Supplementary Information for

Myometrial contraction-activated endometrial PIEZO2 safeguards fertility under maternal stress

Jin-Wen Kang[1,2]†, Yu Zhang[1]†, Hui-Xia Li[1], He Zou[1], Jing-Yi Yang[1], Xiao-Qing Huang[1], Jia-Ying Yu[1], Zi-Wei Ai[3], Ying Zhang[3], Rui Zhang[1], Ying-Nan Liu[1], Zeng-Ming Yang[4]*, Bin Guo[5]*, Hong-Lu Diao[3]*, Ren-Wei Su[1]*

Affiliations:

[1]College of Veterinary Medicine, South China Agricultural University, Guangzhou, China

[2]Reproductive Sciences Center, Division of Developmental Biology, Cincinnati Children's Hospital Medical Center, Cincinnati, OH 45229, USA

[3]Reproductive Medicine Center of Renmin Hospital, Biomedical Engineering College, Hubei University of Medicine, Shiyan, Hubei, China

[4]College of Animal Science, Guizhou University, Guiyang, China

[5]College of Veterinary Medicine, Jilin University, Changchun, China

†These authors contributed equally to this work

***Correspondence**

Ren-Wei Su, 483 Wushan Road, Guangzhou, Guangdong, China, 510642

Email: renweisu@scau.edu.cn

Hong-Lu Diao, PhD.

39 Chaoyang Middle Road, Shiyan, Hubei, China, 442000

Email: hldiao@hbmu.edu.cn

Bin Guo, PhD.

5333 Xi`an Road, Changchun, Jilin, China, 130062

Email: guobin79@jlu.edu.cn

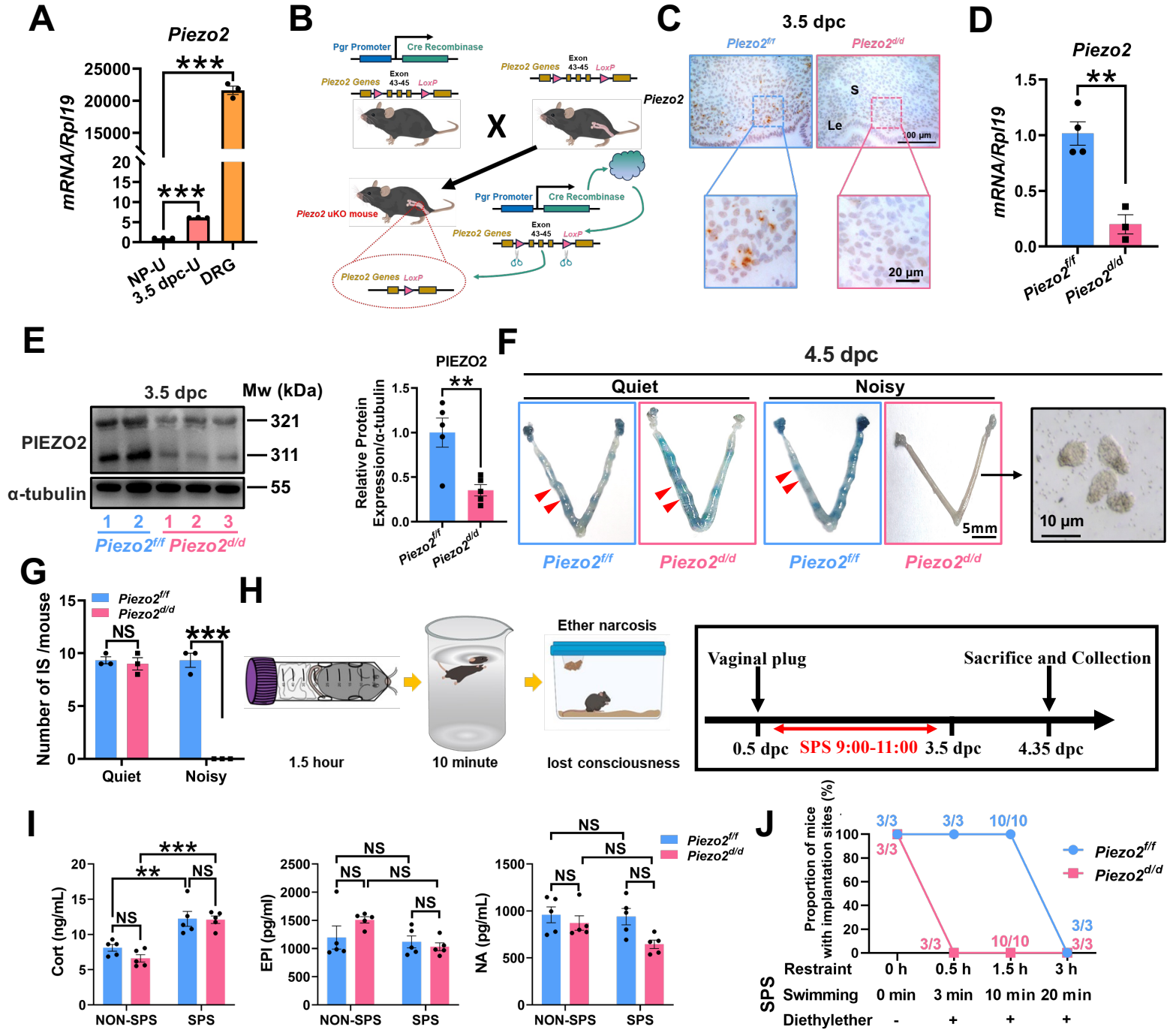
Zeng-Ming Yang, PhD.

2708 Huaxi District, Guiyang, Guizhou, China, 550025

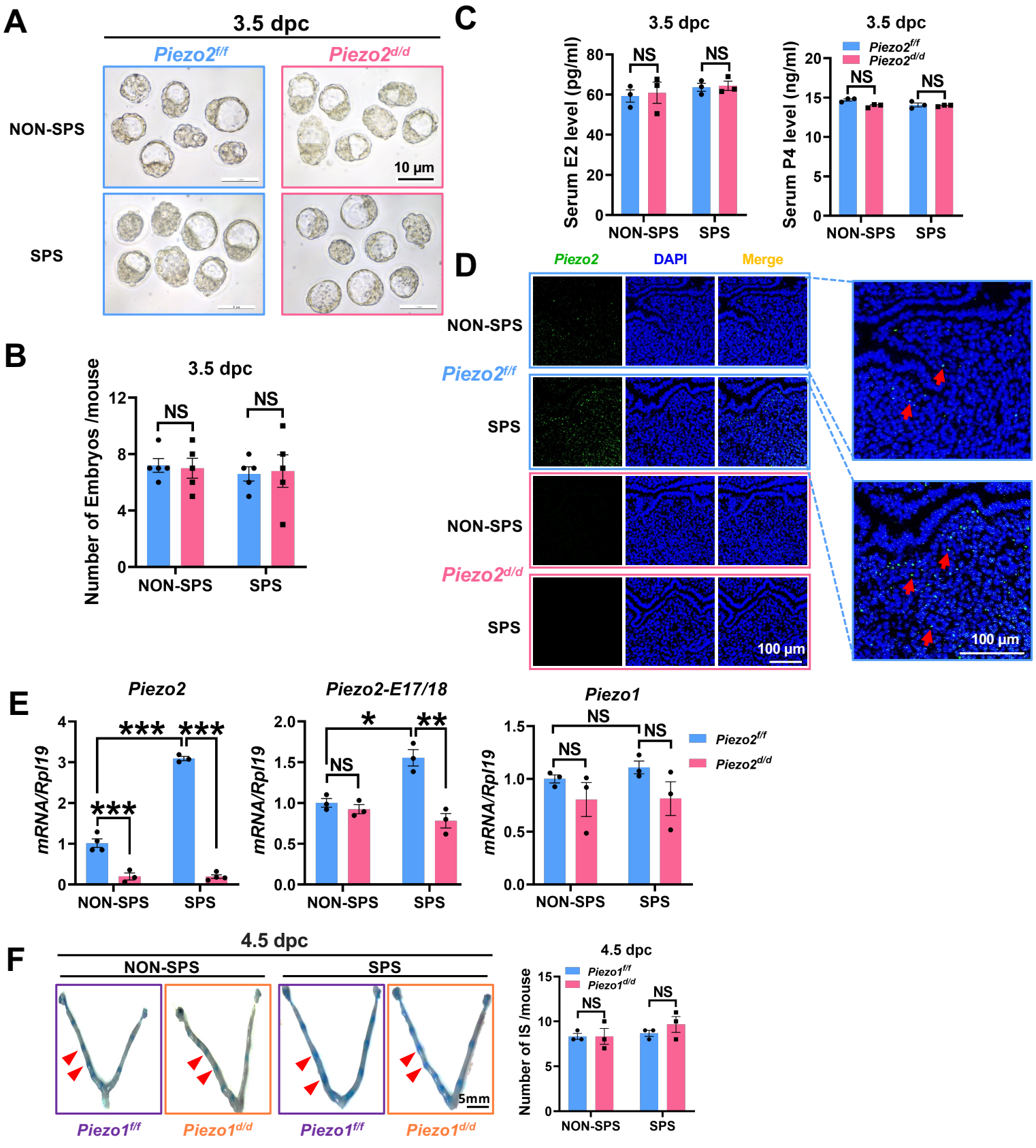
Email: yangzm@gzu.edu.cn

The file includes:

