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Supplemental information

NK-B cell cross talk induces CXCR5 expression on natural killer cells

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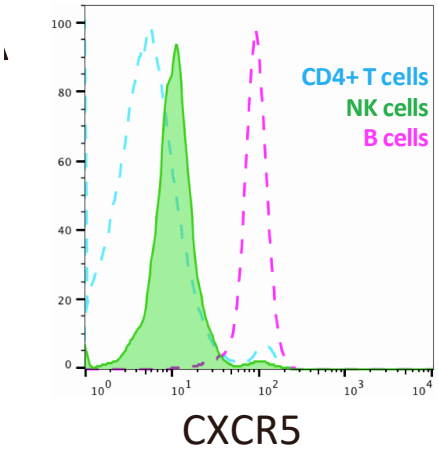
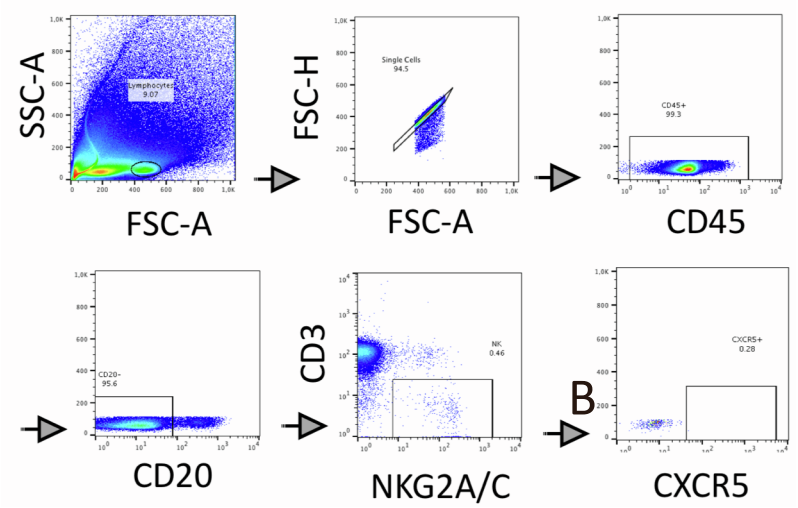
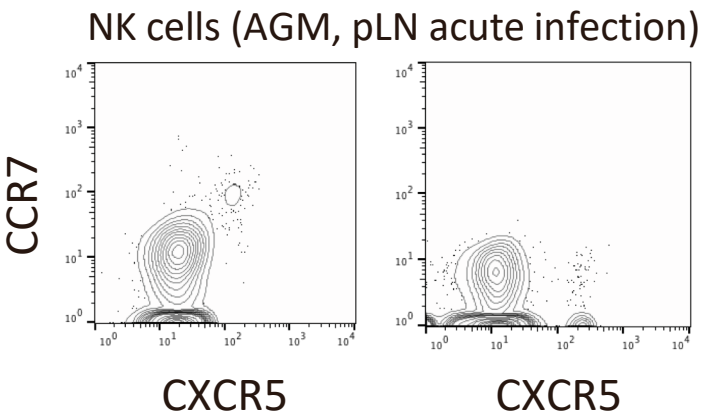
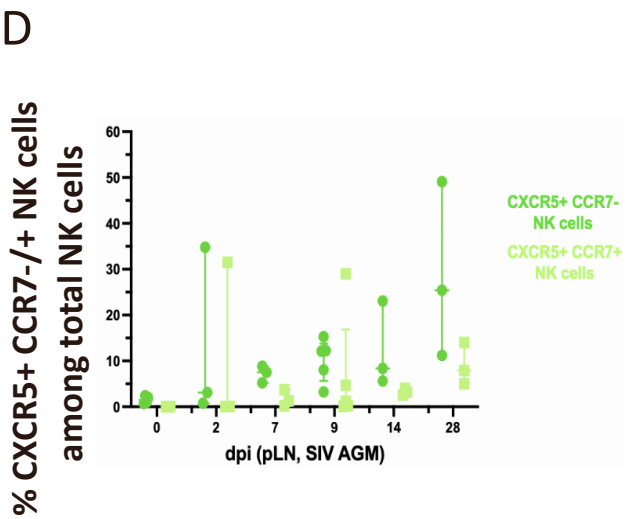

