Transportable, Chemical Genetic Methodology for the Small Molecule-Mediated Inhibition of Heat Shock Factor 1

Christopher L. Moore, Mahender B. Dewal, Emmanuel Nekongo, Sebasthian Santiago, Nancy B. Lu, Stuart S. Levine, Matthew D. Shoulders

EXTENDED EXPERIMENTAL PROCEDURES

Quantitative RT-PCR. The relative mRNA expression levels of selected heat shock response genes were measured using quantitative RT-PCR. Cells were treated as described at 37 °C, harvested by scraping, washed with phosphate-buffered saline (CellGro), and then RNA was extracted using the EZNA Total RNA Kit I (Omega). qPCR reactions were performed on cDNA prepared from 1000 ng of total cellular RNA using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems). The Fast Start Universal SYBR Green Master Mix (Roche) and appropriate primers purchased from Integrated DNA Technologies (**Table S2**) were used for amplifications (6 min at 95 °C then 45 cycles of 10 s at 95 °C, 30 s at 60 °C) in a Light Cycler 480 II Real-Time PCR machine. Primer integrity was assessed by a thermal melt to confirm homogeneity and the absence of primer dimers. Transcripts were normalized to the housekeeping genes *Rplp2* and all measurements were performed at least in triplicate. Data were analyzed using the LightCycler® 480 Software, Version 1.5 (Roche) and data are reported as the mean ±95% confidence interval.

Lentivirus Production. Vesicular stomatitis virus glycoprotein (VSV-G) pseudotyped lentiviral particles were produced by co-transfecting 293FT cells with the structural plasmids necessary for virus production (Rev, RRE, and VSVG) along with the appropriate pLenti-DEST lentivirus constructs. Cells were transfected using Lipofectamine 2000 (Life Technologies) for 24 h, after which the media was removed and replaced with fresh media. Media containing viral particles was collected at 48 h and again at 72 h post-transfection and cell debris was removed by centrifugation at 500 × g for 10 min. Viral supernatant was then aliquoted and stored at –80 °C until use. During lentivirus transductions, cells were treated with 4 μg/mL polybrene (Sigma).

Sample Preparation, Labeling, and Chip Hybridization for Whole Genome Array Analyses. RNA quality was confirmed using an Advanced Analytical Fragment Analyzer, after which 250 ng of total RNA was prepared for microarray analysis using the Nugen Applause 3' Amp Kit (NuGen #5100). 4 μg of labeled cDNA was hybridized overnight (17 h) to Human Primeview Arrays (Affymetrix #901837) following standard Affymetrix protocols. Microarrays were scanned using an Affymetrix GeneChip Scanner 3000 7G Series instrument. Data were extracted using the Affymetrix Expression Console and analyzed using the Transcriptome Analysis Console v3.0. K-Means clustering was performed on genes showing an experiment-wide ANOVA of <0.001 using Cluster 3.0 (http://bonsai.hgc.jp/~mdehoon/software/cluster/software.htm). Data were gene normalized before clustering using 5 nodes and a Euclidean distance similarity metric. Data were visualized using Java TreeView (http://jtreeview.sourceforge.net).[1] Gene ontology analyses were performed using DAVID (http://david.abcc.ncifcrf.gov/summary.jsp) on genes associated to each node.[2,3]

SUPPLEMENTAL REFERENCES

1. Saldanha, A. J. (2004) Java Treeview--extensible visualization of microarray data, *Bioinformatics 20*, 3246-3248.
2. Huang da, W., Sherman, B. T., and Lempicki, R. A. (2009) Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources, *Nat Protoc 4*, 44-57.
3. Huang da, W., Sherman, B. T., and Lempicki, R. A. (2009) Bioinformatics enrichment tools: paths toward the comprehensive functional analysis of large gene lists, *Nucleic Acids Res 37*, 1-13.

SUPPLEMENTAL TABLES

Table S1. Microarray characterization of dn-cHSF1 function (see Excel file).

Table S2. Quantitation of PULSA replicates for HEK293^{HSR} cells expressing polyQ-tdTomato (see **Figure S2** for raw data).

