LncRNAs have been shown to be direct players in chromatin regulation, but little is known about their role at active genomic loci. We investigate the role of lncRNAs in gene activation by profiling the RNA interactome of SMARCB1-containing SWI/SNF complexes in proliferating and senescent conditions. The isolation of SMARCB1-associated transcripts, together with chromatin profiling, shows prevalent association to active regions where SMARCB1 differentially binds locally transcribed RNAs. We identify SWINGN, a lncRNA interacting with SMARCB1 exclusively in proliferating conditions, exerting a pro-oncogenic role in some tumor types. SWINGN is transcribed from an enhancer and modulates the activation of GAS6 oncogene as part of a topologically organized region, as well as a larger network of pro-oncogenic genes by favoring SMARCB1 binding. Our results indicate that SWINGN influences the ability of the SWI/SNF complexes to drive epigenetic activation of specific promoters, suggesting a SWI/SNF-RNA cooperation to achieve optimal transcriptional activation.
very significant portion of the genome can be transcribed upon different stimuli, giving rise to thousands of non-
coding RNAs (ncRNAs). Among them, long noncoding
RNAs (lncRNAs) refer to non-protein-coding transcripts longer
than 200 nucleotides, a broad definition that includes different
types of RNAs. Despite not being translated to proteins lncRNAs
are known to play an active part in multiple cellular processes
during diverse mechanisms. Numerous studies have addressed
the function of lncRNAs in gene silencing, however less is
known about their role at active genomic regions, where many
lncRNAs are transcribed. This is the case of enhancer RNAs
(eRNAs), a particular class of lncRNAs whose production at
enhancers has been directly related to enhancer activity, although
their specific role in this chromatin context is still debated. In addition, it has been proposed that some enhancer-
containing loci harbor lncRNAs with a similar function to
eRNAs', stressing the principle that these ncRNA categories are
not mutually exclusive. However, their function and relationship
with locally bound chromatin factors remain poorly investigated.
SWI/SNF complexes are multimeric ATP-dependent chromatin
remodelers that are critical in maintaining chromatin architecture
and gene expression. They are targeted to regulatory regions, in particular promoters, enhancers and super-enhancers,
playing a widespread role in enhancer activation. Out of the
variety of cellular processes in which SWI/SNF complexes participate, oncogene-induced senescence (OIS) raised particular
interest due to the major chromatin reorganization accompanying
this process. The concomitant gene expression changes aimed at activating this tumor suppressor program are often driven by the cooperative action of different epigenetic complexes, including SWI/SNF. Consistently, genes encoding for components of SWI/SNF are mutated in more than 20% of human cancers, being among the most prominent tumor suppressors in humans. The clearest demonstration of SWI/SNF tumor suppressor role are the malignant rhabdoid tumors (MRTs), specifically driven by the biallelic inactivation of the gene encoding for SMARCBJ SMARCB1 specifi
c role in this chromatin context is still

Results

SMARCBJ specifically binds to distinct transcripts. In order
to increase our understanding of the role of lncRNAs at active
chromatin regions, we set out to investigate the interaction
between lncRNAs and the SWI/SNF complexes. We hypothesized
that relevant interactions should be dynamic through processes
that implicate strong chromatin changes. For this reason, we
initially used as experimental model a controlled cellular process
in which the SWI/SNF complexes exert a major role, i.e., the
induction of cellular senescence, which, once induced by 4-
Hydroxymethylnofen (4OHT) administration, can promote

To investigate whether the observed differential binding was
due to a change in expression upon senescence induction, we
compared the enrichment of SMARCBJ-interacting RNAs with
their differential expression (Supplementary Fig. 1H). The correlation between the differential binding to SMARCBJ and the change in gene expression upon OIS was low (R = 0.22), suggesting that the enrichment in RNA–SMARCBJ interactions could not be explained by the differential availability of the RNA molecules.

We then focused our attention on the ncRNAs bound to
SMARCBJ. We selected a subset of both annotated and una
characterized lncRNAs, confirming their specific enrichment
by RIP-qPCR in proliferating and/or senescence conditions
(Supplementary Fig. 1D). In contrast, the immunoprecipitation of WDR5, a regulatory subunit of the MLL activator complex known to bind RNA, did not retrieve the lncRNAs isolated by SMARCBJ RIP with the same efficiency, although it pulled down its previously reported interactor VOXDS-ASI in proliferating cells (Fig. 1C). To further examine the nature of the observed interactions, we performed SMARCBJ immunoprecipitation coupled with UV irradiation (CLIP). Under these astringent conditions the association between SMARCBJ and some of the identified lncRNAs, including LINC00565, from now on called SWINGN, was maintained, indicating a direct binding between them and SWI/SNF (Supplementary Fig. 1I). On the other hand, CLIP of SUZ12, a core subunit of the Polycomb Repressor Complex 2 (PRC2), failed to enrich for the same SMARCBJ lncRNAs, while it efficiently pulled down lncRNAs previously reported as PRC2 binders (Supplementary Fig. 1J).

These results indicate that the SWI/SNF complexes specifically bind distinct lncRNAs in proliferative and senescent cells, which lead us to hypothesize a potential functional relevance of the interaction between this remodeling complex and a particular set of lncRNAs.

SMARCBJ preferentially localizes to active chromatin.

To investigate whether the interaction between lncRNAs and
SMARCBJ takes place in cis, that is, at DNA loci bound by the
chromatin complexes, we mapped SMARCB1 binding to the chromatin by ChIP-seq, which had only been previously established in some cancer cell lines. In parallel, we demarcated the regulatory regions of the genome by generating ChIP-seq data for the histone marks H3K4me2 and H3K4me3, since their ratio allows distinguishing between enhancer and promoter regions, as well as H3K27ac (enriched at active chromatin). All these studies were performed in proliferating fibroblasts (Fig. 2a), the experimental condition in which we observed a higher number of RNA species interacting with SMARCB1.

SMARCB1 ChIP-seq identified about $47 \times 10^3$ peaks (FDR < 0.01), a number similar to that already found for other components of the SWI/SNF complexes. Motif analysis of SMARCB1 sites revealed a selective enrichment for known motifs such as the one recognized by AP-1 (Fig. 2b and Supplementary Fig. 2A), a sequence-specific transcription factor.
factor already described as involved in SWI/SNF complex recruitment and in enhancer regulation upon senescence induction. These data suggest that SMARCB1 ChIP-seq is mapping SWI/SNF binding loci.

We found that SMARCB1 is enriched at genomic loci marked as promoters (high H3K4me3/H3K4me2 ratio) or enhancers (low H3K4me3/H3K4me2 ratio) (Fig. 2c), presenting a large overlap with H3K27ac signal. Consistently, the chromatin state model generated with ChromHMM showed that SMARCB1 binding peaks present higher percentage of occupancy in regions corresponding to active promoters and strong enhancers (Fig. 2d and Supplementary Fig. 2B).

