

Transcriptional co-regulator *ZMIZ1* modulates estrogen responses that are essential for healthy endometrial function.

Supplemental Figure Legends

Supplemental Figure 1 *ZMIZ1* in Human Endometrium

- A. Chromatin landscapes near mouse (top panel) and human (bottom panel) *ZMIZ1* genes. Mouse tracks include ESR1 (blue) and Histone H3 K27 acetylation (K27Ac, red) ChIPSeq, and loops called from previously published HiC data (1). Potential enhancers (ESR1 and K27Ac peaks) are indicated with dashed outlines; previously described superenhancers (SE) are highlighted in yellow with each one's rank shown below it. Human tracks include proliferative endometrium ESR1 ChIPSeq (blue) (2) and K27Ac (red) and HiC loops from cultured H1644 hESC.
- B. *ZMIZ1* transcript expression level in each endometrial cell type and at multiple menstrual cycle stages from scRNAseq dataset described in Figure 1B.
- C. RT_PCR of *ZMIZ1* levels in THESC cells following 3 days of culture with vehicle (V) or decidualized with EPC or omission of estrogen (PC). N=3 each. ****, ***, ** p<0.0001, 0.001, 0.01, respectively, by ANOVA.
- D. Effect of siNT or si*ZMIZ1* targeting on Ishikawa cells. Left: MTT A595 signal on day 2 after estrogen (E2) treatment was initiated, calculated as % that on the day E2 was added (d0). n=6 for each condition. Center and right: RT-PCR for *ZMIZ1*, *PGR*, or *GREB1* in Ishikawa cells targeted with siNT or si*ZMIZ1* and then treated for 2 days with ethanol vehicle (V) or with 10 nM estradiol (E2). n=3 for all conditions. **, ***, **** p< 0.01, 0.001, 0.0001, respectively, by two way ANOVA.

Supplemental Figure 2 Analysis of targeting *Zmiz1* in mouse uterine tissue.

- A. ZMIZ1 IHC of uterine cross sections taken from ovariectomized *Zmiz1^{ff}* or *Zmiz1^{d/d}* mice injected with estrogen for 2 days, rested for 2 days and then injected with progesterone for 4 days. Scale bars=250 μ m (top) or 200 μ m (bottom).
- B. ZMIZ1 Western blot of total uterine proteins from *Zmiz1^{ff}* (n=2) and *Zmiz1^{d/d}* (n=3) mice. The arrow indicates the 120 kilodalton ZMIZ1 band.
- C. RT-PCR for *Zmiz1* of uterine RNA from *Zmiz1^{ff}* or *Zmiz1^{d/d}* mice treated with V or estradiol (E2) for 2 or 24 hours (h). For *Zmiz1^{ff}* V n=4 for all treatments, for *Zmiz1^{d/d}* V n=5, *Zmiz1^{d/d}* E2, 2h and E2, 24h n=6. **** p< 0.0001, by 2-way ANOVA.

Supplemental Figure 3 Impact of *Zmiz1* deletion on female mouse reproductive tract.

- A. Pups produced during 6 months of continuous breeding of mice with uterine *Zmiz1* deletion (*Zmiz1^{d/d}*; n=6; **** p<0.0001 by unpaired t-test) compared to control (*Zmiz1^{ff}* n=6).
- B. Representative uterine histology and analysis from aged (27-30 weeks old; scale bar=200 μ m) or young (7-14 weeks old) *Zmiz1^{ff}* or *Zmiz1^{d/d}* mice stained with H&E (scale bar=250 μ m) or Masson's trichrome (scale bar=300 μ m).
- C. Estrous cyclicity evaluation of *Zmiz1^{d/d}* females and *Zmiz1^{ff}* littermates using vaginal washes. P=proestrus E=estrus M=metestrus D=diestrus.
- D. Evaluation of ovary function of *Zmiz1^{d/d}* at 3.5 dpc or after superovulation. Graphs show number of oocytes collected following superovulation (n=4 *Zmiz1^{ff}*, 8 *Zmiz1^{d/d}*; **=p<0.01 by unpaired t-test), embryos flushed from uterus at 3.5 dpc (n=17 *Zmiz1^{ff}*, 15 *Zmiz1^{d/d}* ns=non-significant by unpaired t-test), serum prolactin (n=7 *Zmiz1^{ff}*, 8 *Zmiz1^{d/d}* ns=non-

significant by unpaired t-test) or progesterone (n=5 *Zmiz1^{ff}*, 4 *Zmiz1^{d/d}* (from mice with embryos in uterus) $\ast=p<0.05$ by unpaired t-test) at 3.5 dpc. H&E sections of *Zmiz1^{ff}* or *Zmiz1^{d/d}* ovaries from 3.5 dpc (scale bars *Zmiz1^{ff}*=500 μm *Zmiz1^{d/d}*=1000 μm) or superovulation (scale bar=300 μm).

Supplemental Figure 4 *Zmiz1* deletion impairs decidual response.

