Supporting Information for

Cannabidiol modulates PIEZO1 activity to regulate uterine contractility and pregnancy outcome.

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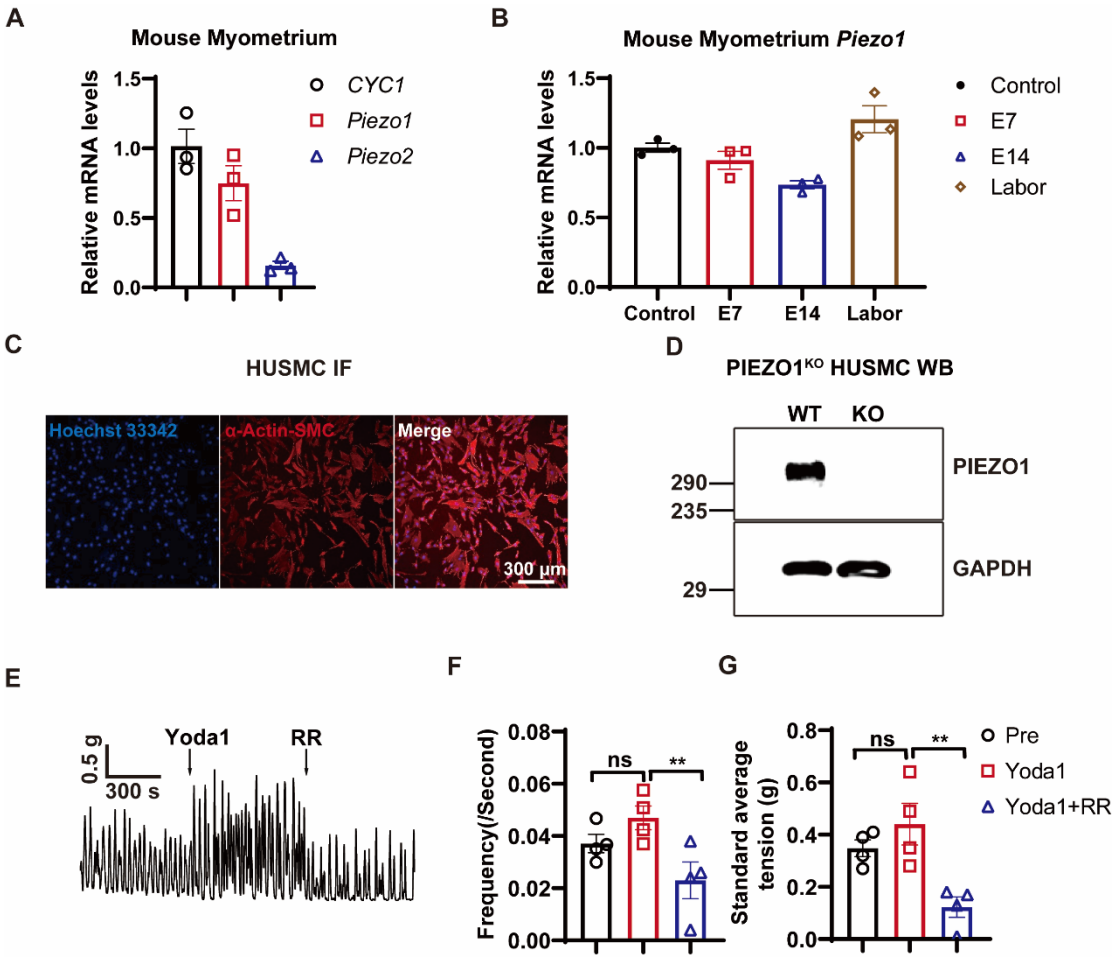

Fig. S1. Mouse myometrium qPCR sequence and immunofluorescence staining of human primary uterine smooth muscle cells.

