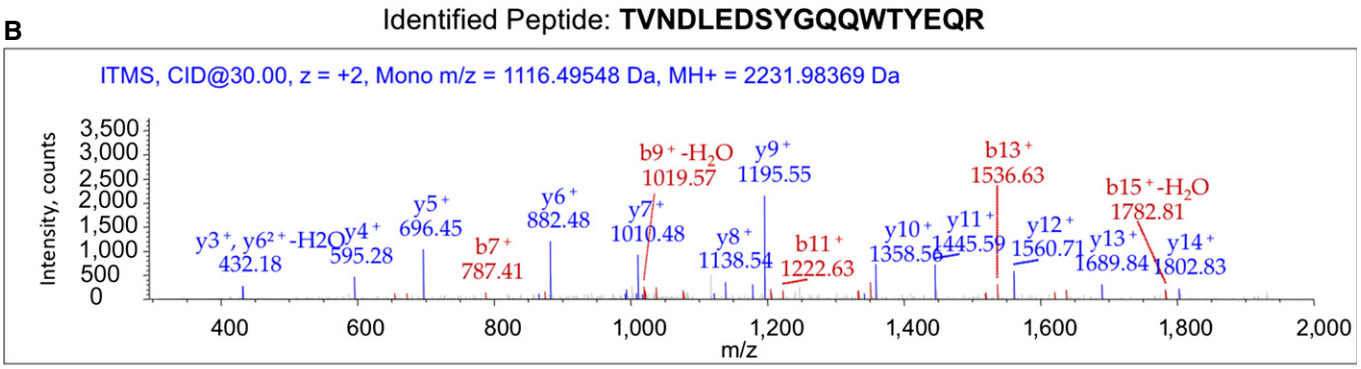
Expanded View Figures

A

Start-End	Mass	Δppm	Amino acid sequence	Modification
44-57	1649.7837	0.34	KYNTDCVQGLTHSK	CAM
203-217	1618.7424	−0.65	VDNSSLTGESEPQTR	
218-230	1540.6576	−0.014	SPDCTHDNPLETR	CAM
436-448	1274.6368	1.11	DVAGDASESALLK	
603-615	1380.7412	−1.7	VIMVTGDHPITAK	
620-637	1828.9146	−1.1	GVGIISEGNETVEDIAAR	
689-697	1086.585	−0.47	LIIVEGCQR	CAM
884-901	2230.9764	−0.13	TVNDLEDSYGQQWTYEQR	
903-930	3337.66135	−2.34	VVEFTCHTAFFVSIVVVQWADLIICKTR	2 CAM
903-928	3267.54015	−1.01	VVEFTCHTAFFVSIVVVQWADLIICK	N-Acetyl, 2 CAM, 1 DTSSP

Identification of Na^+/K^+-ATPase subunit α3 specific peptides by cross-linking/MS

B



C

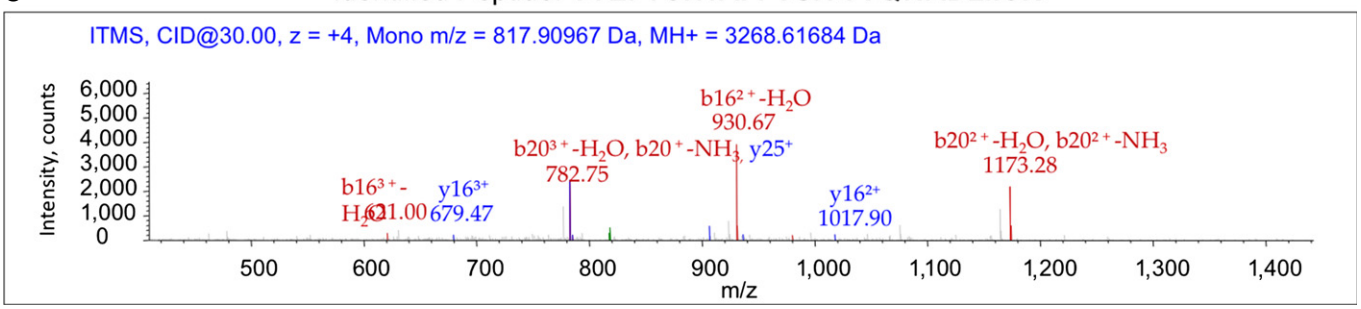


Figure EV1. Assessment of the interaction between α3-NKA and α-syn cross-linking/MS.

