Mechanisms of action and disease: immune synapses

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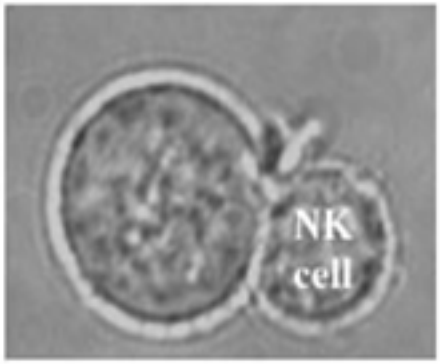
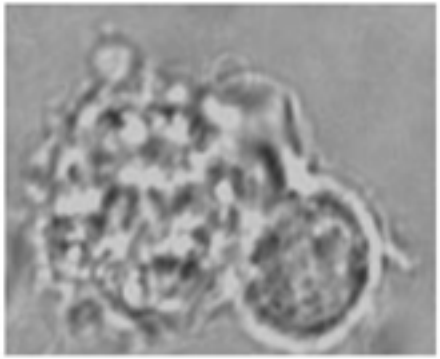
Imperial College London, UK

- Imaging natural killer cell surveillance
- Super-resolution imaging of f-actin organisation at NK cell synapses

0



10 min.



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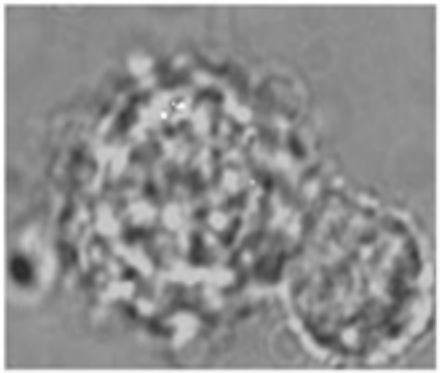
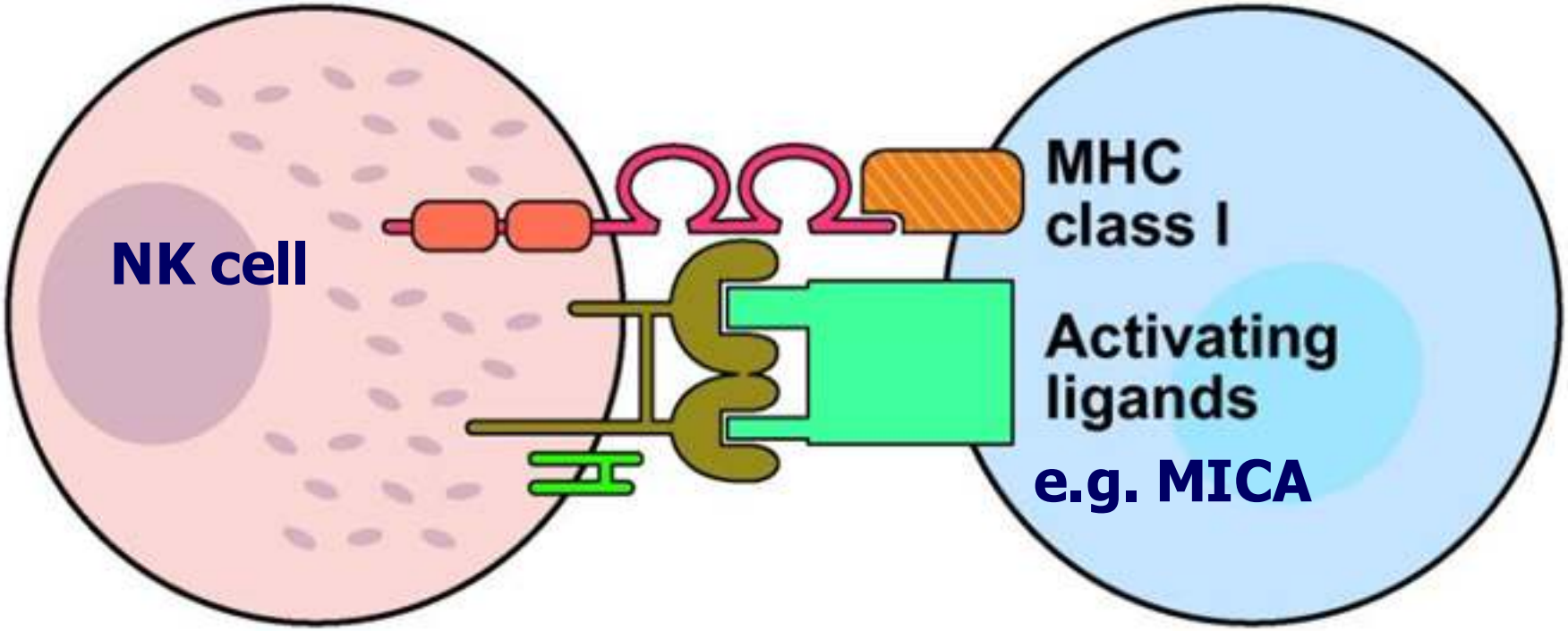
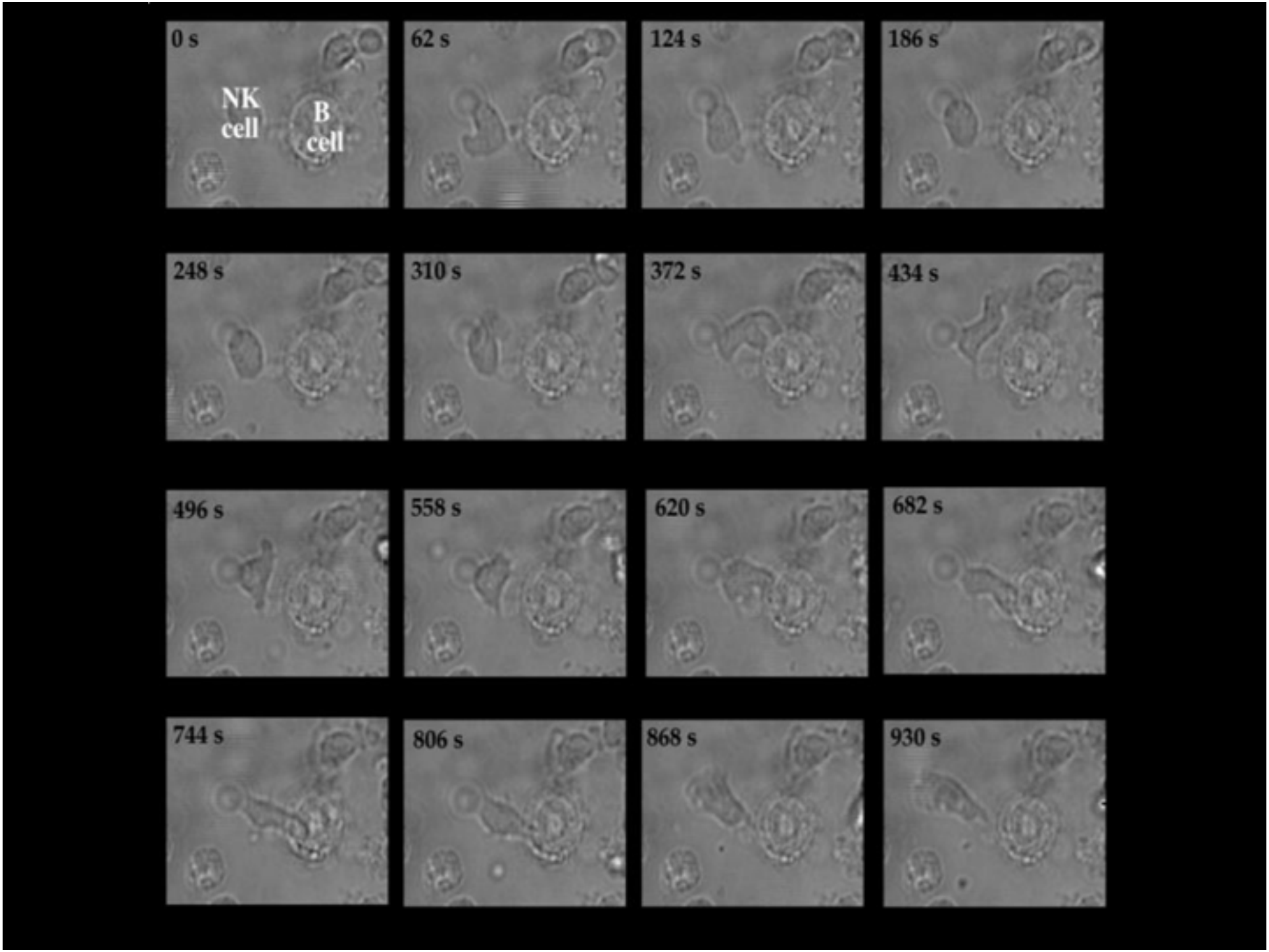


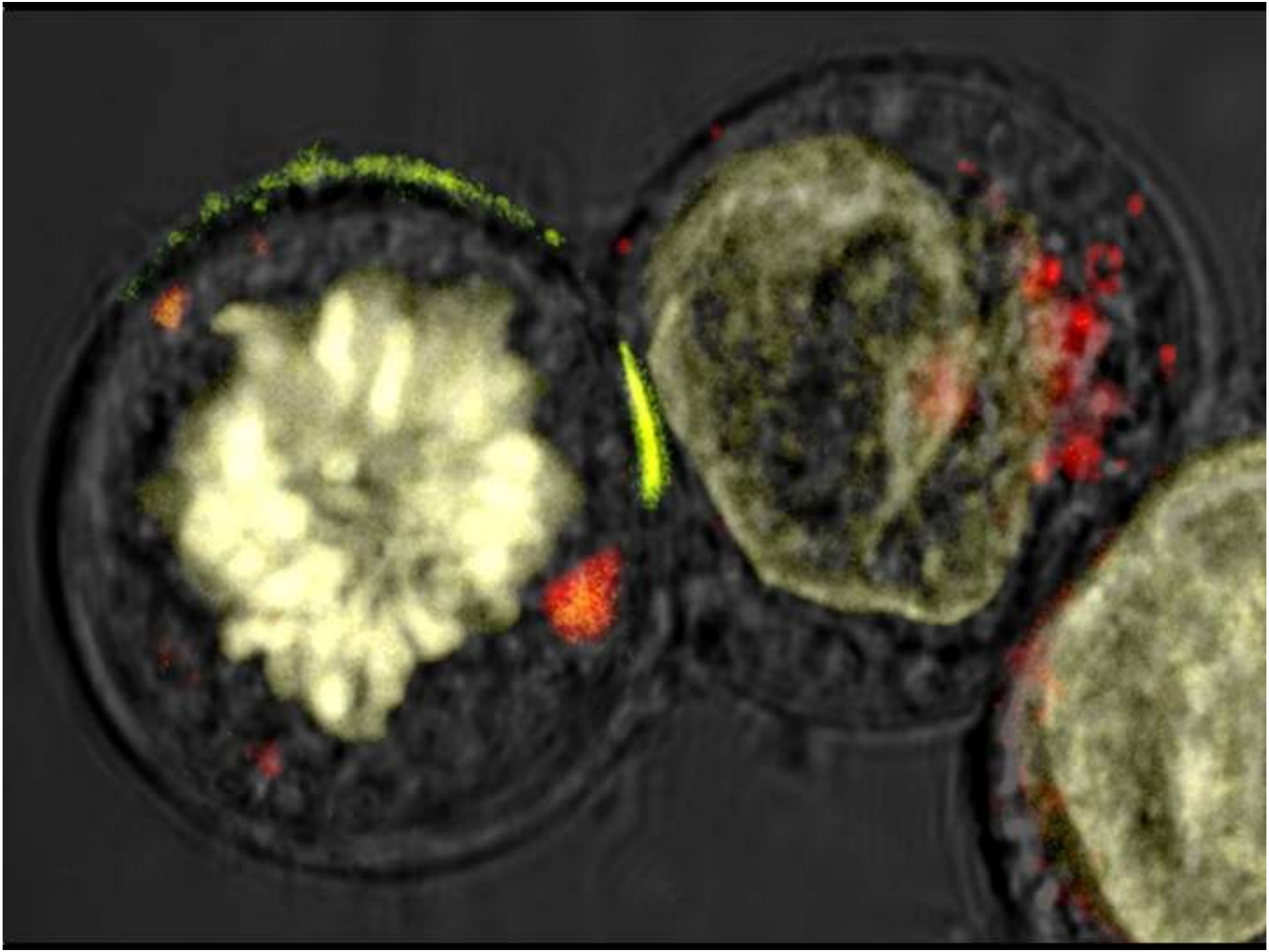
Natural killer cells and T cells can readily kill tumour cells

