YY1 Is Required for Posttranscriptional Stability of SOX2 and OCT4 Proteins

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Abstract

Yinyang1 (YY1) participates in protein-DNA, protein-RNA, and protein-protein interactions and regulates developmental processes and disease mechanisms. YY1 interactions regulate a range of important biological functions, including oocyte maturation, epithelial to mesenchymal transition, and vascular endothelial growth factor (VEGF) signaling. We tested the hypothesis that YY1 is required for inner cell mass (ICM) lineage commitment during preimplantation development. In this study, we document gene expression patterns and protein localization of key transcription factors in *Yy1* global, tissue-specific, and dsRNA-mediated knockout/down embryos. YY1 protein was found in cells of preimplantation and peri-implantation embryos, and adult tissues where two isoforms are observed. In the absence of YY1, OCT4 and SOX2 protein were lost in the ICM during preimplantation and naive neuroectoderm during gastrulation stages, yet no difference in Oct4 or Sox2 mRNA levels was observed. The loss of OCT4 and SOX2 protein occurred specifically in cells that normally express both OCT4 and SOX2 protein. These observations support a role for YY1 mediating and/or regulating the interaction of OCT4 and SOX2 at a posttranscriptional level. Our results suggest that distinct mechanisms of YY1-mediated molecular regulation are present in the early embryo, and may offer insight to promote lineage commitment in *in vitro* cell lines.

Keywords: Yin-yang1, OCT4, SOX2, pluripotency complex, posttranscriptional maintenance, inner cell mass, ES cells, blastocyst, preimplantation, gastrulation

Introduction

THE TRANSCRIPTION FACTOR and epigenetic regulator Yinyang1 (YY1) has been shown to be involved in developmental processes and disease mechanisms through a multitude of protein-DNA, protein-RNA, and protein-protein interactions. Direct interaction of YY1 and its consensus DNA sequence results in positive transcriptional regulation of some genes, yet yields negative regulation of others. The effects of YY1 interaction occur in a cell context-specific manner and include a range of important biological functions, including regulation of Xist transcriptional activation, oligodendrocyte progenitor differentiation, myogenic differentiation, and others (He et al., 2007; Makhoul et al., 2014; Zhou et al., 2015).

Tissue-specific loss of YY1 results in a plethora of developmental phenotypes, as revealed by characterization of mouse models with distinct YY1 abrogation strategies. For example, embryos homozygous for a *Yy1* knock out (KO) allele are peri-implantation lethal (Donohoe et al., 1999), oocyte-specific loss leads to a disruption of oocyte maturation and granulosa cell expansion (Griffith et al., 2011), epiblast-specific loss leads to impaired epithelial to mesenchymal transition and dysregulation of Nodal signaling (Trask et al., 2012), and *Yy1* is required in visceral endoderm to maintain vascular endothelial growth factor (VEGF) signaling and yolk sac development (Rhee et al., 2013).

In the study presented herein, we tested the hypothesis that YY1 is required for inner cell mass (ICM) lineage commitment. We examined gene expression and protein localization of pluripotency factors OCT4 and SOX2 in *Yy1* KO or knockdown blastocysts and tissue-specific and dsRNA-mediated knockdown models. Surprisingly, our results indicate that in cells of the ICM, YY1 is required for both OCT4 and SOX2 protein localization and stability, despite normal levels of mRNA. We confirmed this result in the neuroectoderm of gastrulating embryos where OCT4 and SOX2 are also expressed in the same cells. After epiblast-specific deletion of YY1, OCT4 and SOX2 protein are lost despite no change in mRNA expression levels, but only in cells that normally express both OCT4 and SOX2.

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Our finding that OCT4 localization in the primitive streak does not require YY1 suggests two distinct mechanisms of molecular regulation and supports a role for YY1 in control of OCT4 and SOX2 at the posttranscriptional level. These results also suggest that YY1 may be used as a molecular tool to promote lineage commitment in specific cell lines, and suggest a potential mechanism in which YY1, known to interact with OCT4 in SOX2 in stem cells, stabilizes pluripotency complexes that include OCT4, SOX2, YY1, and other proteins.

