bio-protocol

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Supplementary information for Ex Vivo Assessment of Extracellular Acidification Rate in Murine Intestinal Tissue

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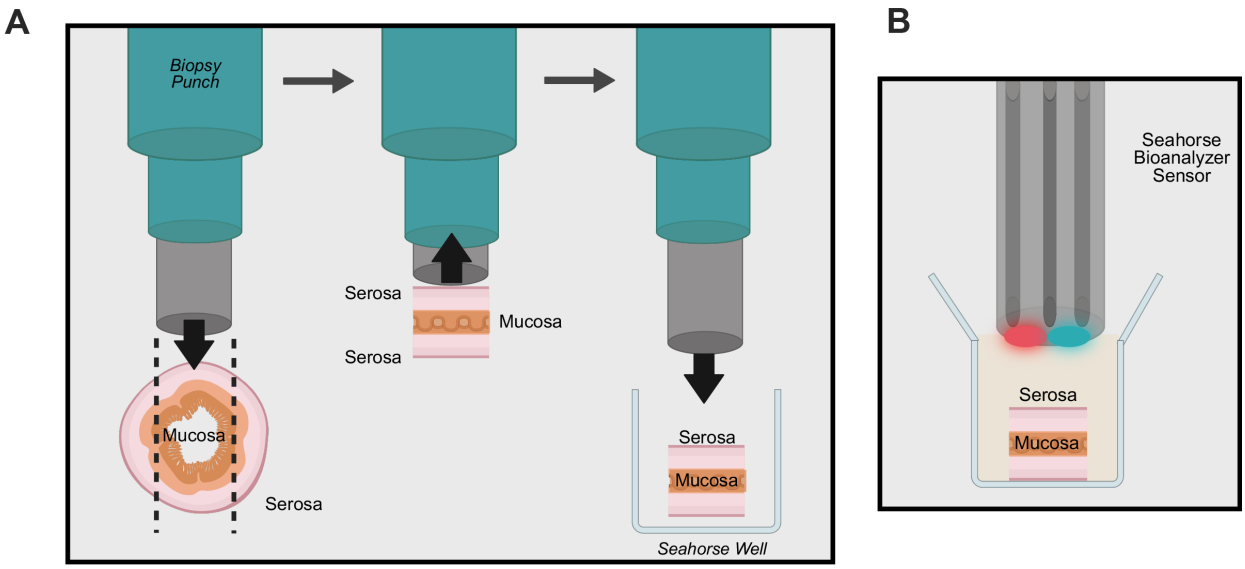

Figure S1. Schematic for tissue biopsy punch orientation. The schematic shows the orientation of a tissue biopsy punch for the described protocol with the serosal side on top (A). Full thickness biopsy punches were obtained such that the serosal side was facing upward. Biopsy punches largely preserve the orientation of the sample. Thus, additional preparation methods can be employed if assessing a mucosal phenotype (i.e. opening and longitudinally bisecting the intestine for investigation of mucosal metabolism). Orientation of sample within the Seahorse Bioanalyzer picturing the oxygen and pH sensors within the machine for a sample well. The Islet Capture screen is not pictured within the well for simplicity of the figure (B).

