

Spot No.	swiss-port No.	Gene name	Protein name	pI	MW	Match. Peptides	Cov. (%)	Score	Subcellular location	Functional ontology	matched peptides	MCF7-Rhein/MCF7	T test	MDAMB231-Rhein/MDAMB231	T test
1588	Q6NUR9	YWHAZ	14-3-3 protein zeta/delta	4.7	27899	13/22	40%	121/56	Cytoplasm	Signal transduction	LAEQAER/EKIETELR	1.02	0.99	1.52	0.022
1564	Q6NUR9	YWHAZ	14-3-3 protein zeta/delta	4.7	27899	10/30	31%	64/56	Cytoplasm	Signal transduction	LAEQAER/SVTEQGAELSNEER	1.29	0.1	1.95	0.021
1200	Q86VC0	RPSA	40S ribosomal protein SA	4.8	32947	6/15	25%	75/56	Cytoplasm	Protein translation	AIVAIENPADVSVISSR/LLVVTDPR	1.13	0.19	1.58	0.016
701	P10809	HSPD1	60 kDa heat shock protein, mitochondrial	5.7	61187	7/12	13%	79/56	Mitochondrion	Protein folding	TVIIEQSWGSPK/GANPVEIR	-1.21	0.59	-2.18	0.011
686	P10809	HSPD1	60 kDa heat shock protein, mitochondrial	5.7	61187	6/12	10%	65/56	Mitochondrion	Protein folding	GANPVEIR/APGFGDNR	-1.5	0.064	-1.51	0.035
689	P10809	HSPD1	60 kDa heat shock protein, mitochondrial	5.7	61187	9/17	19%	110/56	Mitochondrion	Protein folding	TVIIEQSWGSPK/GANPVEIR	-1.53	0.048	-1.07	0.68
525	P11021	HSPA5	78 kDa glucose-regulated protein	5.1	72402	17/28	27%	168/56	ER	Protein folding	VEIIANDQGNR/ITPSYVAFTPEGER	-1.22	0.071	-1.63	0.0022
1148	Q96HG5	ACTB	Actin, cytoplasmic 1	5.3	42052	7/19	18%	74/56	Cytoplasm	Cytoskeleton	AGFAGDDAPR/SYELPDGQVITIGNER	1.6	0.02	1.23	0.016
1154	Q96HG5	ACTB	Actin, cytoplasmic 1	5.3	42052	7/18	18%	67/56	Cytoplasm	Cytoskeleton	AGFAGDDAPR	1.59	0.011	1.34	0.058
1003	Q6GMP2	ENO1	Alpha-enolase	7.0	47481	6/16	11%	67/56	Cytoplasm	Glycolysis	GVSKAVEHINK/SGKYDLD FK	2.2	0.14	-1.64	0.023
980	Q6GMP2	ENO1	Alpha-enolase	7.0	47481	9/27	26%	83/56	Cytoplasm	Glycolysis	AAVPSGASTGIYEALELR	-1.66	0.026	-1.38	0.22

1399	P07355	ANXA2	Annexin A2	7.6	38808	9/18	20%	83/56	Plasma membrane	Calcium regulation	AYTNFDAER/GVDEVTIVNILTNR	1.69	0.4	2.45	0.0042
1449	P12429	ANXA3	Annexin A3	5.6	36524	8/12	23%	72/56	Plasma membrane	Calcium regulation	LTFDEYR/NTPAFLAER	-1.31	0.12	-1.85	0.0017
1291	Q9P291	ARMCX1	Armadillo repeat-containing X-linked protein 1	9.3	49720	7/24	12%	58/56	Plasma membrane	Cytoskeleton regulation	EKAHSGSHSGGLEAK	-1.18	0.4	-1.69	0.014
1669	Q6P1M9	ARMCX5	Armadillo repeat-containing X-linked protein 5	8.9	62649	6/6	12%	66/56	Plasma membrane	Cytoskeleton regulation	ANTGSRPDR/VIAWSRAR	-1.68	0.0071	-1.64	0.077
			Calcium-binding						Calcium Transport						
