Supplementary Material

Uterine macrophages and NK cells exhibit population- and gene-level changes after implantation but maintain pro-invasive properties

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SUPPLEMENTARY FIGURE 1

A

Condition	Patient	Ethnicity	Age	BMI		Prior Losses
MSE/ENDO	1	Asian	23	19.8	-	0
MSE/ENDO	2	White	29	20.9	-	0
MSE/ENDO	3	White	33	23.8	-	0
MSE/ENDO	4	White	23	24	-	0

Condition	Patient	Ethnicity	Age	BMI	EGA	Prior Losses
FT	1	White	32	20.3	5w6d	0
FT	2	African-American	29	23.1	6w3d	0
FT	3	African-American	29	28.5	9w3d	0
FT	4	African-American	24	29.1	11w4d	1

B

C

Endometrium vs. First Trimester Per Library Cell Types
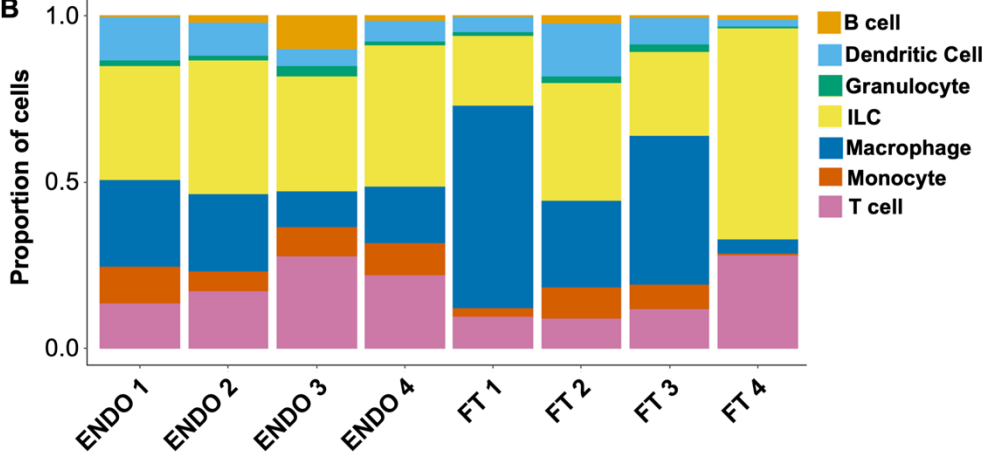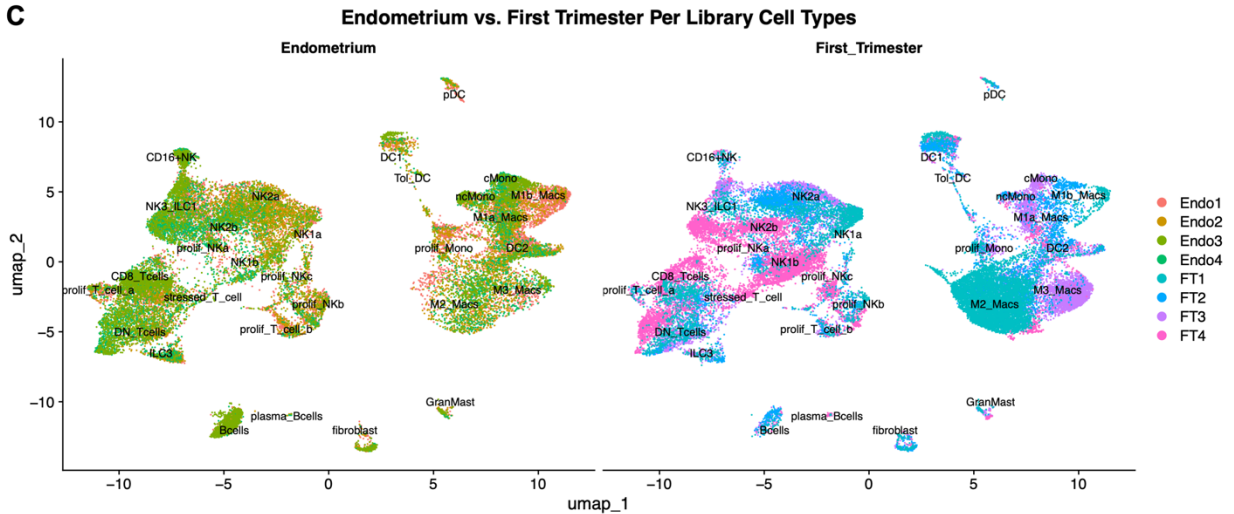
Supplementary Figure 1. Demographics of patients included in this study and immune composition by supercluster of individual samples.

(A) Demographics of subjects from whom CD45+ cells were obtained for scRNAseq. MSE/ENDO, window of implantation (mid-secretory) endometrium. FT, first trimester decidua.

(B) Stacked bar graphs show each supercluster as a percentage of total CD45+ cells in indicated samples of window of implantation (mid-secretory) endometrium (ENDO) and first trimester decidua (FT).

(C) Split UMAPs show the contributions of individual samples to endometrial (Endo) and first trimester (FT) decidual subclusters.

