Figure S1. Verification of Activity and Recruitment using Nuclear Extracts, Related to Figure 1

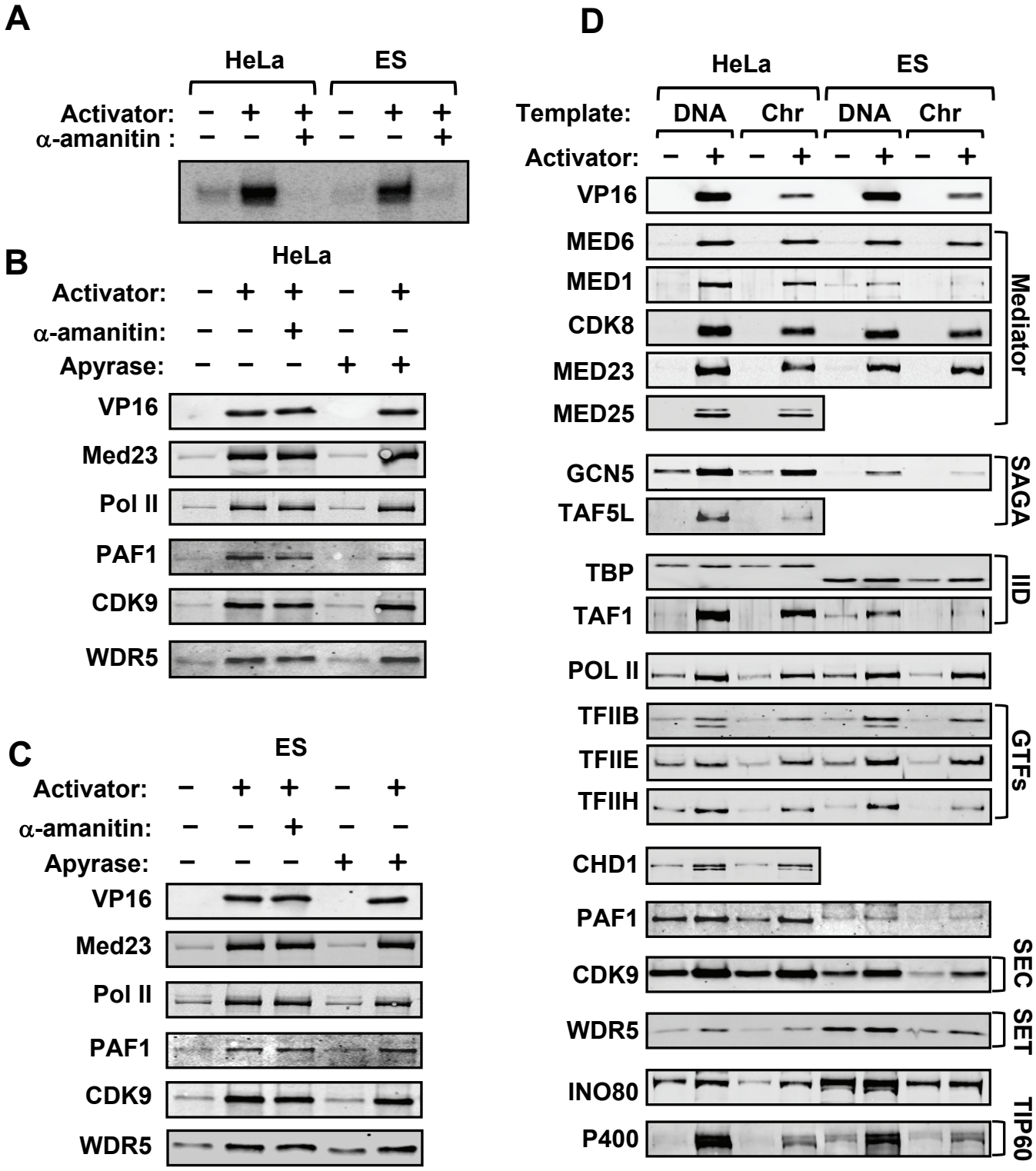

Figure S1. (A) IVT assay showing that α-amanitin inhibits transcription in our system. (B) and (C) IT assays performed in the presence of α-amanitin and apyrase show that SEC/PTEFb (CDK9), PAF1(PAF1) and SET/MLL(WDR5) subunits are recruited to the PIC in both HeLa and mouse ESC extracts under conditions blocking initiation or elongation. (D) Western blot analyzing PIC assembly at the ~60 minute time point from HeLa and mouse ESC nuclear extracts on either naked DNA templates or chromatin (Chr) templates.

