

S1 Table. Primers used for qRT-PCR

Gene	Forward (F) and Reverse (R) Primers	Estimated PCR product size (bp)	Sequence accession number (NCBI and Ensembl)
RPS18	F AGCGGCTGAAGAAGATACGG R TTGGACACACCCACAGTACG	115	XM_003768950.3
HSPA1L (HSP70-1L)	F AGAGACCTGAACAAGAGTATCAACC R AGGTCCTGCACTTTCTCTGATTTAT	80	XM_003768911.3
HSPA2 (HSP70-2)	F GTATTGAAACCGCAGGGGA R GACTACTGCTCTGGTTGTCTG	111	XM_003756345.2
HSPA4L (HSPH3)	F ACATGAAGAGAGACCGAAAGC R CAGGCTTACTTTCAGGCCCA	380	XM_023505624.1
HSPB1 (HSPB1-201, HSP27)	F ACATTTGCTCGGTCACTCCC R AAGCCGTGCTCATCTTGTCT	121	ENSSHAG000000125 27/ENSSHAT000000 14799.1
HSPAA1 (HSP90A)	F TTGGTTACTTCCCCGTGCTG R GCCTTTTGCCGTAGGGTTTC	176	XM_003756541.3
HSP90AB1 (HSP90B)	F TATCCGAGGTGTGGTGGACT R GCTTCCGTA CTGTTCCACA	389	XM_012549073.2
HSP90B1 (GP96)	F TTGTTCCCACTTCTGCTCCC R AGCCGAGTACGGTTGGAATG	355	XM_003772592.2
HSPD1 (HSP60)	F AGGCAAGGGTGAAAAATCCCA R AAGCAATGCACAACCACCAC	259	XM_012544896.2
CALR	F GATGGGGAATGGGAACCACC R CAGGGGTGTA CT CAGGGTTG	133	XM_003760668.3
MHC Class 1 antigen (SAHAI-01)	F CAGATTTCCCGAGTGGAC R GTCGTAGGCGAACTGAAG	144	NM_001280855.1
B2M	F TGTGCATCCTTCCCTACCTGGAGG R CATTGTTGAAAGACAGATCGGACCGC	201	XM_003756064.3

RPS18, ribosomal protein S18.

Supplementary Table 2. Antibodies used in the study

Antibody	Supplier	Antibody Registry number	Dilution
Polyclonal goat anti HSP60	Abcam (Ab82520)	AB_1658326	1:500
Monoclonal mouse anti HSP70	Abcam (Ab2787)	AB_303300	1:1000
Polyclonal rabbit anti HSP90	Abcam (Ab13495)	AB_1269122	1:1000
Monoclonal rat anti Grp94	Abcam (Ab90458)	AB_2295611	1:1000
Polyclonal rabbit anti β -actin	Abcam (Ab8227)	AB_2305186	1:1000
Polyclonal goat anti-mouse Immunoglobulins HRP	DAKO (P0447)	AB_2617137	1:4000
Polyclonal rabbit anti-goat Immunoglobulins HRP	DAKO (P0449)	AB_26177143	1:4000
Polyclonal rabbit anti-rat Immunoglobulin HRP	DAKO (P0450)	AB_2630354	1:4000
Polyclonal goat anti-rabbit Immunoglobulin HRP	DAKO (P0448)	AB_2617138	1:4000

