Supplemental material for

Architectural Mediator subunits are differentially essential for global transcription in *Saccharomyces cerevisiae*

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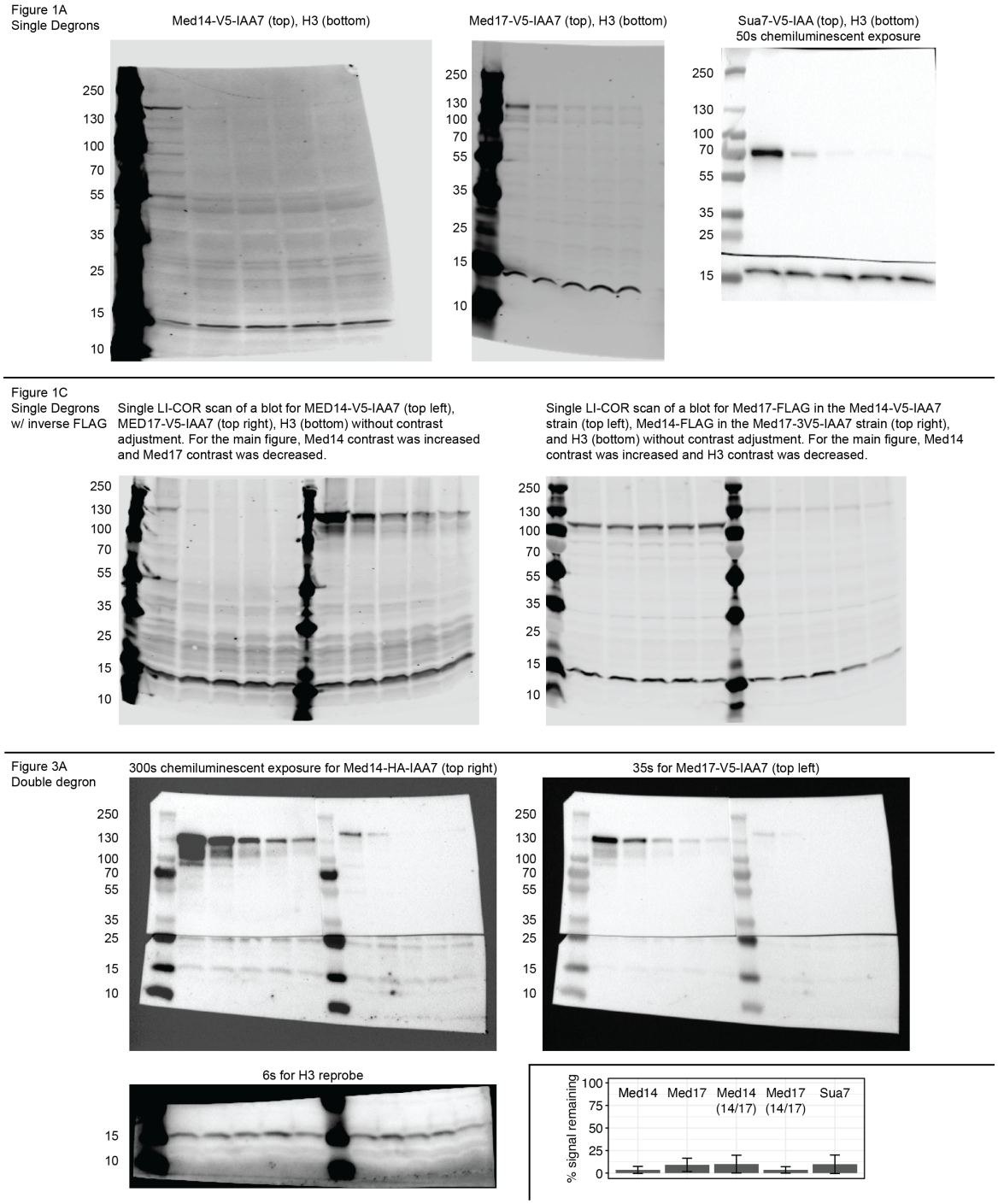

Figure S1. Full western blot scans

Shown at the bottom right of the figure is a barplot of quantification of western blot signal (mean + SD) for the indicated AID-tagged protein following 30 min 3-IAA treatment (14/17 indicates that the protein was quantified in the dual Med14/17-AID strain). We performed two replicates apiece for the single-degron strains (Figure 1A), the degron/FLAG strains (Figure 1C), and the double-degron strain (Figure 3A). We considered the single-degron Mediator and Mediator degron/FLAG strains together (as they are isogenic save for the FLAG tag) for a total of 4 data points. In all cases, depletion is very efficient, but there is relatively large variability between replicates in some cases due to the difficulty of accurately quantifying low signal.

