

A diagram of a cell, represented by a large purple circle with a darker purple nucleus. The cell is surrounded by various microorganisms: several blue, rod-shaped bacteria with flagella, green, oval-shaped bacteria, and numerous small purple dots representing viruses. Some of the bacteria are shown with flagella, indicating they are motile. The overall scene suggests a complex microbial environment interacting with the cell.

Glycolipid

Eggerthellaceae
(*Eggerthella* and
Gordonibacter)

- Gut microbiota strains produce soluble metabolites that activate mast cells.
- *Eggerthella* and *Gordonibacter* strains produce a highly conserved bioactive metabolite that activates mast cells.
- Mast cell response to allergen is exacerbated by preexposure to the bioactive metabolite.
- Chemical and bioinformatic analyses suggest the bioactive is a glycolipid.

- The Balskus lab is interested in elucidating the molecular mechanisms by which the microbiota regulates human health.
- While association studies support a connection between the microbiome and development of atopic diseases¹⁻³, the underlying metabolic mechanisms remain unknown.
- Goal – Identify and characterize gut microbiome-derived metabolites that promote atopic responses.

Screening for bioactive gut microbiota-derived metabolites

Primary Murine Mast Cell Activation Screening

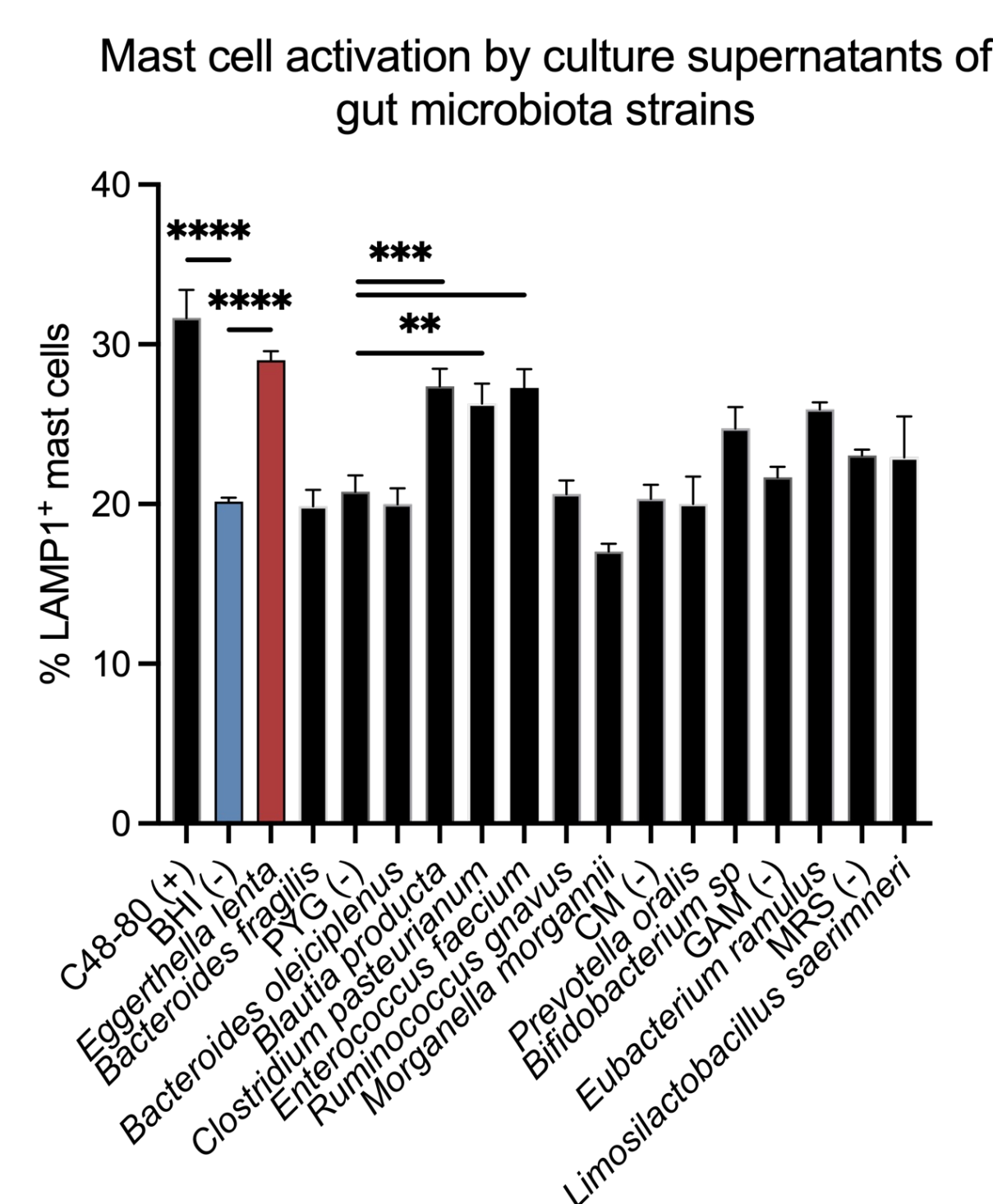
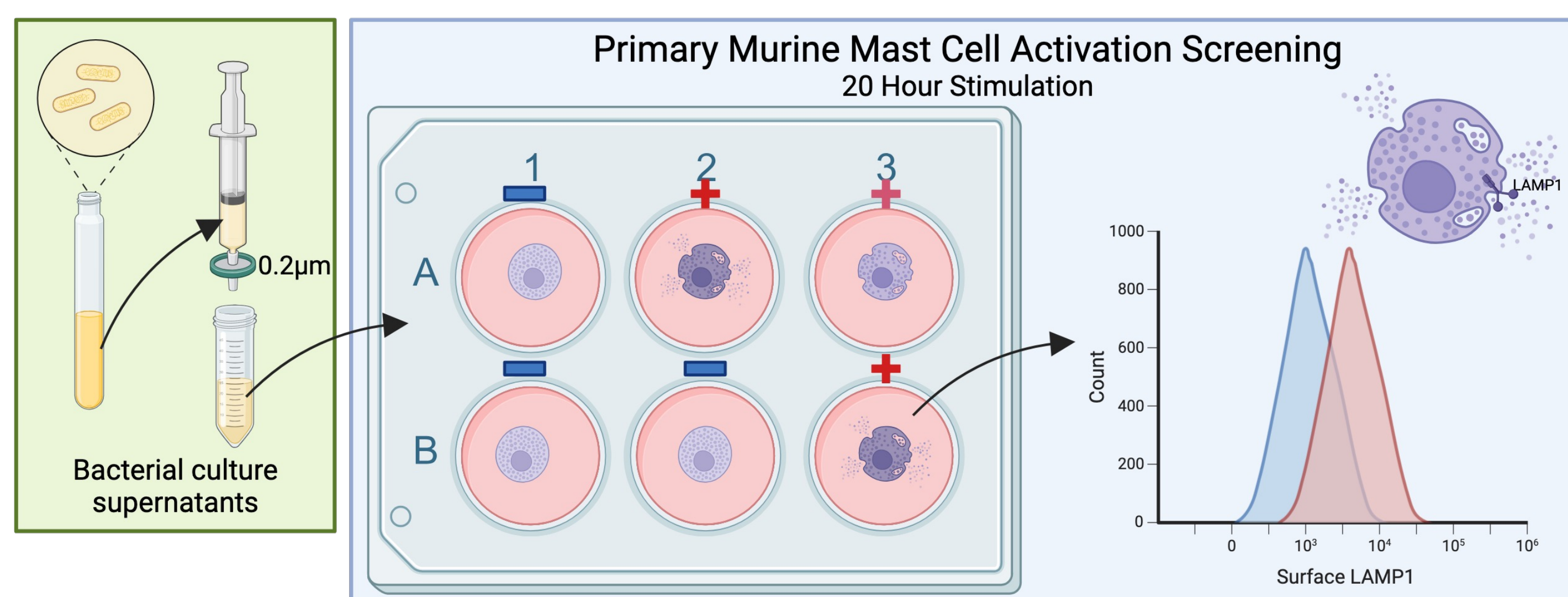


Figure 2. The ability to activate mast cells by culture supernatants is highly conserved among Eggerthellaceae strains.

BMMC were stimulated for 20 hours with filtered culture supernatants from Eggerthellaceae strains. Mast cell activation was evaluated by flow cytometry surface LAMP1 staining. Error bars represent SEM. ANOVA, Sidak's, * $p=0.05$, ** $p=0.01$, *** $p=0.001$, **** $p=0.0001$.

Figure 3. Mast cell activation by *E. lenta* supernatants is dose responsive.

