

Supporting Information for:

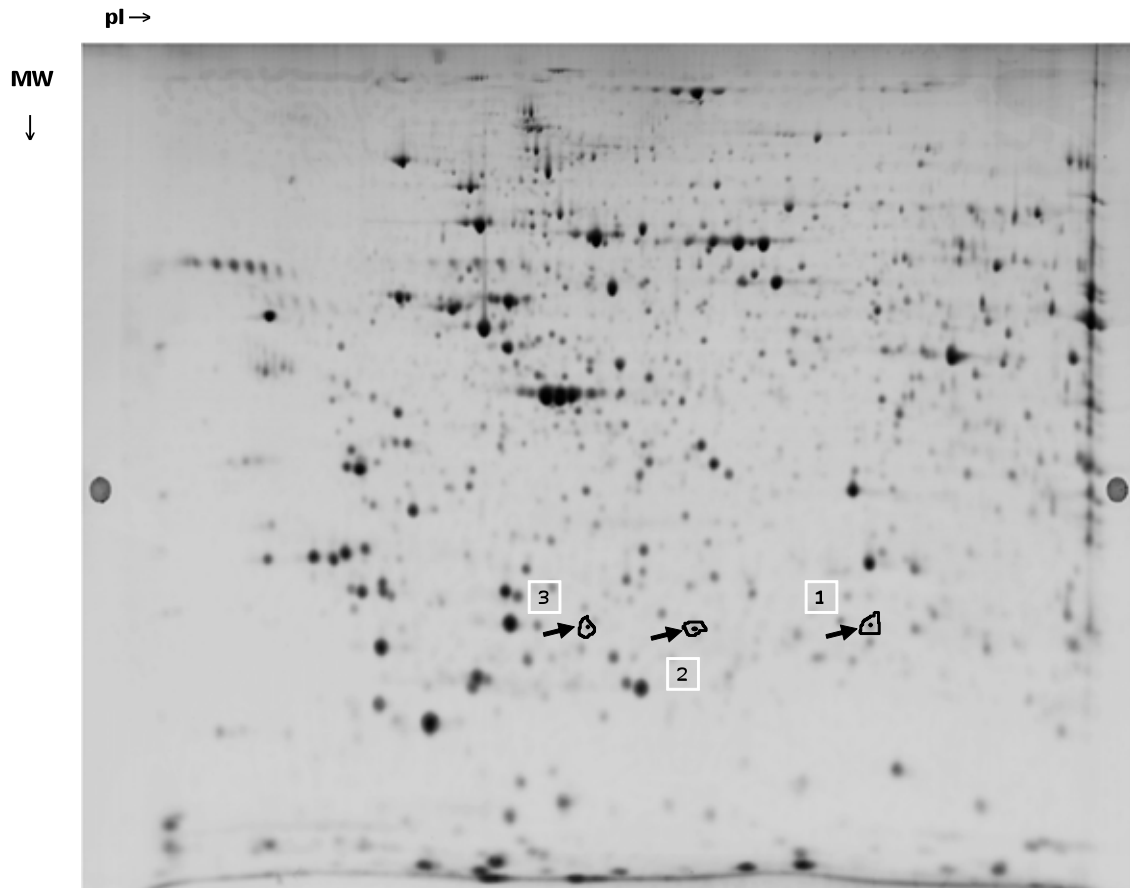
**Proteomic analysis reveals virus-specific Hsp25
modulation in cardiac myocytes**

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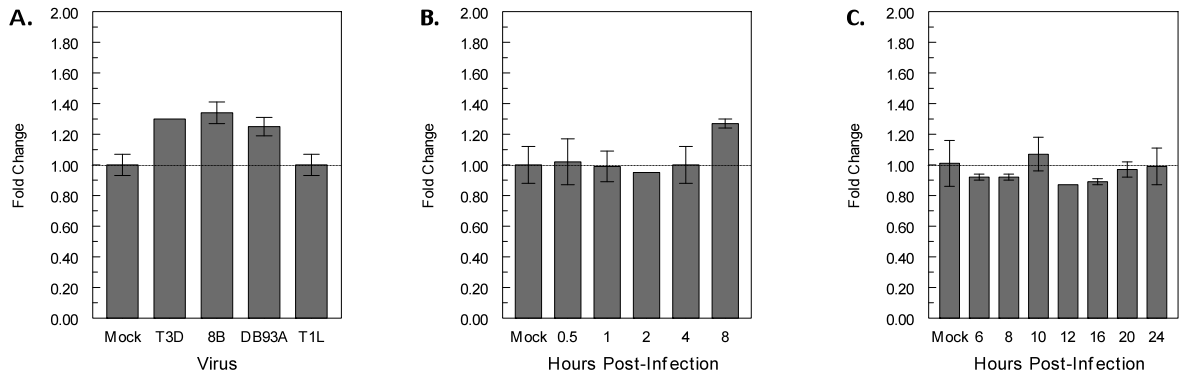
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Supplemental Fig. 1. Hsp25 2D-DIGE gel spots and peptide masses. The three proteins picked and identified as Hsp25 are indicated on this image of the master gel. They have the same molecular weight, but vary from the more basic (protein #1) to more acidic (protein #3).



