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Supplementary Information for

Progesterone Receptor Isoform B Regulates the *Oxtr-Plcl2-Trpc3* Pathway to Suppress Uterine Contractility

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Supplementary text
Figures S1 to S7

Other supplementary materials for this manuscript include the following:

Datasets S1 to S5

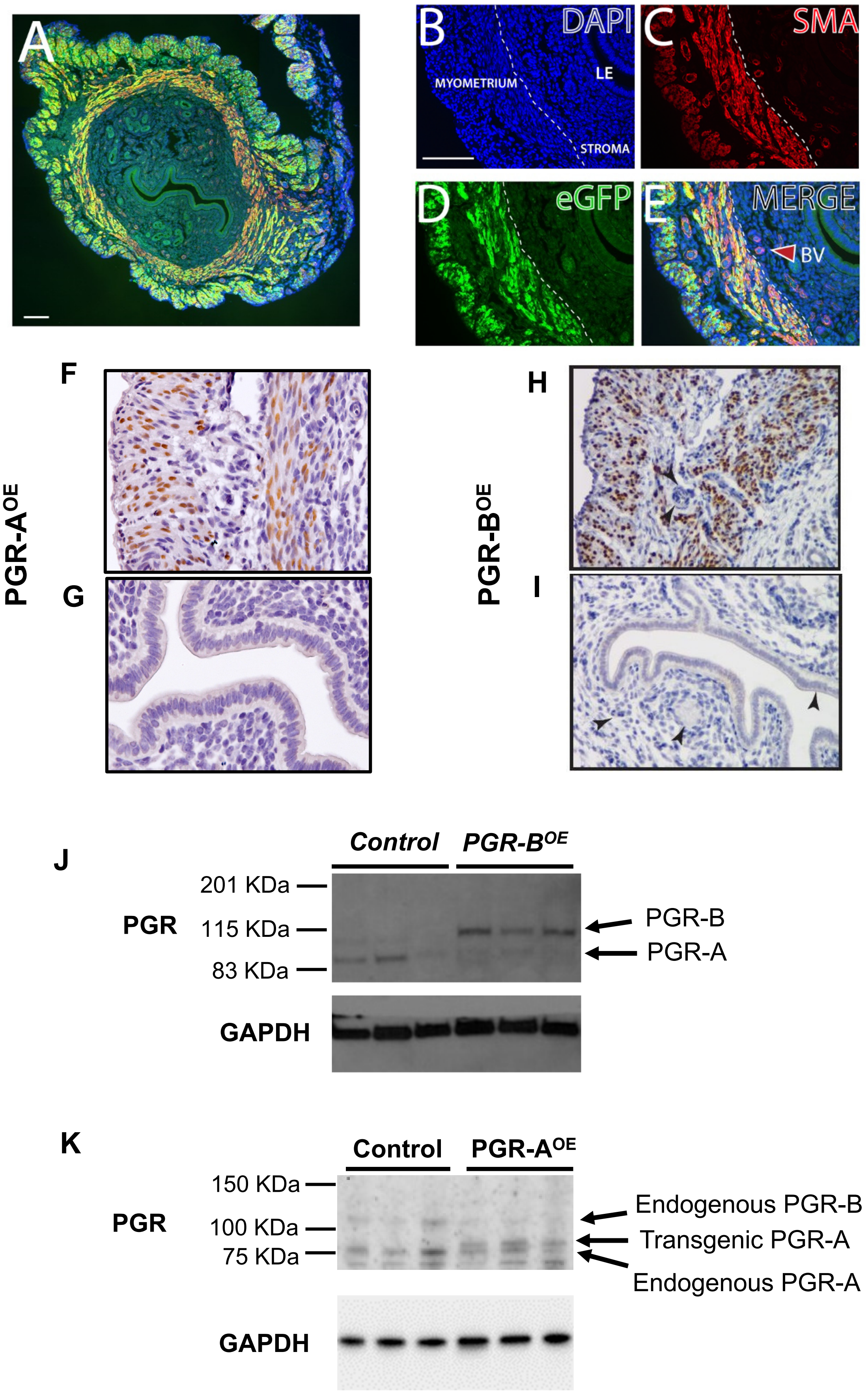
A
B
DAPI
MYOMETRIUM
LE
STROMA
C
SMA
D
eGFP
E
MERGE
BV
F
G
PGR-A^{OE}
H
I
PGR-B^{OE}
J
Control
PGR-B^{OE}
PGR
201 KDa
115 KDa
83 KDa
PGR-B
PGR-A
GAPDH
K
Control
PGR-A^{OE}
PGR
150 KDa
100 KDa
75 KDa
Endogenous PGR-B
Transgenic PGR-A
Endogenous PGR-A
GAPDH

