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Supplementary Information for

In Situ Proximity Labeling Identifies Lewy Pathology Molecular Interactions in the Human Brain

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This PDF file includes:

Other supplementary materials for this manuscript include the following:

Dataset S1 (separate file). Differentially abundant proteins between synucleinopathy and healthy controls.BAR-SYN1.
Dataset S2 (separate file). Differentially abundant proteins between synucleinopathy and healthy controls.BAR-PSER129.
Dataset S3 (separate file). gprofiler output. Significantly enriched pathways. BAR-PSER129.
Dataset S4 (separate file). gprofiler output. Significantly enriched pathways. BAR-SYN1.
Dataset S5 (separate file). Log2 transformed values for all proteins identified in all samples by mass spectrometry. See "table 1" for specimen information. Naming convention for columns are Case #_Capture Condition.

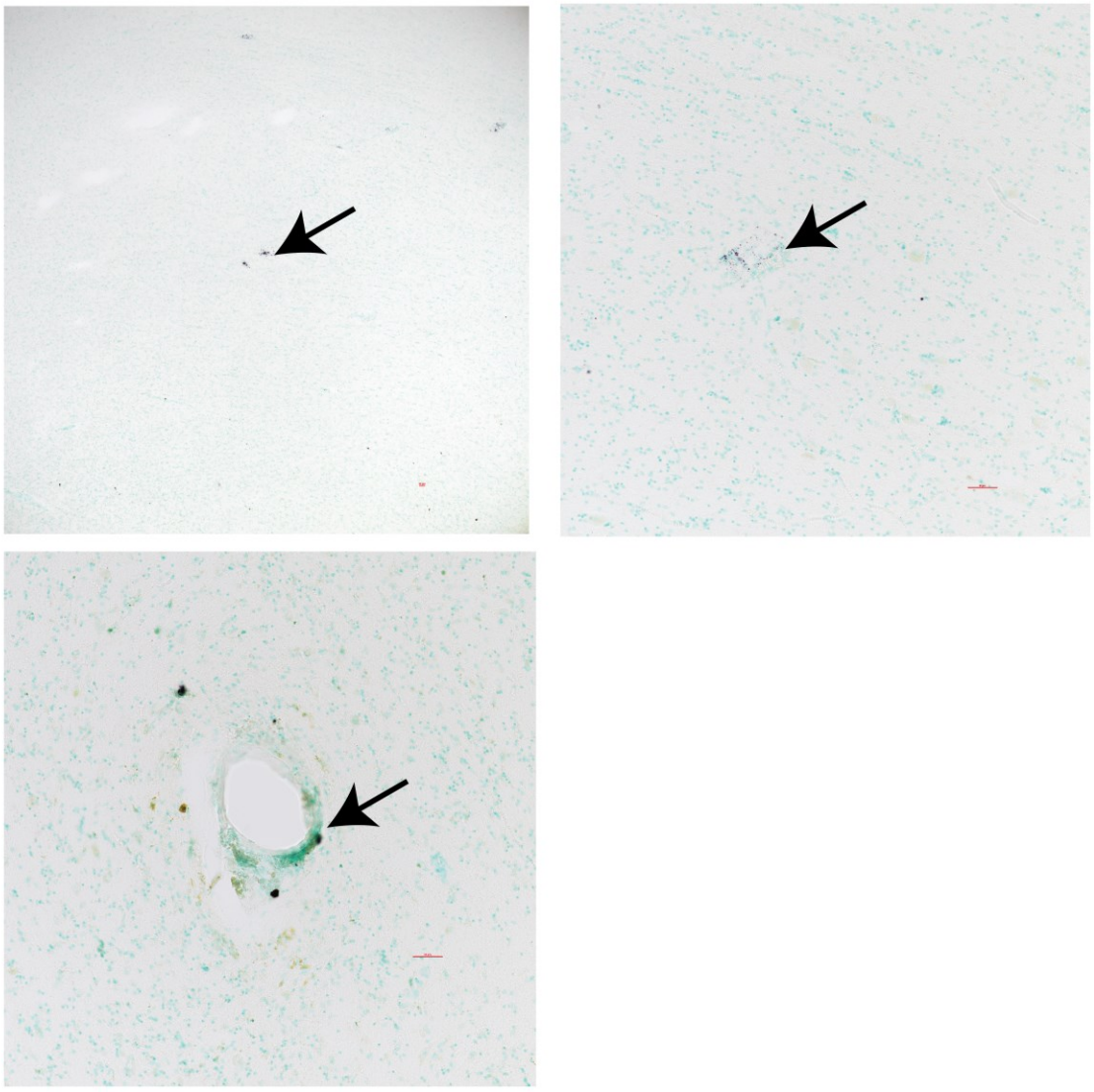

Figure S1. Rare BAR-PSER129 immunoreactivity in healthy controls (HC). Arrows highlight examples of unidentified immunoreactive structures. Red scale bar included for each image. Positive structures appear black, and nuclei are counterstained with methyl green. Similar structures observed in all HC samples. n= 3.

