Microbial-Derived Metabolites Induce Actin Cytoskeletal Rearrangement and Protect Blood-Brain Barrier Function

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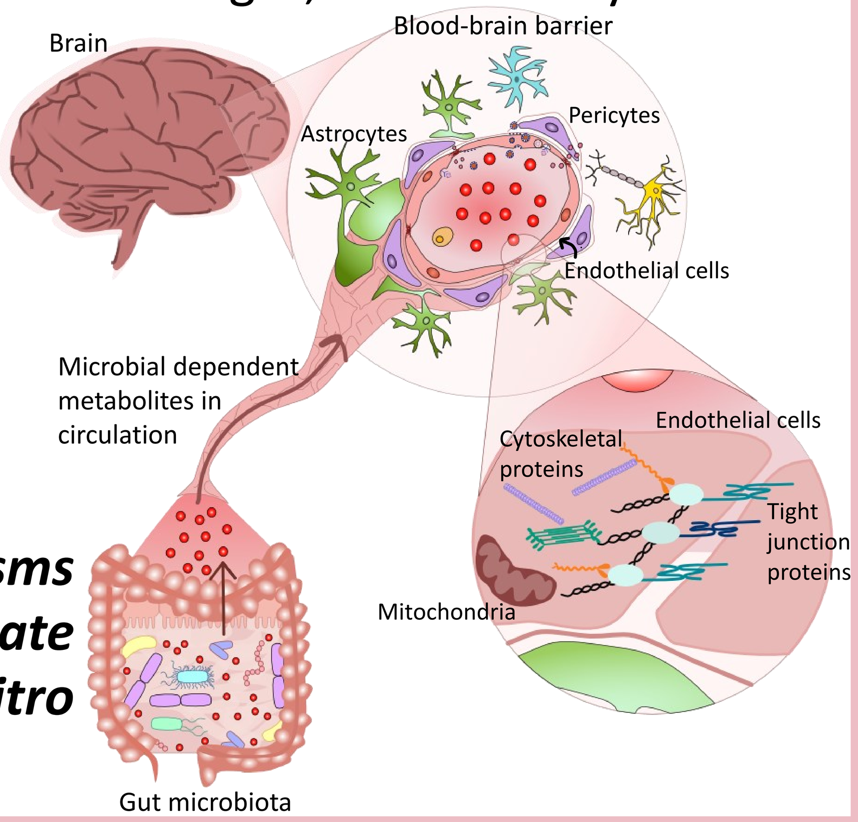
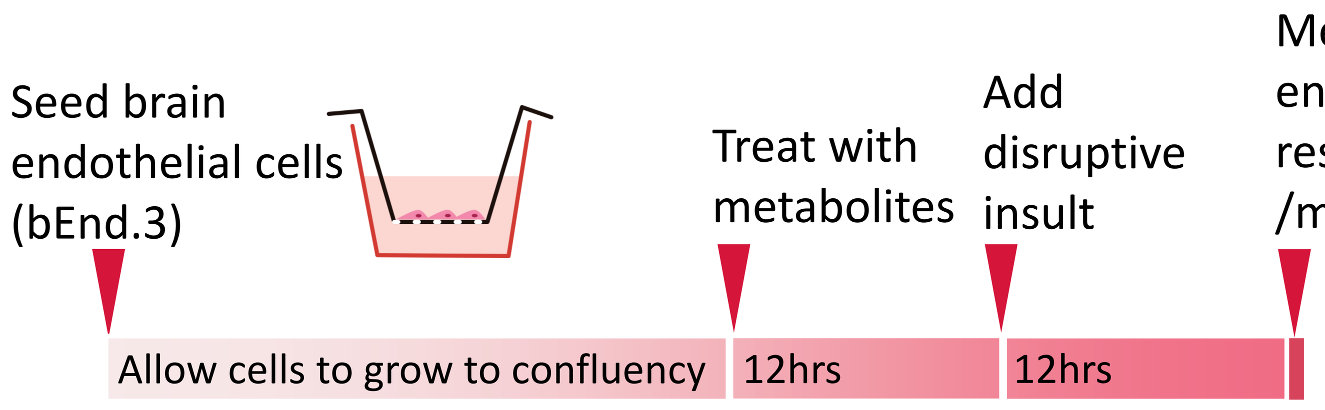
Background