CD4+ T cells
NK cells
B cells
CXCR5


B
SSC-A
FSC-A
FSC-H
FSC-A
CD45
CD20
CD3
NKG2A/C
B
CXCR5


NK cells (AGM, pLN acute infection)
CCR7
CXCR5
CXCR5


D
% CXCR5+ CCR7-/+ NK cells among total NK cells
CXCR5+ CCR7- NK cells
CXCR5+ CCR7+ NK cells
dpi (pLN, SIV AGM)

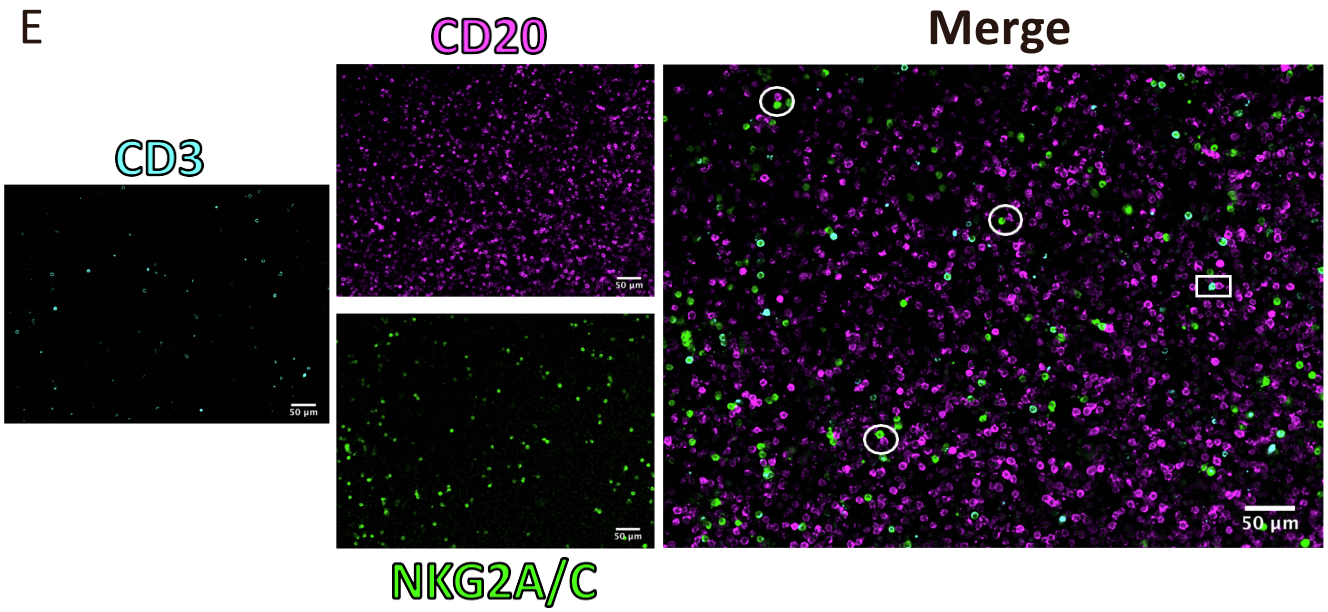
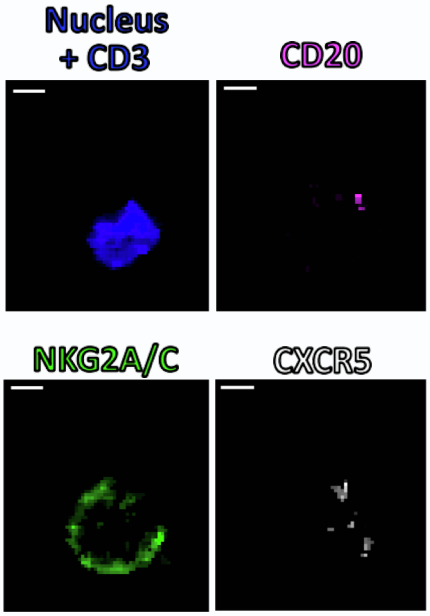
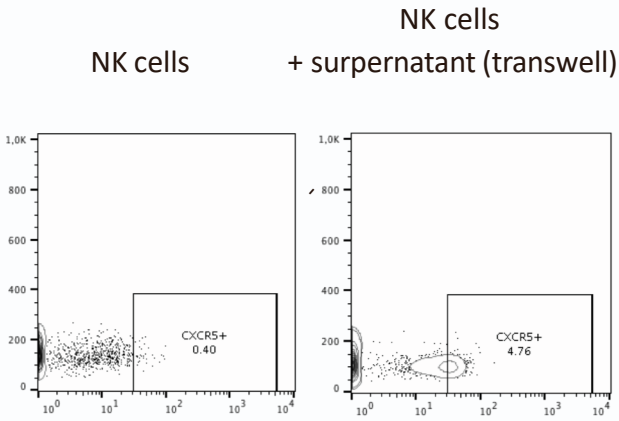

