

Supplemental Materials

Title

Transcriptomics Unveil Canonical and Non-Canonical Heat Shock-Induced Pathways in Human Cell Lines

Authors

Andrew Reinschmidt¹, Luis Solano^{1†}, Yonny Chavez¹, William Drew Hulsy¹, Nikolas Nikolaidis^{1*}

Affiliations

¹ Department of Biological Science, Center for Applied Biotechnology Studies, and Center for Computational and Applied Mathematics, California State University Fullerton, Fullerton, CA, USA.

Andrew Reinschmidt, areinschmidt4@csu.fullerton.edu

Yonny Chavez, Yonny@csu.fullerton.edu

William Drew Hulsy, wdhulsy@csu.fullerton.edu

[†] Current address: Center for Complex Biological Systems, University of California, Irvine
Luis Solano, lesolano@uci.edu

* Corresponding author, Nikolas Nikolaidis, nnikolaidis@fullerton.edu

Supplementary Table 1. Expression of the major heat shock protein genes in HEK293, HeLa, and HepG2 after heat shock.

Please see separate excel file: Supplementary Table 1.

Supplementary Table 2. The function of the conserved enriched gene sets from in Table 1.
Revised Sample Version

| ID | Name | Molecular Function |
|------------|---------------------------------------|--|
| GO:0048018 | Receptor Ligand Activity | The activity of a gene product that interacts with a receptor to effect a change in the activity of the receptor. Ligands may be produced by the same cell that expresses the receptor. Ligands may also be expressed at the plasma membrane of an adjacent cell (e.g., Notch ligands) or be secreted and diffuse extracellularly from their point of origin to the receiving cell (e.g., interleukins). |
| GO:0030545 | Signaling receptor activator activity | Binds to and modulates the activity of a receptor. |
| GO:0044183 | Protein folding chaperone | Binding to a protein or a protein-containing complex to assist the protein folding process. |
| HSA-373076 | Class A/1 (Rhodopsin-like receptors) | Rhodopsin-like receptors (class A/1) are the largest group of GPCRs and are the best studied group from a functional and structural point of view. They show great diversity at the sequence level and thus, can be subdivided into 19 subfamilies (Subfamily A1-19) based on a phylogenetic analysis (Joost P and Methner A, 2002). They represent members that include hormone, light and neurotransmitter receptors and encompass a wide range of functions including many autocrine, paracrine, and endocrine processes. |

Supplementary Table 3. List of the 13 differentially expressed genes ($|\log_2FC| > 0.5$; $p\text{-adj.} < 0.05$) conserved across both batches, all three cell lines, and all condition comparisons, along with descriptions of their associated biological processes.

| HGNC Symbol | Gene Name | Biological Process |
|-------------|--|--|
| CNTF | Ciliary Neurotrophic Factor | Promotes survival of in vitro and in vivo neuronal cell types |
| FGF18 | Fibroblast Growth Factor 18 | Involved in embryonic development, cell growth, morphogenesis, tissue repair, tumor growth, and invasion |
| GNRH1 | Gonadotropin-Releasing Hormone 1 | This gene encodes a proteolytically processed preproprotein to generate a peptide member of the gonadotropin-releasing hormone (GnRH) family of peptides. |
| GNRH2 | Gonadotropin-Releasing Hormone 2 | Codes for a preprotein, however, translation in humans has not yet been shown. |
| HBEGF | Heparin Binding EGF-like Growth Factor | Enables growth factor activity and heparin binding. It is located in the cell surface and extracellular space. |
| JAG1 | Jagged canonical notched ligand 1 | Human jagged 1 is the ligand for the receptor notch 1; the latter is involved in signaling processes. |
| LTA | Lymphotoxin Alpha | The encoded protein, a tumor necrosis factor family member, is a cytokine lymphocytes produce. The protein is highly inducible and secreted and forms heterotrimers with lymphotoxin-beta, which anchor lymphotoxin-alpha to the cell surface. This protein also mediates a large variety of inflammatory, immunostimulatory, and antiviral responses, is involved in the formation of secondary lymphoid organs during development and plays a role in apoptosis. |
| MIA | MIA SH3 domain containing | Predicted to enable growth factor activity. Predicted to be involved in extracellular matrix organization. Predicted to act upstream of or within cell-matrix adhesion. Predicted to be located in extracellular space |
| PGF | Placental Growth Factor | Enables growth factor activity. It is involved in positive regulation of cell population proliferation. It is predicted to be located in the extracellular region. Predicted to be active in extracellular space |
| PSPN | Persephin | This gene encodes a secreted ligand of the GDNF (glial cell line-derived neurotrophic factor) subfamily and TGF-beta (transforming growth factor-beta) superfamily of proteins. The encoded preproprotein is proteolytically processed to generate the mature protein. This protein signals through the RET receptor tyrosine kinase and a GPI-linked coreceptor and promotes the survival of neuronal populations. |
| SEMA4D | Semaphorin 4D | Enables identical protein binding activity, semaphorin receptor binding activity, and transmembrane signaling receptor activity. It involves several processes, including positive phosphatidylinositol 3-kinase signaling, neuron projection development regulation, and phosphate metabolic process regulation. It is an integral component of the plasma membrane. |
| SEMA7A | Semaphorin 7A | This gene encodes a member of the semaphorin family of proteins. The encoded preproprotein is proteolytically processed to generate the mature glycosylphosphatidylinositol (GPI)-anchored membrane glycoprotein. The encoded protein is found on activated lymphocytes and erythrocytes and may be involved in immunomodulatory and neuronal processes. |

| | | |
|-----|-----------------------|--|
| TNF | Tumor Necrosis Factor | This gene encodes a multifunctional proinflammatory cytokine that belongs to the tumor necrosis factor (TNF) superfamily. Macrophages mainly secrete this cytokine. It can bind to and thus function through its TNFRSF1A/TNFR1 and TNFRSF1B/TNFR2 receptors. This cytokine regulates a broad spectrum of biological processes, including cell proliferation, differentiation, apoptosis, lipid metabolism, and coagulation. |
|-----|-----------------------|--|

Supplementary Table 4. Summary table of quality control for Batch 1.

| Sample name | Raw reads | Clean reads | Raw bases | Clean bases | Error rate(%) | Q20(%) | Q30(%) | GC content(%) |
|-------------|-----------|-------------|-----------|-------------|---------------|--------|--------|---------------|
| HeLa1cnt | 35872594 | 35392491 | 10.8G | 10.6G | 0.03 | 97.86 | 94.18 | 50.52 |
| HeLa2cnt | 40588307 | 39763626 | 12.2G | 11.9G | 0.03 | 97.76 | 93.96 | 50.28 |
| HeLa3cnt | 48968978 | 47830612 | 14.7G | 14.3G | 0.03 | 97.85 | 94.19 | 50.58 |
| HeLa1_0R | 31967532 | 31443462 | 9.6G | 9.4G | 0.03 | 97.69 | 93.84 | 50.66 |
| HeLa2_0R | 37072811 | 36192382 | 11.1G | 10.9G | 0.02 | 97.89 | 94.33 | 50.42 |
| HeLa3_0R | 41466226 | 40777276 | 12.4G | 12.2G | 0.03 | 97.8 | 94.11 | 50.71 |
| HeLa1_8R | 30312159 | 29652893 | 9.1G | 8.9G | 0.03 | 97.76 | 93.96 | 51.29 |
| HeLa2_8R | 35227814 | 34635299 | 10.6G | 10.4G | 0.03 | 97.86 | 94.2 | 50.45 |
| HeLa3_8R | 33843797 | 33328748 | 10.2G | 10.0G | 0.03 | 97.86 | 94.21 | 49.7 |
| HEK1cnt | 39926163 | 39126229 | 12.0G | 11.7G | 0.03 | 97.79 | 94.06 | 48.43 |
| HEK2cnt | 34041857 | 33416198 | 10.2G | 10.0G | 0.03 | 97.78 | 94.03 | 48.92 |
| HEK3cnt | 35956039 | 35315742 | 10.8G | 10.6G | 0.03 | 97.73 | 93.89 | 48.31 |
| HEK1_0R | 40629763 | 40099360 | 12.2G | 12.0G | 0.03 | 97.61 | 93.7 | 48.2 |
| HEK2_0R | 38002642 | 37410561 | 11.4G | 11.2G | 0.03 | 97.52 | 93.5 | 48.3 |
| HEK3_0R | 40373617 | 39155548 | 12.1G | 11.7G | 0.03 | 97.72 | 93.96 | 49.14 |
| HEK1_8R | 33442598 | 32604721 | 10.0G | 9.8G | 0.03 | 97.68 | 93.86 | 48.38 |
| HEK2_8R | 35327546 | 34572374 | 10.6G | 10.4G | 0.03 | 97.82 | 94.22 | 48.03 |
| HEK3_8R | 42653168 | 41520036 | 12.8G | 12.5G | 0.03 | 97.67 | 93.83 | 48.5 |
| Hep1cnt | 35856136 | 34755018 | 10.8G | 10.4G | 0.03 | 97.71 | 93.84 | 48.92 |
| Hep2cnt | 32429635 | 31241941 | 9.7G | 9.4G | 0.03 | 97.73 | 93.84 | 45.92 |
| Hep3cnt | 38344302 | 37715012 | 11.5G | 11.3G | 0.03 | 97.7 | 93.87 | 48.28 |
| Hep1_0R | 42554874 | 41628733 | 12.8G | 12.5G | 0.03 | 97.55 | 93.67 | 50.26 |
| Hep2_0R | 38212434 | 37586682 | 11.5G | 11.3G | 0.03 | 97.61 | 93.69 | 49.57 |
| Hep3_0R | 39235855 | 38542215 | 11.8G | 11.6G | 0.03 | 97.56 | 93.66 | 50.12 |
| Hep1_8R | 32004931 | 31142253 | 9.6G | 9.3G | 0.03 | 97.52 | 93.54 | 47.79 |
| Hep2_8R | 43290892 | 42319750 | 13.0G | 12.7G | 0.03 | 97.36 | 93.14 | 48.29 |
| Hep3_8R | 38439567 | 37525468 | 11.5G | 11.3G | 0.03 | 97.43 | 93.33 | 48.47 |

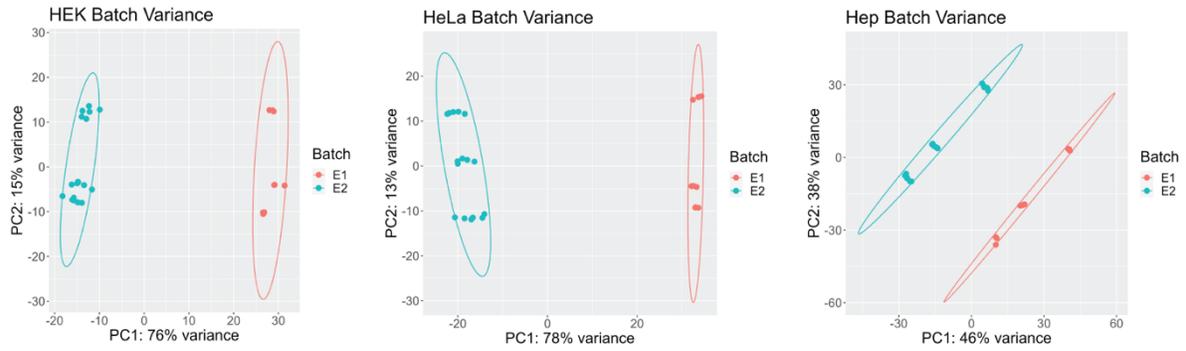
Supplementary Table 5. Summary table of quality control for Batch 2.