Fig. S1. **Activation of *Pgr* isoform transgenes in the uterine smooth muscle.** (A-E) Immunostaining of ACTA2 (red, SMA) and GFP encoded in the Myh11Cre minigene (green) in the transverse mouse uterine section. DAPI in blue serves as the nuclear counter staining. Scale bars denote 100 um. LE, luminal epithelium; BV, blood vessel. (F-I) Immunostaining of the MYC-tag of the expressed transgenic proteins in PGR-A^{OE} (F & G) and PGR-B^{OE} (H & I) mouse uteri. Transverse view. LE, luminal epithelium; BV, blood vessel. (J-K) PGR protein levels in uteri of control and isoform overexpression mice at pseudopregnancy Day 6.

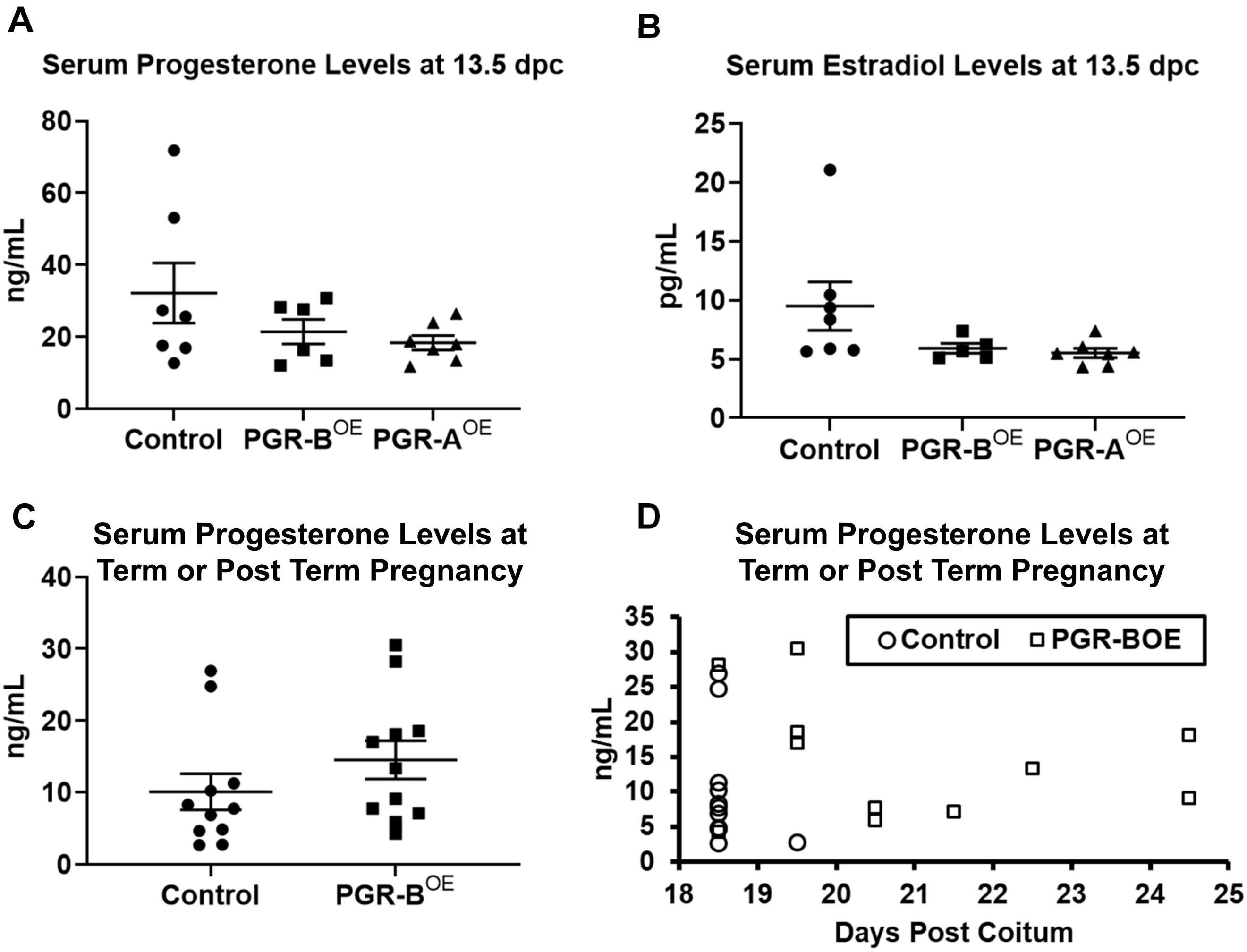