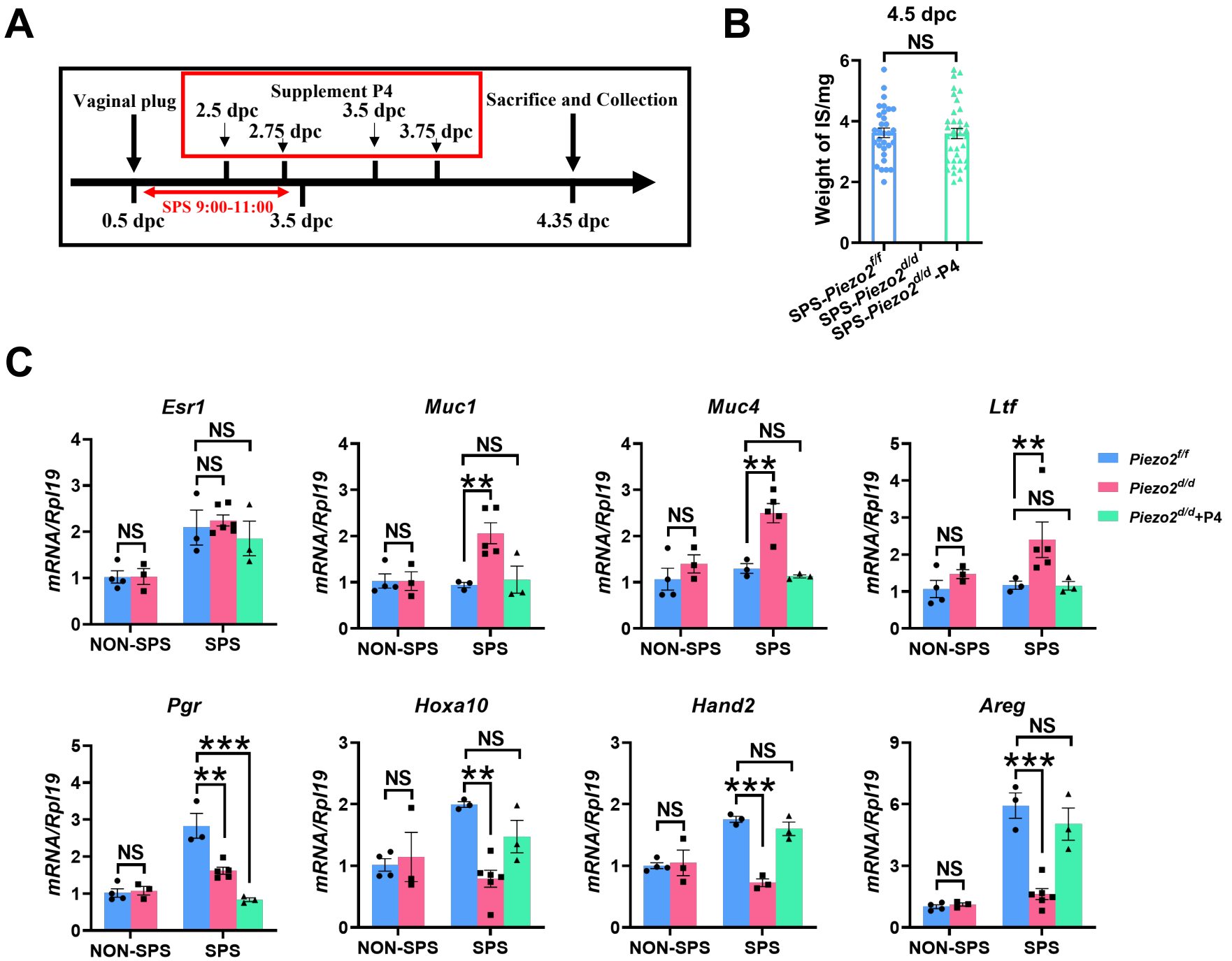
Materials and Methods
Extended Data Figs. 1 to 7
Extended Data Tables 1 to 4


A
Piezo2
mRNA/Rpl19
NP-U
3.5 dpc-U
DRG
B
Pgr Promoter
Cre Recombinase
Piezo2 Genes
Exon 43-45
LoxP
Piezo2
Piezo2 uKO mouse
C
3.5 dpc
Piezo2f/f
Piezo2d/d
S
Le
100 μm
20 μm
D
Piezo2
mRNA/Rpl19
E
3.5 dpc
Mw (kDa)
PIEZO2
α-tubulin
321
311
55
Relative Protein Expression/α-tubulin
F
4.5 dpc
Quiet
Noisy
5mm
10 μm
G
Number of IS /mouse
NS
Quiet
Noisy
H
1.5 hour
10 minute
Ether narcosis
lost consciousness
Vaginal plug
Sacrifice and Collection
0.5 dpc
SPS 9:00-11:00
3.5 dpc
4.35 dpc
I
Cort (ng/mL)
EPI (pg/ml)
NA (pg/mL)
NON-SPS
SPS
J
Proportion of mice with implantation sites (%)
Restraint
Swimming
Diethylether
SPS
0 h
0.5 h
1.5 h
3 h
0 min
3 min
10 min
20 min