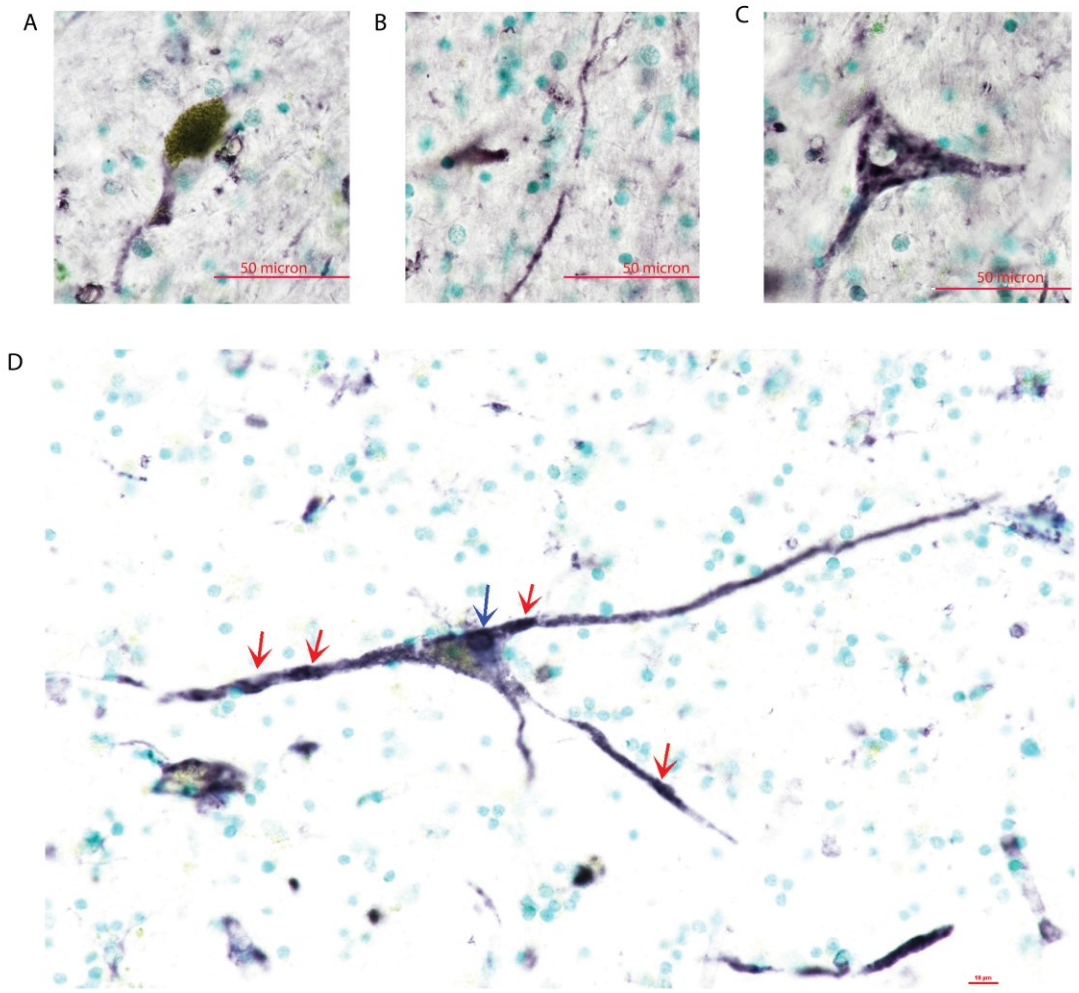

Figure S2. HBB labeled Lewy pathology. Fixed floating human synucleinopathy brain sections were labeled for HBB using the BAR protocol and nickel enhanced DAB detection. High magnification images of midbrain dopamine neurons with apparent HBB labeled Lewy pathologies. (A) Neuromelanin containing neuron with diffuse cytoplasmic labeling, and apparent HBB accumulation in processes. (B) Apparent HBB positive dysmorphic neurite. (C) HBB positive neuron with apparent HBB positive perinuclear accumulations. (D) Neuromelanin containing neuron containing several accumulations in processes (red arrows) and a cytosolic ring shaped HBB positive structure (Blue arrow). n= 3.

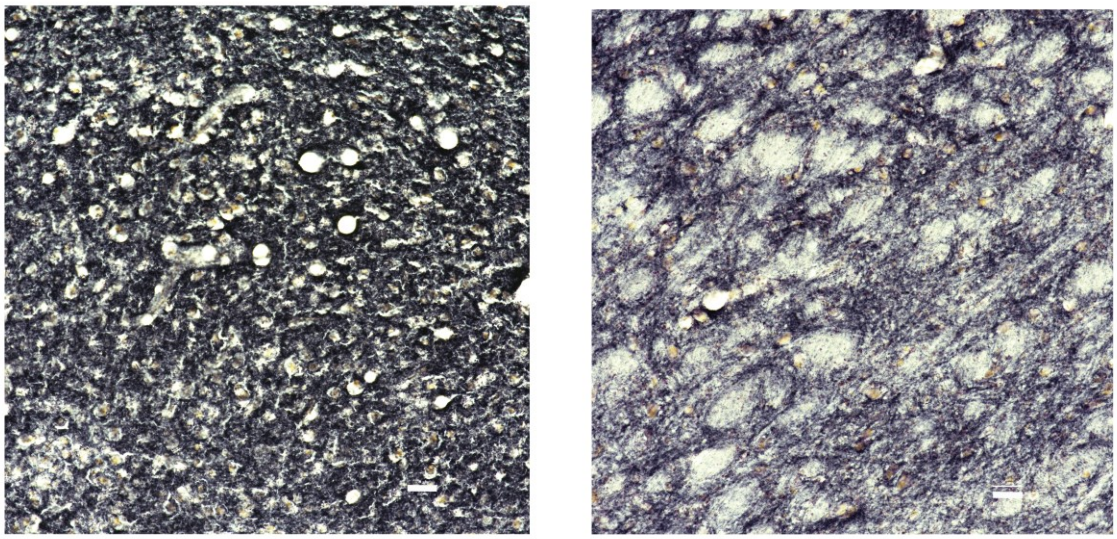

Figure S3. BAR-SYN1 immunoreactivity. Fixed floating human synucleinopathy brain sections were labeled with the BAR-SYN1 protocol. Subsequent nickel enhanced DAB detection of labeled process in cortex (Left Panel) and striatum (Right Panel) n= 2. Scale bars = 50 microns.

