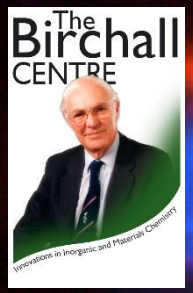
Keele University

THP-1 cells co-cultured (24h) with 25µg/mL AlO(OH)

MRC Medical Research Council

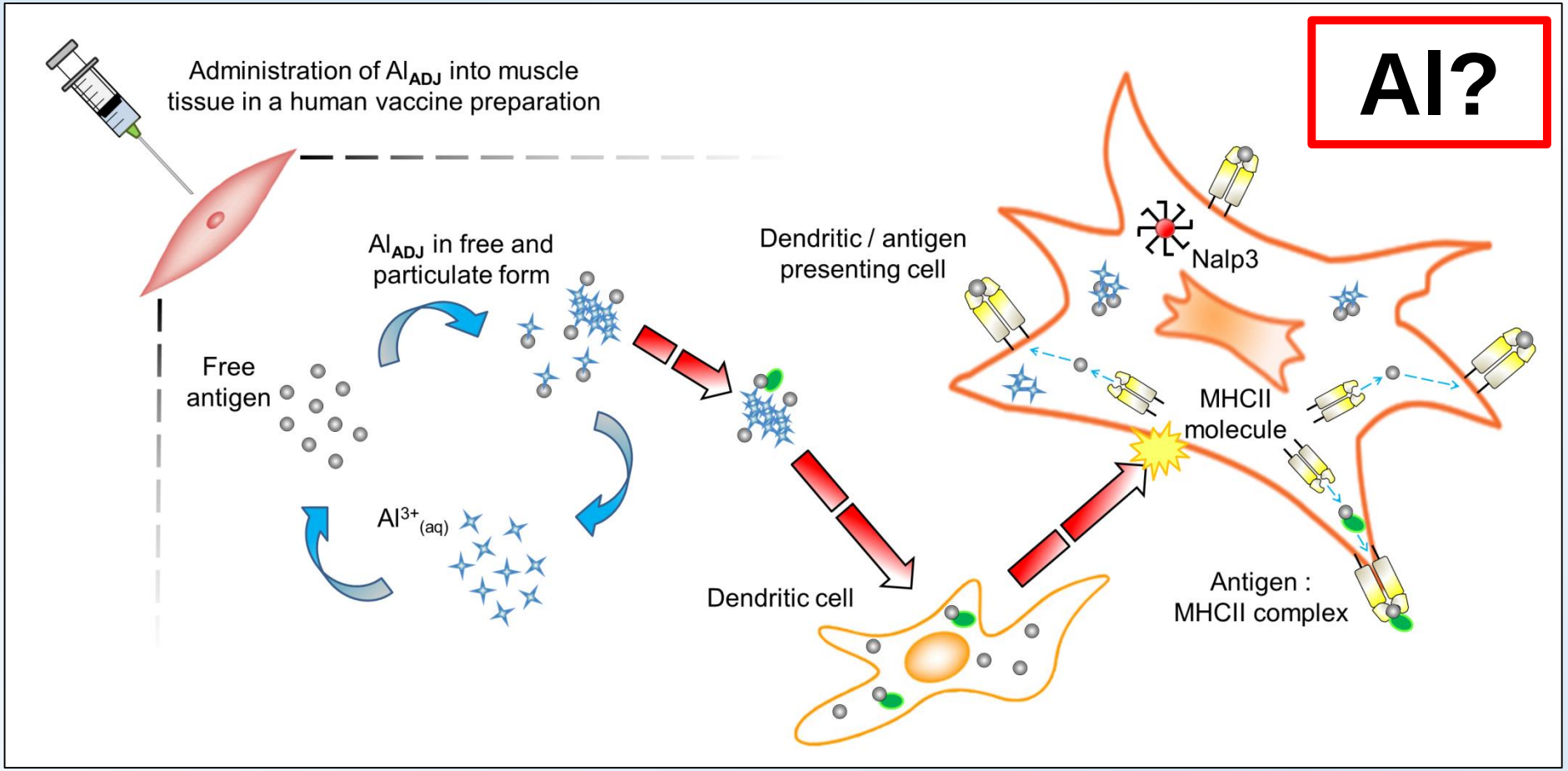
Elucidating upon the cellular uptake of ABA and the $A\beta_{42}$ peptide 'antigen' in a THP-1 cell line

Dr Matthew Mold



11th Keele Meeting on Aluminium, Lille, France 2015

10th Keele Meeting on Aluminium



Current opinion for a mechanism of aluminium adjuvanticity via cognate B-cell activation and T-cell differentiation. *10th Keele Meeting on aluminium, Winchester 2013*.

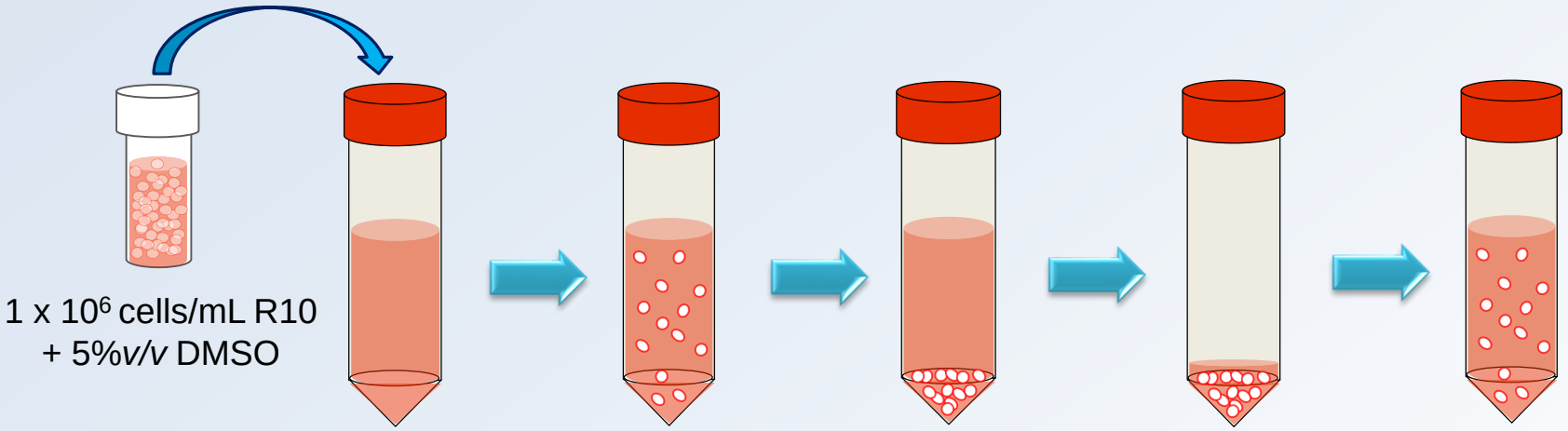
$A\beta_{42}$

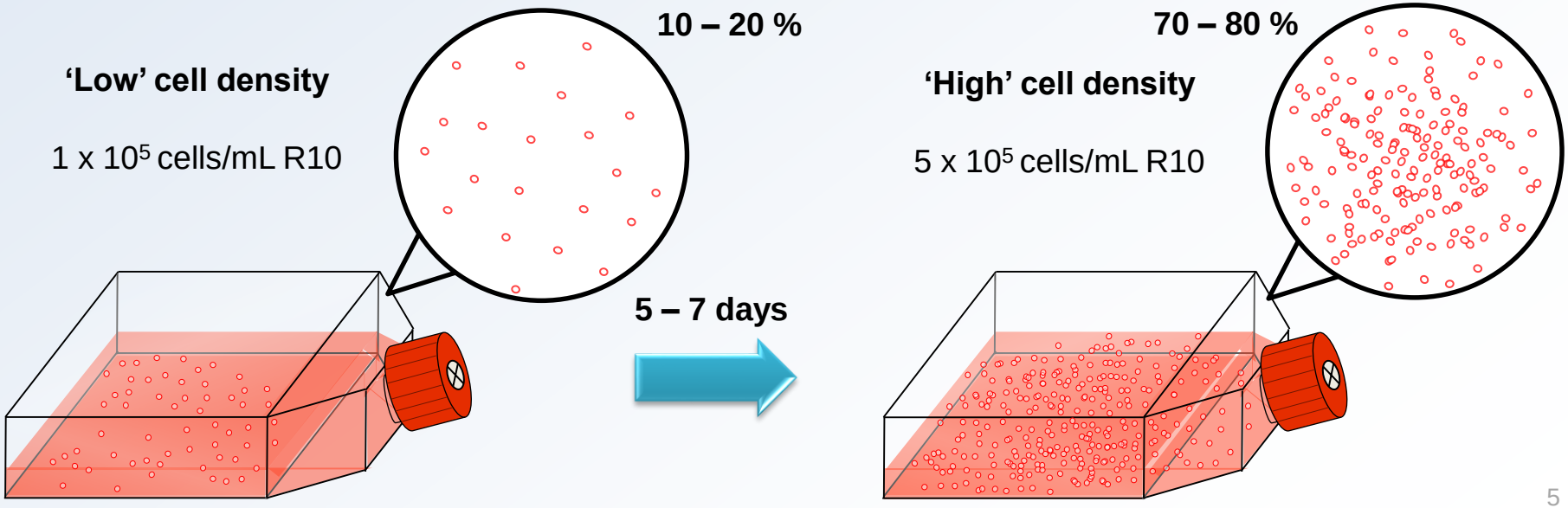
- Amyloidogenic peptide generated by sequential proteolytic cleavage of the amyloid precursor protein (APP) via β- and γ-secretases.
- Predominant form found deposited within senile plaques in Alzheimer's disease (AD).
- Effectiveness as use as an antigen highlighted in a transgenic mice model that overexpresses mutant human APP with reductions in AD-like neuropathologies (**Schenk *et al*., 1999**).
- Translation into clinical trials and the transport of Aβ from the periphery to the brain remain elusive.
- A recent study has suggested that β-amyloid may be transported from distant locations to the brain as evidenced intracellularly in peritoneal monocytes (**Eisele *et al*., 2014**).

Aims and objectives

- Culture of the monocytic T helper 1 (THP-1) cell line and **co-culture** with clinically approved and experimental adjuvant preparations.
- Section THP-1 cells in the presence and absence of aluminium based adjuvants (ABA) using an **agar-paraffin embedding** protocol.
- **Lumogallion** was to be investigated as a fluorescent molecular probe for particulate and amorphous ABA preparations (**Mold *et al*., 2014**).
- Investigate the use of **varying [ABA]** and establish their **cellular uptake**.
- Assessment of the potential **cellular uptake of amyloid** fibrils of $A\beta_{42}$ via use of thioflavin T (ThT).

