Lessons learned from immunodeficiency: Understanding how Wiskott-Aldrich syndrome protein (WASp) stings in immune cells

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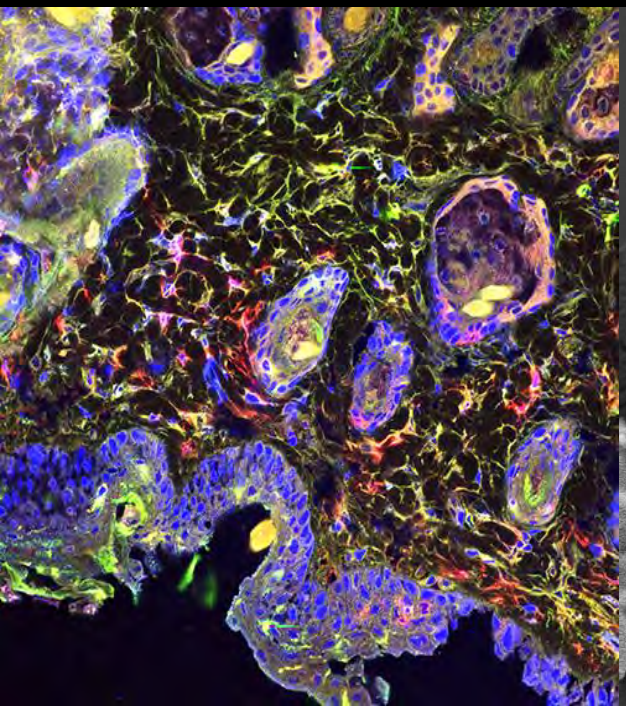
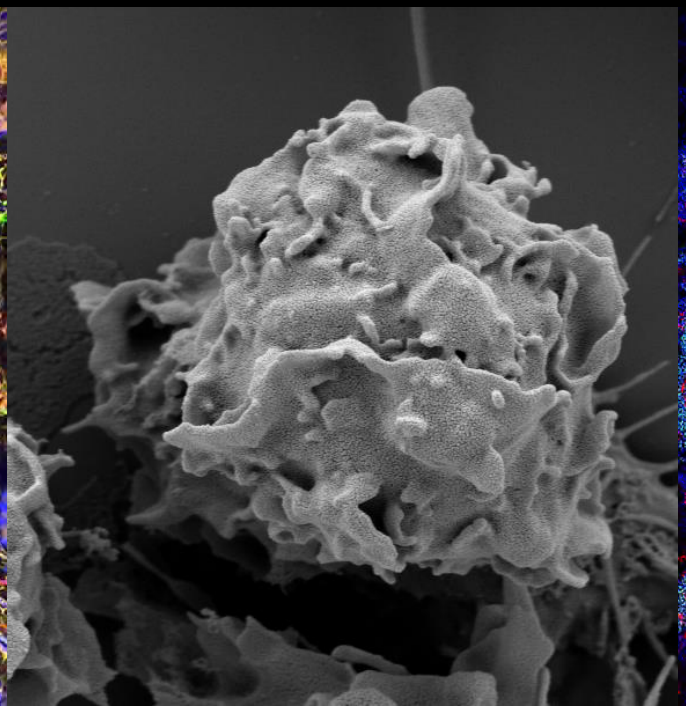
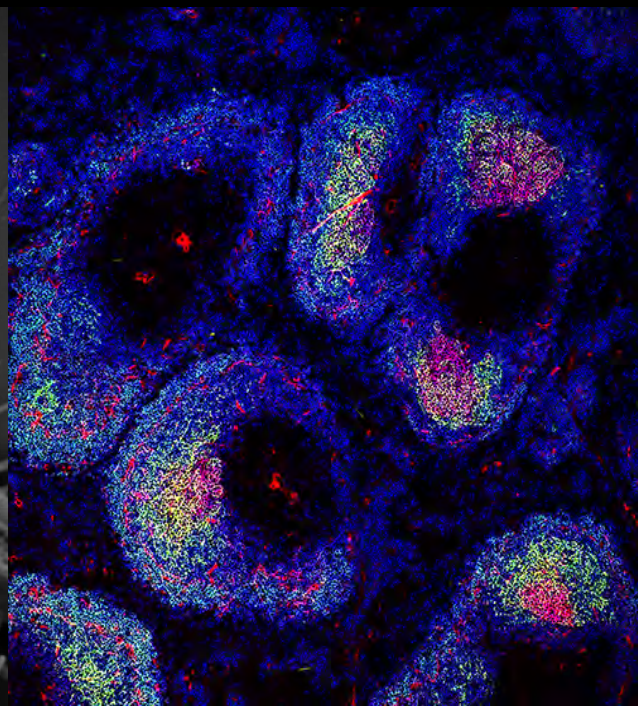
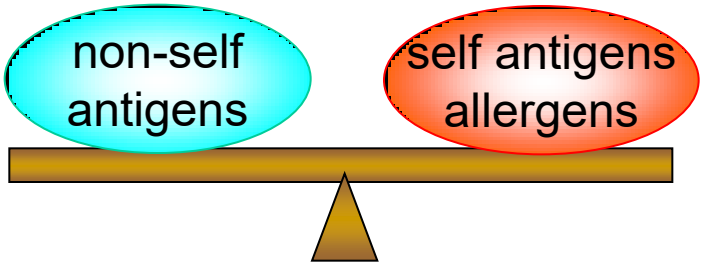
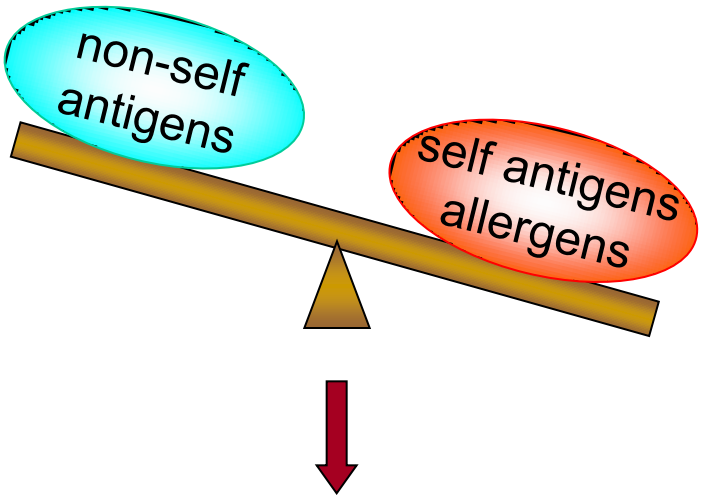
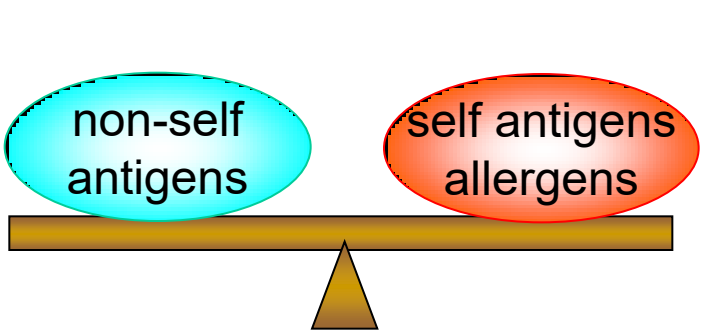
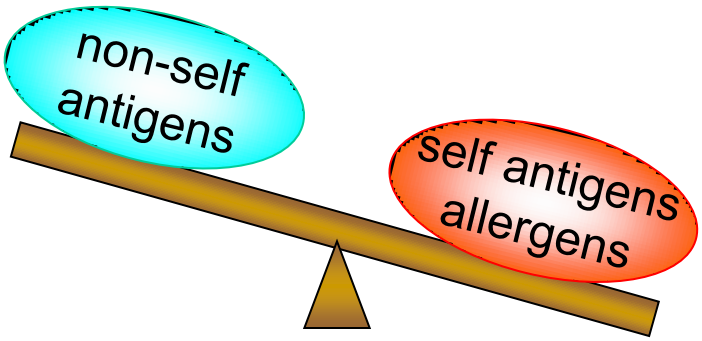








Allergy
Autoimmunity
Cancer





Boy with Wiskott-Aldrich syndrome

Major infections & Allergy
Autoimmunity
Cancer

Conundrum in Immunology:

How can an immune system fail to respond to non-self pathogens while **reacting vigorously to auto-antigens and allergens**?