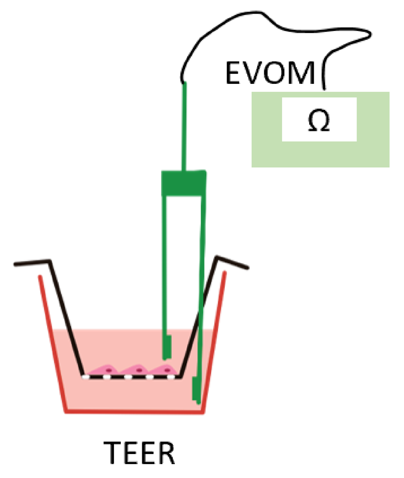
The blood-brain barrier (BBB) is the primary selective barrier between the blood and the brain and is essential for maintaining the optimal microenvironment for brain homeostasis. A compromised BBB is a hallmark of numerous neurodegenerative and stress-related disorders. Recently, the gut microbiota has been identified in having effects on BBB integrity. Gut-microbial dependent metabolites produced in the gut, such as butyrate and propionate, can enter circulation and alter BBB function. Although a proposed communication pathway between the gut microbiota and the BBB have been identified, the mechanism through which these SCFAs interact with the BBB to modulate barrier function is poorly defined

Here, ***we explored the cellular mechanisms involved in butyrate and propionate protection of BBB integrity in an in vitro BBB model.***



Methods





Groups:

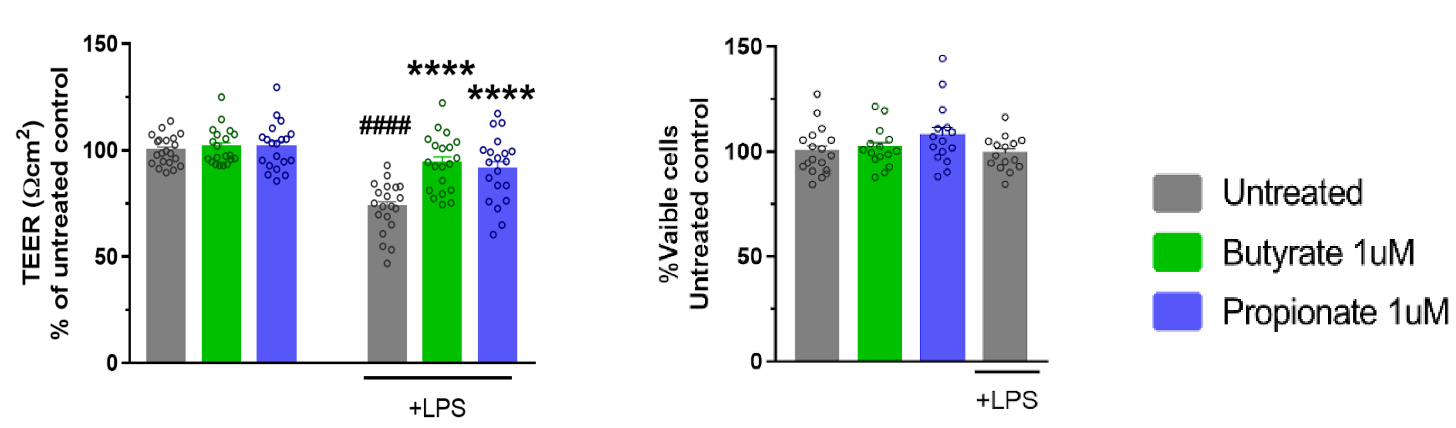
1. Untreated control
2. Butyrate (1uM)
3. Propionate (1uM)
4. LPS (1ug/mL) control
5. Butyrate (1uM) + LPS (1ug/mL)
6. Propionate (1uM) + LPS (1ug/mL)

Disruptive insult: Lipopolysaccharide (LPS) – endotoxin from pathogenic bacteria that induces an immune response and is a common model of inflammation known to disrupt barrier function

Results

Figure 1. The microbial metabolites butyrate and propionate protect against LPS mediated decrease in BBB integrity

