Supplementary material: Warfield et al (2017)

Supplementary Figures S1-S7

Table S1. Primers used (related to Method Details: cDNA Synthesis and Quantitative PCR of Nascent RNA)

Table S2. Yeast Strains (related to Method Details: Strain Construction)

Table S3. Summary of Spike-normalized Pol II ChIP changes after 3-IAA addition (Related to Figures 2-5, 7, S1, S6)

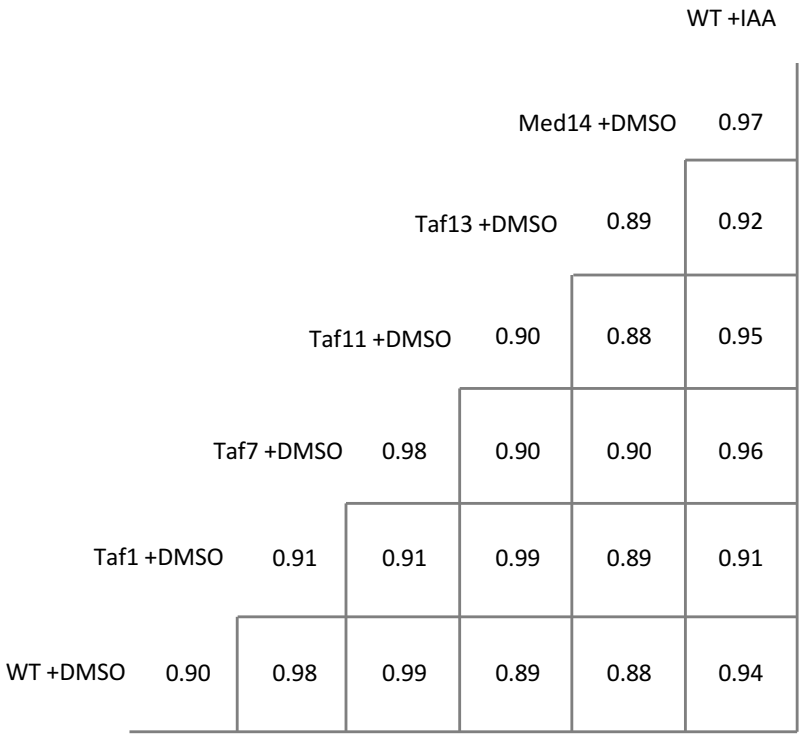
Table S4. TFIID-dependent Gene Clusters (Related to Fig 5)

Table S5. TFIID-dependent GO terms analysis (Related to Fig 5)

