

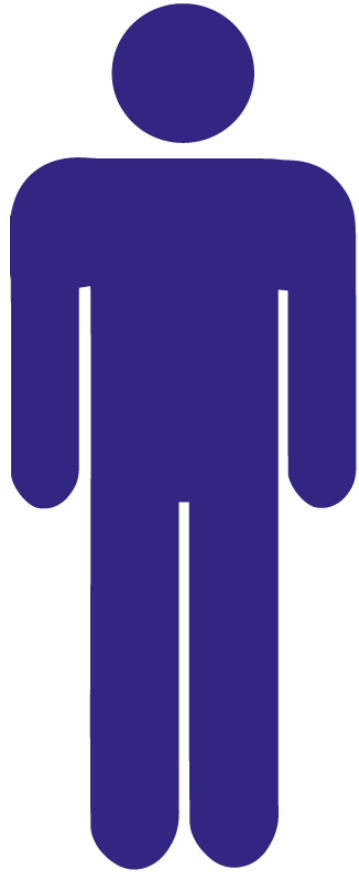
Flowcytometrische analyse van endometriale NK cellen

Renate van der Molen

21-11-2018

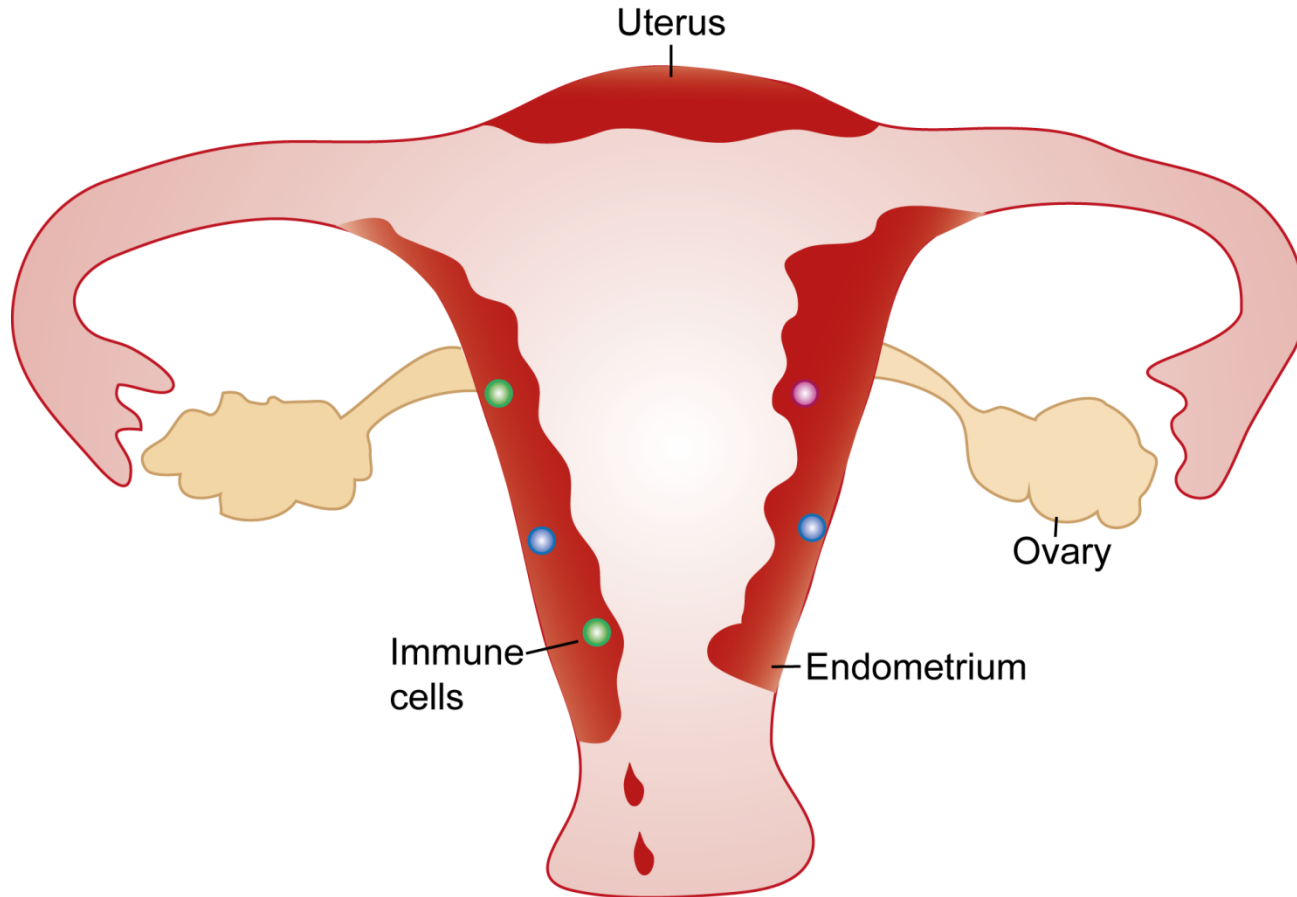
*Afdeling Laboratoriumgeneeskunde, Laboratorium Medische Immunologie,
Radboudumc, Nijmegen*

The immune paradox of pregnancy



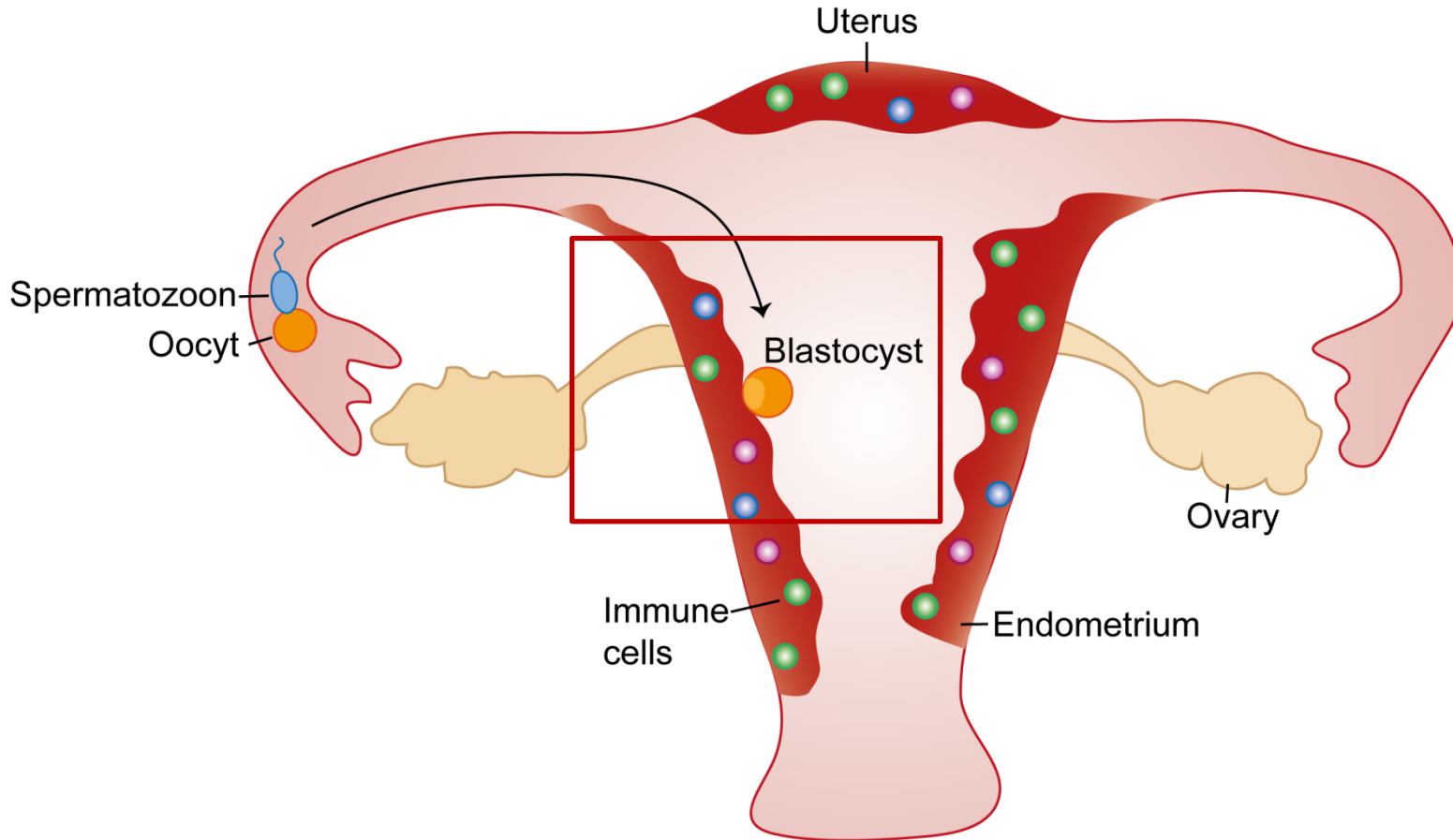
Immunology of pregnancy;

influx of lymphocytes in the endometrium



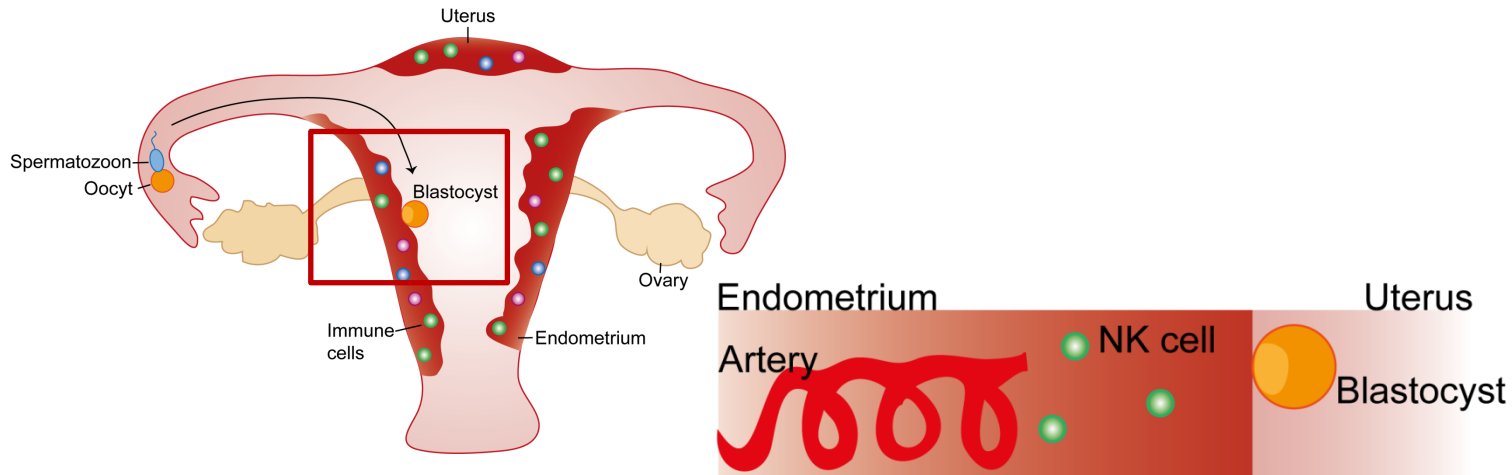
Immunology of pregnancy;

