Tsai et al., Supplemental Tables

Table 1. Female reproductive defects in *Hurp*$^{-/-}$ mice

Parents (♀X♂)	Litter no.	Litter size.	Offspring no. at weaning		
			+/+	+/-	-/-
+/- x +/-	23	6.8 ± 0.93	50 (32%)	73 (46.8%)	33 (21.2%)
+/- x -/-	31	6.1 ± 0.52	-	97 (51.6%)	91 (48.4%)
-/- x +/-	0	0	-	0	0

Table 2. Blastocyst collected from wild-type and *Hurp*$^{-/-}$ female mice

Female genotype*	Mouse no.	Blastocyst no.
+/+	6	7.3 ± 0.8
-/-	4	6.8 ± 0.6

*Wild-type and *Hurp*$^{-/-}$ female mice were plugged by fertile wild-type male mice

Tsai et al., Supplemental Figure S1

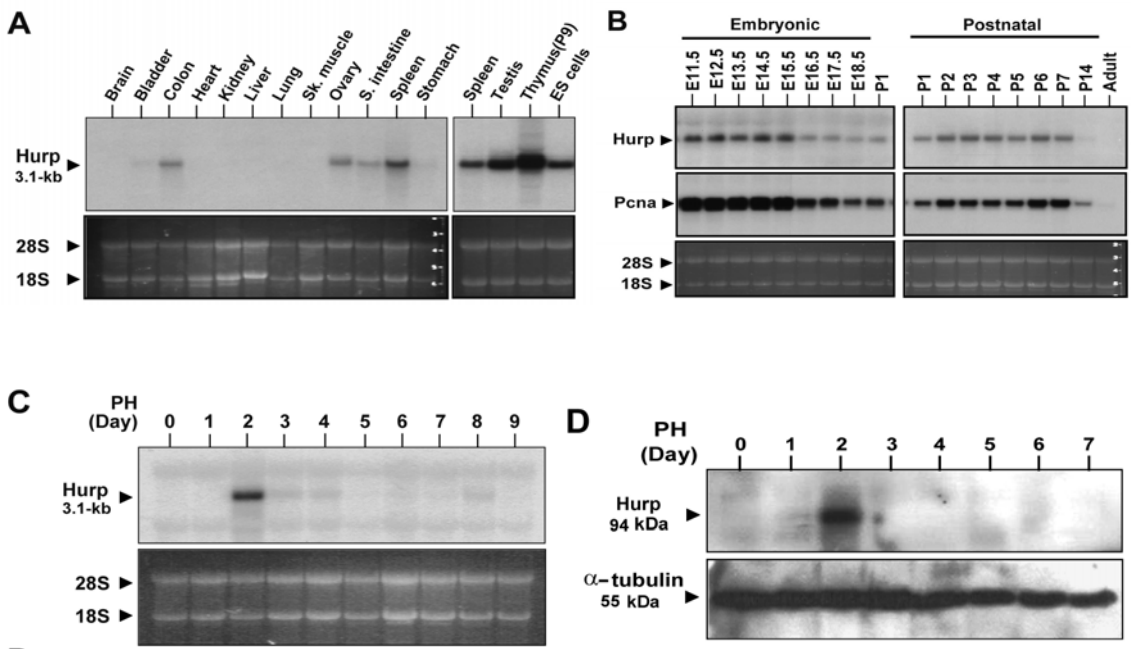


FIGURE S1. Hurp expression coincides with cell cycle progression. *A,* Multiple tissue blot analysis of Hurp mRNA expression in adult mice. All of the tissues were prepared from 2-month old C57BL/6 mice except for the thymus, which was obtained from postnatal day 9 (P9) pups. ES cells, embryonic stem cells. *B,* The mRNA expression of Hurp coincides with the cell proliferation marker Pcna in mouse livers prepared from embryonic day 11.5 (E11.5) to day 18.5 (E18.5) and postnatal day 1 (P1) to day 14 (P14), as well as from adult mice. The intensity of the 28S and 18S rRNA was used as internal control for the total RNA loading. *C,* Northern blot analysis of Hurp mRNA induction during liver regeneration at day 2 post-hepatectomy, which coincides with the G2/M phase during hepatocyte cell cycle progression. Liver regeneration was induced by two-third partial hepatectomy (PH) of 2-month old wild-type C57BL/6 male mice. The intensity of the 28S and 18S rRNA was used as internal controls for total RNA loading. *D,* Validation of specificity of the Hurp polyclonal antibodies was confirmed by western blot analysis of Hurp protein expression during liver regeneration using rabbit anti-mouse Hurp polyclonal antibodies. The same blot was detected using alpha-tubulin antibody as a protein loading control. The protein expression pattern of Hurp is well correlated with the temporal expression of the Hurp mRNA during liver regeneration.

