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Supplementary Information for

Activin-like kinase 5 (ALK5) inactivation in the mouse uterus results in metastatic endometrial carcinoma

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This PDF file includes:

Figs. S1 to S7
Table S1 and S2

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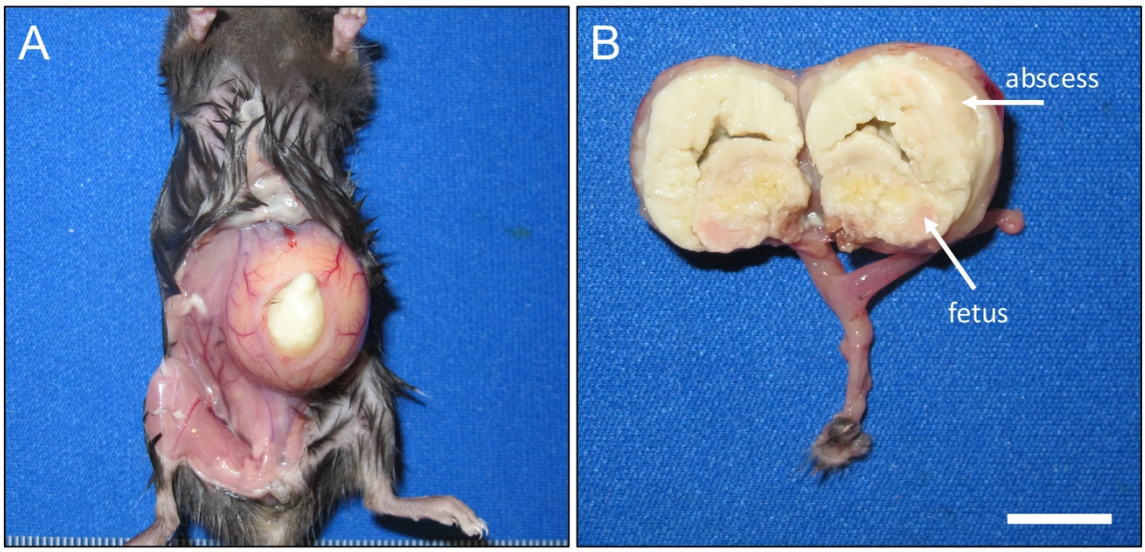

Fig. S1. Mortality of *Alk5* cKO females during pregnancy. A) *Alk5* cKO mouse was sacrificed and dissected at 6.9 months of age due to the presence of an enlarged abdominal mass. This mouse was mated continuously to a WT male until the time of dissection. B) Intrauterine mass shows the presence of an enclosed abscess and a dead fetus. Size bar equals 1 cm.

