Inhibition of MYC by the SMARCB1 tumor suppressor

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SMARCB1 encodes the SNF5 subunit of the SWI/SNF chromatin remodeler. SNF5 also interacts with the oncoprotein transcription factor MYC and is proposed to stimulate MYC activity. The concept that SNF5 is a coactivator for MYC, however, is at odds with its role as a tumor-suppressor, and with observations that loss of SNF5 leads to activation of MYC target genes. Here, we reexamine the relationship between MYC and SNF5 using biochemical and genome-wide approaches. We show that SNF5 inhibits the DNA-binding ability of MYC and impedes target gene recognition by MYC in cells. We further show that MYC regulation by SNF5 is separable from its role in chromatin remodeling, and that reintroduction of SNF5 into SMARCB1-null cells mimics the primary transcriptional effects of MYC inhibition. These observations reveal that SNF5 antagonizes MYC and provide a mechanism to explain how loss of SNF5 can drive malignancy.
SNF5 inhibits target gene recognition by MYC in MRT cells. To look at the impact of SNF5 on MYC in a more tumor-relevant setting, we asked whether SNF5 modulates the interaction of MYC with chromatin in the context of MRT, which is driven by SNF5 loss, and where MYC target gene signatures are repeatedly altered. To determine if MYC is important in MRT cells, we first attenuated its expression and asked how this alters MRT cell viability and anchorage-independent growth. Here, we observed that shRNA-mediated knockdown of MYC significantly decreased both parameters (Supplementary Fig. 2), confirming the importance of MYC to MRT cells and reinforcing the notion that this is an appropriate setting in which to interrogate the influence of SNF5 on MYC.

We next established a system that allowed us to compare the effects of reintroduction of SNF5 in MRT cells with inhibition of MYC in the same setting. G401, an extensively studied (and MYC-dependent; Supplementary Fig. 2) MRT cell line, is an aggressive and often lethal pediatric cancer. Interestingly, loss or inactivation of SMARCB1 is the only recurring mutation in MRT genomes11—pointing to expansive functions of SNF5 in tumor suppression. Loss of SNF5 in MRT compromises SWI/SNF integrity, causing widespread collapse of chromatin at super-enhancers, regulating differentiation, and mobilization of residual SWI/SNF complexes to super-enhancers essential for tumor cell maintenance12. Conversely, reintroduction of wild-type SNF5 into MRT cell lines induces cell cycle arrest, apoptosis, purging of aneuploid cells, and loss of tumorigenicity13–18; demonstrating that the absence of SNF5 remains a driving force in the malignant state of these cells. It is possible that the tumor-suppressive actions of SNF5 are exerted entirely through its role in chromatin remodeling, but given the breadth of impact of SNF5 on cancer-relevant processes, it is equally possible that SNF5 plays a multifaceted role in suppressing tumorigenesis.

In addition to functions within the SWI/SNF complex, SNF5 also binds to c-MYC19–21, an oncoprotein transcription factor with an extensive suite of protumorigenic activities22. SNF5 interacts directly with the carboxy-terminus of MYC19,21 and is proposed to stimulate the ability of MYC to transactivate its target genes19. The concept that SNF5 is a coactivator for MYC, however, conflicts with its well-established role as a tumor suppressor, with a report that SNF5 and MYC oppositely regulate a common set of genes21, with findings that loss of SNF5 in cancer is associated with activation of MYC target gene signatures8–10, and with recent observations that MYC inhibition can restrict rhabdoid tumor growth in vivo23. Given these disparities, it is clear that both the functional significance of the SNF5–MYC interaction—and the underlying mechanisms involved—are unresolved.

