

Supplementary Materials for:
Inferring alterations in cell-to-cell communication in HER2+ breast
cancer using secretome profiling of three cell models

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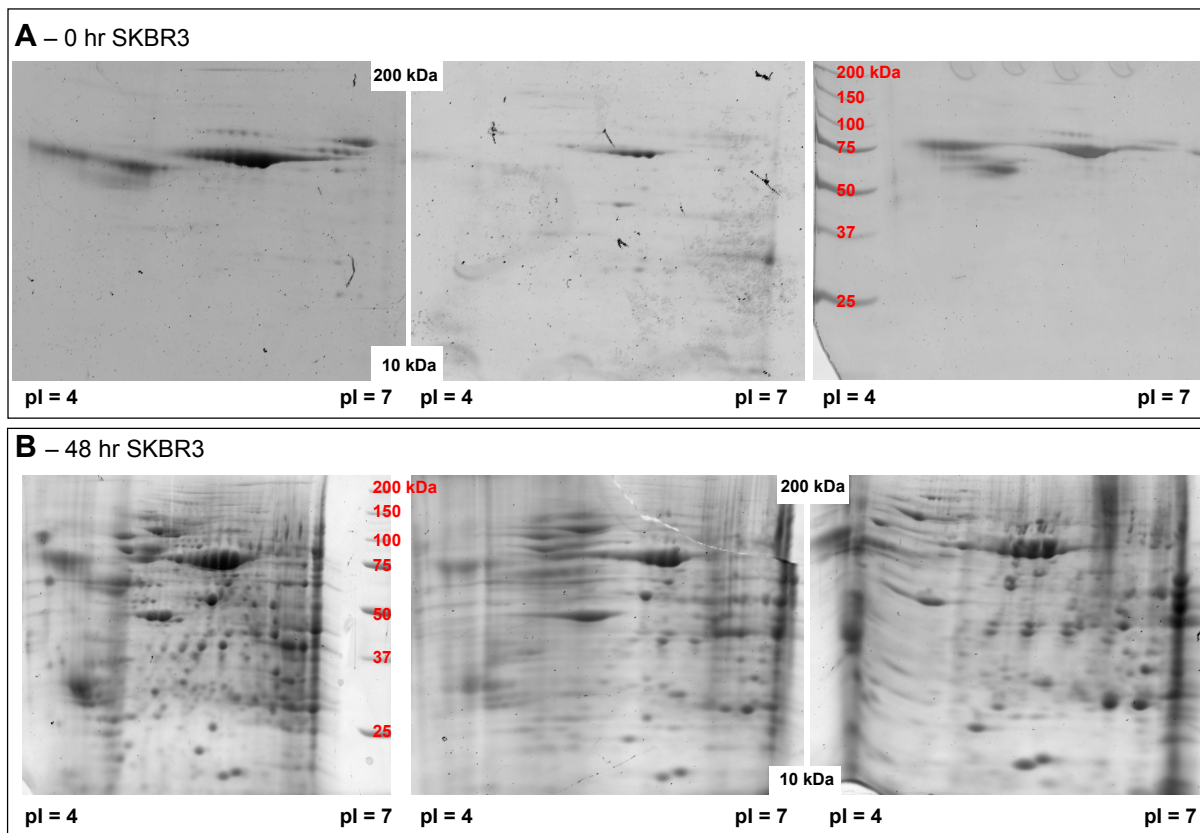


Figure S1: **Three biological replicates of 2-D map of SKBR3 secretome.** (A) Secretome map collected following a brief conditioning period of the media by SKBR3 cells (0 hr sample). Represents residual proteins present in conditioned media following cell wash protocol. (B) Secretome map collected following a 48 hour conditioning period of the media by SKBR3 cells (48 hr sample). All samples were resolved using a IPG strip pH 4-7.

