# Adenovirus-engineered human dendritic cell vaccine induces natural killer cell chemotaxis via CXCL8/IL-8 and CXCL10/IP-10 chemokines

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# Presenter Disclosure Information

Lazar Vujanović, Ph.D.

The following relationships exist related to this presentation:

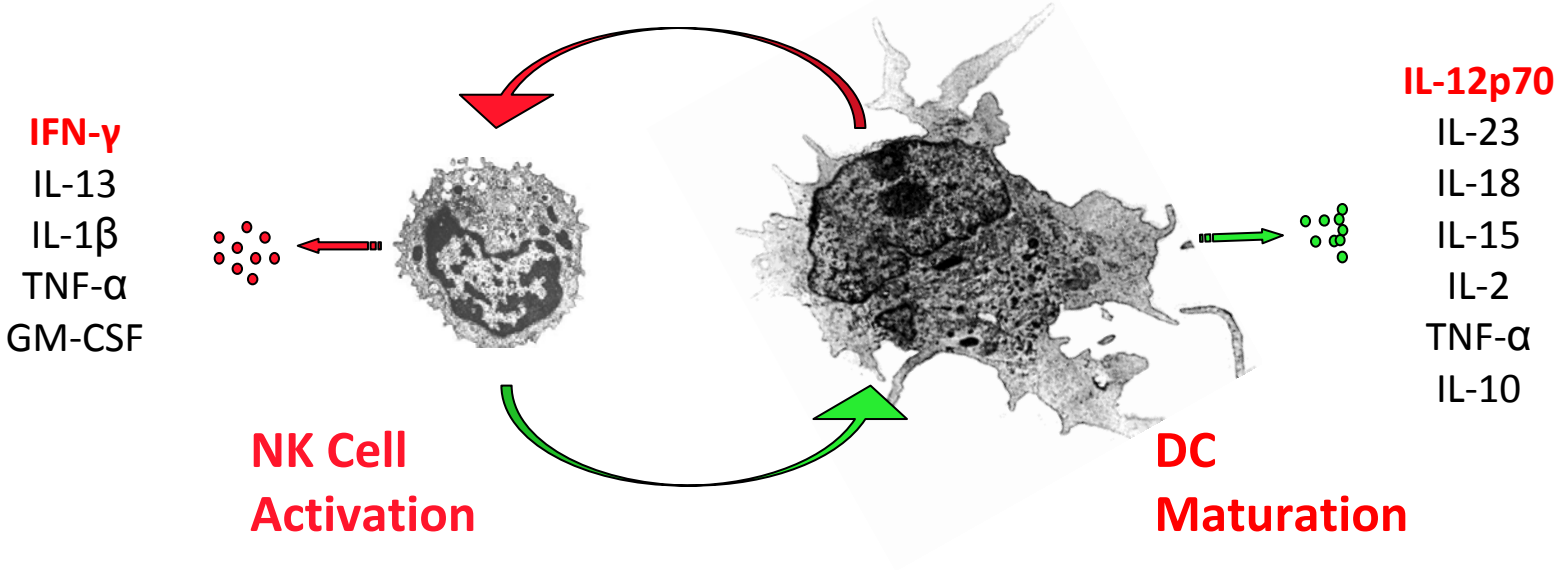
***No Relationships to Disclose***

# Introduction

- Dendritic cells (**DC**) are the most potent antigen presenting cells capable of effective up-take, processing, and presentation of antigenic epitopes

- Natural killer (**NK**) cells are essential effector cells of the innate immunity that play an important role in antitumor and antimicrobial immune defense

- DC and NK cell cross-talk links innate and adaptive immunity, and plays a key role in host immune responses against i[illegible]tious agents and tumors



# Introduction

- Utilize first generation (ΔE1 and ΔE3) recombinant adenoviral vectors (**AdV**) as vehicles for antigen engineering of DC-based tumor vaccines

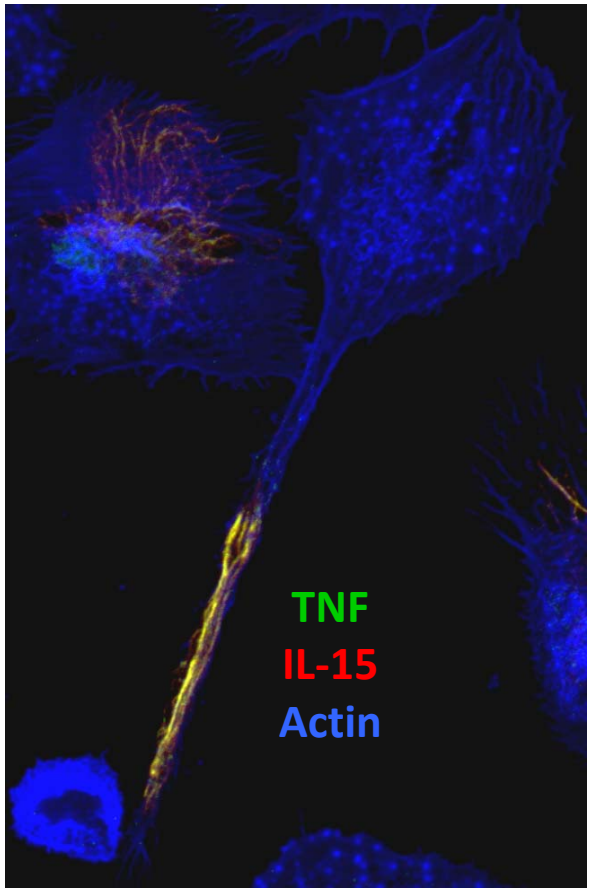
- Monocyte-derived DC can be efficiently transduced with recombinant adenoviral vectors (Ad.DC) and are safe for clinical trials

- AdV infection induces an intermediate level of DC maturation (Vujanovic, L. et al. *Cancer Immunol Immunother*. 2009; 58: 121-133)

- DC transduced with AdV encoding for a tumor antigen stimulate antigen-specific $CD4^+$ and $CD8^+$ T cell responses