| Sample name | Raw reads | Clean reads | Raw bases | Clean bases | Error rate (%) | Q20(%) | Q30(%) | GC content (%) |
|-------------|-----------|-------------|-----------|-------------|----------------|--------|--------|----------------|
| HeLa1cnt | 62016498 | 9.3G | 60845478 | 9.13G | 0.02 | 98.21 | 94.87 | 49.5 |
| HeLa2cnt | 70595362 | 10.59G | 69120548 | 10.37G | 0.02 | 98.23 | 95.01 | 49.52 |
| HeLa3cnt | 60631278 | 9.09G | 59517000 | 8.93G | 0.02 | 98.23 | 95.13 | 50.53 |
| HeLa4cnt | 81419890 | 12.21G | 80080716 | 12.01G | 0.02 | 98.19 | 94.82 | 49.28 |
| HeLa5cnt | 73492284 | 11.02G | 72283092 | 10.84G | 0.02 | 98 | 94.8 | 49.95 |
| HeLa6cnt | 62844008 | 9.43G | 61885638 | 9.28G | 0.02 | 98.24 | 94.99 | 49.85 |
| HeLa1_0R | 61676822 | 9.25G | 60913992 | 9.14G | 0.02 | 98.28 | 95.18 | 50.3 |
| HeLa2_0R | 61288296 | 9.19G | 60459190 | 9.07G | 0.02 | 98.31 | 95.23 | 50.05 |
| HeLa4_0R | 63662714 | 9.55G | 62807390 | 9.42G | 0.02 | 98.31 | 95.24 | 49.68 |
| HeLa5_0R | 61124770 | 9.17G | 60088668 | 9.01G | 0.02 | 98.28 | 95.13 | 50.07 |
| HeLa6_0R | 61395698 | 9.21G | 60252900 | 9.04G | 0.02 | 98.26 | 95.19 | 50.69 |
| HeLa1_8R | 61353524 | 9.2G | 60294048 | 9.04G | 0.02 | 98.19 | 94.94 | 50.38 |
| HeLa3_8R | 60850104 | 9.13G | 59765084 | 8.96G | 0.02 | 98.25 | 95.16 | 50.11 |
| HeLa4_8R | 62198866 | 9.33G | 60957704 | 9.14G | 0.02 | 98.02 | 94.48 | 49.66 |
| HeLa5_8R | 63932420 | 9.59G | 62589710 | 9.39G | 0.02 | 98.08 | 94.7 | 50.37 |
| HeLa6_8R | 61521638 | 9.23G | 60228370 | 9.03G | 0.02 | 98.26 | 95.21 | 50.27 |
| HEK1cnt | 66272392 | 9.94G | 65330354 | 9.8G | 0.02 | 98.3 | 95.17 | 49.92 |
| HEK2cnt | 60035250 | 9.01G | 58712308 | 8.81G | 0.02 | 98.23 | 95.07 | 50.36 |
| HEK3cnt | 64145522 | 9.62G | 63225354 | 9.48G | 0.02 | 98.29 | 95.1 | 49.15 |
| HEK4cnt | 65659452 | 9.85G | 64716964 | 9.71G | 0.02 | 98.35 | 95.26 | 48.98 |
| HEK5cnt | 60758956 | 9.11G | 59607368 | 8.94G | 0.02 | 98.26 | 95 | 48.95 |
| HEK6cnt | 61580132 | 9.24G | 60342074 | 9.05G | 0.02 | 98.4 | 95.42 | 49.15 |
| HEK1_0R | 69441930 | 10.42G | 68057050 | 10.21G | 0.02 | 98.07 | 94.72 | 50.09 |
| HEK2_0R | 67217478 | 10.08G | 65897520 | 9.88G | 0.02 | 98.03 | 94.63 | 49.76 |
| HEK3_0R | 61440470 | 9.22G | 60215412 | 9.03G | 0.02 | 98.25 | 95.16 | 48.6 |
| HEK4_0R | 64208408 | 9.63G | 62884812 | 9.43G | 0.02 | 98.26 | 95.24 | 48.42 |
| HEK5_0R | 60640764 | 9.1G | 59344610 | 8.9G | 0.02 | 98.23 | 95.16 | 48.85 |
| HEK6_0R | 60250308 | 9.04G | 59084584 | 8.86G | 0.02 | 98.24 | 95.19 | 49.16 |
| HEK1_8R | 63929742 | 9.59G | 63030320 | 9.45G | 0.02 | 98.42 | 95.47 | 49.5 |
| HEK2_8R | 63883690 | 9.58G | 62590046 | 9.39G | 0.02 | 98.44 | 95.53 | 49.4 |
| HEK3_8R | 78514742 | 11.78G | 76691164 | 11.5G | 0.02 | 98.41 | 95.46 | 48.93 |
| HEK4_8R | 58987108 | 8.85G | 57708896 | 8.66G | 0.02 | 98.29 | 95.14 | 49.43 |
| HEK5_8R | 63175986 | 9.48G | 62004916 | 9.3G | 0.02 | 98.24 | 95.02 | 49.55 |
| HEK6_8R | 63239640 | 9.49G | 62031320 | 9.3G | 0.02 | 98.39 | 95.39 | 49.39 |
| Hep1cnt | 67481398 | 10.12G | 65973190 | 9.9G | 0.02 | 98.4 | 95.42 | 48.33 |
| Hep2cnt | 79463982 | 11.92G | 77844494 | 11.68G | 0.02 | 98.32 | 95.24 | 47.94 |
| Hep3cnt | 62406870 | 9.36G | 61270232 | 9.19G | 0.02 | 98.25 | 95.03 | 48.08 |
| Hep4cnt | 63241240 | 9.49G | 61807164 | 9.27G | 0.02 | 98.24 | 95.04 | 49.3 |

| | | | | | | | | |
|---------|----------|--------|----------|--------|------|-------|-------|-------|
| Hep5cnt | 65096858 | 9.76G | 63728104 | 9.56G | 0.02 | 98.32 | 95.21 | 48.45 |
| Hep6cnt | 68099944 | 10.21G | 66821570 | 10.02G | 0.02 | 98.19 | 94.86 | 47.62 |
| Hep1_0R | 62107654 | 9.32G | 60784062 | 9.12G | 0.02 | 98.32 | 95.22 | 49.26 |
| Hep2_0R | 60533366 | 9.08G | 59547872 | 8.93G | 0.02 | 98.34 | 95.32 | 49.4 |
| Hep3_0R | 64125286 | 9.62G | 62786732 | 9.42G | 0.02 | 98.32 | 95.22 | 49.1 |
| Hep4_0R | 63549094 | 9.53G | 62115522 | 9.32G | 0.02 | 98.2 | 95.06 | 50 |
| Hep5_0R | 60337954 | 9.05G | 59417138 | 8.91G | 0.02 | 98.17 | 94.86 | 49.09 |
| Hep6_0R | 66796434 | 10.02G | 65204242 | 9.78G | 0.02 | 98.16 | 94.82 | 48.53 |
| Hep1_8R | 65094562 | 9.76G | 63457930 | 9.52G | 0.02 | 98.19 | 94.9 | 48.59 |
| Hep2_8R | 63557536 | 9.53G | 62146416 | 9.32G | 0.02 | 98.15 | 94.85 | 49.11 |
| Hep3_8R | 62153818 | 9.32G | 61130290 | 9.17G | 0.02 | 98.19 | 94.92 | 48.68 |
| Hep4_8R | 64571708 | 9.69G | 62780996 | 9.42G | 0.03 | 97.6 | 93.52 | 48.98 |
| Hep5_8R | 67192788 | 10.08G | 65483534 | 9.82G | 0.03 | 97.69 | 93.66 | 48.87 |
| Hep6_8R | 59336896 | 8.9G | 58500944 | 8.78G | 0.02 | 98.23 | 95.12 | 48.44 |

