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Supplemental Information

Activator-Induced Spread of Poly(ADP-Ribose) Polymerase Promotes Nucleosome Loss at *Hsp70*

Steven J. Petesch and John T. Lis

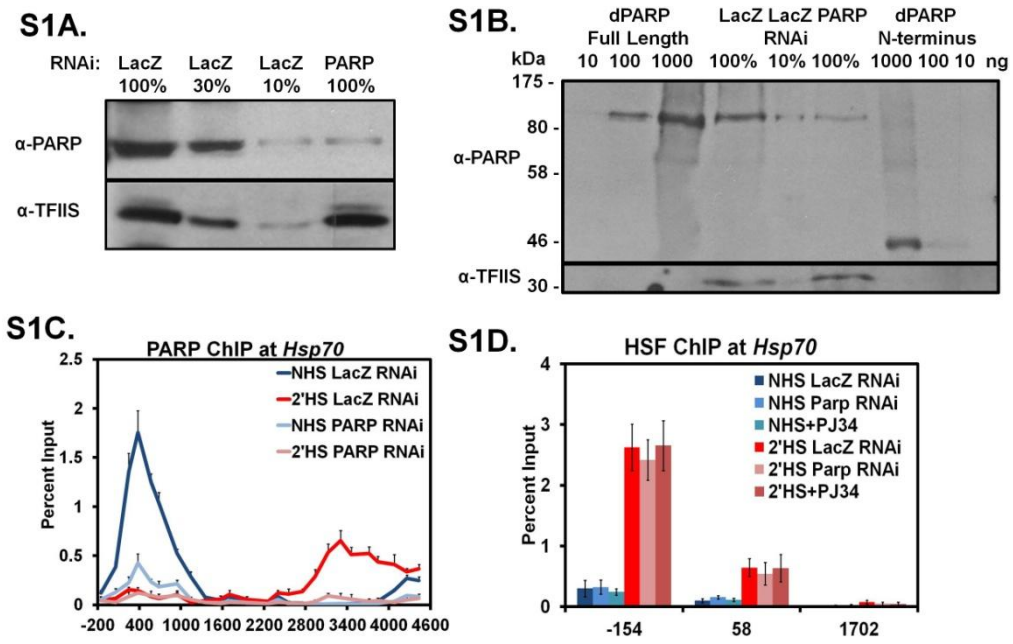

Figure S1, Related to Figure 1.

(A) Western blot confirming the RNAi knockdown of PARP. A serial dilution of the LacZ RNAi control cells were used to help measure the extent of PARP knockdown, shown in the last lane, with 100% equivalent to $5x10^6$ cells. The top panel shows the immunoblot of PARP and bottom panel shows the immunoblot of TFIIS as a loading control.

(B) Western blot showing the specificity of the N-terminal PARP antibody. Increasing amounts of full length, purified His-tagged *Drosophila* PARP (~115 kDa) was loaded in increasing amounts (10, 100, 1000 ng) from left to right and compared to a dilution of LacZ RNAi cells and PARP RNAi cells, with 100% equivalent to $5x10^6$ cells. The antibody is also capable of recognizing the first 43.6 kDa of a His-tagged recombinantly purified N-terminal truncation of *Drosophila* PARP shown in decreasing amounts in the final 3 lanes (1000, 100, 10 ng). The top panel shows the immunoblot of PARP and bottom panel shows the immunoblot of TFIIS as a loading control for the central 3 lanes.

(C) ChIP of PARP at the *Hsp70Ab* HS locus in PARP knockdown cells. S2 cells RNAi depleted of PARP are heat shocked for either 0 (light blue) or 2 minutes (pink) and compared to those

LacZ RNAi NHS (dark blue) and 2’ HS (red) cells. Error bars represent the SEM of 3 independent experiments.

