Materials and Methods

DNA templates. The *HSPA1B* promoter and early transcript including -467 to +216 was amplified from HeLa nuclear extract using a pair of primers (Table S1). The amplified product was cloned into a pCR-Blunt-TOPO plasmid. The biotinylated template was generated by PCR using the cloned vector as a template and a set of primers, one conjugated with biotin at the 5' end. The PCR product was gel-extracted, purified (Qiagen), and sequence-verified before further experiments. After constructing WT *HSPA1B* cloning vector, Quik-change mutagenesis (Stratgaene) with a pair of primers introducing mutations in TATA box (TATAAA → CCCGGG) was performed and sequence-verified. The single stranded non-template oligos with a biotin conjugate at the 5' end were purchased from Integrated DNA Technology (IDT). The sequences of primers and oligos are listed in Table S1.

Proteins, expression vectors, and purification. A full-length HSF1 was amplified from a HSF1 expression vector provided by Dr. Wu at National Cancer Institute and cloned into pET17b including a His^6 tag. Bacterial expression vectors of GST-tagged full-length (1-835) and truncate (1-617) TRIM28 were provided by Dr. Zhu at Florida State University. His^6-HSF1, His^6-Myc, His^6-Max, and GST-TRIM28 expression was induced in BL21(DE3)pLysS *E. coli* cells using 1 mM IPTG final concentration for 3 hours at 37 degrees Celsius. The cells were harvested and lysed with the xTractor bacterial cell lysis buffer (Clontech) or sonication. Untagged HSF1 was purified according to Soncin *et al*. His^6-HSF1 was purified using Ni beads (Invitrogen). The Ni binding buffer included 10 mM Imidazole, 0.5 M NaCl, 10 mM Tris pH 7.6, 2 mM $MgCl_2$, and 10% glycerol. The beads were washed with 80 mM Imidazole added to the binding buffer. Beads-bound proteins were eluted with 500 mM Imidazole buffer. GST-TRIM28 variants were purified with glutathione beads. The beads were washed with High Salt (1M NaCl, 0.5% CHAPS, 50 mM Tris-HCl pH 7.9, 0.5 mM EDTA, 10% glycerol, 0.5% NP-40) and H/E buffer (50 mM Tris-HCl pH 7.9, 500 mM NaCl, 0.5 mM EDTA, 10% glycerol, 0.5% NP-40) followed by 0.15 M HEGN buffer (50 mM HEPES, pH 7.6, 0.15 M KCl, 0.1 mM EDTA, 10% glycerol, 0.02% NP40). GST-TRIM28 was eluted through 30 mM reduced Glutathione in GSH buffer (80 mM Tris-HCl pH 7.9, 0.1 mM EDTA, 10% glycerol, 0.02% NP-40, 150 mM KCl). TRIM28 WT and the mutants S824A and S824D, were cloned into a pMal-c2 (NEB) bacterial expression vector modified to include a N-terminal His^6 tag and a tobacco etch virus (Tev) protease cleavage site between the MBP and TRIM28. The induced proteins were purified using Ni beads (Invitrogen) as described above. The beads were washed with 50 mM Imidazole, 0.5 M NaCl, 10 mM Tris pH 7.6, 2 mM $MgCl_2$, and 10% glycerol. Then, the beads were equilibriated with Buffer A* (10 mM Tris, 150 mM NaCl, 2 mM MgCl2, 10% Glycerol) before incubating with His^6-tagged Tev protease. The supernatant including untagged TRIM28 protein was collected and verified on the gel. All buffers include freshly added protease inhibitors (1 mM Benzamidine, 1 mM DTT (DTT was excluded in Ni beads purification), 0.25 mM PMSF, aprotinin, sigma A6279, in 1:1000, and 1 mM Na metabisulfite).