- A. RT-PCR of uterine RNA from decidualized *Zmiz1^{ff}* or *Zmiz1^{d/d}* 3 days after intraluminal (il) oil injection (+) compared to un-injected uterine horn (-) showing *Bmp2*, the decidual prolactin *Prl8a2* and *Zmiz1*. For all samples n=4; $\ast, \ast\ast$ $p<0.05, 0.01$, respectively, by two-way ANOVA.
- B. Weights of uterine horns from *Zmiz1^{d/d}* mice and control littermates (*Zmiz1^{ff}*) 3 days after il oil injection. For *Zmiz1^{d/d}* and *Zmiz1^{ff}* littermates n=4; $\ast p<0.05$, by unpaired t-test.
- C. H&E stained uterine sections from *Zmiz1^{d/d}* or *Zmiz1^{ff}* littermates 3 days after intraluminal (il) oil injection (+) compared to un-injected uterine horn (-). Scale bars=300 μm or 250 μm . Circled area highlights decidualized cells.
- D. PGR or ESR1 Western blot of uterine proteins and ESR1 IHC from samples 20h after il oil injection (+) or un-injected controls (-). PGR-B and PGR-A are B and A isoforms of PGR, respectively. GAPDH is the loading control. Graphs show quantification of signal in each band (PGR or ESR1 band normalized to GAPDH band). For all conditions n=3. $\ast p<0.05$ by 2-way ANOVA. Scale bar=200 μm .

Supplemental Figure 5 *Zmiz1* Regulates Genes Driving Decidual Responses

- A. Heat map showing the differentially expressed genes in *Zmiz1^{ff}* and *Zmiz1^{d/d}* uterine RNA 20 h following il oil injection (+) or in non-injected controls (-).

- B. Pathway summary shows the impact of il oil injection (+ vs -) in *Zmiz1^{ff}* or *Zmiz1^{d/d}* on activity of pathways impacting the cell cycle. The heatmap shows the relative fold-changes (+ vs – il oil injection) of genes in the cell cycle checkpoint pathway.
- C. RT-PCR of uterine RNA from *Zmiz1^{ff}* or *Zmiz1^{d/d}* mice without (–) or 20 hours following il oil injection (+) of cell cycle genes *Ki67*, *Mcm2*, *Mcm5*, *Foxm1*, *Ccna2*, *Ccnb1*, *Ccnb2*, *Cdc2a* and *Zmiz1*. For *Zmiz1^{ff}* –, n=4; *Zmiz1^{ff}* +, n=4-6; *Zmiz1^{d/d}* –, n=5; *Zmiz1^{d/d}* +, n=3. *, **, ***, **** p<0.05, 0.01, 0.001, 0.0001 by 2-way ANOVA.

Supplemental Figure 6 *Zmiz1* deletion enhances collagen synthesis and inhibits proliferation associated genes

- A. RT-PCR of uterine RNA from *Zmiz1^{ff}* or *Zmiz1^{d/d}* mice of collagen subunits *Col1a1*, *Col5a2*, *Col6a1*, and *Col6a3*. For *Zmiz1^{ff}* V n=9, *Zmiz1^{ff}* E24h n=10, *Zmiz1^{d/d}* V n=8, *Zmiz1^{d/d}* E24h n=9. **, ***, **** p<0.01, 0.001, 0.0001, by 2-way ANOVA.
- B. Representative sections showing Masson's trichrome staining of ovariecomized *Zmiz1^{ff}* (n=3) or *Zmiz1^{d/d}* (n=3) uterus treated for 24h with estradiol (E2). There is no apparent difference between the samples.
- C. Heatmap of fold changes (E24h vs. V) of genes in the DNA synthesis pathway in *Zmiz1^{ff}* and *Zmiz1^{d/d}* mice from analysis in Table 1. RT-PCR of uterine RNA from *Zmiz1^{ff}* or *Zmiz1^{d/d}* mice of *Foxm1*, *Mcm2*, *Mcm5*, *Ccna2*, *Ccnb1*, *Ccnb2* and *Cdc2a*. For *Zmiz1^{ff}* V n=4-9, *Zmiz1^{ff}* E24h n=5-10, *Zmiz1^{d/d}* V n=3-8, *Zmiz1^{d/d}* E24h n=5-9. *, **, **** p<0.05, 0.01, 0.0001, ns=not significant by 2-way ANOVA.
- D. Graphical summary of some of the top enriched signals, functions and pathways following IPA Core Analysis of DEG from RNAseq analysis of *Zmiz1^{d/d}* vs *Zmiz1^{ff}* RNA 24h after estradiol (E24h) injection. Positive or negative z-score suggests *Zmiz1* deletion increases or decreases signaling, respectively.

