

Role of Lymph from Alcohol-Treated Animals on Naïve Perilymphatic Adipose Tissue Immunomodulation

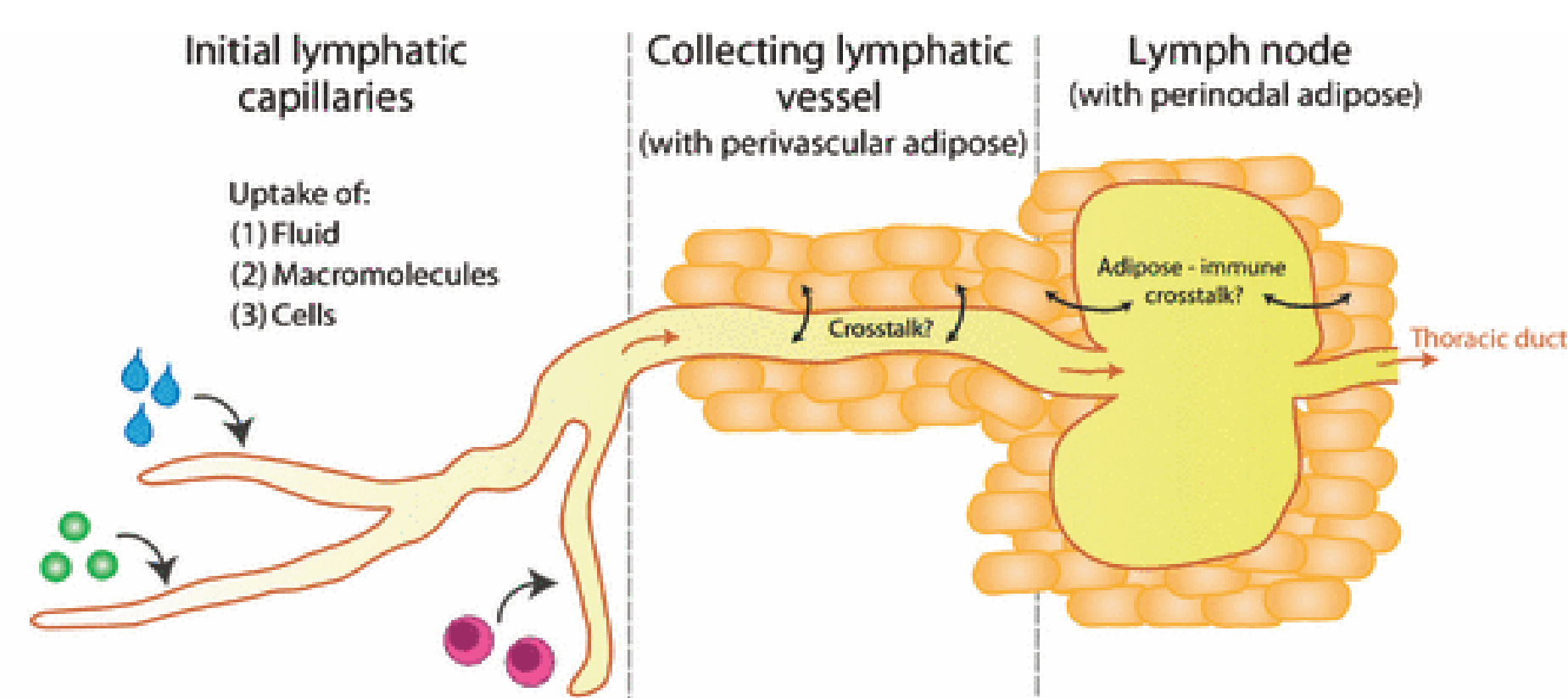
Kourtney Weaver, Flavia Souza-Smith, Ph.D.

