Menin directs regionalized decidual transformation through epigenetically setting PTX3 as an obstacle balancing FGF and BMP signaling

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Article

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1 **Menin directs regionalized decidual transformation through**
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Abstract

During programmed decidualization in rodents, uterine stromal cells undergo extensive cellular and molecular reprogramming into morphologically and functionally distinct decidual cells, forming the discrete regions defined as the primary decidual zone (PDZ), the secondary decidual zone (SDZ) and the layer of undifferentiated stromal cells respectively. However, the underlying mechanism governing this spatiotemporal specificity of decidualization remains elusive. Here, we demonstrated that uterine deletion of *Men1*, a key component of the MLL1/2 histone methyltransferase complex, disrupted the terminal differentiation of stromal cells, resulting in chaotic decidualization and pregnancy failure. Genome-wide epigenetic profile reveals that *Men1* distribution in chromatin recapitulates the enrichment of transcription active modification H3K4me3 orchestrating spatiotemporal decidualization of stromal cells. Further transcriptomic investigation demonstrates that *Men1* directly regulated the expression of PTX3, an extra-cellular trap for FGF2, in a H3K4me3 dependent manner in decidual cells. Decreased Ptx3 upon *Men1* ablation leads to aberrant activation of ERK1/2 in the SDZ due to the unrestrained FGF2 signal emanated from undifferentiated stromal cells, which blunt BMP2 induction and stromal cell differentiation during decidualization. In brief, our study provides genetic evidence and molecular mechanism for epigenetic rewiring mediated decidual regionalization by *Men1* and shed new light in pregnancy maintenance.

44 **Key words:** *Men1*, decidualization, H3K4me3, PTX3, pregnancy failure

Introduction

Successful pregnancy requires rapid remodeling of the endometrium in a process termed decidualization, which requires the blastocyst attachment as a trigger and occurs in a region-specific manner in mice¹. At the initiation of decidualization, stromal cells surrounding the implanted blastocyst undergo proliferation and postmitotic differentiation extensively, forming the primary decidual zone (PDZ) between day 5 (day 1= the day of positive of vaginal plug) and day 6². Subsequently, stromal cells adjacent to the PDZ continue to proliferate and differentiate into decidual cells to form the secondary decidual zone (SDZ) at the antimesometrial pole by day 8. A large portion of decidual cells experience polyploidization, characterized by large mono or bi-nucleated cells that undergo repeated DNA replication without cytokinesis³. In addition, a thin layer of undifferentiated stromal cells lines between the myometrium and the SDZ⁴. It is believed that the regionalized decidual structure is not only important for the supply of nutrition to the developing embryo but also acts as a barrier against excessive invasion of trophoblast cells^{1,5}. While only small incrementation has made about the underlying mechanism directing programmed stromal cells differentiation in recent decades.

Several studies have demonstrated that a set of central genes in evolutionarily conserved signaling pathways during embryogenesis, such as BMP (bone morphogenetic protein), WNT (wingless-type MMTV integration site family), FGF (fibroblast growth factor) signaling and HOX (homeobox) genes,

67 possess the potential function in building the regional pattern of
68 decidualization^{4,6,7}. BMP2, an indispensable factor for the differentiation of
69 stromal cells into decidual cells, is intensively expressed in PDZ and SDZ^{6,8}.
70 The expression of WNT ligand *Wnt4*, which is also critical for the uterine stromal
71 cell differentiation, largely overlapped with *Bmp2*⁴. Meanwhile, *Sfrp4*, a WNT
72 antagonist, is observed in the layer of undecidualized stromal cells outside the
73 SDZ⁴. In addition to *Sfrp4*, *Fgf2*, a member of FGF family, also exhibits a similar
74 expression pattern⁶. The fine-tuned interplay of these regionalization
75 determinants synergistically or antagonistically promotes the tissue
76 homeostasis during decidualization. However, how these genes are
77 coordinated to specify the allocation of decidual cells remains largely unknown.

78 Menin, encoded by the *Men1*, is a multifunctional scaffold protein
79 participating in a variety of cellular functions, whose deficiency contributes to
80 embryonic lethality at E11.5-E13.5 with defects in multiple organs^{9,10}. Menin
81 regulates target genes expression in a cell type-specific manner, including *Hox*
82 genes, *Cdkn1b* and *Cdkn2c*, *Esr1* and *p35*, by interacting with chromatin-
83 modifying enzymes, in particular, the histone methyltransferase (HMT) MLL1/2
84 that catalyze histone 3 lysine 4 trimethylation (H3K4me3)¹¹⁻¹⁵. Regarding that
85 early pregnancy is a precisely regulated process, whether the target genes of
86 Menin-MLL complex and how Menin and its interplay with H3K4me3
87 modification participate in embryo implantation and decidualization remain
88 elusive.

89   In current study, we utilize a combination of genetic, biochemical and
90   pharmacological approaches to investigate the role of *Men1* in peri-implantation
91   uteri and reveal that *Men1* ablation disrupts the appropriated differentiation of
92   decidual stromal cells, resulting in unordered regionalization of decidualization
93   and compromised pregnancy success. The reduction of *Bmp2* caused by
94   aberrantly activated FGF2-ERK1/2 signaling pathway deranges the adequate
95   development of the SDZ due to disorganized genomic H3K4me3 modification
96   in the absence of *Men1*. The previously unappreciated PTX3, an extra-cellular
97   protein trapping FGF2, is identified as a Menin directly regulated downstream
98   gene and provides insightful information for the orchestration of decidualization
99   and pregnancy maintenance in curbing the cross-talk between FGF2-ERK1/2
100   and BMP2.

Results

***Men1* is expressed in the peri-implantation mouse uterus in a spatiotemporal manner**

Highly heterogeneous uterine endometrium is comprised with different cell types that undergo tremendous changes during early pregnancy. To elucidate the physiological significance of *Men1* during early pregnancy, we first examined the cell-specific and temporal expression of *Men1* in peri-implantation uteri using in situ hybridization. There was weak *Men1* expression in pre-implantation uterus on days 1 and 4. After embryo implantation, *Men1* transcript was moderately up-regulated in proliferating stromal cells surrounding the blastocyst on day 5. Accompanied by the initiation of decidualization, stromal cells underlying the implantation chamber exit mitosis and differentiate into epithelioid cells to form the PDZ. The transcript of *Men1* became undetectable in the PDZ but was intensively expressed in proliferating stromal cells outside the PDZ on day 6. After the SDZ was established by day 8, *Men1* expression was restricted in mesometrial pole (Fig. 1a). These observations indicated that *Men1* expression was tightly correlated with the progress of decidualization. Consistent with its mRNA expression pattern, Menin protein was widely expressed in the uterus during decidualization and located mainly in nucleus (Fig 1b). It was interesting to note that Menin protein possessed wider expression than its transcript which perhaps due to the discrepant stability between mRNA and protein. Account for the well

overlapping between *Men1* mRNA and proliferating cells, it was conceivable to speculate that the newly transcribed *Men1* was probably for *Men1* translation for daughter cells during mitosis (Fig 1b). The spatiotemporal expression pattern of *Men1* indicates that it could be a considerable regulator in peri-implantation events.

Mice with uterine deletion of *Men1* show severe subfertility

To explore the functional role of uterine *Men1* in pregnancy, we generated mice with uterine deletion of *Men1* (*Men1*^{d/d}) by crossing *Men1* floxed mice (*Men1*^{f/f}) with PR-Cre mice (*Pgr*^{Cre/+})^{10,16}. These mice show efficient deletion of *Men1* in the pregnant uterus at both mRNA and protein levels (Fig. 1c-e). To interrogate the role of *Men1* in female fertility, *Men1*^{d/d} mice and their littermate controls (*Men1*^{f/f}) were mated with wild type fertile males. Only 25% plug-positive *Men1*^{d/d} mice gave birth to offspring and the litter size was significantly lower than those in littermate *Men1*^{f/f} mice (Fig. 1f, g). These results demonstrated uterine *Men1* was crucial for successful pregnancy.

Ablation of uterine *Men1* causes defective decidualization and miscarriage at mid-gestation

Early pregnancy is a highly programmed dynamic process involving blastocyst attachment, decidualization and placentation. Defects at any stage will give rise to adverse pregnancy outcomes. To dissect out the defects causing

subfertility in *Men1^{d/d}* mice, we first assessed the early pregnancy events on day 5 with increased vascular permeability at the attachment site¹⁷. The number of implantation sites and the embryo spacing were comparable in *Men1^{ff}* and *Men1^{d/d}* mice on day 5 as visualized by intravenous injection of Chicago blue (Fig. 2a, b). The gross morphology and embryo attachment reaction as revealed by HE staining and COX2 expression, respectively, were also similar between *Men1^{ff}* and *Men1^{d/d}* (Fig. 2c and Supplementary Fig. 1a). Stromal cells surrounding the blastocyst undergo extensive proliferation and differentiation into decidual cells under the initiation of BMP2^{2,8}. The semblable proliferation and expression pattern of *Bmp2* and BMP antagonist *Dan* in *Men1^{ff}* and *Men1^{d/d}* implantation sites confirmed that mice with uterine deletion of *Men1* had normal initiation of decidualization (Fig. 2d and Supplementary Fig. 1b-d). Moreover, IGFBP5, which provide a favorable environment for blastocyst growth by coupling with IGF1¹⁸, had a similar subepithelial expression pattern in *Men1^{ff}* and *Men1^{d/d}* implantation sites (Supplementary Fig. 1e).

