We hereby provide the initial portrait of lincNORS, a spliced lincRNA generated by the MIR193BHG locus, entirely distinct from the previously described miR-193b-365a tandem. While inducible by low O₂ in a variety of cells and associated with hypoxia in vivo, our studies show that lincNORS is subject to multiple regulatory inputs, including estrogen signals. Biochemically, this lincRNA fine-tunes cellular sterol/steroid biosynthesis by repressing the expression of multiple pathway components. Mechanistically, the function of lincNORS requires the presence of RALY, an RNA-binding protein recently found to be implicated in cholesterol homeostasis. We also noticed the proximity between this locus and naturally occurring genetic variations highly significant for sterol/steroid-related phenotypes, in particular the age of sexual maturation. An integrative analysis of these variants provided a more formal link between these phenotypes and lincNORS, further strengthening the case for its biological relevance.
Cells monitor intracellular oxygen ($O_2$) tension using specific oxygenase enzymes that act as negative regulators for hypoxia-inducible factors (HIF) and their downstream transcriptional programs. Although the core of this mechanism is known to operate in essentially all metazoans, decreased $O_2$ (hypoxia) also elicits context-specific responses, which remain incompletely understood. Similar to other signaling pathways, valuable insights to this end continue to emerge from noncoding transcriptome studies. During the past decade, various types of non-coding RNAs have been shown to respond to hypoxia, potentially functioning as feedback elements that impact various branches of this response. Among the best documented is miR-210, the prototypical hypoxia microRNA thought to fine-tune mitochondrial activity to environmental $O_2$ availability. More recently, the oxygen-sensitive noncoding transcriptome has been expanded to include hypoxia-inducible lncRNAs, such as NEAT1, MALAT1, MEG3, H19, NLCAT1, HOTAIR, HIF-1-AS2, and MIR210HG. Although these lncRNAs exert biochemical and phenotypical effects during experimental oxygen deprivation, there is extensive evidence to support their involvement in a broader spectrum of responses. Furthermore, their overall relevance in physiology and disease remains insufficiently understood.

Here we present an initial analysis of the mature lincRNA product of MIR193BHG locus, henceforth termed Noncoding Oxygen-Sensitive Regulator of Sterol Homeostasis (lincNORS). This locus was previously investigated exclusively in the context of the miR-193b-365a tandem. Our investigation reveals a connection between lincNORS, oxygen-sensing, and steroid hormone responses, adding to the growing evidence of the interdependence and overlap between these networks. Our study identifies a previously unrecognized modulator of the cellular steroid biosynthesis pathways and points to its relevance for specific human phenotypes.

Results

Identification and characterization of lincNORS. We first screened noncoding transcripts induced by decreased $O_2$ tension in MCF7 breast cancer cells. RNA-seq analysis captured the expected hypoxic coding signature and the increased abundance of NEAT1, MALAT1, MIR210HG, and HIF1A-AS2, known as non-coding hypoxic transcripts (Fig. 1a, Supplementary Fig. 1a, and Supplementary Data 1)6–9. Among the top upregulated noncoding RNAs, we identified a previously uncharacterized lncRNA, ENSG00000262454/RP11-65J21.3/MIR193BH, corresponding to the genomic locus that hosts the miR193b-365a tandem (Supplementary Fig. 1b). The miR-193b and miR-365a sequences are intrinsic to this locus and are therefore lost during splicing, specifying a specific designation for the mature lncRNA. Our decision to focus on this transcript was due to its consistent and wide-range response to oxygen deprivation in a broad spectrum of human cell lines, both transformed and non-transformed (Fig. 1b, c and Supplementary Fig. 1c). Although early studies were performed under severe hypoxia (0.2% $O_2$), predominantly encountered in the tumor environment, subsequent studies were performed under 1–2% $O_2$, in an vitro approximation of physiologically low $O_2$. Interestingly, no significant induction of miR-193b and miR-365a was observed upon $O_2$ deprivation, in contrast to miR-210, which exhibited the expected induction (Fig. 1d). The result reflects published microRNA-seq and RNA-seq data for MCF-7 cells, whereas these miRNAs are rarely reported as hypoxia-inducible. A potential explanation is that miRNA biogenesis is partially impaired during hypoxia.

Analysis of The Cancer Genome Atlas (TCGA) data sets revealed a positive correlation between lincNORS expression and a panel of well-established hypoxia signature genes in several cancers, including triple-negative breast cancer (TNBC) and pancreatic adenocarcinoma (PAAD) (Fig. 1e and Supplementary Data 2). Using a hypoxia score, calculated based on the hypoxia metagenes established by Buffa et al.20, we further showed that in both TNBC and PAAD, the in vivo lincNORS levels were more closely associated with the hypoxia state than NEAT1, an lncRNA known to respond to oxygen deprivation (Supplementary Fig. 1d).

To begin characterization of this lncRNA, we used 5′-RNA ligation-mediated rapid amplification of cDNA ends (5′-RACE) that identified two main transcription start sites (TSS), corresponding to ENST00000579945/MIR193BH-001 and ENST00000634265/MIR193BH-004, matching cap analysis of gene expression (CAGE) information from the FANTOM project (Supplementary Fig. 2a, b)21. Furthermore, the two polyadenylation (PolyA) sites identified by 3′ RACE agree with Cancer Genome PolyA Site & Usage data (Supplementary Fig. 2a, b). A closer examination of lincNORS revealed well-recognized features of a bona fide lncRNA. First, multiple coding-potential analyses produced consistently low scores (Supplementary Fig. 2c), and the SORFS mass spectrometry database (http://sorfs.org/database) did not list any peptides associated with this locus at the time of writing. Second, the locus is separated from the flanking ones. Third, as expected for a lncRNA, lincNORS is expressed in a tissue-specific pattern, with a higher abundance in hormone-responsive tissues and skeletal muscle (Supplementary Fig. 3). Furthermore, GTEx data sets indicate an overall abundance comparable to, or higher than, the well-established lncRNAs HOTAIR, CCAT1, and MIR210HG (Supplementary Fig. 3).

Cell fractionation revealed a predominant, albeit not exclusive, nuclear localization of lincNORS in MCF7 cells (Fig. 1f). Furthermore, the majority of nuclear lincNORS is chromatinnassociated, a relatively common feature of lncRNAs involved in gene expression regulation. Individual lincNORS loci were visualized by hybridization with custom-designed RNAscope® probes, a pattern consistent with nuclear enrichment and hypoxic responsiveness (Supplementary Fig. 1e).

Upstream molecular determinants of lincNORS abundance. To gain deeper insights into the hypoxic induction of lincNORS, we profiled the response at the primary transcript level using the nascent RNA Bru-seq method (Supplementary Data 3)22. As shown in Fig. 2a, the MIR193BH locus exhibited a significantly increased signal in hypoxic cells, consistent with a transcriptional effect. As internal controls for the assay, canonical hypoxia-inducible genes displayed the expected activation (Supplementary Fig. S4a).