Supplementary Figure 2. Cell cycle analysis guides identification of proliferative immune subclusters.
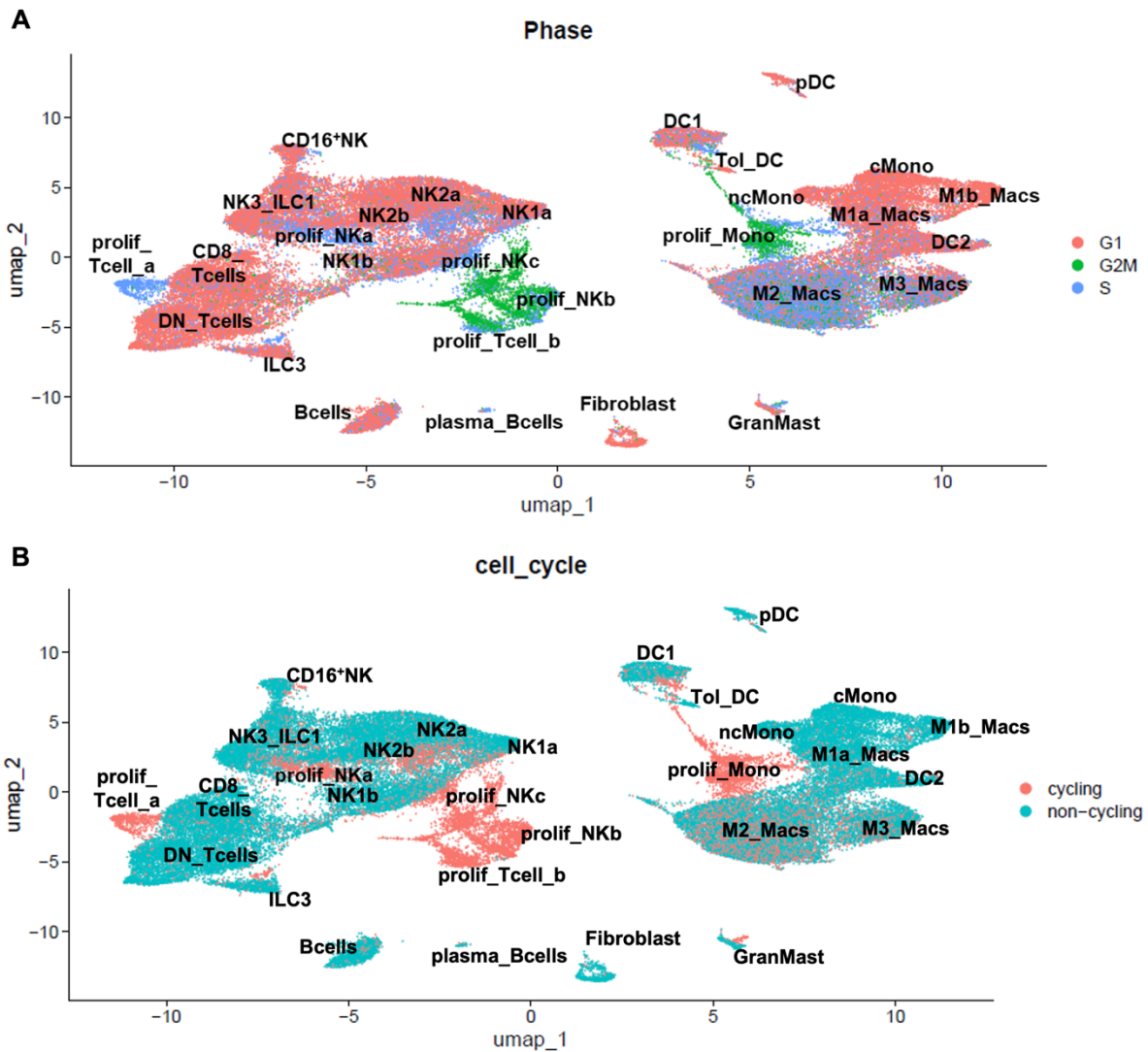
(A) Cells shown in red are predicted to be in the G1 phase of the cell cycle, cells shown in green are predicted to be in G2/M phase, and cells shown in blue are predicted to be in S phase.

(B) Similar to (A), cells shown in red are predicted to have entered the phases of the cell cycle associated with active division, while teal cells are not predicted to be actively dividing.

