

Supplementary data

Table 1S: Proteins differentially expressed between T98G and CCF-STTG1 cell lines ($p < 0.01$ and Fold Change > 1.5).

Spot number	Swiss-Prot or NCBI entry	Protein	Gene name	Theoretical Mr(kDa)/pI	function	Fold Change T98G/CCF
Chaperone and stress response proteins – Cytosolic						
1	P34932	Heat shock 70 kDa protein 4	HSPA4	94.3 / 5.11	Cytosolic stress response protein.	1.72
2	P08238	Heat shock protein HSP 90-beta	HSP90A B1	83.3 / 4.97	Cytosolic molecular chaperone.	1.67
3	P08238	Heat shock protein HSP 90-beta	HSP90A B1	83.3 / 4.97	Cytosolic molecular chaperone.	1.68
4	P09211	Glutathione S-transferase P	GSTP1	23.4 / 5.43	Conjugation of reduced glutathione to a wide number of exogenous and endogenous hydrophobic electrophiles.	2.13
5	Q06830	peroxiredoxin 1	PRDX1	22.1 / 8.27	Involved in redox regulation of the cell.	3.46
6	P04792	Heat shock protein beta-1	HSP27	22.8 / 5.98	Involved in stress resistance and actin organization.	-2.02
Chaperone and stress response proteins – Mitochondrial						
7	Q8N1C8	HSPA9 protein	HSPA9	73.9 / 6.04	protein folding mitochondrion	1.64
Chaperone and stress response proteins – Endoplasmic reticulum						
8	P30101	Protein disulfide-isomerase A3	PDIA3	56.8 / 5.98	Catalyzes the rearrangement of -S-S-bonds in proteins in the endoplasmic reticulum lumen.	-1.71
9	P07237	Protein disulfide-isomerase	P4HB	57.1 / 4.76	This multifunctional protein catalyzes the formation, breakage and rearrangement of disulfide bonds.	-1.67
10	P27797	Calreticulin	CALR	48.1 / 4.29	Molecular calcium-binding chaperone promoting folding, oligomeric assembly and quality control in the ER via the calreticulin/calnexin cycle.	-2.01
11	P11021	78 kDa glucose-regulated protein	GRP78	72.3 / 5.07	Probably plays a role in facilitating the assembly of multimeric protein complexes inside the ER.	-1.79
12	P14625	heat shock protein gp96 precursor	TRA1	90.1 / 4.6	Molecular chaperone functions in endoplasmic reticulum associated degradation (ERAD).	-1.50
Cytoskeletal proteins						
13	P47756	F-actin capping protein beta subunit	CAPZB	31.4 / 5.36	F-actin-capping proteins bind in a Ca ²⁺ -independent manner to the fast growing ends of actin filaments (barbed end) thereby blocking the exchange of subunits at these ends.	-1.57
14	P18206	Vinculin	VCL	116.7 / 5.83	Actin filament (F-actin)-binding protein involved in cell-matrix adhesion and cell-cell adhesion.	-2.26
15	P08670	Vimentin	VIM	53.7 / 5.06	Vimentins are class-III intermediate filaments found in various non-	-5.49

