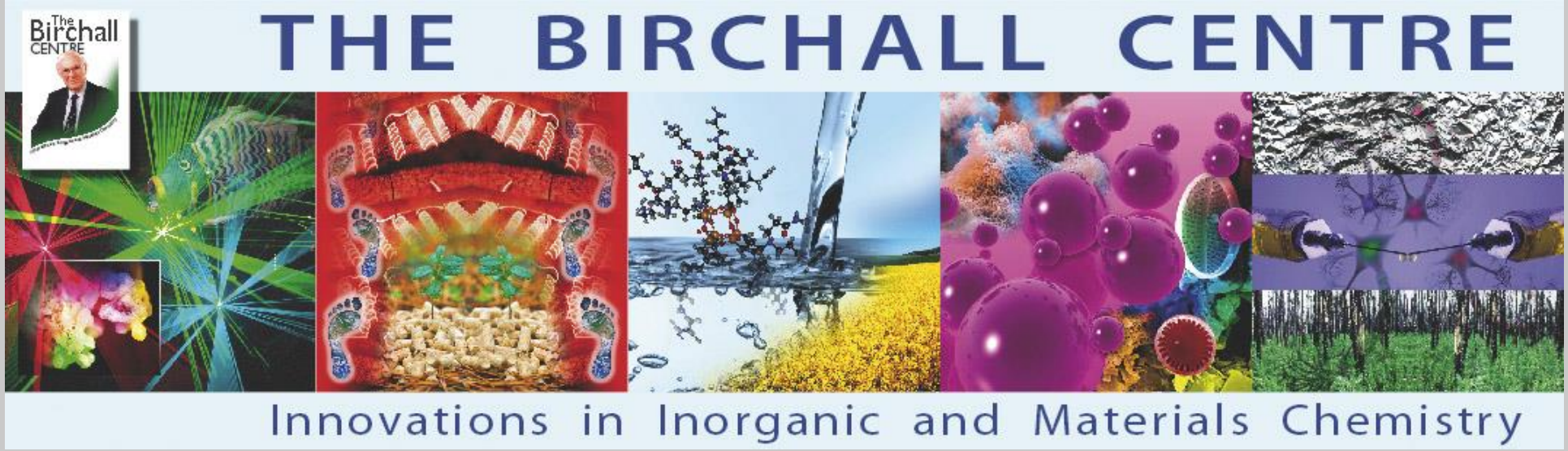

Systemic Toxicity of Aluminium Adjuvants

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http://www.keele.ac.uk/aluminium/

https://www.hippocraticpost.com/?s=Exley

Warsaw, 2019

There are no clinically-approved (aluminium) adjuvants!

There are only clinically-approved vaccines.

The safety of adjuvants is established alongside the safety of vaccines.

So, why are aluminium adjuvants used as placebos in vaccine safety trials?!

For example, in demonstrating the ‘safety’ of HPV vaccines

The NEW ENGLAND JOURNAL of MEDICINE

ESTABLISHED IN 1812 MAY 10, 2007 VOL. 356 NO. 19

Quadrivalent Vaccine against Human Papillomavirus to Prevent High-Grade Cervical Lesions

The FUTURE II Study Group*

Vaccine 29 (2011) 9289

Contents lists available at ScienceDirect

Vaccine

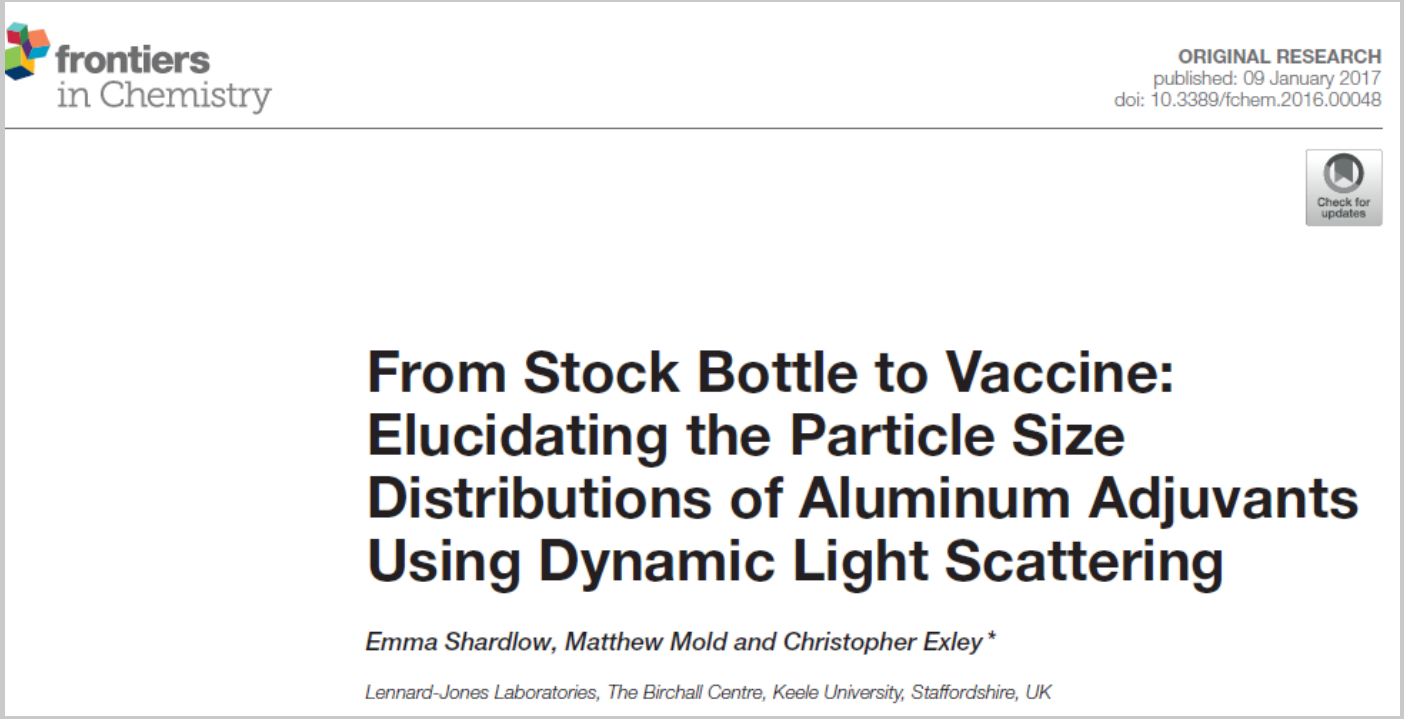
journal homepage: www.elsevier.com/locate/vaccine

Letter to the Editor

Aluminium-based adjuvants should not be used as placebos in clinical trials

[2] Hem SL, Johnston CT, HogenEsch H. Imject Alum is not aluminum hydroxide adjuvant or aluminum phosphate adjuvant. Vaccine 2007;25:4985–6.
[3] Exley C, Siesjö P, Eriksson H. The immunobiology of aluminium adjuvants: how

So, what do we know about aluminium adjuvants that are used in clinically-approved vaccines?



frontiers in Chemistry

ORIGINAL RESEARCH
published: 09 January 2017
doi: 10.3389/fchem.2016.00048

Check for updates

From Stock Bottle to Vaccine: Elucidating the Particle Size Distributions of Aluminum Adjuvants Using Dynamic Light Scattering

*Emma Shardlow, Matthew Mold and Christopher Exley**

Lennard-Jones Laboratories, The Birchall Centre, Keele University, Staffordshire, UK

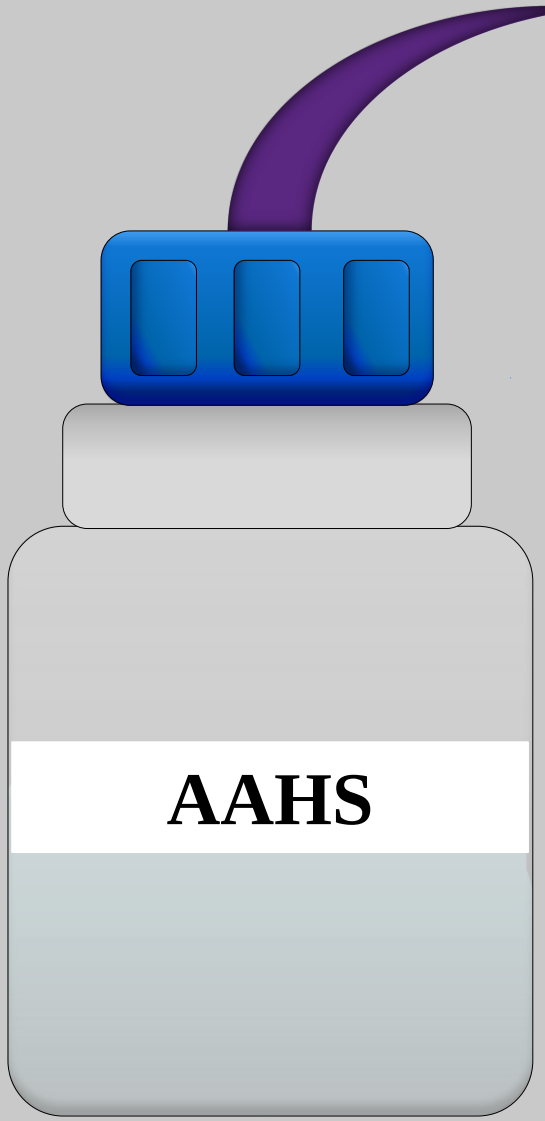
https://www.frontiersin.org/articles/10.3389/fchem.2016.00048/full

- Aluminium oxyhydroxide (boehmite)
- Poorly crystalline – hydrated structure (14.1% H_2O at the surface interface)
- Composed of nanoneedles - 4.5 nm × 2.2 nm × 10 nm in size
- Most frequently used adjuvant in commercial vaccines

Aluminium hydroxyphosphate

Amorphous – hydrated structure (24.2% H_2O at the surface interface)

Composed of platy particles – 50nm in size



Amorphous aluminium hydroxyphosphate-sulphate

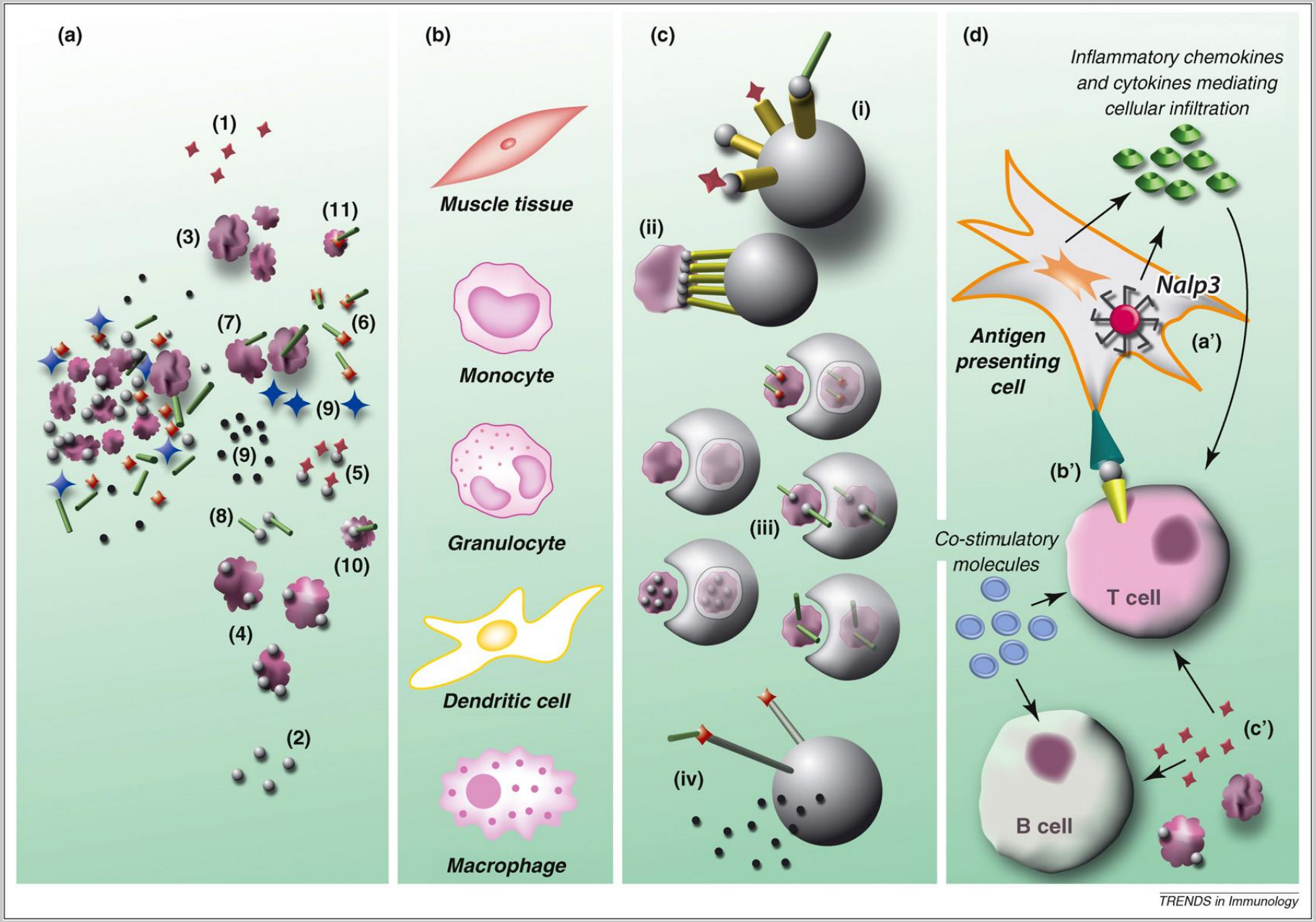
?

?

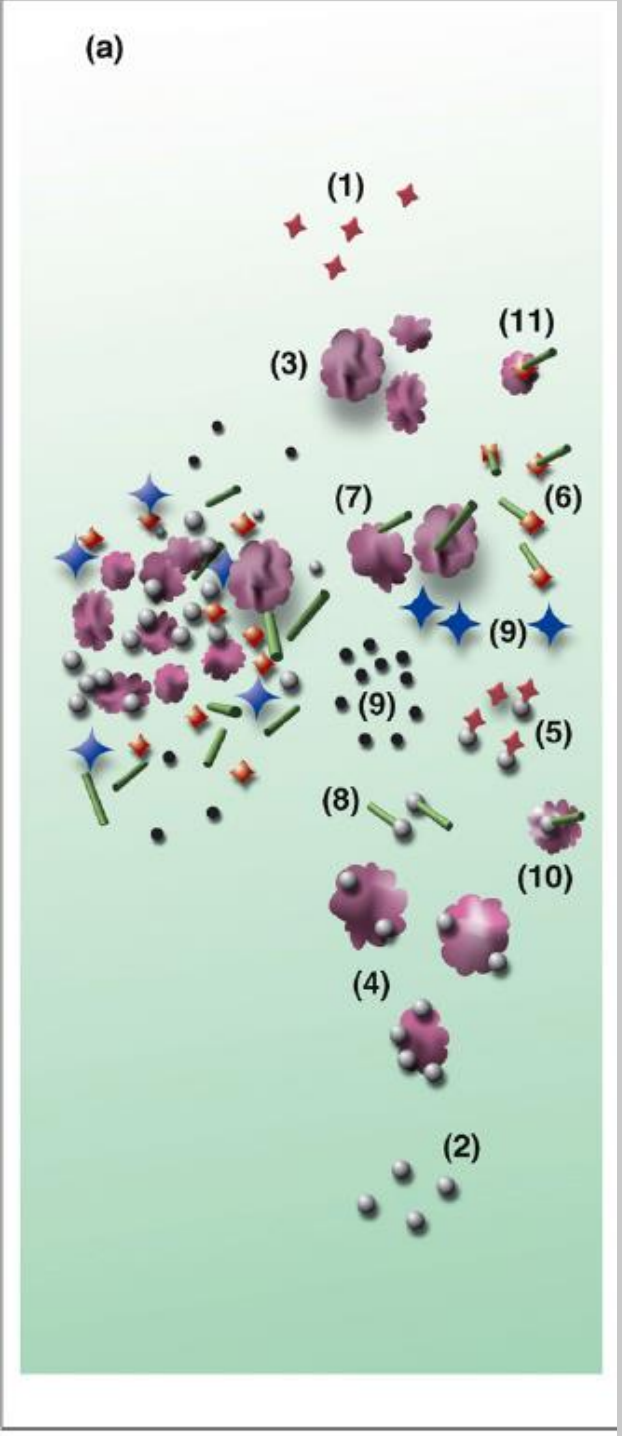
Gardasil!

So, how do aluminium adjuvants work?

How might understanding this also begin to explain the known adverse events associated with their use in vaccines?



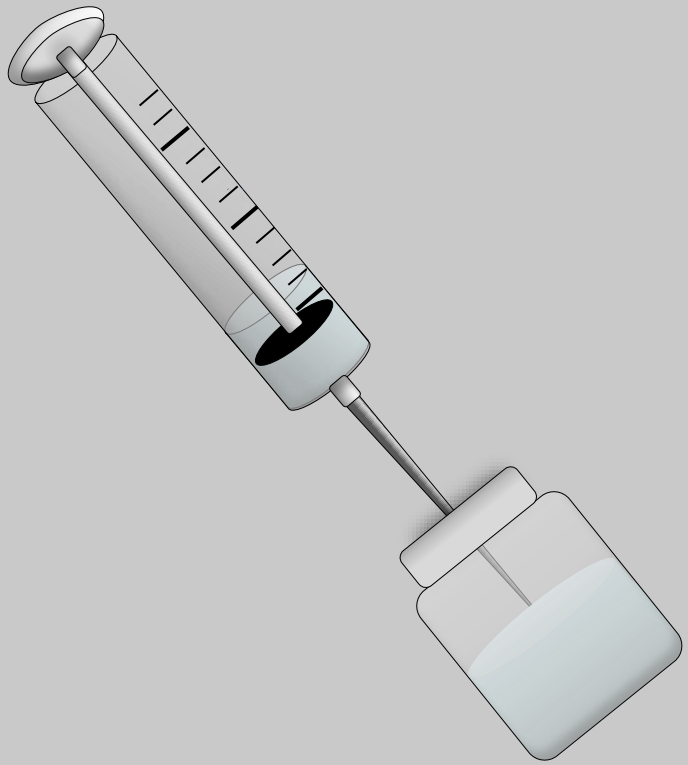
Exley et al.,(2010) The Immunobiology of aluminium adjuvants; how do they really work? Trends in Immunology, 31,103-109



The Critical Environment of the Injection Site

A. Dilution of the vaccine preparation into the muscle interstitial fluid (MIF) results in an array of potential agonists of the immune cascade including; **(1)** $Al^{3+}_{(aq)}$; **(2)** free antigen (AG); **(3)** particulate adjuvant (ADJ); **(4)** ADJ with associated AG; **(5)** AG-Al complex; **(6)** MIF ligand-Al complex; **(7)** ADJ with associated MIF ligand; **(8)** MIF ligand-AG complex; **(9)** particulate iron (as contaminant of adjuvant) either free or with adsorbed Al/AG and resultant reactive oxygen species (ROS); **(10)** ADJ with associated MIF ligand-AG complex; **(11)** ADJ with associated MIF ligand-Al complex. MIF ligands might include biomolecules such as; ATP, albumin, transferrin, citrate, fibrinogen.

