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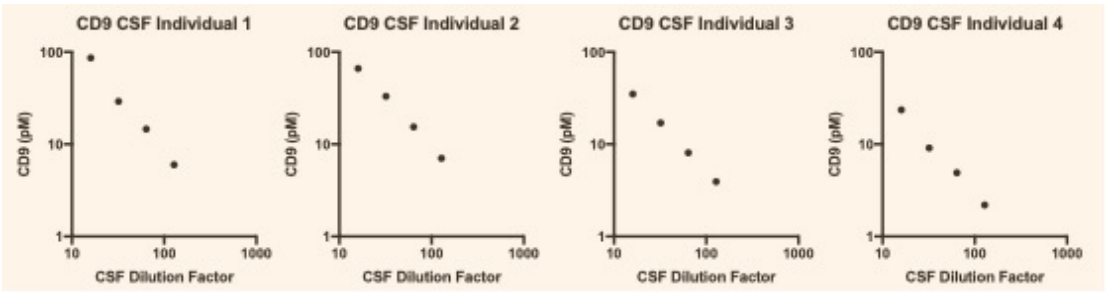
Supplementary information

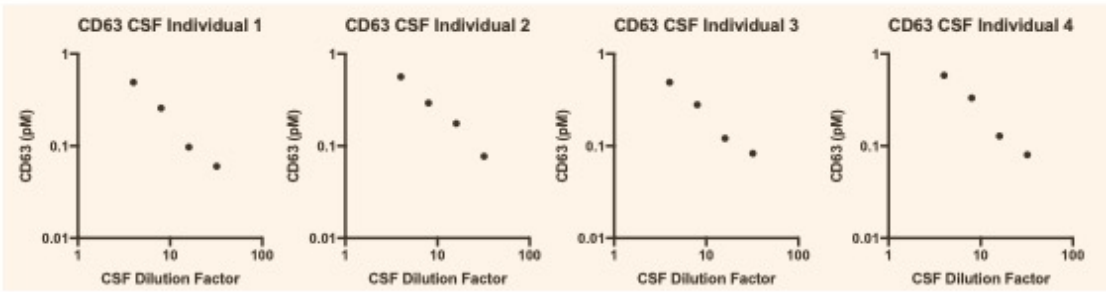
L1CAM is not associated with extracellular vesicles in human cerebrospinal fluid or plasma

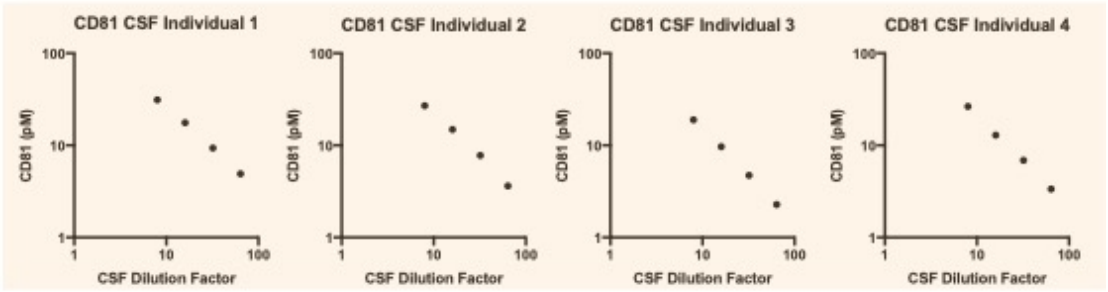
In the format provided by the authors and unedited