16	P08670	Vimentin	VIM	53.7 / 5.06	epithelial cells. Vimentins are class-III intermediate filaments found in various non-epithelial cells.	-7.62
17	P26038	Moesin	MSN	67.8 / 6.08	Probably involved in connections of major cytoskeletal structures to the plasma membrane.	1.72
18	Q15691	microtubule-associated protein, RP/EB family, member 1	MAPRE1	29.9 / 5.02	May be involved in microtubule polymerization, and spindle function. May play a role in cell migration.	1.93
Intracellular signal proteins						
19	P52565	Rho GDP-dissociation inhibitor 1	ARHGAP1	23.2 / 5.03	Regulates the GDP/GTP exchange reaction of the Rho proteins by inhibiting the dissociation of GDP from them, and the subsequent binding of GTP to them	-1.55
20	P50395	Rab GDP dissociation inhibitor beta	GDI2	50.7 / 6.11	Regulates the GDP/GTP exchange reaction of most Rab proteins by inhibiting the dissociation of GDP from them, and the subsequent binding of GTP to them.	-1.66
Protein degradation						
21	Q06323	Proteasome activator complex subunit 1	PSME1	28.7 / 5.78	Implicated in immunoproteasome assembly and required for efficient antigen processing.	-3.41
22	Q9UL46	Proteasome activator complex subunit 2	PSME2	27.0 / 5.54	Implicated in immunoproteasome assembly and required for efficient antigen processing.	-2.26
23	P07339	Cathepsin D	CPSD	44.6 / 6.10	Acid protease active in intracellular protein breakdown. Involved in the pathogenesis of several diseases such as breast cancer and possibly Alzheimer disease.	-5.72
24	P09936	Ubiquitin carboxyl-terminal hydrolase isozyme L1	UCHL1	24.8 / 5.33	Ubiquitin-protein hydrolase involved both in the processing of ubiquitin precursors and of ubiquitinated proteins.	14.86
Metabolic proteins						
25	P18669	Phosphoglycerate mutase 1	PGAM1	28.8 / 6.67	Glycolysis. Interconversion of 3- and 2-phosphoglycerate with 2,3-bisphosphoglycerate as the primer of the reaction.	-1.65
26	P18669	Phosphoglycerate mutase 1	PGAM1	28.8 / 6.67	Glycolysis Interconversion of 3- and 2-phosphoglycerate with 2,3-bisphosphoglycerate as the primer of the reaction.	-1.85
27	P07195	L-lactate dehydrogenase B chain	LDHB	36.6 / 5.71	Belongs to the LDH family; involved in the final step of the anaerobic glycolysis pathway.	1.51
28	P29401	Transketolase	TKT	67.9 / 7.58	Pentose shunt	2.33
29	P00367	Glutamate dehydrogenase 1, mitochondrial	GLUD1	61.4 / 7.66	May be involved in learning and memory reactions by increasing the turnover of the excitatory	-1.53

Other proteins

30	P09382	Galectin-1	LGALS 1	14.7 / 5.33	May regulate apoptosis, cell proliferation and cell differentiation.	-2.18
31	P02792	Ferritin light chain	FTL	20.0 / 5.51	Stores iron in a soluble, non-toxic, readily available form. Important for iron homeostasis. Also plays a role in delivery of iron to cells.	1.75
32	Q15365	Poly(rC)-binding protein 1	PCBP1	37.5 / 6.66	Single-stranded nucleic acid binding protein that binds preferentially to oligo dC.	-1.69
33	P20700	lamin B1	LMNB1	66.4 / 5.11	Lamins are components of the nuclear lamina, a fibrous layer on the nucleoplasmic side of the inner nuclear membrane.	2.22
34	O43852	calumenin	CALU	37.1 / 4.47	Calumenin is a Ca ²⁺ -binding protein that belongs to the CREC superfamily and is localized in endoplasmic reticulum.	-2.02
35	P23526	Adenosylhomocysteinase	AHCY	47.7 / 5.92	Adenosylhomocysteine is a competitive inhibitor of S-adenosyl-L-methionine-dependent methyl transferase reactions.	1.52
36	P23381	Tryptophanyl-tRNA synthetase, cytoplasmic	WARS	53.2 / 5.83	Regulates ERK, Akt, and eNOS activation pathways that are associated with angiogenesis, cytoskeletal reorganization and shear stress-responsive gene expression.	-2.8

Table 2S: Spots were identified by MALDI/MS from T98G and CCF-STTG1 cell protein lysates.

