Supplemental Methods

Immunoblot analysis

Whole-cell protein extracts were isolated from flash-frozen myometrial samples homogenized in 1X RIPA buffer containing proteinase inhibitors (Roche), and 1 mM phenylmethylsulfonyl fluoride (PMSF). Myometrial homogenates were centrifuged at 5000 x*g* for 10 min at 4°C and supernatants were harvested and stored at -80°C. Protein concentrations were quantified using the Bradford assay (BCA Protein Assay Kit, Pierce) and equivalent protein concentrations (25 µg) were added to 4X NuPage LDS Sample Buffer (Invitrogen) following the manufacturers' instructions. Proteins were resolved by electrophoresis in a 4-12% gradient SDS-PAGE (Bio-Rad, Hercules, CA) under reducing conditions and were electroblotted onto nitrocellulose membrane (Milipore Corp., MA). The membrane was blocked for 1 h at room temperature in 5% (wt/vol) nonfat dry milk in 1X PBS, containing 0.1% Tween-20. Primary rabbit polyclonal antibodies against connexin-43 (sc-6560 (Santa Cruz Biotechnology), 1:500) and β-actin (ab8227 (Abcam), 1:5000) were diluted in 'blocking buffer' (PBS, containing 0.1% Tween-20, containing 5% vol/vol bovine serum albumin) and incubated overnight at 4°C. The membrane was washed three times in 1X PBS, containing 0.1% Tween-20, and incubated with horseradish peroxidase-conjugated goat α-rabbit-IgG (ab')2 fragment (GE NA9340V (GE Healthcare), 1:2000) diluted in blocking buffer, followed by incubation at room temperature for 1 h. Enhanced Chemiluminescent Substrate (Thermo Scientific) was used according to the manufacturer's protocol. Densitometric analysis of immunoreactive bands were quantified using ImageQuaNT software (Molecular Dynamics) and normalized to β-actin loading controls.

Supplemental Figures:

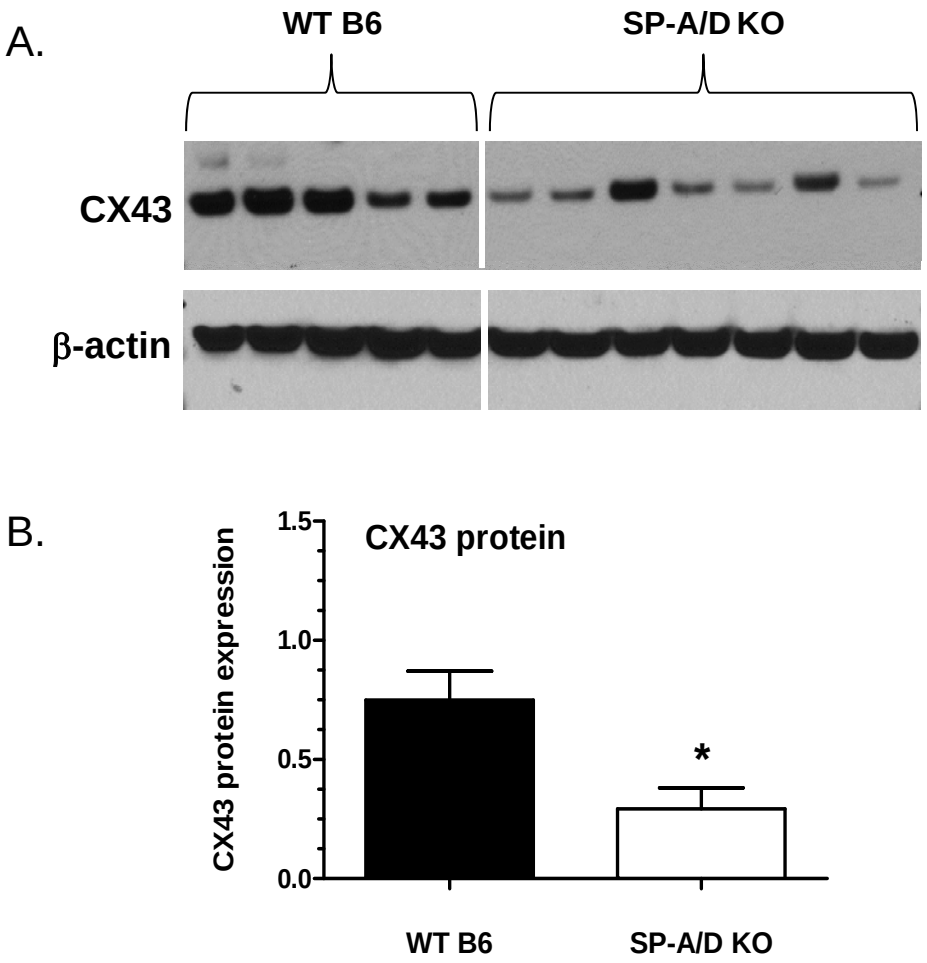

Fig. S1. CX43 protein expression is significantly lower in myometrium of SP-A/D$^{-/-}$ mice during second pregnancies. **(A)** Homogenate proteins (25 μg) of myometrial tissue from 18.5 dpc wild-type (WT) C57BL/6 (B6, n=5) and SP-A/D KO (n=7) mice were analyzed for CX43 protein by immunoblotting (anti-CX43 polyclonal (sc-6560, Santa Cruz), 1:500); β-actin was analyzed as a loading/transfer control (anti-β-actin (ab8227, Abcam), 1:2000). **(B)** Densitometric scans normalized to β-actin revealed that CX43 protein was reduced by ~60% in SP-A/D deficient mouse myometrium compared to WT B6 at 18.5 dpc ($p = 0.01$).