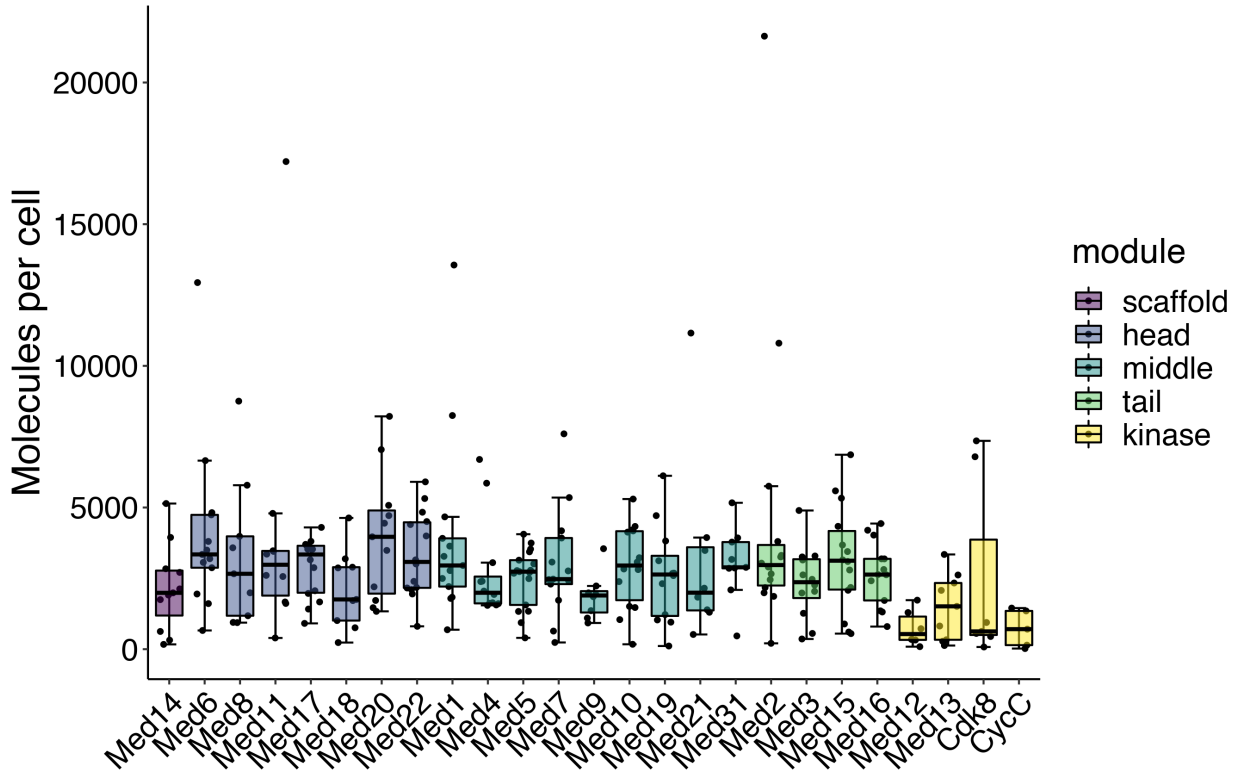

Figure S2. Comparison of Mediator subunit abundances

Box and jitter plot displaying molecules per cell for each Mediator subunit from the proteomic studies summarized in (Ho *et al.* 2018). Whiskers represent the smallest and largest values up to IQR * 1.5. The median abundances of all head subunits were greater than that of Med14 (2,661 – 3,966 molecules per cell) with the exception of Med18 (1,756 molecules per cell). Three middle subunits (Med4, Med9, and Med21) displayed similar abundances to Med14 (1,895 – 1,994 molecules per cell), while the remainder were present at median levels higher than that of Med14 (2,473 – 2,954 molecules/cell). Tail subunits were present at median abundances greater than Med14 (2,364 – 3,123 molecules per cell), while kinase subunits were less abundant than Med14 (534 – 1,512 molecules per cell).

