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The SET oncoprotein promotes estrogen-induced transcription by facilitating establishment of active chromatin

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SET is a multifunctional histone-binding oncoprotein that regulates transcription by an unclear mechanism. Here we show that SET enhances estrogen-dependent transcription. SET knockdown abrogates transcription of estrogen-responsive genes and their enhancer RNAs. In response to 17β-estradiol (E2), SET binds to the estrogen receptor α (ERα) and is recruited to ERα-bound enhancers and promoters at estrogen response elements (EREs). SET functions as a histone H2 chaperone that dynamically associates with H2A.Z via its acidic C-terminal domain and promotes H2A.Z incorporation, ERα, MLL1, and KDM3A loading and modulates histone methylation at EREs. SET depletion diminishes recruitment of condensin complexes to EREs and impairs E2-dependent enhancer-promoter looping. Thus, SET boosts E2-induced gene expression by establishing an active chromatin structure at ERα-bound enhancers and promoters, which is essential for transcriptional activation.

SET | estrogen | transcriptional activation | histone chaperone | breast cancer

Significance

The estrogen receptor ERα regulates expression of genes essential for growth of ERα$^+$ breast cancer. Estrogen-mediated transcription is regulated by epigenetic changes to histones and chromatin architecture. Here, we demonstrate that the SET oncoprotein, an ATP-independent histone chaperone, whose functions in oncogenesis are not well understood, critically regulates ERα-mediated transcription by helping establish an active chromatin structure at key estrogen-regulated promoters and enhancers. SET knock down strongly inhibits estrogen-induced transcription. Because SET interacts with other transcription factors, including p53 and glucocorticoid, androgen, and thyroid receptors, SET also likely regulates transcription of other hormone-responsive genes. Our findings suggest that antagonizing SET is a potential therapeutic strategy for ERα$^+$ breast cancer.

Gene expression in eukaryotes is modulated by changes in chromatin structure, particularly at promoter and enhancer regulatory regions. During transcriptional activation, chromatin structure is modified by histone post-translational modifiers, adenosine 5′ triphosphate (ATP)-dependent chromatin remodelers, chromatin architectural regulators, and histone chaperones that function in combination with one another (1–3). Alterations of chromatin regulators contribute to the establishment and maintenance of pathological cellular states, including cancer. The SE translocation (SET) oncoprotein is homologous to the ATP-independent nucleosome assembly proteins (NAP) that function as histone chaperones (4). Initially identified as a SE translocation-Nucleoporin 214 (SET-CAN) fusion gene in a poorly differentiated myeloid leukemia (5), SET is overexpressed in several cancer types, including breast, nonsmall cell lung, head and neck and hepatocellular cancer. SET inhibition has been suggested as a potential cancer therapy (6). However, how SET contributes to oncogenesis is not well understood. Understanding SET's role as an oncoprotein has been challenging because SET has multiple functions and participates in multiple cellular processes, including cell division, immune defense, apoptosis, epigenetic modifications, DNA repair, transcription, cell migration, and metastasis (7). SET is a potent inhibitor of the tumor suppressor phosphatase protein phosphatase 2A (PP2A) and the metastasis suppressor NM23-H1 (8, 9). In some settings, SET has been shown to potently activate transcription (10). However, SET was also described as a component of an inhibitor of histone acetyltransferase complex, which was postulated to mask histone acetyltransferase targets to inhibit transcriptional activation (11, 12). SET was shown to interact with estrogen receptor α (ERα) and other steroid hormone receptors and suppress hormone-dependent transcription in a reporter assay (13). Recently, SET has been shown to bind in an acetylation-dependent manner to the C-terminal domain of p53 and inhibit its transcriptional activity (14, 15).

To better understand how SET regulates transcription, we focused on the estrogen response. Estrogen receptor signaling activates a highly complex and tightly regulated transcriptional response in which epigenetic modulation has been shown to be important (16–18). ERα functions as a transcription factor, which binds to estrogen response elements (EREs) in enhancers and promoters to orchestrate a finely timed regulated transcriptional response that involves the tethering of several coregulators to specific transcriptional regulatory regions (19). SET participates in an interconnected network of proteins that are recruited to ERα (20). However, there are contradictory data about the role of SET in response to estrogen. On the one hand, SET is overexpressed in 50 to 60% of breast cancers, including ERα$^+$ cells, whose growth and proliferation rely on estrogen, which suggests that SET promotes the estrogen response (21). On the other hand, it has been proposed that SET inhibits hormone-induced transactivation in a breast cancer cell line (13). To investigate SET function in an unbiased way, we studied genome-wide changes in SET localization in response to estrogen in breast cancer cell lines and the

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effect of SET knockdown (KD) on estrogen-dependent transcription and epigenetic modifications in the vicinity of EREs and estrogen-regulated genes.

Here we show that SET promotes estrogen-induced transcriptional activation by altering chromatin around EREs in estrogen-responsive genes. In response to estrogen, SET is rapidly and specifically recruited with ERα to ERE, especially in enhancers. Furthermore, SET is required for enhancer activation. SET KD strongly inhibits ERα binding, enhancer RNA (eRNA) transcription, histone modifications, and condensin complex loading of estrogen-dependent genes, inhibiting their transcriptional activation. Mechanistically, SET acts as a histone chaperone that dynamically associates with the histone variant H2A.Z after estrogen stimulation and promotes H2A.Z incorporation at estrogen-responsive regulatory regions and increases enhancer–promoter interactions. Knocking down SET mimics the transcriptional effect of H2A.Z KD on estrogen-responsive genes. Taken together, we propose that SET functions as a critical regulator to establish an active chromatin structure at key estrogen-regulated regulatory regions to activate transcription of estrogen-induced genes.

Results

Ligand-Activated Recruitment of SET to ERα-Bound Enhancers. To assess the relevance of SET on the clinical outcome in breast cancer, we drew survival plots based on SET expression using patient data from Gene Expression Omnibus (GEO), The European Genome-phenome Archive (EGA), and The Cancer Genome Atlas (TCGA) databases (*SI Appendix*, Fig. S1 *A* and *B*) (22). High SET expression was significantly associated with poor prognosis in ERα$^+$ breast cancer (HR = 1.31, P = 1.1e–5), but not in ERα$^-$ breast cancer (HR = 1.08, P = 0.41). We then analyzed whether expression of estrogen-responsive genes correlates with SET levels. When patients were grouped based on high or low SET expression, 5% of 300 estrogen-responsive genes analyzed showed significant differential expression (10 up, 6 down) using a stringent cutoff (*SI Appendix*, Fig. S1 *C* and *D*). Although this number of differentially expressed genes (DEG) was low, the probability of identifying 16 DEG from a pool of 300 genes with this cutoff by chance was low (P < 1.53e–15 by hypergeometric test). These observations prompted us to investigate the functional role of SET in estrogen-induced transcription in ERα$^+$ breast cancer.

E2-induced ERα binding has been mapped by chromatin immunoprecipitation coupled with next-generation sequencing (ChIP-seq) in several cell lines, including MCF7 (23). To analyze the interplay between SET and ERα on chromatin without bias, we performed SET chromatin immunoprecipitation sequencing (ChIP-seq) in vehicle- and E2-treated MCF7 cells. Because a suitable ChIP-grade SET antibody is not available, we stably expressed FLAG-tagged SET in MCF7 and performed immunoprecipitations with FLAG (a peptide with the sequence DYKDDDDK (where D=aspartic acid, Y=tyrosine, and K=lysine) antibody. We then compared our SET ChIP-seq data [GEO GSE200900] (24) to published ERα ChIP-seq data (25). First, we examined SET enrichment at significant peaks in the absence or presence of E2. Consistent with the idea that SET regulates the estrogen-response program, SET binding to chromatin sites at which it was detected without added estrogen robustly increased after E2 stimulation (Fig. 1*A*). Moreover, the number of significantly bound SET peaks increased after E2 treatment, giving rise to ~700 de novo E2-induced SET-binding sites (Fig. 1*B*). SET and ERα barely colocalized in the absence of E2, but after E2 treatment a significant number of colocalized peaks appeared (Fig. 1 *C* and *D*), consistent with our finding that the interaction between SET and ERα on chromatin is estrogen-dependent. To confirm our ChIP-seq results, we used ChIP-qPCR to examine ERα and SET localization to the regulatory regions of three estrogen-responsive genes (*FOXC1*, *TFF1*, *MYC*) and one control gene (*HBB*, the gene encoding β-globin) (Fig. 1*E*). Both ERα and SET were enriched at the estrogen-responsive gene loci after E2 treatment, but not at the control gene. To further understand the role of SET after E2 induction, we analyzed the genomic localization of SET peaks. SET mainly bound to intergenic regions and gene bodies and only a small fraction was bound to promoters (*SI Appendix*, Fig. S1*E*). These data suggest that SET may preferentially bind to enhancer regions. To test this hypothesis, we re-analyzed the Encyclopedia of DNA Elements (ENCODE) ChIP-seq data of the well-characterized enhancer marks H3K27ac and p300. SET and ERα strikingly colocalized with H3K27ac and p300 after E2 induction (Fig. 1*C*). Importantly, this colocalization was only evident at SET peaks that also colocalized with ERα, suggesting that SET may be involved in regulating estrogen-responding regulatory regions. We examined the locus of *TFF1*, a typical estrogen-responsive gene, in more detail (Fig. 1*F*). After E2 treatment, SET and ERα were significantly enriched together with H3K27ac and p300 at the *TFF1* promoter and an upstream enhancer. A similar occupancy pattern was also observed in other estrogen-responsive gene loci, such as *TOP2A*, *SOX3*, *CDC6*, and *KRT19* (*SI Appendix*, Fig. S1*F*).

To verify our findings in MCF7 cells, we examined ERα and SET binding profiles in another ERα$^+$ breast cancer cell T47D using the CUT&Tag technique [GEO GSE218815] (26, 27). Compared to ChIP-seq, CUT&Tag has the advantages of requiring fewer cells, no formaldehyde fixation, low cost, and high-quality data. E2 significantly increased ERα binding in the genome (4,472 de novo ERα binding sites), while the total number of SET-bound peaks decreased after E2 treatment (31,267 vs. 29,631) (*SI Appendix*, Fig. S2 *A* and *B*). The SET/ERα cobound sites increased after E2 treatment (from 4,969 to 5,826) and E2 treatment promoted both ERα and SET binding at these sites (*SI Appendix*, Fig. S2 *C–H*). After E2 treatment, the occupancies of ERα and SET at multiple ERα-regulated gene loci (*CTSD*, *GREV1*, *B4GALT1*, *TOP2A*, *ADCY9*, and *MLYCD*) were significantly increased. Taken together, our results suggest that SET and ERα interact and colocalize at the regulatory regions of estrogen-responsive genes in response to estrogen stimulation in a genome-wide manner.

