
Figures and figure supplements

The nucleosomal barrier to promoter escape by RNA polymerase II is overcome by the chromatin remodeler Chd1

Peter J Skene, et al.

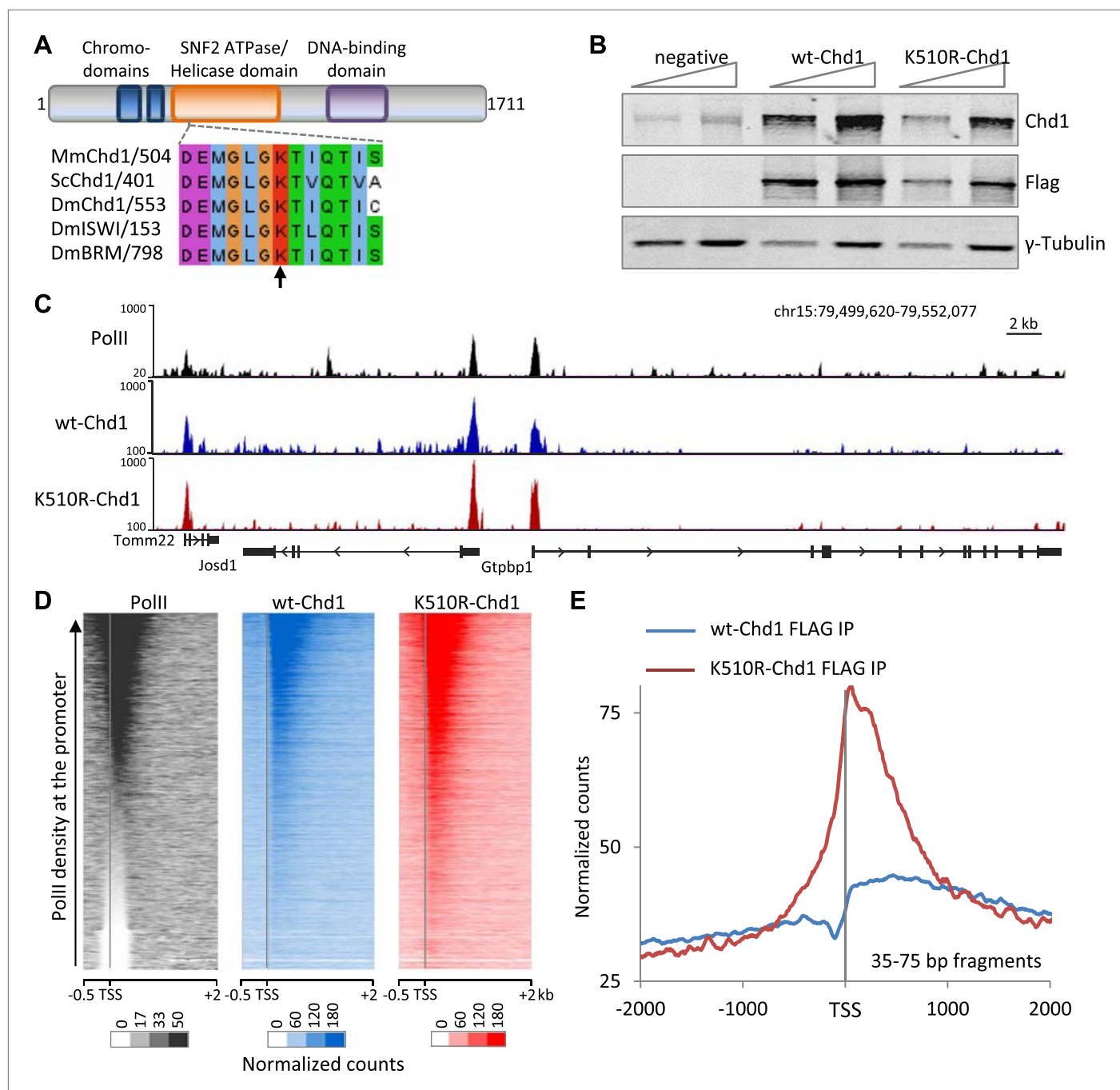


Figure 1. Chd1 is recruited to promoters with high PolII occupancy and requires ATPase activity to extend into the gene body. **(A)** Schematic of the domain structure of the full-length mouse Chd1 (1-1711) used to generate the N-terminal FLAG-tagged construct. A region corresponding to the Chd1 ATP-binding pocket is shown below and aligned to various homologues from *Saccharomyces cerevisiae* and *Drosophila melanogaster*. The arrow indicates the conserved lysine residue mutated to form the dominant negative. **(B)** MEFs stably expressing either FLAG-tagged wild-type Chd1 or the dominant negative K510R were subjected to western analysis with a two-fold dilution series. Untransfected MEFs were used as a reference. **(C)** A representative genome browser snapshot of ChIP-seq data (all fragment lengths) indicating the high occupancy of PolII, wildtype-Chd1 and K510R-Chd1 at gene promoters. PolII distribution was determined in cells expressing wildtype-Chd1 using the N20 antibody. Normalized counts are indicated on the y-axis. **(D)** Chd1 binding to the 5' end of genes was determined by ChIP-seq. Here, all recovered DNA fragments, irrespective of length, were analyzed. Each row of the heatmap represents the binding pattern across the -0.5 kb to +2 kb region flanking the TSS. Genes were ranked by the level of PolII occupancy in the -100 to +300 bp region, as measured by ChIP-seq in the MEFs expressing wildtype Chd1, using the N20 antibody that binds to the N-terminus of the largest subunit of PolII. **(E)** Analyzing only the short DNA fragments recovered (35-75 bp) allows precise quantification of Chd1 binding. *Figure 1. Continued on next page*

Figure 1. Continued

mapping of Chd1, indicating that wildtype Chd1 binding tracks into the gene body, whereas K510R-Chd1 accumulates just downstream of the TSS. The genome-wide average is shown across the 4 kb encompassing the TSS.

DOI: [10.7554/eLife.02042.003](https://doi.org/10.7554/eLife.02042.003)

