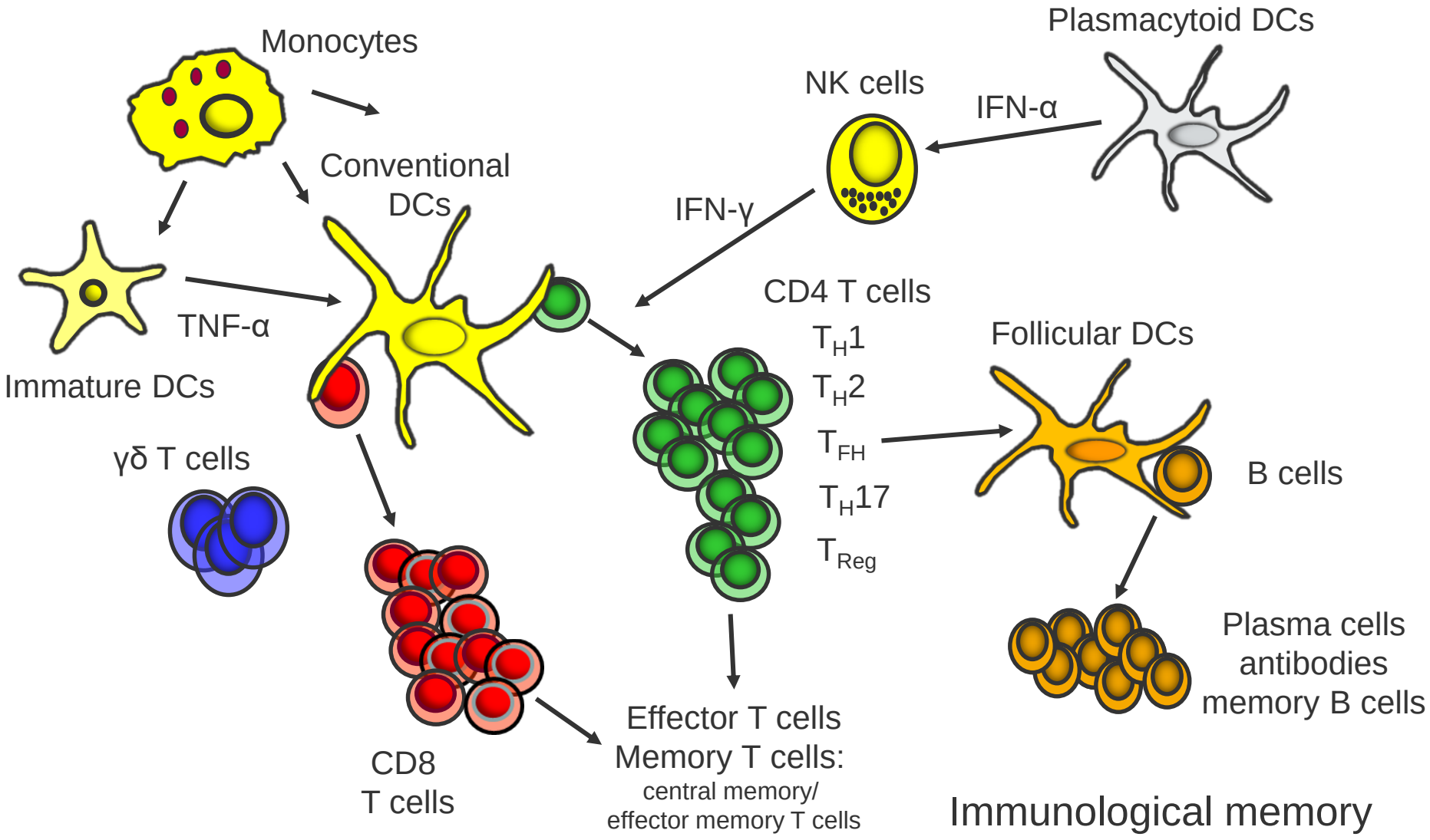
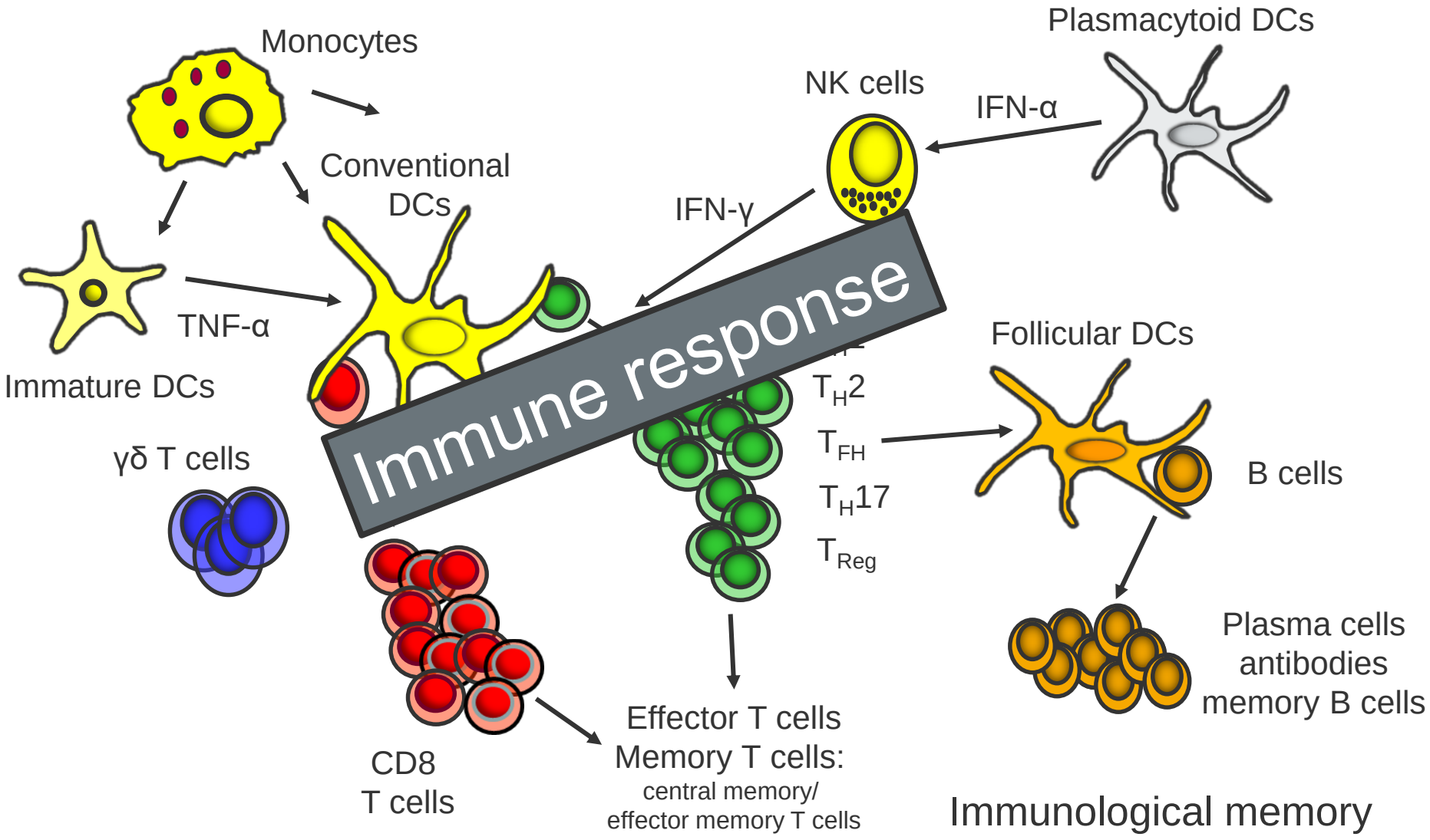
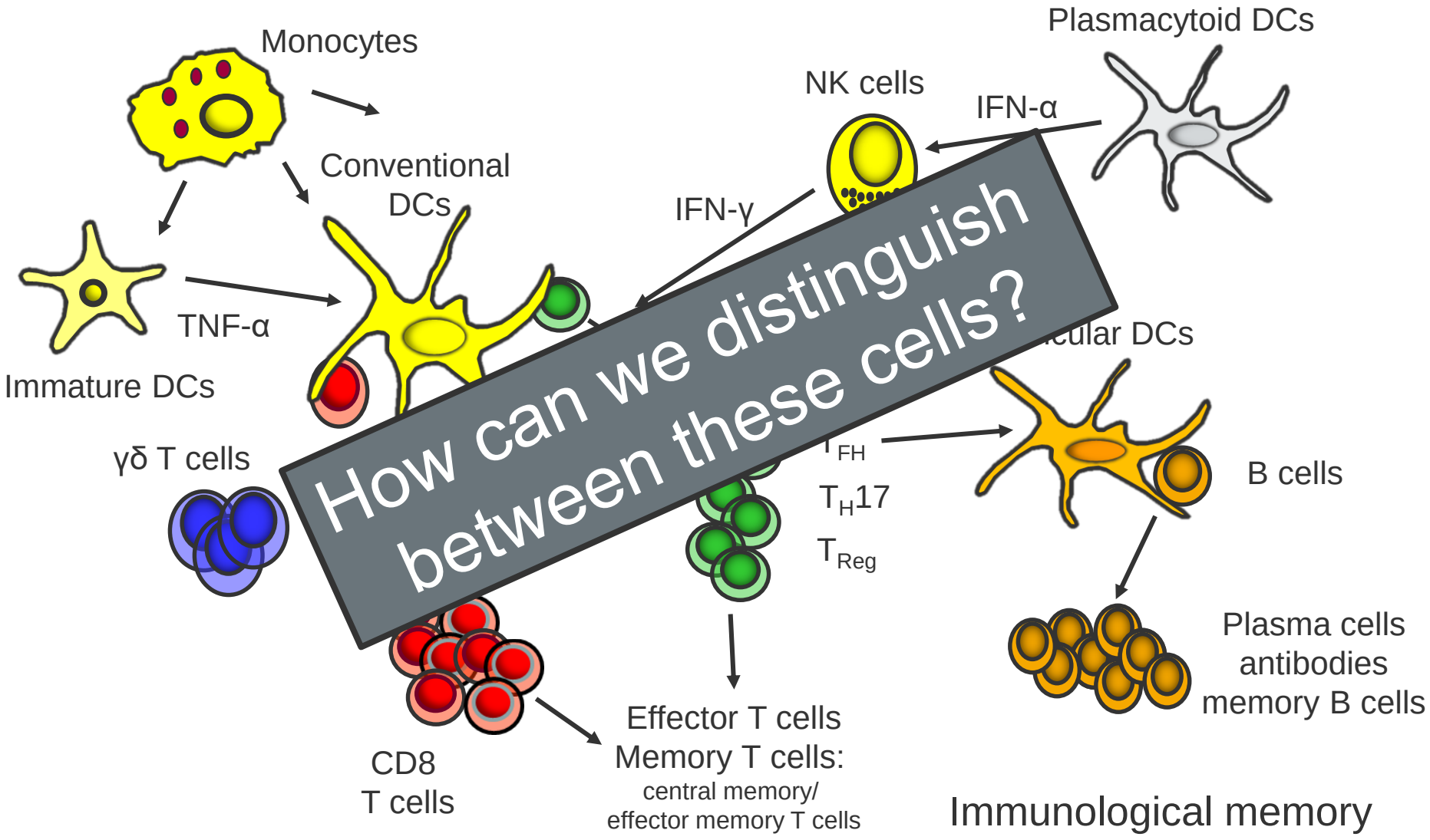
Immune cells - simple overview



