

1 **Supplementary Materials for**

2 **A GREB1-steroid receptor feedforward mechanism governs differential GREB1 action in**
3 **endometrial function and endometriosis**

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10 Supplementary Fig. 1 GREB1 acts as a PR cofactor in human endometrial stromal cells.

11 Supplementary Fig. 2 *Greb1* KO mice have normal ovarian function.

12 Supplementary Fig. 3 Compromised progesterone signaling in the uteri of *Greb1* KO mice.

13 Supplementary Fig. 4 Transcript levels of *Igf1*, *Mcm2*, *Klf4*, and *Klf15* in uteri of mimicked
14 hormonal states of pregnancy.

15 Supplementary Fig. 5 Decidualization is defective in *Greb1* KO mice.

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17 endometriotic females.

18 Supplementary Table 1 List of primers and TaqMan probes

19 Supplementary Table 2 List of antibodies

20 Supplementary Table 3 List of Primers used for Chromatin Immunoprecipitation (ChIP).

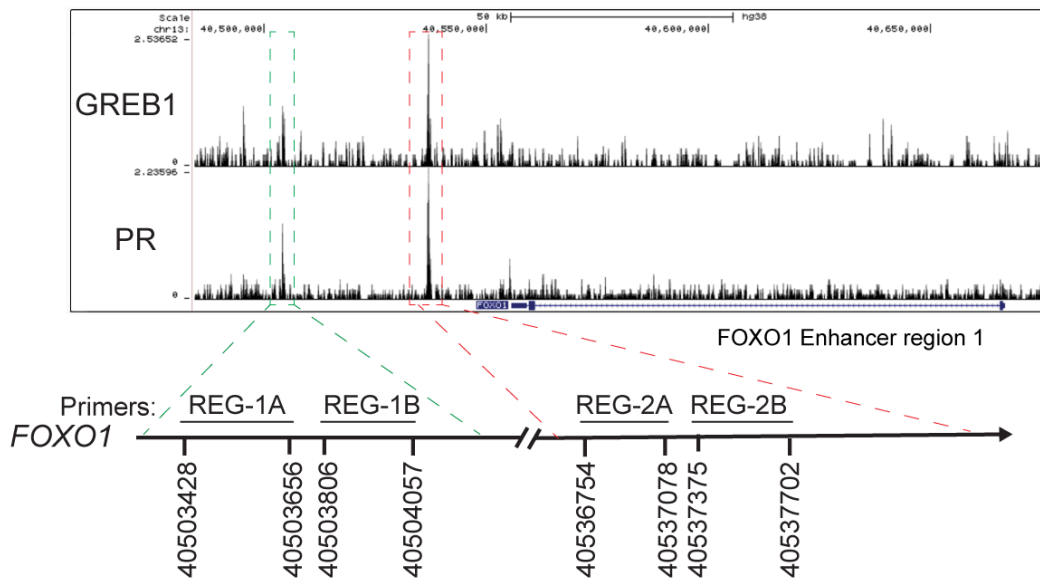
21 **Supplementary figure legends**

22 **Supplementary Fig. 1 GREB1 acts as a PR cofactor in human endometrial stromal cells. a,**

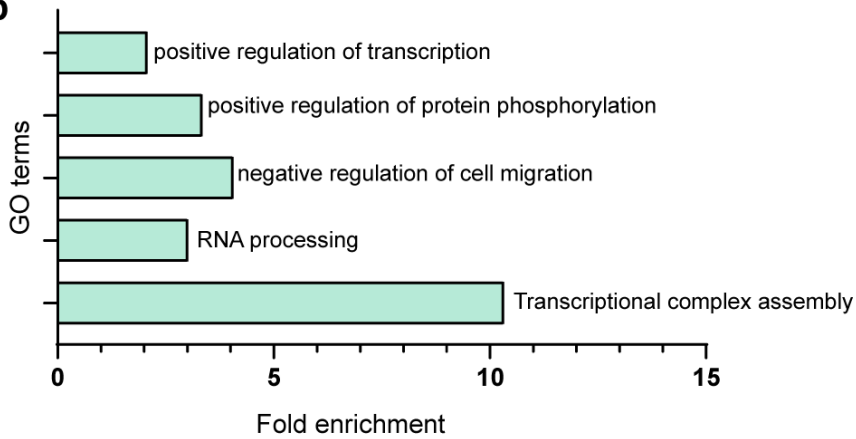
23 Visualization of GREB1 and PR ChIP-seq peaks enriched around the enhancer region of FOXO1.

