An acetylation-enhanced interaction between transcription factor Sox2 and the steroid receptor coactivators facilitates Sox2 transcriptional activity and function

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SRY-box 2 (Sox2) is a transcription factor with critical roles in maintaining embryonic stem (ES) cell and adult stem cell functions and in tumorigenesis. However, how Sox2 exerts its transcriptional function remains unclear. Here, we used an *in vitro* protein–protein interaction assay to discover transcriptional regulators for ES cell core transcription factors (Oct4, Sox2, Klf4, and c-Myc) and identified members of the steroid receptor coactivators (SRCs) as Sox2-specific interacting proteins. The SRC family coactivators have broad roles in transcriptional regulation, but it is unknown whether they also serve as Sox2 coactivators. We demonstrated that these proteins facilitate Sox2 transcriptional activity and act synergistically with p300. Furthermore, we uncovered an acetylation-enhanced interaction between Sox2 and SRC-2/3, but not SRC-1, demonstrating it is Sox2 acetylation that promotes the interaction. We identified putative Sox2 acetylation sites required for acetylation-enhanced interaction between Sox2 and SRC-3 and demonstrated that acetylation on these sites contributes to Sox2 transcriptional activity and recruitment of SRC-3. We showed that activation domains 1 and 2 of SRC-3 both display a preferential binding to acetylated Sox2. Finally, functional analyses in mouse ES cells demonstrated that knockdown of SRC-2/3 but not SRC-1 in mouse ES cells significantly downregulates the transcriptional activities of various Sox2 target genes and impairs ES cell stemness. Taken together, we identify specific SRC family proteins as novel Sox2 coactivators and uncover the role of Sox2 acetylation in promoting coactivator recruitment and Sox2 transcriptional function.

SRY-box 2 (Sox2) is a family member of SRY-related HMG box transcription factors (1). It is best known for its critical role in maintenance of embryonic stem (ES) cell pluripotency and in induction of pluripotent stem cells (1–3). As a transcription factor, Sox2 has been shown to control gene expression of ES core regulatory circuitry cooperatively with Oct4 and Nanog (4–6). A limited number of regulatory proteins have been shown to either enhance or repress Sox2 transcriptional activity. For example, CBP/p300 have been reported to potentiate Sox2 transcriptional activation (7–9), whereas CARM1 can methylate Sox2 and facilitate Sox2-mediated transcriptional activation (10). Using *in vitro*–based transcription assays, the DNA repair XPC complex and the DKC1 complex were identified as coactivators of OCT4 and Sox2 and shown to regulate the expression of key pluripotency genes critical for self-renewal in ES cells (11–13). However, given the critical role of Sox2 in the ES transcriptional circuit and the complexity of transcriptional regulation, additional key coactivators for Sox2 are yet to be identified.

Steroid receptor coactivators (SRCs) were initially identified and characterized as primary coactivators for nuclear hormone receptors (NRs) (14–16). This family of coactivators is therefore also named nuclear receptor coactivators (NCOAs) and includes three structurally and functionally related proteins: SRC-1 (also known as NCOA1) (14), SRC-2 (also known as NCOA2, GRIP1, and TIF2) (17, 18), and SRC-3 (also known as NCOA3, AIB1, p/CIP, ACTR, RAC3, and TRAM-1) (19–23). They function as primary NR-interacting proteins to recruit additional transcription coregulators such as CBP/p300, CARM1, and others (15, 16, 24). SRCs have also been shown to enhance transcriptional activation by numerous non-NR transcription factors including NF-κB, Smad3, and p53 (25–28). In addition, SRC-3 could function as a coactivator for the orphan nuclear receptor Esrrb in ES cells and is critical for sustaining ES cell self-renewal (29, 30). However, it is unknown whether the SRC family coactivators also play a role in transcriptional regulation by ES core transcription factors such as Sox2.

Oct4, Sox2, c-Myc, and Klf4 are ES core transcription factors that are best known as Yamanaka factors for their ability to cooperatively induce the generation of induced pluripotent stem cells (2, 6, 31, 32). To better understand their functions in transcriptional regulation, we previously established *in vitro* pulldown assays using GST fusions of Oct4, Sox2, c-Myc, and Klf4 to screen for their specific interacting transcriptional coregulators (33). Using this assay, we previously reported that

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Oct4 interacts directly with H3K4 methyltransferase SET1A. Here, by using the same approach, we identified the SRC family coactivators as Sox2-interacting proteins and demonstrated that they function as Sox2 coactivators. Interestingly, we found that acetylation of Sox2 by p300 enhances Sox2 interaction with SRC-2 and SRC-3. Functional analysis demonstrated that Sox2 acetylation correlates with Sox2 transcriptional activity and the recruitment of SRC-3. Finally, we showed that both SRC-2 and SRC-3 are required for optimal Sox2 target gene expression and maintenance of ES cell stemness. Thus, we conclude that the SRC family proteins are novel Sox2 coactivators and contribute to maintenance of ES cell pluripotency at least in part by acting as Sox2 coactivators.

Results

Identification of the SRC family coactivators as Sox2-interacting proteins

To identify potential transcriptional coregulators for ES core transcription factors, we characterized the interaction of various transcriptional coregulators with GST fusion of mouse c-Myc, Klf4, Oct4, and Sox2 proteins by *in vitro* pulldown assay as described (33). The coregulators were synthesized and labeled with S^{35}-methionine *via* an *in vitro* transcription/translation-coupled system. Coregulators bound to GST-fusion proteins were separated by SDS-PAGE and visualized by autoradiography. With this approach, we observed preferential binding of SRC-1, SRC-2, and SRC-3 to GST-Sox2 (Fig. 1A). On the other hand, p300 was found to bind better to GST-Klf4, whereas BRG1 bound to GST-Myc, GST-Klf4, and GST-Oct4 but not GST-Sox2. These results reveal differential interaction of coregulators with ES core transcription factors. Given that all three members of the SRC family coactivators exhibit selective binding of Sox2, we next focused our effort to investigate the role of these proteins in Sox2 transcriptional regulation.

To validate the interaction between Sox2 and the SRC family proteins, we performed co-immunoprecipitation (co-IP) assay. As shown by Western blot (WB) results in Figure 1B, FLAG-tagged Sox2 coimmunoprecipitated with Myc-tagged SRC family proteins. Furthermore, using cellular extracts derived from mouse E14 ES cells, we observed that Sox2 coimmunoprecipitated with endogenous SRC-1, SRC-2, and SRC-3 proteins (Fig. 2C). As a positive control, we also observed that Sox2 coimmunoprecipitated with endogenous p300 (Fig. 2C). These co-IP assays substantiate that Sox2 interacts with the SRC family coactivators, and this interaction is at least comparable with the interaction between Sox2 and p300.

To test the physiological relevance of the interaction between Sox2 and the SRC family coactivators, we examined by the chromatin immunoprecipitation (ChIP) assay whether the SRC family coactivators associate with Sox2-regulated genes in mouse E14 ES cells. We first validated by ChIP assay that Sox2 was indeed enriched at the enhancer or promoter regions of well-known Sox2 target genes Sox2, Nanog, Fgf4, Esrrb, and

Dax1 (34) (Fig. 1D). We then carried out the ChIP assay to examine whether SRC-1, SRC-2, SRC-3, and p300 were also enriched at these regions. The results in Figure 1E showed that p300 was variably enriched at these regions. Interestingly, we observed robust enrichment of SRC-2 and SRC-3 at all five genes tested, whereas the association of SRC-1 was barely detected (Fig. 1E). While the failure for SRC-1 detection might be due to the antibody used, these results are consistent with the observed interaction between Sox2 and SRC-2/3 and imply a role of SRC-2/3 in Sox2-regulated gene expression.

SRC family coactivators enhance transcriptional activation by Sox2

We next tested whether the SRC family coactivators were able to promote Sox2 transcriptional activity. To this end, we used transient transfection and luciferase reporter assay by using a well-characterized FGF4-luc reporter, which is driven by a Sox2-responsive FGF4 enhancer (35). The representative results showed that ectopically expressed SRC-1 enhanced Sox2 transcriptional activity in a dose-dependent manner (Fig. 2A). Similarly, we found that both SRC-2 and SRC-3 also enhanced Sox2 transcriptional activation in a similar fashion (Fig. 2, B and C). As p300 is known to function as a Sox2 coactivator (7, 8) and potentiates transcriptional activation by SRC family coactivators (20), we analyzed whether p300 and SRC proteins can synergistically promote Sox2 activation. As shown in Figure 2, D–F, p300 alone is a more potent coactivator than SRC-1, SRC-2, and SRC-3 in stimulating transcriptional activation by Sox2. However, coexpression of p300 with SRC-1, SRC-2, or SRC-3 had a synergistic effect on stimulating Sox2 transcriptional activity. Together these results functionally support the classification of SRC family proteins as Sox2 transcriptional coactivators and suggest that the SRC family coactivators may work together with CBP/p300 to facilitate Sox2 transcriptional activation.

p300-catalyzed Sox2 acetylation enhances the interaction between Sox2 and SRC-2/3

The synergistic effect observed in the luciferase reporter assay as a result of p300 and SRC-3 coexpression suggests a potential role of p300 in promoting Sox2 and SRC-3 interaction. Given that p300 is a lysine acetyltransferase reported to acetylate Sox2 (36), we hypothesized that during transcription, p300 may acetylate Sox2 that in turn may promote the interaction and recruitment of SRC family coactivators. To test this hypothesis, we first confirmed that ectopic coexpression of p300 indeed elevated the levels of Sox2 acetylation (Fig. 3A). Next, we tested whether coexpression of p300 could promote the interaction between Sox2 and SRC-3. As shown in Figure 3B, IP followed by WB analysis demonstrated that coexpression of p300 indeed strongly enhanced the interaction between Sox2 and SRC-3.

