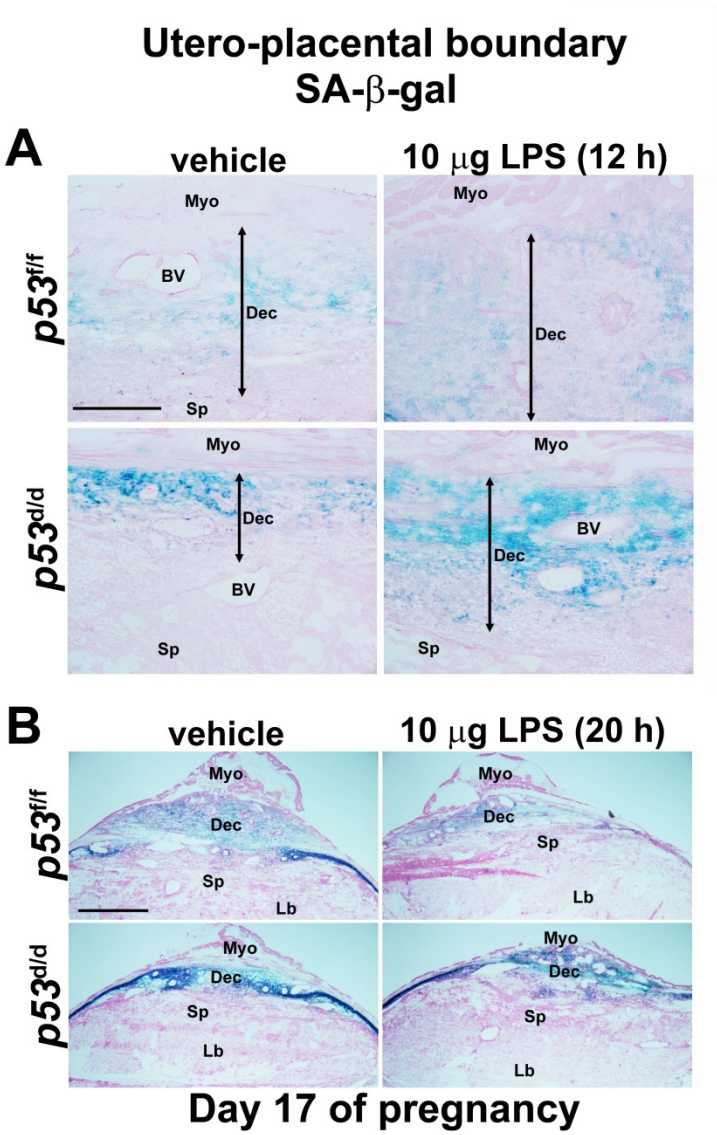
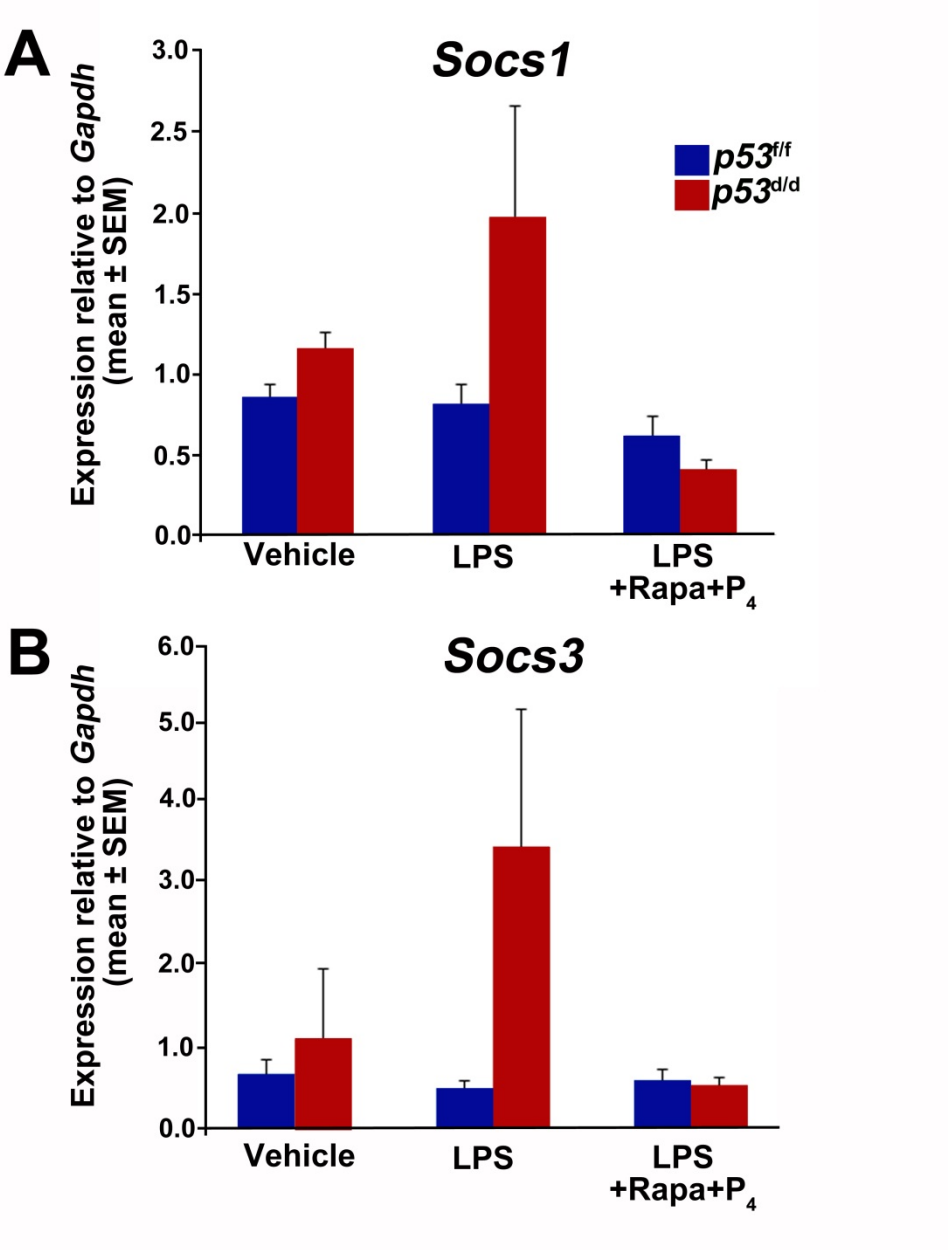
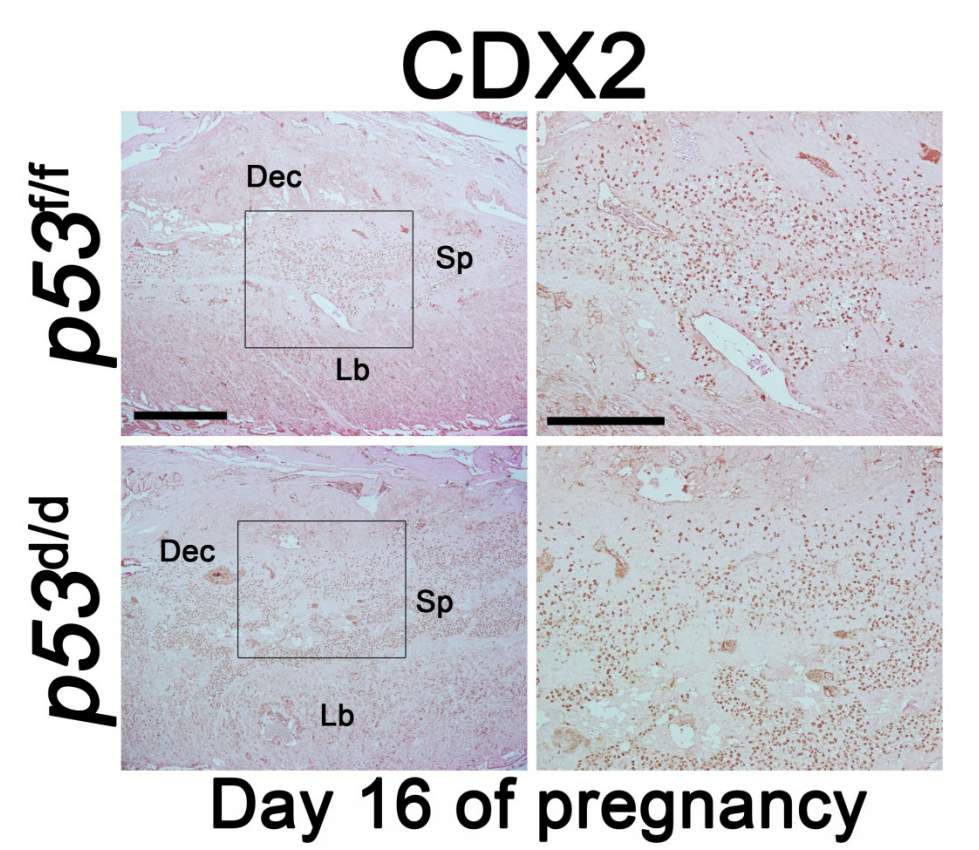