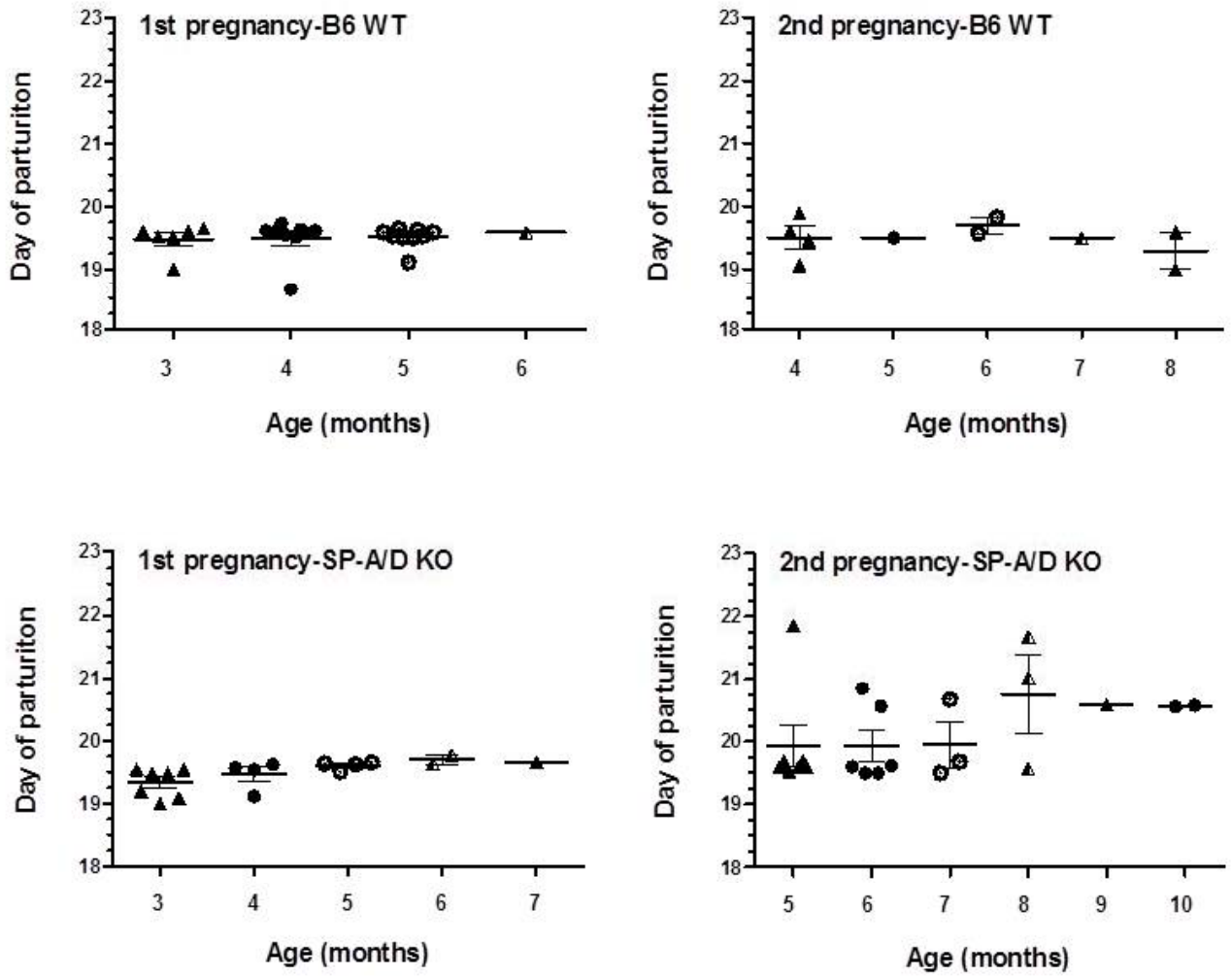

Fig. S2. Age of parity does not influence the time to labor in mice. The age, in months, and the time to labor, listed as day of parturition, is plotted for **(A,C)** primigravid and **(B, D)** multigravid wild-type (WT) C57BL/6 (B6) and SP-A/D deficient mice. Parturition timing was unaffected by age during first **(A)** and second **(B)** pregnancies in WT B6 mice. Similarly, first pregnancies in SP-A/D deficient mice demonstrated no correlation between age and the time to labor **(D)**. During second pregnancies, SP-A/D null mice manifested a similar pattern of delayed parturition across all age groups **(E)**. No statistically significant differences existed in gestation length *vs.* age among SP-A/D KO or WT groups.