Supplementary Information for

Krüppel-like factor 17 upregulates uterine corin expression and promotes spiral artery remodeling in pregnancy

This PDF file includes:

Figures S1 to S9

Table S1

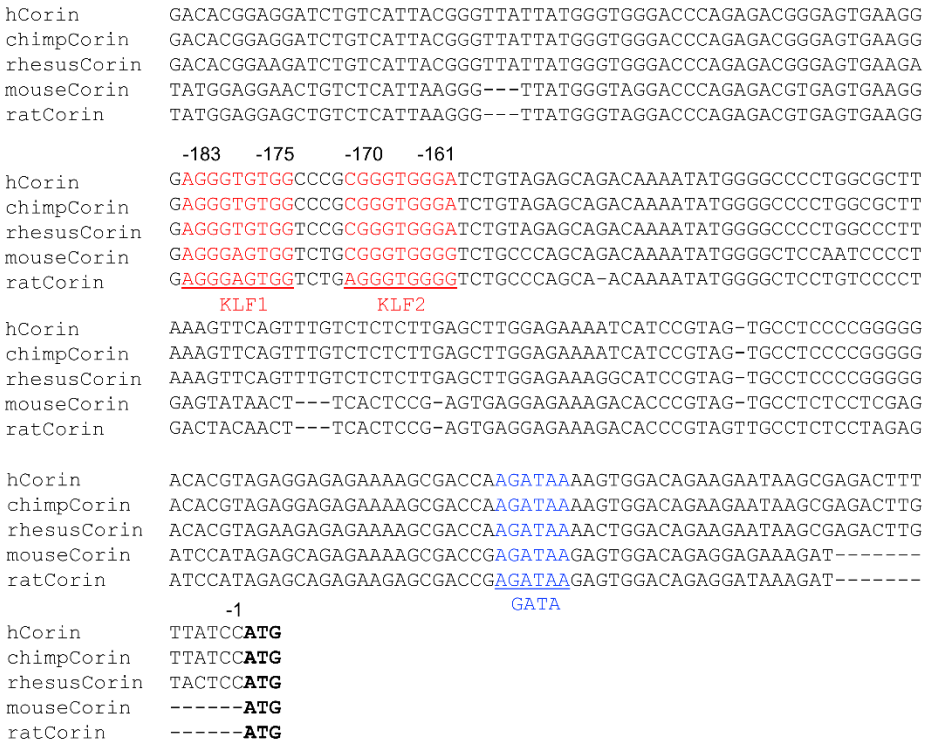

Fig. S1. Conserved KLF- and GATA-binding sites in the *CORIN* promoter. Alignment of the human *CORIN* promoter sequence containing two KLF-binding sites (red) and a GATA-binding site (blue) with 5'-upstream sequences from chimpanzee (chimp), rhesus monkey (rhesus), mouse and rat *Corin* genes. The nucleotide numbering is relative to the translation initiation codon ATG (boldface). The E-box-binding site, which is located further 5'-upstream to the KLF-binding sites, is not included.

