Natural Killer Cell Engagers

innate pharma

Ciml IMMUNOLOGY

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MARSEILLE IMMUNOPÔLE

ANR AGENCE NATIONALE DE LA RECHERCHE

MSD AVENIR améliorer la Vie ENsemble par l'Innovation et la Recherche

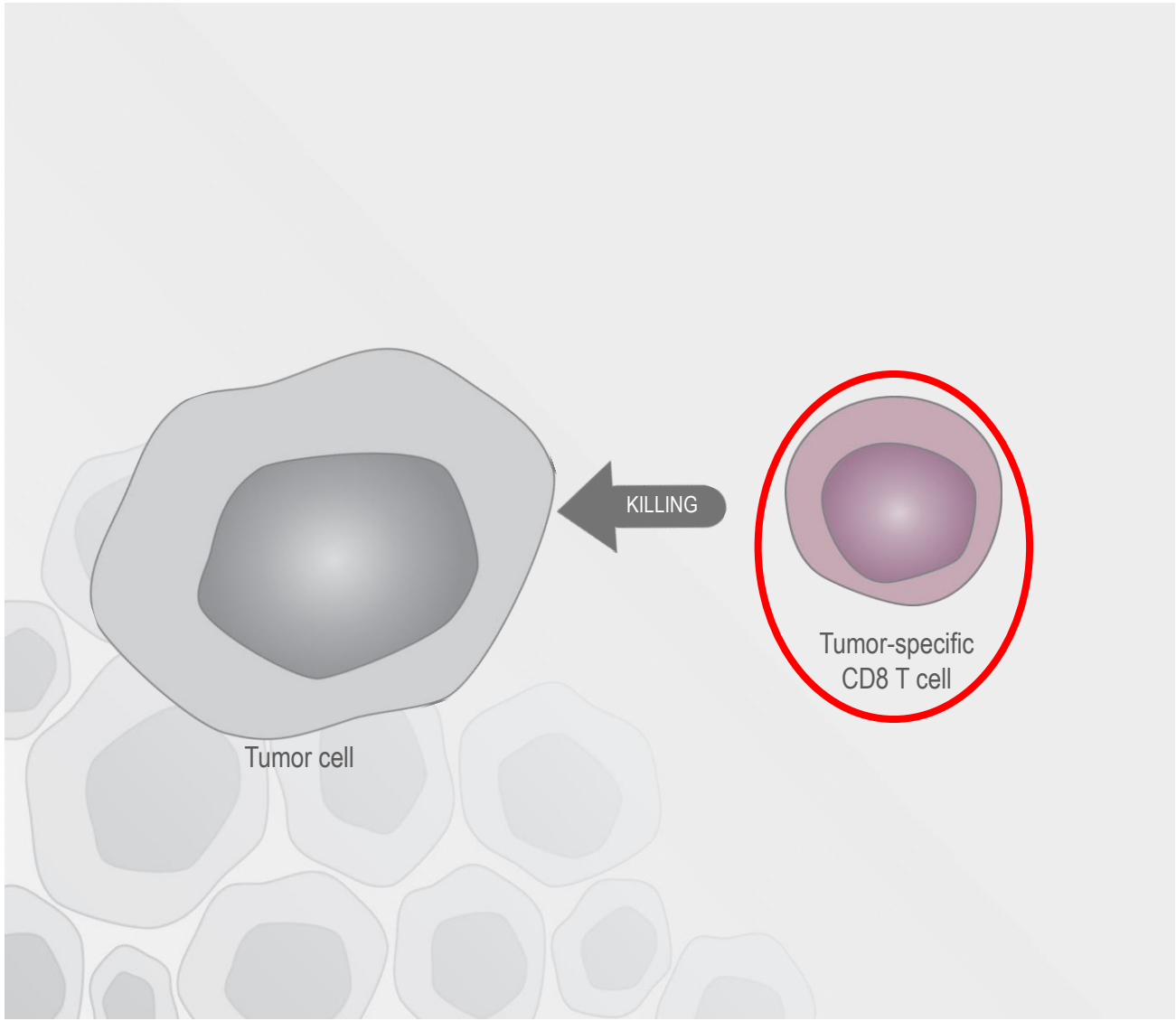
Disclosure

- Innate Pharma
- Co-founder + CSO

Today's presentation

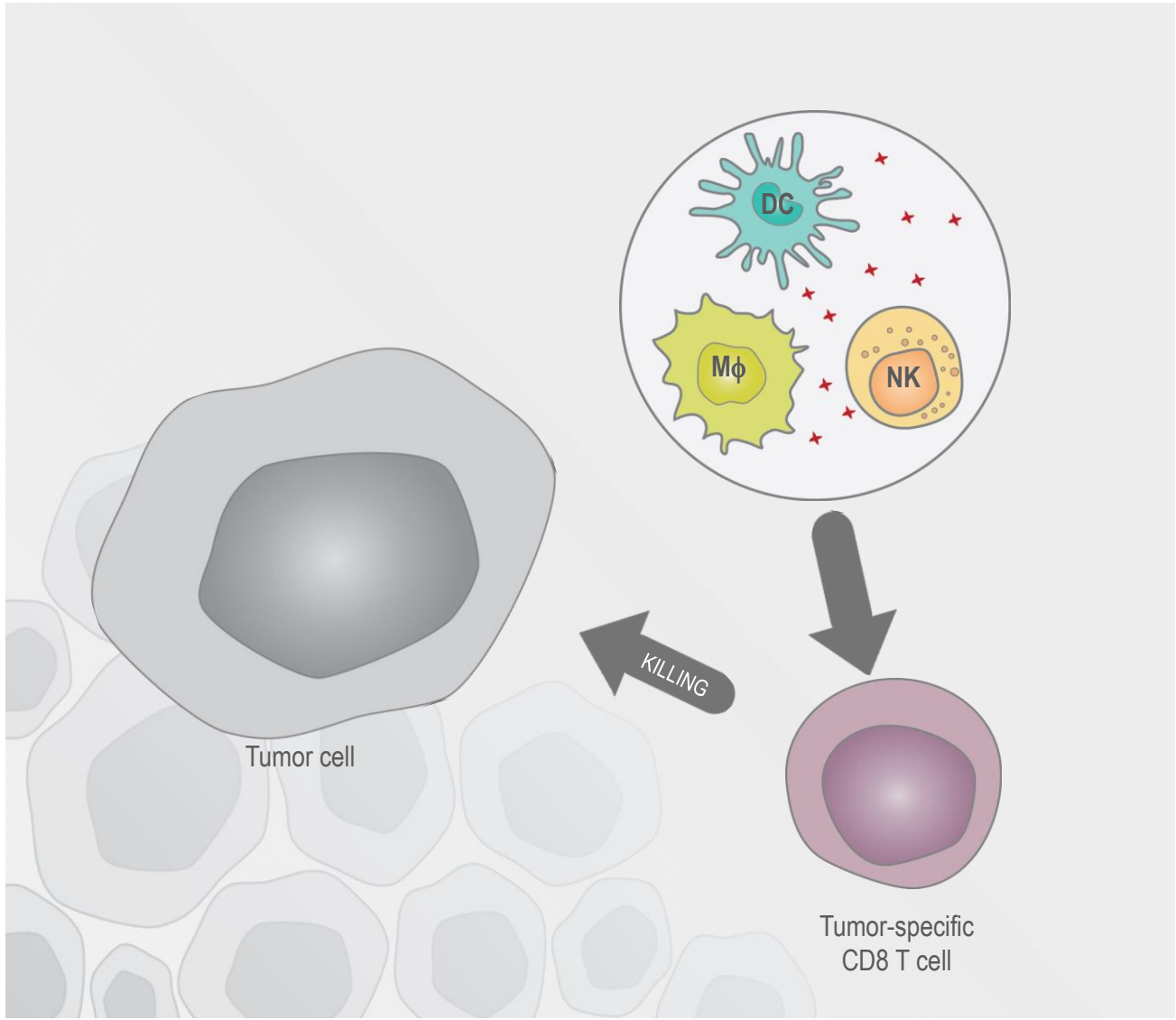
- **The cancer innate immunity cycle**
- **What's Next? Natural Killer Cell Engagers**
  - First generation: NKCE^3
  - NKCE^3 to NKCE^4, new data from next generation technology
- **The ANKET™ platform**
  Antibody-based NK cell engager therapeutics

A pivotal role of T cells in tumor immunity



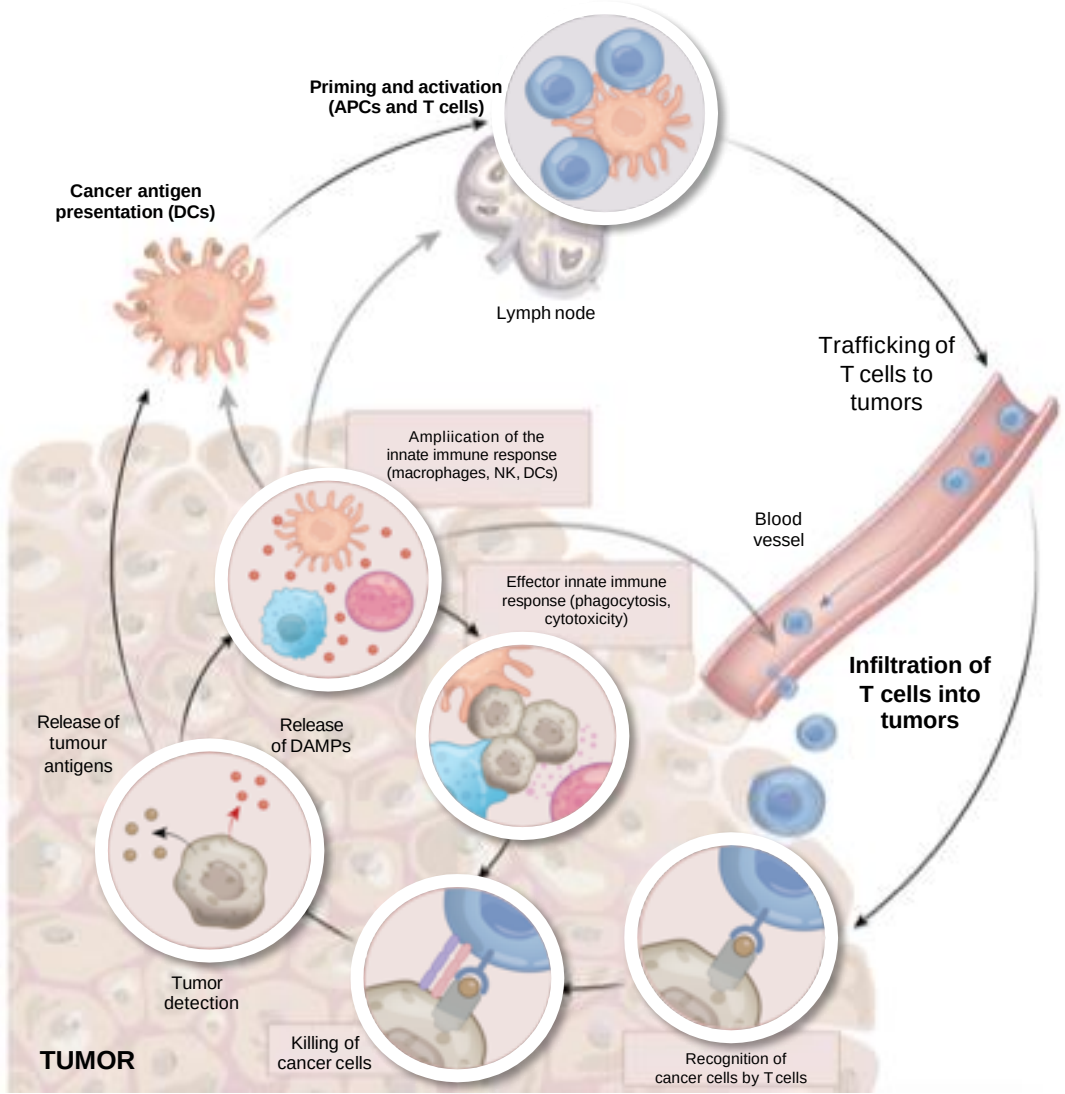
Pages et al., NEJM, 2005
Okazaki & Honjo, Int. Immunol. 2007
Chen & Mellman, Immunity 2013
Schumacher & Schreiber, Science 2015
Sharma & Allison, Science 2015
Chen & Mellman, Nature 2017