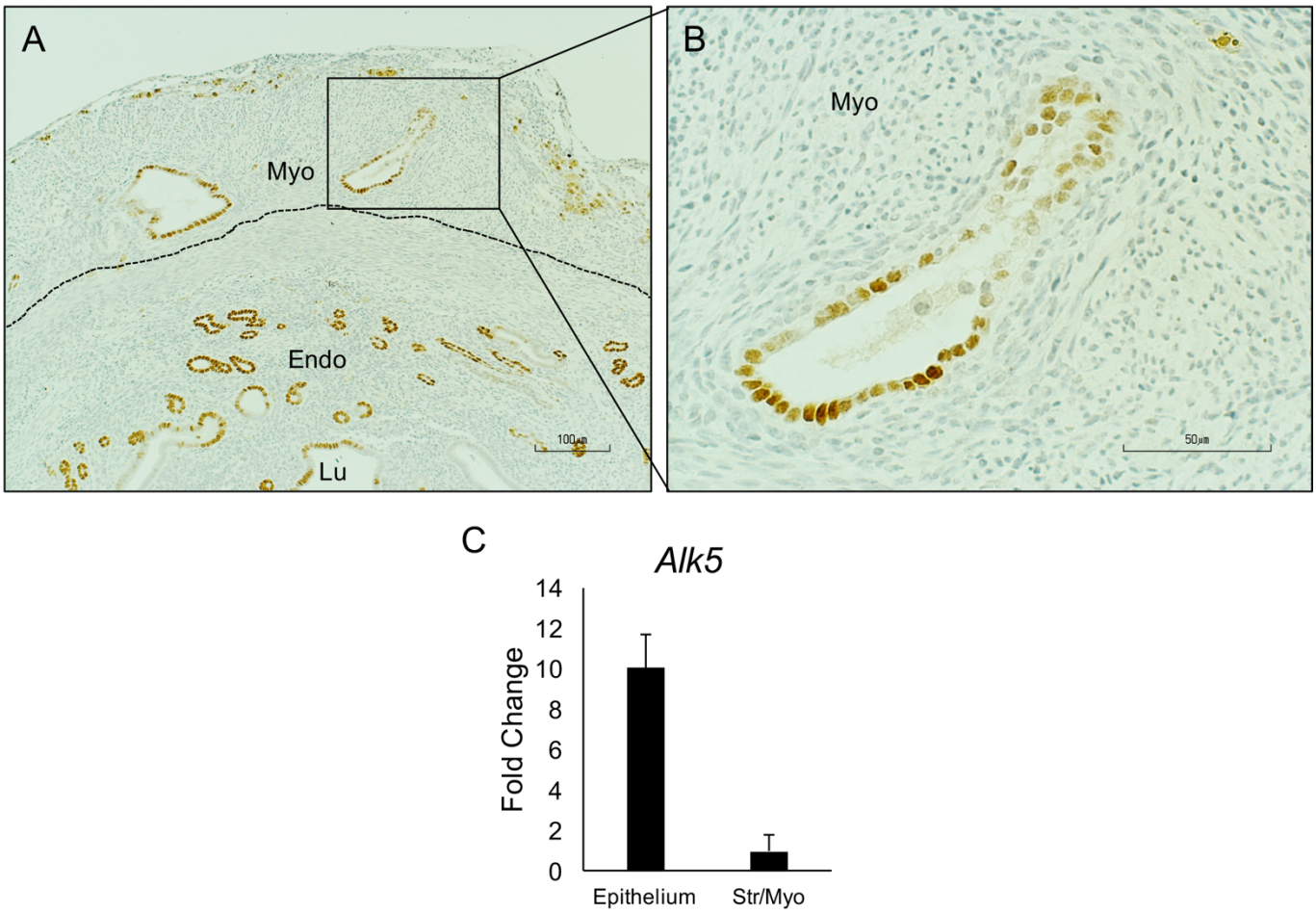

Fig. S2. FOXA2 expression in the glands of *Alk5* cKO mice with cancer and tissue-specific *Alk5/Tgfbr1* expression. A-B) Immunohistochemistry of FOXA2 in the uterine cancers of *Alk5* cKO mice at low (A) and high magnification (B). FOXA2 expression is detected in the normal uterine glands that are within the endometrium and in the invasive glands within the myometrium. C) Comparison of the gene expression of *Alk5/Tgfbr1* in the luminal uterine epithelium and stromal/myometrial compartments of WT mice using quantitative real time PCR.