Natural killer cell immune synapse

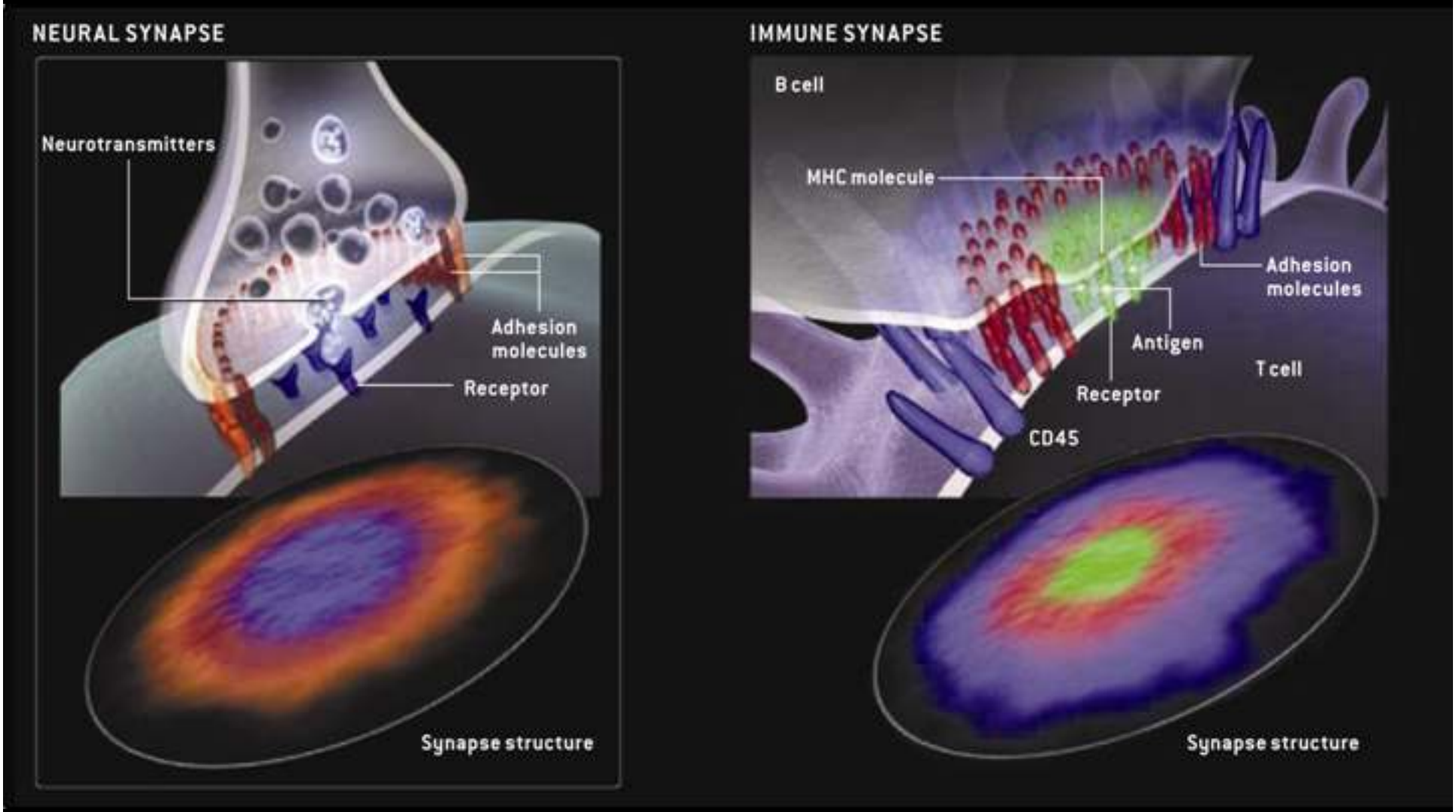


Lanier LL. Annu Rev Immunol. 2005;23:225-74.


0 s
NK cell
B cell
62 s
124 s
186 s
248 s
310 s
372 s
434 s
496 s
558 s
620 s
682 s
744 s
806 s
868 s
930 s

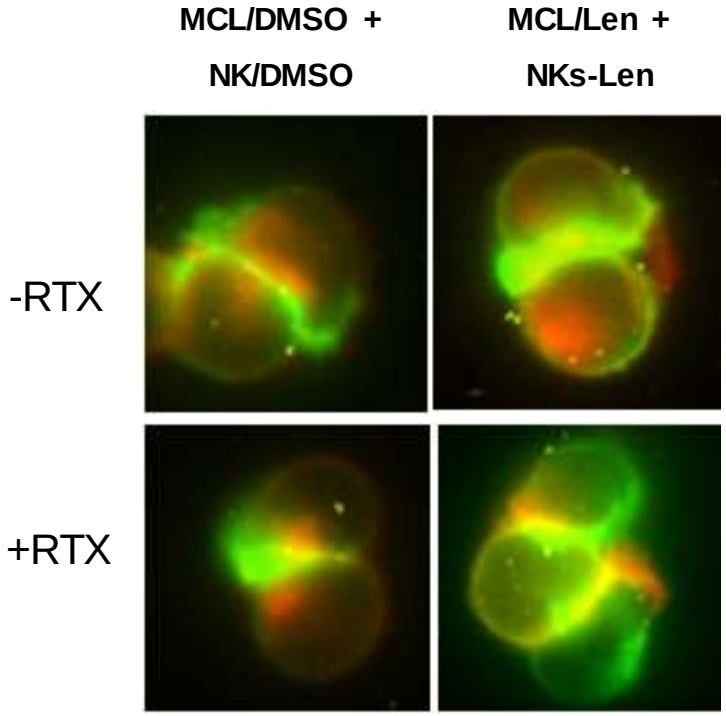


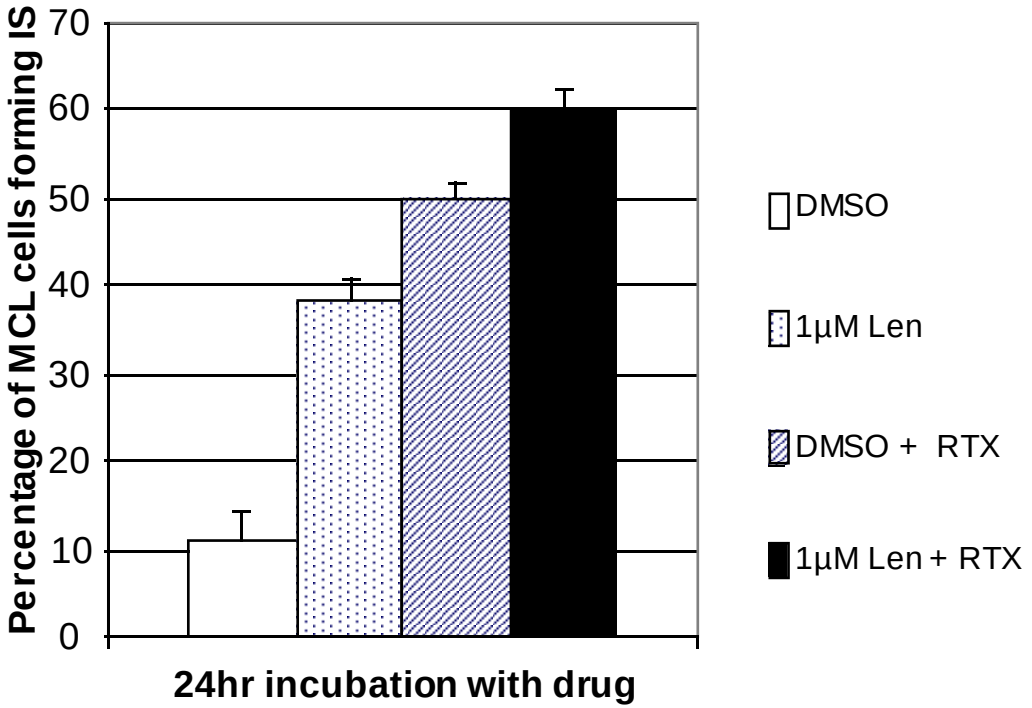
The immune synapse



Davis DM, Scientific American, 2006;294:48-55.

Lenalidomide (Revlimid) increases immune synapse formation between NK cells and mantle cells

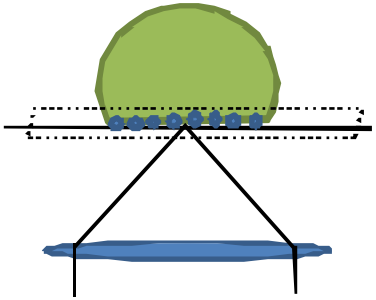




Courtesy of Antonia Lopez – Girona of Celgene

Microtweezers and confocal microscopy

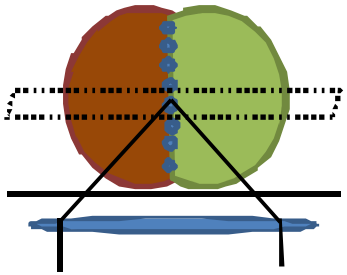
Artificial activating substrates



Microscope focal plane

Microscope objective

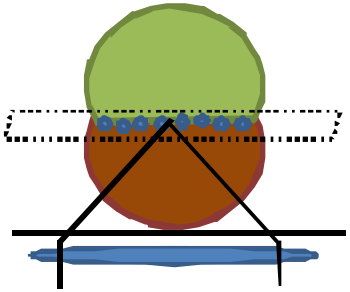
Image reconstruction from confocal z-stack

