Supplementary Figure Legends:

Supplementary Figure S1. Histology of PTENKO tumors and hormone supplementation in treated mice. (A) Cell autonomous loss of PTEN resulted in formation of endometrial carcinoma prior to initiation of therapy. PTENKO tumor attached to kidney (a) and tumor histology assessed by HE (b). Pankeratin marked all tumor epithelia (c) confirmed to be PTEN-null (d). Normal expression of PTEN was seen in the tumor stroma (d). Abundant expression of PR (e) and ER α (f) was detected in both tumor stroma and epithelia. **(B)** Quantification of proliferation index in pre-therapy tumors. Approximately 20% of PTENKO tumor epithelia were proliferating based on Ki67 expression (a&b). **(C)** Measurement of estradiol and progesterone serum levels in experimental animals. High levels of estrogen were detected in all mice supplemented with estrogen pellets (a). High levels of progesterone were only detected with progesterone treatment (b). **(D)** High magnification images of placebo and progesterone treated PTENKO tumors. Nuclei in placebo treated tumors show evidence of atypia (a) while the nuclei of progesterone treated residual tissue were normal (b). Scale bars are 100 μm. Results are mean± SD.

Supplementary Figure S2. Biologic behavior of PTENKO tumors during and after cessation of progesterone therapy. (A) Measurement of proliferation assessed by Ki67 expression. A drop in the proliferation index of tumor epithelia was detected upon treatment with progesterone. **(B)** Measurement of programmed cell death. TUNEL assay (a-e) and cleaved caspase 3 staining (f-j) revealed increased apoptotic cell death with progesterone therapy peaking at four weeks. The differences at four weeks were statistically significant. **(C)** PTENKO tumors recurred upon cessation of progesterone therapy in a hyper-estrogenic hormonal milieu. After 8 weeks of progesterone therapy mice bearing PTENKO implants were split into two groups. In the first group (n=4), progesterone pellets were removed and placebo pellets were implanted. In the second cohort (n=2), fresh progesterone pellets were implanted. Estrogen supplementation was continued in all mice to maintain a hyper-estrogenic state. Within 6 weeks, tumors recurred in placebo treated mice (a-d). With continued progesterone therapy there was no evidence of

tumor tissue (e-h). Scale bars are 100 μm in A&C and 50 μm in B. Results are mean ± SD. For significance, *= P <0.01.

Supplementary Figure S3. Measurement of proliferative index and ERα expression in PTENKO tumors treated under different hormonal conditions. **(A)** Assessment of proliferation by Ki67 staining. As expected treatment of PTENKO tumors with progesterone and estrogen caused a significant decrease in the proliferative index of tumor epithelia (b vs. a). Surprisingly the proliferation index of PTENKO tumors treated with progesterone but no estrogen did not decrease and was similar to estrogen only treated tumors (c vs. a). **(B)** The expression of ERα in PTENKO tumors. In all hormonal conditions predominance of ERα expression was detected in the tumor epithelia. Scale bars are 100 μm. Results are mean ± SD.

Supplementary Figure S4. Stromal or epithelial specific deletion of *PR-A* and *PR-B*. **(A)** Western blot for expression of PR. PR-A and PR-B expression were efficiently lost in Cre-GFP $PR^{loxP/loxP}$ stroma and these cells expressed cre. Normal expression of both isoforms was confirmed in GFP control infected $PR^{loxP/loxP}$ stroma. ERK was used as a loading control. **(B)** Generation of PTENKO tumors with deletion of *PR* only in tumor stroma. Epithelia marked with TROP1 were isolated from $Pten^{loxP/loxP}$ uteri and infected with Cre-RFP inducing deletion of *Pten*. Neonatal stroma harvested from $PR^{loxP/loxP}$ mice was infected with Cre-GFP lentivirus and cultured short term to achieve deletion of *PR*. GFP positive stromal cells were FACS isolated (to ensure inclusion of PR negative stroma only) and combined with PTEN-null epithelia. Histology of regenerated grafts confirms stromal specific loss of PR and cell autonomous loss of PTEN. **(C)** Proliferation index in tumor epithelia was measured by Ki67 expression. When WT PR (GFP-control) was present in the stroma, therapy with progesterone caused a significant drop in the epithelial proliferation compared to placebo treated tissue. With stromal specific loss of PR (Cre-GFP) proliferation of tumor epithelia was unchanged with progesterone therapy. **(D)** Epithelial-specific deletion of *PR* did not affect tumor response to progesterone therapy. To selectively induce loss of PR in tumor

