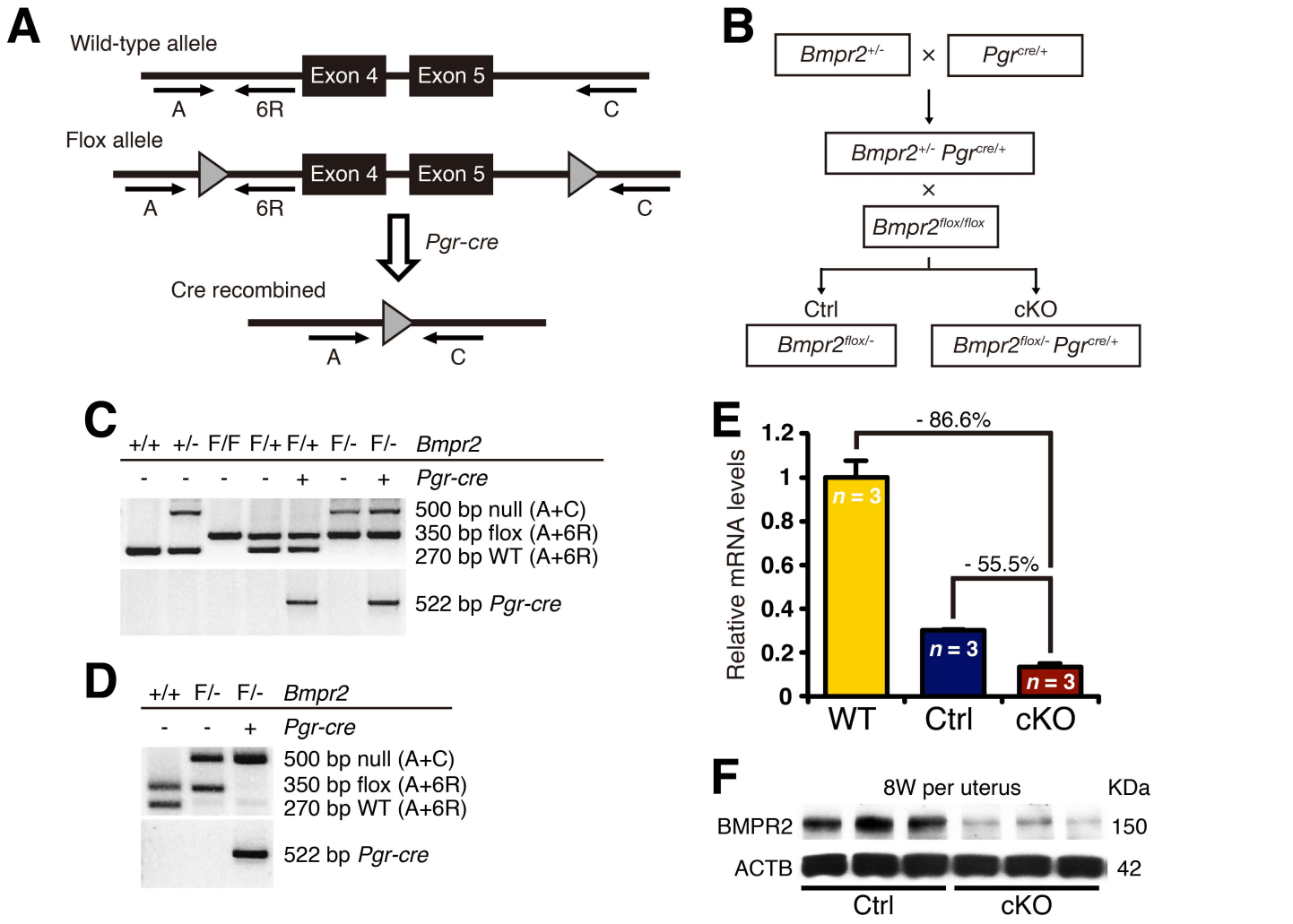
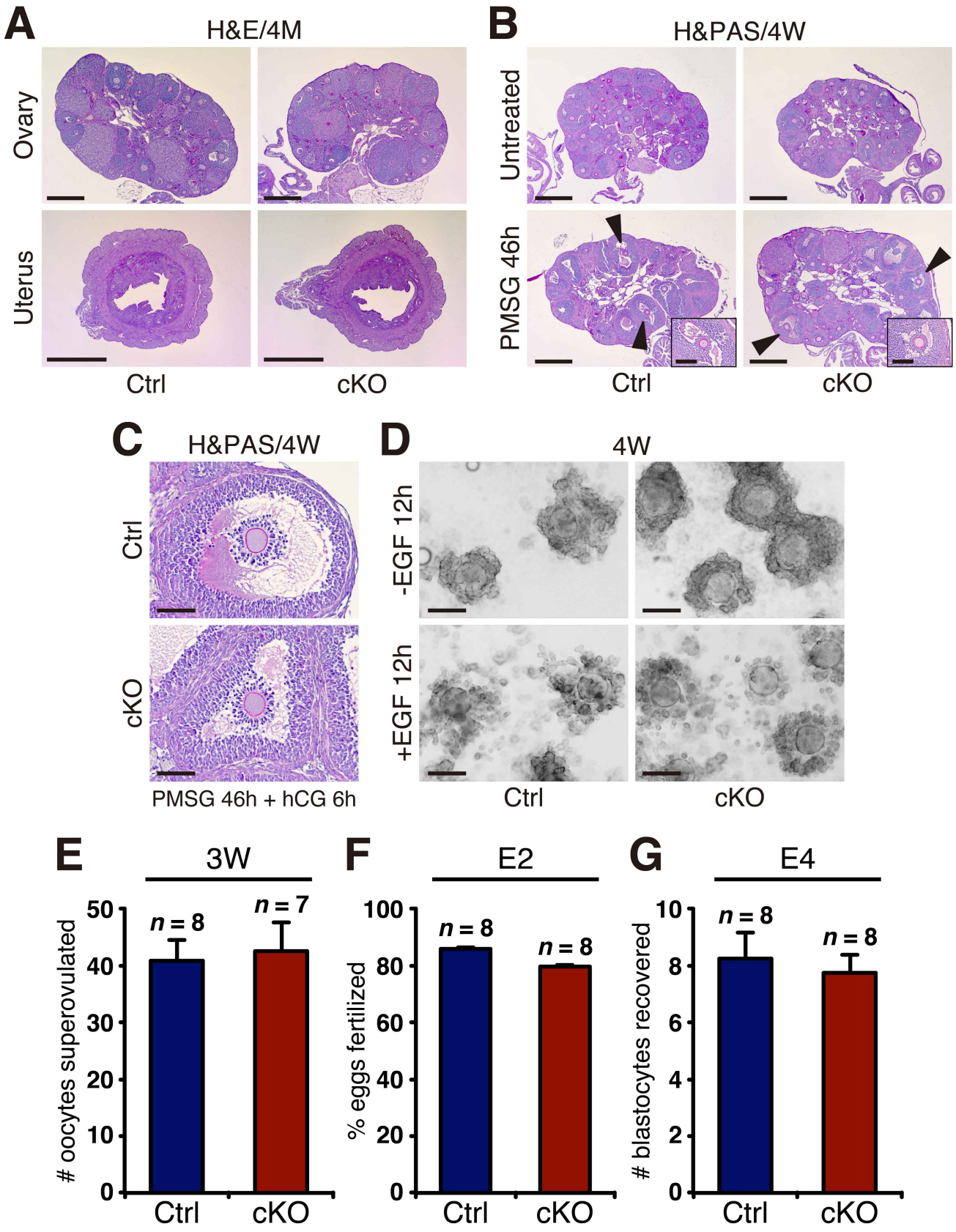