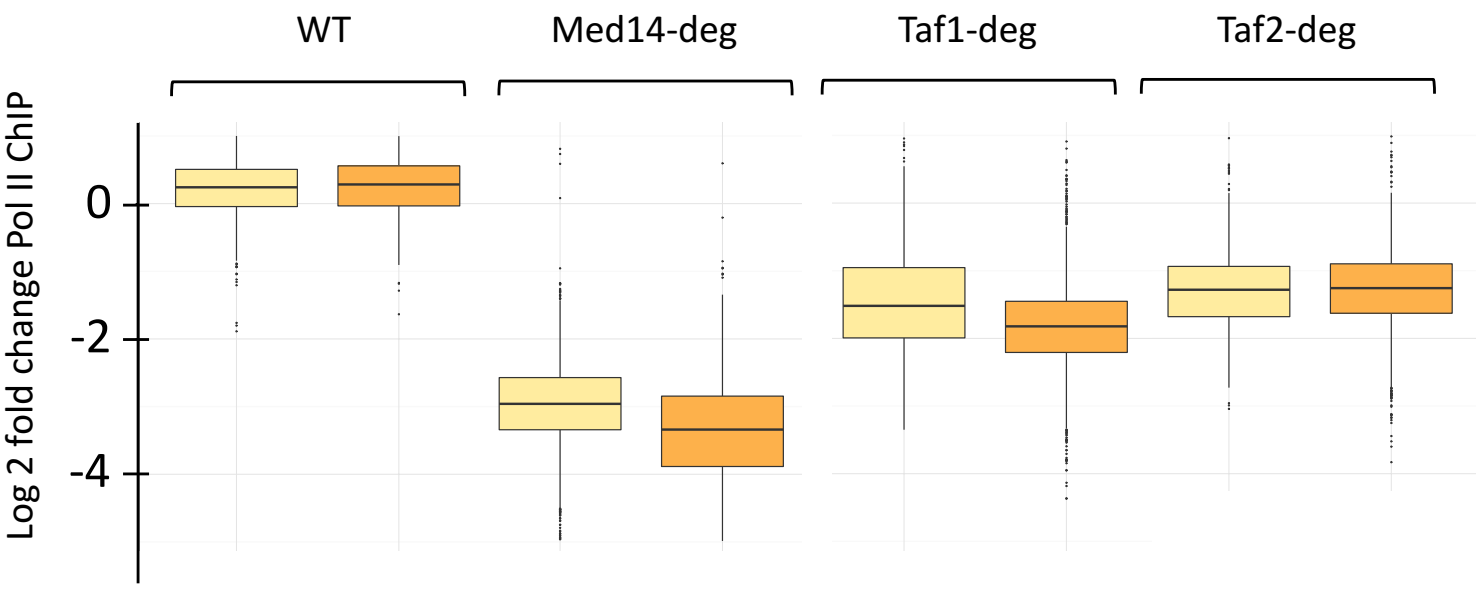
Fig S1

Warfield et al (2017)

A



B



Taf1-enriched = yellow

Taf1-depleted = orange

Fig S2

Warfield et al (2017)

