Supplementary Material 1

Supplementary Methods

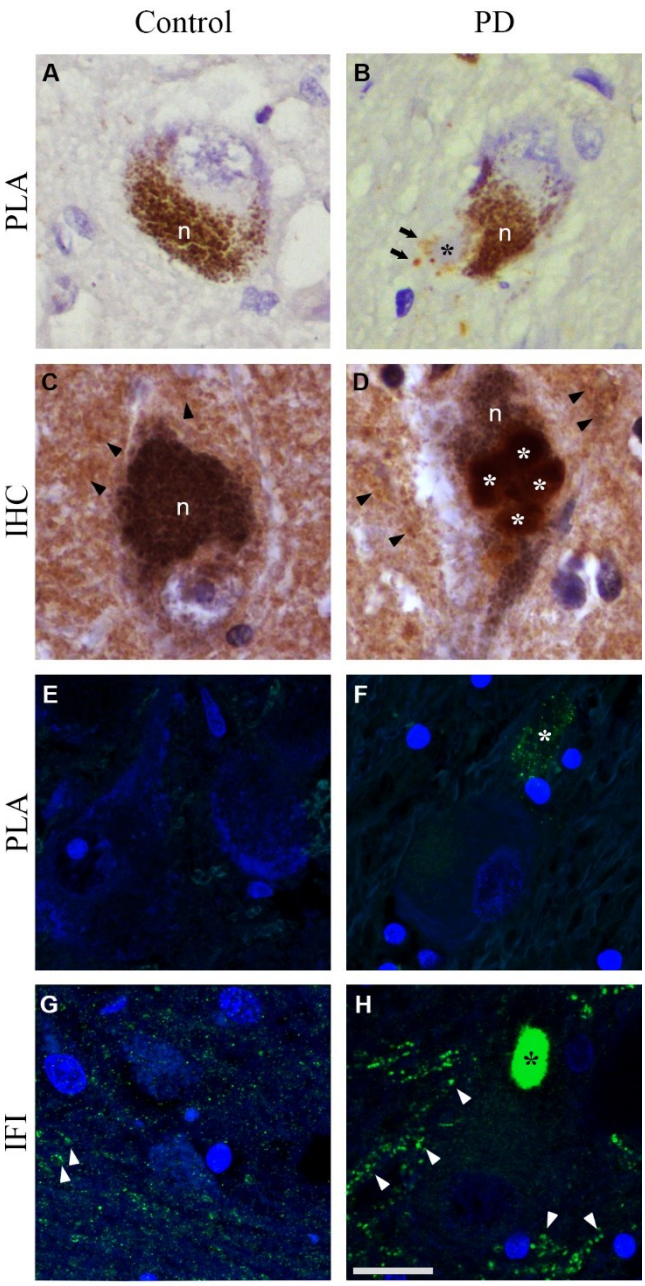
Brain sections of mesencephalic region (control subjects N=3; PD N=4) were obtained from Nervous Tissue Bank of Milan. Written informed consent was obtained prior to acquisition of tissue from all patients. Briefly, the brains were fixed in 10% formaldehyde for at least 1 month at 20°C and paraffin-embedded. The clinical diagnosis was confirmed by neuropathological analysis, done according to the current BrainNet Europe Consortium Guidelines (*Alafuzoff et al., Staging/typing of Lewy body related alpha-synuclein pathology: a study of the BrainNet Europe Consortium. Acta Neuropathol. 2009*). The distribution of α-Synuclein and α-Synuclein PLA staining was performed exactly as described for skin biopsy, with the only difference in pretreatment being that 80% instead of 10% of formic acid was used.