As p300 is also known to interact with Sox2 as well as SRC-3 (7, 9, 16), a caveat in the experiments shown in Figure 3B is that the observed enhanced interaction between Sox2 and SRC-3 might be attributed to the formation of a Sox2/SRC-3/
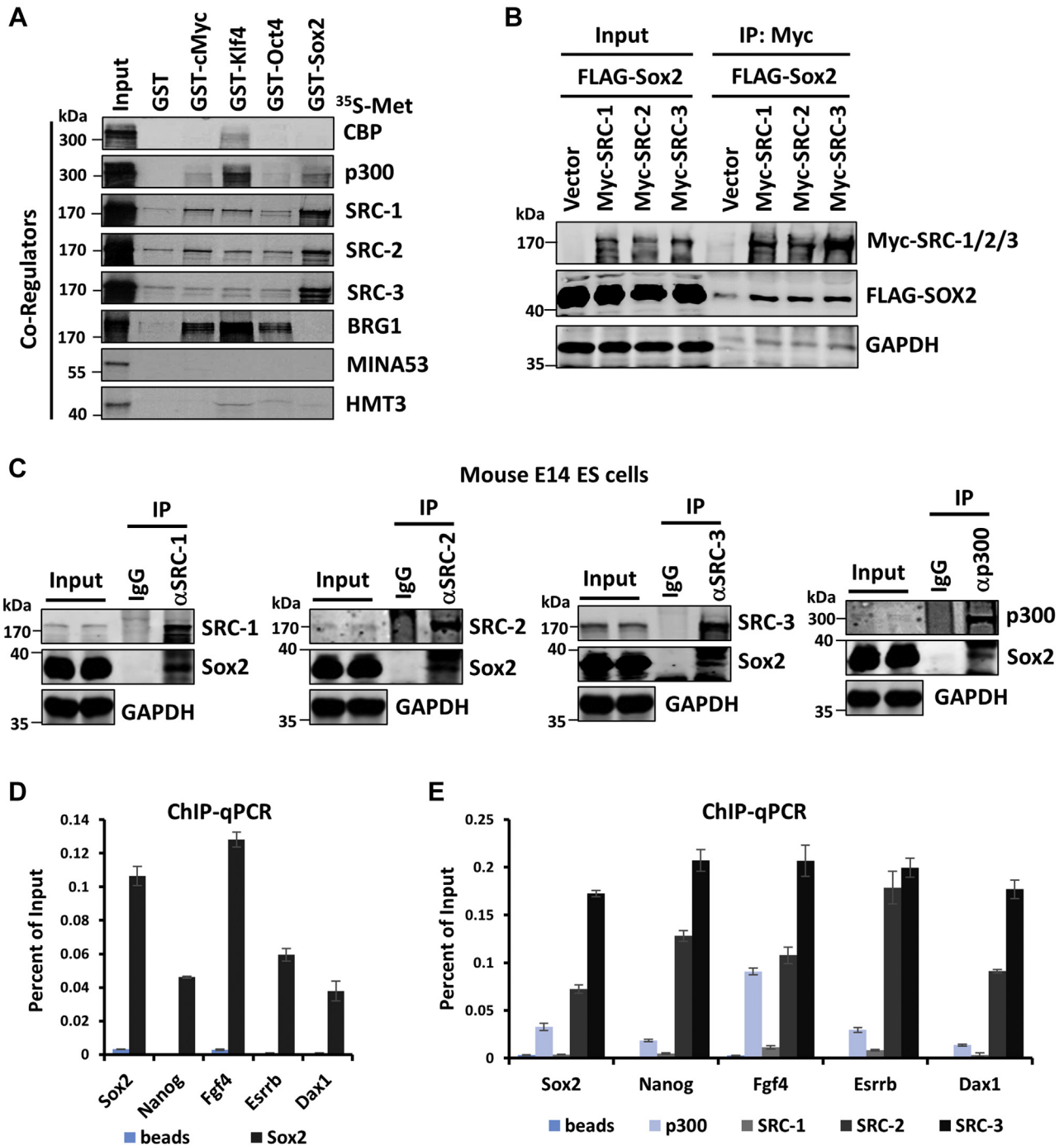
Figure 1. Identification of the SRC family coactivators as Sox2-interacting proteins. *A*, GST-tagged c-Myc, Klf4, Oct4, and Sox2 were used to pulldown ^{35}S-methionine-labeled coregulators synthesized *via in vitro*–coupled transcription and translation reactions. The bound coregulators were subsequently visualized by autoradiography. *B*, co-IP assay confirming the interaction between Sox2 and SRC family coactivators. The plasmids encoding FLAG-Sox2 and Myc-SRCs were cotransfected into HEK293T cells as indicated, and the interaction between Sox2 and each SRC proteins was determined by immuno-precipitation of Myc-SRCs followed by WB analysis using the anti-FLAG antibody. *C*, co-IP assays using mouse E14 ES cell extracts were performed to detect the interaction of endogenous Sox2 with endogenous SRC-1, SRC-2, SRC-3, and p300. *D* and *E*, ChIP assays were performed by analyzing the association of Sox2 (*D*) and p300, SRC-1, SRC-2, and SRC-3 (*E*) with five Sox2 target genes. The ChIP data were relative to the percentage of input. ChIP, chromatin immunoprecipitation; co-IP, co-immunoprecipitation; ES, embryonic stem; Sox2, SRY-box 2; SRCs, steroid receptor coactivators.

p300 ternary complex, not necessarily due to elevated binding of acetylated Sox2 by SRC-3. To distinguish these two possibilities, we coexpressed FLAG-SRC-3 and Myc-Sox2 in HEK293T cells and elevated Sox2 acetylation by treating cells with both histone de-acetylase inhibitor trichostatin A (TSA)

and Sirtuin inhibitor nicotinamide (NAM) without coexpression of p300 (37). As a control, we also treated cells with sodium crotonate (NaCro), which was expected to increase protein crotonylation rather than acetylation (38, 39). TSA plus NAM treatment was expected to elevate acetylation of
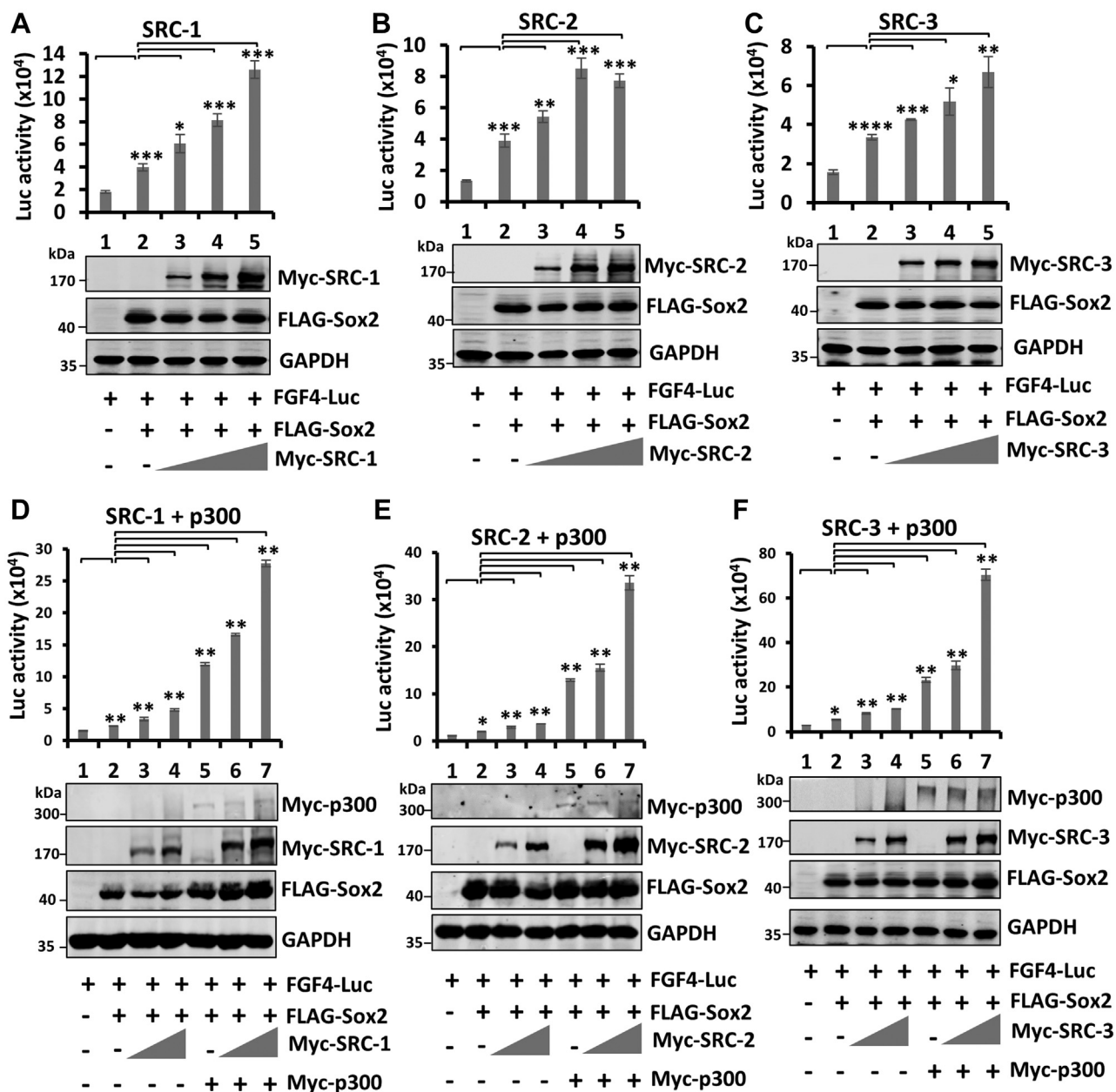
Figure 2. SRC family coactivators enhance Sox2 transcriptional activity. *A–C*, luciferase reporter assay showing that the SRC family coactivators SRC-1 (*A*), SRC-2 (*B*), and SRC-3 (*C*) enhanced transcriptional activation by Sox2 in a dose-dependent manner. Also shown are WB results of ectopically expressed FLAG-Sox2 with Myc-SRC-1, Myc-SRC-2, or Myc-SRC-3, respectively. GAPDH serves as a loading control. FGF4-Luc reporter, 50 ng; FLAG-Sox2, 200 ng; Myc-SRC-1/2/3, 200 ng, 500 ng, and 1000 ng for an increasing dose of SRC expression plasmids. *D–F*, luciferase reporter assay showing that SRC-1 (*D*), SRC-2 (*E*), and SRC-3 (F) promoted Sox2 transcriptional activity synergistically with p300. Also shown are WB results of ectopically expressed FLAG-Sox2, Myc-p300, and Myc-SRC-1, Myc-SRC-2, or Myc-SRC-3, respectively. FGF4-Luc, 50 ng; FLAG-Sox2, 200 ng; Myc-SRC-1/2/3, 200 ng and 500 ng; Myc-p300, 1000 ng. Data are represented as relative luciferase activity ($\times 10^4$) (mean ± SD of three experimental repeats). All statistical analyses were performed using Excel 2016. **p* < 0.05; ***p* < 0.01; ****p* < 0.001; *****p* < 0.0001. Sox2, SRY-box 2; SRCs, steroid receptor coactivators.

ectopically expressed Sox2 by blocking deacetylation, and indeed, this was the case, as shown in Figure 3C. Subsequent IP-WB analysis revealed that TSA plus NAM treatment markedly increased the interaction between Myc-Sox2 and FLAG-SRC-3 and this effect was not observed with NaCro treatment (Fig. 3D). Note TSA plus NAM treatment did not alter the levels of Myc-Sox2 and FLAG-SRC-3, implying the increased interaction is not due to increased protein expression. We also tested if TSA plus NAM could promote the interaction between ectopically expressed FLAG-Sox2 and

endogenous SRC-3. Representative results in Figure 3E showed that TSA plus NAM treatment indeed robustly elevated the interaction between FLAG-Sox2 and endogenous SRC-3. These results suggest that acetylation promotes the interaction between Sox2 and SRC-3.