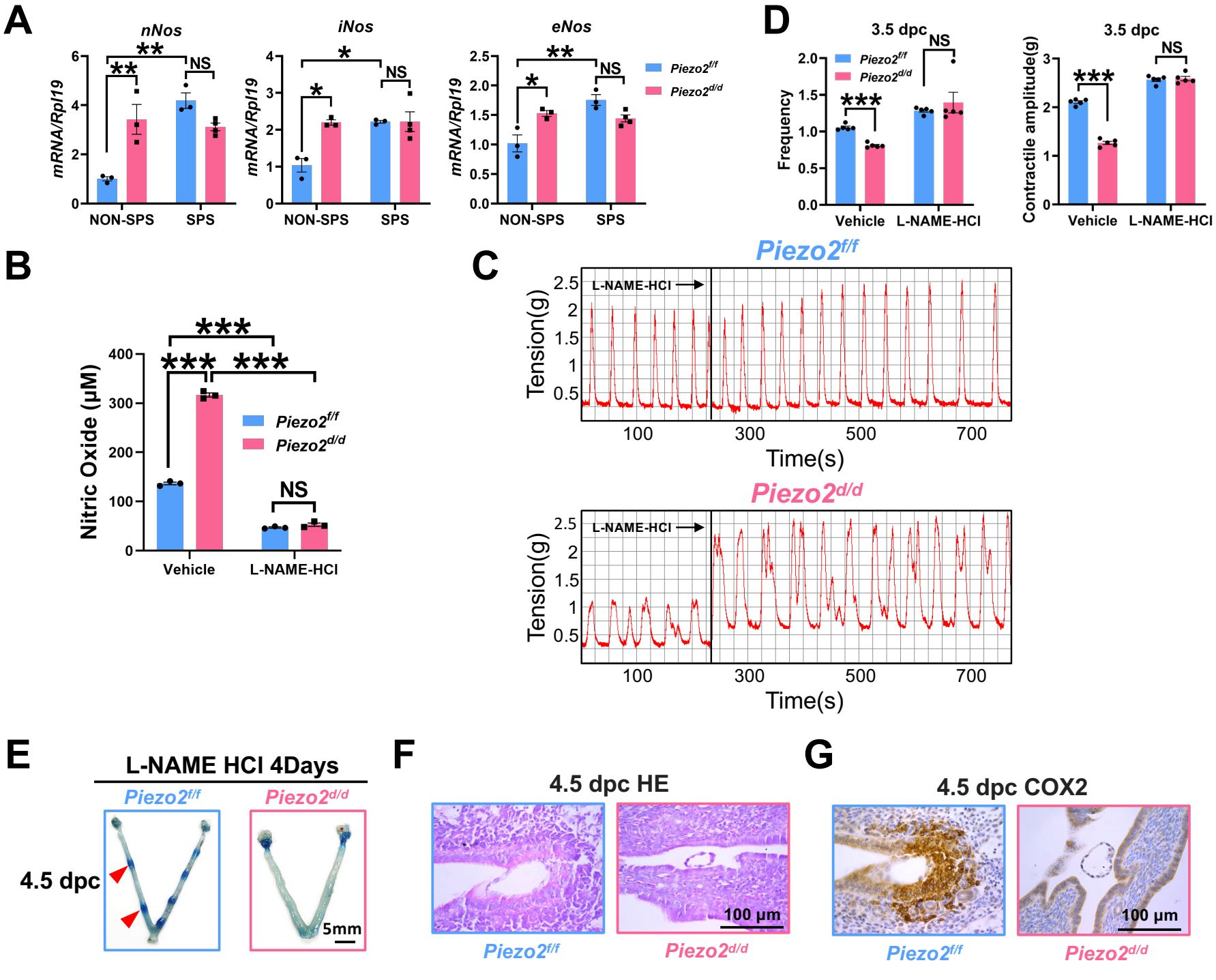
Extended data Fig. 1. Generation and characterization of uterine-specific *Piezo2* conditional knockout mice. (A) *Piezo2* mRNA expression in non-pregnant uterus (NP-U), uterus at 3.5 dpc of pregnancy (D4-U), and dorsal root ganglion (DRG), quantified by RT-qPCR and normalized to Rpl19, n = 3 biological replicates. One-way ANOVA with Tukey's post hoc test. (**B**) Schematic of the conditional *Piezo2* knockout strategy. *Piezo2*$^{f/f}$ mice carrying loxP sites flanking exons 43–45 were crossed with *Pgr*$^{Cre/+}$ transgenic mice to generate uterine-specific *Piezo2* conditional knockout (*Piezo2*$^{d/d}$) females. (**C**) Immunohistochemistry detecting PIEZO2 in uterine cross-sections at 3.5 dpc from *Piezo2*$^{f/f}$ and *Piezo2*$^{d/d}$ mice, confirming the loss of PIEZO2 protein expression in the endometrial stroma of knockout mice. S, stroma; LE, luminal epithelium. Scale bar, 100 μm and 20 μm. (**D**) Quantitative RT-PCR confirming efficient *Piezo2* deletion in *Piezo2*$^{d/d}$ uteri, with *Rpl19* serving as an internal control. Statistical significance determined using a two-sided Student's t-test. (**E**) Western blot analysis of PIEZO2 expression in uterine tissues at 3.5 dpc in *Piezo2*$^{d/d}$ and *Piezo2*$^{f/f}$ (n = 5 each) mice. Expression was normalized to α-tubulin with the quantification shown to the right (two-sided Student's t-test). (**F**) Representative uterine images at 4.5 dpc from *Piezo2*$^{f/f}$ and *Piezo2*$^{d/d}$ females housed under quiet (45 ± 5 dB) or noisy (65 ± 5 dB) acoustic conditions. Red arrowheads indicate implantation sites visualized by Chicago Sky Blue dye injection. Morphologically normal blastocysts were recovered from the uterine lumen of *Piezo2*$^{d/d}$ mice under noisy conditions (right panel). Scale bars, 5 mm and 10 μm. (**G**) Quantification of implantation site number per mouse under quiet and noisy conditions. *Piezo2*$^{d/d}$ mice displayed normal implantation under quiet conditions but complete implantation failure under noisy conditions (two-way ANOVA with Tukey's post hoc test). (**H**) Schematic of the modified Single Prolonged Stress (SPS) protocol. SPS consists of sequential restraint stress (1.5 h), forced swim (10 min), and diethyl ether exposure until loss of consciousness, with this protocol administered daily between 0.5 and 3.5 dpc (9:00–11:00 AM). (**I**) Serum levels of corticosterone (Cort), epinephrine (EPI), and noradrenaline (NA) in *Piezo2*$^{f/f}$ and *Piezo2*$^{d/d}$ mice with or without SPS treatment, confirming successful stress induction. SPS significantly elevated corticosterone in both genotypes (n=5, two-way ANOVA with Tukey's post hoc test). (**J**) Dose–response analysis of stress sensitivity. Mice were subjected to various intensities of SPS by adjusting the exposure durations. *Piezo2*$^{d/d}$*Piezo2*-cKO mice displayed implantation failure at lower stress intensities than controls. Fractions indicate number of mice with implantation sites out of the total. Quantitative data are presented as mean ± SEM. ns, $P > 0.05$; *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.


A
3.5 dpc
Piezo2f/f
Piezo2d/d
NON-SPS
SPS
10 μm
B
3.5 dpc
Number of Embryos /mouse
NS
NON-SPS
SPS
C
3.5 dpc
Serum E2 level (pg/ml)
Serum P4 level (ng/ml)
Piezo2f/f
Piezo2d/d
NS
D
Piezo2
DAPI
Merge
100 μm
E
Piezo2
Piezo2-E17/18
Piezo1
mRNA/Rpl19
F
4.5 dpc
Piezo1f/f
Piezo1d/d
5mm
Number of IS /mouse