24 **b,** Gene ontology of enriched signaling pathways from GREB1 HES cistrome.

a



b



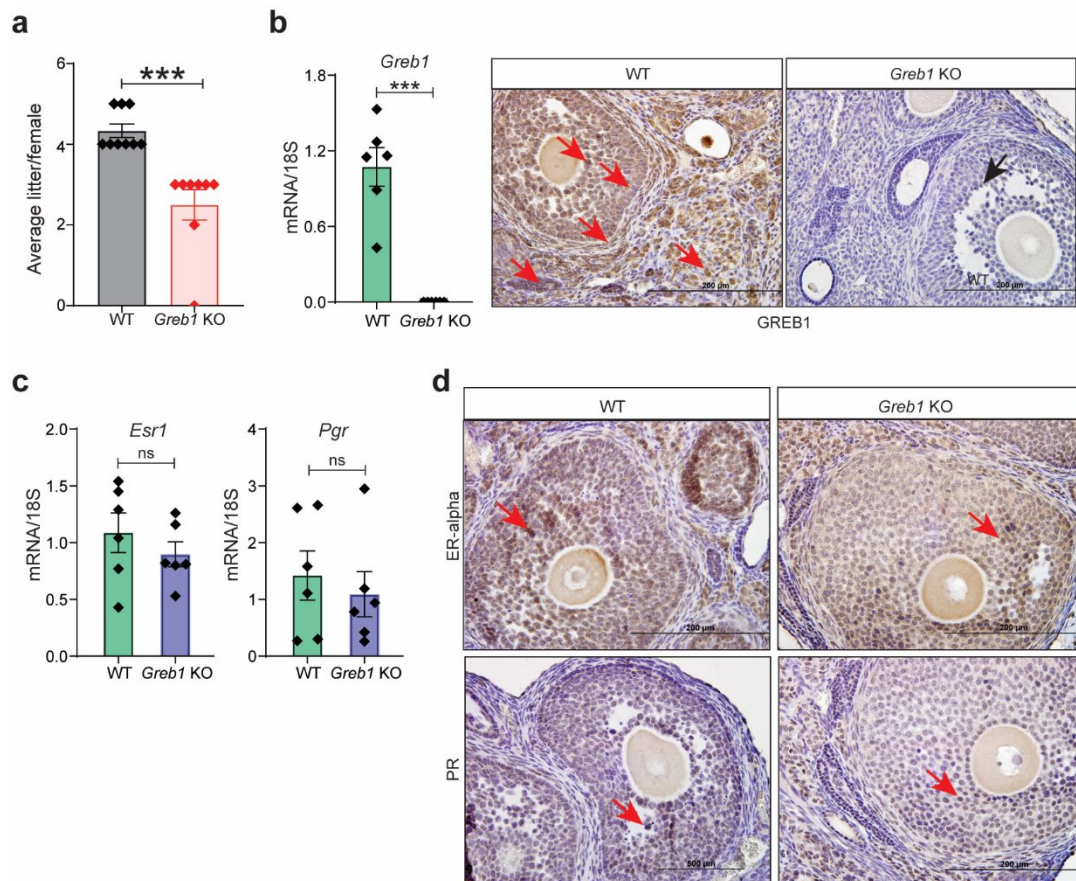
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29 **Supplementary Fig. 2 *Greb1* KO mice have normal ovarian function.** **a**, Graph depicting the
 30 number of litter per female from wild type (n=9) and *Greb1* KO (n=8) in six-month fertility tests.
 31 Paired, two-tailed, t-test. Data reported as the mean \pm SEM. *P< 0.05, ***P< 0.001, ****P< 0.0001
 32 **b**, Analysis of GREB1 transcript and protein levels in ovaries from wild-type (WT) (n=6) and *Greb1*
 33 KO mice (n=6). Paired, two-tailed, t-test. Data reported as the mean \pm SEM. *P< 0.05, ***P<
 34 0.001, ****P< 0.0001. Red arrowhead indicates the GREB1-positive cells and black arrowhead
 35 indicates GREB1-negative cells; scale bar: 200 μ m. **c**, The relative mRNA concentration of *Esr1*
 36 and *Pgr* (determined by QRT-PCR) in the ovary of WT (n=6) and *Greb1* KO mice (n=6). Paired,
 37 two-tailed, t-test. Data reported as the mean \pm SEM. *P< 0.05, ***P< 0.001, ****P< 0.0001. **d**,
 38 Representative cross-sectional images of the ovary from WT and *Greb1* KO mice stained for ER-
 39 alpha and PR; scale bar: 200 μ m. At the time of tissue collection, most of the females from WT or
 40 *Greb1* KO were at the diestrus or metestrus stage of the estrous cycle.



