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Supplementary Materials for

Enhanced drug delivery to the reproductive tract using nanomedicine reveals therapeutic options for prevention of preterm birth

Hannah C. Zierden, Jairo I. Ortiz, Kevin DeLong, Jingqi Yu, Gaoshan Li, Peter Dimitrion, Sabrine Bensouda, Victoria Laney, Anna Bailey, Nicole M. Anders, Morgan Scardina, Mala Mahendroo, Sam Mesiano, Irina Burd, Gunter Wagner, Justin Hanes, Laura M. Ensign*

*Corresponding author. Email: lensign@jhmi.edu

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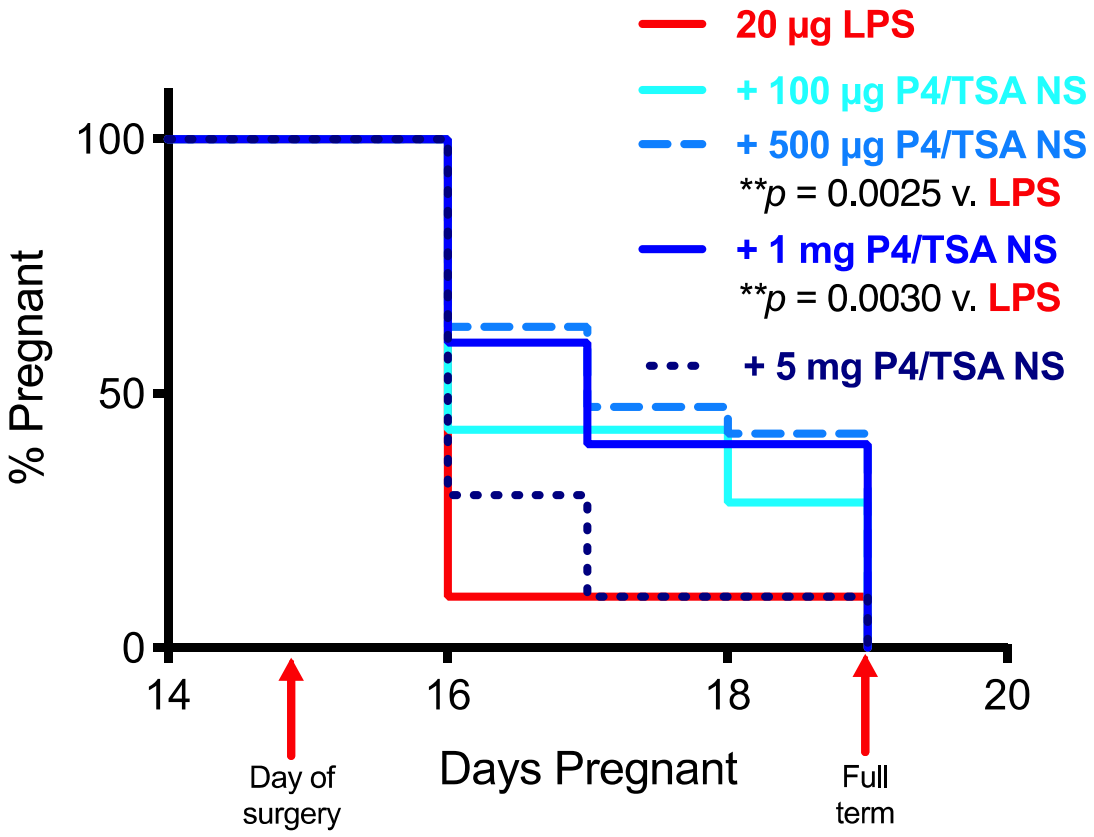

Fig. S1. PTB prevention for various doses of P4 with TSA. Pregnancy survival curves showing the percentage of animals remaining pregnant after DDI of 20 µg LPS (n=20) on E15. Daily vaginal administration of 15 µg TSA NS with either 100 µg P4 NS (n=7), 500 µg P4 NS (n=19), 1 mg P4 NS (n=20, repeated from Figure 3B), or 5 mg P4 NS (n=10). *p <0.01, via Mantel-Cox test.

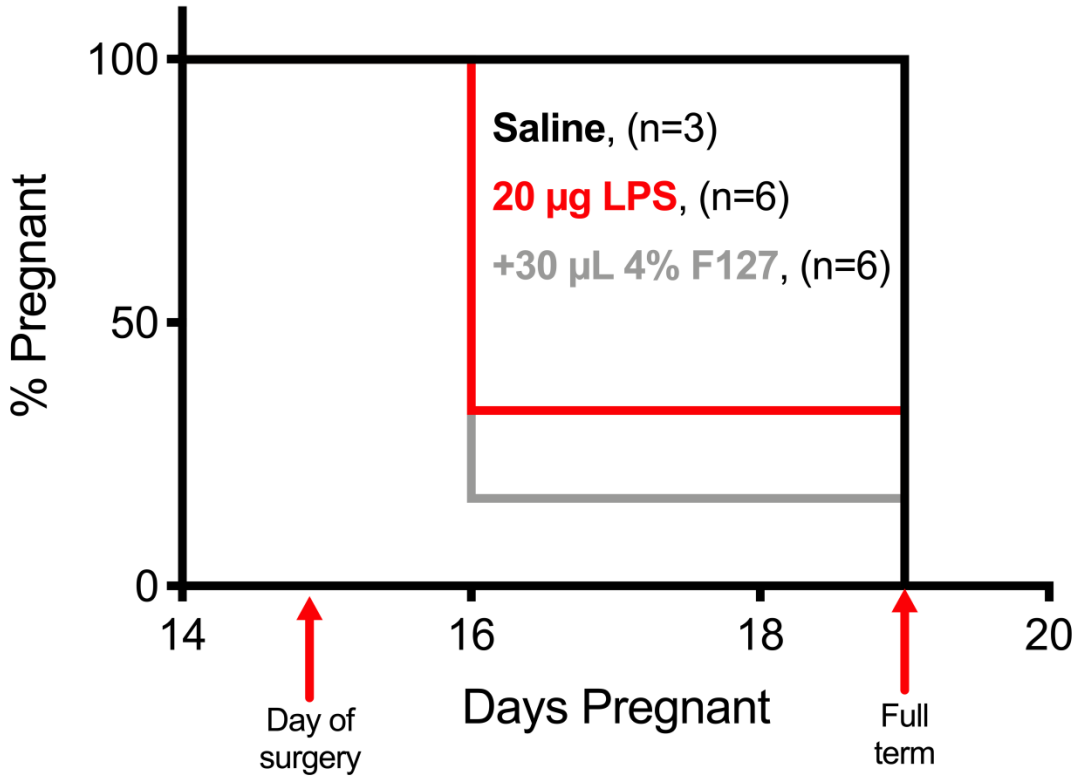

Fig. S2. PTB prevention for F127 vehicle control. Pregnancy survival curves showing the percentage of animals remaining pregnant after DDI of LPS on E15. Daily vaginal administration of 4% F127 vehicle control (n=6) had no effect on PTB induction after 20 µg LPS alone (n=6).

