

YY1 Positively Regulates Transcription by Targeting Promoters and Super-Enhancers through the BAF Complex in Embryonic Stem Cells

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SUMMARY

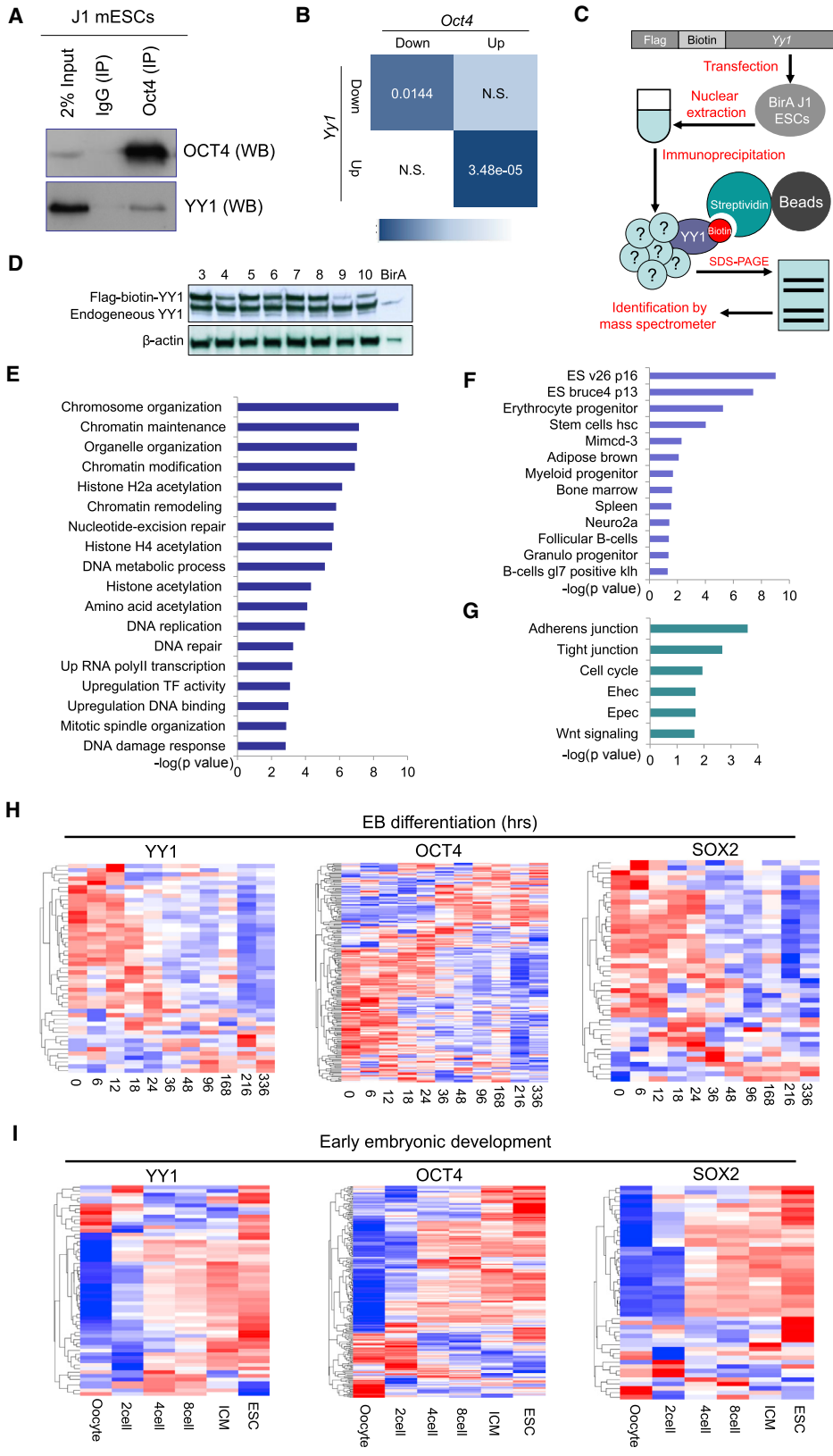
Yin Yang 1 (YY1) regulates early embryogenesis and adult tissue formation. However, the role of YY1 in stem cell regulation remains unclear. YY1 has a Polycomb group (PcG) protein-dependent role in mammalian cells. The PcG-independent functions of YY1 are also reported, although their underlying mechanism is still undefined. This paper reports the role of YY1 and BAF complex in the OCT4-mediated pluripotency network in mouse embryonic stem cells (mESCs). The interaction between YY1 and BAF complex promotes mESC proliferation and pluripotency. Knockdown of *Yy1* or *Smarca4*, the core component of the BAF complex, downregulates pluripotency markers and upregulates several differentiation markers. Moreover, YY1 enriches at both promoter and super-enhancer regions to stimulate transcription. Thus, this study elucidates the role of YY1 in regulating pluripotency through its interaction with OCT4 and the BAF complex and the role of BAF complex in integrating YY1 into the core pluripotency network.

INTRODUCTION

Yin Yang 1 (YY1) is the mammalian ortholog of pleiohomeotic, a transcription factor that binds Polycomb DNA response elements in *Drosophila melanogaster* and recruits Polycomb group (PcG) proteins to DNA (Brown et al., 1998). Like certain DNA sequences (Farcas et al., 2012; Wu et al., 2013), transcription factors (Endoh et al., 2008), pre-existing histone modifications (Bernstein et al., 2006), and non-coding RNA (Kotake et al., 2011), YY1 is also considered as one of the well-accepted DNA binding factors that can recruit PcG proteins to specific chromatin sites (Bracken and Helin, 2009). YY1 was originally identified as a transcriptional repressor due to its interaction with the Polycomb repressive complex 2 (Satijn et al., 2001), which further initiates the tri-methylation of K27 of histone 3 (H3K27me3) to repress specific genes, such as *MHCIIb* and *MCK*, during myoblast differentiation (Caretta et al., 2004). Moreover, YY1 has been reported to regulate cell proliferation and differentiation (Gordon

et al., 2006), and its deficiency in mice caused peri-implantation lethality during embryonic development (Donohoe et al., 1999). Within the B lineage of lymphocytes, YY1 has played critical roles at all stages of B cell differentiation (Kleiman et al., 2016). Conditional deletion of *Yy1* resulted in a blockage at the pro-B cell to pre-B cell stage (Liu et al., 2007).

Several studies also pointed out that YY1 has transcriptional activation functions independent of PcG. Findings by Lee et al. (1995) demonstrated that the association of YY1 with p300 resulted in histone acetylation, which caused gene activation by facilitating the binding of RNA polymerase and transcription factors to promoter regions. Studies by the Seto team revealed that YY1 recruited PRMT1 to mediate histone methylation on lysine and arginine residues, and this PRMT1-mediated histone H4-R3 methylation also induced transcriptional activation (Rezaei-Zadeh et al., 2003). In addition, the association of YY1 with MDM2, PIASy, and UBC9 contributed to protein ubiquitination and sumoylation (Deng et al., 2007; Sui et al.,



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2004). Furthermore, Lu et al. (2013) found no significant co-occupancy between YY1 and Ezh2. They provided evidence that YY1 acts as an activator for many loci, suggesting an Ezh2-independent role of YY1 in muscle cells. Works by the Young group proposed a model wherein YY1 binds to both gene-regulatory elements and their associated RNAs, which further enhances YY1 occupancy at these elements (Sigova et al., 2015). This finding outlined a positive feedback loop that contributed to the stability of gene expression programs regulated by YY1. YY1 also plays a potential role in different cancer types. It was reported that ectopic expression of YY1 results in carcinogenesis through cell-cycle deregulation (Gordon et al., 2006). The dynamic interactions between YY1 and the cell-cycle regulators, such as CDKs, CYCLINs, pRB, and P53, frequently resulted in dysfunctional cell-cycle progression and tumorigenesis (Cicatiello et al., 2004; Parija and Das, 2003; Yakovleva et al., 2004).

