Effects of the Western Diet on Colon Transcriptomics and Histology

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Introduction / Background

- Diets high in fructose and fat are correlated with **Metabolic Dysfunction-Associated Steatotic Liver Disease (MASLD)**
- MASLD affects the liver-gut axis
- **Leaky gut** is characterized by increased intestinal permeability due to dysfunctional tight junctions
- **Goal:** To compare the effects of the COCO diet vs the CONTROL diet on the colon using transcriptomics and histological analyses

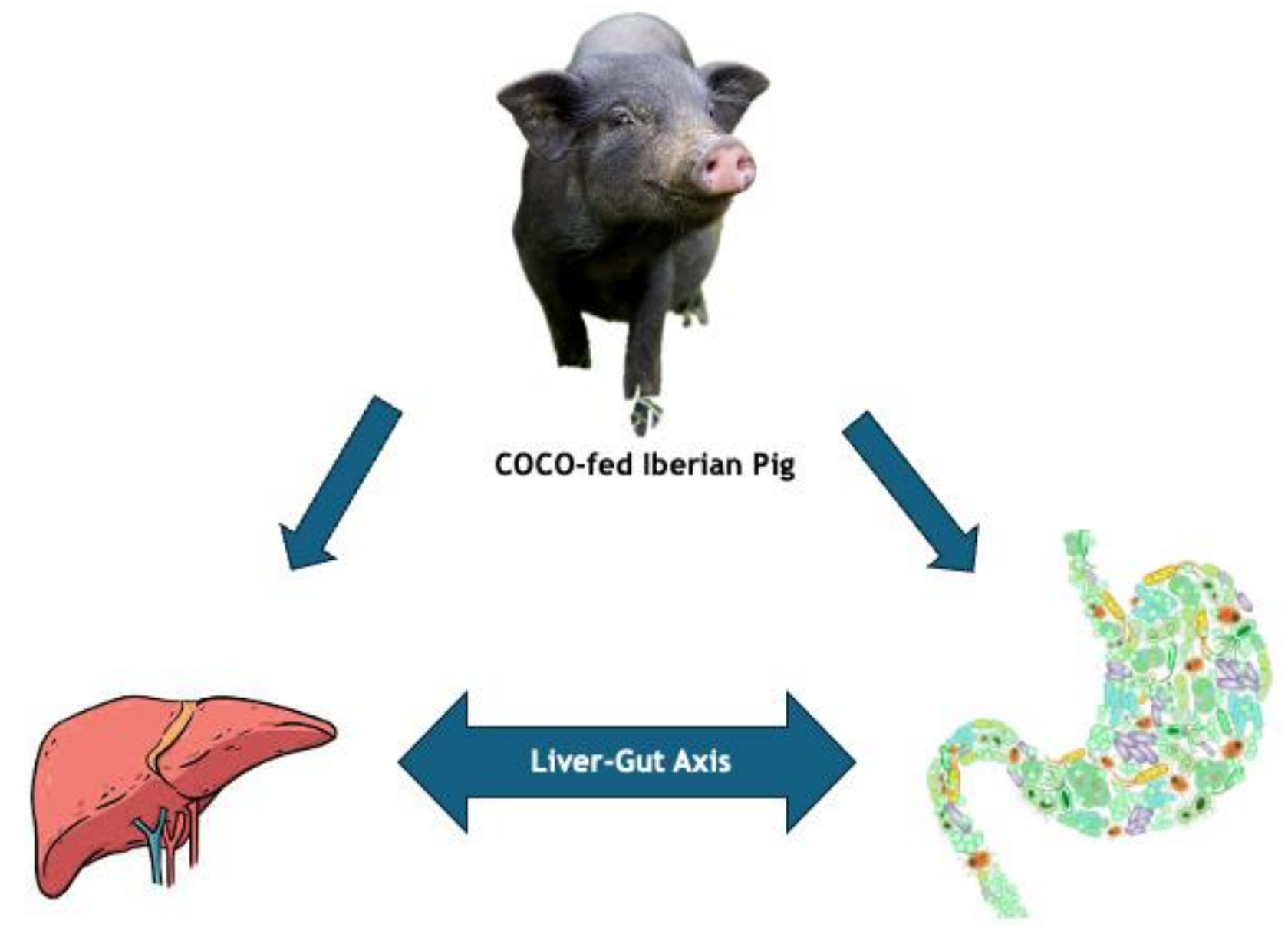


Figure 1. Illustration of the connection between the diet, liver, and gut microbiome.