The cell cytoskeleton – highly dynamic
Regulated by WASp family proteins

Cell-to-cell communication

Migration

Blood circulation

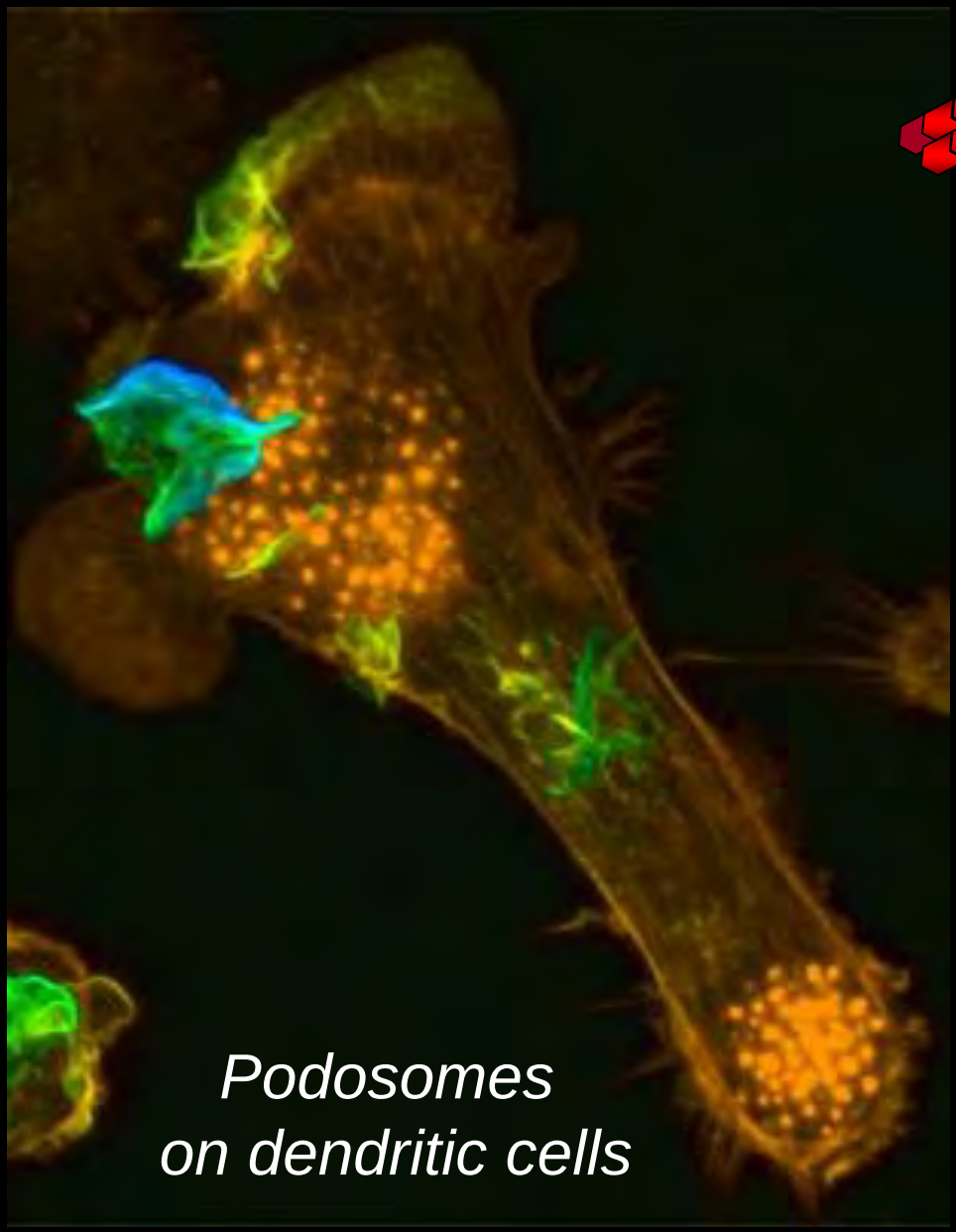
Immunological synapse

Actin Cytoskeleton

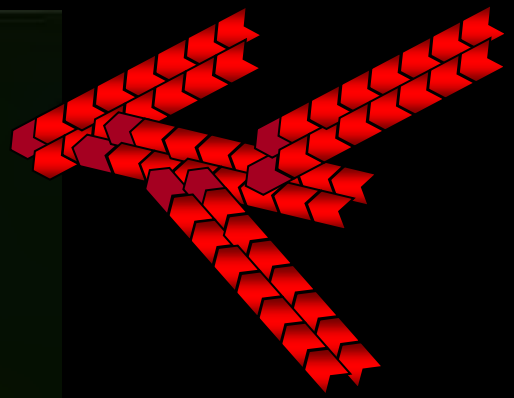
Formin family

WASp family

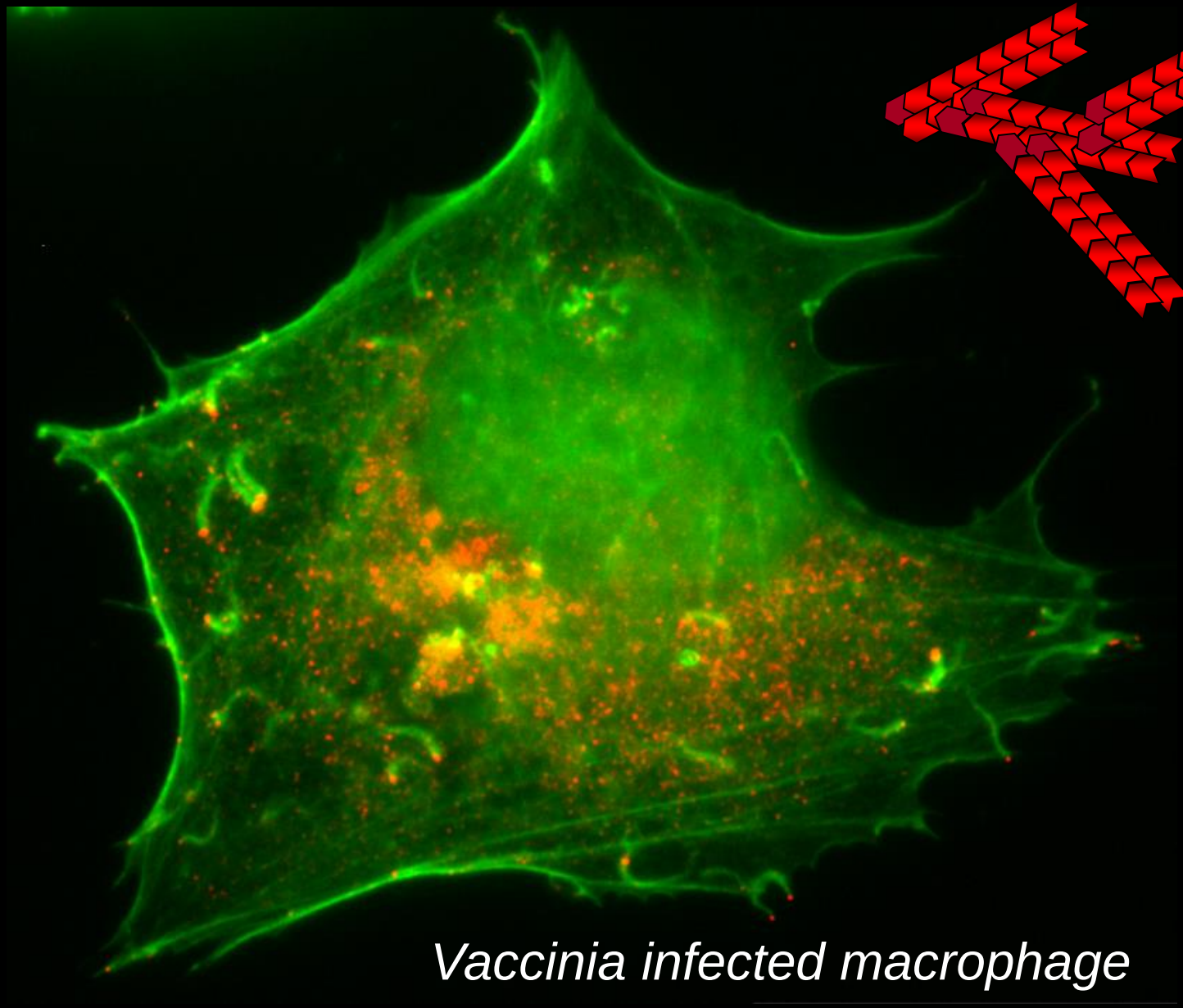
The actin cytoskeleton



Podosomes
on dendritic cells

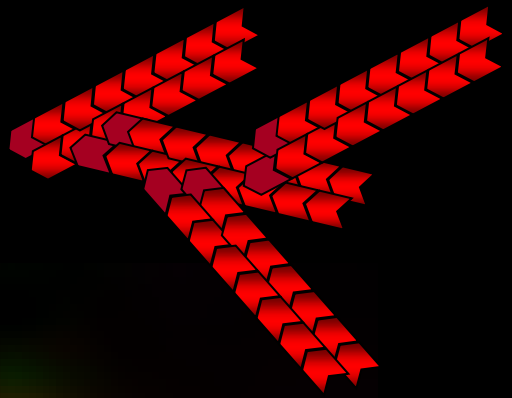


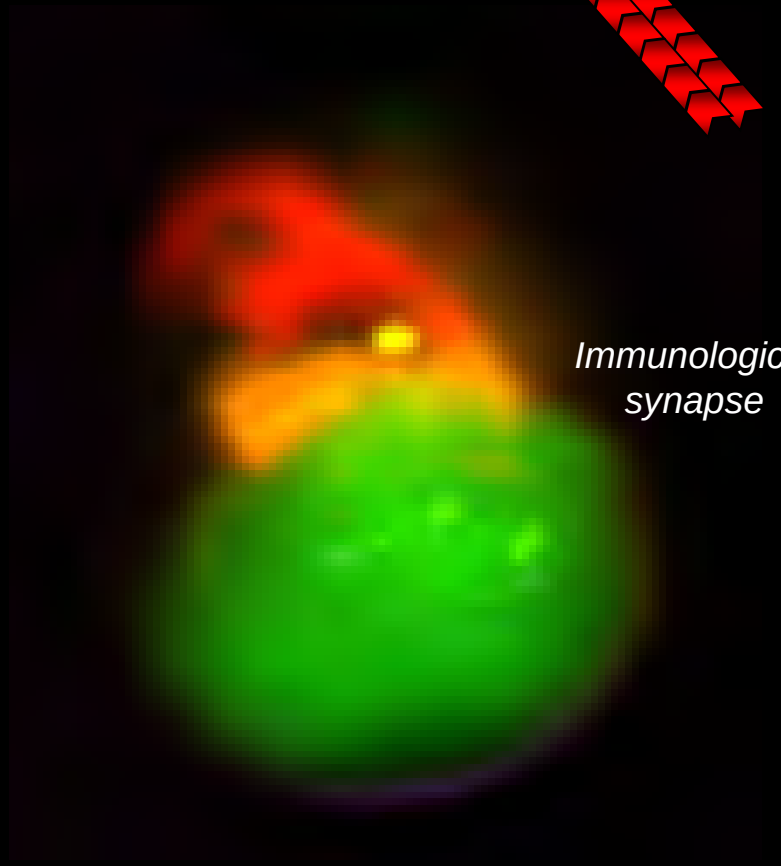
The actin cytoskeleton



Vaccinia infected macrophage

The actin cytoskeleton





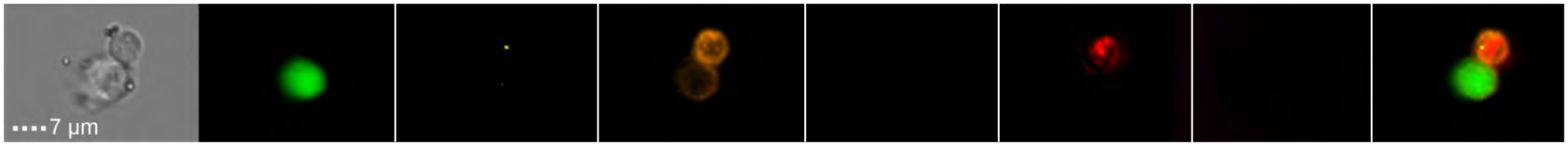
Immunological synapse

Immunological synapse with *ImageStream*
NK cell – Tumor cell

Microscopy

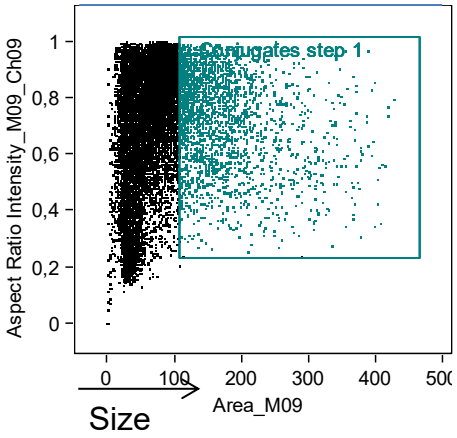
Wildtype NK cells

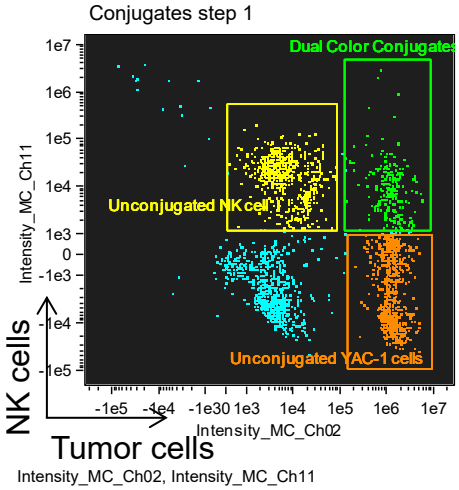
WASP-/- NK cells



YAC-1 | Granzyme B | F-actin | Live/Dead | NK1.1 | CD3 | Merge

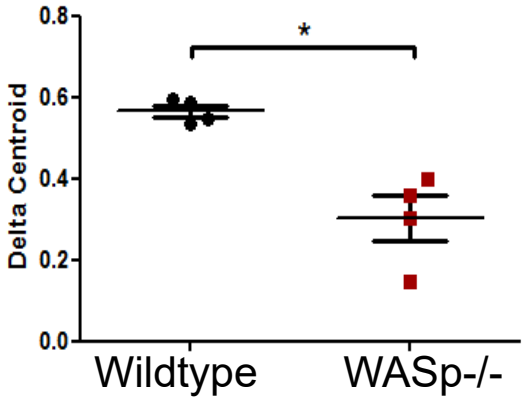
Flow cytometry





Intensity_MC_Ch02, Intensity_MC_Ch11

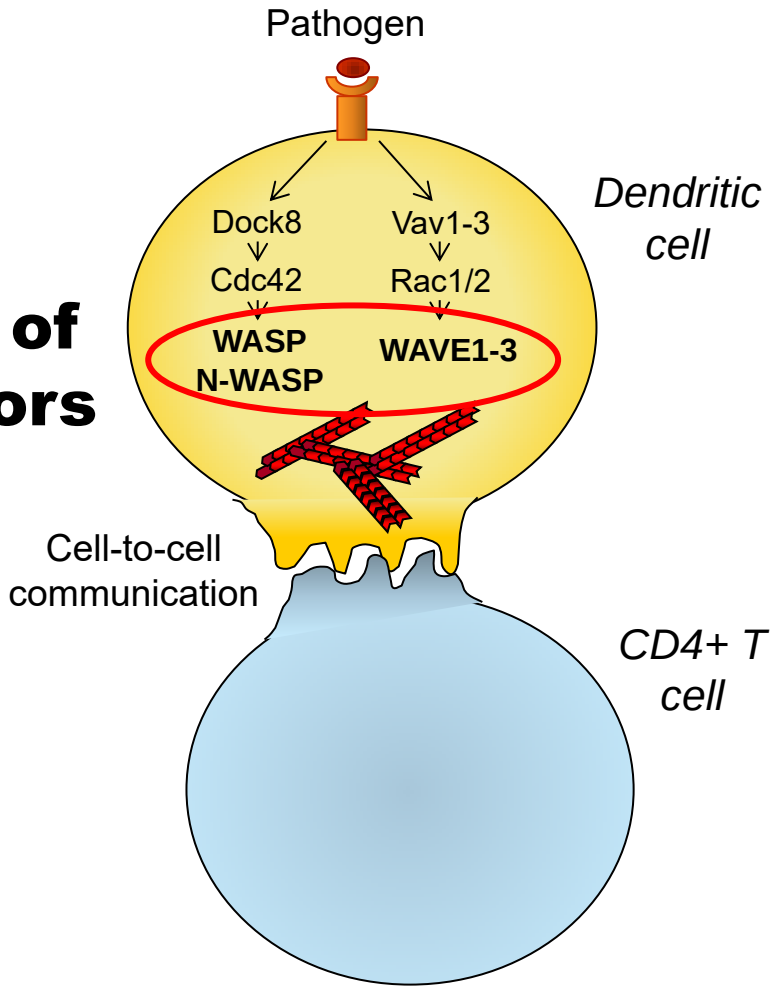
Population	Count	%Total	%Gated
Conjugates step 1	2295	11.5	100



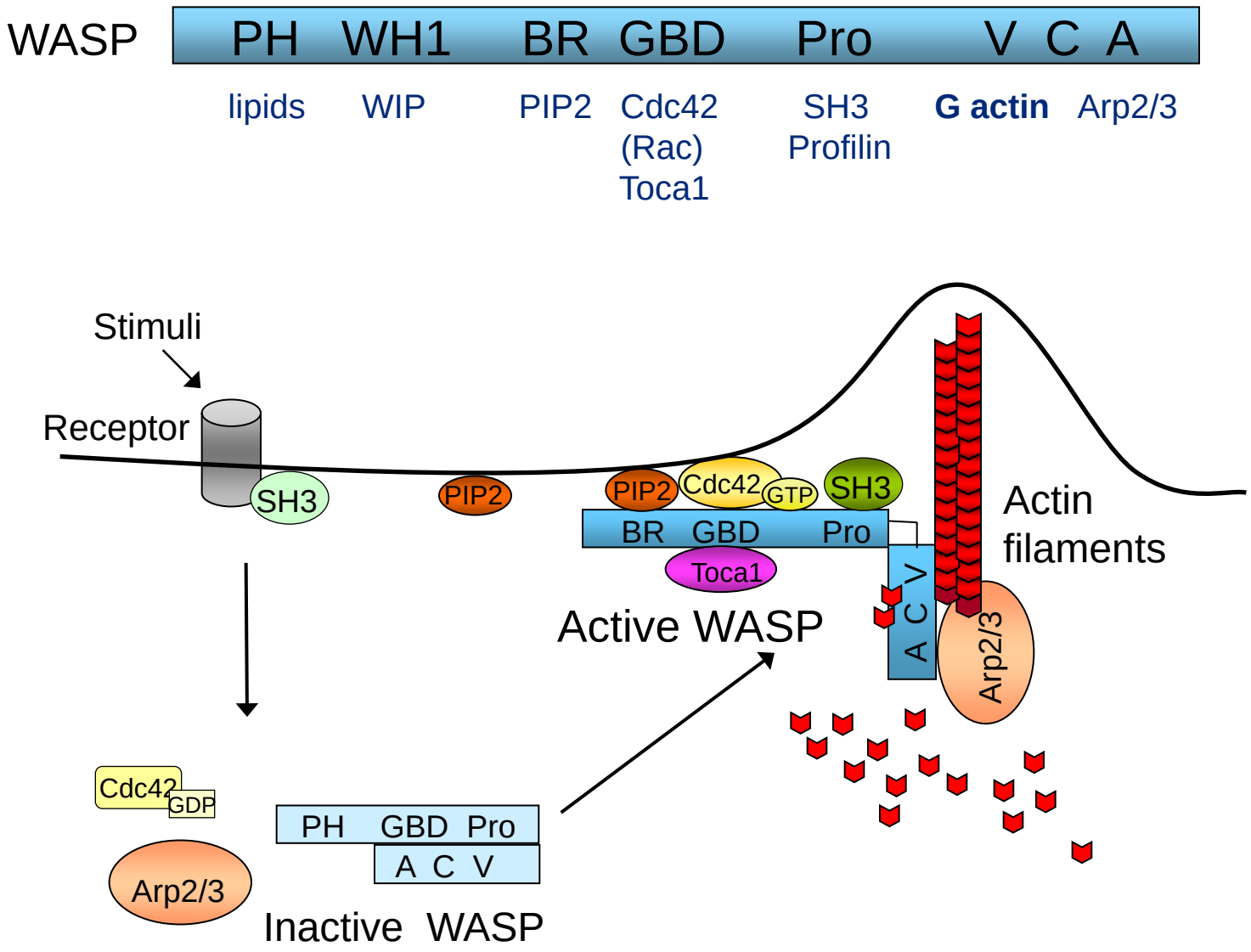
Kritikou et al, Scientific Reports 2016

The cell cytoskeleton – highly dynamic
Regulated by WASp family proteins

WASp family of actin regulators

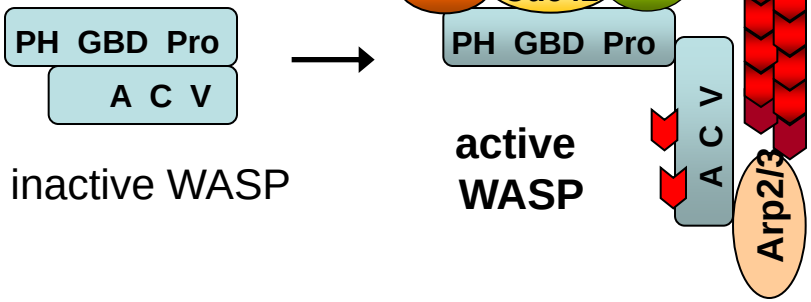


WASp - regulates the cytoskeleton in immune cells



WASp - A key immunological multitasker

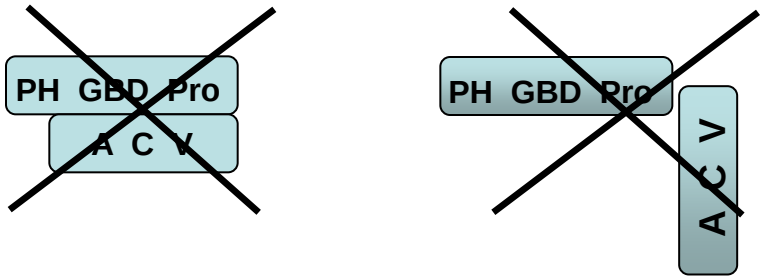
Normal



Actin polymerization

Migration
Adhesion
Cell-cell communication
Intracellular Signaling

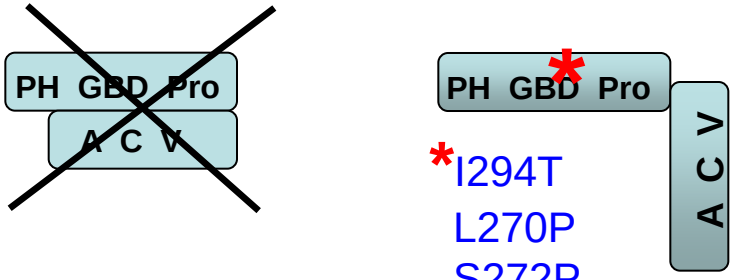
WAS



Immune response ↓

Eczema ↑
Autoimmunity ↑
Cancer ↑

XLN

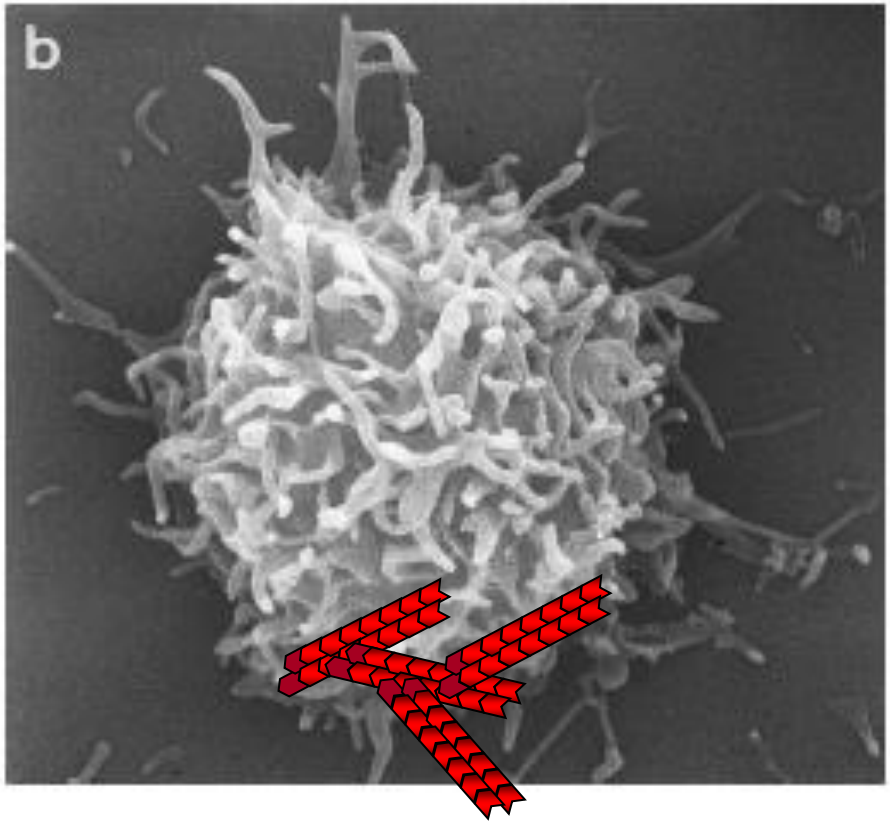


Congenital Neutropenia

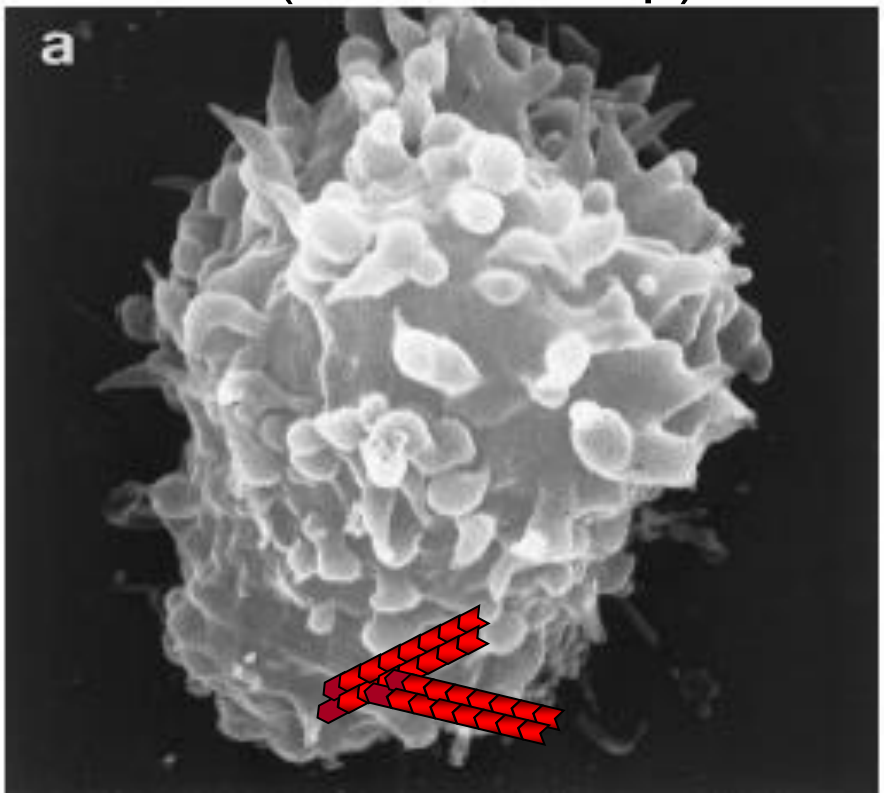
?

WASp-deficient lymphocytes show abnormal cytoarchitecture

Control



WAS (lack of WASp)

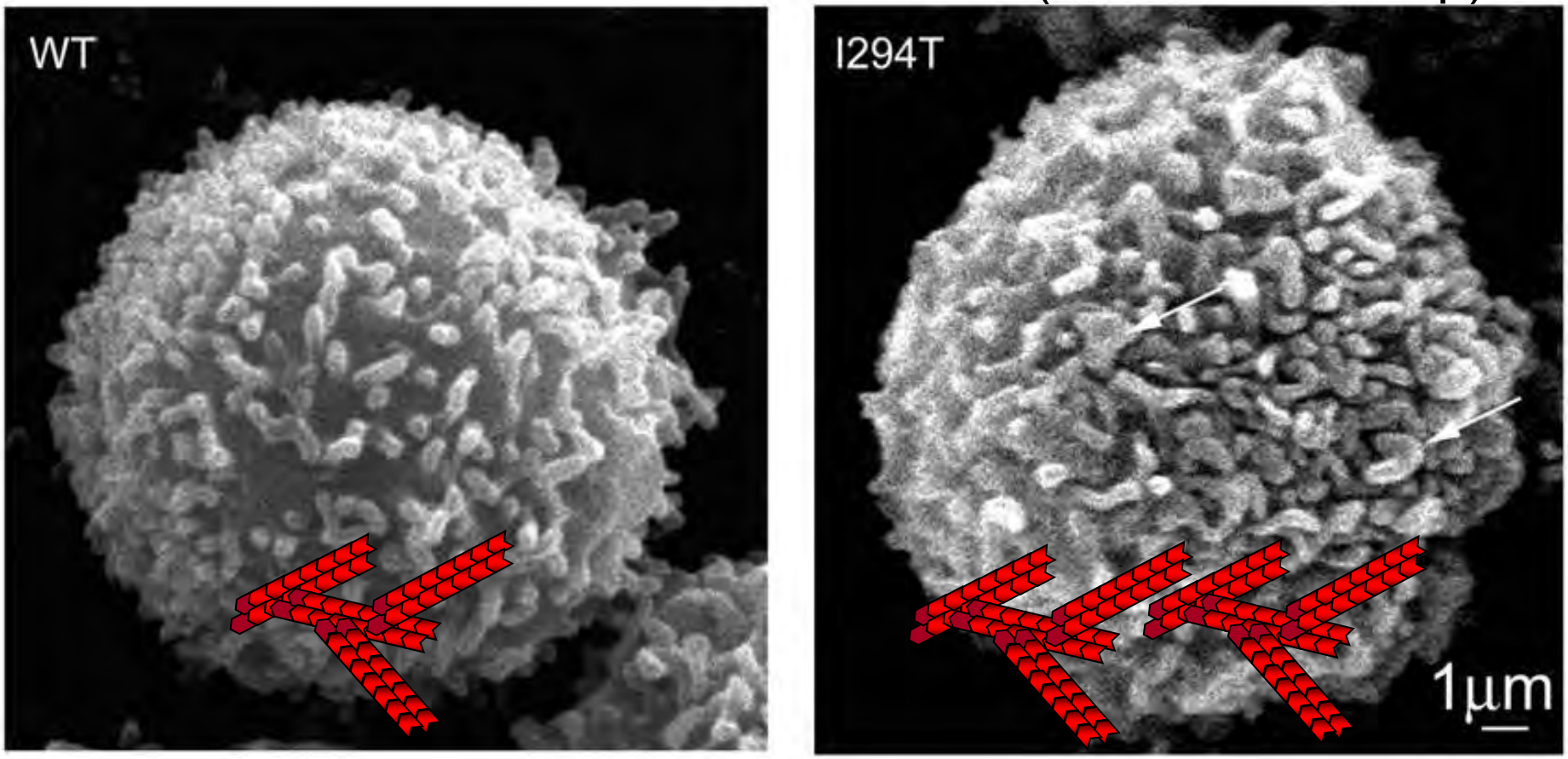


Kenney et al, Blood 1986
Molina et al., Blood 1997
Westerberg et al., Blood 2001, 2005

Lymphocytes expressing overactive WASp show abnormal cytoarchitecture

Control

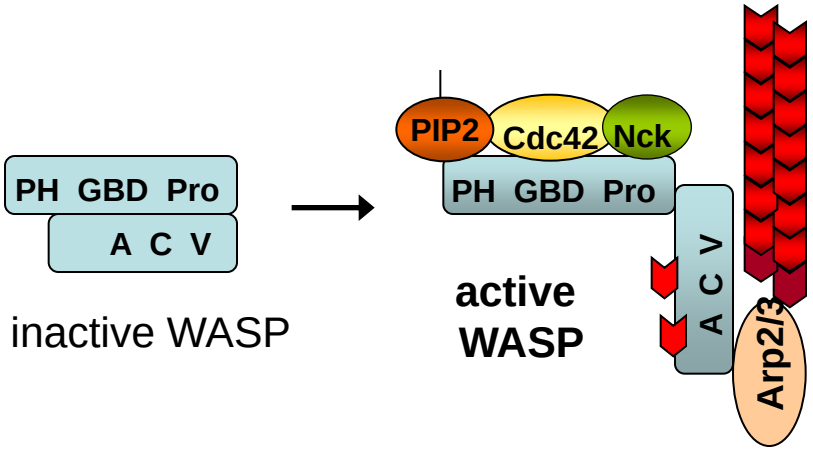
XLN (overactive WASp)



Moulding et al., J Exp Med 2007
Burns et al., Blood 2010
Westerberg et al., J Exp Med 2010

WASp - A key immunological multitasker

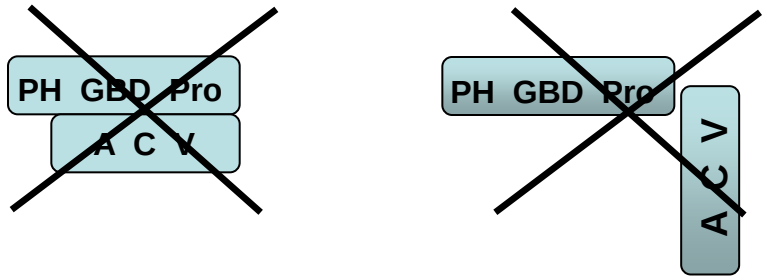
Normal



Actin polymerization

Migration
Adhesion
Cell-cell communication
Intracellular Signaling

WAS



Immune response ↓

Eczema ↑
Autoimmunity ↑
Cancer ↑

XLN

Congenital Neutropenia

?