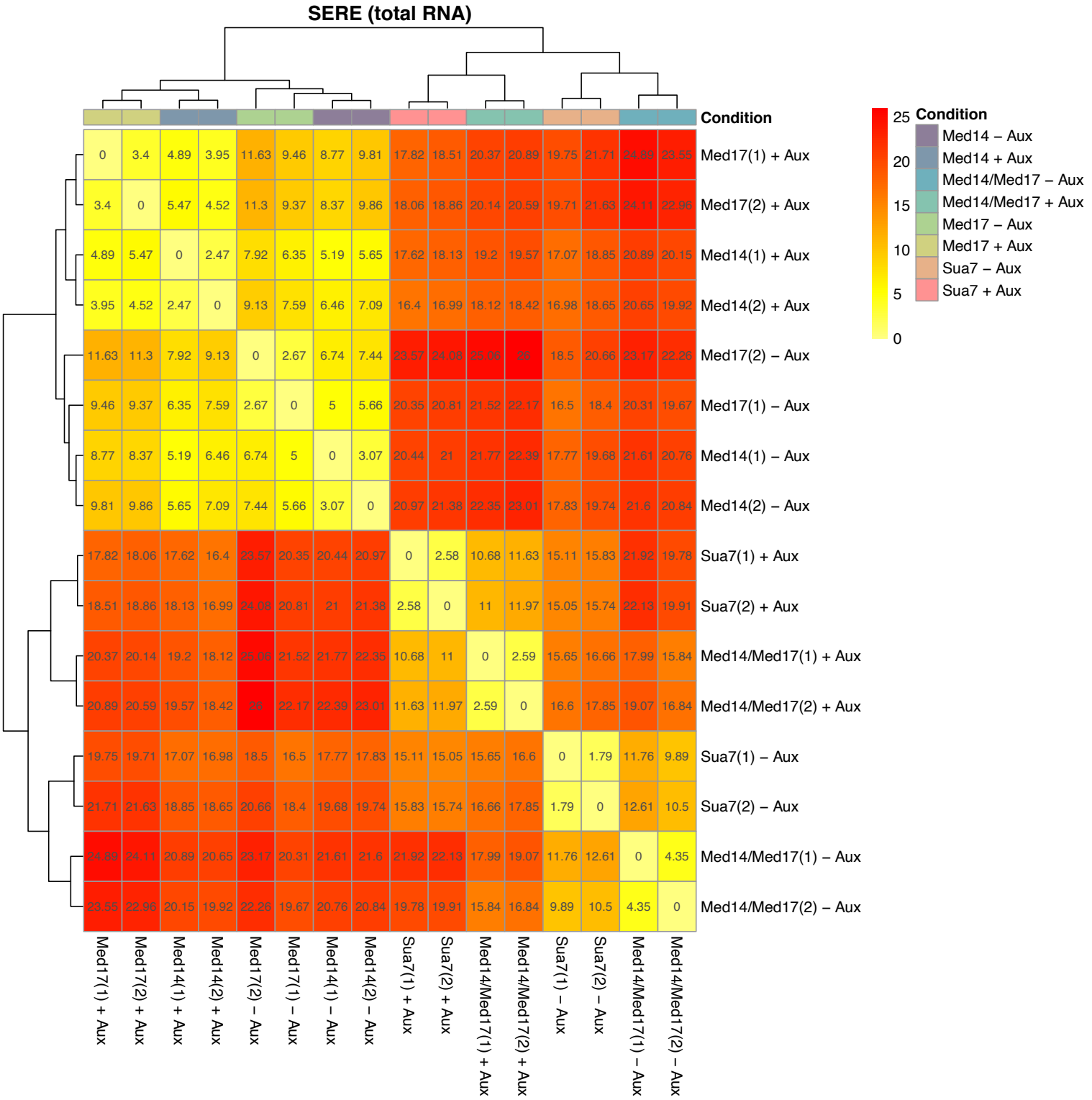

Figure S3. SERE analysis of total RNA-seq datasets

Heatmap of SERE values for all total RNA-seq samples analyzed in this study. We note that the Med14-AID and Med17-AID datasets correlate well with one another while the Sua7-AID and Med14/17-AID datasets cluster separately. This is likely due to the different sequencing configurations used for these datasets (see Materials and Methods).