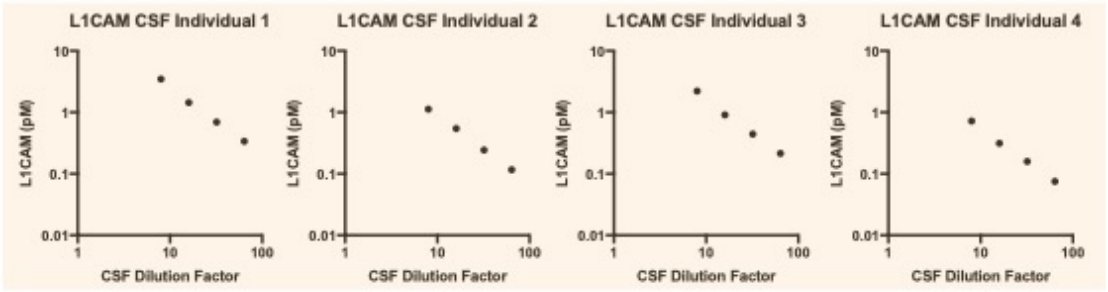
Supplementary Figure 1: Linearity of dilution for Simoa assays
a) Endogenous dilution linearity (parallelism) in human CSF samples for each Simoa assay (CD9, CD63, CD81, L1CAM and albumin). Data represent the average of two technical replicates. Each dilution linearity experiment was performed on CSF from four individuals.
b) Endogenous dilution linearity (parallelism) in human plasma samples for each Simoa assay (CD9, CD63, CD81, L1CAM and albumin). Data represent the average of two technical replicates. Each dilution linearity experiment was performed on plasma from four individuals.

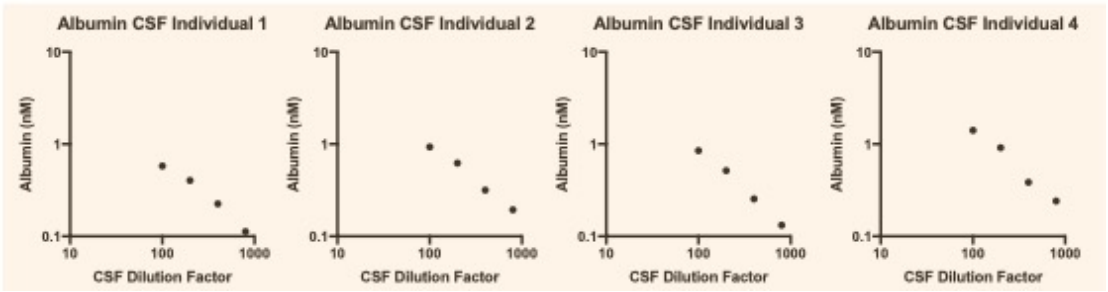
a.


CD9 CSF Individual 1
CD9 CSF Individual 2
CD9 CSF Individual 3
CD9 CSF Individual 4
CD9 (pM)
CSF Dilution Factor

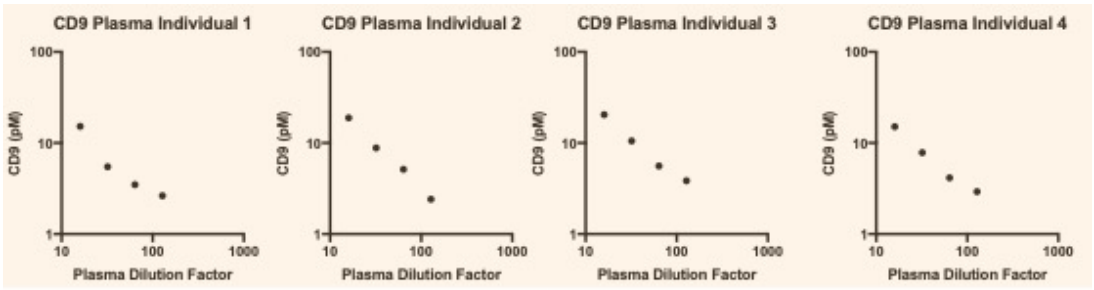

CD63 CSF Individual 1
CD63 CSF Individual 2
CD63 CSF Individual 3
CD63 CSF Individual 4
CD63 (pM)
CSF Dilution Factor

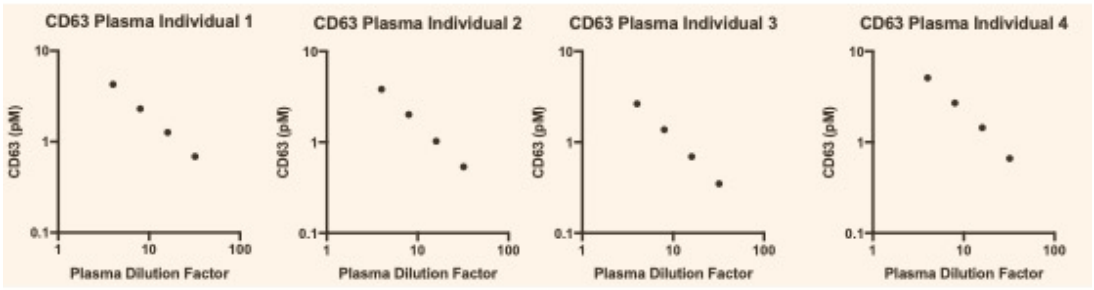

CD81 CSF Individual 1
CD81 CSF Individual 2
CD81 CSF Individual 3
CD81 CSF Individual 4
CD81 (pM)
CSF Dilution Factor

