

Supplemental Table 1. DIGE experimental design

Cytosolic fractions: 9 samples (3 Mock, 3 HSV-1 4h, 3 HSV-1 8h)

GEL	Cy3 (50µg)	Cy5 (50µg)	Cy2 (5.5µg/sample)
1	Mock A	HSV-1 4h B	pooled
2	HSV-1 4h A	Mock B	pooled
3	Mock C	HSV-1 8h A	pooled
4	HSV-1 8h B	HSV-1 4h C	pooled
5	HSV-1 8h C	none	pooled

Microsomal fractions: 9 samples (3 Mock, 3 HSV-1 4h, 3 HSV-1 8h)

GEL	Cy3 (50µg)	Cy5 (50µg)	Cy2 (5.5µg/sample)
1	Mock A	HSV-1 4h B	pooled
2	HSV-1 4h A	Mock B	pooled
3	Mock C	HSV-1 8h A	pooled
4	HSV-1 8h B	HSV-1 4h C	pooled
5	HSV-1 8h C	none	pooled

DIGE experimental design and labelling strategy. Pairs of samples are reverse-labelled in order to eliminate the possibility of dye labelling bias.

Supplemental Table 2. Phenyx submission parameters

Scoring model	ESI-QTOF (Default)	
Parent charge	2,3,4 (trust=yes)	
Rounds	1	2
Modifications	Cys_CAM [fixed] Oxidation_M [variable]	Cys_CAM [fixed] Oxidation_M [variable]
Enzyme	trypsin miss cleav. 1 cleav. Mode= normal	trypsin miss cleav. 2 cleav. Mode= normal
AC score	6	6
Peptide Thresholds	length >=6 score >=5.0 p-value <=1.0E-4	length >=6 score >=5.0 p-value <=1.0E-4
Parent tolerance	50.0ppm	0.1Da
Turbo scoring	tolerance=800.0ppm coverage>=0.2 series=b;b++;y;y++	tolerance=800.0ppm coverage>=0.2 series=b;b++;y;y++

The first round parameters need to be stringent enough to sufficiently validate protein identification. The second round parameters make it possible to open the search criteria to increase the sequence coverage, by searching for combinatorial modifications or other features. A two-round search therefore identifies proteins according to a first set of parameters and then performs a more exhaustive search on the proteins while saving computation time and reducing the random match rate.

Scoring model: refers to the algorithm that generates the score between experimental and theoretical masses, dependent upon the instrument type.

Parent charge: refers to the charge state of the parent ions.

Modifications: Fixed chemical/post-translational modification that occurs for every instance of the modifiable amino acid in the protein sequence; variable modification that may occur or may not occur.

Enzyme: The enzyme used to digest the protein. The error allowance for enzyme inefficiency is included as number of sites per peptide that were not cut (Miss Cleav.) and as occurrence of the digestion according to the cleavage rules (one or both ends of protein) (Cleav mode).

AC score: Refers to the minimum significant value for a protein's accession number score. Protein matches scoring lower than this value (set at 6) are rejected from the identified proteins.

P-value: Statistical measure of the probability that the identification is a random match. (The lower the p-value, the more significant match).

