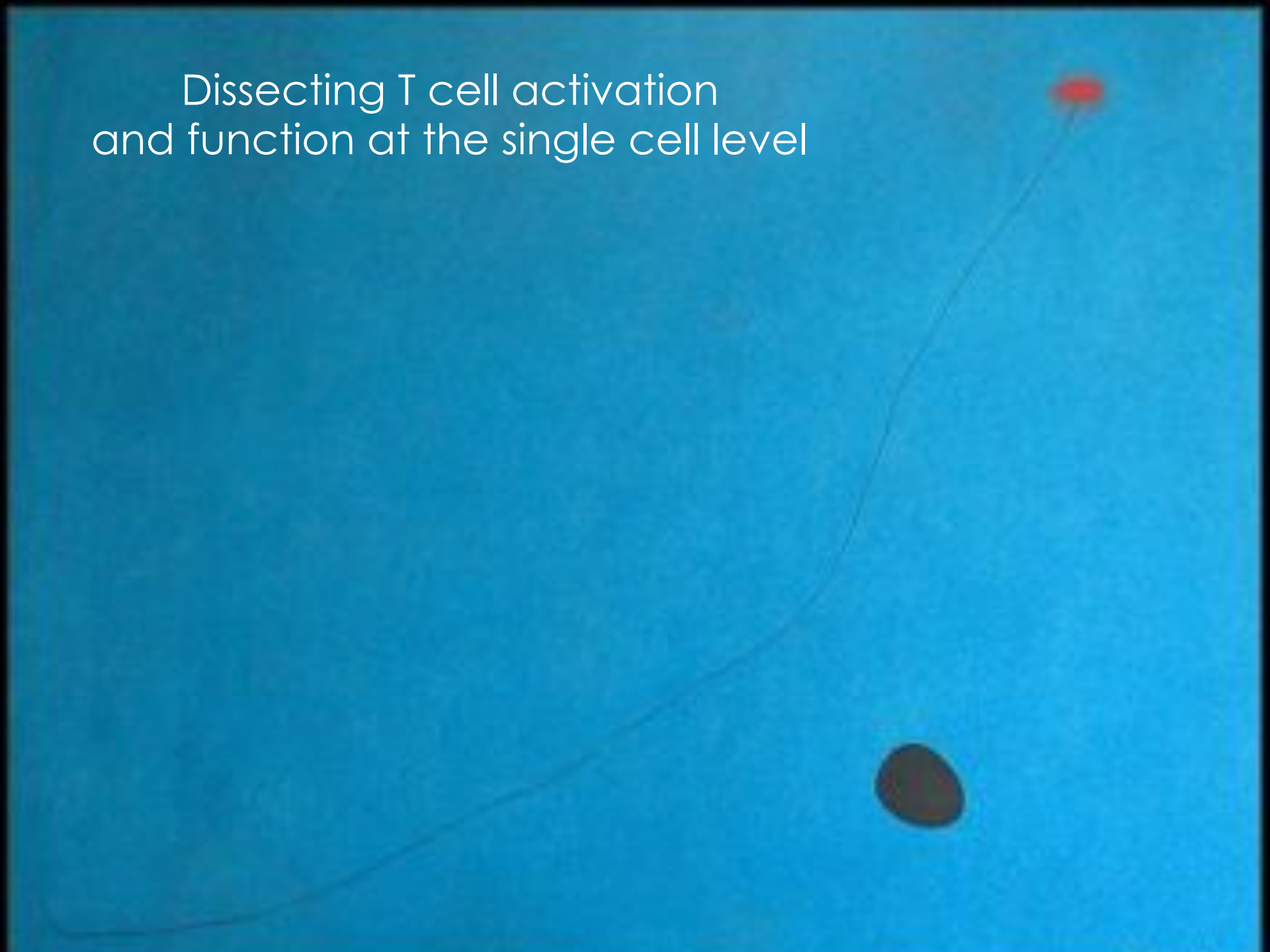
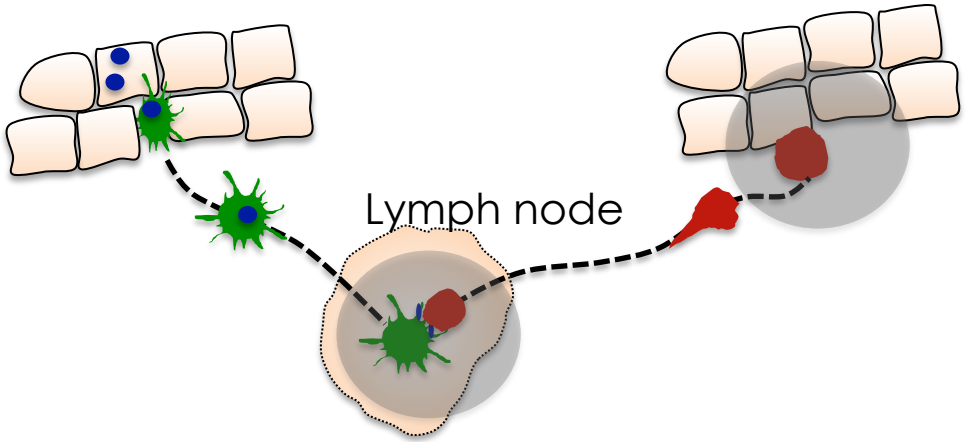
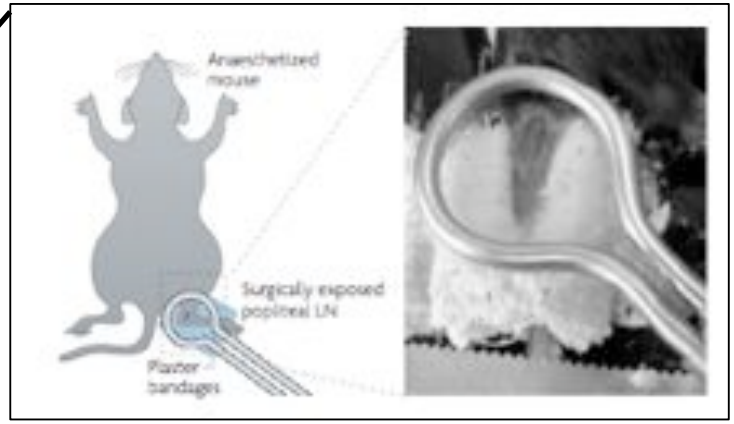
Dissecting T cell activation and function at the single cell level

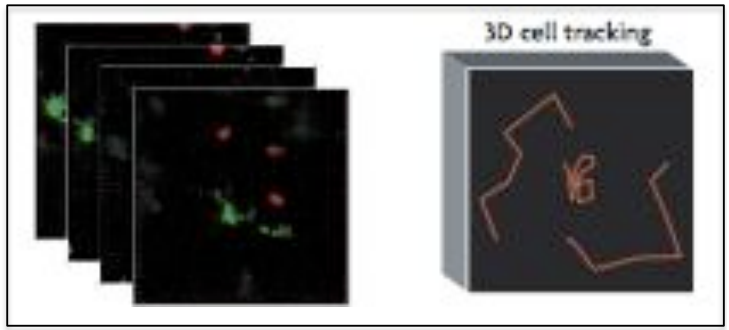


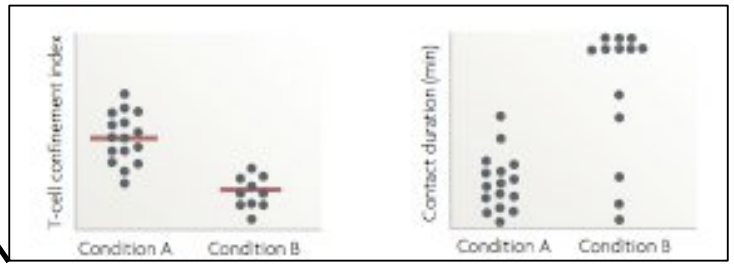
Regulation and outcome of immune cell interactions in vivo



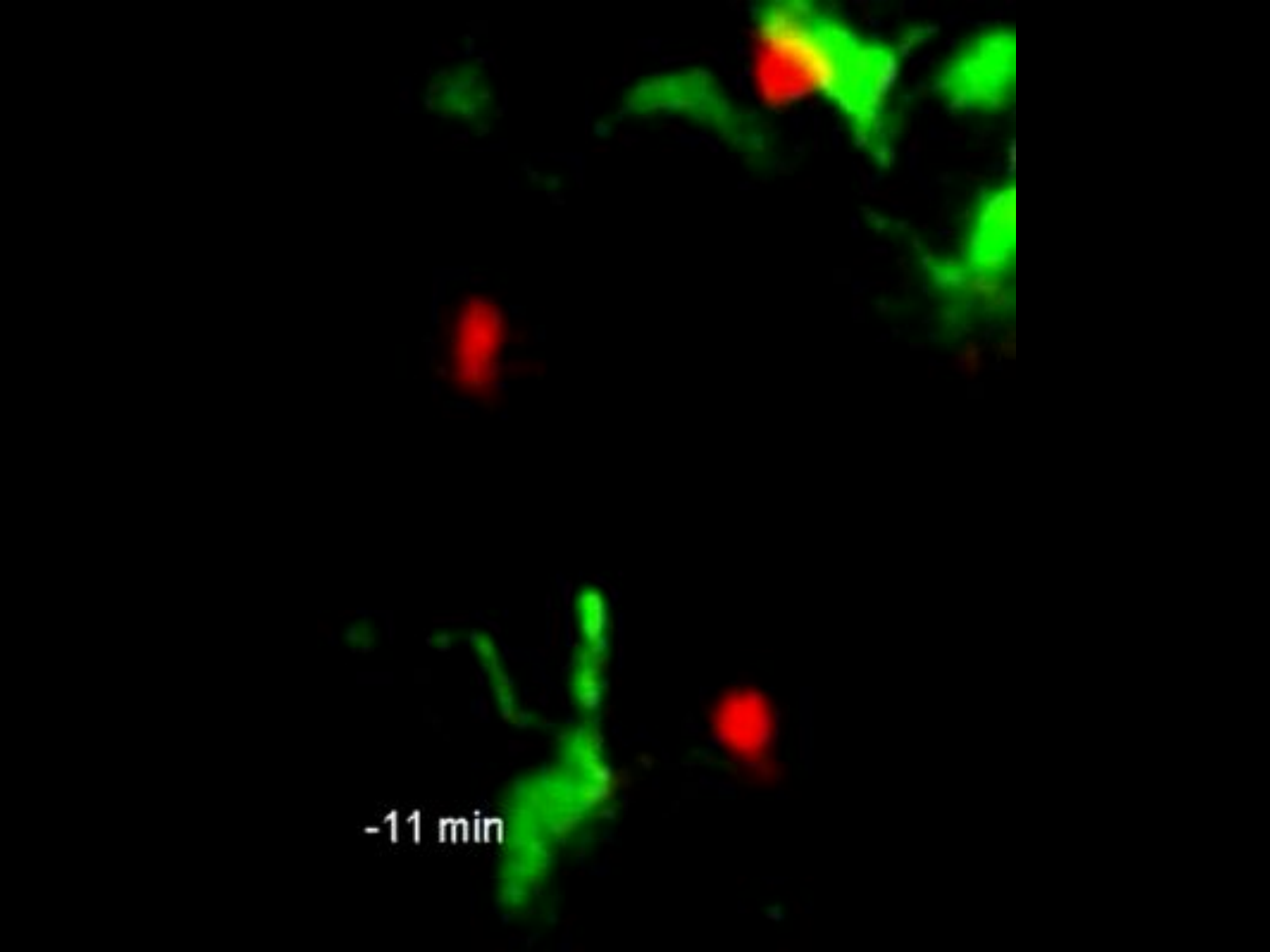
In vivo imaging of immune responses with two-photon microscopy