Since SMARCB1 is present at actively transcribed chromatin regions, we wondered whether the gene loci bound by the SWI/SNF complexes harbored SMARCB1-interacting RNAs identified by RIP approach (Fig. 1c). Indeed, by cross-comparing ChIP and RIP-seq enriched genes, we observed 1217 transcripts interacting with SMARCB1 in cis, including 241 ncRNAs (Fig. 2e and Supplementary Data 3). These represent 74% of SMARCB1-interacting transcripts, a number higher than expected by chance (Supplementary Fig. 2C). Our findings demonstrate that SMARCB1 is able to bind active promoter and enhancer regions and to interact with coding and noncoding RNAs transcribed at some of these loci.

**SWINGN enhancer locus controls GAS6 expression.** The observation that the majority of SMARCB1-associated lncRNAs are transcribed from regions of active chromatin bound by SMARCB1 itself, prompted us to investigate with more detail the regulation of one of these loci. In particular, we focused our attention on the uncharacterized lncRNA, SWINGN, being (i) one of the lncRNAs most significantly interacting with SMARCB1 (Fig. 1e and Supplementary Data 1), (ii) associated to the protein preferentially in proliferating but not in senescence conditions (Fig. 3a), (iii) able to specifically interact with purified SMARCB1 in vitro (Supplementary Fig. 3A, B), and (iv) transcribed from a genomic locus bound by SMARCB1 (Fig. 3b).

SWINGN is an intergenic lncRNA located in the long arm of chromosome 13 presenting two annotated isoforms, a shorter one of 2.3 Kb known as LINC00565 and a longer one of 7.5 Kb annotated as TCONS_0002133, according to GENCODE v31 and the human lincRNA body map catalogs, respectively (Fig. 3a). SWINGN locus presents typical enhancer features in BJ cells. Heat maps are ranked by SMARCB1 occupancy. Right, distribution of the different states in the SMARCB1 binding map compared to the distribution in BJ total genome. Venn diagram representing the overlap between genes enriched in SMARCB1 RIP in proliferating conditions (n = 1641, including 481 ncRNAs, 980 mRNAs, and 180 pseudogenes) and genes presenting a SMARCB1 ChIP peak (26467). Significance (upper cumulative p-value) has been calculated by hypergeometric test.

**Fig. 2 Profiling of SMARCB1 genomic binding map highlights preferential binding at active, transcribed chromatin regions.** a Genomic snapshots of SMARCB1, H3K27ac, H3K4me2 and H3K4me3 ChIP-seq data at ATF3 and SERPINE1 loci in BJ proliferating cells. b Top enriched consensus motifs, as defined by MEME-ChIP motif analysis for the peaks in SMARCB1 ChIP-seq experiment in BJ cells. MEME-ChIP E-value estimates the expected number of motifs with similar features that one would find in a similarly sized set of random sequences. c Heat maps of SMARCB1, H3K4me2, H3K4me3, and H3K27ac occupancy at SMARCB1 peaks identified by SMARCB1 ChIP-seq in BJ cells. Heat maps are ranked by SMARCB1 occupancy. d Left, emissions of the chromatin state model generated by ChromHMM using histone marks ChIP-seq data in BJ cells, representing the percentage of regions assigned to a particular chromatin state (columns) containing a specific histone mark (rows). Right, distribution of the different states in the SMARCB1 binding map compared to the distribution in BJ total genome. e Venn diagram representing the overlap between genes enriched in SMARCB1 RIP in proliferating conditions (n = 1641, including 481 ncRNAs, 980 mRNAs, and 180 pseudogenes) and genes presenting a SMARCB1 ChIP peak (26467). Significance (upper cumulative p-value) has been calculated by hypergeometric test.
that SWINGN is an lncRNA transcribed from an enhancer locus, presenting some of the characteristics previously described for eRNAs.

Since transcriptional enhancers typically regulate genes that are proximally located, we investigated the regulatory interactions involving SWINGN locus. The analysis of public high-resolution Hi-C data of human proliferating fibroblasts showed that SWINGN is located in a topologically-associated domain also containing the protein-coding gene Growth Arrest Specific 6 (GAS6) (Fig. 3c). Both SWINGN and GAS6 showed a strong reduction in their expression levels upon senescence (Fig. 3d and Supplementary Fig. 4A), mainly due to transcriptional inhibition, as shown by Global Run-On sequencing (GRO-seq) data in proliferating and senescent cells (Fig. 3e–h) and as shown by Global Run-On sequencing (GRO-seq) data in proliferating and senescent cells (Fig. 3e–h). This transcriptional reduction is accompanied by a concomitant reduction in their expression levels upon senescence (Fig. 3d and Supplementary Fig. 4A), mainly due to transcriptional inhibition, as shown by Global Run-On sequencing (GRO-seq) data in proliferating and senescent cells (Fig. 3e–h).
Hi-C data showed the strongest contacts between SWINGN gene and two positions corresponding to the transcription start site (TSS) and the gene body of GAS6 (Fig. 3c). The contact between SWINGN and GAS6 TSS was confirmed with more resolution by 3C technique (Supplementary Fig. 4D, E), suggesting that SWINGN locus is an enhancer of GAS6. In addition, SMARCBl is bound to SWINGN as well as to GAS6 promoter and enhancer regions corresponding exactly to the two DNA loci where the strongest chromatin interactions occur (Fig. 3b, c). We therefore hypothesized that the regulation of the protein-coding gene GAS6 is orchestrated by the SWI/SNF complexes. To experimentally test this notion, we depleted SMARCBl or SMARCC1 in BJ fibroblasts and found GAS6 RNA and protein levels strongly affected, indicating that SWI/SNF is required for GAS6 expression (Fig. 3e and Supplementary Fig. 4F, G).

Taken together, these data show that the SWI/SNF complexes control the transcriptional activation of the proto-oncogene GAS6 as part of a topologically organized region that is in physical interaction with the enhancer-like SWINGN locus.

SWINGN controls GAS6 expression in a SWI/SNF dependent manner. It has been proposed that cis-acting noncoding transcripts may be required for optimal activity of enhancers5, so we wondered whether SWINGN transcript would also play a role in this regulation. To test this idea, we depleted the IncRNA in BJ and 1MR90 proliferating fibroblasts using three independent ASOs (Fig. 3f and Supplementary Fig. 5A, B). SWINGN reduction resulted in a consistent decrease in GAS6 expression at both RNA and protein levels (Fig. 3h), indicating that GAS6 is regulated not only by SMARCBl, but also by the RNA product of SWINGN locus. This regulation was specific for GAS6 within this chromatin domain since did not affect other neighbor genes although it did alter the expression of GAS6-AS2, a lncRNA sharing GAS6 promoter (Supplementary Fig. 5A).

To further confirm the functional co-dependency between SMARCBl and SWINGN transcript, we used as control a highly aggressive type of pediatric cancer driven by the biallelic deletion of SMARCBl gene12. In these tumors, SMARCBl loss impairs the chromatin affinity of the deficient SWI/SNF complexes, preventing the activation of specific enhancer regions implicated in differentiation.8 In particular, we focused on atypical teratoid/rhabdoid tumors (AT/RT), an incurable cancer of the central nervous system with loss of SMARCBl and protein levels (Fig. 3f), indicating that GAS6 is regulated not only by SMARCBl, but also by the RNA product of SWINGN locus. This regulation was specific for GAS6 within this chromatin domain since did not affect other neighbor genes although it did alter the expression of GAS6-AS2, a lncRNA sharing GAS6 promoter (Supplementary Fig. 5A).

