

**Supplementary information to:**

**Original article:**

**THE SMALL CHAIN FATTY ACID BUTYRATE ANTAGONIZES  
THE TCR-STIMULATION-INDUCED METABOLIC SHIFT IN  
MURINE EPIDERMAL  $\gamma$  T CELLS**

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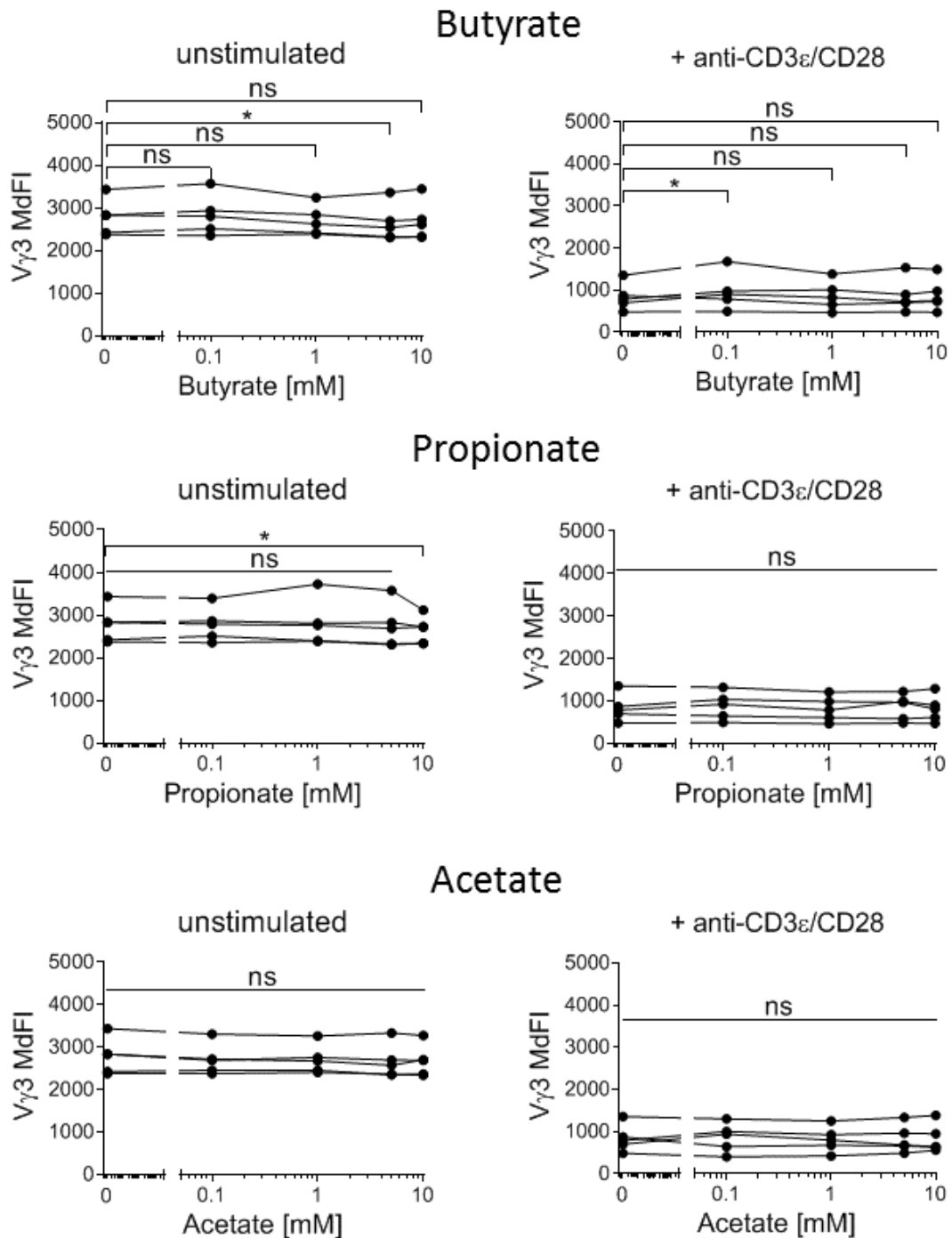
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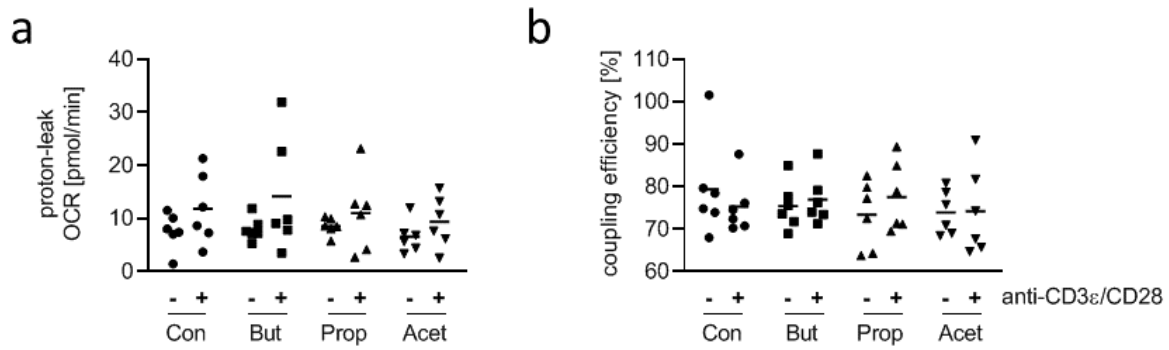
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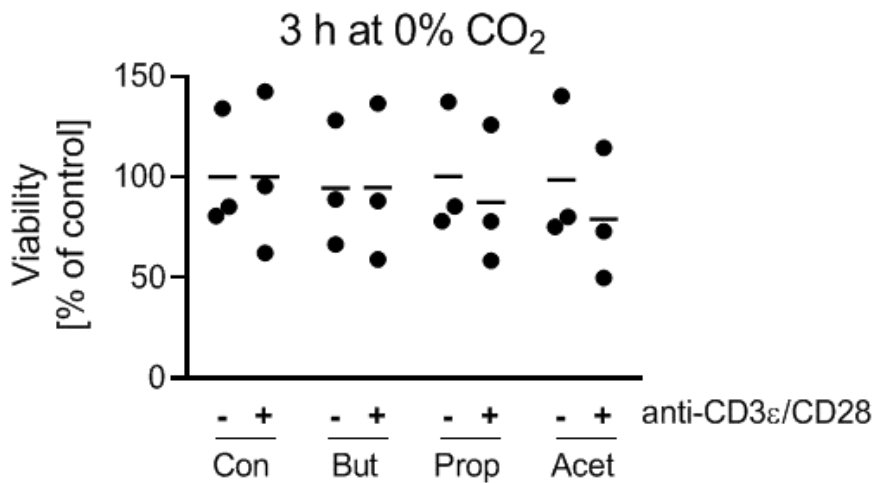
**Supplementary Figure 1: Influence of SCFA on TCR-internalization during stimulation of 7-17 DETC**