epithelia, endometrial epithelia harvested from adult $PR^{loxP/loxP}$ mice was infected with a Cre doxycycline inducible lentiviral vector that co-expresses shPTEN constitutively. Map of this viral vector is shown. Constitutive knock-down of PTEN and inducible expression of Cre was confirmed by western blot in 3T3 infected cells. Infected uterine epithelia were regenerated in vivo, in combination with WT stroma for 6 weeks, during which a tumor was established due to knock down of PTEN. Experimental mice were treated with Doxycycline (n=6) or vehicle (n=4). In this system, administration of Doxycycline results in expression of Cre and deletion of epithelial *PR*. After ten days, sufficient for loss of PR protein, equal numbers of mice in each group were treated with estrogen plus progesterone (progesterone) or estrogen plus placebo (placebo). Progesterone therapy in both doxycycline (epithelial *PR* deleted) and vehicle (epithelial *PR* WT) treated mice resulted in resolution of PTENKO tumors (a-d and i-l). Conversely, tumor was detected in all placebo treated mice regardless of doxycycline administration (e-h & m-p). Epithelial specific loss of PR was confirmed in tissue regenerated in doxycycline treated mice (k&o). Epithelial specific loss of PTEN was confirmed (d,h,l&p). Scale bars are 50 μm in B and 100 μm in C&D. Results are mean ± SD.

Supplementary Figure S5. Activation of KRAS concomitant with PTEN loss (PTENKO/Kras) results in endometrial tumors that invade the kidney parenchyma. (A) Establishment of a PTENKO/Kras tumor was verified prior to initiation of hormonal therapy. PTENKO/Kras tumors are shown attached to the kidney (a). Histology (b) and pankeratin staining (c) confirmed formation of an endometrial carcinoma. Cell autonomous loss of PTEN was confirmed in these tumors (d). **(B)** Evidence of invasion into the kidney parenchyma. Tumor tissue marked with pankeratin (a) is found surrounding kidney glomeruli (a &b) in PTENKO/Kras tumors. These findings confirms tumor invasion into the kidney. **(C)** High magnification images of PTENKO/Kras tumors that were treated with placebo (a) or progesterone (b). **(D)** The RAS pathway was activated in PTENKO/Kras tumors. Phosphorylation of ERK was examined as an indicator for activation of the RAS pathway. Phospho-ERK (pERK) was detected in the epithelia of PTENKO/Kras but not PTENKO tumors (a vs. b). Scale bars equal 100μm