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SWINGN-mediated control of GAS6 has implications in cancer. The regulatory interaction between SWINGN and GAS6 prompted us to investigate the biological consequences of altering this association. In fact, GAS6 is a secreted protein that binds to receptor tyrosine kinases of the TAM family to activate downstream pathways and promote cell growth and survival8. GAS6 function is consistent with the observed decrease of its expression upon OIS (Fig. 3d) and is also in line with a recent report showing that GAS6 overexpression delays senescence onset40. Consistently, the knockdown of SWINGN in BJ fibroblasts impairs their proliferation and increases apoptosis levels (Supplementary Fig. 6A, B). To get insight into SWINGN impact on GAS6 regulation in cancer we analyzed the correlation between SWINGN and GAS6 expression across samples of different tumor types of The Cancer Genome Atlas (TCGA). Their expression presented a positive correlation in several of the tumor types analyzed, such as lung squamous carcinomas (LUSC) or breast invasive carcinoma (BRCA), while there was no correlation in colon adenocarcinoma (COAD) (Fig. 4a and Supplementary Fig. 6C).

To elucidate whether the regulation of GAS6 by SWINGN is relevant in tumorigenesis, we selected H226 LUSC and HCT116 COAD cell lines, having respectively high and low expression of both SWINGN and GAS6 (Supplementary Fig 6D). Similarly to that observed in normal fibroblasts, in H226 lung cell line SMARCBl immunoprecipitation retrieved SWINGN (Supplementary Fig. 6E), and, SWINGN depletion decreased GAS6 expression (Fig. 4b, c). On the other hand, such dependency was not observed in HCT116 colon cells, which didn’t show a significant decrease of GAS6 mRNA even though ASO treatment was effective in reducing SWINGN levels (Supplementary Fig 6F), confirming that the regulation of GAS6 by SWINGN is cell type dependent. As consequence of SWINGN knockdown, H226 cell proliferation and colony formation capacity were affected concomitantly with an increase in the percentage of apoptotic cells (Fig. 4d, e and Supplementary Fig. 6G). Moreover, H226 cells depleted of SWINGN presented a reduced tumor growth rate when injected into immunodeficient mice (Fig. 4f and Supplementary Fig. 6H). On the other hand, SWINGN depletion in HCT116 cells did not affect cell growth (Supplementary Fig. 6I–J), consistent with the notion that the effect observed in cell proliferation is specific and dependent on the regulation of GAS6 by SWINGN. To better characterize the effect of SWINGN we performed gene expression analysis by RNA-seq on H226 cells upon SWINGN inhibition, revealing 1644 differentially expressed genes, whose change was validated in H226 and BJ cells (Fig. 4g, h, Supplementary Fig. 6K and Supplementary Data 4). Gene Ontology analysis on the genes affected showed terms related to apoptosis, cell migration and inflammation (Fig. 4i), in line with the cellular phenotype and the change in expression detected in tumors, indicating the role of SWINGN as a pro-oncogenic lncRNA.

SWINGN regulates SWI/SNF complexes at additional gene loci. Our data demonstrate that SWINGN regulates the oncogene GAS6. However, we observed that the inhibition of GAS6 did not completely phenocopy the loss of SWINGN (Supplementary Fig. 7A–C), and GAS6 overexpression only partially recovered the effect of SWINGN knockdown (Supplementary Fig. 7D). Interestingly, SWINGN inhibition affects a large set of genes (Fig. 4g). While many of these gene expression changes might be secondary to GAS6 downregulation, we speculated that others are caused by SWINGN through a SWI/SNF-dependent mechanism. In support of this hypothesis, the analysis of the promoters of the genes regulated by SWINGN41,42 showed the most significant enrichment in transcription factor binding motifs linked to the SWI/SNF complexes such as AP-1 and NFκB (Fig. 5a). In addition, a highly significant number of these genes have a SMARCBl ChIP-seq peak (p-value = 5.43e−38. Fig. 5b), confirming that genes regulated by SWINGN share their regulation with SWI/SNF.

We excluded that SWINGN had an effect on SWI/SNF stability, since we did not observe alterations of the protein
Fig. 4 SWINGN-mediated GAS6 regulation is relevant in cancer progression. a Correlation plot showing SWINGN and GAS6 expression (as FPKM+ pseudocount values) in LUSC samples (n = 552) from TCGA database (gdc-portal.nci.nih.gov). Correlation p-value is calculated using a t-distribution. b, c RT-qPCR (b) and Western blot (c) analysis of H226 lung squamous carcinoma cells depleted of SWINGN. RNA levels were normalized to ASO_CTRL and detected with two different primer sets. Graphs show mean ± SEM of normalized values (n = 3); image is from a representative experiment. d MTS proliferation assay of control and SWINGN-depleted H226 cells. Absorbance values were normalized to day 0. Graph shows mean ± SEM (n = 3). T-test p-value is calculated with data points at day 4: * < 0.05; ** < 0.01. e Clonogenicity assay of control and SWINGN-depleted H226 cells. Number of colonies (left) and cell density by absorbance (right) are shown. Graph shows mean ± SD of two independent experiments, picture refers to a representative experiment. f Analysis of tumors generated by subcutaneous injection of control or SWINGN-depleted H226 cells in BALB/cA-Rag2−/− mice. Graph shows tumor size mean ± SEM (n = 7); statistical significance was calculated using one-way ANOVA and Bonferroni’s Multiple Comparison Test compared to ASO_CTRL. g Volcano plot of RNA-seq analysis of H226 cells depleted of SWINGN using ASO_LINC#3 or treated with control ASO. Black dots indicate genes with a significant change comparing the two conditions (Adj. p-value < 0.01; logFC ± 0.5). Adj. p-value is calculated by quasi-likelihood (QL) F-test with Benjamini-Hochberg correction. h RT-qPCR validation of a selection of the most affected genes obtained in the RNA-seq analysis in H226 cells. Threshold: p-value < 10−4. p-value derived by random sampling of the whole genome combined with Fisher’s inverse chi-square method. Source data for panels b–h are provided as a Source Data file. Student’s t-test p-values in b–e summarized as follows: * < 0.05; ** < 0.01; *** < 0.001.
levels of different core components upon SWINGN knockdown (Supplementary Fig. 7E). We then speculated that SWINGN might influence SMARCB1 binding and/or activity at these regulated loci. To address this, we investigated the effect of SWINGN depletion on SMARCB1 occupancy in BJ fibroblasts. SWINGN inhibition did not cause a strong global effect on SMARCB1 chromatin binding (p-value = 0.03) (Supplementary Fig. 8A). However, a subset of SMARCB1 peaks was significantly affected (4791 peaks, p-value = 5.28e-05), with SMARCB1 signal reduced in 95% of these regions (Fig. 5c and Supplementary Fig. 8C). In parallel, we performed H3K27ac ChIP-seq under the same experimental conditions. The global analysis of H3K27ac occupancy only showed a slight effect upon SWINGN knockdown (p-value = 0.01) (Supplementary Fig. 8B). In contrast, a stronger effect was observed when focusing on the differential H3K27ac peaks (12,097 peaks, p-value = 1.64e-10), resulting from a drop of
Fig. 5 SWINGN regulates gene activation at additional loci by controlling SWI/SNF activity. a Analysis of transcription factor binding by Gene Set Enrichment Analysis across the Molecular Signatures Database for genes differentially expressed upon SWINGN knockdown. FDR is represented as Benjamini-Hochberg corrected. b Overlap between genes presenting an SMARCB1 ChIP peak and genes differentially expressed upon SWINGN depletion. Significance (upper cumulative $p$-value) has been calculated by hypergeometric test. c, d Metagene plot showing SMARCB1 (c) or H3K27ac (d) differential occupancy in control (ASO_CTRL) and SWINGN knockdown (ASO_LINC) (left) and pie chart illustrating the composition of the peaks (right). e Metagene plot showing H3K27ac differential occupancy in control (ASO_CTRL) and SWINGN knockdown (ASO_LINC) in regions with differential binding of SMARCB1 (left), all regions bound by SMARCB1 (center), and regions with unaffected SMARCB1 binding upon SWINGN knockdown (right).

