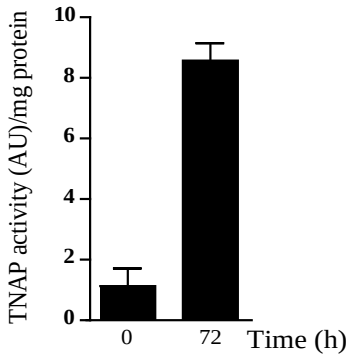
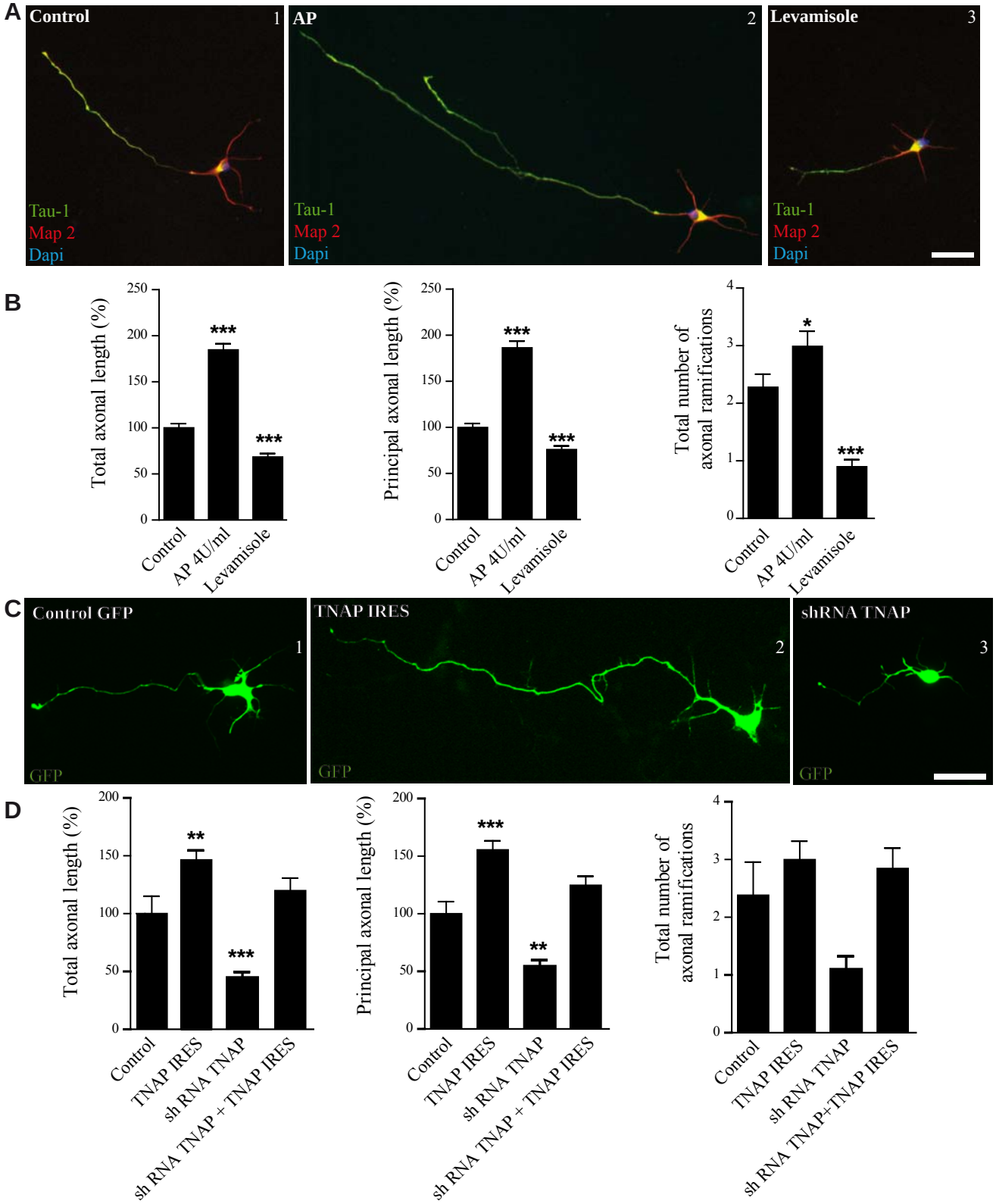
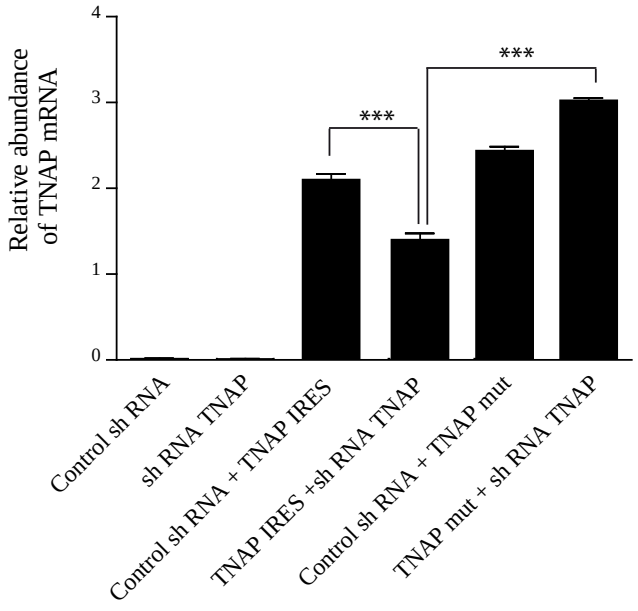