Since the deficiency of *Men1* has no severe defects in the blastocyst attachment reaction and the initiation of decidualization, we next examined the progress of decidualization. The morphology of decidual bulge and the number of implantation sites were comparable between *Men1^{ff}* and *Men1^{d/d}* mice on day 6 with termination of stromal cell proliferation in the established PDZ (Fig. 2e, f and Supplementary Fig. 2a). Our results showed that there were comparable expressions of OCT4, Ki67, *Dtprp*, COX2, *Bmp7*, β -Catenin and

Wnt4 in day 6 uteri, implying normal embryo development, stromal cell proliferation, PDZ formation¹⁹⁻²² (Supplementary Fig. 2b-h). Collectively, these results indicated that uterine *Men1* was dispensable for blastocyst attachment and PDZ formation.

Following the formation of PDZ, stromal cells adjacent to the PDZ continue to proliferate and differentiate to form SDZ until day 8. Reduced average weight of implantation sites with retarded embryos was emerged in *Men1*^{d/d} mice on day 8 in comparison to *Men1*^{f/f} mice (Fig. 2g-i). From day 9 to day 12, more obvious defects appeared at the implantation sites, including abnormal placentation and severe embryo absorption (Fig. 2j-l). These data hinted that the formation of SDZ was hindered from day 7 to day 8. In order to further confirm the effect of *Men1* deletion on the progress of decidualization, an oil-induced artificial decidualization model²³ was applied (Supplementary Fig. 3a). *Men1*^{f/f} mice uteri exhibited a robust decidual response 96 hours after intraluminal oil injection, while the *Men1*^{d/d} uteri showed a remarkably weakened or disappeared decidual response (Supplementary Fig. 3b, c). Collectively, these results demonstrated that uterine specific deletion of *Men1* disturbed the progression of decidualization and ultimately resulted in embryo resorption and miscarriage at mid-gestation.

Uterine deletion of *Men1* leads to inadequate differentiation of decidual cells in the SDZ

The observations of abnormal decidualization on day 8 led us to investigate the cellular defects in *Men1^{d/d}* uteri. Polyploidization, a hallmark of mature decidual cells in the SDZ, was marked by mono or bi-nucleated cells through endoduplication. H3K27me3 was critical epigenetic modification for X chromosome inactivation for dose complementary effect of female somatic cells²⁴. Our results showed that polyploid decidual cells in *Men1^{f/f}* mice possessed more than four puncta H3K27me3 staining, indicating at least four sets of homologous chromosomes in polyploid decidual cells. While the number of polyploid decidual cells was obviously reduced in *Men1^{d/d}* mice (Fig. 3a, b). Consistently, we found aberrantly increased PCNA (DNA replication in S-phase) and BrdU (DNA synthesis in S-phase) in *Men1^{d/d}* decidual cells on day 7.5 of pregnancy (Fig. 3b). Further, flow cytometric analysis of DNA content revealed that 2N cells were significantly increased with a concomitant decreased of > 4N cells in *Men1* deletion decidual tissues on day 8 (Fig. 3c, d). In *Men1^{d/d}* mice, both *Dtprp* mRNA and protein were significantly reduced on day 8 (Fig. 3e, f), suggesting that the terminal differentiation of decidual cells was impaired. These results indicated *Men1* was critical for the progression of polyploidy during decidualization.

On day 8, the expression of COX2 in the presumptive site of placentation was disordered, foreboding impaired placentation in *Men1^{d/d}* mice (Fig. 3g, h). Accompanied by normal decidualization, abundant uterine natural killer (uNK) cells accumulated at the mesometrial pole on day 8²⁵. In contrast to numerous

DBA⁺ uNK cells in *Men1^{ff}* mesometrial pole, *Men1* null uteri exhibited dramatic decrease of uNK cells (Fig. 3h). Aberrant decidualization tends to give rise to abnormal trophoblast invasion and compromised placentation⁵. Eccentric expression pattern of trophoblast giant cells marker PL1 in *Men1^{d/d}* uteri signified shallow trophoblast invasion on day 8 (Fig. 3i). All these observations strongly proved the defective decidualization in the absence of uterine *Men1*.

The morphological and physiological changes of stromal cells during decidualization is mainly regulated by steroid hormones from ovary¹. Progesterone is indispensable for decidualization maintenance through its cognate nuclear receptor progesterone receptor (PR)⁵. There was no significant difference in circulating levels of E2 and P4 in both groups on day 6 with similar expression of progesterone biosynthetic enzymes cytochrome P450 cholesterol side-chain cleavage enzyme (P450_{scc}) and 3 β -hydroxysteroid dehydrogenase (3 β -HSD) in the ovary as well as comparable expression PR and its target genes, *Hand2* and *Hoxa10*, in day 6 and day 8 uterus (Supplementary Fig. 4a, b and Supplementary Fig. 5a-f). These data suggested that the defect decidualization was primary originated from local dysfunction of *Men1* in stromal cells.

Procedures for decidualization dysregulated in the absence of uterine *Men1*

To further excavate the underlying molecular mechanism for the defective

terminal differentiation of decidual cells in the SDZ observed in *Men1^{d/d}* mice, decidual tissues were isolated from *Men1^{fl/fl}* and *Men1^{d/d}* uteri on day 8 and subjected to gene expression profiles analysis using RNA sequencing (RNA-seq). A total of 1,669 genes were differential expressed with 870 down-regulated genes (Supplementary Data 1) and 789 up-regulated genes (Supplementary Data 2) in *Men1^{d/d}* mice in comparison to wild type (Fig. 4a). Decidualization involves a series of biological changes including the proliferation and differentiation of stromal cells, angiogenesis and immunological adaptation^{5,22,26}. Functional annotation revealed that down-regulated genes upon *Men1* deletion mainly enriched in the terms of cell proliferation inhibition, fatty acid metabolic process, multicellular organism development and negative regulation of angiogenesis (Fig. 4b and Supplementary Data 3). In contrast, genes up-regulated in *Men1^{d/d}* decidual tissues were enriched in categories that were resistance to cell differentiation such as positive regulation of ERK1/2 cascade and categories detrimental to the development of decidua such as apoptotic process and inflammatory response (Fig. 4b and Supplementary Data 4).

The down-regulated genes in negative regulation of cell proliferation, including *Bmp2*, *Ptn*, *Fgf10* and *Igf1*, possessed specific expression patterns and were important for decidualization^{6,27,28} (Fig. 4c, d). On the contrary, genes associated with the activation of MAPK activity that promotes cell proliferation (*Fgfr1*, *Pdgfa*) were up-regulated in *Men1^{d/d}* uteri (Fig. 4c). Besides, genes

expressed in the thin layer of undecidualized stromal cells were increased after *Men1* knockout, such as *Sfrp4* and *Prlr* (Fig. 4c, d).

Increased vascular permeability and angiogenesis are crucial to successful decidualization. Angiopoietins (ANGPT1 and ANGPT2) and their receptor TIE2 directs angiogenesis during decidualization^{22,26}. ANGPT1 induces vessel maturation and maintains vessel leakiness, whereas ANGPT2, acting as a natural antagonist of ANGPT1, induces vessel destabilization required for further sprouting and regression of vessels²⁹. Our RNA-seq data indicated several genes in promoting angiogenesis (*Angpt1*, *Edn1*, *Ednra*) were up-regulated and genes in inducing destabilization of blood vessels and endothelial sprouting (*Angpt2*, *Hif3a*, *Mmp2*) were down-regulated due to uterine *Men1* deletion (Fig. 4c, e). Furthermore, NK-specific genes (*Prf1*, *Klrg1*, *Ctsg*, *Il15r*) and several granzyme genes (*Gzma*, *Gzmb*, *Gzmc*, *Gzmd*, *Gzme*, *Gzmg*) were significantly decreased in *Men1*^{d/d} decidua (Fig. 4c), indicating the maturation of uNK was compromised. Moreover, *Corin*, functions in promoting trophoblast invasion and spiral artery remodeling³⁰, was decreased substantially in *Men1*^{d/d} females (Fig. 4c).

Interestingly, loss of *Men1* led to decreased *Cdkn1b* (encoding p27) in both mRNA and protein levels (Fig. 4c, f). Especially, immunohistochemical analysis indicated that the expression of p27 was higher in polyploid cells (Fig. 4f). Previous studies manifest that increased p27 level in liver cells causes a failure to enter mitosis and thereby induces polyploidy³¹. Reduced polyploid decidual

cell in *Men1*^{d/d} decidual tissues might account for the reduction of p27. In addition, gene set enrichment analysis (GSEA) analysis showed that cytokinesis associated genes (*Rhoc*, *Rhob*, *Dstn*)³² were significantly enriched by *Men1* loss (Fig. 4g, h and Supplementary Data 5). Consistently, there were more stromal cells undergoing proliferating in *Men1* knockout SDZ as evidenced by significant increase of mitotic marker pH3 (Fig. 4i, j). Above all, *Men1* loss had a strong negative impact on normal physiological changes in decidualization.