Department of Physiology, Louisiana State University Health Sciences Center, New Orleans, LA

Background

- Alcohol impairs innate and adaptive immune responses, harming host defense against infections
- Chronic alcohol administration has also been shown to impact early manifestations of metabolic dysfunction such as insulin resistance in rodent models
- In our previous studies, we found that alcohol induces mesenteric lymphatic leakage and perilymphatic adipose tissue (PLAT) immunometabolic dysregulation, specifically, it increased PLAT CD4+T cells, Tregs, DCs, IL-6, and IL-1
- Whether the lymph from alcohol-treated animals is directly causing PLAT immunometabolic dysregulation is still to be determined

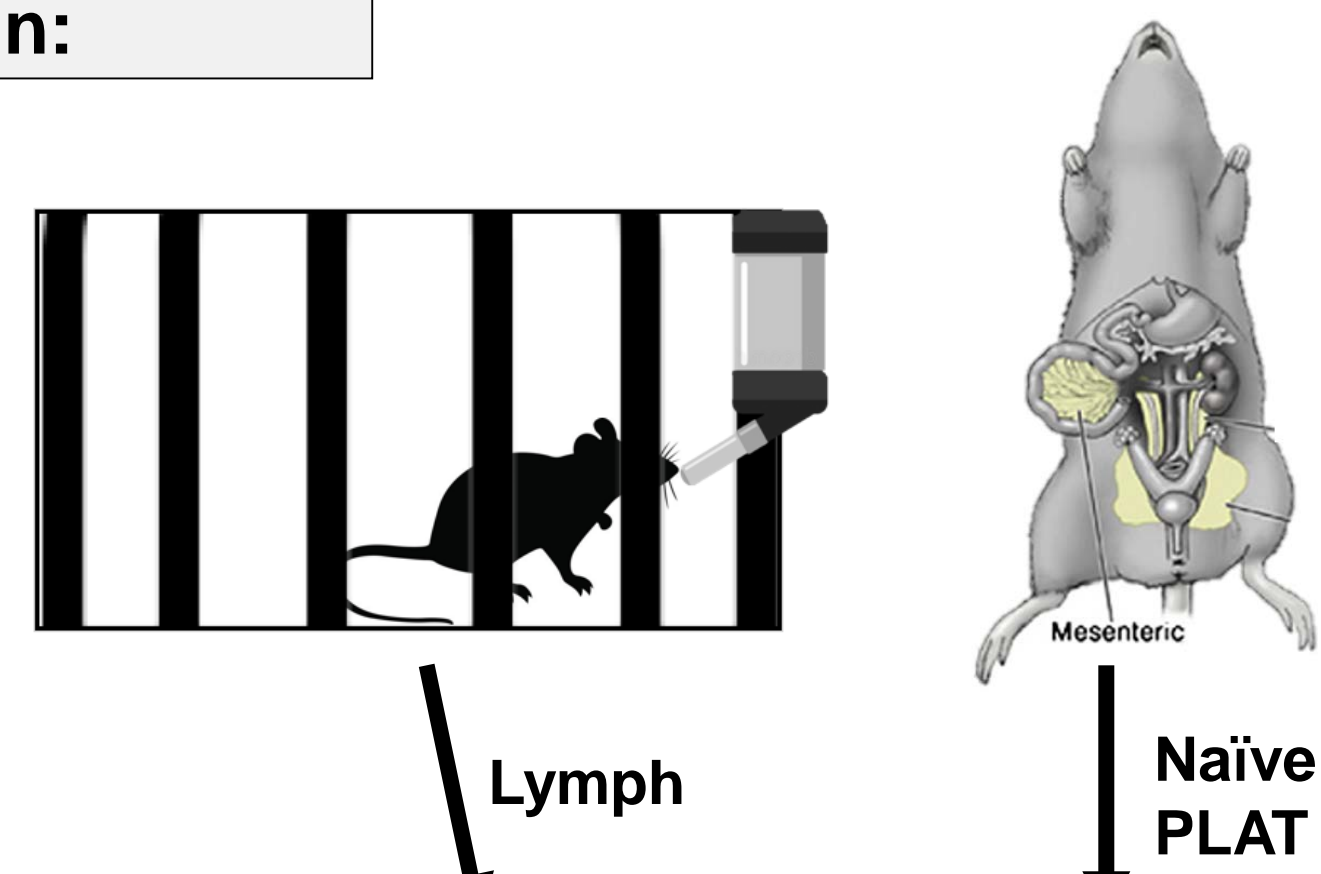
We hypothesize that lymph contents from alcohol-treated animals will immunomodulate naïve PLAT explants.