Fig. S2. **Serum Hormone Levels in Pregnant Mice.** (A-B) serum progesterone (A) and estradiol (B) concentrations in 13.5 dpc mice of denoted groups. $p > 0.05$ by one-way ANOVA for both (A) and (B). (C-D) serum progesterone concentrations at term. $p > 0.05$ by the Mann-Whitney test for (C). (D) serum progesterone levels at the time of euthanasia for each individual mouse. Each datapoint represents the serum hormone concentration from one single mouse.

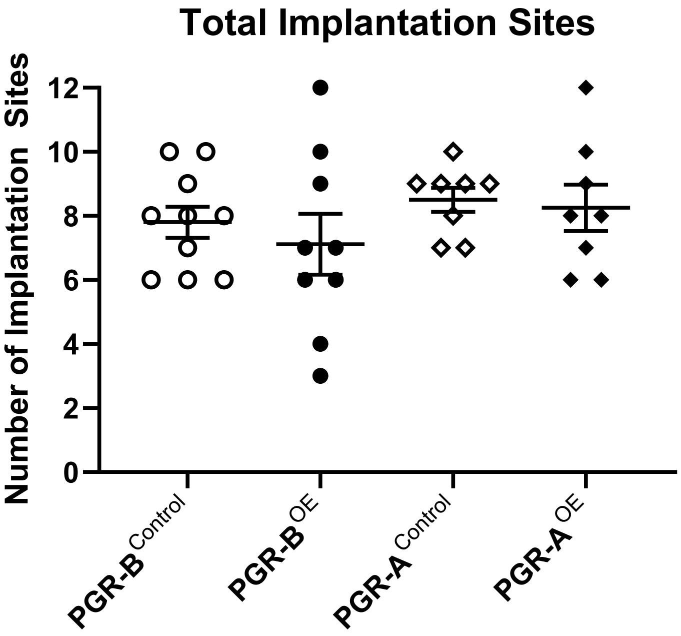

Fig. S3. **Embryo implantation sites identified in each pregnancy.** Total implantation sites, including those of live and resorbed embryos, from each individual animal at their first pregnancy were counted at the end of pregnancy by high frequency ultrasound or gross dissection. P = 0.505 by ordinary one-way ANOVA. Error bars denote standard error of the mean.