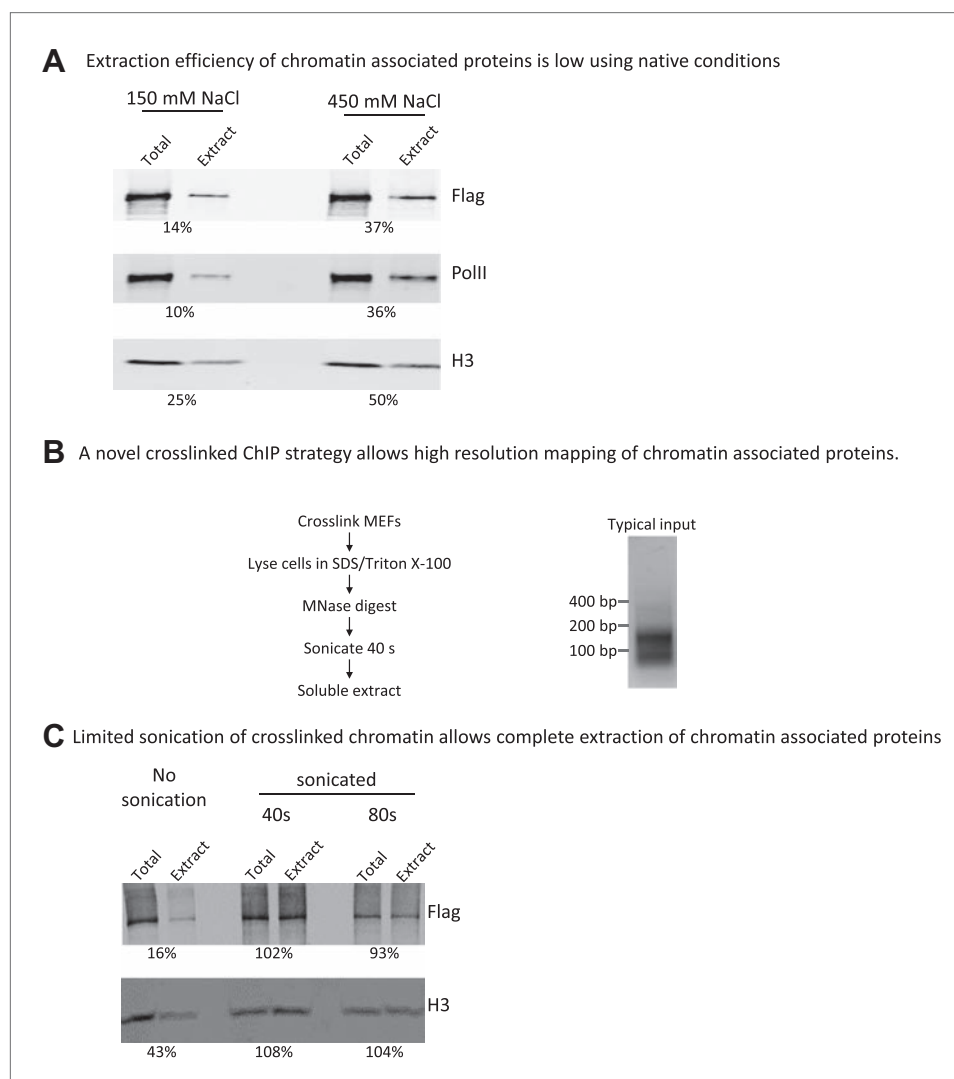


Figure 1—figure supplement 1. A novel crosslinking ChIP strategy allows near-complete extraction of chromatin-associated proteins and high-resolution mapping of binding sites.

DOI: [10.7554/eLife.02042.004](https://doi.org/10.7554/eLife.02042.004)

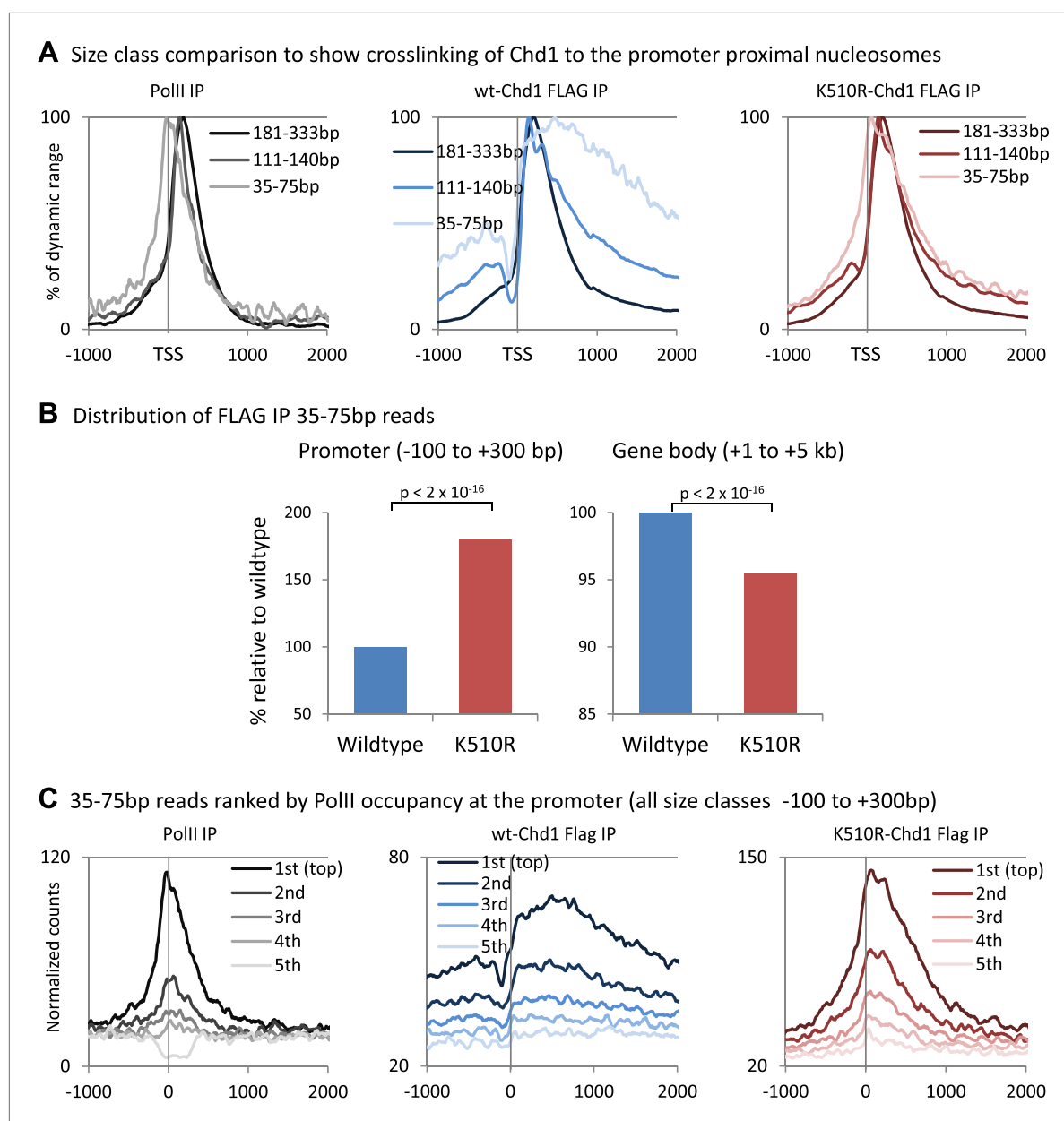


Figure 1—figure supplement 2. Analysis of short fragments provides high-resolution mapping of Chd1 and PolII binding sites at the promoter.

