communications biology

A Nature Portfolio journal

Article

https://doi.org/10.1038/s42003-026-09567-1

HBP1 enhances progesterone receptor activity and IGFBP1 expression driving endometrial decidualization

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Decidualization of human endometrial stromal cells (HESCs) is one of the critical steps in the establishment of human pregnancy. Nevertheless, little is known about the molecular mechanisms underlying human decidualization. In this study, we identified high-mobility group box transcription factor 1 (HBP1) as a key player in the decidualization of HESCs. Knockdown of HBP1 expression significantly reduced the mRNA and protein expression levels of the decidualization markers insulin-like growth factor-binding protein 1 (IGFBP1) and forkhead box O1 (FOXO1). Although progesterone receptor (PGR) expression did not significantly change, the expression levels of PGR-regulated target molecules decreased. Furthermore, ChIP-Seq and RNA-Seq analyses revealed that HBP1 directly transcriptionally regulates IGFBP1 expression. Additionally, overexpression of HBP1 promoted the enrichment of histone H3K4me3 at the promoter regions of *PGR* and its target molecules *FK506 binding protein 5 (FKBP5)*, *Fos-related antigen 2 (FRA-2/FOSL2)*, and *FK506-binding protein 4 (FKBP4)*, which indicated that HBP1 enhances *PGR* transcriptional activity. Clinical specimen analysis further confirmed that the expression of HBP1 and PGR target molecules was significantly downregulated in the endometria of patients with recurrent implantation failure. In conclusion, this study provides evidence that HBP1 plays an important regulatory role in embryo implantation.

Decidualization of HESCs is critically important for the establishment and maintenance of pregnancy[1,2]. This process is a key event during the "implantation window". Studies have shown that insufficient decidualization of HESCs is closely associated with various pregnancy-related disorders, including embryo implantation failure, recurrent spontaneous abortion, intrauterine growth restriction, and preeclampsia[3–6]. Therefore, a deeper understanding of the molecular mechanisms underlying HESC decidualization not only benefits in the prevention of decidualization-related disorders but also provides clinicians with more effective therapeutic strategies, which will improve pregnancy outcomes.

Under the synergistic regulation of oestrogen and progesterone, HESCs undergo cyclic changes. During the proliferative phase, oestrogen plays a dominant role, promoting endometrial proliferation[7]. Following ovulation, as progesterone secretion increases, the endometrium gradually transitions from the proliferative phase to the secretory phase[7,8]. During the establishment of a human pregnancy, the embryo can only be successfully implanted if the endometrium is decidualized and reaches a receptive state. Progesterone (P4) and its receptor (PGR) signalling play pivotal roles in the decidualization process of HESCs[9,10]. As one of the key regulatory factors in decidualization, P4 exerts its biological functions through the PGR-mediated signalling pathway, and the proper response and regulation of P4 signalling by PGR are essential for the successful progression of decidualization[11]. Any factors that affect the P4/PGR signalling pathway may influence the establishment and maintenance of pregnancy[12]. P4/PGR signalling not only directly regulates the expression of decidualization marker genes but also profoundly affects the successful establishment of pregnancy and outcomes through interactions with multiple signalling pathways[13–15]. However, the precise molecular mechanisms governing endometrial decidualization remain incompletely understood and warrant further investigation.

High-mobility group box transcription factor 1 (HBP1) is a widely expressed transcription factor belonging to the sequence-specific HMG

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family[16]. Research has demonstrated that HBP1 plays a crucial role in cellular proliferation and differentiation and has dual functions in transcriptional regulation[17–19]. On the one hand, HBP1 can activate the transcription of specific genes, inducing cellular senescence and apoptosis[20]. On the other hand, HBP1 primarily functions as a transcriptional repressor[16], exerting significant inhibitory effects on the cell cycle in both normal and cancer cells[21]. HBP1 knockout mice show a significant worsening of diabetes; HBP1 regulates glucose and insulin homeostasis by forming an insulin/HBP1/IGFBP1 negative feedback loop, which involves transcriptional activation of IGFBP1 and suppression of the PI3K/AKT signalling pathway[22]. Notably, IGFBP1 is a well-established marker of endometrial decidualization in humans[23]. Recent RNA-Seq analysis of in vitro decidualization in primary endometrial stromal cells revealed that HBP1 expression is significantly upregulated during decidualization[24], suggesting its potential role in regulating this process. Nevertheless, the specific molecular mechanisms of HBP1 in endometrial decidualization, its downstream signalling pathways, and its regulatory relationship with IGFBP1 remain poorly understood and require further investigation.

This study provides evidence that HBP1 plays an important regulatory role in embryo implantation. HBP1 can directly regulate the expression of IGFBP1, a decidualization marker, and can also increase the transcriptional activity of *PGR* through H3K4me3 modification, which plays a key role in endometrial decidualization. In addition, decreased levels of HBP1 expression in the endometrium were closely associated with repeated implantation failure (RIF), suggesting that HBP1 may play an important role in successful embryo implantation.

Results

Dynamic expression of HBP1 in human endometrial stromal cells

RNA-Seq data were obtained from a previous study[24] in which a heatmap analysis of genes differentially expressed in HESCs during in vitro decidualization was performed on Day 3 (D3). *HBP1* expression was significantly upregulated at D3 (Fig. 1A). In the model of in vitro decidualization of HESCs induced by MPA combined with cAMP, the mRNA expression levels of *HBP1*, *IGFBP1*, and *PRL* progressively increased from D0 to D6 (Fig. 1B), with corresponding increases in IGFBP1 and HBP1 protein levels (Fig. 1C, D). IHC analysis of endometrial biopsies from healthy volunteers revealed minimal HBP1 expression during the proliferative phase, with progressive nuclear accumulation in stromal cells throughout the secretory phase (Fig. 1E). IF confirmed the predominant nuclear localization of HBP1 at D4 of decidualization (Fig. 1F). These findings demonstrated that HBP1 is dynamically regulated under progesterone control.

HBP 1 plays an important role in the decidualization of human endometrial stromal cells

Quantitative PCR analysis of *PRL* (Fig. 2A), *IGFBP1* (Fig. 2B), and *FOXO1* (Fig. 2C) revealed time-dependent upregulation during HESC decidualization (D0–D6), with *HBP1* knockdown significantly reducing the expression of these markers. Western blot analysis revealed progressive increases in HBP1, IGFBP1, and FOXO1 protein levels during decidualization (D0–D6), which were markedly suppressed by HBP1 knockdown (Fig. 2D); moreover, HBP1 overexpression significantly increased IGFBP1 protein levels after 2 days of decidualization (Fig. 2E). Immunofluorescence analysis revealed that HBP1-knockdown HESCs maintained a spindle-shaped morphology after 3 days of decidualization and failed to exhibit a typical polygonal morphology (Fig. 2F). MTS assays revealed significantly reduced proliferation in HBP1-overexpressing HESCs (Fig. 2G) and enhanced proliferation in HBP1-knockdown cells (Fig. 2H). Consistently, HBP1 overexpression significantly decreased the number of Ki67-positive cells after 3 days of decidualization (Fig. 2I). These findings demonstrated that HBP1 regulates both decidualization and endometrial homeostasis through controlling proliferation.