Although YY1 has multiple transcriptional regulation functions in various biological processes, few reports have examined the role of YY1 in pluripotency regulation. The Orkin group has classified the ESC transcriptional network into three distinct transcription modules: the core module, the PRC module, and the Myc module (Kim et al., 2010). In that regard, Vella et al. (2012) reported that YY1 did not physically interact with PcG proteins, but extended the MYC-related transcription factor network in embryonic stem cells (ESCs). They found that YY1 binding had a strong correlation with the components of the Myc module, and YY1-regulated pluripotency through gene activation rather than repression, suggesting the involvement of YY1 in Myc-related transcription network. However, the in-depth mechanisms of YY1 in pluripotency regulation, and its role in the core pluripotency network need to be better defined. In the present study, we employed immunoprecipitation (IP) for the affinity purification of YY1 protein complexes in mouse ESCs (mESCs) in combination with mass spectrometry (MS) to construct an YY1

interactome. We report the discovery of the BAF complex as a *bona fide* YY1 partner. Mechanistically, the BAF complex associates with YY1 to activate transcription, promote ESC proliferation, and maintain pluripotency. In the presence of the BAF complex, YY1 participates in the core pluripotent network to regulate ESC pluripotency.

RESULTS

YY1 Is an Interacting Partner of OCT4 in mESCs

OCT4 is a well-known key pluripotency factor that is critical for stem cell pluripotency and somatic cell reprogramming (Niwa et al., 2000; Scholer et al., 1990; Takahashi and Yamanaka, 2006). The pluripotency protein interaction network in ESCs has been identified, and this tight protein network seems to function as a cellular module dedicated to pluripotency (Wang et al., 2006). Although the interaction of YY1 with the pluripotency module has been mentioned by Wang et al. (2006), YY1 was not found to be present in the OCT4-centered network in previous studies (Pardo et al., 2010; van den Berg et al., 2010). Nevertheless, in the work by Pardo et al. (2010), YY1 was identified in one purification of OCT4 complex, but it was not included in the final dataset because of the strict criteria of result reproducibility. Importantly, we observed a physical interaction between YY1 and OCT4 by co-immunoprecipitation, (coIP) in J1 ESCs (Figure 1A). To further inspect the functional interaction between YY1 and OCT4, we extracted the previously published knockdown dataset of *Oct4* or *Yy1* by small hairpin RNAs (shRNAs) in mESCs (Loh et al., 2006; Vella et al., 2012). We found that, among the 292 genes with significant expression changes in response to knockdown of *Yy1*, 42 were also significantly affected by knockdown of *Oct4* (Table S1). Furthermore, among these 42 genes, 29 had a similar expression alteration trend, with 11 genes ($p = 0.014$) downregulated and the other 18 genes ($p = 3.48 \times 10^{-5}$) upregulated

Figure 1. YY1 Participates in OCT4-Mediated Pluripotency Regulation

- (A) CoIP assay shows the interaction between YY1 and the endogenous OCT4 in J1 mESCs.
- (B) Heatmap depicting a significant correlation of gene expression alteration in response to knockdown of *Yy1* or *Oct4*. The correlation with $p < 0.05$ was considered as significant.
- (C) Scheme shows the biotin-dependent IP-MS system for identifying of YY1 interactome.
- (D) Western blotting for the expression of YY1 proteins. Lanes 3–10 represent the 8 cell clones transfected with *Flag-biotin-Yy1*. These cells express both exogenous Flag-biotin-YY1 protein and endogenous YY1 protein. BirA cells without transfection of *Flag-biotin-Yy1* were used as a negative control. They only express endogenous YY1 protein. β -Actin was used as a loading control.
- (E–G) Bar plots of molecular pathways enriched in (E) GO, (F) Mouse Gene Atlas (MGA), and (G) Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways analysis for 56 genes encoding proteins identified as YY1-interacting proteins in (C). These genes are listed in Table S2. Displayed on the x axis are enrichment scores as calculated by $-\log(p \text{ value})$. For MGA analysis, ES v26 p16 means the v26 ESC line at passage 16, while ES bruce4 p13 means the bruce4 ESC line at passage 13.
- (H and I) Heatmaps showing the dynamic expressions of genes encoding proteins identified as YY1, OCT4, and SOX2 interacting proteins during (H) EB differentiation and (I) early embryo development.



A



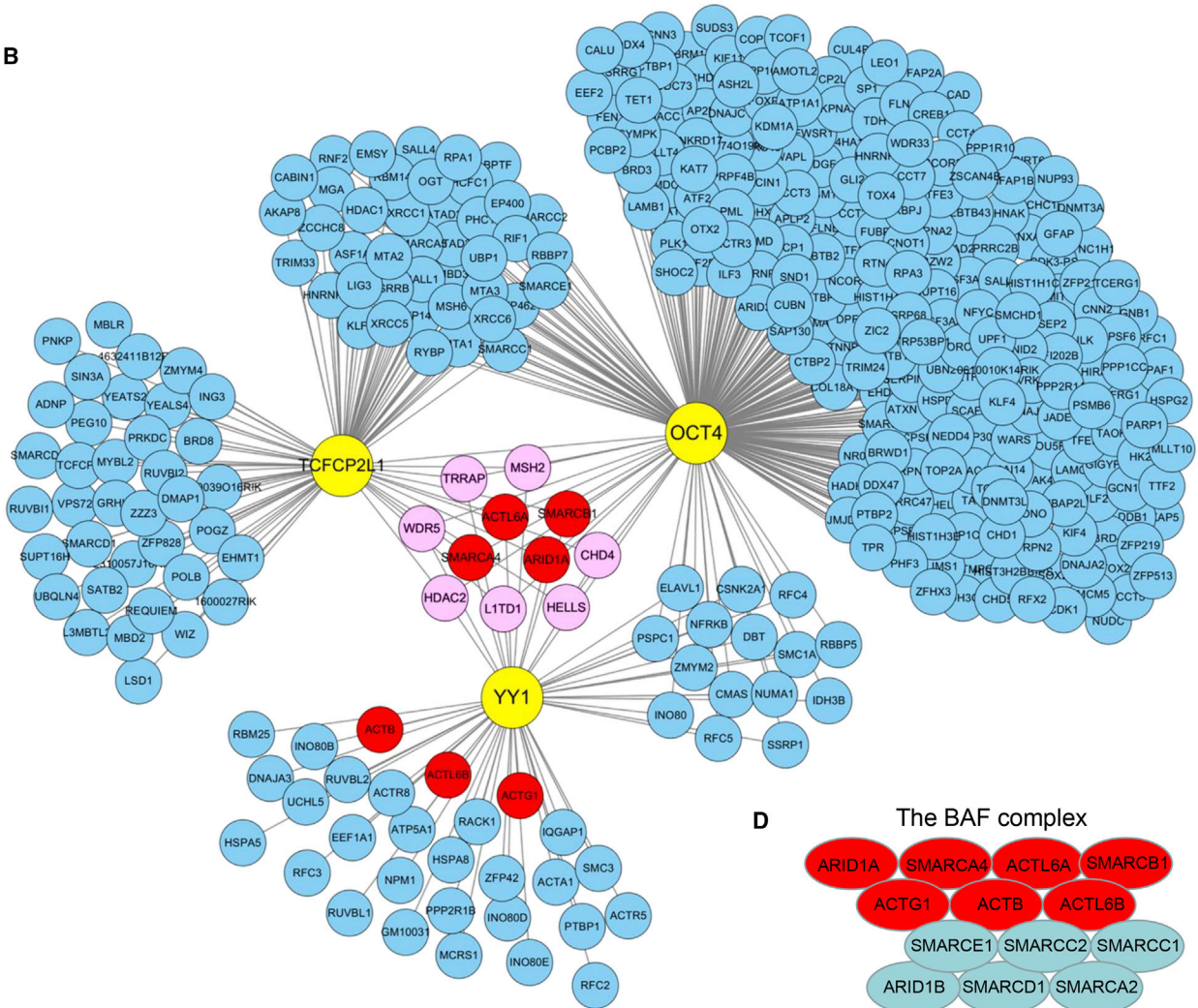
Enrichment Results

Terms	P-value
PROTEIN_POU5 F1-22083510	5.27E-30
PROTEIN_TCFC P2L1-20362541	3.46E-20
PROTEIN_POU5 F1-20362541	9.61E-17
MESC_H3K36ME 3-18692474	5.53E-16
PROTEIN_NANO G-21589869	6.57E-15
PROTEIN_SALL4 -20362541	9.30E-14
RNAISCREEN_1 8614019	2.14E-10
PROTEIN_ESRR B-20362541	7.88E-10

C

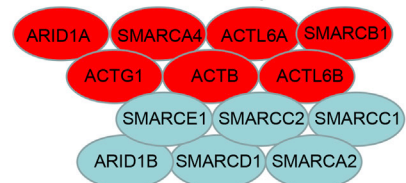
Gene	Peptide no. of YY1B3 (BirA)
ACTL6A	33(5)
CHD4	11(0)
TRRAP	9(0)
SMARCA4	9(4)
HELLS	7(4)
WDR5	3(0)
ARID1A	2(0)
HDAC2	2(0)
L1TD1	2(0)
MSH2	3(1)
SMARCB1	3(1)

B



D

The BAF complex



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(Figure 1B). These results indicate a close correlation between *Yy1* and *Oct4* in regulating gene expression and suggest functional and cooperative roles for YY1 and OCT4 in mESCs.

