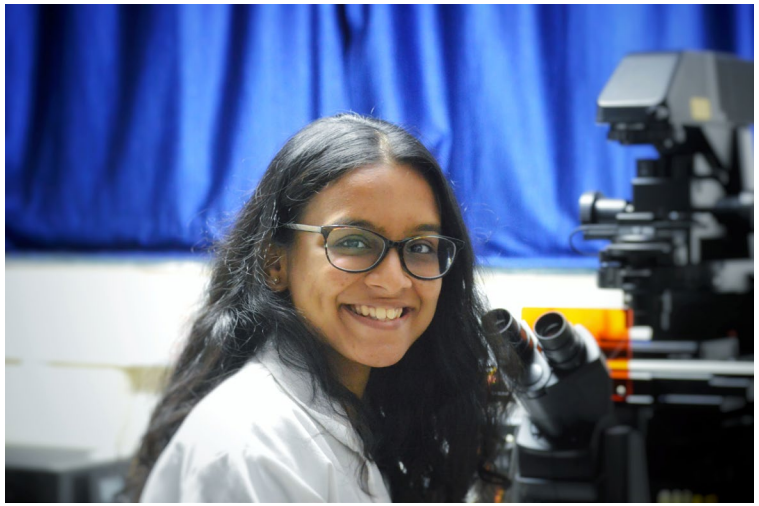
Where are the Particles?



Siddharth Jhunjhunwala

Bioengineering, Indian Institute of Science, Bangalore

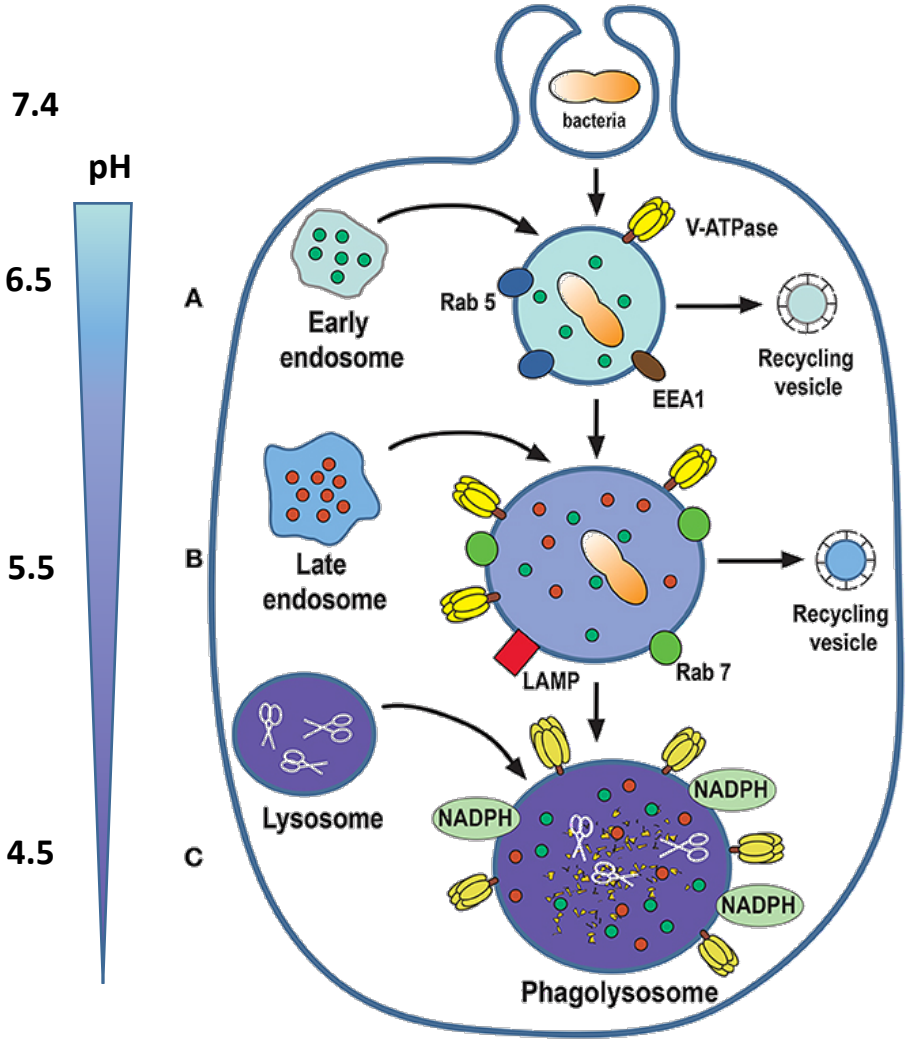
CRS 2023

Show of Hands

- Polymeric/metallic particle, phagocytosed by macrophage. Where do you expect the particle to be 8-24 hr. post phagocytosis?

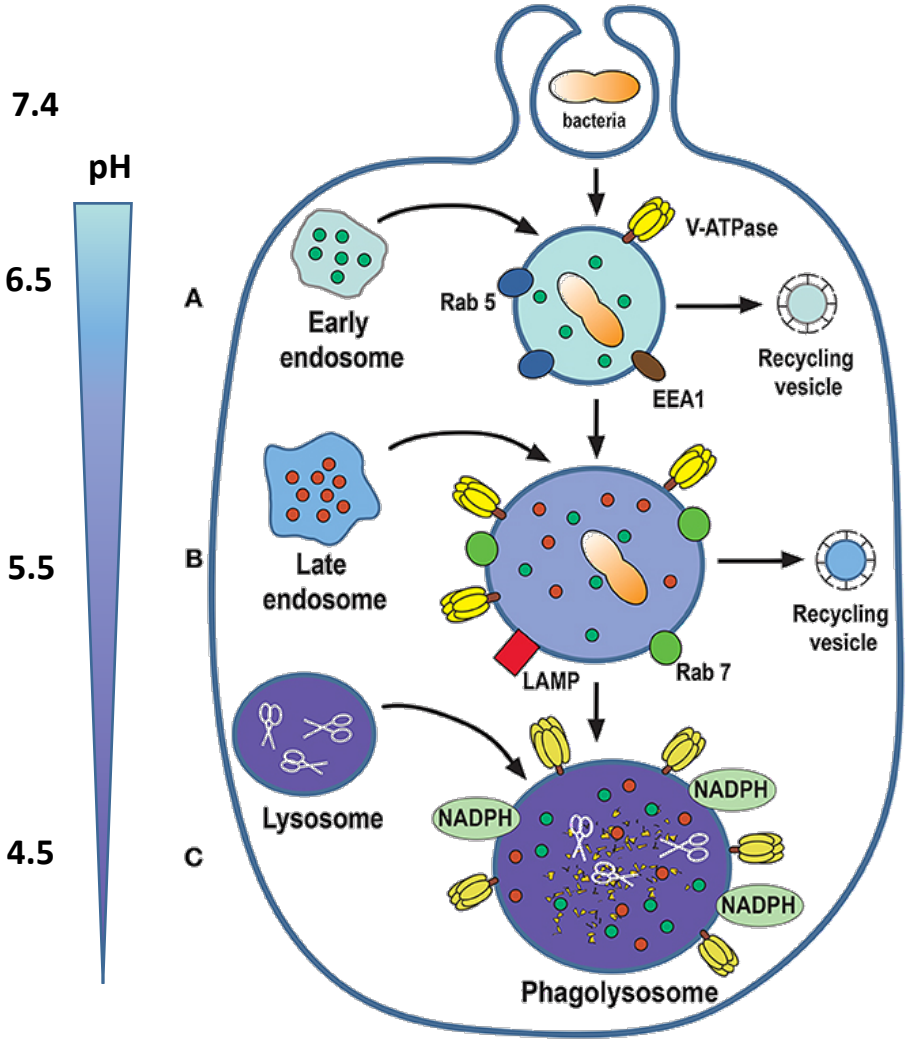
1. Lysosome
2. Endosomes (early)
3. Cytoplasm
4. Other cellular compartments

The Intracellular Journey of a Phagocytosed "Substance"



Adapted from Uribe-Querol and Rosales, 2020. *Front. Immunol.*

The Intracellular Journey of a Phagocytosed "Substance"



Adapted from Uribe-Querol and Rosales, 2020. *Front. Immunol.*

While the ability of phagosomes to acidify has been appreciated for more than a century, since Metchnikoff made his pioneering observations, the underlying determinants and its biological significance remain incompletely understood.

Westman and Grinstein, 2021
Front. Cell. Dev. Biol.

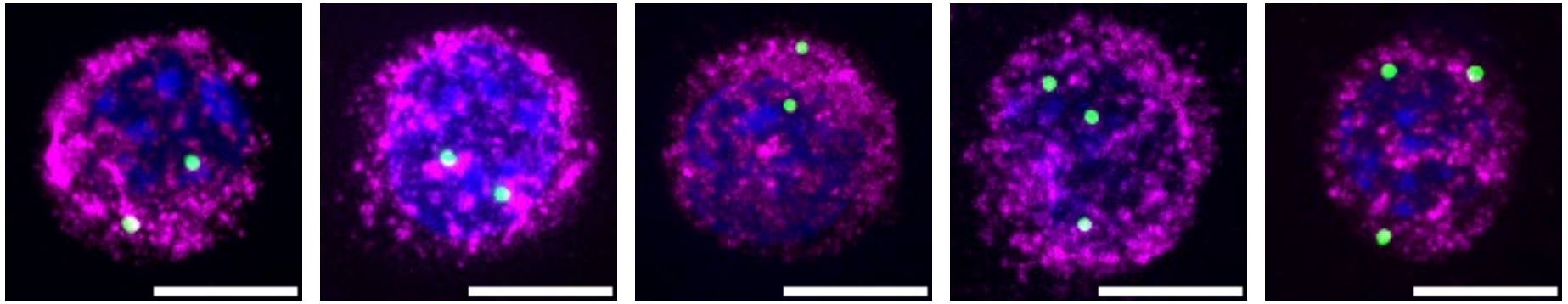
The mechanisms of phagosome – lysosome fusion and acidification of phagosomes remains poorly understood

Do “hard” particles always end up in the lysosome?

