Supporting Information

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SI Text

Animals and Treatments. Females mice were mated with fertile males to induce pregnancy (vaginal plug = day 1 of pregnancy). Embryos at the two-cell stage were recovered by flushing oviducts on day 2 of pregnancy. Embryos at the blastocyst stage were recovered by flushing uteri on day 4. To examine receptivity and implantation, pregnant dams were killed on day 4 (0800–1000 hours) and on day 5 (0900 hours), respectively. Implantation sites were visualized by an i.v. injection of 0.1 mL of 1% Chicago blue dye solution in saline 3–5 min before sacrificing, and the number of implantation sites, demarcated by distinct blue bands, was recorded.

To determine whether experimentally induced decidualization occurs in uteri deleted of Kruppel-like factor 5 (KLF5), *Klf5*$^{d/d}$ females were mated with vasectomized WT males. On day 4, one uterine horn was infused with sesame oil (25 μL), and the noninfused contralateral horn served as a control. Mice were killed on day 8 of pseudopregnancy. Weights of infused (with oil) and noninfused (control) uterine horns were recorded, and fold increase in weight was used as an index of decidualization.

To examine the effects of estrogen and/or P_4 on uterine expression of *Klf5*, adult mice (8–10 wk old) were ovariectomized and rested for 2 wk. Mice were injected with estradiol-17β (E_2, 100 ng/0.1 mL oil/mouse) or P_4 (2 mg/0.1 mL oil/mouse). All steroids were dissolved in sesame oil and injected subcutaneously. The control group of mice received sesame oil (0.1 mL/mouse). They were killed at different times, as indicated in the text and in Fig. S2.

In Situ Hybridization. In situ hybridization was performed as previously described (1). In brief, frozen sections (12 μm) were mounted onto poly-L-lysine–coated slides and fixed in cold 4% (wt/vol) paraformaldehyde in PBS. The sections were prehybridized and hybridized at 45 °C for 4 h in 50% (vol/vol) formamide hybridization buffer containing the ^{35}S-labeled antisense or sense cRNA probes. RNase A-resistant hybrids were detected by autoradiography. Sections were poststained with H&E. Sections hybridized with the sense probes did not exhibit any positive signals and served as negative controls.

Immunostaining. Immunostaining of estrogen receptor α (ERα) (sc-542; Santa Cruz, 1:200), progesterone receptor (PR) (#18–0172; Invitrogen, 1:300), and Ki67 (#RM-9106-S; Neomarkers, 1:300) was visualized using a Histostain-Plus (diaminobenzidine) kit (#2014; Invitrogen). Immunofluorescence for E-cadherin (#4065; Cell Signaling, 1:400) was performed using a secondary antibody Cy2-conjugated donkey anti-rabbit (771-225-152; Jackson ImmunoResearch). Actin staining was performed using rhodamine phalloidin (R415; Invitrogen, 1:500), and nuclear staining was by Hoechst 33342 (H1399; Molecular Probes, 2 μg/mL). Guinea pig anti-KLF5 antibody was produced as previously described (2). Immunofluorescence was performed on fresh-frozen sections. Sections were fixed in 10% neutral buffered formalin and incubated with primary antibody at 4 °C overnight, followed by incubation in secondary antibody for 1 h in PBS. Immunofluorescence was visualized under a confocal microscope (Nikon Eclipse TE2000).

1. Lim H, et al. (1997) Multiple female reproductive failures in cyclooxygenase 2-deficient mice. *Cell* 91:197–208.

2. Wan H, et al. (2008) Kruppel-like factor 5 is required for perinatal lung morphogenesis and function. *Development* 135:2563–2572.

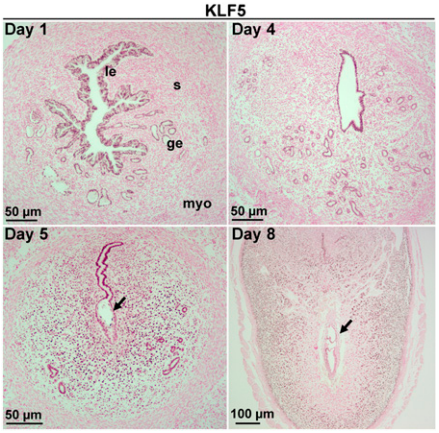

Fig. S1. KLF5 expression in the periimplantation mouse uterus. KLF5 immunostaining in uterine sections showing larger areas. Nuclear KLF5 staining is shown as brownish black deposits on tissue sections counterstained with eosin. KLF5 is present in uterine epithelia of days 1 and 4 of pregnancy. On days 5 and 8, KLF5 is also expressed in decidual cells. le, luminal epithelium; s, stroma; ge, glandular epithelium.

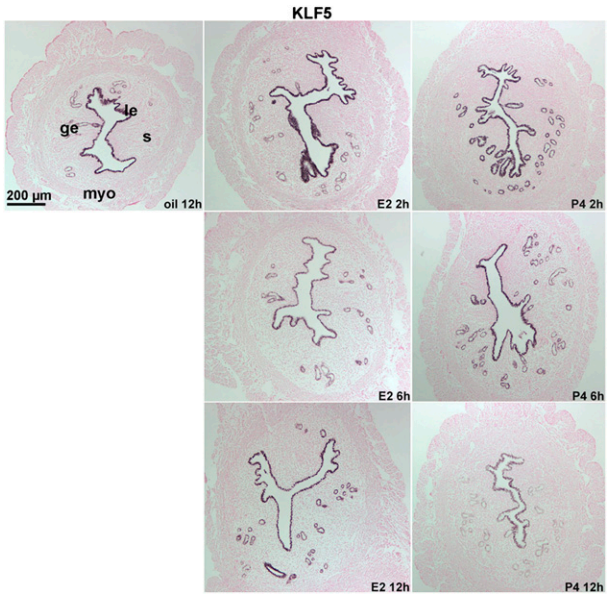

Fig. S2. Ovarian hormones modestly influence KLF5 expression. Immunohistochemistry of KLF5 in uteri of ovariectomized mice treated with E2 and/or P4. WT mice were ovariectomized and rested for 10 d before hormone treatment. le, luminal epithelium; s, stroma; ge, glandular epithelium; myo, myometrium.

