Supplemental Materials and methods

Gobal run-on sequencing (GRO-seq)

The GRO-seq libraries were prepared as in Core et al. 2008 (2), except the sequencing adapter was added using the polyadenylation and reverse transcription method developed in the Weismann lab (3).

Ten million nuclei isolated from 2 biological replicates of LacZ-RNAi and Fcp1-RNAi cells were allowed to run-on for 10 minutes at 30°C in a reaction containing ATP, CTP, GTP, and BrUTP. The RNA was isolated using Trizol and acid phenol extractions. The RNAs were base hydrolyzed to an average length of 100-150 nucleotides, and RNAs with incorporated BrU were purified with 2 immunoprecipitations successive using Anti-BrdUTP agarose. The RNA was treated with PNK and polyadenylated using E. coli polyA polymerase to add 50-100 As to their 3' ends. The polyadenylated RNA was reverse transcribed used with the following bar coded oligonucleotides (one for each replicate):

INOO3:

5'-pTAGAGATCGTCGGACTGTAGAACTCT-iSp18-CAAGCAGAAGACGGCATACGATTTTTTTTTTTTTTTTTTTVN

INOO4:

5'-pTGATGATCGTCGGACTGTAGAACTCT-iSp18-CAAGCAGAAGACGGCATACGATTTTTTTTTTTTTTTTTTTVN

The underlined sequence indicates the bar-code and iSp18 indicates an 18 carbon spacer between two oligo linkers used for Illumina sequencing.

The resulting DNA was gel extracted and circularized using Epicentre CircLigase (catalog # CL4111K), and PCR amplified for 12-15 cycles. The two bar-coded libraries for each treatment were combined and sequenced using Illumina sequencer.

After linker removal, the reads were trimmed to 26 nucleotides, and the reads were mapped to non-repetitive regions of the *Drosophila* genome using Bowtie. The reads were assigned to either the promoter (100bp region with the most reads within 250bp of the TSS) or the gene body (500bp downstream of the TSS to the 3' end of the gene). The gene regions with statistically significant differences in read counts between the LacZ-RNAi and Fcp1-RNAi libraries were determined using edgeR (4).

Laser Scanning Confocal Microscopy

The *Drosophila* salivary glands in Figure S1 were dissected from wandering 3rd instar larvae as previously described (6), immediately transferred to a MatTek glass bottom dish, and a coverslip was placed over the sample. The samples were heat shocked using a preheated C-Apochromat 63x, 1.2 NA, water immersion objective as previously described (7). At 10 minute HS, 2-channel color (RFP and GFP) images were captured using a Carl Ziess 710 laser scanning microscope.

Fly lines used in this study:

Line	Source
6983, salivary gland specific GAL4 driver	(Bloomington stock center)
mRFP-Pol II, 6983	(7)
7017, Gal80-ts line	(Bloomington stock center)
WizFcp1 RNAi line	(5)
GFP-Fcp1	(see below)

Generation of GFP-Fcp1 constructs

The full length Fcp1 cDNA from the pDONR221 vector (see materials and methods in the main text) was transferred to the pTWG vector (Murphy Lab) vector. The GFP-Fcp1 fusion construct was introduced into the *Drosophila* germ line by P-element mediated transformation as previously described (1).

Primer sets used in this study

Fcp1 Cloning (Gateway adapter sequence is underlined)

Forward

GGGGACAAGTTTGTACAAAAAAGCAGGCTTGATGCAGAACATACCGGACGAGG

Reverse

GGGGACCACTTTGTACAAGAAAGCTGGGTACATGTCAAAGTCCTCCAGGCCGAG

Quick change forward (for catalytically dead mutant)

GCGCGCCAGGGGGCGTGGCCGCATCTGG

Quick change reverse (for catalytically dead mutant)

CCAGATGCGGCCACGCCCCCTGGCGCGC

Templates for dsRNAs (T7 promoter sequence is underlined in each primer)

LacZ forward

GAATTAATACGACTCACTATAGGGAGAGATATCCTGCTGATGAAGC

LacZ reverse

GAATTAATACGACTCACTATAGGGAGAGCAGGAGCTCGTTATCGC

Fcp1 (A) forward

GAATTAATACGACTCACTATAGGGATAAGACGGAATCCAGCAGCGAAGT

Fcp1 (A) reverse

GAATTAATACGACTCACTATAGGGATAACCTTGTAGGTGCCTGCGTTGA

Fcp1 (B) forward

GAATTAATACGACTCACTATAGGGACACCAATGGACAAGAAGCTG

Fcp1 (B) reverse

GAATTAATACGACTCACTATAGGGAGCCGAGGAACTCCCTTTCTA

Fcp1 (C) forward

GAATTAATACGACTCACTATAGGGAGTCCAACAGCGAGAAGGAA

Fcp1 (C) reverse

GAATTAATACGACTCACTATAGGGAGTCCGCTGAAACGGAATTTA

CyclinT1 forward

GAATTAATACGACTCACTATAGGGAATGAGTCTCCTAGCCACGCCAA

CyclinT1 reverse

GAATTAATACGACTCACTATAGGGACTTTGACCAGCTGGCAGGTGCG

qPCR primers

Hsp70-154 primer set

Hsp70–200F TGGCAGAAAGAAAACTCGAGAAA

Hsp70–108R GACAGAGTGAGAGAGCAATAGTACAGAGA

Hsp70+96 primer set

Hsp70+56F: ACAAGCGCAGCTGAACAAGCTA

Hsp70+137R: ACTTGGTTGTTGGTTACTTT

Hsp70+379 primer set

Hsp70+334F CACCACGCCGTCCTACGT

Hsp70+423R GGTTCATGGCCACCTGGTT

Hsp70+682 primer set
Hsp70+645F ATATCTGGGCGAGAGCATCACA
Hsp70+718R GTAGCCTGGCGCTGGGAGTC
Hsp70+946 primer set
Hsp70+872F CATCGACGAGGGATCTCTGTTC
Hsp70+1019R GGCGCGAGGGTTGGA
Hsp70+1427 primer set
Hsp70+1363F CTGTGCAGGCCGCTATCC
Hsp70+1490R GCGCTCGATCAGCTTGGT
Hsp70+1702 primer set
Hsp70+1649F GGGTGTGCCCCAGATAGAAG
Hsp70+1754R TGTCGTTCTTGATCGTGATGTTC
Hsp70+1952 primer set
Hsp70Ab+1925F TGGACGAGGCTGACAAGAACT
Hsp70Ab+1978R ACCGGATAGTGTCGTTGCACTT
Hsp70Ab+2211 primer set
Hsp70Ab+2155F GGTCGACTAAGGCCAAAGAGTCTA
Hsp70Ab+2266R TCGATCGAAACATTCTTATCAGTCTCA
Hsp70Ab+2669 primer set
Hsp70Ab+2631F TCGCAGACACCGCATTTGT
Hsp70Ab+2706R ACCAATTGCAACAGAGACTGGAA
Hsp70Ab+4080 primer set
Hsp70Ab+4035F TGGAAACTGCCTCCAACAACTG
Hsp70Ab+4124R AGACGCACGAGACCAATCTGTA
Background
Forward GCAGGGATTTCTCAGCCATA
Reverse CCGGGGAGAAGTAAAGGACT
Hsp26+7 primer set
Hsp26-22F CGAACAGAGCACAGATCGAATTC
Hsp26+36R GAGTTGTTCACTGCTCGA
Hsp26+229 primer set
Hsp26+200F TCGCTTGTGGATGAACTCCA
Hsp26+259R CAATCCCAGTCCAAGCTCGT
Hsp26+624 primer set
Hsp26+580F CAAGGTTCCCGATGGCTACA
Hsp26+667R CTGCGGCTTGGGAATACTGA
Hsp83-45 primer set
Hsp83-100F AAGTTGCATCCCTGGCATCCAGAA
Hsp83+10R TTTCAAGACTCGAACCGGCAAACG
Hsp83+288 primer set
Hsp83+224F AAAGAGTGAATAGTTTATCAGTGGCTATGG
Hsp83+352R GGGTCAAGAACATTCGAGATGCACAG
Hsp83+1268 primer set
Hsp83+1218F GCTTCCGATGCCCTAGACAA
Hsp83+1318R TCCTTGCCAGAGTCCAGCTT