Generation of the YY1 Interactome in mESCs

To investigate the proteins interacting with YY1, we used the YY1B3-ESC line expressing biotinylated YY1. Overexpression of *Yy1* in YY1B3-ESC line versus BirA-ESC line (control ESC line) was validated by qPCR (Figure S1A), and the expressions of endogenous and exogenous biotin-tagged YY1 were further confirmed by western blot (Figure 1D). Similar to the R1 and BirA-ESC lines, the YY1B3-ESC line displayed dome-shaped morphology (Figure S1B), expressed wild-type levels of OCT4 and NANOG (Figure S1C), and exhibited normal ESC clonogenicity (Figures S1D and S1E). These results demonstrated that overexpression of *Yy1* did not change ESC identity. We thus pulled down YY1 protein complexes in this line using streptavidin beads, followed by MS analysis (Figure 1C).

A total of 56 interacting proteins identified by MS analysis were significantly enriched in the biotinylated YY1 purification compared with controls (Table S2). It is important to point out that, although we did uncover OCT4 in the purification (with three peptide sequence both in YY1B3 and BirA cells; Table S2), it was not included in the final candidate list because of our stringent selection criteria. Interestingly, we found 7 components of the BAF complex, 11 components of the INO80 chromatin-remodeling complex, and 4 components of the replication factor C complex (Table S3), which functionally participates in DNA topological changes, chromosomal remodeling, and organization. We also found that YY1 interacted with WDR5, HDAC2, CHD4, and RBBP5, which belong to complexes involved in transcriptional regulation and cell-cycle control (Table S3). These observations demonstrated

that YY1 might participate in various biological processes in mESCs.

Gene ontology (GO) analysis of biological processes showed an enrichment in the regulation of chromatin architecture and epigenetic modification categories (Figure 1E). The Mouse Gene Atlas analysis showed that the encoding genes of YY1-associated proteins have higher expression levels in ESC lines (Figure 1F). In addition, the Kyoto Encyclopedia of Genes and Genomes cellular pathway analysis demonstrated an enrichment of proteins involved in adherent junctions and cell cycle (Figure 1G). Regulation of adherent junctions in ESCs is important to form compact dome-shaped colonies (Stankovich et al., 2011), while cell cycle is necessary for normal self-renewal, proliferation, and division in ESCs (Re et al., 2014).

The YY1 Interactome Is Enriched for Proteins with Critical Roles in mESC Maintenance

We analyzed the expression levels of the genes encoding the YY1-interacting proteins in embryoid body (EB) differentiation, early embryonic development, and somatic cell reprogramming using previous published data (Haileselasse Sene et al., 2007; Heng et al., 2010; Tang et al., 2011). First, the majority of the coding genes for YY1-associated proteins (more than 75%) were highly expressed in ESCs and downregulated during EB differentiation (Figure 1H). Second, single-cell transcriptomic analysis of early mouse embryos (Tang et al., 2011) revealed that a large number of the coding genes for YY1-associated proteins (more than 80%) were upregulated during embryonic development from oocyte to inner cell mass and established ESCs (Figure 1I). These expression characteristics of YY1 interactome are very similar with the characteristics of OCT4 and SOX2 interactomes (Figures 1H and 1I). Third, the encoding genes of the YY1 and OCT4 interactomes exhibited similar expression characteristics during reprogramming, in which half of the genes were upregulated

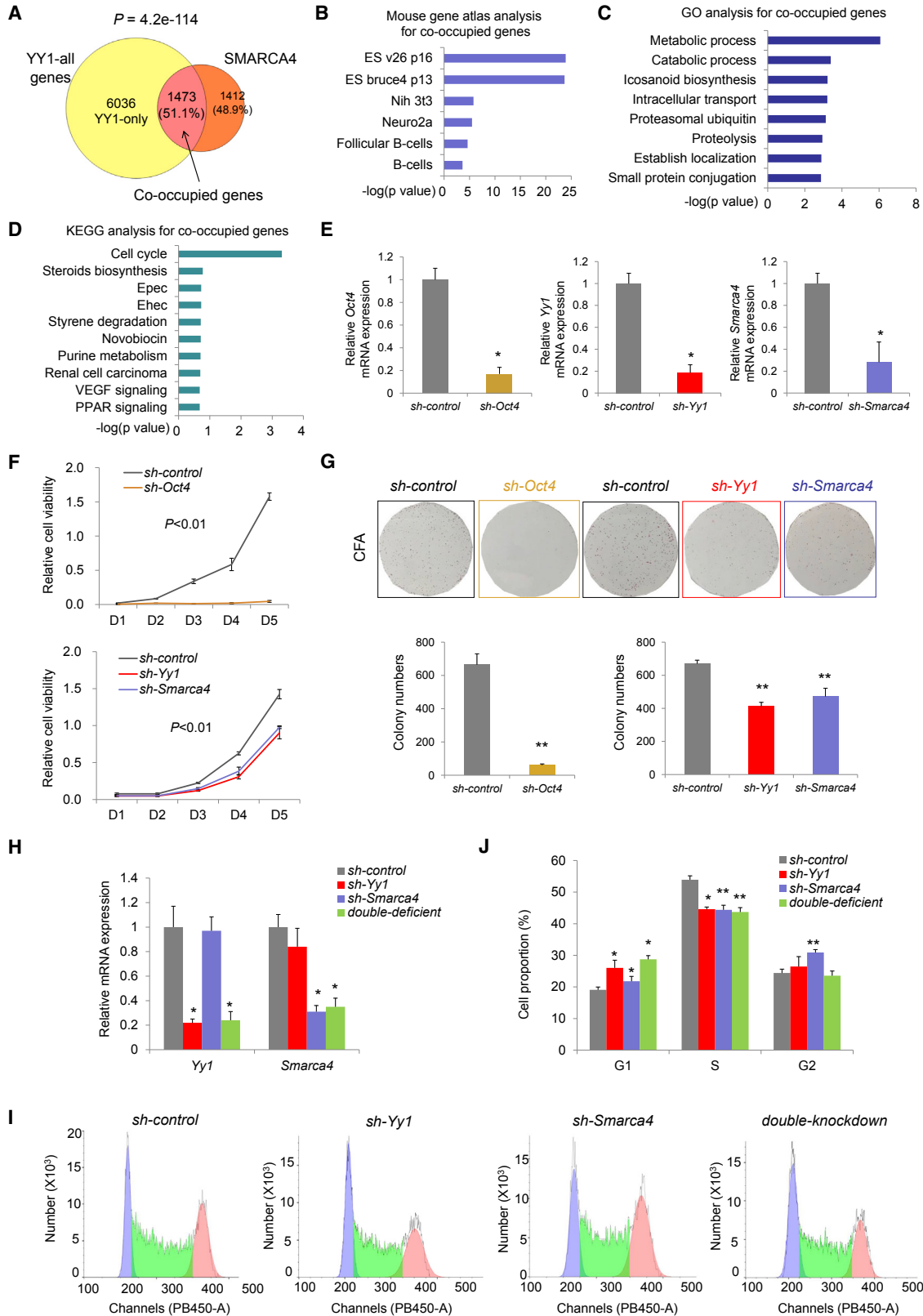
Figure 2. YY1 Is Integrated into the Pluripotency Network through Interacting with the BAF Complex

(A) ESCAPE analysis shows the OCT4 and TCFCP2L1 interactomes are significant similar to the YY1 interactome. Each square represents a list. The color indicates the experiment type, and the brightness indicates the level of local similarity among the lists. We use simulated annealing to try to arrange the lists from the ESCAPE database by their gene content similarity. The enriched terms appear as circles on top of the colored squares representing the gene lists from the ESCAPE database on the canvas: the brighter the circle the more significant the overlap with the input list. The results are also available in a table with the associated p values on the right.