Implantation of the blastocyst



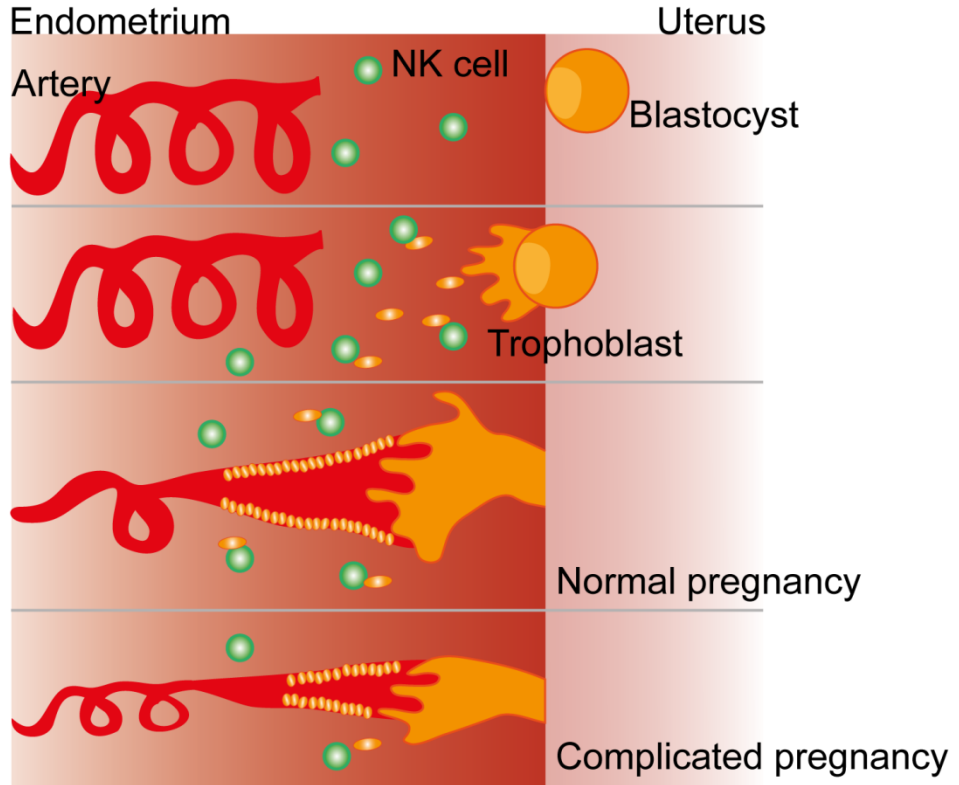
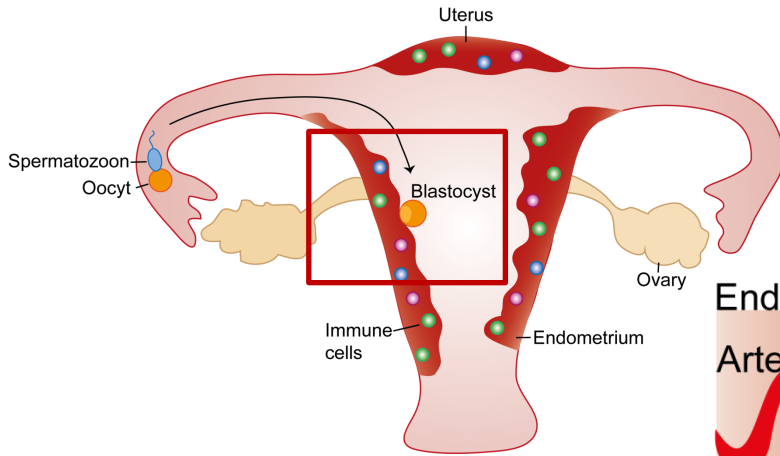
Immunology of pregnancy;

Immune interaction lymphocytes trophoblast cells

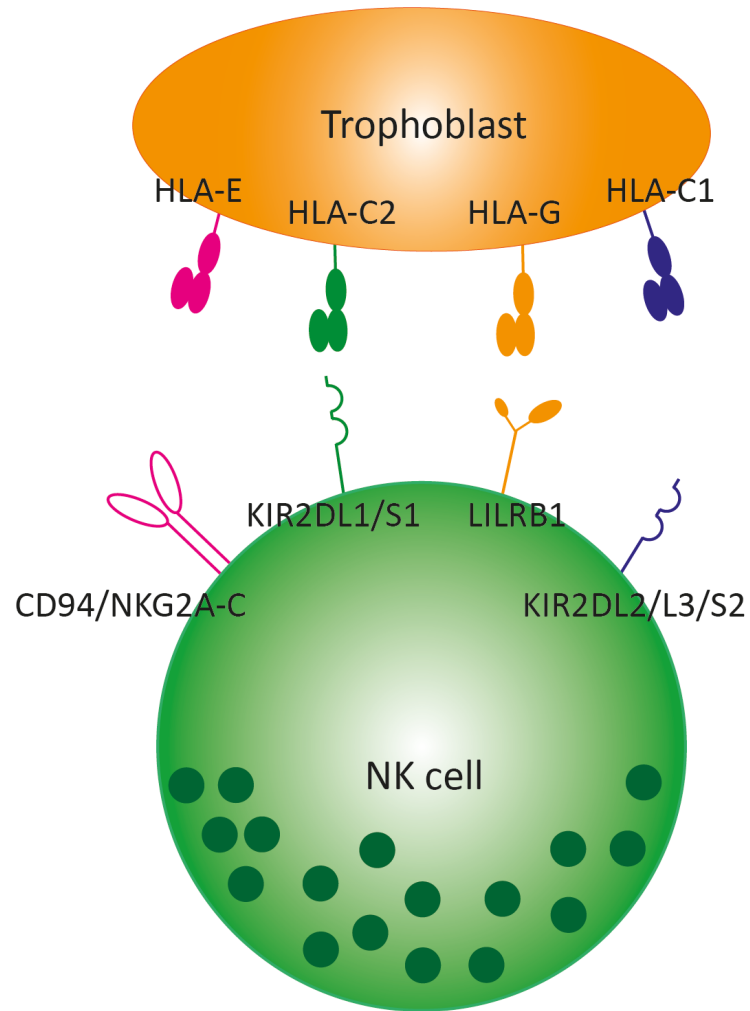
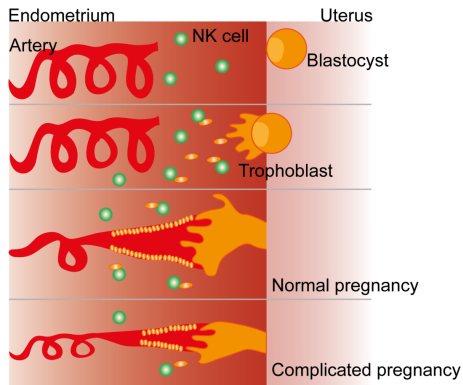


Immunology of pregnancy;

Immune interaction lymphocytes trophoblast cells



Recognition of trophoblasts by NK cells



NK cell receptor repertoire: a balance between inhibitory and activating signals

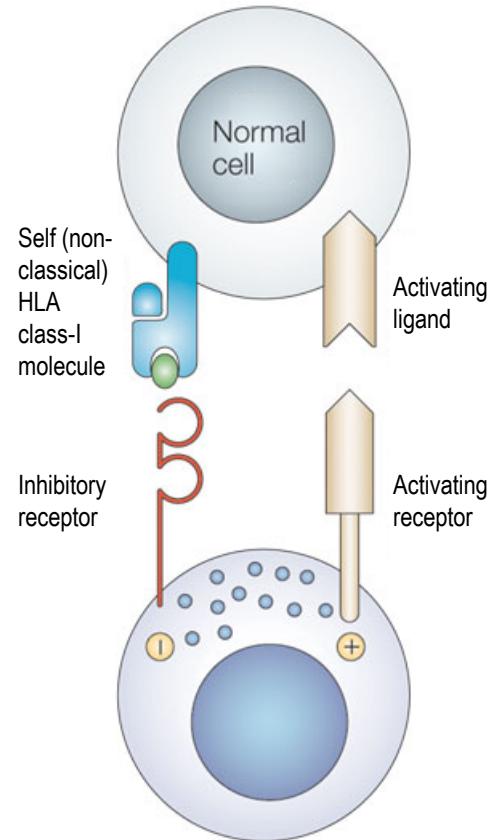
Inhibitory receptors

Killer Immunoglobulin-Like Receptors (KIR):

- KIR2DL1 (HLA-C2)
- KIR2DL2/3 (HLA-C1)
- KIR3DL1 (HLA-Bw4)

