Micro 204
Innate Lymphocytes

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Innate Lymphoid Cells

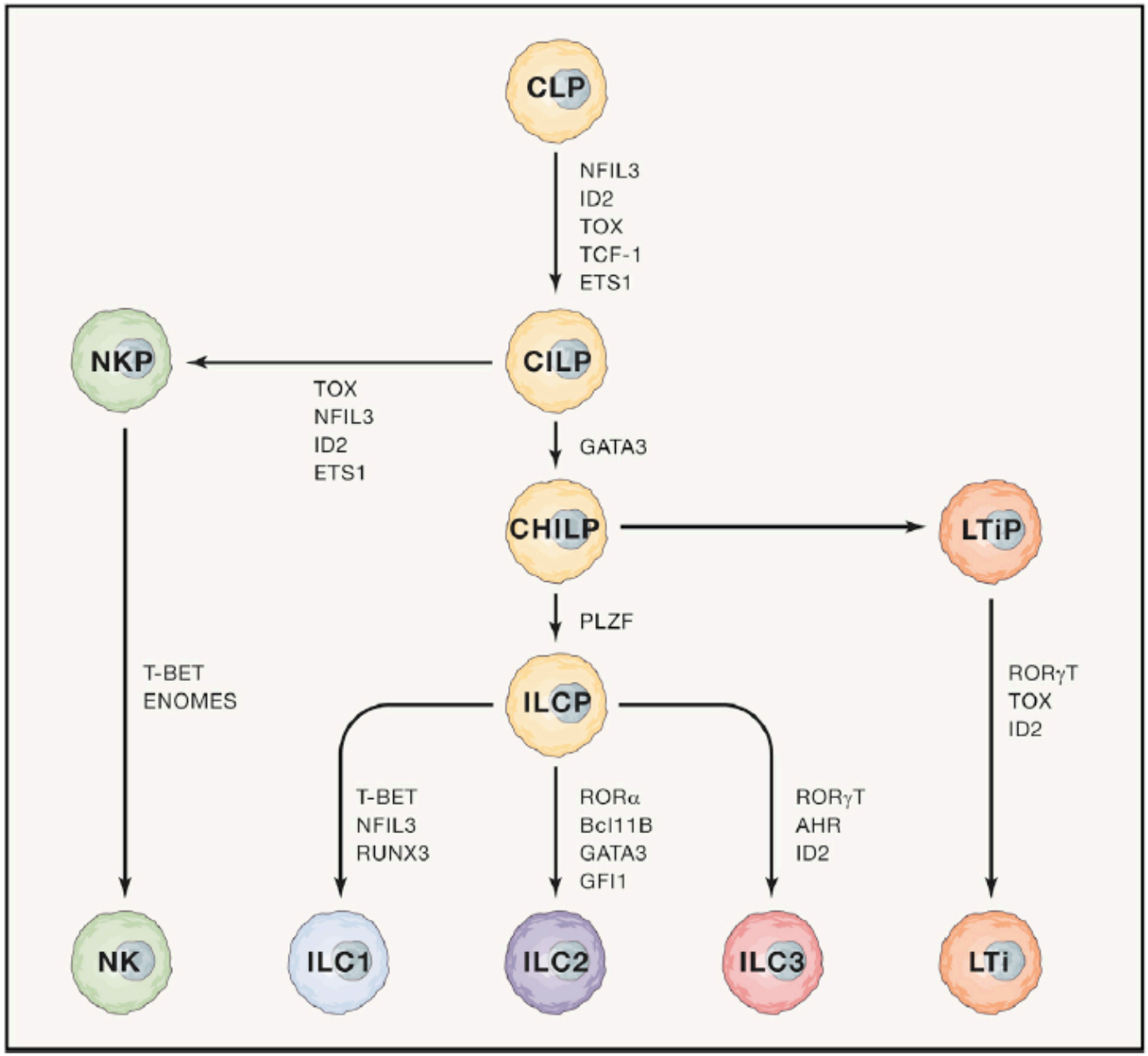
Lineage-negative, Id2-dependent cells that arise from a common lymphoid precursor

Some mediate lymph node organogenesis during fetal development

Some become tissue-resident effector cells expressing cytokines driven by transcriptional modules associated with CD4 helper T subsets (Th1, Th2, Th17)

Roles in homeostasis - establishing commensals, responding to dietary signals, responding to circadian cues, etc.

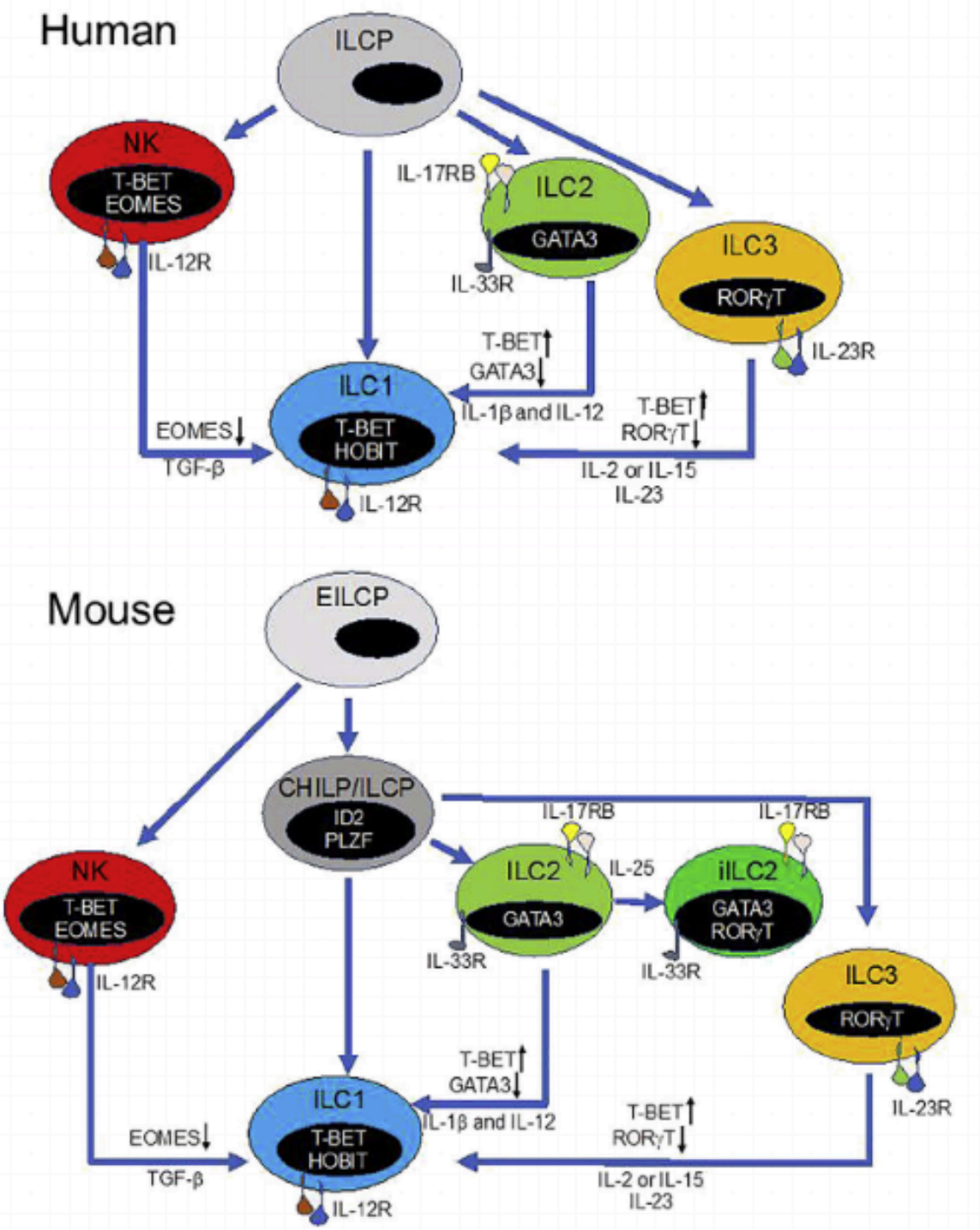
Roles in inflammation and disease



Vivier et al. Cell 2018

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

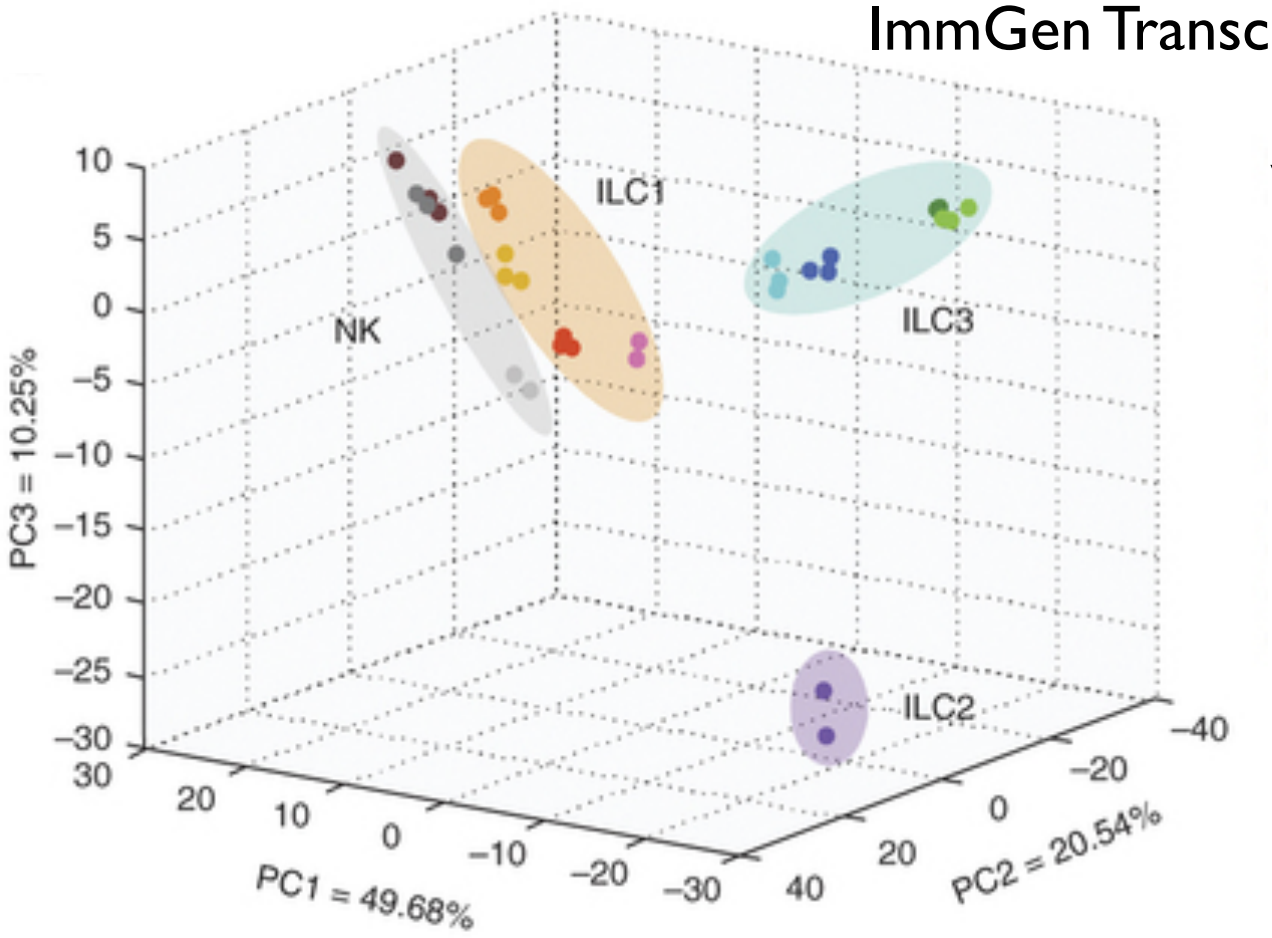
Vivier et al. Cell 2018



Colonna Immunity 2018

ImmGen Transcriptional Analysis

www.immgen.com



Unclear whether ILC1 cells and NK cells are truly distinct lineages or a spectrum of cells within a single lineage that includes ILC1 cells, immature NK cells and mature NK cells

Nature Immunology **16**, 306–317 (2015)

Natural Killer versus Natural Killer T (NKT) cells

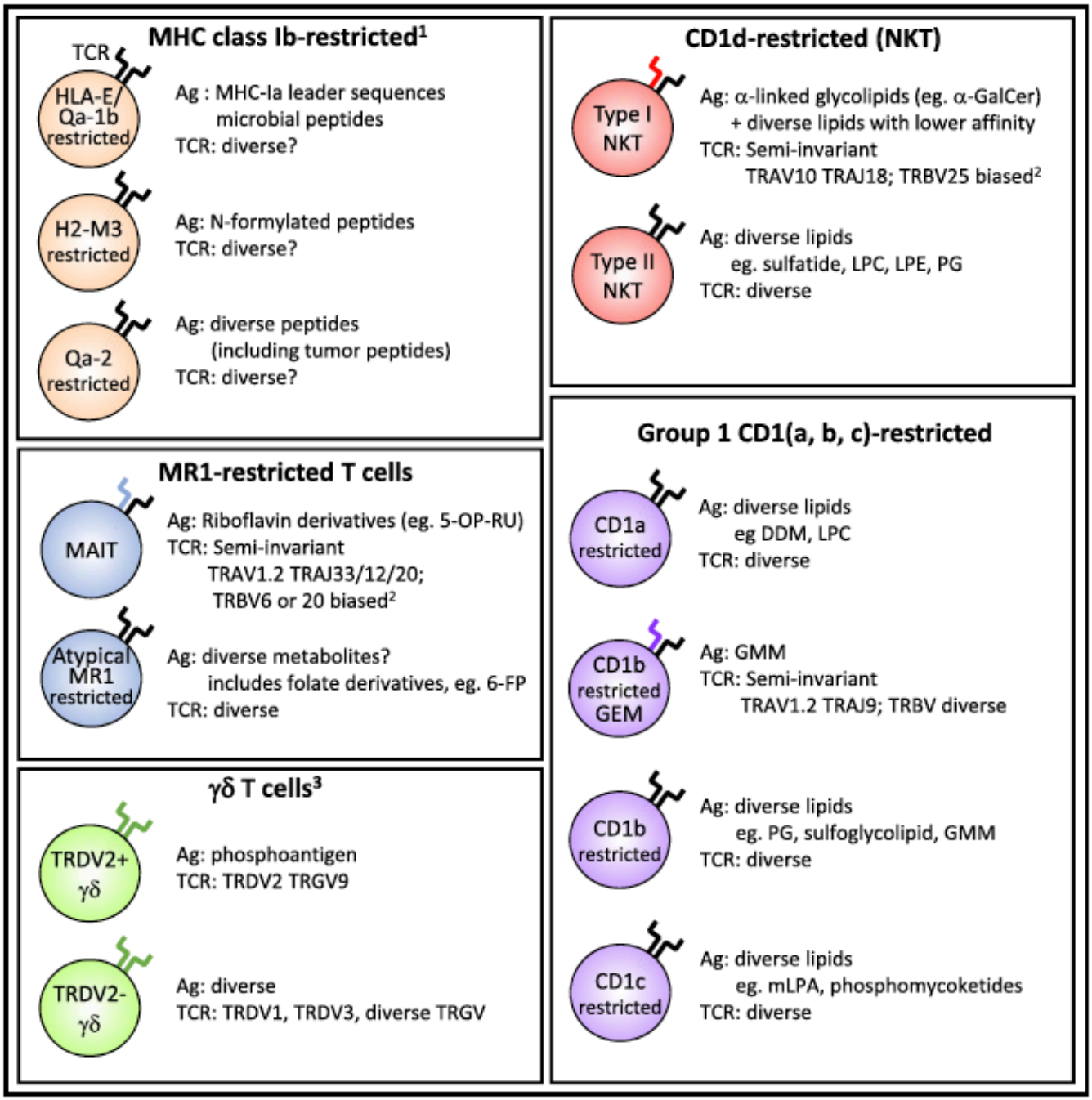
What is the difference?

NKT cells are T cells!

rearrange TcR genes, express an invariant αβ-TcR, and require a thymus for development

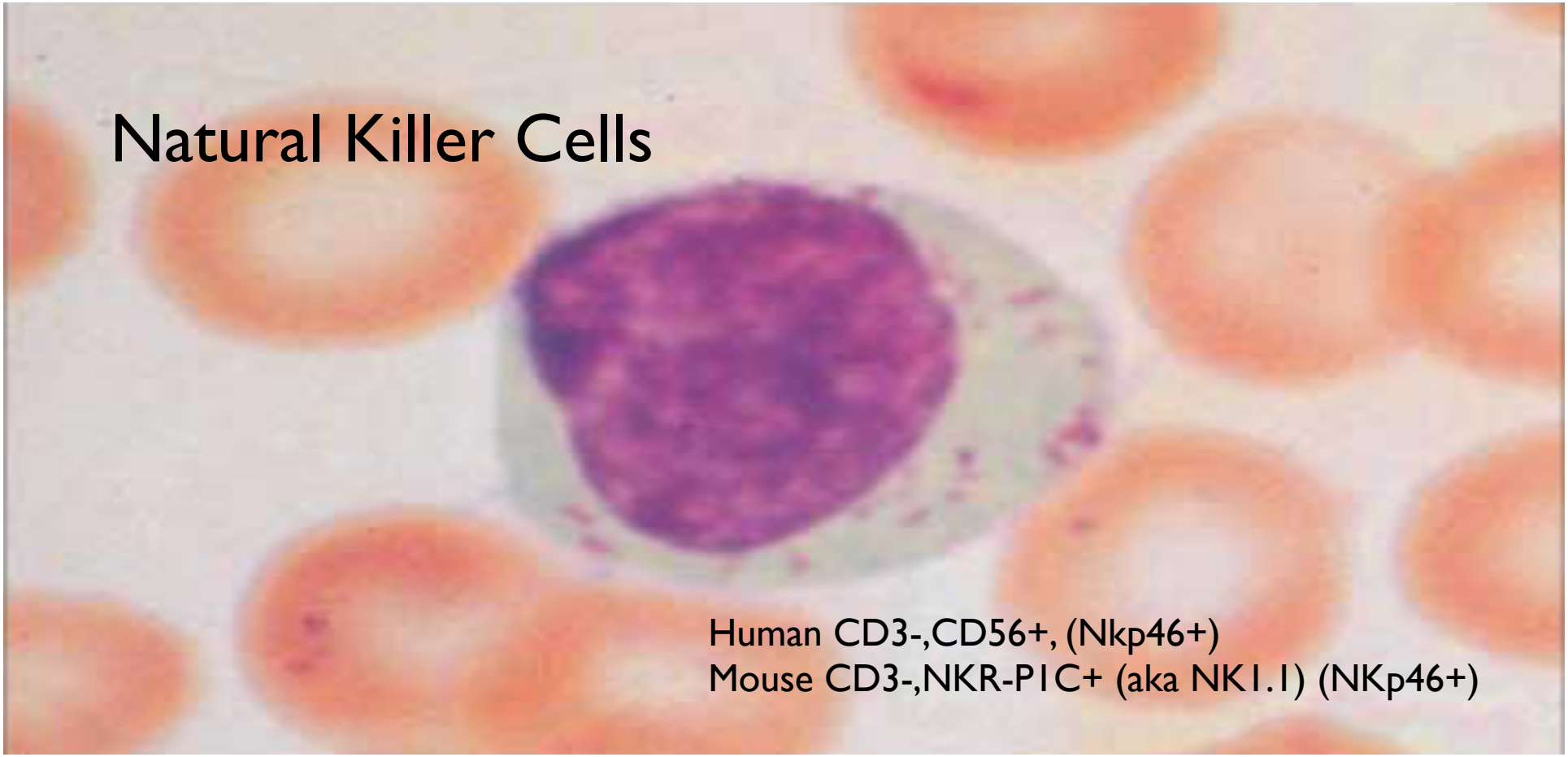
NK cells are not T cells!