c-e Significance has been calculated by t-test (represented as $p$-value) while difference between conditions has been measured by Euclidean distance (ED).
f Distribution of chromatin states at all SMARCB1 peaks (left) and in the regions differentially bound by SMARCB1 upon SWINGN knockdown (right).
g Heat map of selected genes differentially expressed upon SWINGN knockdown (RNA-seq) with a SMARCB1 or H3K27ac binding peak changing concordantly upon SWINGN depletion. h, i Genomic snapshot of SMARCB1 and H3K27ac ChIP-seq signals in control (ASO_CTRL) and SWINGN knockdown (ASO_LINC) conditions at GAS6/SWINGN (h) and PDGFRB (i) loci. Asterisks point out significantly changing peaks. The location of the primers used for ChIRP is indicated below. j RNA enrichment in ChIRP experiments with control (LacZ), CONCR and SWINGN probes determined by RT-qPCR and calculated as percentage of Input for the indicated transcripts. Graph shows mean ± SD of $n=5$ (SWINGN) or $n=2$ (CONCR). k DNA enrichment in ChIRP experiments with control (LacZ), CONCR and SWINGN probes determined by qPCR and calculated as percentage of Input with the indicated primer sets. Graph shows mean ± SEM of $n=4$ (SWINGN) or $n=2$ (CONCR). Source data underlying j–k panels are provided as a Source Data file. Student’s t-test $p$-values are summarized as follows: * $p<0.05$; ** $p<0.01$; *** $p<0.001$.

H3K27ac in 99% of these regions (Fig. 5d and Supplementary Fig. 8D).

To assess the relationship between the decrease in SMARCB1 binding and the H3K27ac reduction caused by SWINGN depletion, we analyzed H2K27ac signal at the 4791 SMARCB1 differentially bound sites upon SWINGN knockdown (Fig. 5c). A significant decrease in H3K27ac occupancy was observed at these sites ($p$-value = 1.7e$^{-10}$) (Fig. 5e). In contrast, the differences were partially reduced when considering all SMARCB1 regions and almost abolished when the SMARCB1 differential peaks were subtracted from the whole set of SMARCB1 regions (Fig. 5e). These data demonstrate that the changes in H3K27ac are mainly associated with the decreased binding of SMARCB1 at specific loci caused by SWINGN knockdown.

Next, since it has been shown that the ablation of SMARCB1 can decrease acetylation at enhancers11, we analyzed H3K27ac changes upon SWINGN knockdown separately at distal enhancer sites and proximal promoter regions. H3K27ac occupancy was mostly altered at regions identified as active promoters (Fig. 5f and Supplementary Fig. 8E, F), suggesting that SWINGN depletion affects preferentially SMARCB1 function in driving promoter acetylation.

Given the effect of SWINGN at specific promoters, we explored the connection between the observed chromatin changes and the alterations in gene expression induced by SWINGN down-regulation. Since we had described the role of SWINGN in the SWI/SNF-mediated activation of GAS6, we focused on genes whose expression and chromatin status were similarly and concordantly affected. Following these premises, we found 85 genes presenting concordantly altered expression, SMARCB1 and H3K27ac binding upon SWINGN knockdown (Fig. 5g and Supplementary Data 5). Of note, 276 genes did not show a change in gene expression concordant with SMARCB1 altered binding when SWINGN was depleted. We speculate that this may be due to the accumulation of opposed transcriptional and posttranscriptional mechanisms that result in an increase of mRNA steady state levels that do not reflect their epigenetic status.

On the other hand, we predicted that the 85 concordantly regulated genes include the primary SWINGN-SMARCB1 targets. As expected, GAS6 was one of them. SMARCB1 signal was significantly reduced at GAS6 enhancer region, accompanied by a decreased H3K27ac occupancy at both GAS6 and SWINGN promoter sites (Fig. 5h), and corresponding with the down-regulation of GAS6 mRNA levels. Besides GAS6, other genes of the same chromosomal region (13q34) appeared in the gene set co-regulated by SWINGN and SMARCB1 (Supplementary Fig. 8G). Intriguingly, the set of affected genes also included genes localized in different chromosomes already known to be dependent on SMARCB1 status, such as Platelet Derived Growth Factor Receptor Beta (PDGFRB). This gene, recently found altered in AT/RT and elected as a novel therapeutic target for these tumor types24, showed similar chromatin changes upon SWINGN knockdown, corroborating the hypothesis of a regulation orchestrated by SMARCB1 and SWINGN (Fig. 5i).

Since our data show that SWINGN and SMARCB1 physically interact and co-regulate several genes, we predicted that the lncRNA should be present at the co-regulated genomic loci. By applying Chromatin Isolation by RNA Purification (ChIRP) technique we found that the probes that specifically pulled down SWINGN (Fig. 5j) not only showed enrichment at its own genomic locus as expected, but also at additional positions where SMARCB1 binds in a SWINGN-dependent manner, including GAS6 and PDGFRB regions (Fig. 5k). In contrast, no significant enrichment was detected at HPRT promoter, bound by SMARCB1 but not regulated by SWINGN, or at a non-expressed intergenic locus (Fig. 5k). On the other hand, ChIRP of CONCR lncRNA, which is expressed at similar levels (Supplementary Fig. 8H), did not show a similar enrichment pattern (Fig. 5j, k).

These data are in accordance with the direct role of SWINGN in promoting SMARCB1 binding at specific loci to promote their activation.

