

# Supplementary Information for

## Myometrial contraction-activated endometrial PIEZO2 safeguards fertility under maternal stress

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

### Affiliations:

<sup>1</sup>College of Veterinary Medicine, South China Agricultural University, Guangzhou, China

<sup>2</sup>Reproductive Medicine Center of Renmin Hospital, Biomedical Engineering College, Hubei University  
of Medicine, Shiyan, Hubei, China

<sup>3</sup>College of Animal Science, Guizhou University, Guiyang, China

<sup>4</sup>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](mailto:renweisu@scau.edu.cn)

Hong-Lu Diao, PhD.

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

Email: [hldiao@hbmh.edu.cn](mailto:hldiao@hbmh.edu.cn)

27 Bin Guo, PhD.

28 5333 Xi'an Road, Changchun, Jilin, China, 130062

29 Email: [guobin79@jlu.edu.cn](mailto:guobin79@jlu.edu.cn)

30

31 Zeng-Ming Yang, PhD.

32 2708 Huaxi District, Guiyang, Guizhou, China, 550025

33 Email: [yangzm@gzu.edu.cn](mailto:yangzm@gzu.edu.cn)

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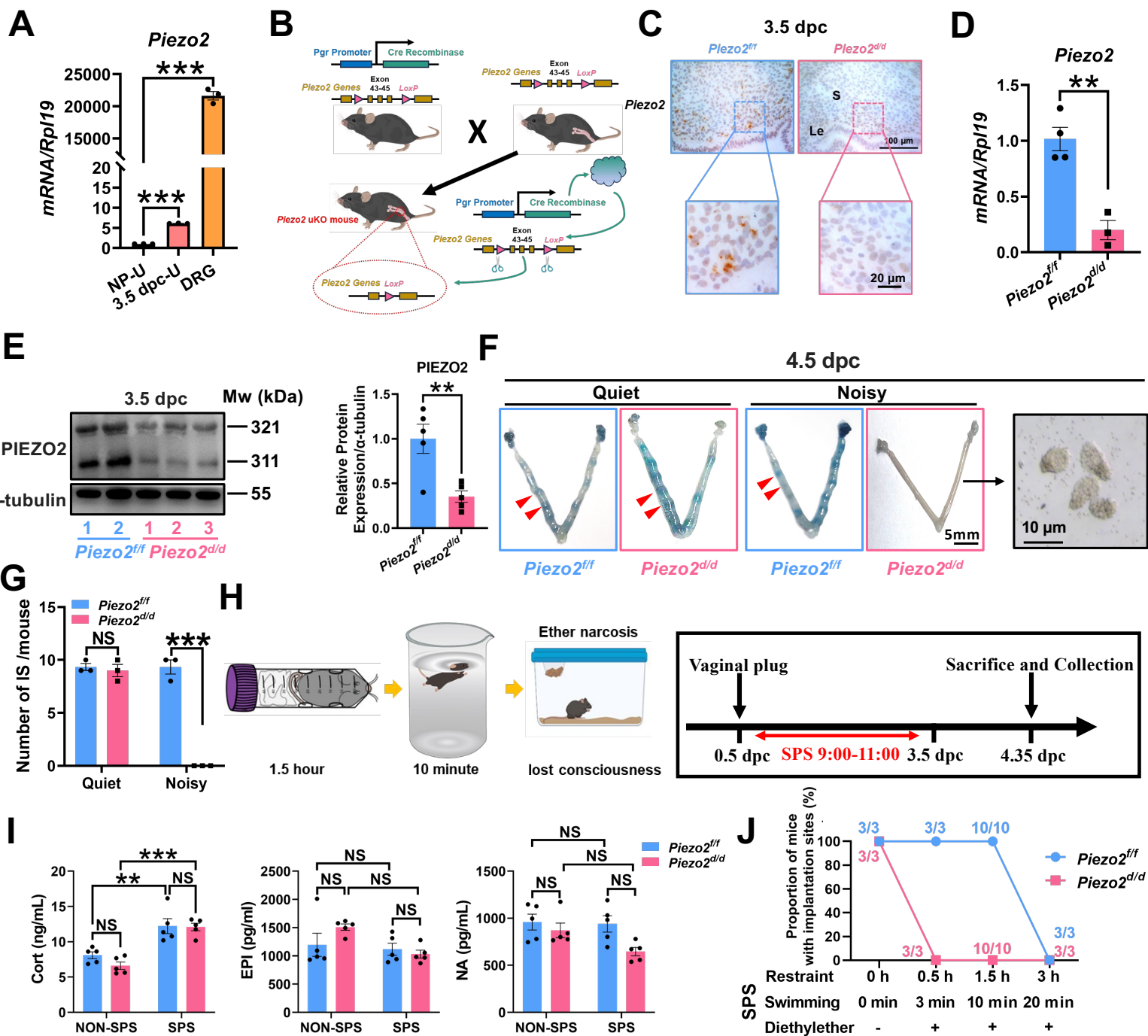
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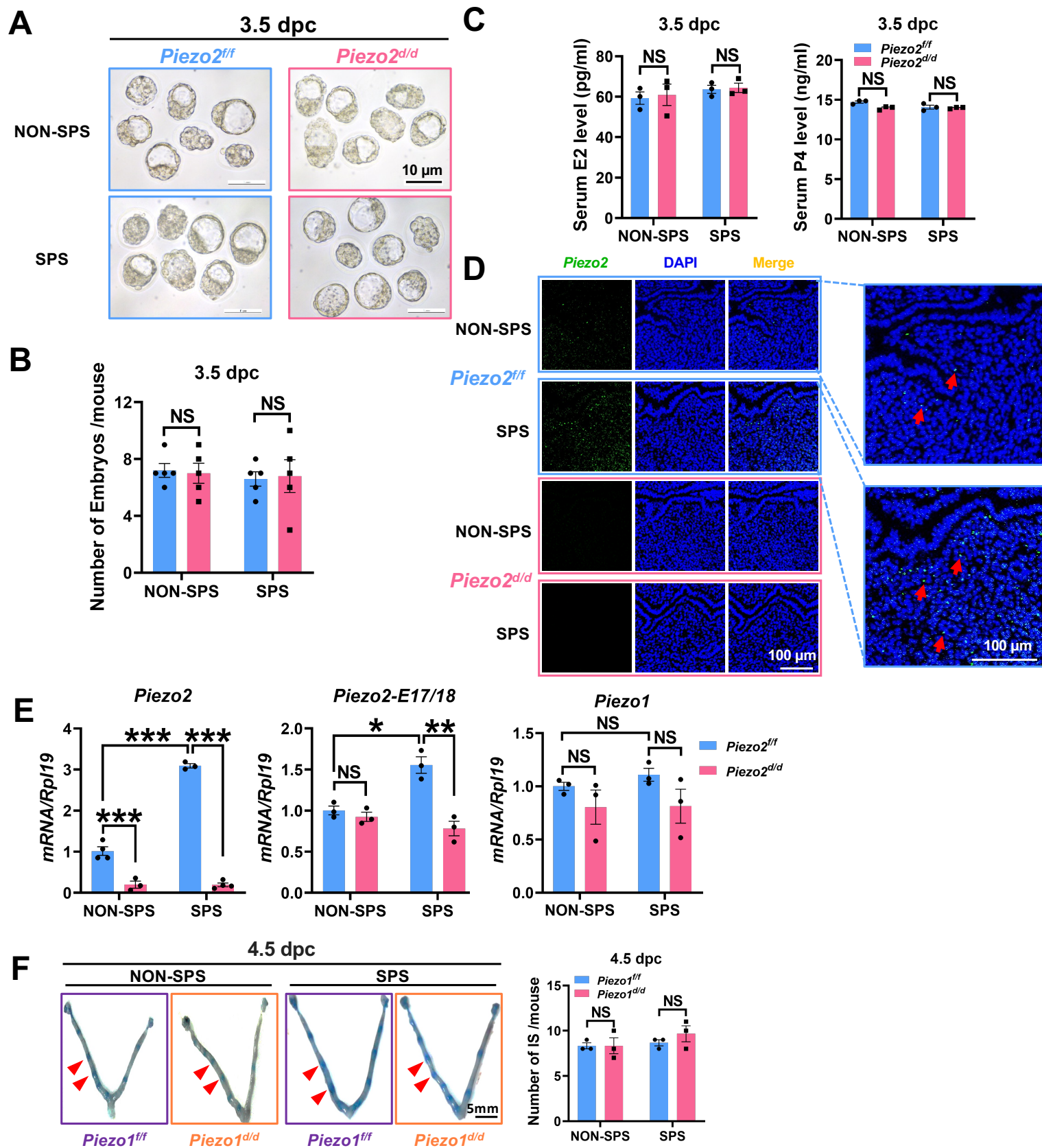
37 Materials and Methods

