Supplemental Information for

The ABL2 kinase regulates an HSF1-dependent transcriptional program required for lung adenocarcinoma brain metastasis

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Materials and Methods

RNA-sequencing analysis

For differential expression analysis of RNA-seq data comparing PC9 parental to PC9-BrM3 cells, total RNA was collected from equally sub-confluent cell cultures using the RNeasy kit (Qiagen) and 1 μg total RNA input was used for each sample. For differential expression analysis comparing PC9-BrM3 Tet-shNTC vs Tet-shHSF1 (clone 7480) knockdown cells, PC9-BrM3 cells were plated overnight and were then treated with 500 ng/mL doxycycline for 48 hrs to induce shRNA knockdown prior to RNA isolation. Libraries were sequenced on an Illumina HiSeq 2000 sequencing system using 50-bp single-end reads by the Duke University Genome Sequencing facility. RNA-seq fastq data files were processed using the TrimGalore toolkit followed by read quality assessment with the Fast-QC analysis tool. Reads were mapped to the GRCh37 version of the human genome and transcriptome using the HISAT2 RNA-seq alignment tool (1). Reads were used for subsequent analysis if they mapped to a single genomic location, and gene counts were compiled using FeatureCounts (2). Normalization and differential expression were carried out using the DESeq2 Bioconductor package (3) in the R statistical programming environment. Datasets containing differential expression analysis corresponding to each RNA-seq experiment are provided as Supplementary File 1 and Supplementary File 2. RNA sequencing raw data have been deposited in the NCBI Gene Expression Omnibus (GEO) under accession number GSE149246.
Gene set enrichment analysis

Following analysis of RNA-seq differential expression data comparing PC9 parental to PC9-BrM3 cells, data were sorted by statistical rank and imported into the GSEA software tool from the Broad Institute (4). The pre-ranked gene list was processed under default settings and size filters for analysis across all signatures contained within the following mSigDB databases: Hallmark (v6.2, 50 gene sets), Positional (v6.2, 259 gene sets), Curated (v6.2, 3,648 gene sets), Motif (v6.2, 776 gene sets), Computational (v6.2, 782 gene sets), Gene Ontology (v6.2, 4,364 gene sets), Oncogenic Signatures (v6.2, 187 gene sets), and Immunologic Signatures (v6.2, 4,872 gene sets).

Generation of HSF1-regulated E2F target gene panel

Following differential expression analysis of RNA-seq data from PC9-BrM3 Tet-shNTC vs Tet-shHSF1 RNA, a list of E2F-related target gene signatures depleted by HSF1 knockdown were assembled and compared for shared gene targets to create what we termed a “conserved” E2F target gene panel. Depleted E2F-related signatures available through the mSigDB that were included in the analysis were the HALLMARK_E2F_TARGETS, KONG_E2F3_TARGETS, E2F_03, E2F_Q3, E2F1_Q6, and E2F_Q4_01 signatures. We included E2F target genes from each of these signatures if they were common to at least five of the six signatures, which generated a final list of 15 E2F targets. The genes included in this final list were: E2F8, MCM7, DNMT1, RANBP1, DCTPP1, MCM2, PRKDC, MCM4, MCM6, ATAD2, SMC1A, SMC3, NASP, MCM3, and CDC6. A list of all E2F target signatures with corresponding genes is provided as Supplementary File 3.
To generate the HSF1 heat shock-induced gene panel, we analyzed HSF1 ChIP-sequencing data from Mendillo et al, 2012 (5). We created a rank order list of genes sorted by peak height values for HSF1 binding in low-malignant BPE cells, high-malignant BPLER derivative cells, or low-malignant BPE cells exposed to 42C heat shock (BPE+HS) as described in the original study. Gene lists were filtered to include gene targets bound by HSF1 under BPE+HS conditions to generate a list of 1569 heat shock-induced gene targets; genes that also scored in BPE control or BPLER samples in addition to BPE+HS were excluded. This filtered list of heat shock-induced genes was then cross-referenced to the present PC9-BrM3 Tet-shHSF1 knockdown RNA-seq dataset to identify log2foldchange values for each gene in the setting of brain metastasis. As a final filtering step, the top 20 genes from the BPE+HS gene list with reads present in the PC9-BrM3 RNA-sequencing data were included to form a representative HSF1 heat shock target gene panel.