LAMP1

500 nm particles

Hoechst



2h 4h 6h 8h 18h

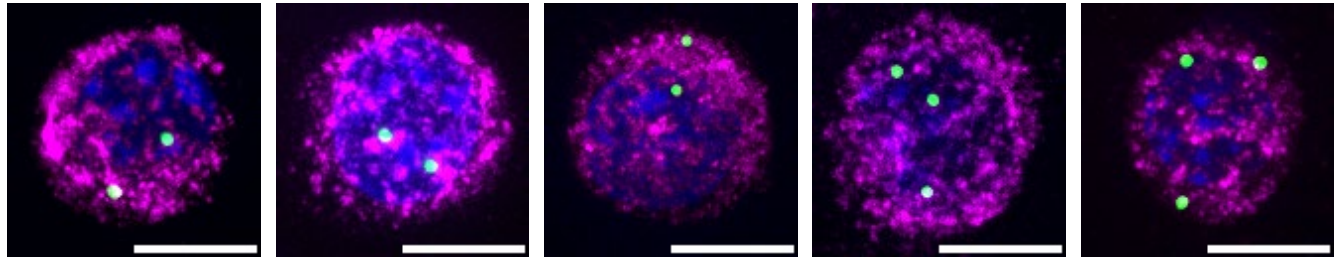
Scale bar: 10 µm

do “hard” particles always end up in the lysosome?

LAMP1

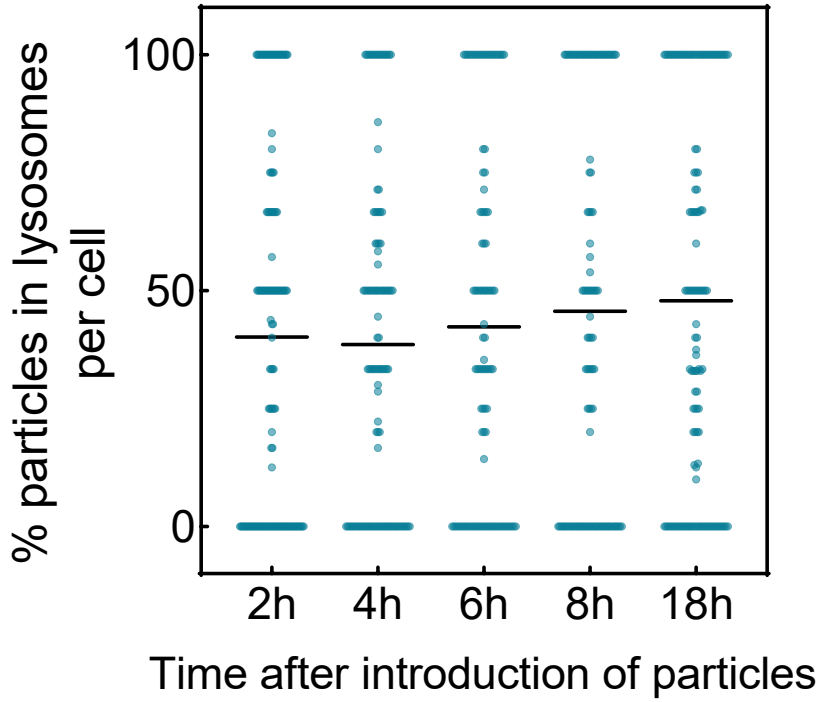
500 nm particles

Hoechst



Scale bar: 10 µm

2h 4h 6h 8h 18h



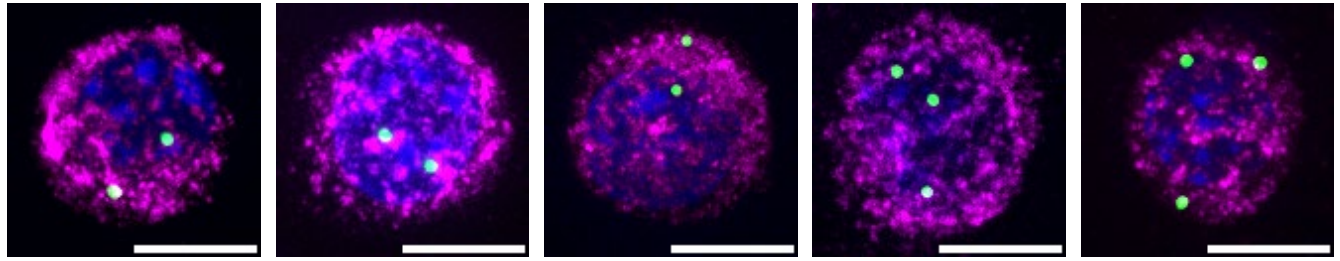
N ≥ 3
n ≥ 42 cells/N

do “hard” particles always end up in the lysosome?