BMDCs were stimulated for 20 hours with *E. lenta* filtered culture supernatants. Mast cell activation was evaluated by flow cytometry surface LAMP1 staining. Error bars represent SEM. ANOVA, Dunnett's, * $p=0.05$, ** $p=0.01$.

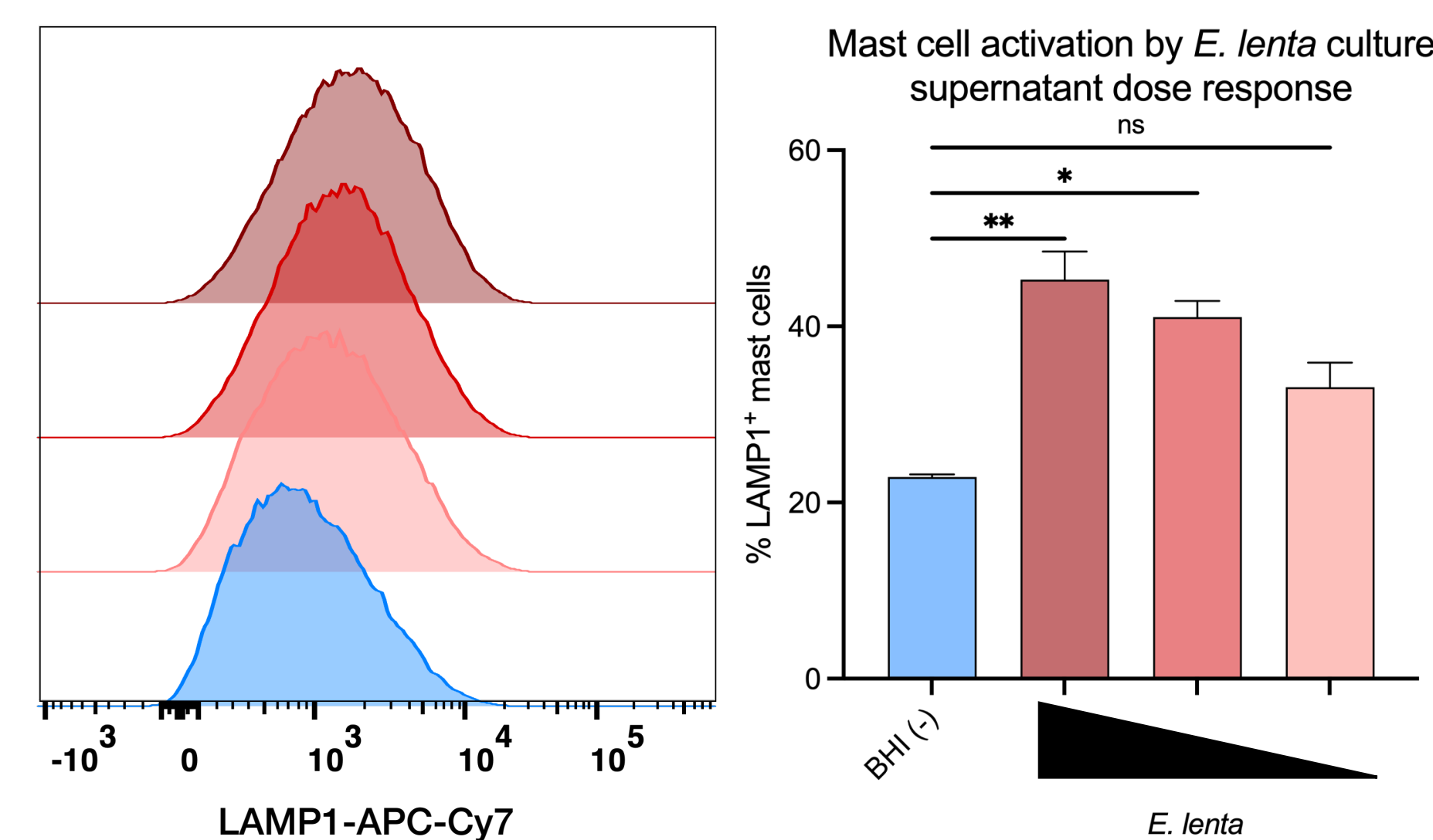
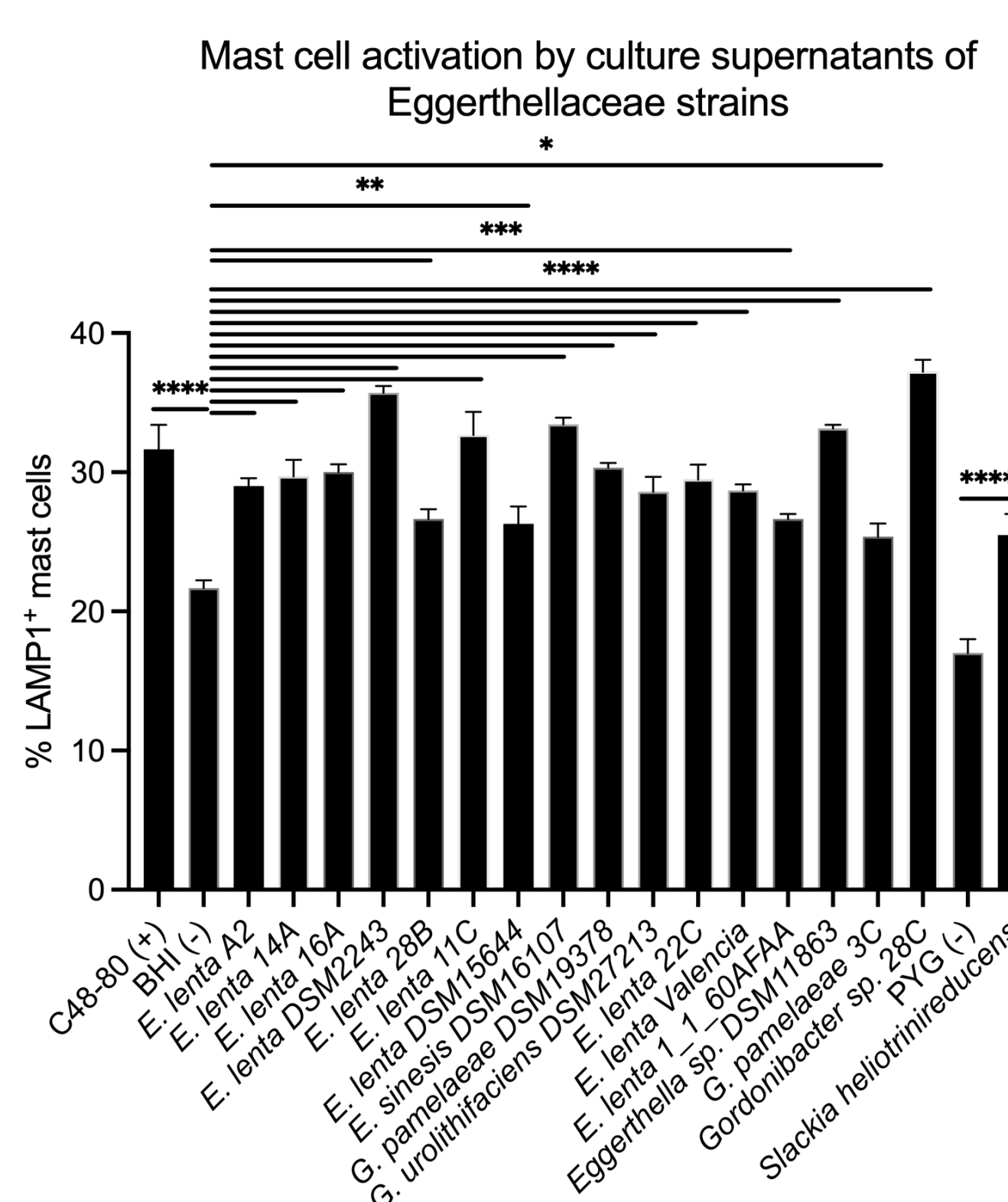