Although the above results support a role of acetylation in promoting the interaction between Sox2 and SRC-3, it is not known whether the elevated interaction is driven by Sox2 acetylation and/or SRC-3 acetylation. To address this question, we transfected HEK293T cells separately with FLAG-Sox2 or

Figure 3. Sox2 acetylation promotes the interaction between Sox2 and SRC-2/3. *A*, IP-WB analysis showing that ectopically expressed p300 elevated Sox2 acetylation. *B*, co-IP assay showing that coexpressed p300 enhanced the interaction between SRC-3 and Sox2. A nonspecific band that overlaps with Myc-Sox2 (*). *C*, IP-WB analysis showing that inhibition of both histone de-acetylase and Sirtuin by TSA and NAM elevated Sox2 acetylation. FLAG-Sox2 was transiently expressed in HEK239T cells, and cells were treated with DMSO or TSA (1 μM) plus NAM (5 mM) for 24 h before being harvested for IP-WB analysis. *D*, co-IP assay showing that TSA plus NAM treatment enhanced the interaction between Sox2 and SRC-3. FLAG-SRC-3 and Myc-Sox2 were coexpressed in HEK239T cells, and cells were treated with TSA (1 μM) plus NAM (5 mM) or sodium crotonate (NaCro, 20 mM) for 24 h before being harvested for co-IP assay using the antibody as indicated. *E*, co-IP assay showing that TSA plus NAM treatment markedly enhanced the interaction between ectopically expressed FLAG-Sox2 and endogenous SRC-3. *F*, the *right panel* showing the experimental scheme. FLAG-Sox2–transfected HEK239T cells were treated with DMSO or

Myc-SRC-3 and treated either FLAG-Sox2– or Myc-SRC-3–transfected cells with DMSO or TSA plus NAM. The whole-cell extracts were prepared independently and then mixed before co-IP was performed. Subsequent IP-WB assays showed that treatment of FLAG-Sox2–transfected cells, but not Myc-SRC-3–transfected cells, with TSA plus NAM resulted in an elevated interaction between FLAG-Sox2 and Myc-SRC-3 (Fig. 3, *F* and *G*). These results suggest that it is Sox2 acetylation that promotes the interaction between Sox2 and SRC-3. By the same approach, we found that Sox2 acetylation also promotes Sox2 interaction with SRC-2, but not SRC-1 (Fig. 3*H*).

To test whether acetylation enhances the interaction between endogenous Sox2 and SRC family proteins, we treated mouse E14 ES cells with TSA plus NAM for 12 h and subsequently carried out the co-IP assay. As shown in Figure 3*I*, although TSA plus NAM variably reduced the protein levels of Sox2, SRC-2, and SRC-3, more Sox2 proteins were found to coimmunoprecipitate with SRC-2 (4.08-fold) and SRC-3 (7.53-fold). IP with an antiacetylated lysine (panKac) antibody followed by WB analysis of Sox2 revealed that TSA plus NAM treatment resulted in a 3.12-fold increase of Sox2 acetylation. Together, these data support the idea that Sox2 acetylation promotes Sox2 interaction with SRC-2/3, but not SRC-1.

Acetylation at Sox2 K97, K105, and K111 enhances Sox2 interaction with SRC-3

Having established that acetylation on Sox2 enhances Sox2 interaction with two of three SRC family coactivators, we next attempted to define the Sox2 acetylation site(s) responsible for this acetylation-driven interaction. A previous study collectively identified 11 acetylation sites from Sox2 treated with recombinant p300 *in vitro*, and the acetylation sites are enriched at the N-terminal region of Sox2 as illustrated in Figure 4*A* upper panel (36). We thus used Sox2–SRC-3 interaction to determine the Sox2 acetylation site(s) required for the acetylation-driven interaction with SRC-3. We first divided Sox2 into three fragments as illustrated in Figure 4*A* lower panel and tested whether their interaction with SRC-3 is enhanced by TSA plus NAM treatment. Representative results in Figure 4*B* revealed that, similar to full-length Sox2, the Sox2 1 to 243aa fragment interacted with SRC-3 in an acetylation-enhanced manner. However, this acetylation-enhanced interaction was not observed for Sox2 112 to 243aa and 152 to 319aa fragments. Together, these results suggest that acetylation at the N-terminal 1 to 111aa region is likely to promote the interaction between Sox2 and SRC-3.

To more precisely define the acetylation site(s) involved in Sox2–SRC-3 interaction, we mutated each reported acetylation sites in the N-terminal region to arginine, which mimics the unacetylated state of lysine. Interestingly, we found that K to R mutation of K97, K105, or K111 abolished TSA plus NAM-stimulated interaction with SRC-3 (Fig. 4*C*). In contrast, mutation of K117 and K119 had no effect on acetylation-enhanced interaction of Sox2 with SRC-3 (Fig. 4*C*). Furthermore, the luciferase reporter assay revealed a reduced transcriptional activity for Sox2 K97R, K105R, and K111R mutants, whereas the transcriptional activity for K117R and K119R mutants was essentially equivalent to WT Sox2 (Fig. 4*D*). To test whether K97R, K105R, and K111R mutations impaired the recruitment of the SRC-3 coactivator by Sox2, the FGF4-luc reporter together with WT Sox2, K97R, K105R, or K111R mutant were transfected into HEK293T cells, and the binding of Sox2 and mutants and the recruitment of endogenous SRC-3 to the FGF4-luc reporter were evaluated by the ChIP assay. Representative results in Figure 4*E* show that K97R, K105R, and K111R mutations slightly reduced the binding of Sox2 to the FGF4 enhancer. However, recruitment of the SRC-3 coactivator was significantly reduced for all three mutants. Note that SRC-3 was barely detected at the FGF4-luc reporter in the absence of Sox2, indicating that the association of SRC-3 with the FGF4-luc reporter is dependent on its interaction with Sox2. We thus conclude that acetylation at K97, K105, and K111 of Sox2 promotes the interaction between Sox2 and SRC-3. As mutation in any of the three sites abolishes acetylation-enhanced interaction between Sox2 and SRC-3, we suggest this acetylation-enhanced interaction may require simultaneous acetylation of all three sites.

SRC-3 contains acetylated Sox2-binding domains

Bromodomain-containing proteins are well characterized for their ability to bind acetylated histones as well as nonhistone proteins (40, 41). However, the SRC family proteins do not contain a typical bromodomain. Nevertheless, the SRC family proteins are multistructural and functional domain proteins (24, 42) (Fig. 5*A*). To define which region(s) of SRC-3 is responsible for binding to acetylated Sox2, we first compared the receptor-interaction domain, activation domain 1 (AD1), and activation domain 2 (AD2) for their ability to bind Sox2 derived from control and TSA plus NAM–treated cells. As shown in Figure 5*B*, we found that the SRC-3–receptor-interaction domain did not interact with Sox2 in an acetylation-enhanced manner. However, both SRC-3–AD1 and SRC-3–AD2 exhibited an acetylation-enhanced
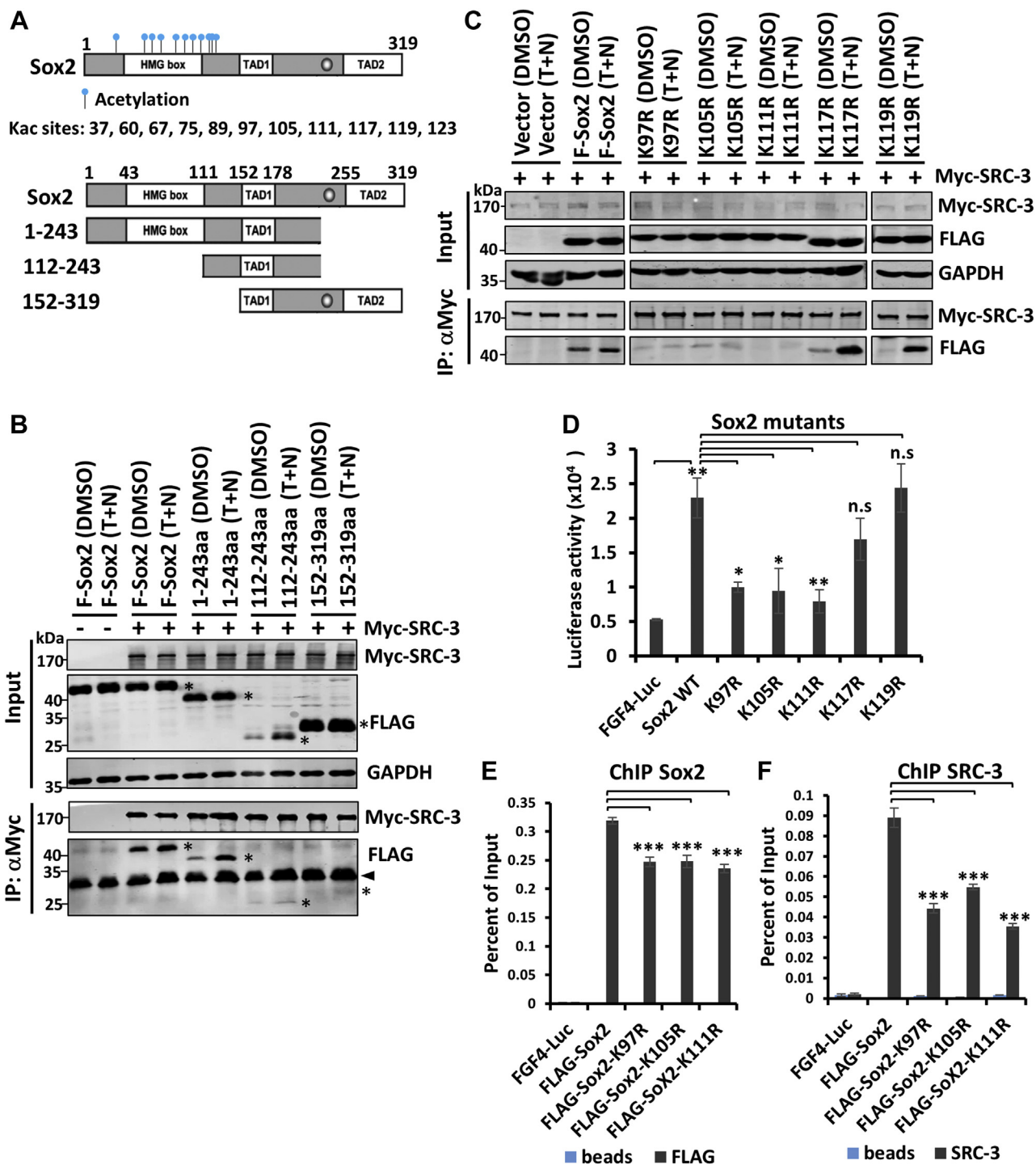
Figure 4. K97, K105, and K111 of Sox2 mediate acetylation-enhanced interaction with SRC-3. *A, top panel* is a schematic diagram showing all mouse Sox2 acetylation sites reported in reference (35). The *lower panel* illustrates the structural organization of full-length and truncated Sox2 mutants used in this study. *B,* co-IP assay mapping the Sox2 region(s) required for acetylation-enhanced interaction with SRC-3. HEK293T cells were transfected with FLAG-tagged Sox2 or truncated mutants and treated with DMSO or TSA (1 μM) plus NAM (5 mM) (T + N) for 24 h before being harvested for preparation of whole cell-extracts, and the resulting extracts were mixed with equal volume of extracts derived from Myc-SRC-3–expressed HEK239T cells for the co-IP assay using antibodies as indicated. Note that only Sox2 1 to 243aa, but not 112 to 243aa and 152 to 319aa, showed enhanced interaction with Myc-SRC-3 upon T + N treatment. Full-length and truncated Sox2 mutants bands (*) and light chain of the antibody (◄). *C,* co-IP assay determining the Sox2 lysine residues required for acetylation-enhanced interaction with SRC-3. The experiments were essentially as in (*B*) except a panel of Sox2 K to R acetylation site mutants was used. *D,* luciferase reporter assay showing that Sox2-K97R, Sox2-K105R, and Sox2-K111R mutants exhibited reduced transcriptional activities. Data are represented as relative luciferase activity ($\times 10^4$) (mean ± SD of three biological repeats). *E,* ChIP assay evaluating the binding of FLAG-Sox2, Sox2-K97R, Sox2-K105R, and Sox2-K111R mutants to the transfected FGF4-luciferase reporter. *F,* ChIP assay showing the recruitment of endogenous SRC-3 to the transfected FGF4-luciferase reporter by FLAG-Sox2, Sox2-K97R, Sox2-K105R, and Sox2-K111R mutants. All statistical analyses were performed using Excel 2016. *$p< 0.05$; **$p < 0.01$; ***$p < 0.001$. co-IP, co-immunoprecipitation; DMSO, dimethyl sulfoxide; NAM, nicotinamide; n.s., no statistical significance; Sox2, SRY-box 2; SRCs, steroid receptor coactivators; TSA, trichostatin A.