(A), Piezo1 and Piezo2 mRNA expression measured via RT-qPCR in the mouse myometrium. All gene expression levels were normalized to GAPDH, and subsequently to Cytochrome C1 (*CYC1*). (*n* = 3 from three mice) (B), Piezo1 mRNA levels in mouse myometrium across pregnancy stages showing increased expression during labor. Expression normalized to *CYC1* and expressed relative to control. (*n* = 3 from three mice) (C), Immunofluorescence of PIEZO1 (red) in primary human myometrial smooth muscle cells (HUSMCs) and counterstained with a nuclear marker (blue). Images are representative of three experiments. Scale bar, 300 μm. All images in (C) are representative of at least three independent experiments. (D), Representative western blot images verified Piezo1 knockout (KO) efficiency in HUSMCs. (E), Representative curve of spontaneous contractions of isolated mouse uterus treated with 3 μM Yoda1 stimulation and 30 μM Ruthenium Red(RR). 20mM tetraethylammonium (TEA) in the bath solution blocked the potassium channel. Each segment was recorded for at least 10 minutes. All traces in (E) are representative of at least three independent experiments. (F and G), Quantification of contractile frequency (F) and standardized mean contractile tension (G) demonstrating RR inhibition of Yoda1-induced contractility. (*n* = 4, ns $P > 0.5$, ** $P < 0.01$, Student's paired *t*-test; mean ± SEM).

