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Supplemental information

**Gestationally dependent immune organization
at the maternal-fetal interface**

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SUPPLEMENTAL TABLES and FIGURES

Table S1. A summary of antibodies used for our mass cytometry analysis. Related to Figure 1. The table shows the antibody target, antibody clone, the element and isotope the antibody was conjugated to, the final concentration of the metal-conjugated antibody used for protein detection, and the vendor from whom the antibodies were purchased.

Antibody target	Clone	Element	Mass	Concentration (ug/ml)	Vendor
Biotin	1D4-C5	In	113	2	BioLegend
CD45	30-F11	see footnote			BioLegend
CD45.2	104	In	115	4	BioLegend
Ter119	TER-119	La	139	2	BioLegend
B220	RA3-6B2	Ce	140	4	BioLegend
Ly-6G	1A8	Pr	141	2	BioLegend
CD11c	N418	Nd	142	1	BioLegend
TCR β	H57-597	Nd	143	1	BioLegend
CD115 (CSF-1R)	AFS98	Nd	144	2	Fluidigm Sciences
CD69	H1.2F3	Nd	145	1	BioLegend
F4/80	BM8	Nd	146	1	BioLegend
CD3	17A2	Sm	147	2	BioLegend
IgD	11-26c.2a	Nd	148	2	BioLegend
CD19	6D5	Sm	149	1	BioLegend
CD25	3C7	Nd	150	4	Fluidigm Sciences
CD64	X54-5/7.1	Eu	151	4	BioLegend
CD80	16-10A1	Sm	152	2	BioLegend
CD8	53-6.7	Eu	153	1	BioLegend
CD11b	M1/70	Sm	154	1	BioLegend
CD40	HM40-3	Gd	155	2	BioLegend
IgM	RMM-1	Gd	156	1	BioLegend
CD117 (c-Kit)	2B8	Gd	157	0.5	BioLegend
TCR $\gamma\delta$	GL3	Tb	159	2	BioLegend
CTLA-4 (CD152)	UC10-4B9	Dy	161	2	BioLegend
Ly-6C	HK1.4	Dy	162	2	BioLegend
CD194 (CCR4)	2G12	Dy	163	2	BioLegend
CD62L	MEL-14	Dy	164	2	BioLegend
PD-L1 (CD274)	10F.9G2	Ho	165	2	BioLegend
Fc ϵ RI- α	MAR-1	Er	166	0.5	BioLegend
CD335 (Nkp46)	29A1.4	Er	167	2	BioLegend
Siglec-F	E50-2440	Er	168	1	BDBiosciences
CD49b	DX5	Er	170	2	BioLegend
CD44	IM7	Yb	171	1	BioLegend
CD4	RM4-5	Yb	172	1	BioLegend
PD-1 (CD279)	29F.1A12	Yb	173	2	BioLegend
MHC-II	M5/114.15.2	Yb	174	1	BioLegend
CD86	GL-1	Lu	175	2	BioLegend
CD45.1	A20	Yb	176	2	BioLegend
FoxP3	FJK-16s	Gd	158	4	Fluidigm Sciences
CD68	FA-11	Tm	169	2	BioLegend

prpS6 Ser235/Ser236	A17020B	Bi	209	2	BioLegend
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Footnote: the anti-CD45 antibody administered retro-orbitally is detected by the anti-biotin antibody (isotope In113)

Table S4. Generalized Estimating Equations coefficients and statistics for regressions of immune cell fractions over embryonic days. Related to Figure 3C. Generalized Estimating Equations (GEE) was used to fit linear models and compare compartments within each cell type. PB was used as the reference.

Coefficient	Estimate	Naive.S.E.	Naive.z	Robust.S.E.	Robust.z	P value	Cell type
Intercept	2.66E-01	6.48E-02	4.11E+00	5.50E-02	4.84E+00	6.41E-07	B cells
Day	3.93E-03	4.38E-03	8.96E-01	3.59E-03	1.09E+00	1.37E-01	B cells
EV	-2.14E-01	9.17E-02	-2.33E+00	6.63E-02	-3.22E+00	6.34E-04	B cells
TIS	-2.26E-01	9.17E-02	-2.46E+00	8.83E-02	-2.56E+00	5.29E-03	B cells
Day:EV	3.81E-03	6.19E-03	6.15E-01	4.51E-03	8.44E-01	1.99E-01	B cells
Day:TIS	-1.45E-03	6.19E-03	-2.35E-01	6.21E-03	-2.34E-01	4.07E-01	B cells
Intercept	2.55E-02	1.68E-02	1.52E+00	1.68E-02	1.52E+00	6.42E-02	Basophils
Day	-7.89E-04	1.14E-03	-6.91E-01	1.03E-03	-7.63E-01	2.23E-01	Basophils
EV	-9.31E-03	2.55E-02	-3.65E-01	1.72E-02	-5.42E-01	2.94E-01	Basophils
TIS	2.67E-03	2.37E-02	1.13E-01	2.46E-02	1.08E-01	4.57E-01	Basophils
Day:EV	4.37E-05	1.72E-03	2.55E-02	1.06E-03	4.13E-02	4.84E-01	Basophils
Day:TIS	1.49E-03	1.60E-03	9.28E-01	1.64E-03	9.07E-01	1.82E-01	Basophils
Intercept	8.86E-02	2.77E-02	3.20E+00	4.79E-02	1.85E+00	3.23E-02	CD4+ T cells
Day	-9.08E-04	1.87E-03	-4.86E-01	3.04E-03	-2.99E-01	3.82E-01	CD4+ T cells
EV	-1.20E-01	3.91E-02	-3.06E+00	4.98E-02	-2.40E+00	8.14E-03	CD4+ T cells
TIS	-7.16E-02	3.91E-02	-1.83E+00	5.24E-02	-1.37E+00	8.58E-02	CD4+ T cells
Day:EV	5.30E-03	2.64E-03	2.01E+00	3.21E-03	1.65E+00	4.93E-02	CD4+ T cells
Day:TIS	1.48E-03	2.64E-03	5.61E-01	3.37E-03	4.39E-01	3.30E-01	CD4+ T cells
Intercept	8.33E-02	2.07E-02	4.03E+00	3.57E-02	2.33E+00	9.84E-03	CD8+ T cells
Day	-1.14E-03	1.40E-03	-8.16E-01	2.30E-03	-4.96E-01	3.10E-01	CD8+ T cells
EV	-1.09E-01	2.92E-02	-3.72E+00	3.66E-02	-2.97E+00	1.51E-03	CD8+ T cells
TIS	-6.50E-02	2.92E-02	-2.23E+00	4.04E-02	-1.61E+00	5.37E-02	CD8+ T cells
Day:EV	4.48E-03	1.97E-03	2.27E+00	2.36E-03	1.90E+00	2.85E-02	CD8+ T cells
Day:TIS	1.18E-03	1.97E-03	5.99E-01	2.67E-03	4.42E-01	3.29E-01	CD8+ T cells
Intercept	3.17E-02	1.36E-02	2.33E+00	1.95E-02	1.62E+00	5.24E-02	Eosinophils
Day	-1.60E-03	9.22E-04	-1.74E+00	1.17E-03	-1.36E+00	8.64E-02	Eosinophils
EV	-4.06E-03	1.91E-02	-2.12E-01	2.26E-02	-1.80E-01	4.29E-01	Eosinophils