T cells are not autonomous in their anti-tumor functions



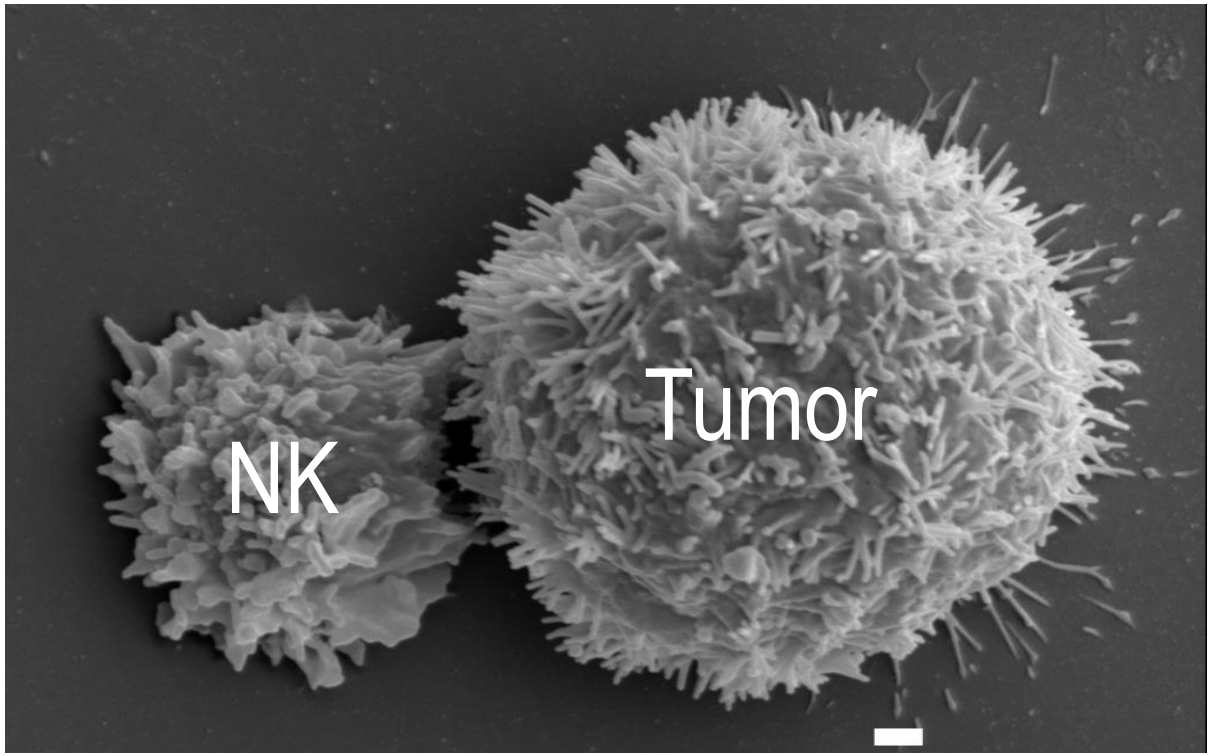
The cancer innate immunity cycle

Demaria et al., Nature 2019



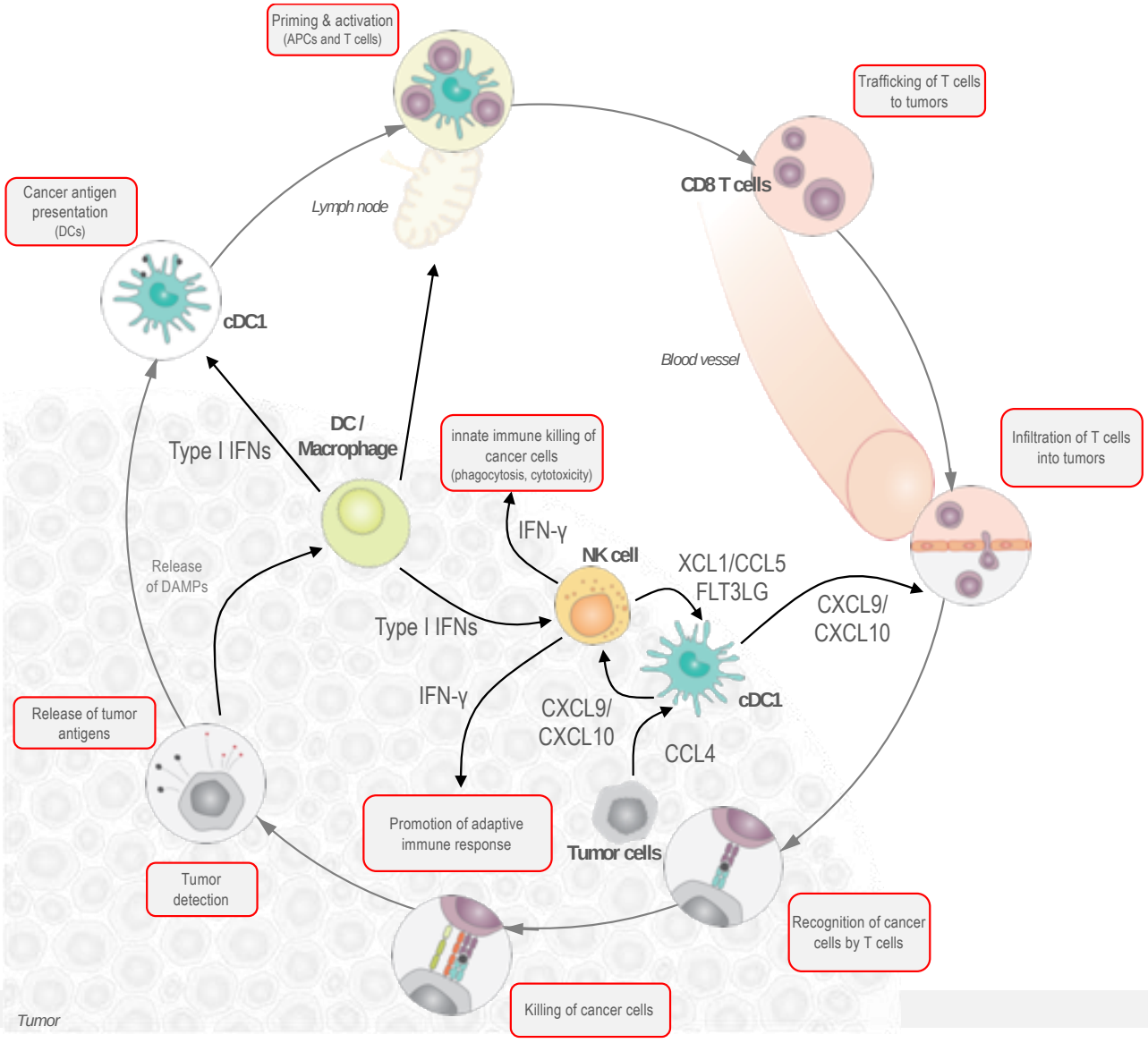
Innate Lymphoid cells

Stimuli		Mediators	Immune function
Tumors, intracellular microbes (Virus, bacteria, parasites)	→ NK, ILC1	IFN-γ Granzymes Perforin	Type 1 immunity (Macrophage activation, cytotoxicity)
Large extracellular parasites and allergens	→ ILC2	IL-4 IL-5 IL-13 IL-9 AREG	Type 2 immunity (Alternative macrophage activation)
Mesenchymal organizer cells (Retinoic acid, CXCL13, RANK-L)	→ LTi	RANK Lymphotoxin TNF IL-17 IL-22	Formation of secondary lymphoid structures
Extracellular microbes (Bacteria, fungi)	→ ILC3	IL-22 IL-17 GM-CSF Lymphotoxin	Type 3 immunity (Phagocytosis, antimicrobial peptides)



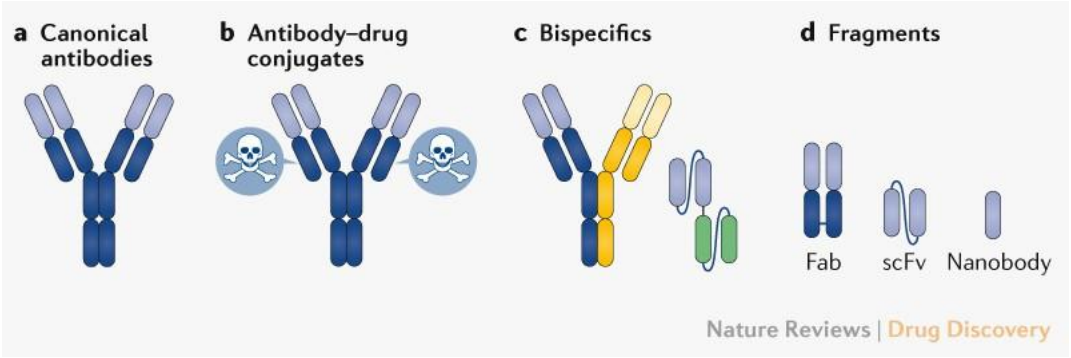
Vivier et al., Nature Immunol. 2008
Vivier et al., Science 2011
Vivier et al., Cell 2018

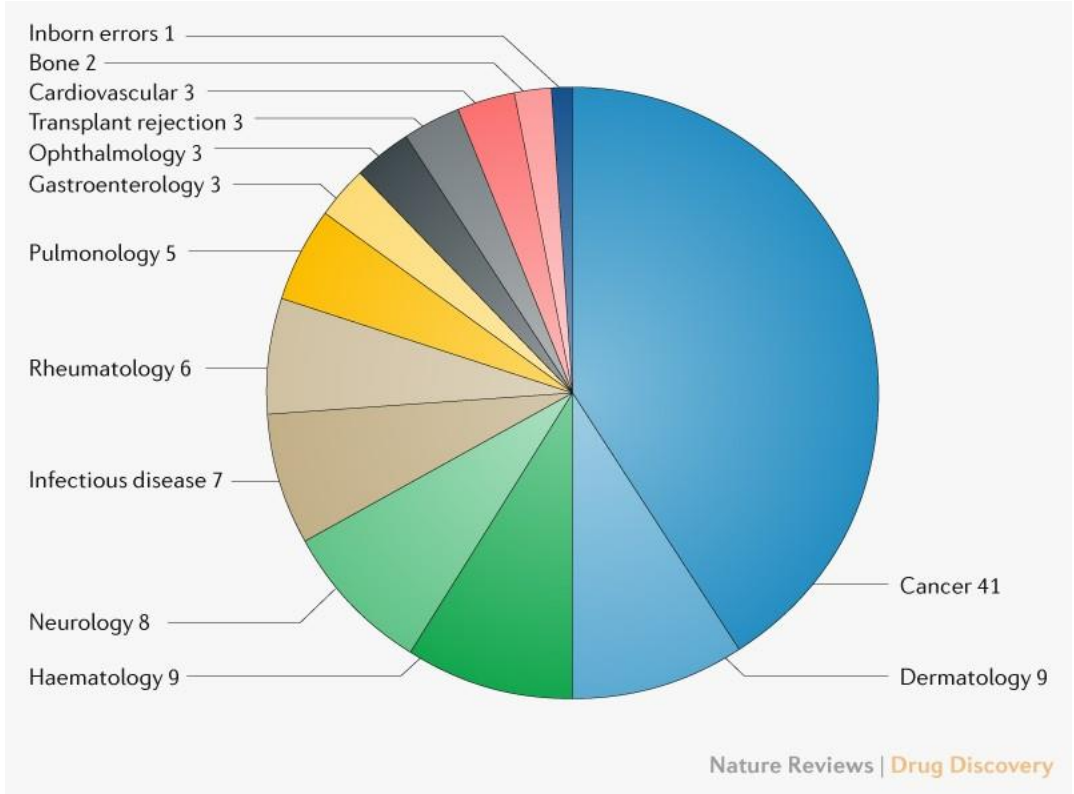
The cancer innate immunity cycle



Demaria, Gajewski, Vivier, SITC in press

Antibodies are great medicines

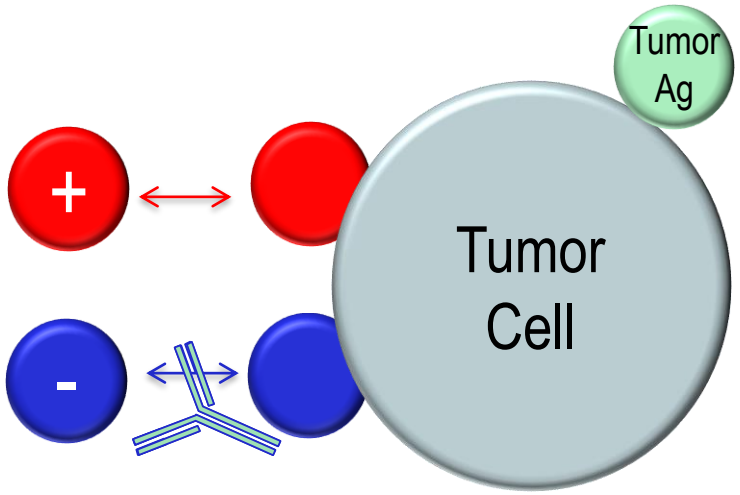




A. Mullard, Nature Reviews Drug Discovery 2021

Antibodies in cancer immunotherapy

- T cells
- NK cells

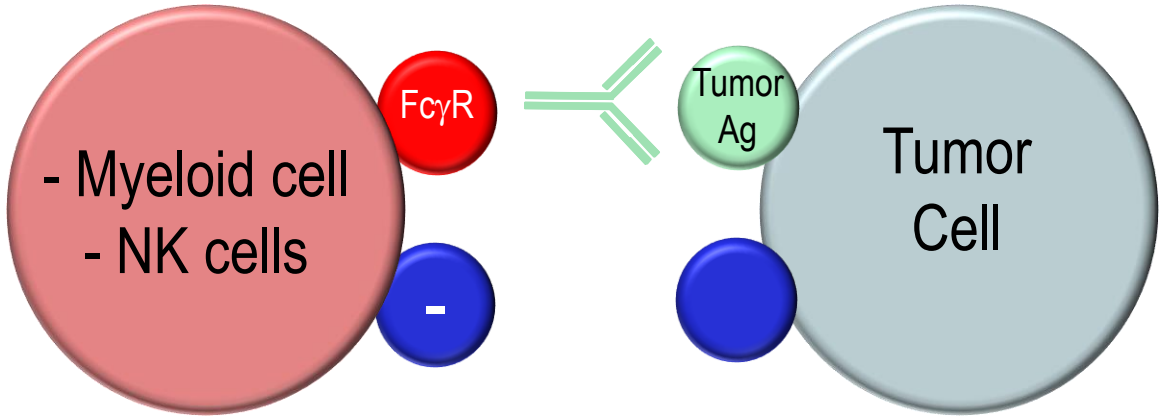


Blocking antibodies

e.g. *anti-PD-(L)1 mAbs*
anti-NKG2A mAbs
anti-TIGIT mAbs

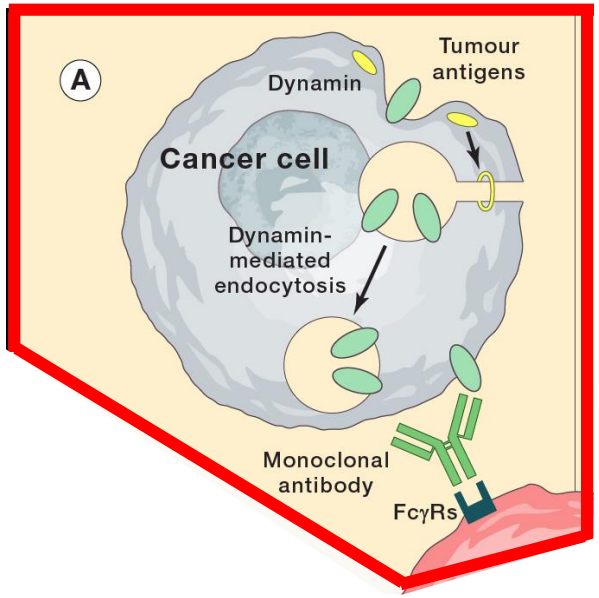
Cytotoxic antibodies

e.g. *anti-CD20 mAbs*
anti-EGFR mAbs
anti-HER2 mAbs

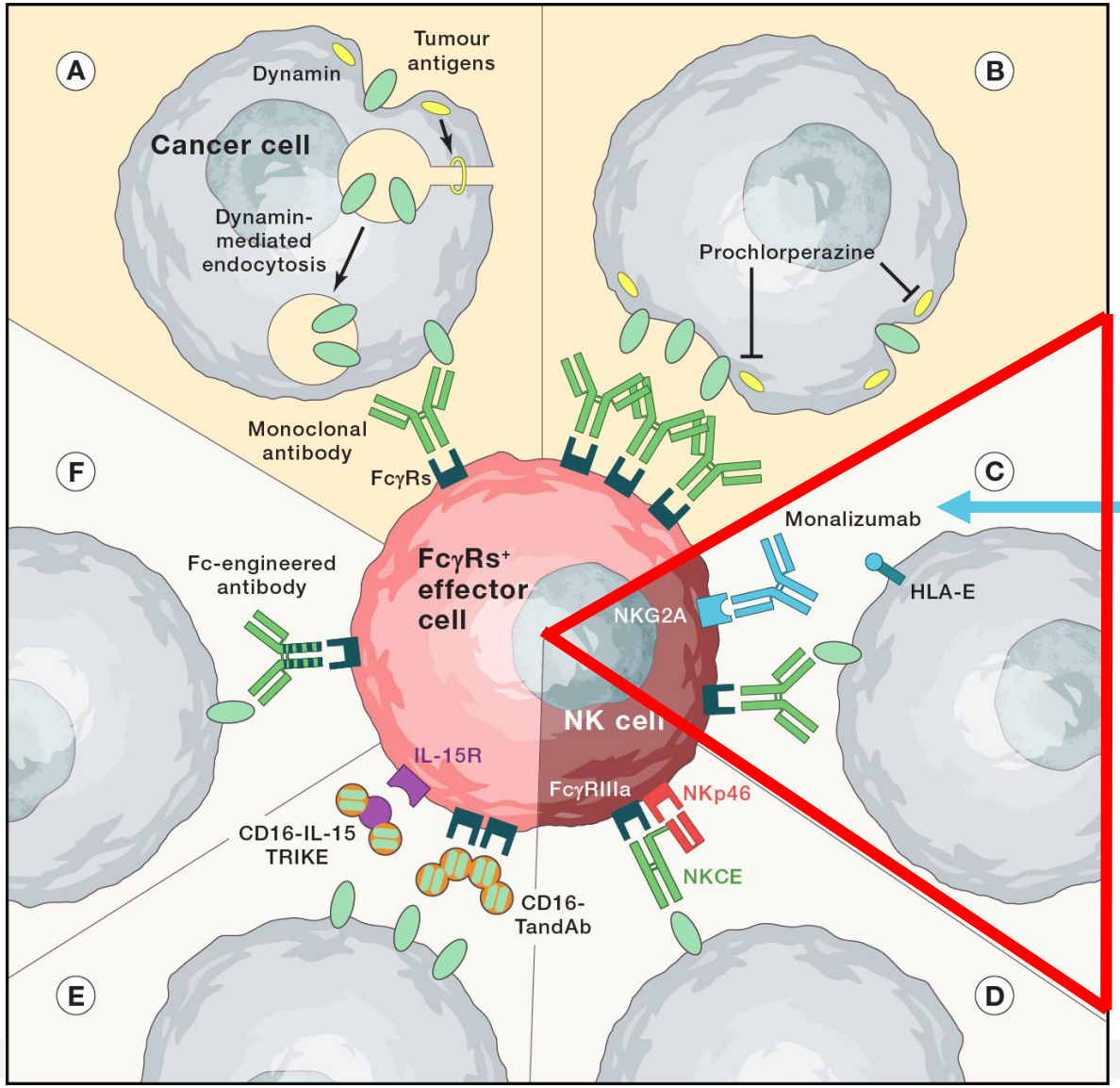


Antibody-dependent cell phagocytosis (ADCP)
Antibody-dependent cell cytotoxicity (ADCC)