Requirement of SET for ERα-Dependent Transcriptional Activation. To link these binding events to transcriptional regulation, we profiled differential estrogen-responsive gene expression in response to E2 using RNA-seq in MCF7 cells knocked down for *SET* [GEO GSE200900] (24). Two SET isoforms (SETα and SETβ) in humans share high homology. CRISPRi was used to KD each SET isoform specifically (*SI Appendix*, Fig. S3*A*). Both isoforms affected estrogen signaling but SETβ KD downregulated expression of all estrogen-responsive genes tested more efficiently than SETα KD (*SI Appendix*, Fig. S3*B*). To explore the impact of total SET on transcriptional regulation, we performed RNA-seq in MCF7 cells in which SET was knocked down by an siRNA pool that knocked down both isoforms (*SI Appendix*, Fig. S4*A*). SET KD did not affect ERα expression (*SI Appendix*, Fig. S4*A*). Hierarchical clustering was used to organize RNA-seq analysis of ~300 known estrogen-responsive genes in triplicate MCF7 samples. SET can both upregulate and downregulate estrogen-responsive gene expression (*SI Appendix*, Fig. S4*B*). The differentially expressed estrogen-responsive genes comparing wild type (WT) and SET KD cells are shown in a volcano plot in *SI Appendix*, Fig. S4*C*. Some estrogen-responsive genes, such as *TOP2A*, *NMU*, *CCNA1*, and *SOX3*, whose expression positively

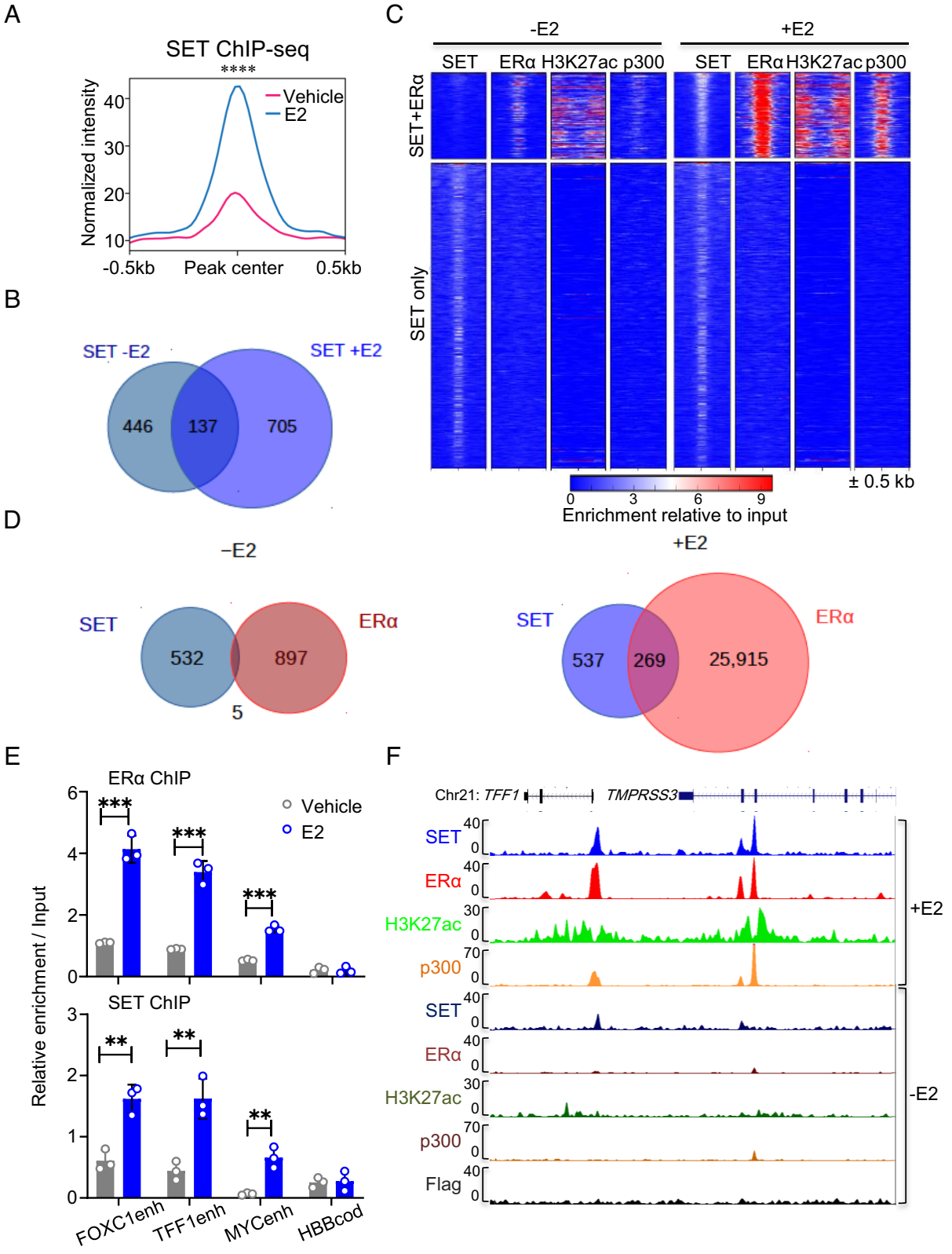

Fig. 1. Estrogen recruits SET to ERα-bound sites in the genome of MCF7 breast cancer cells. (*A*) SET ChIP-seq analysis offset peak enrichment in the MCF7 genome with or without adding 100 nM estradiol (E2) for 35 min. *P* value < 0.0001, Mann–Whitney *U* test. (*B*) Venn diagram of overlap between SET ChIP-seq binding sites in the presence or absence of E2. Overlap probability in E2-treated cells according to hypergeometric test is $P = 3.64e{-}31$. (*C*) Heatmap of SET, ERα, H3K27ac and p300 ChIP-seq signals with or without estradiol. SET ChIP-seq signals were divided into SET+ERα and SET only groups according to whether ERα binding was detected. (*D*) Venn diagrams showing overlap of SET and ERα ChIP-seq peaks in cells treated or not with E2. Overlap significance without and with E2 are $P = 0.22$ and $P = 5.77e{-}15$, respectively, according to the hypergeometric test. (*E*) SET and ERα occupancies at estrogen-responsive elements (EREs) of the *FOXC1*, *TFF1* and *MYC* genes in response to estrogen by ChIP-qPCR. qPCR data show mean ± SD of three biological replicates; $*P < 0.05$; $**P < 0.01$; $***P < 0.001$; Student's *t* test. (*F*) The University of California, Santa Cruz (UCSC) browser snapshot shows the distribution of normalized reads of SET, ERα, H3K27ac, p300, and FLAG at the *TFF1* gene locus with and without E2 treatment. enh, enhancer; cod, coding region.