Supplemental Figure 7 Co-localization of ZMIZ1 and ESR1 in Uterine Cells

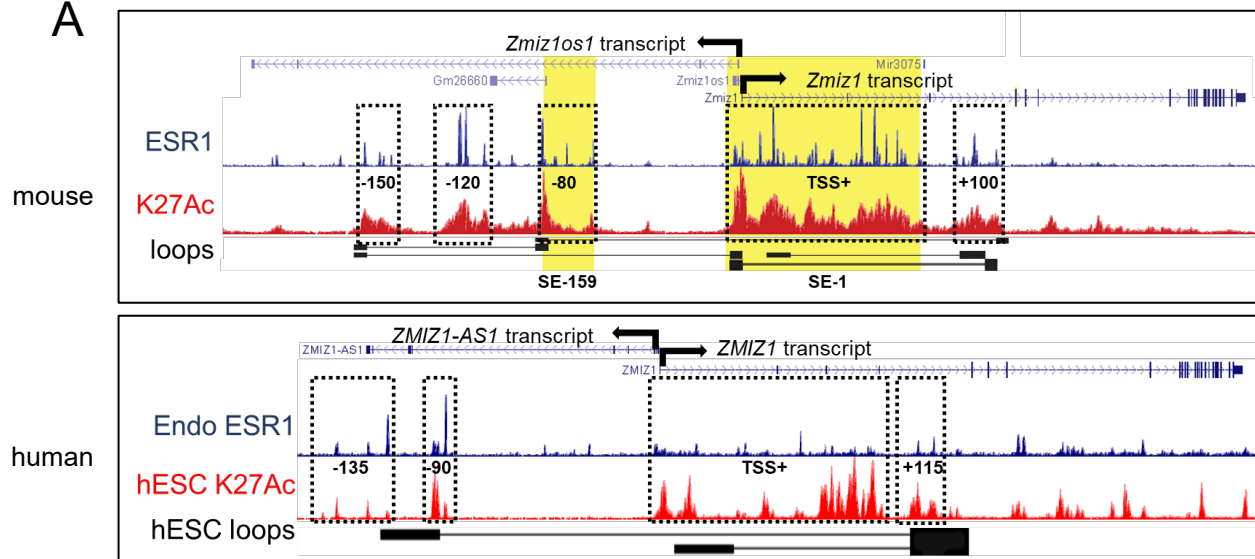
- A. IFA of *Zmiz1^{ff}* or *Zmiz1^{d/d}* uterine sections for ZMIZ1 (green) or ESR1 (red) in uterine tissue from mice treated as in Figure 7A, without oil decidual stimulus, showing localization in nuclei of the same cells. DAPI (blue). Scale bar=10 μ m.
- B. IFA of *Zmiz1^{ff}* or *Zmiz1^{d/d}* uterine sections for ZMIZ1 (green) or ESR1 (red) in uterine tissue from mice treated with E2 for 24h showing ZMIZ1 and ESR1 localization in nuclei of the same cells. Scale bar=10 μ m.

References

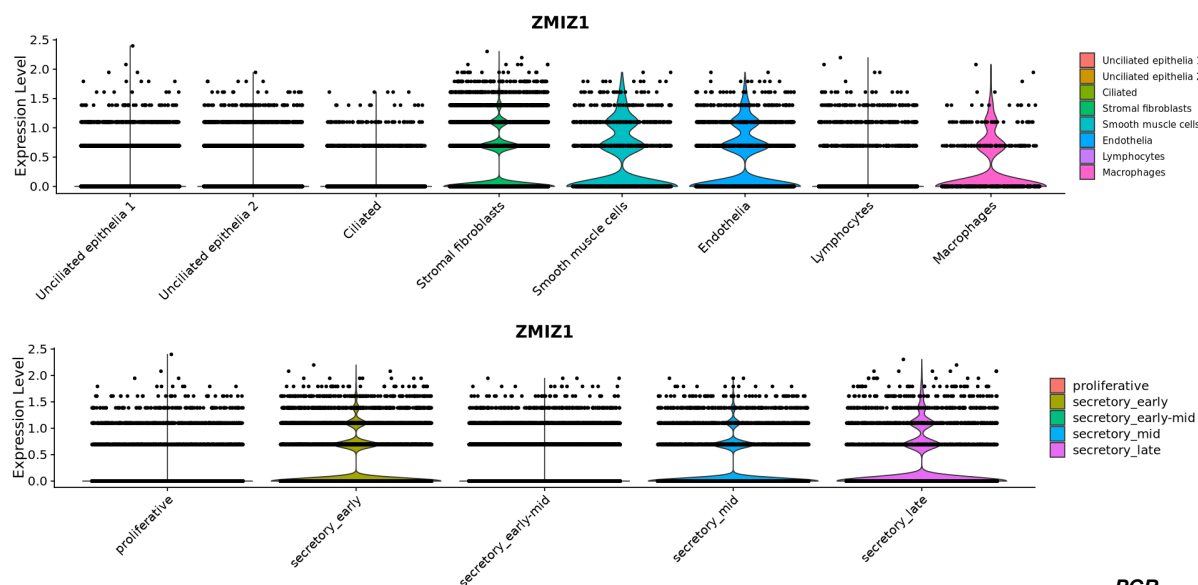
1. Hewitt SC, Grimm SA, Wu SP, DeMayo FJ, and Korach KS. Estrogen receptor alpha (ERalpha)-binding super-enhancers drive key mediators that control uterine estrogen responses in mice. *The Journal of biological chemistry*. 2020;295(25):8387-400.
2. Hewitt SC, Wu SP, Wang T, Ray M, Brolinson M, Young SL, et al. The Estrogen Receptor alpha Cistrome in Human Endometrium and Epithelial Organoids. *Endocrinology*. 2022;163(9):2022.04.19.488787.

FS1

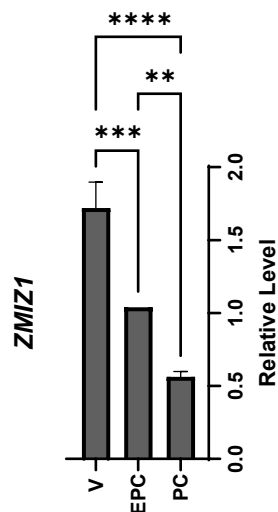
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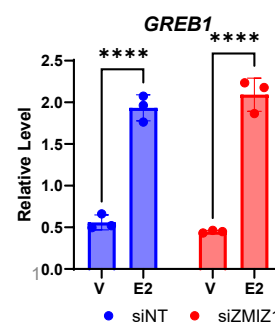
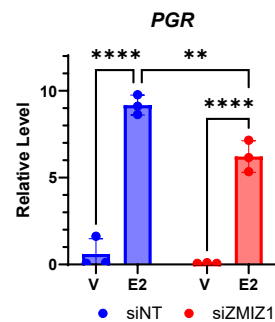
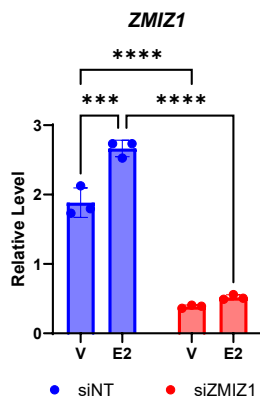
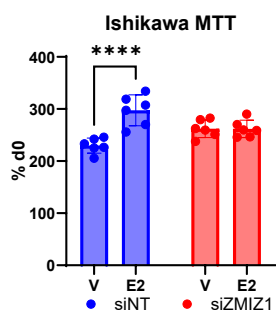
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C