Hsp83+3680 primer set
Hsp83+3628F GCGACCAGTCGAAACAAACAACCA
Hsp83+3732R AACTCGGCCGTAGTAAACTCAG
RpL32+60 primer set
RpL32+10F TCTGGTTTCCGGCAAGGTATGT
RpL32+110R GCAGTTCAACTCGAAACCGCCAAA
RpL32+291 primer set
RpL32+241F ATACTGCCCAAGAAGCTAGCCCAA
RpL32+341R GCACTGACCCACTGGAAATATCAC
RpL32+563 primer set
Rp49+549F CCCAAGGGTATCGACAACAGA
Rp49+613R CGATGTTGGGCATCAGATACTG
B1-tub+44 primer set
B1-tub+5F GCTCTCCAAAGCGAATGCACTA
B1-tub+82R CGCTTATAGCAGTCGAACACAACA
B1-tub+754 primer set
B1-tub+714F TGCCCAGATGGTGAAGGGTACTTT
B1-tub+793R ATGAGCAGCAAGCTCTTATGTGCG
B1-tub+1460 primer set
B1-tub+1423F AACAACCCTCGGAAACGCCTT
B1-tub+1496R TGAGAGCCATACTTGGGCACAACA
B1-tub+2686 primer set
B1-tub+2648F TGTATGCAGCAGATGGTCTAGGCT
B1-tub+2723R GACTTGGCCAATGAGTCATCACAG
Thor+75 primer set
Thor+15F AACAGCCAACGGTGAACACATAGC
Thor+135R TAAGCACACTCGATATGGCTGCGA
Thor+742 primer set
Thor+697F ATGCAACGCAGACCACCTTGACTA
Thor+787R TCTTCATGAAAGCCCGCTCGTAGA
pnr+123 primer set
pnr+76F CATTCCAATCAGAACGCGCACACA
pnr+170R TCGGAATCCAACTGAAGGCAAGGA
pnr+4254 primer set
pnr+4214F TGGCGATATTGCTATCAAGGCGGA
pnr+4294R ATGTCCGGAGATTATGGCTCCCTT
pnr+16473 primer set
pnr+16432F AGTGCAAGGAGGAGCATGGTAAGT
pnr+170R AGATGGGCAAACGGTGATGAGTGT

Supplemental References

1. **Brand, A. H., and N. Perrimon**. 1993. Targeted gene expression as a means of altering cell fates and generating dominant phenotypes. Development (Cambridge, England) **118**:401-15.

2. **Core, L. J., J. J. Waterfall, and J. T. Lis**. 2008. Nascent RNA Sequencing Reveals Widespread Pausing and Divergent Initiation at Human Promoters. Science **322**:1845-1848.

3. **Ingolia, N. T., S. Ghaemmaghami, J. R. S. Newman, and J. S. Weissman**. 2009. Genome-wide analysis in vivo of translation with nucleotide resolution using ribosome profiling. Science (New York, N.Y.) **324**:218-23.

4. **Robinson, M. D., D. J. McCarthy, and G. K. Smyth**. 2010. edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. Bioinformatics (Oxford, England) **26**:139-40.

5. **Tombácz, I., T. Schauer, I. Juhász, O. Komonyi, and I. Boros**. 2009. The RNA Pol II CTD phosphatase Fcp1 is essential for normal development in *Drosophila melanogaster*. Gene **446**:58-67.

6. **Yao, J., K. L. Zobeck, J. T. Lis, and W. W. Webb**. 2008. Imaging transcription dynamics at endogenous genes in living *Drosophila* tissues. Methods (San Diego, Calif.) **45**:233-41.

7. **Zobeck, K. L., M. S. Buckley, W. R. Zipfel, and J. T. Lis**. 2010. Recruitment timing and dynamics of transcription factors at the Hsp70 loci in living cells. Molecular cell. Elsevier Inc. **40**:965-75.

Figure S1

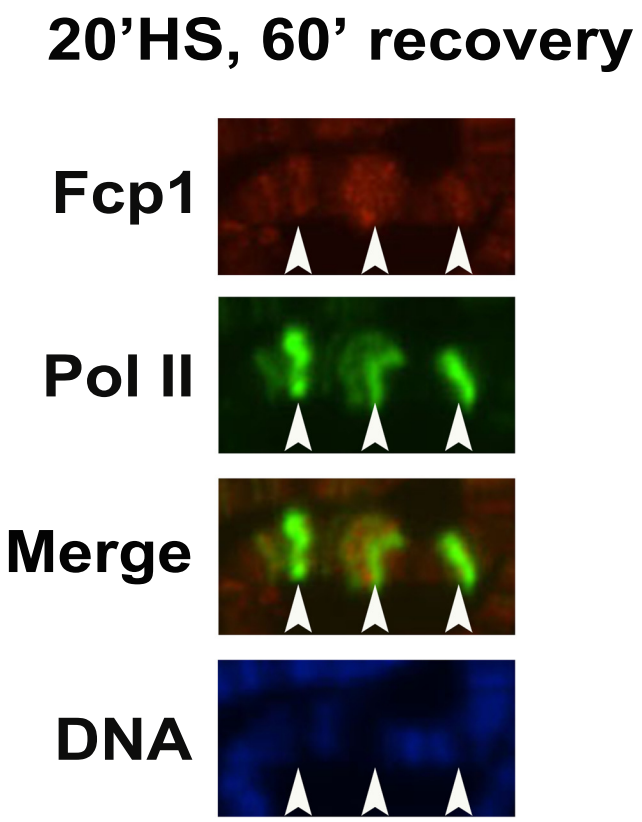


Figure S1. Localization of Fcp1 at *Hsp70* loci after recovery from heat shock. Immunofluorescence staining of polytene chromosomes with antibodies specific to Fcp1 (red) and Serine 5 phosphorylated Pol II CTD (H14 antibody, green) after a 10 minute HS and 60 minutes recovery at room temperature. The arrows indicate the *Hsp70* loci (87A and 87C (endogenous) and a single *Hsp70* transgene at 87E) from left to right. The DNA is stained with DAPI (blue). Merge is an overly of Fcp1 and Pol II.

