Supplementary figures to:

Traumatic brain injury in the presence of $A\beta$ pathology affects neuronal survival, glial activation and autophagy

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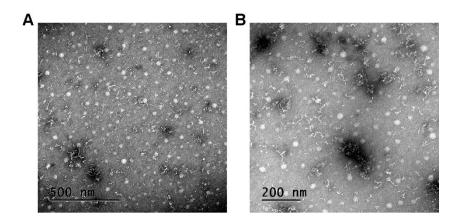
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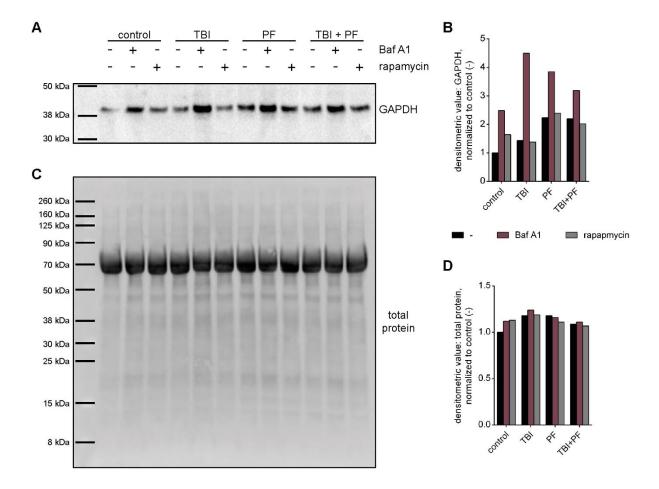
Running title: TBI in the presence of Aβ pathology

Supplementary Figure 1 TEM characterization of Aβ₄₂ protofibrils.



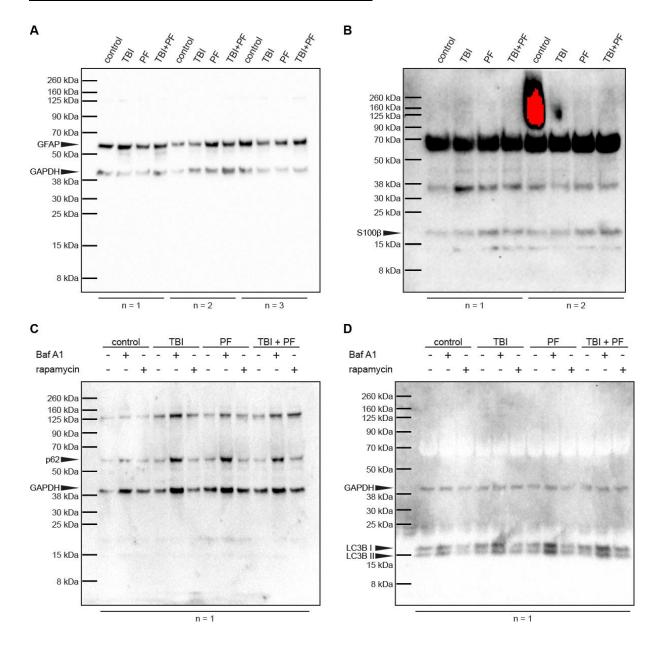
A and B) $A\beta$ protofibril characterization using TEM. Scale bars 500 nm (A) and 200 nm (B). Negative staining of the $A\beta$ protofibrils was performed using the following method: $A\beta$ protofibrils (100 μ M) were diluted 1:10 in Milli-Q H₂O and a 5 μ l drop of the sample was placed on a formvar/carbon coated 200-mesh copper grid (Ted Pella). After 10 s, the excess solution was removed by blotting with filter paper. The sample was then directly stained with 2% uranyl acetate for 5 s. Excess of uranyl acetate was removed by blotting on filter paper. Dried grids were examined by TEM (Tecnai G2 Spirit) operated at 80kV.

Supplementary Figure 2 Western blot loading assessed by housekeeping gene GAPDH and total protein stain



A) Representative blot (mouse monoclonal GAPDH, clone 1D4, #NB300-221, Novus Biologicals; 1:10000) and B) quantification of the detected GAPDH bands. Both, the experimental groups (TBI, PF and TBI+PF) and the use of the autophagy inhibitor Baf A1 (500 nM, 6 h) or inducer rapamycin (200 nM, 24 h), were found to influence the expression level of the housekeeping gene GAPDH and thus unsuitable as loading control and for protein normalization. C) Total protein staining of the membrane with No-Stain Protein Labeling Reagent (#A44717, Thermo Fisher) and D) its quantification confirmed equal loading when using protein concentrations determined by Pierce BCA Protein Assay kit.

Supplementary Figure 3 Full-length western blots



A) Full length blot of the cropped image shown in Figure 3C, GFAP rabbit polyclonal (#Z0334, Agilent DAKO; 1:10000). **B)** Full length blot of the cropped image shown in Figure 3G, S100b mouse monoclonal (clone SH-B1, #S2532, Sigma-Aldrich; 1:500). **C)** Full length blot of the cropped image shown in Figure 6A, p62 rabbit polyclonal (#NBP1-48320, Novus Biologicals; 1:1000). **D)** Full length blot of the cropped image shown in Figure 6A, LC3B rabbit polyclonal (#NB100-2220, Novus Biologicals; 1:1000). When indicated: GAPDH mouse monoclonal (clone 1D4, #NB300-221, Novus Biologicals; 1:10000).