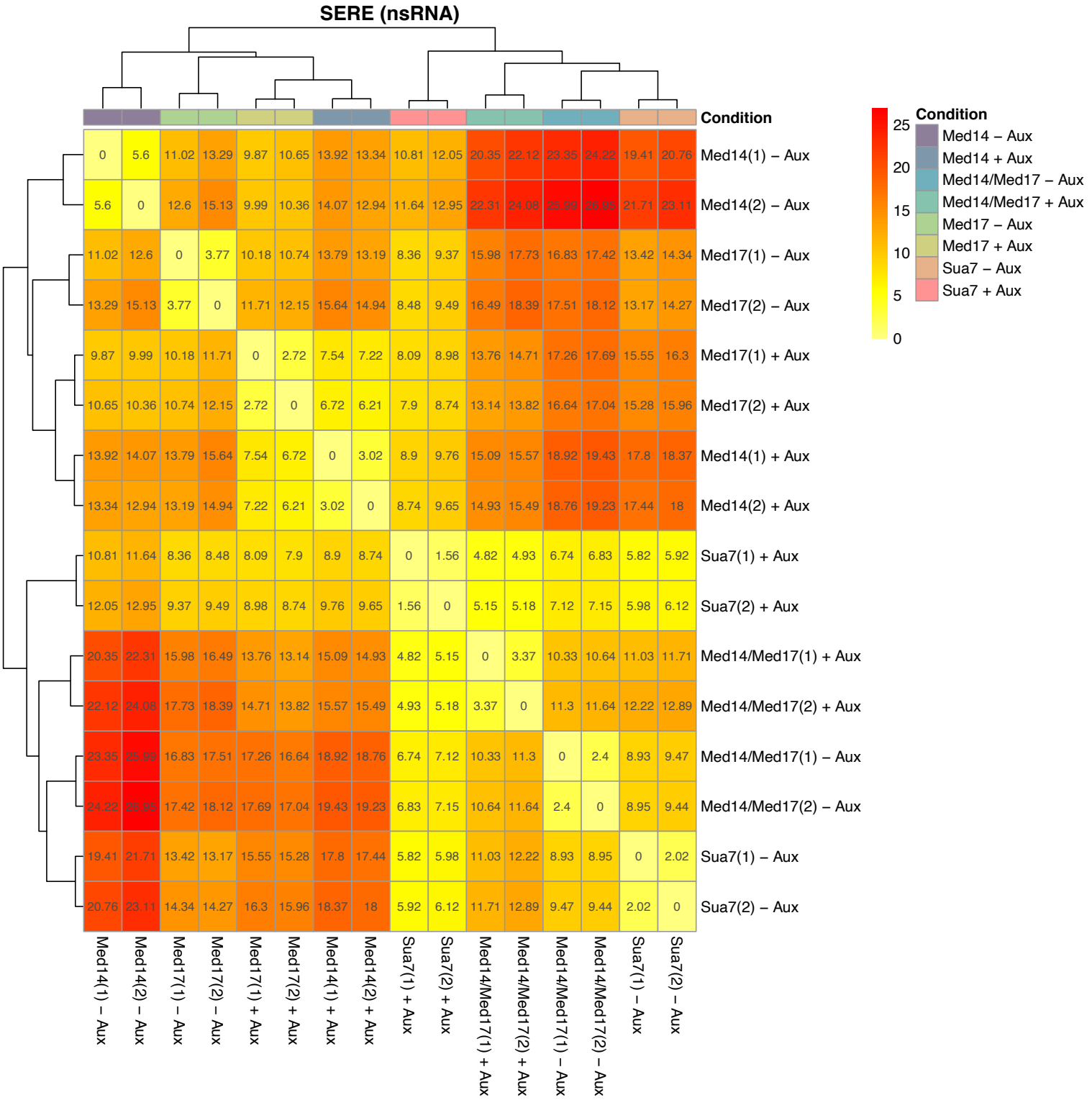

Figure S4. SERE analysis of 4tU-seq datasets

Heatmap of SERE values for all 4tU-seq samples analyzed in this study. We note that the Med14-AID and Med17-AID datasets correlate well with one another while the Sua7-AID and Med14/17-AID datasets cluster separately. This is likely due to the different sequencing configurations used for these datasets (see Materials and Methods).