**Real-time quantitative PCR**

RNA was isolated using the RNeasy RNA isolation kit (Qiagen), and cDNA synthesis was performed using oligo(dT) primers and M-MLV reverse transcriptase (Invitrogen). Samples for RT-qPCR analysis were loaded in triplicate wells with iTaq Universal SYBR Green Supermix (Bio-Rad). All primers were purchased from Sigma Aldrich, and analysis of real-time data was collected using a Bio-Rad CFX384 machine and CFX Maestro software. Expression levels of target genes were normalized to 18S control housekeeping gene expression with the ddCt algorithm. A list of all primer sequences is provided in Supplementary Table S1.
**Immunoblotting procedures**

Cells were lysed in RIPA buffer (50 mM Tris-HCl pH 7.4, 150 mM NaCl, 1 mM EDTA, 1% Triton X-100, 1% sodium deoxycholate and 0.1% SDS) containing protease-phosphatase inhibitor cocktail (Cell Signaling) and rotated at 4°C for 15 minutes followed by microcentrifugation to remove cell debris. Protein concentration was quantified using the DC Protein Assay (Bio-Rad). Equal amounts of protein were separated by SDS/PAGE and transferred onto nitrocellulose membranes using the Transblot Turbo Transfer system (Bio-Rad). For most primary antibodies, membranes were incubated overnight at 4°C at the dilution recommended by the manufacturer followed by incubation with corresponding secondary antibody for 1 h at room temperature. For immunoblot staining of phospho-HSF1 (S326), membranes were incubated with primary antibody at 1:10,000 for 1 h at room temperature prior to incubation with corresponding secondary antibody for 1 h at room temperature. Blots were developed using SuperSignal West PLUS Chemiluminescent Substrate developing solution (Invitrogen) and imaged using either film or a ChemiDoc XRS+ imager (Bio-Rad). The following antibodies used for immunoblot analysis were purchased from Cell Signaling: HSP90 (4877S), beta-Actin (8H10D10) (3700S), Phospho-CrkL (Tyr207) (3181L), PARP (9542S), beta-Tubulin (D2N5G) (15115S), Rabbit Anti-Mouse IgG (Light Chain Specific) (D3V2A) (5880S), Goat anti-Rat IgG, HRP-linked (7077S); Millipore Sigma: ABL1 (8E9) (MAB1130), Anti-PolyHistidine (H1029), Phosphotyrosine (4G10) (05-321); Enzo Life Sciences: HSF1 (ADI-SPA-950-F); Abnova: ABL2 (6D5) (H00000027-M03); Abcam: Phospho-HSF1 (Ser326) (Ab76076); Santa Cruz: Cyclin B1 (H-20) (sc-594), E2F1 (KH95) (sc-251), E2F2 (TFE25) (sc-9967), E2F3 (PG30) (sc-56665), E2F4 (RK-13) (sc-511), E2F6 (TFE61) (sc-
53273), E2F8 (B-9) (sc-514064), CDC6 (180.2) (sc-9964), CRKL (C-20) (sc-319), Normal Mouse IgG (sc-2025); Roche Diagnostics: GFP (11814460001); Jackson Immunoresearch: Peroxidase AffiniPure Goat Anti-Mouse IgG (H+L) (115-035-003), Peroxidase AffiniPure Goat Anti-Rabbit IgG (H+L) (115-035-144).

**Co-immunoprecipitation assays**

Cells were lysed with immunoprecipitation buffer (50 µM Tris-HCl pH 8.0, 150 mM NaCl, 1.0% NP-40, 1x protease inhibitor cocktail) and lysates were incubated for 30 min on ice prior to clearing by centrifugation at 15,000 rpm for 10 min. Supernatants were incubated with antibodies overnight followed by incubation with protein A/G PLUS-agarose beads (Santa Cruz) for 3 h. Following incubation, beads were washed three times with immunoprecipitation buffer. Bound proteins were eluted with 4x Laemmli Sample Buffer (Bio-Rad) and visualized by SDS-PAGE on nitrocellulose membranes followed by immunoblot analysis. Total expression for each protein was visualized by running equal concentrations of whole protein lysate input on an SDS-PAGE gel prior to transfer onto nitrocellulose membranes and subsequent immunoblot analysis.