What Happens to the Aluminium Adjuvant?



Vaccine preparations (adjuvants in 0.9% NaCl)

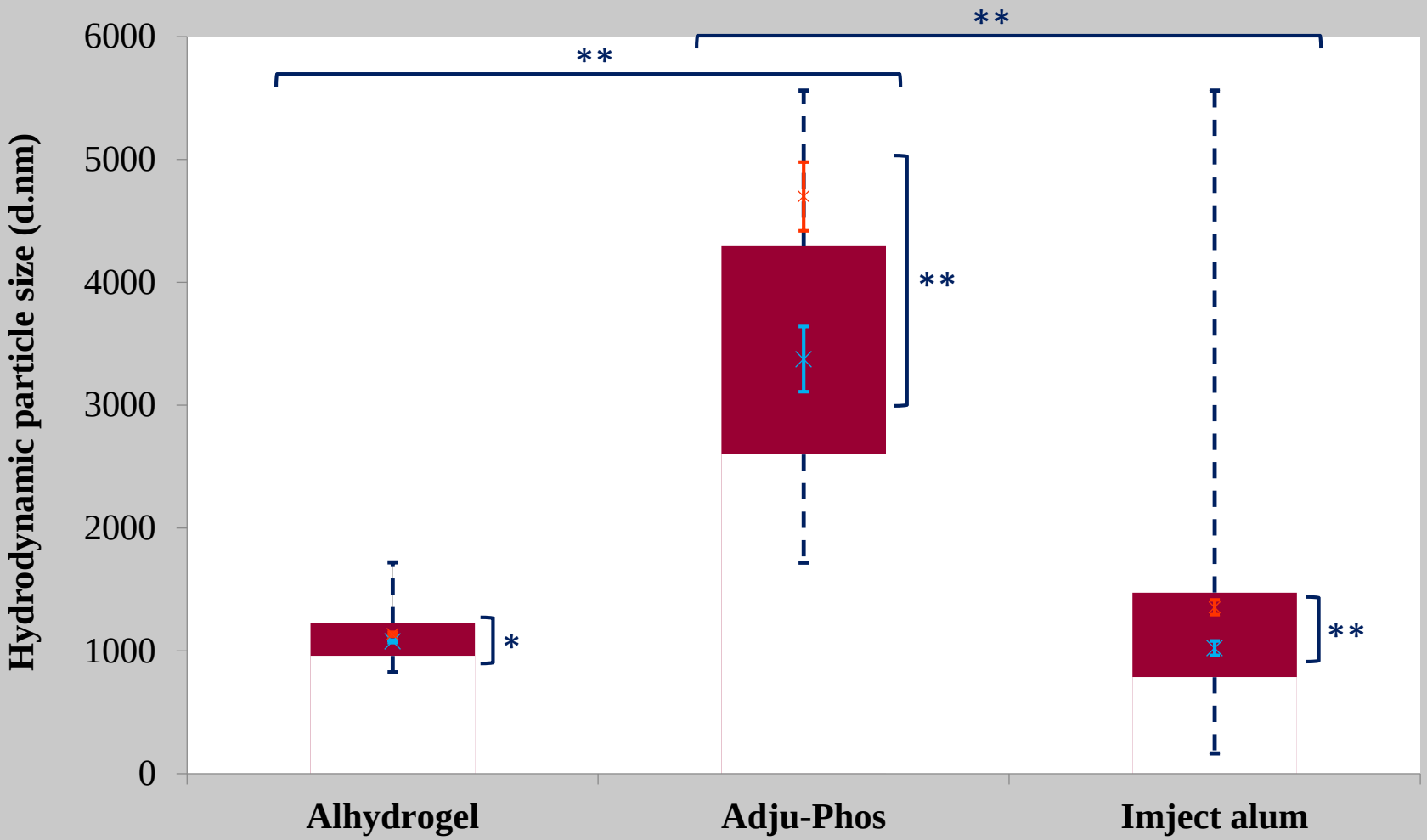
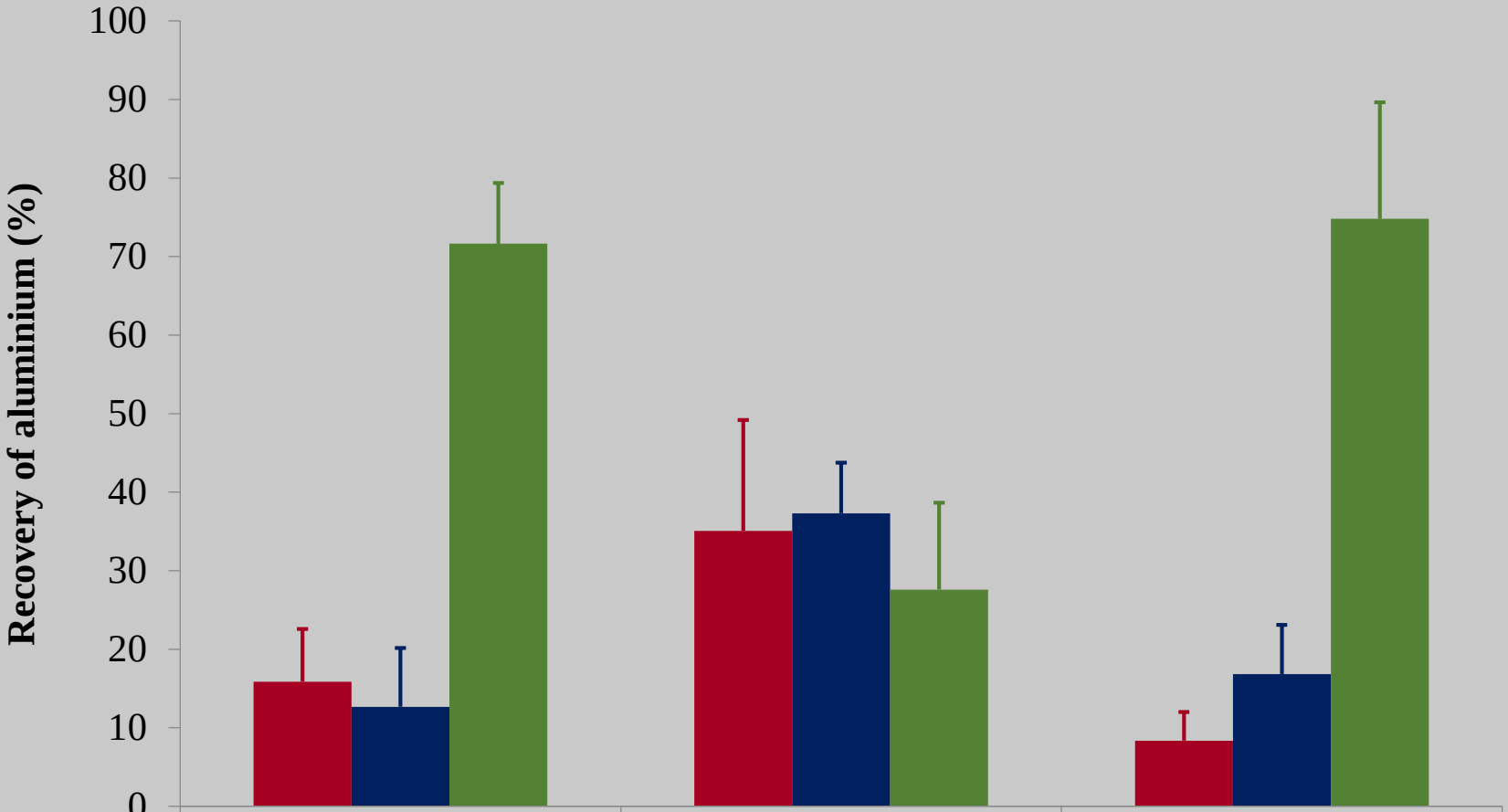


Fig 1: Size distributions of Alhydrogel, Adju-Phos & Imject alum in 0.9% NaCl following initial formulation (0hrs). Box plots are representative of the interquartile range of the data while blue dashed lines indicate the maxima and minima. Orange crosses indicate Z-average cumulant size values (nm) while light blue crosses represent the median peak size value (nm). Error bars represent the ±SE of the measurement where n = 5.



	Alhydrogel	Adju-Phos	Imject alum
>5.6µm	16	35	8
<5.6µm	13	37	17
<2.7µm	72	28	75

Fig 2: Recovery of Al (%) following selective filtration of Alhydrogel, Adju-Phos & Imject alum in 0.9% NaCl post initial formulation (0hrs). Error bars represent the %RSD of the measurement where n = 5.

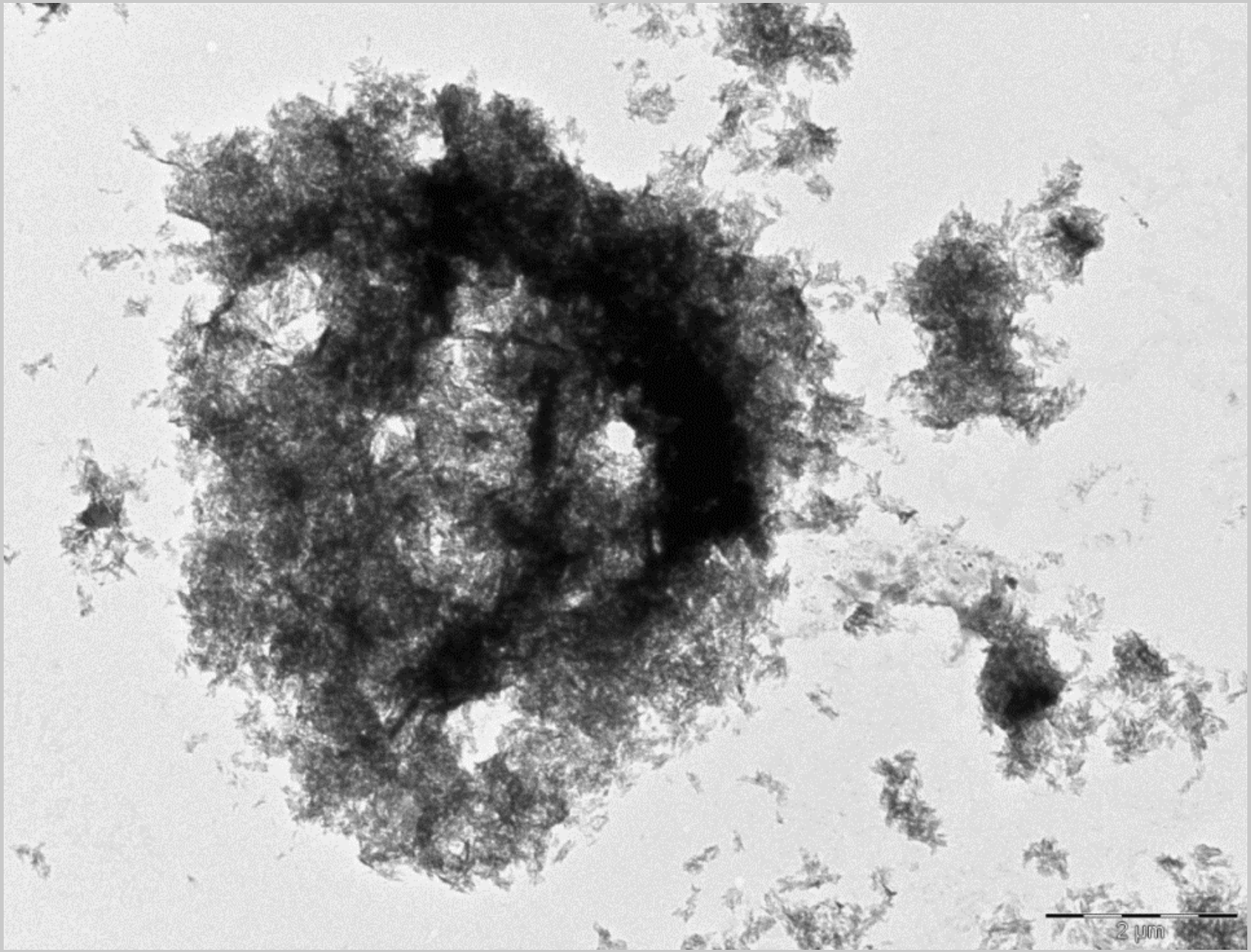


Fig 3: TEM image of Alhydrogel in 0.9% NaCl (0hrs). Mag. 10,000X, scale bar 2µm.

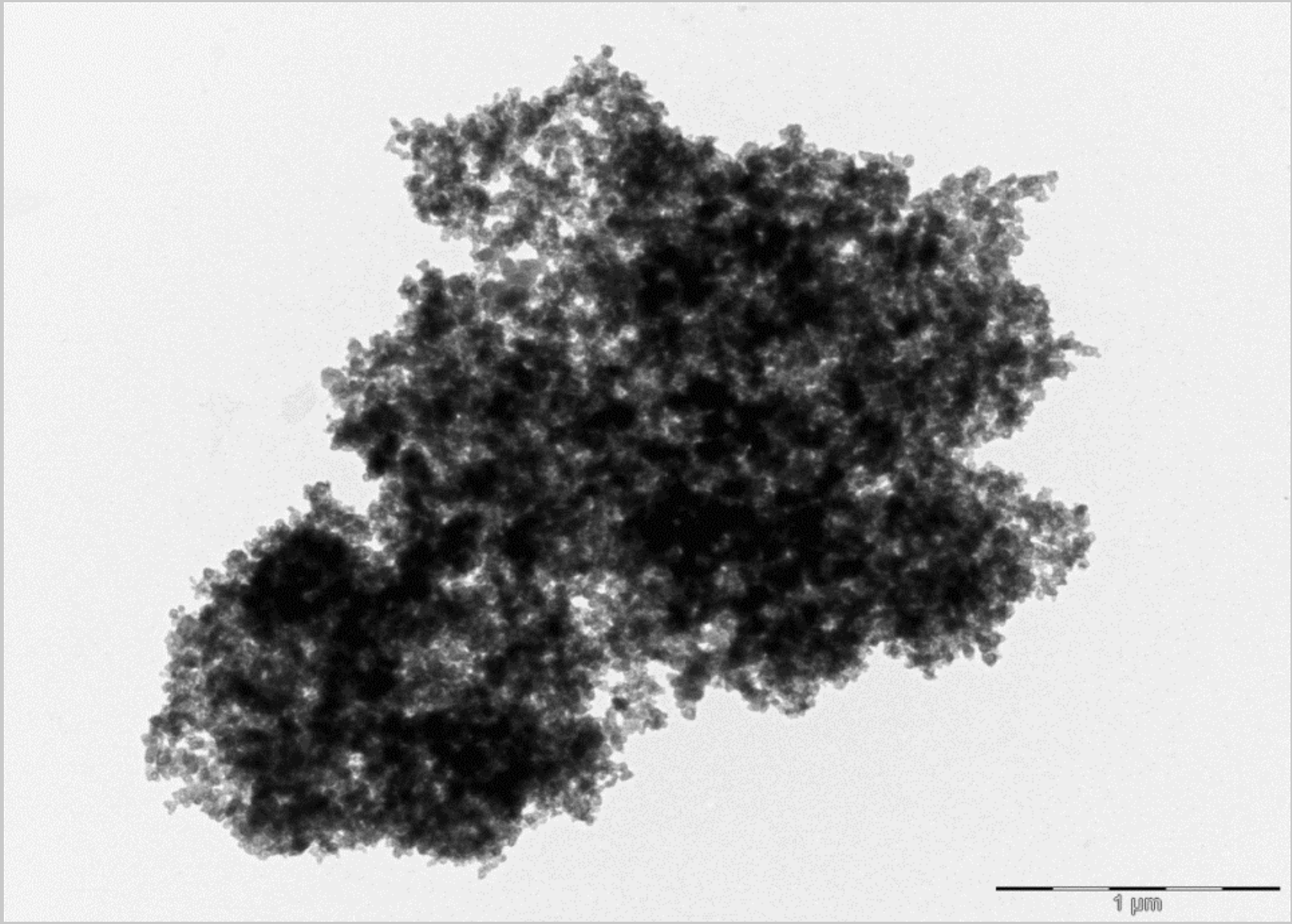


Fig 4: TEM image of Adju-Phos in 0.9% NaCl (0hrs). Mag. 30,000X, scale bar 1µm.