Lectin-like receptors:

- CD94:NKG2A (HLA-E)



Activating receptors

Natural cytotoxicity receptors (NCR):

- NKp30
- NKp44
- NKp46

Lectin-like receptors:

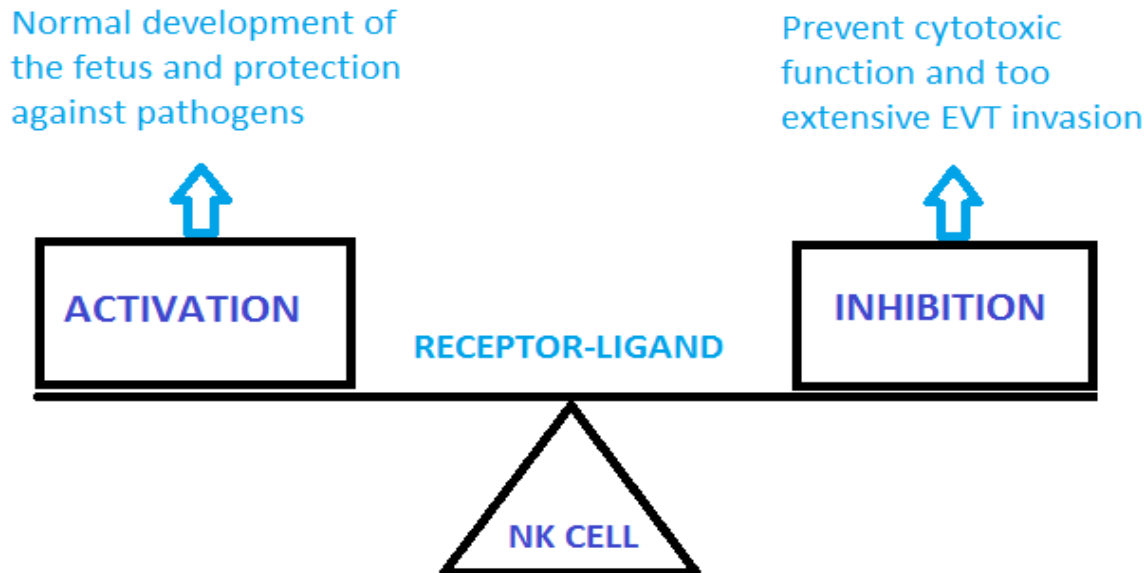
- CD94:NKG2C (HLA-E)
- NKG2D (MIC)

Activating KIR:

- KIR2DS
- KIR3DS

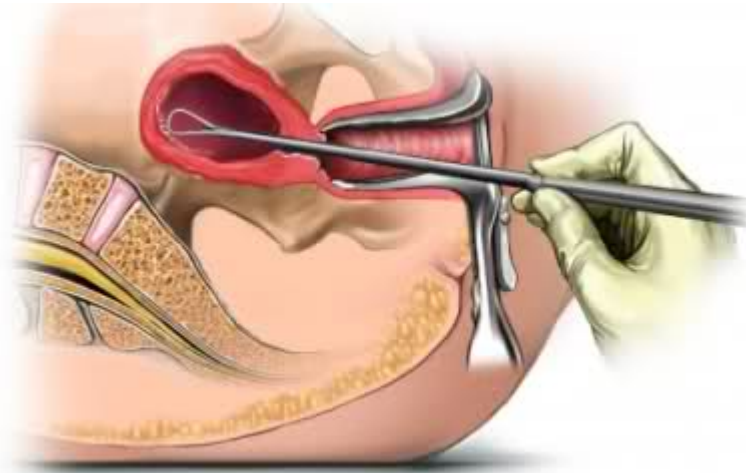
NK cell regulation

- Uterine or decidual NK cells have a key role in early pregnancy
- Activity is tightly regulated
- Major role for activating and inhibiting KIRs expressed on NK cells
 - *KIR AA and HLA-C2 combination associated with complications*

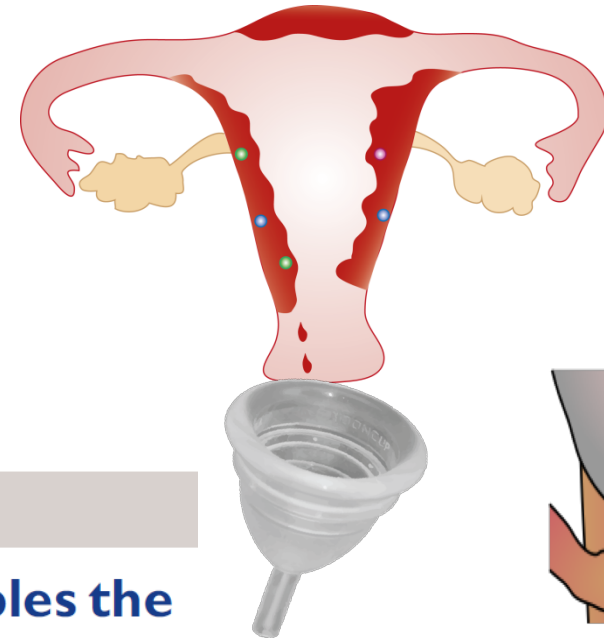


Hiby et al. *J Exp Med* 2004, Hiby et al. *Hum Reprod* 2008, Hiby et al. *J Clin Invest* 2010, Hiby et al. *Jl* 2014

Access to the non-pregnant uterus



Menstrual blood
source endometrial cells



human
reproduction

ORIGINAL ARTICLE *Reproductive biology*

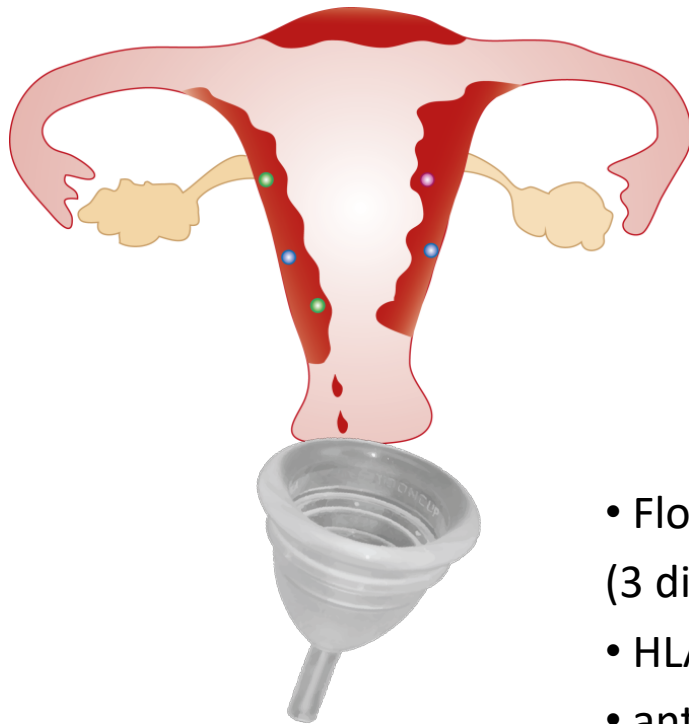
Menstrual blood closely resembles the uterine immune micro-environment and is clearly distinct from peripheral blood

R.G. van der Molen^{1,*}, J.H.F. Schutten¹, B. van Cranenbroek¹,
M. ter Meer¹, J. Donckers², R.R. Scholten², O.W.H. van der Heijden²,
M.E.A. Spaanderman^{2,3}, and I. Joosten¹

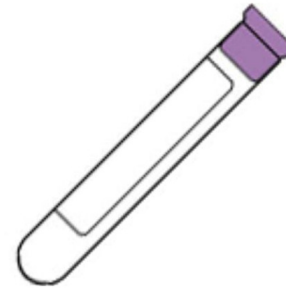
Materials and methods

25 females

Menstrual blood
source endometrial cells

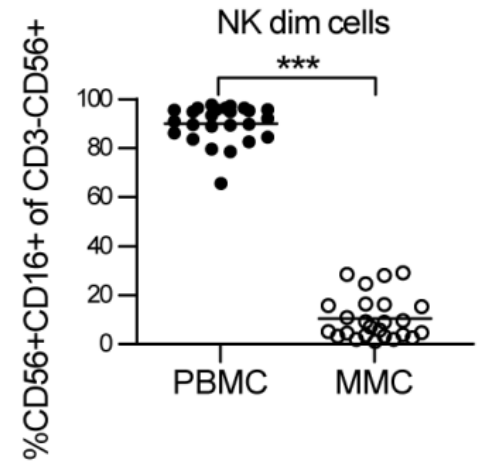
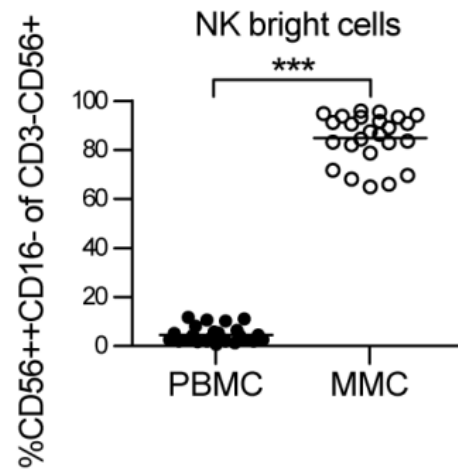
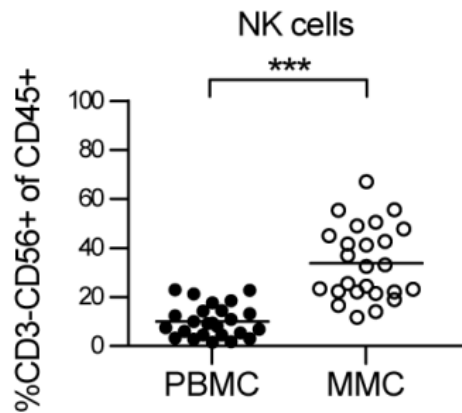
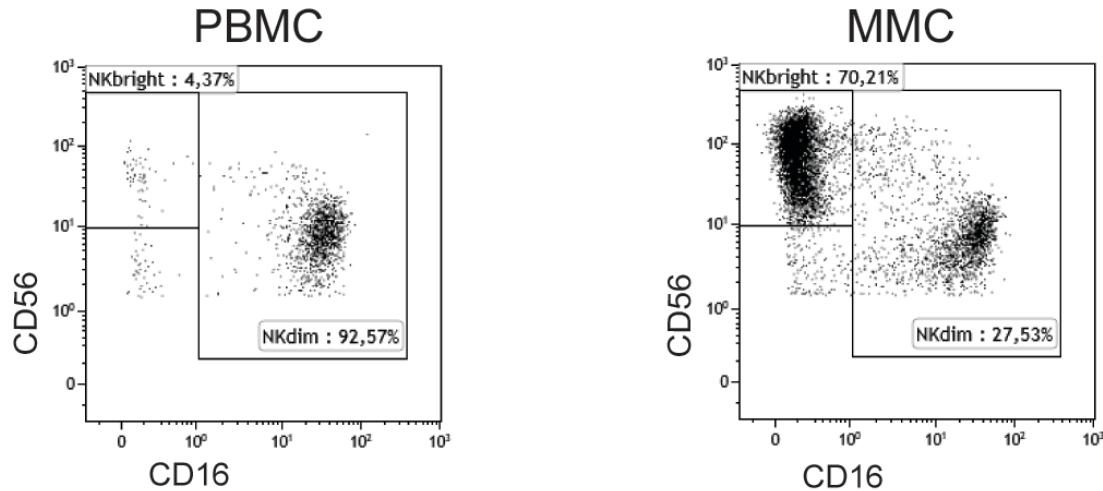