Figure 4. Mast cell inflammatory response to allergen is exacerbated by pre-exposure to *E. lenta* supernatants.

BMMC were stimulated for 20 hours with *E. lenta* supernatants and anti-DNP IgE. Cells were washed and incubated for 30 minutes with 100 ng/mL DNP-HSA in tyrode buffer. Cell culture supernatants were evaluated for cytokine and chemokine production by Luminex multiplex; two analytes are shown. Error bars represent SEM. ANOVA, Sidak's, * $p=0.05$, ** $p=0.01$, *** $p=0.001$, **** $p=0.0001$.

Figure 1. Culture supernatants from *Eggerthella lenta* activate mast cells most significantly compared to other gut microbiota strains.

Bone marrow derived murine mast cells (BMMC) were stimulated for 20 hours with filtered culture supernatants from gut microbiota strains. Mast cell (CD34⁺FcεRI+cKit⁺) activation was evaluated by flow cytometry surface LAMP1 staining. Error bars represent SEM. ANOVA, Sidak's, ** $p=0.01$, *** $p=0.001$, **** $p=0.0001$.



Mast cell activation by *E. lenta* culture supernatant dose response

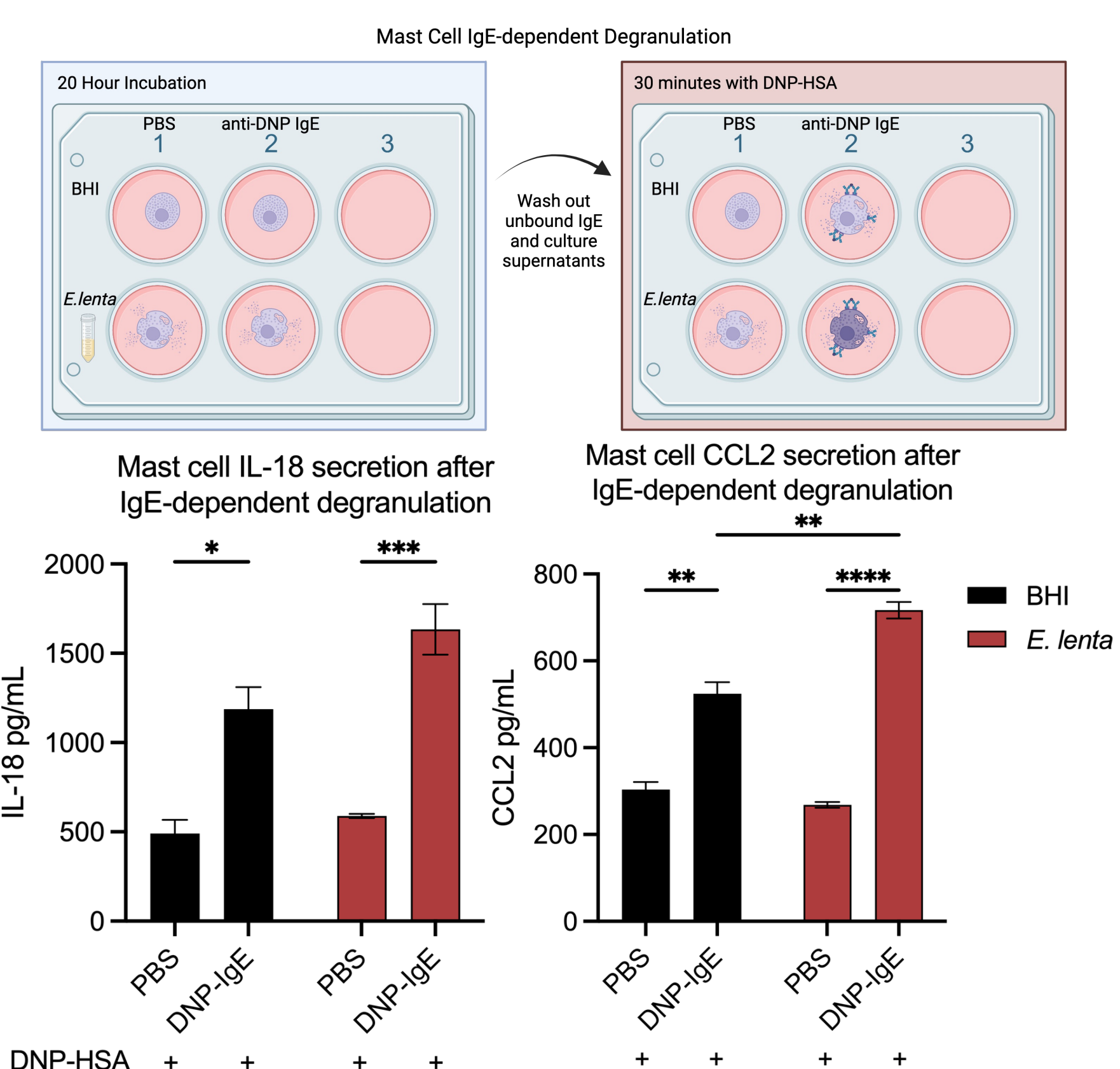
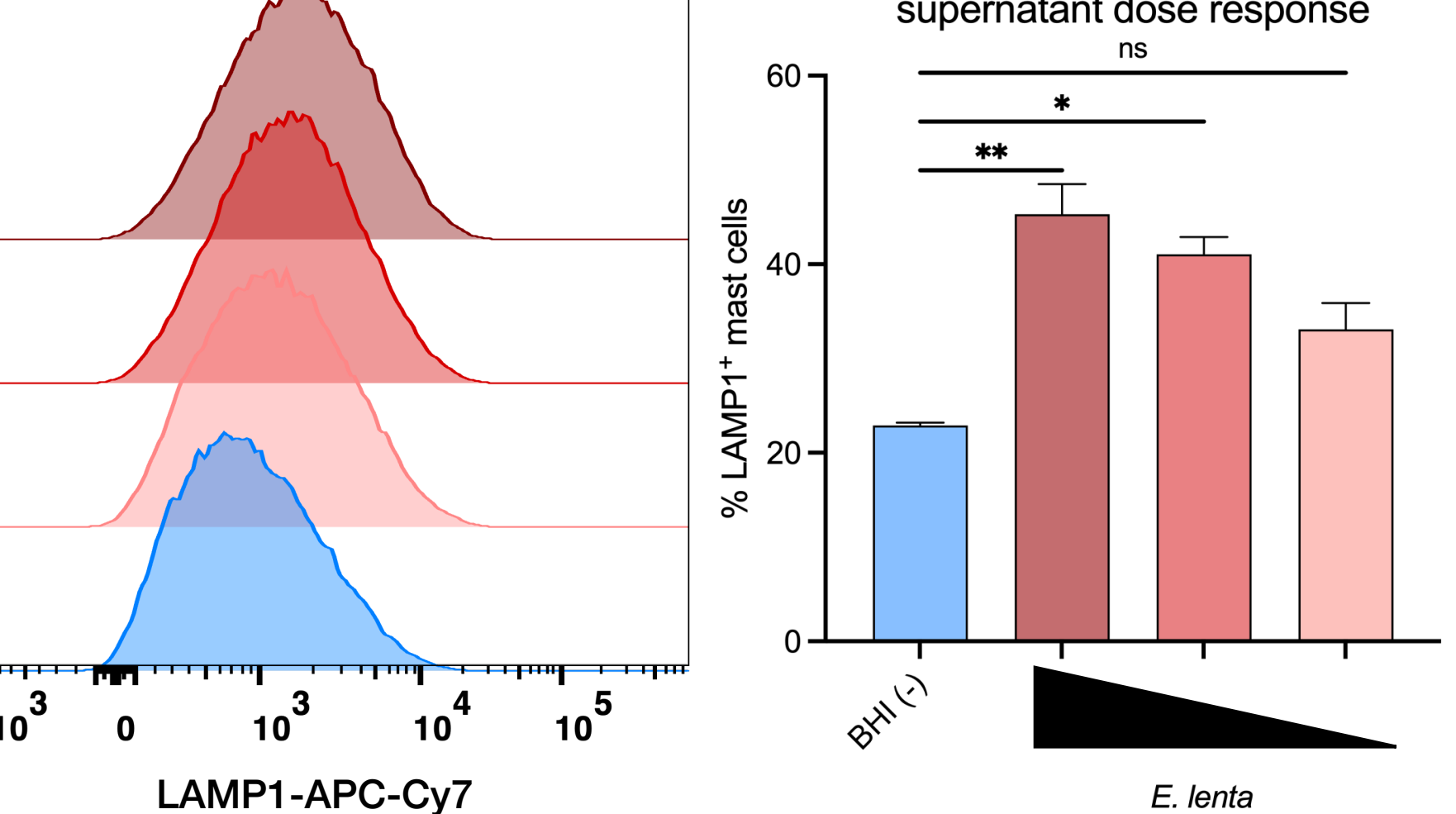


Figure 5. Ongoing chemical and bioinformatic analyses support a small glycolipid as the bioactive metabolite.

Bioactivity-guided fractionation paired with metabolomics and biochemical analyses support a small, polar glycolipid as the bioactive metabolite. Comparative genomics and structural homology modeling have independently identified putative biosynthetic genes for glycolipid formation.