1025	Q9BV35	SLC25A23	mitochondrial carrier protein SCaMC-3	6.9	52573	6/6	11%	64/56	Mitochondrion		ILHSMDR/QEKLTGMWWK	-1.39	0.34	-1.68	0.034
1538	P07339	CTSD	Cathepsin D	6.1	45037	7/16	13%	64/56	Lysosome	Protein degradation	QVFGEATK/QPGITFIAAK	-1.87	0.0047	-1.25	0.61
1971	P29762	CRABP1	Cellular retinoic acid-binding protein 1	5.3	15727	12/33	62%	149/56	Cytoplasm	signal transduction	VGEGFEEETVDGR/KVAVAAASKPHVEIR	-1.2	0.46	-3.14	0.00023
1718	P29762	CRABP1	Cellular retinoic acid-binding protein 1	5.3	15727	6/16	48%	87/56	Cytoplasm	signal transduction	PNFAGTWK/VGEGFEEETVDGR	1.06	0.84	1.9	0.01
1968	P29373	CRABP2	Cellular retinoic acid-binding protein 2	5.4	15854	5/17	36%	66/56	Cytoplasm	signal transduction	PNFSGNWK/SENFEELLK	-1.36	0.071	-1.59	0.023
1860	P23528	CFL1	Cofilin-1	8.2	18719	6/27	26%	63/56	Cytoplasm	Cytoskeleton regulation	AVLFCLSEDKK/MLPKDKCR	-3.16	0.00015	-9.87	2.50E-05
1870	P60981	DSTN	Destrin	8.1	18950	4/5	16%	61/56	Cytoplasm	Cytoskeleton regulation	YALYDASFETK/HFVGMLPEK	-2.76	0.0002	-7.76	5.90E-06

1598	P38117	ETFB	Electron transfer flavoprotein subunit beta	8.2	28054	4/12	14%	60/56	Mitochondrion	Electron transport	VDLVLLGK/LGPLQVAR	-1.5	0.01	-1.43	0.012
1355	P29692	EEF1D	Elongation factor 1-delta	4.9	31217	9/34	24%	78/56	Cytoplasm	Protein translation	FKYDDAER/YDDAERR	1.53	0.63	-1.76	0.0017
1294	P04075	ALDOA	Fructose-bisphosphate aldolase A	8	39851	14/33	41%	132/56	Cytoplasm	Glycolysis	PYQYPALTPEQK	-1.61	0.0092	-1.48	0.019
1293	P04075	ALDOA	Fructose-bisphosphate aldolase A	8	39851	9/33	28%	78/56	Cytoplasm	Glycolysis	PYQYPALTPEQK/ELSDIAHR	1.27	0.17	1.64	0.0038
1118	Q7Z2K8	GPRIN1	G protein-regulated inducer of neurite outgrowth 1	8.3	103134	10/20	8%	65/56	Plasma membrane	Neurite outgrowth	MDPMTVR/ADPMFTGK	1.41	0.34	-1.61	0.024
2082	P09382	LGALS1	Galectin-1 <sup>a</sup>	5.3	15048	1/1	8%	74/30	Secreted	Immuno response	DSNNLCLHFNPFR	-1.34	0.45	-2.12	0.0022
1598	P17931	LGALS3	Galectin-3	8.6	26193	5/17	18%	58/56	Secreted	Immuno response	IALDFQR/GNDVAFHFNPR	-1.5	0.01	-1.43	0.012
1568	P17931	LGALS3	Galectin-3	8.6	26193	8/23	34%	121/56	Secreted	Immuno response	GNDVAFHFNPR/IALDFQR	-1.94	0.0055	-1.17	0.36
1721	Q9CWI7	RAN	GTP-binding nuclear protein Ran	7.0	24579	7/29	32%	71/56	Nucleus	Nucleus tranport	LVLVGDGGTGK/HLTGEFEK	-1.05	0.67	1.63	0.003