Peripheral blood



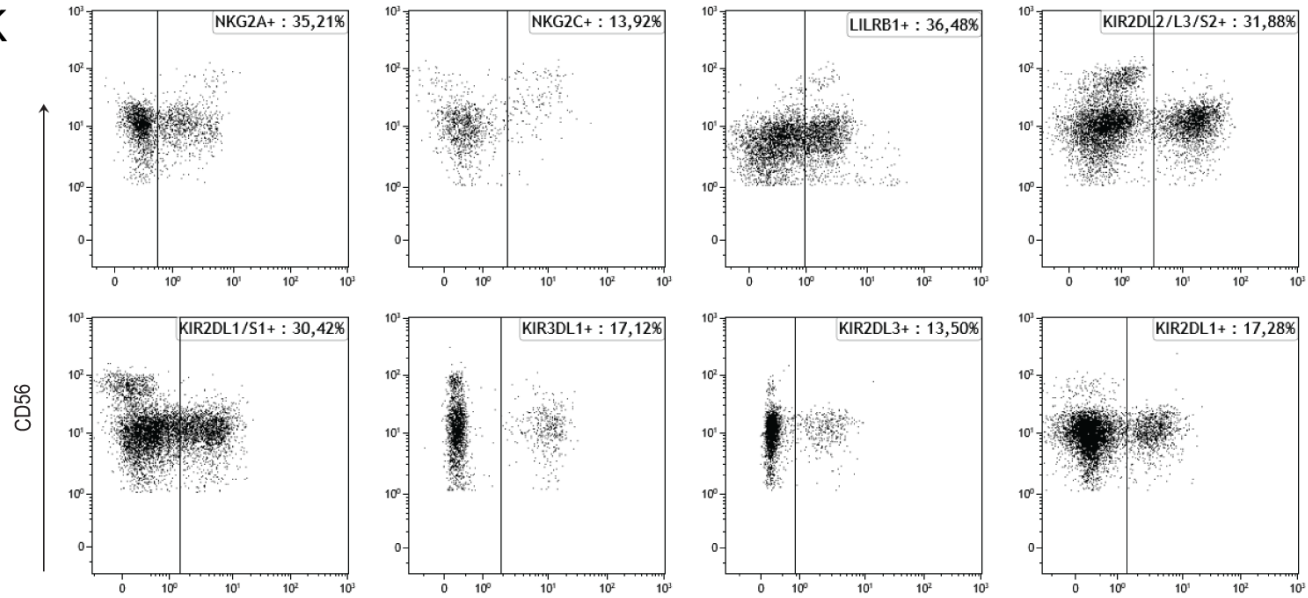
- Flow cytometry: NK cell receptor (NKR) expression (3 different panels with each 10 Abs)
- HLA-C genotype (PCR-SSOP)
- anti-CMV antibodies (plasma; chemiluminescence)

