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Supplemental Information

Dynamics of Chromatin and Transcription during Transient Depletion of the RSC Chromatin Remodeling Complex

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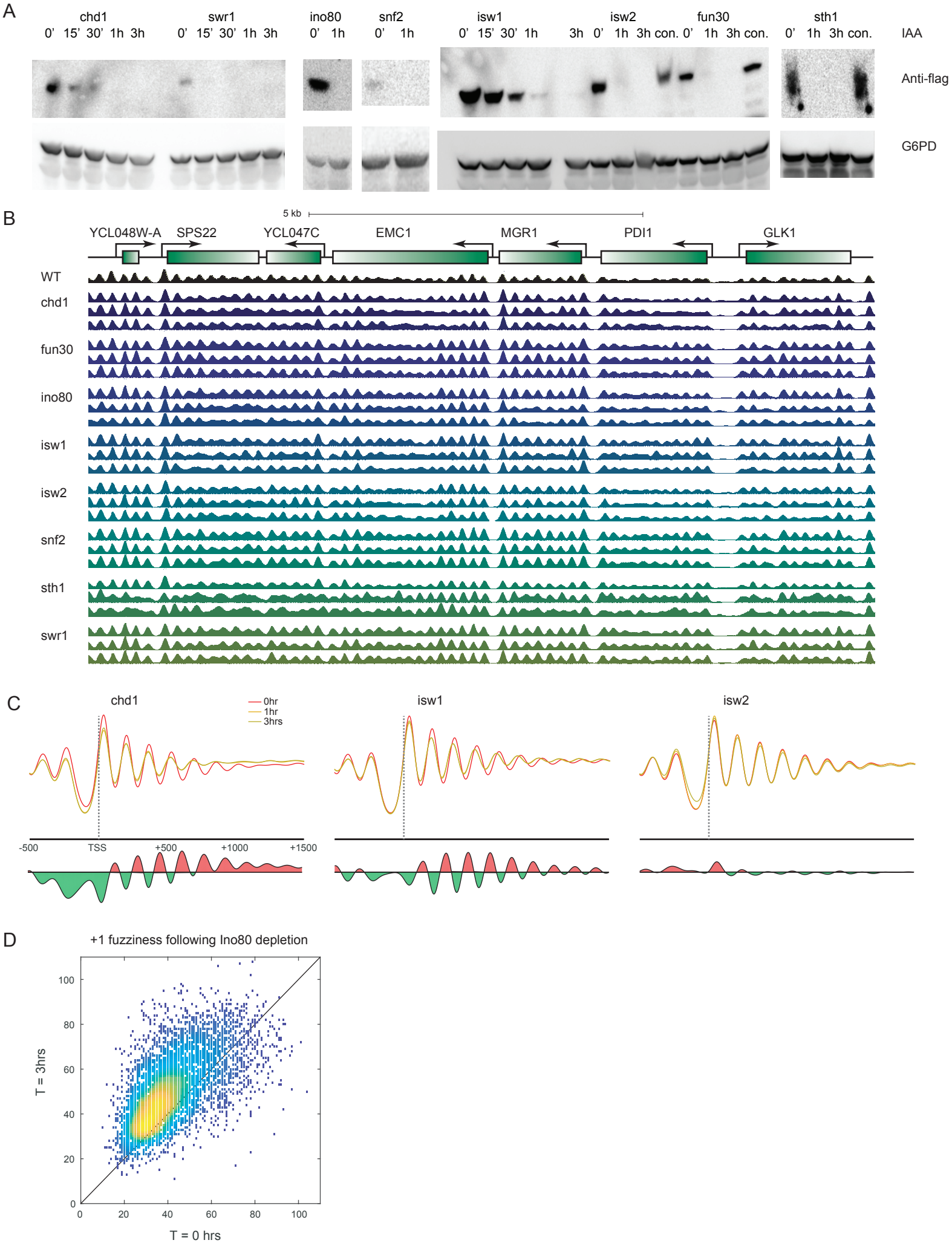

