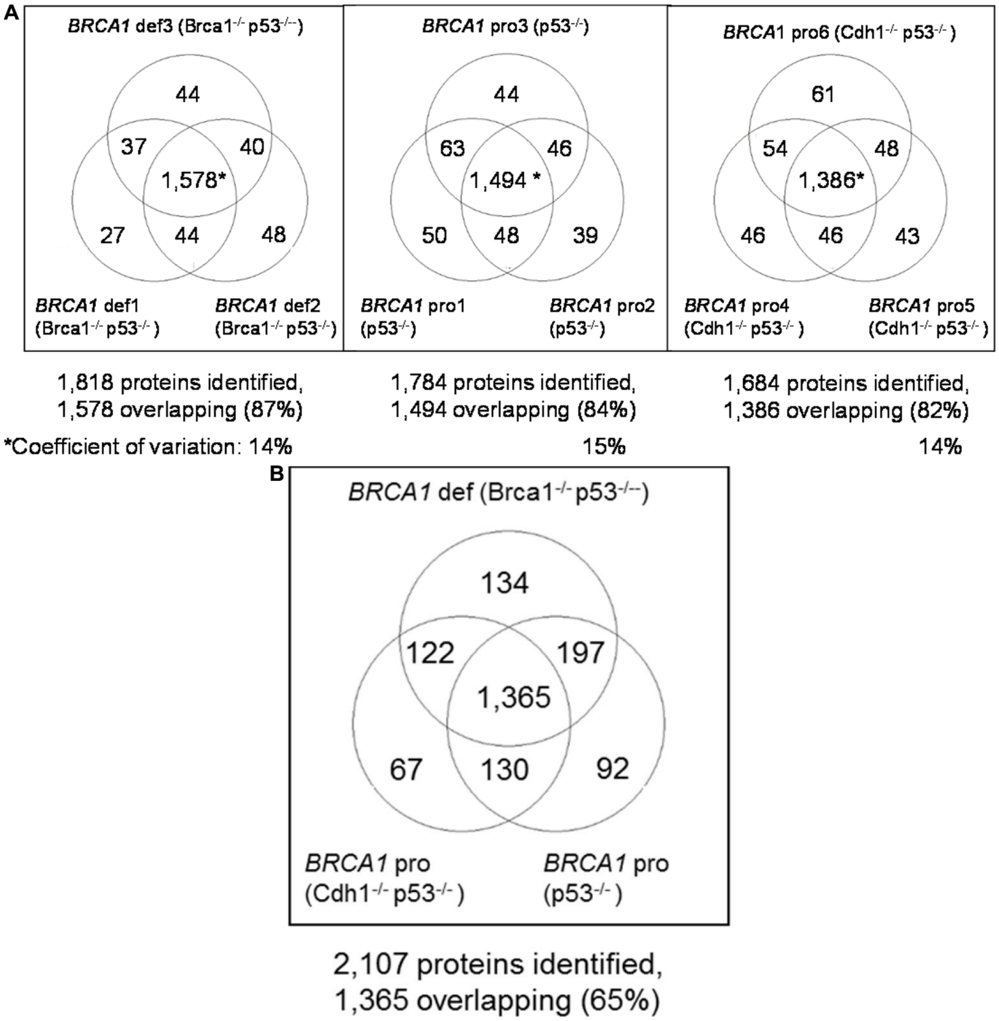
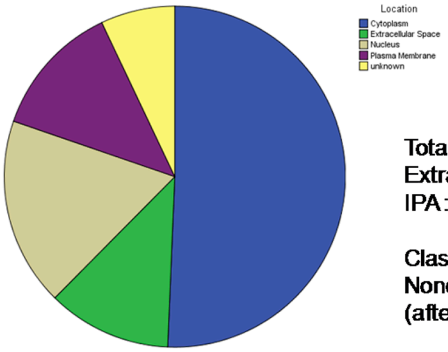
Secretome proteomics reveals candidate non-invasive biomarkers of *BRCA1* deficiency in breast cancer

Supplementary Materials



Supplementary Figure S1: (**A**) Venn diagram of protein identifications in three biological replicates. (**B**) Venn diagram of protein identifications among the three groups. Proteins identified in at least one triplicate sample of each group were used for overlap analysis.

