Proteomic analysis of urinary extracellular vesicles reveal biomarkers for neurologic disease

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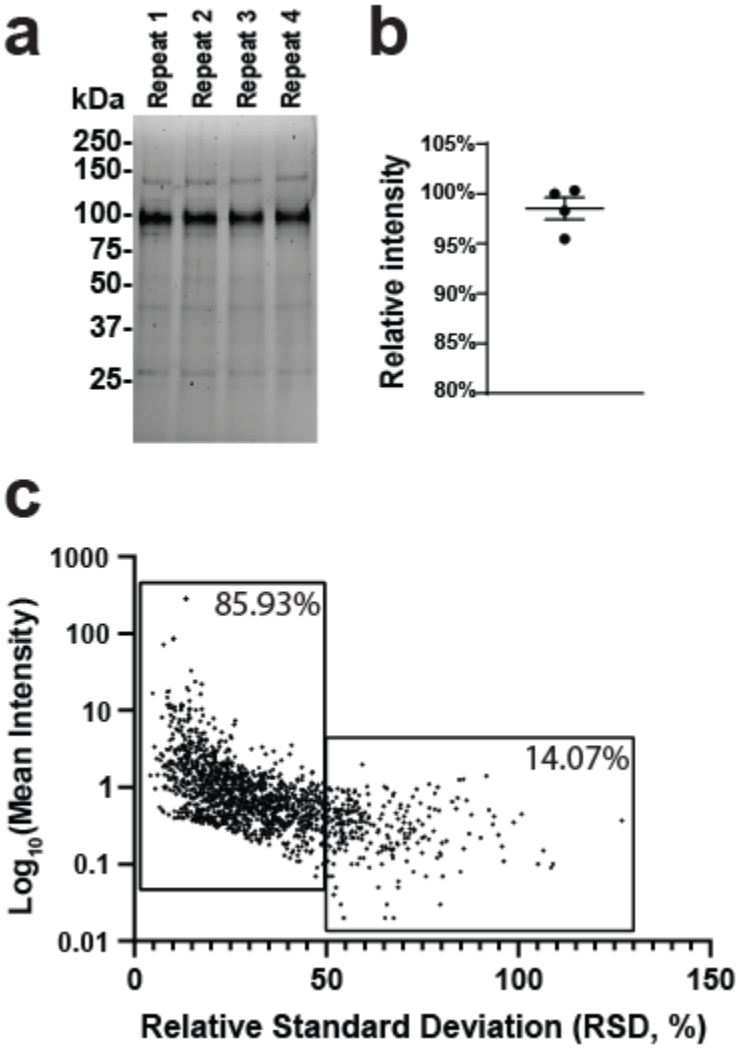
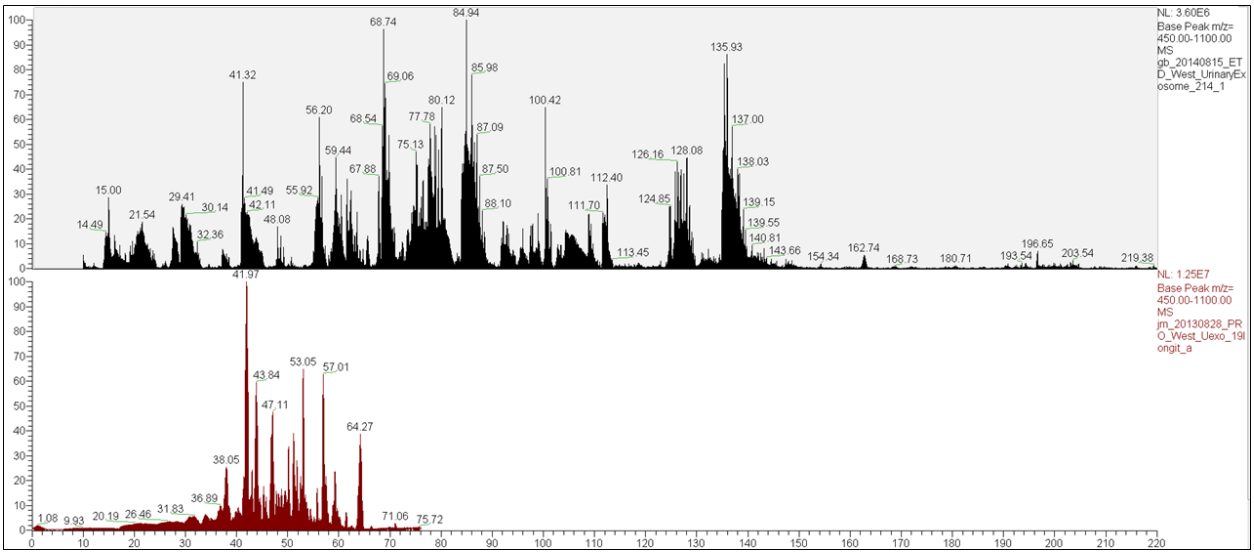
Supporting data