38 Extended Data Figs. 1 to 7

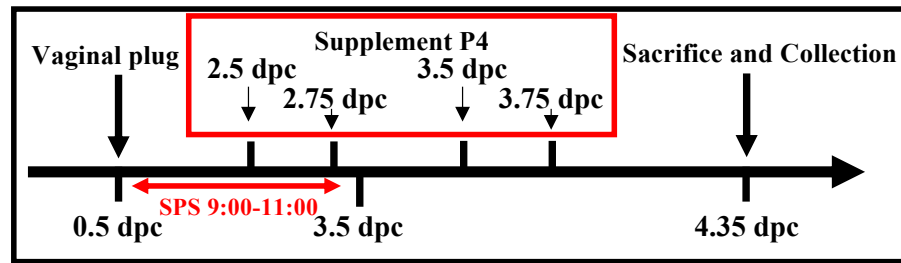
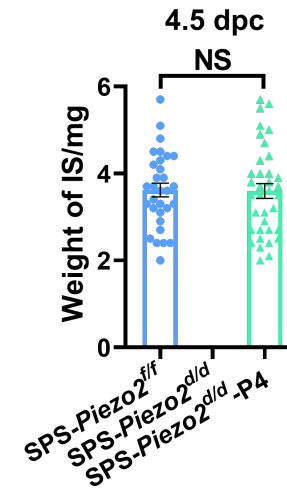
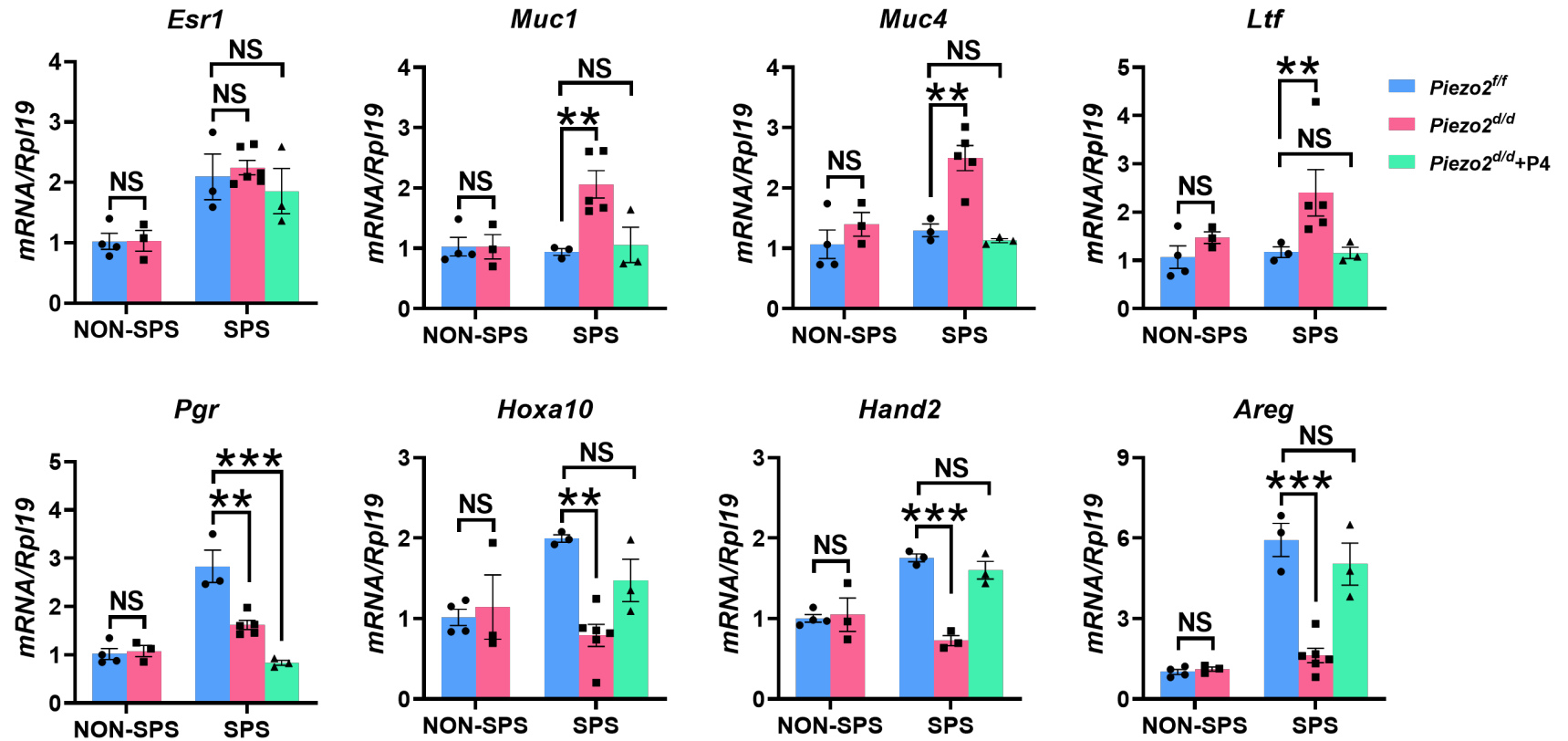
39 Extended Data Tables 1 to 4