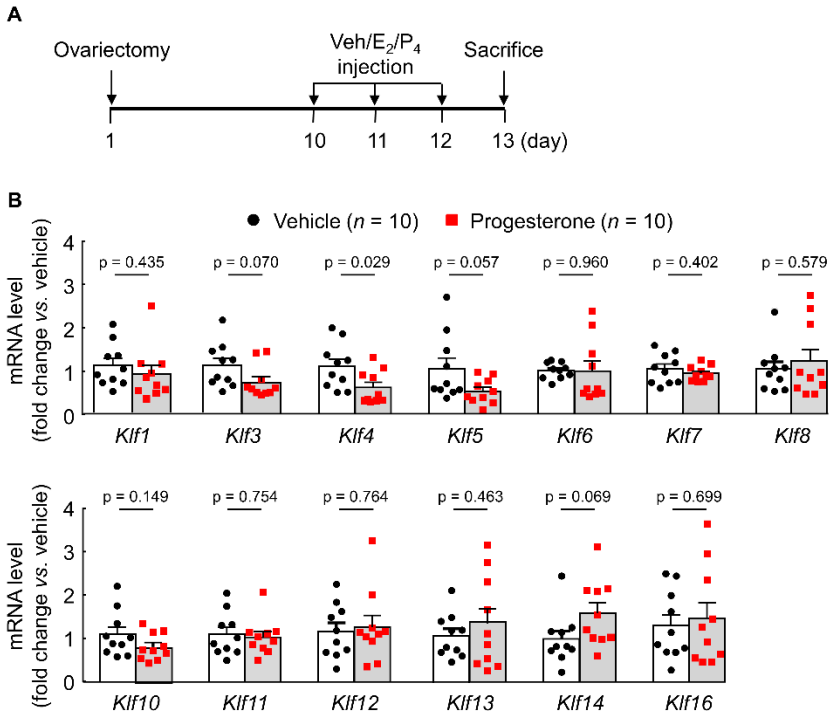

Fig. S2. Uterine expression of *Klf* genes in ovariectomized mice treated with progesterone. (*A*) Schematic illustration of the experiment. Nine days after post-op recovery from ovariectomy, C57BL/6 mice were injected with vehicle (Vec), estrogen (E_2) or progesterone (P_4) (*s.c.,* daily for three days). Uteruses were isolated for *Corin* and *Klf* expression analysis. (*B*) qRT-PCR analysis of mRNA levels for the indicated *Klf* genes. *n* = 10 per group. Data are presented as mean ± SEM; *P* values were analyzed by two-tailed Student's *t* test. ns, not significant.

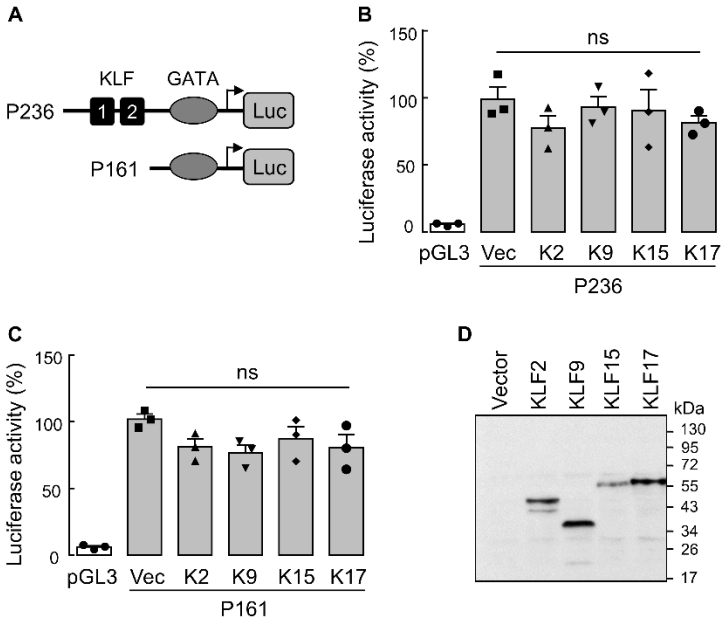

Fig. S3. Effects of recombinant KLF protein expression on *CORIN* promoter activities in HL-1 cardiomyocytes. (*A*) Illustration of *CORIN* promoter constructs used. (*B* and *C*) Luciferase activities in HL-1 cells transfected with pGL3 plasmid (negative control) or co-transfected with the P236 (*B*) or P161 (*C*) *CORIN* promoter construct and a control pCMV vector (Vec) or plasmids expressing human KLF2 (K2), KLF9 (K9), KLF15 (K15) or KLF17 (K17). *n* = 3 per group. Data are presented as mean ± SEM; ns, not significant, as analyzed by ANOVA. (*D*) Western blotting of recombinant KLF proteins in transfected HL-1 cells using an anti-FLAG antibody. Data are representative of three experiments.

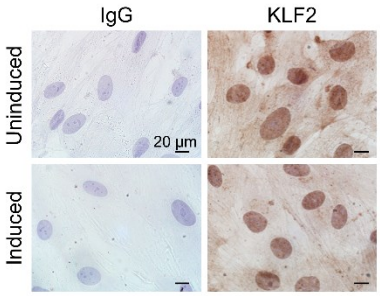

Fig. S4. Immunohistochemistry of endogenous KLF2 proteins in control (uninduced) and decidualized (induced) HESCs. The experiment was done using an anti-KLF2 antibody with normal IgG as a negative control. Similar nuclear staining levels of KLF2 were found in uninduced and induced HESCs. Data are representative of three independent experiments.