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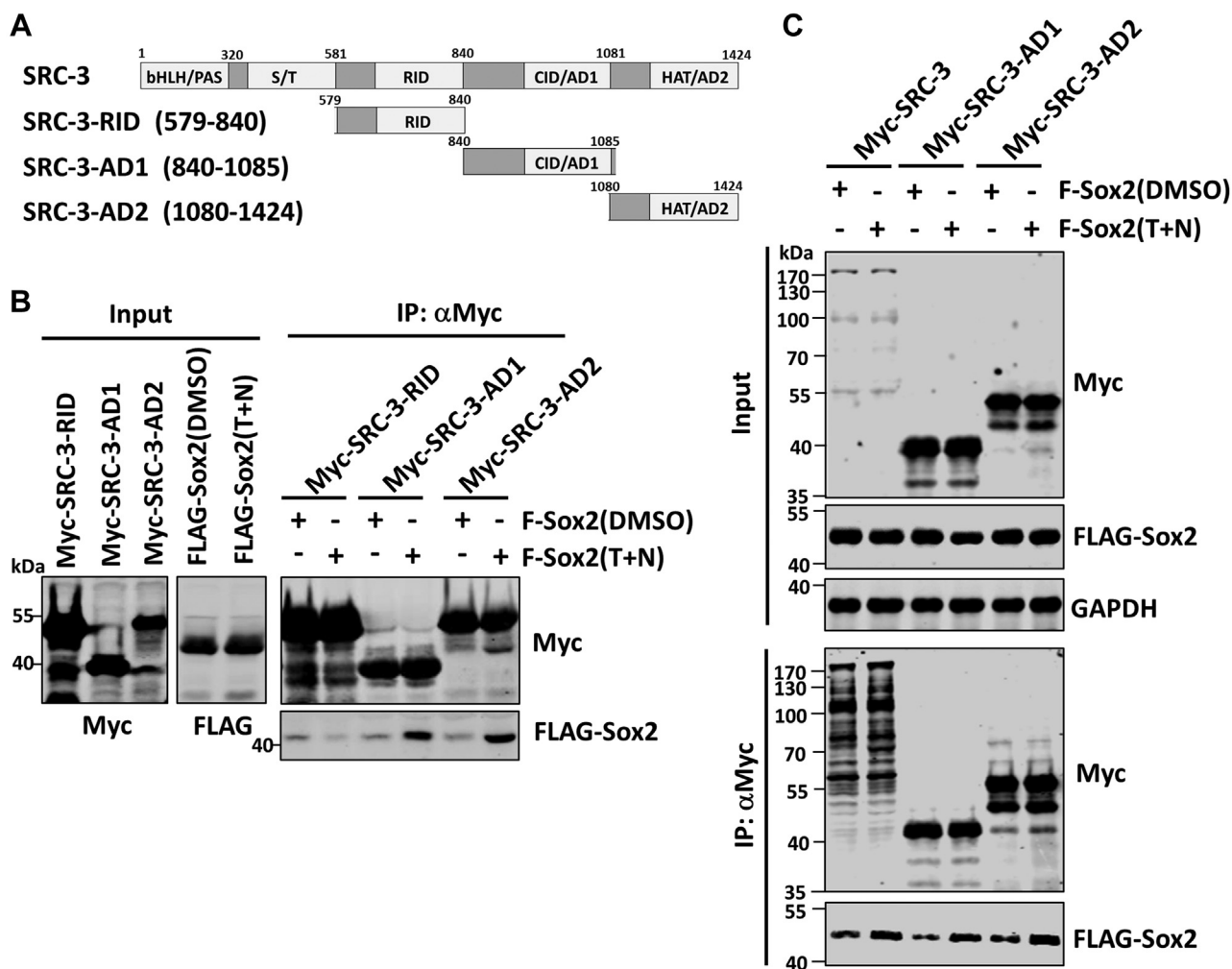*J. Biol. Chem.* (2021) 297(6) 101389 **7**

Figure 5. Both AD1 and AD2 regions of SRC-3 bind preferentially acetylated Sox2. *A*, schematic diagrams showing SRC-3 structural and functional domains and three constructs used in this study. *B*, co-IP assay analyzing the interaction between Sox2 and three distinct regions of SRC-3. HEK293T cells were transfected with FLAG-Sox2 and treated with DMSO or TSA (1 μM) plus NAM (5 mM) (T + N) for 24 h. In addition, HEK293T cells were transfected with Myc-SRC-3-RID, Myc-SRC-3-AD1, or Myc-SRC-3-AD2, respectively. The cellular extracts were prepared and mixed as indicated for IP with anti-Myc and WB analysis using Myc and FLAG antibodies as indicated. Note both SRC-3-AD1 and SRC-3-AD2 regions bind with enhanced affinity for acetylated Sox2. *C*, co-IP assay comparing the binding of acetylated Sox2 to full-length SRC-3 and SRC-3-AD1 and SRC-3-AD2. The experiments were carried out as in (*B*). AD1, activation domain 1; AD2, activation domain 2; co-IP, co-immunoprecipitation; DMSO, dimethyl sulfoxide; IP, immunoprecipitation; NAM, nicotinamide; RID, receptor-interaction domain; Sox2, SRY-box 2; SRCs, steroid receptor coactivators; TSA, trichostatin A.

interaction with Sox2. Further analysis revealed that both SRC-3–AD1 and SRC-3–AD2 bind acetylated Sox2 with a similar affinity as that of full-length SRC-3 (Fig. 5C). We thus conclude that two distinct regions of SRC-3, AD1 and AD2, are capable of selective binding to acetylated Sox2.

SRC-2 and SRC-3 regulate Sox2 target gene transcription and stemness in mouse ES cells

Having demonstrated that SRC-1/2/3 interact with Sox2 and promote Sox2 transcriptional activity in luciferase assay and that Sox2 acetylation enhances Sox2 interaction with SRC-2/3, we next tested whether SRC-1/2/3 contribute to the transcriptional activity of Sox2 target genes and maintenance of self-renewal in mouse ES cells. To this end, we knocked down each of the SRC proteins in mouse CGR8 ES cells using two specific shRNA constructs and validated the efficiency of

knockdown by both quantitative RT-PCR and WB analyses (Fig. 6, A–C). We then examined how knockdown of each SRC protein affected the transcriptional activity of a group of Sox2-activated genes and a group of Sox2-repressed genes. The results in Figure 6, D and E show that knockdown of SRC-1 in general had no significant effect on both Sox2-activated genes (Fig. 6D) and Sox2-repressed genes (Fig. 6E). However, knockdown of either SRC-2 or SRC-3 broadly impaired the transcriptional activity of Sox2-activated genes (Fig. 6, F and H) and derepressed the transcriptional activity of Sox2-repressed genes (Fig. 6, G and I). Collectively, these results reveal a critical role of SRC-2 and SRC-3 in transcriptional regulation by Sox2 in mouse ES cells.