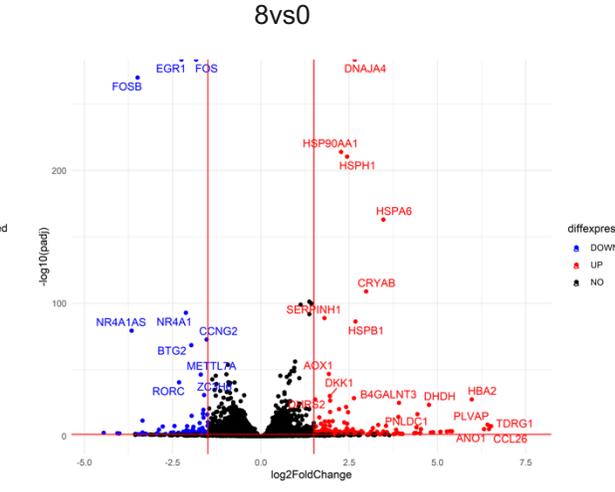
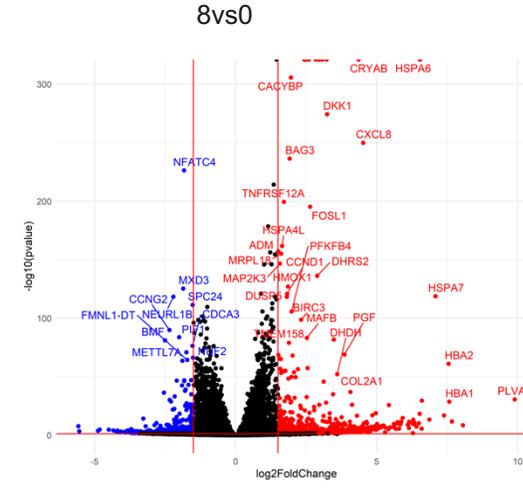
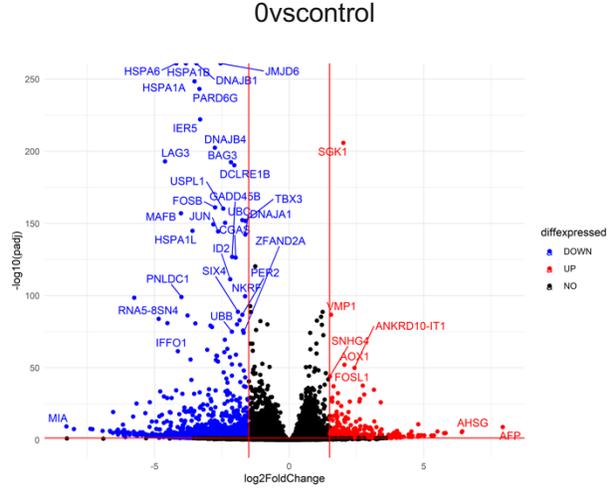
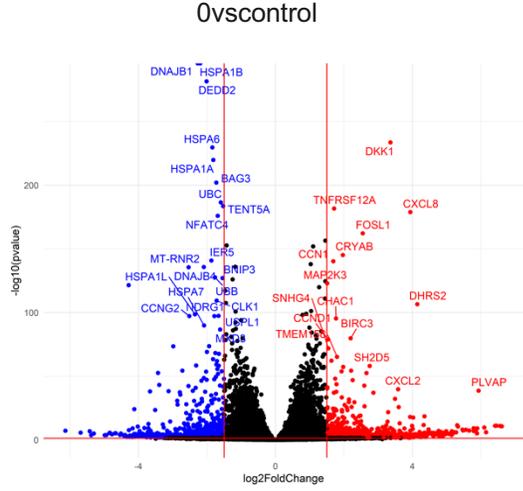
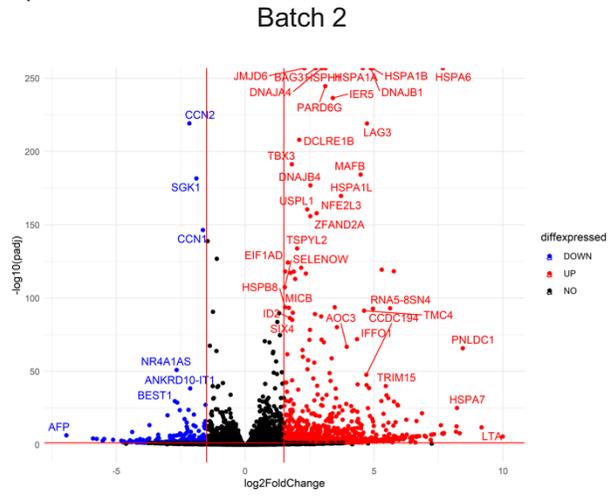
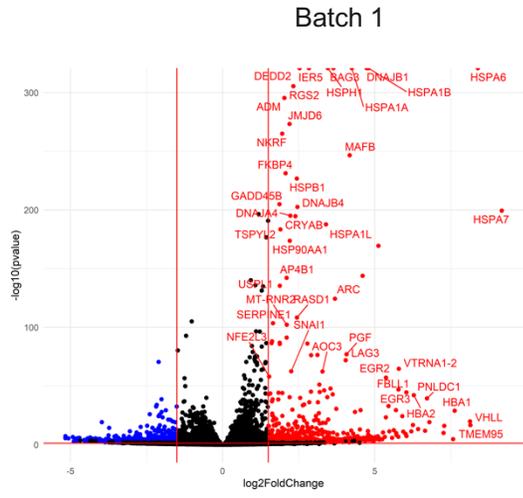
Supplementary Table 6. Primers used in qPCR experiments.

| Classification | HGNC Symbol | Primer Sequence (5'-> 3') | Primer Sequence (3'-5') | Product Size (bp) |
|---------------------------------|-------------|---------------------------|-------------------------|-------------------|
| Signal Receptor Ligand Activity | LTA | ACCATTTTCAGGGGTCGTCAC | GGATGGTTCAGGGAGGTGTG | 131 |
| Signal Receptor Ligand Activity | MIA | CAGGAGTGCAGCCACCCTAT | AAATAGCCCAGGCGAGCAG | 194 |
| Signal Receptor Ligand Activity | TNF | CAAGGACAGCAGAGGACCAG | TCCTTTCCAGGGGAGAGAGG | 156 |
| Heat Acclimation | HSPA1A | AGCTGGAGCAGGTGTGTAAC | CAGCAATCTTGGAAGGCC | 154 |
| Heat Acclimation | HSPA6 | CCAGAGGAACGCCACTATCC | GGAGGGATGCCACTGAGTTC | 156 |
| Heat Acclimation | BAG3 | AAGCCCAGAAGACGCACTAC | GACAGATGACCTGAACGGGG | 131 |
| Heat Acclimation | DNAJB1 | GGCTCACCTGGGCTCG | CCCGGGAATTATCCCAACCC | 129 |
| Negative Control | ACTB | CTTCGCGGGCGACGAT | CCACATAGGAATCCTTCTGACC | 104 |
| Negative Control | GAPDH | CGGGAAGGAAATGAATGGGC | GGAAAAGCATCACCCGGAGG | 148 |

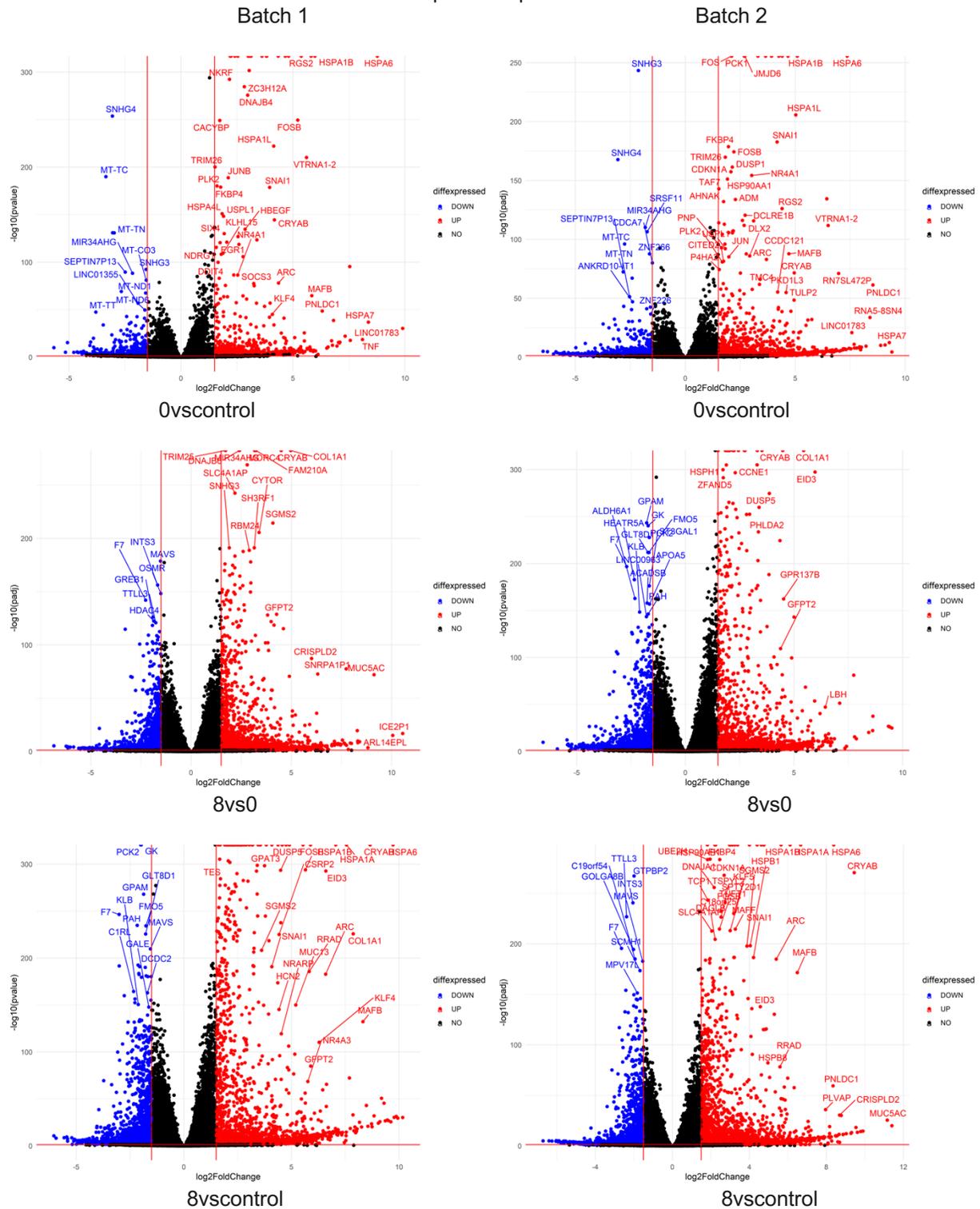


Supplementary Figure 1. PCA Analysis Highlighting Batch Effects in Cell Lines. PCA analyses of VST-normalized counts separated by cell line indicate that system variance is primarily driven by batch effects in HEK293 (Figure 1A), HeLa (Figure 1B), and HepG2 (Figure 1C) cells. The PCA explains 76%, 78%, and 46% of the variance in each cell line, respectively, demonstrating the impact of batch variability on gene expression profiles.

HeLa comparisons



HepG2 comparisons

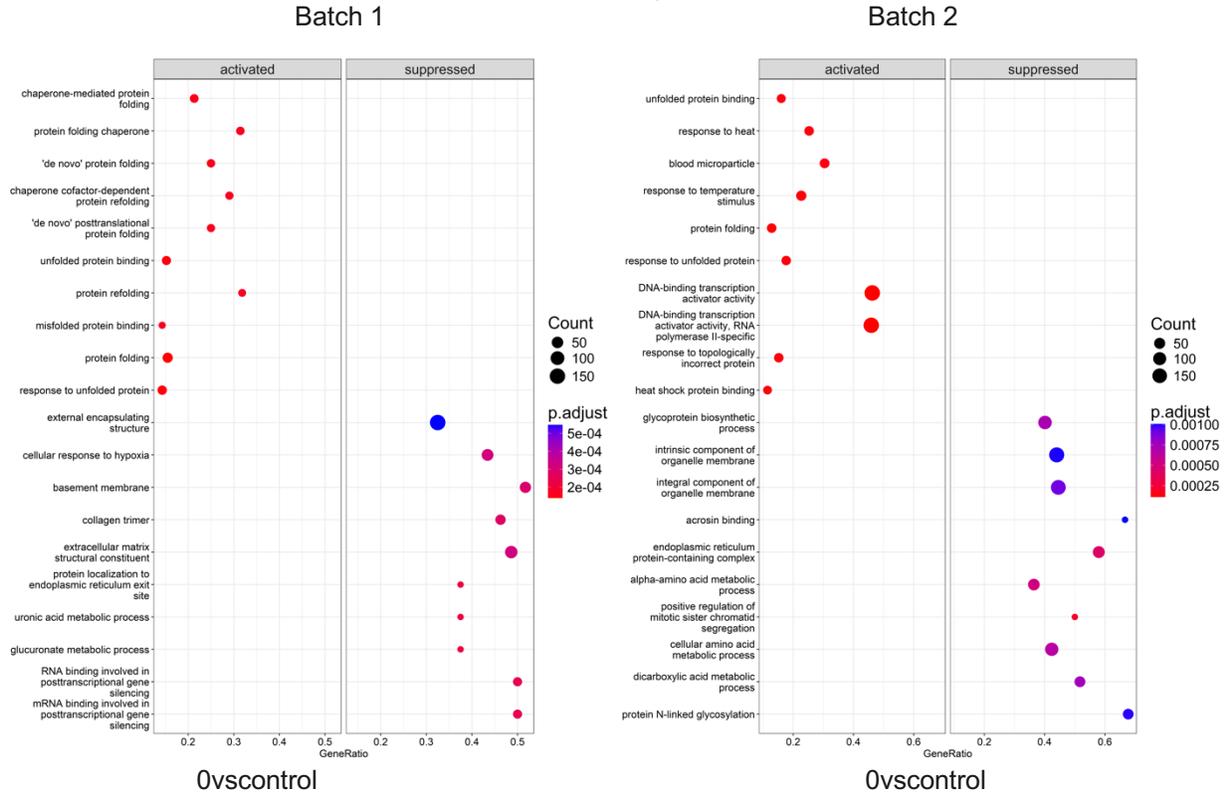


Supplementary Figure 2. Volcano Plots of Differentially Expressed Genes by Batch. Volcano plots illustrate the condition comparisons within each cell line for both Batch 1 and Batch 2. Each dot represents an individual gene, with blue dots indicating downregulated genes (\log_2 fold change < -1) and red dots indicating upregulated genes (\log_2 fold change > 1). Plots show gene expression changes for

HEK293, HeLa, and HepG2 (all comparisons for both batches). Abbreviations are as follows: 0vsControl (0 hours after heat shock vs. Control cells); 8vs0 (8 hours recovery after heat shock vs. 0 hours after heat shock cells); 8vsControl (8 hours recovery after heat shock vs. Control cells).