Here, we use a combination of biochemical and genomic approaches to interrogate how SNF5 impacts MYC. We demonstrate that SNF5 selectively inhibits the ability of MYC to bind DNA in vitro and in cells, and show that reintroduction of SNF5 into MRT cells results in a broad and comprehensive displacement of MYC from chromatin. By comparing SNF5 reintroduction with MYC inhibition, we further demonstrate that the actions of SNF5 on MYC are independent of its effects on chromatin remodeling, and instead are mediated via control of RNA-polymerase pause release at MYC-regulated genes. These observations show that SNF5 tempers target gene recognition by MYC, providing a mechanism to account for enhanced MYC function in MRT and suggesting that the tumor-suppressive functions of SNF5 are mediated, at least in part, by inhibiting MYC.
was engineered to express inducible forms of enhanced green fluorescent protein (EGFP), SNF5, or OmoMYC—a dominant-negative mutant that blocks the productive association of MYC with its target genes.\(^{29-32}\) In this experimental system the level of reintroduced SNF5 was comparable to endogenous SNF5 in other cell lines (Supplementary Fig. 3a). Reintroduced SNF5 interacted with the core SWI/SNF component BAF155 (Supplementary Fig. 3b), and co-migrated with other SWI/SNF complex subunit members in glycerol sedimentation assays (Supplementary Fig. 3c), consistent with its assembly into an SWI/SNF complex.\(^{12}\) SNF5 also suppressed anchorage-independent growth of G401 cells in culture (Supplementary Fig. 3d, e). OmoMYC, as expected, reduced interaction of MYC with chromatin (Supplementary Fig. 3f). Notably, expression of SNF5 did not alter steady-state MYC protein levels (Supplementary Fig. 3g), providing the opportunity to look specifically at the effects of SNF5 on MYC function.

First, we coupled ChIP to next-generation sequencing (ChIP-Seq) to track the distribution of MYC across the genome of G401 cells. We identified ~900 peaks of MYC binding in G401 cells expressing the EGFP control (FDR of 0.01). This number of peaks was relatively low, compared to what has been reported for other cell types,\(^{33}\) but the pattern of binding was observed to be authentic for MYC. Binding sites were enriched in the E-box motif (Fig. 2a), predominantly promoter proximal (Fig. 2b), and enriched in genes linked to the well-established role of MYC in stimulating protein synthesis (Fig. 2c). Comparison with six published ChIP-Seq data sets revealed that more than half of the MYC binding sites we tracked in G401 cells are shared with the other six cell types (Fig. 2d), while a hypergeometric test showed significant overlap of our G401 data with the two MSigDB Hallmark MYC target gene collections (Fig. 2e), both of which contain different MYC target genes. Reducing the analysis FDR to a more relaxed value of 0.1 increased the number of MYC peaks to ~1500, but did not substantively change any of the relevant characteristics (Supplementary Fig. 4). We conclude that the number of MYC binding sites in G401 cells is comparatively low, but that those sites that are bound are strongly connected to the core functions of MYC.

We next compared EGFP with the effects of OmoMYC or SNF5 expression in G401 cells (Fig. 2f–i). Here, OmoMYC or SNF5 reduced detectable MYC binding genome wide, consistent with its known functions.\(^{29-32}\) Importantly, SNF5 also reduced MYC binding. The effects of SNF5 on MYC were genome-wide and the average magnitude of reduction in binding intensity was about threefold (Fig. 2f). As described, steady-state levels of MYC are...
Fig. 2 SNF5 inhibits chromatin binding by MYC in MRT cells. a Known motif enrichment analysis was performed on the ChIP-Seq data from G401 cells expressing EGFP. The top four motifs are shown; all are enriched in the E-box sequence (CACGTG). b MYC peaks in the EGFP-expressing cells were quantified in terms of their distance to the nearest annotated transcriptional start-site (TSS). Peaks within 1 kb of a TSS are called as “promoter” peaks. c MYC peaks located within 1 kb from a TSS were assigned to their nearest gene and GO term enrichment analysis was performed. d Overlap of MYC peaks obtained from EGFP-expressing G401 cells with six published MYC ChIP-seq data sets: NB4 acute promyelocytic leukemia cells (GSM935643), hESC human embryonic stem cells (GSM935509), Ramos Burkitt’s lymphoma cells (GSM762711), A549 lung cancer cells (GSM1003607), K562 chronic myeloid leukemia cells (GSM935516), and MCF7 breast cancer cells (GSM1006866). e MYC peaks that are located within 1 kb from a TSS were assigned to their nearest gene and overlaid with two MSigDB Hallmark MYC target data sets. A hypergeometric test was performed; significance is displayed below the Venn diagram. f Scatterplot of normalized peak read counts for each condition (average of replicates), ranked based on EGFP peak read number. g Heat maps of MYC peak intensity for each condition representing the combined average of normalized peak intensity in 100-bp bins ± 2 kb around the center of peaks. Genes ranked based on EGFP. h Two example genome browser tracks from each condition. i Box-and-whisker plot of log2-fold changes of MYC peaks for OMOMYC or SNF5 samples (FDR < 0.05), relative to EGFP. Box extends from 25th to 75th percentile with median marked by the middle line, whiskers extend from minimum to maximum point. n = 2 independent ChIP-seq experiments.
SNF5 causes changes in chromatin state that are independent of its impact on MYC. a Scatterplots showing log2-fold changes in ATAC-Seq peaks for OmoMYC and SNF5, compared to EGFP samples. b Differential ATAC-Seq peaks were separated based on whether they are TSS-proximal (≤1 kb upstream or 100 bp downstream of the TSS) or TSS-distal (greater than these parameters). c Graph presents a breakdown of the 2491 ATAC-Seq peaks induced by SNF5, according to their distance from the nearest annotated TSS. d GO analysis of genes associated with ATAC-seq peaks induced by SNF5 expression. e De novo motif analysis of ATAC-seq peaks induced by SNF5 expression, showing the top four motifs identified. f Scatterplot showing the log2-fold changes of MYC ChIP-Seq read counts (SNF5/EGFP) on the x-axis and the log2-fold changes of ATAC-Seq read counts (SNF5/EGFP) on the y-axis at all promoters.