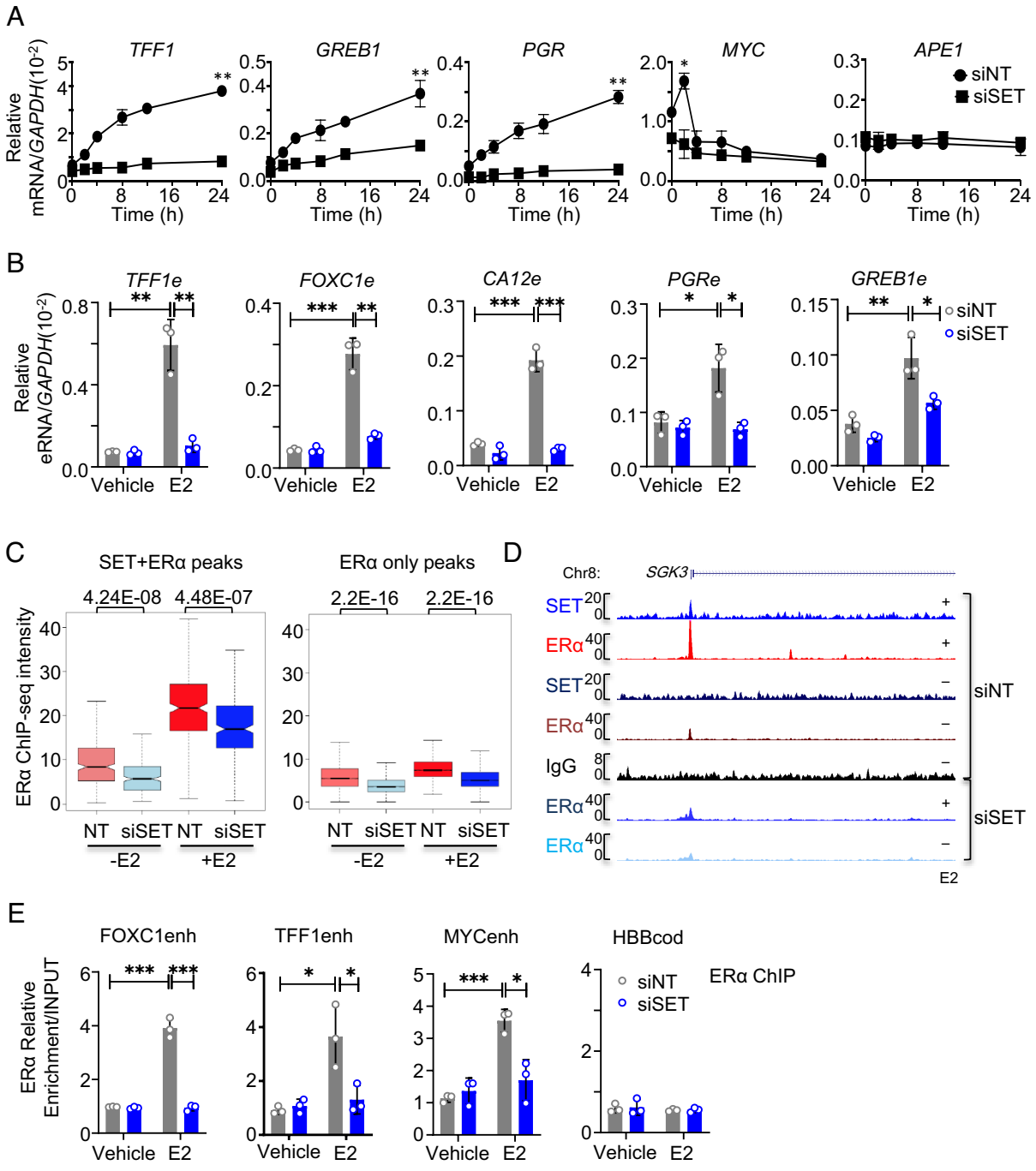

Fig. 2. SET KD blocks ERα-dependent transcription. (*A*) Effect of SET KD on expression of estrogen-responsive genes. MCF7 were transfected with an siGENOME SMARTpool targeting human SET (siSET) or a non-targeting siRNA pool (siNT) and treated with E2 for the indicated times before harvest. Levels of all mRNAs were normalized to *GAPDH* mRNA. *APE1* mRNA served as a control estrogen unresponsive gene. qPCR data show mean ± SD of three biological replicates; **P* < 0.05; ***P* < 0.01; ****P* < 0.001; Student's *t* test. (*B*) Effect of SET KD on E2-induced eRNAs. eRNA levels were normalized to *GAPDH* mRNA. qPCR data show mean ± SD of three biological replicates; **P* < 0.05; ***P* < 0.01; ****P* < 0.001; Student's *t* test. (*C*) Box plots comparing ERα ChIP-seq peak intensity in cells pretreated with siGENOME SMART pool targeting human SET (siSET) or siNT and then treated or not with E2 for 35 min showing ERα peaks that also bound SET (*Left*) or did not bind SET (*Right*). *P* value, Mann–Whitney *U* test. (*D*) UCSC browser snapshot showing the distribution of normalized SET and ERα reads at the *SGK3* locus in SET-depleted MCF7 cells with or without E2 treatment. (*E*) ChIP assay for ERα in SET-depleted or control (siNT) MCF7 cells treated or not with E2. ChIP-qPCR data show mean ± SD of three biological replicates; **P* < 0.05; ***P* < 0.01; ****P* < 0.001; Student's *t* test. enh, ehancer; cod, coding region.

correlated with SET in ERα$^+$ breast cancer, were significantly downregulated by SET KD. We focused on the gene activation signature of SET and verified by qRT-PCR that SET depletion eliminated E2-induced upregulation of four typical estrogen-induced genes (*TFF1*, *MYC*, *GREB1*, and *PGR*) with varying induction kinetics, while an estrogen-unresponsive gene (*APE1*) remained unchanged (Fig. 2*A* and *SI Appendix*, Fig. S4*D*). Thus, SET enhances estrogen-induced transcription of some genes.

Since our bioinformatic analyses suggested that SET binds to ERα-bound enhancers in response to estrogen, we hypothesized that SET might regulate enhancer activation. Noncoding transcripts initiated from enhancer regions (eRNAs) correlate with enhancer activity and are functionally involved in enhancer-promoter looping (16). Therefore, we measured eRNA levels of estrogen-responsive genes (*TFF1*, *FOXC1*, *CA12*, and *PGR*) in control and SET KD (SET-KD) cells. SET KD abrogated the transcriptional induction

of eRNAs of all these estrogen-responsive genes (Fig. 2*B*), suggesting that SET is required for E2-dependent enhancer activation.

After E2 stimulation, ERα is phosphorylated and translocates to the nucleus to activate its target genes (28). To assess whether the failure to induce estrogen-responsive gene expression in SET-KD cells could be attributed to the lack of ERα nuclear translocation or reduced ERα protein, nuclear and cytoplasmic fractions of control and SET-KD MCF7 cells, isolated at different times after adding E2, were analyzed by immunoblot probed for SET, ERα and cytoplasmic (α-tubulin) and nuclear (lamin A/C) loading controls. ERα mRNA and protein levels as well as nuclear translocation in response to estrogen were similar in SET-KD and control cells (*SI Appendix*, Fig. S4 *A* and *E*), suggesting that SET KD did not affect ERα expression or block its translocation. It is worth noting that the ERα immunoblot detected three bands in the nucleus, in accord with known ERα splice variants in MCF7 (29). Except for the main 66 kDa isoform, other potential ERα isoforms corresponding to the lower bands (54 and 48 kDa) became less prominent in the nucleus in SET KD, suggesting that SET might regulate alternative splicing or translation of the ERα mRNA.

SET is also a potent inhibitor of PP2A, a tumor suppressor family of phosphatases, which regulate multiple signaling pathways associated with cancer progression such as Akt, β-catenin, and c-Myc (30). Previous studies reported that PP2A downregulates estrogen-induced gene expression by dephosphorylating ERα at S167 (31). To investigate whether SET regulates the expression of estrogen-response genes predominantly through inhibiting PP2A, we inhibited PP2A with the specific inhibitor fostriecin and analyzed whether PP2A inhibition could rescue E2-induced gene expression in SET-KD cells (*SI Appendix*, Fig. S4*F*). PP2A inhibition had little effect on the transcriptional levels of E2-induced genes and failed to rescue the effect of SET KD, implying that PP2A inhibition is not an important feature of SET regulation of estrogen-responsive genes.

SET and ERα genomic binding profiles suggest that SET could play a role in ERα recruitment to EREs. To investigate the genome-wide effect of SET on E2-induced ERα recruitment, we performed ERα ChIP-seq in control and SET-KD cells, with or without estrogen treatment [GEO GSE 200900] (24). SET depletion significantly reduced estrogen-induced ERα enrichment at SET/ERα cobound sites as well as at ERα-bound sites in which SET binding was not detected by ChIP (Fig. 2*C*). ERα binding to these latter sites was only slightly induced by estrogen. The diminished ERα recruitment is shown by genome browser snapshots for the estrogen-responsive gene *SGK3* as an example (Fig. 2*D*). The suppressive effect of SET KD on E2-induced ERα binding at the enhancers of estrogen-responsive genes was also validated for three genes (*FOXC1*, *TFF1*, *MYC*) by ChIP-qPCR (Fig. 2*E*). Therefore, SET strongly promotes E2-dependent ERα-mediated transcriptional activation.

SET Regulates Histone Methylation at ERα Target Enhancers. Histone modifications and their epigenetic modifiers play important roles in estrogen-induced transcriptional activation (18, 32, 33). Most ERα-bound sites are potential enhancers based on the presence of histone modifications, such as H3K4me1/2 and H3K27ac, which are characteristic signatures of active enhancers (16). Additionally, oxidative DNA damage that occurs during demethylation of histone H3K9me2 may recruit the DNA damage repair machinery to promote E2-induced gene expression by facilitating chromatin remodeling at ERα binding regions (17). In response to estrogen, ERα-bound sites also acquire H3K4me1/2 and have reduced H3K9me2 (34). Given the importance of histone lysine methylation in transcriptional regulation and the known histone chaperone function of SET (1, 2, 10, 35), we hypothesized that SET might be involved in E2-induced histone modifications. SET has been reported to interact with the histone H3K4 methylase MLL1 in HeLa (36) and the histone H3K9me2 demethylases, LSD1(KDM1A), KDM3A, and KDM4B, are reported to modulate estrogen signaling in ERα$^+$ breast cancer cells (17, 32, 37). However, KD of *LSD1* did not affect E2-induced gene expression in MCF7(38). KDM3A and KDM4B cooperatively regulate chromatin transactions of the ERα, and KDM3A plays a central role in recruiting KDM4B (39). Therefore, to assess the effect of SET on histone modifiers, we used coimmunoprecipitation to assess the dynamic association of SET with the histone modifiers MLL1 and KDM3A. Immunoprecipitation (IP) with MLL1 antibody showed that ERα association with MLL1 increased after adding E2 (Fig. 3*A*). Although SET binding to ERα was detected in the absence of exogenous E2, E2 also increased the binding of SET to MLL1 in MCF7 cells. MLL1 binding to both ERα and SET persisted for at least 50 min after adding E2. When the IP was repeated with anti-KDM3A, KDM3A also associated with both ERα and SET 35 min after adding E2, but the interaction was no longer detected 50 min after adding E2 (Fig. 3*B*). As a control, KDM3A did not pulldown the paralogue NAP protein, NAP1L1, in the presence or absence of added E2. Thus, both MLL1 and KDM3A associate with ERα and SET in an E2-dependent manner.

To determine whether SET helps recruit MLL1 and KDM3A to EREs, we used MLL1 and KDM3A ChIP-qPCR to compare MLL1 and KDM3A occupancies at the two EREs and gene body of the *TFF1* gene locus in control and SET-KD MCF7 (Fig. 3*C*). SET KD significantly attenuated E2-induced recruitment of these histone modifiers to the *TFF1* enhancer (ERE2) and promoter (ERE1) EREs but had no effect on background binding to the gene body. Correspondingly, the comparison of H3K4me1 and H3K9me2 ChIP-qPCR in control and SET-KD MCF7 showed that E2-induced enrichment of histone H3K4me1, a mark of both active and poised enhancers, and reduction of H3K9me2, a mark of transcriptional repression, at EREs in *FOXC1*, *TFF1,* and *MYC* enhancers were abolished by SET KD (Fig. 3*D*). Together, these data suggest that SET promotes E2-mediated recruitment of histone modifiers MLL1 and KDM3A to enhancer EREs, resulting in E2-induced histone modifications at ERα target enhancers.

