



Transfection by photoporation enables inflammasome activation studies in targeted single cells

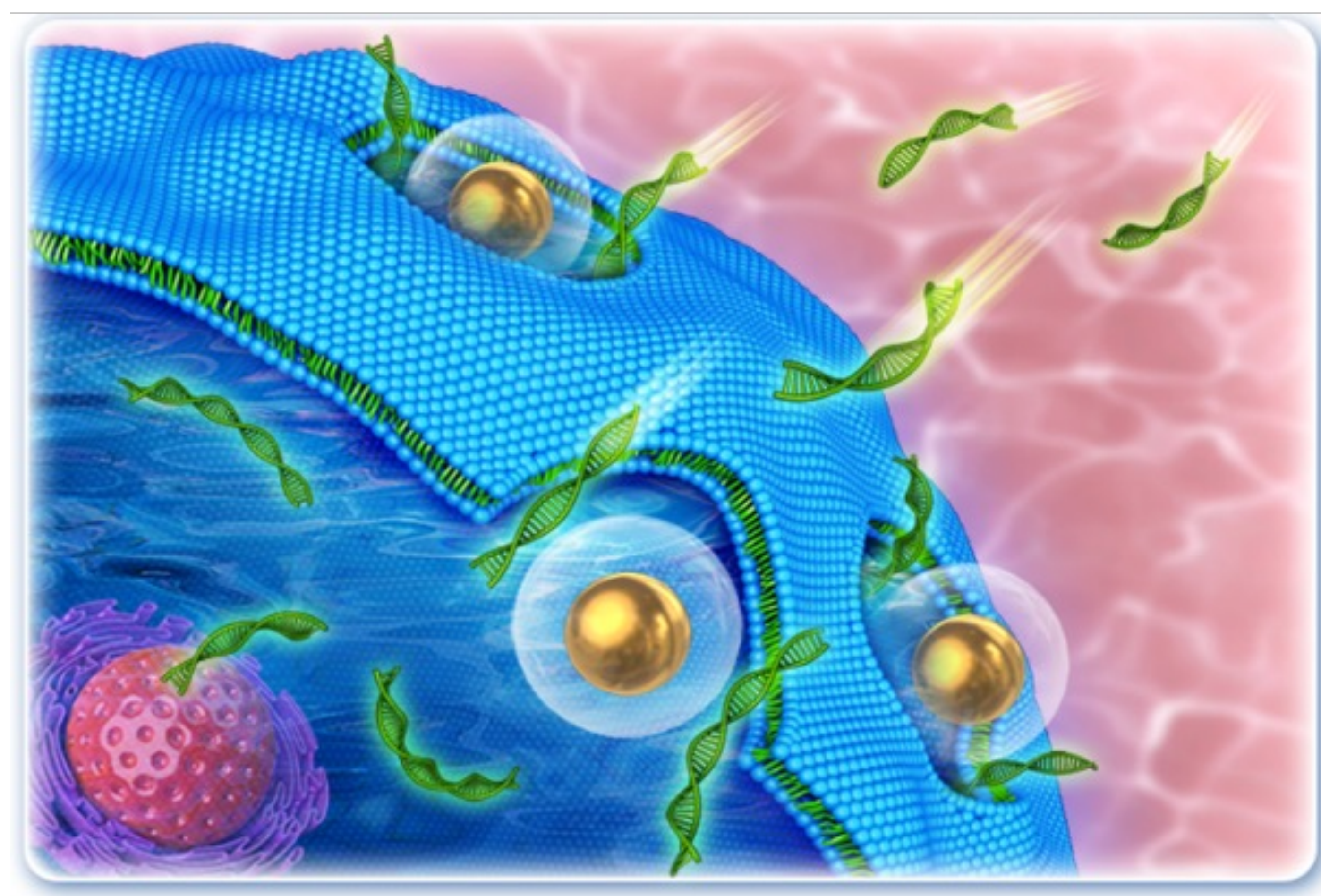
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Introduction