***Men1*-deficient uteri show diminished BMP responsiveness but enhanced FGF responsiveness**

As aforementioned, uterine with *Men1* deletion had an insufficient terminal differentiation of stromal cells in the SDZ. Interestingly, FGF signaling pathway that positively regulated cell proliferation was significantly enriched among the up-regulated genes in *Men1*^{d/d} uteri. In contrast, gene set responding to BMP which motivated differentiation of stromal cells was significantly suppressed in *Men1*^{d/d} uteri (Fig. 5a, b and Supplementary Data 5). During decidualization, *Fgf2* transcript was localized in the undifferentiated stromal cells underneath the myometrium⁶. FGF2 binds and activates the tyrosine kinase receptor complex FGFR1-FRS2 and recruits the downstream GRB2-SOS1 complex to incite ERK1/2 signaling cascade which is a major pathway controlling cell proliferation³³. *Fgfr1* and *Frs2* but not the ligand *Fgf2* were significantly

increased by *Men1* deletion (Fig. 5b, c). BMP2 is a well-known decidual marker critical for the differentiation of stromal cells during decidualization^{6,8}. The expression of *Bmp2* and its target *Id1* was substantially lower in *Men1*^{d/d} mice uteri on day 8 of pregnancy (Fig. 5b, d). BMPs execute their effects by inducing the phosphorylation of SMAD1/5/8 proteins. Accordingly, the protein level of p-SMAD1/5/8 was decreased in *Men1*^{d/d} decidual cells (Fig. 5e). Interestingly, both the significant elevated expression of *Fgfr1* and reduced expression of *Bmp2* caused by *Men1* deletion mainly occurred in the SDZ (Fig. 5f). We also observed an elevation of phosphorylated ERK1/2 in *Men1*^{d/d} stromal cells in the SDZ (Fig. 5g). These observations were consistent with enrichment of ERK1/2 cascade and decreased cell differentiation observed in *Men1*^{d/d} decidual tissues (Fig. 4b, c). Thus, spatiotemporal decidualization regulated by programmed FGF signaling and BMP signaling was disorganized in *Men1* deficiency uterine. Indeed, mutually inhibitory effects between FGF and BMP signaling are common module throughout embryogenesis³⁴. Next, we explored whether there was a cross-talk between BMP2 and FGF2-ERK1/2 signals, and what role Menin played in this communication during the development of the SDZ.

Menin positively regulates the expression of *Ptx3* in a H3K4me3 dependent manner

Menin is an indispensable component of MLL1/2 HMT complex that catalyzes H3K4me3 at gene promoter, which is usually associated with

transcriptional activation of genes⁹. In order to define the distribution of Menin on genome and its relevance to gene expression in decidual cells, we performed ChIP-seq for Menin and H3K4me3 in decidual tissues on day 8. Consistent with the published literature, Menin and H3K4me3 is predominantly positioned at gene promoter (Menin peaks: n=10,230, 76.65% at promoters; H3K4me3 peaks: n=26,002, 70.62% at promoters) (Fig. 6a). Combined with RNA-seq data, we found that Menin and H3K4me3 was significantly enriched at the transcription start site (TSS) of expressed genes (RPKM>1) (Fig. 6b and Supplementary Fig. 6a). H3K4me3 was specifically reduced at TSSs rather than genebodies in the absence of Menin (Fig. 6b). A direct comparison of Menin and H3K4me3 ChIP-seq peaks at gene promoters showed a strong positive correlation ($R^2=0.5888291$) (Fig. 6c). The promoters of 6,800 genes bound by Menin (92% of all genes with promoter bound by Menin) were also H3K4me3 modified (Supplementary Fig. 6b). We therefore focused on the potential functional relationship between Menin and H3K4me3 in gene transcriptional activation during decidualization.

We speculated that Menin activated gene expression through H3K4me3 by interacting with MLL1/2 during decidualization. The *Kmt2a* (encoding MLL1) and *Kmt2b* (encoding MLL2) mRNA levels were not affected by the absence of *Men1* (Supplementary Fig. 6c). We utilized the RIME (Rapid immunoprecipitation mass spectrometry of endogenous proteins)³⁵ to identify the interactome networks of endogenous Menin protein in decidual tissues. As

expected, subunits of MLL1/2 complex, MLL1, MLL2, WDR5, DPY30, OGT and PSIP1 were pulled down by Menin (Fig. 6d and Supplementary Data 6). Albeit total H3K4me3 protein level was not affected by *Men1* ablation (Supplementary Fig. 6d), read coverage of H3K4me3 at promoters was significantly reduced at the absence of *Men1* ($p < 2.2e-16$) (Fig. 6e). Analysis of ChIP-seq data revealed that although Menin directly binding at the promoters of Menin-MLL typical target genes (e.g. *Cdkn1b*, *Cdkn2c*, *Esr1*, *Hoxa* cluster and *Meis1*) in decidual tissue, none of them had H3K4me3 reduction after *Men1* deletion (Fig. 6f). Correspondingly, except for the decreased expression of *Cdkn1b*, the mRNA levels of other Menin target genes had no significant difference in the absence of *Men1* (Fig. 4c, 6f). There might be an alternative mechanism different from H3K4me3 dependent mechanism as previously reported participated in the transcriptional regulation of *Cdkn1b* by Menin¹³. According to these results, we hypothesized that *Men1* regulates the transcription of a subset of decidual-specific target genes through H3K4me3 modification.

In *Men1^{ff}* decidual cells, Menin and H3K4me3 showed prominent co-localization around the transcription start site (TSS) $\pm$ 3 kilobases (kb) (Fig. 6b, g and Supplementary Fig. 6a, e). H3K4me3 peaks were classified into two clusters depending on the changes after *Men1* deletion (Fig. 6g and Supplementary Fig. 6e). We focused on cluster 1 containing loci where H3K4me3 ChIP-seq signal was strongly decreased following *Men1* deletion, representing 6,469 associated genes (Fig. 6g). To identify the direct Menin-

H3K4me3 target genes responsible for the decidualization defects observed in *Men1*^{d/d} uteri, we intersected the data of RNA-seq with the Menin and H3K4me3 ChIP-seq results and found 2,995 potential Menin-H3K4me3 direct target genes. Among them, only 348 exhibited a significant decrease in mRNA levels following *Men1* deletion (Fig. 6h). In these potential target genes, *Ptx3* attracted our attention since it was one of the top ten down-regulated genes upon *Men1* deletion (Fig. 6i). Furthermore, deletion of *Ptx3* resulted in compromised decidualization³⁶. Indeed, *Men1* loss decreased *Ptx3* expression in decidual tissues on day 8 of pregnancy (Fig. 7a). Following *Men1* deletion, the peak of H3K4me3 was significantly reduced at the *Ptx3* locus and RNA-seq tracks for *Ptx3* were profoundly decreased in *Men1*^{d/d} uteri (Fig. 7b). Through Menin ChIP assays using a pair of specific primers targeting the *Ptx3* promoter locus, we further confirmed that *Ptx3* was a direct target gene of Menin in decidual cells (Fig. 7c). Meanwhile, H3K4me3 occupancy at the *Ptx3* promoter locus was dramatically decreased in *Men1*-knockout stromal cells (Fig. 7d). These results suggested that Menin upregulate *Ptx3* expression in a H3K4me3 dependent manner.

Menin inhibits the cross-talk between FGF2-ERK1/2 and BMP2 by regulating the transcription of *Ptx3*

PTX3 has been shown to bind with selected FGFs, including FGF2 and FGF8b, through its N-terminal domain, and sequester them in the extra cellular

matrix, thus inhibiting their biological effects³⁷. As described above, *Men1* loss up-regulated FGF response. We found *Ptx3* specifically expressed in the SDZ close to the thin layer of undifferentiated stromal cells where *Fgf2* located, and significantly decreased following *Men1* deletion (Fig. 7e). During embryogenesis, FGF2 is considered to be a paracrine factor and is essential for tissue patterning and organogenesis³⁴. Based on these results, we considered that the decrease of *Ptx3* caused by *Men1* deficiency contributed to excessive ERK1/2 activation induced by paracrine FGF2 produced by the layer of undifferentiated stromal cells. To confirm this conjecture, primary mouse endometrial stromal cells (mESC) isolated from day 4 uterus of pseudopregnancy were cultured with or without recombinant mouse PTX3 in the presence of FGF2. PTX3 treatment for 72h significantly inhibited the phosphorylation of ERK1/2 induced by FGF2 in mESC compared with cells without PTX3 treatment (Fig. 7f). Consistent with previous studies, the inhibitory effect of PTX3 on FGF2 was conserved during mouse decidualization. Thus, we concluded Menin suppressed the activation of FGF2-ERK1/2 signaling pathway in the SDZ by modulating *Ptx3* transcription through epigenetic H3K4me3 modification.