HBP1 enhances FOXO1 protein expression in the nucleus and promotes human endometrial decidualization through the suppression of AKT phosphorylation

To investigate the molecular mechanisms underlying HBP1-mediated decidualization, we performed RNA-Seq transcriptome analysis on HESCs in *HBP1* knockdown and control groups on Day 3 (D3) of in vitro decidualization. KEGG pathway enrichment analysis of the differentially expressed genes (DEGs) revealed significant activation of the PI3K–AKT signalling pathway (Fig. 3A). Subsequent Western blot analysis demonstrated that although total AKT protein levels remained unchanged upon HBP1 knockdown, phosphorylated AKT (p-AKT) levels were markedly elevated, accompanied by downregulation of IGFBP1 expression (Fig. 3B). Importantly, treatment with the PI3K inhibitor LY294002 in *HBP1* knockdown cells significantly reduced p-AKT levels and restored IGFBP1 expression without affecting total AKT protein levels (Fig. 3B). We reanalyzed the RNA-Seq data for *FOXO1* and *PGR*, which are available in the GEO dataset (GSE 94036)[25]. The analysis revealed no significant overlap between the DEGs from the si*HBP1*, si*PGR* and si*FOXO1* conditions (Supplementary Fig. 1), providing computational evidence that HBP1 operates independently. The results of WB and IF (Fig. 3C, D) suggested that HBP1 overexpression increased the nuclear expression of FOXO1.

HBP1 potentiates progesterone signalling by upregulating PGR target gene expression while maintaining constant PGR levels during human endometrial decidualization

Quantitative PCR analysis revealed significant upregulation of *HBP1* mRNA levels in MPA-treated HESCs, which was markedly attenuated upon *PGR* knockdown (Fig. 4A), indicating positive regulation of HBP1 expression by the P4/PGR signalling pathway. During in vitro decidualization (D2-D6), HBP1 knockdown did not alter PGR expression at either the mRNA (Fig. 4B) or protein level (Fig. 4C). RNA-Seq analysis of the *HBP1*-knockdown and control groups on Day 3 of decidualization revealed distinct clusters of differentially expressed genes (Fig. 4D). Heatmap analysis demonstrated that *HBP1* knockdown significantly downregulated the expression of multiple decidualization markers and *PGR* target genes, whereas PGR expression remained unchanged (Fig. 4E). Subsequent qPCR validation confirmed that *HBP1* knockdown did not significantly affect *PGR* mRNA levels but did significantly reduce the expression of *PGR* target genes, including *FOSL2*, *FKBP4*, *FKBP5*, and *SRC1* (Fig. 4F) ($P < 0.05$). Western blot analysis revealed that HBP1 knockdown significantly decreased the protein levels of FOSL2, FKBP4, and FKBP5 during decidualization (D2–D6) (Fig. 4G). ChIP–qPCR analysis demonstrated stronger recruitment of *PGR* to the *FOXO1* and *HOXA10* promoters in the HBP1-overexpressing group than in the control group (Fig. 4H, I). These findings collectively demonstrated that HBP1 enhances the transcriptional activity of *PGR* and regulates its occupancy at target gene enhancers.

HBP1 directly regulates *IGFBP1* transcription and modulates *PGR* transcriptional activity through H3K4me3 modification

To elucidate the molecular mechanisms underlying HBP1-mediated decidualization, we performed ChIP-Seq analysis on HESCs overexpressing Flag-tagged HBP1 on Day 3 (D3) of in vitro decidualization. Genomic distribution analysis revealed that HBP1 binding sites were predominantly located in promoter regions, distal intergenic regions, and intronic areas (Fig. 5A), whereas H3K4me3 peaks were enriched primarily in promoter regions (Fig. 5B). Integrated analysis of RNA-Seq and ChIP-Seq data demonstrated significant activation of the FOXO signalling pathway among genes that were both downregulated upon HBP1 knockdown and bound by HBP1 (Fig. 5C). The ChIP-Seq data indicated the co-occupancy of HBP1 and H3K4me3 at the *IGFBP1* promoter region, with HBP1 overexpression significantly increasing the H3K4me3 peak intensity at this locus (Fig. 5D). RNA-Seq confirmed that *IGFBP1* expression was downregulated upon HBP1 knockdown, indicating direct transcriptional regulation by the cooperation between HBP1 and H3K4me3.