Cell culture and transfection. HeLa cells grown in suspension or HeLa nuclei purchased from Texcell-North America were used to generate nuclear extracts according to Supplementary data. HEK293 cells were grown in DMEM supplemented with 10% FBS and 1% Penicillin/Streptomycin solution. Scrambled and TRIM28 shRNAs cloned in pGFP-V-RS were

purchased from OriGene (Cat. # TG320633). Scrambled and CDK9 shRNAs cloned in pRS plasmid were purchased from OriGene (Cat. # TR320298A). Transfection of shRNAs into HEK293 cells was performed using the FuGene 6 (Promega) as recommended by the shRNA manufacturer (OriGene). For ChIP-qPCR assays, HEK293 cells grown in a dish were heat-shocked in 43 degree water bath for assigned time durations and cross-linked in 0.75% formaldehyde followed by quenching cross-linking with glycine in a final concentration of 125 mM to media. To test kinase inhibitors for TRIM28 S824 phosphorylation, the cell culture was exchanged with a fresh warm medium including a desired inhibitor (or DMSO for control) an hour prior to heat-shock. As a general rule, media (with or without an inhibitor) was preheated to 43 degree to be supplied to cells starting heat-shock induction. Cells were rinsed with cold PBS twice before scraping them for next steps or snap freezing at -80. Inhibitors used in this study are: NU7441 from Tocris Bioscience (Cat. No 3712), KU55933 from Abcam (ab120637), and KRIBB11 from EMD Chemicals, Inc (385570). Stock solutions (1000 X) were made dissolving the inhibitors as 2 mM, 10 mM, and 10 mM in DMSO to target the final concentrations of 2 μM, 10 μM and 10 μM for NU7441, KU55933, and KRIBB11.

Western blot and immunoprecipitation. Primary antibodies (cat#; experimental dilution used) obtained from Abcam and used in the Western blots are as followed: HSF1 (ab82586; 1:1000), TRIM28 (ab10484; 1:500 or 1:1000), ACACA1 (ab78274; 1:300), PCMT1 (ab55510; 1:500), TBP (ab818; 1:2000), HSPA1B (ab55288; 1:500), and HLTF (ab17984; 1:2000). Antibodies for PC4 (A301-161A; 1:5000) and DHX36 (A300-525A; 1:3000), and Med23 (A300-425A; 1:1,000) were from Bethyl. Antibodies for Pol II (8WG16, sc-899; 1:100, 1:200), TFIIEα (C-17; 1:500), Cyclin T1 (C-20; 1:500), CDK9 (sc-484; 1:500), HSP70 (4E7; 1:1000), β-Tubulin (H-235; 1:200), and TFIID (N-12; 1:1000) were from Santa Cruz. Antibodies for NFκb (#4764; 1:1000) and ERK1/2 (#4695; 1:500) were purchased from Cell Signaling. The antibodies listed above have been validated for the relevant species and applications, and the validation is provided on the manufacturers' websites. PURb antibody (1:2900) was provided by Dr. Kelm Jr at the University of Vermont College of Medicine. For *in vivo* KD assays, HEK293 lysates from scrambled and TRIM28 KD cells were obtained using RIPA buffer and the protein concentration was quantified through Bradford assay before Immunoblotting. TRIM28 was depleted from HeLa NE through Immunoprecipitation. TRIM28 antibody (Bethyl, A300-274A, 3 μg/1 mg lysates) was bound to protein A beads and incubated with HeLa NE for 3, 15, and 2.5 hours consecutively for depletion. The resultant TRIM28 depleted NE was used for transcription assays described below. Immunoprecipitated proteins from the first round of immunoprecipitation were eluted with 0.1 M Glycine, pH 2.75 after washes with 0.5 M HEGN and a final wash with 0.15 M HEGN, and subjected to the SDS-PAGE followed by in-gel digestion of protein bands and MS in Proteomics Core Facility at Beth Israel Deaconess Medical Center (BIDMC). To identify proteins interacting with TRIM28 with low affinity, TRIM28 antibody (Bethyl, A300-274A, 3 μg/1 mg lysates) was bound to protein A beads and incubated with HeLa NE for 3 hours at 4 degree. The validation of these antibodies has been provided on the manufacturer's website. The proteins-beads complex was washed with a 20 beads volume of 0.25 M KCl-HEGN (see above) per each for 4 times and then TRIM28 bound proteins were eluted with 0.5 M KCl-HEGN (see above). The elute was TCA-precipitated to be loaded in a lane for SDS-PAGE. The entire lane was subjected to in-gel digestion for MS in Proteomics Core Facility at BIDMC.