Tsai et al., Supplemental Figure S2

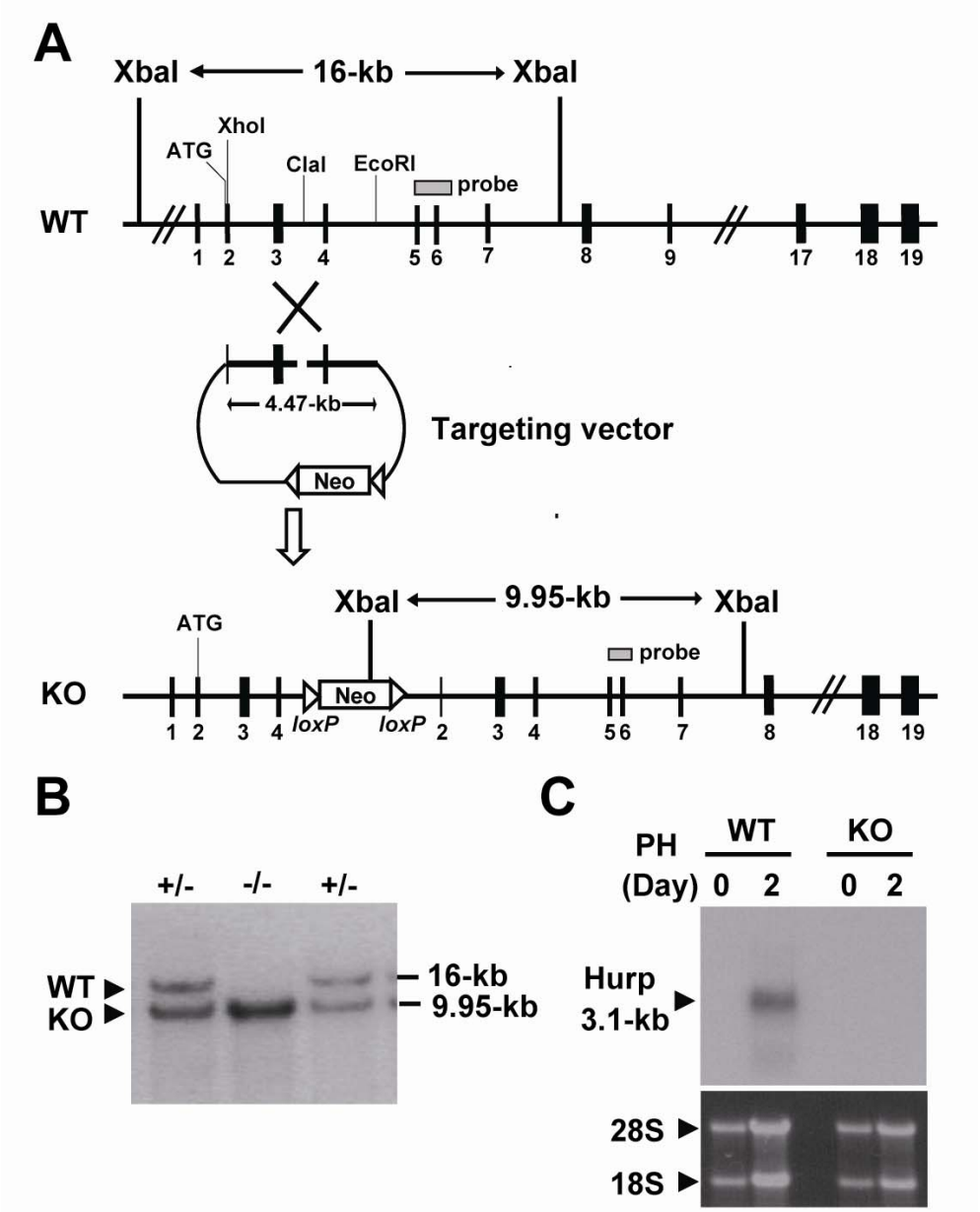


FIGURE S2. Generation of the Hurp knockout allele. *A*, The 4.47-kb genomic DNA extending from within exon 2 to within intron 4 was used as the homologous recombination arm. The targeting vector was linearlized at the unique ClaI site and introduced into the ES cells. Gray box, flanking probe; Neo, neomycin expression cassette. *B*, Southern blot analysis of tail DNA isolated from *Hurp*$^{+/-}$ and *Hurp*$^{-/-}$ mice. The genomic DNA was digested with XbaI. *C*, Northern blot analysis of Hurp mRNA expression in the wild-type (WT) and *Hurp*$^{-/-}$ (KO) mice. The RNA samples were prepared from the resting livers and regenerating livers 2 days after partial hepatectomy (PH) which stimulates quiescent hepatocytes to re-enter cell cycle and induces Hurp expression in wild-type mice. The 567-bp cDNA fragment containing Hurp exon 5-9 was used as probe