(D) ChIP of HSF at *Hsp70* in PARP knockdown and PJ34 treated cells. S2 cells RNAi depleted of LacZ or PARP or treated with 300 nM PJ34 are heat shocked for either 0 (dark blue, medium blue, or light blue, respectively) or 2 minutes (red, pink, or dark pink, respectively). The y-axis represents the percent of input material immunoprecipitated. The x-axis represents the center of the PCR amplicons in base pair units with 0 being the TSS of *Hsp70* and -154 located at the promoter, 58 at the pause site, and 1702 in the body of the gene. Error bars represent the SEM of 3 independent experiments.

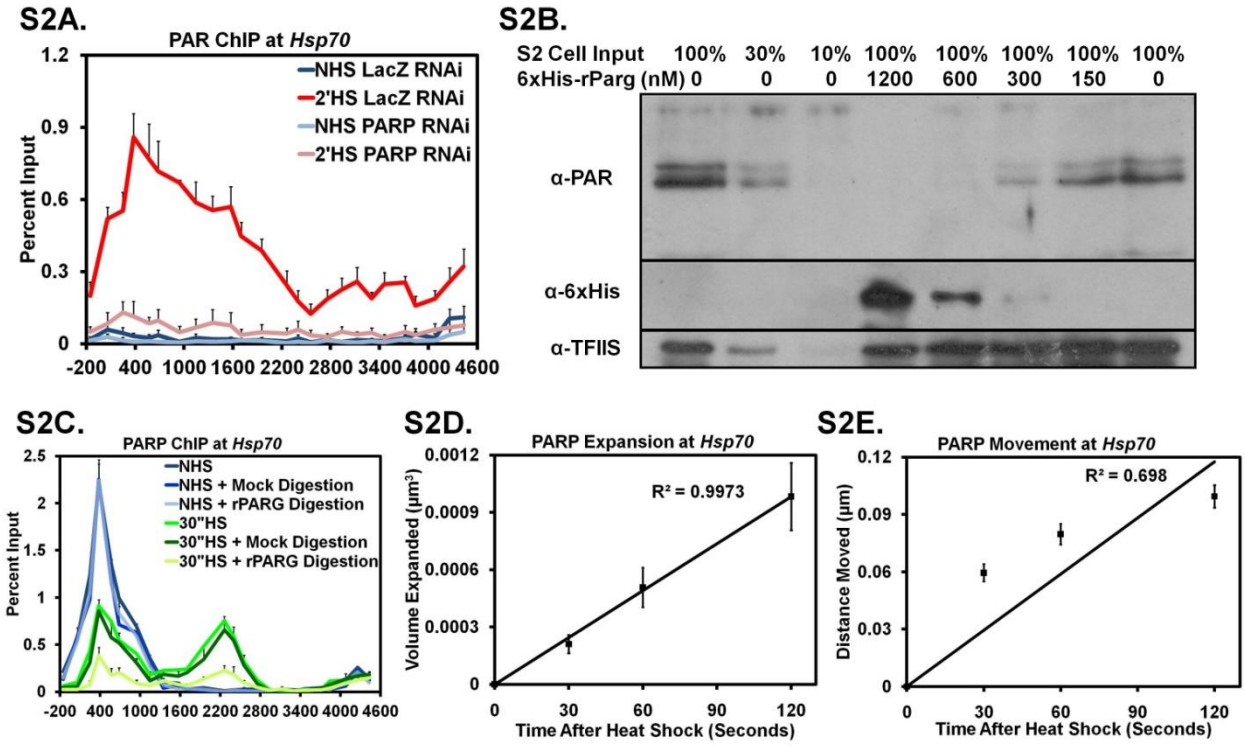

Figure S2, Related to Figure 2.

(A) ChIP of PAR at the *Hsp70Ab* HS locus in PARP knockdown cells. S2 cells RNAi depleted of PARP are heat shocked for either 0 (light blue) or 2 minutes (pink) and compared to those LacZ RNAi NHS (dark blue) and 2'HS (red) cells. Error bars represent the SEM of 3 independent experiments.