**Chromatin immunoprecipitation and quantitative PCR (ChIP-qPCR)**

ChIP-qPCR analysis was performed as previously described (6). Briefly, cells were cross-linked with formaldehyde (1% final concentration) on ice for 5 min with gentle shaking. Formaldehyde was quenched by adding 125 M glycine in PBS for 5 min on ice with gentle shaking. Cells were then lysed in cell lysis buffer (25 mM Tris, pH 7.5, 150 mM NaCl, 1 mM EDTA, 1% Triton X-100, 0.1% SDS) containing protease and phosphatase inhibitors
and transferred to a 15 mL conical tube for 15 min on ice. 1 mL of IP buffer (50 mM Tris, pH 7.5, 150 mM NaCl, 1 mM EDTA, 1% Triton X-100) was added, and lysates were then sonicated ten times for 30 s each on ice using a microprobe on setting 5. Lysates were cleared by spinning down cellular debris at 13,000 RPM at 4°C for 30 min. Samples containing antibody against HSF1 (Enzo) or IgG control were rotated overnight at 4°C, after which Dynabeads Protein G were added to all samples and rotated at 4°C for 43h. Beads were washed twice with IP buffer, twice with IP buffer + 0.5 M NaCl, and twice with TE Buffer (10 mM Tris, pH 7.5, 1 mM EDTA). Proteins were then eluted by adding TE buffer + 1% SDS and incubating at 65°C for 10 min, and beads were spun down at 14,000 rpm for 10 min. Samples were then incubated overnight at 65°C to reverse cross-links, followed by treatment with proteinase K for 1.5 h at 37°C and purification with a PCR cleanup kit (Qiagen) prior to analysis via qPCR. Primer sequences are listed in Supplementary Table S1.

**Nuclear/Cytosolic Subcellular Fractionation Immunoprecipitation**

Cells plated onto 15 cm dishes were harvested by trypsinization and pelleted by spinning at 1,000 RPM for 4 min. The cell pellet was washed with 5 mL PBS, pelleted by centrifugation, and then resuspended in 1 mL PBS, transferred to a 1.5 mL tube, and re-pelleted. The cell pellet was resuspended in 500 µL cytosolic lysis buffer (20 mM Tris-HCl, pH 7.5, 10 mM NaCl, 3 mM MgCl₂) and incubated for 15 min on ice. 25 µL of 10 % NP-40 was added to the cell homogenate and the sample was vortexed for 10 s followed by centrifugation at 3,000 RPM for 10 min at 4°C. The supernatant was then removed and designated as the cytosolic fraction. The remaining nuclear pellet was resuspended in
nuclear lysis buffer (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 1% NP-40) and incubated on ice for 30 min with vortexing at 10 min intervals. The sample was spun down at 14,000 RPM at 4°C for 30 min. The supernatant was transferred to a new 1.5 mL tube and designated the nuclear fraction. 1000 µg of each fraction were used for subsequent immunoprecipitation following the method described above.

**FACS analysis**

Subconfluent monolayers of PC9-BrM3 cells transduced with inducible shRNAs against non-target control (shNTC) or HSF1 (shHSF1 clone 7481) were treated with 500 ng/mL doxycycline for 48 h prior to harvest by trypsinization, fixation by EtOH, and staining with propidium iodide using the Propidium Iodide Flow Cytometry Kit (Abcam #ab139418) following the manufacturer-supplied protocol. Fixed and stained cells were analyzed by FACS on a BD FACSCanto II analyzer. Post-FACS cell cycle analysis was performed using FlowJo 10 software.

**Kaplan-Meier Analysis and Mutual Exclusivity Analysis**

Lung adenocarcinoma patient microarray data were analyzed using the KMplot analysis tool (kmplot.com) (7). Patient groups were stratified by tertile (n=720 total patients across all cohorts). Affimetrix identifiers for each gene were as follows: 228361_at (E2F1), 219990_at (E2F8), 218782_s_at (ATAD2), (CDC6), 210543_s_at (PRKDC), 202482_x_at (RANBP1), 202107_s_at (MCM2), 201555_at (MCM3), 212142_at (MCM4), 210983_s_at (MCM7). Survival data were plotted in GraphPad Prism 8 software. Mutual exclusivity analysis of HSF1-E2F target genes was performed with CBioPortal using the TCGA
Provisional lung adenocarcinoma dataset (n=515 patients) (8, 9). A user-defined list of the 15 gene HSF1-E2F target panel was used, and patients with mRNA expression data (RNAseq, z-score threshold ± 2.0) were included. Heatmaps of patient cohorts were generated using default clustering settings on CBioPortal, and mutual exclusivity analysis heatmaps were plotted using GraphPad Prism 8 software.

**Immunofluorescence and confocal microscopy**

For immunofluorescence staining and imaging of HSF1 protein, $10^5$ PC9-BrM3 lung cancer cells transduced with pLKO-Tet-ON-shNTC or pLKO-Tet-ON-shHSF1 (clone 7480) constructs were plated in triplicate wells on 18 mm glass coverslips in 12-well dishes for 24 h to allow for cell adhesion. The following day, 500 ng/mL dox was added to each well for an additional 48 h. At endpoint, cells were fixed in 4% ice-cold PFA and blocked in 3% BSA in PBS (w/v) prior to staining with anti-HSF1 antibody (1:100, Enzo) overnight at 4°C. The following day, cells were incubated with goat anti-rat Alexa Fluor 568 secondary antibody (1:100, ThermoFisher) for 1 h and nuclei were stained with Hoechst 33342 diluted 1:10,000 in PBS for 15 minutes. Slides were mounted onto coverslips with Dako Fluorescence mounting medium, cells were imaged using a Leica Inverted SP5 confocal microscope, and analysis was performed using ImageJ software (10).