DOI: [10.7554/eLife.02042.005](https://doi.org/10.7554/eLife.02042.005)

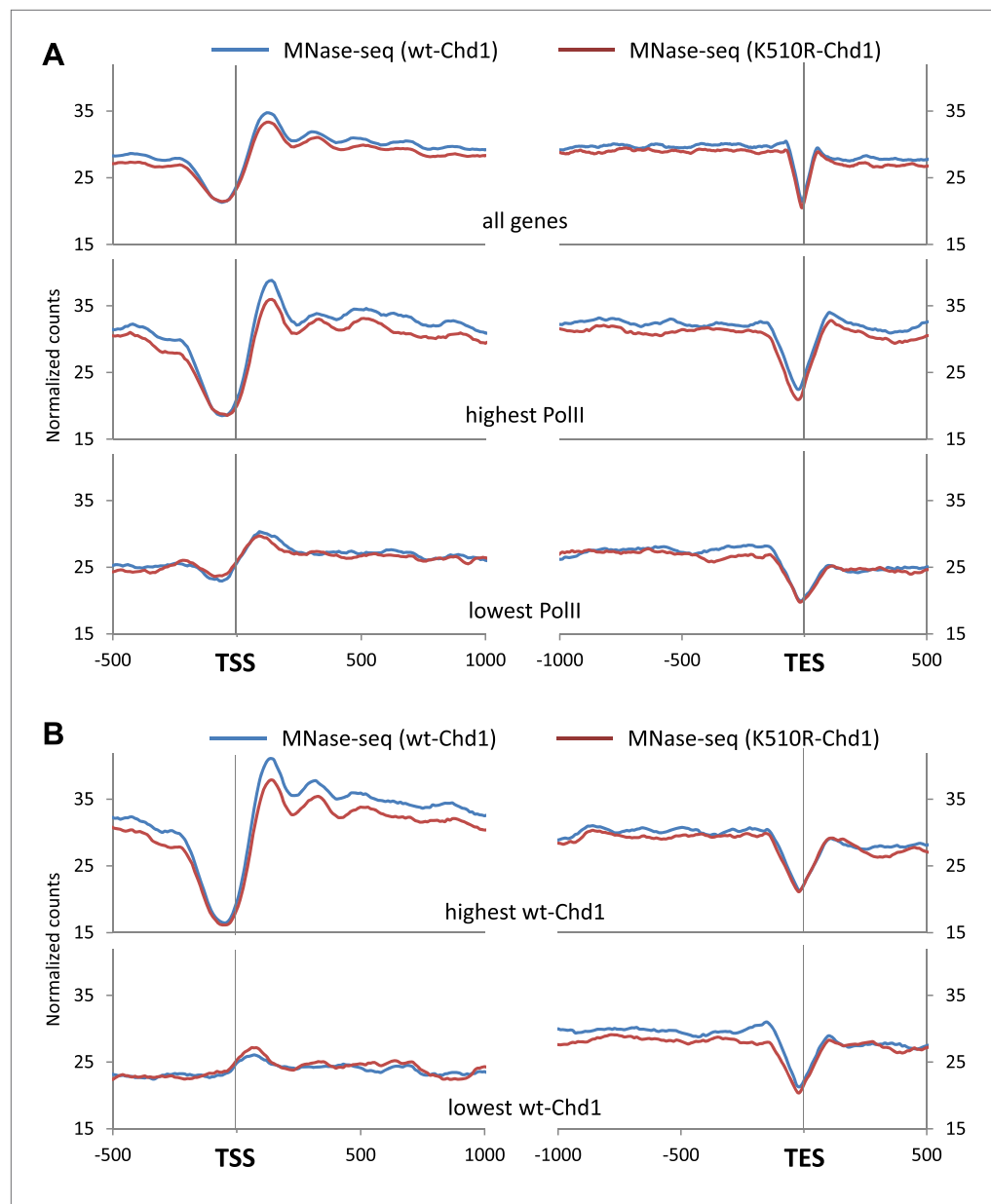


Figure 2. Chd1 activity is required to maintain nucleosome occupancy in the promoter region and the gene body. Cross-linked chromatin was digested with MNase and the DNA fragments were subjected to paired-end sequencing. Mono-nucleosomal fragments (111–140 bp) were aligned relative to the TSS or TES. **(A)** Average nucleosome profile for all genes. Genes were also ranked and split into quintiles based on PolII promoter occupancy (all fragment sizes; density within –100 to +300 bp). The nucleosome map for the highest and lowest quintile is shown. **(B)** Nucleosome profiles for genes with either the highest or lowest quintile of wildtype-Chd1 binding (35–75 bp fragment sizes; density within 0 to +1 kb). Statistical significance was determined using the two sample Kolmogorov–Smirnov (KS) test on the average number of normalized counts within 5 kb downstream of the TSS or upstream of TES. All groups were highly statistically significant ($p < 1 \times 10^{-9}$; TES of genes with the highest Chd1 at the promoter was significant $p < 3 \times 10^{-4}$) with the exception of the TSS and TES of genes with the lowest PolII density and the TSS of genes with the lowest Chd1 density which showed no significant change.

DOI: [10.7554/eLife.02042.006](https://doi.org/10.7554/eLife.02042.006)