Eczema – an enigma of WASp deficiency

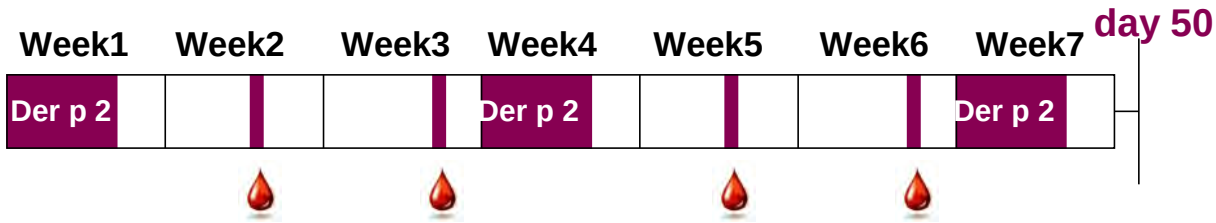
Eczema in WAS patients???

Cell-trafficking disease
Hyporesponsive cells

ause for development of eczema in WAS
zema mouse model

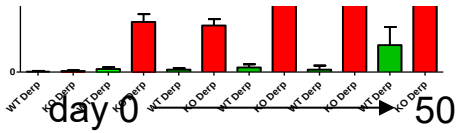
tal model

Marisa Baptista

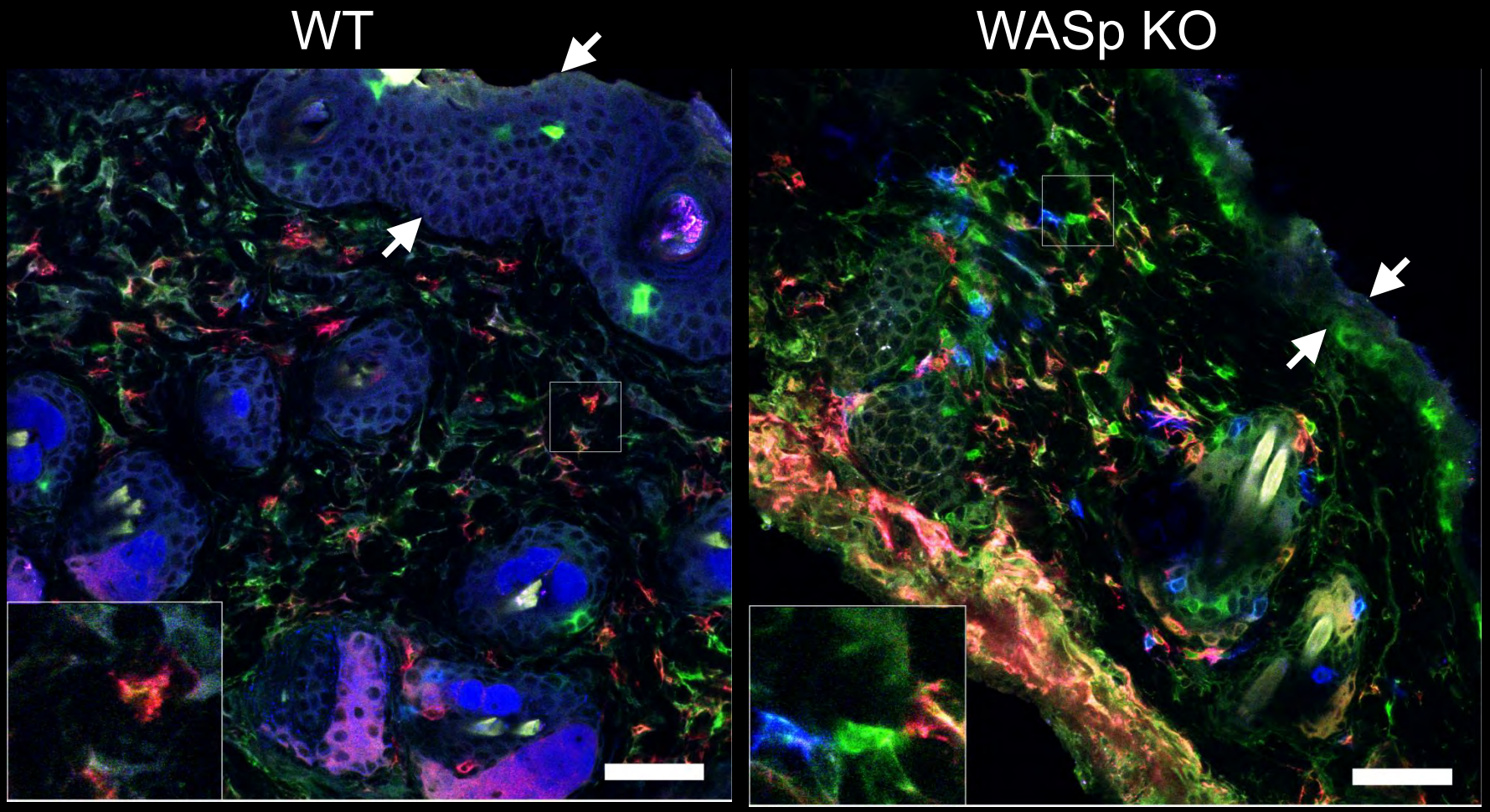


W I

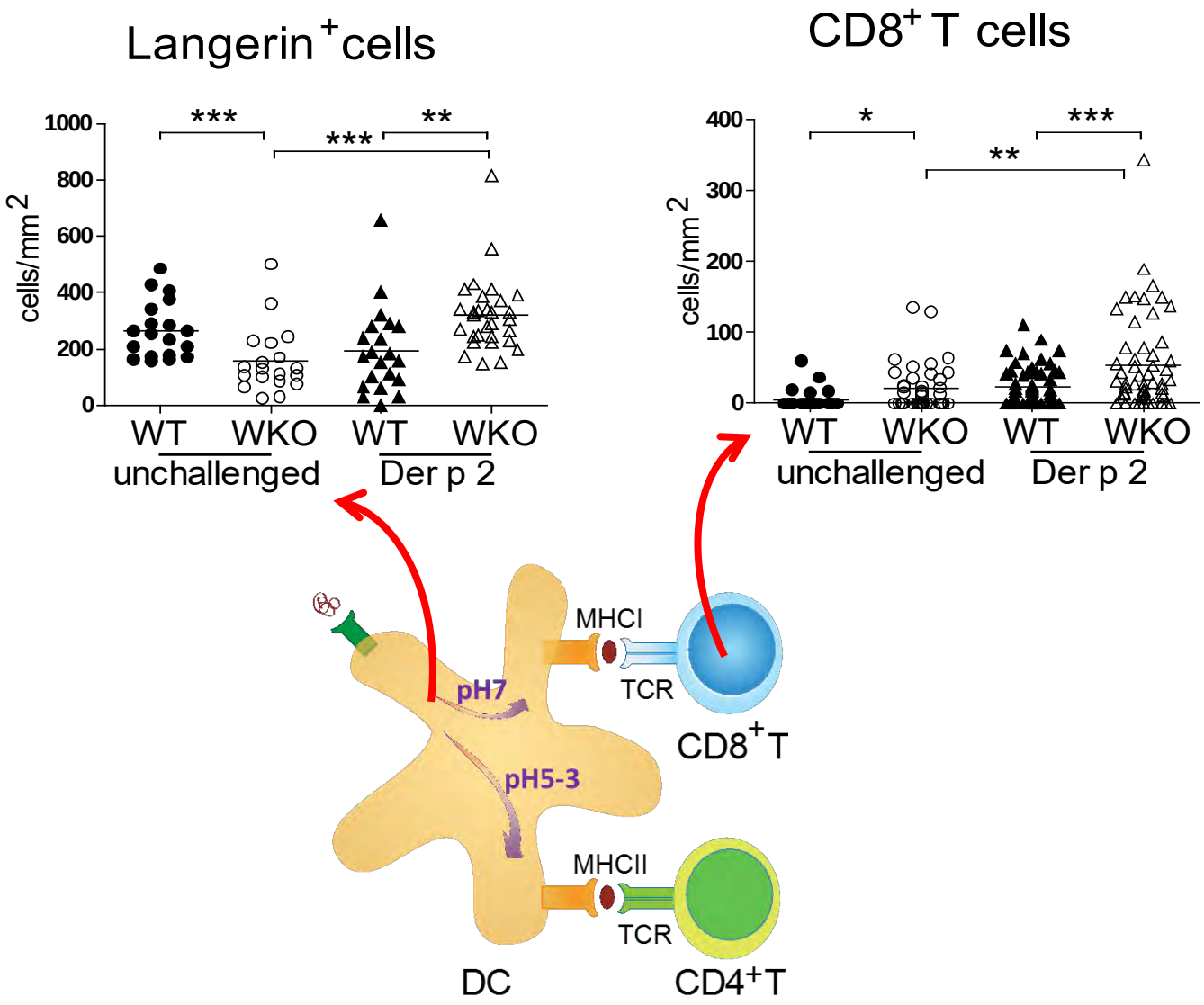
WASP KO



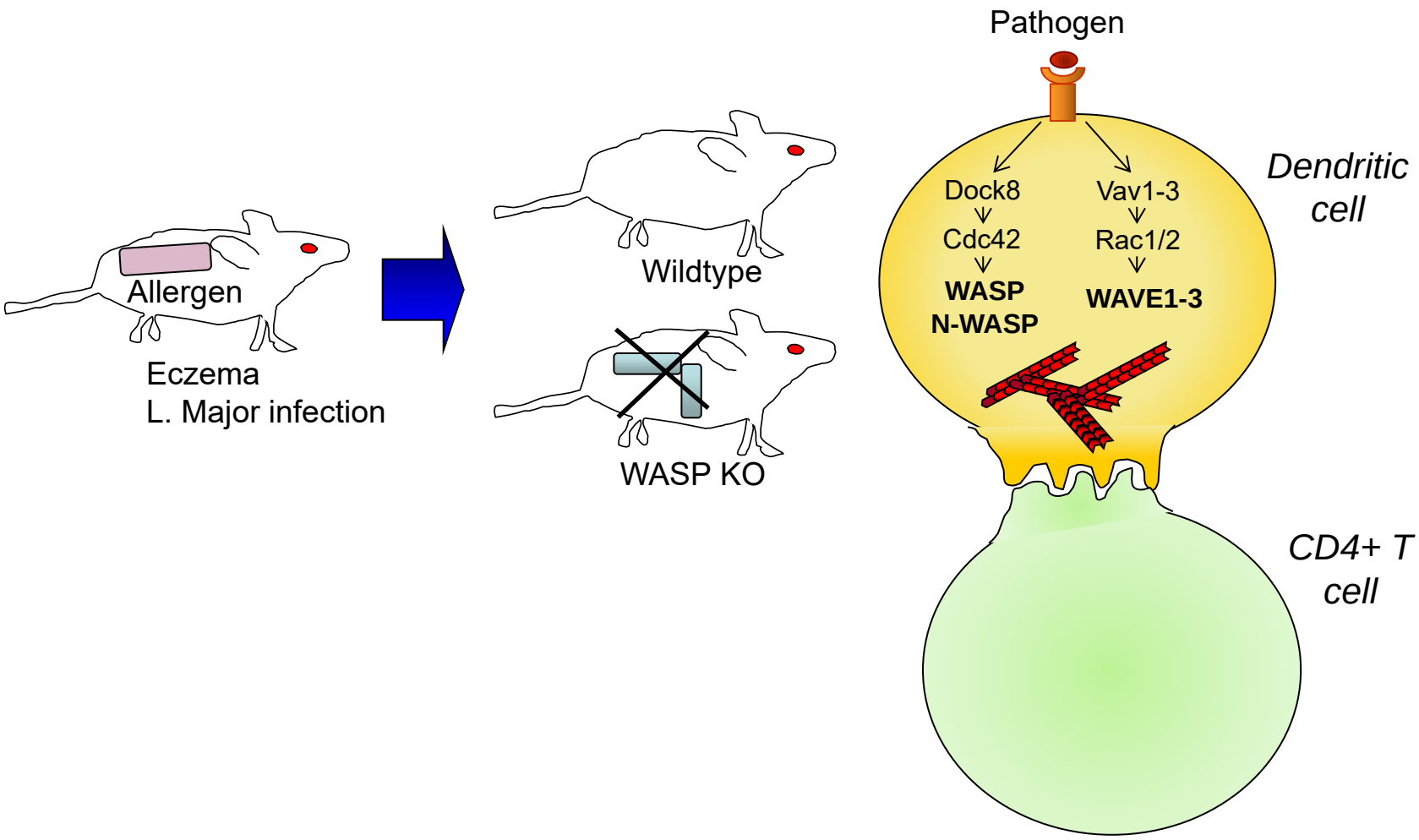
WASp KO mice have reduced epidermal thickening upon Der p 2 challenge



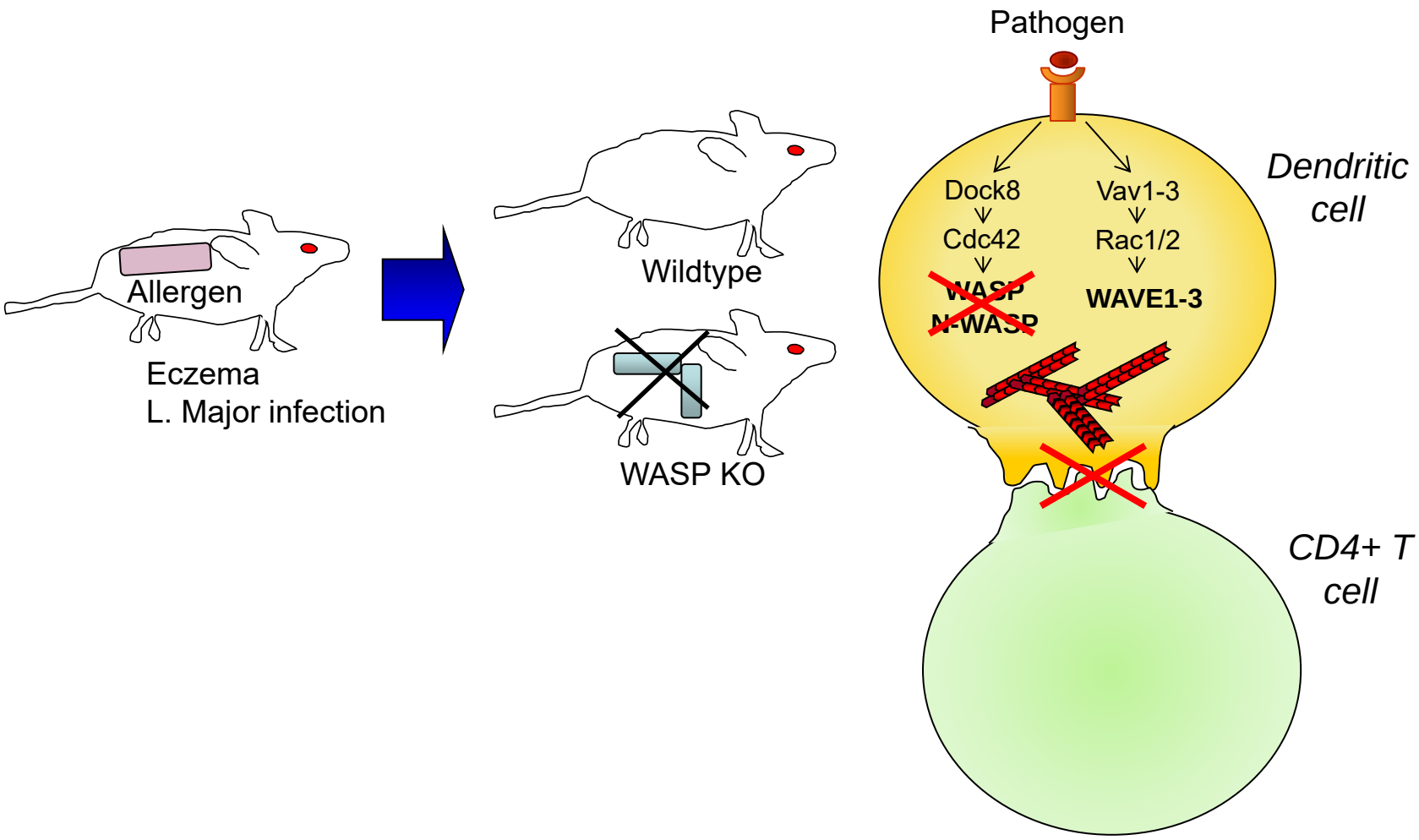
WASp KO mice accumulate dendritic cells and CD8+ T cells in the dermis



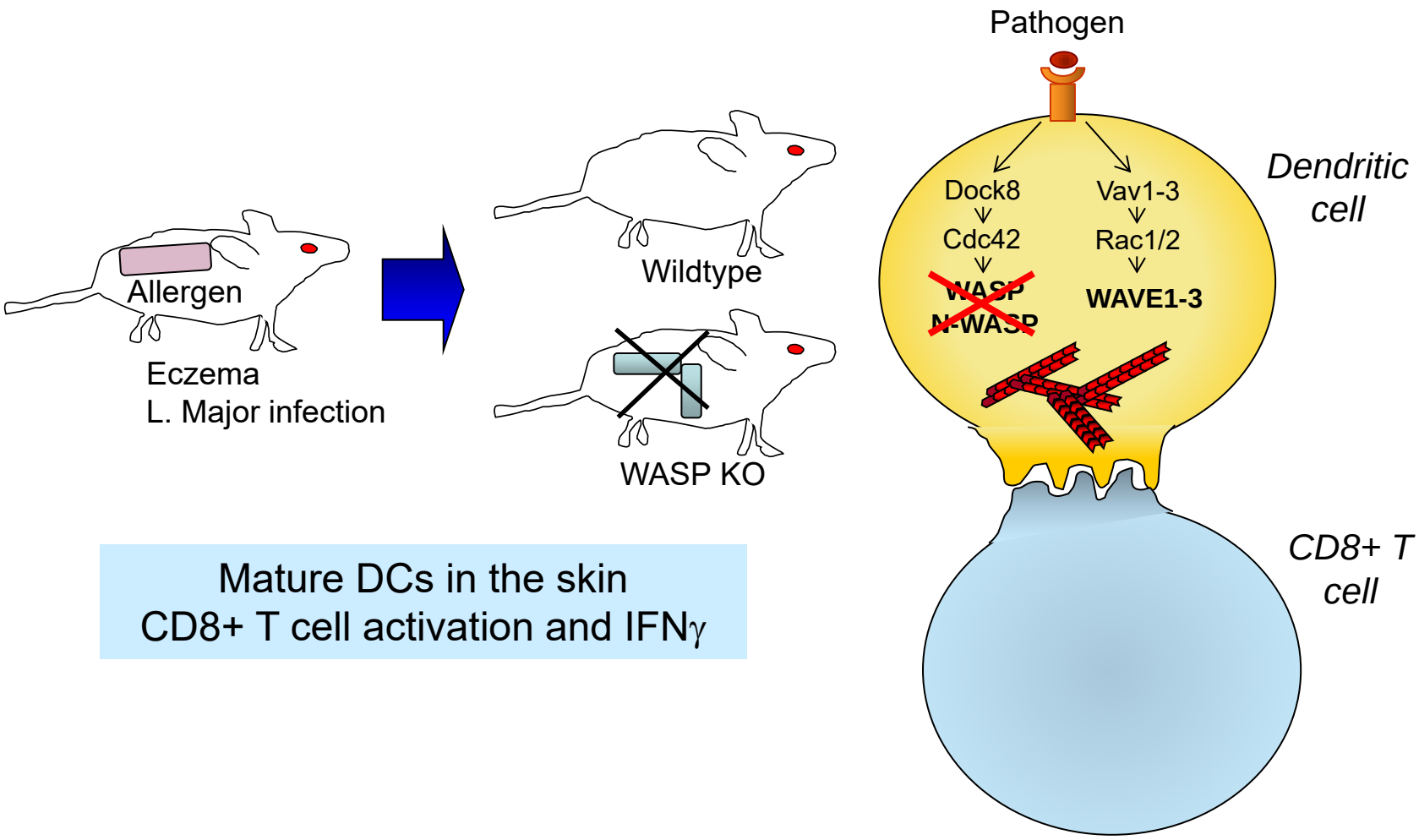
WASP-deficient dendritic cells fail to activate CD4+ T cells