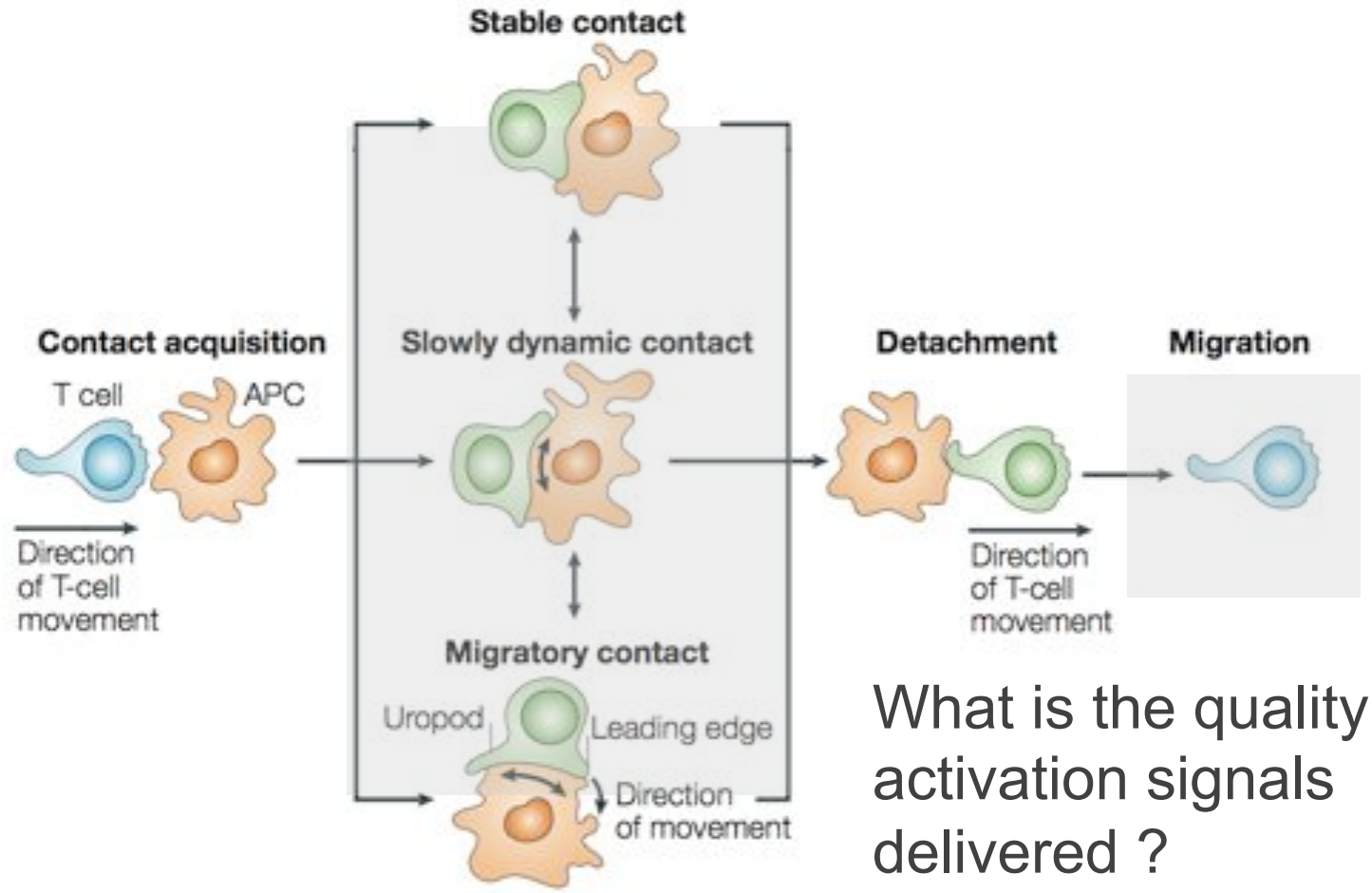
Bousso et al. *Science 2002,* Bousso et al *Nat. Immunol 2003*


-11 min

How T cells sense TCR ligands in vivo ?

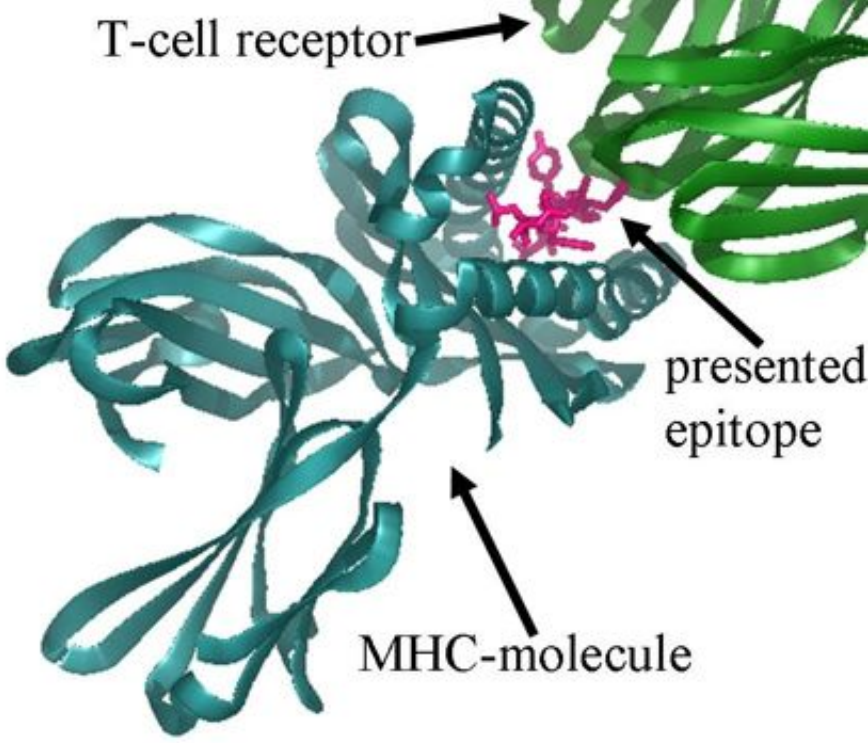
Hélène Moreau, PhD student
(unpublished)

Which parameters dictate the mode of antigen recognition ?



What is the quality of the activation signals delivered ?

P. Friedl et al., *Nature Reviews Immunology*, 2005



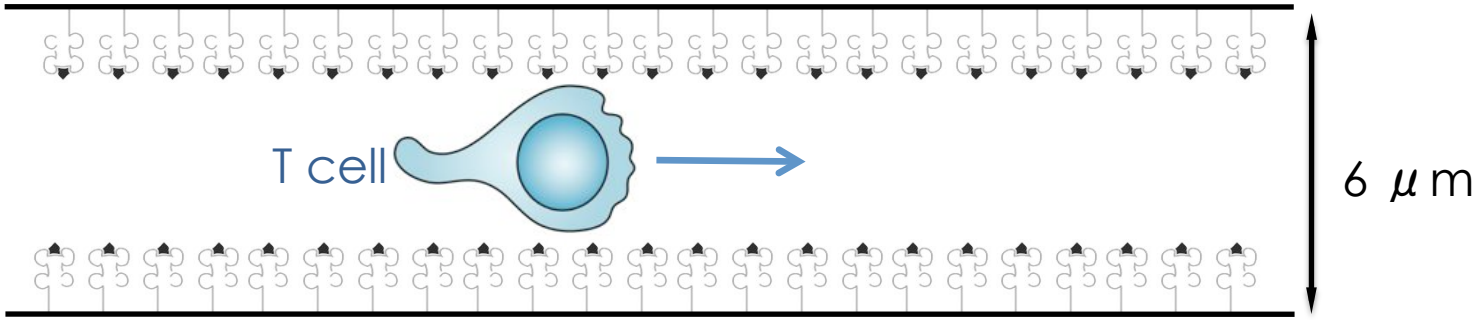
OVA peptide variants

N4 S I I N F E K L

Q4 S I I Q F E K L

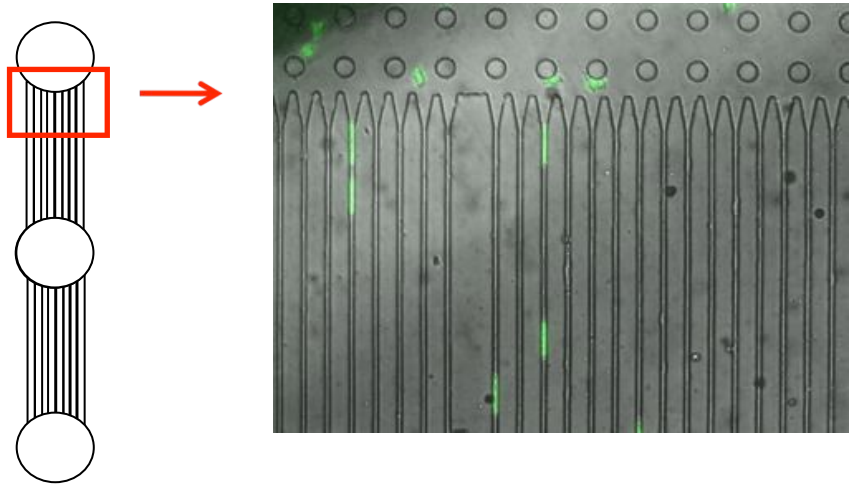
V4 S I I V F E K L

Migration of T cells in micro-channels coated with pMHC



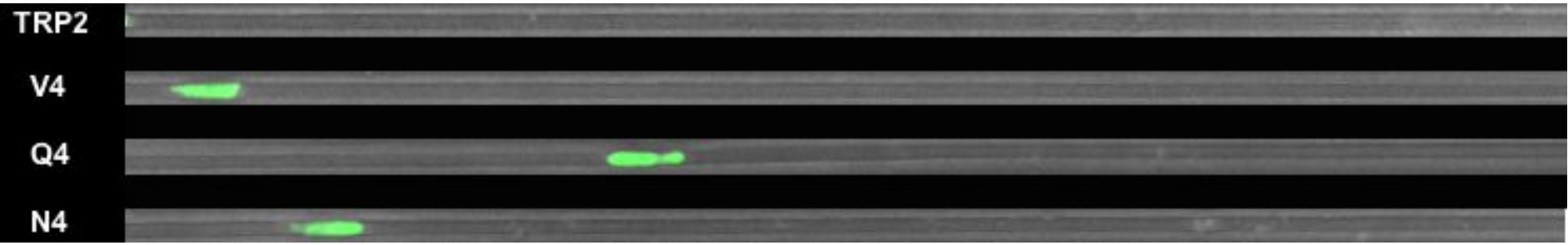
Surface of the micro-channel coated with recombinant pMHC

⇒ Antigen-recognition in a confined environment that promotes motility

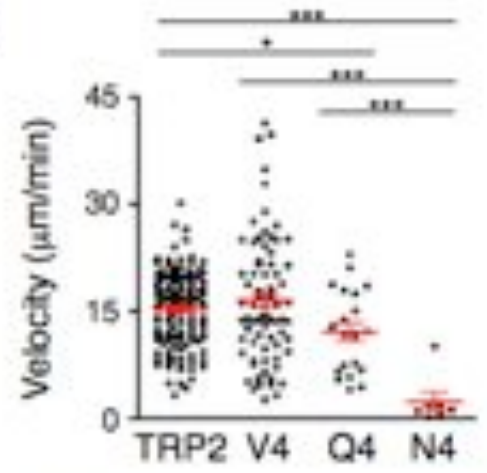


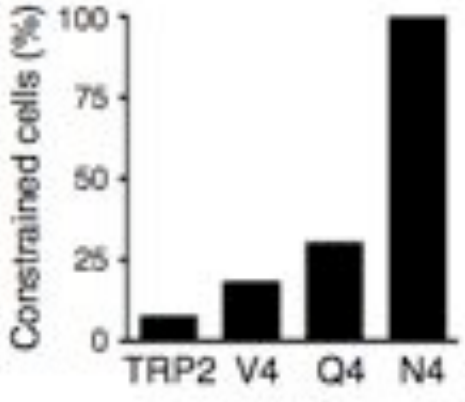
Collaboration with Ana-Maria Lennon-Duménil (INSERM U653) and Matthieu Piel and Emmanuel Terriac (CNRS UMR144), Institut Curie.

Diverse modes of antigen recognition in pMHC coated micro-channels



GFP expressing OT-I T cells migrating in 6 μm micro-channels coated with pMHC





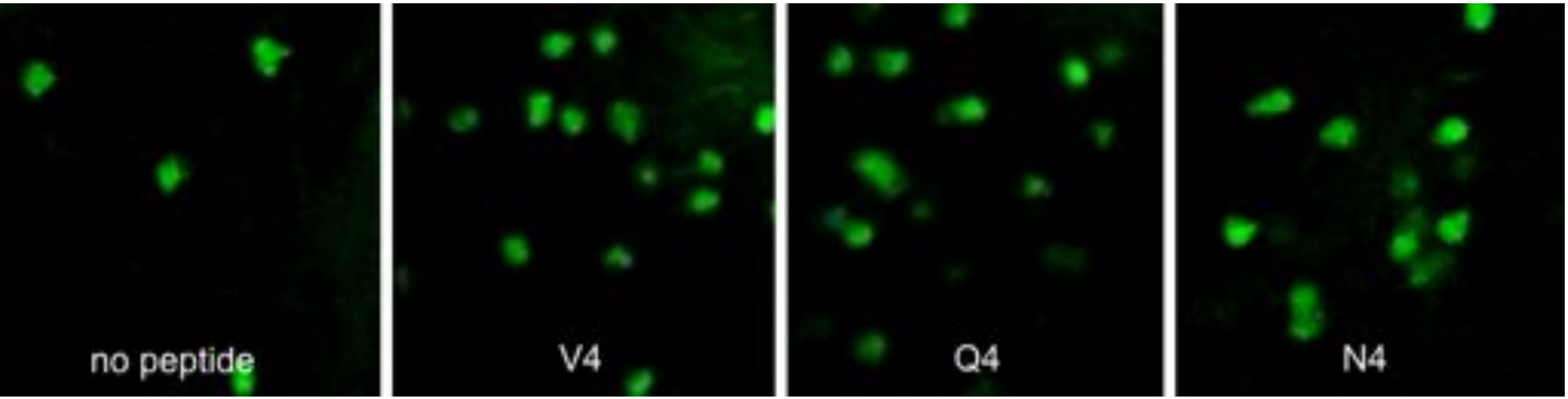
⇒ Only the high affinity peptide (N4) induces complete T cell arrest *in vitro*.
⇒ Q4 and V4 induce dynamic recognition of Ag.