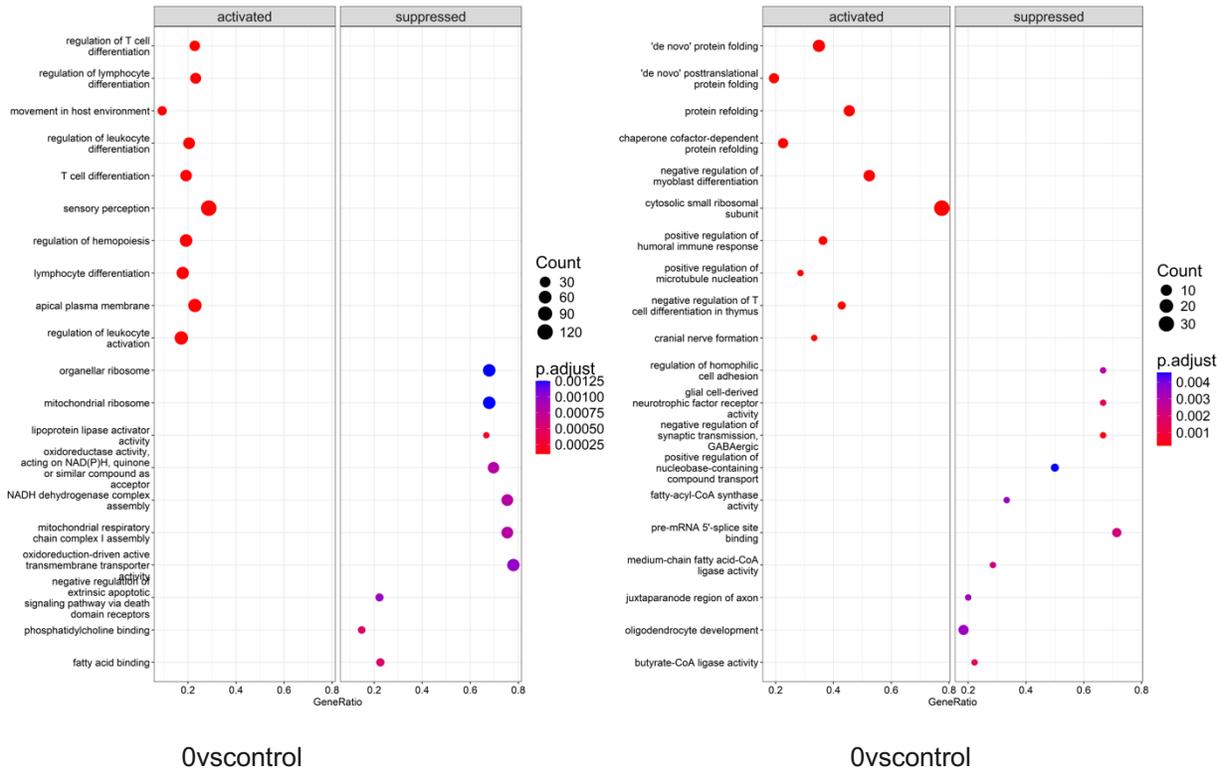
A

HEK293 comparisons



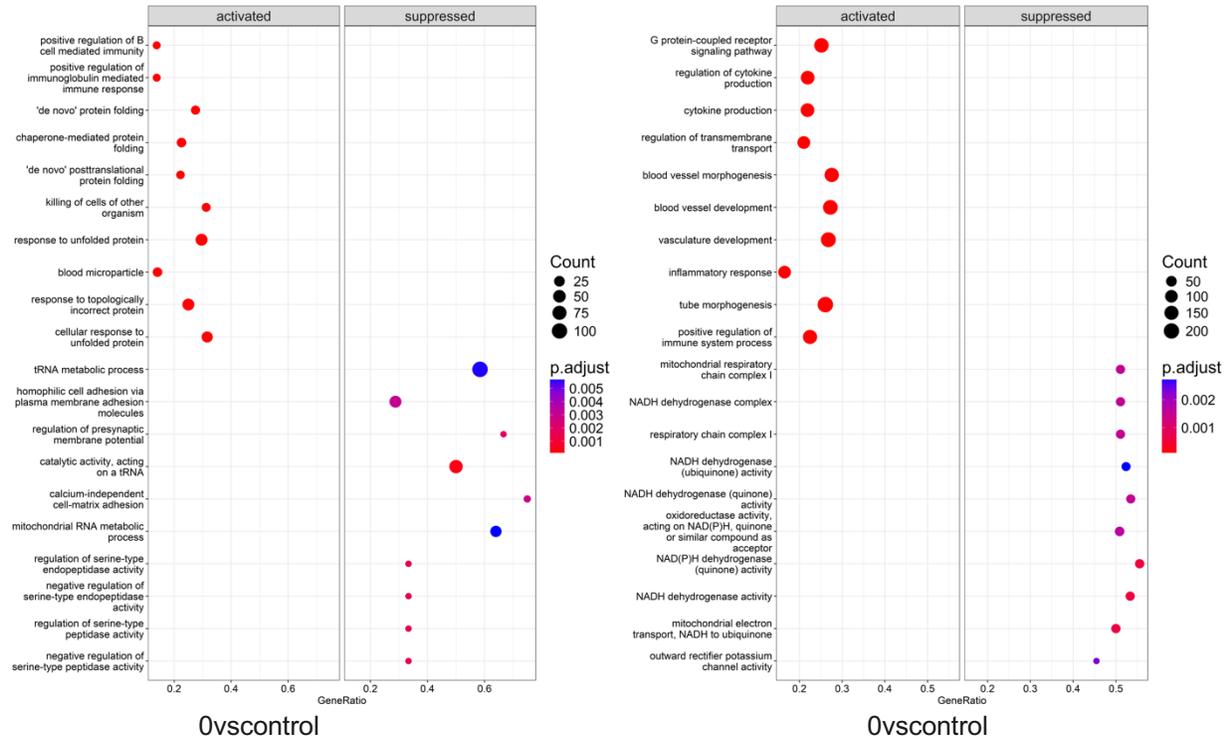
B

HeLa comparisons



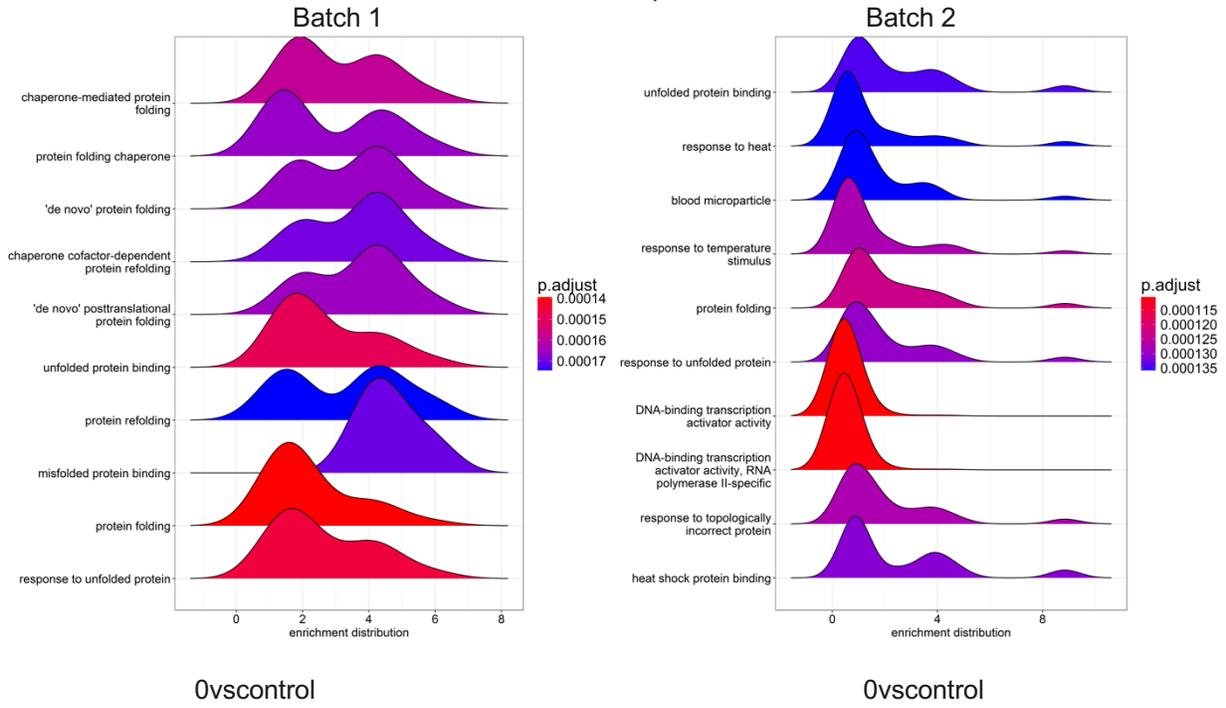
C

HepG2 comparisons

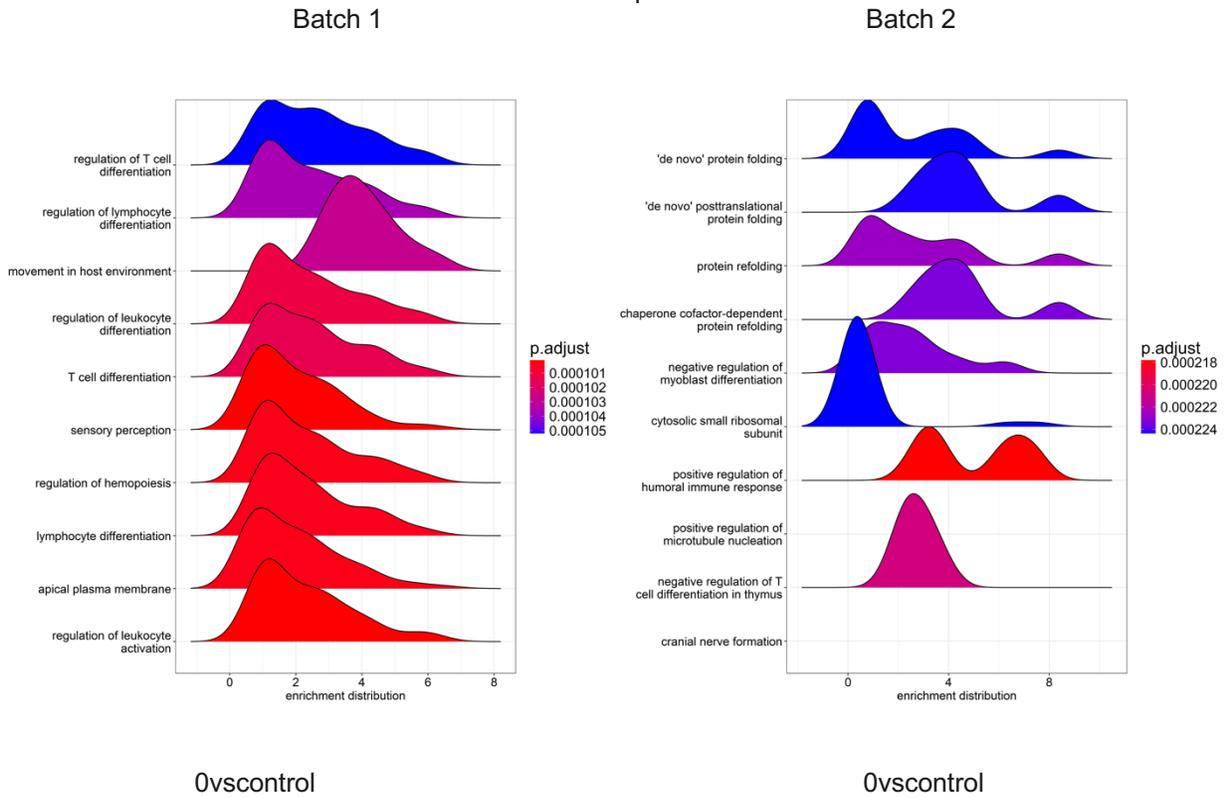


Supplementary Figure 3. Gene Set Enrichment Analysis via Dot Plots. Dot plots visualize Gene Set Enrichment Analysis (GSEA) results for the top 15 positive and top 15 negative normalized enrichment score (NES) hits [using the Human Phenotype Ontology in HEK293 (A), HeLa (B), and HepG2 (C) cell lines at 0 hours vs control cells, providing insights into enriched pathways influenced by heat shock. Abbreviations are 0vsControl (0 hours after heat shock vs. Control cells).