Immune cells - simple overview

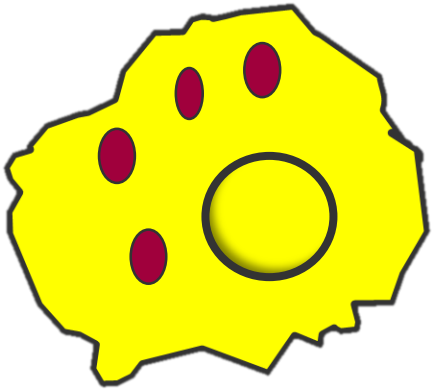


Immune cells - simple overview

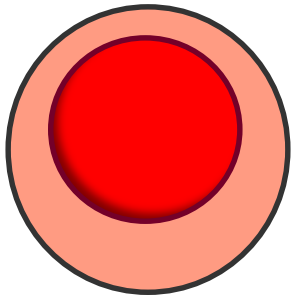


Phenotypes of immune cells

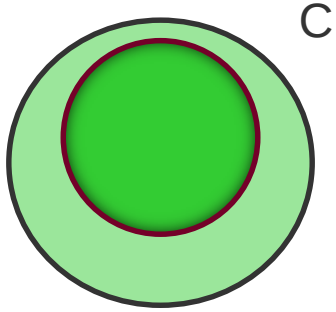
Monocytes

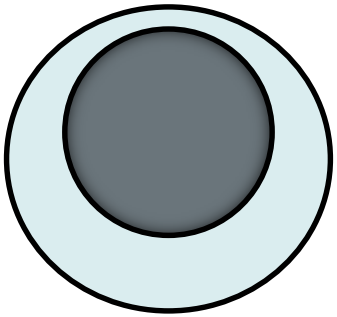


CD8 T cells

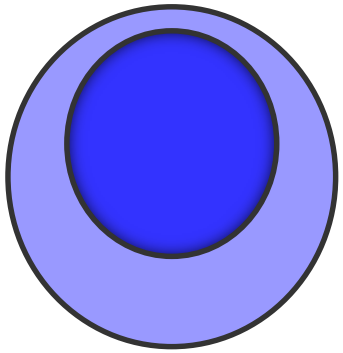


CD4 T cells



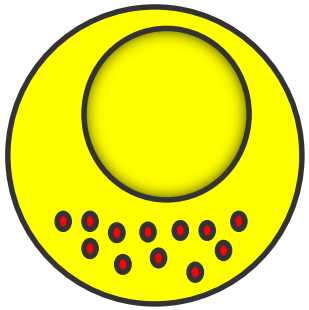


B cells

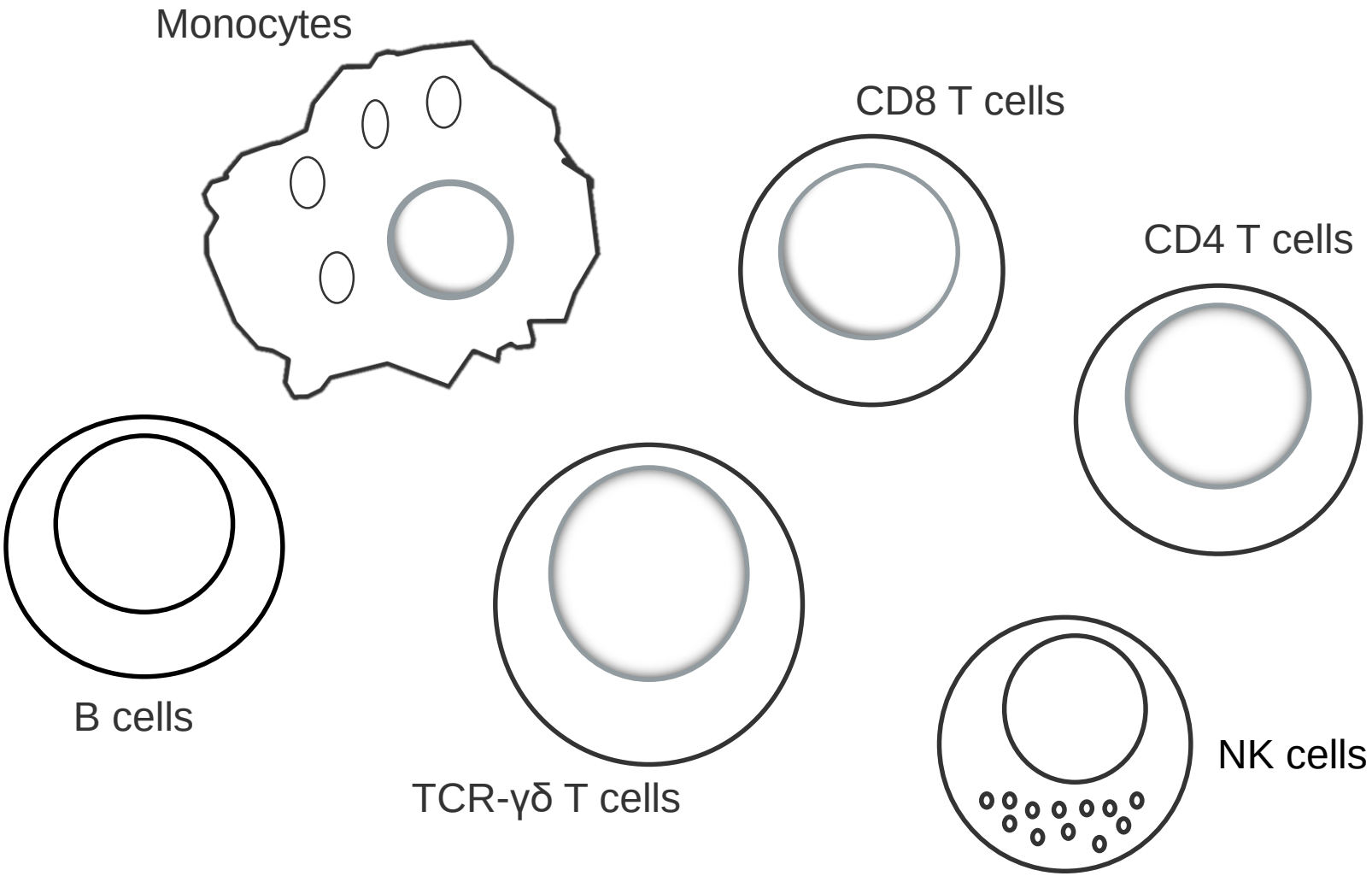


TCR-γδ T cells

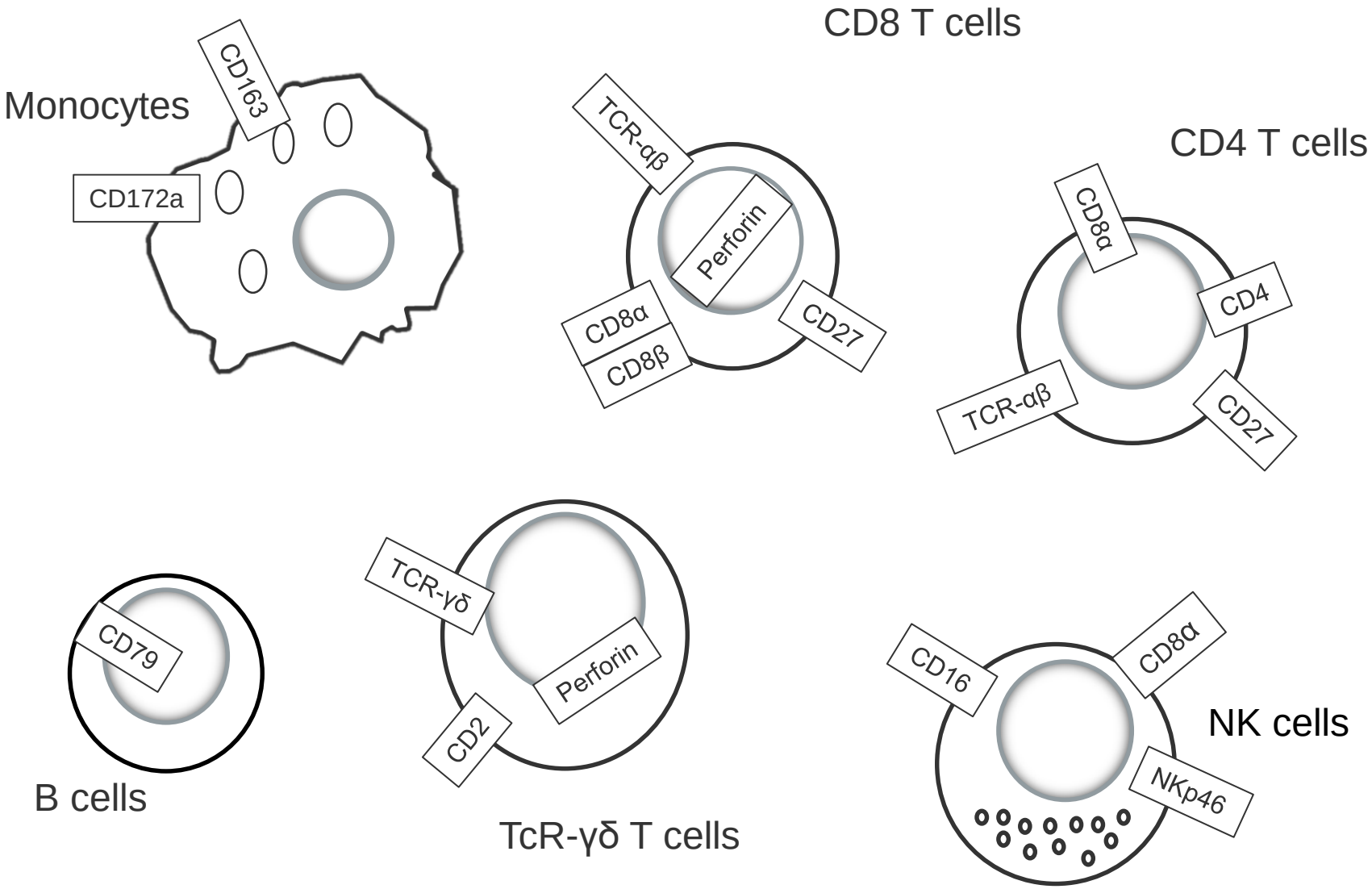
NK cells



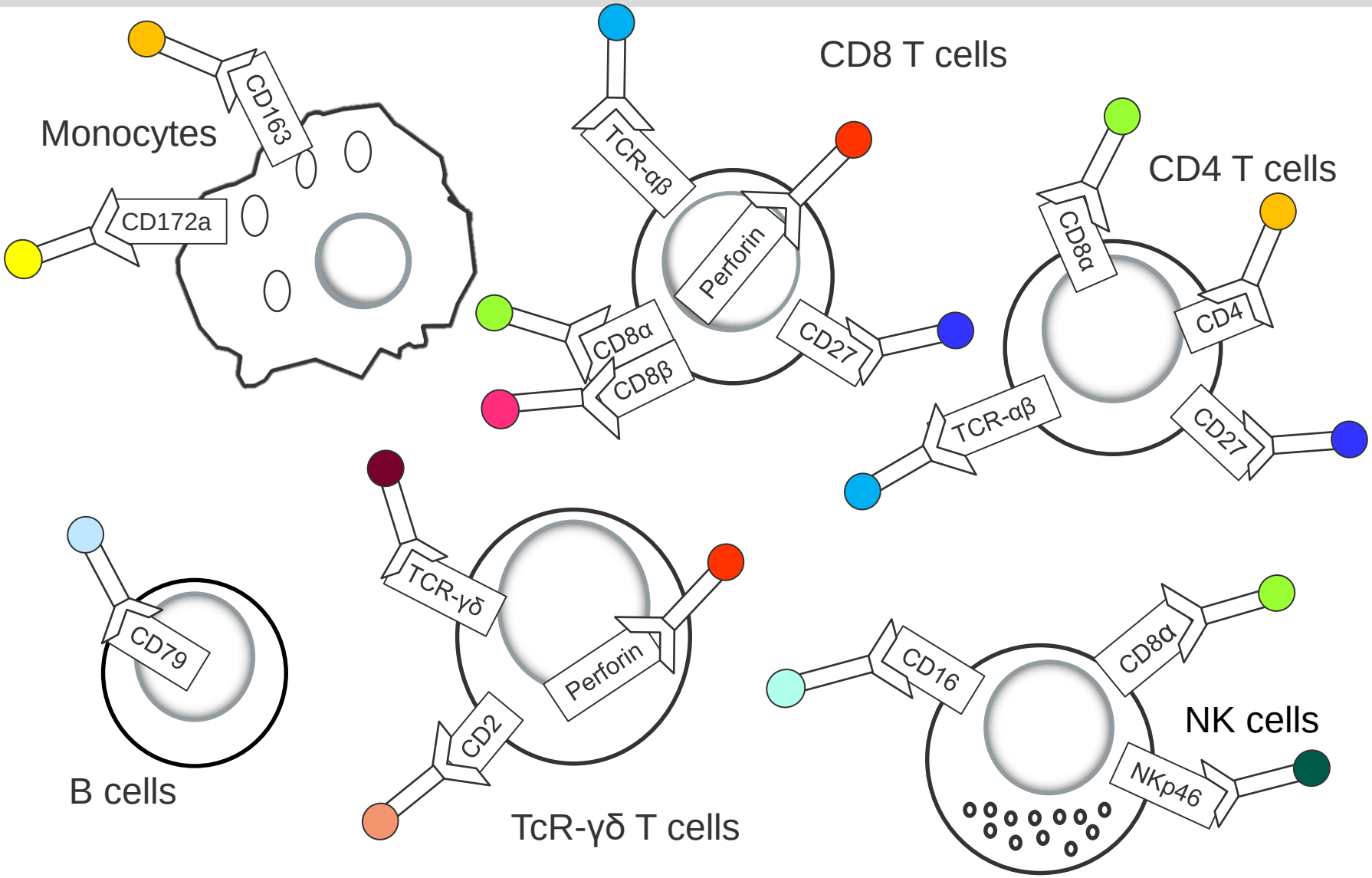
Phenotypes of immune cells



Phenotype-specific antigens

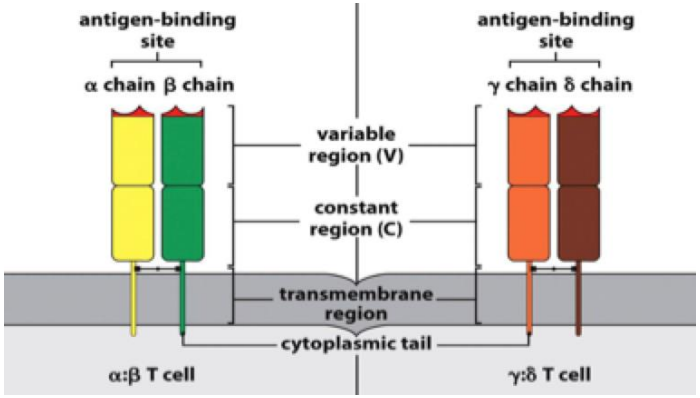


Staining with labelled monoclonal antibodies



T cells – major subsets

- Most T cells: αβ TCR
- αβ T cells restricted by classical MHC molecules
- γδ T cells act MHC independent



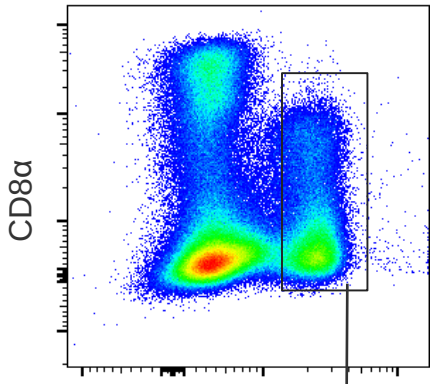
Janeway et al. 2017

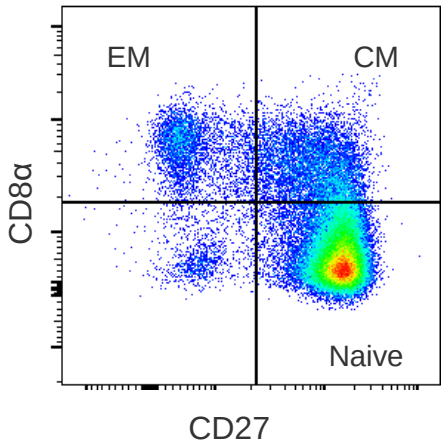
Table 2 Cluster of differentiation (CD) markers on porcine T cells

Population/subset	*Antigen*	*Features*	*References*
T cells	CD1	Progenitors in thymus, not expressed on extrathymic T cells, but on some B cells, monocytes, and DCs	Pescovitz et al. (1984) Saalmüller et al. (1989) Pescovitz et al. (1990)
TcR-αβ T cells	CD2	Expressed on all αβ T cells	Saalmüller et al. (1989) Pescovitz et al. (1994a)
	CD3	All T cells	Saalmüller (1996) Pescovitz et al. (1998)
	CD4	T-helper cells, pDC	Pescovitz et al. (1985) Pescovitz et al. (1994b) Summerfield and McCullough (2009)
	CD5	T cells, not on NK cells, low expression on γδ T cells	Saalmüller et al. (1994a) Saalmüller et al. (1994b)
	CD6	Nearly exclusive expression on αβ T cells	Saalmüller et al. (1994c) Pauly et al. (1996)
	CD8α	Homodimers expressed on NK cells, activated and memory T-helper cells	Saalmüller et al. (1987b) Saalmüller et al. (1994d) Saalmüller et al. (2002)
	CD8β	CD8αβ heterodimers, exclusively expressed on thymocytes and cytolytic T cells	Yang and Parkhouse (1997)