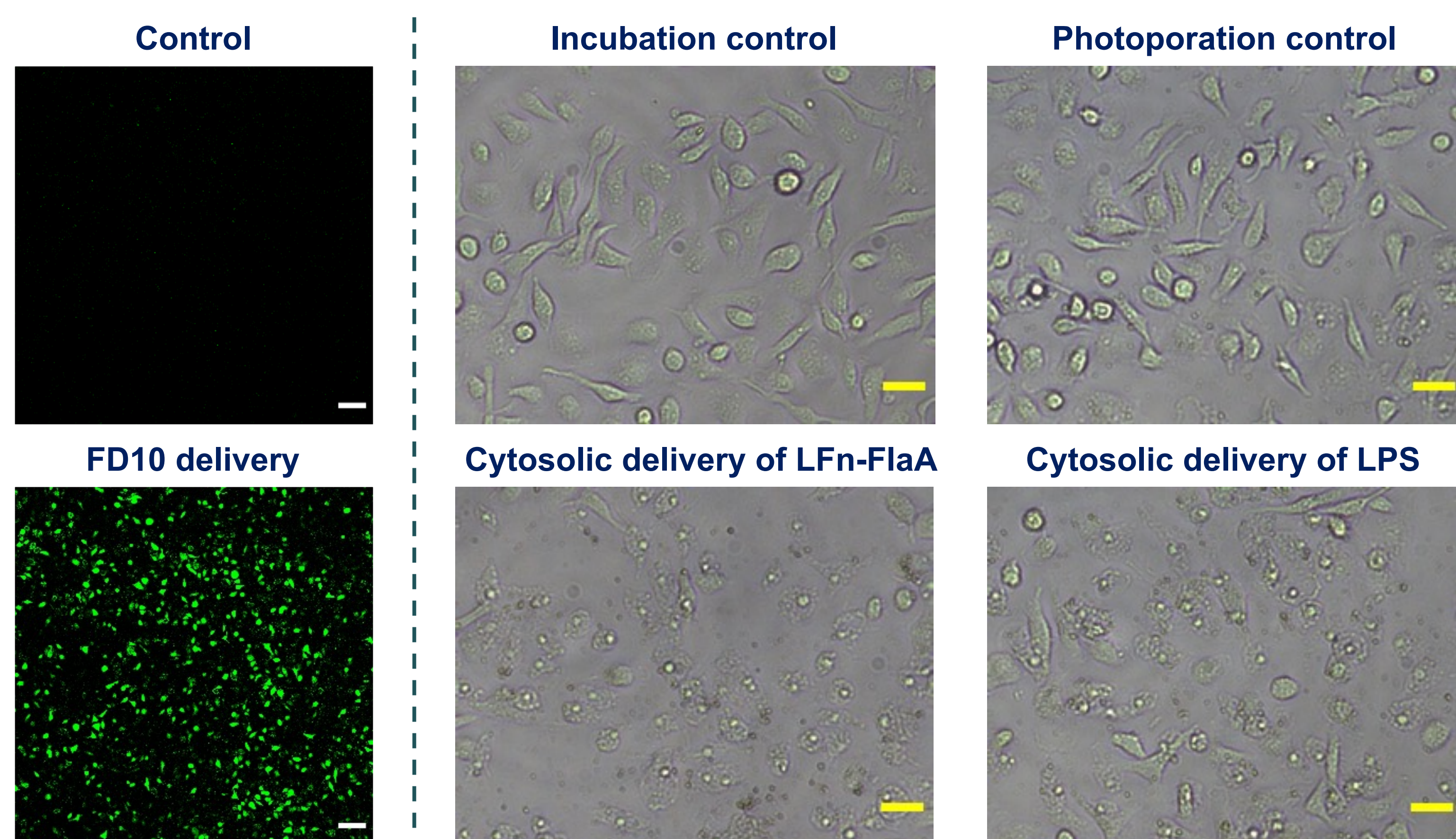
- **Inflammasomes** are multi-protein complexes that guard against cellular stress and microbial infections.
- Inflammasome activation studies frequently require delivery of pathogen-derived virulence factors into the cytosol of macrophages and other innate immune cells.
- Primary myeloid cells such as macrophages are known to be **difficult-to-transfect**.
- **Electroporation** is frequently used but is accompanied with several drawbacks such as **high cell toxicity**, **functional/genomic alterations**.
- Alternative: use of **laser-assisted nanoparticle-sensitized photoporation** for the cytosolic delivery of inflammasome inducing proteins.