SUPPLEMENTARY FIGURE 3

A

Processed fresh

Single cell dissociation	Freezing	CD45⁺ cell sorting	Sequencing	Analysis

B Fresh

UMAP_2 / UMAP_1

Myeloid (Monocyte, Macrophage, DC) — ILC — Bcell — Granulocyte — Tcell

C Frozen

UMAP_2 / UMAP_1

Myeloid (Monocyte, Macrophage, DC) — ILC — Bcell — Granulocyte — Tcell

D

Proportion of cells — Fresh, Frozen

Legend: Bcell, Granulocyte, ILC, Myeloid cell, Tcell

E

Number of DEGs — Bcell, Granulocyte, ILC, Myeloid, Tcell

F
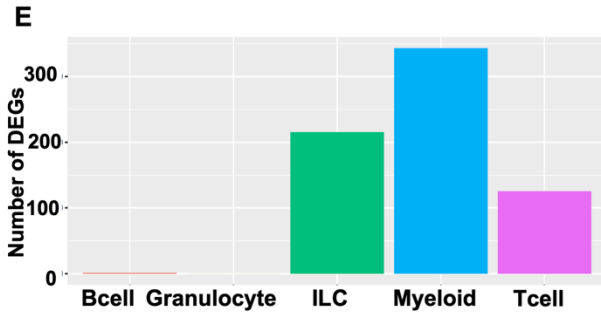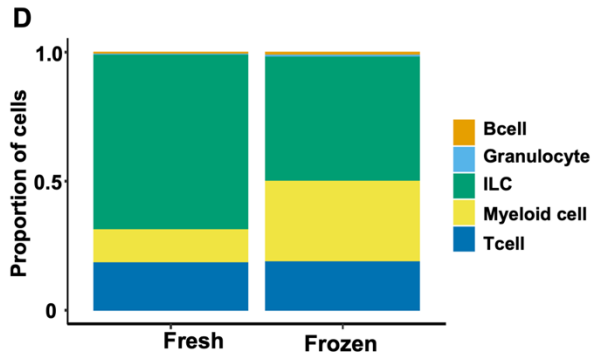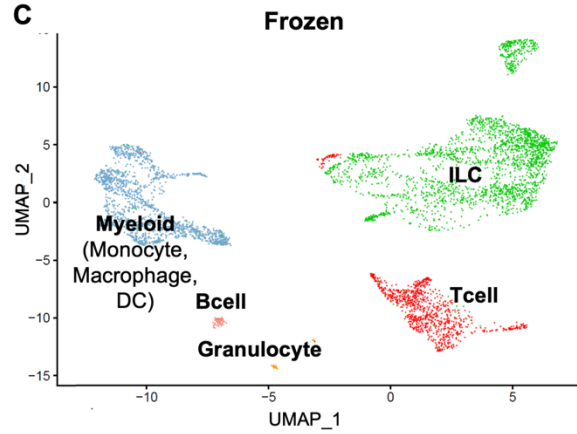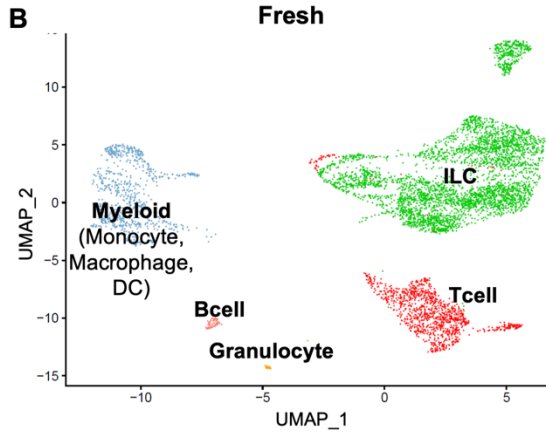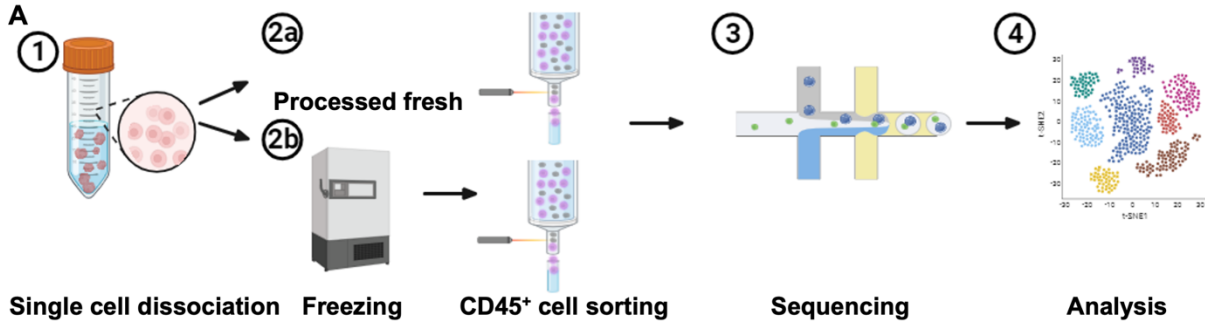
Myeloid

GENE SYMBOL	AVG LOG2FC	ADJ P VALUE
GNLY	2.773140706	0.000100081
NKG7	1.560181824	1.84E-05
GZMA	1.439200012	3.05E-05
IL2RB	1.388372409	4.41E-10
TRDC	1.077737589	8.44E-05
KLRB1	1.00135502	0.0001023
IL32	0.778293254	0.033197277
RPS10	0.668243728	5.65E-77
LGALS2	0.582725433	2.96E-18
CXCR4	0.565182655	0.001314232
CD300E	0.556975733	0.000298315
FCER1A	0.491461162	0.001236627
RPL23	0.472650995	4.02E-40
AREG	0.470100372	1.53E-06
RPS11	0.45288378	7.28E-53
CD1C	0.452081876	0.001355567
RPS20	0.444437081	3.08E-20
RPL7	0.433984623	2.30E-43
RPL31	0.429344851	1.15E-27
FOSL2	0.423073931	3.57E-05
IGF1	-1.39469571	1.88E-15
MSR1	-1.42313905	3.44E-23
FCGBP	-1.45020023	4.11E-18
SLCO2B1	-1.46293799	3.18E-23
A2M	-1.62195104	5.05E-21
STAB1	-1.6320888	3.72E-24
GAL3ST4	-1.67721967	6.59E-27
LYVE1	-1.72912978	1.39E-14
APOC1	-1.76372745	8.74E-20
MAF	-1.82712141	4.80E-24
PLTP	-1.84705175	6.13E-28
HAMP	-1.88080831	4.38E-19
FOLR2	-2.12629776	9.46E-36
DAB2	-2.17925705	3.68E-21
CCL2	-2.22074164	9.45E-20
NRP1	-2.38230962	2.72E-28
APOE	-2.48653915	6.88E-31
SPP1	-2.66783398	9.56E-06
SELENOP	-2.83889601	2.74E-27
RNASE1	-3.7120661	7.34E-24