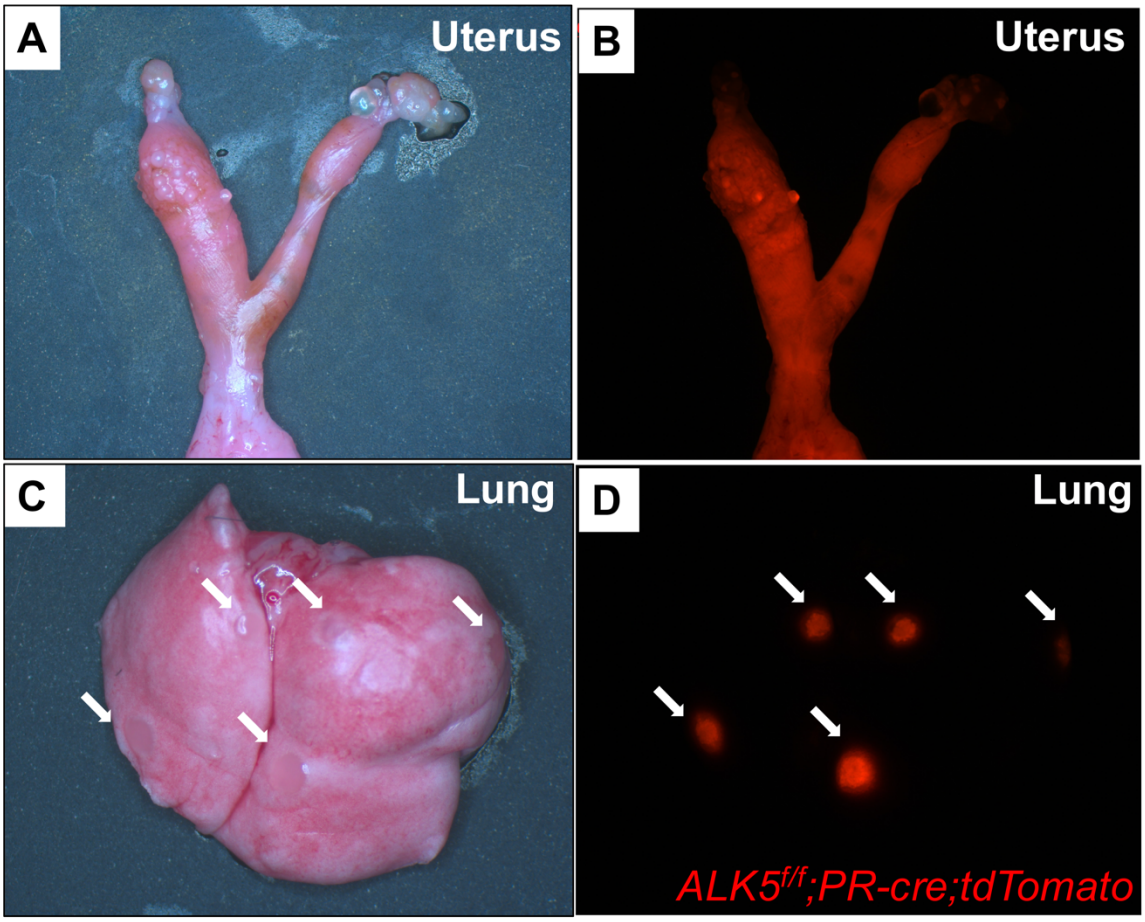

Fig. S3. Lung metastases in *Alk5* cKO mice originate in the reproductive tract. A,C) Gross uterus (A) and lungs (C) from an *Alk5*$^{flox/flox}$-*PRcre*$^{+/-}$-*Rosa26*^{tdTomato} mouse imaged under a light stereomicroscope. B,D) Uterus and lungs from an *Alk5*$^{flox/flox}$-*PRcre*$^{+/-}$-*Rosa26*^{tdTomato} mouse imaged under fluorescence stereomicroscope. Pictured is a uterus from an adult mouse mated continuously for six months beginning at six weeks of age.