***BRCA1* deficient (Brca1$^{-/-}$ p53$^{-/-}$)**

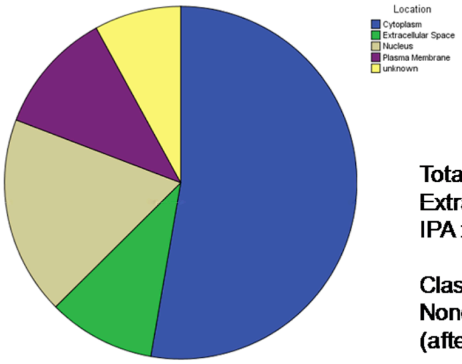


Location

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Cytoplasm	763	50,7	50,7	50,7
	Extracellular Space	177	11,8	11,8	62,4
	Nucleus	269	17,9	17,9	80,3
	Plasma Membrane	191	12,7	12,7	93,0
	unknown	106	7,0	7,0	100,0
	Total	1506	100,0	100,0	

Total proteins	1506
Extracellular proteins	
IPA:	368 (24.4%)
Classical secretion (Signal P):	456 (30.3%)
Nonclassical secretion (SecretomeP):	433 (28.8%)
(after exclusion of signalP+ proteins)	

***BRCA1* proficient (p53$^{-/-}$)**

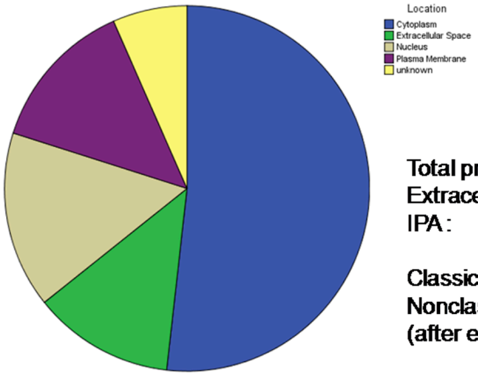


Location

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Cytoplasm	755	52,7	52,7	52,7
	Extracellular Space	141	9,8	9,8	62,6
	Nucleus	261	18,2	18,2	80,8
	Plasma Membrane	161	11,2	11,2	92,0
	unknown	114	8,0	8,0	100,0
	Total	1432	100,0	100,0	

Total proteins	1432
Extracellular proteins	
IPA:	302 (21%)
Classical secretion (Signal P):	344 (24%)
Nonclassical secretion (SecretomeP):	468 (32.7%)
(after exclusion of signalP+ proteins)	