Dynamic Estrogen-Dependent Association of SET with Histone H2A.Z. SET belongs to the ATP-independent nucleosome assembly protein family, which functions as histone H2A-H2B chaperones that regulate chromatin accessibility for DNA replication, DNA repair and gene expression through nucleosome remodeling (3). These chaperones can replace canonical histone H2A with variants H2A.Z, H2A.C, and H2A.X whose incorporation into nucleosomes alters nucleosome stability. In particular, H2A.Z replacement, which occurs at promoters during transcriptional activation, destabilizes nucleosomes at promoter regions to promote nucleosome disassembly and DNA accessibility for transcription initiation (1, 40). To investigate whether SET acts as an E2-regulated histone chaperone, we immunoprecipitated SET from nuclear lysates of E2-treated MCF7 and probed for ERα and histone H2A and its variants and H2B (Fig. 4*A*). SET dynamically associated with ERα in the nucleus, with the strongest interaction observed ~35 min after E2 treatment. In the absence of added estrogen, SET mostly associated with histone H2A and H2A.C was faintly detected, but there was no detectable interaction with H2A.X, H2A.Z, or H2B. Thirty-five minutes after adding E2, H2A binding was replaced by a strong H2A.Z signal, but SET-immunoprecipitation of both H2A and H2A.Z returned to pre-E2 baseline by 50 min. We did not observe

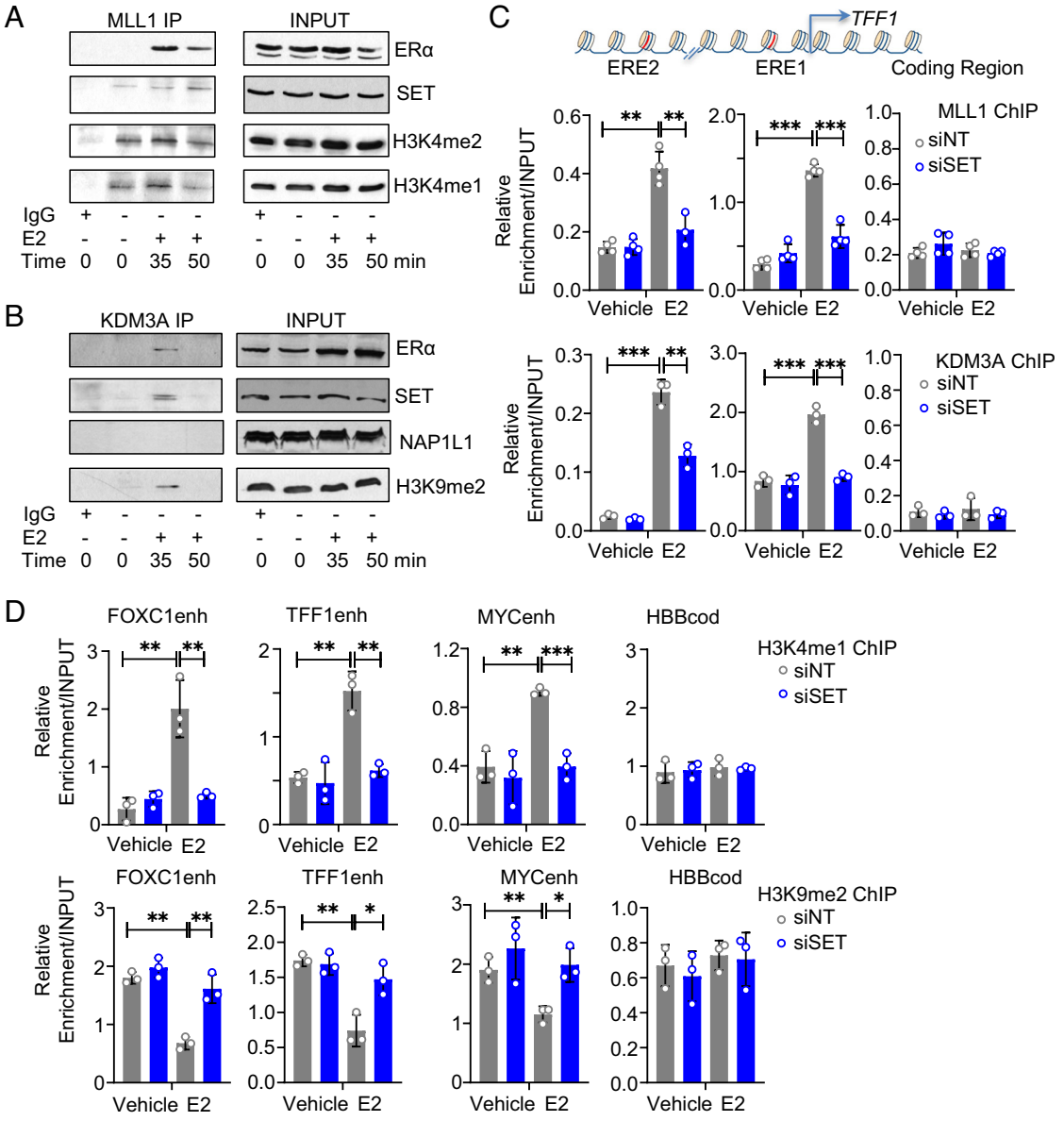

Fig. 3. SET regulates estrogen-dependent histone modifications at EREs. (*A*) Coimmunoprecipitation of the histone H3K4 methyltransferase, MLL1, with ERα, SET and its substrates in response to E2 treatment. After adding E2 for the indicated times, MCF7 cells were harvested and nuclear extracts were prepared. Co-IP with anti-MLL1 was analyzed by immunoblot probed with indicated antibodies. Data are representative of two independent experiments. (*B*) Coimmunoprecipitation of the histone H3K9 demethylase, KDM3A, with ERα, SET, the control nucleosome assembly protein NAP1L1 and the KDM3A histone substrates in response to E2 treatment. After adding E2 for the indicated times, MCF7 cells were harvested, and nuclear extracts were prepared. Co-IP was performed using anti-KDM3A and immunoblots were probed with indicated antibodies. Data are representative of two independent experiments. (*C*) ChIP-qPCR assays for MLL1 and KDM3A at the EREs of the estrogen responsive gene *TFF1* in SET-depleted (siSET) or control (siNT) MCF7 after E2 treatment for 35 min. (*D*) ChIP-qPCR assays showing H3K4me1 and H3K9me2 abundance at the EREs of estrogen responsive genes (*FOXC1, TFF1, MYC*) or unresponsive control gene (*HBB*) in SET-depleted or control MCF-7 cells in response to E2. For *C* and *D*, data show mean ± SD of three biological replicates; **P* < 0.05; ***P* < 0.01; ****P* < 0.001; Student's *t* test. enh, enhancer; cod, coding region.

estrogen-dependent binding of SET to histone variants H2A.C and H2A.X. Our data suggest that SET specifically and dynamically associates with ERα and H2A.Z in an estrogen-dependent manner. It is worth noting that SET ChIP-seq also showed strong E2-independent enrichment for SET selectively at the H2A.Z and H2B gene bodies (*H2AFZ* and *HIST1H2BE*, respectively), but not other histone genes, which coincided with H3K27ac enrichment (*SI Appendix*, Fig. S5*A*). There was no SET or ERα binding at the gene encoding H2A.X (*H2AFX*) or other histone gene bodies even though these genes were also enriched for H3K27ac marks. However, the significance of these interactions is unclear.

H2A.Z has previously been shown to be essential for estrogen signaling (41, 42). In our system, H2A.Z KD, like SET KD, also robustly diminished the expression of all four E2-induced genes we analyzed by qRT-PCR (*TFF1*, *GREB1*, *CTSD*, and *PGR*), but had no effect on SET itself (Fig. 4*B*). Although a previous study (43) demonstrated that estrogen also induces DNA double-strand breaks (DSBs), which activates phosphorylation of H2A.X, termed γH2A.X, at ERα-bound regions, H2A.X KD had a more modest effect on E2-induced gene transcription (*SI Appendix*, Fig. S5*B*), indicating that the histone variant H2A.Z, rather than H2A.X, predominantly regulates hormone-dependent gene expression.

H2A.Z Replacement at EREs Depends on SET. Because both SET and H2A.Z enhance estrogen signaling and SET dynamically associates with H2A.Z, we hypothesized that SET mediates estrogen-dependent H2A.Z replacement of H2A at EREs. To