We also investigated how knockdown of SRC-1/2/3 affected the maintenance of ES cell stemness by examining the capability of CGR8 ES cell colony formation. We observed that knockdown of SRC-2 or SRC-3, but not SRC-1, impaired ES
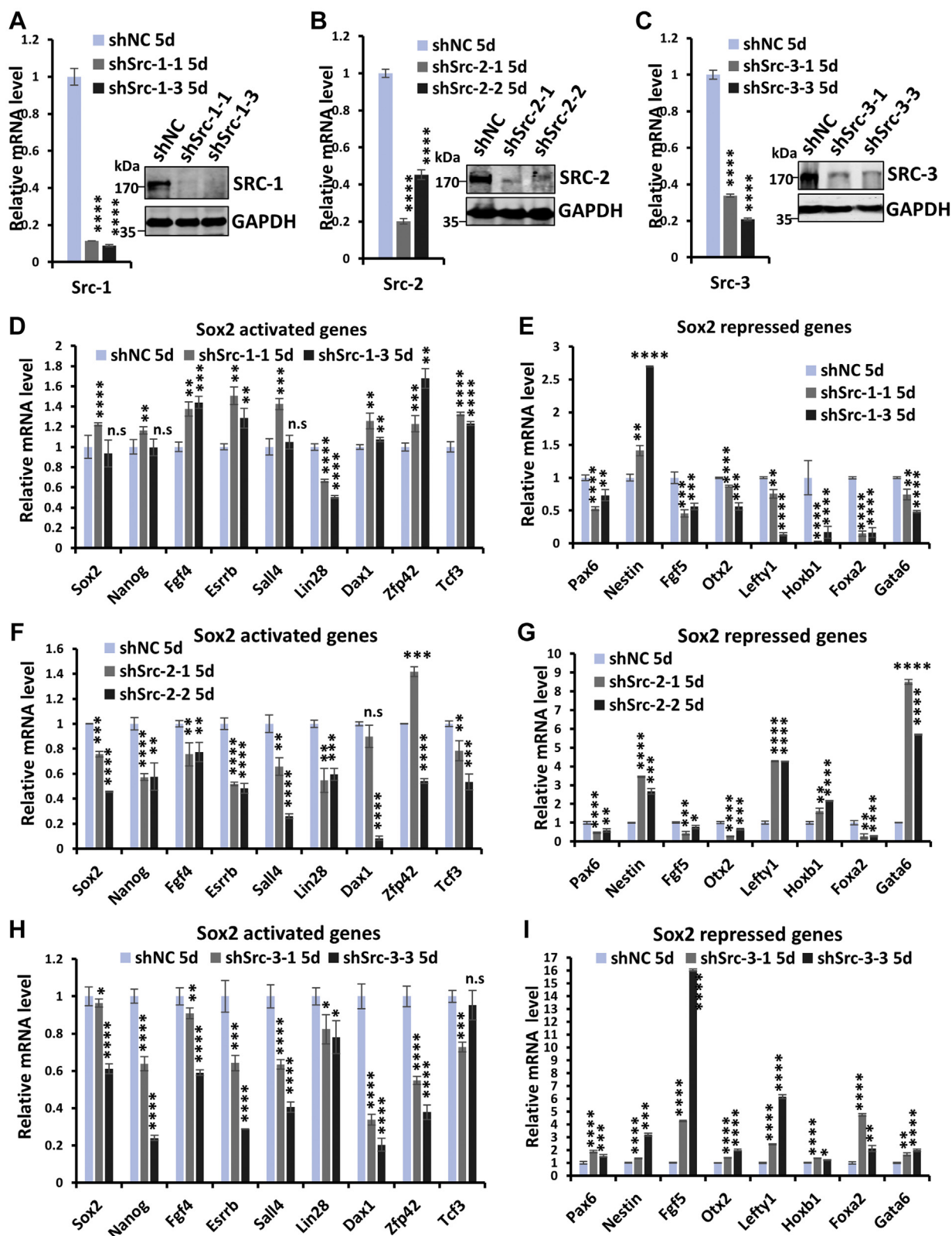
Figure 6. SRC-2 and SRC-3 but not SRC-1 regulate Sox2 target gene expression. *A–C*, knockdown of SRC-1 (*A*), SRC-2 (*B*), and SRC-3 (*C*) in mouse CGR8 ES cells by shRNAs. CGR8 ES cells were transfected with control shNC or two Src-1-, Src-2-, or Src-3-specific shRNAs as indicated. Quantitative RT-PCR and WB analyses were performed 5 days after transfection. *D* and *E*, quantitative RT-PCR analysis of a panel of Sox2-activated genes (*D*) and Sox2-repressed genes (*E*) after knockdown of Src-1 in CGR8 cells as in (*A*). *F* and *G*, quantitative RT-PCR analysis of a panel of Sox2-activated genes (*F*) and Sox2-repressed genes (*G*) after knockdown of Src-2 in CGR8 cells as in (*B*). *H* and *I*, quantitative RT-PCR analysis of a panel of Sox2-activated genes (*H*) and Sox2-repressed genes (*I*) after knockdown of Src-3 in CGR8 cells as in (*C*). All data are represented as the mean ± SD of three biological repeats. All statistical analyses were performed using Excel 2016. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ****$p < 0.0001$. shNC, shVector; Sox2, SRY-box 2; SRCs, steroid receptor coactivators.

cell self-renewal and led to cell differentiation (Fig. 7*A*). Impaired ES stemness activity upon knockdown of SRC-3 observed in our study is consistent with previous reports (29, 30). Thus, our study reveals for the first time a critical role of SRC-2 in transcriptional regulation by Sox2 and in maintenance of ES stemness. Our finding that knockdown of SRC-3 impairs Sox2 transcriptional activity also provides a new explanation as why SRC-3 is essential for maintenance of ES cell pluripotency.

Discussion

In this study, we identified SRC family coactivators as Sox2-interacting proteins and provided evidence that this family of proteins can function as Sox2 coactivators synergistically with p300 (Figs. 1 and 2). Interestingly, we found that acetylation on Sox2 promotes the interaction of Sox2 with both SRC-2 and SRC-3 but not SRC-1 (Fig. 3) and that Sox2 acetylation is required for its optimal transcriptional activity and effective recruitment of SRC-3 (Fig. 4). We show two regions in SRC-3, which correspond to previously identified AD1 and AD2, exhibit increased binding activity for acetylated Sox2 (Fig. 5). Furthermore, functional analyses in mouse ES cells show that knockdown of SRC-2 or SRC-3, but not SRC-1, impaired Sox2 transcriptional activity as well as ES cell stemness (Figs. 6 and 7). Taken together, our data suggest a working model for SRC family coactivators working together with CBP/p300 to promote Sox2 transcriptional activity (Fig. 7*B*). In addition to previously reported interactions with CBP/p300 and other coactivators (7–13), Sox2 also directly interacts with the SRC family coactivators. Thus, in principle, the SRC family coactivators can enhance Sox2 transcriptional activation, possibly *via* their ability to recruit CBP/p300, arginine methyltransferase CARM1, and other transcriptional coregulators, in
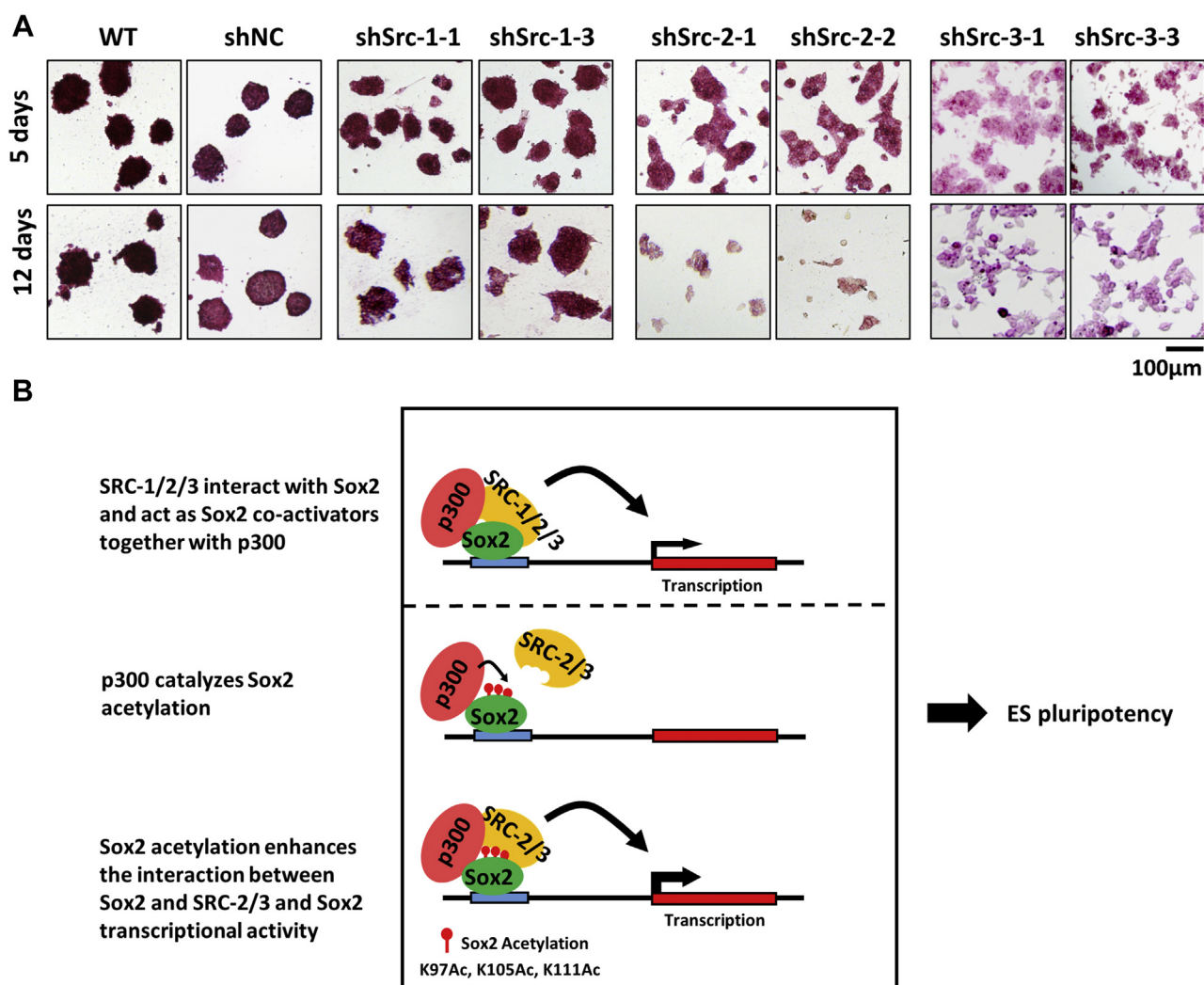

Figure 7. SRC-2 and SRC-3 but not SRC-1 are critical for maintenance of ES self-renewal capability. *A*, representative AP staining images of control and SRC-1-, SRC-2-, or SRC-3-deficient CGR8 ES clones. Knockdown of SRC-1, SRC-2, and SRC-3 was achieved by transfection of CGR8 ES cells with specific shRNAs as indicated. *B*, working model illustrating how SRC family coactivators enhance Sox2 transcriptional activity and maintenance of ES cell self-renewal. The *top panel* illustrates that Sox2 interacts with and recruits p300 as well as the SRC family coactivators. These coactivators, together with other coactivators, mediate transcriptional activation by Sox2. The *lower panel* shows that p300 catalyzes acetylation of Sox2, which in turn enhances the interaction between Sox2 and SRC-2/3 to further augment Sox2 transcriptional activity and function in maintenance of ES pluripotency. AP, alkaline phosphatase; ES, embryonic stem; Sox2, SRY-box 2; SRCs, steroid receptor coactivators.

analogy to their well-characterized primary coactivator function in transcriptional regulation by NRs (15, 16) (Fig. 7B, upper panel). In addition, CBP/p300 can be recruited by either Sox2 or the SRC family coactivators. Besides acetylating histones, CBP/p300 can also acetylate Sox2, which in turn promotes the recruitment of SRC-2/3 and further augments Sox2 transcriptional activity (Fig. 7B, lower panel). Together these events endow Sox2 as a potent transcription factor in maintenance of ES stemness, cell fate reprogramming, and tumorigenesis (4, 43–46).

