Supplemental Materials for:

The death effector domain-containing DEDD is required for uterine decidualization during early pregnancy in mice

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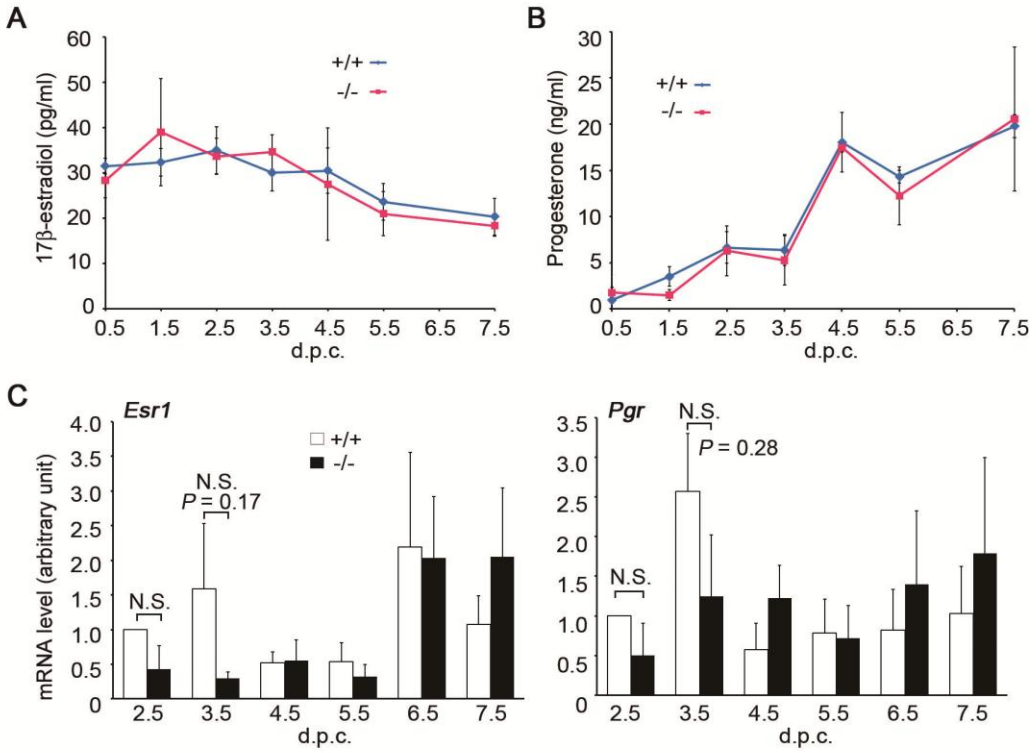
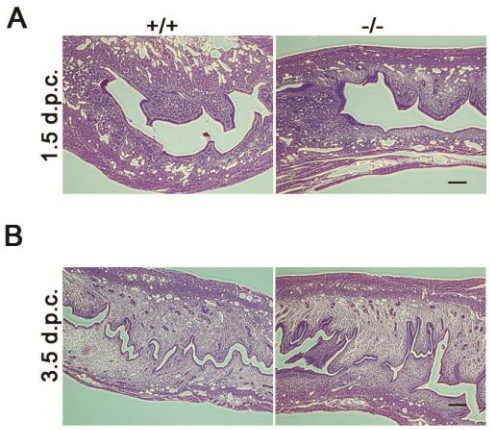
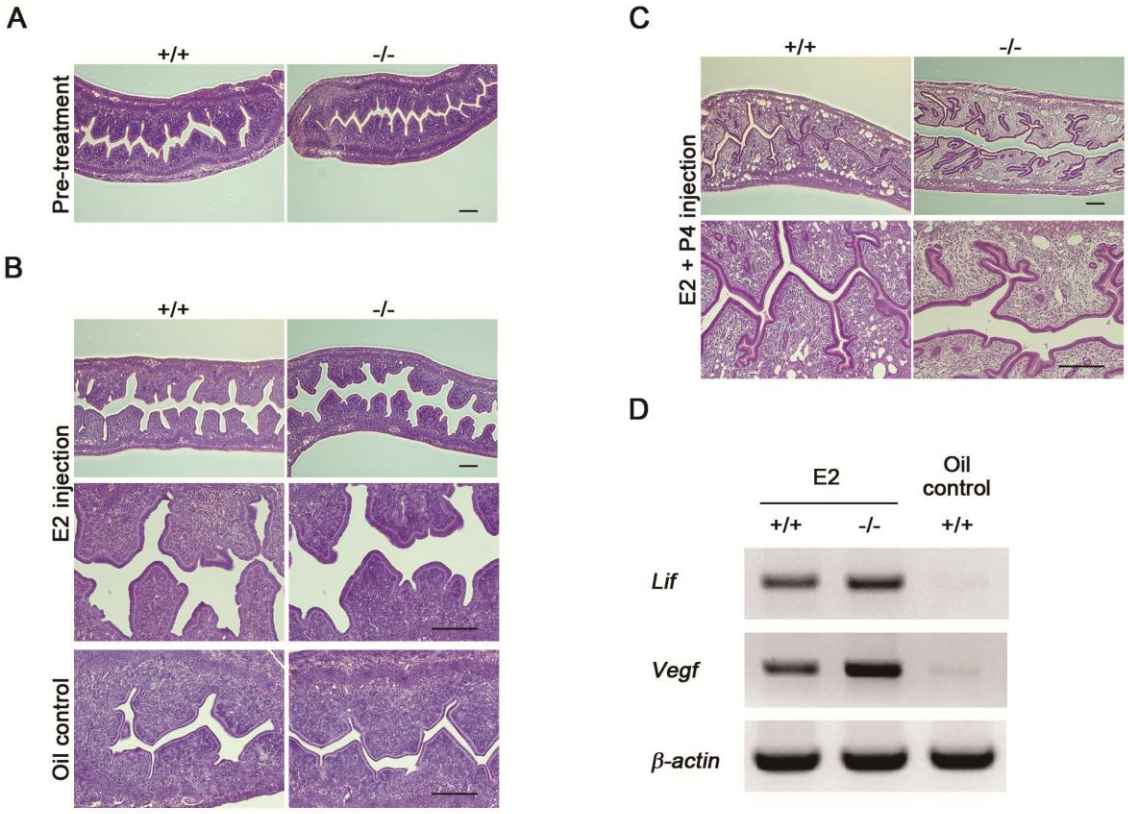