**DNA plasmids**

Sequences for stable shRNAs targeting the ABL kinases were as follows: scrambled shRNA (GGTGTATGGGCTACTATAGAA); ABL2 shRNA (CCTTATCTCACCACCTCTGAA). Dox-inducible shRNA sequences targeting human
HSF1 (Clone 7480: GCAGGTGTTCATAGTCAGAA, cloneID: TRCN0000007480) (Clone 7481: GCACATTCCATGCCCAAGTAT, cloneID: TRCN0000007481), E2F1 (Clone 658: CAGGATGGATATGAGATGGGA, cloneID: TRCN0000039658) (Clone 659: CGCTATGAGACCTCACTGAAT, cloneID: TRCN0000039659) and E2F8 (Clone 429: GCCCAGAAATCAGTCCAAATA, cloneID: TRCN0000017429) (Clone 800: GATGTTGGCTTAGTTTAATTT, cloneID: TRCN00000425800) were cloned into the all-in-one dox-inducible Tet-pLKO-puro vector kindly provided by Dmitri Wiederschain (Addgene plasmid #21915) (11). Briefly, sense and anti-sense oligos for respective shRNA sequences flanked by 5’ AgeI or 3’ EcoRI restriction site overhangs were mixed in 10X annealing buffer (1M NaCl, 100 mM Tris-HCl, pH=7.4) and annealed by placing in boiling water that was allowed to cool naturally to 30°C on a lab bench. Tet-pLKO-puro vector backbone was digested via restriction enzyme digest using AgeI and EcoRI (NEB), followed by gel-purification using the QIAquick Gel Extraction Kit (Qiagen). Gel-purified vector and annealed oligos were then ligated using T4 DNA ligase (NEB) followed by transformation into One Shot Stbl3 chemically competent cells (ThermoFisher). For all cloning products, plasmid DNA extracted from transformed colonies was validated by sequencing prior to transfection and lentiviral transduction into target cells.

Preparation of GST-tagged Proteins

Plasmids expressing GST, GST-ABL2 SH2, or GST-ABL2 SH3-SH2 were transformed in BL21 DE3 Escherichia coli. Protein expression was induced with 1 mM isopropyl-β-d-thiogalactoside for 6 h at 37°C. Cells were lysed by sonication in the following buffer: 125 mM Tris-HCl, pH 7.4, 150 mM NaCl, 1 mM EDTA, 1 mM DTT, 1 % v/v Triton X-
100, and protease inhibitors. GST-fusion proteins were captured on glutathione-Sepharose beads, and bound proteins were washed twice with wash buffer (125 mM Tris-HCl, pH 7.4, 150 mM NaCl, 1 mM EDTA, 1 mM DTT) and eluted with elution buffer (125 mM Tris-HCl, pH 7.4, 150 mM NaCl, 50 mM reduced glutathione, 1 mM DTT, 1 % v/v Triton X-100). The proteins were buffer exchanged into storage buffer (20 mM Tris-HCl, pH 7.4, 150 mM NaCl, 10 % glycerol and 1 mM DTT), and the purified protein was aliquoted, flash frozen in liquid nitrogen, and stored at −80 °C.

**Preparation of His-tagged Proteins**

Human HSF1 was cloned into the pET-32A vector and transformed in BL21 Gold DE3 Escherichia coli cells. Protein expression was induced with 1 mM isopropyl-β-d-thiogalactoside for 6 h at 37°C. Cells were lysed by sonication in the following buffer: 20 mM Tris-HCl, pH 7.5, 400 mM NaCl, 10 mM imidazole, 1 mM DTT, 0.1 % v/v Triton X-100, and protease inhibitors. His fusion proteins were captured on Ni-NTA beads, and bound proteins were washed twice with wash buffer (20 mM Tris-HCl, pH 7.5, 400 mM NaCl, 40 mM imidazole, 1 mM DTT) and eluted with elution buffer (20 mM Tris-HCl, pH 7.5, 200 mM NaCl, 250 mM imidazole, 0.1 % v/v Triton X-100, and 1 mM DTT). The proteins were buffer exchanged into storage buffer (20 mM Tris-HCl, pH 7.5, 200 mM NaCl, 0.1 % v/v Triton X-100, 10 % glycerol and 1 mM DTT), and the purified protein was aliquoted, flash frozen in liquid nitrogen, and stored at −80 °C.