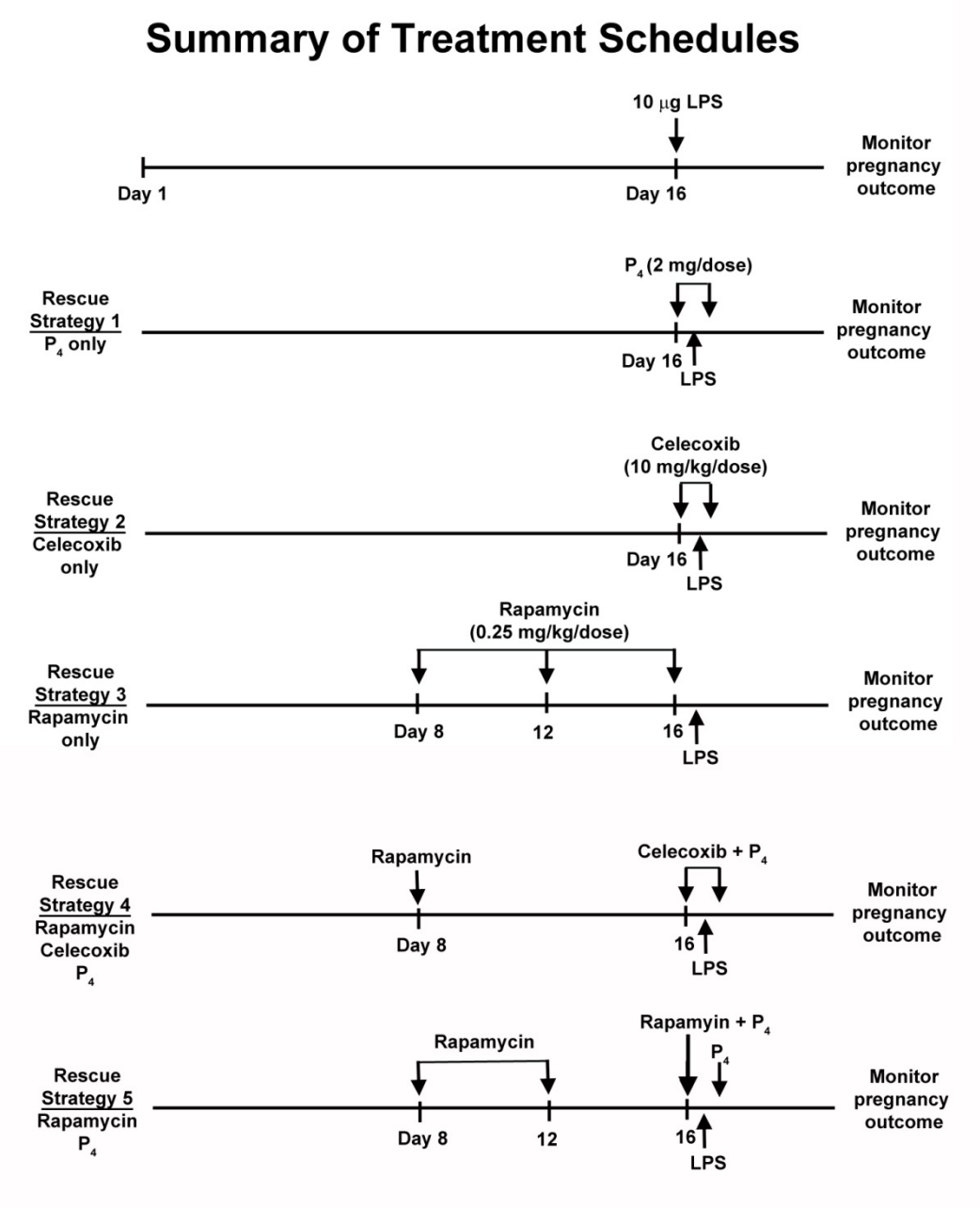
Supplementary Figure 1. Senescence-associated β-galactosidase (SA-β-gal) staining on day 17 of pregnancy after LPS injection. Decidual SA-β-gal staining did not appreciably change after 12 h (**A**) or 20 h (**B**) post-LPS treatment. LPS was injected i.p. on day 16 in *Trp53*$^{loxP/loxP}$ *Pgr*$^{+/+}$ (***p53***$^{\mathbf{f/f}}$) and *Trp53*$^{loxP/loxP}$ *Pgr*$^{Cre/+}$ (***p53***$^{\mathbf{d/d}}$) females, and implantation sites were examined on day 17. As expected, deciduae of *p53*$^{d/d}$ females already showed higher intensity of SA-β-gal staining compared to that of *p53*$^{f/f}$ females. Decidual boundaries are demarcated by double-sided arrows. Myo, myometrium; Dec, decidua; Sp, spongiotrophoblast; Lb, labyrinth; BV, blood vessel. Bar for (**A**), 250 μm; Bar for (**B**), 1 mm.