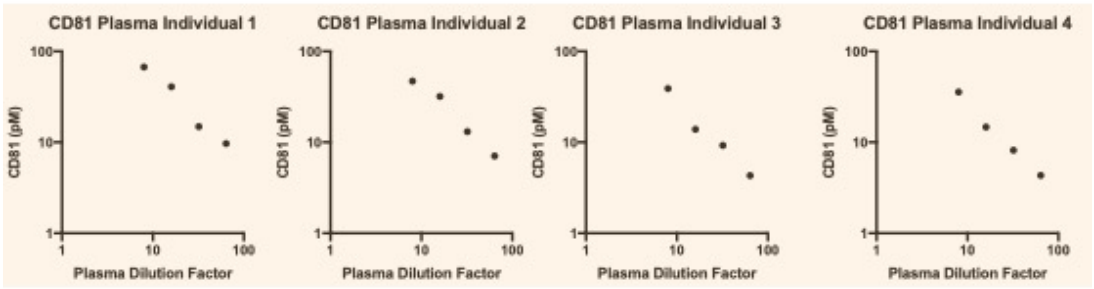

L1CAM CSF Individual 1
L1CAM CSF Individual 2
L1CAM CSF Individual 3
L1CAM CSF Individual 4
L1CAM (pM)
CSF Dilution Factor

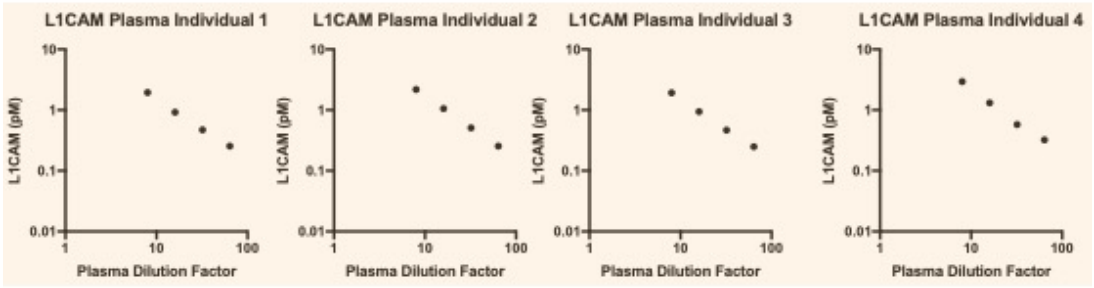

Albumin CSF Individual 1
Albumin CSF Individual 2
Albumin CSF Individual 3
Albumin CSF Individual 4
Albumin (nM)
CSF Dilution Factor

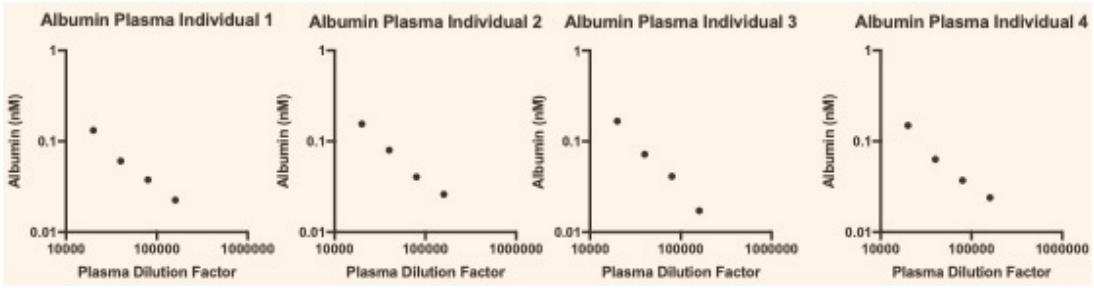
b.