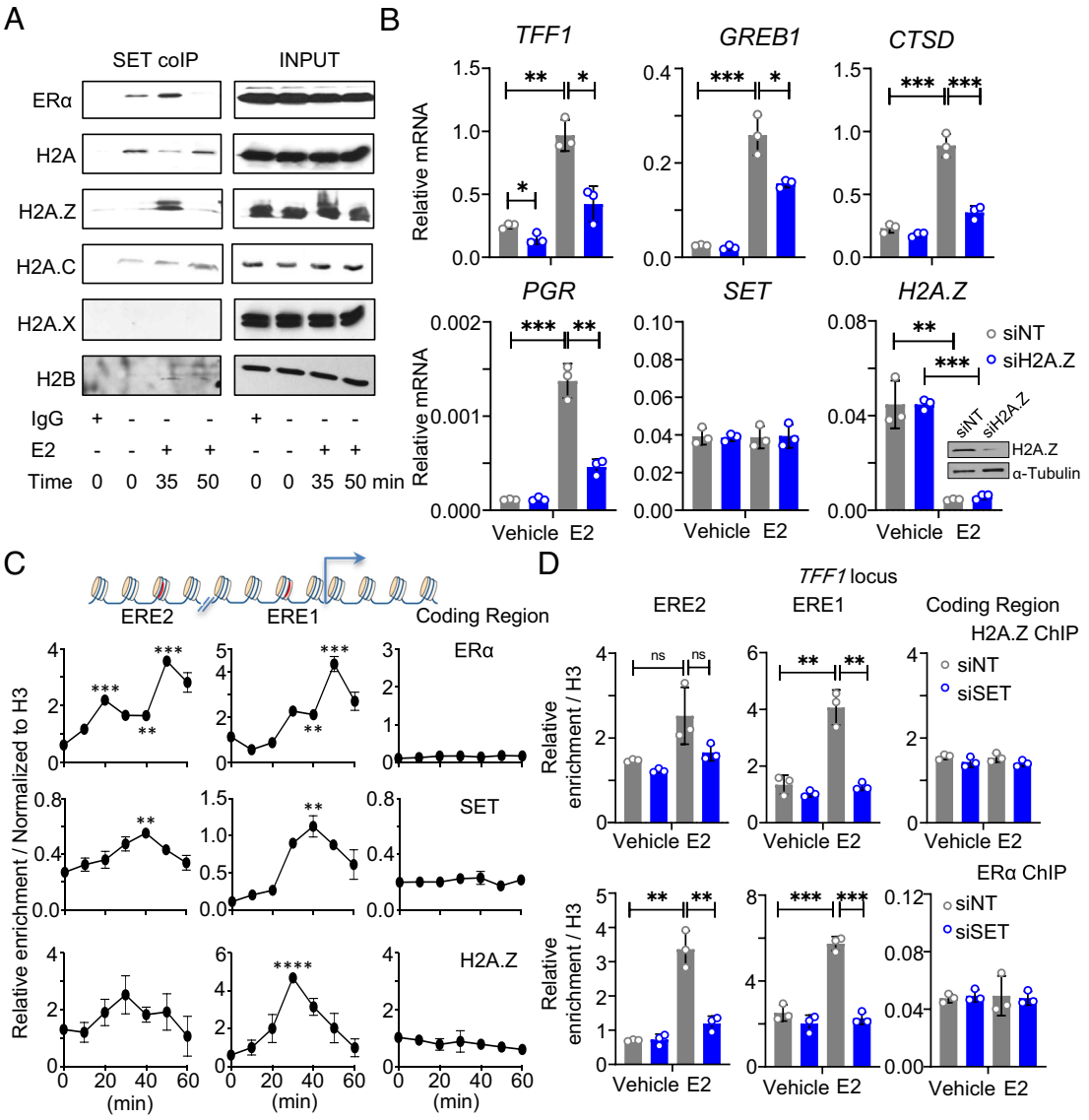

Fig. 4. SET associates with ERα and H2A.Z after estrogen stimulation. (*A*) Coimmunoprecipitation of SET with ERα and histone H2 variants. After adding E2 for the indicated times, MCF7 cells were harvested and nuclear extracts were prepared. SET immunoprecipitations were analyzed by immunoblot probed with indicated antibodies. H2A.Z transiently displaced H2A after adding E2. Immunoblots are representative of duplicate experiments. (*B*) Effect of *H2AFZ* KD on expression of estrogen-responsive genes *TFF1GREB1CTSD* and *PGR*. MCF7 cells, transfected with an siGENOME SMARTpool targeting *H2AFZ* (siH2A.Z) or a non-targeting siRNA pool (siNT) were treated with E2 for 24 h. Levels of all mRNAs determined by qRT-PCR were normalized to *ACTB* mRNA. Shown are mean ± SD of three biological replicates; **P* < 0.05; ***P* < 0.01; ****P* < 0.001; Student's *t* test. (*C*) Dynamics of ERα, SET and H2A.Z occupancy at the enhancer (ERE2), promoter (ERE1) and coding region of the *TFF1* gene after adding E2, assayed by ChIP-qPCR. The drawing at the top shows the approximate positions of primers used in the ChIP-qPCR assay. Enrichment of ERα, SET and H2A.Z were normalized to histone H3. Shown as mean ± SD of three independent replicates; **P* < 0.05; ***P* < 0.01; ****P* < 0.001; Student's *t* test. (*D*) Effect of *SET* KD on E2-induced ERα and H2A.Z occupancy at the enhancer, promoter and coding region of the *TFF1* gene determined by ChIP-qPCR assay. Shown are mean ± SD of three biological replicates; **P* < 0.05; ***P* < 0.01; ****P* < 0.001; Student's *t* test.

test this, we first analyzed by ChIP-qPCR the dynamics of ERα, SET, and H2A.Z binding to the estrogen-responsive *TFF1* gene locus over an hour after adding E2 (Fig. 4*C*). Consistent with previous reports (33, 41), we observed an ordered and cyclic pattern of increased occupancy by ERα, SET and H2A.Z at the *TFF1* ERE1 and ERE2, but not at its gene body, after adding E2. ERα occupancy at the EREs gradually increased after adding E2, peaked at 50 min, and then dropped. H2A.Z occupancy coincided approximately, but not exactly, with SET binding, peaked earlier (30 min for H2A.Z, 40 min for SET) and returned to baseline by 1 h. SET and H2A.Z enrichment was greater at the *TFF1* promoter (ERE1) than at the enhancer (ERE2).

Next, we determined the effect of SET KD on H2A.Z and ERα occupancy at the *TFF1* enhancer and promoter regions (Fig. 4*D*). SET KD significantly decreased estrogen-dependent ERα recruitment and H2A.Z deposition at these regulatory elements, particularly at the promoter region. Taken together, our results suggest that SET promotes E2-induced H2A.Z deposition at estrogen responsive regions.

SET Acts as an E2-Induced H2A.Z Histone Chaperone. To explore further our hypothesis that SET participates in E2-induced H2A.Z incorporation, we aligned SET protein sequence to three previously described H2A.Z-related histone chaperones, human ANP32e and yeast CHP and NAP1 (1). Although those histone chaperones have limited overall homology, the SET acidic C-terminal domain has two regions similar to the known H2A.Z interacting domains (ZID) of ANP32E, which has been implicated in H2A.Z removal (44) (*SI Appendix*, Fig. S6*A*). To test the ability of SET's putative ZID domains to bind H2A.Z, we measured the in vitro and in vivo

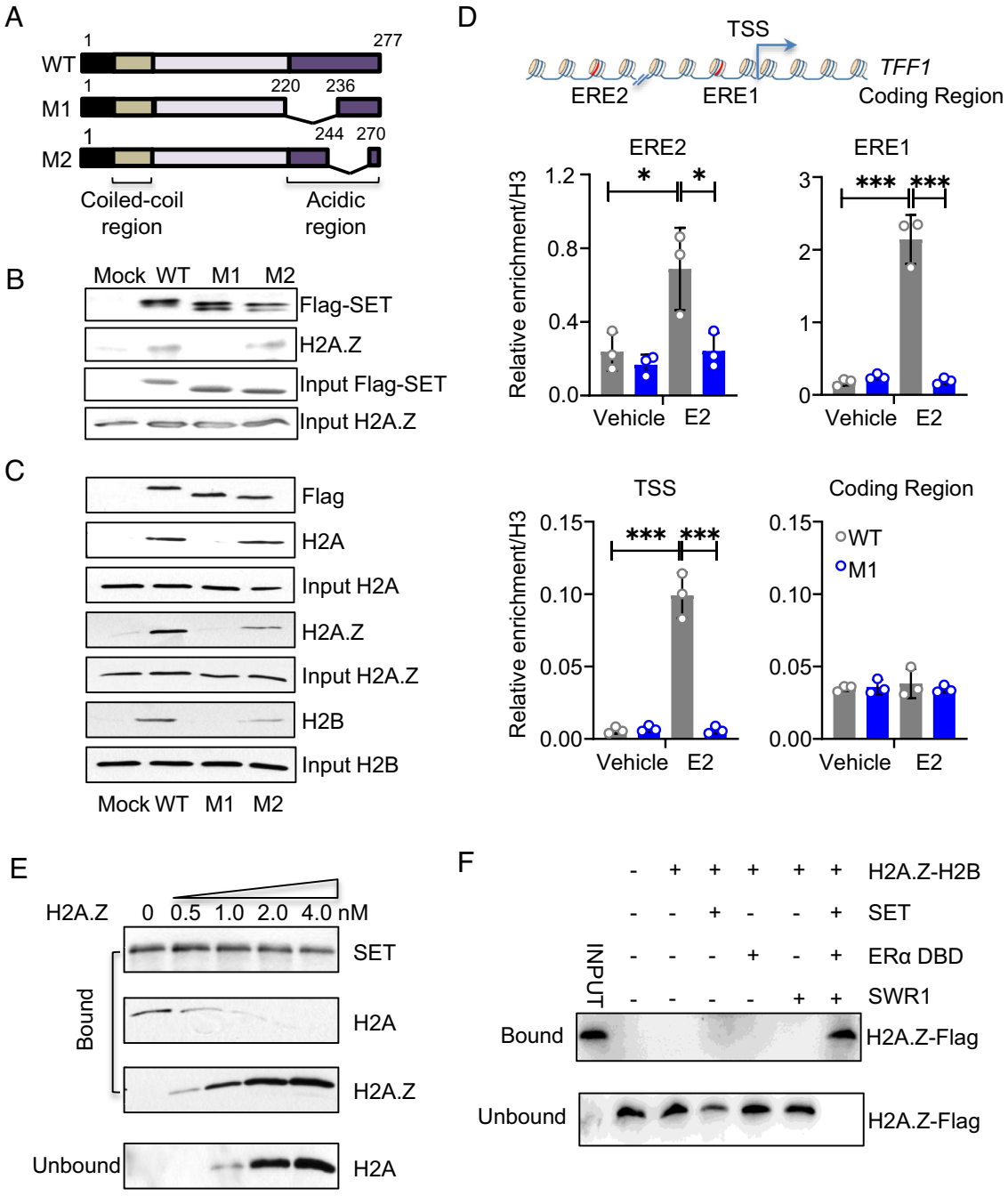

Fig. 5. SET facilitates E2-induced H2A.Z deposition into nucleosomes. (*A*) Schematic of the functional domains of full-length SET (WT) and truncated M1 and M2. (*B*) Coimmunoprecipitation of recombinant purified FLAG-tagged WT and mutant SET with recombinant H2A.Z. (*C*) Coimmunoprecipitation of stably expressed FLAG-tagged WT and mutant SET with H2A.Z in HEK-293T cells. Co-IP using anti-FLAG on nuclear extracts was probed with indicated histone antibodies. (*D*) FLAG ChIP-qPCR assay for stably expressed WT or M1 mutant SET in MCF7 cells, amplified at the indicated regions of the *TFF1* gene before and after adding E2. Relative FLAG ChIP enrichment was normalized to histone H3. qPCR data show mean ± SD of three biological replicates; **P* < 0.05; ***P* < 0.01; ****P* < 0.001; Student's *t* test. (*E*) In vitro competition binding assay to detect effect of adding increasing amounts of histone H2A.Z to mixtures of 1.2 nM SET and 4.2 nM histone H2A. (*F*) In vitro incorporation of H2A.Z in mononucleosomes formed with an ERE containing DNA sequence in the presence of the SWR1 chromatin remodeling complex and SET. H2A.Z-H2B, dimer of H2A.Z and H2B; ERα DBD, ERα DNA binding domain.

association of H2A.Z with FLAG-tagged full-length or truncated versions of SET ($M1^{220}\Delta^{236}$ and $M2^{244}\Delta^{270}$) lacking each of the potential ZIDs (Fig. 5*A*). The M1, but not the M2, deletion abolished the in vitro interaction between recombinant purified SET and H2A.Z (Fig. 5*B*). To examine the SET-H2A.Z interaction in cells, we performed co-IP experiments using HEK-293T stably expressing empty vector or FLAG-tagged full-length SET or the two deletion mutants. Full-length SET and to a lesser extent the M2 mutant immunoprecipitated H2A, H2B, and H2A.Z, while the M1 truncation did not bind to any of the histones tested (Fig. 5*C*). Thus, SET has a functional ZID domain (amino acids 220 to 236) in its acidic C-terminal domain, which is also needed for binding to the canonical H2A and H2B proteins.