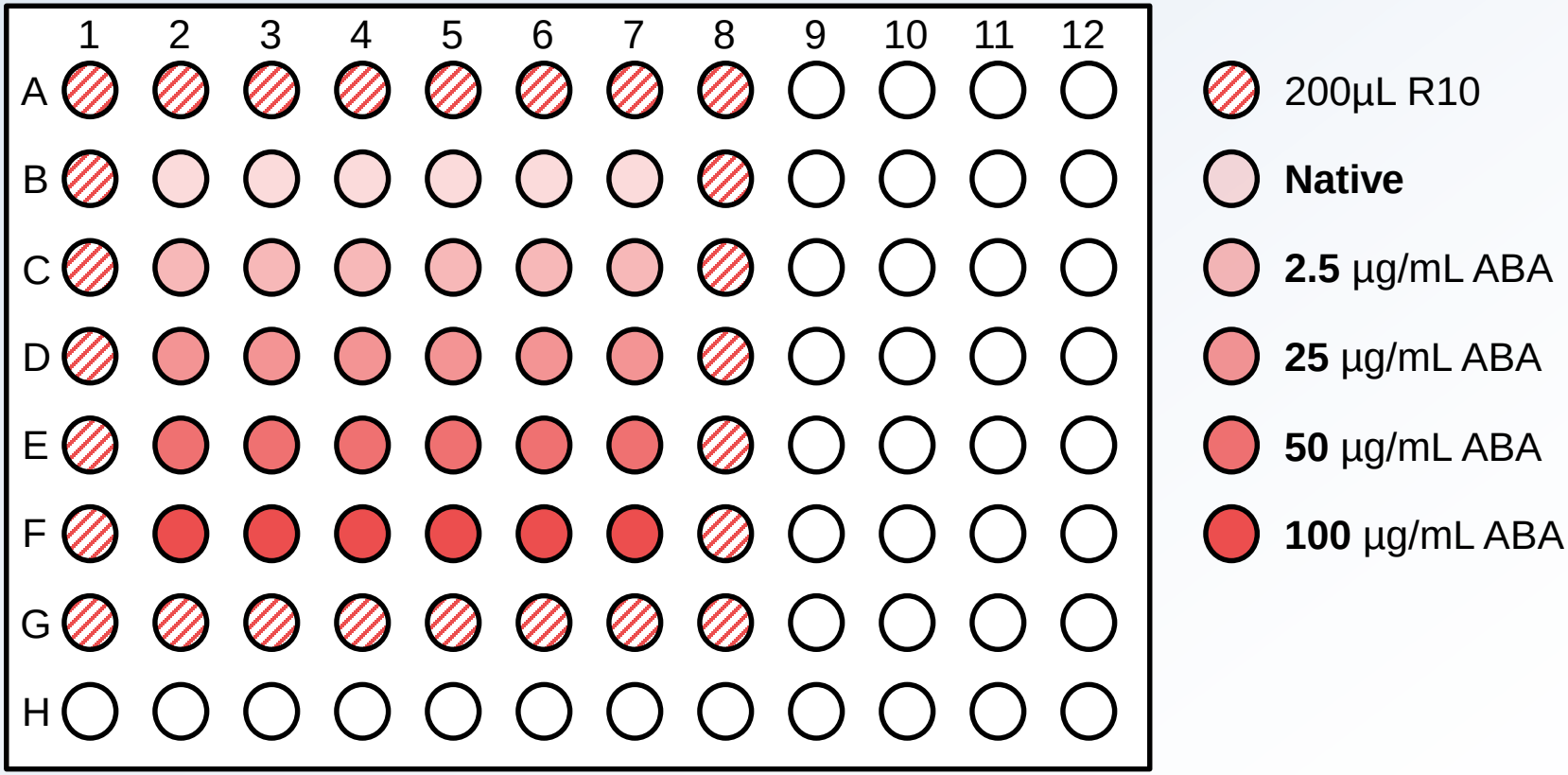
THP-1 cell culture



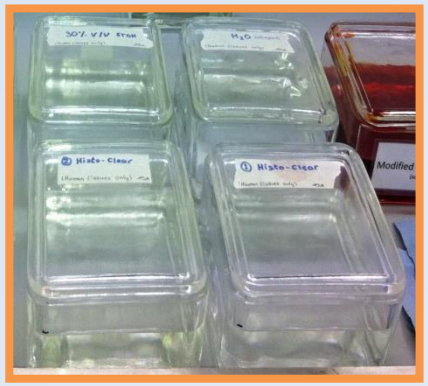


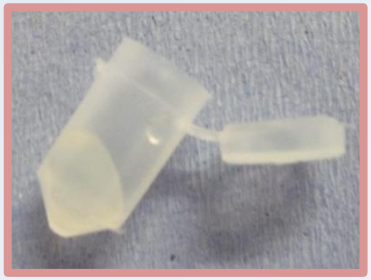
Co-culture of THP-1 cells & ABAs

- THP-1 cells re-suspended in R10 to 5 x 10^5 cells/mL (50,000 per 100µL).
- 100µL 2X [ABA] in R10 added at increasing concentrations to cells.
- 96 well plate format used and plates incubated for 24 h at 37^{o}C + 5% CO_2.