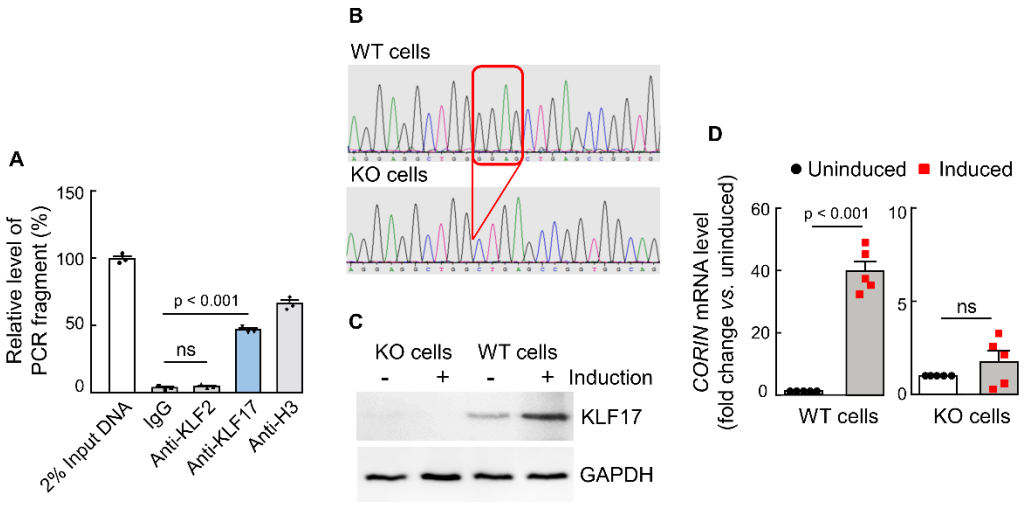

Fig. S5. Additional data from ChIP analysis in HESCs and *KLF17*-KO HESCs. (*A*) ChIP analysis was done in HESCs, as shown in Fig. 5. qPCR was used to quantify levels of PCR products amplified from chromatin fragments that were pulled down with antibodies against KLF2, KLF17 and histone 3 (H3) (positive control) or IgG (negative control). As another positive control, 2% input DNA fragments were used as templates in the experiment. n = 3 per group. Data are presented as mean ± SEM; ns, not significant; $P < 0.001$, as analyzed by ANOVA. (*B*) The *KLF17* gene was disrupted in a second line of HESCs using CRISPR/Cas9. Partial sequencing data from WT and *KLF17*-KO HESCs are shown. Red box indicates the deleted sequence. (*C*) Western blotting of endogenous KLF17 protein in WT and *KLF17*-KO HESCs without (-) or with (+) decidualization (induction). GAPDH was a control. Data are representative of three independent experiments. (*D*) *CORIN* mRNA levels in WT and *KLF17*-KO HESCs without (uninduced) or with (induced) decidualization. n = 5 per group. Data are presented as mean ± SEM; ns, not significant; $P < 0.001$, as analyzed by two-tailed Student's t test.

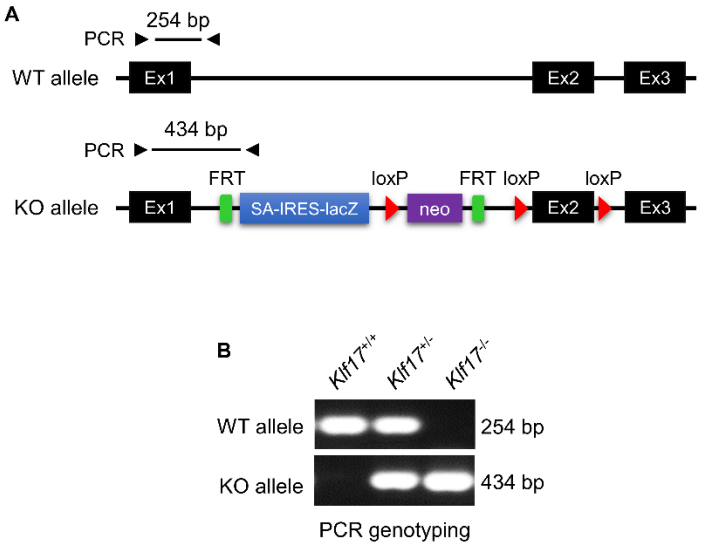

Fig. S6. Generation of *Klf17*$^{-/-}$ mice. (*A*) Illustration of WT and knockout (KO) *Klf17* alleles. Locations of PCR primers used for genotyping the WT and KO alleles are indicated. (*B*) PCR products from *Klf17*$^{+/+}$, *Klf17*$^{+/-}$ *and Klf17*$^{-/-}$ mice with WT (254-bp fragment) and KO (434-bp fragment) alleles.