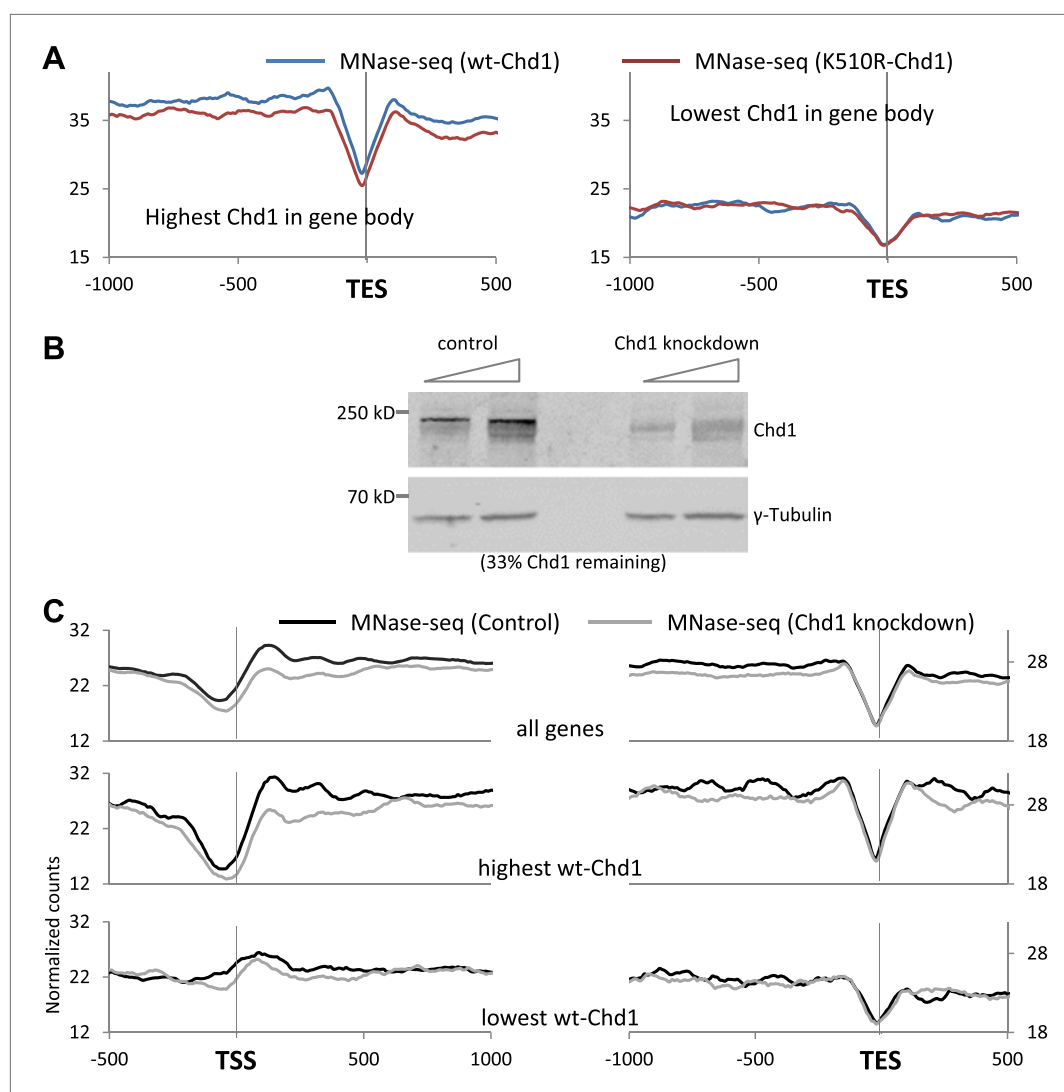


Figure 2—figure supplement 1. Chd1 activity is required to maintain nucleosome occupancy in the promoter region and the gene body.

DOI: [10.7554/eLife.02042.007](https://doi.org/10.7554/eLife.02042.007)

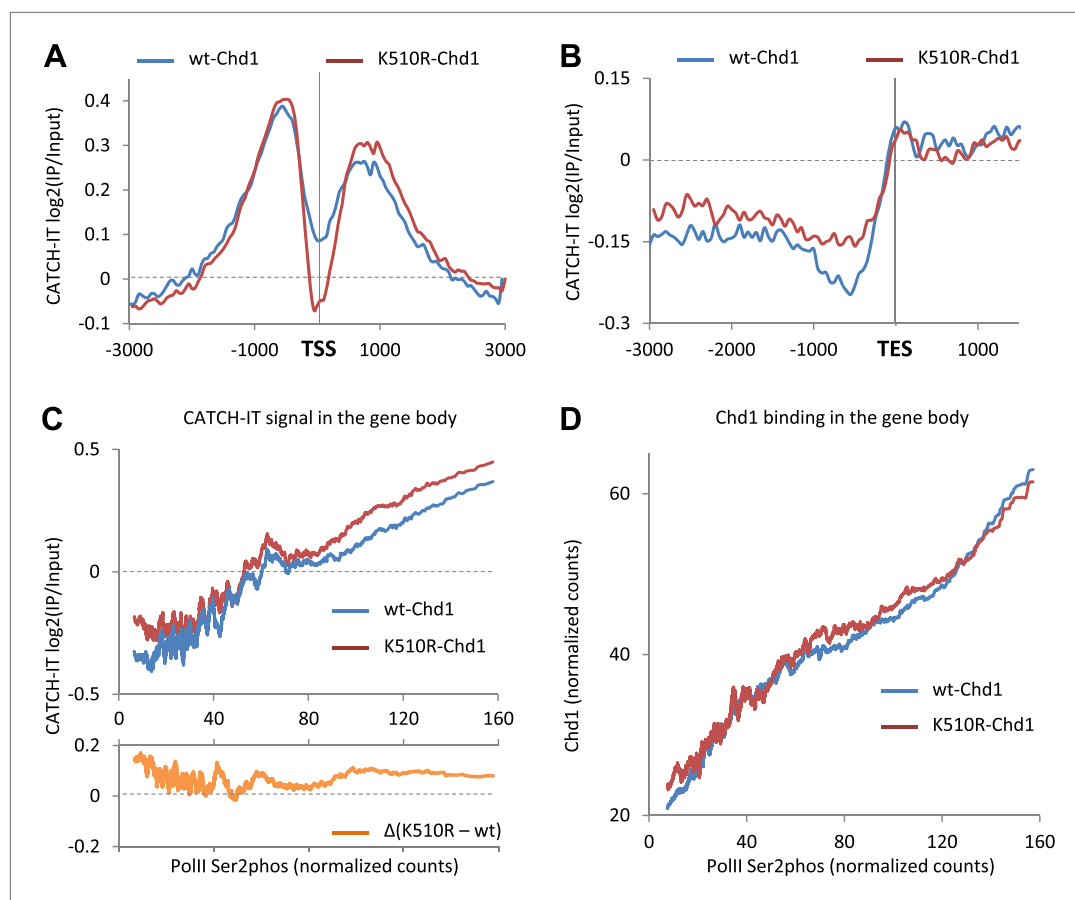


Figure 3. Chd1 activity has opposing effects on nucleosome turnover at the promoter and the gene body. Nucleosome turnover is decreased over the promoter but increased over the gene body. The genome-wide average nucleosome turnover was discerned using CATCH-IT at both the (A) TSS and (B) TES. (C) Nucleosome turnover correlates with elongating PolII density and is increased in cells expressing K510R-Chd1. Genes were ranked by the density of serine-2-phosphorylated PolII within the last 3 kb of the gene body. The average density of elongating PolII was calculated with a sliding window of 500 genes plotted against the average CATCH-IT signal within -3 to -0.5 kb relative to the TES (Pearson correlation coefficient >0.85). The difference for each window was calculated and is shown graphically below. (D) Chd1 is recruited to genes with actively elongating PolII. Genes were ranked as per (C) and plotted against the average ChIP-seq signal for FLAG-tagged wildtype Chd1 (35–75 bp fragment sizes) within the same region. (Pearson correlation coefficient >0.9).

DOI: [10.7554/eLife.02042.008](https://doi.org/10.7554/eLife.02042.008)