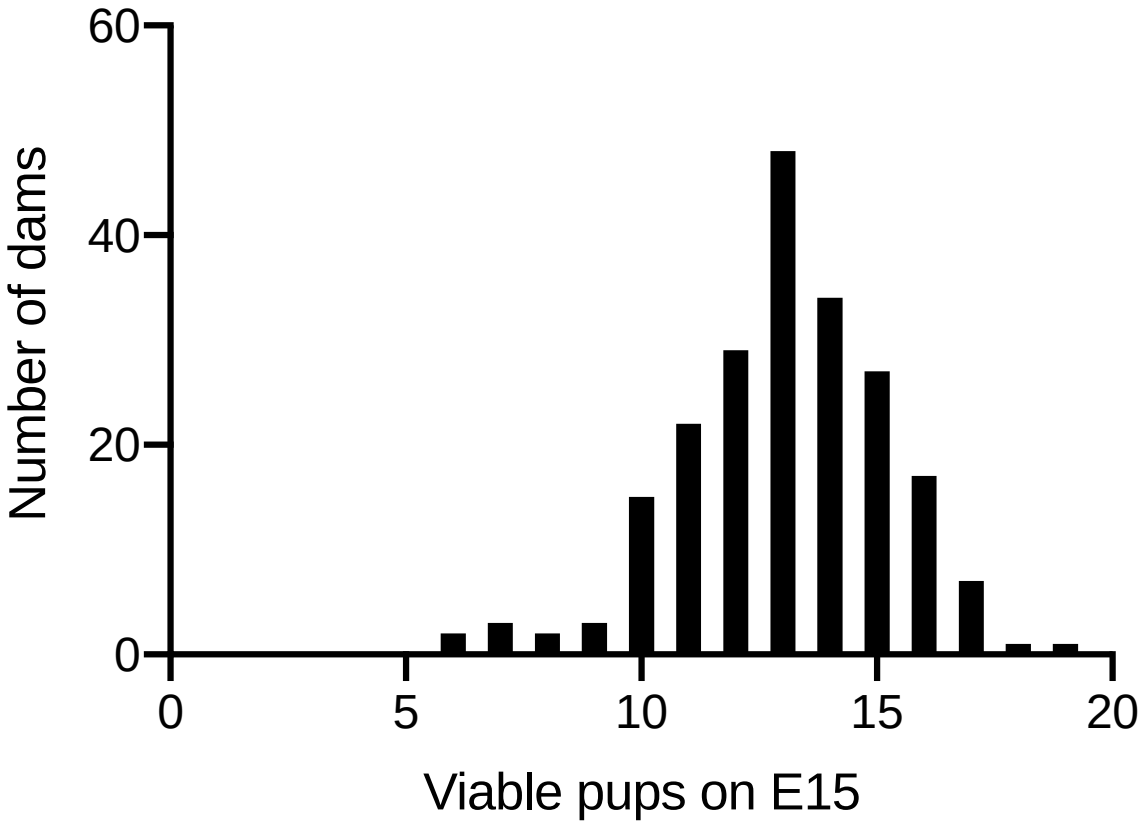

Fig. S3. Histogram of litter sizes. Litter size was counted on E15 at the time of surgery, and included only viable pups.

