Additional File 2

Investigating intestinal epithelium metabolic dysfunction in Celiac Disease using personalized genome-scale models

Chloe McCreery, Drew Alessi, Katarina Mollo, Alessio Fasano, and Ali R. Zomorrodi

Table of Contents

Detailed description of differentially active metabolic tasks

In this section, we present more details about the roles of certain essential metabolic tasks showing significant differential activity between the study groups (**Figure 2** of the main text).

Metabolic tasks involved in mitochondrial metabolism: Mitochondrial aspartate transaminase catalyzes the synthesis of oxaloacetate, an important intermediate in TCA cycle. Mitochondrial malic enzyme, malate dehydrogenase, and citrate synthase support the production of ATP via the TCA cycle. Riboflavin kinase is also an enzyme that is crucial for the metabolism of riboflavin (vitamin B2) and synthesis of FAD, an essential cofactor in the TCA cycle. Additionally, riboflavin itself is known to have antioxidant and anti-inflammatory properties (28). Finally, elevated lactate secretion may suggest increased anaerobic glycolysis is sIECs, which may indicate mitochondrial dysfunction or impaired tissue oxygenation in active CeD due to tissue destruction.

Metabolic tasks involved in nucleotide synthesis and DNA repair: Adenylsuccinate lyase and adenine phosphoribosyltransferase participate in purine biosynthesis and purine salvage pathways, respectively (35, 36). Cytosolic fumarase catalyzes the formation of L-malate from fumarate and contributes to the DNA damage response (37). Thymidylate synthase also participates in DNA synthesis as a key regulator of thymidine (38),. Finally, B-ureidopropionase is involved in the breakdown of pyrimidine, which is crucial for DNA and RNA synthesis.

Metabolic tasks related to amino acid metabolism: L-alanine transaminase is responsible for the intestinal catabolism of dietary compounds to synthesize alanine, an important nitrogen source for extraintestinal tissues (39). 5, 10-methylenetetrahydrofolate reductase is an enzyme implicated in folate cycle and is a key contributor to the production of amino acid homocysteine. Arginase also catalyzes the hydrolysis of arginine to urea and ornithine.

Probing sIECs metabolic adaptability for performing essential functions

A shadow price analysis was conducted for each personalized GEM to evaluate sIECs metabolic adaptability to perturbations for performing the 59 essential metabolic tasks and metabolite secretions. This analysis did not identify a significant difference in the overall sIECs adaptability for performing these functions (the sum of non-zero shadow prices across all tasks or metabolites) between the study groups (Mann-Whitney U, $q < 0.05$; **Additional File 1**). However, when assessing the adaptability for individual metabolic tasks or metabolite secretions, significant differences between the groups were observed for two tasks: ornithine secretion into the bloodstream and carboxylic acid dissociation (**Figure ST1**). The non-zero shadow prices count for ornithine secretion was significantly lower in the active CeD patients, indicating a lower adaptability, compared to the remission CeD. Interestingly, this is despite the elevated secretion of ornithine into blood observed in the active CeD patients (**Figure 2**, main

text). Conversely, the adaptability for carboxylic acid dissociation, as reflected by non-zero shadow prices count, was significantly diminished in both active and remission CeD groups relative to healthy controls. This function is responsible for the production of carbonic acid, which then dissociates into HCO3- ions. HCO3- ions neutralize acids like gastric hydrochloric acid that may harm the gastrointestinal tract. These findings indicate a lower flexibility of active CeD and remission CeD patients to perform metabolic processes related to this function.

The significantly lower adaptability for both of these metabolic functions under active CeD suggests that the sIECs metabolic system in the active CeD state is operating under more genetic or epigenetic constraints, due to inflammation or tissue damage, limiting its ability to utilize alternative pathways or adapt to increased metabolic demands in this state.