	CD25	α-chain of IL-2 receptor, expressed on activated T cells Tregs	Bailey et al. (1992) Käser et al. (2008a,b)
	CD27	Marker to differentiate between $CD4^+CD8\alpha^+CD27^+$ central and $CD27^-$ effector memory T-helper cells, heterogeneous expression on CD8αβ T cells but functionality not yet defined	Reutner et al. (2012) Reutner et al. (2013) Talker et al. (2013) Gerner et al. (2015)
	CD45RC	Differentiation marker, downregulated on activated and memory T cells	Saalmüller et al. (2002) Talker et al. (2013) Gerner et al. (2015)
	CD52	T cell marker, downregulated after *in vitro* stimulation, also expressed on granulocytes and monocytes	Saalmüller et al. (1987a) Leitner et al. (2012)
TcR-γδ T cells	CD2	Homing behavior, characterization of IFN-γ-producing γδ T cells	Saalmüller et al. (1989) Hirt et al. (1990) Saalmüller et al. (1990) Sedlak et al. (2014a,b)
	CD5	Low expression on γδ T cells	Saalmüller et al. (1994a)
	CD8α	Expression on more differentiated γδ T cells	Sedlak et al. (2014a,b)
	SCW5	Expression on subpopulation of $CD2^-$ γδ T cells	Saalmüller (1996) and Sedlak et al. (2014a)
	TcR-δ chain	Detection of all γδ T cells	Yang and Parkhouse (1996) Davis et al. (1998)

Saalmüller & Gerner 2016 The Immune System of Swine. Encyclopedia of Immunobiology, Vol. 1, 538-548.

Differentiation of CD4$^+$ Tcells





CM: central memory T cells
EM: effector memory T cells

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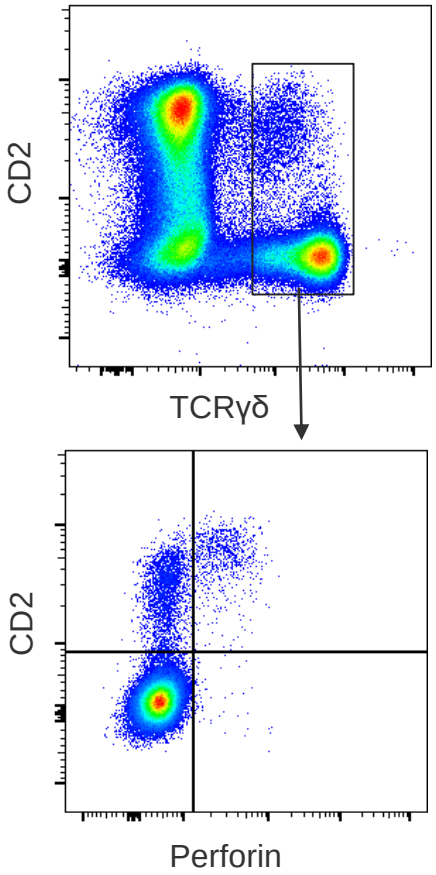
γδ T-cell phenotypes

- γδ T cells



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TcR-γδ T cells	CD2	Homing behavior, characterization of IFN-γ-producing γδ T cells
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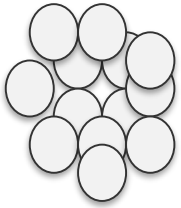
	TcR-δ chain	Detection of all γδ T cells

Saalmüller & Gerner 2016 The Immune System of Swine. Encyclopedia of Immunobiology, Vol. 1, 538-548.