Supplemental material figure 1. Measurement of Beta-gal activity

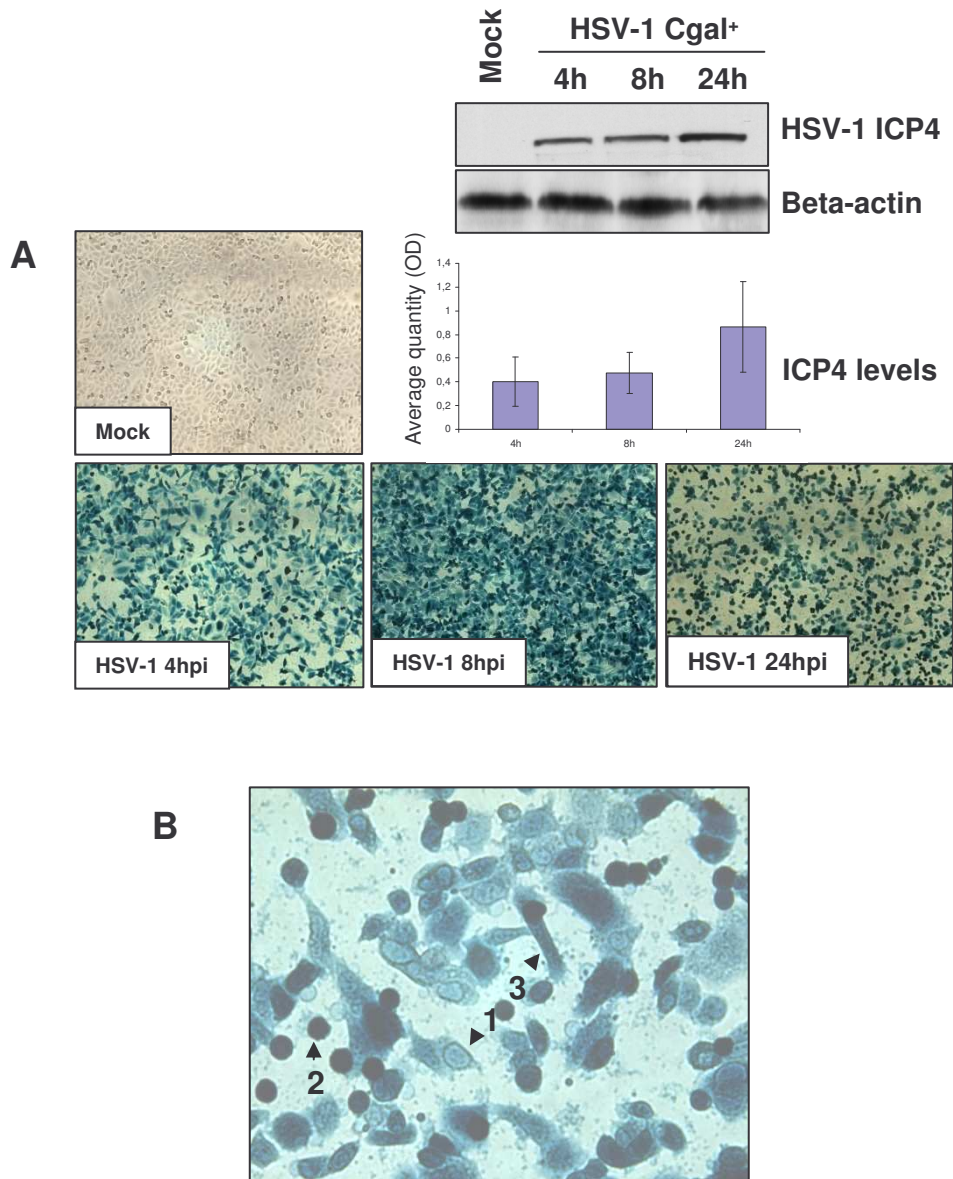
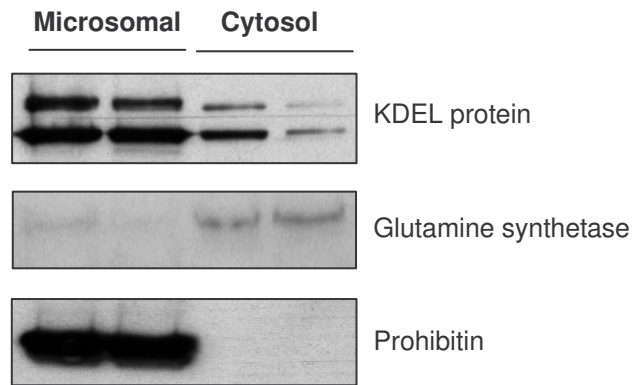


Figure 1. β -galactosidase activity staining of Huh7 cells upon HSV-1 Cgal+ infection. Human hepatoma Huh7 cells were infected with HSV-1 Cgal+ and analyzed 4, 8 and 24 hpi. HSV-1 ICP4 protein was evidenced by Western blot analysis. The bar plot shows an average \pm SD of the OD intensities in all 9 independent experiments (A). Infected cells were positive for b-gal staining 4, 8, and 24 hpi (A). 24 hpi large cytoplasmic vacuoles (1), cell rounding (2), and detachment (3) were observed, indicating the HSV-induced cytopathic effect (B). Equal protein loading was demonstrated using an antibody against b-actin. A representative case of three independent experiments is shown.

Supplemental material figure 2.

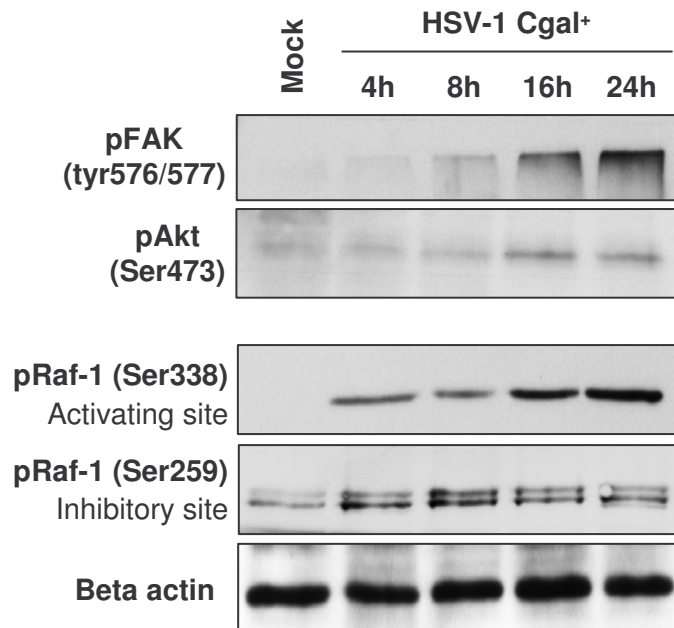


Quality control of isolated organelles in Huh7 cells. Western blotting were performed using specific antibodies against KDEL (ER marker), glutamine synthase (cytoplasmic protein) and prohibitin (mitochondrial marker).