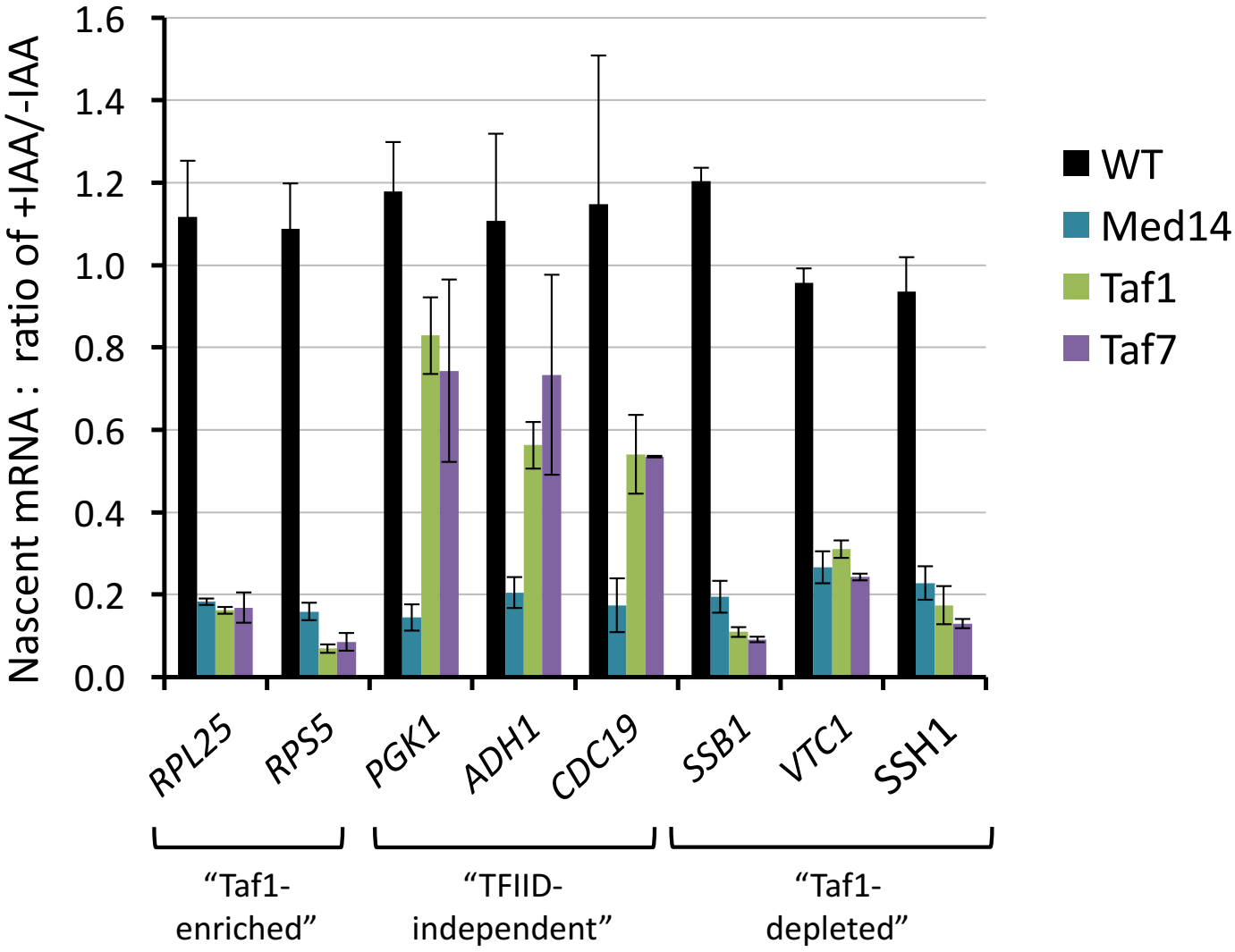
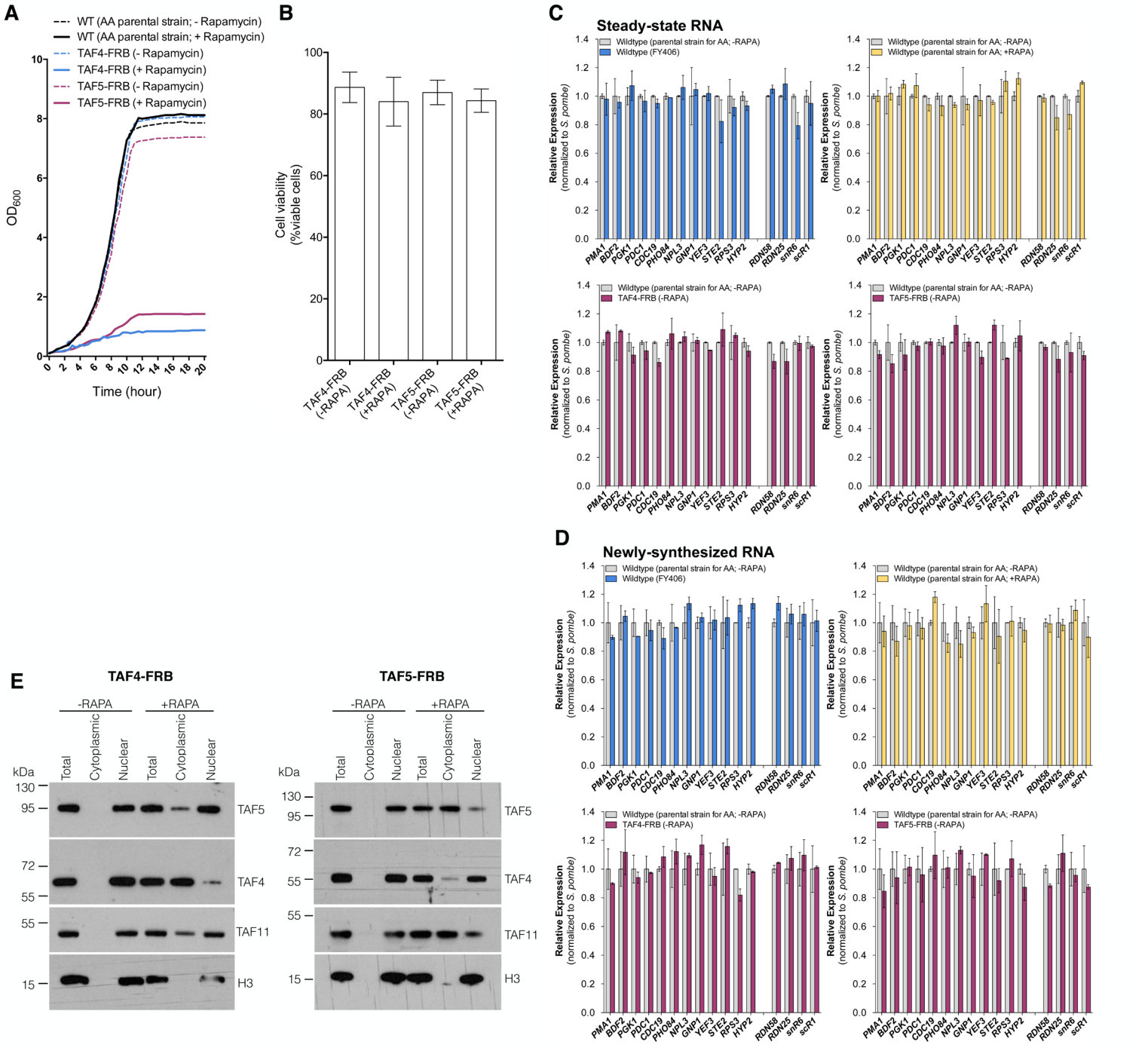