Based on the evidence for a transcriptional response at this locus, we further tested the possible role of HIF-α isoforms in loss-of-function experiments. Although HIF-1α depletion had no measurable effect on lincNORS levels (Supplementary Fig. 4b), HIF-2α knockdown blunted the hypoxic accumulation of lincNORS in breast cancer cells (Fig. 2b). Supporting evidence for the role of HIF-2 was obtained using 786-O kidney cancer cells, which are documented to overexpress HIF-2α and corresponding targets regardless of $O_2$ tension due to pVHL E3 ligase inactivation. Similarly, the sensitivity of lincNORS to low $O_2$ is compromised in VHL-deficient cells and rescued by restoring VHL expression (Fig. 2c). This result is consistent with results reported by Zhang et al. listing MIR193BH (lincNORS) among the HIF-2 targets23. The preferential role of HIF-2 has
**Fig. 1 lincNORS is an oxygen-regulated, nucleus-located lncRNA.** a Heatmap depicting the expression of 47 lincRNAs significantly upregulated (fold change ≥ 2 or ≤ 0.5) in MCF-7 cells under hypoxia (1% $O_2$, 24 h) versus normoxia (21% $O_2$, 24 h). Color intensity represents RPKM scaled by the row. The red color represents a positive correlation while the blue color represents a negative correlation. The size of the dots is correlated with the expression level of lincNORS, NEAT1, and well-established hypoxia signature genes in triple-negative breast cancer (TNBC), pancreatic adenocarcinoma (PAAD), glioblastoma (GBM), and lung adenocarcinoma (LUAD) from TCGA. The significance is defined as **p < 0.01, two-sided Student’s t-test). b** qPCR analysis of lincNORS induction by hypoxia across diverse cell types, including both tumor (0.2% $O_2$) and non-tumorigenic cell lines (1% $O_2$), and fallopian tube epithelial cells (2% $O_2$). lincNORS RNA level was measured by qPCR. All the samples were cultured in hypoxia for 24 h except FTE cells (48 h). Data represent mean ± SD from three biological replicates. c dPCR analysis of miR193b, miR365a, miR210, and lincNORS expression in MCF-7 cells cultured under 21% or 1% $O_2$. A diagram of lincNORS and its intronic miRNAs is shown above. miR210 served as a positive reference for hypoxia-induced miRNA. Data represent mean ± SD from three biological replicates (**p < 0.01, two-sided Student’s t-test). e** Spearman’s correlation between lincNORS and well-established hypoxia signature genes in triple-negative breast cancer (TNBC), pancreatic adenocarcinoma (PAAD), glioblastoma (GBM), and lung adenocarcinoma (LUAD) from TCGA. The size of the dots is correlated with the strength of the Spearman’s coefficient. The red color represents a positive correlation while the blue color represents a negative correlation. The corresponding scale is presented next to the image. “NS” was added inside the dots where the correlation is not significant. The significance is defined as $p < 0.05$. f MCF-7 cells were harvested and fractionized after 24 h culture in 21% $O_2$ or 1% $O_2$. The RNA distribution of lincNORS, NEAT1, and GAPDH in cytoplasmic (CYT), nucleoplasm (NUC), and chromatin-associated (CHR) fractions were assessed by qPCR. NEAT1 and GAPDH were included as nuclear and cytoplasmic controls. Data represent mean ± SD from three biological replicates. Western blot was performed to confirm cell fractions and a representative image from three independent experiments was shown.
been previously described for hypoxia lincRNAs, in particular NEAT1, MALAT1, and NLCUAT1\(^{30,31}\).

A survey of the genomic region surrounding lincNORS using available ChIP-seq data sets (GSE28352) did not reveal functional HIF recruitment sites within the lincNORS proximal regulatory region, in contrast to standard hypoxia responders typically driven by HIF-1 from proximal promoters (e.g., CA9 and mir210/mir210HG) (Fig. 2d).\(^{32}\) Based on the knowledge that HIFs (and in particular HIF-2) sometimes transactivate from distant enhancers, we identified major peaks at the neighboring locus LINC02130 (Fig. 2e and Supplementary Fig. 5a)\(^{33,34}\). This locus is a plausible distal regulatory element based on (1) ENCODE genome segmentation features (Supplementary Fig. 5b); (2) chromatin interaction with the lincNORS proximal regulatory region (RNAPII ChIA-PET GSE33664) (Fig. 2e);\(^{35}\) (3) LINC02130 transcript is much less abundant than lincNORS in tissues and cell lines (Fig. 2e—GTEX track summary and Supplementary Fig. 1b for MCF7 cells); and (4) evidence of activation by hypoxia but the corresponding transcript has very low abundance (Supplementary Fig. 1b). Interestingly, the HIF prolyl-hydroxylase inhibitor DMOG was much less effective in inducing lincNORS than low O\(_2\) (Supplementary Fig. 5c, d).

Despite inducing the expected hypoxic responses (increased HIF-2 protein abundance; CA9 transcript upregulation), DMOG impact on lincNORS was modest in MDA-MB-231 and reproducibly absent in MCF7. In conclusion, while these data indicate that lincNORS is a hypoxia-inducible gene, they also show that it is an atypical one, prompting us to investigate additional details on lincNORS upregulation.

Detailed examination of the lincNORS genomic neighborhood revealed hormonal response hallmarks, including DNase I hypersensitivity peaks that are further stimulated by estradiol (E2) treatment (Supplementary Fig. 6a), as well as ChIP-seq peaks for estrogen receptor (ER), ER pioneer factors (FOXA1, GATA3) and ER coactivators (NCOA3/SRC3) in relevant cell types (Supplementary Fig. 6b). As is often the case for estrogen-responsive genes, the major regional ER peak resides at the putative LINC02130 enhancer (Supplementary Fig. 6a).\(^{27}\) Accordingly, a mega trans ER enhancer was identified at this site in the recent study by Rosenfeld and colleagues (Supplementary Fig. 6c).\(^{28}\) Finally, our analysis of TCGA RNA-seq data sets detected significantly higher lincNORS expression in ER/ progesterone receptor (PR) positive breast tumors compared to their negative counterparts (Fig. 3a). Collectively, this information prompted us to directly test the response of lincNORS to E2 treatment. In short-term experiments, hormonal treatment resulted in reproducible (if not marked) lincNORS induction (Fig. 3b). More strikingly, lincNORS expression was dramatically reduced in long-term estrogen deprivation (LTED) of MCF7 cells (Fig. 3c).

