The conjunctiva as a site for investigation of human mucosal immunology *in situ* – elucidating the mechanisms of immune escape in adenovirus-induced epidemic keratoconjunctivitis (EKC)

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[4]Duke-NUS Graduate Medical School

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Topics

Inflammation of the ocular mucosal surface caused by adenovirus infection – EKC (epidemic keratoconjunctivitis)

Human NK cell populations and their regulation

Profiling NK cells in the conjunctiva mucosa over the course of EKC

Elucidating the mechanisms of immune subversion by adenoviruses

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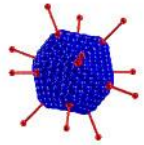
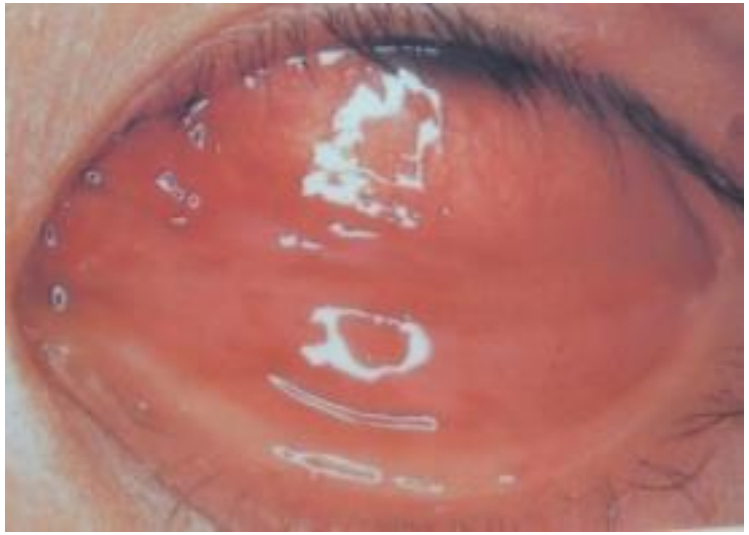
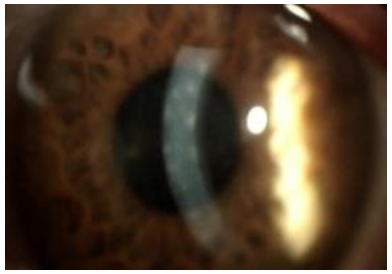
Group D human adenoviruses (HAdV) cause epidemic keratoconjunctivitis (EKC)

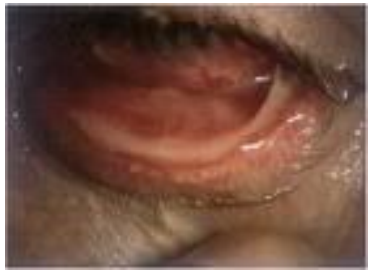




Severe conjunctivitis



Subepithelial keratitis



Pseudomembrane

Human adenovirus types are classified into seven groups

Group	Type	Clinical diseases
A	12, 18, 31	
B	3, 7, 11, 14…	Conjunctivitis Pharyngitis Pneumonia
C	1, 2, 5, 6	Pharyngitis Pneumonia
D	8, 9, 19, 37, 53, 54…	EKC
E	4	Conjunctivitis Pneumonia
F	40, 41	Gastroenteritis
G	52	Gastroenteritis

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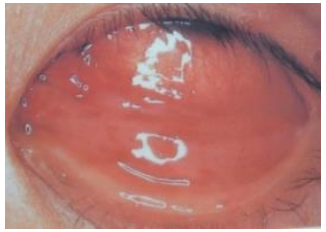
Clinical features of epidemic keratoconjunctivitis (EKC)

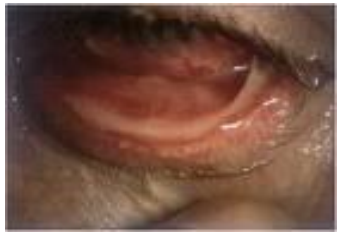
Severe and prolonged inflammation

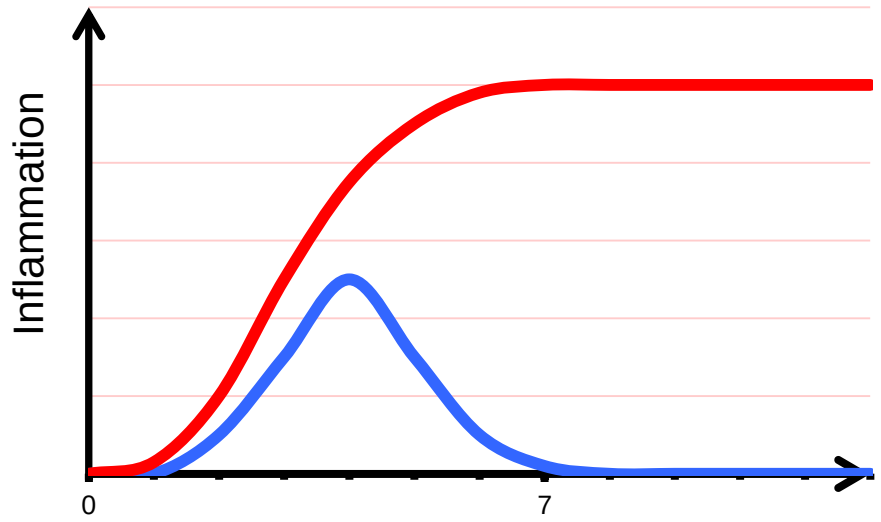
Group D Human adenoviruses (HAdV) cause EKC

Severe conjunctivitis



Pseudomembrane





Mechanisms?

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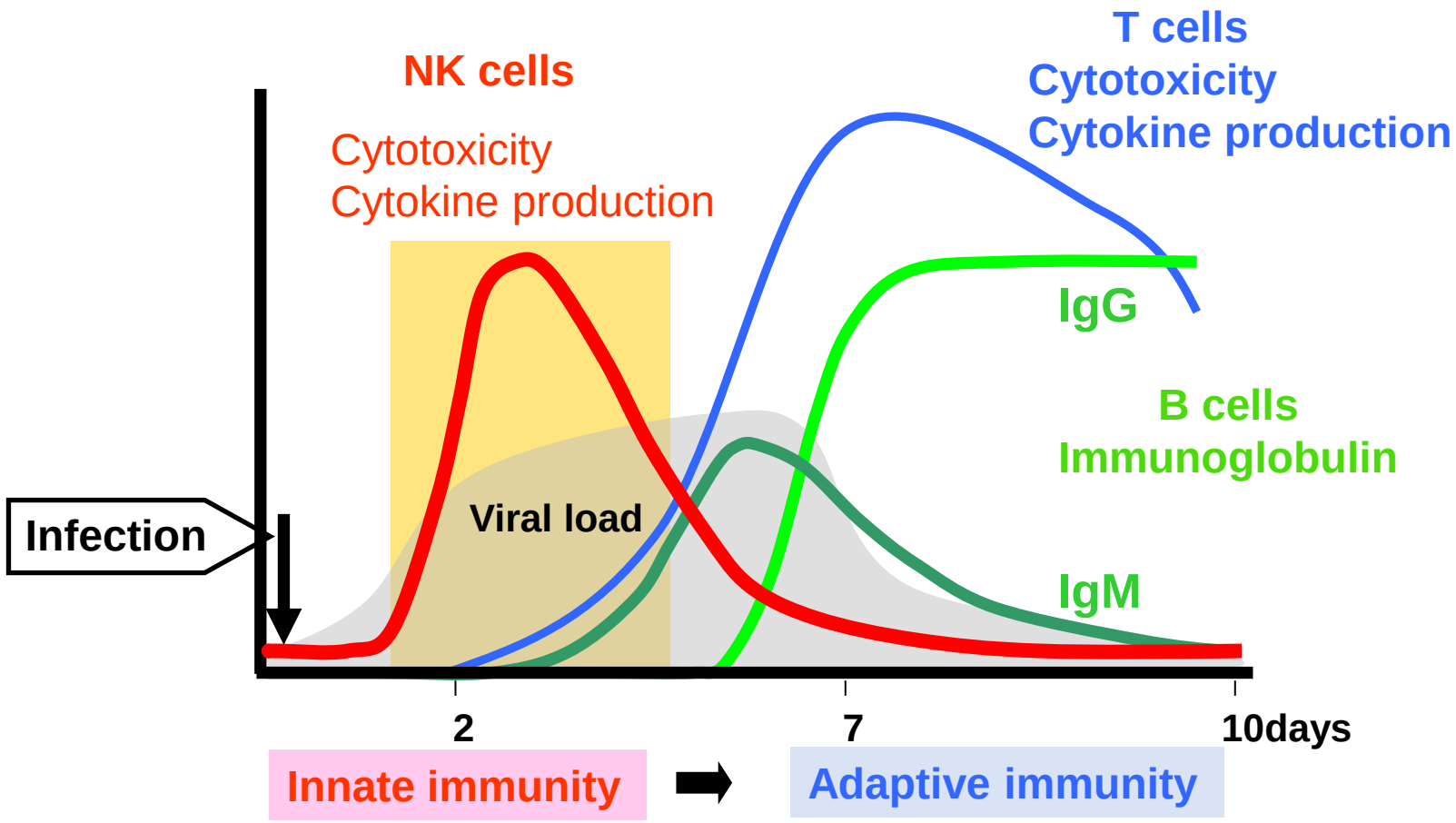
Natural Killer cells provide initial protection against virus infections and prime adaptive immunity