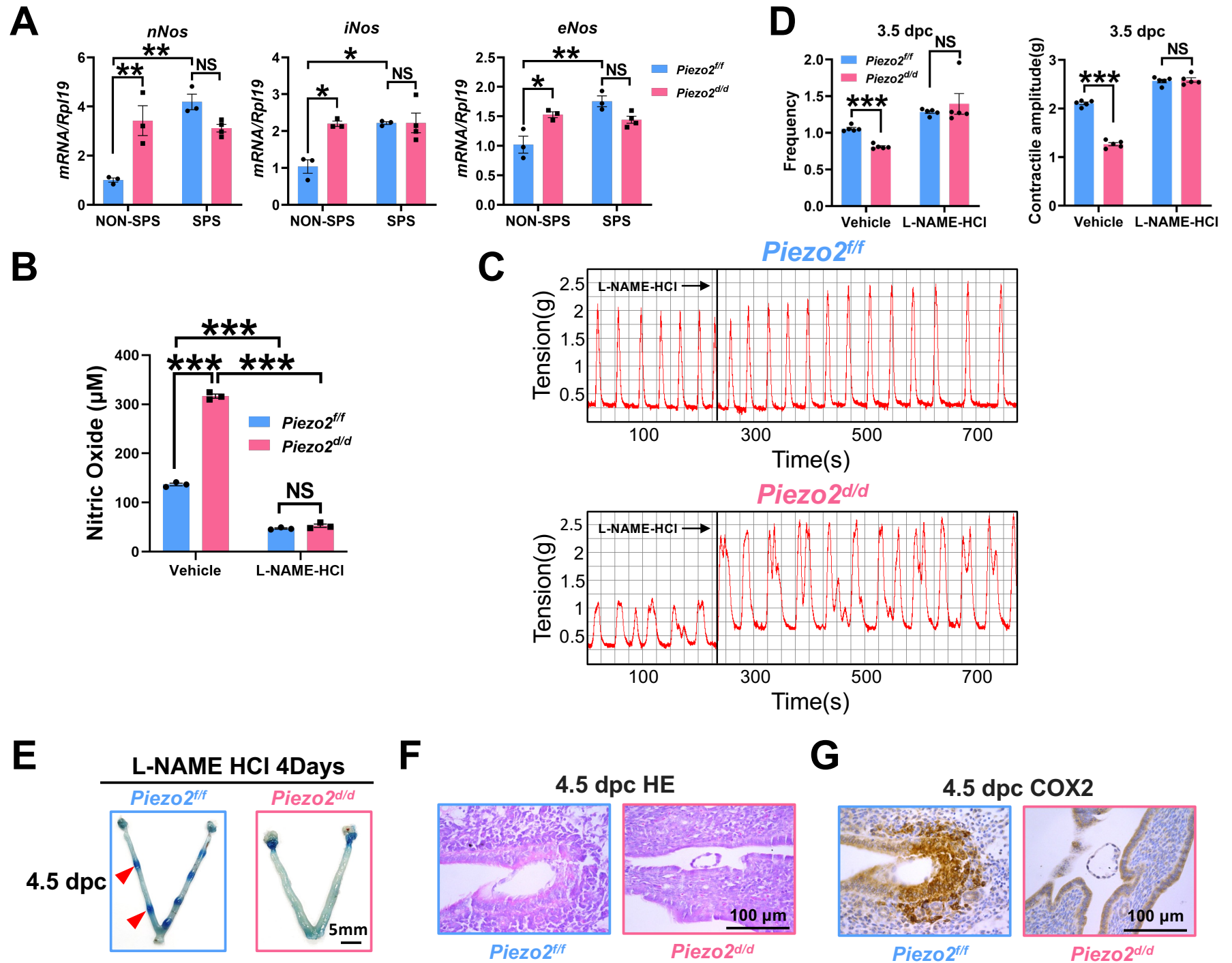
40 **Extended data Fig. 1. Generation and characterization of uterine-specific *Piezo2* conditional**  
41 **knockout mice.** (A) *Piezo2* mRNA expression in non-pregnant uterus (NP-U), uterus at 3.5 dpc of  
42 pregnancy (D4-U), and dorsal root ganglion (DRG), quantified by RT-qPCR and normalized to *Rpl19*,  $n$   
43 = 3 biological replicates. One-way ANOVA with Tukey's post hoc test. (B) Schematic of the conditional  
44 *Piezo2* knockout strategy. *Piezo2<sup>ff</sup>* mice carrying loxP sites flanking exons 43–45 were crossed with  
45 *Pgr<sup>Cre/+</sup>* transgenic mice to generate uterine-specific *Piezo2* conditional knockout (*Piezo2<sup>d/d</sup>*) females. (C)  
46 Immunohistochemistry detecting PIEZO2 in uterine cross-sections at 3.5 dpc from *Piezo2<sup>ff</sup>* and *Piezo2<sup>d/d</sup>*  
47 mice, confirming the loss of PIEZO2 protein expression in the endometrial stroma of knockout mice. S,  
48 stroma; LE, luminal epithelium. Scale bar, 100  $\mu$ m and 20  $\mu$ m. (D) Quantitative RT-PCR confirming  
49 efficient *Piezo2* deletion in *Piezo2<sup>d/d</sup>* uteri, with *Rpl19* serving as an internal control. Statistical  
50 significance determined using a two-sided Student's t-test. (E) Western blot analysis of PIEZO2  
51 expression in uterine tissues at 3.5 dpc in *Piezo2<sup>d/d</sup>* and *Piezo2<sup>ff</sup>* ( $n = 5$  each) mice. Expression was  
52 normalized to  $\alpha$ -tubulin with the quantification shown to the right (two-sided Student's t-test). (F)  
53 Representative uterine images at 4.5 dpc from *Piezo2<sup>ff</sup>* and *Piezo2<sup>d/d</sup>* females housed under quiet ( $45 \pm 5$   
54 dB) or noisy ( $65 \pm 5$  dB) acoustic conditions. Red arrowheads indicate implantation sites visualized by  
55 Chicago Sky Blue dye injection. Morphologically normal blastocysts were recovered from the uterine  
56 lumen of *Piezo2<sup>d/d</sup>* mice under noisy conditions (right panel). Scale bars, 5 mm and 10  $\mu$ m. (G)  
57 Quantification of implantation site number per mouse under quiet and noisy conditions. *Piezo2<sup>d/d</sup>* mice  
58 displayed normal implantation under quiet conditions but complete implantation failure under noisy  
59 conditions (two-way ANOVA with Tukey's post hoc test). (H) Schematic of the modified Single  
60 Prolonged Stress (SPS) protocol. SPS consists of sequential restraint stress (1.5 h), forced swim (10 min),  
61 and diethyl ether exposure until loss of consciousness, with this protocol administered daily between 0.5  
62 and 3.5 dpc (9:00–11:00 AM). (I) Serum levels of corticosterone (Cort), epinephrine (EPI), and  
63 noradrenaline (NA) in *Piezo2<sup>ff</sup>* and *Piezo2<sup>d/d</sup>* mice with or without SPS treatment, confirming successful  
64 stress induction. SPS significantly elevated corticosterone in both genotypes ( $n=5$ , two-way ANOVA with  
65 Tukey's post hoc test). (J) Dose–response analysis of stress sensitivity. Mice were subjected to various  
66 intensities of SPS by adjusting the exposure durations. *Piezo2<sup>d/d</sup>Piezo2-cKO* mice displayed implantation  
67 failure at lower stress intensities than controls. Fractions indicate number of mice with implantation sites  
68 out of the total. Quantitative data are presented as mean  $\pm$  SEM. ns,  $P > 0.05$ ; \* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P$   
69  $< 0.001$ .