Methods

Animal Trial:

- Juvenile Iberian pigs were fed a control diet or a high-fat & high-fructose diet enriched in coconut oil (COCO) for 10 weeks

Transcriptomics:

- RNA was isolated from COCO and control tissues
- Up-or-down regulated genes were identified using DAVID Gene Ontology software
- Heatmaps were created via ClustVis to visualize expression and select proteins for western blots

Histology:

- H&E-stained colon crypts were imaged and measured using Leica microscope
- Ki67 stained crypts were imaged, and Ki67 +/- cells were counted using Fiji and ImageJ software

Protein Expression:

- Proteins were extracted and run on SDS-PAGE gels
- Western blot analysis was performed to look at the expression of four proteins: Claudin-2, Claudin-4, Occludin, and Zonulin 1 (ZO1)
- Protein quantification was performed using Bio-Rad Image Lab Software

Results

Transcriptomics

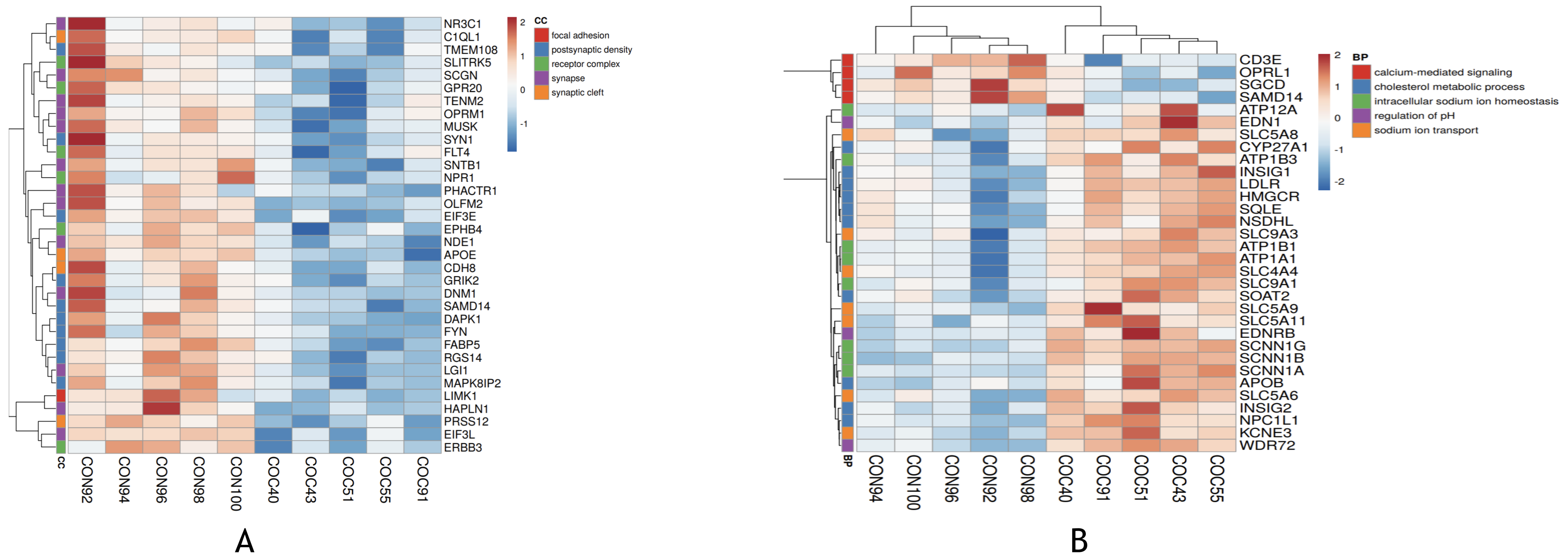
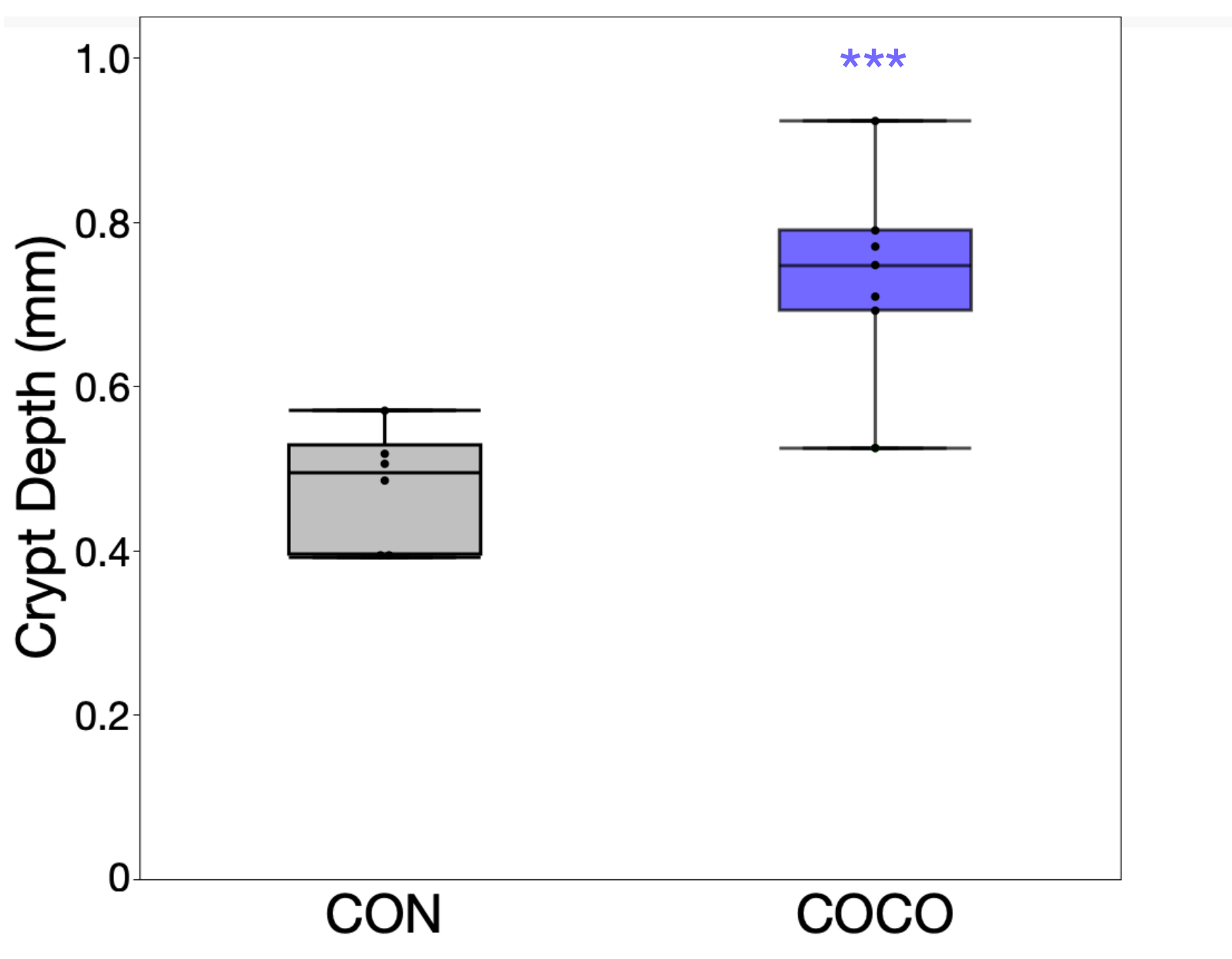


