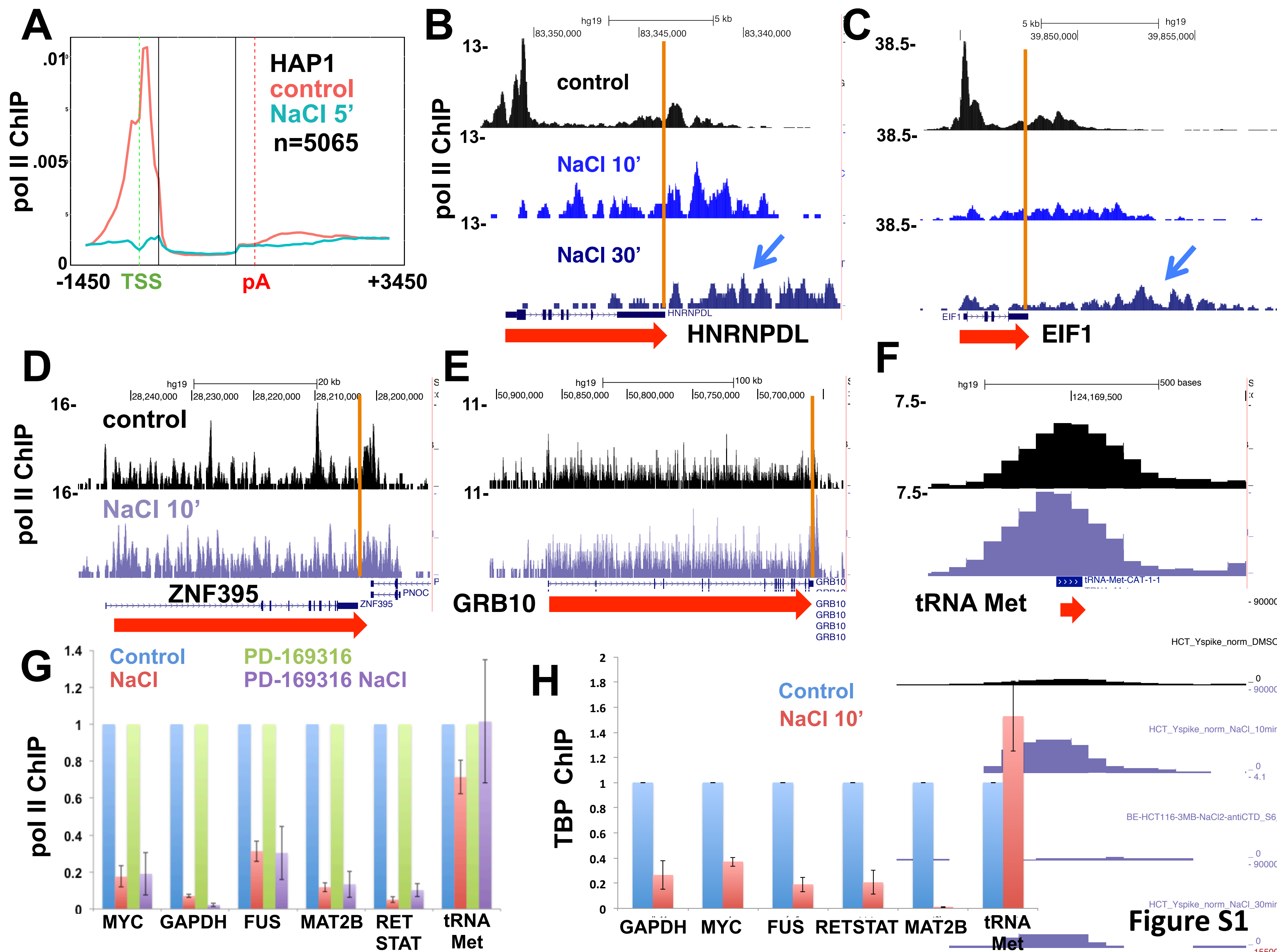


Figure S1

Supplemental Figure Legends

Figure S1. Related to Figures 1, 2.

A. Genome-wide loss of paused pol II in high NaCl in HAP1 myeloid leukemia cells. Anti-pol II ChIP-seq in cells treated -/+ addition of NaCl to 0.5M for 5 min.

B, C. Pol II ChIP normalized to total mapped reads in HCT116 cells treated with 350mM NaCl for 10 and 30 min. Note pol II "creeping" with depletion from 5′ end (blue arrows) as in Fig. 1I.