E
CD20
Merge
CD3
50 µm
NKG2A/C

Figure S1. Identification of CXCR5+ NK cells, Related to Figure 1 and Figure 2. A) Histogram of CXCR5 expression on CD4+ T cells, B cells and NK cells from pLN in acute SIVagm infection. A representative animal is shown (AGM at day 9 p.i.). **B)** Gating strategy of CXCR5+ NK cells in pLN from cynomolgus macaques. An example for cells from SIVmac-infected MAC at day 9 p.i. is shown. **C)** Dot plot of CXCR5+CCR7+ and CXCR5+CCR7- NK cells in pLN from AGM during acute SIVagm infection (day 9 p.i.). **D)** Kinetic of CXCR5+CCR7- and CXCR5+CCR7+ NK cell frequencies in pLN during primary SIVagm infection. Light green rectangles indicate CXCR5+CCR7+ NK cells and dark green circles CXCR5+CCR7- NK cells. (n=3 AGM, except at day 9 pi where n=5). **E)** Analysis of contacts between NK and B cells. Cells were isolated from PBMC of non-infected AGM (n=3). NK cell marker in green, B cell in magenta and CD3 in cyan. The white circles delineate NK and B cells and the white square T and B cells. Each dot bar indicates median values, and error bars indicate interquartile ranges.