TCR-pMHC affinity dictates the dynamics of antigen recognition

OT-I CD8 T cells

Poly I:C + peptide

Intravital 2-photon imaging of the spleen



Immune responses at the single cell level

Two-photon imaging

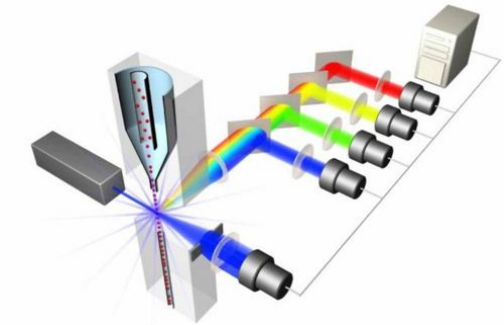
in vivo

Dynamic informations:
Cell motility and interaction

Lack of phenotypic information

Manual/semi-automated analysis

Flow cytometry



http://probes.invitrogen.com

ex vivo

Static informations

Hundreds of phenotypic
and functional markers

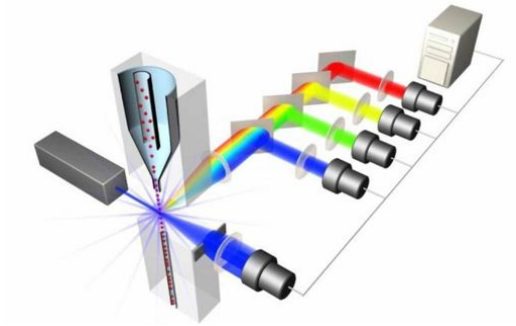
Automated and
multiparametric analysis

Linking phenotype to cell behavior in vivo

Two-photon imaging

+

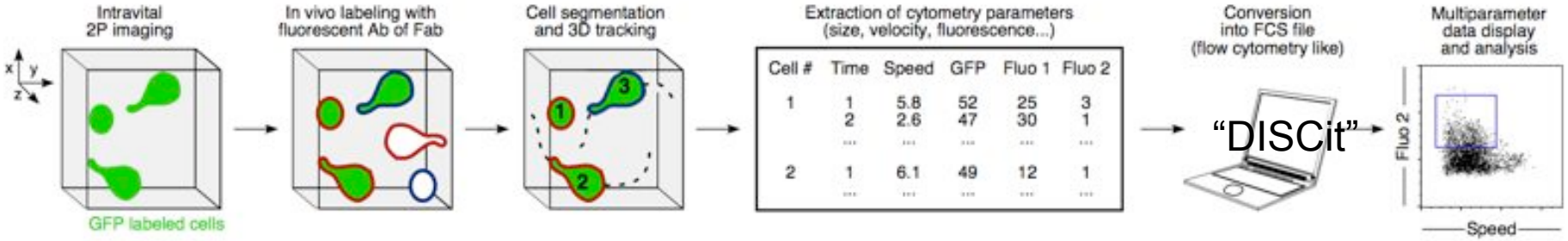
Flow cytometry

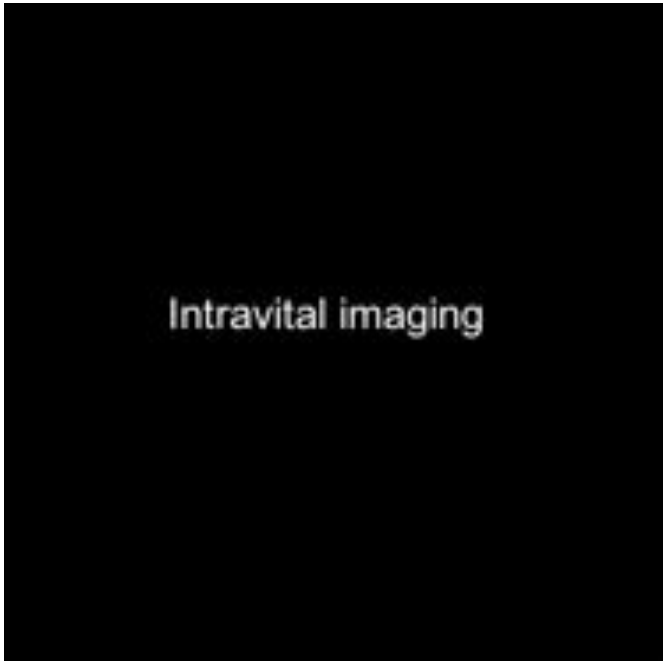


http;//probes.invitrogen.com

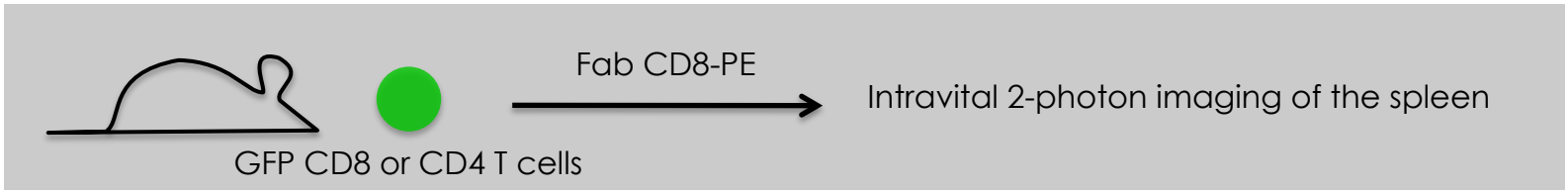
DISC: Dynamic in Situ Cytometry

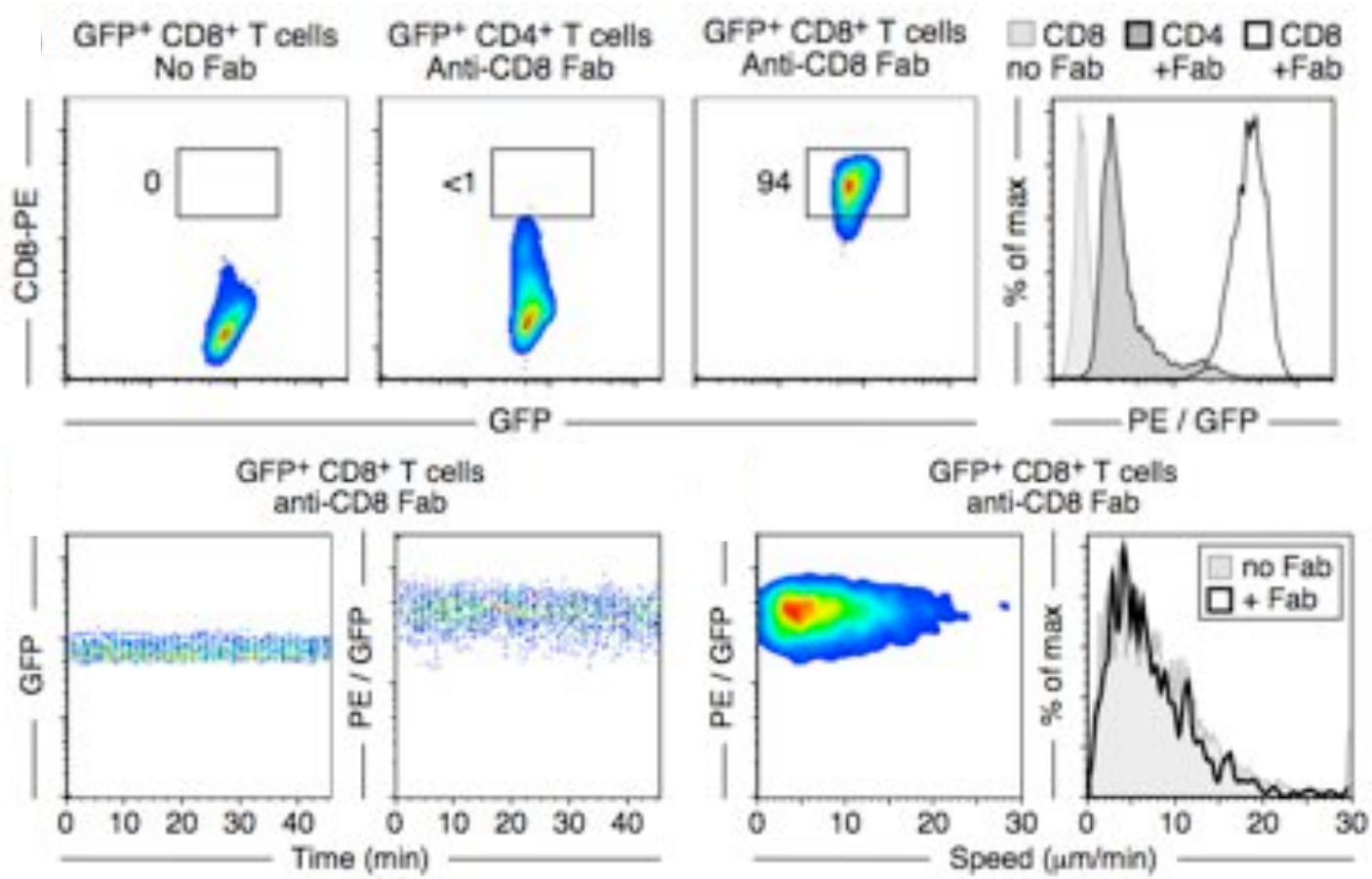
Dynamic In Situ Cytometry (DISC)