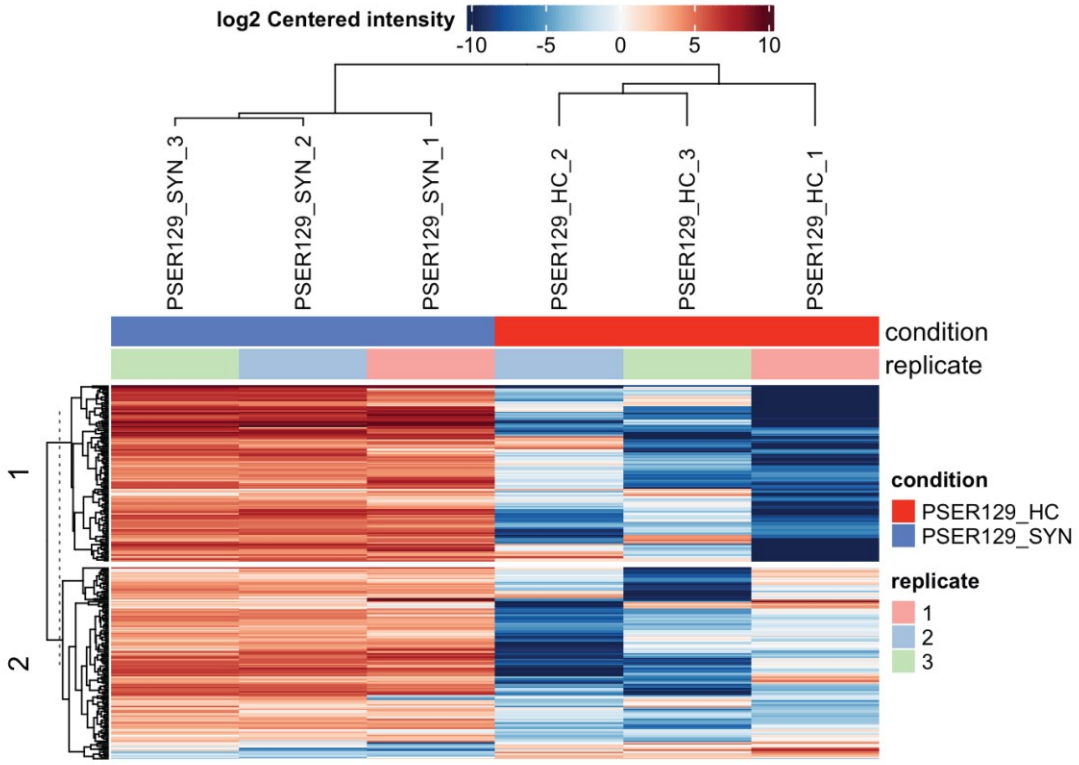

Figure S4. Heatmap of normalized BAR-PSER129 captured proteins. Unbiased hierarchical clustering of normalized values for BAR-PSER129 identified proteins from synucleinopathy and non-synucleinopathy brains. Results show samples are differentiated according to condition (i.e., disease state).