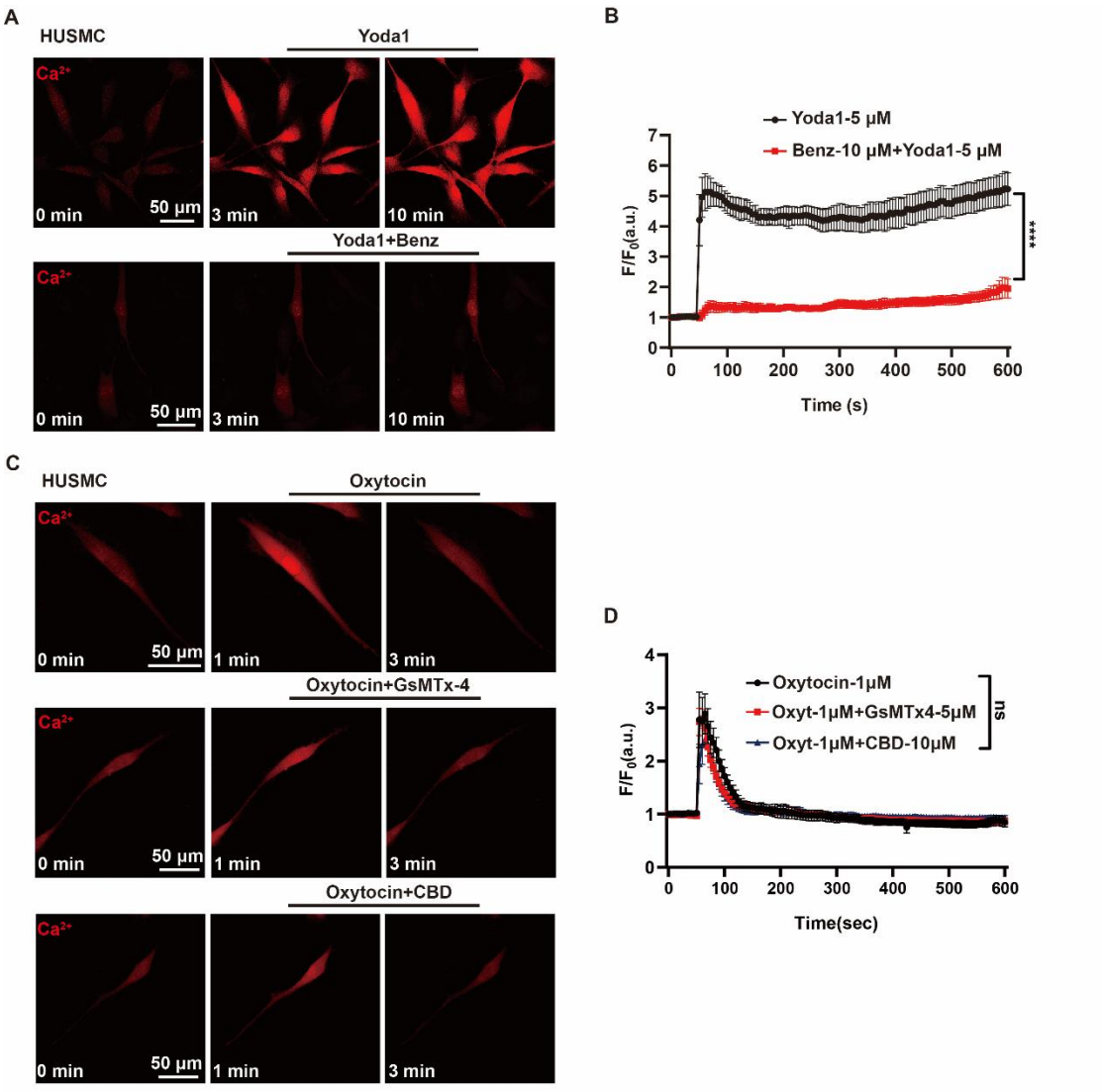

Fig. S2. CBD does not inhibit calcium influx induced by oxytocin.

(A and B), Representative live-cell fluorescence images (A) and quantification (B) of HUSMCs showing transient intracellular Ca^{2+} elevation (red) following Yoda1 (5 μM) treatment. Benzbromarone (10 μM) inhibits Yoda1-induced Ca^{2+} responses. Scale bar, 50 μm. All images in (A) are representative of at least three independent experiments. n = 7 cells. **** $P < 0.0001$, two-way ANOVA. (C and D), Representative live-cell fluorescence images (C) and quantification (D) of HUSMCs showing transient intracellular Ca^{2+} elevation (red) following oxytocin (1 μM) treatment. Co-treatment with GsMTx-4 (5 μM) or cannabidiol (CBD, 10 μM) does not affect oxytocin-induced Ca^{2+} responses. Scale bar, 50 μm. All images in (C) are representative of at least three independent experiments. Oxytocin 1 μM, n = 6 cells; GsMTx-4 5 μM, n = 8 cells; CBD 10 μM, n = 8 cells, *ns* $P > 0.05$, two-way ANOVA.