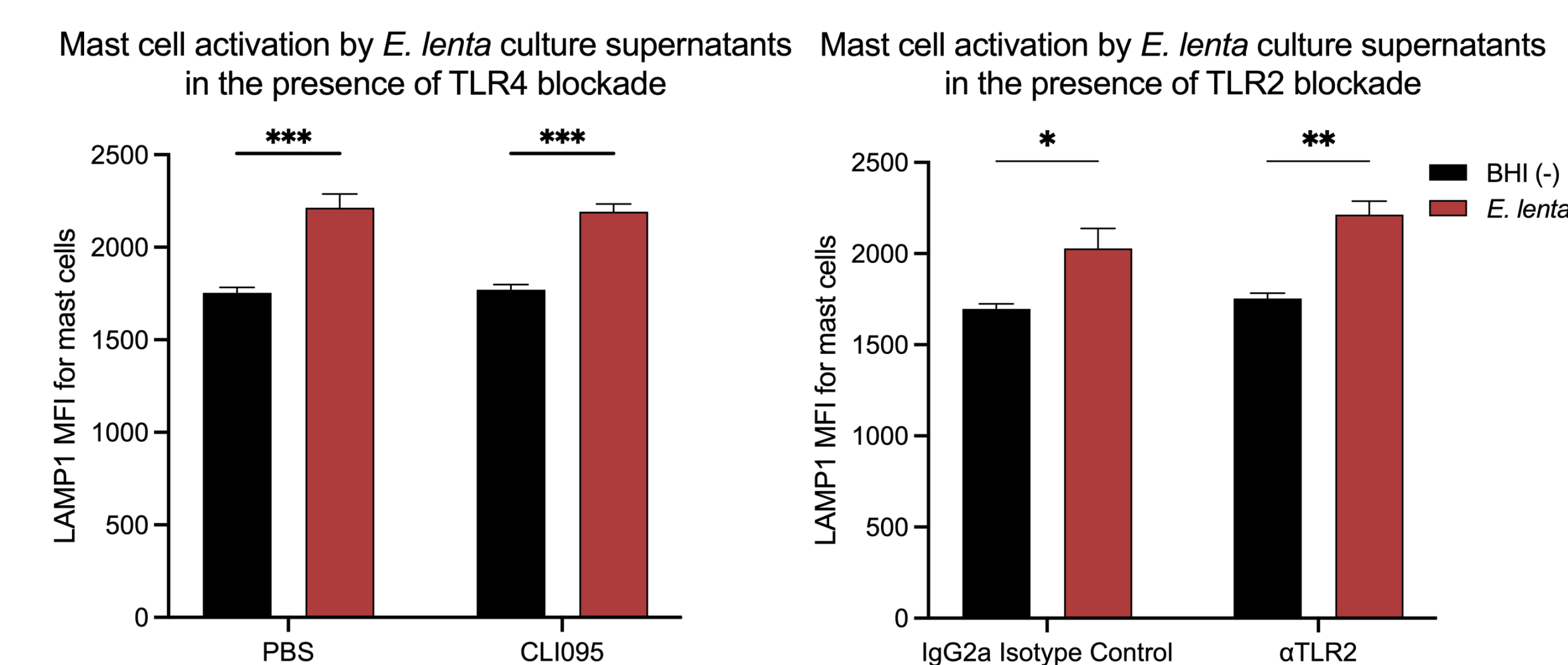
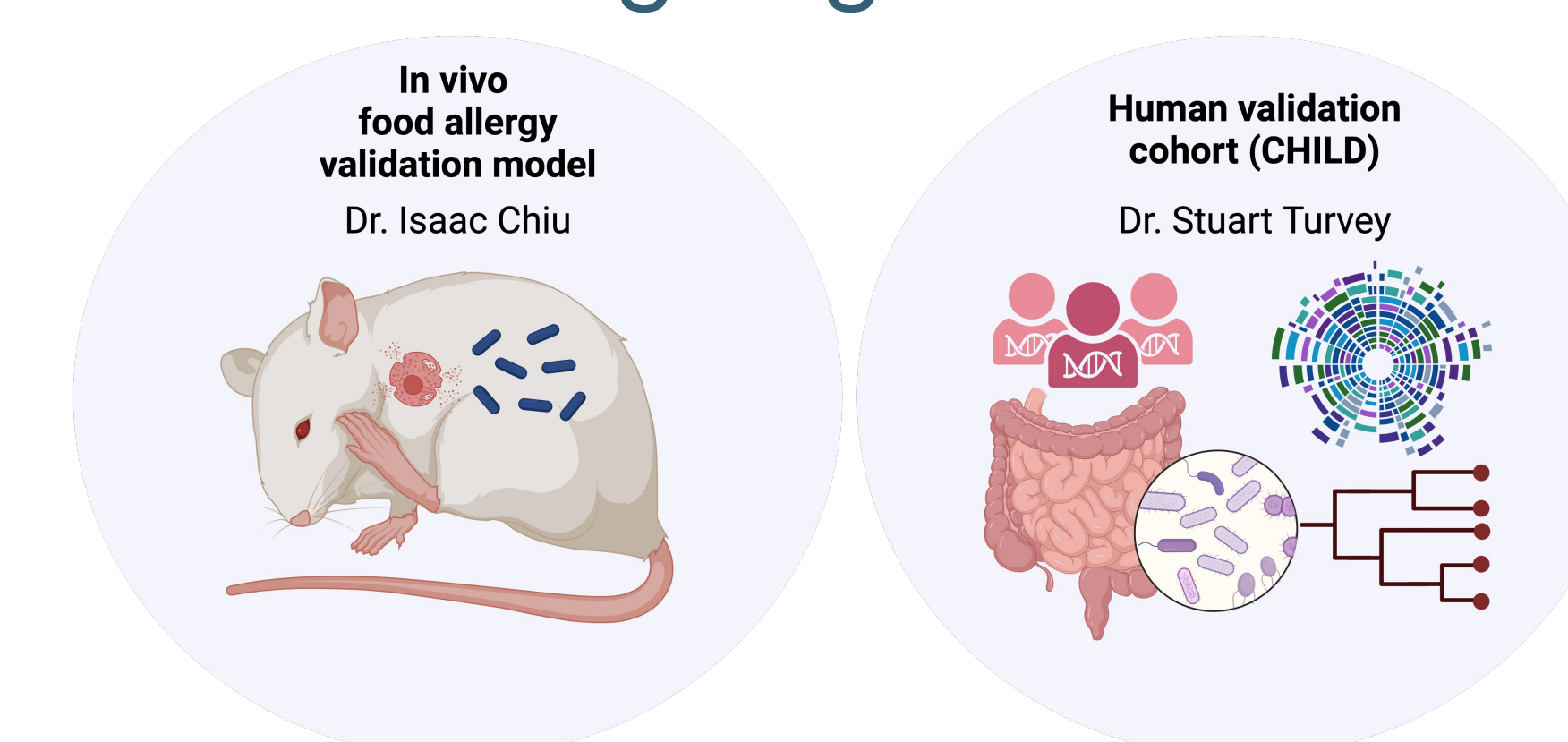