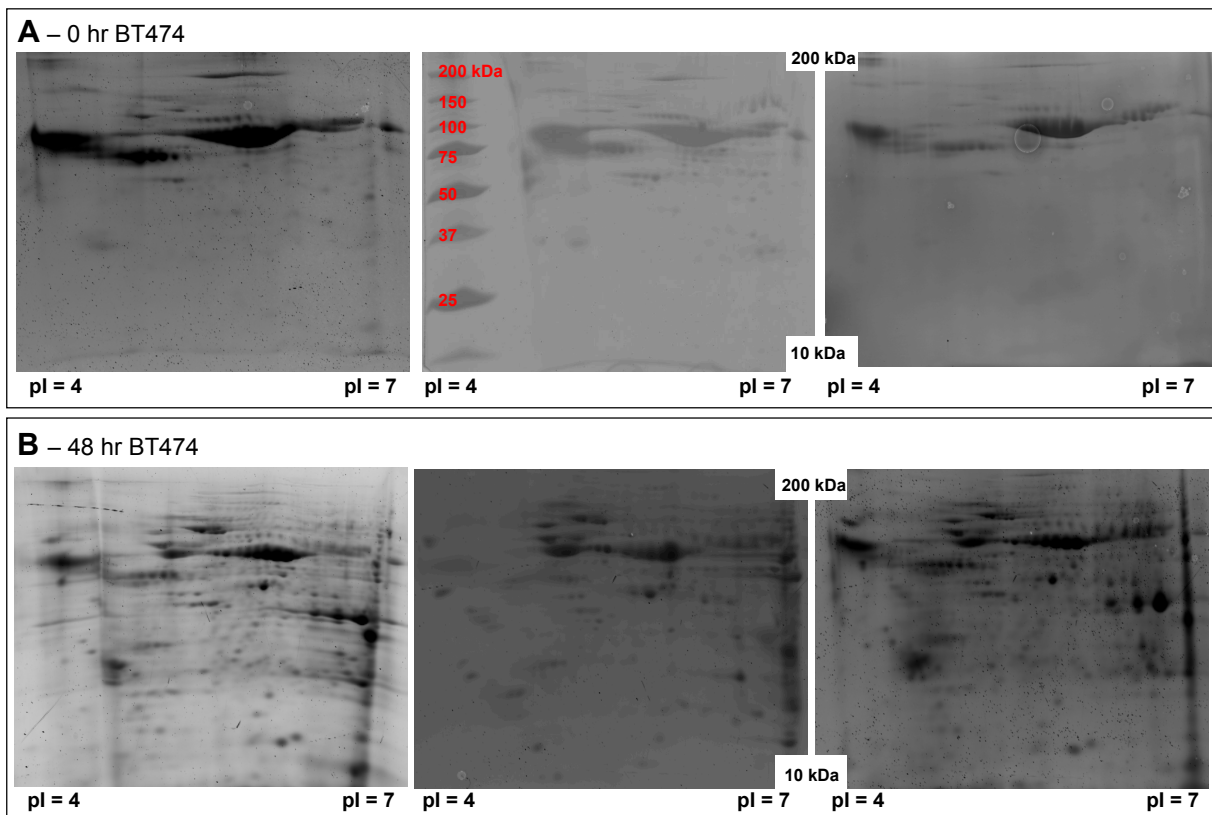


Figure S2: **Three biological replicates of 2-D map of BT474 secretome.** (A) Secretome map collected following a brief conditioning period of the media by BT474 cells (0 hr sample). Represents residual proteins present in conditioned media following cell wash protocol. (B) Secretome map collected following a 48 hour conditioning period of the media by BT474 cells (48 hr sample). All samples were resolved using a IPG strip pH 4-7.