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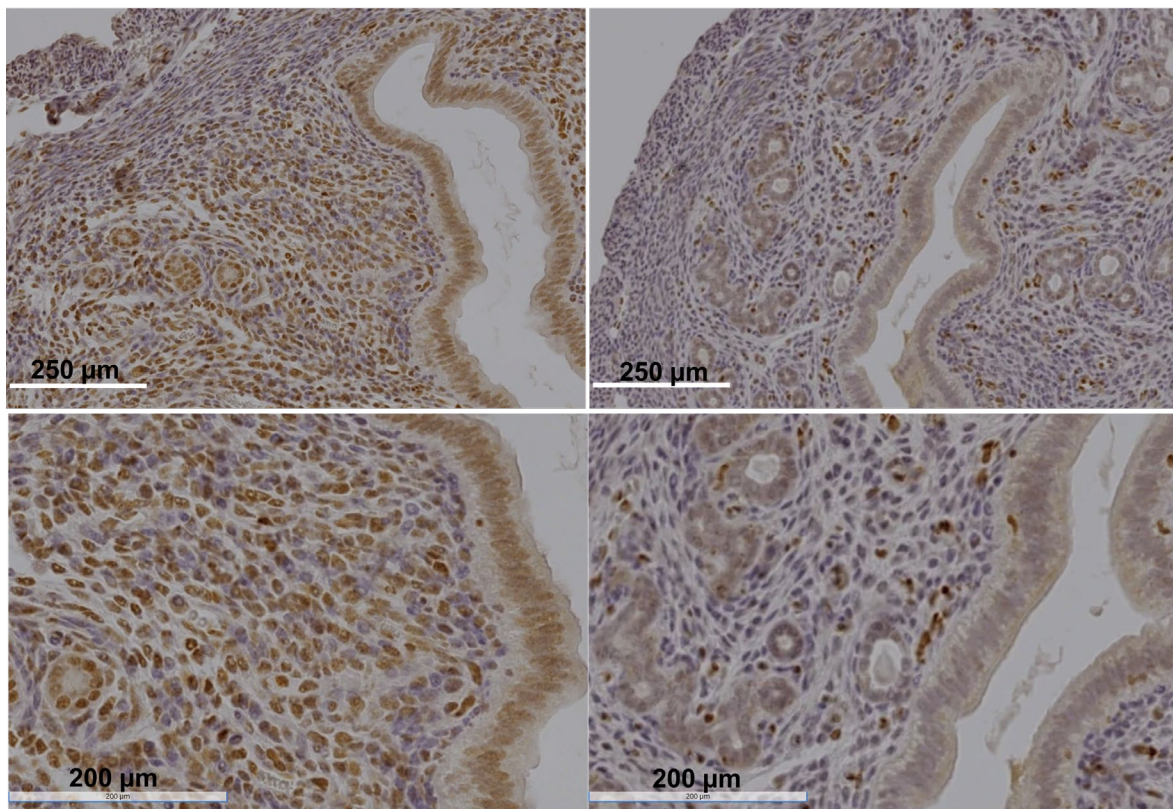