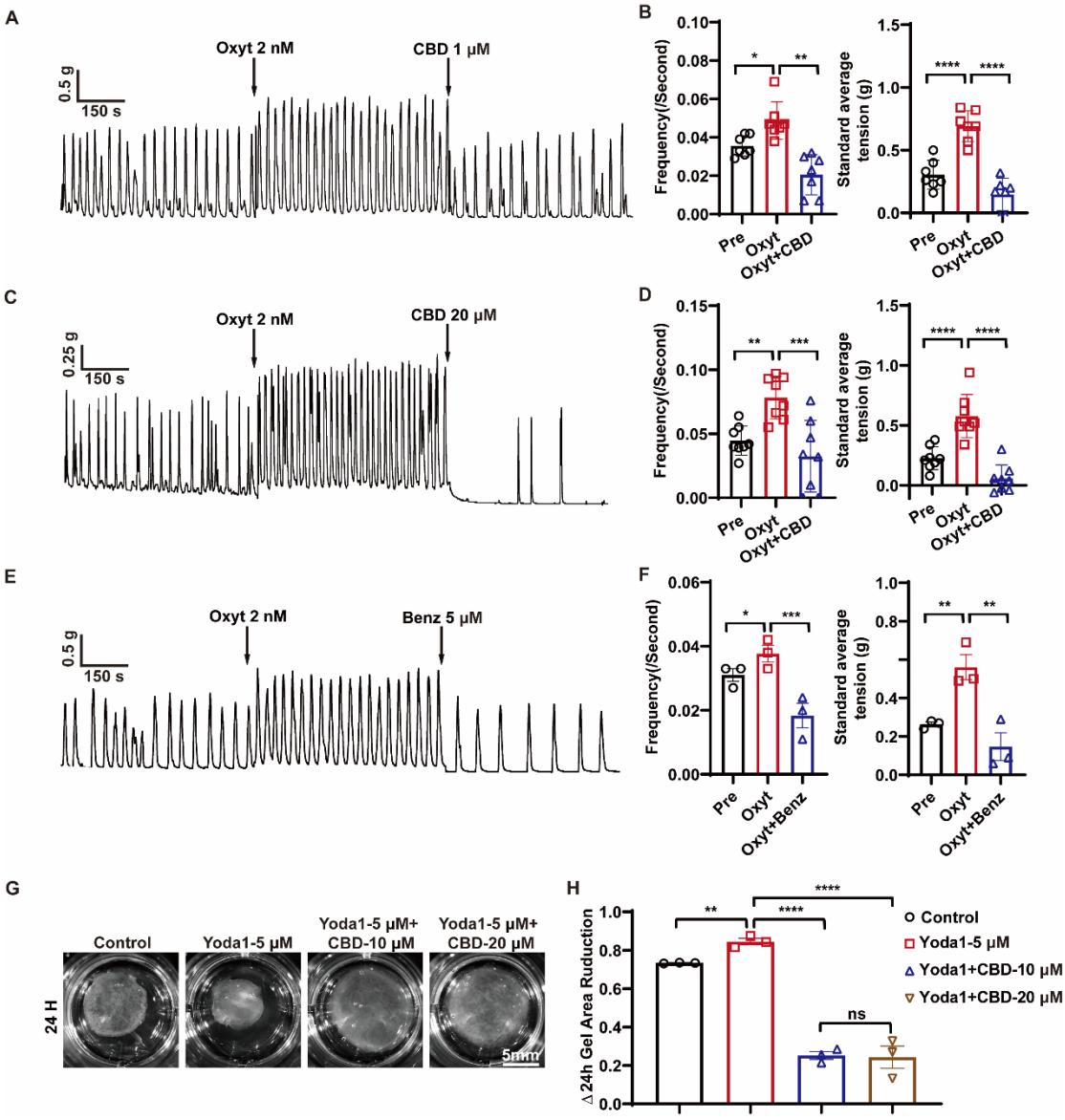

Fig. S3. Effects of different concentrations of CBD on spontaneous uterine contractions induced by oxytocin in isolated mouse uterus.

(A), Representative trace of spontaneous contractions in isolated mouse uterus following oxytocin (2 nM) stimulation, followed by CBD (1 μM) treatment. Recording duration ≥ 10 minutes per segment. All traces in (A) are representative of at least three independent experiments. (B), Quantification of contractile frequency and standardized mean contractile tension from experiments in (A) under different periods (n = 7, * P < 0.05, ** P < 0.01, **** P < 0.0001, Student's paired t-test; mean ± SEM). (C), Representative trace of spontaneous contractions in isolated mouse uterus following oxytocin (2 nM) stimulation, followed by CBD (20 μM) treatment. Recording duration ≥ 10 minutes per segment. All traces in (C) are representative of at least three independent experiments. (D), Quantification of contractile frequency and standardized mean contractile tension from experiments in (C) under different periods (n = 8, ** P < 0.01, *** P < 0.001, **** P < 0.0001, Student's paired t-test; mean ± SEM). (E), Representative trace of spontaneous contractions in isolated mouse uterus following oxytocin (2 nM) stimulation, followed by Benzbromarone (Benz 5 μM) treatment. Recording duration ≥ 10 minutes per segment. All traces in (E) are representative of at least three independent experiments. (F), Quantification of contractile frequency and

standardized mean contractile tension from experiments in (E) under different periods (n = 3, * P < 0.05, ** P < 0.01, *** P < 0.001, Student's paired t-test; mean ± SEM). (G and H), Collagen gel contraction assays after 24 h treatment. Representative images (G) and quantification of gel area (H) show control, Yoda1 (5 μM), Yoda1 (5 μM) + CBD (10 μM), and Yoda1 (5 μM) + CBD (20 μM) conditions. Scale bar, 5 mm. Data representative of three independent experiments. (n = 3 independent experiments, *ns* P > 0.05, ** P < 0.01, **** P < 0.0001, Student's unpaired t-test; mean ± SEM).

