

Table S1. List of antibodies used for immunophenotyping and microscopy.

Murine immunophenotyping and microscopy			
Cell marker	Fluorochrome	Clone	Company
CD45	V450; Alexa Fluor 700	30-F11	BD Biosciences
F4/80	PE; APC	BM8	eBiosciences
Ly6G	APC-Cy7	1A8	BD Biosciences
CD11b	PE-CF594; Alexa Fluor 594	M1/70	BD Biosciences; Biolegend
MHC-II	Alexa Fluor 700	M5/114.15.2	eBiosciences
CD80	V450	16-10A1	BD Biosciences
CD86	APC	GL1	BD Biosciences
CD206	PerCP-Cy5.5	C068C2	BioLegend
CD62L	PE-Cy7	MEL-14	BD Biosciences
IFN γ	PerCP-Cy5.5	XMG1.2	BD Biosciences
TNF α	PE-Cy7	MP6-XT22	BD Biosciences
TGF β	APC	TW7-16B4	BioLegend
IL-10	V450	JES5-16E3	BD Biosciences
H2Kb (MHC-I)	APC	AF6-88.5.5.3	eBiosciences
H2Kd (MHC-I)	eFluor 450	SF1-1.1.1	eBiosciences
Ovalbumin (OVA)*	FITC	Polyclonal	Abcam/Rockland
Rabbit IgG Isotype*	FITC	Polyclonal	Abcam/Rockland
IgG	Alexa Fluor 594	Polyclonal	Life Technologies
Human immunophenotyping and microscopy			
Cell marker	Fluorochrome	Clone	Company
CD15	BV650; Alexa Fluor 647	HI98; W6D3	BD Biosciences
CD14	BUV395	M ϕ P9	BD Biosciences
IgG	Alexa Fluor 594	Polyclonal	Life Technologies

*OVA antibody and isotype from different lots and different manufacturers were used.

