Supporting Information

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SI Methods

Decidualization. Experimentally induced decidualization was performed in non-pregnant mice as described in ref. 6 of the main article. Mice were ovariectomized and 14 d later were s.c. injected with E (2 μg/kg body weight) in 0.1 mL of sesame oil for 3 consecutive days. This was followed by daily injections of P (40 mg/kg body weight) for 3 consecutive days. Decidualization was then initiated in one horn by injection of 20 μL oil. The other horn was left unstimulated. The animals were treated with P or P plus letrozole for an additional 3 d following decidual stimulation and then killed to collect the uterine tissue.

LCM. Sections of uteri collected on d 4, 5, and 6 of pregnancy were subjected to LCM. Frozen sections (10 μm) containing the implantation sites were placed on polyethylene naphthalate membrane slides (Molecular Devices) and dehydrated in ethanol and xylene. Using the Veritas microdissection instrument, the mesometrial and anti-mesometrial regions were excised and the decidual tissues were obtained. RNA was isolated from these tissues using a Pico Pure RNA isolation kit according to the manufacturer's instructions. cDNA was synthesized from the isolated RNA using the Sensiscript reverse transcriptase kit (Qiagen) and subjected to real-time PCR using gene-specific primers.

Microarray Analysis. Mice were treated with or without letrozole and subjected to experimentally induced decidualization. Uteri were collected from untreated or treated animals at 72 h following the application of decidual stimulus (n = 5). Total RNA was prepared from these tissues and subjected to microarray analyses using Affymetrix mouse arrays (GeneChip mouse genome 430 2.0 array) following the Affymetrix protocol as described previously (1).

Real-Time PCR. These assays were performed as described in ref. 9 of the main article. The expression of 36B4 mRNA, encoding a ribosomal protein, was used to normalize the variability of mRNA amounts in the RNA samples analyzed.

Immunohistochemistry. Immunohistochemistry was performed as described in ref. 6 of the main article.

Assay of Aromatase Activity. The aromatase activity in uterine and ovarian homogenates was determined by the tritiated water release assay as described previously (2). Briefly, 250 μL of uterine or ovarian homogenates was incubated with 300 pmol of [1β-^{3}H]androstenedione for 6 h at 37 C. The results were calculated as fmol of [^{3}H]water released per 24 h per milligram of tissue. Aromatase activity is expressed as mean ± SEM of data derived from 3 independent experiments.

1. Cheon YP, Li Q, Demayo FJ, Bagchi IC, Bagchi MK (2002) A genomic approach to identify novel progesterone receptor-regulated pathways in the uterus during implantation. *Mol Endocrinol* 16:2853–2871.

2. Lephart ED, Simpson ER (1991) Assay of aromatase activity. *Methods Enzymol* 206:477–483.

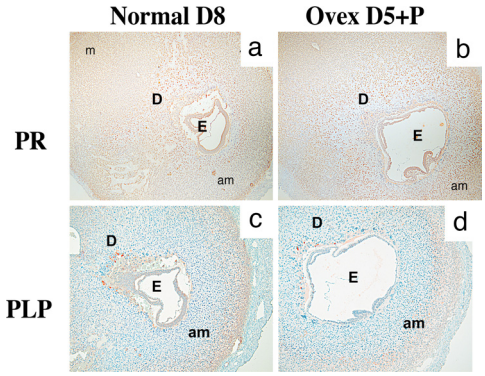

Fig. S1. Sections of ovariectomized P-treated D8 and normal D8 uteri were subjected to immunohistochemical analysis using antibodies specific for PR (*a* and *b*) and prolactin-like protein type B (*PLP-B*; *c* and *d*). The labels am, m, and D denote anti-mesometrial area, mesometrial area, and decidua, respectively; E denotes estrogen.

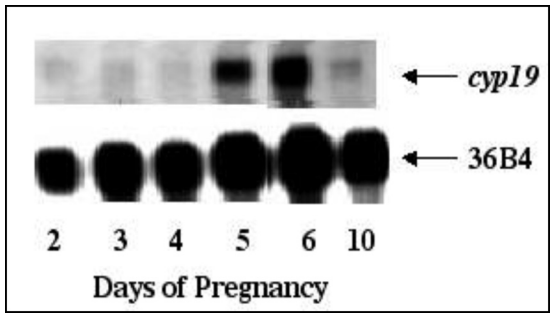

Fig. S2. RNA from d 2 to d 10 of pregnancy was analyzed by Northern blotting with cDNA probes specific for P450 aromatase (*Upper*) and 36B4 (*Lower*).

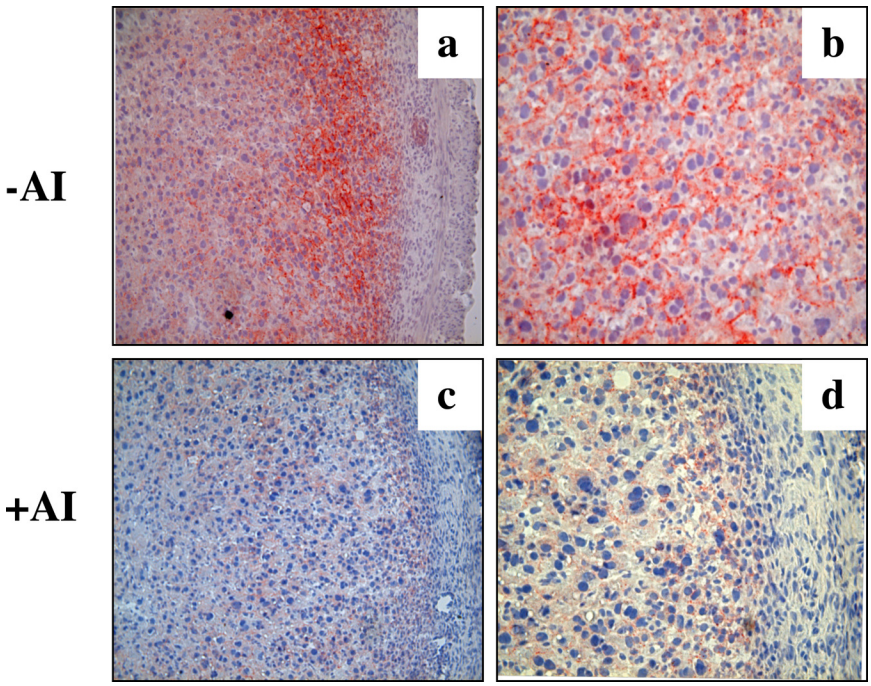

Fig. S3. Immunohistochemical analysis of Cx43 in the uterine sections of mice treated with P (panels *a* and *b*) and P plus letrozole (panels *c* and *d*). M indicates mesometrial area.