1510	Q6FH47	GNB2L1	Guanine nucleotide-binding protein subunit beta-2-like 1 <sup>a</sup>	7.6	35511	10/33	29%	100/56	Plasma membrane	Signal transduction	DETNYGIPQR/LWDLTTGTTTR (MAAPAVSGLSR)	1.45	0.023	1.89	0.0049
568	P11142	HSPA8	Heat shock cognate 71 kDa protein	7.6	35511	(1/1)	(2%)	(34/30)	Plasma membrane	Signal transduction	(DETNYGIPQR)	-1.18	0.24	-1.58	0.006

582	P11142	HSPA8	Heat shock cognate 71 kDa protein	5.4	71082	10/32	20%	70/56	Cytoplasm	Protein folding	VEIIANDQGNR/TTPSYVAFTDTER	-1.2	0.19	-1.55	0.0069
1654	Q9UC36	HSPB1	Heat shock protein beta-1 / HSP-27	5.9	22826	10/33	42%	105/56	Cytoplasm	Protein folding	VPFSLLR/GPSWDPFR	-2.42	0.0017	1.08	0.8
678	P61978	HNRNPK	Heterogeneous nuclear ribonucleoprotein K	5.4	51230	7/15	16%	77/56	Nucleus	Transcription control	GSDFDCELR/VVLIGGKPDR	-1.39	0.091	-1.7	0.011
2085	P22626	HNRNPA2B1	Heterogeneous nuclear ribonucleoproteins A2/B1 <sup>a</sup>	8.9	37464	6/14	16%	61/56	Nucleus	Transcription control	EKEQFR/DYFEEYGK (GGNFQFGDSR)	-1.55	0.0052	-1.55	0.0053
1017	P05783	KRT18	Keratin, type I cytoskeletal 18 <sup>a</sup>	5.3	48029	9/18	18%	94/56	Cytoplasm	Cytoskeleton	STFSTNYR/LASYLDR (AQIFANTVDNAR)	-1.16	0.4	-1.63	0.034
1056	Q9P1Y4	KRT19	Keratin, type I cytoskeletal 19	5.0	44079	5/14	13%	59/56	Cytoplasm	Cytoskeleton	FGPGVAFR/APSIHGGSGGR	1.15	0.12	-2.26	0.013
1135	Q9P1Y4	KRT19	Keratin, type I cytoskeletal 19	5.0	44079	13/38	28%	120/56	Cytoplasm	Cytoskeleton	FGPGVAFR/APSIHGGSGGR	-1.87	0.0044	1.58	0.16
1141	Q9P1Y4	KRT19	Keratin, type I cytoskeletal 19	5.0	44079	25/67	48%	215/56	Cytoplasm	Cytoskeleton	FGPGVAFR/APSIHGGSGGR	-2.22	0.00021	1.78	0.36
1120	Q9P1Y4	KRT19	Keratin, type I cytoskeletal 19	5.0	44079	17/36	37%	169/56	Cytoplasm	Cytoskeleton	FGPGVAFR/APSIHGGSGGR	-1.75	0.0043	1.89	0.14
1134	Q9P1Y4	KRT19	Keratin, type I cytoskeletal 19	5.0	44079	26/59	48%	237/56	Cytoplasm	Cytoskeleton	FGPGVAFR/APSIHGGSGGR	-1.8	0.0019	2.01	0.25
1931	P60660	MYL6	Myosin light polypeptide 6	4.6	17090	6/17	33%	82/56	Cytoplasm	Cytoskeleton regulation	EAFQLFDR/ILYSQCGDVMR	1.15	0.24	-1.74	9.20E-05
1930	P60660	MYL6	Myosin light polypeptide 6	4.6	17090	9/34	55%	111/56	Cytoplasm	Cytoskeleton regulation	EAFQLFDR/ILYSQCGDVMR	-1.59	0.031	-1.53	0.0055
1752	O43678	NDUFA2	NADH dehydrogenase [ubiquinone] 1 alpha	9.6	11029	4/13	26%	60/56	Mitochondrion	Electron transport	GVGAKLGLR/LGLREIR	1.34	0.24	2.93	0.022

Protein Interaction Network Analysis															
ID	Protein ID	Protein Name	Experimental Data				Predicted Function				Statistical Metrics				