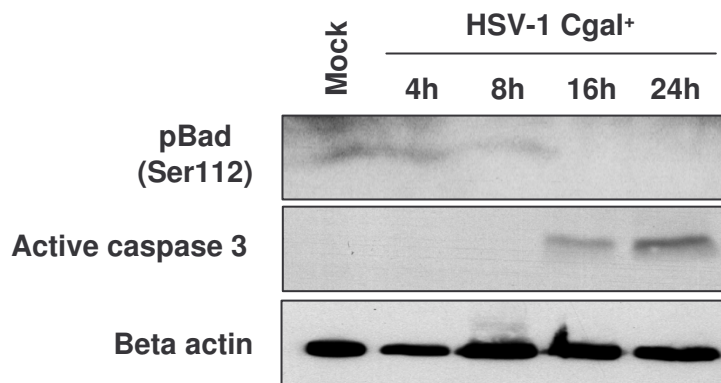
Supplemental material figure 3.

Signaling intermediates altered by HSV-1 Cgal⁺ infection

Survival intermediates altered by HSV-1 Cgal⁺ infection



Apoptotic intermediates altered by HSV-1 Cgal⁺ infection



MS/MS data from differential cytosolic proteins

Spot	AC	Protein name	pl	MW	score	cvg%	peptides	
719	P30154	Serine/threonine-protein phosphatase 2A 65 kDa regulatory subunit A beta isoform	4.84	66214	26,18	6	4	
Sequenced peptides			z	m/z	d m/z	z-score	p-value	position
			2	621,89	-0,015	7,75	8,56E-12	47-58
			2	437,237	-0,012	6,37	9,94E-07	34-40
			2	459,271	-0,014	6,27	1,52E-06	118-125
			2	583,275	-0,019	5,78	2,21E-05	579-588
Spot	AC	Protein name	pl	MW	score	cvg%	peptides	
875	P61978	Heterogeneous nuclear ribonucleoprotein K. [ISOFORM 2]	5.39	50976	36,43	11	5	
Sequenced peptides			z	m/z	d m/z	z-score	p-value	position
			2	561,776	-0,017	8,44	1,28E-13	38-46
			2	549,738	-0,008	6,46	1,28E-07	140-148
			2	597,87	-0,013	6,96	2,99E-09	306-316
			2	437,27	-0,013	6,05	5,33E-06	397-405
			2	630,31	-0,017	7,9	9,33E-12	423-433
Spot	AC	Protein name	pl	MW	score	cvg%	peptides	
978	Q02790	52 kDa FK506-binding protein	5.35	51805	14,11	4	2	
Sequenced peptides			z	m/z	d m/z	z-score	p-value	position
			2	566,782	-0,004	8,27	5,12E-13	344-353
			2	530,269	-0,018	5,84	2,15E-05	409-416
Spot	AC	Protein name	pl	MW	score	cvg%	peptides	
1002	Q02790	52 kDa FK506-binding protein	5.35	51805	56,85	16	7	
Sequenced peptides			z	m/z	d m/z	z-score	p-value	position
			2	566,79	-0,011	9,52	7,65E-18	344-353
			2	719,87	-0,013	9,46	1,17E-17	331-343
			2	690,33	-0,016	9,36	2,41E-17	39-51
			2	531,31	-0,012	9,31	3,21E-17	378-386
			2	530,26	-0,011	6,83	2,56E-08	409-416
			2	484,26	-0,014	6,65	1,01E-07	66-73
			2	561,30	-0,010	5,72	4,91E-05	88-97
Spot	AC	Protein name	pl	MW	score	cvg%	peptides	
1071	Q92599	Septin-8. [ISOFORM 2]	5.89	55756	19,83	6	3	
Sequenced peptides			z	m/z	d m/z	z-score	p-value	position
			2	490,827	0,002	7,17	2,14E-10	179-187
			2	446,72	-0,002	6,35	7,91E-07	283-289
			2	511,27	0	6,3	1,51E-06	332-339
Spot	AC	Protein name	pl	MW	score	cvg%	peptides	
1344	Q9HB07	UPF0160 protein MYG1	6.25	42450	21,39	9	3	
Sequenced peptides			z	m/z	d m/z	z-score	p-value	position
			2	548,41	-0,004	7,82	2,31E-12	189-198
			2	747,87	-0,007	7,14	5,10E-10	220-231
			2	550,32	-0,007	6,44	5,09E-08	244-253
Spot	AC	Protein name	pl	MW	score	cvg%	peptides	
1394	P35237	Serpin B6	5.18	42590	15,28	9	2	
Sequenced peptides			z	m/z	d m/z	z-score	p-value	position
			2	476,28	-0,008	7,22	1,42E-09	82-89
			2	519,27	-0,006	6,18	2,65E-06	364-372
Spot	AC	Protein name	pl	MW	score	cvg%	peptides	
1396	P40121	Macrophage capping protein (Myc basic motif homolog 1)	6.73	39240	15,33	7	2	
Sequenced peptides			z	m/z	d m/z	z-score	p-value	position
			2	640,82	-0,012	7,58	1,40E-10	254-265
			2	801,43	0,015	7,75	2,99E-11	326-339