CD9 Plasma Individual 1
CD9 Plasma Individual 2
CD9 Plasma Individual 3
CD9 Plasma Individual 4
CD9 (pM)
Plasma Dilution Factor


CD63 Plasma Individual 1
CD63 Plasma Individual 2
CD63 Plasma Individual 3
CD63 Plasma Individual 4
CD63 (pM)
Plasma Dilution Factor


CD81 Plasma Individual 1
CD81 Plasma Individual 2
CD81 Plasma Individual 3
CD81 Plasma Individual 4
CD81 (pM)
Plasma Dilution Factor

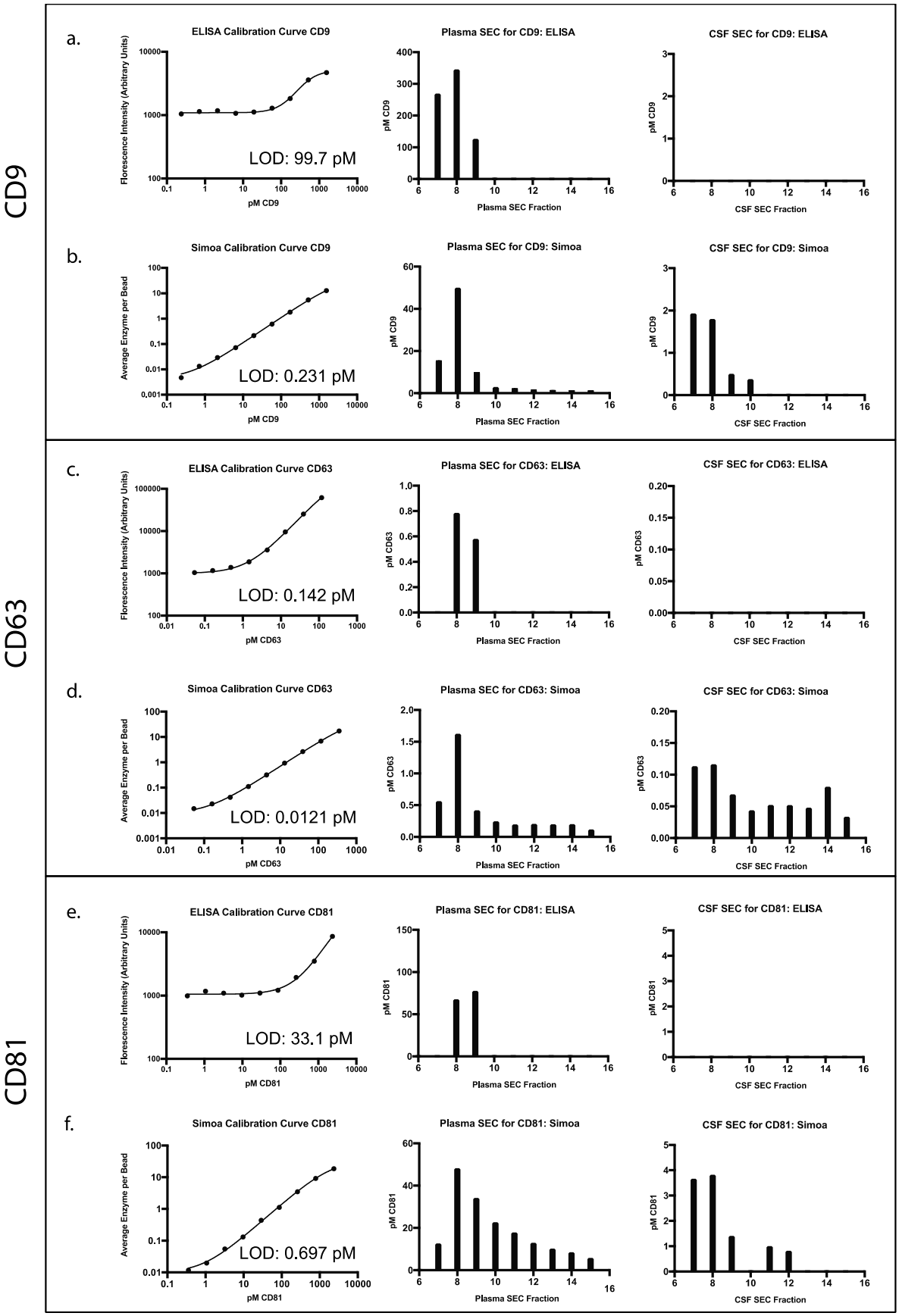

L1CAM Plasma Individual 1
L1CAM Plasma Individual 2
L1CAM Plasma Individual 3
L1CAM Plasma Individual 4
L1CAM (pM)
Plasma Dilution Factor