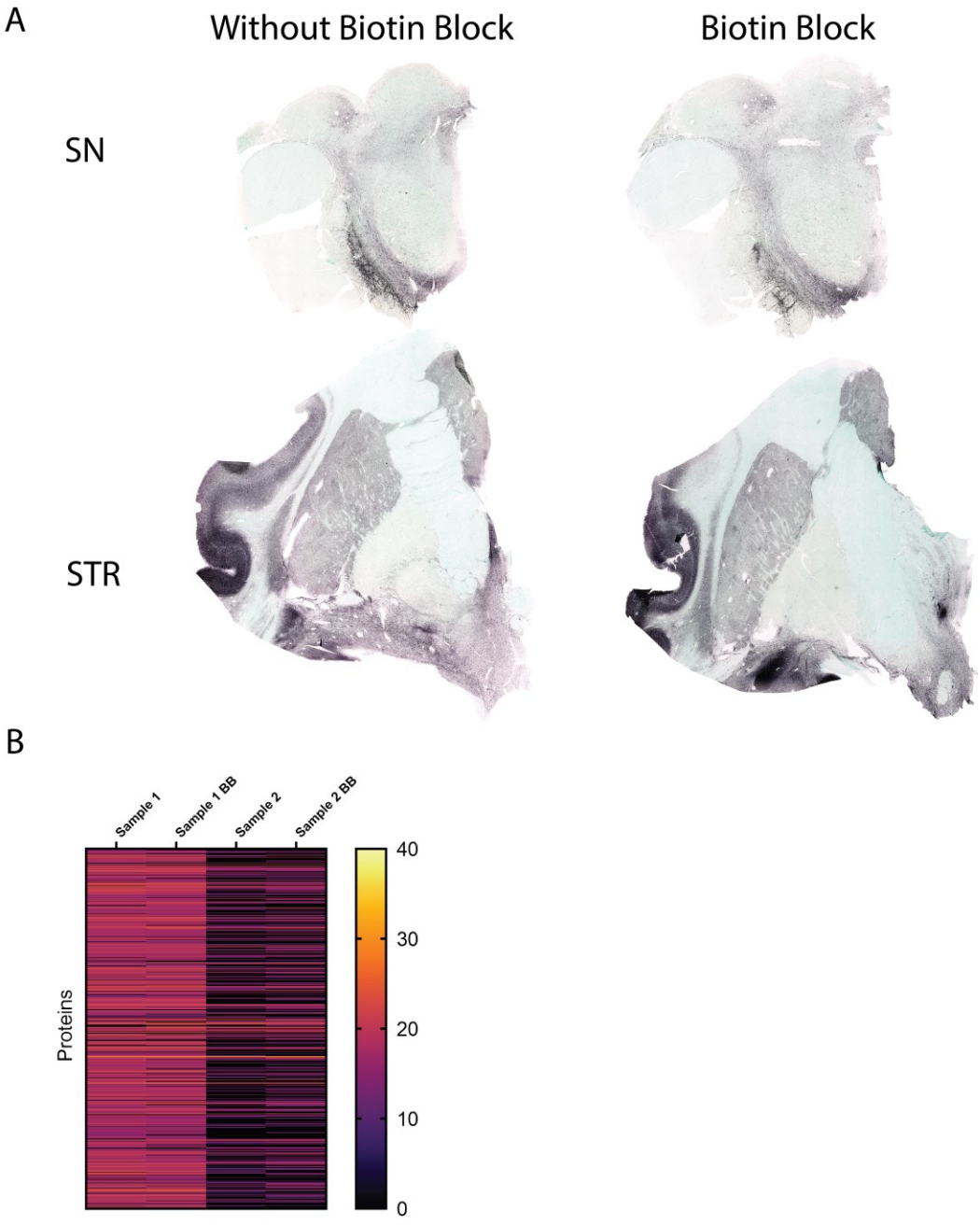

Figure S5. Additional biotin blocking controls. (A) Brain sections from the same individual with Parkinson's disease were labeled using the BAR-PSER129 protocol with and without conducting an endogenous biotin blocking protocol. Whole slide scans containing the substantia nigra (SN) and striatum (STR) demonstrate BAR-PSER129 labeling under both conditions. (B) BAR-PSER129 was conducted on a brain sample from Parkinson's disease ("sample 1") and a brain sample from a neurologically intact individual ("sample 2"). Matched brain samples were processed using the same BAR-PSER129 protocol with the exception that endogenous biotin was blocked (denoted BB). Heat map of proteins identified in each condition is shown. Heatmap scale is log2 transformed protein abundance.