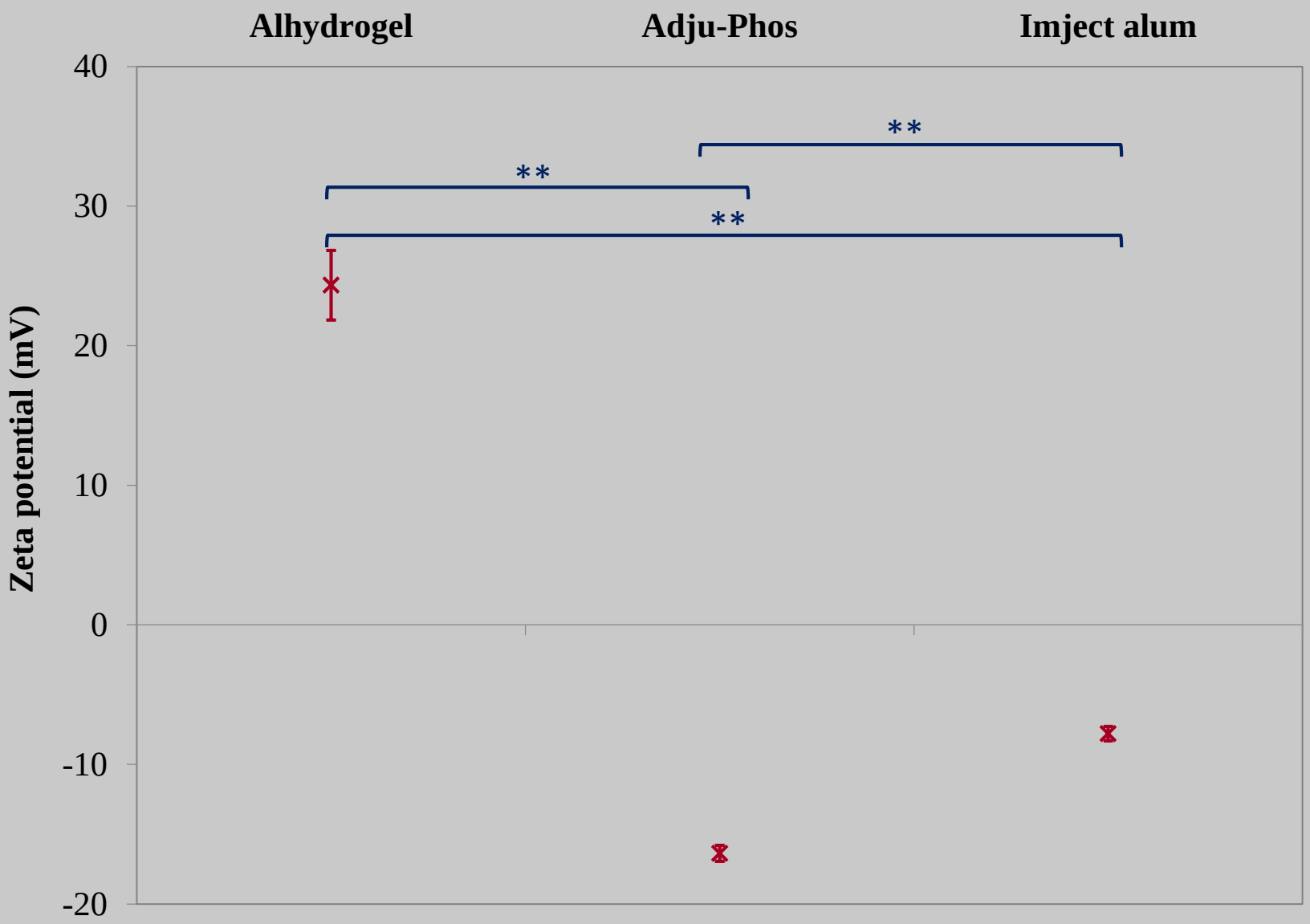
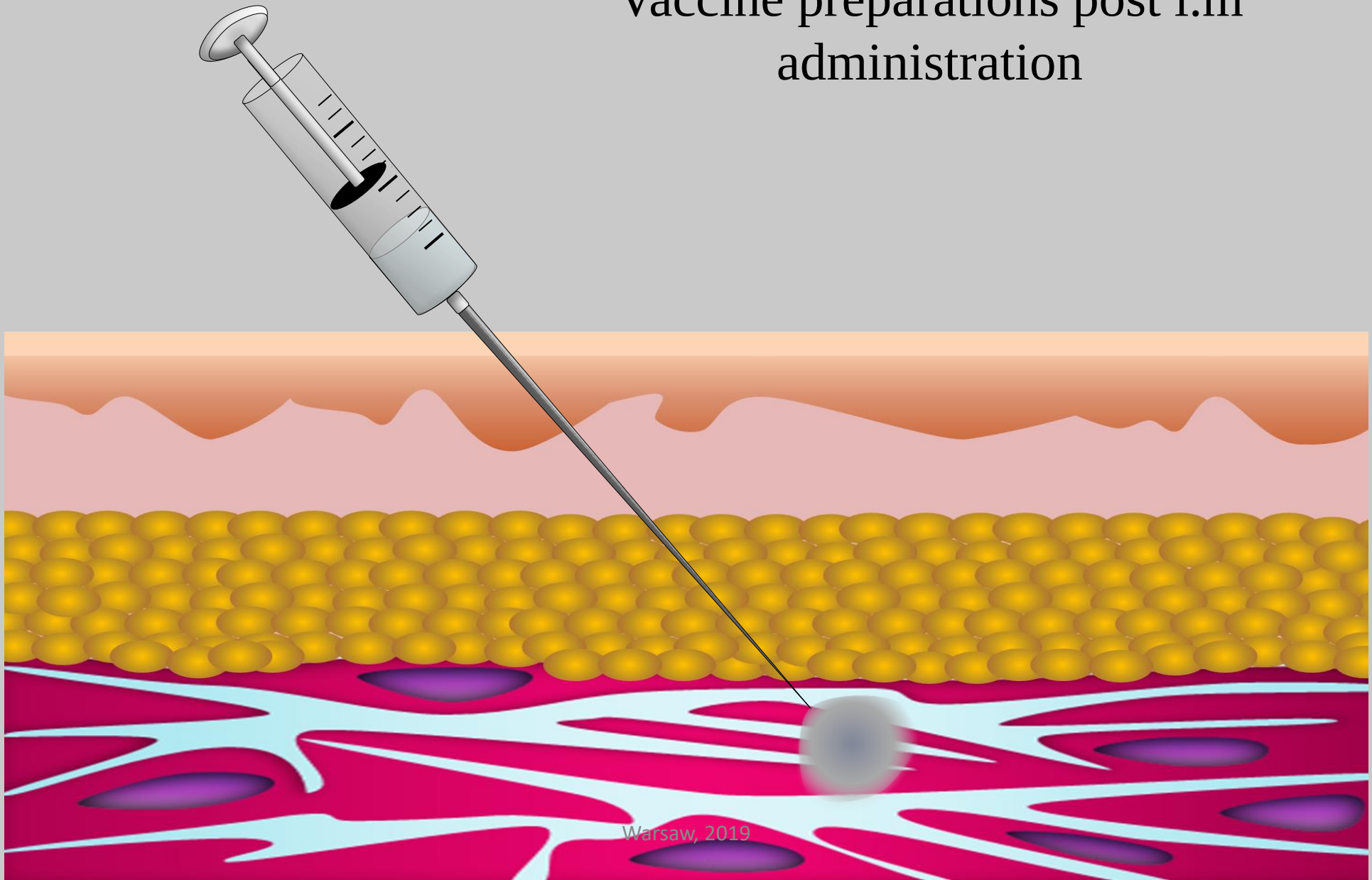


Fig 6: Zeta potential measurements of Alhydrogel, Adju-Phos & Imject alum in 0.9% NaCl following initial formulation (0hrs). Error bars represent the ±SD where n = 5.

Vaccine preparations post i.m administration



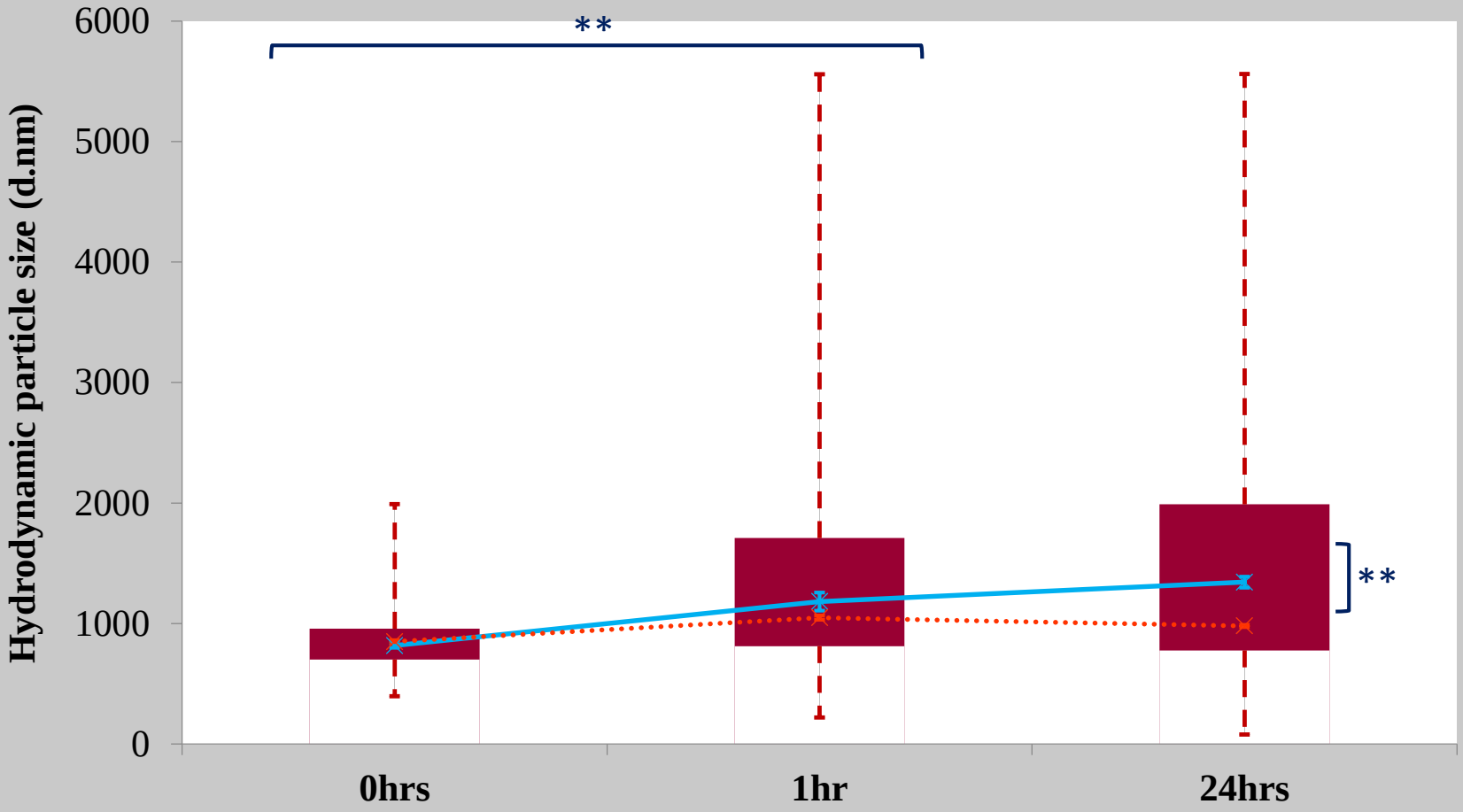
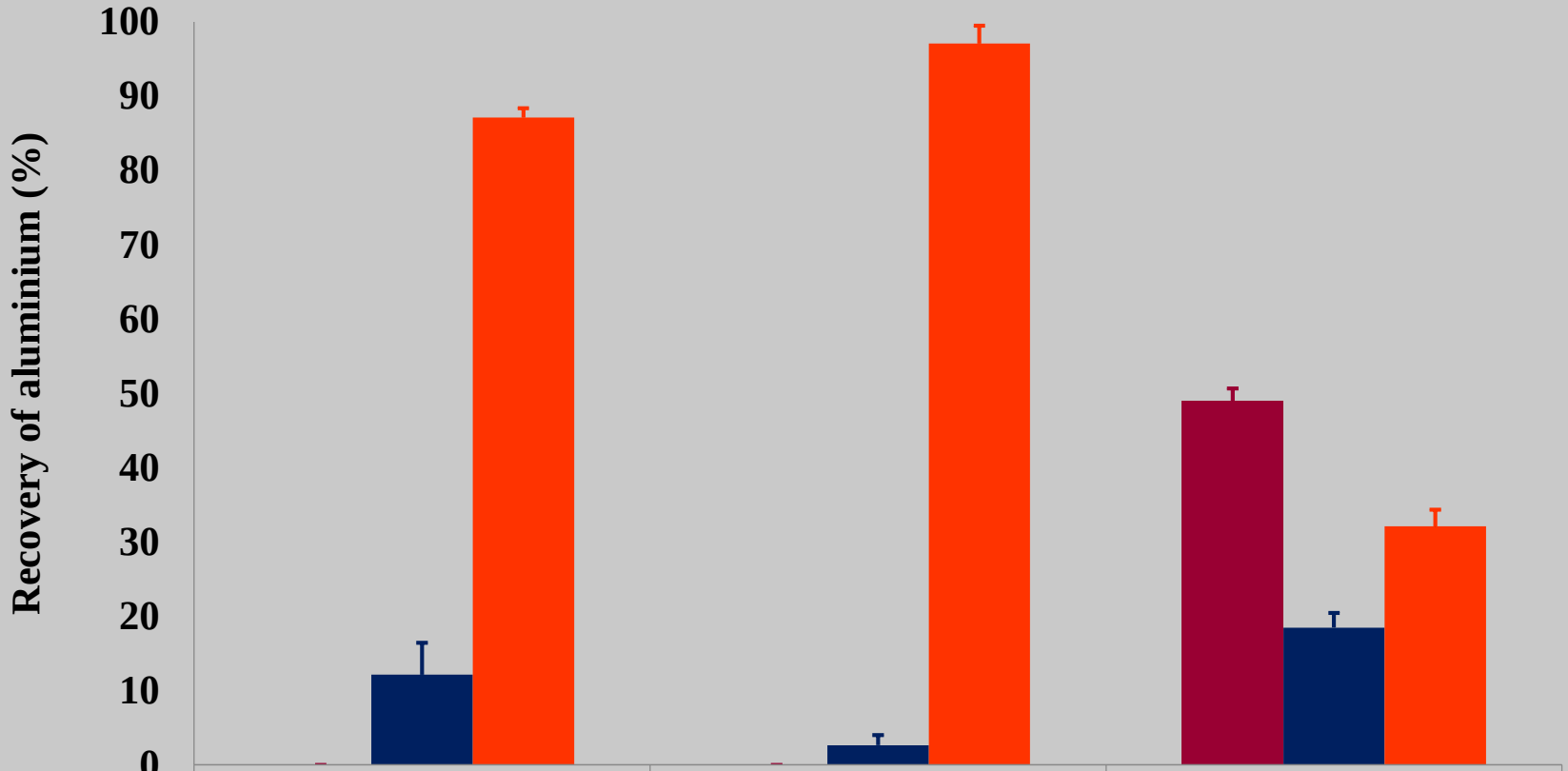


Fig 7: Size distributions of Alhydrogel in R10 medium following 0, 1 & 24hrs incubation (37°C). Box plots are representative of the interquartile range of the data while blue dashed lines indicate the maxima and minima. Orange crosses indicate Z-average cumulant size values (nm) while light blue crosses represent the median peak size value (nm). Error bars represent the ±SE of the measurement where n = 5.



	0hrs	1hr	24hrs
>5.6µm	0	0	49
<5.6µm	12	3	18
<2.7µm	87	97	32

Fig 8: Recovery of Al (%) following selective filtration of Alhydrogel in R10 medium following 0, 1 & 24hrs incubation (37°C). Error bars represent the %RSD of the measurement where n = 5.

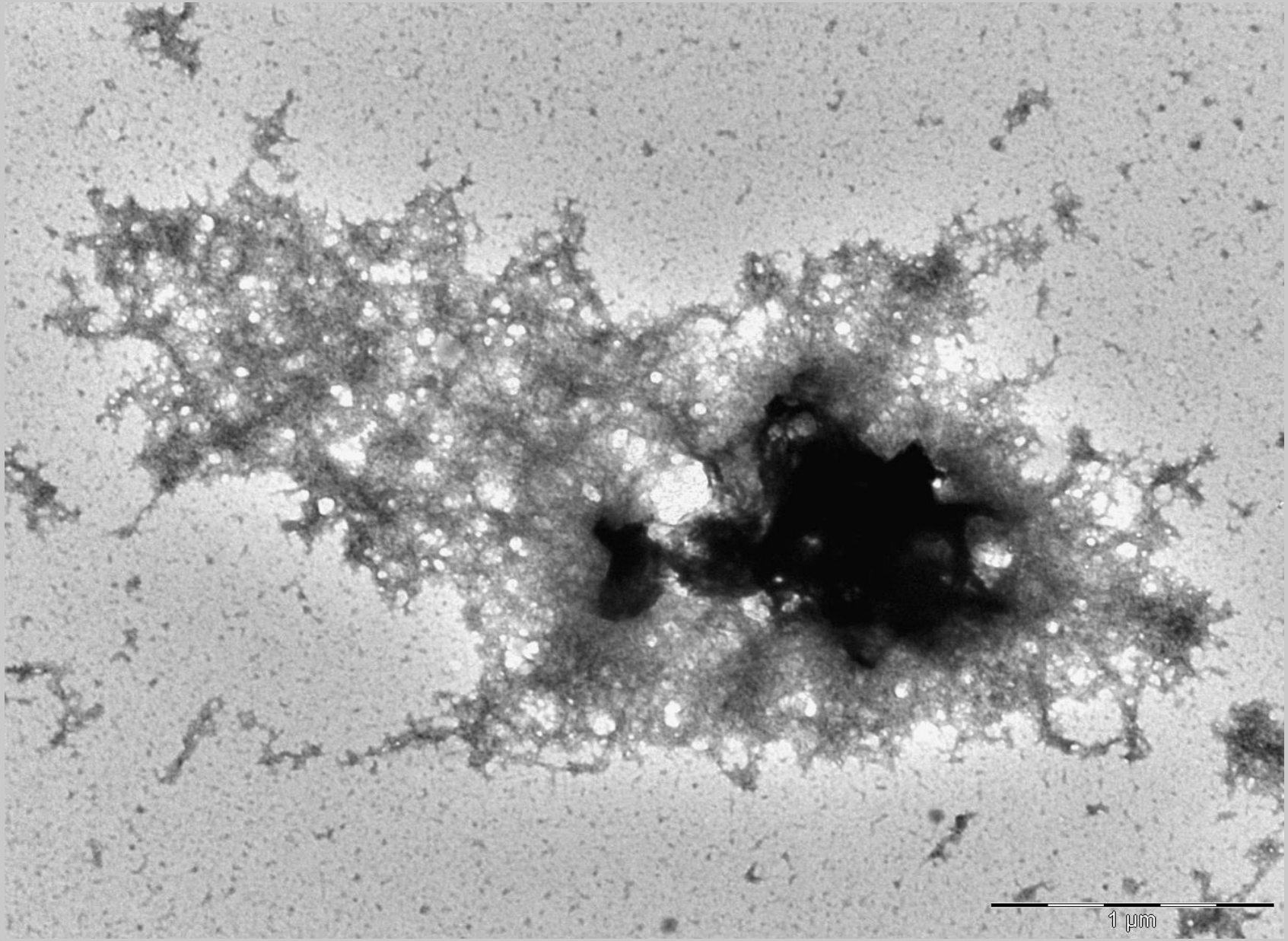


Fig 9: TEM image of Alhydrogel in R10 medium (0hrs). Mag. 30,000X, scale bar 1µm.