Prime Th1 anti-viral response

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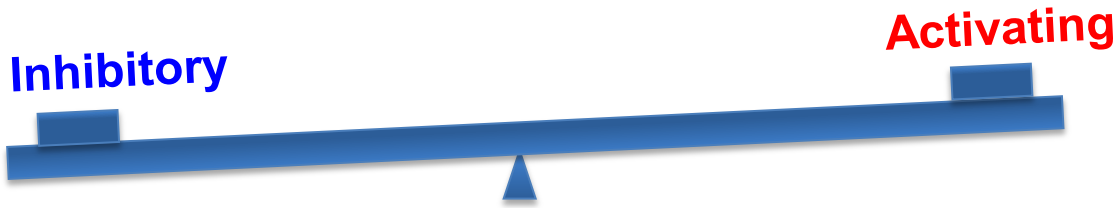
NK cells are controlled through a balance in signaling from inhibitory and activating factors



Cell contact factors	**Killer Cell Immunoglobulin-like Receptors (KIR2DL1/2/3, 3DL1)**	**2DS1, 3DS1**
	NKG2A	**NKG2C**
	LILRB1	**NKG2D**
		DNAM-1
		NKp30/46
		CD16
Soluble factors	**IL-10** **TGF-β**	**IL-12, IL-15, IL-18** **IFN-α** **IL-2**

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HLA class I-specific inhibitory receptors create NK cell heterogeneity

Inhibitory receptors

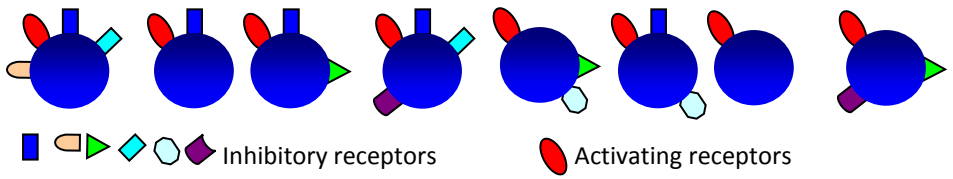
	Receptor	*Ligand*
Variable	KIR2DL1	HLA-C2
	KIR2DL2/3	HLA-C1
	KIR3DL1	HLA-B Bw4
	KIR2DL4	HLA-G
	KIR2DL5A,5B	?
	KIR3DL2	HLA-A3/11
	KIR3DL3	?
Conserved	NKG2A/CD94	HLA-E
	LILRB1 LILRB2	HLA-A,B,C,G

Activating receptors

Receptor	*Ligand*
KIR2DS1,2,3,4,5	HLA-C?
KIR3DS1	?
KIR2DL4	HLA-G
NKG2C	HLA-E
NKG2D	MICA&B/ULBP
CD16	Fcγ
CD160	HLA-C
2B4	CD48
NKp30	BAT3
NKp44	HA
NKp46	HA
DNAM-1	CD112/155

Variegated expression

Homogeneous expression



Yawata et al. Blood 2009

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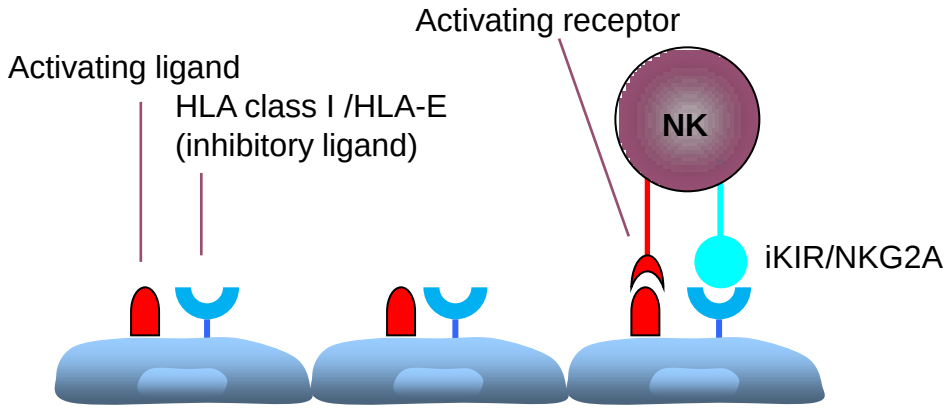
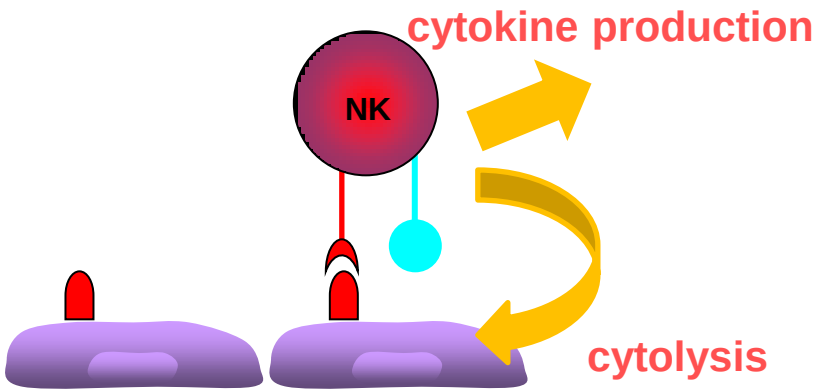
‘Missing-self’ response is unique to NK cells



Normal cells

Reduced inhibition of NK cells through cognate KIR-HLA class I interaction



Abnormal cells

virally infected cells
cancer cells

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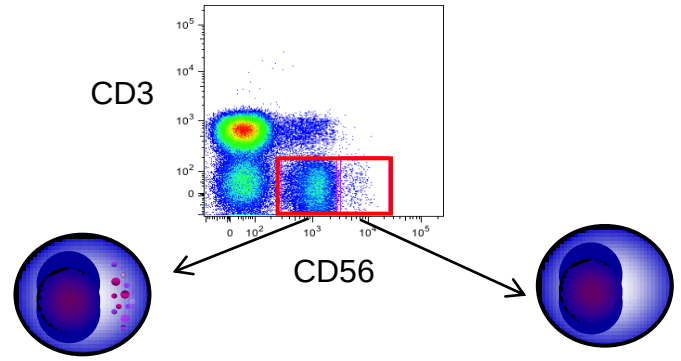
Human natural killer cell subsets have distinct function



	Mature	Immature
Cytotoxicity	+++	+
Cytokine production by monokines	+	+++
Chemokine receptors	**CXCR1, CX3CR1**	**CXCR3, CCR5, CCR7**
HLA specific inhibitory receptors	**KIR, NKG2A**	**NKG2A**
ADCC	**$CD16^{++}$**	**$CD16^{-}$**

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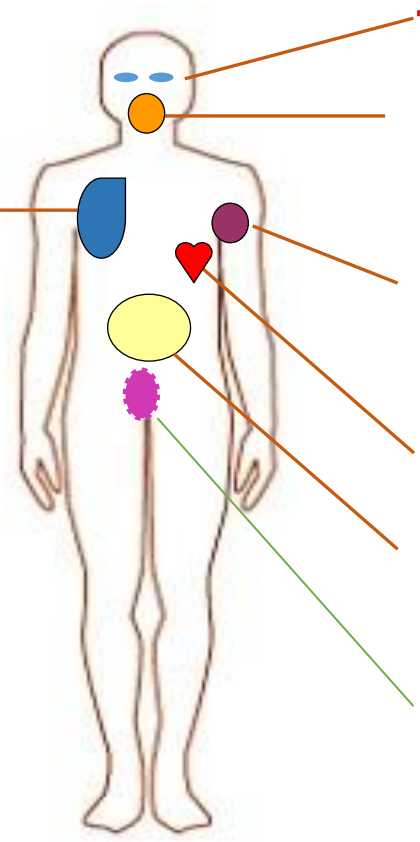
NK cell populations in human organs



The eye: ?