**In vitro GST-pulldown assay**
GST-ABL2 fragments were added dissolved at a final concentration of 1 µM in binding buffer (25 mM Tris HCl, pH 7.5, 100 mM NaCl, 0.1%, 1 mM DTT, and 5% glycerol). A 30 µL aliquot of glutathione-Sepharose beads were added to each sample and incubated overnight at 4°C. An equal molar concentration of recombinant His-HSF1 was added to each sample and rotated at 4°C for 4 h. Reactions were subsequently washed three times with binding buffer and resuspended in Laemmli sample buffer. Interactions were visualized by immunoblotting as described above. The GST-ABL2-SH3 construct was generated from the GST-ABL2-SH3-SH2 construct through site directed mutagenesis by inserting a stop codon at L168. The primers used to generate this construct were FWD 5’-GGTACCAGGAATTTCTCTCATGCTATTAACTGGAGTGATGT-3’; REV 5’-ACATCACTCCAGTAAATAGCTAGGAGAACATTCTGGTACC-3’.

**Transient transfections and site-directed mutagenesis**

The pcDNA-hHSF1 WT plasmid was a gift from Lea Sistonen (Addgene plasmid #71724; http://n2t.net/addgene:71724; RRID:Addgene_71724) (12) and the empty vector pcDNA3 Flag HA plasmid was a gift from William Sellers (Addgene plasmid #10792; http://n2t.net/addgene:10792; RRID:Addgene_10792). The pN1-ABL2-eGFP plasmid (also known as pN1-ARG-eGFP) was a gift from Anthony Koleske (Yale University, New Haven, CT, USA). Transient transfections were performed using Lipofectamine 2000 (Invitrogen) according to the manufacturer’s instructions. Cells were transfected with 1 µg of DNA per plasmid and were incubated for 24 h at 37°C prior to immunoprecipitation. Mutants for pN1-ABL2-eGFP were generated by site directed
mutagenesis using the Q5 Site-Directed Mutagenesis Kit (NEB) according to the manufacturer’s instructions.

Primers used for ABL2 mutagenesis were as follows: **ABL2 R198K** FWD 5’-GCTGCTCTCAGTTCTGTCTTTTGGTACCAGGAAGCTGCCG-3’; REV 5’- GCGGCAGCTTCTGGTCCAAGAGAGTGGACGACGAC-3’. **ABL2 K317M** FWD 5’-CTTCCTTCAGTGTACACAAACCTCGAAGGCTACTT-3’; REV 5’-AAGTACAGCCTACGTGGCTGTGACTGAGACAGGAAG-3’. **ABL2 P158L** FWD 5’-AGTGATGATGATGCTAGACCCCAACCTGCGC-3’; REV 5’-GGACAGGGTTGGTGGCTAAGCAACTACATCAT.

Primers used to generate HSF1 mutants were as follows: **HSF1 1-129** FWD 5’-CGCTGTCTTGCGGATCTTCTAGTCTTTCTCAGGGTG-3’; REV 5’-CACCCCTGAAAGAGTGAAGACTAGAAGATCCGCCAGGACAGCG-3’. **HSF1 1-167** FWD 5’-GGCCACCTCCGCCACAGCTACTTCTCTCAGGTGGCC-3’; REV 5’-ATGAAGCATGAGAATGAGTAGCTGGCAGGGAGGTGCC-3’. **HSF1 1-177** FWD 5’-TGGGCATGCTTGGTAGGCTGGCC-3’; REV 5’-GGCCAGCTTCTCGGTAGAAGCATGCCCA-3’. **HSF1 1-187** FWD 5’-TCAGGAACTGAAATGAGCTTTCTAGACCGCTTTCTGTTGCTGCTG-3’; REV 5’-CCAGCAACAGAAAGTGTGTACAGTACTACAGGAACTAGG-3’. **HSF1 1-197** FWD 5’-AGGATCTCCTGCTTAGAAGCTCAGTGAAGCTAGAAGCAGGATCTTCTGGCC-3’; REV 5’-CCAGATCTCCTGCTTAGAAGCTCAGTGAAGCTAGAAGCAGGATCTTCTGGCC-3’. **HSF1 1-206** FWD 5’-GGGGATCTTTCTCATACCCCAGGACAGGATCC-3’; REV 5’-
GGATCCTGGGGTGTAGAGAAAGATCCCC-3’. **HSF1 1-287 FWD 5’-**
GGGGGCTGCTGGATAGCTACCTCTCGTCTATGCTC-3’; **REV 5’-**
GAGCATAcAGACGAGGTAGCTATCCAGCAGCAGCC-3’. **HSF1 1-379 FWD 5’-**
CACTGAGCTCATTTCTTCAcAGGCAGGCTACGCT-3’; **REV 5’-**
CAGCGTACcGCCTGAGTACAAGAATGAGCTCAGTG-3’. **HSF1 1-441 FWD 5’-**
GCTCCTGcGGGAGAcAGCtACTCTTGGcATAcTGcC-3’; **REV 5’-**
GCCAGTATCCAAGAGTACGCTGTCTccccAGGAGC-3’. **HSF1 187-195del FWD 5’-**
GGTTTGACTGCACCAGGACGACTTTCTGTGC-3’; **REV 5’-**
GCAcACAGAAAGTCGTCCTGGTGcAGTCAAACC-3’. **HSF1 188-193del FWD 5’-**
TGACTGcAcCGATGAGTTGAcGACTTTCTGTGC-3’; **REV 5’-**
CAcACAGAAAGTCGTCAcACATCTcAcTGcAGTC-3’