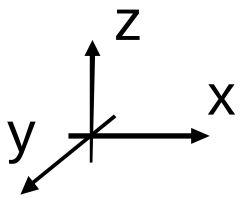


Resolution improved by x5

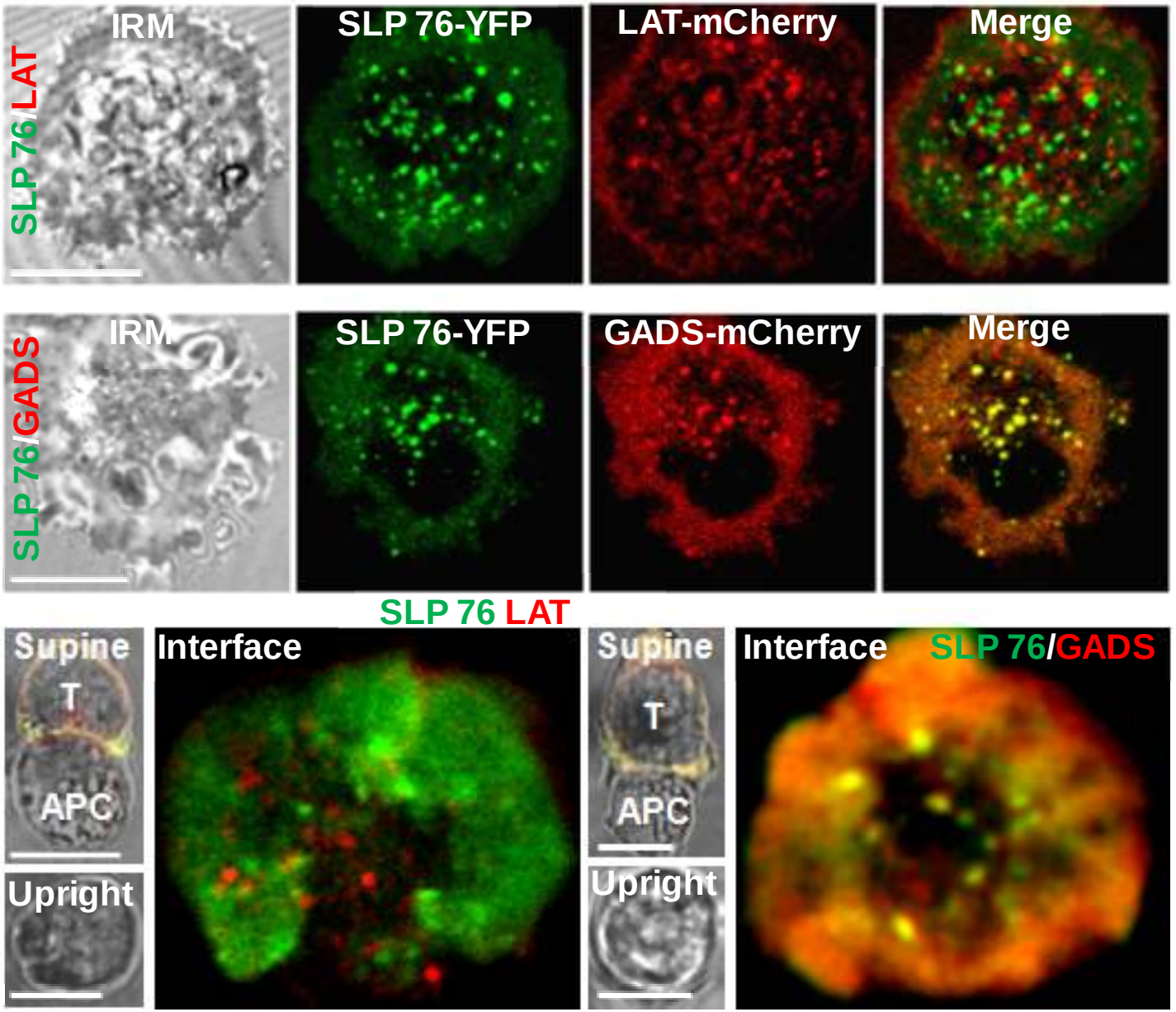
Speed increased 10-100 fold

Microtweezers & confocal microscopy



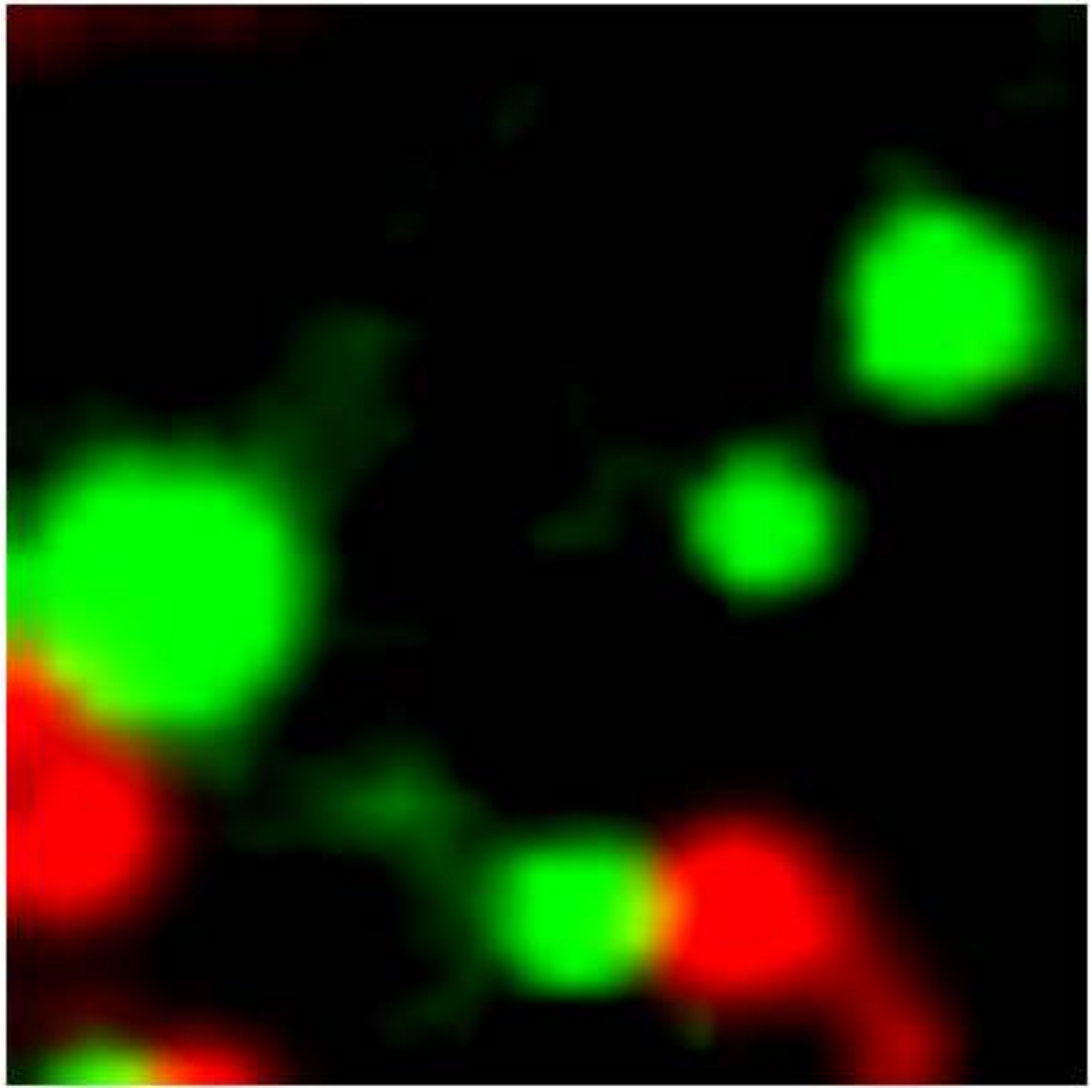


T cell synapses: distinct clusters of LAT and SLP-76

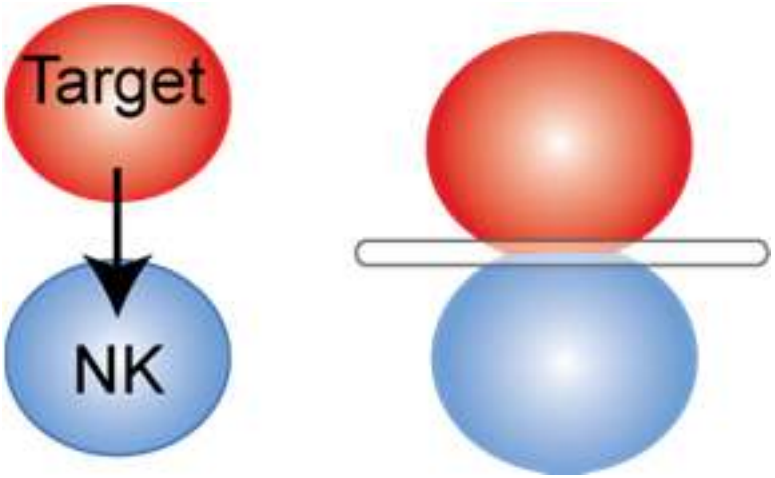


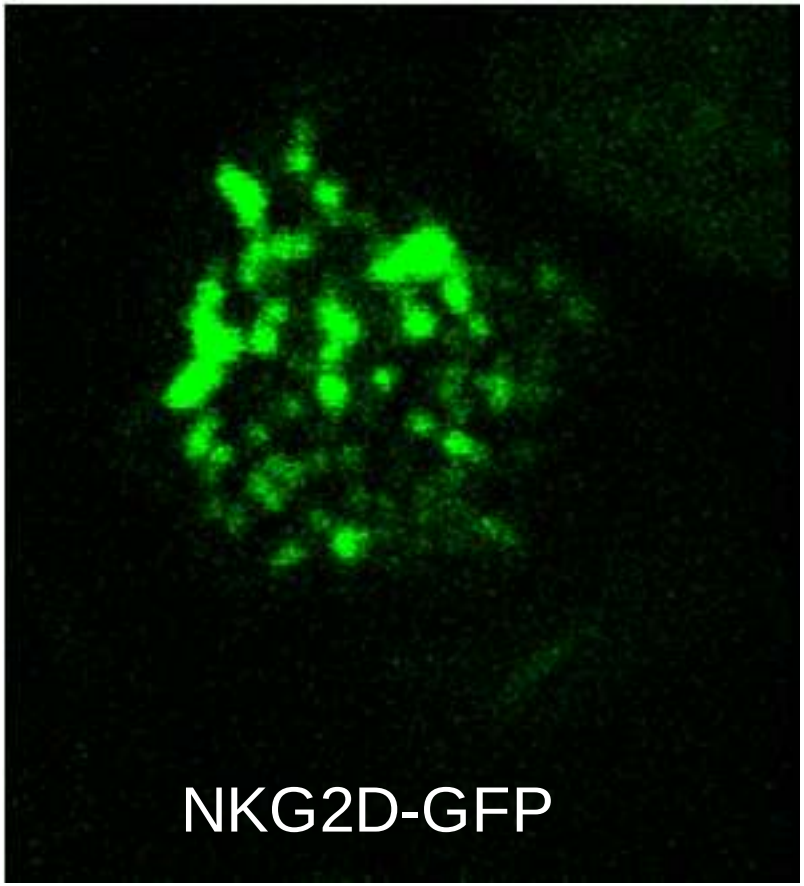
Purbhoo MA et al. Science Signal. 2010;121:ra36.

LAT and SLP-76 Dynamics



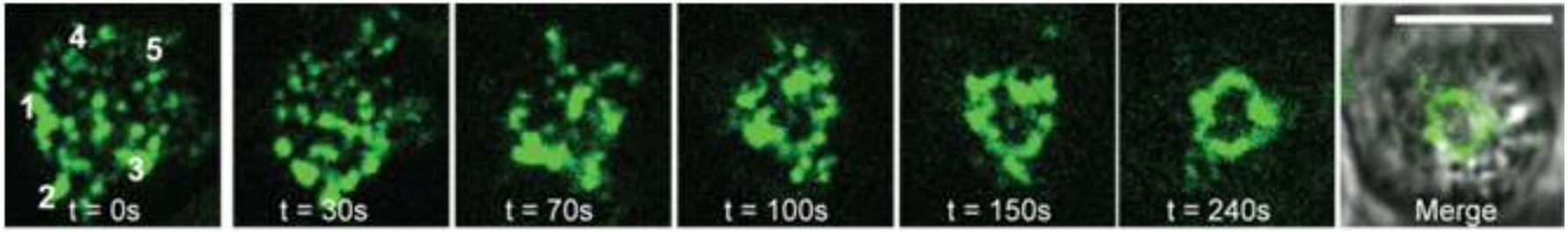
Optical Tweezers for high resolution imaging



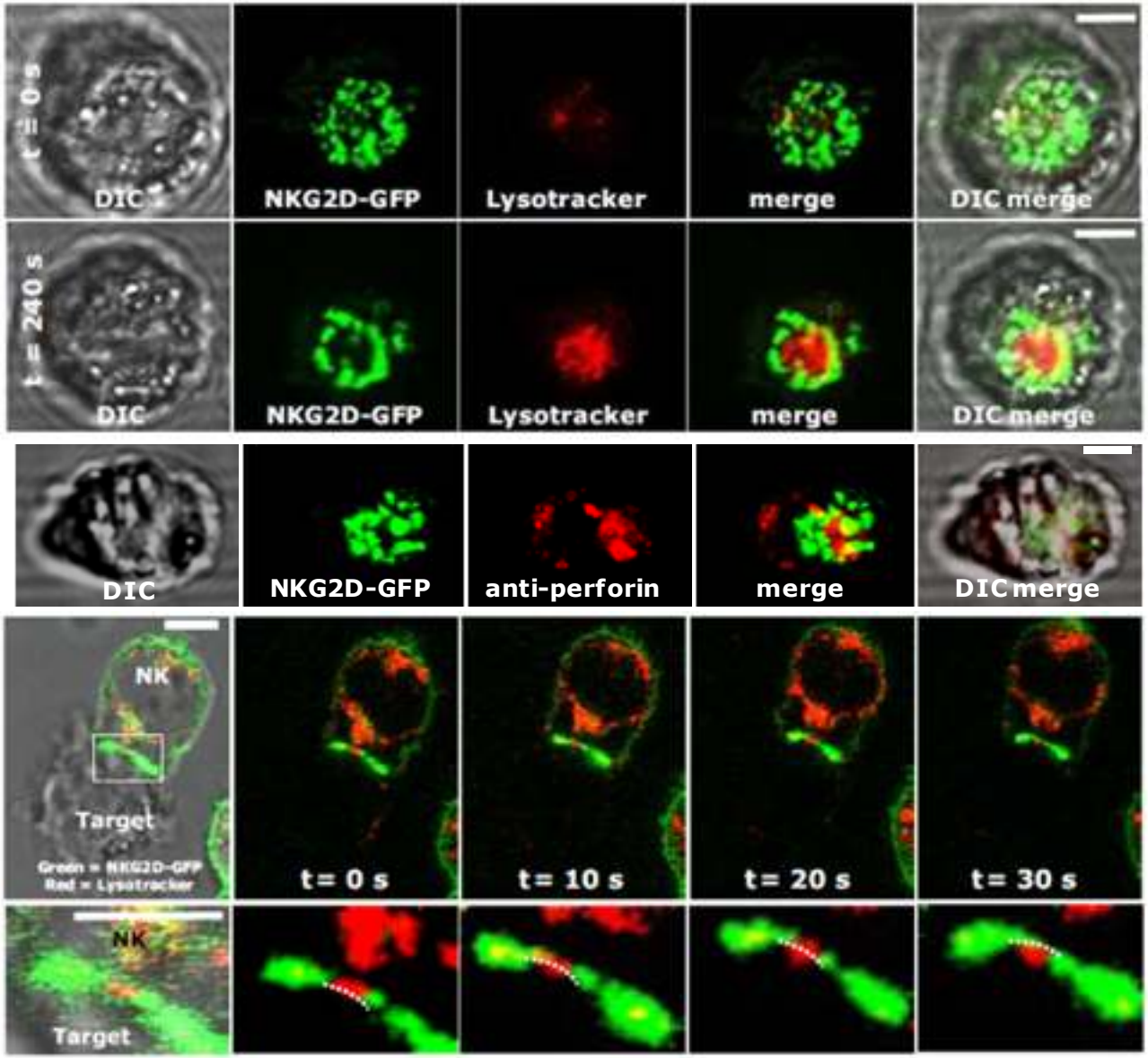


Resolution improved by x5

Speed increased by 10–100 fold

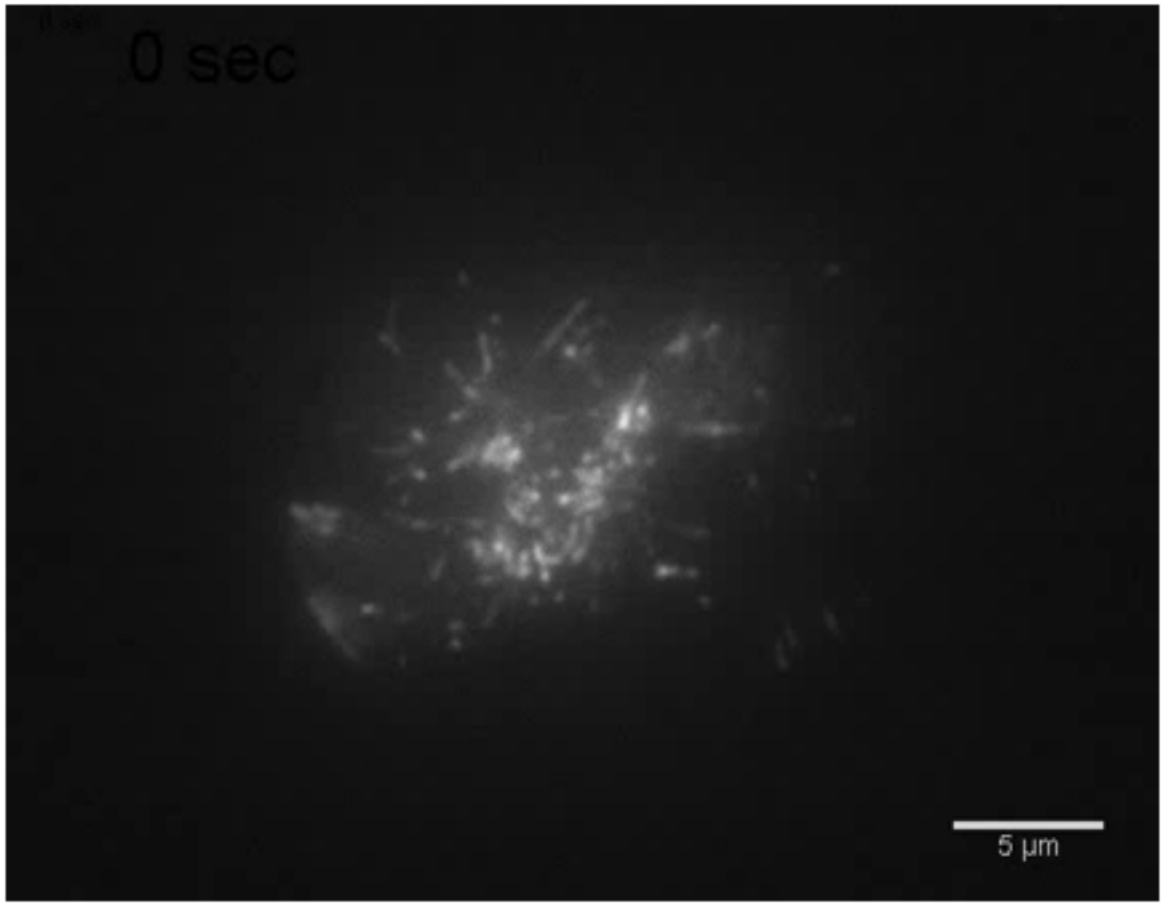


Microclusters at NK cell synapses assemble into rings through which secretion occurs