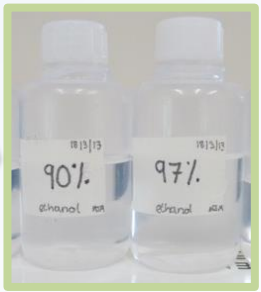


Paraffin embedding

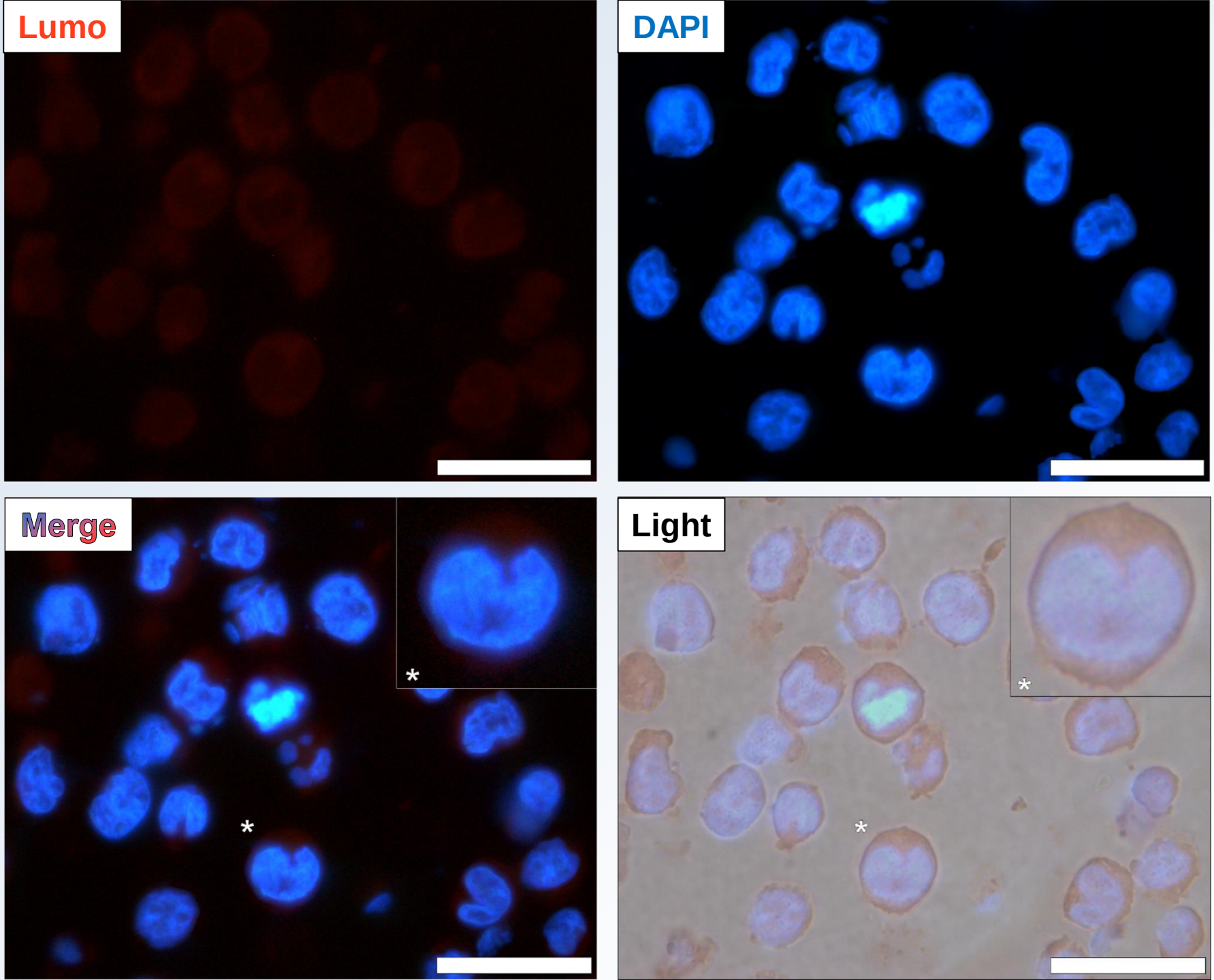




1. Fixation & pre-embedding
2. Dehydration & clearing
3. Infiltration & embedding
4. Microtomy
5. De-waxing & staining

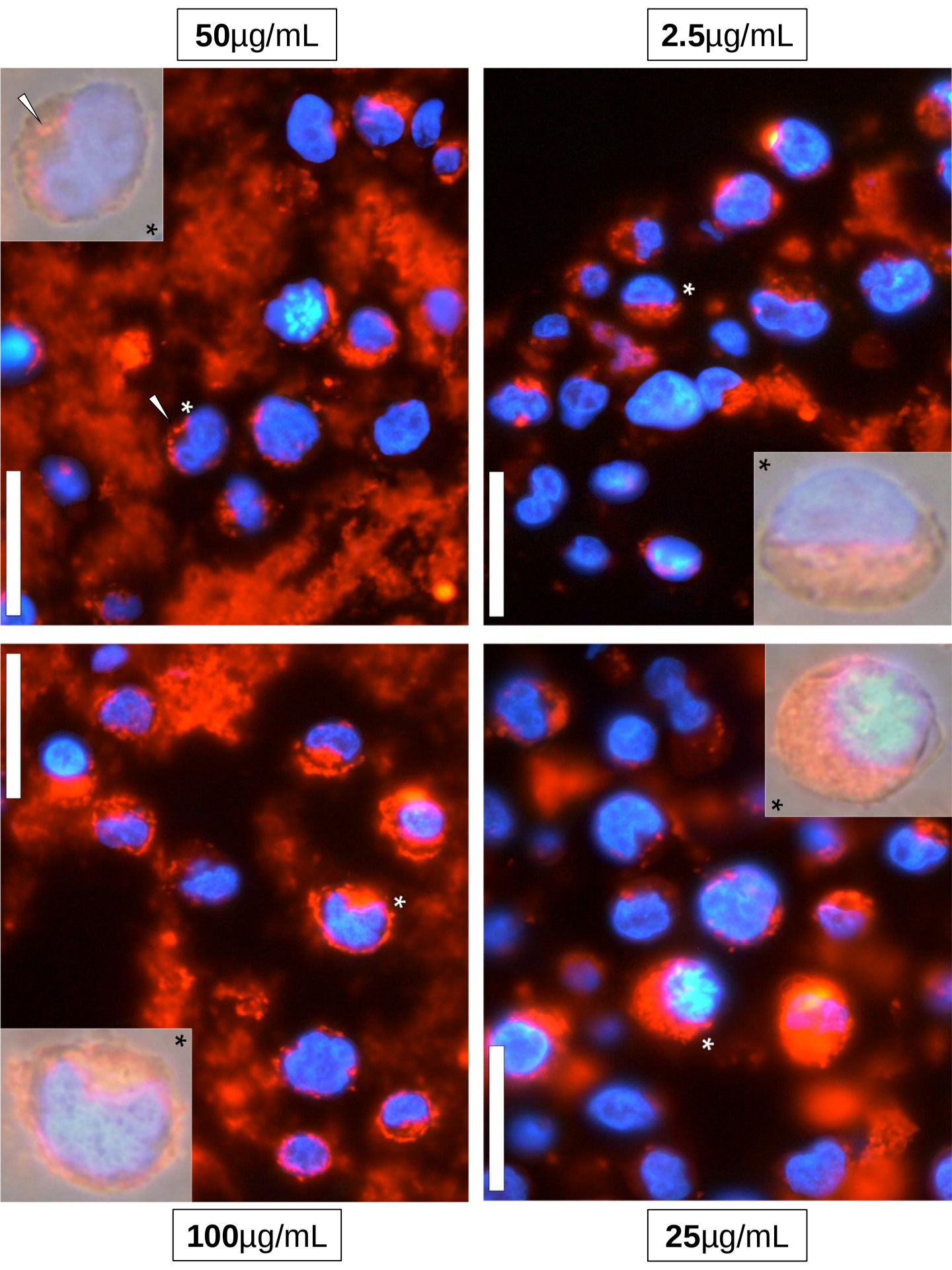


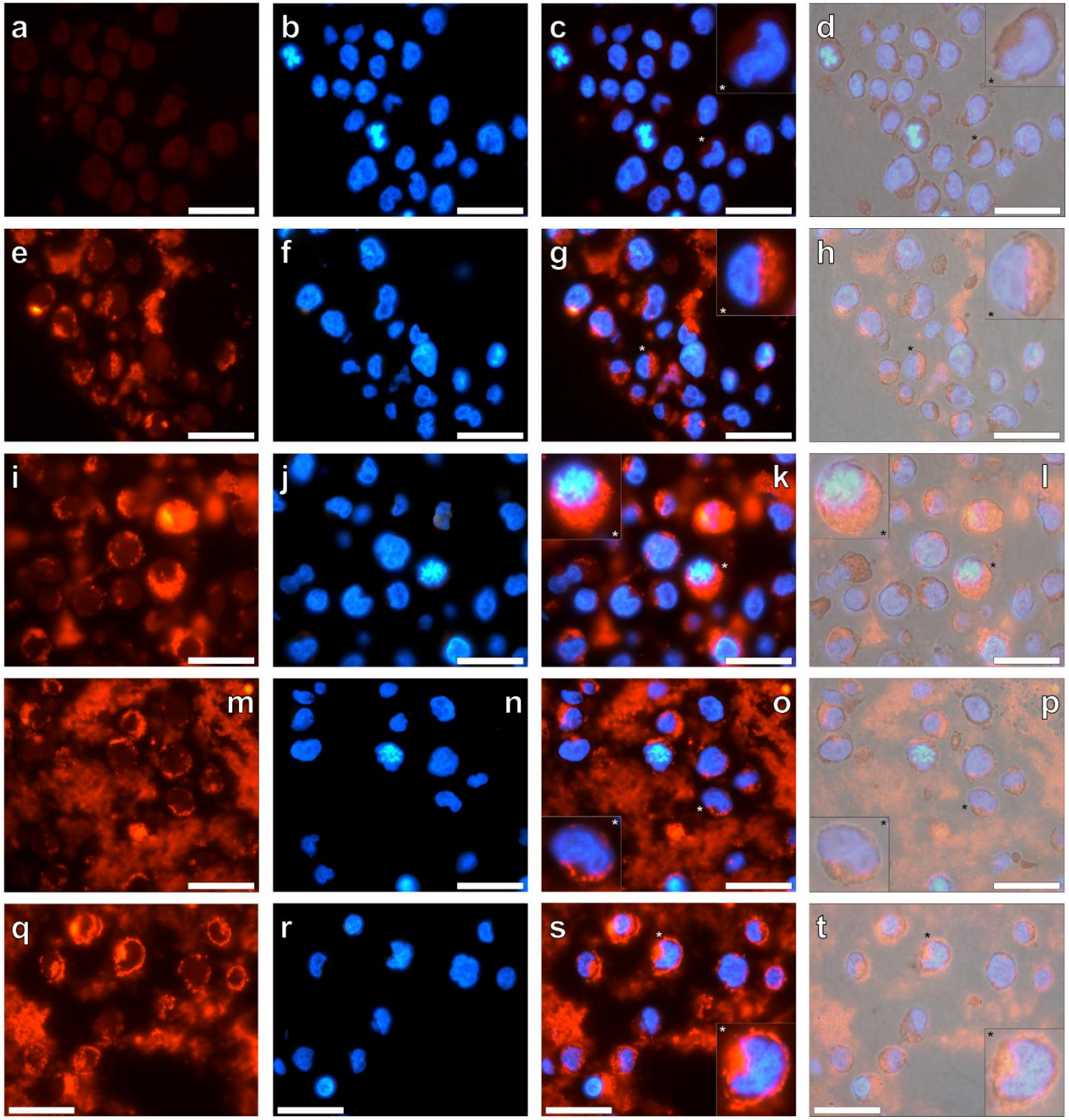
Native THP-1 cells (R10)