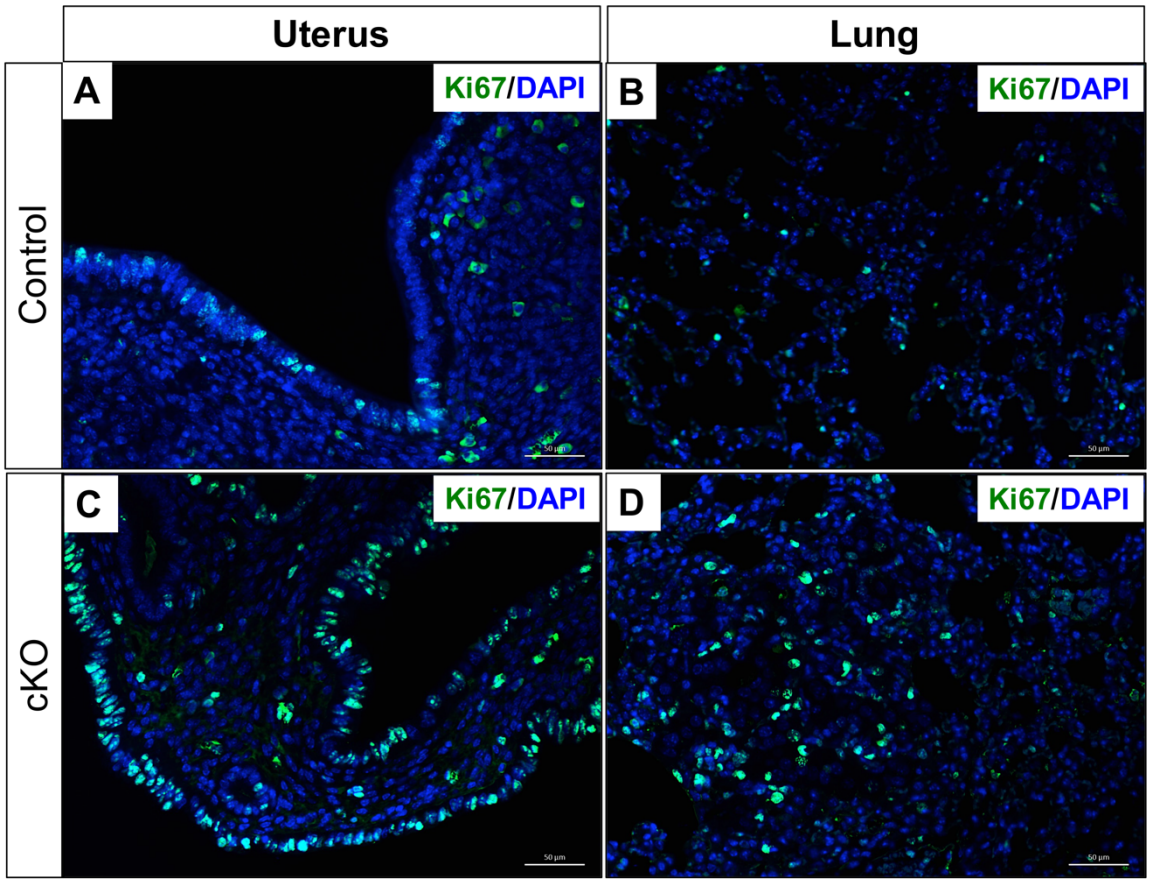

Fig. S4. Uterine and lung tissues from *Alk5* cKO mice show increased proliferation compared to control tissues. A-B) Uterus and lung sections from a control mouse stained with Ki67 and DAPI. C-D) Uterus and lung sections from an *Alk5* cKO mouse stained with Ki67 and DAPI. Tissues were collected from mice at six months of age following continuous mating to male mice beginning at six weeks of age. Size bar equals 50 µm.