WASP-deficient dendritic cells fail to activate CD4+ T cells

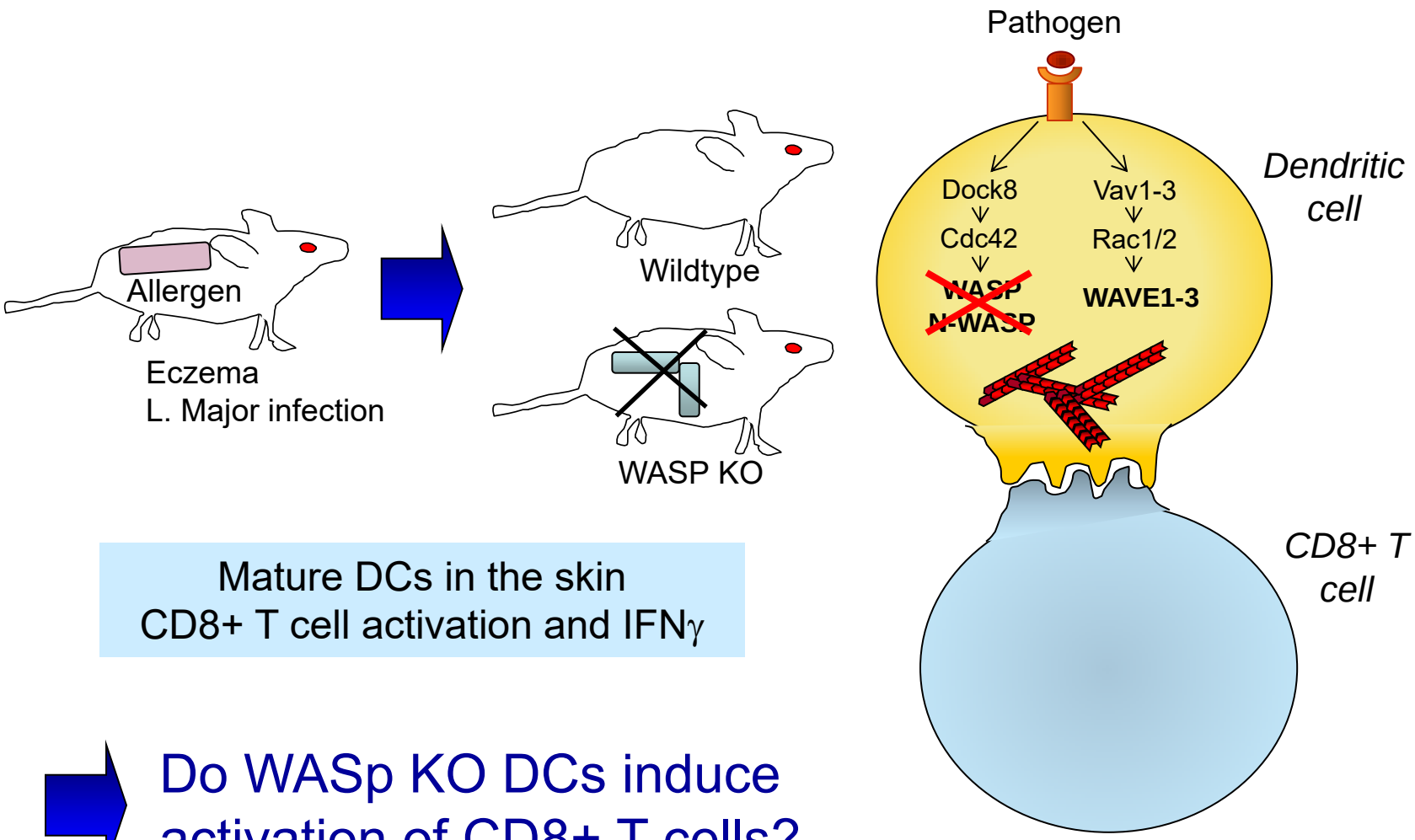


WASP-deficient dendritic cells activate CD8+ T cells



Mature DCs in the skin
CD8+ T cell activation and IFNγ

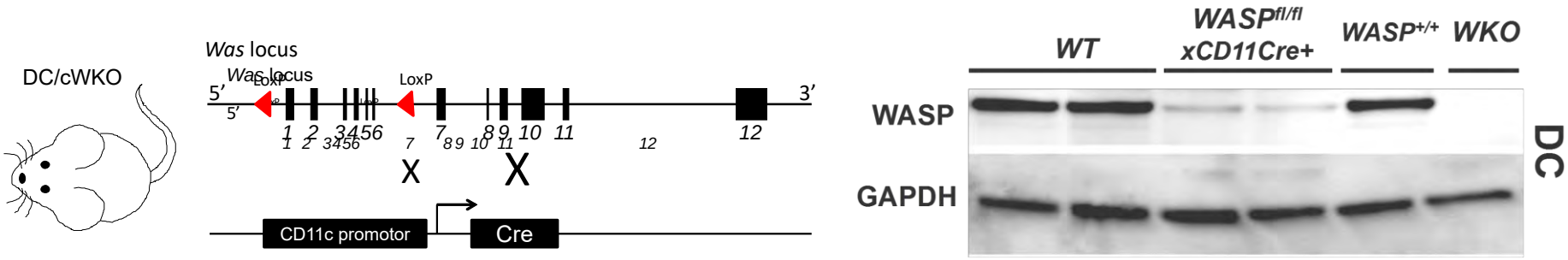
WASP-deficient dendritic cells activate CD8+ T cells



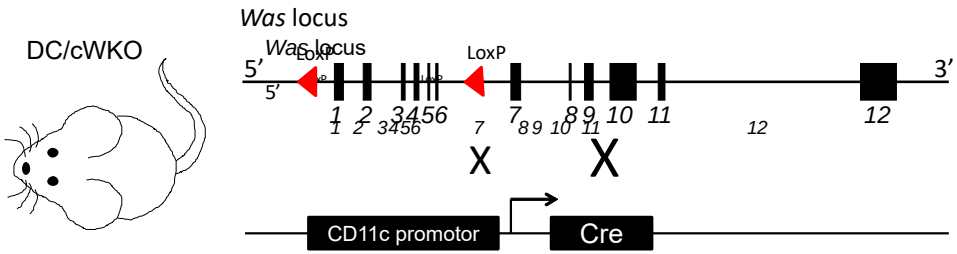
Mature DCs in the skin
CD8+ T cell activation and IFNγ

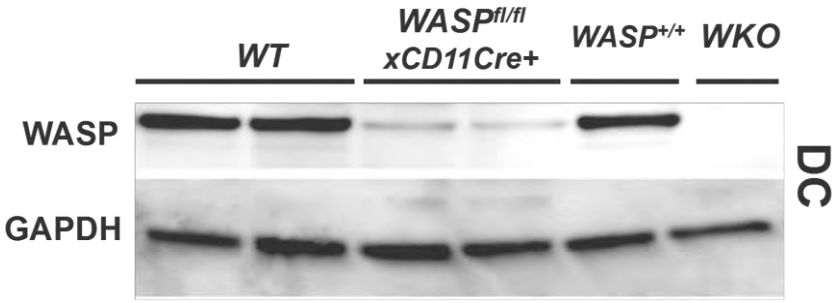
Do WASp KO DCs induce activation of CD8+ T cells?

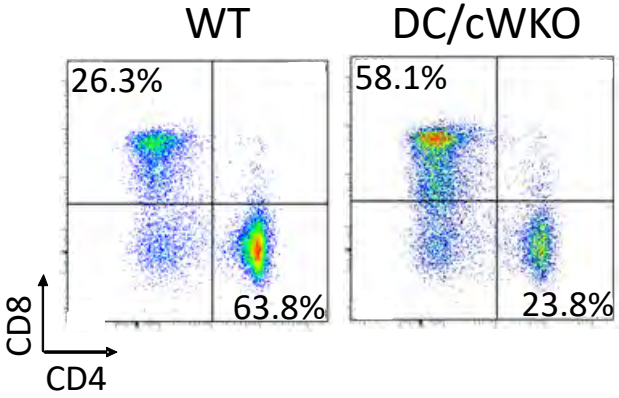
Increased expansion of CD8+ T cells over CD4+ T cells in DC-specific WASp KO mice

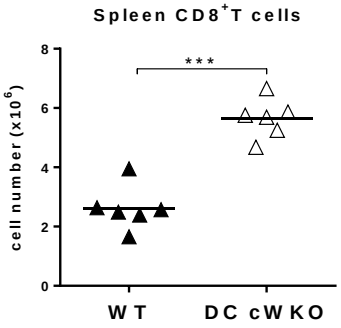


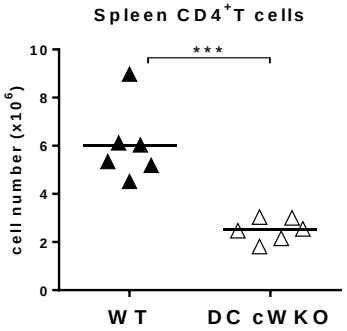
Increased expansion of CD8+ T cells over CD4+ T cells in DC-specific WASp KO mice

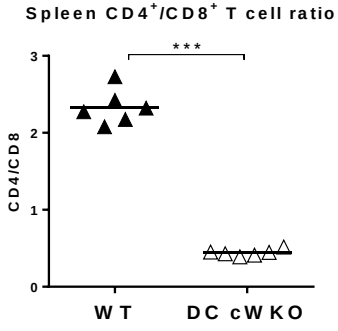






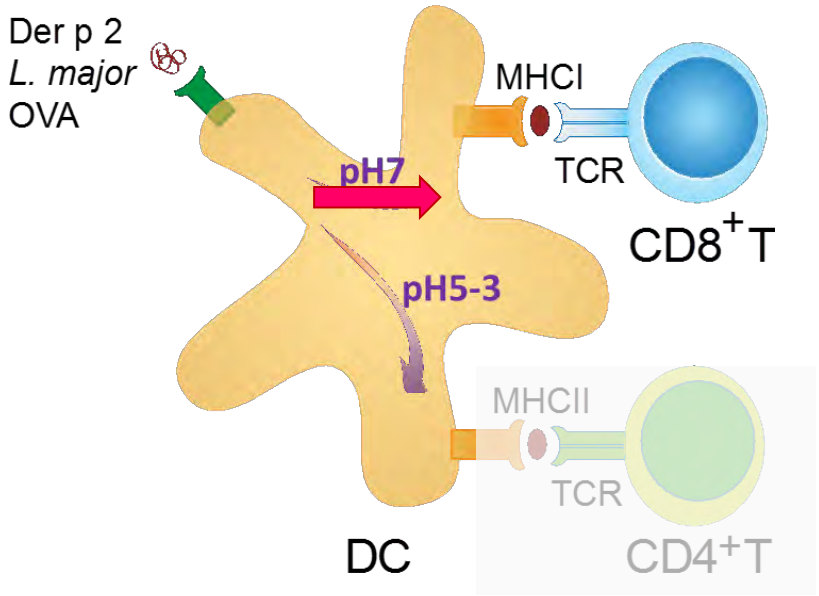






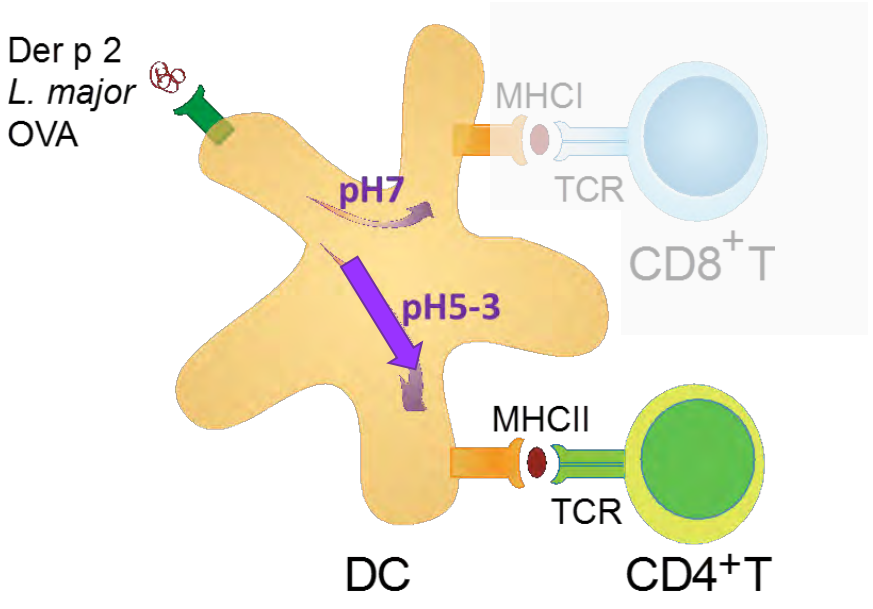
DCs in the wildtype setting

CD8+ DCs



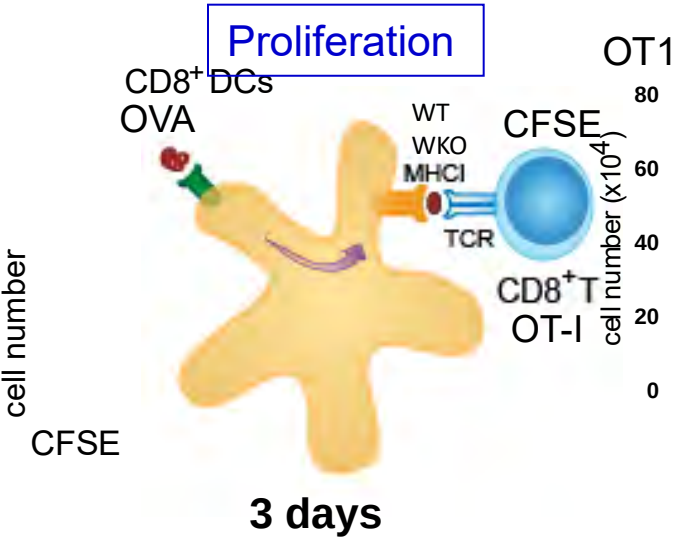
10% of spleen DCs

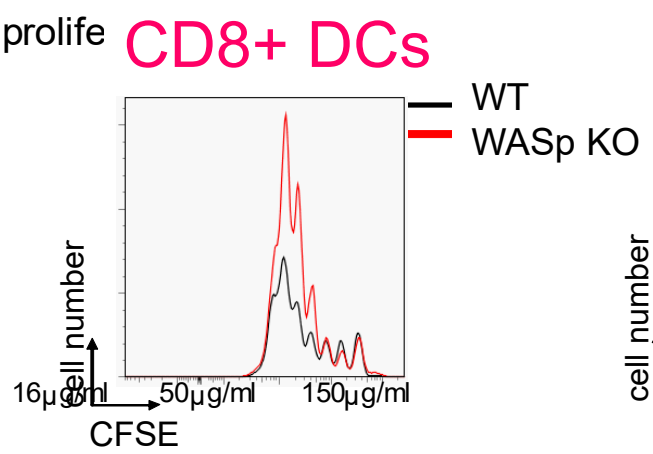
CD8- DCs

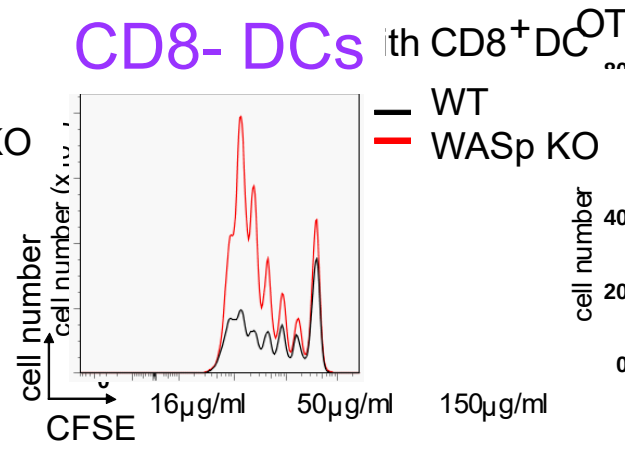


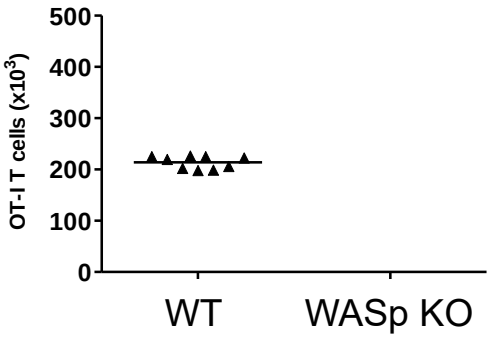
50% of spleen DCs

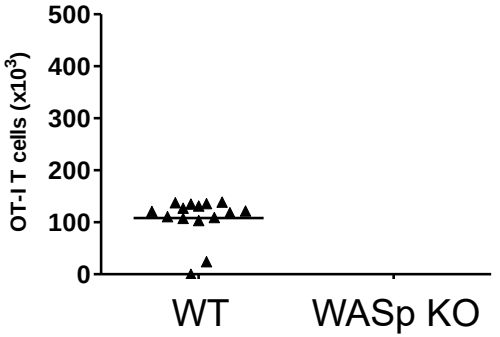
WASp KO CD8- DCs have increased capacity to cross-present antigen



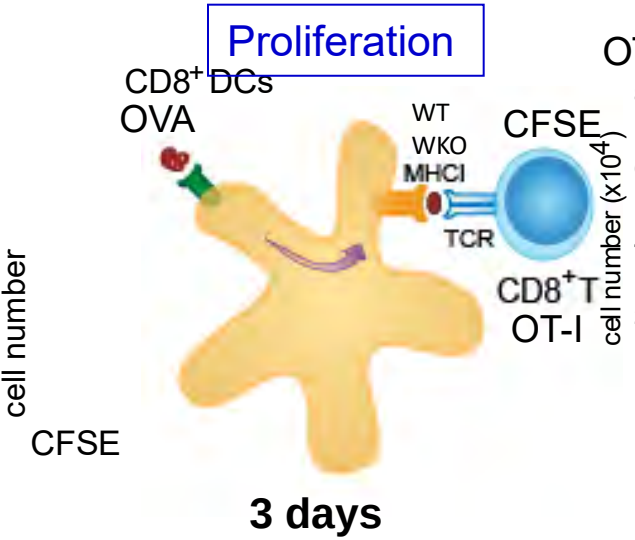


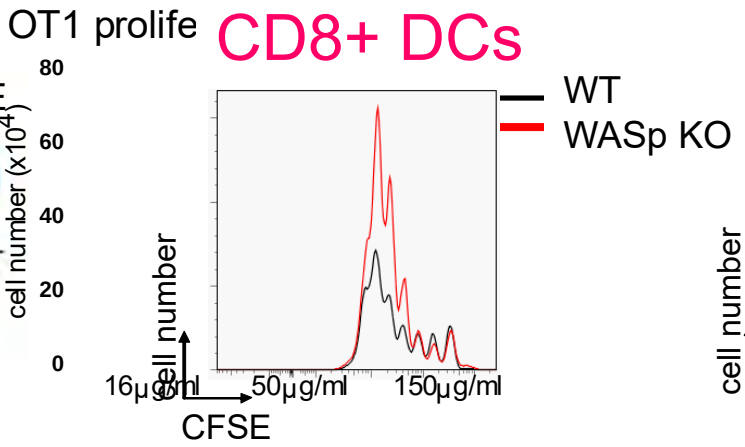


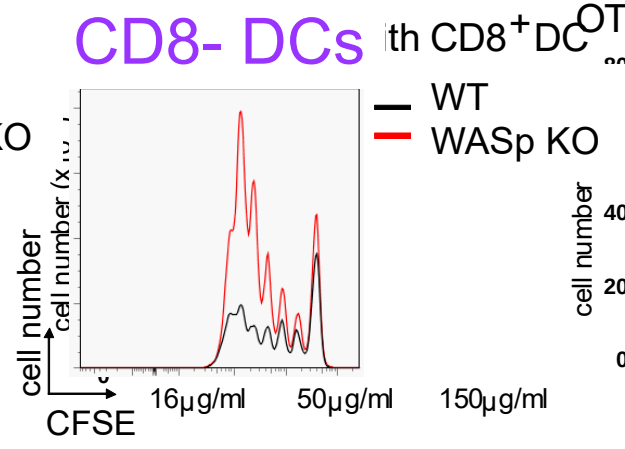


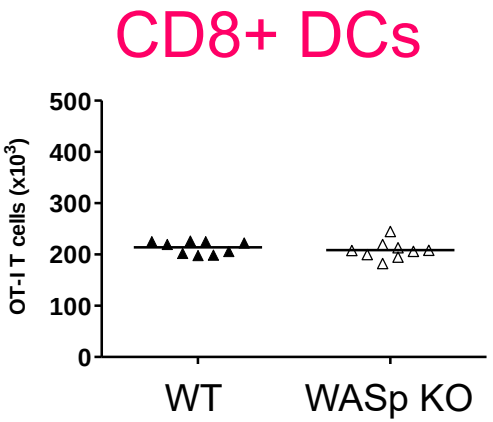


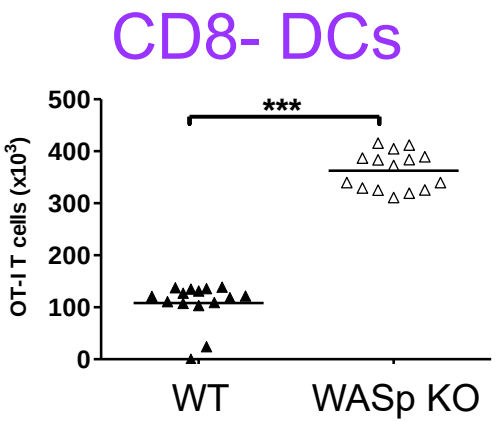
WASp KO CD8- DCs have increased capacity to cross-present antigen