TIS	8.14E-03	1.91E-02	4.25E-01	2.31E-02	3.53E-01	3.62E-01	Eosinophils
Day:EV	5.33E-04	1.30E-03	4.11E-01	1.36E-03	3.93E-01	3.47E-01	Eosinophils
Day:TIS	5.59E-04	1.30E-03	4.31E-01	1.46E-03	3.84E-01	3.51E-01	Eosinophils
Intercept	1.80E-01	7.10E-02	2.53E+00	4.68E-02	3.85E+00	6.00E-05	Monocytes
Day	-4.48E-03	4.80E-03	-9.34E-01	3.04E-03	-1.47E+00	7.06E-02	Monocytes
EV	9.79E-01	1.00E-01	9.75E+00	6.72E-02	1.46E+01	2.02E-48	Monocytes
TIS	3.72E-01	1.00E-01	3.70E+00	8.18E-02	4.54E+00	2.80E-06	Monocytes
Day:EV	-3.91E-02	6.78E-03	-5.77E+00	4.63E-03	-8.44E+00	1.55E-17	Monocytes
Day:TIS	-6.67E-03	6.78E-03	-9.83E-01	5.77E-03	-1.15E+00	1.24E-01	Monocytes
Intercept	2.46E-01	5.42E-02	4.54E+00	7.94E-02	3.10E+00	9.77E-04	NK cells
Day	3.90E-04	3.66E-03	1.06E-01	5.30E-03	7.36E-02	4.71E-01	NK cells
EV	-2.46E-01	7.67E-02	-3.21E+00	8.06E-02	-3.05E+00	1.14E-03	NK cells
TIS	-8.02E-02	7.67E-02	-1.05E+00	8.63E-02	-9.29E-01	1.76E-01	NK cells
Day:EV	2.63E-03	5.18E-03	5.08E-01	5.38E-03	4.90E-01	3.12E-01	NK cells
Day:TIS	-5.82E-03	5.18E-03	-1.12E+00	5.77E-03	-1.01E+00	1.56E-01	NK cells
Intercept	7.59E-02	8.43E-02	9.01E-01	3.75E-02	2.02E+00	2.15E-02	Neutrophils
Day	4.86E-03	5.69E-03	8.54E-01	2.58E-03	1.88E+00	2.98E-02	Neutrophils
EV	-2.70E-01	1.19E-01	-2.26E+00	6.67E-02	-4.04E+00	2.63E-05	Neutrophils
TIS	6.28E-02	1.19E-01	5.26E-01	9.32E-02	6.73E-01	2.50E-01	Neutrophils
Day:EV	2.18E-02	8.05E-03	2.71E+00	4.67E-03	4.67E+00	1.53E-06	Neutrophils
Day:TIS	8.97E-03	8.05E-03	1.11E+00	6.77E-03	1.32E+00	9.26E-02	Neutrophils

Table S5. Generalized Estimating Equations coefficients and statistics for regressions of protein medians in MP and neutrophils over embryonic days. Related to Figure 3G. GEE was used to fit linear models per protein and cell type to compare compartments. PB was used as the reference.

Coefficient	Estimate	Naive.S.E.	Naive.z	Robust.S.E.	Robust.z	P value	Cell/protein
Intercept	7.92E-01	5.71E-01	1.39E+00	3.58E-01	2.21E+00	1.34E-02	MP/PD-L1
Day	-3.01E-02	3.85E-02	-7.80E-01	2.28E-02	-1.32E+00	9.32E-02	MP/PD-L1
EV	1.84E+00	8.07E-01	2.28E+00	9.41E-01	1.95E+00	2.54E-02	MP/PD-L1
TIS	3.65E+00	8.07E-01	4.52E+00	6.28E-01	5.81E+00	3.20E-09	MP/PD-L1
Day:EV	-1.17E-01	5.45E-02	- 2.15E+00	5.85E-02	-2.01E+00	2.25E-02	MP/PD-L1
Day:TIS	-1.76E-01	5.45E-02	- 3.22E+00	4.13E-02	-4.26E+00	1.03E-05	MP/PD-L1
Intercept	3.49E-01	1.45E-01	2.41E+00	1.19E-01	2.94E+00	1.63E-03	Neutrophils/ PD-L1
Day	-1.28E-02	9.78E-03	- 1.30E+00	7.75E-03	-1.65E+00	5.00E-02	Neutrophils/ PD-L1
EV	-7.80E-02	2.05E-01	-3.81E-01	1.77E-01	-4.40E-01	3.30E-01	Neutrophils/ PD-L1
TIS	3.47E-01	2.05E-01	1.69E+00	1.93E-01	1.80E+00	3.59E-02	Neutrophils/ PD-L1
Day:EV	3.70E-03	1.38E-02	2.68E-01	1.08E-02	3.42E-01	3.66E-01	Neutrophils/ PD-L1
Day:TIS	-1.33E-02	1.38E-02	-9.63E-01	1.27E-02	-1.05E+00	1.47E-01	Neutrophils/ PD-L1
Intercept	4.55E+00	6.85E-01	6.64E+00	9.90E-01	4.59E+00	2.20E-06	MP/Ly-6C
Day	8.76E-03	4.63E-02	1.89E-01	6.59E-02	1.33E-01	4.47E-01	MP/Ly-6C
EV	-9.56E-01	9.69E-01	-9.87E-01	9.73E-01	-9.83E-01	1.63E-01	MP/Ly-6C
TIS	1.12E+00	9.69E-01	1.16E+00	9.55E-01	1.18E+00	1.20E-01	MP/Ly-6C
Day:EV	7.25E-02	6.54E-02	1.11E+00	6.62E-02	1.10E+00	1.36E-01	MP/Ly-6C
Day:TIS	-1.10E-01	6.54E-02	- 1.69E+00	6.93E-02	-1.59E+00	5.55E-02	MP/Ly-6C
Intercept	2.97E+00	2.67E-01	1.11E+01	3.89E-01	7.64E+00	1.12E-14	Neutrophils/ Ly-6G
Day	-4.26E-03	1.80E-02	-2.36E-01	2.50E-02	-1.70E-01	4.32E-01	Neutrophils/ Ly-6G
EV	- 1.17E+00	3.77E-01	- 3.09E+00	3.35E-01	-3.48E+00	2.54E-04	Neutrophils/ Ly-6G
TIS	- 1.31E+00	3.77E-01	- 3.47E+00	3.42E-01	-3.83E+00	6.36E-05	Neutrophils/ Ly-6G
Day:EV	6.39E-02	2.55E-02	2.51E+00	2.17E-02	2.95E+00	1.58E-03	Neutrophils/ Ly-6G
Day:TIS	6.30E-02	2.55E-02	2.47E+00	2.31E-02	2.72E+00	3.22E-03	Neutrophils/ Ly-6G

Table S6. Generalized Estimating Equations coefficients and statistics for regressions of immune fractions in MP subsets over embryonic days. Related to Figure 4E and S4G. GEE was used to fit linear models and compare fraction of immune cells in compartments within each MP subset. PB was used as the reference.

Coefficient	Estimate	Naive.S.E.	Naive.z	Robust.S.E.	Robust.z	P value	Cell type
Intercept	6.69E-02	3.97E-02	1.68E+00	3.28E-02	2.04E+00	2.06E-02	Ly-6Chi
Day	-4.63E-04	2.68E-03	-1.73E-01	2.04E-03	-2.27E-01	4.10E-01	Ly-6Chi
EV	1.02E-01	5.61E-02	1.82E+00	5.88E-02	1.74E+00	4.11E-02	Ly-6Chi
TIS	3.41E-02	5.61E-02	6.08E-01	5.01E-02	6.81E-01	2.48E-01	Ly-6Chi
Day:EV	3.34E-03	3.79E-03	8.82E-01	3.86E-03	8.66E-01	1.93E-01	Ly-6Chi
Day:TIS	4.79E-04	3.79E-03	1.26E-01	3.30E-03	1.45E-01	4.42E-01	Ly-6Chi
Intercept	3.98E-02	1.84E-02	2.17E+00	1.09E-02	3.64E+00	1.34E-04	Patrolling
Day	-1.29E-03	1.24E-03	-1.04E+00	7.14E-04	-1.81E+00	3.55E-02	Patrolling
EV	1.73E-01	2.60E-02	6.65E+00	2.96E-02	5.85E+00	2.50E-09	Patrolling
TIS	9.81E-03	2.60E-02	3.77E-01	1.60E-02	6.13E-01	2.70E-01	Patrolling
Day:EV	-9.79E-03	1.76E-03	-5.58E+00	1.91E-03	-5.11E+00	1.57E-07	Patrolling
Day:TIS	-5.33E-04	1.76E-03	-3.03E-01	1.05E-03	-5.05E-01	3.07E-01	Patrolling
Intercept	1.05E-02	3.19E-02	3.30E-01	1.43E-03	7.37E+00	8.71E-14	Phagocytic
Day	-4.30E-04	2.11E-03	-2.04E-01	9.19E-05	-4.68E+00	1.42E-06	Phagocytic
EV	5.79E-02	3.95E-02	1.47E+00	2.16E-02	2.68E+00	3.63E-03	Phagocytic
TIS	1.55E-02	3.95E-02	3.92E-01	2.86E-02	5.41E-01	2.94E-01	Phagocytic
Day:EV	-2.57E-03	2.63E-03	-9.76E-01	1.35E-03	-1.91E+00	2.84E-02	Phagocytic
Day:TIS	3.21E-03	2.63E-03	1.22E+00	2.02E-03	1.59E+00	5.62E-02	Phagocytic
Intercept	-2.30E-04	7.59E-02	-3.03E-03	1.51E-03	-1.53E-01	4.39E-01	MoDC
Day	2.13E-04	5.73E-03	3.72E-02	1.31E-04	1.63E+00	5.20E-02	MoDC