Lumo
DAPI
Merge
Light

Alhydrogel®

2.5 - 100 *µg/mL*

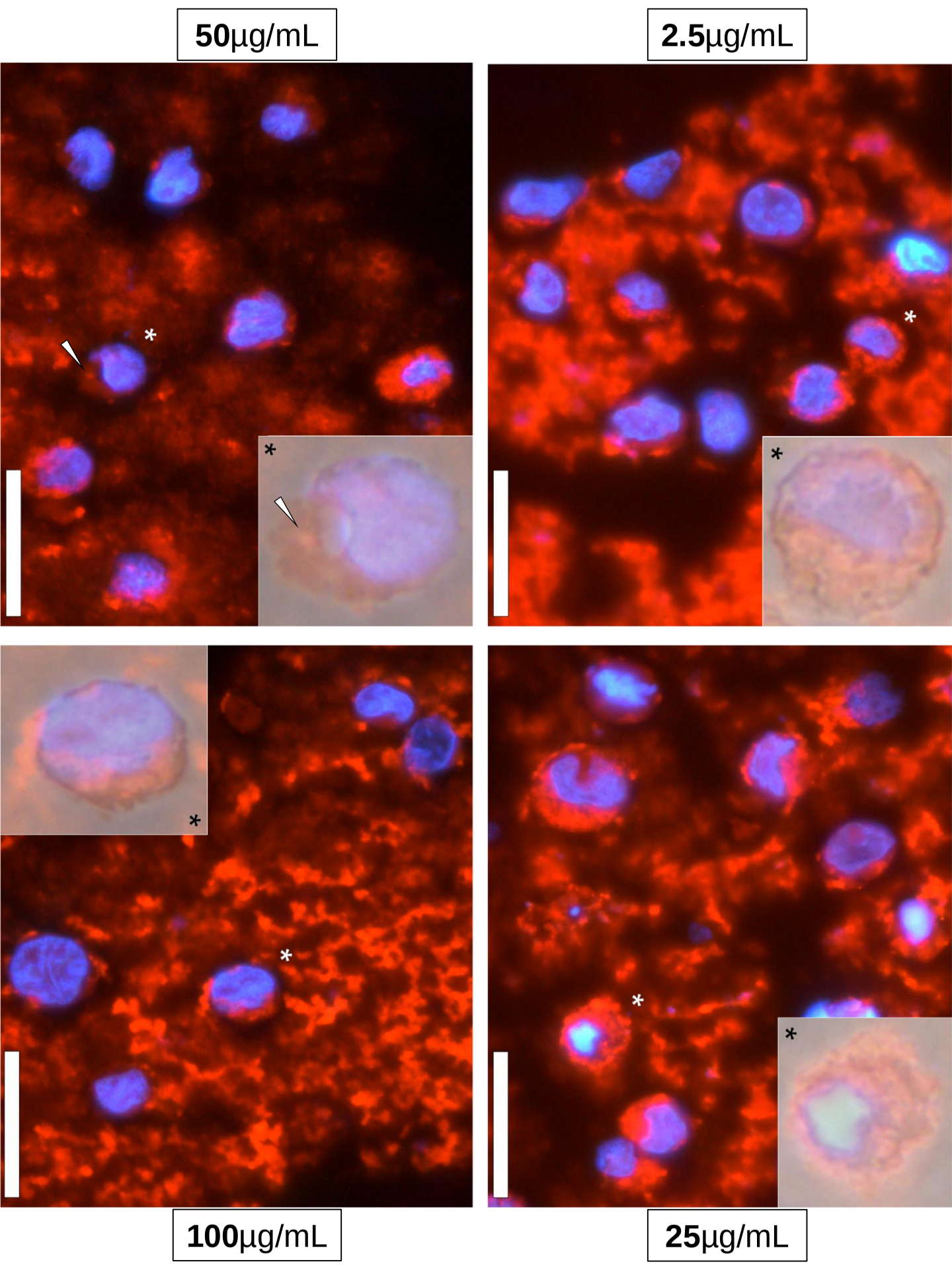

50µg/mL
2.5µg/mL
100µg/mL
25µg/mL

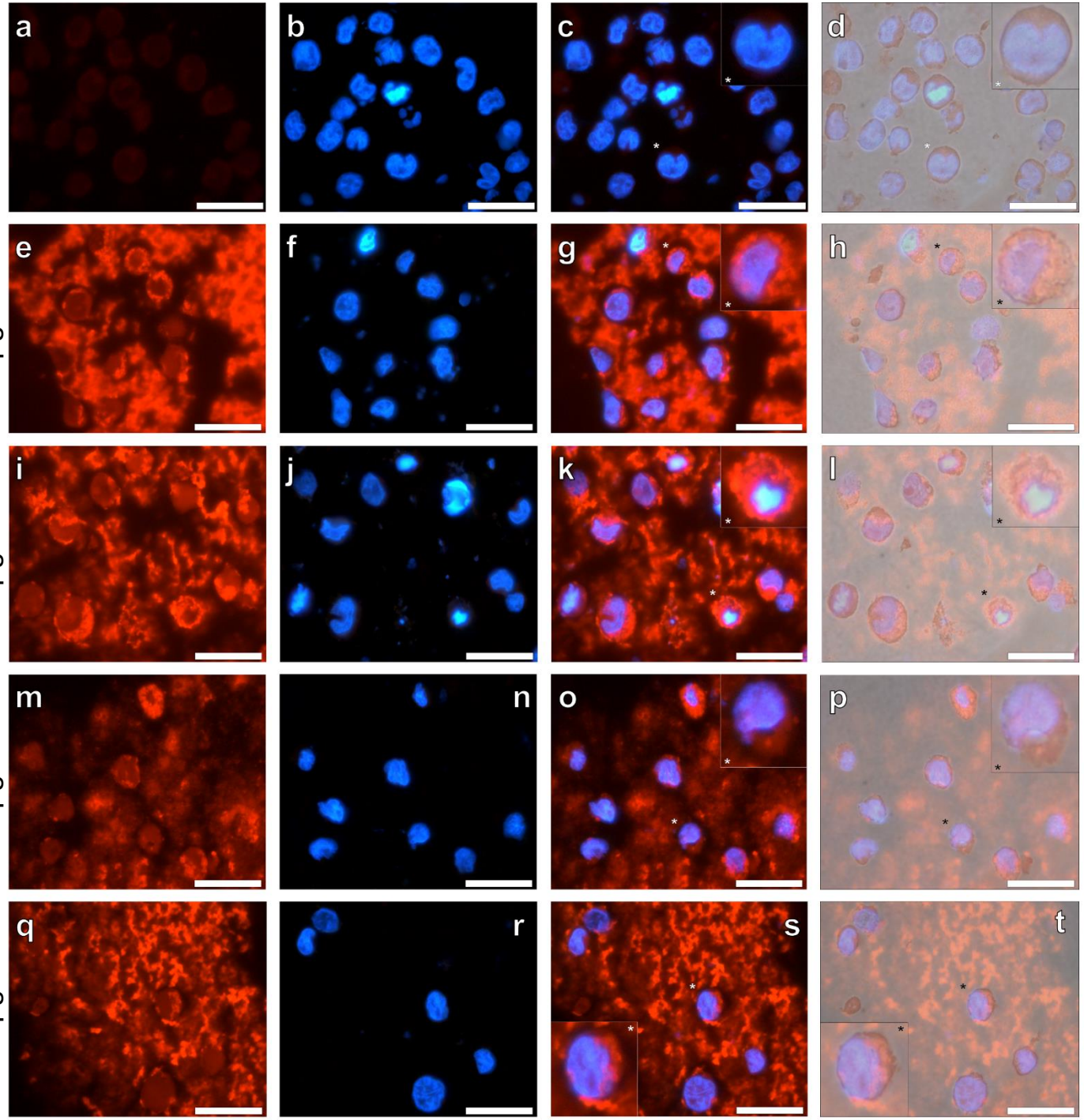


- Alhydrogel® found localised in cell cytoplasm.
- ABA particles were found internalised in THP-1 cells (*ca* 1.0µm).
- Alhydrogel was found readily internalised at all [ABA]s.
- ABA particulates were also found **associated with plasma membranes** at 100µg/mL of the adjuvant.