(B) Diagram depicting the proteins detected by interactome analysis. The three key transcription factors, YY1, OCT4, and TCFCP2L1, are in yellow, the co-interacting proteins by all these three key transcription factors are in pink, the BAF complex subunits are in red, other proteins are in blue.

(C) List of the co-interacting proteins of the three key transcription factors YY1, OCT4, and TCFCP2L1. The peptide count of the 11 proteins represents the interaction reliability with YY1 in YY1B3 versus BirA cells. If the peptide counts of YY1-interacting protein in YY1B3 cells (left of the brackets) is more than 2-fold of that in BirA cells (in the brackets), and simultaneously more than BirA plus two, the interaction was considered to be significant.

(D) The composition of the BAF complex. YY1-interacting components are in red, others are in blue. The four red components in the top layer are co-interacted with YY1, OCT4, and TCFCP2L1. The three red components in the second layer are interacted with YY1 alone.



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while others were downregulated (Figure S2A). GO analysis showed the different functions between the up- and down-regulated genes during reprogramming (Figures S2B and S2C). These results suggest that the encoding genes of YY1 interactome have relative higher expressions in pluripotent cells, but were downregulated when pluripotency was abolished.

The BAF Complex Is Present in the YY1-Mediated Pluripotency Regulatory Network

A high correlation among the interactomes of YY1, OCT4, and TCF2L1 was observed by ESCAPE analysis (Figure 2A). Like OCT4, TCF2L1 is also a pluripotency factor, which stimulates ESC proliferation and self-renewal (Chen et al., 2008; Ivanova et al., 2006). By comparing the previously published OCT4 and TCF2L1 interactomes (Ding et al., 2012; van den Berg et al., 2010) with YY1 interactome in this study, we identified 11 co-associated proteins of the 3 factors (Figure 2B). Interestingly, 4 of the 11 co-interacting proteins belonged to the BAF complex (Figure 2C). Given that another three components of the BAF complex (ACTB, ACTL6B, and ACTG1) were found exclusively in the YY1 interactome, YY1 totally interacted with seven subunits of the BAF complex in mESCs (Figure 2D). These results suggest a strong interaction between the BAF complex and YY1 in mESC.

YY1 Interacts with the BAF Complex to Promote mESC Proliferation

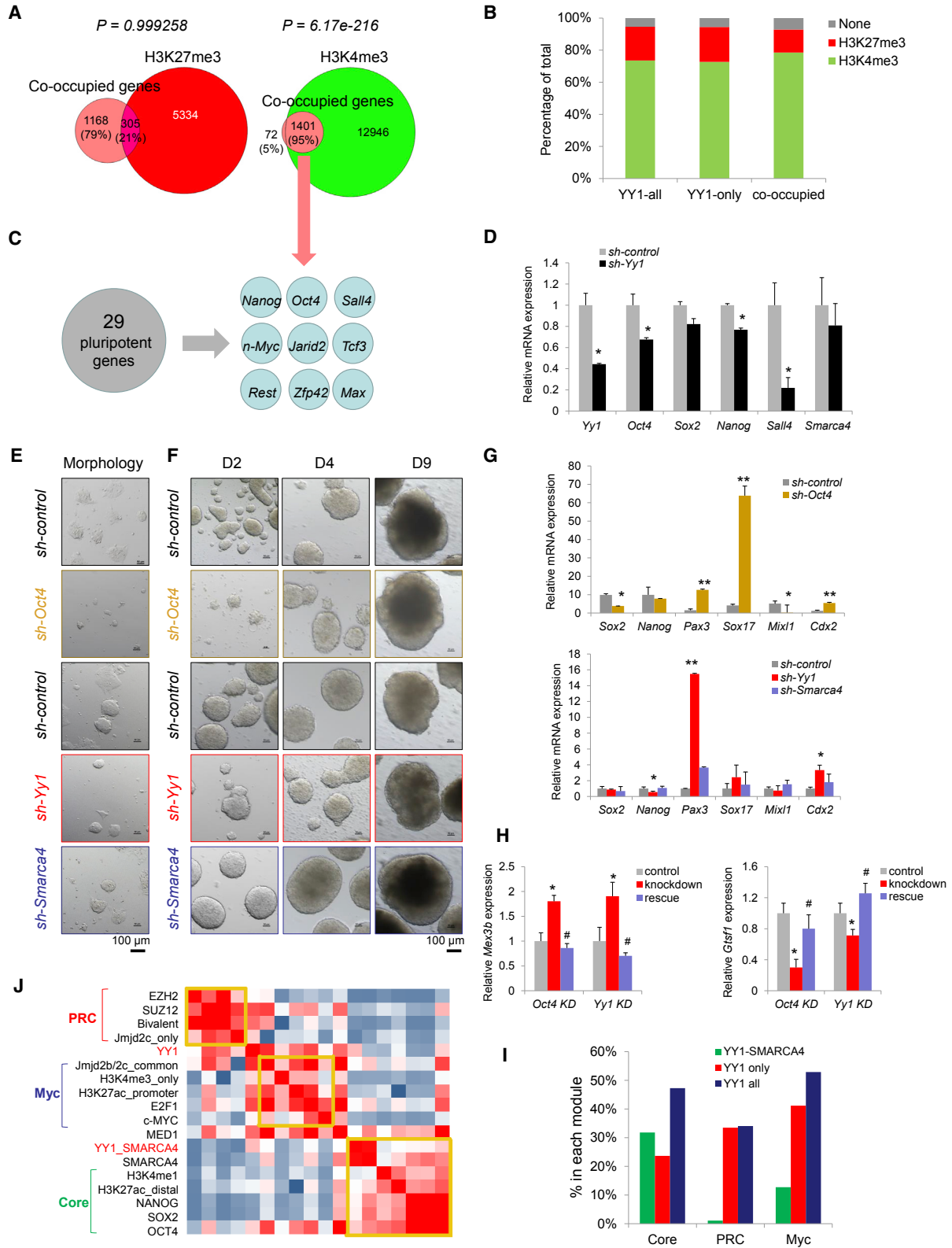
SMARCA4, also known as BRG1, is a core component of the BAF complex. It plays an important role in maintaining pluripotency by upregulating the expression of *Oct4* and other pluripotency genes (Singhal et al., 2014). We inspected the overlap of DNA binding sites between YY1

and SMARCA4, and identified 3,324 common sites (Figure S3A). The distribution analysis demonstrated that the co-binding sites were associated preferentially with promoter regions (about 44.2%), to a lesser extent with intergenic regions (about 36.5%), and only for the remaining 19% with gene body regions (Figure S3A). Nevertheless, among the 18,408 YY1-only binding regions, 35.4%, 43.1%, and 21.5% were localized at promoter sites, intergenic regions, and gene bodies, respectively (Figure S3A).

