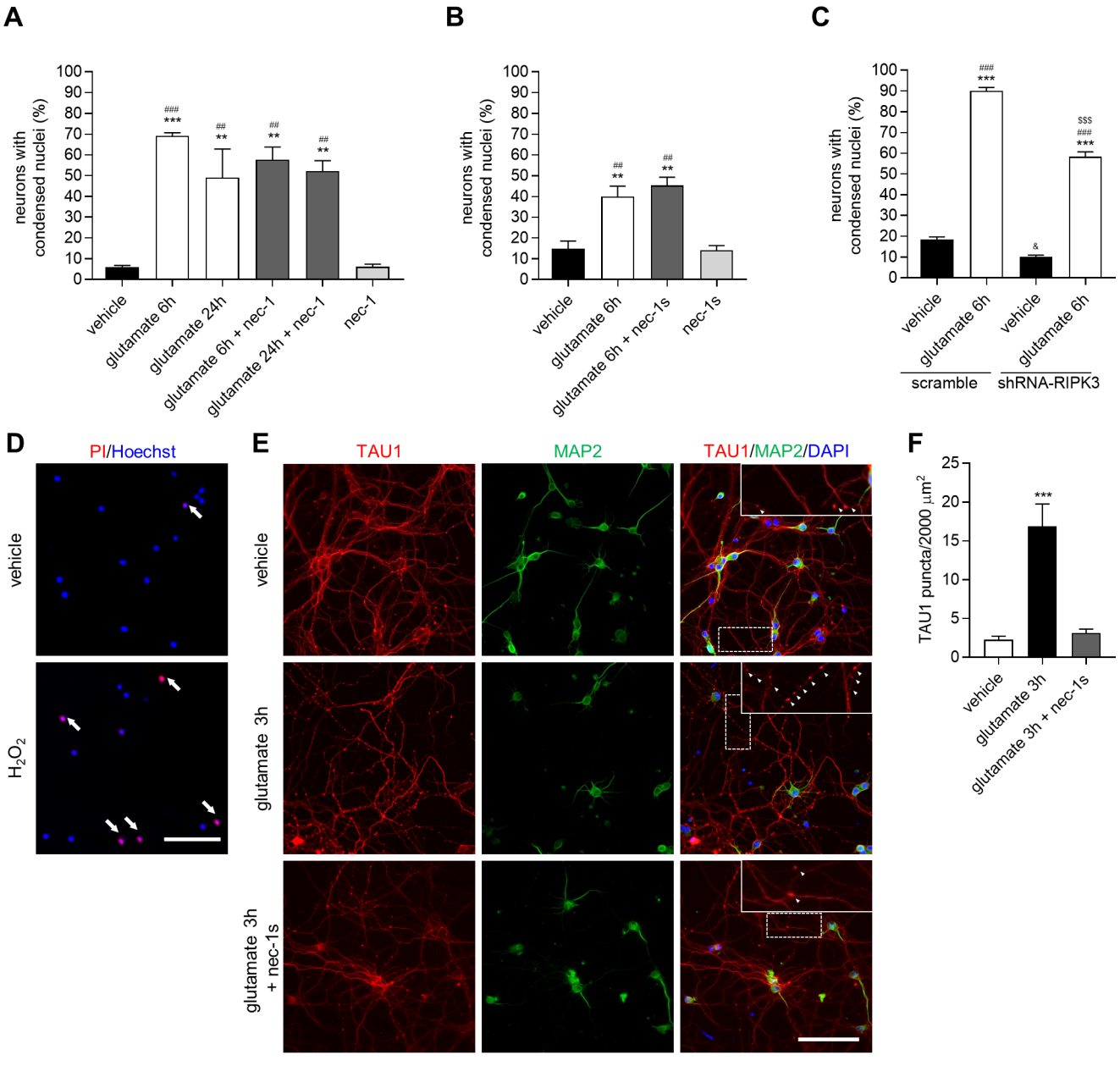
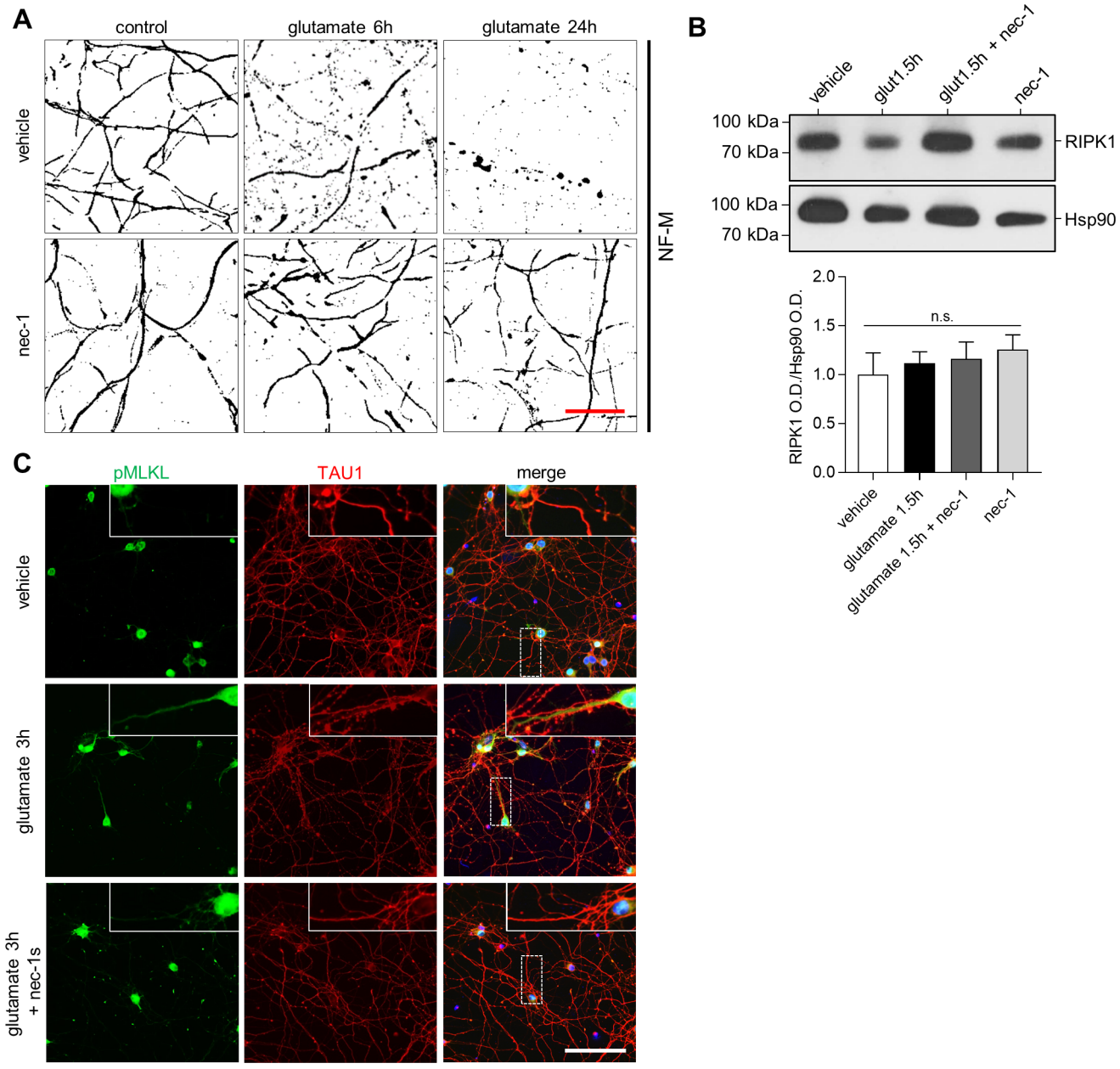
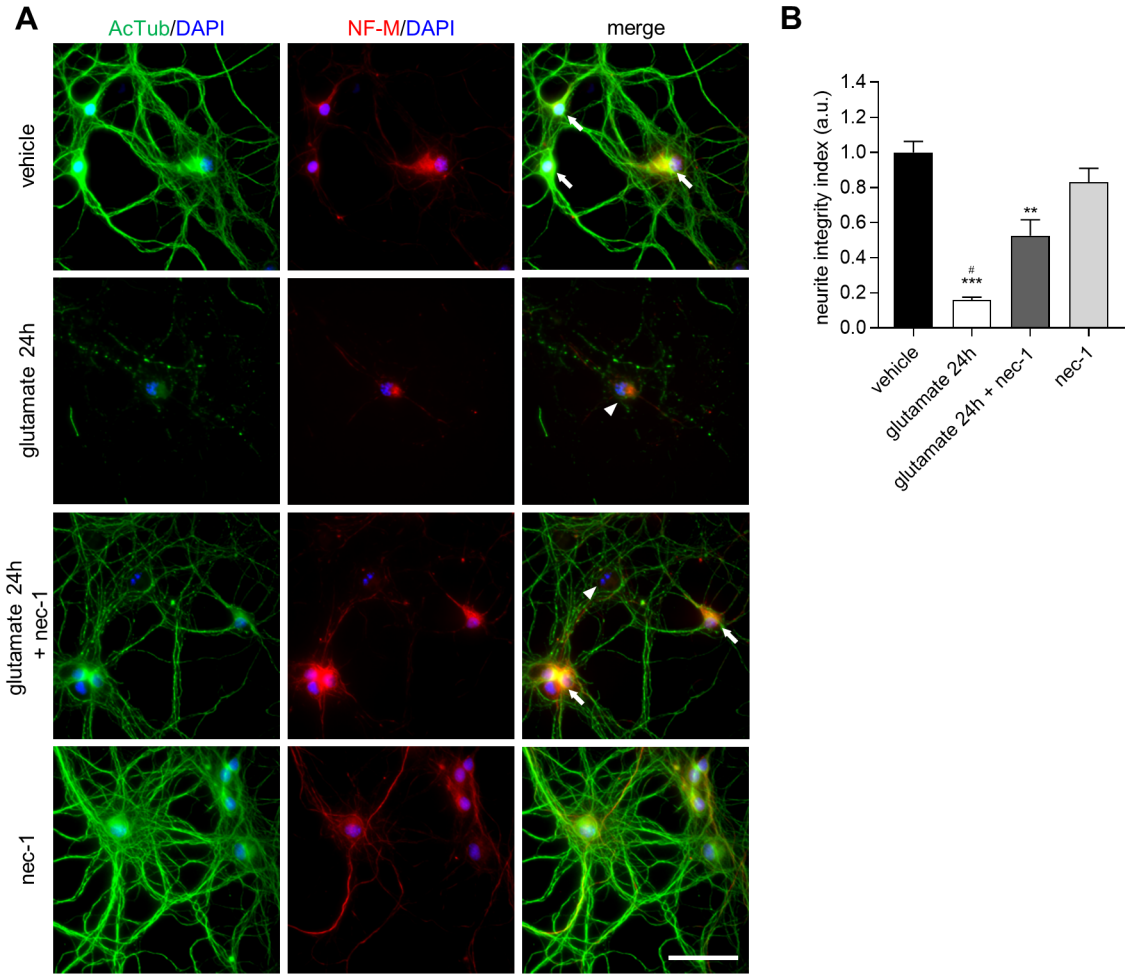


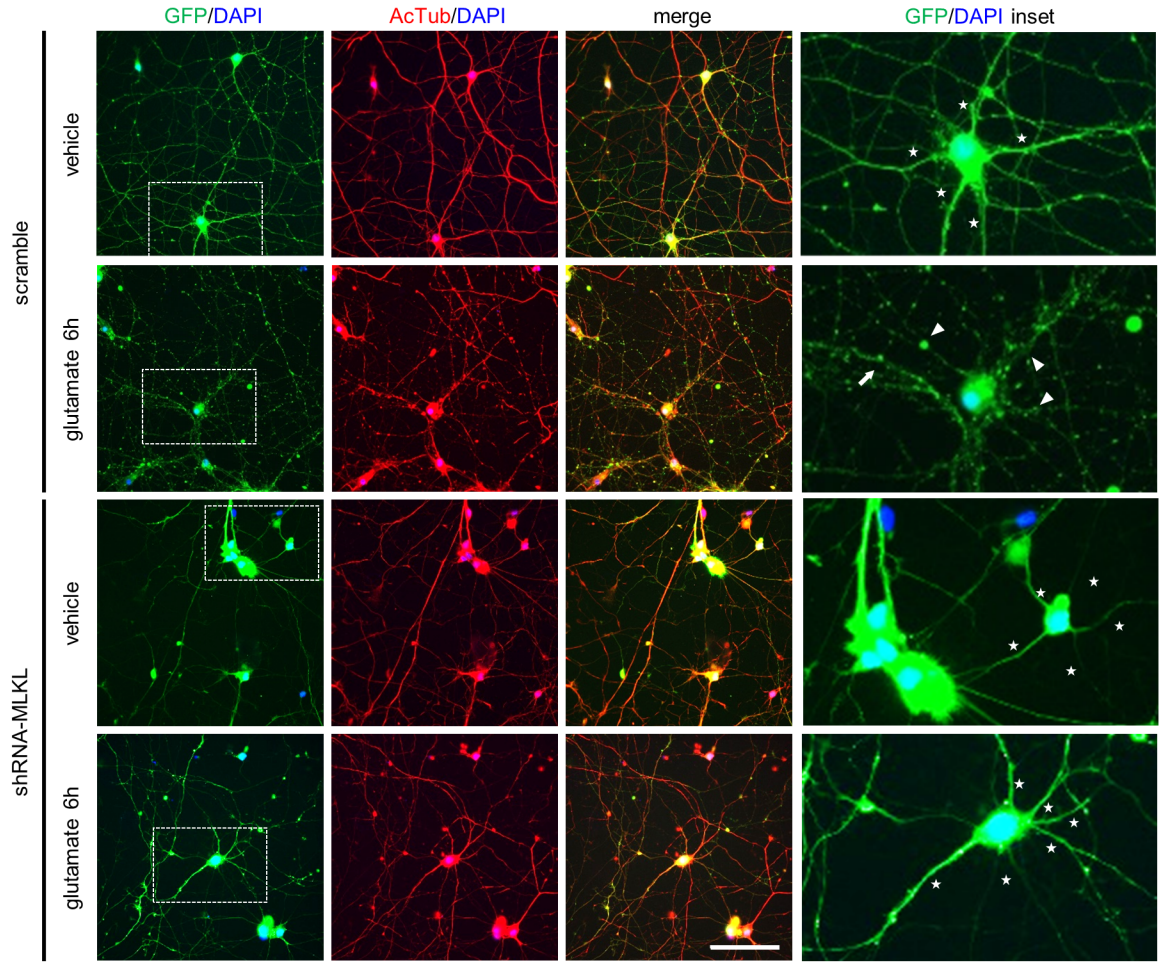
Supplementary Figure 1. (**A**) Mean percentage ± S.E.M. of neurons showing condensed DAPI stain in nec-1 experiments described in **Fig. 1A and Fig. S3A** (n = 3, one-way ANOVA, $^{\#\#}$ and ** P<0.01, $^{\#\#\#}$ and *** P<0.001; Tukey's post-test: *'s show significant differences with vehicle treatment; $^{\#}$'s show significant difference with nec-1 control treatment). (**B**) Mean percentage ± S.E.M. of neurons showing condensed DAPI stain in nec-1s