A

B

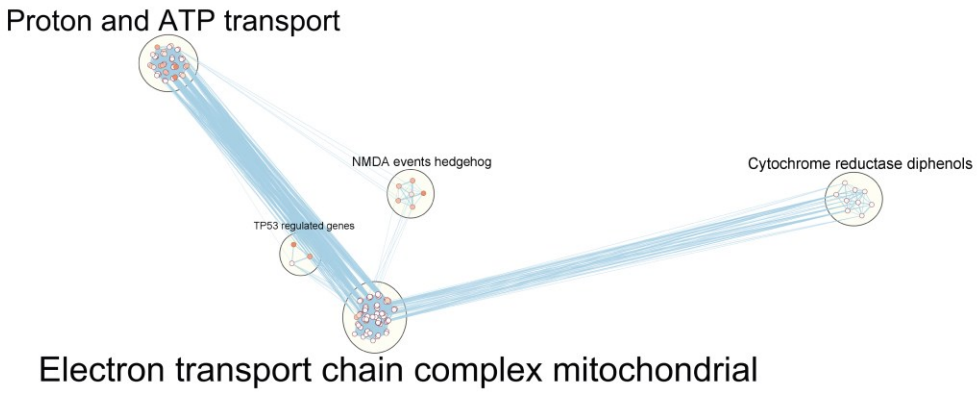

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Figure S6. KEGG Parkinson's disease pathways enrichment. (A) KEGG pathway enrichment following the removal of alpha-synuclein from the BAR-PSER129 identified proteins. Depicted are all resulting enriched pathways with Parkinson's disease denoted by "1." (B) Components of KEGG Parkinson's disease pathway enriched by BAR-PSER129. BAR-PSER129 identified proteins found in the KEGG Parkinson's disease pathway were analyzed by gprofiler using database including GO biological process, GO molecular function, and GO cellular component. Significantly enriched pathways were mapped using cytoscape software. Auto-annotations are displayed above each cluster of nodes.