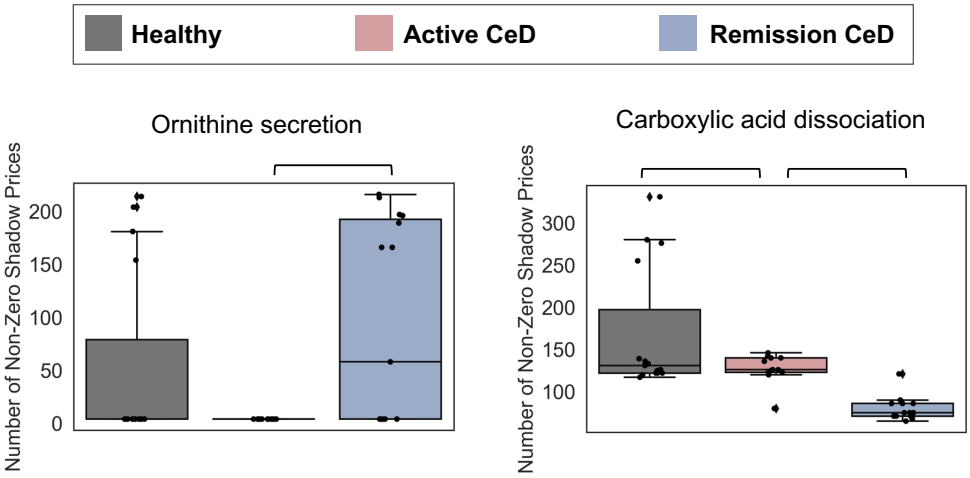

Figure ST1. Analysis of sIECs metabolic adaptability across conditions. In this analysis, a higher count of non-zero shadow prices represent an increased flexibility of the metabolic network for handling perturbations. Metabolic tasks (ornithine secretion, carboxylic acid dissociation) showing significant differential activity between the groups are shown.

Sensitivity analysis

As noted in the main text, one active CeD subject is distinctly positioned away from the main cluster, in the PCA plot for the essential metabolic tasks (see **Figure 1C** in the main text). Further analysis involving a PCA plot generated using standardized data (**Figure ST2, Additional File 1**) confirmed that this data point is an outlier. However, given the lack of any identifiable errors in the collection or handling of biospecimens or data collection for this participant and considering the potential biological relevance of the atypical gene expression and metabolic profiles observed, we opted to retain this subject in all subsequent analyses. Nevertheless, to evaluate if this outlier may distort our statistical analysis of metabolic tasks, we conducted a sensitivity analysis excluding this subject. This exclusion rendered only one metabolic task—lactate secretion into the blood—statistically non-significant for active CeD vs. controls comparison (Mann-Whitney U, $q < 0.05$), and the overall pattern of differential activity for this task remained unchanged.

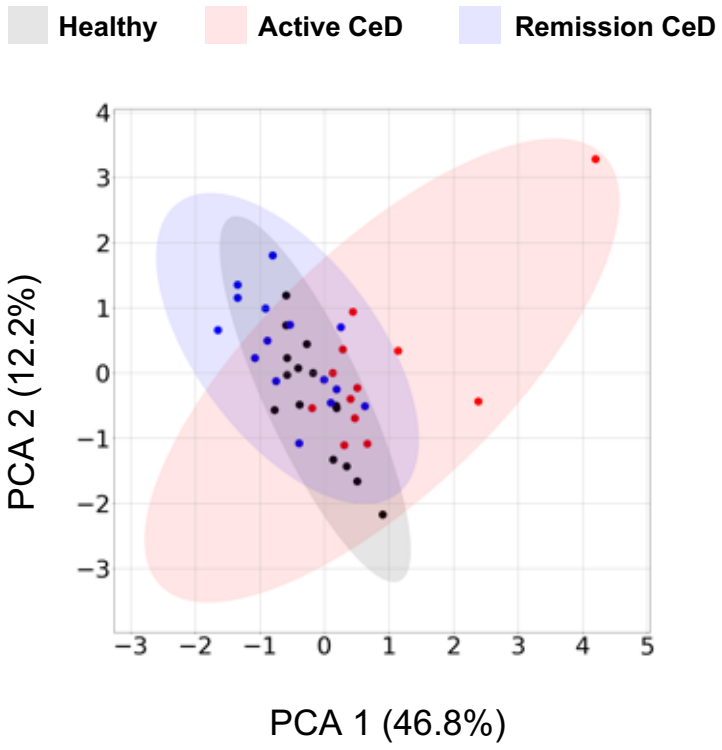

Figure ST2. PCA with standardized data. Essential metabolic tasks were used a features and subjects as samples. Data along the first and second principal components (PC1 and PC2) were standardized independently using z-score transformation. The standardized values were then visualized.

Statistical analysis of sIECs essential metabolic tasks within the validation dataset

We conducted an independent statistical analysis to identify essential sIECs metabolic tasks that show significant differential activity between the study groups in the multi-cohort validation dataset (see the main text for details). This analysis identified 42 essential metabolic tasks showing significantly altered activity between at least one pair of study groups (Mann-Whitney U, $q < 0.05$) as shown in **Figure ST3**. Of these 42 tasks, 24 also showed significant differential activity between the study groups in our primary analysis.