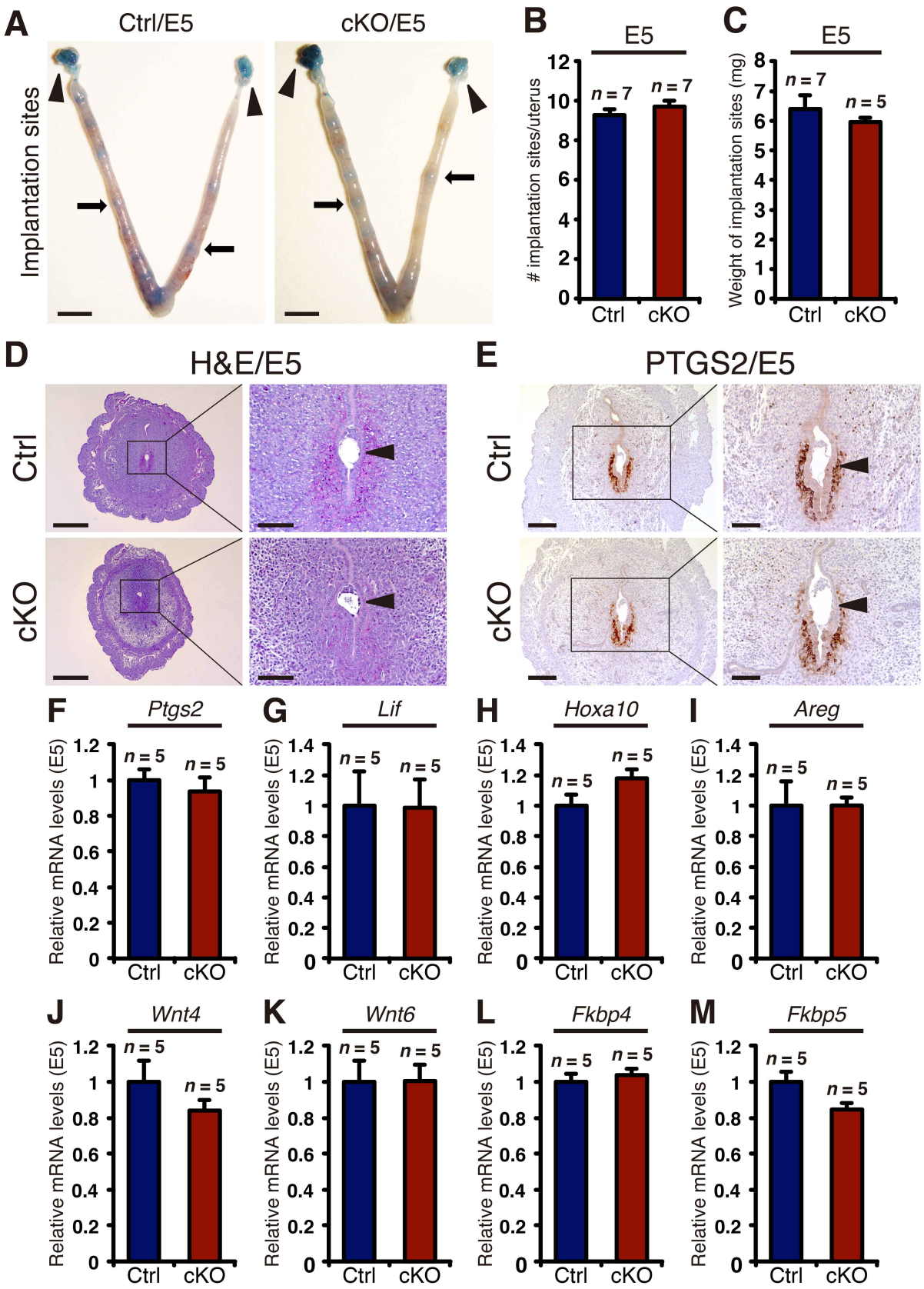
Supplemental Figure 1.

Generation of *Bmpr2* conditional knockout mice. (**A**) Illustration of the *Bmpr2* conditional allele with exon 4 and 5 flanked by two *loxP* sites. Primers A, C, and 6R are used to distinguish the *Bmpr2* WT, *floxed*, and *Cre* recombined alleles. (**B**) Schematic representation of the breeding strategy for generating control and *Bmpr2* cKO mice. The *Bmpr2* null allele was used to achieve maximal deletion of *Bmpr2* gene. (**C**) PCR analyses for *Bmpr2* genotyping. Representative PCR images are shown. *flox*/- and *flox*/+ are abbreviated as F/- and F/+, respectively. (**D**) Recombination of *Bmpr2* floxed allele in the genomic DNA from uterine tissues. (**E**) The *Bmpr2* mRNA levels in WT, control, and *Bmpr2* cKO uteri were measured by real-time quantitative PCR (qPCR). Data are means ± SEM. (**F**) *Bmpr2* cKO uteri showed reduction of BMPR2 protein levels as assessed by Western blot. ACTB was used as an internal control. (**G**) Reduction of BMPR2 protein expression in the decidua but not the smooth muscle of E8 implantation sites from *Bmpr2* cKO females compared with controls. Scale bar: 200 μm (10X panels); 50 μm (40X panels).