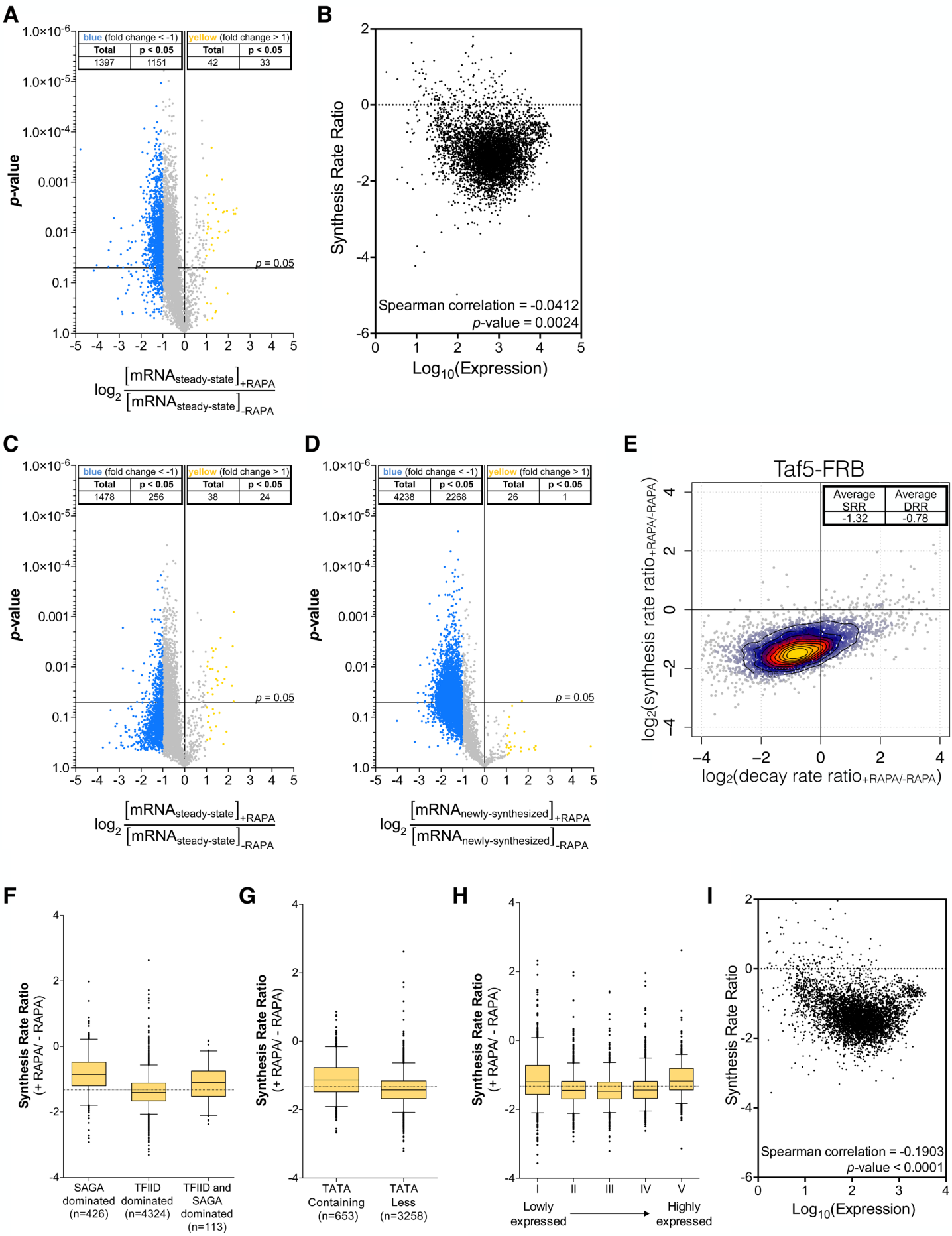