Tonsil: CD56^{dim} < CD56^{bright}
IL-22 & IFN-g

Lung: CD56^{dim}

Lymph node: CD56^{dim} < CD56^{bright}

PBMC/spleen: CD56^{dim} > CD56^{bright}
IFN-g

Intestine: CD56^{bright}
IL-22

Decidua: CD56^{bright}
Angiogenic cytokines

Lower reproductive tract: CD56^{dim}

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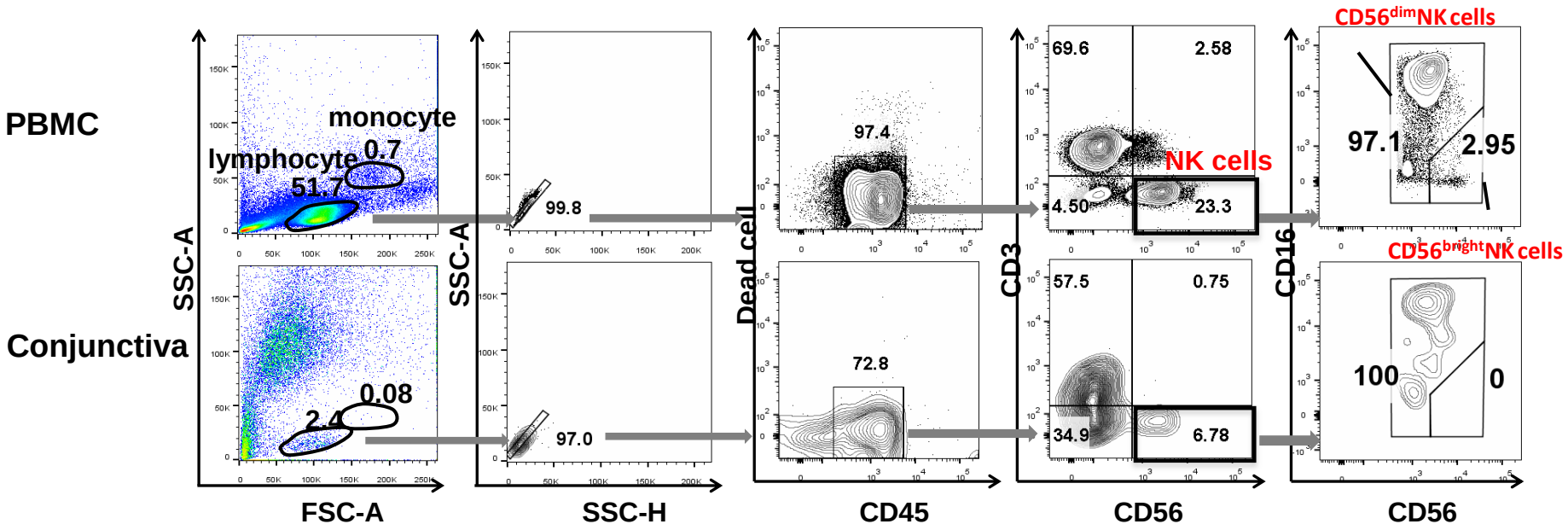
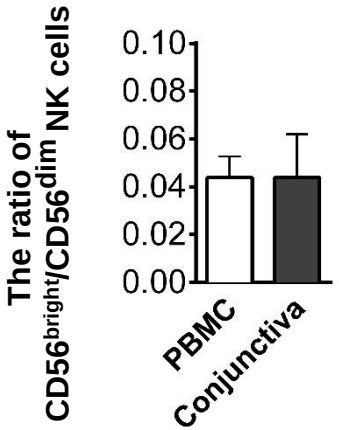
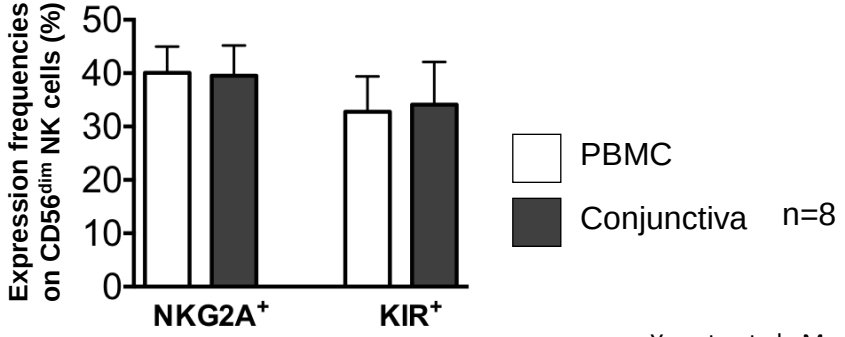
Mature CD56^{dim} NK cells are the dominant type in the conjunctiva







Yawata et al., Mucosal Immunology 2015

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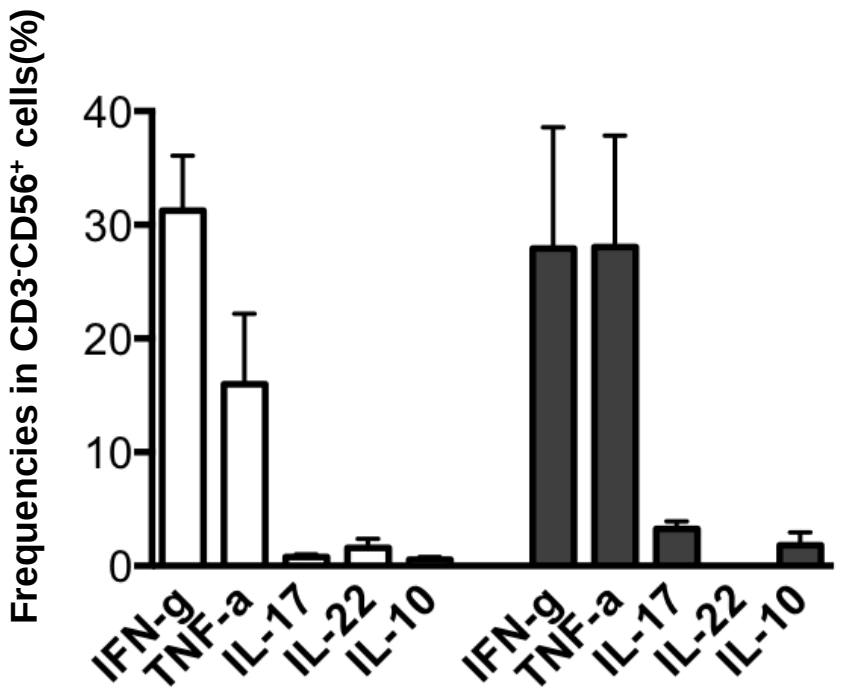
Ocular surface NK cells produce anti-viral cytokines, similar to those in peripheral blood



PBMC

Conjunctiva

n=3

PMA(2ng/ml)
Ionomycin (1ug/ml)
5 hrs

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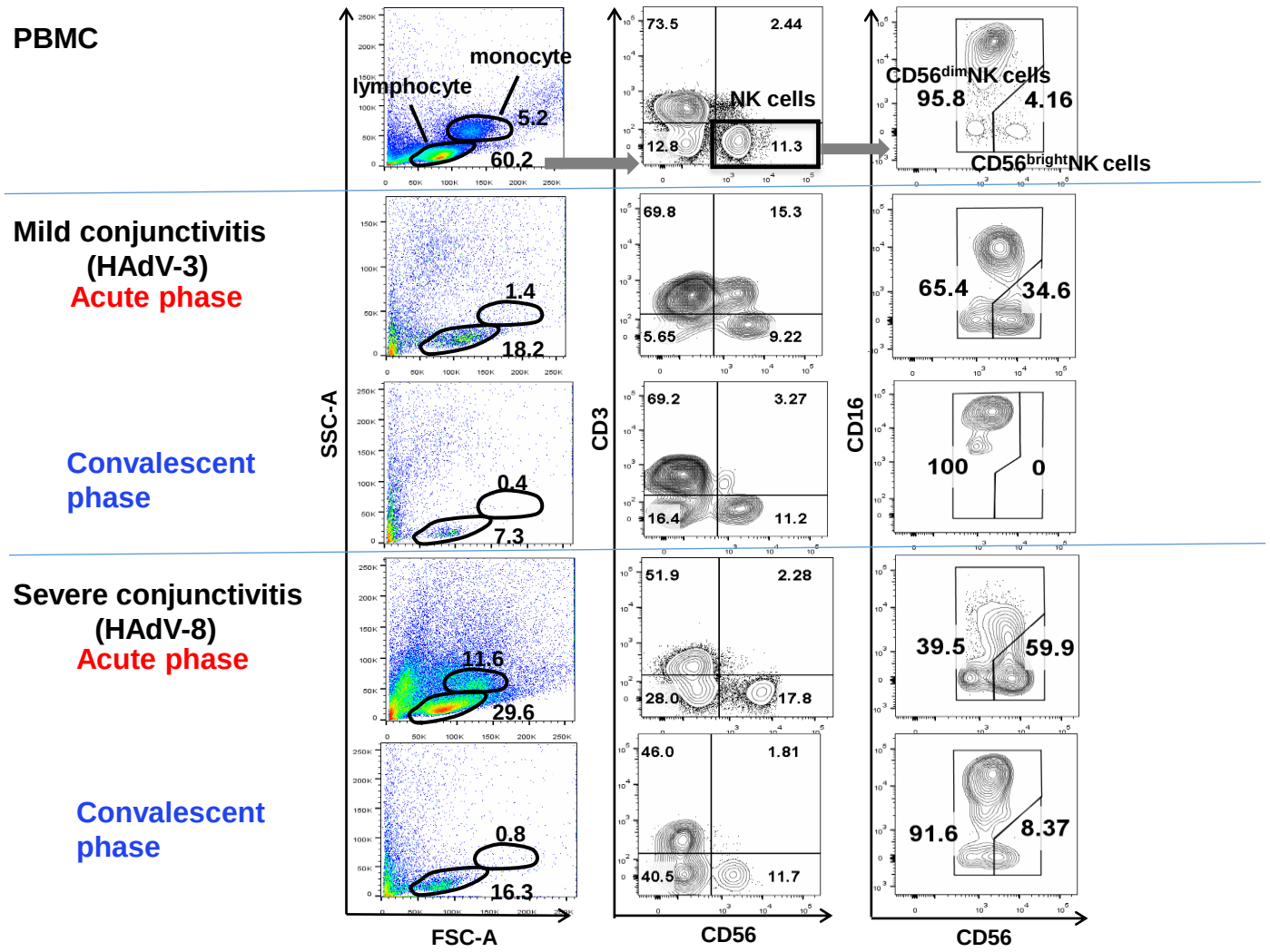
CD56^{bright} NK cells increase in acute adenovirus-induced conjunctivitis