More NK cells, of bright phenotype, in endometrium

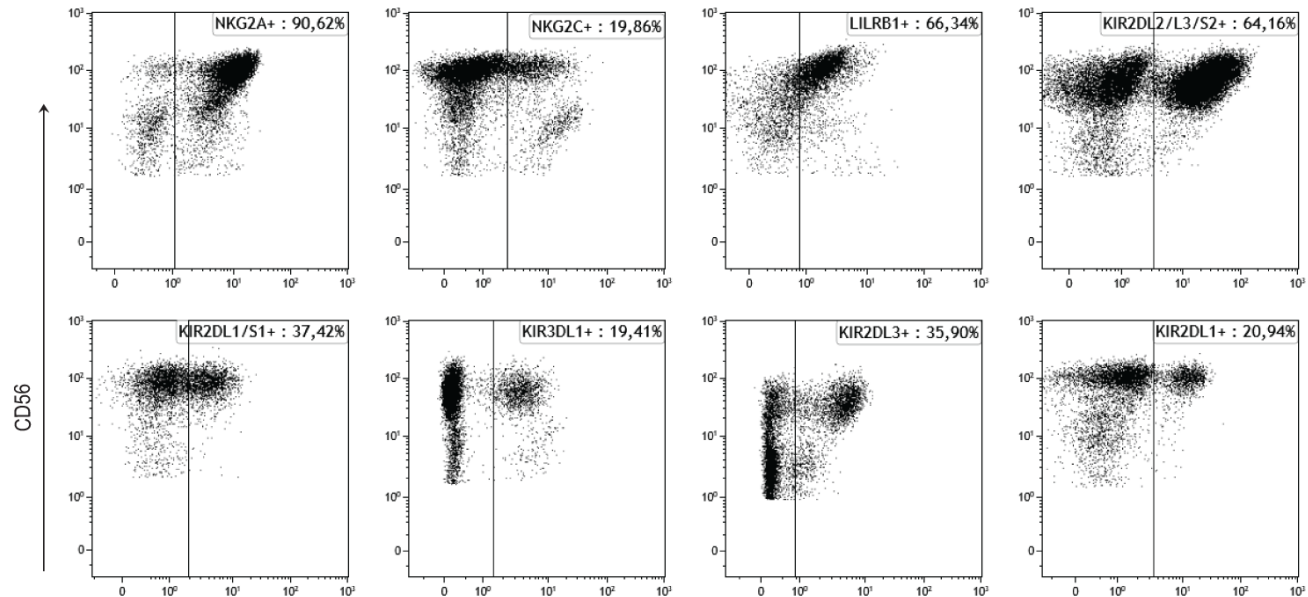


NK cell receptor expression

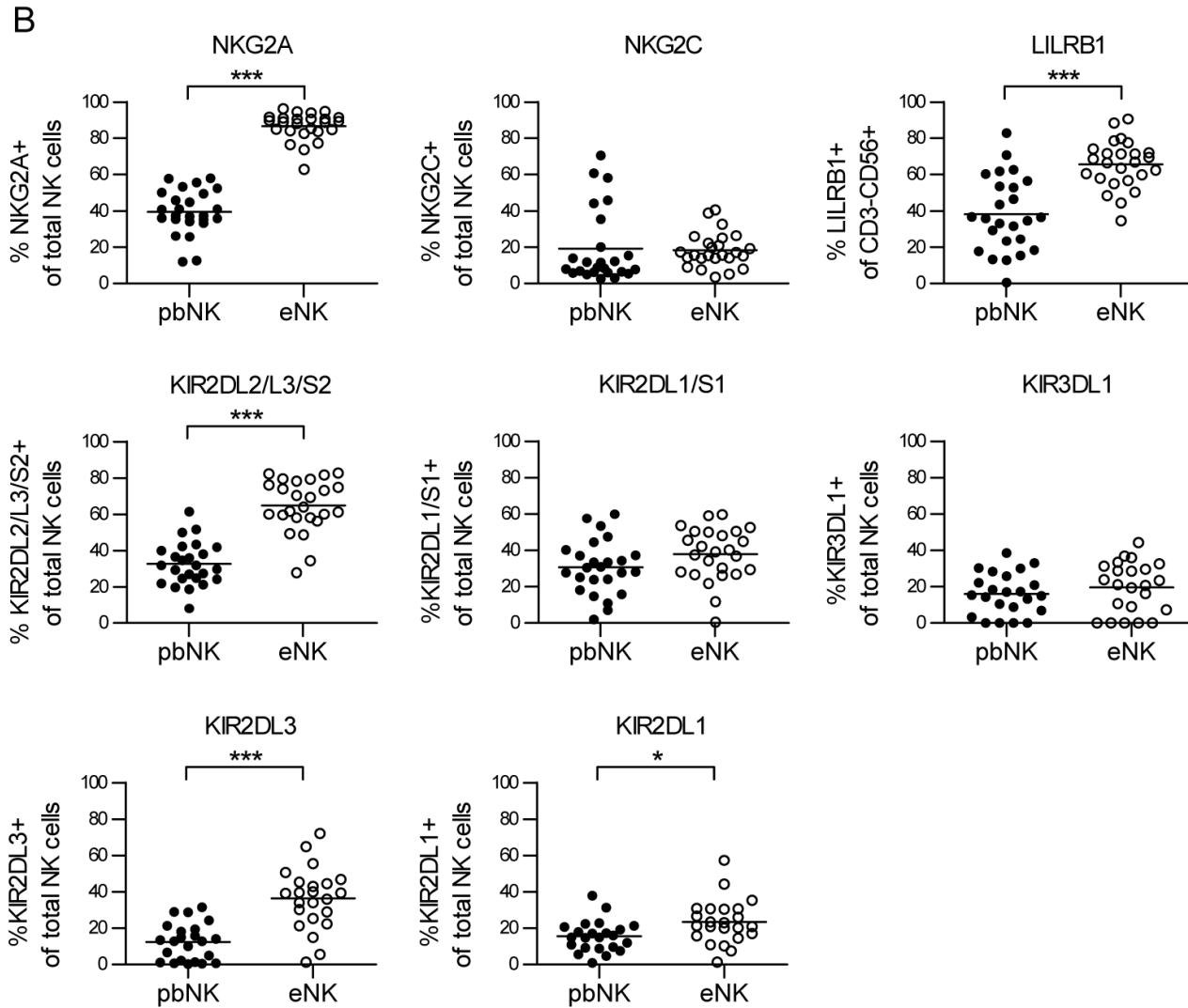
pbNK



eNK



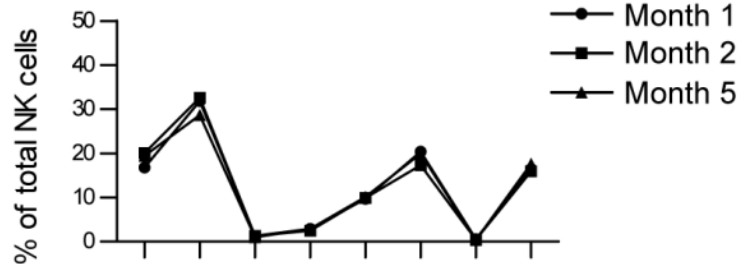
eNK cells contain more NKR expressing cells



KIR repertoire does not differ over consecutive menstrual cycles

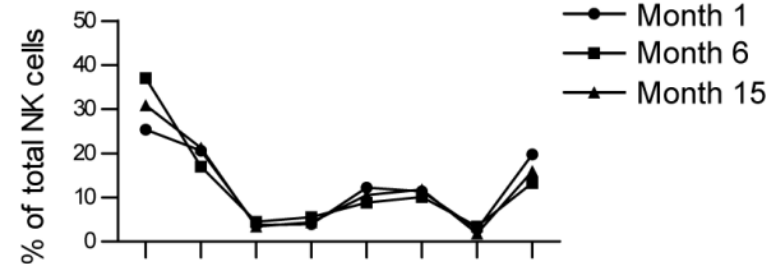
B

Donor MBC16



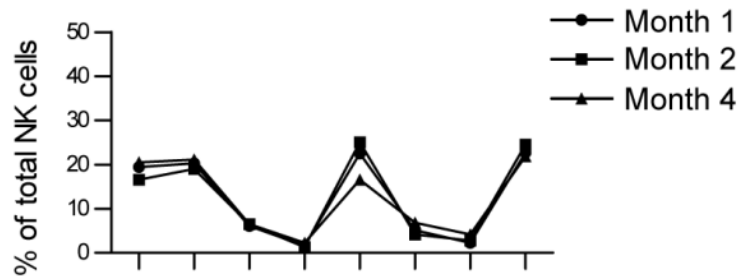
KIR2DL2/L3/S2	-	+	-	-	+	+	-	+
KIR2DL1/S1	-	-	+	-	+	-	+	+
KIR3DL1/S1	-	-	-	+	-	+	+	+

Donor MBC23



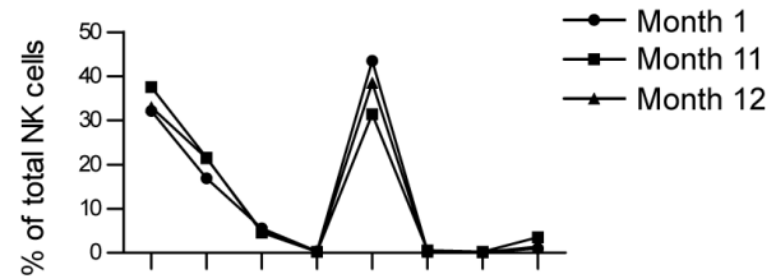
KIR2DL2/L3/S2	-	+	-	-	+	+	-	+
KIR2DL1/S1	-	-	+	-	+	-	+	+
KIR3DL1/S1	-	-	-	+	-	+	+	+

Donor MBC25



KIR2DL2/L3/S2	-	+	-	-	+	+	-	+
KIR2DL1/S1	-	-	+	-	+	-	+	+
KIR3DL1/S1	-	-	-	+	-	+	+	+

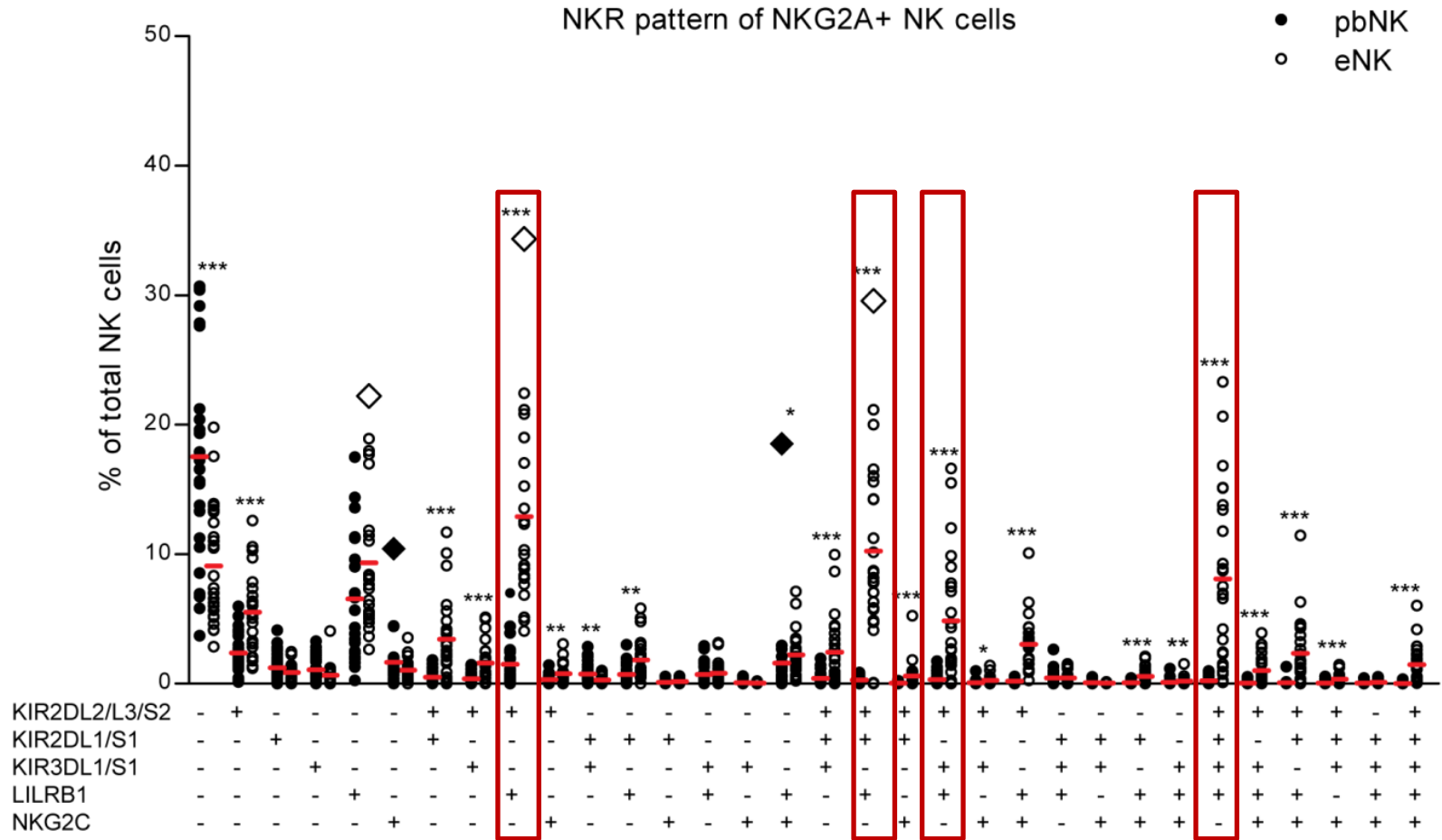
Donor MBC31



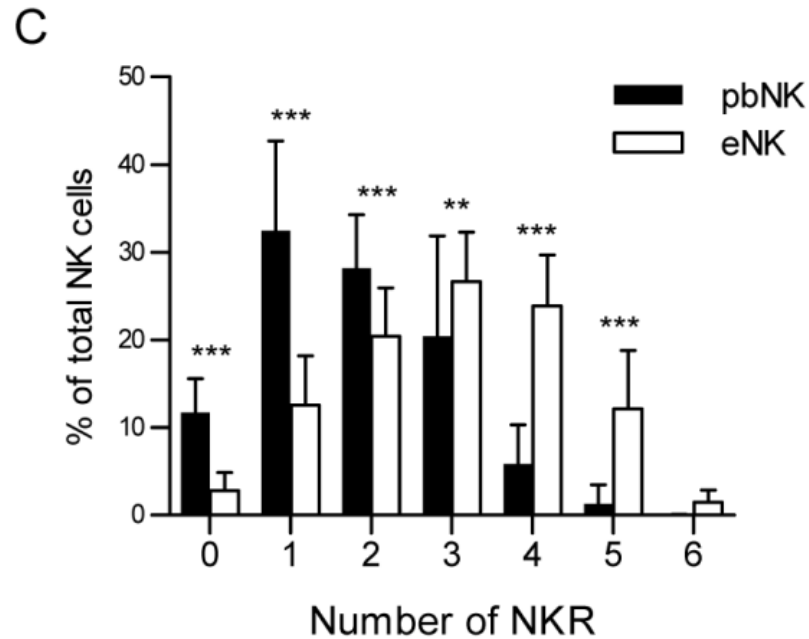
KIR2DL2/L3/S2	-	+	-	-	+	+	-	+
KIR2DL1/S1	-	-	+	-	+	-	+	+
KIR3DL1/S1	-	-	-	+	-	+	+	+