In the preceding results, *Men1* loss decreased BMP response program (Fig. 5). Indeed, suppression of BMP signaling by FGF2 sustained undifferentiated proliferation of human ES cells³⁸. Next, we explored whether the reduction of BMP2 was caused by abnormal highly activated FGF2-ERK1/2 signal in the

409 absence of *Men1*. During in vitro decidualization of mESC induced by E2 and
410 P4, the expression pattern of Menin was almost consistent with that of BMP2
411 in spite of the slight decrease expression of Menin after 96h of decidualization
412 (Supplementary Fig. 7a). MI-503, a Menin inhibitor, blocks the Menin/MLL
413 interaction and trigger Menin protein degradation via ubiquitin-proteasome
414 pathway³⁹. MI-503 treatment for 48h reduced the expression of Menin protein
415 without affecting the mRNA level of *Men1* during the decidualization of mESC
416 (Supplementary Fig. 7b, c). The expression of Menin target gene *Ptx3* was also
417 significantly reduced upon MI-503 treatment (Fig. 7g). However, MI-503
418 treatment for 72h did not affect the expression of BMP2 and the activity of
419 ERK1/2 during mESC decidualization (Supplementary Fig. 7d), implying *Bmp2*
420 was not a direct target of Menin and consistent with the ChIP data showing no
421 Menin occupying and comparable H3K4me3 modification at *Bmp2* promoter
422 upon *Men1* knockout (Supplementary Fig. 7e). While in the presence of
423 exogenous FGF2, MI-503 treatment resulted in decreased BMP2 and elevated
424 ERK1/2 phosphorylation (Fig. 7h). Meanwhile co-treatment with MEK inhibitor
425 PD0325901 reversed the reduction of BMP2 induced by MI-503 (Fig. 7h).
426 These results demonstrated FGF2 acting as a paracrine factor had an inhibitory
427 effect on BMP2 expression during decidualization. Supplementation of
428 exogenous PTX3 also inhibited excessive activation of ERK1/2 and recovered
429 BMP2 level (Fig. 7h). These results suggested Menin regulated the expression
430 of BMP2 during decidualization by setting PTX3 as a trap for FGF2-ERK1/2

431 pathway, thereby ensuring the success of decidualization.

Discussion

The regulatory machinery of endometrial heterogeneity and decidual regionalization after embryo implantation disturb scientists for long time due to the scarcity of suitable mouse model. Developmentally associated transcription factors *HOXA10*, whose expression expanded throughout the PDZ and SDZ, have been reported playing an important role in establishing region-specific decidual tissue during decidualization^{7,40}. While, other potential participants in decidual regionalization remained largely unknown. Here, we demonstrated that uterine *Men1*, which was spatiotemporally expressed during decidualization, guided the stromal cells towards the fate of terminal differentiation by directing histone modification rewiring, guaranteeing the expansion of the SDZ boundary during regionalization of decidualization.

Previous studies indicated that spatiotemporal expression pattern of *Bmp2* with the progression of decidualization contributed to highly heterogeneous of decidual cells^{1,6}. At the initiation of decidualization, distinct accumulation of *Bmp2* in the subepithelial stroma was essential for blastocyst attachment reaction at the implantation site^{2,6,8}. With the spatiotemporal progression of decidualization, expanded *Bmp2* expression in the PDZ and SDZ was critical for the terminal differentiation of stromal cells^{6,8}. A significant decrease of *Bmp2* in the SDZ on day 8 caused by *Men1* deficiency disturbed the terminal differentiation of stromal cells during the expansion of SDZ without affecting the blastocyst attachment reaction and the PDZ formation.

Menin has yielded cell-specific results in terms of H3K4me3 regulation and the expression of target genes including *Hoxa* genes, *Cdkn1b* and *Cdkn2c*, *Esr1* and *p35* through connecting with histone methyltransferases MLL1/2¹¹⁻¹⁵. There was a dramatic whole genomic chromatin alteration in human endometrium decidualization marked by histone modification and chromatin accessibility^{41,42}. However, the role of H3K4me3 modification in decidual regionalization remained elusive. Distinctive histone methyltransferases in large COMPASS-like complexes performed non-redundant histone modifications in various cell types⁴³. The SET1A/B family is responsible for genome-wide bulk H3K4me2/H3K4me3 modification and the MLL3/4 complex family mediates H3K4me1 modification at enhancers and super-enhancer⁴³. MLL1/2 complex, containing unique subunit Menin, is responsible for H3K4me3 modification at certain genes in cell specific manner^{11,43}. In this study, we depicted the H3K4me3 profiling in regionalized decidualization during early pregnancy and unraveled its regulatory apparatus by Menin.

Among the direct targets marked by Menin-H3K4me3 during stroma decidualization, *Ptx3* involved in decidualization regulation possesses specific expression pattern in the SDZ³⁶, but its physiological significance in stromal cell differentiation remains intangible. Several pieces of evidences showed that PTX3 acted as a natural FGF2 trap through its N-terminal domain to sequester FGF2-ERK1/2 signaling from promoting cell proliferation³⁷. Interestingly, our investigation demonstrated that *Men1*-deficient uteri showed augmented

FGF2-FGFR1-ERK1/2 signaling activity in the SDZ at the absence of PTX3. This abnormality subsequently reduced *Bmp2* and blocked cell differentiation in the SDZ. Consistent with previous studies, the inhibitory effect of PTX3 on FGF2 was conserved during mouse decidualization. Indeed, the homeostasis between BMP and FGF has been described as “constituting a common module” throughout embryogenesis^{34,44}. FGF signaling inhibited the expression of BMPs in dorsal mesoderm, thus limiting their expression to ventral mesoderm for proper dorsoventral axis specification⁴⁵. Inhibition of BMP activity by the FGF signal promoted posterior neural development⁴⁶. Furthermore, suppression of BMP signaling by FGF2 sustained undifferentiated proliferation of human ES cells³⁸. Consistently, we evidenced that the discouraged cross-talk between BMP2 and FGF2 coordinated by Menin regulated PTX3 facilitated the maturation of decidua, which was further supported by the mutually exclusive expression of *Bmp2* and *Fgf2* in the SDZ and undifferentiated stromal cells, respectively. Together, we provided insightful evidence that the uncomfortable cross-talk between stromal cells in different regions through paracrine manner was curbed under the precise orchestrating of PTX3.

Moreover, *Men1* deficiency disrupted the polyploidization in the SDZ with reduced large mono or bi-nucleated polyploid cells as evidenced by H3K27me3. In female somatic cells, the punctate immunostaining of H3K27me3 stand for inactivated X chromosome²⁴. Somatic cells with two or more punctate H3K27me3 in nuclei was considered as polyploid cells. Currently, flow

cytometry was the major way to evaluate the polyploid stromal cells. Here, we established a new visible method to estimate the polyploidization which will greatly advance the mechanism study for polyploidy.

Previously studies suggested that endoreduplication and the absence of cytokinesis were the primary reasons for polyploidization of stromal cells which were mediated by various cyclin associated proteins^{3,5,26}. Here, we found that cytokinesis program was significantly enriched in *Men1*^{d/d} decidual cells. We also provided previously unappreciated mechanism for this black box. *Cdkn1b* (encoding p27), a recognized Menin-MLL1/2 target gene¹³, was decreased in *Men1*^{d/d} uteri. Furthermore, multiple observations proved that female mice lacking functional p27 were infertile⁴⁷⁻⁴⁹. Furthermore, based on the observation that p27 induced polyploidy of liver cells without cytokinesis^{31,50}, we speculated p27 also participated in regulating decidual cell polyploidization. Though the mRNA of *Cdkn1b* was reduced, the H3K4me3 modification at the promoter of *Cdkn1b* between *Men1*^{ff} and *Men1*^{d/d} stroma was comparable, indicating an alternative regulation of Menin on *Cdkn1b* which deserved further investigation.

Collectively, our results provided a new perspective that the patterning of decidual cells into disparate fates through the concerted activity of BMP and FGF signaling pathways underpinned the progression of decidualization. Results reported here revealed that the blunt induction of BMP2 caused by unrestrained FGF2-ERK1/2 signaling activity deranged the development of SDZ due to disorganized genomic H3K4me3 distribution in the absence of

520 *Men1*. The previously unappreciated PTX3, an extra-cellular trap for FGF2, was
521 identified as a critical Menin-H3K4me3 regulated downstream gene in decidual
522 regionalization and pregnancy maintenance by curbing the cross-talk between
523 FGF2-ERK1/2 and BMP2. Our finding will remarkably advance our knowledge
524 on current model of decidualization and inspire future studies for human fertility
525 improvement.

Methods and Materials

Animals

Men1-floxed mouse line (*Men1^{ff}*) was constructed as previously described¹⁰. Uterine-specific knockout mice (*Men1^{d/d}*) were generated by mating *Men1^{ff}* females with *Pgr^{cre/+}* males¹⁶. Mice were housed in the animal care facility of Xiamen University according to the guidelines for the care and use of laboratory animals. All experimental procedures were approved by the Animal Welfare Committee of Research Organization (X200811), Xiamen University.

Analysis of pregnancy events

Pregnancy events were analyzed as described previously¹⁷. Briefly, 2-month-old female mice were mated with fertile wild type males to induce pregnancy (vaginal plug = day 1 of pregnancy). Plug-positive females were kept separately for pregnant experiments. Pregnancy rate and litter size were monitored for the whole pregnancy process. For day 5 and day 6 pregnant mice, implantation sites were visualized by an intravenous injection of 100 μ L of 1% Chicago blue in saline. The number and average weight of implantation sites demarcated by distinct blue bands was recorded. Uterine horns were flushed to check for the presence of embryos if no blue band was observed. Mouse blood samples were collected on day 6 and serum E2 as well as P4 levels were measured by radioimmunoassay. At least three mice were used for every individual experiment in each mouse model.

Artificial decidualization model

Pseudopregnant mice were obtained by mating females with vasectomized males of the same strain. To induce artificial decidualization, one uterine horn of pseudopregnant mice was infused with sesame oil (25 µl) on day 4; the non-infused contralateral horn taken as a control. The weight of infused and non-infused uterine horns was recorded 4 days after the oil infusion and the fold increase in uterine weights was served as an index of decidualization²³.

