



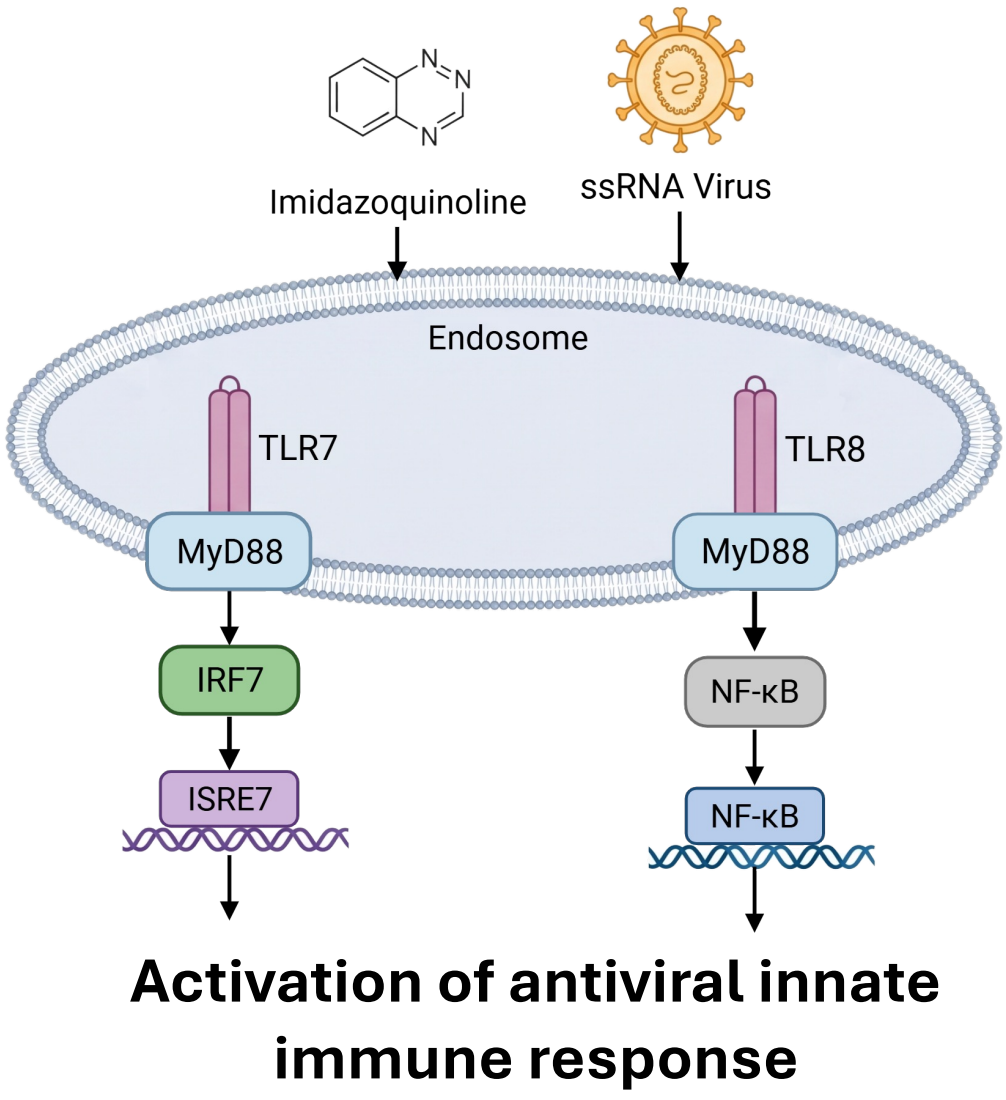
PapMV Nanoparticle: A New Generation of TLR7/8 Agonists