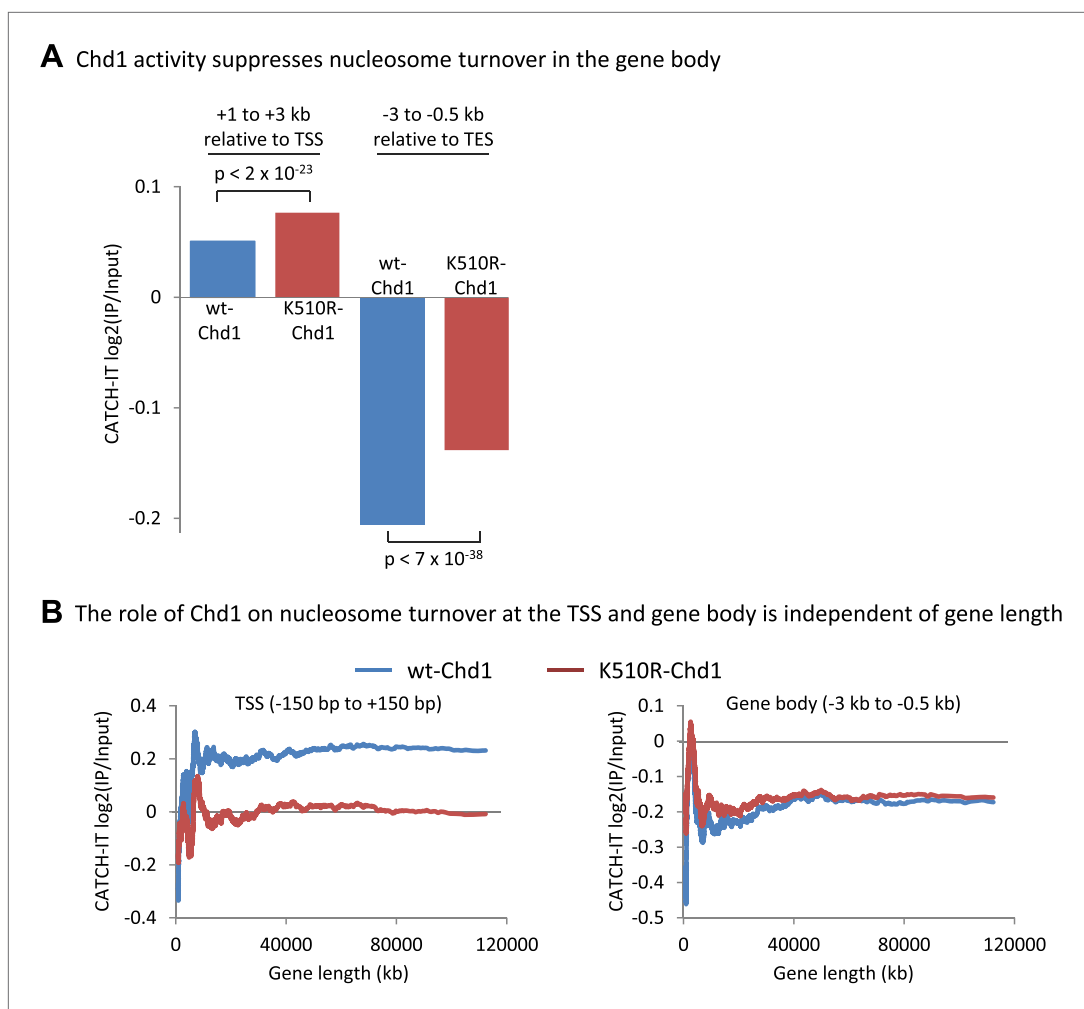


Figure 3—figure supplement 1. Chd1 activity suppresses nucleosome turnover in the gene body, and the role of Chd1 is independent of gene length.

DOI: [10.7554/eLife.02042.009](https://doi.org/10.7554/eLife.02042.009)

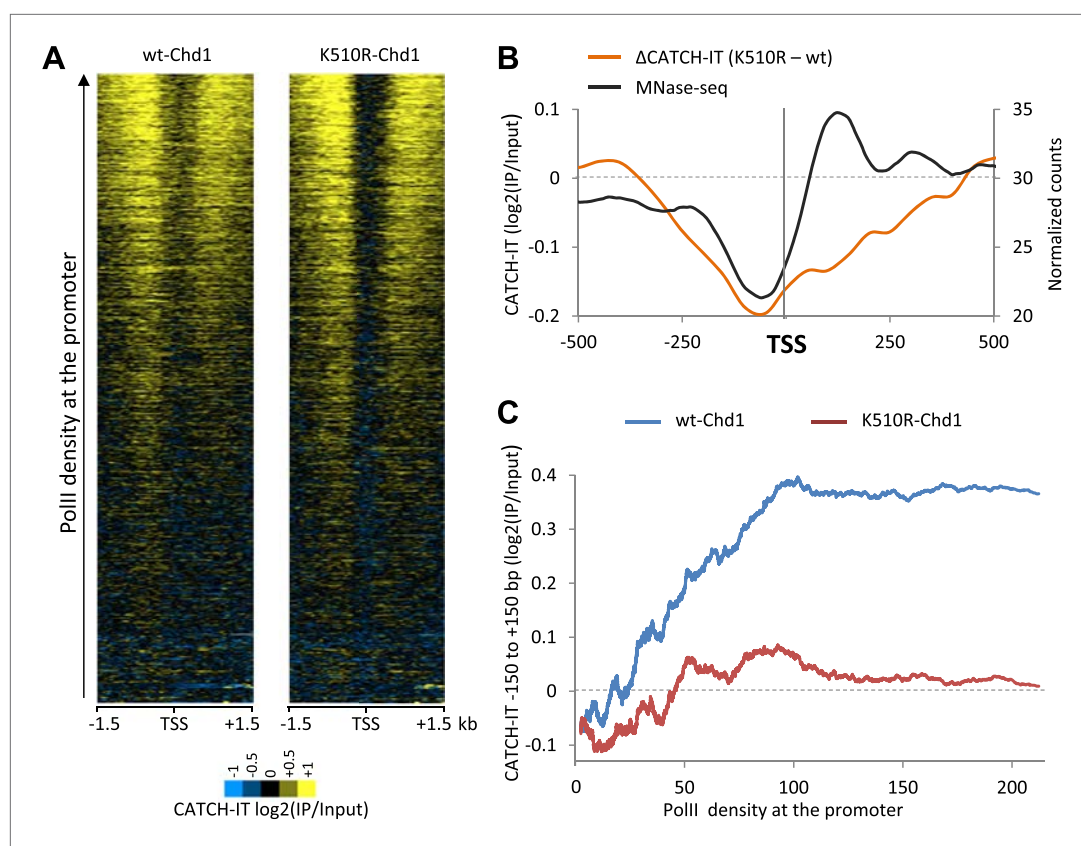


Figure 4. Chd1 activity is responsible for PolII-directed nucleosome turnover at the promoter. **(A)** CATCH-IT data represented as a heatmap for the ± 1.5 kb surrounding the TSS. Genes were ranked by the level of PolII occupancy at the promoter (all fragment sizes; density within -100 to $+300$ bp). **(B)** Nucleosome turnover is reduced over the promoter proximal region in cells expressing K510R-Chd1. The difference in CATCH-IT signal between cells expressing wildtype and K510R-Chd1 at the TSS ± 500 bp is plotted. The genome-wide average nucleosome occupancy is shown for reference for cells expressing wildtype-Chd1. **(C)** Chd1 is required for PolII-directed turnover at the promoter proximal region. Genes were ranked by PolII promoter density in cells expressing wildtype-Chd1 and plotted against the average CATCH-IT signal in the promoter proximal region with a sliding window of 600 genes. DOI: [10.7554/eLife.02042.010](https://doi.org/10.7554/eLife.02042.010)

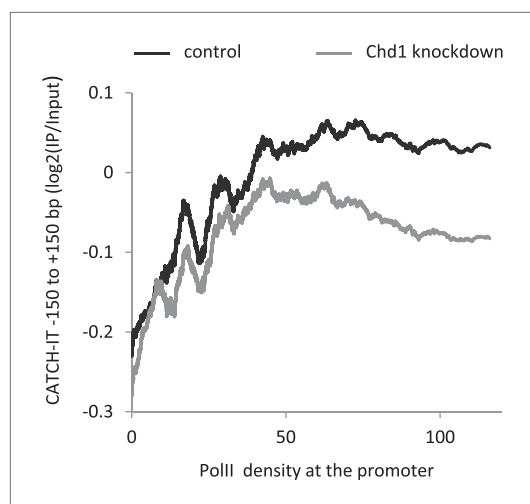


Figure 4—figure supplement 1. Knockdown of endogenous Chd1 shows a partial reduction in nucleosome turnover at the promoter.