Quantitative PCR. RNA molecules longer than 18 nt were extracted using miRNeasy mini kit (Qiagen) as instructed by the manufacturer. From independent KD experiments, 700 ng or 1.5 μg of RNA was converted to cDNA by reverse transcription using High Capacity cDNA Reverse Transcription kit (Applied Biosystems). qPCR was carried out with equal amount of resultant cDNAs and indicated primers through Platinum Tag DNA Polymerase High Fidelity (Invitrogen) under the thermal cycling as 2 minutes at 94 degree followed by 25 cycles of 20 seconds at 94 degree, 30 seconds at 55 degree, and 1 min at 68 degree.

Mouse ESC Culture, Transfection and Chip-seq sample preparation. Mouse ESCs, E14Tg2a, were obtained from Mutant Mouse Research Resource Centers and American Type Culture Collection. They were cultured on gelatin-coated plates in M15 medium: DMEM (Invitrogen) supplemented with 10% FBS, 10 μM 2-mercaptoethanol, 0.1 mM nonessential amino acids (Invitrogen), 1× EmbryoMax nucleosides (Millipore), 1,000 U of ESGRO (Millipore).
To determine the knockdown efficiency of siRNAs, 50×10^3 ESCs were transfected with siRNAs at 50 nM in one well of a 24-well plate. Cells were harvested 48 hours after transfection for qRT-PCR, and cells were harvested 24, 36, 48 and 60 hours for Western blot. For ChIP-seq or ChIP-qPCR sample preparation, ESCs (2.5×10^6) were transfected with control or Trim28 siRNAs (SiRNA D-040800-02, si2; D-040800-03, si3, Thermo scientific) at 50 nM using lipofectamine 2000 (Invitrogen) in one 10 cm plate for each replicate. After 48 hours transfection, ESCs were harvested and processed according to the protocol in Nat Protoc.2006:1(2): 729-728 for ChIP-seq analysis. For ChIP-qPCR anaylsis, harvested mES cells were processed as described below.

Chromatin Immunoprecipitation & Sequencing. ChIP experiment was conducted using the IP-star (Diagenode, Denville, NJ) and according to the manufacturer's protocol. Briefly, 5 million cells were immunoprecipitated using an antibody, sc-899 (Santa Cruz Biotechnology, 3 μg/each IP) or ab5095 (Abcam, 3 μg/each IP) against whole Pol II or S2 Pol II respectively. Illumina libraries were constructed using the SPRI-TE Nucleic Acid Extractor (Beckman Coulter, Indianapolis, IN) with a size selection of 200-400bp. The samples were sequenced on a HiSeq sequencing platform and resulting short reads were aligned against the mm9 reference mouse genome. The antibodies listed above have been validated for the relevant species and applications, and the validation is provided on the manufacturers' websites.

Chromatin Immunoprecipitation & qPCR. ChIP experiment was conducted following Abcam X-ChIP protocol with mild modifications. Cell lysis buffer includes 5 mM PIPES (pH 8.0), 85 mM KCl, 0.5% NP-40 and fresh protease inhibitors described above. Nuclei lysis buffer including 50 mM Tris-Cl (pH 8.0), 10 mM EDTA, 1% SDS was added before sonication. Sonication was performed at 12% amplitude for 30 sec with 2 mins intervals on ice, Sonic Dismembrator Model 500, Fisher Scientific and was optimized to produce DNA segments ranging between +100 and +1,000 bp on a DNA gel. Antibodies used in IP were TRIM28 antibody from Bethyl A300-274A (3 μg/each IP), Phospho-TRIM28 (S824) antibody from Bethyl A300-767A (5 μg/each IP), total Pol II antibody from Santa Cruz Biotechnology sc-899 (5 μg/each IP), and HSF1 antibody from Cell Signaling #4356 (5 μl/each IP). After IP and reverse cross-linking, DNA was purified through Qiagen PCR purification kit. Input DNAs were quantified for qPCR analysis. qPCR was performed as described above except for 30 times of

cycles and 30 seconds for extension at 68 degree. The antibodies listed above have been validated for the relevant species and applications, and the validation is provided on the manufacturers' websites.