Spot number	Accession number	GI number	Name	Mr [kDa]	pI	MASCOT protein score	Coverage	Matched/ searched peaks
1	P18206	gi 4507877 gi 3832703	vinculin isoform VCL [Homo sapiens]	116,6	5,7	72,2	16,5	13/42
2	P34932	9	Heat shock 70 kDa protein 4[Homo sapiens]	94,3	5,0	212,0	29,3	20/26
3	P08238	gi 2014959 4	Heat shock protein HSP 90-beta [Homo sapiens]	83,2	4,8	193,0	35,9	24/49
4	P26038	gi 4505257 gi 2104038	moesin [Homo sapiens]	67,8	6,0	121,0	27,4	16/37
5	Q8N1C8	6	HSPA9 protein [Homo sapiens]	73,8	6,0	106,0	26	12/29
6	P20700	gi 5031877	lamin B1 [Homo sapiens]	66,4	5,0	143,0	25,6	15/24
7	P29401	gi 4507521 gi 2136165	transketolase [Homo sapiens]	67,8	8,5	142,0	27,0	12/18
8	P30101	7	Protein disulfide-isomerase A3 [Homo sapiens]	56,7	5,9	227,0	41,8	23/42
9	P23381	gi 340368	transfer RNA-Trp synthetase	53,1	5,7	90,0	18,5	8/17
10	P00367	gi 4885281	glutamate dehydrogenase 1 [Homo sapiens]	61,4	8,5	132,0	27,4	15/30
11	P50395	gi 6598323	GDP dissociation inhibitor 2 [Homo sapiens]	50,6	6,1	201,0	44,0	19/28
12	O43852	gi 2809324 gi 6241428	calumenin [Homo sapiens]	37,0	4,3	135,0	29,8	11/21
13	P08670	9	vimentin [Homo sapiens]	53,6	4,9	292,0	47,2	28/45
14	P23526	gi 9951915 gi 6241428	S-adenosylhomocysteine hydrolase [Homo sapiens]	47,7	5,9	214,0	32,4	15/20
15	P08670	9	vimentin [Homo sapiens]	53,6	4,9	188,0	44,8	19/38
16	Q15365	gi 5453854	poly(rC) binding protein 1 [Homo sapiens]	37,5	6,8	134	35,1	11/16
17	Q15691	gi 6912494 gi 3041079	microtubule-associated protein, RP/EB family, member 1 [Homo sapiens]	30,0	4,9	111,0	38,1	9/26
18	Q9UL46	2	proteasome activator subunit 2 [Homo sapiens]	27,4	5,4	121,0	40,6	12/28
19	Q06323	gi 3058114 1	proteasome activator subunit 1 isoform 2 [Homo sapiens]	28,6	6,3	79,2	34,8	8/25
20	P18669	gi 4505753	phosphoglycerate mutase 1 (brain) [Homo sapiens]	28,8	6,8	161	49,6	10/15
21	P18669	gi 4505753	phosphoglycerate mutase 1 (brain) [Homo sapiens]	28,8	6,8	140	49,6	12/28
22	P04792	gi 4504517	Heat shock protein beta-1 [Homo sapiens]	22,8	6,0	132,0	52,2	9/20
23	P52565	gi 4757768	Rho GDP dissociation inhibitor (GDI) alpha [Homo sapiens]	23,2	4,9	128,0	40,7	11/21
24	P09936	gi 4185720	ubiquitin carboxy-terminal hydrolase L1 [Homo sapiens]	23,0	5,2	77,8	37,0	6/20
25	P09211	gi 2204207	Glutathione S-transferase P [Homo sapiens]	23,4	5,3	82,0	34,3	6/18
26	Q06830	gi 4505591	peroxiredoxin 1 [Homo sapiens]	22,1	9,2	107,0	44,7	7/15
27	P09382	gi 4504981	beta-galactoside-binding lectin precursor [Homo sapiens]	14,7	5,2	122,0	61,5	9/24
28	P27797	gi 4757900 gi 2007012	Calreticulin [Homo sapiens]	48,1	4,1	191,0	48,2	17/43
29	P07237	5	Protein disulfide-isomerase [Homo sapiens]	57,1	4,6	265,0	51,6	24/43
30	P02792	gi 182516	ferritin light subunit [Homo sapiens]	16,4	5,6	132,0	49,0	10/16
31	P07339	gi 4503143	cathepsin D preproprotein [Homo sapiens]	44,5	6,1	123,0	27,2	12/18
32	P47756	gi 4826659 gi 2014959	F-actin capping protein beta subunit [Homo sapiens]	30,6	5,6	207,0	48,9	17/29
33	P08238	4	Heat shock protein HSP 90-beta [Homo sapiens]	83,2	4,8	202,0	27,5	20/28
34	P11021	gi 1650723 7	78 kDa glucose-regulated protein [Homo sapiens]	72,3	4,9	339,0	50,5	30/41
35	P07195	gi 4557032	lactate dehydrogenase B [Homo sapiens]	36,6	5,7	183,0	40,1	15/24
36	P49588	gi 1015321 gi 2330857	alanyl-tRNA synthetase [Homo sapiens]	106,7	5,2	125,0	22,7	17/38
37	O43175	7	D-3-phosphoglycerate dehydrogenase	56,6	6,3	127,0	21,4	13/27
38	Q2VF42	gi 2622479 0	Glucose-6-phosphate 1-dehydrogenase	54,8	7,0	269,0	42,3	22/28
39	P06733	gi 693933	2-phosphopyruvate-hydratase alpha-enolase; carbonate dehydratase [Homo sapiens]	47,1	7,7	90,6	27,6	11/30
40	P12004	gi 4505641	Proliferating cell nuclear antigen [Homo sapiens]	28,8	4,4	114,0	36,0	8/16