To evaluate the importance of the ZID domain for the E2-dependent ERα interaction and recruitment of SET to ERα-bound regions, we generated MCF7 stably expressing FLAG-tagged M1 SET. M1 SET still interacted with ERα in an ordered and cyclical fashion after adding E2 (*SI Appendix*, Fig. S6*B*), indicating that the ZID domain is not required for the E2-induced SET/ERα interaction. However, despite its ability to bind to ERα, M1 SET

was not recruited to its binding sites in the *TFF1* locus (Fig. 5*D*). Correspondingly, overexpression of M1 SET reduced E2-dependent expression of the tested genes, *TFF1*, *GREB1*, *CTSD*, and *CCND1*, compared to WT SET (*SI Appendix*, Fig. S6*C*). Collectively, these results suggest that the H2A.Z-interacting domain of SET contributed to its estrogen-dependent induction of transcription.

Since SET interacts with both H2A and H2A.Z, we next evaluated whether H2A.Z competes with H2A for SET binding in vitro. To address this question, we developed an in vitro competition binding assay to assess the binding preference of SET for H2A or H2A.Z. Recombinant FLAG-tagged SET was pre-incubated with an excess of purified H2A and varying amounts of purified H2A.Z were added and allowed to compete with H2A for SET binding (Fig. 5*E*). SET complexes with H2A or H2A.Z were immunoprecipitated with anti-FLAG and the amount of each histone in the bound and unbound fractions was analyzed by immunoblot. H2A.Z outcompeted H2A for SET binding in a dose-dependent manner.

Previous studies in yeast demonstrated that histone chaperones NAP1 and Chz1 take part in H2A.Z incorporation into nucleosomes catalyzed by the SWR1 chromatin remodeling complex

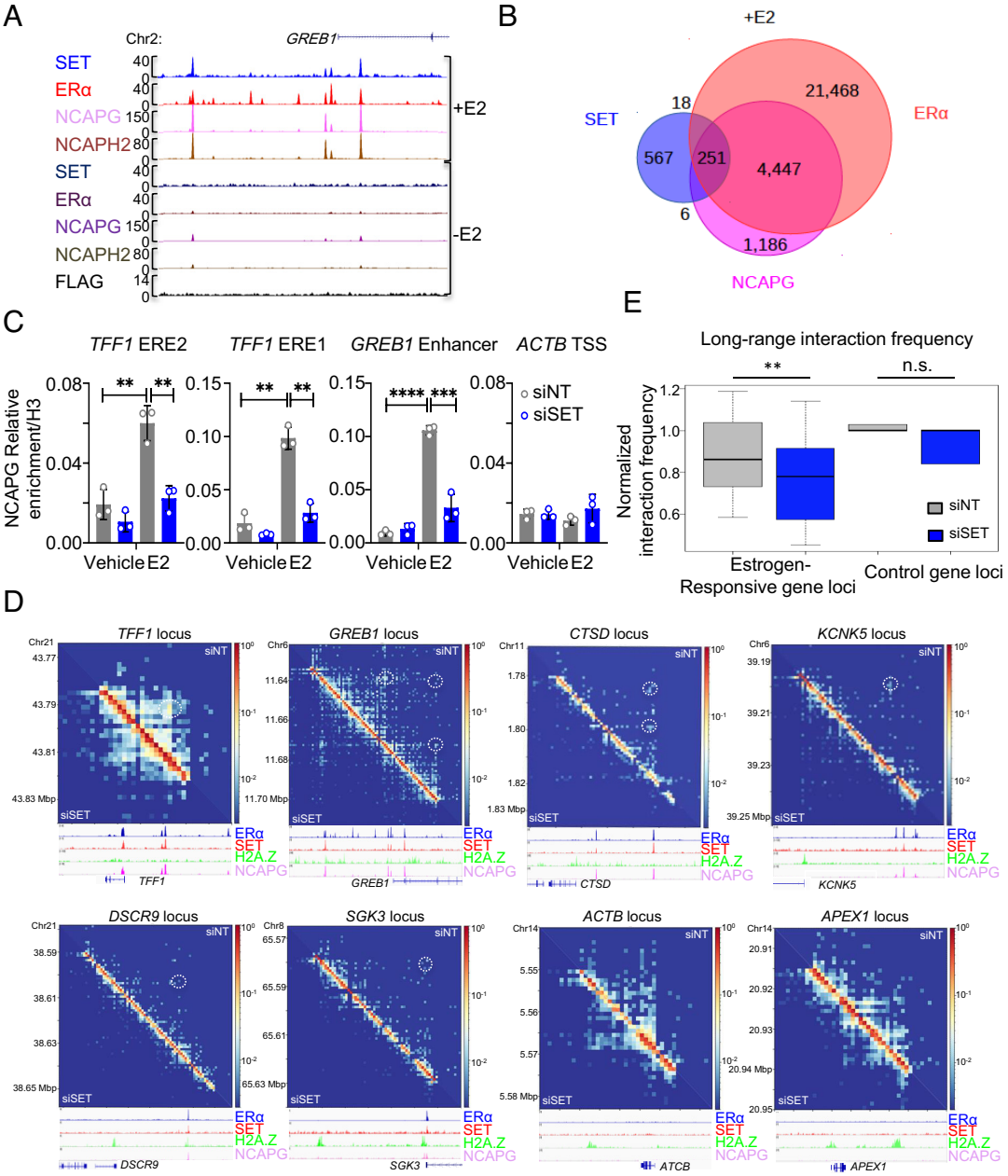

Fig. 6. Colocalization of SET and condensin complexes. (*A*) UCSC browser snapshot showing the distribution of normalized reads of SET, ERα, NCAPG, NCAPH2 and FLAG at the *GREB1* gene locus in MCF7 cells treated or not with E2. (*B*) Venn diagram showing numbers of genome-wide ChIP peaks containing SET, ERα and NCAPG and their overlap in MCF7 cells after adding E2. Overlap *P* values according to hypergeometric test: NCAPG-SET: 5.91e−126, SET-ERa: 5.77e−15, NCAPG-ERa overlap: 1e−999 (*C*) E2-induced recruitment of NCAPG to the *TFF1* and *GREB1* EREs compared to the *ACTB* TSS determined by ChIP-qPCR assay of MCF7 cells treated or not with E2. qPCR data show mean ± SD of three biological replicates; $*P < 0.05$; $**P < 0.01$; $***P < 0.001$; Student's *t* test. (*D*) HiC matrix of chromatin interaction profiles at high resolution in defined regions of estrogen-responsive *GREB1, SGK3, TFF1, DSCR9, CTSD* and *KCNK5* loci and estrogen-unresponsive *ACTB* and *APEX1* loci in SET-depleted MCF7 cells (siSET, *Bottom Left*) or control (siNT, *Top Right*) after adding E2. The interaction profiles were determined using Capture-C. All interaction values are calculated by the log1p method. Each color block represents the sum of the interaction values within 1,000 bp. Red indicates a stronger interaction as indicated on the color scale. The horizontal and vertical coordinates are labeled by chromosomal location. The occupancy of ERα, SET, H2A.Z, and NCAPG at each locus is shown under the heat map. (*E*) Box plot comparing the mean of normalized long-range interaction frequencies within nine estrogen responsive gene loci and two control unresponsive loci in control (siNT) and SET-depleted (siSET) MCF7 cells analyzed using 5 kb sized windows. A paired two sample Wilcoxon test was used for analysis. $*P < 0.05$; $**P < 0.01$.

(45). In mammalian cells, p400, the homologue of the SWR1 protein, is also recruited to ERα-bound regions (41). To investigate whether SET is also involved in E2-induced H2A.Z deposition, we performed in vitro histone H2A.Z replacement assays using a method that was similar to what was used to analyze the SWR1 chromatin remodeling complex. Recombinant FLAG-SET promoted nucleosome assembly in vitro (*SI Appendix*, Fig. S6*D*). To determine whether SET is able to incorporate H2A.Z, a biotin-labeled 282 bp PCR product containing the TFF1 promoter ERE1 was assembled into mononucleosomes using purified histone octamers. Reconstituted nucleosomes were incubated with H2A.Z-H2B, SET and SWR1 purified from *Saccharomyces cerevisiae* (*SI Appendix*, Fig. S6*E*). Nucleosomes were immunoprecipitated and the amount of incorporated H2A.Z was measured by immunoblot (Fig. 5*F*). H2A.Z was efficiently deposited into the core histone when both SET and SWR1 were present. Taken together these results indicate that SET acts as an H2A.Z histone chaperone and participates in E2-induced H2A.Z incorporation catalyzed by the SWR1 ATP-dependent chromatin remodeling complex.

SET Promotes E2-Dependent Enhancer–Promoter Looping. Although our data suggest that SET is required for E2-induced H2A.Z deposition at EREs, bioinformatic analyses failed to show any significant correlation between E2-induced SET occupancy and published H2A.Z ChIP-seq binding profiles (42). One plausible explanation for this lack of correlation is that most of the SET/ERα cobound regions are enhancers, while H2A.Z preferentially localizes at promoters genome-wide. However, we hypothesized that the SET-H2A.Z interaction might promote long-distance interactions of SET/ERα cobound enhancers to H2A.Z-bound promoters. The chromatin architectural regulators condensin I and condensin II are recruited to ERα-bound sites and increase ligand-dependent enhancer activation by promoting the formation of enhancer-promoter looping and eRNA transcription (46). The STRING database, which includes known and predicted protein–protein interactions, suggests that SET can interact with several members of the condensin complex, including SMC2, SMC4, NCAPG, and NCAPH2 (*SI Appendix*, Fig. S7*A*). Co-IP verified that SET dynamically associated with NCAPG, with the strongest interaction observed ~30 min after E2 treatment (*SI Appendix*, Fig. S7*B*). To investigate whether SET colocalizes with condensins at ERα-bound sites, we compared our SET ChIP-seq data with publicly available ChIP-seq data for NCAPG and NCAPH2, members of the condensin I and II complexes, respectively. SET showed a significant E2-dependent colocalization with both NCAPG and NCAPH2 genome-wide (Fig. 6*A* and *SI Appendix*, Fig. S7*C*). To determine whether the condensin–SET cobound peaks were also occupied by ERα, we analyzed their peak overlap after E2 treatment. Most of the condensin–SET cobound peaks coincided with ERα peaks (Fig. 6*B*), suggesting that they may form a complex that facilitates ERα-dependent enhancer–promoter contacts.