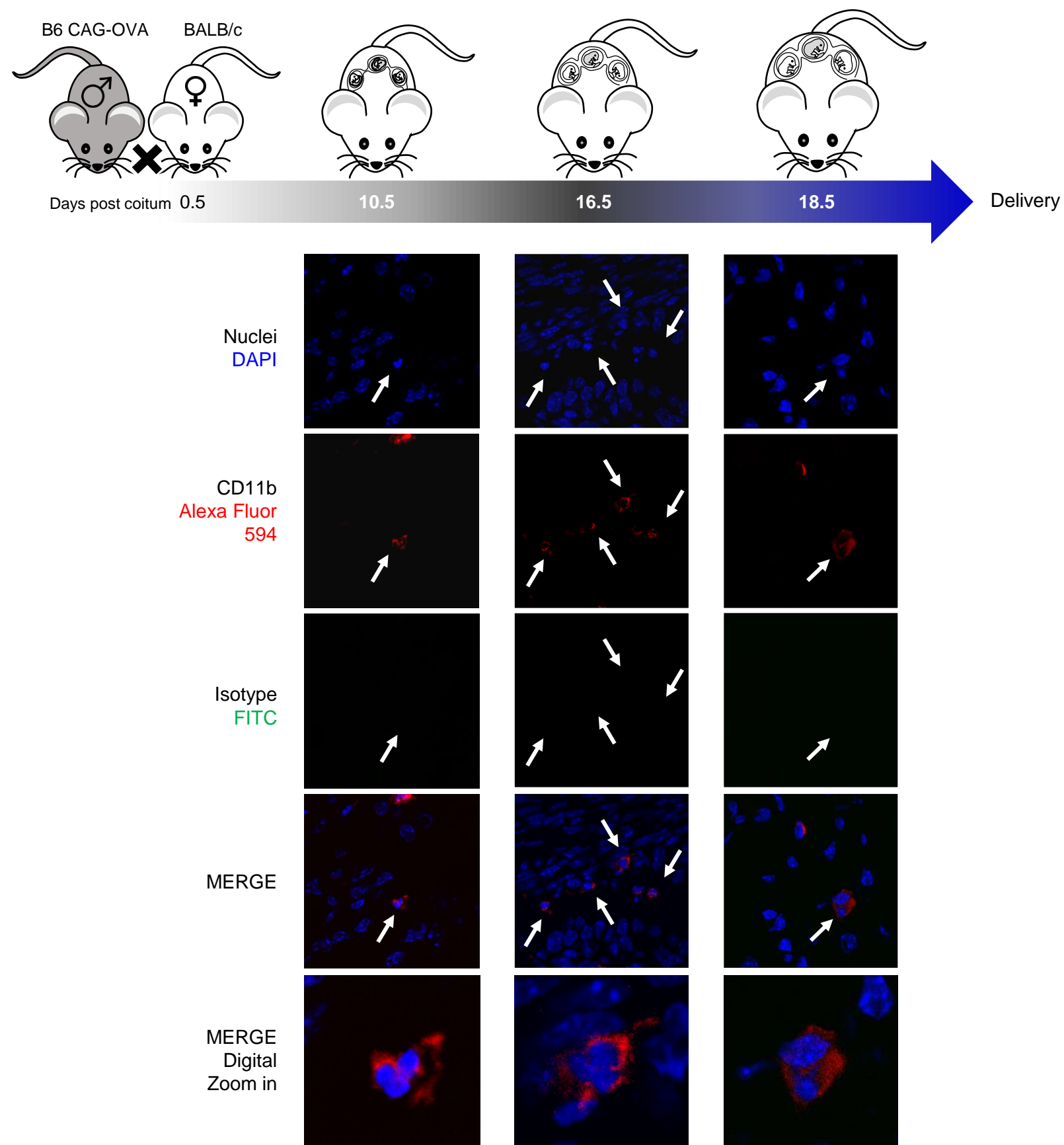


Fig. S1. Isotype staining control for the localization of Ly6G+ and F4/80+ cells in the myometrial tissues in the second half of pregnancy. BALB/c females were mated with B6 CAG-OVA males, and fetuses with surrounding myometrial tissues were collected at 10.5 days *post coitum* (dpc), 16.5 dpc, or 18.5 dpc. Confocal microscopy imaging of DAPI+CD11b+ cells (indicated by white arrows) with lack of staining for FITC isotype control in the myometrial tissues (100x magnification with digital zoom) (n = 10 each).