DOI: [10.7554/eLife.02042.011](https://doi.org/10.7554/eLife.02042.011)

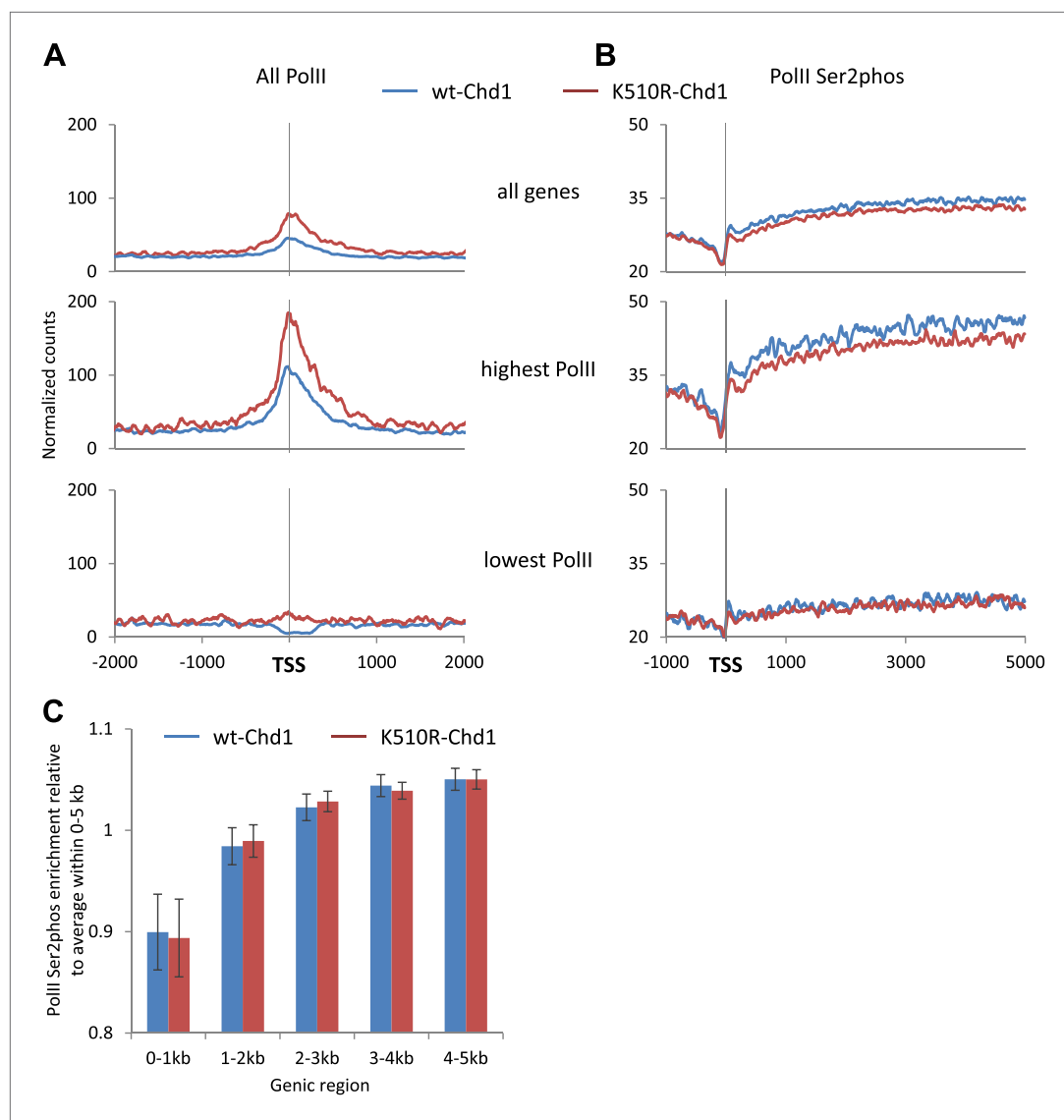


Figure 5. Chd1 activity is required to enable PolII to efficiently escape the promoter proximal region. Genome-wide distribution of PolII was determined using ChIP-seq with antibodies against **(A)** the N-terminus of Rpb1 to map total PolII and **(B)** PolII Ser2phos to map elongating PolII. The recovered short reads (35–75 bp) were mapped and the genome-wide average was plotted. Genes were also ranked and split into quintiles based on total PolII promoter occupancy in cells expressing wildtype-Chd1 (all fragment sizes; density within –100 to +300 bp). The PolII distribution is shown for the highest and lowest quintiles. Statistical significance was determined using the KS test on the average counts within **(A)** –100 to +300 bp and **(B)** +1 kb to +5 kb relative to the TSS. Differences between wt-Chd1 and K510R-Chd1 were significant ($p < 1 \times 10^{-9}$) with the exception of elongating PolII density in genes the lowest promoter PolII occupancy. **(C)** The elongation rate within the gene body is unaffected by the expression of K510R-Chd1. The fold enrichment in PolII Ser2phos density within 1 kb genic windows was calculated relative to the average density within the first 5 kb of the gene. Error bars represent standard deviation.

DOI: [10.7554/eLife.02042.012](https://doi.org/10.7554/eLife.02042.012)