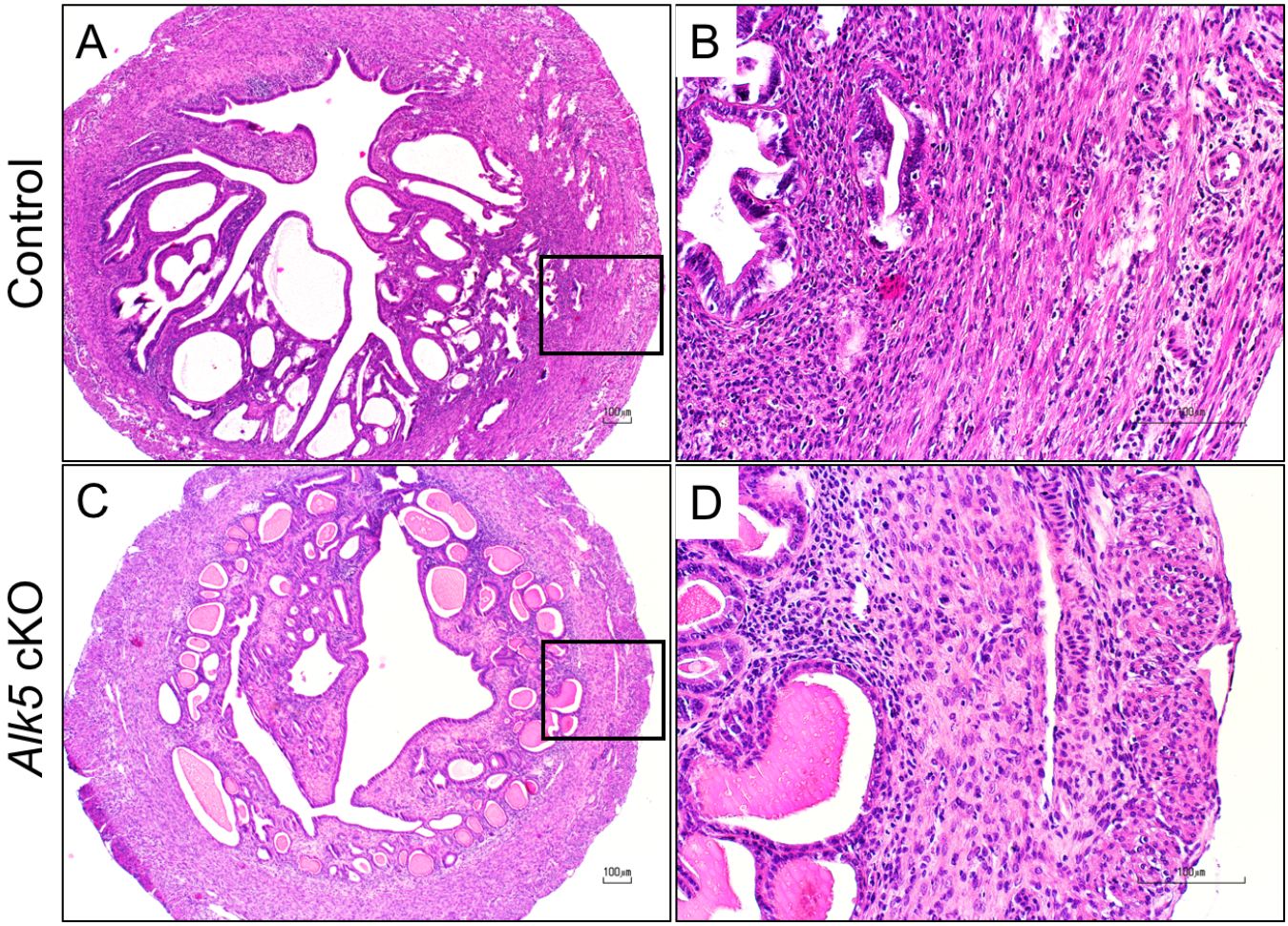

Fig. S5. Uteri from ovariectomized control and *Alk5* cKO mice after long-term E2 treatment. A-D) Uterine cross-sections from control (A-B) and *Alk5* cKO (C-D) mice. Mice were ovariectomized, then treated with an E2-secreting pellet for three months (0.025mg/pellet/90days). Tissues were sectioned and stained with H&E. Size bar equals 100 µm.