As a core transcription factor in the ES transcription-regulatory circuit, how Sox2 regulates transcription is of great interest and has been actively investigated. While proteomic approaches have led to identification of several hundreds of Sox2-interacting proteins (7, 47, 48), only a limited number of proteins has so far been characterized as Sox2 transcriptional coactivators. These include the ubiquitous coactivators CBP/p300 and arginine methyltransferase CARM1, the DNA repair XPC complex, and the DKC1 complex (8, 11, 13). To identify specific coactivators for ES core transcription factors, we explored a panel of histone-modification enzymes and transcriptional coregulators using an *in vitro*–based pulldown assay (33). Here, the SRC family coactivators were found to selectively bind Sox2 in comparison with Oct4, Klf4, and Myc (Fig. 1A). Interestingly, although Sox2 interacts with all three SRC proteins in pulldown and co-IP assays (Fig. 1, A−C), ChIP analysis with mouse ES cells only detected enrichment of both SRC-2 and SRC-3, but not SRC-1, at multiple Sox2 target genes (Fig. 1E). Although the failure in SRC-1 detection by ChIP could be potentially due to technical issues such as the quality of the SRC-1 antibody used, we found that knockdown of SRC-2 or SRC-3, but not SRC-1, in mouse E14 cells impaired Sox2 transcriptional activity and ES stemness (Figs. 6 and 7), suggesting that SRC-1 is dispensable for Sox2 transcriptional function. As Sox2 has been linked to tumorigenesis in multiple tissues (45, 49, 50), it will be of interest to determine whether SRC-2/3 also have critical roles in Sox2-driving oncogenic processes in the future.

In addition to its effect on chromatin structure, histone acetylation also serves as an affinity tag for binding of a panel of effector proteins containing a bromodomain or YEATS domain (51, 52). Small-molecule inhibitors that bind competitively to the acetyl–lysine recognition motif, the bromodomain, have been used in clinical trials for treatment of various cancers (53, 54). Increasing evidence indicates that transcription factors can also be acetylated, and similar to acetylation on histones, acetylation on transcription factors can also regulate the interaction with bromodomain-containing proteins. For example, acetylation on MyoD was first shown to mediate the interaction between MyoD and CBP/p300, and this acetylation-dependent interaction requires the bromodomain of CBP/p300 (55, 56). p53 is the first reported acetylated nonhistone protein, and p53 acetylation has been shown to be recognized by CBP as well as PBRM1, a double-bromodomain protein in the SWI/SNF complex (57, 58). Similarly, acetylation of STAT3 also facilitates its

interaction with CBP/p300 and transcriptional activity (59). In addition, an elegant study in Twist shows that Twist contains a "histone H4-mimic" motif that is diacetylated by Tip60. Diacetylated Twist binds the bromodomain protein BRD4, and pharmacologic inhibition of the Twist–BRD4 association suppresses tumorigenesis in basal-like breast cancer (60). In this study, we show that acetylation on Sox2 promotes the binding of SRC-2/3 but not SRC-1. Mutation of either one of the three previously identified acetylated lysine residues, namely K97, K105, and K111, impairs acetylation-enhanced Sox2–SRC-3 interaction, suggesting acetylation on these sites likely promotes Sox2 interaction with SRC-3. Currently, how acetylation on these sites promotes Sox2 interaction with SRC-3 is not clear. As single K to R mutation in any of the three sites is sufficient to abolish acetylation-enhanced interaction, one possibility is that acetylation on all three sites is required for the observed acetylation-enhanced interaction. Alternatively, some of these lysine residues may be required for acetylation and not necessarily involved directly in the interaction. Given that Sox2 acetylation promotes Sox2 interaction with SRC-2 and SRC-3 but not SRC-1 and that SRC-1 is not detected in Sox2 target genes (Fig. 1E) and is dispensable for Sox2 transcriptional activity and function in stemness, it is tempting to suggest that the Sox2 acetylation–driven SOX2–SRC-2/3 interaction is likely essential for effective recruitment of SRC-2/3 and subsequent Sox2 transcriptional function. In support of this, we detected impaired SRC-3 recruitment by the Sox2 mutant with K97R, K105R, or K111R mutation (Fig. 4).

A more intriguing question raised by our study is how the SRC family coactivators selectively bind acetylated Sox2, as they contain neither a bromodomain nor a YEATS domain. Our preliminary analysis demonstrated that both the AD1 and AD2 regions of SRC-3 exhibit selective binding of acetylated Sox2 (Fig. 5). Thus, it is of great interest to determine in the future the structural basis for this acetylation-enhanced interaction between Sox2 and SRC-3, as this may reveal novel acetylation recognition motifs in the SRC family coactivators. As these family coactivators have broad roles in transcriptional regulation and other biological processes (24, 42), our finding they function as readers of acetylated proteins may have roles extending beyond transcriptional regulation by Sox2.

Experimental procedures

Cells, plasmids, and antibodies

HeLa S3 and HEK293T cells were cultured in Dulbecco's modified Eagle's medium (DMEM) (GIBCO, Thermo Fisher) with 10% fetal bovine serum (GIBCO), and CGR8 cells were cultured in Glasgow's minimum essential medium (GIBCO) with 15% fetal bovine serum (GIBCO), 1000 U/ml LIF, 2 mM GlutaMAX, and 0.1 mM β-mercaptoethanol at 37 °C in air atmosphere with 5% CO_2. Mouse E14 ES cells were cultured in DMEM (GIBCO, Thermo Fisher) with 15% fetal bovine serum (GIBCO), 1000 U/ml cytokine leukemia inhibitory factor (LIF),

2i (1 μM PD0325901 and 1 μM CHIR-99021), 2 mM GlutaMAX, and 0.1 mM β-mercaptoethanol, without feeder cells, at 37 °C in air atmosphere with 5% CO_2. Mouse CGR8 ES cells were cultured as above but without addition of 2i.

The following plasmids were used in our study: pGEST-4T-1-c-Myc, pGEST-4T-1-Sox2, pGEST-4T-1-Oct4, pGEST-4T-1-Klf4, pSG5-FLAG-Sox2, pSG5-FLAG-Sox2 (1–243aa), pSG5-FLAG-Sox2 (112–243aa), pSG5-FLAG-Sox2 (152–319aa), pcDNA3.1-Myc-Sox2, pSG5-FLAG-SRC-1, pSG5-FLAG-SRC-2, pSG5-FLAG-SRC-3, pcDNA3.1-Myc-SRC-1, pcDNA3.1-Myc-SRC-2, pcDNA3.1-Myc-SRC-3, pCMV-p300-Myc, pGL3-FGF4-Luc, pLKO.1-puro-shSRC-1-1, pLKO.1-puro-shSRC-1-3, pLKO.1-puro-shSRC-2-1, pLKO.1-puro-shSRC-2-2, pLKO.1-puro-shSRC-3-1, pLKO.1-puro-shSRC-3-3, pLKO.1-puro-shp300-1, pLKO.1-puro-shp300-2, and pLKO.1-puro-p300-3. All plasmids were verified by DNA sequencing.

The following antibodies were used in our study: anti-FLAG antibody (Sigma, F1804, rabbit), anti-FLAG antibody (Sigma, F7425, mouse), anti-Myc antibody (HUABIO, R1208-1, rabbit), anti-Myc antibody (Abmart, 284067, mouse), anti-pan-Kac antibody (CST, 9441S, rabbit), anti-SRC-1 antibody (CST, 2191, rabbit), anti-SRC-2 antibody (CST, 96687, rabbit), anti-SRC-3 antibody (CST, 2126, rabbit), anti-p300 antibody (CST, 54062, rabbit), anti-GAPDH antibody (Abcam, M20006L, mouse), and anti-Sox2 antibody (Abcam, ab92494, rabbit).

WB analysis

WB analysis was performed by the standard protocol using antibodies as indicated. In brief, cells or immunoprecipitated protein samples were directly lysed by 1× SDS buffer (65 mM Tris-HCL, pH 6.8, 2% w/v SDS, 10% glycerol, 2.5% v/v β-mercaptoethanol, and 0.05% w/v bromophenol blue) and boiled at 100 °C for 10 min. The proteins samples were separated on SDS-PAGE gels and then transferred to the nitrocellulose membrane (GE Healthcare Life Sciences). After blocked in 5% to 8% milk for 1 to 3 h at room temperature (RT) or overnight at 4 °C with rocking, the membranes were incubated with the primary antibody overnight at 4 °C or 2 to 4 h at RT with rocking. The membranes were washed three times with PBST buffer (0.1% Tween-20 in 1 × PBS buffer) before incubated with an appropriate secondary antibody (Alexa Fluor 680 goat anti-rabbit or Alexa Fluor 790 goat anti-mouse antibody, Jackson ImmunoResearch, dilution: 1:20,000) at RT for 1 h. The membranes were visualized using the Odyssey CLx Imaging System (LI-COR Biosciences).

Co-IP assay

For exogenous protein co-IP, the indicated plasmid(s) was transfected into HEK293T cells. The cells were collected after 48 h of transfection and then lysed in the IP lysis buffer (50 mM Tris HCl, pH 7.5, 150 mM NaCl, 1% Triton X-100, 1 mM EDTA, 10% glycerol, 1× protease inhibitor cocktail (MCE), and 1 mM DTT (Amresco)) on ice. After centrifuged at 12,000 rpm for 20 min at 4 °C, the supernatants were collected and diluted three times with the IP binding buffer (20 mM Tris HCl, pH 7.5, 150 mM NaCl, 1 mM EDTA, 10%, glycerol, 1× protease inhibitor cocktail (MCE), and 1 mM DTT (Amresco)) and then incubated with anti-FLAG beads (GenScript) or anti-Myc beads (GenScript) for 6 to 8 h at 4 °C. After washing with the IP washing buffer (20 mM Tris HCl, pH 7.5, 150 mM NaCl, 1 mM EDTA, 0.1% Triton X-100, 1× protease inhibitor cocktail (MCE), and 1 mM DTT (Amresco)) four times, the precipitates were boiled in 1× SDS loading buffer and analyzed by SDS-PAGE.

For co-IP of endogenous proteins, antibodies were added at a concentration of 1 μg/mg of protein lysates and incubated overnight or 6 to 8 h at 4 °C, followed by antibody–protein complex capture with Protein G/Protein A Sepharose beads (Santa Cruz).