Albumin Plasma Individual 1
Albumin Plasma Individual 2
Albumin Plasma Individual 3
Albumin Plasma Individual 4
Albumin (nM)
Plasma Dilution Factor

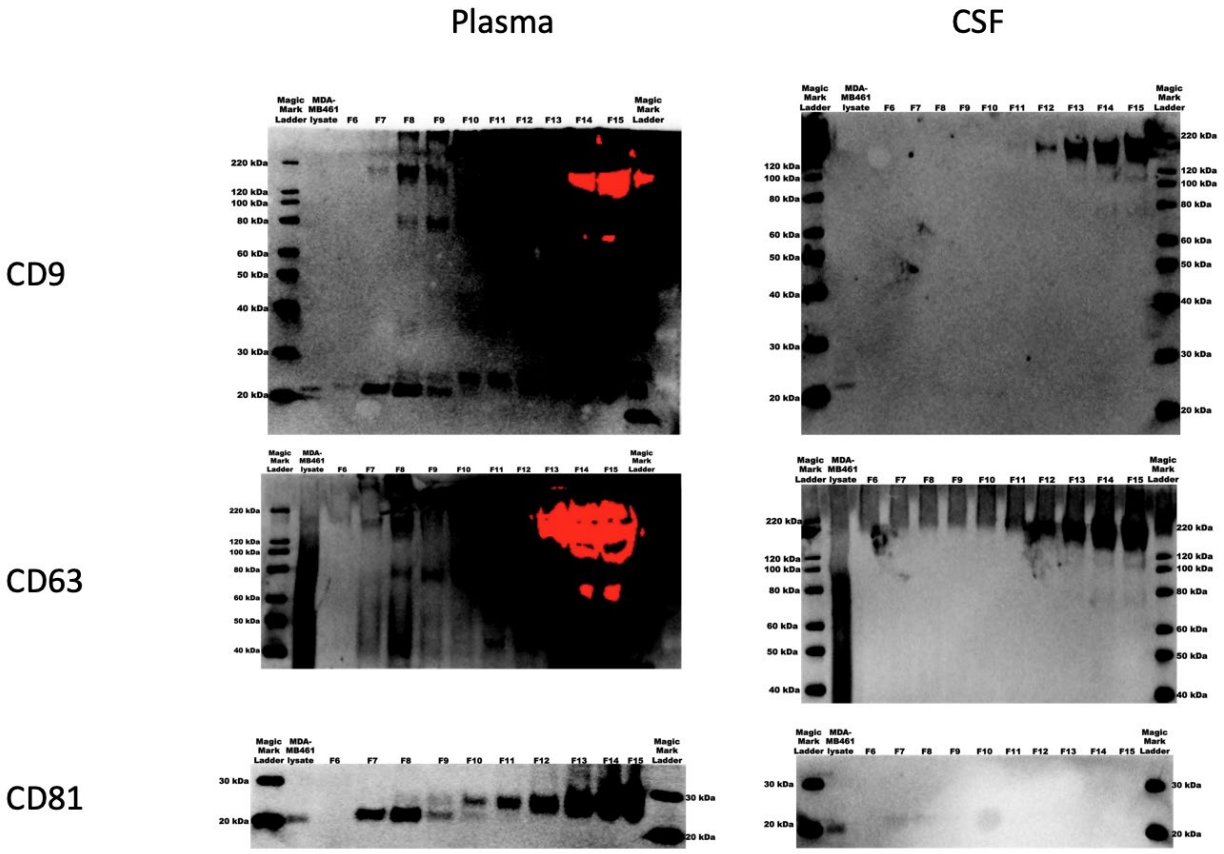
Supplementary Table 1: Spike and recovery for Simoa assays
Spike and recovery for each Simoa assay (CD9, CD63, CD81, L1CAM and albumin) in human plasma and CSF. Data are the average of four individual samples and two technical replicates.