ILCs

GENE SYMBOL	AVG LOG2FC	ADJ P VALUE
CYP26A1	1.182911436	9.08E-41
SPINK2	1.068238959	1.16E-23
GNLY	1.058311845	6.12E-94
IGFBP7	1.056597177	2.09E-47
KIR2DL3	0.971549397	2.79E-47
ADAMTS1	0.919063879	2.96E-68
AFAP1L2	0.882989684	1.17E-44
ITGAD	0.84760035	2.98E-45
SLC12A8	0.833331168	2.42E-31
CSF1	0.830772466	2.72E-25
KIR2DL1	0.801509892	7.51E-23
EPAS1	0.784828344	2.65E-29
IL2RB	0.752076257	5.40E-149
MYO1E	0.715569388	1.33E-25
SLC44A1	0.710101712	5.49E-27
ACY3	0.702408294	7.00E-17
GZMB	0.699019509	1.02E-58
KIR3DL1	0.697150963	6.05E-17
ABHD2	0.631028183	3.51E-28
HIPK2	0.613581866	1.09E-25
AC253572.2	-0.59914772	7.46E-11
MTRNR2L8	-0.6021063	5.84E-08
PFN1	-0.61602268	4.52E-161
MT-ATP8	-0.62418172	2.11E-44
TUBA1A	-0.64166352	3.79E-37
SEPTIN1	-0.65149936	1.13E-34
ITM2C	-0.65716653	1.47E-24
IFITM1	-0.69523371	2.83E-24
ALOX5AP	-0.71741684	1.47E-63
RGS2	-0.71891451	1.02E-27
LTB	-0.73504358	4.38E-23
LINC01871	-0.75624479	1.40E-25
ARF5	-0.80881552	1.96E-35
LYZ	-1.14345605	4.20E-68
CST3	-1.16993438	1.64E-85
HLA-DPB1	-1.23909116	1.21E-80
CD74	-1.28848108	2.37E-151
HLA-DPA1	-1.33397876	2.59E-107
HLA-DRB1	-1.76235973	1.66E-190
HLA-DRA	-1.89840892	1.98E-216

Increased in Fresh

Increased in Frozen

Supplementary Figure 3. Similar clustering, similar gene expression profiles, and modest relative decrease in ILC representation when sequencing previously frozen uterine immune cells.

(A) Graphical representation of workflow to dissociate MSE tissue, process and sort CD45+ cells fresh or frozen/thawed, and perform scRNAseq.

(B, C) UMAP plots showing superclustering of CD45+ cells meeting quality metrics, recovered from fresh (B) and frozen/thawed (C) MSE. ILC, innate lymphoid cells.

(D) Stacked bar charts showing proportion of total CD45+ cells represented by each supercluster in fresh and frozen/thawed MSE.

(E) Bar graph showing absolute number of significant, differentially expressed genes (DEGs) in indicated fresh versus frozen/thawed superclusters. Significant genes are defined as having an adjusted p value of <0.05 and an absolute fold change of 25% or greater (either ≥25% enriched in fresh or ≥25% in frozen).

(F) List of the top 20 DEGs enriched in fresh (highlighted in pale red) or frozen (highlighted in pale yellow) myeloid cells (left) and ILCs (right). Official gene symbol, average log2 fold change, and adjusted p values are shown.