Spot	AC	Protein name	pl	MW	score	cvg%	peptides	
1511	Q13347	Eukaryotic translation initiation factor 3 subunit 2 (eIF-3 beta) (eIF3 p36).	5.38	36502	39,46	15	5	
Sequenced peptides			z	m/z	d m/z	z-score	p-value	position
EGDLLFTVAK			2	546,81	-0,009	7,37	5,96E-10	21-30
SGEVLVNVK			2	472,79	-0,014	8,28	4,16E-13	177-185
QINDIQLSR			2	543,8	-0,0015	7,37	7,12E-10	190-198
DMTMFVTASK			2	581,76	-0,001	9,86	1E-19	199-208
SYSSGGEDGYVR			2	638,78	-0,002	6,57	5,63E-08	299-310

Spot	AC	Protein name	pl	MW	score	cvg%	peptides	
2011	P63029	Translationally-controlled tumor protein (TCTP)	4.76	19462	66,69	37	6	
Sequenced peptides			z	m/z	d m/z	z-score	p-value	position
DLISHDELFSDIYK			3	847,94	-0,020	8,4	0,00132	6-19
YIKDYMK			2	488,76	-0,012	6,87	3,39E-08	91-97
GKLEEQKPER			3	405,23	-0,008	6,21	1,19E-07	101-110
VKPFMTGAAEQIK			3	479,27	-0,009	6,66	8,73E-08	111-123
EDGVTPFMIFFK			2	723,87	-0,019	8,42	6,59E-05	153-164
DGLEMEK			2	419,192	-0,0055	6,1	2,49E-06	165-171

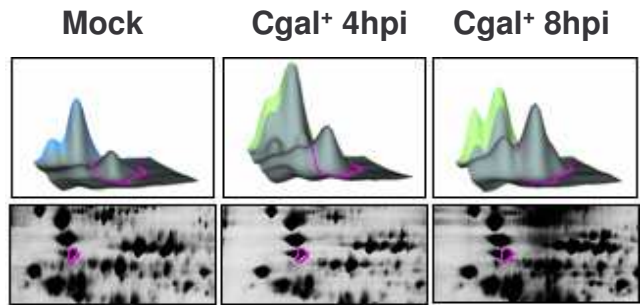
Spot	AC	Protein name	pl	MW	score	cvg%	peptides	
2088	P04906	Glutathione S-transferase P (GST class-pi).	6.89	23439	16,69	10	2	
Sequenced peptides			z	m/z	d m/z	z-score	p-value	position
PPYTIVYFPVR			2	676,38	-0,008	7,73	2,89E-11	1-11
STCLYGQLPK			2	583,81	-0,015	8,97	1,12E-15	45-54

Spot	AC	Protein name	pl	MW	score	cvg%	peptides	
2853	P09382	Galectin-1 (Lectin galactoside-binding soluble 1).	5.33	14716	8,68	12	2	
Sequenced peptides			z	m/z	d m/z	z-score	p-value	position
SFVLNLGK			2	439,27	-0,008	8,66	9,41E-15	29-36
LPDGYEFK			2	471,73	0,003	6,22	5,27E-05	101-108

Differential Cytosolic proteins (I)

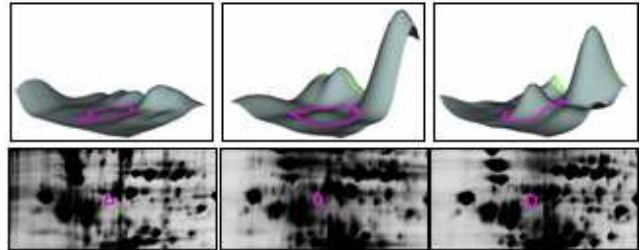
Ser/thre PP2A 65KDa regulatory subunit A