**Viral transduction procedures**

Lentiviral shRNAs against ABL2 and corresponding scramble plasmids were packaged in 3rd generation lentiviral packaging vectors (pMDL, pCMV-VSV-G, pRSV-REV) and contained a GFP-tag enabling sorting for stably-expressing clones. For lentiviral expression of shRNAs in the pLKO.1-puro backbone against non-target control (NTC) and **HSF1**, 2nd generation lentiviral packaging vectors (pMD2.G and PSPAX2) were used. Briefly, 293T cells were transfected with packaging DNAs and corresponding DNAs using FuGENE6 reagent (Promega). Culture supernatants containing virus were harvested and filtered 24 hours and 48 hours after transfection and were added to target cell cultures in the presence of 8 µg/ml polybrene (Sigma-Aldrich) for a minimum of 6 h. Stably-
transduced cells expressing shRNAs cloned in the pLKO.1 backbone were selected with puromycin (1 μg/ml) for five days.

**Cell viability assay**

Cells transduced with inducible lentiviral shRNAs as described in figure legends were seeded in 96-well plates in at least triplicate and measured each day using CellTiter-Glo reagent (Promega). Plates were read on a Tecan Infinite M1000 Microplate Reader and results were analyzed in GraphPad Prism 8 software. For inducible constructs, cells were seeded (500 cells/well for PC9, 200 cells/well H2030) at the beginning of the study and 500 ng/mL doxycycline was added every 24 hrs to corresponding wells to examine the impact of knockdown on cell growth.

**Promoter analysis of predicted degenerate HSF1 binding sites at E2F target genes**

To identify predicted degenerate HSEs located near promoter regions of canonical E2F targets identified in the 15-gene HSF1-regulated E2F target gene panel, we utilized Ensembl to download reference sequences for each gene containing a minimum of 5kb base pairs upstream of the predicted transcriptional start site (TSS) for each gene. We used the coding sequence (CDS) regions as deposited on GenBank (NCBI) to determine the TSS for each gene. We validated each gene for the presence of the canonical E2F DNA binding motif sequence, $TTTnnCGC$, where “$n$” represents either a C or a G nucleotide. For the canonical HSE nucleotide sequence, we used the following established sequence containing triple tandem inverted repeats: $nGAAnnTTCnnGAAn$, where “$n$” represents any nucleotide A, T, C, or G. We then created sequence search strings for partial variations of
the canonical HSE, which we termed a “degenerate” HSE, that contained slight variations in nucleotide sequences near the 5’ or 3’ end of the canonical HSE. This approach was based off of the findings published by Li et al, 2016 (13), where a degenerate HSE was identified in *C. elegans*. The following search strings were used for the degenerate HSEs: \textit{GAAnnTTC}, \textit{AAnnTTCnnGA}, and \textit{TTCnnGAA}. Degenerate HSE sequences were then used to search the promoter regions of genes from the E2F target panel.
Supplementary Table S1

List of human primers used for real-time quantitative-PCR (RT-qPCR) and ChIP-qPCR (bottom). All primers were purchased from Sigma.