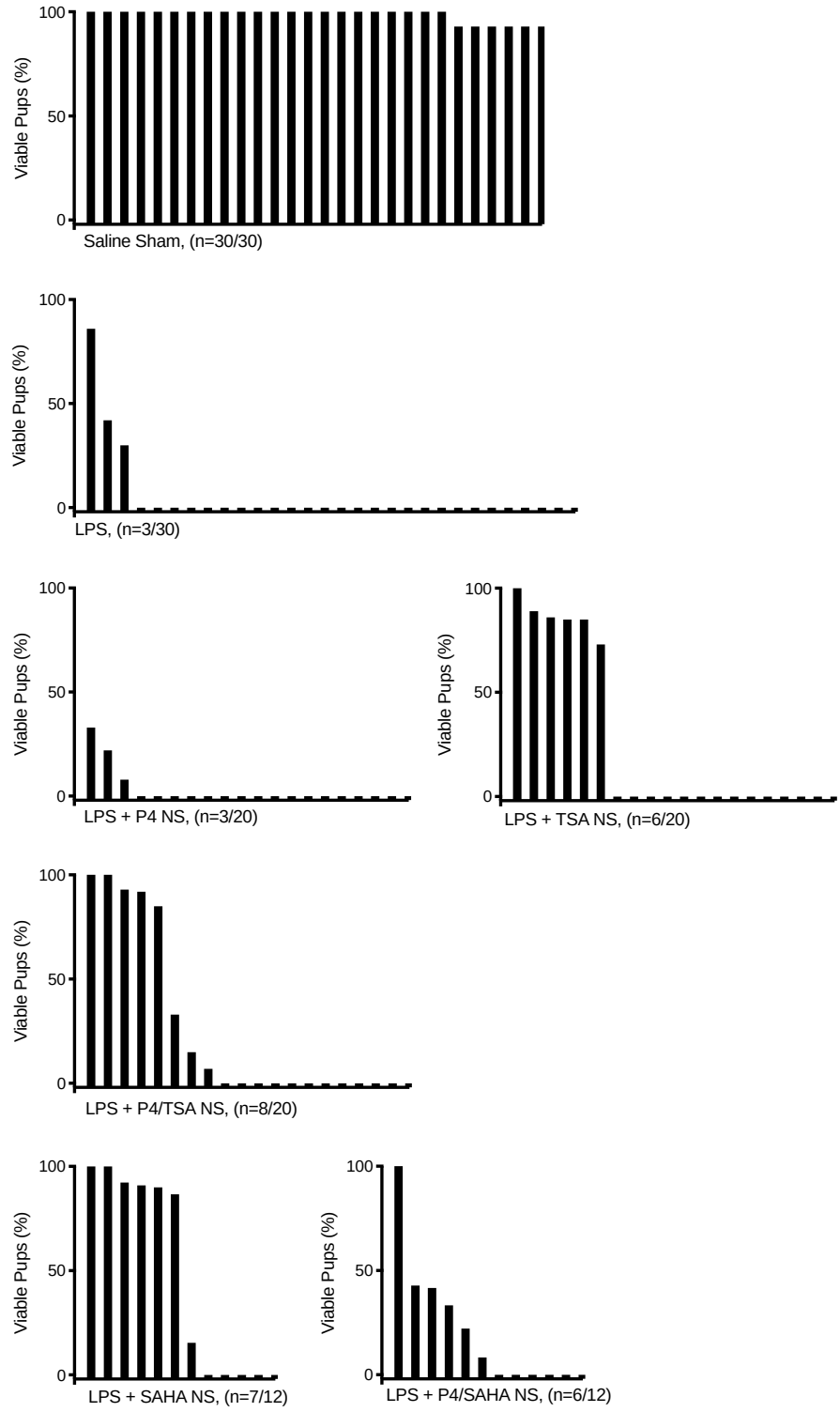

Fig. S4. Individual litter pup viability. For term litters, percentage of pups born live compared to viable pups counted on E15. Each bar represents an individual litter for saline sham, LPS (20 µg), LPS + P4 NS (1 mg), LPS + TSA NS (15 µg), LPS + P4/TSA NS (1 mg/15 µg), LPS + SAHA NS (15 µg), or LPS + P4/SAHA NS (1 mg/15 µg). Litters delivered prematurely are shown as 0% viability. The number of live litters out of the total is shown below each plot.

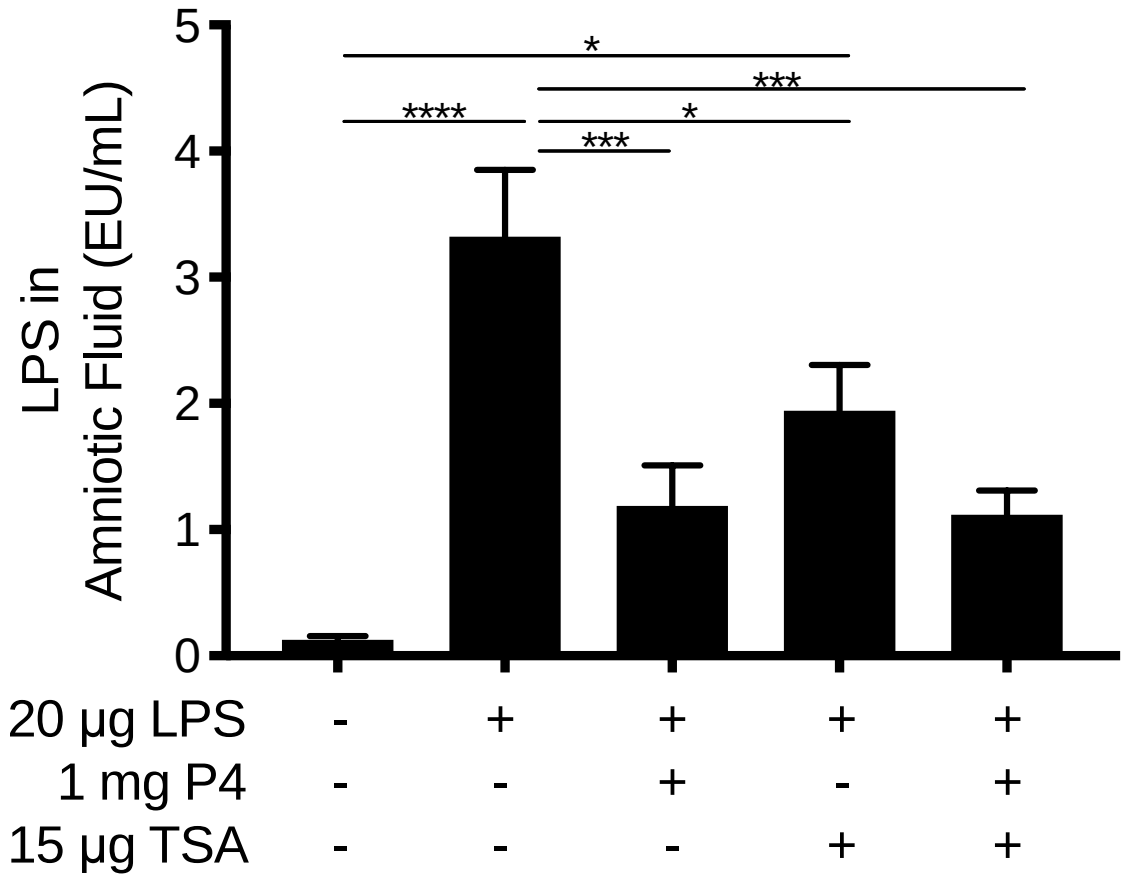

Fig. S5. Amniotic fluid endotoxin quantification. Endotoxin quantification from pooled amniotic fluid collected 8 h after surgery. Data are shown as mean ± SEM (n ≥ 8 dams). *$p < 0.05$, *** $p < 0.001$, **** $p < 0.0001$.