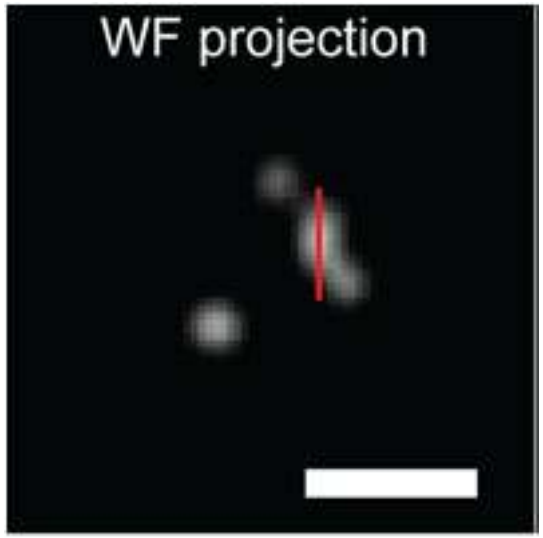
How do lytic granules pass through the actin mesh that underlies cell membranes?

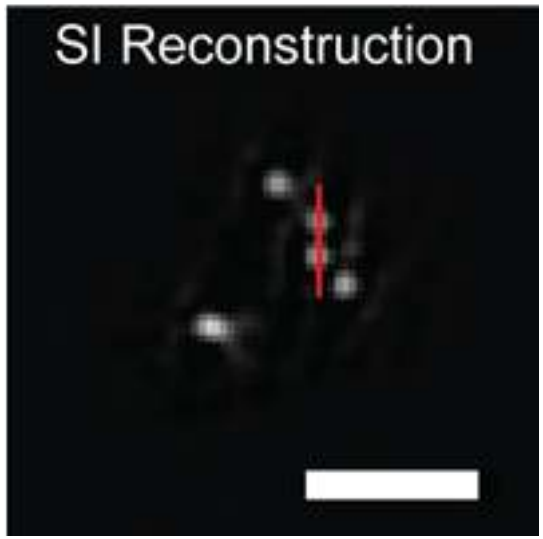
- Actin is cleared from the centre of the synapse

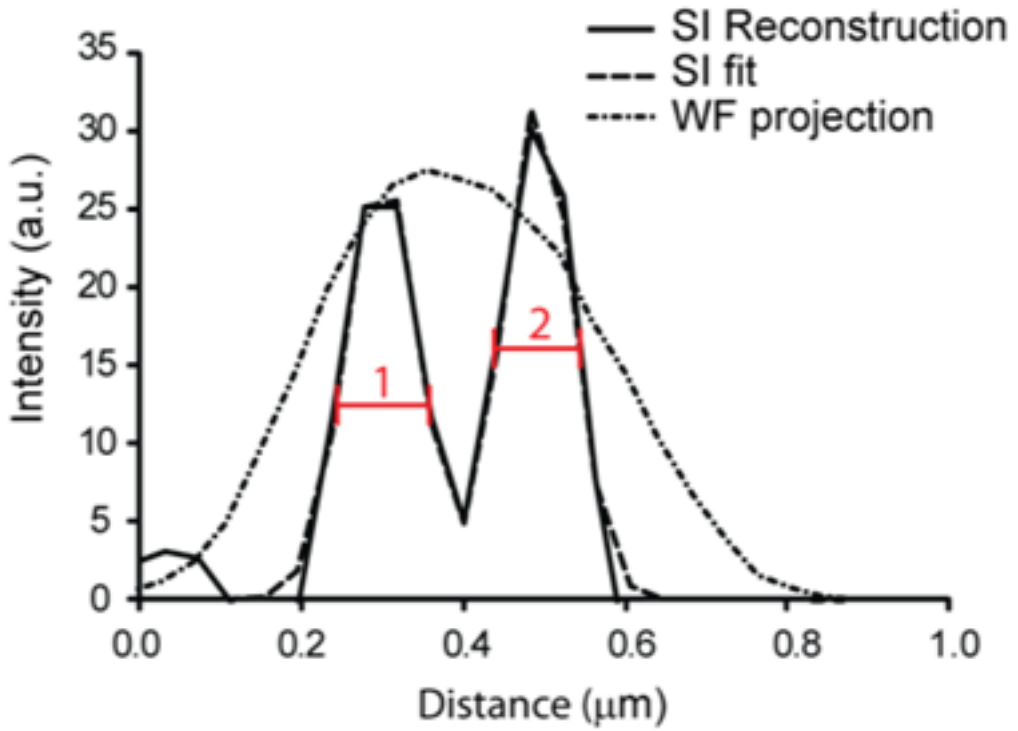


Structured Illumination Microscopy (OMX)