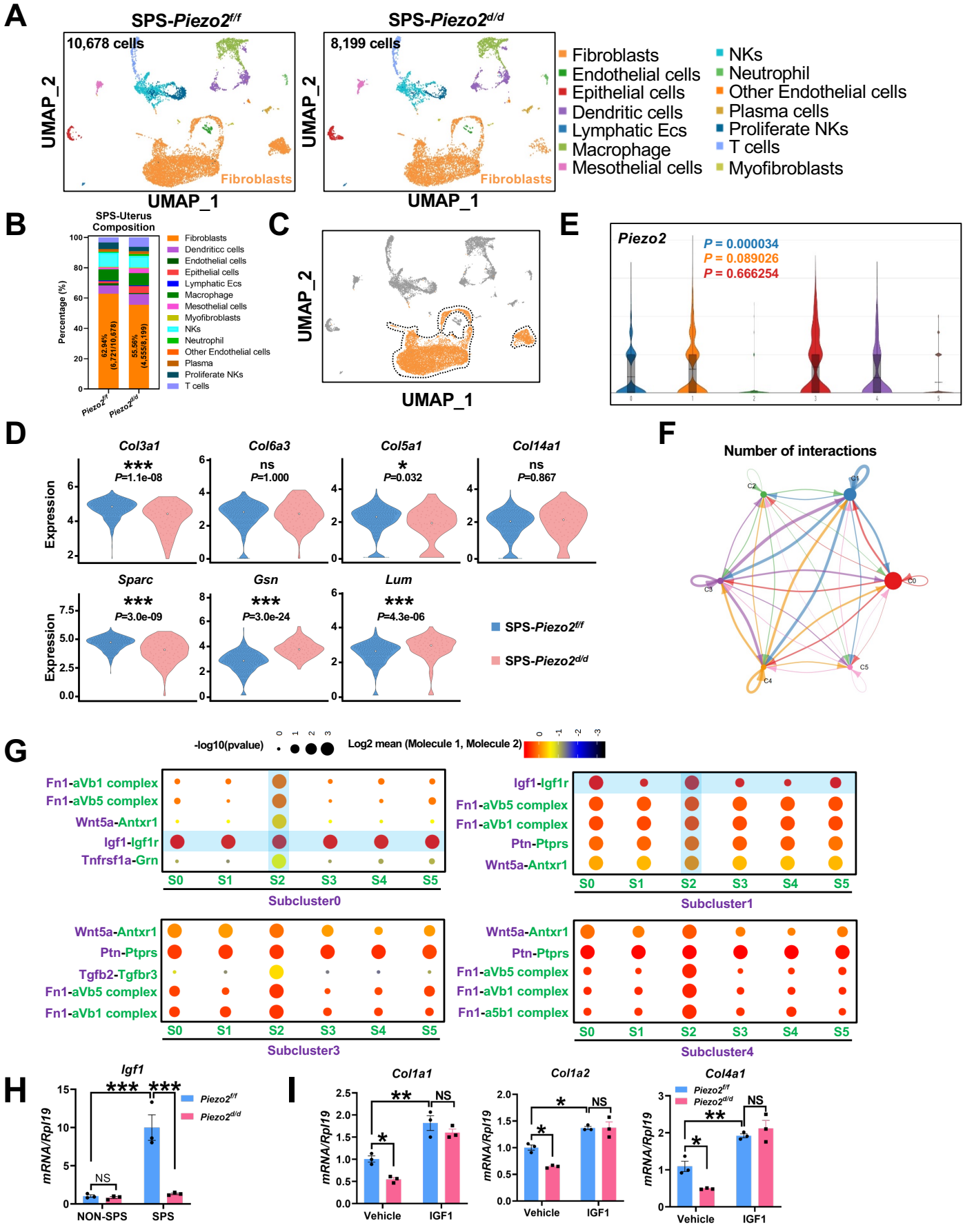
Extended data Fig. 2. Ovarian function in response to stress and differential regulation in *Piezo2*/*Piezo1*. (**A**) Representative images of blastocysts recovered at 3.5 dpc from *Piezo2*$^{f/f}$ and *Piezo2*$^{d/d}$ mice with or without SPS treatment. Blastocyst morphology was comparable across all groups. Scale bar, 10 μm. (**B**) Quantification of blastocyst number per mouse at 3.5 dpc across all experimental groups. No significant differences were observed (n=5, two-way ANOVA with Tukey's post hoc test). (**C**) Serum estradiol (E2) and progesterone (P4) levels at 3.5 dpc in *Piezo2*$^{f/f}$ and *Piezo2*$^{d/d}$ mice with or without SPS treatment. No significant differences were observed between genotypes irrespective of stress conditions (n = 3, two-way ANOVA with Tukey's post hoc test). (**D**) Single-molecule fluorescence *in situ* hybridization (smFISH) of *Piezo2* mRNA in uterine sections from *Piezo2*$^{f/f}$ and *Piezo2*$^{d/d}$ mice with or without SPS treatment. SPS selectively upregulated *Piezo2* expression in the endometrial stroma of *Piezo2*$^{f/f}$ mice. Arrows indicate *Piezo2*-positive cells. Scale bar, 100 μm. (**E**) Quantitative RT-PCR analysis of *Piezo2* (left), *Piezo2* exons 17–18 (middle, upstream of the floxed region), and *Piezo1* (right) expression in *Piezo2*$^{f/f}$ and *Piezo2*$^{d/d}$ uteri with or without SPS treatment. SPS increased *Piezo2* transcription only when functional PIEZO2 protein was present (left, middle panels). n=3 or 4. Statistical significance was determined via two-way ANOVA with Tukey's post hoc test. (**F**) Representative uterine images and quantification of implantation sites in *Piezo1*$^{f/f}$ (control) and *Piezo2*$^{d/d}$ (*Pgr*$^{Cre/+}$*Piezo1*$^{f/f}$) mice with or without SPS treatment. Unlike *Piezo2*-cKO mice, *Piezo1*-cKO mice showed stable implantation that was unaffected by SPS (two-way ANOVA with Tukey's post hoc test). Scale bar, 5 mm. Quantitative data are presented as mean ± SEM. ns, $P > 0.05$; *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.


A
Vaginal plug
Supplement P4
2.5 dpc
2.75 dpc
3.5 dpc
3.75 dpc
Sacrifice and Collection
SPS 9:00-11:00
0.5 dpc
3.5 dpc
4.35 dpc
B
4.5 dpc
NS
Weight of IS/mg
SPS-Piezo2f/f
SPS-Piezo2d/d
SPS-Piezo2d/d -P4
C
Esr1
Muc1
Muc4
Ltf
Pgr
Hoxa10
Hand2
Areg
mRNA/Rpl19
NON-SPS
SPS
NS
**
***
Piezo2f/f
Piezo2d/d
Piezo2d/d+P4

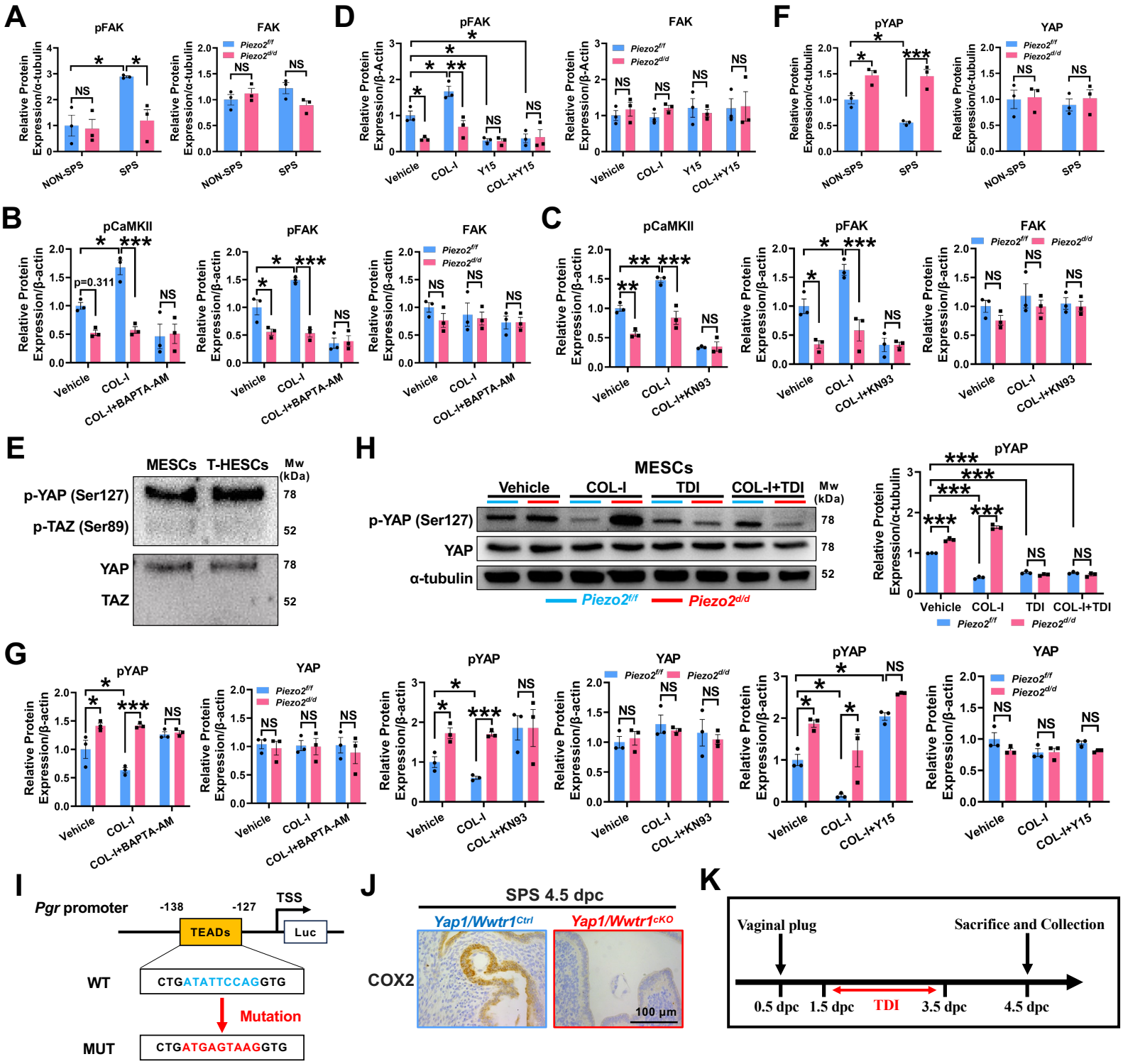
Extended data Fig. 3. Exogenous progesterone supplementation normalizes gene expression in SPS-Piezo2-deficient mice. (**A**) Schematic of the progesterone (P4) supplementation protocol. Exogenous progesterone (P4) was administered subcutaneously to SPS-*Piezo2*$^{d/d}$ mice at 2.5, 2.75, 3.5, and 3.75 dpc during the peri-implantation period. The SPS protocol was followed daily from 0.5−3.5 dpc and administered from 9:00−11:00 a.m. (**B**) Quantification of implantation site number (left) and implantation site weight (right) at 4.5 dpc in SPS-*Piezo2*$^{f/f}$, SPS-*Piezo2*$^{d/d}$, and SPS-*Piezo2*$^{d/d}$+P4 mice (n = 10 per group). (**C**) Quantitative RT-PCR analysis of estrogen-responsive genes (*Esr1*, *Muc1*, *Muc4*, *Ltf*) and progesterone-responsive genes (*Pgr*, *Hoxa10*, *Hand2*, *Areg*) in non-SPS and SPS-treated *Piezo2*$^{f/f}$, *Piezo2*$^{d/d}$, and *Piezo2*$^{d/d}$+P4 uteri at 3.5 dpc. Expression was normalized to *Rpl19* (n = 4 or 3 per group). Quantitative data are presented as mean ± SEM. Statistical significance was determined using a two-way ANOVA with Tukey's post hoc test. ns, $P > 0.05$; *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.