***BRCA1* proficient (Cdh1$^{-/-}$ p53$^{-/-}$)**

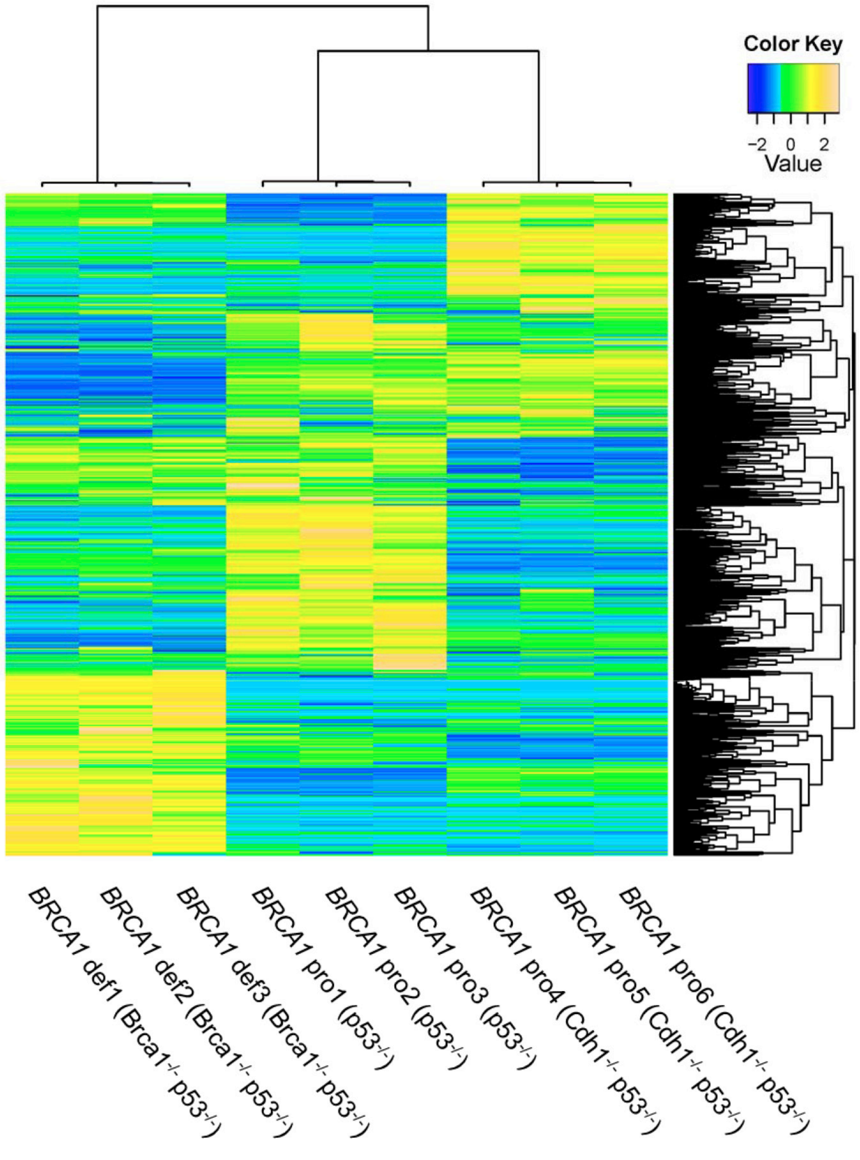


Location

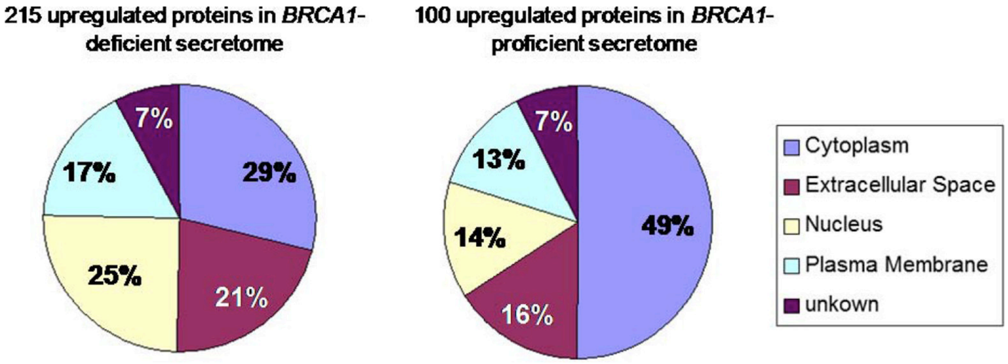
		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Cytoplasm	683	51,8	51,8	51,8
	Extracellular Space	165	12,5	12,5	64,3
	Nucleus	206	15,6	15,6	79,9
	Plasma Membrane	178	13,5	13,5	93,4
	unknown	87	6,6	6,6	100,0
	Total	1319	100,0	100,0	

Total proteins	1319
Extracellular proteins	
IPA:	343 (26%)
Classical secretion (Signal P):	418 (31.7%)
Nonclassical secretion (SecretomeP):	402 (30.5%)
(after exclusion of signalP+ proteins)	