To test whether SET regulates E2-induced recruitment of condensin complexes to EREs, we compared NCAPG ChIP-qPCR at the regulatory regions of two estrogen-responsive gene (*TFF1* and *GREB1*) in control and SET KD cells (Fig. 6*C*). As previously reported (46), NCAPG occupancy increased in response to E2 in control cells. However, SET KD cells showed strongly reduced E2-dependent NCAPG binding at *TFF1* and *GREB1* regulatory regions, suggesting that SET recruitment is needed for appropriate condensin complex assembly at ERα-activated genes.

We next investigated whether SET KD impairs E2-dependent 3D chromatin reorganization by performing high-resolution Capture-C assays on specific estrogen-response genes in control and SET KD cells [GEO GSE200900] (24). A biotinylated RNA bait library was used to capture six estrogen-responsive gene loci, including enhancer, promoter and coding regions (*TFF1*, *GREB1*, *CSTD*, *DSCR9*, *SGK3*, and *KCNK5*), and two ERα-independent housekeeping gene loci (*APE1* and *ACTB*) as controls of estrogen nonresponsive genes. As expected, E2 treatment significantly increased specific promoter–enhancer interactions in all estrogen-responsive gene loci but had no significant effect on the housekeeping genes (Fig. 6*D*). SET KD cells showed significantly fewer E2-dependent long-range interactions in estrogen-responsive genes, but not in control estrogen unresponsive loci. This trend was maintained independently of the window size used for the analysis (Fig. 6*E* and *SI Appendix*, Fig. S7*D*). These data suggest that SET promotes enhancer–promoter looping in response to estrogen specifically at estrogen-responsive loci. To gain further insight on the roles of SET and other factors in spatial chromatin organization, we compared the 3D organization in control and SET KD cells of these gene loci where SET and ERα colocalize (Fig. 6*D*). At the *SGK3* locus, SET/ERα colocalize only at the promoter region; at the *TFF1*, *GREB1* and *CTSD* loci, both promoters and enhancers have strong SET/ERα binding; and at the *KCNK5* and *DSCR9* region, SET/ERα only localize at enhancers. In addition to ERα-mediated promoter–enhancer interactions, we also observed significantly increased long-range interactions between ERα-SET bound sites at the *SGK3*, *DSCR9* and *KCNK5* loci and other H2A.Z bound sites that do not bind ERα or SET, likely indicating looping of SET containing complexes with other chromatin bound structures (marked as white dotted circles in Fig. 6*D*). Similar long-range interactions are also present in publicly available ERα Chromatin Interaction Analysis by Paired-End Tag Sequencing (ChIA-PET) data. Taken together, our data indicate that SET plays an important role in ERα-mediated changes in chromatin architecture.

Discussion

Here we show that SET is necessary for estrogen-dependent transcription. In response to E2, SET is recruited to a subset of E2-regulated enhancers and promoters, where it promotes ERα binding, aids in depositing the histone variant H2A.Z, recruits histone writers and condensin complexes and promotes enhancer–promoter looping, resulting in an active chromatin state for transcription (*SI Appendix*, Fig. S8). SET KD showed that SET is required for E2-dependent transcriptional upregulation and eRNA transcription of estrogen-responsive genes.

In eukaryotic cells, nucleosomes encode epigenetic information that governs multiple physiological processes including DNA replication, DNA repair, and gene transcription. With the aid of histone chaperones, nucleosome assembly and disassembly modulate gene transcription by controlling chromatin accessibility. As a histone chaperone, the oncoprotein SET regulates gene transcription. However, the precise regulatory mechanisms by which SET modulates transcription have been unclear, even controversial. To study the functions of SET in transcriptional regulation in a mechanistic and unbiased way, our study defined, for the first time to our knowledge, the genome-wide distribution of SET in ERα$^+$ breast cancer cells, revealing that SET is recruited to ERα-bound cis-regulatory elements in an estrogen-dependent manner. SET was needed to establish an active chromatin structure at EREs in response to estrogen. The order in which chromatin changes occur is uncertain. Because SET is an H2A-H2B chaperone and we found that it substitutes

H2A.Z for H2A, this substitution, which opens up chromatin, could be the event that initiates SET-dependent reorganization of chromatin at EREs. Here we identified a 16 aa stretch in the acidic C-terminal domain of SET as important for SET binding to H2A, H2A.Z, and H2B and its recruitment to EREs. This was surprising since a previous study suggested that the acidic C-terminal tail of SET was not required for its role in nucleosome assembly (4, 47). The functional impairment of the SET M1 deletion mutant was not simply a result of its charge, since deletion of a similarly acidic domain distal to M1 (M2) had no effect on SET histone binding. It is worth noting that our experimental analysis focused on genes that are transcriptionally activated by estrogen, but do not rule out a role for SET in establishing a repressive chromatin structure at genes whose expression is repressed by estrogen, as has been suggested by prior studies (48), since we did not study genes whose transcription was repressed by estrogen.

SET has also been reported to bind to other steroid hormone and glucocorticoid receptors and regulate the expression of glucocorticoid receptor target genes (11). Further work is needed to determine whether SET also activates transcription involving nonsteroid transcription factors or whether its recruitment to steroid hormone receptors mediates its specificity. It also remains unclear how SET is recruited to its functionally diverse target sequences. According to the crystallographic structure of SET lacking its C-terminal domain, the bottom surface of its "earmuff" domain can bind both core histones and double stranded DNA without sequence preference (4). Our bioinformatic analyses showed coimmunoprecipitation and colocalization of SET and ERα in response to estrogen. This suggests that recruitment of SET to its target regions relies, at least partially, on ERα, consistent with a SET and ERα physical interaction (13). The binding dynamics of ERα and SET to the promoter and enhancer regions of *TFF1* indicate that association of SET with ERα is temporally and spatially regulated. Moreover, SET lacking its histone interacting domain maintained its ability to bind ERα but did not bind to EREs (*SI Appendix*, Fig. S5*B* and Fig. 5*D*), suggesting that ERα is necessary but not sufficient for SET recruitment to EREs and that H2A.Z binding may also play a role in recruiting SET. However, there are still a lot of missing pieces needed to build a detailed model. Unlike a previous report that suggested that SET interacts with unoccupied, but not estrogen-occupied, ERα in MCF7 (13), our data support E2-induced spatiotemporal association of SET with ERα, but do not indicate whether ERα and SET interact directly or indirectly via other factors. We also do not know whether they dynamically coassociate before they bind to chromatin and then bind coordinately or whether one or the other binds first. SET has also been shown to be recruited to DSBs to moderate the DNA damage response (49). DSBs are indeed detected at EREs during the estrogen response, although the origin of DSBs is not precisely defined (17, 50). ERα binding to EREs or transcription is required for E2-induced DSB generation. However, the fact that SET specifically regulates transcriptional activation of some estrogen-responsive genes and E2-induced SET-ERα colocalized sites only occupy 1% of ERα-binding sites in the genome makes it unlikely that SET recruitment to EREs is mainly mediated by DNA damage. Further experimental work is needed to define the key factor(s) that contribute to SET recruitment to EREs.

SET regulates gene expression in multiple ways. Besides facilitating the establishment of fully active chromatin structures, as we show here, SET is also able to remove histone H1 which promotes chromatin accessibility (51). SET is a potent natural inhibitor of NM23-H1 (an endonuclease that has been suggested to cleave structured regions of DNA in regulatory regions to allow duplex DNA to form for transcription factor binding) and PP2A, which negatively regulates ERα-mediated transcription (8, 9). It is likely that SET inhibition of NM23-H1 and PP2A also promotes the ERα-mediated response, although we couldn't rescue the transcriptional effects of SET KD by chemically inhibiting PP2A. Additionally, ERα antagonizes the pro-apoptotic function of p53 by modulating a subset of p53-regulated genes in ERα$^+$ breast cancer (52). SET has been shown to inhibit p53 acetylation and repress p53-mediated gene transcription, which also impairs p53-dependent apoptosis and enhances estrogen-dependent cell growth and survival.

SET is frequently overexpressed in acute myeloid leukemia, head and neck squamous cell carcinoma, Wilms' tumor, colorectal cancer, and human breast cancer and overexpression is associated with tumor progression and poor prognosis (6). As an inhibitor of PP2A, a tumor suppressor phosphatase that negatively regulates multiple pro-growth/pro-survival oncogenic kinase signaling pathways associated with cancer progression, SET has been considered as a potential target in cancer therapy (6, 21). Our study gives new insight into how SET promotes estrogen-induced transcriptional activation in breast cancer and provides additional molecular evidence to support investigating antagonizing SET as a therapeutic strategy in ERα$^+$ breast cancer.

Materials and Methods

Cell Culture. MCF7 and T47D cells were purchased from American Type Culture Collection (ATCC) and maintained in Dulbecco's Modified Eagle Medium (DMEM) high glucose medium (Life Technologies) supplemented with 10% fetal bovine serum (FBS) (Homeland), and penicillin/streptomycin. For experiments related to estrogen stimulus, cells were washed with phosphate-buffered saline (PBS) and kept in phenol-red free DMEM for 2 h and then cultured in phenol-red free DMEM supplemented with 5% charcoal/dextran stripped FBS (Hyclone) for at least 72 h. Lentiviral-infected cells were selected using 2 mM puromycin for 2 wk.

Methods and any associated references are available in the *SI Appendix*.

Data, Materials, and Software Availability

Anonymized (RNA-seq, ChIP-seq, capture-C) data have been deposited in GEO (GSE200900; GSE218815) (24, 27).