ChIP-seq data sets also suggest a potential role of FOSL2 in the regulation of lincNORS, a hypoxia-inducible AP-1 family member (Supplementary Fig. 5a).\(^{29}\) Using a loss-of-function approach, we confirmed the positive impact of FOSL2 on lincNORS expression in breast cancer cells (Supplementary Fig. 5c), predominantly at a lower oxygen concentration. Based on the evidence that FOSL2 responds to multiple stimuli, including steroid hormones, its role in the regulation of lincNORS may not be limited to oxygen-related responses.\(^{30,31}\)

**lincNORS coordinately modulates the sterol biosynthesis program.** To gain insight into lincNORS biochemical functions, we downregulated it using a combination of siRNA/shRNA, similarly to the recently published studies on the lincRNAs CHROME and SCAT (Supplementary Fig. 7a).\(^{32,33}\) For transient interventions, we first validated non-overlapping siRNAs targeting the major exon of lincNORS (present in all isoforms). Based on their differential ability to knockdown lincNORS expression, these will be henceforth referred to as siNORS_s (strong) and siNORS_w (weak) (Supplementary Fig. 7b).

We next performed RNA-seq analysis in hypoxic MCF7 cells to determine the consequences of lincNORS downregulation (Supplementary Fig. 8a and Supplementary Data 4). Differentially regulated transcripts were subjected to Gene Set Enrichment Analysis (GSEA) and Ingenuity Pathway Analysis (IPA), both of which identified the upregulation of cholesterol biosynthesis as the top transcriptomic signature (Fig. 4a and Supplementary Fig. 8b). As illustrated in Fig. 4b and Supplementary Fig. 8c, the program responsible for the de novo synthesis of cholesterol and related steroids was coordinately upregulated by lincNORS siRNA, with significant upregulation of many individual genes. A similar effect was evidenced in ER-negative MDA-MB-468 cells using the AmpliSeq platform (Fig. 4c, d and Supplementary Data 5). Individual genes were validated by qPCR in a diverse cell panel (Fig. 4f and Supplementary Fig. 8d). The impact of lincNORS may extend beyond biosynthesis, with negative effects on the STARD4 transport regulator and potentially other steroid network components (Fig. 4g and Supplementary Fig. 8f). Similar effects (Fig. 4g) were demonstrated on SCD (stearoyl CoA desaturase), a gene often coregulated with a sterol biosynthesis program. Evidence that lincNORS was successfully repressed came from several lines of evidence: (1) The knockdown efficiency of siNORS_s was greater than that of siNORS_w and overall it exhibited a stronger impact on sterol biosynthesis transcripts; (2) Although this response is also detectable under high O\(_2\), it was generally more obvious at lower oxygenation, as expected based on lincNORS abundance; (3) lincNORS downregulation with a non-overlapping shRNA elicited similar effects on sterol biosynthesis genes (Supplementary Fig. 8d).

We then performed gain-of-function studies and we stably expressed full-length lincNORS (NORS OE) or vector-only control and performed RNA-seq on cells growing under ambient O\(_2\) (Supplementary Fig. 9a and Supplementary Data 6). Both GSEA and IPA identified cholesterol biosynthesis among the top suppressed signatures (Supplementary Fig. 9b, c), with multiple components downregulated (Supplementary Fig. 9d). We confirmed by qPCR the downregulation of MSMO1, SQLE, and HMGCR as a result of lincNORS transcript induction (Supplementary Fig. 9e). As expected, this intervention did not significantly affect miR-193b or miR-365a levels (Supplementary Fig. 7b). Based on RNA-seq data, lincNORS downregulation and overexpression did not significantly affect the expression of flanking genes MKL2/MRTFB and PARN, thus indicating a predominantly trans activity (Supplementary Fig. 8e).

We found that the overall effect of lincNORS on total cellular cholesterol reflected the changes in transcript abundance. These were demonstrated in both loss- and gain-of-function studies, using two cell types (MCF7 and MDA-MB-231) and with two quantification methods (mass spectrometry and a commercial kit) (Fig. 4h and Supplementary Fig. 8g). Furthermore, the effect of lincNORS on cholesterol/lanosterol ratio supports the notion that it affects predominantly the distal products of the sterol/ steroid biosynthesis than lower molecular weight precursors. The hydroxylated derivative 25-OH cholesterol did not appear to change significantly, consistent with the lack of response of the transcript for the conversion enzyme (Fig. 4i and Supplementary Fig. 8h).

**The impact of lincNORS on cellular phenotypes.** Functionally, the importance of sterol biosynthesis is not restricted to
hepatocyte physiology, being also essential for steroid hormone production, as well as for cellular growth and differentiation programs. Furthermore, it is intricately connected to steroid hormone signaling and has been recently recognized as critical for neoplastic cell escape from steroid hormone dependence.

Given the diverse biological roles of this pathway, we hypothesized that the experimental manipulation of lincNORS would have consequences on phenotype.

To test this possibility, we examined the effects of lincNORS upregulation and downregulation in standard assays for cell viability and invasion under different environmental conditions. lincNORS knockdown had consistent pro-invasive consequences, with a probable contribution from deregulated sterol biogenesis, as suggested by the mitigating effect of simvastatin (Fig. 5a, b and Supplementary Fig. 10a, b). A similar, although more modest effect was observed in cell viability assays (Fig. 5c and Supplementary...
Consistent evidence was observed in MDA-MB-231 xenografts with stably suppressed or overexpressed lincNORS. Knockdown resulted in increased metastatic load, whereas stable overexpression had the opposite effect (Fig. 5d, e). However, lincNORS status in xenografts did not significantly affect primary tumor size (Supplementary Fig. 10e). The effect of shRNA on lincNORS expression was still detectable in the harvested tumors, while miR-193b expression remained unchanged (Fig. 5f). Steroid biosynthesis transcripts underwent changes similar to those observed in tissue culture (Fig. 5g).

Mechanistic insights into lincNORS effects. We next investigated whether lincNORS impacts steroid biosynthesis genes by...
suppressing transcription or by altering mRNA turnover. Following siRNA-mediated lincNORS knockdown, nascent transcripts were labeled for 30 min with bromouridine (BrU), collected by immunoprecipitation with anti-BrU antibody, and quantified by qPCR analysis. Consistent with a specific posttranscriptional effect, siRNA treatment lowered total lincNORS transcript, while exhibiting no significant effect in the BrU-labeled pool (Supplementary Fig. 11a). We performed similar quantifications for steroid biosynthesis components and identified significant enrichment in BrU-labeled transcripts following lincNORS-depletion (Supplementary Fig. 11a).
In contrast, we did not observe significant changes in transcript stability, suggesting a predominantly transcriptional impact of lincNORS (Fig. S11b).