48 hr 184A1

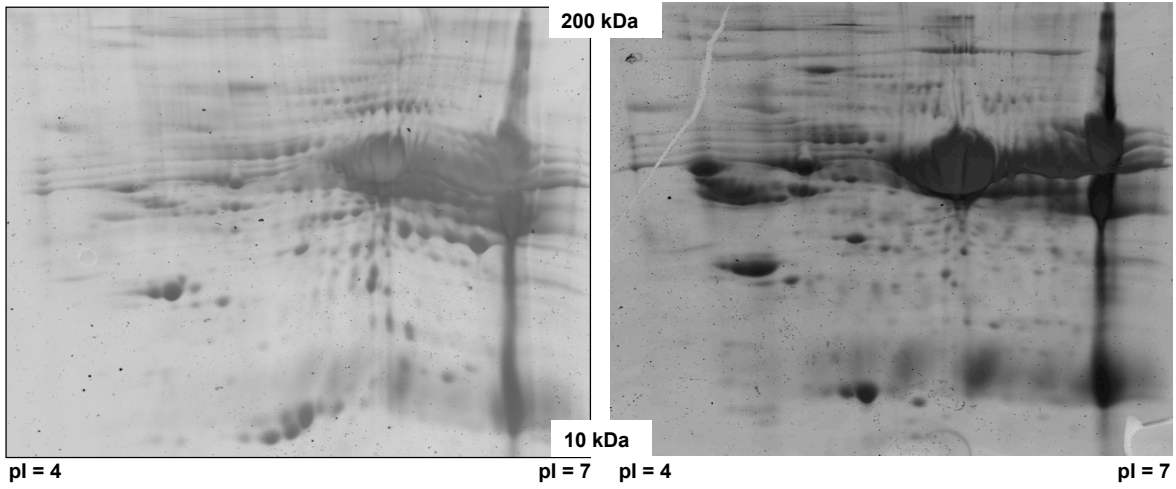


Figure S3: **Two technical replicates of 2-D map of 184A1 secretome.** Secretome maps collected following a 48 hour conditioning period of the media by 184A1 cells (48 hr sample). All samples were resolved using a IPG strip pH 4-7.