Figure S2

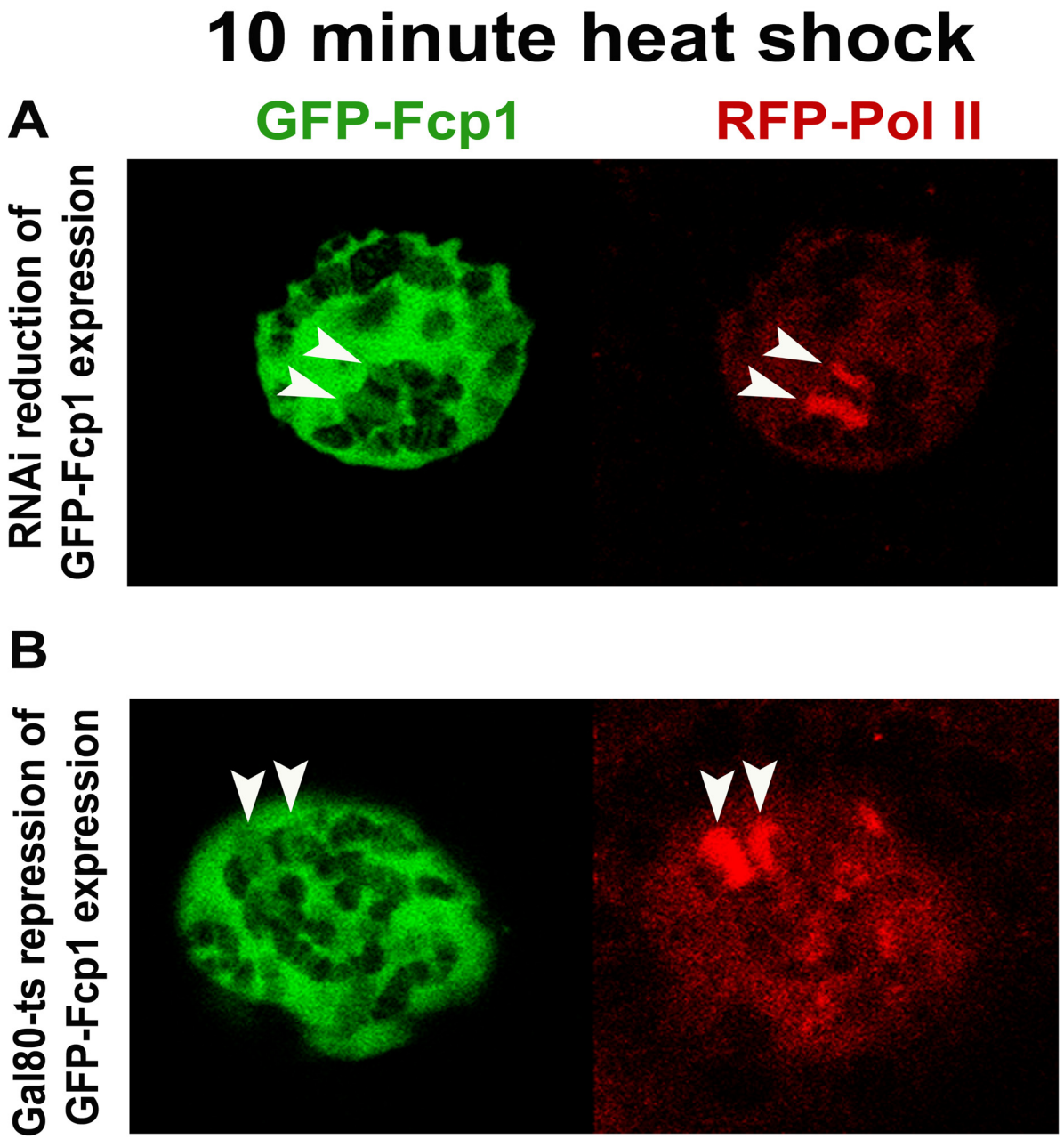


Figure S2. GFP-Fcp1 localizes to the induced *Hsp70* loci in living cells. Laser scanning confocal microscopy images of polytene nuclei co-expressing GFP-Fcp1 (green) and mRFP-Pol II (red) at 10 minute HS. Overexpression of GFP-Fcp1 in salivary glands using the Gal4-UAS system results in a small salivary gland phenotype that does not allow imaging (data not shown). In order to image GFP-Fcp1 in living cells, Fcp1 protein levels were reduced by RNAi depletion (**A**, GFP-Fcp1/+;Rpb3RN, 6983/WizFcp1RNAi or using the Gal80-ts repressor (**B**, GFP-Fcp1/+;Rpb3RN, 6983/7017) grown at room temperature. Arrows highlight the *Hsp70* loci.