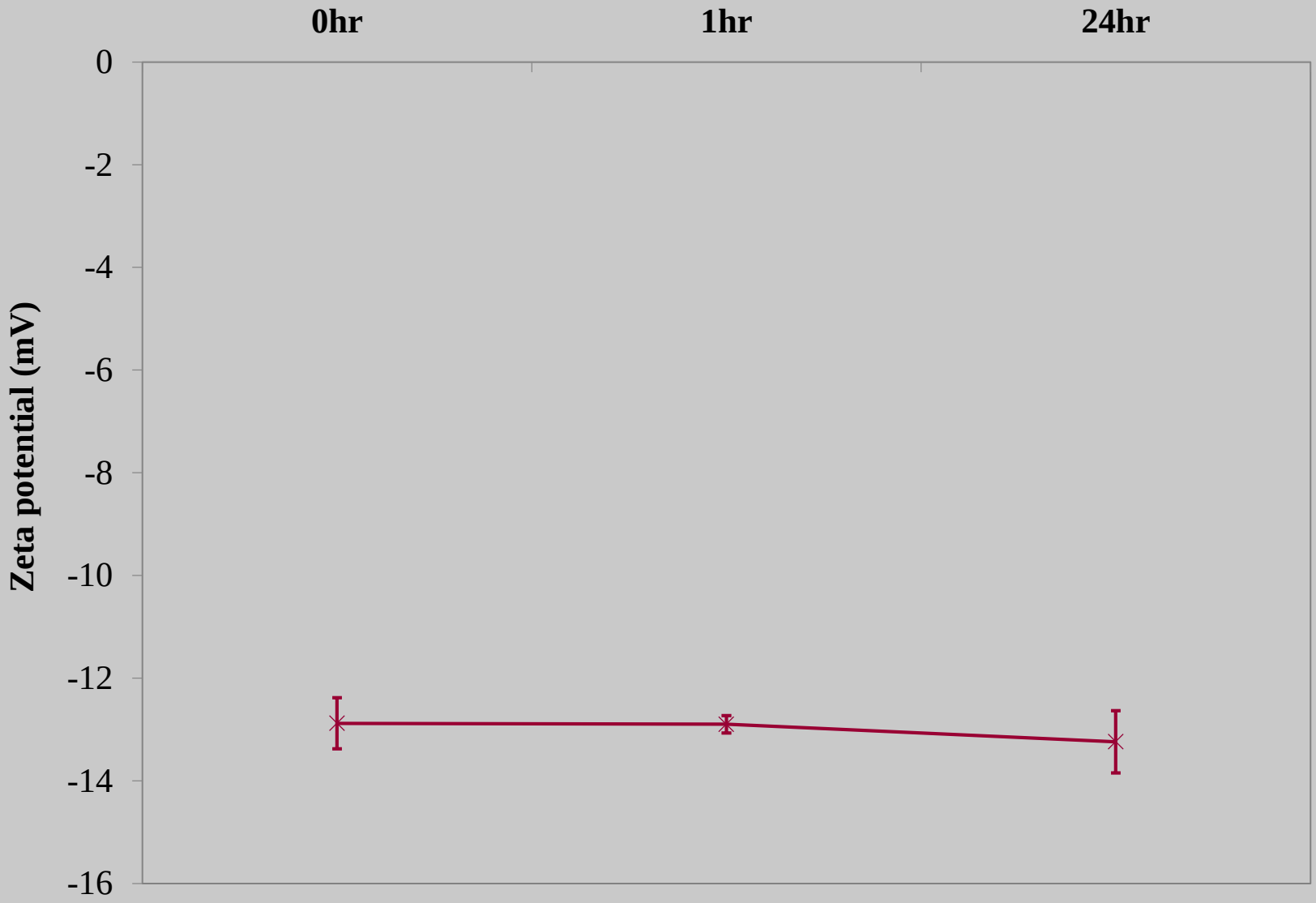


Fig 10: Zeta potential measurements of Alhydrogel in R10 medium following 0, 1 & 24hrs incubation (37°C). Error bars represent the ±SD where n = 5.

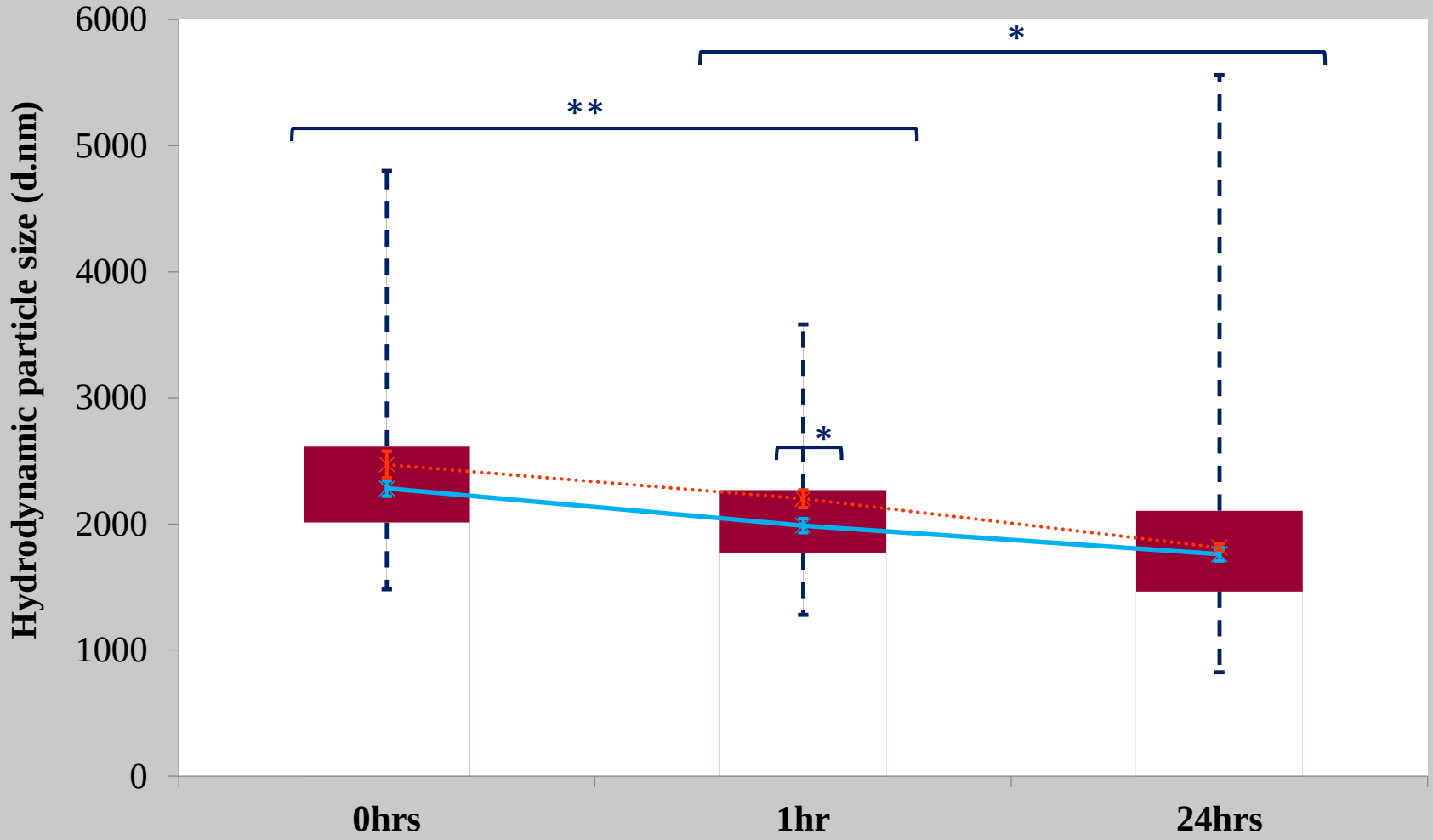
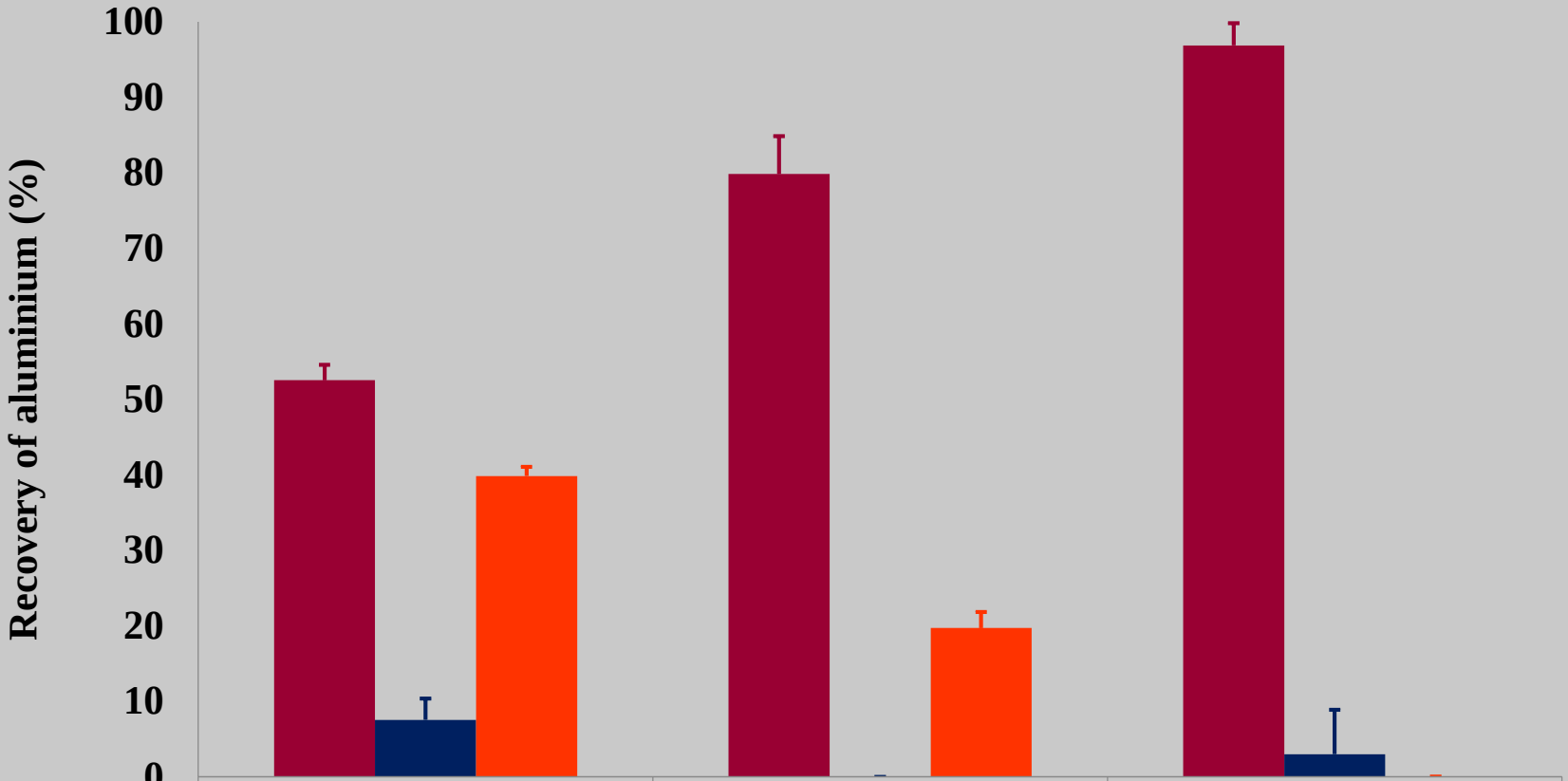


Fig 11: Size distributions of Adju-Phos in R10 medium following 0, 1 & 24hrs incubation (37°C). Box plots are representative of the interquartile range of the data while blue dashed lines indicate the maxima and minima. Orange crosses indicate Z-average cumulant size values (nm) while light blue crosses represent the median peak size value (nm). Error bars represent the ±SE of the measurement where n = 5.



	0hrs	1hr	24hrs
>5.6µm	53	80	97
<5.6µm	8	0	3
<2.7µm	40	20	0

Fig 12: Recovery of Al (%) following selective filtration of Adju-Phos in R10 medium following 0, 1 & 24hrs incubation (37°C). Error bars represent the %RSD of the measurement where n = 5.

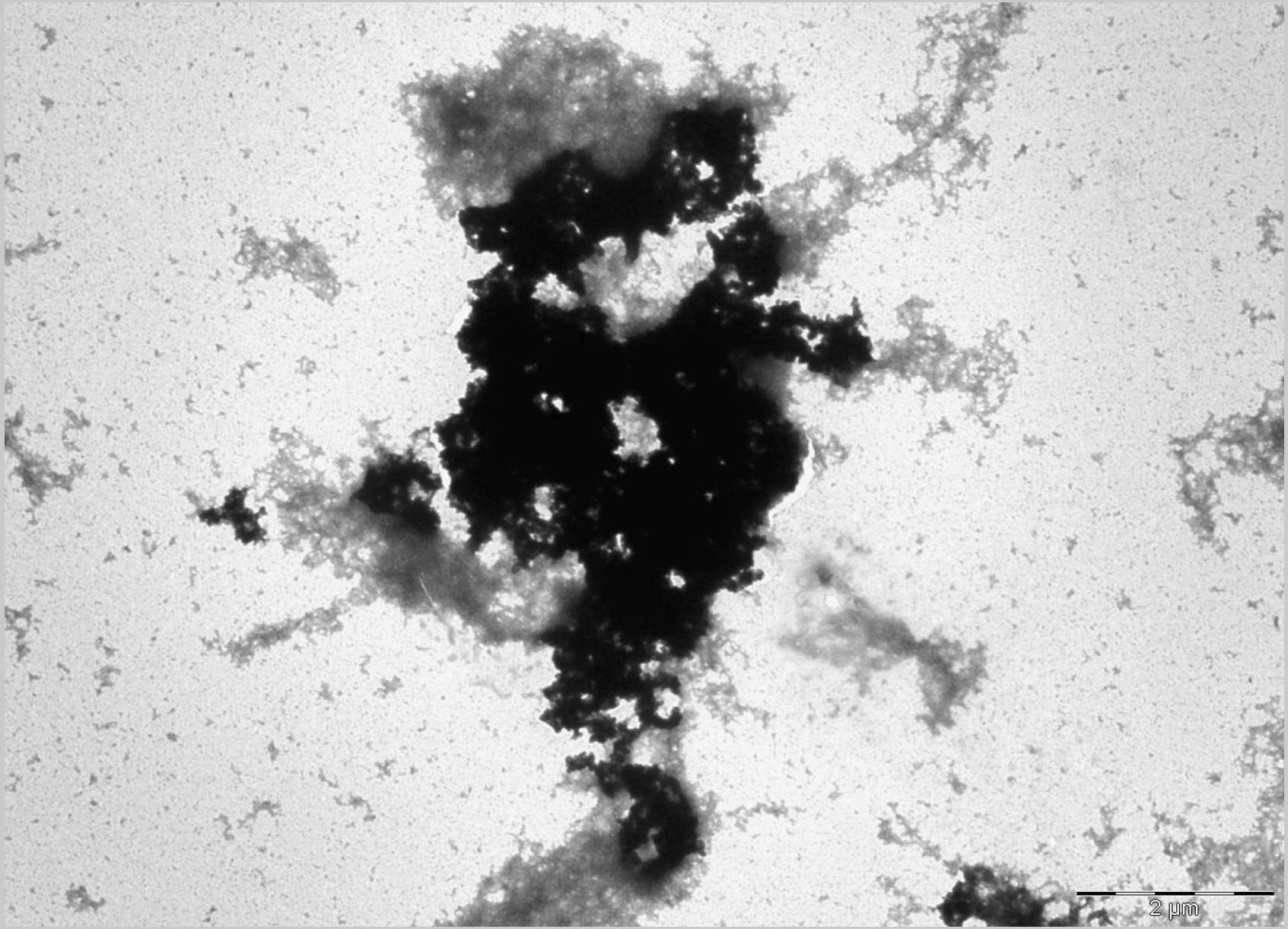


Fig 13: TEM image of Adju-Phos in R10 medium (0hrs). Mag. 30,000X, scale bar 2µm.

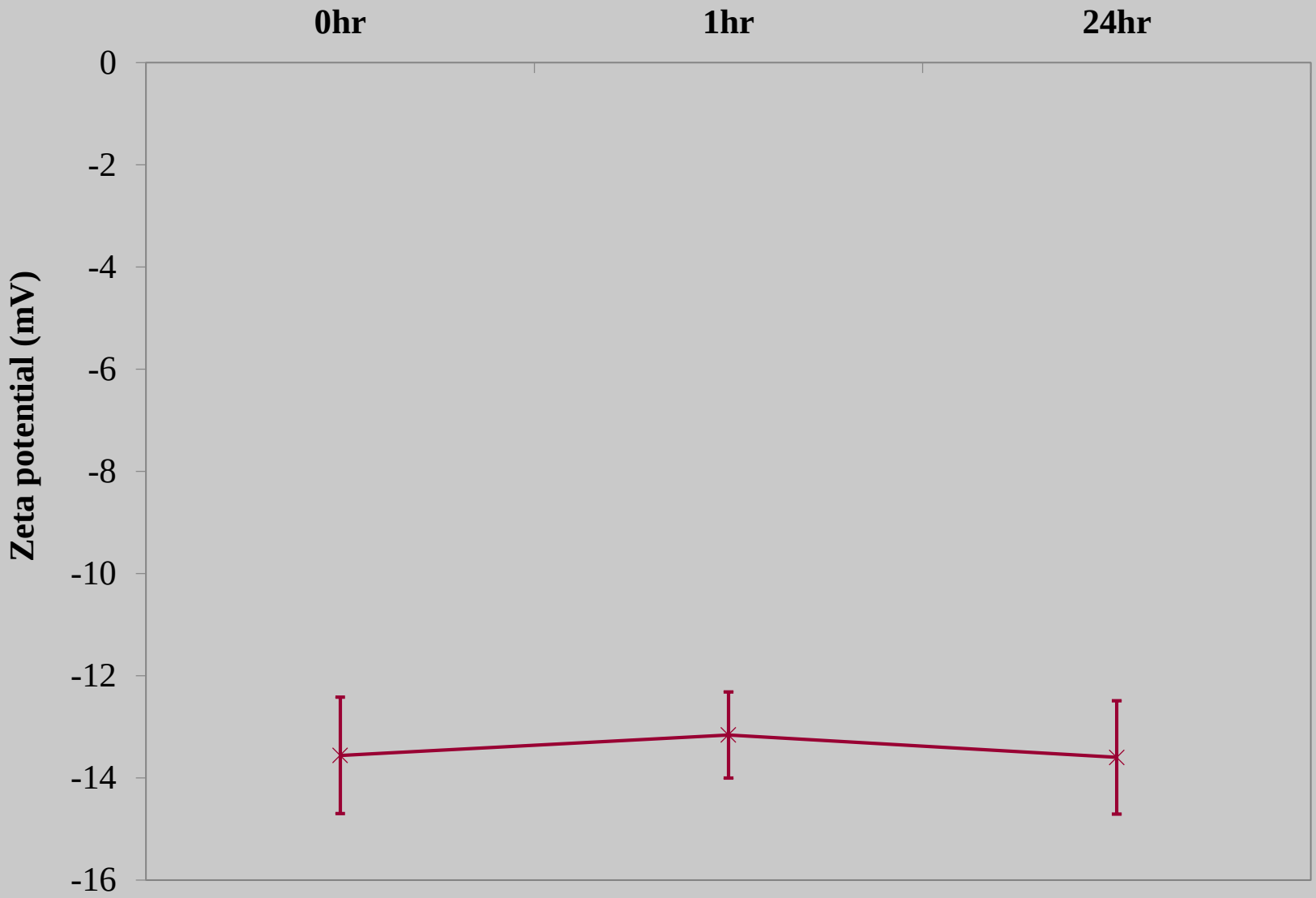


Fig 14: Zeta potential measurements of Adju-Phos in R10 medium following 0, 1 & 24hrs incubation (37°C). Error bars represent the ±SD where n = 5.