Methods

Experimental Design:

- **Alcohol-Treated Group**
Male Fischer 344 rats received Lieber-DeCarli liquid diet containing 36% of calories from alcohol for 10 weeks
- **Control Group**
Male Fischer 344 rats received isocaloric Lieber-DeCarli liquid diet and were pair-fed for 10 weeks



RNA Isolation & qPCR → Measure gene expression of CD3 & CD4 T cells, CD26 & CD38 (CD4 T cell activation markers), FOXP3 (Tregs), & IL-6, IL-1B, IL-10 cytokines

Protein Extraction & ELISA → Measure protein concentrations of adiponectin, IL-6, and IL-1B



Lymph from alcohol and control animals were collected and co-cultured with naïve PLAT explants from 5 age-matched animals for 48 hours.

Results

Gene expression of PLAT stimulated with lymph

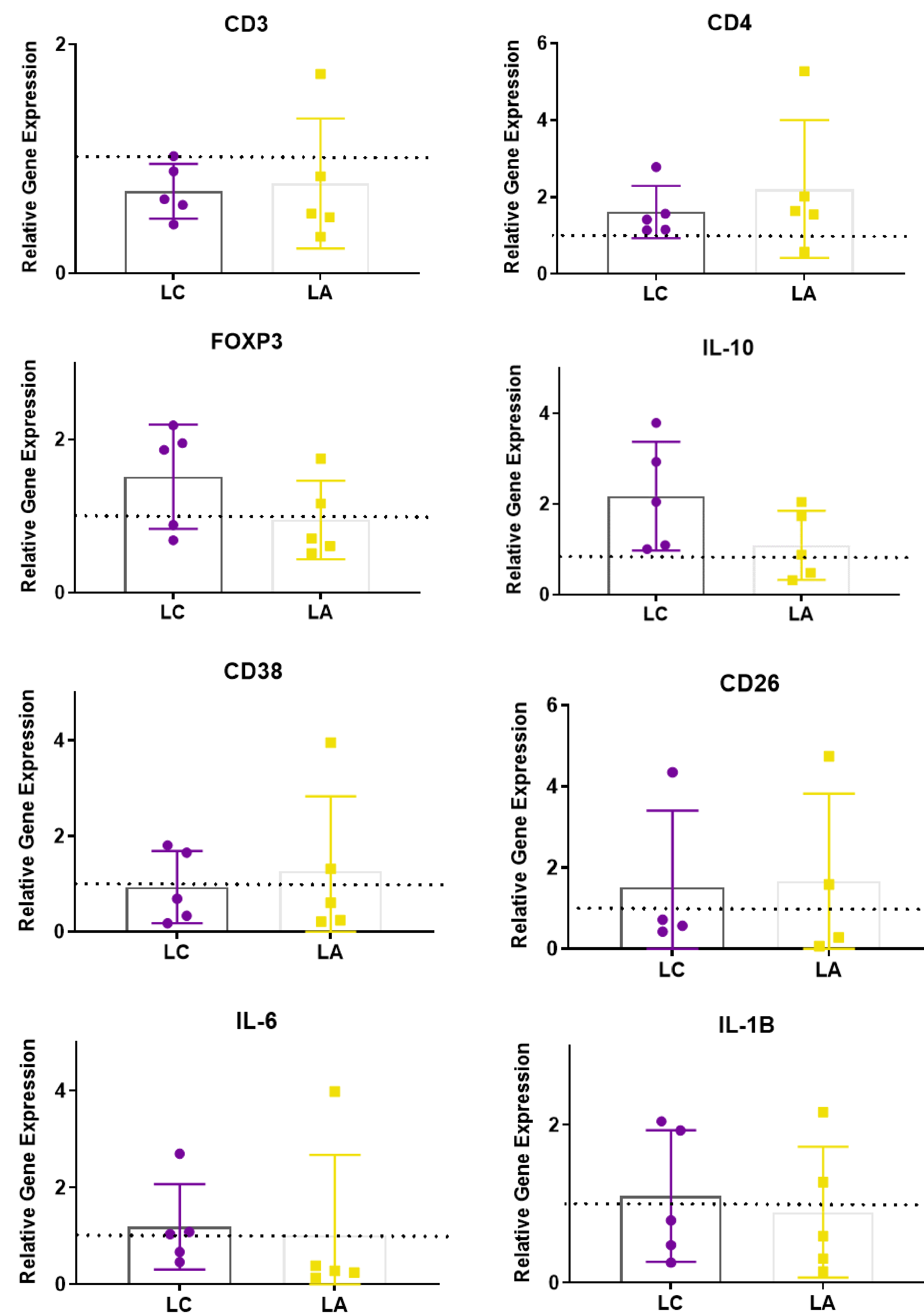


Figure 1: Gene expression was compared between PLAT stimulated with lymph from control animals (LC) and PLAT stimulated with lymph from alcohol-treated animals (LA) relative to the negative control. These data represent values that were averaged from three qPCR runs. T-test $p > 0.05$. One-way ANOVA $p > 0.05$.