Fig S3

Warfield et al (2017)




A
blue (fold change < -1)
Total
p < 0.05
1397
1151
yellow (fold change > 1)
Total
p < 0.05
42
33
p-value
p = 0.05
log2 [mRNAsteady-state]+RAPA / [mRNAsteady-state]-RAPA
B
Synthesis Rate Ratio
Spearman correlation = -0.0412
p-value = 0.0024
Log10(Expression)
C
blue (fold change < -1)
Total
p < 0.05
1478
256
yellow (fold change > 1)
Total
p < 0.05
38
24
p-value
p = 0.05
log2 [mRNAsteady-state]+RAPA / [mRNAsteady-state]-RAPA
D
blue (fold change < -1)
Total
p < 0.05
4238
2268
yellow (fold change > 1)
Total
p < 0.05
26
1
p-value
p = 0.05
log2 [mRNAnewly-synthesized]+RAPA / [mRNAnewly-synthesized]-RAPA
E
Taf5-FRB
Average SRR
-1.32
Average DRR
-0.78
log2(synthesis rate ratio+RAPA/-RAPA)
log2(decay rate ratio+RAPA/-RAPA)
F
Synthesis Rate Ratio (+ RAPA/ - RAPA)
SAGA dominated (n=426)
TFIID dominated (n=4324)
TFIID and SAGA dominated (n=113)
G
Synthesis Rate Ratio (+ RAPA/ - RAPA)
TATA Containing (n=653)
TATA Less (n=3258)
H
Synthesis Rate Ratio (+ RAPA/ - RAPA)
I
II
III
IV
V
Lowly expressed
Highly expressed
I
Synthesis Rate Ratio
Spearman correlation = -0.1903
p-value < 0.0001
Log10(Expression)