Conclusions

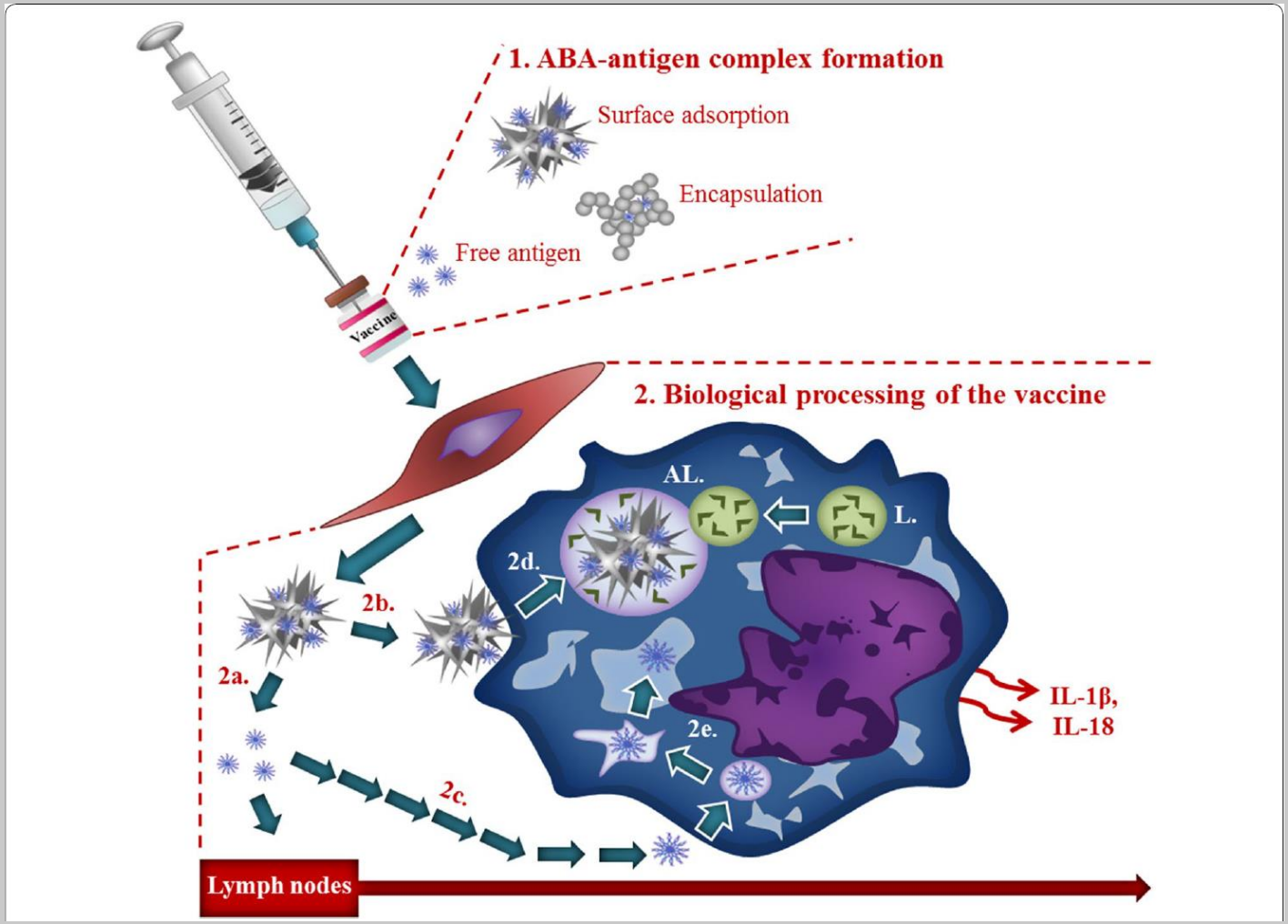
- In 0.9% NaCl, negatively charged Adju-Phos has a larger overall particle size than positively charged Alhydrogel
  - Alh - ~ 72% ≤ 2.7μm
  - Adj - ~ 28% ≤ 2.7μm

- At the site of injection both adjuvants become negatively charged upon administration

- Following administration Alhydrogel has a larger abundance of particles available for phagocytosis.
  - Alh - ~ 97% ≤ 2.7μm
  - Adj - ~ 20% ≤ 2.7μm

What About the Cellular Response to Aluminium Adjuvants?

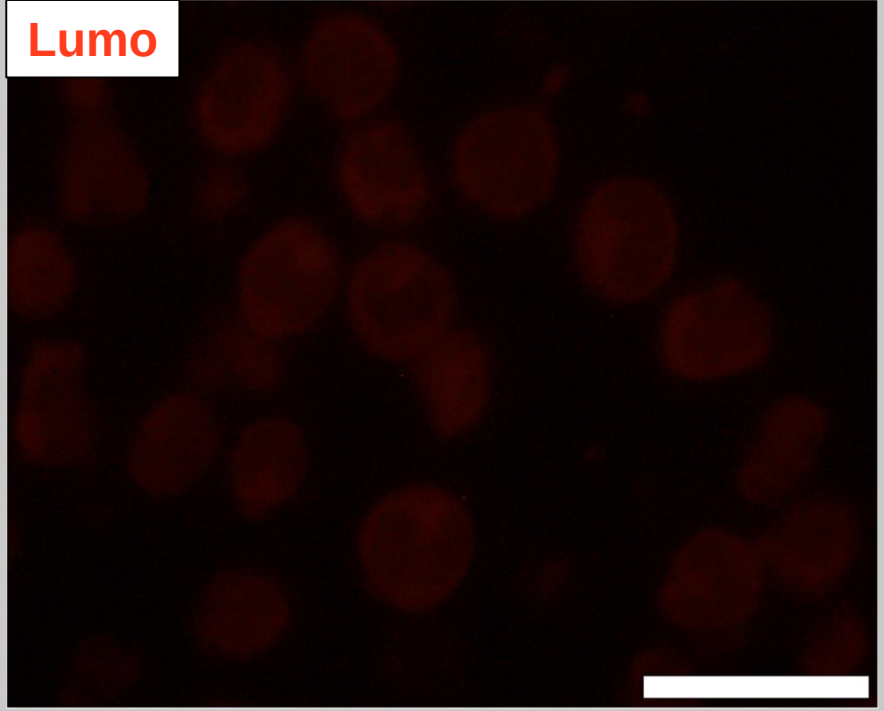
https://aacijournal.biomedcentral.com/articles/10.1186/s13223-018-0305-2

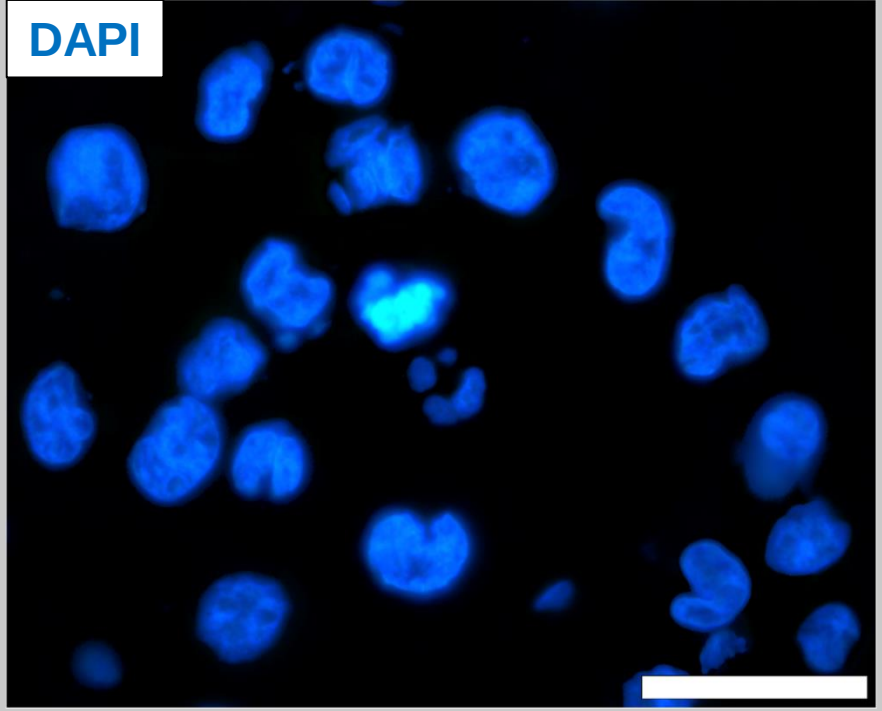


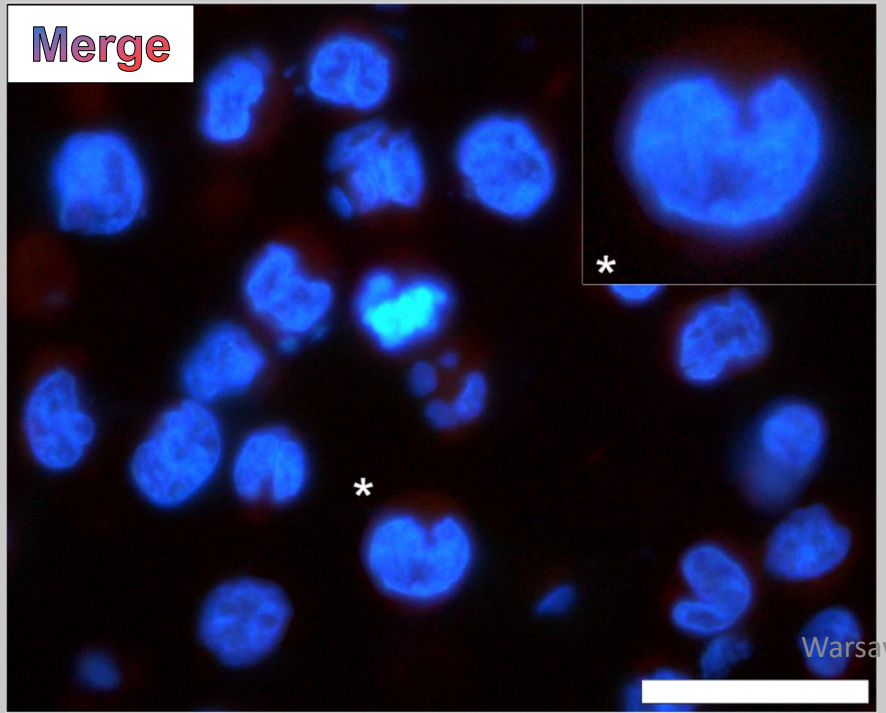
https://www.nature.com/articles/srep31578

https://www.nature.com/articles/srep06287

Native THP-1 cells (R10)


Lumo


DAPI


Merge
*
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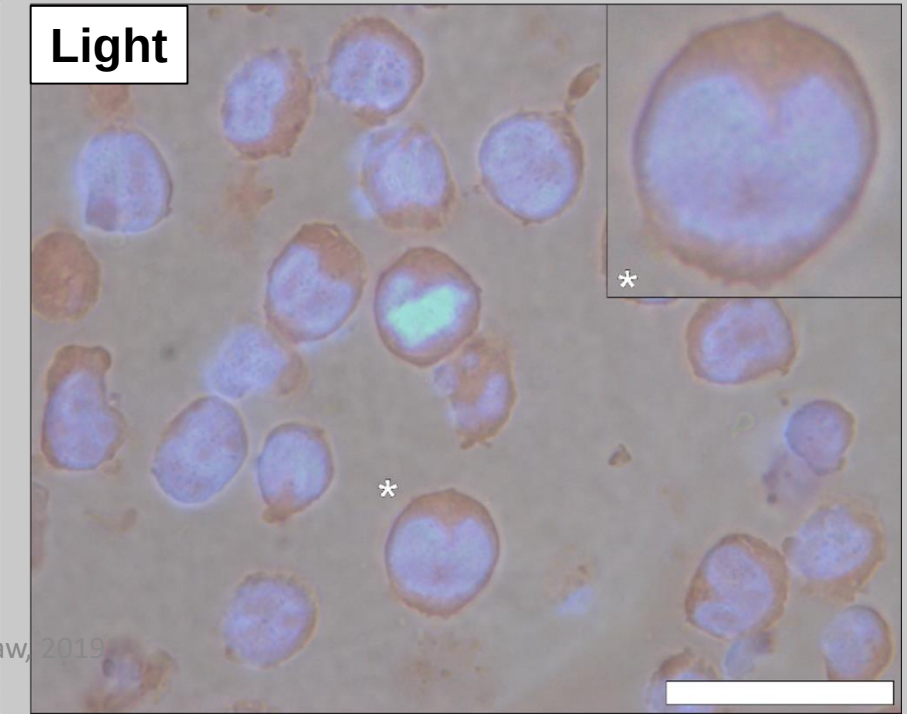
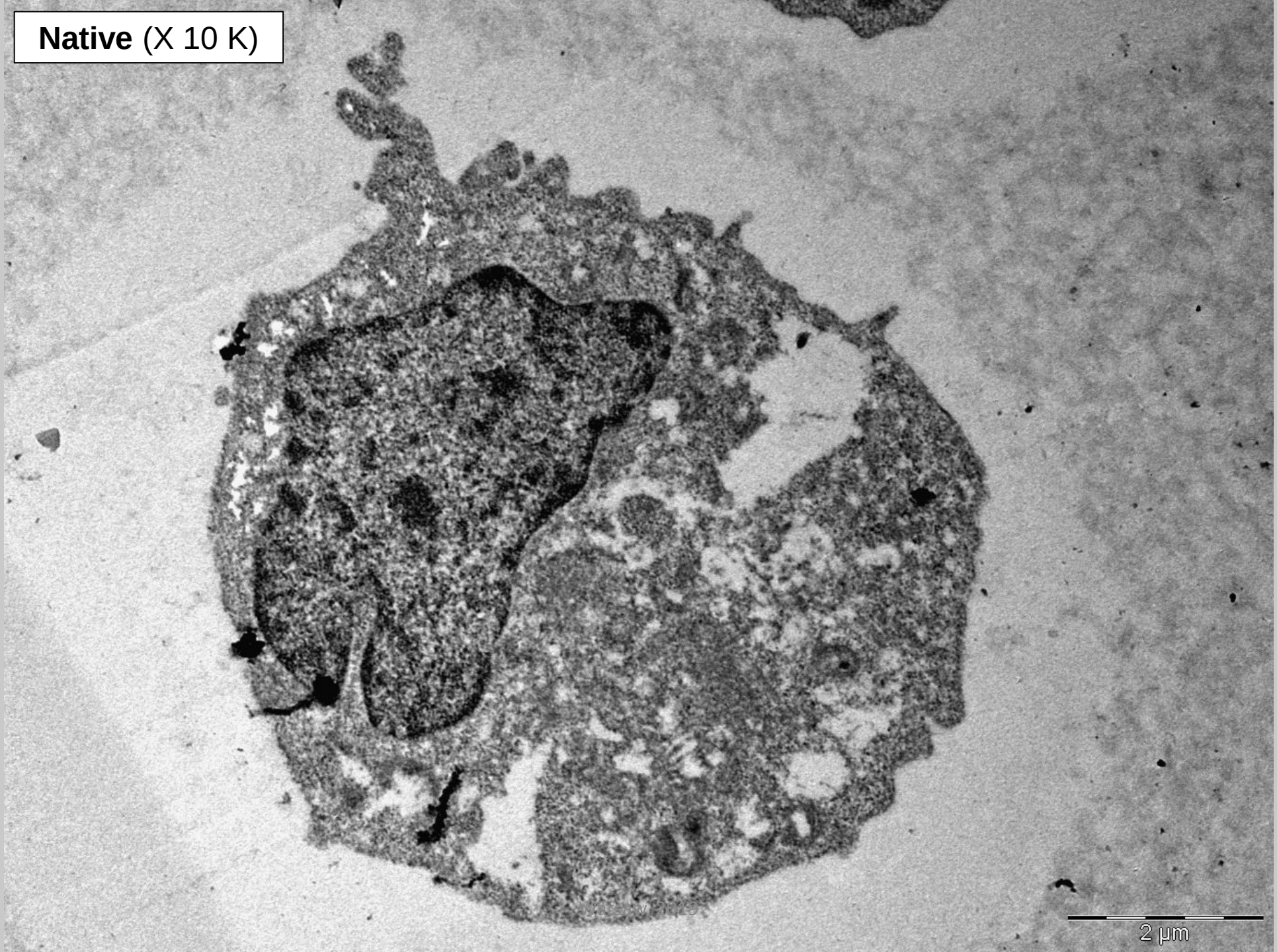
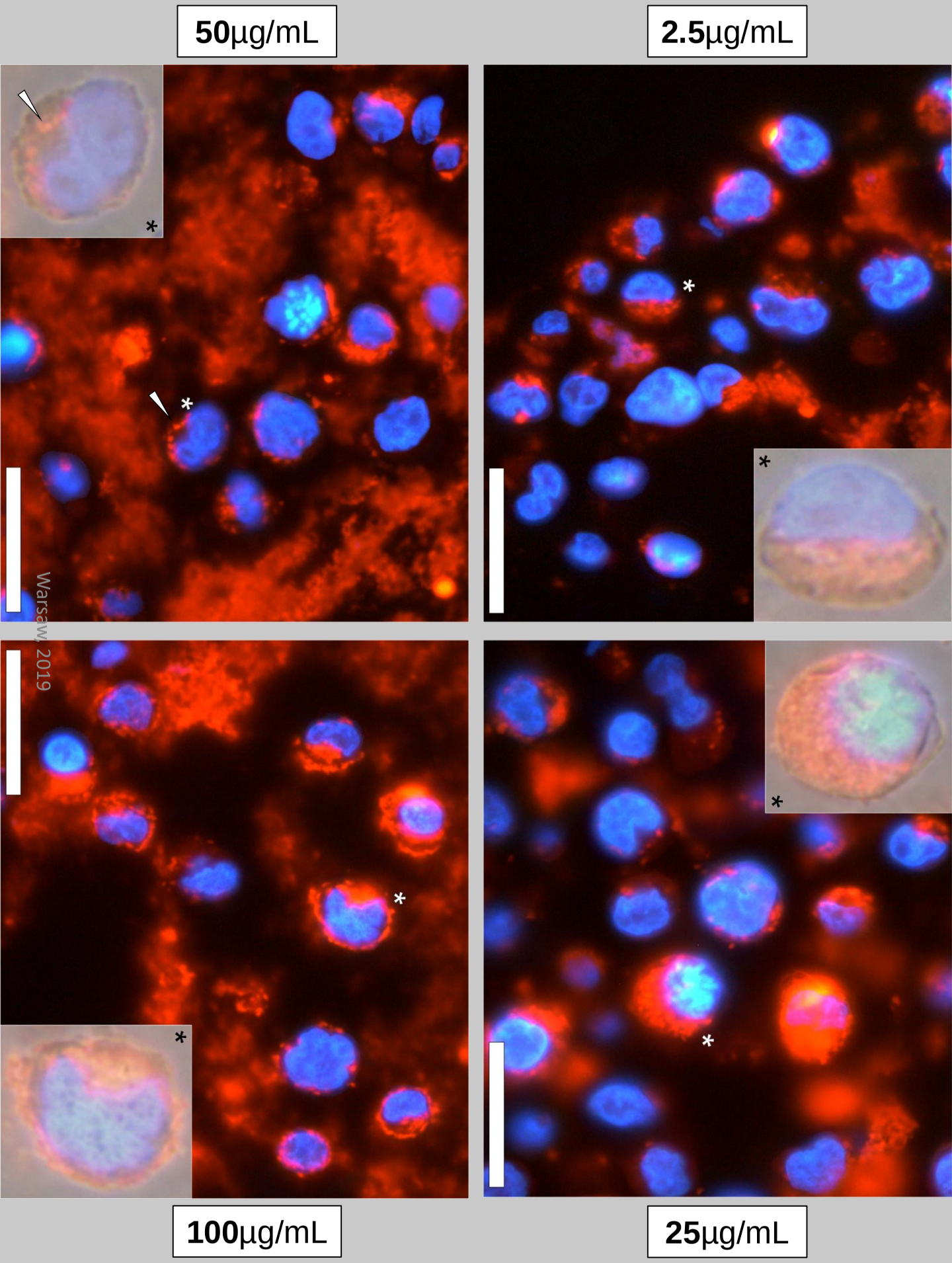
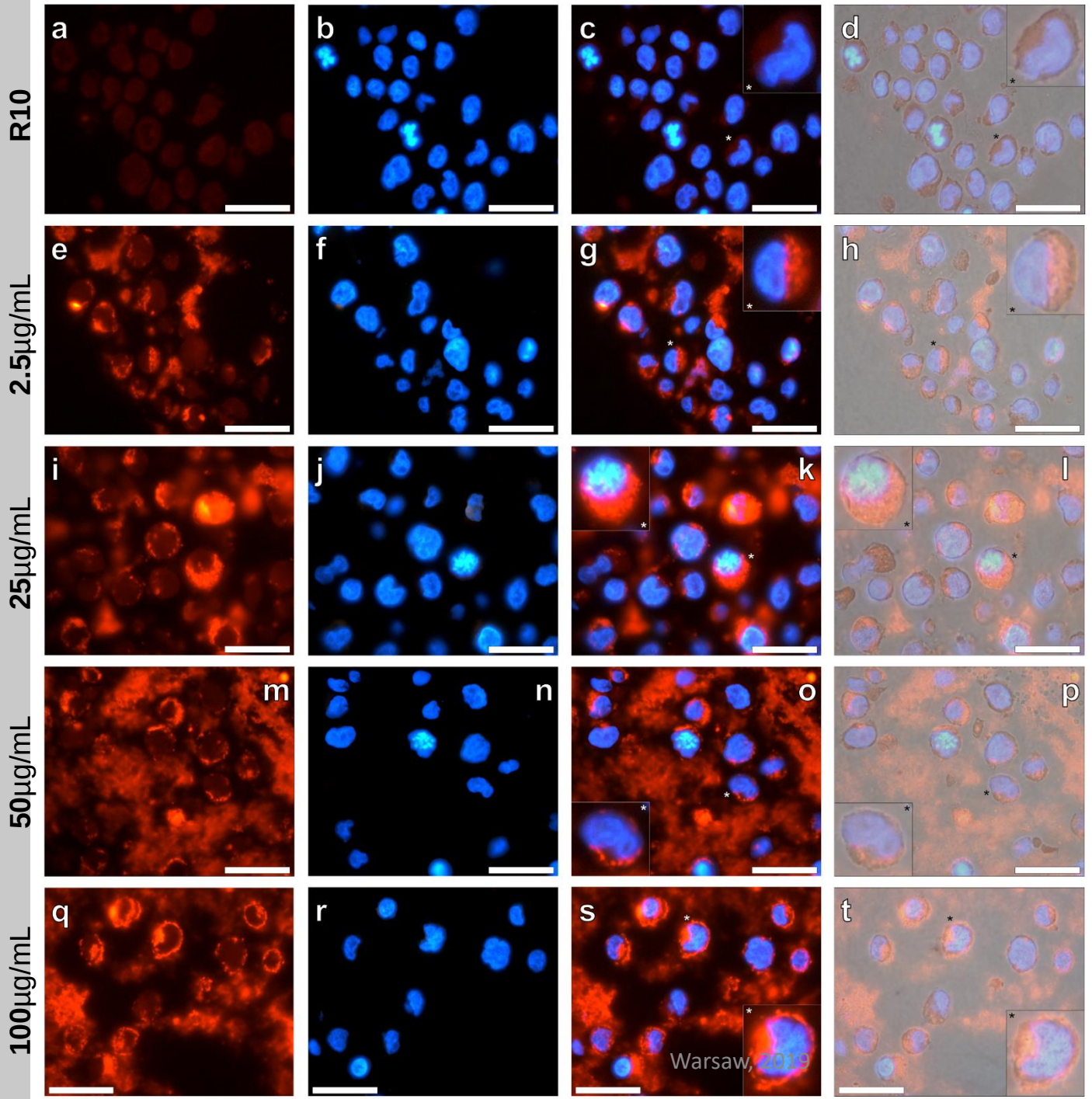