Supplemental Fig. 2. Reovirus modulation of Hsp25 is post-transcriptional.

Primary cardiac myocyte cultures were mock- or reovirus-infected (moi 10 pfu per cell), and RNA was harvested at the indicated times post-infection for qRT-PCR. Copy number for each sample was first normalized to GAPDH, and then samples from reovirus-infected cultures were normalized to those from mock-infected cultures at that same time point. Results are expressed as the average of duplicate wells \pm standard deviation. A) Infected with indicated reovirus strain and harvested at 10 hours post-infection. B and C) Infected with T3D and harvested at indicated time post-infection.

Supplemental Table 1. Biological function of differentially expressed proteins in 8B-infected primary cardiac myocyte cultures.

Category	P-value	Gene Name for Protein
Inflammatory Response	1.05E-02 - 3.63E-02	GSN, DCTN2
Post-Translational Modification	5.58E-07 - 1.05E-02	PLOD2, P4HB, PDIA3, PPP1CB, HSPA5, GSN, HSPA4, P4HA1, GSPT1, ERP29, PDIA6, UBA1, PLOD3
Protein Folding	7.75E-03 - 7.75E-03	ERP29, PDIA6, HSPA5
Free Radical Scavenging	1.57E-02 - 1.57E-02	GSN
Amino Acid Metabolism	5.58E-07 - 5.27E-03	P4HA1, P4HB, PLOD3
Organismal Development	5.27E-03 - 4.14E-02	TOP1, RAD23B, DCTN2, PRKAR1A
Reproductive Development and Function	2.09E-02 - 2.09E-02	TOP1
Organismal Functions	1.57E-02 - 1.57E-02	DCTN2
Respiratory Disease	6.94E-06 - 3.11E-02	ALDH1B1, P4HB, HSP90B1, TOP1, ENO1, PDIA3, HSPA5, GSN
Gastrointestinal Disease	5.27E-03 - 3.43E-02	PLOD2, COL1A1, P4HA1, HSP90B1, P4HB, TOP1, FSCN1, CALD1, UBA1, HSPA5, GSN, GLG1
Renal and Urological Disease	4.89E-03 - 2.09E-02	TOP1, HSPA1B, PRKAR1A

Supplemental Table 2. Mascot analysis (combined PMF- MS/MS) of Spot #1 ¹

Observed Mr ²	Exper. Mr	Calc. Mr	Delta	AA Start	AA End	Miss	Ions	Peptide
927.60	926.59	926.48	0.11	193	202	0	-	AQIGGPEAGK
931.60	930.59	930.50	0.09	119	127	0	-	EGVVEITGK
1005.57	1004.56	1004.47	0.09	13	20	0	48	SPSWEPFR
1031.57	1030.56	1030.46	0.10	21	28	0	49	DWYPAHSR
1075.67	1074.67	1074.57	0.10	84	93	0	28	QLSSGVSEIR
1104.61	1103.60	1103.50	0.10	132	140	0	62	QDEHGYISR
1149.71	1148.71	1148.60	0.11	29	38	0	91	LFDQAFGVPR
1798.10	1797.10	1796.93	0.17	101	116	0	49	VSLDVNHFAPEELTVK
1833.14	1832.13	1831.97	0.17	176	192	0	81	AVTQSAEITIPVTFEAR

¹ Most basic spot; putative unphosphorylated Hsp25: (P14602) Heat shock 27 kDa protein (Hsp 27, Hsp 25), Mass: 23000, Protein Score: 459

² Additional peptides for which there was no match (and which could include post-translationally modified Hsp25 peptides): 1009.57, 1018.57, 1021.57, 1021.57, 1035.56, 1037.57, 1037.57, 1047.56, 1051.56, 1053.56, 1058.65, 1062.58, 1063.57, 1079.59, 1087.58, 1095.62, 1126.66, 1134.72, 1148.68, 1161.72, 1171.70, 1175.73, 1187.67, 1203.64, 1314.91, 1332.86, 1424.89, 1499.93, 1528.96, 1630.01, 1634.08, 1701.05, 1820.09, 1847.15, 1923.11, 1941.12, 1985.25, 1987.26, 2003.27, 2079.31, 2079.31, 2091.33, 2230.41, 2233.41, 2285.38, 2288.29, 2304.27, 2316.32, 2319.22, 2331.24, 2334.50, 2435.47, 2440.26, 2596.41, 2679.57, 3065.97, 3110.93, 3194.04, 3347.02