Chromatin regulation by SNF5 is distinct from effects on MYC. Given that reintroduction of SNF5 into MRT cells will reconstitute SWI/SNF12, it is possible that the effects we observe on MYC binding in our G401 system are due to alterations in chromatin accessibility triggered by the SWI/SNF complex. To ask whether SNF5 alters the chromatin landscape at or around MYC target genes, we used the assay for transposase accessible chromatin followed by next-generation sequencing (ATAC-Seq)34 to identify changes in chromatin accessibility induced by EGFP, SNF5, and OmoMYC expression. Overall, we identified ∼25,000 sites of open chromatin in the EGFP-expressing cells. None of these sites were significantly affected by OmoMYC expression (Fig. 3a), indicating that displacement of MYC from its target genes in G401 cells does not substantively alter open chromatin status. In contrast, SNF5 expression resulted in a profound increase in chromatin accessibility, causing ∼2500 new open chromatin sites to be formed (Fig. 3a). Only seven sites showed decreased accessibility upon SNF5 reintroduction (Fig. 3a). The majority of new open chromatin sites were transcription start site (TSS)-distal (Fig. 3b), with 90% being at least 5 kb from the nearest TSS, and half more than 50 kb away (Fig. 3c). Assignment of gained open chromatin peaks to their nearest gene showed a strong enrichment of genes involved in signal transduction, development, and differentiation (Fig. 3d).

Intracellular signal transduction
Cell development
Cell migration
Localization of cell
Reg. of multicellular development
Cell proliferation
Regulation of signaling
Regulation of cell differentiation
Regulation of molecular function
Angiogenesis

De novo motif analysis of ATAC-seq peaks induced by SNF5 expression, showing the top four motifs identified.
were entirely separable from MYC; there was no correlation between fold changes of MYC ChIP-Seq read counts (SNF5/EGFP) and ATAC-Seq read counts (SNF5/EGFP) at all promoters (Fig. 3f, \( r = 0.03 \)), and no enrichment for E-box motifs in the ATAC-peak sequences (Supplementary Table 1). We conclude that reintroduction of SNF5 in G401 cells alters the chromatin landscape in a way that is consistent with its known functions, but that these effects are physically separable from its actions on MYC.

**SNF5 inhibits RNA polymerase pause release at MYC targets.**

A key transcriptional function of MYC is to modulate release of paused RNA polymerases at its target genes\(^{38} \). To determine if the ability of SNF5 to temper MYC binding to chromatin impacts this activity, we used PRO-Seq\(^{39} \), a global nuclear run-on approach, to compare how SNF5 and OmoMYC alter the distribution of active RNA polymerases in G401 cells, genome-wide and at near-nucleotide level resolution. PRO-Seq also allowed us to follow primary transcriptional effects, and at the same time point (24 h postinduction) as our ChIP- and ATAC-Seq experiments.