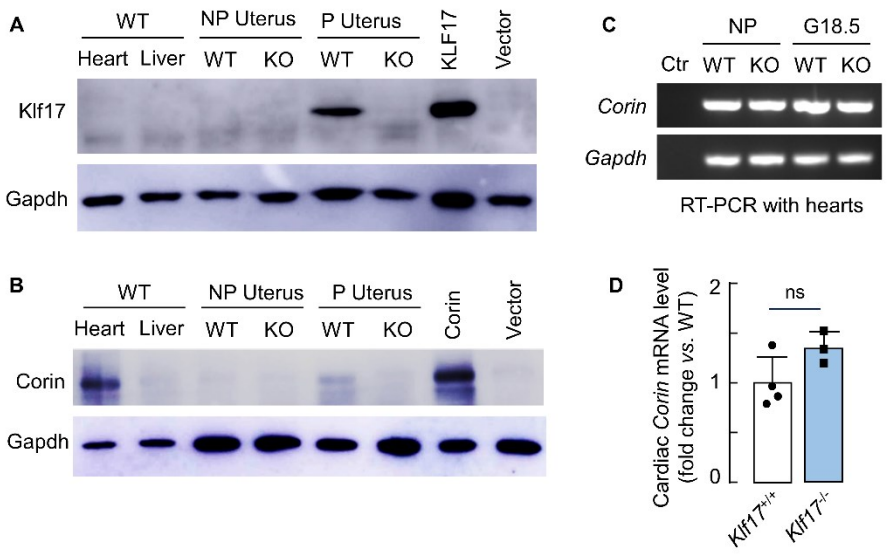

Fig. S7. Analysis of Klf17 and corin expression in *Klf17*$^{+/+}$ (WT) and *Klf17*$^{-/-}$ (KO) mice. (*A* and *B*) Uterine Klf17 (*A*) and corin (*B*) expression in non-pregnant (NP) and pregnant (P) (G12.5) WT and KO mice was analyzed by western blotting. Samples from hearts and livers were used as controls. Recombinant KLF17 and corin expressed in transfected HEK293 cells were used as additional controls. GAPDH was a protein loading control. Data are representative of three experiments. (*C*) Cardiac *Corin* expression was analyzed by RT-PCR with hearts from NP and P (G18.5) WT and KO mice. *Gapdh* was a positive control. Samples, in which cDNA templates were omitted, were used as a negative control (Ctr). (*D*) qRT-PCR was done to verify *Corin* mRNA levels in hearts from NP *Klf17*$^{+/+}$ and *Klf17*$^{-/-}$ mice. *n* = 3-4 per group. Data are presented as mean ± SEM; ns, not significant, as analyzed by two-tailed Student's *t* test.

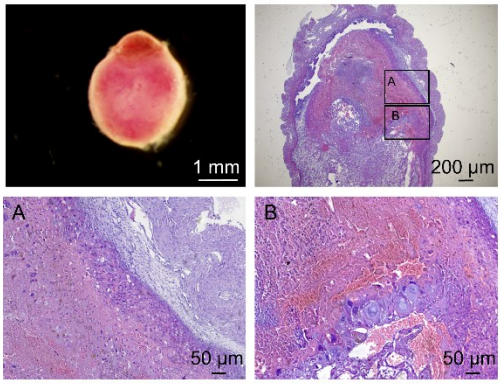

Fig. S8. Histological analysis of an abnormal embryo from *Klf17*$^{-/-}$ mice. *Klf17*$^{-/-}$ female and male mice were mated. At G12.5 day, embryos were isolated. Top left panel shows an abnormal embryo. Histological analysis was done in H&E-stained tissue sections, revealing tissue necrosis, as shown in an image with low magnification (x 25) (top right panel) and higher magnification (x 100) (bottom two panels).