7-17 cells were treated with the indicated concentrations of butyrate, propionate or acetate and simultaneously stimulated with anti-CD3ε/CD28 antibodies for 6 hours. Subsequently, cells were detached, stained with anti-Vγ3-FITC antibodies and analyzed by flow cytometry. The median fluorescence intensity (MdfI) of live 7-17 cells, defined by a negative DAPI staining, is shown (n = 5; individual biological replicates are connected by lines; two-way ANOVA (biological replicates are paired without Geisser-Greenhouse correction) followed by a Dunnett's multiple comparisons test, which compared SCFA-treated samples with controls separately within unstimulated or stimulated samples; \*\*\* p < 0.001, \*\* p < 0.01, \* p < 0.05, ns = not significant)



**Supplementary Figure 2: Influence of SCFA on the metabolic parameters proton-leak and coupling efficiency in 7-17 DETC**

7-17 cells were stimulated with anti-CD3 $\epsilon$ /CD28 antibodies for 20 hours or left unstimulated and subsequently treated with 5 mM of butyrate, propionate or acetate for one hour. After that the oxygen consumption rate (OCR) and extracellular acidification rate was measured by the Seahorse device (Agilent). (a) Proton-leak was calculated as the difference between OCR after oligomycin and OCR after antimycin A / rotenon injection and (b) coupling efficiency was calculated from the OCR values before and after oligomycin treatment as the proportion of O<sub>2</sub>-usage for ATP-production from basal respiration in % according to manufacturer's protocol (a, b) (n = 6)



**Supplementary Figure 3: Influence of SCFA on viability of 7-17 DETC under Seahorse-measurement conditions**

7-17 cells were stimulated with anti-CD3 $\epsilon$ /CD28 antibodies for 20 hours or left unstimulated. Subsequently, the cells were transferred into an incubator with 0 % CO<sub>2</sub> and treated with 5 mM of butyrate, propionate or acetate for 3 hours in order to simulate the conditions during the metabolic profiling in the Seahorse-device. Viability was assessed using an MTT-assay. The measured absorbance-values were normalized to the mean of the untreated cells (= 100 % viability) of all biological replicates (n = 3).