for the Northern blot analysis. This probe was designed to detect the normally spliced Hurp mRNA and other aberrantly spliced variants in the knockout mice as predicted from the genomic structure of the targeted allele such as aberrant mRNA containing duplicated exon 3-4. However, our result revealed that there was no detectable hybridization signal in the knockout mice, which suggests that the mRNA transcribed from the Hurp targeted allele is likely to be very unstable. The intensity of the 28S and 18S rRNA was used as internal control for total RNA loading.

Tsai et al., Supplemental Figure S3

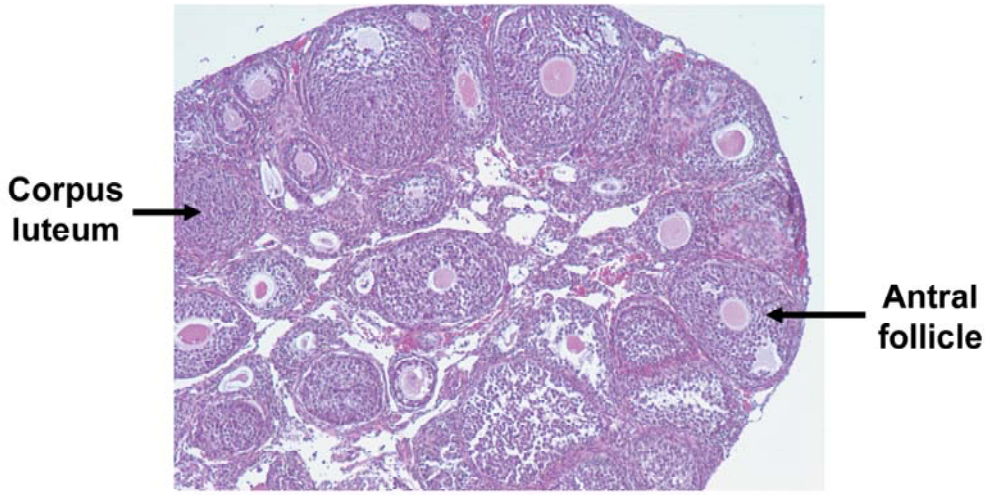


FIGURE S3. Normal ovary histology of the *Hurp*$^{-/-}$ female mice. Histology of the ovary was examined by H&E staining of paraffin-embedded ovarian sections prepared from 6-week old *Hurp*$^{-/-}$ female mice. Different stages of oocytes and corpus luteum can be observed in this ovary.