42 **Supplementary Fig. 3 Compromised progesterone signaling in the uteri of *Greb1* KO mice.**

43 **a**, Relative transcript levels of *Muc1* in uterine tissues of dpc 4 from wild type (n=5) and *Greb1*

44 KO mice (n=5). Paired, two-tailed, t-test. Data reported as the mean \pm SEM. *P< 0.05, ***P<

45 0.001, ****P< 0.0001. **b**, Relative mRNA levels of *Greb1*, *Pgr*, and *Esr1* in the uteri of *Greb1* KO

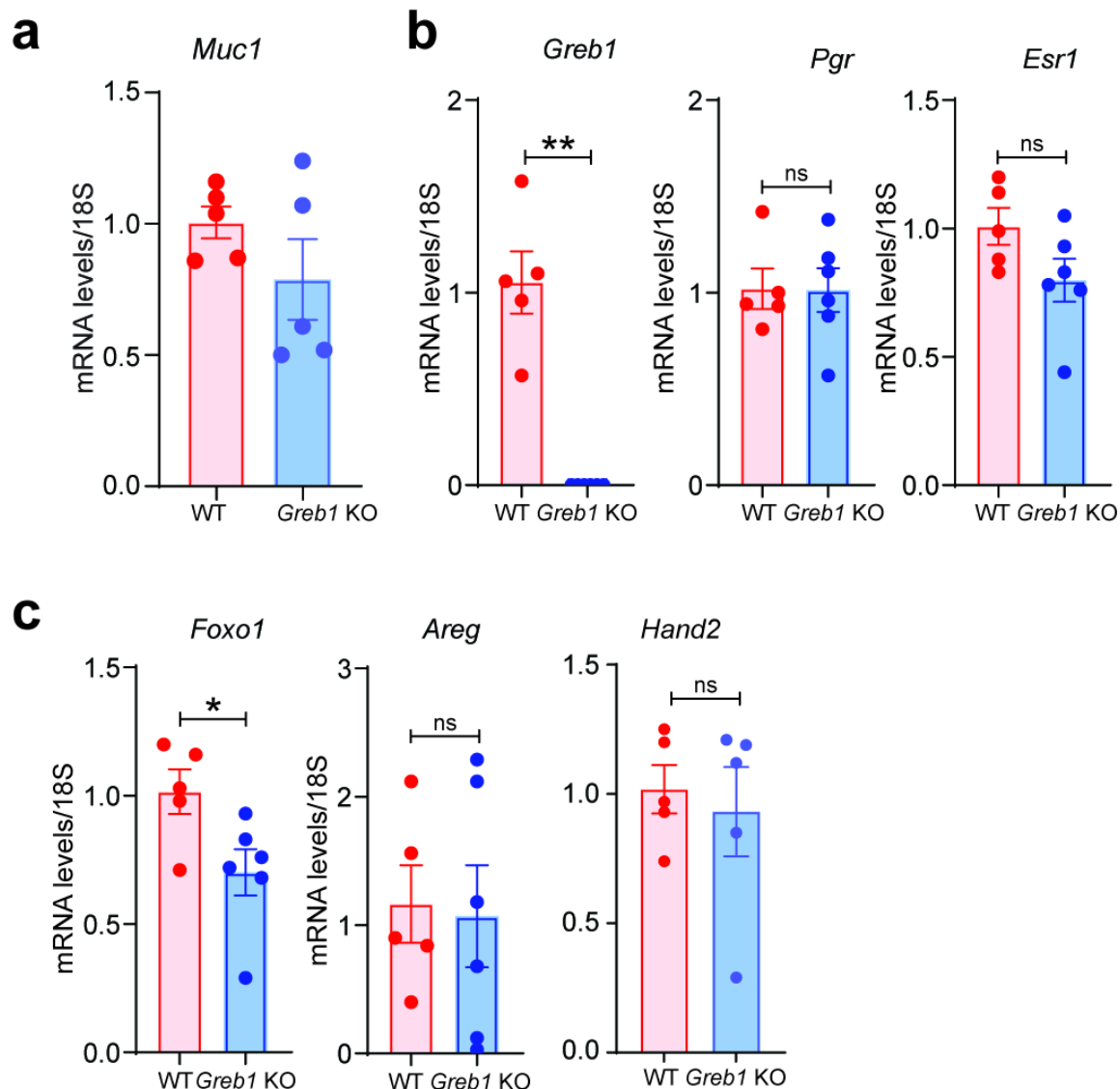
46 and WT mice at dpc 4 (n=5 for each genotype). Paired, two-tailed, t-test. Data reported as the