LAMP1

500nm particles

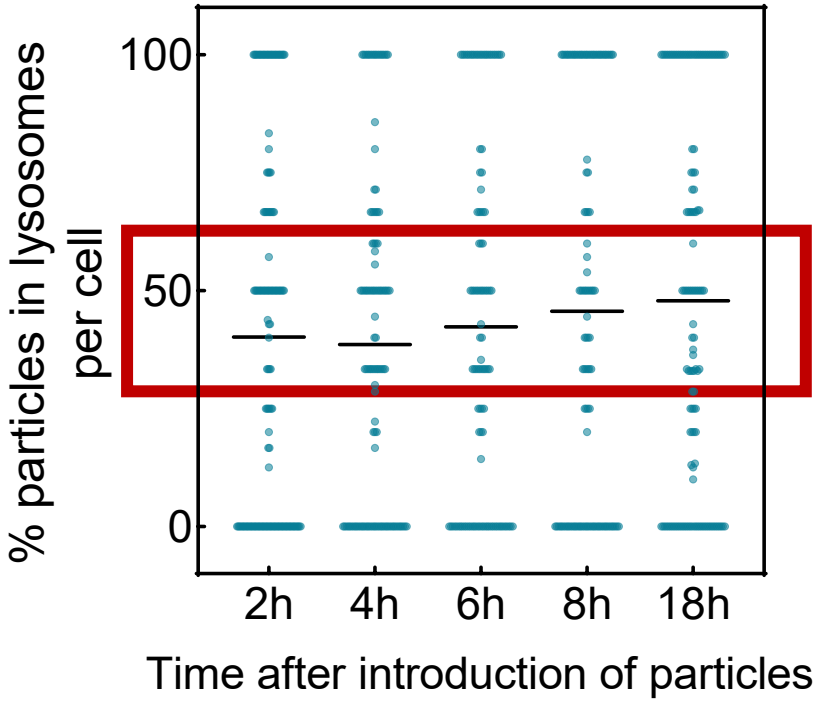
Hoechst



Scale bar: 10 µm

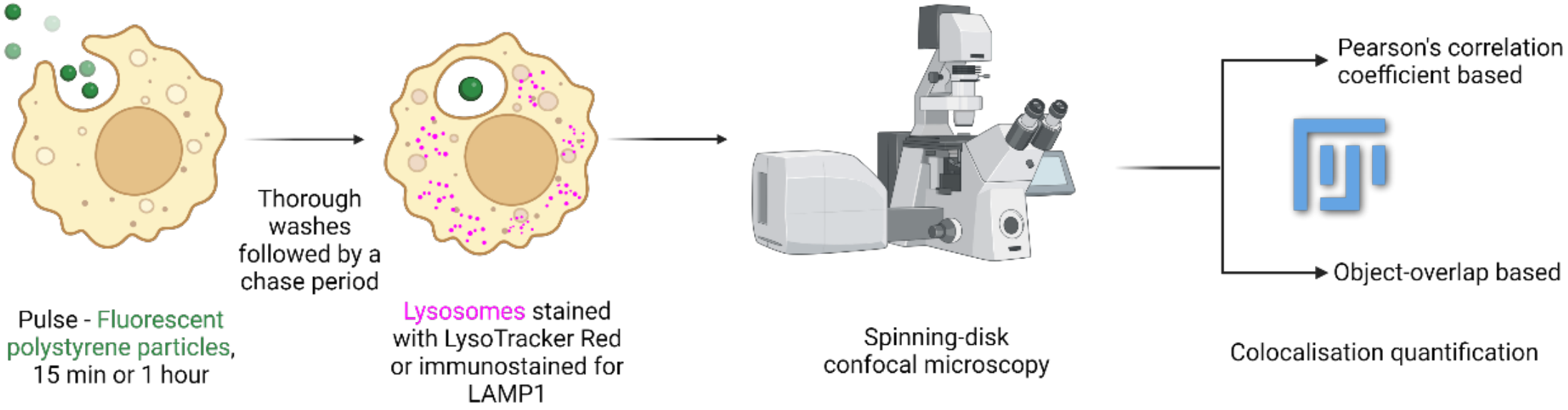
2h 4h 6h 8h 18h

Observation: < 50% particles found in LAMP1-associated compartments



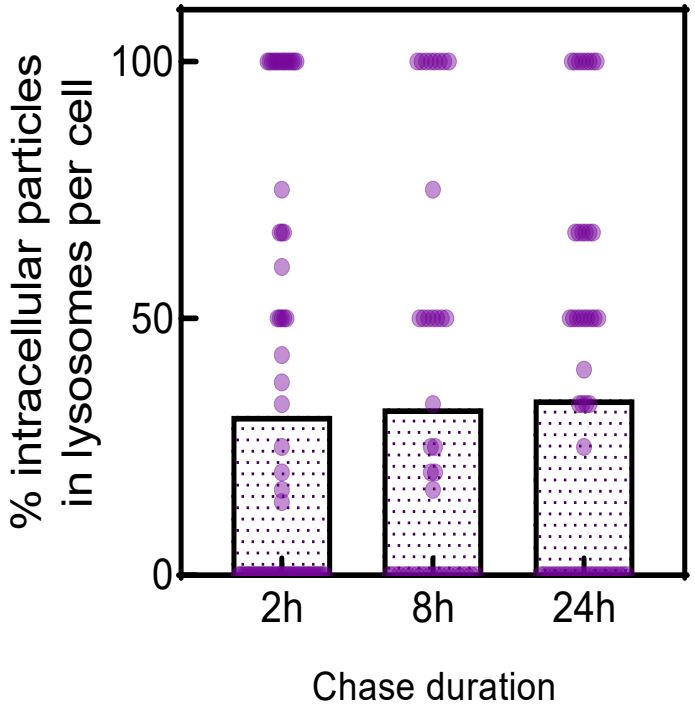
N ≥ 3
n ≥ 42 cells/N

The Method – imaging

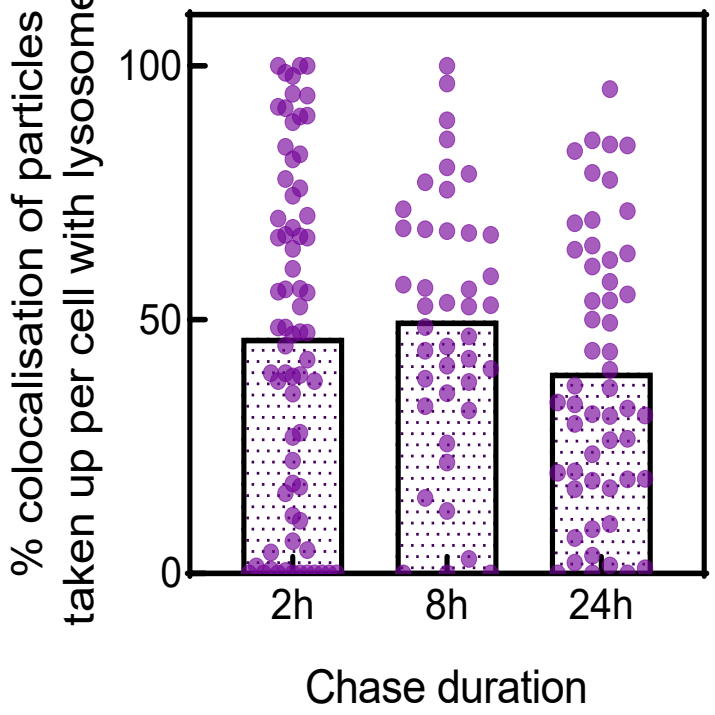


The Method – imaging

Pearson's Correlation Co-efficient based method



Object-overlap based method



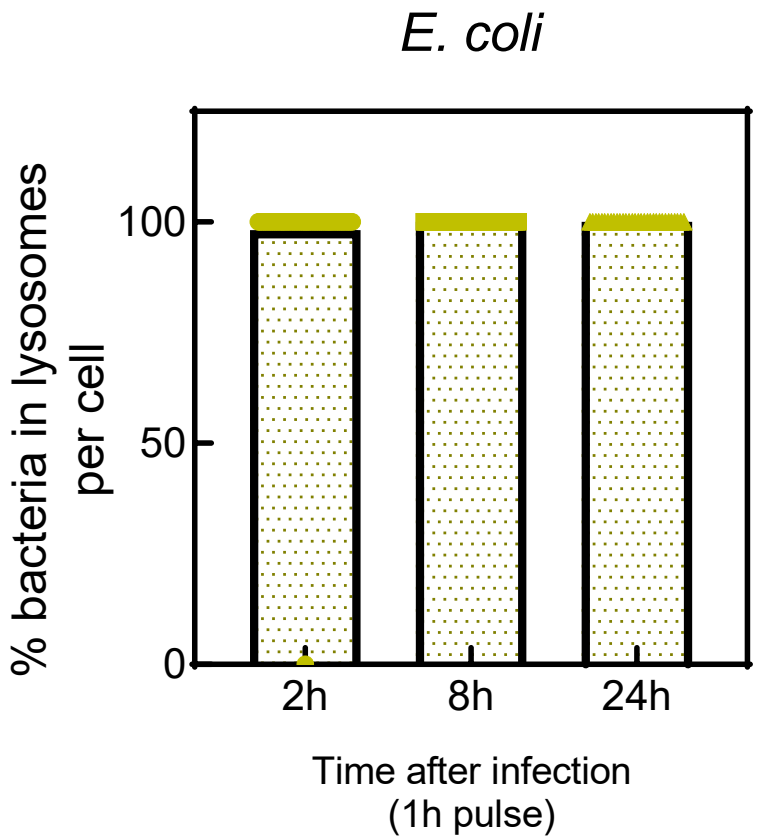
N ≥ 3
n ≥ 14 cells/N

That Can’t Be True

That Can't Be True

LysoTracker Red
GFP-expressing
E. coli

N = 3
n ≥ 14 cells/N



All bacteria reach lysosomes, but the signals for phagosome maturation remain to be understood

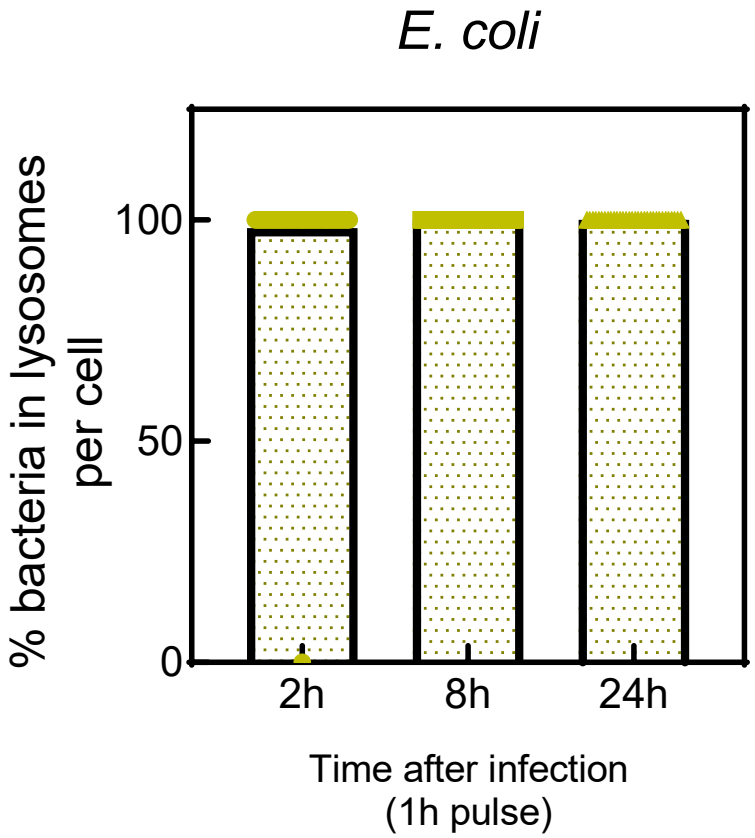
LysoTracker Red
GFP-expressing
E. coli

Outstanding Questions

What are the molecular signaling pathways to and from the phagosome and how are they regulated?

Are immune signals able to modulate phagosome maturation upon triggering of a single signal transduction pathway or are multiple pathways involved? Is it possible to identify common key molecules?