Tsai et al., Supplemental Figure S4

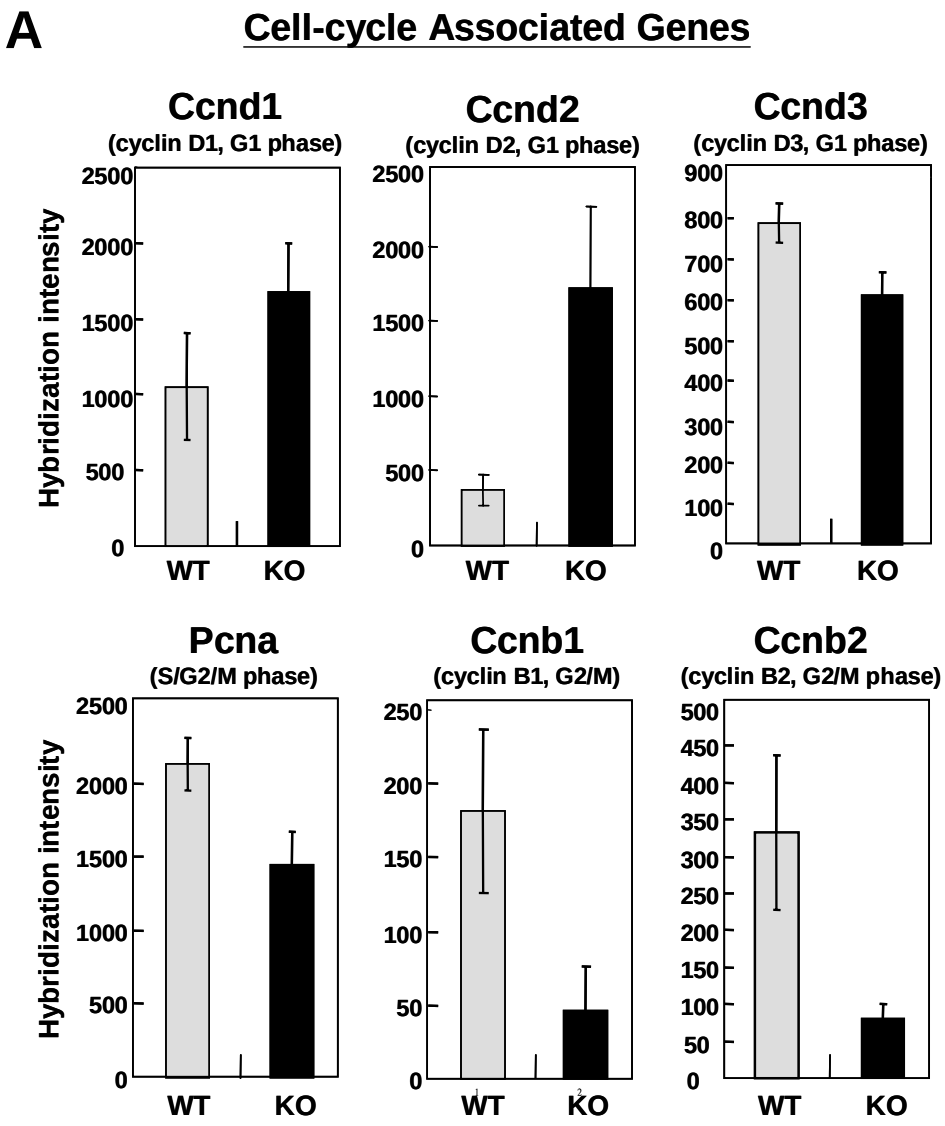