D, E. UCSC genome browser screenshots of pol II ChIP on the ZNF395 and GRB10 genes in HCT116 cells that lack a 5′ peak of paused pol II. Note pol II resistance to high salt at genes that lack 5′ paused pol II as in Fig. 1

F. Pol II ChIP on a tRNA Met gene is resistant to eviction in high salt.

G. Loss of pol II from 5′ ends in high salt is unaffected by blocking the hypertonic stress response. HCT116 cells were treated with the p38 kinase inhibitor PD-169316 (15μM) for 1 hr. then NaCl was added for (10 min.) then mouse M12 cells were spiked in and pol II occupancy assayed at the 5′ ends with normalization to the mouse actin 5′ end. ChIP qPCR signals were normalized to the - NaCl control. Mean and SEM are shown for 4 PCR reactions.

H. Loss of TBP from 5′ ends in high salt. NaCl was added to HCT116 cells (10 min.) then mouse M12 cells were spiked in and TBP occupancy assayed at the 5′ ends with normalization to the mouse actin 5′ end. ChIP qPCR signals were normalized to - NaCl control. Note that TBP at the tRNAMet gene (see F) is resistant to high salt. Mean and SEM are shown for 4 PCR reactions.

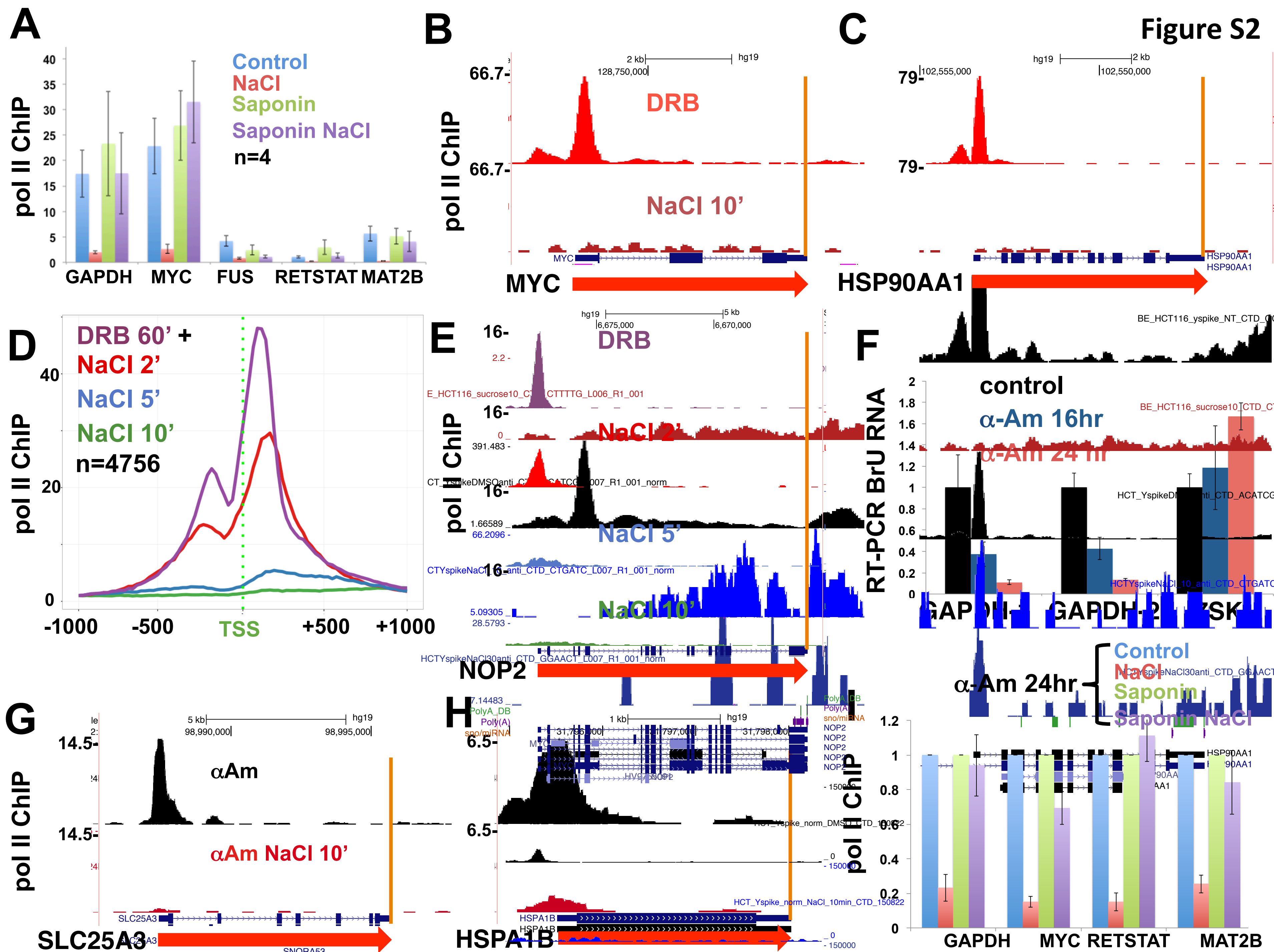


Figure S2
A
pol II ChIP
Control
NaCl
Saponin
Saponin NaCl
n=4
GAPDH
MYC
FUS
RETSTAT
MAT2B
B
pol II ChIP
DRB
NaCl 10'
MYC
C
HSP90AA1
D
DRB 60' +
NaCl 2'
NaCl 5'
NaCl 10'
n=4756
pol II ChIP
-1000
-500
TSS
+500
+1000
E
pol II ChIP
DRB
NaCl 2'
NaCl 5'
NaCl 10'
NOP2
F
RT-PCR BrU RNA
control
