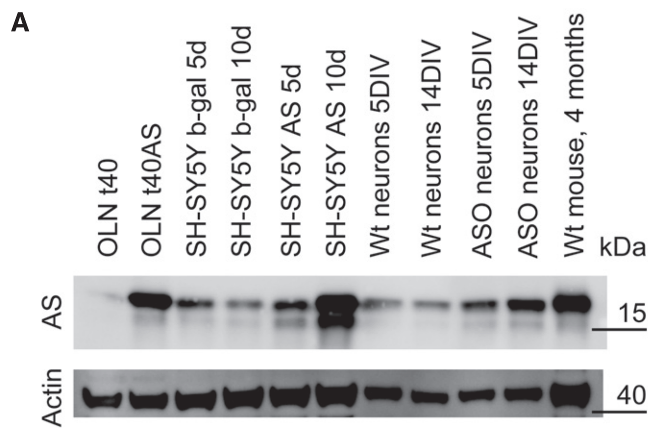
Expanded View Figures



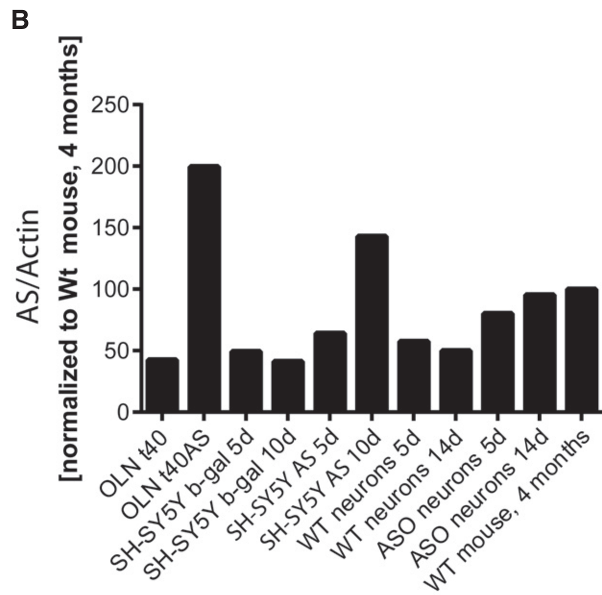


Figure EV1. Evaluation of the AS level in cell models used for study.

A About 10 μg total lysate from the different cell models, primary mouse hippocampal neurons from wild-type and AS-transgenic mice (ASO), and total brain homogenate from a wild-type C57BL/6J mouse were resolved by 10–16% SDS–PAGE probed for α-syn (ASY-1), and actin.

B The level of AS was quantified and normalised to the actin level. The data are presented with the level in total brain homogenate from 4-month-old C57BL/6J mouse arbitrarily numbered as 100.

 © 2018 The Authors

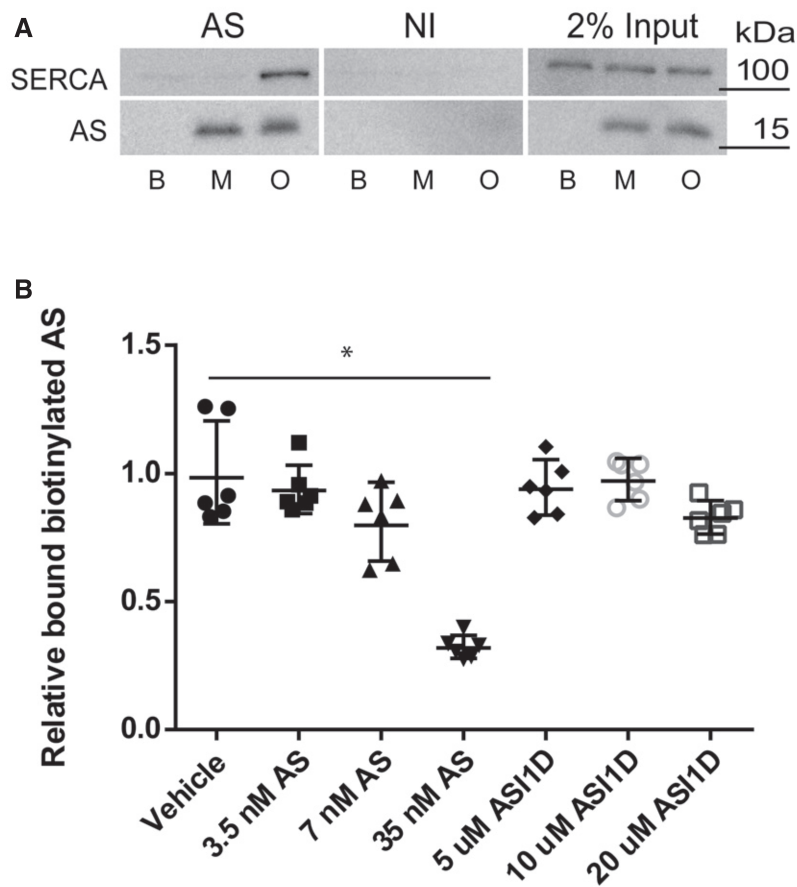


Figure EV2. Aggregated AS interacts with SERCA..