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INTRODUCTION/BACKGROUND

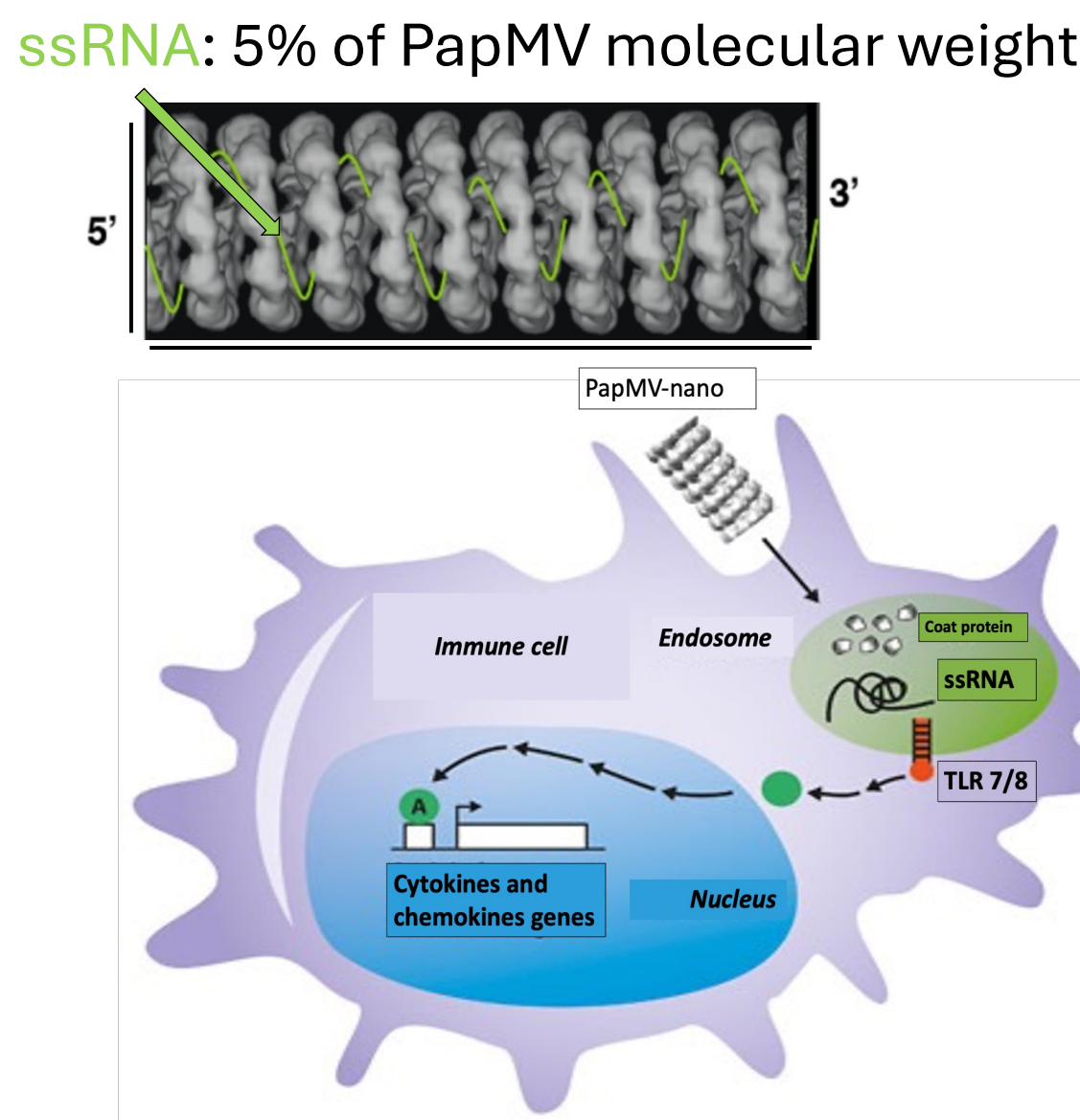
Toll-Like Receptors (TLR) 7/8

- Location:** Highly expressed in endosomes of immune cells.
- Role:** Detect viral single stranded RNA (ssRNA) and activate innate antiviral responses.
- Synthetic agonists:** Imidazoquinolines (IMDs) as Imiquimod (IMQ), R848, CL075. Restricted to topical application due to adverse effects.
- Challenge:** Developing effective TLR7/8 agonists that stimulate antiviral immunity while minimizing systemic toxicity.