Supplementary Figure S6. Analysis of epithelial and stromal populations from PTENKO and PTENKO/Kras tumors. (A) Schema for isolation of tumor epithelia and stroma. PTENKO and PTENKO/Kras tumor epithelia expressed RFP as they were infected with a Cre-RFP expressing lentivirus during tumor generation. To differentially color mark the stroma, WT stromal cells were infected with a lentivirus expressing GFP in short-term culture. Stromal cells expressing high levels of GFP were FACS isolated and combined with Cre-RFP infected epithelia. Resulting PTENKO and PTENKO/Kras tumors were dissected off the kidney and dissociated into single cells. FACS analysis showed segregation of these tumor cells into clear RFP or GFP positive populations. RFP positive cells were harvested as tumor epithelia while GFP positive cells were harvested as tumor stroma. **(B)** Full length image of western blot shown in Fig. 6B. **(C)** Location of primers used to amplify *PR-A* and *PR-B* transcripts. Given the complete overlap of *PR-A* transcript with *PR-B*, two sets of primers were utilized. First the collective transcripts of *PR-A* and *PR-B* were measured (PR_{A+B} primers). Then transcripts of *PR-B* alone were quantified (PR_B primers). No significant difference between transcript levels of either *PR* isoform was detected in the epithelia of PTENKO vs. PTENKO/Kras tumors. Results are mean ± SD. **(D)** Methylation specific PCR was performed to screen for methylation of the *PR-A* gene promoter. Isolated DNA was treated with bisulfite to convert unmethylated cytosines to uracils. Primer sets were designed to specifically amplify either unmethylated or methylated *PR* DNA. **(E)** Methylation specific PCR of the *PR-A* promoter in isolated tumor stroma. An increase in the ratio of methylated vs. un-methylated PCR product was detected in stroma of PTENKO/Kras vs. PTENKO tumors. DNA isolated from murine lymph nodes untreated or treated with Sss I CpG methyltransferase were used as controls.

Supplementary Figure S7. Over-expression of the human progesterone receptor (hPR) with a lentivirus in the tumor stroma. (A) The human progesterone receptor was expressed from a constitutively active ubiquitin promoter using a GFP color tagged lentiviral construct (a). HeLa cells lacking endogenous expression of PR were infected with hPR-GFP or control GFP lentivirus. Western

blot confirmed expression of PR-A and PR-B isoforms from this construct. ERK was used as a loading control (b). Over-expression of PR-A and PR-B was confirmed in neonatal stroma infected with the hPR-GFP virus by western blot. Expression of ERK was used as a loading control (c). (**B)** PTENKO/Kras tumors developed when GFP (a&b) or hPR-GFP (f&g) was expressed in the tumor stroma. Loss of PTEN was confirmed in the tumor epithelia (c&h). Phospho-ERK was detected as a downstream target of Ras activation (d&i). Stromal PR was diminished in GFP pre-therapy tumors (e). Conversely, abundant PR was detected in stroma of hPR-GFP pre-therapy tumors (j). **(C)** Loss of PTEN and activation of the RAS pathways was confirmed in tumor epithelia. **(D)** The histology of PTENKO/Kras tumors with stromal hPR-GFP treated with progesterone or placebo. Obvious regression of tumor was observed in PTENKO/Kras tumors with progesterone therapy when hPR was expressed in tumor stroma (a&b) compared to placebo treated counterparts (c&d). Scale bars equal 100μm except where noted.

Supplementary Methods

Construction of hPR-GFP construct: Human *PR* (*PGR*) was chosen for the PR over-expression experiments to minimize the possibility of silencing through endogenous regulatory pathways. To generate hPR-GFP, the entire coding sequence of human *PR* (encompassing *PR-B* and *PR-A*) was first amplified by PCR from pENTR223.1 (OpenBiosystems clone 100016179, Thermo Scientific) using the forward primer TCTAGAACCATGACTGAGCTGAAGGC and the reverse primer GAATTCTCACTTTTTATGAAAGAGAAGGGGTTTC. PCR was performed using Easy A polymerase (Agilent), and products were subcloned into TopoTA pCR2.1 (Invitrogen). The EcoRI fragment containing the *PR* gene was transferred to the same sites in the FU-CGW lentivirus construct.