A
nNos
iNos
eNos
mRNA/Rpl19
NON-SPS
SPS
NS
Piezo2f/f
Piezo2d/d
B
Nitric Oxide (μM)
Vehicle
L-NAME-HCl
C
Piezo2f/f
Piezo2d/d
L-NAME-HCl
Tension(g)
Time(s)
D
3.5 dpc
Frequency
Contractile amplitude(g)
Vehicle
L-NAME-HCl
E
L-NAME HCl 4Days
4.5 dpc
5mm
F
4.5 dpc HE
100 μm
G
4.5 dpc COX2
100 μm

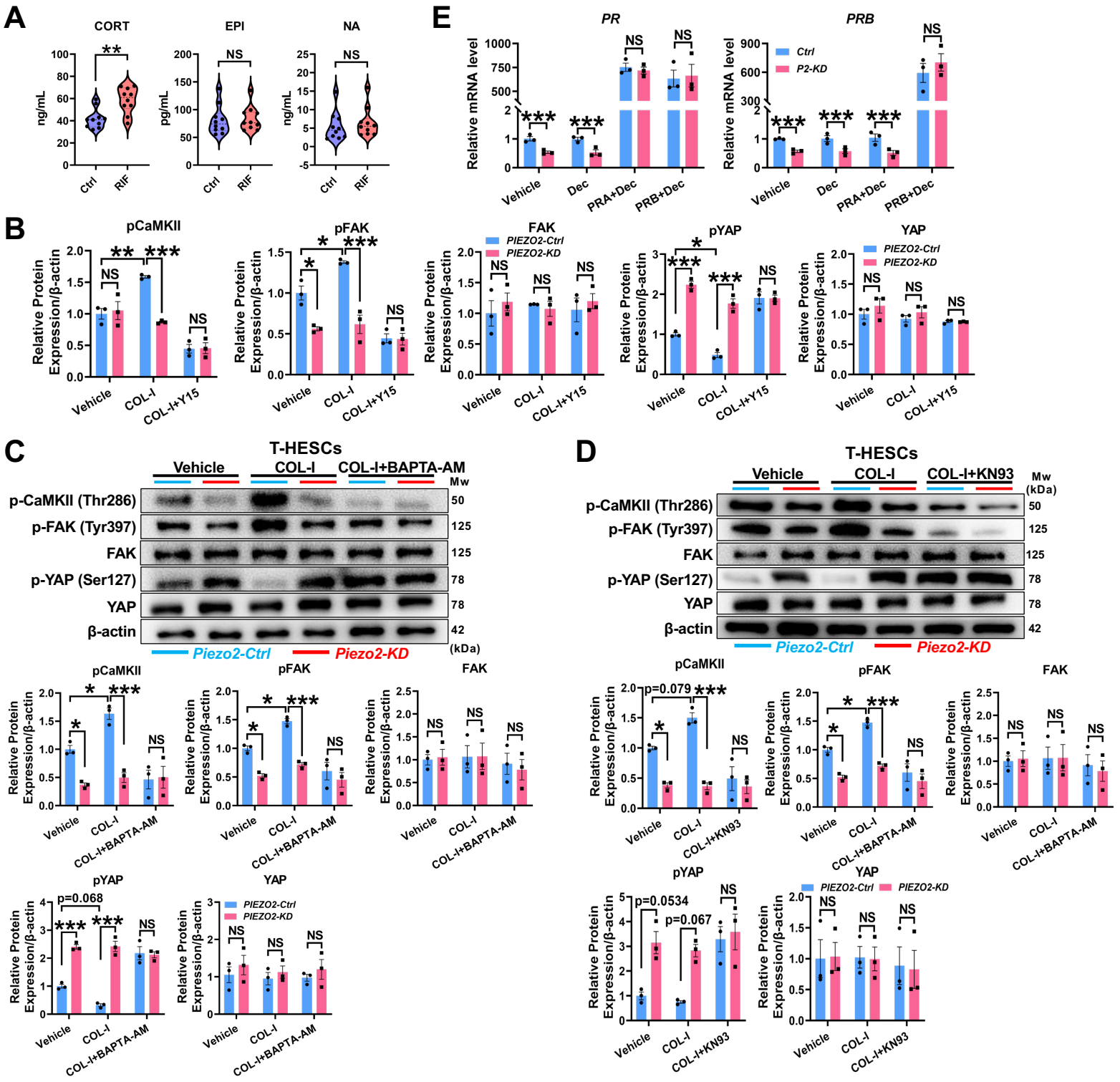
Extended data Fig. 4. NOS-mediated paracrine regulation of uterine contractility in PIEZO2-deficient endometrium. (**A**) Quantitative RT-PCR analysis of *nNos*, *iNos*, and *eNos* expression in *Piezo2*$^{f/f}$ and *Piezo2*$^{d/d}$ uteri with or without SPS treatment. Expression was normalized to *Rpl19* (n = 3 per group), with NOS isoforms significantly upregulated in *Piezo2*$^{d/d}$ endometrium. (**B**) Nitric oxide (NO) levels in endometrial tissue from *Piezo2*$^{f/f}$ and *Piezo2*$^{d/d}$ mice treated with vehicle or NOS inhibitor (L-NAME HCl). Injections were administered daily from 0.5 to 3.5 dpc, with NOS levels assessed on. 3.5 dpc (n = 3 per group). (**C and D**) Representative *ex vivo* tension traces from *Piezo2*$^{f/f}$ (top) and *Piezo2*$^{d/d}$ (bottom) uteri before and after L-NAME HCl treatment (**C**), and quantification of contraction frequency and amplitude following L-NAME HCl treatment (**D**) collected at 3.5 dpc (n = 5 per group). (**E**) Representative uterine images at 4.5 dpc from *Piezo2*$^{f/f}$ and *Piezo2*$^{d/d}$ mice treated with L-NAME HCl daily for 4 days. Implantation sites were visualized by intravenous injection of Chicago Sky Blue dye (blue spots) and indicated with red arrowheads. Scale bar, 5 mm. (**F, G**) Histological analysis (H&E staining) (F) and immunohistochemistry detecting COX2 (**G**) in uterine sections at 4.5 dpc following L-NAME HCl treatment. Scale bar, 100 μm. Quantitative data are presented as mean ± SEM. Statistical significance was determined using a two-way ANOVA with Tukey's post hoc test. ns, $P > 0.05$; *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.