Figure S3

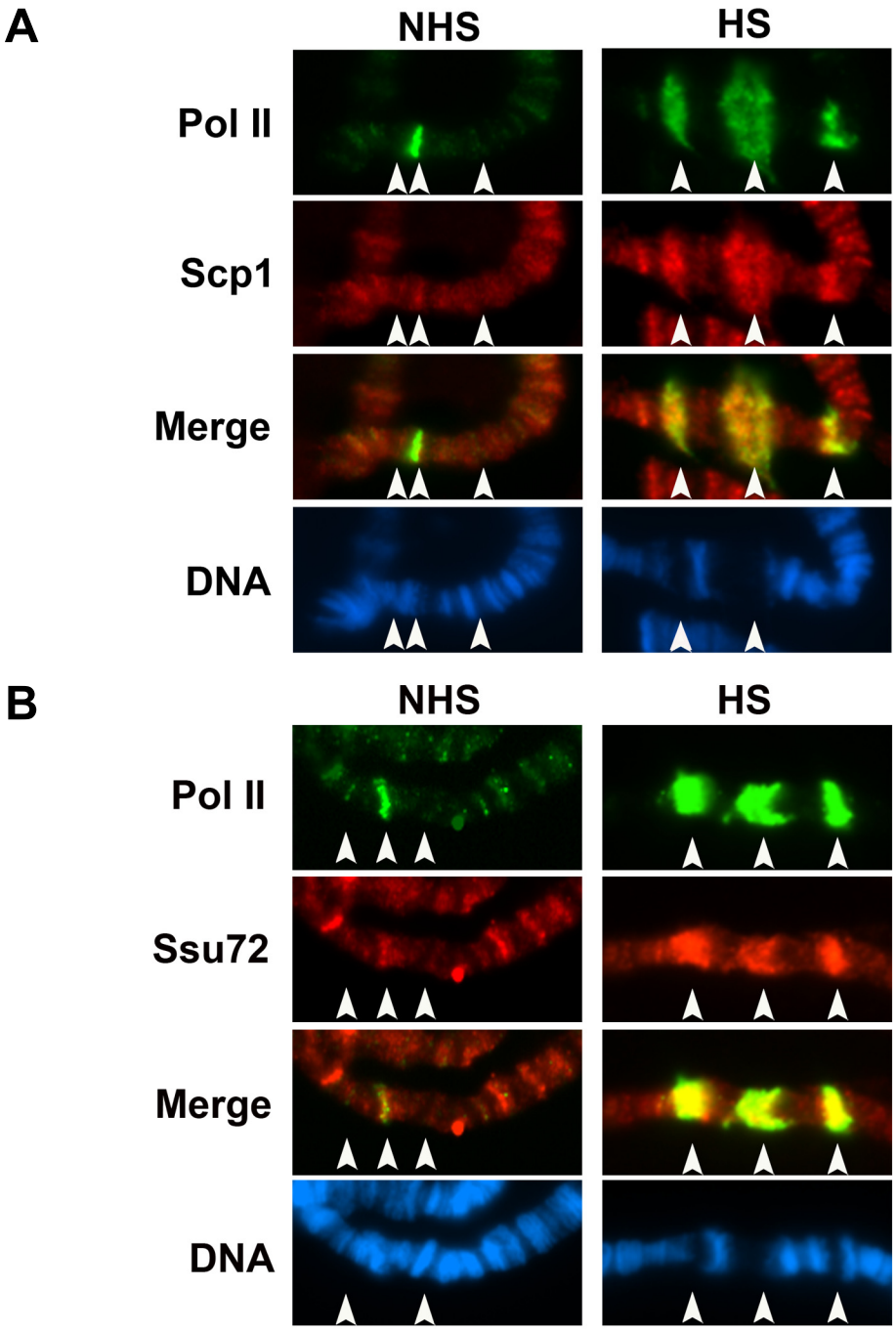


Figure S3. CTD phosphatases Scp1 and Ssu72 localize to active *Hsp70* loci. Immunostaining of polytene chromosomes with **(A)** Scp1 or **(B)** Ssu72 antibodies (red) and Serine 5 phosphorylated Pol II CTD (H14 antibody, green) at the *Hsp70* loci (87A and 87C (endogenous) and a single *Hsp70* transgene at 87E) from left to right (indicated with the arrows) under NHS and HS conditions. The DNA is stained with DAPI (blue). Merge is an overlay of Ssu72 or Scp1 with Pol II.

Figure S4

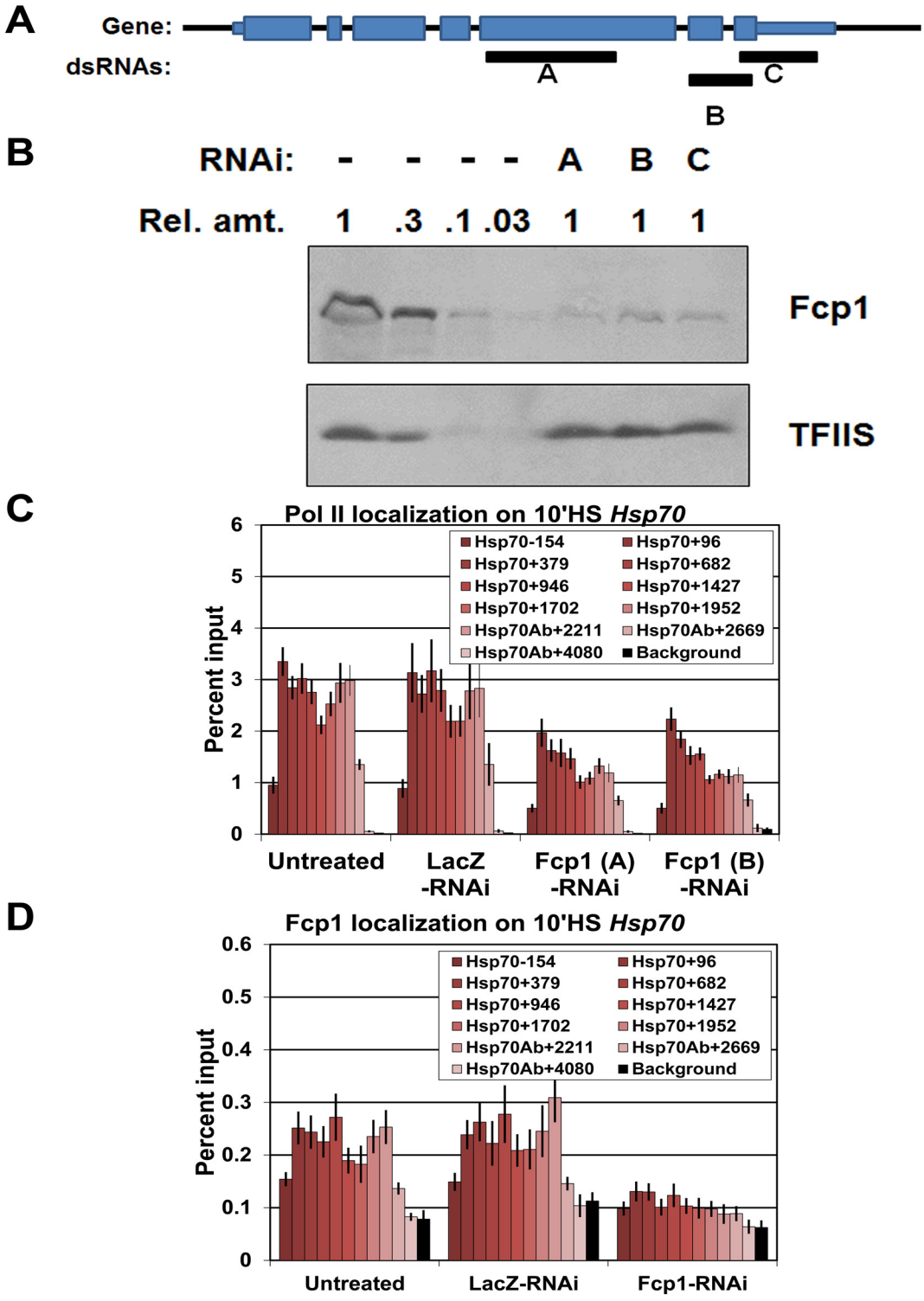


Figure S4. Multiple dsRNAs result in similar depletion of Fcp1. (A) The location of the three dsRNAs used to knock-down Fcp1 (A: +1502 to +2290, B: +2720 to +3103, C: +3017 to +3486, relative to the TSS). The blue areas represent the region included in the mRNA, with the thicker regions indicating the coding region and the narrow regions indicating the UTRs.**(B)** Western blots of whole cell extracts from control (-) and each Fcp1 RNAi (A-C) probed with antibodies for Fcp1 (lab stock, 1:1000) and TFIIS (lab stock loading control, 1:3000). The relative amount loaded is indicated (1=1x106cells). **(C)** ChIP results for the Pol II subunit Rpb3 enrichment on the *Hsp70* gene in Untreated, LacZ-RNAi, and Fcp1-RNAi (A and B dsRNAs) S2 cells at 10 minute HS. **(D)** ChIP results for the Fcp1 enrichment on the *Hsp70* gene in Untreated, LacZ-RNAi and Fcp1-RNAi (A dsRNA) S2 cells at 10 minute HS. The legends indicate the midpoint of each PCR fragment (*Hsp70Ab* indicates primer sets specific for the Ab copy of *Hsp70*). The error bars indicate the SEM of at least three biological replicates.