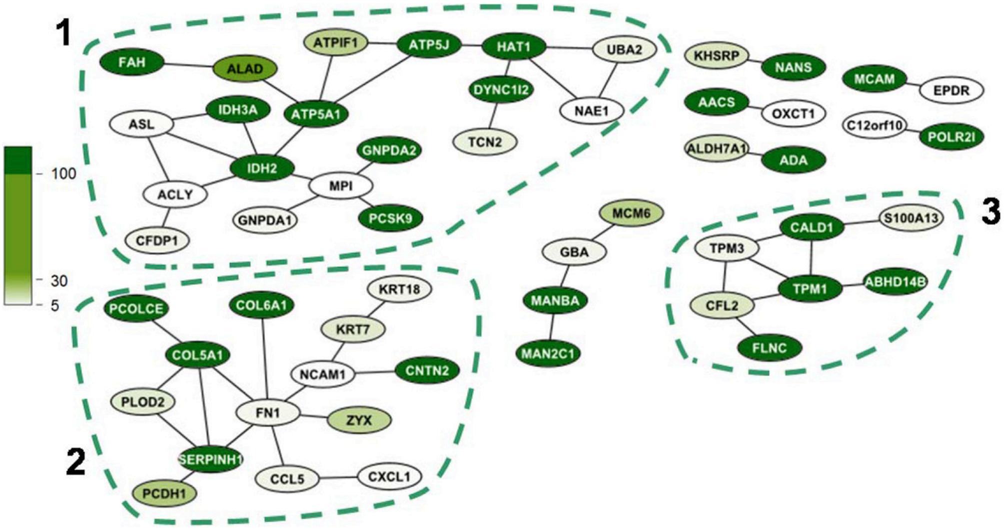
Supplementary Figure S2: Analysis of subcellular location, SignalP and SecretomeP.



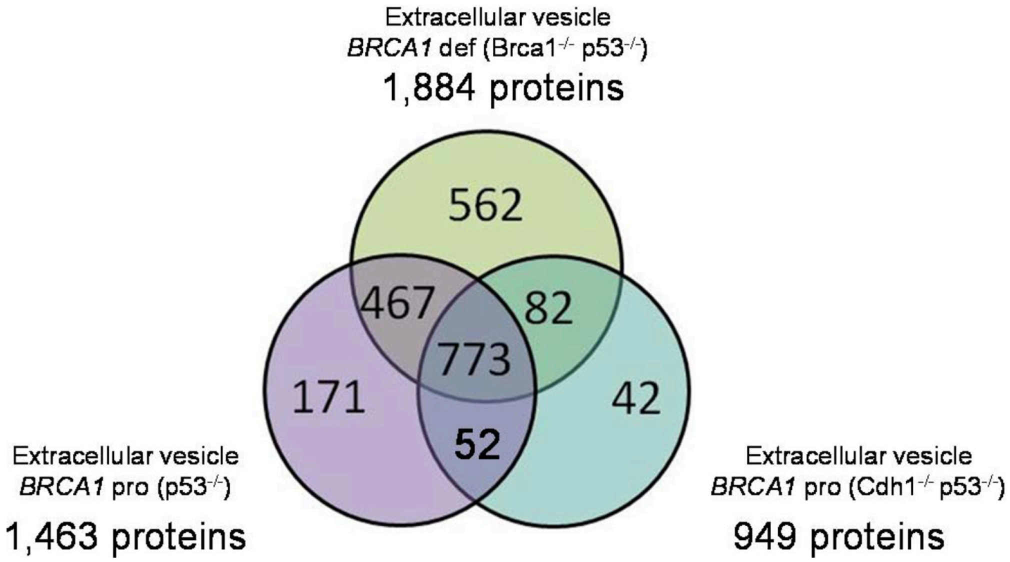
Supplementary Figure S3: Unsupervised cluster analysis on all proteins identified in secretomes shows three distinct groups based on the mutational status.