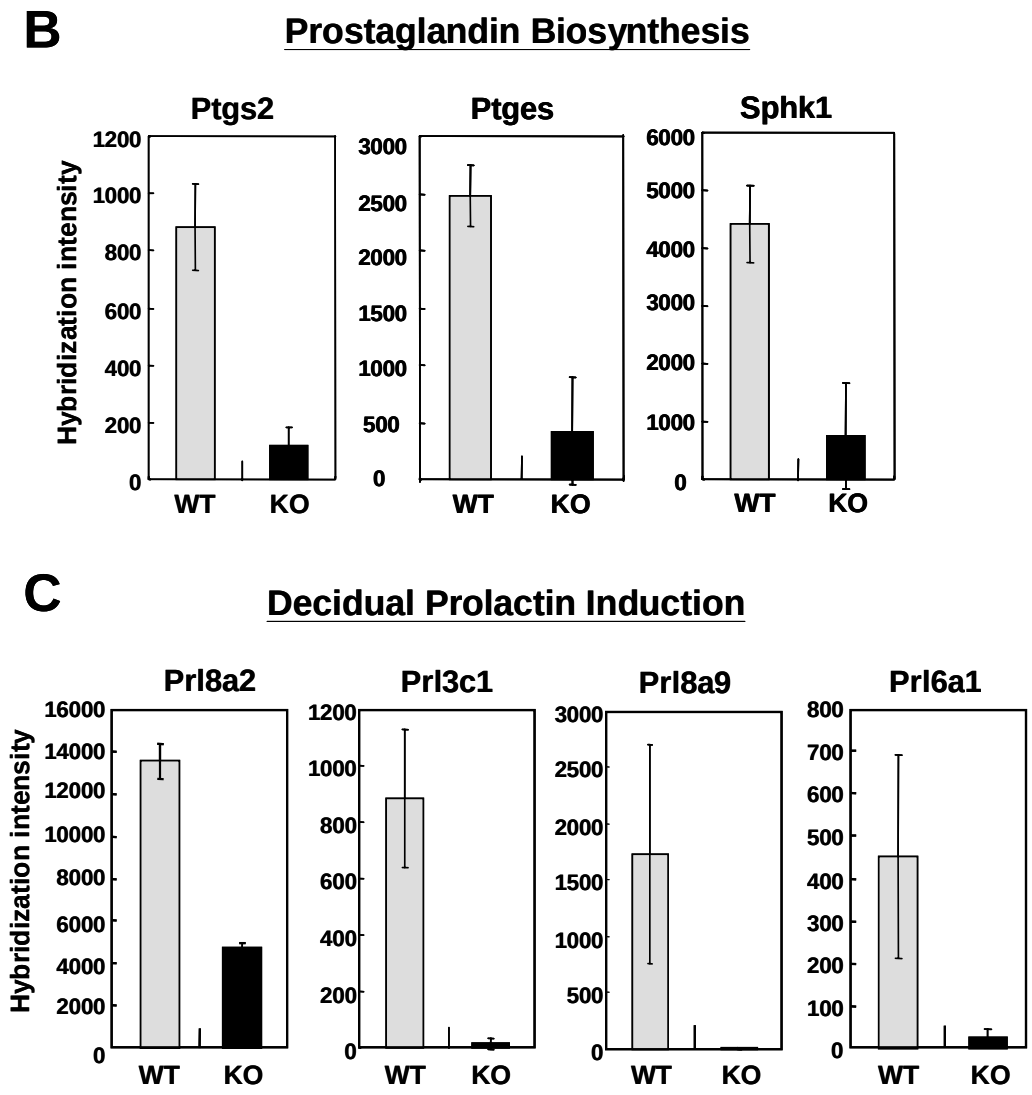


