Cell Metabolism, Volume *30*

Supplemental Information

Lactate Buildup at the Site of Chronic Inflammation Promotes Disease by Inducing $CD4^+$ T Cell Metabolic Rewiring

Valentina Pucino, Michelangelo Certo, Vinay Bulusu, Danilo Cucchi, Katriona Goldmann, Elena Pontarini, Robert Haas, Joanne Smith, Sarah E. Headland, Kevin Blighe, Massimiliano Ruscica, Frances Humby, Myles J. Lewis, Jurre J. Kamphorst, Michele Bombardieri, Costantino Pitzalis, and Claudio Mauro

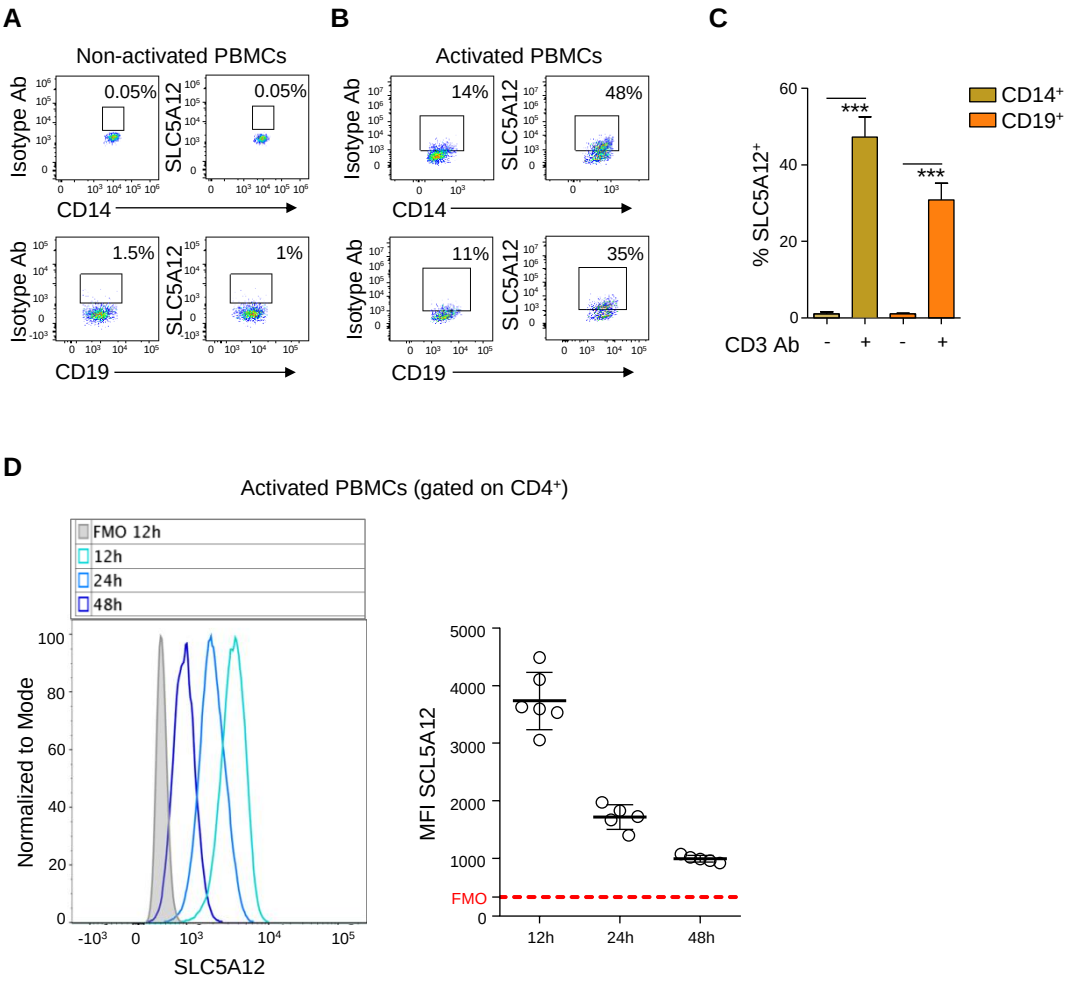


Figure S1. Related to Figure 1. SLC5A12 expression and kinetic in immune cells from human peripheral blood.

(A-C) Representative flow cytometry plots of SLC5A12 expression by CD14$^+$ monocytes or CD19$^+$ B cells from non-activated (n=3; A) or anti-CD3 mAb-activated (n=6; B) HC PBMCs. Quantification shown in (C). One-way ANOVA (C). (D) Kinetic of SLC5A12 expression by HC PBMCs CD4$^+$ T cells (n=5-6) activated for the indicated time points. Data expressed as mean ± s.e.m. ***P ≤ 0.001.