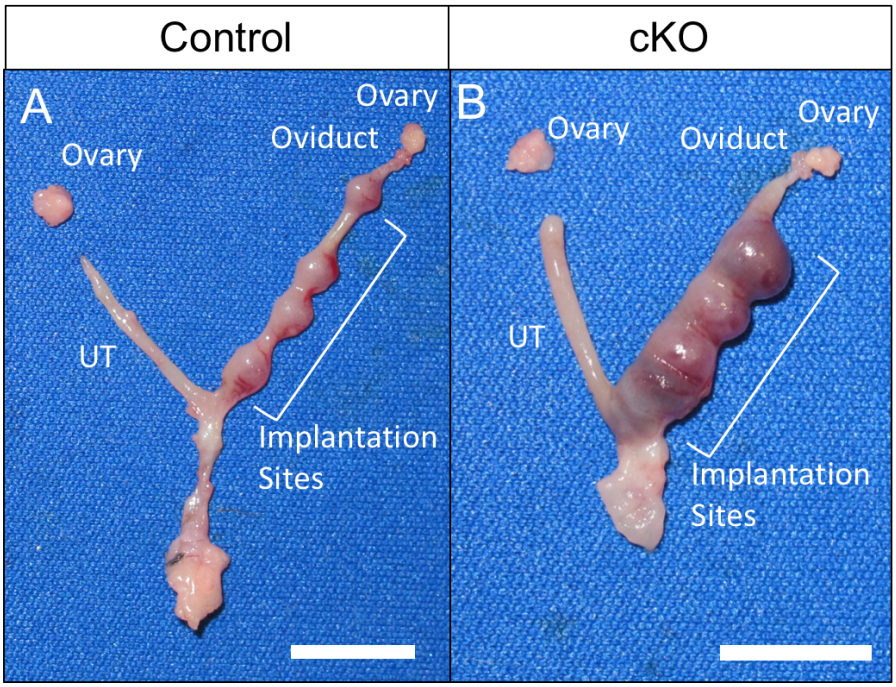

Fig. S6. Unilateral oviduct removal in control and *Alk5* cKO mice. The oviduct was removed from the left uterine horn in both control (A) and *Alk5* cKO (B) mice. Implantation sites are observed in the uterine horns with the intact oviduct from both the control and *Alk5* cKO mice. Uteri were dissected from 4.8-month-old mice mated continuously for three months. Size bar equals 1cm.