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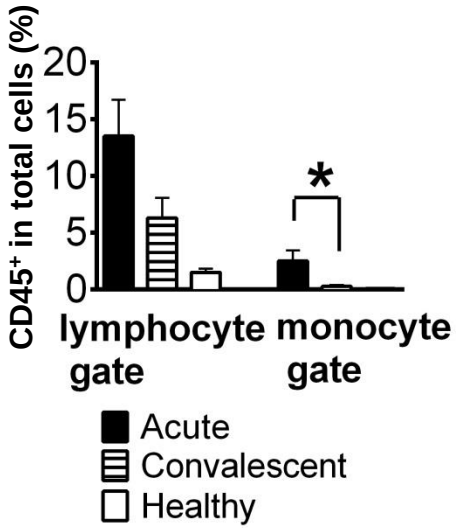
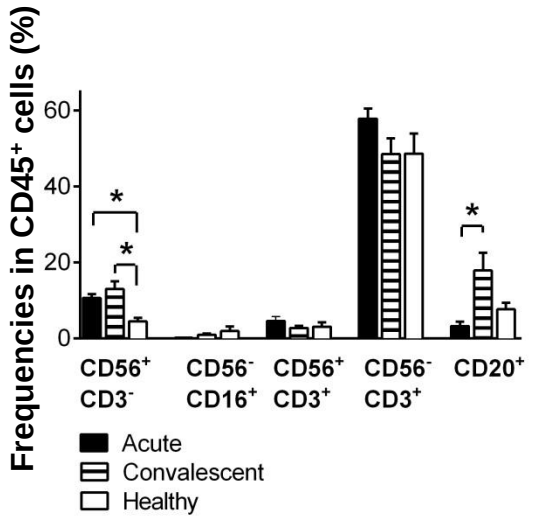
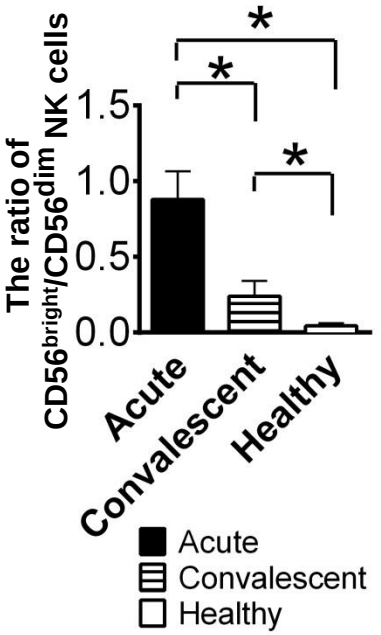
CD56^{bright} NK cells increase in adenovirus-induced conjunctivitis







n=12

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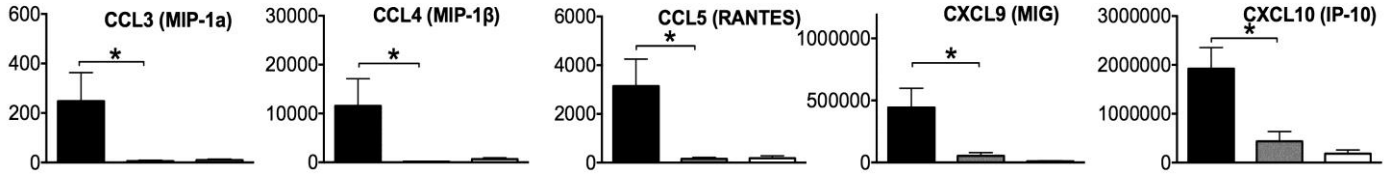
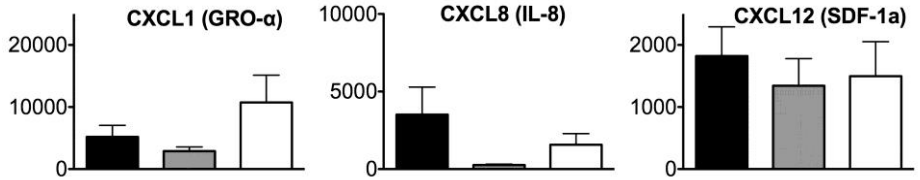
Elevated levels of chemokines that recruit CD56^{bright} NK cells are identified in the tear fluid in the acute phase of adenovirus-infected conjunctiva

Chemokines attracting CD56^{bright} NK cells (CXCR3, CCR5)



Chemokines attracting CD56^{dim} NK cells (CXCR1, CXCR2, CXCR4)



Acute phase

Convalescent phase

Healthy control

n=13 Multiplex beads assay

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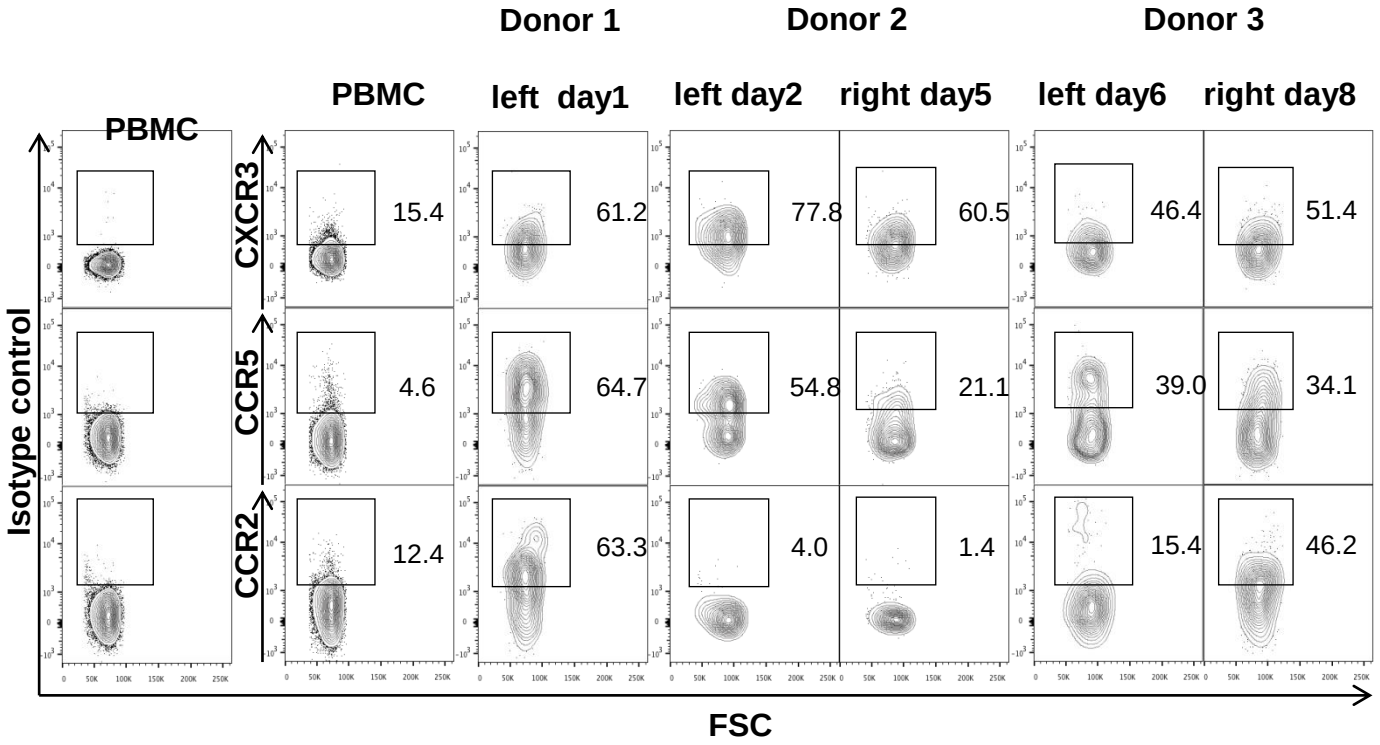
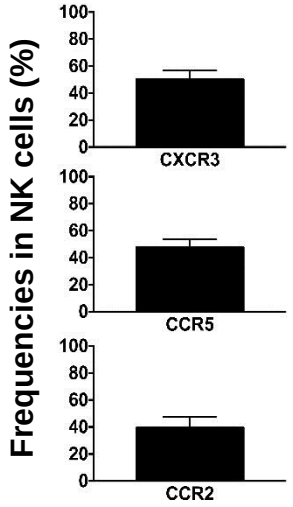
The CD56^{bright}, immature NK cells express the receptors CXCR3 and CCR5 in the acute phase of viral inflammation