40nm Beads



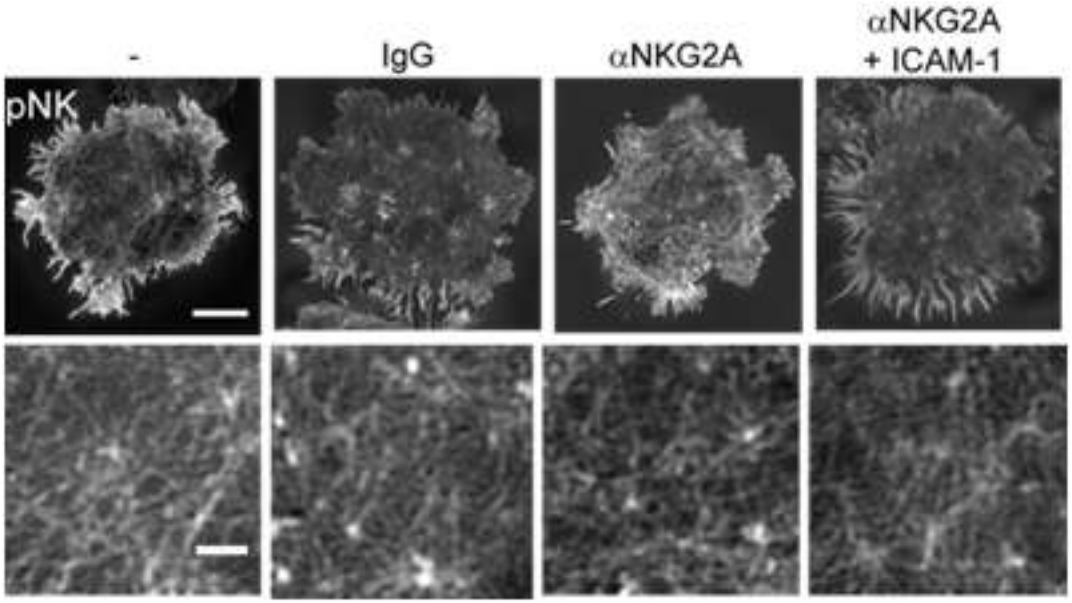




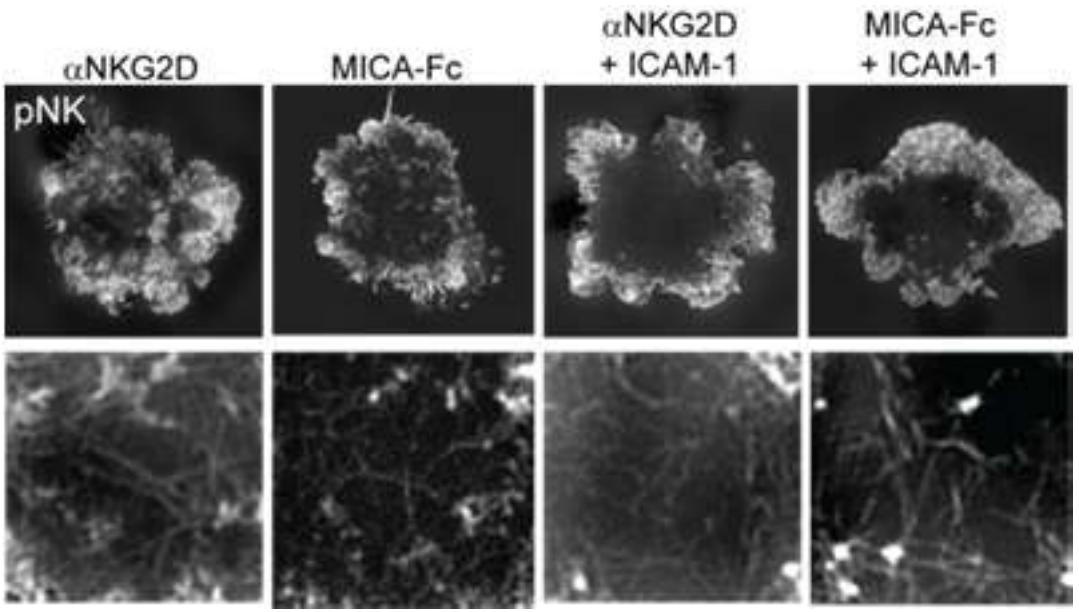
1. FWHM ~105nm
2. FWHM ~98nm

Actin Organisation at the Immune Synpase

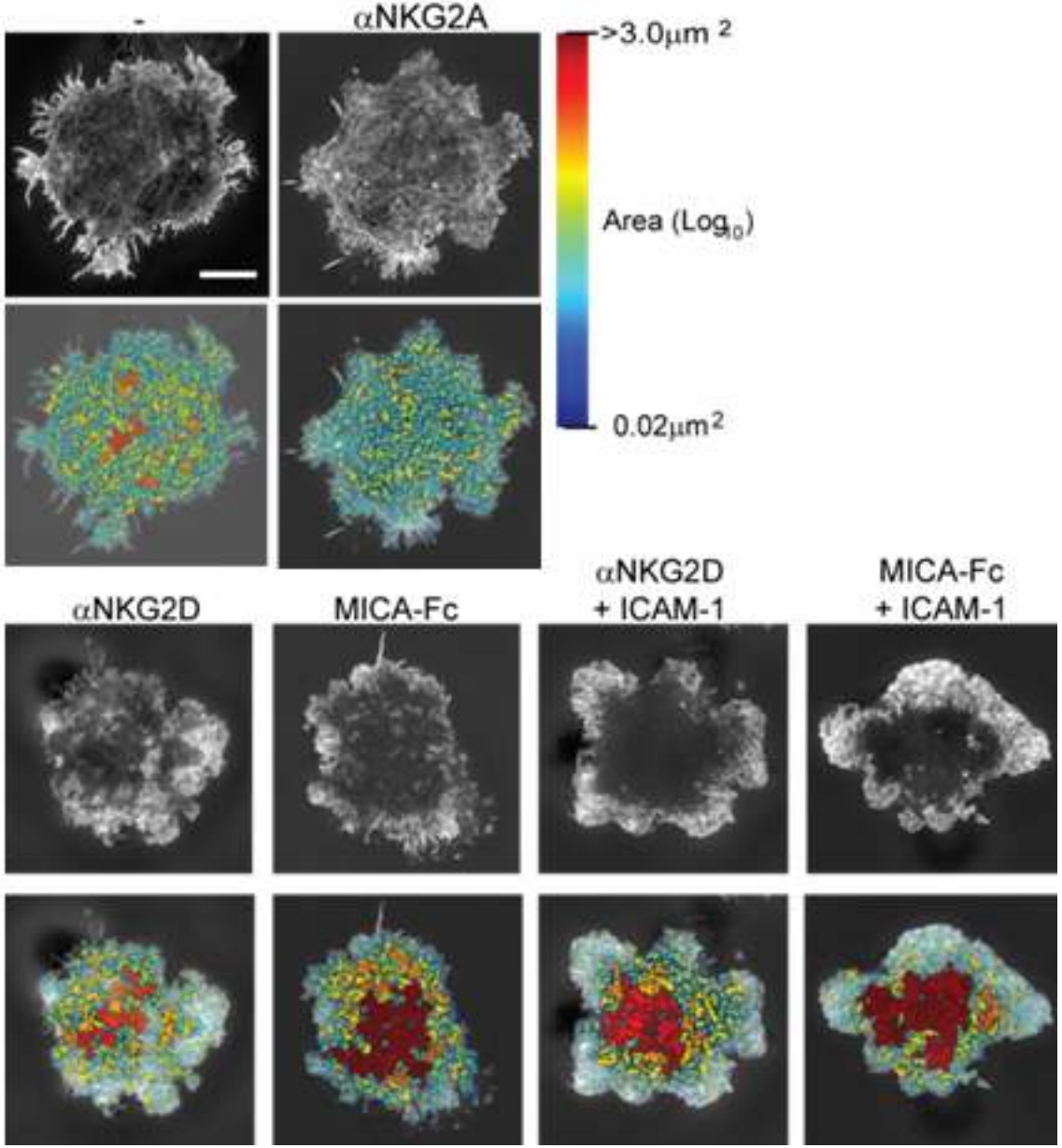
Inhibitory

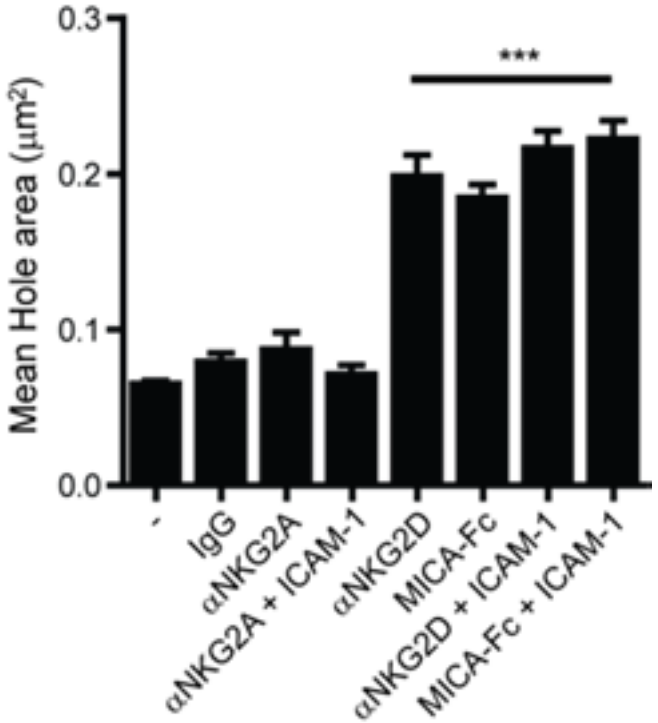


Activating



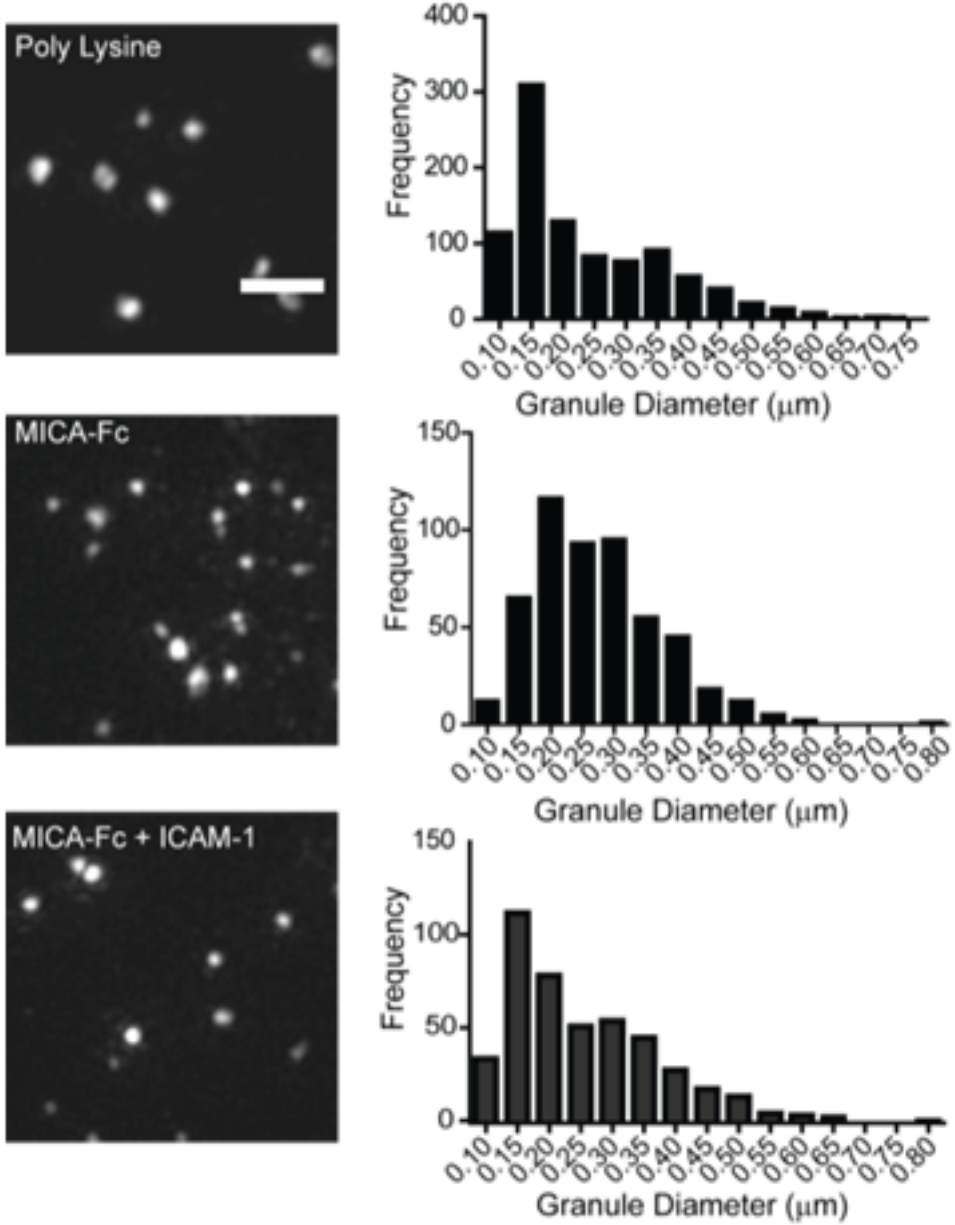
Organisation of Actin Mesh

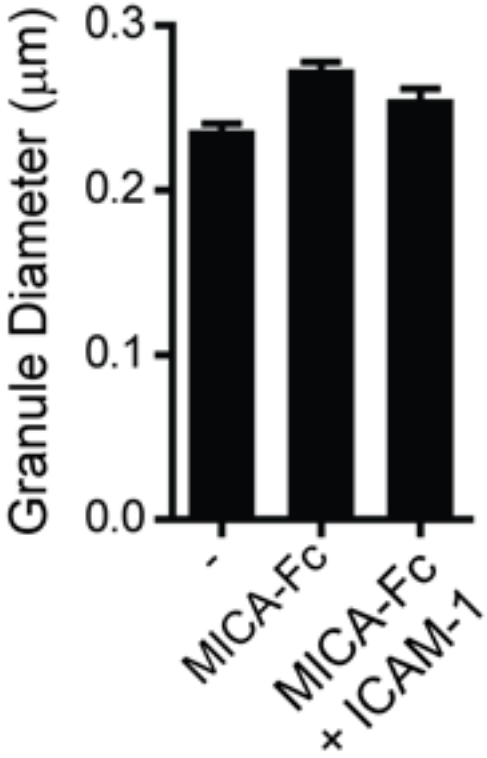




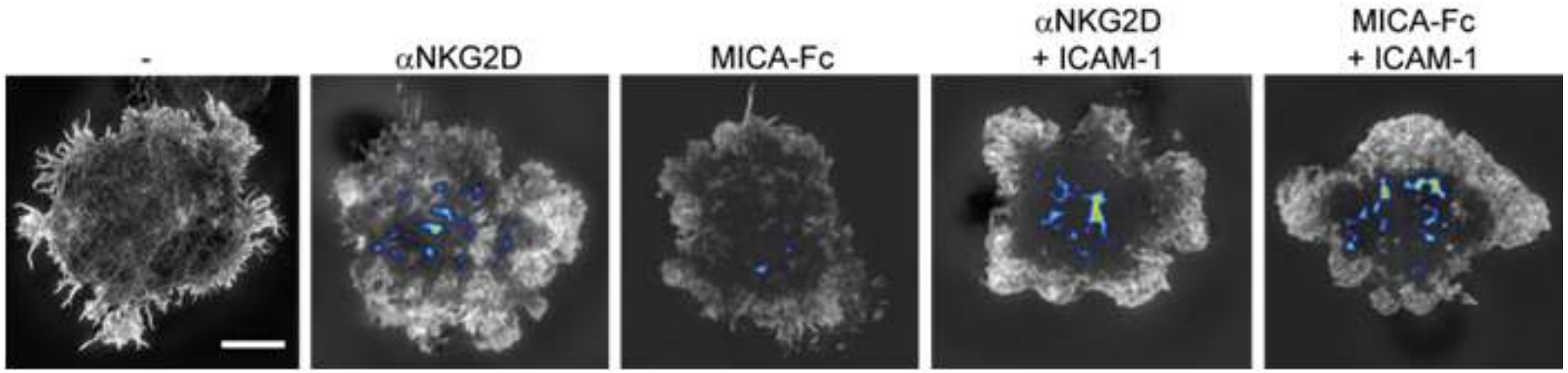
The mean hole length is increased to ~250nm