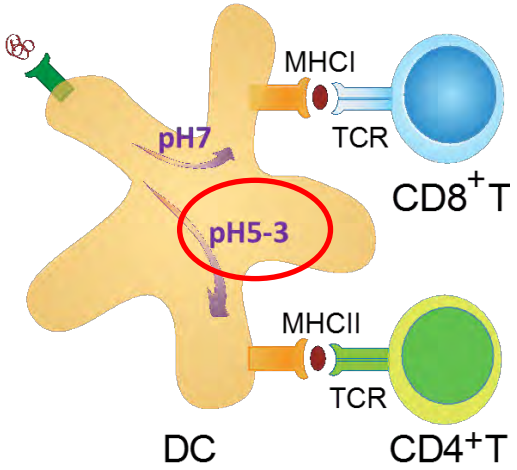




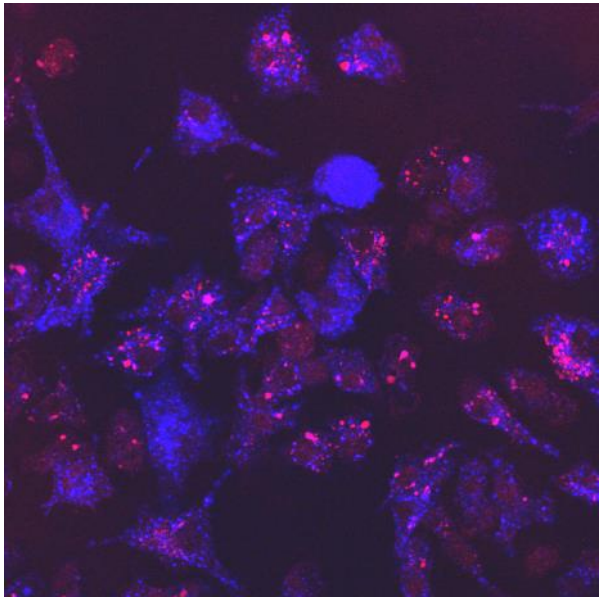


WASp KO CD8- DCs have decreased capacity to acidify exogenous antigen

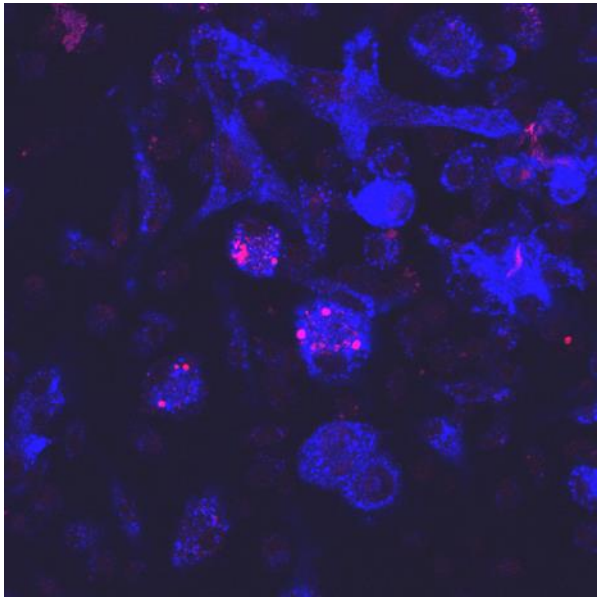
Acidification



wildtype



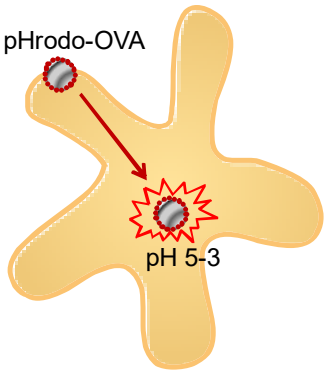
WASp KO

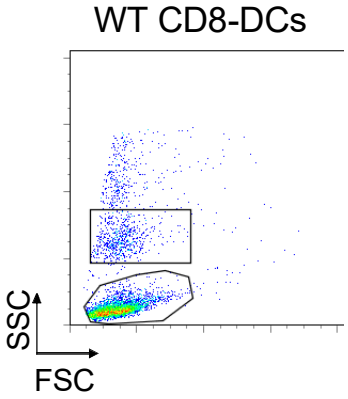


CD11c (dendritic cells) pHrodo-OVA (acidic vesicles)

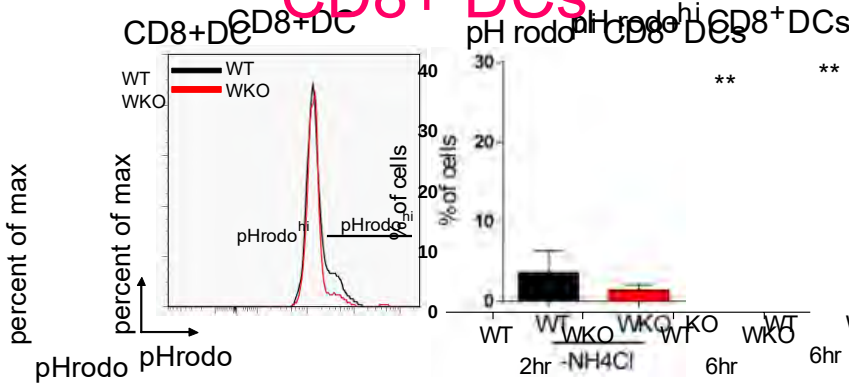
WASp KO CD8- DCs have decreased capacity to acidify endocytic vesicles

Acidification

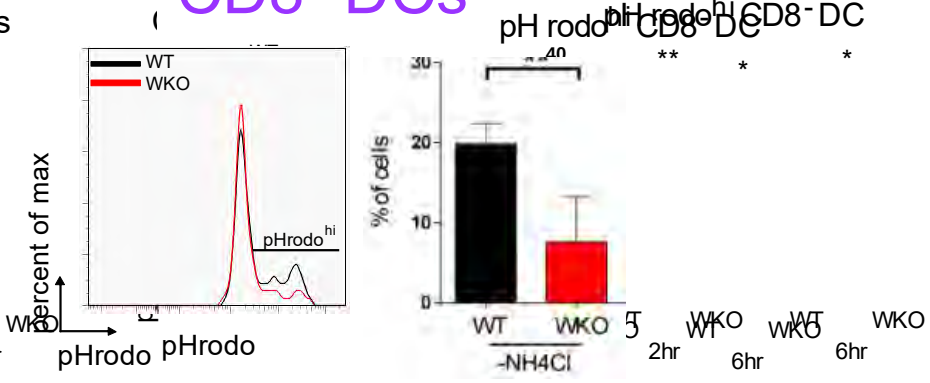




CD8+ DCs



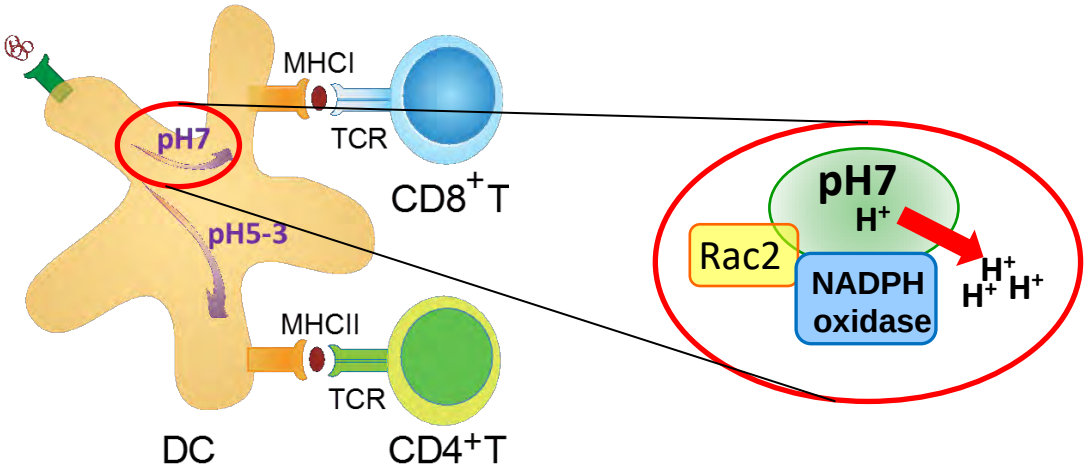
CD8- DCs



WT
WASp KO

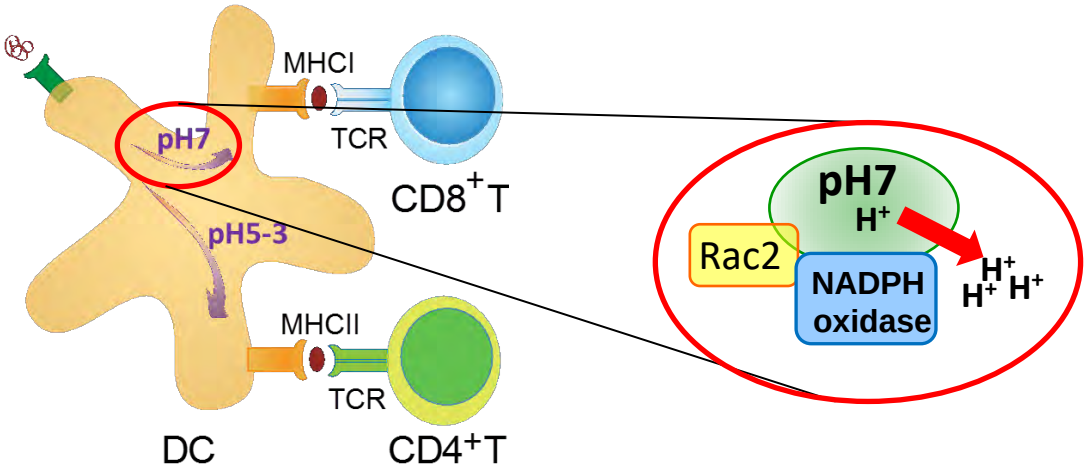
WASP-deficient CD8- DCs have increased expression of Rac2

CD8+ DC

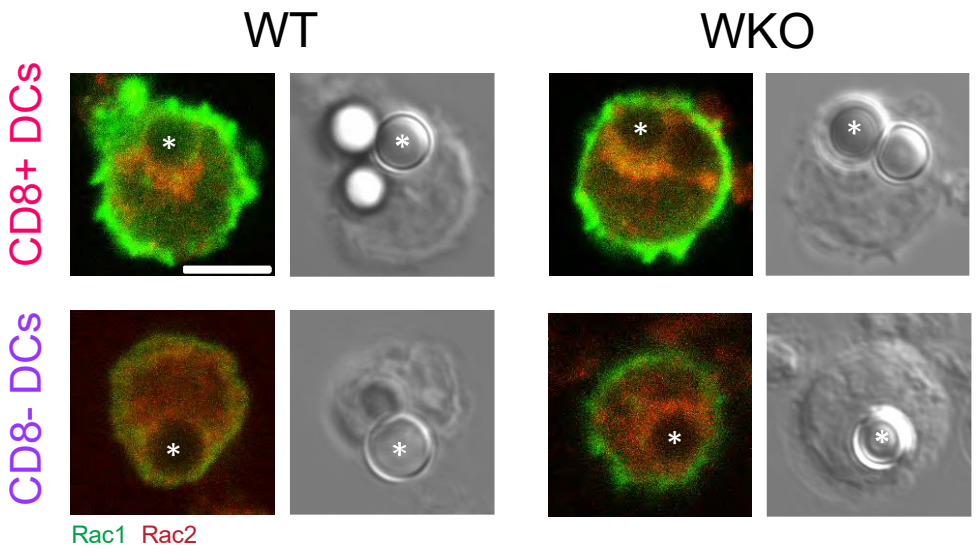


WASP-deficient CD8- DCs can efficiently recruit Rac2 to their phagosomal membrane

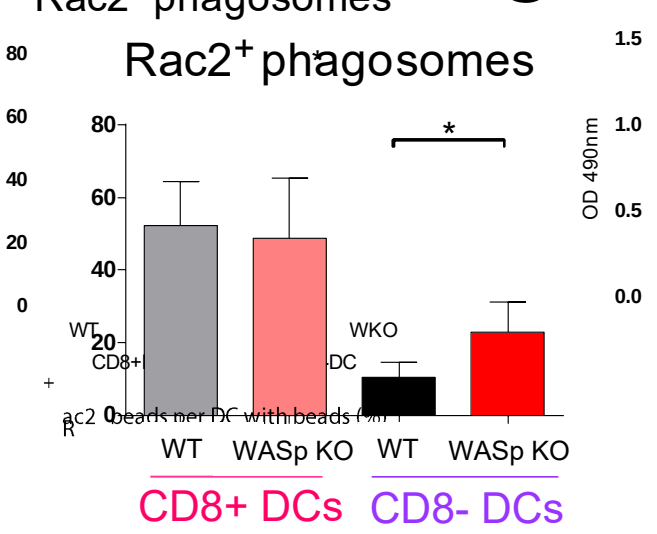
CD8+ DC



Rac2 localization

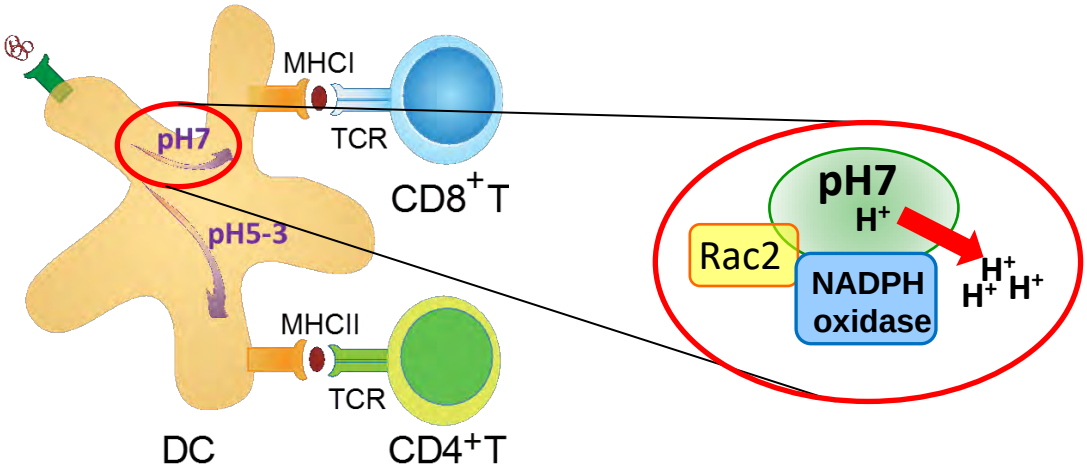


C

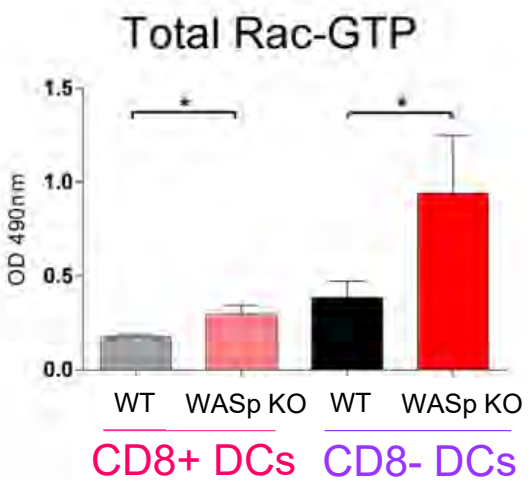


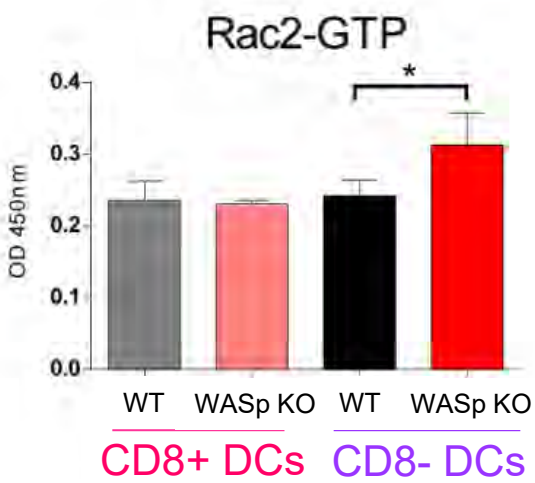
WASP-deficient CD8- DCs can efficiently recruit Rac2 to their phagosomal membrane

CD8+ DC



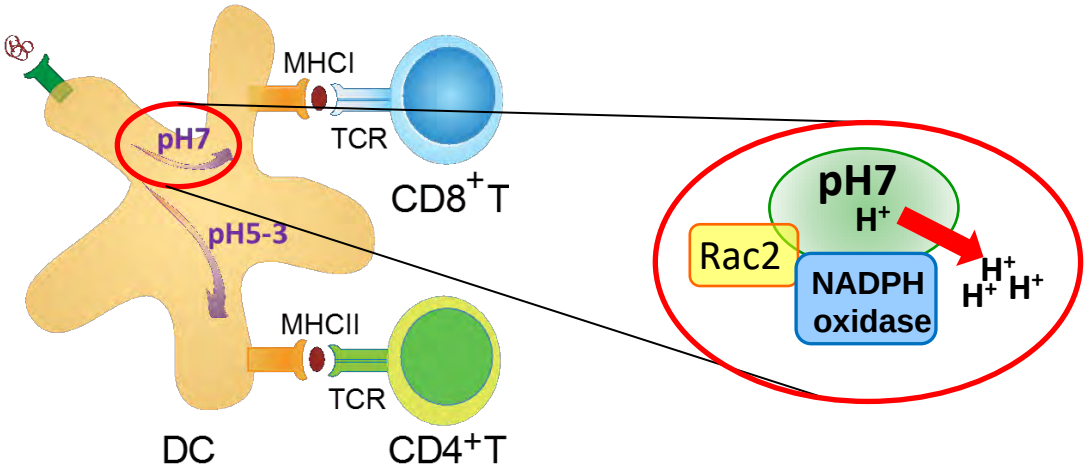
Rac2 activation





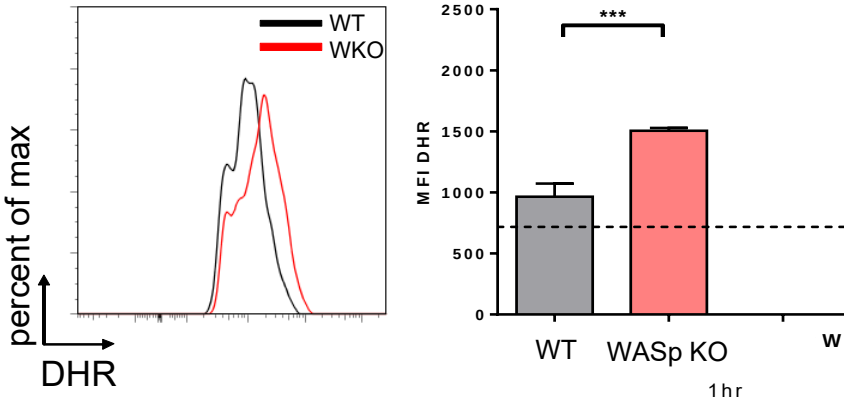
WASP-deficient DCs have increased ROS production