eNK cells have a distinct NKR expression pattern

A

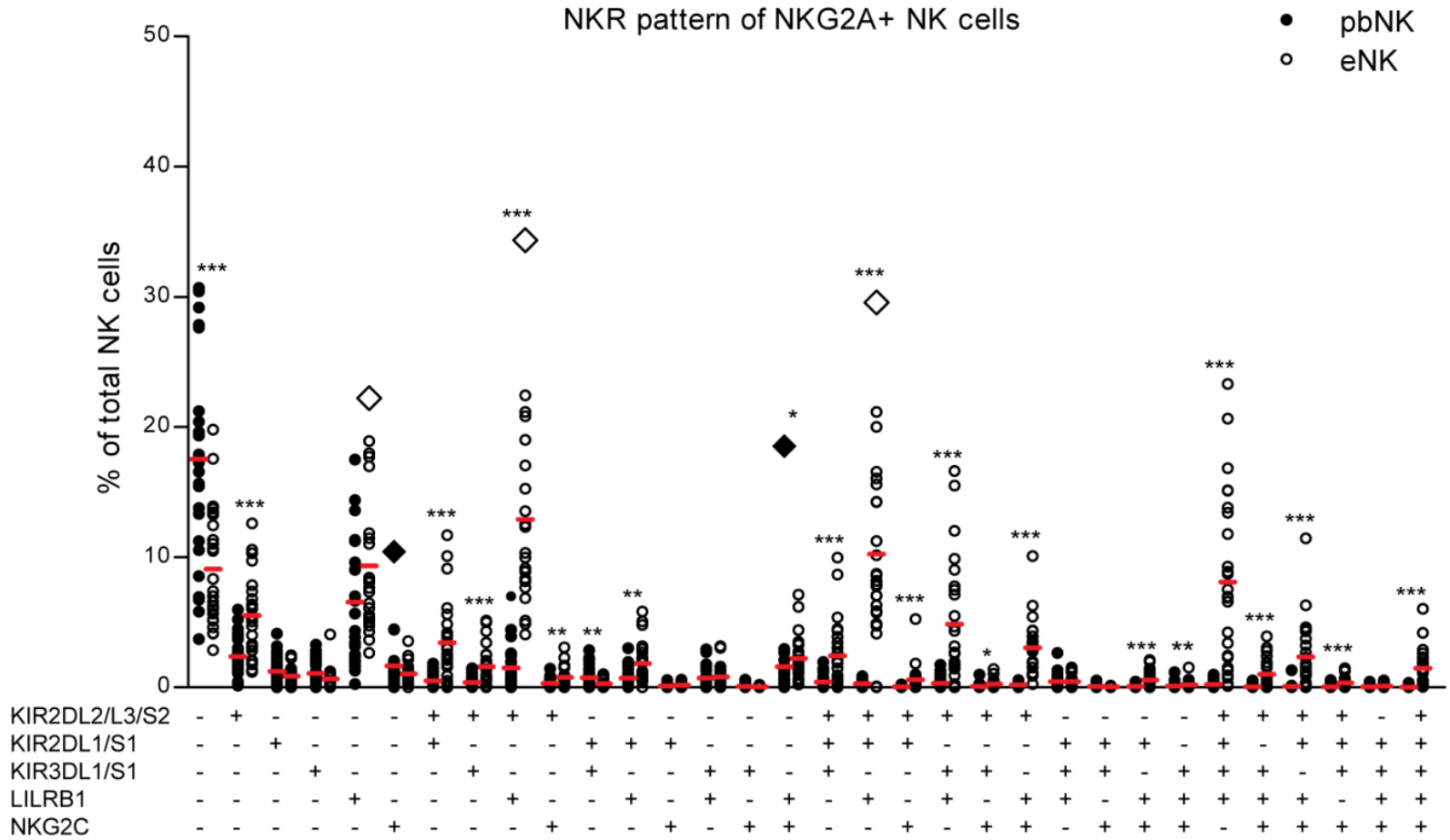


eNK cells co-express multiple NKR



eNK subpopulation expansions

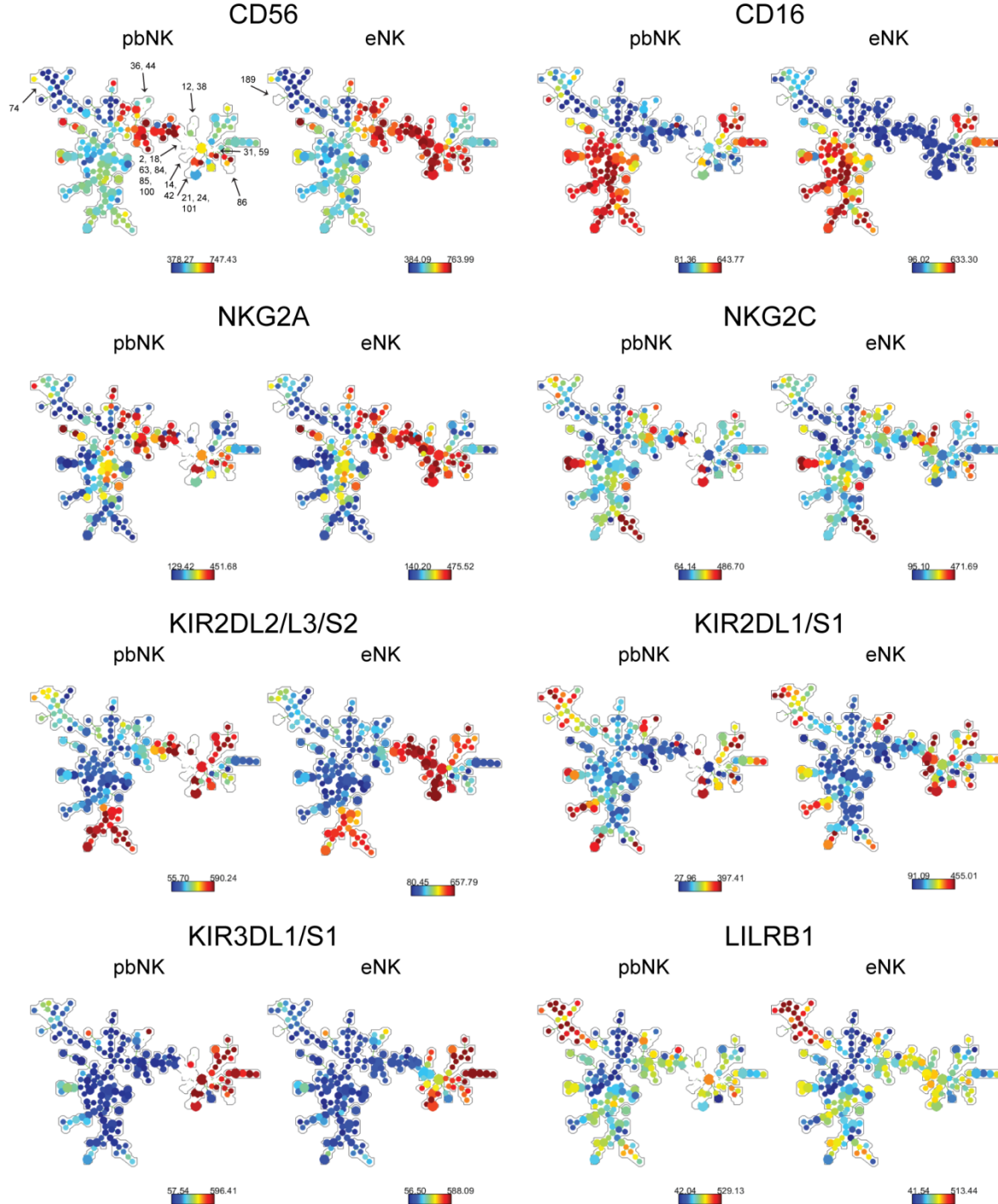
A



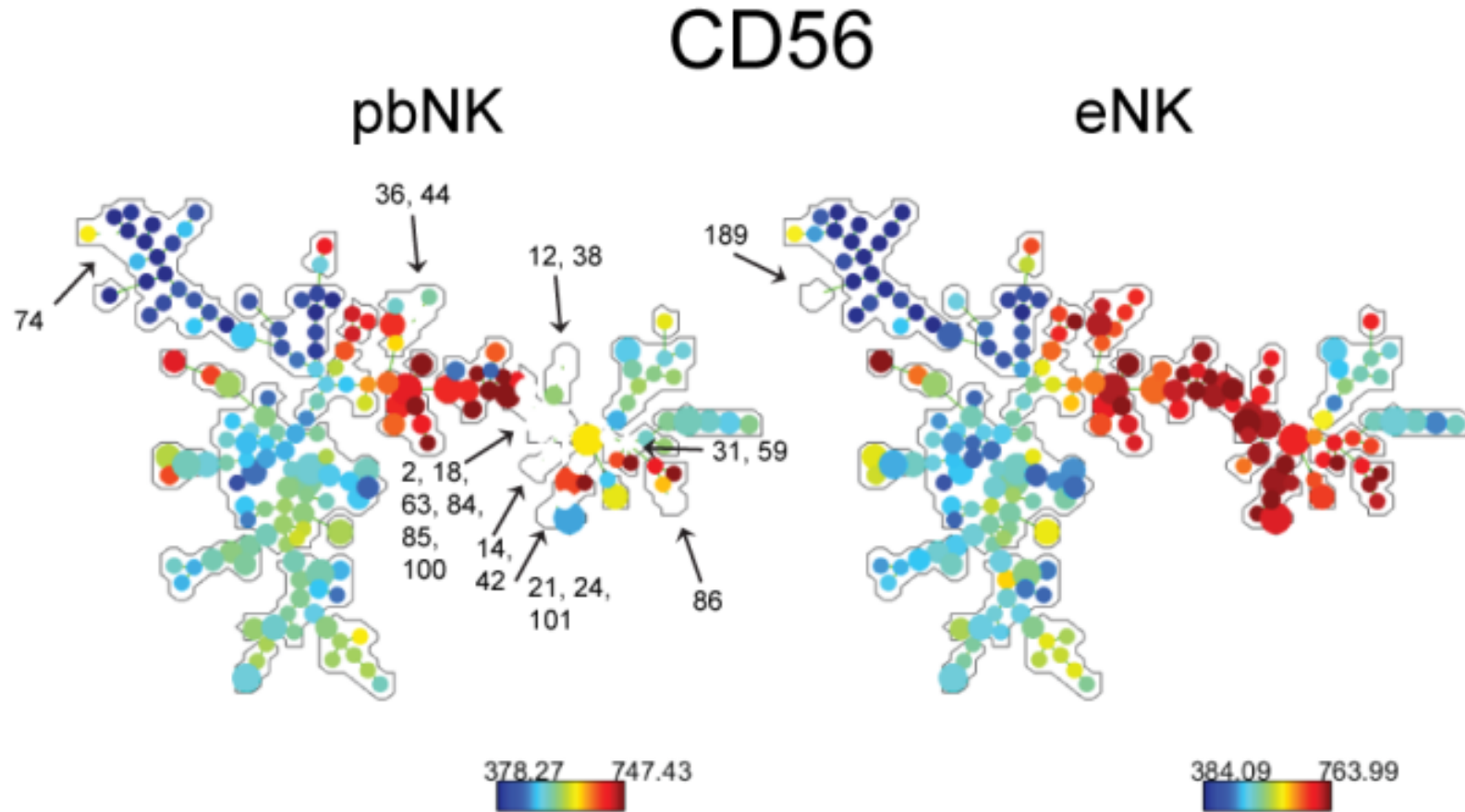
Tissue-specific eNK subpopulation expansions, independent of HLA-C and CMV

Donor	Expansion	CMV seropositivity	HLA-C	Self-specific KIR expansion?
<i>A: Endometrium</i>				
MBC14	NKG2A+LILRBI+ KIR2DL2/L3/S2+	–	C2C2	No
MBC31	NKG2A+LILRBI+ KIR2DL2/L3/S2+ KIR2DL1/SI+	–	C1C2	Yes
MBC33	LILRBI+KIR2DL1/SI+ NKG2C+	+	C2C2	Yes
MBC34	NKG2C+	+	C1C2	No
MBC40	null	–	C1C1	No
MBC46	NKG2A+LILRBI+	–	C1C1	No
MBC50	Null	–	C2C2	No
	KIR2DL2/L3/S2+			
<i>B: Peripheral blood</i>				
MBC14	LILRBI+	–	C2C2	No
	LILRBI+ KIR2DL2/L3/S2+			
MBC16	NKG2A+NKG2C+	+	C1C1	Yes