PapMV nanoparticles (nano)

- Structure:** Flexuous rod-shaped nanoparticles formed by papaya mosaic virus (PapMV) recombinant coat proteins self-assembled onto ssRNA.
- Mechanism:** Internalized by immune cells; ssRNA activates TLR7/8.
- Potential:** Antiviral, anticancer and adjuvant properties.
- Safety:** Demonstrated safety in humans through systemic administration.



PapMV nano Vs IMDs

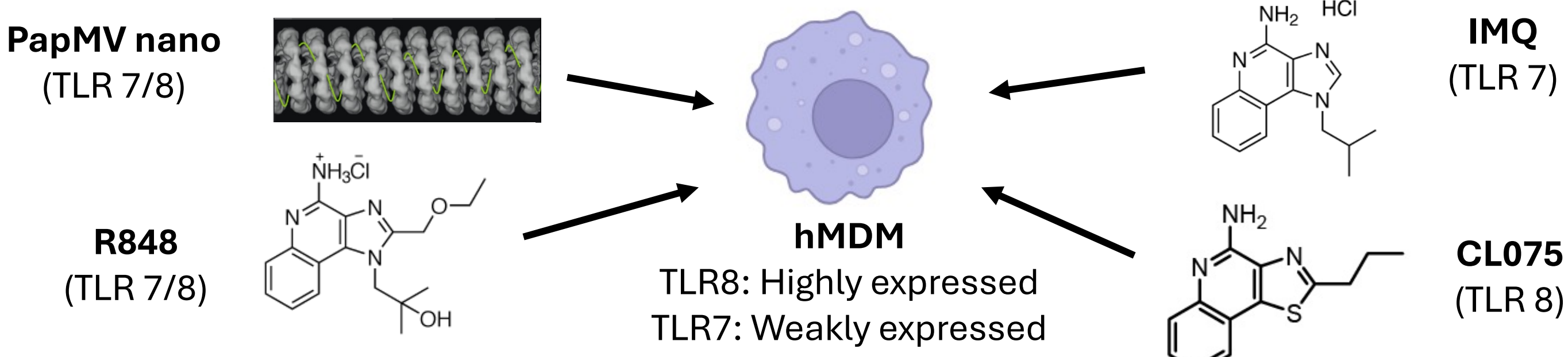
Parameters	PapMV nano	IMDs
1. Composition	Protein and ssRNA (1517 nt)	Low molecular weight synthetic compounds
2. Size	Nanoparticle: 80 nm (length), 14 nm (diameter)	Approximately 1.5nm
3. Cell entry	Internalization	Membrane diffusion

Objectives

- Compare the immunomodulatory effects of PapMV nano and synthetic TLR7/8 agonists (IMQ, R848, and CL075) on human monocyte-derived macrophages (hMDMs).
- Evaluate the protective effect of PapMV nano-induced innate immunity against SARS-CoV-2 infection.