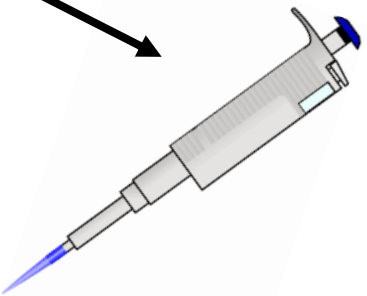
Immune system - Postnatal development

PBMC
isolation

Leukocyte
counting



FCM
staining

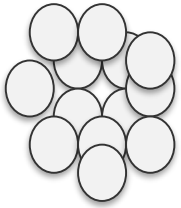


FCM analysis

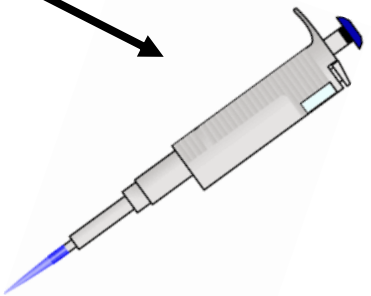
Immune system - Postnatal development

PBMC
isolation

Leukocyte counting



FCM
staining

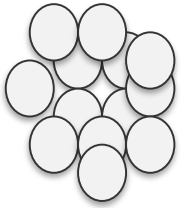


FCM analysis

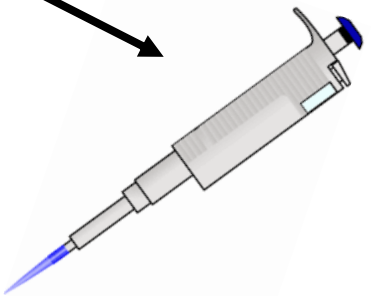
Immune system - Postnatal development

PBMC isolation