Figure 6. Mast cell activation by *E. lenta* supernatants occurs independently of two glycolipid-sensing receptors TLR2 and TLR4.

BMMC were treated with anti-TLR2-IgG2a or TLR4 signaling inhibitor CLI-095 for 1 hour before being stimulated for 20 hours with *E. lenta* supernatants. Mast cell activation was evaluated by flow cytometry surface LAMP1 staining. Error bars represent SEM. ANOVA, Sidak's, * $p=0.05$, ** $p=0.01$, *** $p=0.001$.

Additional Ongoing and Future Work



Conclusions

- Gut microbiota strains produce soluble metabolites that activate mast cells.
- Eggerthellaceae family genera including *Eggerthella* and *Gordonibacter* produce a highly conserved bioactive metabolite that activates mast cells.
- Activation of mast cells with the bioactive metabolite is dose responsive.
- Mast cell response to allergen is exacerbated by preexposure to the bioactive metabolite.
- Chemical and bioinformatic analyses suggests that the bioactive metabolite is a small, polar glycolipid.

References and Funding

1. Bao, R. et al. Fecal microbiome and metabolome differ in healthy and food-allergic twins. *Journal of Clinical Investigation* 131, e141935 (2021).
2. Feehley, T. et al. Healthy infants harbor intestinal bacteria that protect against food allergy. *Nat Med* 25, 448–453 (2019).
3. Hoskinson, C. et al. Delayed gut microbiota maturation in the first year of life is a hallmark of pediatric allergic disease. *Nat Commun* 14, 4785 (2023).