(B) Titration of the amount of purified recombinant, purified rat 6xHis-PARG (rPARG) using ChIP extracts. Western blots of sonicated ChIP extracts mock treated (first 3 lanes) or those treated with various amounts of rPARG (last 5 lanes) for 30 minutes at 37 °C to titrate the amount of rPARG needed to digest the bulk PAR. The first three panels are a serial dilution of the mock treated sample to help quantify the extent of digestion, with 100% of the input corresponding to $2.5x10^6$ S2 cells. The last five lanes contain the same amount of ChIP extracts as in lane 1 but varying amounts of rPARG, from 1200, 600, 300, 150, to 0 nM. Top panel: immunoblot of PAR showing the PAR signal associated with bands migrating at the molecular weight of PARP (~114 kDa). Middle panel: immunoblot for 6x-His to detect the amount of rPARG added to the ChIP extract. Bottom panel: immunoblot of TFIIS as a loading control.

(C) ChIP of PARP at the *Hsp70Ab* HS locus with PARG digestion. ChIP extracts prepared for PARP IP were treated with mock treatment or with 1200 nM of rPARG prior to IP with either NHS (light blue) or 30" HS samples (pale green-yellow). These values were compared to control NHS (medium blue) or 30" HS (dark green) samples that were mock treated at 37 °C for 30 minutes. The values of the mock treatment were not significantly different from those obtained without any additional treatment for NHS (dark blue) or 30" HS (bright green). Error bars for the mock and rPARG treated samples represent the SEM of 3 independent experiments. Error bars represent the SEM of 3 independent experiments.

(D) PARP's movement follows a constant rate of three-dimensional expansion at *Hsp70Ab*. The average distance of movement (from the center of the peak of PARP) was measured in base pairs for each time point and then subtracted from the NHS value. No significant difference was found between the 0 and 5 second time point. The distance between the 0 (+480) and the 30 (+2230), 60 (+2820), and 120 (+3400) second time points were used as a first order measure of linear distance. The base pair units were converted to nanometer distances by the fact that 1 base pair of DNA measures 0.34 nm. As the DNA is compacted into chromatin, the values were also divided by a compaction ratio of 10, typical of most 10-30 nm chromatin fibers. (Linear distances are plotted in Fig S2E, which shows a non-linear relationship versus time.) The nanometer distances were then cubed and plotted on the y-axis to model the volume encompassed in μm^3. The x-axis represents the time elapsed following HS in seconds. The error bars represent the propagated error in volume from the SEM of each percent input used in measuring the average base pairs of movement.

(E) The average distance of movement in base pairs was measured for each time point and then subtracted from the NHS value. The distance between the 0 (+480) and the 30 (+2230), 60 (+2820), and 120 (+3400) second time points were first converted to nanometer distances by the fact that 1 base pair of DNA measures 0.34 nm. Since this DNA is compacted into chromatin, the value was also adjusted by a compaction ratio of 10, typical of most 10-30 nm chromatin fibers. The y-axis represents the linear distance traveled in μm. The x-axis represents the time following HS in seconds. The error bars represent the propagated error from the SEM of each percent input used in measuring the average base pairs of movement. The linear R^2 value is depicted on the graph.

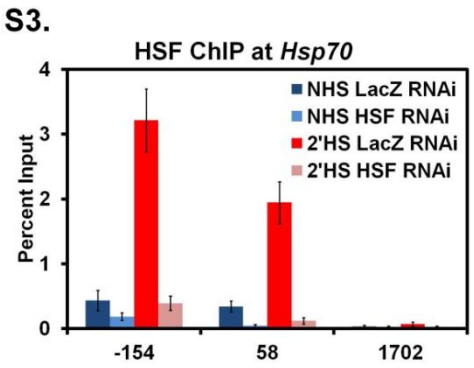

Figure S3, Related to Figure 3.

ChIP of HSF at *Hsp70*. S2 cells RNAi depleted of LacZ or HSF are heat shocked for either 0 (dark blue or light blue respectively) or 2 minutes (red or pink respectively). The y-axis represents the percent of input material immunoprecipitated. The x-axis represents the center of the PCR amplicons in base pair units with 0 being the TSS of *Hsp70* with -154 located at the promoter, 58 at the pause site, and 1702 in the body of the gene. Error bars represent the SEM of 3 independent experiments.