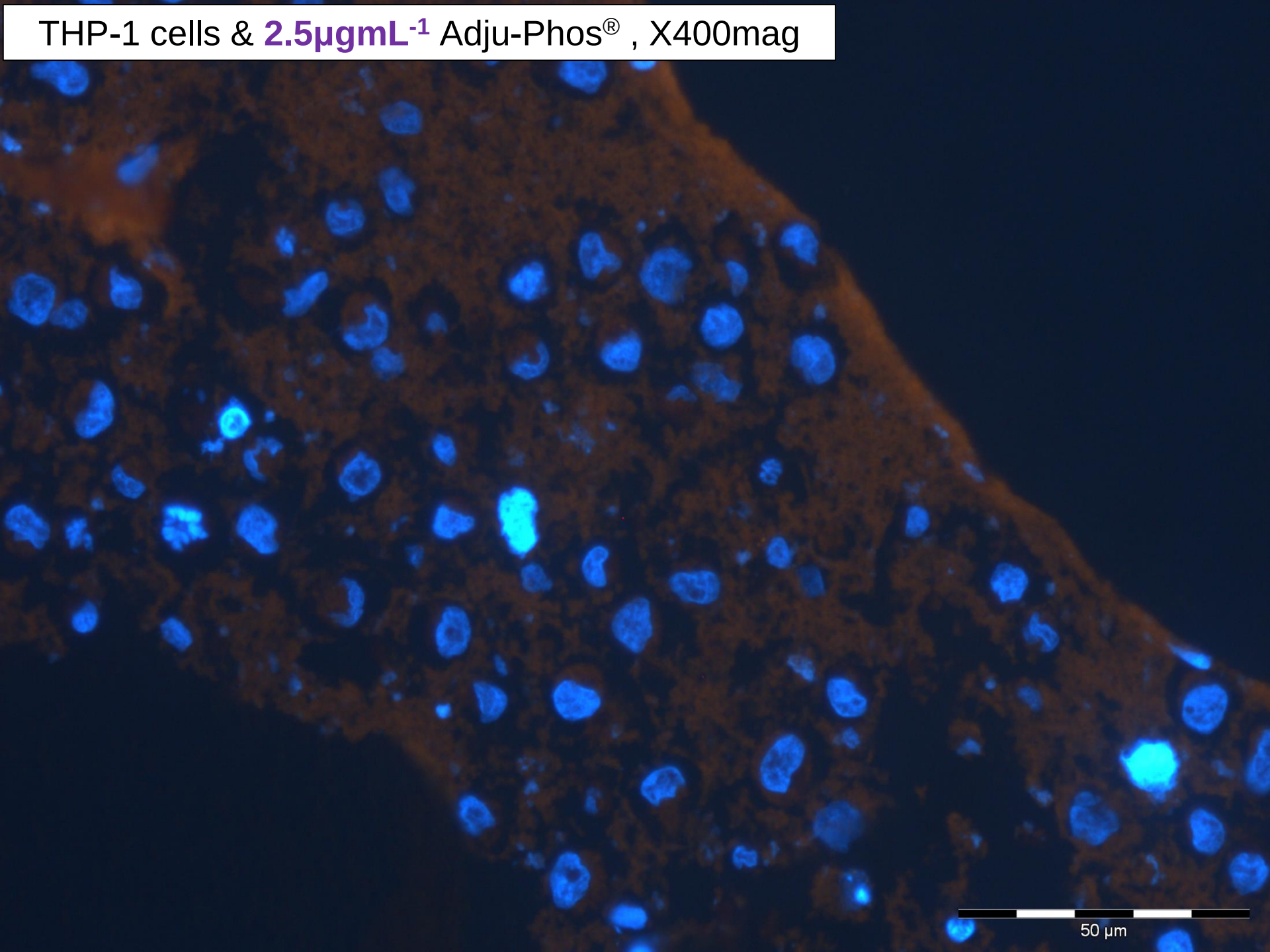
Adju-Phos®

2.5 - 100 *µg/mL*


50µg/mL
2.5µg/mL
100µg/mL
25µg/mL

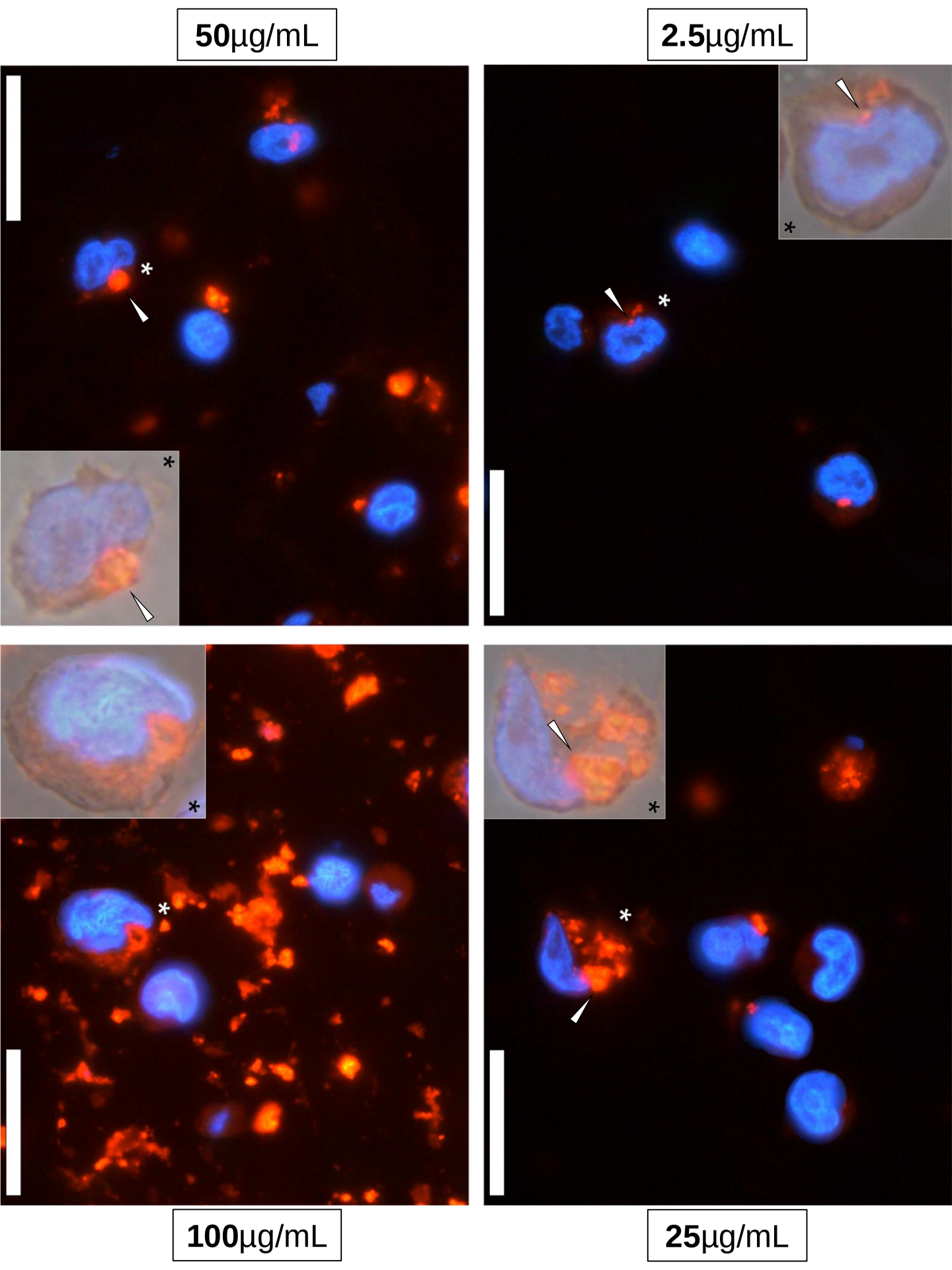


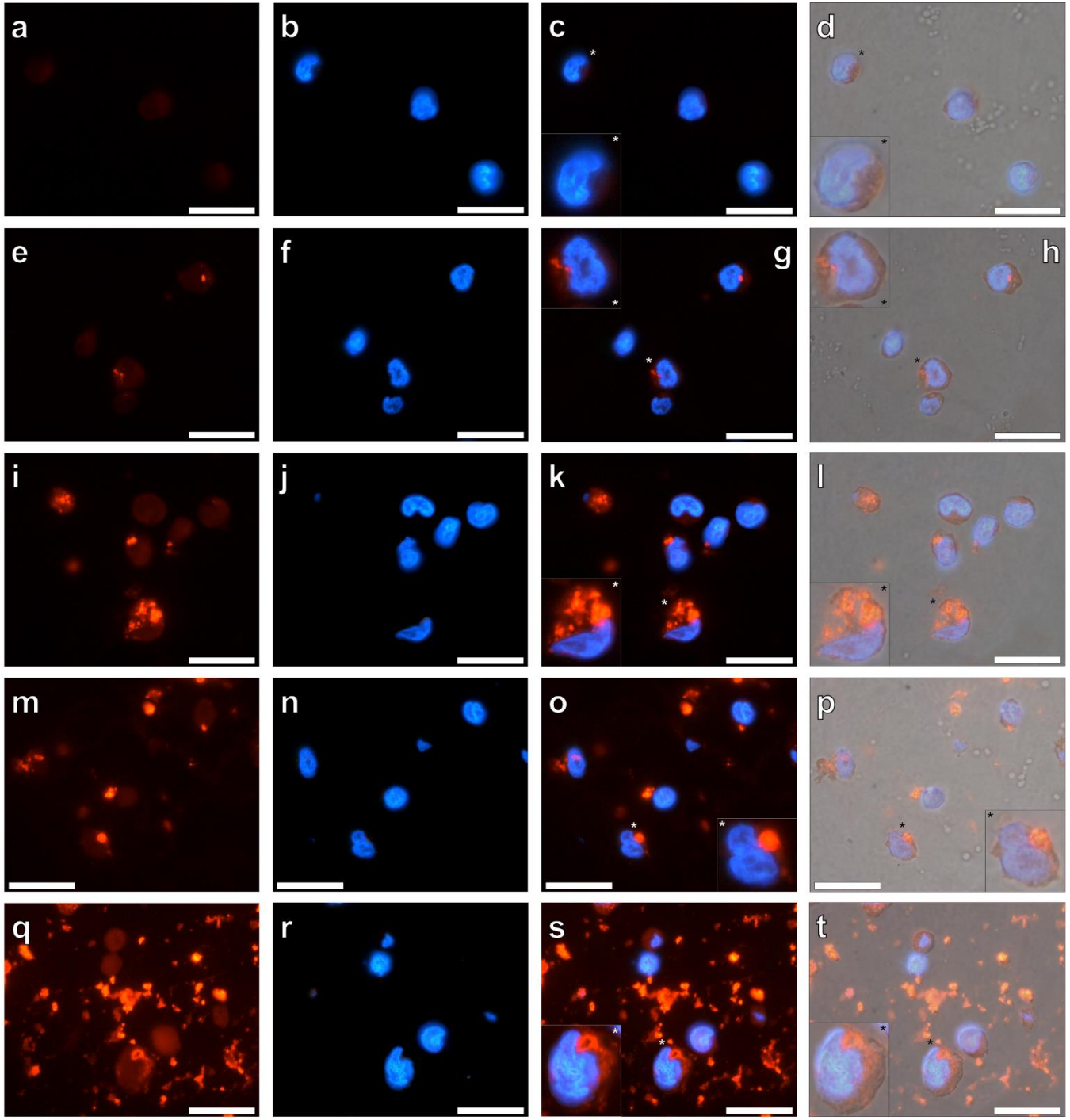
- Adju-Phos® found localised in cell cytoplasm only.
- Discreet ABA particles were found internalised in THP-1 cells, however their identification were sometimes difficult.
- Adju-Phos was readily internalised at 2.5 and 25µg/mL of the ABA.
- Uptake less pronounced at 50 and 100µg/mL of the adjuvant.


THP-1 cells & 2.5µgmL-1 Adju-Phos® , X400mag
50 µm

Imject® Alum

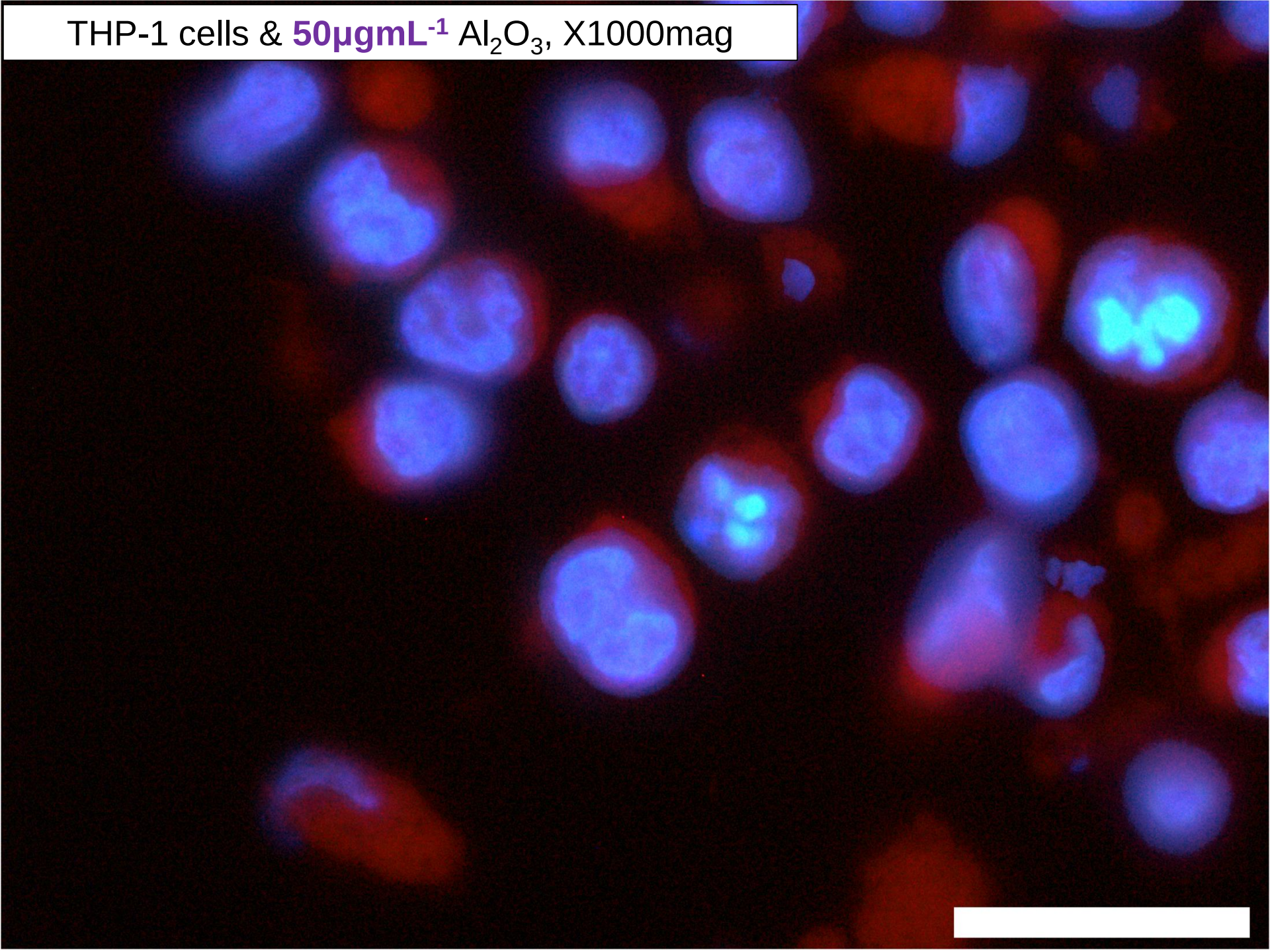
2.5 - 100 *µg/mL*

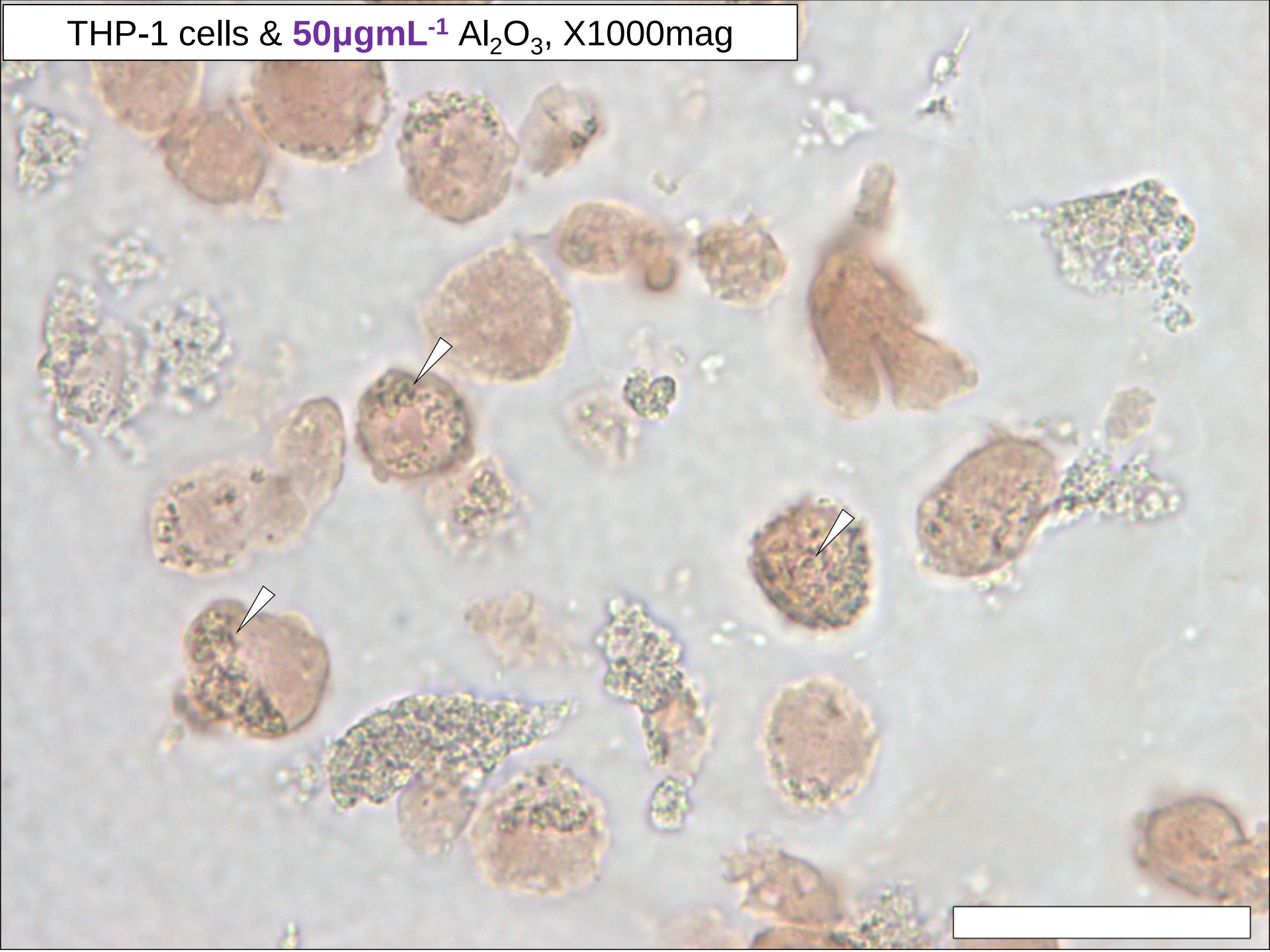

50µg/mL
2.5µg/mL
100µg/mL
25µg/mL



- Imject® Alum found localised in cell cytoplasm only.
- ABA particles as large as *ca* 2.0µm were found internalised in THP-1 cells.
- Imject® Alum was found internalised at 2.5 – 50µg/mL of the ABA.
- Uptake less pronounced at 100µg/mL of the adjuvant.