47 mean \pm SEM. *P< 0.05, ***P< 0.001, ****P< 0.0001. **b**, Relative mRNA levels of progesterone

48 target genes, *Foxo1*, *Areg*, and *Hand2* in the uteri of *Greb1* KO and WT mice at dpc 4 (n=5 for

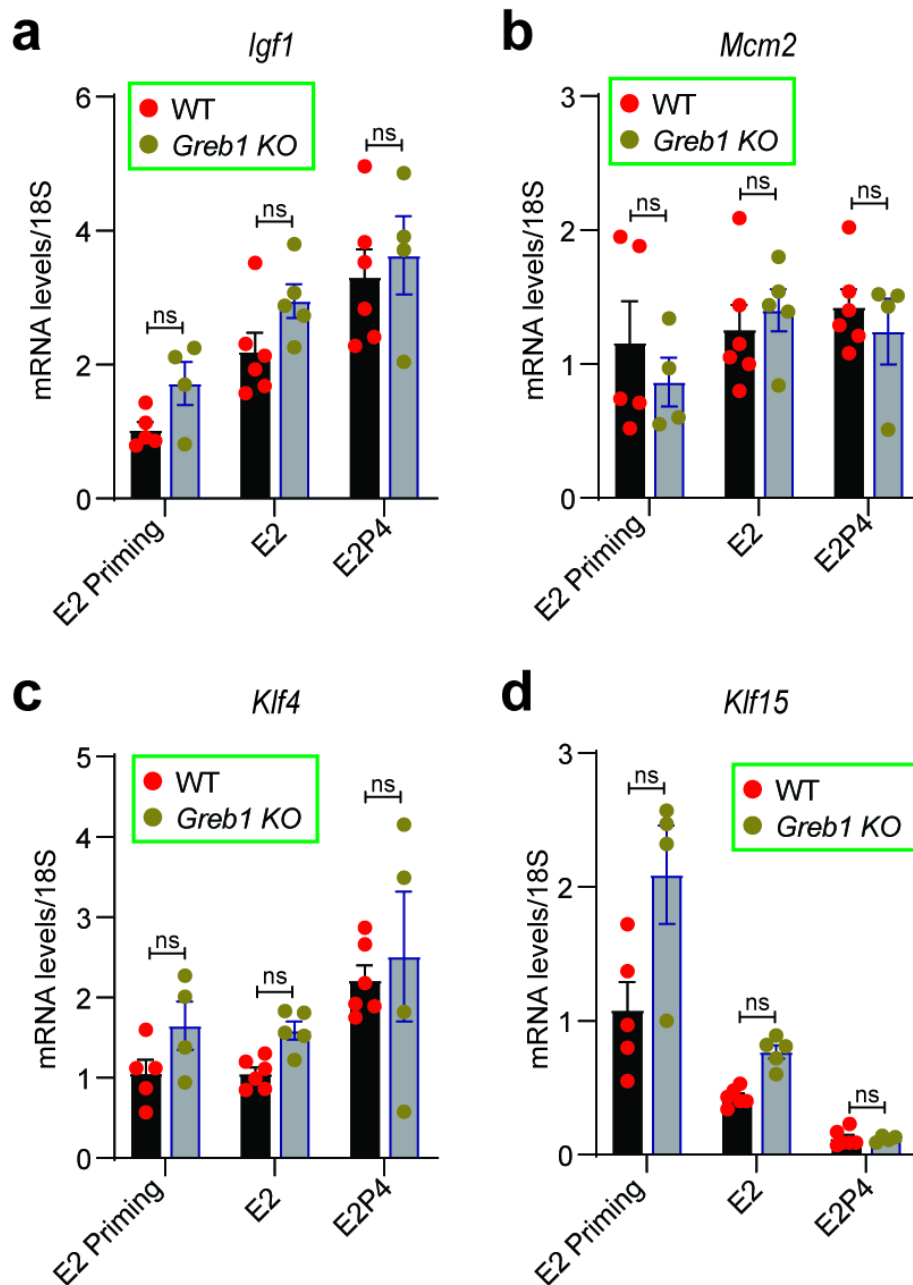
49 each genotype). Paired, two-tailed, t-test. Data reported as the mean \pm SEM. *P< 0.05, ***P<