EV	2.23E-01	8.31E-02	2.68E+00	5.44E-02	4.10E+00	2.06E-05	MoDC
TIS	1.83E-01	8.31E-02	2.20E+00	2.18E-02	8.36E+00	3.01E-17	MoDC
Day:EV	-1.24E-02	6.17E-03	-2.01E+00	3.39E-03	-3.66E+00	1.29E-04	MoDC
Day:TIS	-8.41E-03	6.17E-03	-1.36E+00	1.49E-03	-5.63E+00	8.93E-09	MoDC
Intercept	7.84E-03	1.12E-02	7.01E-01	4.87E-03	1.61E+00	5.36E-02	DC
Day	-1.98E-04	7.80E-04	-2.54E-01	3.24E-04	-6.11E-01	2.71E-01	DC
EV	2.42E-02	1.53E-02	1.58E+00	1.32E-02	1.84E+00	3.32E-02	DC
TIS	8.80E-03	1.53E-02	5.76E-01	9.62E-03	9.15E-01	1.80E-01	DC
Day:EV	2.77E-05	1.05E-03	2.64E-02	8.49E-04	3.26E-02	4.87E-01	DC
Day:TIS	4.00E-04	1.05E-03	3.81E-01	6.84E-04	5.84E-01	2.80E-01	DC
Intercept	6.01E-02	2.68E-02	2.25E+00	1.00E-02	5.99E+00	1.07E-09	Presenting
Day	-2.78E-03	1.81E-03	-1.54E+00	6.34E-04	-4.39E+00	5.73E-06	Presenting
EV	2.60E-01	3.78E-02	6.87E+00	3.30E-02	7.86E+00	1.86E-15	Presenting
TIS	1.71E-02	3.78E-02	4.52E-01	2.54E-02	6.73E-01	2.50E-01	Presenting
Day:EV	-9.92E-03	2.56E-03	-3.88E+00	2.26E-03	-4.39E+00	5.54E-06	Presenting
Day:TIS	1.48E-03	2.56E-03	5.81E-01	1.81E-03	8.19E-01	2.06E-01	Presenting
Intercept	4.58E-03	1.84E-02	2.49E-01	1.94E-03	2.36E+00	9.05E-03	Proliferating
Day	-1.58E-04	1.29E-03	-1.23E-01	1.25E-04	-1.27E+00	1.02E-01	Proliferating
EV	4.80E-02	2.04E-02	2.35E+00	7.05E-03	6.81E+00	4.75E-12	Proliferating
TIS	5.72E-02	2.03E-02	2.82E+00	1.08E-02	5.29E+00	6.20E-08	Proliferating
Day:EV	-2.86E-03	1.43E-03	-2.00E+00	4.55E-04	-6.28E+00	1.71E-10	Proliferating
Day:TIS	-3.11E-03	1.42E-03	-2.19E+00	6.85E-04	-4.54E+00	2.77E-06	Proliferating
Intercept	5.55E-03	1.11E-01	4.99E-02	9.78E-02	5.68E-02	4.77E-01	F4/80hi
Day	5.95E-03	7.44E-03	8.00E-01	7.33E-03	8.12E-01	2.08E-01	F4/80hi

Table S7. Generalized Estimating Equations coefficients and statistics for regressions of PD-L1+ MP fractions in PD-L1+ MP subsets over embryonic days. Related to Figure 4F.

GEE was used to fit linear models and compare fraction of PD-L1+ MPs in compartments within each PD-L1+ MP subset. PB was used as the reference.

Coefficient	Estimate	Naive.S.E.	Naive.z	Robust.S.E.	Robust.z	P value	Cell type
Intercept	8.05E-01	1.41E-01	5.72E+00	1.30E-01	6.19E+00	3.06E-10	Patrolling
Day	5.23E-03	9.50E-03	5.51E-01	8.42E-03	6.21E-01	2.67E-01	Patrolling
EV	-4.08E-01	1.99E-01	-2.05E+00	2.13E-01	-1.92E+00	2.75E-02	Patrolling
TIS	-6.24E-01	1.99E-01	-3.14E+00	1.60E-01	-3.91E+00	4.64E-05	Patrolling
Day:EV	-2.53E-03	1.34E-02	-1.89E-01	1.38E-02	-1.84E-01	4.27E-01	Patrolling
Day:TIS	-6.35E-03	1.34E-02	-4.73E-01	1.07E-02	-5.93E-01	2.77E-01	Patrolling
Intercept	3.28E-01	1.33E-01	2.46E+00	6.66E-02	4.92E+00	4.32E-07	Phagocytic
Day	-1.14E-02	8.81E-03	-1.30E+00	4.31E-03	-2.65E+00	4.00E-03	Phagocytic
EV	-3.40E-01	1.65E-01	-2.06E+00	9.87E-02	-3.45E+00	2.82E-04	Phagocytic
TIS	-4.65E-01	1.65E-01	-2.82E+00	1.24E-01	-3.75E+00	8.97E-05	Phagocytic
Day:EV	2.80E-02	1.10E-02	2.55E+00	6.41E-03	4.37E+00	6.26E-06	Phagocytic
Day:TIS	5.12E-02	1.10E-02	4.66E+00	8.86E-03	5.78E+00	3.69E-09	Phagocytic
Intercept	-1.44E-01	2.26E-01	-6.35E-01	9.58E-02	-1.50E+00	6.67E-02	MoDC
Day	1.80E-02	1.71E-02	1.05E+00	7.76E-03	2.31E+00	1.03E-02	MoDC
EV	7.60E-01	2.48E-01	3.07E+00	1.62E-01	4.68E+00	1.44E-06	MoDC
TIS	1.10E+00	2.48E-01	4.44E+00	1.20E-01	9.19E+00	2.02E-20	MoDC
Day:EV	-3.73E-02	1.84E-02	-2.03E+00	1.18E-02	-3.17E+00	7.63E-04	MoDC
Day:TIS	-5.66E-02	1.84E-02	-3.08E+00	9.27E-03	-6.11E+00	5.04E-10	MoDC

Table S8. Generalized Estimating Equations coefficients and statistics for regressions of fractions out of MPs in MP subsets over embryonic days. Related to Figure 4 and S4H. GEE was used to fit linear models and compare fraction out of MPs in compartments within each MP subset. PB was used as the reference.

Coefficient	Estimate	Naive.S.E.	Naive.z	Robust.S.E.	Robust.z	P value	Cell type
Intercept	2.12E-01	1.03E-01	2.05E+00	1.44E-01	1.47E+00	7.09E-02	Ly-6Chi
Day	2.07E-02	6.98E-03	2.96E+00	9.84E-03	2.10E+00	1.77E-02	Ly-6Chi
EV	-3.02E-01	1.46E-01	-2.07E+00	1.62E-01	-1.86E+00	3.13E-02	Ly-6Chi
TIS	-7.74E-02	1.46E-01	-5.29E-01	1.70E-01	-4.54E-01	3.25E-01	Ly-6Chi
Day:EV	1.58E-02	9.88E-03	1.60E+00	1.11E-02	1.42E+00	7.85E-02	Ly-6Chi
Day:TIS	-9.38E-03	9.88E-03	-9.50E-01	1.17E-02	-8.05E-01	2.10E-01	Ly-6Chi
Intercept	2.34E-01	6.50E-02	3.59E+00	8.53E-02	2.74E+00	3.09E-03	Patrolling
Day	-3.19E-03	4.39E-03	-7.26E-01	5.89E-03	-5.42E-01	2.94E-01	Patrolling
EV	3.10E-02	9.20E-02	3.37E-01	9.53E-02	3.25E-01	3.73E-01	Patrolling
TIS	-1.38E-01	9.20E-02	-1.50E+00	9.24E-02	-1.50E+00	6.73E-02	Patrolling
Day:EV	-8.52E-03	6.21E-03	-1.37E+00	6.53E-03	-1.30E+00	9.61E-02	Patrolling
Day:TIS	1.39E-03	6.21E-03	2.24E-01	6.39E-03	2.18E-01	4.14E-01	Patrolling
Intercept	6.55E-02	6.59E-02	9.94E-01	2.01E-02	3.25E+00	5.72E-04	Phagocytic
Day	-2.10E-03	4.36E-03	-4.81E-01	1.39E-03	-1.50E+00	6.62E-02	Phagocytic
EV	7.10E-03	8.16E-02	8.71E-02	3.76E-02	1.89E-01	4.25E-01	Phagocytic
TIS	-8.16E-02	8.16E-02	-1.00E+00	7.37E-02	-1.11E+00	1.34E-01	Phagocytic
Day:EV	4.18E-04	5.43E-03	7.70E-02	2.46E-03	1.70E-01	4.33E-01	Phagocytic
Day:TIS	1.65E-02	5.43E-03	3.04E+00	5.35E-03	3.09E+00	1.01E-03	Phagocytic
Intercept	-1.11E-02	1.24E-01	-9.00E-02	1.34E-02	-8.29E-01	2.04E-01	MoDC