Boosting cytotoxic antibodies against cancer



Boosting cytotoxic antibodies against cancer

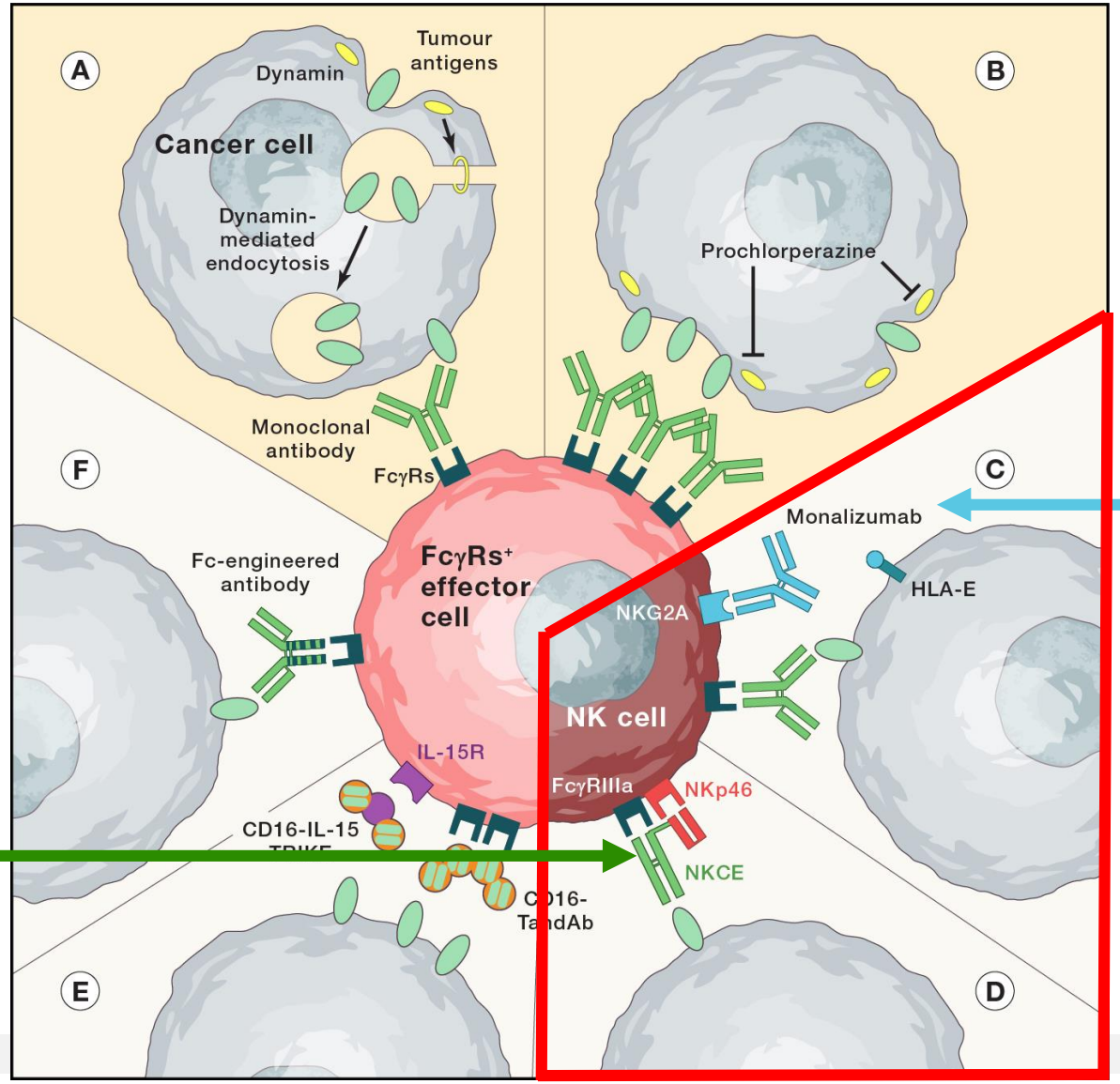


Blocking mAbs: Anti-NKG2A mAbs (Monalizumab)

André et al. Cell 2018

Gauthier & Vivier, Cell 2020

Boosting cytotoxic antibodies against cancer

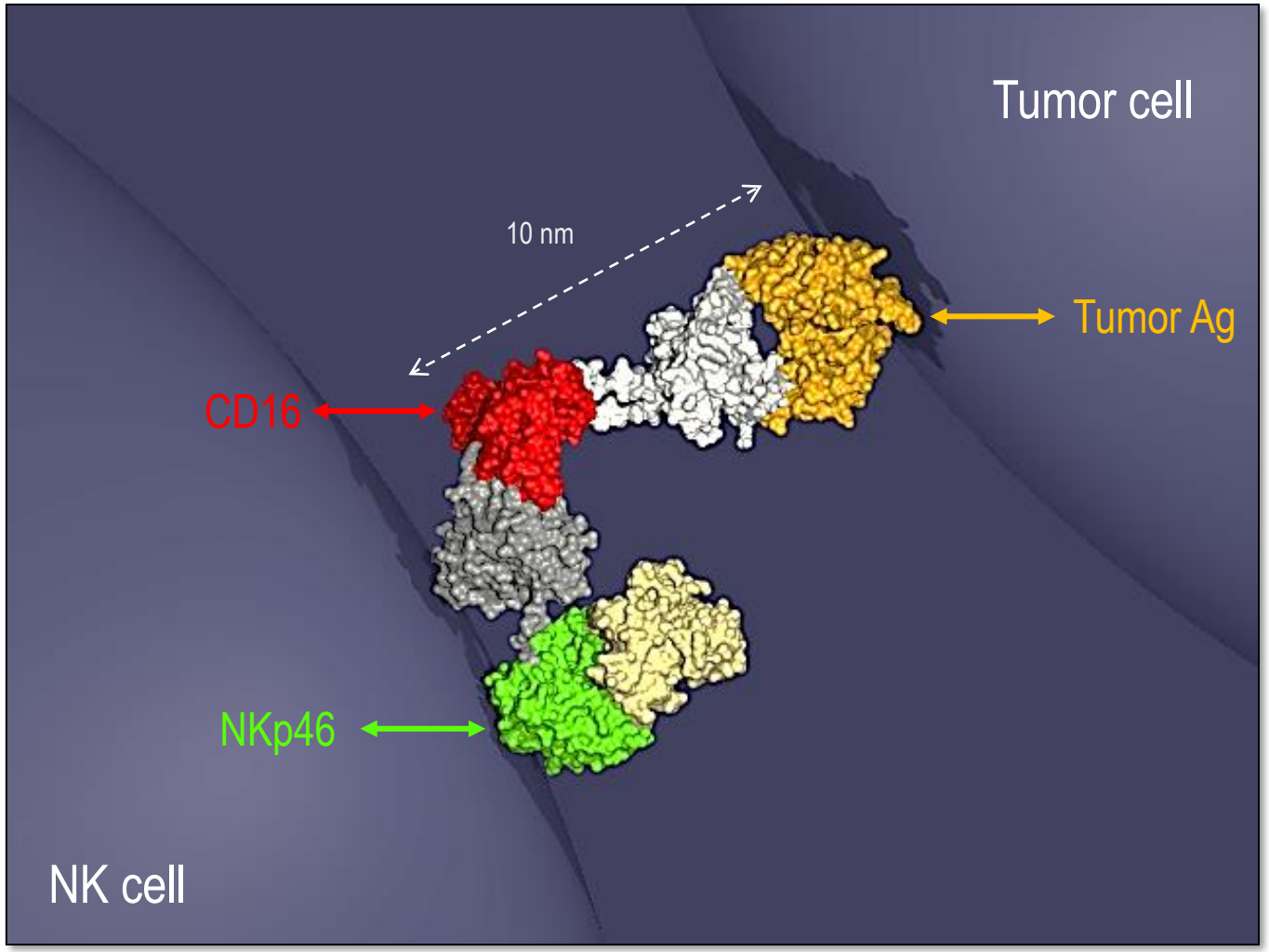


Blocking mAbs:
Anti-NKG2A mAbs
(Monalizumab)
André et al. Cell 2018

Agonist mAbs:
NKp46 NK cell engagers
Gauthier et al. Cell 2019

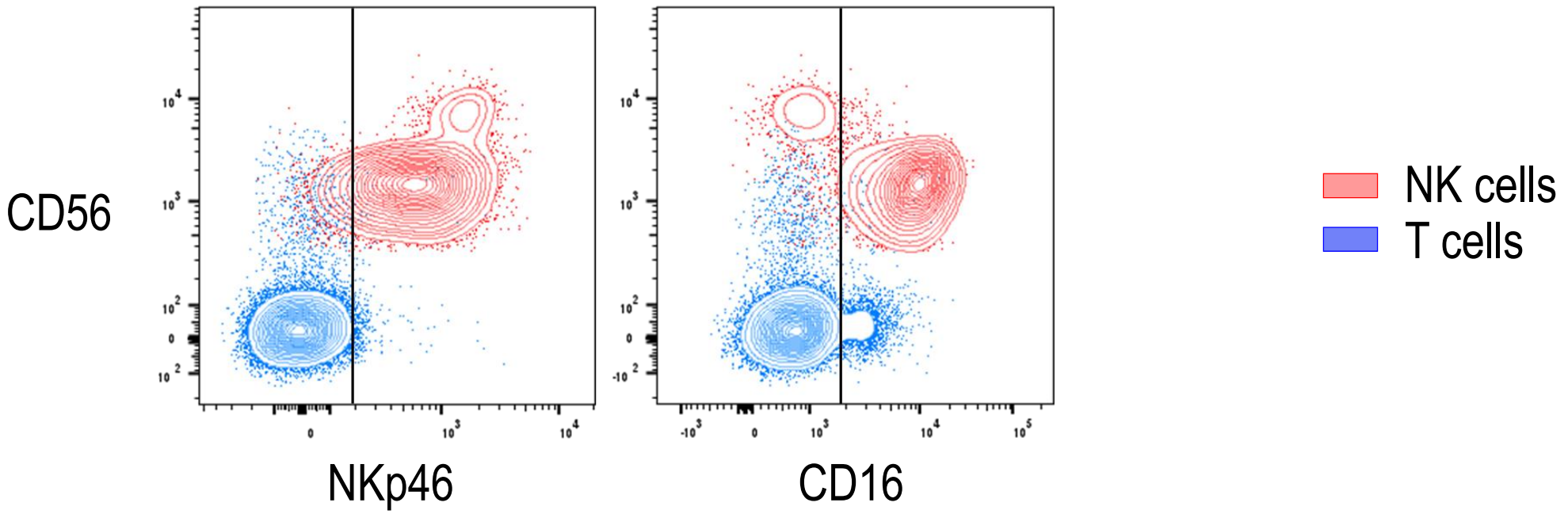
Gauthier & Vivier, Cell 2020

Trifunctional Natural Killer Cell Engagers (NKCE^3)



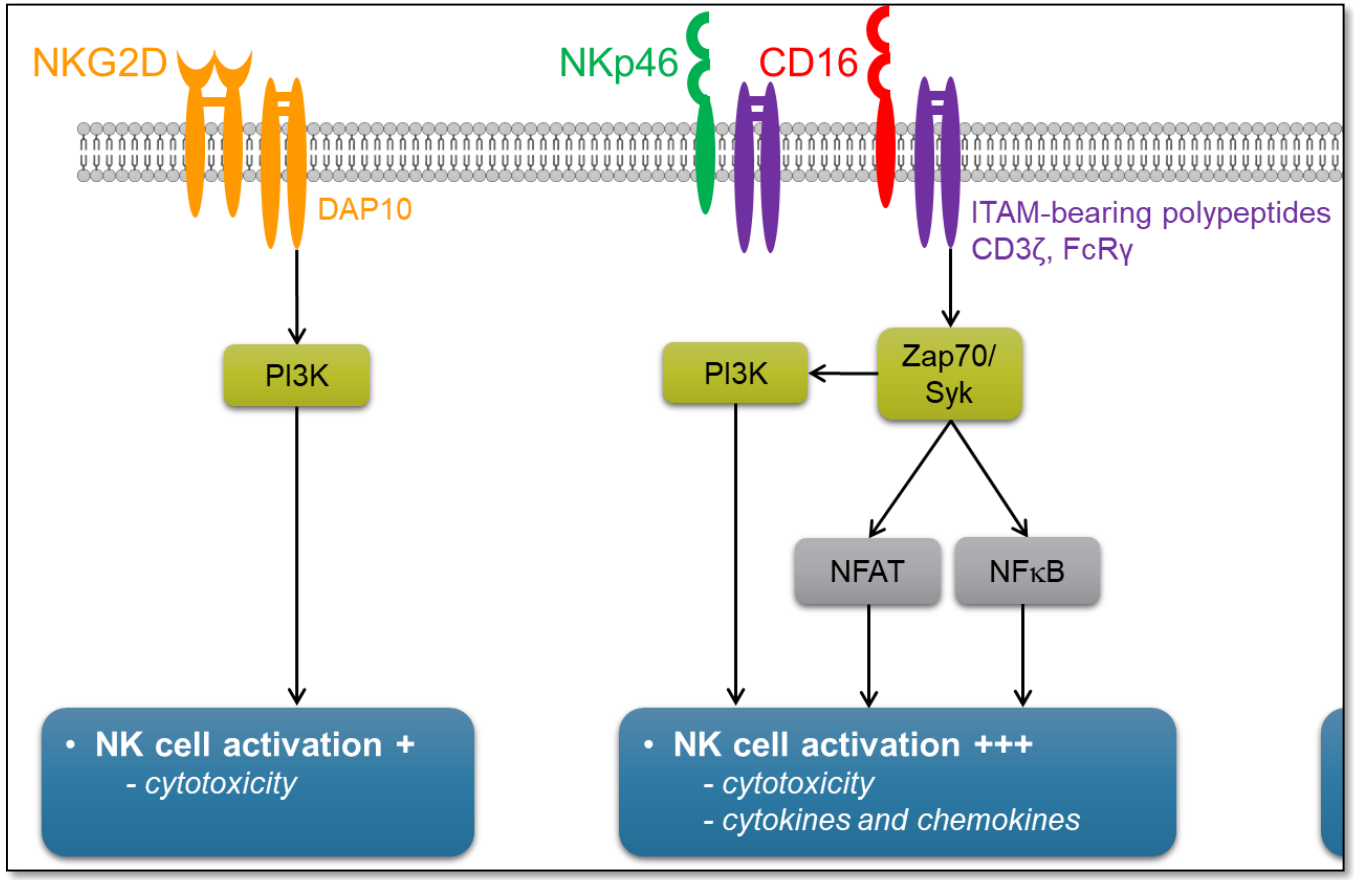
Gauthier & Vivier, Cell 2019

Rationale for engaging NK cells through NKp46 1/3



NKp46 is expressed by all NK cells

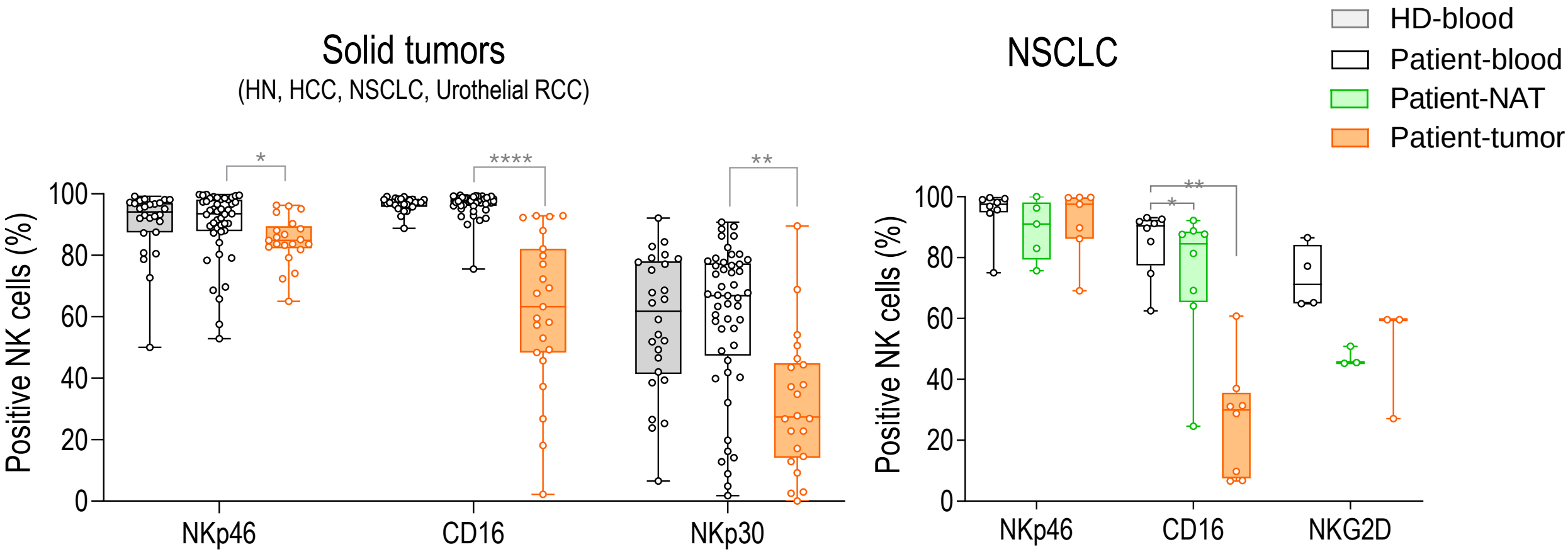
Rationale for engaging NK cells through NKp46 2/3



