

Supplemental Figure 1. C-truncated asyn aggregates extensively *in-vitro*. (A) Coomasie stained SDS-PAGE gels showing the amount of soluble vs insoluble asyn for each C-truncation of asyn at the 96-hour time point of *in-vitro* incubation; C-truncated asyn samples were prepared at 150 μ M (n=4). Mobilities of molecular mass markers in kDa are indicated. (B) Densitometric analysis of the gels in A; one-way ANOVA with Dunnet's test determined a large significant increase in the final extent of aggregation for truncations 1-129 and shorter at 96 hours. Error bars = std. * = p ≤ 0.05 , **** = p ≤ 0.0001 .



Supplementary Figure 2. *In-vitro* **proteolytic digestion profiles differ for fibrils comprised of different C-truncated forms of αsyn.** (A) Coomasie stained SDS-PAGE gels showing for each truncation the undigested αsyn fibrils along with equal amounts of digested fibrils (1 mg/mL) using either trypsin or proteinase K at two concentrations (0.05 mg/mL or 0.025 mg/mL for trypsin and 0.005 mg/mL or 0.0025 mg/mL for proteinase K) for 30 minutes at 37 °C. Digestion profiles differed between the shorter and longer C-truncated forms of αsyn as well as FL αsyn, indicating differences in αsyn fibril structure.



Supplemental Figure 3. New 5G9 mouse monoclonal antibody is specific for mouse α syn and preferentially binds aggregated α syn. (A)Western blots loaded with 200 ng of recombinant mouse (M) and human (H) α syn, or 25 μ g of lysate from HEK293T cells transfected to expressed mouse or human α syn. The 5G9 antibody detects only mouse α syn and not human α syn in both cases. (B) Immunohistochemical staining of a non-transgenic mouse induced to develop pathologic α syn inclusions in the ipsilateral entorhinal cortex when intra-striatally injected with pre-formed mouse α syn fibrils. The 5G9 antibody preferentially binds to mouse α syn located within inclusions and not physiologic mouse α syn as demonstrated by staining in the ipsilateral but not contralateral entorhinal cortex. The staining pattern of 5G9 paralleled that of antibody EP1536Y specific for pSer129 which is a marker of aggregated α syn. Scale bar 50 μ m.



Supplemental Figure 4. Predicted biochemical properties of C-truncated α syn. The theoretical Alipathic index and protein isoelectric point of each carboxy-truncation and its 1-20 truncated counterpart. The biochemical properties of α syn are dominated by carboxy and not amino truncation, as progressive C-truncation leads to a large increase in protein isoelectric point (pI) and related increase in alipathic index due to loss of acidic residues.