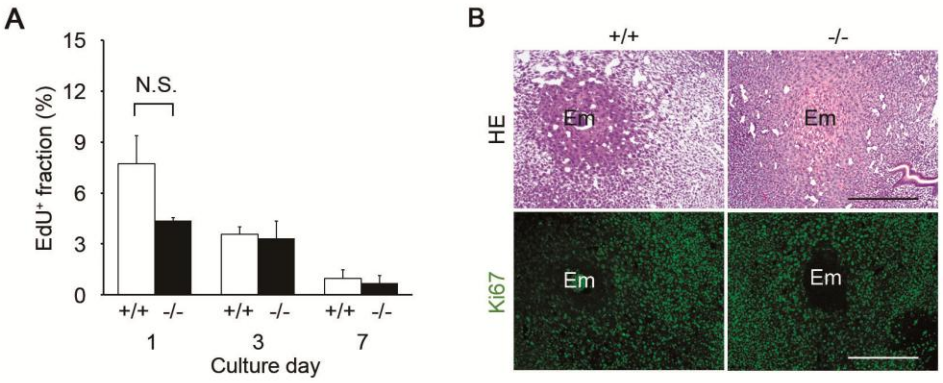
Supplemental Figure 1: Comparable blood hormone levels in *DEDD*$^{+/+}$ and *DEDD*$^{-/-}$ mice. **A** and **B**: Levels for serum steroid hormones in pregnant female mice during early pregnancy (0.5 – 7.5 d.p.c.). A: 17β-estradiol (Estrogen); B: Progesterone. n = 3 for each. Error bar: SEM. **C**: The mRNA levels of *Esr1* (estrogen receptor α) and *Pgr* (progesterone receptor) were assessed by QPCR using uterine RNA at indicated d.p.c. period. n=3 for each. Error bar: SEM.



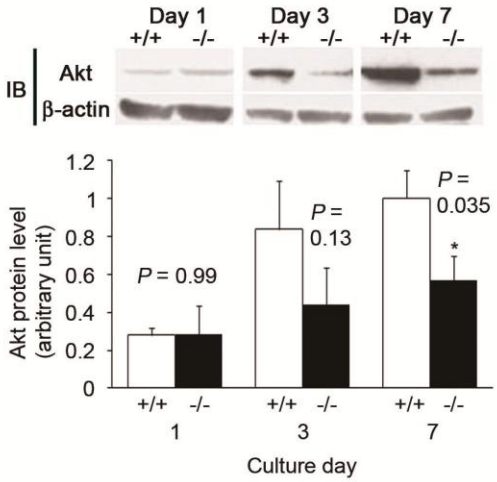
Supplemental Figure 2: Histological analysis of uteri during preimplantation period. A: 1.5 d.p.c. uteri from naturally mated female mice. Apparent epithelial hyperplasia was observed in *DEDD*$^{+/+}$ and *DEDD*$^{-/-}$ mice. **B**: 3.5 d.p.c. uteri. Proliferation of stromal cells and the edematous reaction, concomitantly with luminal closure were observed in *DEDD*$^{+/+}$ and *DEDD*$^{-/-}$ uteri at equivalent levels. Scale bar: 200 μm.