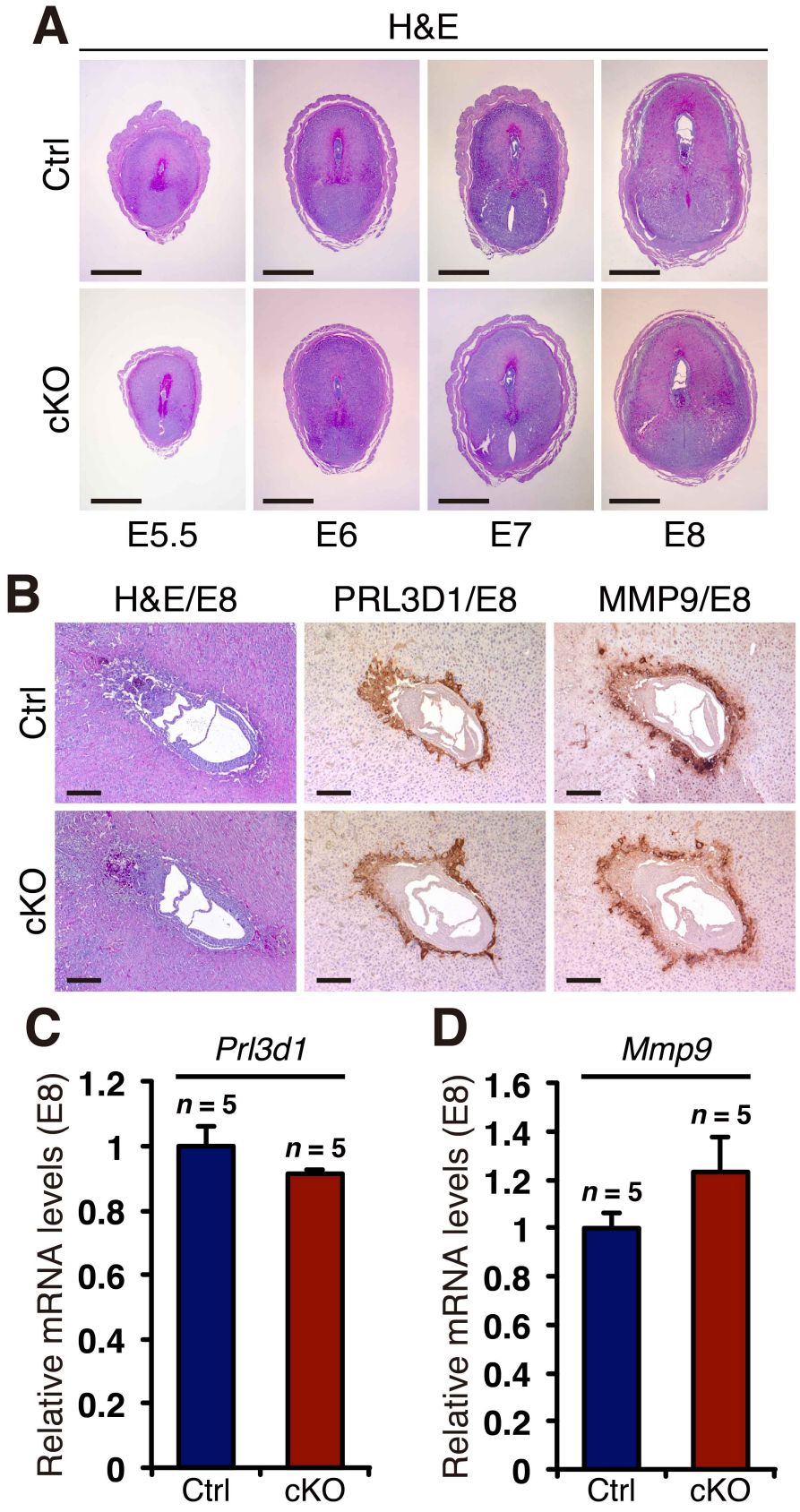
Supplemental Figure 2.

Normal ovarian and uterine histology and normal ovarian functions, fertilization, and preimplantation development in *Bmpr2* cKO female mice. (**A**) Normal ovarian and uterine histology of *Bmpr2* cKO female mice compared with controls. Note that all developmental stage follicles and corpus lutea were present in the ovaries from *Bmpr2* cKO mice. Scale bar; 1 mm (uterus); 500 µm (ovary). (**B**) Normal follicle development in the ovaries from control and *Bmpr2* cKO females after hormone stimulation. Arrowheads indicate preovulatory follicles. Small boxes show higher magnifications. Scale bar; 500 µm (ovary); 100 µm (inset). (**C**) In vivo cumulus expansion analyses revealed normal cumulus cell expansion in the ovaries from control and *Bmpr2* cKO female mice. Scale bar: 100 µm. (**D**) In vitro cumulus expansion analyses from EGF stimulation showed normal cumulus cell expansion in cumulus-oocyte complexes obtained from control and *Bmpr2* cKO female mice. Scale bar: 100 µm. (**E**) Number of ovulated oocytes was comparable between hormone-stimulated immature control and *Bmpr2* cKO female mice (Control, 40.9 ± 3.6; *Bmpr2* cKO, 42.6 ± 5.0). Data are means ± SEM. (**F**) *Bmpr2* cKO female mice showed normal fertilization rate, as determined by the number of recovered 2-cell embryos (Control, 85.9 ± 3.9%; *Bmpr2* cKO, 79.7 ± 2.6%). Data are means ± SEM. (**G**) *Bmpr2* cKO female mice showed a normal development of preimplantation embryos, as determined by the number of recovered blastocysts (Control, 8.3 ± 0.9; *Bmpr2* cKO, 7.8 ± 0.6). Data are means ± SEM.



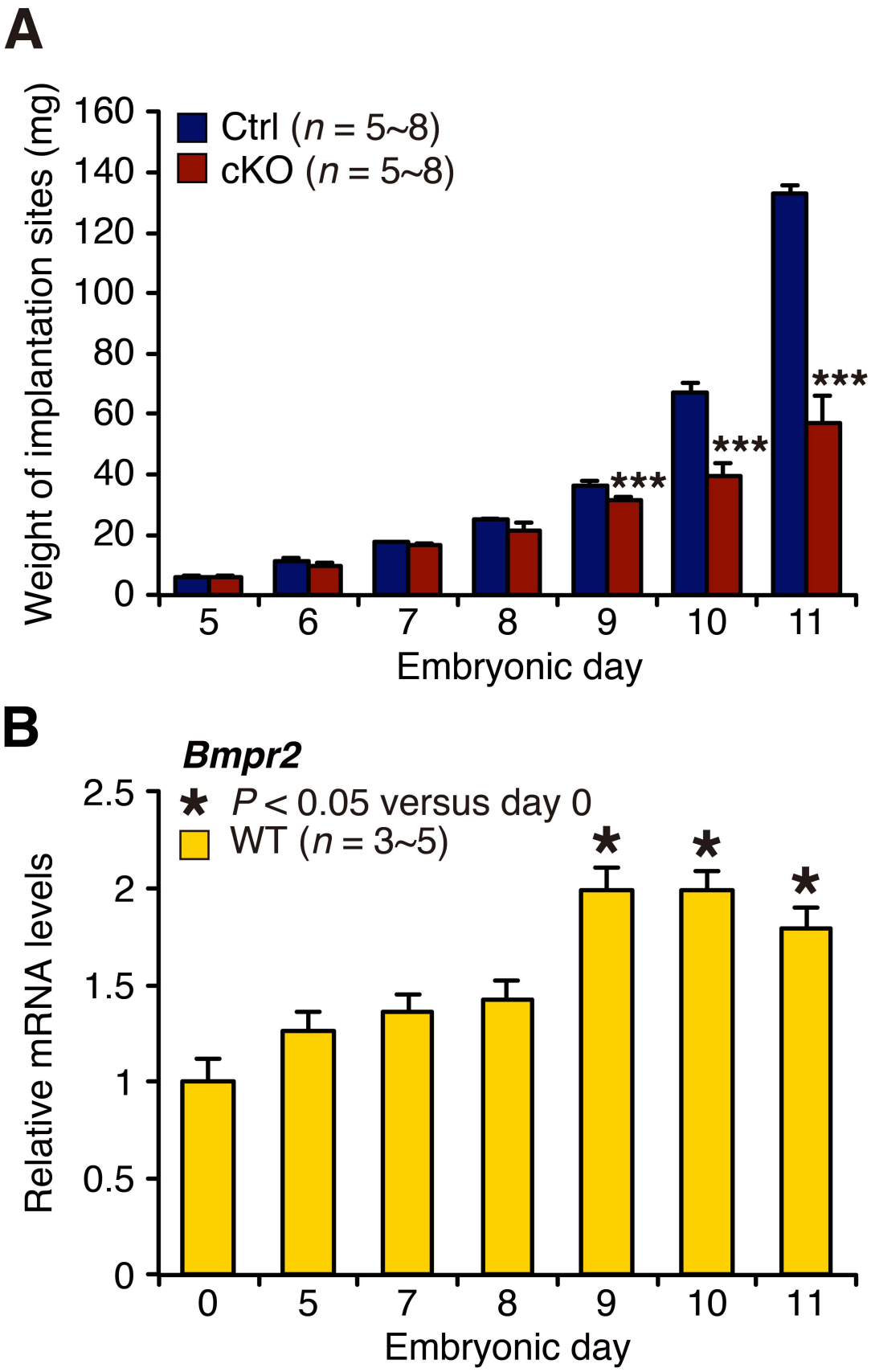
Supplemental Figure 3.