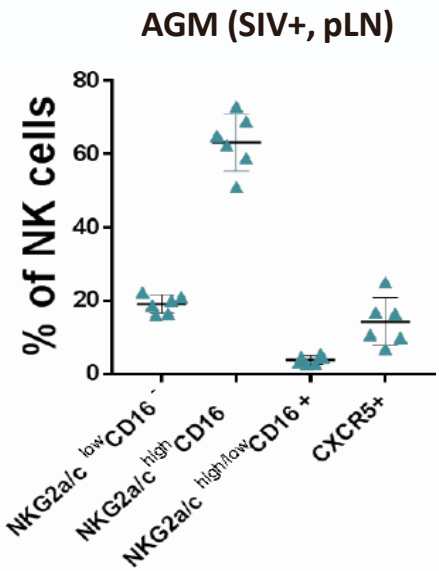
A


Nucleus
+ CD3
CD20
NKG2A/C
CXCR5

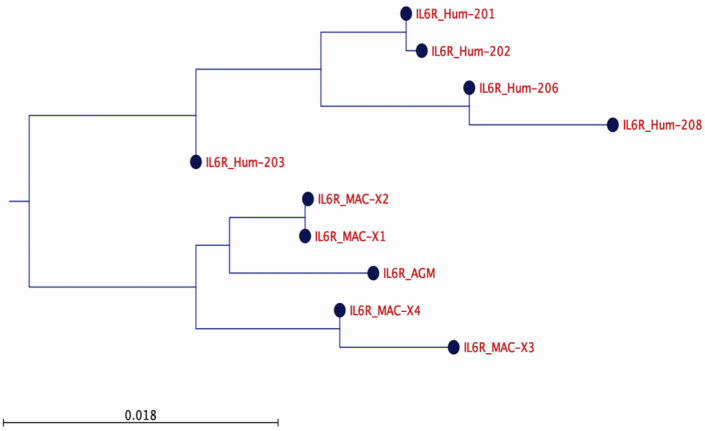
B


NK cells
NK cells
+ surpernatant (transwell)
CXCR5+
0.40
CXCR5+
4.76

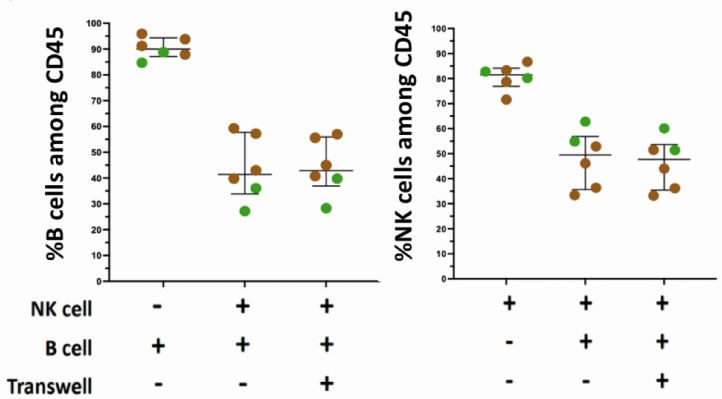
C


AGM (SIV+, pLN)
% of NK cells
NKG2a/c low CD16 -
NKG2a/c high CD16
NKG2a/c high/low CD16 +
CXCR5+

D


IL6R_Hum-201
IL6R_Hum-202
IL6R_Hum-206
IL6R_Hum-208
IL6R_Hum-203
IL6R_MAC-X2
IL6R_MAC-X1
IL6R_AGM
IL6R_MAC-X4
IL6R_MAC-X3
0.018

E


%B cells among CD45
%NK cells among CD45
NK cell
B cell
Transwell

F

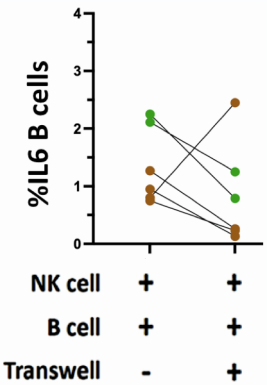
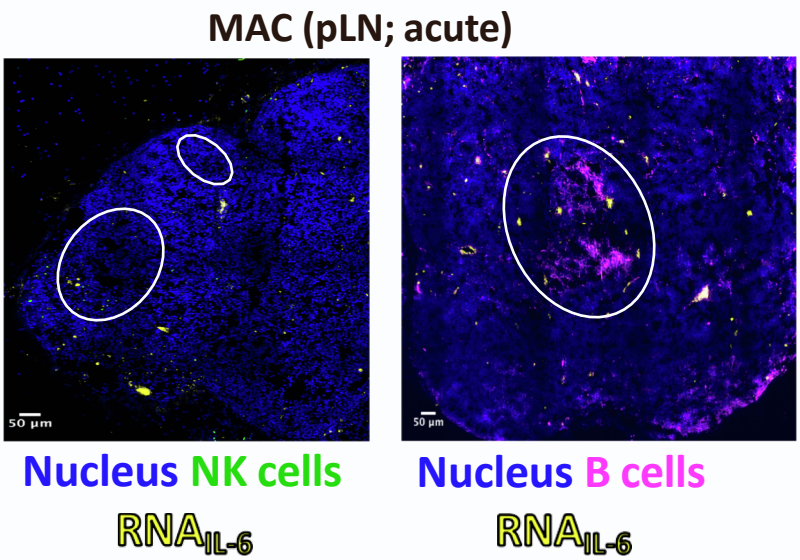