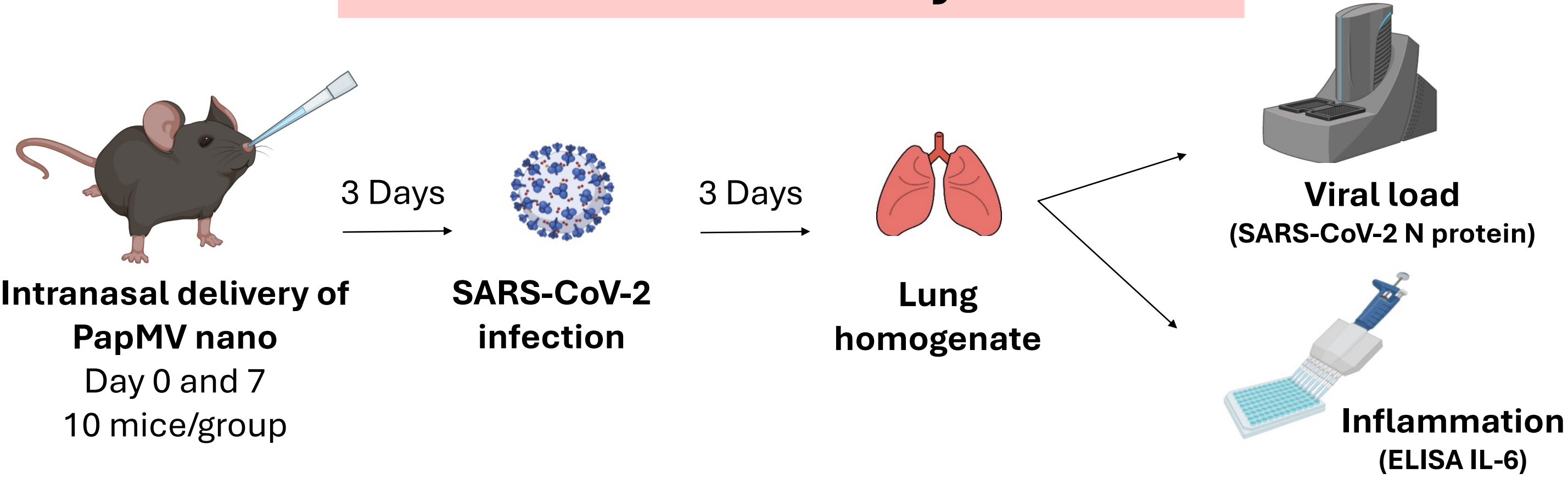
METHODS

In Vitro Studies



	hMDM (cell/well)	Test system	Stimulation time (h)	End point
Motility	4.5x10 ⁴	Automated live-cell imaging system	18	Single-cell tracking (number of pixels in 10 minutes, n=150)
Energy metabolism	2.5x10 ⁴	Extracellular flux analyzer system	12	Oxygen consumption rate (OCR) /extracellular acidification rate (ECAR) (n=6)
Apoptosis	2.0x10 ⁵	FITC annexin V apoptosis detection kit with 7-AAD and flow cytometry	24	Induction of apoptosis (n=3)