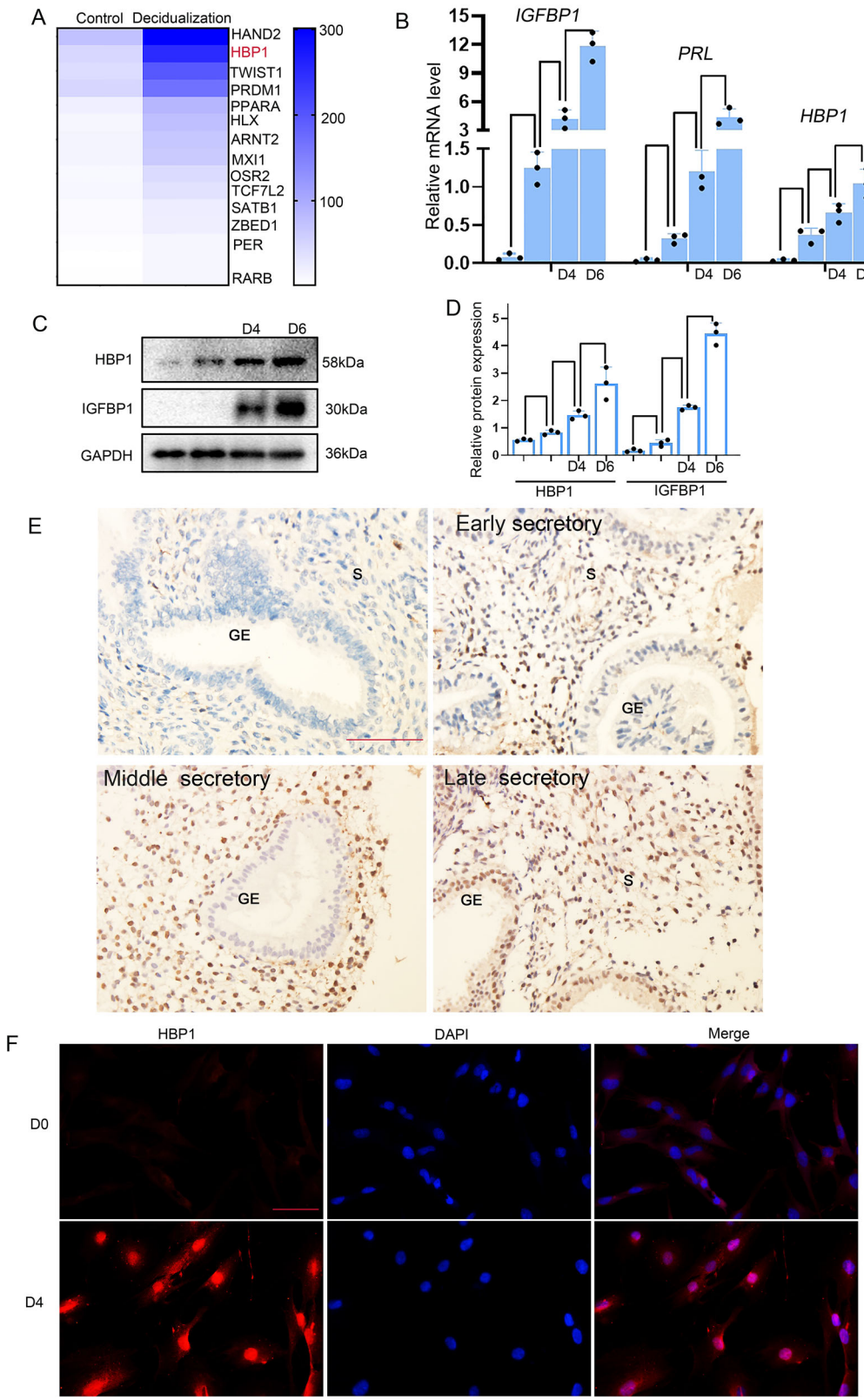


Fig. 1 | Dynamic expression of HBP1 in human endometrial stromal cells. **A** Heatmap analysis of differentially expressed genes in nondecidualized (D0) and decidualized (D3) human endometrial stromal cells using RNA-Seq data from the literature[24]. **B** qPCR analysis of IGFBP1, PRL, and HBP1 mRNA expression levels at D0, D2, D4, and D6 after in vitro decidualization of HESCs treated with MPA plus cAMP. $n = 3$ independent samples. **C**, **D** Western blot analysis of IGFBP1 and HBP1 protein expression levels in HESCs at different time points after treatment with MPA plus cAMP. **E** Immunohistochemical (IHC) analysis of HBP1 protein expression during the proliferative, early secretory, mid-secretory, and late secretory phases of the menstrual cycle. GE glandular epithelium, S stroma. Scale bar: 100 μm. **F** Immunofluorescence (IF) analysis of HBP1 protein localization in non-decidualized (D0) and decidualized (D4) human endometrial stromal cells. Scale bar: 100 μm. All the data are presented as the mean ± standard error of the mean (SEM). $^{*}P < 0.05$, $^{**}P < 0.01$.

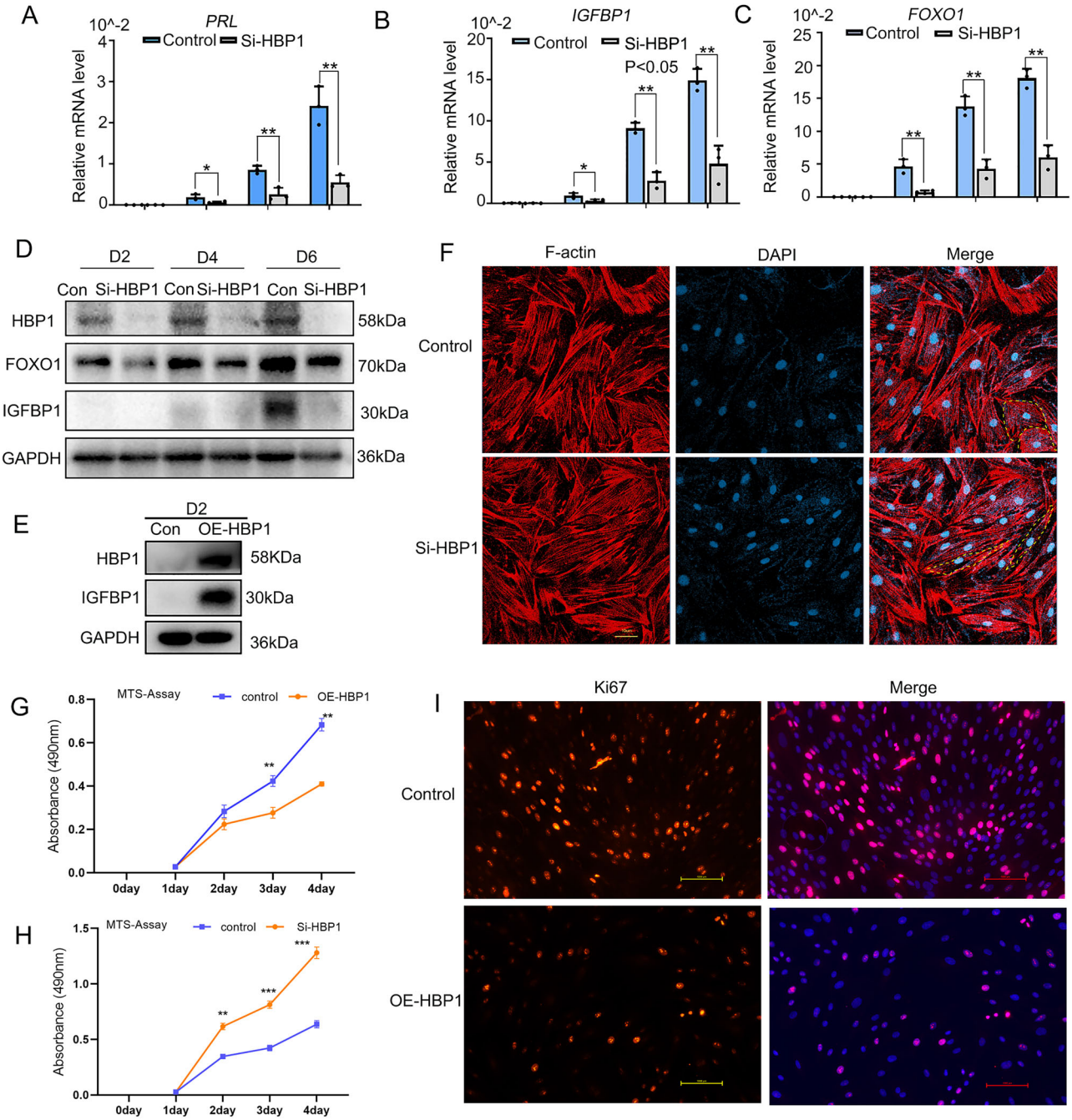


Fig. 2 | HBP1 regulates genes during the decidualization of human endometrial stromal cells. qPCR analysis of *PRL* (**A**), *IGFBP1* (**B**), and *FOXO1* **C** mRNA levels at D0, D2, D4, and D6 in HESCs treated with MPA plus cAMP for in vitro decidualization following HBP1 knockdown (si-HBP1); $n = 3$ independent samples. **D** Western blot analysis of IGFBP1 and FOXO1 protein levels at D2, D4, and D6 during in vitro decidualization of HESCs following HBP1 knockdown (si-HBP1). **E** Western blot analysis of IGFBP1 levels at D2 during in vitro decidualization of HESCs following HBP1 overexpression (OE-HBP1). **F** Immunofluorescence (IF) analysis of F-actin in the control and Si-HBP1 groups of HESCs after 3 days of in vitro decidualization. Scale bar: 10 μm. **G**, **H** Line graphs showing the results of the MTS assay for HESCs treated with OE-HBP1 (**G**) and Si-HBP1 (**H**) during in vitro decidualization at Days 0, 1, 2, 3, 4, and 5. **I** IF analysis of Ki67 expression in control and OE-HBP1 groups of HESCs after 3 days of in vitro decidualization (D3); scale bar: 100 μm. All the data are presented as the mean ± standard error of the mean (SEM). $^{*}P < 0.05$, $^{**}P < 0.01$.

