



Figure S6: Higher HSF binding levels and positioning upstream and proximal to the TSS are important for the induction of HSF's target genes. (A) Histogram with the distribution of distances between the closest HSF ChIP-seq peak and the TSS of HSF-bound genes with HSF-dependent (left panel, n=27) or independent (right panel, n=17) HS activation. The distances are plotted in 200 bp bins from ± 1000 bp to the TSS. **(B)** Box-plot showing the distribution of HSF ChIP-seq binding intensities for the two classes of genes described in A. **(C)** DESeq2 analysis to determine the effect of HSF-RNAi treatment on the PRO-seq pausing region reads before (NHS) and after 20min HS (HS). DESeq2 was used to identify significantly changed genes between HSF-RNAi and LacZ-RNAi cells and the results are displayed as MA plots. Significantly changed genes were defined using an FDR of 0.001. HSF-bound genes are labeled in purple, significantly changed genes (according to DESeq2) are labeled in green and genes that are both HSF-bound and significantly changed are labeled in orange.