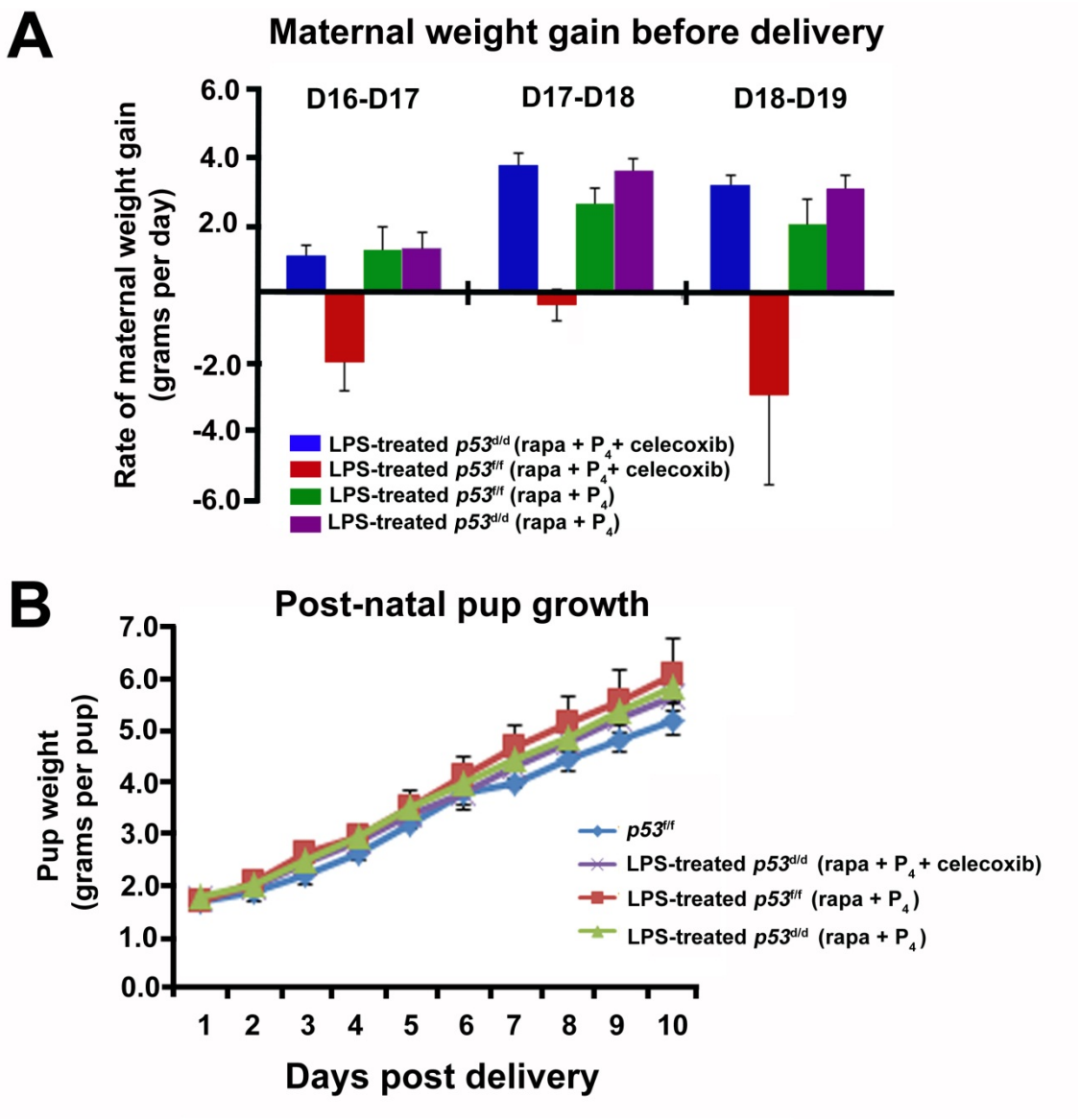
Supplementary Figure 2. qPCR results of ovarian *Socs1* and *Socs3* expression levels. qPCR results show upregulation of *Socs1* (**A**) and *Socs3* (**B**) in ovaries of *Trp53*$^{loxP/loxP}$ *Pgr*$^{Cre/+}$ (***p53***$^{\mathbf{d/d}}$) females 12 h post-LPS injection compared to those in *Trp53*$^{loxP/loxP}$ *Pgr*$^{+/+}$ (***p53***$^{\mathbf{f/f}}$) littermates. This upregulation was attenuated by rapamycin (Rapa) and P_4 treatment (n=3 independent samples per group).



Supplementary Figure 3. Immunolocalization of trophoblast-enriched marker Cdx2 on day 16 of pregnancy in *p53*$^{f/f}$ and *p53*$^{d/d}$ females. Areas enclosed within boxes in left panels are shown at higher magnification in right panels for respective genotypes. Dec, decidua; Sp, spongiotrophoblast; Lb, labyrinth. Bars, 500 µm (left panels) and 250 µm (right panels).