Construction of inducible Cre-shPTEN-GFP construct: Since epithelial deletion of *PR* may impact estrogen induced proliferation of endometrial epithelium (1), a vector was designed to permit conditional

deletion of *PR* in a background PTEN loss. This vector, designed to allow tetracycline regulated expression of genes from a single lentiviral vector (pSTV), is based on the pSLIK vector design by Shin and colleagues (2). The *GFP* open reading frame was PCR amplified using the primers, 5'-ACTGACCTCGAGTCTAGAGCCACCATGGTGAGCAAG and 5'-ACTGACAAGCTTCTTGTACAGCTCGTC, and cloned upstream of a P2A "self-cleaving" peptide sequence in a pBluescript holding vector using the XhoI and HindIII restriction sites. The *rtTA 2S-M2* open reading frame was PCR amplified using the primers, 5'-ATATGCTAGCATGTCTAGACTGGACAAGAGCAAAG and 5'-CGTAGAATTCCCCGGGGAGCATG, and cloned downstream of the P2A "self-cleaving" peptide sequence using the NheI and EcoRI restriction sites resulting in the formation of the GFP-P2A-rtTA cassette. Next the FUW vector was made from the previously described FU-GW vector (*3*). Then the CMV/tetracycline response element hybrid promoter was cloned into FUW using the 5'-ATATTTAATTAATCGAGGTCGACGG and 5'- ATGCAGCGCTGGATCCCCAGGCGATCTGACGG primer sets yielding the FTUW construct. The GFP-P2A-rtTA cassette was then cloned into the FTUW backbone downstream of the human ubiquitin-C promoter using XbaI restriction sites to yield the tetracycline inducible vector (pSTV).

To produce inducible Cre-GFP, Cre recombinase was PCR amplified using the primers, 5'-ACTGACAGATCTATGTCCAATTTACTGACCGTACAC and 5'-GATGATAGCGCTCTAATCGCCATCTTCCAGCAG, and cloned into the pSTV construct using BglII/BamHI and AfeI restriction sites. A Kozak sequence was later added by Stratagene QuickChange XL kit using the primers, 5'- AGATCGCCTGGGGATCTGCCACCATGTCCAATTTACTGACCG and 5'- GTCAGTAAATTGGACATGGTGGCAGATCCCCAGGCGATCTGAC. A *PTEN* short-hairpin RNA sequence (shPTEN) controlled by the H1 promoter (4) was cloned into the PacI restriction sites of inducible Cre-GFP to yield the vector inducible Cre-shPTEN-GFP.

Tumor Generation: WT cultured neonatal stroma was used to generate most tumors except for PTENKO tumors with stromal *PR* deletion. To achieve deletion of stromal *PR*, neonatal $PR^{loxP/loxP}$ endometrial stromal cells were infected with Cre-GFP lentivirus or a control virus expressing only GFP. In experiments with color marked tumor stroma, WT endometrial stromal cells were infected with GFP expressing lentivirus. After 3 days in short-term culture (to allow for transgene expression), GFP positive stromal cells were isolated by FACS. $Pten^{loxP/loxP}$ or $Pten^{loxP/loxP}Kras^{LSL\text{-}G12D/WT}$ FACS isolated epithelia were infected with Cre-RFP lentivirus at an MOI of 40 by centrifugation for 2 hr at 1700 rpm at RT.

Methylation Specific PCR and Bisulfite Sequencing: DNA was extracted from isolated tumor cell fractions using the Allprep DNA/RNA Mico kit (Qiagen) according to the manufacturer's instructions. DNA extracted from lymph nodes was used as an unmethylated DNA control. Lymph node DNA treated with SssI CpG methyltransferase (New England Biolabs) was a positive control for DNA methylation. Bisulfite conversion was performed on 500 ng of DNA using the EpiTect Fast DNA Bisulfite kit (Qiagen). PCR reactions were perform with 150ng bisulfite treated DNA using primer pairs designed to bind to methylated or unmethylated sequences within the promoter region of *PR-A*. PCR mixtures contained 1X PCR buffer, 1.5 mM MgCl2, 200 mM dNTPs, 0.5 M each primer and 1 U Taq polymerase (TaKara). Cycling conditions were 94 C for 5 minute followed by 35 cycles of denaturation at 94 C for 45 s, annealing at 55.5 (methylated primers) or 54 (unmethylated primers) for 45 s and extension at 72C for 45 s, with a terminal extension at 72 C for 10 min. PCR products were visualized on a 3 % agarose gel. Primers are listed in Supplementary Tables.