don't rearrange TcR genes or express CD3 on the cell surface and do not require a thymus



Godfrey, Immunity, 2018

Natural Killer Cells

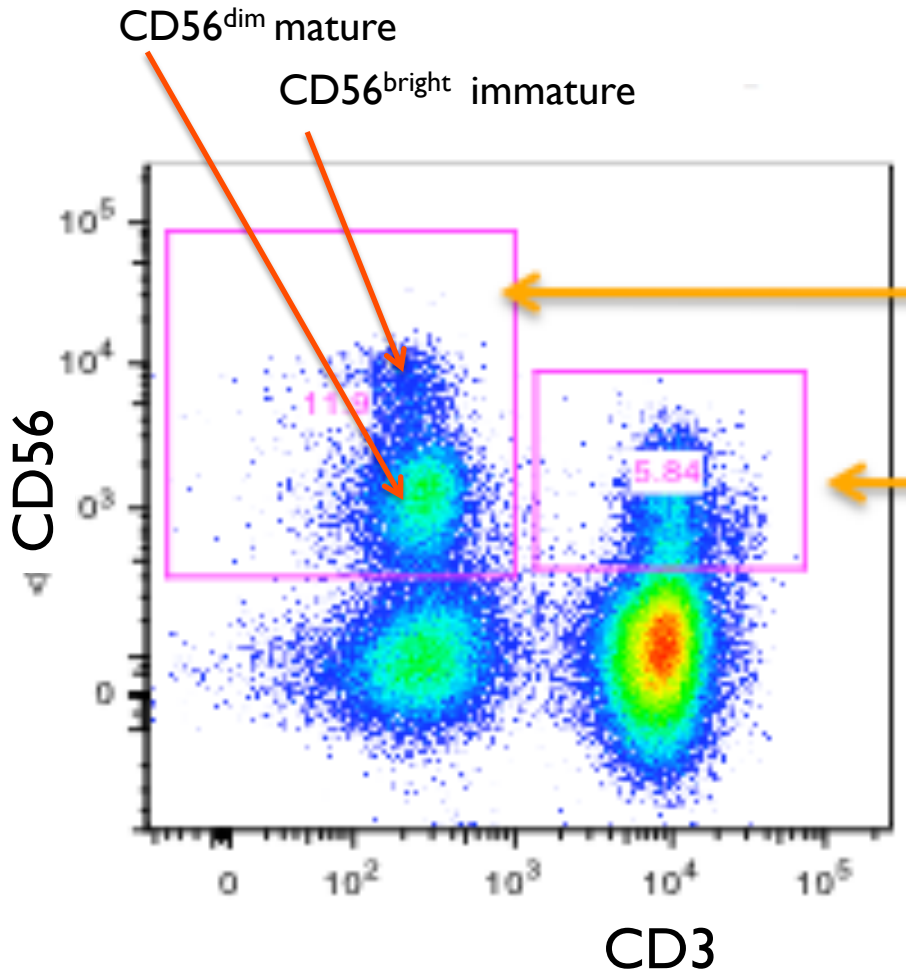


3rd lineage of lymphocytes

Function in innate immunity to protect against viruses, bacteria, parasites, fungi, & tumors

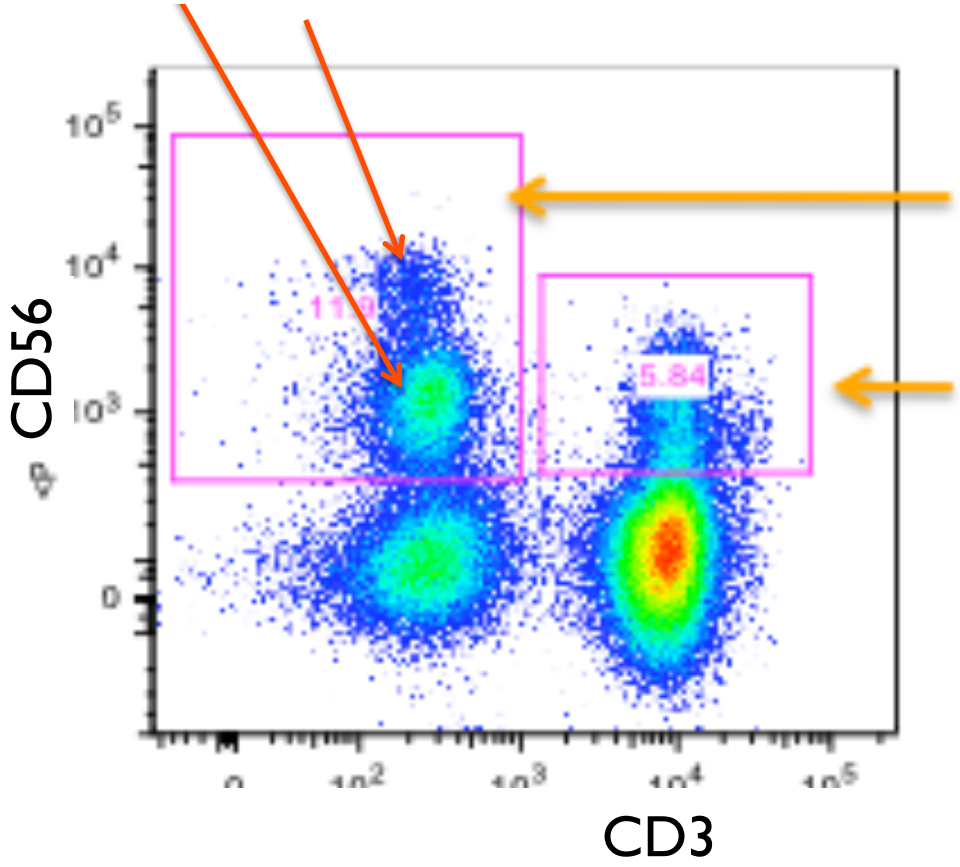
Produce cytokines & kill abnormal cells

Detecting NK cells in human peripheral blood



CD3-CD56+ NK cells

CD3+CD56+ T cells



CD3+CD56+ T cells

These are NOT NKT cells!

They are just activated T cells

MEDICAL INTELLIGENCE

SEVERE HERPESVIRUS INFECTIONS IN AN ADOLESCENT WITHOUT NATURAL KILLER CELLS

CHRISTINE A. BIRON, PH.D., KEVIN S. BYRON, AND JOHN L. SULLIVAN, M.D.

Physiological role of NK cells is to protect against viral infections and cancer

Humans lacking NK cells are particularly susceptible to:

- Epstein-Barr Virus Fleisher, J. Pediatrics 1982

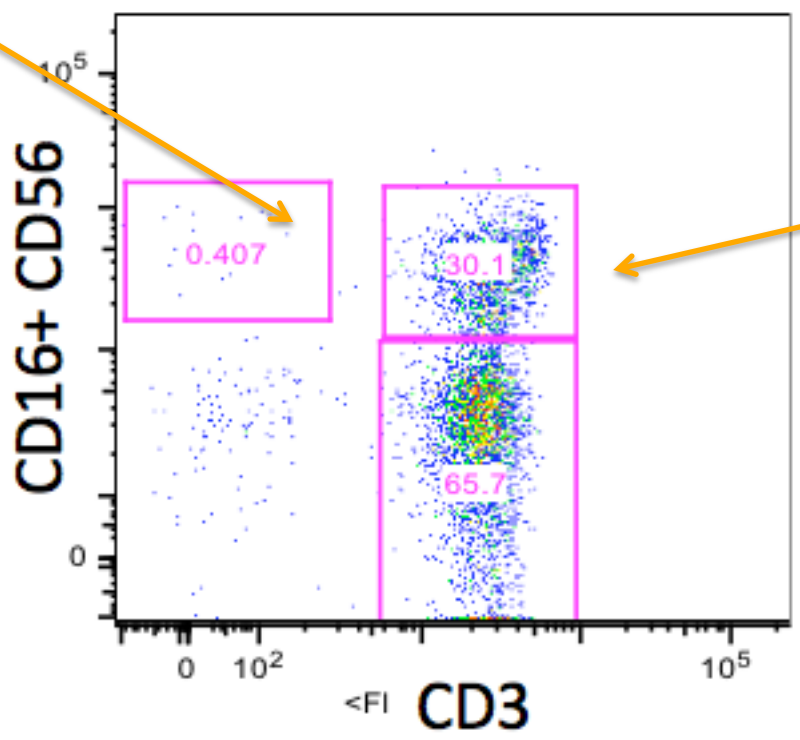
- Cytomegalovirus and other herpesviruses Biron, NEJM 1989

- Papillomavirus (cervical cancer) and Herpes Simplex Virus
  Ballas, J. Allergy Clinical Immuno 1991

- Varicela Zoster Virus Etzioni, J. Peditrics 2005

NK cell-deficient patient

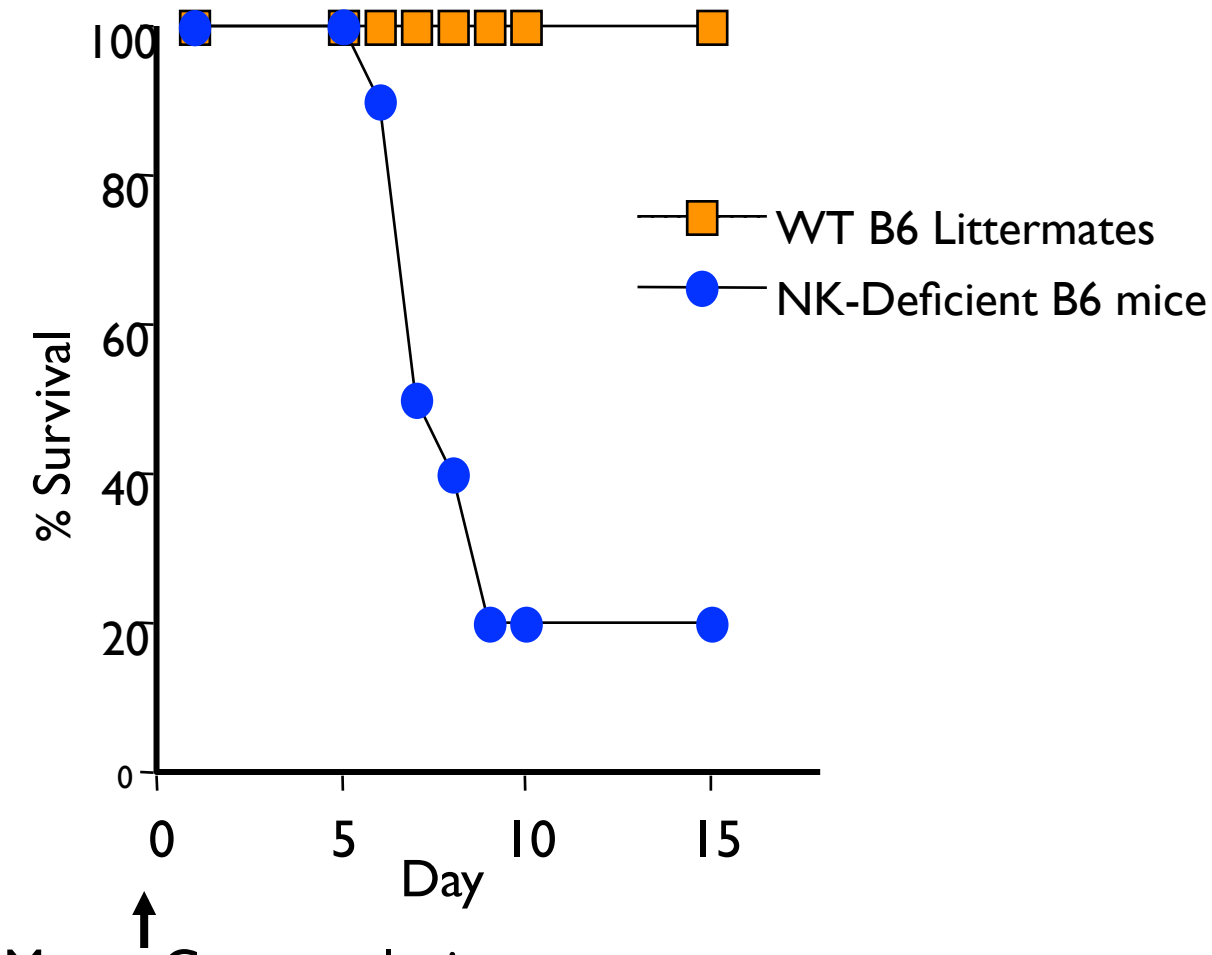
CD3$^-$CD56$^+$ NK cells



CD3$^+$CD56$^+$ T cells

caused by heterozygous loss of *GATA2*

NK cells are critical to early innate host defense against pathogens



Loh JVI 2005

NK Cells - Where do they live?

~5-20% peripheral blood lymphocytes

~5% lymphocytes in spleen, abundant in liver

Low frequency in thymus, bone marrow, uninfected lymph nodes and lymphatics – but increase with infection

>70% of lymphocytes in decidual tissue

NK Cells - What do they do?

Cell mediated-cytotoxicity – "natural killing"

Antibody-dependent cellular cytotoxicity

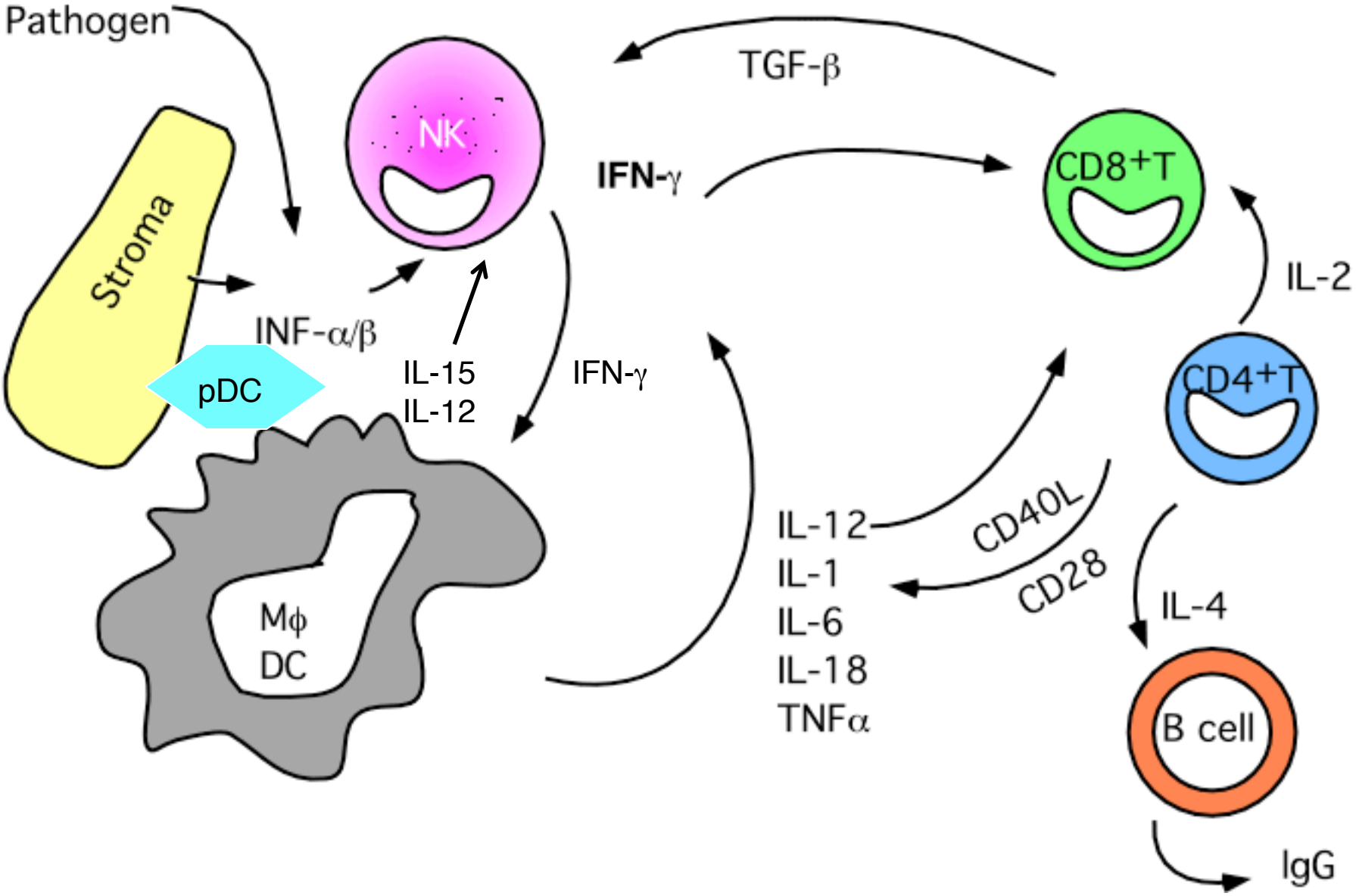
(kill antibody-coated cells via activating Fc receptor CD16)

Early γ-interferon production

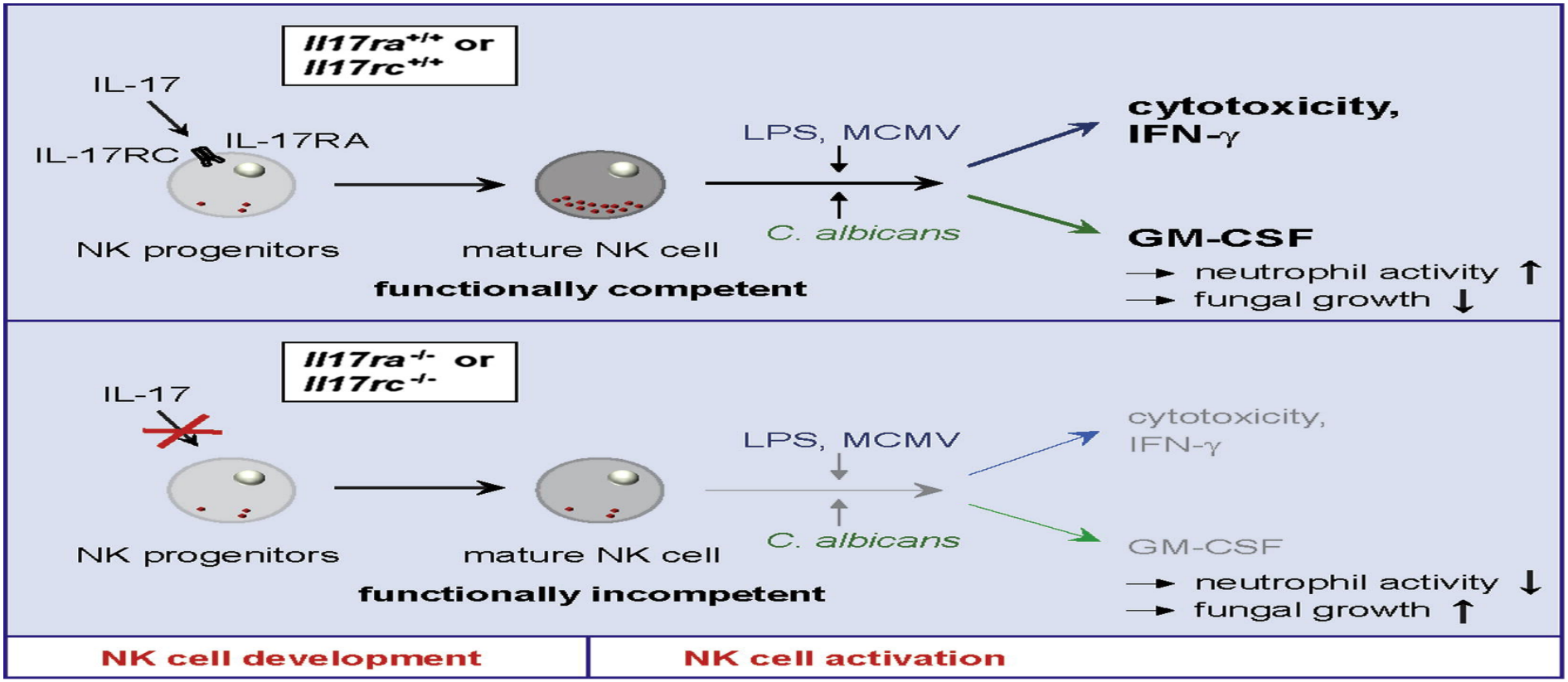
Secrete TNFα, LTα, GM-CSF, IL-3, M-CSF, IL-10, MIP-1a, MIP-1b, RANTES, etc.