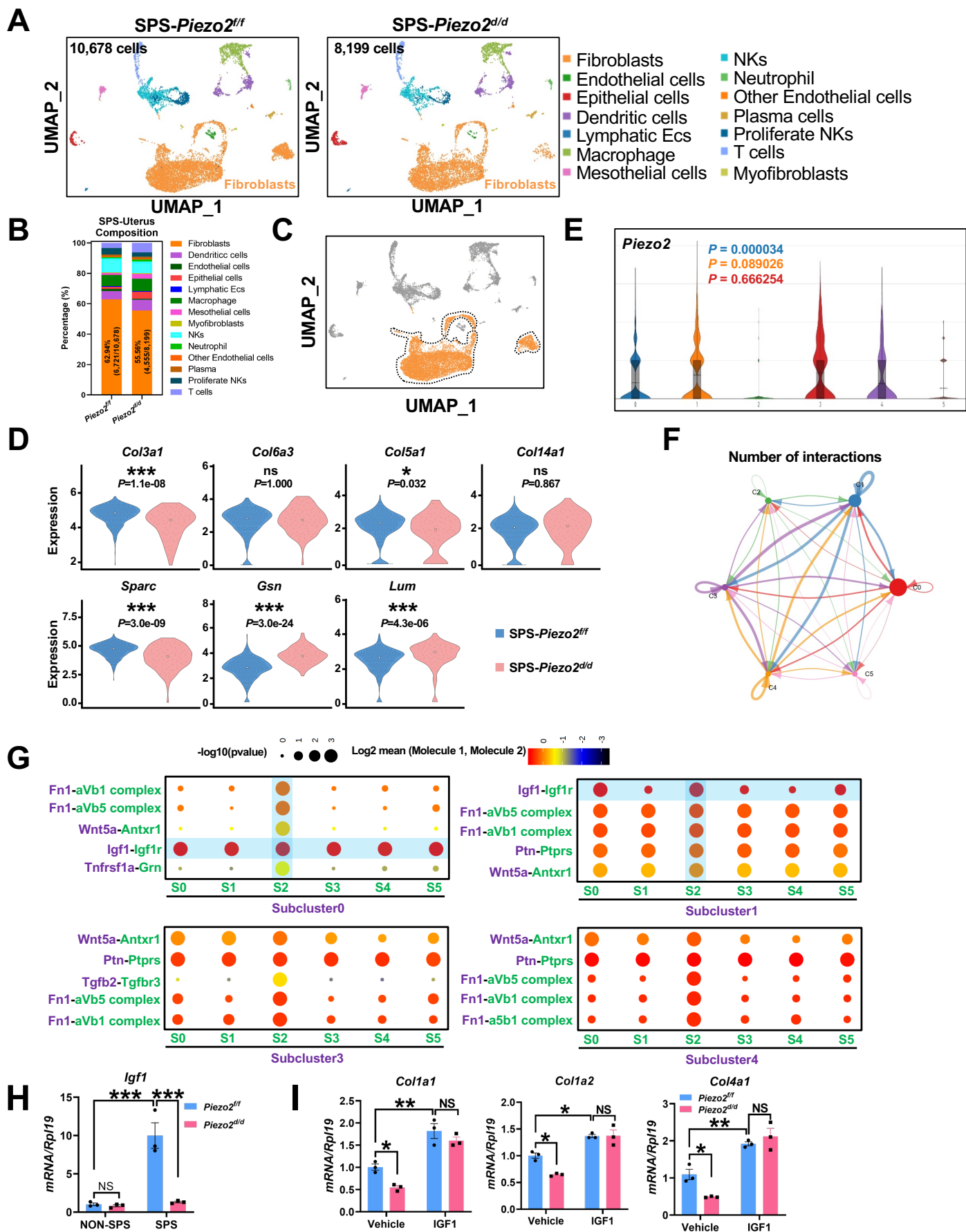
70 **Extended data Fig. 2. Ovarian function in response to stress and differential regulation in**  
71 ***Piezo2/Piezo1*.** (A) Representative images of blastocysts recovered at 3.5 dpc from *Piezo2<sup>ff/ff</sup>* and *Piezo2<sup>d/d</sup>*  
72 mice with or without SPS treatment. Blastocyst morphology was comparable across all groups. Scale bar,  
73 10  $\mu$ m. (B) Quantification of blastocyst number per mouse at 3.5 dpc across all experimental groups. No  
74 significant differences were observed (n=5, two-way ANOVA with Tukey's post hoc test). (C) Serum  
75 estradiol (E2) and progesterone (P4) levels at 3.5 dpc in *Piezo2<sup>ff/ff</sup>* and *Piezo2<sup>d/d</sup>* mice with or without SPS  
76 treatment. No significant differences were observed between genotypes irrespective of stress conditions  
77 (n = 3, two-way ANOVA with Tukey's post hoc test). (D) Single-molecule fluorescence *in situ*  
78 hybridization (smFISH) of *Piezo2* mRNA in uterine sections from *Piezo2<sup>ff/ff</sup>* and *Piezo2<sup>d/d</sup>* mice with or  
79 without SPS treatment. SPS selectively upregulated *Piezo2* expression in the endometrial stroma of  
80 *Piezo2<sup>ff/ff</sup>* mice. Arrows indicate *Piezo2*-positive cells. Scale bar, 100  $\mu$ m. (E) Quantitative RT-PCR  
81 analysis of *Piezo2* (left), *Piezo2* exons 17–18 (middle, upstream of the floxed region), and *Piezo1* (right)  
82 expression in *Piezo2<sup>ff/ff</sup>* and *Piezo2<sup>d/d</sup>* uteri with or without SPS treatment. SPS increased *Piezo2*  
83 transcription only when functional PIEZO2 protein was present (left, middle panels). n=3 or 4. Statistical  
84 significance was determined via two-way ANOVA with Tukey's post hoc test. (F) Representative uterine  
85 images and quantification of implantation sites in *Piezo1<sup>ff/ff</sup>* (control) and *Piezo2<sup>d/d</sup>* (*Pgr<sup>Cre/+</sup> Piezo1<sup>ff/ff</sup>*) mice  
86 with or without SPS treatment. Unlike *Piezo2*-cKO mice, *Piezo1*-cKO mice showed stable implantation  
87 that was unaffected by SPS (two-way ANOVA with Tukey's post hoc test). Scale bar, 5 mm. Quantitative  
88 data are presented as mean  $\pm$  SEM. ns,  $P > 0.05$ ; \* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ .

