

Supporting information for:

Hypoxanthine is a checkpoint stress metabolite in colonic epithelial energy modulation and barrier function

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**Running title:** *Epithelial adenylate metabolites and barrier function*

**Key words:** Epithelia, barrier function, energy metabolism, actin

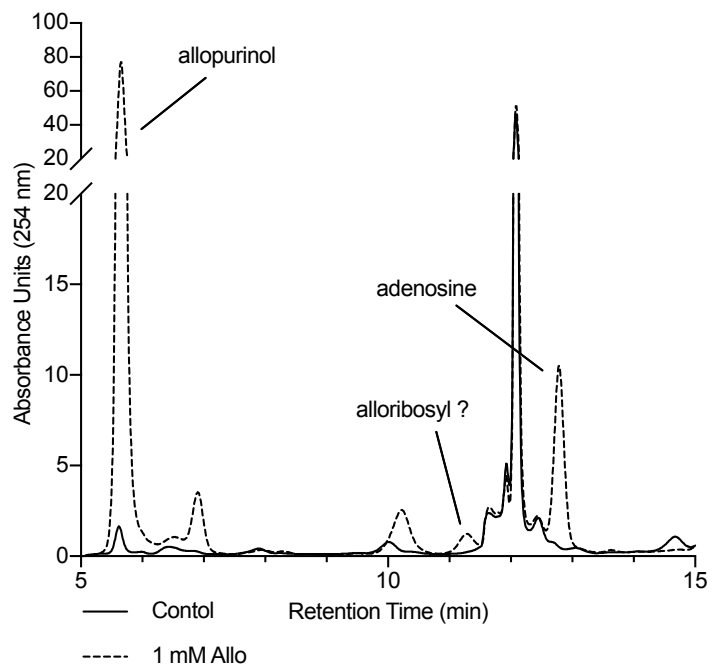
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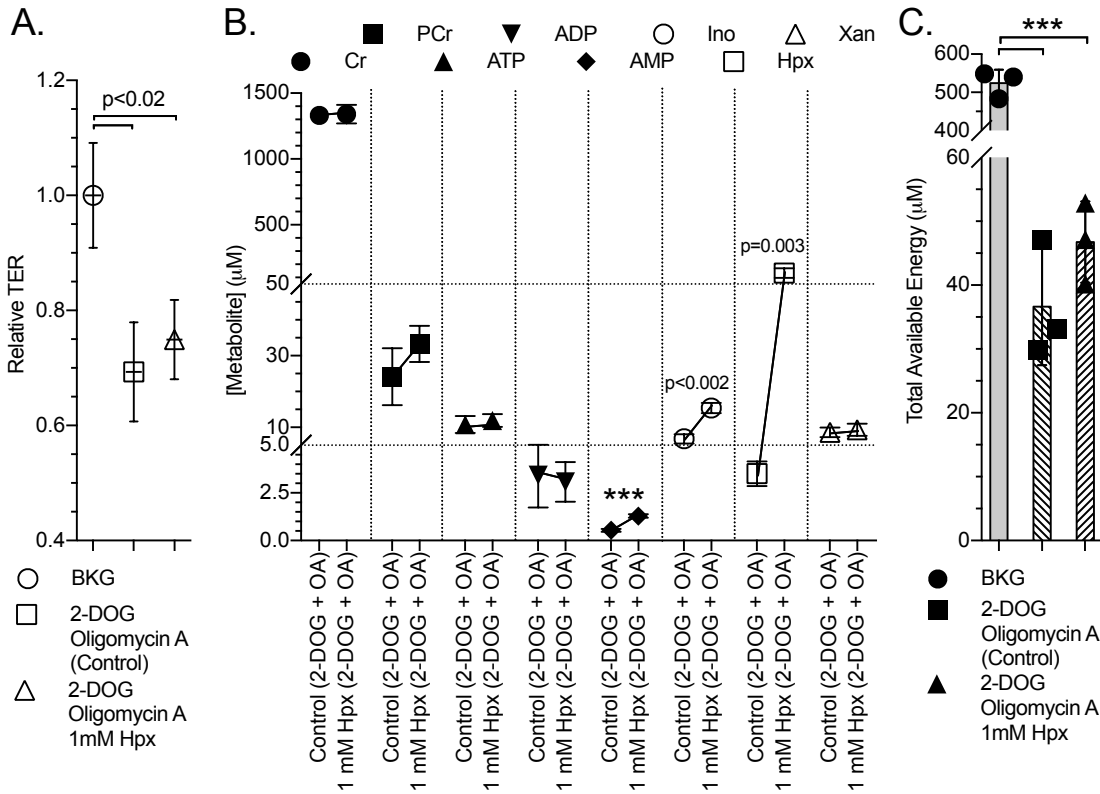
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**Figure S1.** HPLC traces of sub-confluent control and 1 mM allopurinol-treated cells. Indicated are retention times for allopurinol, adenosine, and the proposed alloribosyl. For comparison, retention time for inosine is ~8 min.



**Figure S2.** Barrier and metabolite responses to 2-deoxyglucose + oligomycin A treatments. (A and B) Barrier and metabolite responses of T84 cells to 2-deoxyglucose (20 mM) + oligomycin A (2  $\mu\text{M}$ ) with and without hypoxanthine (1 mM) as measured by HPLC;  $n = 3$ ,  $t = 30$  min. (C) Total available energy of results depicted in panel B. Data presented as mean  $\pm$  SD; TER, transepithelial resistance; 2-DOG, 2-deoxyglucose; BKG, background (untreated); Total available energy =  $\text{PCr} + \text{ATP} + (0.5 \cdot \text{ADP})$ ; Cr, creatine; PCr, phosphocreatine; ATP, adenosine triphosphate; ADP, adenosine diphosphate; AMP, adenosine monophosphate; Ino, inosine; Hpx, hypoxanthine; Xan, xanthine; \*\*\* indicates  $p < 0.001$ .