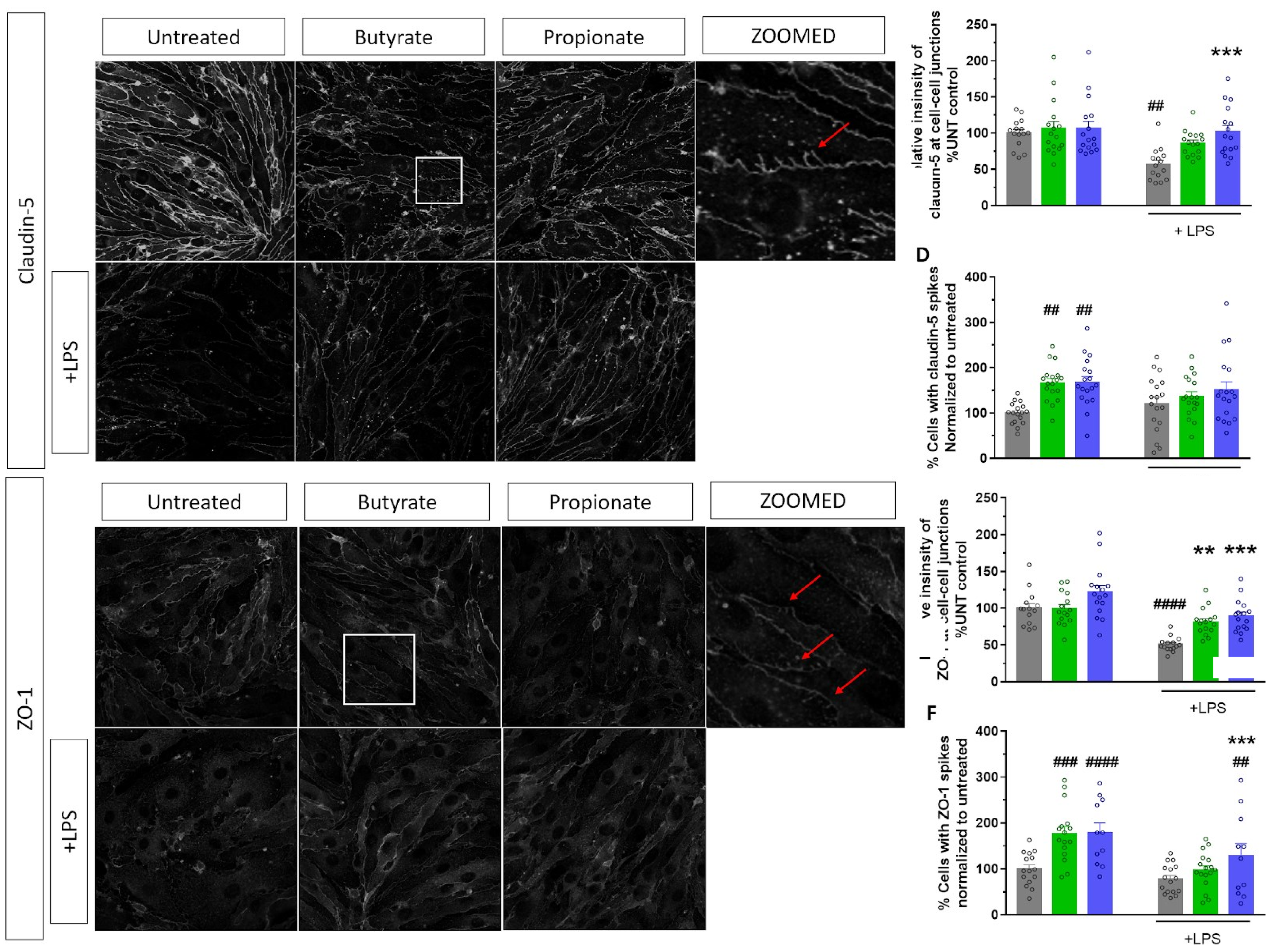
A. Protection of barrier integrity **B. MTT cell viability**



*(A) LPS decreased trans-endothelial electrical resistance (TEER) and butyrate or propionate pre-treatment protected from the decrease. (B) Cell viability is not reduced. LPS vs. untreated (#### p<0.0001), metabolite vs. LPS (****p<0.0001, two-way ANOVA followed by Tukey's multiple comparisons test). Data are presented as mean ± SEM.*

Figure 2. Butyrate and propionate protect against LPS mediated decrease in tight junction protein expression and induced tight junction protein spikes