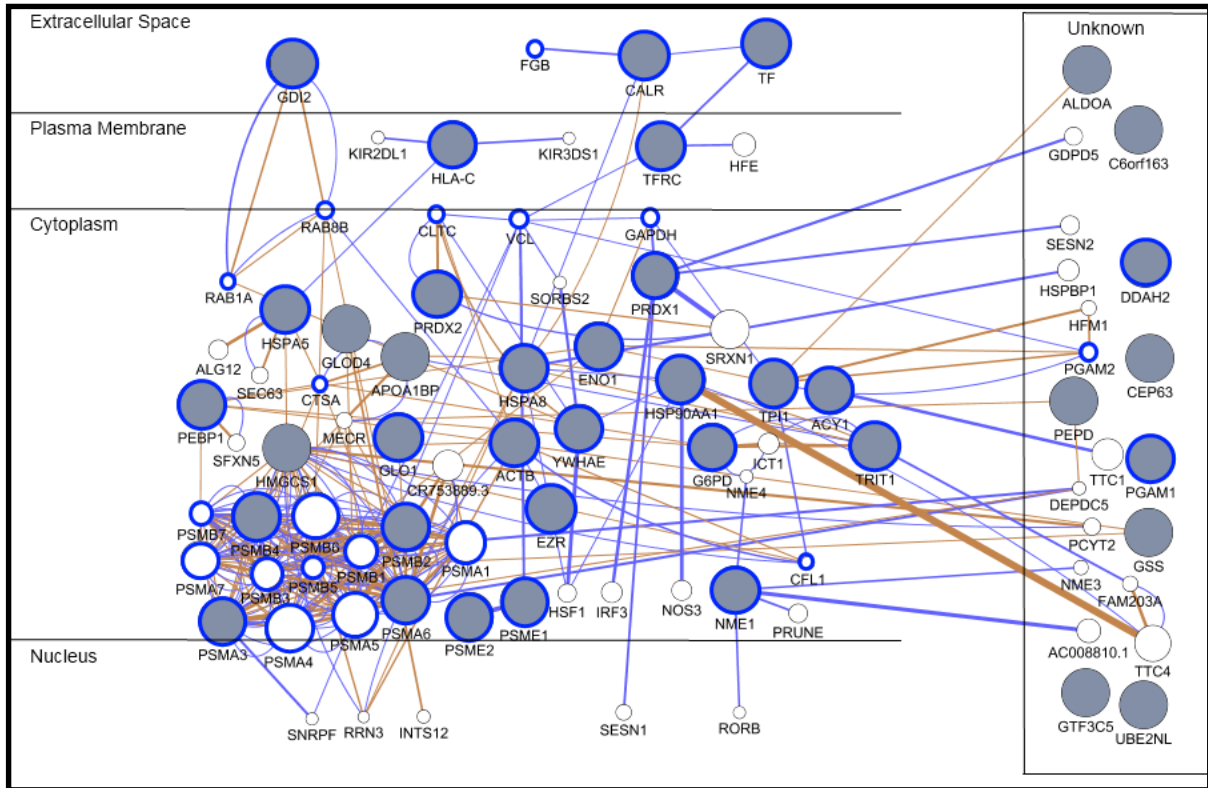


Figure S4: **Protein-protein interaction network inferred from proteins identified in SKBR3 secretome.** Protein-protein interaction networks were constructed in GeneMania using the set of all identified proteins in SKBR3 secretome. The proteins were arranged based upon similarity in subcellular location. Gray circles indicate identified proteins while white circles indicate proteins inferred based upon known interactions with identified proteins. The blue edges indicate experimentally observed protein-protein interactions in Homo Sapiens, while brown edges are predicted protein-protein interactions based on homology with other species. Nodes encircled in blue are found in the Exocarta database, which suggests that they are associated with exosomes.