(but <u>NOT</u> IL-2, IL-4, IL-17, or IL-22)

Innate/Adaptive Immune System



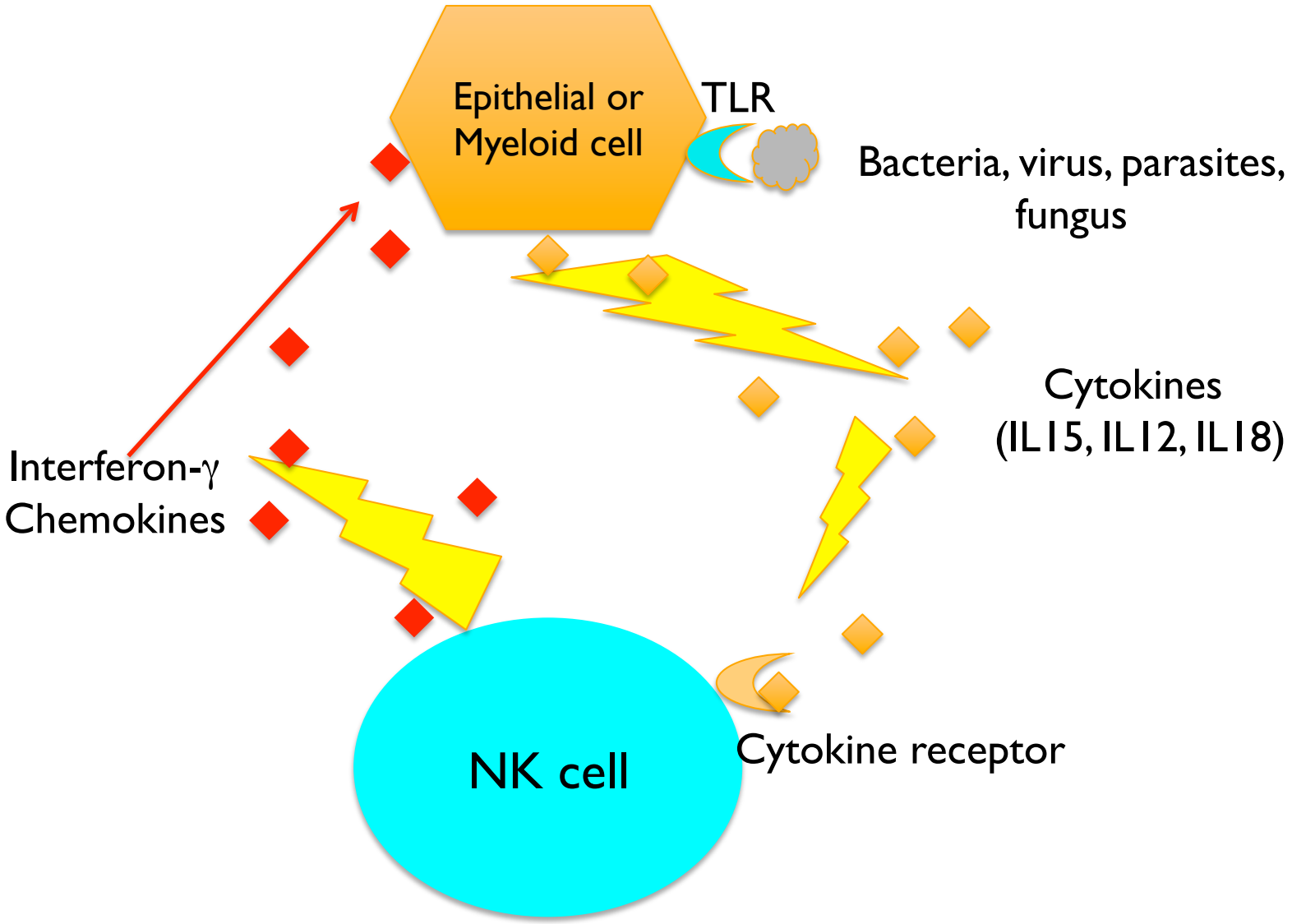
In fungal infections, NK cells respond to IL-17 – producing GM-CSF to recruit granulocytes to control infection



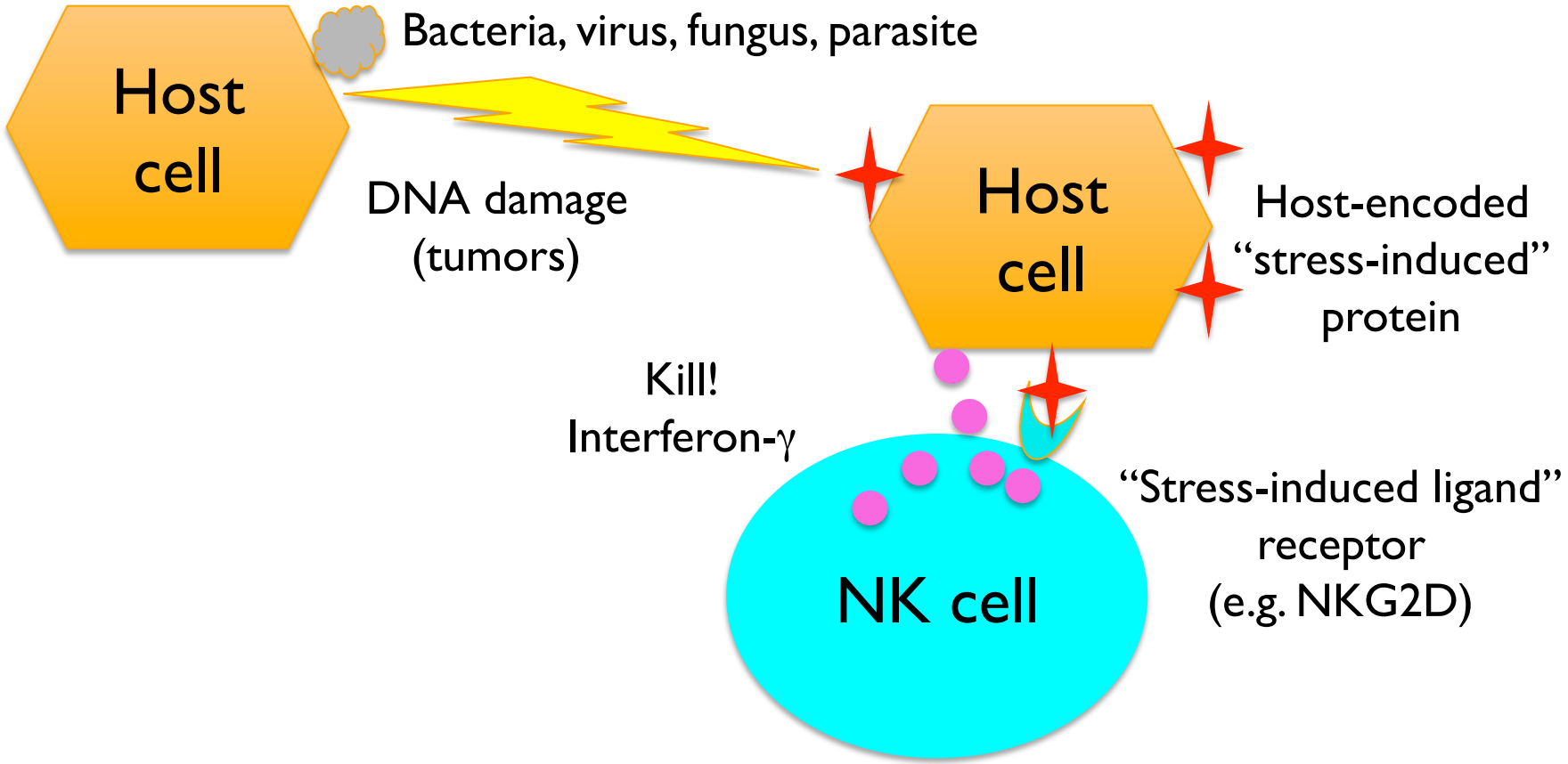
Immunity, Volume 40, Issue 1, 2014, 117 - 127

How do NK cells sense their environment?

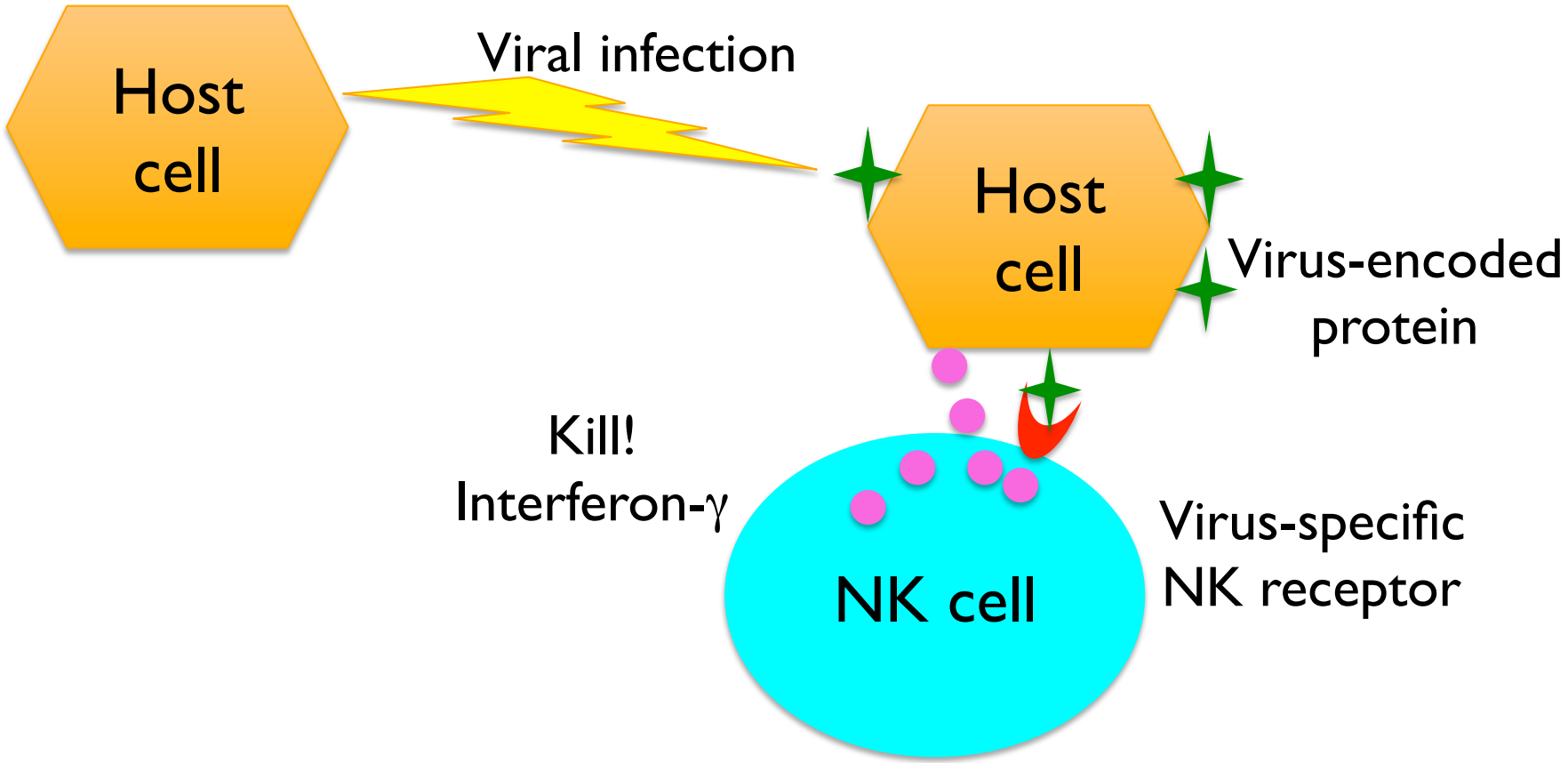
Cytokines produced by infected epithelial or myeloid cells



"Stressed" cells – up regulate host-encoded ligands for activating NK receptors

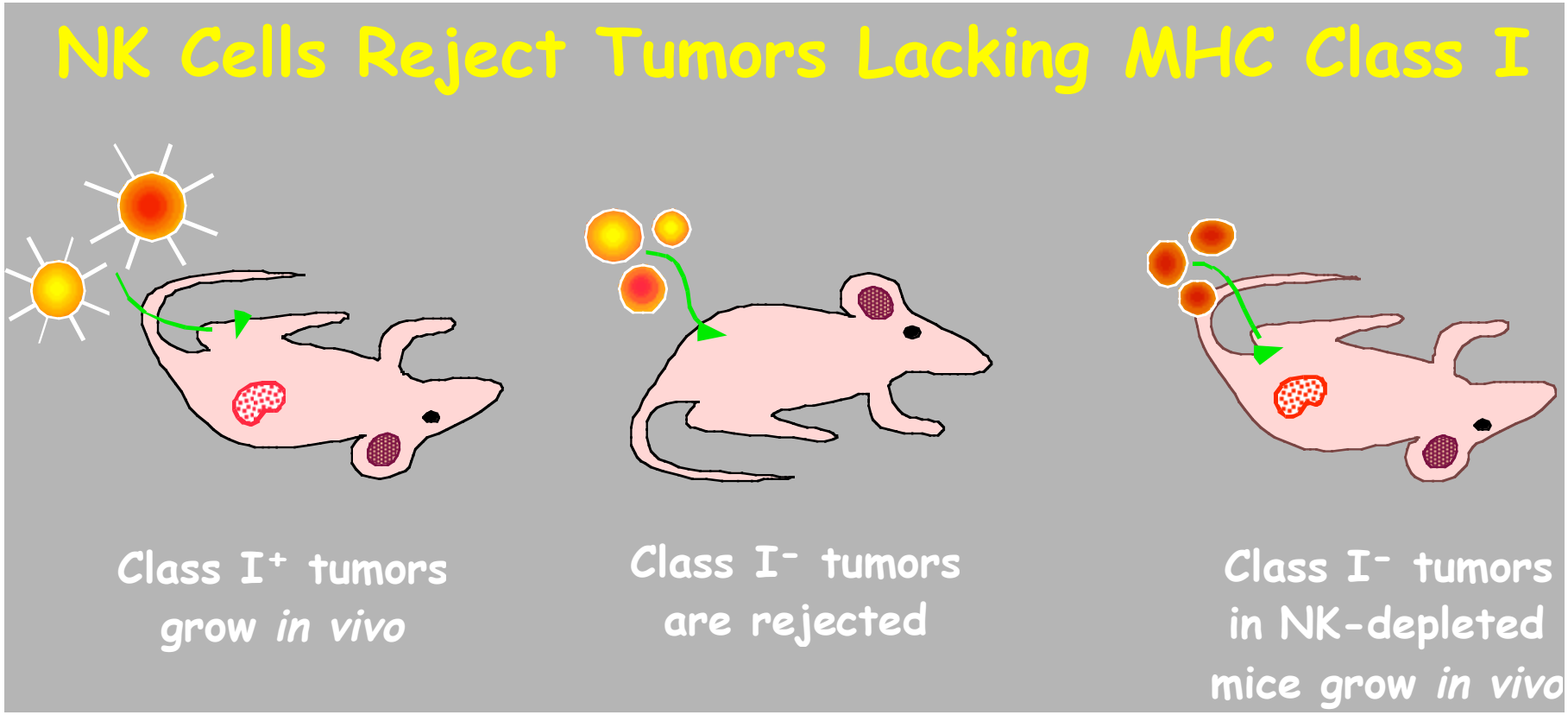


Infected cells express virus-encoded ligands for activating NK receptors



How are NK cell responses regulated?