Day	2.49E-03	9.34E-03	2.67E-01	1.19E-03	2.08E+00	1.85E-02	MoDC
EV	3.15E-01	1.35E-01	2.32E+00	7.60E-02	4.14E+00	1.74E-05	MoDC
TIS	4.66E-01	1.35E-01	3.44E+00	5.76E-02	8.09E+00	2.94E-16	MoDC
Day:EV	-1.78E-02	1.01E-02	-1.77E+00	4.83E-03	-3.69E+00	1.11E-04	MoDC
Day:TIS	-2.17E-02	1.01E-02	-2.16E+00	3.80E-03	-5.72E+00	5.30E-09	MoDC
Intercept	5.21E-02	4.09E-02	1.28E+00	7.02E-02	7.42E-01	2.29E-01	DC
Day	1.50E-04	2.85E-03	5.27E-02	4.79E-03	3.13E-02	4.88E-01	DC
EV	-4.59E-02	5.58E-02	-8.22E-01	7.36E-02	-6.23E-01	2.67E-01	DC
TIS	-3.79E-02	5.58E-02	-6.80E-01	7.49E-02	-5.06E-01	3.06E-01	DC
Day:EV	3.57E-03	3.83E-03	9.31E-01	5.03E-03	7.10E-01	2.39E-01	DC
Day:TIS	2.81E-03	3.83E-03	7.35E-01	5.16E-03	5.45E-01	2.93E-01	DC
Intercept	4.32E-01	5.78E-02	7.46E+00	6.25E-02	6.91E+00	2.43E-12	Presenting
Day	-1.81E-02	3.91E-03	-4.63E+00	3.97E-03	-4.55E+00	2.65E-06	Presenting
EV	-6.63E-02	8.18E-02	-8.10E-01	8.53E-02	-7.76E-01	2.19E-01	Presenting
TIS	-2.72E-01	8.18E-02	-3.33E+00	7.65E-02	-3.56E+00	1.86E-04	Presenting
Day:EV	1.10E-02	5.52E-03	1.99E+00	5.60E-03	1.97E+00	2.46E-02	Presenting
Day:TIS	1.86E-02	5.52E-03	3.37E+00	4.99E-03	3.73E+00	9.53E-05	Presenting
Intercept	2.25E-02	4.56E-02	4.92E-01	6.07E-03	3.70E+00	1.09E-04	Proliferating
Day	-5.00E-04	3.20E-03	-1.56E-01	4.24E-04	-1.18E+00	1.20E-01	Proliferating
EV	5.50E-02	5.08E-02	1.08E+00	1.35E-02	4.07E+00	2.31E-05	Proliferating
TIS	1.39E-01	5.05E-02	2.75E+00	3.23E-02	4.29E+00	8.89E-06	Proliferating

Day:EV	-3.78E-03	3.56E-03	-1.06E+00	8.79E-04	-4.30E+00	8.53E-06	Proliferating
Day:TIS	-7.85E-03	3.52E-03	-2.23E+00	2.05E-03	-3.82E+00	6.56E-05	Proliferating
Intercept	5.55E-03	1.11E-01	4.99E-02	9.78E-02	5.68E-02	4.77E-01	F4/80hi
Day	5.95E-03	7.44E-03	8.00E-01	7.33E-03	8.12E-01	2.08E-01	F4/80hi

Table S9. Generalized Estimating Equations coefficients and statistics for regressions of immune fractions in neutrophil subsets over embryonic days. Related to Figure 5F. GEE was used to fit linear models to all but immunosuppressive neutrophil subset to compare fraction of immune cells in compartments within each neutrophil subset. Immunosuppressive subset was fitted with a quadratic model. PB was used as the reference.

Coefficient	Estimate	Naive.S.E.	Naive.z	Robust.S.E.	Robust.z	P value	Cell type
Intercept	2.83E-03	8.35E-03	3.38E-01	4.80E-03	5.89E-01	2.78E-01	CD80
Day	6.48E-04	5.68E-04	1.14E+00	3.00E-04	2.16E+00	1.55E-02	CD80
EV	-1.93E-02	1.21E-02	- 1.60E+00	6.56E-03	- 2.94E+00	1.62E-03	CD80
TIS	-2.39E-02	1.17E-02	- 2.04E+00	1.03E-02	- 2.31E+00	1.05E-02	CD80
Day:EV	1.34E-03	8.14E-04	1.65E+00	4.27E-04	3.14E+00	8.31E-04	CD80
Day:TIS	1.89E-03	7.94E-04	2.39E+00	7.54E-04	2.51E+00	6.04E-03	CD80
Intercept	1.34E-02	3.93E-02	3.40E-01	2.19E-02	6.11E-01	2.71E-01	Conventional
Day	2.81E-03	2.66E-03	1.06E+00	1.52E-03	1.84E+00	3.26E-02	Conventional
EV	-7.82E-02	5.56E-02	- 1.41E+00	3.92E-02	- 2.00E+00	2.29E-02	Conventional
TIS	2.87E-02	5.56E-02	5.16E-01	3.16E-02	9.08E-01	1.82E-01	Conventional
Day:EV	7.16E-03	3.76E-03	1.91E+00	2.75E-03	2.60E+00	4.60E-03	Conventional
Day:TIS	7.74E-04	3.76E-03	2.06E-01	2.20E-03	3.51E-01	3.63E-01	Conventional
Intercept	2.77E-02	3.04E-02	9.10E-01	2.04E-02	1.36E+00	8.74E-02	Presenting
Day	2.09E-03	2.05E-03	1.02E+00	1.36E-03	1.54E+00	6.16E-02	Presenting
EV	-1.56E-01	4.30E-02	- 3.62E+00	2.68E-02	- 5.81E+00	3.17E-09	Presenting
TIS	-1.04E-01	4.30E-02	- 2.42E+00	3.94E-02	- 2.65E+00	4.08E-03	Presenting
Day:EV	1.18E-02	2.90E-03	4.07E+00	1.85E-03	6.39E+00	8.57E-11	Presenting
Day:TIS	9.11E-03	2.90E-03	3.14E+00	2.92E-03	3.12E+00	8.99E-04	Presenting
Intercept	1.00E-02	1.05E-02	9.56E-01	6.92E-03	1.45E+00	7.38E-02	Proliferating
Day	-1.16E-04	7.01E-04	-1.66E-01	4.39E-04	-2.65E-01	3.95E-01	Proliferating
EV	1.17E-02	1.49E-02	7.84E-01	8.93E-03	1.31E+00	9.56E-02	Proliferating

TIS	9.64E-02	1.59E-02	6.07E+00	1.53E-02	6.29E+00	1.59E-10	Proliferating
Day:EV	-8.77E-04	1.02E-03	-8.62E-01	5.77E-04	-1.52E+00	6.43E-02	Proliferating
Day:TIS	-5.30E-03	1.11E-03	-4.78E+00	1.01E-03	-5.27E+00	6.68E-08	Proliferating
Intercept	1.33E-02	1.25E-02	1.07E+00	7.80E-03	1.71E+00	4.37E-02	Immunosuppressive
Day	-4.71E-04	8.56E-04	-5.50E-01	4.97E-04	-9.47E-01	1.72E-01	Immunosuppressive
EV	-3.19E-03	1.86E-02	-1.72E-01	9.10E-03	-3.51E-01	3.63E-01	Immunosuppressive
TIS	-4.92E-01	7.36E-02	-6.69E+00	1.03E-01	-4.79E+00	8.49E-07	Immunosuppressive
Day:EV	3.92E-04	1.26E-03	3.11E-01	5.76E-04	6.80E-01	2.48E-01	Immunosuppressive
Day:TIS	8.02E-02	1.03E-02	7.80E+00	1.40E-02	5.75E+00	4.45E-09	Immunosuppressive
Day-sq:TIS	-2.84E-03	3.53E-04	-8.07E+00	4.59E-04	-6.20E+00	2.83E-10	Immunosuppressive

Table S10. Generalized Estimating Equations coefficients and statistics for regressions of fractions out of neutrophils in neutrophil subsets over embryonic days. Related to Figure 5G. GEE was used to fit linear models to all but immunosuppressive neutrophil subset to compare fraction out of neutrophils in compartments within each neutrophil subset. Immunosuppressive subset was fitted with a quadratic model. PB was used as the reference.