Bmpr2 cKO female mice display normal implantation. (**A**) Implantation sites were visualized by injection of Chicago blue dye in pregnant control and *Bmpr2* cKO female mice. Arrowheads indicate ovaries, and arrows indicate implantation sites. Scale bar: 5 mm. (**B**) Number of E5 implantation sites was comparable between control and *Bmpr2* cKO female mice (Control, 9.3 ± 0.3; *Bmpr2* cKO, 9.7 ± 0.3). Data are means ± SEM. (**C**) Weight of E5 implantation sites was comparable between control (6.4 ± 0.5 mg) and *Bmpr2* cKO mice (6.0 ± 0.1 mg). Data are means ± SEM. (**D**) Representative H&E-stained E5 implantation sites of pregnant control and *Bmpr2* cKO uteri. Arrowheads indicate the location of implanting blastocysts. Higher-magnification views of the boxed regions are shown on the right. Scale bars: 500 μm (left); 100 μm (right). (**E**) Normal PTGS2 protein expression was detected in E5 implantation sites from pregnant *Bmpr2* cKO female mice. Scale bar: 200 μm (left); 100 μm (right). (**F-M**) mRNA levels of implantation-related genes are comparable between pregnant control and *Bmpr2* cKO uteri. Data are means ± SEM. (**D** and **E**) Higher-magnification images of the boxed regions are shown at right.



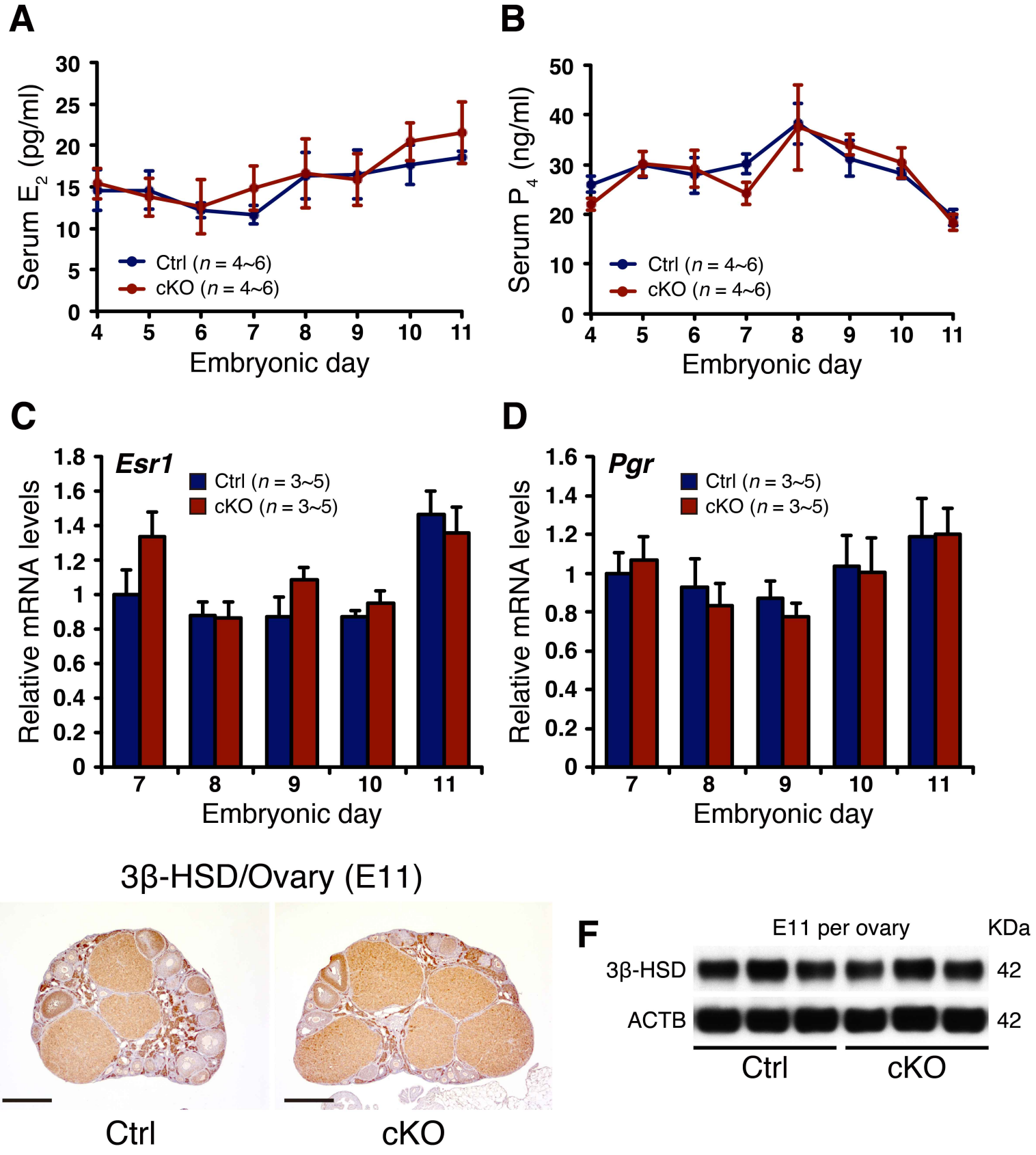
Supplemental Figure 4.

Bmpr2 cKO female mice demonstrate normal fetal development and decidual changes during early pregnancy. (**A**) Morphological changes in H&E-stained implantation sites were comparable between pregnant control and *Bmpr2* cKO female mice until E8. Scale bar: 1 mm. (**B**) Similar development of the embryonic and extraembryonic structures were detected in H&E-stained E8 implantation sites from control and *Bmpr2* cKO uteri. Similar patterns of PRL3D1 and MMP9 protein expression in trophoblast giant cells were observed by immunostaining of E8 implantation sites from pregnant control and *Bmpr2* cKO uteri. Scale bar: 200 µm. (**C** and **D**) Similar *Prl3d1* and *Mmp9* mRNA levels were detected in E8 implantation sites from control and *Bmpr2* cKO uteri. Data are means ± SEM.