A

ZMIZ1 IHC

Zmiz1^{f/f}

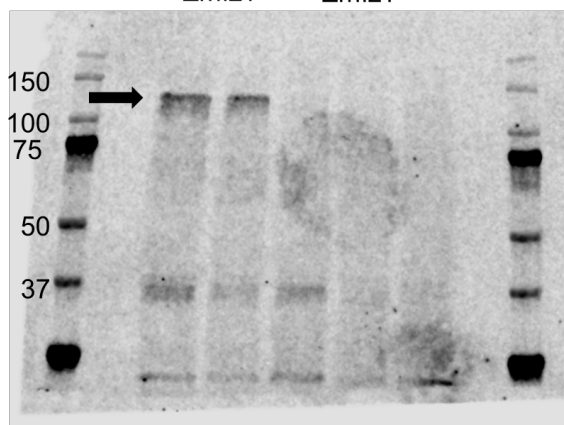
Zmiz1^{d/d}



B

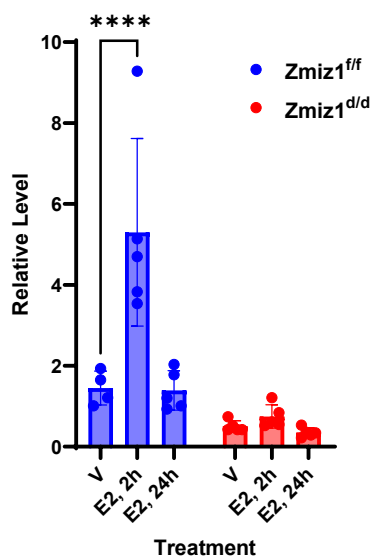
Zmiz1^{f/f}

Zmiz1^{dd}

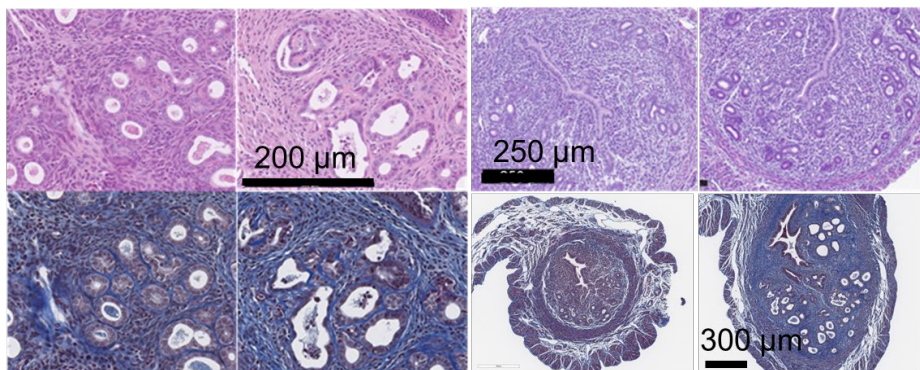
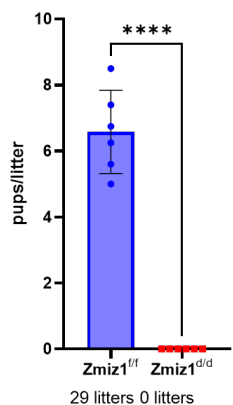


C

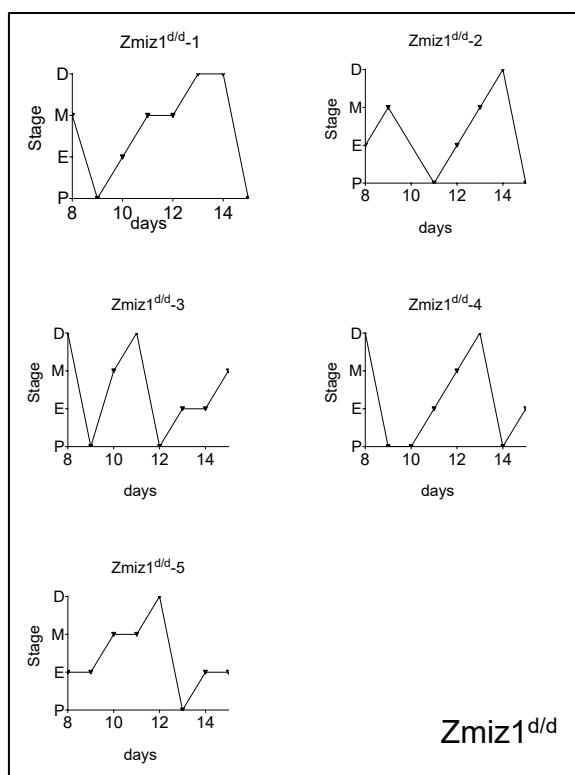
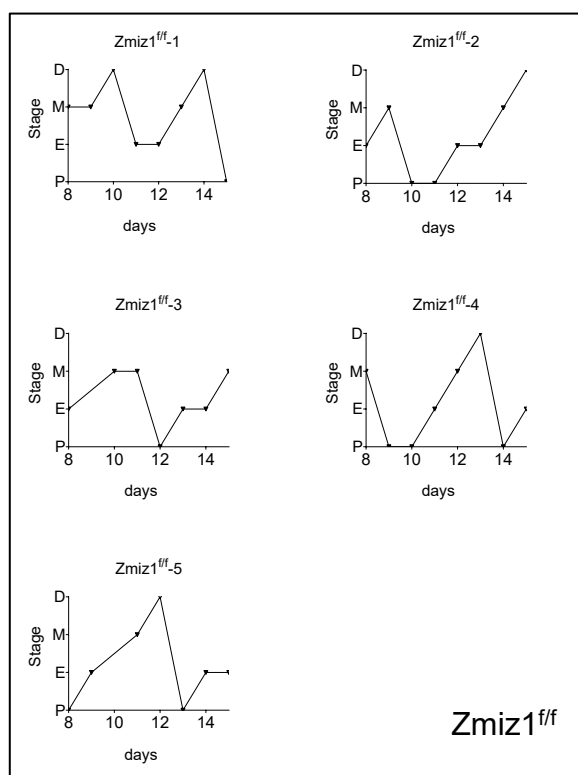
Zmiz1



Sample Group	27-30 weeks old Zmiz1 ^{ff}	27-30 weeks old Zmiz1 ^{d/d}	7-14 weeks old Zmiz1 ^{ff}	7-14 weeks old Zmiz1 ^{d/d}
Atypical Glands	0/6	6/8	0/13	1/11 (mild)
Collagen Deposition	2/6	8/8	0/13	0/11