Supplemental Figure 3: Hormone-responsiveness of uteri. Estradiol (E2) alone, or both E2 and progesterone (P4) were injected into ovariectomized *DEDD*$^{-/-}$ and *DEDD*$^{+/+}$ female mice, and the uteri were analyzed for the responses to the hormones (1). (**A-C**): HE staining of hormone-stimulated uteri. **A**: pre-treatment (control); **B**: 24h after the E2- or a vehicle (mineral oil) - injection; **C**: E2 injection for 3 days plus E2 + P4 injection for following 3 days. **D**: The mRNA expression of *LIF* and *VEGF*, both of which are E2-responsive genes (2, 3), were determined 2hrs after the E2 treatment by the semi-quantitative RT-PCR. Overall, both types of mice revealed similar responses to the hormones in terms of morphological change and gene expression. Scale bar: 200 μm.



Supplemental Figure 4: Comparable proliferative state in *DEDD*$^{+/+}$ and *DEDD*$^{-/-}$ decidual cells. **A**: In vitro decidualizing uterine stromal cells from *DEDD*$^{+/+}$ (white bars) and *DEDD*$^{-/-}$ (black bars) mice were challenged with EdU for 2 hours. Cells were harvested, stained with Hoechst, and analyzed by flow cytometry. n=3 each. Error bar: SEM. **B**: Proliferating cells were detected by staining for Ki67, a nuclear protein expressed in from late G1 through M phase during the cell cycle. Scale bar: 200 μm.



Supplemental Figure 5: Akt protein levels in cultured uterine stromal cells. Lysates were prepared from in vitro decidualizing uterine stromal cells, and used for immuno-blotting with anti-Akt (all isoforms) antibody. Three independent experiments were performed and representative photos are presented. Quantification of each signal was performed using NIH Image J software, and the results are also presented. The values are presented as relative protein levels to that from *DEDD*$^{+/+}$ cells at day 7. Error bar: SEM.

Supplemental Table 1: Primer sequences for QPCR.

Name	Sequence (5'- 3')
For β-actin	CTAAGGCCAACCGTGAAAAG
Rev β-actin	ACCAGAGGCATACAGGGACA
For Dedd	TCTGGAGGAAACATCAATTCG
Rev Dedd	GCTGGCCGCTTACTACACAT
For human GAPDH	AGCCACATCGCTCAGACA
Rev human GAPDH	GCCCAATACGACCAAATCC
For human Dedd	AGCCCTCAGTGATCCCAGAAC
Rev human Dedd	GGCAACACACCACAGGATAG
For Il-11	CGCCGTTTACAGCTCTTGA
Rev Il-11	CAGGGGGATCACAGGTTG
For Timp-3	CACGGAAGCCTCTGAAAGTC
Rev Timp-3	TCCCACCTCTCCACAAAGTT
For Prl-r	AATCCCTGGTATGGCAGACTT
Rev Prl-r	TTCAGGGTTCATGTGCAAAA
For Plp-j	TTCTGGAGGGAGCAAAAGC
Rev Plp-j	CCACCTGTCAGGCTCGTTAT
For Dprp	TGCCATTGAGTCAACCTCAC
Rev Dprp	AGCTTTCTCCCACAGAAGCA
For Igfbp-1	CTGCCAAACTGCAACAAGAA
Rev Igfbp-1	TCCATGGGTAGACACACCAG
For HOXA10	CCTTCAGAAAACAGTAAAGCTTCG
Rev HOXA10	AAGGGCAGCGTTTCTTCC
For Bmp2	GATGCGGTGGACCACACA
Rev Bmp2	GGGCACTTCCACCACAA
For cyclin D3	GGAAGATGCTGGCATACTGG
Rev cyclin D3	GGTAGCGATCCAGGTAGTTCA
For Esr1	GCTCCTAACTTGCTCCTGGAC
Rev Esr2	CAGCAACATGTCAAAGATCTCC
For Pgr	TGCACCTGATCTAATCCTAAATGA
Rev Pgr	GATTGGCACAGCGAGTAGAA

Supplemental Table 2: Primer sequences for semi-quantitative RT-PCR.

Name	Sequence (5'- 3')
For β-actin	GTGGCTACAGCTTCACCACCACAG
Rev β-actin	AGTAATCTCCTTCTGCATCCTGTC
For Lif	ATTGTGCCCTTACTGCTGCT
Rev Lif	CCACACGGTACTTGTTGCAC
For Vegfa	GGTTCCAGAAGGGAGAGGAG
Rev Vegfa	GCATTCACATCTGCTGTGCT

Supplemental References

1. Lydon JP, *et al.* Mice lacking progesterone receptor exhibit pleiotropic reproductive abnormalities. *Genes Dev.* 1995;9(18):2266-78.

2. Sherwin JR, *et al.* Identification of genes regulated by leukemia-inhibitory factor in the mouse uterus at the time of implantation. *Mol. Endocrinol.* 2004;18(9):2185-95.

3. Hastings JM, Licence DR, Burton G.J, Charnock-Jones DS, Smith SK. Soluble vascular endothelial growth factor receptor 1 inhibits edema and epithelial proliferation induced by 17beta-estradiol in the mouse uterus. *Endocrinology.* 2003;144(1):326-34.