Figure S5

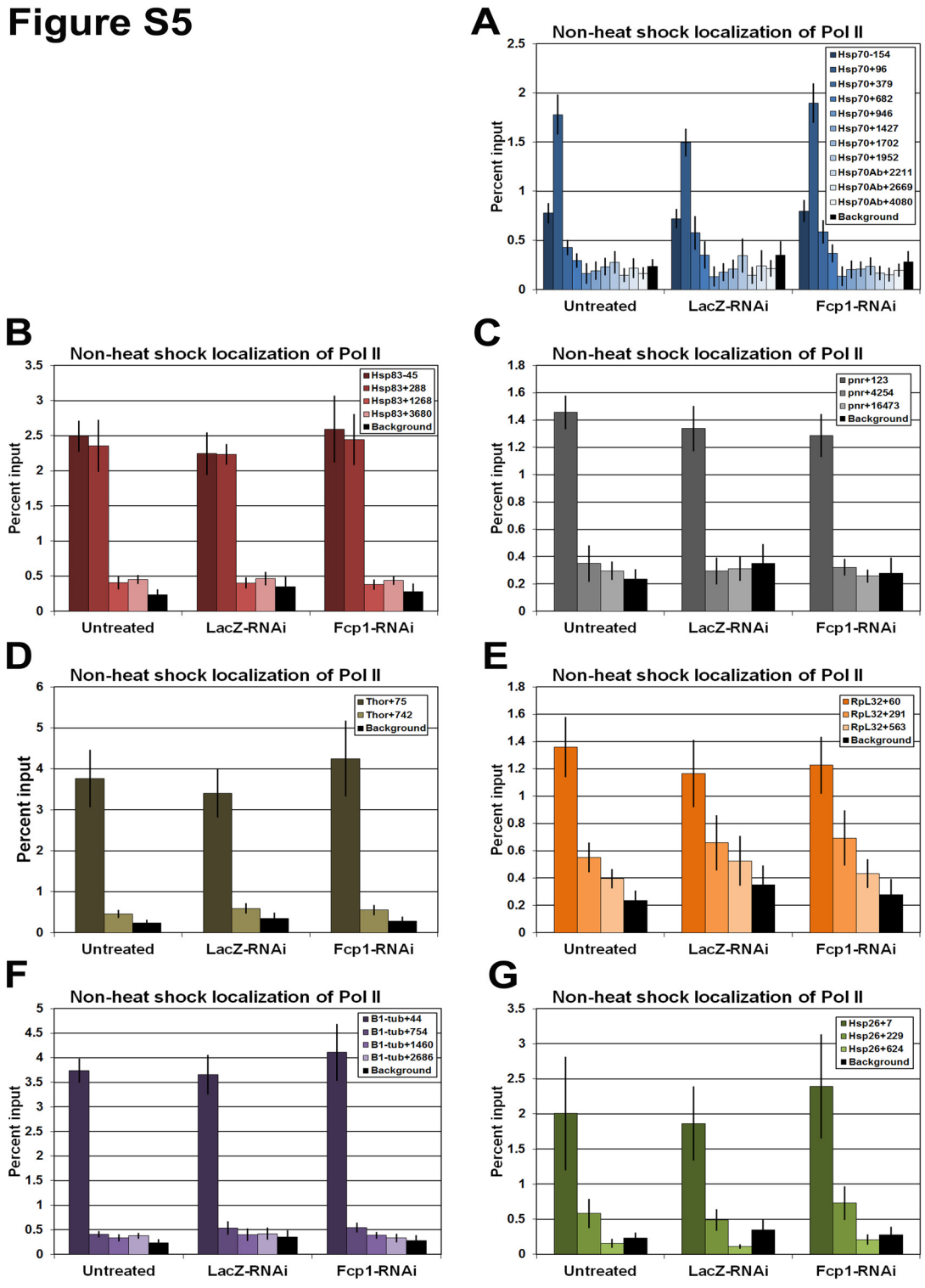
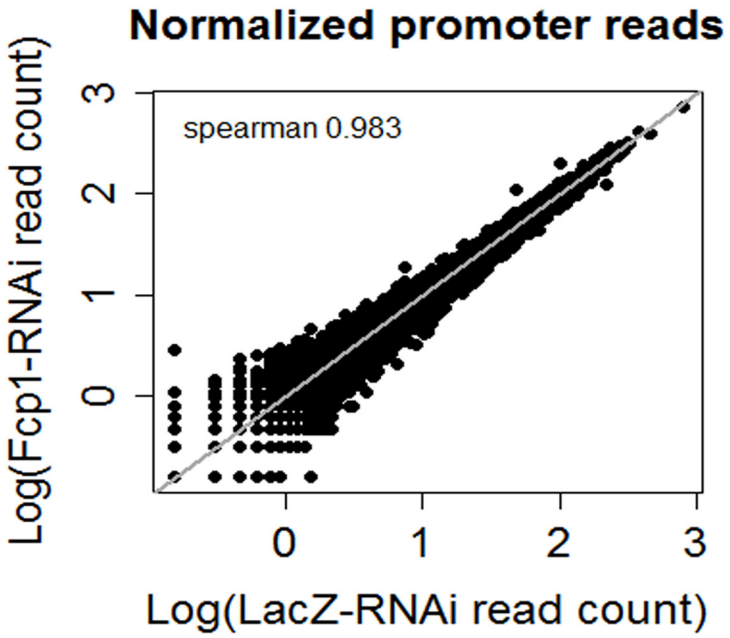


Figure S5. Constitutively expressed genes are not detectably affected by Fcp1 knock-down. **(A-E)** ChIP results for the Pol II subunit Rpb3 enrichment in Untreated, LacZ-RNAi, and Fcp1-RNAi S2 cells at the **(A)** *Hsp70*, **(B)** *Hsp83*, **(C)** *pnr*, **(D)** *Thor*, **(E)** *RpL32*, **(F)** *β-1-tubulin*, and **(G)** *Hsp26* genes under NHS conditions. The legends indicate the midpoint of each PCR fragment (*Hsp70Ab* indicates primer sets specific for the Ab copy of *Hsp70*). The error bars indicate the SEM of at least three biological replicates.