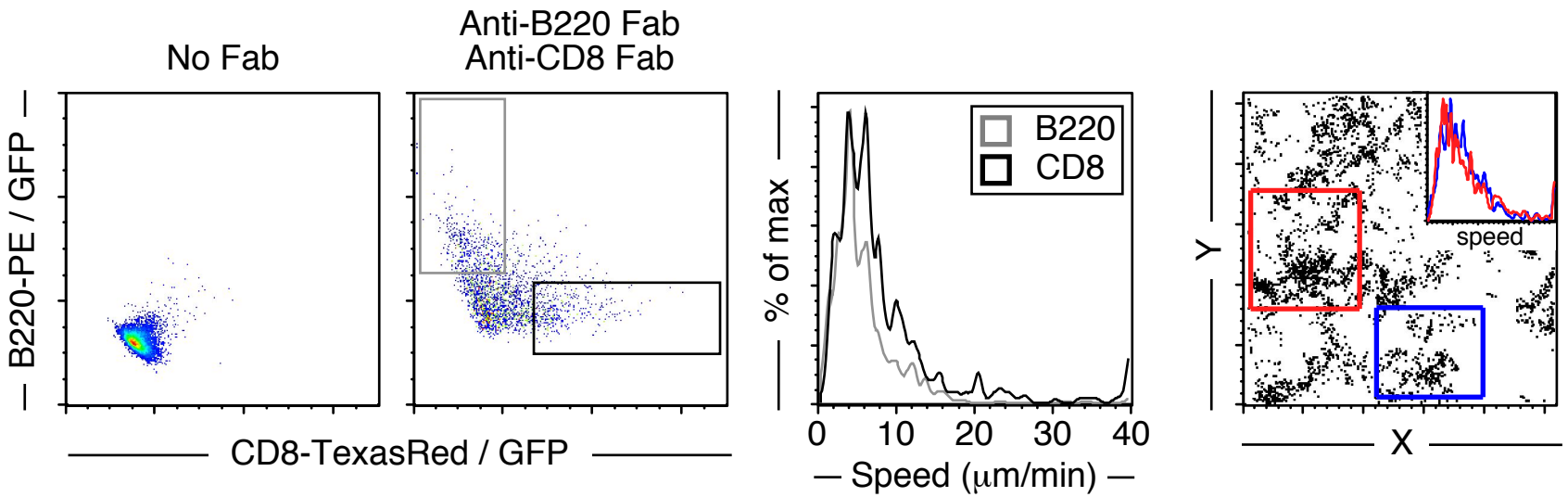


In vivo staining is specific, stable in time, and does not affect dynamics



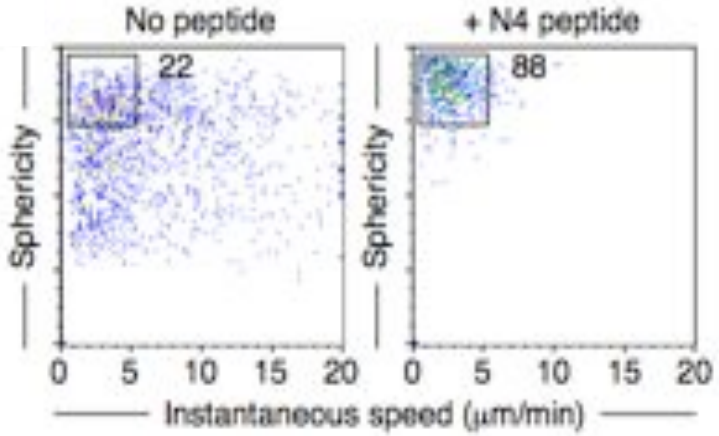


Gating strategies using DISC

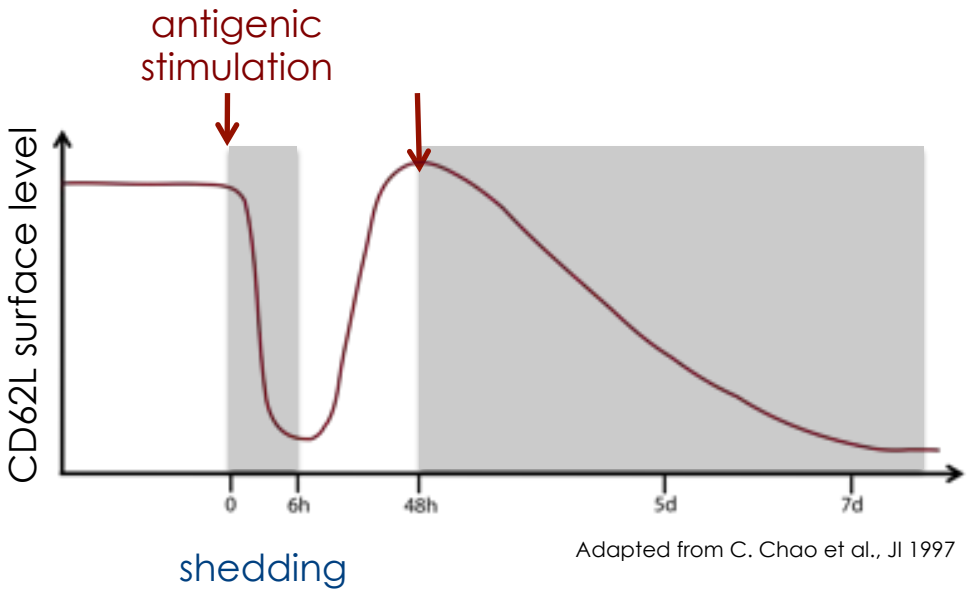


In vivo staining is specific, stable in time, and does not affect dynamics

Intravital 2-photon imaging of the spleen



Exploiting CD62L shedding for tracking TCR signaling

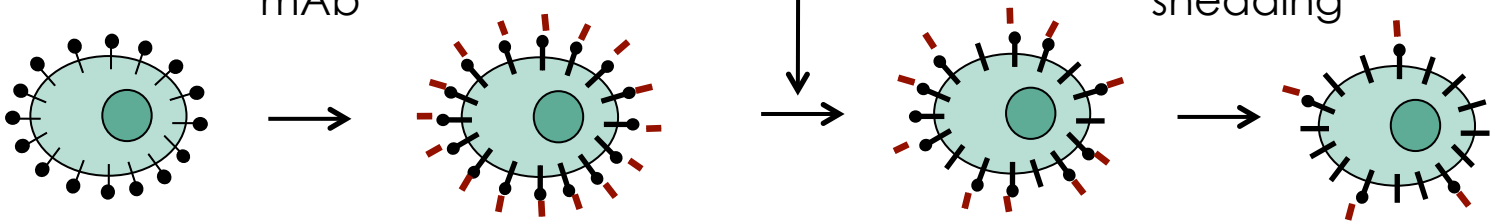


Adapted from C. Chao et al., JI 1997

1. Prestaining with anti CD62L – PE mAb

2. Antigenic stimulation

3. Real time quantification of CD62L shedding



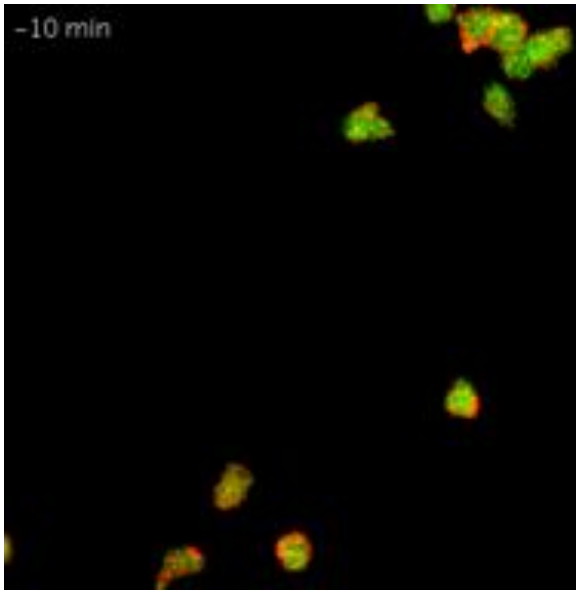
Visualizing TCR signaling *in vivo*

CD62L stained
GFP-expressing T cells

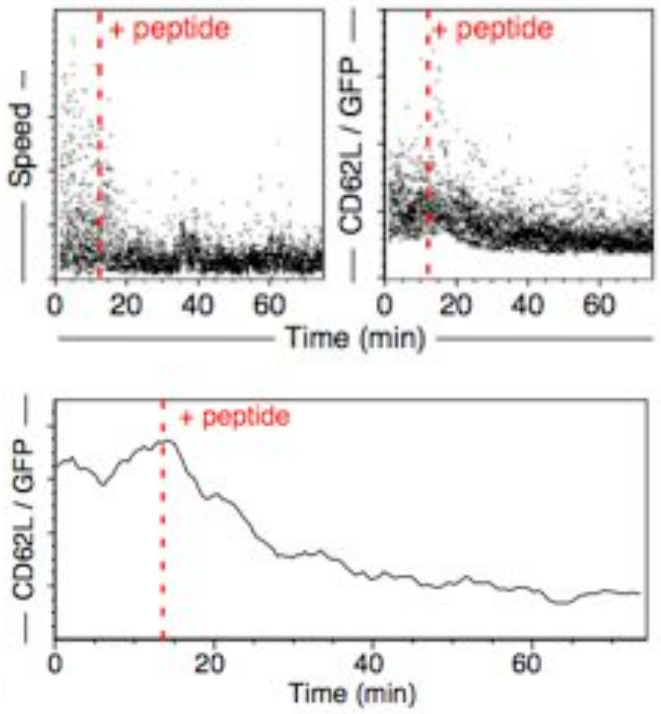
+ antigenic peptide

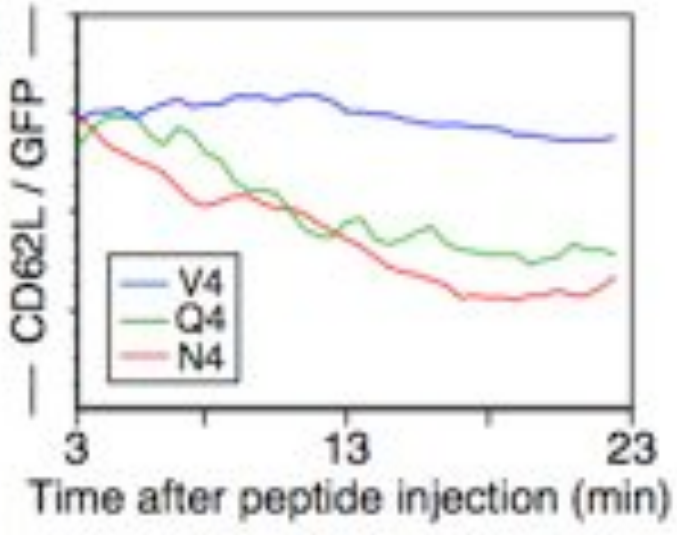
CD62L Shedding ?