NKp46 triggers potent signaling pathways

Lanier et al., Nature Immunol 2008
Vivier et al., Science, 2004

Rationale for engaging NK cells through NKp46 3/3

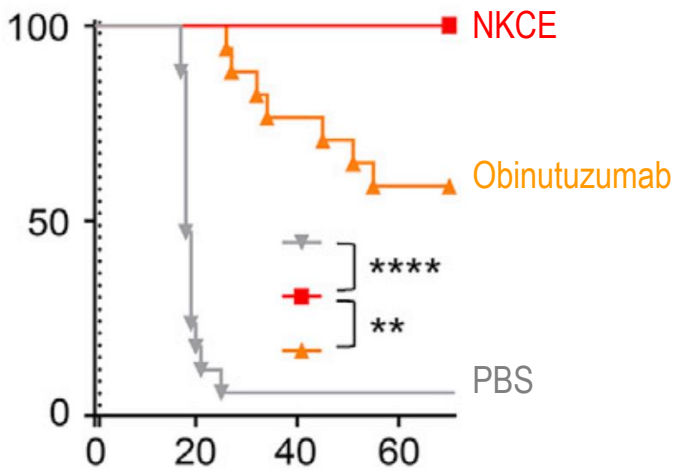


NKp46 expression is conserved on tumor-infiltrating NK cells

NKCE^3 : the first generation

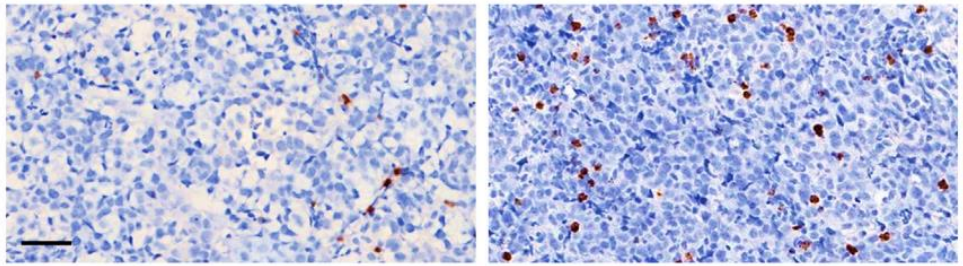
Efficacy

- Activity in preclinical in vivo models
- Efficacy NKCE^3 > approved benchmark antibodies in a cancer model in vivo



Mode of Action

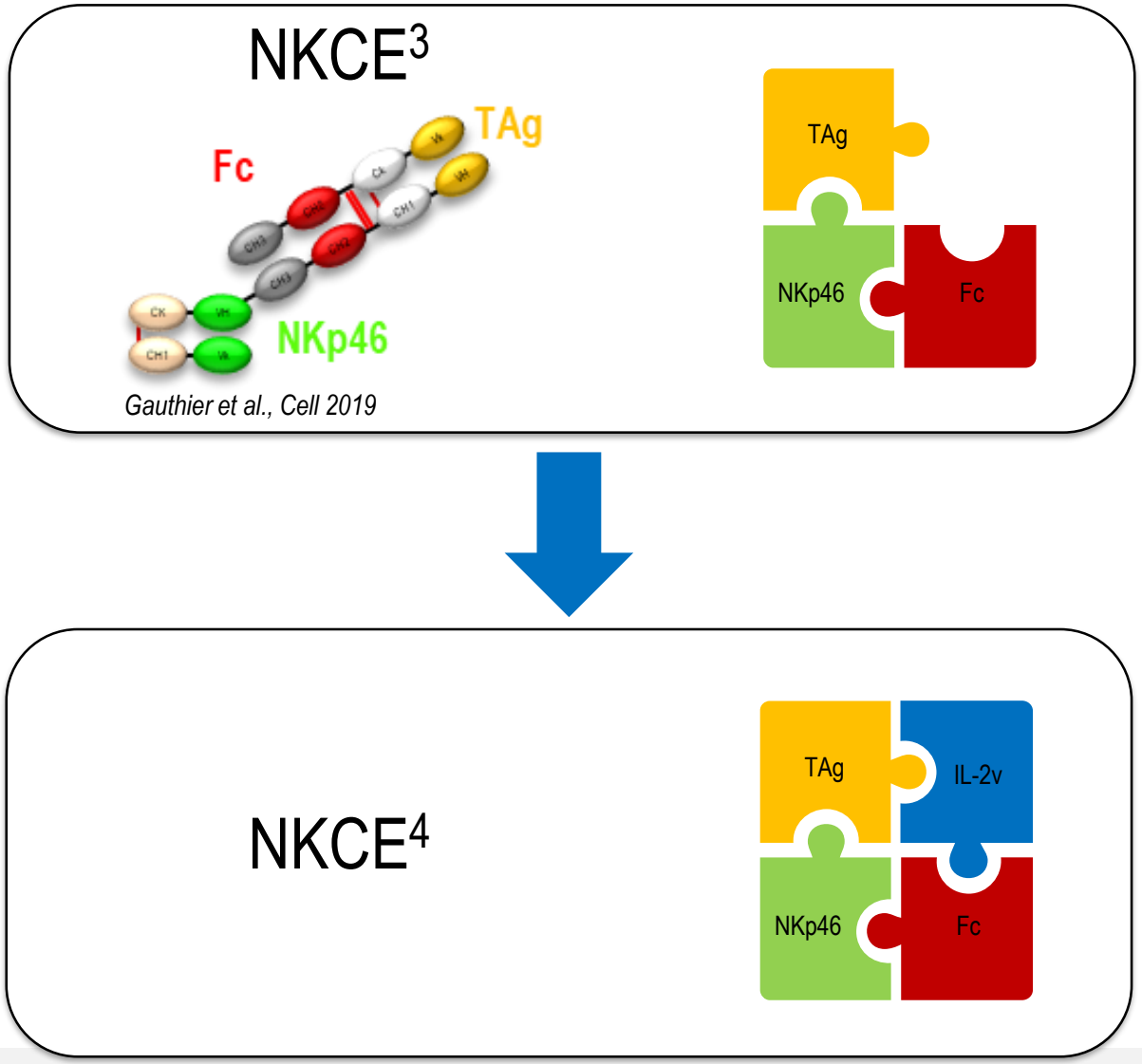
- Optimized killing activation by co-engagement of NKp46 and CD16
- Increased NK cell number in the tumor



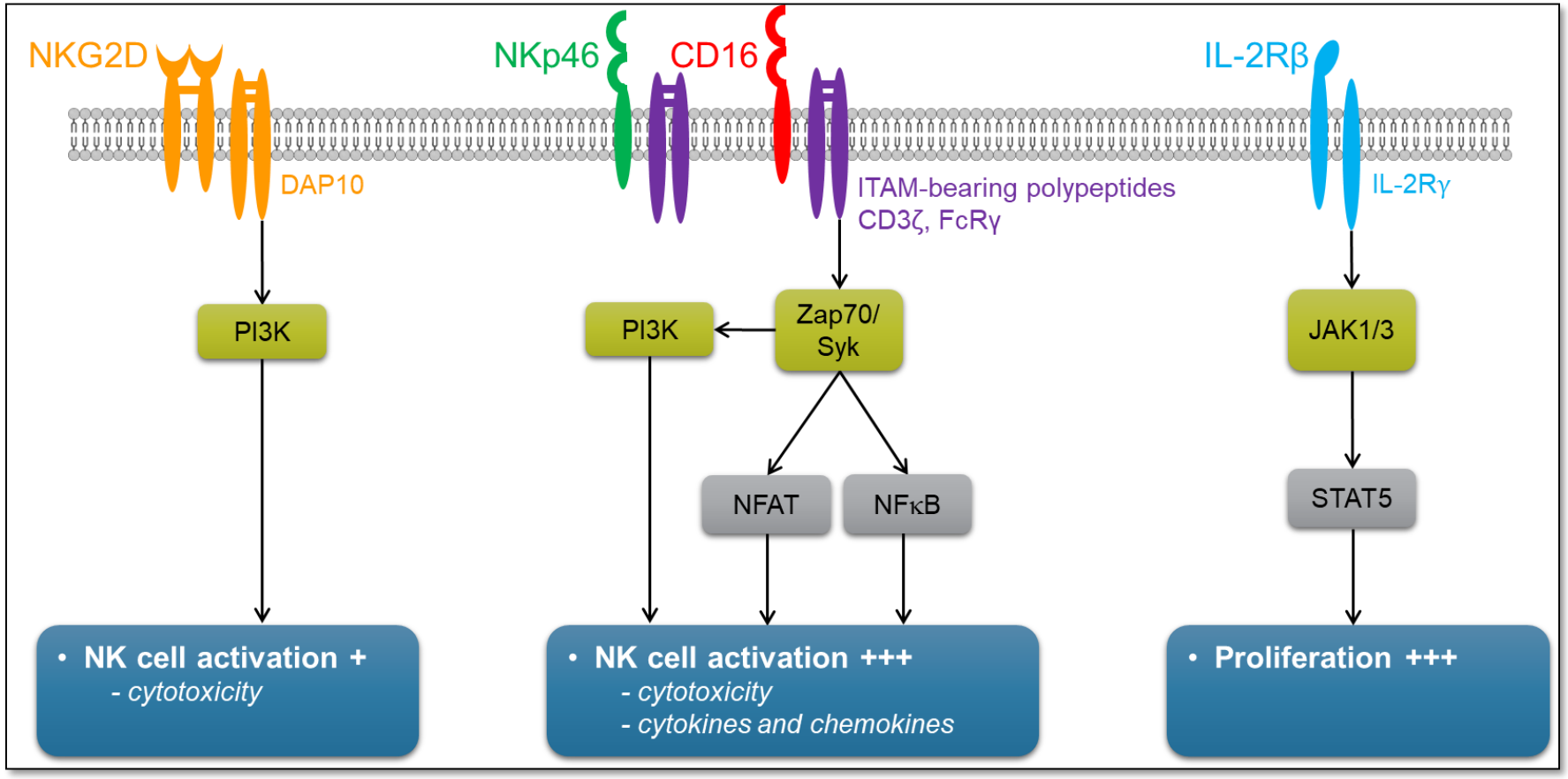
Control NKCE

Gauthier et al., Cell 2019

From NKCE^3 to NKCE^4

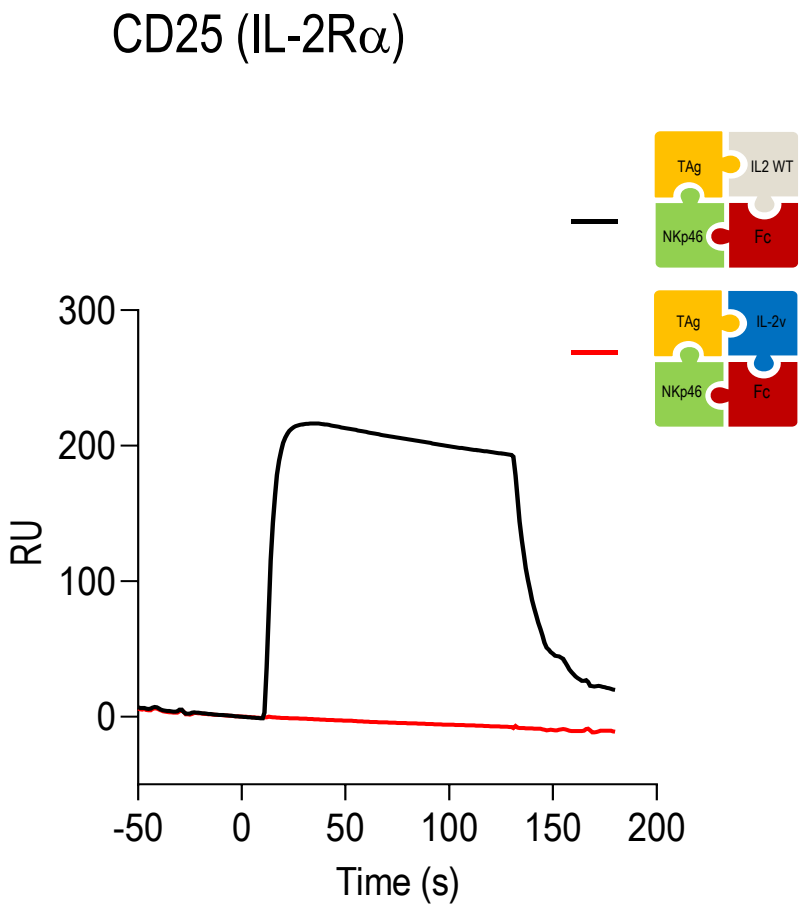


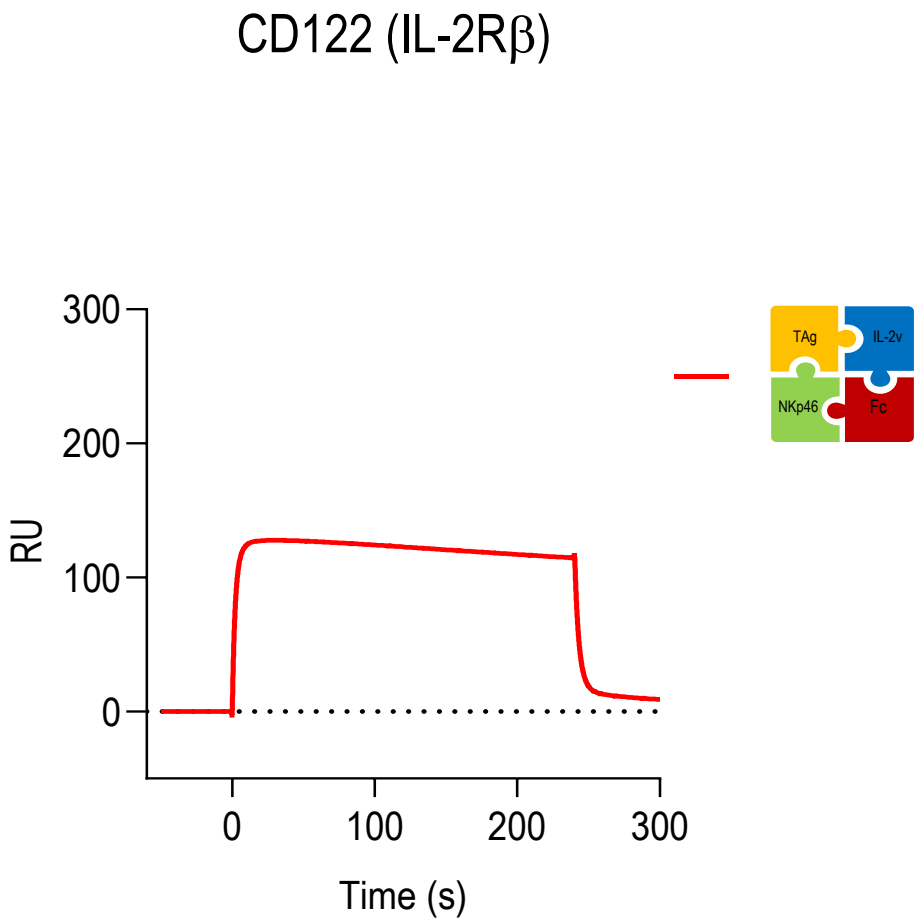
Rationale for including IL-2v in NKCE[4]



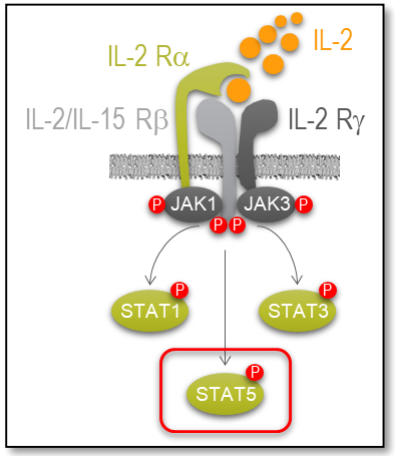
Complementarity between ITAM and IL-2Rγ (γc) signaling pathways

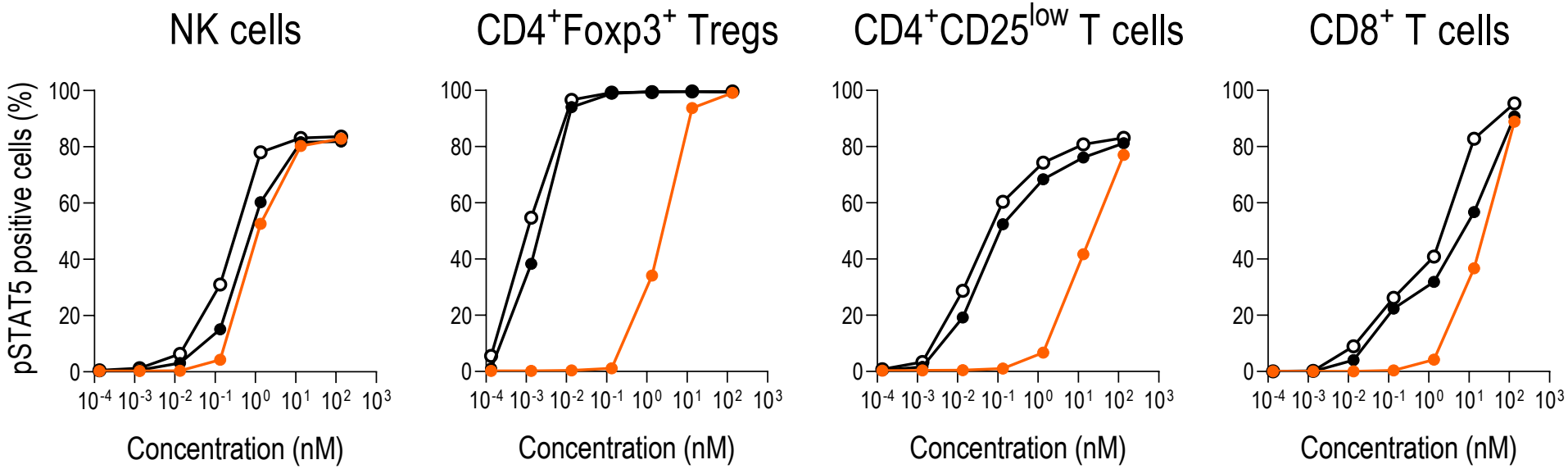
IL-2v in NKCE^4 format does not bind to IL-2Ra but still binds to IL-2Rb