Leukocyte counting

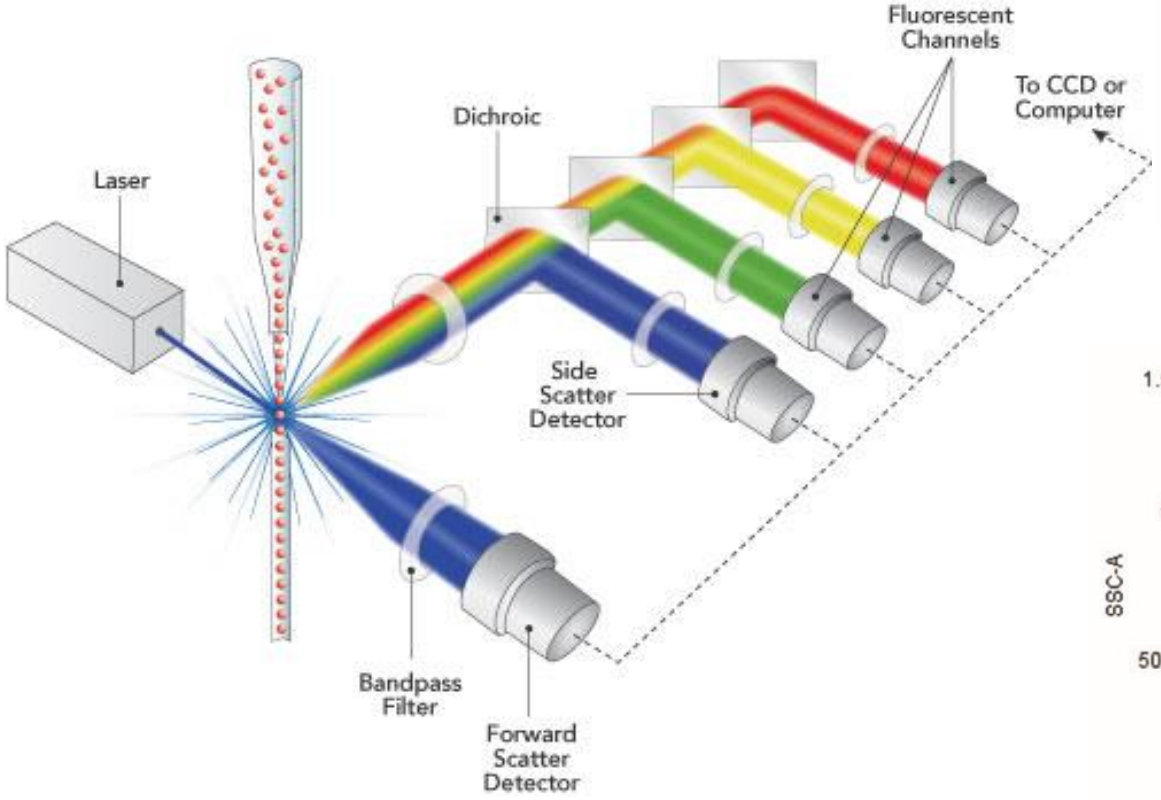


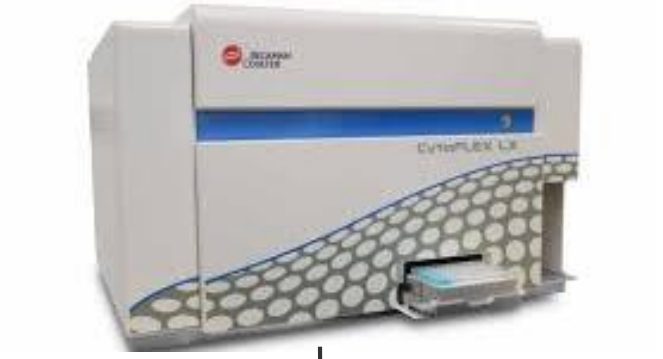
FCM staining

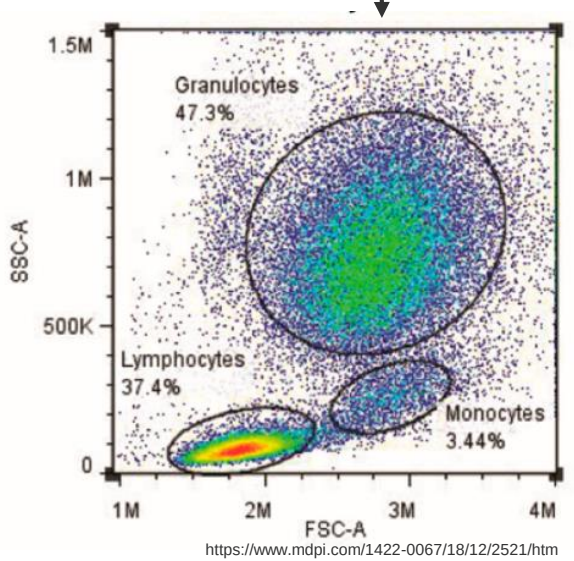


FCM analysis

Multi-colour flow cytometry

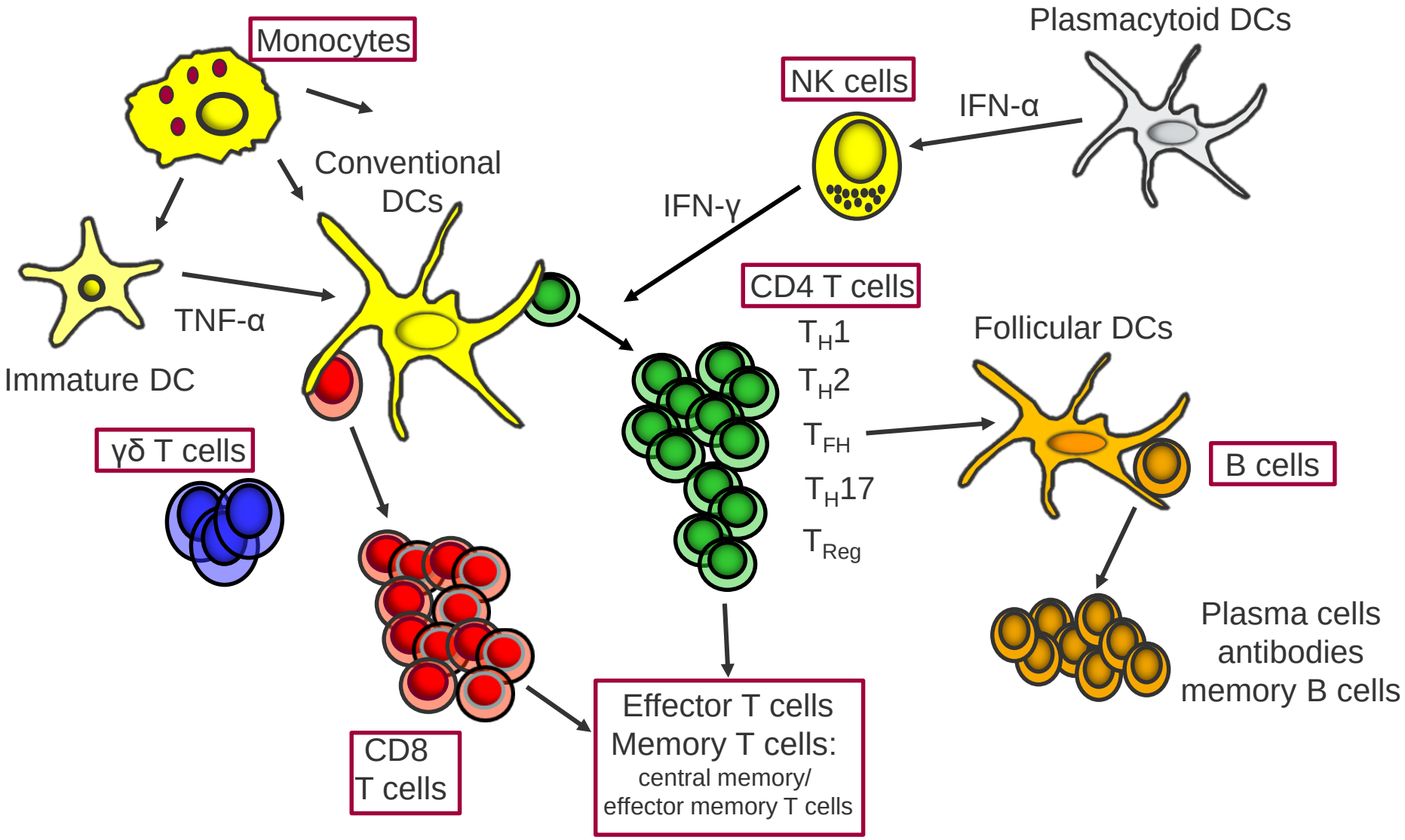




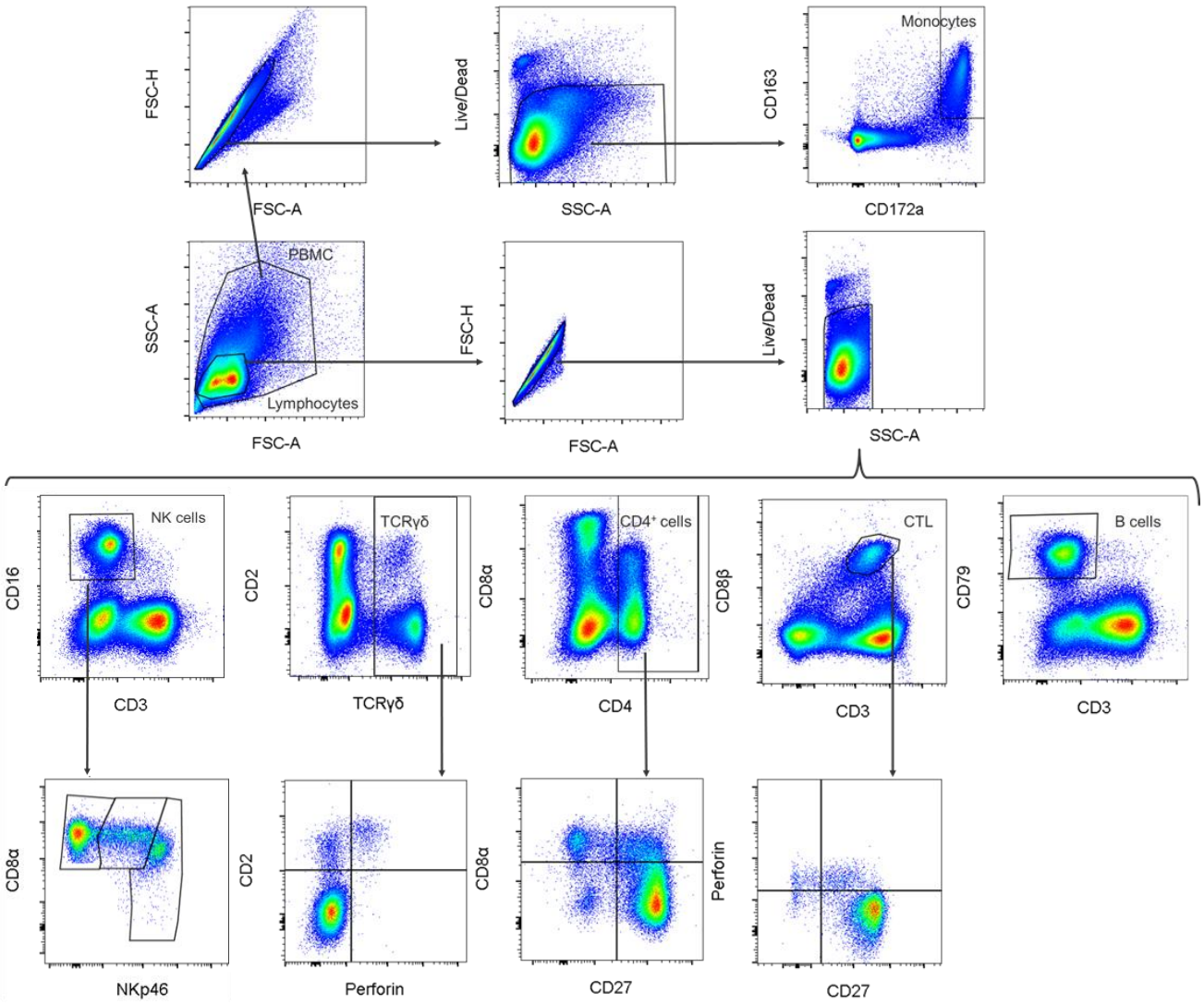


https://www.mdpi.com/1422-0067/18/12/2521/htm

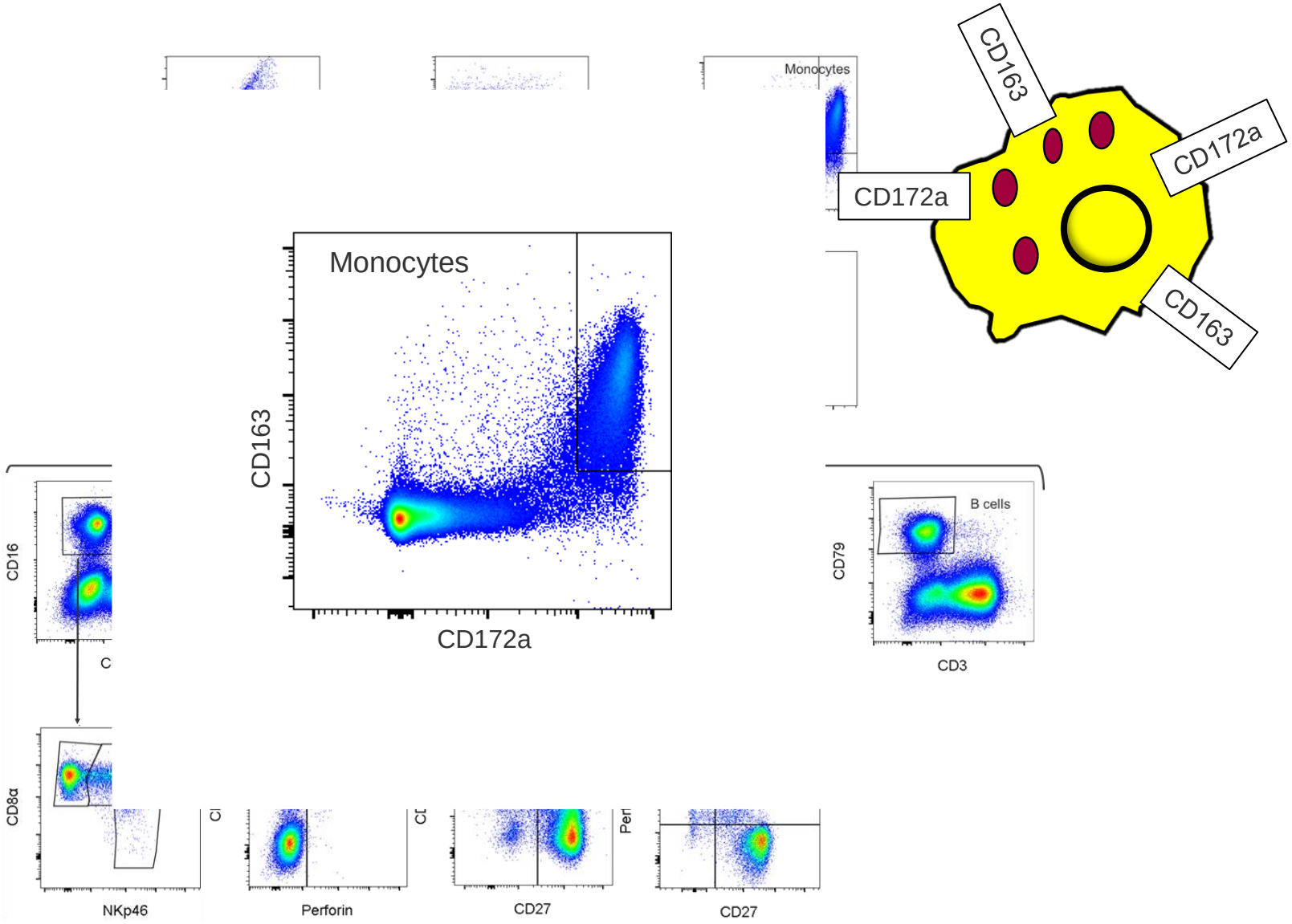
Leukocyte populations



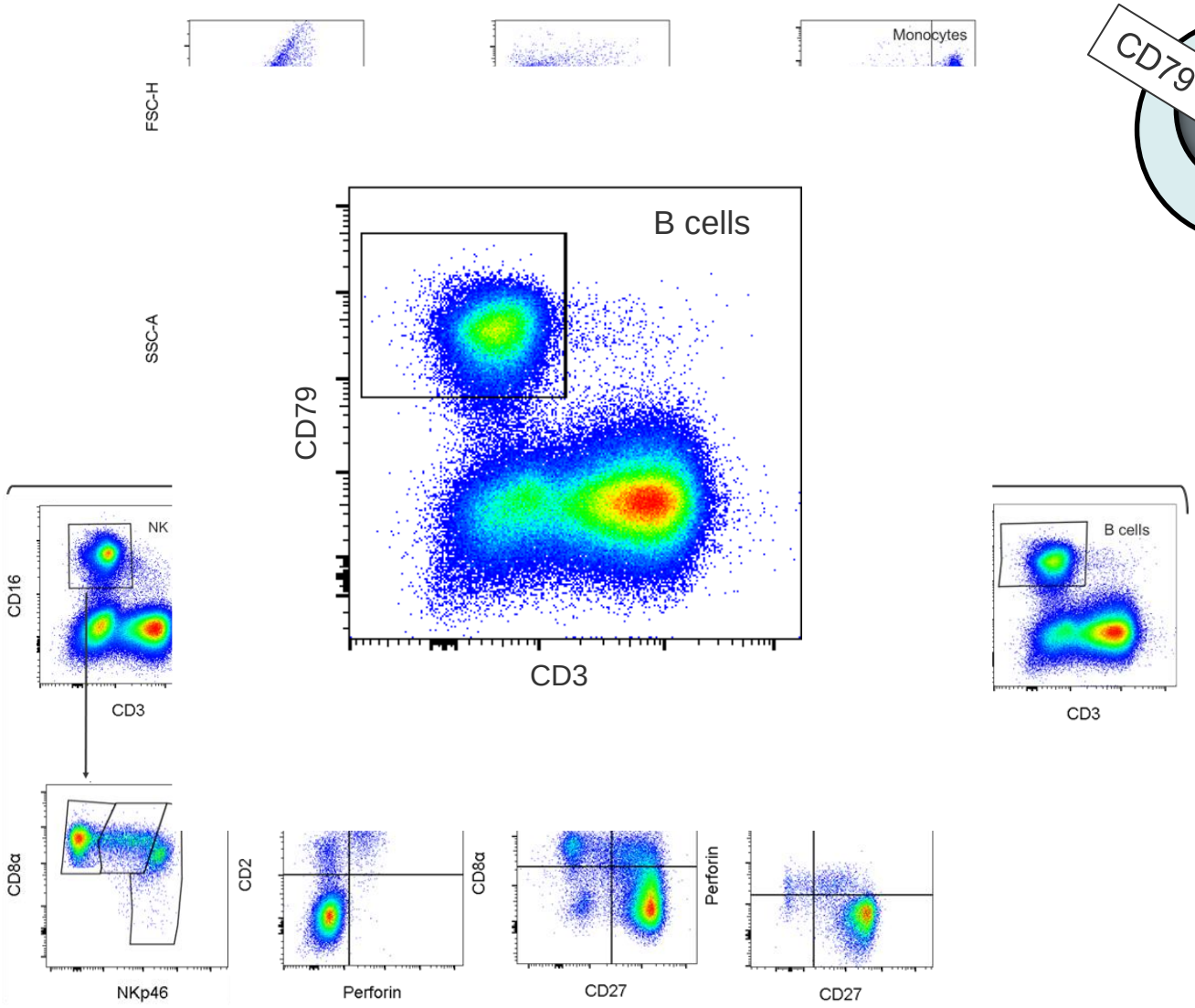
Identification of major leukocyte subsets