Supplementary figure 1. TNAP activity in hippocampal neurons in cultured. Quantification of TNAP activity in hippocampal neurons along the days in cultured. Graph represent, as arbitrary units (AU), the TNAP activity at 24 and 72 hours after neurons were plated The data represent the mean ± s..e.m. obtained from three independent experiments.



Supplementary figure 2. Effect of TNAP on axonal growth in cortical neurons. A, Representative images of cortical neurons (immunostained for Tau-1 [green] and MAP-2 [red] to differentiate between axons and dendrites) cultured for 3 DIV in the absence (1) or presence of alkaline phosphatase (4 U/ml, 2) and of levamisole (500 µM, 3). DAPI-stained nuclei are in blue. Scale bar, 50 µm. B, Quantification of axonal length and the number of ramifications from experiments shown in (A). The total axonal length (corresponding to the principal axon plus ramifications), the length of the principal axon and the number of axonal ramifications were analyzed. The 100% values in control neurons for total axonal length and principal axon length correspond to 425.3 ± 21.07 µm and 329.6 ± 14.86 µm, respectively. The number of axonal ramifications in control neuron was 2.27 ± 0.22. Values represent the means ± s.e.m. (n =3) with at least 50 neurons analyzed in each experiment. * $p<0.05$, *** $p < 0.001$, versus control (one way ANOVA). C, Cortical neurons transfected at 1 DIV with pEGFP (1), TNAP IRES (2) or shRNA TNAP (3) or and fixed at 3 DIV. Scale bar, 50 µm. D, Effect of TNAP overexpression and silencing on the axon length and number of ramifications in cortical neurons. The total axonal length (corresponding to the principal axon plus ramifications), the length of the principal axon and the number of axonal ramifications were analyzed. The 100% values in control neurons for total axonal length of EGFP-transfected neurons and principal axon length of EGFP-transfected neurons correspond to 619.9 ± 93.73 µm and 427.9 ± 45.11 µm, respectively. The number of axonal ramifications in EGFP neuron was 2.38 ± 0.58. Values represent means ± s.e.m. (n = 3) with at least 40 neurons analyzed in each experiment. **$p<0,01$, ***$p<0,001$ (one way ANOVA).



Supplementary figure 3. Knockdown of TNAP enzyme by specific shRNA; Quantification of the mRNA levels of mouse TNAP by quantitative RT-PCR in HEK 293T cells transfected with control shRNA, shRNA TNAP, TNAP IRES, TNAPmut, TNAP IRES plus shRNA TNAP and TNAPmut plus shRNA TNAP. Results were normalized to values obtained for human GAPDH mRNA and expressed as the means ± s.e.m. (n = 3). *** $p < 0.001$ (one way ANOVA).