%IL6 B cells
NK cell
B cell
Transwell

Figure S2. *In vitro* and *in vivo* NK cell population, Related to Figure 2 and Figure 3. A) Staining of NK cells after 1 day of culture. NK cell marker in green, B cell marker in magenta, Nucleus and CD3 in cyan, CXCR5 in grey. Scale bare upper left correspond to 2 micrometers. **B)** Dot plot representative of CXCR5+ NK cells from AGM after culture with the supernatant of NK-B cell cocultures in transwell chambers. **C)** Frequencies of NK cell subpopulations in pLN of chronically SIVagm-infected AGM (n=6 AGM). **D)** Phylogenetic analysis of IL6R genes (variants) was performed for human, macaque and AGM species in phylogram representation (the ladder is in number of substitution/sites). **E)** Percentage of B cells and NK cells. **(F)** IL-6+ B cells after co- culture with autologous cells or co-culture with autologous cells separated by a transwell membrane. B and NK cells were isolated from PBMC of uninfected AGMs (n= 2, green) and MACs (n=4, brown). Non-significative differences (n=6, Wilcoxon test). Each dot bar indicates median values, and error bars indicate interquartile ranges.

A


MAC (pLN; acute)
50 µm
50 µm
Nucleus NK cells
RNA IL-6
Nucleus B cells
RNA IL-6

B

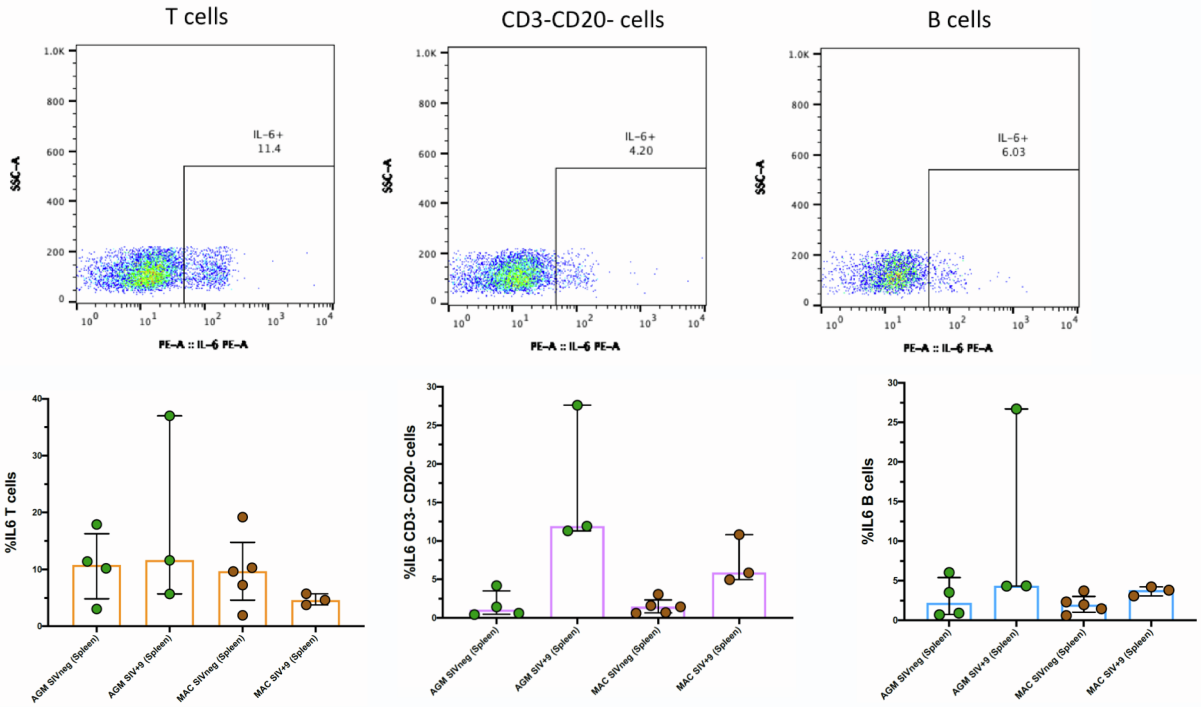

T cells
CD3-CD20- cells
B cells
IL-6+
11.4
IL-6+
4.20
IL-6+
6.03
SSC-A
PE-A :: IL-6 PE-A
%IL6 T cells
%IL6 CD3- CD20- cells
%IL6 B cells
AGM SIVneg (Spleen)
AGM SIV+9 (Spleen)
MAC SIVneg (Spleen)
MAC SIV+9 (Spleen)

Figure S3. In situ and ex vivo IL-6 production in secondary lymphoid organs, Related to Figure 4 and Figure 5. A) *IL-6* mRNA expression in pLN from MAC during acute SIVmac infection (at day 9 p.i.) analyzed by microscopy. Representative B follicles are shown. NK cell marker in green, B cell marker in magenta, $RNA_{IL\text{-}6}$ in yellow and nucleus in blue (n=3 MAC). **B)** Analysis of ex vivo IL-6 expression by flow cytometry. Upper panel: Dot plot representative of IL-6 intracellular *ex vivo* staining in T cells (CD3+), B cells (CD20+) and $CD3^{-}CD20^{-}$ cells from spleen. Lower panel: Frequencies detected in uninfected and acutely (day 9 p.i.) SIVagm and SIV mac infected animals. Each circle indicates an individual animal. Green: AGM, brown: macaque. Each dot bar indicates median values, and error bars indicate interquartile ranges.