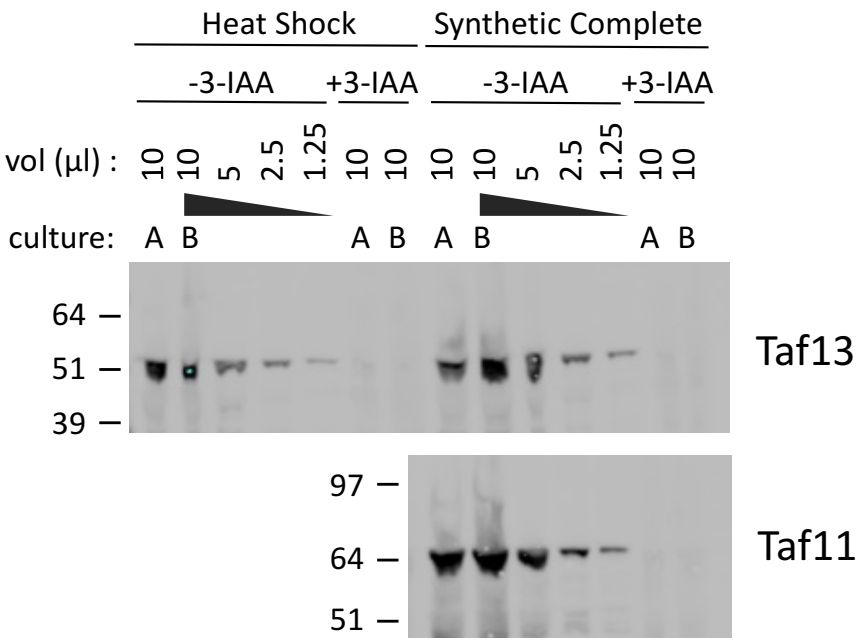
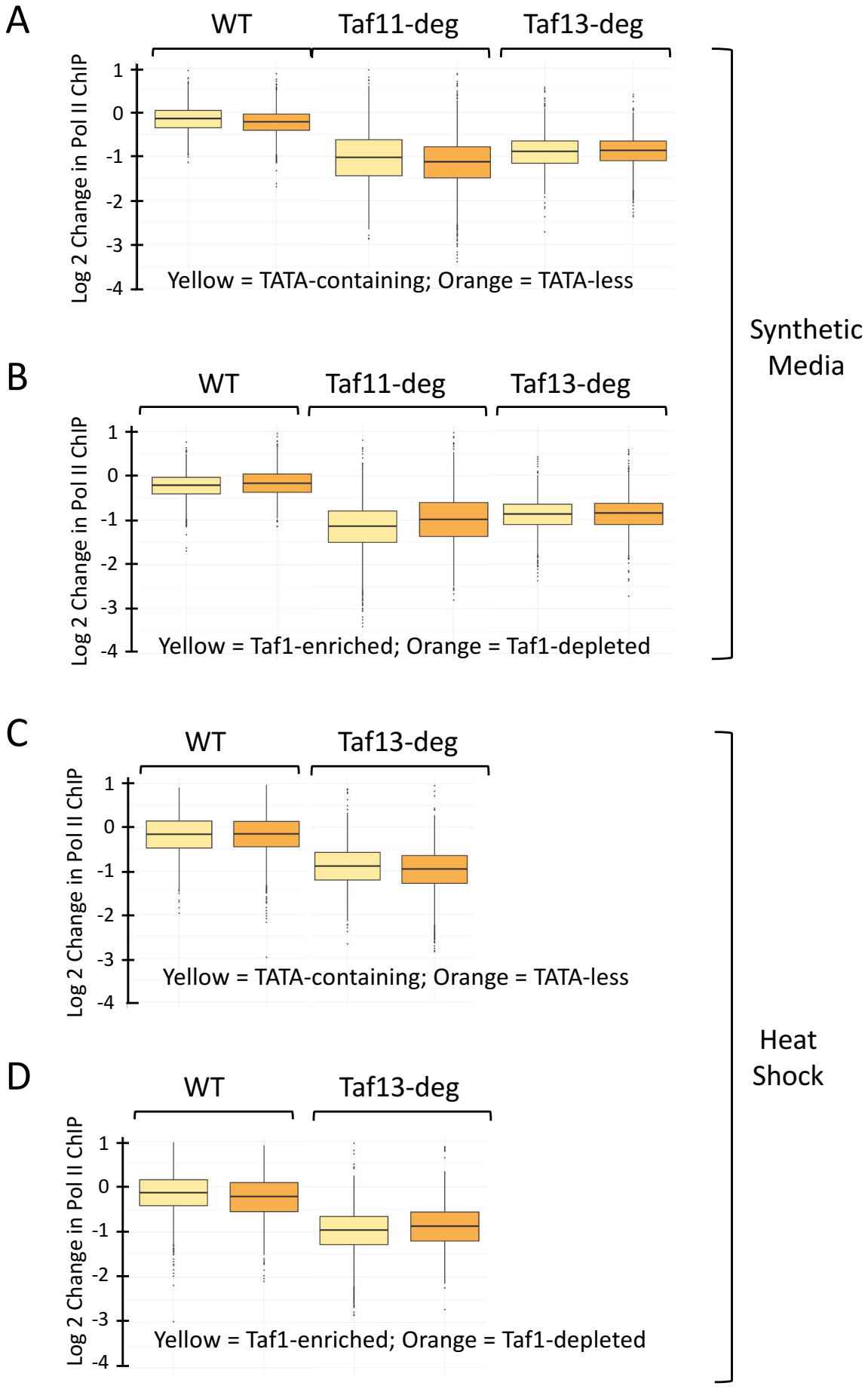
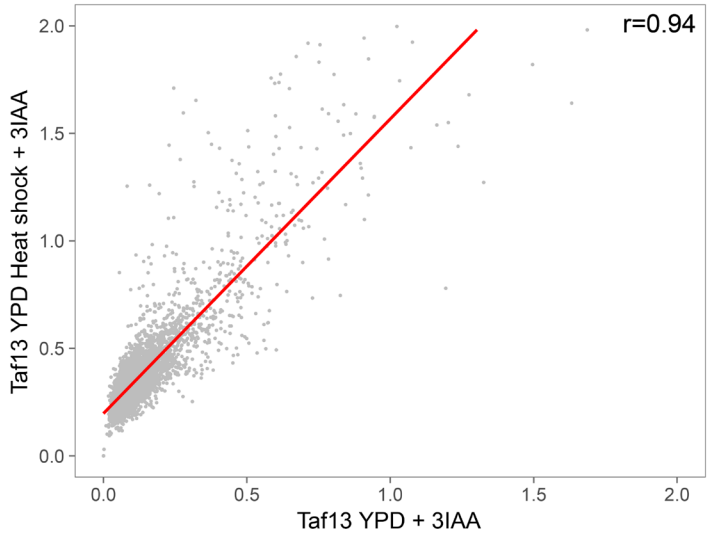
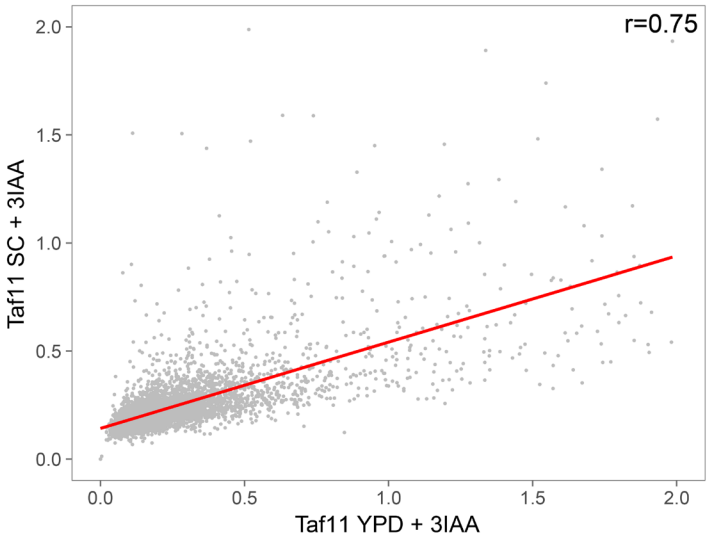