SUPPLEMENTARY FIGURE 4

Macrophages

M1a			M1b			M2			M3		
GENE SYMBOL	AVG LOG2FC	ADJ P VALUE	GENE SYMBOL	AVG LOG2FC	ADJ P VALUE	GENE SYMBOL	AVG LOG2FC	ADJ P VALUE	GENE SYMBOL	AVG LOG2FC	ADJ P VALUE
SPP1	-4.638317418	2.98E-240	SELENOP	-3.4415097	2.77E-49	SELENOP	-2.439964321	0	SPP1	-3.09564238	7.32E-139
APOE	-3.422933287	3.13E-125	HLA-DRB5	-3.243605499	5.73E-66	IFI44L	-2.183662157	3.29E-174	RNASE1	-3.0524065	4.45E-174
RNASE1	-2.942427864	2.10E-134	RNASE1	-3.046674328	3.60E-45	MTSS1	-2.092628258	2.20E-219	CD28	-2.342372045	6.96E-62
PLIN2	-2.435063017	7.52E-107	C1QC	-2.331358802	5.53E-39	JUN	-2.033339645	0	SELENOP	-2.220786186	8.43E-108
IFIT2	-2.315354314	1.75E-75	C1QB	-2.212345034	8.04E-33	CXCL12	-1.978001633	1.37E-113	IFI44L	-2.125124548	6.47E-60
CTSL	-2.273440363	8.15E-125	AC079753.2	-2.103720904	5.92E-49	EGR1	-1.88574926	1.00E-206	AC253572.2	-2.124581801	3.73E-34
MSR1	-2.266002664	1.60E-153	C1QA	-1.94455187	1.75E-25	PLTP	-1.677683422	5.27E-204	MSR1	-2.110509923	4.34E-130
IFIT3	-2.165358975	9.49E-109	OTUD1	-1.517991706	2.93E-26	RNASE1	-1.648905398	2.15E-294	PLTP	-2.091330361	9.91E-92
ISG15	-2.144800948	1.40E-92	GBP2	-1.25724121	1.70E-22	SERPING1	-1.62588744	2.88E-116	MX1	-2.008745289	1.25E-70
IFIT1	-2.070112891	1.32E-77	TMEM176B	-1.251622022	3.85E-20	GYPC	-1.556851464	7.72E-194	IFIT2	-1.997888471	1.95E-27
FABP5	-2.053047327	4.33E-65	TNFAIP8	-1.16969046	4.94E-13	C3	-1.453697847	5.64E-205	TMIGD3	-1.991682969	6.79E-29
APOC1	-2.04401613	1.58E-22	PLCG2	-1.154105498	1.46E-41	F13A1	-1.450850343	2.74E-229	SDC2	-1.989267739	2.24E-35
FN1	-1.911606611	3.07E-16	TNF	-1.151543835	1.18E-26	IGF1	-1.446924203	6.08E-164	FABP5	-1.84693005	1.44E-52
IFITM3	-1.774934547	2.90E-162	KCNJ2	-1.12453467	2.15E-09	TNFAIP3	-1.446276681	3.52E-117	IFIT3	-1.817953622	3.83E-37
C15orf48	-1.739616828	3.29E-118	AC253572.2	-1.106470433	2.95E-11	DUSP6	-1.427105908	8.84E-187	AL021155.5	-1.813318542	1.35E-20
IFI6	-1.721137884	3.52E-100	AC016831.1	-1.106252747	2.78E-10	SIGLEC1	-1.402423088	2.20E-116	AC007952.4	-1.798364341	6.22E-42
MX1	-1.685606731	8.60E-90	PELI1	-1.066061856	3.83E-09	KLF4	-1.37306504	3.78E-148	C2	-1.796847478	1.23E-65
C1QC	-1.520482531	1.98E-68	CCL4L2	-1.030213141	5.46E-15	AC020916.1	-1.372529963	4.49E-170	ITSN1	-1.757379875	7.62E-51
GPNMB	-1.517720264	2.45E-34	TMEM176A	-0.979282339	3.16E-10	FOSB	-1.366707814	9.40E-260	IFIT1	-1.750805244	5.89E-30
IFI44L	-1.507481269	1.81E-80	IFI30	1.014130679	3.31E-29	AZIN1-AS1	-1.338123165	3.09E-43	CADM1	-1.725718181	3.99E-33
SELENOK	1.115505488	1.96E-56	ARL4A	1.072553529	7.75E-07	RPS26	1.245287923	1.40E-231	ALDH2	1.408923465	2.68E-50
MEGF9	1.122443393	3.32E-42	AL034397.3	1.072708793	6.69E-06	SH3BGRL3	1.255977852	4.56E-270	FGL2	1.426427944	4.71E-80
CYCS	1.13597871	1.05E-59	CAPG	1.089375807	5.07E-15	LST1	1.293226532	7.20E-288	WARS	1.429078129	5.50E-36
OSM	1.220625209	1.14E-32	ATP1B3	1.101648473	4.71E-15	CKLF	1.298491742	4.89E-130	RGS2	1.493943124	2.11E-42
RGS2	1.22407144	2.18E-118	RNASE2	1.14674881	4.77E-05	FABP5	1.305355244	4.42E-127	JAML	1.504359769	1.33E-49
LTA4H	1.255422013	2.00E-90	LGALS1	1.158075546	2.45E-41	CAMK1	1.314733217	2.17E-159	AC020656.1	1.559001279	1.56E-39
HBEGF	1.269804034	7.06E-92	SELENOK	1.17034100	5.93E-12	OLR1	1.321581445	5.52E-125	CD52	1.586117229	5.08E-43
S100A9	1.282570643	1.81E-93	TIMP1	1.301102966	2.51E-32	CD48	1.332224142	5.23E-133	LST1	1.611174948	5.19E-106
FCN1	1.290366926	3.23E-187	SMIM25	1.319207694	9.97E-16	IFI30	1.367408348	0	ALOX5AP	1.687785984	1.48E-68
CD1D	1.329404422	8.40E-76	RPS26	1.319979273	2.73E-51	CAPG	1.392313109	9.39E-251	IL1B	1.701500487	1.71E-16
HSPA1B	1.340120503	1.69E-82	ALOX5AP	1.339827513	2.94E-06	PKIB	1.494995422	2.49E-160	HLA-DQB1	1.919486116	3.43E-162
HLA-DRB5	1.346718089	8.57E-19	MARCO	1.37225202	5.63E-08	LSP1	1.512817154	2.63E-180	LTA4H	1.936691276	1.04E-62
S100A8	1.523703371	1.41E-97	LYZ	1.372532812	9.79E-88	S100A6	1.583122281	9.50E-163	CYP27A1	2.16774256	6.45E-79
FBP1	1.611252697	3.54E-124	TWISTNB	1.609231467	1.93E-05	TIMP1	1.61011874	3.61E-187	GPR183	2.217896581	1.56E-112
ALOX5AP	1.67504248	3.89E-131	PLAC8	1.690365705	2.14E-12	S100A4	1.684824959	9.15E-264	OLR1	2.248277274	4.93E-94
CD52	1.768854927	6.33E-169	HBEGF	2.043722294	5.39E-35	APOC2	1.851674482	9.06E-59	HLA-DQA1	2.521097506	1.15E-213
MCEMP1	1.916024607	8.06E-84	MCEMP1	2.081796817	9.00E-23	PPA1	1.866487525	1.74E-283	CLEC10A	2.562361323	1.26E-173
LYZ	1.976816344	0	FBP1	2.348415738	6.54E-19	LGALS1	2.015593415	0	HLA-DRB5	2.675710706	2.99E-45
PLAC8	2.216325805	7.42E-148	RETN	2.976923464	5.06E-14	CLEC10A	2.201682858	1.07E-292	LYZ	3.234862239	2.61E-161
RETN	2.750379612	1.08E-112				LYZ	3.180809276	0	FCN1	3.347605528	3.60E-144