# Introduction, Cont.

- Ad.DC effectively activate both $CD56^{lo}CD16^{+}$ and $CD56^{hi}CD16^{-}$ NK cell subsets
- Ad.DC induce NK cell activation as shown by increased expression of activation marker (CD69), proliferation, IFN-γ secretion, tumoricidal activity *in vitro*, and importantly strong antitumor activity *in vivo*
- Ad.DC-induced NK cell activation is mediated by cell-to-cell contact
- Ad.DC and mDC-mediated NK cell activation is mediated by *trans*-presented IL-15 and transmembrane TNF

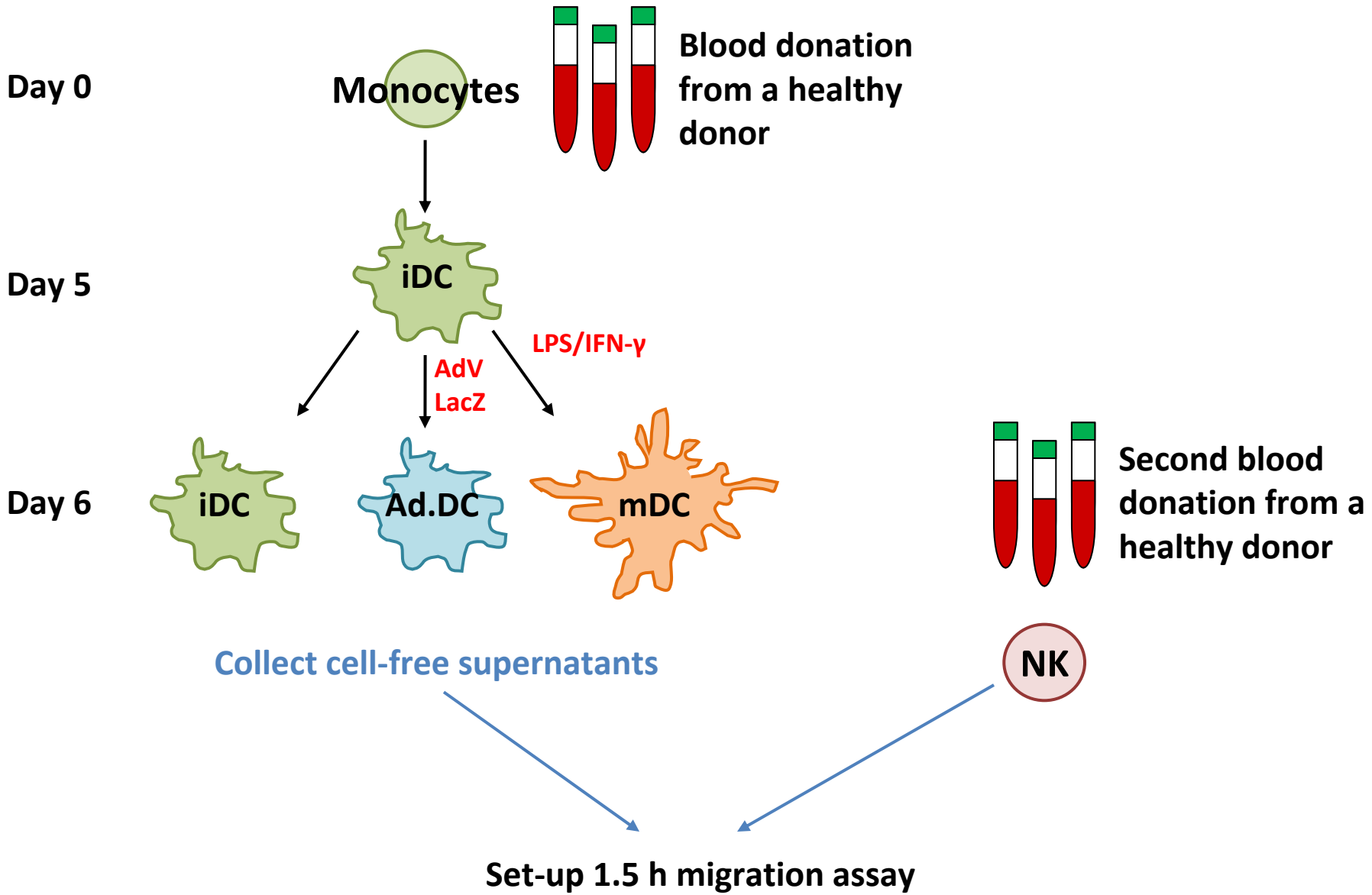



Vujanovic, L., et al. *Blood.* 2010. 116 (4): 575-583.
Butterfield, LH et al. *J Immunother*. 2008. 31 (3): 294-309.
Xu, J et al. *Blood*, 2007, 109 (8): 3333-3341.