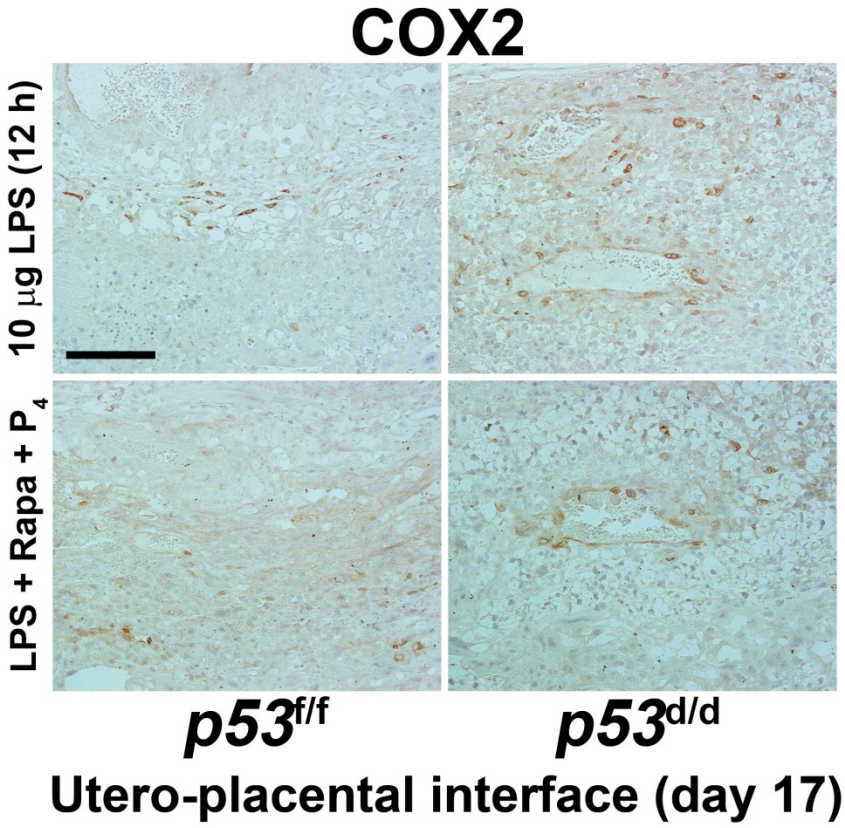


Supplementary Figure 4. Treatment strategies in attempt to rescue preterm birth in mice. 10 μg LPS was injected (i.p.) at 1200 h on day 16 of pregnancy in all cases. Both floxed and deleted mice received an oral gavage of rapamycin (0.25 mg/kg BW) in the mornings of days 8, 12, and 16 of pregnancy. Celecoxib (10 mg/kg BW) was administered as an oral gavage twice on day 16, once 3 h prior to LPS injection and again 4 h after LPS. P_4 (2 mg/dose, s.c.) was injected twice on day 16 at similar time points as celecoxib.

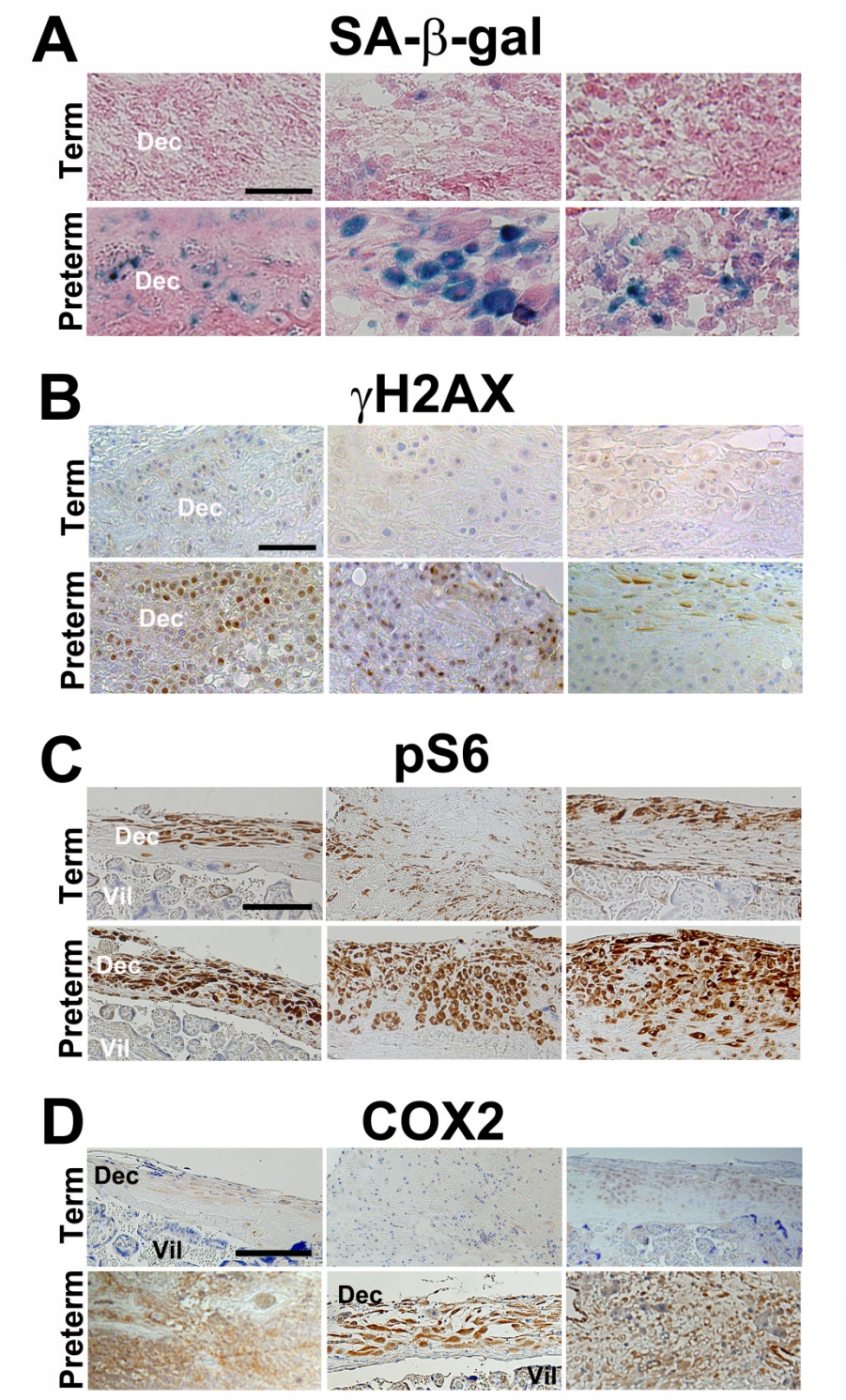


Supplementary Figure 5. Treatment with rapamycin (rapa) and P_4 did not interfere with maternal weight gain from day 16 of pregnancy onward nor did it interfere with neonatal pup growth. (**A**), Maternal weight gain was recorded from days 16 through 19 of pregnancy, and differences in weight gain between consecutive days were graphed (mean ± SEM). Pregnant *Trp53*$^{loxP/loxP}$ *Pgr*$^{+/+}$ (***p53***$^{\mathbf{f/f}}$) females treated with rapamycin, P_4, and celecoxib showed daily loss in maternal weight indicative of resorption, which was confirmed after termination of experiments. Weight loss was not seen in rapamycin and P_4 treated *p53*$^{f/f}$ and *Trp53*$^{loxP/loxP}$ *Pgr*$^{Cre/+}$ (***p53***$^{\mathbf{d/d}}$) females. At least five pregnant dams per treatment group and genotype were monitored and weighed on the indicated days of pregnancy. (**B**), Neonatal pup weight was monitored daily from the day of delivery through post-natal day 10 as an index of neonatal growth