Materials and Methods

Mice

All mouse work was performed with approval from the University of Massachusetts IACUC. Wild-type, *Yy1*^{-/+}, *Yy1* fl/fl, and *Yy1*^{-/+}; Sox2Cre + mice were bred to produced control, null, or epiblast-specific KO embryos and adult tissue protein lysates. Embryonic and adult tissue were stored at -80°C for RNA extraction, processed immediately for protein extraction, or fixed with paraformaldehyde (PFA) for histology. Blastocysts were flushed from uterine tubes and fixed with 4% PFA for 20 minutes at room temperature. Peri-implantation uterus tissue and gastrulating embryos were fixed in 4% PFA at 4°C overnight. At least three embryos per genotype were analyzed for each experiment; specific numbers are noted throughout.

Embryo culture

B6D2F1 female mice (8–10-week old, Jax No. 100006) were superovulated with 10 IU PMSG (Sigma) followed by 10 IU hCG (Sigma) 48 hours later. Zygotes were collected at 21 hours post-hCG treatment from B6D2F1 female mice mated to B6D2F1 males. Zygotes were cultured in the KSOM medium (EmbryoMax[®]; Millipore) and incubated at 37°C in 5% CO₂/5% O₂ balanced in N₂.

dsRNA preparation and microinjection

DNA templates for T7-RNA polymerase-mediated dsRNA production were amplified from genomic DNA or preimplantation embryo cDNA using primers that contained the T7 binding sequences followed by gene-specific sequences as follows: dsGfp forward: TAATACGACTCACTATAGGGCACA TGAAGCAGCAGCTT and reverse: TAATACGACTCAC TATAGGGTGCTCAGGTAGTGGTTGTCG, dsYy1 forward: TAATACGACTCACTATAGGGAATAAGAAGTGGGAGC AGAAG (JM-YY1-T7F1) and reverse: TAATACGACTCA CTATAGGGCAGATGCTTTCTCATAGCAGAGTT (JM-T7YY1-R1). Polymerase chain reaction (PCR) products were purified by gel extraction (Qiagen spin column). *In vitro* transcription was performed using the T7 MEGascript Kit (Ambion) and 0.5 µL of TURBO RNase-free DNase was added to each 10 µL reaction to remove the DNA template.

dsRNA was treated with NucAway Spin Columns (Ambion) to recover the dsRNA, while removing salts and unincorporated nucleotide. The dsRNA was then extracted with phenol:chloroform, precipitated with 70% ethanol, and resuspended in RNase-free water. The quality of dsRNA was confirmed twice by electrophoresis (after *in vitro* transcription and after precipitation). The concentration of dsRNA was measured by NanoDrop, diluted to 1 µg/µL, and stored at -80°C until use. dsRNA was microinjected into the cytoplasm

of zygotes, 2-cell blastomeres, or meiosis II (MII) oocytes using a Piezo-drill (Prime Tech) and Eppendorf Transferrman micromanipulators. 1 µg/µL dsRNA was loaded into a microinjection pipette and constant flow was adjusted to allow successful microinjection. Approximately, 5–10 picoliters of RNA was injected into the cytoplasm of each embryo or oocyte.

RNA extraction, cDNA synthesis, and quantitative PCR

RNA was extracted using the Roche High Purity RNA Isolation Kit according to the manufacturer's directions (Roche, 11828665001). cDNA was made with 1000 µg of total RNA per sample. Quantitative reverse transcription-PCR (RT-PCR) was performed using TaqMan Gene Expression Assays (Oct4: Mm00656129_gH and Sox2: Mm00488369) and PerfeCTa qPCR SuperMix, Low ROX (Quanta Biosciences No. 95052-02K), and run on a Stratagene 3001mx Q-PCR machine using Quanta's recommended cycling conditions. Quantification was normalized to GAPDH (4352339E-0806018; Applied Biosystems).

Immunoblotting

Embryo lysates were collected and treated with a protease inhibitor cocktail (Roche). Lysates were denatured at 90°C in Laemmli buffer containing β-mercaptoethanol, and run in a 10% sodium dodecyl sulfate–polyacrylamide gel electrophoresis gel. Forty micrograms of protein was loaded per well. Proteins were then transferred to a polyvinylidene difluoride membrane, and blots were blocked with 5% milk. YY1 antibody (Santa Cruz; sc1703, 1:1000) was detected with peroxidase-conjugated anti-rabbit secondary antibody (1:5000). Enhanced chemiluminescence (ECL) was used to detect horseradish peroxidase (HRP) signal. To determine loading control values, the Western blot was stripped with Restore Plus Western Blot Stripping Buffer (Thermo Scientific PI-46430), reprobed with GAPDH (Millipore MAB374; 1:1000), and detected with peroxidase-conjugated anti-mouse secondary antibody (1:5000). Densitometry was performed with ImageJ.