Notably, compared with the controls, HBP1 overexpression significantly increased the H3K4me3 peak intensities at the promoters of *PGR* and its target genes (*FKBP5*, *FOSL2*, and *FKBP4*) (Fig. 5E), suggesting that HBP1 may increase the transcriptional activity of *PGR* and its targets through the modulation of H3K4me3 modification. Further analysis of HBP1 and H3K4me3 binding patterns revealed predominant localization within ± 3 kb regions surrounding the transcription start sites (TSSs) (Fig. 5F), with significantly higher H3K4me3 read counts in HBP1-overexpressing cells (Fig. 5G). GO functional enrichment analysis indicated that H3K4me3-bound upregulated genes following HBP1 overexpression were primarily involved in cellular differentiation processes (Fig. 5H), and KEGG pathway analysis demonstrated significant enrichment in the progesterone response signalling pathway (Fig. 5I). These results collectively demonstrated that HBP1 promotes endometrial decidualization by regulating *PGR* transcriptional activity through the modulation of H3K4me3 histone modifications.

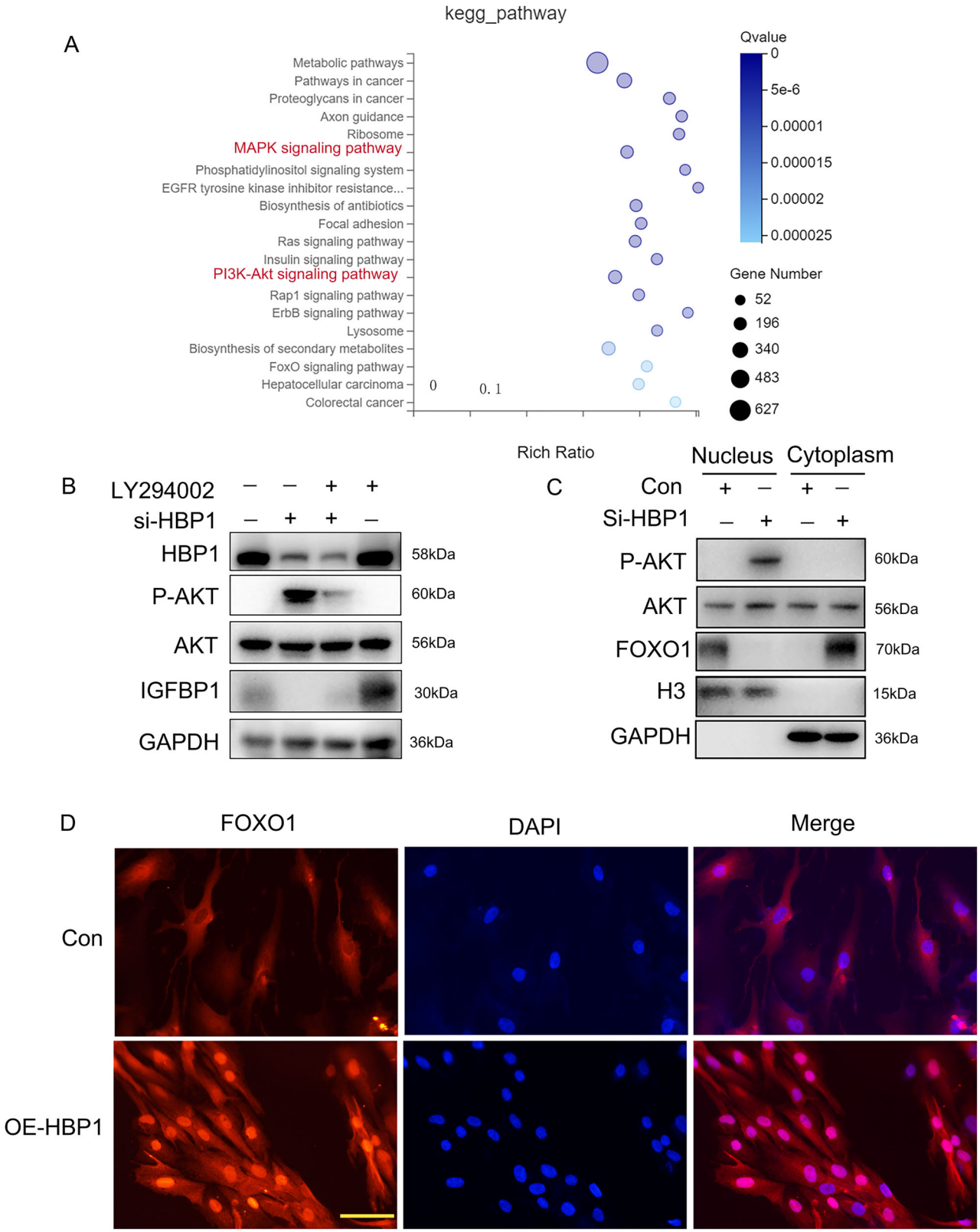
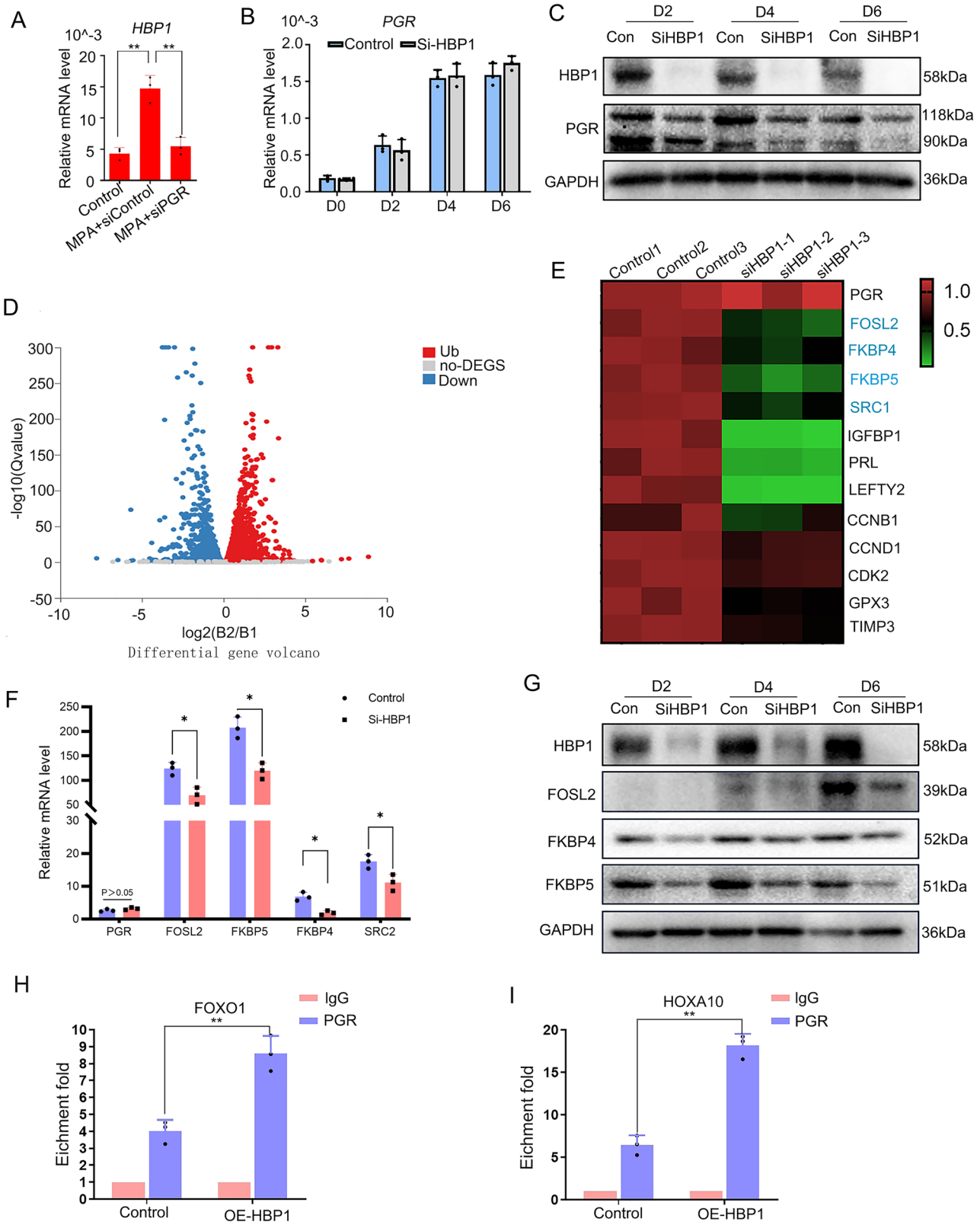