A
chd1
0' 15' 30' 1h 3h
swr1
0' 15' 30' 1h 3h
ino80
0' 1h
snf2
0' 1h
isw1
0' 15' 30' 1h
isw2
3h 0' 1h 3h con.
fun30
0' 1h 3h con.
sth1
0' 1h 3h con.
IAA
Anti-flag
G6PD
B
5 kb
YCL048W-A
SPS22
YCL047C
EMC1
MGR1
PDI1
GLK1
WT
chd1
fun30
ino80
isw1
isw2
snf2
sth1
swr1
C
chd1
0hr
1hr
3hrs
isw1
isw2
-500
TSS
+500
+1000
+1500
D
+1 fuzziness following Ino80 depletion
T = 3hrs
T = 0 hrs
0
20
40
60
80
100

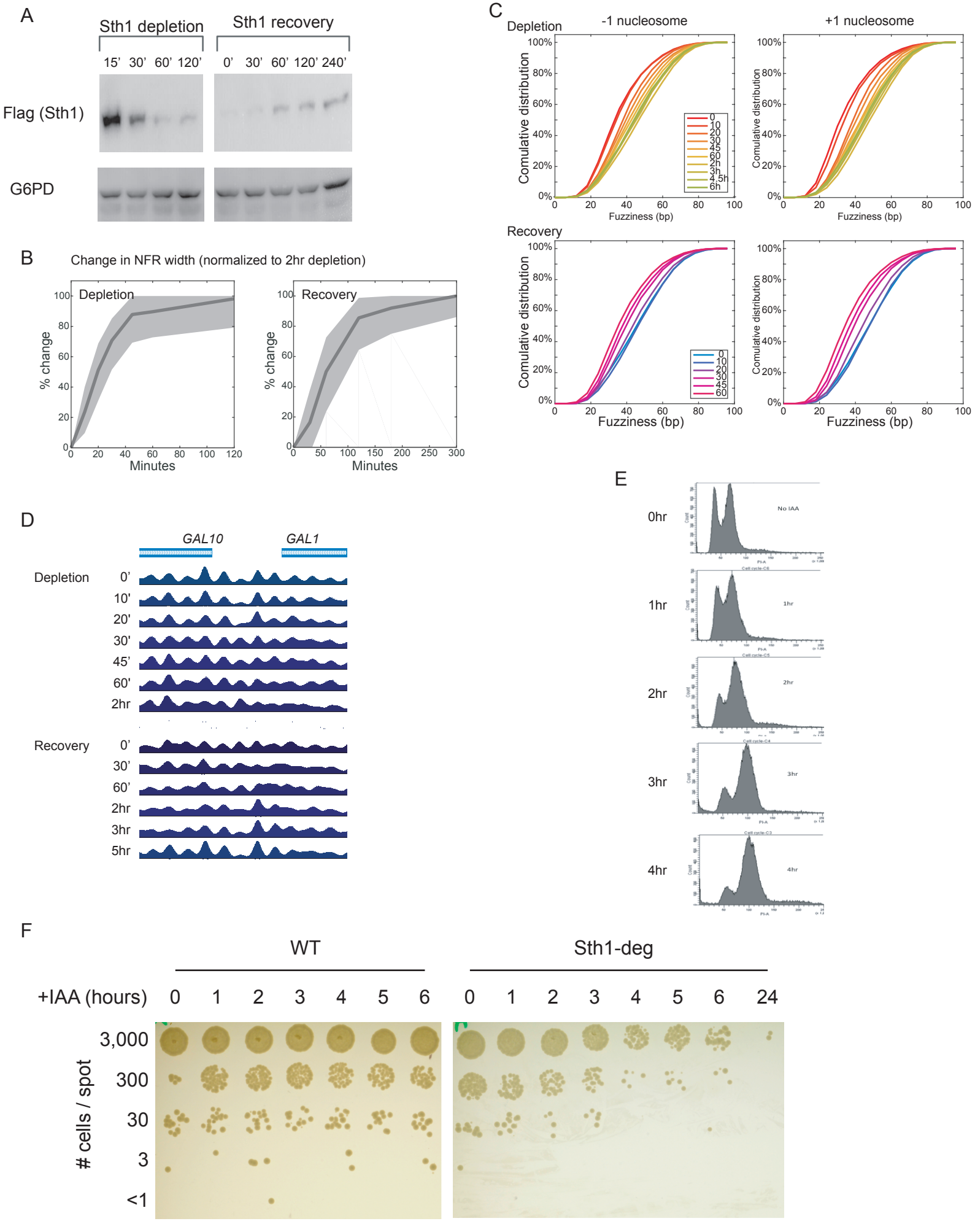
Supplementary Figure 1: Induced knockdown screen of ATP-dependent chromatin remodelers, related to Figure 1

A. Western blot analysis of all 8 ATPAse subunits show drastic reduction of flag-degron-tagged proteins after introduction of auxin.