NK cells like to kill cells lacking MHC class I – "missing-self"

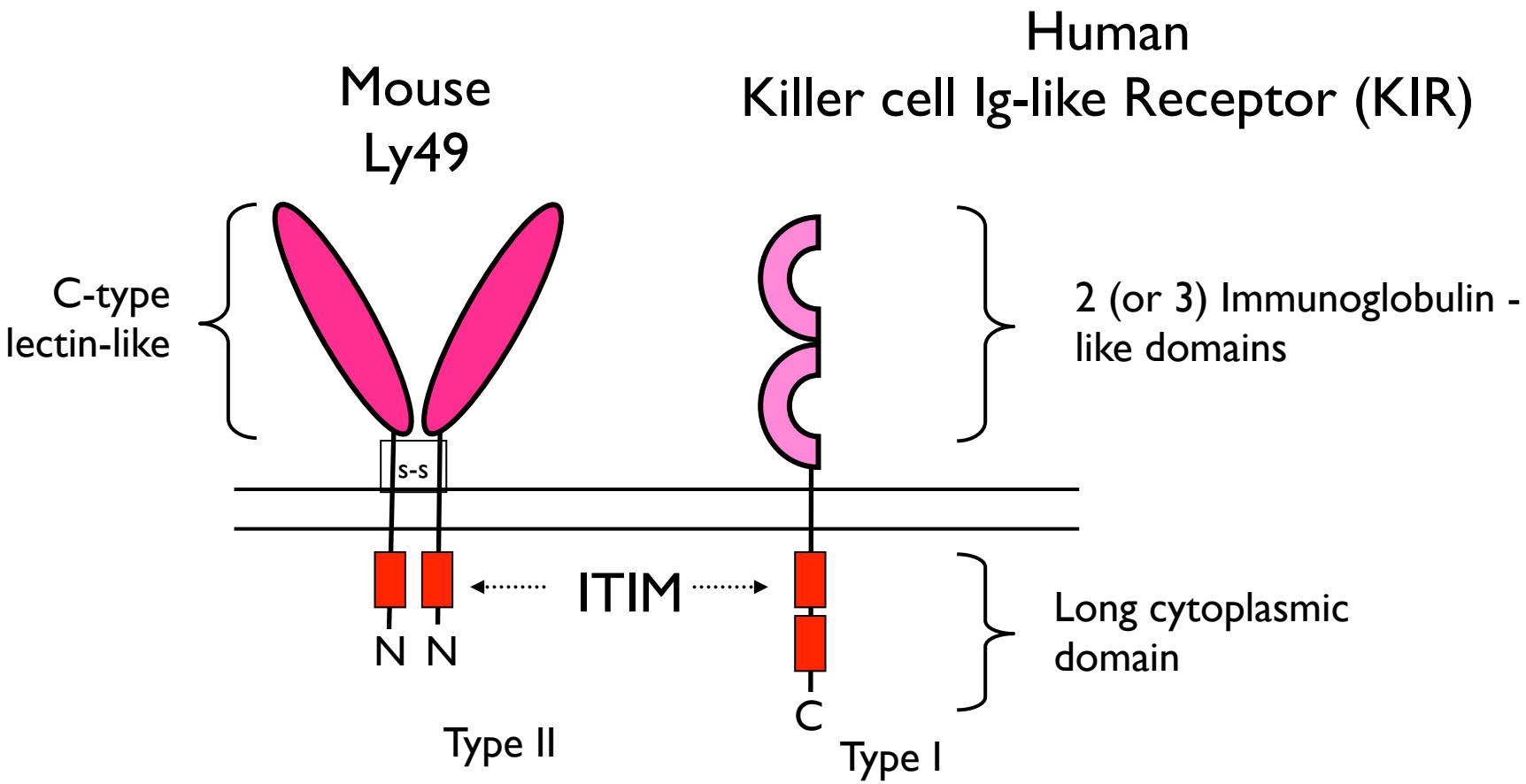


Karre *et al. 1986* Nature 319:675

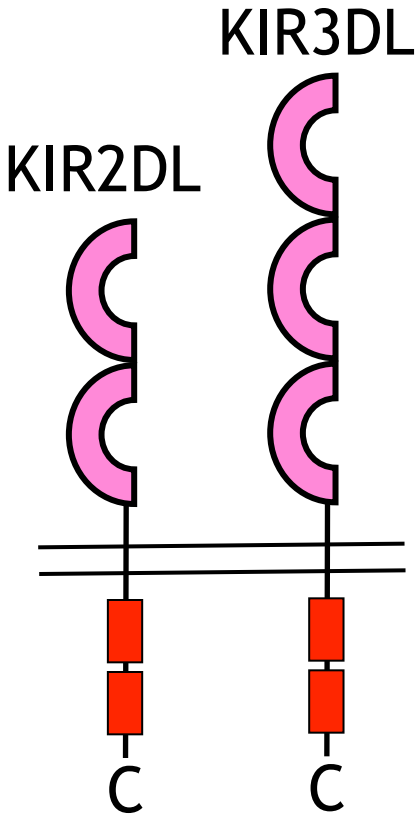
Physiological role for NK cell inhibitory receptors for MHC class I- Detection of virus-infected cells?

Virus	Protein	Effect on class I
Adenovirus	E3-k19	Retain in ER
HSV-1,2	ICP47	Blocks TAP
EBV	EBNA1	Block peptide generation
HCMV	US2, US11	ER to cytosol
HCMV	US3	Retain in ER
HCMV	US6	Blocks TAP
HCMV	US10	Degrades HLA-G
MCMV	m152	Retain in ER
MCMV	m04	Associates with H-2
MCMV	m06	Lysosomal degradation
HHV8	K3, K5	Endocytosis
HIV-1	Nef	Endocytosis

Structural differences between MHC class I-specific inhibitory receptors in mice and humans



HLA specificities of human KIRs



KIR2DL1 : HLA-C2 allotypes
(Cw2, 4, 5, 6=Lys80)

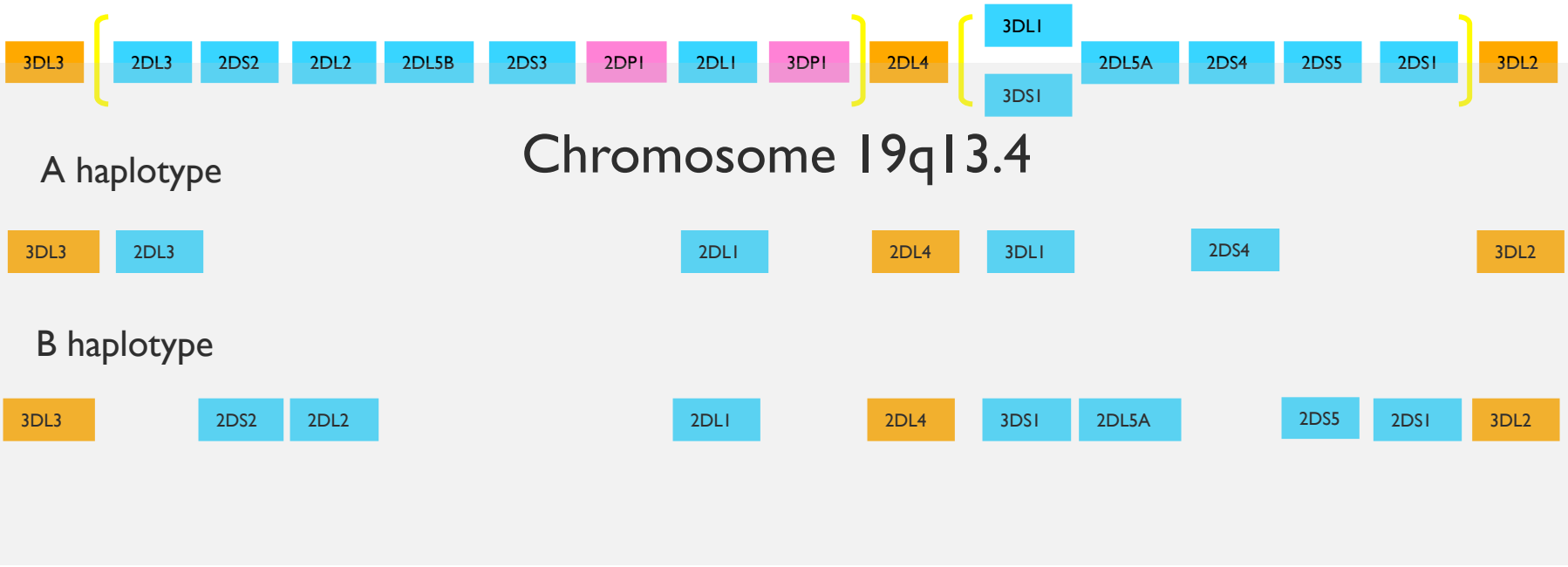
KIR2DL2 & KIR2DL3 : HLA-C1 allotypes
(Cw1, 3, 7, 8=Asn80)

KIR3DL1 : HLA-Bw4

KIR3DL2: HLA-A3

Lanier, *Ann Rev Imm* 2005

Different people have different KIR genes



KIR genes are highly polymorphic!

Gene	2DL1	2DL2	2DL3	2DL4	2DL5	2DS1	2DS2	2DS3
Alleles	59	31	59	65	51	16	23	16
Proteins	34	13	34	37	22	8	9	7
Nulls	2	0	1	0	0	0	0	1

Gene	2DS4	2DS5	3DL1	3DS1	3DL2	3DL3	2DP1	3DP1
Alleles	35	23	137	39	158	126	40	29
Proteins	16	17	84	22	109	69	0	0
Nulls	0	0	3	1	1	0	0	0

907 alleles at 14 KIR loci

https://www.ebi.ac.uk/ipd/kir/stats.html

Killer Cell Ig-like Receptors (KIR)

Ig superfamily

7-12 functional genes on human chromosome 19q

Extensive allelic polymorphism 698 alleles!) no rearrangement; mono-allelic expression possible

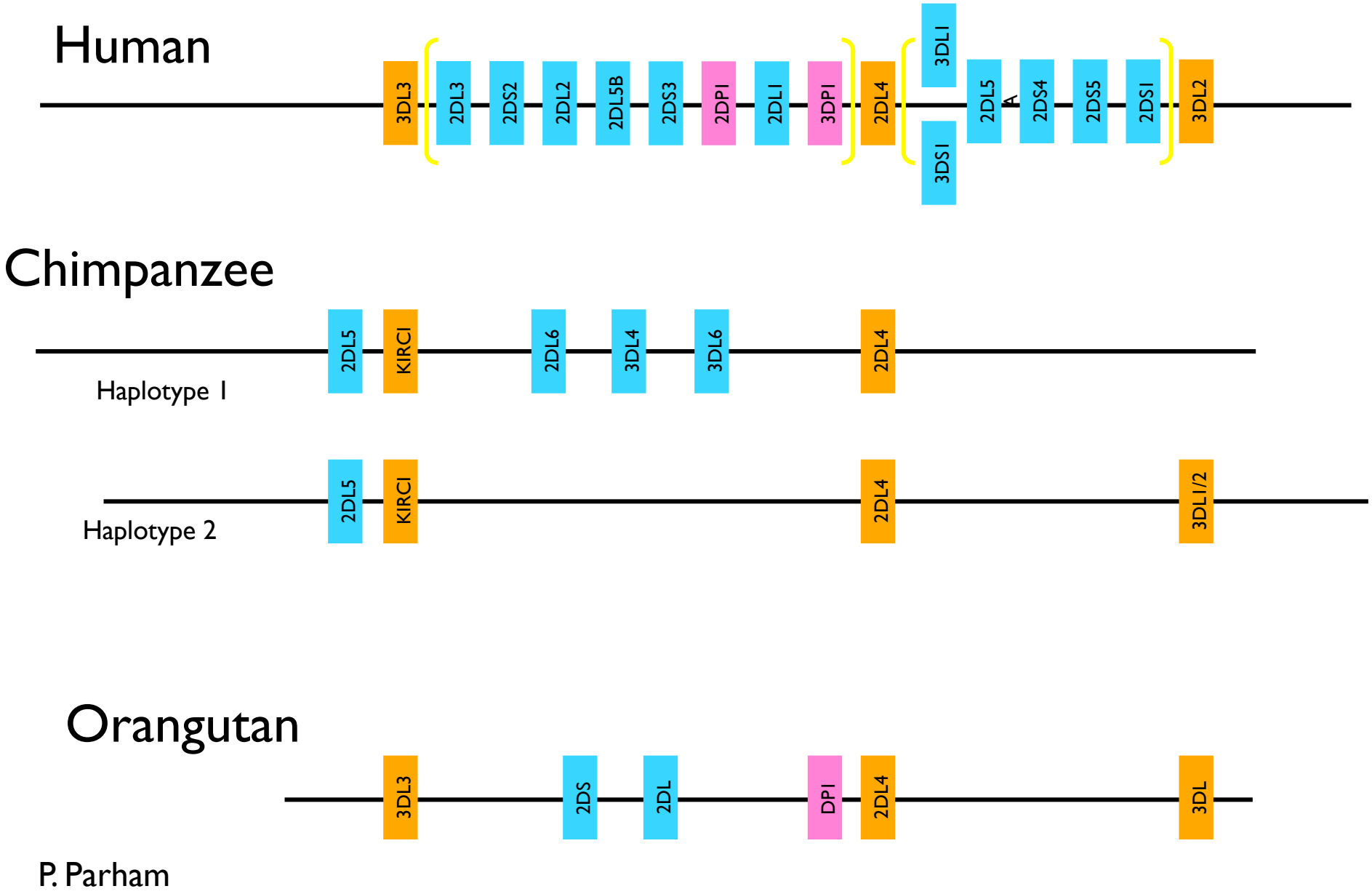
Expressed by subsets of NK cells and memory T cells (mostly $CD8^+$ T cells, but also $CD4^+$ T cells)

Inhibitory KIR2DL recognize HLA-C; KIR3DL recognize HLA-A, -B

Activating KIR - no intrinsic signaling

-associate with DAP12 ITAM-adapter protein

Rapid expansion of KIR genes in primates



P. Parham

Ly49 Receptors

C-type lectin-like superfamily (but don't bind sugars)

10-20 functional genes on mouse chromosome 6

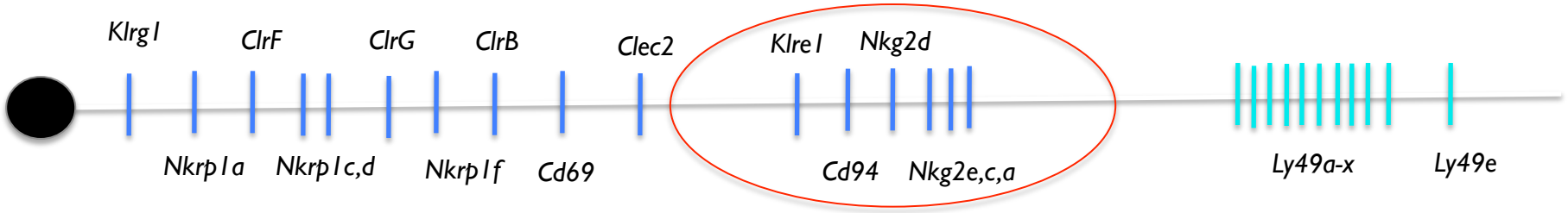
Extensive allelic polymorphism (no rearrangement)
mono-allelic expression possible

Expressed by subsets of NK cells and memory T cells
(usually $CD8^+$ T cells)

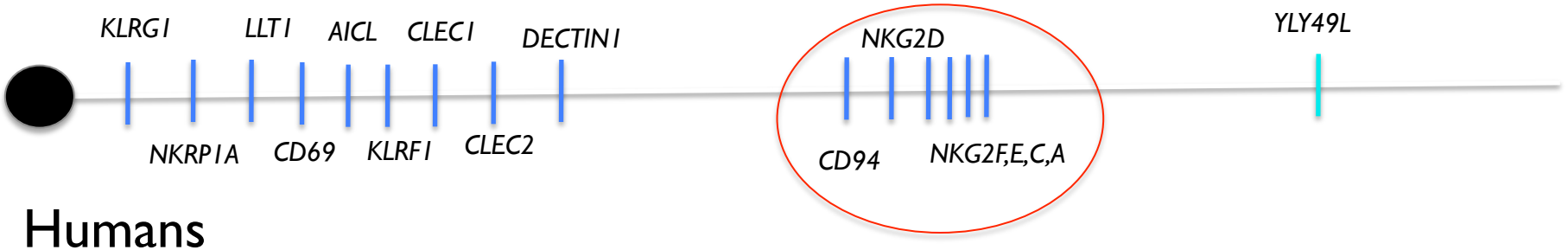
Inhibitory Ly49 recognize polymorphic H-2D and H-2K

Activating Ly49 receptors - no intrinsic signaling
-associate with DAP12 ITAM-adapter protein

Conserved NKG2 genes on mouse chromosome 6 and human chromosome 12p13.1



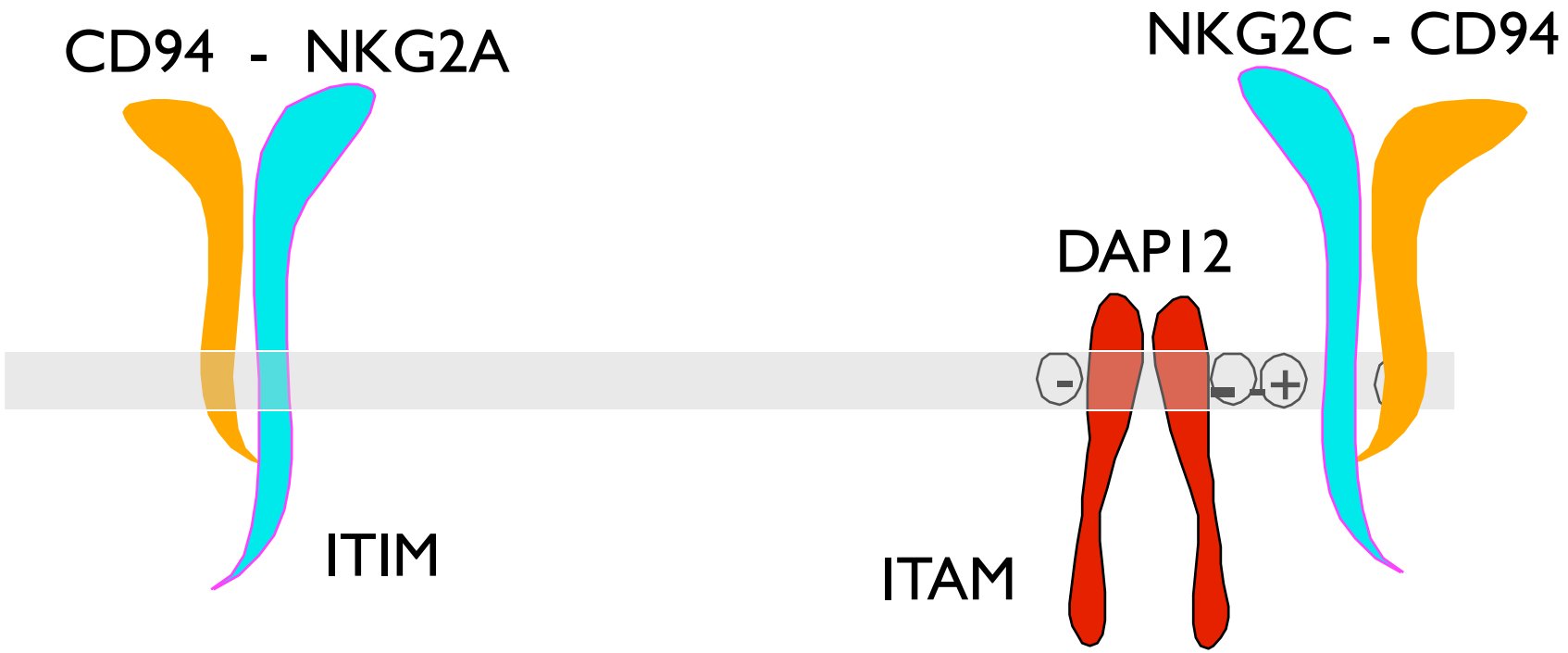
Mice



Humans

Inhibitory CD94-NKG2A and activating CD94-NKG2C-DAP12 receptors

Ligand = MHC class I HLA-E



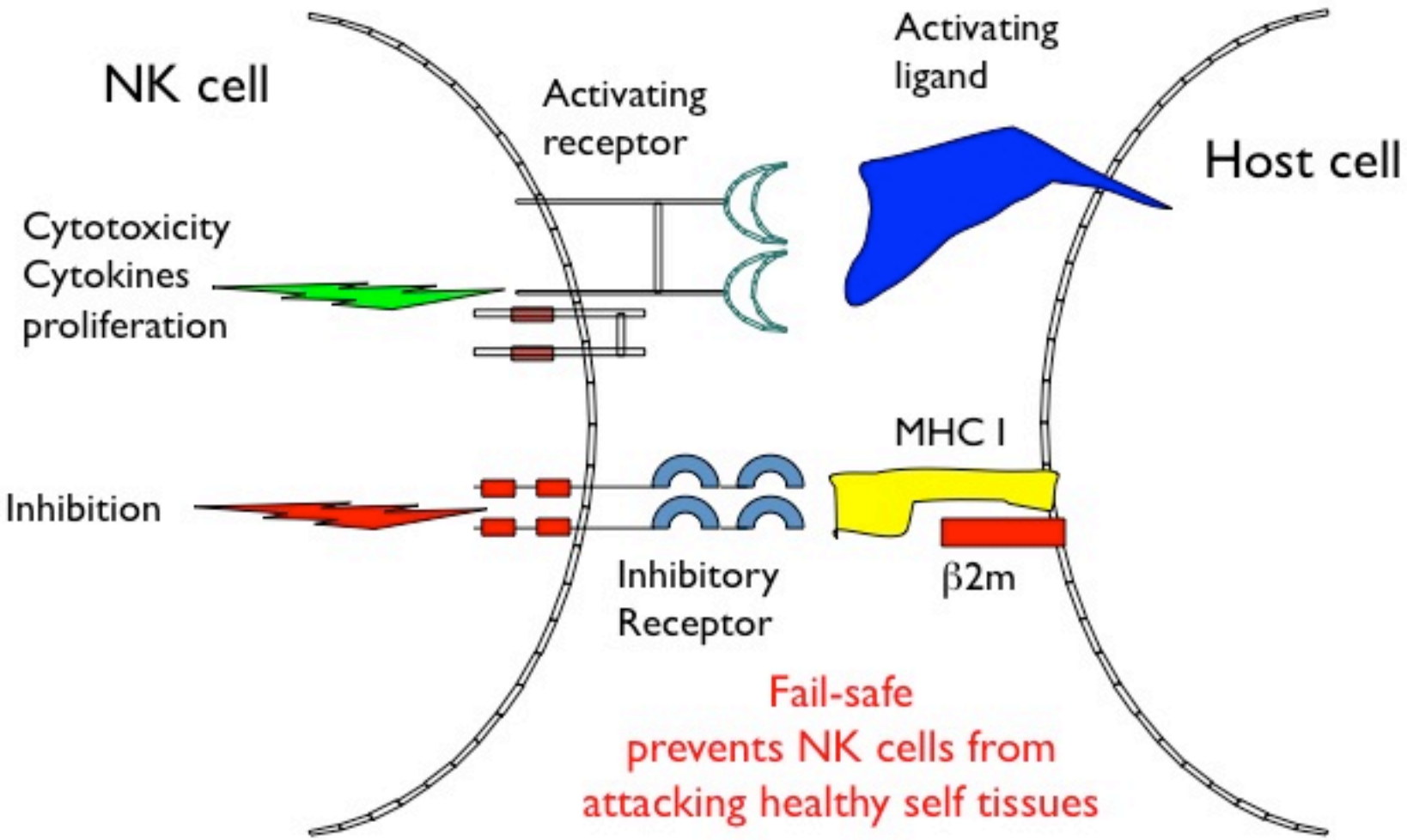
Recruit phosphatases - SHP-1 -inhibit responses