Immunostaining

Samples were dissected and fixed in 4% PFA, dehydrated into 100% EtOH, treated with xylenes, and embedded in paraffin wax at 60°C. Sections were cut at 7 µm and dried overnight before further processing. Immunofluorescence (IF) was performed with heat mediated antigen retrieval in 0.01 M Tris base pH 10.0, followed by treatment with a 0.5% milk block and overnight antibody incubations at the concentrations listed below. Secondary antibodies were incubated for 1 hour at room temperature. Tissue was counterstained with DAPI dilactate before mounting and curing with ProLong Gold Antifade Reagent (Invitrogen). Digital images were taken with a Nikon Eclipse TE-2000-S microscope (Nikon) and Nikon Elements software. Cell locations were counted manually by visual observation of their morphological position in blastocysts.

The following antibodies and antibody concentrations were used: CDX2 (BioGenex; AM392-5 M, 1:200); E-cadherin (BD Biosciences; 610181, 1:250); OCT3/4 (Santa Cruz; sc5279 1:200); SOX2 (Santa Cruz; sc17320, 1:200); YY1 (Santa Cruz; sc1703, 1:100); 488 donkey anti-mouse (Invitrogen; A-21202, 1:500); 488 donkey anti-rabbit (Invitrogen; A-21206, 1:500);

FIG. 1. Nuclear YY1 protein is detected in the preimplantation and peri-implantation mouse embryo by IF. Zygotes were collected and cultured to specific preimplantation stages, fixed, and whole-mount IF with DAPI and YY1 antibody (A–E). Stages examined included the following: 2-cell (A), 4-cell (B), 8-cell (C), morula (D), and blastocyst (E). Peri-implantation YY1 protein localization was assessed on E4.5 uterine sections counterstained with E-cadherin antibody in red and DAPI nuclear stain in blue (F). A'–F' show single channel localization of YY1. IF, immunofluorescence; YY1, Yinyang1.

546 donkey anti-mouse (Invitrogen; A-10036, 1:500); and 546 donkey anti-rabbit (Invitrogen; A-10040, 1:500).

Results

To test the hypothesis that YY1 is required for ICM lineage commitment, we examined gene expression and protein localization of heterodimerizing pluripotency factors OCT4 and SOX2 in Yy1 global, tissue-specific, and dsRNA-mediated knockdown models.

We first examined YY1 protein localization patterns in the preimplantation and peri-implantation embryo (Fig. 1). We observed that YY1 primarily localized to the cytoplasm of 2-cell embryos, and displayed predominantly nuclear localization

in 4-cell, 8-cell, morula, and blastocyst-staged embryos (Fig. 1A–E). Nuclear YY1 was also observed in cells of the implanting mouse blastocyst, where staining appeared stronger than it did in the surrounding uterine luminal epithelium and decidual cells (Fig. 1F). We then assessed presence of YY1 by Western blotting. We assayed protein lysates generated from MII, 1-cell, 2-cell, 8-cell, morula, blastocyst-staged, embryonic day (E) 11.5, and E14.5 embryos, as well as 3T3 cells and several adult tissues (brain, liver, heart, uterus, and ovary).

Consistent with IF, YY1 was detected in all tissues examined, with the exception of 1-cell embryos (Fig. 2A). The YY1 antibody detected two discrete bands in the majority of tissue lysates, one at ~60 kDa and one at ~55 kDa. The relative intensity of the isoforms varied across the tissues.

FIG. 2. YY1 is detected in embryonic and adult tissue by immunoblotting. YY1 was detected in embryonic tissue, 3T3 cells, and adult tissues, and GAPDH was used as a loading control (A). Two bands at ~60 and ~55 kDa were observed with varying degrees of expression in different tissues (A). YY1 protein isoforms were assessed across tissues by densitometry (A, B). Initially, only the larger YY1 band is observed (B). The lower 55 kDa band appears as development progresses, and all adult tissues examined contain both forms of YY1 (B).