B. Nucleosome positions in all 8 AID strains before and after induction of auxin in a 10Kb genomic region.

C. Average MNase coverage positioned relative to the TSS, (as in Figure 1C,extended to gene body)

D. Fuzziness of nucleosome +1 of all genes before and 3 hours after auxin induction in Ino80 AID strain. Fuzzyness is defined as the distance between the 20%th and 80%th centers of reads assigned to the same nucleosome peak.


A
Sth1 depletion
Sth1 recovery
15' 30' 60' 120'
0' 30' 60' 120' 240'
Flag (Sth1)
G6PD
B
Change in NFR width (normalized to 2hr depletion)
Depletion
Recovery
% change
Minutes
C
Depletion
-1 nucleosome
+1 nucleosome
Recovery
Comulative distribution
Fuzziness (bp)
0
10
20
30
45
60
2h
3h
4.5h
6h
D
GAL10
GAL1
Depletion
0'
10'
20'
30'
45'
60'
2hr
Recovery
0'
30'
60'
2hr
3hr
5hr
E
0hr
1hr
2hr
3hr
4hr
No IAA
F
WT
Sth1-deg
+IAA (hours)
0 1 2 3 4 5 6 0 1 2 3 4 5 6 24
cells / spot
3,000
300
30
3
<1

Supplementary Figure 2: Dynamics of Sth1 depletion and recovery show massive yet reversible disruptions in chromatin organization, related to Figure 2

A. Western blot analysis of Degron-tagged-Sth1 during addition (Sth1-depletion) and removal (Sth1-recovery) of auxin shows drastic reduction in protein levels after introduction of auxin and partial recovery of the protein following removal of auxin from the media.

B. Percent of change in NFR width at each time point out of the maximal change following Sth1 depletion (left) and recovery (right). Lines are medians, shaded areas include 20-80% of genes

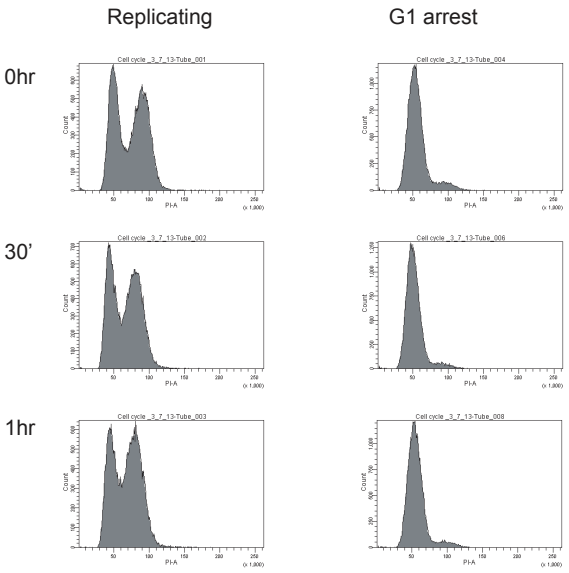
C. Cumulative distribution of the fuzziness of -1 (left) and +1 (right) nucleosomes following Sth1 depletion and recovery.

D. Coverage following Sth1 depletion and recovery in Gal1 and Gal10 promoter area.

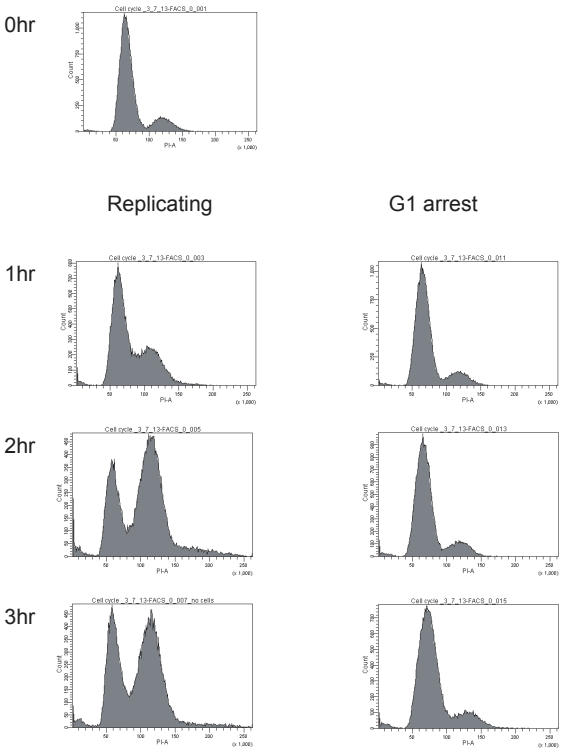
E. Cellular DNA content measured by FACS following RSC depletion. Cells are arrested following Sth1 depletion.