4hpi/Mock		8hpi/Mock	
T-test	Ratio	T-test	Ratio
0,0012	1,71	0,0041	1,83



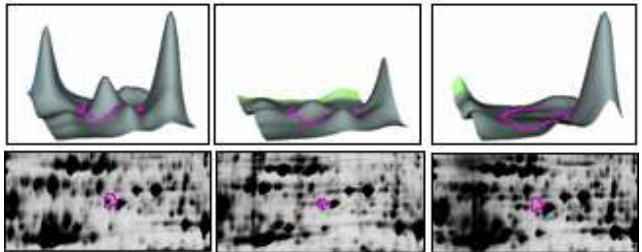
Heterogeneous nuclear ribonucleoprotein K

4hpi/Mock		8hpi/Mock	
T-test	Ratio	T-test	Ratio
0,022	1,62	0,0033	2,97



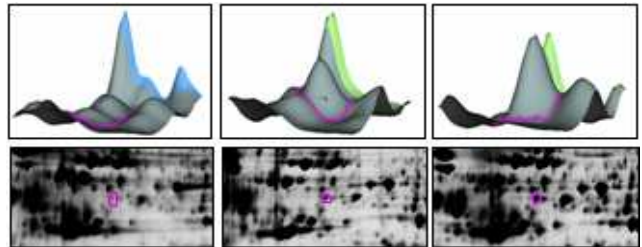
52KDa FK506-binding protein

4hpi/Mock		8hpi/Mock	
T-test	Ratio	T-test	Ratio
0,035	-2,37	8,80E-05	-4,8



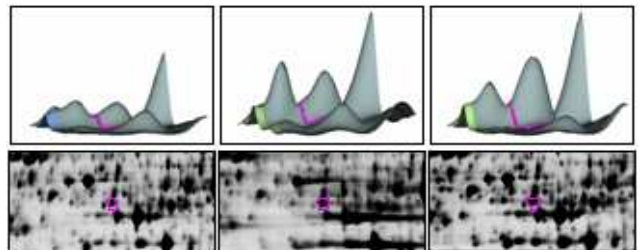
52KDa FK506-binding protein

4hpi/Mock		8hpi/Mock	
T-test	Ratio	T-test	Ratio
0,0071	1,72	0,0071	2,24



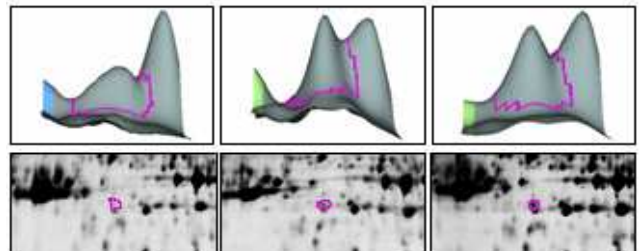
Septin 8

4hpi/Mock		8hpi/Mock	
T-test	Ratio	T-test	Ratio
0,034	1,87	0,0031	2,26



UPF0160MYG1

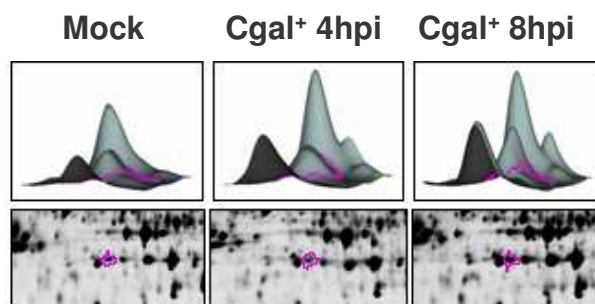
4hpi/Mock		8hpi/Mock	
T-test	Ratio	T-test	Ratio
0,028	1,71	0,0024	1,67



Differential Cytosolic proteins (II)