Supplementary Table 3. HSPs identified in the proteome of a DFTD cell line

Gene symbol*	Name*	Number of unique peptides
HSP70 superfamily: HSPA (HSP70) and HSPH (HSP110) families		
HSPA Family		
HSPA2	Heat shock protein family A (Hsp70) member 2	17
HSPA4	Heat shock protein family A (Hsp70) member 4	20
HSPA5	Heat shock protein family A (Hsp70) member 5	33
HSPA8	Heat shock protein family A (Hsp70) member 8	37
HSPA9	Heat shock protein family A (Hsp70) member 9	28
HSPH Family		
HSPH1	Heat shock protein family H (Hsp110) member 1	28
HSP90 family		
HSP90AA1	Heat shock protein 90 alpha family class A member 1	24
HSP90AB1	Heat shock protein 90 alpha family class B member 1	40
HSP90B1	Heat shock protein 90 beta family member 1	44
Chaperonins family		
HSPD1	Heat shock protein family D (Hsp60) member 1	35
HSPE1	Heat shock protein family E (Hsp10) member 1	5
CCT2	Chaperonin containing TCP1 subunit 2	29
CCT3	Chaperonin containing TCP1 subunit 3	21
CCT4	Chaperonin containing TCP1 subunit 4	20
CCT5	Chaperonin containing TCP1 subunit 5	19
CCT6B	CCT6B	9
CCT7	Chaperonin containing TCP1 subunit 7	15
CCT8	Chaperonin containing TCP1 subunit 8	25
DNAJ (HSP40) family		
DNAJA1	DnaJ heat shock protein family (Hsp40) member A1	4
DNAJA2	DnaJ heat shock protein family (Hsp40) member A2	9
DNAJA3	DnaJ heat shock protein family (Hsp40) member A3	2
DNAJB11	DnaJ heat shock protein family (Hsp40) member B11	5
DNAJC8	DnaJ heat shock protein family (Hsp40) member C8	2

*Nomenclature according to the HUGO Gene Nomenclature Committee (HGNC).

Supplementary Table 4. Biological relevance of selected cognate DFTD antigens identified by mass spectrometry analyses

Predicted protein

14-3-3 protein. Spots 7,8,9,10

Function:

Contributes to regulate cell cycle, cell growth, differentiation, survival, apoptosis, migration and spreading.

Associations to cancer:

14-3-3 protein is involved in human gastric cancer cell progression and potential diagnostic and prognostic biomarker in liver and renal cancer. 14-3-3zeta protein is currently undergoing extensive investigation as a novel therapeutic target. 14-3-3 theta protein has been identified as an antigen that induces a humoral response in lung cancer.

References:

(1-9).

60 kDa heat shock protein (HSP60), mitochondrial. Spot 1

Function:

Protein mostly localized in the mitochondrial matrix and outer mitochondrial membrane, constitutively expressed under normal condition, and induced by heat shock, mitochondrial damage, and mitochondrial DNA depletion.

Associations to cancer:

Overexpression reported in various cancers such as adrenal tumours and human breast, large bowel, bronchial, exocervical, ovarian, gastric and prostate cancers. HSP60 is actively secreted by tumour cells and plays a role in transformation, promotion of angiogenesis and metastases. Serum antibodies against HSP60 are elevated in patients with osteosarcoma.

References:

(10-22).

ATP synthase subunit beta, mitochondrial. Spots 4,18

Function:

Enzyme that catalyses ATP synthesis. It also exists outside the cell membrane.

Associations to cancer:

Ectopic (outside the cell membrane) the enzyme has been proposed as a marker for tumour target therapy. The down-regulation of the catalytic subunit of the enzyme is a hallmark of most human carcinomas.

References:

(23-27).

Predicted protein

Cathepsin B. Spot 12

Function:

The protein is a lysosomal cysteine proteinase. It is also known as amyloid precursor protein secretase and is involved in the proteolytic processing of amyloid precursor protein (APP).

Associations to cancer:

The expression and subcellular localization of cathepsins changes during cancer progression and cathepsins are involved in various aspects of tumourigenesis including metastasis and aggressive behaviour. Cathepsin B has been proposed as potential biomarker and therapeutic target in human cancers such as breast, human pancreatic ductal adenocarcinoma (PDA), cervical, colon, endometrial and pancreatic cancers.

References:

(28-35)

Cellular retinoic acid-binding protein 1. Spot 11

Function:

Specific binding protein for a vitamin A and is thought to play an important role in retinoic acid-mediated differentiation and proliferation processes.

Associations to cancer:

It has been found overexpressed in ovarian carcinoma tissues.