Protein expression of PLAT stimulated with lymph

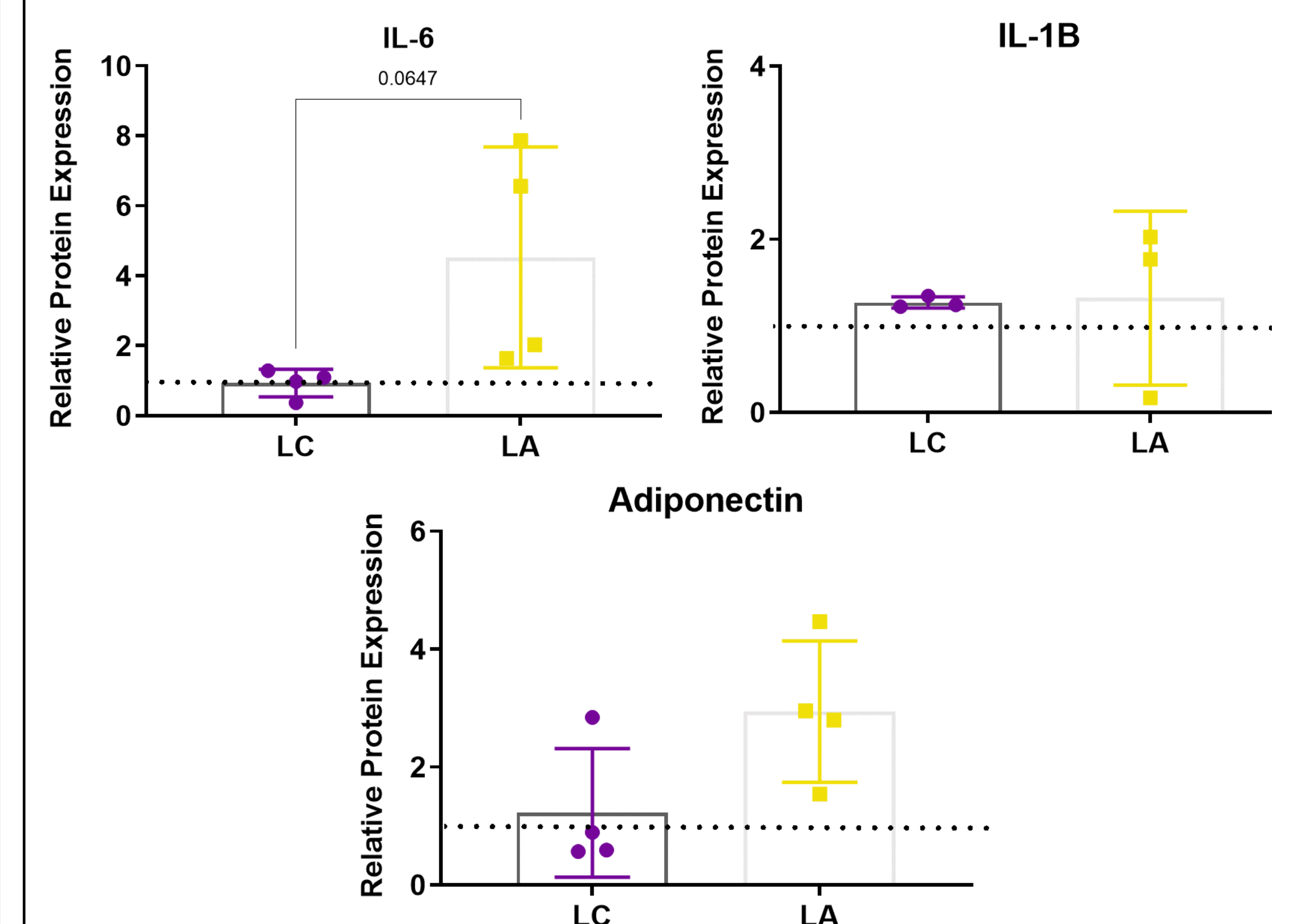


Figure 2: Protein expression was compared between PLAT stimulated with lymph from control animals (LC) and PLAT stimulated with lymph from alcohol-treated animals (LA) relative to the negative control. Values shown in IL-6 graph were averaged between two assays. T-test $p > 0.05$. One-way ANOVA $p > 0.05$.

Conclusions

- We observed a trend of an alcohol-induced increase of CD4 gene expression in PLAT samples
- FOXP3 and IL-10 gene expression were higher, but not significant, in PLAT samples cultured with lymph from control animals compared to alcohol-treated animals
- There was increased expression of IL-6 in PLAT cultured with lymph from alcohol-treated animals consistent with increased IL-6 protein expression in PLAT from alcohol-treated animals seen in our previous *in vivo* studies
- Neither gene expression nor protein were detectable in supernatant samples
- Future studies with this model include repeating these experiments with a second cohort of animals to increase sample size
- Proteomic analysis of lymph contents from control and alcohol-treated animals will also be conducted

Overall, these studies were the first step in understanding the consequences of chronic alcohol on lymph and its effects on PLAT immune cell milieu