F. Survival test of WT and degron-Sth1 strains following auxin induction. Cells were incubated in the presence of auxin for the indicated times, serial diluted and spotted on YPD plates. Plates were incubated for 2-3 days in 30^0C.

A Depletion



B Recovery

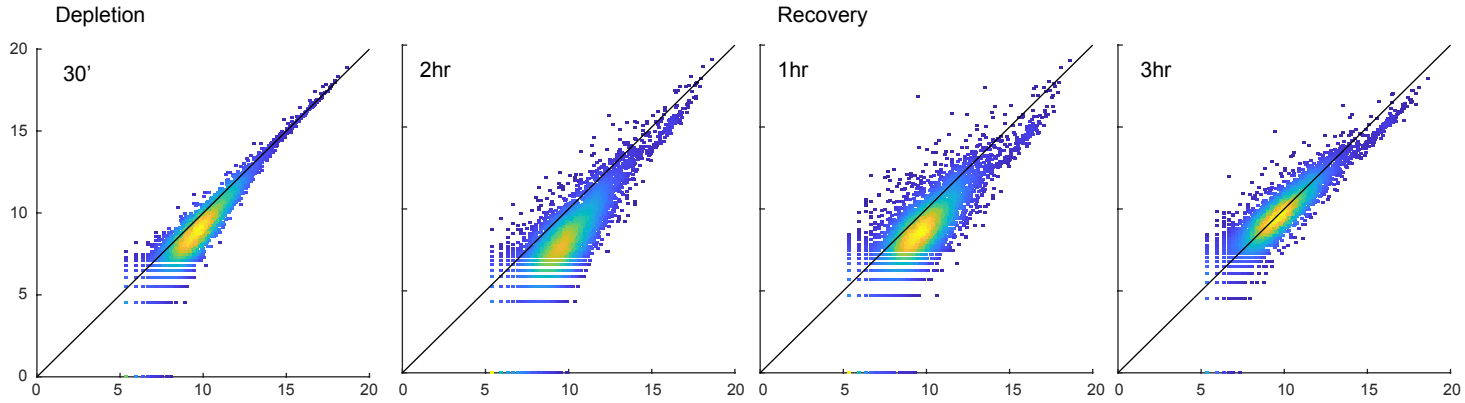


Supplementary Figure 3: Sth1-dependent NFR clearing is replication independent, related to Figure 3

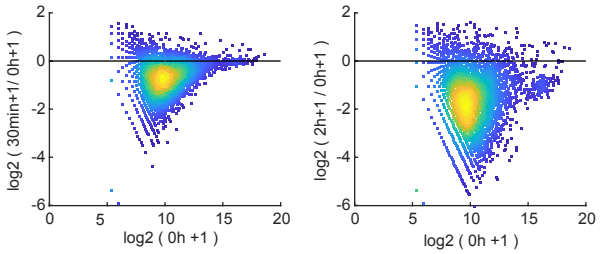
A. Cellular DNA content measured by FACS following RSC depletion in G1 arrested cells and in replicating cells.

B. Cellular DNA content measured by FACS following RSC recovery in G1 arrested cells and in replicating cells.

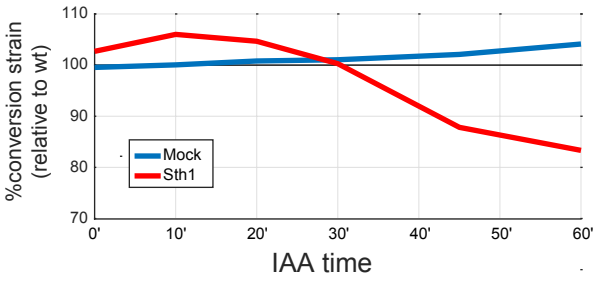
A Gene expression levels compared to pre IAA addition expression (log2)