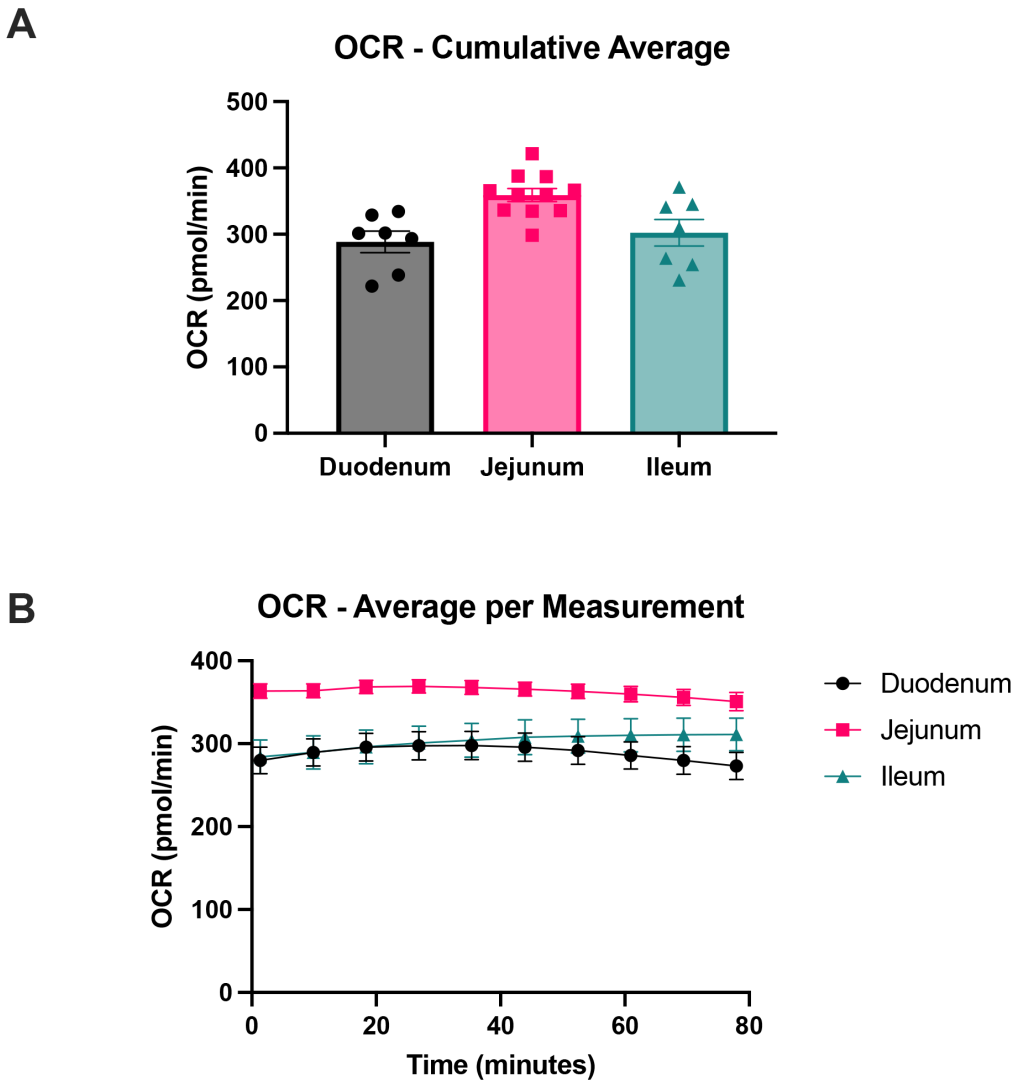

Figure S2. Ex vivo assessment of oxygen consumption rate in murine intestinal tissue. (A) Average oxygen consumption rate (OCR) from wildtype murine duodenum (n = 7), jejunum (n = 11), and ileum (n = 7). These data represent the cumulative average of replicates and measurements such that each point represents one animal. (B) Average OCR for each measurement cycle throughout the duration of the assay from wildtype murine duodenum (n = 7), jejunum (n = 11), and ileum (n = 7). Data are presented as average ± standard error of the mean (SEM). These experiments utilized samples from wildtype male and female mice, approximately 8–12 weeks of age. 5–7 biopsy punches were used per intestinal section per animal.