C



Superovulated oocytes

Genotype	Oocytes/mouse
<i>Zmiz1^{fl/fl}</i>	~26
<i>Zmiz1^{fl/did}</i>	~12

Embryos in uterus 3.5 dpc

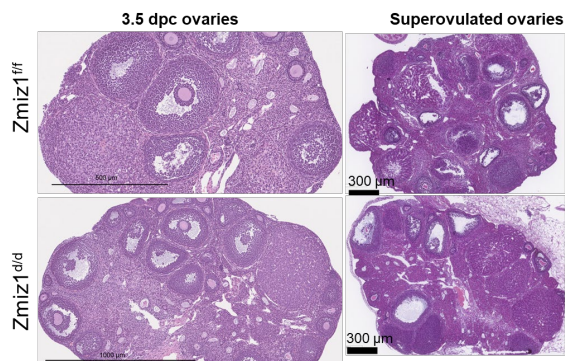
Genotype	Number of Embryos
<i>Zmiz1^{fl/fl}</i>	~2
<i>Zmiz1^{fl/did}</i>	~1

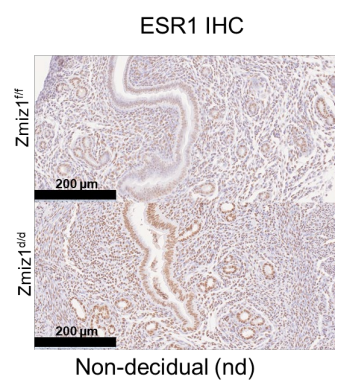
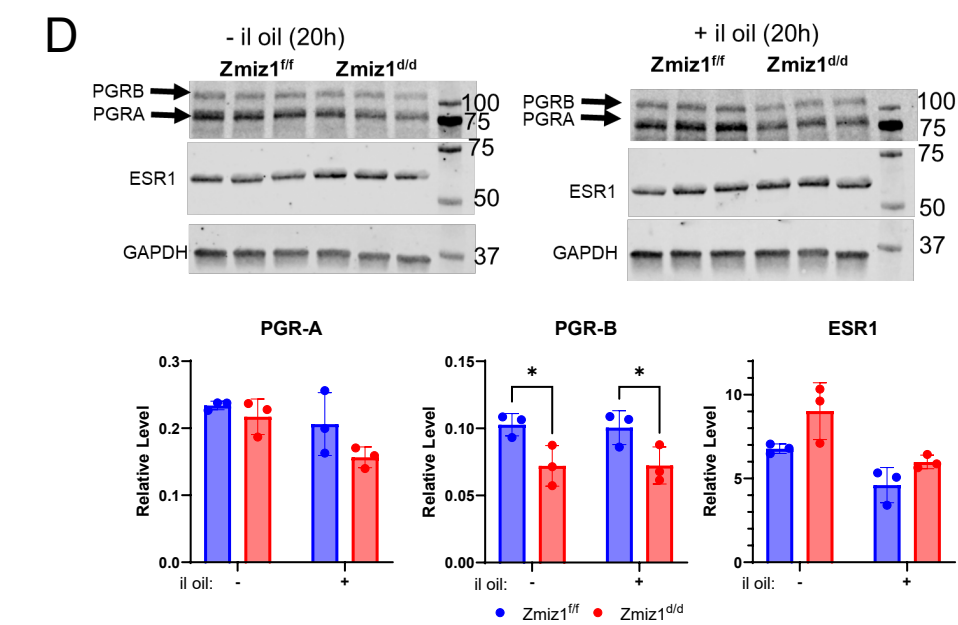
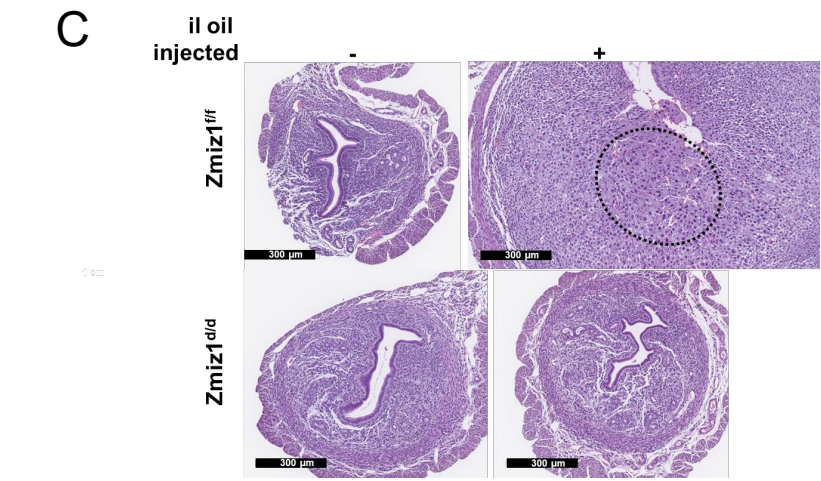
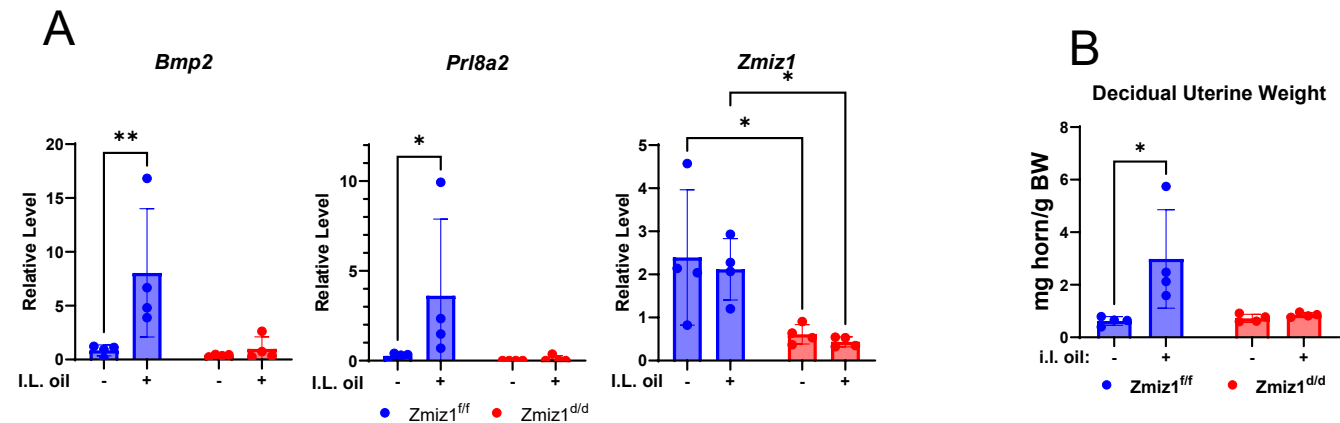
Serum progesterone, 3.5 dpc

