Supplemental material 1. Antibodies used in study

Hsp70
- Rabbit polyclonal antibody recognizing full length Hsp70 was developed by Moravian Biotechnology by immunization of human recombinant Hsp70 purified from E. coli.
- Mouse monoclonal antibody clone C92F3A-5 (Stressgen)
- Mouse monoclonal antibody clone GGS2.1 (Moravian Biotechnology) recognizing phosphorylated C-terminal threonine of Hsp70 GSGP(pT)IEEVD.

Hsp90
- Rabbit polyclonal antibody recognizing full length Hsp90 was developed by Moravian Biotechnology by immunization of mixture of human recombinant Hsp90α and Hsp90β purified from E. coli.
- Rat monoclonal antibody clone 9D2 recognizing Hsp90α (Stressgen)
- Mouse monoclonal antibody clone GDD8.2 (Moravian Biotechnology) recognizing phosphorylated C-terminal serine and threonine of Hsp90α GDDD(pT)(pS)RMEEVD.
- Mouse monoclonal antipody clone AC88 recognizing both Hsp90α and Hsp90β (Stressgen),

CHIP
- Mouse monoclonal antibody clone MBCHIP-3 recognizing epitope FDPVTR of U-box domain (Moravian Biotechnology)

HOP
- Mouse monoclonal antibody clone MBHOP-1 recognizing epitope PQAMKHYT of human HOP protein (Moravian Biotechnology)

GST
- Anti-Glutathione-S-Transferase (GST)–Peroxidase Conjugate antibody produced in rabbit (Sigma Aldrich)

P53
- Mouse monoclonal antibody clone DO-1.

Estrogen receptor
- Rabbit monoclonal antibody clone SP1 (Thermo-Labvision).

CDK4
- Mouse monoclonal antibody clone DCS-35 (Thermo-Labvision).

Development of phospho-specific Hsp70 and Hsp90 antibodies and CHIP and HOP antibodies
The C-terminal phosphopeptides of Hsp90α GDDD(pT)(pS)RMEEVD and Hsp70 GGSGSGP(pT)IEEVD were synthesized along with their non-phosphorylated counterparts (Clonestar). Phosphopeptides were coupled at their N-termini to keyhole limpet hemocyanin (KLH) using glutaraldehyde for use as immunogens. Purified proteins HOP and CHIP were also used for development of monoclonal antibodies (Moravian Biotechnology). Monoclonal antibodies to CHIP (MBCHIP-3) and HOP (MBHOP-1) proteins and to phosphorylated Hsp70 (GGS2.1) and phosphorylated Hsp90 (GDD8.2) were developed in Moravian-Biotechnology using the method described (Vojtesek B. et al.: J Immunol Methods. 1992 Jul 6;151(1-2):237-44) The specificity of monoclonal antibodies was tested using peptide ELISA, western blotting and phosphomimetic mutants of Hsp70 and Hsp90.
ELISA test of phosphospecific monoclonal antibodies:
The phospho-specificity of selected clones were tested by peptide ELISA using biotinylated peptides corresponding to phosphorylated and non-phosphorylated peptides of Hsp70 and Hsp90a C-termini.
(A) The clone GGS2.1 specifically recognizes phosphorylated C-terminal peptide of Hsp70 GGSGSGP(pT)IEEVD
(B) The clone GDD8.2 specifically recognizes double phosphorylation of Hsp90α GDDDP(T)(pS)RMEEVD

The test of phosphospecific antibodies on dephosphorylated cell lysate
The whole cell lysate of MCF-7 cells was dephosphorylated by calf intestine phosphatase (CIP). The clones GDD2.1 and GDD8.2 specifically recognize phosphorylated Hsp70 and Hsp90.