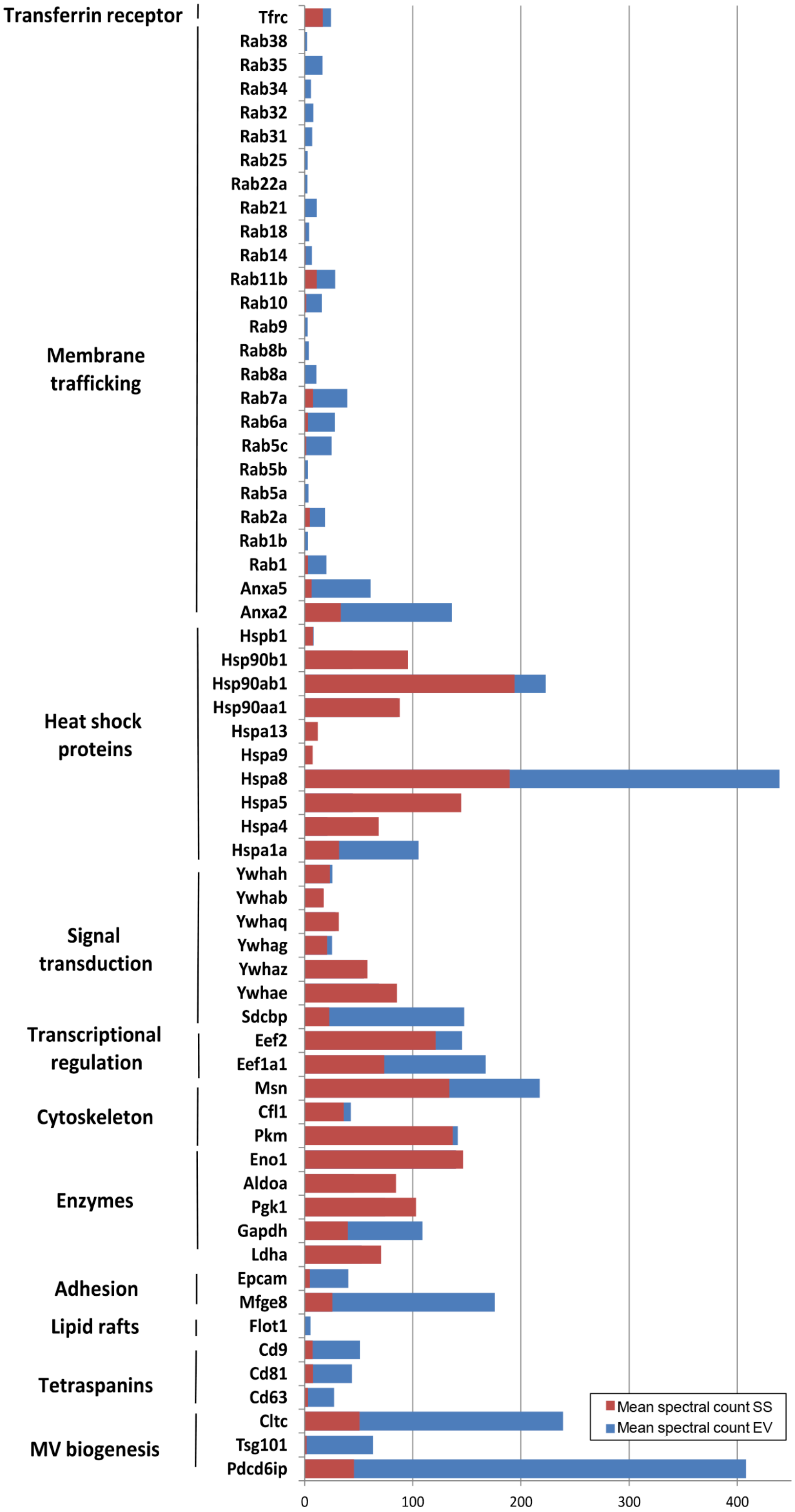
Supplementary Figure S4: Gene Ontology analysis of subcellular localization of upregulated (*n* = 215) and downregulated (*n* = 100) proteins in BRCA1-deficient secretomes.