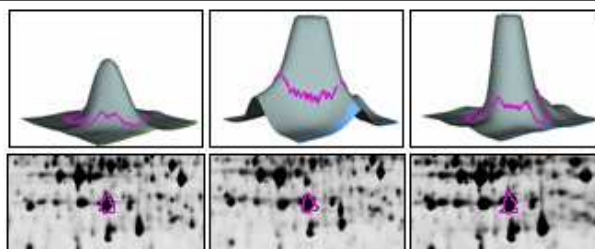
Serpin B6

4hpi/Mock		8hpi/Mock	
T-test	Ratio	T-test	Ratio
n.s	n.d	0,013	1,77



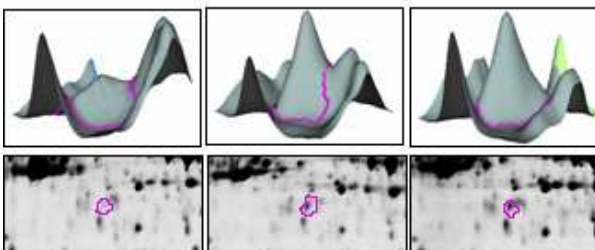
Macrophage capping protein

4hpi/Mock		8hpi/Mock	
T-test	Ratio	T-test	Ratio
n.s	n.d	0,0022	1,78



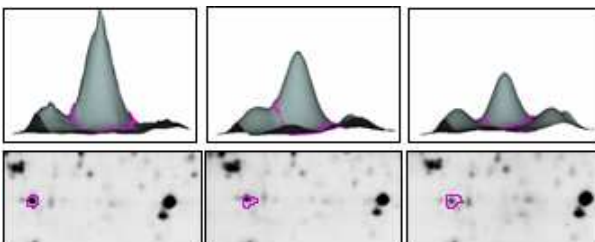
Eukaryotic translation initiation factor 3 subunit 2