References:

(36-39) <http://www.ncbi.nlm.nih.gov/gene/1381>

Heterogeneous nuclear ribonucleoprotein. Spot 1

Function:

Transcription factor and has a role during cell cycle progression.

Associations to cancer:

The protein has been implicated in tumourigenesis. It was found overexpressed in melanoma and colorectal, oral, lung, nasopharyngeal, pancreatic, prostate and liver cancers.

References:

(40-46).

Perilipin-3. Spot 18

Function:

Protein required for endosome-to-Golgi transport.

Associations to cancer:

Protein strongly expressed in invasive tumours and in lymph node metastasis in cervical dysplasia and invasive carcinoma.

References:

(47-49)

Predicted protein

Rho GDP-dissociation inhibitor 1. Spot 10

Function:

Down-regulator of Rho family GTPases. It prevents nucleotide exchange and membrane association.

Associations to cancer:

The expression of the protein is altered in a variety of cancers including oral squamous cell carcinoma and colorectal cancer. Overexpression of the protein promotes cell motility and lymph node metastasis. Higher frequency of autoantibodies against Rho-GDP proteins was found in nasopharyngeal and acute leukaemia patients.

References:

(50-56).

Stathmin. Spot 16

Function:

Stathmin is a member of a family of microtubule-destabilizing proteins that regulate the dynamics of microtubule polymerization and depolymerisation.

Associations to cancer:

Stathmin is overexpressed across a broad range of human malignancies including leukaemia, lymphoma, neuroblastoma, ovarian, prostatic, breast and lung cancers and mesothelioma. Stathmin is a potential target in cancer therapies that disrupt the mitotic apparatus.

Stathmin is also upregulated in normally proliferating cell lines. In normal cells, stathmin is upregulated in neurons and anterior pituitary cells. In glial cells, stathmin is a constituent of the myelin Sheath.

References:

(57-66)

Tubulin. Spots 4,16,18

Function:

Tubulin is an integral component of microtubules. It occurs mostly as soluble heterodimers consisting primarily of α - and β -tubulin isoforms or as assembled tubulin polymers that form microtubules.

Associations to cancer:

Autoantibodies against tubulin-alpha and tubulin-beta were detected in sera of renal and oral cell carcinoma and chronic myeloid leukaemia patients.

References:

(67-72).

Predicted protein

Vimentin. Spots 1,3,4,5,6,18

Function

Vimentin is a major constituent of the intermediate filament family of proteins. It is ubiquitously expressed in normal mesenchymal cells; it helps maintaining cellular integrity and provides resistance against stress.