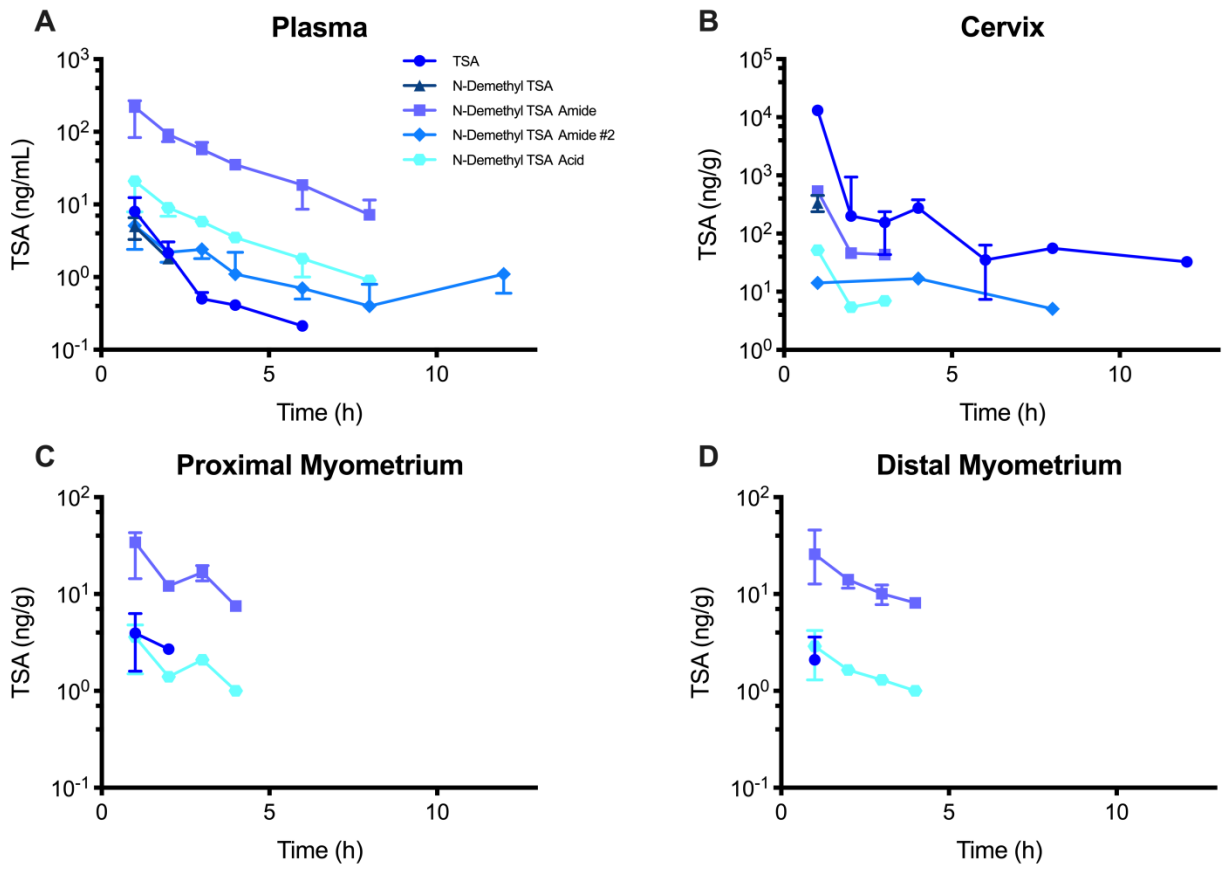

Fig. S6. TSA metabolite PK. Approximate concentrations of active metabolites of TSA in (A) plasma, (B) cervical tissue, (C) proximal myometrial tissue, and (D) distal myometrial tissue collected from healthy dams after a single vaginal P4/TSA (1 mg/15 µg) NS dose on E15. All metabolites were analyzed at all time points. Omission of data at later timepoints indicates that metabolite concentrations were below the lower limit of quantification. Data are presented as median ± interquartile range (n=3).

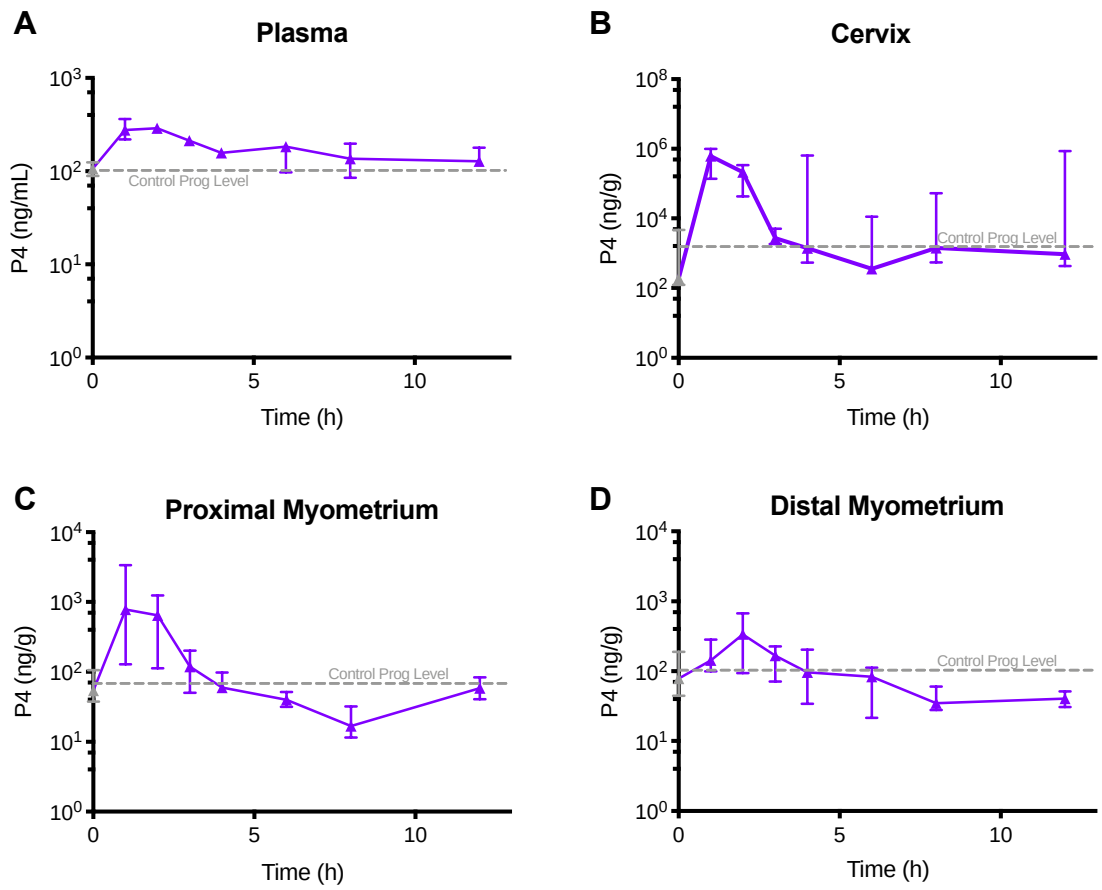

Fig. S7. PK for 1 mg of P4 dosed alone. P4 concentrations in (A) plasma, (B) cervical tissue, (C) proximal myometrial tissue, and (D) distal myometrial tissue collected from healthy dams after a single vaginal P4 (1 mg) NS dose E15. Gray dashed lines represent mean endogenous concentrations of P4 from untreated healthy E15 dams. Data are presented as median ± interquartile range (n=3).