To establish the mechanistic link between lincNORS and sterol biosynthesis, we searched for protein partners bridging this gap. To this end, we followed a well-established RNA affinity purification approach named MS2-TRAP, based on the tagging of lincNORS by addition of MS2 RNA hairpins. We collected complexes binding to MS2-lincNORS or control MS2 RNA in MCF7 cells and subjected them to mass spectrometry, followed by a selection of proteins with no binding to the MS2 RNA (Supplementary Fig. 12a and Supplementary Data 7). Among the high number of proteins identified, we did not find nuclear receptors directly or indirectly affecting sterol biogenesis (e.g., SREBP1, SREBP2, ESR1, NR5A1), but we observed many proteins implicated directly or indirectly in controlling gene expression programs (Supplementary Data 7). However, this data set, as a whole, was not validated and due to the experimental caveats of MS2 purification, may contain false positives. The candidates were further filtered based on several criteria: (1) documented RNA-binding protein status (RBP); (2) predominantly nuclear localization; (3) predicted to bind motifs within lincNORS by RBPmap; and (4) documented connection to sterol/steroid pathways. Based on these criteria, we focused on two members of a known complex: RBP-associated with lethal yellow mutation (RALY) and its frequent partner heterogeneous nuclear ribonucleoprotein C (HNRNPC) (Fig. 6a). The former is of particular interest for multiple reasons: (1) a recent study in mice by Tontonoz’ group described a role for Raly in the regulation of cholesterol biosynthesis, in partnership with a lncRNA termed LeXis; (2) Nabs, a putative yeast homolog of Raly has been shown to regulate the ergosterol pathway; (3) the RALY protein is located in the nucleus, predominantly in the chromatin-associated fraction, which is the same subcellular location of lincNORS (Fig. 6b); (4) lastly, lincNORS is present among the significantly enriched RALY-associated transcripts in MCF7 cells (under the designation RP11-65J21.3) 

Evidence of lincNORS biological relevance from GWAS. Although phenotypic associations of naturally occurring genetic variations can provide uniquely valuable information about gene function, noncoding loci remain insufficiently interrogated using this approach. During the past decade, multiple independent genome-wide association studies (GWAS) have reported highly significant phenotypic associations for a cluster of single-nucleotide polymorphisms (SNPs) situated between the MKL2 and PARN loci (Table 1 and Fig. 7a). Although these studies typically list MKL2 as the name of the locus since it is the closest coding gene, we provide multiple arguments for lincNORS as the more plausible functional element.

From a biochemical perspective, most phenotypes listed in Table 1 are tied to the actions of sex steroids, thus plausibly affected by a modulator of sterol/steroid biosynthesis expressed mainly in hormone-responsive tissues. Furthermore, an examination of the MKL2-PARN genomic region immediately revealed that these SNPs are more likely to impact MIR193BHG/lincNORS than MKL2 (or PARN). First, the top GWAS-reported SNP for puberty onset (rs246185) is very close to the TSS of lincNORS and overlaps with histone acetylation/methylation peaks (Fig. 7a). Second, expression quantitative trait loci (eQTL) analysis in the GTEx database shows that these genetic variations are particularly robust during childhood and adolescence (Supplementary Data 8).

Finally, based on ARIES mQTL information, these variants also exhibit significant associations with the methylation of the MIR193BHG locus rather than MKL2. Interestingly, these mQTLs are particularly robust during childhood and adolescence (Supplementary Fig. 13a), consistent with this locus being particularly important during a specific developmental stage (i.e., during childhood).
rs181766, which is in high linkage disequilibrium with rs246185 and exhibits similarly significant phenotypic associations according to the UK Biobank database (Fig. 8a). However, based on ChIP-seq and open chromatin by Formaldehyde Assisted Isolation of Regulatory Element (FAIRE-seq) data sets from ENCODE, rs181766 represents a potentially stronger causal effector SNP candidate (Supplementary Fig. 13b). Sequences corresponding to the reference or alternative alleles of rs246185 (T/C) and rs181766 (T/C) were subcloned into pGL4 vectors and reporter activity was assayed in MCF7 cells. Although the Alt rs246185 (C) variant had no measurable impact, substitution of the rs181766-C allele for the T allele had a significant effect on...
Data are plotted as mean ± SD (two-sided Student's t-test). Representative images (>200 magnified) from three independent experiments were shown. c MDA-MB-231 cells with stable expression of miR193bh shRNA or control shRNA were plated in a Boyden chamber with Matrigel in the presence of 1 μM simvastatin or DMSO. The cells that invaded through Matrigel were quantified 20 h after plating. Data are shown as mean ± SD from three biological replicates (two-sided Student's t-test). Representative images (>200 magnified) from three independent experiments were shown. d MDA-MB-231 cells with stable expression of MIR193BH shRNA or control shRNA were cultured in 1% O2 for 48 h in the presence of simvastatin or DMSO. Cell viability was measured by MTT assay. Data are shown as mean ± SD from four independent experiments (two-sided Student's t-test).

**Discussion**

Recent noncoding transcriptome studies have revealed new regulatory mechanisms for lipid metabolism based on lncRNAs such as LincLSTR, HULC, H19, LeXis, MeXis, and MALAT1[^54]. Our study expands this group with another regulator of the steroid network program, termed lincNORS.

Understanding how physiological low O2 impacts the homeostasis of steroids and steroid derivatives has broad implications on physiology and disease[^55-^58]. Accordingly, knowledge of tissue-specific modulators, such as lncRNAs, should be particularly informative. Although highly conserved between primates, the regions corresponding to the exons and surrounding regulatory elements of lincNORS exhibit rather limited conservation between the human and mouse genome. It could be argued therefore that the relevance of a mouse knockout model for human biology would be limited. On the other hand, naturally occurring genetic variations in human populations deliver a consistent message about this locus. As described above, single-nucleotide variations close to the TSS of lincNORS are significantly associated with sex hormone-related phenotypes such as the timing of puberty, height growth, and genital development. In this study, we mapped the GWAS signal to a plausible causal SNP and its target effector transcript.

How does the insight from GWAS come together in a cohesive model with lincNORS biochemistry? At this stage, we can only propose a simplistic scenario, with further details anticipated to emerge from future studies. For example, the variant of the minor allele could trigger increased baseline lincNORS expression, leading to more potent suppression of steroid biosynthesis in hormonally-responsive tissues and ultimately to a delay in sexual maturation. Based on the directionality of eQTLs and the nature of transcriptional regulators (both negative and positive) recruited in the proximity of rs181766, the causal alternative allele may exert a positive or negative impact on lincNORS expression, depending on tissue context.