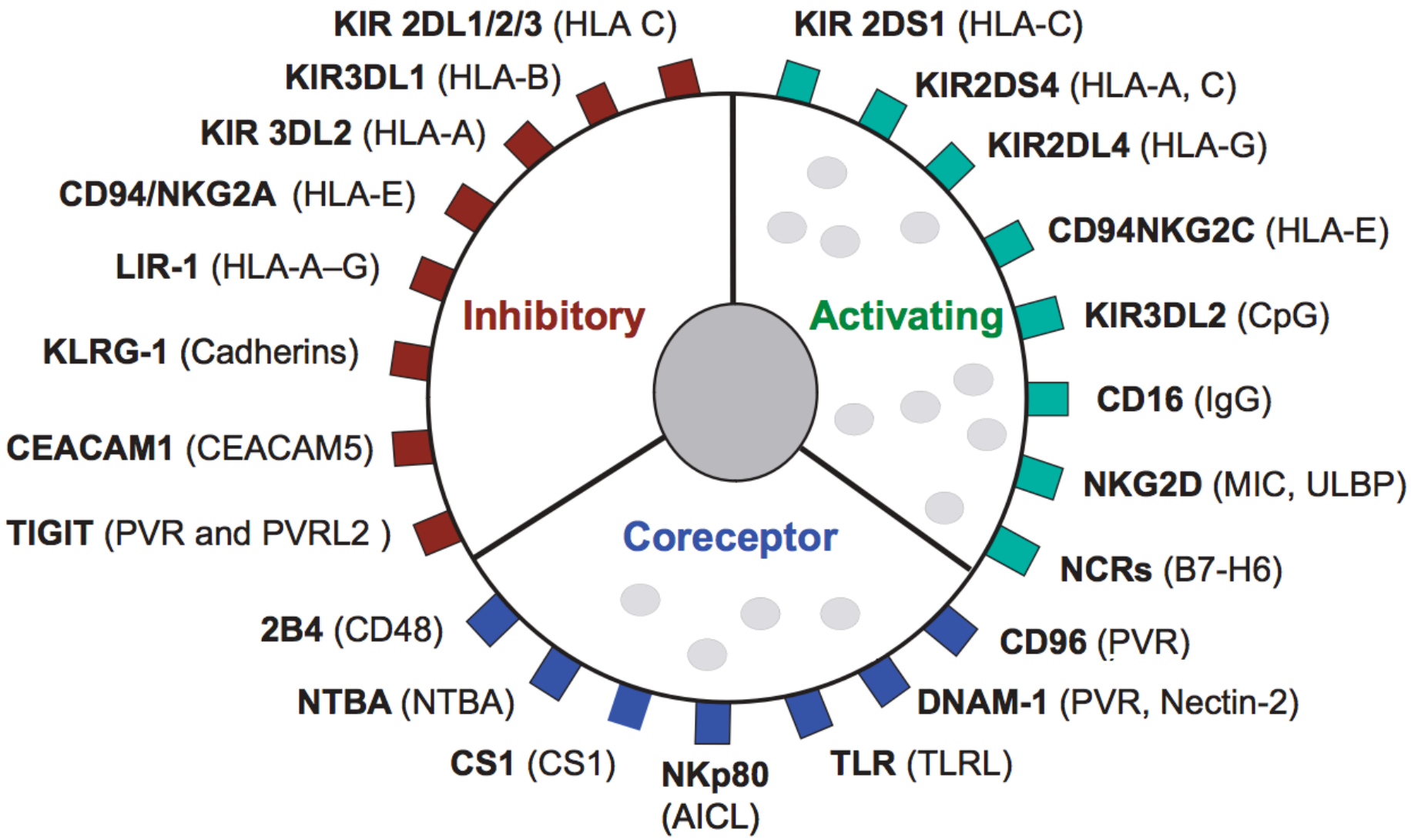
Recruit Syk & Zap70 kinases - activate

"Missing-self" MHC on a cell is not sufficient for an NK cell to attack

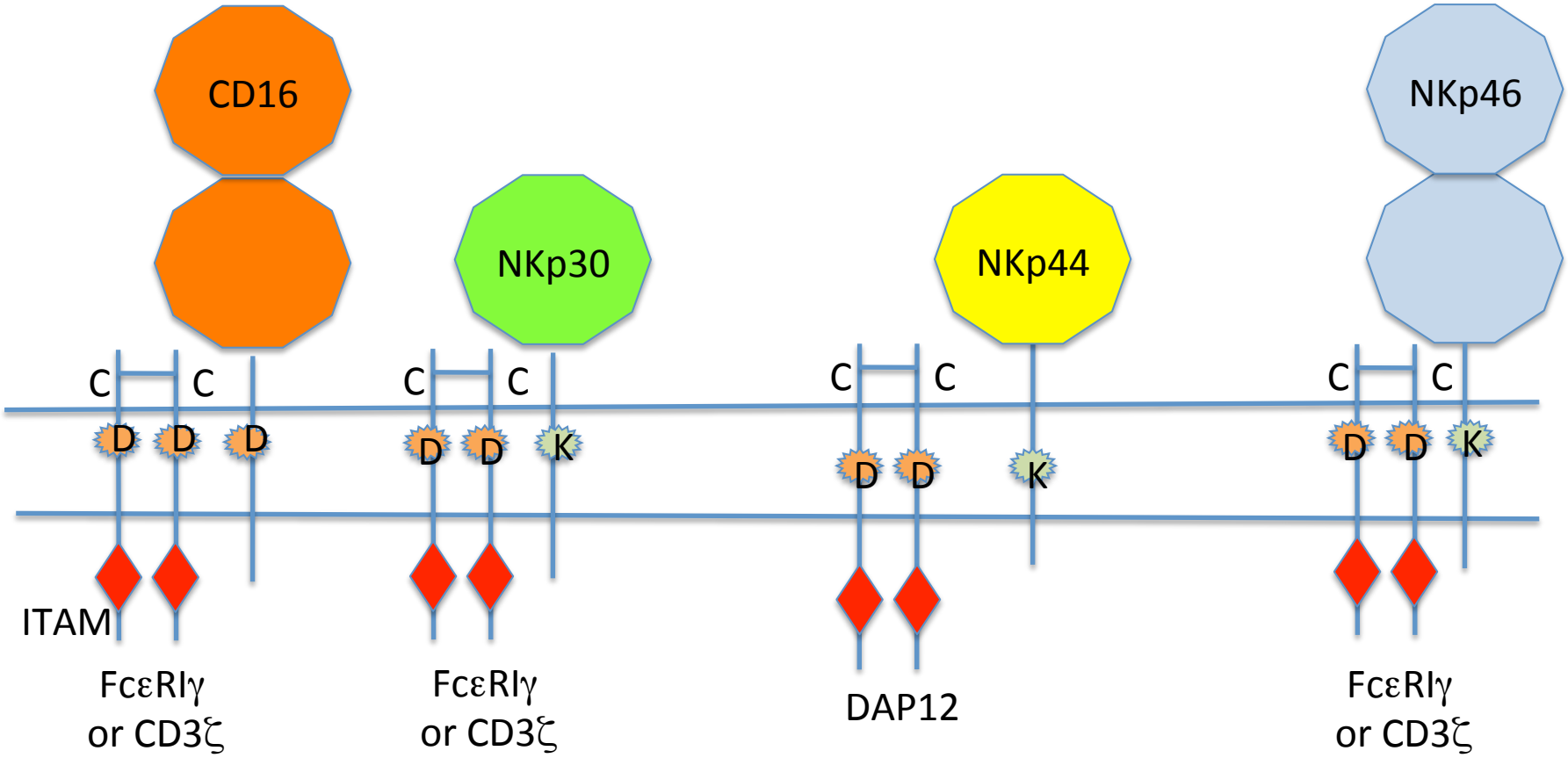
NK cells require activating receptors to detect ligands on the target cell to initiate a response

NK cell functions are controlled by a balance of inhibitory and activating receptors



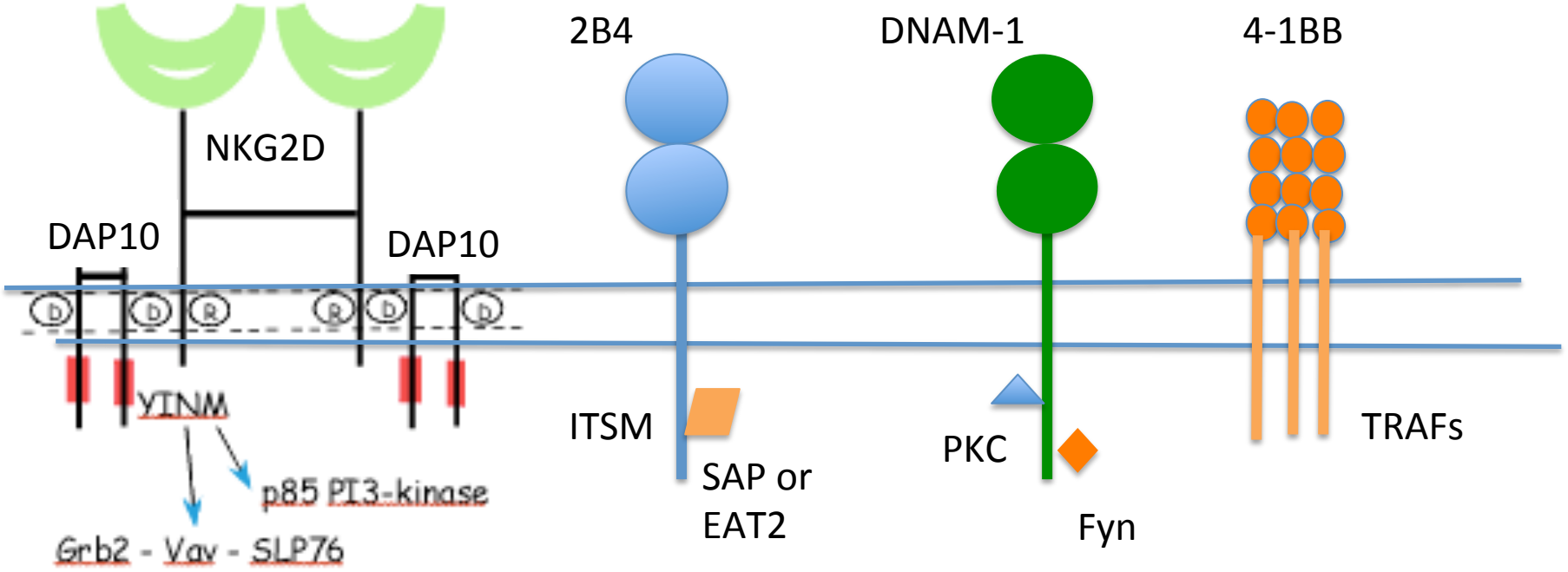

KIR 2DL1/2/3 (HLA C)
KIR3DL1 (HLA-B)
KIR 3DL2 (HLA-A)
CD94/NKG2A (HLA-E)
LIR-1 (HLA-A–G)
KLRG-1 (Cadherins)
CEACAM1 (CEACAM5)
TIGIT (PVR and PVRL2)
2B4 (CD48)
NTBA (NTBA)
CS1 (CS1)
NKp80 (AICL)
TLR (TLRL)
DNAM-1 (PVR, Nectin-2)
CD96 (PVR)
NCRs (B7-H6)
NKG2D (MIC, ULBP)
CD16 (IgG)
KIR3DL2 (CpG)
CD94NKG2C (HLA-E)
KIR2DL4 (HLA-G)
KIR2DS4 (HLA-A, C)
KIR 2DS1 (HLA-C)
Inhibitory
Activating
Coreceptor

ITAM-based Activating NK receptors



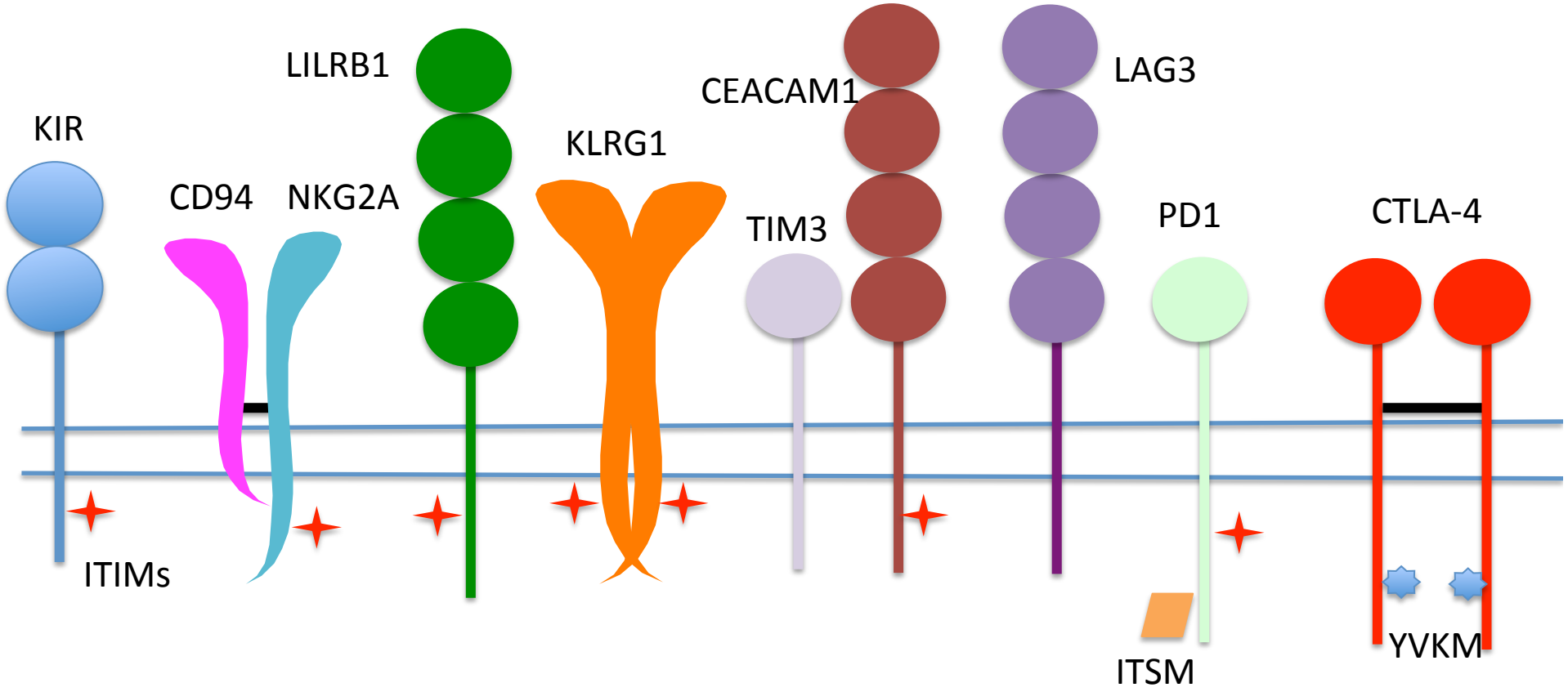
Miller & Lanier Ann Rev Cancer Biology 2019