The interaction is inhibited by aggregate inhibitor, ASI-1D, when analyzed by proximity ligation assay. The inhibition of interaction is not due to ASI-1D blocking the anti-AS antibody quenching the proximity ligation signal.

A Aggregated AS interacts with SERCA 1A. Purified AS oligomers (O) and monomers (M) were incubated with detergent extract of SERCA1a from sarcoplasmic reticuli isolated from rabbit muscle before being subjected to co-immunoprecipitation (co-IP) using anti-AS (AS) and non-immune rabbit IgG (NI) coupled to sepharose. PBS was used as additional negative control. 2% input of each sample was used as input control. The co-IP samples were analysed by immunoblotting using rabbit polyclonal anti-SERCA (J15.5) and rabbit polyclonal anti-AS (ASY-1). A representative blot of three replicates is shown.

B ELISA shows that the aggregation inhibitor ASI-1D does not bind the primary anti-AS antibody and thereby quench the proximity ligation assay. The aggregation inhibitor ASI-1D was able to completely abolish the signal from the proximity ligation assay (Fig 3B); therefore, we tested if the binding of the Syn211 antibody to AS can be inhibited by the aggregate inhibitor ASI-1D. An ELISA assay with 0.7 nM biotinylated AS was developed to test whether increasing amounts of ASI-1D can compete with biotinylated AS bound to Syn211. Increasing amounts of ASI-1D cannot compete against the binding of biotinylated AS to Syn211 even at a concentration of 20 μM. As control for the competition, increasing amounts of non-biotinylated AS were tested, and a concentration of 7 nM AS resulted in ~20% less bound biotinylated AS, and 35 nM AS reduced the biotinylated AS to ~30%. This shows that the effect of the aggregate inhibitor in our PLA assay is not caused by inhibition of the binding between Syn211/AS by ASI-1D. Bars represent mean absorbance ± SD (Kruskal–Wallis one-way rank test with Dunn's *post hoc* test, $*P = 0.001$). $N = 3$.