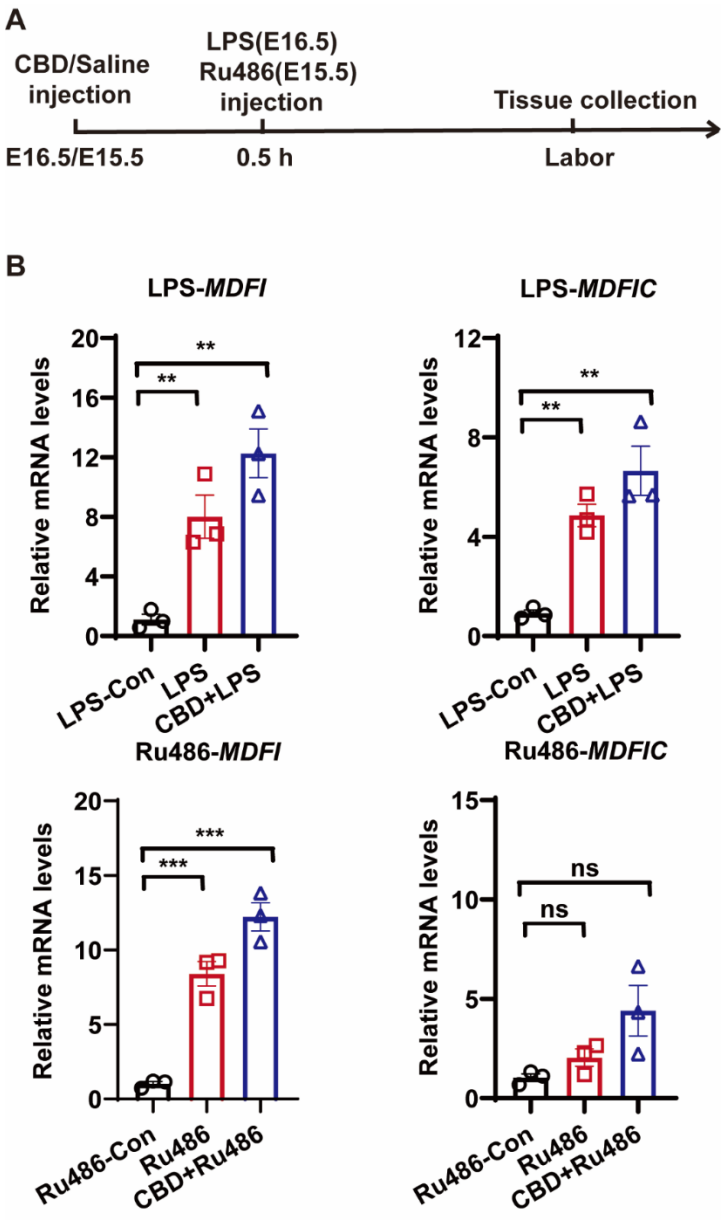

Fig. S4. Preterm Birth Model and qPCR Analysis of Preterm Birth Models in Mouse Myometrium.

(A), Flow chart representation of the preterm birth model. (B), Relative mRNA levels of mMDFI and mMDFIC in uterine tissues from the myometrium of mice from preterm birth models. *n* = 3 mice/group. *ns* $P > 0.05$, ** $P < 0.01$, *** $P < 0.001$, Student's unpaired *t*-test; mean ± SEM.

Table S1. Primers used for q-PCR

Primer	Sequence
hPIEZO1-F1	GCCGAGAGACAGAGAAGAAATAC
hPIEZO1-R1	GCGATGAGGAAGAGGATGATG
hPIEZO2-F1	CGGGAGGATGAACCAATCAA
hPIEZO2-R1	CCAGAAATAGGACCCAGGTAAAT
hCYC1-F1	CAGCTTCCATTGCGGACAC
hCYC1-R1	GGCACTCACGGCAGAATGAA
hMDFI-F1	CTGGAGGTAGTAACAGGATCCACTC
hMDFI-R1	CGATGTTGCACAGCGTCAGGAACTC
hMDFIC-F1	GGAAATCCTTCGGATGGTGAACTC
hMDFIC-R1	CAAGCAAGCCAGGATACAGTGGACAC
mPieoz1-F1	GCAGCCGAGAGACAGAGAAG
mPiezo1-R1	CACAGAGCGGATCAGTGACA