Light
*
*


Native (X 10 K)
2 µm

Alhydrogel®

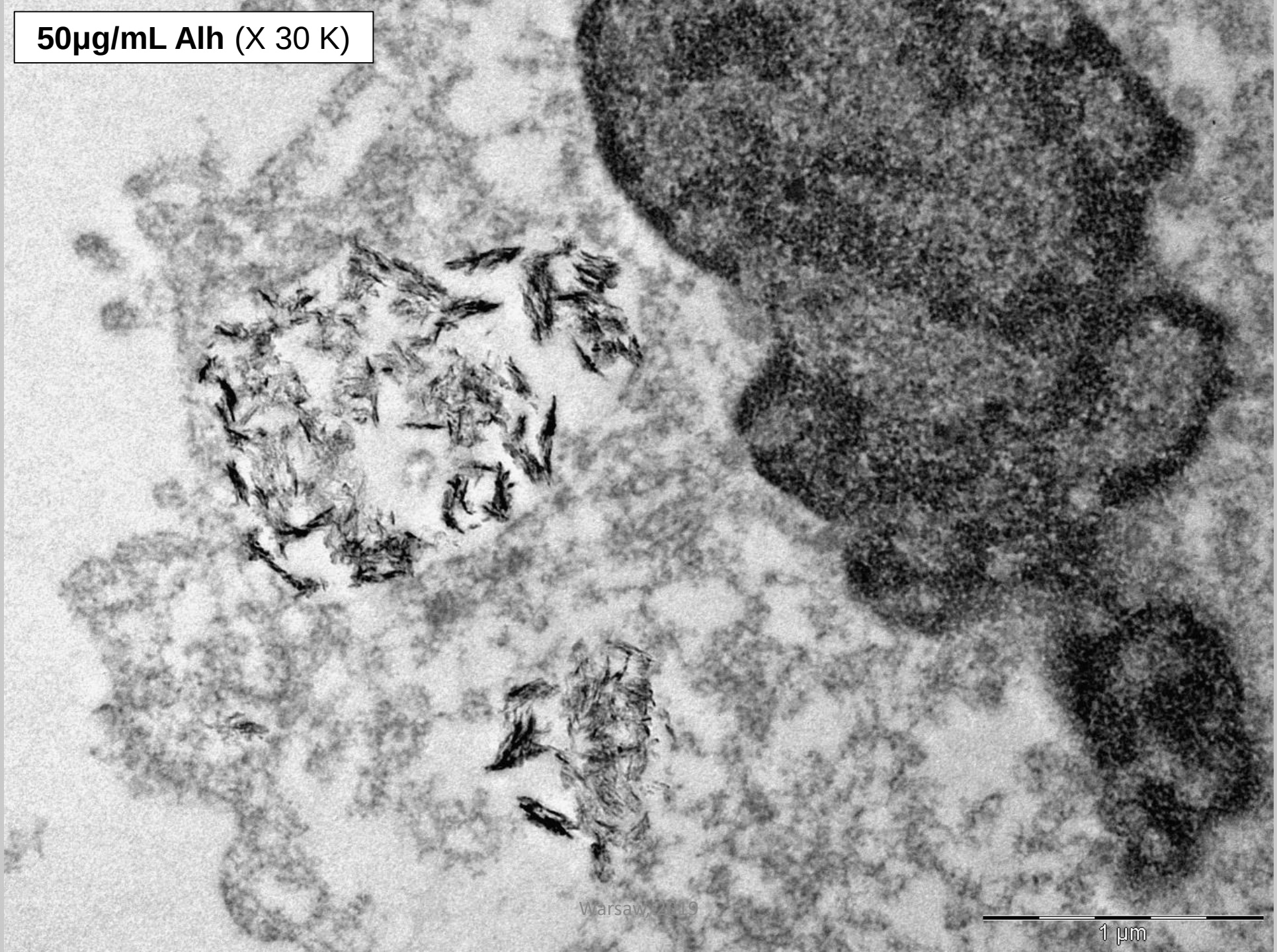
2.5 - 100 *µg/mL*


50µg/mL
2.5µg/mL
100µg/mL
25µg/mL
Warsaw 2019



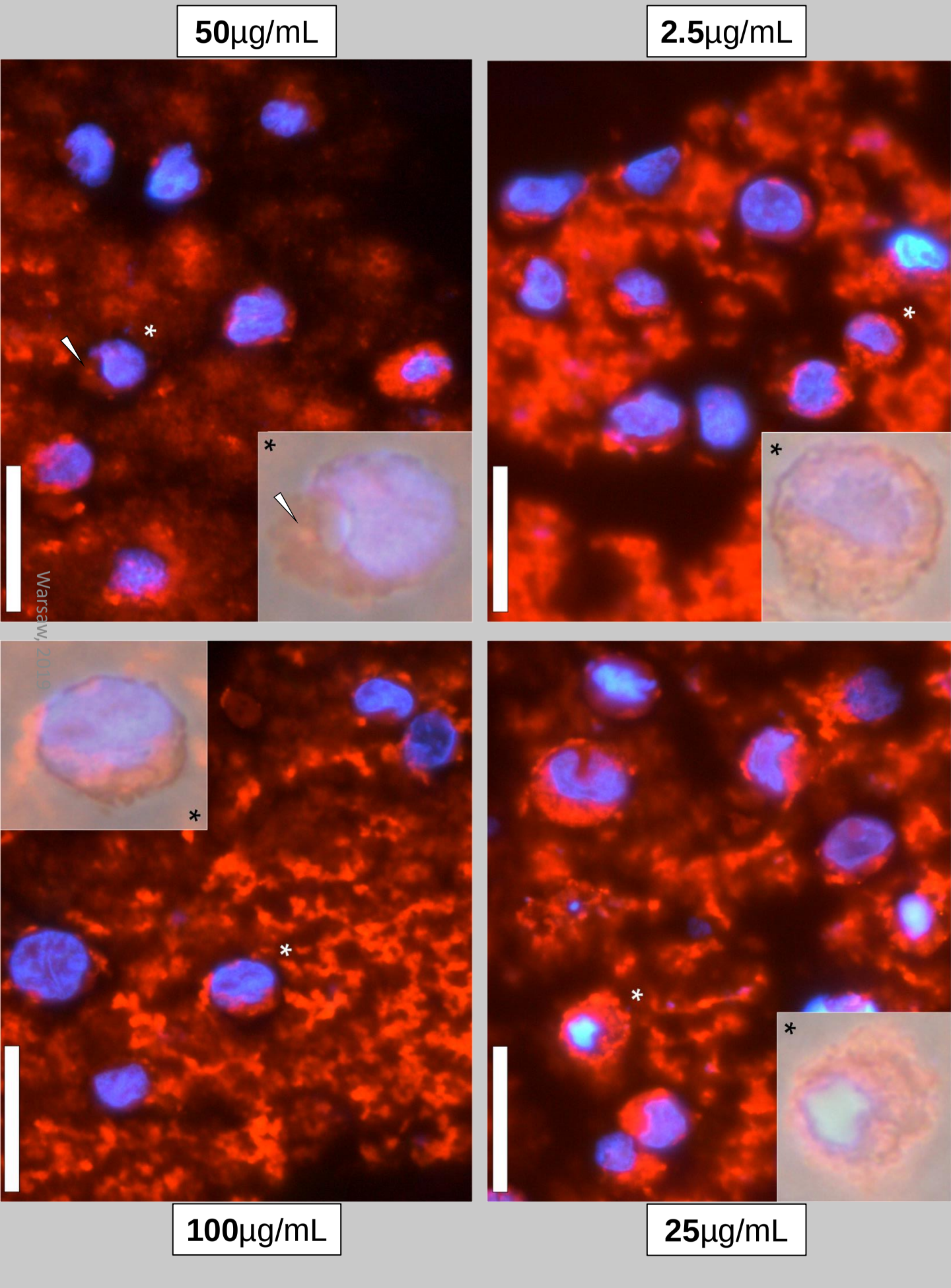
- Alhydrogel® found localised in cell cytoplasm.
- ABA particles were found internalised in THP-1 cells (*ca* 1.0µm).
- Alhydrogel was found readily internalised at all [ABA]s.
- ABA particulates were also found **associated with plasma membranes** at 100µg/mL of the adjuvant.