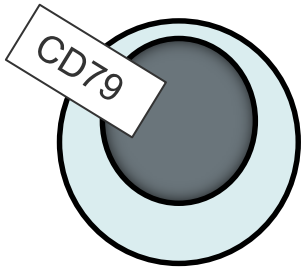


Identification of major leukocyte subsets

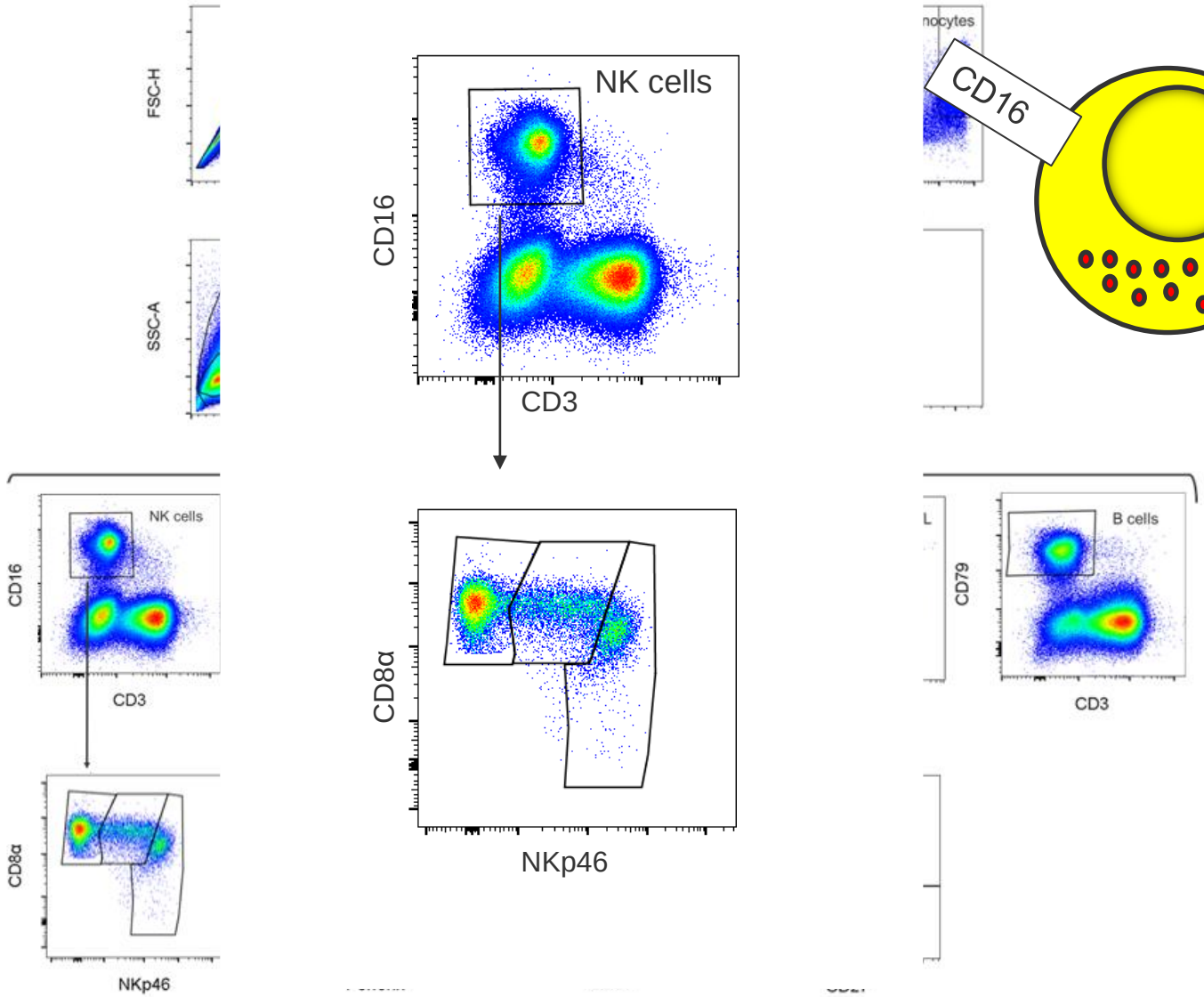


Identification of B cells

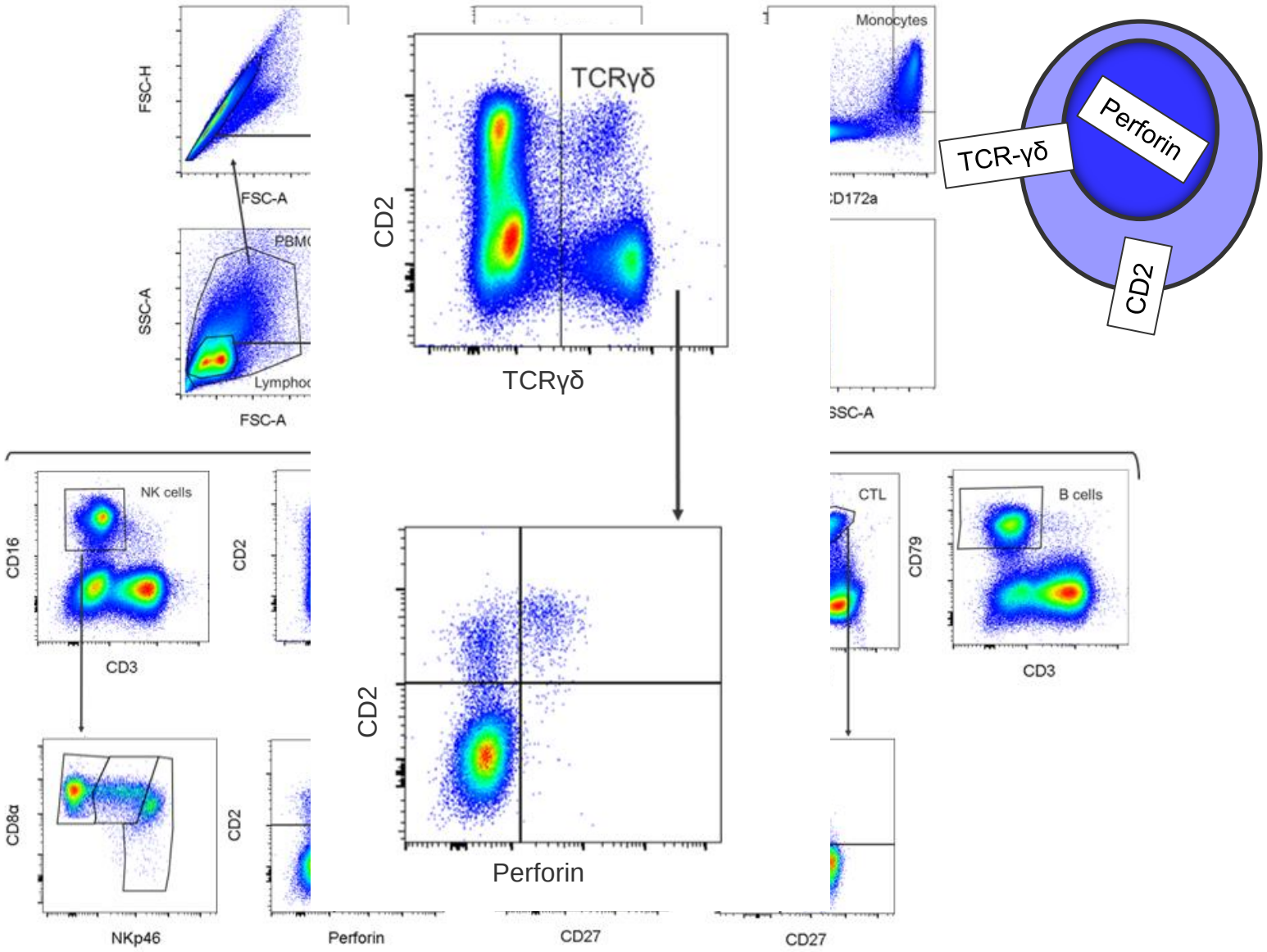




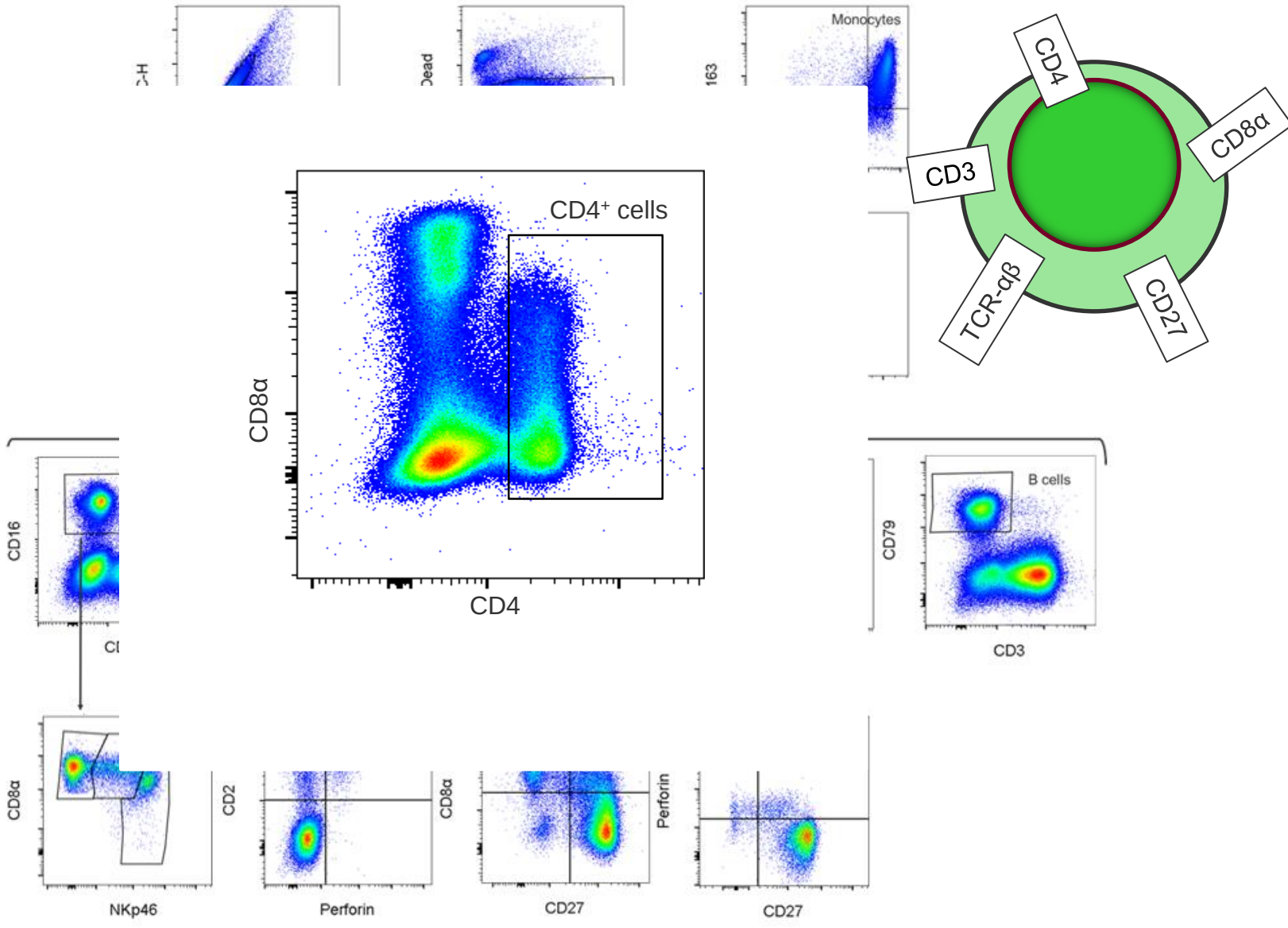
Identification of NK cells



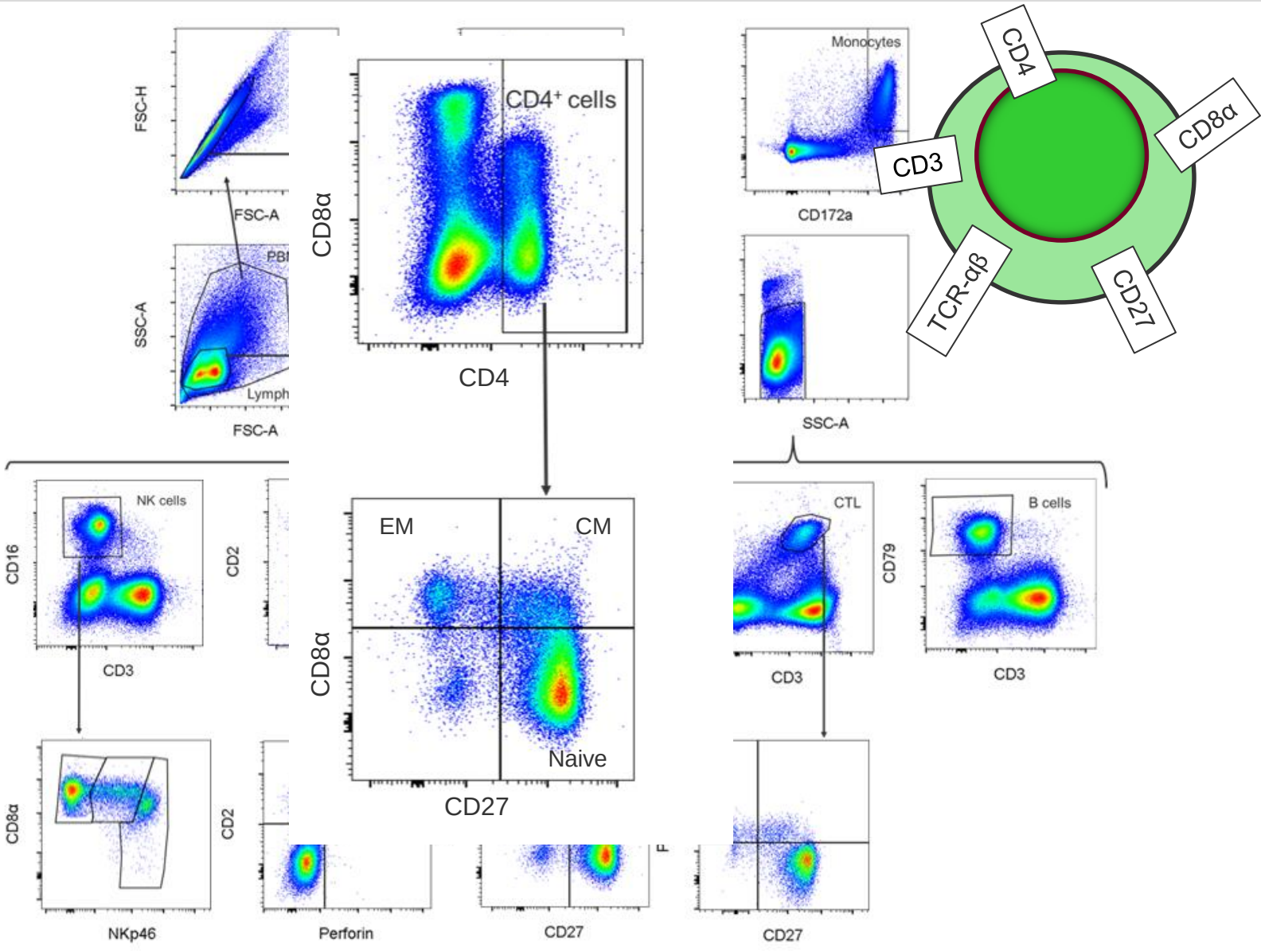
Identification of TCR-γδ T cells



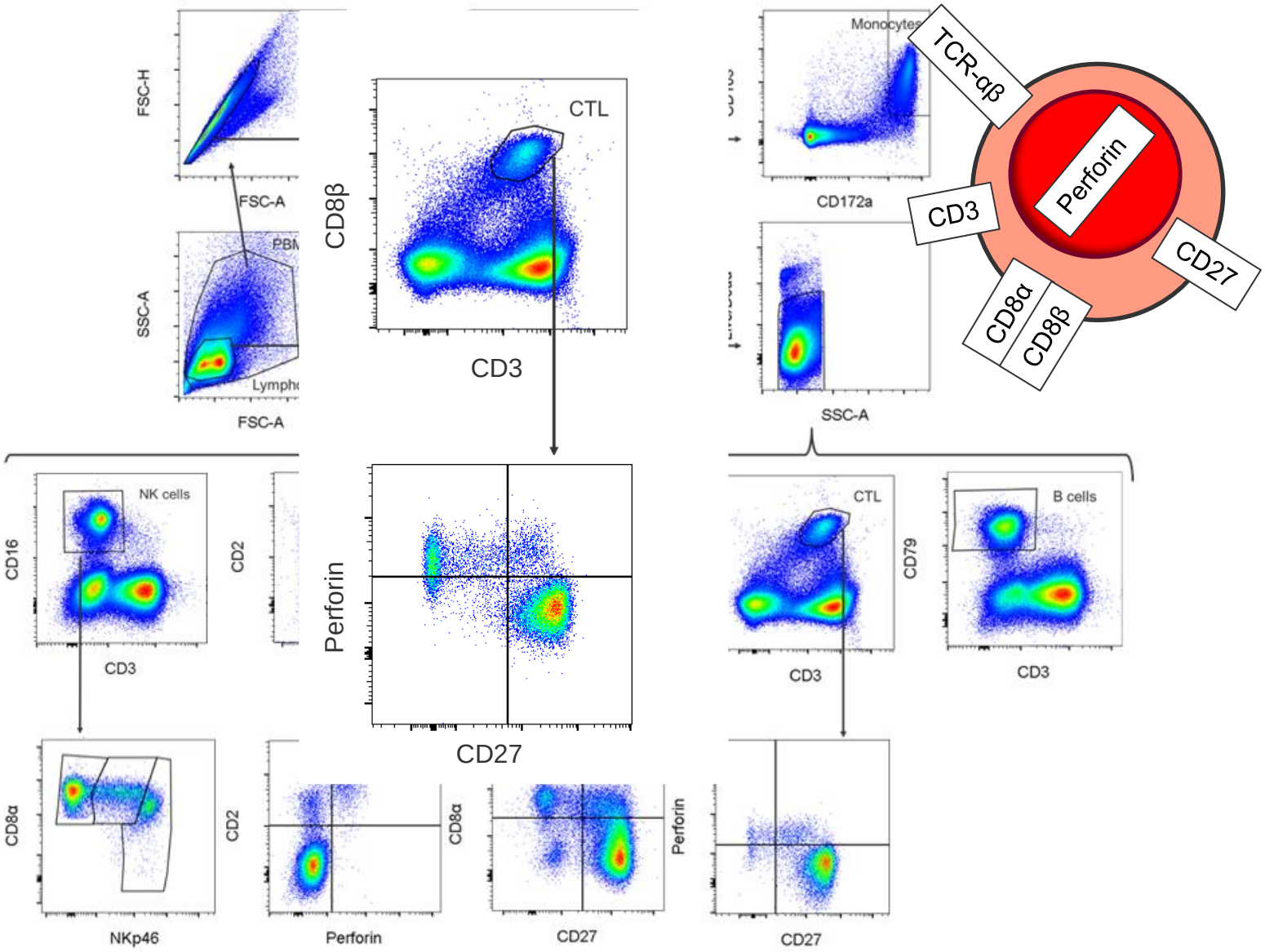
Identification of CD4⁺ T cells



Identification of CD4⁺ T-cell subsets



Identification of CD8β⁺ T-cell subsets