A List of α3-NKA peptides identified from neurons exposed for 10 min to fibrillar α-syn, cross-linked with DTSSP, pulled-down, reduced and alkylated, digested by trypsin, and analyzed by MS/MS through an α3-NKA-targeted identification with SEQUEST.

B MS/MS spectrum of α3-NKA peptide TVNDLEDSYGQQWTYEQR [884-901].

C MS/MS spectrum of α3-NKA peptide VVEFTCHTAFFVSIVVVQWADLIICK [903-928] with one N-terminal acetylation (+42 Da), two carbamidomethyl cysteines (Cys908 and Cys927, +57 Da), and one DTSSP cross-linked residue (S915 or K928, +145 Da).

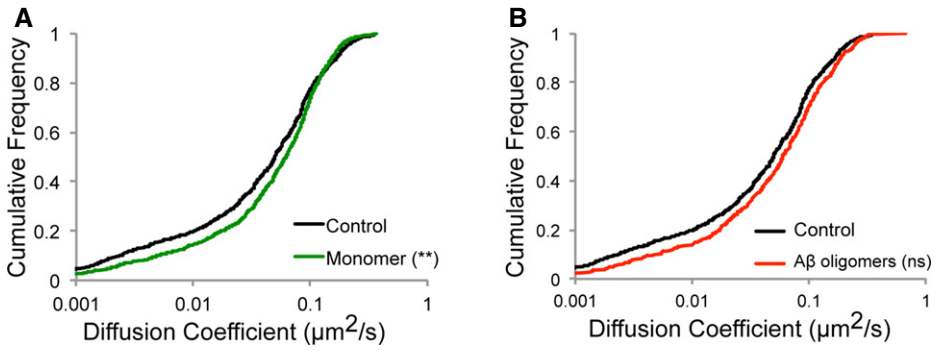


Figure EV2. α3-NKA diffusion in the presence of monomeric α-syn and Aβ oligomers.

A, B Monomeric α-syn-exposed (50 nM, 60 min, green) neurons showed no slowdown in diffusion coefficient of pHluorin-α3-NKA (A) (number of QDs: control, 674; monomer, 865; Kolmogorov–Smirnov test, $^{**}P < 0.01$). (B) No slowdown in the diffusion coefficient of α3-NKA following exposure (200 nM, 60 min) to Aβ oligomers (number of QDs: control, 674; Aβ oligomers, 450; Kolmogorov–Smirnov test, ns = non-significant).