A
SPS-Piezo2f/f
SPS-Piezo2d/d
10,678 cells
8,199 cells
UMAP_2
UMAP_1
Fibroblasts
Fibroblasts
Fibroblasts
Endothelial cells
Epithelial cells
Dendritic cells
Lymphatic Ecs
Macrophage
Mesothelial cells
NKs
Neutrophil
Other Endothelial cells
Plasma cells
Proliferate NKs
T cells
Myofibroblasts
B
SPS-Uterus Composition
Percentage (%)
62.94% (6,721/10,678)
55.56% (4,555/8,199)
Piezo2f/f
Piezo2d/d
Fibroblasts
Dendritic cells
Endothelial cells
Epithelial cells
Lymphatic Ecs
Macrophage
Mesothelial cells
Myofibroblasts
NKs
Neutrophil
Other Endothelial cells
Plasma
Proliferate NKs
T cells
C
UMAP_2
UMAP_1
E
Piezo2
P = 0.000034
P = 0.089026
P = 0.666254
D
Col3a1
*** P=1.1e-08
Col6a3
ns P=1.000
Col5a1
* P=0.032
Col14a1
ns P=0.867
Sparc
*** P=3.0e-09
Gsn
*** P=3.0e-24
Lum
*** P=4.3e-06
Expression
SPS-Piezo2f/f
SPS-Piezo2d/d
F
Number of interactions
C0
C1
C2
C3
C4
C5
G
-log10(pvalue)
Log2 mean (Molecule 1, Molecule 2)
Fn1-aVb1 complex
Fn1-aVb5 complex
Wnt5a-Antxr1
Igf1-Igf1r
Tnfrsf1a-Grn
S0 S1 S2 S3 S4 S5
Subcluster0
Igf1-Igf1r
Fn1-aVb5 complex
Fn1-aVb1 complex
Ptn-Ptprs
Wnt5a-Antxr1
Subcluster1
Wnt5a-Antxr1
Ptn-Ptprs
Tgfb2-Tgfbr3
Fn1-aVb5 complex
Fn1-aVb1 complex
Subcluster3
Wnt5a-Antxr1
Ptn-Ptprs
Fn1-aVb5 complex
Fn1-aVb1 complex
Fn1-a5b1 complex
Subcluster4
H
Igf1
mRNA/Rpl19
*** ***
NS
Piezo2f/f
Piezo2d/d
NON-SPS
SPS
I
Col1a1
Col1a2
Col4a1
mRNA/Rpl19
** NS
* NS
* NS
Vehicle
IGF1

Extended data Fig. 5. scRNA-seq-based characterization of uterine cell populations and stromal paracrine signaling. (**A**) UMAP visualization of cell clusters identified by scRNA-seq in SPS-*Piezo2*$^{f/f}$ (10,678 cells) and SPS-*Piezo2*$^{d/d}$ (8,199 cells) uteri at 3.5 dpc. Each point represents a single cell and is colored according to its assigned cell cluster (n = 14). (**B**) Stacked bar plot showing the distribution of each cell type identified by scRNA-seq in SPS-*Piezo2*$^{f/f}$ versus SPS-*Piezo2*$^{d/d}$ uteri. Stromal fibroblasts were the largest population in both genotypes but were reduced in PIEZO2-deficient uteri relative to controls (62.94% vs. 55.56%). (**C**) UMAP visualization of uterine stromal cell (uSC) subclusters after fibroblast sub-clustering. Dashed circle indicates the uterine stromal cells. (**D**) Violin plots showing collagen and extracellular matrix-associated gene expression in mesenchymal fibroblast in subcluster 2 from SPS-*Piezo2*$^{f/f}$ (Ctrl, blue) and SPS-*Piezo2*$^{d/d}$ (KO, pink) uteri. Statistical significance was determined by Wilcoxon rank-sum test with Bonferroni correction. (**E**) Violin plots showing *Piezo2* expression levels across the six fibroblast subclusters. (**F**) Chord diagram of predicted ligand–receptor interactions among fibroblast subclusters generated with CellPhoneDB. Node size represents the relative abundance of predicted interactions for each subcluster, and line thickness indicates the relative interaction strength. (**G**) Dot plots showing the top five most significant ligand–receptor pairs for each PIEZO2-expressing fibroblast subcluster compared to all other subclusters. Dot size represents the –log10(P value), while the color gradient represents the communication probability (log2 mean expression of the ligand–receptor pair). Blue shading highlights targeted paracrine signaling directed toward the ECM-producing subcluster 2. (**H**) Quantitative RT-PCR analysis of *Igf1* expression in *Piezo2*$^{f/f}$ and *Piezo2*$^{d/d}$ uteri with or without SPS treatment. Expression was normalized to *Rpl19* (n = 3 per group, two-way ANOVA with Tukey's post hoc test). (**I**) Quantitative RT-PCR analysis of collagen gene expression (*Col1a1*, *Col1a2*, *Col4a1*) in *Piezo2*$^{f/f}$ and *Piezo2*$^{d/d}$ cells supplemented with IGF1. Cells were cultured with IGF1 (100 ng/mL) or vehicle for 24 h prior to RNA extraction. Expression was normalized to *Rpl19* (n = 3 per group, two-way ANOVA with Tukey's post hoc test). Quantified data are presented as mean ± SEM. ns, $P > 0.05$; *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.


A
pFAK
FAK
Relative Protein Expression/α-tubulin
NON-SPS
SPS
NS
Piezo2f/f
Piezo2d/d
D
pFAK
FAK
Relative Protein Expression/β-Actin
Vehicle
COL-I
Y15
COL-I+Y15
F
pYAP
YAP
B
pCaMKII
pFAK
FAK
Relative Protein Expression/β-actin
p=0.311
COL-I+BAPTA-AM
C
pCaMKII
pFAK
FAK
COL-I+KN93
E
MESCs
T-HESCs
Mw (kDa)
p-YAP (Ser127)
p-TAZ (Ser89)
YAP
TAZ
78
52
H
MESCs
Vehicle
COL-I
TDI
COL-I+TDI
p-YAP (Ser127)
YAP
α-tubulin
pYAP
G
pYAP
YAP
COL-I+BAPTA-AM
COL-I+KN93
COL-I+Y15
I
Pgr promoter
-138
-127
TSS
TEADs
Luc
WT
CTGATATTCCAGGTG
Mutation
MUT
CTGATGAGTAAGGTG
J
SPS 4.5 dpc
Yap1/Wwtr1Ctrl
Yap1/Wwtr1cKO
COX2
100 μm
K
Vaginal plug
Sacrifice and Collection
0.5 dpc
1.5 dpc
TDI
3.5 dpc
4.5 dpc

Extended data Fig. 6. Hippo–YAP pathway validation and Pgr promoter analysis. (A) Quantification of p-FAK (Tyr397) and FAK protein levels normalized to α-tubulin, corresponding to the Western blot shown in Fig. 5A. n = 3 biological replicates. (B) Quantification of p-CaMKII, p-FAK (Tyr397), and FAK protein levels normalized to β-actin, corresponding to the Western blot shown in Fig. 5B. n = 3 biological replicates. (C) Quantification of p-CaMKII, p-FAK (Tyr397), and FAK protein levels normalized to β-actin, corresponding to the Western blot shown in Fig. 5C. n = 3 biological replicates. (D) Quantification of p-FAK (Tyr397) and FAK protein levels normalized to β-actin, corresponding to the Western blot shown in Fig. 5E. n = 3 biological replicates. (E) Western blot analysis of p-YAP (Ser127) and p-TAZ (Ser89) protein levels, along with total YAP and TAZ, in mouse endometrial stromal cells (MESCs) and human endometrial stromal cells (T-HESCs). (F) Quantification of p-YAP (Ser127) and YAP protein levels normalized to α-tubulin, corresponding to the Western blot shown in Fig. 5G. n = 3 biological replicates. (G) Quantification of p-YAP (Ser127) and YAP protein levels normalized to α-tubulin, corresponding to the Western blot shown in Fig. 5I. n = 3 biological replicates. (**H**) Western blot analysis of p-YAP (Ser127) and total YAP in primary mouse endometrial stromal cells (MESCs) from *Piezo2*$^{f/f}$ and *Piezo2*$^{d/d}$ mice treated with TDI. Cells were cultured with COL-I coating, TDI (no COL-I), or COL-I+TDI, with a control cultured without COL-I coating or treatment (Vehicle). Cultures were maintained for 48 h and then lysed. Relative expression was determined by normalizing to α-tubulin and based on *n* = 3 per group (two-way ANOVA with Tukey's post hoc test, graph on right). Data are presented as mean ± SEM. ns, $P > 0.05$; ***$P < 0.001$. (**I**) Schematic of the *Pgr* promoter region showing the predicted TEAD-binding element (TRE) located at positions –138 to –127 relative to the transcription start site (TSS), as identified using the JASPAR transcription factor binding site database (https://jaspar.elixir.eu). The wild-type sequence (AGGTGGGAAG) and mutant sequence (AGGATTTAAG) used in luciferase reporter assays are indicated. (**J**) Schematic of the TDI-011536 (TDI) treatment protocol for *in vivo* rescue experiments. TDI (3 μM) was administered daily from 1.5 to 3.5 dpc, and implantation was assessed at 4.5 dpc. Quantitative data are presented as mean ± SEM. Statistical significance was determined using a two-way ANOVA with Tukey's post hoc test. ns, $P > 0.05$; *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.