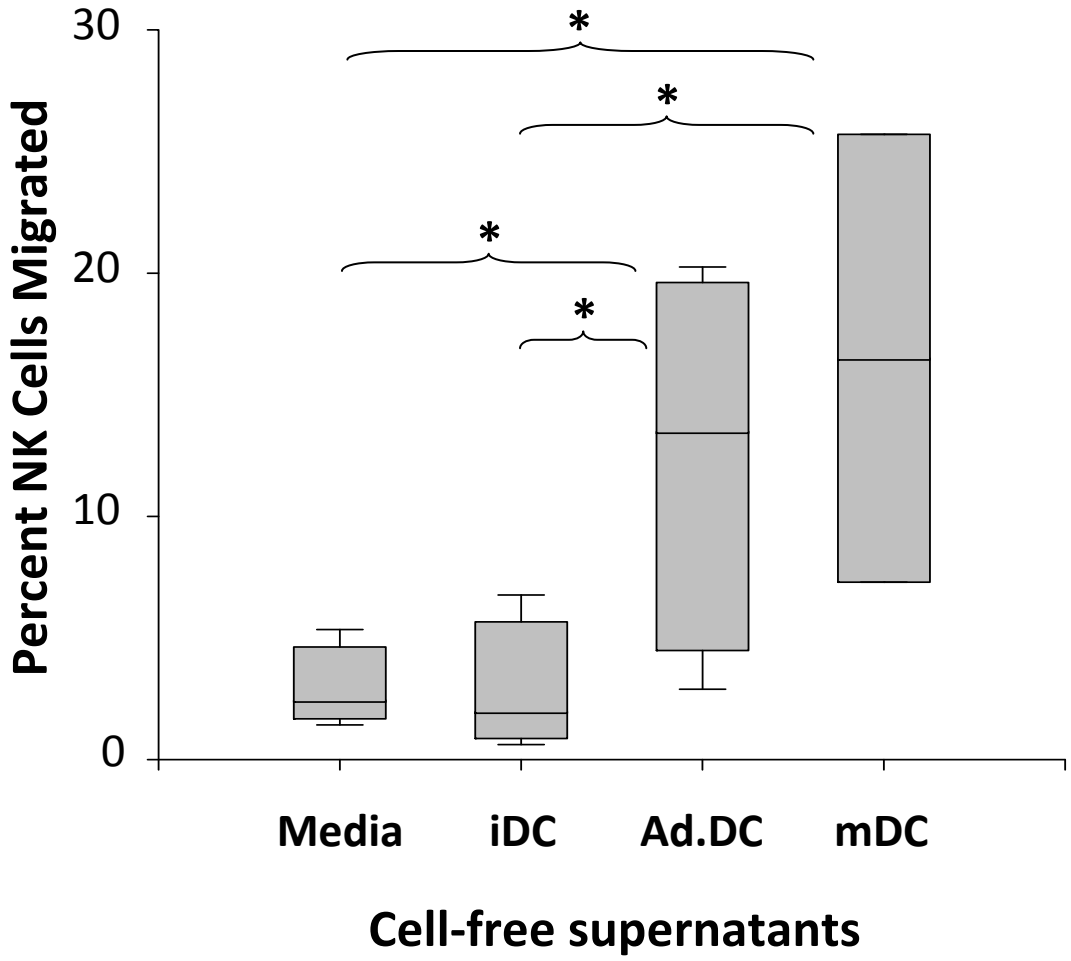
# Major Question: Can Ad.DC recruit NK cells and how?

- Can Ad.DC recruit NK cells in vitro and in vivo?
- Which chemokines Ad.DC produce?
- Which chemokine receptors NK cells express?
- Which chemokines produced by Ad.DC effectively induce NK cell recruitment?

# *In vitro* Experimental set-up



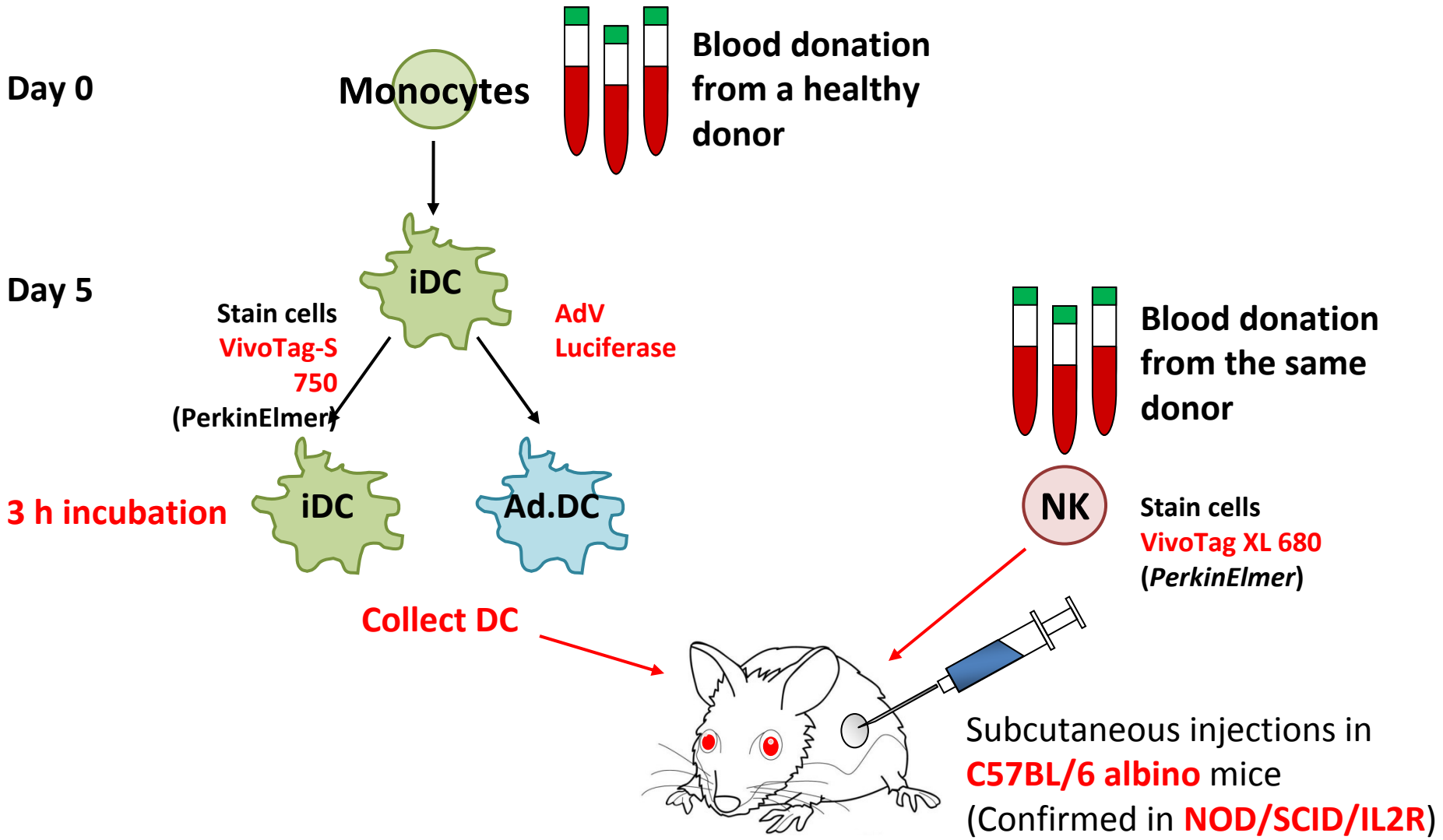
# Ad.DC have the ability to recruit NK cells *in vitro*