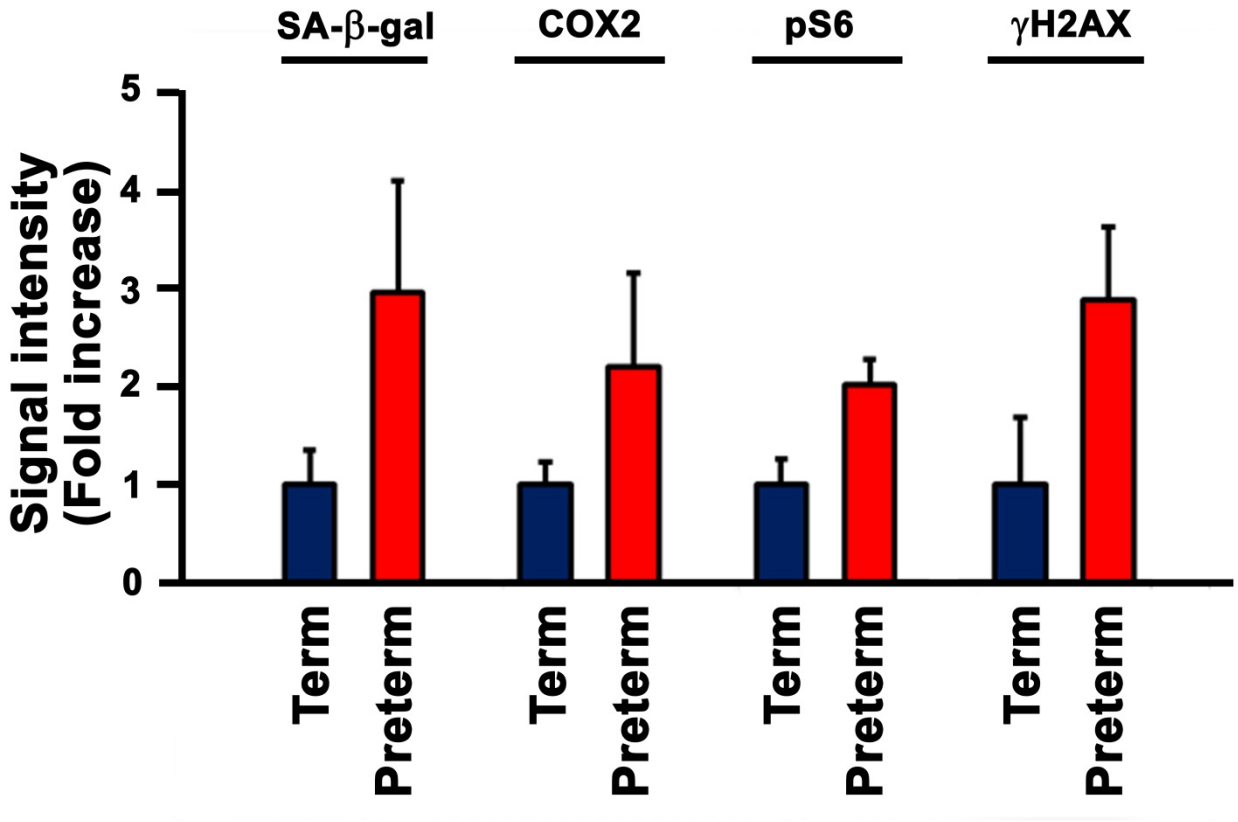
rate (mean ± SEM). Females treated with rapamycin and P_4 showed similar neonatal pup growth rates as untreated floxed females. Neonatal pups from at least four litters per treatment group and genotype were monitored and weighed on the post-natal days indicated.



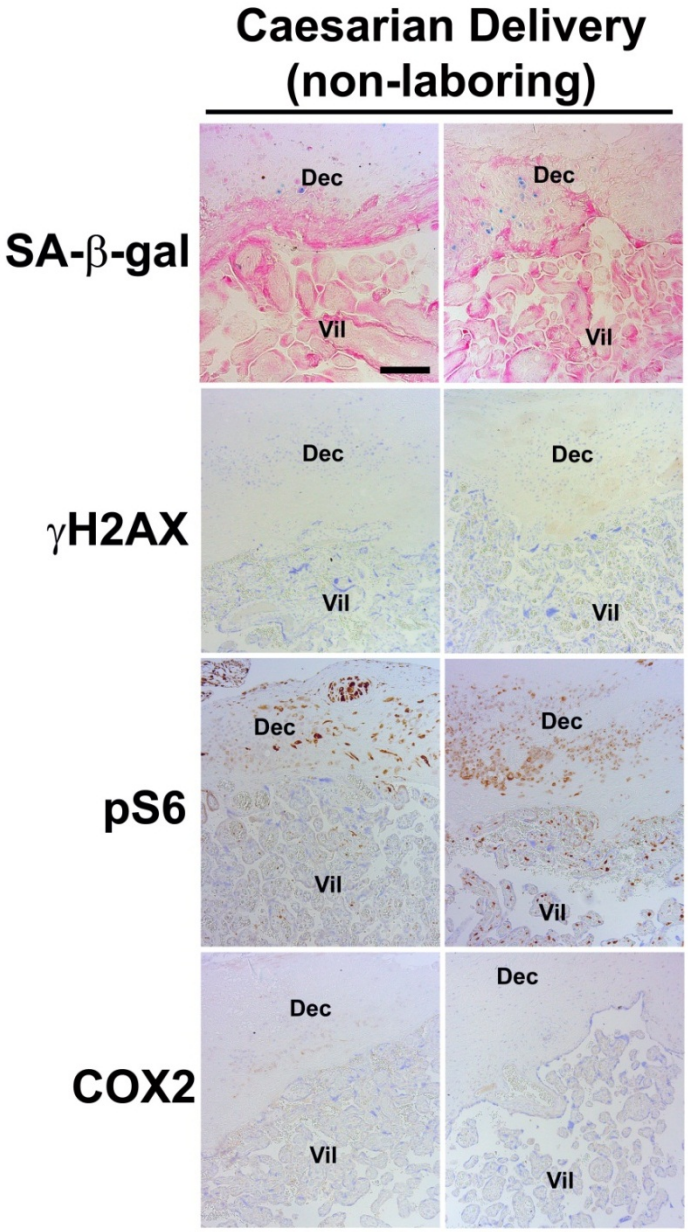
Supplementary Figure 6. Images of higher magnification for decidual expression of Cox2 in *p53*$^{f/f}$ and *p53*$^{d/d}$ females. Immunohistochemistry for Cox2 in LPS-treated *Trp53*$^{loxP/loxP}$ *Pgr*$^{+/+}$ (***p53***$^{f/f}$) and *Trp53*$^{loxP/loxP}$ *Pgr*$^{Cre/+}$ (***p53***$^{d/d}$) females with or without rapamycin (Rapa) and P_4 administration on day 16 of pregnancy and examined 12 h later on day 17. Schedule of treatment is described in Supplementary Figure 4. Bar, 100 μm.