Fig. 3 | HBP1 enhances FOXO1 protein expression in the nucleus and promotes human endometrial decidualization by inhibiting AKT phosphorylation. **A** KEGG pathway enrichment analysis of differentially expressed genes identified by RNA-Seq. **B** Rescue experiment in Si-HBP1 cells. Western blot analysis demonstrated that si-HBP1 attenuated IGFBP1 levels through the activation of AKT phosphorylation, whereas the PI3K inhibitor LY294002 restored IGFBP1 levels by suppressing AKT phosphorylation. **C** Nuclear and cytoplasmic extracts were collected from cells, and Western blot analysis was performed using P-AKT, AKT, and FOXO1 antibodies. H3 and GAPDH were used as controls for nuclear and cytoplasmic protein loading, respectively. **D** IF analysis of FOXO1 expression in the control and OE-HBP1 groups of HESCs. Scale bar: 100 μm.



The decreased expression of HBP1 and PGR target molecules in the endometrium is related to the occurrence of RIF

Comparative analyses of HBP1, PGR, IGFBP1, FKBP5, and FOSL2 were performed in mid-secretory endometrial tissues obtained from healthy controls ($n = 12$) and patients with RIF ($n = 12$) using IHC, qPCR, and WB. Quantitative analysis revealed significant downregulation of HBP1, IGFBP1, FKBP5, and FOSL2 at both the transcriptional (Fig. 6B, D–F) and translational levels (Fig. 6A, G) in the patients with RIF compared with healthy controls. In contrast, PGR expression remained comparable between the groups at the mRNA (Fig. 6C) and protein levels (Fig. 6A, G). These findings, supported by comprehensive in vivo and in vitro functional studies, demonstrated that HBP1 serves as a critical regulator of endometrial

Fig. 4 | HBP1 regulates PGR transcriptional activity by mediating P4/RGR signalling. A qPCR analysis of HBP1 mRNA levels in HESCs under control conditions, after MPA treatment, and after MPA treatment following Si-PGR transfection. The expression levels were normalized to those of GAPDH ($n = 3$ independent samples). **B** qPCR analysis of PGR mRNA levels in HESCs during in vitro decidualization at Days 0, 2, 4, and 6 in the control and Si-HBP1 groups. The data were normalized to those of GAPDH. **C** Western blot analysis of PGR protein levels in HESCs during in vitro decidualization at Days 2, 4, and 6 in the control and Si-HBP1 groups. GAPDH served as a loading control. **D** Volcano plot and heatmap of differentially expressed genes. **E** RNA-Seq analysis of HESCs under control and Si-HBP1 conditions during 3 days of in vitro decidualization. **F** qPCR analysis of the mRNA expression levels of *PGR*, *FOSL2*, *FKBP4*, *FKBP5*, and *SRC1* in HESCs during 3 days of in vitro decidualization in the control and Si-HBP1 groups; $n = 3$ independent samples. **G** Western blot analysis of the protein levels of HBP1, FOSL2, FKBP4, and FKBP5 in HESCs during in vitro decidualization at Days 2, 4, and 6 in the control and Si-HBP1 groups. GAPDH was used as the loading control. ChIP–qPCR assay of PGR binding to *FOXO1* **H** and *HOXA10* **I** in control and *HBP1*-overexpressing HESCs on Day 3 of in vitro decidualization. All the data are presented as the mean ± standard error of the mean (SEM); $n = 3$ independent samples; $^{*}P < 0.05$, $^{**}P < 0.01$.

decidualization and implantation competence, with its dysregulation potentially contributing to the pathogenesis of RIF.

Discussion

Embryo implantation failure remains a significant clinical challenge in reproductive medicine. Emerging evidence suggests that impaired endometrial decidualization may contribute more substantially to reproductive dysfunction than embryo quality alone[26], although the underlying molecular mechanisms remain incompletely understood[27]. Our findings demonstrated that the transcription factor HBP1 plays a pivotal role in endometrial decidualization through direct transcriptional regulation of IGFBP1, a well-established decidualization marker, and modulation of H3K4me3 histone modification to increase *PGR* transcriptional activity. Furthermore, we observed that downregulation of HBP1 and its downstream targets (FKBP5 and FOSL2) in the endometrium may disrupt decidualization processes, potentially contributing to the pathogenesis of implantation failure.

The PGR plays critical roles in embryo implantation and pregnancy maintenance[28]. Impaired endometrial decidualization has been implicated in both unexplained infertility and RIF[29]. Clinical evidence suggests that progesterone resistance, rather than absolute hormone levels, represents a key determinant of decidualization competence, with PGR expression and functionality serving as the primary mediators[30,31]. PGR activity is regulated through interactions with coregulatory proteins, including steroid receptor coactivator 1 (SRC1), FOSL2, FKBP4, and FKBP5[32–36]. Specifically, SRC1 enhances progesterone receptor-dependent initiation and reinitiation of transcription from chromatin[37]; FOSL2 functions as both a transcriptional coregulator and downstream target of PGR and directly binds to regulatory regions of decidualization-associated genes[33]; FKBP4 has been shown to regulate decidualization through the modulation of IGFBP1 expression, with *FKBP4* siRNA treatment reducing IGFBP1 levels by 60% in HESCs[38]; knockdown of FKBP5 in ESCs impaired the secretion of prolactin and IGFBP1 during in vitro decidualization[39]; and FKBP5 contributes to decidualization through the formation of functional complexes with PGR-B and MAGE-11 in response to progesterone signalling[40]. Our experimental data demonstrated that HBP1 knockdown in HESCs does not affect PGR expression at either the mRNA or protein level during in vitro decidualization. However, it significantly downregulated key PGR target molecules in the progesterone response pathway, including FOSL2, FKBP4, and FKBP5. These findings suggest that HBP1 modulates *PGR* transcriptional activity primarily through the regulation of downstream targets, providing a mechanistic explanation for impaired decidualization in HESCs.