To determine if acetylation on Sox2 or SRC-3 enhances the interaction between Sox2 and SRC-3, HEK293T cells were transfected with FLAG-Sox2 or Myc-SRC-3 or their derivative plasmids separately, and transfected cells were then cultured in DMEM with 1 μM TSA and 5 mM NAM (TSA + NAM) or DMSO for 24 h. Cells were harvested to make whole-cell extracts, and co-IPs were performed by mixing TSA + NAM–treated FLAG-Sox2 and DMSO-treated Myc-SRC-3 or vice versa.

In vitro pulldown assay

For *in vitro* pulldown assay to assess the interaction of candidate coregulators with GST fusions of ES core transcription factors, all coregulators were synthesized by *in vitro* coupled transcription–translation system from Promega Biotech Co Ltd with ^{35}S-methionine labeling. GST-c-Myc, GST-Klf4, GST-Oct4, and GST-Sox2 were expressed and purified from *Escherichia coli* as described (33). GST-c-Myc, GST-Klf4, GST-Oct4, and GST-Sox2 were incubated with Glutathione-Sepharose beads in the IP binding buffer for 2 h at 4 °C, andthen, different coregulators were added to the pulldown system and incubated 4 to 6 h at 4 °C. After washing with the IP washing buffer for four times, the beads were boiled in 1× SDS loading buffer and analyzed by SDS-PAGE. The presence of ^{35}S-methionine-labeled coregulators was detected by autoradiography.

Luciferase reporter assay

To assay Sox2 transcriptional activity by the luciferase reporter assay, the FGF4-Luc reporter plasmid and the plasmids encoding Sox2 and/or SRC family coactivators were transfected into HEK293T cells. The quantities of plasmids used are indicated in figure legends. The luciferase reporter assay was performed using a Dual-Luciferase Reporter assay kit from Promega. All luciferase reporter assay statistical analyses were performed using Excel 2016.

ChIP assay

FGF4-luc and FLAG-Sox2 or Sox2mut were transfected into HEK293T cells as indicated in the figure legends. Twenty-four hours after transfection, cells were incubated in the culture

medium with 1% formaldehyde on a shaking device for 15 min at RT. Then 1 M glycine was added to a final concentration of 125 mM and incubated for 5 min to stop crosslinking. Cells were washed with PBS once and lysed with addition of 500 μl ChIP buffer (0.1% SDS, 1% Triton X-100, 1 mM EDTA, 0.1% NaDOC, 0.3 M NaCl, 10 mM Tris HCl, pH 7.5, and protease inhibitors). Subsequent ChIP assay for binding of Sox2 and SRC-3 to the FGF4 enhancer was essentially as described (61).

For ChIP analysis of Sox2 target genes, mouse E14 ES cells cultured with LIF plus 2i without feeder cells were used. Mouse E14 ES cells were fixed initially with 1.5 mM EGS (ACMEC, E56310) at RT for 30 min and subsequently with 0.4% formaldehyde at RT for 10 min. Subsequent ChIP assay for binding of Sox2, SRC-1/2/3, and p300 to Sox2 target genes was essentially as described (62). The primers for Sox2 target genes were designed according to Sox2 ChIP-seq peak data in a previous publication (34), and their sequence information is provided in Table S1.

Alkaline phosphatase staining assay

For ES cell colony-formation assay, CGR8 cells transfected with shSrc-3 were cultured up to 5 and 12 days. Alkaline phosphatase staining was performed using the alkaline phosphatase detection kit (Millipore; SCR004) as described previously (61) and according to the manufacturer's instructions.

Total RNA extraction and quantitative RT-PCR analysis

Total RNA was extracted from cells using the TRIzol extraction kit from TAKARA (D9108A) according to the manufacturer's instructions. All cDNAs were synthesized using the TransScript One-Step gDNA Removal and cDNA Synthesis SuperMix Kit (TransGen Biotech, AT311-02). Subsequent quantitative RT-PCRs were performed using Trans-Start Tip Green quantitative PCR SuperMix (TransGen Biotech, AQ141) on CFX96 Touch Real-Time PCR Detection System (Bio-Rad), and the results were normalized to β-actin mRNA levels. All quantitative RT-PCR statistical analyses were performed by comparative delta-delta Ct method and t test using Excel 2016, and the data represent the mean ± SD for three biological repeats. $p < 0.05$ represents statistically significant of differences (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ****$p < 0.0001$). Primers for quantitative RT-PCR are listed in Table S1.

Data availability

All the data supporting our conclusions are presented in this article and information for primers used in the Table S1. All material is available upon request.

Abbreviations—The abbreviations used are: AD1, activation domain 1; AD2, activation domain 2; ChIP, chromatin immunoprecipitation; co-IP, co-immunoprecipitation; ES, embryonic stem; NaCro, sodium crotonate; NAM, nicotinamide; NCOAs, nuclear receptor coactivators; NRs, nuclear hormone receptors; Sox2, SRY-box 2; SRCs, steroid receptor coactivators; TSA, trichostatin A; WB, Western blot.

References

1. Sarkar, A., and Hochedlinger, K. (2013) The sox family of transcription factors: Versatile regulators of stem and progenitor cell fate. *Cell Stem Cell* **12**, 15–30
2. Takahashi, K., and Yamanaka, S. (2006) Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell* **126**, 663–676
3. Takahashi, K., Tanabe, K., Ohnuki, M., Narita, M., Ichisaka, T., Tomoda, K., and Yamanaka, S. (2007) Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell* **131**, 861–872
4. Boyer, L. A., Lee, T. I., Cole, M. F., Johnstone, S. E., Levine, S. S., Zucker, J. P., Guenther, M. G., Kumar, R. M., Murray, H. L., Jenner, R. G., Gifford, D. K., Melton, D. A., Jaenisch, R., and Young, R. A. (2005) Core transcriptional regulatory circuitry in human embryonic stem cells. *Cell* **122**, 947–956
5. Heng, J. C., and Ng, H. H. (2010) Transcriptional regulation in embryonic stem cells. *Adv. Exp. Med. Biol.* **695**, 76–91
6. Young, R. A. (2011) Control of the embryonic stem cell state. *Cell* **144**, 940–954
7. Kim, B. R., Coyaud, E., Laurent, E. M. N., St-Germain, J., Van de Laar, E., Tsao, M. S., Raught, B., and Moghal, N. (2017) Identification of the SOX2 interactome by BioID reveals EP300 as a mediator of SOX2-dependent squamous differentiation and lung squamous cell carcinoma growth. *Mol. Cell. Proteomics* **16**, 1864–1888
8. Nowling, T. K., Johnson, L. R., Wiebe, M. S., and Rizzino, A. (2000) Identification of the transactivation domain of the transcription factor Sox-2 and an associated co-activator. *J. Biol. Chem.* **275**, 3810–3818
9. Nowling, T., Bernadt, C., Johnson, L., Desler, M., and Rizzino, A. (2003) The co-activator p300 associates physically with and can mediate the action of the distal enhancer of the FGF-4 gene. *J. Biol. Chem.* **278**, 13696–13705
10. Zhao, H. Y., Zhang, Y. J., Dai, H., Zhang, Y., and Shen, Y. F. (2011) CARM1 mediates modulation of Sox2. *PLoS One* **6**, e27026
11. Fong, Y. W., Inouye, C., Yamaguchi, T., Cattoglio, C., Grubisic, I., and Tjian, R. (2011) A DNA repair complex functions as an Oct4/Sox2 coactivator in embryonic stem cells. *Cell* **147**, 120–131
12. Cattoglio, C., Zhang, E. T., Grubisic, I., Chiba, K., Fong, Y. W., and Tjian, R. (2015) Functional and mechanistic studies of XPC DNA-repair

complex as transcriptional coactivator in embryonic stem cells. *Proc. Natl. Acad. Sci. U. S. A.* **112**, E2317–E2326

13. Fong, Y. W., Ho, J. J., Inouye, C., and Tjian, R. (2014) The dyskerin ribonucleoprotein complex as an OCT4/SOX2 coactivator in embryonic stem cells. *Elife* **3**, e03573

14. Onate, S. A., Tsai, S. Y., Tsai, M. J., and O'Malley, B. W. (1995) Sequence and characterization of a coactivator for the steroid hormone receptor superfamily. *Science* **270**, 1354–1357

15. Dasgupta, S., Lonard, D. M., and O'Malley, B. W. (2014) Nuclear receptor coactivators: Master regulators of human health and disease. *Annu. Rev. Med.* **65**, 279–292

16. Johnson, A. B., and O'Malley, B. W. (2012) Steroid receptor coactivators 1, 2, and 3: Critical regulators of nuclear receptor activity and steroid receptor modulator (SRM)-based cancer therapy. *Mol. Cell. Endocrinol.* **348**, 430–439

17. Hong, H., Kohli, K., Trivedi, A., Johnson, D. L., and Stallcup, M. R. (1996) GRIP1, a novel mouse protein that serves as a transcriptional coactivator in yeast for the hormone binding domains of steroid receptors. *Proc. Natl. Acad. Sci. U. S. A.* **93**, 4948–4952

18. Voegel, J. J., Heine, M. J., Zechel, C., Chambon, P., and Gronemeyer, H. (1996) TIF2, a 160 kDa transcriptional mediator for the ligand-dependent activation function AF-2 of nuclear receptors. *EMBO J.* **15**, 3667–3675

19. Anzick, S. L., Kononen, J., Walker, R. L., Azorsa, D. O., Tanner, M. M., Guan, X. Y., Sauter, G., Kallioniemi, O. P., Trent, J. M., and Meltzer, P. S. (1997) AIB1, a steroid receptor coactivator amplified in breast and ovarian cancer. *Science* **277**, 965–968

20. Torchia, J., Rose, D. W., Inostroza, J., Kamei, Y., Westin, S., Glass, C. K., and Rosenfeld, M. G. (1997) The transcriptional co-activator p/CIP binds CBP and mediates nuclear-receptor function. *Nature* **387**, 677–684

21. Chen, H., Lin, R. J., Schiltz, R. L., Chakravarti, D., Nash, A., Nagy, L., Privalsky, M. L., Nakatani, Y., and Evans, R. M. (1997) Nuclear receptor coactivator ACTR is a novel histone acetyltransferase and forms a multimeric activation complex with P/CAF and CBP/p300. *Cell* **90**, 569–580

22. Li, H., Gomes, P. J., and Chen, J. D. (1997) RAC3, a steroid/nuclear receptor-associated coactivator that is related to SRC-1 and TIF2. *Proc. Natl. Acad. Sci. U. S. A.* **94**, 8479–8484

23. Takeshita, A., Cardona, G. R., Koibuchi, N., Suen, C. S., and Chin, W. W. (1997) TRAM-1, a novel 160-kDa thyroid hormone receptor activator molecule, exhibits distinct properties from steroid receptor coactivator-1. *J. Biol. Chem.* **272**, 27629–27634

24. York, B., and O'Malley, B. W. (2010) Steroid receptor coactivator (SRC) family: Masters of systems biology. *J. Biol. Chem.* **285**, 38743–38750