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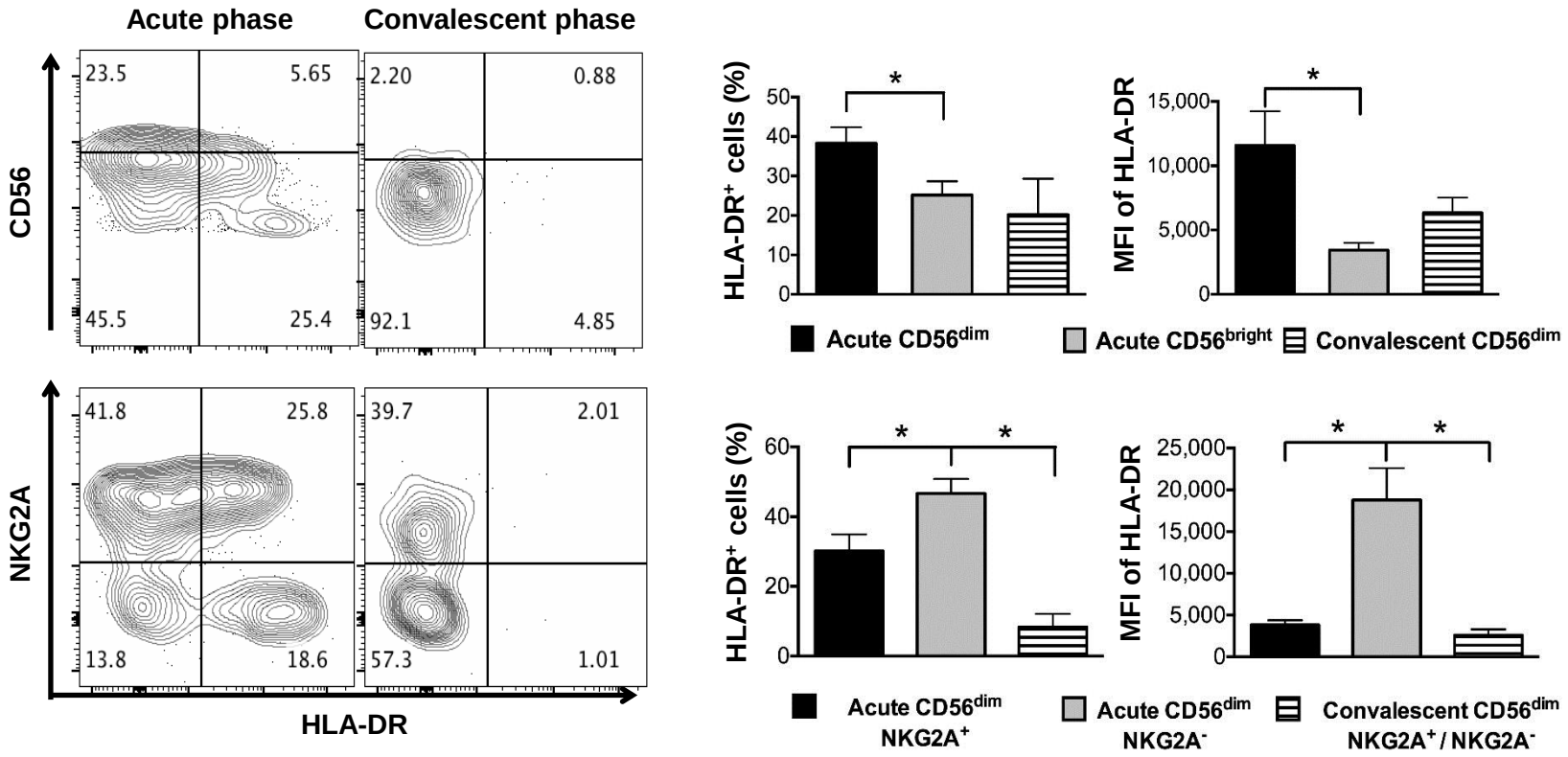
The conjunctiva NK cells recruited to the conjunctiva in adenovirus infection (CD56^{bright} NK cells and NKG2A^{+}NK cells) display are functionally suppressed



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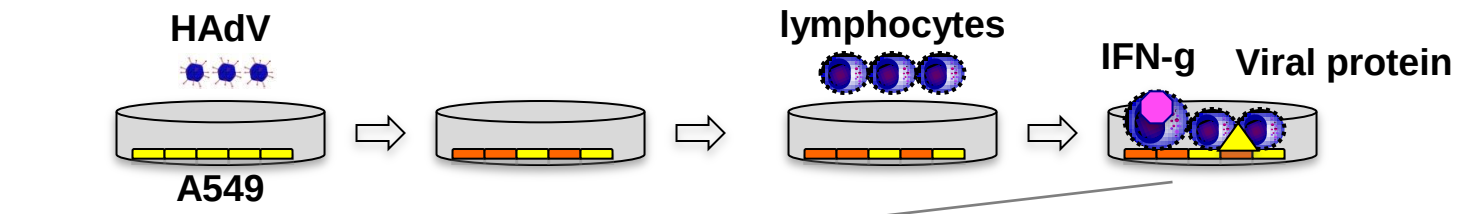
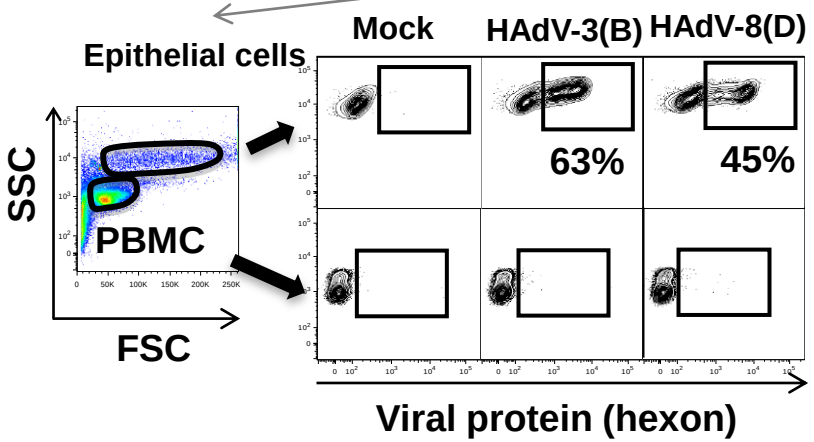
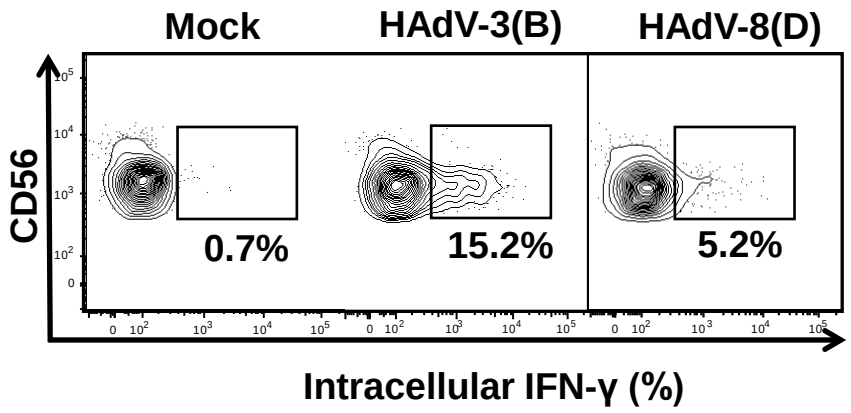
Summary from *ex vivo* study

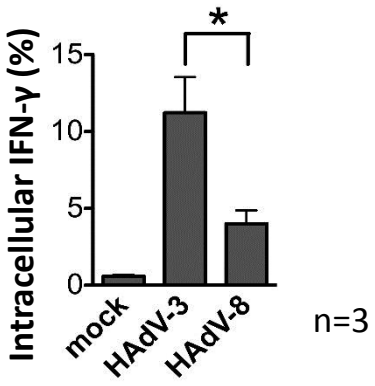
1. Mature NK cells are the dominant NK cell type in the conjunctiva.
2. Immature NK cells are recruited during adenovirus infection.
3. $NKG2A^{+}$NK cells increase in severe conjunctival inflammation.
4. Mature $NKG2A^{-}$NK cells display an activated state.