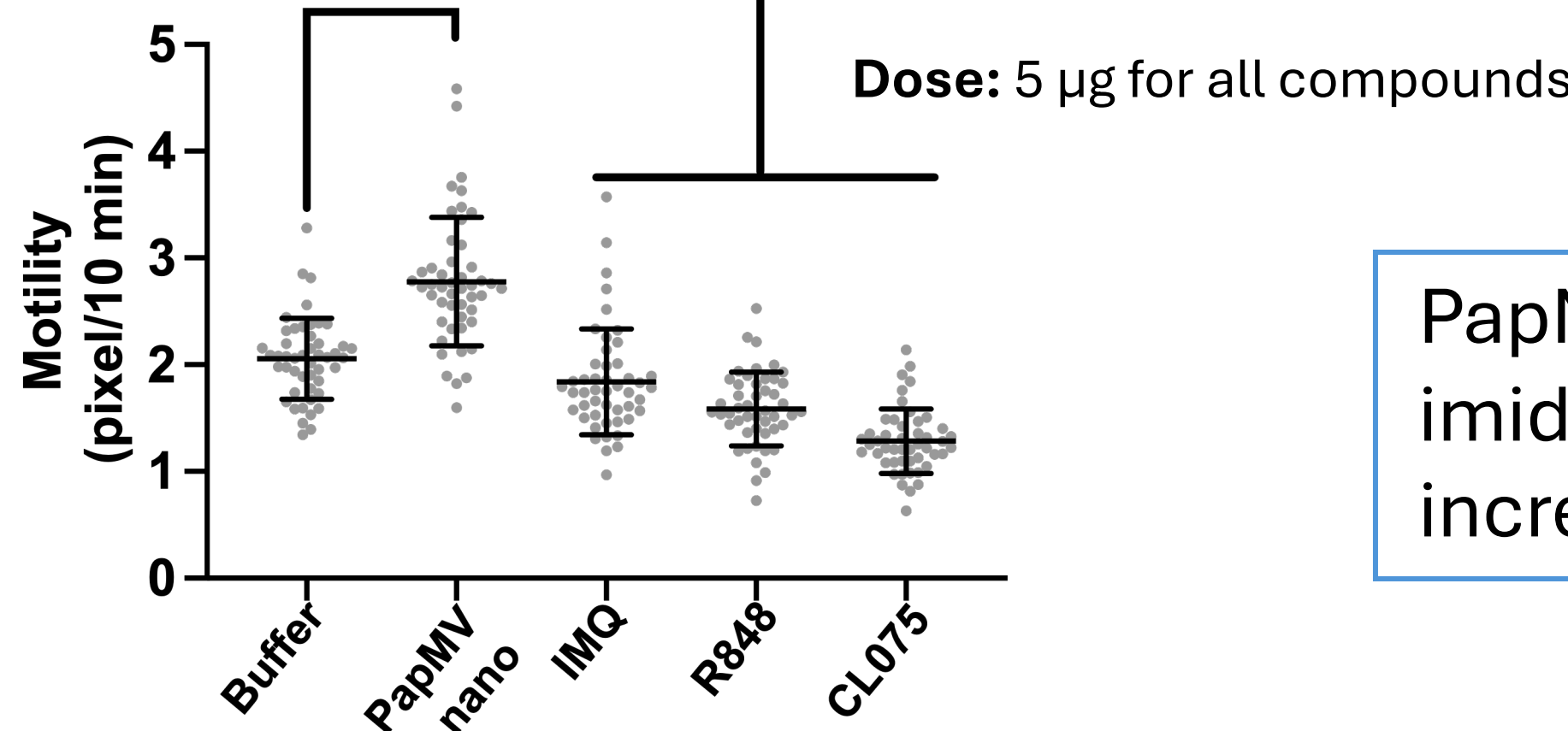
In Vivo study



RESULTS

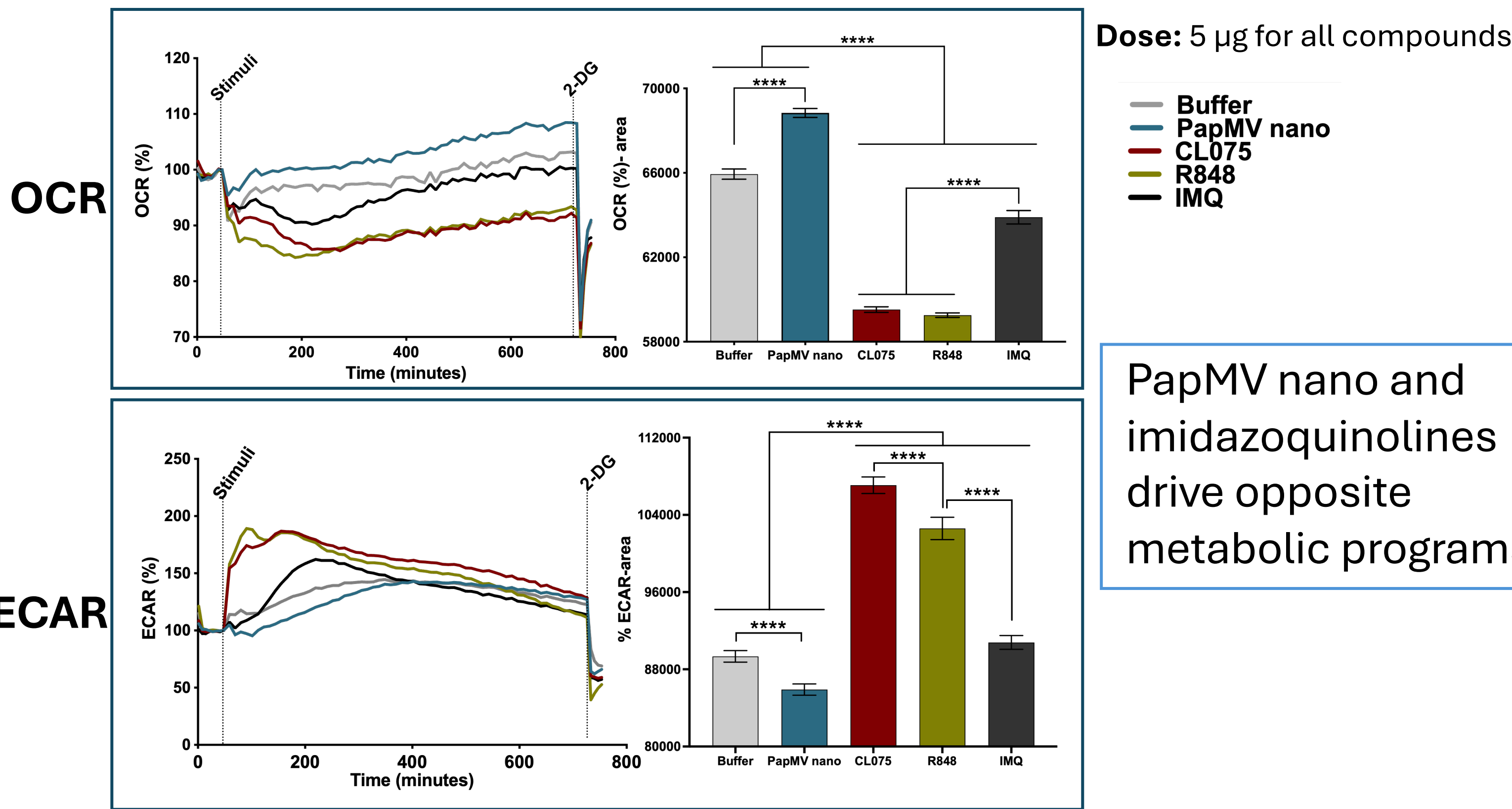
In vitro: PapMV nano and IMDs induce distinct innate immune responses