Coefficient	Estimate	Naive.S.E.	Naive.z	Robust.S.E.	Robust.z	P value	Cell type
Intercept	4.88E-02	3.22E-02	1.52E+00	3.50E-02	1.39E+00	8.18E-02	CD80 Neu
Day	2.35E-03	2.19E-03	1.07E+00	2.24E-03	1.05E+00	1.47E-01	CD80 Neu
EV	5.05E-04	4.65E-02	1.08E-02	4.67E-02	1.08E-02	4.96E-01	CD80 Neu
TIS	-9.49E-02	4.51E-02	-2.11E+00	4.08E-02	-2.33E+00	9.93E-03	CD80 Neu
Day:EV	-1.16E-03	3.14E-03	-3.69E-01	3.01E-03	-3.85E-01	3.50E-01	CD80 Neu
Day:TIS	4.34E-03	3.06E-03	1.42E+00	2.70E-03	1.61E+00	5.39E-02	CD80 Neu
Intercept	2.54E-01	8.77E-02	2.89E+00	9.10E-02	2.79E+00	2.64E-03	Conventional
Day	7.11E-03	5.92E-03	1.20E+00	5.78E-03	1.23E+00	1.10E-01	Conventional
EV	1.07E-01	1.24E-01	8.64E-01	1.28E-01	8.36E-01	2.02E-01	Conventional
TIS	3.76E-02	1.24E-01	3.03E-01	1.04E-01	3.60E-01	3.59E-01	Conventional
Day:EV	-4.32E-03	8.38E-03	-5.16E-01	8.25E-03	-5.24E-01	3.00E-01	Conventional
Day:TIS	-5.32E-03	8.38E-03	-6.36E-01	6.64E-03	-8.02E-01	2.11E-01	Conventional
Intercept	3.72E-01	7.89E-02	4.71E+00	1.11E-01	3.36E+00	3.90E-04	Presenting
Day	1.67E-03	5.33E-03	3.13E-01	7.35E-03	2.27E-01	4.10E-01	Presenting
EV	-3.11E-01	1.12E-01	-2.79E+00	1.31E-01	-2.38E+00	8.67E-03	Presenting
TIS	-4.85E-01	1.12E-01	-4.35E+00	1.26E-01	-3.84E+00	6.14E-05	Presenting
Day:EV	2.04E-02	7.53E-03	2.71E+00	8.65E-03	2.36E+00	9.16E-03	Presenting
Day:TIS	2.60E-02	7.53E-03	3.45E+00	8.49E-03	3.06E+00	1.11E-03	Presenting
Intercept	1.03E-01	6.89E-02	1.50E+00	5.62E-02	1.84E+00	3.30E-02	Proliferating
Day	-3.11E-03	4.61E-03	-6.75E-01	3.57E-03	-8.72E-01	1.92E-01	Proliferating
EV	3.30E-01	9.79E-02	3.37E+00	1.44E-01	2.29E+00	1.09E-02	Proliferating
TIS	3.72E-01	1.04E-01	3.56E+00	7.69E-02	4.84E+00	6.62E-07	Proliferating
Day:EV	-2.22E-02	6.69E-03	-3.32E+00	9.11E-03	-2.44E+00	7.38E-03	Proliferating
Day:TIS	-2.31E-02	7.29E-03	-3.16E+00	5.04E-03	-4.58E+00	2.32E-06	Proliferating
Intercept	1.17E-01	5.51E-02	2.12E+00	5.96E-02	1.96E+00	2.48E-02	Immunosuppressive
Day	-4.61E-03	3.79E-03	-1.22E+00	3.86E-03	-1.19E+00	1.17E-01	Immunosuppressive
EV	9.24E-02	8.21E-02	1.13E+00	7.87E-02	1.17E+00	1.20E-01	Immunosuppressive
TIS	-1.06E+00	3.25E-01	-3.25E+00	3.57E-01	-2.96E+00	1.54E-03	Immunosuppressive
Day:EV	-5.73E-03	5.56E-03	-1.03E+00	4.93E-03	-1.16E+00	1.23E-01	Immunosuppressive
Day:TIS	1.84E-01	4.54E-02	4.04E+00	4.86E-02	3.78E+00	7.82E-05	Immunosuppressive
Day-sq:TIS	-6.67E-03	1.56E-03	-4.28E+00	1.61E-03	-4.15E+00	1.67E-05	Immunosuppressive

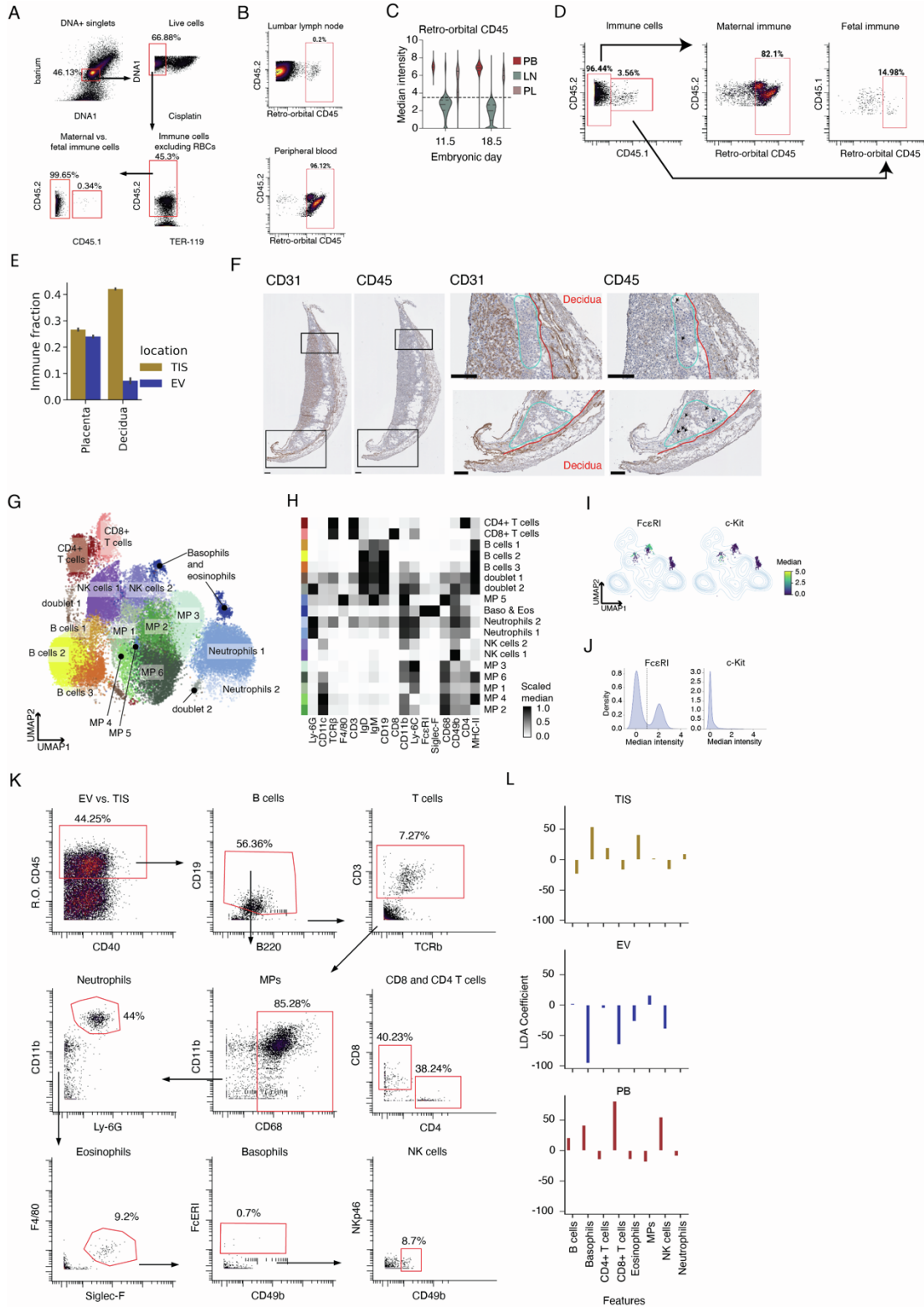


Figure S1. Maternal immune clusters identified by Leiden, determination of endovascular and tissue compartments, and gating strategy. Related to Figure 1. (A) Mass cytometry gating strategy to isolate maternal and fetal immune cells. (B) Representative biaxial plots of immune cells in paired lumbar lymph node and peripheral blood in mice retro-orbitally injected

