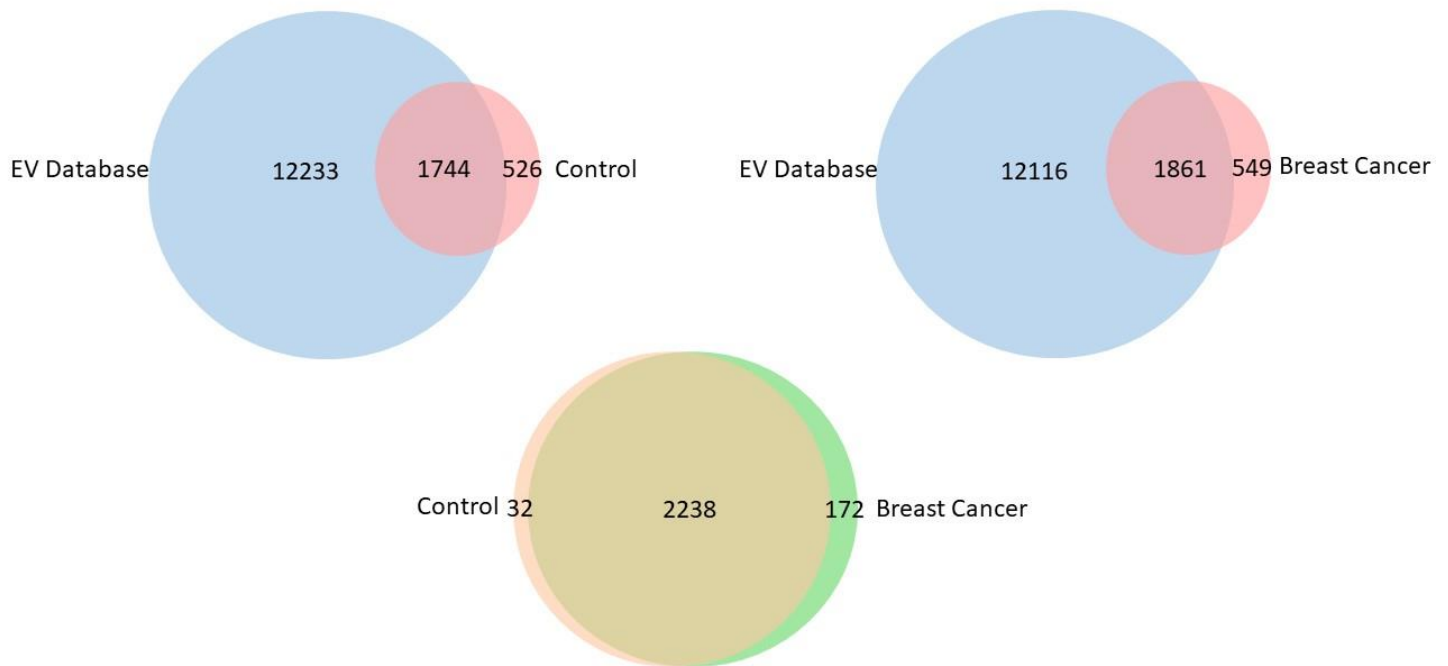


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Sequential phosphoproteomics and N-glycoproteomics of plasma-derived extracellular vesicles

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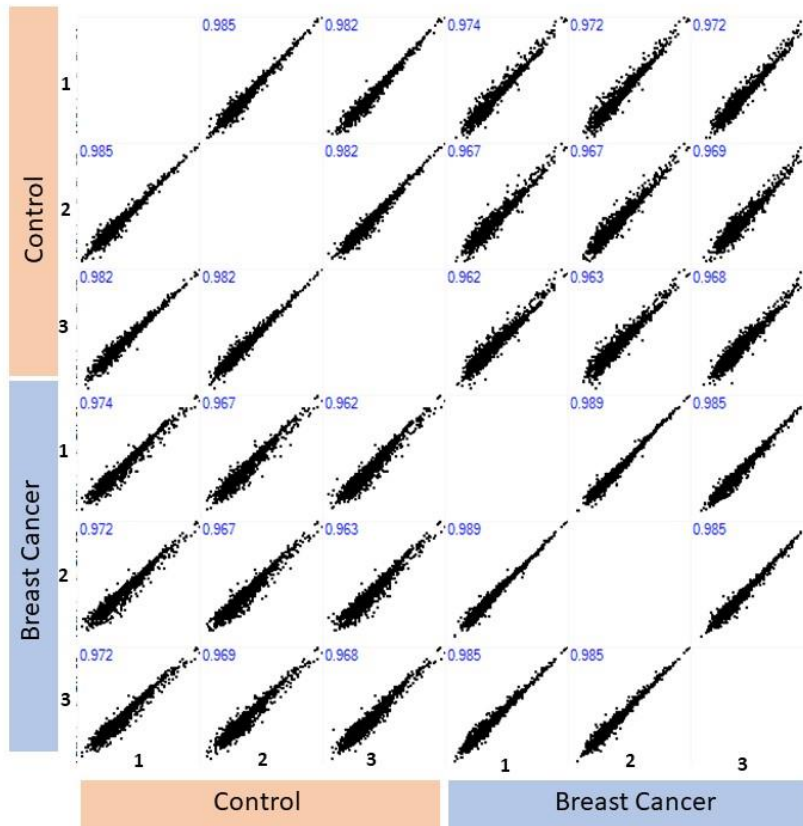
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Supplementary Figure 1

Venn diagrams showing the overlap between the EV database from Vesiclepedia and EV data from control and breast cancer.