Heat Shock
Synthetic Complete
-3-IAA
+3-IAA
-3-IAA
+3-IAA
vol (µl) :
10 10 5 2.5 1.25 10 10 10 10 5 2.5 1.25 10 10
culture:
A B A B A B A B
64
51
39
Taf13
97
64
51
Taf11

Fig S6

Warfield et al (2017)




r=0.94
Taf13 YPD Heat shock + 3IAA
Taf13 YPD + 3IAA
0.0
0.5
1.0
1.5
2.0


r=0.75
Taf11 SC + 3IAA
Taf11 YPD + 3IAA
0.0
0.5
1.0
1.5
2.0

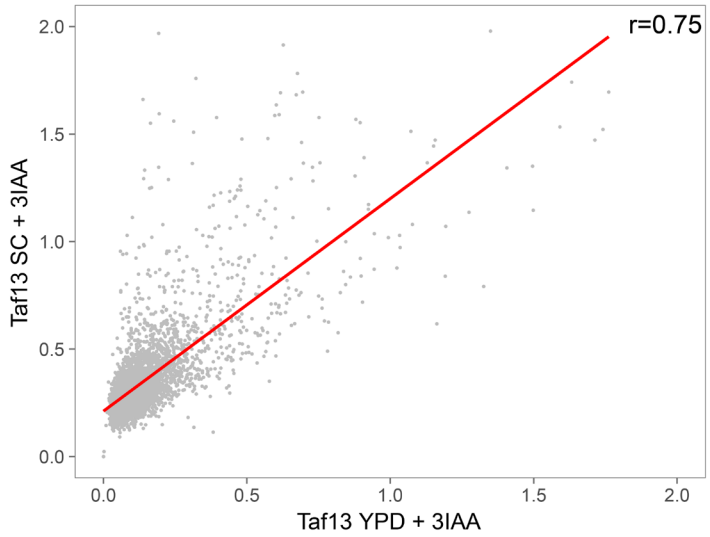

r=0.75
Taf13 SC + 3IAA
Taf13 YPD + 3IAA
0.0
0.5
1.0
1.5
2.0

Figure S1 (related to Figures 1- 4). (**A**) Shown is the Pearson correlation coefficient of Pol II ChIP score between WT + DMSO, WT + 3-IAA and all Degron strains + DMSO. We observe high correlation across the 4808 gene list that we used for all our analysis, indicating that adding the degron tag did not affect transcription in the absence of 3-IAA. (**B**) Genome-wide changes in transcription upon Taf1 and Taf2 depletion. Box plots show the change in native Pol II ChIP on a log2 scale from 4807 genes comparing DMSO and 3-IAA addition. WT and Med14-degron data from Fig 3 are shown for comparison.

Figure S2 (related to Figures 2-5). Analysis of changes in nascent mRNA levels upon Mediator and TFIID-depletion. RT qPCR analysis of 4-thio Uracil labeled mRNAs purified from cells treated with either DMSO or 3-IAA in the indicated degron-tagged strains. Samples were normalized by spike-in of labeled *S. pombe* cells before mRNA isolation. Genes are grouped by previously published categories (Kuras et al., 2000; Li et al., 2000; Rhee and Pugh, 2012).

Figure S3. (related to Figure 6) Impact and depletion efficiency in Taf4 and Taf5 anchor-away strains. **(A)** Addition of FRB domain to either Taf4 or Taf5 does not affect growth of these strains in the absence of rapamycin, while long term exposure to rapamycin leads to lethality. **(B)** After 30 min of exposure to rapamycin, viability of cells in log-phase is not affected, in comparison to its counterpart with vehicle only. **(C-D)** Fusion of FRB domain to either Taf4 or Taf5 does not affect RNA Pol II transcription, both at the steady-state (**C**) or newly-synthesized RNA (**D**) levels. **(E)** Cell fractionation depicting efficient nuclear depletion of both Taf4 and Taf5 upon exposure to rapamycin.

Figure S4 (related to Figure 6). cDTA analyses of Taf4 and Taf5 anchor-away. (**A**) Volcano plot showing changes in steady-state mRNA levels for Taf4 depletion by anchor-away. (**B**) Scatter plot showing a lack of correlation between changes in synthesis rates upon Taf4 depletion and gene expression levels in the wild-type strain. (**C-I**) Nuclear depletion of Taf5 reduced the transcription of a large number of genes. Volcano plots showing changes in steady-state (**C**) and newly-synthesized (**D**) mRNA levels in a *TAF5-FRB* strain treated or

untreated with rapamycin. Genome wide changes in mRNA synthesis rates and decay rates upon Taf5 nuclear depletion (**E**). Changes in synthesis rates according to the gene category were analyzed as in Fig. 6 (**F-I**). All results were obtained from two independent biological replicates.