with anti-CD45. Gated populations of immune cells labeled by retro-orbital anti-CD45. (C) Arcsinh transformed single cell median expression of retro-orbitally injected anti-CD45 in matched peripheral blood (PB), lumbar lymph nodes (LN), and placenta (PL). Plot includes the threshold set to distinguish EV vs. TIS compartments in PL. (D) Representative gating scheme of maternal vs. fetal immune cells in E18.5 maternal-fetal interface sample. Maternal or fetal immune cells labeled by retro-orbital anti-CD45 are gated in subsequent plots. (E) Fraction of maternal immune cells in TIS or EV space in mechanically dissociated placenta from decidua. E12.5, n = 2. (F) IHC of mouse maternal-fetal interface at E13.5. Serial sections were stained with CD31 or CD45. Red line on zoomed in sections indicate the decidua. Cyan region indicates absence of vasculature within the placenta. Arrows point to maternal immune cells in the placenta. Scale bar is 250 μ m. (G) Leiden clusters overlaid on UMAP graph of maternal immune cells. Leiden clusters were named based on their phenotypic marker expression. (H) Scaled median expression of protein markers used to generate UMAP graph and Leiden clusters are shown across all Leiden clusters identified. (I) UMAP indicating arcsinh transformed cellular median intensity of Fc ϵ RI and c-Kit in the Leiden cluster identified as "Basophils and Eosinophils". (J) Kernel density plots of arcsinh transformed Fc ϵ RI and c-Kit medians in the Leiden cluster identified as "Basophils and Eosinophils". Fc ϵ RI plot includes the threshold set to distinguish basophils from eosinophils. (K) Gating strategy to define EV vs. TIS in placenta E12.5 sample, along with the maternal immune cell types identified. (L) Linear discriminant analysis (LDA) coefficients indicating the weight each feature (cell type) was given to identify each class (compartments TIS, EV, and PB).

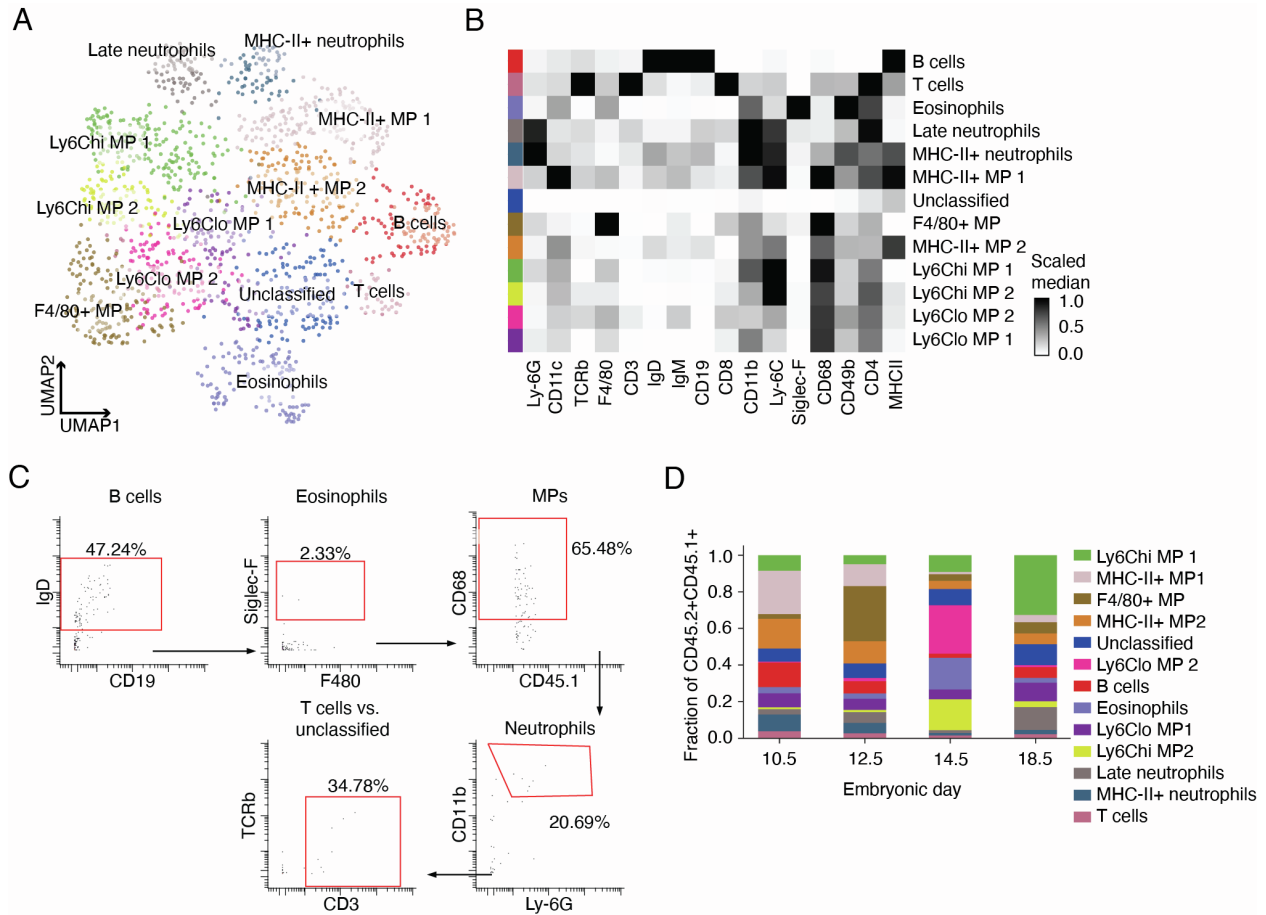


Figure S2. Fetal immune clusters identified by Leiden and their gating strategy. Related to Figure 2. (A) Leiden clusters overlaid on UMAP graph of fetal immune cells. Leiden clusters were named based on their phenotypic marker expression. (B) Scaled median expression of protein markers used to generate UMAP graph. Proteins are shown across all Leiden clusters identified. (C) Gating strategy to identify fetal immune cell types. (D) Fraction of fetal immune cells at E10.5, 12.5, 14.5, and 18.5 across Leiden clusters.