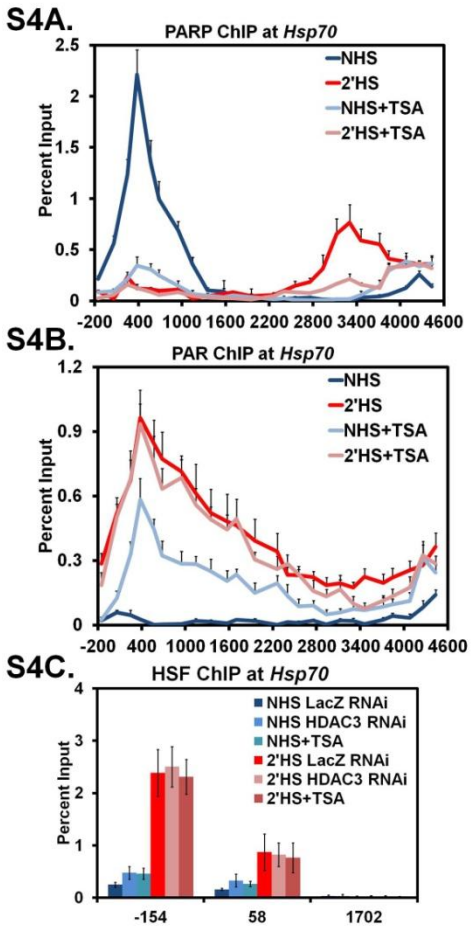

Figure S4, Related to Figure 4.

(A) ChIP of PARP at the *Hsp70Ab* HS locus in the presence of the HDAC inhibitor, TSA. Untreated NHS (dark blue) and 2' HS (red) S2 cells are compared to those NHS (light blue) and 2' HS (pink) cells pretreated with 3 μM TSA for 30 minutes. Error bars represent the SEM of 3 independent experiments.

(B) ChIP of PAR at the *Hsp70Ab* HS locus in the presence of TSA as in (A). Error bars represent the SEM of 3 independent experiments.

(C) ChIP of HSF at *Hsp70* in HDAC3 knockdown and TSA treated cells. S2 cells RNAi depleted of LacZ or HDAC3 dsRNA or treated with 3 μM TSA are heat shocked for either 0 (dark blue, medium blue, or light blue respectively) or 2 minutes (red, pink, or dark pink respectively). The y-axis represents the percent of input material immunoprecipitated. The x-axis represents the center of the PCR amplicons in base pair units with 0 being the TSS of *Hsp70* and -154 located

at the promoter, 58 at the pause site, and 1702 in the body of the gene. Error bars represent the SEM of 3 independent experiments.