			Peptides	Count	Success (%)	Failure (%)	Peptides	Count	Success (%)	Failure (%)	Mean Score	SD Score	Median Score	P-value	
757	Q02818	NUCB1	Nucleobindin-1	5.2	53846	7/18	15%	64/56	Golgi apparatus	Calcium homeostasis	YEMLKEHER/APAAHPEGQLK	1.61	0.12	-1.54	0.05
1835	Q7L3E2	NOL8	Nucleolar protein 8	6.7	132448	8/8	6%	71/56	Nucleus	Transcription control	ENNLKP/SDGPETTTQCK	1.11	0.83	1.67	0.048
1898	P22392	NME2	Nucleoside diphosphate kinase B	8.5	17401	7/13	48%	104/56	Nucleus	Nucleotide biosynthesis	GLVGEIIKR/QHYIDLK	-1.48	0.084	-1.81	0.0053
1698	P0CW26	PNMA6C	Paraneoplastic antigen-like protein 6C	5.2	44304	5/12	12%	61/56	Unknown	Unknown	LEVLLQK/RLLEGRL	-1.59	0.036	-1.37	0.057
1913	Q9UC61	PPIA	Peptidyl-prolyl cis-trans isomerase A	7.7	18229	13/34	60%	129/56	Cytoplasm	Protein folding	VSFELFADK/FEDENFILK	-1.59	0.027	-1.65	0.0068
1895	Q9UC61	PPIA	Peptidyl-prolyl cis-trans isomerase A	7.7	18229	9/40	35%	73/56	Cytoplasm	Protein folding	VSFELFADK/TEWLDGK	-1.28	0.1	-1.64	0.0092
1715	Q06830	PRDX1	Peroxiredoxin-1	8.3	22324	8/16	37%	120/56	Cytoplasm	Redox regulation	IGHPAPNFK/QITVNDLPVGR	3.32	0.0015	5.5	0.00055
1710	Q06830	PRDX1	Peroxiredoxin-1 <sup>a</sup>	8.3	22324	(1/1)	(5%)	(53/30)	Cytoplasm	Redox regulation	IGHPAPNFK/ATAVMPDGQFK (QITVNDLPVGR) (TIAQDYGVLK)	-1.2	0.21	-2.95	0.00061
747	P30101	PDIA3	Protein disulfide-isomerase A3	5.9	57146	7/13	12%	62/56	Cytoplasm	Protein folding	LAPEYEAAATR/VDCTANTNTCNK	1.35	0.29	-1.89	0.0021
1420	Q8TEQ0	SNX29	Sorting nexin-29	5.9	91596	9/29	10%	62/56	Cytoplasm	Vesicle transport	KEIASDSDSR/FAPTVSDLKK	1.15	0.92	-1.55	0.02

1856	P16949	STMN1	Stathmin <sup>a</sup>	5.8	17292	6/26 (1/1)	32% (8%)	118/56 (84/30)	Cytoplasm	Signal transduction	ASGQAFELILSPR	-1.56	0.0015	-3.23	0.00084
580	Q9UC56	HSPA9	Stress-70 protein, mitochondrial	5.9	73920	13/30	21%	100/56	Mitochondrion	Protein folding	VLENAEGAR/RYDDPEVQK	-1.39	0.09	-1.59	0.0053
706	P31948	STIP1	Stress-induced-phosphoprotein 1	6.4	63227	12/20	16%	103/56	Cytoplasm	Stress response	AAALEFLNR/KAAALEFLNR	-1.29	0.03	-1.69	0.00098
1380	A8MYJ7	TTC34	Tetratricopeptide repeat protein 34	7.0	61777	8/12	10%	59/56	Unknown	Unknown	RPQAAPVLAR/ALLVALSR	1.48	0.63	-2.3	0.0013
1841	A8MYJ7	TTC34	Tetratricopeptide repeat protein 34	7.0	61777	6/14	9%	59/56	Unknown	Unknown	ALLVALSR/KGDVPGAAAR	1.19	0.11	1.56	0.011