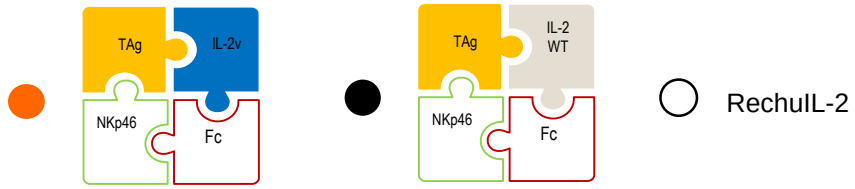




IL-2v is functional in NKCE[4] format and limits Treg activation

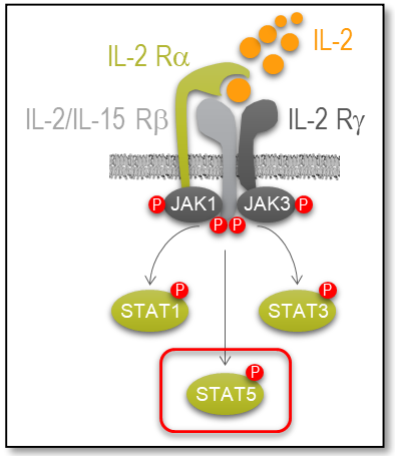


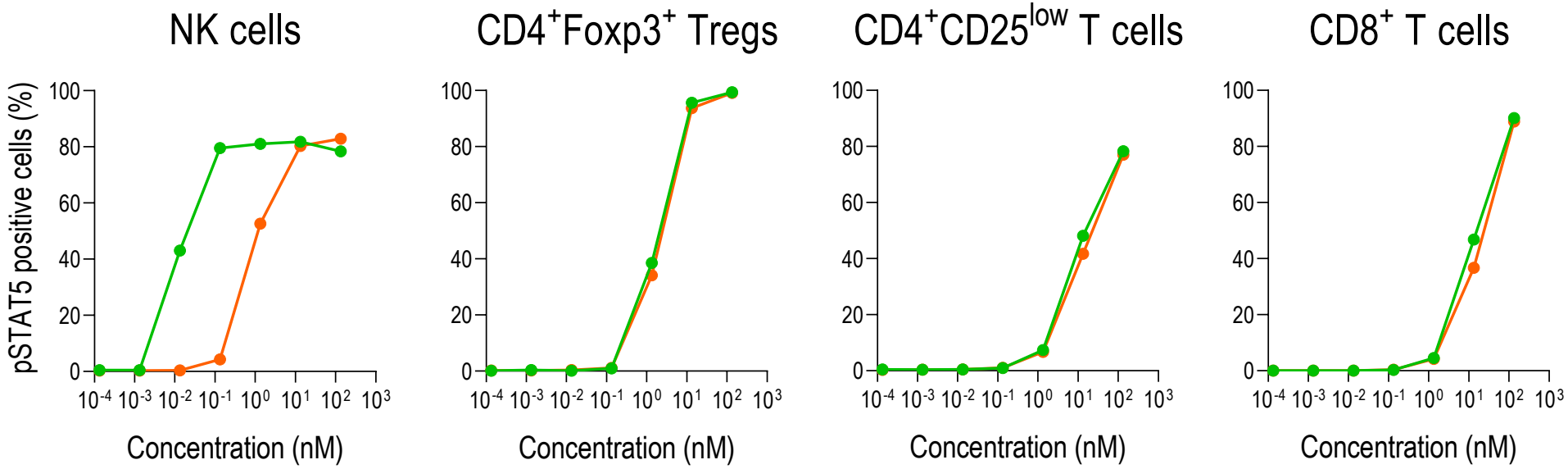


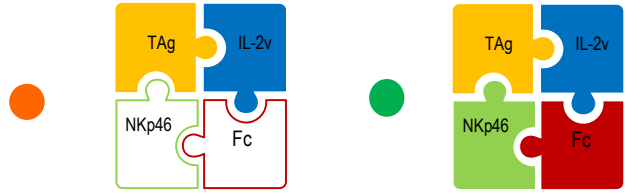


- *Cells: PBMC from HD*
- *Stimulation: 20 min*
- *Read-out: STAT5 phosphorylation by flow cytometry*

NKCE[4] promotes specifically IL-2R activation on NK cells

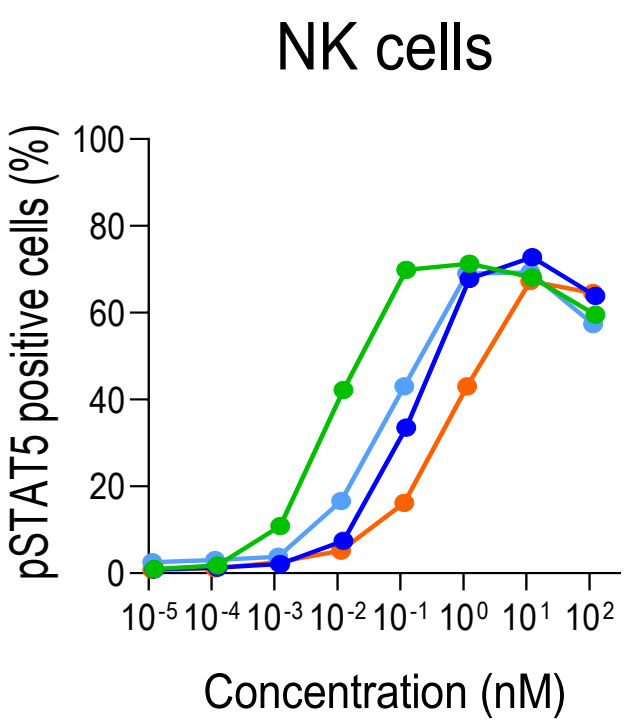


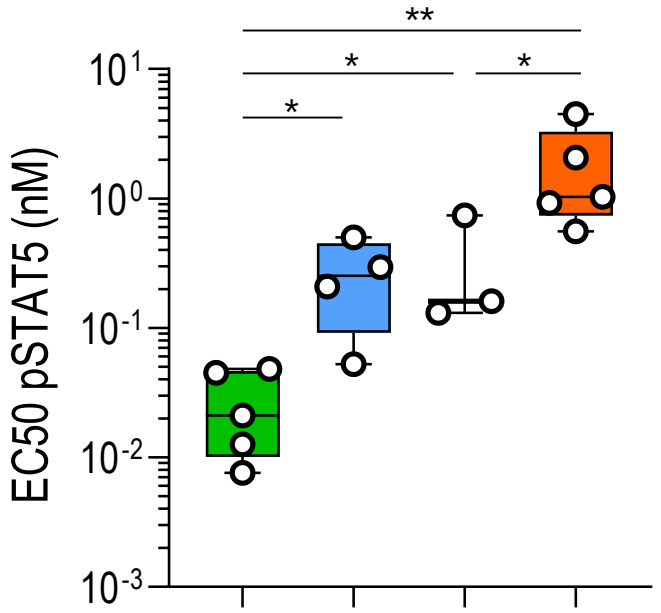


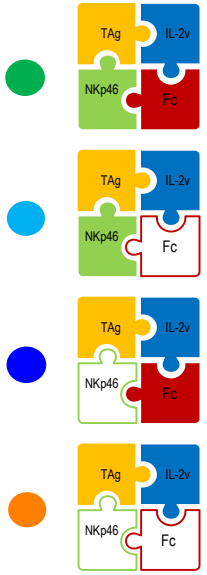


- *Cells: PBMC from HD*
- *Stimulation: 20 min*
- *Read-out: STAT5 phosphorylation by flow cytometry*

Binding to CD16 and NKp46 is required for optimal IL-2R signaling by NKCE[4]

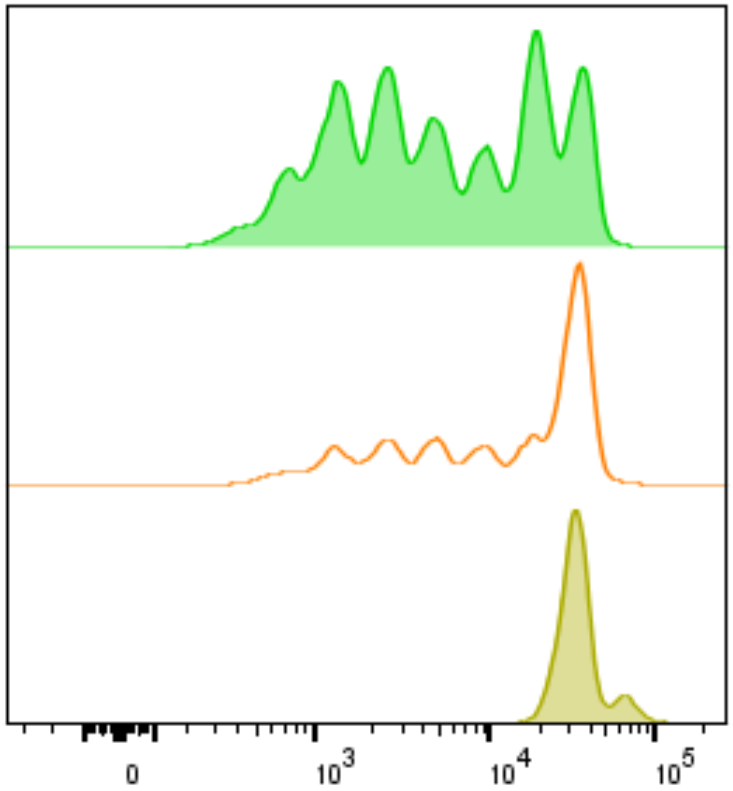


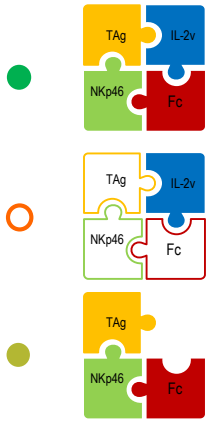




- *Cells: PBMC from HD*
- *Stimulation: 20 min*
- *Read-out: STAT5 phosphorylation by flow cytometry*

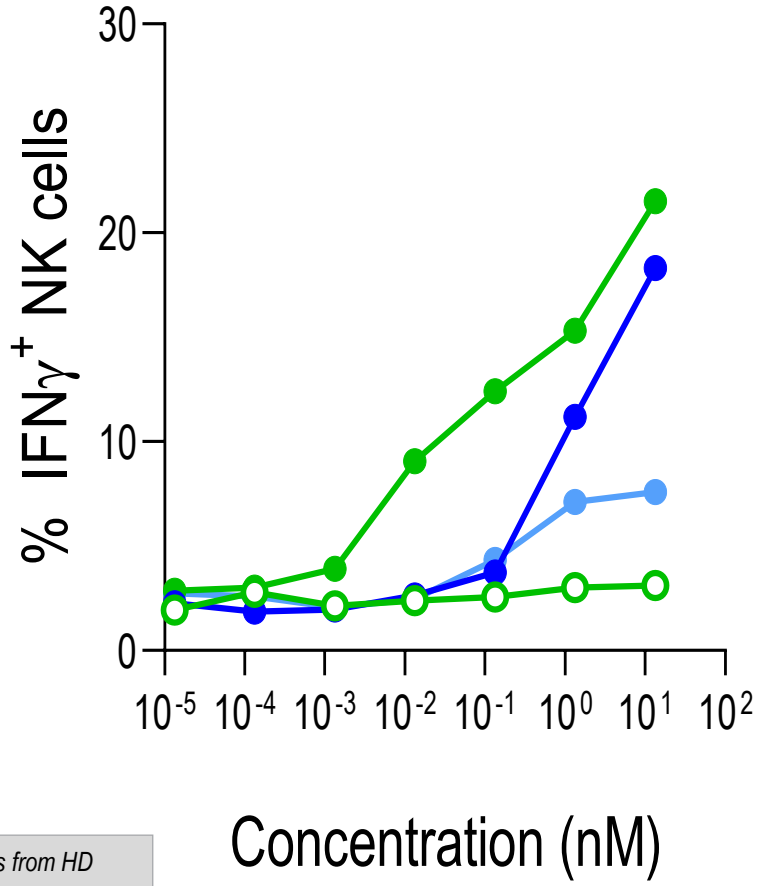
NKCE^4 induces NK cell proliferation

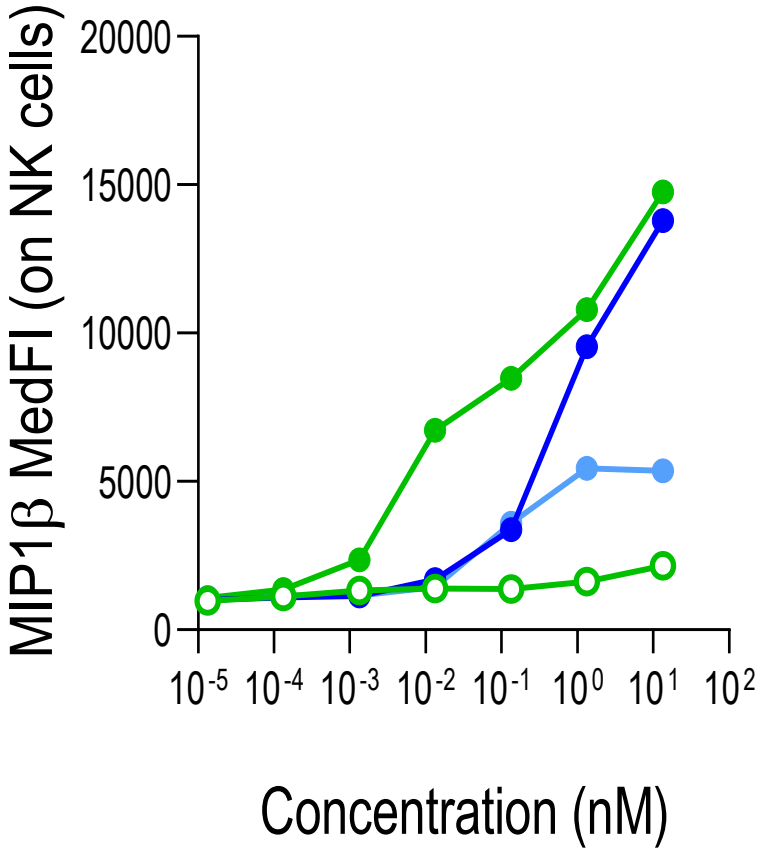


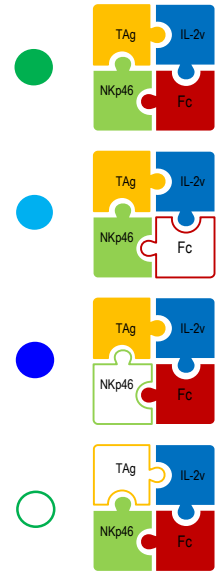


- *Cells: purified NK cells*
- *Stimulation: 5 days*
- *Read out: proliferation, CTV diltution*