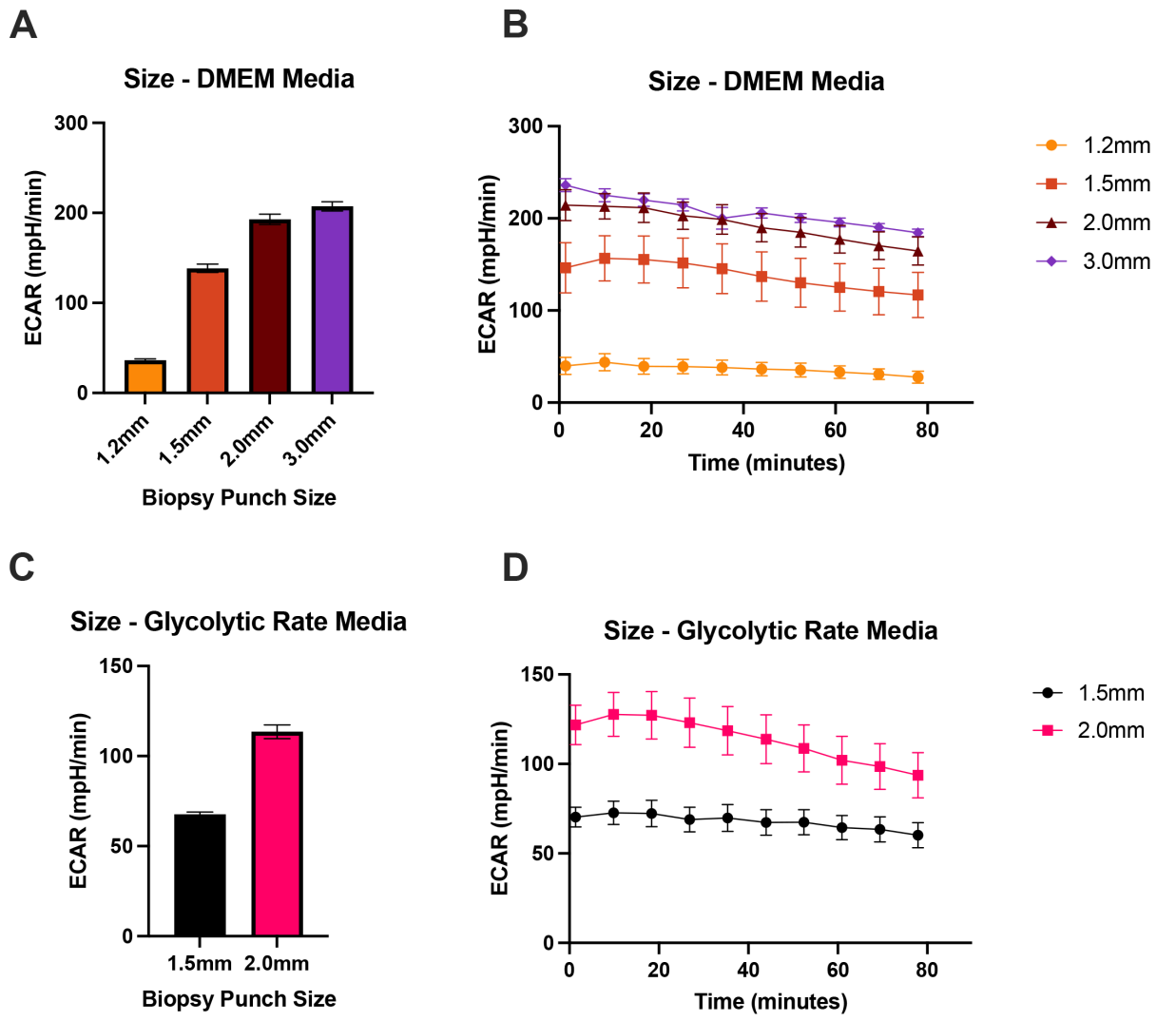

Figure S3. ECAR by biopsy punch size. Extracellular acidification rate (ECAR) for each biopsy punch size using Agilent's DMEM based media (A, B) and Glycolytic Rate media (C, D). Average ECAR from all measurements (A, C) and average for each of the 10 measurement cycles (B, D). Samples from wildtype murine jejunum. 5–10 replicates from 1–2 wildtype mice were used for each condition for these initial optimization tests. Data represent average ± standard error of the mean (SEM).