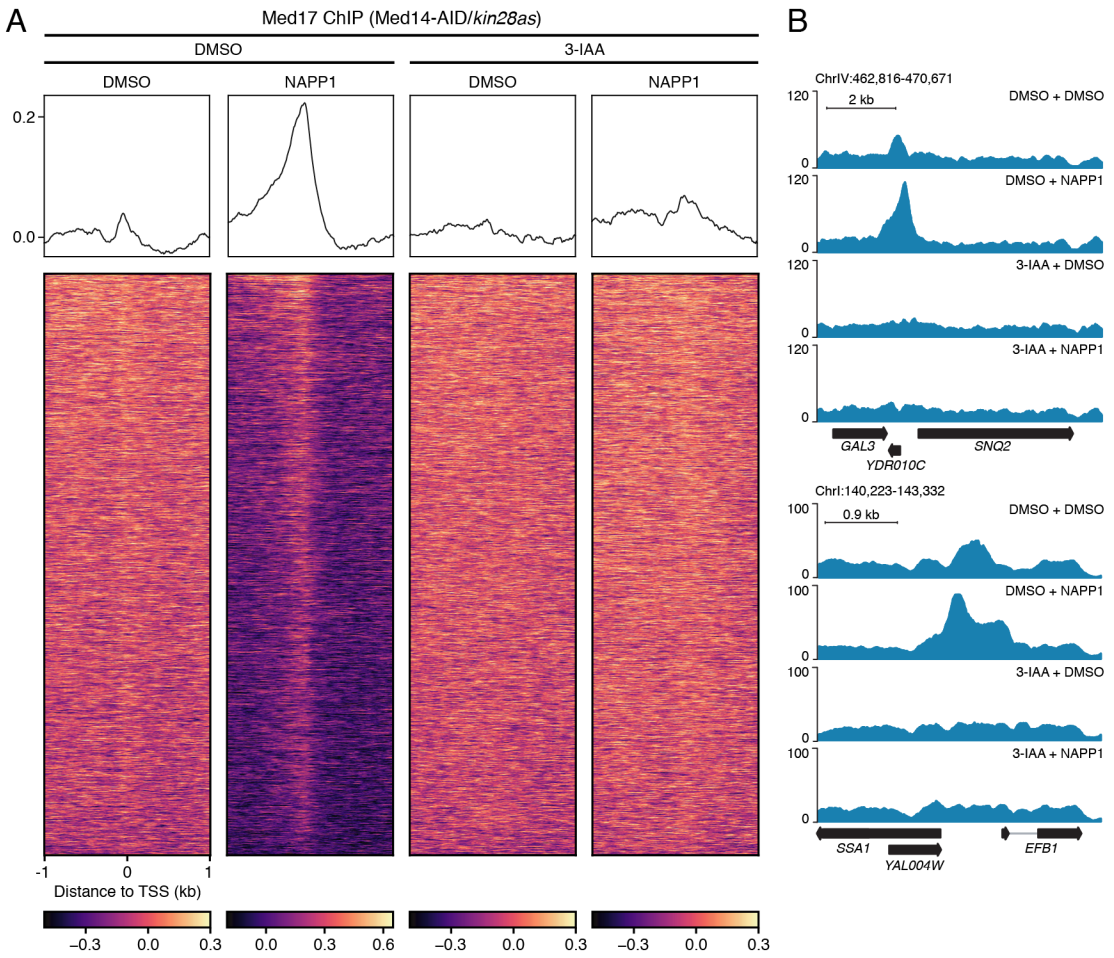

Figure S5. Med14 depletion removes Med17 from promoters

(A) Average plots and heatmaps of $\log_2$(Med17-FLAG/no-tag) ChIP-seq signal around TSSs following one of the four indicated pre-crosslinking treatments: 15 min DMSO or 500 μM 3-IAA followed by 15 min of DMSO or 6 μM NAPP1. (B) Visualization of CPM-normalized Med17 ChIP-seq data for each of the four treatments at two representative regions of the yeast genome.