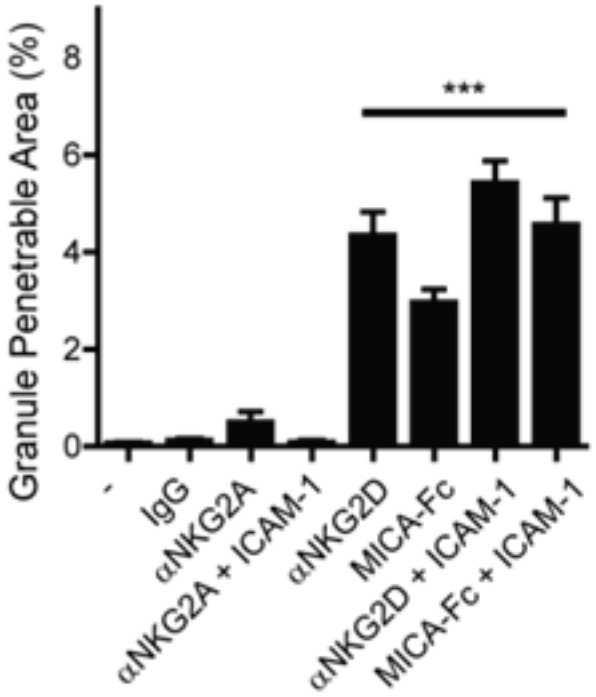
Determination of lytic granule size



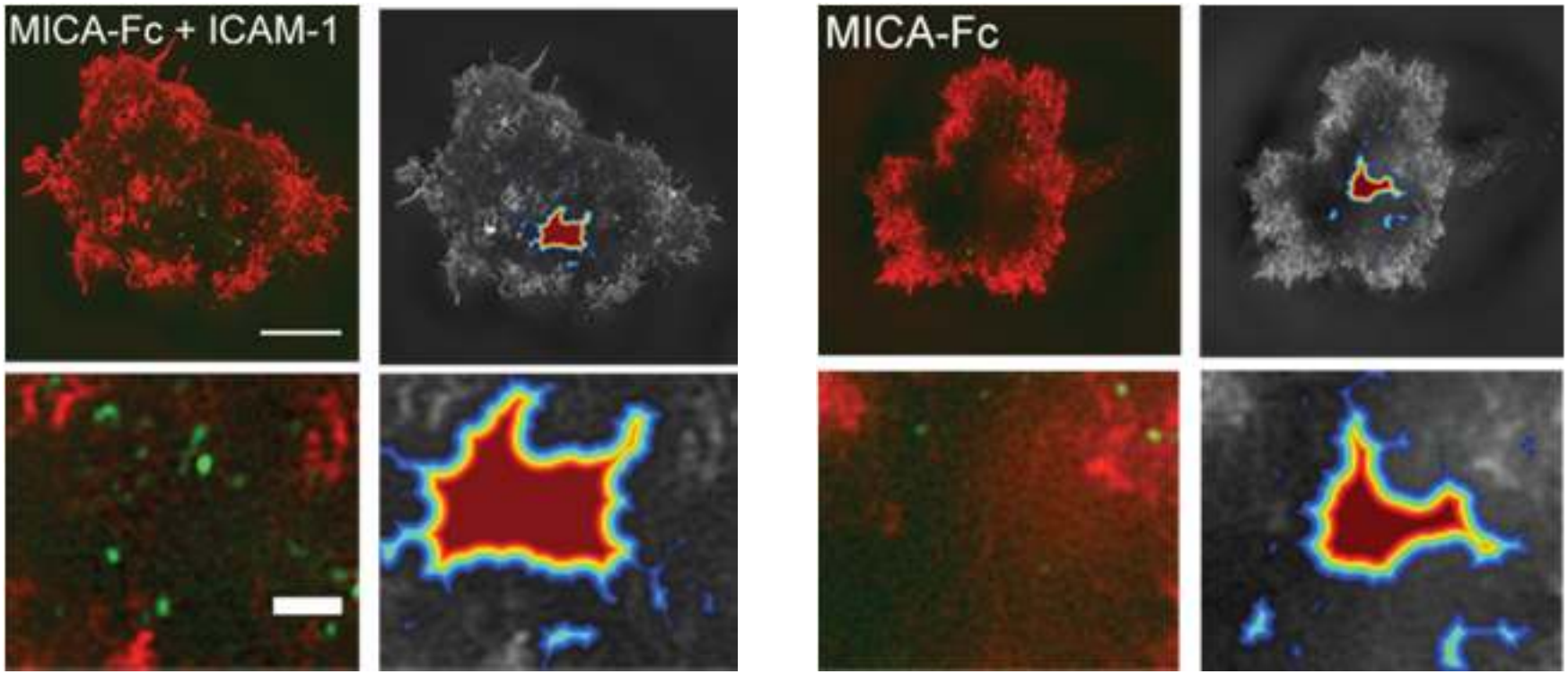


Granule penetrable areas



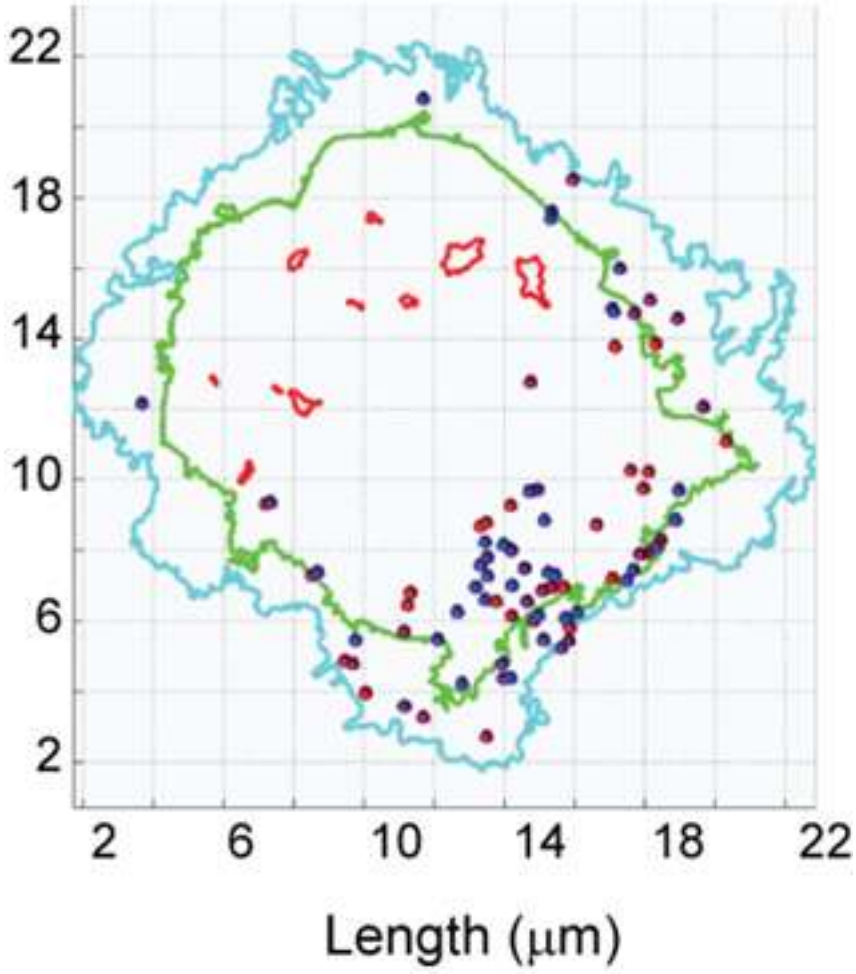


Two colour 3D super-resolution imaging to simultaneously image f-actin and perforrin

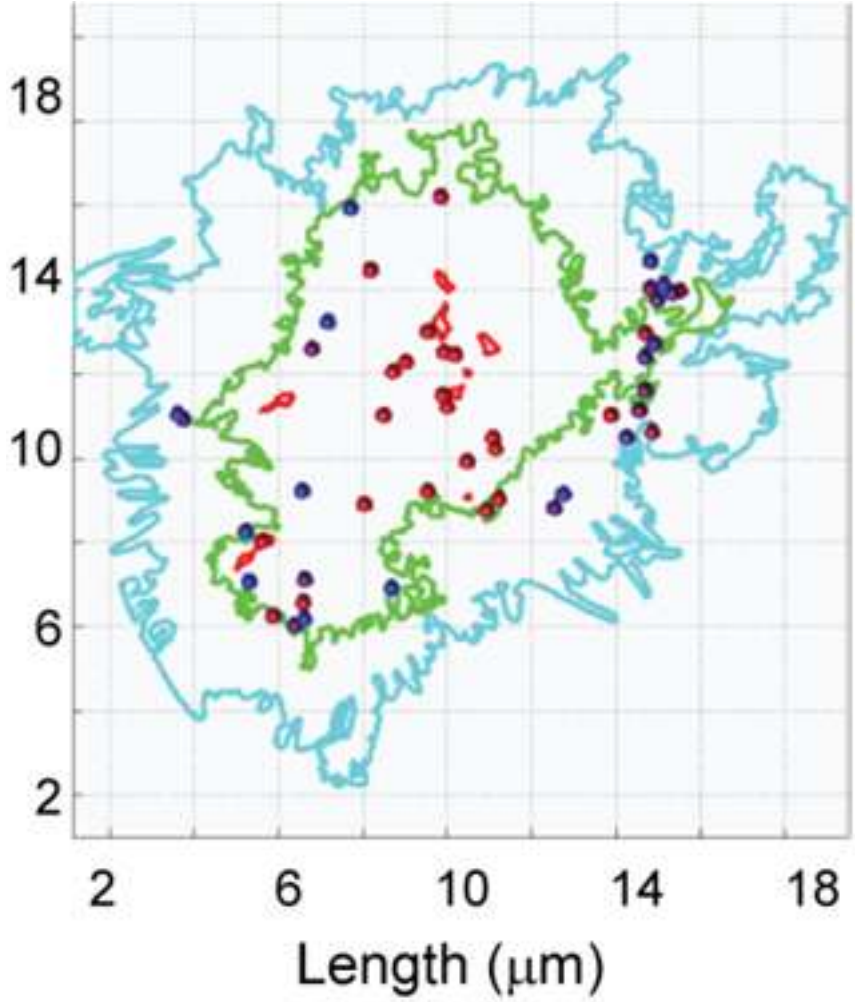


Granules polarize to predicted penetrable areas

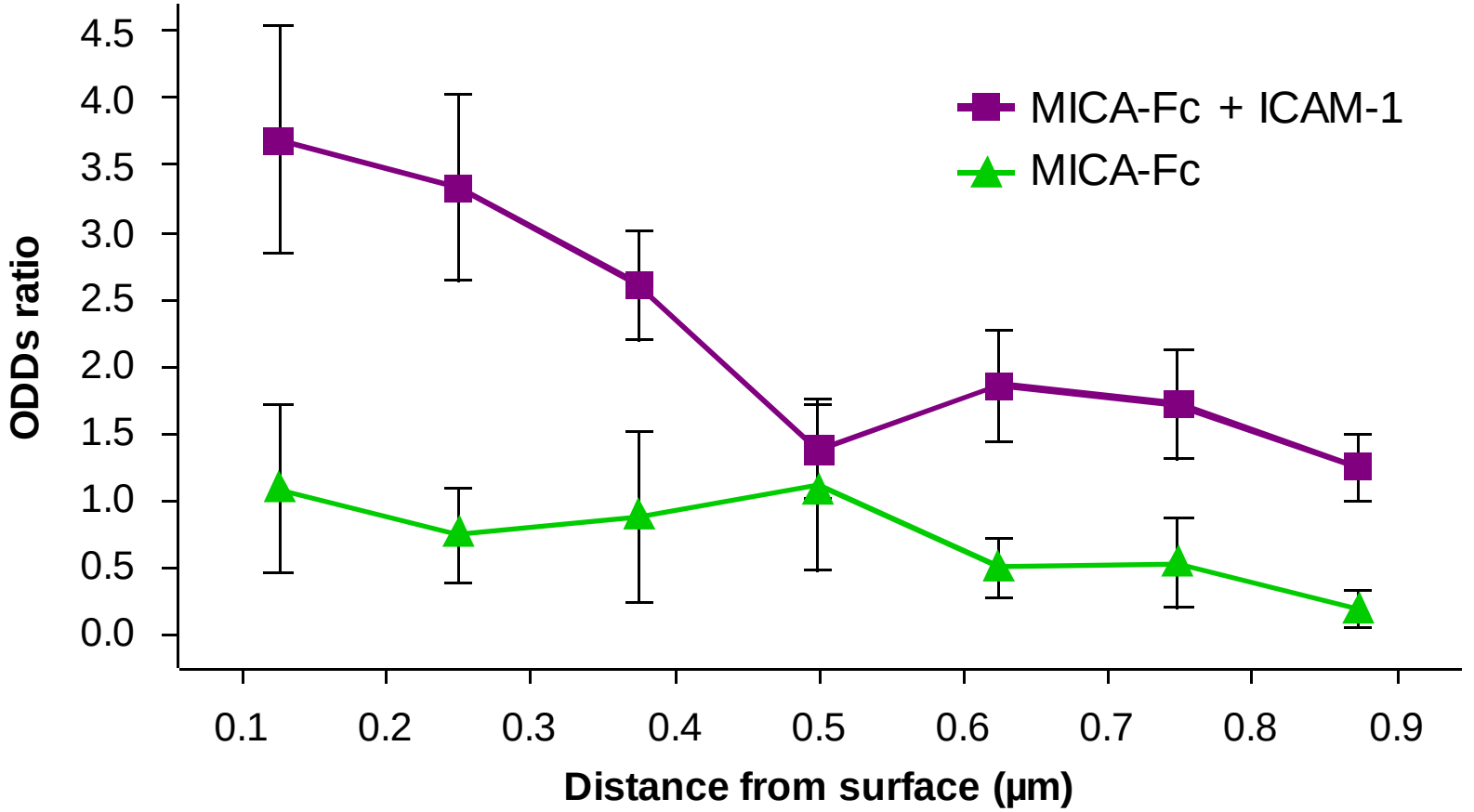
MICA-Fc



MICA-Fc + ICAM-1



Periodicity in actin structure defines secretory domains

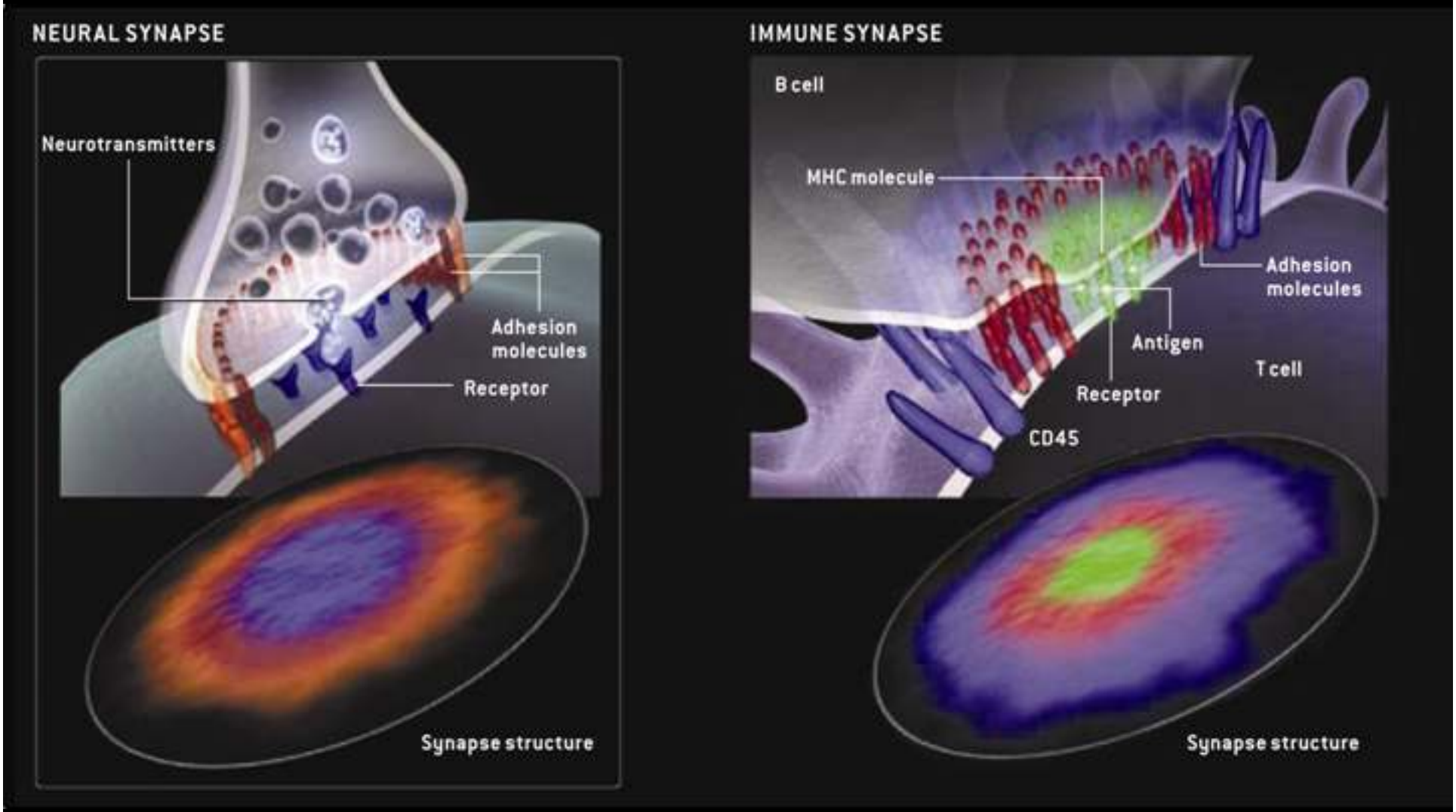


$$\text{Odds ratio} = \frac{\text{No. of granules falling on penetrative area / penetrative area}}{\text{No. of granules falling on non-penetrative area / non-penetrative area}}$$