Photoporation method

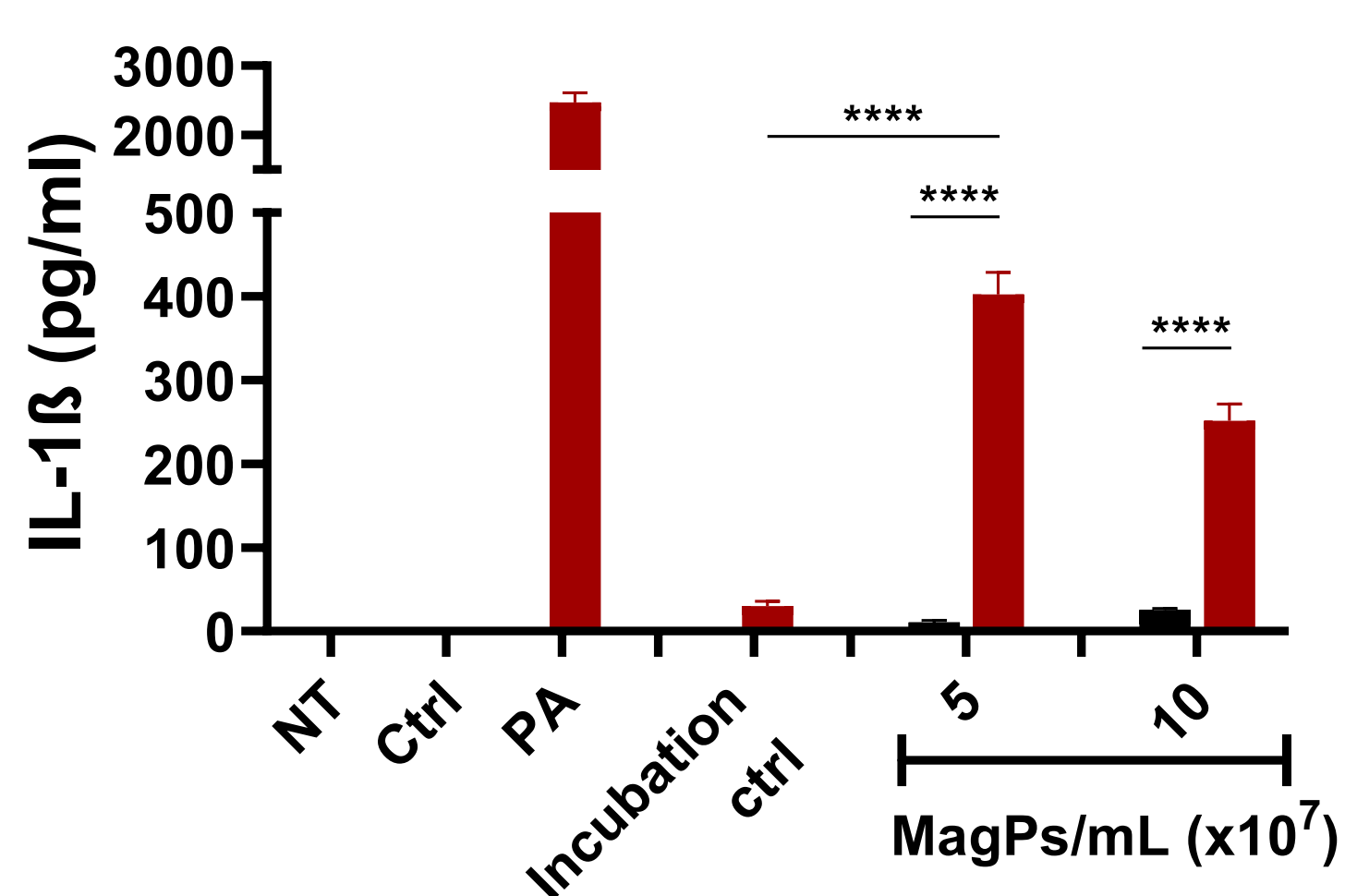


1. Nanosensitizers are adsorbed to cells.
2. Pulsed laser irradiation causing heating or VNBs.
3. Formation of transient pores which allows passage of macromolecules such as toxins into the cytosol.