Immunohistochemistry

3-µm skin and 5-µm brain sections from both healthy controls and PD patients were deparaffinized and submitted to classical immunohistochemical assays. After the rehydration procedure samples (sections from skin biopsies and mesencephalic brain sections) were pre-treated in formic acid (10%, 10 minutes for skin samples and 80%, 20 minutes for brain samples). The sections were then incubated with: i) a blocking solution (1% bovine serum albumin, BSA, in 0.01 M PBS plus 0.1% Triton X-100, 20 minutes, room temperature), ii) a mixture of primary antibodies containing anti-α-Synuclein antibody (Sigma-Aldrich, S3062, rabbit, which recognizes a sequence near the C-terminus of human α-Synuclein, amino acids 111-132) diluted 1:2000 plus anti-Synaptophysin antibody (Dako, clone DakSynap, mouse) diluted 1:100 for 2 hours at 37°C with 1% BSA in 0.01M PBS plus 0.1% Triton X-100. For the indirect immunofluorescence procedure, antigens were revealed with the secondary donkey anti-mouse antibodies conjugated to Alexa Fluor®-568 (Molecular Probes) and donkey anti-rabbit antibodies conjugated to Alexa Fluor®-488 (Molecular Probes), diluted respectively 1:200 and 1:100 in 0.1% BSA in 0.01 M PBS (2 hours, 37°C). Finally, sections were counterstained with TO-PRO-3 (Molecular Probes) 1:1000 in 0.01 M PBS for 10 minutes and mounted with PBS plus glycerol (dilution ratio 1:2). For the immunoenzymatic method, antigens were revealed with Envision®-HRP Rabbit secondary antibody (Dako, 1:2 in 0.01 M PBS, 1 hour, RT) and the enzymatic reaction was accomplished with DAB substrate. In the end, nuclei were counterstained with a 10 second incubation in haematoxylin, then samples were dehydrated and mounted with Eukitt® mounting medium (Kaltek). The specificity of anti-α-Synuclein S3062 antibody was assessed by incubating control and PD sections in anti-α-Synuclein antibody preincubated for 4 hours at 37°C with 2 µM human α-Synuclein purified from *E.coli* according to

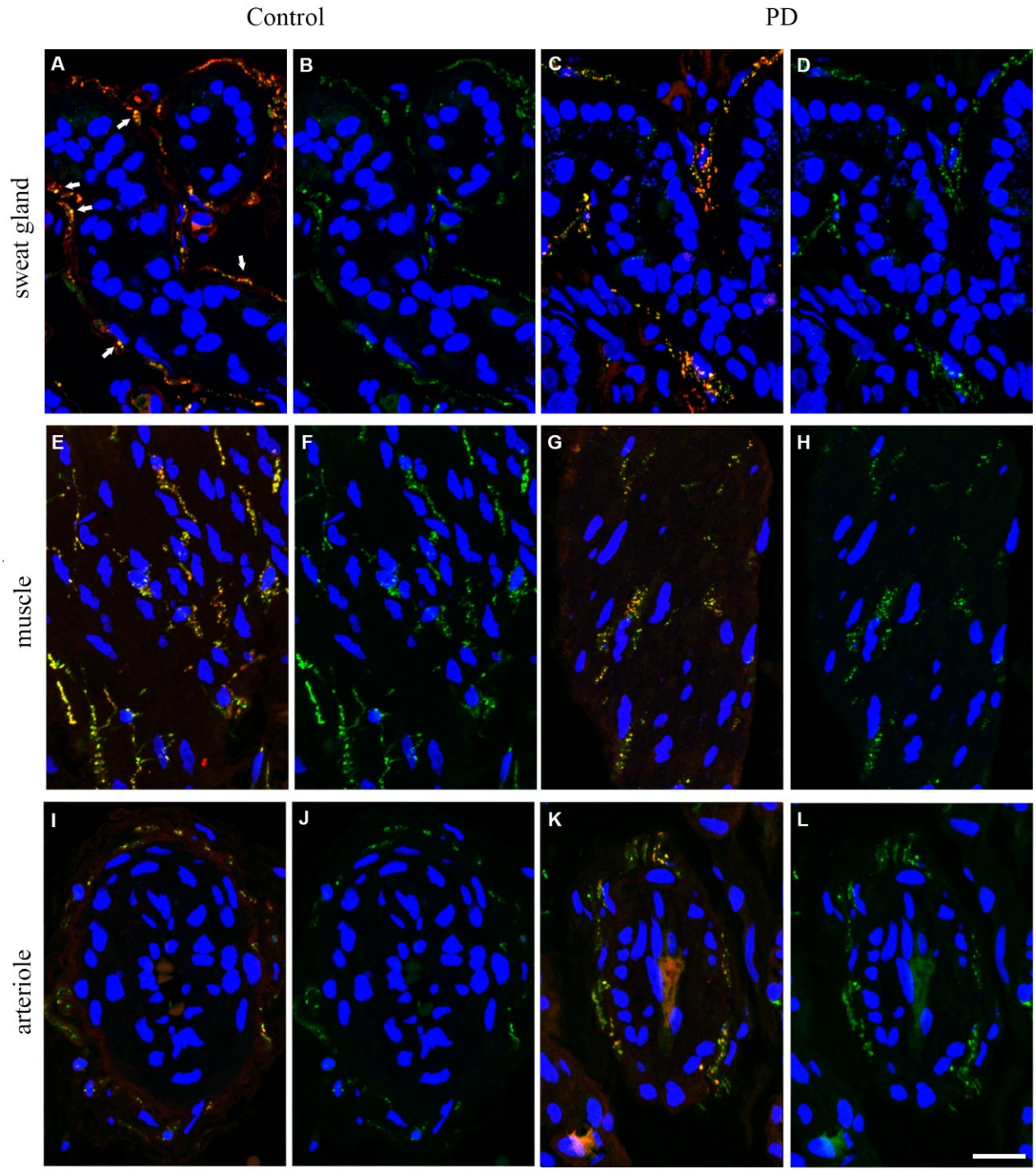
(*Martinez et al., Parkinson's Disease-associated α-Synuclein is a calmodulin substrate. The Journal of Biological Chemistry 2003*) as reported in Supplementary Fig. 3.