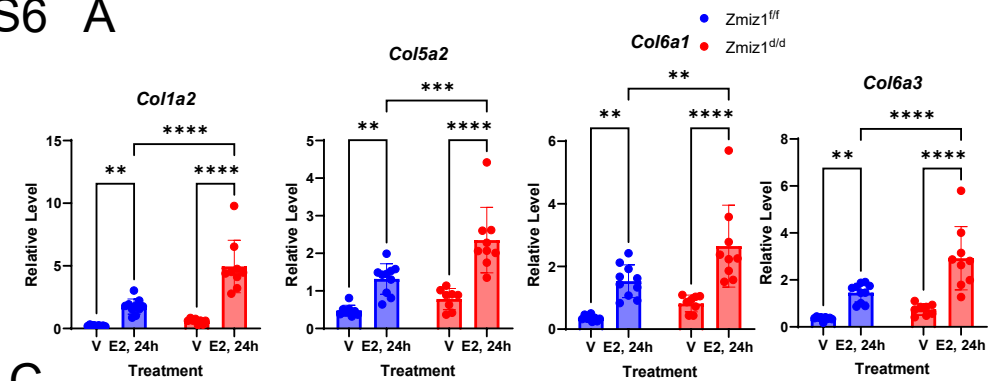
Genotype	Progesterone (ng/ml)
<i>Zmiz1^{fl/fl}</i>	~6
<i>Zmiz1^{fl/did}</i>	~2

Serum Prolactin 3.5 dpc

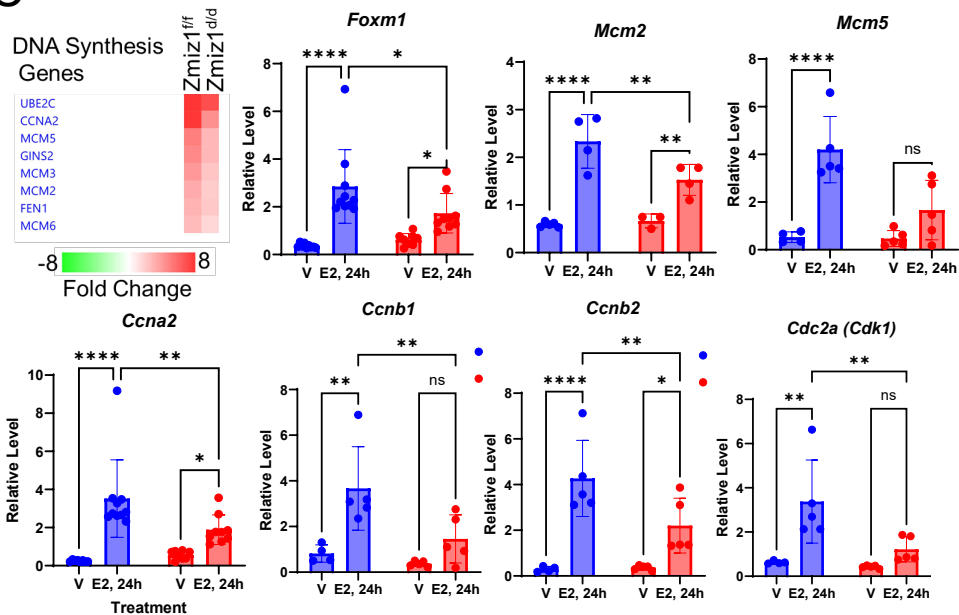
Genotype	Prolactin (ng/ml)
<i>Zmiz1^{fl/fl}</i>	~9
<i>Zmiz1^{fl/did}</i>	~2