α-Am 16hr
α-Am 24 hr
GAPDH-1
GAPDH-2
TSK
α-Am 24hr
Control
NaCl
Saponin
Saponin NaCl
pol II ChIP
GAPDH
MYC
RETSTAT
MAT2B
G
pol II ChIP
αAm
αAm NaCl 10'
SLC25A3
H
HSPA1B

Figure S2. Related to Figure 2, 3.

A. Pol II eviction from 5′ ends in high salt is an active process. HCT116 cells were untreated (control) or permeabilized (saponin 0.3mg/ml, 5 min.) plus 150μM NaN3 (Saponin) and then NaCl was added for 10 min. followed by addition of mouse M12 spike and cross-linking. Pol II occupancy at 5′ ends of 5 genes was assayed by ChIP normalized to mouse actin. Note saponin+azide does not reduce pol II occupancy at pause sites and loss of pol II in high salt requires cell viability. Mean and SEM for 4 PCR reactions are shown. Data is the same as for Fig. 2F.

B, C. Turnover of DRB-arrested pol II at 5′ ends of genes. HCT116 cells were treated with DRB (100μM, 60 min.) then NaCl was added and pol II occupancy assayed by ChIP-seq after 10 minutes normalized to a yeast spike-in. This experiment as in Fig. 3C and is a biological replicate of that in Fig. 3A, B.

D. Turnover of DRB-arrested pol II at 5′ ends of genes. HCT116 cells were treated with DRB (100μM, 1 hr.) then NaCl (350 mM final monovalent cations) was added and pol II occupancy assayed by ChIP-seq after 2, 5, and 10 minutes. Metaplots with 25 bp bins of mean pol II ChIP-seq normalized to total mapped reads are shown. This experiment is a biological replicate of that in Fig. 3A, B