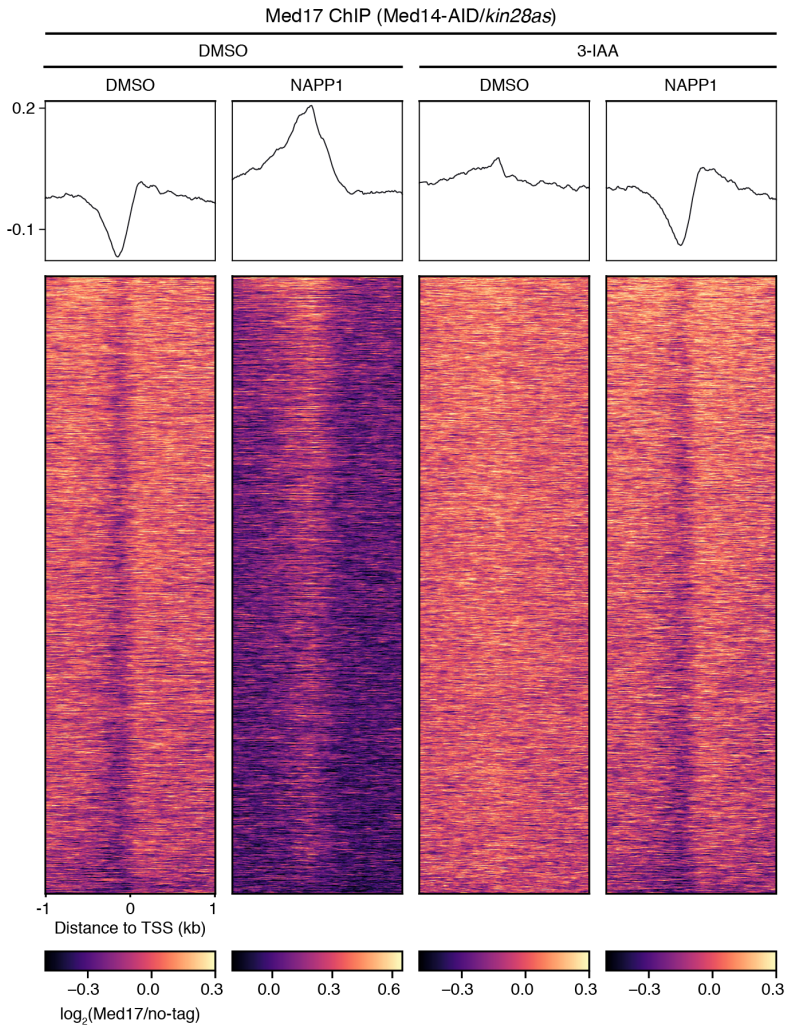

Figure S6. Med17 ChIP-seq replicate analysis

Average plots and heatmaps of replicate log_2(Med17/no-tag) ChIP-seq signal around TSSs following treatment as in Figure S5.