Pauwels *et al*, 2017. *Trends. Immunol.*

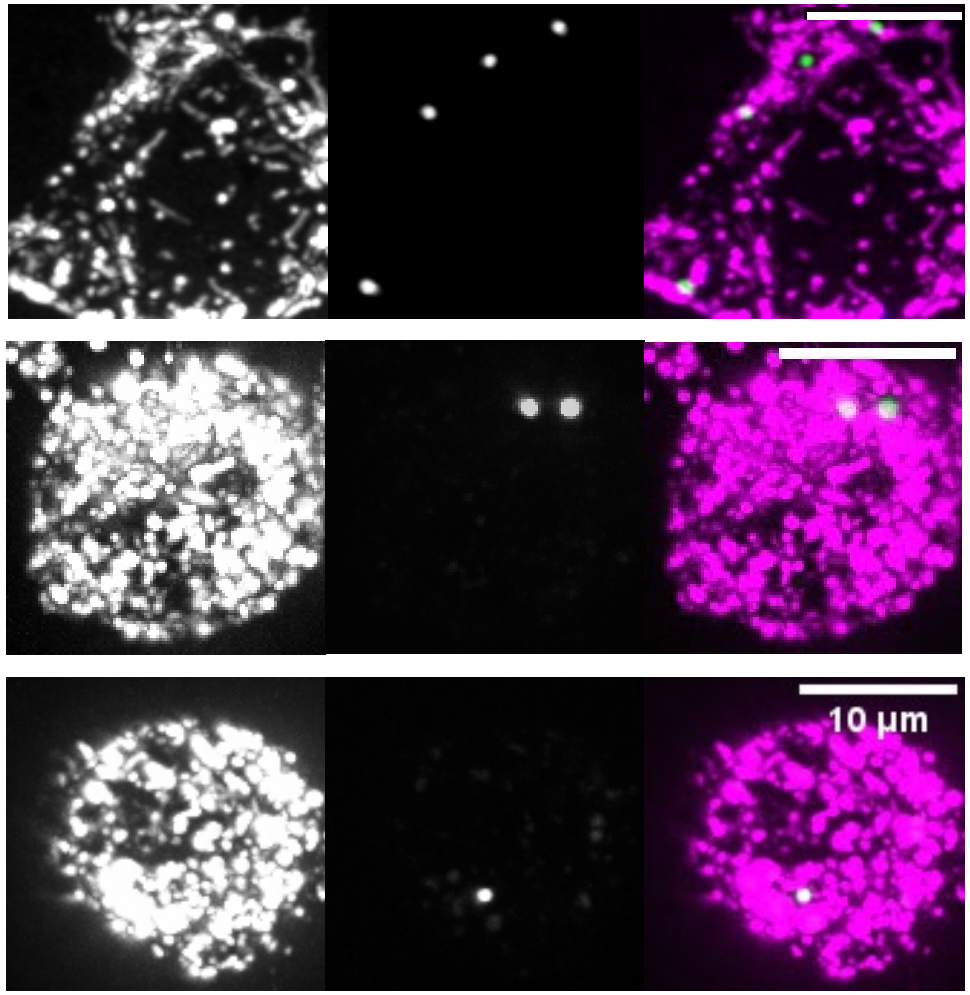


What Does *E. coli* have that Particles Do Not?

Control

Cells pre-treated with LPS

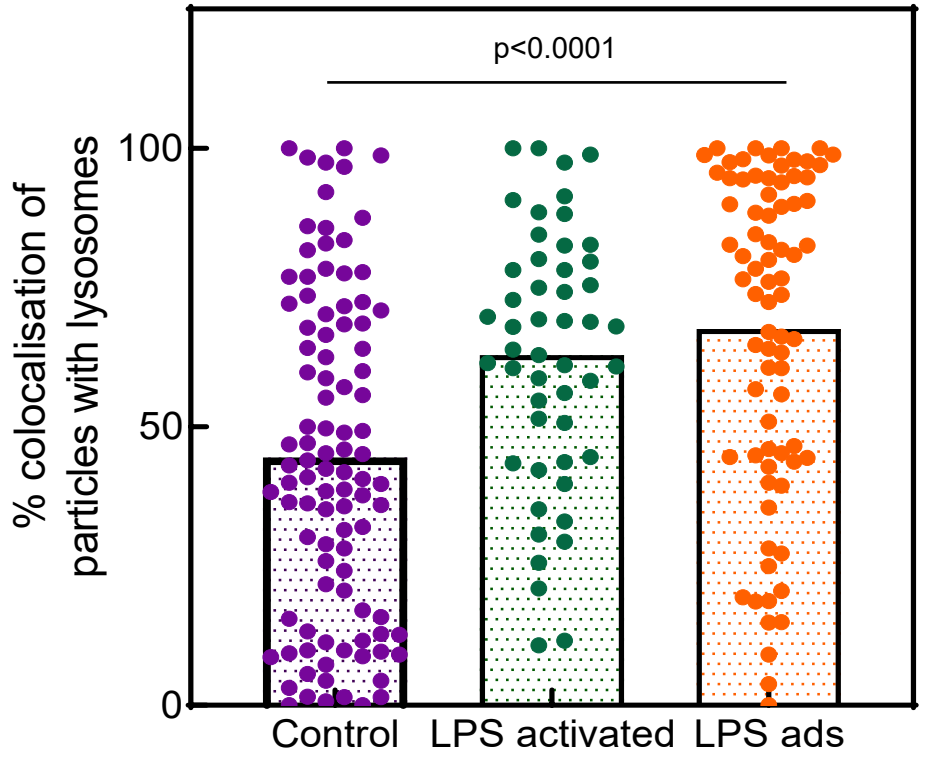
LPS adsorbed particles



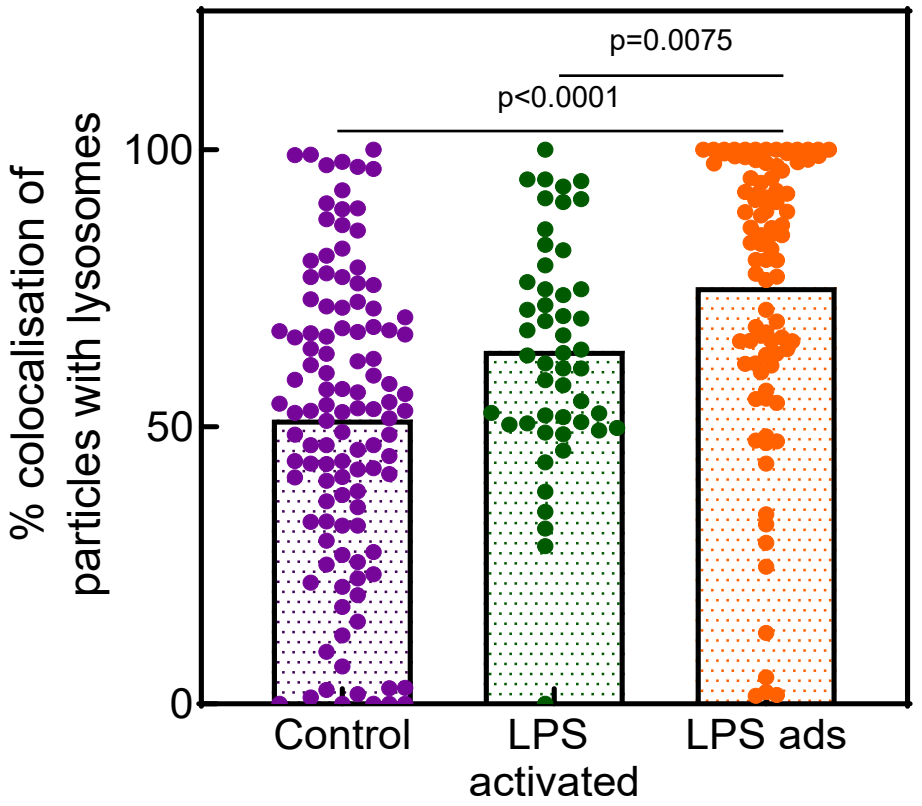
LAMP1 500nm particles Merge

What Does *E. coli* have that Particles Do Not?