50 0.001, ****P< 0.0001.



52 **Supplementary Fig. 4 Transcript levels of *Igf1*, *Mcm2*, *Klf4*, and *Klf15* in uteri of mimicked**
 53 **hormonal states of pregnancy. a-d, Relative mRNA levels of *Igf1* a, *Mcm2* b, *Klf4* c, and *Klf15***
 54 **d, from uteri from WT and *Greb1* KO mice in the indicated treatment groups. Data was analyzed**
 55 **by one-way ANOVA with Tukey's multiple comparisons post-test and presented as mean \pm SEM**
 56 **(n =4-6), P< 0.05, **P<0.01, ***P< 0.001, and ns, non-significant.**

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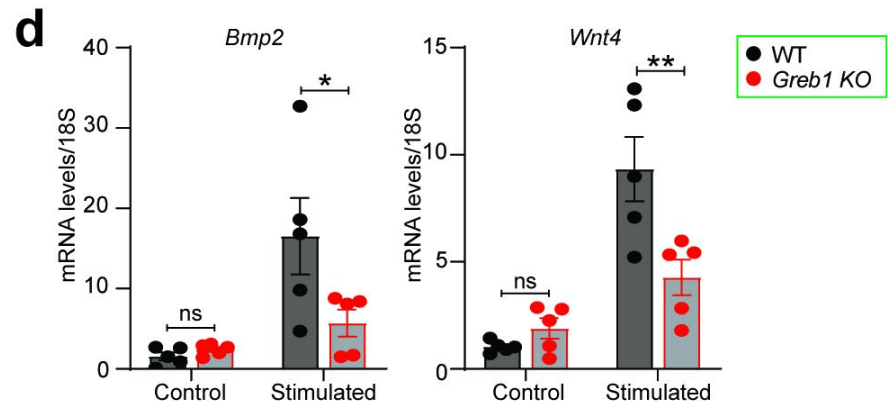
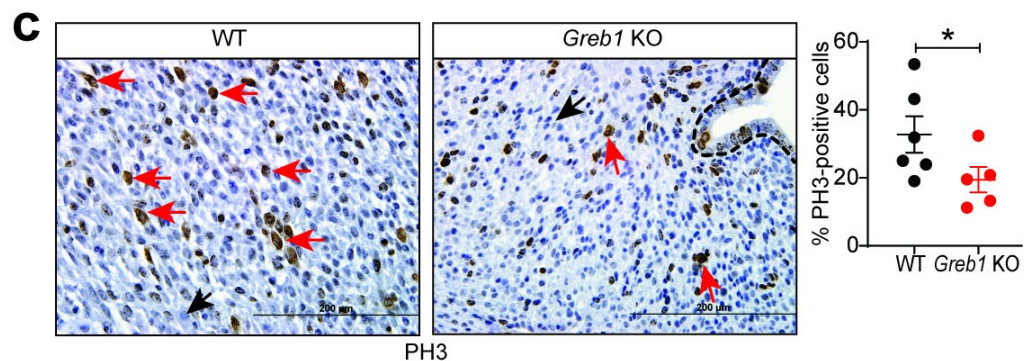
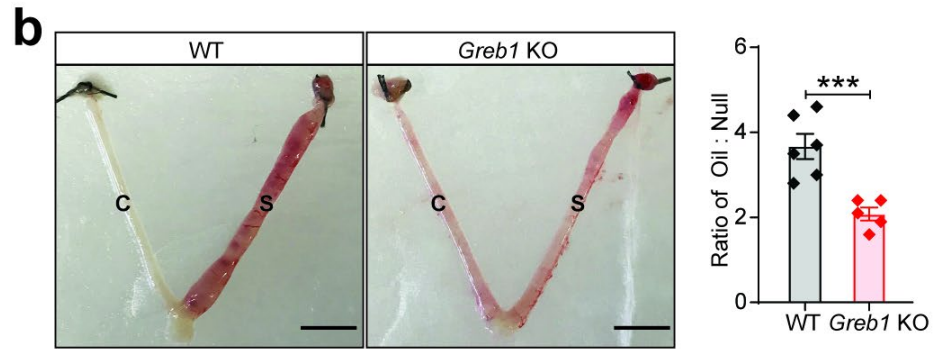
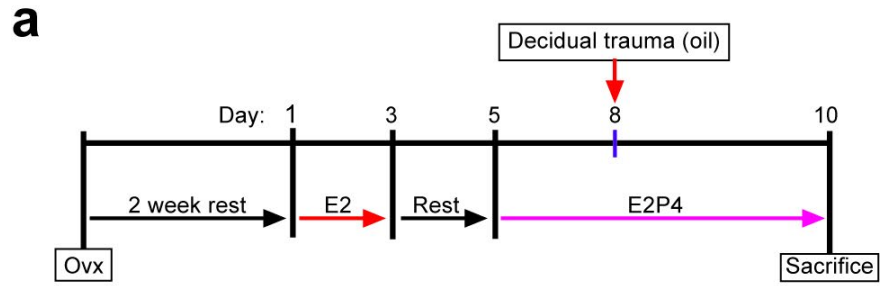
58 **Supplementary Fig. 5 Decidualization is defective in *Greb1* KO mice.** **a**, Timeline for induction
59 of artificial decidualization in ovariectomized mice. **b**, Representative images showing the gross
60 morphology of C: control and S: stimulated (oil-treated) uterine horns from WT and *Greb1* KO
61 mice collected 2 days after oil injection. The ratio between the wet weight of the oil-treated horn
62 to the wet weight of the untreated horn was calculated for each WT and *Greb1* KO (n = 5-6)