The test of phospho-specific antibodies using phospho-mimetic mutants of Hsp70, Hsp90α and Hsp90β. The antibodies were tested on whole lysate of H1299 cells which were transfected with wild-type (wt) Hsps and their point mutants mimicking phosphorylated (D) or non-phosphorylated (A) status. Both GDD8.2 and GGS2.1 specifically recognize transfected phospho-mimetic mutants of Hsp90α and Hsp70 respectively. The antibodies also weakly recognize the endogenous Hsps.
Supplemental material 2. Analysis of Hsp90α phosphorylation by mass spectrometry

**HSP 90α – digestion by endoproteinase Asp-N**

HSP 90α – TiO₂ enrichment; expected [M+H]⁺ of C-terminal P-peptide: singly-phosphorylated = 1046.39 Da; doubly-phosphorylated = 1126.35 Da
HSP 90 alpha – TiO2 enrichment; MALDI-TOF/TOF MS/MS
singly-phosphorylated = 1391.62 Da

HSP 90 alpha – TiO2 enrichment; MALDI-TOF/TOF MS/MS
doubly-phosphorylated = 1471.52 Da
Supplemental material 3. Fluorescence polarization binding assay

**Fluorescence polarization binding assay**
The equilibrium bindings between a fixed concentration (30 nM) of a fluorescent ligand and increasing concentrations of either HOP or CHIP in buffer containing 150 mM NaCl, 50 mM HEPES pH 7.2, 1 mM DTT and 0.05% Tween-20 were monitored by both total fluorescence intensity and fluorescence polarization on the plate reader Filtermax F5 (Molecular Devices). The ligands corresponding to C-terminal peptide of Hsp70 (GGSGSPTIEEVD), phospho-Hsp70 (GGSGSP(pT)IEEVD), Hsp90α (GDDTSRMEEVD) or phospho-Hsp90α (GDDD(pT)(pS)RMEEVD) were labeled by fluorescein on its N-terminus. The equilibrium dissociation constant (Kd) was then calculated by fitting the sigmoidal dose-dependent FP increases as a function of protein concentrations using Graphpad Prism.
Supplemental material 4. Proximity ligation assay

The individual complexes of Hsp70-CHIP, HSP70-HOP, Hsp90-HOP and Hsp90-CHIP were analyzed using rabbit polyclonal antibodies recognizing Hsp70 or Hsp90 and mouse monoclonal antibodies recognizing HOP or CHIP. The complexes were visualized using proximity ligation assay in situ and rolling circle amplification.

The complexes were quantified as a median number of signals per cell.

### Number of signals per cell

<table>
<thead>
<tr>
<th></th>
<th>Hsp70-HOP Median (StDev)</th>
<th>Hsp70-CHIP Median (StDev)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>66.2 (3.6)</td>
<td>83.9 (5.5)</td>
</tr>
<tr>
<td>17AAG</td>
<td>63.1 (10.9)</td>
<td>83.6 (6.8)</td>
</tr>
<tr>
<td>Starvation</td>
<td>10.6 (0.7)</td>
<td>47.3 (4.7)</td>
</tr>
<tr>
<td>Starvation 17AAG</td>
<td>11.3 (1.2)</td>
<td>42.9 (3.0)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Hsp90-HOP Median (StDev)</th>
<th>Hsp90-CHIP Median (StDev)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>25.3 (3.0)</td>
<td>66.3 (5.7)</td>
</tr>
<tr>
<td>17AAG</td>
<td>58.7 (6.6)</td>
<td>74.2 (10.5)</td>
</tr>
<tr>
<td>Starvation</td>
<td>6.3 (1.4)</td>
<td>64.9 (5.5)</td>
</tr>
<tr>
<td>Starvation 17AAG</td>
<td>11.7 (1.0)</td>
<td>60.6 (8.8)</td>
</tr>
</tbody>
</table>

**Complexes with Hsp70**

**Complexes with Hsp90**
Supplemental material 5. The effect of Hsps point mutation on cell proliferation

Growth curve of cell line HEK 293 transfected by Hsp90α, Hsp90β, Hsp70, their point mutants and EmGFP.

Y0 is the Y value when X (time) is zero. It is expressed in the same units as Y, K is the rate constant, expressed in reciprocal of the X axis time units. If X is in minutes, then K is expressed in inverse minutes. 