2h post introduction of particles



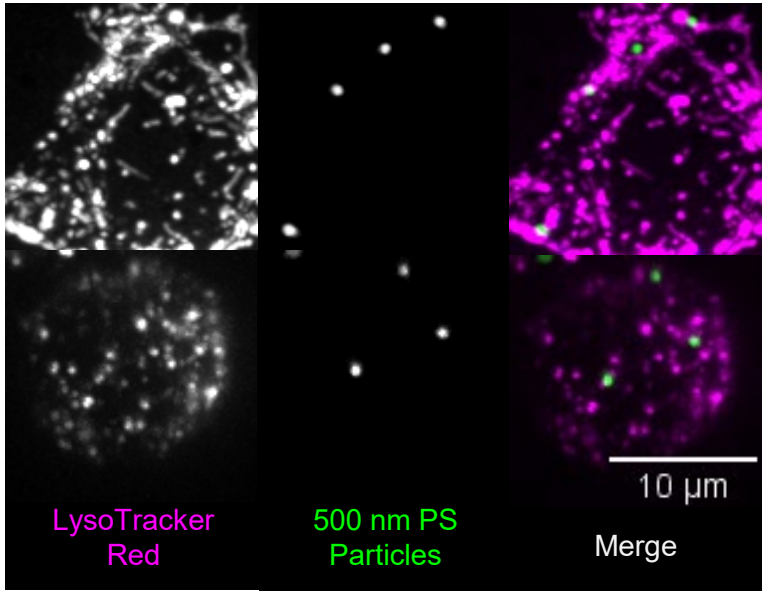
8h post introduction of particles

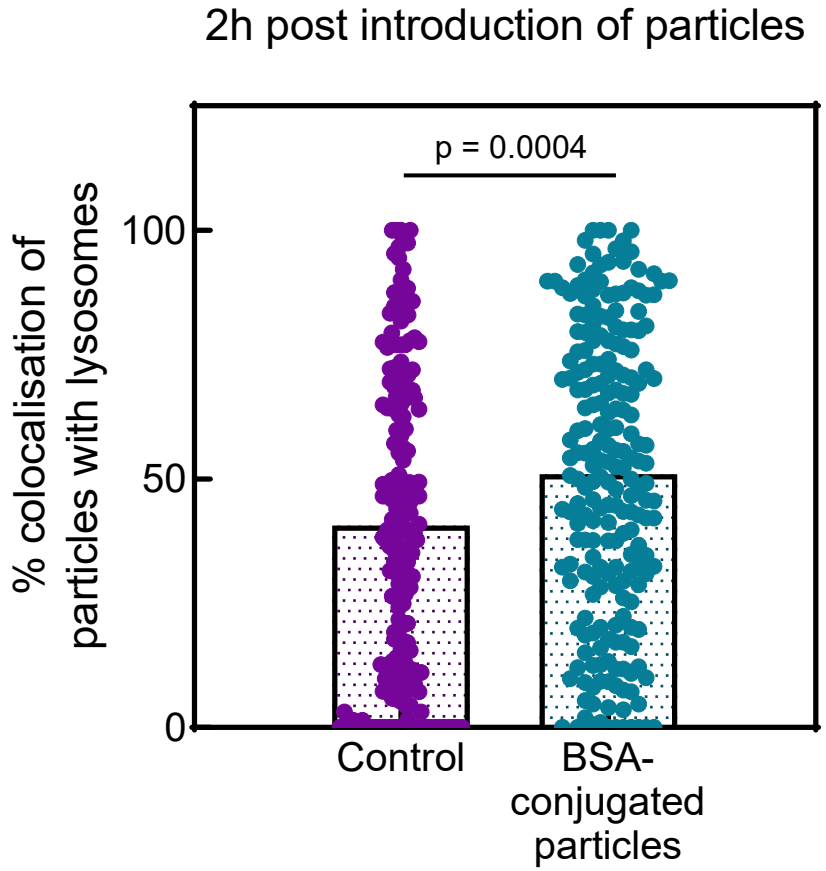


N = 3
n ≥ 17 cells/N

Similar results obtained in primary cells from mice, and ongoing experiments in mice (particle injections)

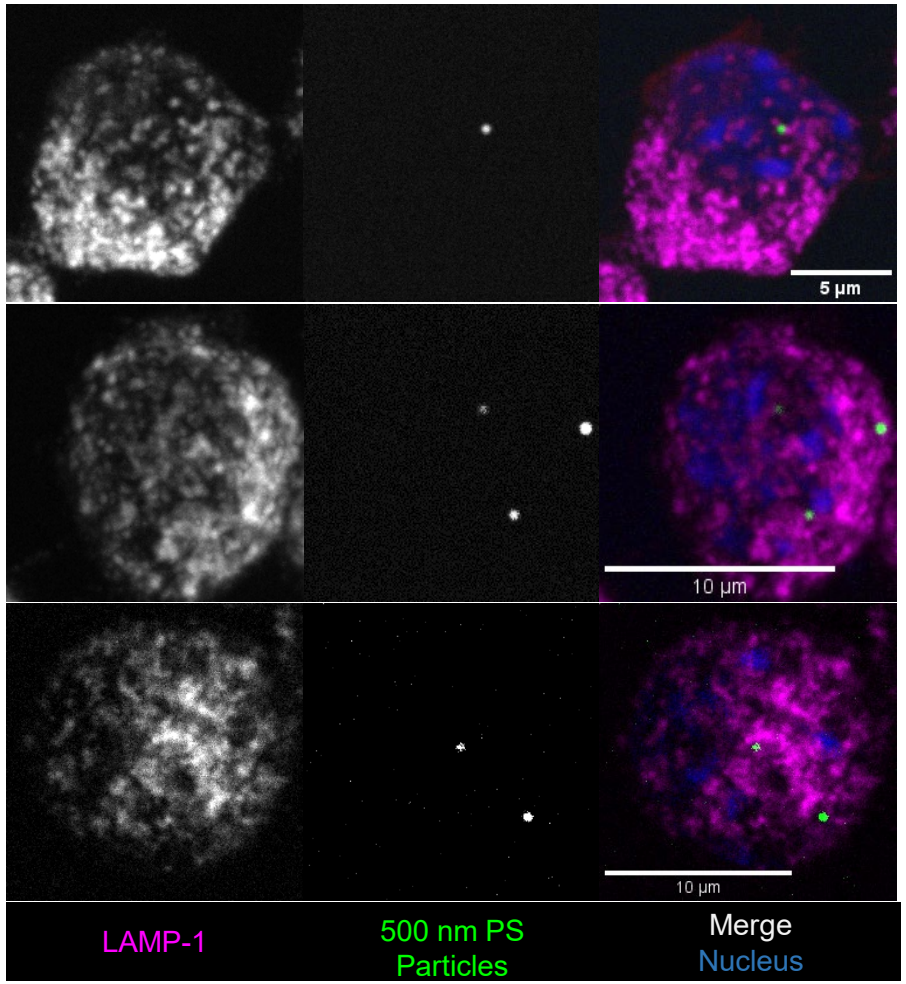
Are Other Signals Involved?

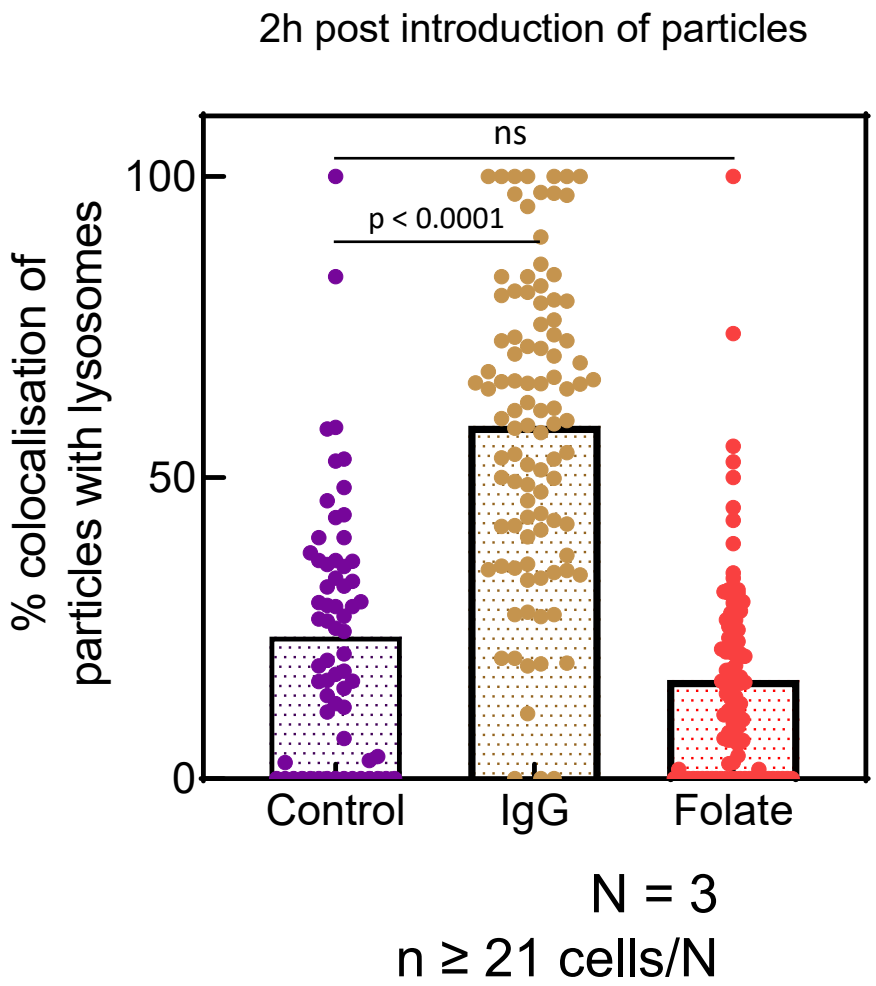




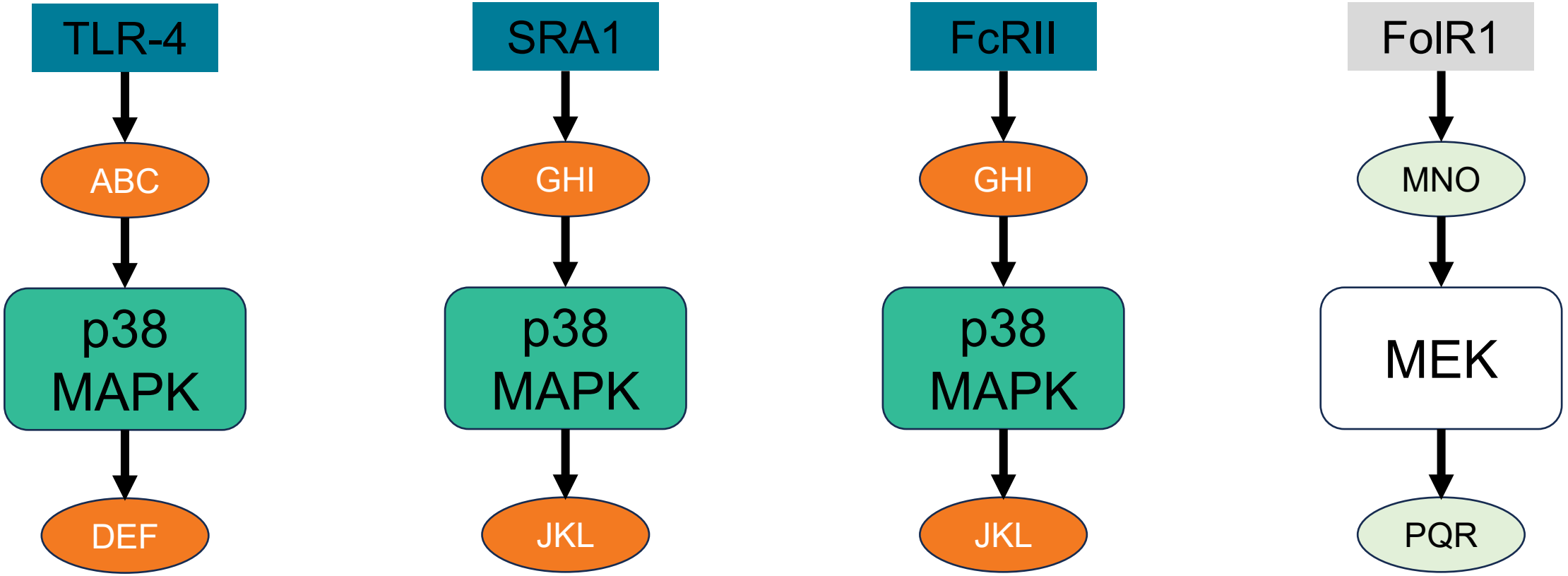
N = 6
n ≥ 32 cells/N

Are Other Signals Involved?