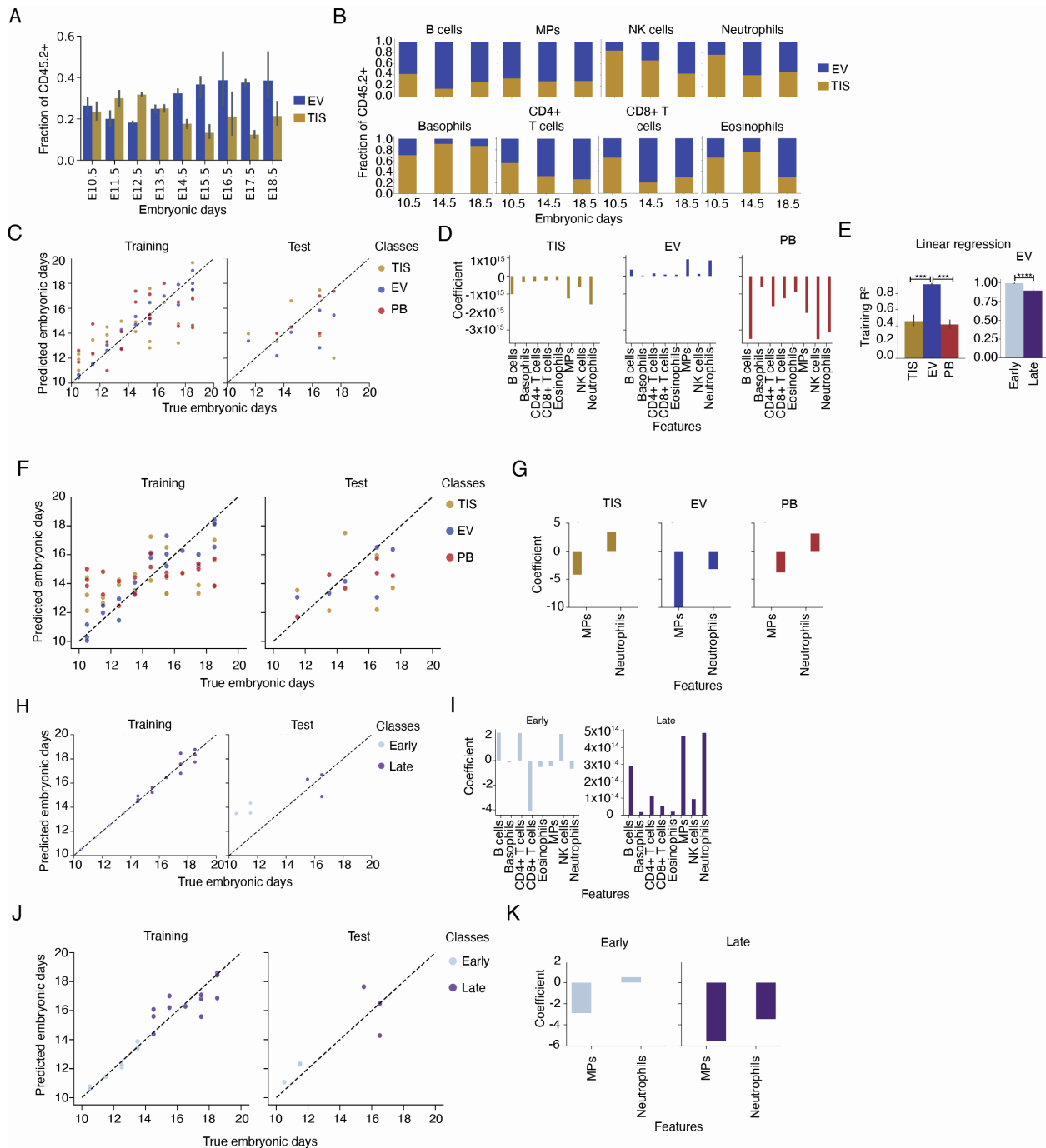


Figure S3. Frequencies of maternal immune cell subsets show predictive dynamics throughout gestation. Related to Figure 3. (A) Fraction of maternal immune cells in the maternal-fetal-interface TIS and EV compartments across embryonic days. (B) Fraction of maternal immune cell subsets in the maternal-fetal-interface TIS and EV compartments across embryonic days 10.5, 14.5, and 18.5. (C) Linear regression results by maternal compartment (classes) based on maternal immune cell fractions out of all immune cells in the given compartment (features). The training set is made up of previously seen data, and the test set is made up of data that has not been seen by the linear regression algorithm. (D) Linear regression coefficients for features, in this case cell type, within the specified class, or maternal compartment. (E) Training accuracy as determined by R^2 of linear regression across each

compartment and EV period based on cell fractions across embryonic days. *** $p \leq 0.001$, **** $p \leq 0.0001$ (one-way ANOVA for comparing compartments, unpaired t test for early and late stages). (F) Linear regression results by maternal compartment (classes) based on maternal immune cell fractions of mononuclear phagocytes and neutrophils in the given compartment (features). (G) Linear regression coefficients for features, in this case cell type, within the specified class, or maternal compartment. (H) Cell fractions of EV compartment were split into early (E10.5 - 13.5) and late (E14.5 – 18.5) and linear regression was run independently for the early and late periods. Linear regression results on the training and test sets based on maternal immune cell fractions (features). (I) Linear regression coefficients for features, in this case cell type, within the specified class, or EV embryonic period. (J) Cell fractions of mononuclear phagocytes and neutrophils EV compartment were split into early and late and linear regression was run independently for the early and late periods. (K) Linear regression coefficients for features, in this case cell type, within the specified class, or EV embryonic period.

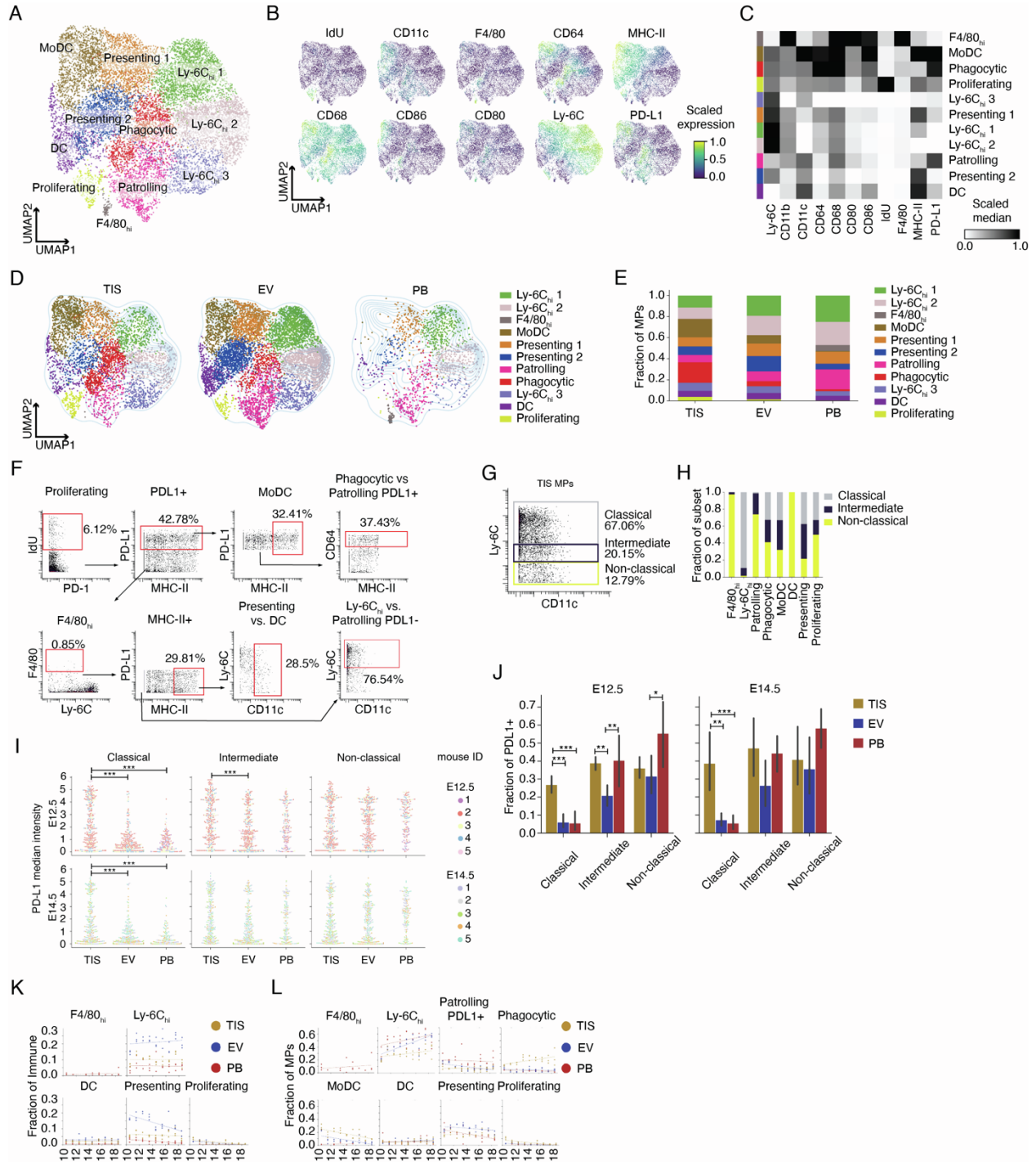


Figure S4. Maternal mononuclear phagocyte clusters identified by Leiden, their gating strategy, and canonical monocyte subset phenotypes. Related to Figure 4. (A) Leiden clusters overlaid on composite UMAP graph of maternal mononuclear phagocytes (MPs) in placenta and peripheral blood across E10.5 to 18.5. Leiden clusters were named based on their phenotypic marker expression. (B) Scaled cellular median intensity of MP phenotypic markers. (C) Scaled median expression of protein markers are shown across all Leiden clusters identified. (D) UMAP plots showing distribution of MP clusters identified by Leiden across compartments. (E) Fraction of MP clusters out of total MPs in each compartment. Embryonic days were aggregated. (F) Gating strategy to identify MP subsets. (G) Gating strategy to identify canonical MP subsets, classical, intermediate, and non-classical. (H) Fraction of canonical MP classification for each MP subset. (I) Single cell arcsinh median intensity of PD-L1 in canonical MP subsets across compartments and at E12.5 and 14.5. To clearly show single cell distribution, samples were capped at 30 cells per mouse. (J) PD-L1 positive fraction of each canonical MP subsets across compartments and at E12.5 and 14.5. For (I) and (J), significance is shown as * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$ (one-way ANOVA per cell type). (K) Fraction of PD-L1 negative MP subsets out of maternal immune cells were fitted with GEE using a linear model comparing TIS, EV, and maternal PB from E10.5 to 18.5. (L) GEE was used to fit a linear model to cell fractions of MP subsets out of all MPs comparing TIS, EV, and PB from E10.5 to 18.5.