n = 4

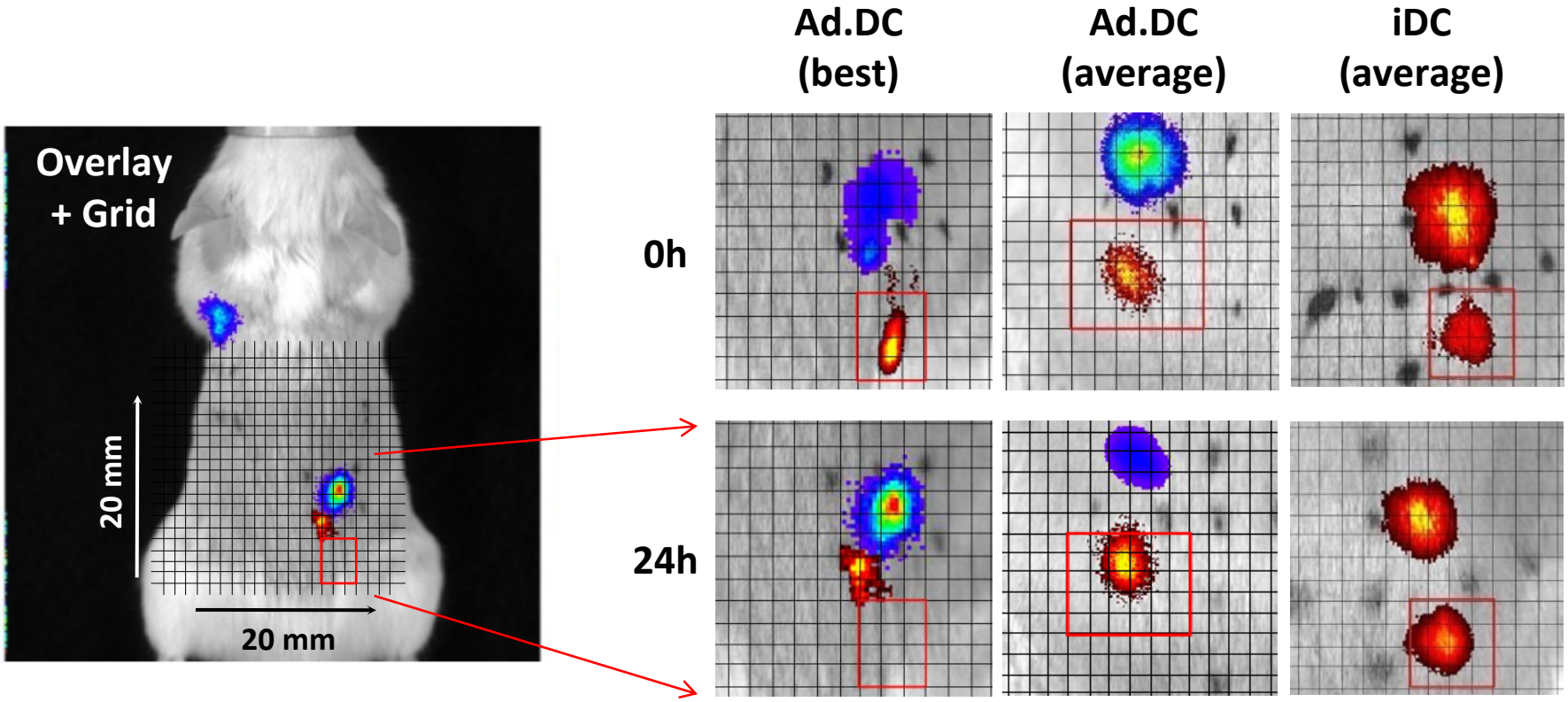
* $p \leq 0.05$

# *In vivo* experimental set-up



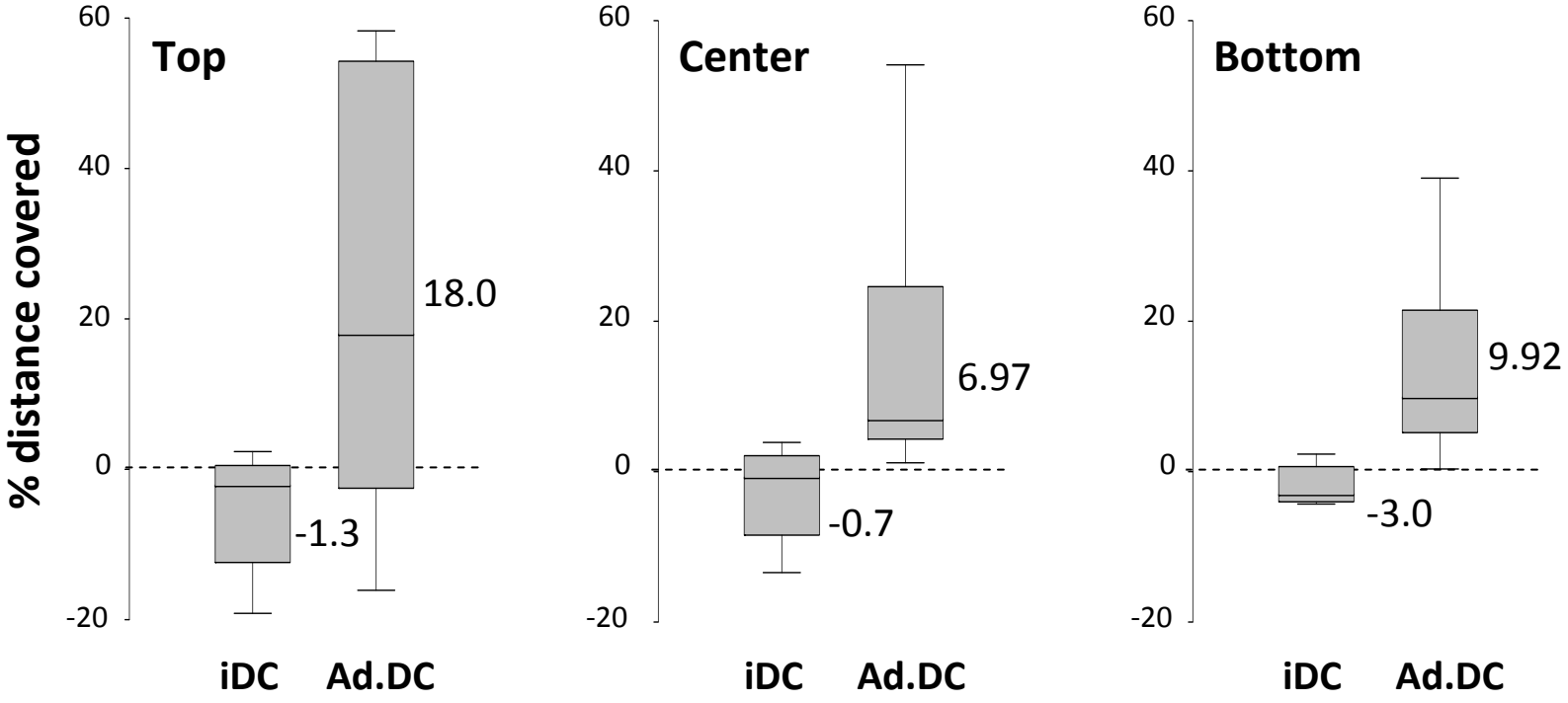

Small animals optical imaging was performed using the **IVIS optical imaging system** at the time of injection (0h) and 24h post-injection

# Enlarged image overlays of the best and average examples of