63 mouse; then ratios were averaged and compared across genotypes by using Paired, two-tailed,
64 t-test. Data reported as the mean \pm SEM. *P< 0.05, ***P< 0.001, ****P< 0.0001. **c**, Representative
65 cross-sectional images of the oil-treated uterus stained for Phospho-Histone H3 (PH3) from WT
66 and *Greb1* KO mice. Red arrowhead indicates the PH3-positive cells and black arrowhead
67 indicates PH3-negative cells; scale bar: 200 μ m. The graph on the right depicts the percentage
68 of ESCs (Endometrial stromal cells) undergoing proliferation in the stimulated horn of the WT
69 (n=6) and *Greb1* KO (n=5) mice two days following receipt of the decidualogenic stimulus.
70 Percentage of PH3 positive cells was calculated by counting positive cells in four separate fields
71 at 400X magnification in uteri by an investigator blinded to treatment groups and plotted as
72 percent positive cells relative to total cells. Paired, two-tailed, t-test. Data reported as the mean \pm
73 SEM. *P< 0.05, ***P< 0.001, ****P< 0.0001. **d**, Relative mRNA levels of decidualization marker
74 genes (*Bmp2* and *Wnt4*) assayed by qRT-PCR. Data was analyzed by one-way ANOVA with
75 Tukey's multiple comparisons post-test and presented as mean \pm SEM (n=4-6), P< 0.05,
76 **P<0.01, ***P< 0.001, and ns, non-significant.