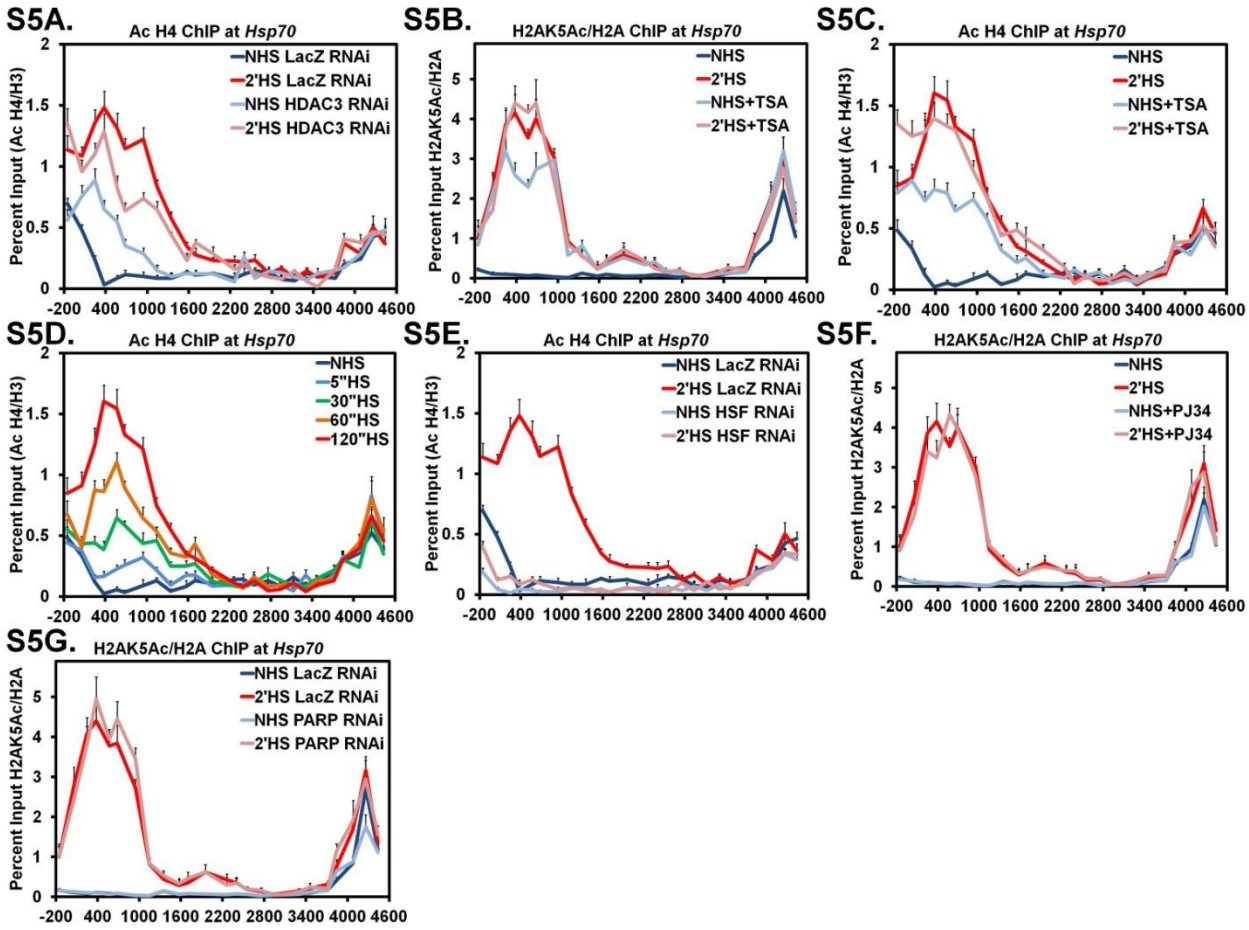

Figure S5, Related to Figure 5.

(A) ChIP of tetra acetylated H4 (antibody can recognize H4K5, K8, K12, and K16 acetylation) at the *Hsp70Ab* locus normalized to histone H3 and plotted on the y-axis. Cells RNAi depleted of HDAC3 and heat shocked for either 0 (light blue) or 2 minutes (pink) are compared to LacZ RNAi cells not heat shocked (dark blue) or heat shocked for 2 minutes (red). Error bars represent the SEM of 3 independent experiments.

(B) ChIP of H2AK5 acetylation at the *Hsp70Ab* locus in untreated NHS (dark blue) and 2' HS (red) S2 cells are compared to those NHS (light blue) and 2' HS (pink) cells pretreated with 3 μM TSA for 30 minutes. The level of H2AK5 acetylation is normalized to ChIP values for histone H2A and plotted on the y-axis. Error bars represent the SEM of 3 independent experiments.

(C) ChIP of tetra acetylated H4 as in (A) but untreated NHS (dark blue) and 2’ HS (red) S2 cells are compared to those NHS (light blue) and 2’ HS (pink) cells pretreated with 3 μM TSA for 30 minutes. Error bars represent the SEM of 3 independent experiments.

(D) Kinetic ChIP analysis of tetra acetylated H4 as in (A). S2 cells are heat shocked for 0 (dark blue), 5 (light blue), 30 (green), 60 (orange), or 120 seconds (red). Error bars represent the SEM of 3 independent experiments.

(E) ChIP of tetra acetylated H4 as in (A) but cells RNAi depleted of HSF and heat shocked for either 0 (light blue) or 2 minutes (pink) are compared to LacZ RNAi cells not heat shocked (dark blue) or heat shocked for 2 minutes (red). Error bars represent the SEM of 3 independent experiments.