NK cell migration towards Ad.DC and iDC



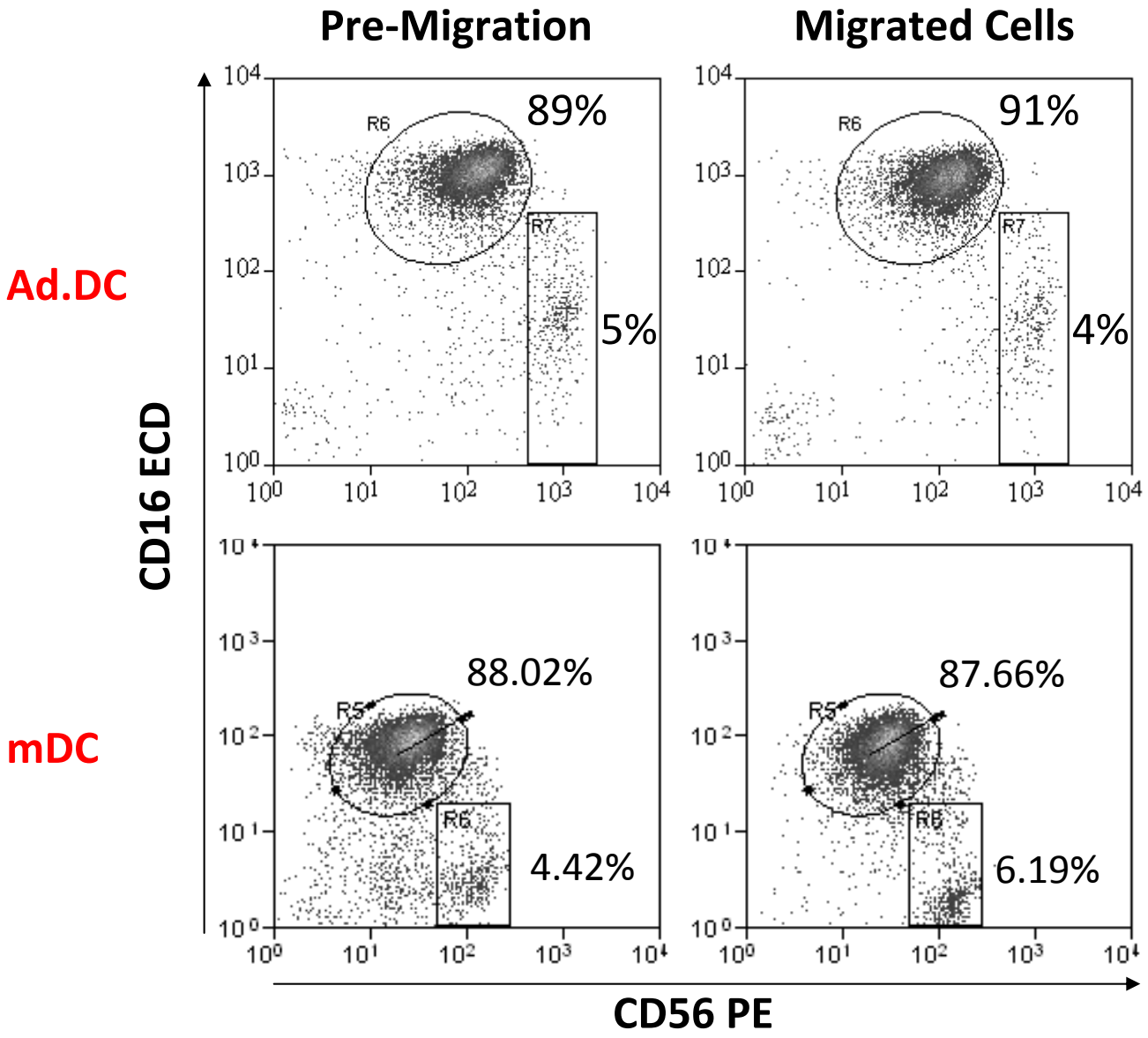

Chemotaxis was quantified by measuring the distance between a DC signal focus to the apex (**Top**), focus (**Center**), and bottom edge (**Bottom**) of an NK cell signal. The data were standardized by calculating the percent change in the determined distance.