CD56^{dim} NK cells produce IFN-g against HAdV-infection









n=3

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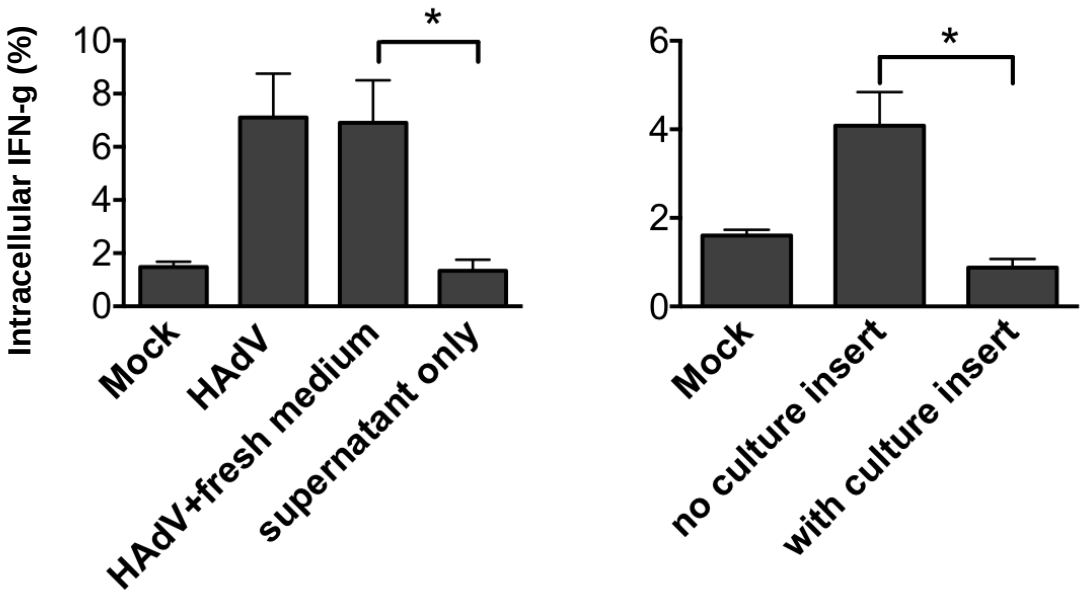
Cell contact-dependent NK cell activation in HAdV infection



Inhibitory receptor–ligand interactions?

Activating receptor–ligand interactions?

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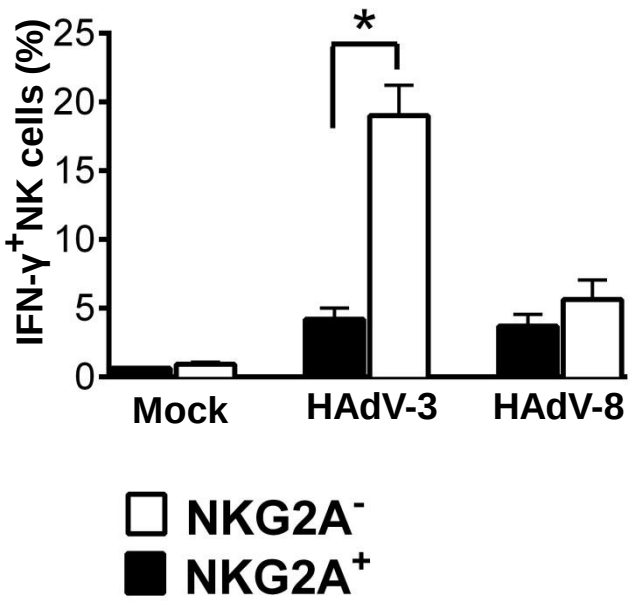
Weaker IFN-g production by NKG2A^{+}NK cells



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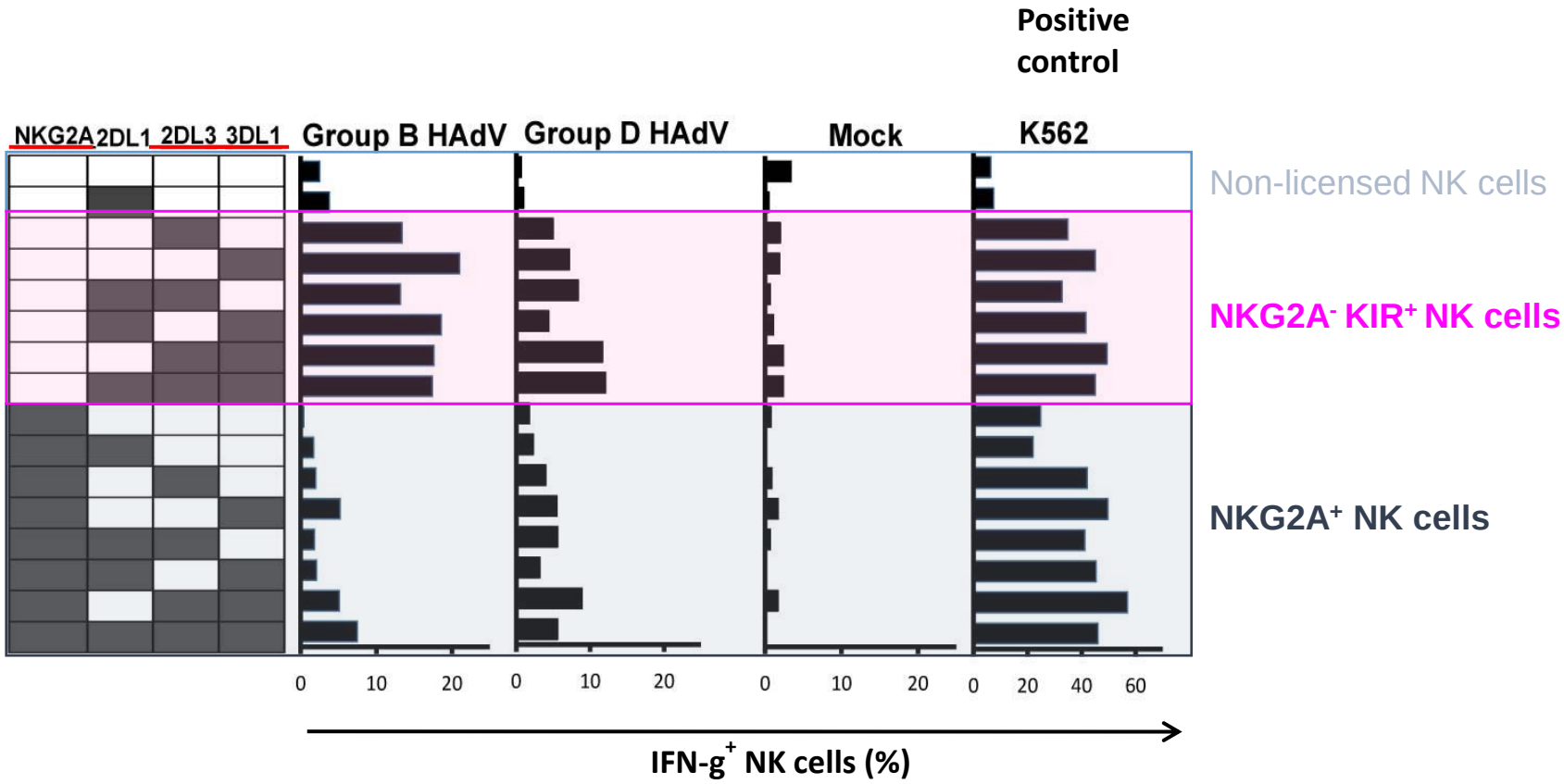
NKG2A$^+$ NK cells showed lower response against HAdV-infected cells



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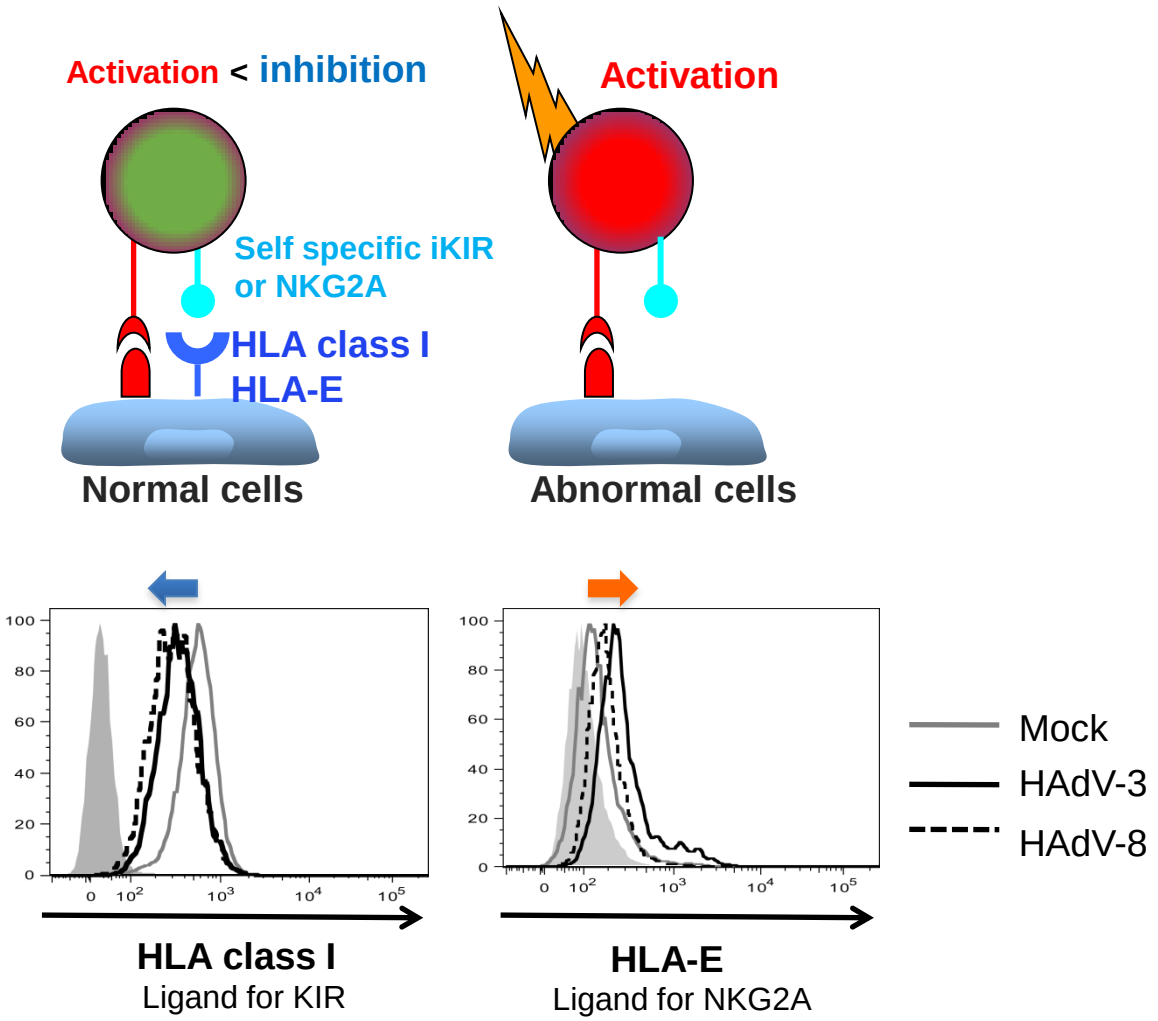