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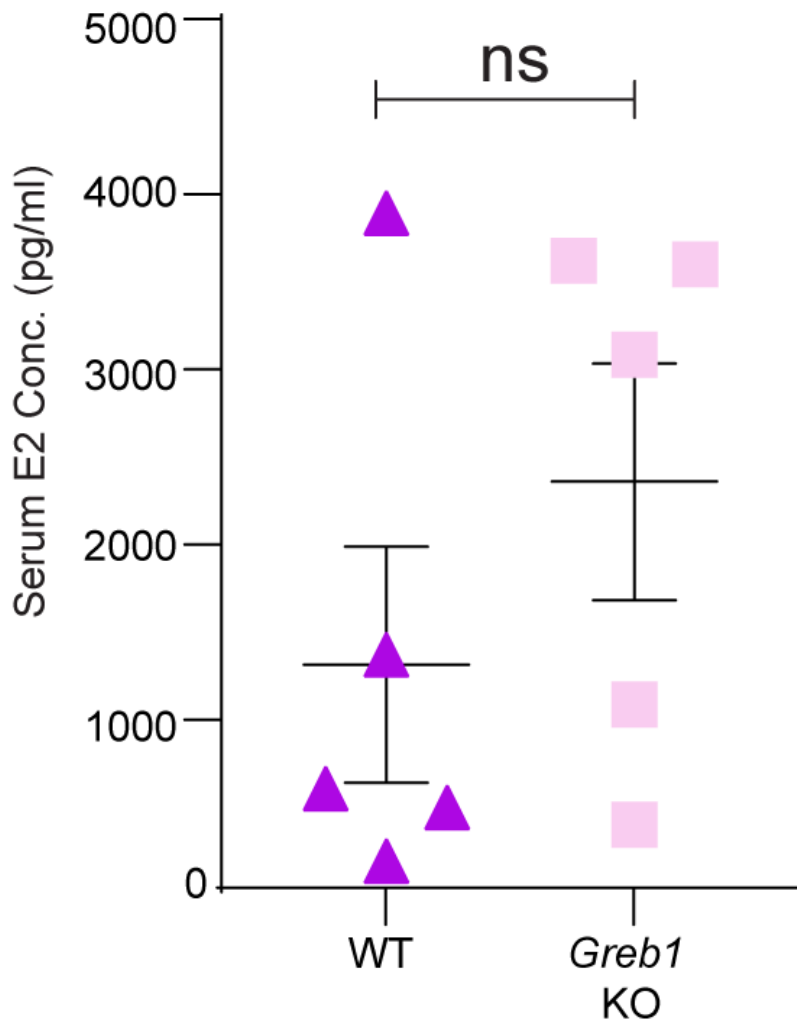
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94 **Supplementary Fig. 6 Estradiol (E2) levels are unaltered in serum collected from control or**
95 ***Greb1* endometriotic females.** Graphs depicting the level of estrogen from WT (n=5) or *Greb1*
96 endometriotic KO (n=5) mice. Paired, two-tailed, t-test. Data reported as the mean \pm SEM. *P<
97 0.05, ***P< 0.001, ****P< 0.0001.



99 **Supplementary Table 1 List of primers and TaqMan probes**

Gene name	Species	Application, Chemistry	Company	Sequence/Cat. No.
Greb1 KO	Mouse	Genotyping	IDT	P1: GACAGGGTGTTCCTTTTGC P2: TTAGGCCACCATTGGAAACT P3: ACAACCTCAGGCTGCAATTT
<i>Greb1</i>	Mouse	qPCR, TaqMan	ABI	Mm00479259_m1
<i>Esr1</i>	Mouse	qPCR, TaqMan	ABI	Mm00433149_m1
<i>Pgr</i>	Mouse	qPCR, TaqMan	ABI	Mm00435628_m1
<i>Fgf18</i>	Mouse	qPCR, TaqMan	ABI	Mm00433286_m1
<i>Areg</i>	Mouse	qPCR, TaqMan	ABI	Mm01354339_m1
<i>Bmp2</i>	Mouse	qPCR, TaqMan	ABI	Mm01340178_m1
<i>Igf1</i>	Mouse	qPCR, TaqMan	ABI	Mm00439560_m1
<i>Klf4</i>	Mouse	qPCR, TaqMan	ABI	Mm00516104_m1
<i>Klf15</i>	Mouse	qPCR, TaqMan	ABI	Mm00517792_m1
<i>Ccnd1</i>	Mouse	qPCR, TaqMan	ABI	Mm00432359_m1
<i>Mcm2</i>	Mouse	qPCR, TaqMan	ABI	Mm00484804_m1
<i>Wnt4</i>	Mouse	qPCR, TaqMan	ABI	Mm01194003_m1
<i>Ihh</i>	Mouse	qPCR, TaqMan	ABI	Mm00439613_m1
<i>Foxo1</i>	Mouse	qPCR, TaqMan	ABI	Mm00490671_m1
<i>Cyp26a1</i>	Mouse	qPCR, TaqMan	ABI	Mm00514486_m1
<i>Il13ra2</i>	Mouse	qPCR, TaqMan	ABI	Mm00515166_m1
<i>Muc1</i>	Mouse	qPCR, TaqMan	ABI	Mm00449604_m1
<i>Hand2</i>	Mouse	qPCR, TaqMan	ABI	Mm00439247_m1
<i>GREB1</i>	Human	qPCR, TaqMan	ABI	Hs00536409_m1
<i>FOXO1</i>	Human	qPCR, TaqMan	ABI	Hs00231106_m1
<i>CCND1</i>	Human	qPCR, TaqMan	ABI	Hs00765553_m1
<i>IGF1</i>	Human	qPCR, TaqMan	ABI	Hs01547656_m1
<i>ESR1</i>	Human	qPCR, TaqMan	ABI	Hs01046816_m1
<i>18S</i>	Human Mouse	qPCR, TaqMan	ABI	4318839