Increased in FT

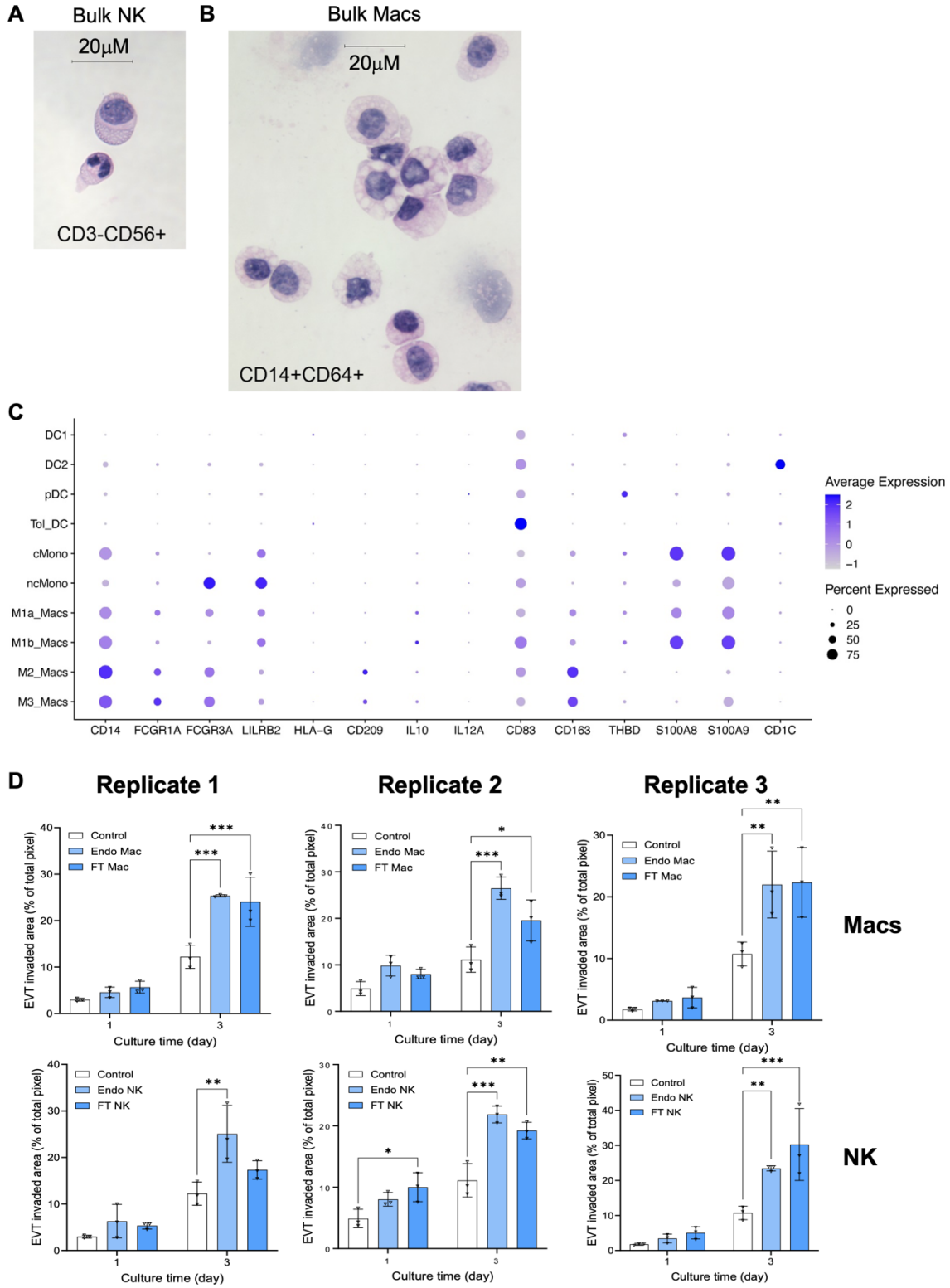Increased in Endo

Supplementary Figure 4. Top genes differentially expressed between endometrial and first trimester decidual macrophages.

List of the top 20 DEGs enriched in first trimester decidual (highlighted in pale blue) or pre-implantation endometrial (white cells, unhighlighted) macrophage subclusters. Official gene symbol, average log2 fold change, and adjusted p values are shown.