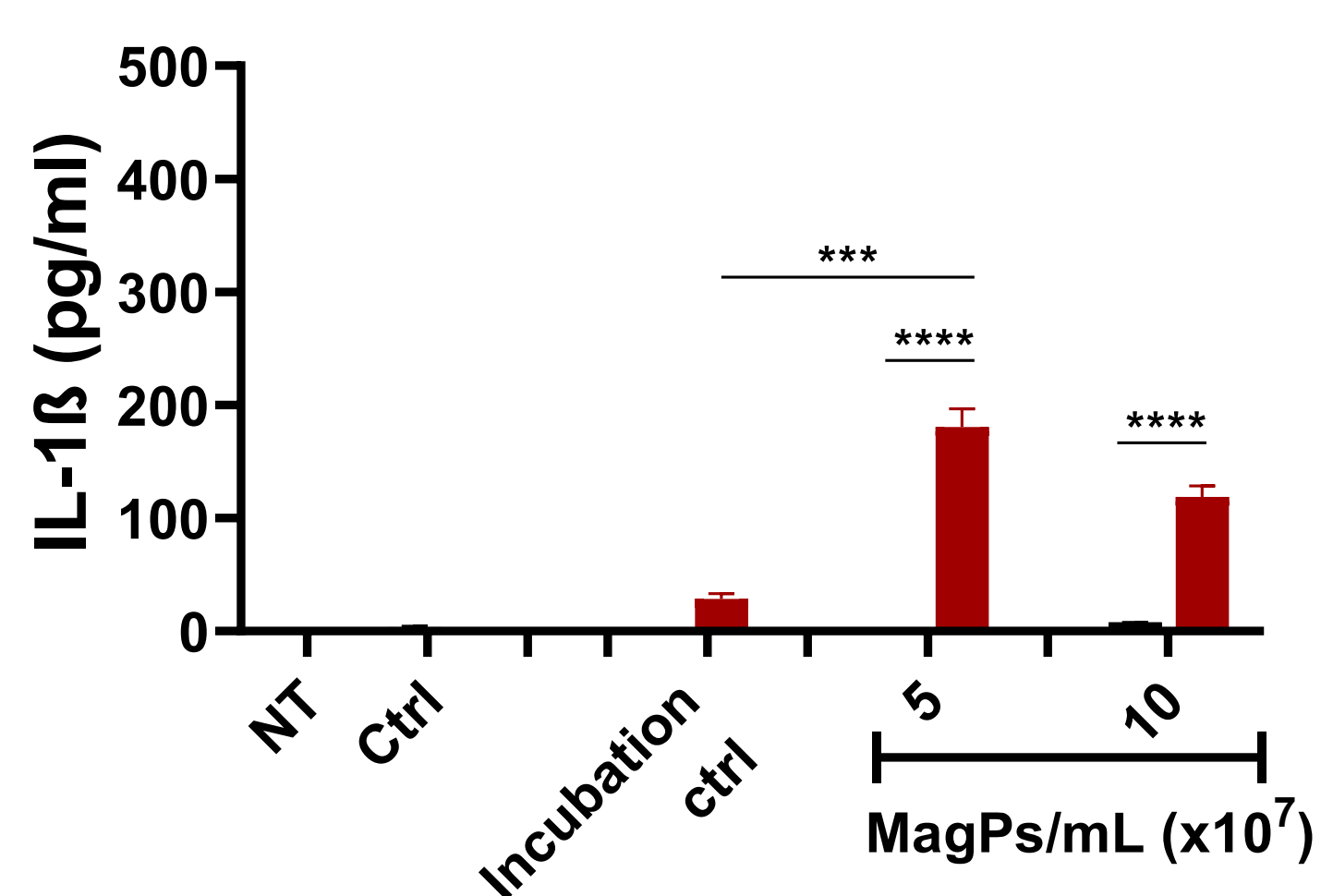
Cytosolic delivery of LPS and Flagellin