**SWINGN activates a pro-oncogenic gene network.** We hypothesized that the subset of genes co-regulated by SWINGN and SMARCB1 might contribute to the observed role of the lncRNA in promoting the proliferation of cancer cells. Interestingly, we observed a positive correlation between SWINGN and several of its predicted targets across LUSC TCGA data set, including PDGFRB as well as Collagen Type 1 Alpha 1 Chain (COL1A1), another gene with known oncogenic function (Fig. 6a and Supplementary Fig. 9A)33–45. The transcriptional activation of these genes is highly dependent on SWINGN expression, as we demonstrated by upregulating its levels by CRISPR activation (CRISPRa) in H226 lung cancer cells. The induction of SWINGN resulted in a concomitant upregulation of not only GAS6, but also PDGFRB and COL1A1 mRNAs without affecting the expression of control genes (Fig. 6b). In line with the oncogenic role of these genes, we observed a decrease in proliferation of H226 cells when either PDGFRB or COL1A1 were inhibited, additive to the effect achieved with GAS6 downregulation alone (Fig. 6c–e and...
Finally, to explore the potential clinical relevance of the SMARCB1 and SWINGN-activated gene network, we selected the 27 top genes out of the 85 co-regulated by SWINGN and SMARCB1, and computed their expression across the LUSC cohort of TCGA, which includes 503 tumors and 49 samples of adjacent healthy tissue. Principal component analysis (PCA) and k-means algorithm based on the expression of this signature separated the samples into two clusters (Fig. 6f). Notably, cluster#1 contained all of the healthy tissues plus only 13 tumors, while cluster#2 included 490 tumors but not healthy tissue samples (Fig. 6f), suggesting a relationship between the transformed status and the expression of the SMARCB1-SWINGN- signature. In agreement with the SWINGN pro-proliferative role associated with gene activation, the average expression of the signature was elevated in the tumor samples conforming cluster#2 as compared to cluster#1 samples, mostly composed of normal tissue samples (Fig. 6g).

Thus, as a whole, our data indicate that SWINGN is necessary to drive SMARCB1 and the SWI/SNF complexes on a particular set of genes to promote their transcriptional activation, and the
coordinated regulation of this gene set by SWINGN uncovers a gene expression network that contributes to the transformed phenotype of cancer cells.

Discussion

The nature of the molecular signals driving the chromatin changes at certain genes while excluding others is still debated, in particular in cellular contexts where potent chromatin changes reshape gene expression, as in the case of oncogene-induced senescence. In this scenario, IncRNAs have raised great interest due to their specificity in expression and diversification of functions, including their capacity to interact with chromatin remodeling complexes to fine-tune their activities in a wide range of cellular contexts.

Our study takes an unbiased approach to isolate RNA molecules interacting with SMARCB1, a core component of the multimeric SWI/SNF complexes. While different studies reported the RNA binding capacity of SMARCA4, the main ATPase of SWI/SNF complexes, it is possible that other proteins of SWI/SNF complex, including SMARCB1, can directly bind RNA through non-canonical RNA-binding domains. Interestingly, analyses of SMARCB1 protein sequence and structure point to this potential; predictor algorithms as FFPred assign RNA binding function terms to SMARCB1, in line with the presence of a low complexity region (LCR), a type of domain often related with RNA binding capacity of proteins. We found that the association between SMARCB1 and some of the identified RNA interactors is maintained under UV-crosslinking conditions in vivo, while in vitro, purified SMARCB1 binds to SWINGN with more affinity than other unrelated RNAs, supporting the hypothesis that SMARCB1 has the capacity to establish direct interactions with some RNA partners. The biological specificity of the in vivo interaction is likely the combination of an intrinsic biochemical affinity as well as the physical proximity of the RNA and SWI/SNF in the nucleus. Importantly, the identification of IncRNAs differentially bound to SWI/SNF in the dynamic context of senescence induction, suggests the functional relevance for at least some of these interactions.

The RNA-binding capacity of SWI/SNF complexes suggests that RNA-SWI/SNF interactions probably are a prevalent phenomenon, RNAs transcribed from cis-regulatory elements having the potential of establishing a mechanism of SWI/SNF-dependent gene regulation. Emerging studies indicate that the interactions between RNA and SWI/SNF can have opposite roles. While Xist antagonizes the ATPase activity of the SWI/SNF core subunit SMARCA4, 7SK small nuclear RNA directs the SWI/SNF complexes to enhancer elements. We speculate that distinct RNA structures or SWI/SNF associated factors as well as specific chromatin and transcriptional contexts might be key to the different outcomes. Although it is difficult to find a unifying mechanism, our study provides additional evidence of the functional crosstalk between RNA and SWI/SNF complexes.

SWINGN, which associates to the chromatin complexes in proliferating but not in senescent cells, represents a relevant example of a SMARCB1-interacting IncRNA. Our detailed study of SWINGN locus in human fibroblasts reveals the existence of a regulatory hub formed by the enhancer element harboring SWINGN, the SWI/SNF complexes and the IncRNA itself in a genomic region topologically-associated to GAS6 gene. The presence of SMARCB1, ensuring the formation of intact SWI/SNF complexes, as well as an appropriate chromatin conformation allowing the physical proximity between SWINGN and the regulated loci, are necessary for this regulatory mechanism, as suggested by the absence of SWINGN-SMARCB1 crosstalk in other cell lines such as AT/RT or HCT116.

Intriguingly, besides GAS6 regulation, SWINGN affects SMARCB1 binding and transcriptional activation at additional distant gene loci, with a more prominent effect at proximal sites, where it is accompanied by a concomitant change in H3K27ac. This finding led us to propose that this IncRNA can influence the ability of the SWI/SNF complexes to regulate promoter activation, possibly by establishing contacts with multiple loci. This hypothesis is supported by the analysis of the genes subject to SMARCB1-SWINGN regulation, which not only include GAS6, but additional targets belonging to the same chromosomal band, suggesting that SWINGN enhancer locus extends its control to longer distances, as already demonstrated for other IncRNA-producing enhancers.

Intriguingly, some of the most relevant SMARCB1-SWINGN targets, such as PDGFRB and COL1A1, are located in different chromosomes. The detection of SWINGN enrichment at PDGFRB by ChIP supports the notion that SMARCB1 and SWINGN-containing complexes participate in the regulation of these loci. We propose that a precise spatial chromatin organization around SWINGN enhancer region could bring together multiple distant genomic loci, allowing their co-regulation by a small number of SWINGN molecules. Some of our conclusions are supported by gene expression and ChIP-seq analyses upon SWINGN depletion, but applying ligation-free methods to capture DNA interactions at unforeseen distance or even between different chromosomes would greatly contribute to shedding light on the long-distance regulatory function of this IncRNA.

The unveiled role of SWINGN in the establishment of its regulatory network provides an epigenetic signature for tumor
progression linked to SMARCB1 alterations. While the global tumor-suppressor activity of SWI/SNF is well established, we have uncovered a subjacent oncogenic set of SMARCB1-SWINGN targets, including GAS6, PDGFRB, and COL1A1. In tumors such as lung squamous cancers, where the SWINGN-SWI/SNF axis is active and the regulatory connection is maintained, SWINGN may represent an interesting therapeutic target.

In conclusion, our study reveals that the SWI/SNF complexes can specifically bind IncRNAs such as SWINGN, which can influence their ability to drive activation of specific promoters. Furthermore, they suggest the possibility of a general mechanism in which SWI/SNF complexes cooperate with RNA to achieve transcriptional activation.

Methods

Cell culture, retroviral-lentiviral infection, and treatments. The following human cell lines were used for this study: B) hTERT foreskin fibroblasts (kindly provided by Dr. J. Gil laboratory), IMR90 lung fibroblasts and HEK-293T (ATCC), H226 lung carcinoma cells, HCT11 Colon adenocarcinoma and CHLA-28 thyroid cancer cells.

B) hTERT, IMR90 and HEK-293T cell lines were cultured in DMEM medium (GIBCO), supplemented with 10% fetal bovine serum (GIBCO) and 1× penicillin/streptomycin (Lonza). H226 cells were maintained in RPMI-1640 medium (GIBCO). See Table 1 for detailed culture conditions.