Supplementary Figures

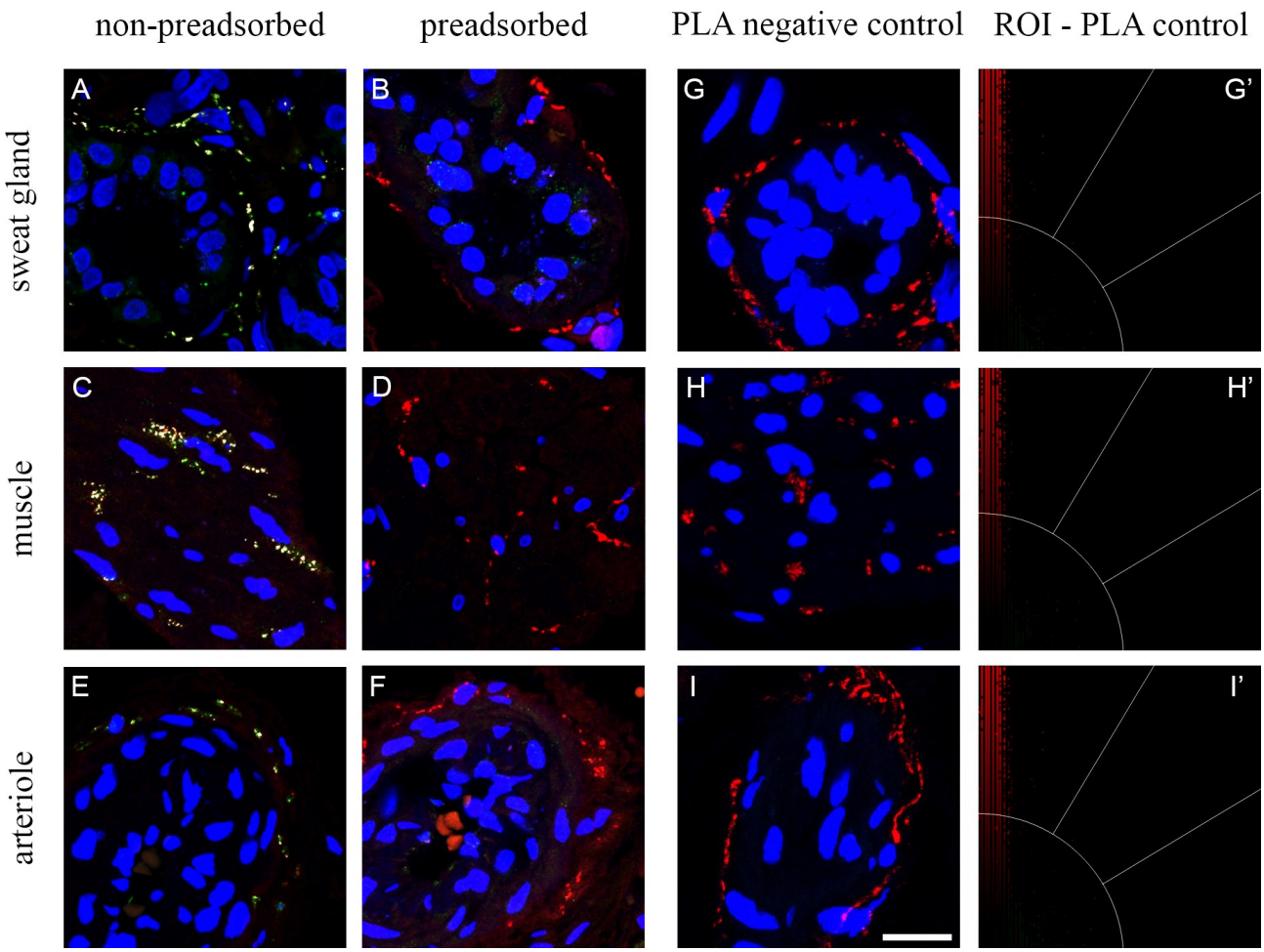


Supplementary Fig. 1. Total and oligomeric α-Synuclein in *substantia nigra.* α-Synuclein-PLA (**A, B, E, F**) and total α-Synuclein (**C, D, G, H**) staining in *substantia nigra* obtained from *post-mortem* human brains of controls (**A, C, E, G**) and Parkinson's disease patients (**B, D, F, H**). Total α-Synuclein was stained using an immunoenzymatic procedure (**C**, **D**; brown signal) or using indirect immunofluorescence (**G**, **H**; green signal), revealing its typical synaptic pattern both in controls and patients (arrowheads). Furthermore, in PD brains (**D**, **H**) it marked Lewy bodies (asterisks), which

were absent in controls. α-Synuclein-PLA signal revelation was performed both with Duolink® detection brightfield (**A**, **B**; brown dots) and with Duolink® detection green (**E**, **F**; green dots). α-Synuclein-PLA does not recognize physiological widespread α-Synuclein, it points out the pathological oligomeric forms in the immediate vicinity of pale bodies (**B**, **F**). 4-step images were collected and reconstructed in a Z-stack. n = neuromelanin. Scale bar, 20 µm.



Supplementary Fig. 2. Total α-Synuclein staining within synaptic terminals in skin biopsies. In sweat glands (**A**, **B**, **C**, **D**), *arrector pilorum* muscles (**E**, **F**, **G**, **H**) and blood vessels (**I**, **L**, **M**, **N**) α-Synuclein (green) is constitutively expressed within the synaptic terminals (synaptophysin-positive, red), both in healthy subjects (**A**, **B**, **E**, **F**, **I**, **J**) and Parkinson's disease patients (**C**, **D**, **G**, **H**, **K**, **L**). Nuclei were counterstained with TOPRO-3 (blue). The resulting co-localization of the two antigens (yellow signal) is shown in **A**, **C**, **E**, **G**, **I**, **K**. Total α-Synuclein distribution is shown, respectively, in **B**, **D**, **F**, **H**, **J**, **L**. Scale bar, 20 μm.



Supplementary Fig. 3. Negative controls. A-F show the staining in immunofluorescence for assessing the specificity of anti-α-Synuclein antibody. In **A, C, E** sections were incubated with non-preadsorbed antibody. α-Synuclein signal (green) is visible and, as expected, colocalizes with synaptophysin (red). Colocalization area is highlighted by white dots. In **B, D, F** sections were incubated with preadsorbed antibody, as described in Supplementary methods. No α-Synuclein is visible within synaptophysin signal (no white area). **G-I** show the negative control for PLA staining, performed by omitting one of the two probes (no white area). In **G'-I',** the region of interest (ROI),

which is defined for colocalization (**G'-I'**) obtained with Leica sp8 LasX software, is shown and no green signal is visible. Scale bar, 20 µm.