## Additional file 1, supplementary figures

## Global SUMOylation on active chromatin is an acute heat stress response restricting transcription

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Figure S1 Comparison of SUMO2/3 peaks in K562 and VCaP cells. (A) Venn diagrams showing overlap of K562 and VCaP SUMO2/3 peaks in control (C) and HS conditions. (B) Distribution of SUMO2/3 peaks shared between K562 and VCaP in control (blue) or HS (red) conditions in annotated genomic loci.



Figure S2 Venn diagram showing co-occurrence of K562 cell SUMO2/2 control condition unique peaks with CTCF, Rad21, and ZNF143 peaks.



-1kb TSS +1kb

Figure S3 Promoter SUMO2/3 ChIP-seq signal correlates with Pol2 and serine 5-phosphorylated Pol2 (s5p). Heat map showing ChIP-seq signal intensities of K562 SUMO2/3 in control (C) and HS conditions, and Pol2 and Pol2-s5p at ±2kb window centered at transcription start sites (TSSs). TSSs are arranged according to Pol2 signal. Intensity is shown as a false-color scale (intensity increases from darker to brighter colors). Line profile of the average signal from the same ChIP-seq data sets at ±1kb region surrounding TSSs. Intensity of SUMO2/3 signal peaks in the "valley" between Pol2 local maxima.



Figure S4 Chromatin environment surrounding intergenic K562 SUMO2/3 peaks. Heat map showing ChIPseq and GRO-seq signal intensities at ±2kb window centered at intergenic K562 SUMO2/3 peaks in control (blue) and HS (red) conditions. K562 cell-specific DNasel, PoI2, PoI2-s5p, histone, TRIM28, CTCF, and Rad21 data are from the ENCODE project (<u>www.encodeproject.org</u>). Intensities are depicted as a false-color scale (intensity increases from darker to brighter colors) for DNasel and ChIP-seq data. GRO-seq signal is shown separately for negative (cyan) and positive (magenta) direction reads.



Figure S5 Average GRO-seq signal along the annotated genes in K562 cells. Average GRO-seq signal from positive-strand reads in control (blue) or HS (red) is shown at the first 4-kb region. GRO-seq signal peaked at the first 250 bp from the TSS and this region was used as the promoter proximal region when determining Pol2 promoter-pausing pausing index (PPI). The rest of the gene was used as the gene body signal in analysis of transcription.



Figure S6 Association of SUMO2/3 peaks with transcriptionally active genes in K562 cells. Transcribed genes were divided according to their association to SUMO2/3 peaks: promoter SUMO2/3-associated (SUMO2/3 peak within -1kb to +100 from the annotated TSS), enhancer SUMO2/3-associated (intergenic SUMO2/3 peak within 100kb of the TSS), and intragenic SUMO2/-associated (intragenic SUMO2/3 peak). Venn diagrams indicate the number of genes in each SUMO2/3 association category in control (SUMO2/3-C) or HS (SUMO2/3-HS) conditions. Transcribed genes had gene body GRO-seq RPKM >0.5 in control or HS conditions.