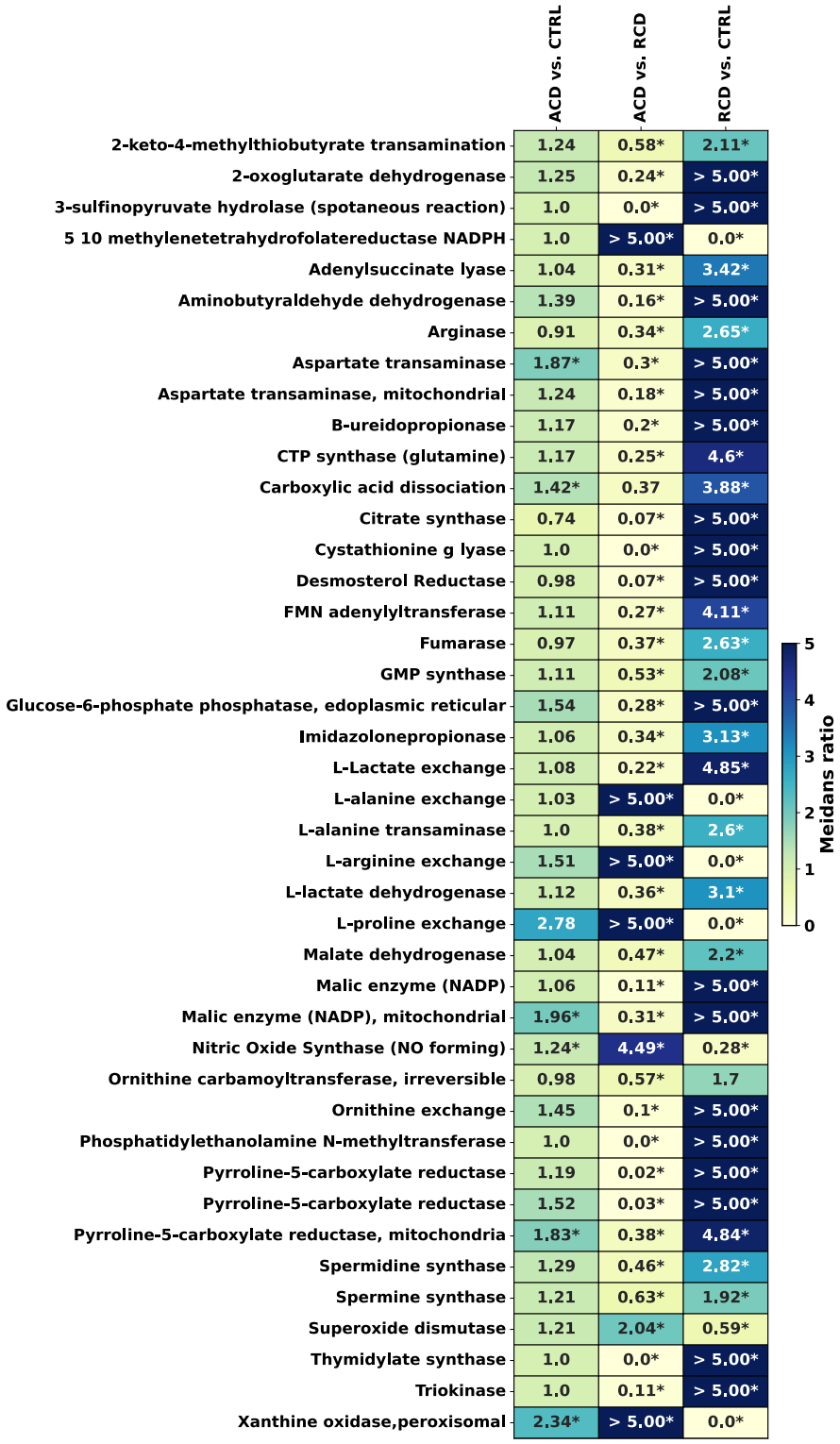

Figure ST3. Differentially active sIECs essential metabolic tasks in the validation dataset. Essential metabolic tasks showing significant differential activity between at least one pair of study groups are shown. Heatmap annotations denote the ratio of medians in the two study groups (ACD/CTRL, ACD/RCD, and RCD/CTRL), and asterisks indicate statistical significance (Mann-Whitney U test, $q < 0.05$). ACD: Active CeD; RCD: Remission CeD; CTRL: Controls.