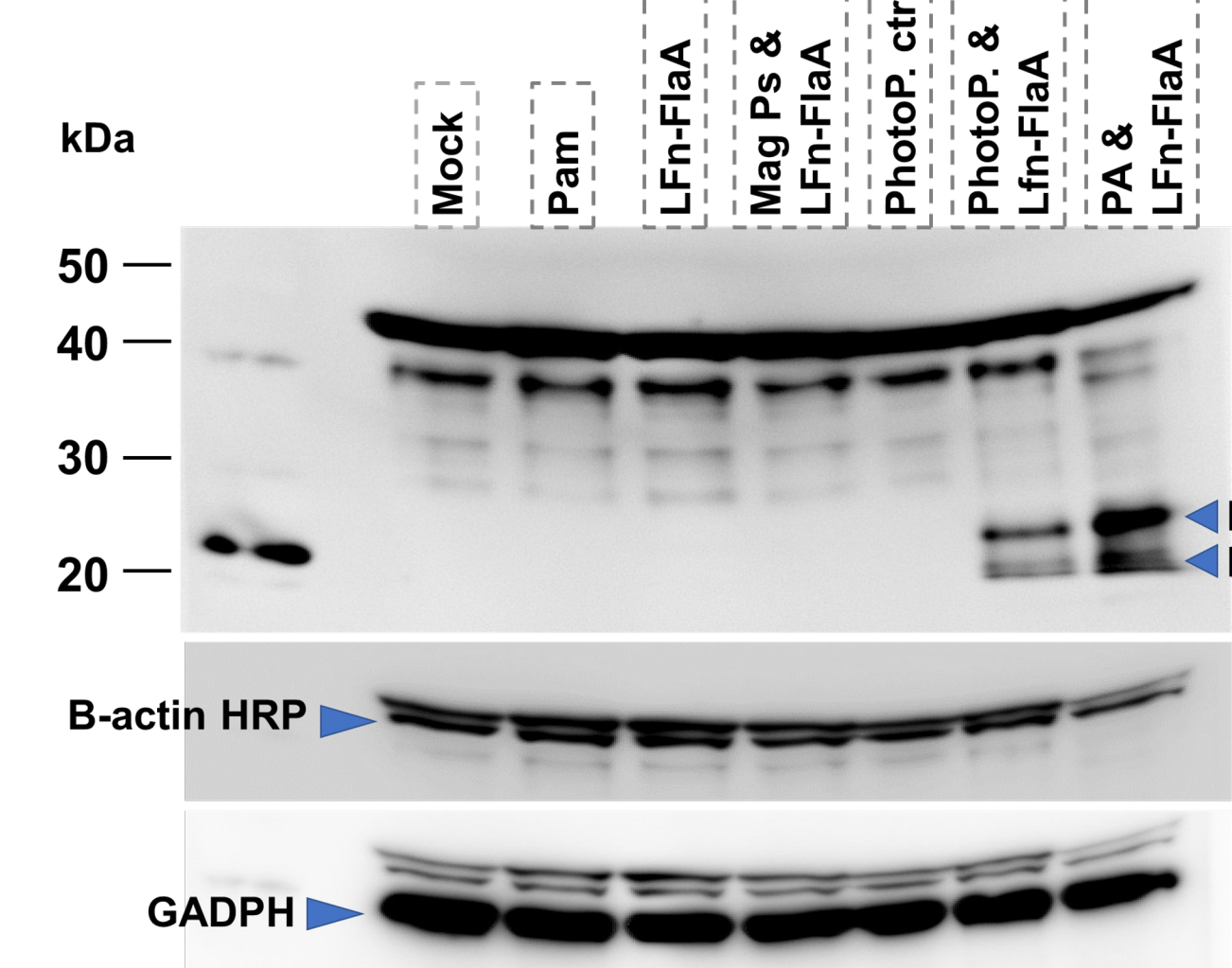
Cytosolic delivery of LFn-FlaA