Spot number	Acession number	GI number	Name	MW [kDa]	pI	MASCOT protein score	Coverage	Matched/ searched peaks
41	P18669	gi 4505753	phosphoglycerate mutase 1 (brain) [Homo sapiens]	28,8	6,8	85,5	33,5	6/12
42	O75489	gi 5138999	NADH-Ubiquinone reductase [Homo sapiens]	30,3	8,8	113,0	31,2	9/24
43	P60174	gi 4507645	Triosephosphate isomerase [Homo sapiens]	26,7	6,5	153,0	54,2	11/25
44	P60174	gi 4507645	Triosephosphate isomerase [Homo sapiens]	26,7	6,5	237,0	79,1	16/26
45	P51571	gi 5454090	Translocon-associated protein subunit delta [Homo sapiens]	19,0	5,7	68,7	36,4	5/17
46	P52895	gi 4503285	aldo-keto reductase family 1, member C2 [Homo sapiens]	36,7	7,8	209,0	57,0	15/24
47	P14625	gi 15010550	Endoplasmin [Homo sapiens]	90,1	4,6	160,0	24,8	18/31

Figure 1S: Representative 2D spot maps of CCF-STTG1 (Panel A) and T98G (Panel B). Proteins separation was performed on pH 3-10 NL 24 cm IPG strips in the first dimension and on 12%T-2.5%C PAGE gels in the second dimension. The identified and statistically changed spots in the comparison of T98G respect CCF-STTG1 cell line and in comparisons with cell lines treated with nitric oxide are numbered.