With the phenotypic connection in mind, it only makes sense that lincNORS responds to estrogen treatment and that its genomic neighborhood appears to be a hub for hormone receptor recruitment. How does hypoxia, the original context that brought lincNORS to our attention, fit into this context? In recent years the importance of HIFs and physiologically low O2 for reproductive organ physiology is coming to light from multiple directions[^59-^62]. In summary, one could imagine lincNORS as an integrator of microenvironmental and hormonal cues that modulates key aspects of sterol/steroid homeostasis that are essential for growth and development.

In hindsight, lincNORS' participation in both low oxygen and estrogen response should not come as a surprise, as these networks are increasingly recognized as interconnected[^12]. Evidence for a similar dual facet already exists for other hypoxia-inducible lncRNAs such as NEAT1 and MALAT1[^53,^64]. In another similarity to lincNORS, in hypoxic cells, it was previously demonstrated that NEAT1 and MALAT1 were controlled predominantly by HIF-2, recruited to distal enhancer elements rather than proximal promoter[^6].

Our study may also expand the emerging link between RALY and steroid/steroid biosynthesis[^43]. It is plausible that the specific noncoding elements alter the function of RALY-containing complexes depending on the cellular context. For example, in hepatocytes in interaction with LeXis RALY becomes relevant for the “classic” cholesterol biosynthesis. On the other hand, by partnering with lincNORS in hormonally-responsive tissues RALY becomes involved in the extrahepatic facets of this pathway[^41]. As RALY has the potential to regulate gene expression at multiple steps (positively or negatively), noncoding RNA interactors may be critical for action specificity[^44,^65].

**Methods**

**Cell culture and reagents.** Human cancer cell lines MCF-7, MDA-MB-231, MDA-MB-468, T47D, A549, HT-29, SKOV3, MIA-PaCa2, and U118 were purchased from American Type Culture Collection (ATCC) and maintained at 37 °C in 5% CO2 with DMEM/High glucose medium supplemented with 10% (v/v) FBS (Biowest) and antibiotics.

All cell lines used were routinely tested for mycoplasma contamination (MycoAlert® Mycoplasma Detection Kit, Lonza). Long-term estrogen-deprived (LTED) MCF7 cells were developed as previously described[^66]. Briefly, MCF7 cells were maintained in medium with 10% dextran-coated charcoal-stripped FBS (Hyclone Laboratories, Inc., Logan, UT) for 6 months to generate LTED cells. Renal clear cell carcinoma cells 786-O with empty vector (VHL−) and 786-O with reconstituted VHL (VHL+) were obtained from Dr. Maria Czyzyk-Krzeska (University of Cincinnati). Patient-derived pancreatic cancer cell lines Pa03C, Panc10.05, Panc198, as well as cancer-associated fibroblast cell line CAF19, were obtained from Dr. Anirban Maitra at Johns Hopkins University[^67]. Human lung fibroblast cell line WI-38 was obtained from Dr. Shadia Jalal’s lab (Indiana University School of Medicine). Fallopian tube FTE282 epithelial cells (a gift from Dr. Ronny Drapkin, University of Pennsylvania) were cultured in DMEM-Ham’s F12 media (Corning) supplemented with 2% Ultrasor G (Crescent Chemical Company) by Dr. Sumegha Mitra. Hypoxia cell culture was performed at 0.2%, 1%, and 2% O2 for the indicated time in an InVivo200 hypoxia workstation (Ruskin, Inc., Cincinnati, OH). Dimethyloxalyglycine (DMOG, Frontier Scientific D1070-100 mg) was dissolved in DMSO and used at 1 mM. Simvastatin (Sigma S6196) was used at indicated concentrations. Estradiol (Sigma E8875) was dissolved in ethanol and used at 10 nM. In estradiol experiments, cells were plated and maintained in medium with 10% dextran-coated charcoal-stripped FBS. Actinomycin D (Sigma A1410) was used at 2.5 μg/ml.

**siRNA transfection.** Silencer® Select siRNAs were purchased from ThermoFisher Scientific and transfected at 10 nM final concentration using RNAiMAX reagent (Invitrogen, CA) with a reverse transfection protocol. siRNAs used in the study...
**Fig. 6** lincNORS regulates cholesterol synthesis via interaction with RALY. 

**a** The level of hnRNPC, RALY, TRIM28, SREBP2, and MS2-GST in the MS2 pulldown material and input lysate was detected by western blot. The data presented are a representative image from two to three independent experiments.

**b** Subcellular location of RALY was detected by western blot. Tubulin, Lamin-B1, and acetyl-histone H3 were included as controls for cytoplasmic, nuclear, and chromatin-associated fractions. A representative image from three independent experiments is shown.

**c** RNA immunoprecipitation (RIP) analysis of RALY. The presence of lincNORS and 18S rRNA in the precipitated complex was detected by qPCR. Data represent mean ± SD from three biological replicates (two-sided Student’s t-test).

**d** qPCR analysis of NORS, MSMO1, and SQLE expression in MCF-7 cells transfected with control, lincNORS siRNA, and/or RALY siRNA. Data represent mean ± SD from four biological replicates (two-sided Student’s t-test). RALY knockdown was confirmed by western blot in each experiment and a representative image is shown on the right.

**e** Enrichment of RNA of cholesterol synthesis genes in RALY-containing immunoprecipitated complex. The barplot displays the mean fold enrichment ± S.E.M. from three independent RALY RIP-Seq vs control experiments in MCF7 cells. The statistical significance of the enrichment was determined with CuffDiff v2.2.1 (*FDR < 0.05, **FDR < 0.01, ***FDR < 0.001). Source data are provided as a Source Data file.

**f** Enrichment of MSMO1, SQLE, H1FX, and GAPDH in RALY-containing immunoprecipitated complex after lincNORS or hnRNPC knockdown was detected by qPCR. Data represent mean ± SD from four biological replicates (two-sided Student’s t-test).
Table 1 Summary of genome-wide association studies reported SNPs in lincNORS promoter.

| SNP.Id      | CHR | POS_hg38 | Distance | MAPPED_TRAIT | RISK_ALLELE | RAIβ | p-value | Beta | Pubmed_id | Journal         |
|-------------|-----|----------|----------|--------------|-------------|------|---------|------|-----------|----------------|
| rs1659127   | 16  | 14294448 | 7840     | Age at menarche | rs1659127-A | 0.34 | 4.00E-09 | 2.4c | 21102462 | Nat Genet       |
| rs1704528   | 16  | 14294893 | 7395     | Age at menarche | rs1704528-? | NR   | 6.00E-12 | -0.033 | 27182965 | Nat Genet       |
| rs246185    | 16  | 14301575 | 713      | Tanner puberty stage | rs246185-C | 0.33 | 6.80E-16 | 0.04  | 25321870 | Nature          |
| rs1704529   | 16  | 14294769 | 7519     | Androgenetic alopaeia | rs1704529-C | NR   | 7.10E-14 | 0.141 | 24770850 | Hum Mol Genet   |
| rs246180    | 16  | 14298066 | 4222     | Androgenetic alopaeia | rs246180-? | NR   | 8.00E-09 | 0.141 | 27182965 | Nat Genet       |
| rs246185    | 16  | 14301575 | 713      | Androgenetic alopaeia | rs246185-C | 0.33 | 2.65E-10 | 0.14  | 28277246 | Nat Genet       |
| rs1659127   | 16  | 14294448 | 7840     | Body height | rs1659127-A | 0.48 | 6.70E-10 | 0.041 | 25429064 | Hum Mol Genet   |
| rs1659127   | 16  | 14301575 | 713      | Body height | rs1659127-A | 0.34 | 1.10E-11 | 0.027 | 20881960 | Nature          |