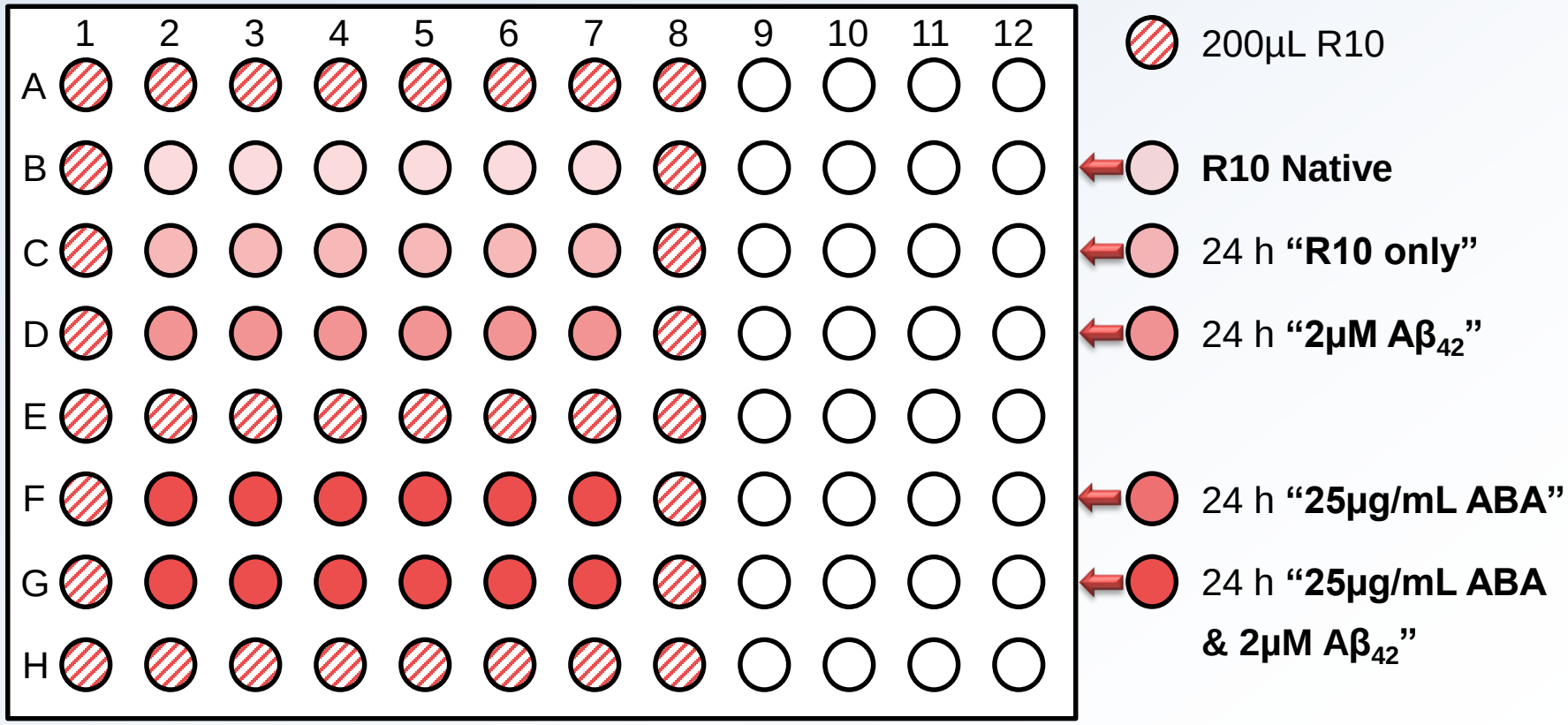
Al_2O_3 nanoparticles

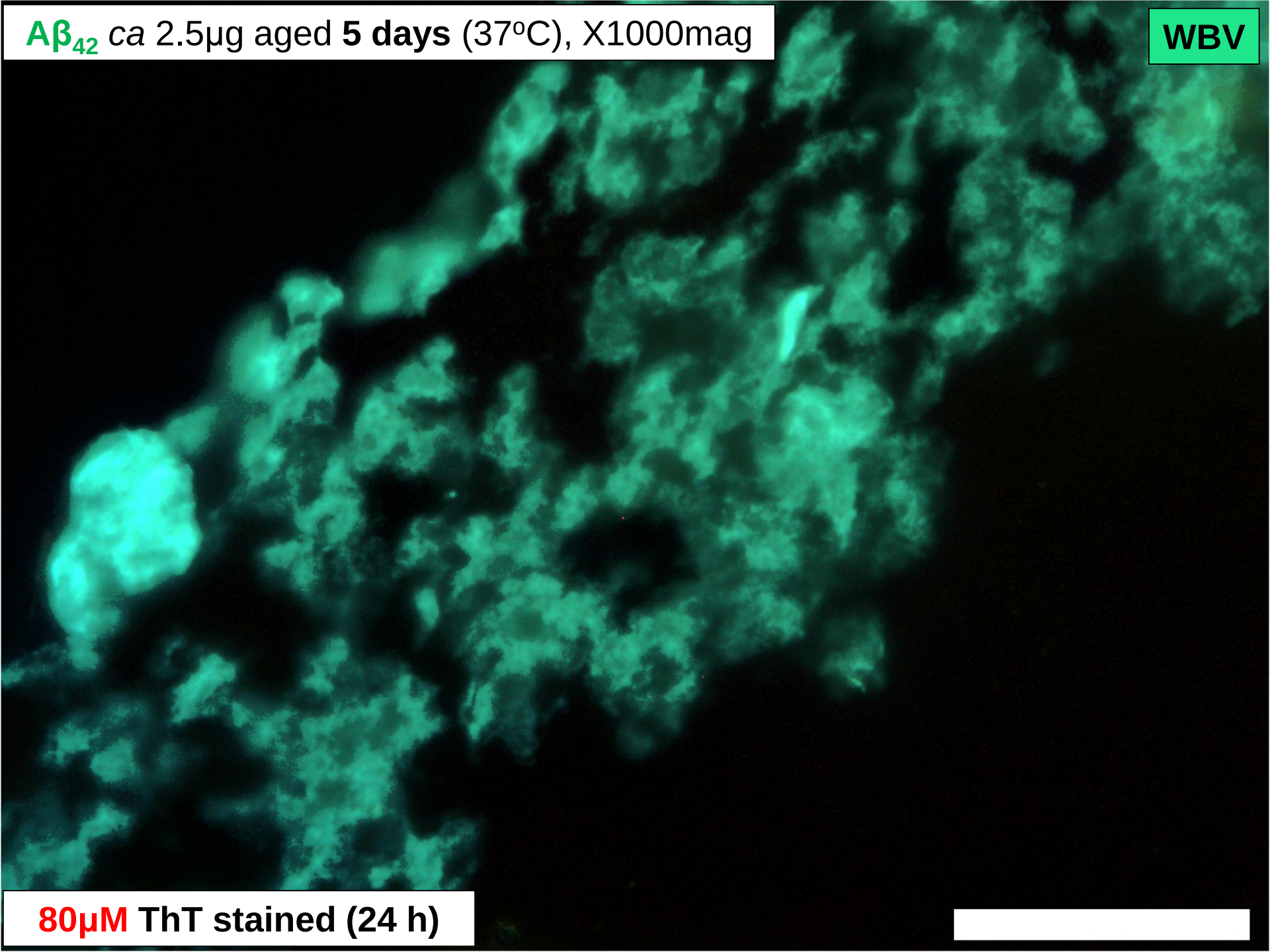

THP-1 cells & 50µgmL-1 Al2O3, X1000mag

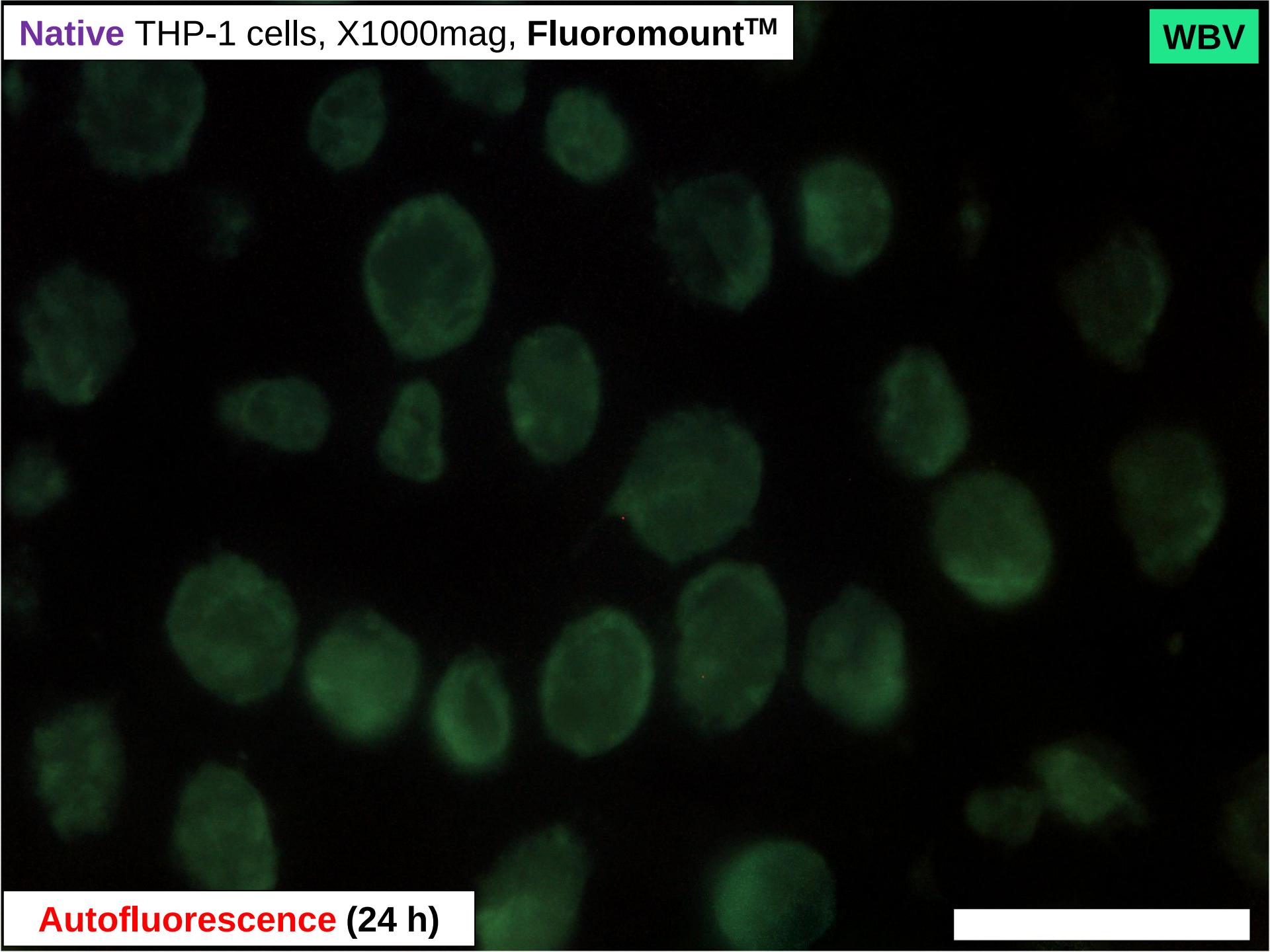

THP-1 cells & 50µgmL-1 Al2O3, X1000mag

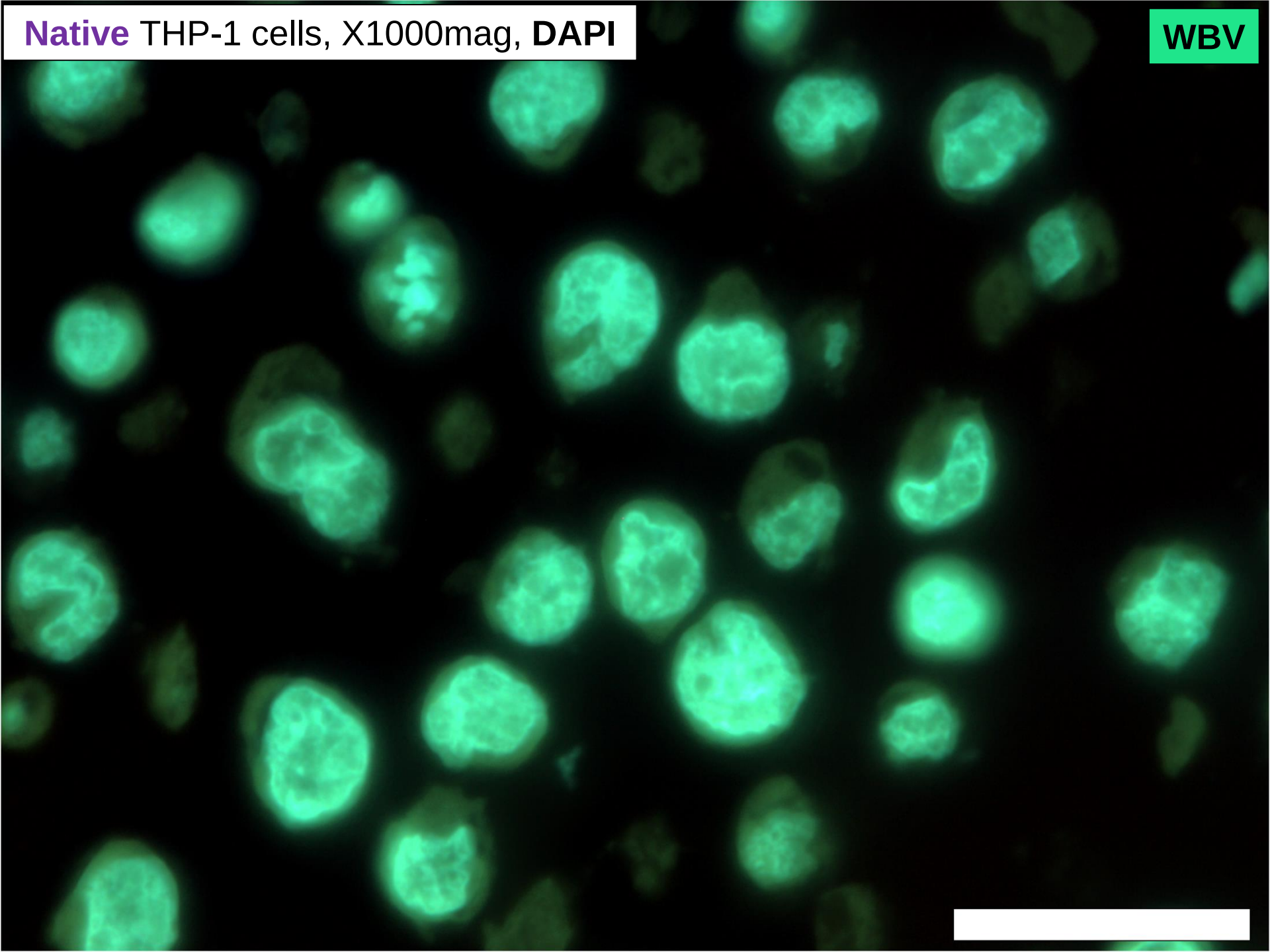
ABA & $A\beta_{42}$ co-culture

- THP-1 cells re-suspended in R10 to 5 x 10^5 cells/mL (50,000 per 100µL).