Figure 2. Heat maps of differentially expressed genes in the colon of juvenile female Iberian pigs fed a control diet or a high-fat, high-fructose diet enriched in coconut oil (COCO) for 10 weeks: (A) cellular components and (B) biological processes associated with downregulated genes. Individual pigs are represented in each column, and rows show each gene's log-transformed counts per million. Blue coloring indicates the row minimum, and red coloring indicates the row maximum.

Histology



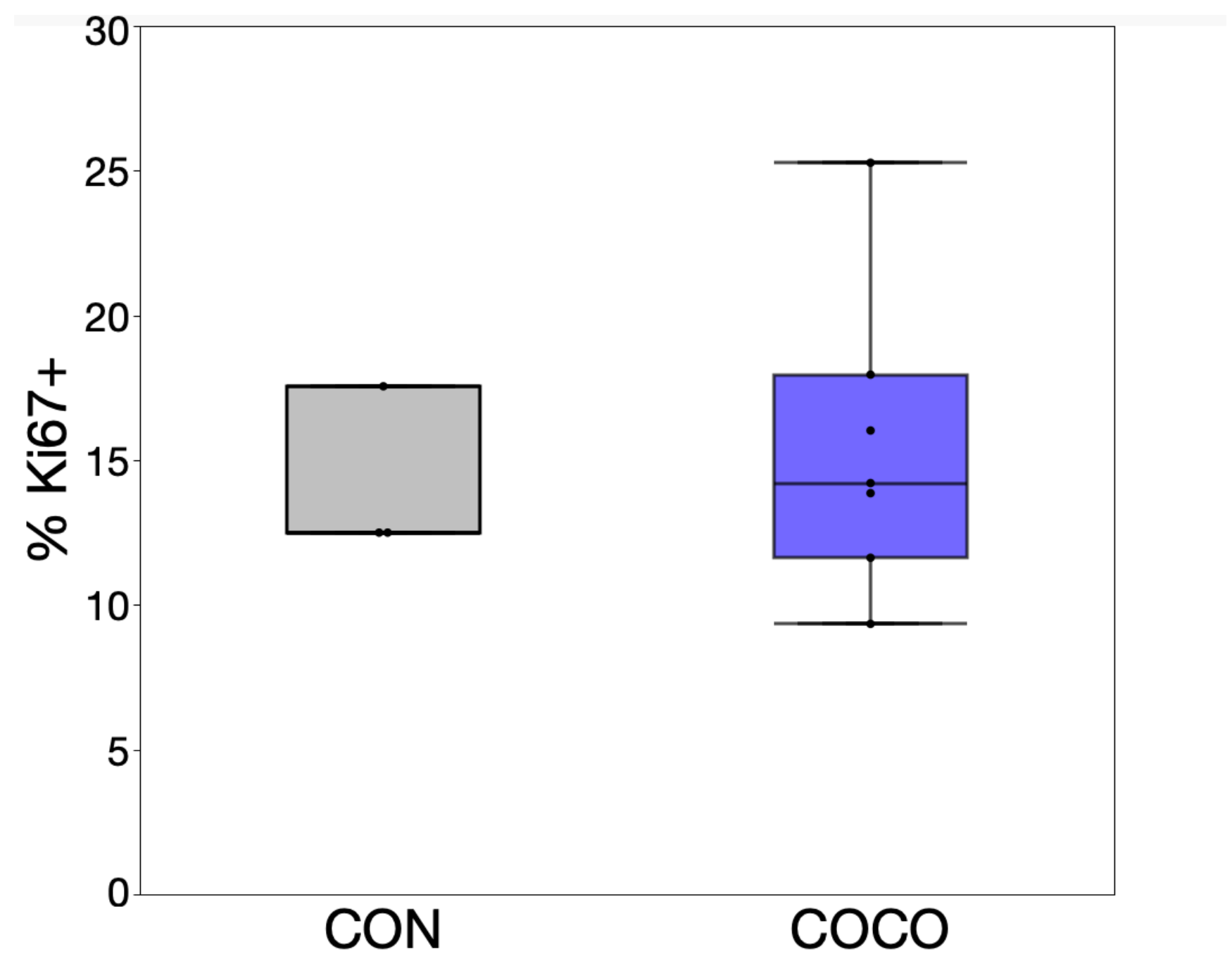
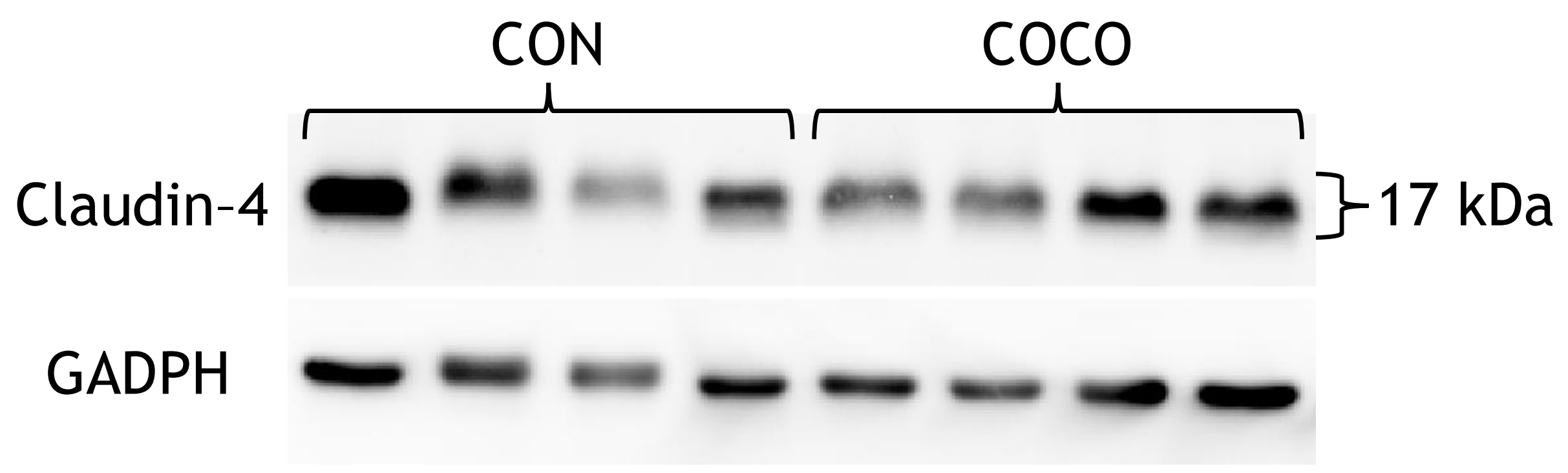