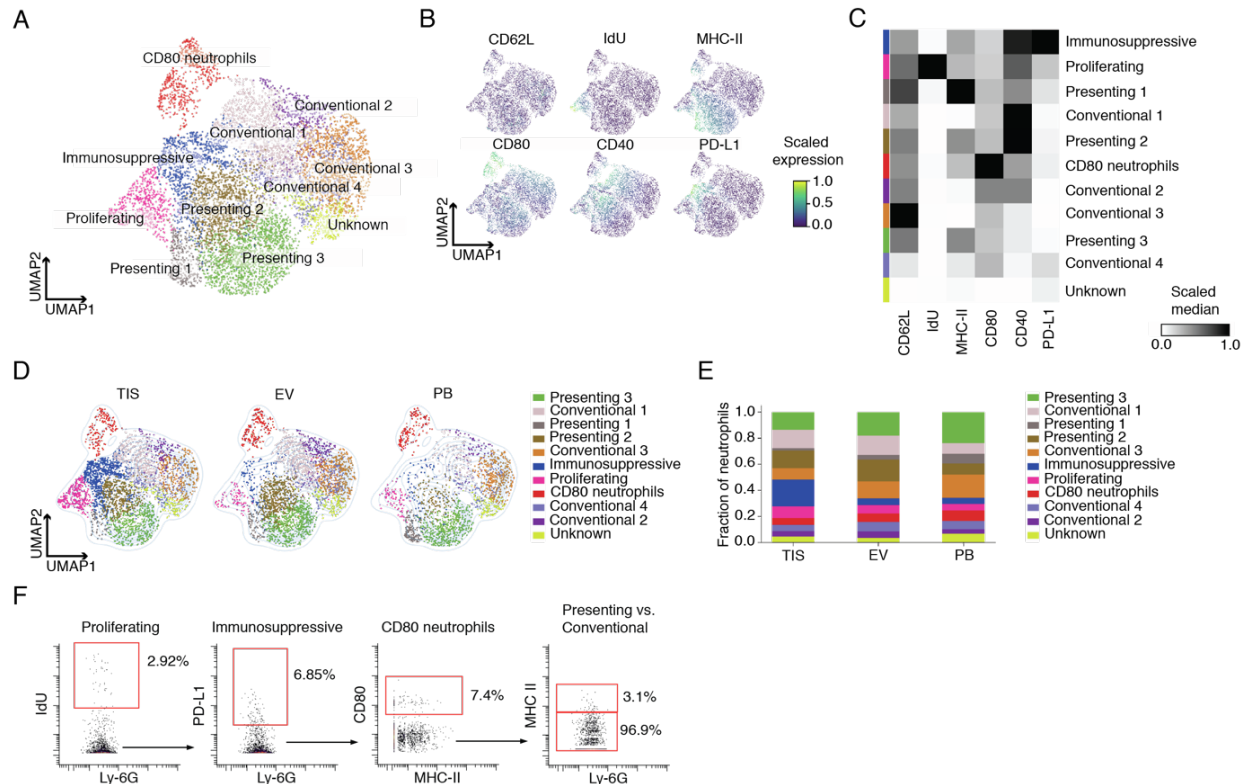


Figure S5. Maternal neutrophil clusters identified by Leiden and their gating strategy. Related to Figure 5. (A) Leiden clusters overlaid on composite UMAP graph of maternal neutrophils in placenta and peripheral blood across E10.5 to 18.5. Leiden clusters were named based on their phenotypic marker expression. (B) Scaled cellular median intensity of neutrophil phenotypic markers. (C) Scaled median expression of protein markers are shown across all Leiden clusters identified. (D) UMAP plots showing distribution of neutrophil clusters identified by Leiden across compartments. (E) Fraction of neutrophil clusters out of total neutrophils in each compartment. Embryonic days were aggregated. (F) Gating strategy to identify neutrophil subsets.

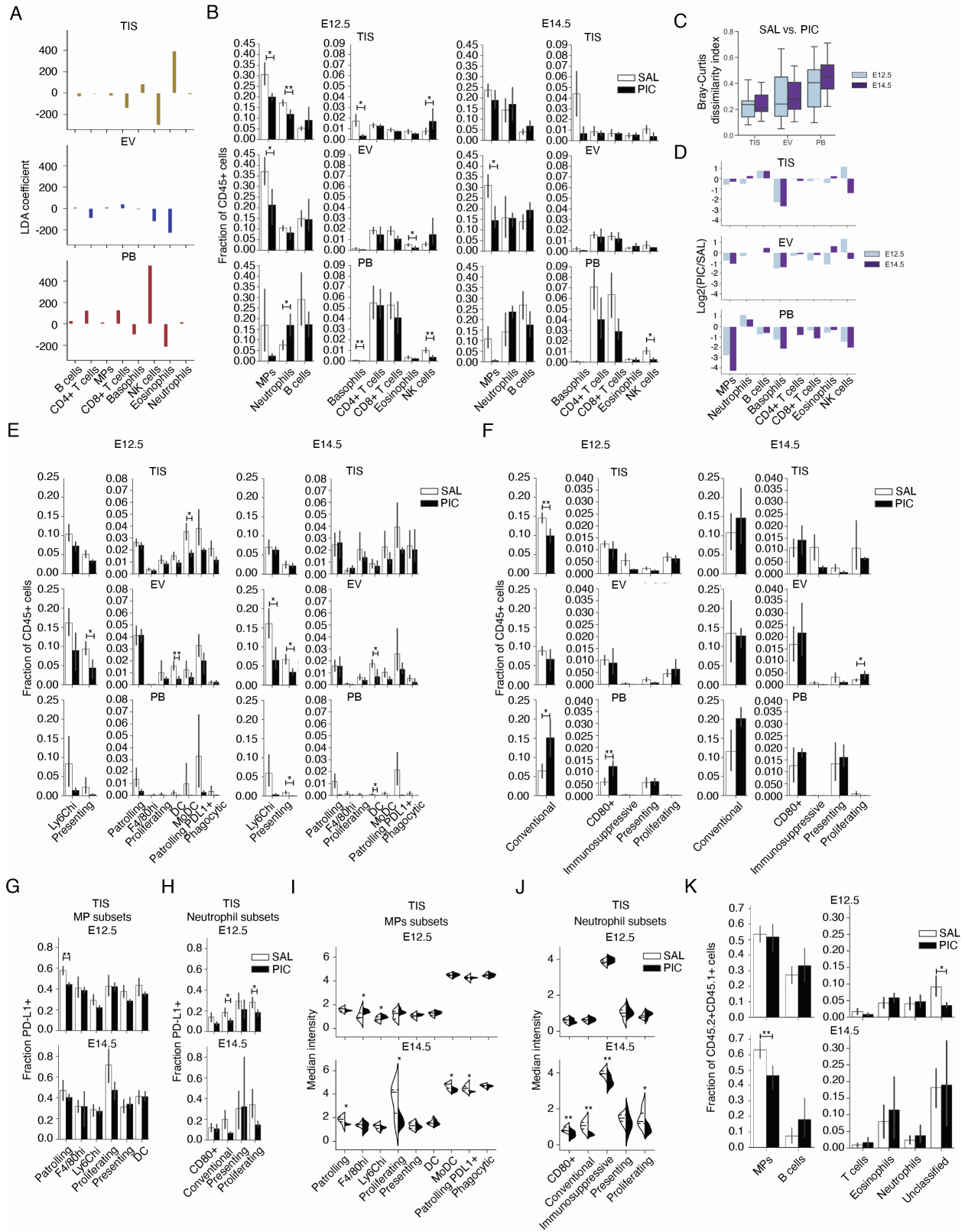


Figure S6. Maternal and fetal immune responses to maternally, systemically administered Poly(I:C) at mid gestation. Related to Figure 6. (A) Linear discriminant analysis (LDA) coefficients for cell type (features), for each compartment (class). LDA was trained on baseline

data comprised of E12.5 and 14.5. (B) For E12.5 and 14.5, maternal immune cell type fractions across compartments when challenged with saline (SAL) or Poly(I:C) (PIC). (C) Bray-Curtis dissimilarity between PIC and SAL treated animals for each compartment across E12.5 and E14.5. (D) Log2 ratio between PIC and SAL across compartment and cell type comparing E12.5 and E14.5. (E) For E12.5 and 14.5, fractions of maternal MP subsets across compartments when challenged with SAL or PIC. (F) For E12.5 and 14.5, fractions of maternal neutrophil subsets across compartments when challenged with SAL or PIC. (G) PD-L1 positive fractions of PD-L1 low maternal MP subsets at E12.5 and 14.5, in TIS compartment when challenged with SAL or PIC. (H) PD-L1 positive fractions of PD-L1 low maternal neutrophil subsets at E12.5 and 14.5, in TIS compartment when challenged with SAL or PIC. (I) Arcsinh transformed median intensity of PD-L1 for MP subsets in TIS at E12.5 and 14.5 upon SAL or PIC challenge. (J) Median intensity of PD-L1 for neutrophil subsets in TIS at E12.5 and 14.5 upon SAL or PIC challenge. (K) For E12.5 and 14.5, fetal immune cell fraction was compared for immune challenge with SAL or PIC. For (A) and (E) through (K), significance is shown as * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$ (unpaired t test).