Costimulatory NK receptors



Miller & Lanier Ann Rev Cancer Biology 2019

Inhibitory NK Receptors



Miller & Lanier Ann Rev Cancer Biology 2019

NATURE BIOTECHNOLOGY | NEWS AND VIEWS

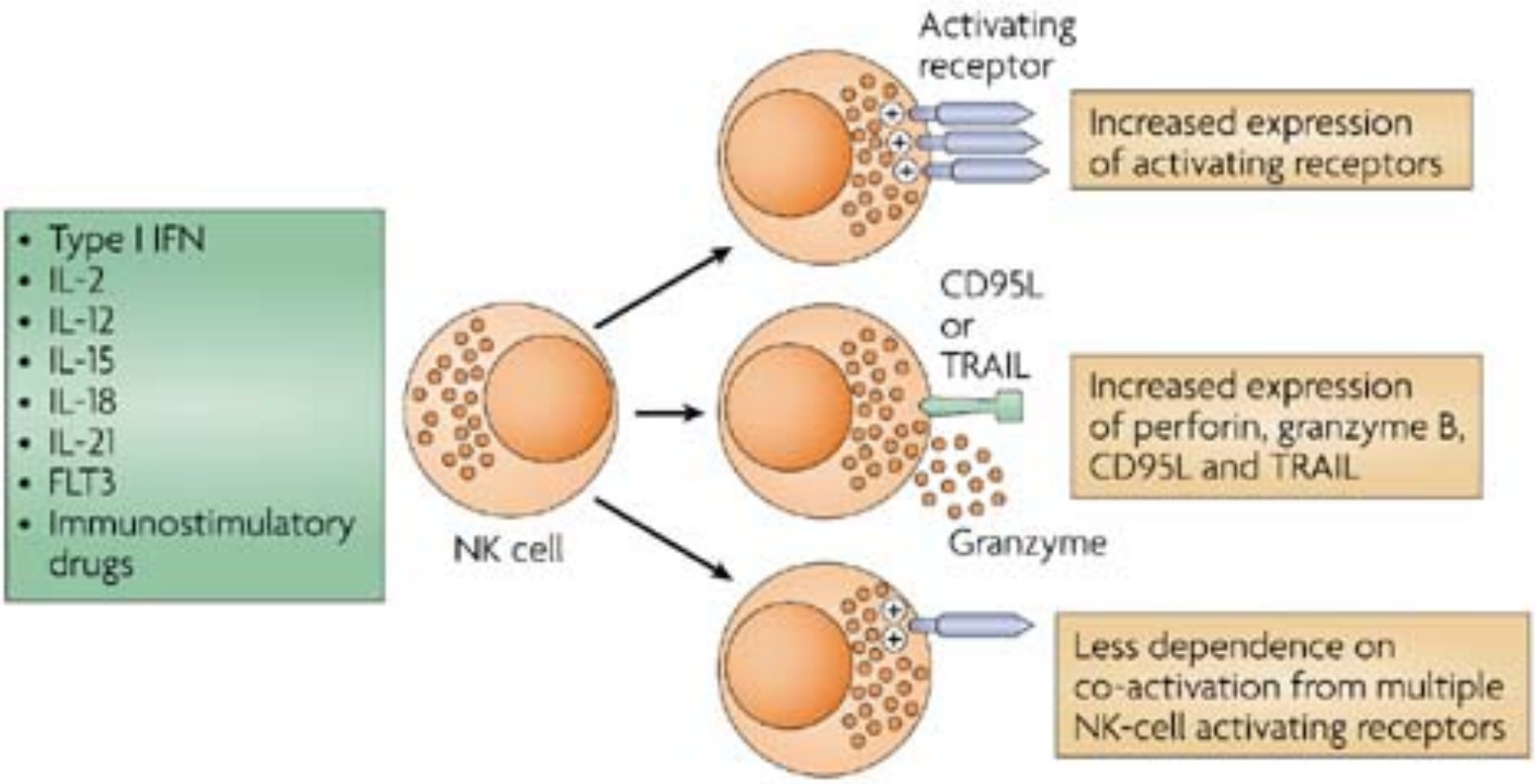
Of snowflakes and natural killer cell subsets

Lewis L Lanier

Nature Biotechnology **32**, 140–142 (2014) | doi:10.1038/nbt.2810
Published online 07 February 2014

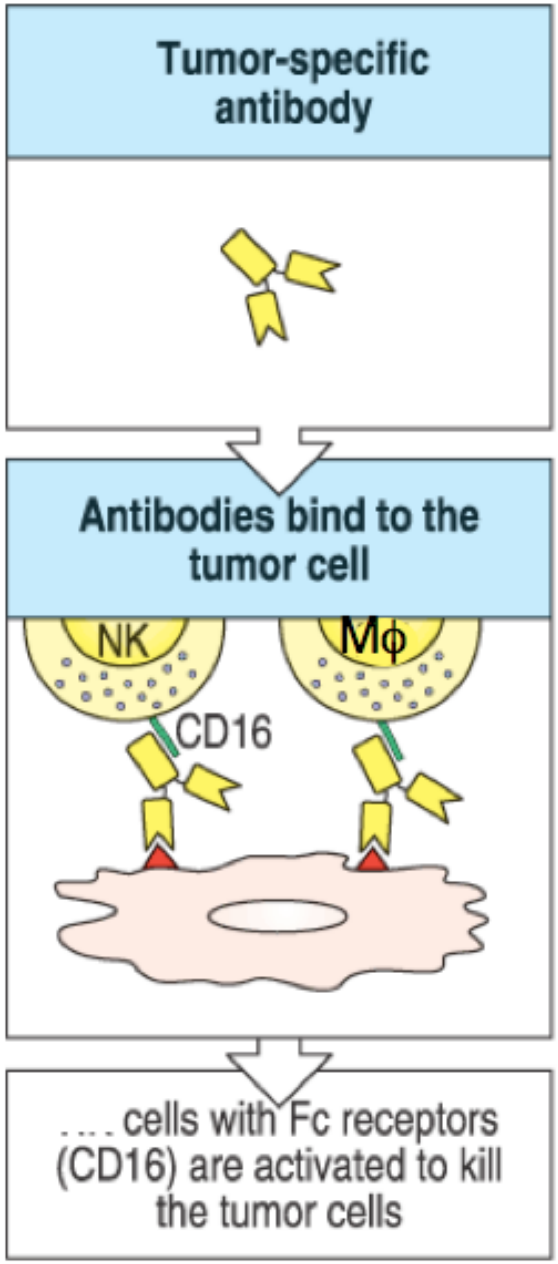
You may have more than 30,000 NK cells subsets in your blood
- CyTOF analysis by Catherine Blish (Stanford)

Factors boosting NK cell lytic activity

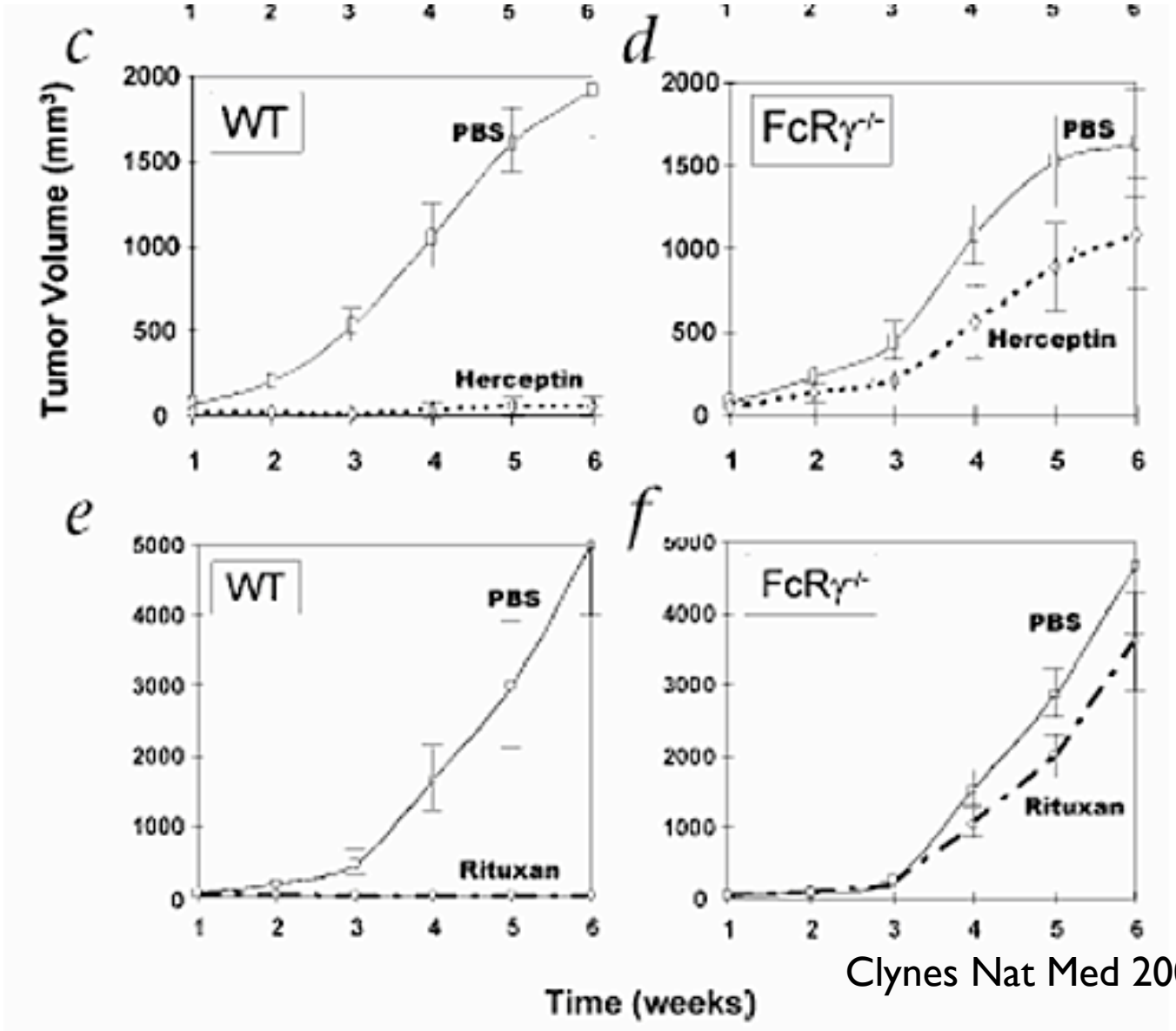


Ljunggren & Malmberg NRI 2007

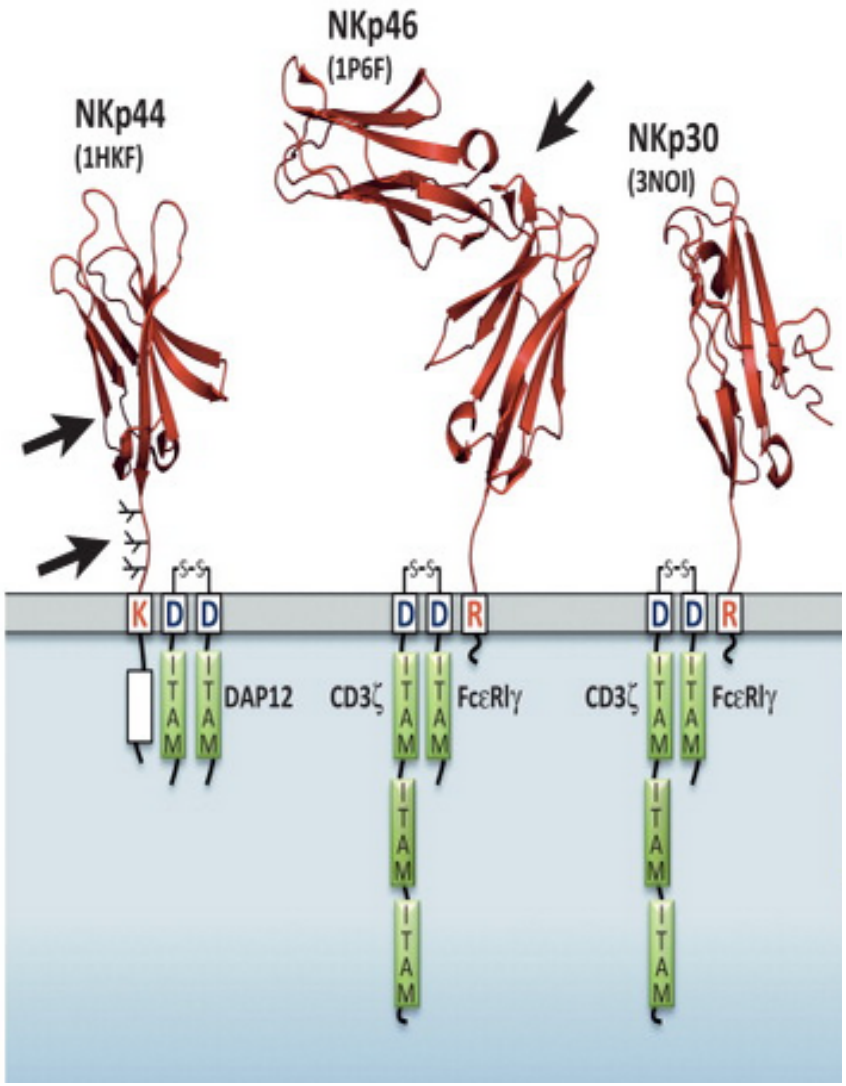
Antibody-dependent cellular cytotoxicity



Activating CD16 Fc receptor on NK cells and macrophages mediates Herceptin- and Rituxan-induced human tumor elimination



Clynes Nat Med 2000



Koch Trends Imm 2013

Natural Cytotoxicity Receptors

ITAM-coupled activating receptor

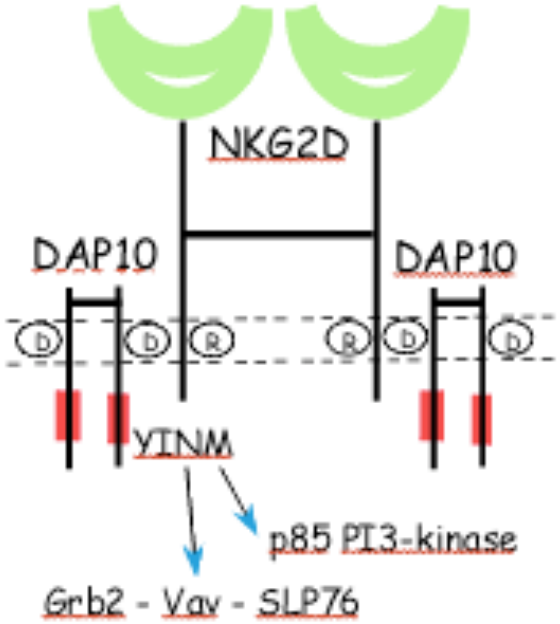
NKp46 expressed by most NK cells in humans and mice

NKp46 also expressed by some ILC and γδ T cells

NKp30 & NKp44 in humans, not mice

Involved in recognition and killing of certain tumors

Ligands poorly defined – broadly distributed (except B7-H6 for NKp30)

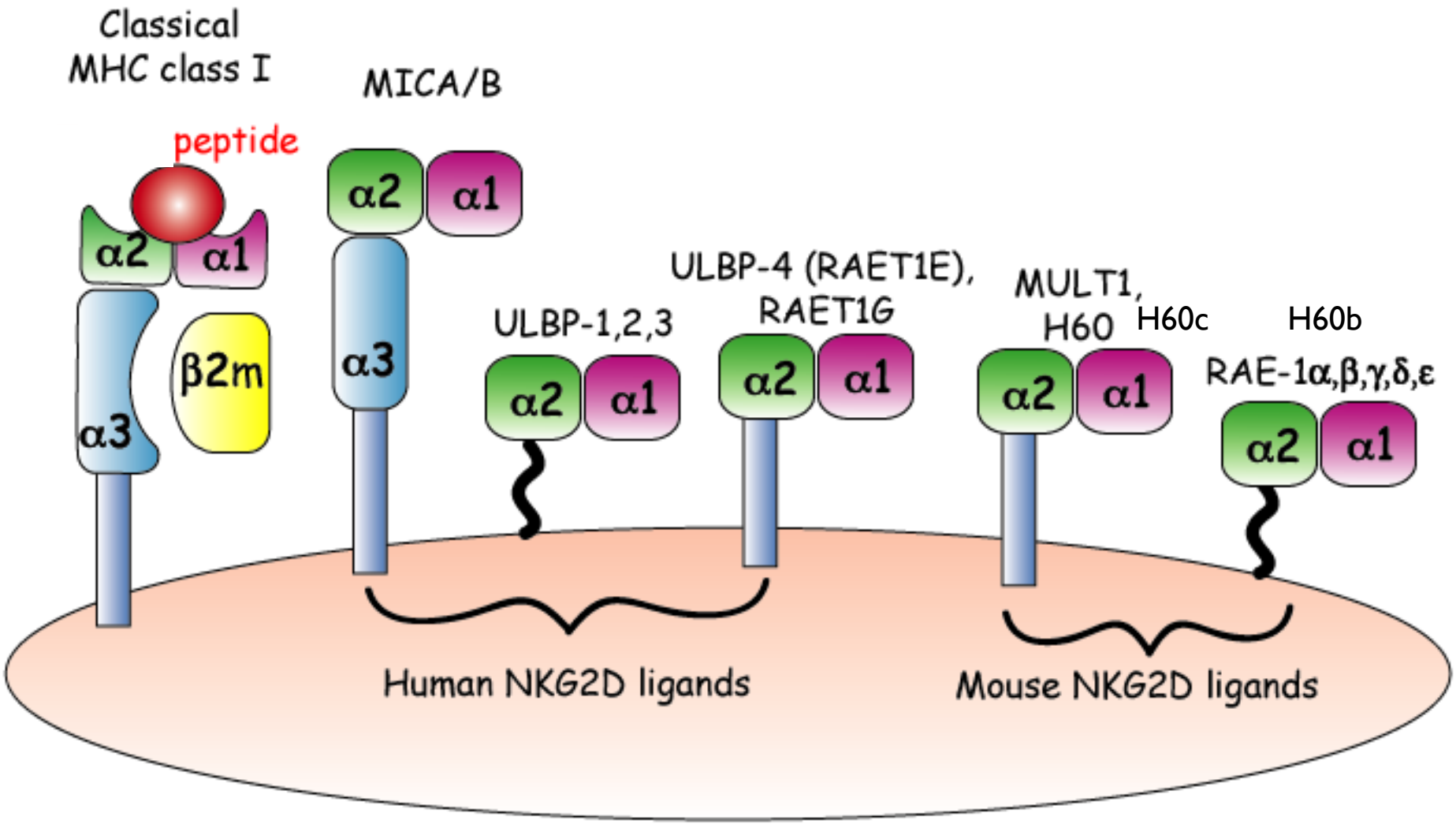


NKG2D

- C-type lectin-like superfamily
- 1 gene, non-polymorphic, conserved mice - humans
- Homodimer expressed on all NK cells, γδ T cells, and $CD8^+$ T cells
- R in transmembrane associates with D in DAP10 transmembrane