Animal #	Species	Gender	Age at inclusion (years)	SIV Negative infection	SIV Acute infection (vRNA, copies/mL plasma)	SIV Chronic infection (vRNA, copies/mL plasma)
AGM01	Chlorocebus sabaeus	Female	10,3		2,00E+07	
AGM02	Chlorocebus sabaeus	Male	5,0		1,30E+07	1,20E+04
AGM03	Chlorocebus sabaeus	Female	5,0		1,30E+07	1,20E+04
AGM04	Chlorocebus sabaeus	Female	6,0		8,20E+06	1,20E+04
AGM05	Chlorocebus sabaeus	Female	5,2		3,60E+08	2,30E+04
AGM06	Chlorocebus sabaeus	Male	4,3		3,60E+08	2,50E+04
AGM07	Chlorocebus sabaeus	Female	5,2		2,80E+08	5,60E+04
AGM08	Chlorocebus sabaeus	Male	6,6			5,00E+05
AGM09	Chlorocebus sabaeus	Male	4,4	n.a.		
AGM10	Chlorocebus sabaeus	Male	6,2	n.a.	3,50E+06	
AGM11	Chlorocebus sabaeus	Male	6,6	n.a.		1,30E+06
AGM12	Chlorocebus sabaeus	Male	5,2			1,90E+06
AGM13	Chlorocebus sabaeus	Male	6,2		7,70E+05	
AGM14	Chlorocebus sabaeus	Male	6,4	n.a.		
AGM15	Chlorocebus sabaeus	Male	4,0	n.a.		
AGM16	Chlorocebus sabaeus	Female	7,0	n.a.		
AGM17	Chlorocebus sabaeus	Male	5,1	n.a.		
AGM18	Chlorocebus sabaeus	Male	4,5	n.a.		
AGM19	Chlorocebus sabaeus	Female	5,2	n.a.		
MAC01	Macaca fascicularis	Male	6,5	n.a.		
MAC02	Macaca fascicularis	Male	8,0	n.a.		
MAC03	Macaca fascicularis	Male	7,8	n.a.		
MAC04	Macaca fascicularis	Male	6,4	n.a.		
MAC05	Macaca fascicularis	Male	6,9	n.a.		
MAC06	Macaca fascicularis	Male	7,6	n.a.		
MAC07	Macaca fascicularis	Male	8,5	n.a.		
MAC08	Macaca fascicularis	Male	9,9	n.a.		
MAC09	Macaca fascicularis	Female	8,4	n.a.		
MAC10	Macaca fascicularis	Female	3,8		5,60E+06	2,50E+04
MAC11	Macaca fascicularis	Female	3,8		2,00E+06	2,60E+04
MAC12	Macaca fascicularis	Female	3,8		3,00E+06	3,30E+04
MAC13	Macaca fascicularis	Male	2,6		2,00E+06	2,50E+06
MAC14	Macaca fascicularis	Male	8,6	n.a.		
MAC15	Macaca fascicularis	Male	8,4	n.a.		
MAC16	Macaca fascicularis	Male	5,7	n.a.		
MAC17	Macaca fascicularis	Female	3,3		1,90E+06	
MAC18	Macaca fascicularis	Male	3,1		9,90E+05	
MAC19	Macaca fascicularis	Male	3,1		1,20E+06	
MAC20	Macaca fascicularis	Female	3,1		2,00E+06	1,50E+03