The Venn diagrams show a 77% overlap between both, the EV database and control EV proteins, and the EV database and EV breast cancer proteins. Overlap of the proteome from the control and the breast cancer EV data is also shown.

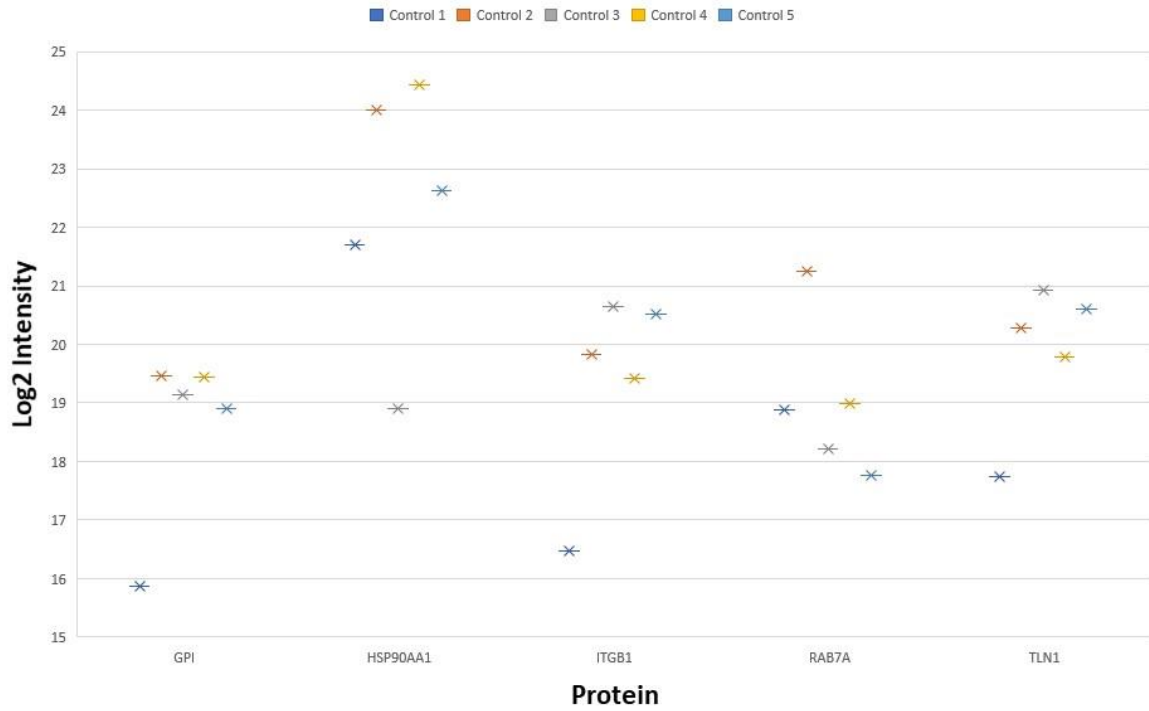


Supplementary Figure 2

Quantitation results from MaxQuant and Perseus showing Pearson correlations from proteome analysis across each condition and replicate.

Scatterplots and Pearson correlation coefficients depicting the log₂-transformed intensities from proteome analysis of control and breast cancer samples, each in triplicate.

Variation Between Same Cohort



Supplementary Figure 3

Scatterplots representing the targeted proteomic (PRM) analysis.

Raw intensities from five individual control samples corresponding to five phosphopeptides from five EV proteins were plotted. See Table S1 for details.

Supplementary Table 1

Comparison of EV phosphopeptides, phosphoproteins, N-glycopeptides and N-glycoproteins using sequential or separate procedures.

Sample	#phosphopeptides	#total peptides	Selectivity	#phosphoproteins
Sequential 1	3947	6110	65%	1298
Sequential 2	3864	5963	65%	1289
Sequential 3	3845	5948	65%	1276
Separate 1	4156	6022	69%	1361
Separate 2	4176	5997	70%	1371
Separate 3	4186	5973	70%	1372

Sample	#glycopeptides	#total peptides	Selectivity	#glycoproteins
Sequential 1	1003	2389	42%	653
Sequential 2	1005	2439	41%	638
Sequential 3	1004	2407	42%	638
Separate 1	1042	1960	53%	673
Separate 2	1013	1893	54%	656
Separate 3	1043	1943	54%	660

Supplementary Table 2

Raw intensities from five individual control samples monitoring five peptides corresponding to five EV proteins, including mean, SD and CV (%).

Proteins selected are in the top 100 EV proteins from Vesiclepedia.

Protein Gene	Peptide	Peptide Modified Sequence Average Masses	Precursor Charge	Control 1	Control 2	Control 3	Control 4	Control 5	Mean	SD	CV (%)
GPI	SNTPILVDGK	SNT[+79.979901]PILVDGK	2	59865	718569	575007	712412	487559	510682.4	241586.6	47.30662
HSP90AA1	EVSDDEAEK	EVS[+79.979901]DDEAEK	2	3382085	16903736	488350	22667768	6458404	9980069	8425475	84.42301
ITGB1	WDTGENPIYK	WDT[+79.979901]GENPIYK	2	90336	930164	1637321	698009	1506258	972417.6	562536.3	57.84925
RAB7A	FQSLGVAFYR	FQS[+79.979901]LGVAFYR	2	486314	2491084	302097	521000	221576	804414.2	850692.6	105.7531
TLN1	VLVQNAAGSQEK	VLVQNAAGS[+79.979901]QEK	2	219752	1279873	1986830	902402	1582806	1194333	603541.5	50.53379