DAP10

- 10 kd homodimer
- Cytoplasmic YINM recruits Grb2 & p85 PI3-kinase

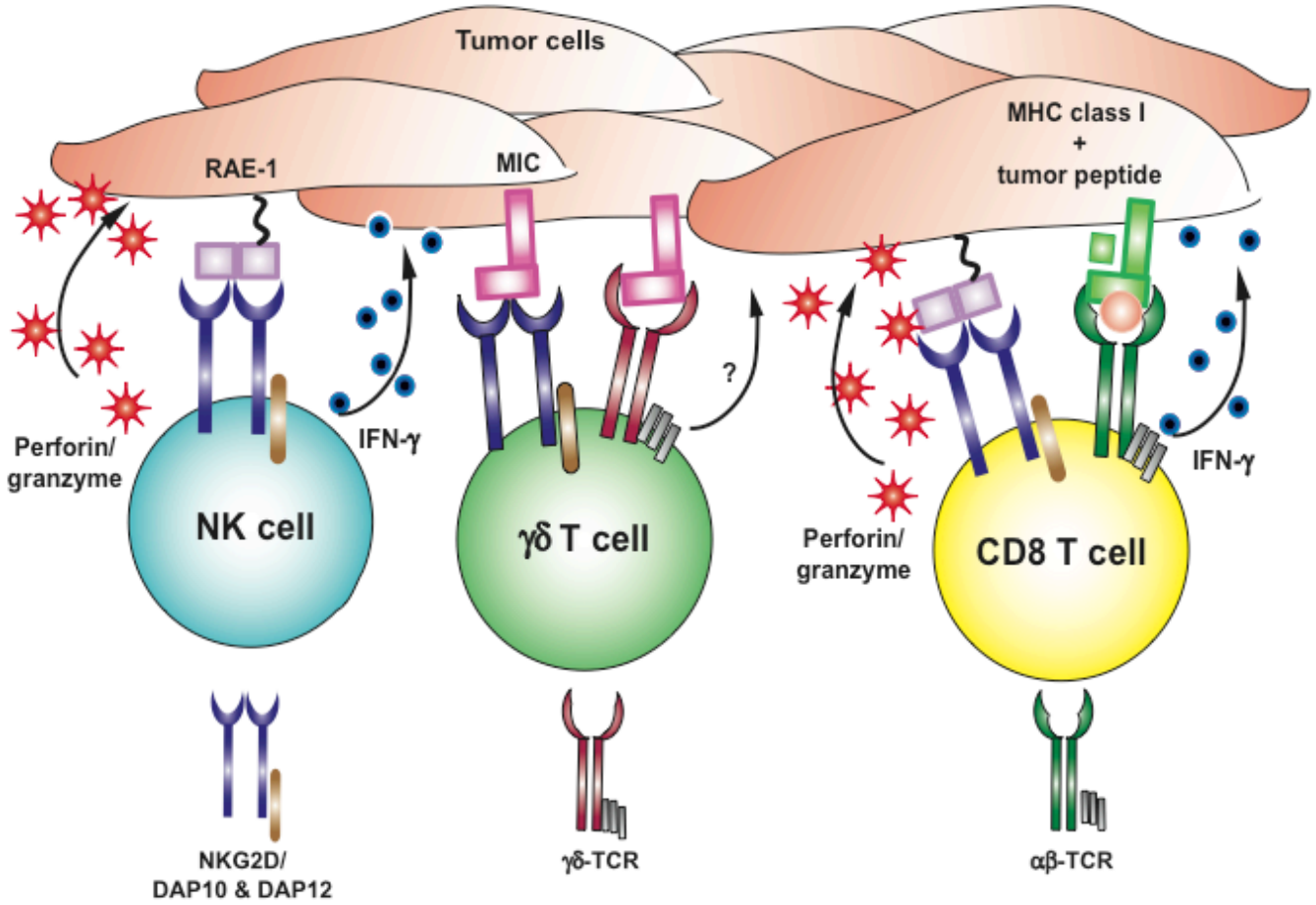

Classical
MHC class I
peptide
α2
α1
β2m
α3
MICA/B
α2
α1
α3
ULBP-1,2,3
α2
α1
ULBP-4 (RAET1E),
RAET1G
α2
α1
MULT1,
H60
H60c
H60b
α2
α1
RAE-1α,β,γ,δ,ε
α2
α1
Human NKG2D ligands
Mouse NKG2D ligands

NKG2D ligands

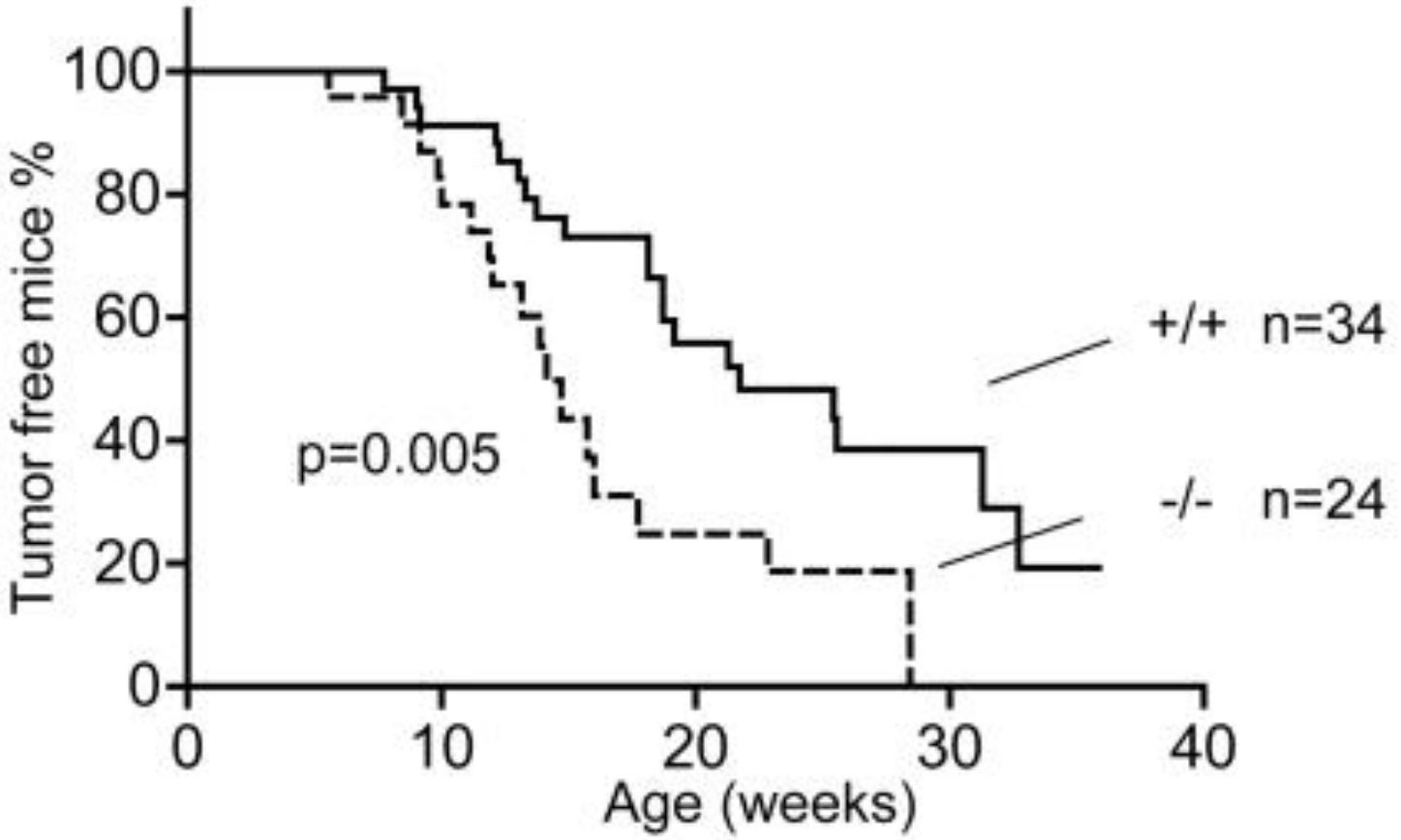
- MHC class I-like
  - don't require peptide or b2-microglobulin
- Bind with nM affinity to NKG2D
- Low levels expressed on healthy tissues
- Induced on virus-infected cells and tumor cells
- Induced by DNA damage (ATM/ATR pathway)
- Elevated in autoimmune diseases
- (rheumatoid arthritis, celiac disease, diabetes, atherosclerosis)*

*Disclosure – I have licensed patents on blocking NKG2D in autoimmune disease

NKG2D on NK cells, γδ T cells and CD8$^+$ T cells detect NKG2D ligands on abnormal cells



Increased incidence of myc oncogene-induced B cell lymphomas in NKG2D-deficient mice



Guerra Immunity 2008

NK cells and Cancer

Caveat

All NK receptors are expressed by other cell types –

No mouse models exist that exclusively lack NK cells without effects on other cell lineages

How do we engage NK cells in new immunotherapies for cancer?

THERE'S A

NATURAL KILLER

INSIDE EVERYONE

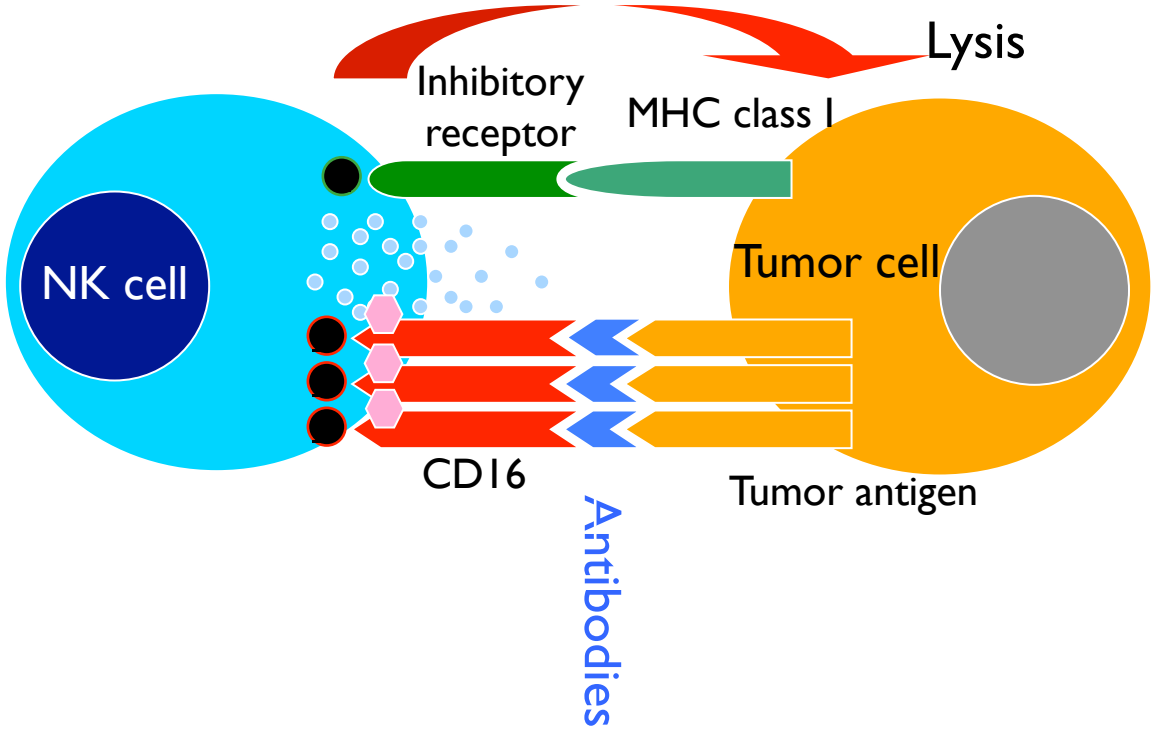
WITH THE POTENTIAL TO TAKE ON

MULTIPLE MYELOMA

Bristol-Myers Squibb

Immuno-Oncology

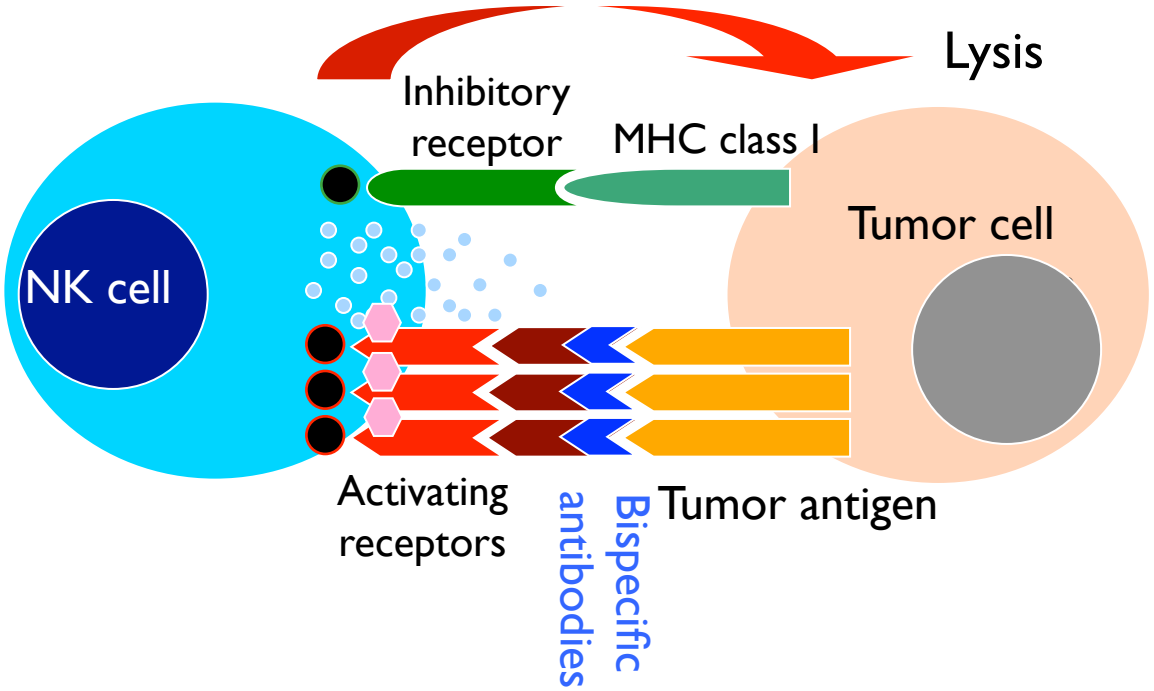
Antibody-dependent cellular cytotoxicity



Rituxan, Herceptin, Erbitux, Daratumumab

Bispecific antibodies

– anti-tumor x anti-NK activating receptor



Checkpoint blockade therapies

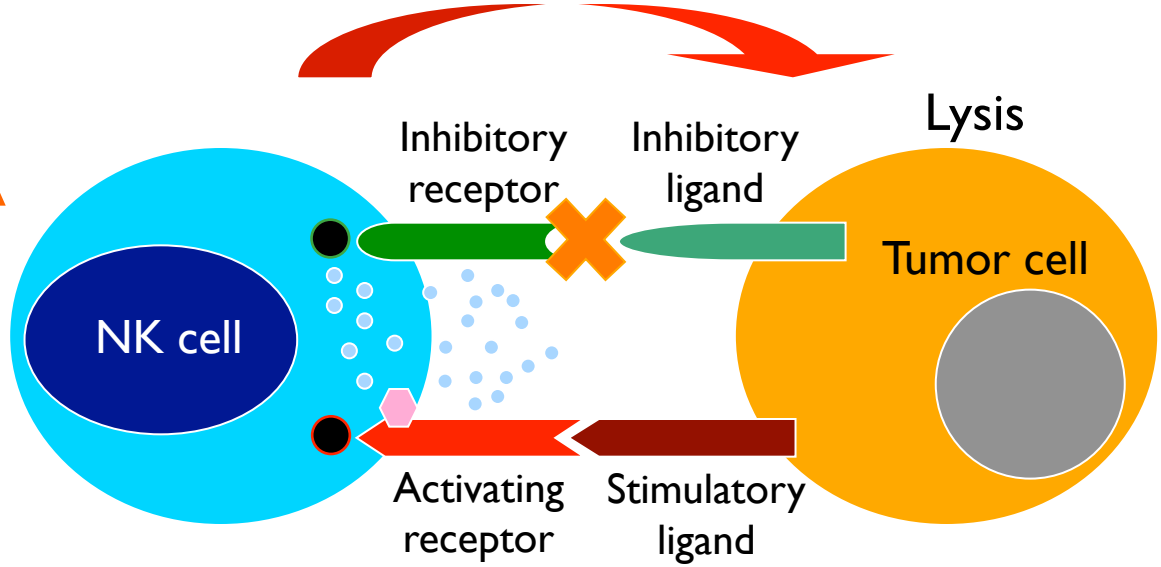
anti-KIR

anti-NKG2A

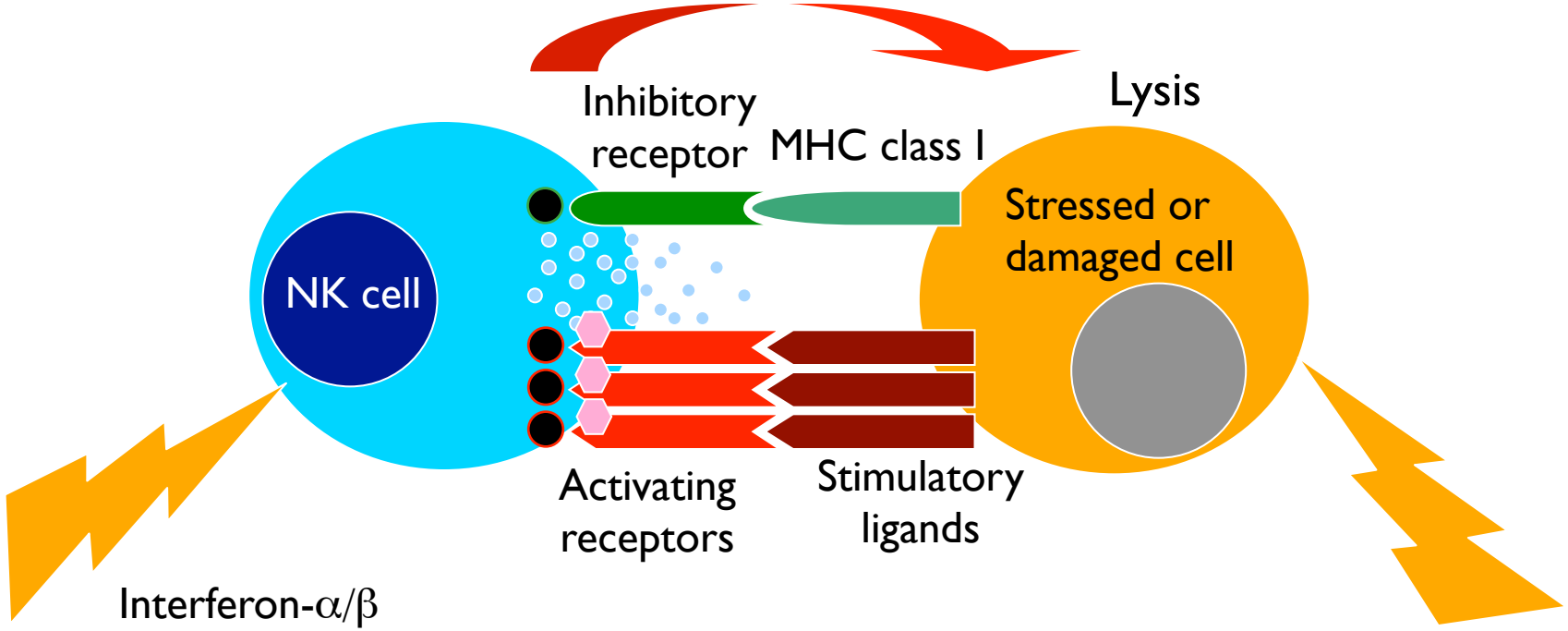
anti-PD1

anti-Tim3

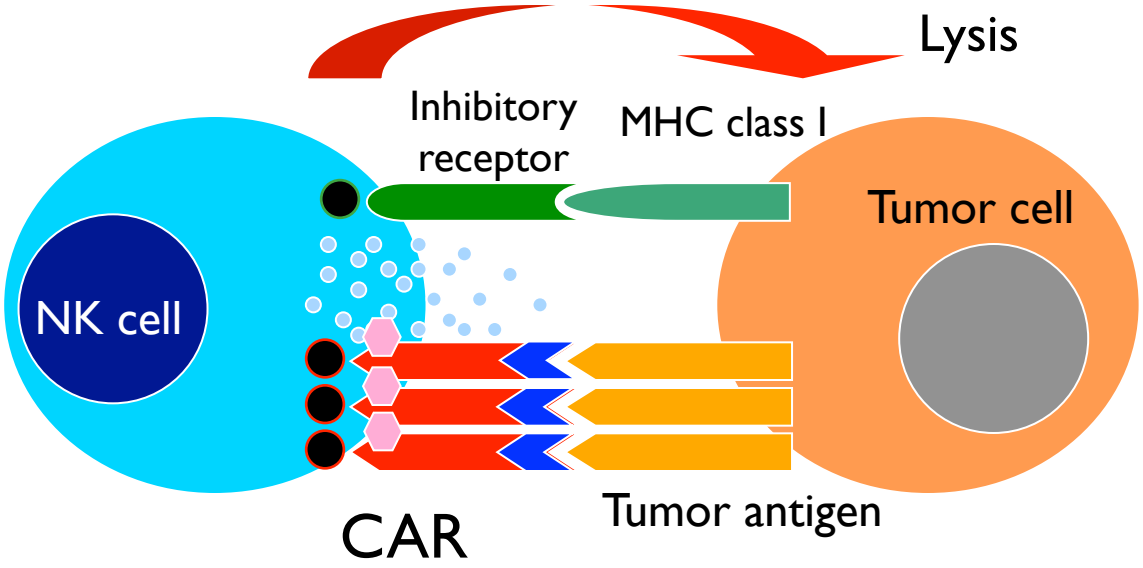
anti-LAG3



Therapies that up-regulate stress-induced ligands on tumors or agents that activate NK cells