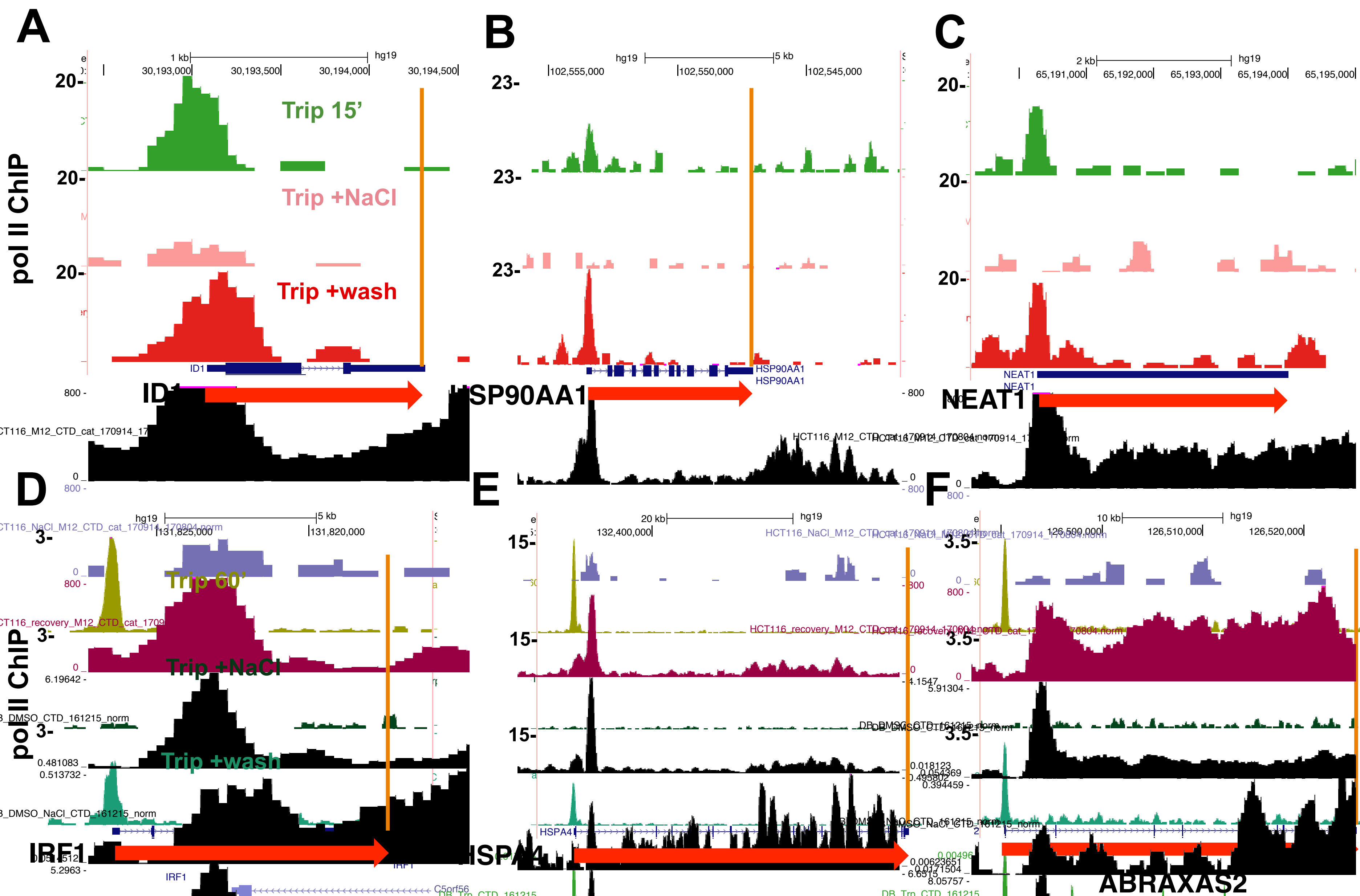
E. UCSC genome browser shot of pol II ChIP-seq signals on NOP2 as in D. Note pol II pile up at the 5′ end and clearance from the gene body in DRB.

F. Inhibition of pol II transcription by α-amanitin (α-Am, 5 μg/ml 16hr or 24hr). Cells were pulse labeled with Bromouridine (2mM, 30 min) and labeled RNA immunoprecipitated with anti-BrdU antibody. BrU labeled RNA was analyzed by qRT-PCR with primer pairs from GAPDH and 7SK transcribed by pol III.

Values are normalized to 7SKsignal then the – amanitin control. Means for 2 PCR reactions are shown.

G-H. Turnover of α-amanitin arrested pol II at 5′ ends of genes. HCT116 cells were treated with α-amanitin (α-Am, 5 μg/ml 24hr) then NaCl was added for 10 min. and assayed by pol II ChIP-seq normalized to a mouse M12 cell spike-in. UCSC genome browser shots are shown.

I. Eviction of α-amanitin arrested pol II from 5′ ends in high salt is an active process. HCT116 cells + α-amanitin (5 μg/ml 24hr) were untreated (control) or permeabilized (saponin 0.3mg/ml, + NaN3 150μM, 5 min.) (saponin) and then NaCl was added (350mM final) for 10 min. Mouse M12 cells were spiked in and pol II occupancy assayed by ChIP-qPCR at the 5′ ends with normalization to the mouse actin 5′ end and - NaCl controls. Mean and SEM for $n \geq 4$ technical replicates.



A
B
C
pol II ChIP
Trip 15'
Trip +NaCl
Trip +wash
ID1
HSP90AA1
NEAT1
D
E
F
Trip 60'
Trip +NaCl
Trip +wash
IRF1
HSPA4
ABRAXAS2
Figure S3

Figure S3. Related to Figure 4.

A-C. Accumulation of "poised" pre-initiation complexes at the TSS during recovery from hypertonic shock in triptolide. HCT116 were treated with triptolide (Trip, 10 μM, 15 min.), then NaCl was added for 10 min. followed by washout with medium containing 10 μM triptolide for 15 min. Pol II ChIP-seq is normalized to M12 spike-in. UCSC genome browser shots are shown as in Fig. 4C.

D-F. UCSC genome browser shots of a biological replicate of the experiment in A-C. Reads are normalized to total mapped reads. Triptolide treatment was for 60 min.