Figure S6

A



B

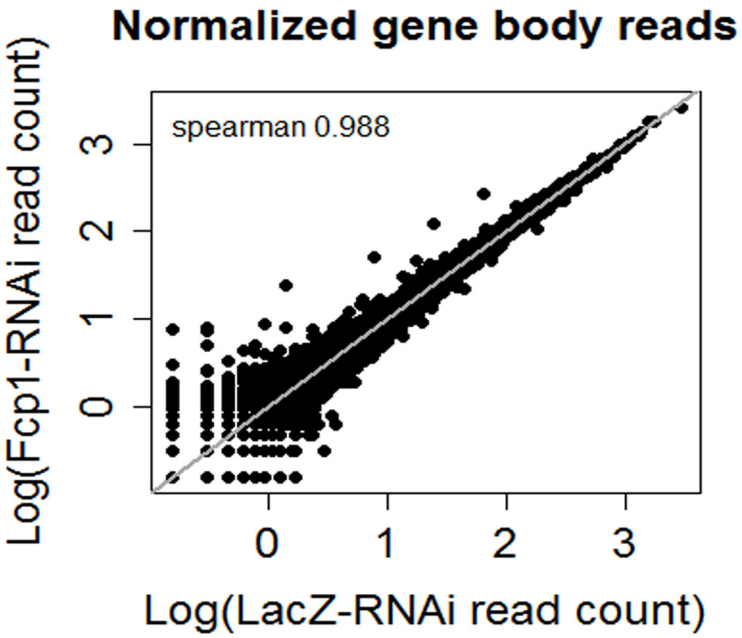


Figure S6. A genome-wide assay shows constitutively expressed genes are not detectably affected by Fcp1 knock-down. GRO-seq was performed in LacZ-RNAi and Fcp1-RNAi cells under NHS conditions to determine the levels of transcriptionally engaged polymerase. **(A)** Normalized read density (log10) from global run-on sequencing (GRO-seq) in promoter regions from LacZ-RNAi and Fcp1-RNAi cells under NHS conditions. The gray line indicates a one-to-one ratio of reads. The spearman correlation between the two datasets is 0.983. **(B)** Normalized GRO-seq read density (log10) in the gene body from LacZ-RNAi and Fcp1-RNAi cells under NHS conditions. The gray line indicates a one-to-one ratio of reads. The spearman correlation between the two datasets is 0.988. Edge-R was used to identify genes with significantly different read counts between LacZ-RNAi and Fcp1-RNAi (4), and no genes had significantly lower read counts in either the promoter or gene body regions of the Fcp1-RNAi library.

Figure S7

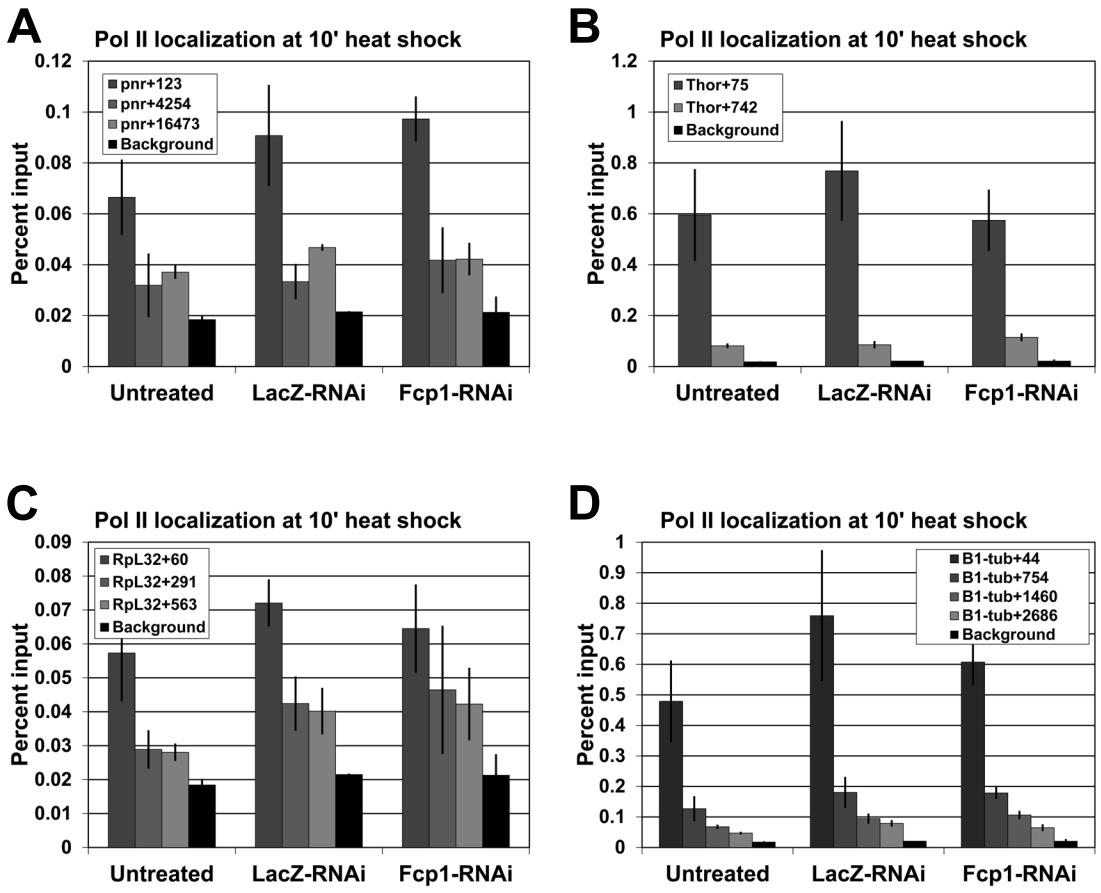


Figure S7. Constitutively expressed genes are not detectably affected by Fcp1 knock-down during heat shock. ChIP results for the Pol II subunit Rpb3 enrichment on the **(A)** *pnr*, **(B)** *Thor*, **(C)** *RpL32*, **(D)** *β-1-tubulin* genes at 10 minutes of heat shock in Untreated, LacZ-RNAi, and Fcp1-RNAi S2 cells. The legends indicate the midpoint of each PCR fragment. The error bars indicate the SEM of at least three biological replicates.