- 100µL 2X [ABA / $A\beta_{42}$] in R10 added following **24 h** incubation (37°C).
- 96 well plate format used and plates incubated for 24 h at 37°C + 5% CO_2.

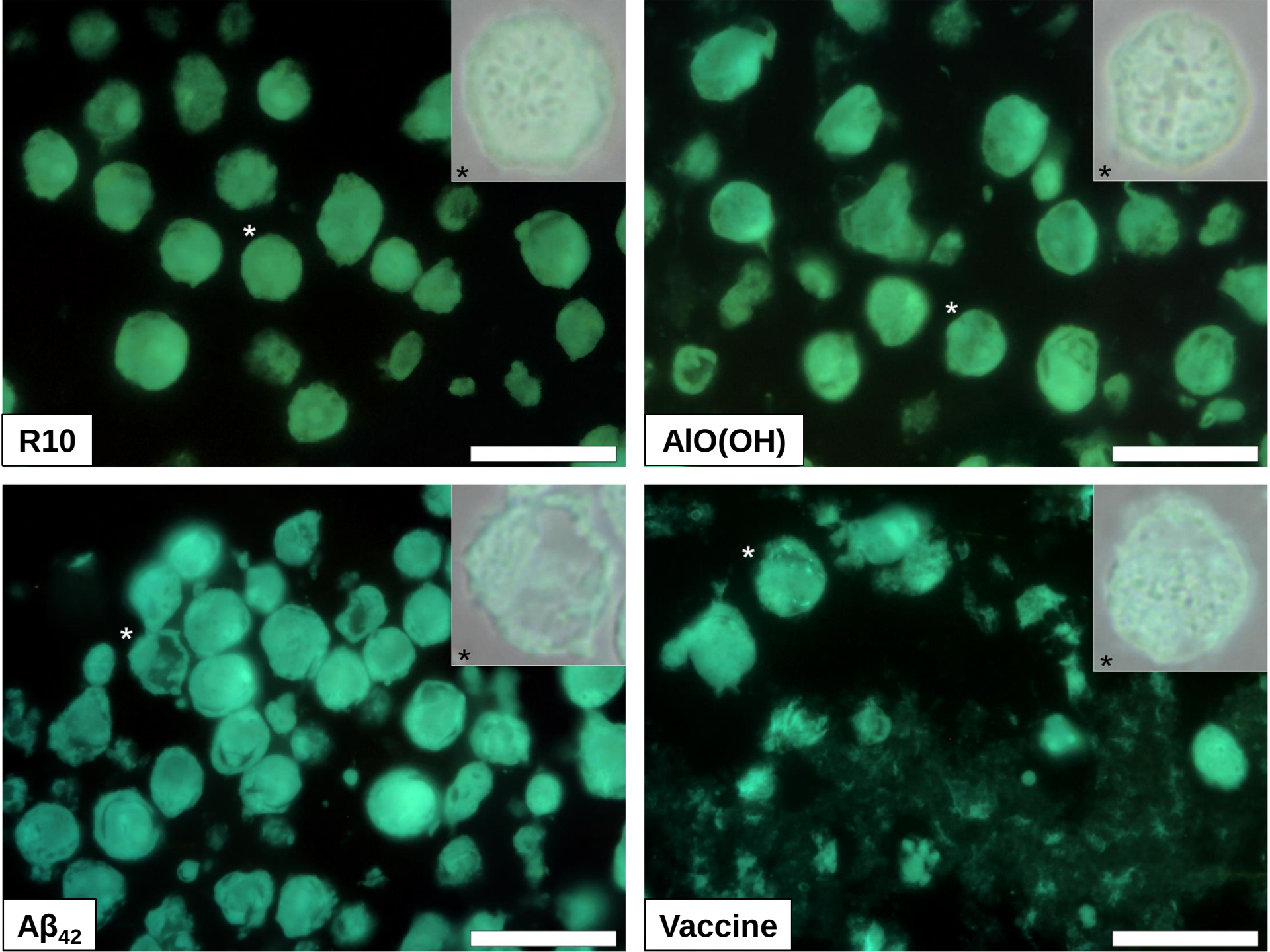



Aβ42 ca 2.5µg aged 5 days (37ºC), X1000mag
WBV
80µM ThT stained (24 h)

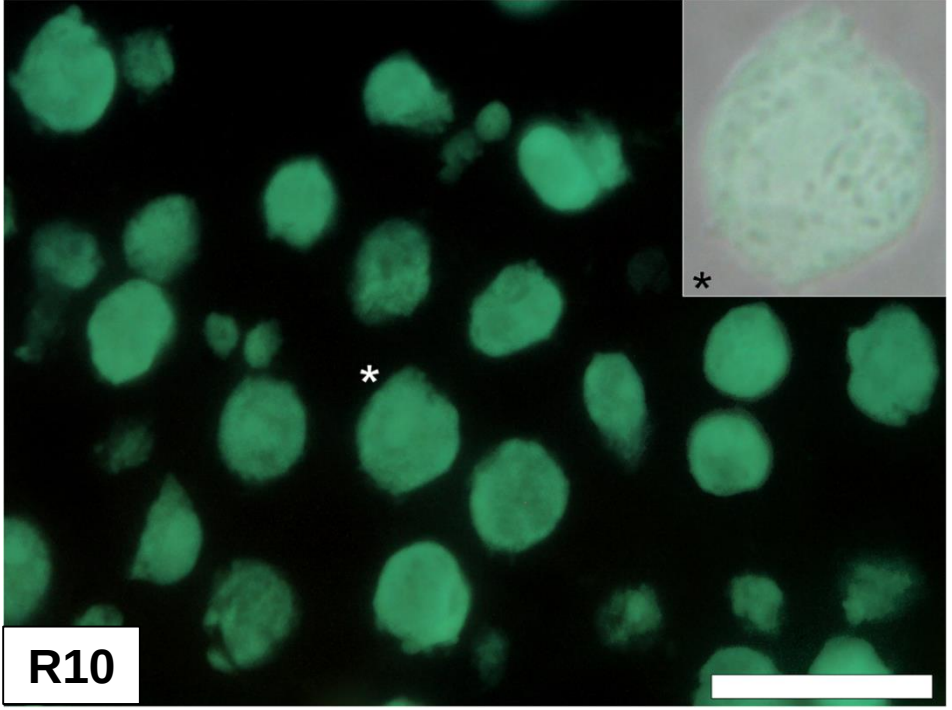

Native THP-1 cells, X1000mag, Fluoromount™
WBV
Autofluorescence (24 h)