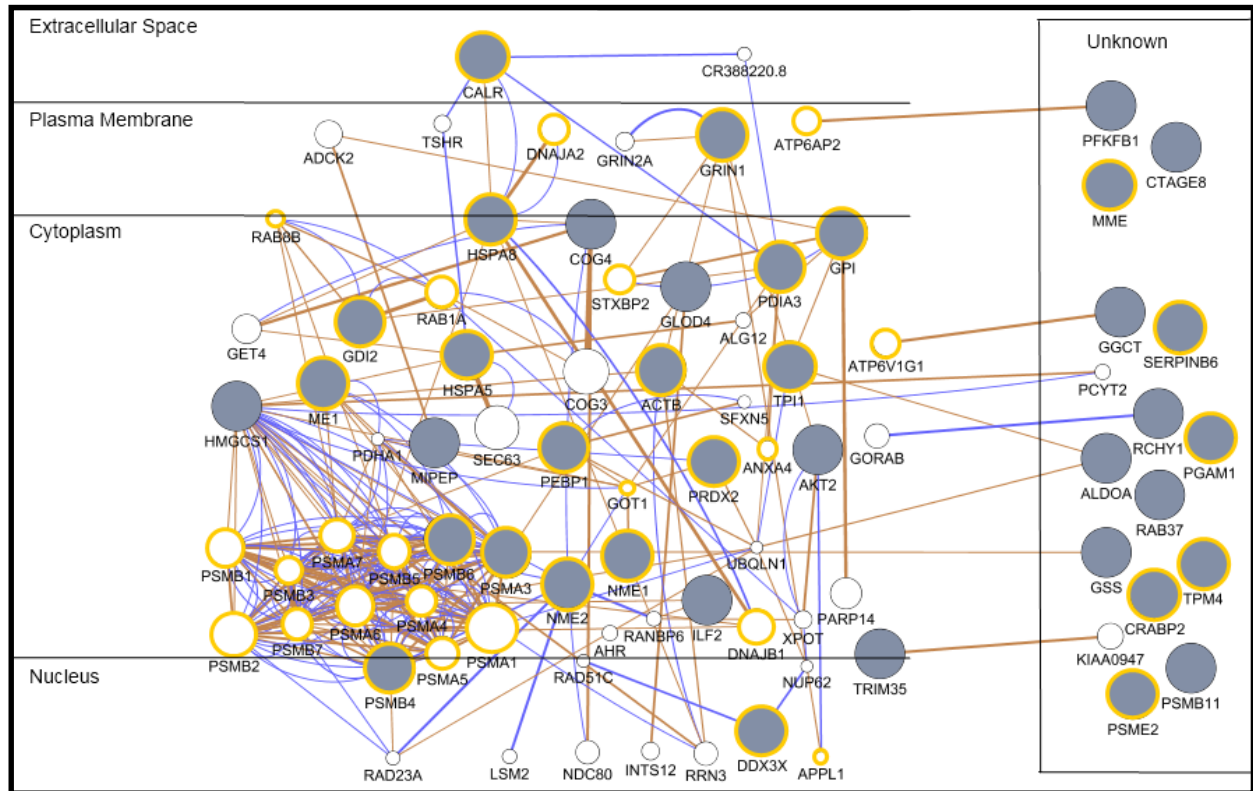


Figure S5: **Protein-protein interaction network inferred from proteins identified in BT474 secretome.** Protein-protein interaction networks were constructed in GeneMania using the set of all identified proteins in BT474 secretome. The proteins were arranged based upon similarity in subcellular location. Gray circles indicate identified proteins while white circles indicate proteins inferred based upon known interactions with identified proteins. The blue edges indicate experimentally observed protein-protein interactions in Homo Sapiens, while brown edges are predicted protein-protein interactions based on homology with other species. Nodes encircled in yellow are found in the Exocarta database, which suggests that they are associated with exosomes.