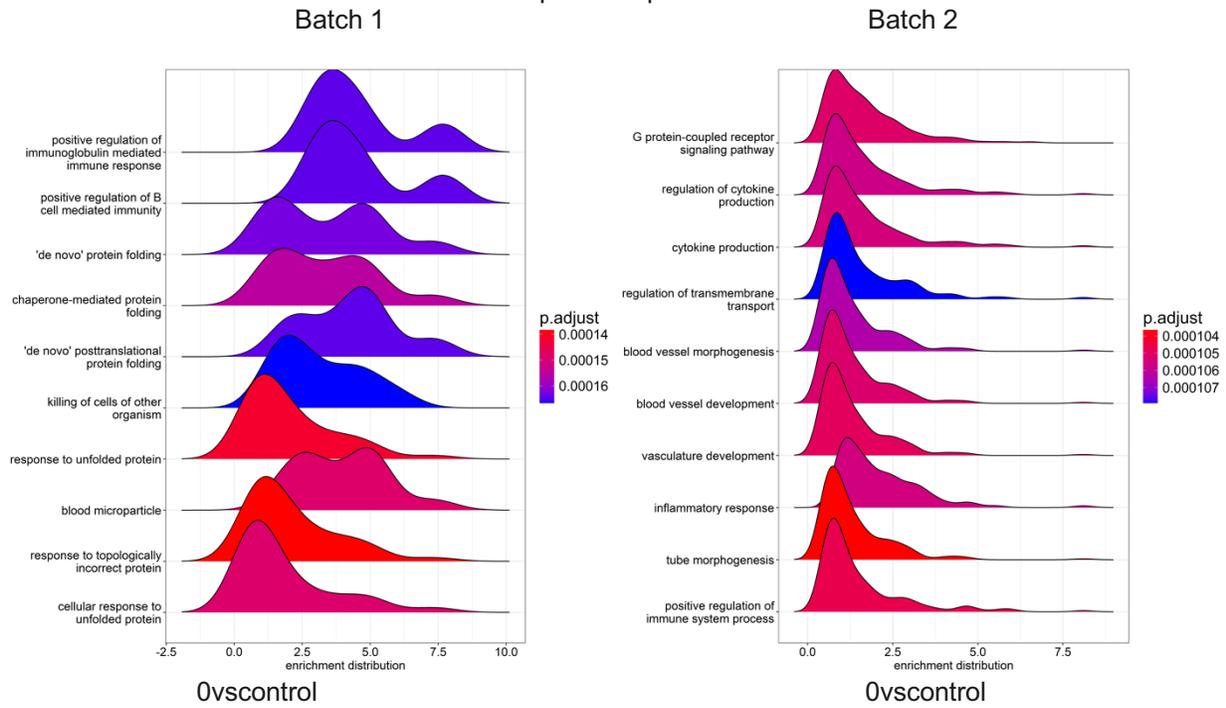
HEK293 comparisons



HeLa comparisons

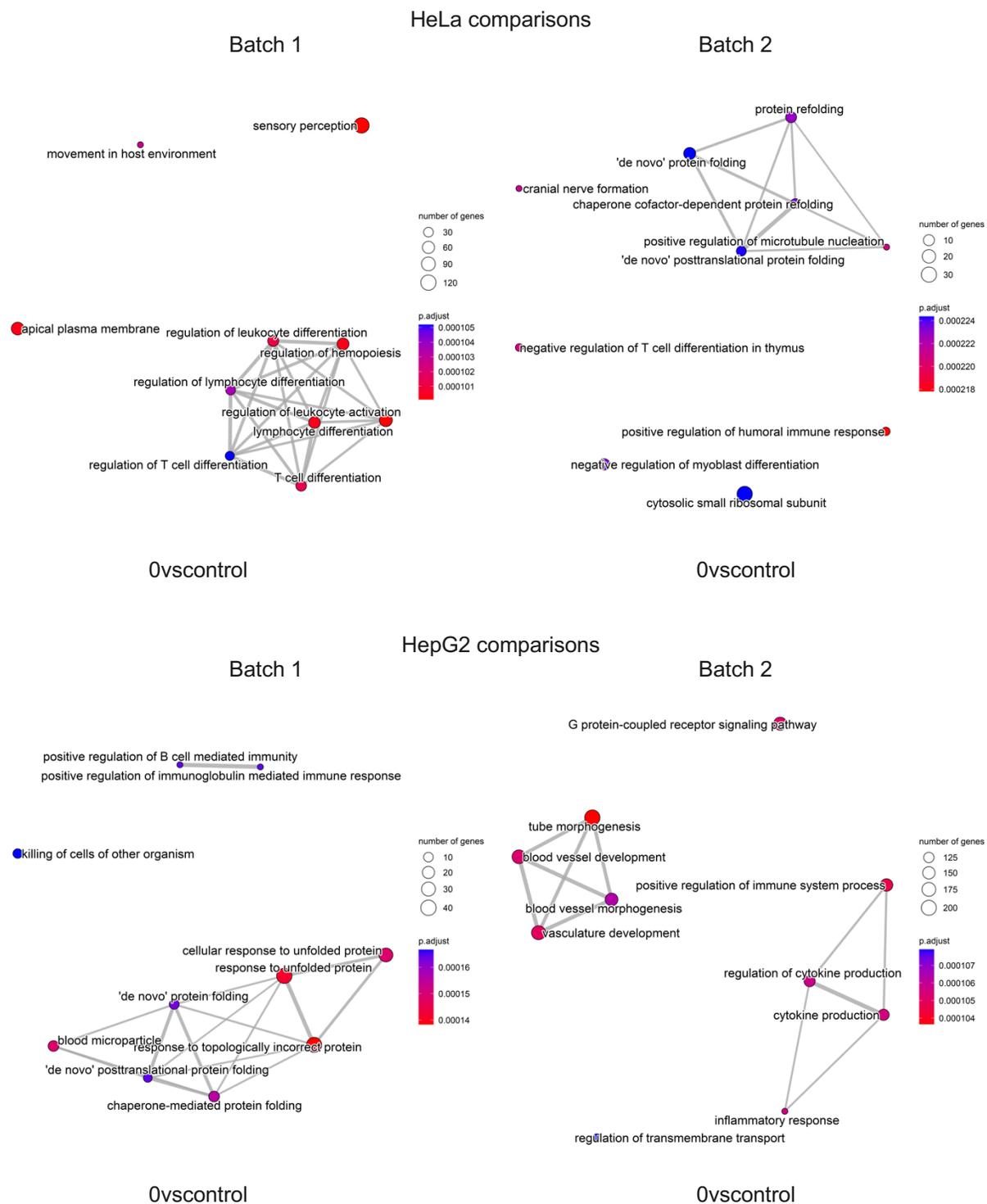


HepG2 comparisons

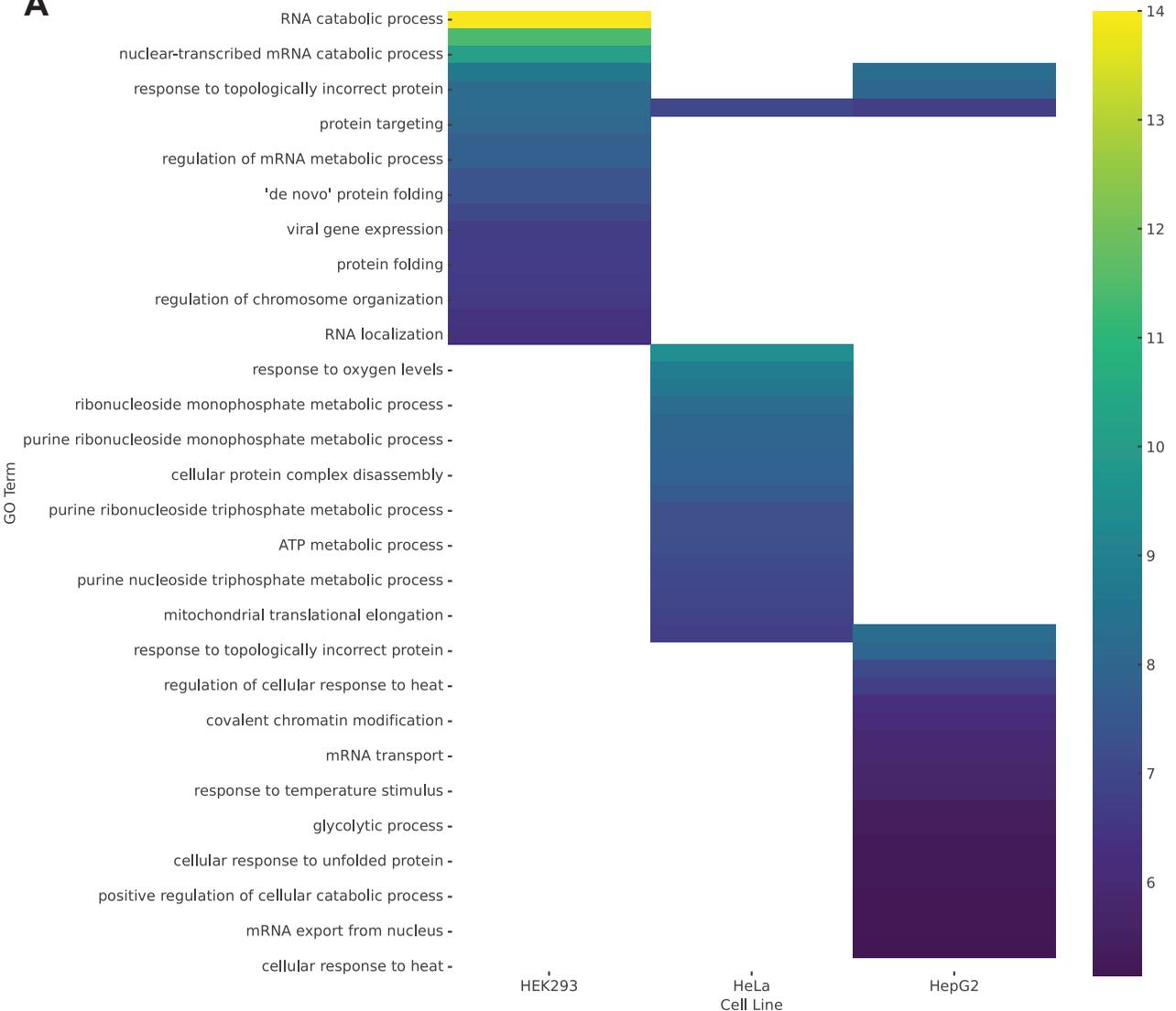
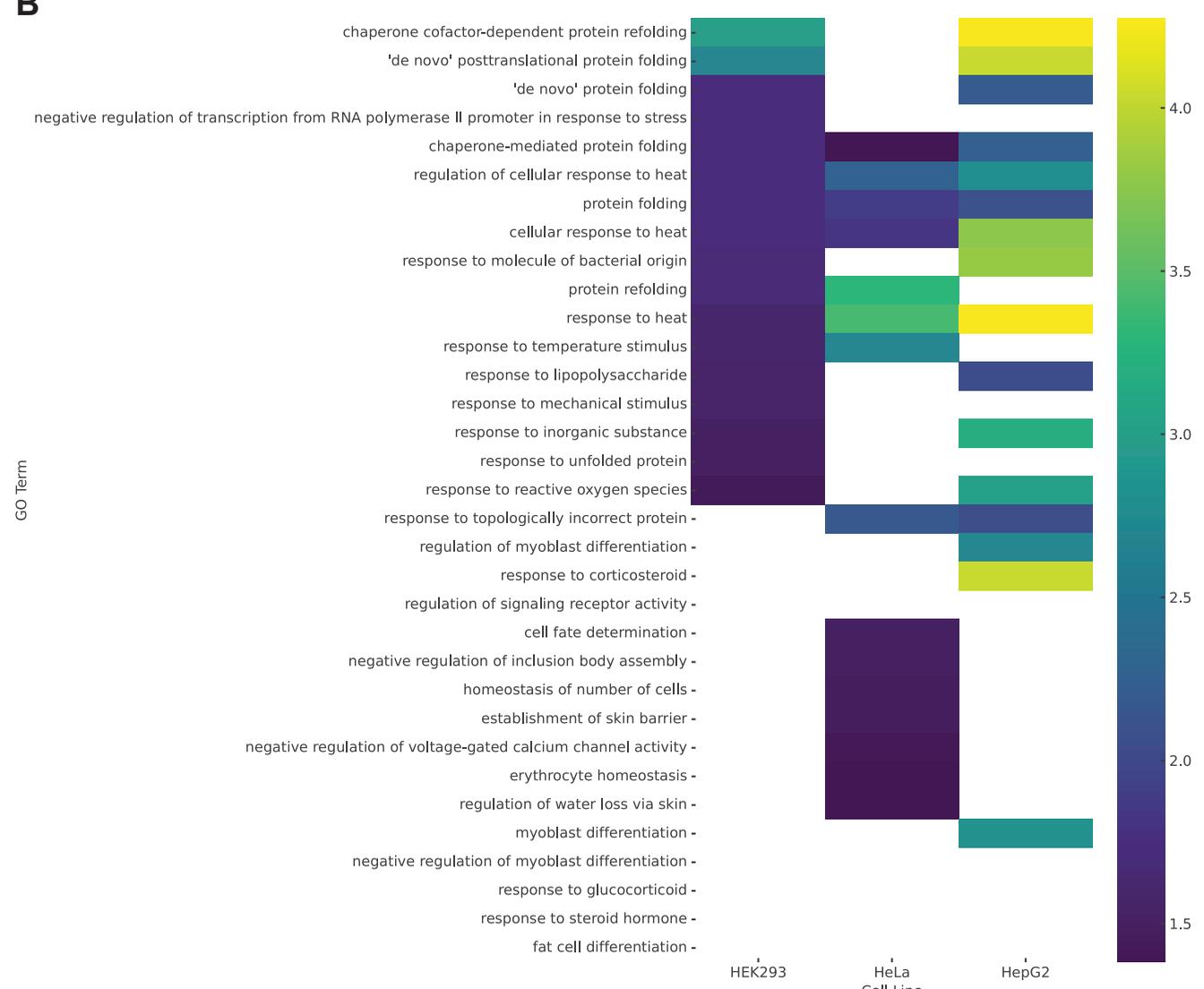


Supplementary Figure 4. Distribution of Enriched Genes Across Top GSEA Hits.

Distribution plots of log₂ fold changes for genes in the top 15 positively enriched GSEA pathways for HEK293, HeLa, and HepG2 cell lines at 0 hours vs. Control. Peak height indicates the number of enriched genes within each log₂ fold change range. Abbreviations are as follows: 0vsControl (0 hours after heat shock vs. Control cells).