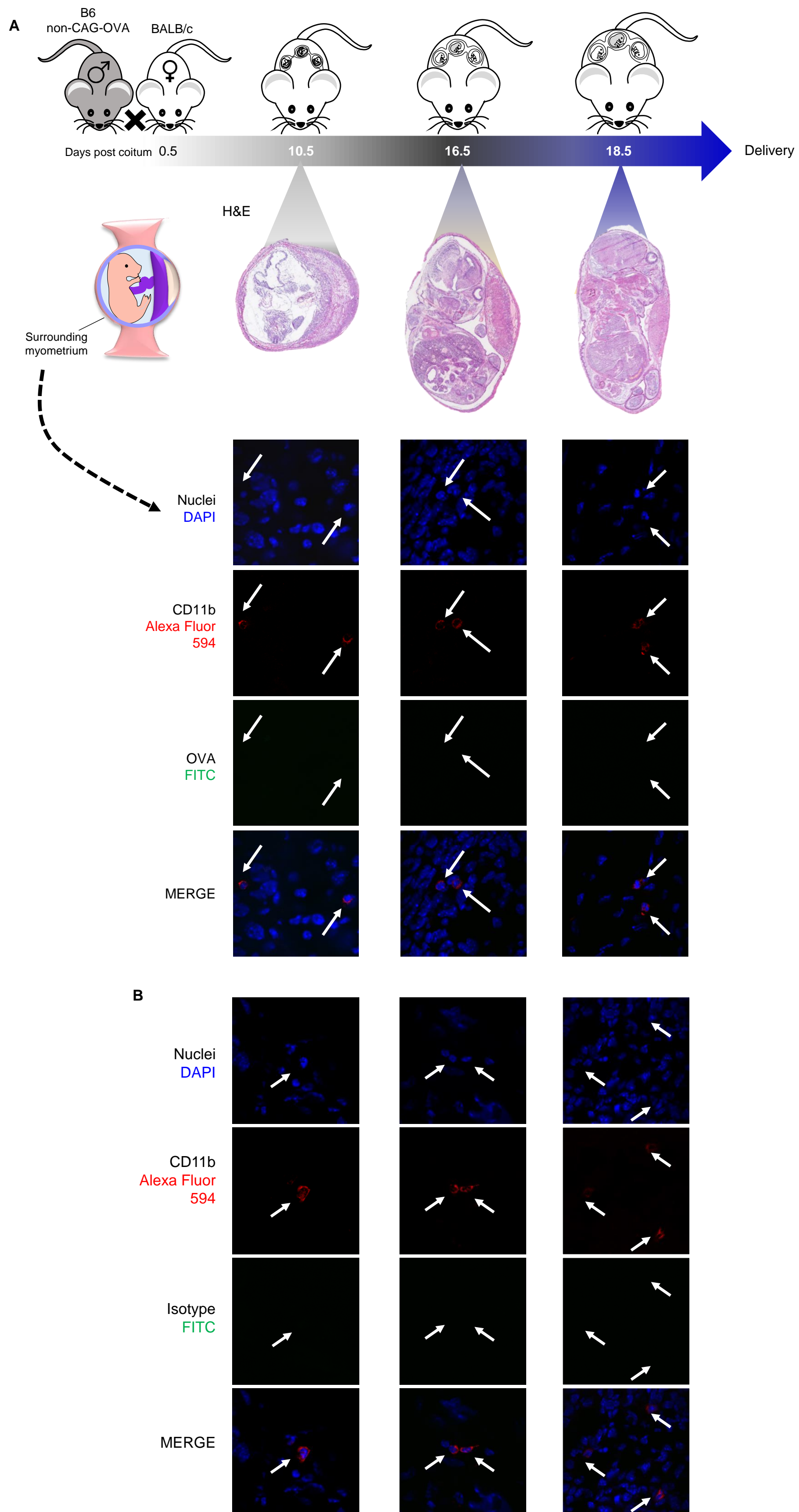


Fig. S2. Ly6G+ and F4/80+ cells in the myometrial tissues in the second half of pregnancy (OVA antibody staining control) (A & B) BALB/c females were mated with B6 non-CAG-OVA males, and the fetuses with surrounding myometrial tissues were collected at 10.5 days post coitum (dpc), 16.5 dpc, or 18.5 dpc. Representative images of hematoxylin & eosin (H&E) staining (4x magnification), and confocal microscopy imaging of DAPI+CD11b+ cells (indicated by white arrows) with lack of staining for (A) OVA-FITC or (B) isotype-FITC control in the myometrial tissues (100x magnification with digital zoom) (n = 3 each).