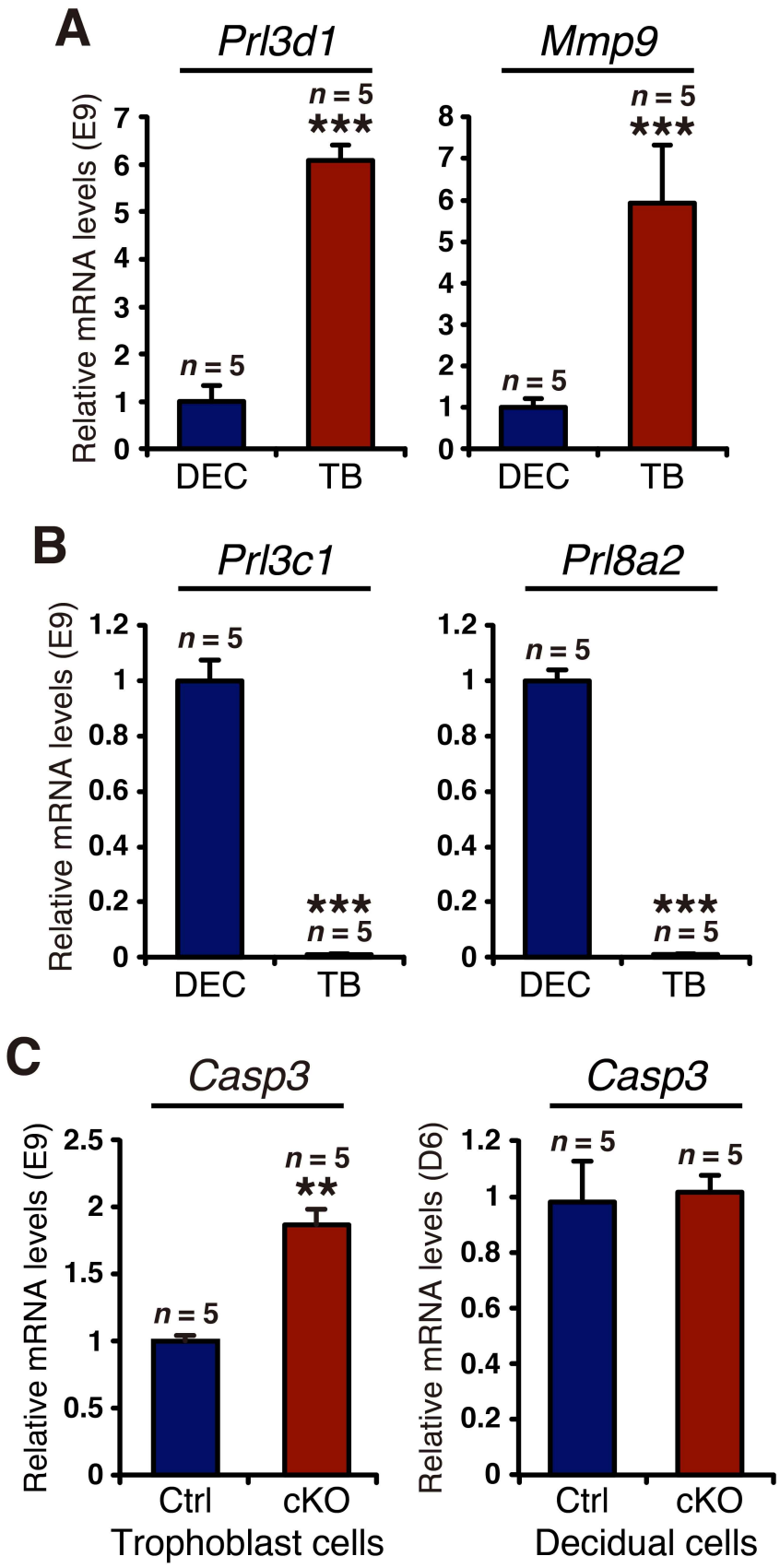
Supplemental Figure 5.

Reduced weight of implantation sites from pregnant *Bmpr2* cKO female mice and time-dependent change of *Bmpr2* expression during pregnancy. (**A**) Weight of implantation sites were statistically lower in *Bmpr2* cKO uteri from E9 (***$P < 0.001$). Data are means ± SEM. (**B**) *Bmpr2* mRNA levels are increased in decidual cells after E9 (*$P < 0.05$). Data are means ± SEM.



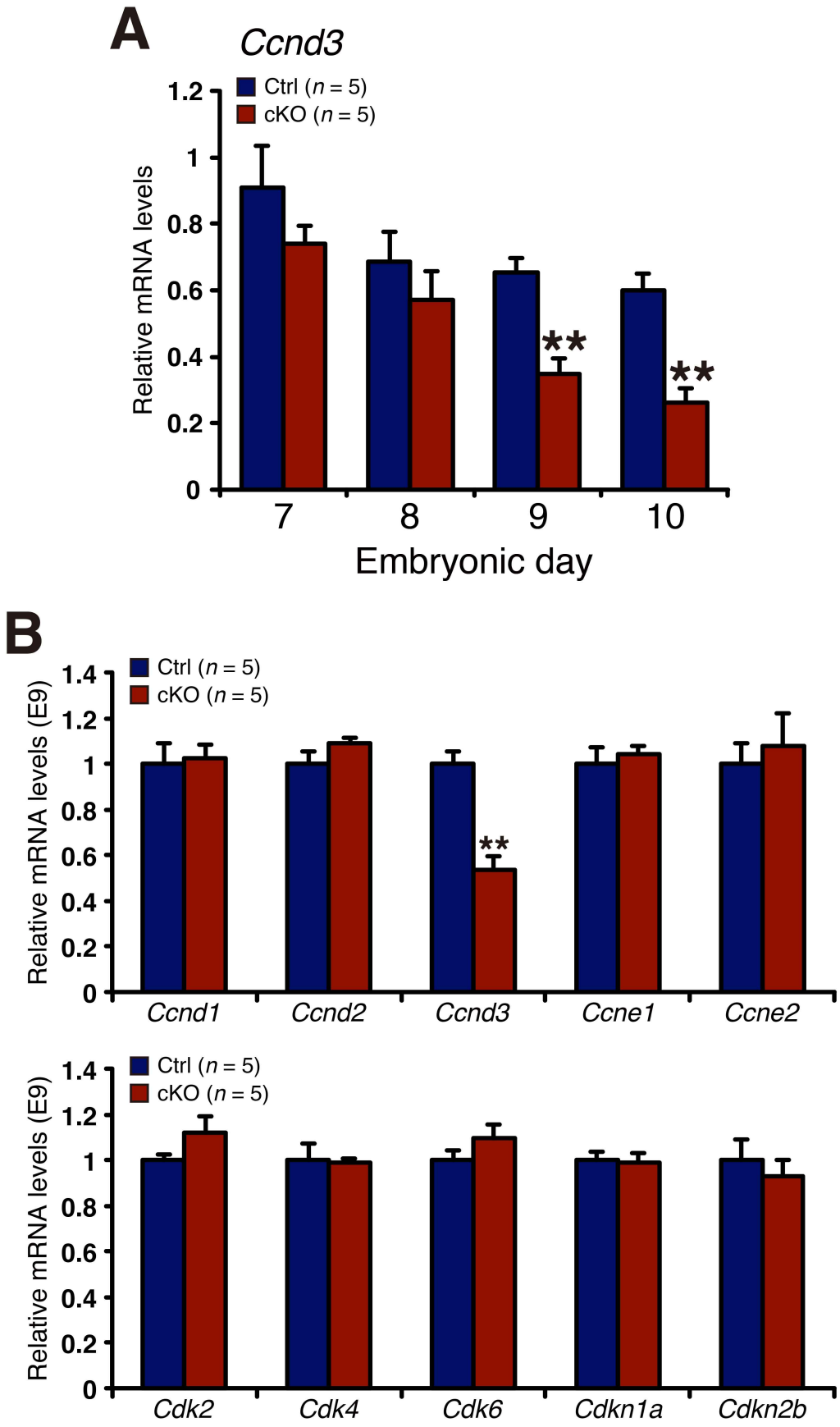
Supplemental Figure 6.