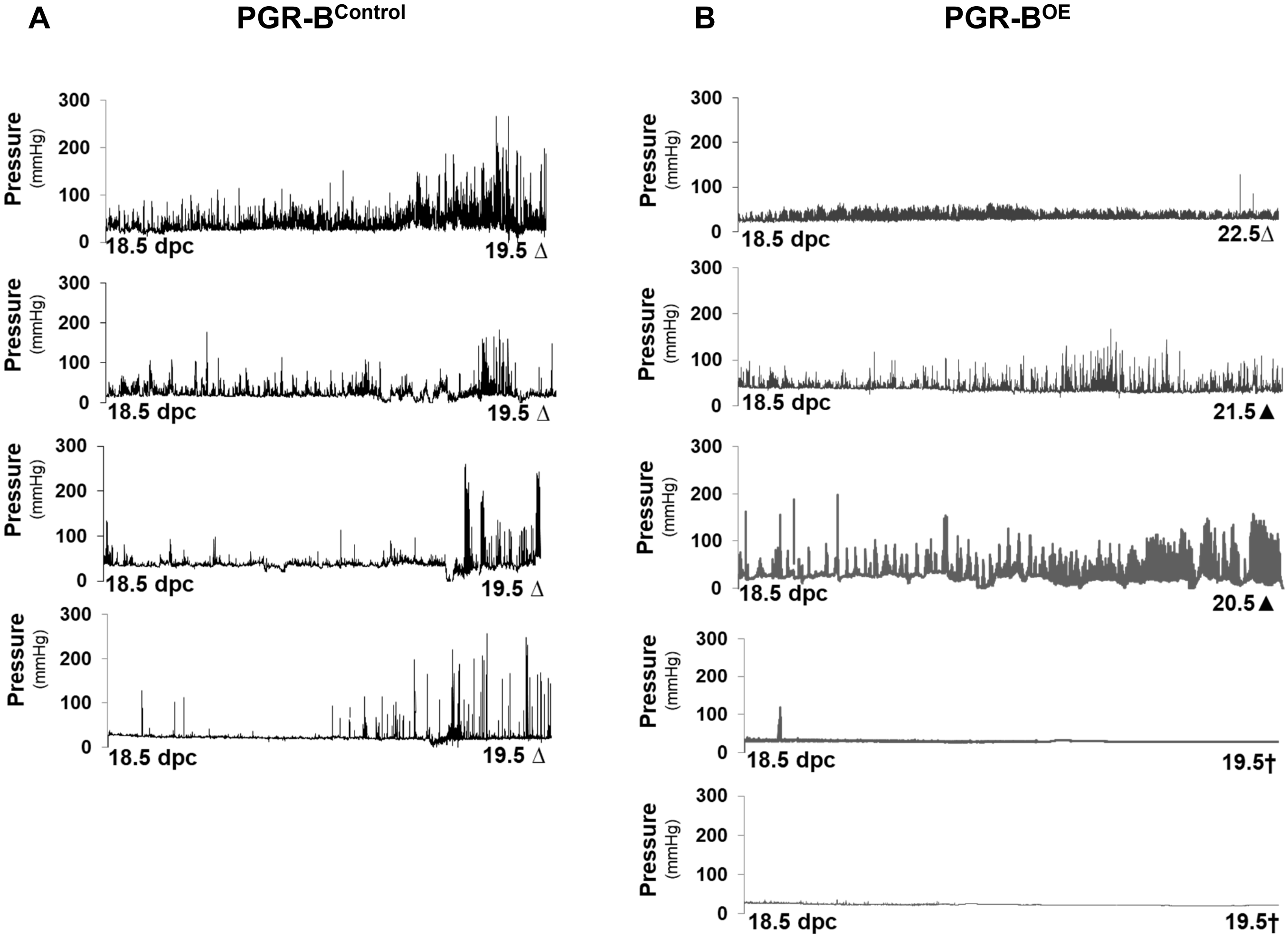

Fig. S4 Intrauterine pressure recordings. Pressure wave forms from each single PGR-B^{control} (A) and PGR-B^{OE}(B) dams examined. Intrauterine pressure measurement recordings began on the morning of 18.5 dpc of pregnancy, and continued until the occurrence of successful parturition (Δ), observation of labor dystocia requiring euthanizing the dam (▲), or spontaneous death of dam (†). Panel A represents the individual pressure readings of four control dams from 18.5 dpc until successful parturition at 19.5 dpc. Panel B represents the individual pressure readings of five PGR-B^{OE} dams; each reading began on the morning of 18.5 dpc and were completed on the day of pregnancy outcome, which ranged from 19.5 to 22.5 dpc.