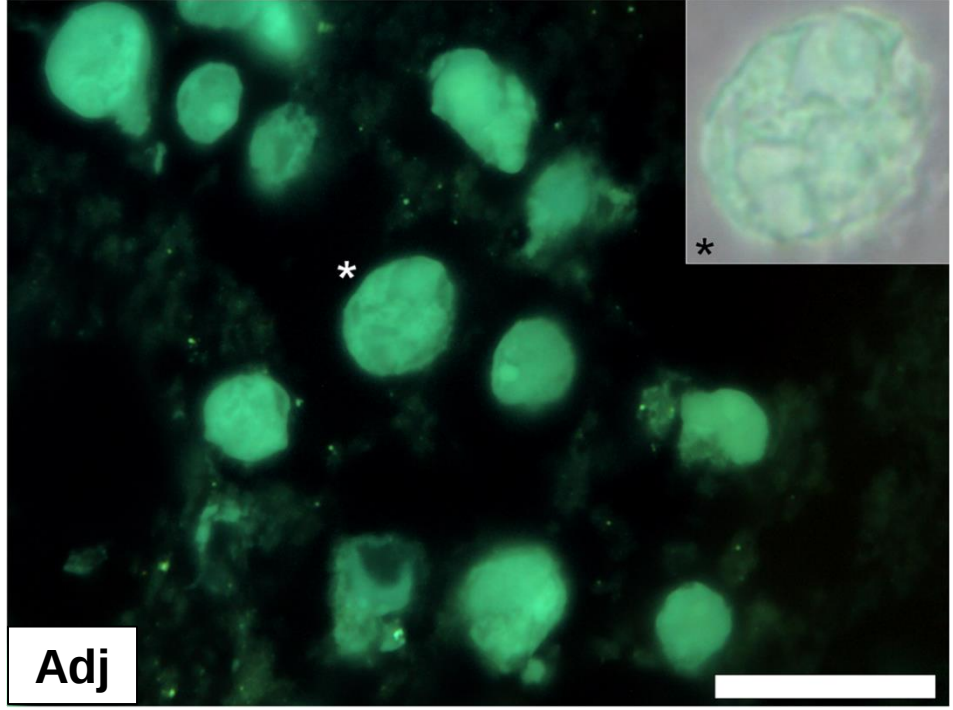

Native THP-1 cells, X1000mag, DAPI
WBV

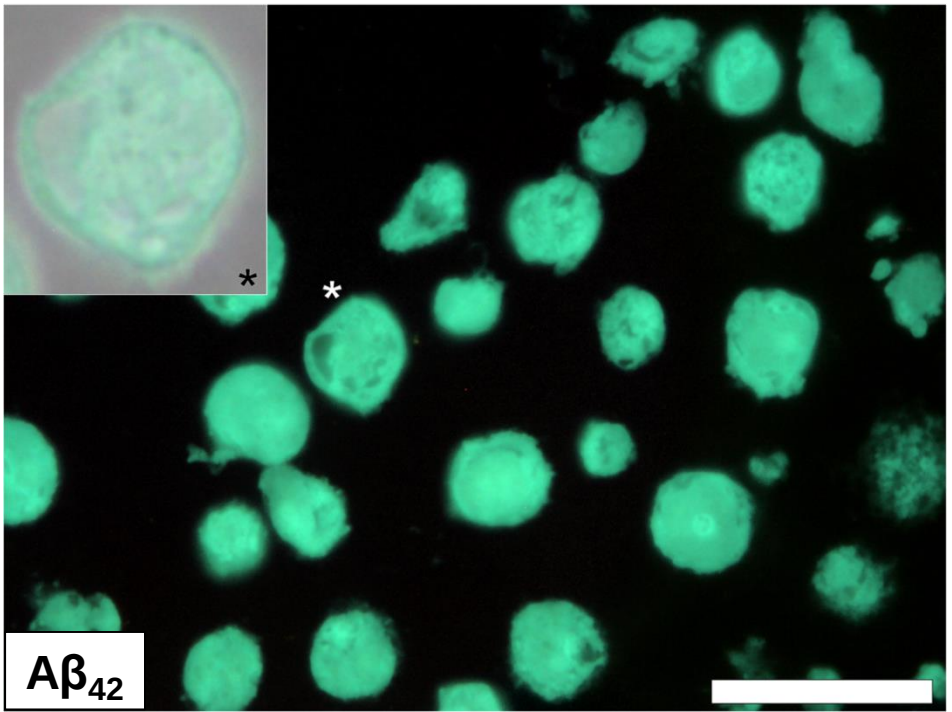
Alhydrogel® & $A\beta_{42}$

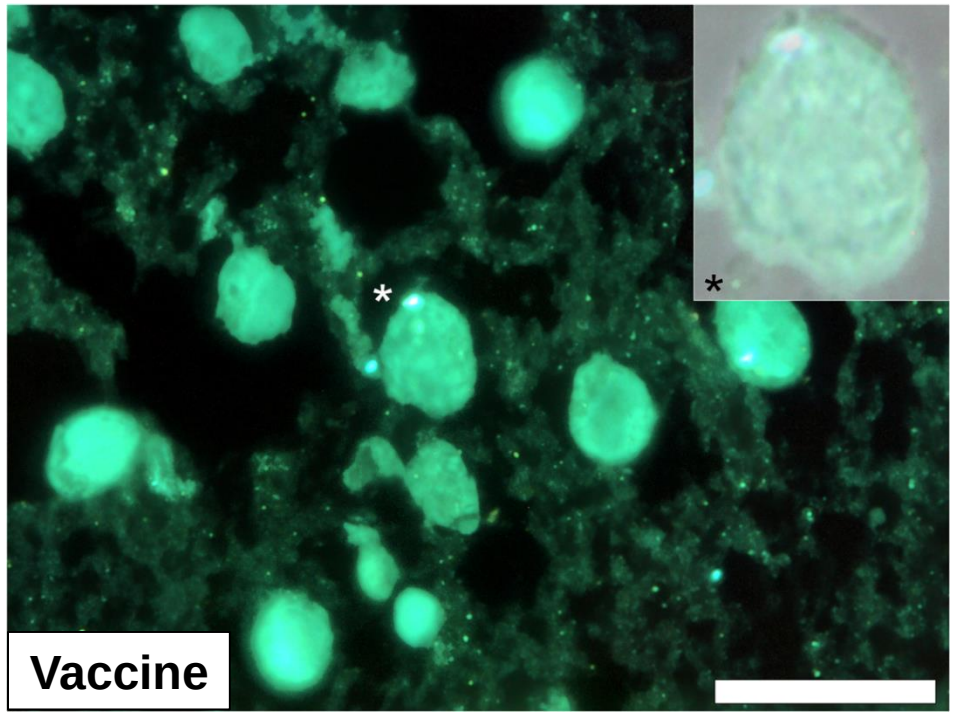

R10
AlO(OH)
Aβ42
Vaccine

Adju-Phos® & $A\beta_{42}$


R10


Adj


Aβ42


Vaccine

Conclusions

- For the two **clinically approved** Alhydrogel® and Adju-Phos® ABA, **intracellular** particulates were observed localised in cell cytoplasm only.
- Only co-culture with Adju-Phos® resulted in the release of **extracellular** genetic material.
- The **experimental** Imject® Alum ABA also exhibited particulates localised in the cell cytoplasm of THP-1 cells, typically **larger** in size.
- Higher [ABA] showed **reduced cellular uptake** (50 & 100µg/mL Adju-Phos® and 100µg/mL Imject® Alum).
- **LM** analyses of Al_2O_3 nanoparticles suggested their internalisation in **both** cell cytoplasm and cell nuclei.

Conclusions

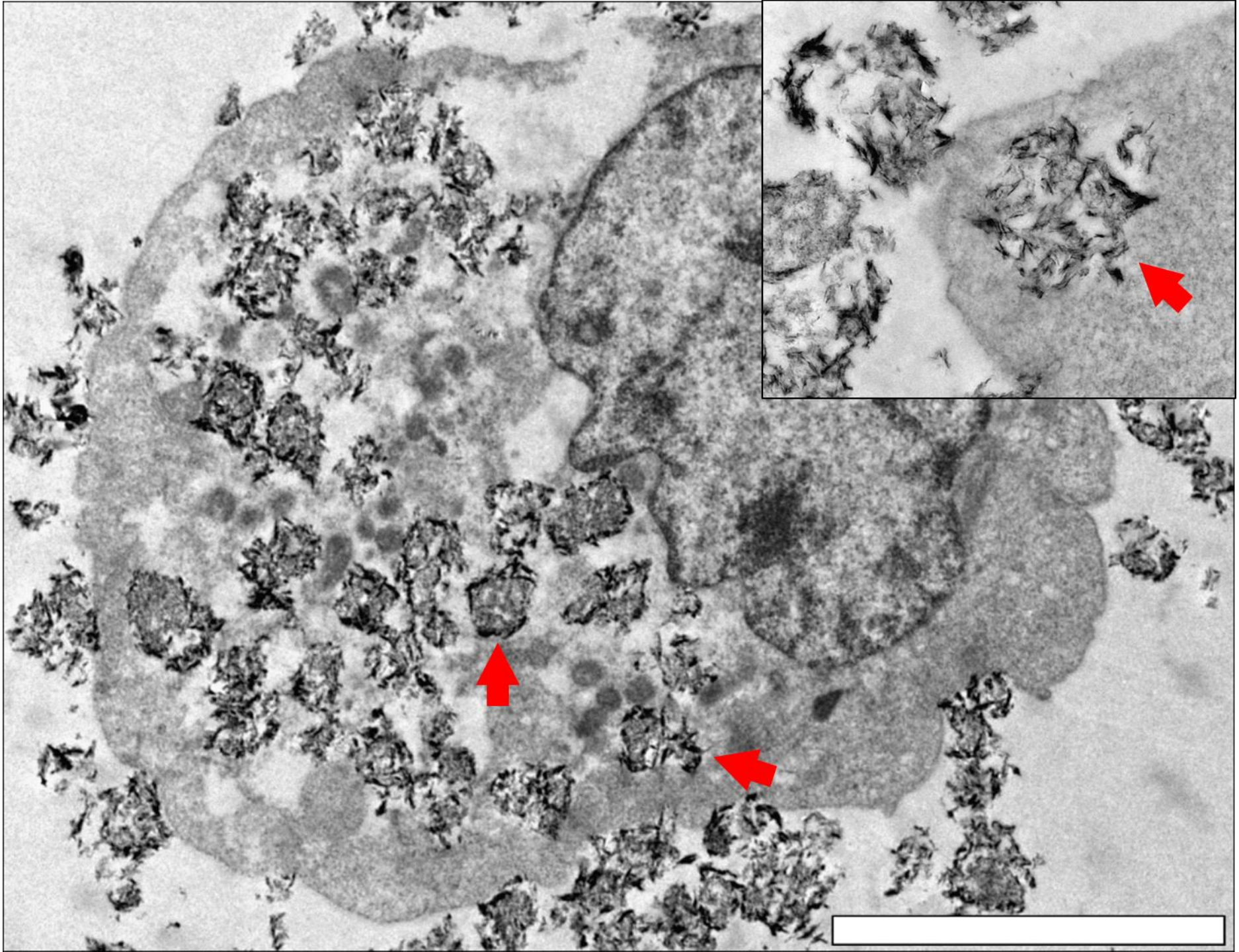
- **Autofluorescence** from native THP-1 cells produced a **green** fluorescence emission.

- Co-culture of THP-1 cells with **$A\beta_{42}$** stained with ThT produced an enhanced **blue-green** fluorescence emission.

- Unequivocal identification of internalised $A\beta_{42}$ in THP-1 cells using ThT however has thus far not been possible due to a predominantly green fluorescence emission from native THP-1 cells only, stained under identical conditions.

Future work

- Optimise **ThT staining protocol** for the unequivocal identification of $A\beta_{42}$ in a THP-1 cell line.
- Optimise **$A\beta_{42}$ concentrations** and ageing of the amyloidogenic peptide for inclusion in THP-1 cell co-cultures.
- Assess the use of **Congo red** for the visualisation of potentially internalised $A\beta_{42}$.
- Investigate the cellular uptake of ABA and $A\beta_{42}$ investigated herein using resin-embedding and **transmission electron microscopy** (especially useful for identification of potentially internalised Al_2O_3 nanoparticles).



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- **Differentiate** THP-1 cells into monocyte derived macrophages using phorobol 12-myristate 13-acetate and assess cellular uptake of the ABA investigated herein and their potential localisation with cellular organelles.

References

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- Dr Edward Visse
- Emma Sandén

- Dr David Mazzocchi Jones
- Dr Helen Price

- Karen Walker (EM unit)