Comparable serum hormone levels and progesterone synthesis between pregnant control and *Bmpr2* cKO female mice. (**A** and **B**) Serum levels of E2 and P4 are comparable between control and *Bmpr2* cKO female mice from E4 to E11. Data are means ± SEM. (**C** and **D**) mRNA levels of *Esr1* and *Pgr* are comparable in implantation sites within pregnant control and *Bmpr2* cKO uteri at E7-E11. Data are means ± SEM. (**E**) Normal protein expression of 3β-HSD in corpus lutea was detected by immunostaining between control and *Bmpr2* cKO female mice at E11. Scale bar: 200 μm. (**F**) Ovarian tissues from pregnant *Bmpr2* cKO female mice show similar 3β-HSD protein levels, which were assessed by Western blot. ACTB expression was used as an internal control.



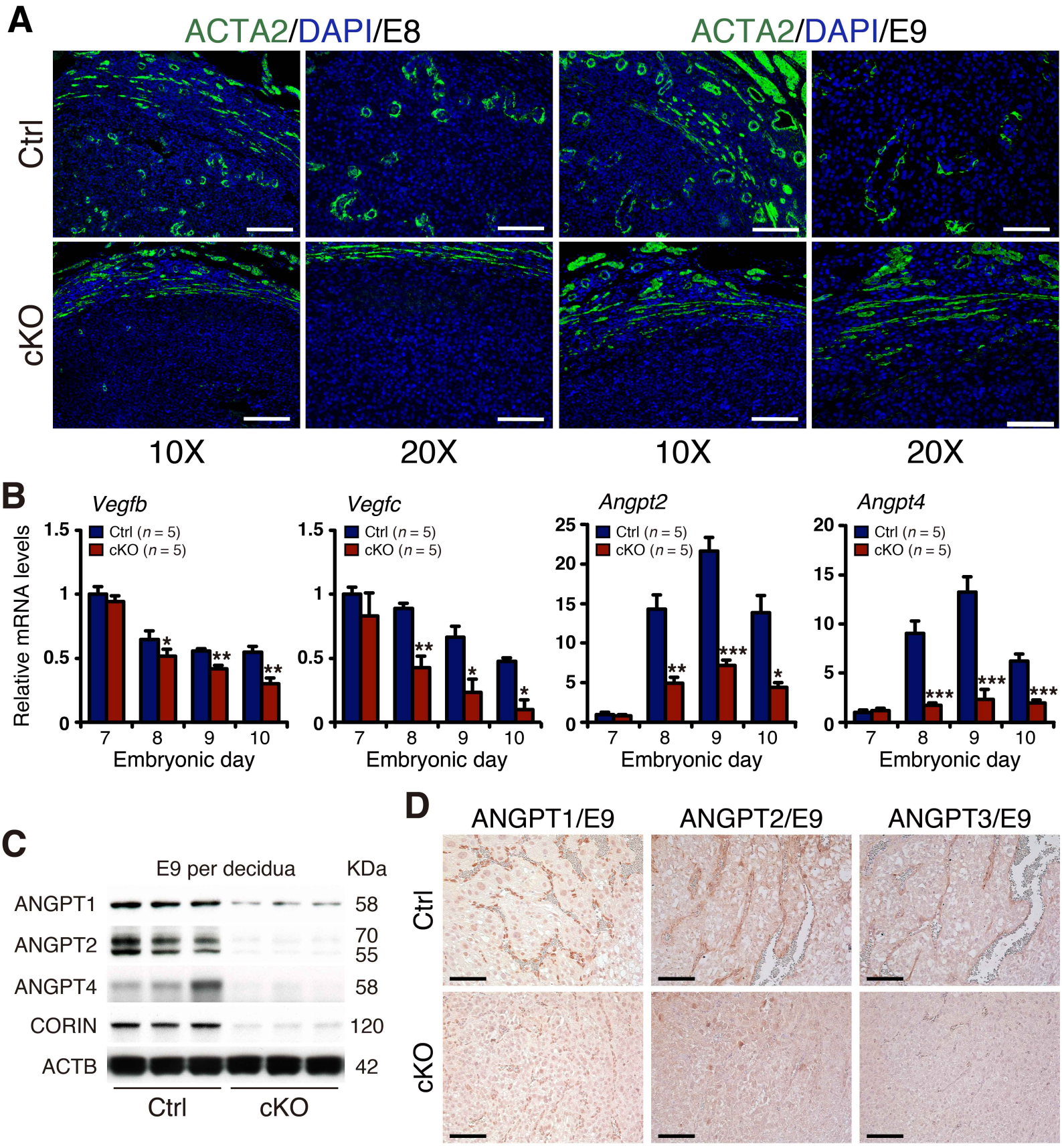
Supplemental Figure 7.

Molecular validation of isolated trophoblast and decidual cells from pregnant control and *Bmpr2* cKO uteri and analysis of *Casp3* mRNA levels. (**A** and **B**) qPCR analyses were conducted to confirm levels of specific genes (**A**) for isolated trophoblast cells, *Prl3d1* and *Mmp9*, (**B**) and for isolated decidual cells, *Prl3c1* and *Prl8a2*, using samples from control and *Bmpr2* cKO female mice. DEC, isolated decidual cells; TB, isolated trophoblast cells (***$P < 0.001$). Data are means ± SEM. (**C**) *Casp3* mRNA levels were elevated in isolated trophoblast cells, but not isolated decidual cells, from *Bmpr2* cKO female mice (**$P < 0.01$). Data are means ± SEM.



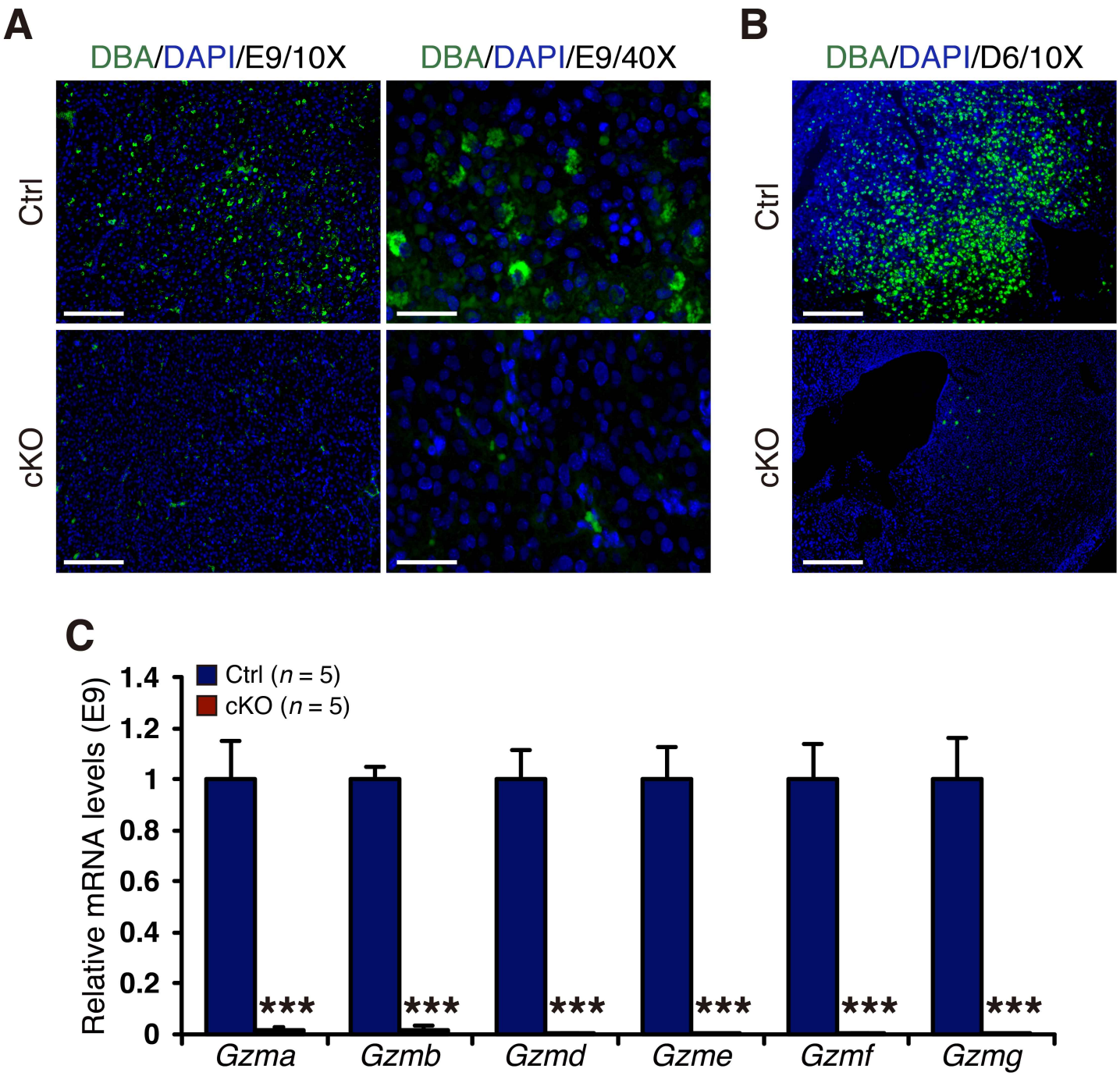
Supplemental Figure 8.