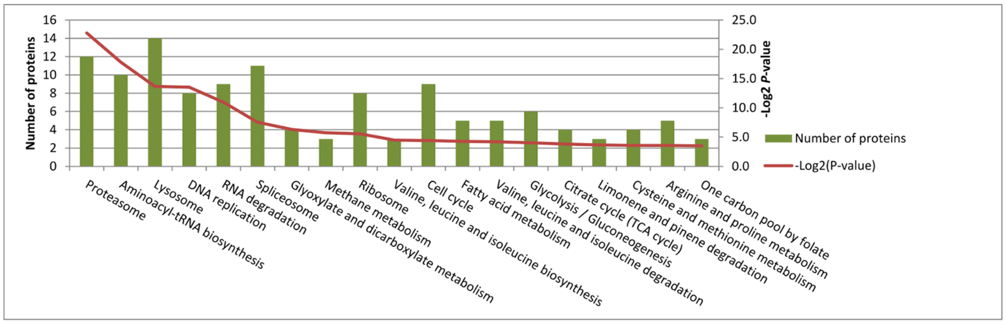
Supplementary Figure S5: Biological networks of 100 highly downregulated proteins in BRCA1-deficient secretome.



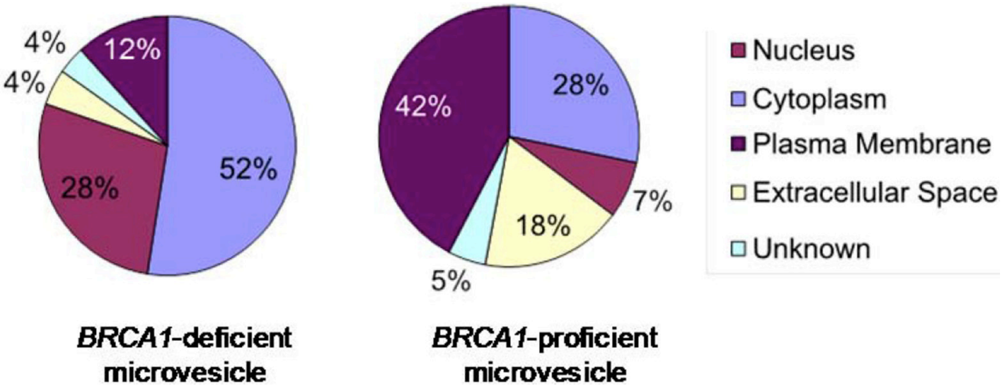
Supplementary Figure S6: Venn diagram representing the total number of uniquely identified proteins in extracellular vesicles across three groups.



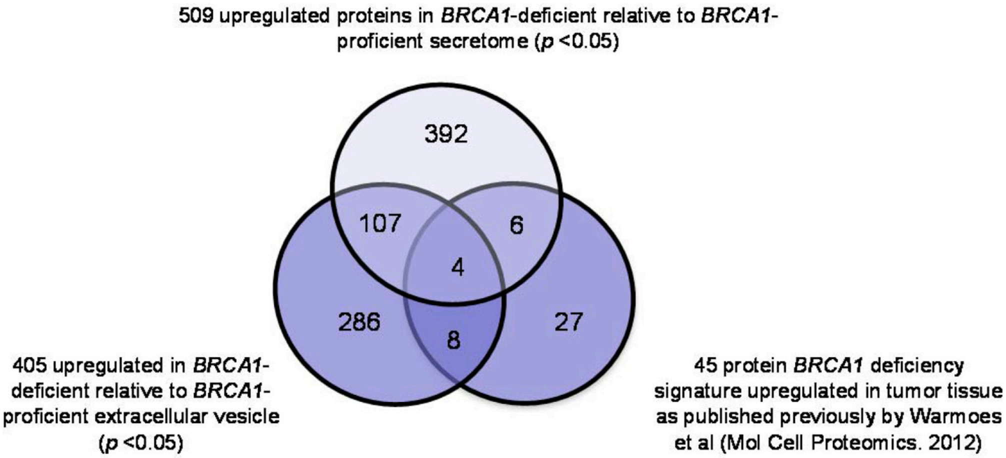
Supplementary Figure S7: Distribution of microvesicle-associated proteins in extracellular vesicle (EV) and soluble secretome (SS) fractions identified across three experiments.