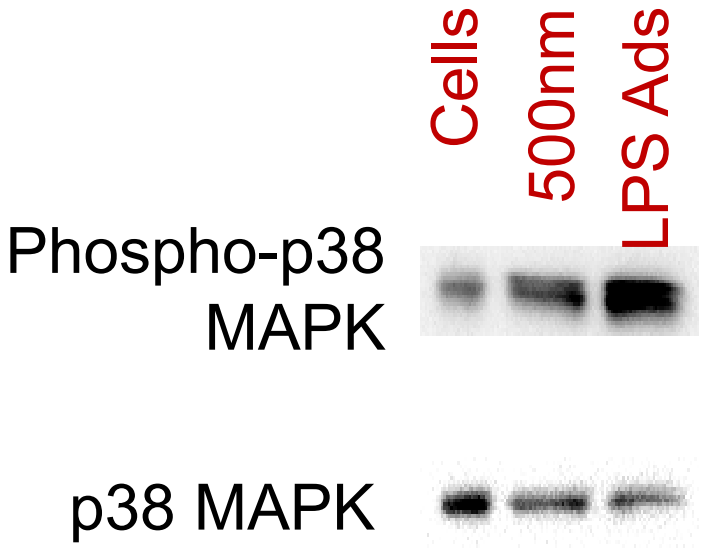


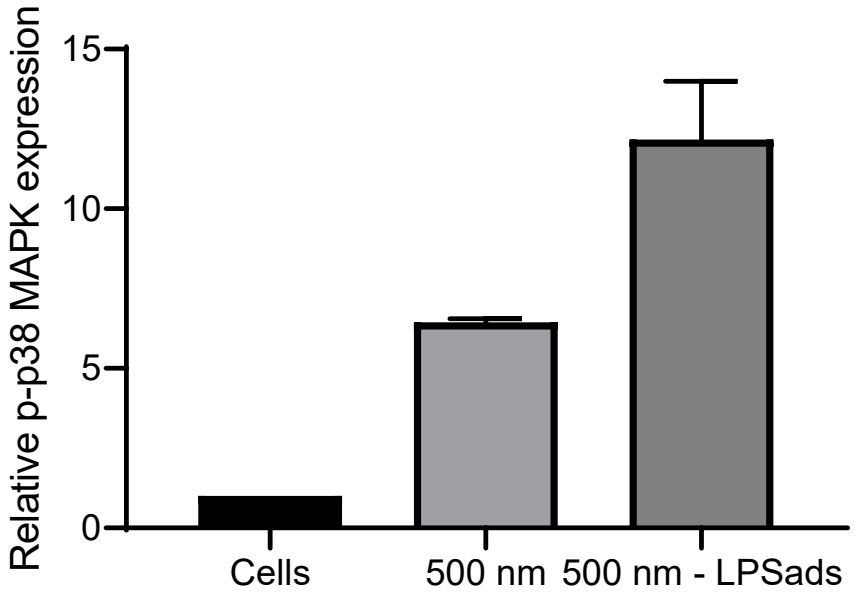


Potential role for p38 MAPK



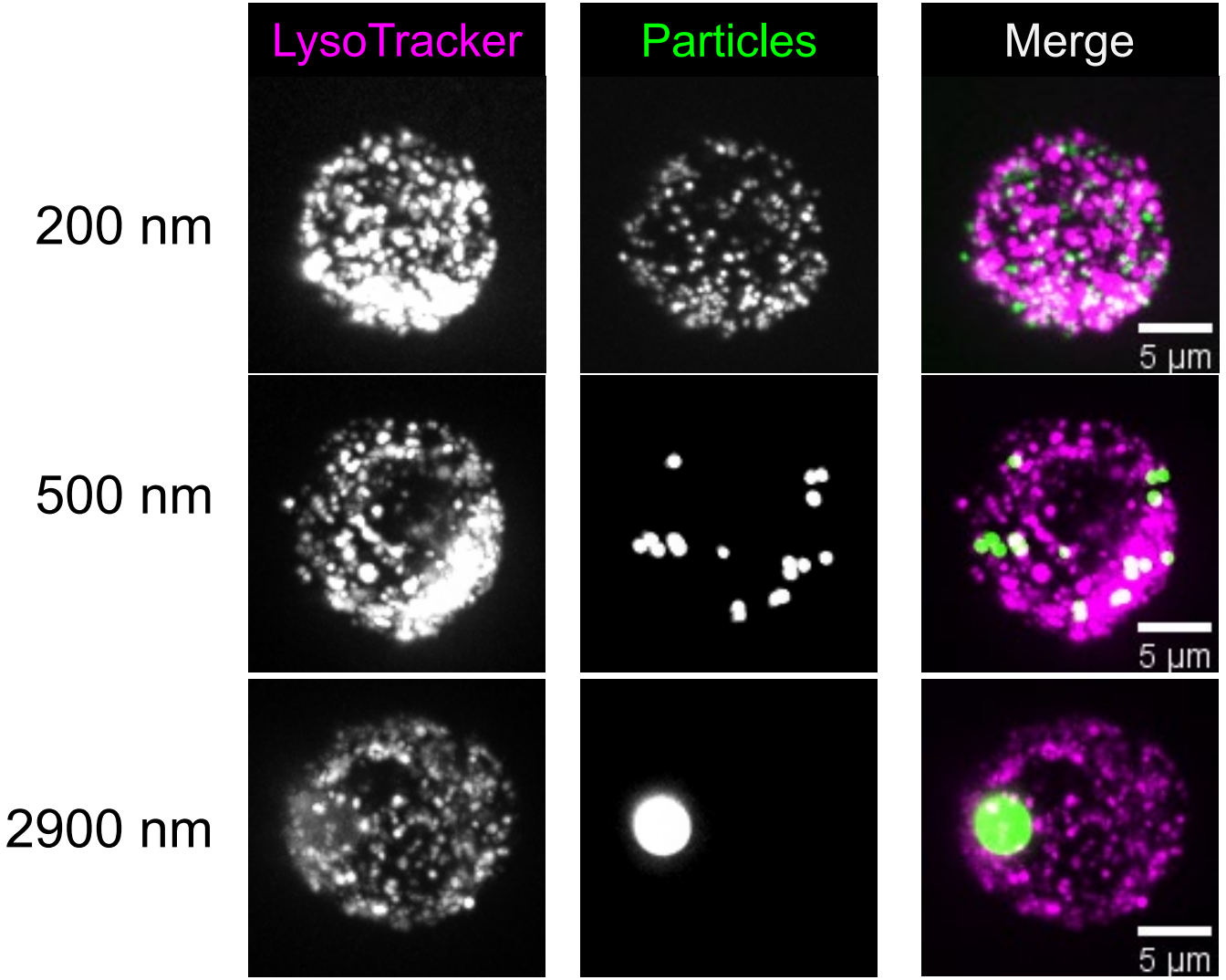
p38 Phosphorylation Essential?