(F) ChIP of H2AK5 acetylation as in (B) but untreated NHS (dark blue) and 2’ HS (red) S2 cells are compared to those NHS (light blue) and 2’ HS (pink) cells pretreated with 300 nM PJ34 for 10 minutes. Error bars represent the SEM of 3 independent experiments.

(G) ChIP of H2AK5 acetylation as in (B) but with S2 cells RNAi depleted of PARP and heat shocked for either 0 (light blue) or 2 minutes (pink) and compared to LacZ RNAi cells that were not heat shocked (dark blue) or heat shocked for 2 minutes (red). Error bars represent the SEM of 3 independent experiments.

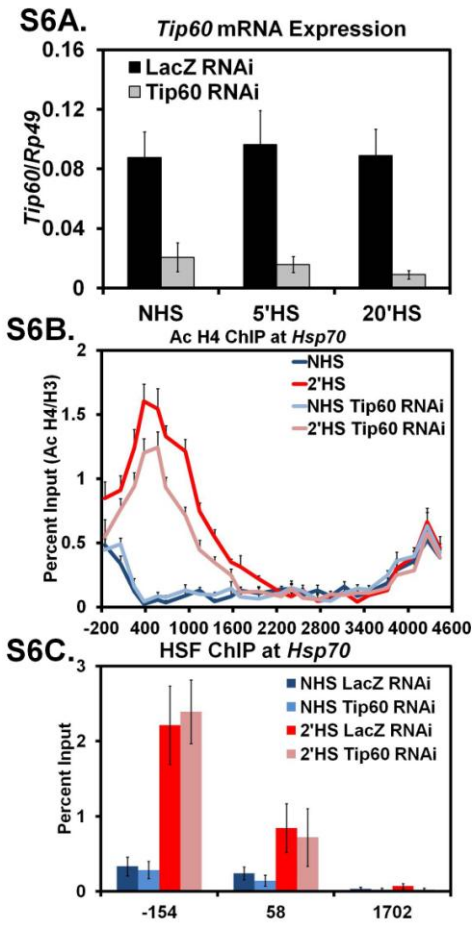

Figure S6, Related to Figure 6.

(A)*Tip60* mRNA levels following 0, 5, and 20 minutes of HS were measured for S2 cells RNAi depleted of LacZ (black) or Tip60 (gray). *Tip60* expression levels were measured by oligo dT primed reverse transcription followed by qPCR using*Tip60* specific primers. *Tip60* mRNA levels are normalized to the *Rp49* gene with error bars representing the SEM of 3 replicates.

(B) ChIP for tetra acetylated H4 (antibody can recognize H4 K5, K8, K12, and K16 acetylation) normalized to histone H3 was performed using S2 cells RNAi depleted of Tip60 and heat shocked for either 0 (light blue) or 2 minutes (pink) and compared to LacZ RNAi cells that were not heat shocked (dark blue) or heat shocked for 2 minutes (red).

(C) ChIP of HSF at *Hsp70*. S2 cells RNAi depleted of LacZ or Tip60 are heat shocked for either 0 (dark blue or light blue respectively) or 2 minutes (red or pink respectively). The y-axis represents the percent of input material immunoprecipitated. The x-axis represents the center of the PCR amplicons in base pair units with 0 being the TSS of *Hsp70* with -154 located at the

promoter, 58 at the pause site, and 1702 in the body of the gene. Error bars represent the SEM of 3 independent experiments.