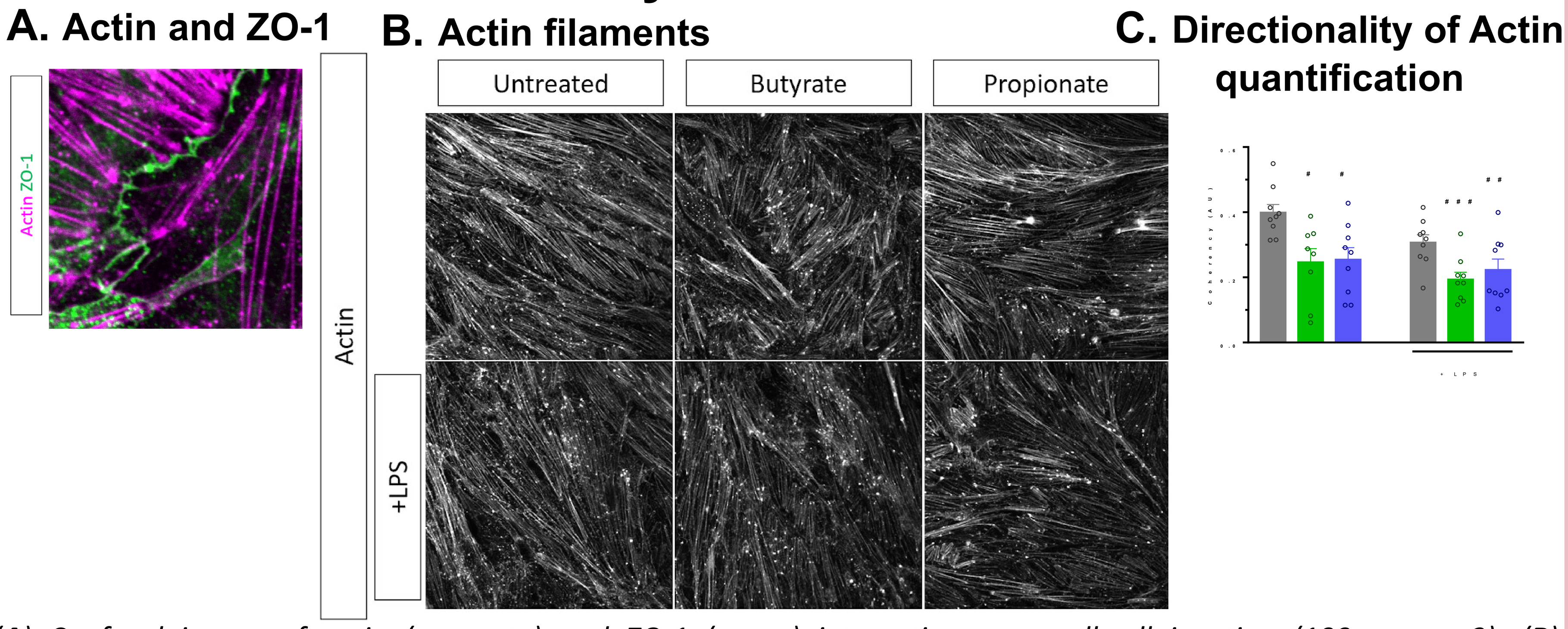
A. Tight junction (TJ) protein expression **B. Quantification of TJ protein expression and spikes**



*(A) Representative confocal images of tight junction proteins claudin-5 and ZO-1 (60x). Arrows indicate protein spikes. (B) LPS decreased the relative intensity of claudin-5 and ZO-1 at cell-cell junctions and butyrate or propionate treatment protect from the decrease. Butyrate or propionate increase the percentage of cells with tight junction protein spikes. Treatment vs. untreated (## p<0.01, ### p<0.001, #### p<0.0001), metabolite vs. LPS (**p<0.01, ***p<0.001, two-way ANOVA followed by Tukey's multiple comparisons test). Data are presented as mean ± SEM.*

Figure 3. Butyrate and propionate decrease directionality coherency of actin filaments

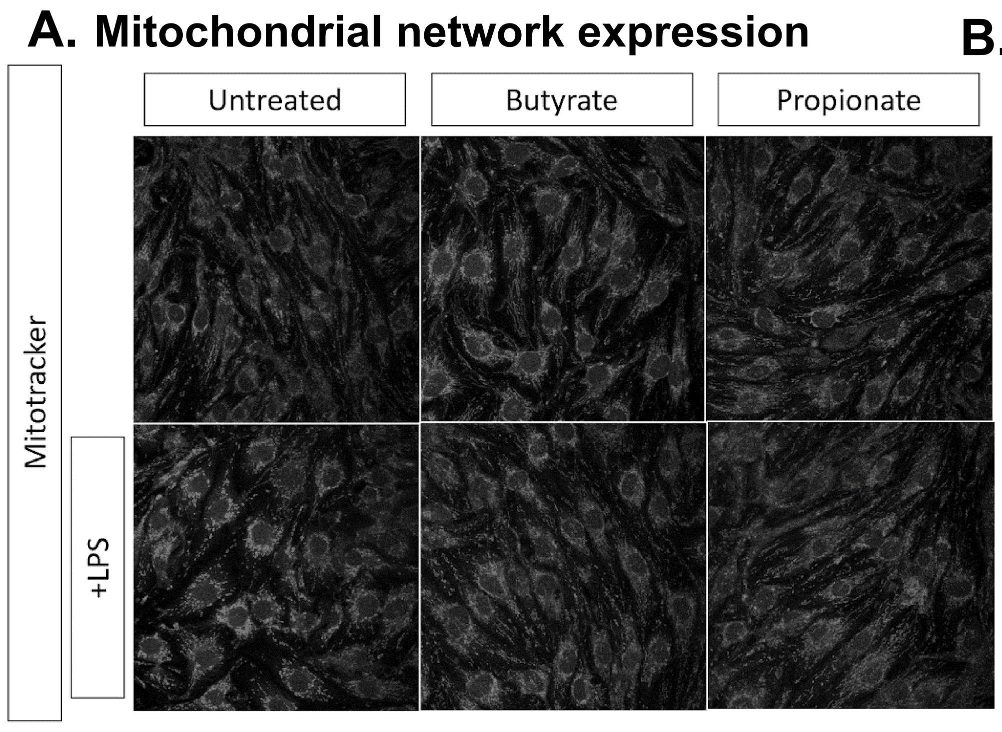
A. Actin and ZO-1 **B. Actin filaments** **C. Directionality of Actin quantification**