In situ hybridization

In situ hybridization with isotopes or digoxigenin (DIG) was modified according to the previously described method²³. Frozen sections (10 µm) were mounted onto poly-L-lysine coated slides and stored at -80°C until used. After removal from -80°C, the slides were placed on a slide warmer (37°C) for 3 minutes and then fixed in 4% paraformaldehyde in PBS for 15 minutes at 4°C. Following prehybridization, uterine sections were hybridized to specific cRNA probes overnight at 65°C. After hybridization, the slides were incubated with RNase A (10 mg/ml) at 37°C for 30 minutes. RNase A-resistant hybrids were detected by autoradiography using liquid emulsion or AP labeled anti-DIG antibody. Mouse-specific cRNA probes labeled with isotope or digoxin for *Men1*, *Bmp2*, *Dan*, *Igfbp5*, *Bmp7*, *Wnt4*, *Prl8a2*, *Hoxa10*, *Ptn*, *Sfrp4*, *Ptx3* and *Fgfr1* were used for hybridization.

Immunostaining

The tissue specimens were fixed overnight in 10% neutral buffered formalin and then dehydrated in increasing concentrations of ethyl alcohol, followed by clearing of alcohol by xylene. Uterine slices were deparaffinized and incubated in citrate buffer for antigen retrieval by hyperbaric heating and then incubated overnight at 4°C with the primary antibodies including Menin (Bethyl, 1:1000), COX2 (Santa Cruz, 1:200), Dtpip (homemade, 1:200), Ki-67 (Abcam, 1:200), PL1 (Santa Cruz, 1:200), 3 β -HSD (Santa Cruz, 1:200), p450scc (Santa Cruz, 1:200), PR (CST, 1:200), HAND2 (Santa Cruz, 1:200), p27(abcam, 1:100), pH3 (CST, 1:200), ERK1/2 (CST, 1:200) and p-ERK1/2 (CST, 1:200). A Histostain-SP Kit (Zhongshan Golden Bridge Biotechnology) was applied to visualize the antigen. Immunofluorescence staining for OCT4 (CST, 1:200), β -Catenin (Epitomics, 1:200), H3K27me3 (CST, 1:200), BrdU (Abcam, 1:500) and PCNA (Epitomics, 1:200) was performed in paraffin-fixed sections and secondary antibodies conjugated with Cy3 dyes were used. The images were captured by Leica DM2500 light microscope. Antibodies with detailed information are listed in Supplementary Table 1.

Western blot

Decidual cells were isolated as described previously¹⁷. The proteins were separated by using 10% SDS-PAGE and transferred to polyvinylidene difluoride membranes. And then the membranes with proteins were blocked with 5% skim

milk in TBST for 1 h at room temperature (RT) and incubated overnight at 4°C with primary antibody for Menin (Bethyl, 1:2000), β -Actin (Bioworld, 1:3000), Dtrp (homemade, 1:2000), p27 (Abcam, 1:500), ERK1/2 (CST, 1:1000), p-ERK1/2 (CST, 1:1000), BMP2 (Abcam, 1:500), SMAD1/5/8 (CST, 1:1000), p-SMAD1/5/8 (CST, 1:1000), H3K4me3 (Abcam, 1:2000) and H3 (Abmart, 1:2000). After incubation with primary antibody, membranes incubated with specific secondary antibodies (Zhongshan Golden Bridge Biotechnology, 1:5000) for 2 h at RT. Bands were visualized using Supersignal West Pico (Thermo Scientific) according to the manufacturer's instructions. Antibodies with detailed information are listed in Supplementary Table 1.

Quantitative real-time PCR

Quantitative real-time PCR was performed as described previously¹⁷. Total RNA was extracted from uterine tissues or cells using TRIzol reagent (Invitrogen) following the manufacturer's protocol. About 1 μ g RNA was used to synthesize cDNA. Expression levels of the genes were validated by quantitative real-time PCR analysis with SYBR Green (TAKARA). All assays were performed at least three times. All PCR primers are listed in Supplementary Table 2.

Flow cytometry

Flow cytometric analysis was performed as previously described²³. Day 8 decidual cells were digested and harvested. The cell pellet was suspended in

0.25 ml PBS after centrifugation; 1 ml of cold 80% ethanol was added dropwise under constant and gentle vortexing. Samples were incubated for 30 min on ice and subsequently overnight at -20°C before subjected to staining. Cell sediments were suspended in staining solution (PBS containing 5mg/ml Propidium Iodide and 2 mg/ml DNase-free RNase A) the next day. Samples were incubated for 30 min at 37°C in the dark. They were then returned to RT and subjected to flow cytometry. The experiments were repeated three times.

RNA-seq

Total RNA was extracted from day 8 decidua (dissect out the embryo and muscle layer) of *Men1^{ff}* and littermates *Men1^{d/d}* using TRIzol reagent (Invitrogen) according to the manufacturer's protocol. Purified RNA was prepared and subjected to RNA sequencing using BGISEQ-500 platform (China, BGI). RNA-seq raw data were initially filtered to obtain clean data after quality control by Trimgalore. High-quality clean data were aligned to the mouse reference genome (mm10) using HISAT2.

RNA-Seq data analysis

Differential expression genes were normalized to fragments per kilobase of exon model per million mapped reads (RPKM) using EdgeR package in R with the criteria of fold change significantly greater than 1.5 and $p < 0.05$. Gene ontology (GO) enrichment analysis of differential expression genes (DEGs) was

analyzed by using DAVID (the Database for Annotation, Visualization and Integrated Discovery, <https://david.ncifcrf.gov>). GO terms with corrected $p < 0.05$ were considered significantly enriched. Gene Set Enrichment Analysis (GSEA) was performed to determine whether a defined set of genes shows statistically significant differences between *Men1^{ff}* and littermates *Men1^{d/d}* decidual tissues.

ChIP-Seq

Chromatin immunoprecipitation (ChIP) was modified and performed according to the previous described standard protocol⁵¹. Decidual tissue wetted with PBS quickly cut into small pieces with an iris scissor. Snipped tissue was fixed with 1% formaldehyde (CST) for 10 mins at RT (for H3K4me3 ChIP), or fixed with disuccinimidyl glutarate (DSG, Santa Cruz, 2 mM) for 30 mins at RT and then double fixed with 1% formaldehyde for another 10 mins at RT (for Menin ChIP). Fixation was stopped by glycine (0.125 M). Tissue homogenate obtained by using Dounce Tissue Grinder was filtered through a 200 μ m cell strainer to remove connective tissue. Fixed cells were lysed by Lysis Buffer 1 (50 mM HEPES pH 7.5, 1 mM EDTA, 140 mM NaCl, 0.5% NP-40, 10% glycerol, 0.25% Triton X-100), Lysis Buffer 2 (10 mM Tris-HCl pH8.0, 1 mM EDTA, 0.5 mM EGTA, 200 mM NaCl) and Lysis Buffer 3 (10 mM Tris-HCl pH8.0, 1 mM EDTA, 0.5 mM EGTA, 100 mM NaCl, 0.1% Sodium Deoxycholate, 0.1% N-lauroylsarcosine). All lysis buffers should be supplemented with protease inhibitors cocktail

(Roche) before use. Chromatin DNA was sheared to average 300-500 bp by using a BioRuptor sonicator (Diagenode). Solubilized chromatin was centrifuged to remove debris and incubated overnight at 4°C with antibody Menin (Bethyl, 10 µg) and H3K4me3 (Abcam, 1 µg) bound to 20 µl protein A magnetic beads (Invitrogen). After washing and elution, the protein-DNA complex was reversed by heating at 65°C overnight. Immunoprecipitated DNA was purified by using QIAquick spin columns (Qiagen). Immunoprecipitated and input DNA were quantified using Qubit 4.0 fluorometer. ChIP-seq libraries were prepared by using the KAPA DNA Hyper Prep Kit (KK8502) following the manufacturer's instruction and sequenced with an Illumina Nova PE150. For ChIP-qPCR, chromatin was sheared by sonication until the average length of 500-1000 bp. Specific primers were used to detect immunoprecipitated chromatin fragments, as well as input chromatin.

RIME

Rapid immunoprecipitation mass spectrometry of endogenous proteins (RIME) for analysis of complexes interact with Menin was performed as previously described³⁵. Snipped decidual tissue was fixed with 2 mM DSG for 30 mins at RT followed by incubation in 1% formaldehyde for 10 min. Crosslinking was quenched by glycine (0.125 M). Tissue homogenate obtained by using Dounce Tissue Grinder was filtered through a 200 µm cell strainer to remove connective tissue. Fixed cells were lysed by Lysis Buffer 1 (50 mM HEPES pH 7.5, 1 mM

EDTA, 140 mM NaCl, 0.5% NP-40, 10% glycerol, 0.25% Triton X-100), Lysis Buffer 2 (10 mM Tris-HCl pH8.0, 1 mM EDTA, 0.5 mM EGTA, 200 mM NaCl) and Lysis Buffer 3 (10 mM Tris-HCl pH8.0, 1 mM EDTA, 0.5 mM EGTA, 100 mM NaCl, 0.1% Sodium Deoxycholate, 0.1% N-lauroylsarcosine). All lysis buffers should be supplemented with protease inhibitors cocktail (Roche) before use. Chromatin was sheared by sonication to produce DNA fragments of 500-1000 bp. 20 µl Protein A (Invitrogen) and 5 µg Menin antibody (Bethyl) or rabbit IgG antibody (CST) were used for each sample. The bead-bound antibody and chromatin were incubated overnight at 4 °C. The next day the beads were washed 10 times with 1ml RIPA buffer and twice with 500 µl 100mM AMBIC (Ammonium Bicarbonate). A volume of 10 µl trypsin solution (Pierce, 15 ng/µl) in 100mM AMBIC was added to the beads followed by overnight incubation at 37 °C. The beads adsorbed protein-antibody complex directly placed into formic acid to give a final concentration of 5%. MS experiments were performed on Thermo Scientific™ Orbitrap Fusion™ Lumos™ Tribrid™.