CD8+ DC

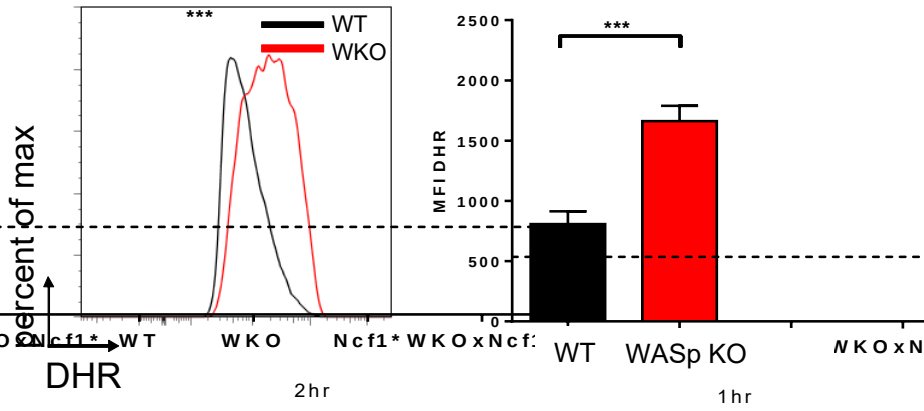


NADPH activity and ROS production

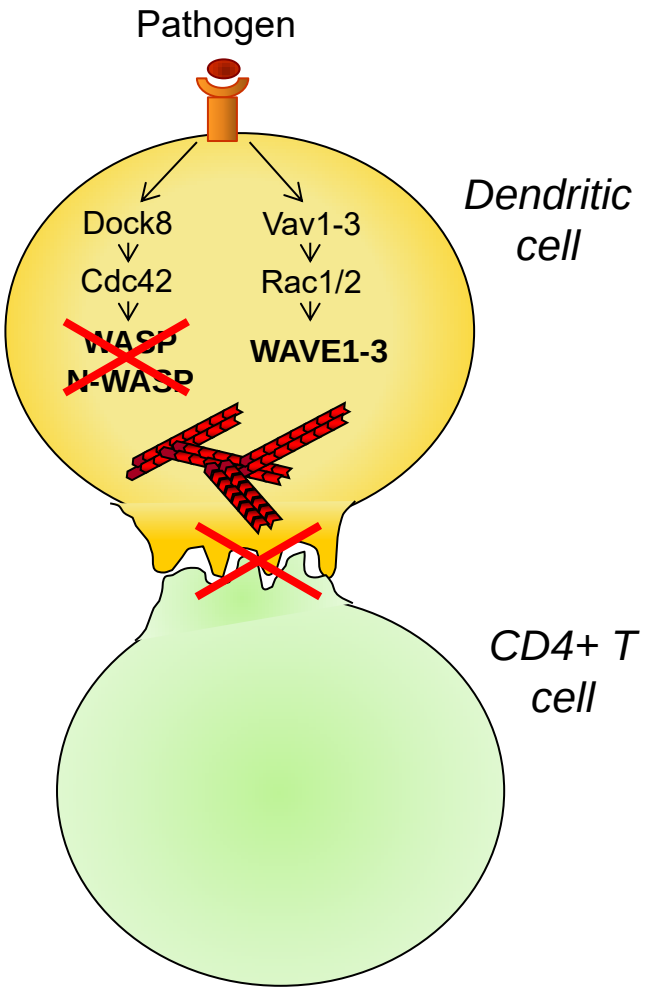
CD8+ DCs



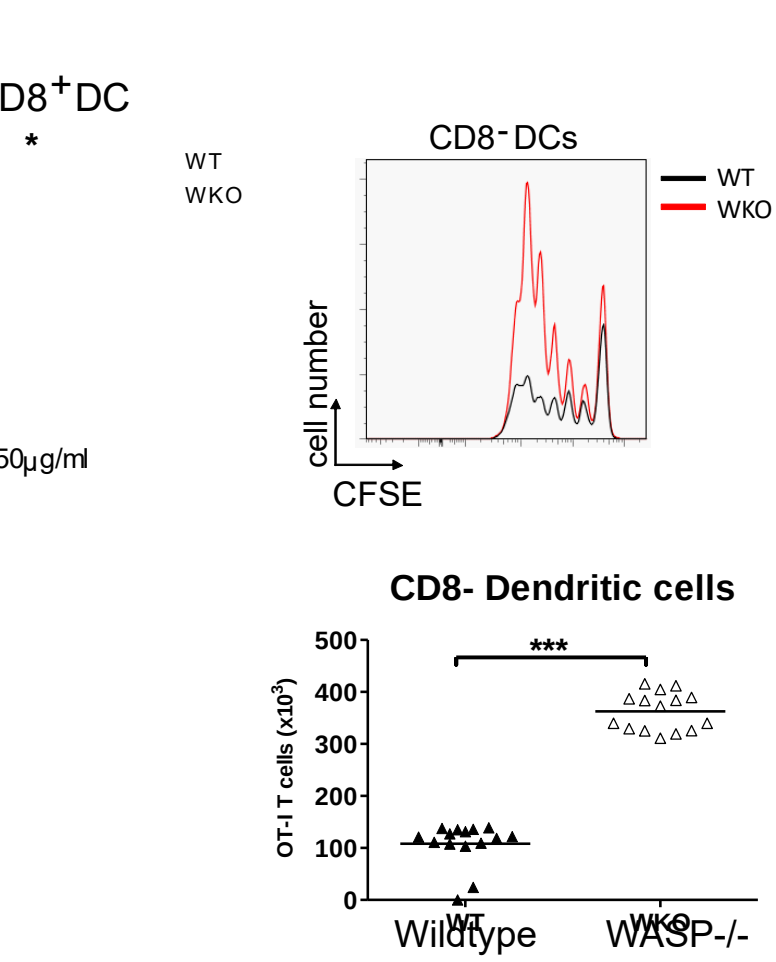
CD8- DCs

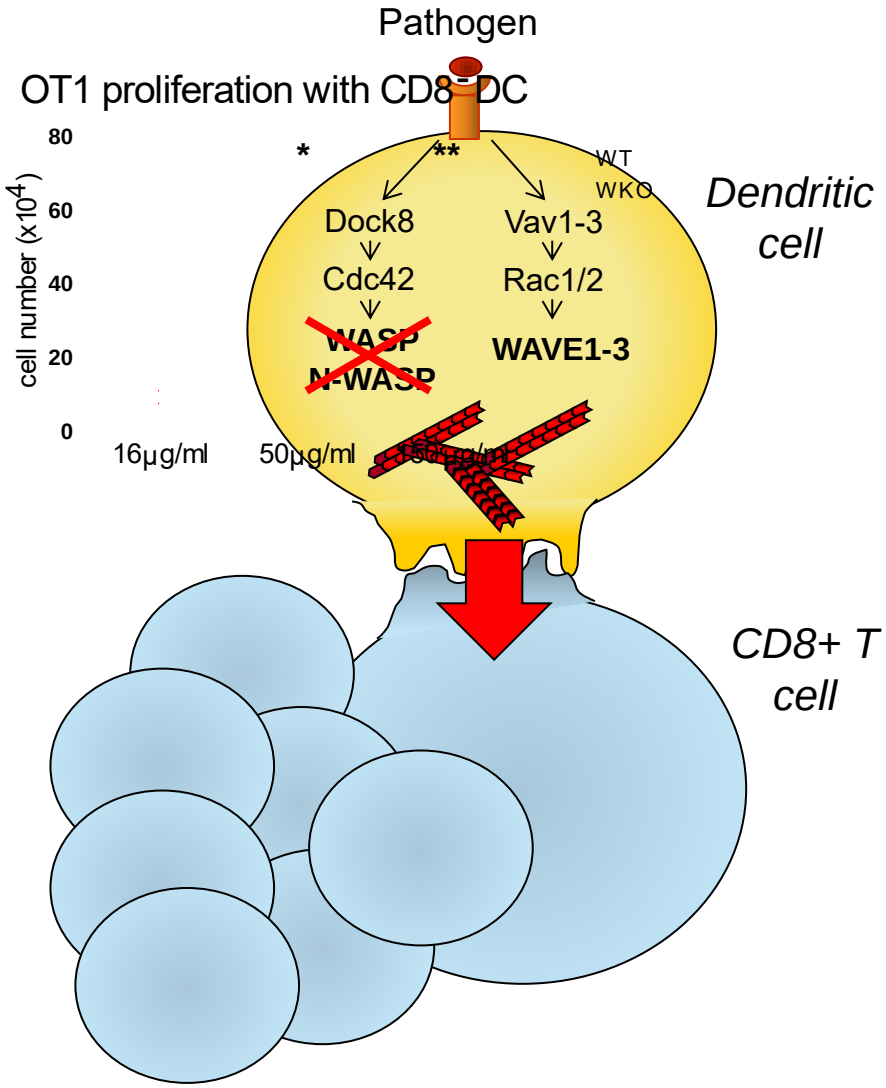


WASP-deficient dendritic cells fail to activate CD4+ T cells



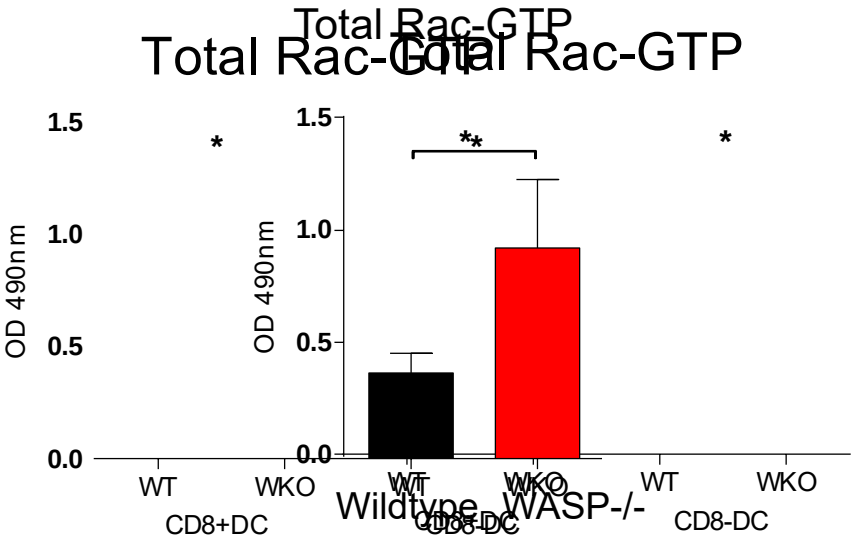
WASP-deficient dendritic cells Induce activation of CD8+ T cells

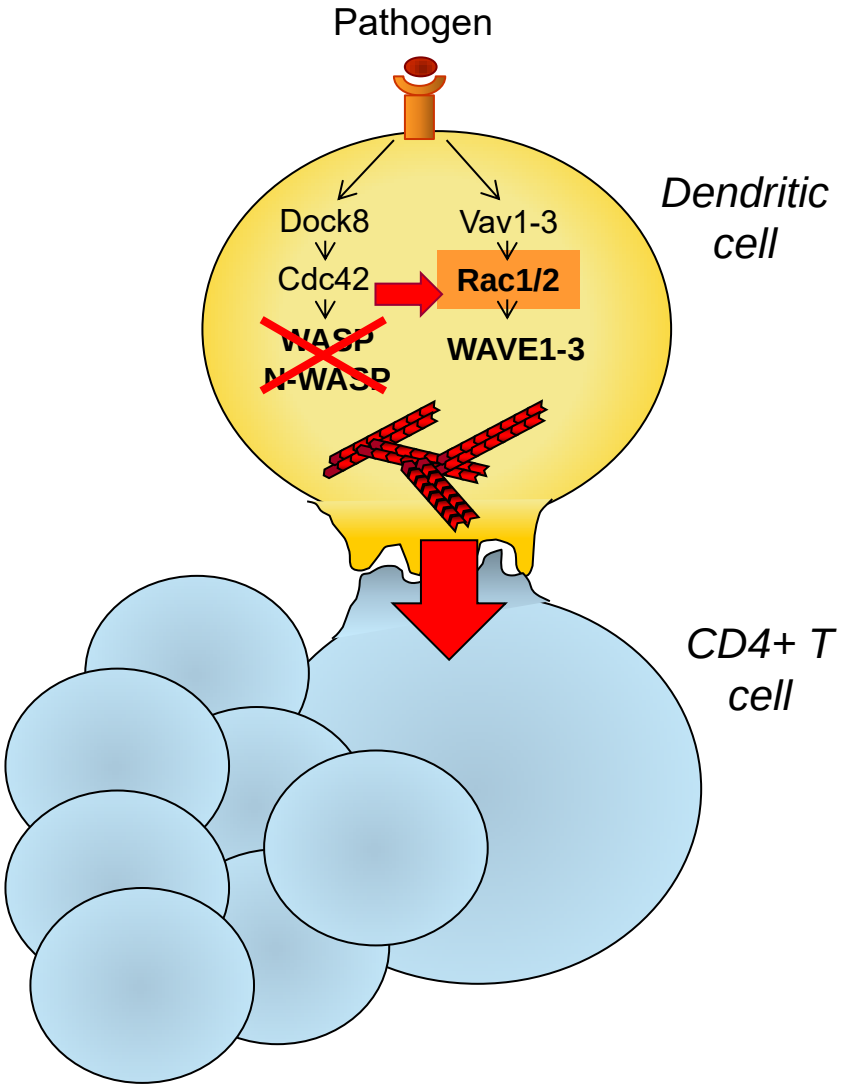




Baptista et al, Nature Communications 2016

Deletion of WASP triggers Rac2 activation and increased cross-presentation by dendritic cells

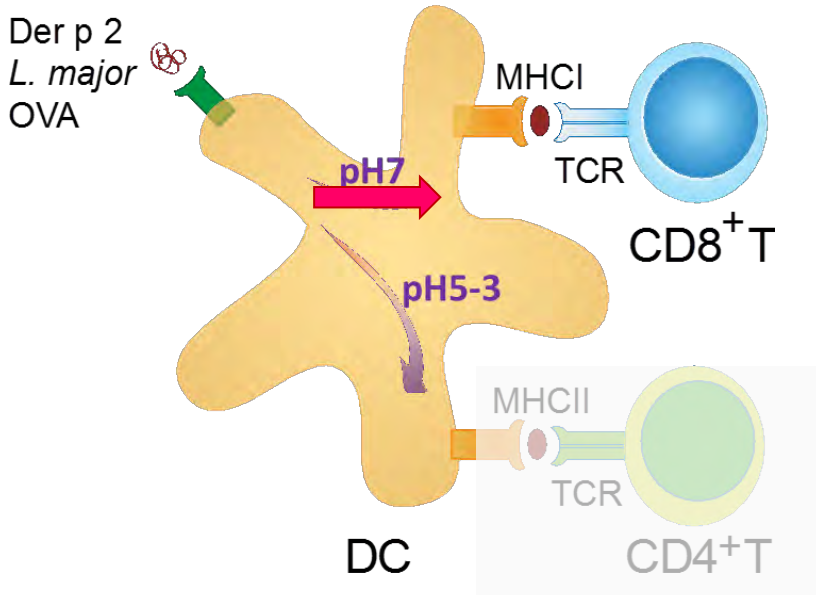




Baptista et al, Nature Communications 2016

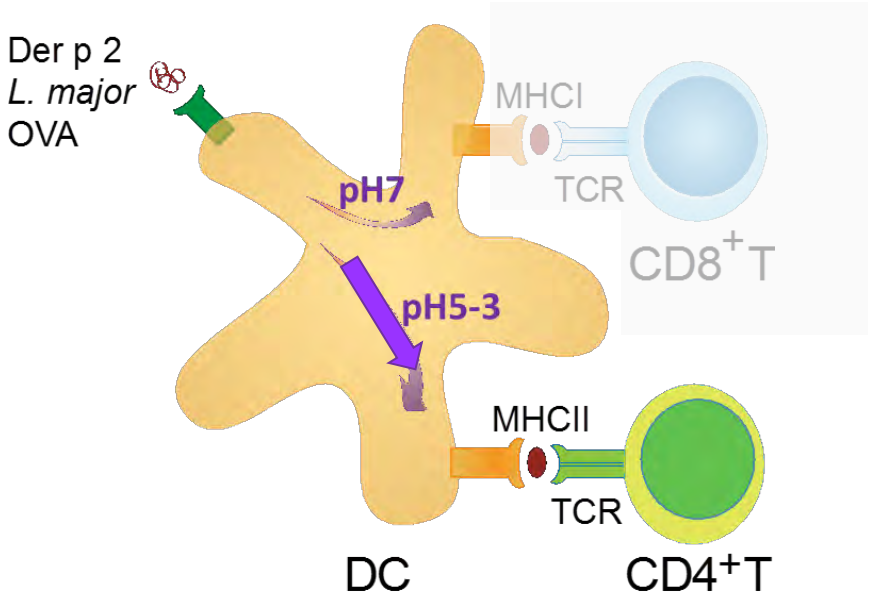
DCs in the wildtype setting

CD8+ DCs



10% of spleen DCs

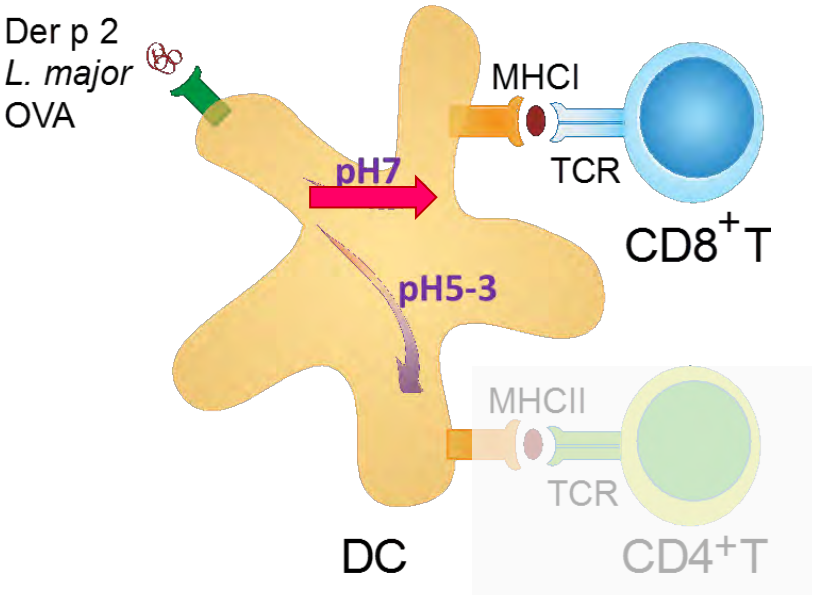
CD8- DCs



50% of spleen DCs

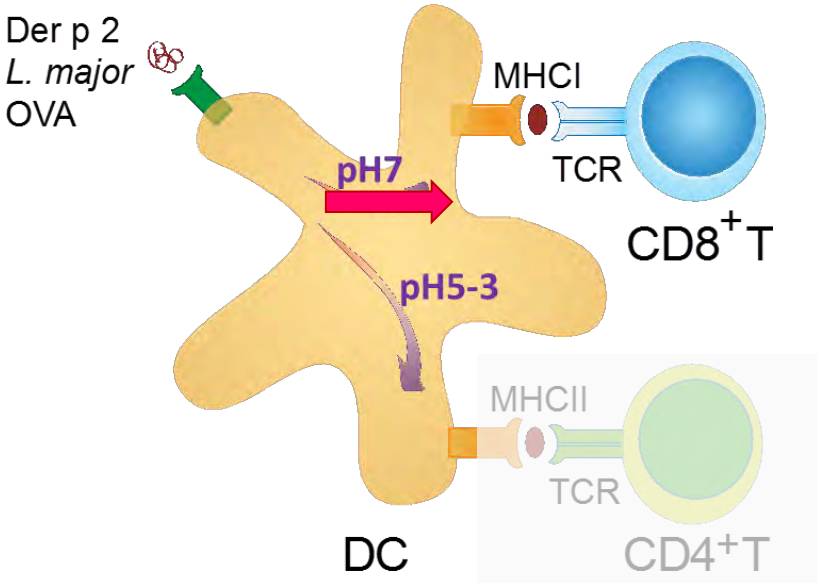
DCs in WASp deficiency

CD8+ DCs



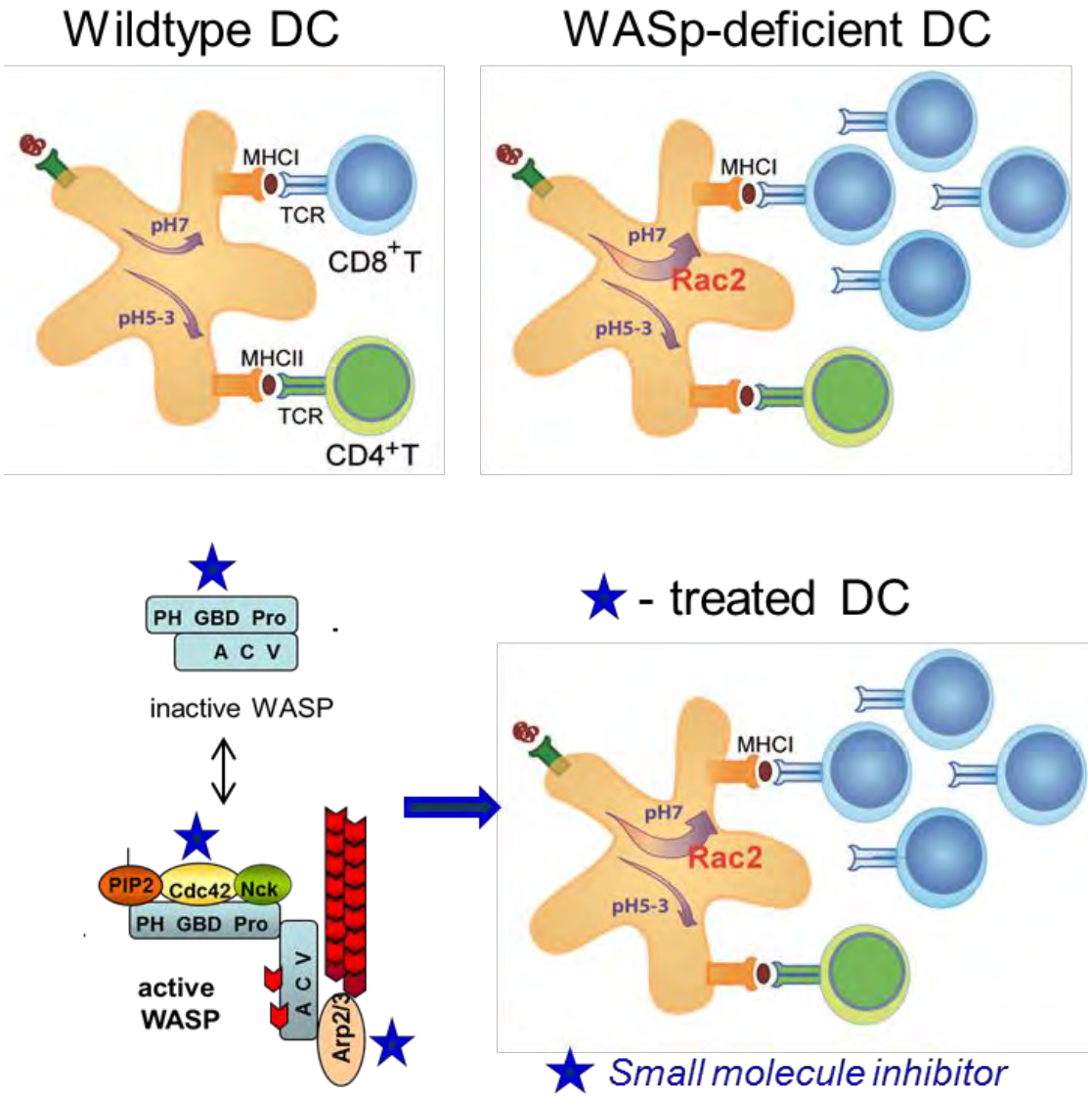
10% of spleen DCs

CD8- DCs

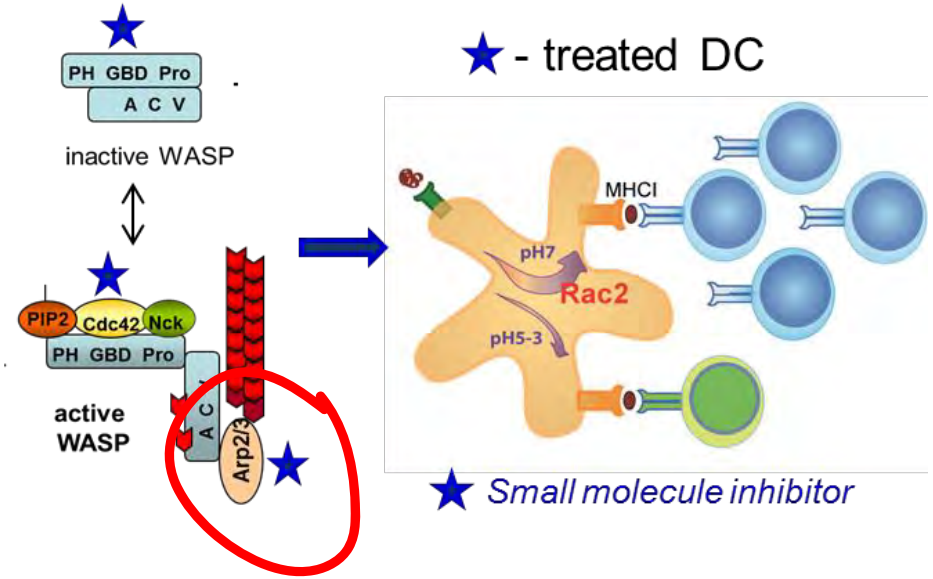


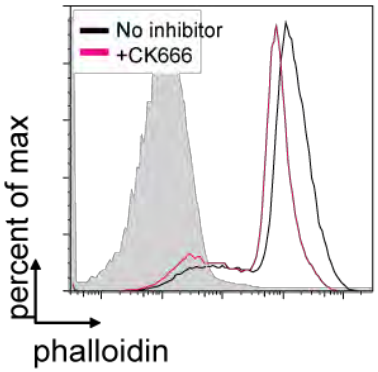
50% of spleen DCs

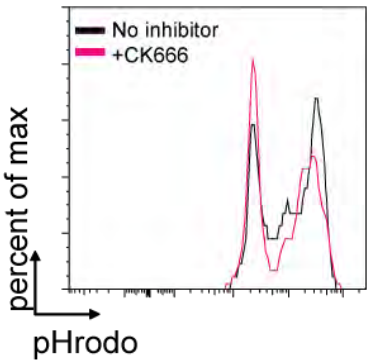
Can tumor antigens be routed into the cross-presentation pathway by targeting the actin cytoskeleton?