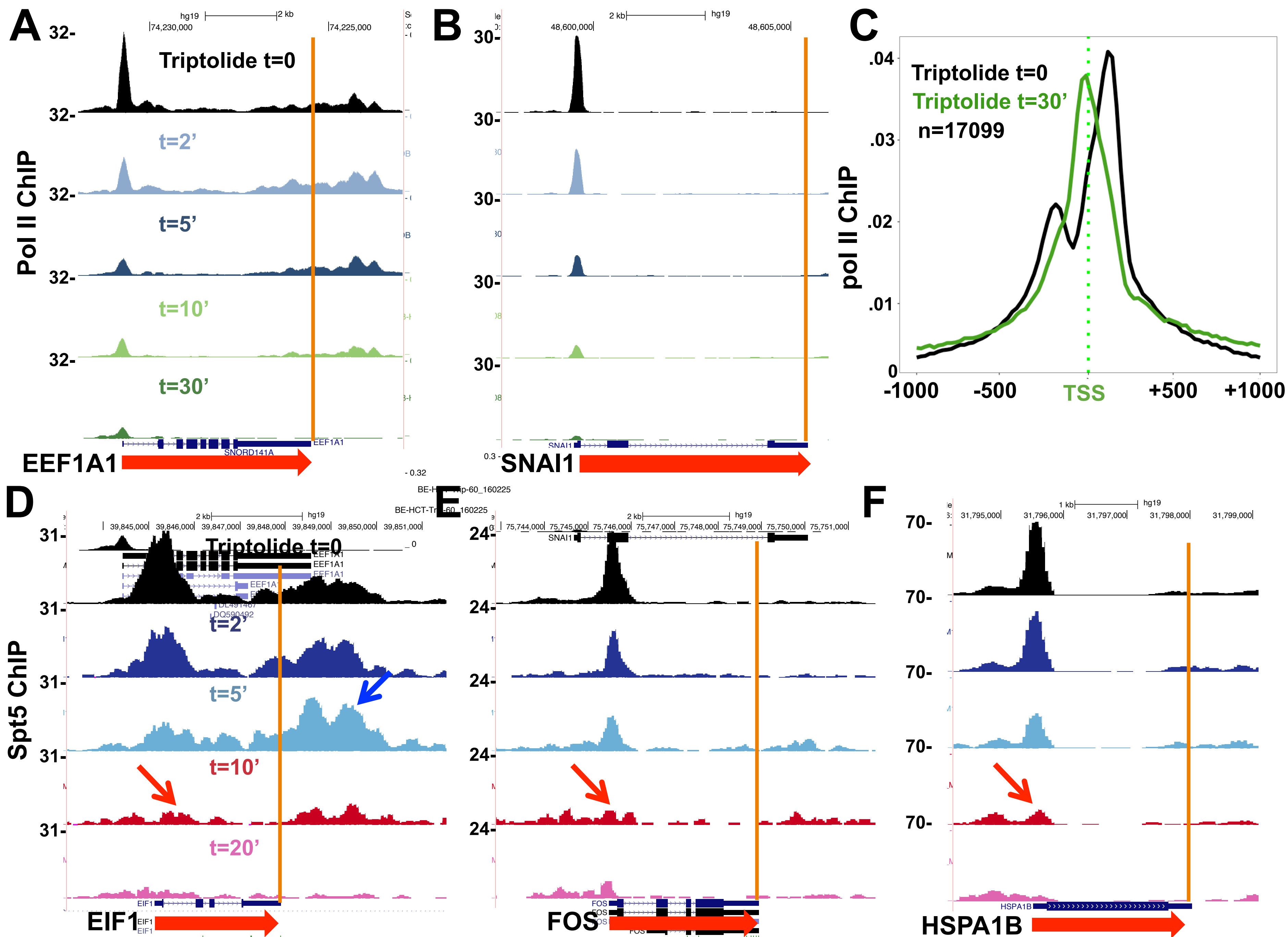


Figure S4

Figure S4. Related to Figure 5.

A, B. Pol II ChIP-seq at time points after adding triptolide (10 μM) to HCT116 cells is normalized to mouse M12 spike-ins as in Fig. 5A-C. UCSC genome browser shots are shown.

C. Metaplots of mean pol II ChIP-signals with 25 bp bins as in A after subsampling so that a total of 0.595 million reads are plotted for each data set. Note that the shift in pol II to be centered over the TSS triptolide is not an artefact of fewer mapped reads.

D-F. Spt5 ChIP-seq at time points after adding triptolide (10 μM) to HCT116 cells normalized to M12 spike-ins. Note rapid loss of Spt5 from 5′ ends (red arrows) relative to the 3′ end of a highly expressed gene (blue arrow). UCSC genome browser shots are shown.