Deletion of uterine *Bmpr2* decreases *Ccnd3* transcription level in decidual tissues from E9. (**A**) *Ccnd3* mRNA levels trended lower at E7 and E8 and are statistically decreased at E9 and E10 in decidual tissues from *Bmpr2* cKO female mice. Data are means ± SEM. (**B**) With the exception of *Ccnd3*, mRNA levels of G1-phase cell cycle regulator genes are comparable in pregnant control and *Bmpr2* cKO uteri (**$P < 0.01$). Data are means ± SEM.



Supplemental Figure 9.

Deletion of uterine *Bmpr2* reduces *Vegf* and *Angpt* mRNA levels and decreases ANGPT and CORIN protein levels secondary to vascular defects in decidual tissues. (**A**) Decreased vasculature in the decidua of pregnant *Bmpr2* cKO uteri at E8 and E9 as revealed by immunofluorescence with an antibody against ACTA2 and nuclear staining with DAPI. Scale bar: 200 µm (10X panels); 100 µm (20X panels). (**B**) Deletion of uterine *Bmpr2* reduces *Vegfb*, *Vegfc*, *Angpt2*, and *Angpt4* mRNA levels in decidual tissues after E7 (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$). Data are means ± SEM. (**C**) Western blot analysis shows that ANGPT and CORIN protein levels were reduced in E9 implantation sites of *Bmpr2* cKO female mice. (**D**) Secondary to vascular defects, ANGPT-positive endothelial cells were significantly decreased in E9 decidual tissues from *Bmpr2* cKO female mice. Scale bar: 100 µm.



Supplemental Figure 10.

Immunofluorescent and molecular analysis of uNK cells in uterine decidua of control and *Bmpr2* cKO females. (**A** and **B**) Using DBA, uNK cells were abundant in the deciduum of pregnant control uteri (**A**) and artificially decidualized uteri (**B**) but were rare in cKO decidua (**A** and **B**). Scale bar: 200 μm (10X panels); 50 μm (40X panels). (**C**) mRNA levels of *Gzms* are significantly decreased in E9 decidual tissues from *Bmpr2* cKO female mice (***$P < 0.001$). Data are means ± SEM.

Supplemental Table 1. Serum hormone levels of 4-month-old control and *Bmpr2* cKO female mice

Mouse genotype	Mean hormone levels ± SEM (*n*)			
	FSH (ng/ml)	LH (ng/ml)	E_2 (pg/ml)	P_4 (ng/ml)
Bmpr2$^{flox/-}$	7.40 ± 1.46 (12)	0.33 ± 0.05 (6)	27.78 ± 2.48 (12)	7.78 ± 1.34 (10)
Bmpr2$^{flox/-}$*Pgr*$^{cre/+}$	6.96 ± 0.90 (11)	0.31 ± 0.07 (8)	24.64 ± 3.25 (13)	7.73 ± 1.34 (13)

Supplemental Table 2. Primer sequence information

Primer sequences for genotyping PCR

Gene name	Primer name	Primer sequences
Bmpr2	Prime A	CACACCAGCCTTATACTCTAGATAC
	Prime c	TTATTGTAAGTACACTGTTGCTGTC
	Prime 6R	CACATATCTGTTATGAAACTTGAG
Gene name	**Forward primers (5'-3')**	**Reverse primer (5'-3')**
Pgr-cre	TATACCGATCTCCCTGGACG	ATGTTTAGCTGGCCCAAATG