| GENE     | PRIMER SEQUENCE       |
|----------|-----------------------|
| ATAD2    | AAGGAGTGTAACACCTACCCACCG |
| ATAD2    | GCAAAGTTGCTCCGTTATTTC |
| CDC6     | CCAAGGCACAGCTCAAGTACAG |
| CDC6     | AACAGTTACGTTTGGAGCAATT |
| DCP1P1    | CCCTTGCTGTGCTGAGTTTGC |
| DCP1P1    | CCCTTGCTGTGCTGAGTTTGC |
| DNMT1    | CCTAGCCCCAGGATACCAAG |
| DNMT1    | ACTCATCCGATTTGGCTTTC |
| E2F1     | ACGTGAAGTTCAGGGAGCCT |
| E2F1     | CTGCTGCTTATTTGGCTTTC |
| E2F1     | GCTGCTGCTTATTTGGCTTTC |
| E2F2     | GCCGAAGTTCATACCGAGTCTT |
| E2F2     | AGGAAGCGGTCATACGTTACTT |
| E2F3     | TGCACTCGATGTCAGCTC |
| E2F3     | ATGGGCTAATGAGAAAGTAC |
| E2F4     | TGCTGCTTCATTTGGCTTTC |
| E2F4     | TCATTCGAGCTATCCAGTACG |
| E2F5     | GTCACTCAGGTCAAGGACTTG |
| E2F6     | TCAGAAGATACGTGCTATTC |
| E2F6     | TCCGTTGGTCTCCTATAGTG |
| E2F7     | AAAGGAGCTATTCCGACCAT |
| E2F7     | GCTGATAGCGAGCAGGAGAACT |
| E2F8     | AAGTACGGGTCTCTGAGATT |
| E2F8     | ATGCTGTGGGTCTTACGTTG |
| HSFL     | CCAATGAAGCATGAGAATGAGGC |
| HSFL     | CTTTGTTGACGACTTTCT GC |
| MCM2     | ATGGCGGAATCATCGGAATCC |
| MCM2     | GGAGGTGAGGGCATCAGTACG |
| MCM3     | TCAGAGAGATACGTGCTATTC |
| MCM3     | TCAGCCGTAATTGGTCTAC |
| MCM4     | GCAGTTAGGCGAGGATTC |
| MCM4     | GCTGGGAGTGGCGTATGTC |
| MCM6     | GAGAGAAATCTGGTTCCTCAGA |
| MCM6     | CAAAGGCACGGCAACAGTTAG |
| MCM7     | GCCGTGGGAAGATATCCCTCG |
| MCM7     | GTACACCTGTGGGAACCC |
| MCM8     | AGATGGGGAATCTAGGCTTC |
| MCM8     | ACTTCTCCGAGTTAAAGATGTG |
| MCM9     | CTCGCGCGTCACTCATCTG |
| MCM9     | AGTAAAGTGGCAGATCTTCTTG |
| RANBP1   | AAATACAGAACAGGTCAC |
| RANBP1   | GAAGTCTTGGGGCTTAAAGCCCT |
| SMC1A    | GCAGAAGAGGTACGTTTCAAG |
| SMC1A    | ATGCGGGAAGTCACTGTGGA |
| SMC3     | GCAAGAAGATGAACCTTCAGG |
| SMC3     | GCAGAAGAGGTACGTTTCAAG |
| 18S      | GAGGAGATGGGAGGAAAGOT |
| 18S      | AGAAGTGAGCGAGCCCTCCTA |
| ATAD2    | TTGGACTAGCCATTGGCATTCTC |
| ATAD2    | TTGGACTAGCCATTGGCATTCTC |
| E2F8     | TCTCTTGTCTGATACCTGCT |
| E2F8     | TCTCTTGTCTGATACCTGCT |
| E2F8     | CCCCAATCCCGAGTTGGTCA |
| SMC1A    | TACCTCAGTTCGCGGCGTA |
| SMC1A    | GATGCGGTAACACCTTCGAA |
| HBB      | GCTTCTGACACAACCTGGTGTCATAGC |
| HBB      | CACCAACCTCATTCCCAGTTCACC |
DATASET S1: RNA-seq differential expression dataset induced by loss of HSF1 in brain metastatic PC9-BrM3 lung cancer cells

DATASET S2: RNA-seq differential expression dataset of PC9 parental versus brain metastatic PC9-BrM3 lung adenocarcinoma cells.

DATASET S3: List of E2F target gene signatures and individual vs. shared gene targets used for derivation of HSF1-dependent E2F target gene signature.
References