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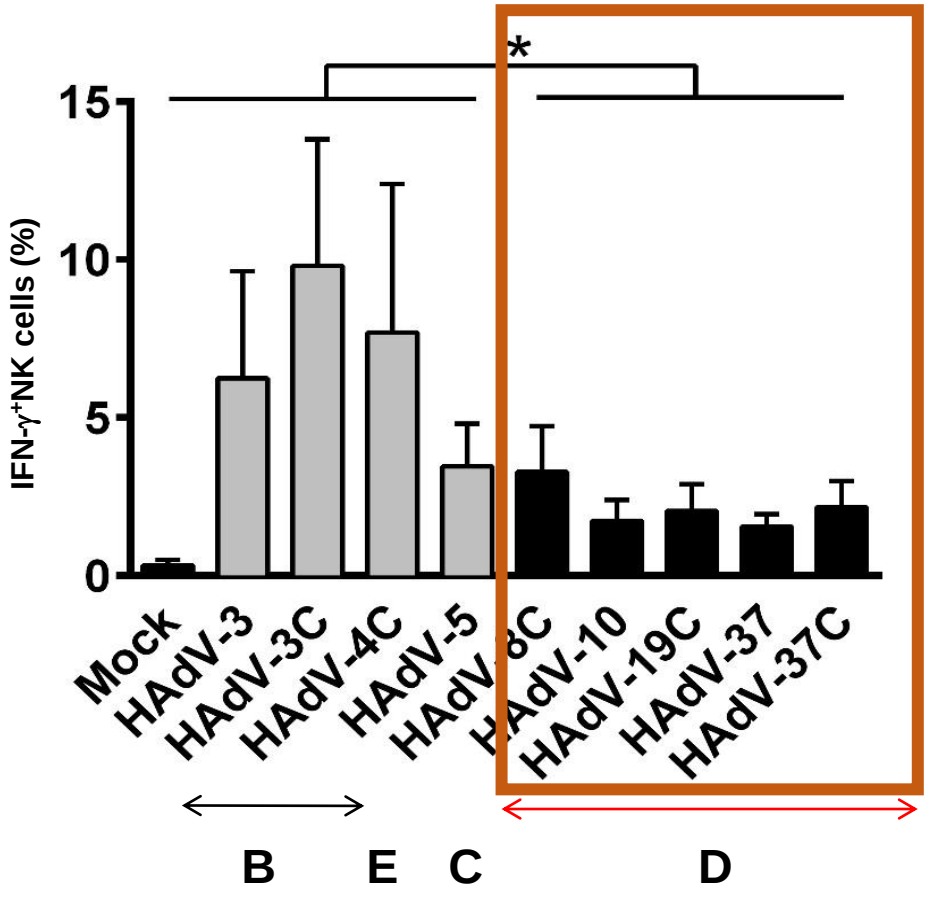
Lower IFN-g response by NK cells against group D HAdVs



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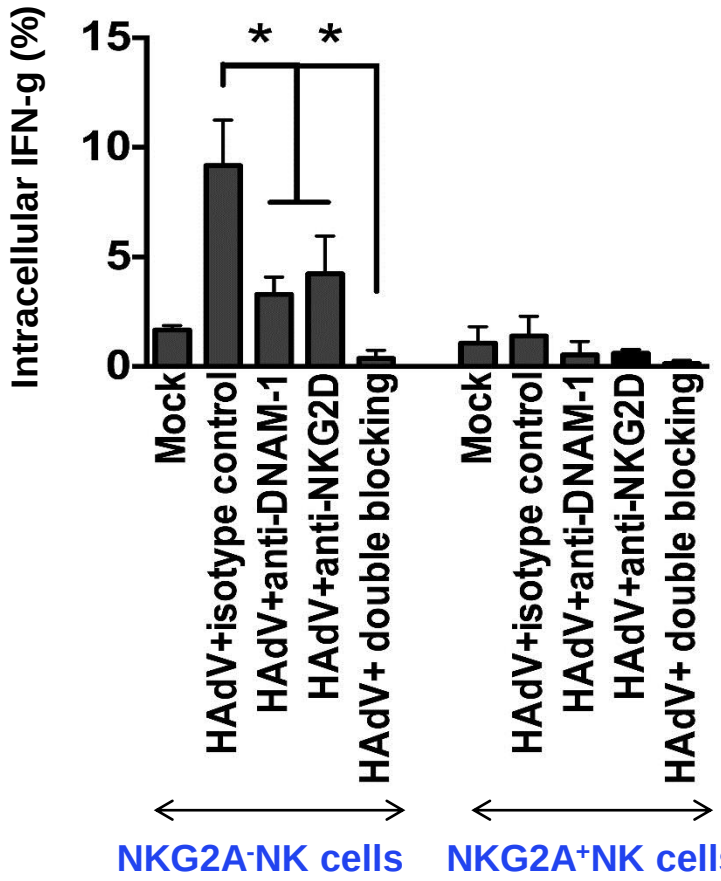
DNAM-1 and NKG2D are key for NK cell activation against HAdV infection



n=3

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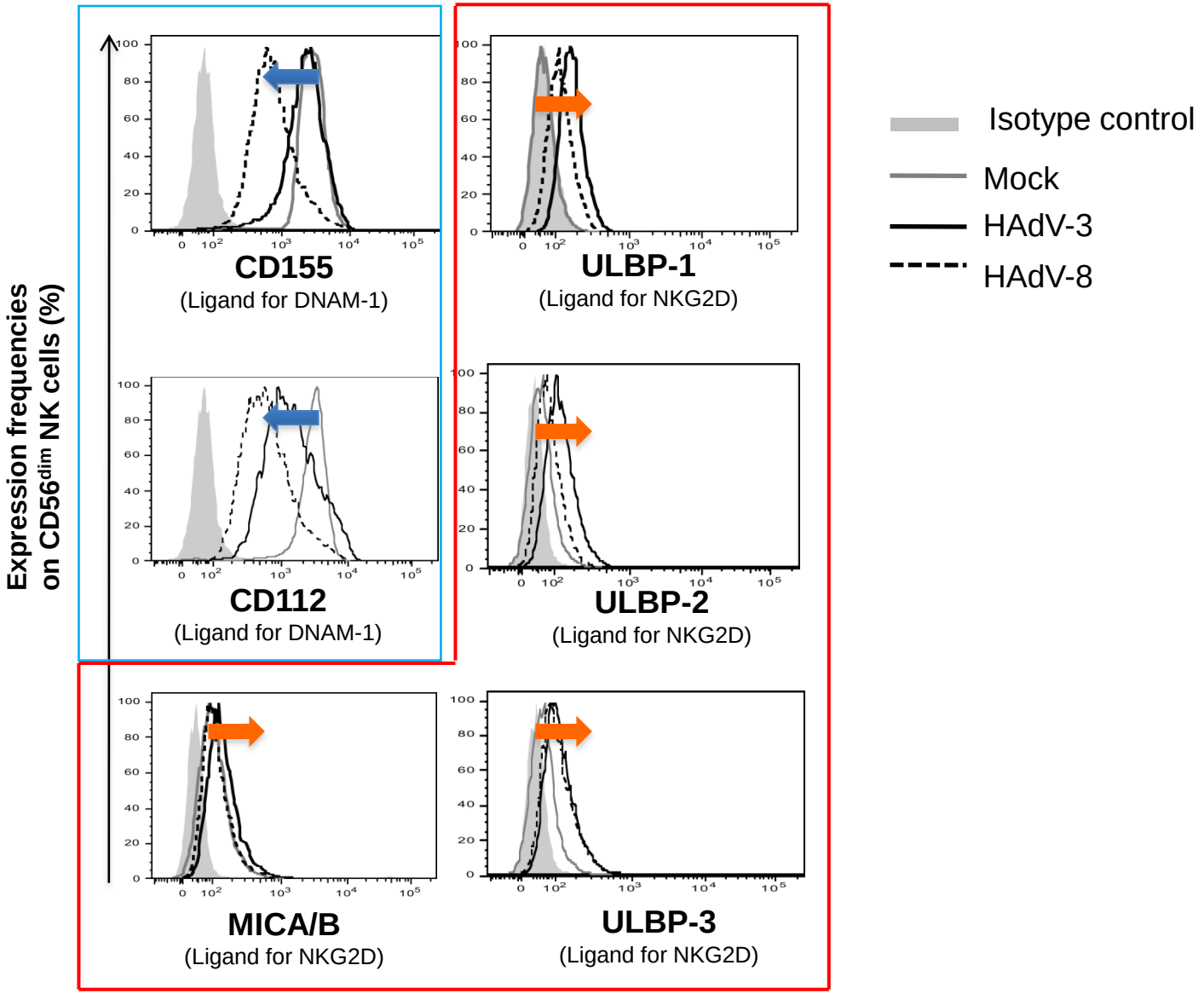
Ligands for DNAM-1 are reduced in HAdV-8 infection