Supplemental Table 3. Mascot analysis (combined PMF- MS/MS) of Spot #2 ¹

Observed Mr ²	Exper. Mr	Calc. Mr	Delta	AA Start	AA End	Miss	Ions	Peptide
927.58	926.58	926.48	0.09	193	202	0	----	AQIGGPEAGK
1005.56	1004.55	1004.47	0.08	13	20	0	36	SPSWEFPR
1031.55	1030.54	1030.46	0.08	21	28	0	51	DWYPAHSR
1075.66	1074.65	1074.57	0.08	84	93	0	2	QLSSGVSEIR
1104.59	1103.59	1103.50	0.09	132	140	0	60	QDEHGYISR
1149.70	1148.69	1148.60	0.09	29	38	0	73	LFDQAFGVPR
1798.08	1797.07	1796.93	0.14	101	116	0	50	VSLDVNHFAPEELTVK
1833.11	1832.11	1831.97	0.14	176	192	0	74	AVTQSAEITIPVTFEAR

¹ Middle spot; putative mono-phosphorylated Hsp25: (P14602) Heat shock 27 kDa protein (Hsp 27, Hsp 25), Mass: 23000, Score: 388

² Additional peptides for which there was no match (and which could include post-translationally modified Hsp25 peptides): 990.36, 1009.56, 1021.56, 1035.55, 1037.55, 1047.57, 1058.63, 1061.57, 1063.55, 1063.55, **1085.53**, 1087.56, 1089.56, 1101.52, 1117.52, 1126.65, 1148.65, 1165.68, 1171.69, 1175.72, 1187.65, 1194.75, 1203.62, 1314.87, 1345.85, 1424.88, 1528.93, 1569.98, 1629.97, 1634.06, 1701.03, 1720.99, 1794.95, 1820.07, 1923.07, 1941.10, 1969.21, 1987.23, 1987.23, 2003.23, 2079.28, 2079.28, 2091.30, 2230.38, 2265.22, 2297.39, 2316.30, 2317.24, 2334.47, 2408.25, 2564.39, 2663.59, 2679.56, 3065.95, 3094.89, 3111.90, 3194.05, 3339.01, 3346.95, 3355.06. The peptide in **bold** is absent in the putative non-phosphorylated Hsp25 (Supplemental Table 1), is present in both this putative mono-phosphorylated Hsp25 and di-phosphorylated Hsp25 (Supplemental Table 3), matches the Exper. Mr for a peptide comprised of residues 13 – 20 with a single phosphorylation, is predicted to be phosphorylated on serine 15

by NetPhos 2.0 software, and includes a serine residue previously shown by others to be phosphorylated (serine 15).

Supplemental Table 4. Mascot analysis (combined PMF- MS/MS) of Spot #3 ¹

Observed Mr ²	Exper. Mr	Calc. Mr	Delta	AA Start	AA End	Miss	Ions	Peptide
927.58	926.57	926.48	0.09	193	202	0	----	AQIGGPEAGK
931.59	930.58	930.50	0.08	119	127	0	----	EGVVEITGK
932.57	931.56	931.46	0.10	94	100	1	----	QTADRWR
1031.55	1030.55	1030.46	0.08	21	28	0	48	DWYPAHSR
1104.59	1103.59	1103.50	0.09	132	140	0	38	QDEHGYSR
1149.70	1148.69	1148.60	0.09	29	38	0	68	LFDQAFGVPR
1798.08	1797.07	1796.93	0.14	101	116	0	49	VSLDVNHFAPEELTVK
1833.12	1832.11	1831.97	0.14	176	192	0	106	AVTQSAEITIPVTFEAR
2018.08	2017.08	2016.92	0.15	13	28	1	----	SPSWEPFRDWYPAHSR

¹ Most acidic spot; putative di-phosphorylated Hsp25: (P14602) Heat shock 27 kDa protein (Hsp 27, Hsp 25), Mass: 23000, Score: 359

² Additional peptides for which there was no match (and which could include post-translationally modified Hsp25 peptides): 1035.55, 1047.55, 1061.58, 1063.55, 1071.72, **1085.53**, 1087.56, 1089.56, 1101.53, 1108.64, 1117.52, 1126.65, 1148.67, **1155.63**, 1165.68, 1171.68, 1175.72, 1179.69, 1187.67, 1277.80, 1282.78, 1307.78, 1314.88, 1316.87, 1424.88, 1425.87, 1475.87, 1528.93, 1701.03, 1794.95, 1820.07, 1923.07, 1941.09, 1941.09, 1969.22, 1985.22, 1987.23, 1987.23, 2003.23, 2079.28, 2079.28, 2091.31, 2230.38, 2262.34, 2297.37, 2334.47, 2408.24, 2419.44, 2565.38, 2662.57, 2663.58, 2679.58, 2914.76, 3094.89, 3111.93, 3194.00, 3339.00, 3339.00, 3346.89, 3347.98, 3355.05. The peptides in **bold** include the one in Table 2 (1085.53), but also a second one (1155.63) which is absent in both the putative non-phosphorylated Hsp25

(Supplemental Table 1) and mono-phosphorylated Hsp25 (Supplemental Table 2), matches the Exper. Mr for a peptide comprised of residues 84 – 93 with one phosphorylation, is predicted to be phosphorylated on serine 86 by NetPhos 2.0 software, and includes a serine residue previously shown by others to be phosphorylated (serine 86).