Supplemental Figures 1-7



Supplemental figure 1. Reproducibility of urine extracellular vesicle (EV) isolation and mass spectrometry detection. (a) 40 mL of urine sample was equally divided into four fractions and urine EVs were processed at the same time (repeat 1-4). Protein-stained gel was shown for total protein detection and **(b)** quantification. Bars show mean and S.E.M. **(c)** RSD and mean intensity of each protein measured (see Methods) from six EV isolations from the same sample. 85.93% of proteins has RSD lower than 50%.



Supplemental figure 2. Comparison of single random LCMS runs from the longitudinal study and discovery cohort. Upper half: Discovery cohort - LTQXL 220min nLCMS run, Triple Play (~20K Res, no exclusions), 46,261 MS2 (450 protein ID's). Lower half: Longitudinal study - Velos Pro Orbitrap 75 min nLCMS run, MS1 HiRes (~60K Res, exclude +1 m/z), 14,629 MS2 (667 protein ID's). Search Engine used standard MASCOT only.

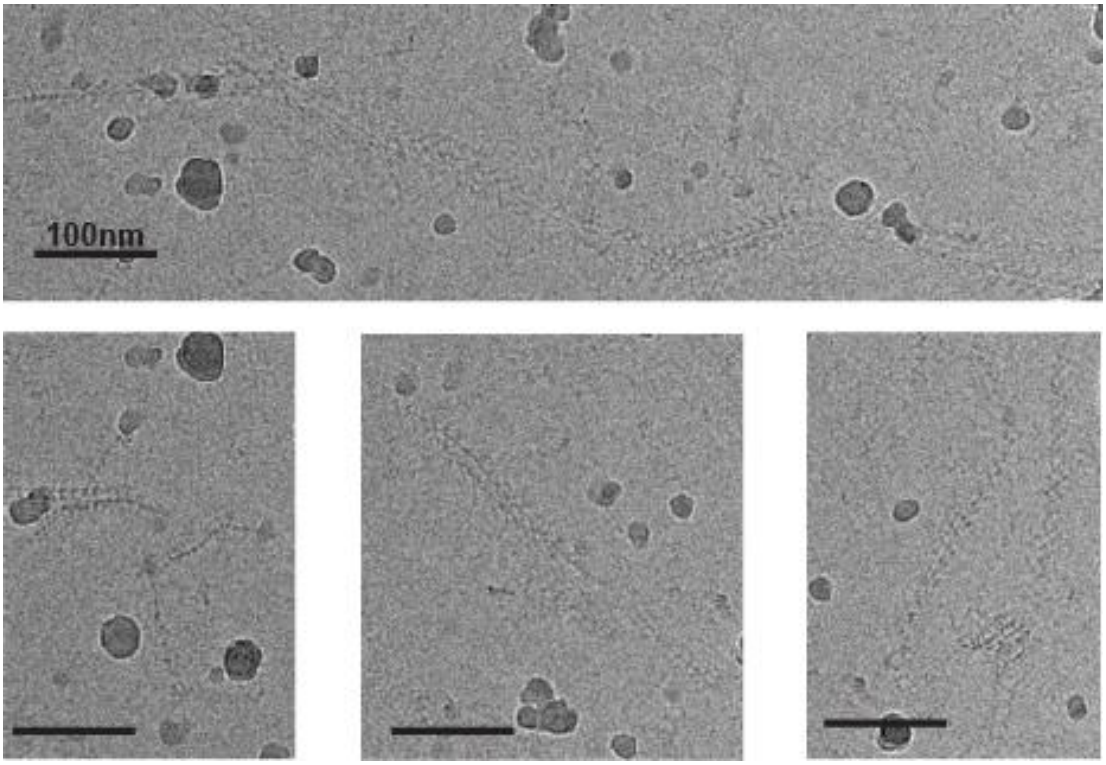
a

Top Pathways	Fold Enrichement	*p* value (FDR corrected)
Huntington Disease	+9.7	$1.3x10^{-12}$
Cytoskeletal regulation by Rho GTPase	+12.2	$4.65x10^{-10}$
Integrin signalling pathway	+6.7	$4.3x10^{-9}$
Ras pathway	+10.3	$2.7x10^{-7}$
Parkinson Disease	+8.1	$8.0x10^{-7}$