Compared to the EGFP control, OmoMYC and SNF5 induced a large number of transcriptional changes in the distribution of active RNA polymerases, both proximal to promoters and further inside gene bodies (Fig. 4a). Consistent with the role of MYC in promoting RNA polymerase pause release, OmoMYC increased the pausing index—the ratio of active polymerases at the promoter versus the gene body\(^{40} \)—at \(~4500\) genes (Fig. 4b), with a smaller number (\(~2000\)) of genes showing a decrease in this ratio. SNF5, in contrast, produced an almost equal number of increases (\(~3500\)) and decreases (\(~3400\)) in pausing index. Notably, when we compared these two data sets, we found that \(~70\%) of the genes that show a change in pausing index with SNF5 are also changed with OmoMYC (Fig. 4c). Separating these genes according to the direction of change, we observed a highly significant correlation between the extent to which polymerase pausing was altered by OmoMYC and SNF5, both for genes showing a gain (Fig. 4d), as well as a loss (Fig. 4e), of pause. In total, \(~70\%) of genes gaining a pause with SNF5 also gained a pause with OmoMYC (Fig. 4f), and \(~35\%) of genes losing a pause with SNF5 lost a pause with OmoMYC (Fig. 4g). Principal component analysis of pausing indices revealed that the transcriptional effects of OmoMYC and SNF5 cluster more closely, and therefore have a similar effect, at MYC-bound, compared to MYC-unbound, genes (Supplementary Fig. 5a). And quantitative comparison of pausing index differences indicated that changes in pausing index were similar between OmoMYC and SNF5 at MYC-bound genes, but different at MYC-unbound loci (Supplementary Fig. 5b). Eighty percent of the MYC targets that gain a pause with SNF5 gain a pause with OmoMYC (Fig. 4h), a significantly higher level of overlap than for non-MYC targets (Supplementary Fig. 6a). In contrast, only 35% of MYC target genes experience a loss of pause under both conditions (Supplementary Fig. 6b). In general, the extent and significance of overlap between SNF5 and OmoMYC was higher for genes experiencing RNA polymerase pause induction, and the types of genes regulated in each direction were different (Supplementary Fig. 6c, d), with pause-induced genes being enriched in those connected to canonical MYC functions, including protein synthesis. Based on these data, we conclude that reintroduction of SNF5 in G401 cells mimics many of the transcriptional effects of MYC inhibition, and that a major impact of SNF5 on transcriptional events is to promote pausing of RNA polymerase at genes regulated by MYC.

Finally, we asked if these changes in RNA polymerase distribution correlate with the known effects of SNF5 reintroduction on the transcriptome of G401 cells. Comparing our PRO-Seq data to published RNA-Seq (RNA-Seq) data sets gathered at 3 or 7 days after reconstitution of G401 cells with SNF5\(^{32} \), we observed highly significant correlations between the two: At day 3, \(~30\%) of the genes that gain a pause with both SNF5 and OmoMYC in our experiments showed reduced RNA levels by RNA-Seq (Supplementary Fig. 6e), and at day 7 this overlap was \(~40\%) (Supplementary Fig. 6f). In contrast, there was no significant overlap between genes that lose a pause and those showing an increase in RNA levels at either time point (Supplementary Fig. 6g, h). The correlation between our PRO-Seq data and these published RNA-Seq data strongly implies that the ability of SNF5 to induce RNA polymerase pausing at genes regulated by MYC represents a significant mechanism through which it shapes the transcriptome.

**Discussion**

Given the frequency with which SWI/SNF components are perturbed in malignancy\(^2 \), understanding the mechanisms through which alterations in SWI/SNF drive tumorigenesis is fundamental to understanding how many cancers form and how they can be treated. Among SWI/SNF-mutant cancers, those defined by loss of SNF5 are particularly intriguing. On one hand, these cancers have an unusually simple genetic profile, with a single driver mutation—loss of SMARCB1/SNF5—and little if any evidence of collaborating oncogenic events. On the other hand\(^{11} \), these cancers are early onset malignancies\(^{41} \) that are difficult to treat and most often lethal. The contrast between the genetic simplicity of cancers like MRT and their aggressive nature implies that loss of SNF5 leads to a multitude of pro-oncogenic effects. Here, we provide evidence that one part of tumor suppression by SNF5 is to temper MYC binding to DNA. The direct connection between SNF5 and MYC explains the recurring activation of MYC target gene signatures in MRT\(^8,10 \) and, because of the broad suite of oncogenic activities possessed by MYC\(^22 \), can help rationalize how loss of a single tumor suppressor can have such profound effects on cellular pathophysiology.