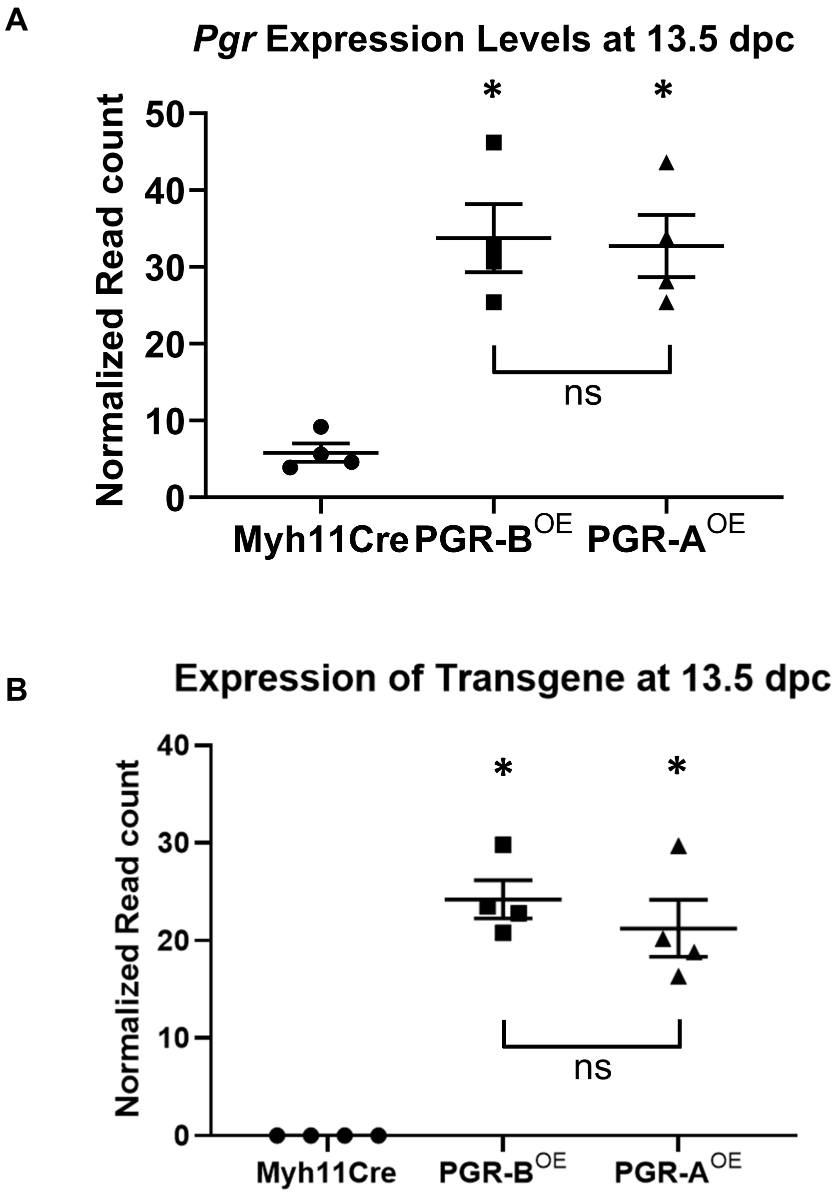

Fig. S5. **Expression levels of *Pgr* transcripts and mRNA from the transgenes in 13.5 dpc mouse uterine tissues.** Normalized read counts of fragments of the *Pgr* exon 2 (A) and the tag sequence of the transgenes (B) were calculated from the RNAseq dataset of denoted groups. *, $p < 0.05$ compared with the Myh11Cre group; ns, $p > 0.05$. The two-tailed Mann Whitney test was used for statistical analyses. Error bars denote standard error of the mean.