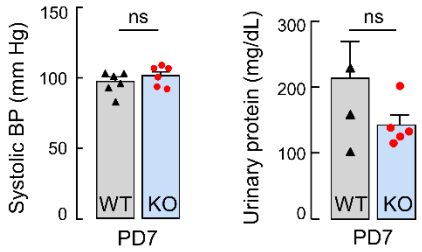

Fig. S9. Blood pressure and urinary protein levels in postpartum *Klf17*$^{+/+}$ (WT) and *Klf17*$^{-/-}$ (KO) mice. Systolic blood pressure (BP) (left) and urinary protein levels (right) were measured in WT and KO mice on postpartum day (PD) 7. n = 3-6 per group. Data are presented as mean ± SEM; *P* values were analyzed by two-tailed Student's *t* test; ns, not significant.

Table S1. Primers used in RT-PCR and qRT-PCR

Gene	Locus	Primer	Sequence	Size (bp)
Corin	NM_016869.3	forward	GGTCCGCATTATCCCTCTGG	231
		reverse	ACACTCCAGGTCCCAGAACT	
Corin	NM_016869.3	forward	CAAGTCTGAGGTCAACTGC	64
		reverse	TGTCCACTTCTGCATTCCAC	
Klf1	NM_010635.3	forward	CAAGAGCTCGCACCTCAAG	92
		reverse	GAGCGAACCTCCAGTCACA	
Klf2	NM_008452.2	forward	GGATCTTTGGAGGAATCACG	61
		reverse	TGTCCCCTCAGGACCTACC	
Klf3	NM_008453.5	forward	TCGCACTTGAAAGCACACA	52
		reverse	TCCCAGGTGCATTTGTACG	
Klf4	NM_010637.3	forward	GCTCCTCTACAGCCGAGAATC	60
		reverse	ATGTCCGCCAGGTTGAAG	
Klf5	NM_009769.4	forward	CCGGAGACGATCTGAAACAC	116
		reverse	CAGATACTTCTCCATTTCACATCTTG	
Klf6	NM_011803.2	forward	ACGAAAAGCTCCCACTTGAA	78
		reverse	ACAACCTTCCCATGAGCATC	
Klf7	NM_033563.2	forward	CCGGCTACTTCTCAGCTTTG	72
		reverse	GGTAGCGTTCCAACTCAAGG	
Klf8	NM_173780.5	forward	GGGCTGCAAGGAAAGGAT	97
		reverse	GTATCTGACTTTCCAAGCCACTG	
Klf9	NM_010638.5	forward	CTCAGAACTGCTTTTAACATTAGGG	61
		reverse	AACACTTTCCTTTTTAGCTCGTG	
Klf10	NM_013692.3	forward	AGCCAACCATGCTCAACTTC	76
		reverse	GGCTTTTCAGAAATTAGTTCCATT	
Klf11	NM_178357.3	forward	CTTGCCACAGAACACCTTCC	65
		reverse	TATTTCCAATGGCCATGACAC	
Klf12	NM_010636.3	forward	CCTTAGATAGCGTTAATGAAACTGG	76
		reverse	GGGGATGGATGTACCTCTTGTA	
Klf13	NM_021366.3	forward	CTGGGCACATTGTACTGGAC	60
		reverse	GGAAGCCGGATAAAACAACA	
Klf14	NM_001135093.1	forward	TGATCGAGTACCGAGGTCGT	86
		reverse	GCTGGAGTCGGAACCAGAG	
Klf15	NM_001355668.1	forward	TGTGGGCCAGAAGTTTCC	62
		reverse	AAGTGCATTTGTGCATTTTGAG	
Klf16	NM_078477.2	forward	CACCTGCGGACTCACACA	76
		reverse	CAGAACGGGCGAACTTCTT	
Klf17	NM_029416.2	forward	GCAGCCATTCTGACACGTTA	77
		reverse	GACTTCTGCTATCCCTAACCTCAG	
Klf17	NM_029416.2	forward	GGCGTAGTAGTGTTGGGACC	199
		reverse	TGGCTGATGAAATCCGCTGT	

Gene	Locus	Primer	Sequence	Size (bp)
Gapdh	NM_001289726.1	forward	TGTTCCTACCCCCAATGTGT	138
		reverse	GGTCCTCAGTGTAGCCCAAG	
CORIN	NM_006587.4	forward	GCAAGCAGATGGGTTTAGGA	92
		reverse	CCAGTTGGAGTGTAATGTCAGC	
PRL	NM_000948.6	forward	CAAAGGATCGCCATGGAA	60
		reverse	CACAGGAGCAGGTTTGACAC	
GAPDH	NM_001256799.3	forward	GGTCTCCTCTGACTTCAACA	116
		reverse	AGCCAAATTCGTTGTCATAC	