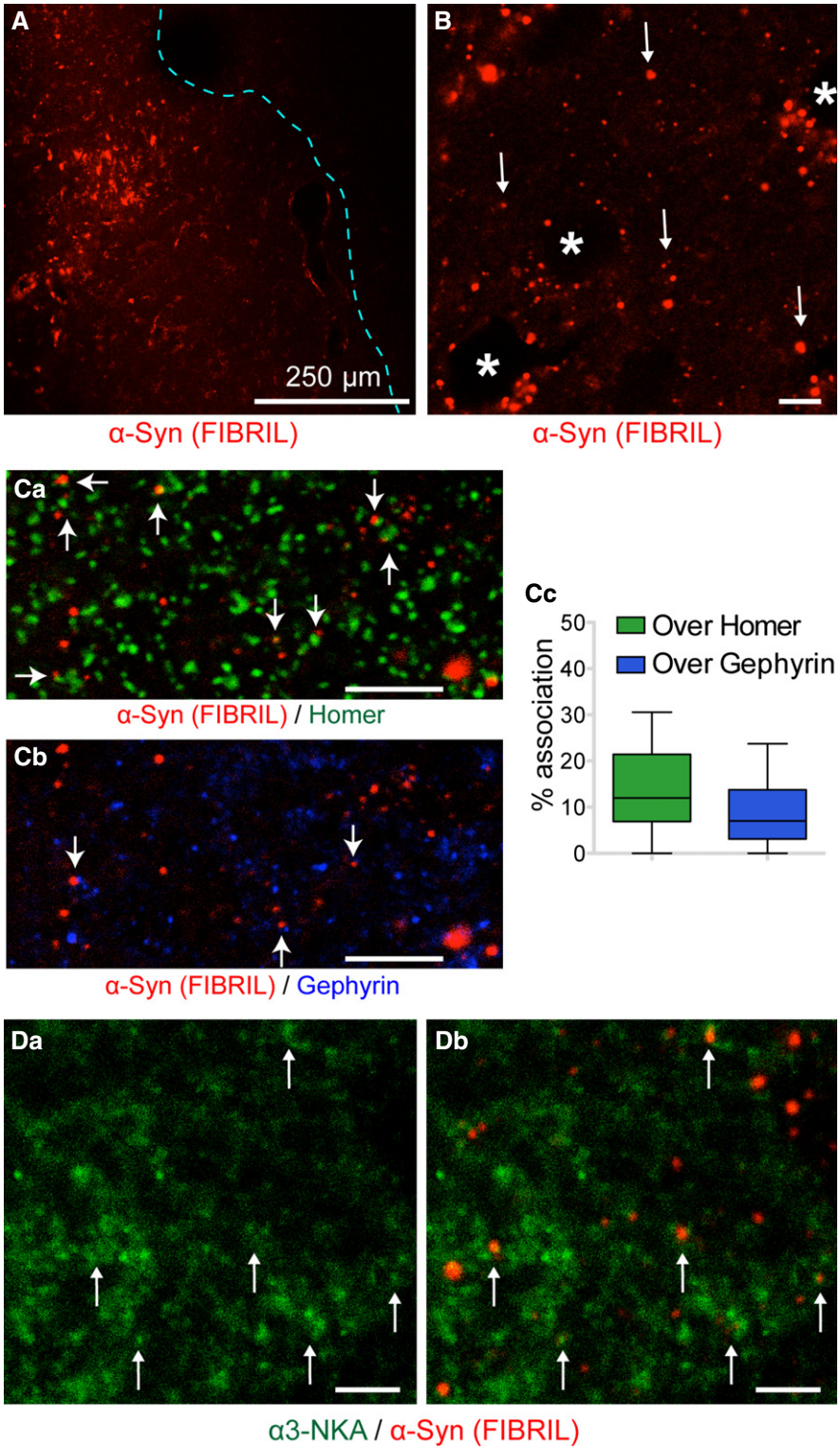


Figure EV3. *In vivo* synaptic clustering of fibrillar α-syn and association with α3-NKA following intra-striatal injection.

A–D Distribution of fibrillar α-syn-ATTO-550 (red) 24 h after injection. (A) Limited spread (outlined by a dashed line) showing ATTO-550 signal. (B) Clusters of fibrillar α-syn (arrow). Note that the clusters are excluded from striato-pallidonigral axon bundles (*). Some of the fibrillar α-syn clusters co-localized with homer (Ca, Cc green) or gephyrin (Cb, Cc, blue) (plot: median, quartile, and min. to max. distribution). (Da-Db) Association of fibrillar α-syn clusters (red, Db) and α3-NKA (green, Da, Db) can be seen (arrow). Unless indicated, scale bars: 5 μm.