Primary uterine stromal cell culture

Primary uterine stromal cells were isolated and cultured as previously described²³. Three to four pseudopregnant day 4 mouse uterine horns were cut into small pieces (2-3 mm). Tissue pieces were first digested in 3 ml fresh medium (HBSS antibiotic; Gibco) containing 6 mg/ml dispase (Gibco) and 25 mg/ml pancreatin (Sigma), and then incubated in fresh medium containing

0.5 mg/ml collagenase (Sigma) at 37 °C for 30 min. The digested cells were passed through a 70 µm filter to obtain the stromal cells. Cells were plated at 60 mm dishes or 6-wells plates, containing phenol red-free Dulbecco modified Eagle medium (DMEM) and Ham F12 nutrient mixture (1:1) (Gibco) with 10% charcoal-stripped fetal bovine serum (CS-FBS) and antibiotic. Two hours later, the medium was replaced with fresh medium (DMEM/F12, 1:1) with 10% CS-FBS. The next morning, the medium was replaced with DMEM/F12 containing 1% C-FBS, E2 (Sigma, 10 nM), P4 (Sigma, 1 µM) and antibiotic to induce decidualization. The media was changed every 48 h. For the treatment of primary uterine stromal cells, MI-503 (2 µM; MedChemExpress), recombinant murine FGF2 (PeproTech, 20 ng/ml), recombinant mouse PTX3 (R&D systems, 100 ng/ml) and MEK inhibitor PD0325901 (Selleck, 5 µM) were used.

Statistical analysis

All data are presented as mean ± SEM. Each experiment included at least three independent samples. Comparison between two groups were made by two-tailed unpaired Student's t-test. $p < 0.05$ was considered to indicate a significant result.

Data availability

The datasets generated and analyzed in the study are available in the NCBI Gene Expression Omnibus (GEO). RNA-seq and ChIP-Seq data sets

generated in this study have been deposited at the Gene Expression Omnibus database under accession number GSE182525 (token: uvqrgucctdqrxy) and GSE182539 (token: ufuxseswvbkzpep), respectively. All custom scripts can be accessed upon request to the corresponding author.

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Author contributions

M.L., W.D., L.T., M.L., and C.G. performed experiments and prepared figures. M.L., W.D., J.L., S.K., H.W. and Z.L. designed experiments. C.Z. provided the *Men1* loxp mouse model. M.L., W.D. and S.K. analyzed data. M.L., H.B., W.D., S.K., H.W. and Z.L. wrote the manuscript.

Competing interests

The authors declare that they have no competing interests.

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Fig. 1 Uterine-specific deletion of *Men1* leads to female subfertility. (a) In situ hybridization of *Men1* in WT uteri on days 1, 4, 5, 6 and 8 of pregnancy. Scale bars: 500 μ m. (b) Immunohistochemical analysis of Menin in day 4 uteri and day 5, 6, 8 implantation sites. Le, luminal epithelium; Ge, glandular epithelium; S, stroma; Bl, blastocyst; Em, embryo. Scale bar: 200 μ m. (c) Immunohistochemical analysis of Menin in *Men1^{f/f}* and *Men1^{d/d}* implantation sites on day 6. S, stroma; Em, embryo. Scale bar: 200 μ m. (d) Quantitative real-time PCR analysis of *Men1* mRNA levels in *Men1^{f/f}* and *Men1^{d/d}* implantation sites on day 6. The values are normalized to *Gapdh* and indicated as the mean $\pm$ SEM. Two-tailed unpaired Student's t test **** p <0.0001. (e) Immunoblotting

895 analysis of Menin protein in *Men1^{ff}* and *Men1^{d/d}* implantation sites on day 6. β -
896 Actin were used as loading controls. **(f)** Pregnancy rates in *Men1^{ff}* and *Men1^{d/d}*
897 female mice. The number within brackets indicates females with pups over total
898 number of plug-positive females. **(g)** Average litter sizes in *Men1^{ff}* and *Men1^{d/d}*
899 mice. Data represent the mean $\pm$ SEM. Two-tailed unpaired Student's t test
900 **** $p < 0.0001$.

Fig. 2 Mice with uterine *Men1* deletion show defective embryo development at post-implantation stage. (a) Implantation sites marked by Chicago blue dye solution in *Men1^{fl/fl}* and *Men1^{d/d}* mice on day 5. Scale bar: 5 mm. **(b)** The average number of implantation sites in *Men1^{fl/fl}* and *Men1^{d/d}* mice

906 on day 5. Data represent the mean $\pm$ SEM. Two-tailed unpaired Student's t test.
907 ns, not significant. **(c)** Immunohistochemical analysis of embryo attachment
908 reaction marker COX2 in *Men1^{ff}* and *Men1^{d/d}* implantation sites on day 5. Scale
909 bar: 200 μ m. **(d)** In situ hybridization of *Bmp2* in *Men1^{ff}* and *Men1^{d/d}*
910 implantation sites on day 5. Scale bar: 200 μ m. **(e)** Implantation sites marked
911 by Chicago blue dye solution in *Men1^{ff}* and *Men1^{d/d}* mice on day 6. Scale bar:
912 5 mm. **(f)** The average number of implantation sites in *Men1^{ff}* and *Men1^{d/d}* mice
913 on day 6. Data represent the mean $\pm$ SEM. Two-tailed unpaired Student's t test.
914 ns, not significant. **(g)** The average weight of the implantation sites in *Men1^{ff}*
915 and *Men1^{d/d}* mice on days 6, 7, 8 and 9 of pregnancy. Data represent the mean
916 $\pm$ SEM. Two-tailed unpaired Student's t test *** p <0.001. **(h)** Representative
917 images of day 8 pregnant uteri in *Men1^{ff}* and *Men1^{d/d}* mice. Scale bar: 5 mm.
918 **(i)** Histology of embryo development in *Men1^{ff}* and *Men1^{d/d}* implantation sites
919 on day 8 of pregnancy. Scale bar: 200 μ m. **(j)** Representative images of day 9
920 pregnant uteri in *Men1^{ff}* and *Men1^{d/d}* mice. Scale bar: 5 mm. **(k)** Histology of
921 implantation sites in *Men1^{ff}* and *Men1^{d/d}* mice on day 9 of pregnancy. Scale bar:
922 200 μ m. **(l)** Representative images of day 12 pregnant uteri in *Men1^{ff}* and
923 *Men1^{d/d}* mice. Scale bar: 5 mm.

Fig. 3 Uterine *Men1* deletion compromises the terminal differentiation of stromal cells with less polyploid decidual cells in the SDZ. (a) Histology of day 8 implantation sites and polyploid stromal cells in *Men1*^{fl/fl} and *Men1*^{d/d} mice.

928 Scale bars: 200 μ m. SDZ, secondary decidual zone. **(b)** Immunofluorescence
 929 analysis of H3K27me3, PCNA and BrdU in the SDZ of *Men1^{ff}* and *Men1^{d/d}* mice
 930 on day 7.5. Scale bars: 200 μ m. **(c)** DNA content quantification of decidual cells
 931 by FACS from *Men1^{ff}* and *Men1^{d/d}* mice on day 8. **(d)** Cellular distribution for
 932 2N, 4N, and >4N populations of stromal cells in *Men1^{ff}* and *Men1^{d/d}* implantation
 933 sites on day 8. Data represent the mean $\pm$ SEM. Two-tailed unpaired Student's
 934 t test * p <0.05, *** p <0.001. **(e)** The expression of decidual marker Dtprp in
 935 *Men1^{ff}* and *Men1^{d/d}* implantation sites on day 8. In situ hybridization indicates
 936 the expression of *Prl8a2* mRNA (top panel). Immunohistochemical staining
 937 indicates the expression of protein Dtprp (bottom panel). Scale bar: 200 μ m. **(f)**
 938 Immunoblotting analysis of Dtprp in *Men1^{ff}* and *Men1^{d/d}* implantation sites on
 939 day 8. β -Actin were used as loading controls. **(g)** Quantitative real-time PCR
 940 analysis of *Ptgs2* in *Men1^{ff}* and *Men1^{d/d}* implantation sites on day 6 and day 8.
 941 The values are normalized to *Gapdh* and indicated as the mean $\pm$ SEM. Two-
 942 tailed unpaired Student's t test *** p <0.001. **(h)** Immunohistochemical analysis
 943 of COX2 and DBA staining in *Men1^{ff}* and *Men1^{d/d}* implantation sites on day 8.
 944 Scale bar: 200 μ m. **(i)** Immunohistochemical analysis of PL1 in *Men1^{ff}* and
 945 *Men1^{d/d}* implantation sites on day 8. Scale bar: 200 μ m.

Fig. 4 Procedures for decidualization deregulated by uterine *Men1* loss.