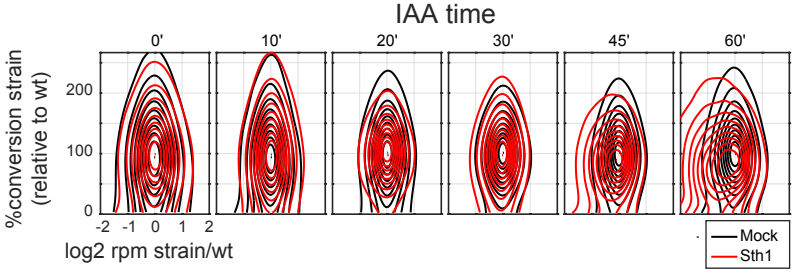
B



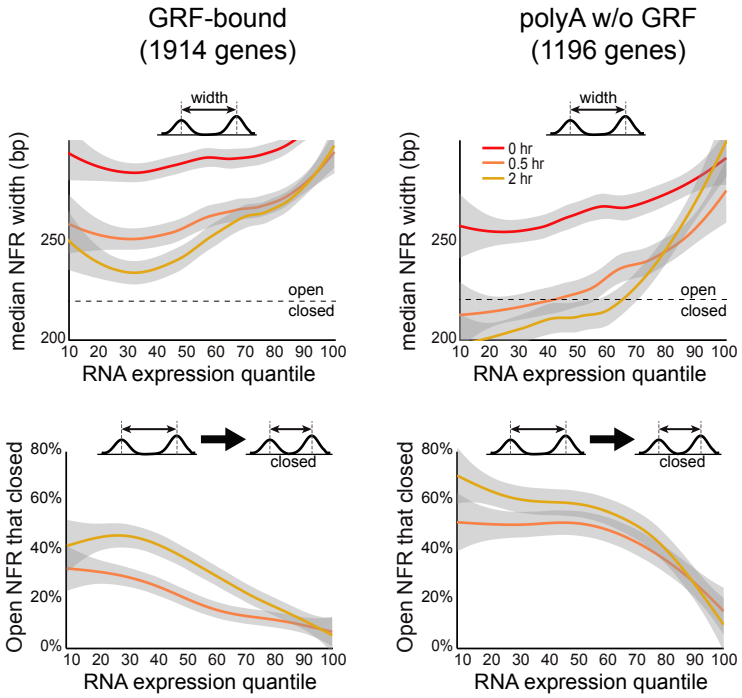
C SLAM-seq conversion rates (total mRNA)



SLAM-seq conversion rates (individual genes)