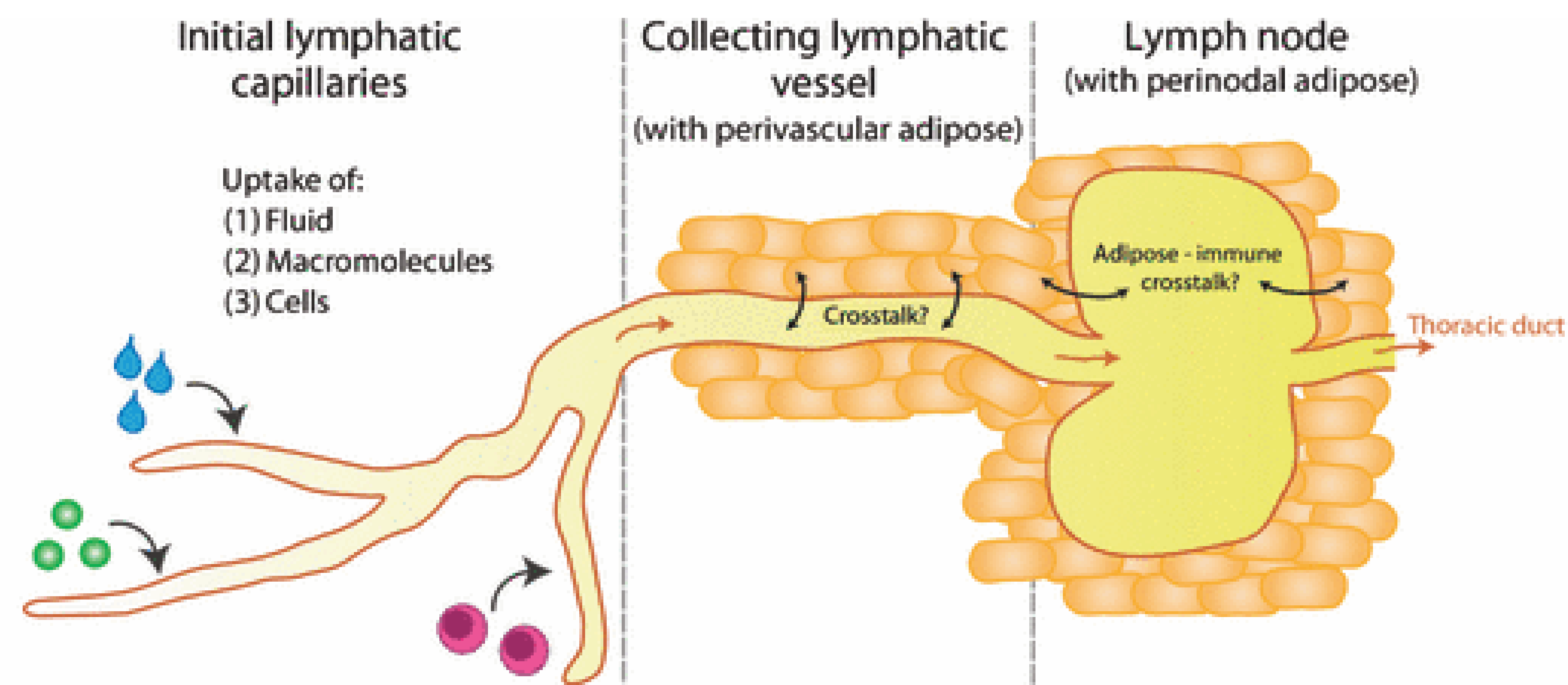
Acknowledgements

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