MAC21	Macaca fascicularis	Male	2,6		2,10E+07	2,20E+03
MAC22	Macaca fascicularis	Female	5,6	n.a.		
MAC23	Macaca fascicularis	Female	5,0	n.a.		
MAC24	Macaca fascicularis	Female	7,2	n.a.		
MAC25	Macaca fascicularis	Female	8,4	n.a.		
MAC26	Macaca fascicularis	Female	8,8	n.a.		
MAC27	Macaca fascicularis	Male	3,6			1,45E+05
MAC28	Macaca fascicularis	Male	5,3			4,58E+02

Table S1. Profile of non-human primates used for this study, Related to Figure 1-5. Description of species, gender, plasma viremia levels corresponding at the different time points (n.a: not applicable).

	Name	Fluorochrome	Clone	Isotype	Supplier	Reference	Antibody dilution used per 10^6cells
LSR II	CD45	PerCP	D058-1283	IgG1	BD	558411	1:20
	CD3	V500	SP34-2	IgG1	BD	560770	1:20
	CD16	ECD	3G8	IgG1	Beckman	A33098	1:20
	NKG2A	PE	Z199	IgG2	Beckman	PNA60797	1:20
	CD20	PE-Cy5	2H7	IgG1	BD	555624	1:10
	Granzyme B	V450	GB11	IgG1	BD	561151	1:20
	CXCR5	FITC	MU5UBEE	IgG1	MABTech	11-9185-42	1:5
	PD-1	APC-Cy7	EH12.2H7	IgG1	Biolegend	329922	1:10
	CD4	V450	L200	IgG1	BD	560811	1:10
	MHC-E	PE	3D12	IgG1	Biolegend	342603	1:10
	HLA-DR	Alexa 700	L243	IgG1	OZYME	307626	1:20
	IFNg	APC	B27	IgG1	BD	554702	1:20
	IL-10	PE-Cy7	JES3-9D1	IgG1	Biolegend	501420	1:5
	IL6	PE	MQ2-6A3	IgG2a	BD	559331	1:5
	CD8	APC-Cy7	130-113-155	IgG2a	Miltenyi	BW135/80	1:10
	CD3	V450	SP34-2	IgG1	BD	560770	1:20
	CD20	Alexa 700	2H7	IgG1	BD	560631	1:10
	IgD	ECD	polyclonal	IgG1	Southern Biotech	2030-07	1:5
	CD27	PE-Cy7	M-T271	IgG1	BD	560609	1:5
	CD107a	PE-Cy5	H4A3	IgG1	BD	555802	1:5
	IgG	FITC	G18-145	IgG1	BD	555786	1:10
	IgM	PE-Cy5	G20-127	IgG1	BD	562031	1:20
	Ki67	PE	B56	IgG1	BD	556027	1:5
Aria II							
	CD45	PerCP	D058-1283	IgG1	BD	558411	1:20
	CD3	V500	SP34-2	IgG1	BD	560770	1:20
	CD16	ECD	3G8	IgG1	Beckman	A33098	1:20
	NKG2A	PE	Z199	IgG2	Beckman	PNA60797	1:20
	CD8	APC-Cy7	130-113-155	IgG2a	Miltenyi	BW135/80	1:10
	CD20	Alexa 700	2H7	IgG1	BD	560631	1:10
	CD14	PE	M5E2	IgG2a	BD	557154	1:10
	CXCR5	FITC	MU5UBEE	IgG1	MABTech	11-9185-42	1:5