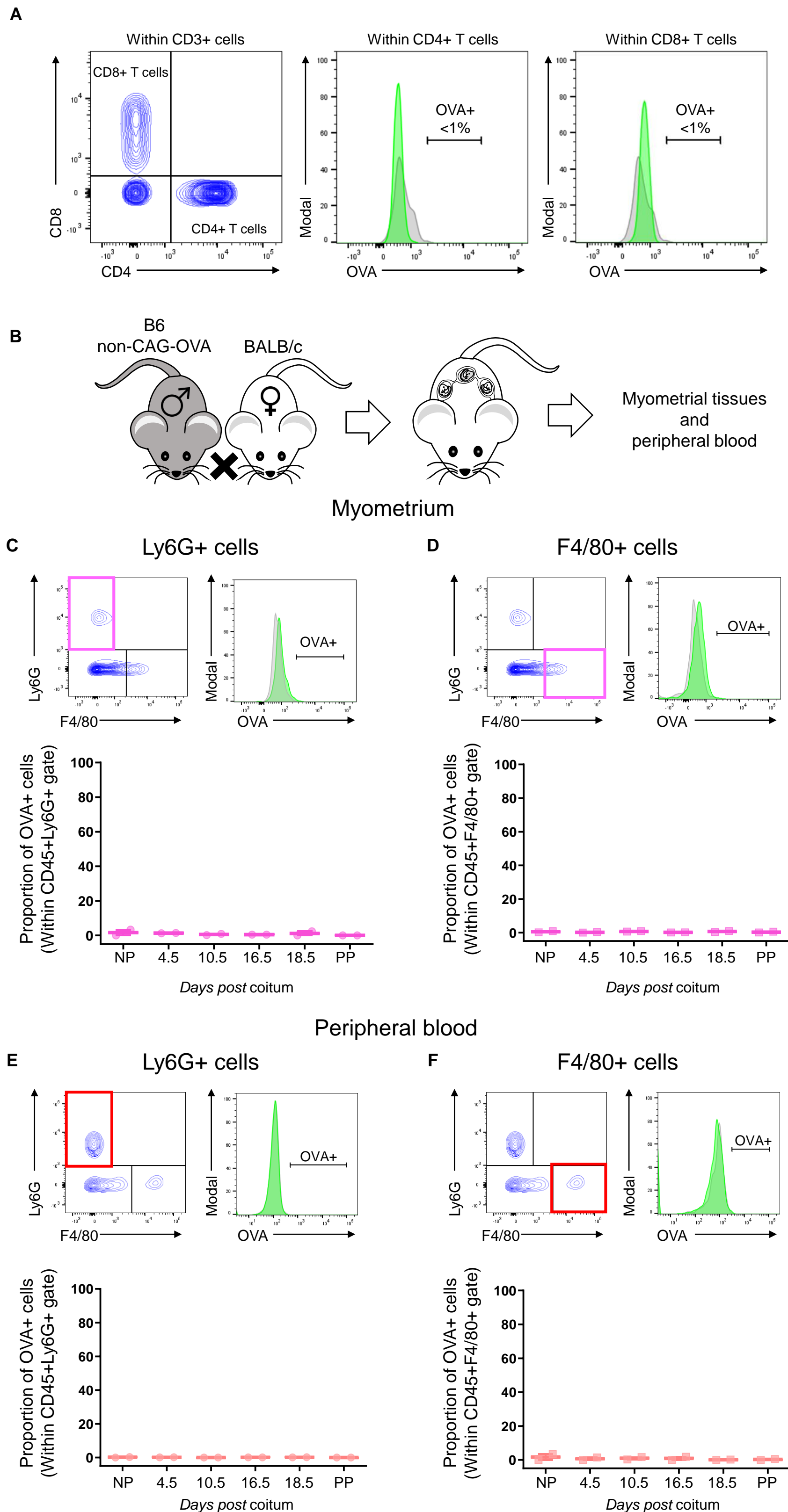


Fig. S3. Expression of OVA in maternal circulating T cells in pregnant dams; and proportions of Ly6G+ and F4/80+ cells in the myometrial tissues and maternal circulation from non-pregnant, pregnant, and post-partum dams. (A) Representative gating strategy for CD4+ and CD8+ T cells within the CD3+ cell population. Grey histograms indicate the isotype control and green histograms indicate OVA antibody staining. T cells do not express the OVA antigen. N = 10. **(B)** BALB/c females mated with B6 non-CAG-OVA males at 4.5 days *post coitum* (dpc), 10.5 dpc, 16.5 dpc, or 18.5 dpc and postpartum (PP). **(C & D)** Representative gating strategies and proportions of **(B)** CD45+Ly6G+OVA+ and **(C)** CD45+F4/80+OVA+ cells in the myometrial tissues from non-pregnant (NP) dams and dams at 4.5 days dpc, 10.5 dpc, 16.5. dpc, 18.5 dpc and PP (n = 2 each). **(E & F)** Representative gating strategies and proportions of **(E)** CD45+Ly6G+OVA+ and **(F)** CD45+F4/80+OVA+ cells in the maternal circulation (peripheral blood) from NP dams and dams at 4.5 dpc, 10.5 dpc, 16.5. dpc, 18.5 dpc and PP (n = 2 each). Data are shown as box-and-whisker plots where midlines indicate medians, boxes indicate interquartile ranges, and whiskers indicate minimum and maximum ranges.