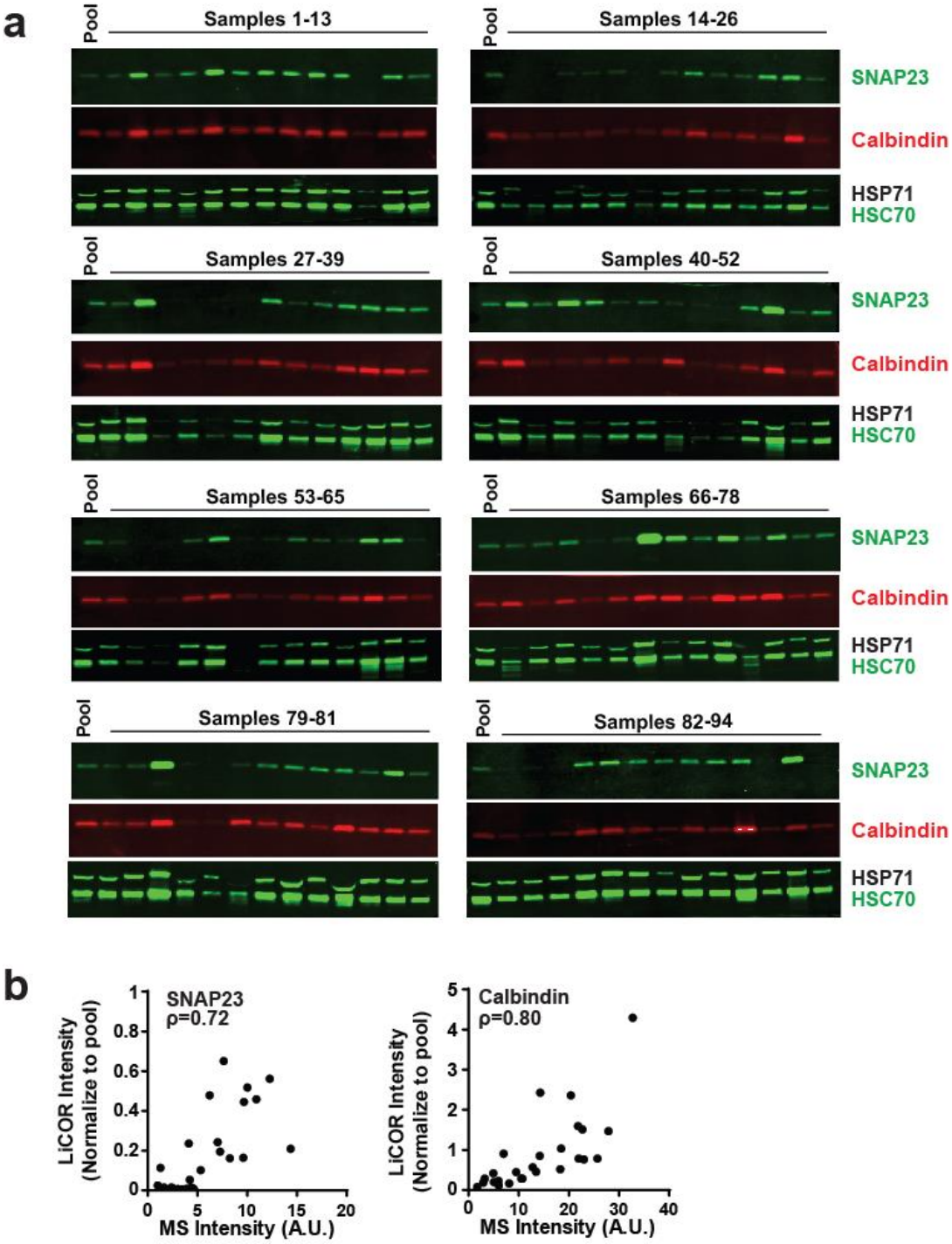
b

Top Pathways	Fold Enrichement	*p* value (FDR corrected)
Cognitive trait	+6.5	$3.6x10^{-5}$
Aging/Telomere Length	+6.5	$4.0x10^{-5}$
Nephrotic Syndrome	+13.0	$2.0x10^{-2}$
HIV	+3.7	$5.1x10^{-2}$

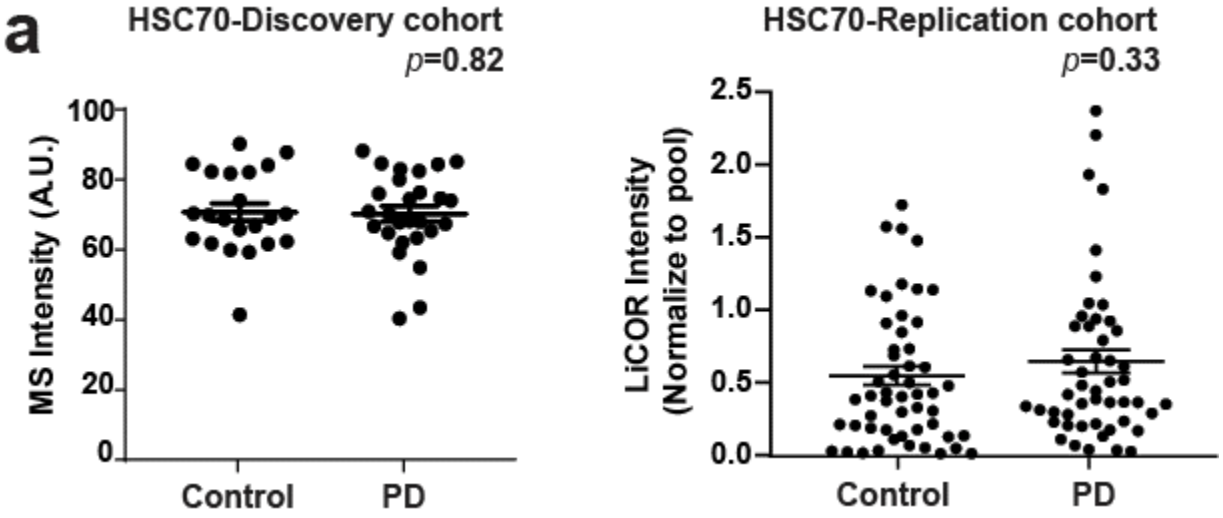
Supplemental figure 3. Pathway analysis for stable proteins. (a). Tissue enrichment analysis (DAVID bioinformatics resources 6.8.) and **(b).** pathway analysis (PANTHER version 13.1) for the most stable proteins (RSD<50%) in urinary EVs.