IGFBP1, a well-established decidualization marker[41], is regulated by a complex network of transcription factors that bind to its promoter region, including FOXO1[42], Wilms tumor 1 (WT1)[43], Relaxin (RLX)[44], CCAAT enhancer-Binding protein β (C/EBPβ)[45], receptor gamma coactivator 1-alpha (PGC-1α)[46], and FOXA1/2[47]. FOXO1 is also considered to be a decidualization marker, mainly because it regulates the transcription of the decidual *prolactin* and *IGFBP1* genes in endometrial stromal cells[48,49]. Additionally, STAT1 and STAT3 have been implicated in IGFBP1 regulation under inflammatory or stress conditions, with cytokines such as IL-6 modulating IGFBP1 expression through STAT3 activation[3]. Our findings identified HBP1 as a novel transcriptional regulator of *IGFBP1*, expanding the known regulatory network of this critical decidualization marker. The AKT signalling pathway has been extensively implicated in HESC decidualization[50–52]. Our results demonstrated that HBP1 promotes decidualization through direct transcriptional regulation of IGFBP1 expression and inhibition of AKT phosphorylation. These findings provide new insights into the molecular mechanisms governing endometrial decidualization and establish HBP1 as a key regulatory component in this process.

Recent studies have shown the crucial role of epigenetic regulation in endometrial decidualization, with histone modifications serving as key mediators of gene expression during this process[53]. Among various epigenetic mechanisms, histone methylation and acetylation have been particularly implicated in the precise regulation of decidualization-associated genes[54]. Notably, the active chromatin markers H3K27ac and H3K4me3 are significantly enriched during HESC decidualization[55,56]. Our ChIP-Seq analysis revealed that HBP1 orchestrates decidualization through direct binding to the TSS of *IGFBP1* and the facilitation of H3K4me3 modification at the *IGFBP1* locus. Furthermore, we observed that HBP1 enhances H3K4me3 enrichment at the transcriptional regulatory regions of *PGR* and its downstream targets, thereby potentiating progesterone responsiveness. These findings position HBP1 as a critical epigenetic regulator in endometrial decidualization.

In summary, our study demonstrated that the transcription factor HBP1 plays a pivotal role in endometrial decidualization through direct transcriptional regulation of *IGFBP1* and enhanced *PGR* transcriptional activity via the modulation of H3K4me3 modifications. Furthermore, the downregulation of HBP1 and its downstream PGR targets in the endometrium was determined to be significantly associated with RIF. These findings not only advance our understanding of the molecular mechanisms underlying endometrial decidualization but also identify potential diagnostic biomarkers and therapeutic targets for RIF management.

Methods

Sample of clinical cases

The specimens for this study were obtained from patients with RIF and healthy multiparous women recruited from Liuzhou Maternal and Child Health Hospital in China. All ethical regulations relevant to human research participants were followed. All the specimens were collected from the endometrium during the "implantation window". This study was approved by the Ethics Committee of Liuzhou Maternal and Child Health Hospital (2021-079), and written informed consent was obtained from all participants prior to enrolment. Participants were aged 20–38 years, with a body mass index (BMI) of 18–23 kg/m², regular menstrual cycles, and normal endocrine function. Patients with polycystic ovary syndrome, endometrial polyps, chronic endometritis, hydrosalpinx, salpingitis, endometriosis, adenomyosis, chromosomal abnormalities, or autoimmune diseases were excluded. The diagnostic criterion for RIF was the failure of at least three transfers of euploid embryos (or an equivalent number of unscreened embryos adjusted for patient age)[57].

Isolation and culture of primary HESCs

Primary HESCs were isolated from endometrial tissues of healthy reproductive-aged female volunteers with regular menstrual cycles. This study was approved by the Ethics Committee of Liuzhou Maternal and