Table S1. The sequences of oligos and primers used in this study

For Non-Template DNA Pull-Down Assay	
GREB1, +1 to +50	Biotin-AAATAAAGTTTTTTCAATGGAAGGCTTGC AGCTCTTGAGGACCTGCCAAA
HSPA1B, +1 to +50	Biotin-GGAAAACGGCCAGCCTGAGGAGCTGCT GCGAGGGTCCGCTTCGTCTTTCG
HSPA1B, +1 to +80	Biotin-GGAAAACGGCCAGCCTGAGGAGCTGCT GCGAGGGTCCGCTTCGTCTTTCGAGAGTGACTCCCGC GGTCCCAA GGCTTTCC
For Protein Expression	
HSF1 forward	CGC GCGCATATGGATCTGCCCGTGGGCCCCGGCG CGGCGGGGCCCAGCAACGTCCC
HSF1 reverse	GCGCTCGAGTTAATGATGATGATGATGGTGGGAGAC AGTGGGGTCCTTGGCTTTGGGAGGCTCCGAGCC
For HSPA1B template DNA construction	
***HSPA1B* cloning forward**	CTCCTTCCCATTAAGACGGAAAAA ACATCCGGGAG AGCCGGTCCG
***HSPA1B* cloning forward**	ACCTTGCCGTGTTGGAACACCCCCACGCAGGAGTA GGTGGTGCCCAGGTC
***HSPA1B* forward from -467**	Biotin-GAAAGGACCCAAGGCTGCTCCGTCCTTCAC
***HSPA1B* reverse from +216**	GCCGGTGCCCTGCTCTGTGGGCTCCGC
***TATA* mutant forward**	GGCGGGTCTCCACGACGACTCCCGGGAGCCGAG GGGCGCGCGGTCC
***TATA* mutant reverse**	GGACCGCGCGCCCCTCGGCTCCCGGGAGTCGTC GTGGAGACCGCGC
For Quantitative PCR	
HSPA1B forward from +1	GGAAAACGGCCAGCCTGAGGAGCTG
HSPA1B reverse from +65	CGCGGGAGTCACTCTCGAAAGACG
HSPA1B forward from +125	GGCGTTCCGAAGGACTGAGCTCTTGTCGCGG
HSPA1B reverse from +313	GGTCGTTGGCGATGATCTCCACCTTGCC
HSPA1B forward from +852	CGACGACGGCATCTTCGAGGTGAAGGCC
HSPA1B reverse from +1180	GCACCCTGGAGCCCGTGGAGAAGGCTCTGC
HSPA1B forward from +1861	GGTGTCAGCCAAGAACGCCCTGGAGTCC
HSPA1B reverse from +2130	GGAGGGTCTGGGTCAGGCCCTACCATTGAG
GAPDH forward	AGAAGGCTGGGGCTCATTTG
GAPDH reverse	AGGGGCCATCCACAGTCTTC
NFκB forward primer #1 (5' end)	CCGGATTCCGGGCAGTGACGCGACG
NFκB reverse primer #1 (5' end)	GGGGTGGGTCTTGGTGGTATCTGTGCTCC
NFκB forward primer #2 (3' end)	CCCCAAGCCAGCACCCCAGCCCTATCCC
NFκB reverse primer #2 (3' end)	GGCCCCCAGGTCTTCATCATCAAACTGC
ERK1 forward primer #1 (5' end)	GGCAGGCGGGAAGGGGCGGGGCCTCGGG
ERK1 reverse primer #1 (5' end)	CCCGGAGCGTGCGCTGGCAGTAGG
ERK1 forward primer #2 (3' end)	CCAAAGCCCTTGACCTGCTGGACCGG
ERK1 reverse primer #2 (3' end)	GGCACAGTGTCCATTTTCTAACAGTCTGGCGGG
IKBα forward primer #1 (5' end)	CCGCCAGCGCCCCAGCGAGGAAGCAGCG
IKBα reverse primer #1 (5' end)	GGATGATGGCCAAGTGCAGGAACGAGTCCC
IKBα forward primer #2 (3' end)	CCCGGATACAGCAGCAGCTGGGCCAGC
IKBα reverse primer #2 (3' end)	CGTTTTGGGCCAGGCAGTGTGCAGTGTGG
HSPA1B forward from -167	GCGGCACCCTGCCCTCTGATTGGTCCAAGGAAGGC