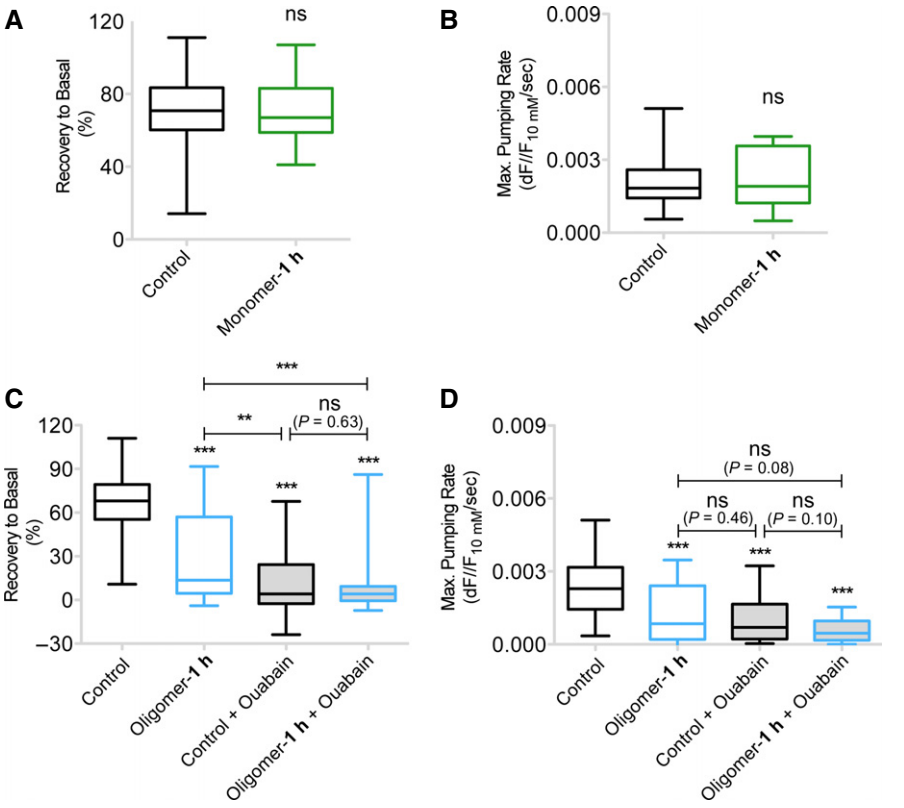


Figure EV4. α3-NKA-dependent Na^+ deregulation by oligomeric but not monomeric α-syn.

A, B Neurons exposed to monomeric α-syn recover to basal level (A) and exhibit no change in Na^+ pumping rate (B).

C, D Na^+ imaging in the presence of ouabain (1 μM, 3–5 min before 0 mM K^+ application) on control or oligomeric α-syn (25 nM) exposed cells. Both oligomeric α-syn-exposed (column 2) and ouabain-treated (column 3) neurons show reduction in the recovery (C) and Na^+ pumping rate (D). Note no additive effect of ouabain on oligomeric α-syn-exposed (column 4) compared to control (column 3) neurons.

Data information: Plots represent median, quartile, and 0–100% distribution; Mann–Whitney *U*-test: $*P < 0.05$, $**P < 0.01$, $***P < 0.001$; two (A, B) or three (C, D) independent experiments have been performed.

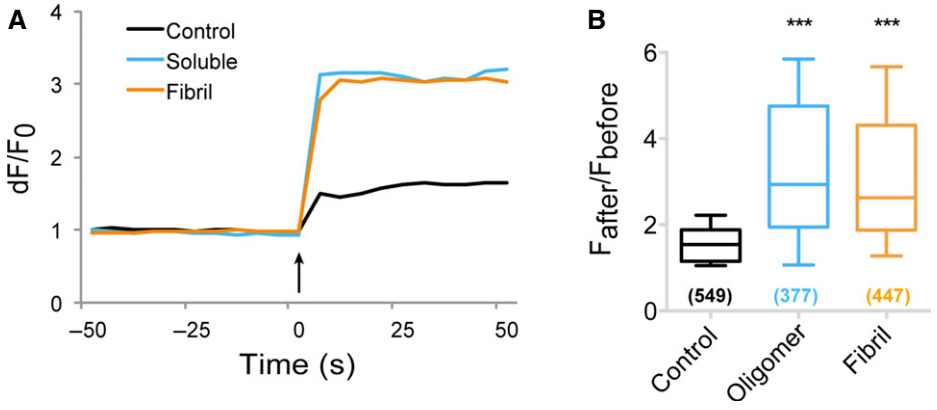


Figure EV5. Increase in glutamate-induced Ca^{2+} influx following α-syn exposure.

A, B DIV 16–19 striatal neurons were exposed (1 h) to oligomeric (25 nM, blue) or fibrillar (0.03 nM, orange) unlabeled α-syn. Unexposed control is shown in black. Glutamate-evoked (arrow, 100 μM, A) Ca^{2+} rise was measured following Fluo-4-AM dye labeling. Note a larger Ca^{2+} influx in cells exposed to α-syn assemblies. Averaged normalized (dF/F_0) fluorescence intensity for all cells is shown (B). Box plot in (B) shows the distribution of change in fluorescence intensity for all cells (box plot: median, quartile, and 10–90% distribution; Mann–Whitney *U*-test: $***P < 0.001$; number of cells (*n*) is shown in parentheses in B; five independent experiments).