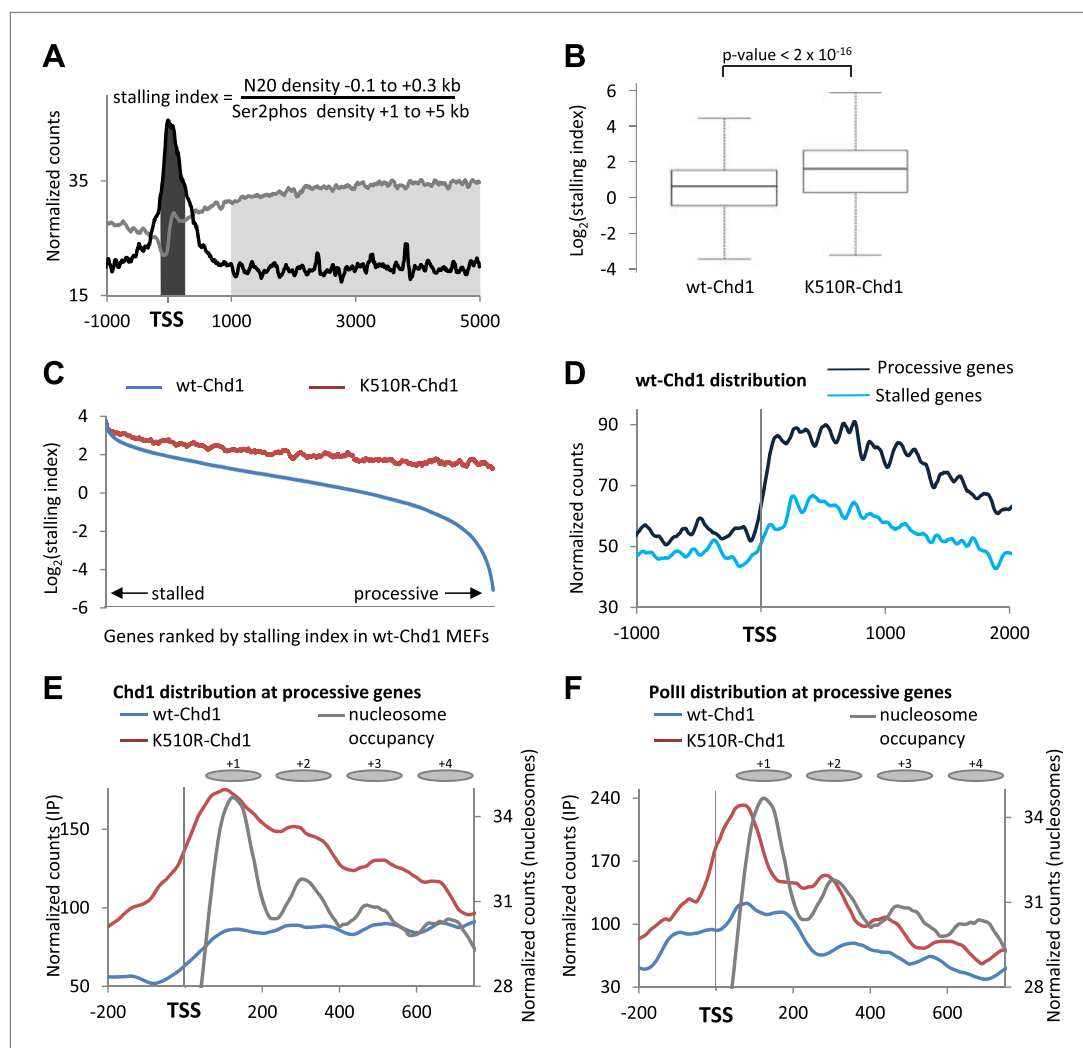


Figure 6. Chd1 functions to alleviate the nucleosomal barrier to PolII transit at highly processive genes. **(A)** Schematic describing the calculation of the stalling index. The dark gray shading indicates the total PolII density at the promoter (-100 to +300 bp) as calculated from the ChIP-seq data using the N20 antibody against the N-terminus of Rpb1 (black line). The light gray shading indicates the density of actively elongating PolII in the gene body (+1 to +5 kb or the end of the gene), calculated from the PolII Ser2phos ChIP-seq data (gray line). The stalling index is the ratio of these two values. **(B)** The stalling index is increased ~twofold in cells expressing K510R-Chd1. Box plot comparing the distribution of stalling indices for all genes. Statistical significance was determined using the Kolmogorov-Smirnov test. **(C)** Lack of Chd1 leads to increased PolII stalling, preferentially affecting genes with processive transcription. Genes were ranked by stalling index and plotted against the stalling index in cells expressing wildtype or K510R Chd1 with a sliding window of 200 genes. **(D)** Chd1 is preferentially recruited to the promoter region of genes with processive transcription compared to stalled genes. Wildtype-Chd1 binding profile across highly stalled and processive genes from the highest quintile of PolII promoter occupancy. **(E)** K510R-Chd1 accumulates near the dyad axis of the promoter proximal nucleosomes. The wildtype and K510R Chd1 binding profiles at these highly processive genes are shown. Nucleosome occupancy in cells expressing wildtype-Chd1 is shown for reference with gray ovals indicating the 147 bp of DNA protected by the nucleosomes. **(F)** PolII stalls in front of promoter proximal nucleosomes in the absence of Chd1 activity. PolII distribution at highly processive genes, as measured by ChIP-seq using the N20 antibody is shown. Nucleosome occupancy in cells expressing wildtype-Chd1 is shown for reference with gray ovals indicating the 147 bp of DNA protected by the nucleosomes. DOI: [10.7554/eLife.02042.013](https://doi.org/10.7554/eLife.02042.013)

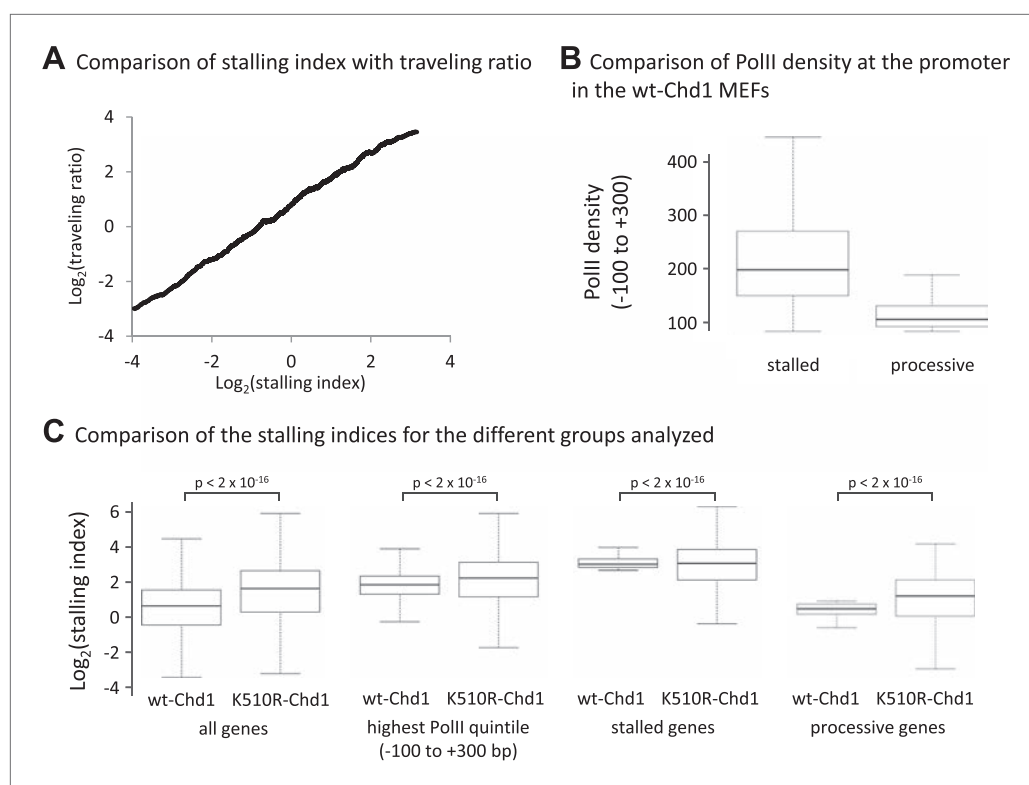


Figure 6—figure supplement 1. Loss of Chd1 results in an increase in the stalling index, predominantly at processive genes.