FIG. 3. YY1-null peri-implantation blastocysts are negative for OCT4 and SOX2 protein. Uterus sections containing day 4 peri-implantation embryos were stained with OCT4, YY1, and DAPI (A–H) or SOX2, YY1, and DAPI (I–P). YY1-null embryos were identified by the lack of YY1 protein, and maternal tissue was used as an intrinsic positive antibody control (E–H, M–P). YY1-null embryo displayed a loss of OCT4 and SOX2 protein, compared to controls. Both a small blastocoel cavity (*arrowhead*) and small ICM-like clusters of cells (*arrow*) were observed in YY1-null embryos. A'–D', DAPI; A''–D'', YY1; A'''–B''', OCT4; C'''–D''', SOX2.

Total YY1 protein was assessed by densitometry of both YY1 bands and GAPDH. These data suggested that YY1 protein levels are highest at the blastocyst stage. Relative proportions of the two YY1 isoforms indicate that early embryos primarily contain the upper band, whereas adult tissues contain both bands (Fig. 2B).

The abundance of YY1 protein in the mouse blastocyst, coupled with the reported peri-implantation lethality of YY1-null embryos, supports that YY1 plays a critical role in the blastocyst where appropriate lineage allocation is required for successful development. To assess lineage commitment of ICM cells that lacked YY1, Yy1 heterozygous

FIG. 4. Absence of OCT4 and SOX2 is recapitulated in dsYy1-injected embryos. Mouse zygotes were injected with either dsYy1 or dsGFP and cultured to day 4. Blastocysts were stained whole mount with OCT4, YY1, and DAPI (A–H) or SOX2, CDX2, and DAPI (I–P). Despite specification of CDX2-negative, ICM-like cluster of cells (*arrow*), dsYy1 embryos lack normal OCT4 and SOX2 protein localization (E–H, M–P).

mice were intercrossed and blastocysts were examined both *in utero* and *ex vivo* after removal from the uterus. Heterozygous intercrosses generated presumptive YY1 mutant blastocysts at expected ratios (~25% in which no YY1 protein was detected, Supplementary Fig. S1; Supplementary Data are available online at www.liebertpub.com/cell).

We also collected uterine tissue from heterozygous intercrosses 4 days after mating, and examined YY1-null embryos *in utero*. We observed a remarkable loss of both OCT4 and SOX2 protein in YY1-null embryos (presumptive YY1 KO, Fig. 3). Although the YY1 negative embryos appeared smaller and misshapen, a cluster of ICM-like cells and a small blastocoel were present (Fig. 3B, D). To generate higher numbers of embryos and to confirm these observations, we utilized a tractable RNA interference (RNAi)-induced YY1 knockdown to reliably identify YY1-null embryos (see Materials and Methods section for zygote RNAi).

Both dsYY1 and control injections (dsGFP) displayed similar blastocyst development rates (Supplementary Fig. S2). Importantly, dsYy1 embryos recapitulated the loss of OCT4 and SOX2 protein observed in YY1-null blastocysts (Fig. 4A–P). We analyzed the total number of embryonic

cells, the number of OCT4+ cells, and the number of SOX2+ cells in dsGFP and dsYy1 embryos. Although dsYY1 embryos had no change in the total number of cells, Oct4+/Sox2+ double-positive cells were significantly decreased, as was the percentage of Sox2+ cells per embryo (Fig. 5A–F).

We next examined mRNA levels of Oct4 and Sox2 to determine if YY1 exerts transcriptional or posttranscriptional changes. Surprisingly, we observed no loss of Sox2 or Oct4 mRNA on day 3 or 4, following dsRNA injection (Fig. 5G, H), indicating that the loss of OCT4 and SOX2 protein in the absence of YY1 is posttranscriptional in nature.

To confirm these findings, we also examined embryos with a conditional deletion of YY1 specifically in the epiblast. During gastrulation, Oct4+ epiblast cells ingress through the primitive streak, resulting in the formation of the three germ layers: ectoderm, mesoderm, and endoderm. At E7.5, the primitive streak is Oct4+ and Sox2–, but the anterior ectoderm (prospective neuroectoderm) is Oct4 and Sox2 double positive. We generated E7.5 wild-type and epiblast-specific Yy1-null embryos through the use of a *Loxp/Cre* system with floxed Yy1 mice and Sox2Cre mice, as described in detail in a previous publication from our

FIG. 5. The number of OCT4- and SOX2-positive cells is reduced in dsYy1 blastocysts, but no difference in RNA levels is observed. The total number OCT4+ and Sox2+ cells was reduced in dsYy1 embryos (A, D) and a slightly reduced percent of OCT4+/SOX2+ cells was observed (B, E), but there was no difference in total number of cells per embryo (C, F). Oct4 and Sox2 mRNA did not differ between control dsGFP and dsYy1 embryos on day 3 or 4 (G, H). Asterisks indicate $p < 0.05$.