*Distance: distance from SNP to lincNORS transcription start site.
*RAl: risk allele frequency.
*Per allele change in age at menarche (weeks).

include: Silencer*Select negative control siRNA (4390843), FOSL2 siRNAs (Supplementary Data 9), RALLY siRNA (s (5’-GACGG-GUAUAAAACGAACTTT-3’), NORS siRNA_w (5’-GCAAGAUGUUUGUCAAGATT-3’) and lincNORPC siRNA (5’-CAACGGGACUAUUAUGAATdT-3’).

Stable cell line generation and RACE. The shRNA for lincNORS was designed to target the sequence 5’-GATTAAGAACACAGTATTC-3’. The hairpin template oligonucleotides were synthesized by Integrated DNA Technologies and subsequently cloned into lentiviral vector plKO1-puro. The scramble shRNA control was purchased from Sigma-Aldrich (SHC002). Lentivirus containing shRNA were constructed using Lipofectamine 2000 transfection reagent. Lentivirus was produced in 293T cells and titrated by infecting human HEK293T cells with serial dilutions of the supernatant. Supernatant was collected 48 h post-transfection. Supernatant was produced at a MOI of 100 and used to infect HEK293T cells at a MOI of 10 to generate lentiviral particles.

RNASeq. Detection of lincNORS expression and subcellular location in MCF-7 cells with lincNORS-specific RNAseq® probe (designed by Advanced Cell Diagnostics, ACD) were performed using RNAseq® Multiplex Fluorescent Reagent kit (ACD 320850) according to the manufacturer’s instructions. A negative control probe (ACD 32350) was also purchased from ACD. Images were visualized with Olympus BX43 microscope equipped with Olympus U-RFL-T as a power supply unit.

Invader assay. Invasion assays were performed in a 24-well plate with BD Falcon cell culture inserts (Corning 333097) coated with Matrigel (BD Bioscience 356237). Cells were starved in serum-free medium overnight prior to the experiment. At the start of the assay, cells were washed with serum-free medium and plated into the upper chamber at 1 × 10^5 cells per well in 0.5 mL serum-free DMEM medium. Complete medium (0.75 mL) with 10% PBS was added to the lower chamber. Cells were allowed to invade for 20 h under either normoxic or hypoxic conditions. After incubation, the medium in the upper chamber was aspirated and non-invaded cells on the upper surface of the filter were removed with a cotton swab. The transwell membranes were fixed and stained with 0.25% crystal violet in formaldehyde-methanol (1:9) solution for 30 min. After staining, the membranes were washed three times with water and air-dried. Invaded cells were visualized using light microscopy. 150 µL 2% SDS was then added to the membrane, incubated for 20 min to solubilize the crystal violet from stained invaded cells, then transferred to a new 96-well plate. The relative invasion was determined using absorbance readings at 550 nm.

Cell viability assay. Cell viability was measured by MTT assay. Cells were plated in 96-well plates (10,000–20,000 cells per well) and relevant treatment (siRNA transcription or drug treatment) was performed as described in each experiment. 4 h prior to the endpoints, 10 µL of MTT (5 mg/mL in PBS, Sigma-Aldrich M5655) were added to each well containing 100 µL medium and incubated for 4 h. The medium was aspirated and 100 µL acid isopropanol (0.04 N HCl in isopropanol) was added to dissolve the insoluble purple formazan product into a colored solution. Absorbance was recorded at 570 nm, with a reference at 630 nm serving as the blank.

RNA extraction and qPCR analysis. For RNA extraction, cells were lysed using QIAzol Lysis Reagent (QIAGEN) and total RNA was isolated using the RNeasy kit (QIAGEN) following the manufacturer’s instructions. Reverse transcription was performed using TaqMan® Reverse Transcription Reagent (Applied Biosystems, CA) from 1 µg of RNA, and qPCR was performed using gene-specific primers (Supplementary Data 9) and SYBR green master mix (Applied Biosystem, CA). The expression of coding lincNORS was amplified using RNAscope® probe (designed by Advanced Cell Diagnostics, ACD) were performed using RNAscope® Multiplex Fluorescent Reagent kit (ACD 320850) according to the manufacturer’s instructions. A negative control probe (ACD 32350) was also purchased from ACD. Images were visualized with Olympus BX43 microscope equipped with Olympus U-RFL-T as a power supply unit.
Expression Master Mix. U6 or RNU48 expression was used as an endogenous control.

**Xenograft model.** All animal experiments were approved and carried out in accordance with the Institutional Animal Care and Utilization Committee at Indiana University School of Medicine. 1 × 10⁶ cells were injected into the mammary fat pad of 10 ~4 weeks old female NSG (NOD.Cg-Prkdcscid Il2rgtm1Wjl/SzJ) mice. Tumor growth was monitored twice a week by measurement of the long and short diameters of the tumor using digital calipers. At the end of the experiments, animals were killed and the primary tumor, lung, and liver were resected. Lung and liver tissue were formalin-fixed, paraffin-embedded, sectioned, and stained with hematoxylin and eosin (H&E) for analysis of metastatic burden. The percentage of lung or liver tissue containing tumor metastasis was analyzed using Aperio (Leica Biosystems).

**RNA-seq and data analysis.** The initial RNA-seq used for identifying hypoxia-induced lncRNAs was performed on Illumina NextSeq500 platform. Sequenced reads were mapped to genome with TopHat2 version 2.1.1. And differential expression analysis was carried out using the DESeq2 package (version 1.12.3) in R/Bioconductor (R version 3.3.1)⁷¹. For analysis of lncNORS function, RNA sequencing was performed using The Ion PI Sequencing 200 Kit v2 Kit (4485149, Life Technologies) on an Ion Proton semiconductor sequencer according to the kit instructions using the Ion PI Chip Kit v2 (4482321, Life Technologies). Reads were aligned using STAR (version 2.3.0) and differential expression was analyzed using the EdgeR (version 3.8.6) package in R (version 3.0.1)⁷²,⁷³. Differential expression with FDR < 0.05 was considered significant.