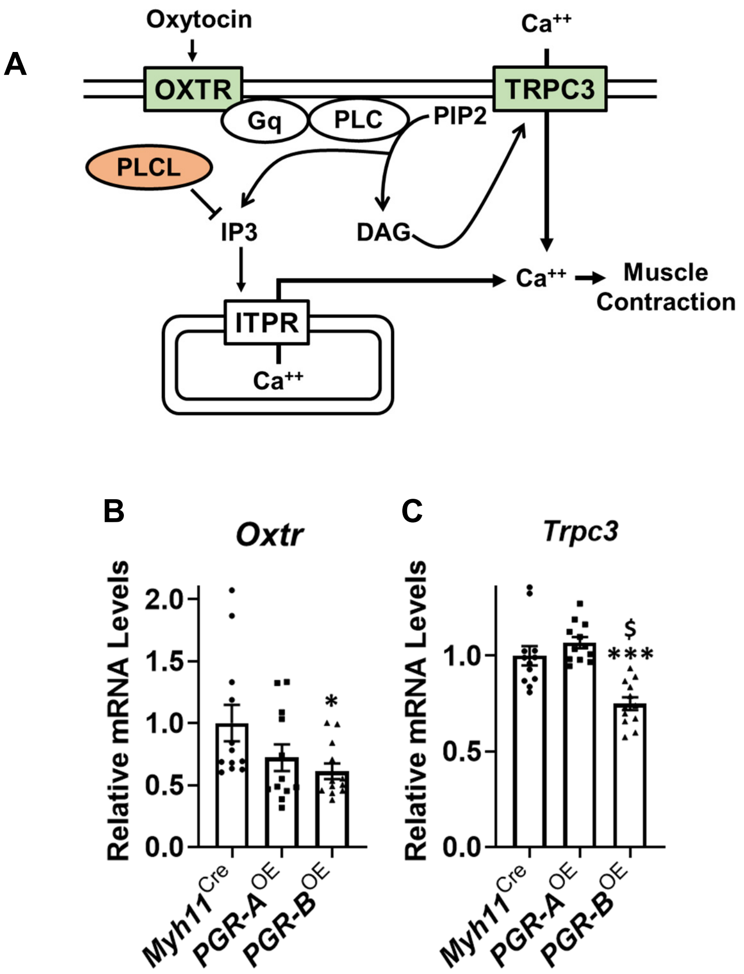

Fig. S6. Altered expression of the phospholipase C pathway members by PGR isoform overexpression in 13.5 dpc uterine specimens. (A) A diagram delineates the pathway of interest. Green and orange depict down- and up-regulation by PGR-B^{OE}, respectively. (B-C) RT-qPCR validation of relative mRNA levels of denoted genes. *, $p < 0.05$ and ***, $p < 0.001$ compared with the $\mathrm{Myh11}^{\mathrm{Cre}}$ group. $, $p < 0.001$ compared with the PGR-A^{OE} group. Two-tailed Mann Whitney test was used. Each group had 6 biological replicates and each biological replicates had two PCR technical duplicates. Error bars denote standard error of the mean.