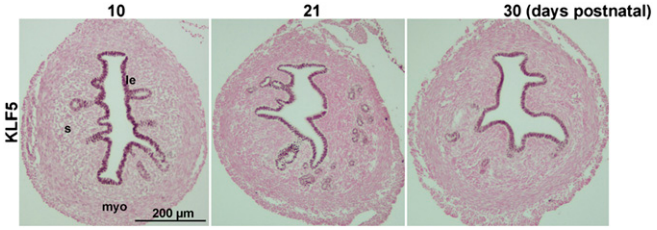

Fig. S3. KLF5 is present in WT uteri of neonatal mice. Immunohistochemistry of KLF5 in uteri of 10-, 21-, and 30-d-old females.

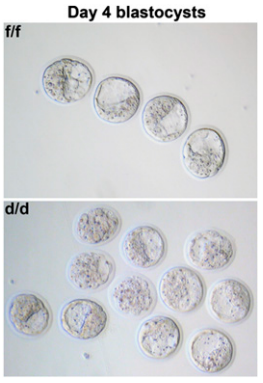

Fig. S4. Preimplantation embryo development is normal in *Klf5*$^{d/d}$ uteri. A comparable number of blastocysts was recovered from *Klf5*$^{f/f}$ and *Klf5*$^{d/d}$ uteri on day 4 of pregnancy.

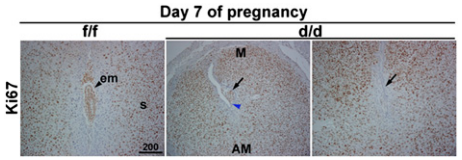

Fig. S5. Blastocysts entrapped within the lumen of *Klf5*$^{d/d}$ mice fail to implant and degenerate. Ki67 immunostaining in sections of *Klf5*$^{f/f}$ and *Klf5*$^{d/d}$ implantation sites showing larger areas on day 7. s, stroma; em, embryo; M, mesometrial pole; AM, antimesometrial pole. Arrowheads indicate the location of blastocysts, and the blue arrowhead indicates the luminal epithelium in which a blastocyst is retained. Arrows indicate the degenerating embryos or residue of embryo debris.

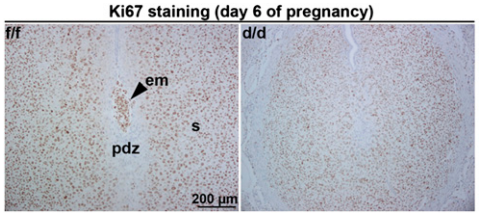

Fig. S6. *Klf5*$^{d/d}$ stromal cells undergo proliferation in the absence of trophectoderm invasion. Immunostaining of Ki67 in *Klf5*$^{f/f}$ and *Klf5*$^{d/d}$ uteri at the site of blastocysts on day 6 of pregnancy. em, embryo; s, stroma; PDZ, primary decidual zone.

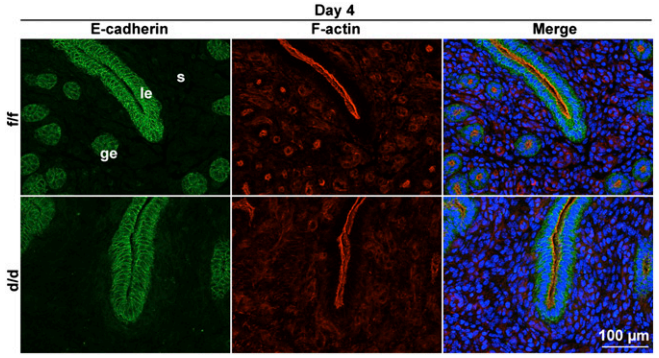

Fig. S7. Actin levels are low in stromal cells before decidualization. Confocal images of E-cadherin and actin colocalization on day 4 uteri of *Klf5*$^{f/f}$ and *Klf5*$^{d/d}$ females. Nuclei are shown in blue. le, luminal epithelium; s, stroma; ge, glandular epithelium.

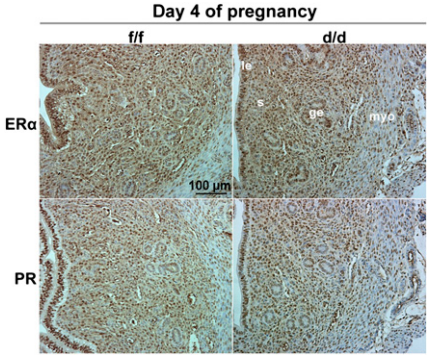

Fig. S8. *Klf5*$^{f/f}$ and *Klf5*$^{d/d}$ mice have normal expression of ERα and PR on day 4 uteri. le, luminal epithelium; s, stroma; ge, glandular epithelium; myo, myometrium.