Protein	CSF Dilution Factor/Spike	Average Recovery (4 Individuals)	Plasma Dilution Factor/Spike	Average Recovery (4 Individuals)
CD9	32x+500	86%	32x+500	79%
	32x+1000	101%	32x+1000	74%
CD63	16x+10	106%	16x+10	83%
	16x+50	105%	16x+50	74%
CD81	32x+500	104%	32x+500	106%
	32x+1000	101%	32x+1000	94%
Albumin	400x+50000	90%	80000x+50000	85%
	400x+100000	85%	80000x+100000	90%
L1CAM	32x+500	106%	64x+500	102%
	32x+1000	107%	64x+1000	98%

Supplementary Figure 2: Comparison of ELISA and Simoa for EV quantification
a) Standard ELISA calibration curve for CD9 (left) and measurement of plasma (center) and CSF (right) SEC fractions using this assay. Data represent the average of two technical replicates.
b) Simoa calibration curve for CD9 (left) and measurement of plasma (center) and CSF (right) SEC fractions using this assay. Data represent the average of two technical replicates.
c) Standard ELISA calibration curve for CD63 (left) and measurement of plasma (center) and CSF (right) SEC fractions using this assay. Data represent the average of two technical replicates.
d) Simoa calibration curve for CD63 (left) and measurement of plasma (center) and CSF (right) SEC fractions using this assay. Data represent the average of two technical replicates.
e) Standard ELISA calibration curve for CD81 (left) and measurement of plasma (center) and CSF (right) SEC fractions using this assay. Data represent the average of two technical replicates.
f) Simoa calibration curve for CD81 (left) and measurement of plasma (center) and CSF (right) SEC fractions using this assay. Data represent the average of two technical replicates.
g) Western blots of plasma (left) and CSF (right) SEC fractions.


CD9
a.
ELISA Calibration Curve CD9
Florescence Intensity (Arbitrary Units)
pM CD9
LOD: 99.7 pM
Plasma SEC for CD9: ELISA
pM CD9
Plasma SEC Fraction
CSF SEC for CD9: ELISA
pM CD9
CSF SEC Fraction
b.
Simoa Calibration Curve CD9
Average Enzyme per Bead
pM CD9
LOD: 0.231 pM
Plasma SEC for CD9: Simoa
pM CD9
Plasma SEC Fraction
CSF SEC for CD9: Simoa
pM CD9
CSF SEC Fraction
CD63
c.
ELISA Calibration Curve CD63
Florescence Intensity (Arbitrary Units)
pM CD63
LOD: 0.142 pM
Plasma SEC for CD63: ELISA
pM CD63
Plasma SEC Fraction
CSF SEC for CD63: ELISA
pM CD63
CSF SEC Fraction
d.
Simoa Calibration Curve CD63
Average Enzyme per Bead
pM CD63
LOD: 0.0121 pM
Plasma SEC for CD63: Simoa
pM CD63
Plasma SEC Fraction
CSF SEC for CD63: Simoa
pM CD63
CSF SEC Fraction
CD81
e.
ELISA Calibration Curve CD81
Florescence Intensity (Arbitrary Units)
pM CD81
LOD: 33.1 pM
Plasma SEC for CD81: ELISA
pM CD81
Plasma SEC Fraction
CSF SEC for CD81: ELISA
pM CD81
CSF SEC Fraction
f.
Simoa Calibration Curve CD81
Average Enzyme per Bead
pM CD81
LOD: 0.697 pM
Plasma SEC for CD81: Simoa
pM CD81
Plasma SEC Fraction
CSF SEC for CD81: Simoa
pM CD81
CSF SEC Fraction

g.



Supplementary Note 1: Citations for manuscripts that utilize L1CAM as a handle for NDEV isolation[1-45]

1 Shi, M. *et al.* Plasma exosomal alpha-synuclein is likely CNS-derived and increased in Parkinson's disease. *Acta Neuropathol* **128**, 639-650, doi:10.1007/s00401-014-1314-y (2014).

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