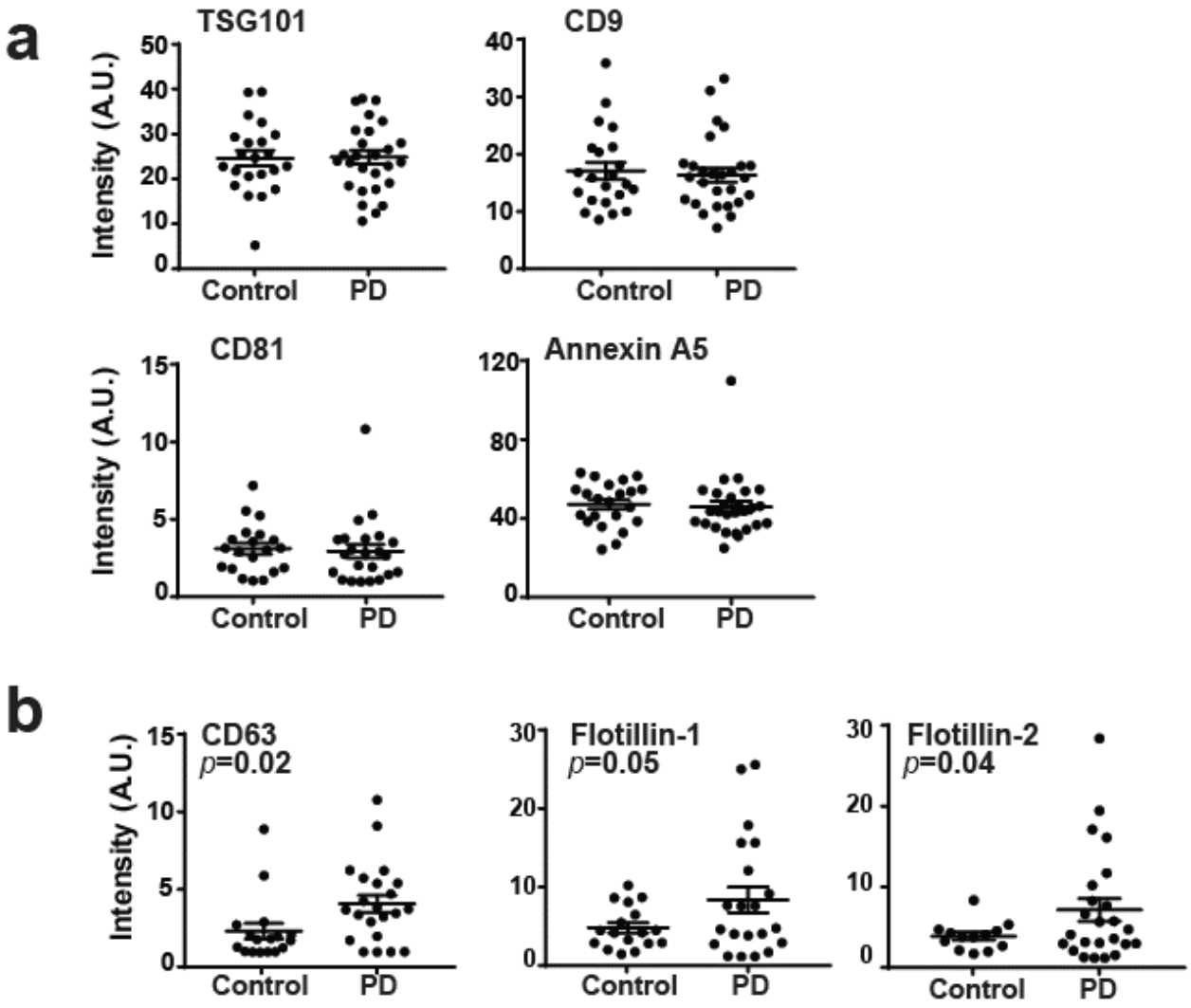
Supplemental figure 4. Protein fibrils and aggregates under cryo-electron microscopy (EM). Representative images of different types of protein fibrils under cryo-EM. Scale bar is 100 nm.



Supplemental figure 5. LiCOR replication with MS measurement. a. Representative blots from part of replication cohort using LiCOR assay. SNAP23, Calbindin and HSPA8 are detected from the same PVDF membrane that was cut in half. Pooled sample (Pool) was loaded in first lane of each gel as internal control. **b.** Correlation between the mass spec intensities (A.U.) and LiCOR measurements for Calbindin and SNAP23 from the same subjects in discovery cohort. Correlation was calculated using Spearman's ρ.



Supplemental figure 6. HSC70 in discovery cohort and replication cohort. a. Plots showing HSPA8 intensity in PD and control groups in discovery cohort measured using mass spectrometry. Bars showing the mean value with error bars showing S.E.M. b**.** Plots showing Calbindin intensity and SNAP23 intensity in PD and control groups in replication cohort measured using LiCOR assay. Bars showing the mean value with error bars showing S.E.M. *p*-value was calculated using student t-test.



Supplemental figure 7. Extracellular vesicle (EV) markers in discovery cohort measured using mass spectrometry. (a). Plots showing EV markers intensity that are significantly different between PD and control groups in discovery cohort. Bars showing the mean value with error bars showing S.E.M. **(b).** Plots showing EV markers that are significantly different between PD and control groups in discovery cohort. Bars showing the mean value with error bars showing S.E.M. *p*-value was calculated using student t-test.