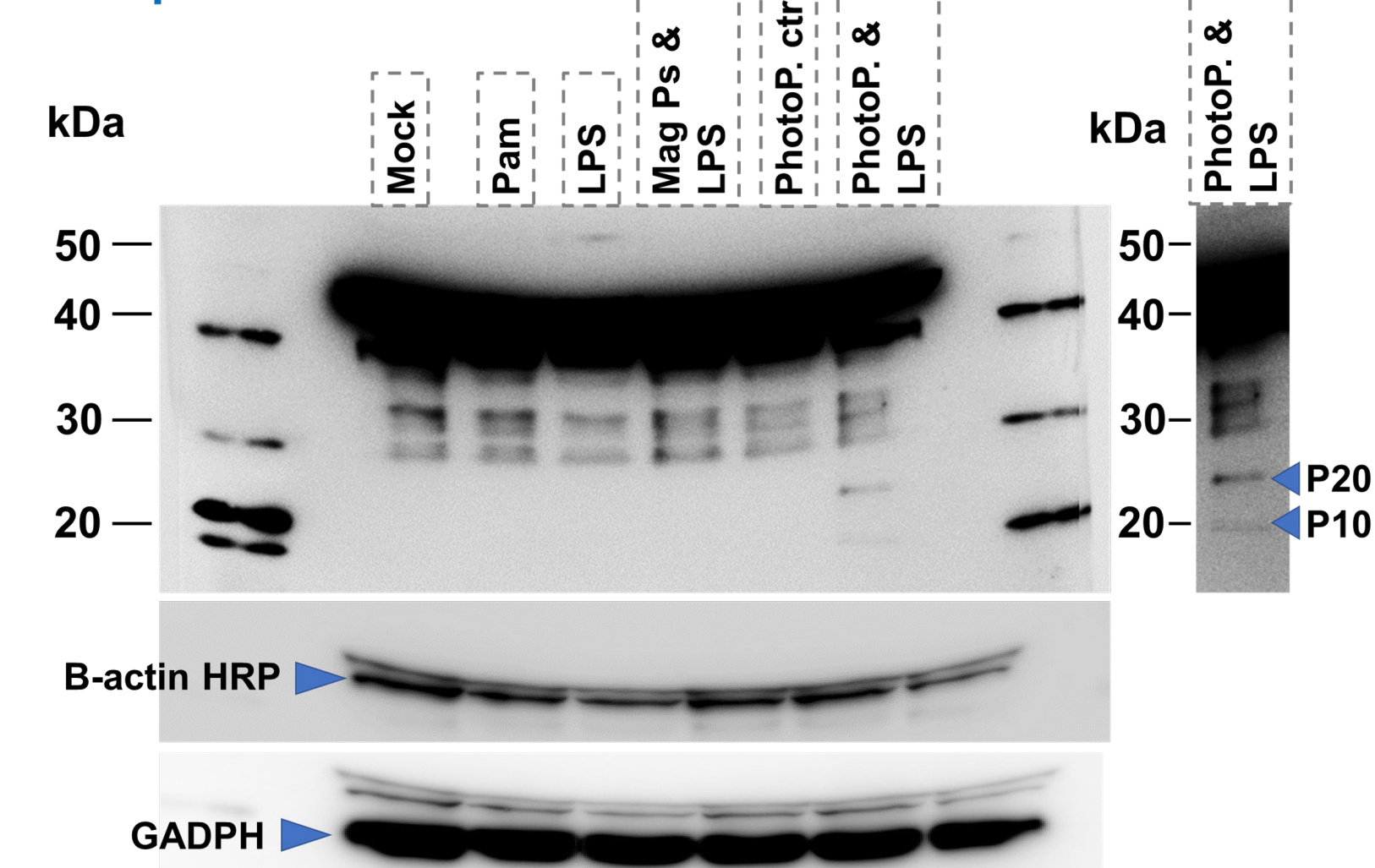
Cytosolic delivery of LPS



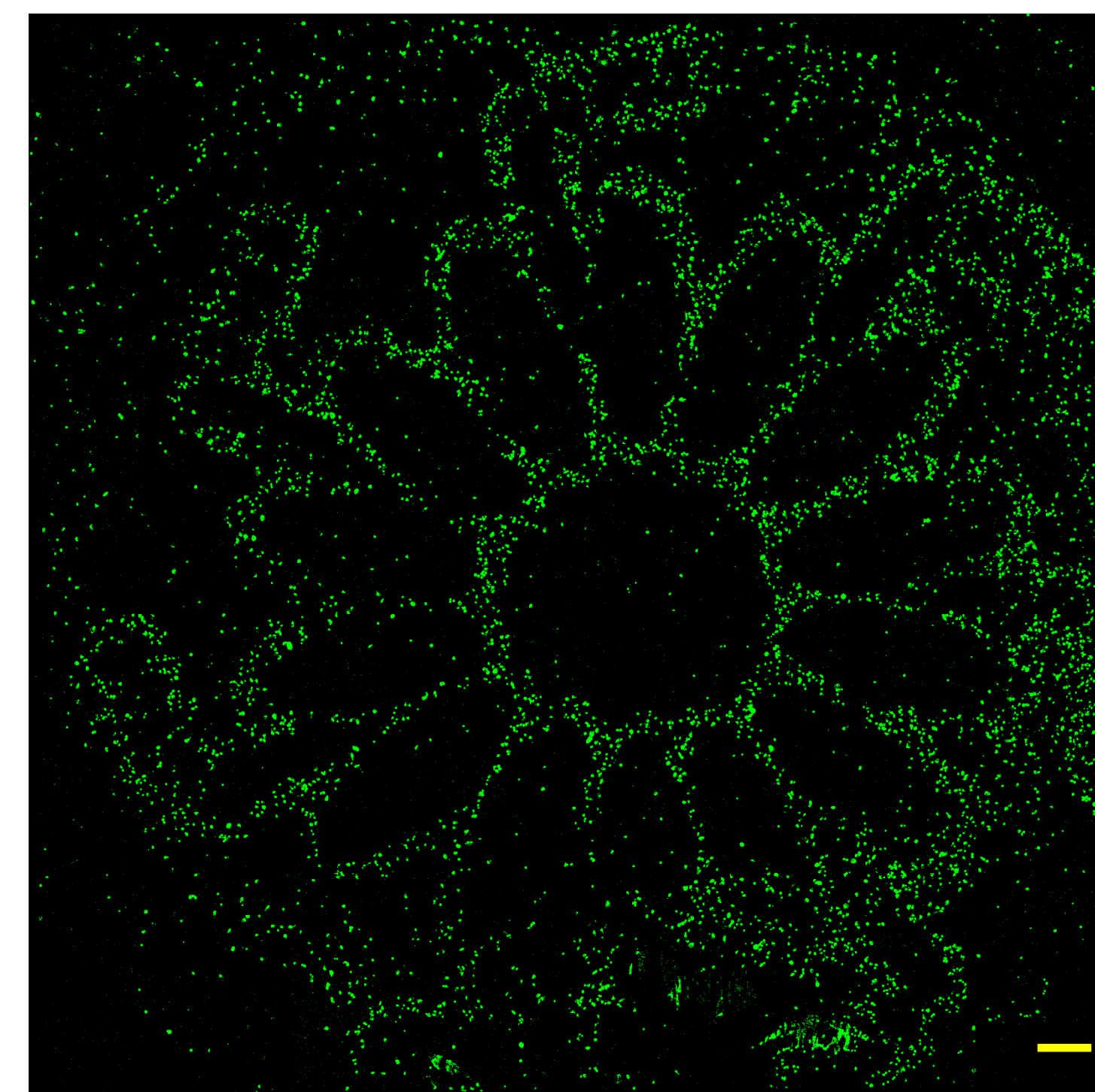