NKCE[4] induces cytokine production upon target engagement

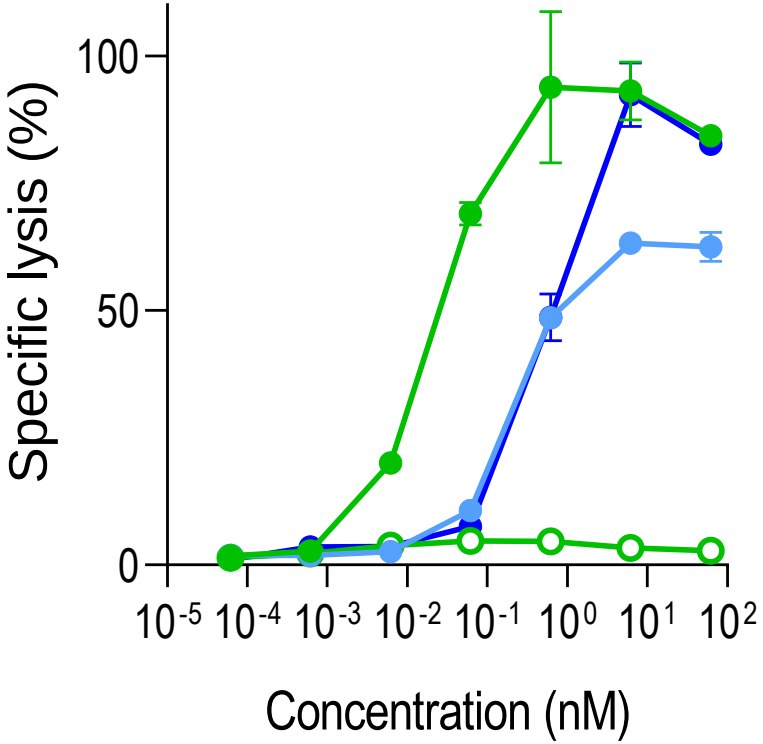


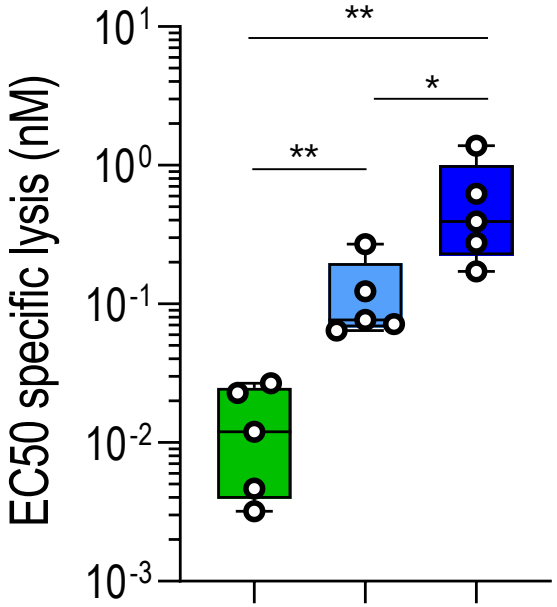


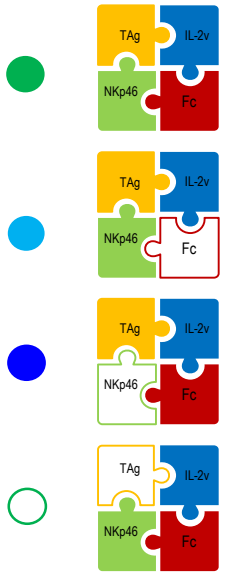


- *Effector: Freshly purified NK cells from HD*
- *Target: Raji B cell line*
- *Incubation: 4h with NKCE + Golgi Stop, E:T=0.5:1*
- *Read out: Intracellular FACS*

NKCE[4] induces cytokine production upon target engagement

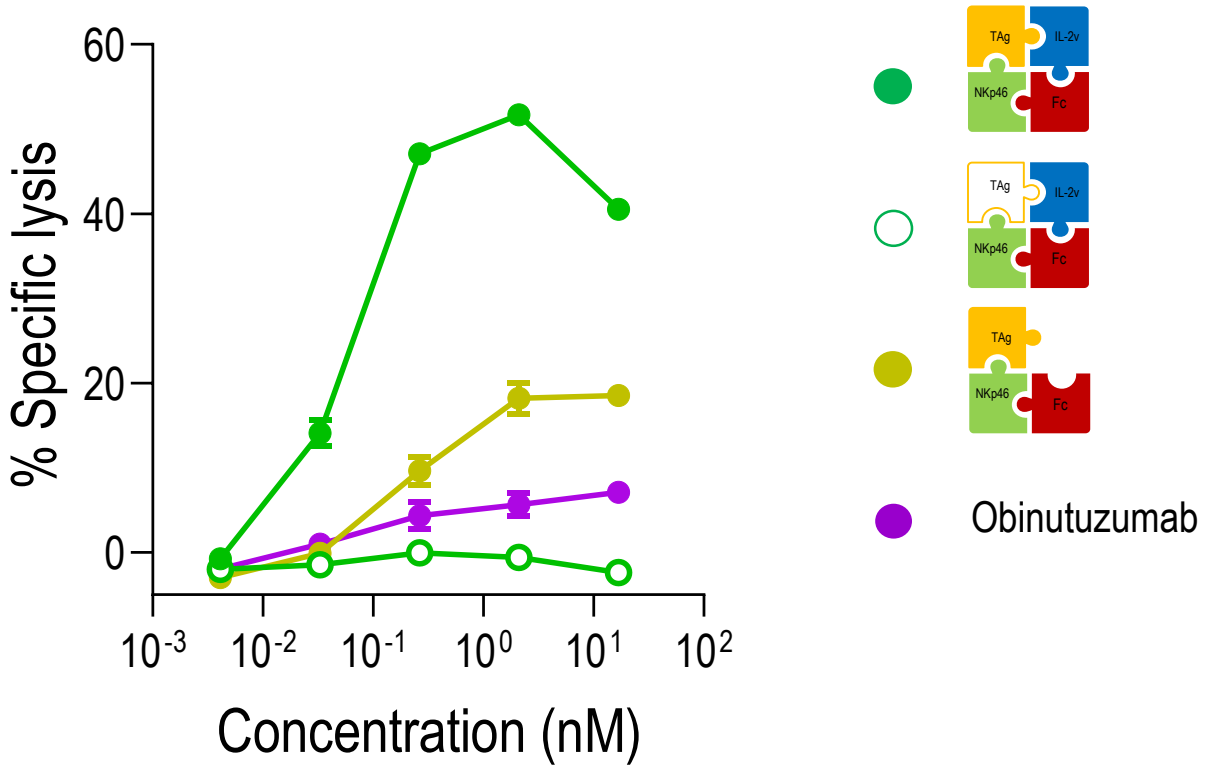






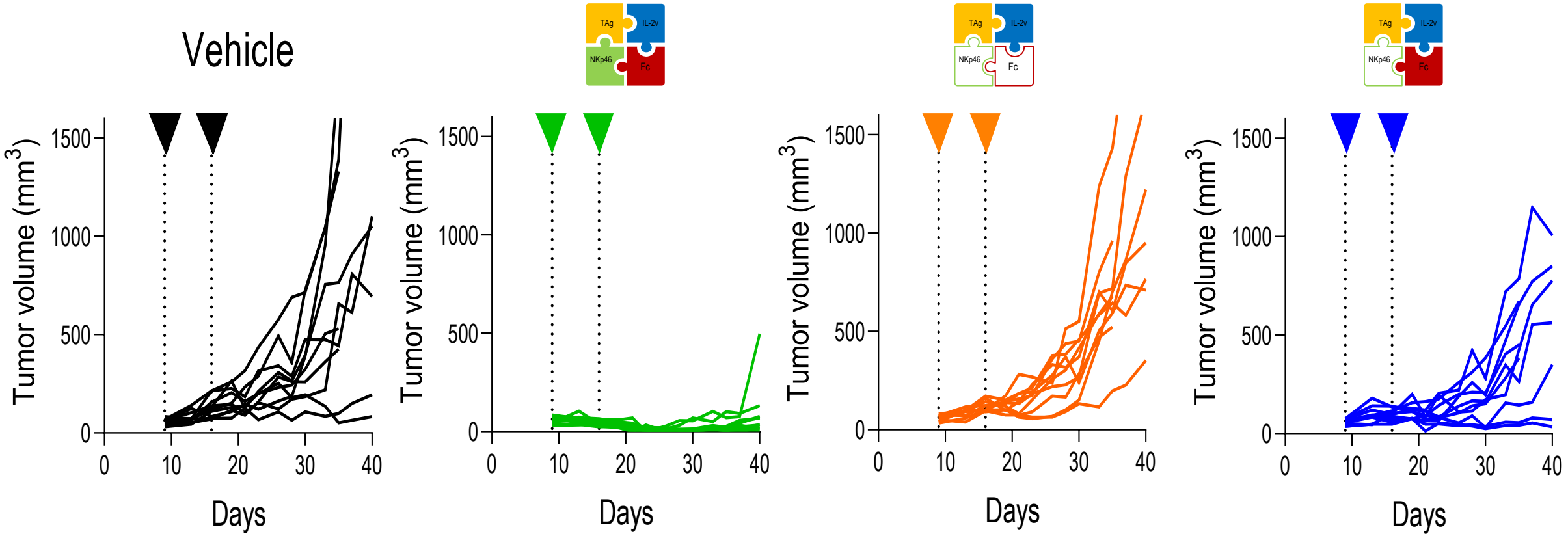
- *Effector: purified NK cells from HD*
- *Target: Raji B cell line*
- *Read out: Calcein release assay, 4h, E:T=10:1*

NKCE[4] induces potent cytotoxicity by mouse NK cells



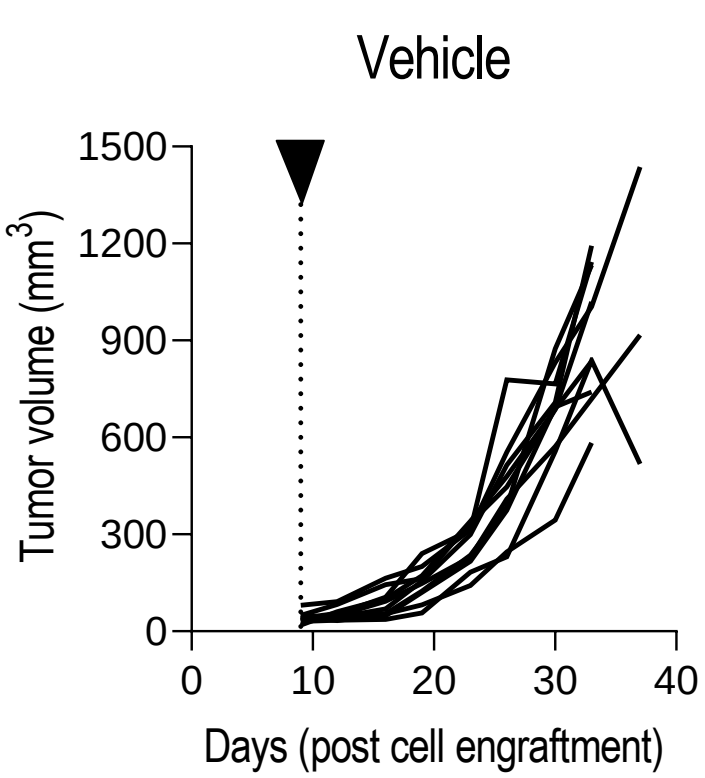
- *Effector: purified NK cells from HD*
- *Target: Raji B cell line*
- *Read out: Calcein release assay, 4h, E:T=10:1*

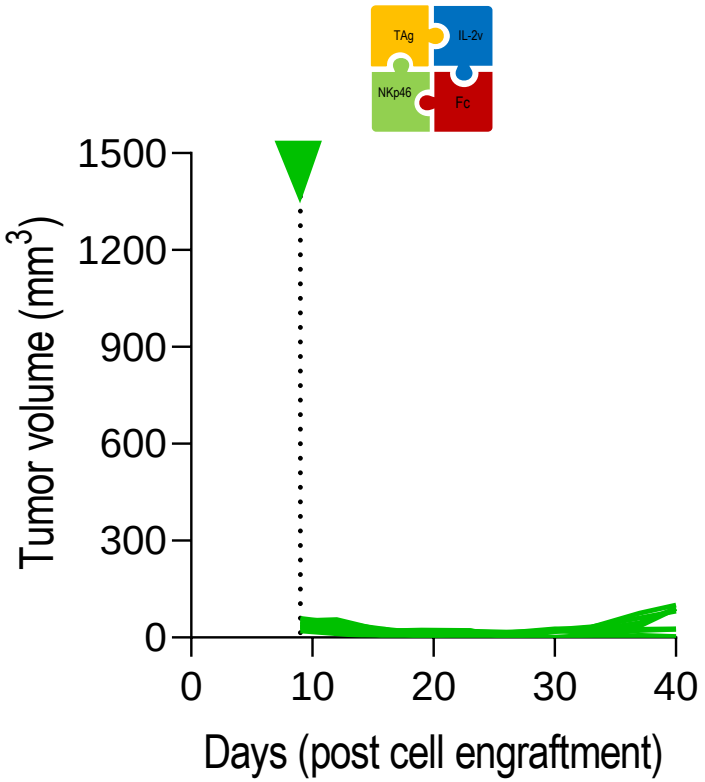
NKCE[4] anti-tumor efficacy: solid tumor model