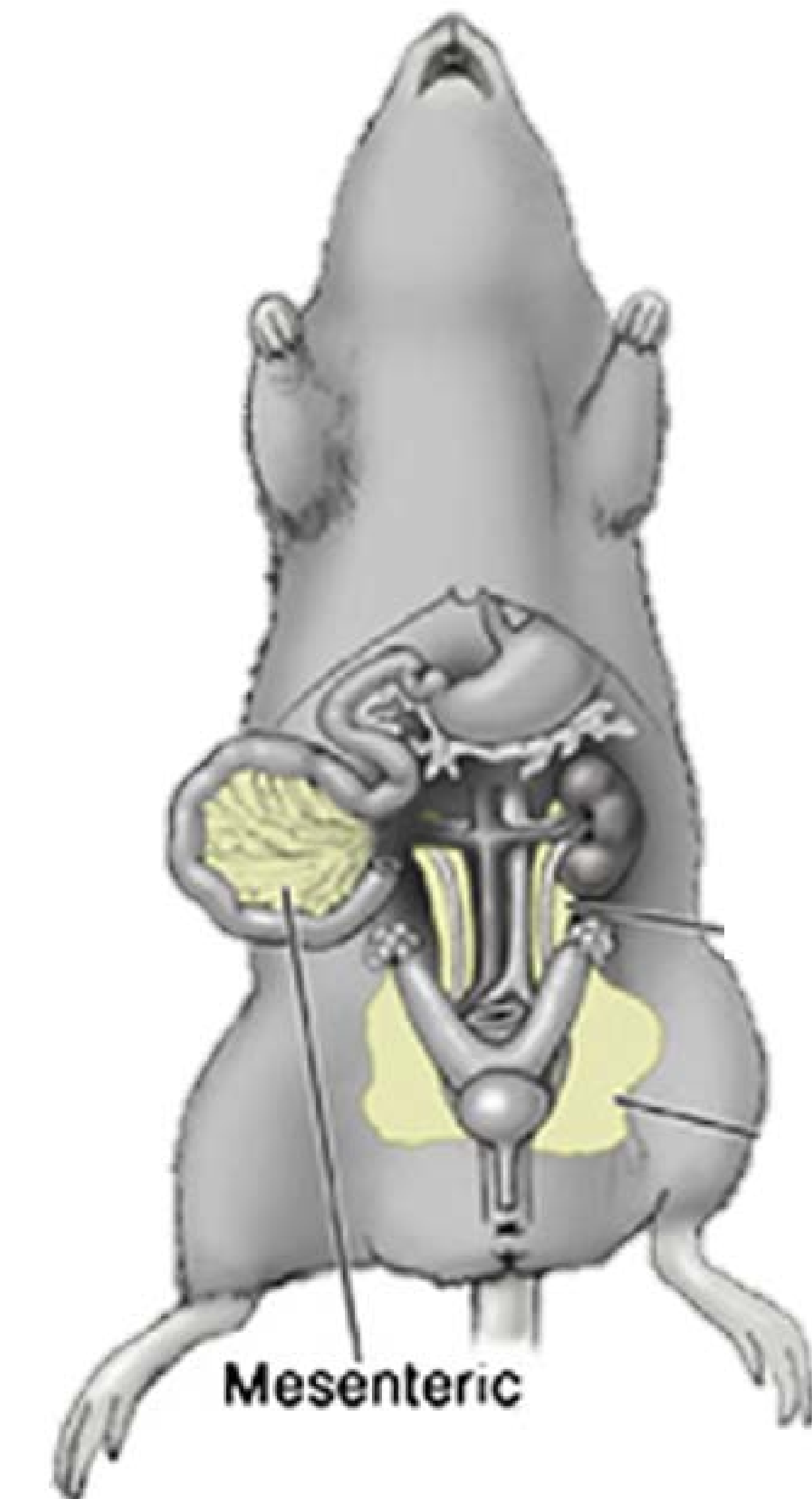
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Lymph