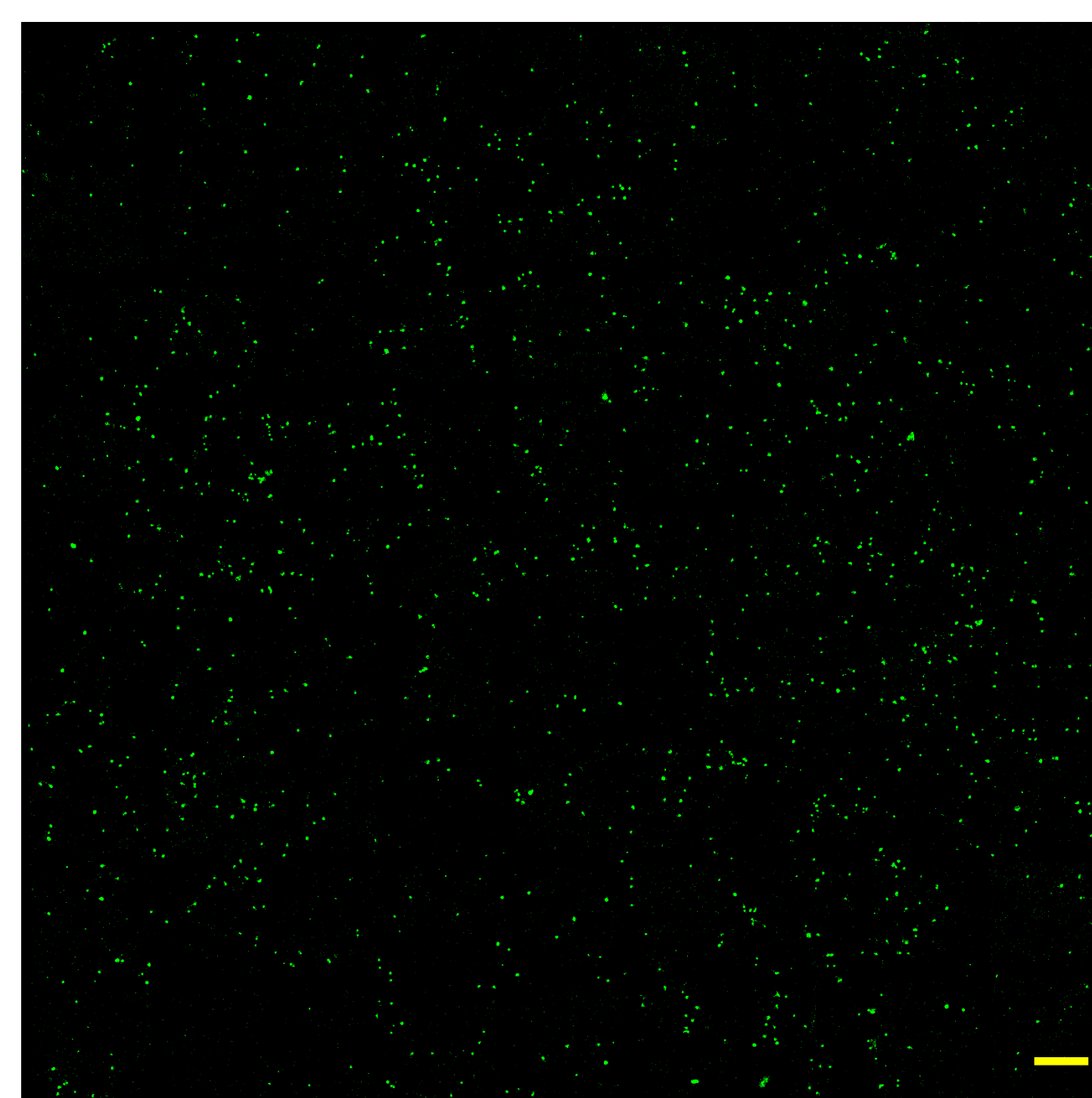
mCaspase-1: LFn-FlaA