RNA extraction, RT-qPCR analysis, and ddPCR analysis

To generate OIS cell systems, B) hTERT fibroblasts. Cells were selected with Neomycin-G418 (Sigma) at a final concentration of 400 μg/ml for at least one week. For senescence induction studies, B) hTERT ER-RAS fibroblasts were treated with 200 nM of 4-hydroxy-tamoxifen (4OHT) for six days replacing medium at 25 passages were used for experiments.

To cluster regularly interspaced short palindromic repeat (CRISPR) activation studies, H226 cells were first engineered to stably express a nucleolarlyinacitve cas9 protein (dCas9) fused to VP64 transcriptional activator (Addgene Plasmid #61425). Lentiviral production in HEK-293T cells and H226 cells were transfected with 1× B-27, 20 μg/ml EGF and 20 μg/ml FGF (all GIBCO).

Transfection reaction was carried out in opti-MEM medium (GIBCO) using Lipofectamine 3000 (Invitrogen), following manufacturer’s instructions. Forty-eight hours after transfection, filtered supernatant supplemented with 4 μg/ml polybrene (Santa Cruz), was used to transduce low passage BJ hTERT ER:RAS cells. For senescence induction studies, B) hTERT ER-RAS fibroblasts were treated with 200 nM of 4-hydroxy-tamoxifen (4OHT) for six days replacing medium at day 3, unless specified otherwise in the text. Only fibroblasts cell lines with less than 20–25 passages were used for experiments.

Stable AT/RT cell lines with inducible re-expression of SMARCB1 were established as previously reported39. Briefly, CHLA-06 cells were transduced with pnducr-21-SMARCB1 or empty pnducr-21 lentiviral vectors, kindly provided by Charles Roberts’s lab. Seventy-two hours after transduction, GEP-positive cells were sorted and treated or not with doxycycline (1 μg/ml) for 48 h to induce SMARCB1 re-expression.

For exogenous GAS6 treatment, recombinant human GAS6 (rGAS6; R&D system) was added to cell medium of IMR90 or H226 cells to a final concentration of 250 ng/ml and incubation was prolonged for 48 h.

For Actinomycin D (ActD) treatment, B) hTERT ER-RAS cells were grown for different time points with a final concentration of 10 μg/ml of ActD solution (Sigma).

For transfection with pLNC-ER:RAS or pLNCX (Empty) vectors (gift from Dr. Gil and described in the ref. 32), 6 μg of gag-pol plasmid and 3 μg of pVSVG vector.

For senescence induction studies, B) hTERT ER-RAS fibroblasts were treated with 200 nM of 4-hydroxy-tamoxifen (4OHT) for six days replacing medium at day 3, unless specified otherwise in the text. Only fibroblasts cell lines with less than 20–25 passages were used for experiments.

All siRNAs employed in this study were designed using BLOCK-iT™ RNAi Designer webpage (https://rnaidesigner.thermos Fisher.com/rnaexpress/) and purchased from Sigma.

All SWINGN-targeting and CTRL ASOs were designed and synthesized by Ionis Pharmaceuticals. ASOs shown in this study were selected from a larger panel of oligonucleotides based on high levels of on-target inhibition and low levels of off-target and toxicity effect.

All siRNAs used in this study are listed in Supplementary Data 6.

Nuclear/cyttoplasm fractionation

For proliferation assay, 1000 cells/well were plated in 96-well plates and cell proliferation was measured over 3 or 4 days with a CellTiter96 Aqueous Non-Radioactive Cell Proliferation Assay (MTS) Kit (Promega), following manufacturer’s advice. Four hundred and ninety nanomolar absorbance was measured by spectrophotometry using the SPECRoStar Nano 96-well plate reader (BMG Labtech).

For clonogenicity assay, 500–1000 cells were plated in 6-well plates and grown for 8–10 days in normal medium. Cells were fixed with 0.5% glutaraldehyde for 15 min and stained with 0.1% Crystal Violet solution (Sigma) for 30 min.

To assess colony formation capacity, plates were air-dried and colonies were counted manually. To quantify cell density, cells were incubated with 500 μl of 10% acetic acid (Sigma) and collected in ELISA plates. Absorbance was measured by spectrophotometry at 570 nm in a SPECRoStar Nano equipment.

For BrdU incorporation assay, cells were labeled with 50 μM BrdU solution (BD Pharmingen) for 16–18 h. In case of senescent cells, OIS was previously induced for 5 days before starting BrdU assay. Then, cells were harvested by trypsinization and BrdU staining was performed using BrdU Flow Kits (BD Pharmingen), according to manufacturer’s instructions. Amount of BrdU incorporation was measured by flow cytometry using a FACSAccuri Cell Analyzer (BD Bioscience).

To measure apoptosis, cells were quickly detached using Accutase solution (Lonza) and kept on ice. Apoptosis was assayed by Annexin V and 7-AAD staining using the Apoptosis Detection Kit I (BD Biosciences) and FACSAccuri flow cytometer, following manufacturer’s recommendations.

Flow cytometry data were recorded by BD CellQuest program and analyzed using the FlowJo software.

Xenograft model

1.5 × 106 H226 cells transfected with SWINGN-specific or CTRL ASOs were suspended in 180 μl of complete medium and mixed with Matrigel (Corning) in a ratio of 1:3. The resultant mix was injected subcutaneously in the flank of 6–7-week old female BALB/cA-Rag2−/−/Il2rg−/− immunodeficient mice (n = 7 per condition). Tumor size was measured over 39 days at the indicated times in a blinded fashion using an electronic precision caliper. The tumor volume (V) was calculated using the formula: V = n/6 × width2 × length.

Protein extraction and immunoblot analysis

Cells were lysed for 15 min in rotation at 4 °C using RIPA buffer (150 mM NaCl, 25 mM Tris HCl [pH 7.5], 2 mM EDTA, 0.1% sodium deoxycholate [Na-DOC], 0.1% SDS, 1% Triton X-100) supplemented with 1× Complete Protease Inhibitor Cocktail [Roche]. Lysed cells were centrifuged at max speed for 10 min at 4 °C and the insoluble pellet was discarded. Protein concentration was estimated by Pierce BCA Protein Assay Kit, using BSA curve as reference and according to manufacturer’s instructions. Proteins were separated on denaturing SDS-PAGE gels and transferred to a nitrocellulose membrane [Biorad] following standard procedures. Membranes were blocked using skim milk or BSA (VWR) and probed first for primary and then for HRP-conjugated secondary antibody. Western Lightening ECL-Plus (Perkin Elmer) was employed for chemiluminescence detection of proteins.

The list of antibodies used in this study can be found in Supplementary Data 6.

Uncovered scans of the most important blots are supplied in the Source Data file.

RNA extraction, RT-qPCR analysis, and ddPCR analysis

Total RNA was isolated using TRIzol reagent (Sigma), treated with DNAse I (Invitrogen) and reverse-transcribed using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystem) with random hexamer primers following manufacturer’s instructions. For reverse-transcription of only poly-A (+) RNAs, DNase I-treated total RNA was converted into cDNA using the SuperScript II Reverse Transcriptase Kit (Invitrogen) with 2.5 μM of Oligo(dT)18. The obtained cDNA was analyzed by quantitative PCR using iTag Universal SYBR Green (Bio-Rad) with kit ViA™ 7 Real-Time PCR System machine (Thermo-Fisher). All reactions were performed...
in triplicate or quadruplicate and HPR1 (Hypoxanthine Phosphoribosyl transferase 1) RNA levels were used for normalization, unless specified otherwise in Figure legend.