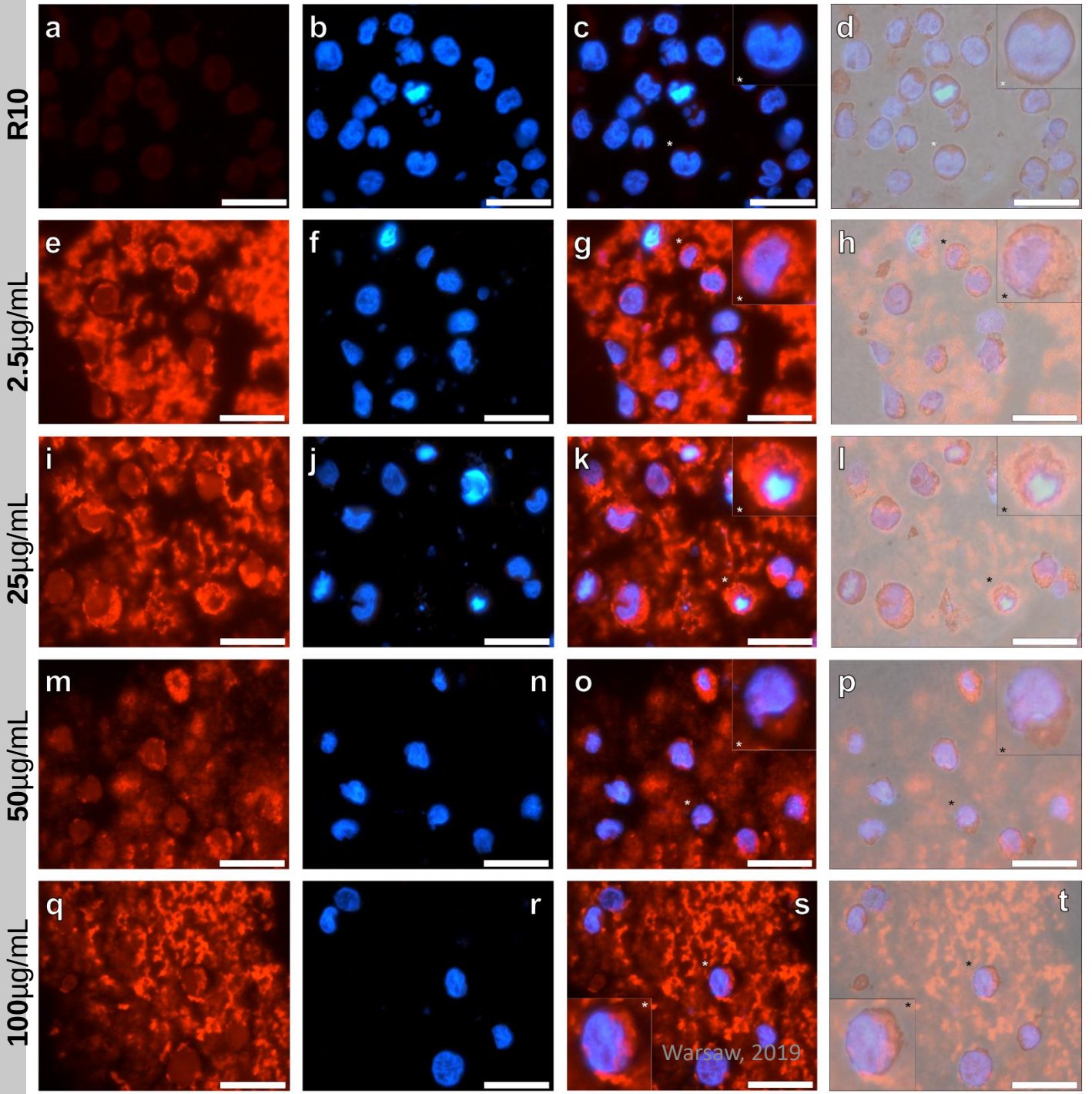

50µg/mL Alh (X 8 K)
Warsaw, 2019
5 µm


50µg/mL Alh (X 30 K)
Warsaw, 2019
1 µm

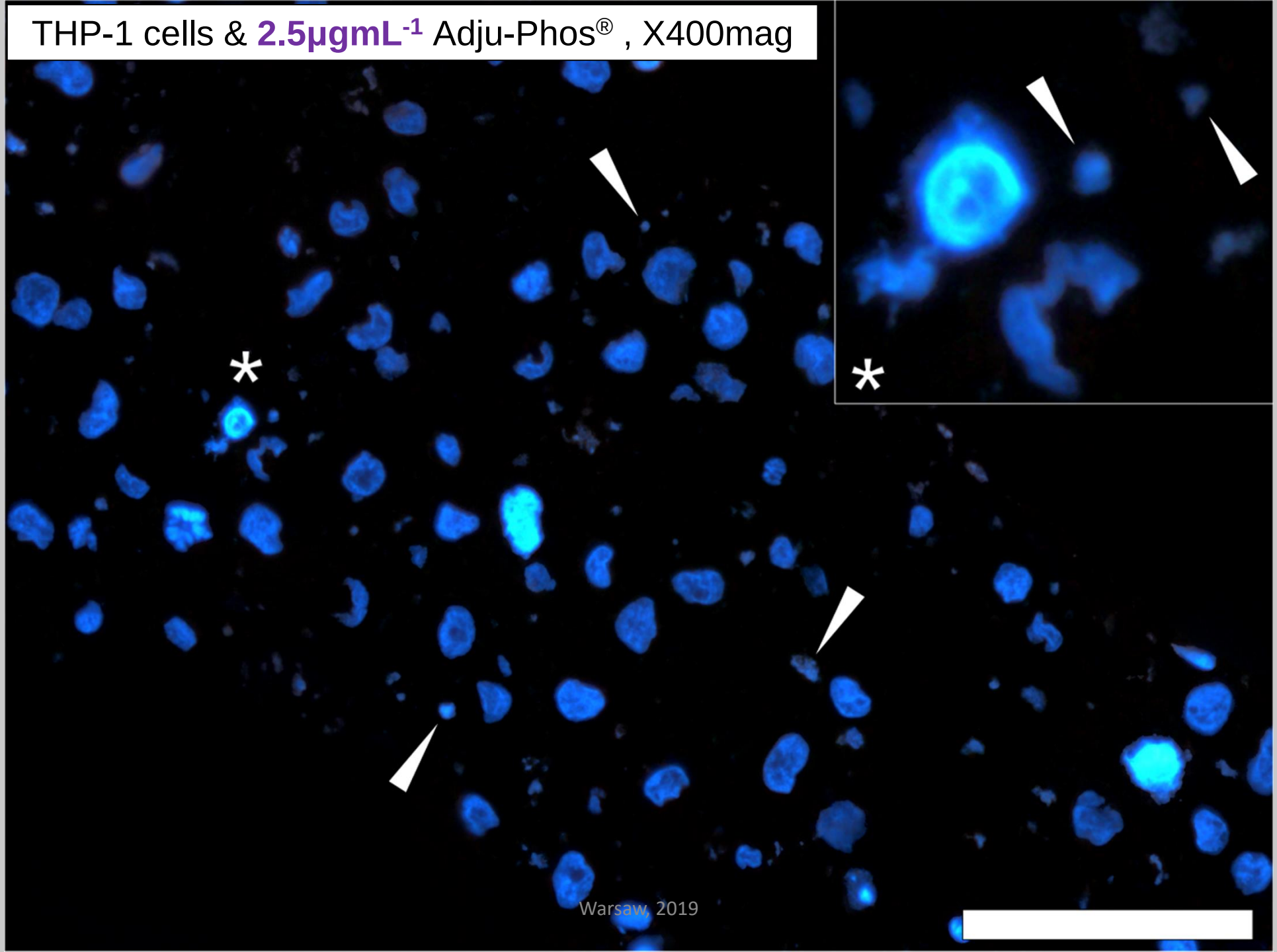
Adju-Phos®

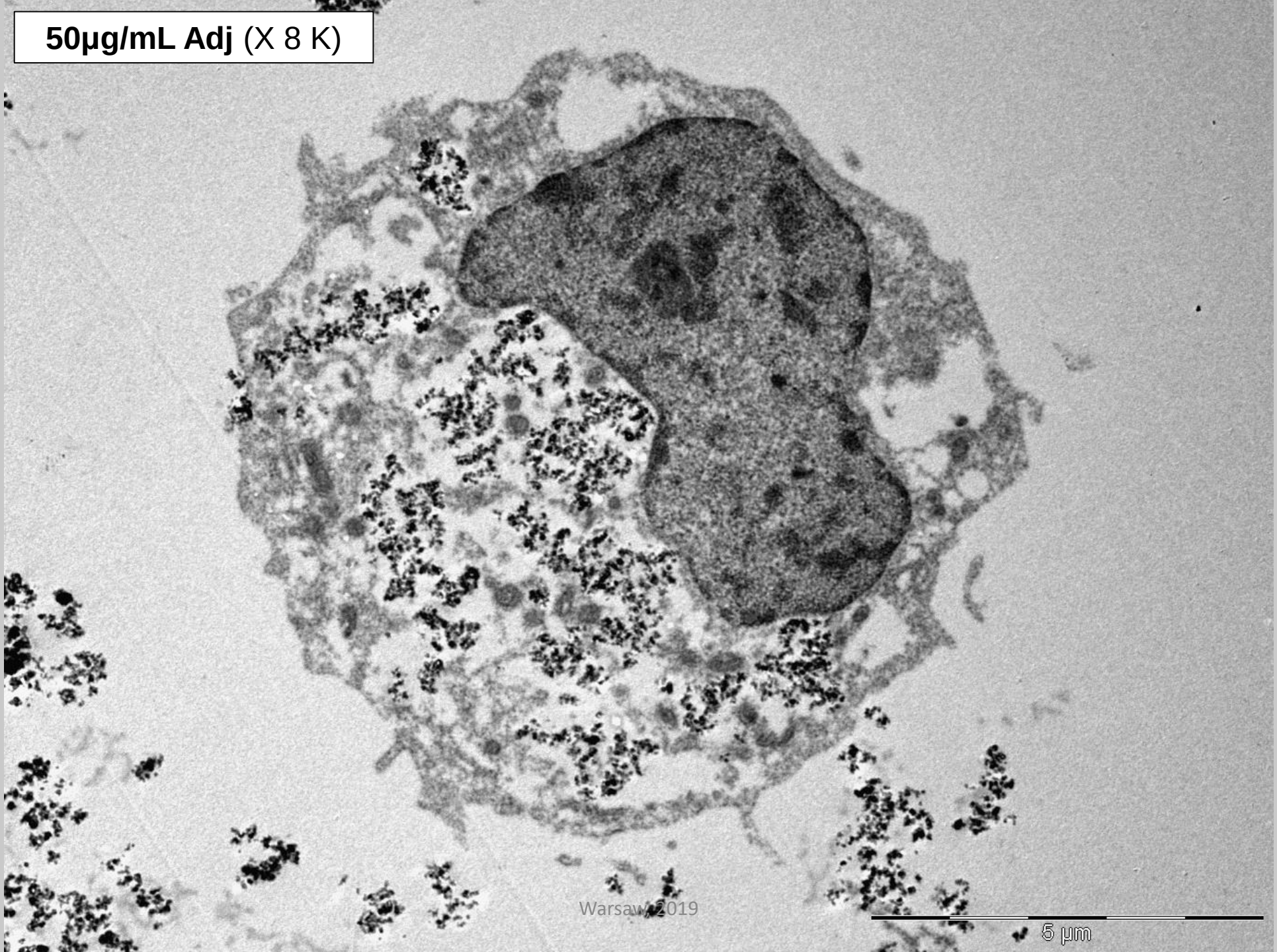
2.5 - 100 *µg/mL*

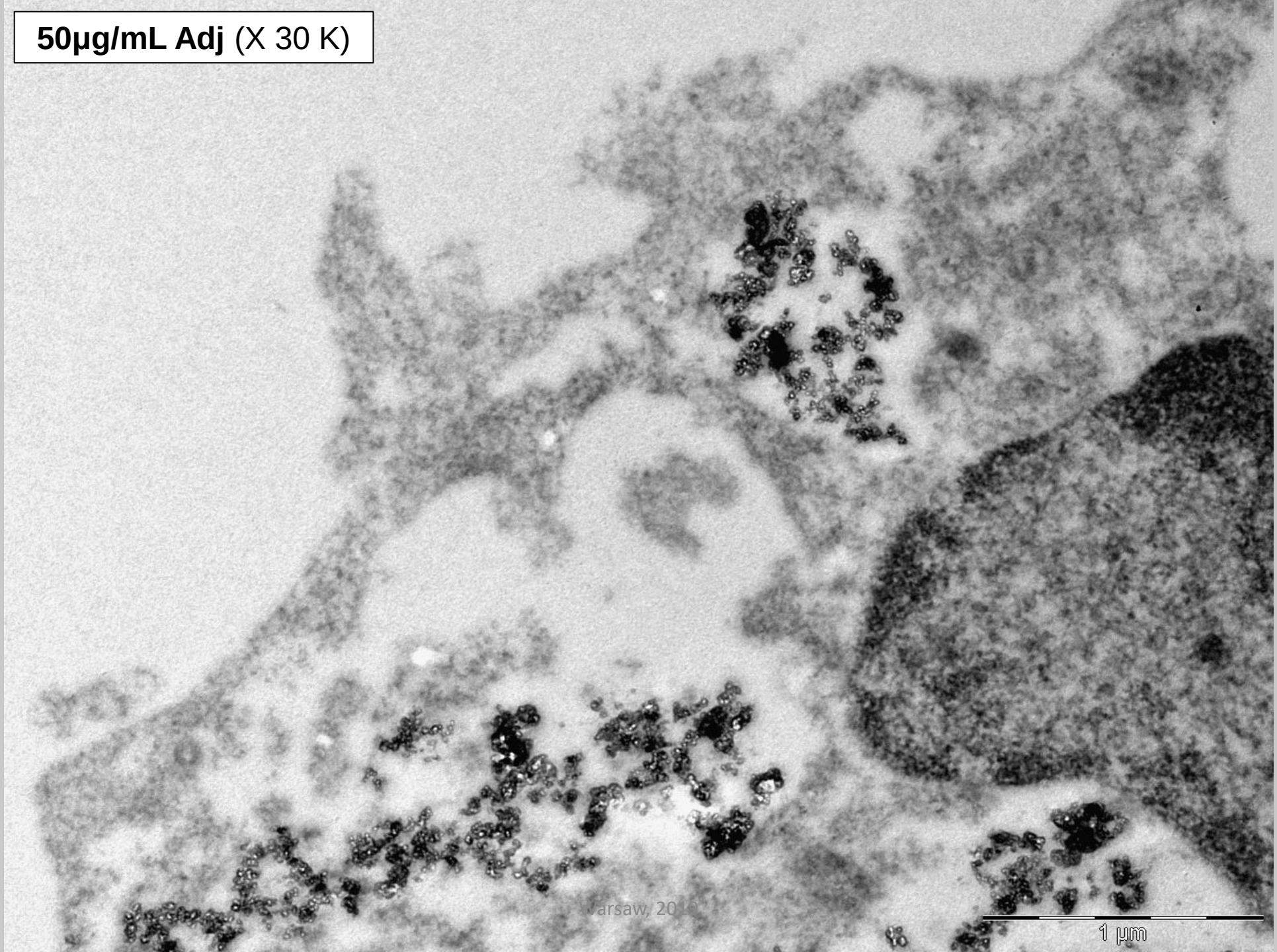

50µg/mL
2.5µg/mL
100µg/mL
25µg/mL



- 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.

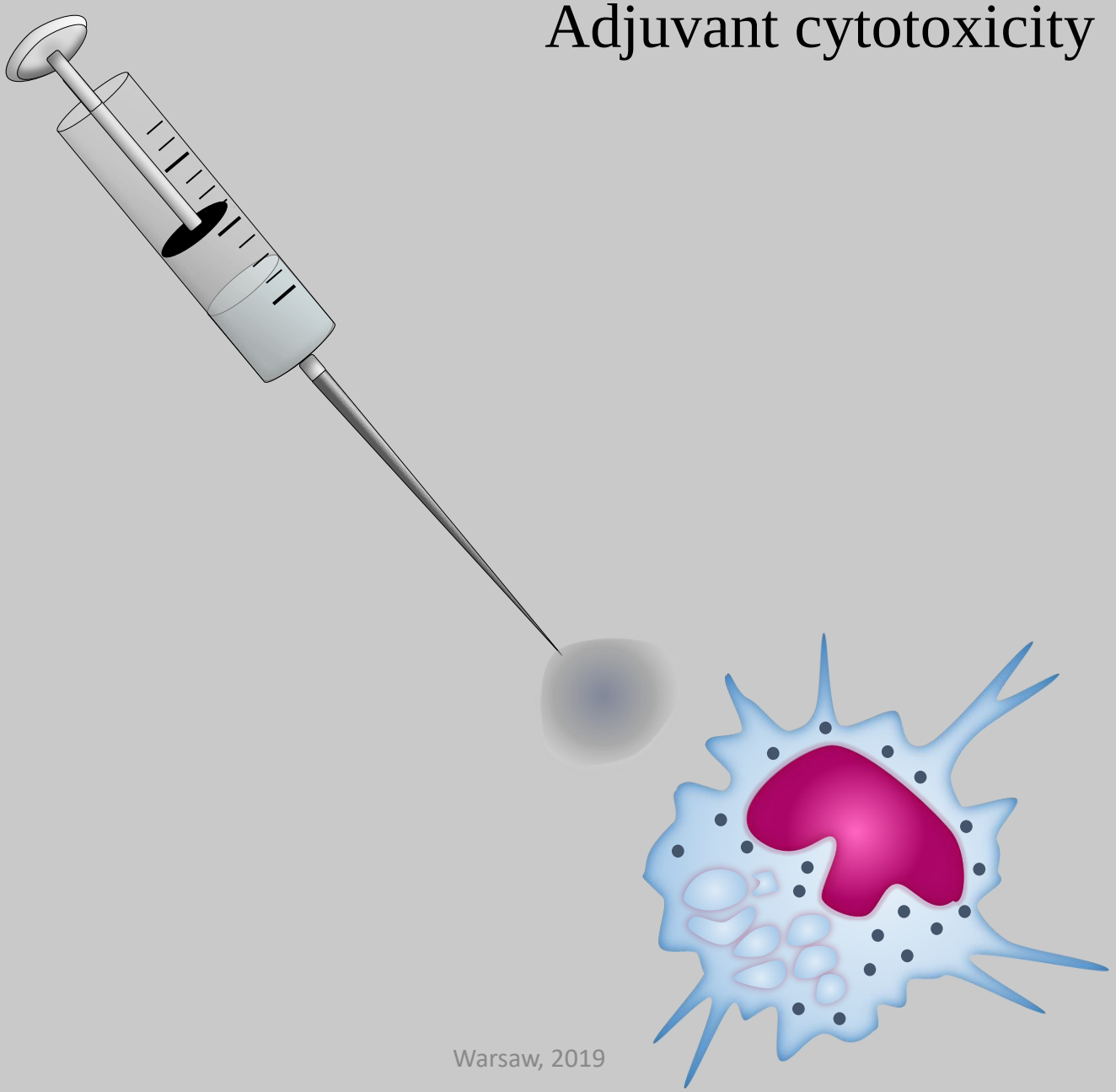

THP-1 cells & 2.5µgmL-1 Adju-Phos® , X400mag
Warsaw, 2019


50µg/mL Adj (X 8 K)
5 µm


50µg/mL Adj (X 30 K)
1 µm

So, What About the Toxicity of Aluminium Adjuvants?

Adjuvant cytotoxicity



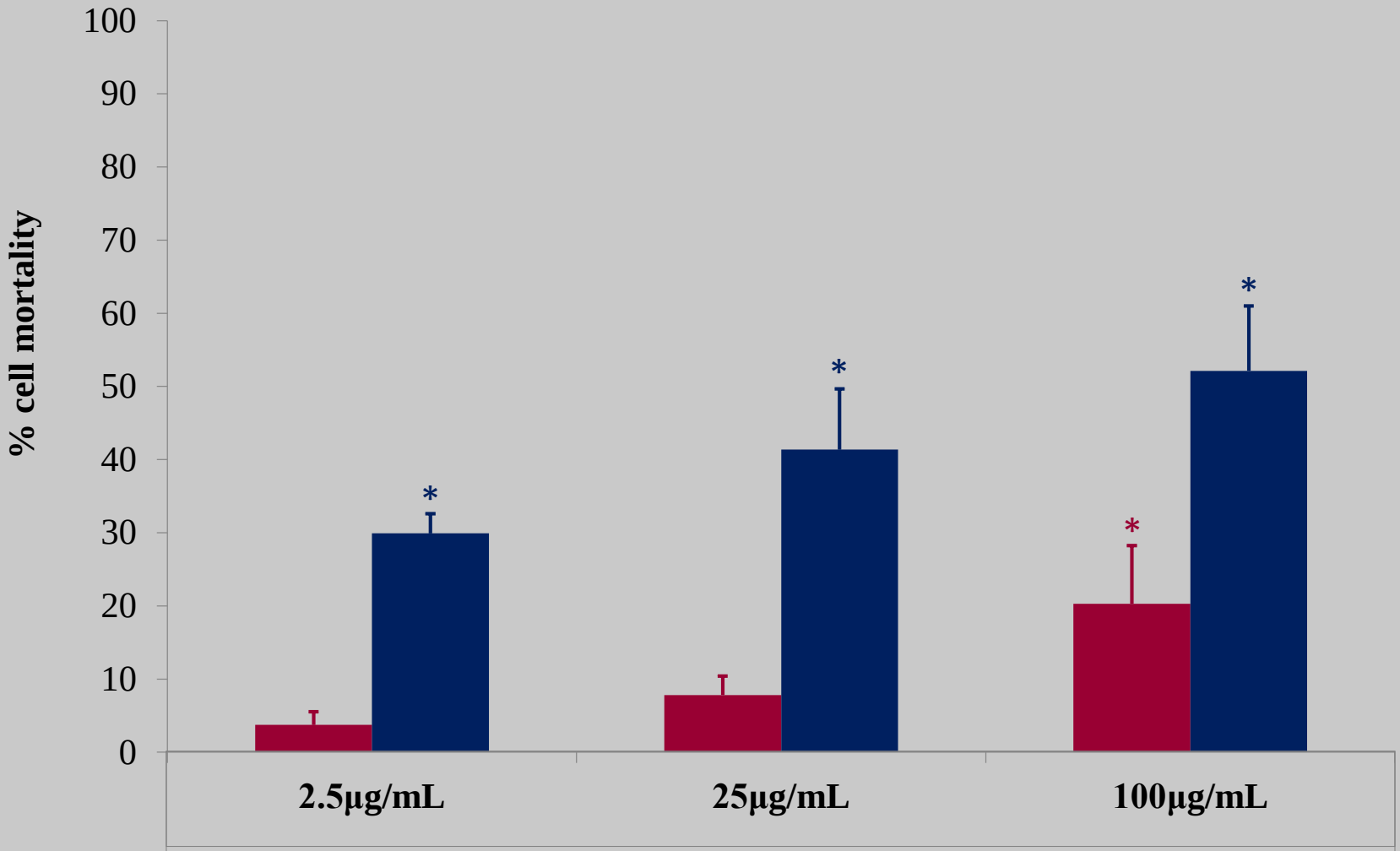


Fig 9: The % mortality experienced in THP-1 cell populations upon exposure to various concentrations of aluminium adjuvants relative to the control group, as elucidated using the live/dead cytotoxicity assay. Plum and blue bars represent Alhydrogel and Adju-Phos respectively. Error bars are representative of ±SD of 3 individual replicates and statistical significance is shown between treatments and respective control groups

Conclusions

For the two aluminium adjuvants used in clinically approved vaccines, intracellular particulates of Alhydrogel® and Adju-Phos®, were observed localised in cell cytoplasm only.