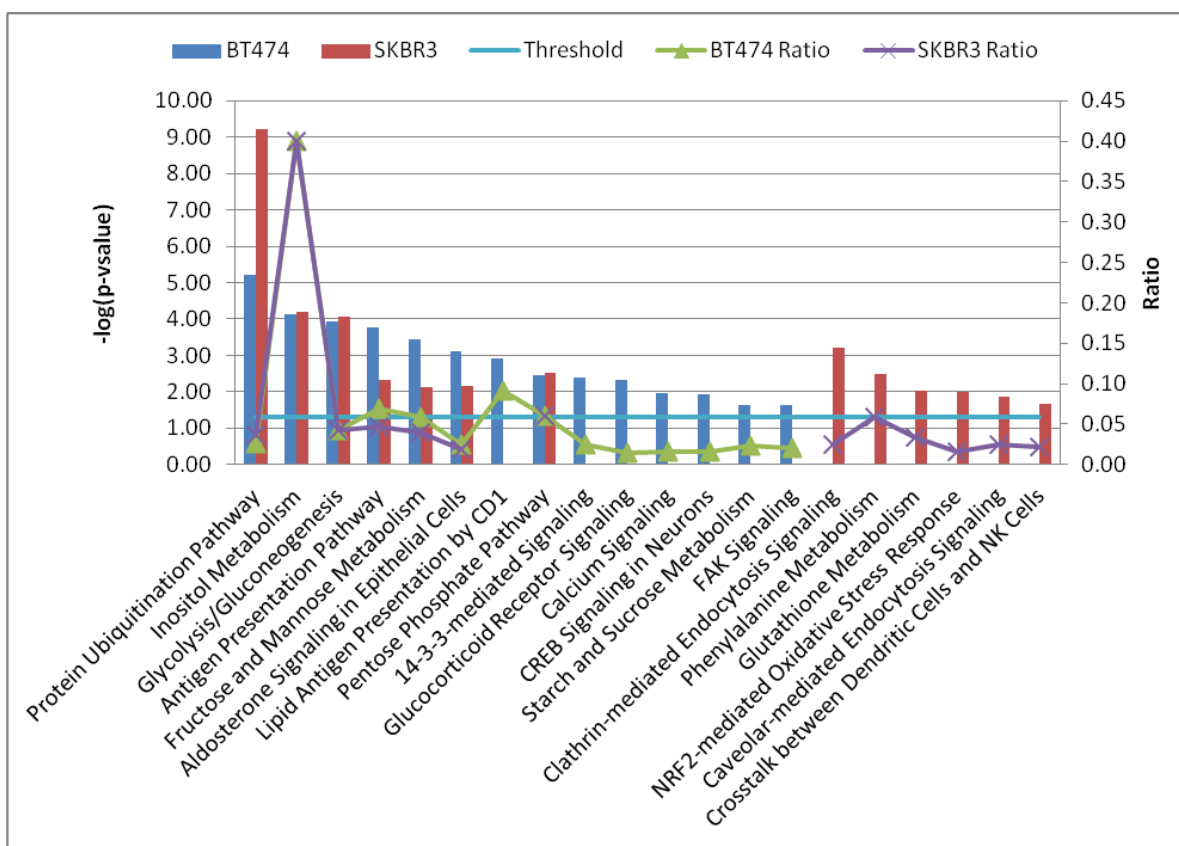


Figure S6: **Significant canonical pathways enriched in the BT474 and SKBR3 secretomes.** Ingenuity Pathway analysis was used to determine enrichment of canonical pathways. The negative of the \log_{10} (p-value) and ratio (number of focus molecules involved in the pathway/total number of molecules in the pathway) are plotted on the primary and secondary Y-axis, respectively. The significance of enriched pathways for BT474 secretome are indicated by blue columns and the corresponding ratios are indicated by the green triangles. Similarly, significance of enriched pathways for SKBR3 are indicated by red columns and corresponding ratios are indicated by purple X's.

Table S1. Proteins identified in 184A1 secretome.

Protein Name	Gene Symbol
3-ketoacyl-CoA thiolase, mitochondrial	ACAA2 [‡]
4-hydroxyphenylpyruvate dioxygenase	HPD [‡]
Adapter molecule crk	CRK [‡]
Alpha-ketoglutarate-dependent dioxygenase alkB homolog 3	ALKBH3
DPY30 domain-containing protein 2	DYDC2
ELMO domain-containing protein 1	ELMOD1
Endomucin	EMCN
Exportin-6	XPO6
Glutamate decarboxylase-like protein 1	GADL1
Glutathione S-transferase Mu 3	GSTM3 [‡]
Heat shock factor-binding protein 1	HSBP1
Heat shock protein beta-6	HSPB6 [‡]
Hepatoma-derived growth factor	HDGF
HLA class I histocompatibility antigen, B-41 alpha chain	HLA-B [‡]
HLA class I histocompatibility antigen, B-82 alpha chain	HLA-B [‡]
Homocysteine-responsive endoplasmic reticulum-resident ubiquitin-like domain member 1 protein	HERPUD1
Kinectin	KTN1
Leucine-rich alpha-2-glycoprotein	LRG1 [‡]
Magnesium transporter protein 1	MAGT1
Mitochondrial Pyruvate dehydrogenase [acetyl-transferring]-phosphatase 1	PDP1
NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 4-like 2	NDUFA4L2
Peroxiredoxin-4	PRDX4 [‡]
PITH domain-containing protein 1	PITHD1
Proteasome activator complex subunit 1	PSME1 [‡]
Protein diaphanous homolog 3	DIAPH3
Protein kinase C delta type	PRKCD [‡]
Protein OSCP1	OSCP1
Putative beclin-1-like protein	BECN1P1
Putative homeobox protein Meis3-like 2	MEIS3P2
Putative uncharacterized protein ENSP00000344348	
Regulator complex protein LAMTOR1	LAMTOR1 [‡]
Ras-GEF domain-containing family member 1B	RASGEF1B
Ras-related protein Rab-7a	RAB7A [‡]
Receptor tyrosine-protein kinase erbB-2	ERBB2 [‡]
Serpin B6	SERPINB6 [‡]
SH3 domain and tetratricopeptide repeat-containing protein 1	SH3TC1
Transferrin receptor protein 1	TFRC [‡]
Transmembrane emp24 domain-containing protein 10	TMED10 [‡]
Transmembrane protein 154	TMEM154
Transmembrane protein 233	TMEM233
Transthyretin	TTR [‡]
UDP-glucuronosyltransferase 1-8	UGT1A8 [‡]
UDP-N-acetylhexosamine pyrophosphorylase	UAP1
Uncharacterized protein C10orf68	C10orf68
Uncharacterized protein C10orf82	C10orf82
Vesicle-associated membrane protein-associated protein A	VAPA [‡]
Zinc finger protein 397	ZNF397
Zinc finger protein 442	ZNF442
Zinc finger protein 713	ZNF713

[‡] indicates identified in exosomes.