4hpi/Mock		8hpi/Mock	
T-test	Ratio	T-test	Ratio
0,0042	1,95	0,0011	1,93



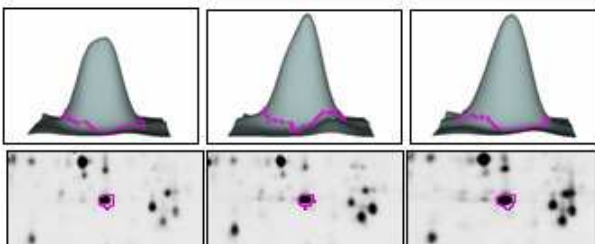
fortilin

4hpi/Mock		8hpi/Mock	
T-test	Ratio	T-test	Ratio
0,0037	-1,54	0,035	-1,55



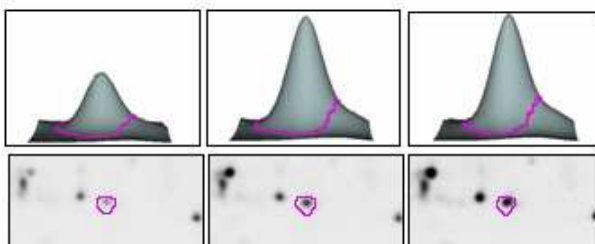
Glutathione S-transferase P

4hpi/Mock		8hpi/Mock	
T-test	Ratio	T-test	Ratio
n.s	n.d	0,0077	1,56

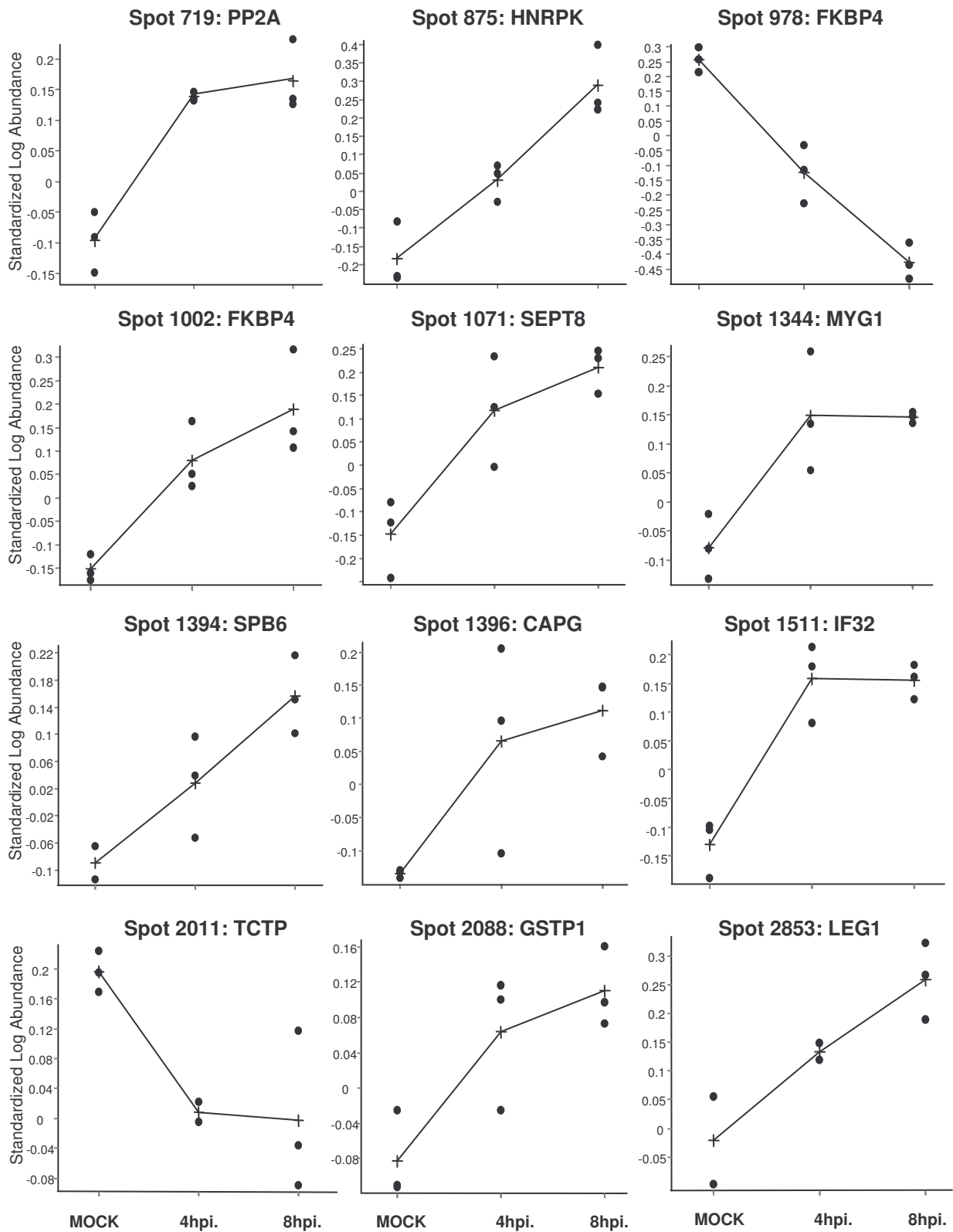


Galectin-1

4hpi/Mock		8hpi/Mock	
T-test	Ratio	T-test	Ratio
n.s	n.d	0,034	1,89



Differential cytosolic proteins in HSV-1 Cgal⁺-infected Huh7 cells



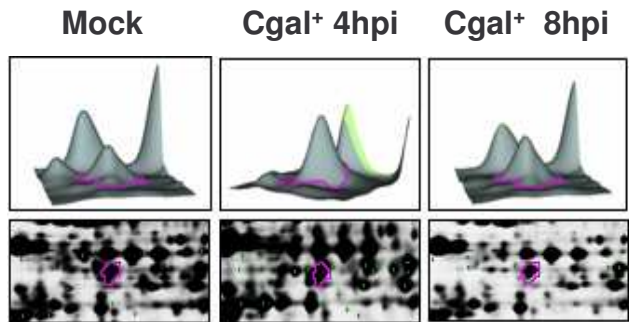
MS/MS data from differential microsomal proteins

Spot	AC	Protein name	pI	MW	score	cvg%	peptides	
1231	Q9Y230	RuvB-like 2 (p47 protein)	5.49	51157	31,84	10	4	
Sequenced peptides			z	m/z	d m/z	z-score	p-value	position
			2	578,31	-0,008	9,02	6,11E-16	29-29
			2	660,83	-0,018	7,2	2,46E-09	223-233
			2	882,45	-0,017	6,8	3,67E-08	314-329
			2	614,82	-0,005	8,82	4,46E-15	428-437
Spot	AC	Protein name	pI	MW	score	cvg%	peptides	
1611	Q9Y371	SH3 domain GRB2-like protein B1 (Bax- interacting factor 1) (Bif-1).	5.78	40796	15,6	5	2	
Sequenced peptides			z	m/z	d m/z	z-score	p-value	position
			2	561,31	-0,005	8,98	1,16E-15	11--21
			2	498,25	-0,006	6,62	1,36E-07	197-204
Spot	AC	Protein name	pI	MW	score	cvg%	peptides	
1627	Q9P2R7	Succinyl-CoA ligase [ADP-forming] beta-chain, mitochondrial precursor	7.05	50317	12,4	4	2	
Sequenced peptides			z	m/z	d m/z	z-score	p-value	position
			2	669,82	-0,010	5,65	4,67E-05	166-175
			2	593,78	-0,006	6,71	5,21E-08	275-284
Spot	AC	Protein name	pI	MW	score	cvg%	peptides	
1628	Q96I99	Succinyl-CoA ligase [GDP-forming] beta-chain, mitochondrial precursor	6.15	46511	58,79	18	7	
Sequenced peptides			z	m/z	d m/z	z-score	p-value	position
			2	608,78	0,028	10	2,2E-20	356-366
			2	594,28	0,042	8,97	8,06E-16	290-301
			2	542,25	0,029	8,83	2,25E-15	302-310
			2	561,76	0,035	8,28	3,3E-13	1--9
			2	620,77	0,044	8,12	7,93E-13	114-123
			2	596,27	0,044	7,79	1,38E-11	154-163
			2	620,74	0,047	6,63	1,45E-08	223-232
Spot	AC	Protein name	pI	MW	score	cvg%	peptides	
1633	Q94905	SPFH domain-containing protein 2 precursor (Erlin-2)	5.47	37840	23,670	10	3	
Sequenced peptides			z	m/z	d m/z	z-score	p-value	position
			2	653,87	-0,015	10	2,83E-20	221-232
			2	579,77	-0,015	7,36	3,34E-10	182-190
			2	674,86	-0,008	6,26	1,64E-06	61-72
Spot	AC	Protein name	pI	MW	score	cvg%	peptides	
1635	Q96I99	Succinyl-CoA ligase [GDP-forming] beta-chain, mitochondrial precursor	6.15	46511	21,63	8	3	
Sequenced peptides			z	m/z	d m/z	z-score	p-value	position
			2	608,82	-0,007	7,85	1,83E-11	356-366
			2	620,796	-0,0125	7,26	1,06E-09	223-232
			2	561,80	-0,009	6,51	3,14E-07	1--9