D



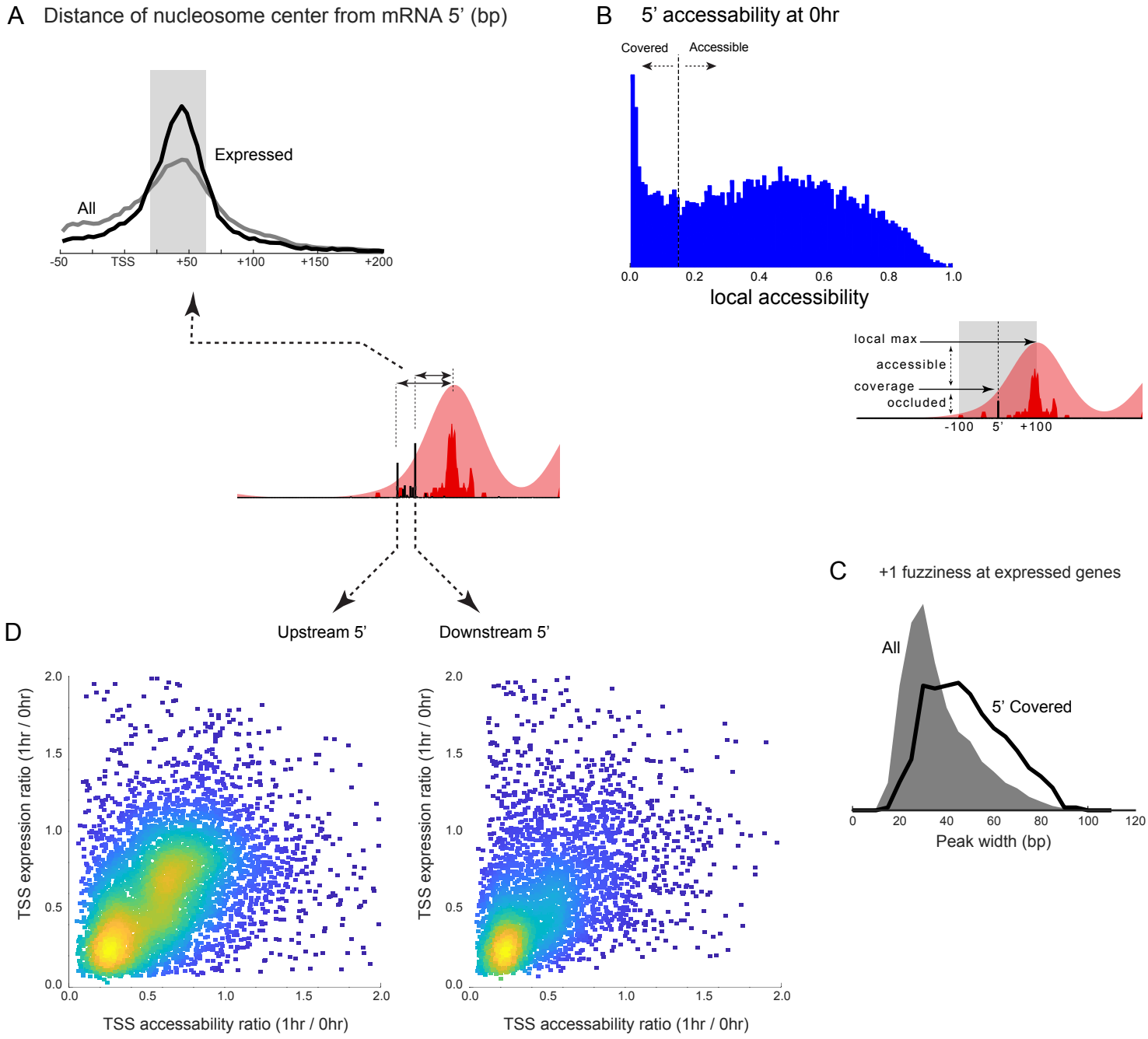
Supplementary Figure 4: RSC maintains open NFRs in lowly-expressed genes but not necessary for acute transcriptional response, related to Figure 4

A. RNA level during Sth1 depletion and recovery vs. RNA level before auxin was added . RNA level was normalized with *K. lactis* spike-in.

B. RNA fold change during Sth1 depletion and recovery vs. RNA level before auxin was added. RNA level was normalized with *K. lactis* spike-in.

C. Changes in rates of RNA synthesis during Sth1 depletion using SLAM-seq (Methods). Changes in percent new RNA (reads w/ converted base) for Sth1 depletion and mock strain in the total mRNA population (left) and distribution of changes for individual genes (right). All changes are shown relative to WT strain processed in parallel.

D. Same as Figure 4C, broken for the two groups of genes shown in Figure 2E.


A
Distance of nucleosome center from mRNA 5' (bp)
Expressed
All
-50
TSS
+50
+100
+150
+200
B
5' accessability at 0hr
Covered
Accessible
0.0
0.2
0.4
0.6
0.8
1.0
local accessibility
local max
accessible
coverage
occluded
-100
5'
+100
Upstream 5'
Downstream 5'
C
+1 fuzziness at expressed genes
All
5' Covered
0
20
40
60
80
100
120
Peak width (bp)
D
TSS expression ratio (1hr / 0hr)
TSS accessability ratio (1hr / 0hr)
0.0
0.5
1.0
1.5
2.0