Primer sequences for real-time quantitative PCR

Target gene	GeneBank accession No.	Forward primers (5'-3')	Reverse primer (5'-3')
Casp3	NM_009810.2	CGATCTGGTACAGACGTG	GCCATGTCATCCTCA
Ptgs2	NM_011198.3	TCCATTGACCAGAGCAGAGA	TCTGGACGAGGTTTTTCCAC
Lif	NM_008501.2	AAAAGCTATGTGCGCCTAACA	GTATGCGACCATCCGATACAG
Hoxa10	NM_008263.3	GCCCCTTCAGAAAACAGTAAAG	AGGTGGACGCTACGGCTGATCTCTA
Areg	NM_009704.3	GGGGACTACGACTACTCAGAG	TCTTGGGCTTAATCACCTGTTC
Wnt4	NM_009523.2	GAGAAGTGTGGCTGTGACCGG	ATGTTGTCCGAGCATCCTGACC
Wnt6	NM_009526.3	TGCCCGAGGCGCAAGACTG	ATTGCAAACACGAAAGCTGTCTCTC
Fkbp4	NM_010219.3	Taqman Assay: Mm00487391_m1*	
Fkbp5	NM_010220.3	Taqman Assay: Mm00487401_m1*	
Prl3d1	NM_001205322.1	GTCTTGAGGTGCCGAGTTGTC	CTGGGTGGGCACTCAACATT
Mmp9	NM_013599.2	CCATGCACTGGGCTTAGATCA	GGCCTTGGGTCAGGCTTAGA
Esr1	NM_007956.4	GCTCCTAACTTGCTCCTGGAC	CAGCAACATGTCAAAGATCTCC
Pgr	NM_008829.2	GCTTGCATGATCTTGTGAAACAGC	GGAAATTCCACAGCCAGTGTCC
Cnr1	NM_007726.3	TGCAGGCCTTCCTACCACTT	TGTGCAGGCAGTCTGAGTCC
Faah	NM_010173.4	AGGATTTGTTCCGCTTGG	CTGCTTGGTCTCCATCACAG
Tpbpa	NM_009411.4	GCCAGTTGTTGATGACCCTGA	CCCATCGCCACTCTCTGTGT
Prl3c1	NM_013766.2	Taqman Assay: Mm00479148_m1*	
Prl8a2	NM_010088.1	Taqman Assay: Mm01135453_m1*	
Ccnd1	NM_007631.2	CATCAAGTGTGACCCGGACTG	CCTCCTCCTCAGTGGCCTTG
Ccnd2	NM_009829.3	GAGTGGGAACTGGTAGTGTTG	CGCACAGAGCGATGAAGGT
Ccnd3	NM_007632.2	CCAGCGTGTCCTGCAGAGTT	CCTTTTGCACGCACTGGAAG
Ccne1	NM_007633.2	TGTTACAGATGGCGCTTGCTC	TTCAGCCAGGACACAATGGTC
Ccne2	NM_001037134.1	TGCTGCCGCCTTATGTCATT	TCCGAGATGTCATCCCATTCC
Cdk2	NM_183417.3	CTGCCATTCTCACCGTGTCC	AGCTTGATGGACCCCTCTGC
Cdk4	NM_009870.3	CCCAATGTTGTACGGCTGA	GGAGGTGCTTTGTCCAGGTA
Cdk6	NM_009873.2	GCTTCGTGGCTCTGAAGCGCG	TGGTTTCTGTGGGTACGCCGG
Cdkn1a	NM_007669.4	CCAGGCCAAGATGGTGTCTT	TGAGAAAGGATCAGCCATTGC
Cdkn2b	NM_007670.4	CCCTGCCACCCTTACCAGA	CAGATACCTCGCAATGTCACG
Vegfa	NM_001025250.3	CACGACAGAAGGAGAGCAGAAG	CTCAATCGGACGGCAGTAGC
Vegfb	NM_011697.3	GAAGAAAGTGGTGCCATGGATAG	CCCATGAGTTCCATGCTCAGA
Vegfc	NM_009506.2	GAGGTCAAGGCTTTTGAAGGC	CTGTCCTGGTATTGAGGGTGG
Prf1	NM_011073.3	TCTCCTCCTATGGCACGCAC	TGTAAGGACCGAGATGCGG
Klrg1	NM_016970.1	TTTGGGGCTTTTGACTGTGAT	TGTAAGGAGATGTGAGCCTTTGT
Il15	NM_008357.2	ACATCCATCTCGTGCTACTTGT	GCCTCTGTTTTAGGGAGACCT
Il15ra	NM_008358.1	Taqman Assay: Mm00500457_m1	
Gzma	NM_010370.2	AGACCGTATATGGCTCTACT	CCCTCACGTGTATATTCATC
Gzmb	NM_013542.2	ATCAAGGATCAGCAGCCTGA	TGATGTCATTGGAGAATGTCT
Gzmd	NM_010372.2	AAACAGCTCTGTCCAAAGCTC	CAAATCTCTGTGGTCTCAGTG
Gzme	NM_010373.3	CTGCTCACTGCAGGAACAGGA	CAAATCTCTGTGGTCTCAGTG
Gzmf	NM_010374.3	TGAGGTTTGTGAAAGATAATG	TCACTGGTGTTGTCCTTATC
Gzmg	NM_010375.2	CATTCCCCATCCAGCTTTTA	GATCTGCGTGGTCTTGGAAT
Ankrd37	NM_001039562.1	Taqman Assay: Mm01200803_m1*	
Egln1	NM_053207.2	GCCCAGTTTGCTGACATTGAAC	CCCTCACACCTTTCTCACCTGTTAG
Hif1a	NM_010431	ACCTTCATCGGAAACTCCAAAG	CTGTTAGGCTGGGAAAAGTTAGG
Hif2a	NM_010137	CTGAGGAAGGAGAAATCCCGT	TGTGTCCGAAGGAAGCTGATG
Angpt1	NM_009640.3	GGGGGAGGTTGGACAGTAA	CATCAGCTCAATCCTCAGC
Angpt2	NM_007426.3	GATCTTCCTCCAGCCCCTAC	TTTGTGCTGCTGTCTGGTTC
Angpt4	NM_009641.1	CAGCCAGCTATGCTACTAGATGG	CCTCTGGAGGCTATTGGAGC
Tie1	NM_011587.2	CAAGGTCACACACACGGTGAA	GCCAGTCTAGGGTATTGAAGTAGG
Tek	NM_013690.2	TGGAGTCAGCTTGCTCCTTT	ACCTCCAGTGGATCTTGGTG
Corin	NM_016869.3	GCTGGTGACTGCTAACTTGCT	CCCATCAGTGACCAAAGGTTC
Gapdh	NM_008084.2	CAATGTGTCCGTCGTGGATCT	GCCTGCTTCACCACCTTCTT
Gapdh	NM_008084.2	Taqman Assay: Mm03302249_g1	

Supplemental Table 3. Antibody information

Antibodies for immunohistochemistry

Antibody name	Manufacturer (city & state or country)	Product No.	Species
PRL3D1	Santa Cruz Biotechnology (Santa Cruz, California)	sc-34713	Goat
MMP9	R&D Systems (Minneapolis, Minnesota)	AF909	Goat
KRT8	Abcam (Cambridge, United Kingdom)	ab59400	Rabbit
PTGS2	Thermo Fisher Scientific (Waltham, Massachusetts)	RB-9072	Rabbit
laminin	Sigma-Aldrich (St. Louis, Missouri)	L9393	Rabbit
MKI67	BD Pharmingen (San Diego, California)	550609	Mouse
CCND3	Thermo Fisher Scientific (Waltham, Massachusetts)	MS-215	Mouse
PECAM1	BD Pharmingen (San Diego, California)	550274	Rat
ANGPT1	Millipore (Billerica, Massachusetts)	AB3120	Rabbit
ANGPT2	Millipore (Billerica, Massachusetts)	AB3121	Rabbit
ANGPT4	Santa Cruz Biotechnology (Santa Cruz, California)	sc-9355	Goat
3ß-HSD	Santa Cruz Biotechnology (Santa Cruz, California)	sc-30820	Goat

Antibodies for immunofluorescence

Antibody name	Manufacturer (city & state or country)	Product No.	Species
BMPR2	Santa Cruz Biotechnology (Santa Cruz, California)	SC-5682	Goat
ACTA2	Abcam (Cambridge, United Kingdom)	ab5694	Rabbit
CD45	BD Pharmingen (San Diego, California)	550539	Rat
ACTB	Santa Cruz Biotechnology (Santa Cruz, California)	sc-1616	Goat

Antibodies for Western blot

Antibody name	Manufacturer (city & state or country)	Product No.	Species
BMPR2	Santa Cruz Biotechnology (Santa Cruz, California)	SC-5683	Goat
CCND3	Thermo Fisher Scientific (Waltham, Massachusetts)	MS-215	Mouse
pAKT	Cell Signaling Technology (Danvers, Massachusetts)	#4060	Rabbit
tAKT	Cell Signaling Technology (Danvers, Massachusetts)	#4691	Rabbit
ANGPT1	Millipore (Billerica, Massachusetts)	AB3120	Rabbit
ANGPT2	Millipore (Billerica, Massachusetts)	AB3121	Rabbit
ANGPT4	Santa Cruz Biotechnology (Santa Cruz, California)	sc-9355	Goat
3ß-HSD	Santa Cruz Biotechnology (Santa Cruz, California)	sc-30820	Goat
ACTB	Sigma-Aldrich (St. Louis, Missouri)	A3853	Mouse