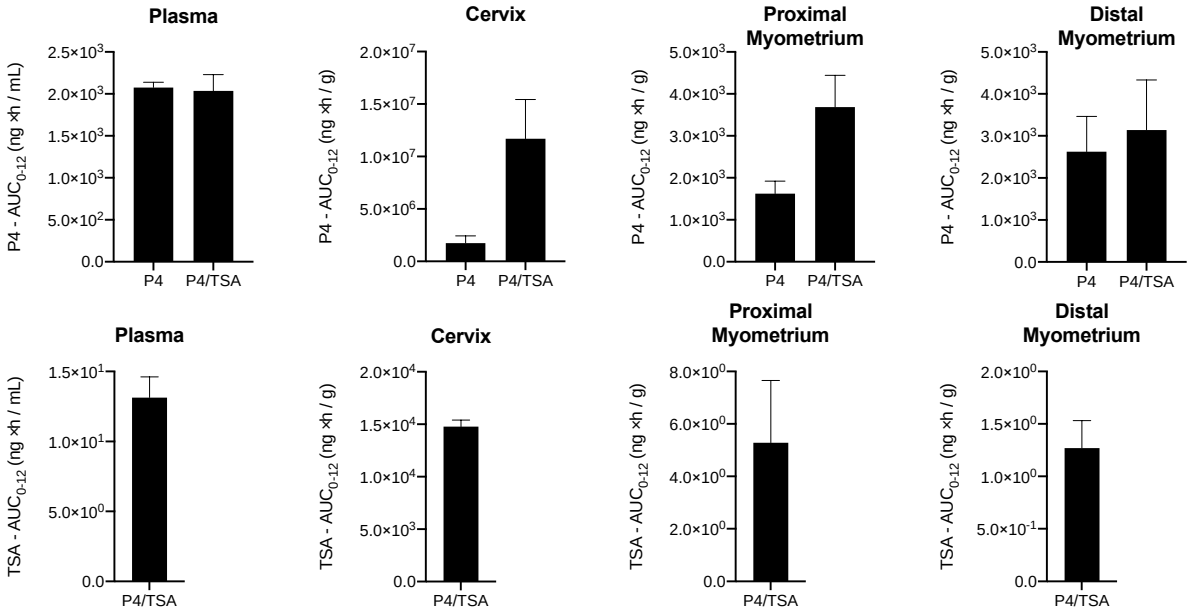

Fig. S8. AUC for PK studies. AUC for P4 (top row) or TSA (bottom row) over 12 h ($AUC_{0\text{-}12}$) after a single vaginal dose of P4 (1 mg) or P4/TSA (1 mg/15 µg). AUC calculations were performed using WinNonLin software. Data are presented as mean ± SEM (n=3).

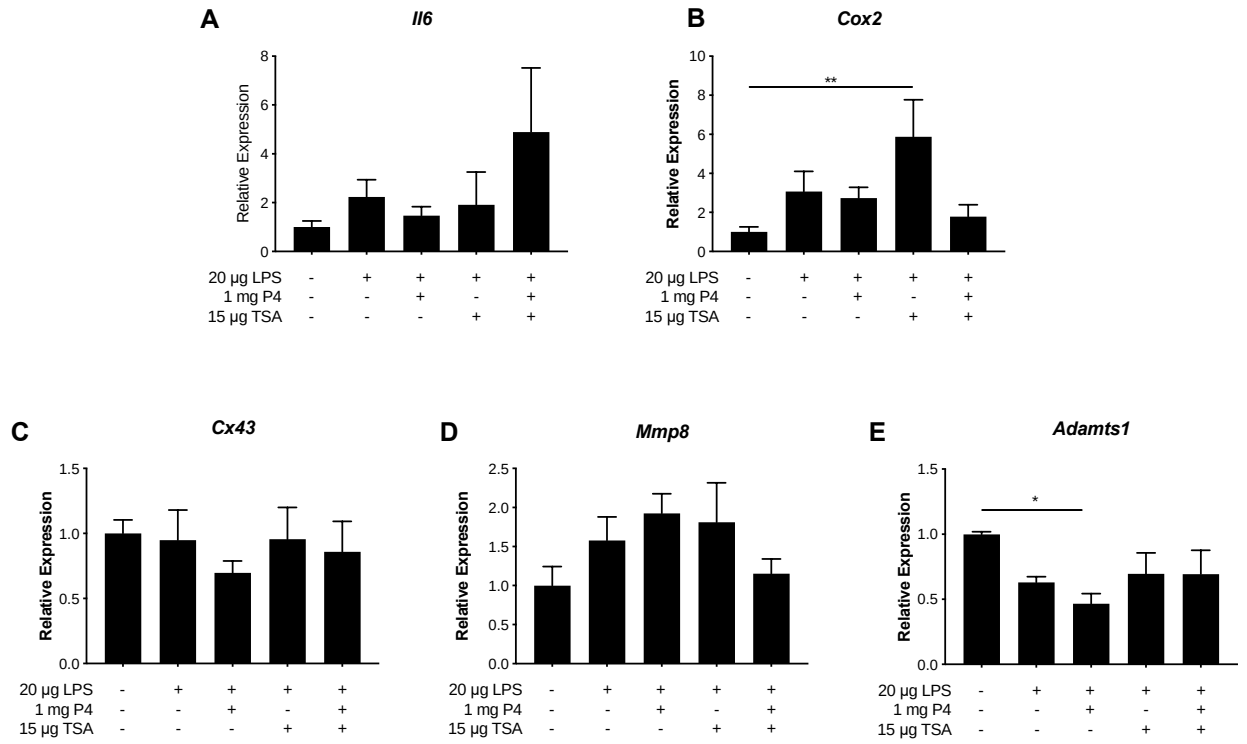

Fig. S9. Cervical gene expression. Relative gene expression of (A) *Il6*, (B) *Cox2*, (C) *Cx43*, (D) *Mmp8*, and (E) *Adamts1* from cervical tissue from dams receiving a DDI of saline or DDI of LPS with no treatment, vaginal P4 NS (1 mg), TSA NS (15 µg), or P4/TSA NS (1 mg/15 µg). Treatment occurred immediately after surgery. Tissue was collected 8 h post-surgery. Gene expression was analyzed using the ΔΔCt method, using *Rplp0* as the housekeeping gene. Results are presented as expression relative to the saline sham group. Data are shown as mean ± SEM (n ≥ 5). * $p < 0.05$, ** $p < 0.01$.