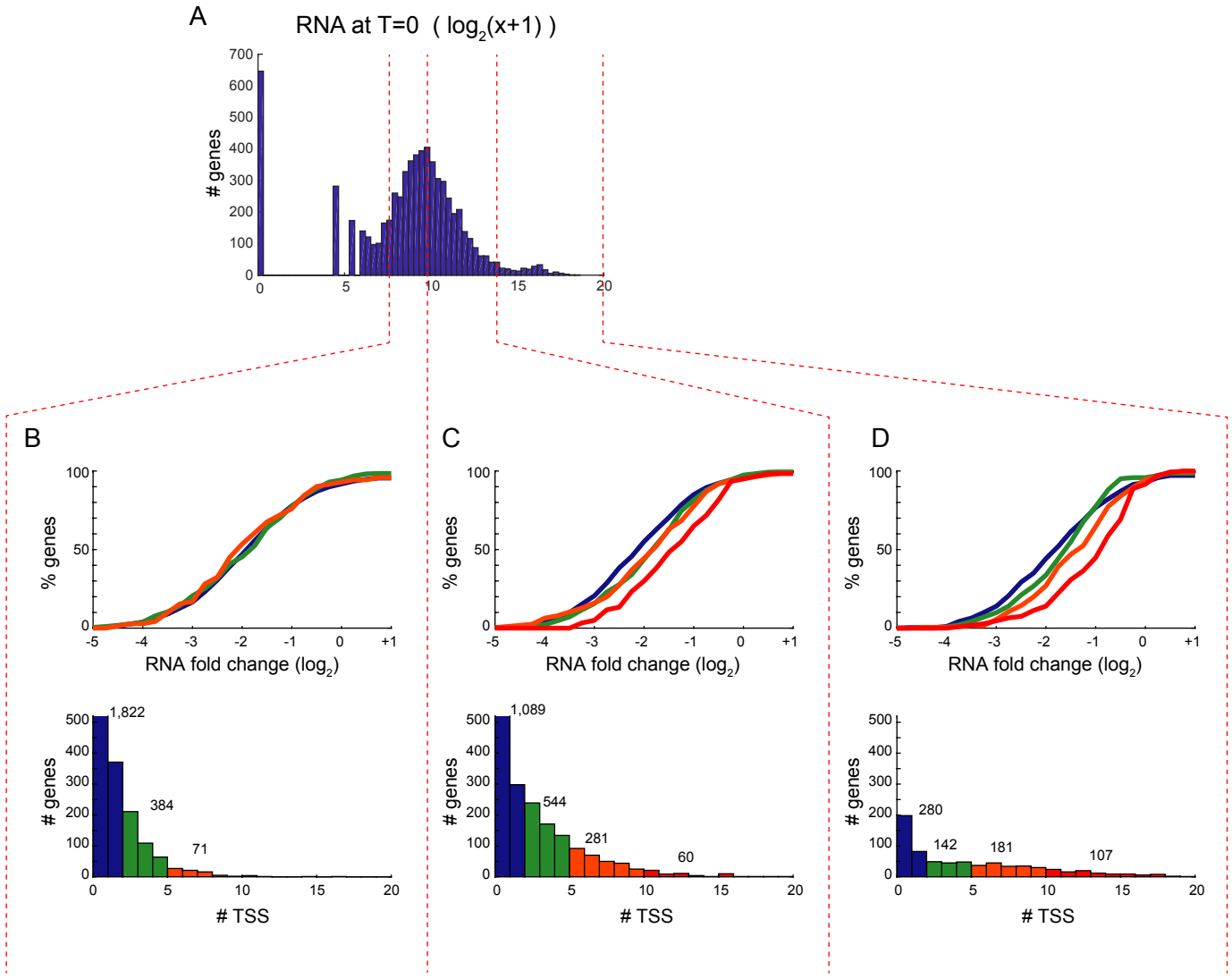
Supplementary Figure 5: Changes in +1 nucleosome position are reflected in TSS usage, related to Figure 5

A. Distribution of the distance of nucleosome center from mRNA 5' position for all genes (grey line) and a subset of expressed gene (black line).

B. Histogram of TSS accessibility measure used to define transcription start sites that are covered by nucleosome.

C. Fuzziness of +1 nucleosome for genes whose 5' locations are covered.

D: As in Figure 5E for two subsets of 5' positions, defined by their location relative to other TSSs of the same gene. Left panel for downstream and right panel for upstream TSS.


A
RNA at T=0 (log2(x+1))
genes
B
C
D
% genes
RNA fold change (log2)
genes
TSS
1,822
384
71
1,089
544
281
60
280
142
181
107

Supplementary Figure 6: Changes in 5' TSS accessibility are indicative of changes in gene expression levels, related to Figure 6

A. Histogram of expression levels (x-axis) of genes, based on 3' RNA-seq before depletion. Three ranges of expression are marked (low, mid, high expressed).

B-D. For each range of expression shown is the cumulative distribution of fold change in RNA following depletion (log base 2) broken down to four classes of genes (top). Also shown is the histogram of number of genes in the range broken down according the number of TSS positions for the gene before depletion.