Only co-culture with Adju-Phos® resulted in the release of extracellular genetic material.

Higher concentrations of aluminium adjuvants co-cultured with THP-1 cells were observed to result in their reduced cellular uptake (50 & 100µg/mL Adju-Phos®).

Conclusions cont.

The cytotoxicities of the two aluminium adjuvants used in clinically-approved vaccines are significantly different with Adju-Phos® expected to induce greater toxicity at the injection site.

The observed lower toxicity of Alhydrogel® despite its high intracellular burden may predispose this adjuvant to its translocation to (potentially) target tissues/ organs away from the injection site.

Serious Adverse Events?

Khan *et al. BMC Medicine* 2013, **11**:99
http://www.biomedcentral.com/1741-7015/11/99

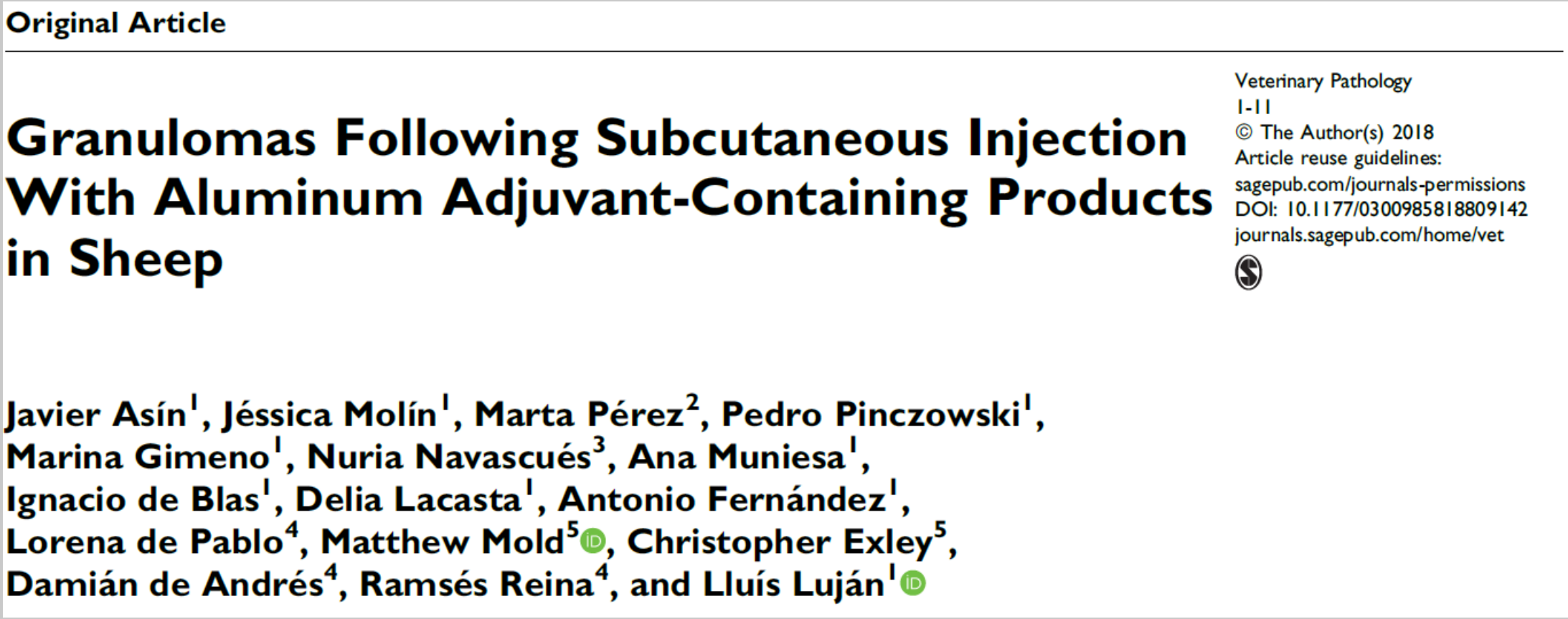
BMC Medicine

RESEARCH ARTICLE Open Access

Slow CCL2-dependent translocation of biopersistent particles from muscle to brain

Zakir Khan[1,2], Christophe Combadière[3,4,5], François-Jérôme Authier[1,2,6], Valérie Itier[1,2,11], François Lux[7,8], Christopher Exley[9], Meriem Mahrouf-Yorgov[1,2,11], Xavier Decrouy[1,2], Philippe Moretto[10], Olivier Tillement[7,8], Romain K Gherardi[1,2,6,12*†] and Josette Cadusseau[1,2,11,12*†]

Serious Adverse Events?



Original Article

Veterinary Pathology
1-11
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DOI: 10.1177/0300985818809142
journals.sagepub.com/home/vet

Granulomas Following Subcutaneous Injection With Aluminum Adjuvant-Containing Products in Sheep

Javier Asín[1], Jéssica Molín[1], Marta Pérez[2], Pedro Pinczowski[1], Marina Gimeno[1], Nuria Navascués[3], Ana Muniesa[1], Ignacio de Blas[1], Delia Lacasta[1], Antonio Fernández[1], Lorena de Pablo[4], Matthew Mold[5], Christopher Exley[5], Damián de Andrés[4], Ramsés Reina[4], and Lluís Luján[1]

http://journals.sagepub.com/doi/10.1177/0300985818809142

New and important research on sheep and recently published in the journal Veterinary Pathology now provides direct evidence of the fate of aluminium adjuvants following sub-cutaneous injection. The research confirms the **accumulation of aluminium adjuvant in lymph glands**. However, it also shows that while lymph glands are a target destination for aluminium adjuvant for the whole vaccine this is not the case when only the aluminium adjuvant is injected. Essentially the handling of aluminium adjuvant is different between whole vaccine and that which is mainly used as the control or placebo in vaccine safety trials. These seminal data for sheep raise new and important questions about how vaccine safety trials are conducted in humans and offer further insight into the role of aluminium adjuvants in serious adverse events following vaccination.

Serious Adverse Events?

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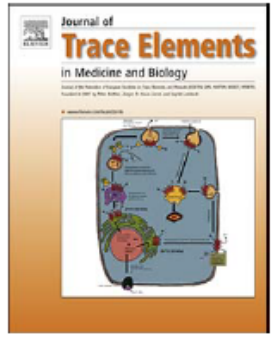

Aluminium in brain tissue in autism

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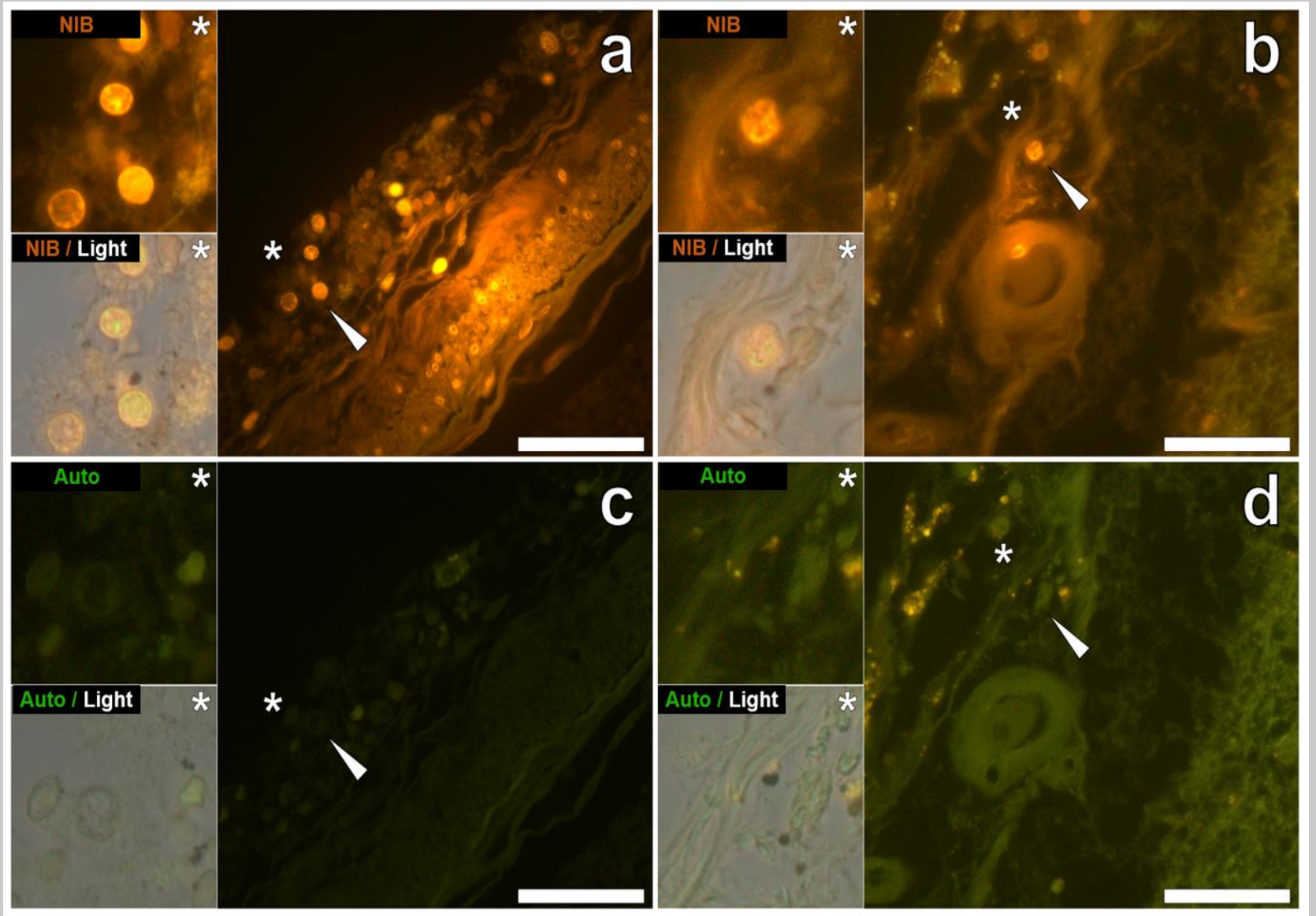

Intrameningeal lumogallion-reactive aluminium identified in the hippocampus **(a & c)** and frontal lobe **(b & d)** of a 50-year-old male donor diagnosed with autism.
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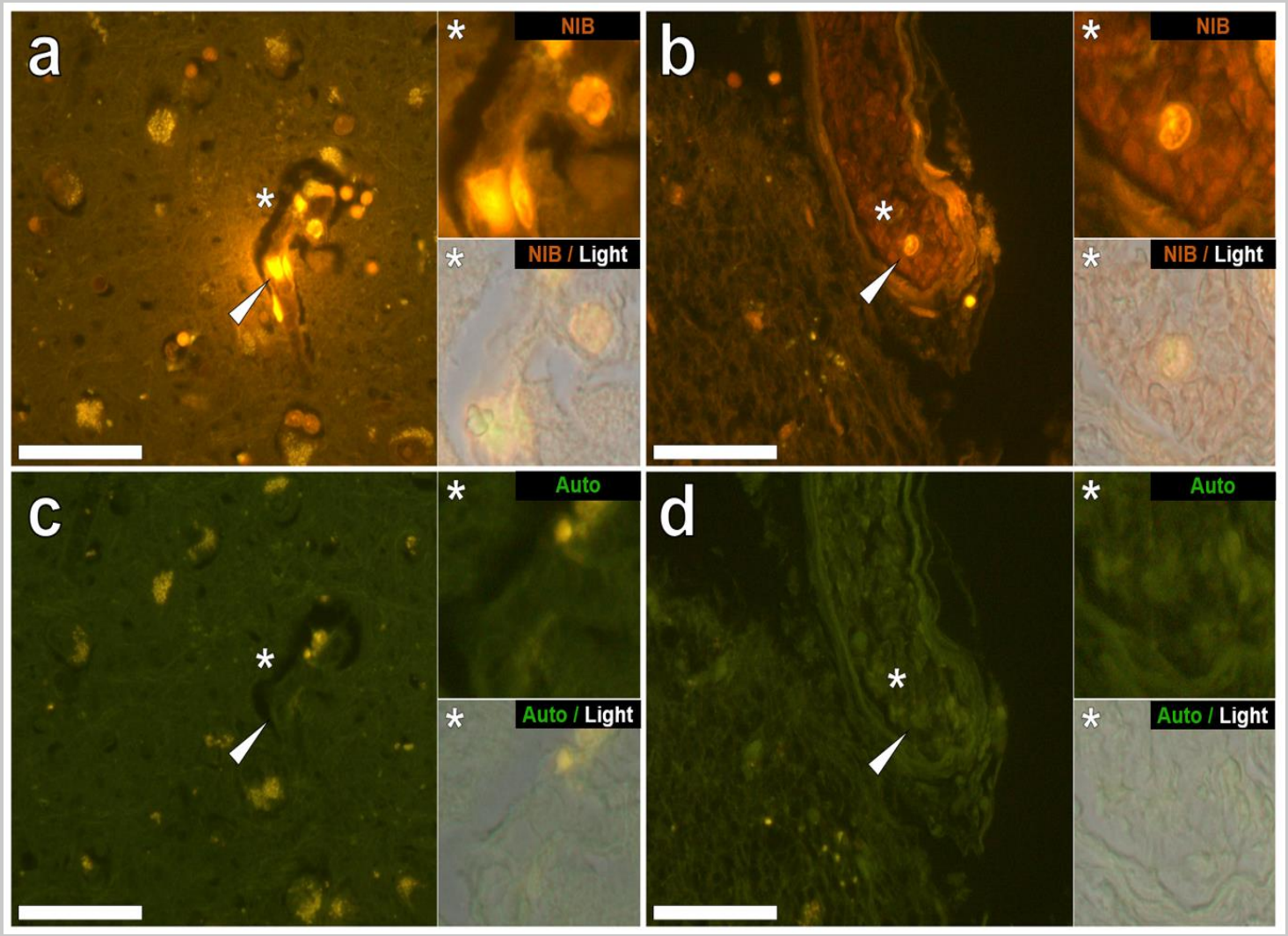
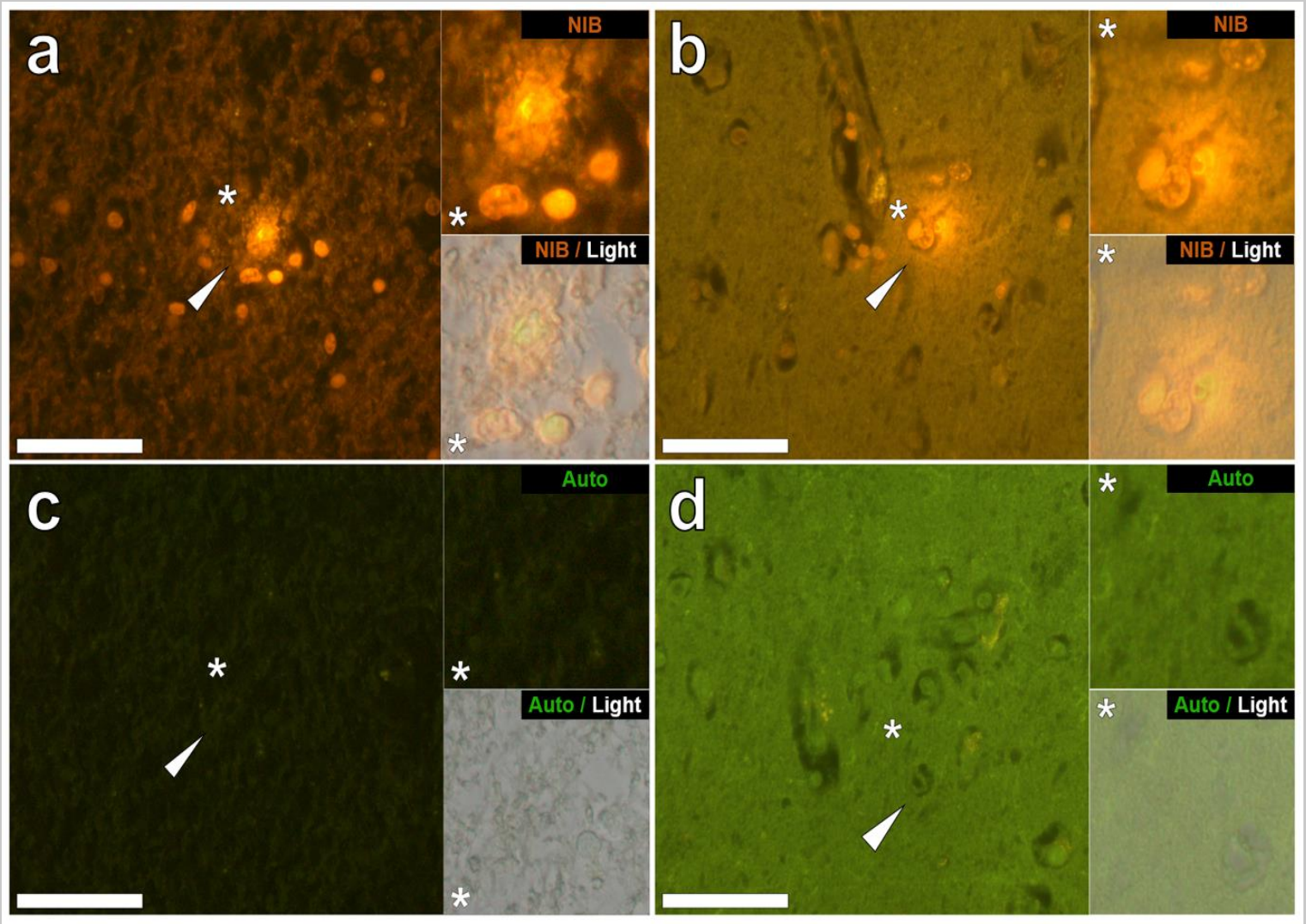


Intravasculature lumogallion-reactive aluminium identified in the hippocampus **(a – d)** of a 50-year-old male donor diagnosed with autism.
https://www.sciencedirect.com/science/article/pii/S0946672X17308763



Lumogallion-reactive aluminium identified in the hippocampus **(a & c)** and parietal **(b & d)** lobe of a 15-year-old male donor diagnosed with autism.

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The Birchall Centre, Keele University and
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