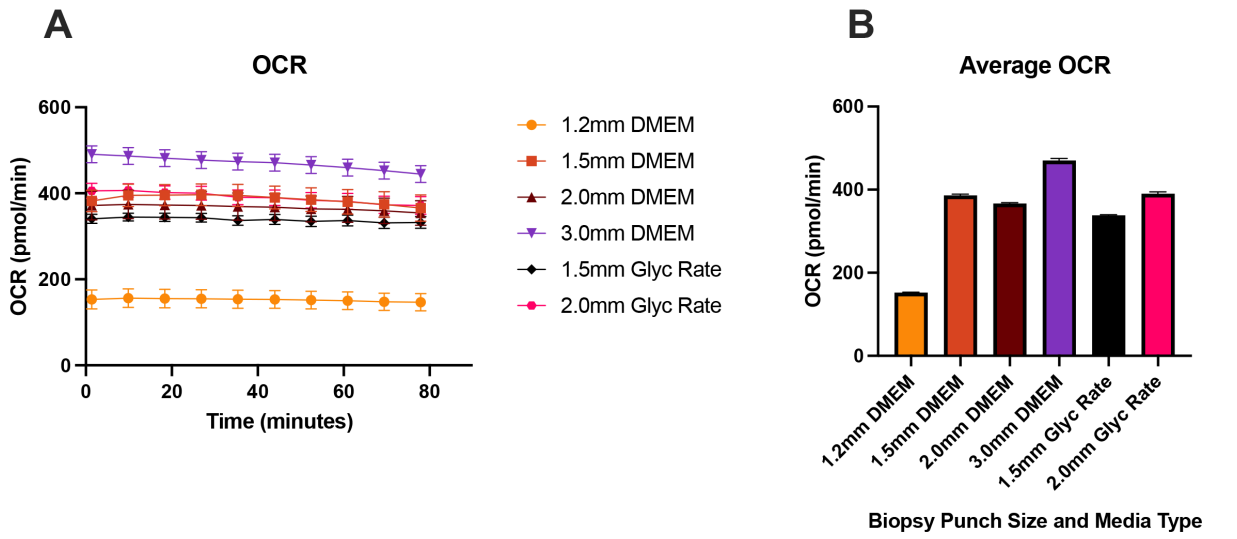

Figure S4. OCR by biopsy punch size. Oxygen consumption rate (OCR) for each biopsy punch size utilizing Agilent's DMEM based media and Glycolytic Rate (Glyc Rate) media. Average OCR across 10 measurement cycles for each biopsy punch size (A) and average OCR of all measurements (B). Samples from wildtype murine jejunum. 5–10 replicates from 1–2 wildtype mice were used for each condition for these initial optimization tests. Data represent average ± standard error of the mean (SEM).

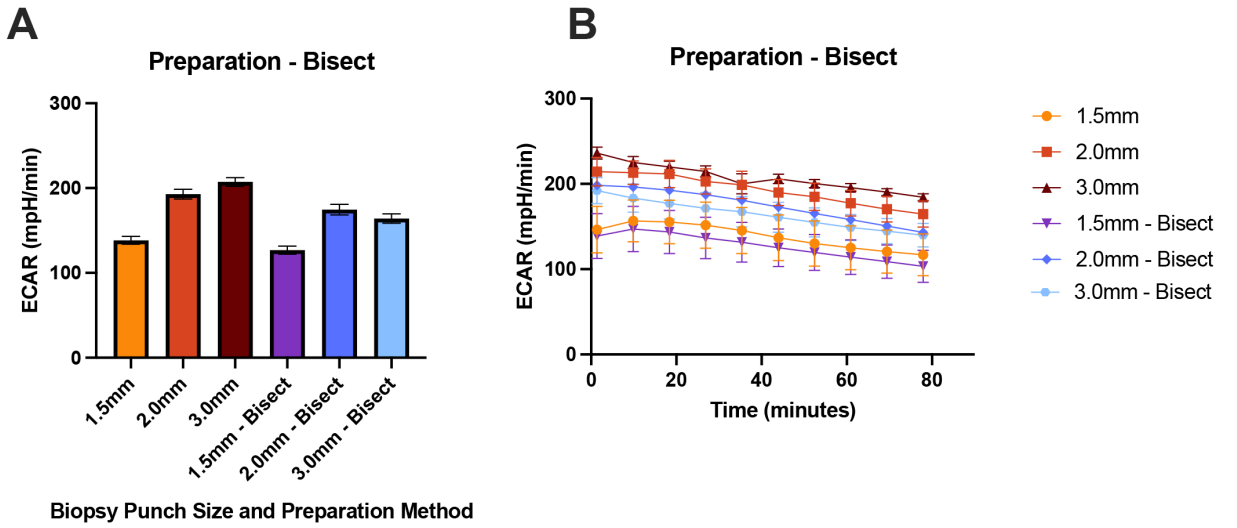

Figure S5. ECAR by preparation method. Extracellular acidification rate (ECAR) for each biopsy punch size using preparation featured in the main text protocol or an additional preparation method where the intestine was first cut in half longitudinally then biopsy punched (Bisect). Average ECAR from all measurements (A) and average for each of the 10 measurement cycles (B). Samples from wildtype murine jejunum using Agilent's DMEM based media. 5–10 replicates from 1–2 wildtype mice were used for each condition for these initial optimization tests. Data represent average ± standard error of the mean (SEM).