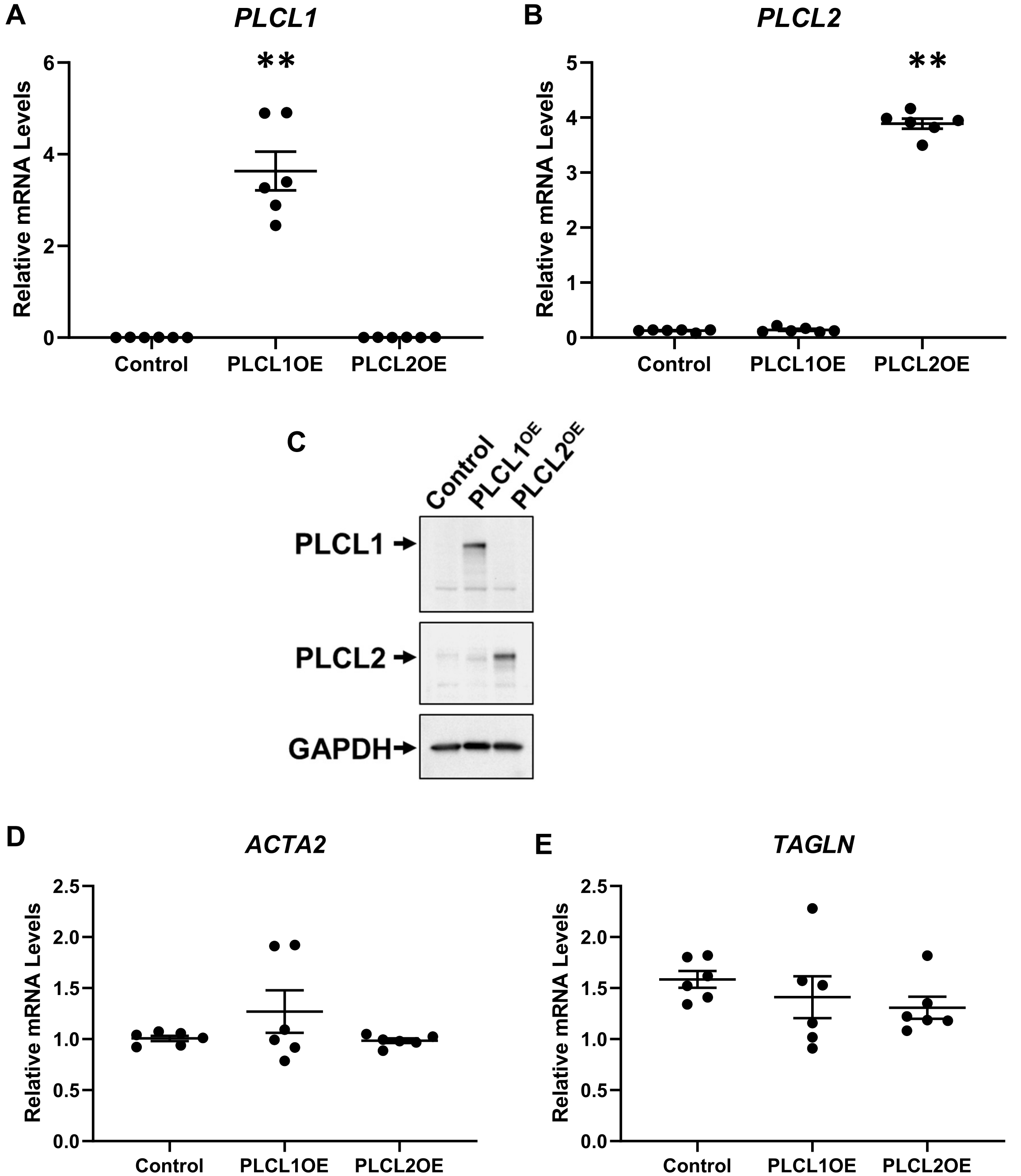

Fig. S7. *PLCL1* and *PLCL2* Overexpression in hTERT-HM Cells by CRISPRa. *PLCL1* (A) and *PLCL2* (B) mRNA levels to 18S rRNA in denoted groups relative were measured by qRT-PCR. RNA from cells of 3 separated wells in each group were subject to assaying in technical duplicates of qRT-PCR. **, $p < 0.01$ by two-tailed Mann Whitney test. Error bars denote standard error of the mean. (C) PLCL1 and PLCL2 protein abundance in denoted groups of cells. GAPDH serves as the loading control. Arrows denote anticipated sizes of target proteins. (D-E) Relative mRNA levels of smooth muscle markers *ACTA2* (D) and *TAGLN* (E) to 18S rRNA in denoted groups. P =0.215 and 0.397 for *ACTA2* and *TAGLN*, respectively, by ordinary one-way ANOVA.

Dataset S1 (separate file). Enriched functional annotations and pathways in uterine genes that are differentially expressed between the two stages of pregnancy and virgin mice (GSE17021).

Dataset S2 (separate file)**.** Complete list of the gene expression profiles in Myh11Cre, PGR-A^{OE} and PGR-B^{OE} uterine specimens at 13.5 dpc (FPKM >=1).

Dataset S3 (separate file)**.** Differentially expressed genes between PGR-A^{OE} and Myh11Cre as well as between PGR-B^{OE} and Myh11Cre.

Dataset S4 (separate file)**.** Enriched terms of molecular functions and diseases and estimated molecular activities in PGR-A$^{OE\_DEG}$ and PGR-B$^{OE\_DEG}$. Data was generated by IPA.

Dataset S5 (separate file). Overrepresented annotations on biological processes in genes that are differentially expressed between Term pregnant and nonpregnant human myometrial tissues.