**A****B****C**

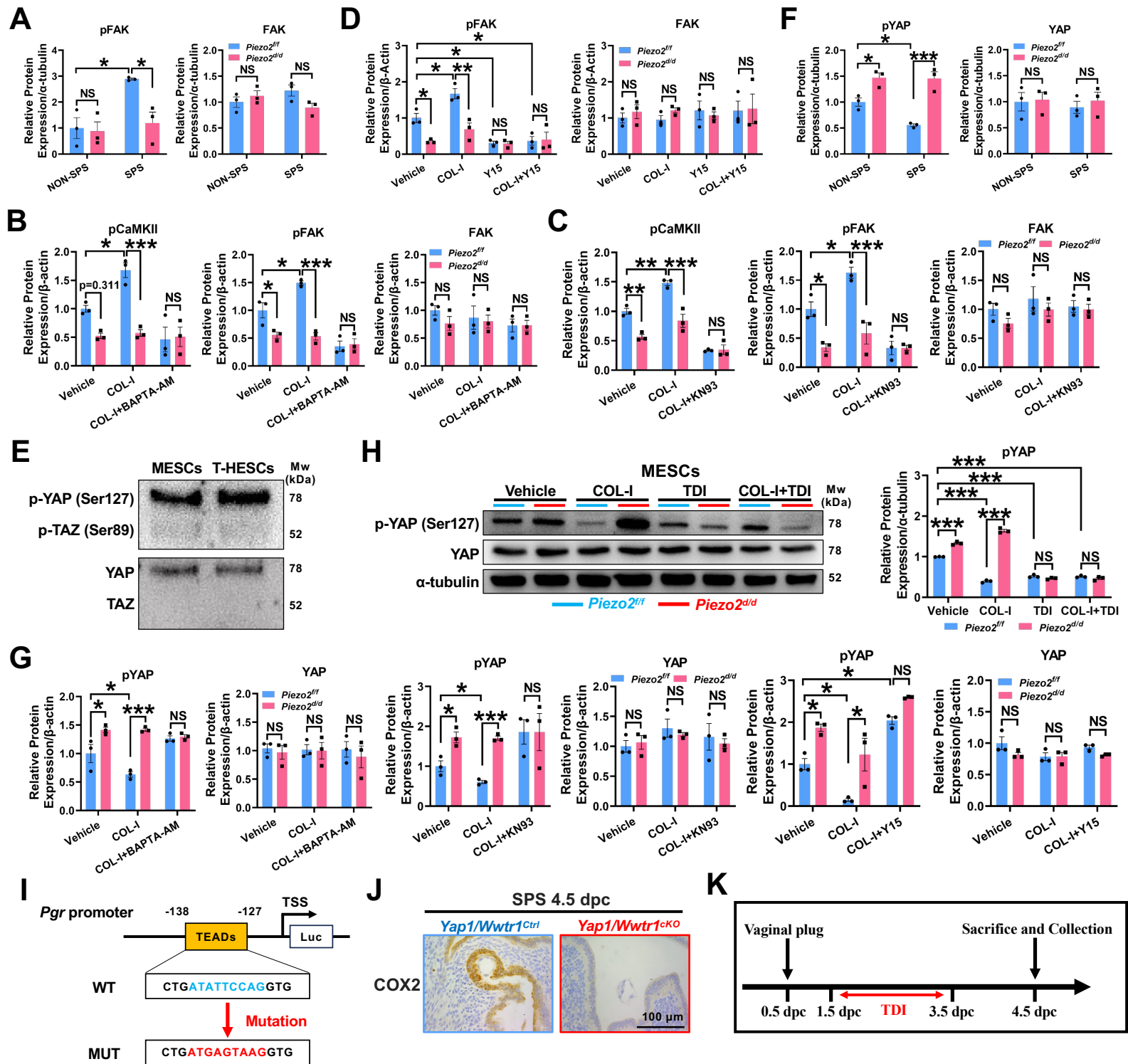
89 **Extended data Fig. 3. Exogenous progesterone supplementation normalizes gene expression in SPS-**  
90 **Piezo2-deficient mice. (A)** Schematic of the progesterone (P4) supplementation protocol. Exogenous  
91 progesterone (P4) was administered subcutaneously to SPS-*Piezo2<sup>d/d</sup>* mice at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



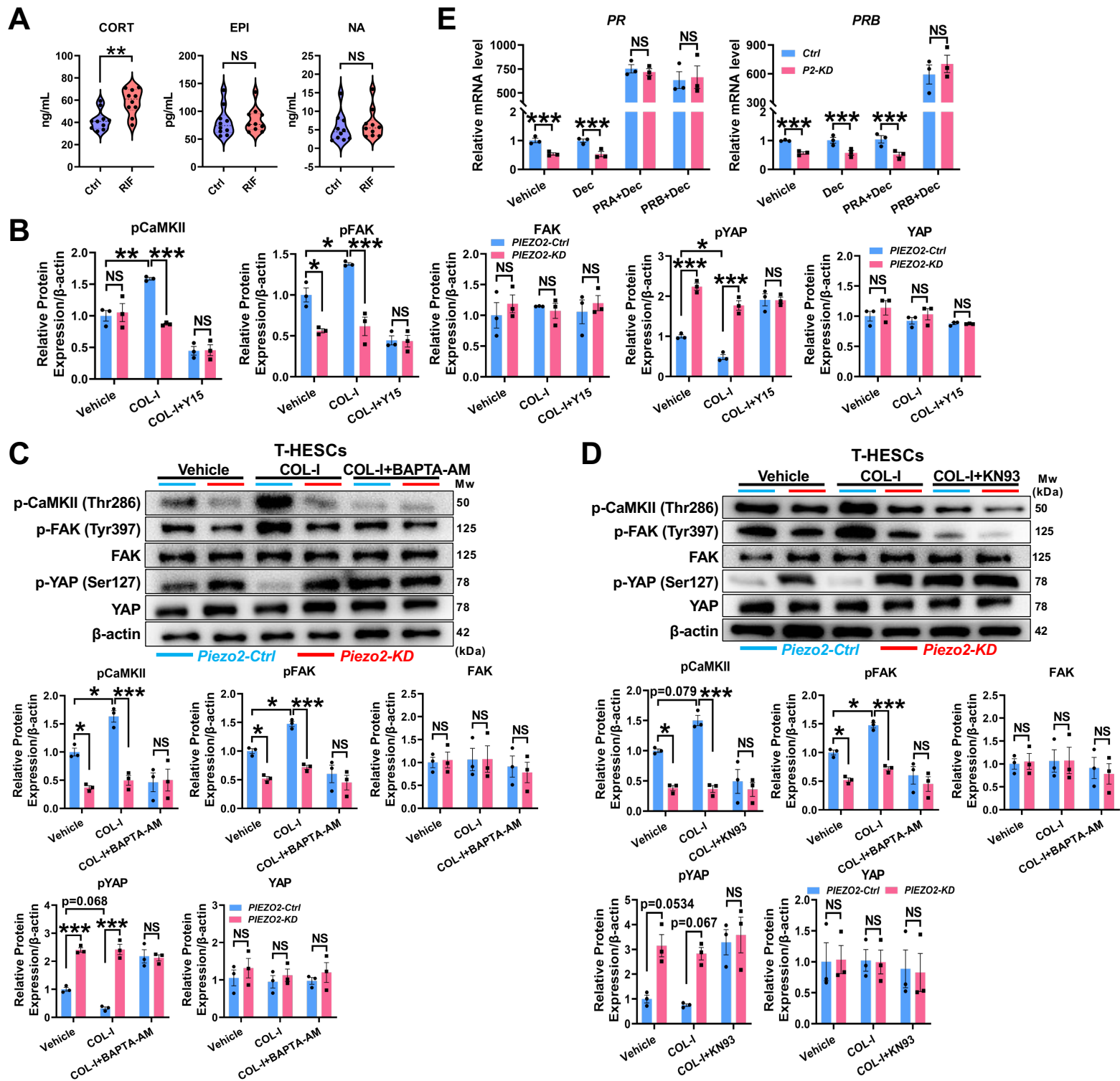
100 **Extended data Fig. 4. NOS-mediated paracrine regulation of uterine contractility in PIEZO2-**  
101 **deficient endometrium. (A)** Quantitative RT-PCR analysis of *nNos*, *iNos*, and *eNos* expression in  
102 *Piezo2<sup>ff/ff</sup>* and *Piezo2<sup>d/d</sup>* uteri with or without SPS treatment. Expression was normalized to *Rpl19* ( $n = 3$   
103 per group), with NOS isoforms significantly upregulated in *Piezo2<sup>d/d</sup>* endometrium. **(B)** Nitric oxide (NO)  
104 levels in endometrial tissue from *Piezo2<sup>ff/ff</sup>* and *Piezo2<sup>d/d</sup>* mice treated with vehicle or NOS inhibitor (L-  
105 NAME HCl). Injections were administered daily from 0.5 to 3.5 dpc, with NOS levels assessed on 3.5  
106 dpc ( $n = 3$  per group). **(C and D)** Representative *ex vivo* tension traces from *Piezo2<sup>ff/ff</sup>* (top) and *Piezo2<sup>d/d</sup>*  
107 (bottom) uteri before and after L-NAME HCl treatment **(C)**, and quantification of contraction frequency  
108 and amplitude following L-NAME HCl treatment **(D)** collected at 3.5 dpc ( $n = 5$  per group). **(E)**  
109 Representative uterine images at 4.5 dpc from *Piezo2<sup>ff/ff</sup>* and *Piezo2<sup>d/d</sup>* mice treated with L-NAME HCl  
110 daily for 4 days. Implantation sites were visualized by intravenous injection of Chicago Sky Blue dye  
111 (blue spots) and indicated with red arrowheads. Scale bar, 5 mm. **(F, G)** Histological analysis (H&E  
112 staining) **(F)** and immunohistochemistry detecting COX2 **(G)** in uterine sections at 4.5 dpc following L-  
113 NAME HCl treatment. Scale bar, 100  $\mu$ m. Quantitative data are presented as mean  $\pm$  SEM. Statistical  
114 significance was determined using a two-way ANOVA with Tukey's post hoc test. ns,  $P > 0.05$ ; \* $P <$   
115  $0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ .



116 **Extended data Fig. 5. scRNA-seq-based characterization of uterine cell populations and stromal**  
117 **paracrine signaling.** (A) UMAP visualization of cell clusters identified by scRNA-seq in SPS-*Piezo2<sup>ff/ff</sup>*  
118 (10,678 cells) and SPS-*Piezo2<sup>d/d</sup>* (8,199 cells) uteri at 3.5 dpc. Each point represents a single cell and is  
119 colored according to its assigned cell cluster ( $n = 14$ ). (B) Stacked bar plot showing the distribution of  
120 each cell type identified by scRNA-seq in SPS-*Piezo2<sup>ff/ff</sup>* versus SPS-*Piezo2<sup>d/d</sup>* uteri. Stromal fibroblasts  
121 were the largest population in both genotypes but were reduced in PIEZO2-deficient uteri relative to  
122 controls (62.94% vs. 55.56%). (C) UMAP visualization of uterine stromal cell (uSC) subclusters after  
123 fibroblast sub-clustering. Dashed circle indicates the uterine stromal cells. (D) Violin plots showing  
124 collagen and extracellular matrix-associated gene expression in mesenchymal fibroblast in subcluster 2  
125 from SPS-*Piezo2<sup>ff/ff</sup>* (Ctrl, blue) and SPS-*Piezo2<sup>d/d</sup>* (KO, pink) uteri. Statistical significance was determined  
126 by Wilcoxon rank-sum test with Bonferroni correction. (E) Violin plots showing *Piezo2* expression levels  
127 across the six fibroblast subclusters. (F) Chord diagram of predicted ligand–receptor interactions among  
128 fibroblast subclusters generated with CellPhoneDB. Node size represents the relative abundance of  
129 predicted interactions for each subcluster, and line thickness indicates the relative interaction strength. (G)  
130 Dot plots showing the top five most significant ligand–receptor pairs for each PIEZO2-expressing  
131 fibroblast subcluster compared to all other subclusters. Dot size represents the  $-\log_{10}(P \text{ value})$ , while the  
132 color gradient represents the communication probability ( $\log_2$  mean expression of the ligand–receptor  
133 pair). Blue shading highlights targeted paracrine signaling directed toward the ECM-producing subcluster  
134 2. (H) Quantitative RT-PCR analysis of *Igfl* expression in *Piezo2<sup>ff/ff</sup>* and *Piezo2<sup>d/d</sup>* uteri with or without  
135 SPS treatment. Expression was normalized to *Rpl19* ( $n = 3$  per group, two-way ANOVA with Tukey's  
136 post hoc test). (I) Quantitative RT-PCR analysis of collagen gene expression (*Colla1*, *Colla2*, *Col4a1*)  
137 in *Piezo2<sup>ff/ff</sup>* and *Piezo2<sup>d/d</sup>* cells supplemented with IGF1. Cells were cultured with IGF1 (100 ng/mL) or  
138 vehicle for 24 h prior to RNA extraction. Expression was normalized to *Rpl19* ( $n = 3$  per group, two-way  
139 ANOVA with Tukey's post hoc test). Quantified data are presented as mean  $\pm$  SEM. ns,  $P > 0.05$ ; \* $P <$   
140 0.05; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ .



141 **Extended data Fig. 6. Hippo–YAP pathway validation and Pgr promoter analysis.** (A) Quantification  
142 of p-FAK (Tyr397) and FAK protein levels normalized to  $\alpha$ -tubulin, corresponding to the Western blot  
143 shown in Fig. 5A.  $n = 3$  biological replicates. (B) Quantification of p-CaMKII, p-FAK (Tyr397), and FAK  
144 protein levels normalized to  $\beta$ -actin, corresponding to the Western blot shown in Fig. 5B.  $n = 3$  biological  
145 replicates. (C) Quantification of p-CaMKII, p-FAK (Tyr397), and FAK protein levels normalized to  $\beta$ -  
146 actin, corresponding to the Western blot shown in Fig. 5C.  $n = 3$  biological replicates. (D) Quantification  
147 of p-FAK (Tyr397) and FAK protein levels normalized to  $\beta$ -actin, corresponding to the Western blot  
148 shown in Fig. 5E.  $n = 3$  biological replicates. (E) Western blot analysis of p-YAP (Ser127) and p-TAZ  
149 (Ser89) protein levels, along with total YAP and TAZ, in mouse endometrial stromal cells (MESC)s and  
150 human endometrial stromal cells (T-HESC)s. (F) Quantification of p-YAP (Ser127) and YAP protein  
151 levels normalized to  $\alpha$ -tubulin, corresponding to the Western blot shown in Fig. 5G.  $n = 3$  biological  
152 replicates. (G) Quantification of p-YAP (Ser127) and YAP protein levels normalized to  $\alpha$ -tubulin,  
153 corresponding to the Western blot shown in Fig. 5I.  $n = 3$  biological replicates. (H) Western blot analysis  
154 of p-YAP (Ser127) and total YAP in primary mouse endometrial stromal cells (MESC)s from *Piezo2<sup>fl/fl</sup>*  
155 and *Piezo2<sup>d/d</sup>* mice treated with TDI. Cells were cultured with COL-I coating, TDI (no COL-I), or COL-  
156 I+TDI, with a control cultured without COL-I coating or treatment (Vehicle). Cultures were maintained  
157 for 48 h and then lysed. Relative expression was determined by normalizing to  $\alpha$ -tubulin and based on  $n$   
158  $= 3$  per group (two-way ANOVA with Tukey's post hoc test, graph on right). Data are presented as mean  
159  $\pm$  SEM. ns,  $P > 0.05$ ; \*\*\* $P < 0.001$ . (I) Schematic of the *Pgr* promoter region showing the predicted  
160 TEAD-binding element (TRE) located at positions  $-138$  to  $-127$  relative to the transcription start site  
161 (TSS), as identified using the JASPAR transcription factor binding site database (<https://jaspar.elixir.eu>).  
162 The wild-type sequence (AGGTGGGAAG) and mutant sequence (AGGATTTAAG) used in luciferase  
163 reporter assays are indicated. (J) Schematic of the TDI-011536 (TDI) treatment protocol for *in vivo* rescue  
164 experiments. TDI ( $3 \mu\text{M}$ ) was administered daily from 1.5 to 3.5 dpc, and implantation was assessed at  
165 4.5 dpc. Quantitative data are presented as mean  $\pm$  SEM. Statistical significance was determined using a  
166 two-way ANOVA with Tukey's post hoc test. ns,  $P > 0.05$ ; \* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ .



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

183 **Extended data Table 1. Fertility assessment of *Piezo2<sup>ff</sup>* and *Piezo2<sup>d/d</sup>* females**

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

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

**Extended data Table 2. Patient clinical characteristics**

| Category           | ID  | Age | BMI<br>(Kg/m <sup>2</sup> ) | Fertility<br>history | Pathology                                 | Time of<br>embryo<br>transfer | Sample<br>collection<br>phase                      | Embryo<br>transfer<br>day                              | Numbers<br>of live<br>births | Embryo<br>quality<br>grade | Endocrine<br>metabolic<br>abnormality |
|--------------------|-----|-----|-----------------------------|----------------------|-------------------------------------------|-------------------------------|----------------------------------------------------|--------------------------------------------------------|------------------------------|----------------------------|---------------------------------------|
| Pregnant<br>(n=18) | B1  | 33  | 22.24                       | Yes                  | mechanical<br>obstruction<br>of fallopian | 1                             | HRT cycle<br>mid-<br>secretory<br>phase<br>(LHP+7) | LHP+5<br>(Next<br>cycle after<br>sample<br>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<br>(n=18)      | B9  | 35  | 21.88                       | No                   | Unexplained                               | 4                             | HRT cycle<br>mid-<br>secretory<br>phase<br>(LHP+7) | LHP+5<br>(Next<br>cycle after<br>sample<br>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       |
| <i>p</i> -value | 0.3016 | 0.2752 | <0.0001 |

| Gene            | Primer application | Forward sequence               | Reverse sequence               |
|-----------------|--------------------|--------------------------------|--------------------------------|
| <i>mPiezo2</i>  | qPCR               | 5'-CCCGGAACAGTAGCCAGAAA-3'     | 5'-TTCCCAAAGGCCCCAGAATCC-3'    |
| <i>mPiezo2-</i> | qPCR               | 5'-ACTCGTCTGCATCCTACACC-3'     | 5'-GGTACACGCGACCATTGACT-3'     |
| <i>E17/18</i>   | qPCR               | 5'-GCTGGCTTCTGGAGGTGACTTG-3'   | 5'-GGTACACGCGACCATTGACT-3'     |
| <i>mPiezo1</i>  |                    |                                |                                |
| <i>mPgr</i>     | qPCR               | 5'-GCAATGGAAGGGCAGCATAAC-3'    | 5'-CTTACGACCTCCAAGGACCA-3'     |
| <i>mEsr1</i>    | qPCR               | 5'-GGAAGCTCCTGTTTGCTCCT-3'     | 5'-AACCGACTTGACGTAGCCAG-3'     |
| <i>mRpl19</i>   | qPCR               | 5'-TCATGGAGCACATCCACAAGCTGA-3' | 5'-CGCTTTCGTGCTTCCTTGGTCTTA-3' |
| <i>mLif</i>     | qPCR               | 5'-ATTGAGCTTGACCTGGAGGC-3'     | 5'-GGTGGAGCTGTATCGGATGG-3'     |
| <i>mNos1</i>    | qPCR               | 5'-CCTTCACAGGGGATGGAACC-3'     | 5'-AGATCGACAGCTTTGGTGGG-3'     |
| <i>mNos2</i>    | qPCR               | 5'-AGAGCCACAGTCCTCTTGC-3'      | 5'-CTGGTCCATGCAGACAACCT-3'     |
| <i>mNos3</i>    | qPCR               | 5'-ATTGGCATGAGGGACCTGTG-3'     | 5'-GGGATGAGGTTGTCCTGGTG-3'     |
| <i>mLtf</i>     | qPCR               | 5'-CCCTACAACTGCGACCTGT-3'      | 5'-CACGACTGCTACCGCATAGT-3'     |
| <i>mMuc1</i>    | qPCR               | 5'-TCACCCCAGTTGTCTGTTGG-3'     | 5'-AGTAGTTGGAGCTGGGGTCT-3'     |
| <i>mMuc4</i>    | qPCR               | 5'-ATGGCTACAAAGGCTACCACC-3'    | 5'-CCTCACTACATGGGGACACAC-3'    |
| <i>mHand2</i>   | qPCR               | 5'-CCAGCTACATCGCCTACCTC-3'     | 5'-GGTCTTCTTGATCTCCGCCT-3'     |
| <i>mHoxa10</i>  | qPCR               | 5'-TCCAAAGGCGAAAATGCAGC-3'     | 5'-TCTGGTGCTTCGTGTAAGGG-3'     |
| <i>mAreg</i>    | qPCR               | 5'-ACAGCGAGGATGACAAGGAC-3'     | 5'-GCCAATAGCTGCGAGGATGA-3'     |
| <i>mIhh</i>     | qPCR               | 5'-TCTGTCTGTTCCCTGCTGCTG-3'    | 5'-TTGTAGGCAAGAGGCACGAG-3'     |
| <i>mBmp2</i>    | qPCR               | 5'-AAGCCAAACACAAACAGCGG-3'     | 5'-CGATCCAGTCATTCCACCCC-3'     |
| <i>mPrl8a2</i>  | qPCR               | 5'-GGTGCATGGATCACTCCTGT-3'     | 5'-ACCCACGTAAGGTCATCATGG-3'    |
| <i>mPrl3c1</i>  | qPCR               | 5'-ACTGCCACACGATATGACCG-3'     | 5'-TGTGACGAGAAGAGGAAAGCA-3'    |
| <i>mColla1</i>  | qPCR               | 5'-AGCACGTCTGGTTTGGAGAG-3'     | 5'-GACATTAGGCGCAGGAAGGT-3'     |
| <i>mColla2</i>  | qPCR               | 5'-CTAGCCAACCGTGCTTCTCA-3'     | 5'-TCTCCTCATCCAGGTACGCA-3'     |
| <i>mCol3a1</i>  | qPCR               | 5'-ACGTAAGCACTGGTGGACAG-3'     | 5'-CAGGAGGGCCATAGCTGAAC-3'     |
| <i>mCol4a1</i>  | qPCR               | 5'-TCATTAGCAGGTGTGCGGTT-3'     | 5'-GTTAGGGCACTGCGGAATCT-3'     |
| <i>mIl1b</i>    | qPCR               | 5'-ATGCCACCTTTTGACAGTGAT-3'    | 5'-AGCCCTTCATCTTTTGGGGT-3'     |
| <i>mIl6</i>     | qPCR               | 5'-CCCCAATTTCGAATGCTCTCC-3'    | 5'-GGATGGTCTTGGTCCTTAGCC-3'    |
| <i>mTnfa</i>    | qPCR               | 5'-GATCGGTCCCCAAAGGGATG-3'     | 5'-CCACTTGGTGGTTTGTGAGTG-3'    |
| <i>mIgfl</i>    | qPCR               | 5'-TGGGGAAAATCAGCAGCCTT-3'     | 5'-CAGAGCGCCAGGTAGAAGAG-3'     |
| <i>hGAPDH</i>   | qPCR               | 5'-GGCAAATTCCATGGCACCG-3'      | 5'-AGCATCGCCCCACTTGATTT-3'     |
| <i>hPIEZO2</i>  | qPCR               | 5'-AAGGCAGTCCACCCTTGATG-3'     | 5'-TGGGCTCGCTTGATGCTAAA-3'     |
| <i>hPGR-B</i>   | qPCR               | 5'-GGAATGGGCTGTACCGAGAG-3'     | 5'-CGGCTCCTTTATCTCCCGAC-3'     |
| <i>hPGR</i>     | qPCR               | 5'-CCGGGCACTGAGTGTTGAAT-3'     | 5'-GAAGGGGTTTCACCATCCCT-3'     |