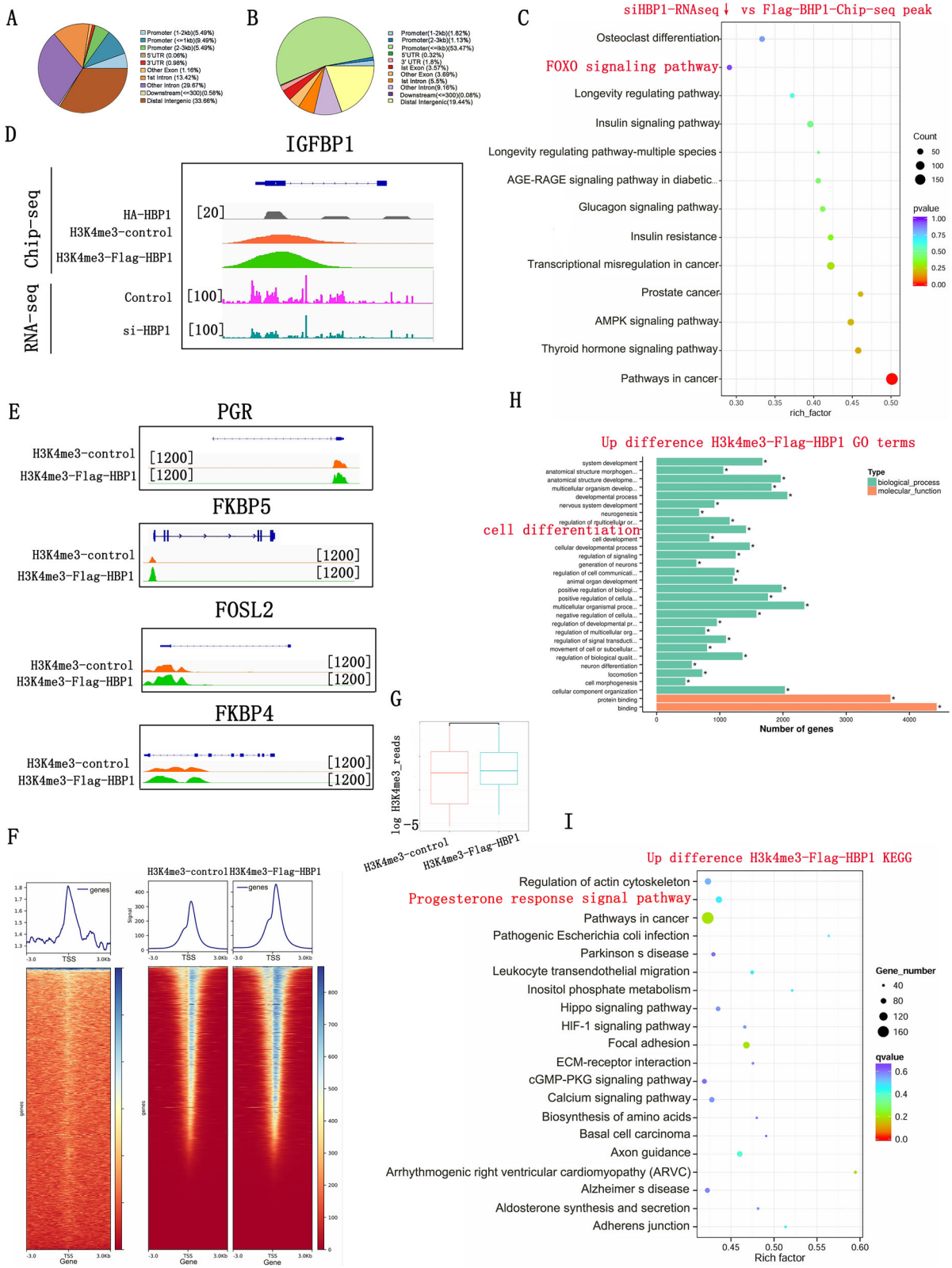


Fig. 5 | HBP1 directly transcriptionally regulates IGFBP1 expression. Distribution patterns of gene structural elements for HBP1 (**A**) and H3K4me3 (**B**) in HESCs during 3 days of in vitro decidualization, as determined by ChIP-Seq analysis. **C** Bubble plot of KEGG pathway enrichment analysis for genes regulated by siHBP1-RNAseq and Flag-HBP1-ChIP-Seq. **D** ChIP-Seq peak profiles of HBP1 and H3K4me3 at the IGFBP1 locus in the control and Flag-HBP1 groups; RNA-Seq read distribution of IGFBP1 gene expression in the control and Si-HBP1 groups. **E** ChIP-Seq peak profiles of H3K4me3 at binding sites of PGR, FKBP5, FOSL2, and FKBP4 in the control and Flag-HBP1 groups. **F** Top: Distribution of HBP1 and H3K4me3 binding within ±3.0 kb regions around transcription start sites (TSSs) in the control and Flag-HBP1 groups, as determined by ChIP-Seq. Bottom: Enrichment heatmap of HBP1 and H3K4me3 binding sites in the control and Flag-HBP1 groups. **G** Comparison of H3K4me3 read profiles between the control and Flag-HBP1 groups. **H** GO analysis bar plot and KEGG pathway enrichment bubble plot **I** of genes upregulated by H3K4me3-Flag-HBP1.

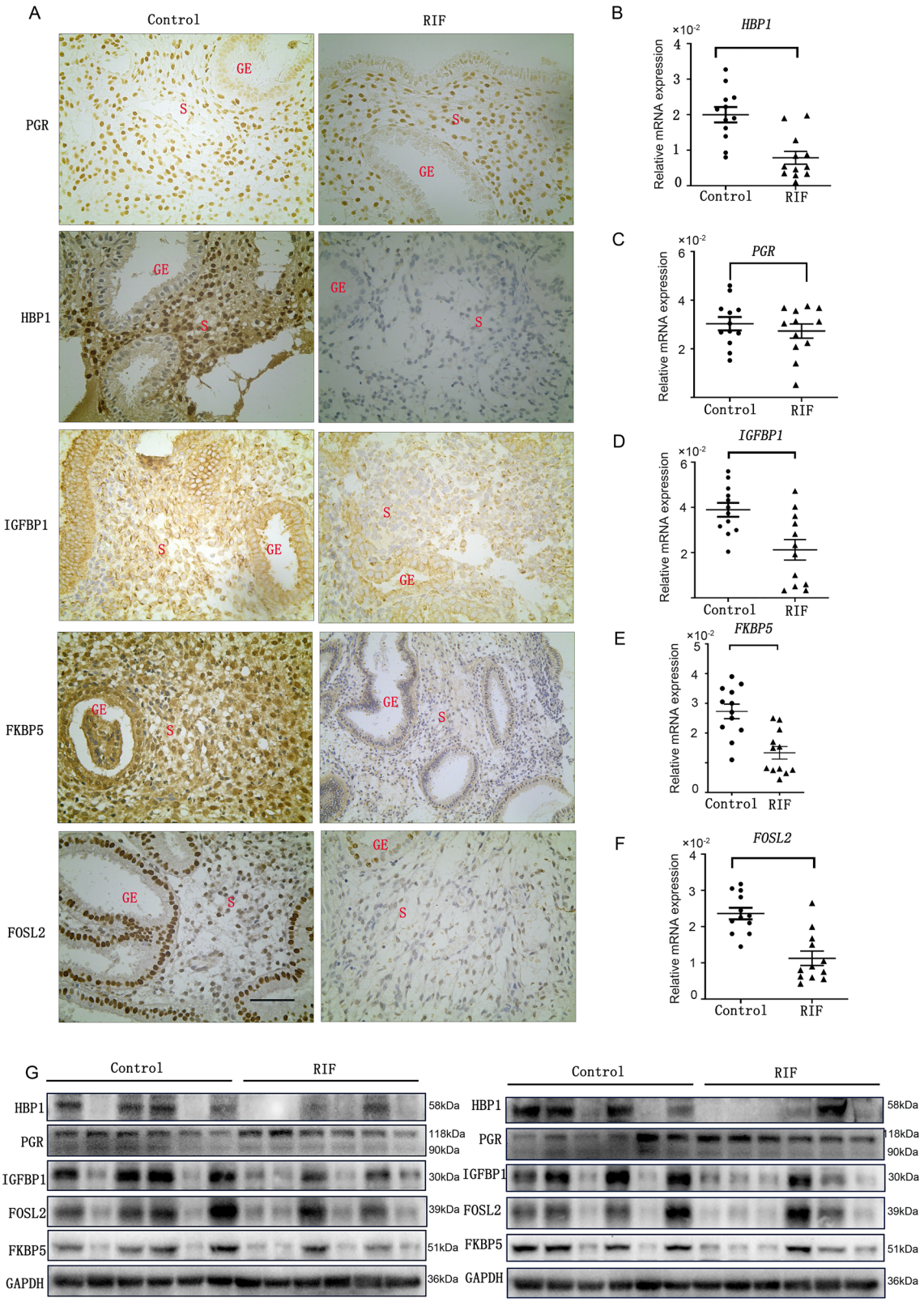


Fig. 6 | The expression levels of HBP1, PGR, IGFBP1, FOSL2, and FKBP5 in endometrial tissues obtained from patients with RIF. The expression patterns of FGR, HBP1, IGFBP1, FKBP5, and FOSL2 were evaluated in secretory-phase endometrial tissues through immunohistochemistry (**A**), qPCR (**B–F**), and Western blot **G**. Comparative analysis was performed between control subjects ($n = 12$) and patients with RIF ($n = 12$ independent samples). GE: glandular epithelium; S: stroma. Scale bar: 100 μm. All the data are presented as the mean ± standard error of the mean (SEM). $^{*}P < 0.05$, $^{**}P < 0.01$.

Child Health Hospital, and informed consent was obtained from all participants prior to sample collection. All ethical regulations relevant to human research participants were followed. The isolation procedure was as follows: endometrial tissues were minced and digested with 2% collagenase for 1 hour, after which the medium was replaced to remove floating cells. The digested mixture was sequentially filtered through 100 μm and 40 μm cell strainers. The filtered cells were cultured overnight at 37 °C with 5% CO_2 in complete medium supplemented with DMEM/F12 (Thermo), 1% penicillin/streptomycin (Solarbio), and 10% foetal bovine serum (Vivacell). The next day, the HESCs exhibited a typical spindle-shaped, fibroblast-like morphology.