Figure S8

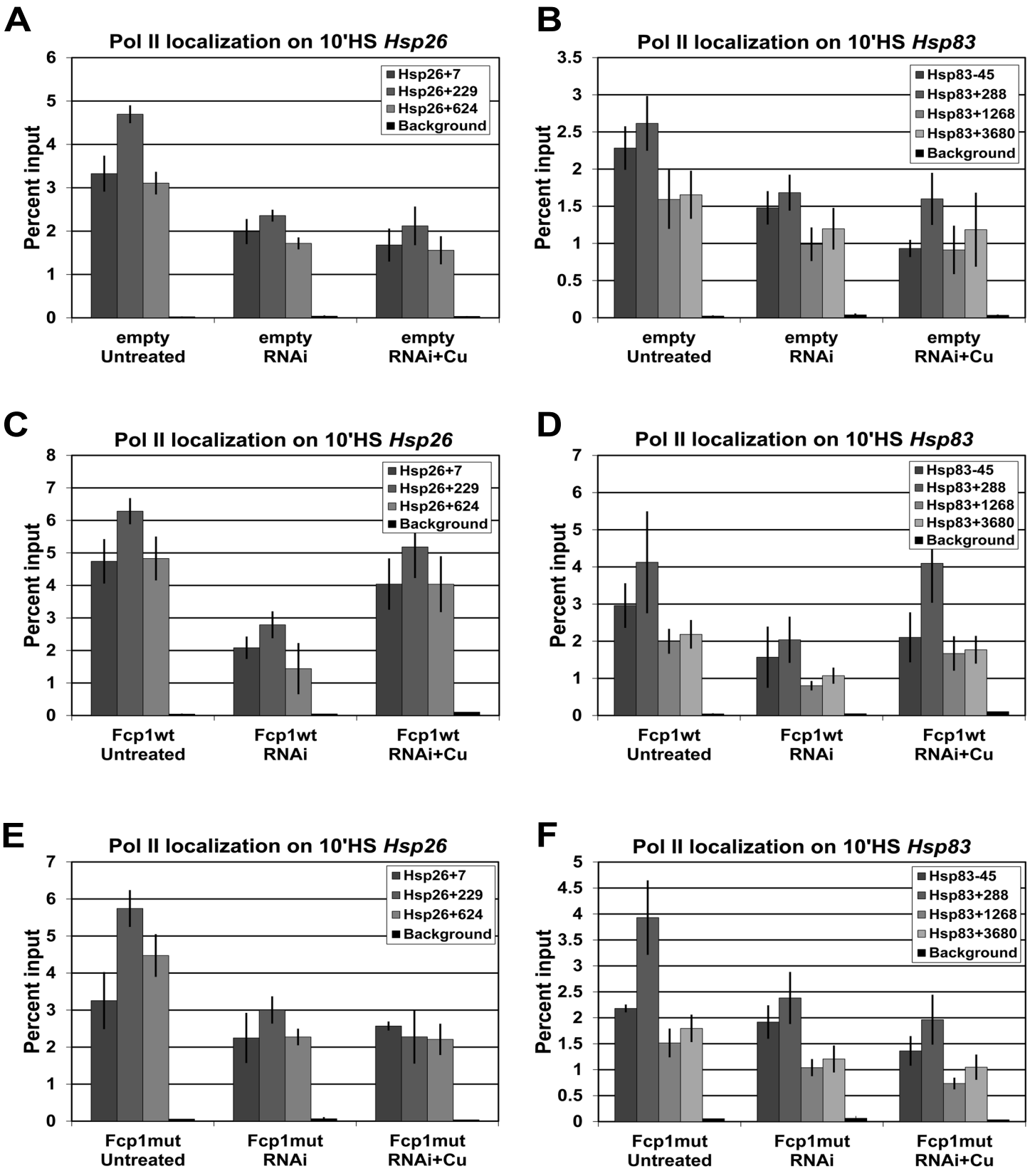


Figure S8. Re-expression of wild type, but not catalytically dead, Fcp1 partially restores Pol II levels on heat shock induced *Hsp26* and *Hsp83*. ChIP results for the Pol II subunit Rpb3 enrichment on **(A,C,E)** *Hsp26* and **(B,D,F)** *Hsp83* at 10 minutes of heat shock +/- Fcp1-RNAi and +/- Cu induction of the transgene from **(A,B)** control (empty vector), **(C,D)** FLAG-tagged wild type Fcp1 transgenic (Fcp1wt) cells, and **(E,F)** FLAG-tagged catalytically dead mutant Fcp1 transgenic (Fcp1mut) cells. The legends indicate the midpoint of each PCR fragment. The error bars indicate the SEM of at least three biological replicates.

Figure S9

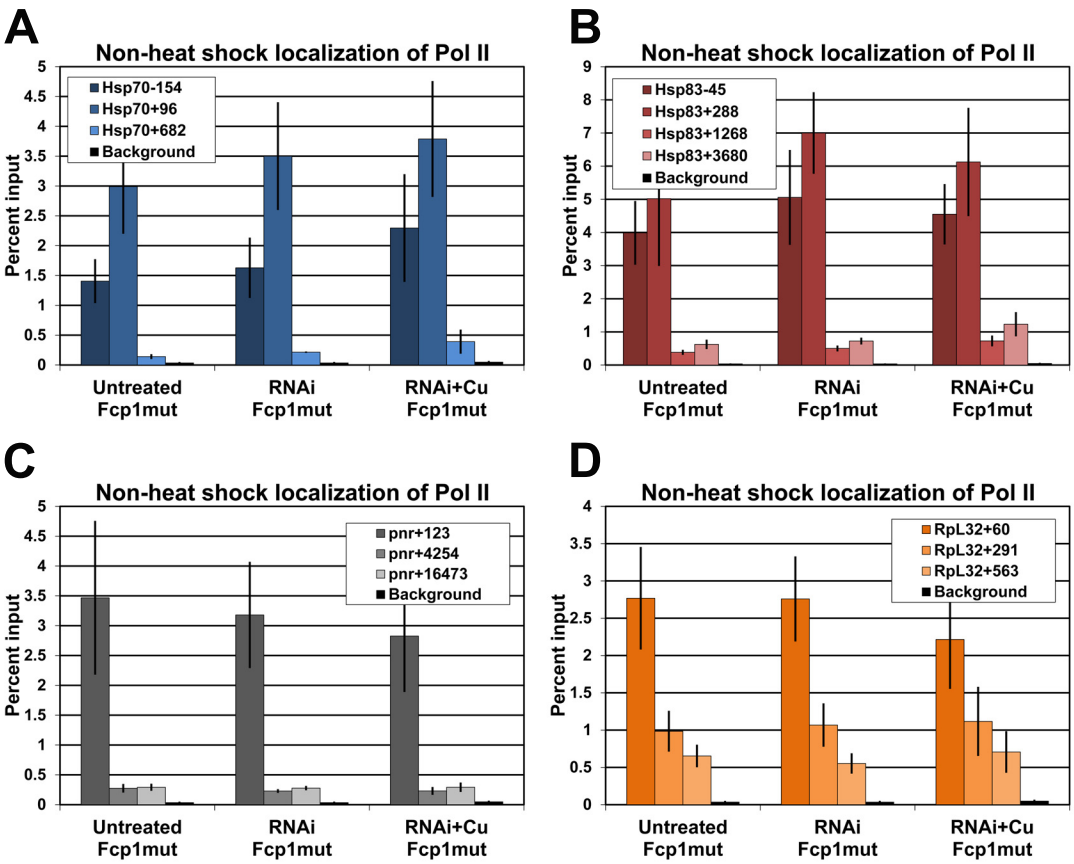


Figure S9. Re-expression of the catalytically dead Fcp1 mutant does change levels of Pol II on constitutively expressed genes. ChIP results for the Pol II subunit Rpb3 enrichment on **(A)** *Hsp70*, **(B)** *Hsp83*, **(C)** *pnr*, and **(D)** *RpL32* genes under non-heat shock conditions +/- Fcp1-RNAi and +/- Cu induction of the FLAG-tagged catalytically dead mutant Fcp1 transgene The legends indicate the midpoint of each PCR fragment. The error bars indicate the SEM of at least three biological replicates.