DOI: [10.7554/eLife.02042.014](https://doi.org/10.7554/eLife.02042.014)

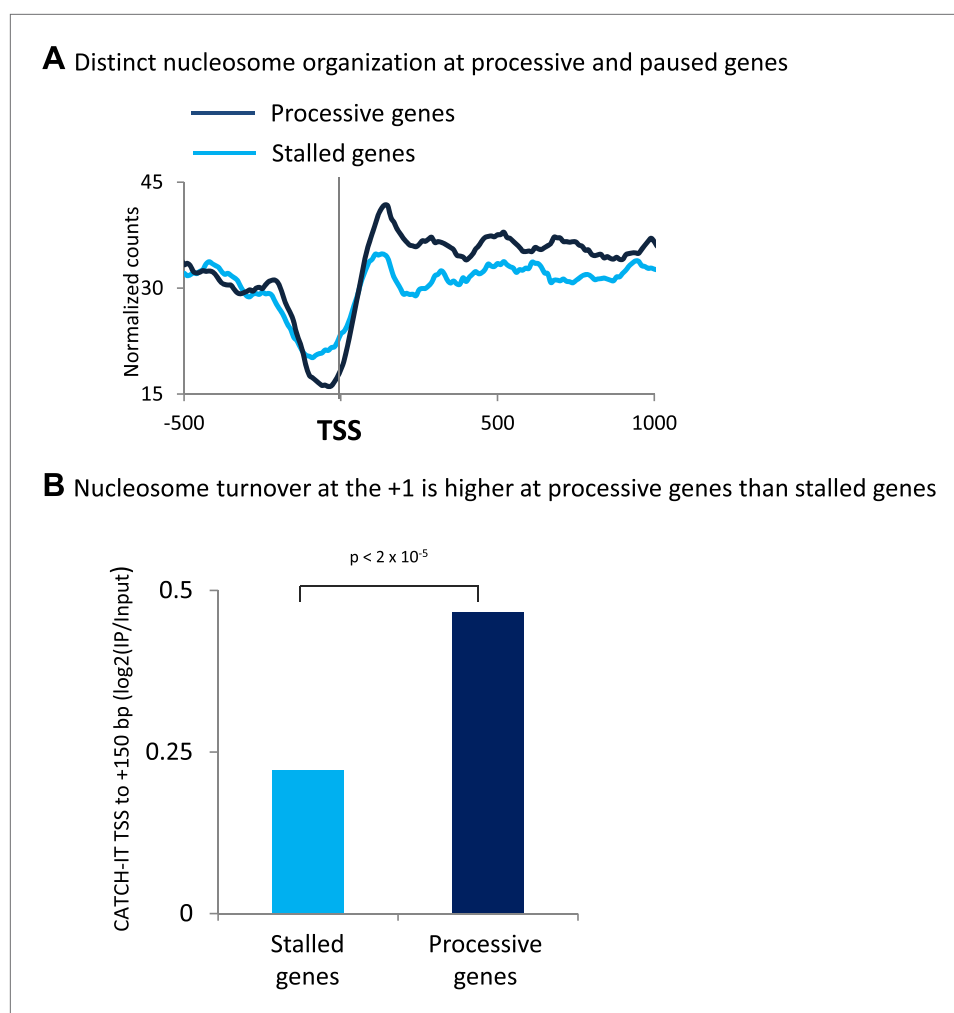


Figure 6—figure supplement 2. The promoters of processive genes have a highly dynamic chromatin barrier to transcription.

DOI: [10.7554/eLife.02042.015](https://doi.org/10.7554/eLife.02042.015)

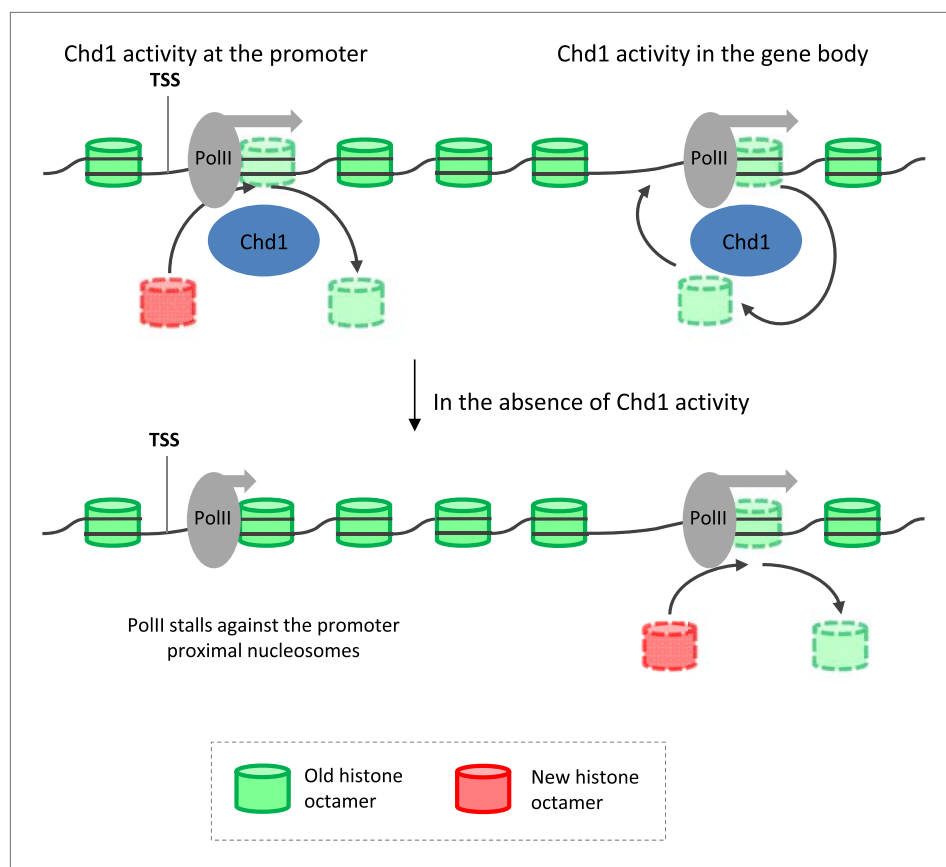


Figure 7. Model depicting the role of Chd1 in defining nucleosome dynamics during transcription. At the promoter of processive genes, Chd1 is recruited directly to the promoter proximal nucleosomes, where it is required for both eviction of existing histone octamers (green) and deposition of new octamers (red). In the absence of Chd1 activity, PolII stalls close to the leading edge of the nucleosome. In the gene body, Chd1 occupancy correlates with elongating PolII, where it reassembles histone octamers in cis behind the transiting PolII. The lack of Chd1 activity leads to increased deposition of new histone octamers through an unknown factor.

DOI: [10.7554/eLife.02042.016](https://doi.org/10.7554/eLife.02042.016)