experiments described in **Fig. 1A** (n = 3, one-way ANOVA, $^{\#\#}$ and ** P<0.01; Tukey's post-test: ** show significant differences with vehicle treatment; $^{\#\#}$ show significant difference with nec-1s control treatment). (**C**) Mean percentage ± S.E.M. of neurons showing condensed DAPI stain in experiments described in **Fig. 1B** (n = 3, one-way ANOVA, $^{\&}$ P<0.05, **, $^{\#\#\#}$ and $^{\$\$\$}$ P<0.001; Tukey's post-test: *'s show significant differences with vehicle treatment with scramble shRNA; $^{\#}$'s show significant differences with vehicle RIPK3-shRNA; $^{\$}$'s show significant differences between glutamate shRNA scramble and shRNA-RIPK3 treatments; $^{\&}$'s show significant differences between vehicle shRNA-scramble and vehicle shRNA-RIPK3 treatments. Quantifications were done from three independent cultures, each one with three replicates for each treatment. (**D**) Representative images of vehicle and H_2O_2 (50 μM for 5 hours) treated neurons for PI-exclusion assay experiment described in **Fig. 4A,C.** Arrows depict PI positive nuclei. Scale bar, 100 μm. (**E**) Representative immunofluorescence images of the effect of glutamate treatment over TAU1 (red; axonal) and MAP2 (green; dendritic) immunostainings together with DAPI for nuclear staining. 7-8DIV cultured hippocampal neurons were treated with vehicle or glutamate (20 μM for 3 hours) with or without RIPK1 inhibition (nec-1s; 100 μM for 18h). Insets show detailed images of axons. Arrowheads point puncta structures along the axons. Scale bar, 100 μm. (**F**) Mean number of puncta/2000 μm^2 ± S.E.M. of axons from experiment described in panel E (n=3; one-way ANOVA, *** P<0.001; Tukey's post-test: *** show significant differences with vehicle treatment). Quantifications were done from three independent cultures, each one with three replicates for each treatment.