FIG. 6. Epiblast-specific YY1 loss results in loss of OCT4 and SOX2 protein in overlapping domains (**B, C, E, F, H, J**). Both Oct4 and Sox2 mRNA expression were normal in control and epiblast-specific Yy1-knockout mice. Sox2 was observed in the anterior neuroectoderm (**A, D**) and Oct4 mRNA was observed in anterior neuroectoderm, epiblast, and the primitive streak (**G, I**). Despite normal protein localization in discrete domains (arrows show OCT4 in the primitive streak), neuroectoderm, an overlapping expression domain, lacked OCT4 and SOX2 protein (arrows in **E, J**). Results are summarized in (**K**).

laboratory (Trask et al., 2012). IF was performed on transverse E7.5 sections with anti-Yy1 antibody, and specific loss in the epiblast and epiblast derivatives was observed as expected (Supplementary Fig. S3).

We then examined Oct4 and Sox2 RNA localization by *in situ* hybridization and protein localization by IF. Similar to the preimplantation results, the mRNA patterns for both genes appeared normal in epiblast-specific KO embryos, but OCT4 and SOX2 protein could not be detected specifically in the anterior ectoderm (arrows in Fig. 6). Interestingly, OCT4 protein was maintained in the YY1-null primitive streak cells where Sox2 is not normally expressed (Fig. 6J), and conversely, SOX2 protein was observed in the chorion of both control and epiblast-specific KO embryos (Fig. 6C, F). Together, these results indicate that in the absence of YY1, loss of OCT4 and SOX2 protein occurs at the post-transcriptional level and only in tissues that normally express all three proteins (Yy1, Oct4, and Sox2).

Discussion

OCT4 and SOX2 are well-known regulators of pluripotency in the preimplantation embryo and *in vitro* embryonic stem cells, but the molecular mechanisms regulating their activity and expression are complex. Preimplantation embryo culture studies have shed light on specific and distinct roles for OCT4 and SOX2 in ICM lineage commitment. For example, both are essential for normal primitive endoderm differentiation mediated by FGF4, yet SOX2 promotes primitive endoderm development in a noncell-

autonomous manner (Wicklow et al., 2014), while OCT4 promotes primitive endoderm development in a cell-autonomous manner downstream of FGF4 (Frum et al., 2013). Distinct mechanisms of regulation have been supported for Oct4 and Sox2 expression, as well. CDX2 helps restrict OCT4 expression to the ICM, but HIPPO pathway members restrict SOX2 expression to the ICM (Wicklow et al., 2014), indicating distinct mechanisms are in place to functionally repress each protein. Recent single-cell transcriptomes have also led to the identification of previously unknown factors that may mediate heterogeneous expression, including SOX21 and CARM1 (Goolam et al., 2016).

The results presented in this study indicate that YY1 is required for stability and/or cellular localization of both OCT4 and SOX2 proteins, but only in cells that normally coexpress all three. We found that YY1 loss did not alter mRNA levels of either locus, but in the absence of YY1, OCT4 and SOX2 protein could not be detected in overlapping expression domains. This was observed by two distinct methodologies (KO and RNAi knockdown) in mouse blastocysts as well as anterior neuroectoderm of the gastrulating mouse embryo using an epiblast-specific Yy1 deletion strategy.

The gastrulating mouse embryos provided insight into germ layer-specific roles for YY1 (Trask et al., 2012). Similar to what we observe in the blastocyst ICM cells, Oct4 and Sox2 mRNA were produced normally in the absence of YY1, including both anterior neuroectoderm and cells ingressing through the primitive streak (Fig. 6). In contrast, OCT4 protein localization was maintained in the primitive

streak (which does not normally express SOX2), but OCT4 and SOX2 protein could not be detected in the neuroectoderm, where both are expressed in control embryos.