25. Na, S. Y., Lee, S. K., Han, S. J., Choi, H. S., Im, S. Y., and Lee, J. W. (1998) Steroid receptor coactivator-1 interacts with the p50 subunit and coactivates nuclear factor kappaB-mediated transactivations. *J. Biol. Chem.* **273**, 10831–10834

26. Yanagisawa, J., Yanagi, Y., Masuhiro, Y., Suzawa, M., Watanabe, M., Kashiwagi, K., Toriyabe, T., Kawabata, M., Miyazono, K., and Kato, S. (1999) Convergence of transforming growth factor-beta and vitamin D signaling pathways on SMAD transcriptional coactivators. *Science* **283**, 1317–1321

27. Dennler, S., Pendaries, V., Tacheau, C., Costas, M. A., Mauviel, A., and Verrecchia, F. (2005) The steroid receptor co-activator-1 (SRC-1) potentiates TGF-beta/Smad signaling: Role of p300/CBP. *Oncogene* **24**, 1936–1945

28. Livengood, J. A., Scoggin, K. E., Van Orden, K., McBryant, S. J., Edayathumangalam, R. S., Laybourn, P. J., and Nyborg, J. K. (2002) p53 Transcriptional activity is mediated through the SRC1-interacting domain of CBP/p300. *J. Biol. Chem.* **277**, 9054–9061

29. Percharde, M., Lavial, F., Ng, J. H., Kumar, V., Tomaz, R. A., Martin, N., Yeo, J. C., Gil, J., Prabhakar, S., Ng, H. H., Parker, M. G., and Azuara, V. (2012) Ncoa3 functions as an essential Esrrb coactivator to sustain embryonic stem cell self-renewal and reprogramming. *Genes Dev.* **26**, 2286–2298

30. Wu, Z., Yang, M., Liu, H., Guo, H., Wang, Y., Cheng, H., and Chen, L. (2012) Role of nuclear receptor coactivator 3 (Ncoa3) in pluripotency maintenance. *J. Biol. Chem.* **287**, 38295–38304

31. Chen, X., Vega, V. B., and Ng, H. H. (2008) Transcriptional regulatory networks in embryonic stem cells. *Cold Spring Harb. Symp. Quant. Biol.* **73**, 203–209

32. Papp, B., and Plath, K. (2011) Reprogramming to pluripotency: Stepwise resetting of the epigenetic landscape. *Cell Res.* **21**, 486–501

33. Fang, L., Zhang, J., Zhang, H., Yang, X., Jin, X., Zhang, L., Skalnik, D. G., Jin, Y., Zhang, Y., Huang, X., Li, J., and Wong, J. (2016) H3K4 methyltransferase Set1a is a key Oct4 coactivator essential for generation of Oct4 positive inner cell mass. *Stem Cells* **34**, 565–580

34. Chronis, C., Fiziev, P., Papp, B., Butz, S., Bonora, G., Sabri, S., Ernst, J., and Plath, K. (2017) Cooperative binding of transcription factors orchestrates reprogramming. *Cell* **168**, 442–459.e20

35. Dailey, L., Yuan, H., and Basilico, C. (1994) Interaction between a novel F9-specific factor and octamer-binding proteins is required for cell-type-restricted activity of the fibroblast growth factor 4 enhancer. *Mol. Cell. Biol.* **14**, 7758–7769

36. Baltus, G. A., Kowalski, M. P., Zhai, H., Tutter, A. V., Quinn, D., Wall, D., and Kadam, S. (2009) Acetylation of sox2 induces its nuclear export in embryonic stem cells. *Stem Cells* **27**, 2175–2184

37. Cole, P. A. (2008) Chemical probes for histone-modifying enzymes. *Nat. Chem. Biol.* **4**, 590–597

38. Sabari, B. R., Tang, Z., Huang, H., Yong-Gonzalez, V., Molina, H., Kong, H. E., Dai, L., Shimada, M., Cross, J. R., Zhao, Y., Roeder, R. G., and Allis, C. D. (2015) Intracellular crotonyl-CoA stimulates transcription through p300-catalyzed histone crotonylation. *Mol. Cell* **58**, 203–215

39. Wei, W., Liu, X., Chen, J., Gao, S., Lu, L., Zhang, H., Ding, G., Wang, Z., Chen, Z., Shi, T., Li, J., Yu, J., and Wong, J. (2017) Class I histone deacetylases are major histone decrotonylases: Evidence for critical and broad function of histone crotonylation in transcription. *Cell Res.* **27**, 898–915

40. Yang, X. J. (2004) Lysine acetylation and the bromodomain: A new partnership for signaling. *Bioessays* **26**, 1076–1087

41. Bannister, A. J., and Miska, E. A. (2000) Regulation of gene expression by transcription factor acetylation. *Cell. Mol. Life Sci.* **57**, 1184–1192

42. Stashi, E., York, B., and O'Malley, B. W. (2014) Steroid receptor coactivators: Servants and masters for control of systems metabolism. *Trends Endocrinol. Metab.* **25**, 337–347

43. Arnold, K., Sarkar, A., Yram, M. A., Polo, J. M., Bronson, R., Sengupta, S., Seandel, M., Geijsen, N., and Hochedlinger, K. (2011) Sox2(+) adult stem and progenitor cells are important for tissue regeneration and survival of mice. *Cell Stem Cell* **9**, 317–329

44. Andoniadou, C. L., Matsushima, D., Mousavy Gharavy, S. N., Signore, M., Mackintosh, A. I., Schaeffer, M., Gaston-Massuet, C., Mollard, P., Jacques, T. S., Le Tissier, P., Dattani, M. T., Pevny, L. H., and Martinez-Barbera, J. P. (2013) Sox2(+) stem/progenitor cells in the adult mouse pituitary support organ homeostasis and have tumor-inducing potential. *Cell Stem Cell* **13**, 433–445

45. Tam, W. L., and Ng, H. H. (2014) Sox2: Masterminding the root of cancer. *Cancer Cell* **26**, 3–5

46. Schaefer, T., and Lengerke, C. (2020) SOX2 protein biochemistry in stemness, reprogramming, and cancer: I PI3K/AKT/SOX2 axis and beyond. *Oncogene* **39**, 278–292

47. Fang, X., Yoon, J. G., Li, L., Tsai, Y. S., Zheng, S., Hood, L., Goodlett, D. R., Foltz, G., and Lin, B. (2011) Landscape of the SOX2 protein-protein interactome. *Proteomics* **11**, 921–934

48. Cox, J. L., Wilder, P. J., Gilmore, J. M., Wuebben, E. L., Washburn, M. P., and Rizzino, A. (2013) The SOX2-interactome in brain cancer cells identifies the requirement of MSI2 and USP9X for the growth of brain tumor cells. *PLoS One* **8**, e62857

49. Bass, A. J., Watanabe, H., Mermel, C. H., Yu, S., Perner, S., Verhaak, R. G., Kim, S. Y., Wardwell, L., Tamayo, P., Gat-Viks, I., Ramos, A. H., Woo, M. S., Weir, B. A., Getz, G., Beroukhim, R., *et al.* (2009) SOX2 is an amplified lineage-survival oncogene in lung and esophageal squamous cell carcinomas. *Nat. Genet.* **41**, 1238–1242

50. Wuebben, E. L., and Rizzino, A. (2017) The dark side of SOX2: Cancer - a comprehensive overview. *Oncotarget* **8**, 44917–44943

51. Zeng, L., and Zhou, M. M. (2002) Bromodomain: An acetyl-lysine binding domain. *FEBS Lett.* **513**, 124–128

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52. Li, Y., Wen, H., Xi, Y., Tanaka, K., Wang, H., Peng, D., Ren, Y., Jin, Q., Dent, S. Y., Li, W., Li, H., and Shi, X. (2014) AF9 YEATS domain links histone acetylation to DOT1L-mediated H3K79 methylation. *Cell* **159**, 558–571

53. Zaware, N., and Zhou, M. M. (2019) Bromodomain biology and drug discovery. *Nat. Struct. Mol. Biol.* **26**, 870–879

54. Perez-Salvia, M., and Esteller, M. (2017) Bromodomain inhibitors and cancer therapy: From structures to applications. *Epigenetics* **12**, 323–339

55. Polesskaya, A., Duquet, A., Naguibneva, I., Weise, C., Vervisch, A., Bengal, E., Hucho, F., Robin, P., and Harel-Bellan, A. (2000) CREB-binding protein/p300 activates MyoD by acetylation. *J. Biol. Chem.* **275**, 34359–34364

56. Polesskaya, A., Naguibneva, I., Duquet, A., Bengal, E., Robin, P., and Harel-Bellan, A. (2001) Interaction between acetylated MyoD and the bromodomain of CBP and/or p300. *Mol. Cell. Biol.* **21**, 5312–5320

57. Mujtaba, S., He, Y., Zeng, L., Yan, S., Plotnikova, O., Sachchidanand, Sanchez, R., Zeleznik-Le, N. J., Ronai, Z., and Zhou, M. M. (2004) Structural mechanism of the bromodomain of the coactivator CBP in p53 transcriptional activation. *Mol. Cell* **13**, 251–263

58. Cai, W., Su, L., Liao, L., Liu, Z. Z., Langbein, L., Dulaimi, E., Testa, J. R., Uzzo, R. G., Zhong, Z., Jiang, W., Yan, Q., Zhang, Q., and Yang, H. (2019) PBRM1 acts as a p53 lysine-acetylation reader to suppress renal tumor growth. *Nat. Commun.* **10**, 5800

59. Hou, T., Ray, S., Lee, C., and Brasier, A. R. (2008) The STAT3 NH2-terminal domain stabilizes enhanceosome assembly by interacting with the p300 bromodomain. *J. Biol. Chem.* **283**, 30725–30734

60. Shi, J., Wang, Y., Zeng, L., Wu, Y., Deng, J., Zhang, Q., Lin, Y., Li, J., Kang, T., Tao, M., Rusinova, E., Zhang, G., Wang, C., Zhu, H., Yao, J., *et al.* (2014) Disrupting the interaction of BRD4 with diacetylated twist suppresses tumorigenesis in basal-like breast cancer. *Cancer Cell* **25**, 210–225

61. Fang, L., Zhang, L., Wei, W., Jin, X., Wang, P., Tong, Y., Li, J., Du, J. X., and Wong, J. (2014) A methylation-phosphorylation switch determines Sox2 stability and function in ESC maintenance or differentiation. *Mol. Cell* **55**, 537–551

62. Yu, M., Yang, W., Ni, T., Tang, Z., Nakadai, T., Zhu, J., and Roeder, R. G. (2015) RNA polymerase II-associated factor 1 regulates the release and phosphorylation of paused RNA polymerase II. *Science* **350**, 1383–1386