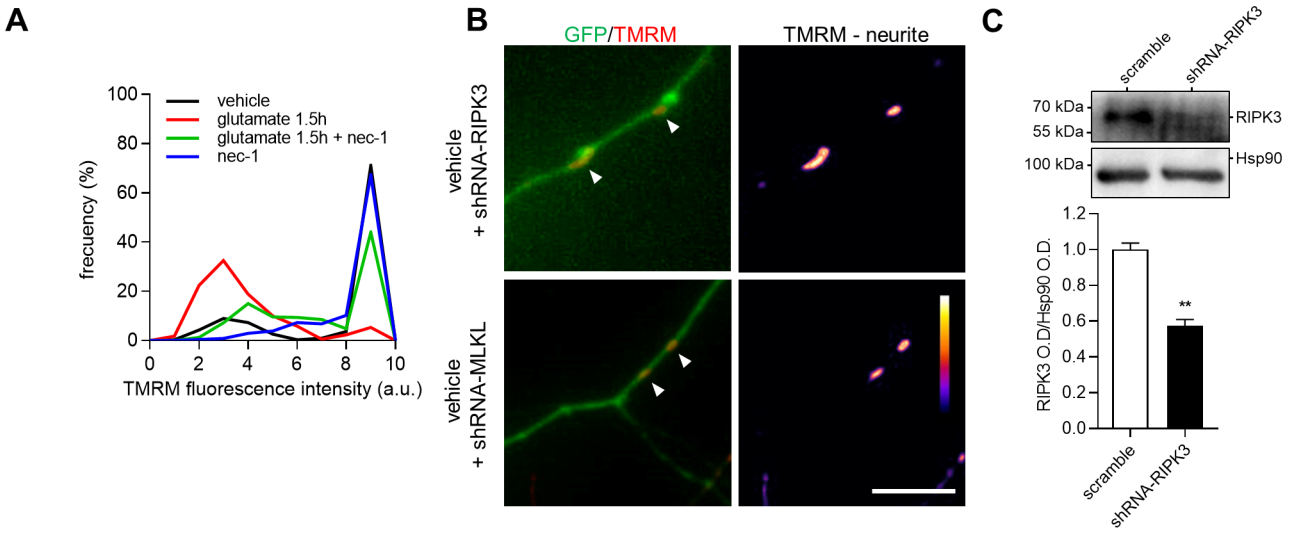
Supplementary Figure 2. (**A**) Binary images of neurites used for neurite degeneration index quantification. Binary images were obtained from primary 7-8DIV hippocampal neuron cultures, exposed to vehicle or glutamate treatment (20 µM for 6 or 24 hours) with or without nec-1 pre-treatment (100 µM for 18 hours), stained for NF-M and binarized (see methods) using imageJ to calculate the neurite integrity index. Scale bar, 20 µm. (**B**) **Top**: RIPK1 western-blot from protein lysates of seven 7-8DIV hippocampal neuronal cultures treated as described in **Fig. 1C**. Hsp90 western-blot was used as loading control. **Bottom**: Densitometric analysis of the relative amount of RIPK1 normalized by Hsp90 ± S.E.M (n = 3, one-way ANOVA, n.s.: non-significant differences). Quantifications were done from three independent cultures, each one with three replicates for each treatment. (**C**) Full images of experiment detailed in **Fig. 1D**. Rectangles show the areas used for the top inset in each image and the images shown in **Fig. 1D**. Scale bar, 100 µm.