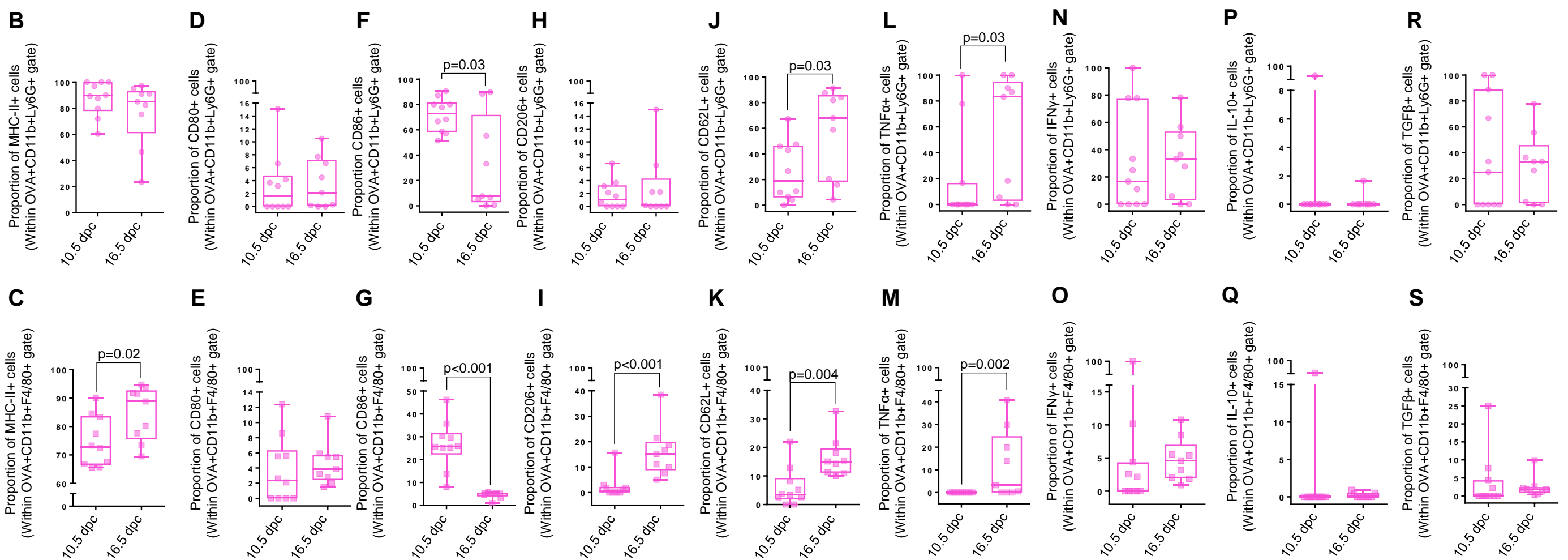
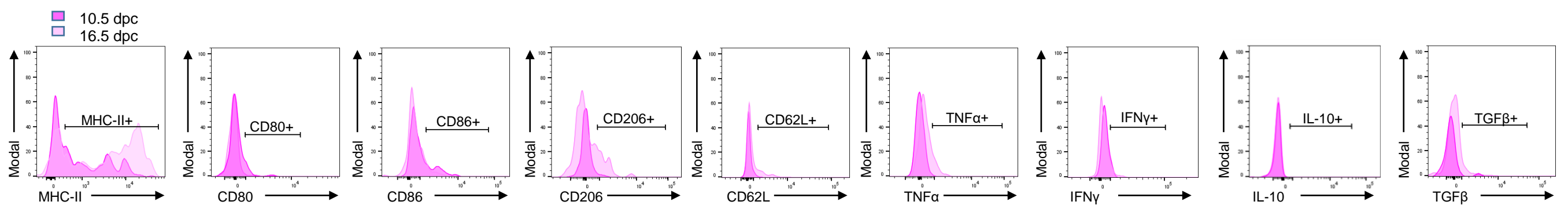
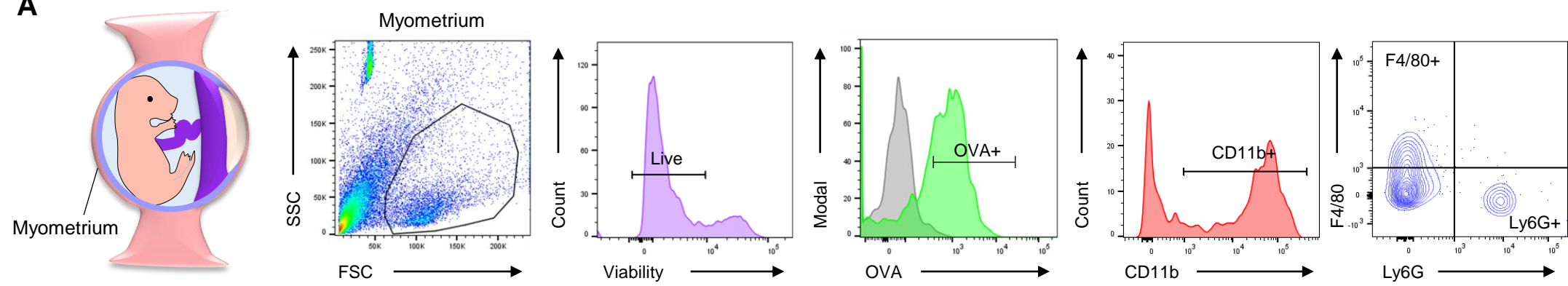
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Fig. S4. Immunophenotyping of fetal antigen-carrying Ly6G⁺ and F4/80⁺ cells in the myometrial tissues during mid and late gestation. (A) Flow cytometry gating strategy used to determine the Ly6G⁺OVA⁺ and F4/80⁺OVA⁺ cells (green histogram) in the myometrium. Proportions of CD11b⁺Ly6G⁺OVA⁺ and CD11b⁺F4/80⁺OVA⁺ cells expressing **(B & C)** MHC-II, **(D & E)** CD80, **(F & G)** CD86, **(H & I)** CD206, **(J & K)** CD62L, **(L & M)** TNF α , **(N & O)** IFN γ , **(P & Q)** IL-10, or **(R & S)** TGF β in the myometrial tissues at 10.5 days *post coitum* (dpc) and 16.5 dpc ($n = 9 - 11$ each). Data are shown as box-and-whisker plots where midlines indicate medians, boxes indicate interquartile ranges, and whiskers indicate minimum and maximum ranges. The p-values were determined using Mann-Whitney U-tests.

