

Supplementary Tables and Figures

Conditioned media from the PC3 and Raw264.7 cells co-culture						
Protein	Gene	Theoretical molecular mass (Da)	Coverage %	No. of peptides	PSMs	
SCO-spondin	SSPO	547.5	0.80	1	1	
Metabotropic glutamate receptor 2	GRM2	95.5	4.24	1	1	
Dystonin	DST	190.2	2.33	1	1	
Coiled-coil domain-containing protein 191	KIAA1407	110.5	4.38	1	1	
Keratin, type I cytoskeletal 27	KRT27	49.8	8.93	1	1	
Baculoviral IAP repeat-containing protein 1	NAIP	159.5	2.35	1	1	
E3 ubiquitin-protein ligase RNF34	RNF34	4.7	77.78	1	2	
Receptor-type tyrosine-protein phosphatase U	PTPRU	162.3	2.14	1	1	
BMP/retinoic acid-inducible neural-specific protein 2	BRINP2	88.9	4.34	1	1	
Olfactory receptor 8D2	OR8D2	34.8	12.86	1	1	
ATP-dependent RNA helicase DDX18	DDX18	75.4	5.82	1	1	
Obscurin	OBSCN	972.4	0.30	1	1	
Neuronal-specific septin-3	SEPTIN3	40.7	12.29	1	1	
Urokinase-type plasminogen activator	PLAU	48.5	3.02	1	1	
Ethanolamine-phosphate phospho-lyase	ETNPPL	55.6	8.82	1	1	
Coiled-coil domain-containing protein 65	CCDC65	57.3	2.07	1	1	
Immunoglobulin heavy variable 3-53	IGHV3-53	12.8	40.52	1	1	
DDB1- and CUL4-associated factor 5	DCAF5	103.9	4.56	1	1	
Lamin tail domain-containing protein 2	LMNTD2	70.3	5.21	1	1	
Thioredoxin domain-containing protein 11	TXNDC11	110.5	3.15	1	1	
Alkylglycerol monooxygenase	AGMO	51.5	9.21	1	2	
Importin subunit alpha-3	KPNA4	57.9	5.57	1	1	
Zinc finger protein 585A	ZNF585A	87.9	5.20	1	1	
Ankyrin repeat domain-containing protein 36B	ANKRD36B	153.5	2.37	1	1	
Syntabulin	SYBU	73	5.24	1	1	
Retinoic acid-induced protein 3	GPRC5A	40.2	10.64	1	1	
CapZ-interacting protein	RCS1	44.1	10.44	1	1	
Transmembrane protein 184A	TMEM184A	46.8	9.41	1	1	
Chloride channel protein 2	CLCN2	97.1	4.93	1	1	
Enolase-phosphatase E1	ENOPH1	28.6	16.34	1	1	
Thymic stromal cotransporter protein	SLC46A2	52	8.98	1	1	
Protein Gm9923	-	67.4	6.21	1	1	
DEP domain-containing protein 5	DEPDC5	180.3	2.01	1	1	
Protein Fam3c	FAM3C	27	12.15	1	1	
Keratin, type II cytoskeletal 5	KRT5	61.7	8.45	1	1	
Ubiquinone biosynthesis protein COQ4 homolog, mitochondrial	COQ4	30.1	5.26	1	1	
Espin	ESPN	154.4	1.92	1	1	
Dynamin-like 120 kDa protein, mitochondrial	OPA1	113.2	4.29	1	1	
Striatin-4	STRN4	23.9	16.89	1	1	
Transcription factor Dp-2	DP2	42.9	11.40	1	1	
Serine/threonine-protein kinase PINK1, mitochondrial	PINK1	63.1	6.90	1	1	
E3 ubiquitin-protein ligase HERC2	HERC2	527.1	0.99	1	1	
Contactin-associated protein 1	CNTNAP1	156.2	2.45	1	1	
Cornifin-A	SPRR1A	15.8	25.00	1	1	
GRIP1-associated protein 1	GRIPAP1	95.9	3.46	1	1	
Ribonuclease P protein subunit p40	RPP40	41.5	9.92	1	1	
Protein Ift140	IFT140	165.8	3.07	1	1	
GTP-binding nuclear protein Ran	RAN	24.3	19.91	1	1	
ATPase, H ⁺ transporting, lysosomal V1 subunit B1	ATP6V1B1	56.8	6.24	1	1	
Cullin-3	CUL3	88.9	5.08	1	2	
Fibrinogen gamma chain	FGG	52.3	1.95	1	1	
Elongation factor 1-alpha 2	EEF1A2	50.4	2.38	1	1	
Protein KIAA0100	KIAA0100	253.5	1.74	1	1	
Echinoderm microtubule-associated protein-like 4	EML4	110.1	3.83	1	1	

Table S1. List of differential proteins for the conditioned media of PC3 cells co-cultured with RAW264.7 cells identified by LC ESI-MS/MS analysis and matched against human (orange), murine (yellow) or both (green) protein databases.