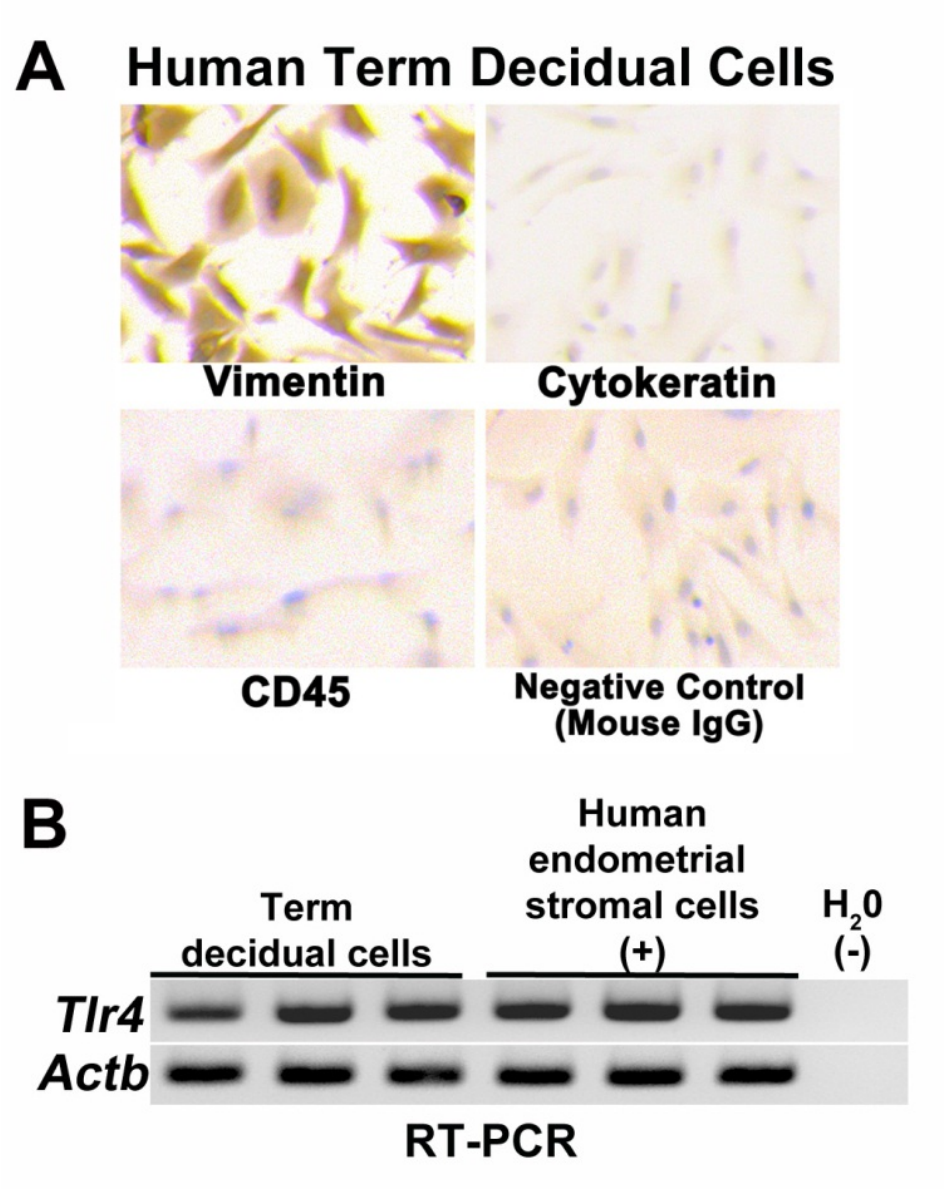
Supplementary Figure 7. Higher magnification images of term and preterm decidua-placental samples from Figure 4 for SA-β-gal staining and immunostaining for γH2AX, pS6, and COX2. Dec, decidua; Vil, villous trophoblast. Bars, 100μm.



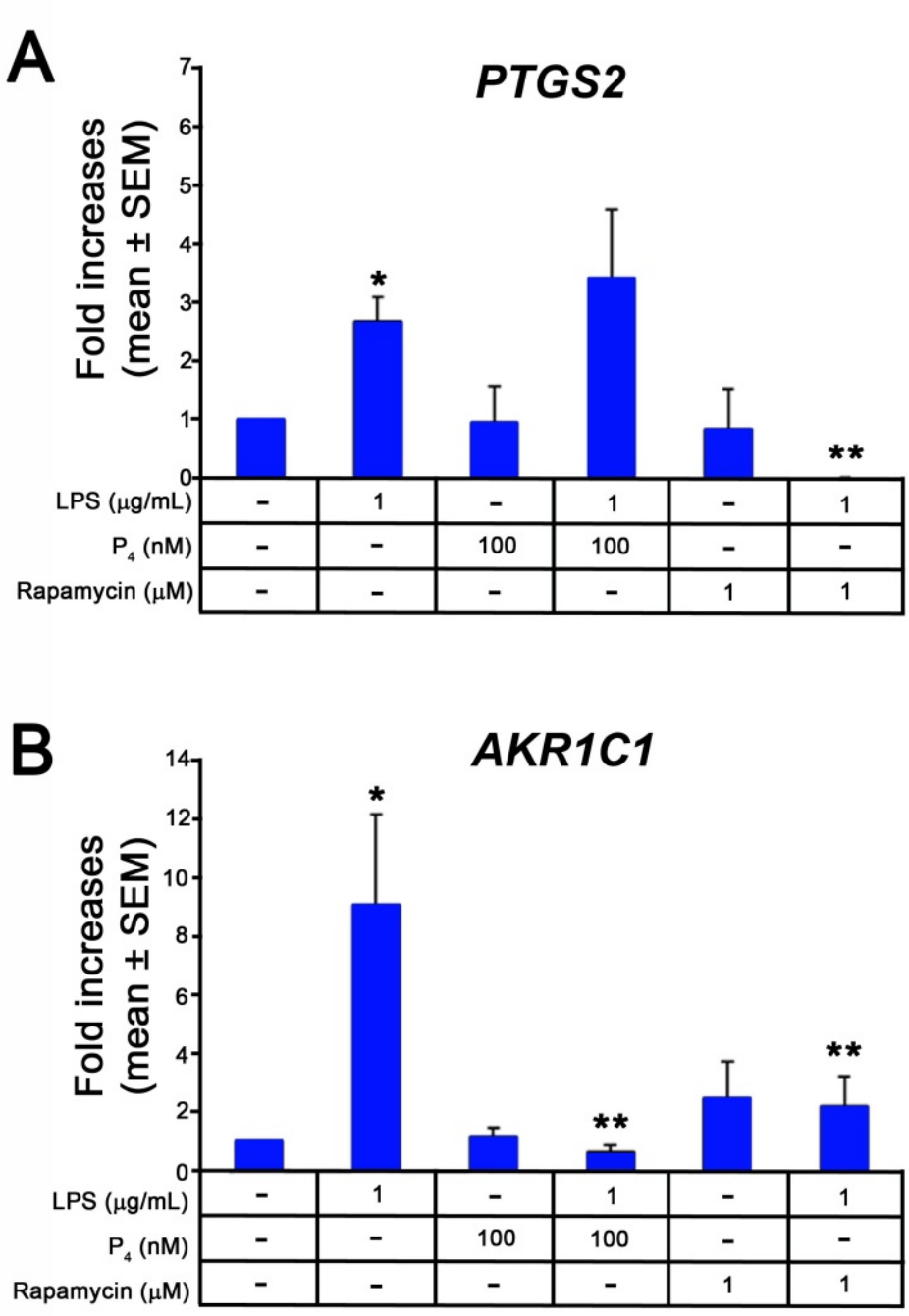
Supplementary Figure 8. Densitometry results for signal intensity of staining for SA-β-gal, COX2, pS6, and γH2AX in human term and preterm decidua-placental samples shown in Figure 4. Three representative images of each staining group from term and preterm samples were analyzed by densitometry software as described in the Methods (mean ± SEM).