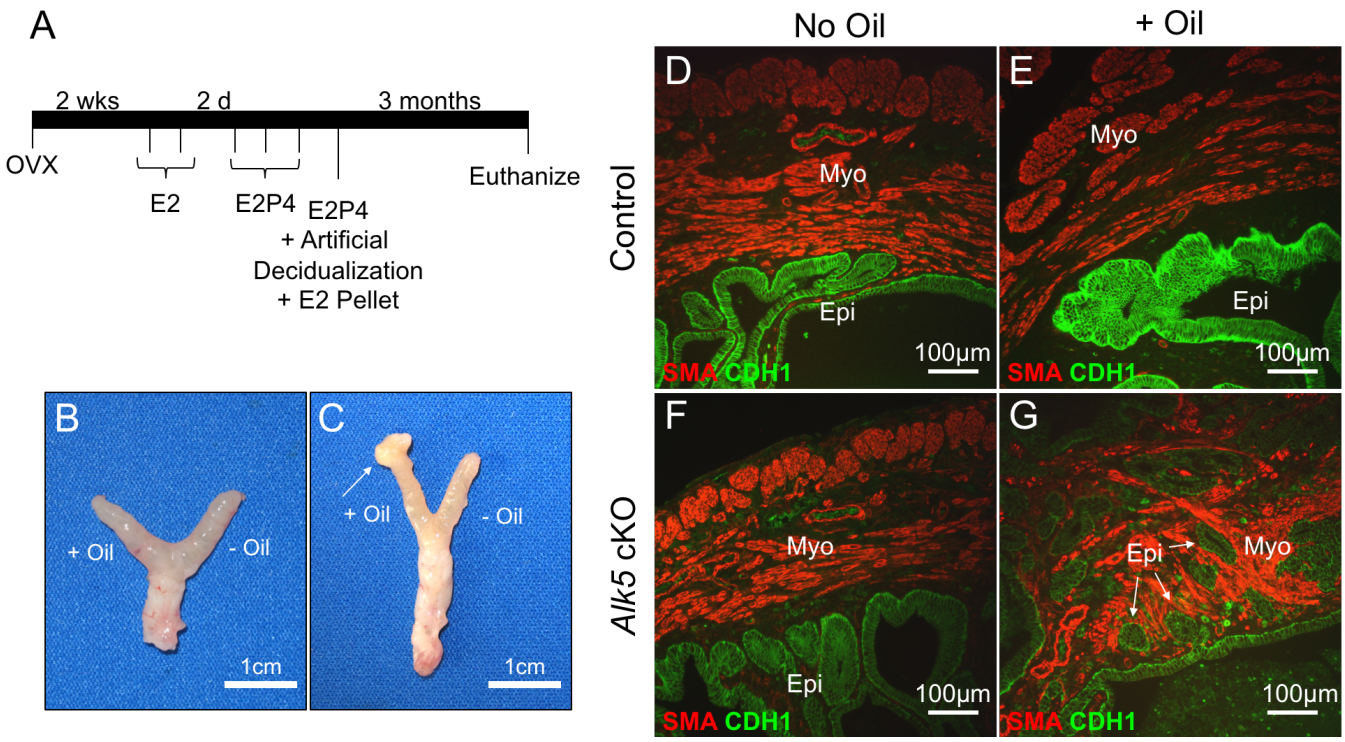

Fig. S7. Cancer development in a model of artificial decidualization. A) Experimental scheme used to induce artificial decidualization. Mice were ovariectomized (OVX), treated with 100 ng E2 (2 days), 1mg P4 + 6.7 ng E2 (4 days) and on the 4th day, injected (intrauterine) with oil, followed by a long-term E2 regimen (0.025mg/pellet/90days). B-C) Uteri from control (B) and *Alk5* cKO mice (C) after an artificial decidual stimulus (Oil) was delivered to one uterine horn. D-G) Uterine cross-sections from control (D-E) and *Alk5* cKO (F-G) mice stained with smooth muscle actin (SMA, red) and E-cadherin (CDH1, green). Uterine horns pictured in D, F did not receive the artificial stimulus. Uterine horns in E, G received an oil injection as the decidual stimulus. Size bar equals 1cm (B,C) or 100 µm (D-G).

Table 1. *Alk5* cKO mice develop endometrial tumors and lung metastases

Group	Genotype	Age Mating Began (weeks)	Days until death	Endometrial tumors	Lung Metastases
Virgin mice	Control	not mated	dissect at 120	no	no
	Control	not mated	dissect at 225	no	no
	Control	not mated	dissect at 320	no	no
	Control	not mated	dissect at 320	no	no
	Alk5 cKO	not mated	dissect at 120	no	no
	Alk5 cKO	not mated	dissect at 120	no	no
	Alk5 cKO	not mated	dissect at 225	no	no
Mated to fertile males	Control	6	dissect at 240	no	no
	Control	6	dissect at 240	no	no
	Control	6	dissect at 320	no	no
	Control	6	dissect at 320	no	no
	***Alk5* cKO**	**6**	**dissect at 240**	**yes**	**yes**
	***Alk5* cKO**	**6**	**dissect at 225**	**yes**	**yes**
	***Alk5* cKO**	**6**	**dissect at 180**	**yes**	**yes**
	***Alk5* cKO**	**6**	**dissect at 150**	**yes**	**yes**
	***Alk5* cKO**	**6**	**dissect at 165**	**yes**	**yes**
	Alk5 cKO*	6	28	no	no
	Alk5 cKO*	6	105	no	no
	Alk5 cKO*	6	21	no	no
	Alk5 cKO*	6	31	no	no
	Alk5 cKO*	7	112	no	no
	Alk5 cKO*	6	142	no	no
	Alk5 cKO*	6	28	no	no
	Alk5 cKO*	7	112	no	no
	Alk5 cKO*	8	80	no	no
Mated to vasectomized males	Control	6	dissect at 240	no	no
	Control	6	dissect at 240	no	no
	Control	6	dissect at 240	no	no
	Alk5 cKO**	6	dissect at 240	no	no
	Alk5 cKO**	6	dissect at 240	no	no
	Alk5 cKO**	6	dissect at 240	no	no
	Alk5 cKO**	6	dissect at 240	no	no

Ovariectomy + E2 for three months	Control	not mated	dissect after 3 months E2 pellet	no	no
	Control	not mated	dissect after 3 months E2 pellet	no	no
	Alk5 cKO	not mated	dissect after 3 months E2 pellet	no	no
	Alk5 cKO	not mated	dissect after 3 months E2 pellet	no	no
	Alk5 cKO	not mated	dissect after 3 months E2 pellet	no	no

*mice died during pregnancy with intrauterine abscess or hemorrhage
**mice developed a cervical/vaginal mass

Table S2. List of Antibodies and dilutions

Antibody	Catalog Number	Vendor	Species	Dilution
TTF-1	sc-13040	Santa Cruz	Rabbit	1:200
KRT8	TROMA-I	Developmental Studies Hybridoma Bank	Rat	1:50
Ki67	550609	BD	Mouse	1:300
ERα	MS-354-P0	Thermo Fisher	Mouse	1:500
PAX8	ACI438	BioCare	Mouse	1:200
SMA/ACTA2	ab5694	Abcam	Rabbit	1:200
PR	sc-7208	Santa Cruz	Rabbit	1:200
FOXA2	ab108422	Abcam	Rabbit	1:1000
E-Cadherin	610181	BD Biosciences	Mouse	1:200