A
CORT
EPI
NA
ng/mL
pg/mL
Ctrl
RIF
B
pCaMKII
pFAK
FAK
pYAP
YAP
Relative Protein Expression/β-actin
PIEZO2-Ctrl
PIEZO2-KD
Vehicle
COL-I
COL-I+Y15
C
T-HESCs
Vehicle
COL-I
COL-I+BAPTA-AM
p-CaMKII (Thr286)
p-FAK (Tyr397)
FAK
p-YAP (Ser127)
YAP
β-actin
Mw
(kDa)
Piezo2-Ctrl
Piezo2-KD
D
COL-I+KN93
E
PR
PRB
Relative mRNA level
Ctrl
P2-KD
Dec
PRA+Dec
PRB+Dec
NS

Extended data Fig. 7. Human endometrial stromal cell signaling cascade and PGR isoform-specific rescue. (**A**) Serum levels of cortisol (CORT), epinephrine (EPI), and noradrenaline (NA) in fertile controls (Ctrl) and patients with recurrent implantation failure (RIF). Individual data points are displayed for Ctrl and RIF endometrial samples (n = 8 per group, two-sided Student's t-test). (B) Quantification of p-CaMKII, p-FAK (Tyr397), FAK, p-YAP (Ser127), and YAP protein levels normalized to β-actin, corresponding to the Western blot shown in Fig. 6E. n = 3 biological replicates. (**C, D**) Western blot analysis evaluating p-CaMKII (Thr286), p-FAK (Tyr397), total FAK, p-YAP (Ser127), and total YAP in T-HESCs. PIEZO2-KD and control cells were cultured on COL-I, or without COL-1 (Vehicle), with or without BAPTA-AM (**C**) or with or without KN93 (**D**). Lysates were obtained at 48 h post-treatment, and expression was normalized to β-actin (n = 3 independent experiments, two-way ANOVA with Tukey's post hoc test), with graphical quantification shown on the right. (**E**) Quantitative RT-PCR analysis of *PR* (total PGR) and *PRB* (PGR-B isoform) expression in T-HESCs. PIEZO2-KD and control cells were cultured as non-decidualized cells (Vehicle), decidualized (Dec) cells, or Dec cells with a vector overexpressing PGR-A (PRA+Dec) or PGR-B (PRB+Dec). After 48 h, RNA was extracted and expression was normalized to *GAPDH* (n = 3 per group, two-way ANOVA with Tukey's post hoc test). Quantitative data are presented as mean ± SEM. ns, $P > 0.05$; *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

Extended data Table 1. Fertility assessment of *Piezo2*$^{f/f}$ and *Piezo2*$^{d/d}$ females

Genotype	Females	Pups	Litters	Litters/Female	Pups/Female	Pups/Litter	Weight/Pup	Gestation Days
Piezo2$^{f/f}$	8	351	45	5.63±0.183	43.88±1.747	7.80±0.122	1.37±0.002	19.13±0.072
Piezo2$^{d/d}$	8	250	42	5.25±0.164	31.25±1.461	5.95±0.125	1.16±0.005	21.08±0.150
P value	/	/	/	0.1489	**7.23×10^{-5}**	**4.31×10^{-8}**	**3.00×10^{-15}**	**4.94×10^{-7}**

Data are presented as mean ± SEM. Statistical comparisons were performed using a two-sided Student's *t*-test. Bold values indicate significant differences between genotypes.

Extended data Table 2. Patient clinical characteristics

Category	ID	Age	BMI (Kg/m^2)	Fertility history	Pathology	Time of embryo transfer	Sample collection phase	Embryo transfer day	Numbers of live births	Embryo quality grade	Endocrine metabolic abnormality
Pregnant (n=18)	B1	33	22.24	Yes	mechanical obstruction of fallopian	1	HRT cycle mid-secretory phase (LHP+7)	LHP+5 (Next cycle after sample collection)	1	I-II	Without
	B2	32	23.24								
	B3	39	23.07								
	B4	31	21.56								
	B5	32	19.47								
	B6	35	21.41								
	B7	34	22.50								
	B8	29	22.18								
	S1	31	22.31								
	S2	36	23.26								
	S3	27	19.47								
	S4	33	20.14								
	S5	38	22.27								
	S6	30	22.47								
	S7	31	23.14								
	S8	32	21.57								
	S9	34	22.21								
	S10	30	21.63								
RIF (n=18)	B9	35	21.88	No	Unexplained	4	HRT cycle mid-secretory phase (LHP+7)	LHP+5 (Next cycle after sample collection)	0	I-II	Without
	B10	31	20.21			3					
	B11	38	21.99			3					
	B12	27	20.07			4					
	B13	30	19.10			3					
	B14	34	22.67			2					
	B15	36	20.70			3					
	B16	34	23.75			4					
	S11	26	19.65			3					
	S12	29	20.83			3					
	S13	30	25.61			2					
	S14	29	29.69			2					
	S15	32	29.24			5					
	S16	29	23.71			3					

	S17	35	27.06	2
	S18	30	24.00	2
	S19	32	22.37	2
	S20	31	26.84	4
p-value		0.3016	0.2752	<0.0001