Tau is the time constant, expressed in the same units as the X axis. It is computed as the reciprocal of K. 

Doubling-time is in the time units of the X axis. It is computed as ln(2)/K.

<table>
<thead>
<tr>
<th>Y=Y0<em>exp(k</em>X)</th>
<th>Hsp90α-wt</th>
<th>Hsp90α-AA</th>
<th>Hsp90α-DD</th>
<th>Hsp90α-wt</th>
<th>Hsp90β-wt</th>
<th>Hsp90β-A</th>
<th>Hsp90β-D</th>
<th>EmGFP</th>
<th>Global values *</th>
</tr>
</thead>
<tbody>
<tr>
<td>Y0</td>
<td>0.585</td>
<td>0.645</td>
<td>0.575</td>
<td>0.606</td>
<td>0.618</td>
<td>0.603</td>
<td>0.607</td>
<td>0.607</td>
<td>0.592 0.6074</td>
</tr>
<tr>
<td>k</td>
<td>0.046</td>
<td>0.034</td>
<td>0.048</td>
<td>0.044</td>
<td>0.042</td>
<td>0.046</td>
<td>0.045</td>
<td>0.045</td>
<td>0.043 0.0432</td>
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<tr>
<td>Doubling Time</td>
<td>3.074E-03</td>
<td>7.567E-03</td>
<td>1.412E-02</td>
<td>6.016E-03</td>
<td>8.630E-03</td>
<td>7.488E-03</td>
<td>3.654E-03</td>
<td>5.465E-03</td>
<td>5.786E-03 1.085E-02 0.00593</td>
</tr>
<tr>
<td>Lower 95% conf. limit</td>
<td>0.579</td>
<td>0.630</td>
<td>0.547</td>
<td>0.594</td>
<td>0.601</td>
<td>0.589</td>
<td>0.590</td>
<td>0.668</td>
<td>0.571 0.5965</td>
</tr>
<tr>
<td>Upper 95% conf. limit</td>
<td>0.591</td>
<td>0.660</td>
<td>0.603</td>
<td>0.618</td>
<td>0.635</td>
<td>0.618</td>
<td>0.604</td>
<td>0.689</td>
<td>0.613 0.6182</td>
</tr>
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</table>