Summary

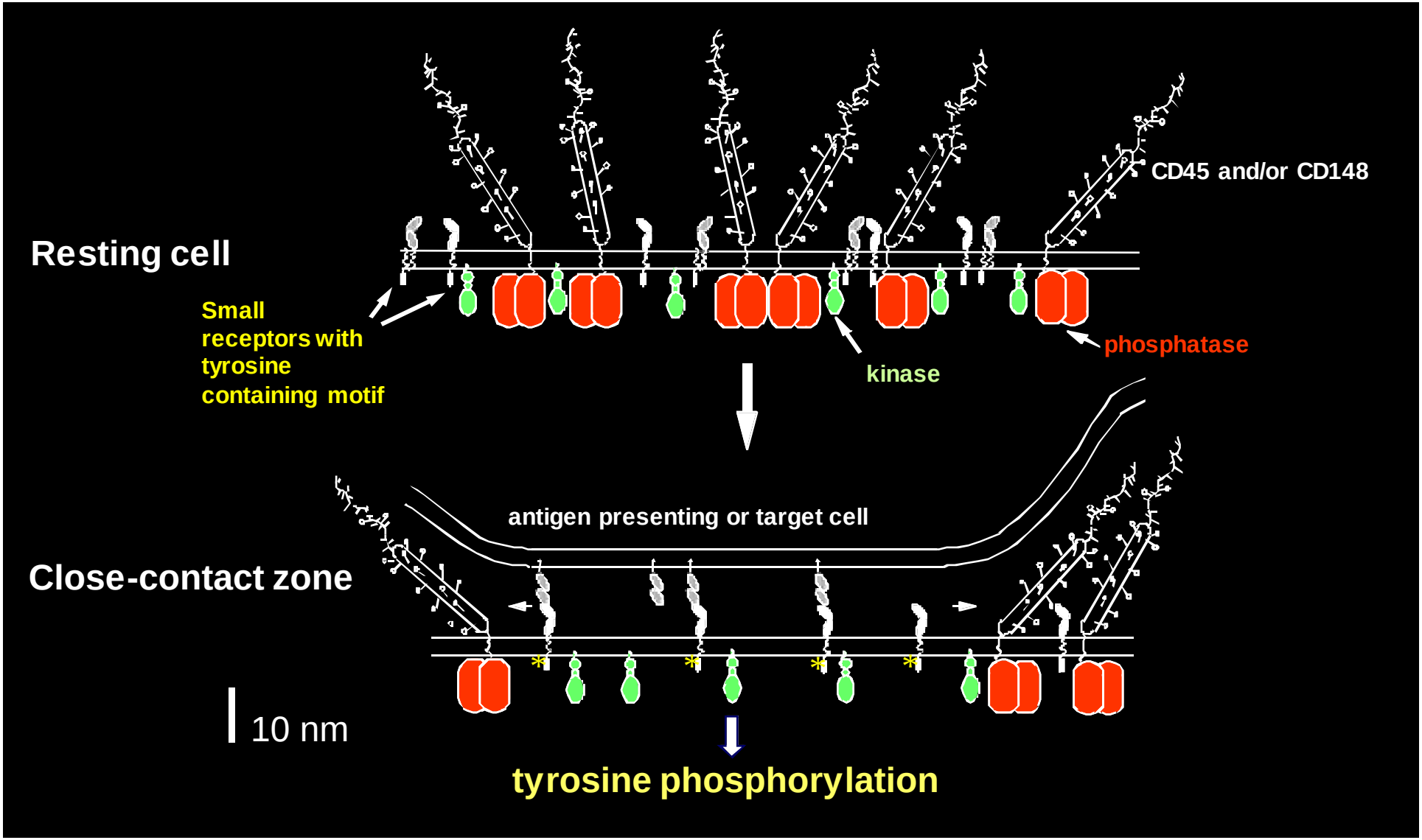
- Signalling microclusters of NKG2D organize around a central secretory domain
- Cortical actin is not cleared from the centre of the NK cell synapse
- Periodicity of cortical actin opens up in the central synapse, where lytic granules dock

The immune synapse



Davis DM, Scientific American, 2006;294:48-55.

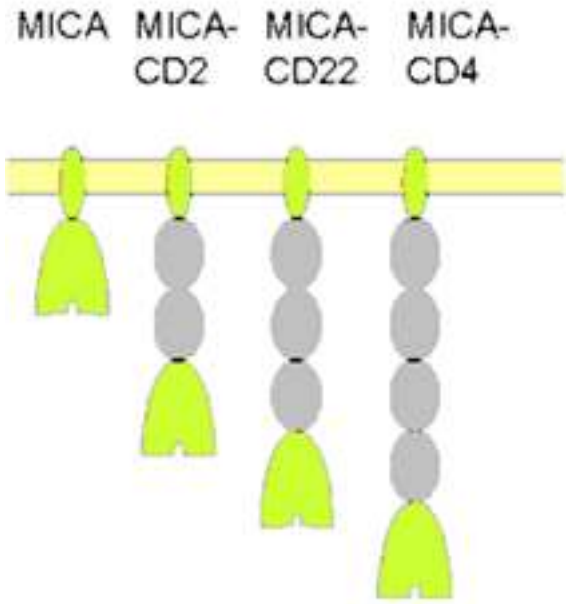
The kinetic-segregation model for receptor triggering



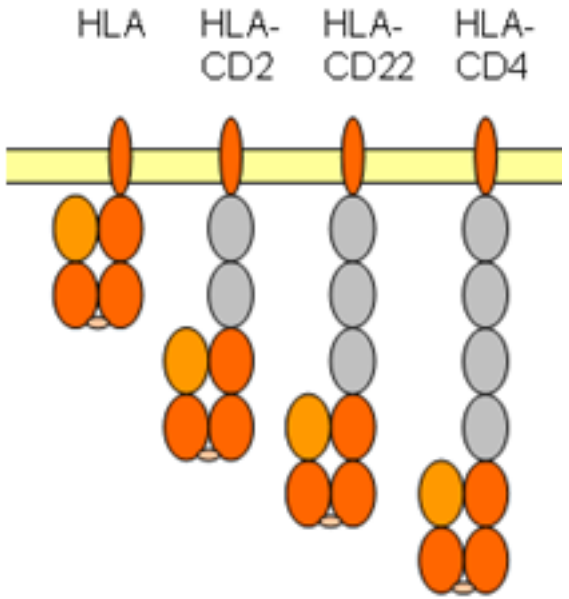
Tim Springer, expanded by Simon Davis and Anton van der Merwe.

Modifying the extracellular-domain size of NK ligands

MICA (activating ligand of NKG2D)



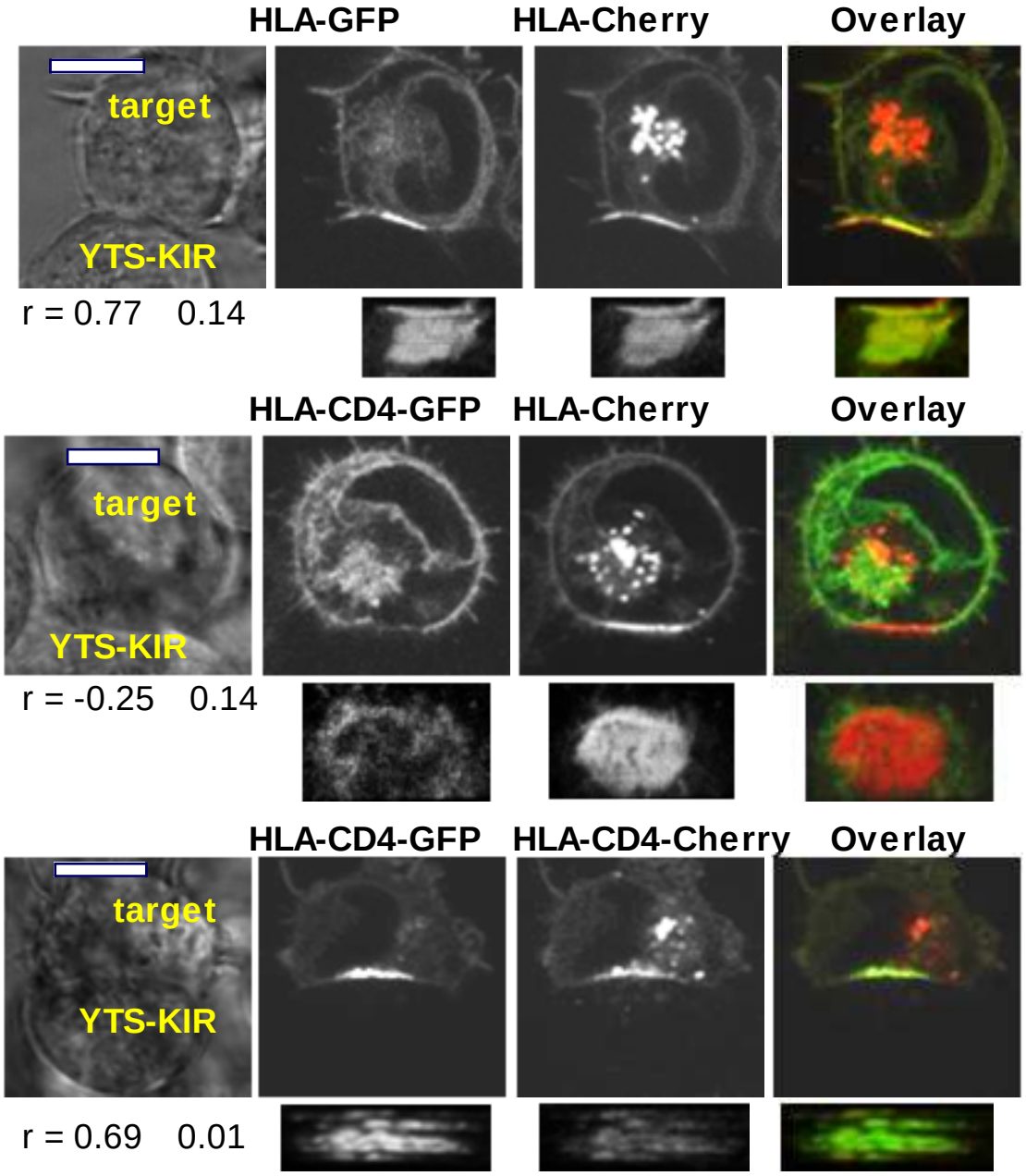
HLA-C (inhibiting ligand of KIR)

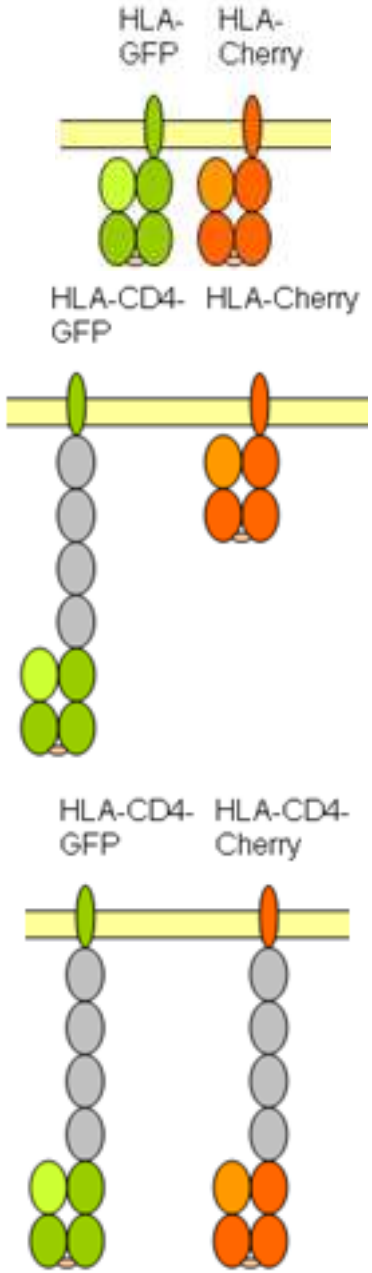


HLA-single-chain trimer construct (SCT):