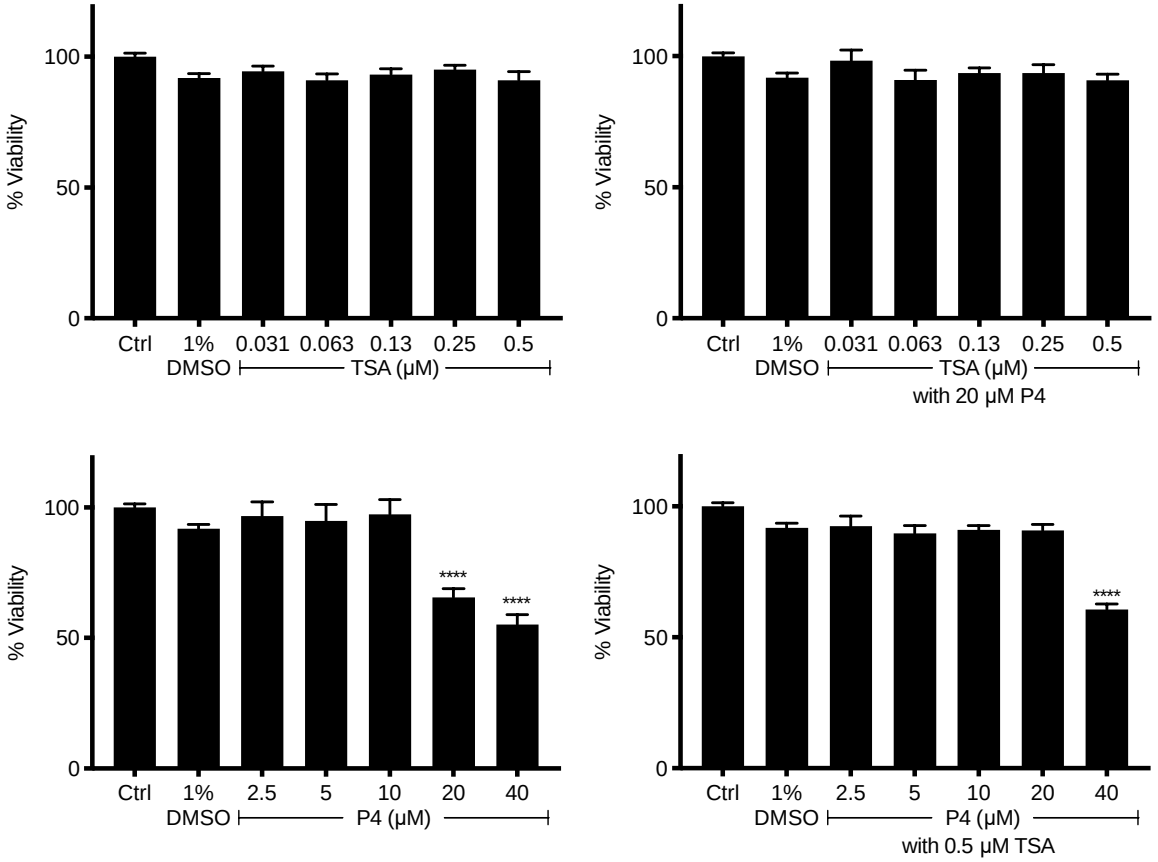

Fig. S10. PHM1-41 cell viability. PHM1-41 viability after 24 h incubation with P4, TSA, or P4 with TSA. Data are shown as mean ± SEM (n ≥ 6). **** $p < 0.0001$ compared to untreated cells (Ctrl).

Table S1. Formulation optimization for TSA NS. Formulation no. 20 was used for studies described in the main manuscript. Pluronic F127 and additional stabilizer percentages are weight/volume in water. Size, PDI, and ζ-potential are represented as mean ± SD (n=3).