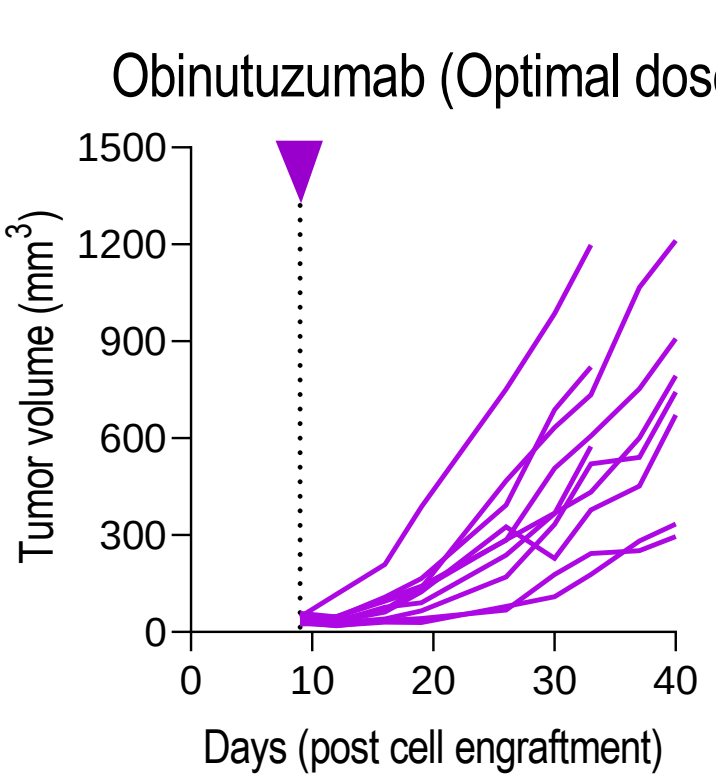


- *Model: Raji, 5x10[6] in matrigel, sc*
- *Mice: CB17 SCID*

CD20-NKCE[4] is more potent than obinutuzumab

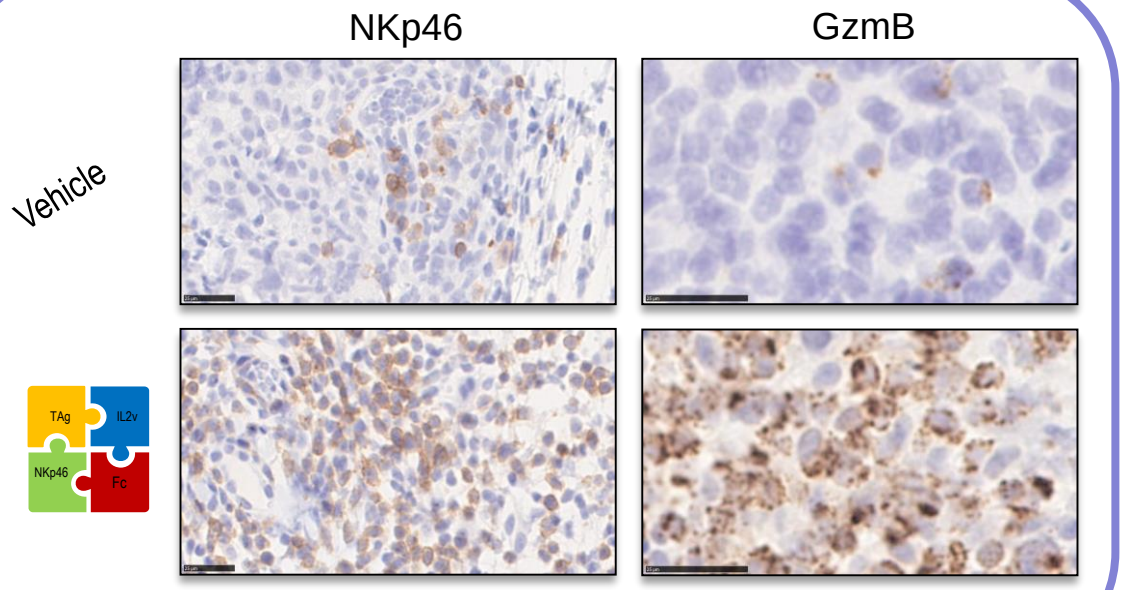


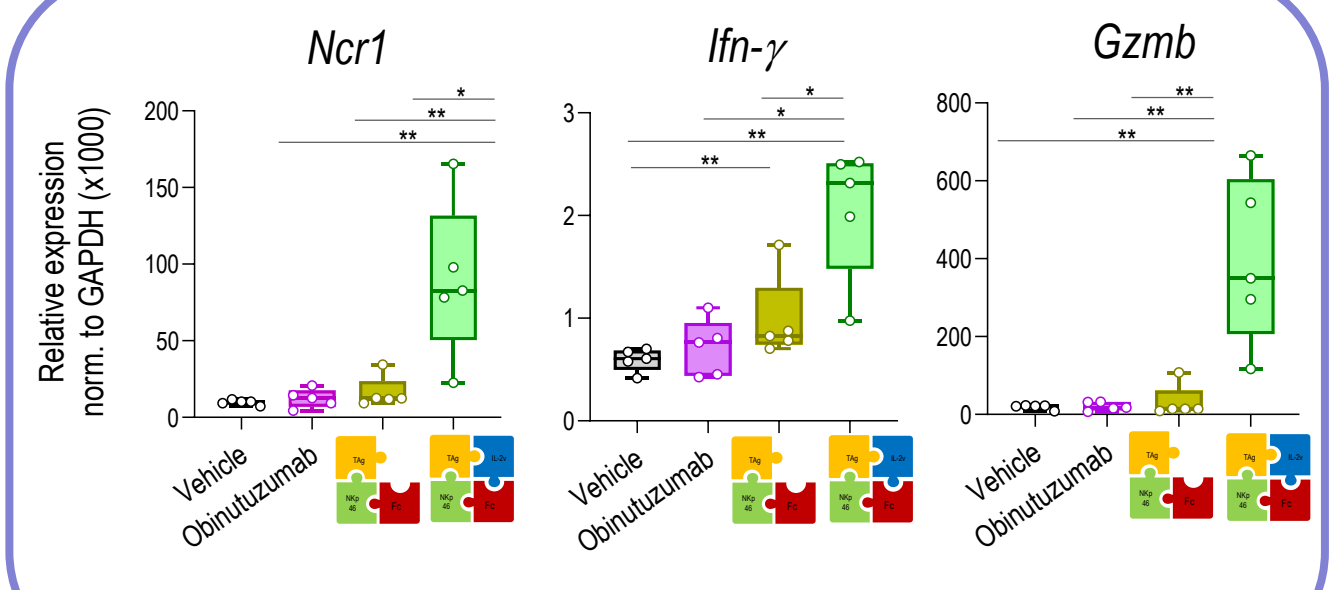




- *Model: Raji, 5x10[6] in matrigel, sc*
- *Mice: CB17 SCID*

Mechanisms of NKCE^4 anti-tumor efficacy. 1

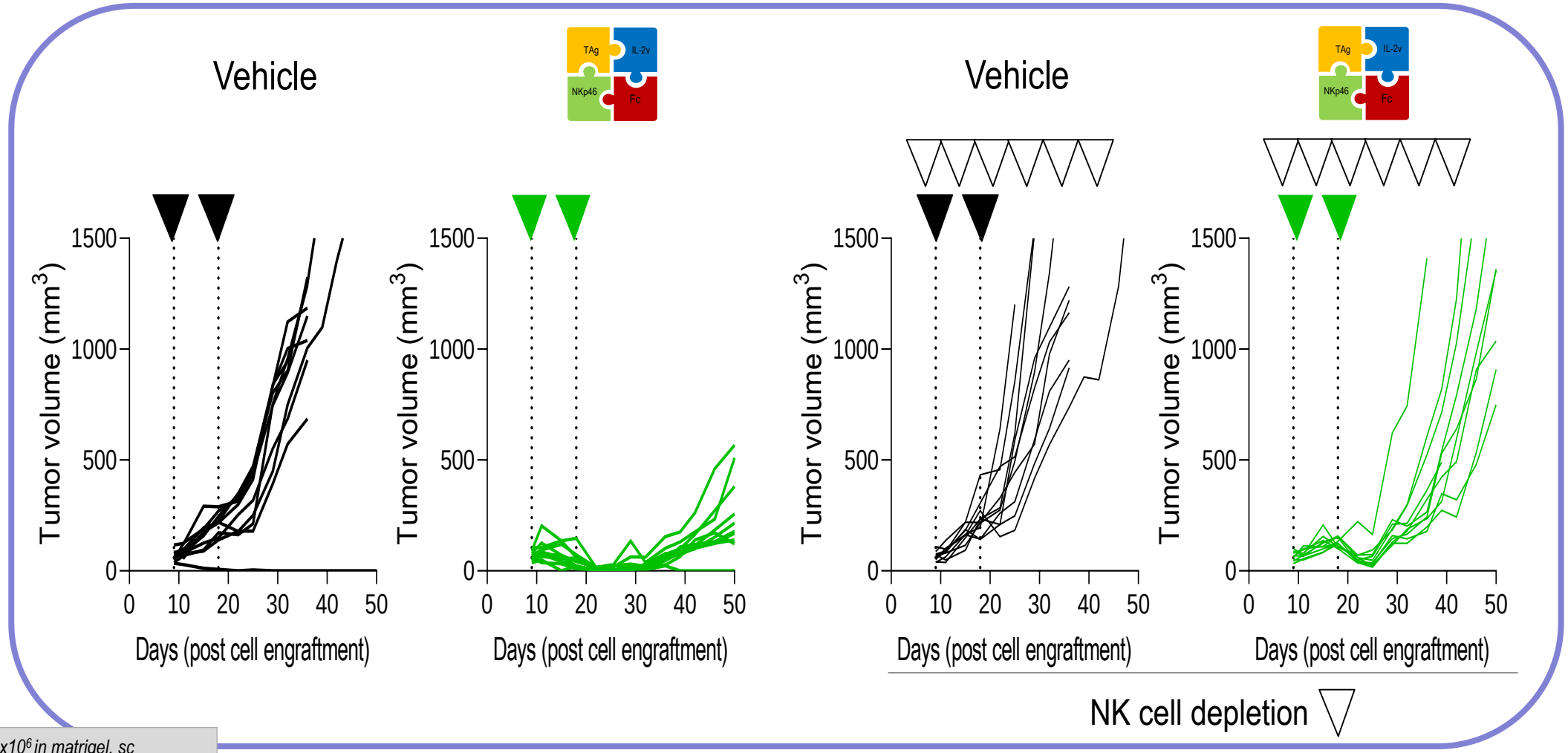




NKCE^4 induces accumulation of activated NK cells at the tumor bed

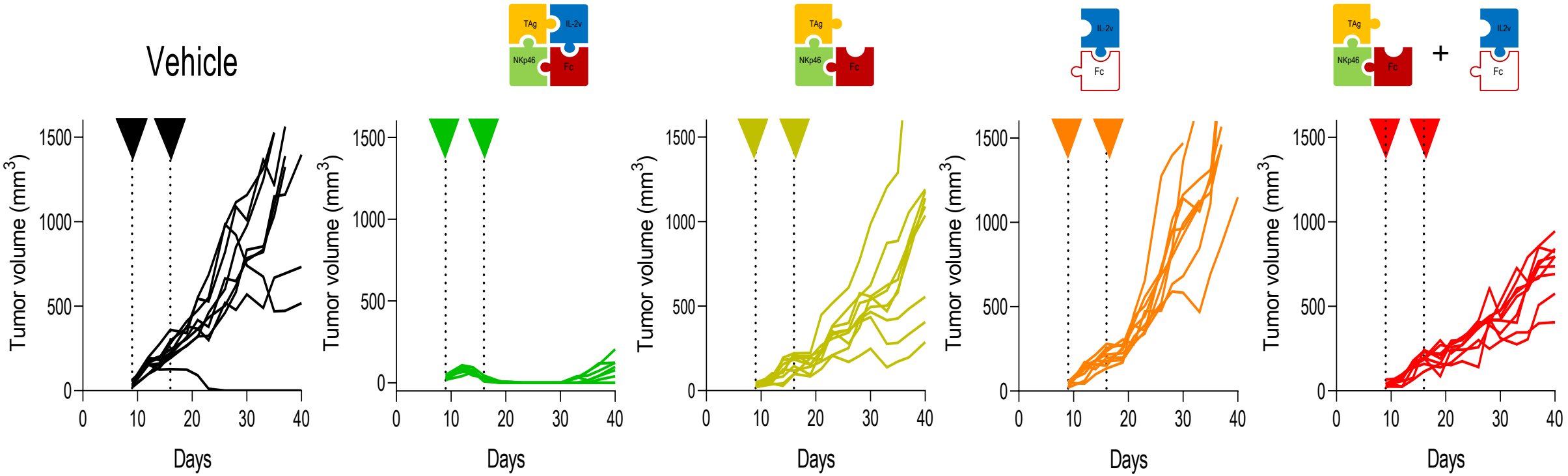
- *Model: Raji, 5x10^6 in matrigel, sc*
- *Mice: CB17 SCID or RAGko huNKp46Tg (immunodeficient)*
- *Treatment: single injection 3 days before analysis*

Mechanisms of NKCE[4] anti-tumor efficacy. 2



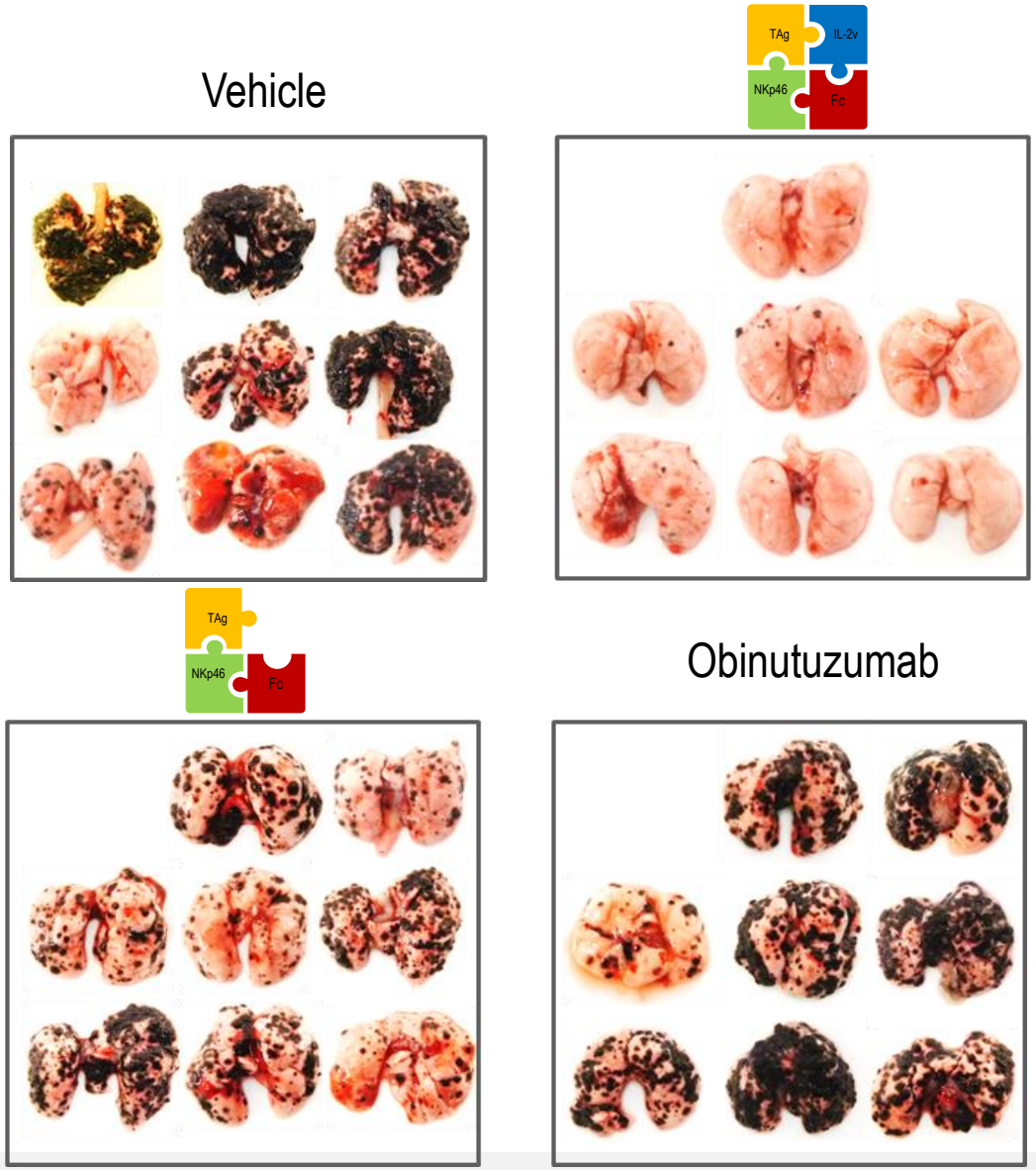
- *Model: Raji, 5x10[6] in matrigel, sc*
- *Mice: CB17 SCID*
- *NK depletion: anti-asialo GM1, q1w*

Mechanisms of NKCE^4 anti-tumor efficacy. 3



Optimal efficacy requires all arms in a single NKCE^4 molecule

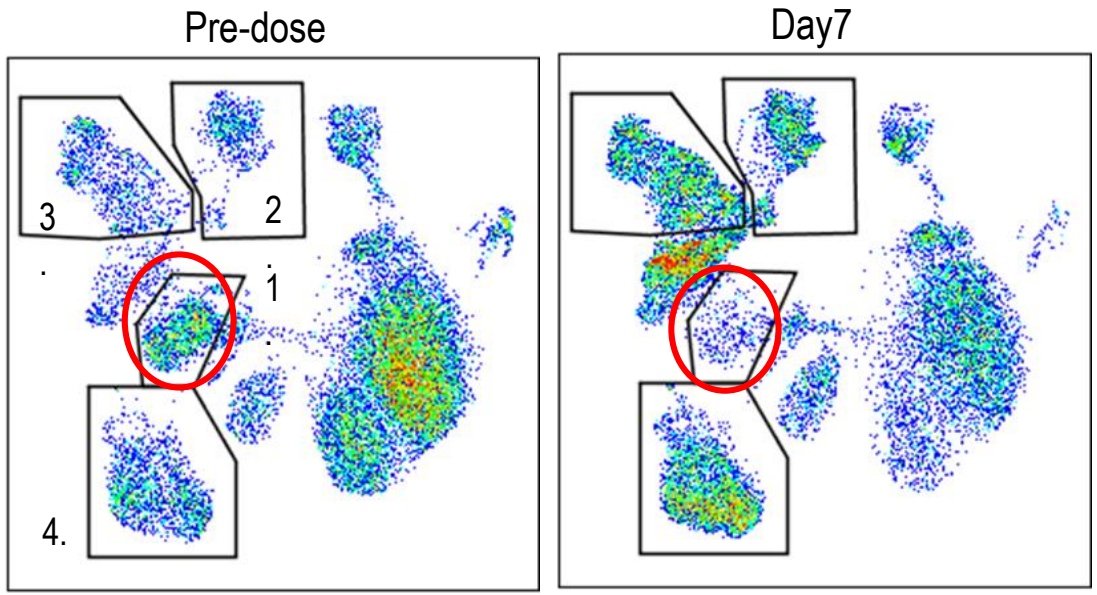
NKCE[4] anti-tumor efficacy: disseminated tumor model



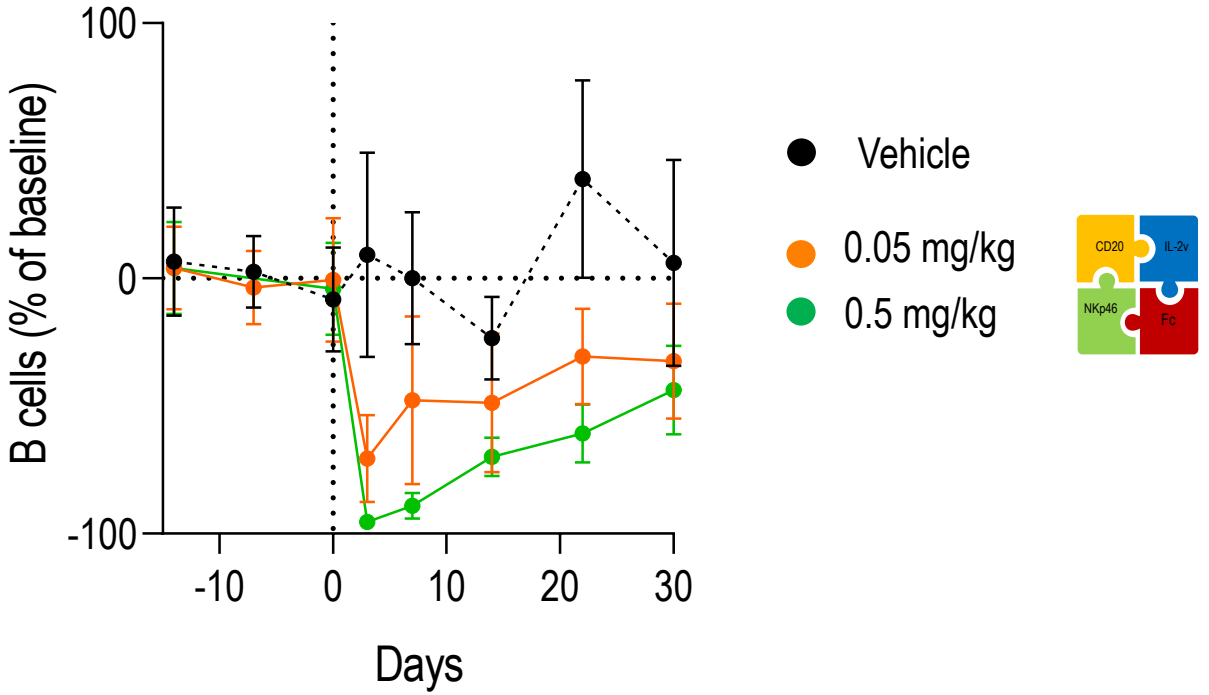
- *Model: huCD20-B16F10, 5x10[5], i.v.*
- *Mice: C57BL/6*
- *Treatment: D1*
- *Lung analysis: D13*