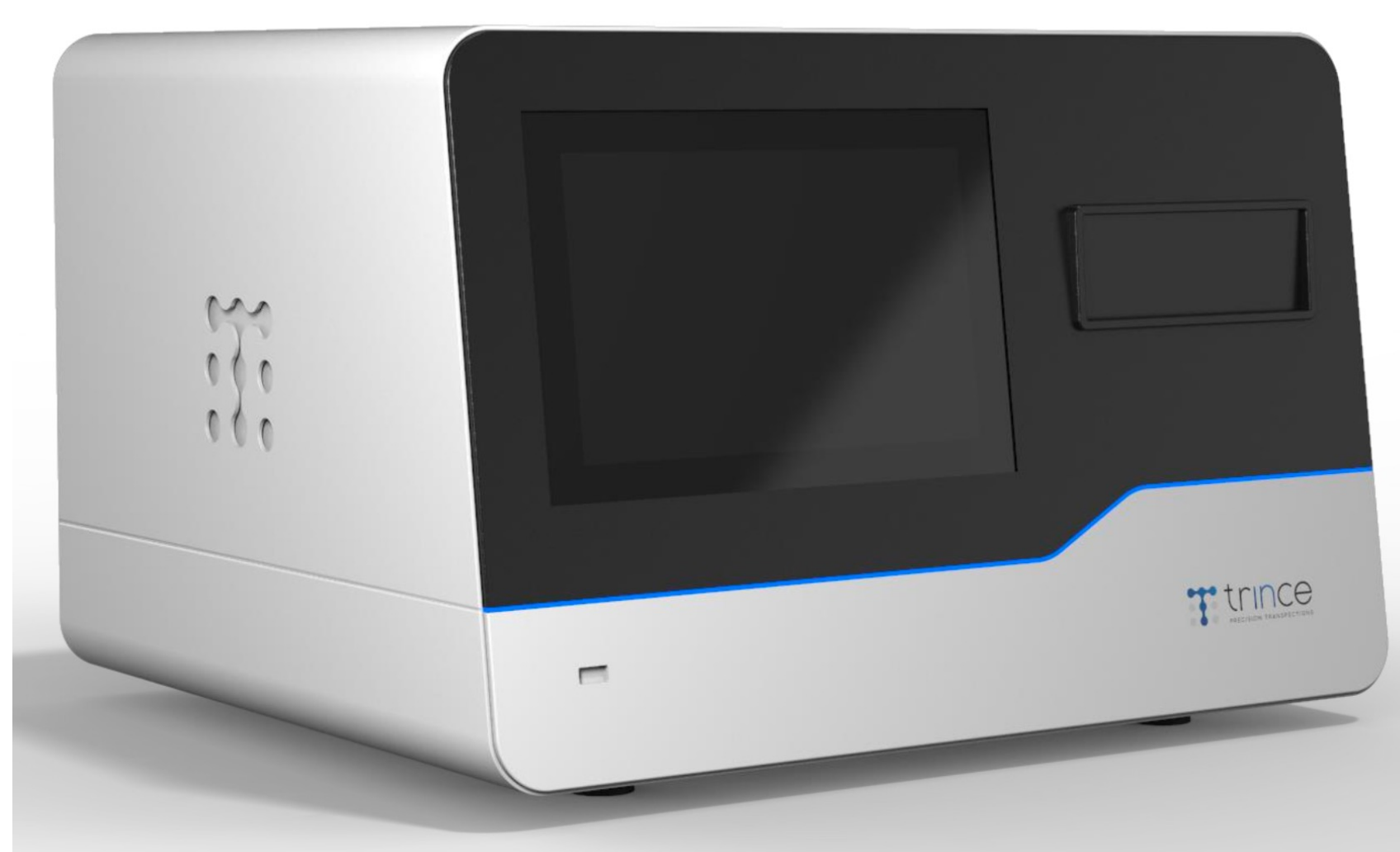
mCaspase-1: LPS



Spatial selective photoporation



LumiPore photoporation device



Recently a **UGent spin-off** company 'Trince' was established to bring photoporation to the **market** as a **new generation of transfection technology**.

Conclusion

1. Photoporation can deliver both proteinaceous and non-proteinaceous inflammasome stimuli into primary macrophages
2. Spatial photoporation is a unique feature of the technology which allows to dissect the heterogeneity of inflammasome responses in single cells and to
3. Photoporation enables efficient research of immune responses in individual immune cells in numerous contexts.

References

1. Harizaj A et al, Nanoscale, 2021, 13.
2. Xiong R et al, Nature Nanotechnology, 2021, 16, 1281–1291

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