Intravital 2-photon imaging of the spleen

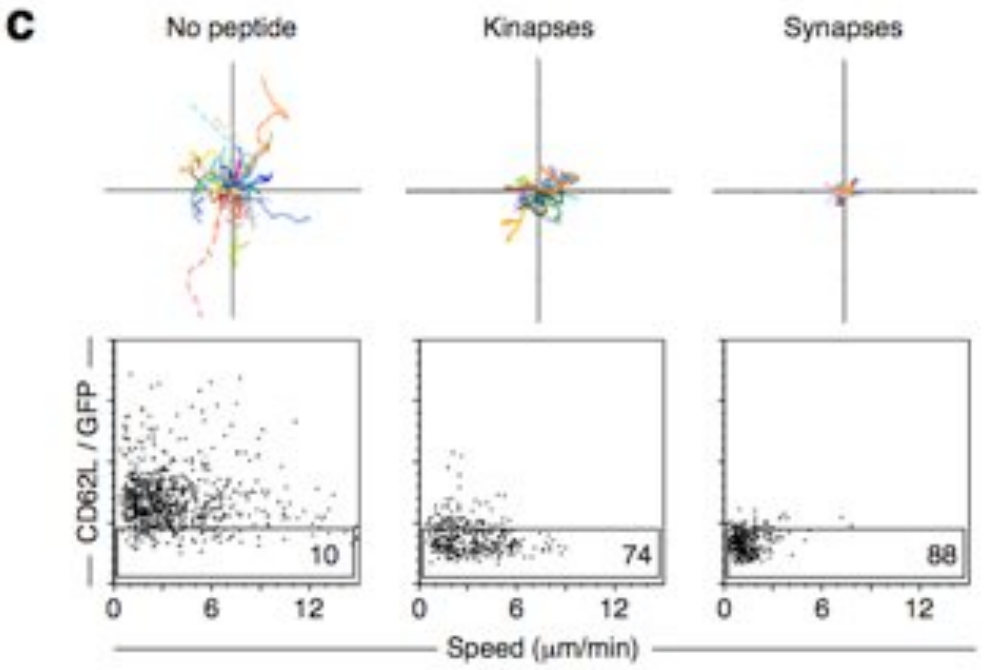


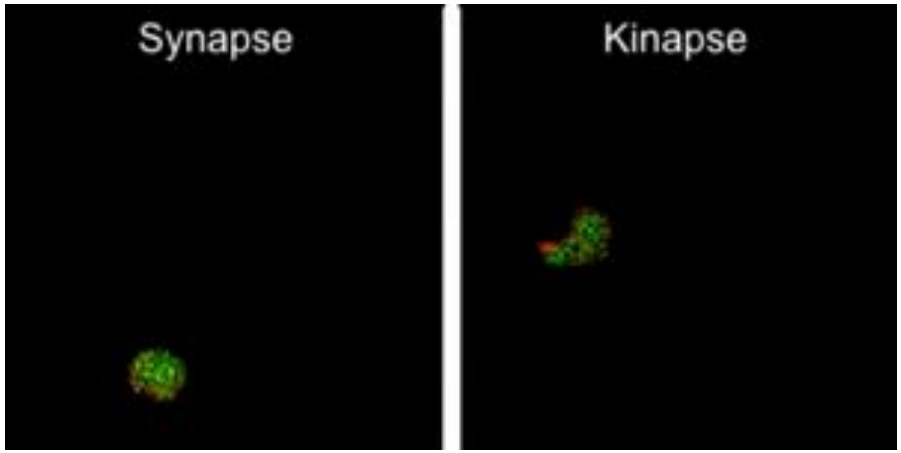
Visualizing TCR signaling *in vivo*



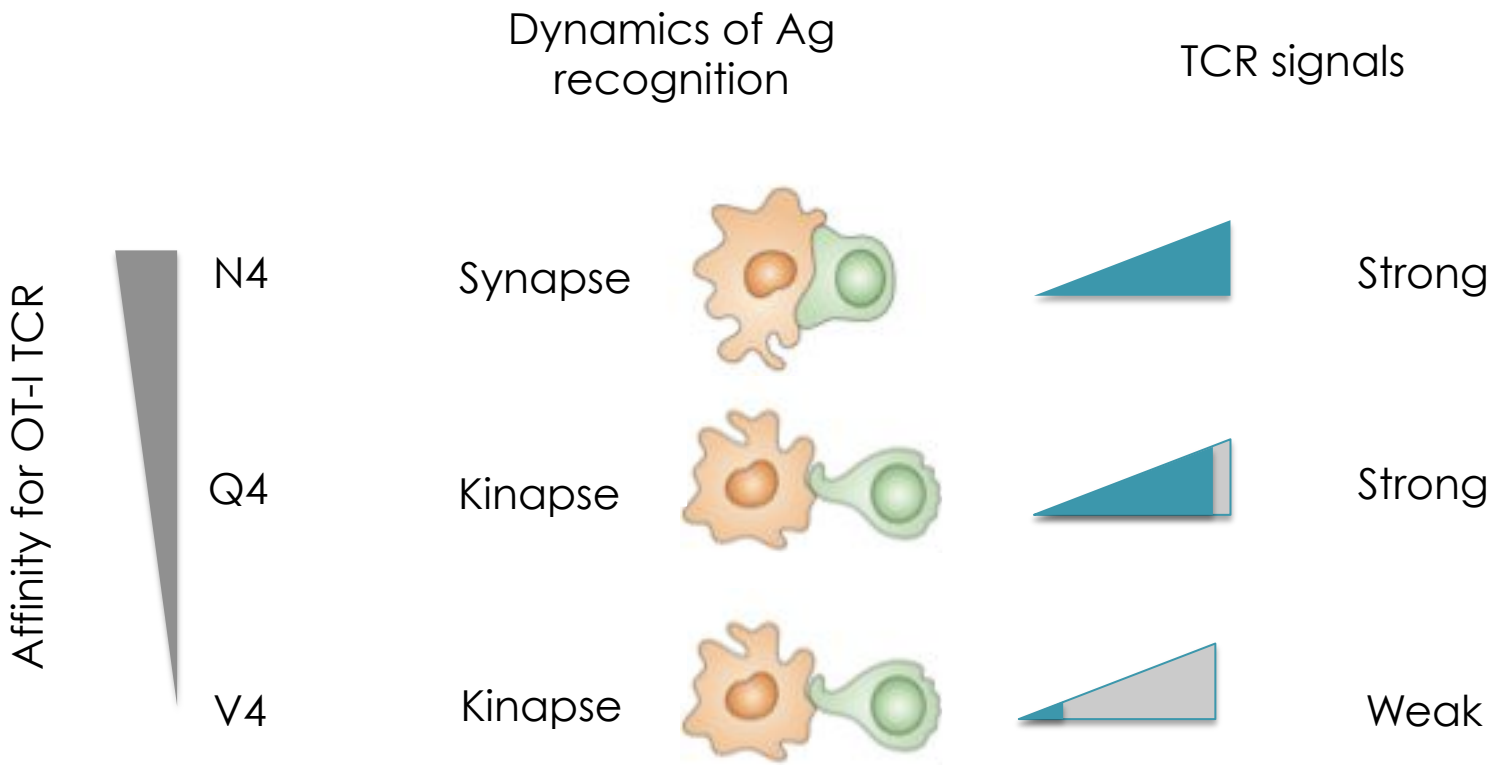


Role of pMHC affinity on early TCR signals *in vivo*



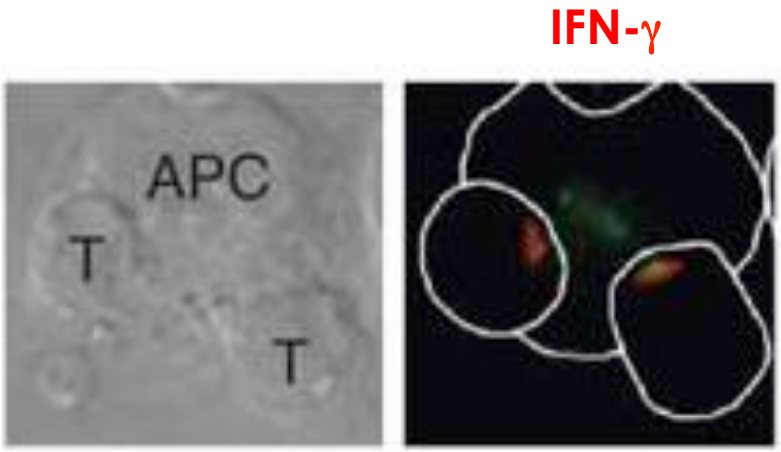


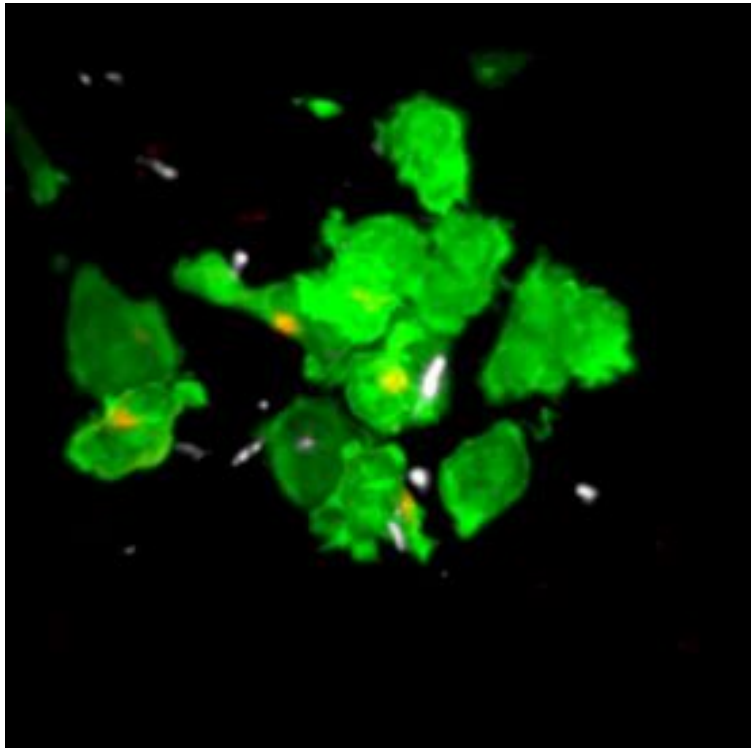
pMHC affinity: dual role with distinct thresholds



DISC: An approach to simultaneously assess cell dynamics and phenotype
A tool for quantifying imaging data
DISCit software freely available upon request

Targeted secretion of IFN-γ by CD4+ T cells at the immunological synapse





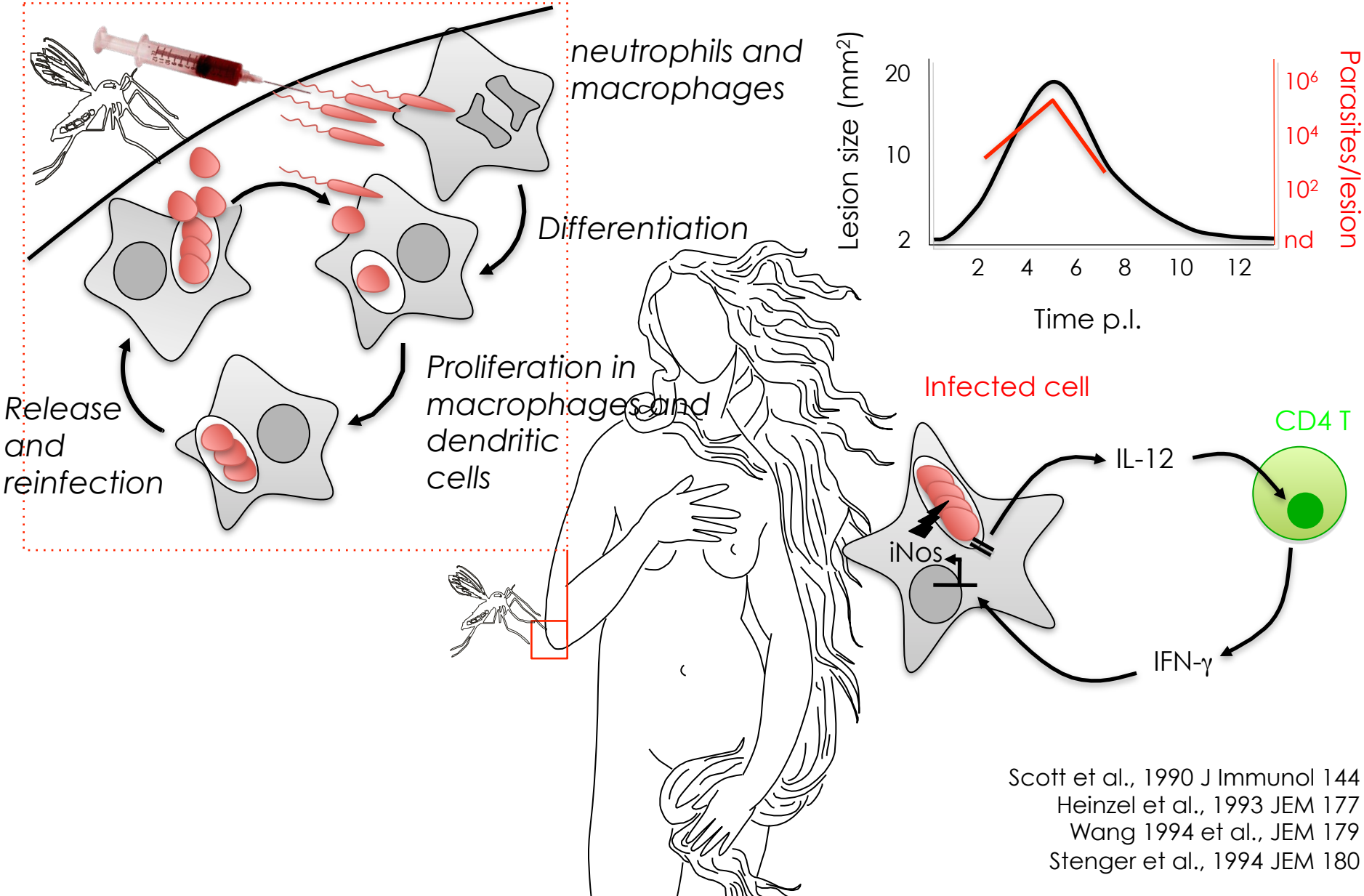
Egen *et al. Immunity* 2011

Do CD4+ T cell effector functions

extend beyond the immunological synapse *in vivo* ?

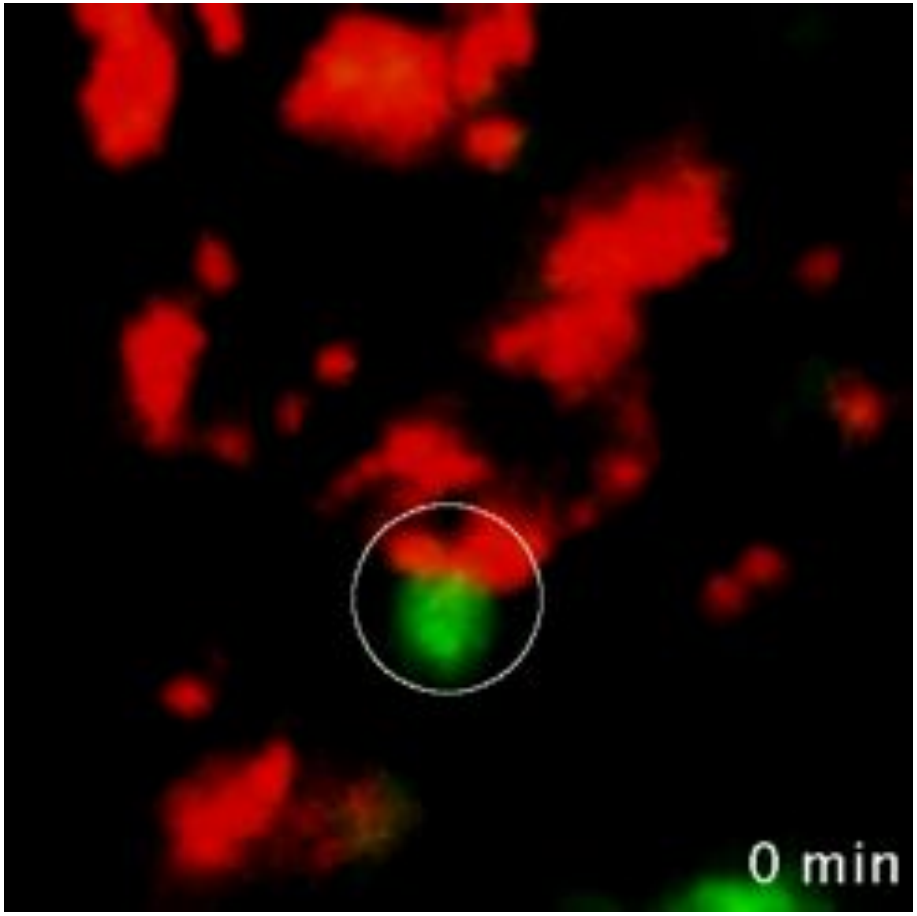
Andreas Müller, postdoctoral fellow

L. major life and death



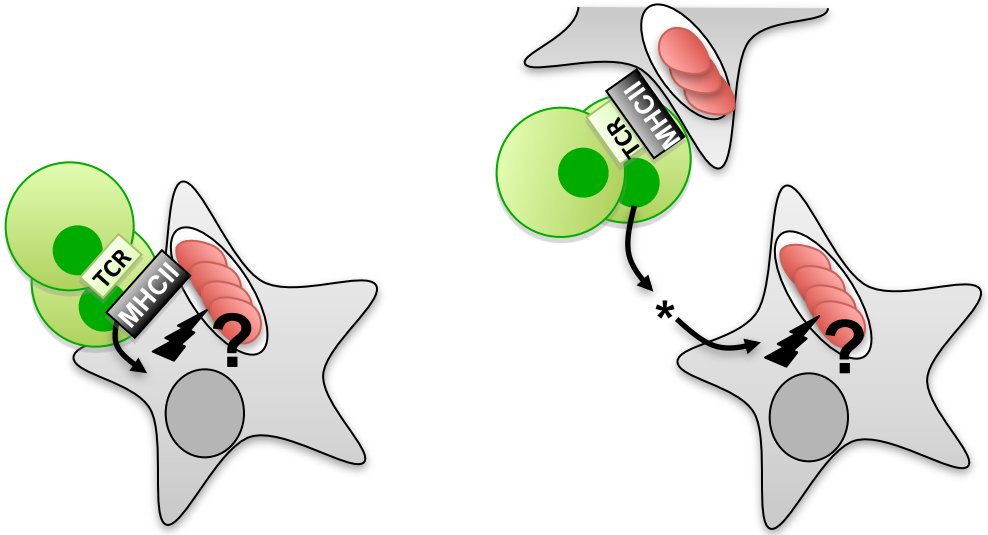
Scott et al., 1990 J Immunol 144
Heinzel et al., 1993 JEM 177
Wang 1994 et al., JEM 179
Stenger et al., 1994 JEM 180

Stable interactions between parasite-specific effector CD4+ T cells and infected cells are rare.