We first analyzed the YY1 binding sites at promoter regions. For the genes with their promoters only occupied by YY1 and not SMARCA4 (YY1-only genes) (Figure 3A; Table S4), functional analysis showed significant expression enrichment in ESC lines, with the roles mainly focused on the regulation of RNA metabolism, amino acid degradation, and Wnt signaling pathway (Figures S3B–S3D). Definitely, 1,473 genes with promoters co-occupied by both YY1 and SMARCA4 were identified as co-occupied genes (Figure 3A; Table S4). Like the YY1-only genes, co-occupied genes also displayed significant expression enrichment in ESC lines (Figure 3B). However, these genes enriched for various biological processes, such as cell-cycle progression (Figures 3C and 3D), which was quite different from those of YY1-only genes. The outstanding functional enrichment on cell cycle indicates the co-occupied genes may regulate cell proliferation. To prove this hypothesis, we knocked down *Oct4*, *Yy1*, or *Smarca4* by shRNA, respectively. The knockdown efficiency was measured by qPCR (Figure 3E). Knockdown of *Oct4*, *Yy1*, or *Smarca4* significantly inhibited cell viability and colony formation (Figures 3F and 3G). Moreover, deficiency of either *Yy1* or *Smarca4*, or both (Figure 3H), significantly decreased S-phase cell population but increased G1- or/and G2-phase cell population (Figures 3I and 3J). Cell-cycle arrest is

Figure 3. YY1 Interacts with the BAF Complex to Promote Cell Proliferation

(A–D) Overlapping of two groups of genes whose promoters (± 3 kb surrounding the TSS) were bound by YY1 (yellow) or SMARCA4 (orange), respectively. YY1-SMARCA4 co-occupied genes are the 1,473 genes with promoters co-targeted by YY1 and SMARCA4. YY1-only genes are the 6,036 genes with promoters targeted only by YY1 but not by SMARCA4. YY1-all genes represent all the 7,509 YY1 bound genes comprising YY1-only genes and YY1-SMARCA4 co-occupied genes. Bar plots of molecular pathways enriched in (B) MGA, (C) GO, and (D) KEGG pathways analysis for YY1-SMARCA4 co-occupied genes. Displayed on the x axis are enrichment scores as calculated by $-\log(p \text{ value})$. (E) Knockdown efficiency of *Oct4*, *Yy1*, or *Smarca4* in mESCs was validated by qPCR. Data are presented as mean \pm SD from three independent replicates. * $p < 0.05$ compared with control cells. (F) Cell proliferation was evaluated by cell counting kit 8 cell viability assay. Knockdown of *Oct4*, *Yy1*, or *Smarca4* significantly inhibited mESCs growth. The experiments were performed three times in triplicate. Data are presented as means \pm SD from three independent replicate experiments. (G) Knockdown of *Oct4*, *Yy1*, or *Smarca4* significantly suppressed colony formation of mESCs. Data are presented as mean \pm SD from three independent replicate experiments. ** $p < 0.01$ compared with control cells. (H) Knockdown efficiency of mESCs treated with sh-*Yy1*, sh-*Smarca4*, or double, respectively. Experiments were conducted in three independent replicates and results shown as mean \pm SD * $p < 0.05$ compared with control cells. (I) Distribution of cell population in G1, S, and G2 phase. Cells were stained by DAPI. The experiments were performed three times in triplicate. Blue, green, and red represent G1, S, and G2, respectively. (J) Deficiency of *Yy1*, *Smarca4*, or double decreased S-phase cell population but increased G1- or/and G2-phase cell population. Data are depicted as mean \pm SD * $p < 0.05$, ** $p < 0.01$ compared with control cells.



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consistent with the decreased cell proliferation caused by deficiency of *Yy1* or *Smarca4*. These results demonstrated that the interaction of YY1 and the BAF complex contributed to mESC proliferation.

YY1 Interacts with the BAF Complex to Maintain Pluripotency

YY1 and SMARCA4 co-occupied genes were significantly enriched for H3K4me3 (1,401 genes, 95.1%) while mildly enriched for H3K27me3 (305 genes, 20.7%), indicating that YY1 and SMARCA4 may co-occupy promoters to activate transcription in mESCs (Figure 4A). An enrichment comparison among all YY1-bound genes (YY1-all genes), YY1-only genes and co-occupied genes with representative histone markers revealed large enrichments of all the three groups for H3K4me3. Interestingly, co-occupation by SMARCA4 further elevated the H3K4me3 enrichment at YY1 binding genes (Figure 4B). Within 29 pluripotency genes (Ding et al., 2015) (Table S4), the promoters of 9 genes were co-occupied by YY1 and SMARCA4 (Figure 4C). Moreover, knockdown of *Yy1* significantly suppressed the expression of several key pluripotent genes such as *Oct4*, *Nanog*, and *Sall4* (Figure 4D), supporting the fact that YY1 is important for maintenance of pluripotency.

We evaluated the influence of *Oct4*, *Yy1*, and *Smarca4* deficiency on differentiation. Morphology analysis showed that knockdown of *Oct4*, *Yy1*, or *Smarca4* in mESCs resulted in similarly smaller and irregular colonies compared with control (Figure 4E). Knockdown of either *Oct4*, *Yy1*, or *Smarca4* did not influence the formation of EBs (Figure 4F). As expected, since OCT4 inhibits neural ectoderm and trophoblast differentiation (Niwa et al., 2000; Thomson et al., 2011), but promotes mesendoderm differentiation in the presence of BMP4 (Wang et al., 2012), knockdown of *Oct4* suppressed the expression of *Sox2* and *Nanog*, and

the mesoderm markers *Mixl1*. Simultaneously, it significantly induced the endoderm marker *Sox17*, the ectoderm marker *Pax3*, and the trophoblast marker *Cdx2* (Figure 4G, top panel). Importantly, knockdown of *Yy1* and *Smarca4* induced similar expression alterations of these markers (Figure 4G, bottom panel). To further test the correlation of YY1 and OCT4 in regulating gene expression, we investigated whether the gene expression changes resulting from either *Oct4* or *Yy1* knockdown would be similarly rescued by the re-introduction of *Smarca4*. qRT-PCR results showed that re-expression of *Smarca4* rescued the expression of *Mex3b* and *Gtsf1* (Figure 4H). Taken together, these results support that YY1 and SMARCA4 regulate pluripotency, to some extent, in an OCT4-dependent manner.

YY1 Participates in the Core Pluripotency Regulation Network through the BAF Complex

As described previously, the pluripotency transcriptional network can be classified into three distinct modules (Kim et al., 2010). We analyzed the enrichment of the members in each module for YY1-all, YY1-only, and YY1-SMARCA4 co-occupied genes, respectively. We observed that the three modules were equivalently enriched for YY1-all and YY1-only genes. However, very few genes in the PRC module, but a large portion of genes in the core module, were enriched for co-occupied genes (Figure 4I), indicating that the BAF complex may draw YY1 into the core pluripotency network. Furthermore, we collated genome-wide targets for a global view of YY1 and YY1-SMARCA4 co-bound regions along with transcription factors and histone marks (Das et al., 2014). The hierarchical clustering tree and heatmap representation of occupancy correlation revealed these three distinct ESC regulatory modules (Figure 4J). Interestingly, YY1-all regions mainly correlated with the PRC and Myc modules, whereas

Figure 4. YY1 Interacts with the BAF Complex to Promote Pluripotency

- (A) Overlapping of the YY1-SMARCA4 co-occupied genes (light red) with genes enriched for H3K27me3 (red) or H3K4me3 (Sato et al., 2004), respectively.
- (B) Histograms showing percentages of genomic regions in mESC promoters where YY1 (YY1-all, YY1-only, or co-occupied with SMARCA4) overlaps with H3K4me3 or H3K27Me3 peaks.
- (C) The pluripotent genes with active histone marker H3K4me3 are bound by YY1 and SMARCA4 simultaneously in mESCs.
- (D) Knockdown of *Yy1* decreased several pluripotency genes expression. Data are presented as mean \pm SD from three independent replicates. * $p < 0.05$ compared with control cells.
- (E) mESC morphology in response to deficiency of *Oct4*, *Yy1*, or *Smarca4*, respectively.
- (F) The influence of *Oct4*, *Yy1*, or *Smarca4* deficiency on the formation of embryoid bodies. Scale bar represents 100 μ m.
- (G) The influence of *Oct4*, *Yy1*, or *Smarca4* deficiency on the expression of the representative pluripotency and differentiation markers. Results are shown as mean \pm SD from three independent replicates. * $p < 0.05$, ** $p < 0.01$ compared with control cells.
- (H) Expression changes of *Mex3b* and *Gtsf1* in response to knockdown of *Oct4* or *Yy1* were rescued by re-introduction of *Smarca4*. Data are depicted as mean \pm SD from three independent replicates. * $p < 0.05$ compared with control cells. # $p < 0.05$ compared with knockdown cells.
- (I) Histograms showing percentages of the promoter targets of YY1 (YY1-all, YY1-only, or co-occupied with SMARCA4) overlapping with the core, PRC or Myc transcription modules.
- (J) Correlation map of binding loci showing the degree of co-occupancy between each pair of the selected proteins representative of the well-defined three modules. Red indicates more frequent co-localization of each pair of proteins.