		Fraction of Cells Containing an Inclusion Body (percent out of 10,000; Population P4 in PULSA; see **Figure S2**)		
		Replicate 1	**Replicate 2**	**Replicate 3**
Q_0-tdTomato	vehicle	0.4%	0.7%	1.1%
	dox	1.5%	0.7%	1.0%
	Shield-1	0.9%	0.7%	0.5%
polyQ$_{67}$-tdTomato	vehicle	5.3%	4.6%	5.5%
	dox	8.4%	9.1%	9.2%
	Shield-1	5.1%	4.9%	5.1%

Table S3. Compilation of primers used for qPCR.

Transcript	**Forward**	**Reverse**
HSP90AA1	5′- GATAAACCCTGACCATTCC -3′	5′- AAGACAGGAGCGCAGTTTCATAAA -3′
HSPA1A	5′- GGAGGCGGAGAAGTACA -3′	5′- GCTGATGATGGGGTTACA -3′
DNAJB1	5′- TGTGTGGCTGCACAGTGAAC -3′	5′- ACGTTTCTCGGGTGTTTTGG -3′
Rplp2	5′- CCATTCAGCTCACTGATAACCTTG -3′	5′- CGTCGCCTCCTACCTGCT -3′

SUPPLEMENTAL FIGURE LEGENDS

Figure S1. STA-9090- and 17-AAG-mediated activation of the heat shock response.

Immunoblot of HEK293T-Rex cells upon treatment with increasing concentrations of the HSP90 inhibitors STA-9090 and 17-AAG, showing that the heat shock response is activated even at concentrations incapable of inducing Akt degradation, a marker for successful HSP90 inhibition.

Figure S2. PULSA flow cytometry data for HEK293^{HSR} cells expressing polyQ-tdTomato.

Representative primary data produced from the PULSA flow cytometry method when performed on HEK293^{HSR} cells transiently expressing Q_0-tdTomato or polyQ$_{67}$-tdTomato in different cytosolic protein folding environments, including vehicle, and chronic repression of HSF1 (dn-cHSF1, induced by dox), and chronic activation of HSF1 (FKBP.cHSF1, induced by Shield-1) as in **Figures 6F** and **6G**.