# Ad.DC have the ability to recruit NK cells *in vivo*

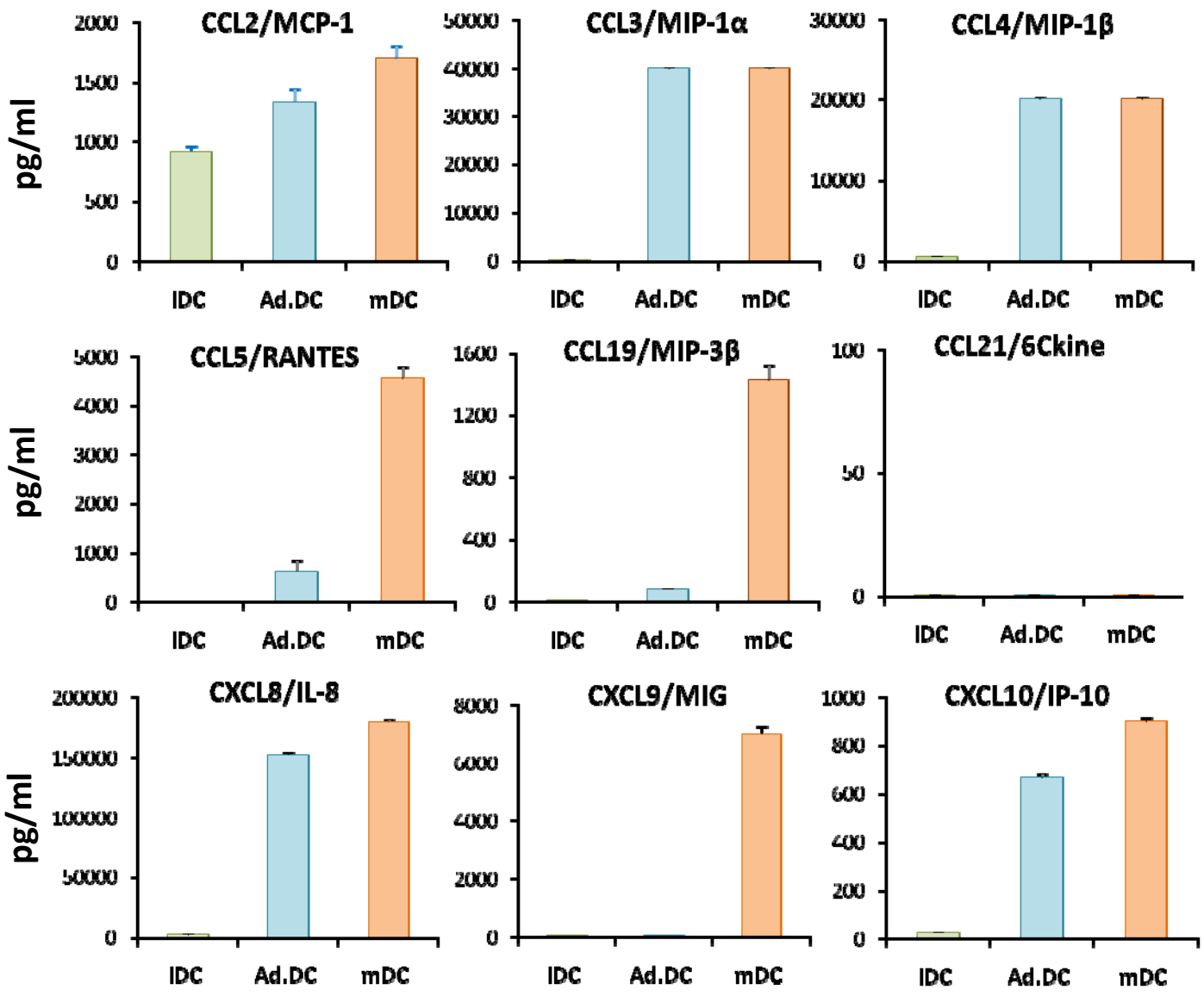



| | |
|---|---|
| **Top** | Apex |
| **Center** | Focus |
| **Bottom** | Bottom edge |