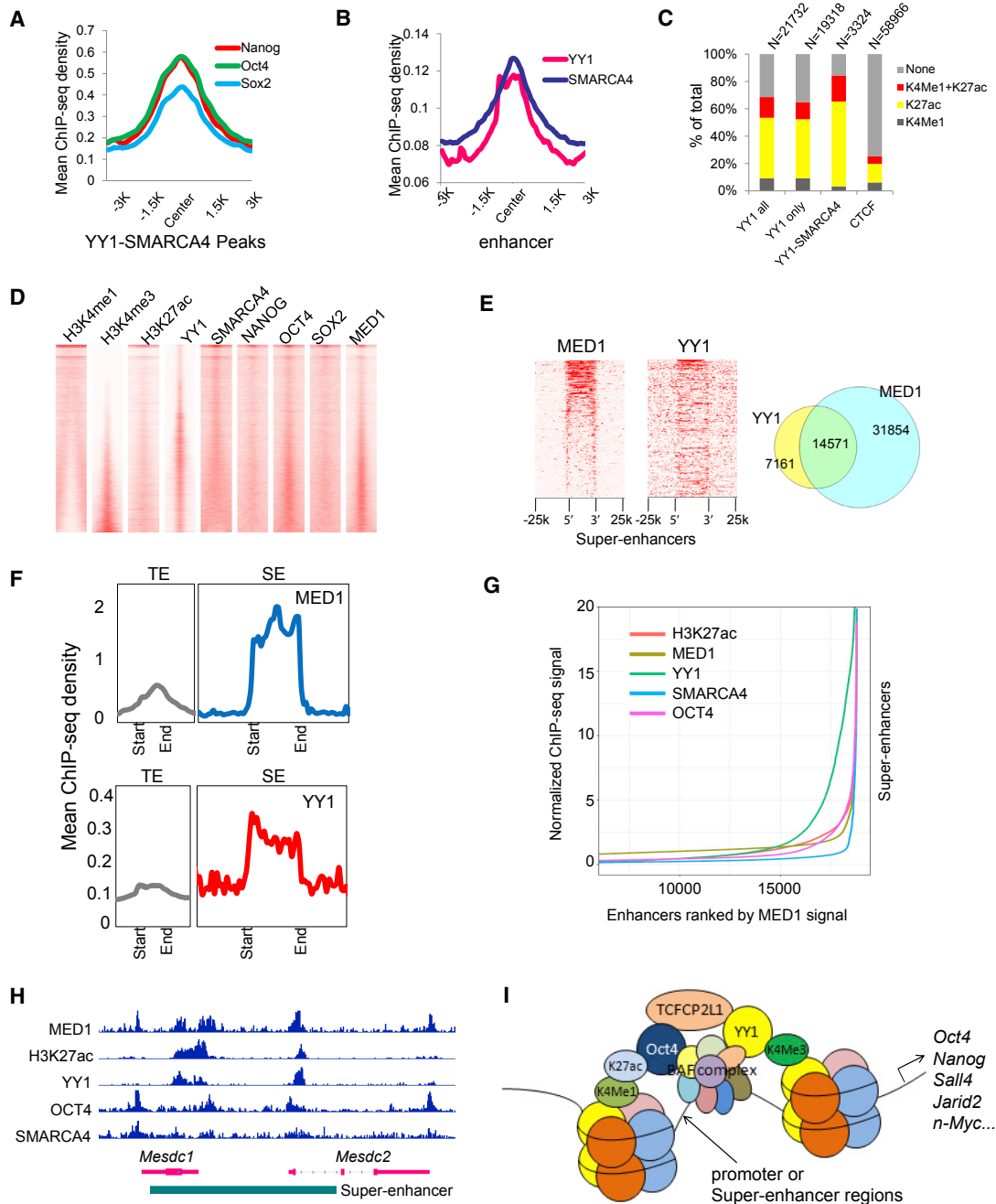


Figure 5. YY1 Positively Regulates Super-Enhancer Activity in mESCs

- (A) Average ChIP-seq read density for OSN around the YY1-SMARCA4 peaks' center.
- (B) YY1 and SMARCA4 bound regions are enriched for enhancers.
- (C) Histograms showing percentages of genomic regions in mESCs where YY1 (all, only, or together with SMARCA4) and CTCF ChIP-seq peaks overlap with H3K4me1 (K4me1), H3K27ac (K27ac), or both (K4me1 and K27ac) peaks.
- (D) Heatmaps of YY1 binding loci sorted for the enhancer mark H3K4me1 and the active promoter mark H3K4me3. H3K27ac is an active mark for both enhancers and promoters. mESC-specific SEs are co-bound by OSN, MED1, and SMARCA4.
- (E) Heatmap of YY1 and MED1 intensity at 231 mESC SEs. YY1 shares the majority of its targets with MED1.
- (F) ChIP-seq density diagrams for MED1 and YY1 showing enrichment in SE regions.

(legend continued on next page)



YY1-SMARCA4 co-bound regions segregated into the core module (Figure 4J).

We evaluated the enrichment of OCT4 or c-MYC on YY1 targets. We observed that SMARCA4 co-occupancy increased the OCT4 enrichment at YY1-bound regions from 67.4% to 80.9% (Figure S3E). Moreover, we compared the OCT4/YY1 co-target genes with the c-MYC/YY1 co-bound genes. Two gene pools, the OCT4-only (6,749 genes) and the c-MYC-only (994 genes) were established by analysis of chromatin immunoprecipitation sequencing (ChIP-seq) data (Figure S3F). The overlapping rate of c-MYC only genes with YY1-all genes was 51.7%, which is higher than that of YY1-all genes with OCT4-only genes (39.3%) (Figure S3G). Interestingly, in the presence of the BAF complex, the overlapping rate of c-MYC only genes with YY1-SMARCA4 co-occupied genes decreased to 10.6%, while the overlapping rate of YY1-SMARCA4 co-occupied genes with OCT4-only genes increased to 44.3% (Figure S3H), supportive of YY1 participation into the OCT4-mediated network via the BAF complex. Taken together, our observations indicate that transcriptional activation rather than repression of genes in the core pluripotency network is the main outcome of the interaction between YY1 and the BAF complex in pluripotent stem cells.

YY1 Positively Regulates ESC-Specific Super-Enhancers in mESCs

Active ESC enhancers are often co-occupied by multiple core pluripotency factors, including OCT4, SOX2, and NANOG (OSN) (Kagey et al., 2010; Whyte et al., 2013). Interestingly, we found that YY1 and SMARCA4 co-bound regions were enriched for OSN (Figure 5A), suggesting that YY1 may interact with the BAF complex to positively regulate enhancer activity in mESCs. We selected the enhancers based on their histone marks and if located farther than 3 kb from the transcription start site (TSS). We observed that both YY1 and SMARCA4 were enriched at enhancer regions in mESCs (Figure 5B). Importantly, YY1 and SMARCA4 co-binding regions were greatly enriched for H3K27ac and the combination of both marks (H3K4me1 and H3K27ac) (Figure 5C), suggesting a SMARCA4-dependent function of YY1 in regulating ESC enhancer activity. We sorted YY1 peaks by H3K4me1 and H3K4me3 to distinguish enhancers from promoters, and observed that both enhancer and promoter regions enriched for YY1 were

co-bound by SMARCA4 and OSN (Figure 5D). Compared with typical enhancers (TEs), ESC super-enhancers (SEs) have a larger size and an increased capability to activate transcription. They are enriched for MED1 and OSN in ESCs (Whyte et al., 2013). In our study, we observed that 67.0% (14,571 out of 21,732) of YY1 binding peaks were also occupied by MED1 (Figure 5E). Similarly, YY1 was also enriched at SEs (Figure 5F). Moreover, YY1 had a significantly stronger enrichment density in SEs than TEs (Figures 5F–5H). Therefore, our results suggest a role of YY1 as a positive regulator of gene expression through regulation of SEs in mESCs. Taken together, our data on the global transcriptional cooperation between YY1 and the core transcription network provide a regulatory mechanism whereby YY1 targets both promoters and SEs to activate the transcription of pluripotency genes (Figure 5I).