**AmpliSeq and data analysis.** Automated library preparation was performed according to the protocol available with the Ion Chef (MAN0013432) and using the

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![Fig. 7 GWAS-reported SNPs in lincNORS promoter support its roles in steroid regulation.](image-url)

**Fig. 7 GWAS-reported SNPs in lincNORS promoter support its roles in steroid regulation.** a Locations of SNPs in lincNORS promoter reported in NHGRI-EBI catalog of published genome-wide association studies were visualized in UCSC genome browser. b eQTL boxplot showing genotype-expression relationships between rs246185, rs246180, rs1704528, and rs1659129 with lincNORS (top) or the closest protein-coding gene MKL2 (bottom) in skeletal muscle.
Fig. 8 The impact of rs181766 on lincNORS promoter activity. a List of physical traits that are reported as significantly associated with rs181766 in UK Biobank (https://genetics.opentargets.org/). b The effect of two selected SNPs rs246185, rs181766 on lincNORS expression was examined using luciferase assay. A diagram displaying the lincNORS promoter cloning was shown on the right. Data represent mean ± SD from 4 to 11 biological replicates (*p < 0.05, **p < 0.005, one-way ANOVA followed by Dunnett’s multiple comparisons test against REF). c eQTL boxplot showing genotype-expression relationships between rs181766 and lincNORS (top) or the closest protein-coding gene MKL2 (bottom).

Gene set enrichment analysis. Gene set enrichment analysis was performed following GSEA User Guide (The Broad Institute). Briefly, each condition was considered as a group and gene list was ranked with GSEA default ranking metrics. Gene sets from the Molecular Signature Database were used in the analysis to identify the pathways significantly enriched in each group. Gene sets were pre-ranked 1000 times to obtain empirical FDR corrected p-values.

Western blot analysis. Cell lysates were prepared in RIPA buffer containing protease inhibitor cocktail (ThermoFisher Scientific). For HIIF western blot, cells were fractionated using RNLI buffer (10 mM Tris–HCl pH 8.0, 140 mM NaCl, 1.5 mM MgCl₂, 0.5% NP-40, protease inhibitor cocktail) and the nuclear fraction was collected for analysis. Proteins were separated by SDS-polyacrylamide electrophoresis (SDS-PAGE), then transferred onto PVDF membranes (Biorad). Primary antibodies used in this study included those that recognized HIF-1α (R&D AF1935), HIF-2α (R&D AF2886; Cell Signaling #7096), β-actin (Thermo MA5-15739; Santa Cruz sc-1615), β-tubulin (Santa Cruz sc-5274), acetyl-histone H3 (Millipore 06-599), Lamin-B1 (Abcam ab16048), SREBP2 (R&D AF7119), hnRNPC (Santa Cruz sc-32308), TRIM28 (Abcam ab10483), RALY (Abcam ab170105), and Enterobacteriace Phage MS2 coat protein (Millipore ABE76). For a complete list of antibodies used please see Supplementary Data 10.

Total cholesterol quantification. Cellular cholesterol was measured using the Wako Cholesterol assay kit (WAKO, #439-17501) and liquid chromatography-mass spectrometry. For the measurement using Wako Cholesterol assay kit, cellular lipids were extracted using 750 µL chloroform: methanol (2:1) solution. After extraction for 30 min on a rocker, samples were centrifuged for 10 min at 1500 × g at 4 °C, then the lower chloroform phase was collected and air-dried in a fume hood until all liquid evaporated. The lipids were resuspended in 10% Triton-X100 in isopropanol. Total cholesterol content was measured using the Wako Cholesterol assay kit (WAKO, #439-17501) according to the manufacturer’s instructions. For the measurement using liquid chromatography-mass spectrometry, Oxyesters were extracted from cells using a modified sterol extraction method from McDonald et al. Briefly, cell pellets were extracted with 1 mL of dichloromethane/methanol 50/50 (v/v) containing 5 µg of butylated hydroxytoluene (BHT) and 200 ng of each deuterated internal standard. 0.2 mL of water were added after mixing and vortexed for 15 s and centrifuged. The organic layer was transferred to a new tube and saponified with 0.6 mL of ethanolic KOH (1 N). The tubes were flushed with nitrogen, capped, and heated to 35 °C for 1 h. 2 mL of water were added and 2 mL dichloromethane. The aqueous fraction was extracted twice with dichloromethane then the two organic fractions were combined and dried with nitrogen gas at room temperature. The samples were then derivatized according to Honda et al. The derivatization reaction mixture was composed of 100 mg of 2-methyl-6-nitrobenzoic anhydride, 30 mg of 4-diethylaminopyridine, 80 mg of picolinic acid, 1.5 mL of tetrahedralfluoruran (THF). 0.15 mL of this mixture was added to each sample along with 0.02 mL of triethylamine (TEA). The reaction proceeded at room temperature for 1 h, then the samples were dried and subsequently reconstituted in 0.1 mL of acetonitrile just prior to LC/MS/MS analysis. The analysis was done with an Agilent 1200 liquid chromatography system coupled to an Agilent 6460 QQQ mass spectrometer (Santa Clara, CA) in Bindley Bioscience Center at Purdue University. Data were processed using Agilent MassHunter Quantitative analysis software (V.B.06).

Bromouridine (BrU)-labeled RNA Immunoprecipitation and Bru-Seq. BrU labeling of newly synthesized RNA and BrU-RNA isolation were performed following the method established by Ljungman’s group. Briefly, MCF-7 cells were cultured in RPMI medium supplemented with 10% fetal bovine serum. For each sample, the cells were treated with 0.1 mM BrU (R&D Systems, #5129) for 1 h. After treatment, the cells were washed and lysed with lysis buffer. The RNA was extracted using the RNeasy Mini kit (Qiagen). RNA was end-3′ terminal dT labeling using TdT and biotin-dUTP (Roche) following provided protocol. The labeled RNA was immunoprecipitated using Protein-G magnetic beads (ThermoFisher) and incubated with antibodies against H3K4me1 or 2 and H3K36me1 or 2. The precipitated RNA was eluted and reverse transcribed using the SuperScript III First-Strand Synthesis System (ThermoFisher) and the primers. The resulting cDNA was PCR amplified using primers specific for lincNORS. The PCR products were then labeled with 5-bromo-2′-deoxyuridine (BrU) and sequenced using a standard 454 Genome Sequencer FLX system (Roche). The sequences were aligned with the human genome (NCBI Build 37) using the Bowtie2 tool and the number of reads per 100 bases of genomic DNA was calculated using the Bedtools tool.
transfected with either control siRNA or lincNORS siRNA were cultured in hypoxic conditions. BrU (Sigma-Aldrich #850187) at a final concentration of 2 mM was added to the plate when cells reached ~80% confluency. After labeling for 30 min, cells were harvested for total RNA extraction. BrU-labeled RNA was immuno-purified from the total RNA using anti-BrU antibody (BD Pharmingen, 555627) conjugated with Dynabeads Goat anti-mouse IgG beads (Invitrogen #11033). After 1-h incubation, beads were washed three times with 0.1% BSA in DEPC-PBS to remove unbound RNA then heated at 95 °C for 10 min to recover the BrU-RNA from the beads. The BrU-RNA and the input RNA were analyzed using qPCR. The same BrU-RNA isolation procedure was performed with MCF-7 cells cultured in 21% or 0.2% O2 and the BrU-labeled RNA was sequenced at the University of Michigan Sequencing Core using Illumina HiSeq2000 sequencer.