© 2018 The Authors

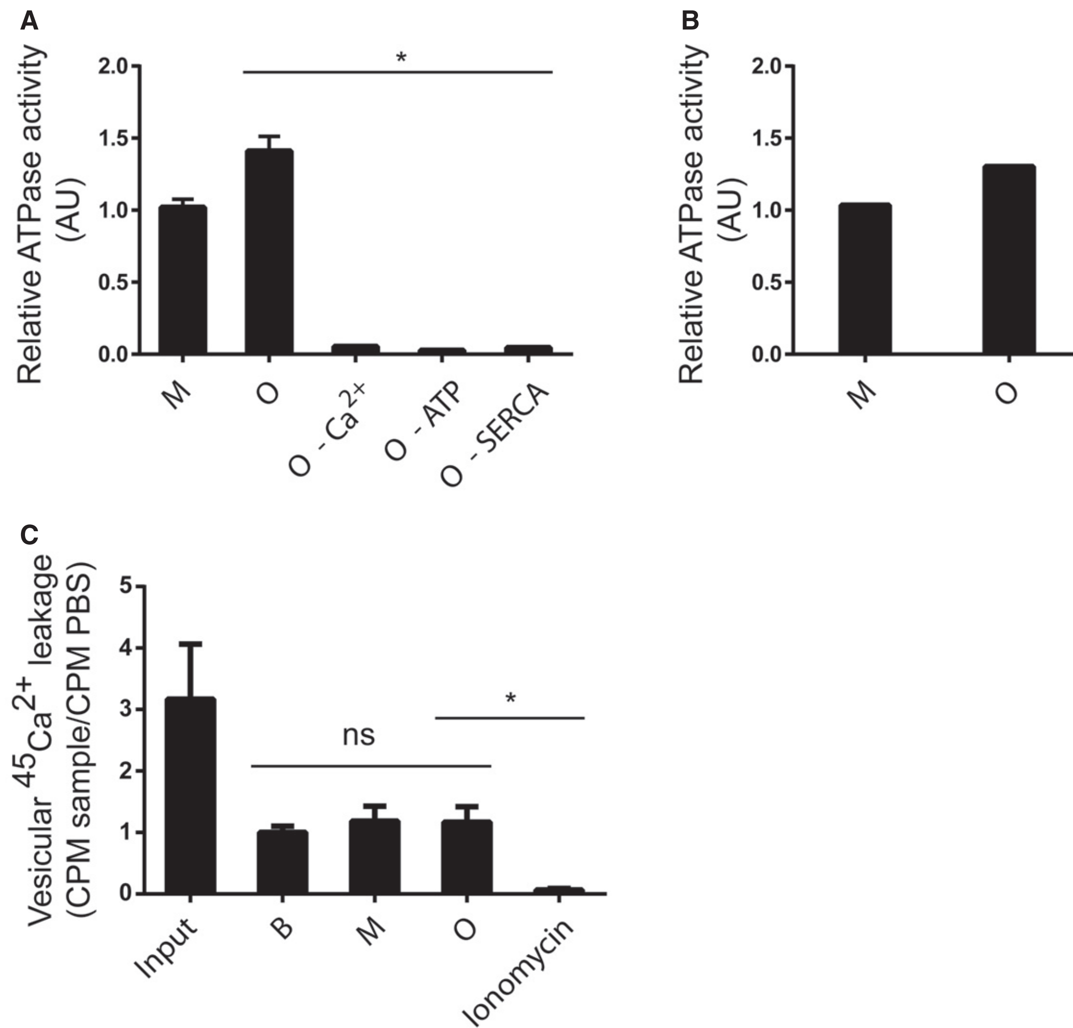


Figure EV3. AS oligomers can only stimulate SERCA activity when Ca^{2+} and ATP are present, and AS oligomer cannot by itself hydrolyse ATP.

A SERCAs ATPase activity was measured as hydrolysis of ATP and release of inorganic phosphate as in Fig 4A–C. Sarcoplasmic reticulum (SR) vesicles isolated from rabbit skeletal muscle containing SERCA1a were mixed with 50 μg/ml AS monomer (M), AS oligomer (O), AS oligomer without Ca^{2+} (oligomer–Ca^{2+}), AS oligomer in the absence of ATP (oligomer–ATP) or AS oligomer in the absence of SERCA (O–SERCA). Bars represent the average relative ATP hydrolysis ± SD normalised to buffer (Kruskal–Wallis one-way rank test with Dunn's *post hoc* test, *P = 0.01). N = 3.

B Sarcoplasmic reticulum is a highly specialised muscle form of endoplasmic reticulum; and to validate that the activity is not dependent on this, the SERCA ATPase activity was also measured in ER microsomes extracted from COS cells overexpressing SERCA1a in the presence of 50 μg/ml AS monomer (M) or AS oligomer (O). Bars represent the average relative ATP hydrolysis normalised to buffer from three individual experiments (Wilcoxon signed rank test, *P = 0.0286).

C AS oligomers have previously been proposed as forming pores in membranes leading to a leakage of Ca^{2+}. The ability of AS oligomers to form Ca^{2+} permeable pores was studied in intact microsomes from extracted rabbit muscle preloaded with $^{45}Ca^{2+}$. The microsomes were incubated with PBS (B), monomer (M) or oligomer (O) for 1 min before analysis. Ionomycin-treated microsomes served as positive control for completely permeabilised membranes. No difference in $^{45}Ca^{2+}$ efflux could be demonstrated between PBS and the AS preparations in contrast to ionomycin that caused release of all $^{45}Ca^{2+}$ from the microsomes. Bars represent the average $^{45}Ca^{2+}$ CPM ± SD remaining after 5 min normalised to PBS from three individual experiments (Kruskal–Wallis one-way rank test with Dunn's *post hoc* test, *P = 0.0427).