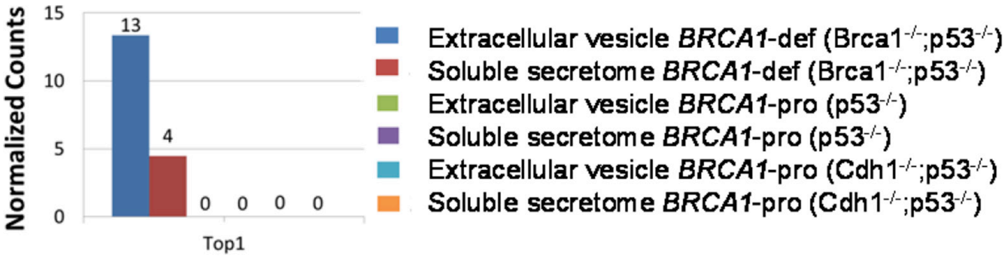
Supplementary Figure S8: Significant biological processes in 405 upregulated proteins in extracellular vesicles of *BRCA1*-deficient breast tumor cell lines .



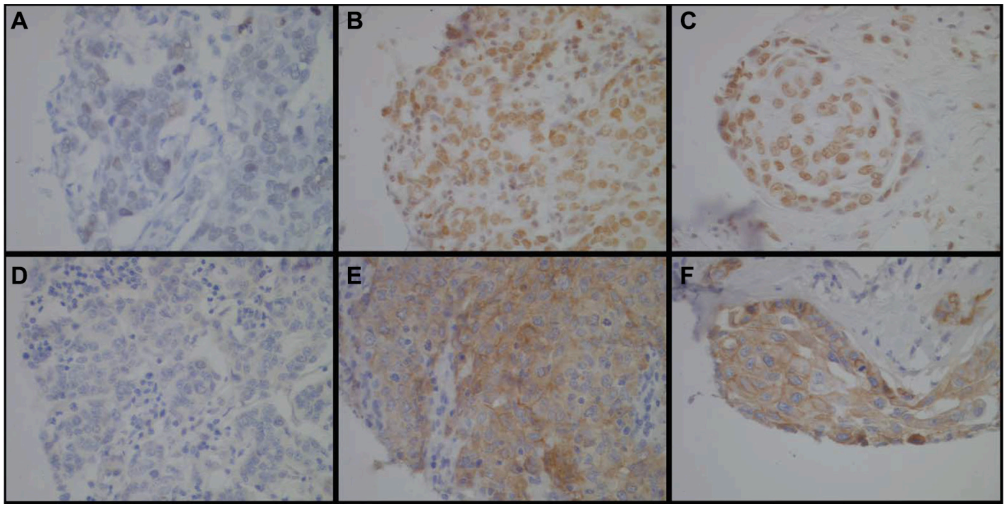
Supplementary Figure S9: Subcellular localization of proteins enriched in *BRCA1*-deficient extracellular vesicles (*n* = 405, left panel) and in BRCA1-proficient extracellular vesicles (*n* = 85, right panel).



Supplementary Figure S10: Overlap analysis of proteins enriched in *BRCA1*-deficient secretome, extracellular vesicle and *BRCA1*-deficient tissue signature.



Supplementary Figure S11: Levels of TOP1 in soluble secretome and microvesicle factions of individual samples.



Supplementary Figure S12: Representative TOP1 and CDH3 staining. Representative TOP1 IHC positive staining of a sporadic (**A**) *BRCA1*- (**B**) and *BRCA2*-mutated (**C**) human breast cancer case and representative CDH3 positive staining of a sporadic (**D**), *BRCA1*-(**E**) and *BRCA2*-mutated (**F**) human breast cancer case.

Supplementary Table S1: List of proteins detected in BRCA1-deficient and -proficient secretomes harvested from breast tumor cell lines and spectral count quantification data. See Supplementary_Table_S1

Supplementary Table S2: 509 upregulated proteins in BRCA1-deficient relative to BRCA1-proficient secretomes. See Supplementary_Table_S2

Supplementary Table S3: BinGO gene ontology of biological process analysis on sub-networks. See Supplementary_Table_S3

Supplementary Table S4: List of proteins detected in BRCA1-deficient and -proficient extracellular vesicles and soluble secretome fractions and spectral count quantification data. See Supplementary_Table_S4