SUPPLEMENTARY FIGURE 5

ILCs

Increased in FT → / Increased in Endo →

NK1a GENE SYMBOL	AVG LOG2FC	ADJ P VALUE	NK1b GENE SYMBOL	AVG LOG2FC	ADJ P VALUE	NK2a GENE SYMBOL	AVG LOG2FC	ADJ P VALUE	NK2b GENE SYMBOL	AVG LOG2FC	ADJ P VALUE	NK3/ILC1 GENE SYMBOL	AVG LOG2FC	ADJ P VALUE
SELENOP	-3.286487378	5.13E-70	IGKC	-3.38274942	3.38E-134	AREG	-2.279868729	6.91E-240	IGKC	-2.76513771	1.16E-92	IGKC	-2.32510205	1.36E-171
RNASE1	-3.082556211	4.46E-61	IFI44L	-2.74549926	3.20E-56	TSC22D3	-1.762126651	3.20E-211	IGFBP7	-2.1002317	1.12E-49	ISG20	-1.91911875	2.65E-145
APOE	-2.811170483	2.65E-47	ERAP2	-1.75157023	1.84E-52	APOC2	-1.521658905	7.28E-83	IFITM1	-1.86353654	2.04E-82	IFITM1	-1.63162994	7.61E-132
C1QB	-2.483396056	6.89E-94	C1QA	-1.51257796	6.33E-41	FTL	-1.213380877	3.38E-211	ERAP2	-1.68379408	3.22E-35	TSC22D3	-1.41498207	2.17E-39
C1QA	-2.465858114	1.86E-97	GOLGA8O	-1.48837148	1.47E-29	SOCS1	-1.211820709	1.20E-121	SLC44A1	-1.57205007	2.18E-31	CMC1	-1.34506182	1.71E-125
C1QC	-2.313473019	1.37E-72	MT-ND4L	-1.48494501	6.68E-160	CDHR1	-1.180227896	3.63E-70	CMC1	-1.47057453	3.32E-73	MT2A	-1.34032774	6.15E-70
CD14	-1.946570705	1.04E-52	MTRNR2L8	-1.39460687	2.39E-50	DDIT4	-1.070720681	1.79E-108	CD2	-1.46758877	3.60E-74	ISG15	-1.27872845	7.17E-68
SPTSSB	-1.531807209	8.40E-29	SLFN5	-1.29036196	7.39E-83	JUN	-1.03059738	4.62E-16	ISG20	-1.44961106	5.14E-33	STAT1	-1.19715075	1.96E-43
CTSB	-1.512068103	8.61E-37	IL32	-1.284252	7.40E-79	ZFP36L2	-1.027237395	1.69E-192	ITGAD	-1.43657197	3.53E-31	XAF1	-1.14773989	1.21E-45
JUN	-1.47534051	6.80E-34	LINC00426	-1.28308916	4.87E-20	TXNIP	-1.021000935	5.30E-69	PLCG2	-1.40772035	3.13E-155	IFITM3	-1.10027929	7.98E-36
ENTPD1	-1.465226928	5.19E-31	CD2	-1.26964224	5.36E-40	DUSP1	-0.956729478	3.00E-58	MX1	-1.40755471	1.01E-20	CD2	-1.08141106	1.09E-43
CD74	-1.386473001	6.75E-49	XAF1	-1.24371811	1.16E-31	CD74	-0.920117619	8.46E-84	APOC2	-1.35097484	3.96E-18	PLCG2	-1.05224044	1.27E-139
CDHR1	-1.362644826	2.80E-13	SLC44A1	-1.22749697	4.22E-27	CEBPD	-0.85906764	2.52E-35	MT-ATP8	-1.33955565	1.01E-69	EPSTI1	-0.97696566	2.58E-33
FTL	-1.347393971	3.60E-89	POLR2J3	-1.21289231	4.04E-35	JUNB	-0.847355756	2.75E-50	GZMB	-1.29668484	4.10E-46	CEBPD	-0.95331126	1.27E-45
GRN	-1.297783985	9.27E-23	C1QB	-1.16784134	3.00E-27	PNRC1	-0.81463365	5.95E-76	IFITM3	-1.28376097	5.02E-27	GBP5	-0.94104852	1.16E-27
TTN	-1.26245793	0.006650051	BCL2	-1.1576661	5.37E-20	IFITM1	-0.759141732	1.96E-15	MT-ND4L	-1.26651283	3.43E-85	GZMK	-0.92335229	1.05E-32
AC020916.1	-1.218361039	9.09E-07	SNHG14	-1.14450564	1.65E-16	TTN	-0.75374794	1.04E-17	XAF1	-1.2592379	9.39E-25	SLFN5	-0.89997747	1.44E-58
AL627171.2	-1.216333816	8.70E-34	ABCA2	-1.09315197	4.50E-19	IGFBP7	-0.731554468	3.10E-32	GBP5	-1.12039969	2.03E-15	RPS26	-0.83310219	2.41E-127
SPINK2	-1.175458988	1.67E-06	DTHD1	-1.08233797	1.14E-22	ID2	-0.710936116	1.33E-78	AFAP1L2	-1.11894635	1.12E-14	LYST	-0.80538633	1.10E-21
AREG	-1.128427028	0.768305101	ZNF683	1.46348116	5.54E-41	CD247	0.703307808	2.97E-52	ISG15	-1.06081309	1.37E-21	CD74	-0.77972827	7.33E-48
H2AFZ	0.972416938	7.95E-35	CRIP1	1.46352185	1.77E-102	DUT	0.707683957	2.43E-16	BTG2	1.421672573	1.32E-31	MAP3K8	0.860412769	4.02E-29
MCM7	0.999571253	2.54E-24	FOSB	1.496860774	3.34E-62	TUBB	0.720117146	2.24E-29	LTB	1.446373815	6.84E-20	SDF2L1	0.862016341	1.44E-23
PCNA	1.063885837	5.13E-25	VPS37B	1.497263836	7.00E-53	NME1	0.725185111	1.05E-29	CCL4L2	1.495645685	3.07E-24	ITGA1	0.866028079	3.71E-31
RGCC	1.068351157	6.11E-27	REL	1.551583527	9.21E-65	RGCC	0.729395112	1.24E-43	ALOX5AP	1.496245685	3.21E-35	MANF	0.906615418	8.56E-26
HSPE1	1.086633619	8.01E-53	CD52	1.554015097	5.49E-109	KRT81	0.733557734	2.83E-75	RGCC	1.555652971	7.85E-32	ZNF331	0.909762846	6.26E-40
TUBB	1.088009826	1.72E-28	FOSL2	1.619735028	1.22E-67	STMN1	0.740151364	2.86E-20	FOSB	1.68133614	2.27E-35	CAPG	0.923168201	2.17E-48
CRIP1	1.094339067	7.31E-71	DNAJB1	1.651133531	9.87E-55	SRM	0.747474673	2.49E-31	DUSP1	1.743210527	4.62E-29	CCL4	0.979949737	4.30E-161
LGALS1	1.103660131	5.19E-59	LDLRAD4	1.737624112	8.68E-54	DCTPP1	0.75578065	1.81E-34	CCL3	1.837317794	1.35E-26	JUND	1.03444784	3.39E-186
CCL4	1.134483705	4.00E-58	RASGEF1B	1.752732865	9.83E-63	CXXC5	0.761034302	4.71E-30	TNFAIP3	1.871572495	7.55E-48	FASLG	1.050403673	9.39E-39
MCM6	1.141489496	1.47E-31	AC020916.1	1.758993431	1.89E-72	IFI27L2	0.764337886	1.39E-43	ZNF331	1.891877686	2.59E-37	EZR	1.055925139	1.33E-52
MCM3	1.156073113	8.26E-29	CD69	1.882197153	4.51E-87	CRIP1	0.796084557	1.85E-117	JUN	1.961590153	6.11E-41	ITM2C	1.057525532	4.31E-68
HELLS	1.169755773	7.42E-31	TNFAIP3	2.018311	4.96E-82	RGS16	0.825089379	4.06E-22	CD160	2.030034669	1.11E-30	LDLRAD4	1.14215048	1.03E-38
STMN1	1.20292107	1.67E-32	RGS1	2.105997426	3.38E-111	TNFRSF18	0.837062373	1.46E-101	RGS1	2.052482398	2.12E-55	IGFBP2	1.156271131	2.23E-74
KRT81	1.217706336	6.89E-40	CCL4	2.112263296	3.92E-176	KRT86	0.914678537	1.01E-86	FOS	2.074518818	8.73E-70	NR4A2	1.175737921	9.53E-82
MT2A	1.329581252	1.48E-34	NR4A1	2.167304966	1.38E-95	FASLG	1.018460434	5.37E-68	JUNB	2.148435508	2.32E-55	TNFRSF18	1.183732138	4.72E-68
KRT86	1.332648024	1.55E-42	ZNF331	2.335125629	3.82E-92	CYBA	1.050259811	2.20E-85	CD69	2.257855946	2.78E-91	CD52	1.442583377	3.35E-96
XCL2	1.378559642	1.55E-72	LYZ	2.444041285	1.74E-198	CD52	1.170277439	3.33E-193	CCL4	2.271248312	3.70E-108	KRT86	1.519965014	7.37E-130
XCL1	1.878432772	5.49E-99	DUSP2	2.492049672	2.74E-139	CD9	1.248095843	5.88E-112	CXCR4	2.423474534	1.06E-54	KRT81	1.628185489	1.26E-141
CD9	1.893770747	3.67E-88	NR4A2	3.4433553	1.57E-298	LTB	1.435621191	5.99E-68	CD9	2.661673804	1.07E-76	LYZ	1.766641967	1.11E-75
LYZ	1.897424676	2.34E-39							DUSP2	2.686693559	6.03E-100	CD9	2.09086451	3.08E-135