Figure S2. Western Blotting of Depleted Extracts, Related to Figure 2

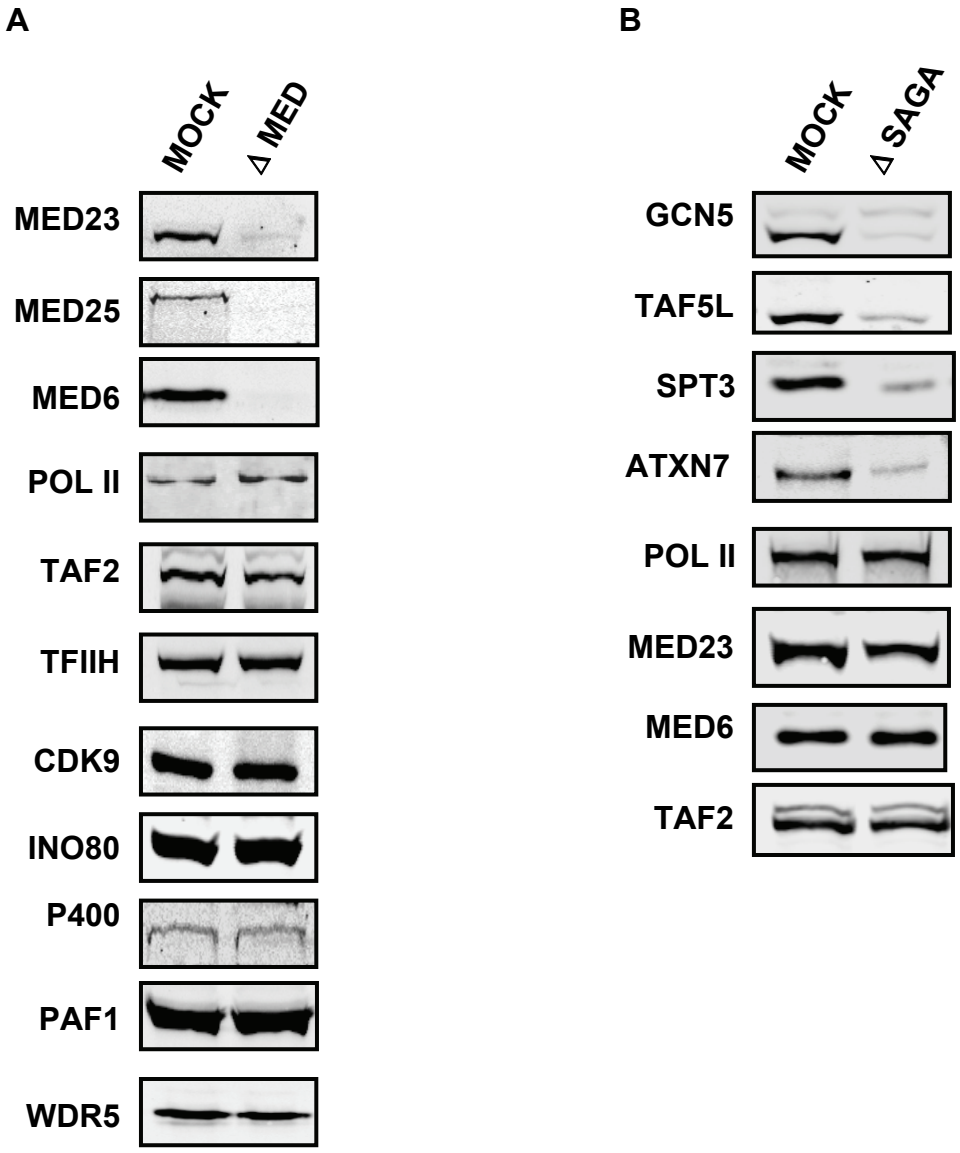

Figure S2. Depleted Mediator (A) or SAGA (B) and mock nuclear extracts were immuno blotted to identify immuno depleted proteins.