# Ad.DC and mDC induce chemotaxis of both $CD56^{lo}CD16^{+}$ and $CD56^{hi}CD16^{-}$ NK cells



# Ad.DC secrete a number of inflammation-associated chemokines

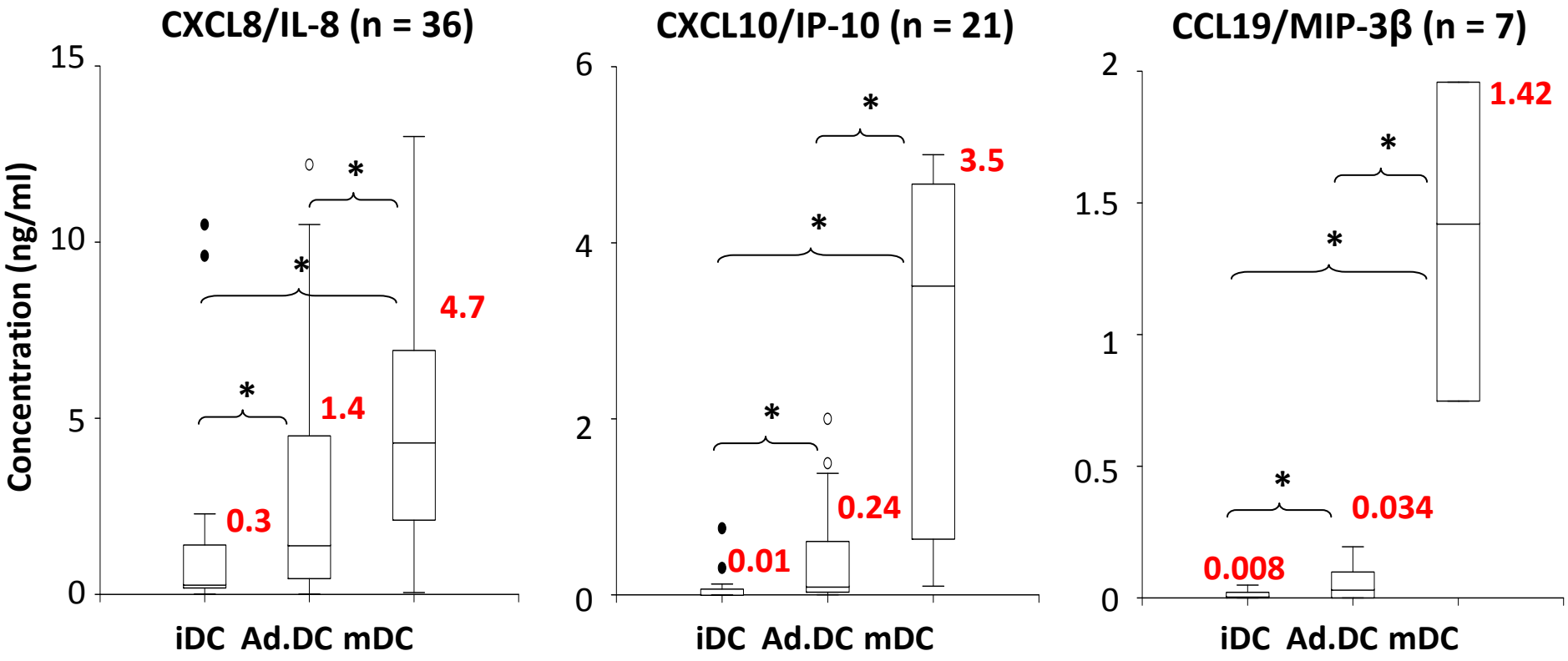


# Chemokine receptors tested on circulating NK cells by FACS

| Ligand | Receptor | $CD56^{lo}CD16^{+}$ | $CD56^{hi}CD16^{-}$ | $CD56^{lo}CD16^{-}$ |
|---|---|---|---|---|
| CCL2/MCP-1 | CCR2 | - | - | + |
| CCL5/RANTES | CCR3 | - | - | ++ |
| CCL4/MIP-1β, CCL2, CCL5 | CCR4 | - | - | + |
| CCL3/MIP-1α, CCL4, CCL5 | CCR5 | - | - | + |
| **CCL19/MIP-3β CCL21/6Ckine** | **CCR7** | **++** | **++** | **+++** |
| **CXCL8/IL-8** | **CXCR1** | **++** | **-** | **++** |
| **CXCL9/MIG, CXCL10/IP-10** | **CXCR3** | **+** | **+++** | **++** |