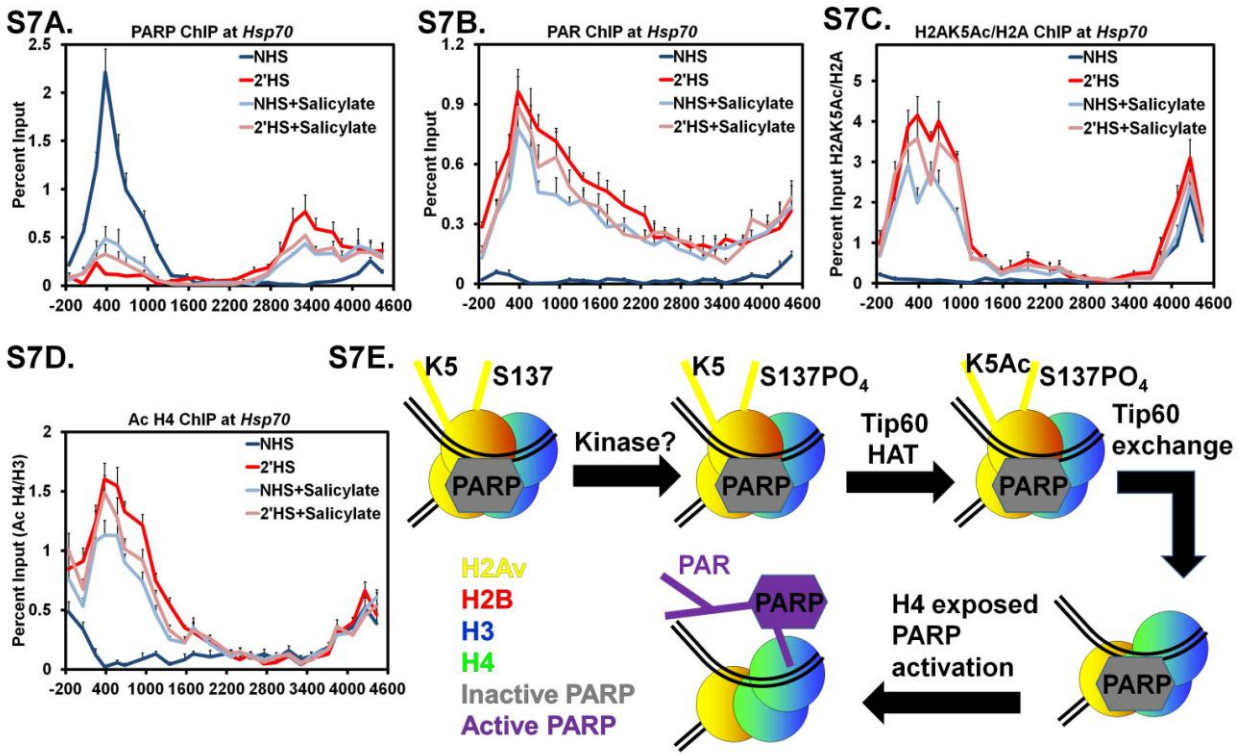

Figure S7, Related to Figure 7.

(A) ChIP of PARP at the *Hsp70Ab* HS locus in the presence of sodium salicylate. Untreated NHS (dark blue) and 2' HS (red) S2 cells are compared to those NHS (light blue) and 2' HS (pink) cells pretreated with 10 mM sodium salicylate for 30 minutes.

(B) ChIP of PAR at the *Hsp70Ab* HS locus in the presence of sodium salicylate as in (A). Error bars represent the SEM of 3 independent experiments.

(C) ChIP of H2AK5 acetylation normalized to H2A in the presence of sodium salicylate as in (A). Error bars represent the SEM of 3 independent experiments.

(D) ChIP of tetra acetylated H4 normalized to H3 in the presence of sodium salicylate as in (A). Error bars represent the SEM of 3 independent experiments.

(E) Model depicting how dTip60 activates PARP. A single nucleosome, representing the first nucleosomes at *Hsp70* before HS, is depicted with H2Av in yellow, H2B in red, H3 in blue, and H4 in green with inactive PARP bound depicted in gray. The N-and C-terminal tails of H2Av are shown as yellow protrusions from H2Av with K5 and S137 highlighted. An unknown kinase is

responsible for phosphorylating the C-terminal domain of H2Av at S137. Upon HS, Tip60 is recruited to *Hsp70* and the acetyltransferase activity is stimulated by the presence of S137 phosphorylation, leading to H2AvK5Ac. The acetylation stimulates the dTip60 complex to exchange the modified H2Av-H2B dimer, thereby exposing the H4 within the nucleosome. The exposure of H4 within the nucleosome can provide an epitope that stimulates the activity of PARP leading to its activation, shown in purple.