Comparison of Fits F-Test

Null hypothesis One curve for all data sets

Alternative hypothesis Different curve for each data set

P value < 0.0001

Conclusion (alpha = 0.05) Rejected null hypothesis

Preferred model Different curve for each data set

F (DFn, DFd) 360.8 (16,1740)
<table>
<thead>
<tr>
<th></th>
<th>Hsp90α-AA</th>
<th>Hsp90α-DD</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Null hypothesis</strong></td>
<td>$k(Hsp90\alpha-wt) = k(Hsp90\alpha-AA)$</td>
<td>$k(Hsp90\alpha-wt) = k(Hsp90\alpha-DD)$</td>
</tr>
<tr>
<td><strong>Alternative hypothesis</strong></td>
<td>$k$ unconstrained</td>
<td>$k$ unconstrained</td>
</tr>
<tr>
<td><strong>P value</strong></td>
<td>$&lt; 0.0001$</td>
<td>$0.0305$</td>
</tr>
<tr>
<td><strong>Conclusion (alpha = 0.05)</strong></td>
<td>Reject null hypothesis</td>
<td>Reject null hypothesis</td>
</tr>
<tr>
<td><strong>Preferred model</strong></td>
<td>$k$ unconstrained</td>
<td>$k$ unconstrained</td>
</tr>
<tr>
<td><strong>F (DFn, DFd)</strong></td>
<td>$351.5 (1,174)$</td>
<td>$4.758 (1,174)$</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Hsp90β-A</th>
<th>Hsp90β-D</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Null hypothesis</strong></td>
<td>$k(Hsp90\beta-wt) = k(Hsp90\beta-A)$</td>
<td>$k(Hsp90\beta-wt) = k(Hsp90\beta-D)$</td>
</tr>
<tr>
<td><strong>Alternative hypothesis</strong></td>
<td>$k$ unconstrained</td>
<td>$k$ unconstrained</td>
</tr>
<tr>
<td><strong>P value</strong></td>
<td>$&lt; 0.0001$</td>
<td>$&lt; 0.0001$</td>
</tr>
<tr>
<td><strong>Conclusion (alpha = 0.05)</strong></td>
<td>Reject null hypothesis</td>
<td>Reject null hypothesis</td>
</tr>
<tr>
<td><strong>Preferred model</strong></td>
<td>$k$ unconstrained</td>
<td>$k$ unconstrained</td>
</tr>
<tr>
<td><strong>F (DFn, DFd)</strong></td>
<td>$16.03 (1,174)$</td>
<td>$29.59 (1,174)$</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Hsp70-A</th>
<th>Hsp70-D</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Null hypothesis</strong></td>
<td>$k(Hsp70-wt) = k(Hsp70-A)$</td>
<td>$k(Hsp70-wt) = k(Hsp70-D)$</td>
</tr>
<tr>
<td><strong>Alternative hypothesis</strong></td>
<td>$k$ unconstrained</td>
<td>$k$ unconstrained</td>
</tr>
<tr>
<td><strong>P value</strong></td>
<td>$&lt; 0.0001$</td>
<td>$0.697$</td>
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<tr>
<td><strong>Conclusion (alpha = 0.05)</strong></td>
<td>Reject null hypothesis</td>
<td>Do not reject null hypothesis</td>
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<tr>
<td><strong>Preferred model</strong></td>
<td>$k$ unconstrained</td>
<td>$k = 0.045$</td>
</tr>
<tr>
<td><strong>F (DFn, DFd)</strong></td>
<td>$1415 (1,174)$</td>
<td>$0.1521 (1,174)$</td>
</tr>
</tbody>
</table>
Supplemental material 6. The expression of STIP1 (HOP) and STUB1 (CHIP) mRNA in common human cancers.

The expression of STIP1 (HOP) mRNA in cancer vs. normal tissues

**Pei Pancreas**
- Pancreas (16)
- Pancreatic Carcinoma (36)

**P-value**: 2.47E-7
**t-Test**: 6.141
**Fold Change**: 1.801

**Talantov Melanoma**
- Skin (7)
- Cutaneous Melanoma (45)

**P-value**: 6.56E-23
**t-Test**: 23.767
**Fold Change**: 22.922

**DErrico Gastric**
- Gastric Mucosa (31)
- Gastric Intestinal Type Adenocarcinoma (26)

**P-value**: 4.83E-11
**t-Test**: 8.156
**Fold Change**: 2.709

**Landi Lung**
- Lung (49)
- Lung Adenocarcinoma (58)

**P-value**: 2.87E-16
**t-Test**: 9.601
**Fold Change**: 1.499

**Dyrskjot Bladder 3**
- Bladder (9)
- Bladder Mucosa (5)
- Superficial Bladder Cancer (28)

**P-value**: 1.05E-9
**t-Test**: 8.614
**Fold Change**: 2.560

**Radvanyi Breast**
- Breast (8)
- Invasive Ductal Breast Carcinoma (32)

**P-value**: 0.027
**t-Test**: 2.300
**Fold Change**: 3.705
The expression of STUB1 (CHIP) mRNA in cancer vs. normal tissues

**Pei Pancreas**

1. Pancreas (16)
2. Pancreatic Carcinoma (36)

**Talantov Melanoma**

1. Skin (7)
2. Cutaneous Melanoma (45)

**DErrico Gastric**

1. Gastric Mucosa (31)
2. Gastric Intestinal Type Adenocarcinoma (26)

**Landi Lung**

1. Lung (49)
2. Lung Adenocarcinoma (58)

**Dyrskjot Bladder 3**

1. Bladder (9)
2. Bladder Mucosa (5)
3. Superficial Bladder Cancer (28)

**Radvanyi Breast**

1. Breast (8)
2. Invasive Ductal Breast Carcinoma (32)