YY1 and the BAF Complex Regulate Different Metabolic Pathways in mESCs and Lineage Development

Lu et al. (2013) found an EZH2-independent function of YY1, in which YY1 acts as an activator for many loci in myoblasts. Consistent with Lu et al., we observed that YY1 target genes were not enriched for either EZH2 or H3K27me3 (only 3.3% and 5.9%, respectively) in mouse myoblasts (Figure S4A). Instead, they were enriched for H3K4me3 (more than 90%) (Figure S4B), indicating that, in mouse myoblasts, YY1 functioned mostly as a transcriptional activator similarly as in mESCs.

In mESCs, the genes targeted by YY1 partially overlapped with those targeted by H3K27me3 (26.3%) (Figure 6A), whereas they highly overlapped with those targeted by H3K4me3 (93.7%) (Figure 6B). The YY1 targets were also enriched by H3K4me3 in mouse myoblasts (Figure 6B). Interestingly, the genes co-bound by YY1 and H3K4me3 in myoblasts highly overlapped (88%) with those in mESCs (Figure 6B), indicating that YY1 may play similar roles in the two cell types via transcriptional activation. For these genes co-bound by YY1 and H3K4me3 in both cell types, GO analyses showed functional enrichments in RNA and protein metabolism (Figure S4C). On the contrary, for these genes specific to mESCs, the functional enrichments were associated with DNA metabolism (Figure S4D). YY1 also targeted certain genes in myotubes, but the amount and functions were very limited (Figures 6B and S4E).

(G) Distribution of YY1 (Sato et al.), OCT4 (pink), MED1 (yellow), SMARCA4 (blue), and H3K27ac (Chudin et al., 2002) normalized ChIP-seq density across a subset of mESC enhancers defined by MED1 ChIP-seq signals. For each ChIP-seq data point, we normalized the plot by dividing the ChIP-seq signals by the maximum signals individually, and we sorted them in an ascending order.

(H) ChIP-seq binding profiles for MED1, H3K27ac, YY1, OCT4, and SMARCA4 at the *Mesdc1/2* locus in mESCs. Gene models are depicted below the binding profiles. The SEs and scale bars are depicted below the binding profiles.

(I) The mechanistic model of YY1 in regulating pluripotency in mESCs. YY1 interacts with the BAF complex to bind the SE regions to promote pluripotent genes expression. OCT4 and TCF2L1 also participate in this process.

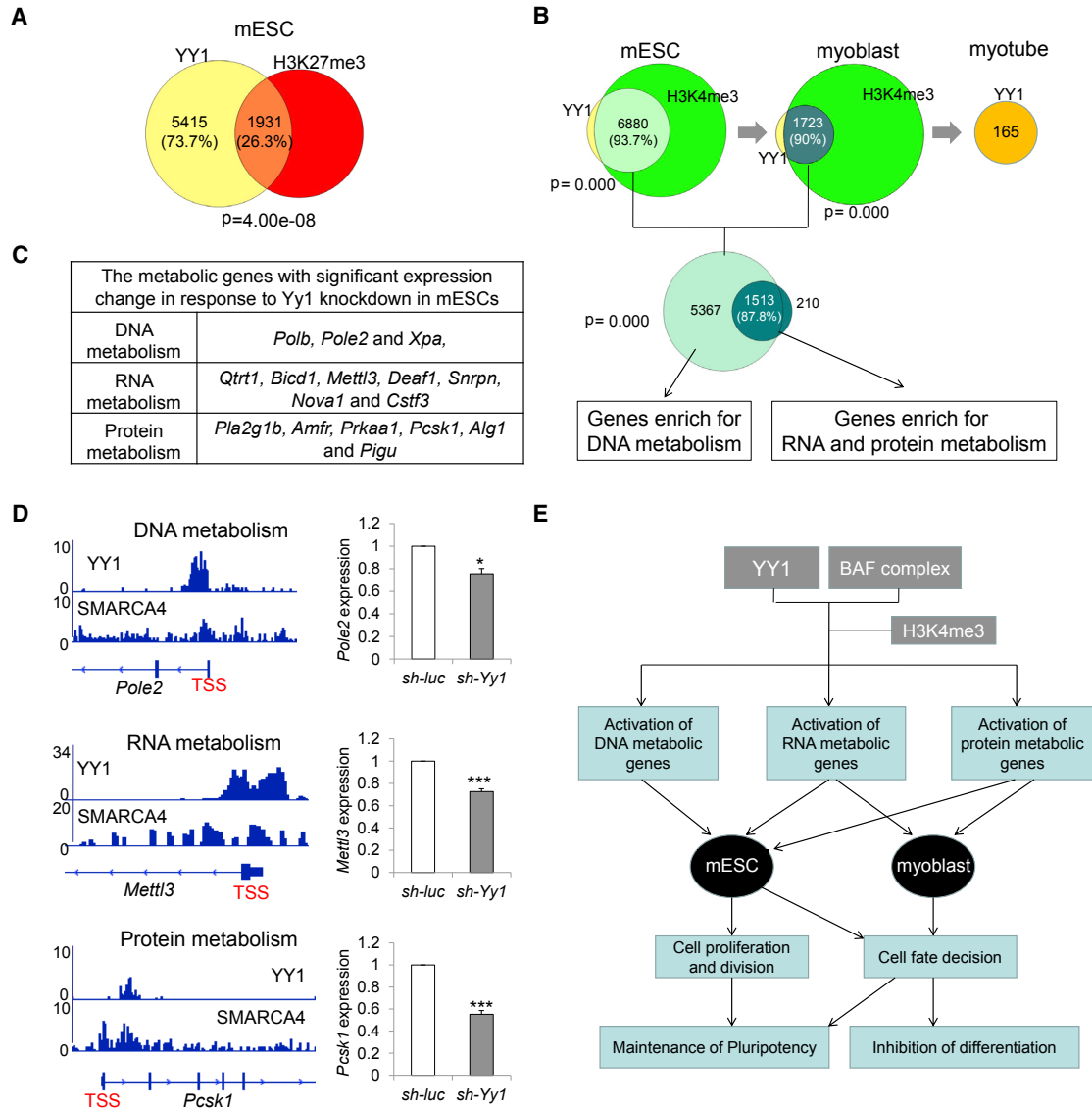


Figure 6. YY1 Regulates Different Metabolic Pathways in mESCs and Lineage Development with the BAF Complex

(A) Overlapping of YY1 (yellow) and H3K27me3 (red) targets in mESCs.

(B) Venn diagrams shows the overlap between YY1 target genes (yellow) and H3K4me3 enrichment (Sato et al.) in mESCs (upper left) and mouse myoblasts (upper middle). YY1 only targets few genes in mouse myotube (yellow, upper right). The bottom panel shows the overlap between genes co-bound by YY1 and H3K4me3 in mESCs (light green) and in myoblasts (deep green).

(C) The list of metabolic genes with significant expression change in response to Yy1 knockdown in mESCs.

(D) ChIP-seq binding profiles for YY1 and SMARCA4 at the loci of representative genes for DNA, RNA, and protein metabolism in mESCs (left). The TSS is marked in red at the beginning of each individual gene. The impact of Yy1 knockdown on gene expression is shown for each ChIP-seq binding profile (right). The statistical significance was obtained by the t test. * $p < 0.05$, *** $p < 0.001$ compared with control cells.

(E) Schematic diagram of the molecular mechanism of YY1-BAF complex in positive regulation of specific genes involving in maintenance of pluripotency and cell fate decision in different cells.

Knockdown of YY1 in mESCs resulted in notable expression changes of several genes involved in the DNA, RNA, and protein metabolism (Figure 6C). These genes which were co-bound by YY1 and SMARCA4 were downregulated

in response to Yy1 knockdown (Figure 6D). These results demonstrate that YY1 cooperates with the BAF complex to induce transcriptional activation of metabolic genes to control pluripotency and cell fate in stem cells (Figure 6E).