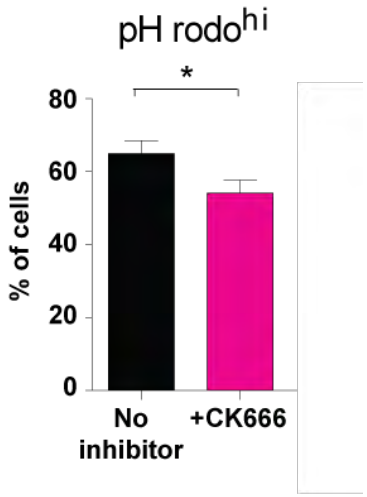


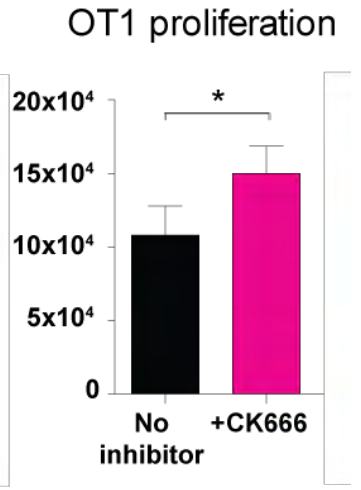
Can tumor antigens be routed into the cross-presentation pathway by targeting the actin cytoskeleton? – Yes!







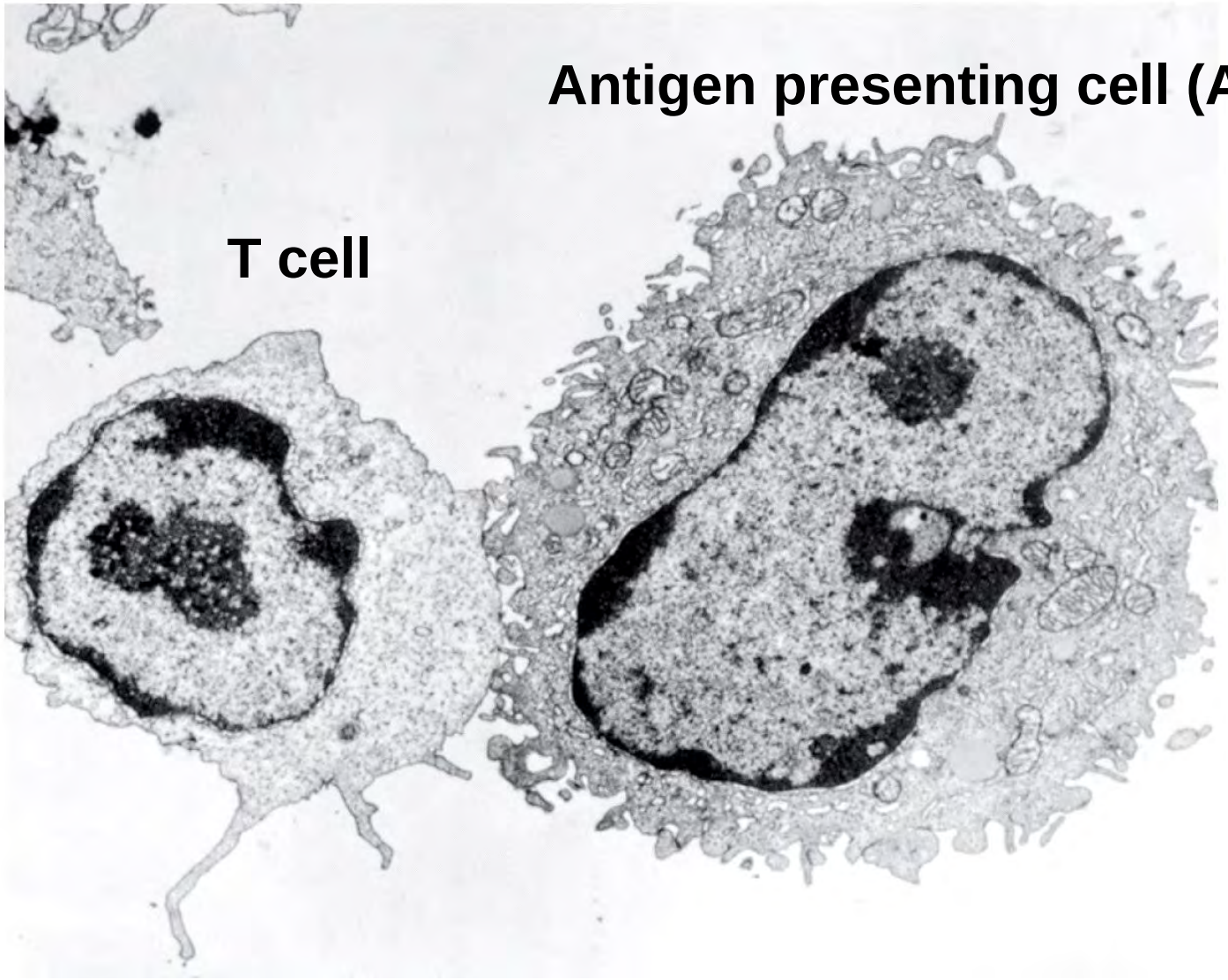




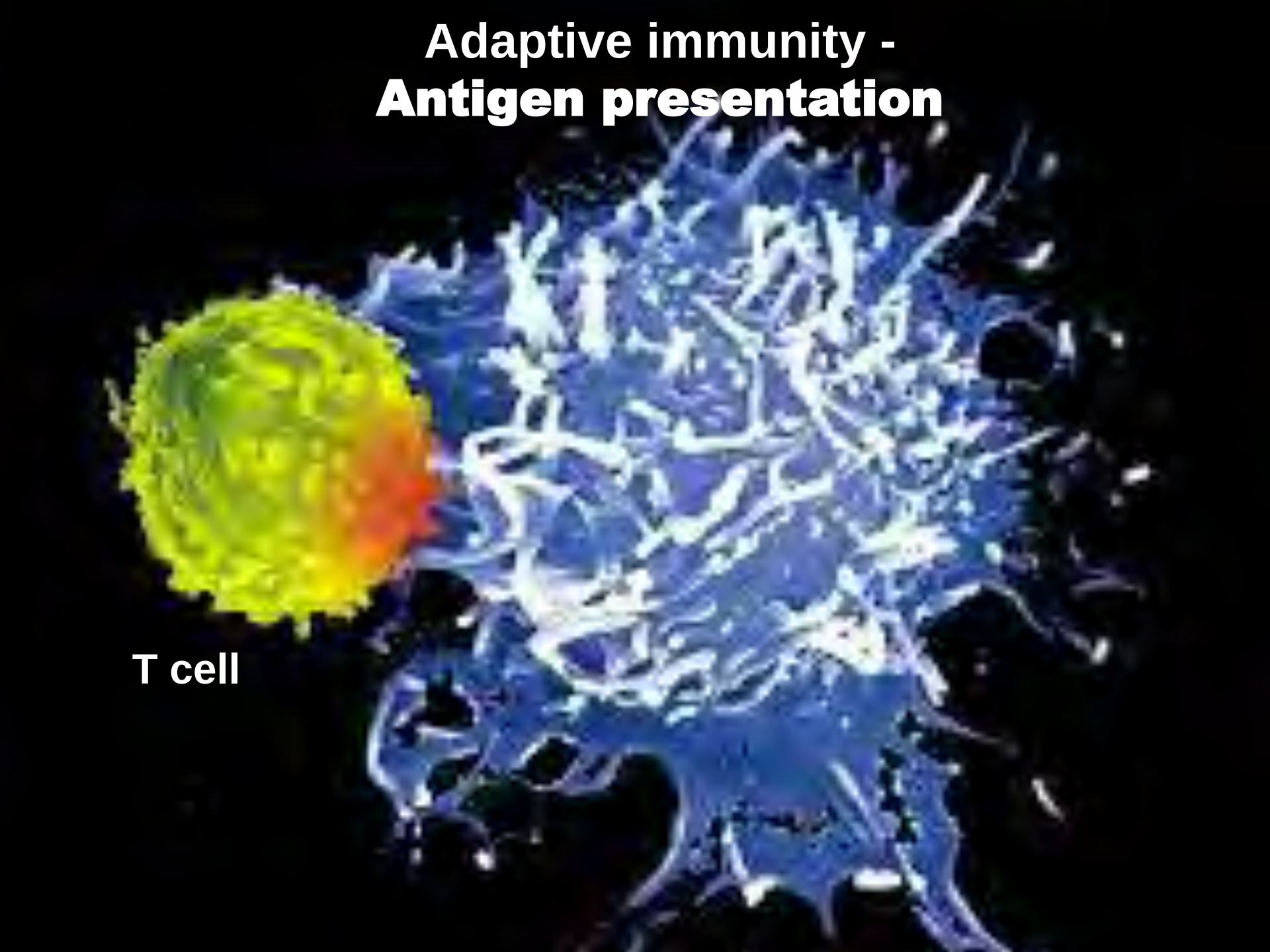
WT
WT + CK666

Oliveira and Westerberg, unpublished

Adaptive immunity -
Antigen presentation

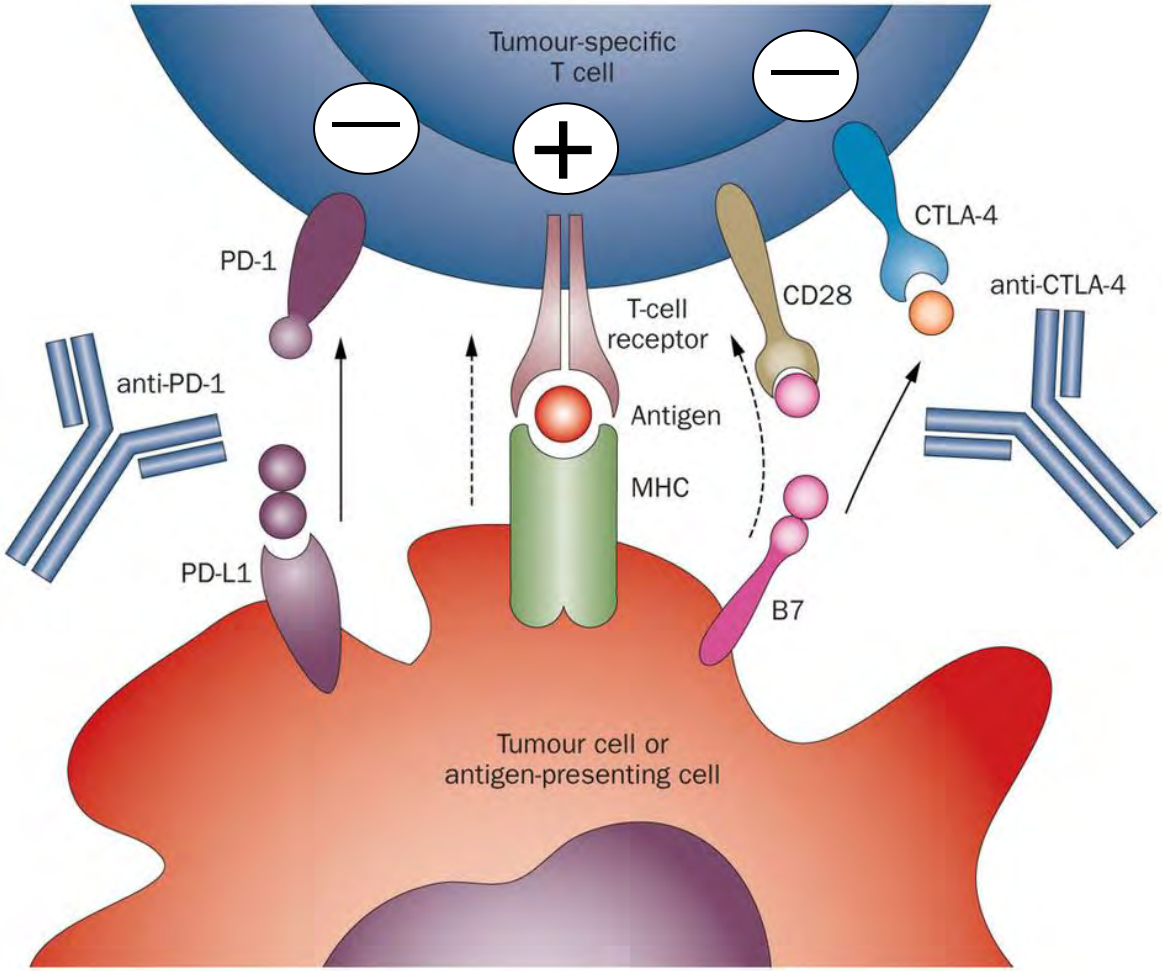


Adaptive immunity - **Antigen presentation**



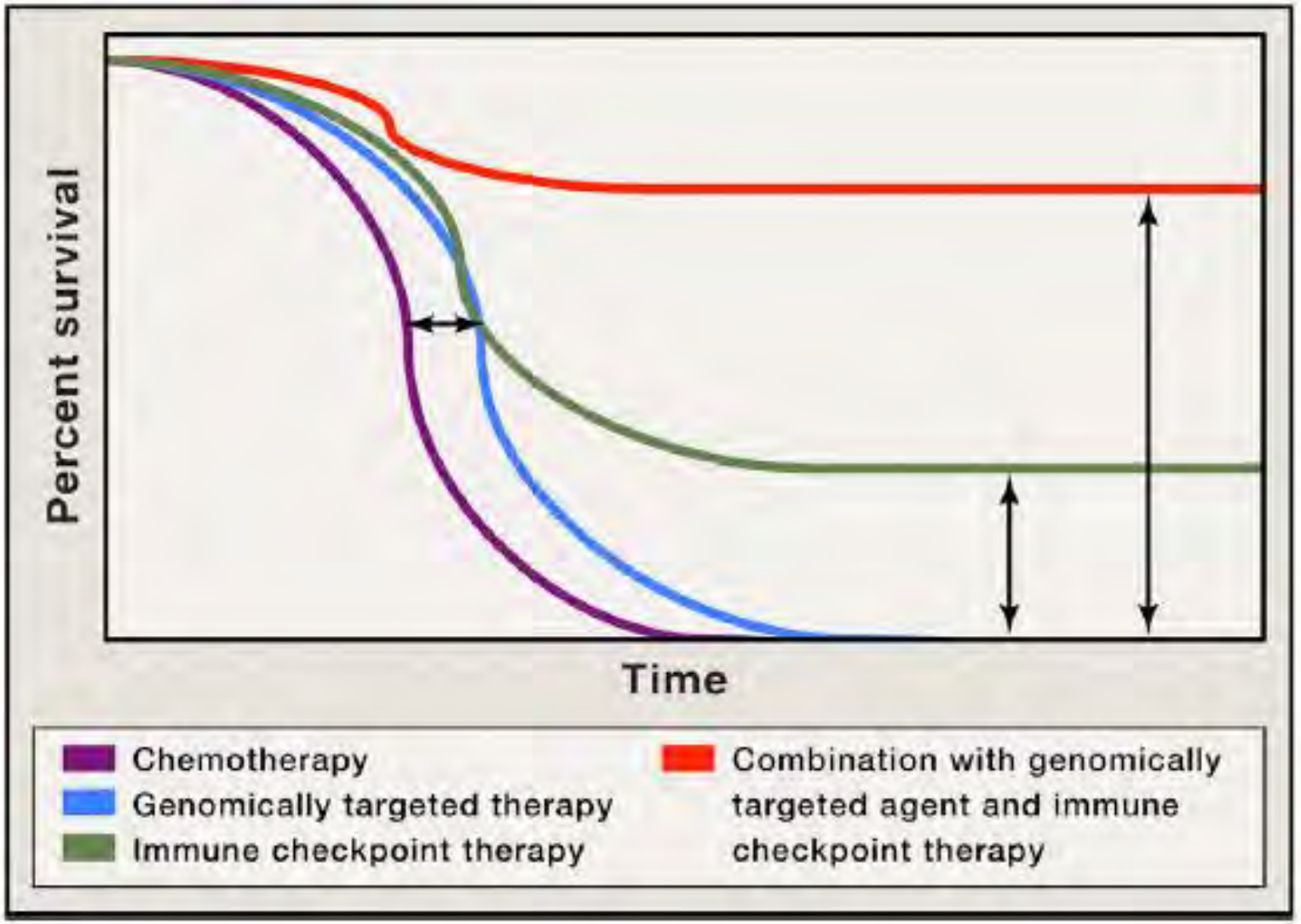
Checkpoint therapy (anti-CTLA4 and anti-PD-1 Ab):

”Releasing the breaks”



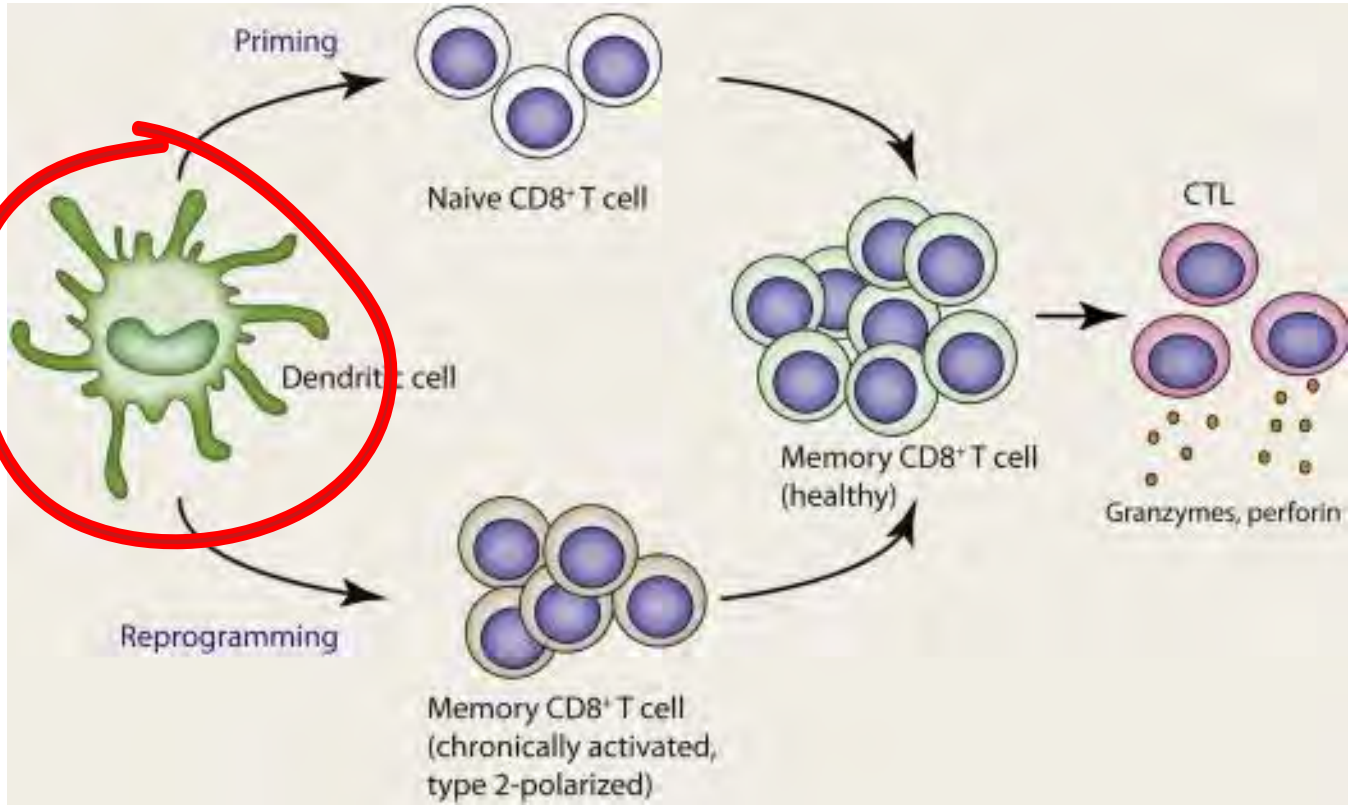
Lawrence et al, Nature 2013
Palucka and Banchereau, Immunity 2013
Sharma and Allison Cell 2015, Science 2015