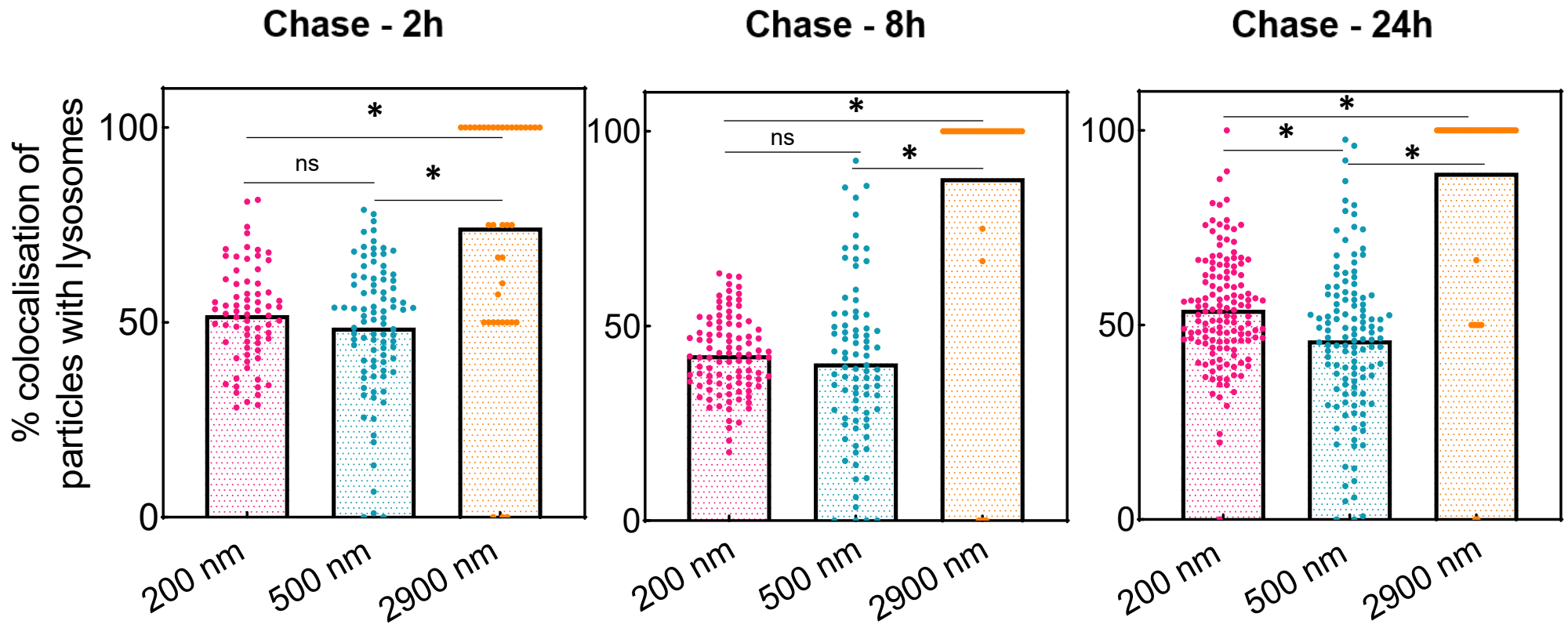
N = 2

What About Bio-Physical Cues?



Images are representative MIPs after an 8h chase

Larger Particles Associate with Lysosomes

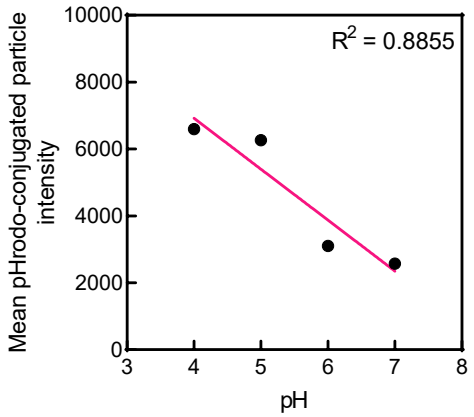


N = 3
n > 17 cells / N

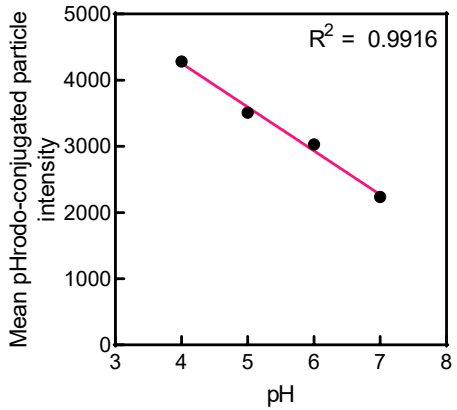
* = $p < 0.05$
Kruskal-Wallis, Dunn's Multiple Comparison Tests

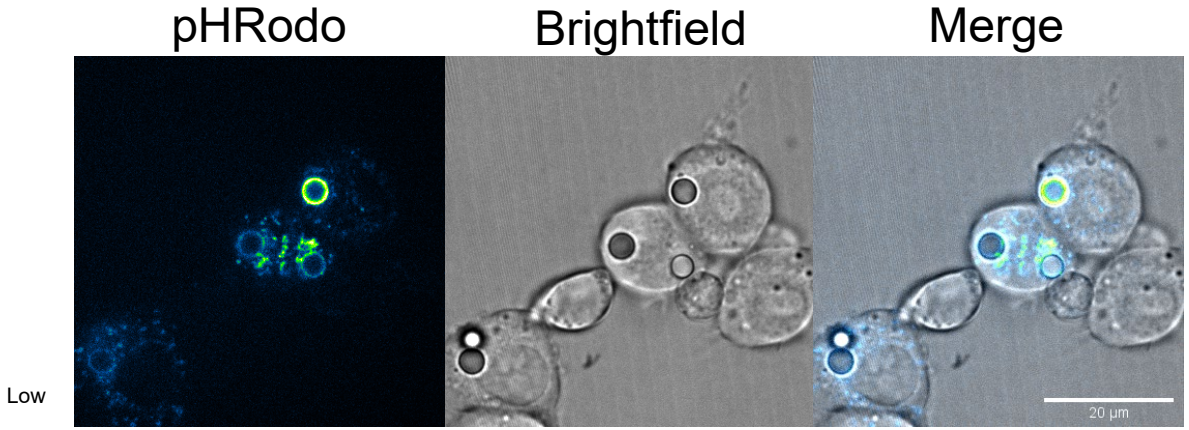
Confirmation – pH of Particle Compartments