Supplementary Figure 9. SA-β-gal staining and immunostaining for γH2AX, pS6, and COX2 for decidua-placental samples from non-laboring Caesarian deliveries prior to term. Caesarian deliveries were performed between 31-36 weeks of gestation due to various pathologies including preeclampsia (5/11), placenta previa (2/11), placental abruption, or fetal distress/anomalies (4/11). Six independent samples were analyzed for SA-β-gal staining while five independent samples were analyzed for each immunostaining. Dec, decidua; Vil, villous trophoblast. Bar, 200μm.



Supplementary Figure 10. Isolation and culture of human term decidual cells. (**A**)**,** Human term decidual cells were isolated to ~99% purity, as assessed by immunostaining for vimentin (stromal cell), cytokeratin (epithelia-derived trophoblast and amnion cells) and CD45 (pan-immune cell marker). (**B**), RT-PCR results show that TLR4 is expressed in human term decidual cells. Human endometrial stromal cells served as positive (+) controls.



Supplementary Figure 11. Expression of *PTGS2* and *AKR1C1* in cultured human term decidual cells following exposure to LPS in presence or absence of P_4 or rapamycin. (**A**)**,** qPCR results showed increases in decidual *PTGS2* expression levels 6 h post-LPS exposure, which was downregulated by rapamycin, but not P_4, treatment. (**B**), qPCR showed increases in decidual *AKR1C1* expression levels 6 h post-LPS exposure, which were downregulated by rapamycin or P_4 treatment. Experiments were repeated in three independent samples (mean ± SEM; **P* < 0.05 compared to control; ***P* < 0.05 compared to LPS-treated cells).

Supplementary Table 1. Combined treatments with rapamycin, celecoxib and/or P_4 supplementation that failed to rescue preterm birth in *p53*$^{d/d}$ females under low grade inflammation.

Genotype	Challenge	Treatment	Time of delivery	No. of dams	Rate of preterm birth	Rate of dystocia	% dead pups and resorptions over total number of pups
p53$^{d/d}$	**10 µg LPS**	**Cele + P_4**	Day 16 1700 h to day 17 0600 h	1	9% (1)	9% (1)	100% (6/6)
			Day 18 0600 h to day 18 1800 h	1	9% (1)	0	100% (8/8)
			Day 19 0600 h to day 19 1800 h	3	0	9% (1)	81% (13/16)
			Day 20 0600 h to day 21 0800 h	5	0	0	19% (5/27)
			Did not deliver	1[A]	-	-	-
			Total	**11**	**18% (2)**	**18% (2)**	**50% (25/50)**
		Cele + Rapa	Day 16 1700 h to day 17 0600 h	1	25% (1)	0	100% (7/7)
			Day 17 0600 h to day 17 1800 h	2	50% (2)	25% (1)	100% (15/15)
			Day 19 0600 h to day 19 1800 h	1	0	25% (1)	43% (3/7)
			Total	**4**	**75% (3)**	**50% (2)**	**86% (25/29)**
		Rapa	Day 17 0600 h to day 17 1800 h	3	43% (3)	14% (1)	100% (13/13)
			Day 18 0600 h to day 18 1800 h	2	29% (2)	14% (1[B])	100% (11/11)
			Day 20 0600 h to day 21 0800 h	2	0	0	0% (0/13)
			Total	**7**	**71% (5)**	**29% (2)**	**65% (24/37)**
		Cele	Day 17 0600 h to day 17 1800 h	**4**	**100% (4)**	**50% (2)**	**83% (19/23)**
		P_4	Day 19 0600 h to day 19 1800 h	6	0	38% (3)	30% (13/44)
			Day 20 0600 h to day 21 0800 h	2	0	0	70% (7/10)
			Total	**8**	**0% (0)**	**38% (3)**	**37% (20/54)**

Trp53$^{loxP/loxP}$ *Pgr*$^{Cre/+}$ (***p53***$^{d/d}$) dams were used. Time of delivery is defined as the day of pregnancy when the dam first started delivering pups. Preterm birth is defined as delivery occurring before day 19 of pregnancy. Dystocia is defined as difficult delivery lasting more than 12 h. LPS was administered i.p. on day 16 of pregnancy at 1200h. Cele, celecoxib (10 mg/kg BW/dose); Rapa, rapamycin (0.25 mg/kg BW/dose); P_4, progesterone (2 mg/dose). The rates of females showing preterm birth and dystocia were calculated over the total number of females examined within a treatment group, while numbers in parentheses indicate the number of females showing the phenotype. The rate of dead pups and resorptions were calculated over the total number of pups delivered, while numbers in parentheses indicate their numbers.

[A]eight resorption sites.

[B]one pup crowned starting from 0700h on day 18 until the afternoon; female was distressed, and experiment was terminated.

Supplementary Table 2. Combined treatments with rapamycin, P_4, and/or celecoxib in *p53*$^{f/f}$ and *p53*$^{d/d}$ females that rescued preterm birth in LPS-treated *p53*$^{d/d}$ females.