Checkpoint therapy – survival in Melanoma



Sharma and Allison,Cell 2015

Therapeutic vaccine candidates

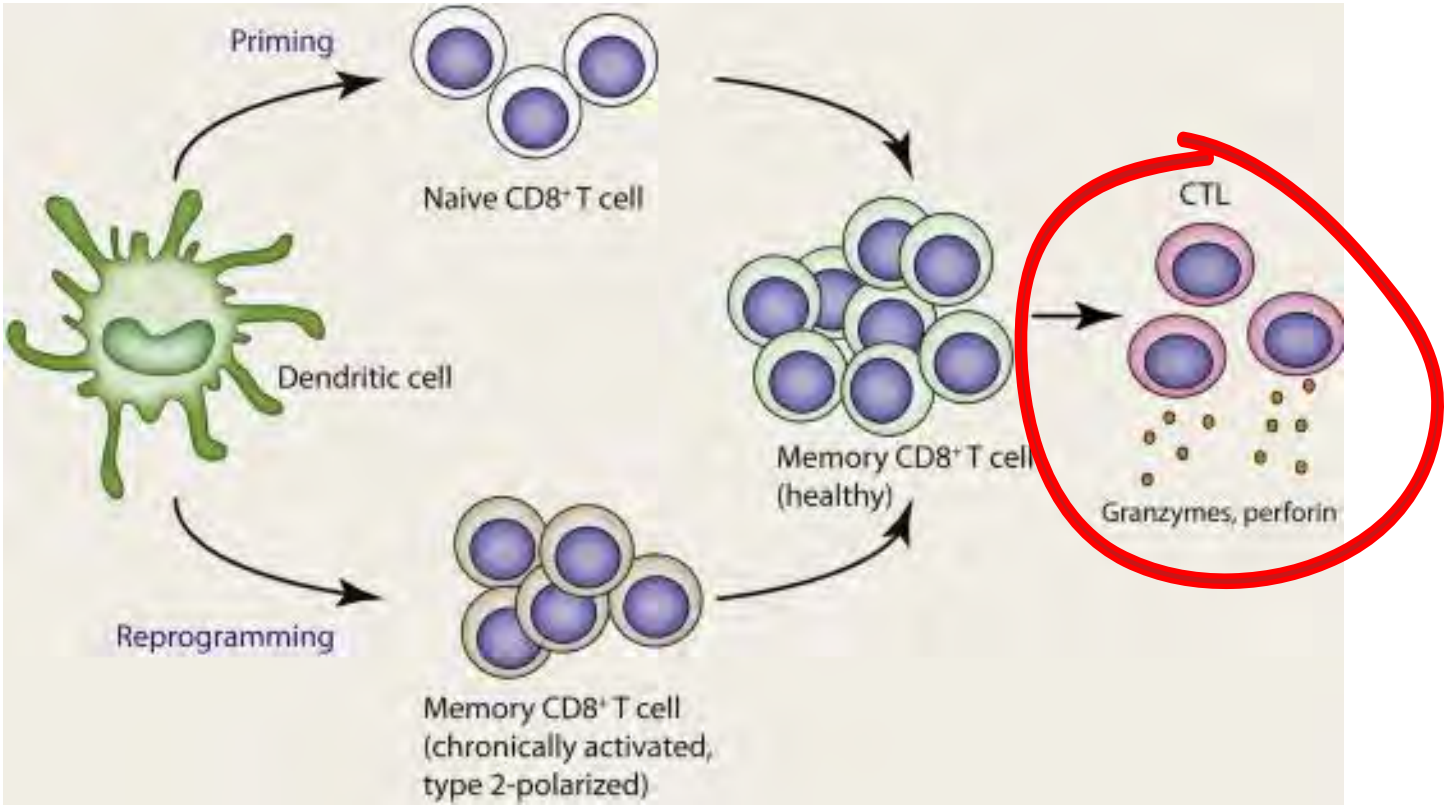


Dendritic cell

Nucleic acid (DNA, RNA)
Synthetic peptides (short long)
Recombinant proteins (antigen, antigens fused with anti-DC Ab)
Tumor cells (Genetically modified and/or killed)
Viral vectors (oncolytic, antigen cassetes)
Bacteria

Palucka and Banchereau, Immunity 2013

Therapeutic vaccine candidates

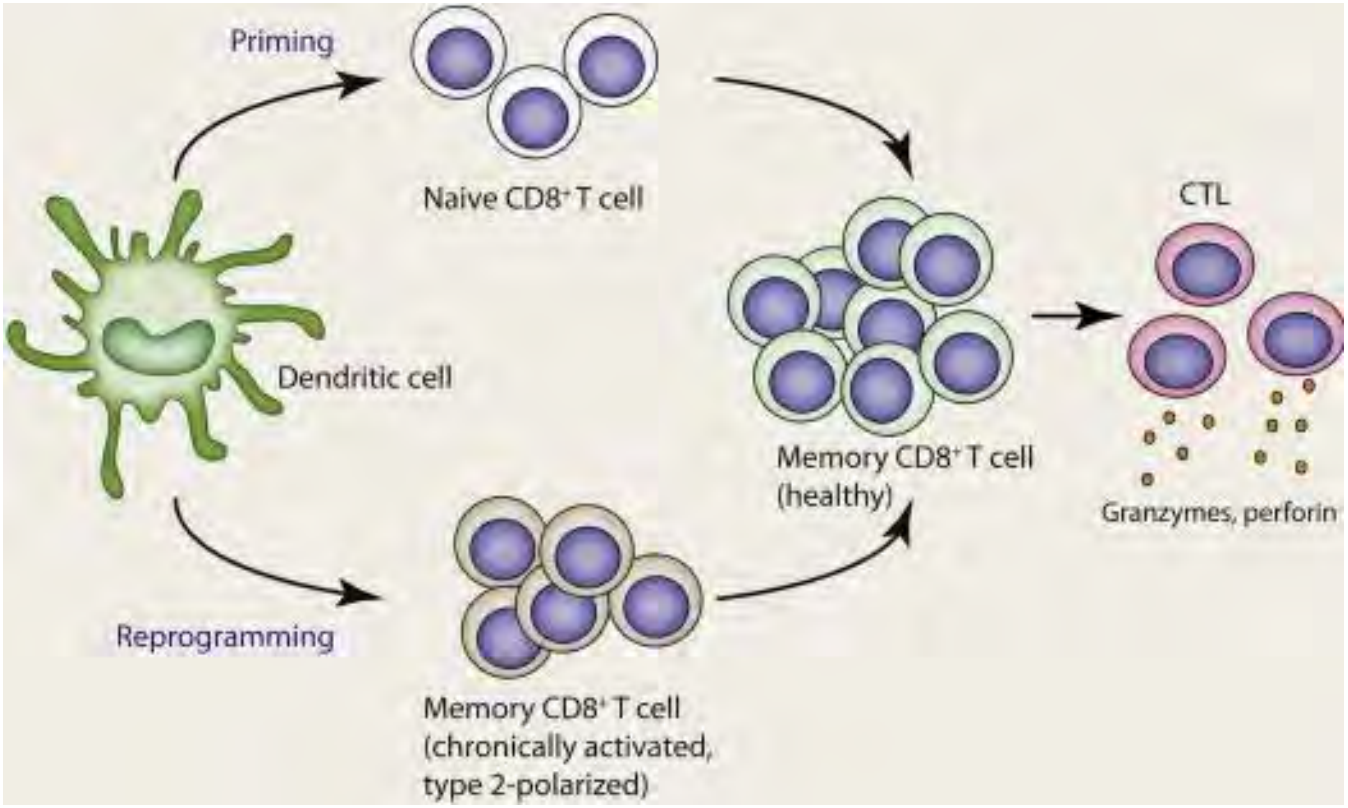


Desired properties of CD8+ T cells

High TCR affinity, T cell avidity
High granzyme, perforin
High proliferation potential
Express chemokine receptors
Express integrins CD49a, CD103

Palucka and Banchereau, Immunity 2013

Therapeutic vaccine candidates



Hurdles to overcome

T cell intrinsic regulators: CTLA4, PD-1, LAG-3, TIM-3, 2B4

T cell extrinsic regulators: suppressor cells (Treg cells, MDSC, macrophage)

soluble factors (IL-10, TGF-β, IL-13)

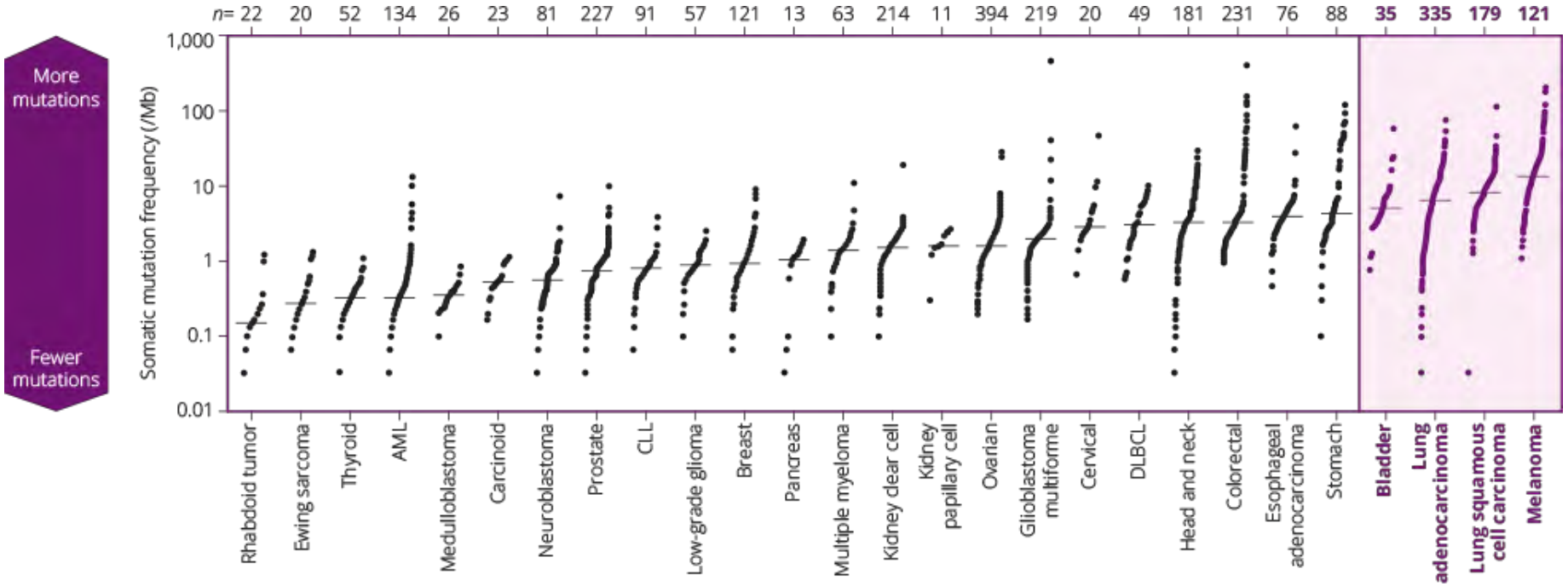
tumor derived suppressive surface factors (B7 family, IDO, ILT, HLA-E)

Access to tumor site: loss of tumor antigen and MHC class I, lack of chemokines, adhesion molecules, tumor vascularization

Palucka and Banchereau, Immunity 2013

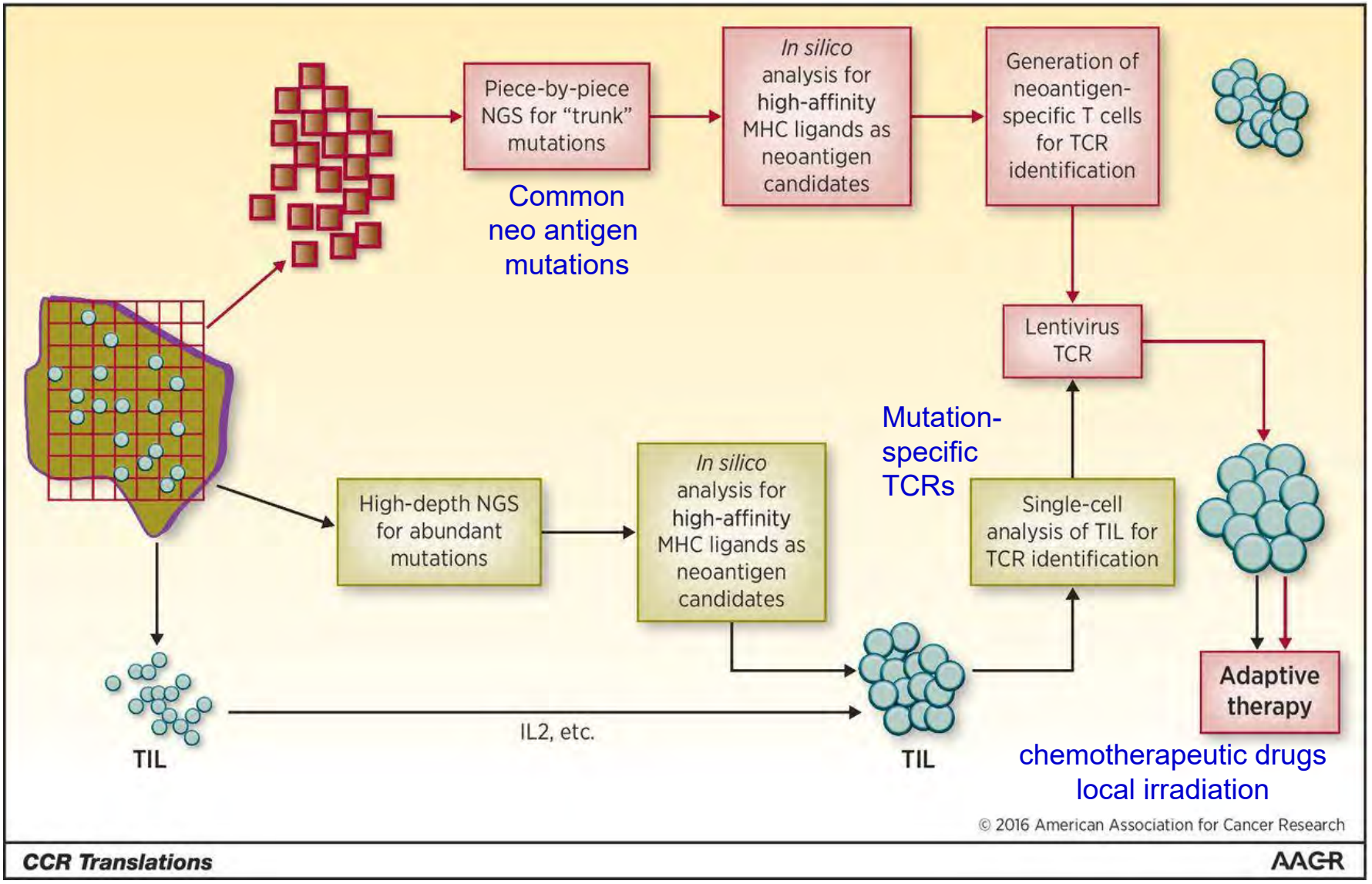
Neoantigen: A Long March toward Cancer Immunotherapy

Somatic mutations in cancer give rise to neoantigens



Lawrence et al, Nature 2013

Design of mutation-specific TCR gene therapy for the clinic



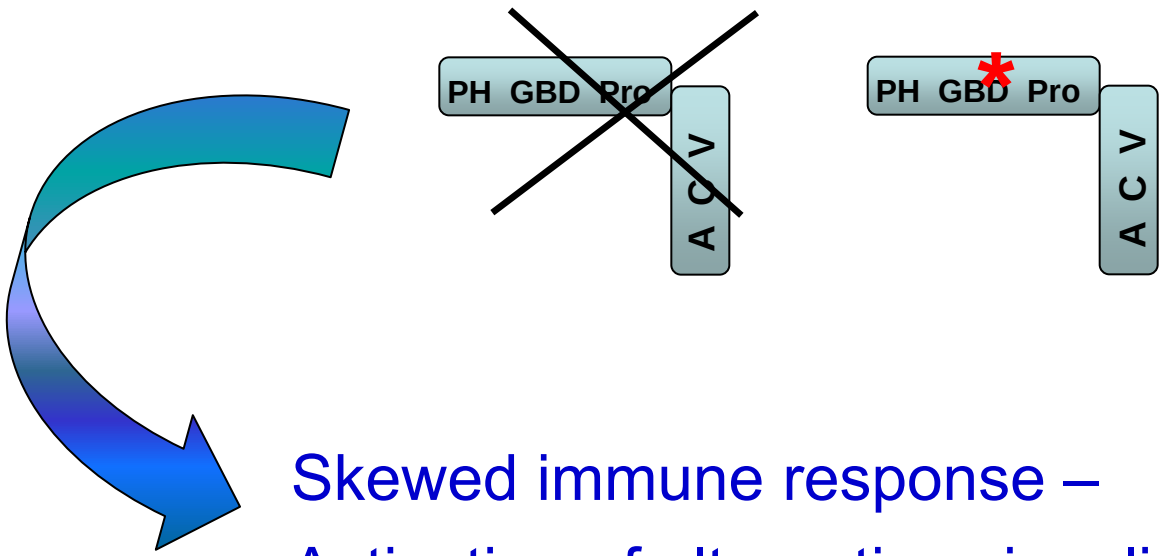
Leisegang et al, Clinical Cancer Research 2016

Conundrum in Immunology:

How can an immune system fail to respond to non-self pathogens while **reacting vigorously to auto-antigens and allergens**?

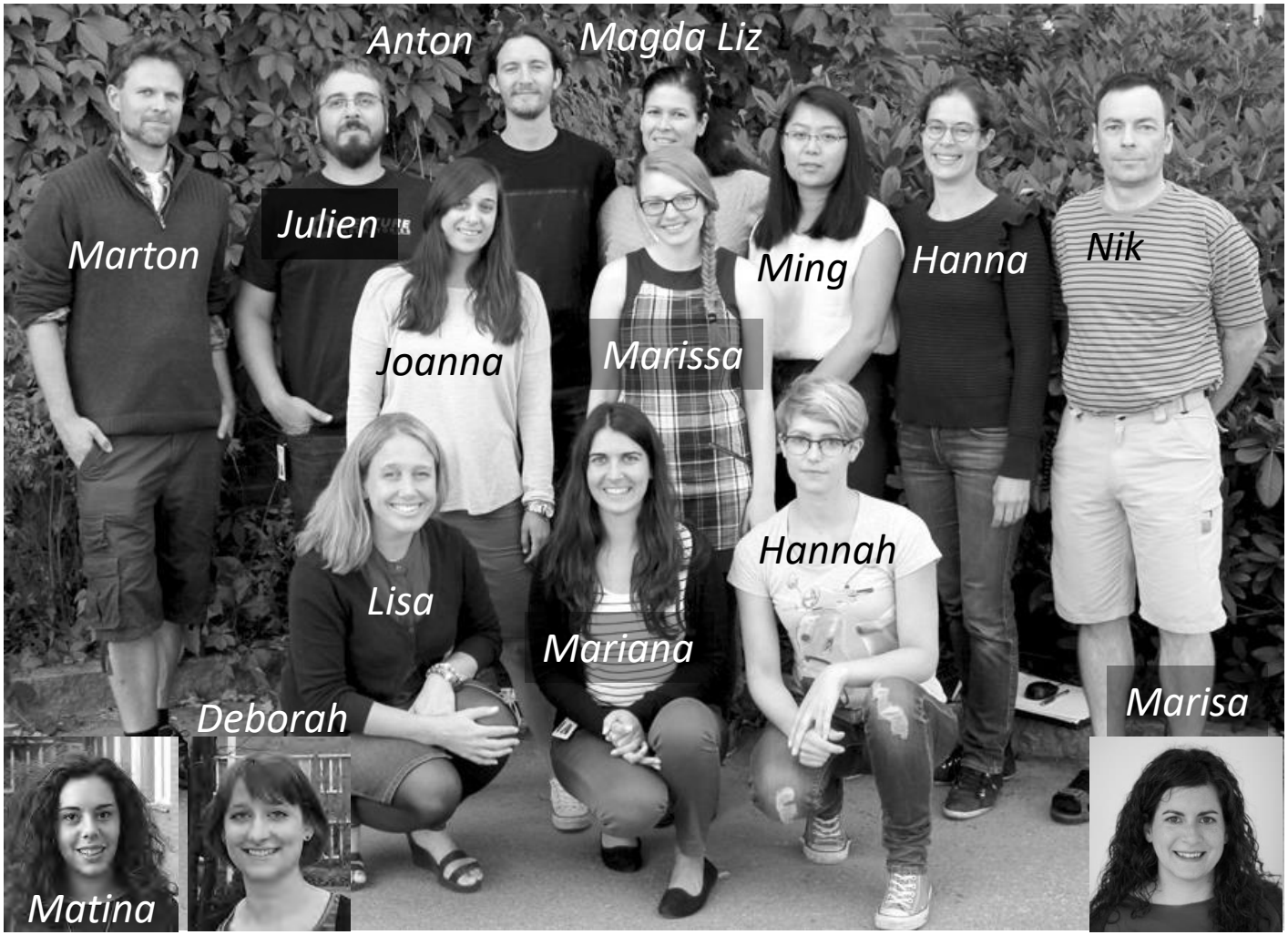
Conundrum in Immunology:

How can an immune system fail to respond to non-self pathogens while **reacting vigorously to auto-antigens and allergens**?



Skewed immune response –

Activation of alternative signaling pathways!



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Luigi Notarangelo
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WASPSTINGS Network