CD20-NKCE[4] induces circulating B cell depletion in NHP

1. B cells
2. NK cells
3. CD8 T cells
4. CD4 T cells

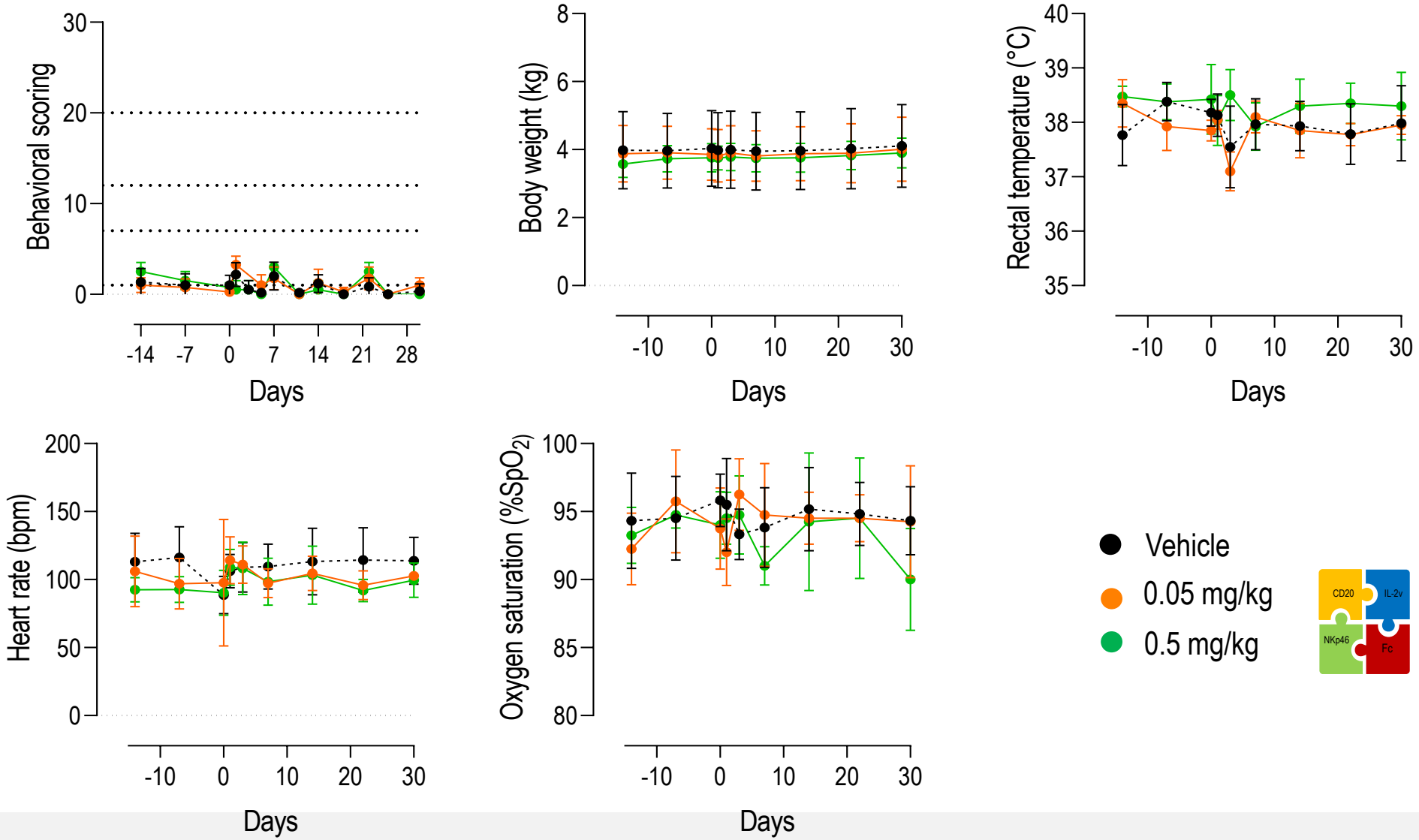


UMAP whole blood NHP treated with CD20 NKCE[4] 0.5 mg/kg, n=4



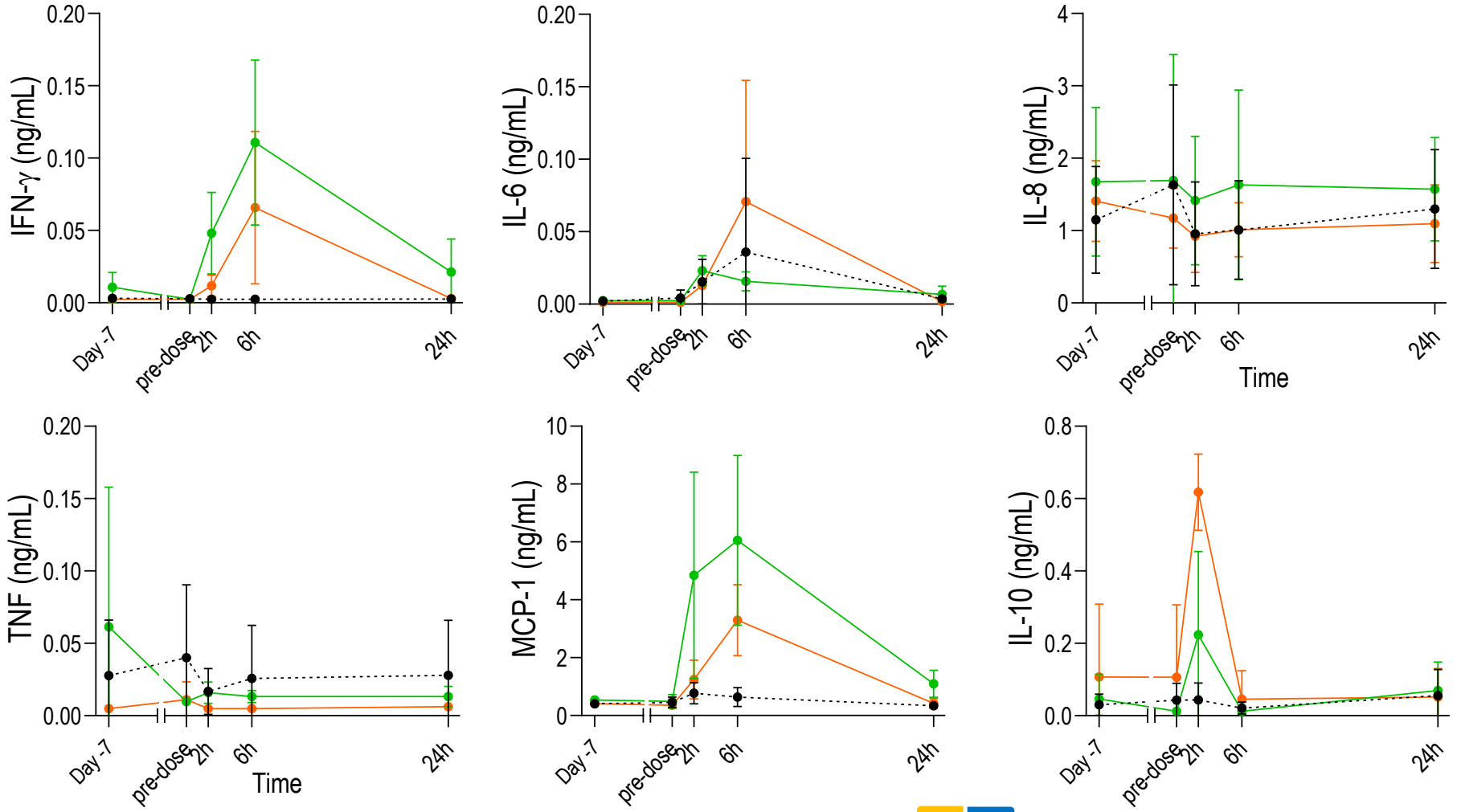
Absence of CD20-NKCE[4] toxicity in NHP

Clinical data



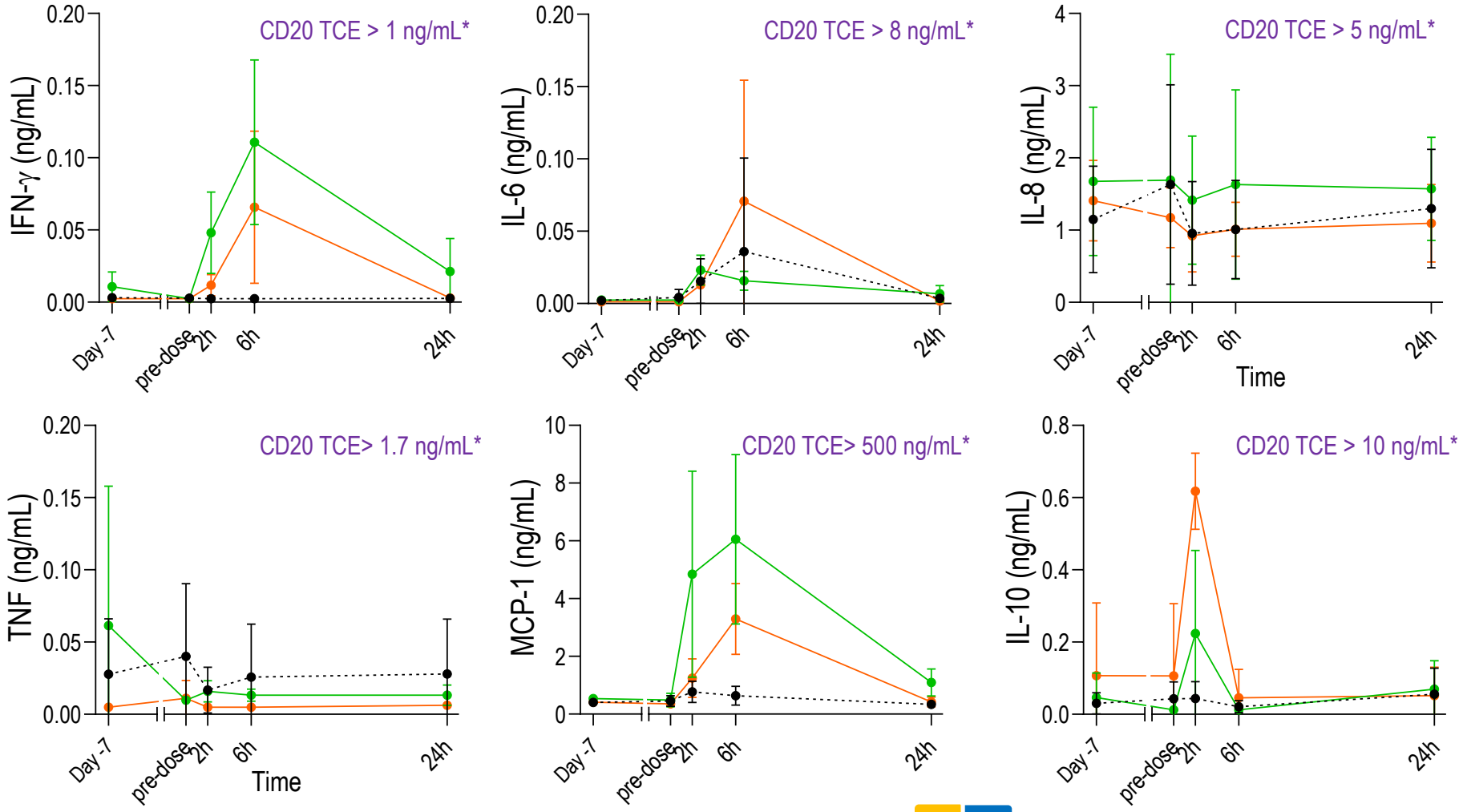
Absence of CD20-NKCE[4] toxicity in NHP

Biological data



Absence of CD20-NKCE[4] toxicity in NHP

Biological data

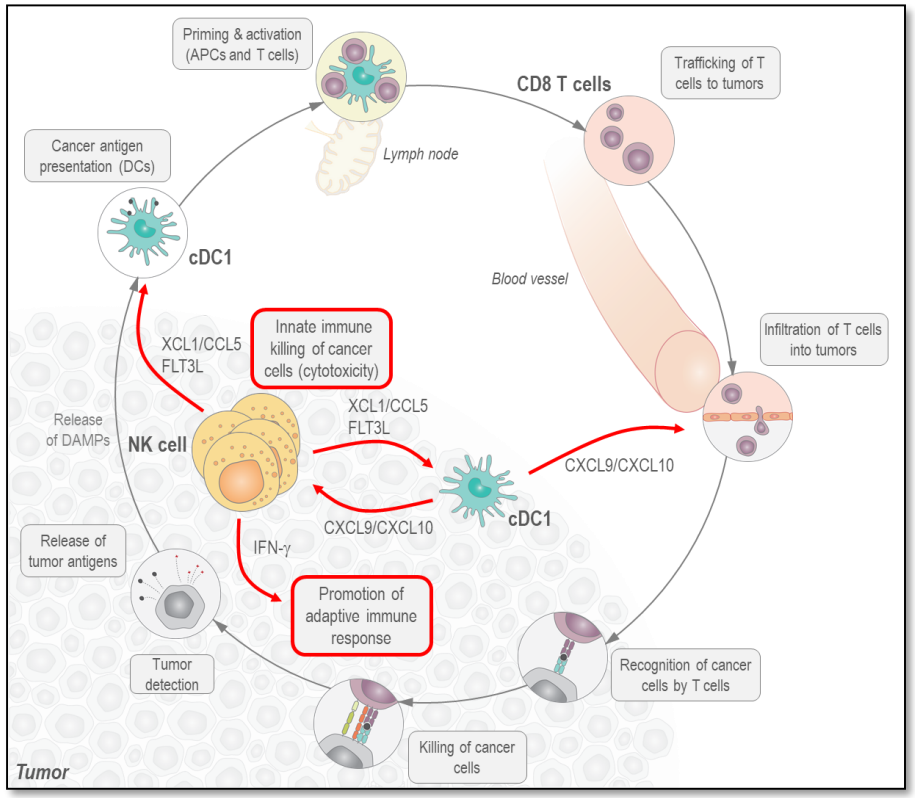


MCP-1 = CCL2

**Engelberts et al., EBioMedicine, 2020*

Natural Killer cell engagers

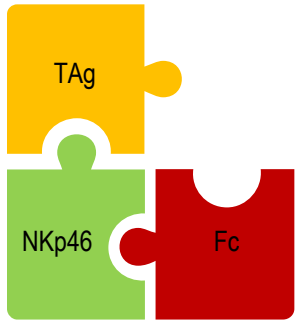
- **NKCE technology provides efficient engagement of NK cells against tumors**
- **NKCE engage NK cells through NKp46, the most NK cell-specific activating receptor**
- Trifunctional NKCE[3] **co-engage NKp46 and CD16 on NK cells** and a tumor antigen on cancer cells; **this leads to potent NK cell activation, cytotoxicity and efficient control of tumor growth** in various preclinical mouse models (*Gauthier et al., Cell, 2019*).
- **NKCE[4] also induce NK cell proliferation and in vitro cytolytic activity against malignant cells expressing the targeted antigen.**
- **NKCE[4] have long PK and show in vivo anti-tumor efficacy in several preclinical tumor models**



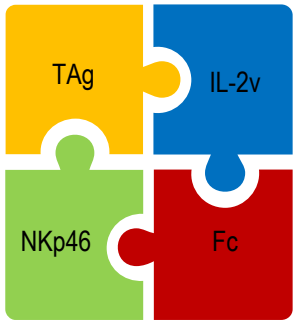
ANKET™

Antibody-based NK cell Engager Therapeutics

NKCE^3



NKCE^4



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Anti-tumor immunity induced by tetrafunctional Natural Killer cell engagers armed with not-alpha IL-2 variant

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