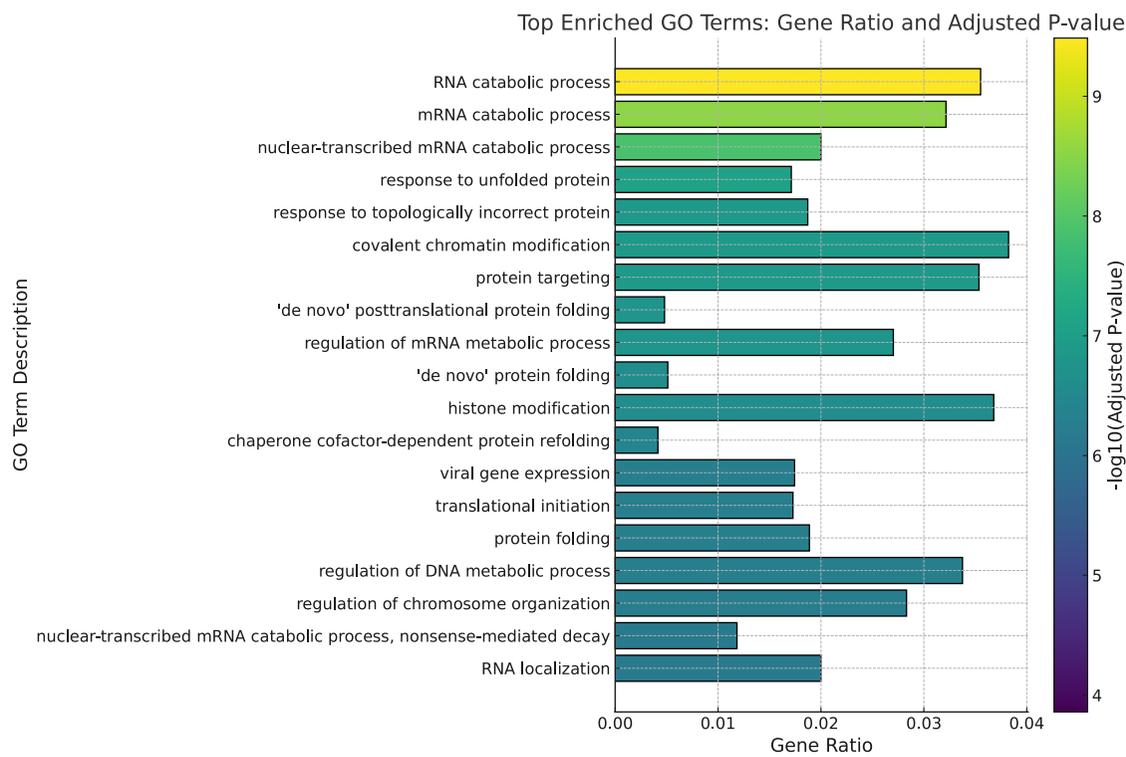
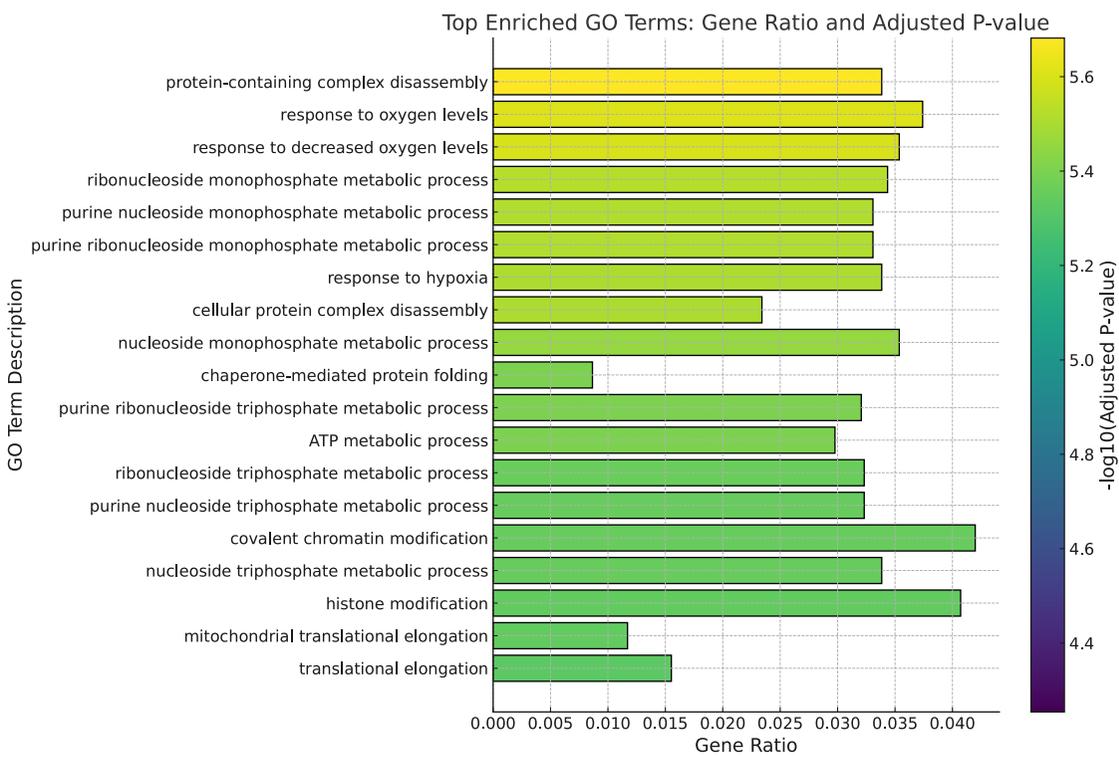
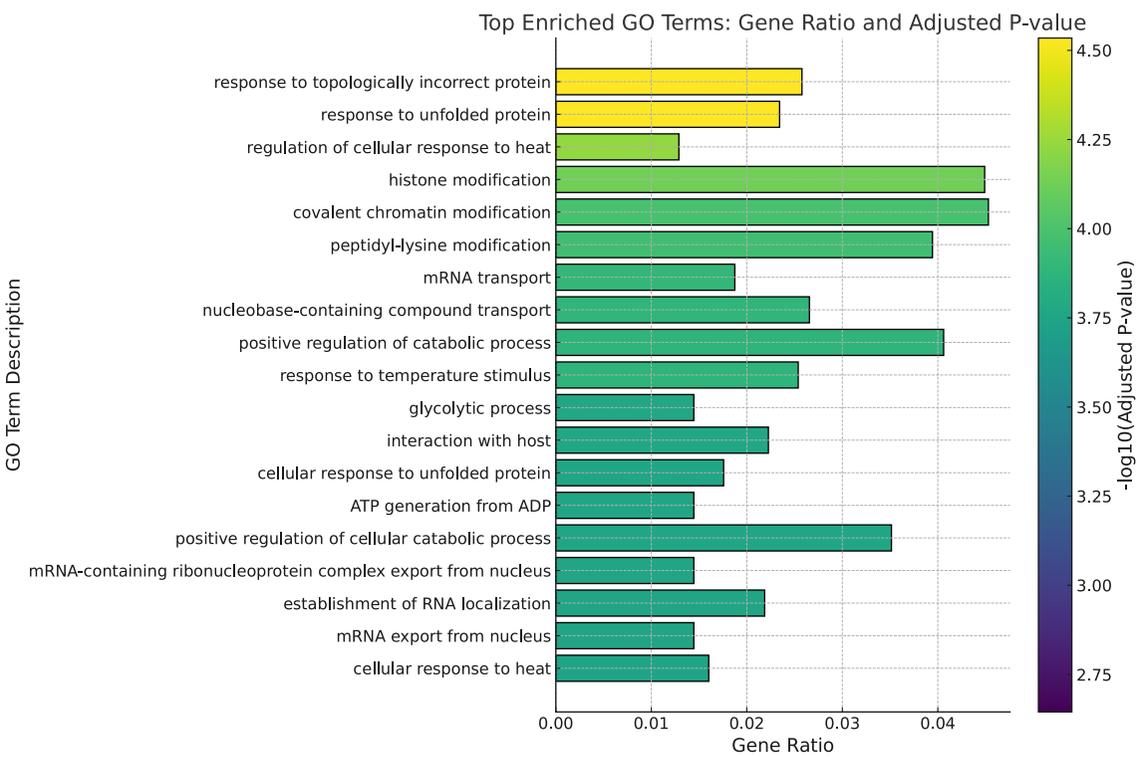
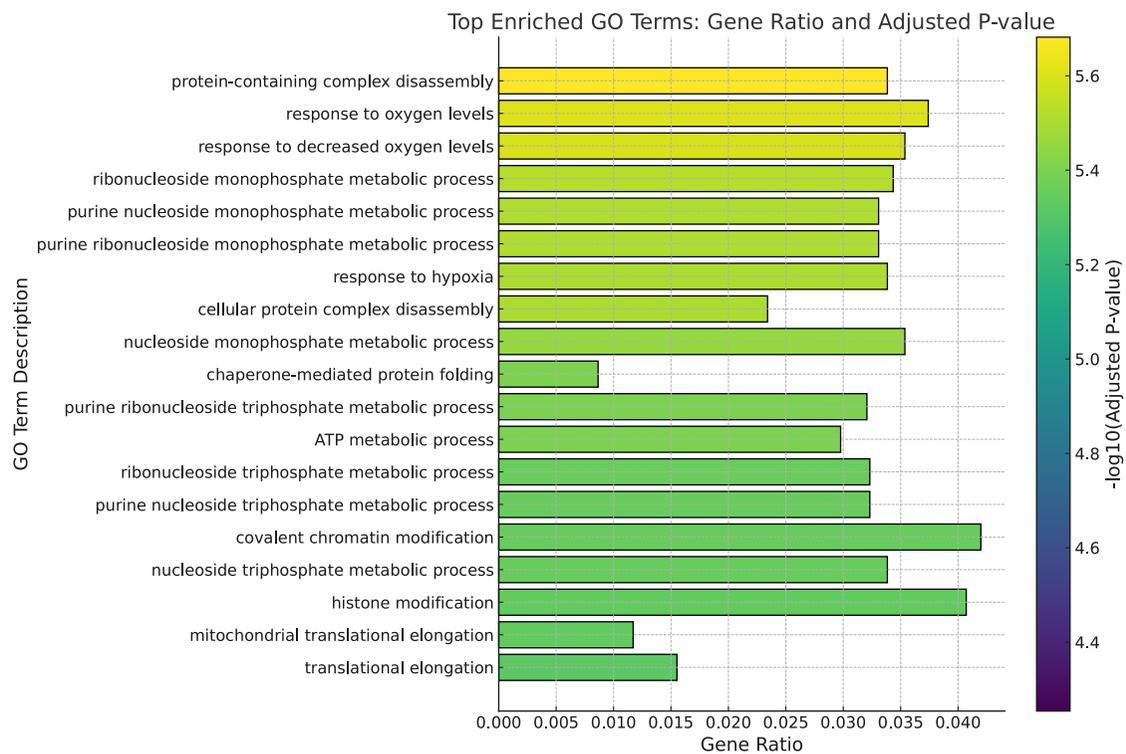
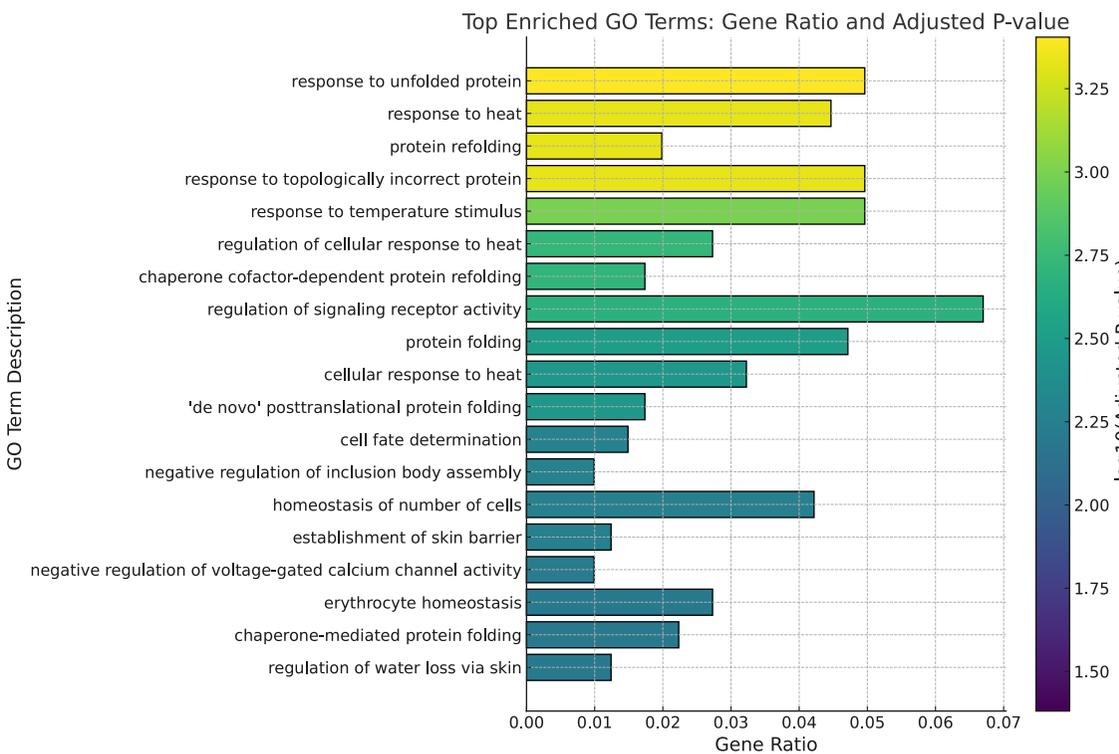
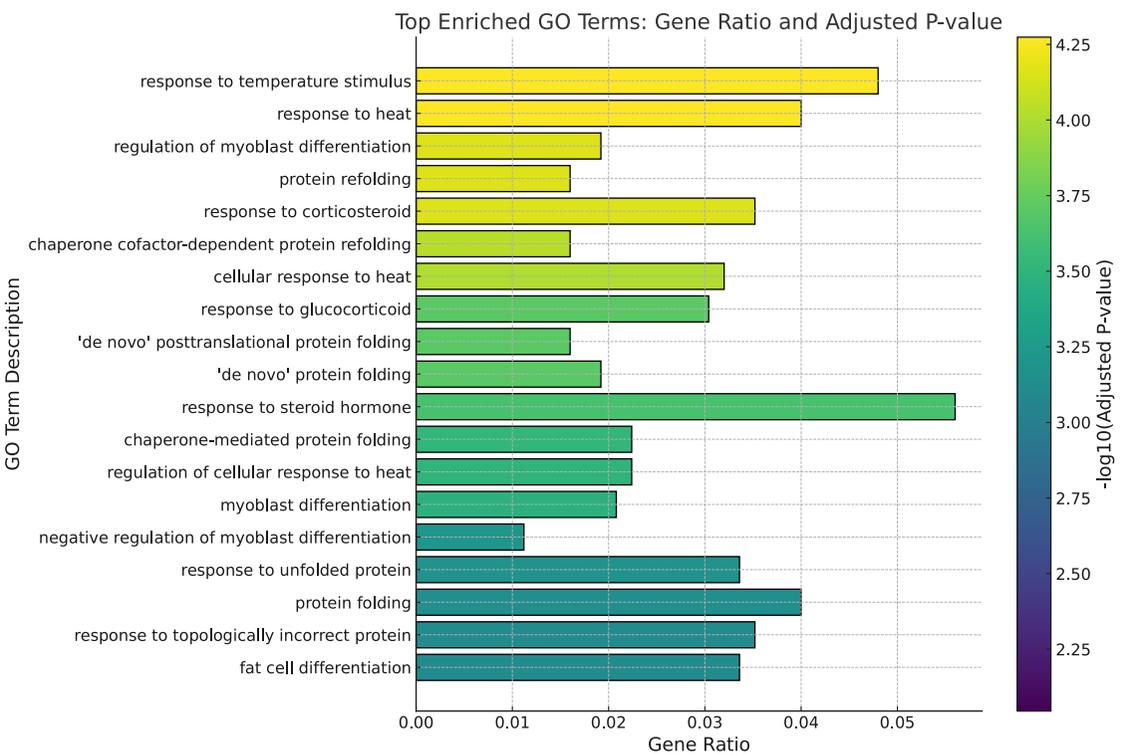
Supplementary Figure 5. Enrichment Maps of GSEA Hits for HeLa and HepG2 Cells. Network enrichment maps of the top 10 positive GSEA NES hits for HeLa and HepG2 cell lines at 0 hours vs. Control. Nodes represent enriched gene sets, while edges indicate shared genes between sets. Maps are shown for HeLa (Batch 1, top left panel; Batch 2, top right panel) and HepG2 (Batch 1, bottom left panel; Batch 2, bottom right panel), providing a systems-level perspective on pathway relationships under heat shock conditions.