187

188 **Extended data Table 3. List of primers and other sequences**

Gene	Primer application	Forward sequence	Reverse sequence
mPiezo2	qPCR	5′-CCCGGAACAGTAGCCAGAAA-3′	5′-TTCCCAAAGGCCCAGAATCC-3′
mPiezo2-E17/18	qPCR	5′-ACTCGTCTGCATCCTACACC-3′	5′-GGTACACGCGACCATTGACT-3′
mPiezo1	qPCR	5′-GCTGGCTTCTGGAGGTGACTTG-3′	5′-GGTACACGCGACCATTGACT-3′
mPgr	qPCR	5′-GCAATGGAAGGGCAGCATAAC-3′	5′-CTTACGACCTCCAAGGACCA-3′
mEsr1	qPCR	5′-GGAAGCTCCTGTTTGCTCCT-3′	5′-AACCGACTTGACGTAGCCAG-3′
mRpl19	qPCR	5′-TCATGGAGCACATCCACAAGCTGA-3′	5′-CGCTTTCGTGCTTCCTTGGTCTTA-3′
mLif	qPCR	5′-ATTGAGCTTGACCTGGAGGC-3′	5′-GGTGGAGCTGTATCGGATGG-3′
mNos1	qPCR	5′-CCTTCACAGGGGATGGAACC-3′	5′-AGATCGACAGCTTTGGTGGG-3′
mNos2	qPCR	5′-AGAGCCACAGTCCTCTTTGC-3′	5′-CTGGTCCATGCAGACAACCT-3′
mNos3	qPCR	5′-ATTGGCATGAGGGACCTGTG-3′	5′-GGGATGAGGTTGTCCTGGTG-3′
mLtf	qPCR	5′-CCCTACAAACTGCGACCTGT-3′	5′-CACGACTGCTACCGCATAGT-3′
mMuc1	qPCR	5′-TCACCCCAGTTGTCTGTTGG-3′	5′-AGTAGTTGGAGCTGGGGTCT-3′
mMuc4	qPCR	5′-ATGGCTACAAAGGCTACCACC-3′	5′-CCTCACTACATGGGGACACAC-3′
mHand2	qPCR	5′-CCAGCTACATCGCCTACCTC-3′	5′-GGTCTTCTTGATCTCCGCCT-3′
mHoxa10	qPCR	5′-TCCAAAGGCGAAAATGCAGC-3′	5′-TCTGGTGCTTCGTGTAAGGG-3′
mAreg	qPCR	5′-ACAGCGAGGATGACAAGGAC-3′	5′-GCCAATAGCTGCGAGGATGA-3′
mIhh	qPCR	5′-TCTGTCTGTTCCTGCTGCTG-3′	5′-TTGTAGGCAAGAGGCACGAG-3′
mBmp2	qPCR	5′-AAGCCAAACACAAACAGCGG-3′	5′-CGATCCAGTCATTCCACCCC-3′
mPrl8a2	qPCR	5′-GGTGCATGGATCACTCCTGT-3′	5′-ACCCACGTAAGGTCATCATGG-3′
mPrl3c1	qPCR	5′-ACTGCCACACGATATGACCG-3′	5′-TGTGACGAGAAGAGGAAAGCA-3′
mCol1a1	qPCR	5′-AGCACGTCTGGTTTGGAGAG-3′	5′-GACATTAGGCGCAGGAAGGT-3′
mCol1a2	qPCR	5′-CTAGCCAACCGTGCTTCTCA-3′	5′-TCTCCTCATCCAGGTACGCA-3′
mCol3a1	qPCR	5′-ACGTAAGCACTGGTGGACAG-3′	5′-CAGGAGGGCCATAGCTGAAC-3′
mCol4a1	qPCR	5′-TCATTAGCAGGTGTGCGGTT-3′	5′-GTTAGGGCACTGCGGAATCT-3′
mIl1b	qPCR	5′-ATGCCACCTTTTGACAGTGAT-3′	5′-AGCCCTTCATCTTTTGGGGT-3′
mIl6	qPCR	5′-CCCCAATTTCCAATGCTCTCC-3′	5′-GGATGGTCTTGGTCCTTAGCC-3′
mTnfa	qPCR	5′-GATCGGTCCCCAAAGGGATG-3′	5′-CCACTTGGTGGTTTGTGAGTG-3′
mIgf1	qPCR	5′-TGGGGAAAATCAGCAGCCTT-3′	5′-CAGAGCGCCAGGTAGAAGAG-3′
hGAPDH	qPCR	5′-GGCAAATTCCATGGCACCG-3′	5′-AGCATCGCCCCACTTGATTT-3′
hPIEZO2	qPCR	5′-AAGGCAGTCCACCCTTGATG-3′	5′-TGGGCTCGCTTGATGCTAAA-3′
hPGR-B	qPCR	5′-GGAATGGGCTGTACCGAGAG-3′	5′-CGGCTCCTTTATCTCCCGAC-3′
hPGR	qPCR	5′-CCGGGCACTGAGTGTTGAAT-3′	5′-GAAGGGGTTTCACCATCCCT-3′

hCOL1A1	qPCR	5′-GGGAGATGCTGGTCCTGCT-3′	5′-GCACCATCATTTCCACGAGC-3′
hIGFBP1	qPCR	5′-TGTGACGAGAAGAGGAAAGCA-3′	5′-CTCCTGATGTCTCCTGTGCC-3′
hFOXO1	qPCR	5′-TGGAGATCGACCCGGACTTC-3′	5′-CCGAGTTGGACTGGCTAAACT-3′
hPRL	qPCR	5′-GGGGTTCATTACCAAGGCCA-3′	5′-TTGGGCTTGCTCCTTGTCTT-3′
hIGF1	qPCR	5′-ATGTATTGCGCACCCCTCAA-3′	5′-GCACTCCCTCTACTTGCGTT-3′
siPIEZO2	RNA interference	5′-GCTTGAGGATCTTGGCTTA-3′	
Padlock Probe-PIEZO2	sm-ISH	5′-TCTTTATGGTAAACGCCTTTGTTCCTTTTACGACCTCAATG CTGCTGCTGTACTACTCTTGACATAGATCTGTAGAGTCT-3′	
Oligo-dT	sm-ISH	5′-AAGCAGTGGTATCAACGCAGAGTACTTTTTTTTTTTTTTTTTTTTTTTTTTTVN-3′	
RCA primer	sm-ISH	5′-ACAGCAGCAGCATTGAGG*T*C-3′ Note: *= thiophosphate modifications	
HRP labeled detection probe	sm-ISH	5′- HRP-CCTCAATGCTGCTGCTGTACTAC-3′	
Pgr Promoter-WT	Dual-Luciferase Reporter Assay	5′-GCTTTTTATAGGGTGGGGCTGGCATGCTTCTTAATTCAGTACAAATTCTGATATGTTT AGATTTCTTCTCAAAGCATCTGATATTCCAGGTGGAATCAGACTAGTGAGTAGCTGG AATTCCAACGCCAGAGATTTAGATCTAGCCAGTGATTGGCTAGGGAGGGGCTTTGGG CGGGCCTTCCTAGAGCGCCAACGCTTGCTAGAAAGCTATGGAGCCAGTCTAGACTGT CACTATCAGTCTTTGTAGTATTTACGGGTGCGAGGATTC-3′	
Pgr Promoter-Mut	Dual-Luciferase Reporter Assay	5′-GCTTTTTATAGGGTGGGGCTGGCATGCTTCTTAATTCAGTACAAATTCTGATATGTTT AGATTTCTTCTCAAAGCATCTGATGAGTAAGGTGGAATCAGACTAGTGAGTAGCTGG AATTCCAACGCCAGAGATTTAGATCTAGCCAGTGATTGGCTAGGGAGGGGCTTTGGG CGGGCCTTCCTAGAGCGCCAACGCTTGCTAGAAAGCTATGGAGCCAGTCTAGACTGT CACTATCAGTCTTTGTAGTATTTACGGGTGCGAGGATTC-3′	
Piezo2-WT	Genotyping	5′-ACTTAGATGGGGCAGGTGCT-3′	5′-ACTTCCCTACCCACCCATTC-3′
Piezo2-flox	Genotyping	5′-GCCGCTCTAGAACTAGTGGA-3′	5′-ATCTACCACGGGGGCTCTCTC-3
Pgr-WT	Genotyping	5′-TATACCGATCTCCCTGGACG-3′	5′-CCCAAAGAGACACCAGGAAG-3′
Pgr-Cre	Genotyping	Same as *Pgr-WT*	5′-ACTTAGATGGGGCAGGTGCT-3′

189

Extended data Table 4. List of antibodies

Antibody	Type	Manufacturer	Catalog no.	Application	Dilution
Ki67	Rabbit IgG	Santa Cruz	SC-23900	IHC	1:200
Cox2	Rabbit IgG	Cell Signaling	4842	IHC	1:200
Hand2	Rabbit IgG	Abcam	ab200040	IHC	1:200
Muc1	Rabbit IgG	Cell Signaling	16564S	IHC	1:200
Pgr	Rabbit IgG	Invitrogen	MA5-16393	IHC	1:200
ERα	Rabbit IgG	Santa Cruz Biotechnology	sc7207	IHC	1:200
Biotinylated secondary	Goat anti-Rabbit IgG	Vactorlabs	BA-1000	IHC	1:200
Collagen I	Rabbit IgG	Abways	ab138492	IF	1:100
Fluorescent secondaries	Goat anti-Rabbit IgG	Jackson	711-225-152	IF	1:200
Piezo2	Rabbit IgG	NOVUS	NBP1-78624	WB	1:1000
FAK	Rabbit IgG	PTM BIO	PTM-5717	WB	1:1000
p-FAK	Rabbit IgG	Cell Signaling	8556T	WB	1:1000
p-CaMKII	Rabbit IgG	Affinity Biosciences	AF3493	WB	1:1000
α-Tubulin	Rabbit IgG	Cell Signaling	2144	WB	1:1000
YAP/TAZ	Rabbit IgG	Cell Signaling	93622S	WB	1:1000
p-YAP	Rabbit IgG	Cell Signaling	4911S	WB	1:1000
β-actin	Mouse IgG	Abmart	M20011XS	WB	1:1000
Streptavidin–HRP conjugate	N/A	Invitrogen	434323	IHC	1:1000
GAPDH	Mouse IgG	Abmart	M20050XS	WB	1:1000

IHC, immunohistochemistry; IF, immunofluorescence; WB, western blot.