Filipe-Santos et al., 2009
Cell Host & Microbe

T cell-APC interactions conferring *L. major* clearance



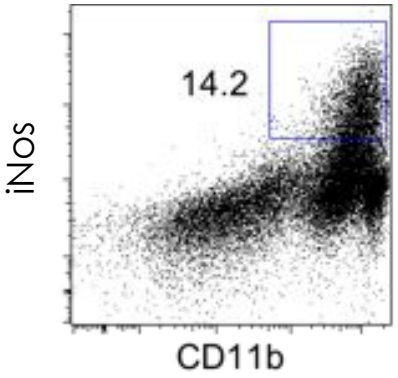
Which interactions result in the activation

of defense mechanisms in the infected APC?

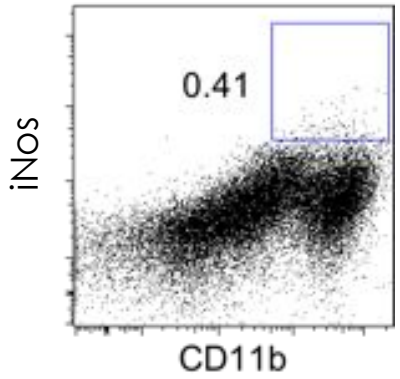
MHCII expression is required for iNos induction

L. major ear infection:
Day 17 p.l.

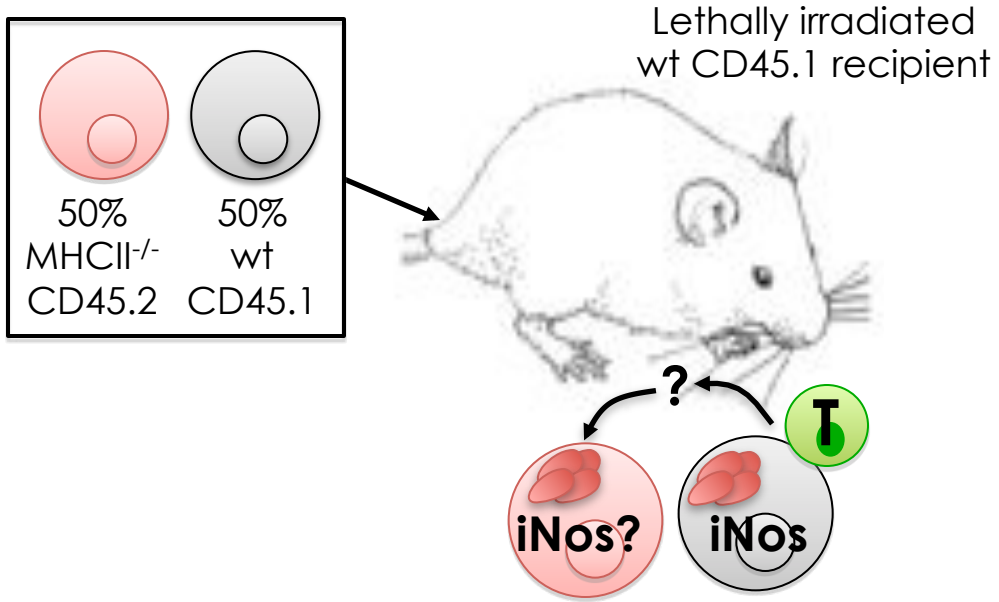
wild type



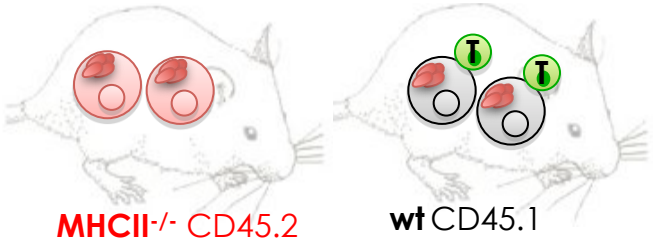
class II MHC$^{-/-}$

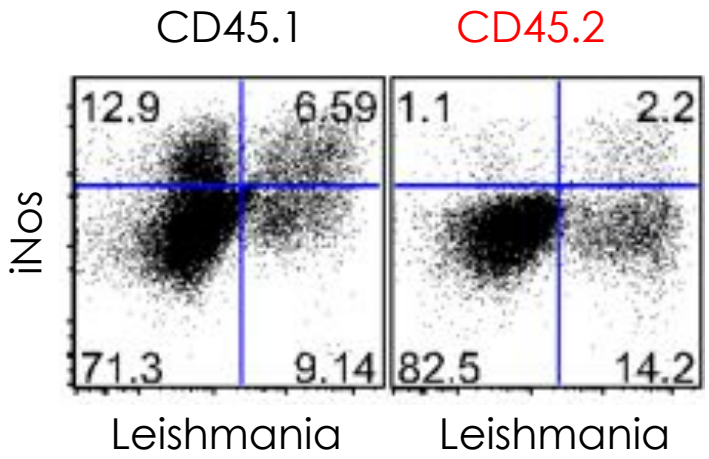


Is MHCII required in all infected cells for iNos induction?

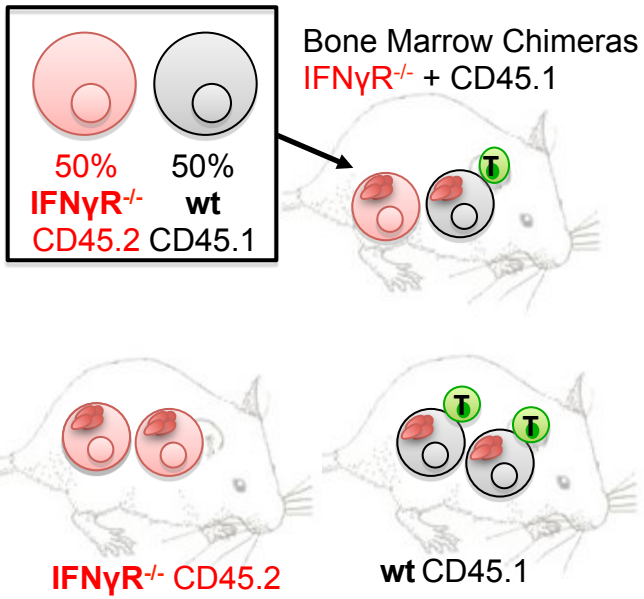


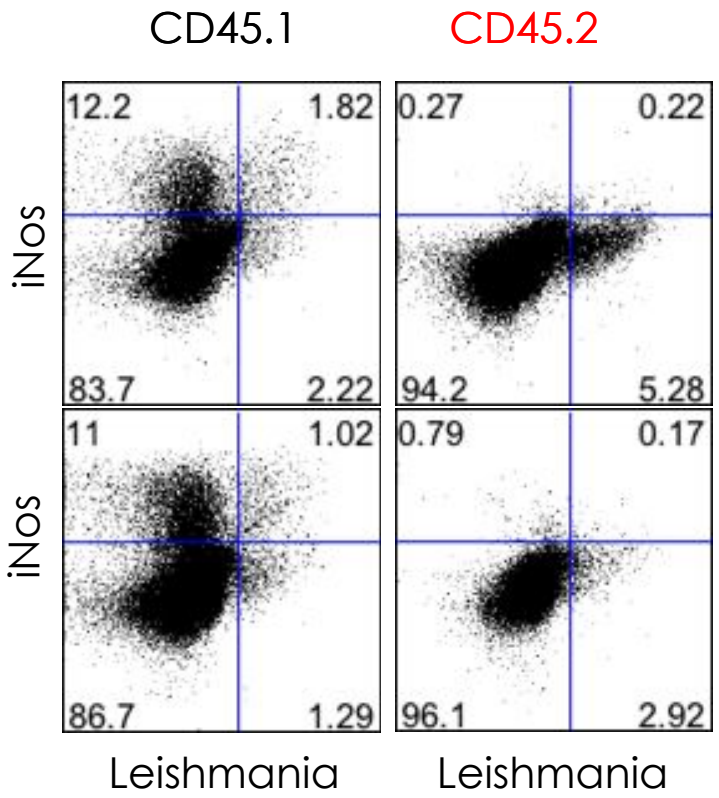
MHCII-driven interactions rescue iNos expression in bystander cells