FIGURE S4. Deregulation of gene expression associated with cell proliferation and implantation in the artificially decidualized uteri of *Hurp*$^{-/-}$ (KO) mice. *A,* Cell-cycle associated genes. *B,* Genes involved in prostaglandin biosynthesis (1, 2). *C,* Decidual prolactin genes (3, 4). RNA samples isolated from wild-type (WT) and *Hurp*$^{-/-}$ uteri 5 days after the decidual stimulus were systematically compared for changes in gene expression by Affymetrix microarray. Genes related to cell proliferation and implantation were identified based on KEGG pathway database (http://www.genome.jp/kegg/pathway.html).

Tsai et al., Supplemental Figure S4 continued



Tsai et al., Supplemental Figure S5

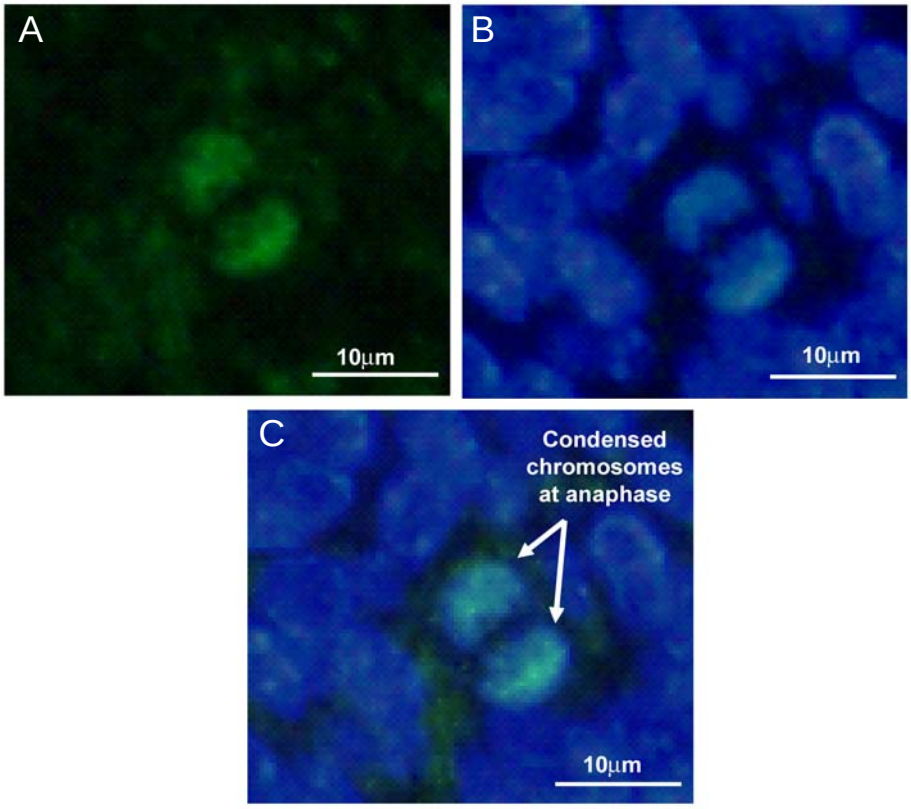


FIGURE S5. Co-localization of Hurp protein with condensed chromosomes during mitosis of the endometrial stromal cells in wild-type mice. *A*, IHC staining of Hurp protein in the uterine section prepared from the ovariectomized wild-type mice that had received an i.p. injection of estrogen E2 and sacrificed at 72h after injection. *B*, Nuclei and condensed chromosomes were counter stained blue with DAPI. *C*, Merge of IHC staining of Hurp and DAPI. Stromal cells with mitotic figures and visible condensed chromosomes (about 0.1%, 4/4000) could be observed in uterine sections of the E2 treated wild-type mice. Our data revealed that Hurp protein co-localized with condensed chromosomes during mitosis, in this case the anaphase chromosomes, under physiological condition. This observation is consistent with the role of Hurp being involved in stabilizing and targeting K-fibers to the kinetochore of chromosomes. Magnification 2,000X.

SUPPLEMEMTAL METHODS

Generation of Hurp knockout mouse

A Lambda FIXII library (Stratagene) derived from strain 129Sv/Ev was screened with mouse Hurp cDNA probe. A 4.47-kb XhoI-EcoRI DNA fragment, which extends from within exon 2 to intron 4, was used as the homologous recombination arm for construction of an "insertion-type" targeting vector. The Hurp targeting vector was linearlized within the arm using ClaI and transfected into the AB2.2 ES cells using electroporation (5). Selection medium containing neomycin was applied after 24h and maintained for 7 days. The DNA extracted from individual ES clone was examined by mini-Southern blot analysis using a flanking probe (6). The targeted ES cells were injected into C57BL/6 blastocysts. Chimeric male mice were bred with C57BL/6 females for germline transmission. All of the mice were housed in a specific pathogen free facility. All of the animal protocols are consistent with the recommendations outlined in the "Guide for the Care and Use of Laboratory Animals" (Washington, DC, National Academy Press, 1996) and strictly follow the rules issued by Institutional Animal Care and Use Committees of the National Yang-Ming University.