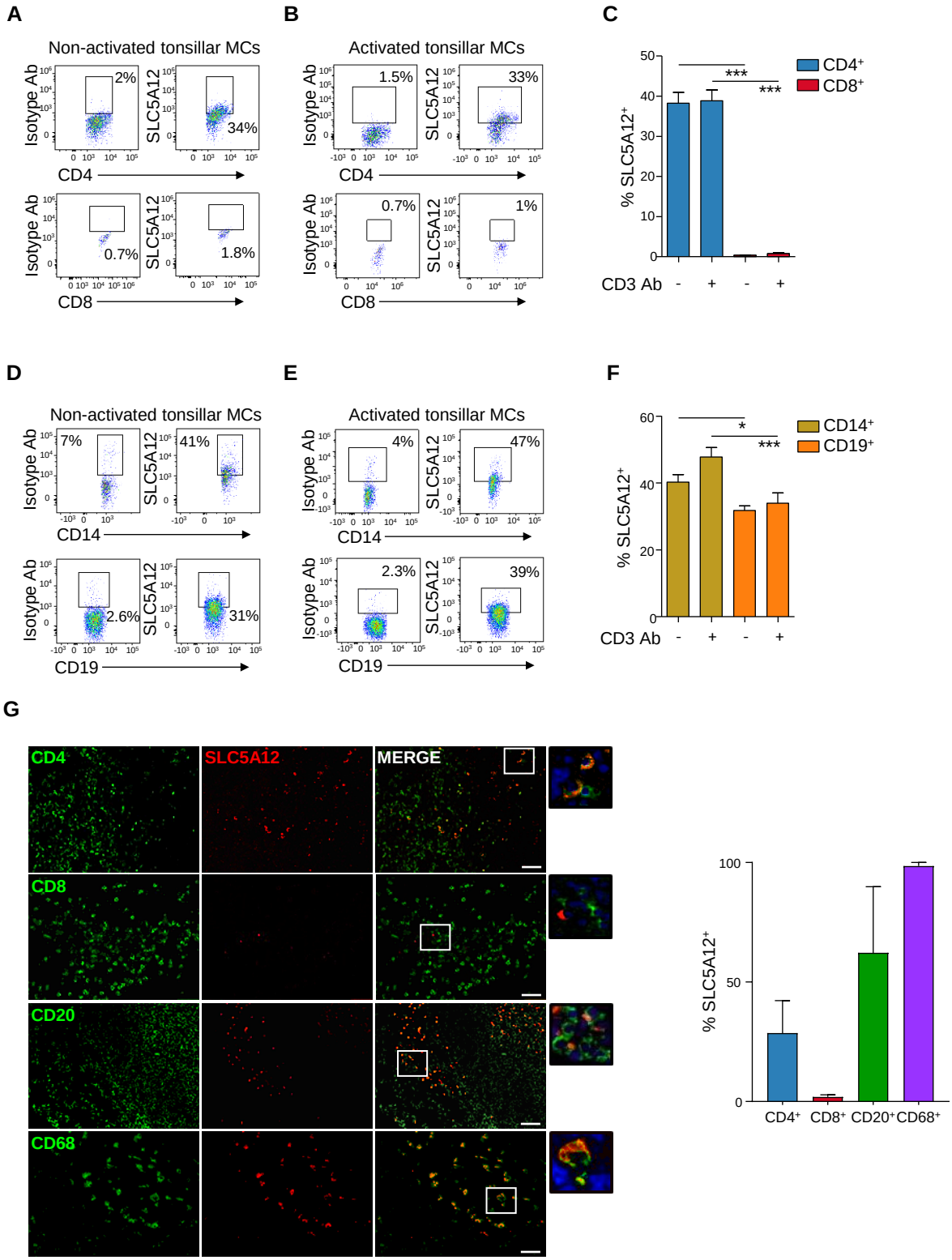


Figure S2. Related to Figure 1. SLC5A12 expression in immune cells from human tonsils.

(A-F) Representative flow cytometry plots of SLC5A12 expression by non-activated (n=4; A, D) or anti-CD3 mAb-activated (n=4; B, E) tonsil MCs gated for $CD4^+$, $CD8^+$ (A-B), $CD14^+$ and $CD19^+$ (D-E). Quantification shown in (C, F). One-way ANOVA. Data expressed as mean ± s.e.m. *P ≤ 0.05; ***P ≤ 0.001. (G) Representative immunofluorescence images of tonsils and related quantification. Co-staining for SLC5A12 (red), CD3, CD4, CD20 or CD68 (green), and DAPI (blue). Scale bar: 50µm.