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101 *All primer sequences are written 5' to 3'

102 ABI-applied biosystems

103 IDT-integrated DNA technologies.

104 **Supplementary Table 2 List of antibodies**

Antibody	Company, Catalogue number	Application and Titer
GREB1	Abcam, ab72999	Immunoblotting (1:1000), ChIP (3 ug)
GREB1	ThermoFisher Scientific PA5-50673	Immunohistochemistry (1:100) Immunofluorescence (1:100)
Ki-67	Abcam, ab15580	Immunohistochemistry (1:100)
ER- α	Abcam, ab75635	Immunohistochemistry (1:100)
Cyclin D1	CST, #55506	Immunohistochemistry (1:100)
Phospho-Histone H3	Millipore Sigma, 06-570	Immunohistochemistry Immunofluorescence (1:100)
MUC1	Abcam, ab15481	Immunofluorescence (1:100)
PR Antibody (F-4) AC	SCBT, sc-166169 AC	Immunoprecipitation (3 ug)
normal mouse IgG-AC	SCBT, sc-2343	Immunoprecipitation (3 ug)
PR	SCBT, PR (H-190) sc-7208	Immunohistochemistry (1:100) Immunoblotting (1:1000) ChIP (3 ug)
Normal Rabbit IgG	CST, #2729	Immunohistochemistry (1:1000) ChIP (3 ug)
Goat anti-Rabbit IgG (H+L)	ThermoFisher Scientific, A32731	Immunofluorescence (1:100)
GAPDH	CST, #2118S	Immunoblotting (1:5000)
Anti-rabbit IgG, HRP-linked	CST, #7074	Immunoblotting (1:3000)

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106 **Supplementary Table 3 List of Primers used for Chromatin Immunoprecipitation (ChIP)**

Primers	Company	Sequence
FOXO1 Region 1A Forward	Sigma-Aldrich	GGTCAACCAATGCACACAATG
FOXO1 Region 1A Reverse	Sigma-Aldrich	CGTAGGTCCCTGGATGAGTATAA
FOXO1 Region 1B Forward	Sigma-Aldrich	CAATGCCGACGTCCAGAG
FOXO1 Region 1B Reverse	Sigma-Aldrich	GGGATTCCTGAGAACACTAAGC
FOXO1 Region 2A Forward	Sigma-Aldrich	GCTCCCAAGAATCATGTGTTATG
FOXO1 Region 2A Reverse	Sigma-Aldrich	GTGTTTGTGAAGCAGGTTGG
FOXO1 Region 2B Forward	Sigma-Aldrich	GGAGCTGGTTCACAGAAAGT
FOXO1 Region 2B Reverse	Sigma-Aldrich	CCCATCAAAGTAATCAGGGACA
UNTR Forward	Sigma-Aldrich	CTGTACCTGGGGTTCATTCATT
UNTR Reverse	Sigma-Aldrich	CAGTAAGCCGTTCACTCTCACA

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108 *All primer sequences are written 5' to 3'