

## **Deciphering age-related alterations in the ileal immune system shaping the microbiome landscape: A comprehensive Mass Cytometry Analysis**

As individuals age, the gut microbiome undergoes significant shifts in its diversity and composition, which have been linked to various age-related tissue dysfunctions and diseases. Our recent investigations have demonstrated that changes in the aged immune system are a crucial factor contributing to these intricate shifts within the microbiome upon aging. This study, seeks to characterize the specific changes within the aged immune system that act as driving forces behind the observed changes in the microbiome and to elucidate the underlying mechanisms governing their actions.

Employing an extensive immune phenotyping panel, we conducted a meticulous analysis of cells extracted from the small intestine of both young and aged mice. Leveraging cutting-edge CyTOF technology for high-dimensional profiling, we enabled an in-depth exploration of the implicated immune cell populations. To discern quantitative differences in the abundance and frequencies of immune cells in the ileum during aging, the CITRUS algorithm was applied.

Our findings indeed revealed distinctive shifts in immune cell populations within the aged ileum. Notably, we observed a decrease in the proportion of naïve B cells, accompanied by an elevation in innate lymphoid cells, specifically the ILC3 subpopulation. Furthermore, an enrichment of CD4<sup>+</sup> memory T cells was identified in the aged ileum compared to their younger counterparts. These results mark a significant advancement in our understanding of the immune landscape within the ileum during aging.

In summary, this study sheds light on immunological signatures associated with age-related alterations in the ileum, providing a foundation for future investigations into their potential contribution to microbiome shifts and the underlying mechanisms through which immune cells orchestrate changes in the microbiome landscape.