The means by which YY1 regulates a wide array of biological processes has remained enigmatic, and likely involves posttranslational modifications of YY1 itself (Trask and Mager, 2010). YY1 is a multifaceted protein with acidic and basic residues, four C2H2-type zinc finger domains, two glycine-rich domains, and a histidine-rich domain. YY1 can be posttranslationally modified and contains motifs that are known targets for acetylation, phosphorylation, S-nitrosation, and O-linked glycosylation, as well as calpain or caspase-mediated posttranslational processing (Hiromura et al., 2003; Riman et al., 2012; Rizkallah and Hurt, 2009; Takasaki et al., 2007). It is probable that posttranslational modifications of YY1 enable or prevent interactions with combinations of binding partners that affect tissue-specific roles (Trask and Mager, 2010). Our data support these notions as we observe different isoform intensities of YY1 during specific stages and tissues, which may offer insight into their functional importance.

The gastrulating mouse embryo may provide a novel system for protein biochemistry that may yield insight into whether differentially modified forms of YY1 in primitive streak and neuroectoderm may mediate OCT4 and SOX2 interaction. Of note, previous studies have determined that YY1 is acetylated in anterior tissue and not acetylated in posterior tissue of the gastrulating mouse (Takasaki et al., 2007), suggesting that perhaps acetylated YY1 may specifically regulate OCT4/SOX2 complexes. Targeted point mutations and/or deacetylation may be used to provide insight on this testable hypothesis. Generation of differentially posttranslationally modified forms of YY1 protein may lead to mechanistic understanding of how YY1 regulates OCT4 and SOX2, which would be of great value in many stem cell and iPS approaches that require precise regulation of these proteins.

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Author Disclosure Statement

The authors declare that no conflicting financial interests exist.

References

Donohoe, M., et al. (1999). Targeted disruption of mouse Yin Yang 1 transcription factor results in peri-implantation lethality. *Mol. Cell. Biol.* 19, 7237–7244.

- Frum, T., et al. (2013). Primitive endoderm development in the mouse blastocyst. *Dev. Cell.* 25, 610–622.
- Goolam, M., et al. (2016). Heterogeneity in Oct4 and Sox2 targets biases cell fate in 4-cell mouse embryos article heterogeneity in Oct4 and Sox2 targets biases cell fate in 4-cell mouse embryos. *Cell.* 165, 61–74.
- Griffith, G., et al. (2011). Yin-Yang1 is required in the mammalian oocyte for follicle expansion. *Biol. Reprod.* 663, 654–663.
- He, Y., et al. (2007). The transcription factor Yin Yang 1 is essential for oligodendrocyte progenitor differentiation. *Neuron.* 55, 217–230.
- Hiromura, M., et al. (2003). YY1 is regulated by O-linked N-acetylglucosaminylation (O-GlcNAcylation). *J. Biol. Chem.* 278, 14046–14052.
- Makhlouf, M., et al. (2014). A prominent and conserved role for YY1 in xist transcriptional activation. *Nat. Commun.* 5, 1–12.
- Rhee, S., et al. (2013). Visceral endoderm expression of Yin-Yang1 (YY1) is required for VEGFA maintenance and yolk sac development. *PLoS One.* 8, e58828.
- Riman, S., et al. (2012). Phosphorylation of the transcription factor YY1 by CK2 prevents cleavage by caspase 7 during apoptosis. *Mol. Cell. Biol.* 32, 797–807.
- Rizkallah, R., and Hurt, M. (2009). Regulation of the transcription factor YY1 in mitosis through phosphorylation of its DNA-binding domain. *Mol. Biol. Cell.* 20, 4766–4776.
- Takasaki, N., et al. (2007). Acetylated YY1 regulates Otx2 expression in anterior neuroectoderm at two Cis-sites 90 Kb apart. *EMBO J.* 26, 1649–1659.
- Trask, M., and Mager, J. (2010). Complexity of polycomb group function: Diverse mechanisms of target specificity. *J. Cell. Physiol.* 226, 1719–1721.
- Trask, M., Tremblay, K., and Mager, J. (2012). Yin-Yang1 is required for epithelial-to-mesenchymal transition and regulation of nodal signaling during mammalian gastrulation. *Dev. Biol.* 368, 273–282.
- Wicklow, E., et al. (2014). HIPPO pathway members restrict SOX2 to the inner cell mass where it promotes ICM fates in the mouse blastocyst. *PLoS Genet.* 10, 1–13.
- Zhou, L., et al. (2015). Linc-YY1 promotes myogenic differentiation and muscle regeneration through an interaction with the transcription factor YY1. *Nat. Commun.* 6, 10026.

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