To evaluate the absolute number of SWINGN RNA molecules per cell, total RNA was isolated from 0.5×10^6 BJ or H226 cells accurately counted using a Countess Automated Cell Counter (Thermo Fisher). RNA extraction and cDNA generation were performed as described above using 1 μg of RNA. Droplet digital PCR (ddPCR) and qPCR analysis was performed using the QX200™ Droplet Digital PCR System (Bio-Rad) with the 20 μl reaction mixtures containing 0.9 μM primers, 0.25 μM probes, 1X ddPCR Supermix for Probes No dUTP (Bio-Rad) and 30 ng of cDNA as final concentration. PCR amplification settings were optimized using different temperatures and PCR final assay was performed with the following cycling conditions: 10 min at 95°C; 40 cycles of 30 sec at 94°C and 60 sec at 56°C; and 1 cycle of 10 min at 98°C with a 2°C/s ramp rate. Positive droplet populations were separated from negative droplets and quantified automatically with QuantaSoft droplet reader software (Bio-Rad) as copies/μl. Probes targeting long or short SWINGN isoforms were designed in the regions amplified by set1F or set1F primer sets, respectively (details in Supplementary Fig. 3). Probes carrying a 5′-FAM (Fluorescein) fluorophore, a 3′-BIQ (Iowa Black Quencer) and an internal ZEN quenchers, were synthesized by IDT. All qPCR primer and ddPCR probe sequences are provided in Supplementary Data 6.

**RNA sequencing and data analysis.** For RNA-seq of BJ cells depleted or not of SWINGN, total RNA was isolated and DNAse I-treated using the Trizol Simply RNA kit (Promega). ASO_LINC3 was used to knockdown SWINGN expression. One microgram of quality-verified RNA was used for library preparation and sequencing on Illumina Nextseq 500 (75 bp single-end mode, 10× reads/sample). Sequencing data were aligned to the genome assembly hg19 using STAR55 with default parameters. Differential expression analysis was carried out by using the R package edgeR5 in Bioconductor and significant genes were selected applying the following filtering: adjusted p-value < 0.01; |log2foldchange| > 0.5.

**RNA immunoprecipitation and sequencing (RIP-seq).** Native RNA immunoprecipitation was performed as previously described2 with minor modifications. Briefly, 1.5×10^7 BJ fibroblasts were treated to induce senescence as indicated in the text, harvested and lysed. Nuclear extract was isolated and homogenized by dounce tissue grinder (pH 7.4), followed by the addition of Dynabeads Protein A or G beads and incubated with specific antibodies (listed in Supplementary Data 6) overnight. 40 μl of Protein A or G beads were used to recover antibody-protein complexes, which were then split into two fractions (for protein and RNA detection). To isolate proteins, beads were mixed in 2X Protein Loading Buffer supplemented with DTT to 50 and 10 min load and incubated overnight at 4°C for western blot analysis. To retrieve RNA, beads were re-suspended in 1 ml of TRI reagent and RNA extraction was performed as stated above.

For RIP-qPCR, equal volume of Input and Immunoprecipitated RNA were treated with DNAse I and reverse-transcribed. qPCR results were normalized and represented as percentage of Input.

For RIP-seq assay, BJ fibroblasts were previously UV crosslinked at 1500×100 μm^2/cell in a UVc500-Hoefer UV crosslinker

For RIP-seq analysis, SMARC1 native RIP experiment was performed in biological triplicate. RNA quality was verified by Experion RNA analysis kit (Bio-Rad) and the integrity of the RNA measured by Qubit and mean RNA fragment size was assessed with a 4200 TapeStation Automated Electrophoresis System (Agilent Technologies). Multiplexed libraries were sequenced on an Illumina Nextseq500 platform in a 75 bp paired-end mode, with a depth of at least 10× reads/sample. CTRL and SWINGN knocked down ChIP-seq experiment was performed in biological duplicate.

For in vitro pulldown assay, BJ or H226 cells were collected 36 h after transfection. ChIP-seq analysis was run with no cut off filters and all RIP-seq peaks detected were visualized as a volcano plot (Fig. 1b).

In vitro RNA pulldown assay. To analyze SMARC1 capacity of RNA binding, 30 pmol of biotinylated SMARC1 C-terminal MYC/DDK-tagged protein (Supplementary Fig. TP317885) were attached to 40 μl anti-FLAG M2 Magnetic Beads (Sigma) in Binding Buffer (150 mM NaCl; 50 mM Tris HCl [pH 7.5]; 1 mM EDTA; 0.5% NP-40; 10% glycerol and supplemented with protease inhibitors) for 2 h at 4°C. Total RNA was extracted from BJ fibroblasts as described above, denatured by heating at 70°C for 10 min and then cool down slowly to 4°C to allow proper folding. One million of renatured RNA was used to bead-coating SMARC1 protein for RT for 3 h along with RNAse inhibitors. RNA-protein complexes were washed four times with the same incubation buffer with a concentration of 300 mM NaCl. RNA were eluted and extracted with TRI reagent and analyzed by RT-qPCR as previously described. An aliquot of starting RNA material was processed in parallel and used as input RNA for normalization. The position of the different primer sets spanning SWINGN sequence is illustrated in Supplementary Fig. 3.

**Chromatin immunoprecipitation (ChIP).** HJ527ac ChIP qPCR was performed as previously described29. SMARC1 and histone marks ChIP was performed as described38 with some modifications. Briefly, 4×10^7 BJ cells were double-crosslinked in 2 mM dithiothreitol (DTT) and fixed at 2°C. Crosslinking was performed for 45 cycles (30″ each) in 150 mM NaCl, 0.1% sodium dodecyl sulphate (SDS). Fixed cells were washed with PBS and DNA was extracted with TRIzol (M) and chloroform. A 4μg microgram of DNA was isolated and concentrated to 100 ng by using a GeneClean II kit (Biorobot). DNA quality was verified by Experion RNA analysis kit (Bio-Rad) and the integrity of the DNA measured by Qubit and mean DNA fragment size was assessed with a 4200 TapeStation Automated Electrophoresis System (Agilent Technologies).

For UV-RIP assay, BJ or H226 cell DNA was precipitated by UV (for different temperatures and different exposure, settings were optimized using standard UV-transfection cells) and isolated in DNE PAGE gel for western blot analysis. To retrieve RNA, cells were incubated overnight at 4°C for 100 min load and incubated overnight at 4°C for western blot analysis. To retrieve RNA, cells were incubated overnight at 4°C for 100 min load and incubated overnight at 4°C for western blot analysis. To retrieve RNA, cells were incubated overnight at 4°C for 100 min load and incubated overnight at 4°C for western blot analysis.

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For ChIP-seq analysis of cells depleted of SWINGN, BJ fibroblasts were transfected with CTRL or LINC3 ASOs and cells were collected 36 h after transfection.