No.	Tube Size	Bead Type	Bead Mass	F127 Concentration	Additional Stabilizer	Size (nm)	PDI	ζ-potential (mV)
1	2mL tube	ZSBO5	2.0 g	4%	-	192.3 ± 4.9	0.19 ± 0.07	2.12 ± 0.39
2	2mL tube	ZrOBO5	2.0 g	4%	-	175.67 ± 31.5	0.45 ± 0.13	-1.11 ± 0.54
3	2mL tube	ZrOBO15	2.0 g	4%	-	139.37 ± 22.7	0.42 ± 0.11	-4.69 ± 1.82
4	1.5mL tube	ZSBO5	1.0 g	4%	-	214.23 ± 6.4	0.22 ± 0.04	-2.08 ± 0.26
5	1.5mL tube	ZrOBO5	1.0 g	4%	-	304.63 ± 149.6	0.6 ± 0.25	-0.9 ± 0.46
6	1.5mL tube	ZrOBO15	1.0 g	4%	-	309.9 ± 77	0.33 ± 0.06	1.08 ± 1.97
7	0.5mL tube	ZSBO5	0.5 g	4%	-	323.73 ± 23.9	0.32 ± 0.02	1.68 ± 0.4
8	0.5mL tube	ZrOBO5	0.5 g	4%	-	305.1 ± 30.3	0.42 ± 0.13	-0.94 ± 1.22
9	0.5mL tube	ZrOBO15	0.5 g	4%	-	337.6 ± 47.4	0.39 ± 0.04	-0.86 ± 1.62
10	2mL tube	ZSBO5	0.2 g	4%	-	246.93 ± 20.2	0.31 ± 0.13	-1.04 ± 0.32
11	2mL tube	ZSBO5	0.5 g	4%	-	233.27 ± 7.3	0.27 ± 0.12	-0.22 ± 0.27
12	2mL tube	ZSBO5	1.0 g	4%	-	218.13 ± 4.4	0.25 ± 0.12	0.1 ± 0.38
13	2mL tube	ZSBO5	1.5 g	4%	-	212.73 ± 7.4	0.22 ± 0.02	-0.16 ± 0.23
14	2mL tube	ZrOBO15	0.2 g	4%	-	473.17 ± 72.9	0.39 ± 0.01	-1.11 ± 0.7
15	2mL tube	ZrOBO15	0.5 g	4%	-	621.13 ± 491.7	0.58 ± 0.36	-0.14 ± 0.37
16	2mL tube	ZrOBO15	1.0 g	4%	-	186.97 ± 9.8	0.64 ± 0.07	0.2 ± 0.21
17	2mL tube	ZrOBO15	1.5 g	4%	-	1066.63 ± 1295.3	0.57 ± 0.13	-0.61 ± 1.24
18	1.5mL tube	ZSBO5	0.2 g	4%	-	238.57 ± 22.4	0.28 ± 0.09	-0.41 ± 0.58
19	1.5mL tube	ZSBO5	0.5 g	4%	-	236.1 ± 9.3	0.22 ± 0.07	-0.11 ± 0.39
20	**1.5mL tube**	**ZSBO5**	**1.5 g**	**4%**	**-**	**215.9 ± 12.9**	**0.12 ± 0.07**	**-0.06 ± 0.04**
21	1.5mL tube	ZSBO5	2.0 g	4%	-	219.8 ± 10.7	0.27 ± 0.05	0 ± 0.39
22	2mL tube	ZSBO5	1.5 g	1%	-	252.67 ± 8.9	0.26 ± 0.03	-2.35 ± 0.56
23	2mL tube	ZSBO5	1.5 g	2%	-	948.57 ± 44.9	0.43 ± 0.04	-4.54 ± 1.66
24	2mL tube	ZSBO5	1.5 g	4%	-	347.37 ± 4.1	0.17 ± 0.09	-4.61 ± 2.39
25	2mL tube	ZSBO5	1.5 g	6%	-	213.3 ± 5.9	0.2 ± 0.02	-2.35 ± 1.2
26	1.5mL tube	ZSBO5	0.5 g	1%	-	483.9 ± 82.2	0.4 ± 0.05	-4.07 ± 1.95
27	1.5mL tube	ZSBO5	0.5 g	2%	-	338.17 ± 27.6	0.32 ± 0.04	-2.59 ± 1.11
28	1.5mL tube	ZSBO5	0.5 g	4%	-	319.43 ± 26.2	0.11 ± 0.01	-1.42 ± 0.14
29	1.5mL tube	ZSBO5	0.5 g	6%	-	462.13 ± 25.3	0.35 ± 0.03	-0.48 ± 0.25
30	2mL tube	ZSBO5	1.5 g	4%	1% HS15	212.1 ± 4.6	0.3 ± 0.02	0.16 ± 0.35
31	2mL tube	ZSBO5	1.5 g	4%	2% HS15	199.93 ± 3.2	0.29 ± 0.02	-0.04 ± 0.35
32	2mL tube	ZSBO5	1.5 g	4%	3% HS15	238.03 ± 8.9	0.49 ± 0.06	0.12 ± 0.07
33	2mL tube	ZSBO5	1.5 g	4%	1% F68	201.5 ± 1.5	0.16 ± 0.03	-0.39 ± 0.04
34	2mL tube	ZSBO5	1.5 g	4%	2% F68	229.63 ± 1	0.15 ± 0.05	0.12 ± 0.62
35	2mL tube	ZSBO5	1.5 g	4%	3% F68	328.7 ± 19.2	0.42 ± 0.04	0.24 ± 0.6
36	2mL tube	ZSBO5	1.5 g	4%	1% TPGS	206.33 ± 4.2	0.17 ± 0.05	0.52 ± 0.96
37	2mL tube	ZSBO5	1.5 g	4%	2% TPGS	217.83 ± 4.5	0.25 ± 0.02	1 ± 0.57
38	2mL tube	ZSBO5	1.5 g	4%	3% TPGS	199.53 ± 0.8	0.18 ± 0	-0.55 ± 0.16
39	1.5mL tube	ZSBO5	0.5 g	2%	1% HS15	213.33 ± 9	0.35 ± 0.05	-0.38 ± 0.67
40	1.5mL tube	ZSBO5	0.5 g	2%	2% HS15	304.47 ± 15.3	0.53 ± 0.07	-0.01 ± 0.05
41	1.5mL tube	ZSBO5	0.5 g	2%	3% HS15	256.53 ± 11.3	0.6 ± 0.11	0.92 ± 0.44
42	1.5mL tube	ZSBO5	0.5 g	2%	1% F68	252.93 ± 6	0.38 ± 0.04	0.77 ± 0.64
43	1.5mL tube	ZSBO5	0.5 g	2%	2% F68	255.97 ± 12.5	0.35 ± 0.12	0.43 ± 0.13
44	1.5mL tube	ZSBO5	0.5 g	2%	3% F68	237.43 ± 3.3	0.22 ± 0.02	-0.09 ± 0.34
45	1.5mL tube	ZSBO5	0.5 g	2%	1% TPGS	293.4 ± 12.3	0.47 ± 0.02	0.72 ± 0.58
46	1.5mL tube	ZSBO5	0.5 g	2%	2% TPGS	329.4 ± 22.6	0.55 ± 0.03	0.5 ± 0.85
47	1.5mL tube	ZSBO5	0.5 g	2%	3% TPGS	269.13 ± 4.9	0.51 ± 0.04	1.08 ± 0.4
48	1.5mL tube	ZSBO5	0.5 g	6%	1% HS15	374.87 ± 105.6	0.6 ± 0.35	-7.78 ± 3.77
49	1.5mL tube	ZSBO5	0.5 g	6%	2% HS15	440.3 ± 312.4	0.66 ± 0.3	-16.53 ± 1.21
50	1.5mL tube	ZSBO5	0.5 g	6%	3% HS15	251.1 ± 23.2	0.66 ± 0.08	-11.91 ± 2.92
51	1.5mL tube	ZSBO5	0.5 g	6%	1% F68	346.87 ± 28.2	0.31 ± 0.04	-10.46 ± 3.68
52	1.5mL tube	ZSBO5	0.5 g	6%	2% F68	241.23 ± 9.9	0.2 ± 0.05	-0.96 ± 0.75
53	1.5mL tube	ZSBO5	0.5 g	6%	3% F68	220.9 ± 2.8	0.2 ± 0.06	-1.22 ± 1.26
54	1.5mL tube	ZSBO5	0.5 g	6%	1% TPGS	214.47 ± 20.2	0.31 ± 0.13	-8.34 ± 1.73
55	1.5mL tube	ZSBO5	0.5 g	6%	2% TPGS	229.07 ± 12.2	0.54 ± 0.08	-10.7 ± 1.66
56	1.5mL tube	ZSBO5	0.5 g	6%	3% TPGS	214 ± 1.9	0.52 ± 0.07	-13.53 ± 1.7