(a) Volcano plot of differentially expressed genes in *Men1^{ff}* and *Men1^{d/d}* decidual tissues on day 8 as determined by RNA-seq. Blue and red dots represent genes that are differentially expressed ($p < 0.05$) between *Men1^{ff}* and *Men1^{d/d}* decidual tissues. Genes not significantly differentially expressed are shown in black. **(b)** Gene Ontology ($p < 0.05$) functional analysis for differentially expressed genes determined by DAVID. n, number of genes. **(c)** Heatmap of physiological function associated genes differentially regulated between *Men1^{ff}* and *Men1^{d/d}* mice uteri on day 8. RPKM of each gene from different samples was normalized to Z-score. **(d)** In situ hybridization of *Ptn* and *Sfrp4* in *Men1^{ff}* and *Men1^{d/d}* implantation sites on day 8. Scale bar: 200 μ m. **(e)** Quantitative real-time PCR analysis of *Angpt1* and *Angpt2* mRNA levels in *Men1^{ff}* and *Men1^{d/d}* implantation sites on day 8. The values are normalized to *Gapdh* and indicated as the mean $\pm$ SEM. Two-tailed unpaired Student's t test $*p < 0.05$. **(f)** Immunohistochemical analysis of p27 in the SDZ of *Men1^{ff}* and *Men1^{d/d}* implantation sites on day 8. Scale bar: 200 μ m. **(g)** GSEA plot showing the enrichment of cytokinesis related genes (n=115) in *Men1^{d/d}* uteri compared with *Men1^{ff}* uteri on day 8. NES, normalized enrichment score. **(h)** Heatmap of cytokinesis associated genes differentially expressed between *Men1^{ff}* and *Men1^{d/d}* mice uteri on day 8. RPKM of each gene from different samples was normalized to Z-score. **(i)** Immunohistochemical analysis of pH3 in *Men1^{ff}* and *Men1^{d/d}* implantation sites on day 8. Scale bars: 200 μ m. **(j)** Quantitative

969 analyses of pH3 positively stained cells in the SDZ on day 8. Data represent
970 the mean $\pm$ SEM. Two-tailed unpaired Student's t test $**p < 0.01$.

971

972 **Fig. 5 Decidual cells in *Men1*-deficient uteri show diminished BMP**

973 **responsiveness and enhanced Fgf2 responsiveness. (a) GSEA plot**

974 **evaluating the changes in the indicated gene signatures of response to FGF**

975 (n=46) and response to BMP (n=57) in *Men1^{ff}* uteri compared with *Men1^{d/d}* uteri.
976 NES, normalized enrichment score. **(b)** Heatmap of differentially expressed
977 genes between *Men1^{ff}* and *Men1^{d/d}* mice uteri on day 8. RPKM of each gene
978 from different samples was normalized to Z-score. **(c, d)** Quantitative real-time
979 PCR analysis of *Bmp2*, *Id2*, *Fgfr1* and *Fgf2* mRNA levels in *Men1^{ff}* and *Men1^{d/d}*
980 uteri on day 8. The values are normalized to *Gapdh* and indicated as the mean
981 $\pm$ SEM. Two-tailed unpaired Student's t test * $p < 0.05$, ** $p < 0.01$. ns, not
982 significant. **(e)** Immunoblotting analysis of BMP2 and phosphorylated
983 SMAD1/5/8 in *Men1^{ff}* and *Men1^{d/d}* uteri. Total SMAD1/5/8 and β -Actin were
984 used as loading controls. **(f)** In situ hybridization of *Bmp2* and *Fgfr1* in *Men1^{ff}*
985 and *Men1^{d/d}* uteri on day 8. AM, antimesometrial pole. Scale bar: 200 μ m. **(g)**
986 Immunohistochemical staining of phosphorylated ERK1/2 and total ERK1/2 in
987 *Men1^{ff}* and *Men1^{d/d}* uteri on day 8. Scale bar: 200 μ m.

988

989 **Fig.6 Menin directly regulated the transcription of a small number of**

990 **decidual genes in a H3K4me3 dependent manner. (a) Genomic distribution**

991 **of Menin and H3K4me3 peaks in decidual tissues on day 8. (b) Menin and**

992 H3K4me3 ChIP-seq signal density at TSS. TSS, transcriptional start site. **(c)**
 993 Correlation between Menin and H3K4me3 ChIP-seq signal in gene promoters.
 994 $R^2=0.5888291$. **(d)** Proteomic analyses of endogenous Menin-binding partners.
 995 **(e)** Change of H3K4me3 reads number of in gene promoters between *Men1^{fl/fl}*
 996 and *Men1^{d/d}* decidual tissues on day 8. $p<2.2e-16$. **(f)** Genome browser view of
 997 normalized ChIP-seq signals of Menin and H3K4me3 and RNA-seq tracks of
 998 known target genes in *Men1^{fl/fl}* and *Men1^{d/d}* decidual tissues on day 8. **(g)**
 999 Coverage profiles for Menin and H3K4me3. Heatmaps are centered at TSS.
 1000 6,469 genes show significantly decreased H3K4me3 following *Men1* deletion.
 1001 TSS, transcriptional start site. **(h)** Venn diagram showing overlap between
 1002 genes with reduction H3K4me3 in promoters by *Men1* loss (n =6,469), genes
 1003 with promoter bound by Menin (n=7,364) and genes differentially expressed (n
 1004 =1,659) upon *Men1* loss (n =1,659). **(i)** Volcano plot show the top ten down-
 1005 regulated genes upon *Men1* deletion among the overlapped genes (n=348)
 1006 from (h).

Fig.7 Menin regulates the expression of BMP2 during decidualization by setting PTX3 as a trap for FGF2. (a) Quantitative real-time PCR analysis of *Ptx3* in *Men1^{f/f}* and *Men1^{d/d}* uteri on day 8. The values are normalized to *Gapdh* and indicated as the mean $\pm$ SEM. Two-tailed unpaired Student's t test $*p < 0.05$. **(b)** Genome browser view of normalized ChIP-seq signals of Menin and H3K4me3 at the *Ptx3* promoter locus and RNA-seq tracks for *Ptx3* in *Men1^{f/f}*

1014 and *Men1*^{d/d} decidual tissues. **(c)** Quantitative ChIP analysis of Menin in *Ptx3*
1015 promoter in uterine decidual tissue. Data represent the mean $\pm$ SEM. Two-tailed
1016 unpaired Student's t test * $p=0.0282$. **(d)** Quantitative ChIP analysis of H3K4me3
1017 in *Ptx3* promoter in *Men1*^{f/f} and *Men1*^{d/d} uterine decidual tissue. Data represent
1018 the mean $\pm$ SEM. Two-tailed unpaired Student's t test * $p=0.0238$. **(e)** In situ
1019 hybridization of *Ptx3* in *Men1*^{f/f} and *Men1*^{d/d} uteri on day 8. Scale bar: 200 μ m.
1020 **(f)** Immunoblotting analysis of the phosphorylation of ERK1/2 in mouse primary
1021 endometrial stromal cells treatment with FGF2 (20 ng/ml) in the absence or
1022 presence of PTX3 (100 ng/ml). Total ERK1/2 and β -Actin were used as loading
1023 controls. **(g)** Quantitative real-time PCR analysis of *Ptx3* in primary mESC
1024 treated with DMSO or MI-503(2 μ M) in vitro. The values are normalized to
1025 *Gapdh* and indicated as the mean $\pm$ SEM. Two-tailed unpaired Student's t test
1026 * $p<0.05$. **(h)** Immunoblotting analysis of Menin, BMP2 and phosphorylated
1027 ERK1/2 after the addition of PTX3 (100 ng/ml) or PD0325901 (5 μ M) in mESC
1028 treatment with MI-503 (2 μ M) in the presence of FGF2 (20 ng/ml). Total ERK1/2
1029 and β -Actin were used as loading controls.

Fig. 8 A proposed schematic diagram showing the function of uterine *Men1* during decidualization. (a) Uterine deletion of *Men1* leads to abnormal polyploidization of decidual cells in the SDZ, accompanied by impeded maturation of uNK cells, increased inflammatory response and shallow trophoblast invasion. **(b)** Menin positively regulates the expression of *Ptx3* in a H3K4me3 dependent manner. PTX3, trapping FGF2 from undifferentiated stromal cells, ensures the expression of BMP2 in the SDZ by inhibiting the activation of FGF2-ERK1/2.

1039

1040 **Supplementary Fig. 1 Attachment reaction of blastocyst is comparable**

1041 **between *Men1*^{f/f} and *Men1*^{d/d} females in day 5 uteri. (a) Histology of day 5**

1042 **implantation sites in *Men1*^{f/f} and *Men1*^{d/d} mice. Scale bar: 200 μm. (b) In situ**

1043 **hybridization of *Dan* in *Men1*^{f/f} and *Men1*^{d/d} uteri on day 5. Scale bar: 200 μm.**

1044 **(c, d) Immunohistochemistry of Ki67 and BrdU in *Men1*^{f/f} and *Men1*^{d/d}**

1045 **implantation sites on day 5. Scale bar: 200 μm. (e) In situ hybridization of *Igfbp5***

1046 **in *Men1*^{f/f} and *Men1*^{d/d} implantation sites on day 5. Scale bar: 200 μm.**

Supplementary Fig. 2 Initiation of decidualization is comparable between

***Men1^{f/f}* and *Men1^{d/d}* uteri on day 6. (a)** Immunohistochemistry of Ki67 in *Men1^{f/f}* and *Men1^{d/d}* implantation sites on day 6. Scale bar: 200 μm. **(b)** Immunofluorescence analysis of OCT4 in *Men1^{f/f}* and *Men1^{d/d}* implantation sites on day 6. Scale bar: 200 μm. **(c, d)** Immunohistochemistry of COX2 and Dtprp in *Men1^{f/f}* and *Men1^{d/d}* implantation sites on day 6. Scale bar: 200 μm. **(e)** In situ hybridization of *Bmp7* in *Men1^{f/f}* and *Men1^{d/d}* implantation sites on day 6. Scale bar: 200 μm. **(f)** Immunofluorescence analysis of β-Catenin in *Men1^{f/f}* and *Men1^{d/d}* implantation sites on day 6. Scale bar: 200 μm. **(g)** In situ hybridization

1057 of *Wnt4* in *Men1^{ff}* and *Men1^{d/d}* implantation sites on day 6. Scale bar: 200 μ m.
1058 **(h)** Quantitative real-time PCR analysis of *Wnt4* in *Men1^{ff}* and *Men1^{d/d}*
1059 implantation sites on day 6. The values are normalized to *Gapdh* and indicated
1060 as the mean $\pm$ SEM. Two-tailed unpaired Student's t test. ns, not significant.