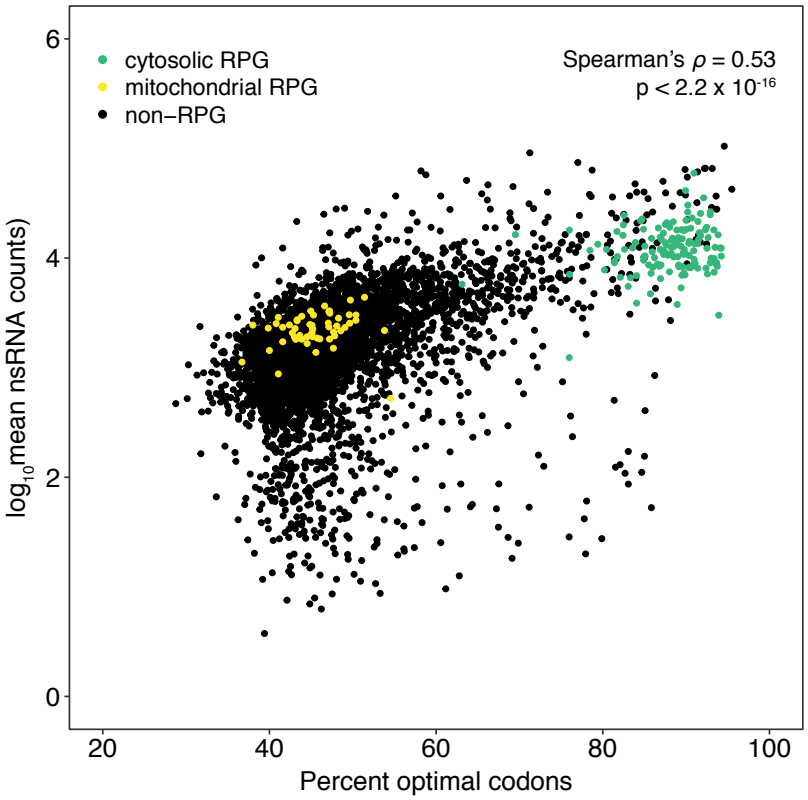

Figure S7. Comparison of codon optimality and transcription

Scatterplot comparing the optimal codon percentage and average normalized nsRNA levels of mRNA genes (n = 5,011) across all DMSO-treated samples. Cytosolic and mitochondrial RPGs are highlighted as control gene groups with high and low codon optimality, respectively.