Conditioned media from the hemin pre-treated PC3 and Raw264.7 cells co-culture					
Protein	Gene	Theoretical molecular mass (kDa)	Coverage %	No. of peptides	PSMs
ADP-dependent glucokinase	ADPGK	54.1	9.26	1	1
Alkylglycerol monoxygenase	AGMO	51.5	9.21	1	4
ATP-binding cassette sub-family A member 1	ABCA1	13.8	31.71	1	1
AT-rich interactive domain-containing protein 1A	ARID1A	241.9	1.18	1	1
Bifunctional epoxide hydrolase 2	EPHX2	62.6	5.41	1	1
CD44 antigen	CD44	81.5	1.62	1	1
Chloride intracellular channel protein 6	CLIC6	73	4.26	1	1
Collagen alpha-1(XVI) chain	COL16A1	157.7	2.06	1	1
Complement component C9	C9	63.1	1.61	1	1
Cyto kinase-dependent hematopoietic cell linker	CLNK	49.5	4.21	1	1
E3 ubiquitin-protein ligase HACE1	HACE1	102.3	4.40	1	1
Enoyl-CoA hydratase, mitochondrial	ECHS1	31.4	12.41	1	1
Fornin-2	FMN2	180	1.34	1	1
Gelsolin	GSN	85.6	1.02	1	1
Importin subunit alpha-3	KPNA4	57.9	5.57	1	1
Integrator complex subunit 8	INTS8	113	3.22	1	1
Kelch-like protein 26	KLHL26	68.1	5.37	1	1
Keratin, type II cytoskeletal 5	KRT5	23.1	19.40	1	1
Klotho	KL	116.1	2.96	1	1
Membrane-associated phosphatidylinositol transfer protein 3	PITPNM3	13.1	35.34	1	1
Membrane-spanning 4-domains subfamily A member 7	MS4A7	26.1	7.08	1	1
Methylcrotonoyl-CoA carboxylase subunit alpha, mitochondrial	MCCCL1	66.4	5.02	1	3
Neuron navigator 2	NAV2	268.1	1.12	1	1
Nuclear receptor co-binding protein 2	NRBP2	57.8	7.39	1	1
Nucleoporin SEH1	SEH1L	39.6	10.56	1	1
OTU domain-containing protein 1	OTUD1	51	6.08	1	1
Phospholipase B1, membrane-associated	PLB1	56.6	5.69	1	1
PRA1 family protein 3	ARL6IP5	21.6	14.36	1	1
Ran-binding protein 30	RANBP10	70.4	5.85	1	1
Retinoic acid-induced protein 3	GPRCSA	40.2	10.64	1	1
RNA-binding protein 4	RBM4	40.3	10.16	1	1
Roundabout homolog 3	ROBO3	148.1	3.61	1	1
Scavenger receptor class A member 5	SCARA5	54	5.66	1	2
Thrombospondin-4	THBS4	105.8	1.04	1	1
Tubulin beta-5 chain	TUBB6	49.8	3.14	1	1
Tyrosine-protein kinase JAK3	JAK3	125	2.22	1	1
Uncharacterized protein KIAA1551	KIAA1551	194.7	2.18	1	1
Zinc finger and BTB domain-containing protein 17	ZBTB17	87.9	4.86	1	1
Zinc finger protein 544	ZNF544	81.7	5.17	1	1
Retrotransposon gag domain-containing protein 1	RGAG1	144.2	1.87	1	1
Neuronal-specific septin-3	SEPTIN3	40.7	12.29	1	1
Putative deoxyribonuclease TATDN1	TATDN1	33.5	7.64	1	1
Serine/threonine-protein kinase PAK 6	PAK6	74.8	6.75	1	1
Vimentin-type intermediate filament-associated coiled-coil protein	VMAC	18.3	15.38	1	1
Voltage-dependent L-type calcium channel subunit alpha	CACNA1S	246	0.37	1	1
Zinc finger CCHC domain-containing protein 5	ZCCHC5	52.8	8.84	1	1
Protein I600014C10Rik	-	5.6	60.00	1	1
MCG142225	-	34.1	8.33	1	1
MCG134312, isoform CRA_a	-	77.2	5.19	1	1
MCG142459	-	19.5	22.94	1	1
5'-AMP-activated protein kinase subunit gamma-2	PRKAG2	62.9	6.54	1	1