1752	Q01995	TAGLN	Transgelin	8.9	22653	9/22	35%	78/56	Cytoplasm	Cytoskeleton regulation	GPSYGMCSR/TDMFQTVDLFEKG	1.34	0.21	2.93	0.022
381	P55072	VCP	Transitional endoplasmic reticulum ATPase	5.2	89950	12/22	12%	95/56	ER	Vesicle transport	KGDIFLVR/GDIFLVR	-1.32	0.036	-1.61	0.0021
1650	P60174	TPI1	Triosephosphate isomerase	5.7	31057	7/16	22%	89/56	Cytoplasm	Glycolysis	VVFHQTK/IAVAAQNCYK	-1.36	0.23	-2.59	0.0046
1638	P60174	TPI1	Triosephosphate isomerase <sup>a</sup>	5.7	31057	14/28 (1/1)	59% (3%)	200/56 (41/30)	Cytoplasm	Glycolysis	KFFVGGNWK/DCGATWVVLGHSER (IAVAAQNCYK)	-1.56	0.041	-1.27	0.35
1303	Q9NV66	TYW1	tRNA wybutosine-synthesizing protein 1 homolog	6.4	84732	8/16	9%	73/56	Cytoplasm	Translation control	TQGKNLQEK/DIFVSGVK	-1.68	0.019	-1.83	0.14
1454	Q96IK2	TPM1	Tropomyosin alpha-1 chain	4.7	32746	14/44	30%	117/56	Cytoplasm	Cytoskeleton regulation	LDKENALDR/KATDAEADVASLNR	-1.43	0.1	-1.93	0.015

1417	Q96IK2	TPM1	Tropomyosin alpha-1 chain	4.7	32746	11/33	23%	95/56	Cytoplasm	Cytoskeleton regulation	LDKENALDR/RQLVEEELDR	-1.16	0.51	-1.79	0.0064
1476	P06753	TPM3	Tropomyosin alpha-3 chain	4.7	32856	8/31	19%	59/56	Cytoplasm	Cytoskeleton regulation	AADAEAEVASLNR	-1.81	0.0031	-2.77	0.00063
1482	Q9UCS3	TPM4	Tropomyosin alpha-4 chain	4.7	28619	19/45	36%	70/56	Cytoplasm	Cytoskeleton regulation	IQALQQQADEAEDR/EKAEGDVAALNR	-1.66	0.0021	-1.67	0.016
1289	P07951	TPM2	Tropomyosin beta chain	4.7	32945	12/33	32%	102/56	Cytoplasm	Cytoskeleton regulation	LDKENAIDR/QLEEEQQALQK	-1.12	0.69	-2.07	0.0019
1497	P21796	VDAC1	Voltage-dependent anion-selective channel protein 1	8.6	30868	5/9	22%	72/56	Mitochondrion	Transport	GYGFGLIK/VTQSNFAVGYK	-1.53	0.015	-1.37	0.02
1526	Q9UC06	ZNF70	Zinc finger protein 70	8.7	52480	5/7	10%	66/56	Nucleus	Transcription control	ECGKAQFSQSSHLLR/SFNQSSGLSQHR	2.25	0.042	1.65	0.0042

**Supplementary Table 1. List of rhein-induced differentially expressed MCF-7 and MDA-MB-231 proteins identified by MALDI-TOF peptide mass fingerprinting and MALDI-TOF/TOF sequence analysis after 2D-DIGE analysis.** Proteins displaying rhein-induced differential labeling of lysines using Cy2 dye, Cy3 dye and Cy5 dye, respectively, were identified by MALDI-TOF peptide mass mapping and MS/MS sequence analysis. Proteins displaying an average fold-difference of  $\geq 1.5$ -fold where  $p < 0.05$  and spots matched in all images are shaded grey. <sup>a</sup>Proteins identified by MALDI-TOF/TOF sequence analysis.