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Figure S1. Loss of HSF1 preferentially impairs growth of brain-metastatic versus parental lung adenocarcinoma cells in vitro. A) FACS analysis of propidium iodide staining in fixed PC9-BrM3 cells transduced with inducible shNTC or shHSF1 treated with 500 ng/mL doxycycline for 48 h. Pie chart percentages for each cell cycle phase (G1, S, G2/M) are provided as the average of 3 biological replicates per condition, and statistical analysis performed using 2-way ANOVA followed by Tukey’s multiple comparison posthoc testing. **** p-value < 0.0001. B-E) Cell-Titer Glo assay measuring cell viability in B-C) H2030 parental vs H2030-BrM3 or D-E) HCC4006 parental vs HCC4006-BrM cells transduced with shRNAs against non-target control or HSF1 clone 7480 (B, D) or clone 7481 (C, E) and treated with doxycycline for the indicated timepoints. For B-C, n=3 replicates per condition. For D-E, n=6 replicates per condition. Statistical analysis performed by two-way ANOVA followed by Fisher’s multiple comparison posthoc testing. * p-value < 0.05, ** p-value < 0.01, **** p-value < 0.001, ns = not significant.
Figure S2. HSF1-dependent regulation of E2F target and G2M checkpoint gene signatures. A-B) Volcano plots showing relative depletion vs enrichment of target genes within the hallmark E2F Targets (A) or G2M Checkpoint (B) gene sets. C) Venn diagram depicting overlapping depleted transcripts between E2F Target and G2M Checkpoint gene sets in HSF1 knockdown cells. D-E) RT-PCR analysis of E2F target gene expression in PC9-BrM3 cells transduced with inducible shRNAs against non-target control (shNTC), and two independent shRNAs against either E2F1 (shE2F1) (D) or E2F8 (shE2F8) (E). Cells were treated with 500 ng/mL doxycycline for 72 h. n = 3 biological replicates. Statistical analysis performed by ANOVA followed by Tukey’s multiple comparison posthoc testing. * p-value < 0.05, ** p-value < 0.01, *** p-value < 0.001, **** p-value < 0.0001, ns = not significant.
Figure S3. E2F target and G2M checkpoint gene signatures are enriched in brain-metastatic cells relative to parental cells. A-B) GSEA plots showing E2F Target (A) and G2M Checkpoint (B) enrichment signatures in PC9-BrM3 compared to PC9 parental cells. C) Venn diagram depicting overlapping enriched genes between E2F Target and G2M Checkpoint gene sets in PC9-BrM3 cells. D) RT-qPCR analysis of mRNA expression of E2F family genes in PC9 parental vs PC9-BrM3 cells. n=3 biological replicates. Statistical analysis of RT-qPCR data performed using two-way ANOVA followed by Fisher’s multiple comparison post-hoc testing. **** p value < 0.0001, ns = not significant. E-F) Kaplan-Meier survival analysis correlating overall survival with mRNA expression for E2F1 or E2F8 in human lung adenocarcinoma patients. Survival groups were separated into tertiles based on mRNA expression (n = 720 total patients), and statistical analysis was performed using Log-Rank Mantel Cox test.
Figure S4. Analysis of E2F gene target promoters reveals presence of degenerate heat shock elements. A) Sequence motifs used for promoter analysis of genes containing E2F binding sites, degenerate HSEs and canonical HSEs. B) Promoter analysis of the E2F target gene ATAD2 with E2F (blue) and predicted degenerate HSE (purple) binding motifs highlighted. TSS = transcriptional start site. C-D) Promoter analysis of the E2F target genes MCM4 and E2F8 with E2F (blue) and predicted degenerate HSE (purple) binding motifs highlighted. E-F) Promoter analysis of the heat shock responsive target genes HSP90AB1 (E) or HSPA6 (F) with canonical HSE sequences indicated in yellow. For HSP90AB1 and HSPA6 promoters, no E2F binding motifs were identified.
Figure S5. Subcellular localization of ABL2 and HSF1 protein-protein interactions. A) Co-immunoprecipitation pulldown assay of Flag-HSF1 and immunoblots of indicated proteins in nuclear vs cytoplasmic subcellular fractions harvested from 293T cells. B) GST in vitro pulldown assay of purified HSF1 (His) incubated with purified ABL2 SH2 or ABL2 SH3-SH2 protein domains. C) Diagram depicting HSF1 truncation mutants used in co-immunoprecipitation pulldown assays. DBD = DNA binding domain, LZ = leucine zipper domains, RD = regulatory domain, AD = activation domain. D) Co-immunoprecipitation pulldown assay of HSF1 truncation mutants and immunoblots for corresponding proteins in 293T cells co-transfected with HSF1 truncation mutants and ABL2-GFP. E) Co-immunoprecipitation pulldown assay of HSF1 truncation mutants and immunoblots for corresponding proteins in 293T cells co-transfected with HSF1 1-166 through 1-206 truncation mutants and ABL2-GFP. WCL = whole cell lysate.
Figure S6. ABL allosteric inhibition regulates HSF1 expression in a transcription- and proteasomal-independent manner. A) RT-qPCR analysis of HSF1 mRNA expression in PC9-BrM3 cells treated with 10 µM ABL001 for the indicated timepoints. HSF1 mRNA expression was normalized to mRNA expression of housekeeping gene 18S. n=3 biological replicates. Statistical analysis performed by two-way ANOVA followed by Tukey’s multiple comparison post-hoc testing. ** p-value < 0.01, *** p-value < 0.005, **** p-value < 0.001, ns = not significant. B) Immunobotts of PC9-BrM3 cells treated with either DMSO or the ABL allosteric inhibitor GNF5 (24h) in addition to DMSO or MG132 at the indicated concentrations.