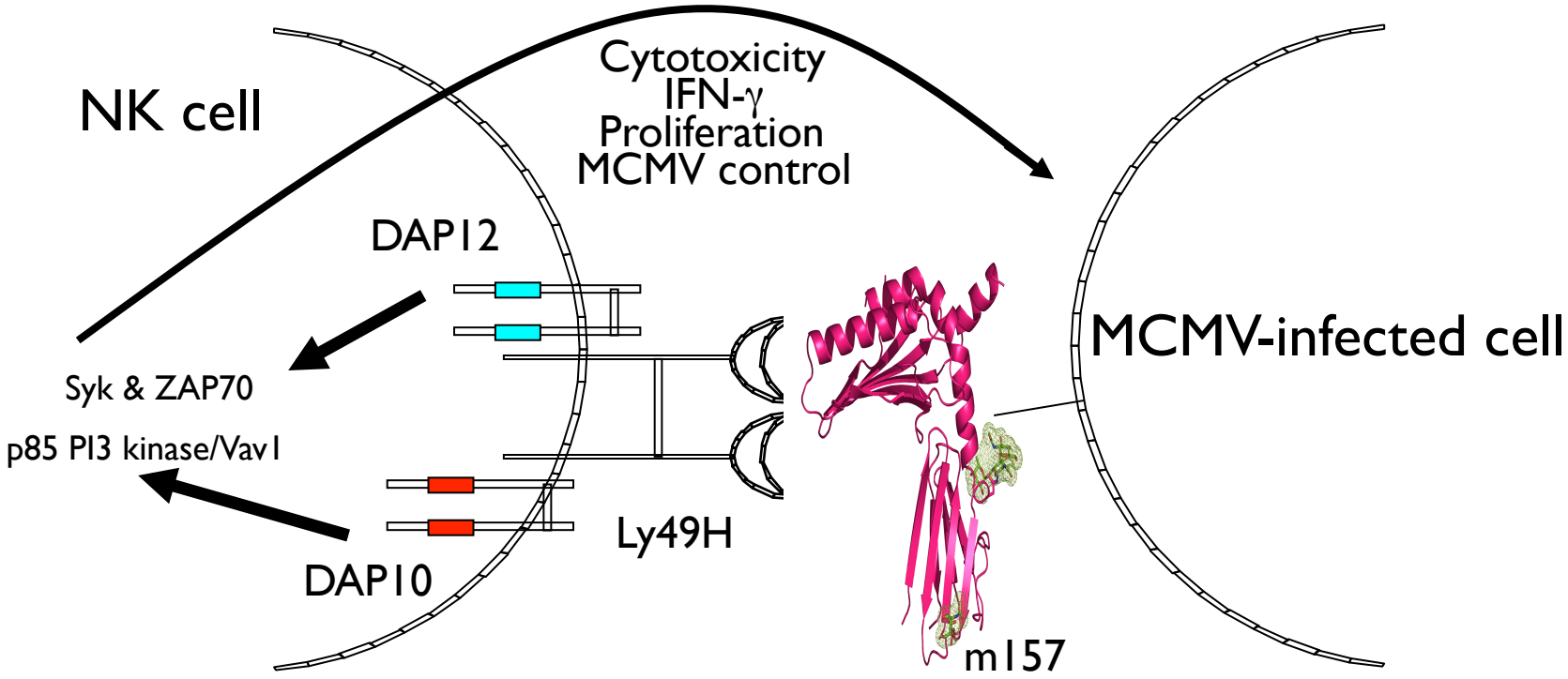


Chimeric antigen receptors

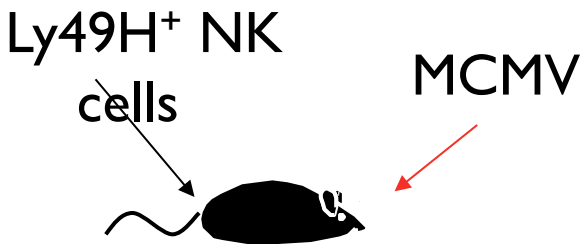


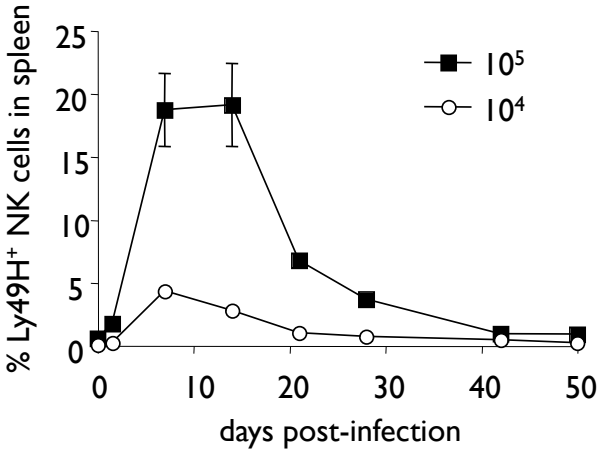
anti-CD19-cytoplamic CD137-CD3ζ

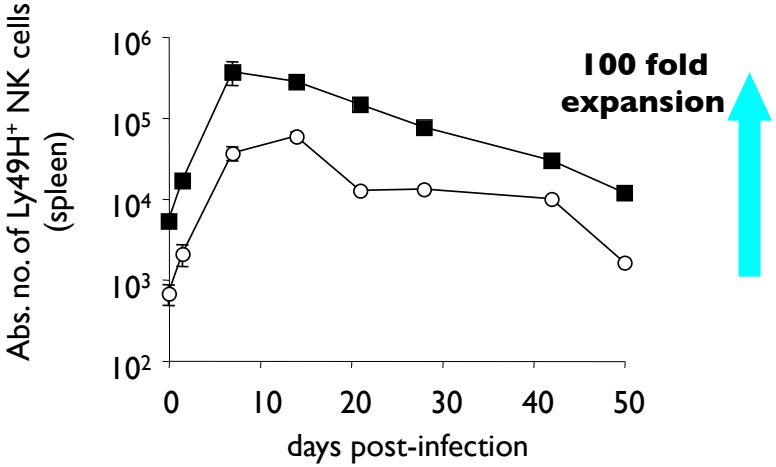
Ly49H drives NK cell responses to cytomegalovirus in mice

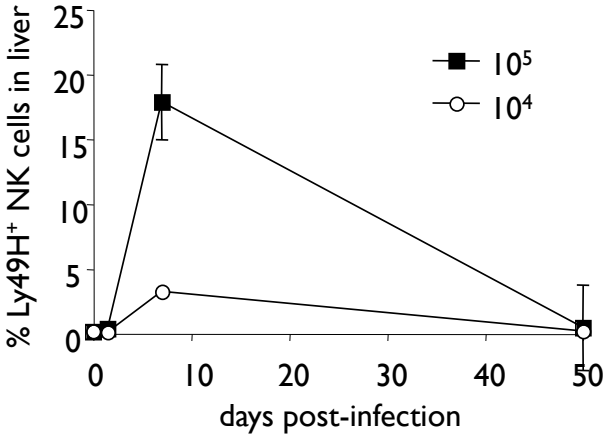


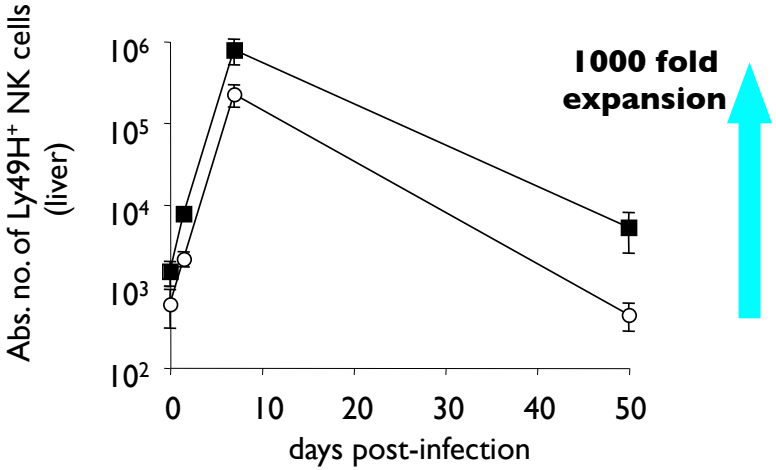
Expansion and contraction of Ly49H$^+$ NK cells during MCMV infection









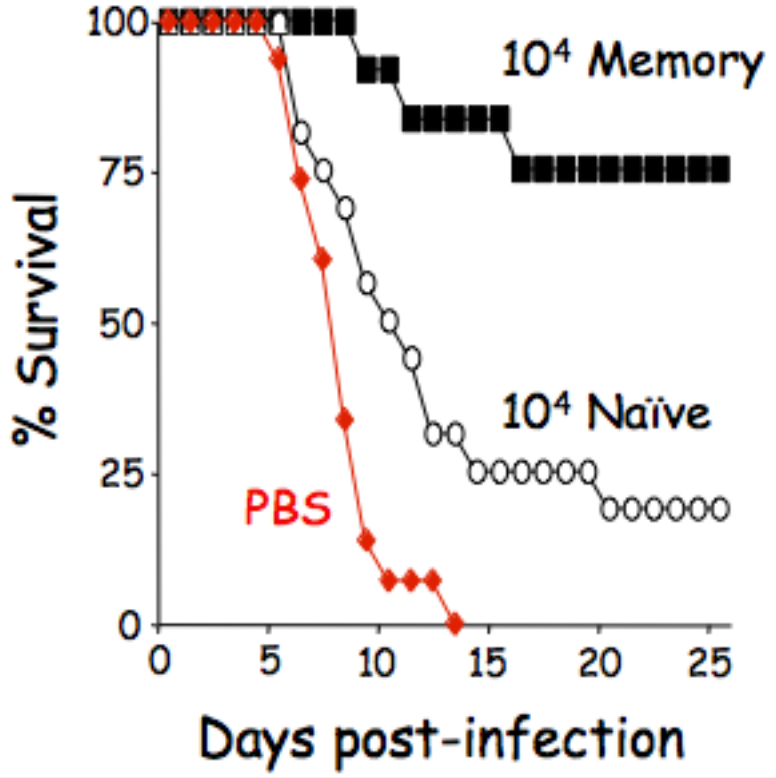


Long-lived memory NK cells protect neonatal mice against MCMV infection better than naïve NK cells

Adult naïve or memory NK cells

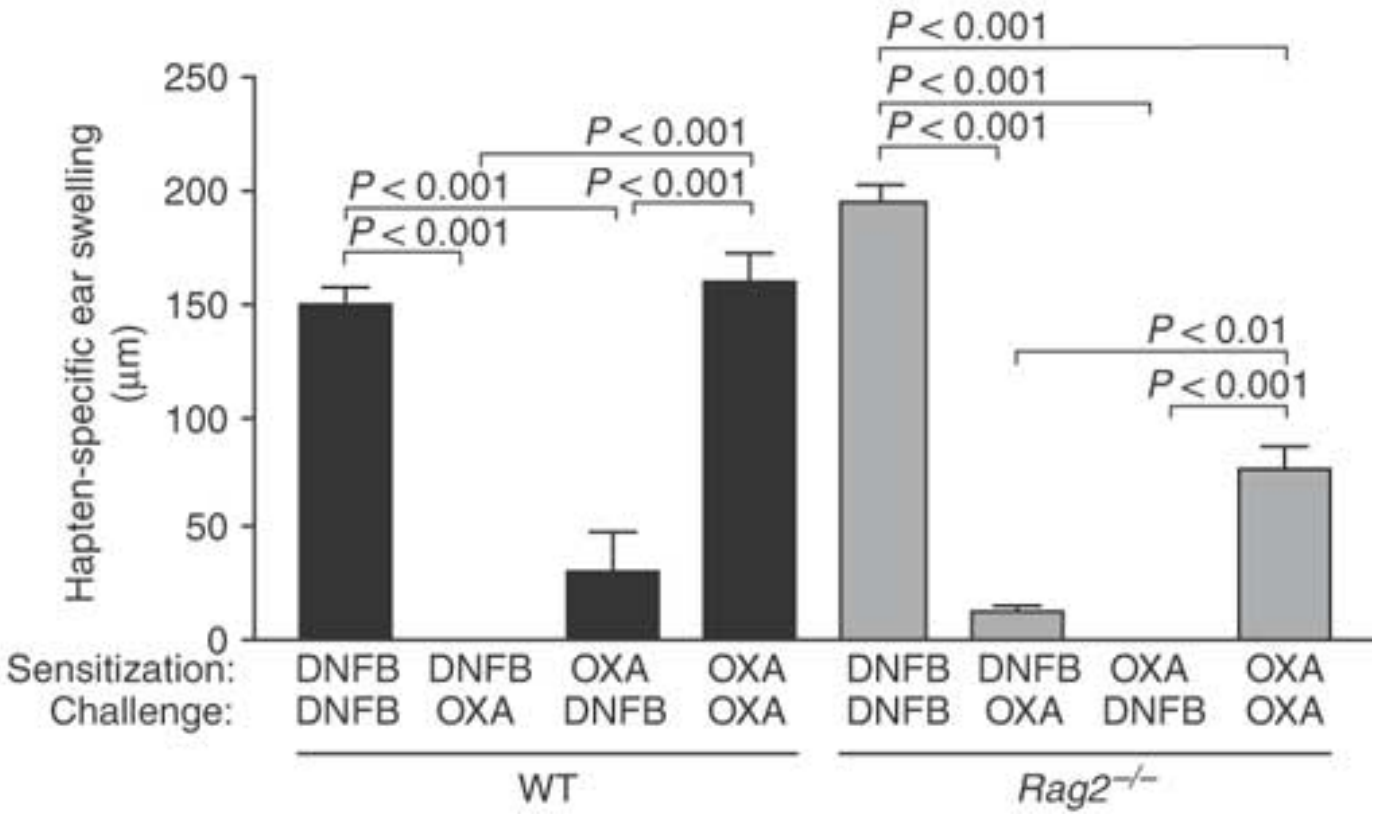
MCMV

Newborn MCMV-susceptible mice



Memory NK cells kill better and make more cytokine on a per cell basis than naïve NK cells in vitro

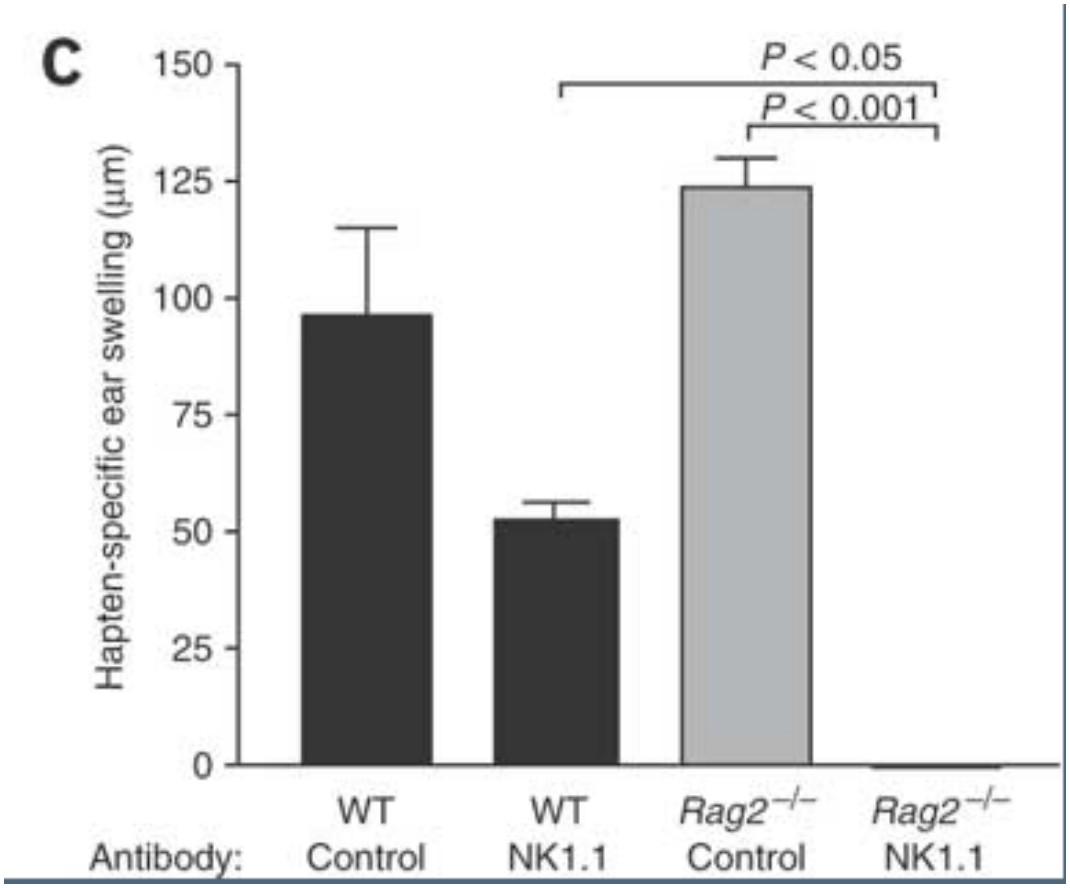
Hapten-specific contact hypersensitivity responses in RAG-deficient mice



day 0 & 1 paint hapten on back skin
day 6 or 30 – challenge ear – measure swelling

O'Leary NI 2006

Depletion of NK cells eliminates contact hypersensitivity responses in RAG-deficient mice



day 0 & 1 paint hapten on back skin
day 6 deplete NK cells day 6 – challenge ear – measure swelling

O'Leary NI 2006

Antigen-specific NK cell memory

*induced against haptens, HIV-I, influenza, VSV, HSV-I

*adoptively transfer hapten-specific contact hypersensitivity response to unimmunized recipients

*reside exclusively in liver

*require CXCR6 to mediate function

*receptors responsible for antigen recognition unknown

NK cells

- Keep you alive during certain viral infections
- Regulated by inhibitory and activating receptors
- Receptors are evolving rapidly
- Possess immunological memory

Recent Reviews

ILC
Colonna Immunity 48:1104, 2018

NK
Cerwenka & Lanier Nature Review Immunology 16:112, 2016

NK cell Immunotherapy
Miller & Lanier Ann Rev Cancer Biology 2019