	LILRBI+ KIR2DL2/L3/S2+ NKG2C+			
MBC19	LILRBI+ KIR2DL1/SI+ NKG2C+	+	C2C2	Yes
MBC27	KIR2DL2/L3/S2+ NKG2C+	?	C1C2	Yes
	LILRBI+ KIR2DL2/L3/S2+ NKG2C+			
MBC33	LILRBI+ KIR2DL1/SI+ NKG2C+	+	C2C2	Yes
MBC34	LILRBI+ KIR2DL1/SI+ NKG2C+	+	C1C2	Yes
MBC35	KIR2DL2/L3/S2+ KIR2DL1/SI+	+	C1C2	Yes
MBC48	KIR2DL2/L3/S2+ KIR2DL1/SI+ KIR3DL1/SI+NKG2C+	+	C2C2	Yes
	LILRBI+ KIR2DL2/L3/S2+ KIR2DL1/SI+NKG2C+			
	LILRBI+KIR2DL2/L3/S2+ KIR2DL1/SI+ KIR3DL1/SI+ NKG2C+			
MBC50	KIR2DL2/L3/S2+ KIR2DL1/SI+	–	C2C2	Yes

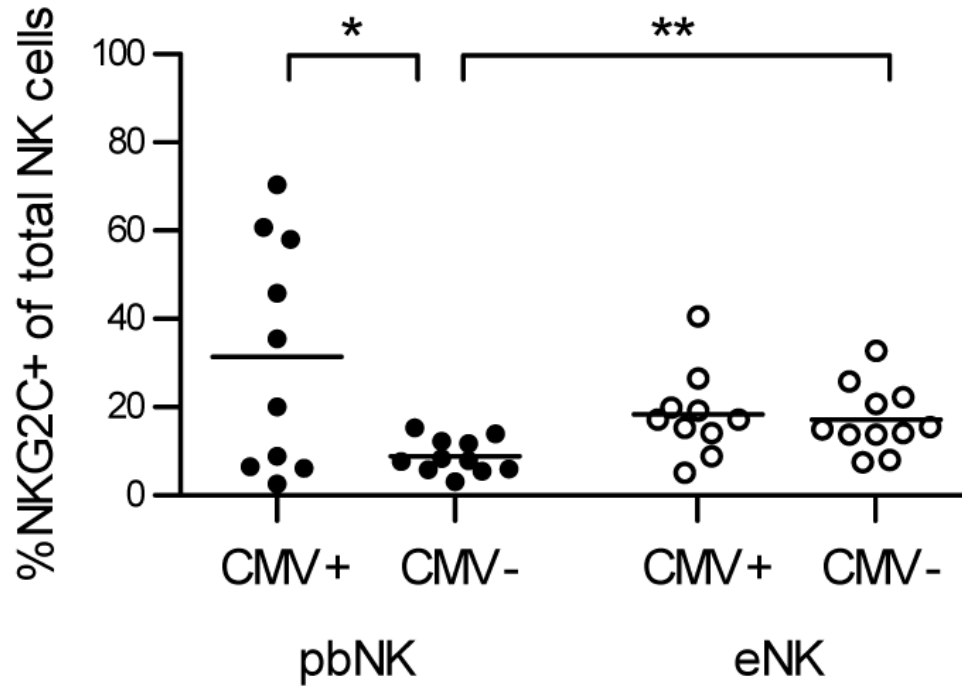
SPADE analysis



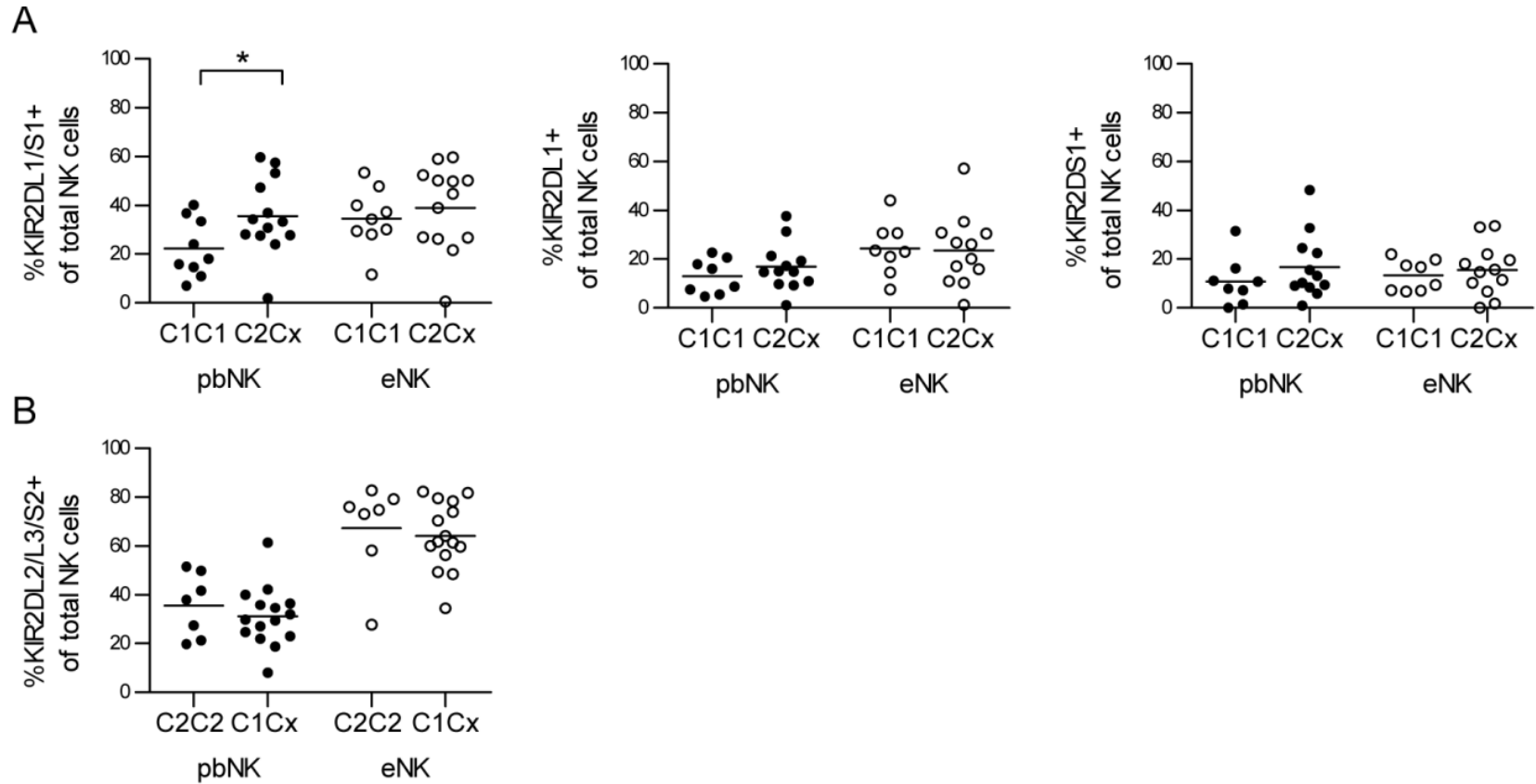
Higher diversity in eNK cell population



CMV has no imprint on eNK cell phenotype



HLA-C genotype does not influence KIR expression on eNK



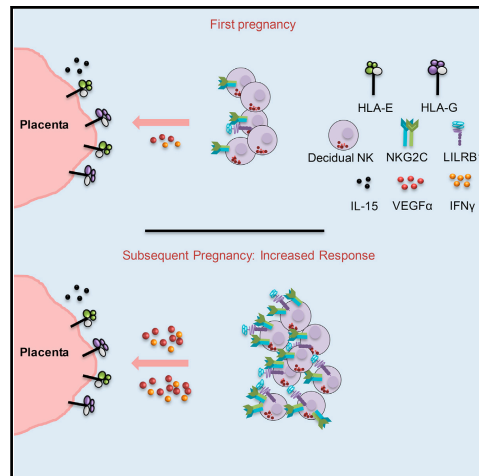
Pregnancy trained memory eNK cells?