**ChIP sequencing (ChIP-seq) and data analysis.** ChIP-seq libraries were prepared with at least 5 ng of DNA, following the protocol described in the ref.39 including a final 0.6× SPRI beads clean-up for fragment size selection. Pooled ChIP-seq library concentration was measured by Qubit and mean DNA fragment size was assessed with a 4200 TapeStation Automated Electrophoresis System (Agilent Technologies). Multiplexed libraries were sequenced on an Illumina Nextseq500 platform in a 75 bp paired-end mode, with a depth of at least 10× reads/sample. CTRL and SWINGN knockdow ChIP-seq experiment was performed in biological duplicate.

For ChIP-seq analysis, the human genome assembly hg19 (BAM) files were sorted and PCR replicates were removed using bowtie2 (parameters:--no-discardant--no-mixed--X 1000 very-sensitive), PICARD and samtools (parameters: -q 15 -bh -F 1028)59. BedGraph and BigWig files were generated using bedtools60 and bedGraphToBigWig tools. ChIP-seq peaks were determined by performing MACS259. MACS2 was run with following cut offs: for H3K27ac ChIP, FDR < 0.001; for SMARCB1 ChIP, FDR < 0.001. Selected ChIP-seq peaks were used for visualization using IGV viewer (Broad Institute).

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reads. Regions were sorted in descending order based on the mean value of the ChIP-seq signal per region and visualized using deepTools plotHeatmap. SMARCB1 and H3K27ac signals (as.bigwig) derived from merged BAM ChIP-seq signal per region and visualized using deepTools plotHeatmap. Reads. Regions were sorted in descending order based on the mean value of the

**Chromatin conformation capture (3C) analysis.** Quantitative Chromosome Conformation Capture (3C-qPCR) assay was performed following a previously published protocol with minor modifications. Digestion efficiency was measured by qPCR quantification through multiple restriction sites in undigested and digested samples, by using PCR primers that amplified across HindIII restriction sites. To analyze ligation products forward anchor primer was used in combination with forward primers designed across HindIII cutting sites over SWINGN/Genes locus. Interaction frequencies were normalized to HPRT or intergenic region controls. Primer position is depicted in Supplementary Fig. 4E and their sequence can be found in Supplementary Data 6.

**ChIPR assay.** ChIPR assay has been performed in BJ fibroblasts by using cross-linked nuclear extract from 9 × 10⁶ cells, as previously described. Briefly, BJ cells were crosslinked in 1% formaldehyde and then lysed using Lyss Buffer (50 mM Tris HC1 [pH 7.5], 10 mM EDTA, 0.5% SDS). Cells extract was sonicated in a microtome device for 15 cycles (30″ON/45″OFF) and centrifuged at max speed to eliminate insoluble chromatin. One percent of cleared extract was saved as input while the remaining material was diluted with Hybridization Buffer (15% formamide, 500 mM NaCl, 1 mM EDTA, 0.5% SDS, supplemented with protease and RNase inhibitors, incubated with specific probes and rotated O/N at RT. The following day 400 μl of Streptavidin magnetic beads (Dynabeads MyOne Streptavidin C1-Thermo Fisher) were added to each elution condition and incubated 4 h at RT in rotation. Beads were then washed five times with Wash Buffer (2x saline-sodium citrate [SSC], 0.5% SDS) while elution was carried out using PK buffer (100 mM NaCl, 1 mM EDTA, 0.5% SDS, 10 mM Tris HC1 [pH 7] or [pH 8] for RNA or DNA, respectively). 10% of ChIPR samples was used to analyze RNA enrichment by RTPCR after standard RNA extraction while the remaining material was employed to amplified probes and probes O/N at RT. A pool of 3 different biotinylated oligonucleotides was used to pull down SWINGN RNA while two probes targeting CONC and LacZ biotinylated oligos. All biotinylated probes were purchased from IDT and listed in Supplementary Data 6.

**Gene ontology and pathway analysis.** To identify enriched molecular pathways associated with differences in gene expression, Ingenuity Pathway Analysis (IPA) (http://www.ingenuity.com/) was performed using filtered differentially expressed genes as indicated in the text. This analysis was implemented with Gene Ontology (GO) functional enrichment analysis, which was carried out through TopGene web application, part of the TopGene portal. Representative significantly-enriched categories were selected with a Benjamini-Hochberg corrected false discovery rate (FDR B&H) threshold of 0.05.

**Expression analysis in tumor samples.** Transcriptomic data (aligned with hg38 genome assembly) were obtained from the TCGA Data Portal (https://tcga-data.nci.nih.gov). SWINGN expression levels were evaluated in those cancer types presenting at least five normal and five tumor samples. Log2FPKM values were used for differential expression analysis, which was carried out with LIMMA. For enrichment studies, log2FPKM gene expression values in TCGA data of different cancer types were used. The distribution of TCGA RNA-seq data for the genes of interest was evaluated by Shapiro-Wilk normality test and resulted normal. Therefore, Spearman correlation was used to compare expression data. For logarithmic representation, a pseudocount was added representing the lowest FPKM expression value for each dataset.

To analyze SWINGN oncogenic signature, filtering was applied to select genes with affected SMARCB1 and H3K27ac binding upon IncRNA deletion, as well as highly changing in gene expression (log2FC) > 1. The expression of this gene subset was evaluated in lung squamous carcinoma (LUSC) TCGA dataset to generate an expression matrix across the 552 LUSC samples (49 normal and 503 tumors). Principal component analysis followed by k-means algorithm was used to identify the different clusters. Silhouette function was used to determine the optimal number of clusters before applying the k-means method. Average expression for each sample was calculated separately for each cluster and represented as box plots.

All the analyses were performed in R/Bioconductor and statistical significance was determined by unpaired Student’s t-test.

**Statistical analysis.** Experimental data were represented as mean ± standard deviation of at least three biological replicates (unless specified otherwise in Figure legends) and significance was determined by two-tailed unpaired Student’s t-test using GraphPad software. Significant p-values were summarized as follows: not significant (ns); p-value < 0.05 (*); p-value < 0.01 (**); p-value < 0.001 (***)

**Reporting summary.** Further information on research design is available in the Nature Research Reporting Summary linked to this article.

**Data availability**

A reporting summary for this Article is available as a Supplementary Information file. RIP-seq, ChIP-seq and RNA-seq data reported that support the findings of this study have been deposited in Gene Expression Omnibus (GEO) repository under the accession number GSE128327. The raw data (including uncropped western blot images) underlying Figs. 1E, 3D–H, 4B–H, 5J, 6B–E and Supplementary Figs. 1A–E, 1I, 1J, 1H, 2A, C, G, 5A, B, 6A, E, 7A, B, E, 9B–D are provided as a source data file. All the other data supporting the findings of this study are available from the corresponding author upon reasonable request.

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Author contributions
E.Gr. and M.H. conceived and designed the experiments; E.Gr. and I.R. performed experiments and performed data analyses; J.G. and F.P.M. provided experimental support; E.Gr. performed bioinformatics analyses; V.C. and J.I.M.S. supervised chromatin state analysis; S.G. contributed with design, testing and production of reagents; E.Gr. and M.H. wrote the manuscript; M.H. supervised the work.

Competing interests
The authors declare no competing interests.

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