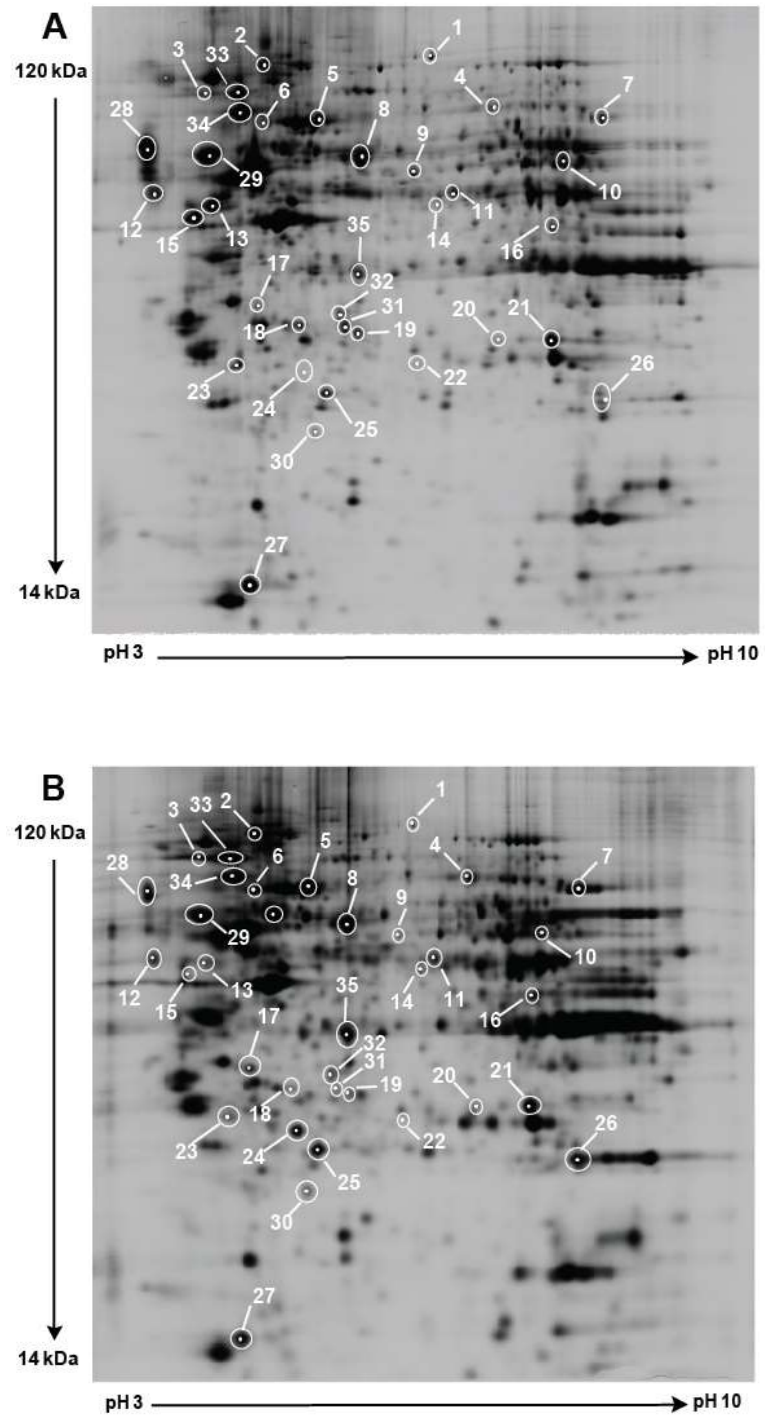


Figure 2S: Validation of LC/MS/MS identified proteins by immunoblotting. Immunoblottings of selected proteins differentially expressed from T98G and CCF-STTG1 cell lines: HSP 70 (Heat shock 70 kDa), GSTP1 (Glutathione S-transferase P), UCHL1 (Ubiquitin carboxyl-terminal hydrolase isozyme L1), VIM (Vimentin) and PRDX1 (peroxiredoxin 1). Histograms represent the mean volumes and the standard deviations calculated for the specific band of each experimental group. Data with asterisks indicates a significant differences between two cell lines with a p value < 0.05. Desitometric measures were normalized against actin. Primary antibodies used are: polyclonal anti HSP70 (Cell Signaling #4872) 1:1000, polyclonal anti GSTP1 (Santa Cruz Biotechnology sc-28494) 1:500, polyclonal anti UCHL1 (Santa Cruz Biotechnology sc-25800) 1:500, polyclonal anti Vimentin (Monosan MON3005) 1:500, polyclonal anti PRXI (Santa Cruz Biotechnology sc-7381) 1:500 and polyclonal anti actin (Santa Cruz Biotechnology sc-7210).