Challenge	Treatment	Genotype	Time of delivery	No. of dams	Rate of preterm birth	% dead pups and resorptions over total number of pups	
10 µg LPS	Rapa + P_4 +Cele	*p53*$^{f/f}$	Day 19 0600 h to day 19 1800 h	1	0	29%	(2/7)
			Day 20 0600 h to day 21 0800 h	4[A]	0	53%	(9/17)
			Did not deliver	1[B]	-	100%	(5/5)
			Total	**6**	**0**	**67%**	**(16/24)**
		p53$^{d/d}$	Day 19 0600 h to day 19 1800 h	2	0	18%	(3/17)[C]
			Day 20 0600 h to day 21 0800 h	3	0	4%	(1/27)
			Total	**5**	**0**	**9%**	**(4/44)**
	Rapa + P_4	*p53*$^{f/f}$	Day 19 0600 h to day 19 1800 h	3	0	16%	(6/38)[D]
			Day 20 0600 h to day 21 0800 h	4	0	0%	(0/30)
			Total	**7**	**0**	**9%**	**(6/68)**
		p53$^{d/d}$	Day 19 0600 h to day 19 1800 h	2	0	19%	(3/16)[E]
			Day 20 0600 h to day 21 0800 h	4	0	3%	(1/31)
			Total	**6**	**0**	**9%**	**(4/47)**

Littermate *p53*$^{f/f}$ (*Trp53*$^{loxP/loxP}$ *Pgr*$^{+/+}$) and *p53*$^{d/d}$ (*Trp53*$^{loxP/loxP}$ *Pgr*$^{Cre/+}$) dams were used. Time of delivery is defined as the day of pregnancy when the dam first started delivering pups. Preterm birth is defined as delivery occurring before day 19 of pregnancy. LPS was administered i.p. on day 16 of pregnancy at 1200h. Rapamycin (Rapa, 0.25 mg/kg BW/dose) was given as an oral gavage on days 8, 12, and 16 of pregnancy. Celecoxib (Cele, 10 mg/kg BW) was administered as oral gavages twice on day 16, once 3 h prior to LPS injection and again 4 h after LPS. Progesterone (P_4 , 2 mg/dose, s.c.) was injected twice on day 16 at similar time points as celecoxib. The rates of females showing preterm birth were calculated over the total number of females examined within a treatment group. The rates of dead pups and resorptions were calculated over the total number of pups delivered, while numbers in parentheses indicate their numbers.

[A]two females lost weight daily from days 16 through 20 of pregnancy; both females showed resorptions.
[B]one female did not deliver and lost weight daily from days 16 through 20 of pregnancy showing resorptions.
[C]one dam delivered eight pups, three of which died post-delivery.
[D]one dam delivered eight pups, six of which died post-delivery.
[E]one dam delivered eight pups, three of which died post-delivery.

Supplementary Table 3. An alternative schedule of rapamycin treatment with P_4 rescues preterm birth in LPS-treated *p53*$^{d/d}$ females.

Challenge	Treatment	Dam genotype	Time of delivery	No. of dams	Rate of preterm birth	% dead pups and resorptions over total number of pups
10 µg LPS	Rapa (Days 8, 10, 12) + P_4 (Day 16)	*p53*$^{f/f}$	Day 19 1600 h to Day 20 1700 h	7	0%	0% (0/41)
		p53$^{d/d}$	Day 20 0700 h – 1700 h	6	0%	0% (0/33)

Littermate *p53*$^{f/f}$ (*Trp53*$^{loxP/loxP}$ *Pgr*$^{+/+}$) and *p53*$^{d/d}$ (*Trp53*$^{loxP/loxP}$ *Pgr*$^{Cre/+}$) dams were used. Time of delivery is defined as the day of pregnancy when the dam first started delivering pups. Preterm birth is defined as delivery occurring before day 19 of pregnancy. LPS was administered i.p. on day 16 of pregnancy at 1200h. Rapamycin (Rapa, 0.25 mg/kg BW/dose) was given as an oral gavage on days 8, 10, and 12 of pregnancy. Progesterone (P_4, 2 mg/dose) was administered subcutaneously on day 16 of pregnancy 3 h prior to LPS injection (0900h), then again 4 h after LPS injection (1600h). The rates of females showing preterm birth were calculated over the total number of females examined within a treatment group. The rates of dead pups and resorptions were calculated over the total number of pups delivered, while numbers in parentheses indicate their numbers.

Supplementary Table 4. Patient information for term and preterm decidua-placental samples.

Variable	Term Delivery		Preterm Delivery	
	Mean (Min-Max)	SD	Mean (Min-Max)	SD
Number	28	-	20	-
Vaginal delivery	28/28	-	20/20	-
Cesarean section	0/28	-	0/20	-
Gestational weeks of delivery	39.0 (37-41)	0.9	32.2 (25-36)	3.1
Age	31.8 (21-42)	5.0	31.1 (18-39)	6.1
Parity	0.5 (0-2)	0.6	0.7 (0-2)	0.7
Chorioamnionitis	0/28	-	8/20	-
Premature rupture of the membranes	0/28	-	8/20	-
Fetal growth retardation	0/28	-	0/20	-
Birth Weight (g)	3058 (2480-3755)	334	1919 (655-2751)	552

Values are given as mean (minimum-maximum range). Term and preterm placentae from women with singleton, vaginal deliveries were examined. Women undergoing term vaginal delivery did not show any clinical or pathological signs of preterm delivery, infection, or other maternal or placental diseases. Women with singleton preterm vaginal delivery were not medically indicated and did not show clinical or pathological signs of other maternal or placental diseases apart from preterm delivery. Newborns did not have any apparent birth or chromosomal abnormalities. SD, standard deviation.

Supplementary Table 5. Patient information for placental samples from non-laboring women undergoing Cesarean section.

Variable	Caesarian Delivery	
	Mean (Min-Max)	SD
Number	11	-
Vaginal delivery	0/11	-
Cesarean section	11/11	-
Gestational weeks of delivery	33.5 (31-36)	1.8
Age	31.1 (18-39)	5.0
Parity	0.9 (0-2)	1.0
Chorioamnionitis	0/11	-
Premature rupture of the membranes	0/11	-
Fetal growth retardation	0/11	-
Preeclampsia	5/11	-
Placenta Previa	2/11	-
Placental abruption or fetal distress	4/11	-
Birth Weight (g)	1807 (1498-2516)	321

Values are given as mean (minimum-maximum range). Placentae from women with singleton deliveries were examined. Women undergoing Cesarean delivery without labor did not show any clinical or pathological signs of preterm delivery and infection, but showed clinical or pathological signs of preeclampsia, placenta previa, abrupt placenta, or fetal distress/anomaly. Newborns did not have any apparent birth or chromosomal abnormalities. SD, standard deviation.