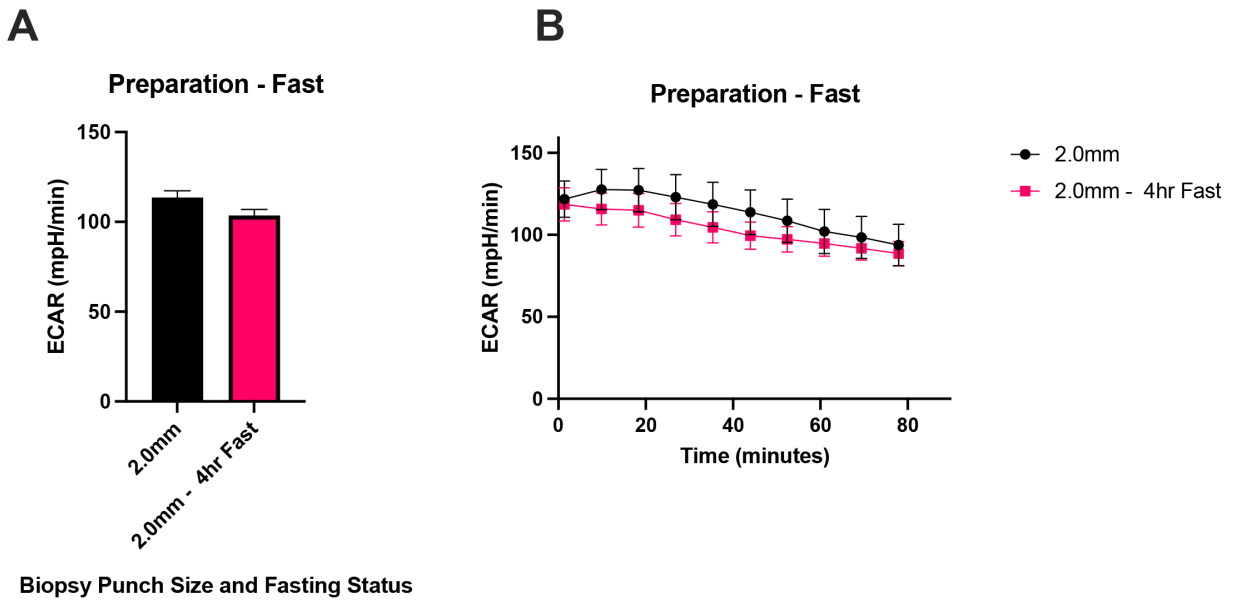

Figure S6. ECAR by fasting status. Extracellular acidification rate (ECAR) for 2 mm biopsy punches collected from fed or 4 h fasted mice. Average ECAR from all measurements (A) and average for each of the 10 measurement cycles (B). Samples from wildtype murine jejunum using Agilent's Glycolytic Rate media. 5–10 replicates from 1–2 wildtype mice were used for each condition for these initial optimization tests. Data represent average ± standard error of the mean (SEM).

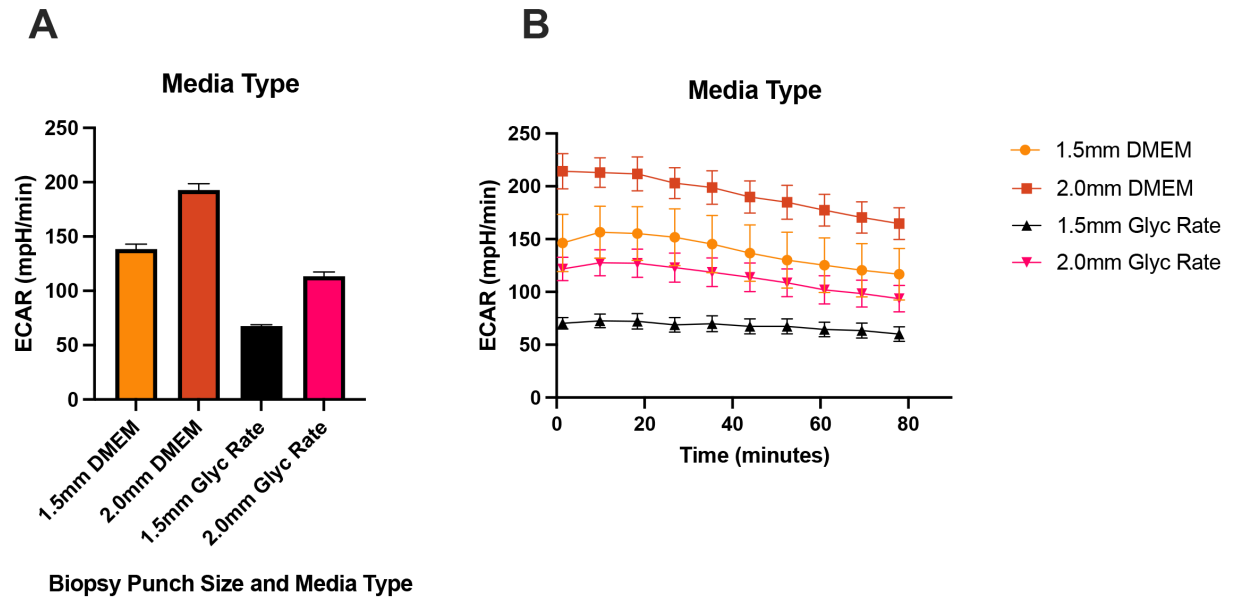

Figure S7. ECAR by media type. Extracellular acidification rate (ECAR) for each biopsy punch size using Agilent's DMEM based media and Glycolytic Rate media (Glyc Rate). Average ECAR from all measurements (A) and average for each of the 10 measurement cycles (B). Samples from wildtype murine jejunum. 5–10 replicates from 1–2 wildtype mice were used for each condition for these initial optimization tests. Data represent average ± standard error of the mean (SEM).