FIGURE S1

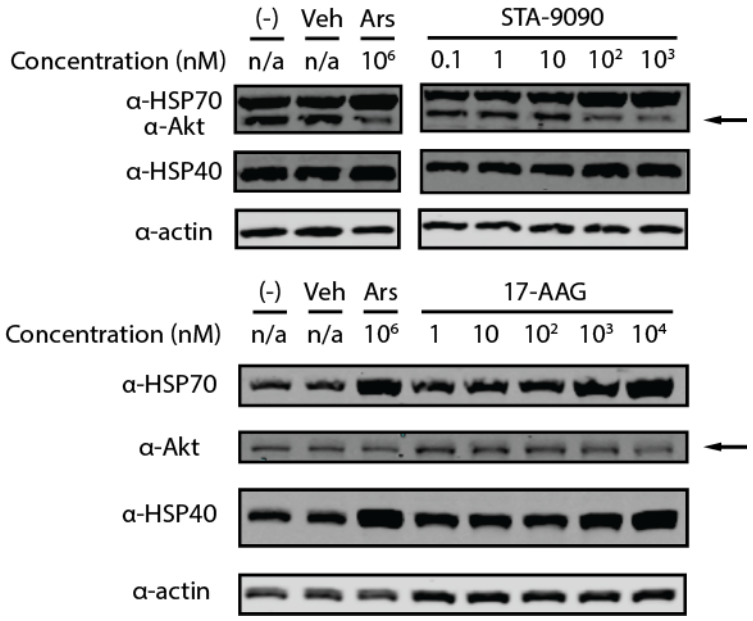
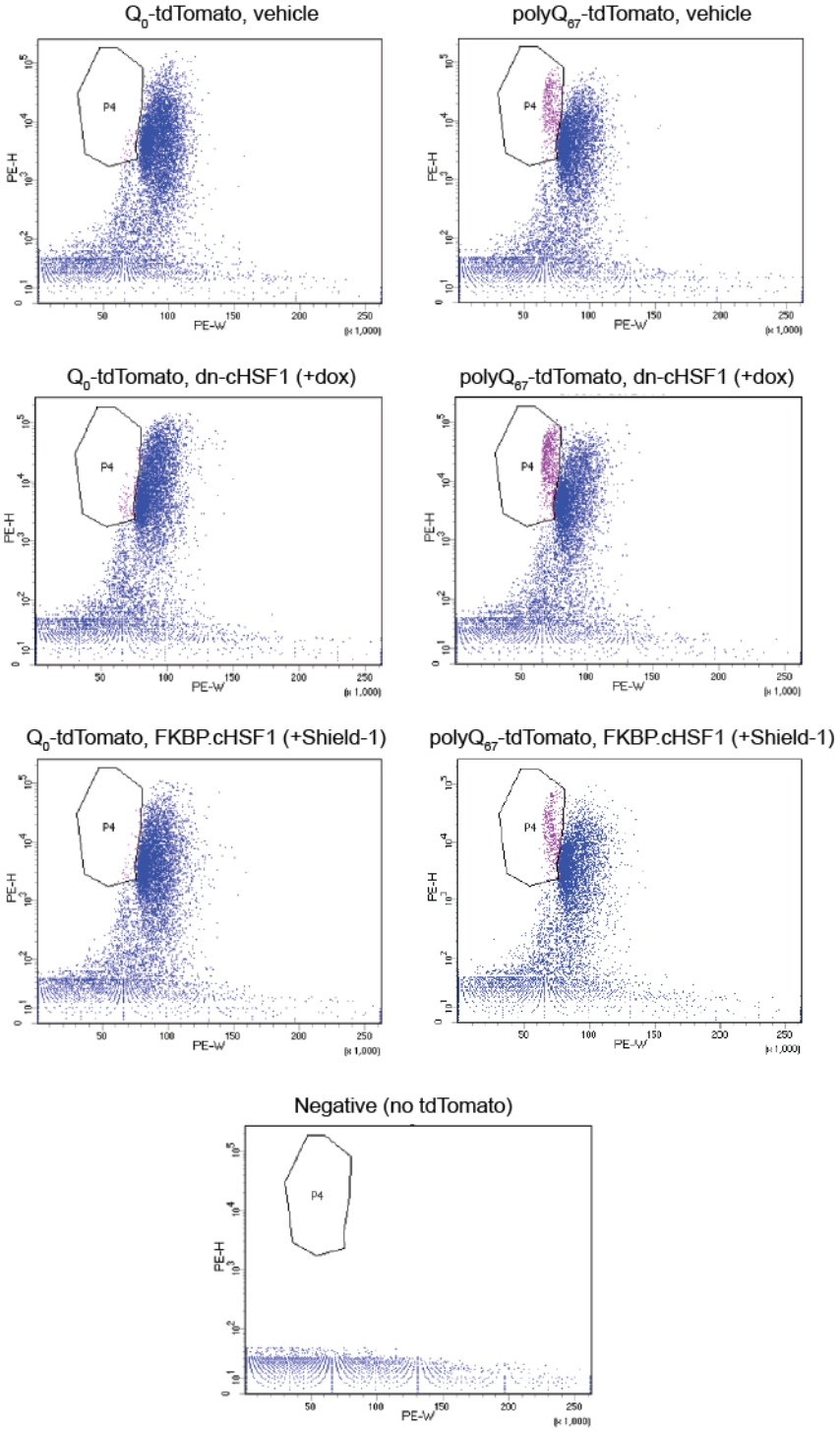

(-)
Veh
Ars
STA-9090
Concentration (nM)
n/a
n/a
10⁶
0.1
1
10
10²
10³
α-HSP70
α-Akt
α-HSP40
α-actin
(-)
Veh
Ars
17-AAG
Concentration (nM)
n/a
n/a
10⁶
1
10
10²
10³
10⁴
α-HSP70
α-Akt
α-HSP40
α-actin

FIGURE S2


Q0-tdTomato, vehicle
polyQ67-tdTomato, vehicle
Q0-tdTomato, dn-cHSF1 (+dox)
polyQ67-tdTomato, dn-cHSF1 (+dox)
Q0-tdTomato, FKBP.cHSF1 (+Shield-1)
polyQ67-tdTomato, FKBP.cHSF1 (+Shield-1)
Negative (no tdTomato)
P4
PE-H
PE-W
(x 1,000)