The evidence that SNF5 directly impedes DNA binding by MYC is compelling (Fig. 1), and in line with recent NMR-based studies showing that the imperfect repeats of SNF5—which are required for this activity (Fig. 1b)—recognize the DNA-binding surface of the MYC:MAX bHLHZip heterodimer in a manner that is mutually exclusive with DNA recognition\(^{42} \). What we do not know, however, is the biochemical context in which SNF5—tempers MYC in cells. We see (Supplementary Fig. 3c), as others have reported\(^{12,37} \), that SNF5 that is reintroduced into MRT cells is incorporated into an intact SWI/SNF complex, suggesting there is little unincorporated SNF5 in our experiments. We also see in these experiments changes in chromatin accessibility (Fig. 3) that are consistent with functional reconstitution of SWI/SNF. We cannot, however, exclude the possibility that there is some low level of free SNF5 that inhibits MYC binding, or that SWI/SNF transiently donates SNF5 for MYC inhibition. Regardless of the context, however, the ability of SNF5 to interfere with target recognition by MYC in vitro and in two different cellular systems demonstrates a clear biochemical mechanism through which SNF5 antagonizes a key MYC activity.

The data presented here show that reintroduction of SNF5 into MRT cells leads to changes in TSS-distal chromatin accessibility at sites connected to differentiation and development (Fig. 3), very much in line with recently documented functions of SNF5 in maintaining lineage-specific enhancers and cell identity\(^{12,36,37} \) and activating bivalent promoters at developmentally important genes\(^37 \). By comparing these activities with the location of MYC in G401 MRT cells, we show that SNF5 has a second set of
activities—against MYC—that are almost exclusively promoter-proximal (Fig. 2), and induce RNA polymerase pause arrest at genes regulated by MYC (Fig. 4). Our ability to physically separate the canonical functions of SNF5 from its anti-MYC activities reveals that SNF5 does not modulate MYC binding via changes in chromatin accessibility, and supports a revised model in which dual regulation of both chromatin accessibility (at TSS-distal enhancers) and control of MYC (at TSS-proximal promoters) are part of the SNF5 tumor-suppression program (Fig. 5).

What is the functional significance of a dual tumor-suppression mechanism for SNF5? In cells such as G401, which possess a modest number of MYC binding sites, almost all of which are promoter-proximal (Fig. 2), these two mechanisms would impact distinct sites in the genome, allowing SNF5 to
support both the broad transcriptional patterning achieved by enhancer/super-enhancer regulation, as well as the gene-specific control that can be achieved via targeting a sequence-specific transcription factor such as MYC. By directly modulating MYC at promoters, SNF5 would have the ability to couple enhancer-mediated cell identity determination with essential ancillary processes such as regulation of the cell cycle and biomass production (Supplementary Fig. 6c); an activity that would be critical during processes such as development—where SNF5 plays a key role in maintaining the balance between pluripotency and differentiation—but upon SNF5 loss could readily conspire to drive tumorigenesis. Indeed, although the promoter-specific function of SNF5 in MRT has not previously been reported, our PRO-Seq studies clearly show that SNF5 impacts primary transcriptional events at MYC target genes in G401 cells and mimics MYC inhibition. Many of these changes in polymerase distribution correlate with changes in transcript levels, implying that the changes we see are relevant to disease processes in MRT. We also point out that the separable functions of SNF5 in regulating chromatin structure and tempering MYC do not necessarily have to operate at distinct regions of the genome. High levels of MYC overexpression lead to broad invasion of promoters as well as enhancers by MYC, and are associated with MYC binding to degenerate E-box elements. In cells with a high MYC burden, therefore, MYC that is bound at enhancers may be particularly sensitive to inhibition, and the tumor-suppressive functions of SNF5 could act within the context of SWI/SNF to both maintain normal patterns of open chromatin status at key enhancers and to resist cooption of enhancer function by ectopic MYC.

One of the challenges in treating cancers such as MRT is that loss of a key tumor suppressor such as SNF5 does not readily reveal a strategy where drug-like molecules—most of which are inhibitors—could be effective. Most children diagnosed with MRT die quickly from highly metastatic disease, despite treatment regimens that can involve combinations of surgery, chemotherapy, and radiation. Some modest improvements in patient survival have been made in recent years, but there is currently no standard of care for treating MRT sufferers, and the chances that a child diagnosed with MRT will survive a year, let alone 5 years, is very small. Our data strongly imply that loss of SNF5 drives MRT, in part, by derepressing MYC. Although there are no drug-like MYC inhibitors available at present, the importance of MYC to human cancer fuels intense interest in their discovery, and a variety of direct and indirect methods to target MYC in the clinic are being moved forward. Many of these approaches target the interaction of MYC with chromatin. OmoMYC itself is being developed in this capacity, and has in vivo action against atypical teratoid rhabdoid tumors, which (like MRT) are caused by SNF5 loss. The striking parallels we see between SNF5...
reintroduction and OmoMYC expression in G401 cells—as well as our finding that MRT cell lines depend on MYC for viability—lays a strong conceptual foundation for the idea that MYC inhibition would be unexpectedly effective in treating this malignancy, and others driven through inactivation of SNF5.