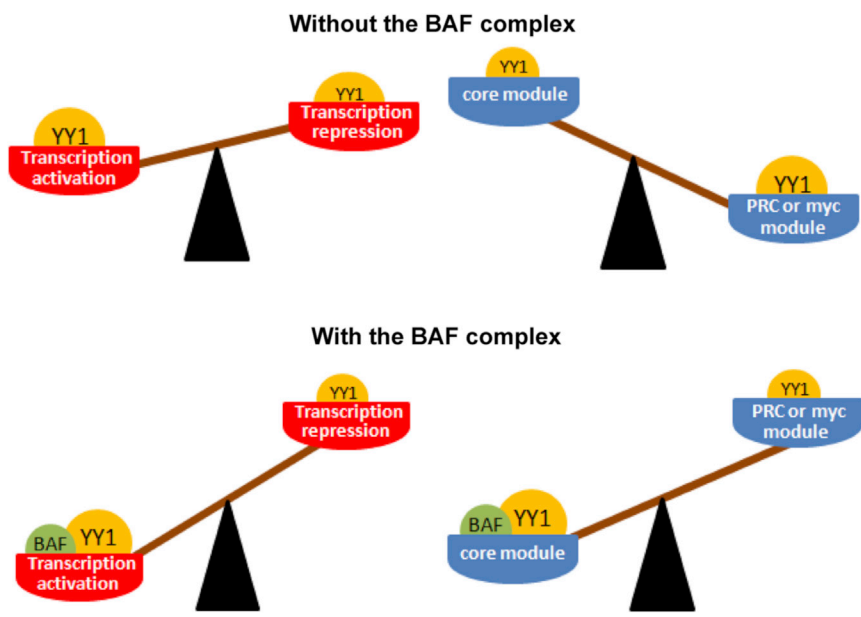


Figure 7. The Function Model for YY1 in the Absence or Presence of the BAF Complex

Without the interaction with the BAF complex, YY1 prefers to promote transcription activation, and is involved in the PRC- or MYC-related transcription factor network in mESCs (upper panel). With the interaction with the BAF complex, the transcriptional activation function of YY1 is enhanced, and the regulatory mode shifts to the core pluripotency network (lower panel).

DISCUSSION

Our study validated YY1 as an interacting partner of OCT4 for the regulation of pluripotency in mESCs, and identified the BAF complex among YY1 interactors. The BAF complex has been shown to be not only essential for maintenance of pluripotency, but also paramount in enhancing the efficiency of somatic cell reprogramming (Kadoch et al., 2013; Singhal et al., 2010). We demonstrated that the BAF complex participated in the YY1-mediated pluripotency regulation, promoted ESC proliferation, and was necessary for YY1 to modulate its activity in two ways (Figure 7). The first manner was to enhance YY1-mediated transcription activation. Our data strongly support that YY1 promotes transcription of the pluripotency genes to maintain mESCs, largely through the interaction with the BAF complex. ESCs are characterized by hyperdynamic and open chromatin, which is compacted when these cells differentiate to multiple lineages (Meshorer et al., 2006). To maintain the pluripotent state, chromatin remodelers are required to prevent this chromatin compaction, therefore facilitating the expression of pluripotency genes. Evidence showed that chromatin remodelers, such as the BAF and INO80 complexes, are crucial for the reversal of development and the reactivation of pluripotency genes such as *Oct4* and *Nanog*, which occurs during the nuclear reprogramming of a somatic cell back into an ES-like cell state (Ho and Crabtree, 2010). Importantly, we found YY1 interacted with many components of the BAF and INO80 complex, indicating chromatin remodeling played key role in YY1-mediated transcriptional activation.

Here we proposed a second role for the BAF complex, which is to anchor YY1 into OCT4-mediated core pluripotency network. Although a recent paper reported that YY1 participated in the MYC-related transcriptional network in regulating pluripotency (Vella et al., 2012), these findings are not incompatible with our observations since our study takes into consideration the presence of the BAF complex in regulating YY1's function. By associating with the BAF complex, YY1 preferentially formed transcriptional activation complexes with OCT4 to bind gene promoters, enhancers, and, in particular, SEs. This role of YY1 would further induce the expression of core pluripotency genes including *Oct4*, *Nanog*, and *Sall4*, thereby forming a positive regulatory loop in the core pluripotent network.

Further, we provided multiple lines of evidence, which integrate YY1 into the core pluripotency network rather than the MYC- or PRC-related network. First, we identified a direct interaction between YY1 and OCT4 by coIP assays, and our interactome analysis showed a noticeable correlation between YY1 and OCT4 interactomes. Second, mESCs express a complex called esBAF, which is mainly characterized by containing the BAF subunit SMARCA4 rather than BRM (Ho et al., 2009a). This complex regulates the core pluripotency transcriptional network in mESCs (Ho et al., 2009b). Third, the promoter and SE regions co-occupied by YY1 and the BAF complex were also enriched for OCT4 and H3K4me3, indicating an OCT4-dependent transcriptional activation function of YY1 in mESCs. Last, by collating YY1 and SMARCA4 co-transcriptome with the three pluripotency modules, we found a relative higher overlap of the YY1/SMARCA4 co-transcriptome with the core module. Taken together, we conclude that YY1 is



integrated into the core network for pluripotency regulation through the BAF complex.

In summary, our study provides strong evidence that YY1 is integrated into the core pluripotency network through the BAF complex. YY1 occupies the promoter and SE regions of its target genes and initiates their transcription. YY1 regulates DNA, RNA, and protein metabolic processes in stem cells together with the participation of the BAF complex.

EXPERIMENTAL PROCEDURES

Cell Line Generation, Manipulation, and Culture

All mouse ESC lines, including J1, R1, ZHBTc4, BirA, and YY1B3, were cultured in the defined medium as described in [Supplemental Experimental Procedures](#).

coIP and Western Blot Analysis

Nuclear extracts were prepared from J1 mESCs. Endogenous OCT4 was immunoprecipitated with 5 μ g of OCT4 antibody (Santa Cruz, sc5279) pre-bound to Protein G agarose beads (Roche Diagnostics), and co-immunoprecipitated YY1 was identified by western blot with a YY1 antibody (Santa Cruz, sc281). TUBULIN (Abcam, ab6046) and β -ACTIN (Sigma, A5441) were used as loading control.

Nuclear Extraction, Affinity Purification, and MS Analysis

We employed a YY1 transgene with a Flag-biotin dual tag for the affinity purification of YY1 protein complexes using our well-established protocols ([Faiola et al., 2014](#)). Detailed procedures are provided in [Supplemental Experimental Procedures](#).

Vector Construction

The shRNAs used in this study to knockdown *Oct4*, *Yy1*, or *Smarca4* are *plko-puro-shOct4*, *plko-BSD-shYy1*, and *plko-BSD-shSmarca4*. The detailed information for vector construction is described in [Supplemental Experimental Procedures](#).

RNA Extraction and Real-Time qPCR

Total RNA was extracted from cell pellets using RNeasy reagent (MRC, Cincinnati, OH, USA). Detailed procedures are provided in [Supplemental Experimental Procedures](#). The primers are listed in [Table S5](#).

Cell-Viability Assay

The cell counting kit 8 (Dojindo, Japan) was used for the determination of the number of viable cells. Detailed procedures are provided in [Supplemental Experimental Procedures](#).

Colony-Formation Assay

On the fourth day after infection by shRNA lentivirus, 1,000 R1 mESCs with knockdown of *Oct4*, *Yy1*, or *Smarca4* were seeded into individual wells of a 6-well plate. After a 5-day culture, the cell colonies were stained for alkaline phosphatase. Experiments were performed three times in triplicates.

Cell-Cycle Analysis

Yy1-, *Smarca4*-, and double-deficient R1 mESCs were used for cell-cycle analysis. Detailed procedures are provided in [Supplemental Experimental Procedures](#).

Binding Site Analysis

As input for binding site analysis, previously published raw ChIP-seq datasets were used, and the corresponding GSE numbers are summarized in [Table S6](#). Detailed analysis procedures are provided in [Supplemental Experimental Procedures](#). The called peaks and their annotations are listed in [Table S7](#).

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures, four figures, and seven tables and can be found with this article online at <https://doi.org/10.1016/j.stemcr.2018.02.004>.

AUTHOR CONTRIBUTIONS

J.W., X.W., and C.W. designed and performed experiments, analyzed data, and wrote the manuscript. X.H., T.H., D.P., L.C., L.W., and J.R. provided bioinformatics support. Q.M., X.H., X.L., and J.S. helped to complete the supplemental experiments. F.F., D.G., M.F., H.Y., X.L., X.C., and X.C. helped with raw data collection, manuscript writing, and helpful discussion. J.D. and J.W. conceived the project, designed the experiments, analyzed data, and prepared and approved the manuscript.

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