HSPA1B reverse from +10	GCCGTTTTCCGGACCGCGCGCCCCTCGGC
HSPA1B forward from -19	GCCGAGGGGCGCGCGGTCCGGAAAACG
HSPA1B reverse from +103	CGGAACCGGGGAAACTCAACACGCCGGTGCC
HSPA1B forward from +163	CCGCCGTTTCCAGCCCCCAGTCTCAGAGCGG
HSPA1B reverse from +313	GGTCGTTGGCGATGATCTCCACCTTGCCGTGTTGGAA CACCCC
HSPA1B reverse from +2010	CTCCTTCCTCTTGTGCTCAAACTCGTCCTTCTC
Mouse HSPA1B TSS-p1 forward	CCA GAC GCT GAC AGC TAC TCA GAA TCA AAT CTG G
Mouse HSPA1B TSS-p1 reverse	CCG CGG CAG GGA TGC TCC TGG GAA GG
Mouse HSPA1B TSS-p2 forward	GCC CAA GGT GCA GGT GAA CTA CAA GGG CG
Mouse HSPA1B TSS-p2 reverse	CCT GCC GCT GAG AGT CGT TGA AGT AGG CGG
Mouse HSPA1B Gene body forward	GCG CCG TGG AGG ACG AGG GTC TCA AGG GC
Mouse HSPA1B Gene body reverse	GCA CAC CCG CTC CAG CTC CTC CCG C
Mouse JUN TSS-p1 forward	GGC CAA CCC GGT CGG CCG CGG ACT CCG G
Mouse JUN TSS-p1 reverse	GCG CCC GGA CTT GTG AGC TTC TTC TCC G
Mouse JUN TSS-p2 forward	CCG AGA GCG GTG CCT ACG GCT ACA GTA ACC C
Mouse JUN TSS-p2 reverse	GCT CCG GCG ACG CCA GCT TGA GCA GCC CG
Mouse JUN Gene body forward	GCG GCC TAT TGG CCG GCA GAC TTT GCG GAC GGG C
Mouse JUN Gene body reverse	GCA ATC TAC AGT CTC TAT TGC AGT TTG TAA CCC CTC CC
Mouse ERK1 TSS-p1 forward	GGG AGG CGG GAG GAG TGG AGA TGG CGG CGG
Mouse ERK1 TSS-p1 reverse	CGC CGA TGT ACT GCA GCT GCG TGT AGC GTG G
Mouse ERK1 TSS-p2 forward	GCC CCT GGC AAG GCT AGG GGC CCA AAG TAG GG
Mouse ERK1 TSS-p2 reverse	GGG GCA GGC AGG TCT CCC TGT GCG G
Mouse ERK1 Gene body forward	GGA GTT GAT CTT CCA GGA GAC AGC CCG CTT CCA GCC
Mouse ERK1 Gene body reverse	GCA GAG ATG ATG CAG CAG CAC CCA GAC TGG ACA GCC
Mouse EGR1 TSS-p1 forward	CCC AGC GCG CAG AAC TTG GGG AGC CG
Mouse EGR1 TSS-p1 reverse	GCG GGG TGC AGG GGC ACA CTG CGG GG
Mouse EGR1 TSS-p2 forward	GGG GAG CCG AGC GAA CAA CCC TAT GAG C
Mouse EGR1 TSS-p2 reverse	GCT GTG TGA AAT AGA GGG AAA CCC CAG TCC CAT CCC
Mouse EGR1 Gene body forward	GCC TGA AAC AGC CAT GTC CAA GTT CTT CAC CTC TAT CC
Mouse EGR1 Gene body reverse	GGG CAG GGT TCT GAG GGC CCA AAC CC

Additional Supplementary Materials:

1. ChIP-seq material preparation- mES TRIM28 KD and ChIP material preparation
2. TRIM28 interacting proteins- A list of proteins co-immunodepleted along with TRIM28 from HeLa NE
3. TRIM28 interacting proteins #2- A list of proteins co-immunodepleted along with TRIM28 from HeLa NE
4. TRIM28 interacting proteins with low affinity- A list of proteins immuno-precipitated with TRIM28 through low-salt washes and mild elution
5. TRIM28 supplementary data set 1- Uncropped gels and images to supplement main figures