Figure S3. SAGA and Mediator Recruitment are Independent, Related to Figure 3

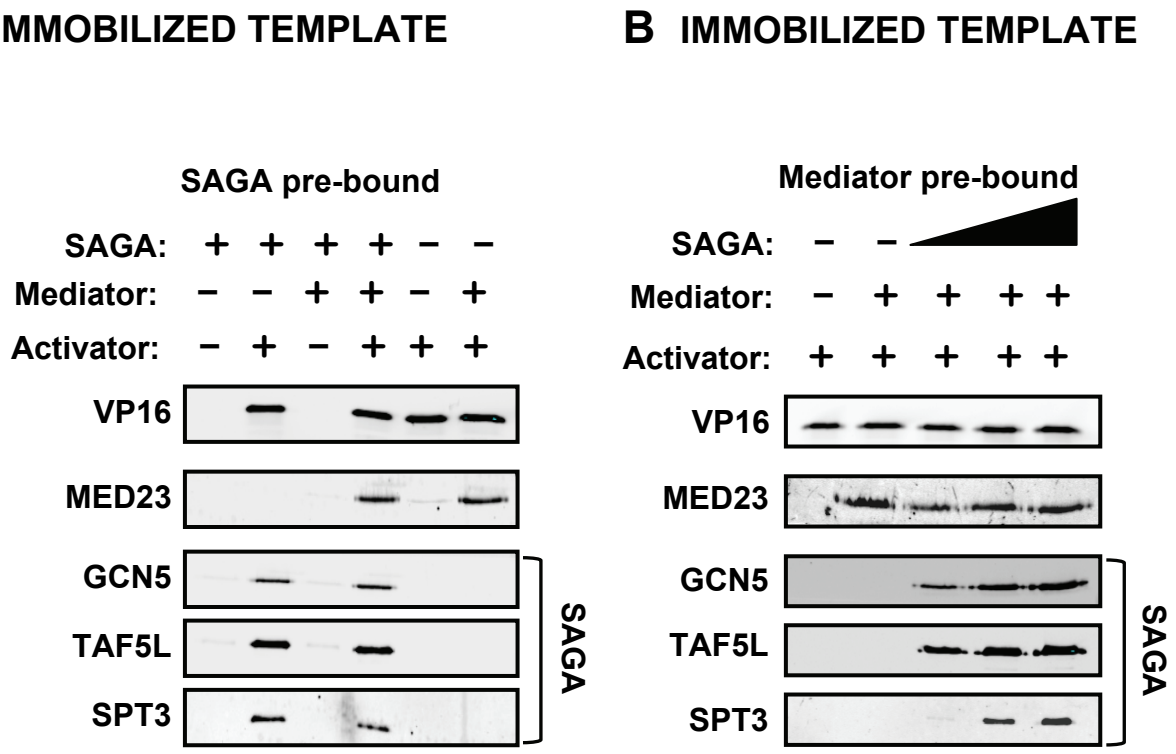

Figure S3. Immobilized template assays demonstrate that SAGA and Mediator are recruited independently by activator and do not compete. In (A) SAGA was prebound to GAL4-VP16 and Mediator was added. In (B) Mediator was prebound and increasing amounts of SAGA were added. Note that SAGA saturates at the middle point.

Extended Experimental Procedures

IT Recruitment Assay

Antibodies used in immunoblotting included MED23 (BD Pharmingen, 550429), MED25 (Carey Lab Stock), Pol II CTD 8WG16 (QED Bioscience), TFIIB (Tantin et al., 1996), TAF5L (Sigma, SAB4501194), CHD1 (Bethyl, A301-217A), PAF1 (Bethyl, A300-173A), WDR5 (Bethyl, A302-429A), INOC1 (Bethyl, A303-370A), EP400 (Bethyl, A300-541A), GAL4-VP16 (Carey Lab Stock), TBP (Santa Cruz, SC-204), TAF1 (Santa Cruz, SC-735), MED1 (Santa Cruz, SC-8998), MED6 (Santa Cruz, SC-9434), CDK8 (Santa Cruz, SC-1521), TFIIE-α (Santa Cruz, SC-237), CDK9 (Santa Cruz, SC-484), TFIIH-p89 (Santa Cruz, SC-293), GCN5 (Santa Cruz, SC-20698X).

