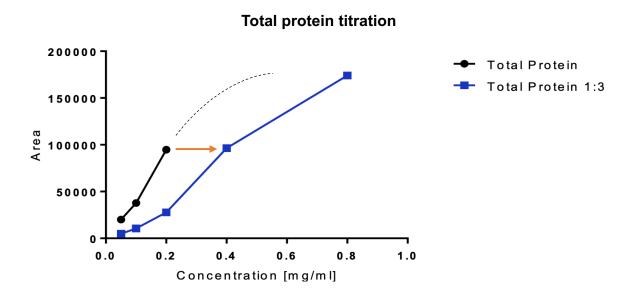
Supplementary Material

Automated Capillary Electrophoresis Immunoblot for the Detection of Alpha-Synuclein in Mouse Tissue

Supplementary Figure 1. Titration of total protein by CE (Simple Western) immunoblot using midbrain tissue. Tissue lysates in concentrations ranging from 0.05 mg/ml to 0.8 mg/ml were incubated with total protein solution in its undiluted form, as well as in a dilution of 1:3 with distilled H₂O. For incubation with undiluted total protein, midbrain samples with protein concentrations between 0.05 mg/ml and 0.2 mg/ml were used. The rest of the curve was estimated (*dotted line*). Deviation of the linear range is shown by the orange arrow.



Supplementary Figure 2. aSyn protein levels in mouse tissues using SDS-PAGE immunoblot. Tissue lysates (25 µg) of 15-month-old WT and a 5-week-old aSyn null mice were subjected to SDS-PAGE, followed by incubation with 4% PFA for 1 h and incubated with anti-total aSyn antibody solution overnight, and subsequently with an HRP-conjugated secondary antibody. Signals were detected using chemiluminescence. A single band corresponding to aSyn at around 18-19 kDa was evident in the cortex, midbrain, and lung tissues, while a faint signal was observed in the remaining organ lysates, with the exception of KO tissue lysates (A). On a second membrane, the same experimental procedure was performed without the primary anti-total aSyn antibody, and as expected, no aSyn signal was detected at around 19 kDa (B). Bands visualized at higher molecular weight correspond to unspecific binding sites of the secondary antibody.