Naïve
PLAT



PLAT explant + no lymph (negative control) PLAT explant + control lymph PLAT explant + alcohol lymph

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Results

Gene expression of PLAT stimulated with lymph

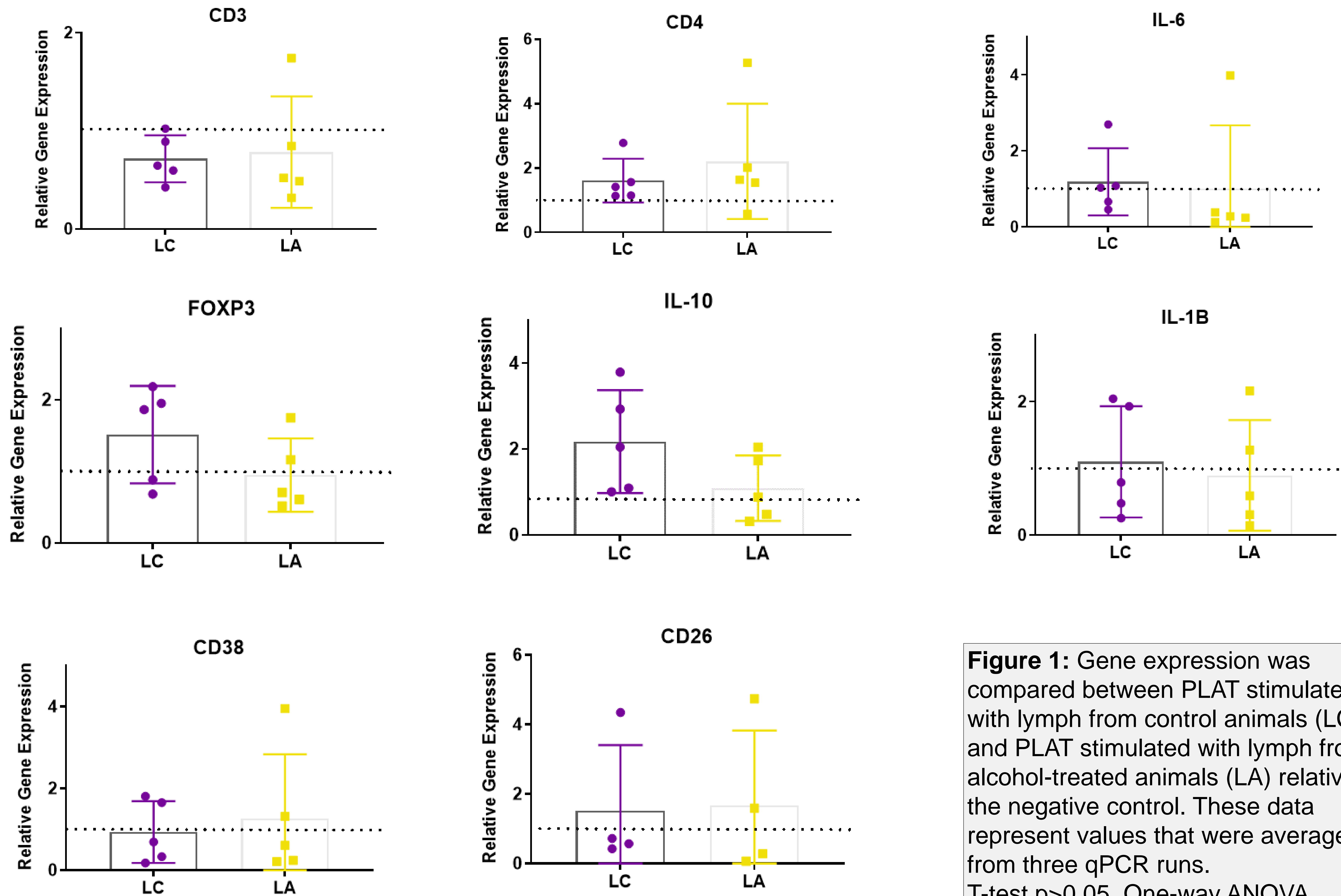


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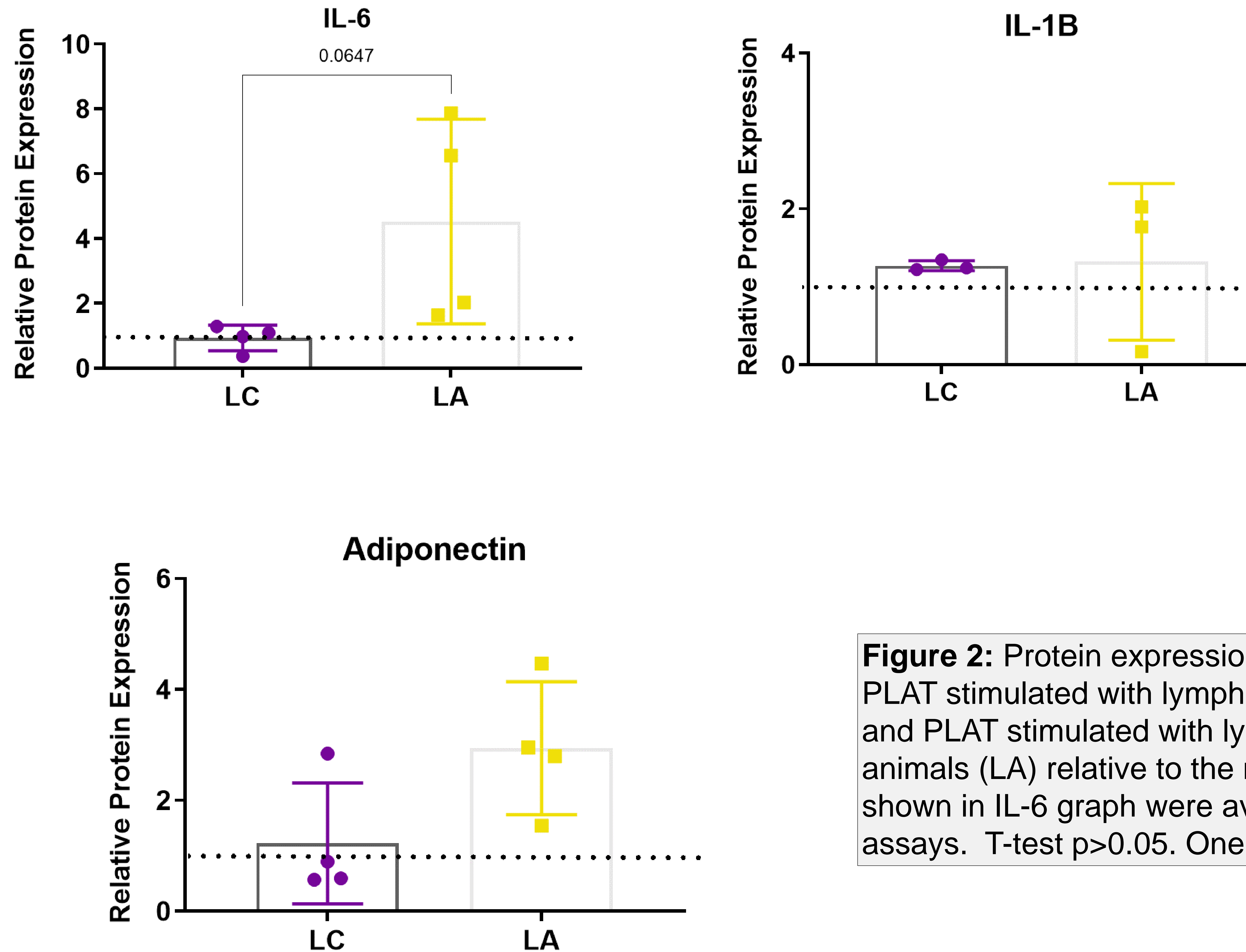


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