1. MOTILITY



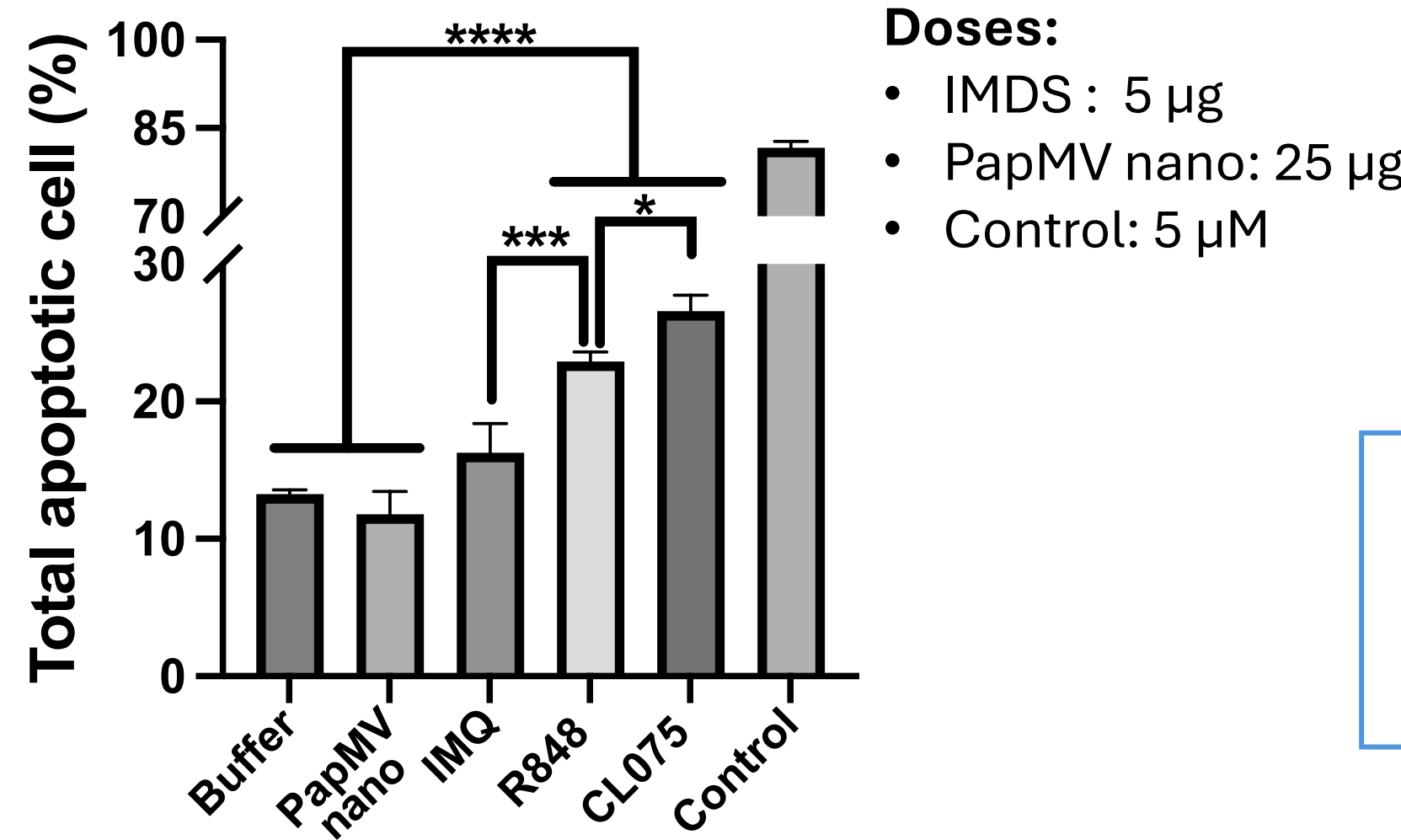
PapMV nano, but not imidazoquinolines, increases hMDM motility