Acrosin-binding protein	ACRBP	61.1	6.48	1	1
Adhesion G protein-coupled receptor L4	EILTD1	82.2	3.92	1	1
Angiomotin-like protein 1	AMOTL1	107.9	3.62	1	1
Apolipoprotein A4	APOA1	30.6	3.79	1	1
ATP synthase subunit O, mitochondrial	ATPSO	23.3	5.63	1	1
Breast cancer anti-estrogen resistance protein 3	BCAR3	92.2	4.88	1	1
Centriolar coiled-coil protein of 110 kDa	CCP110	111.1	3.39	1	1
Cytochrome b5	CYB5A	15.2	32.09	1	1
F-actin-capping protein subunit alpha-3	CAPZ3	34.9	8.03	1	1
FERM domain-containing protein 3	FRMD3	68.4	5.55	1	1
Furin	FURIN	86.7	3.53	1	1
Gamma-tubulin complex component 2	TUBGCP2	103.2	3.09	1	1
Netrin-5	NTN5	48.8	3.32	1	1
Protein AF-9	MLLT3	63.3	3.51	1	1
Putative E3 ubiquitin-protein ligase UNKL	UNKL	11	35.19	1	1
Rho GTPase-activating protein 1	ARHGAP1	54.4	9.39	1	1
SIC-transferring protein 1	SHC1	62.6	8.12	1	1
SID1 transmembrane family member 2	SIDT2	94.4	4.58	1	1
Sodium-dependent neutral amino acid transporter B(O)AT3	SLOC6A8	69.2	4.88	1	1
Tubulin beta-4B chain	TUBB4B	49.8	3.15	1	1
Tubulin polyglutamylase TTL4	TTL4	132.4	2.77	1	1
Type 1 phosphatidylinositol 4,5-bisphosphate 4-phosphatase	TMEM558	30	17.25	1	1
Unconventional myosin-1xb	MYO9B	240.2	2.07	1	1
Vesicle-associated membrane protein 2	VAMP2	17.9	28.83	1	1
YTH domain-containing family protein 3	YTHDF3	63.9	8.08	1	1
Zinc finger protein 536	ZNF536	141.5	3.61	1	1
MANSC domain-containing protein 4	MANSC4	36.8	11.87	1	1
Olfactory receptor 24	OLFR24	34.8	8.68	1	1
Microtubule-associated serine/threonine-protein kinase 3	MAST3	11.1	33.02	1	1
Uncharacterized protein C11orf84 homolog	C11orf84	41.2	10.24	1	1
Protein Myo15b	MYO15B	332.8	0.79	1	1
Type-1B angiotensin II receptor	AGTR1	40.9	6.96	1	1
Carbonyl reductase [NADPH] 2	CBR2	25.9	14.75	1	1
Opioid growth factor receptor-like protein 1	OGFR1	52.2	7.38	1	1
Protein Scml2	SCML2	106.7	4.02	1	1
Protein Zfp108	ZFP108	73.1	4.83	1	1
Core-binding factor, runt domain, alpha subunit 2, translocated to, 3	CBFA2T3	67.9	6.61	1	1
Tripartite motif-containing protein 438	TRIM438	52.2	5.17	1	1
Testis-expressed sequence 11 protein	TEX11	109.6	2.85	1	1
Differentially expressed in FDCP 6 homolog	DEF6	73.9	1.48	1	1
Vigilin	HDLBP	141.4	2.13	1	1
A-kinase anchor protein SPHKAP	SPHKAP	186.3	2.41	1	1
Alpha-enolase	ENO 1	47.1	5.53	1	1
CREB-binding protein	CREBBP	265.2	3.36	2	2
Elongation factor 1-alpha 2	EEF1A2	50.4	2.38	1	1
GDNF family receptor alpha-2	GFRA2	51.5	7.54	1	1
Peptidyl-prolyl cis-trans isomerase A	PPIA	18	8.48	1	1
Phosphoglycerate mutase 1	PGAM1	28.8	8.27	1	1
Probable ATP-dependent RNA helicase DDX59	DDX59	68.8	5.49	1	1
Prosaposin receptor GPR37L1	GPR37L1	52.7	7.07	1	1
Zinc finger protein 608	ZNF608	162.1	2.45	1	1
Triosephosphate isomerase 1	TRIP1	30.8	3.50	1	1
60S ribosomal protein L30	RPL30	12.8	24.35	1	1
Calcium/calmodulin-dependent protein kinase kinase 2	CAMKK2	64.7	2.38	1	1