Figure S10

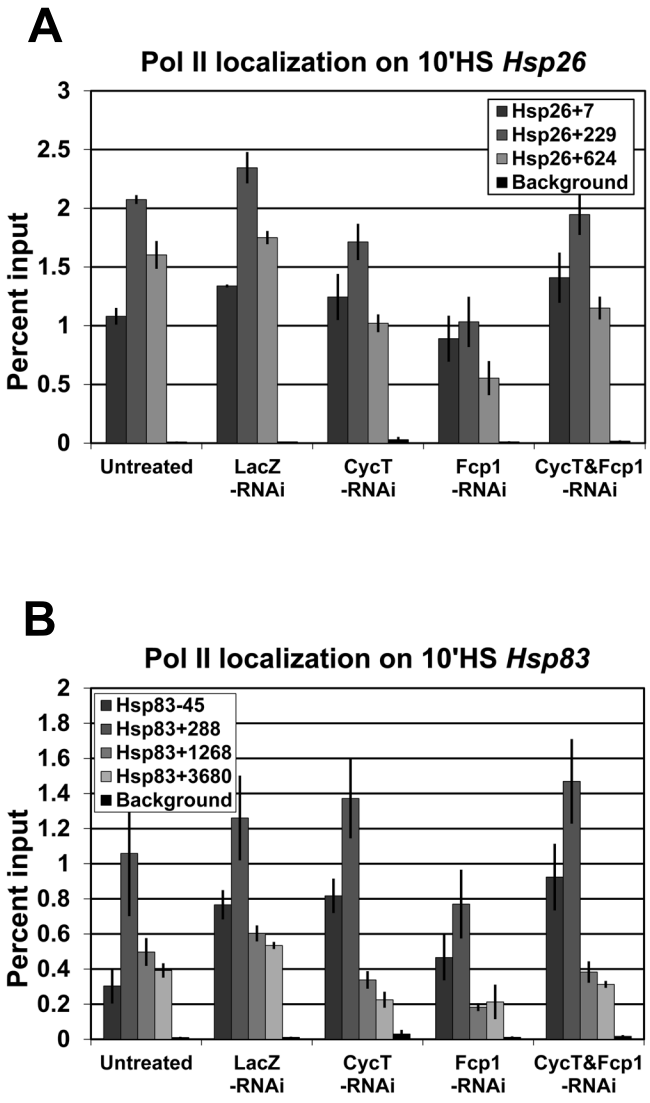


Figure S10. Co-depletion of Cyclin T1 with Fcp1 partially restores Pol II levels on heat shock induced *Hsp26* and *Hsp83*. ChIP results for the Pol II subunit Rpb3 enrichment on **(A)** *Hsp26* and **(B)** *Hsp83* genes at 10 minutes of heat shock in Untreated, LacZ-RNAi, Cyclin T1-RNAi, Fcp1-RNAi, and CyclinT1+Fcp1-RNAi S2 cells. The legends indicate the midpoint of each PCR fragment. The error bars indicate the SEM of at least three biological replicates.

Figure S11

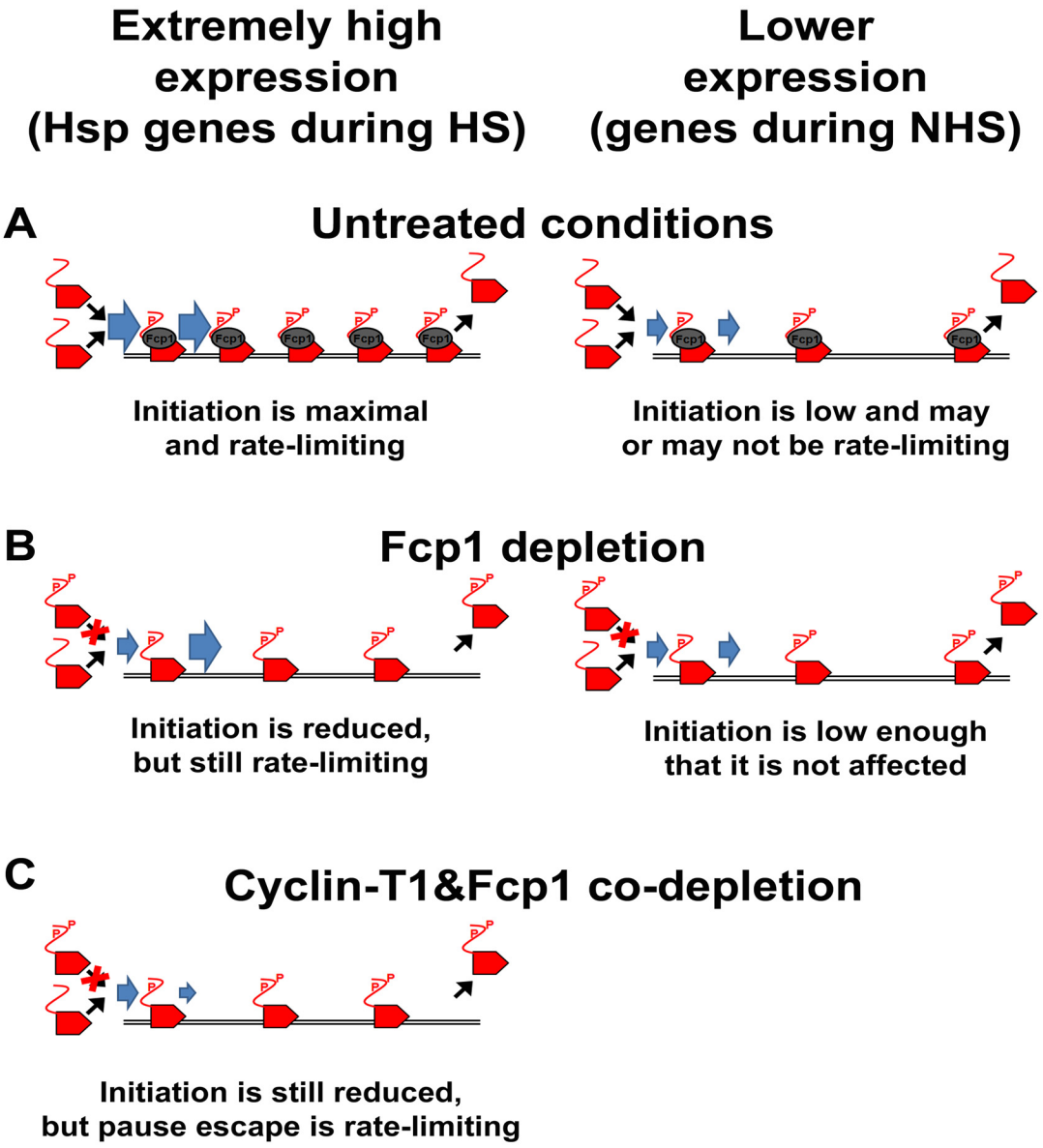


Figure S11. Model for results. (A) Highly expressed heat shock genes rapid ly recruit and release Pol II, and other genes have much lower rates of Pol II recruitment and release (Pol II is represented by the red rocket with CTD tail, and the rates of initiation and pause release are indicated by the size of the blue arrows). **(B)** Fcp1 knock-down increases the amount of phosphorylated free Pol II (represented by Ps on the CTD tail). Because the phosphorylated Pol II cannot initiate transcription, this reduces Pol II initiation on heat shock genes, but the lower initiation rates on other genes allows enough time to bind an initiation-competent Pol II. **(C)** Cyclin-T1 knock-down lowers the release rate, and the pause region is fully occupied by Pol II in co-depleted cells because the reduced release rate allows the heat shock genes enough time to bind an initiation-competent Pol II.