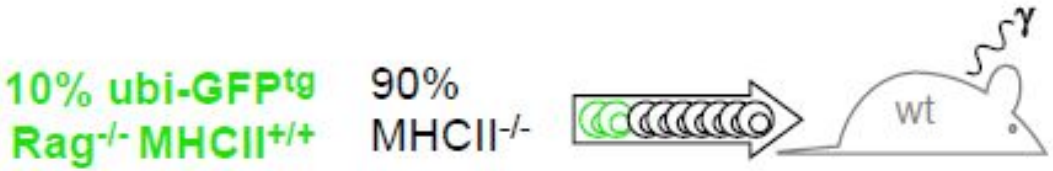


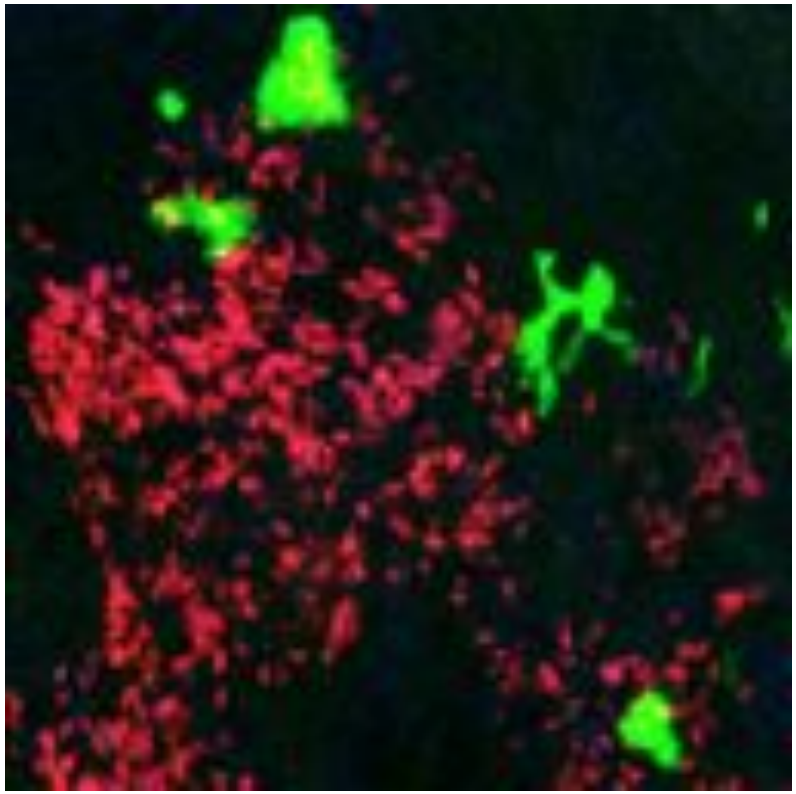
IFN-γ mediates the bystander activity



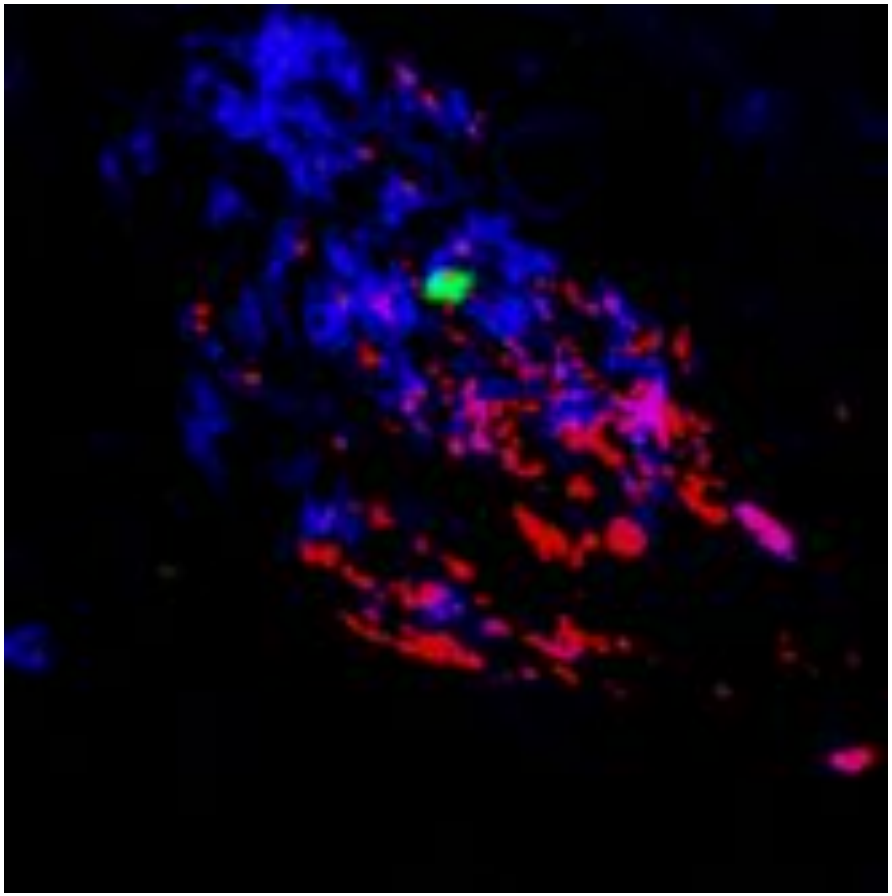


Visualizing bystander effector T cell activity



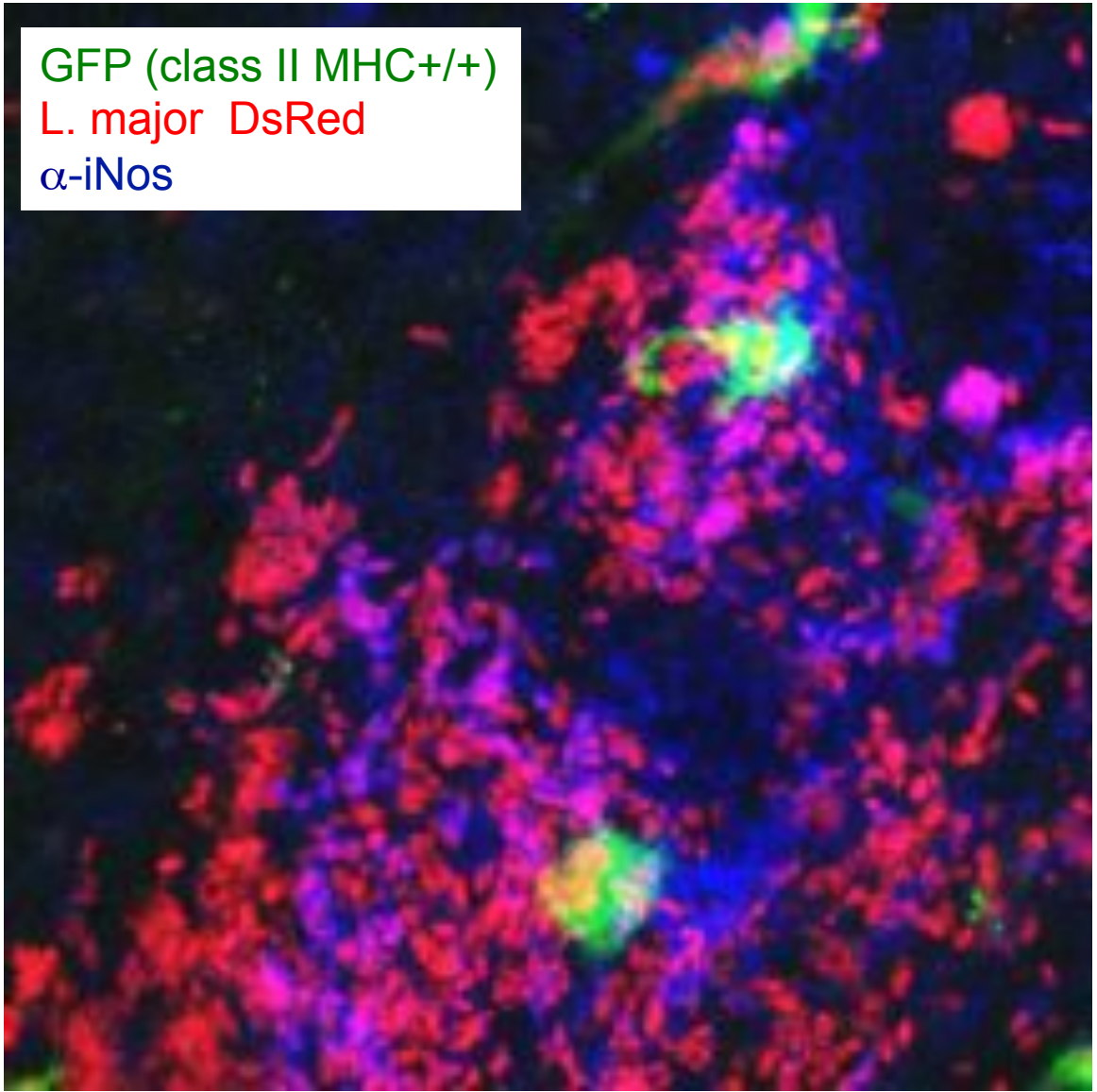


Visualizing bystander effector T cell activity



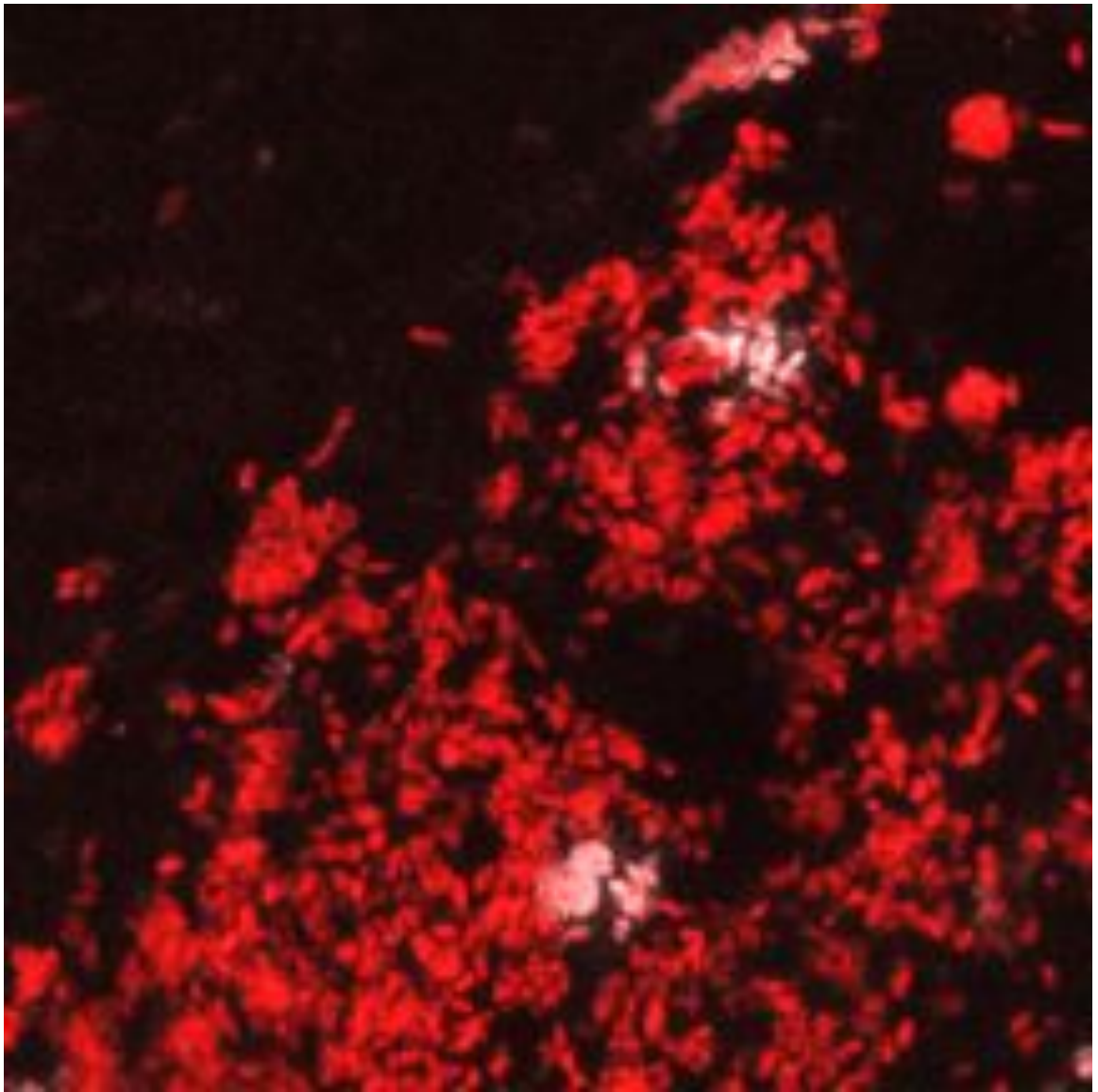
GFP (class II MHC+/+)
L. major DsRed
iNos