2. ENERGY METABOLISM



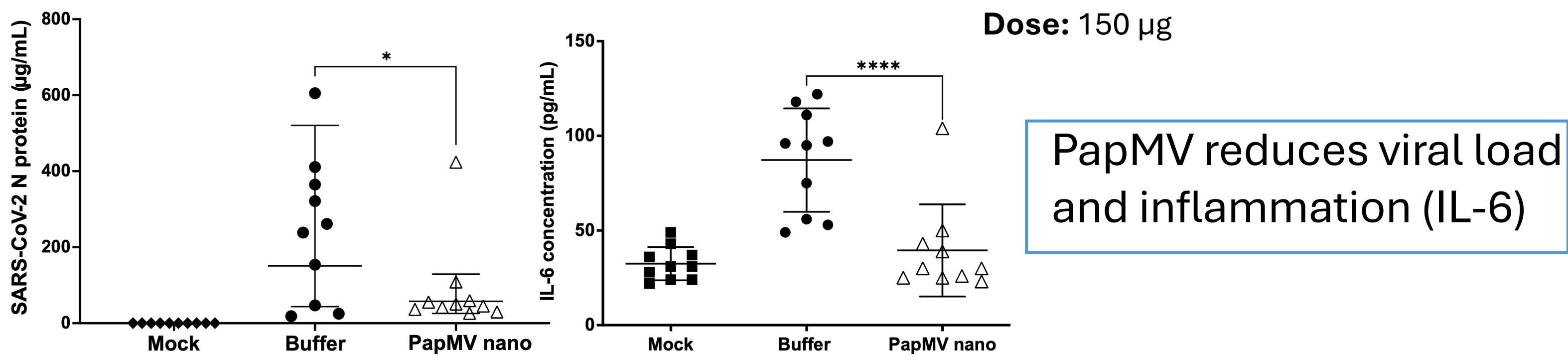
PapMV nano and imidazoquinolines drive opposite metabolic programs

3. APOPTOSIS



PapMV nano does not induce apoptosis, unlike imidazoquinolines

In vivo: PapMV nano mitigate SARS-CoV-2 infection



PapMV reduces viral load and inflammation (IL-6)

CONCLUSIONS

- PapMV nano and IMDs both activate TLR7/8 and can elicit antiviral immunity, but they induce distinct cellular programs in human macrophages: **PapMV nano promotes motility and mitochondrial respiration without triggering apoptosis**, while IMDs favor glycolysis and apoptosis.
- Intranasal PapMV nano significantly **reduced SARS-CoV-2 lung viral load and inflammation** (IL-6) in a transgenic mouse model.
- Together with its favorable safety profile, these results **support PapMV nano as a promising, safer alternative to imidazoquinolines** for stimulating innate antiviral immunity in the lungs with potential for broad protection against respiratory viruses.

ACKNOWLEDGMENTS