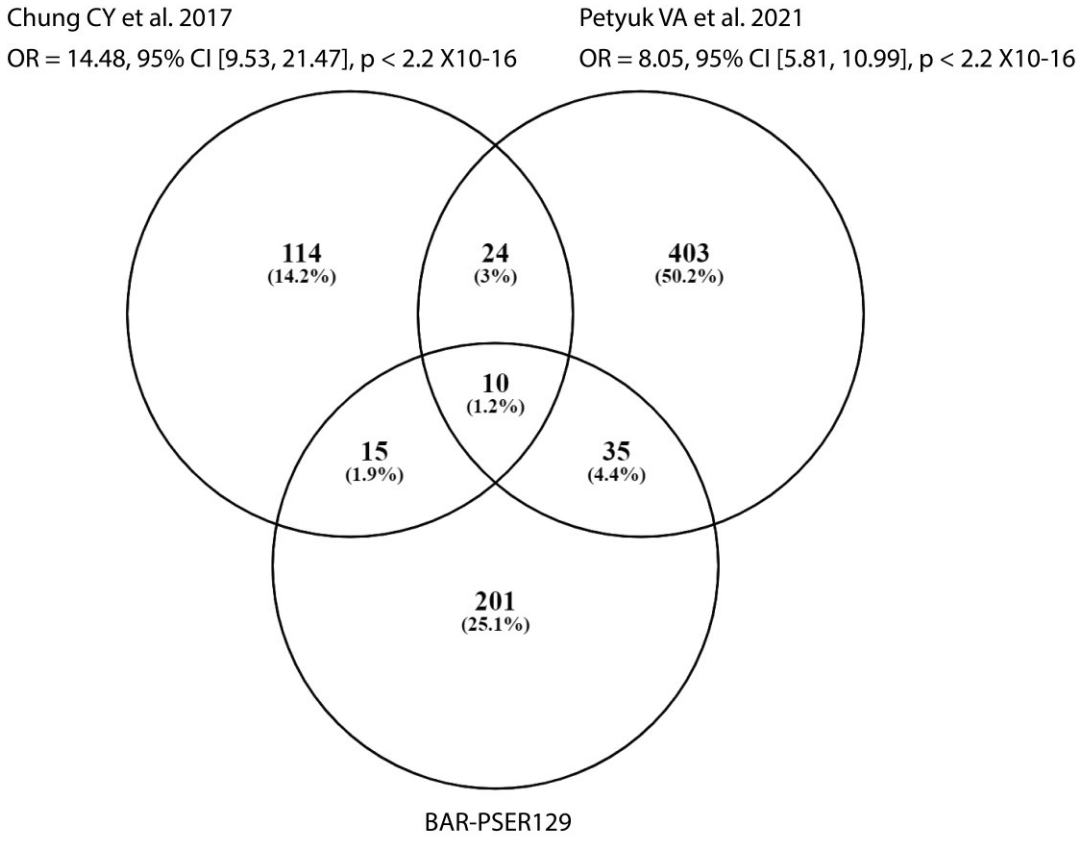

Figure S7. Overlap of BAR-PSER129 identified proteins with two relevant data sets. The first data set Chung CY et al. 2017 used in vivo proximity labeling to identify alpha-synuclein interacting proteins in primary rat cortical neurons (1). The second comparison data set Petyuk VA et al. 2021 performed mass spectrometry on lysates from healthy and Parkinson's disease substantia nigra tissues(2). For both comparison data sets, significantly enriched proteins were selected as identified by the original analysis. Depicted in the Venn diagram are the number of proteins and percentage of all proteins in parenthesis. Venn diagram was generated using Venny 2.0. The calculated odds ratio (a measure of enrichment) is included for each comparison data set. Fishers exact test was used to calculate significance of each odds ratio.

SI References

1. Chung CY, *et al.* (2017) In Situ Peroxidase Labeling and Mass-Spectrometry Connects Alpha-Synuclein Directly to Endocytic Trafficking and mRNA Metabolism in Neurons. *Cell Syst* 4(2):242-250 e244.
2. Petyuk VA, *et al.* (2021) Proteomic Profiling of the Substantia Nigra to Identify Determinants of Lewy Body Pathology and Dopaminergic Neuronal Loss. *J Proteome Res* 20(5):2266-2282.