Table S2. Flow cytometry antibody list, Related to Figure 1-5. Antibodies used to stain NK cells and B cells by flow cytometry using LSRII and antibodies used to stain NK cells during cell sorting by Aria II.

In situ	Name	Fluorochrome	Clone	Isotype	Supplier	Reference
	Anti-NKG2A	n.a	Polyclonal	IgG-Rabbit	Abcam	ab170844
	Anti-Ki67	n.a	MIB-1	IgG1-Mouse	Santa Cruz	sc-101861
	Anti-CD20	n.a	L26	IgG2a-Mouse	Cell Marque	120M-84
	Anti-CD138	n.a	MI15	IgG1k-Mouse	Biolegend	356501
	Nucleus	DAPI	n.a	n.a	Sigma	D9542-10MG
	Anti-Mouse IgG2a	AlexaFluor 568	Polyclonal	IgG-Goat	Invitrogen	A-21134
	Anti-Mouse IgG H&L	AlexaFluor 568	Polyclonal	IgG-Donkey	Abcam	ab175472
	Anti-Rabbit IgG H&L	AlexaFluor 488	Polyclonal	IgG-Donkey	Abcam	ab150061
In vitro	**Name**	**Fluorochrome**	**Clone**	**Isotype**	**Supplier**	**Reference**
	NKG2A	APC	Z199	IgG2	Beckman	PNA60797
	CD20	FITC	2H7	IgG1	BD	560631
	CD3	V450	SP34-2	IgG1	BD	560770
	CD20	Alexa 700	2H7	IgG1	BD	560631
	CXCR5	FITC	MU5UBEE	IgG1	MABTech	11-9185-42
	Nucleus	DAPI	n.a	n.a	Sigma	D9542-10MG

Table S3. Microscopy antibody list, Related to Figure 1-5. Antibodies used to stain NK cells and B cells by microscopy in *in situ* and *in vitro* conditions (n.a: not applicable).

Monkey (NK cells):	combined with	Supernatant from monkey:
AGM14	->	AGM15
AGM14	->	AGM18
AGM17	->	AGM14
AGM17	->	AGM15
AGM17	->	AGM18
AGM18	->	AGM14
AGM18	->	AGM15

Table S5. AGM IDs combination between NK and supernatant condition, Related to Figure 3.
Animals from which supernatants were collected and animals for which fresh NK cells were cultured with the respective supernatants (Figure 3E).

ID	Gender
745	Female
746	Male
771	Female
772	Male
773	Female
774	Female
775	Female
776	Female
777	Female
778	Female
779	Male
780	Female
781	Male

Table S6. Blood healthy donor used for this study, Related to Figure 4. Description of healthy donors according to ID (gender).