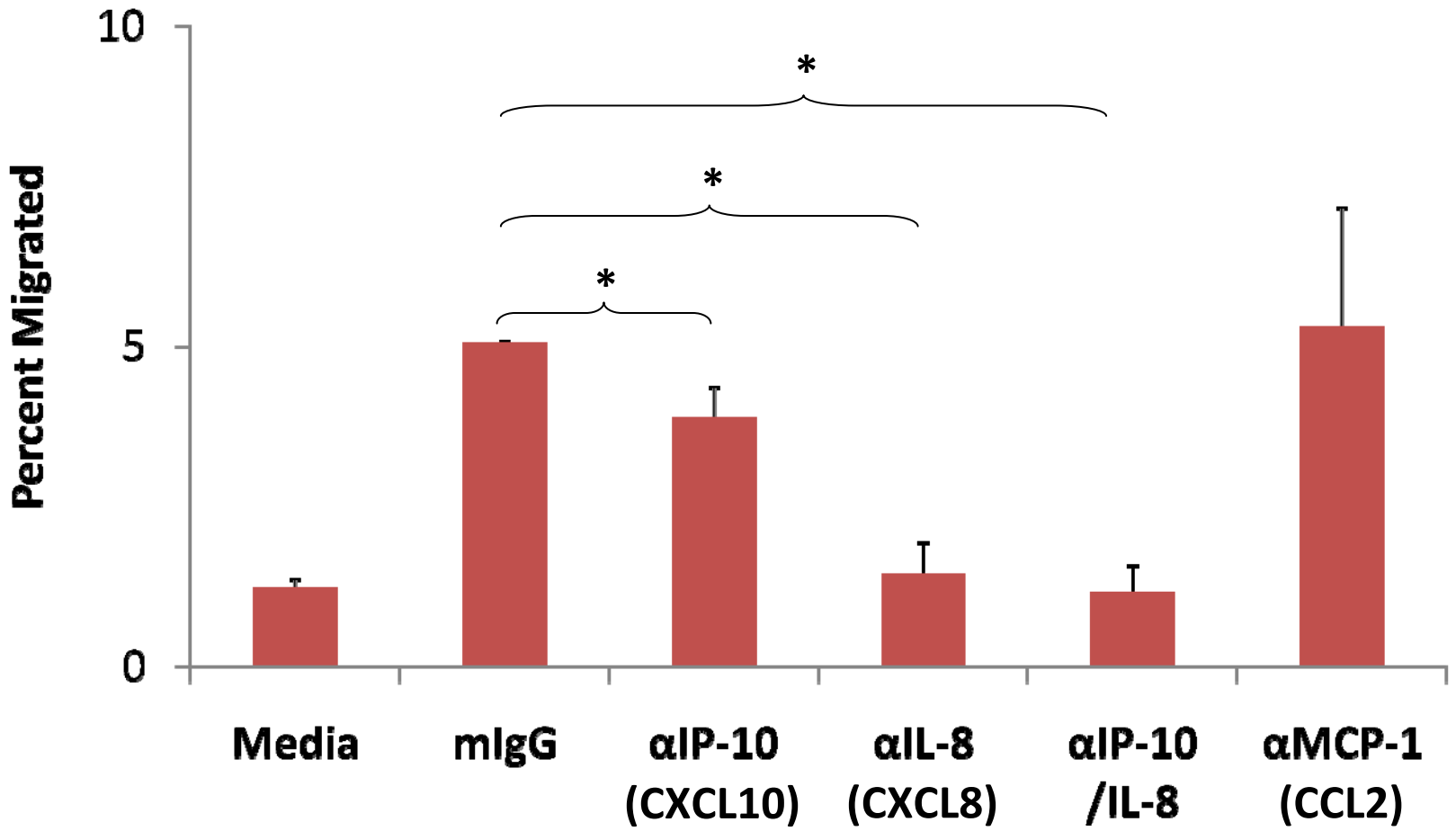
- 0-2%  + 2-30%  ++ 30-50%  +++ >50%

# Ad.DC secrete increased amounts of CXCL8/IL-8, CXCL10/IP-10 and CCL19/MIP-3β



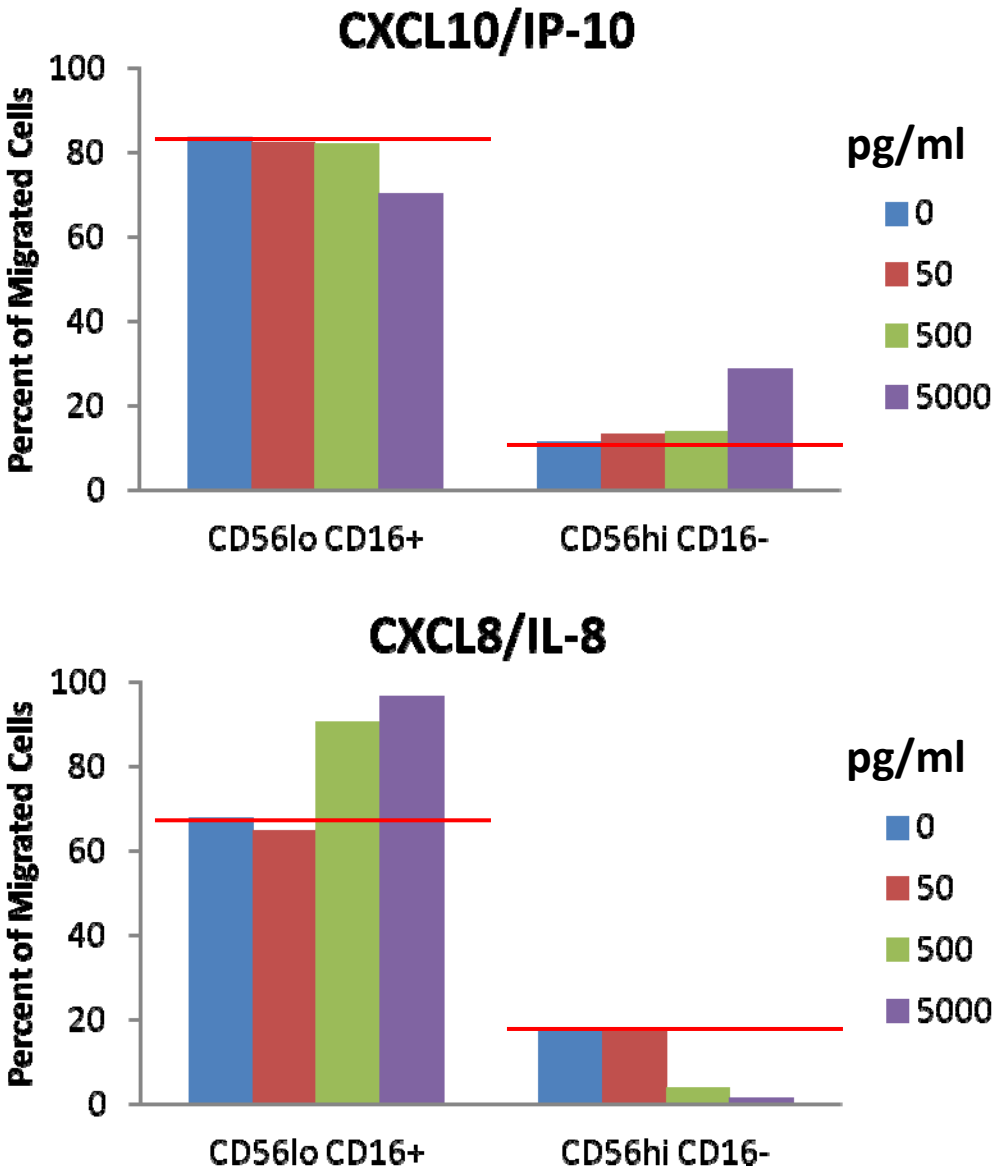

* $p < 0.05$

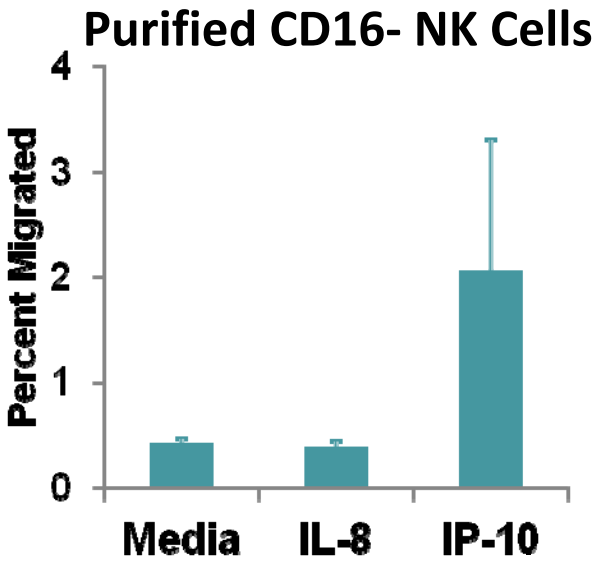
# Ad.DC recruit NK cells via CXCL8/IL-8 and CXCL10/IP-10




* $p < 0.05$

# CXCL8/IL-8 selectively recruits CD56$^{lo}$ while CXCL10/IP-10 recruits CD56$^{hi}$ NK cell subsets



# Conclusions

- Ad.DC effectively recruit NK cells *in vitro* and, more importantly, *in vivo*
- Ad.DC secrete a number of inflammation-associated chemokines
- Ad.DC mediate recruitment of NK cells by CXCL8/IL-8 and CXCL10/IP-10
- CXCL8/IL-8 selectively recruits $CD56^{lo}$ while CXCL10/IP-10 selectively recruits $CD56^{hi}$ NK cell subsets

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