Figure S5 (related to Figure 7). Degron efficiency in alternative growth conditions. Western blot of degron efficiency in cells grown under heat shock stress and in synthetic complete media. Experiment was done in biological duplicate (A and B cultures). Variable amounts of the -3-IAA "B" samples were loaded to allow quantitation of degron efficiency. The positions of molecular weight markers are indicated.

Figure S6 (related to Figure 7). Nearly all genes are sensitive to TFIID depletion in cells grown in synthetic media and undergoing heat shock stress. (**A and B**) Data from cells grown in synthetic media from Fig 7A were split into TATA-containing, TATA-less, "Taf1-enriched" and "Taf1-depleted" gene categories and plotted as in Fig 4. (**C and D**) same as A and B except data from cells under heat shock stress from data in Fig 7B.

Figure S7 (related to Fig 7) Comparison of genome-wide transcription after Taf-depletion in different growth conditions. Shown in the scatterplot are the spike-normalized Pol II ChIP signals after 3-IAA addition to the indicated degron strains. Pol II ChIP signals from synthetic complete media (SC) and heat shock in YPD are compared to strains grown in YPD at 30 deg.

Table S1. Primers (related to Method Details: cDNA Synthesis and Quantitative PCR of Nascent RNA)

Gene	Primer Name	Sequence
RPS5	RPS5_F	GTCGTCTTGGCTACTCCAATTC
	RPS5_R	GTCAACCAAAGAAGCATCCTTAAC
RPL25	RPL25_F	GCTCCATCTGGTATGTGAACTG
	RPL25_R	GCACTATTCTTGTCGTCGGATAG
PGK1	PGK1_F	AGCGTGTCTTCATCAGAG
	PGK1_R	TGGCAAAGCAGCAACAA
ADH1	ADH1_F	CTTCTACGAATCCCACGGTAAG
	ADH1_R	GTGTGACAGACACCAGAGTATT
CDC19	CDC19_F	CAAAGACCAACAACCCAGAAAC
	CDC19_R	GGTATTCGTAAGAACCGTGAGAG
SSB1	SSB1_F	CGTCATTACTGTCCCAGCTTAC
	SSB1_R	GGCAGTAGGTTCGTTGATGATA
SSH1	SSH1_F	CCCAAAGCTACCACACCTAAT
	SSH1_R	ACCCACTAGAAATGTTGGGAAA
VTC1	VTC1_F	TGCCAATGAGCGTACCTTT
	VTC1_R	TGCACTGACCCTACCTATCT
S. pombe tubulin (NDA3)	Tubulin_F	CCGCTGGTGGAAAGTATGTT
	Tubulin_R	GCCAATTCAGCACCTTCAGT

Table S2. Yeast Strains (related to Method Details: Strain Construction)

Strain	Background	Description
SHY1058	972h-	*S. pombe*: RPB3-3xFlag::KanMX
SHY1035	BY4705	*S. cerevisiae*: *mat alpha delta ade2::hisG his3 delta 200 leu2 delta 0 lys2 delta 0 met15 delta 0 trp1 delta 63 ura3 delta 0 RPB3-3X Flag::NatMX*
SHY1036	SHY1035	*S. cerevisiae*: *RPB3-3x Flag::NatMX, pGPD1-OSTIR::HIS3 (constitutively expressed OSTIR integrated at HIS3)*
SHY1039	SHY1036	*S. cerevisiae*: *TAF1-3xV5 IAA7::KanMX, RPB3-3x Flag::NatMX, pGPD1-OSTIR::HIS3*
SHY1041	SHY1036	*S. cerevisiae*: *TAF7-3xV5 IAA7::KanMX, RPB3-3x Flag::NatMX, pGPD1-OSTIR::HIS3*
SHY1042	SHY1036	*S. cerevisiae*: *TAF11-3xV5 IAA7::KanMX, RPB3-3x Flag::NatMX, pGPD1-OSTIR::HIS3*
SHY1043	SHY1036	*S. cerevisiae*: *TAF13-3xV5 IAA7::KanMX, RPB3-3x Flag::NatMX, pGPD1-OSTIR::HIS3*
SHY1054	SHY1036	*S. cerevisiae*: *MED14-3xV5 IAA7::KanMX, RPB3-3x Flag::NatMX, pGPD1-OSTIR::HIS3*
MJE110	W303	*S. cerevisiae: MATalpha tor1-1; fpr1::NAT; RPL13A-2xFKB12::TRP1; TAF4-FRB::kanMX6*
MJE112	W303	*S. cerevisiae: MATalpha tor1-1; fpr1::NAT; RPL13A-2xFKB12::TRP1; TAF4-FRB::kanMX6*