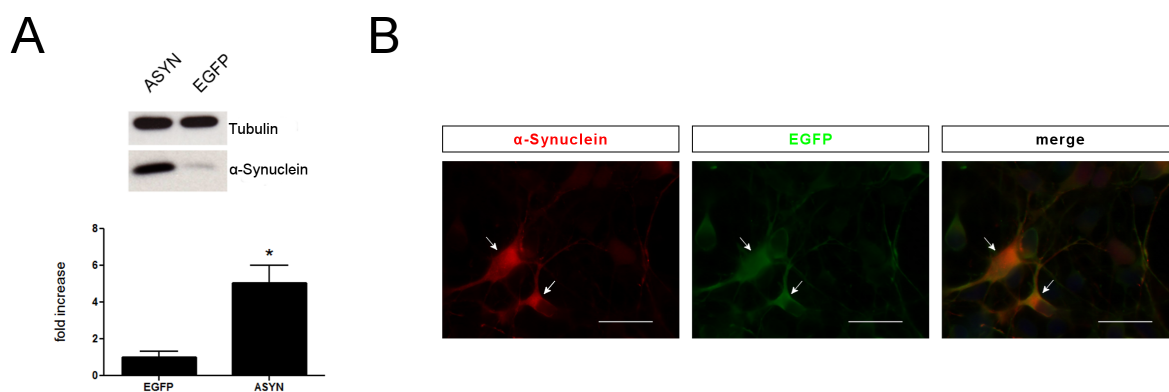


Alpha-synuclein regulates neuronal levels of manganese and calcium.

Ducic T, Carboni E, Lai B, Chen S, Michalke B, Lázaro DF, Outeiro TF, Bähr M, Barski E, Lingor P.

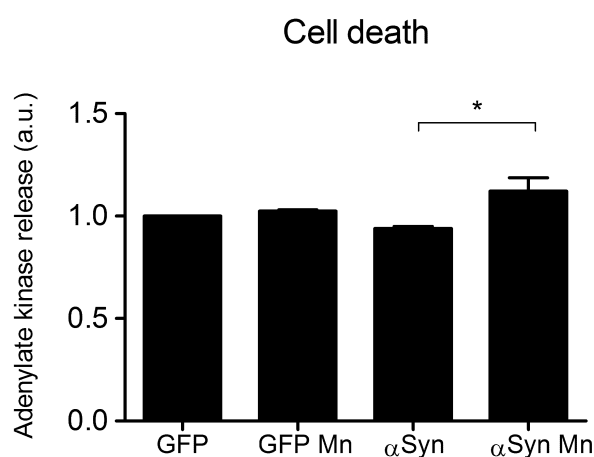
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Supporting information



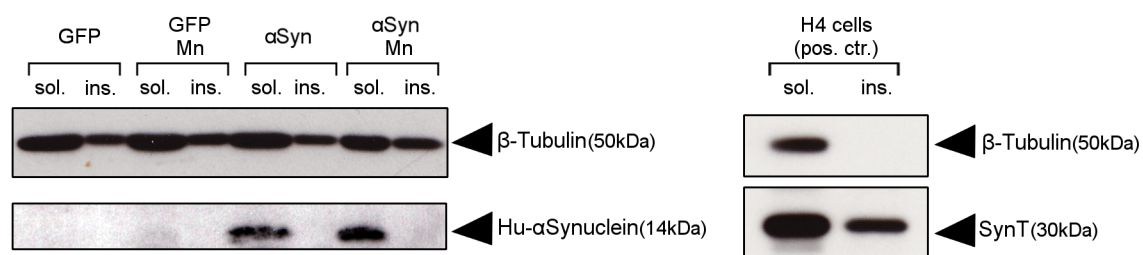
Supporting Fig. S1: Over-expression of α Syn in primary midbrain neuron cultures.

PMN cultures were transfected with p. α Syn-WT while control cultures were transfected with p.EGFP. The protein blot detection was made with a pan- α -synuclein antibody showing that transfected cells had a significantly higher α -synuclein expression (A). Representative images of a PMN culture transfected with p. α Syn-WT immunostained with a pan- α -synuclein antibody. EGFP fluorescence (co-expressed by the p. α Syn-WT) confirms transfection. Scale bars: 20 μ m (B).



Supporting Fig. S2: Cytotoxicity of α Syn overexpression and Mn treatment.

Cytotoxicity was quantified by assessment of adenylate kinase release. Values are shown as arbitrary unites relative to GFP (+SEM). One way-ANOVA and Tukey post-hoc test reveal that the presence of Mn in cells overexpressing α Syn lead to an increased adenylate kinase release compared to α Syn-treatment alone (* P=0.03).



Supporting Fig. S3: Analysis of the solubility state of αSyn.

Left: Representative image of the Triton X-100 solubility assay in PMNs overexpressing αSyn for 3 DIV. In PMNs overexpressing αSyn 100% of the protein is in the soluble fraction, which does not depend on the presence of Mn. Right: The positive control (H4 cells transfected with SynT/Synph-1 plasmids) shows that ~32% of the total αSyn is present in the insoluble fraction.