Minipigs as animal model

Are Minipigs a potential model for immune modulatory drug testing?

Usability of Minipigs

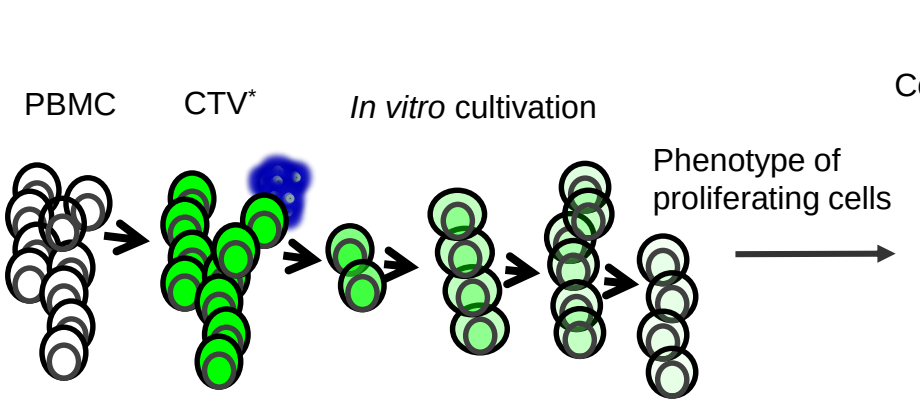
? **For**
- ✓ immunomodulatory drug candidates

? **How**
- ✓ Established test systems
  - T-cell proliferation
  - Cytokine detection (Elispot, ICS*, Luminex)

*ICS = intracellular cytokine staining

? **Why**
- ✓ Usability for development and studies
- ✓ Additional animal model to rodents
- ✓ Comparison to humans