Supplemental Experimental Procedures

ChIP

Additional ChIP performed in supplemental figures beyond what is shown in the main document was performed using 2 µL of rabbit anti-HSF (Boehm et al., 2003), 2 µL of rabbit anti-tetra acetyl-Histone H4 (Millipore 06-598), and 2 µL of rabbit anti-Histone H3 ChIP grade (Abcam ab1791).

Quantitative Real-Time PCR Analysis

ChIP and RT-qPCR primer sets are provided in Table S1. Real-Time PCR was performed as in (Petesch and Lis, 2008). For ChIP samples, a standard curve was generated by serially diluting input samples to quantify IP samples. For MNase digests, a fold difference was calculated between MNase treated and untreated samples. All values used were collected from the linear range of amplification.

Chemical Treatments

PJ34 was added to S2 cells in media to a final concentration of 300 nM and allowed to mix for 10' at room temperature. Cells were then collected following NHS or 2' HS conditions outlined in the ChIP section. Additional chemical treatments included in supplementary figures not performed in the main document were performed by adding both final concentrations of 3 µM TSA or 10 mM sodium salicylate directly to the S2 cells while still in media either 30 or 10 minutes respectively prior to HS for either 0 or 2 minutes.

RNAi Treatments

All RNAi treatments were performed as in (Petesch and Lis, 2008). RNAi primers and RefSeq DNA Identifiers for all knockdowns are provided in Table S1. Briefly, S2 cells were treated with double stranded RNA, designed using the Ambion MEGAscript manual, targeting either the coding sequence of the listed factor or β-galactosidase (LacZ, as a negative control). Cells were

collected and split into NHS and 2’ HS samples to be processed using the ChIP or high-resolution MNase assay.

mRNA Expression Analysis

All mRNA expression analyses were as performed in (Petesch and Lis, 2008). Briefly, total RNA was isolated (Qiagen RNeasy) from Tip60 and LacZ RNAi S2 cells following 0, 5, and 20 minutes of HS. *Hsp70*and *Tip60* levels were determined from oligo dT mediated quantitative real-time reverse transcription-PCR using primers targeting either *Hsp70* or *Tip60*. The stable ribosomal protein RpL32 gene (*Rp49*) was used to internally standardize for the amount of RNA.

High-Resolution MNase Mapping

MNase mapping was preformed as in (Petesch and Lis, 2008). Briefly, nuclei isolation, followed by chromatin isolation was performed using cross-linked S2 cells. Samples were split into equal portions and treated with either 0 or 500 total units of MNase (USB) for 30 minutes at room temperature and their DNA recovered.

PARG and PARP Purification

Recombinant, rat, 6xHis-N-terminally tagged PARG was a gift of W. Lee Kraus and was purified as in (Kim et al., 2004). *Drosophila* full length and N-terminus (corresponding to the first 383 amino acids) PARP were cloned into pET-19b harboring a 6xHis-N-terminal tag. Briefly, transformed BL21-CodonPlus (DE3)-RIPL *E. coli* cells (Agilent Technologies) were induced with 1 mM IPTG for 3 hours at 30 °C. 6xHis tagged proteins were purified using conventional Ni-NTA Agarose (Qiagen) methods.

Western Blots

Western blots were performed using standard conditions, and input dilutions were used as a quantitative indication of signal linearity. Antibody lab stocks of HSF (Shopland et al., 1995)

and TFIIS (Adelman et al., 2005) were used at dilutions of 1:2000 and 1:3000 respectively. Rabbit anti-HDAC3 antibody (Santa Cruz sc-11417) was used at a 1:500 dilution. Additional Western blots were performed using rabbit anti-Parp serum raised to recognize the N-terminus (Kim et al., 2004) was a gift of W. Lee Kraus, mouse anti-PAR (Trevigen 4335), and mouse anti-6xHis (Santa Cruz sc-8036) at 1:1000, 1:500, and 1:1000 dilutions respectively.

Supplemental References

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