Immunohistochemical (IHC) staining

All endometrial biopsy samples were fixed in formalin and embedded in paraffin. The section preparation process was as follows: the paraffin sections were dewaxed and rehydrated, and 5 μm thick endometrial sections were placed in 10 mM citrate buffer (pH 6.0) for antigen retrieval by autoclaving for 10–15 min. The sections were subsequently incubated with 3% hydrogen peroxide to inactivate endogenous peroxidase activity, followed by blocking with 5% bovine serum albumin (BSA) for 1 hour. The sections were incubated overnight (24 h) at 4 °C with primary antibodies (HBP1, PGR, IGFBP1, FOSL2, and FKBP5) and antibody dilution solution (HBP1 negative control for the IHC results), followed by incubation with secondary antibodies at room temperature for 1 h. Finally, the sections were developed using DAB, counterstained with haematoxylin, and mounted after dehydration and clearing.

Immunofluorescence (IF) staining

Cells were fixed with 4% paraformaldehyde and permeabilized with 0.1% Triton X-100. After permeabilization, the cells were incubated with primary antibodies (HBP1, F-actin, and Ki67) and a negative control (antibody dilution solution) overnight at 4 °C. The next day, the cells were incubated with a fluorescence-labelled secondary antibody for 1 h, and the nuclei were counterstained with DAPI. Finally, images were captured and analysed using a fluorescence microscope.

Cell proliferation assay

Cell viability and proliferation were evaluated with the MTS colorimetric assay. Cells in the logarithmic growth phase were harvested and seeded into 96-well plates at densities ranging from 1×10^4 to 1×10^5 cells per well (in 100 μL medium per well), with each condition performed in triplicate. After overnight incubation at 37 °C in a 5% CO_2 humidified atmosphere to ensure cell attachment, 10 μL of MTS reagent was added directly to each well at the specified time points. Plates were subsequently incubated for 2 to 4 h at 37 °C. Finally, the optical density (OD) at 490 nm was recorded for each well using a microplate reader.

Real-time qPCR

Total RNA was extracted from either endometrial biopsy tissues or cultured cells using TRIzol reagent (Invitrogen). RNA integrity was assessed by 1% agarose gel electrophoresis. The RNA concentration was measured using a Nanodrop spectrophotometer. One microgram of total RNA was reverse-transcribed into cDNA using a reverse transcription kit. qPCR analysis was subsequently performed using an ABI Q5 real-time PCR system with SYBR Green dye (Takara). The mRNA expression levels of all target genes were normalized to those of the housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*). All the PCR primers used are listed in Supplementary Data 1.

Western blot analysis

Total protein was extracted from either endometrial tissue samples or cultured cells using RIPA lysis buffer (Thermo) supplemented with 10% protease inhibitor cocktail (MCE). The primary antibodies used in the experiments included those against HBP1, IGFBP1, PGR, FOXO1, FOSL2, AKT, P-AKT, FKBP4, and FKBP5, with GAPDH serving as the internal control. Following enhanced chemiluminescence analysis for P-AKT, the membrane was stripped using stripping buffer (Solarbio) and subsequently reprobed with an AKT antibody. The band intensities of target proteins were quantified using ImageJ software, and relative protein expression levels were calculated by normalization to those of GAPDH[58]. All the catalogue numbers and antibodies are listed in Supplementary Data 1.

RNA-Seq

Total RNA was extracted from cultured HESCs (decidualized for 3 days) using TRIzol® Reagent (Invitrogen), followed by quality control with Qubit and Bioanalyzer to assess concentration and integrity. Qualified RNA underwent rRNA depletion, fragmentation, and reverse transcription into cDNA. Sequencing adapters were ligated to construct libraries, which were quantified, normalized, and pooled for paired-end sequencing on BGI (China). Differentially expressed genes were identified using a multiple-test-corrected *P*-value combined with fold-change criteria. Statistical significance was defined as a corrected P-value < 0.05 and an absolute fold change >2.

ChIP-Seq and ChIP–qPCR

HESCs (decidualized for 3 days) were cross-linked with 1% formaldehyde for 10 minutes, followed by chromatin shearing via sonication in lysis buffer. The lysates were incubated overnight at 4 °C with anti-IgG (negative control) or antibodies (H3K4me3 and PGR), and immunoprecipitation was performed using protein A/G agarose beads. The precipitated complexes were reverse-crosslinked at 65 °C for 4 h, and the immunoprecipitated DNA fragments were extracted using phenol/chloroform/isoamyl alcohol (25:24:1). Immunoprecipitated and input DNA samples were quantified using a Qubit 4.0 fluorometer and sequenced with an Illumina Nova-PE150. All the PCR primers used for ChIP-qPCR are listed in Supplementary Data 1.

Statistics and reproducibility

The experimental data are presented as the mean ± standard error of the mean (SEM). All in vivo and in vitro functional and phenotypic experiments were performed in at least independent biological triplicate to ensure reproducibility. All replication attempts were successful. Statistical analyses were performed using Prism 9.0 software (GraphPad, San Diego, CA). Comparisons between groups were conducted using Student's t test or one-way analysis of variance (ANOVA), and rate comparisons were assessed using the chi-square test. $P < 0.05$ was considered to indicate statistical significance.

Ethical approval

This study was approved by the Ethics Committee of Liuzhou Maternal and Child Health Hospital (2021-079), and written informed consent was obtained from all participants prior to enrolment.

Reporting summary

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

Data availability

The RNA sequencing data obtained during this study have been deposited in the NCBI Sequence Read Archive (accession: PRJNA1233012; https://www.ncbi.nlm.nih.gov/sra/PRJNA1233012). Furthermore, the ChIP-Seq data are available from the China National Center for Bioinformation (accession number HRA014463) (https://ngdc.cncb.ac.cn/gsa-human/browse/HRA014463). Additional supporting datasets and methodological details have been archived and can be retrieved via the aforementioned accession links. All source data underlying the graphs presented in the main Figs. are available in Supplementary Data 2. All uncropped and unedited blot/gel images are available in Supplementary Fig. 2. The representative negative control for the IHC results is available in Supplementary Fig. 3. Approval Letter for Medical Ethics Committee of Liuzhou is available in

Supplementary Fig. 4. All other data are available from the corresponding authors (Aiping Qin or Pinxiu Huang) upon reasonable request.

Code availability

The Methods section provides information on the publicly accessible software used in the study. There was no application of any proprietary code or mathematical algorithm thought to be essential to the findings.

Received: 18 March 2025; Accepted: 12 January 2026;
Published online: 18 January 2026

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Acknowledgements

This work was supported by the National Natural Science Foundation of China (82160296 and 82460309) and the Guangxi Medical and Health Key Discipline Construction Project.

Author contributions

P.H. and A.Q. designed the experiments. Y.G., W.T., and C.N. performed the experiments. H.F. and J.H. collected the human endometrial tissues. Z.C. and Z.L. performed the statistical analyses. Y.G. wrote the manuscript. P.H., A.Q., and Z.L. revised the manuscript. All the authors reviewed the manuscript.

Competing interests

The authors declare no competing interests.

Additional information

Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s42003-026-09567-1.

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Peer review information *Communications Biology* thanks Francesco (J) DeMayo, Peng-Chao Wang and Vinay Shukla for their contribution to the peer review of this work. Primary Handling Editors: Kuangyu Yen and Kaliya Georgieva. A peer review file is available.