**Methods**

**Cell culture and transductions.** G401, A204, and HEK293 cell lines were obtained from ATCC and maintained in Dulbecco’s Modified Eagle Medium (DMEM) supplemented with 10% FBS and 1% penicillin/streptomycin, or RPMI with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin, respectively. HEK293 cells were maintained in DMEM supplemented with 10% FBS and 1% penicillin/ streptomycin. All cultures were maintained in a mycoplasma-negative atmosphere.

For inducible constructs, lentiviral transductions were performed by transfecting HEK293 cells with the appropriate inducible construct, the pMD2.G envelope expressing plasmid, and the psPAX2 packaging plasmid, which were gifts from Didier Trono (Addgene plasmid #12259 and #12260, respectively). Viral supernatants were collected and resuspended in DMEM with no phenol red. Flow cytometry analysis was performed on the population with puromycin and selected single cells. The number of GFP-positive cells in the population was determined using an HEMG buffer (10 mM HEPES, pH 7.6, 3.5 mM MgCl2, 100 mM KCl, 0.1 mM EDTA, 10% glycerol) with Protease Inhibitor Cocktail (Roche). Nuclei were pelleted at 500 g for 5 min. Excess over MYC:MAX or MAX:MAX and incubated for 30 min at room temperature before the biotin-E box probe was added. SNF5-null clones were selected with puromycin. The number of GFP-positive cells in the population was determined using an HEMG buffer (10 mM HEPES, pH 7.6, 3.5 mM MgCl2, 100 mM KCl, 0.1 mM EDTA, 10% glycerol, 1 mM DTT with Protease Inhibitor Cocktail, Roche), and then lysed by addition of ammonium sulfate at a final concentration of 0.3 M. Insoluble chromatin fraction was removed by centrifugation (100,000 × g for 20 min) at 4°C. Proteins were separated on a 5% to 20% gradient gel and stained with 0.05% crystal violet in 70% methanol overnight at room temperature and then destained with extensive washes with 70% methanol. For Western blotting and antibodies. G401 cells induced with 1 μg/ml doxycycline for 24 h (and other cells used in comparing SNF5 levels) were collected in a lysis buffer (150 mM Tris-HEPES pH 8.0, 150 mM NaCl, 5 mM EDTA, 1% Triton X-100 with Protease Inhibitor Cocktail, Roche), sonicated at 25% power for 15 s, and centrifuged at 4°C, 0.2 μm filter and rediluted with 0.5× Laemmli buffer. Western blots were performed using the LightShift Chemiluminescent EMSA kit (ThermoFisher Scientific) according to the manufacturer’s instructions.

**Flow cytometry analysis.** G401 cells transduced with inducible SNF5-HA were plated at 10 × 10^6 per plate and treated with 1 μg/ml doxycycline for 24 h. Cells were collected and lysed by denaturing in Buffer A (10 mM HEPES, pH 7.6, 3.5 mM MgCl2, 100 mM KCl, 0.1 mM EDTA, 10% glycerol, 1 mM DTT with Protease Inhibitor Cocktail, Roche). Nuclei were pelleted at 500 × g for 5 min, resuspended in Buffer B (10 mM HEPES, pH 7.6, 3.5 mM MgCl2, 100 mM KCl, 0.1 mM EDTA, 10% glycerol, 1 mM DTT with Protease Inhibitor Cocktail, Roche), and then lysed by addition of ammonium sulfate at a final concentration of 0.3 M. Insoluble chromatin fraction was removed by centrifugation (100,000 × g for 20 min) at 4°C. Proteins were separated on a 5% to 20% gradient gel and stained with 0.05% crystal violet in 70% methanol overnight at room temperature and then destained with extensive washes with 70% methanol. For Western blotting and antibodies. G401 cells induced with 1 μg/ml doxycycline for 24 h (and other cells used in comparing SNF5 levels) were collected in a lysis buffer (150 mM Tris-HEPES pH 8.0, 150 mM NaCl, 5 mM EDTA, 1% Triton X-100 with Protease Inhibitor Cocktail, Roche), sonicated at 25% power for 15 s, and centrifuged at 4°C, 0.2 μm filter and rediluted with 0.5× Laemmli buffer. Western blots were performed using the LightShift Chemiluminescent EMSA kit (ThermoFisher Scientific) according to the manufacturer’s instructions.