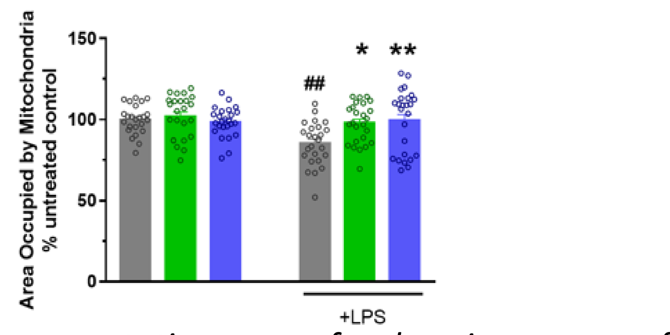


(A) Confocal image of actin (magenta) and ZO-1 (green) interaction at a cell-cell junction (100x zoom 3). (B) Representative confocal images of actin landscape (60x). (C) Butyrate or propionate with and without LPS decrease the coherency of the directionality of the actin filaments. Metabolite vs. untreated (#p<0.05, ##p<0.01 ####p < 0.0001), two-way ANOVA followed by Tukey's multiple comparisons test). Data are presented as mean ± SEM.

Figure 4. Butyrate and propionate protect against LPS mediated decrease in mitochondrial footprint

A. Mitochondrial network expression **B. Mitochondrial footprint quantification**





*(A) Representative confocal images of mitochondrial networks (60x). (B) LPS decreased the mitochondrial footprint and butyrate or propionate protect against the decrease. LPS vs. untreated (## p<0.01), metabolite vs. LPS (*p<0.05, **p<0.01, two-way ANOVA followed by Tukey's multiple comparisons test). Data are presented as mean ± SEM.*

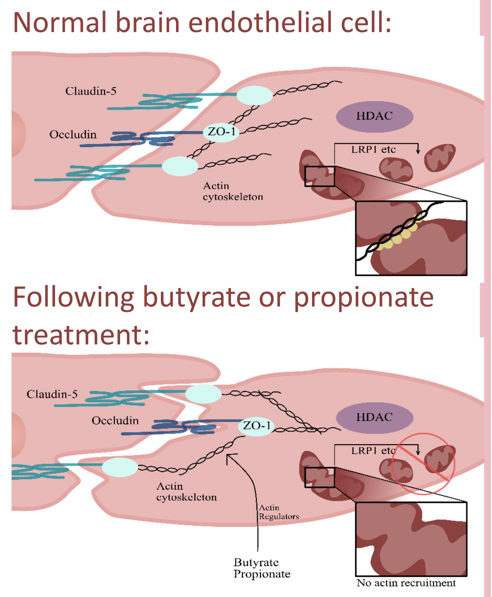
Conclusion

Microbial metabolites butyrate and propionate:

- directly alter and protect blood-brain barrier integrity and physiology
- modulate the actin cytoskeleton which is reflected in
  - increased tight junction protein spikes
  - protection of the mitochondrial network

This emphasizes that microbial dependent metabolite interaction with the blood-brain barrier is an important **communication pathway** in the gut microbiota-brain axis.

Since BBB integrity is compromised in a variety of pathological states including neurodegenerative and stress-related disorders, this is a desirable protective effect and future research will determine if this could be achieved via a **dietary intervention design**



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