Phagocytosis of *Escherichia coli*
(Positive control)

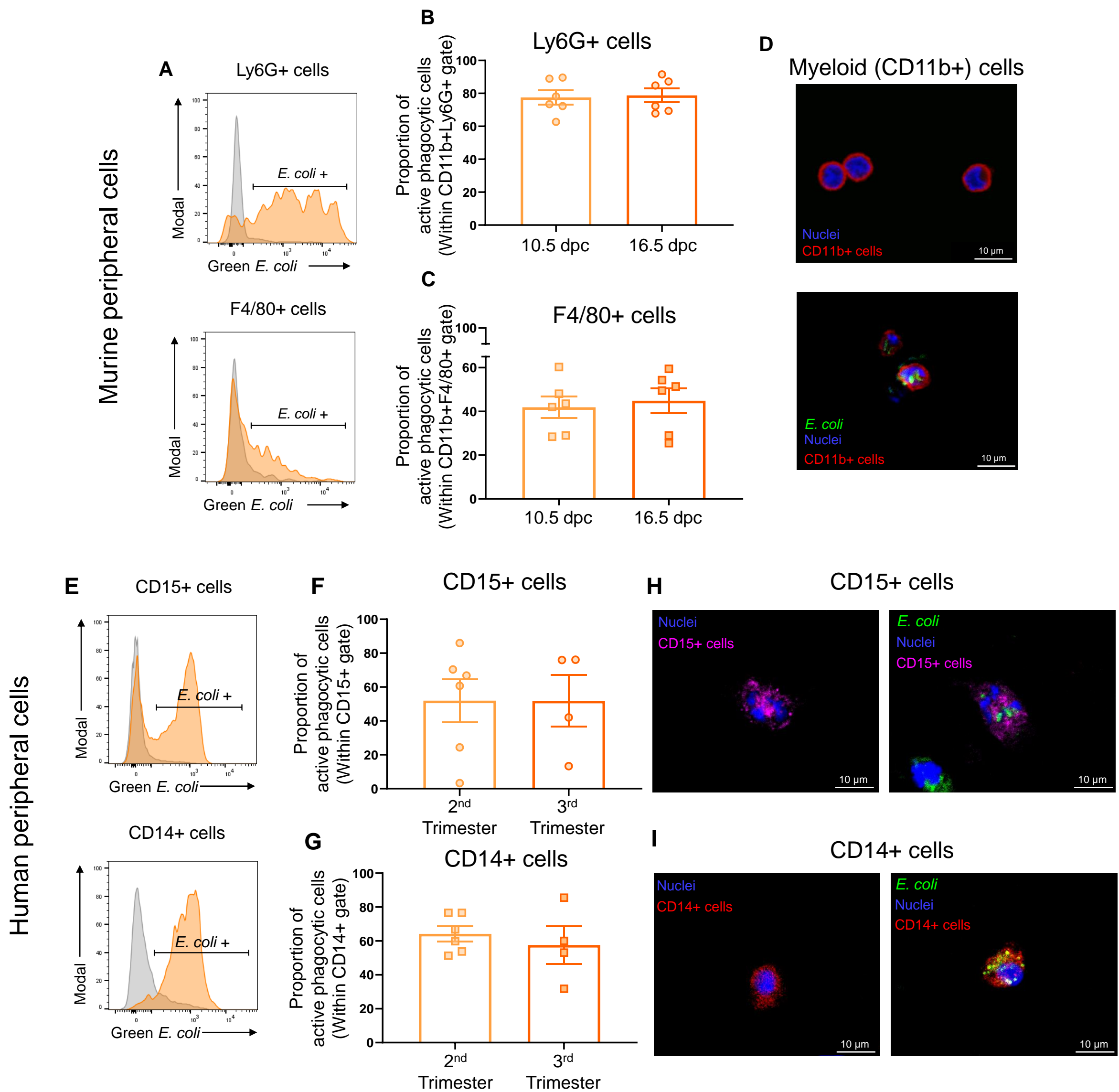


Fig. S5. Phagocytosis of *Escherichia coli* by murine maternal Ly6G+ or F4/80+ cells and human maternal CD15+ or CD14+ cells in the second and third trimester of pregnancy. Murine maternal peripheral Ly6G+ or F4/80+ cells and human maternal peripheral CD14+ or CD15+ cells were collected during the second trimester/10.5 days *post coitum* (dpc) or third trimester/16.5 dpc and cultured with pHrodo Green-labelled *Escherichia coli* (*E. coli*) (n = 4 – 6 each). The uptake of labelled *E. coli* by murine Ly6G+ or F4/80+ cells and human CD15+ or CD14+ cells was evaluated by flow cytometry. **(A & E)** Representative gating strategy showing the uptake of labelled *E. coli* by murine Ly6G+ or F4/80+ cells and human CD15+ or CD14+ cells. Proportions of active **(B)** murine Ly6G+ and **(F)** human CD15+ cells or **(C)** murine F4/80+ and **(G)** human CD14+ cells that phagocytosed labelled *E. coli*. Data are shown as scatter dot plots where bars indicate the mean and whiskers indicate the standard error of the mean. P-values were determined using Mann-Whitney U-tests. **(D)** Representative confocal microscopy images showing murine maternal peripheral CD11b+ (indicated in red) cells alone or after phagocytosing green *E. coli*. Blue immunofluorescence indicates DAPI (nuclei). Scale bars represent 10 μm. **(H & I)** Representative confocal microscopy images showing human maternal peripheral CD15+ or CD14+ cells alone or after phagocytosing labelled *E. coli*. Blue immunofluorescence indicates DAPI (nuclei), pink indicates CD15, red indicates CD14, and green indicates *E. coli*. Scale bars represent 10 μm.