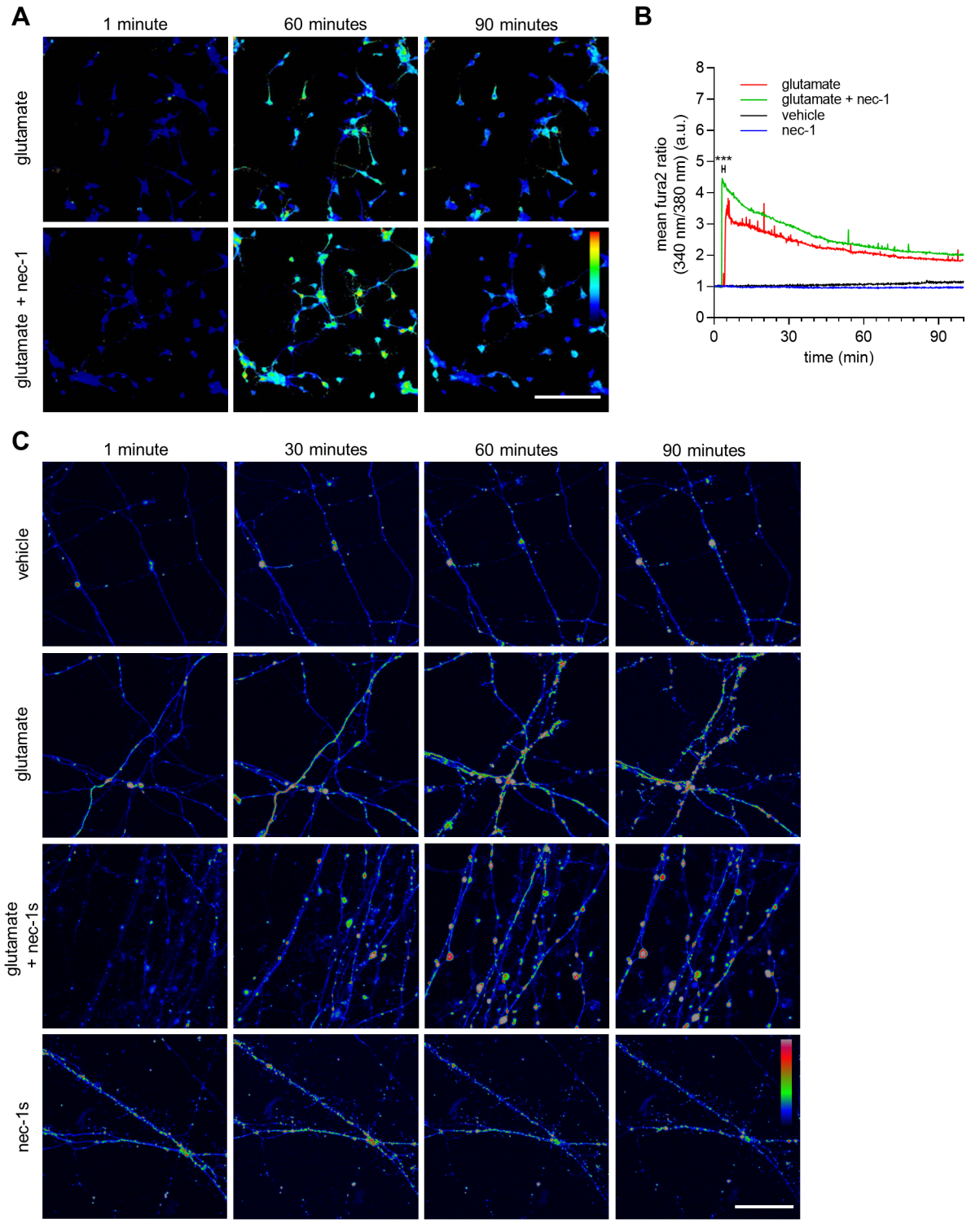
Supplementary Figure 3. (**A**) Dissociated cultured hippocampal neurons immunostained for acetylated tubulin (Ac-Tub, green), neurofilament medium polypeptide (NF-M, red) and DAPI nuclear staining (blue). Cultures were treated with vehicle or glutamate (20 µM for 24 hours) with or without nec-1 pre-treatment (100 µM for 18 hours). Arrows and arrowheads indicate healthy and condensed neuronal somas, respectively. Scale bar, 50 µm. (**B**) Neurite integrity quantification from immunostained neurons as in panel A, evaluated with the mean neurite integrity index ± S.E.M. (n = 3, one-way ANOVA, $^{\#}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$; Tukey's post-test: *'s show significant differences with vehicle treatment; $^{\#}$'s show significant differences with nec-1 control treatments).