Table S2. List of differential proteins for the conditioned media of hemin pre-treated PC3 cells co-cultured with RAW264.7 cells identified by LC ESI-MS/MS analysis and matched against human (orange), murine (yellow) or both (green) protein databases.

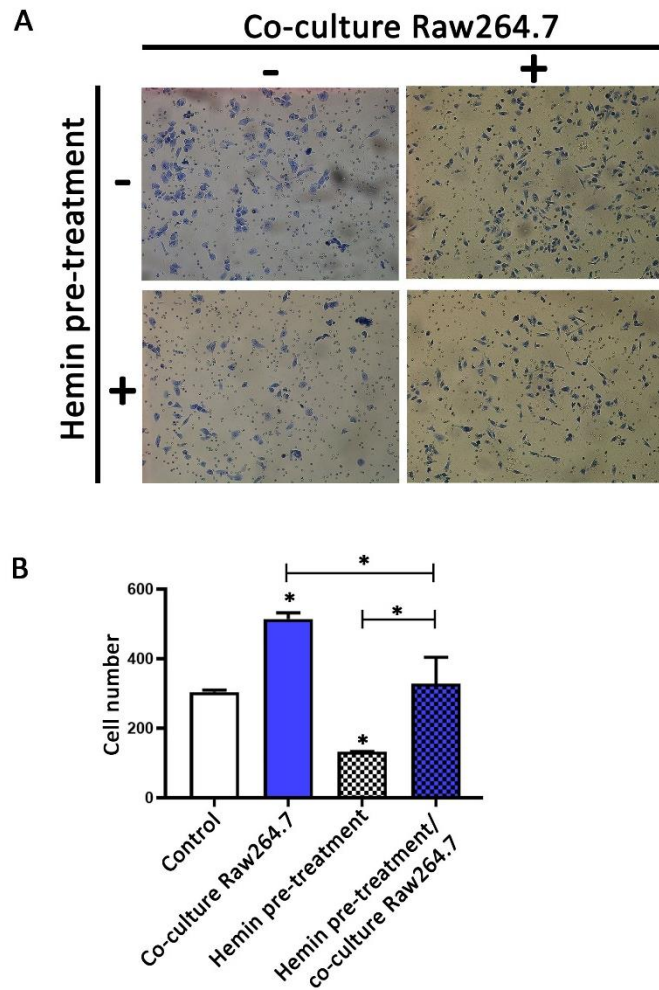


Figure S1. PC3 cells grown in co-culture with Raw246.7 cells increase invasive properties. **(A)** PC3 cells pre-treated or not with hemin were plated on Matrigel in a transwell insert and incubated alone or in co-culture with Raw264.7 cells. **(B)** Cell invasion was measured using Matrigel-coated transwell inserts. Cells that had invaded to the underside of the inserts after 24 h of incubation were counted by light microscopy. Four fields of view from each insert were counted. One representative from at least three independent experiments is shown (columns, mean of eight fields of view; bars, SEM; * $p < 0.05$, significant difference).