Automated measurement of iNos induction

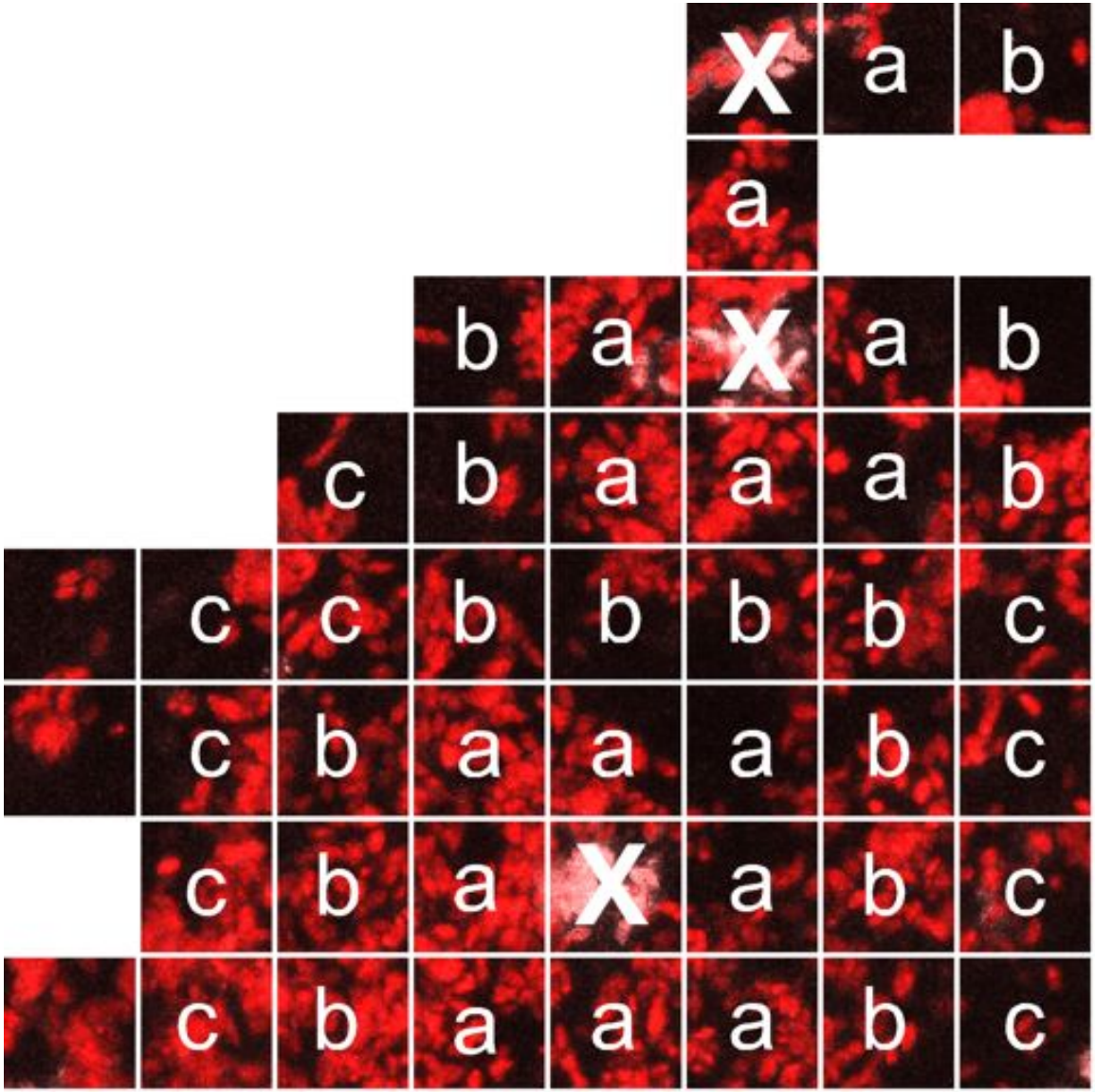


Automated measurement of iNos induction

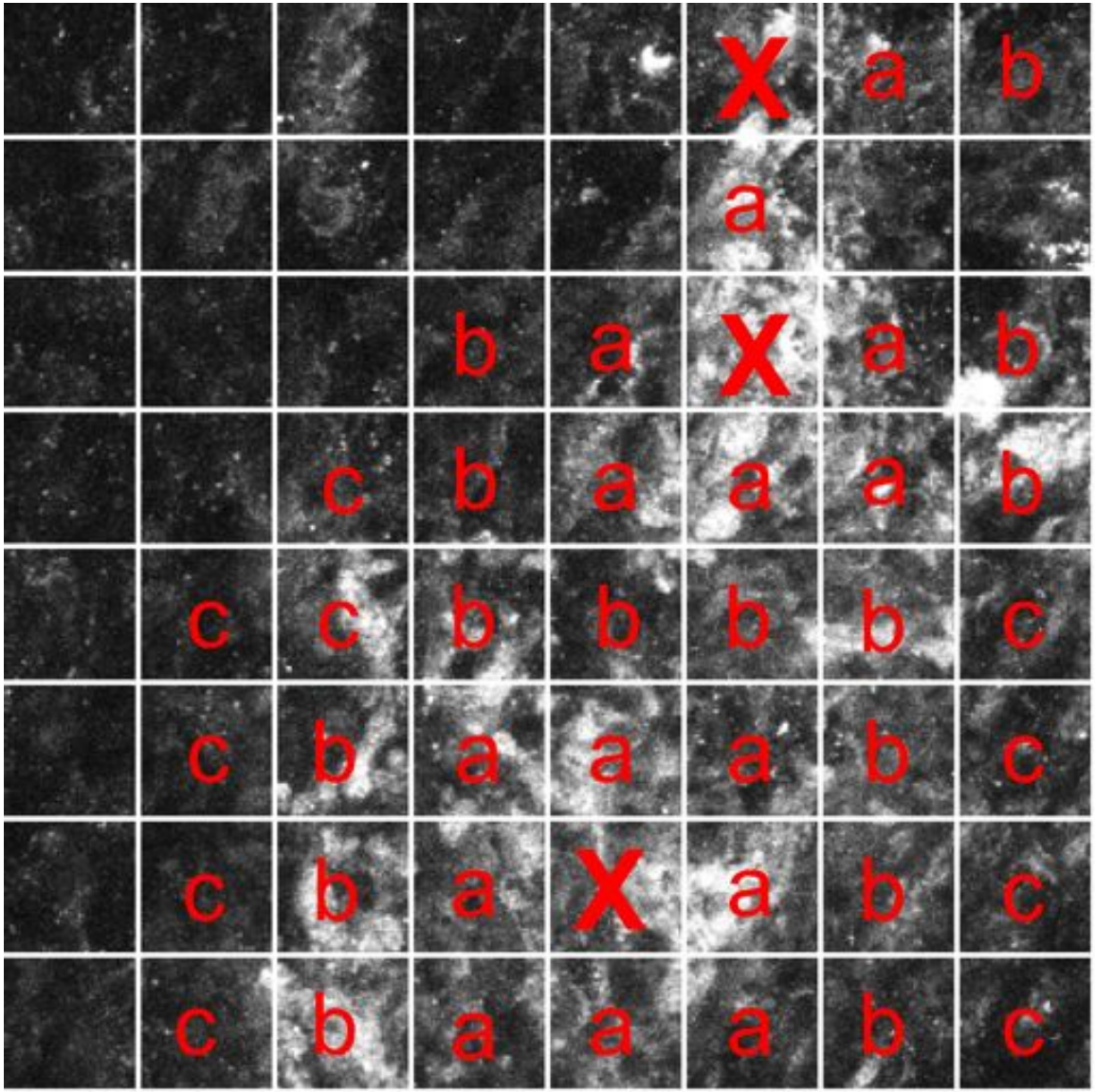
Determination of infected class II MHC+/+ cells



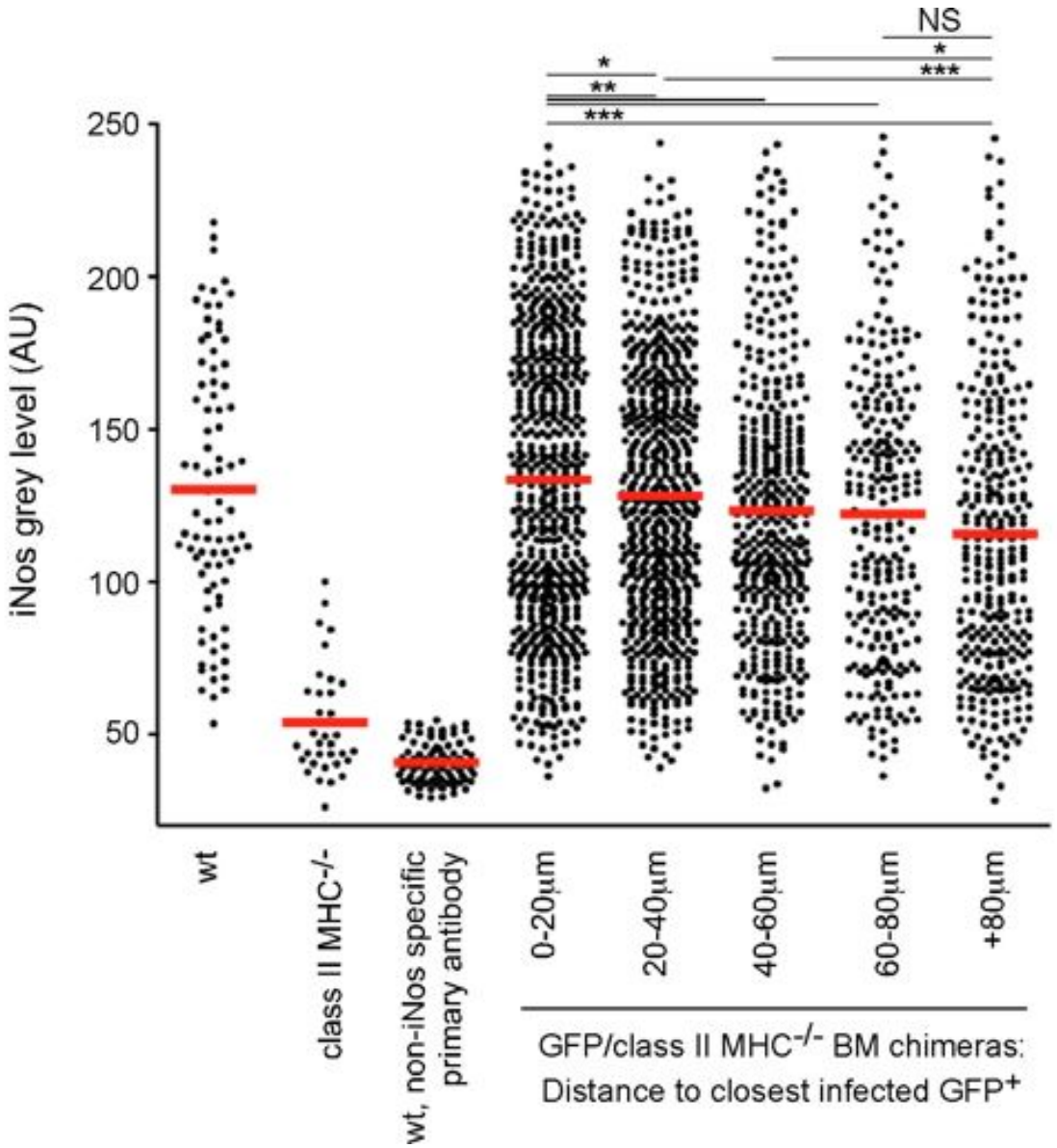
Distance of neighboring parasite-containing regions:
a=0-20µm
b=20-40µm
c=40-60µm

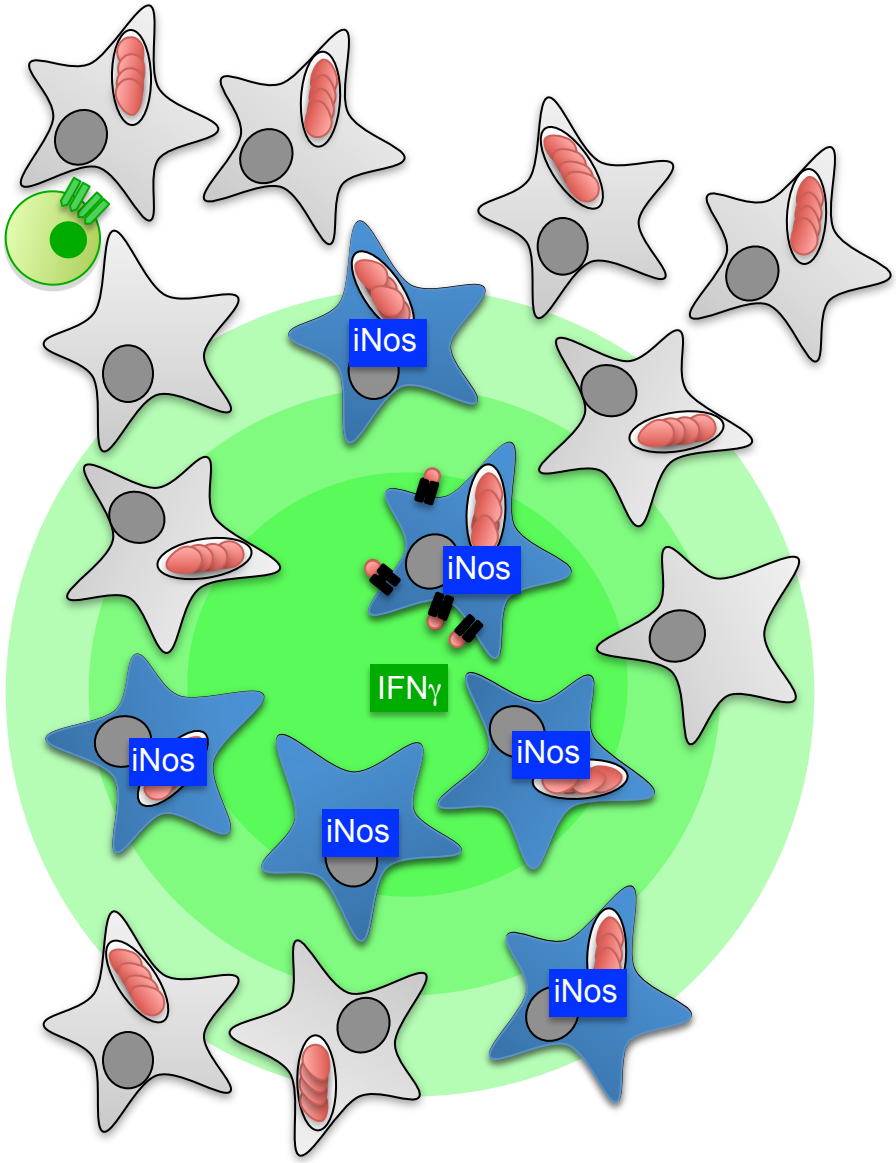


Determination of iNos associated with parasite-containing regions.



Spatial diffusion of CD4+ T cell effector activity





Antigen-specific CD4 T cells form limited number of stable contacts with infected cells (presumably due to limited antigen presentation)

CD4 T cells effector functions occurs beyond the immunoligical synapse, reaching not only the antigen-presenting cell but also non-presenting bystander cells (range >100 μm)

CD4 T cells can control an infection by engaging <10% of infected cells

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