Table S2. Pup behavior fail rates. Percentage and number of pups failing each task are shown. Failures were not included in data shown in Figure 3F-K or in Figure 7E-J.

Cliff Aversion	**PND 5**	**PND 9**	**PND 15**	**PND 17**	**PND 22**
Saline	27% (n=16)	0% (n=0)	-	-	-
LPS + P4/TSA NS	24% (n=7)	0% (n=0)	-	-	-
Negative Geotaxis	**PND 5**	**PND 9**	**PND 15**	**PND 17**	**PND 22**
Saline	93% (n=55)	51% (n=30)	-	-	-
LPS + P4/TSA NS	96% (n=29)	58% (n=17)	-	-	-
Surface Righting	**PND 5**	**PND 9**	**PND 15**	**PND 17**	**PND 22**
Saline	12% (n=7)	0% (n=0)	-	-	-
LPS + P4/TSA NS	14% (n=4)	0% (n=0)	-	-	-
Open Field	**PND 9**	**PND 13**	**PND 15**	**PND 17**	**PND 22**
Saline	55% (n=32)	7% (n=4)	0% (n=0)	3% (n=2)	0% (n=0)
LPS + P4/TSA NS	52% (n=16)	0% (n=0)	3% (n=1)	0% (n=0)	0% (n=0)
Cliff Aversion	**PND 8**	**PND 10**	**PND 15**	**PND 17**	**PND 22**
Saline	6% (n=3)	-	44% (n=19)	-	-
LPS + SAHA NS	0% (n=0)	-	32% (n=15)	-	-
LPS + P4/SAHA NS	0% (n=0)	-	23% (n=12)	-	-
Negative Geotaxis	**PND 8**	**PND 10**	**PND 15**	**PND 17**	**PND 22**
Saline	69% (n=30)	-	6% (n=3)	-	-
LPS + SAHA NS	21% (n=10)	-	4% (n=2)	-	-
LPS + P4/SAHA NS	31% (n=12)	-	0% (n=0)	-	-
Surface Righting	**PND 8**	**PND 10**	**PND 15**	**PND 17**	**PND 22**
Saline	0% (n=0)	-	0% (n=0)	-	-
LPS + SAHA NS	0% (n=0)	-	0% (n=0)	-	-
LPS + P4/SAHA NS	0% (n=0)	-	0% (n=0)	-	-
Open Field	**PND 8**	**PND 10**	**PND 15**	**PND 17**	**PND 22**
Saline	75% (n=33)	19% (n=8)	0% (n=0)	0% (n=0)	0% (n=0)
LPS + SAHA NS	68% (n=33)	18% (n=9)	0% (n=0)	0% (n=0)	0% (n=0)
LPS + P4/SAHA NS	69% (n=27)	53% (n=21)	0% (n=0)	0% (n=0)	0% (n=0)

Table S3. Antibodies used in Western blotting. Primary and secondary antibodies used for western blotting. * indicates an antibody that was tested, but failed to visualize murine PR.

Antigen	Antibody	Dilution	Supplier
GAPDH	sc-32233	1:1000 in 1% BSA	Santa Cruz
Mouse IgG	715-035-150	1:10000 in 1% BSA	Jackson ImmunoResearch
PR	M356801-2	1:1000 in 1% BSA	Agilent
PR *	MA5-12658	1:400 in 1% BSA	Invitrogen
PR *	sc-810	1:4000 in 1% BSA	Santa Cruz
PR *	#8757	1:1000 in 1% BSA	Cell Signal

Table S4. Primer sequences. Forward and reverse primer sequences used in RT-qPCR experiments.

Rplp0	F:	5'- CAC TGG TCT AGG ACC CGA GAA G -3'
	R:	5'- GGT GCC TCT GGA GAT TTT CG -3'
Adamts1	F:	5'- AGT TAC CTC CAA TGC AGC TCT CA -3'
	R:	5'- ATC CCG AGA GTG TCA CAC GTG T-3'
Ccl3	F:	5'- AGC TGA CAC CCC GAC TGC CT -3'
	R:	5'- TCA GGA AAA TGA CAC CTG GCT GGG A -3'
Cx43	F:	5'- GCC CGA ACT CTC CTT TTC CT -3'
	R:	5'- CAT GTC TGG GCA CCT CTC TTT -3'
Cox2	F:	5'- CAG CCA GGC AGC AAA TCC -3'
	R:	5'- ACA TTC CCC ACG GTT TTG AC -3'
Il1β	F:	5'- GAA ATG CCA CCT TTT GAC AGT G -3'
	R:	5'- TGG ATG CTC TCA TCA GGA CAG -3'
Il6	F:	5'- CCT CTC TGC AAG AGA CTT CC -3'
	R:	5'- CTC CGG ACT TGT GAA GTA GG -3'
Mmp8	F:	5'- CAC GCA CCC TAT GAG GAC AA-3'
	R:	5'- GCA GGA CAC GTG GGA TGA GT-3'
Nfκb1	F:	5'- ATG GCA GAC GAT GAT CCC TAC -3'
	R:	5'- CGG AAT CGA AAT CCC CTC TGT T -3'
Oxtr	F:	5'- GAT CAC GCT CGC CGT CTA C -3'
	R:	5'- CCG TCT TGA GTC GCA GAT TC -3'
Zeb1	F:	5'- ACT GCA AGA AAC GGT TTT CCC -3'
	R:	5'- GGC GAG GAA CAC TGA GAT GT -3'