Article

Immunity

Trained Memory of Human Uterine NK Cells Enhances Their Function in Subsequent Pregnancies

Graphical Abstract



Authors

Moriya Gamliel, Debra Goldman-Wohl, Batya Isaacson, ..., Jacob H. Hanna, Simcha Yagel, Ofer Mandelboim

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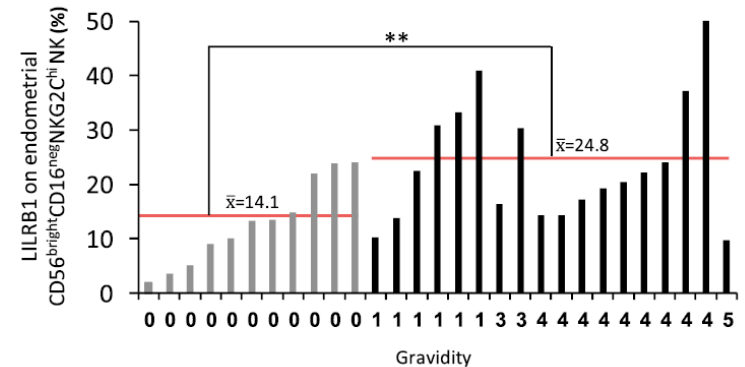
In Brief

Natural killer cells are present in the human decidua, regulating trophoblast invasion and angiogenesis. Here, Gamliel et al. report on a special subset of human decidua natural killer cells, which “remember” pregnancy and better support subsequent pregnancies. This might explain why first pregnancies are at increased risk of developing diseases of poor placentation.

Highlights

- A unique subset of human natural killer cells exists in repeated pregnancies
- These NK cells, termed PTdNKs, express increased amounts of NKG2C and LILRB1
- PTdNKs secrete increased levels of IFN- γ and VEGF α ; the latter supports vascularization

D

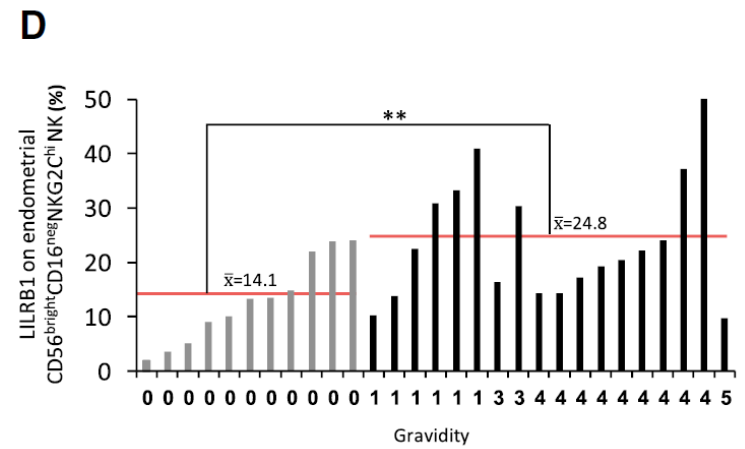
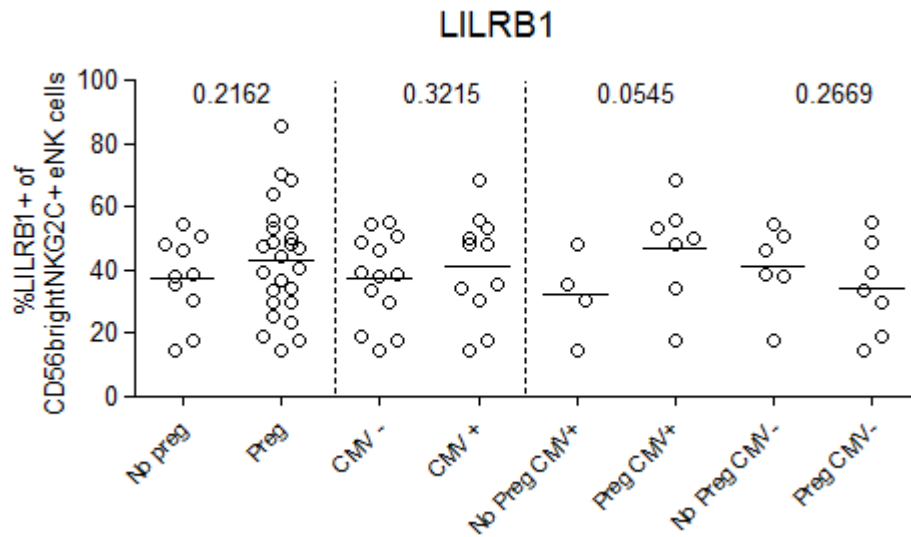


Gamliel et al., 2018, *Immunity* 48, 951–962
May 15, 2018 © 2018 Elsevier Inc.
<https://doi.org/10.1016/j.immuni.2018.03.030>

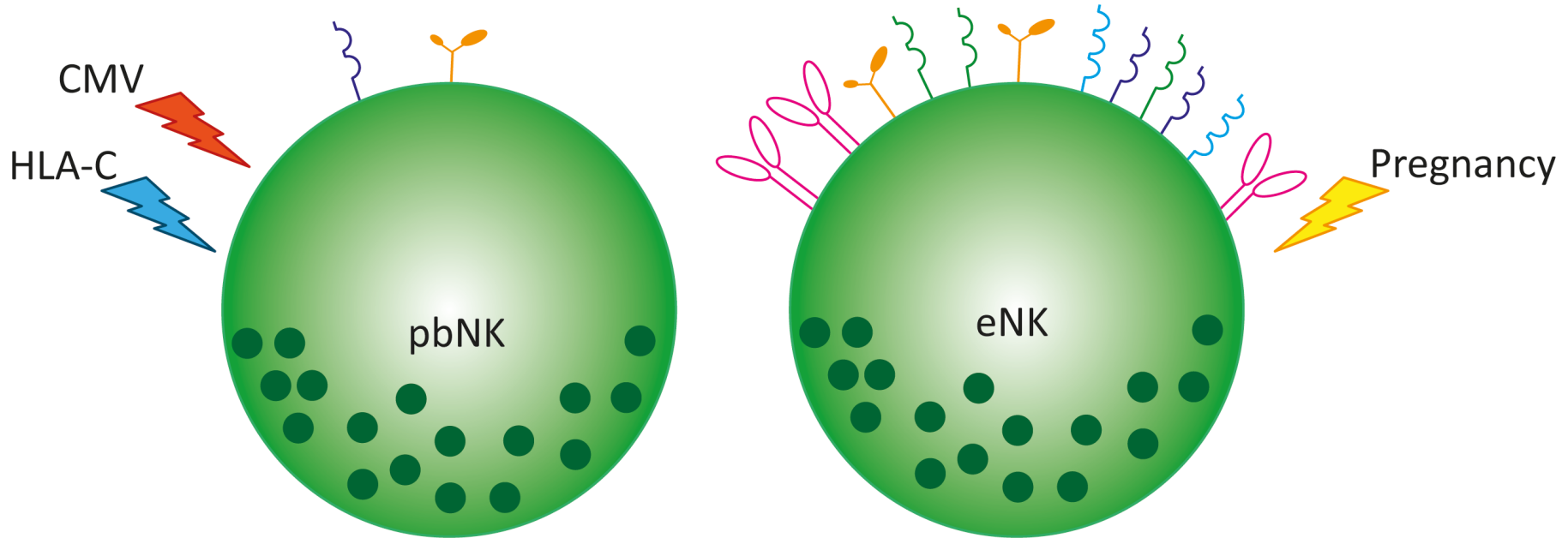
CellPress

Radboudumc

Pregnancy trained memory eNK cells?



Conclusion



NK cells in endometrium have unique, tissue-specific phenotype, independent of CMV status and *HLA-C* genotype, but influenced by previous pregnancy

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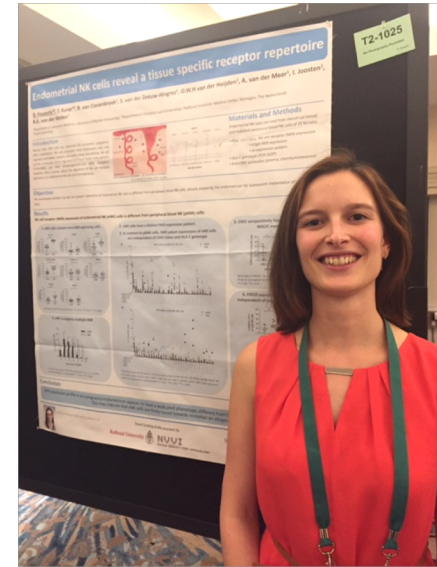
Department of Gynaecology and Obstetrics

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Includeren deelnemers op poli:

Dr. Freke Wilmink

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