|                                    |                                |                                                                                                                                                                                                                                                                                                      |                             |
|------------------------------------|--------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------|
| <i>hCOL1A1</i>                     | qPCR                           | 5'-GGGAGATGCTGGTCCTGCT-3'                                                                                                                                                                                                                                                                            | 5'-GCACCATCATTTCCACGAGC-3'  |
| <i>hIGFBP1</i>                     | qPCR                           | 5'-TGTGACGAGAAGAGGAAAGCA-3'                                                                                                                                                                                                                                                                          | 5'-CTCCTGATGTCTCCTGTGCC-3'  |
| <i>hFOXO1</i>                      | qPCR                           | 5'-TGGAGATCGACCCGGACTTC-3'                                                                                                                                                                                                                                                                           | 5'-CCGAGTTGGACTGGCTAAACT-3' |
| <i>hPRL</i>                        | qPCR                           | 5'-GGGGTTCATTACCAAGGCCA-3'                                                                                                                                                                                                                                                                           | 5'-TTGGGCTTGCTCCTTGTCTT-3'  |
| <i>hIGF1</i>                       | qPCR                           | 5'-ATGTATTGCGCACCCCTCAA-3'                                                                                                                                                                                                                                                                           | 5'-GCACTCCCTCTACTTGCGTT-3'  |
| <i>siPIEZO2</i>                    | RNA interference               | 5'-GCTTGAGGATCTTGGCTTA-3'                                                                                                                                                                                                                                                                            |                             |
| <i>Padlock</i>                     | sm-ISH                         | 5'-TCTTTATGGTAAACGCCTTTGTTCCCTTTTACGACCTCAATG                                                                                                                                                                                                                                                        |                             |
| <i>Probe-PIEZO2</i>                |                                | CTGCTGCTGTACTACTCTTGACATAGATCTGTAGAGTCT-3'                                                                                                                                                                                                                                                           |                             |
| <i>Oligo-dT</i>                    | sm-ISH                         | 5'-AAGCAGTGGTATCAACGCAGAGTACTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTVN-3'                                                                                                                                                                                                                                  |                             |
| <i>RCA primer</i>                  | sm-ISH                         | 5'-ACAGCAGCAGCATTGAGG*T*C-3' Note: *= thiophosphate modifications                                                                                                                                                                                                                                    |                             |
| <i>HRP labeled detection probe</i> | sm-ISH                         | 5'- HRP-CCTCAATGCTGCTGCTGTACTAC-3'                                                                                                                                                                                                                                                                   |                             |
| <i>Pgr Promoter-WT</i>             | Dual-Luciferase Reporter Assay | 5'-<br>GCTTTTTATAGGGTGGGGCTGGCATGCTTCTTAATTCAGTACAAATTCTGATATGTTT<br>AGATTTCTTCTCAAAGCATCTGATATTCAGGTGGAATCAGACTAGTGAGTAGCTGG<br>AATCCAACGCCAGAGATTAGATCTAGCCAGTGATTGGCTAGGGAGGGGCTTTGGG<br>CGGGCCTTCCTAGAGCGCCAACGCTTGCTAGAAAGCTATGGAGCCAGTCTAGACTGT<br>CACTATCAGTCTTTGTAGTATTTACGGGTGCGAGGATTC-3'  |                             |
| <i>Pgr Promoter-Mut</i>            | Dual-Luciferase Reporter Assay | 5'-<br>GCTTTTTATAGGGTGGGGCTGGCATGCTTCTTAATTCAGTACAAATTCTGATATGTTT<br>AGATTTCTTCTCAAAGCATCTGATGAGTAAGGTGGAATCAGACTAGTGAGTAGCTGG<br>AATCCAACGCCAGAGATTAGATCTAGCCAGTGATTGGCTAGGGAGGGGCTTTGGG<br>CGGGCCTTCCTAGAGCGCCAACGCTTGCTAGAAAGCTATGGAGCCAGTCTAGACTGT<br>CACTATCAGTCTTTGTAGTATTTACGGGTGCGAGGATTC-3' |                             |
| <i>Piezo2-WT</i>                   | Genotyping                     | 5'-ACTTAGATGGGGCAGGTGCT-3'                                                                                                                                                                                                                                                                           | 5'-ACTTCCCTACCCACCCATTC-3'  |
| <i>Piezo2-flox</i>                 | Genotyping                     | 5'-GCCGCTCTAGAACTAGTGGA-3'                                                                                                                                                                                                                                                                           | 5'-ATCTACCACGGGGGCTCTCTC-3' |
| <i>Pgr-WT</i>                      | Genotyping                     | 5'-TATACCGATCTCCCTGGACG-3'                                                                                                                                                                                                                                                                           | 5'-CCCAAAGAGACACCAGGAAG-3'  |
| <i>Pgr-Cre</i>                     | Genotyping                     | Same as <i>Pgr-WT</i>                                                                                                                                                                                                                                                                                |                             |

**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    |
| ER $\alpha$                | Rabbit IgG | Santa Cruz           | sc7207      | IHC         | 1:200    |
|                            |            | Biotechnology        |             |             |          |
| Biotinylated secondary     | Goat anti- | Vactorlabs           | BA-1000     | IHC         | 1:200    |
|                            | Rabbit IgG |                      |             |             |          |
| Collagen I                 | Rabbit IgG | Abways               | ab138492    | IF          | 1:100    |
| Fluorescent secondaries    | Goat anti- | Jackson              | 711-225-152 | IF          | 1:200    |
|                            | Rabbit IgG |                      |             |             |          |
| 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   |
| $\alpha$ -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   |
| $\beta$ -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.