500 nm



3 µm



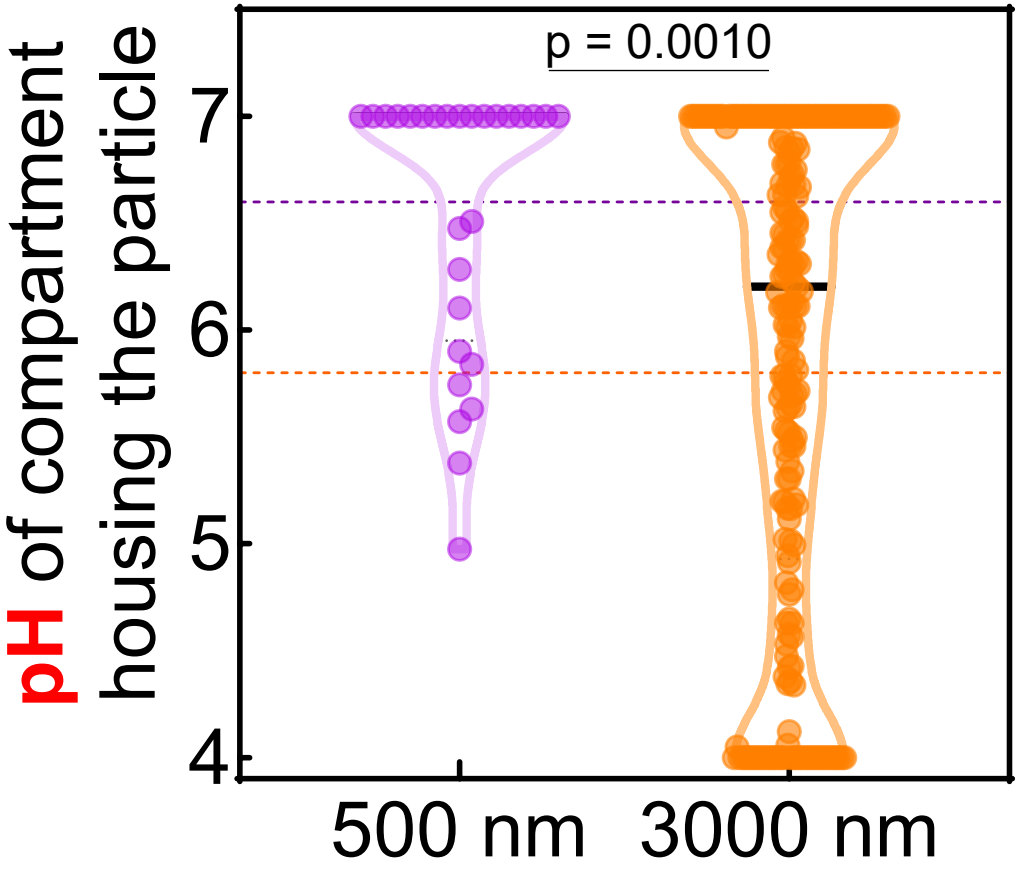


Low

High

pHrodo intensity

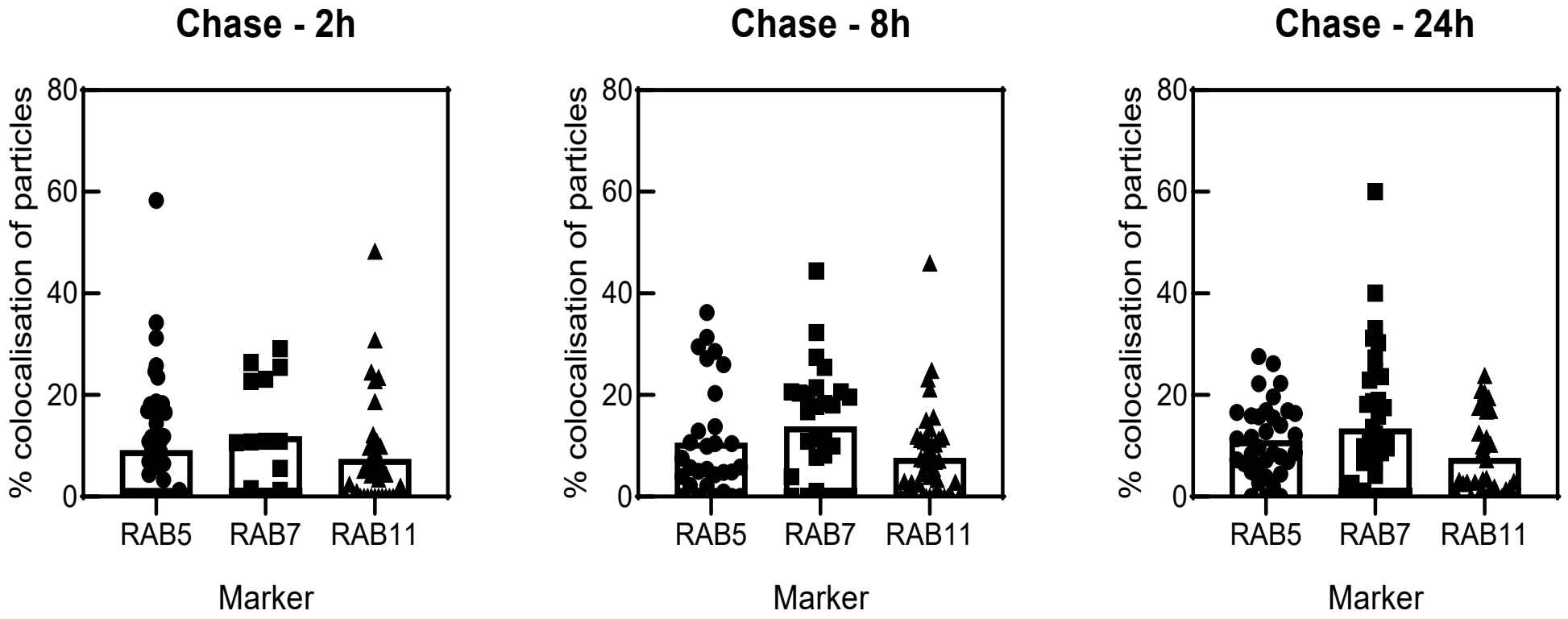
Confirmation – pH of Particle Compartments



N = 3
n > 10 / N

8 hours post uptake

Where are the Particles?



N = 3

n > 10 cells/N

So What?

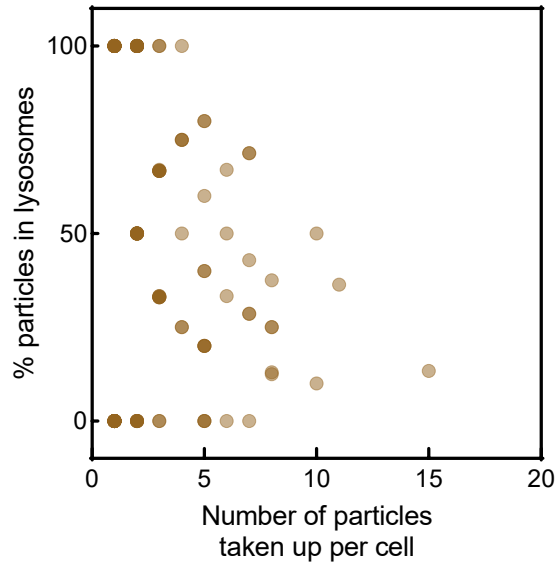
- Phagocytosed particles do not always end up in the lysosome
- **Application:** we can control delivery to specific endosomal compartments, which can be exploited for delivery of peptides for vaccines or delivery of pH-sensitive small molecules
- **Toxicology:** particles are unlikely to be degraded completely by phagocytic immune cells, so they may accumulate in the body

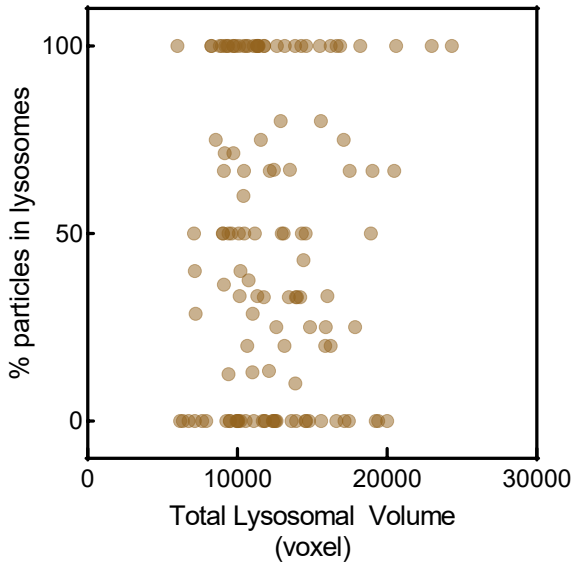
Acknowledgements

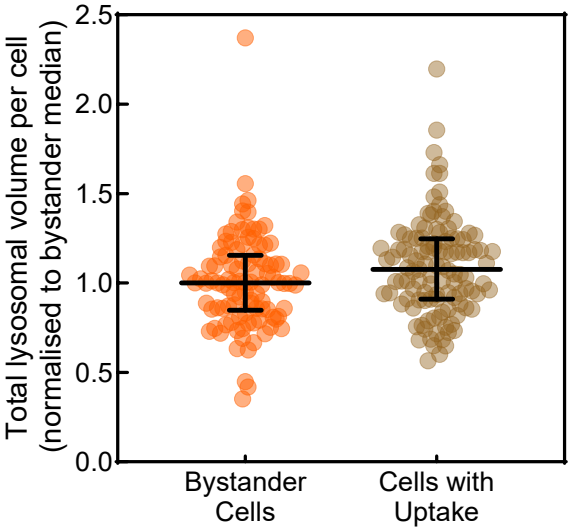
IndiaAlliance
DBT wellcome

INDIAN INSTITUTE OF SCIENCE
भारतीय विज्ञान संस्थान

Not a Function of Particle Number or Lysosomal Volume



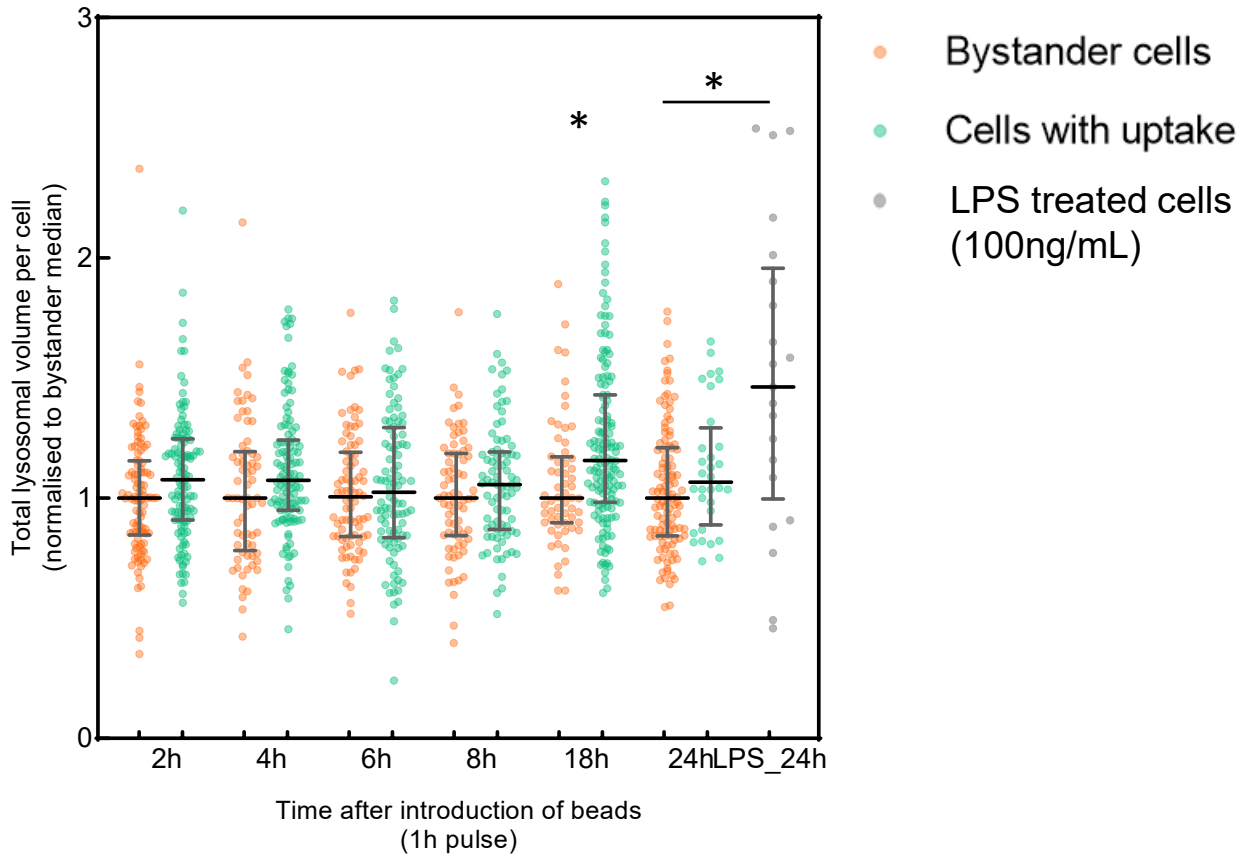




N = 3, n = 128 cells
Holds true across other time points as well

N = 3
avg n = 108 cells/group

No change in lysosomal volumes of cells that take up particles



Number of particles taken up per cell