RNA analysis

Total RNA was isolated using TRIzol (Invitrogen Life Technologies) from the tissues of each genotype. Semi-quantitative RT-PCR and Northern blot analysis were used to determine mRNA expression (7). The cDNA probes used for the Northern blot hybridization were: mouse Hurp (NM_144553, from base 807 to 1373; covering from within exon 5 to within exon 9) and mouse Pcna (NM_011045, from base 174 to 856; covering from within exon 1 to within exon 6). We execute reverse transcription with 2μg of total RNA using oligo-d(T) as primer and Superscript III reverse transcriptase (Invitrogen Life Technologies). The real-time quantitative PCR was carried out on a Roche LightCycler 480 Real-time PCR instrument using a TaqMan probe searched at the Universal ProbeLibrary (Roche Applied Science) and LightCycler TaqMan Master (Roche Applied Science). The program for the real-time PCR was pre-incubation for 10 sec at 95^{o}C, followed by 65 cycles of 5 sec at 95^{o}C, 20 sec at 59^{o}C, and 2 sec at 72^{o}C. Fluorescence was acquired at each elongation step during amplification and analyzed using the Light Cycler Software 4.05. All amplifications were carried out in triplicate for each RNA sample and primer set, and all real-time quantitative PCR measurements were done using RNA samples from three individual mice. The amount of total input cDNA was normalized using Hprt as an internal control.

Induction of artificial decidualization

Artificial induction of decidual reaction was performed as described previously (8). Ovariectomized female mice (4-5 weeks old) were treated with 3 daily subcutaneous (s.c.) injections of 100ng of estrogen (E2, 17α-Estrodiol, Sigma-Aldrich E8750) prepared in sesame oil (Sigma-Aldrich S3547). After two days of rest, mice were treated with daily s.c. injections of 1mg of progesterone (P4, Sigma-Aldrich P0130) and 6.7ng of E2. Six hours after the P4 and E2 injection on the third day, one uterine horn was infused with 30μl of sesame oil to induce decidualization. The daily injections of P4 and E2 were continued until the day of sampling. The animals were sacrificed and the uterine weights of the oil-infused and control horns were measured on day 5 after oil infusion.

Estrogen treatment

Female mice were ovariectomized at 4-5 week-old and hormone treatment was started 10 days later as previously described (9). Ovariectomized female mice were i.p. injected with E2 (100 ng/animal prepared in 0.1 ml sesame oil) or an oil control. Mice were sacrificed at 2h, 24h and 72h after E2 or oil injection and the uterine samples were collected for RNA and IHC analysis.

Generation of rabbit anti-mouse Hurp antibody

A full-length cDNA clone (I.M.A.G.E. clone 6816660) was used as the template for PCR amplification of the mouse Hurp cDNA fragment. The Hurp cDNA (amino acids 62-266) fragment was subcloned into the pQE-32 (Qiagen) vector, which contains a His-tag sequence. This His-Hurp fusion protein was expressed in bacteria and purified for injection into rabbits to generate anti-sera containing polyclonal antibodies against the mouse Hurp protein.

Immunohistochemistry (IHC), DNA synthesis and mitotic index

IHC staining of paraffin-embedded uterine sections (3 μm) were performed as described previously (10). IHC of BrdU and phospho-histone H3 was detected by the Chemicon IHC Select^{TM} System (DAB150) and LSAB^{TM} Kit (DakoCytomation K0690). IHC of Hurp was detected by biotinylated secondary antibodies (1:300, Vector Laboratories) and Alexa Fluor 488-conjugated Tyramide signal-amplification kit (Molecular Probes). The number of S-phase cells was estimated by bromodeoxyuridine (BrdU) incorporation into nuclei. Mice were injected with BrdU (Sigma B5002, 100mg/kg) i.p. 2h before sacrifice. The IHC analysis was performed on paraffin-embedded uterine sections using anti-BrdU antibodies (DAKO M0744) and counterstained with haematoxylin. To measure endometrial mitotic activity, anti-phospho-histone H3 antibodies (Upstate 06-570) were used for IHC staining. About 1000 stromal cells in random fields of the IHC staining slides were examined for the presence of positive cells for each mouse.

Statistics

The results are presented as mean ± s.d. from at least three independent experiments. Comparisons between two groups were done using a Student's t test to calculate probability values. A p value of <0.05 was considered significant.

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