**MS2-tagged RNA affinity purification**. RNA pulldown using MS2-tagged RNA affinity purification was performed according to the previously described protocol with slight adaptation. lincNORS cDNA was cloned into the pMS2 vector upstream of the MS2 tag. MCF-7 cells were co-transfected with either lincNORS-MS2 vector or the empty vector and the pMS2-GST vector. 48 h after transfection, cells were lysed and proteins were quantified. Cell lysate containing 2 mg of protein was incubated with 60 µL Glutathione sepharose beads (GE Healthcare) for 3 h at 4 °C, followed by five washes with the lysis buffer to remove nonspecific bound proteins. Beads were further treated with 20 units of DNase-free DNase (QIAGEN) for 1 min at 14,000 × g. The supernatant was loaded on an SDS-PAGE gel. For RNA analysis, 700 µL QIAzol lysis buffer were added onto the beads, and RNA was isolated using RNeasy kit (Qiagen). RNA immunoprecipitation performed by Macchi lab followed the protocol established by Keene et al., with some modifications. Briefly, 2 × 10⁶ MCF7 cells were lysed in lysis buffer (10 mM HEPES pH 7.4, 100 mM KCl, 5 mM MgCl₂, 0.5% NP-40, 1 mM DTT plus RNAse and proteinase inhibitors) for 3 h at ~80 °C and centrifuged at 12,000 × g for 30 min at 4 °C. The supernatants were collected and 1% of each sample was set aside as input while the remaining was incubated for 4 h at 4 °C with protein A Magnetic beads (Invitrogen) coated either with 3 µg of an anti-RALLY antibody (Bethyl, #A302-069A) or with 3 µg of Rabbit IgG (Millipore). The beads were then washed four times with NT2 buffer (50 mM Tris-HCl pH 7.5, 150 mM NaCl, 1 mM MgCl₂, 0.1% NP-40, 1% urea) and RNA was isolated using TRIzol (Invitrogen). Purified RNA was then converted to cDNA using the RevertAid First Strand cDNA Synthesis Kit (Thermo Scientific), following the manufacturer’s instructions. The qPCRIBio SYGreen Mix (PCR Biosystems) was used as a master mix for qRT-PCR. The reactions were performed on a CFX384 thermal cycler and run in triplicate for each target gene for a total of three independent biological replicates. RALY RIP-qPCR data were previously published by Macchi lab and re-analyzed for lincRNA and cholesterol gene enrichment using the same method as previously described.

**Luciferase assay**. Oligos of lincNORS promoter region containing rs246185 and rs181766 reference or alternative allele were ordered as gBlocks fragments from IDT and inserted into pGL4 vectors. MC7 were seeded at 70–80% confluency in 24-well tissue culture plates 24 h before transfection. Luciferase construct (1 µg) and pSvβ-β-galactosidase vector (0.1 µg), or their corresponding empty vector controls were transfected into cells according to manufacturer’s protocol using Superfect reagent (Qiagen) with a DNA/Superfect ratio of 1:2.5 (wt/wt). The promoter activity was calculated from the ratio of firefly luciferase to β- galactosidase levels and expressed as arbitrary units.

**Colocalization analysis**. Colocalization analysis was performed to calculate the likelihood that the same causal SNP drives both lincNORS expression in human artery and skeletal muscle as well as the age of menarche GWAS signal at this locus. We queried the GTEx online server (https://www.gtexportal.org) to identify the tissues in which rs181766 is an eQTL for lincNORS. For each tissue with a lincNORS eQTL at rs181766 (p<0.05), we performed a two-tailed Student’s t-test or ANOVA with post hoc comparison where appropriate unless otherwise noted in the table. The data are presented as mean ± SD, unless stated otherwise.

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

**Data availability**. RNA-seq and Bru-seq data that support the findings of this study have been deposited in the National Center for Biotechnology Information Gene Expression Omnibus (GEO) and are accessible through the GEO Series accession numbers GSE152793 and GSE152799, respectively. All other relevant data are available from the corresponding author upon reasonable request. Source data are provided with this paper.

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**Statistical analysis**. Statistical analysis was performed in Excel, R (version 3.0.1, http://www.rproject.org/) or GraphPad Prism (version 6). Statistical significance of differences in means was determined by Mann-Whitney U-test or ANOVA with post hoc comparison where appropriate unless otherwise noted in the figure legend. The difference was considered statistically significant when p-value was <0.05. Data are presented as mean ± SD, unless stated otherwise.

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Author contributions
X.W. and M.I. conceived and designed the experiments. X.W., C.M.N., A.R., E.B., M.K.W., O.M.T., D.N.P., R.M., A.M. (McIntyre), A.B., M.P.B., S.M., and H.K. contributed to the
in vitro experiments. A.S.J performed LC-MS analysis on total cholesterol content and metabolites ratio measurement. D.L.C and W.P.B performed an analysis of lincNORS-associated SNPs. X.Z., F.F., and K.P.N generated and analyzed LTED-MCF7. S.M. generated samples from FTE cells. M.L.F. generated samples from patient-derived cell lines. M.G. contributed to the RNA pulldown experiment and A.M. (Mosley) performed the mass spectrometry analysis. A.R., T.T., and P.M. independently validated lincNORS interaction with RALY. The mouse experiments were performed by X.W. with assistance from IU Simon Cancer Center In Vivo therapeutics (IVT) Core and the tumor metastasis was analyzed by G.E.S. B.A.H., M.R., and K.P.N performed the AmpliSeq and RNA-seq. M.T.P. and M.L. performed the sequencing and data analysis of Bru-seq. X.W., A.B. (Buecheilin), C. I., J.R., and S.O. contributed to the bioinformatic analysis. X.W. prepared the manuscript, X.W., C.M.N., M.B.P., and M.I. drafted and edited the manuscript with input from all authors.

Competing interests
The authors declare no competing interests.

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