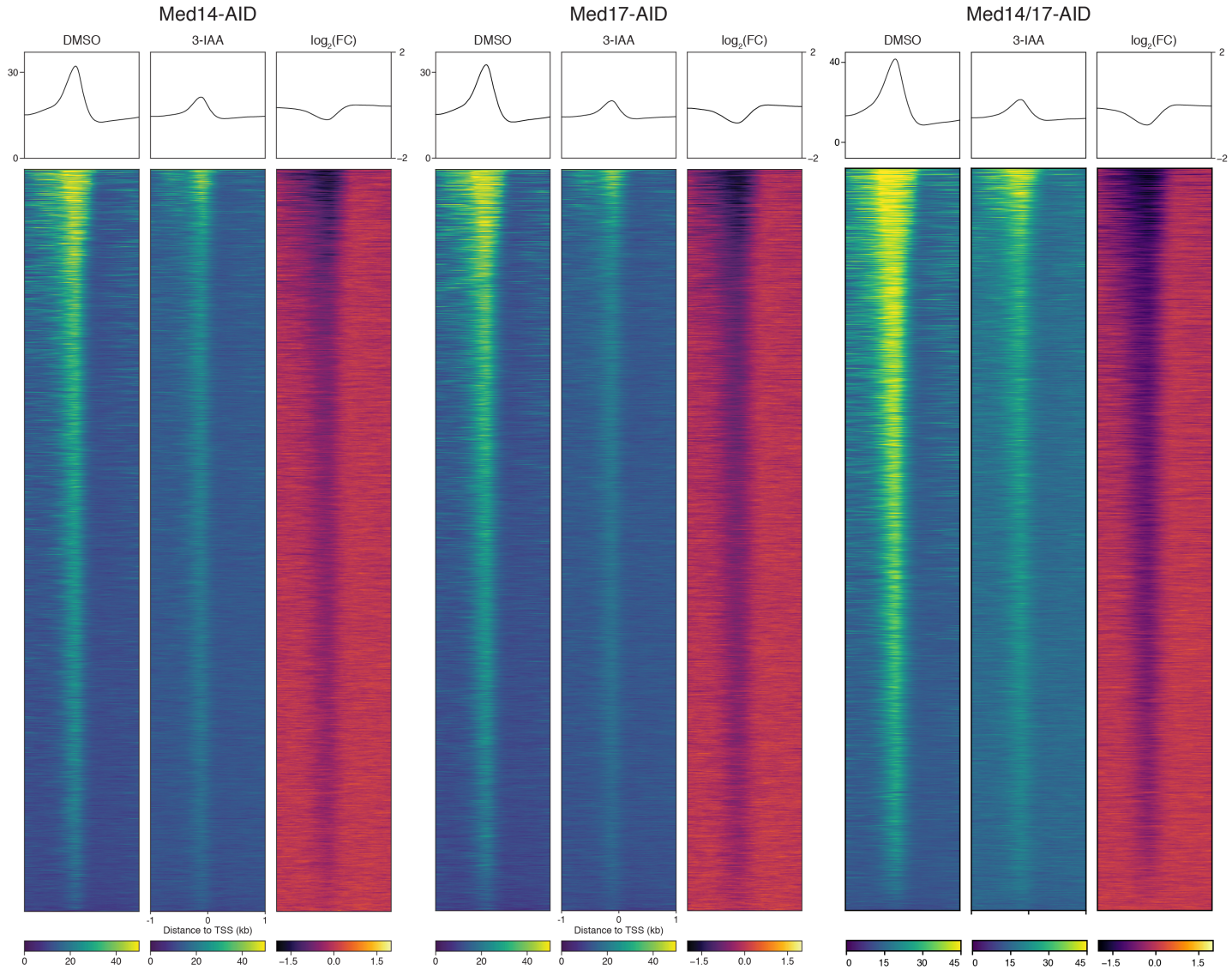

Figure S8. Sua7 ChIP-seq replicate analysis

Average plots and heatmaps of replicate Sua7 ChIP-seq signal around TSSs following DMSO or 3-IAA treatment of the indicated strain. An average plot and heatmap of the log_2(3-IAA/DMSO) ChIP-seq signal is also shown for each sample.

Strain	Genotype	Source
DHP43 (*S. pombe*)	*h-*	D. Devys
GZY191	*MATα ade2Δ::hisG his3Δ200 leu2Δ0 lys2Δ0 met15Δ0 trp1Δ63 ura3Δ0 his3::pGPD1-OsTIR1-HIS3*	(TOURIGNY *et al.* 2018)
GZY219	GZY191 *MED14-3V5-IAA7-kanMX6*	(TOURIGNY *et al.* 2018)
GZY269	GZY191 *MED17-3V5-IAA7-kanMX6*	This work
GZY272	GZY269 *SUA7-6GLY-3FLAG-hphMX4*	This work
GZY337	GZY219 *MED14-6GLY-3FLAG-TRP1*	This work
GZY341	GZY269 *MED17-6GLY-3FLAG-TRP1*	This work
GZY364	GZY191 *SUA7-3V5-IAA7-kanMX6*	This work
GZY379	*his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 RPB3-TAP::HIS3MX6 kin28as(L83G) leu2::pGPD1-OsTIR1-LEU2 MED14-3HA-IAA7-kanMX6 MED17-6GLY-3FLAG-URA3*	This work
GZY380	GZY269 *MED14-3HA-IAA7-URA3*	This work
GZY388	GZY380 *SUA7-6GLY-3FLAG-TRP1*	This work
GZY425	*his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 RPB3-TAP::HIS3MX6 kin28as(L83G) leu2::pGPD1-OsTIR1-LEU2 MED14-3HA-IAA7-kanMX6*	This work

Table S1. Yeast strains used in this study

All *S. cerevisiae* strains were constructed in the BY4705 background except for GZY421 and GZY425, which are in the BY4741 background.

Table S2. Information on total RNA-seq and 4tU-seq results

This Excel spreadsheet contains mean $\log_2$(fold changes), p-values, and adjusted p-values for the 5,080 genes analyzed in total RNA-seq and 4tU-seq experiments. Gene classification information (e.g. coactivator dependence) is also provided.

Supplemental references

Ho, B., A. Baryshnikova and G. W. Brown, 2018 Unification of Protein Abundance Datasets Yields a Quantitative *Saccharomyces cerevisiae* Proteome. Cell Systems 6**:** 192-205.e193.

Tourigny, J. P., M. M. Saleh, K. Schumacher, D. Devys and G. E. Zentner, 2018 Mediator Is Essential for Small Nuclear and Nucleolar RNA Transcription in Yeast. Mol. Cell. Biol. 38**:** e00296-00218.