**Glycerol sedimentation assay.** G401 cells expressing inducible SNF5-HA were plated at 10 × 10^6 per plate and treated with 1 μg/ml doxycycline for 24 h. Cells were collected and lysed by denaturing in Buffer A (10 mM HEPES, pH 7.6, 3.5 mM MgCl2, 100 mM KCl, 0.1 mM EDTA, 10% glycerol, 1 mM DTT with Protease Inhibitor Cocktail, Roche). Nuclei were pelleted at 500 × g for 5 min, resuspended in Buffer B (10 mM HEPES, pH 7.6, 3.5 mM MgCl2, 100 mM KCl, 0.1 mM EDTA, 10% glycerol, 1 mM DTT with Protease Inhibitor Cocktail, Roche), and then lysed by addition of ammonium sulfate at a final concentration of 0.3 M. Insoluble chromatin fraction was removed by centrifugation (100,000 × g for 20 min) at 4°C. Proteins were separated on a 5% to 20% gradient gel and stained with 0.05% crystal violet in 70% methanol overnight at room temperature and then destained with extensive washes with 70% methanol. For Western blots, membranes were washed with water. All plates were analyzed with Odyssey (Li-Cor Biosciences). Western blots were performed using the LightShift Chemiluminescent EMSA kit (ThermoFisher Scientific) according to the manufacturer’s instructions.
ChiP and ChiP-Seq. Transduced cells were plated at 10 × 10^6 per plate and treated with 1 μg/ml doxycycline for 24 h. Cells were crosslinked using 1% formaldehyde for 10 min, quenched with 0.125 M glycine for 10 min, washed with ice-cold PBS two times, and collected by centrifugation. Cells were extracted in 10 mM HEPES, pH 7.9, 10 mM KCl, 0.1% NP-40 and then incubated in 1× TE (1 mM Tris, pH 8.0, 1 mM EDTA) for 15 min at 37 °C. Reactions were stopped by adding Trizol with Trizol and subsequently purified using chloroform and isopropanol. RNA adaptors (IDT) were then added to the 3′ side biotinylated RNA and following a second round of biotin-RNA purification, 5′ RNA caps were removed using CAP CLIP (CellScript, C-C1011H). 5′ RNA adaptors (IDT) were then added. One additional round of RNA purification was performed, and an RNA adapter was in a reverse transcription reaction to generate cDNA. Libraries were amplified using the generated cDNA and a PCR cycle number determined from a test analysis of a portion of sample. Library amplification was performed with Phusion high-fidelity polymerase (NEB) and customized Illumina-index based index primers (IDT). Sequencing data were obtained on an Illumina NextSeq500 with 75× single reads. Sequencing was performed by the VANTAGE Core at Vanderbilt University.

ChiP-Seq processing and analysis. ChiP-Seq reads were aligned to the human genome using Bowtie2. Peaks in each sample were called using MACS2 with q value of 0.01. Peaks were annotated using Homer command annotatePeaks, and enriched motifs were identified by Homer command findMotifGenome with the default region size and the motif length (-size 200 and -len 8, 10, 12). Transcription factor motifs were visualized using ChromaSharp (ChromaSharp, https://chromasharp.shinyapps.io/ChromaSharp/). Peaks were annotated using Homer command annotatePeaks to determine whether peaks were near TSS promoter or far away from TSS (TSS-distal). Enriched motifs were identified by Homer command findMotifGenome with the default region size and the motif length (-size 200 and -len 8, 10, 12) (https://bioinfo.vanderbilt.edu/NRSA/). Consensus peaks in each condition were identified using DiffBind, where peaks occurring at least two replicates were included. Peaked identified across conditions were combined into a final peak set and ChiP read counts were calculated for the final peak set. Read counts were normalized to the total mapped reads, and differential peaks were determined by DESeq2, which calculated the log2 fold changes, Wald test p values, and adjusted p values (False Discovery Rate, FDR) by the Benjamini–Hochberg procedure. The significantly changed peaks were assessed with an FDR < 0.05. Hypergeometric test was used to estimate the enrichment of MYC target genes in MysdB Hallmark data sets using all human genes as background. The overlap of EGP MYC peaks with published MYC peaks was calculated by first belong all putative EGP peaks which were generated by the average normalized peak intensity within ±2 kb from peak center with 100 bp bin size. The peak read graph showed the average normalized peak intensity in EGP, SNF5 and OMOMYC, where peaks were ranked by the normalized peak intensity. Transcription factor motifs were visualized using ChromaSharp (ChromaSharp, https://chromasharp.shinyapps.io/ChromaSharp/). Peaks that fell within 1 kb of promoter using function annotation clustering through DAVID (https://david.ncifcrf.gov/). Correlation of ChiP-Seq replicates on normalized counts of all promoters is presented in Supplementary Fig. 8a.