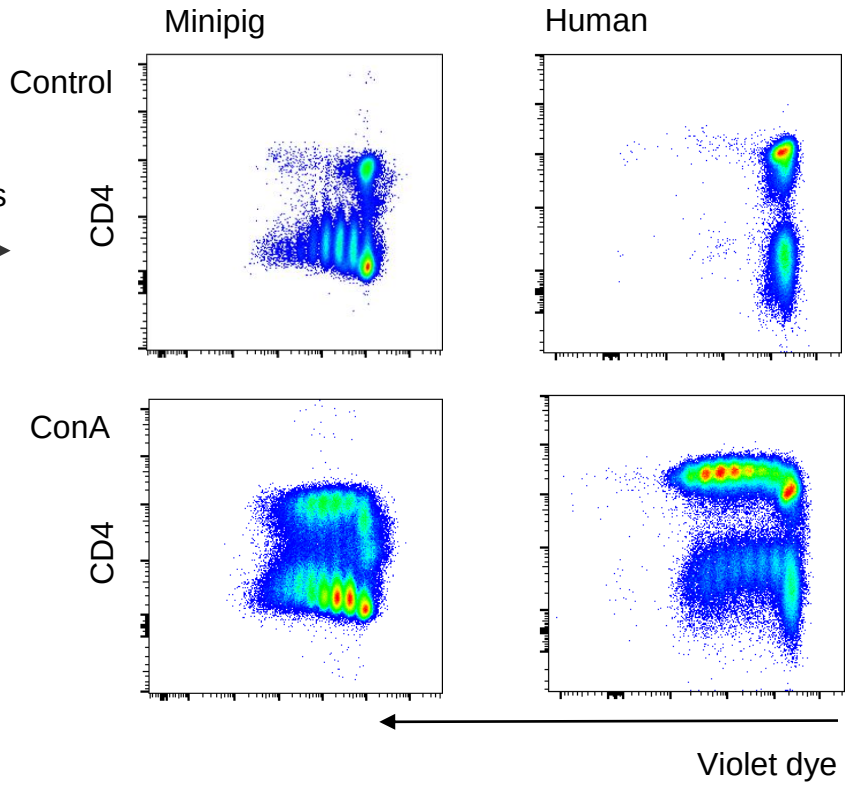
Proliferation assays



Stimulation
- polyclonal (mitogen)
- vaccine antigen

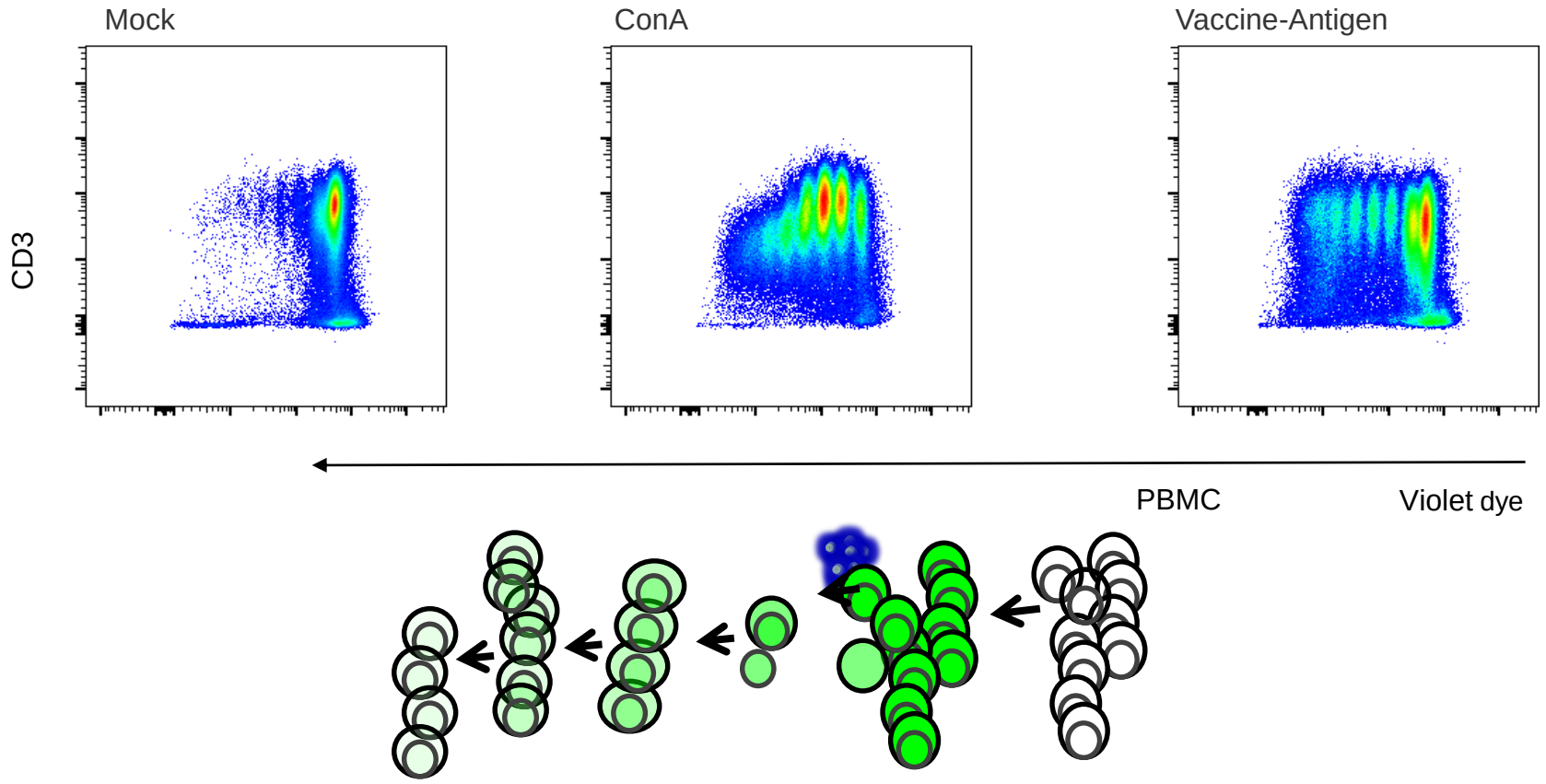
Proliferation
- polyclonal
- antigen-specific

*CTV = Cell trace-violet staining



✓ Minipig PBMCs behave comparable to human PBMCs

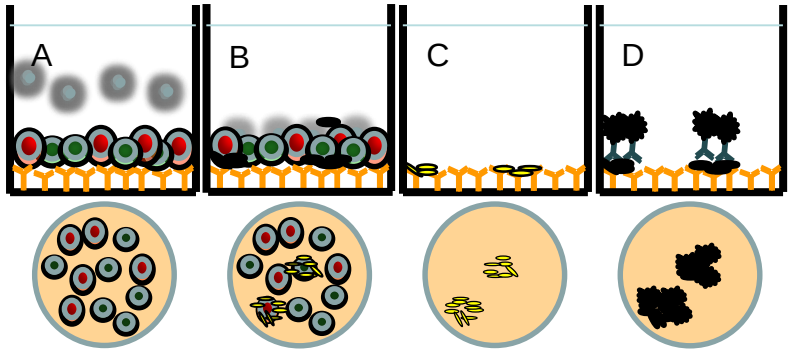
Antigen-specific recall response after vaccination



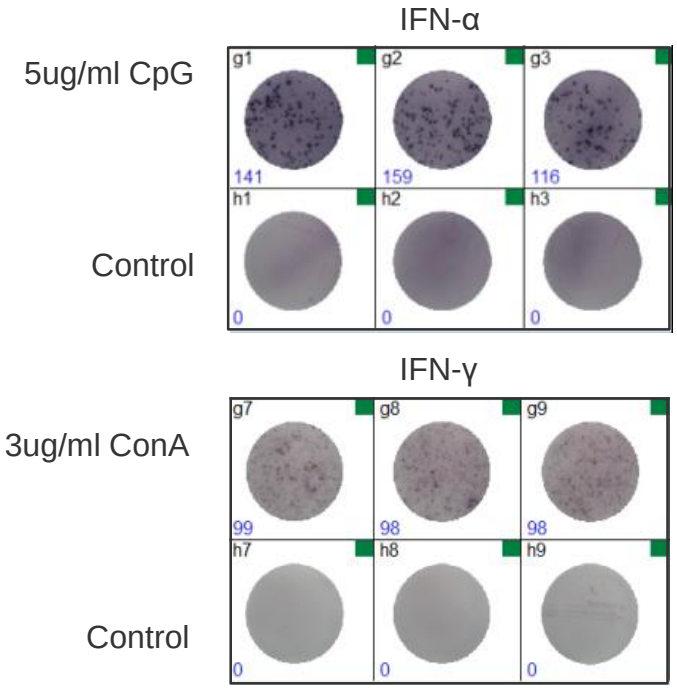
✓ Minipig PBMCs show an antigen-specific T-cell response

Detection of cytokines

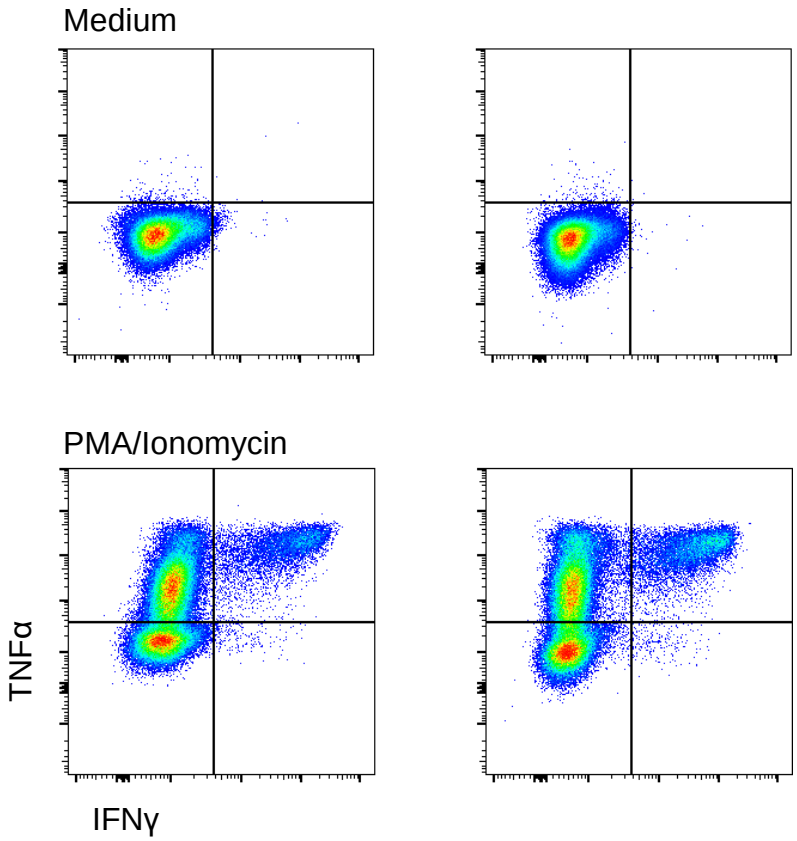
Elispot assays



Koinig et al. 2014



Intracellular cytokine staining



✓ Minipig cytokines can be detected in ELISpot assays and via ICS

Summary and take home message

- ✓ Leukocytes from Minipigs can be further analysed in detail
- ✓ mAbs established for the characterisation of leukocyte populations of domestic pigs are useable for Minipigs
- ✓ An impressive panel of mAbs against porcine CD molecules is available
- ✓ Cytokines from Minipigs can be detected with specific mAb with ELISpot assays, intracellular cytokine staining, Luminex assays
- ✓ Data demonstrated that the development of the Minipig immune system and antigen-specific immune response can be analysed
- ✓ Cell culture assays with Minipig PBMCs are established
- ✓ *In vitro* testing of immunomodulatory drugs with Minipig PBMCs should be possible
- ✓ Comparison to the reactivity of human PBMCs is feasible

Acknowledgments

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Department of Pathobiology

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Kerstin Mair
Jemma Milburn
Martina Patzl
Sandra Podhadsky
Helene Rabl
Selma Schmidt
Maria Stadler
Sonja K

University Clinic for Swine
Department for Farm Animals
and Veterinary Public Health

Andrea Ladinig
Christian Knecht
Sophie Dürlinger
Heinrich Kreutzmann
Michaela K

Thank you very much for your attention!

Sven Jäckel
Michael W. Schmitt