Extract and Protein Preparation

Immunodepletion of Mediator was performed as described (Lin et al., 2011). Briefly, antibodies were cross-linked to protein A and G paramagnetic beads (Invitrogen) using 20 mM dimethylpimelimidate in 0.1 M Sodium Borate buffer, pH 9, and washed extensively with 50 mM Glycine pH 2.5. The cross-linked beads were equilibrated in buffer D (20 mM Hepes, pH 7.9, 0.1 mM EDTA, 20% glycerol, 0.1 M KCl) and incubated with HeLa nuclear extract in buffer D, for 4 hours at 4°C. The supernatant was isolated and used for immobilized template analysis as described above. The conditions used for purification are cited in the legends.

Genomewide Analysis

Reads were mapped to the Mouse (mm19) genome using Bowtie software. Only reads that aligned to a unique position in the genome with no more than two sequence mismatches were retained for further analysis. Duplicate reads that mapped to the same exact location in the

genome were counted only once to reduce clonal amplification effects. The genome was tiled into 50-bp windows. Each read was extended by 150 bases (we refer to tags as the extended read counts within a bin) and was counted as one read to each window to which it partially or fully matched. The total counts of the input and ChIP samples were normalized to each other. The input sample was used to estimate the expected counts in a window; the average value for all windows was assigned to windows with zero counts. Finally, we used the Poisson distribution to estimate the probability of observing the ChIP counts within a window given the expected counts in the input sample window. We considered all windows with P-values less than 10^{-5} to have significant peaks. A p-value < 10^{-5} was chosen to give a False Discovery Rate (FDR) of <1.5%. The FDR was calculated by applying the same statistic described above to the two halves of the same input library. Heat maps for promoter regions spanning a 10 kb window surrounding the TSS were generate by plotting the –log poisson p-val (-logP) obtained for each 50 bp intervals. Only –logP ≥ 5 were considered significant and thus genes considered bound. Genes were ranked based on the average –logP for the binding at the promoter regions.

MuDPIT Analysis of Immobilized Templates and Purified Proteins

For MuDPIT analysis, the equivalent of 150 to 300 immobilized template reactions, on 50 ng naked DNA template per reaction, were performed, washed, pooled together, and eluted as described previously for MuDPIT analysis of PICs on chromatin templates (Lin et al., 2011). MuDPIT of Mediator employed the equivalent of protein obtained from approximately 5E9 cells. Note: In Figure 1B, “CHD family” as a single entry represents the average of all CHD-family proteins due to the high degree of conservation between family members. Also, in some instances, we eliminated specific subunits from our calculations when we felt that its NSAF values were significantly over-represented relative to other detected subunits, occasionally due to their multimeric composition. For example, RuvB, a component of both TIP60 and INO80, is known to be a double hexamer, and inclusion in those complexes dramatically increases and

almost certainly over-represents the amount of those complexes using our quantitation method. Also inclusion of actin related subunits would dramatically over-represent the same complexes. We do present these data in Supplemental Table 1.

MS Sort Analysis of MuDPIT Data

Analysis of the MuDPIT data was performed using a custom built R function termed MS Sort (www.r-project.org/). MS Sort queries the original data, identifies and sums the NSAFs for all detected subunits of a given complex within the input file and divides by the total number of known subunits to calculate an average NSAF per complex. Briefly, identified proteins were matched to an input list of proteins involved in the regulation of transcription grouped by complex. The subunits and UniProt ID numbers used for each complex are presented in Supplemental Table 1. The input files and program are available on request.

Comparison of Protein Complex and Protein Detection Reproducibility

To assess the reproducibility of protein complex and individual subunit detection across the PIC samples, Pearson correlation coefficients were calculated and the *r* value for each pair-wise comparison reported (Mosley et al., 2011).

Supplemental Figure Titles and Legends

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Supplementary Table 1: Immobilized Template MuDPIT full dataset table

Supplementary Table 2: Mediator purification MuDPIT full dataset table

Supplementary Table 3: SAGA purification MuDPIT full dataset table

Supplemental References

Lin, J. J., Lehmann, L. W., et al. (2011) Mediator coordinates PIC assembly with recruitment of CHD1. Genes Dev*, 25***,** 2198-209.

Mosley, A. L., Sardiu, M. E., et al. (2011) Highly reproducible label free quantitative proteomic analysis of RNA polymerase complexes. Mol Cell Proteomics*, 10***,** M110 000687.

Tantin, D., Chi, T., et al. (1996) Biochemical mechanism of transcriptional activation by GAL4-VP16. Methods Enzymol*., 274***,** 133-49.