A**B**

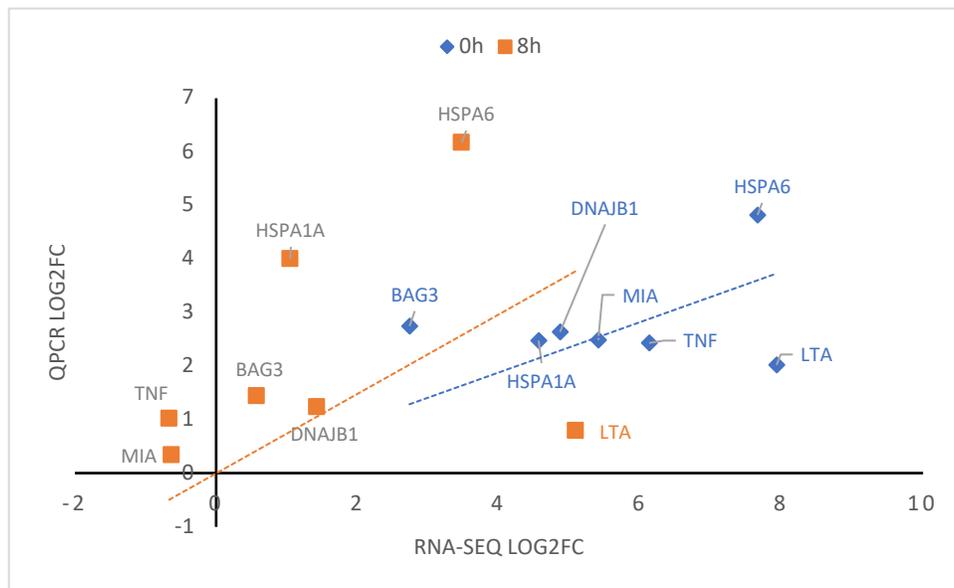
Supplementary Figure 6. Comparative Heatmap of $-\log_{10}(\text{p-values})$ for Enriched GO Terms Across HEK293, HeLa, and HepG2 Cell Lines for Batch 1 (A) and Batch 2 (B). The heatmaps depict the $-\log_{10}(\text{p-values})$ for the top 19 enriched Gene Ontology (GO) Biological Process (BP) terms across three cell lines: HEK293, HeLa, and HepG2. Each row corresponds to a unique GO term, and each column represents one of the cell lines. The color intensity reflects the statistical significance of enrichment, with darker colors indicating higher significance [$-\log_{10}(\text{p-value})$ values]. Missing values, where a GO term was not significantly enriched, are masked, and appear as blank cells in the heatmap. The complete data tables used are located in Appendix 2.

Batch 1: In HEK293, enriched GO terms are RNA catabolic process (GO:0006401); mRNA catabolic process (GO:0006402); nuclear-transcribed mRNA catabolic process (GO:0000956); response to unfolded protein (GO:0006986); response to topologically incorrect protein (GO:0035966); covalent chromatin modification (GO:0016569); protein targeting (GO:0006605); 'de novo' posttranslational protein folding (GO:0051084); regulation of mRNA metabolic process (GO:1903311); histone modification (GO:0016570); 'de novo' protein folding (GO:0006458); chaperone cofactor-dependent protein refolding (GO:0051085); viral gene expression (GO:0019080); translational initiation (GO:0006413); protein folding (GO:0006457); regulation of DNA metabolic process (GO:0051052); regulation of chromosome organization (GO:0033044); nuclear-transcribed mRNA catabolic process, nonsense-mediated decay (GO:0000184); and RNA localization (GO:0006403). In HeLa, enriched GO terms are protein-containing complex disassembly (GO:0032984); response to oxygen levels (GO:0070482); response to decreased oxygen levels (GO:0036293); ribonucleoside monophosphate metabolic process (GO:0009161); purine nucleoside monophosphate metabolic process (GO:0009126); purine ribonucleoside monophosphate metabolic process (GO:0009167); response to hypoxia (GO:0001666); cellular protein complex disassembly (GO:0043624); nucleoside monophosphate metabolic process (GO:0009123); purine ribonucleoside triphosphate metabolic process (GO:0009205); chaperone-mediated protein folding (GO:0061077); ATP metabolic process (GO:0046034); ribonucleoside triphosphate metabolic process (GO:0009199); purine nucleoside triphosphate metabolic process (GO:0009144); covalent chromatin modification (GO:0016569); nucleoside triphosphate metabolic process (GO:0009141); histone modification (GO:0016570); mitochondrial translational elongation (GO:0070125); and translational elongation (GO:0006414). In HepG2, enriched GO terms are response to topologically incorrect protein (GO:0035966); response to unfolded protein (GO:0006986); regulation of cellular response to heat (GO:1900034); histone modification (GO:0016570); covalent chromatin modification (GO:0016569); peptidyl-lysine modification (GO:0018205); mRNA transport (GO:0051028); nucleobase-containing compound transport (GO:0015931); response to temperature stimulus (GO:0009266); positive regulation of catabolic process (GO:0009896); glycolytic process (GO:0006096); interaction with host (GO:0051701); cellular response to unfolded protein (GO:0034620); ATP generation from ADP (GO:0006757); positive regulation of cellular catabolic process (GO:0031331); establishment of RNA localization (GO:0051236); mRNA export from nucleus (GO:0006406); mRNA-containing ribonucleoprotein complex export from nucleus (GO:0071427); and cellular response to heat (GO:0034605).

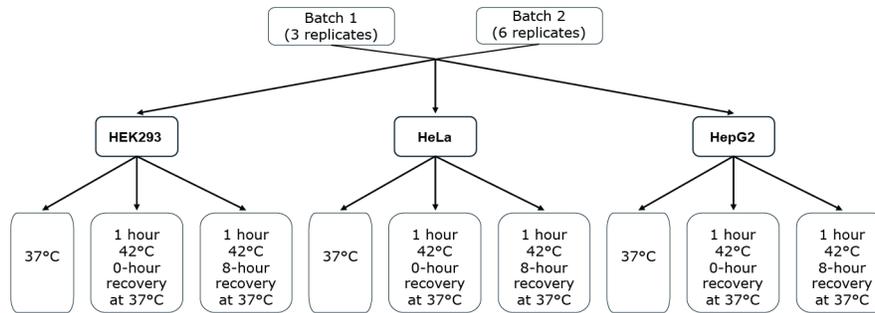
Batch 2: In HEK293 cells, enriched GO terms include chaperone cofactor-dependent protein refolding (GO:0051085), 'de novo' posttranslational protein folding (GO:0051084), negative regulation of transcription from RNA polymerase II promoter in response to stress (GO:0097201), regulation of cellular response to heat (GO:1900034), response to molecule of bacterial origin (GO:0002237), response to lipopolysaccharide (GO:0032496), response to reactive oxygen species (GO:0000302), and several others related to protein folding and stress responses. In HeLa cells, enriched GO terms highlight processes such as response to unfolded protein (GO:0006986), response to heat (GO:0009408), protein refolding (GO:0042026), response to topologically incorrect protein (GO:0035966), regulation of signaling receptor activity (GO:0010469), homeostasis of number of cells (GO:0048872), establishment of skin barrier (GO:0061436), negative regulation of voltage-gated calcium channel activity (GO:1901386), erythrocyte homeostasis (GO:0034101), and others involved in cellular adaptation, differentiation, and homeostasis. In HepG2 cells, enriched GO terms include response to temperature stimulus (GO:0009266), response to corticosteroid (GO:0031960), response to glucocorticoid (GO:0051384), response to steroid hormone (GO:0048545), regulation of myoblast differentiation (GO:0045661), negative regulation of myoblast differentiation (GO:0045662), fat cell differentiation (GO:0045444), and others emphasizing differentiation, hormonal response, and protein folding.

A**B****C****D****E****F**

Supplementary Figure 7. Individual Top Enriched GO Terms after heat shock (0 h). The bar charts illustrate the top 19 enriched Gene Ontology (GO) terms in HEK293 (A, Batch 1 and D, Batch 2), HeLa (B, Batch 1 and E, Batch 2), and HepG2 (C, Batch 1 and F, Batch 2) cells after heat shock. Each bar represents a GO term, with the gene ratio (proportion of genes associated with the term) shown on the x-axis. The color intensity corresponds to the statistical significance of the enrichment, expressed as $-\log_{10}(\text{adjusted p-value})$, with brighter colors indicating higher significance. The data used are located in Appendix 2 and were used to generate the comparative figure (Supplementary Figure 6). The GO annotations are provided in Supplementary Figure 6.



Supplementary Figure 8. Scatter plot comparing RNA-seq and qPCR fold change values. The scatter plot displays log₂ fold change (log₂FC) values obtained from RNA-seq (x-axis) and qPCR (y-axis) analyses for genes HSPA1A, HSPA6, BAG3, DNAJB1, LTA, MIA, and TNF at 0 hours (blue circles) and 8 hours (orange squares) post-heat shock. Data points near the diagonal indicate strong agreement between RNA-seq and qPCR measurements, while points further from the diagonal represent differences in the magnitude of fold change between the two methods, suggesting potential differences in sensitivity or dynamic range.



Supplementary Figure 9. Flowchart diagram illustrating the experimental design and treatment conditions. Two experimental batches were analyzed, consisting of 3 and 6 replicates for HEK293, HeLa, and HepG2 cell lines. Samples were subjected to a 1-hour mild heat shock at 42°C and allowed to recover for either 0 hours or 8 hours at 37°C. Control samples were maintained at 37°C for the entire experiment. This experimental design ensures a robust comparison of gene expression changes induced by heat shock across replicates and batches.