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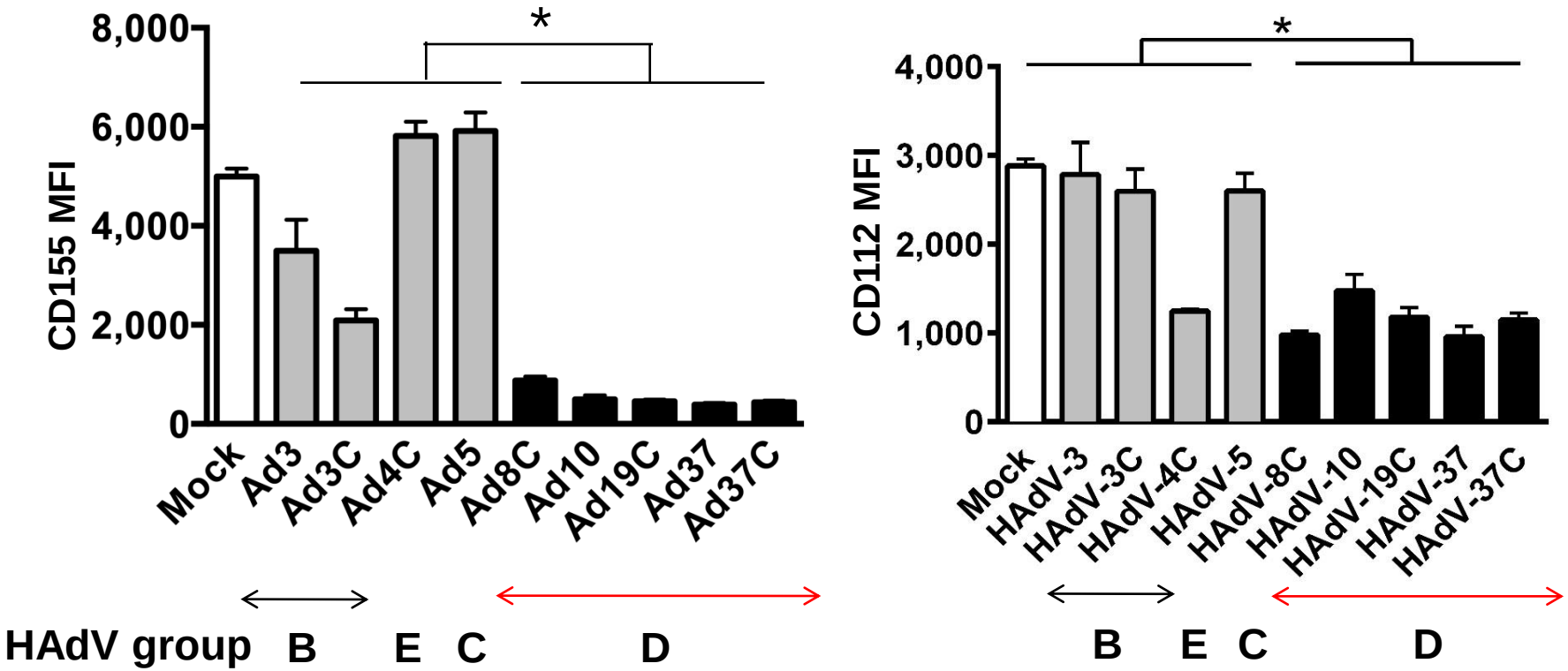
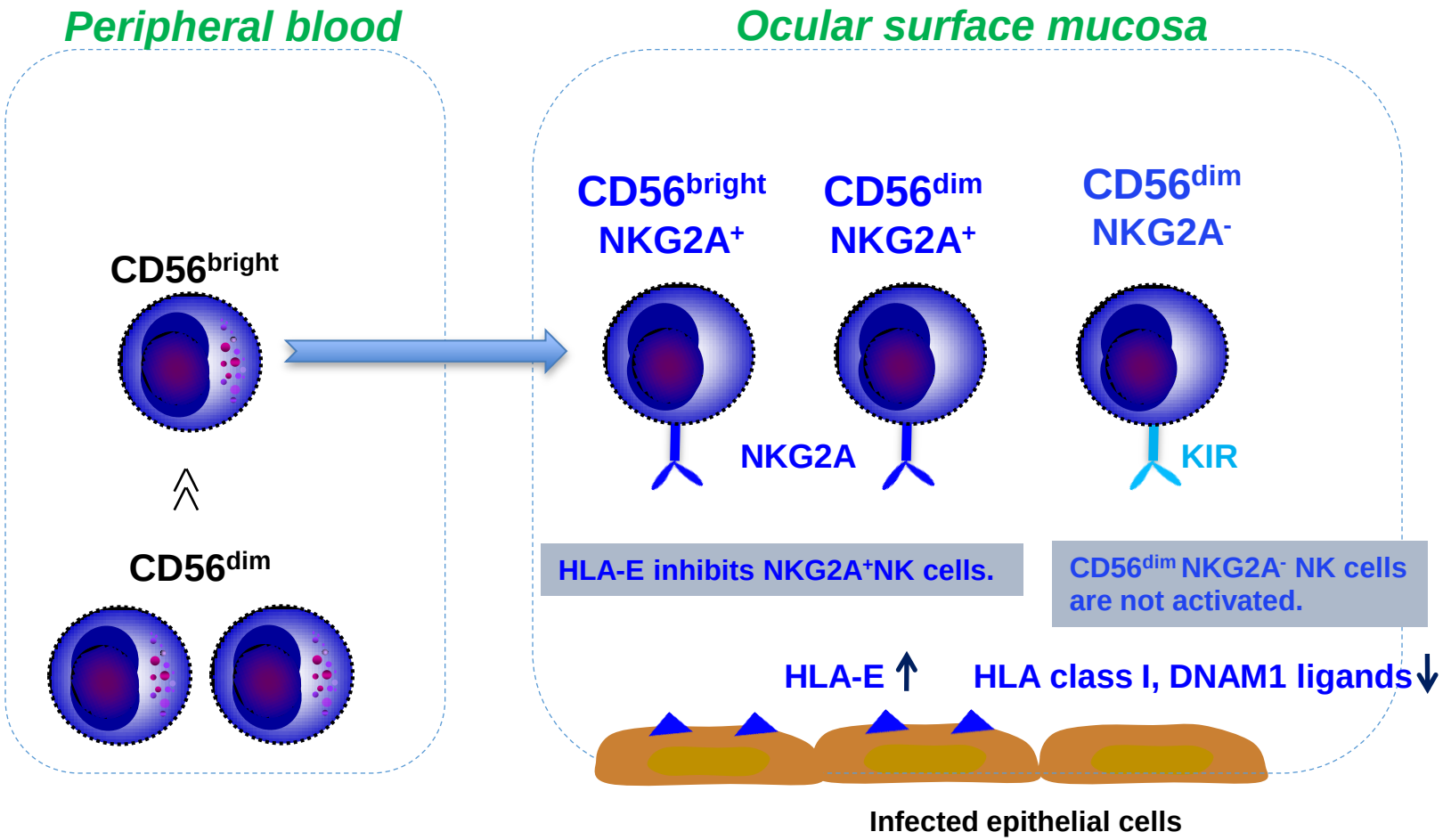
Group D HAdVs down-regulate specific activating ligands on infected epithelia and dampen NK cell responses



Yawata et al., Mucosal Immunology 2015

Summary

- Immature CD56^{bright}NK cells and NKG2A^{+}mature CD56^{dim}NK cells are inhibited by up-regulated inhibitory ligand on HAdV-infected epithelium.
- Group D HAdV escape from mature NK cell anti-viral response by down-regulating activating ligands on infected cells.



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