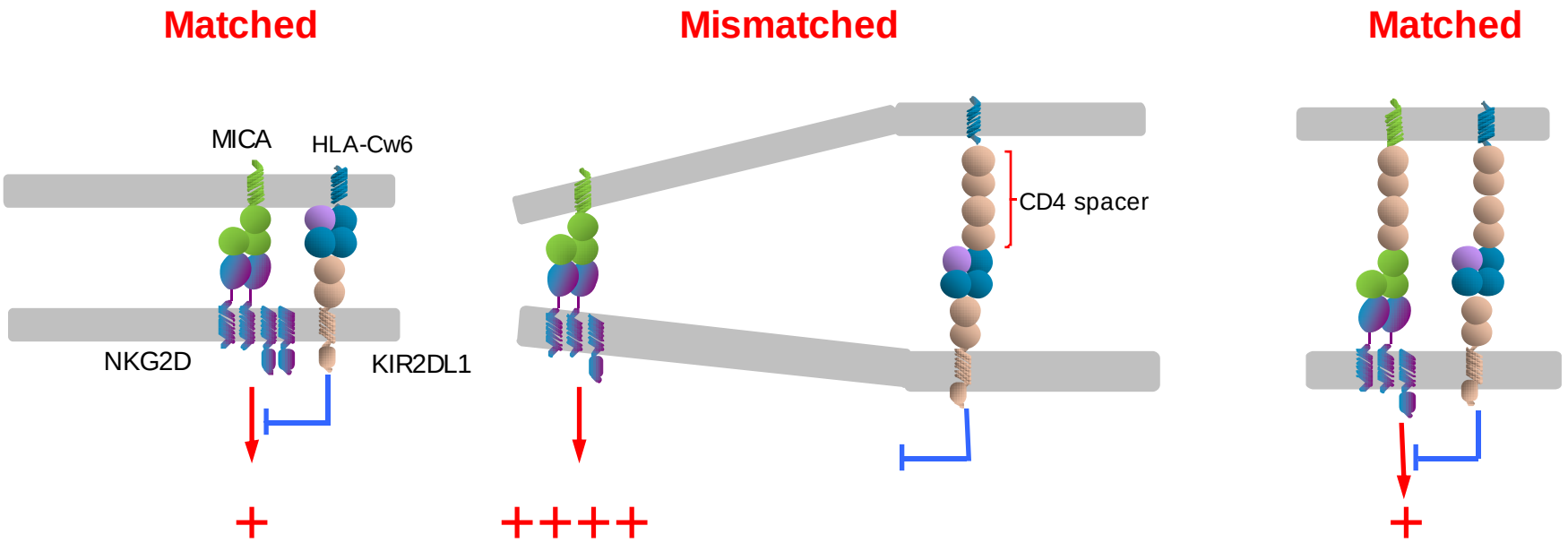
- HLA-Cw6
- Peptide
- β_2-microglobulin

Colocalization requires matched protein sizes





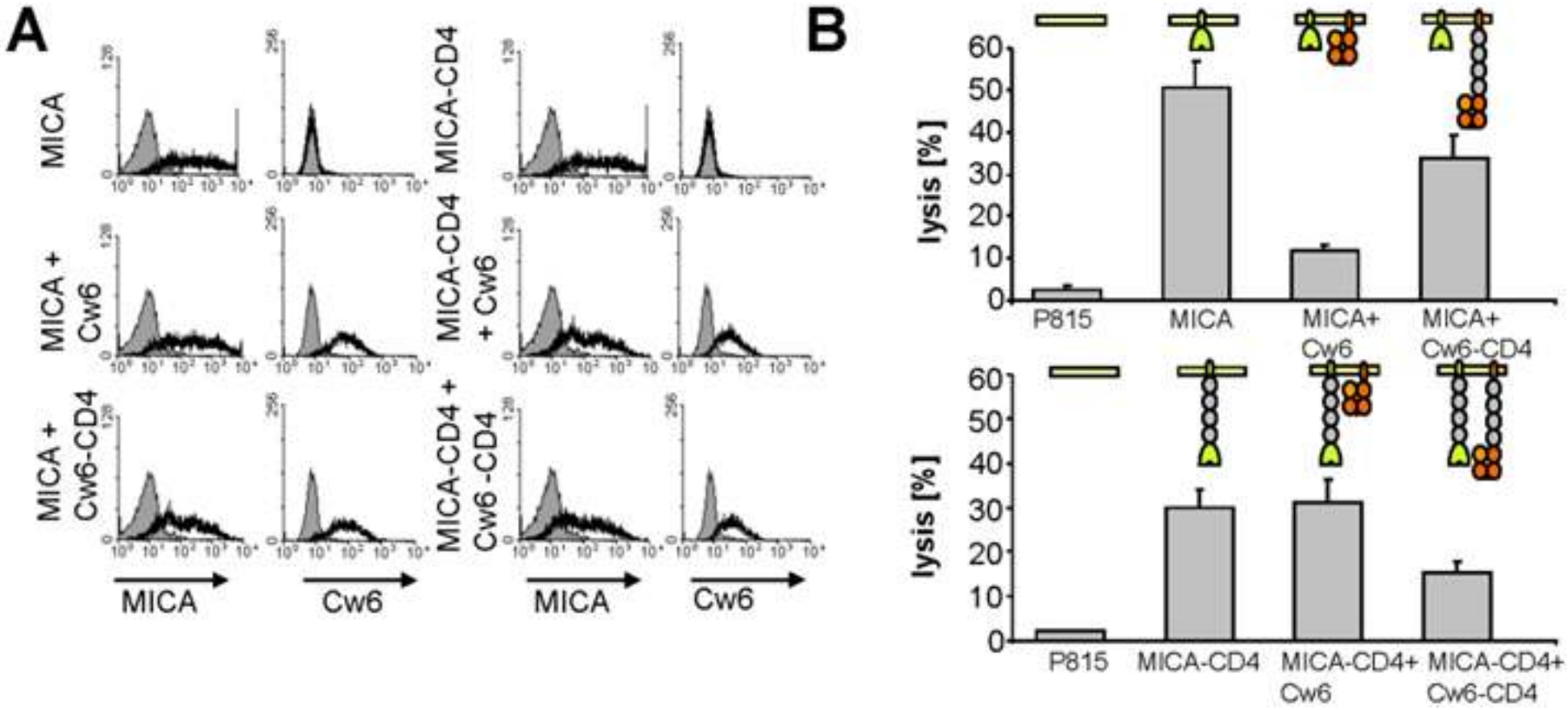
Importance of matched size



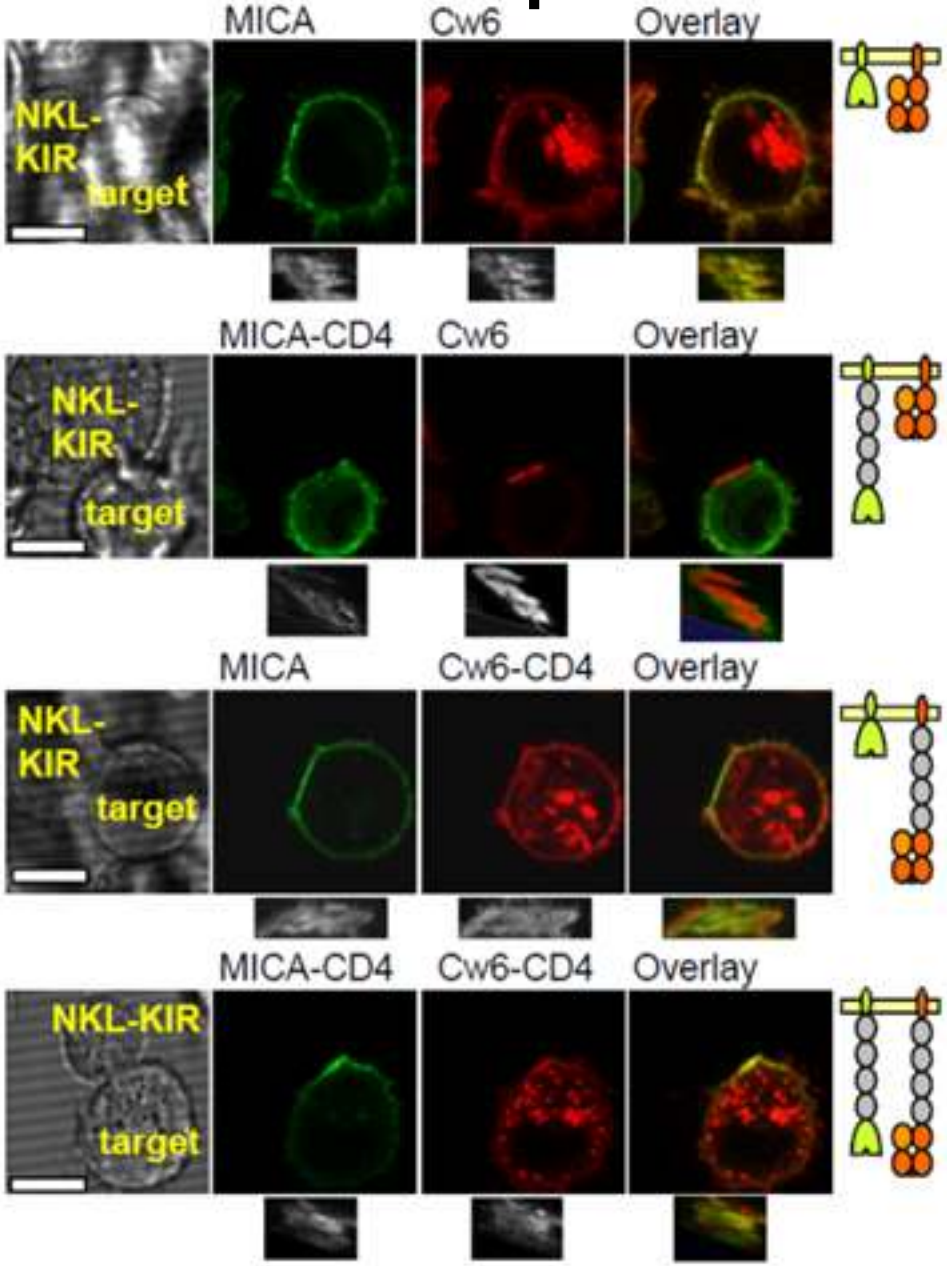
matched size integration → **colocalization** → **good signal**

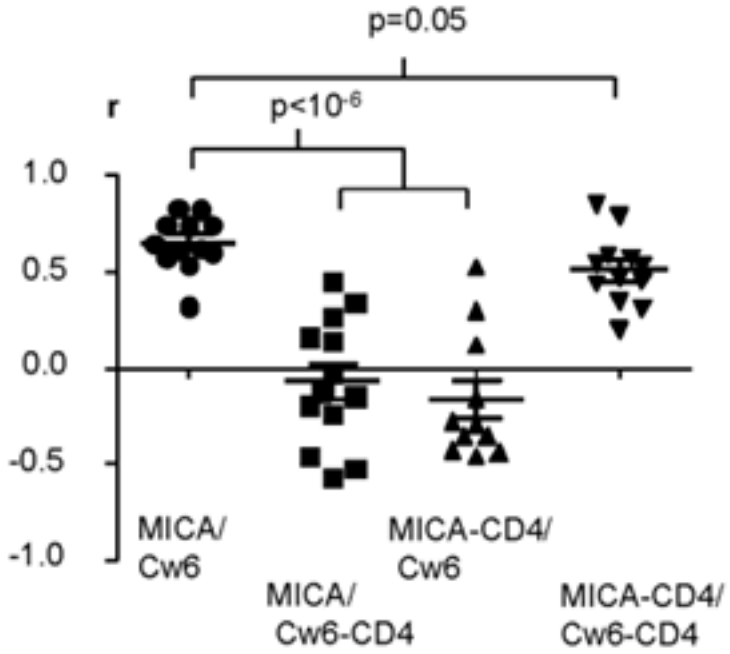
mismatched size → **poor colocalization** → **poor signal integration**

Optimal inhibition requires matched sizes



Colocalization of MICA and HLA-Cw6 requires matching size





Conclusions

- Elongation of NK cell ligands abrogates their ability to trigger receptor-signaling
- Synaptic location of proteins can be determined by their size
- Optimal signal integration of inhibitory and activatory receptors requires matching receptor/ligand dimensions

Summary

- The cortical actin mesh opens up for lytic granule secretion
- Activating and inhibitory ligand sizes are critical for NK synapse organisation and signal integration
- Drugs, e.g. lenalidomide, could act by influencing the supramolecular dynamics at synapses

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Sophie Pageon
Dominika Rudnicka
Alex Savell

Funding: MRC, BBSRC,
Lister Institute, Royal Society

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whose work I presented:

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Karsten Köhler

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P. Anton van der Merwe, Shiqiu Xiong (Oxford)
Nigel J. Burroughs, (Warwick)
Ilan Davis, Ian Dobbie (Oxford)
Mark Howarth (Oxford)

Paul French, Mark Neil, Chris Dunsby (Imperial)
Eleanor Riley (LSHTM)
Quentin Sattentau, Clare Joly (Oxford)
Jeremy Griggs (GSK)
Eric Vivier (Marseille)
Petter Hoglund (Karolinska)
Matt Sleeman (MedImmune)
Salim Khakoo (St Mary's)
Chris Rudd, Hebin Liu (Cambridge)
Colin Hopkins, Otto Berninghausen (Imperial)
Martin Spitaler (Imaging facility)

The Continuum of Care for the Multiple Myeloma Patient

Wednesday 4 May 2011
10:30–12:30
Paris, France

13th IMW 2011 PARIS
www.myeloma-paris2011.com

A Celgene-sponsored satellite symposium
at the 13th International Myeloma Workshop

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