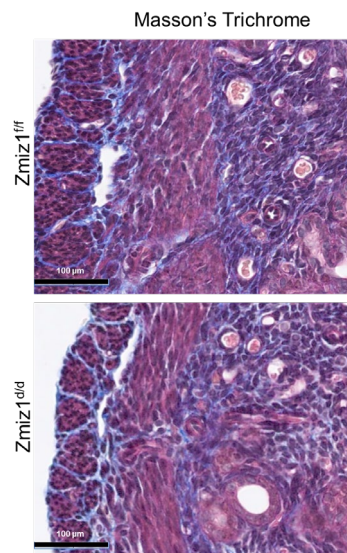




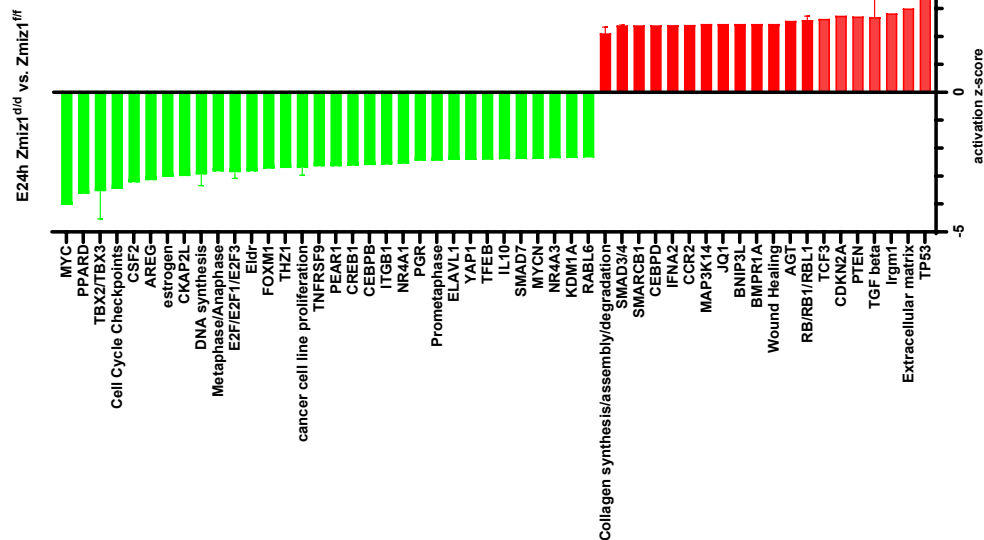
C



B



D



A

- il oil

Zmiz1^{f/f}

Zmiz1^{d/d}

ZMIZ1
&
ESR1

ZMIZ1

ESR1

DAPI

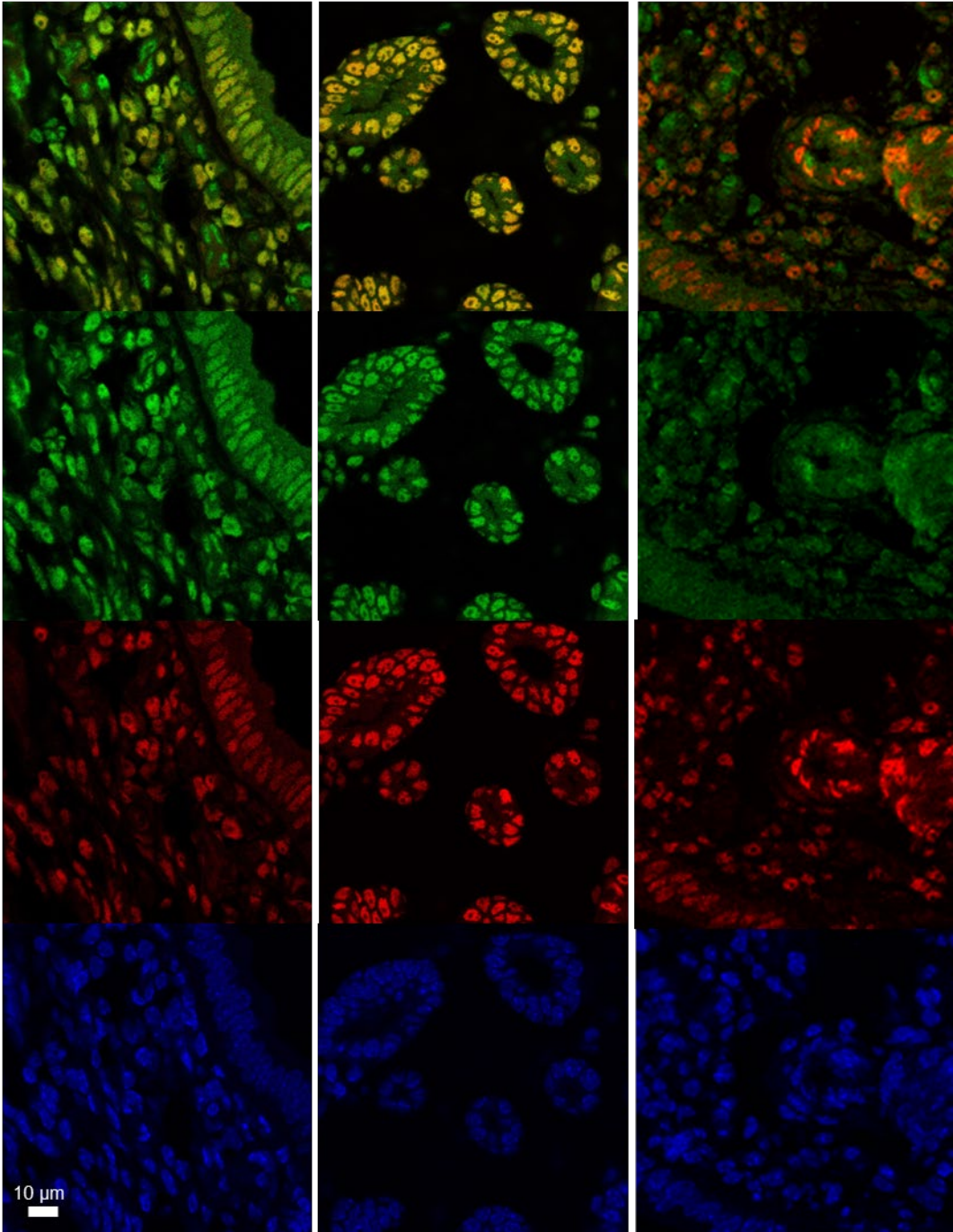


Fig S7