Supplementary Figure 4. Representative images of experiment quantified in **Fig. 3E**. 7-8DIV cultured hippocampal neurons were infected with lentivirus that express shRNA to knock-down MLKL (shRNA-MLKL) or a scramble sequence (scramble) and co-express GFP as reporter gene. Cultures were exposed to vehicle or glutamate (20 μM for 6 hours), fixed and immunostained against GFP (green) and AcTub (red) and stained with DAPI for nuclei visualization. Rectangles in first column detail the origin of the insets shown in the 4th column. Stars depict the presence of neurites with a continuous morphology, arrows show beaded neurites and arrowheads fragmented neurites. Scale bar, 50 μm.



Supplementary Figure 5. (**A**) Frequency distribution histogram of mitochondrial TMRM fluorescence relative intensity for all the treatments described in **Fig. 6A**. Bimodal distribution for al treatments are consistent with significant Shapiro-Wilk normality Test (P<0.001), that show TMRM data distribution for all treatments is non-parametric. (**B**) Representative images of shRNA-RIPK3 and shRNA-MLKL vehicle treatment controls of experiment explained in **Fig. 6C,D. Left column**: Fluorescence of GFP (green) and TMRM (red). Arrowheads depict TMRM positive mitochondria in GFP positive neurites. Arrowheads show TMRM positive mitochondria in GFP positive neurites. **Right column**: TMRM intensity profile images for all the described treatments. Color map: relative level of TMRM fluorescence form less (blue) to high (white) fluorescence intensity. Scale bar, 10 μm. (**C**) **Top**: Western-blot of RIPK3 from 7-8 DIV protein lysates of hippocampal neuron cultures, infected with lentivirus that express scramble shRNA or RIPK3-shRNA. Hsp90 western-blot was used as loading control. **Bottom**: Densitometric analysis of the relative RIPK3 levels normalized by Hsp90 ± S.E.M (n = 3, unpaired, two-tailed, t-Test, **P<0.01). Quantifications were done from three independent cultures, each one with three replicates for each treatment.



Supplementary Figure 6. (**A**) Calcium dynamics in neuronal soma obtained from time-lapse recordings using the same treatment protocol as in **Fig. 8A.** Time-lapse representative images of neuronal soma stained with Fura-2 cytoplasmic Ca^{2+} sensitive dye, at 1, 60 and 90 minutes of recording. Neuronal cultures were treated with vehicle or glutamate (20 μM at 3 minutes of recording) with or without nec-1 pre-treatment (100 μM for ~18 hours before the start of time-lapse recording). Color bar: Ratiometric fluorescence intensity of fura-2 from less (blue) to higher (red) levels. Scale bar, 100 μm. (**B**) Mean ratiometric fura-2 fluorescence intensity levels, from time-lapse recording obtained from the different treatments indicated in **Fig. S6A** (n = 3, two-way ANOVA, ***P<0.001; Bonferroni's post-test: *'s, show significant differences between vehicle and other treatments). (**C**) Complete panel with representative images from experiment described in **Fig. 8C,D**. Color bar, fluorescence intensity profile of fluo3 from less (blue) to high (brown) levels. Scale bar, 50 μm.