Supplementary Fig. 3 Loss of *Men1* impairs uterine decidualization in oil-induced model. (a) Schematic diagram of surgical regimen used to induce decidual response. Mice were treated with oil (25 μ l) in one uterine horn on day 4 of pseudopregnancy. Uteri were collected on day 8. (b) Gross morphology of the decidual response in *Men1^{ff}* and *Men1^{d/d}* uteri on day 8 of pseudopregnancy. Scale bar: 5 mm. (c) Ratio of the decidual horn to the control horn weight of *Men1^{ff}* and *Men1^{d/d}* uteri on day 8 of pseudopregnancy. Data represent the mean $\pm$ SEM. Two-tailed unpaired Student's t test *** p <0.001.

Supplementary Fig. 4 Ovarian steroid hormone secretion is comparable between *Men1^{fl/fl}* and *Men1^{d/d}* mice. (a) Immunohistochemistry staining of Menin, 3 β -HSD and P450scc in day 6 *Men1^{fl/fl}* and *Men1^{d/d}* ovaries. Scale bar: 200 μ m. CL, corpus luteum; F, follicle. **(b)** Serum estradiol-17 β (E2) and progesterone (P4) levels in *Men1^{fl/fl}* and *Men1^{d/d}* mice on day 6. Data represent the mean $\pm$ SEM. Two-tailed unpaired Student's t test. ns, not significant.

Supplementary Fig. 5 Uterine deficiency of *Men1* has no adverse effect on progesterone signaling pathway. (a) Quantitative real-time PCR analysis of *Pgr* in *Men1^{fl/fl}* and *Men1^{d/d}* uteri on day 6. The values are normalized to *Gapdh* and indicated as the mean $\pm$ SEM. Two-tailed unpaired Student's t test. ns, not significant. **(b)** Immunohistochemistry of PR in the implantation sites of *Men1^{fl/fl}* and *Men1^{d/d}* mice on day 6 and day 8 of pregnancy. Scale bars: 200 μ m. **(c, d)** Quantitative real-time PCR analysis of *Hand2* and *Hoxa10* in *Men1^{fl/fl}* and

1085 *Men1*^{d/d} uteri on day 6 and day 8. The values are normalized to *Gapdh* and
1086 indicated as the mean $\pm$ SEM. Two-tailed unpaired Student's t test. ns, not
1087 significant. **(e)** In situ hybridization of *Hoxa10* in *Men1*^{ff} and *Men1*^{d/d}
1088 implantation sites on day 6 and day 8. Scale bar: 200 μ m. **(f)**
1089 Immunohistochemistry of HAND2 in *Men1*^{ff} and *Men1*^{d/d} implantation sites on
1090 day 6 and day 8. Scale bars: 200 μ m.

Supplementary Fig. 6 Uterine specific deletion of *Men1* does not affect the global H3K4me3 level. (a) Menin and H3K4me3 ChIP-seq signal density in the genome. (b) Venn diagram of genes with promoter enriched by H3K4me3 and Menin. (c) Quantitative real-time PCR analysis of *Kmt2a* and *Kmt2b* mRNA levels in *Men1^{f/f}* and *Men1^{d/d}* implantation sites on day 8. The values are normalized to *Gapdh* and indicated as the mean $\pm$ SEM. Two-tailed unpaired Student's t test. ns, not significant. (d) Immunoblotting analysis of H3K4me3 in *Men1^{f/f}* and *Men1^{d/d}* decidual tissues on day 8. H3 was used as loading controls.

1100 **(e)** Coverage profiles for Menin and H3K4me3. Heatmaps are centered at TSS.
1101 5,644 genes in cluster 2 have no significant difference of H3K4me3 modification
1102 following *Men1* deletion. TSS, transcriptional start site.

Supplementary Fig. 7 MI-503 Treatment has no effect on the expression of

Bmp2 in the absence of Fgf2. (a) Immunoblotting analysis of Menin, BMP2

and Dtpnp expression for mouse primary endometrial stromal cells during in

vitro decidualization. β-Actin was used as loading control. **(b)** Immunoblotting

analysis of Menin expression in the presence of 0, 1, 2, or 4 μM MI-503 in mESC.

β-Actin was used as loading control. **(c)** Quantitative real-time PCR analysis of

Men1 in mESC during in vitro decidualization with or without treatment of 2 μM

MI-503 for 72h. The values are normalized to *Gapdh* and indicated as the mean

± SEM. Two-tailed unpaired Student's t test. ns, not significant. **(d)**

Immunoblotting analysis of Menin, BMP2 and p-ERK1/2 in mESC treatment

with MI-503 (2 μM) for 72h during in vitro decidualization. Total ERK1/2 and β-

Actin were used as loading controls. **(e)** Genome browser view of normalized

ChIP-seq signals of Menin and H3K4me3 at the *Bmp2* promoter locus and

RNA-seq tracks for *Bmp2* in *Men1^{fl/fl}* and *Men1^{d/d}* decidual tissues.

1118 **Supplementary Table 1:** Antibody information

Antibodies	Company	Catalog No.
Menin	Bethyl	A300-105A
Dtprp	homemade	N/A
p27	Abcam	ab32034
COX2	Santa Cruz	sc-1746
Ki67	Abcam	ab15580
PR	CST	8757
P450-scc	Santa Cruz	sc-18043
3 β -HSD	Santa Cruz	sc-30820
HAND2	Santa Cruz	sc-9409
PL1	Santa Cruz	sc-34713
pH3	CST	9701
OCT4	CST	2840
β -Catenin	Epitomics	1247-1
H3K27me3	CST	9733
BrdU	Abcam	ab6326
PCNA	Epitomics	2714-1
p-ERK1/2	CST	4370
ERK1/2	CST	4695
BMP2	Abcam	ab32034
p-SMAD1/5/8	CST	9516

SMAD1/5/8	CST	9743
H3K4me3	Abcam	ab8580
H3	Abmart	TD6932
β -Actin	Bioworld	AP0060

1119

1120 **Supplementary Table 2.** Primer list.

1121 Quantitative real time PCR primer

Gene	Forward primer (5'-3')	Reverse primer (5'-3')
<i>Men1</i>	CCCACTCACCCCTTTATCA	CGGCAGTAGTTGTAGTCT
<i>Gapdh</i>	TGGCAAAGTGGAGATTGTTGCC	AAGATGGTGATGGGCTTCCCG
<i>Wnt4</i>	AGGAGTGCCAATACCAGT	GCTGAAGAGATGGCGTATAC
<i>Ptgs2</i>	GTCTGGTGCCTGGTCTGATGAT	GTGGTAACCGCTCAGGTGTTG
<i>Pgr</i>	ACCTGATCTAATCCTAAATGA	ATTGTGTTAAGAAGTAGTAAGAC
<i>Hoxa10</i>	GGCAGTTCCAAAGGCGAAAA	CAAAAAAAGCCAGAACAAC
<i>Hand2</i>	TCGGTTATCTAGTGCTGTC	ATACTTACAATGTTTACACCTTCA
<i>Angpt1</i>	TTCTTCGCTGCCATTCTGACTC	CGTTGGTGTGTACTGCTCTG
<i>Angpt2</i>	ACGGTCAACAACCTCGCTCCT	TTCCGCACAGTCTCTGAAGGT
<i>Cdkn1b</i>	TCAAACGTGAGAGTGTCTAACG	CCGGGCCGAAGAGATTTCTG
<i>Fgfr1</i>	ACCTCACCGCTCTACCTGGA	TCTGGCTATGGAAGTCGCTCTT
<i>Fgf2</i>	AGAACGGCGGCTTCTTCCT	TGGCACACACTCCCTTGATAGA
<i>Bmp2</i>	GATCTGTACCGCAGGCACTCA	AGTTCCTCCACGGCTTCTTCG
<i>Id1</i>	CGAGGTGGTACTTGGTCTGT	CCTTGAGGCGTGAGTAGCA

<i>Ptx3</i>	AGCGTGCATCCTGTGAGACCA	TCCTTTCCACCCACCACCAACA
<i>Kmt2a</i>	GAGCAGCCATCGCAGAAGGA	GAGCCAGAGCATCAGAGGAGAG
<i>Kmt2b</i>	GCAAGAGGAAGAGGAGGAGACT	GCTGGAGGTGGAGGAGATGTT

1122

1123 ChIP assay primers

Gene	Forward primer (5'-3')	Reverse primer (5'-3')
<i>Ptx3</i>	CCAGGACAAGCTGATAACACTT	GCATCTGCGAGTTCTCCAG

1124