© 2018 The Authors

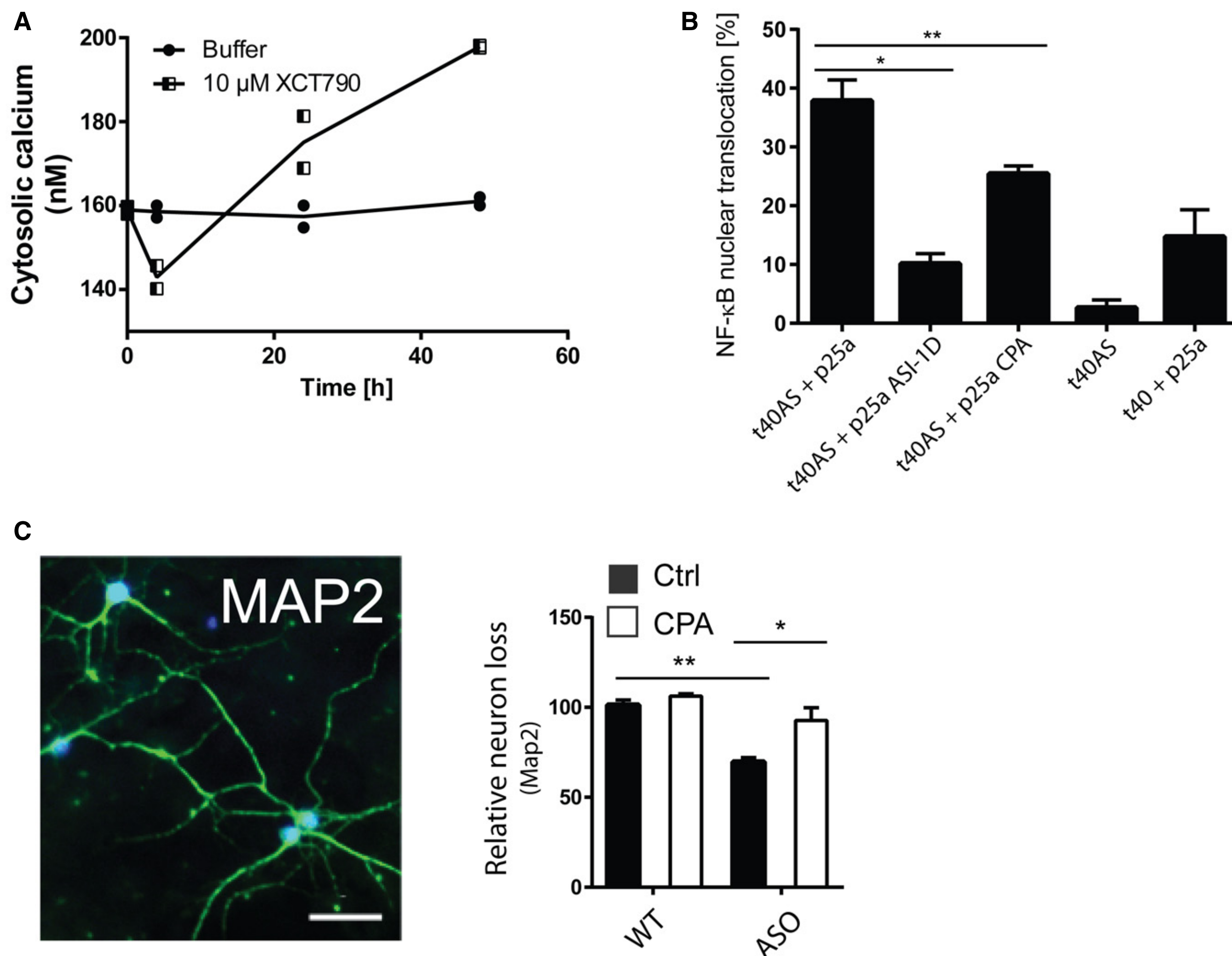


Figure EV4. Allosteric activation of SERCA mimics the Ca^{2+} dysregulation of aggregated AS. CPA reduces NF-κB nuclear translocation in OLN cells and increases survival in neurons.

A Allosteric activation of SERCA by XCT790 mimics the early decrease and later increase in Ca^{2+} observed upon intracellular AS aggregation. Cytosolic Ca^{2+} levels were quantified by the Ca^{2+} sensor Fura-2 and converted to absolute values using the Fura-2 Calcium Imaging Calibration Kit. Non-mitotic SH-SY5Y wt cells were generated by treatment with 10 μM retinoic acid for 2 days before treatment with SERCA activator, 10 μM XCT790 (Sigma-Aldrich). The cytosolic Ca^{2+} in SH-SY5Y cells was measured after 4, 24 and 48 h. Points represent Ca^{2+} concentrations as mean ± SD, $N = 2$.

B CPA reduces NF-κB nuclear translocation in OLN cells. Aggregation of AS in OLN cells by co-expression of AS and p25a generates a cell stress that increases nuclear translocation of NF-κB (p65), which can be rescued by ASI-1D treatment [10]. Bars represent the average percentage of transfected cells with NF-κB translocated to the nucleus ± SD in > 100 transfected cells in each experiment (one-way ANOVA multiple comparisons with Sidak *post hoc* test, $^{*}P = 0.0001$ and $^{**}P = 0.0006$). $N = 3$.

C Survival of neurons from day 6 to day 14 was quantified by counting MAP2-positive cells. Representative MAP2 staining pattern (green) of primary hippocampal neurons with NeuN marking neuronal nuclei. Scale bar is 50 μm. Bars represent remaining MAP-2-positive neurons at day 14 normalised to the number present at day 6, presented as means of three individual experiments ± SD each of > 300 neurons (one-way ANOVA multiple comparisons with Sidak *post hoc* test, $^{*}P = 0.0003$, $^{**}P = 0.0001$).

© 2018 The Authors