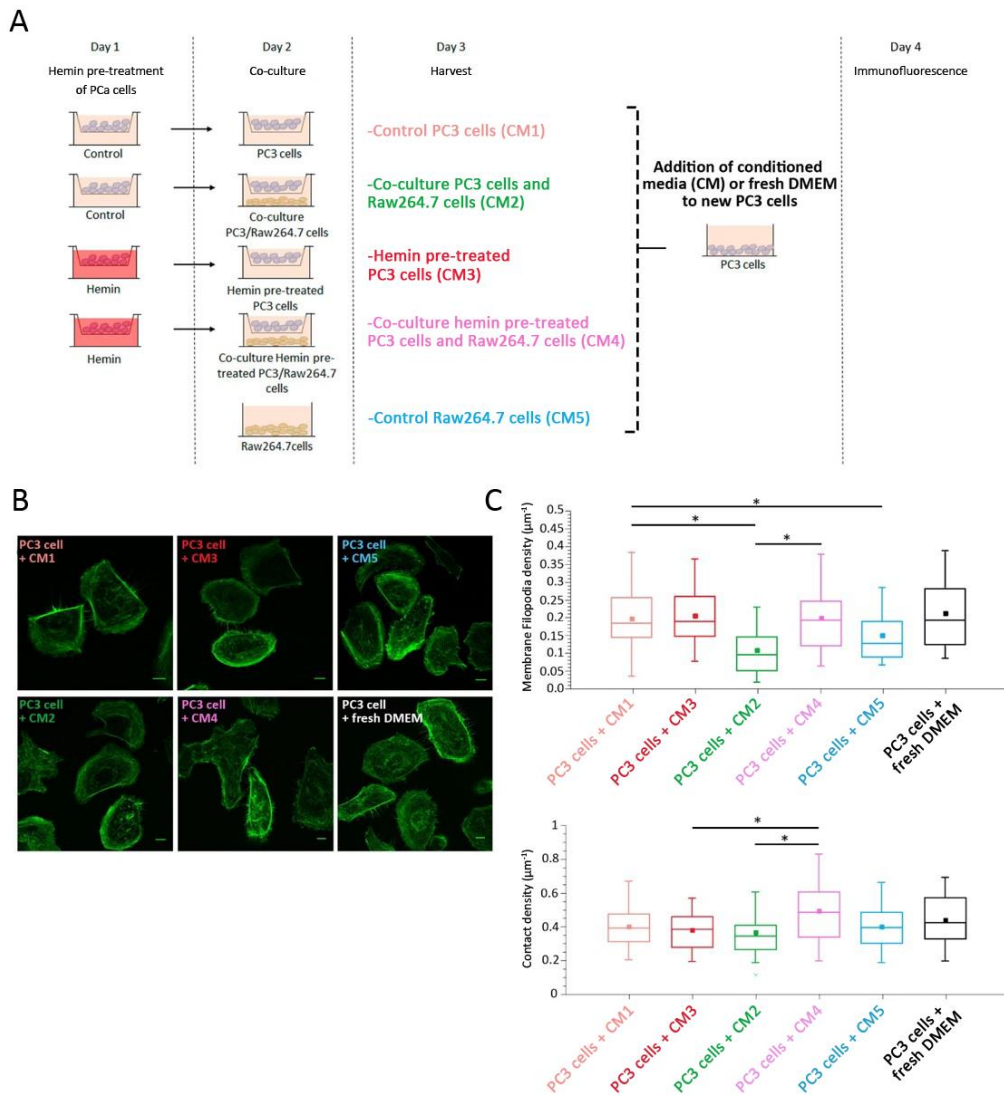


Figure S2. Analysis of cell contact density in PC3 cells cultured with conditioned media (CM) from PC3-Raw246.7 co-culture systems. PC3 cells were treated or not with hemin (50 μM , 24 h) and then co-cultured with or without Raw246.7 cells (24 h), in different compartments (insert membrane and well). CM from the different experimental conditions was then added to PC3 cells for 24 h. **(A)** Schematic representation of the experimental design. **(B)** Cells were fixed and stained with rhodamine-phalloidin and imaged by confocal microscopy to assess contact density and number of protrusions (scale bars: 10 μm). One representative image for each group is shown. **(C)** Boxplot comparing the measured filopodia density per cell for the different experimental conditions. Error bars represent standard error ($n \geq 36$ cells per condition; $*p < 0.001$). CM1 (pink): CM from PC3 grown alone; CM2 (green): CM from PC3-Raw246.7 co-culture; CM3 (red): CM from PC3 grown alone pre-treated with hemin; CM4 (purple): CM from PC3 pre-treated with hemin and co-cultured with Raw246.7; CM5 (light blue): CM from Raw246.7 grown alone (upper panel). Boxplot comparing cell-cell contacts densities for the different experimental conditions. An intensity profile for each of these sectors was determined using a custom-made algorithm to count contacts (Matlab) ($n \geq 37$ contact regions per condition; $*p < 0.001$) (lower panel).