Differential Microsomal proteins

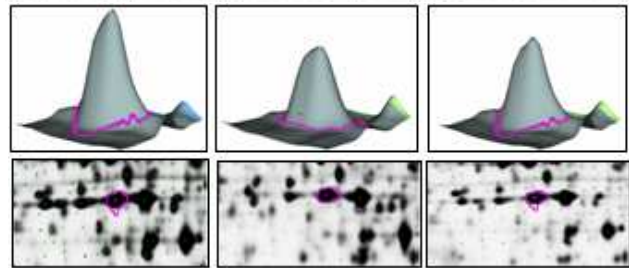
RuvB-like 2

4hpi/Mock		8hpi/Mock	
T-test	Ratio	T-test	Ratio
0,0028	1,73	n.s	n.d



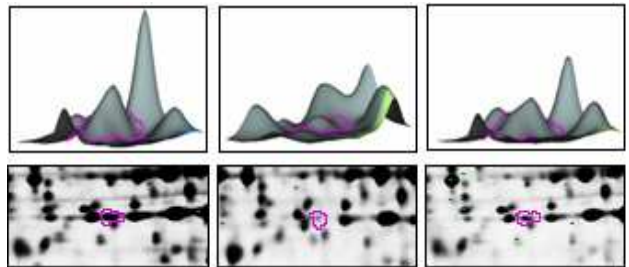
Bax-interacting factor 1 (Bif-1)

4hpi/Mock		8hpi/Mock	
T-test	Ratio	T-test	Ratio
0,045	-1,57	0,034	-1,46



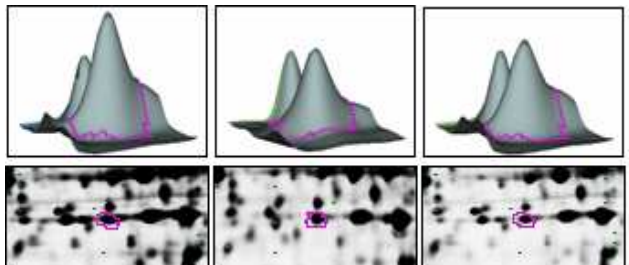
Succinyl-CoA ligase [ADP forming] beta chain

4hpi/Mock		8hpi/Mock	
T-test	Ratio	T-test	Ratio
0,031	-1,81	0,026	-1,54



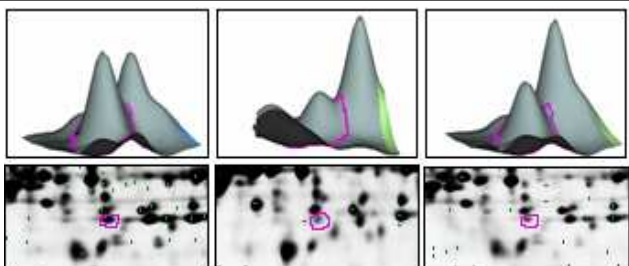
Succinyl-CoA ligase [GDP forming] beta chain

4hpi/Mock		8hpi/Mock	
T-test	Ratio	T-test	Ratio
0,034	-1,48	0,028	-1,53



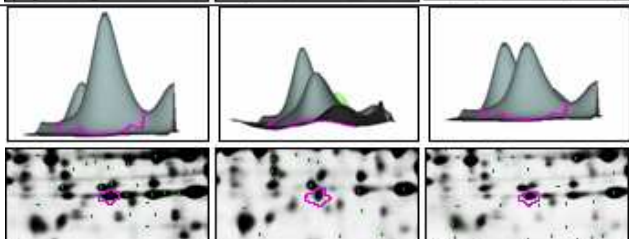
Erlin-2

4hpi/Mock		8hpi/Mock	
T-test	Ratio	T-test	Ratio
0,045	-1,7	0,012	-1,7



Succinyl-CoA ligase [GDP forming] beta chain

4hpi/Mock		8hpi/Mock	
T-test	Ratio	T-test	Ratio
0,0069	-2,17	0,0064	-1,75



Differential microsomal proteins in HSV-1 Cgal⁺-infected Huh7 cells