ATAC-Seq processing and analysis. Adapter sequences of ATAC-Seq reads were trimmed by cutadapt (cutadapt -a CTGTCATCTTATAACATCATCC-minimum-length 15 -paired-output), then aligned to the human genome using Bowtie2 (bowtie2 -p 8 -X 2000 -q-no-mixed-no-discard). Peaks in each sample were called using MACS2 (call peak with at least 2× number mapped reads and FDR < 0.102). Peaks were annotated using Homer command annotatePeaks to determine whether peaks were near TSS promoter or far away from TSS (TSS-distal). Enriched motifs were identified by Homer command findMotifGenome with the default region size and the motif length (-size 200 and -len 8, 10, 12) (https://bioinfo.vanderbilt.edu/NRSA/). Consensus peaks in each condition were identified using DiffBind, where peaks occurring at least two replicates were included. Peaks identified across conditions were combined into a final peak set and ATAC-Seq read counts for the final peak set were calculated using DiffBind. Read counts were normalized by the RLE method, and differential peaks were identified by DESeq2. Transcription factor motifs were visualized using ChromaSharp (ChromaSharp, https://chromasharp.shinyapps.io/ChromaSharp/). Transcription factor motifs were visualized using ChromaSharp (ChromaSharp, https://chromasharp.shinyapps.io/ChromaSharp/). Peaks that fell within 1 kb of promoter using function annotation clustering through DAVID (https://david.ncifcrf.gov/). Correlation of ATAC-Seq replicates on normalized counts of all promoters is presented in Supplementary Fig. 8b.

Pro-Seq processing and analysis. After adapter trimming and low-quality segment removal by cutadapt, Pro-Seq reads longer than 15 bp were reversed complemented using FastX tools. Reversed complemented reads were aligned to human genome using Bowtie2. Reads mapped to rRNA loci and reads with mapping quality less than 10 were removed. The reads were normalized by the RLE implemented in the DESeq2. NRSA (http://bioinfo.vanderbilt.edu/NRSA/), a tool to provide a comprehensive list of known TSSs. For known genes, was used to estimate RNA polymerase abundance in proximal-promoter and gene body regions of genes, to calculate pausing index and pausing index alterations. Briefly, the promoter-proximal region is defined by examining each 50 bp window with a 5 bp sliding step along the coding strand spanning ±500 bp from known TSS. The region with the highest number of reads is considered as the promoter-proximal region and its read density is calculated. Gene body is defined as the region from +1 kb downstream of a TSS to its transcription termination site (TTS). Pausing index for each gene is calculated as the ratio of promoter-proximal density over gene body density and the significance of pausing is evaluated by Fisher's exact test. DESeq2 was implemented to detect significant transcriptional changes for promoter-proximal and gene body regions accounting for the batch effect. The significantly transcriptional changes were assessed with an FDR < 0.05 or <0.0001 as described in figure legends. GO term analysis was performed on the overapped set of genes with an increased pausing using homer (https://homer.ucsd.edu/homer/). Consensus peaks in each condition were identified using DiffBind through DAVID (https://david.ncifcrf.gov/). Correlation of Pro-Seq replicates on gene body densities is presented in Supplementary Fig. 8c.
Reporting summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability. All sequencing data have been deposited at GEO with the accession number GSE109310. Routine metrics for all next-generation sequencing data are presented in Supplementary Table 2. Any other data supporting the findings in this study are available upon request. Uncropped scans for all blots are presented in Supplementary Fig. 9.

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Author contributions
A.M.W. and W.P.T. designed the experiments, interpreted the data, and wrote the manuscript. A.M.W., S.L.L. and G.C.H. performed analyses of data. J.W. and Q.L. performed and oversaw all computational and statistical analyses. E.M. contributed to experimental design and provided key reagents.

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