For bisulfite sequencing, PCR products obtained from the stroma of three separate PTENKO and PTENKO/Kras tumors were cloned into pCR2.1 using the TOPO-TA kit (Invitrogen). Clones containing inserts were identified by PCR using M13 forward and reverse primers supplied with the kit. Ten positive colonies were sequenced for each condition. Samples were aligned to the bisulfite converted *PR-A*

promoter sequence using BLAST. Results were screened to ensure absence of non-conversion and sibling clone errors. Primers are listed in Supplementary Tables.

Supplementary Tables

Supplementary Table 1. Antibodies used for FACS sorting

Anti-mouse Trop1-PE	eBioscience	12-5791
Anti-moue CD90-APC	eBioscience	17-0902
Anti-mouse CD31-FITC	eBioscience	11-0311
Anti-mouse CD45-FITC	eBioscience	11-0451
Anti-mouse Ter119-FITC	eBioscience	11-5921

Supplementary Table 2. Antibodies used for IHC

Anti-pankeratin (clone PCK-26)	Sigma, C1801	1:750
Anti-estrogen receptor α (ERα clone 1D5)	Dako, M7047	1:100
Anti-progesterone receptor (PR, clone SP2)	Labvision, RM-9102-S0	1:250
Anti-PTEN (clone 138G6)	Cell Signaling Technology, 9559	1:100
Anti-Ki67	Vector Laboratories, VP-K451	1:1000
Anti-phospho-ERK Tyr202 (clone D13.14.4E)	Cell Signaling Technology, 4370	1:400
Anti-cleaved caspase 3	Cell Signaling Technology, 9664	1:800
Biotinylated anti-mouse	Vector, BMK-2202	1:1000
Biotinylated anti-rabbit	Jackson IR, 111-065-003	1:1000
SA-Horseradish peroxidase	Jackson IR, 016-010-084	1:1000
TUNEL kit	Millipore, 17-141	

Supplementary Table 3. Primers used for QPCR

PR A+B F	5'-TGGAAGAAATGACTGCATCG-3'
PR A+B R	5'-TAGGGCTTGGCTTTCATTTG-3'
PR B F	5'-ACACCTTGCCTGAAGTTTCG-3'
PR B R	5'-CTGTCCTTTTCTGGGGGACT-3'
GAPDH F	5'-TGTTCCTACCCCCAATGTGT-3'
GAPDH R	5'-TGTTCCTACCCCCAATGTGT-3'

Supplementary Table 4. Antibodies used for Western blotting

Anti-progesterone receptor (PR, clone SP2)	Labvision, RM-9102-S	1:400
Anti-Cre	Novagen, 69050	1:10000
Anti-ERK2 (C-14)	Santa Cruz, sc-154	1:5000
HRP-anti-rabbit	Bio-Rad, 170-6515	1:10000

Supplementary Table 5. Primers used for Methylation Specific PCR

Unmethylated Forward	5'-AGAAGAAATATGAAAAAAAGTTTTTT-3'
Unmethylated Reverse	5'-AATAAATATAAAATCACAAAACCCAA-3'
Methylated Forward	5'-GAAGAAATACGAAAAAAAGTTTTTC-3'
Methylated Reverse	5'-ATAAATATAAAATCGCAAAACCCG-3'
Bisulfite Forward	5'-AGAAGAAATAYGAAAAAAAGTTTTTY-3'
Bisulfite Reverse	5'-AATAAATATAAAATCRCAAAACCCRA-3'

References:

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3. Lois C, Hong EJ, Pease S, Brown EJ, Baltimore D. Germline transmission and tissue-specific expression of transgenes delivered by lentiviral vectors. Science 2002;295:868-72.
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