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1. R. J. Burgess, Z. Zhang, Histone chaperones in nucleosome assembly and human disease. *Nat. Struct. Mol. Biol.* **20**, 14–22 (2013).
2. H. J. Kim, J. H. Seol, J. W. Han, H. D. Youn, E. J. Cho, Histone chaperones regulate histone exchange during transcription. *EMBO J.* **26**, 4467–4474 (2007).
3. S. Venkatesh, J. L. Workman, Histone exchange, chromatin structure and the regulation of transcription. *Nat. Rev. Mol. Cell Biol.* **16**, 178–189 (2015).
4. S. Muto *et al.*, Relationship between the structure of SET/TAF-Ibeta/INHAT and its histone chaperone activity. *Proc. Natl. Acad. Sci. U.S.A.* **104**, 4285–4290 (2007).
5. Y. Adachi, G. N. Pavlakis, T. D. Copeland, Identification and characterization of SET, a nuclear phosphoprotein encoded by the translocation break point in acute undifferentiated leukemia. *J. Biol. Chem.* **269**, 2258–2262 (1994).
6. C. H. Switzer *et al.*, Targeting SET/I(2)PP2A oncoprotein functions as a multi-pathway strategy for cancer therapy. *Oncogene* **30**, 2504–2513 (2011).
7. S. W. Jiang, S. Xu, H. J. Chen, P. Duan. Liu, Oncogenic role of SET/I2PP2A for gynecologic cancers. *Curr. Drug. Targets.* **18**, 1152–1157 (2017).
8. M. Li, A. Makkinje, Z. Damuni, The myeloid leukemia-associated protein SET is a potent inhibitor of protein phosphatase 2A. *J. Biol. Chem.* **271**, 11059–11062 (1996).
9. Z. Fan, P. J. Beresford, D. Y. Oh, D. Zhang, J. Lieberman, Tumor suppressor NM23-H1 is a granzyme A-activated DNase during CTL-mediated apoptosis, and the nucleosome assembly protein SET is its inhibitor. *Cell* **112**, 659–672 (2003).
10. M. J. Gamble, H. Erdjument-Bromage, P. Tempst, L. P. Freedman, R. P. Fisher, The histone chaperone TAF-I/SET/INHAT is required for transcription in vitro of chromatin templates. *Mol. Cell Biol.* **25**, 797–807 (2005).
11. T. Ichijo, G. P. Chrousos, T. Kino, Activated glucocorticoid receptor interacts with the INHAT component Set/TAF-Ibeta and releases it from a glucocorticoid-responsive gene promoter, relieving repression: Implications for the pathogenesis of glucocorticoid resistance in acute undifferentiated leukemia with Set-Can translocation. *Mol. Cell Endocrinol.* **283**, 19–31 (2008).
12. S. B. Seo *et al.*, Regulation of histone acetylation and transcription by INHAT, a human cellular complex containing the set oncoprotein. *Cell* **104**, 119–130 (2001).
13. M. A. Loven, N. Muster, J. R. Yates, A. M. Nardulli, A novel estrogen receptor alpha-associated protein, template-activating factor Ibeta, inhibits acetylation and transactivation. *Mol. Endocrinol.* **17**, 67–78 (2003).
14. J. Y. Kim *et al.*, Inhibition of p53 acetylation by INHAT subunit SET/TAF-Ibeta represses p53 activity. *Nucleic Acids Res.* **40**, 75–87 (2012).
15. D. Wang *et al.*, Acetylation-regulated interaction between p53 and SET reveals a widespread regulatory mode. *Nature* **538**, 118–122 (2016).
16. W. Li *et al.*, Functional roles of enhancer RNAs for oestrogen-dependent transcriptional activation. *Nature* **498**, 516–520 (2013).
17. B. Perillo *et al.*, DNA oxidation as triggered by H3K9me2 demethylation drives estrogen-induced gene expression. *Science* **319**, 202–206 (2008).
18. P. Tropberger *et al.*, Regulation of transcription through acetylation of H3K122 on the lateral surface of the histone octamer. *Cell* **152**, 859–872 (2013).
19. J. S. Carroll, Mechanisms of oestrogen receptor (ER) gene regulation in breast cancer. *Eur. J. Endocrinol.* **175**, R41–49 (2016).
20. J. R. Schultz-Norton, Y. S. Ziegler, A. M. Nardulli, ERalpha-associated protein networks. *Trends Endocrinol. Metab.* **22**, 124–129 (2011).
21. M. Janghorban *et al.*, Targeting c-MYC by antagonizing PP2A inhibitors in breast cancer. *Proc. Natl. Acad. Sci. U.S.A.* **111**, 9157–9162 (2014).
22. B. Győrffy, Survival analysis across the entire transcriptome identifies biomarkers with the highest prognostic power in breast cancer. *Comput. Struct. Biotechnol. J.* **19**, 4101–4109 (2021).
23. S. A. Krum *et al.*, Unique ERalpha cistromes control cell type-specific gene regulation. *Mol. Endocrinol.* **22**, 2393–2406 (2008).
24. D. Valle-Garcia, C. Guo, K. F. Meza-Sosa, J. Lieberman. The SET Oncoprotein Promotes Estrogen-Induced Transcription by Facilitating Establishment of Active Chromatin. NCBI *Gene Expression Omnibus*. https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE200900. Deposited 15 April 2022.
25. J. D. Stender *et al.*, Genome-wide analysis of estrogen receptor alpha DNA binding and tethering mechanisms identifies Runx1 as a novel tethering factor in receptor-mediated transcriptional activation. *Mol. Cell Biol.* **30**, 3943–3955 (2010).
26. H. S. Kaya-Okur *et al.*, CUT&Tag for efficient epigenomic profiling of small samples and single cells. *Nat. Commun.* **10**, 1930 (2019).
27. C. Guo *et al.*, The SET Oncoprotein Promotes Estrogen-Induced Transcription by Facilitating Establishment of Active Chromatin. NCBI *Gene Expression Omnibus*. https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE218815. Deposited 26 November 2022.
28. A. C. Tecalco-Cruz, I. A. Perez-Alvarado, J. O. Ramirez-Jarquin, L. Rocha-Zavaleta, Nucleo-cytoplasmic transport of estrogen receptor alpha in breast cancer cells. *Cell Signal.* **34**, 121–132 (2017).
29. M. Al-Bader, C. Ford, B. Al-Ayadhy, I. Francis, Analysis of estrogen receptor isoforms and variants in breast cancer cell lines. *Exp. Ther. Med.* **2**, 537–544 (2011).
30. P. Seshacharyulu, P. Pandey, K. Datta, S. K. Batra, Phosphatase: PP2A structural importance, regulation and its aberrant expression in cancer. *Cancer Lett.* **335**, 9–18 (2013).
31. J. C. Keen *et al.*, Protein phosphatase 2A regulates estrogen receptor alpha (ER) expression through modulation of ER mRNA stability. *J. Biol. Chem.* **280**, 29519–29524 (2005).
32. M. A. Wade *et al.*, The histone demethylase enzyme KDM3A is a key estrogen receptor regulator in breast cancer. *Nucleic Acids Res.* **43**, 196–207 (2015).
33. K. W. Jeong *et al.*, Recognition of enhancer element-specific histone methylation by TIP60 in transcriptional activation. *Nat. Struct. Mol. Biol.* **18**, 1358–1365 (2011).
34. W. W. Gao *et al.*, JMJD6 licenses ERalpha-dependent enhancer and coding gene activation by modulating the recruitment of the CARM1/MED12 co-activator complex. *Mol. Cell* **70**, 340–357. e348 (2018).
35. Y. Zhang, D. Reinberg, Transcription regulation by histone methylation: Interplay between different covalent modifications of the core histone tails. *Genes Dev.* **15**, 2343–2360 (2001).
36. T. Shimoyama, K. Kato, M. Miyaji-Yamaguchi, K. Nagata, Synergistic action of MLL, a TRX protein with template activating factor-I, a histone chaperone. *FEBS Lett.* **579**, 757–762 (2005).
37. L. Shi *et al.*, Histone demethylase JMJD2B coordinates H3K4/H3K9 methylation and promotes hormonally responsive breast carcinogenesis. *Proc. Natl. Acad. Sci. U.S.A.* **108**, 7541–7546 (2011).
38. S. Zhang *et al.*, Dynamics of estrogen-induced ROS and DNA strand break generation in estrogen receptor alpha-positive breast cancer. *Biochem. Biophys. Res. Commun.* **602**, 170–178 (2022).
39. D. Jones, L. Wilson, H. Thomas, L. Gaughan, M. A. Wade, The Histone demethylase enzymes KDM3A and KDM4B co-operatively regulate chromatin transactions of the estrogen receptor in breast cancer. *Cancers (Basel)* **11**, 1122 (2019).
40. S. S. Gadad, J. Shandilya, V. Swaminathan, T. K. Kundu, Histone chaperone as coactivator of chromatin transcription: Role of acetylation. *Methods Mol. Biol.* **523**, 263–278(2009).
41. N. Gevry *et al.*, Histone H2A.Z is essential for estrogen receptor signaling. *Genes Dev.* **23**, 1522–1533 (2009).
42. M. Brunelle *et al.*, The histone variant H2A.Z is an important regulator of enhancer activity. *Nucleic Acids Res.* **43**, 9742–9756 (2015).
43. M. Periyasamy *et al.*, APOBEC3B-mediated cytidine deamination is required for estrogen receptor action in breast cancer. *Cell Rep.* **13**, 108–121 (2015).
44. A. Obri *et al.*, ANP32E is a histone chaperone that removes H2A.Z from chromatin. *Nature* **505**, 648–653 (2014).
45. K. Yen, V. Vinayachandran, B. F. Pugh, SWR-C and INO80 chromatin remodelers recognize nucleosome-free regions near +1 nucleosomes. *Cell* **154**, 1246–1256 (2013).
46. W. Li *et al.*, Condensin I and II complexes license full estrogen receptor alpha-dependent enhancer activation. *Mol. Cell* **59**, 188–202 (2015).
47. Z. Karetsou *et al.*, Identification of distinct SET/TAF-Ibeta domains required for core histone binding and quantitative characterisation of the interaction. *BMC Biochem.* **10**, 10 (2009).
48. K. Linher-Melville, S. Zantinge, G. Singh, Liver kinase B1 expression (LKB1) is repressed by estrogen receptor alpha (ERalpha) in MCF-7 human breast cancer cells. *Biochem. Biophys. Res. Commun.* **417**, 1063–1068 (2012).
49. A. Kalousi *et al.*, The nuclear oncogene SET controls DNA repair by KAP1 and HP1 retention to chromatin. *Cell Rep.* **11**, 149–163 (2015).
50. B. G. Ju *et al.*, A topoisomerase IIbeta-mediated dsDNA break required for regulated transcription. *Science* **312**, 1798–1802 (2006).
51. S. Kadota, K. Nagata, Silencing of IFN-stimulated gene transcription is regulated by histone H1 and its chaperone TAF-I. *Nucleic Acids Res.* **42**, 7642–7653 (2014).
52. S. T. Bailey, H. Shin, T. Westerling, X. S. Liu, M. Brown, Estrogen receptor prevents p53-dependent apoptosis in breast cancer. *Proc. Natl. Acad. Sci. U.S.A.* **109**, 18060–18065 (2012).