Supplementary Figure 5. Top genes differentially expressed between endometrial and first trimester decidual ILCs.

List of the top 20 DEGs enriched in first trimester decidual (highlighted in pale blue) or pre-implantation endometrial (white cells, unhighlighted) ILC subclusters. Official gene symbol, average log2 fold change, and adjusted p values are shown.

SUPPLEMENTARY FIGURE 6

A Bulk NK
20μM
CD3-CD56+

B Bulk Macs
20μM
CD14+CD64+

C

DC1, DC2, pDC, Tol_DC, cMono, ncMono, M1a_Macs, M1b_Macs, M2_Macs, M3_Macs

CD14, FCGR1A, FCGR3A, LILRB2, HLA–G, CD209, IL10, IL12A, CD83, CD163, THBD, S100A8, S100A9, CD1C

Average Expression
2
1
0
-1

Percent Expressed
0
25
50
75

D

Replicate 1   Replicate 2   Replicate 3

EVT invaded area (% of total pixel)
Control
Endo Mac
FT Mac
Culture time (day)
Macs

EVT invaded area (% of total pixel)
Control
Endo NK
FT NK
Culture time (day)
NK

Supplementary Figure 6. Pre- and post-implantation bulk CD14+CD64+ macrophages promote invasion of primary EVTs as strongly as pre- and post-implantation bulk NK cells ex vivo on an implantation-on-a-chip device.

(A,B) H&E-stained cytospins were prepared from cryopreserved first-trimester decidual flow-sorted cells and imaged with a Leica brightfield microscope at 63x. The scale bar shown is 20 microns. Shown are (A) CD3-CD56+ "Bulk NK cells" that appear lymphocytic with abundant fine cytotoxic granules. In (B), CD14+CD64+ "Bulk Macrophages" are predominantly large, heavily vacuolated cells that resemble tissue macrophages.

(C) Dot plot representation of DC10-associated genes expressed among indicated myeloid subclusters.

(D) Quantification of individual biological replicates on the implantation-on-a-chip (IOC). Three technical replicates per biological replicate are shown. Invasion area of EVTs beyond the fetal channel at days 1 and 3 of culture are shown. One-way ANOVA with Tukey's multiple comparison test was performed. Data are presented as mean ± SD. *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$.