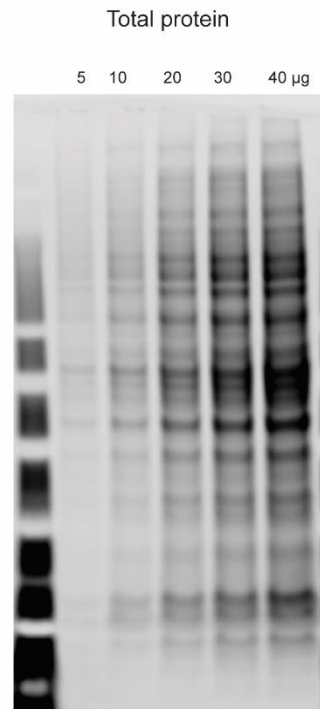
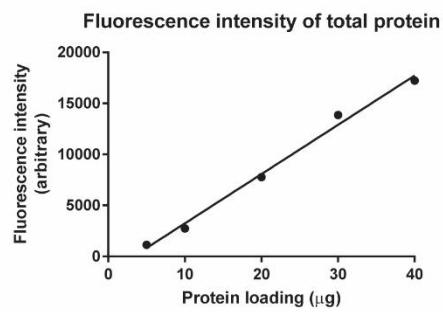
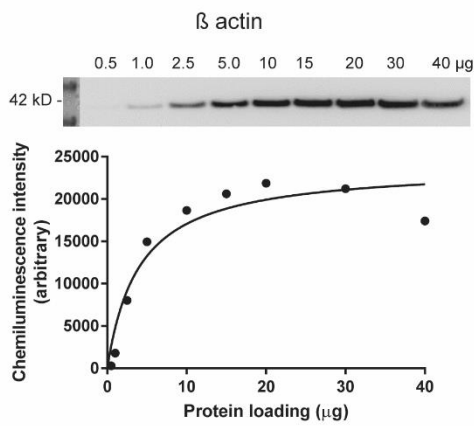
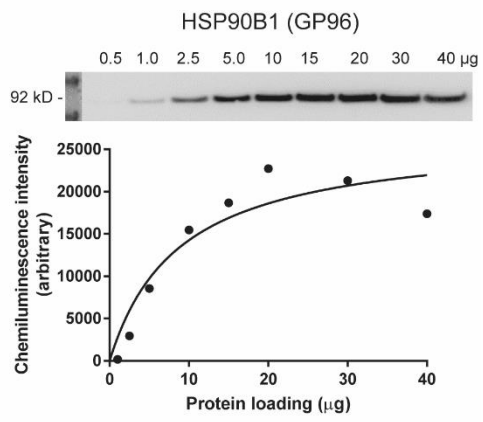
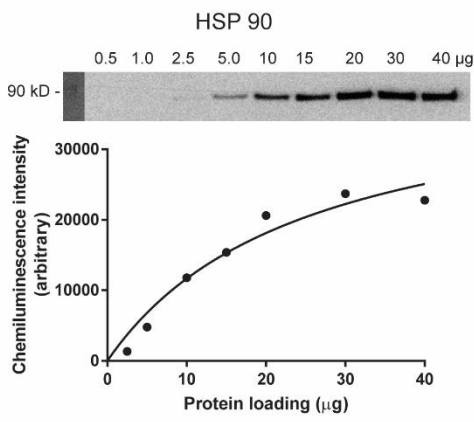
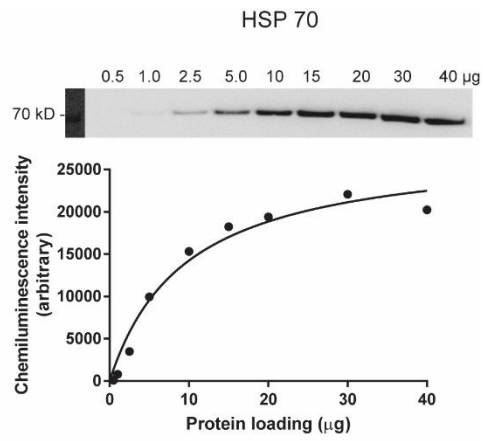
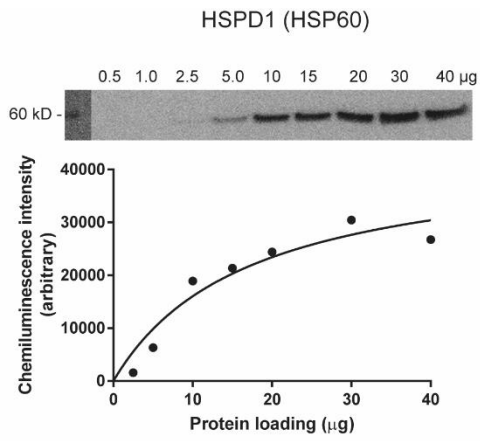
Associations to cancer:

Vimentin is overexpressed in various epithelial cancers, including prostate cancer, gastrointestinal tumours, tumours of the central nervous system, breast cancer, malignant melanoma, and lung cancer. Overexpression in cancer correlates well with accelerated tumour growth, invasion, and poor prognosis. Autoantibodies against vimentin were detected in sera from patients with pancreatic cancer. Anti-vimentin therapeutic approaches have also been proposed.

References:

(73-77).

Supplementary Figure 1



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