Figure 3. H&E staining of colon crypt depth of CON vs. COCO treated pigs (n=13). COCO crypts were significantly deeper than control (p<0.001).

Figure 4. % Ki67 positive cells per crypt in CON vs. COCO treated pigs (n=9).

Protein Expression



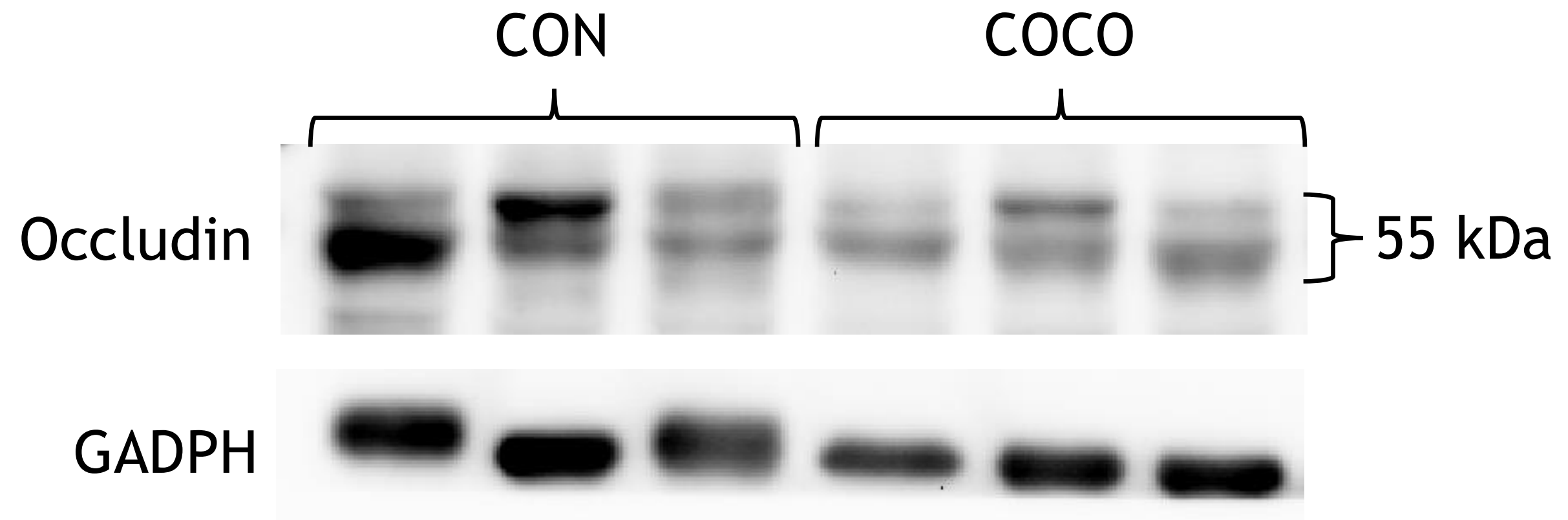


Figure 5. Representative Western blots to measure the expression of Claudin-4 (top bands) and GAPDH (bottom bands).

Figure 6. Representative Western blots to measure the expression of Occludin (top bands) and GAPDH (bottom bands).

Conclusion

- Transcriptomics revealed down and up regulation of biological processes, metabolic function, and cellular components in COCO diet.
- COCO pigs had significantly deeper colon crypts, indicative of tissue damage (p = 0.0007).
- Ki67+ analysis is being performed to determine if there is a higher level of proliferative cells in response to tissue damage.
- Tight junction protein expression was not significantly different between the COCO and CON diets.

References

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- Mahon, Kyle et al. "Transcriptomic Changes in the Frontal Cortex of Juvenile Pigs with Diet-Induced Metabolic Dysfunction-Associated Liver Disease." *Biomedicines* vol. 13,7 1567. 26 Jun. 2025, doi:10.3390/biomedicines13071567

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