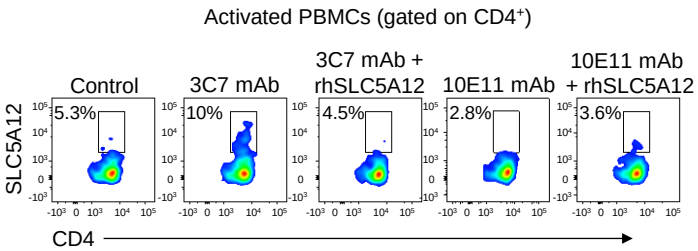


Figure S3. Related to Figure 1. SLC5A12 monoclonal antibodies binding in human CD4⁺ T cells.

Representative flow cytometry plots of SLC5A12 expression by CD4⁺ T cells from anti-CD3 mAb-activated HC PBMCs. Cells were pre-incubated for 1 hour in the presence or absence of SLC5A12 recombinant peptide (1:100) before a further incubation with SLC5A12 mAbs (3C7 IgG or 10E11 IgG). Alexa Fluor 555 goat anti-rat (1:1000, Invitrogen) was used as secondary antibody.

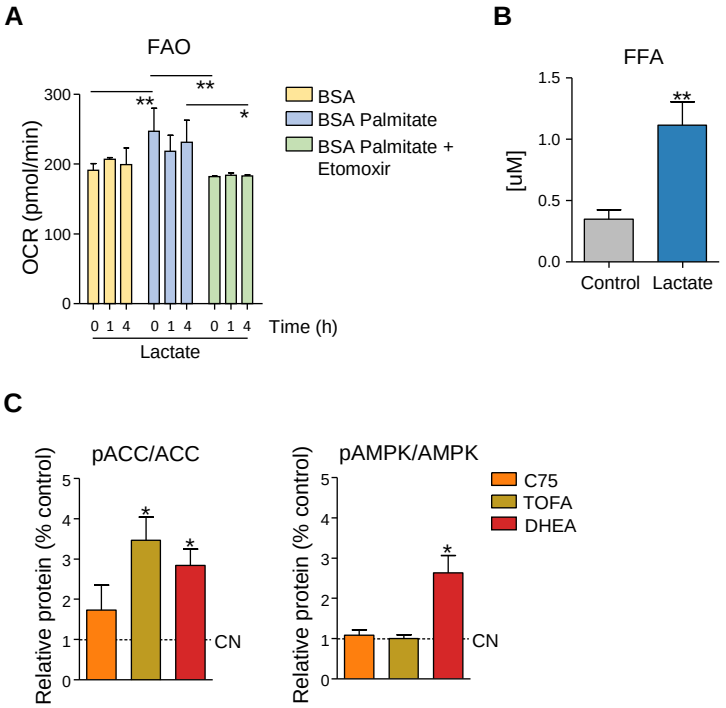


Figure S4. Related to Figure 4. Effects of lactate on CD4⁺ T cells lipid metabolism.
(A) Seahorse measurements of fatty acid oxidation (FAO)-driven oxygen consumption rates (OCR) by activated CD4⁺ T cells treated with sodium lactate (10mM) for 1 or 4 hours in the presence of Glucose (2.5mM) with BSA, BSA-palmitate (167μM) or BSA-palmitate plus etomoxir (40uM), (n=3). (B) Free fatty acid (FFA) intracellular levels in activated CD4⁺ T cells (n=4) treated with sodium lactate (10mM) for 4 hours. (C) Densitometric quantification of western blot analysis (n=2) of P-ACC, ACC, P-AMPK and AMPK expression by activated CD4⁺ T cells treated with C75 (10uM), TOFA (20uM) or DHEA (20uM), or left untreated. Untreated CD4⁺ T cells (Ctrl - dotted line) set to 1.
Two-way ANOVA (A) or two-tailed Student's t-test (B-C). Data expressed as mean ± s.e.m. *P ≤ 0.05; **P ≤ 0.01.

A

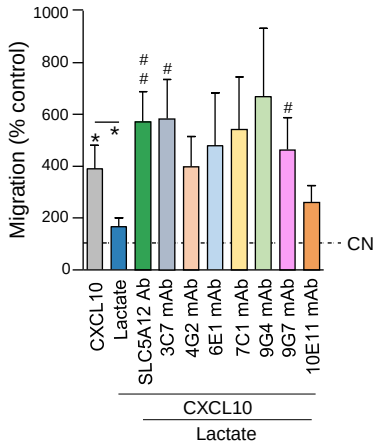


Figure S5. Related to Figure 5. Effects of SLC5A12 mAbs on human CD4⁺ T cell migration.

(A) In vitro chemokinesis of activated CD4⁺ T cells (n=5) in response to CXCL10 (300 ng/mL; 4 hours) in the presence of sodium lactate (10mM) with or without SLC5A12 Ab or the mAb clones 3C7, 4G2, 6E1, 7C1, 9G4, 9G7 or 10E11. Untreated CD4+ T cells (w/o CXCL10 - dotted line) were set to 100. Two-tailed Student's t-test. Data expressed as mean ± s.e.m. *P ≤ 0.05; #P ≤ 0.05 versus lactate.

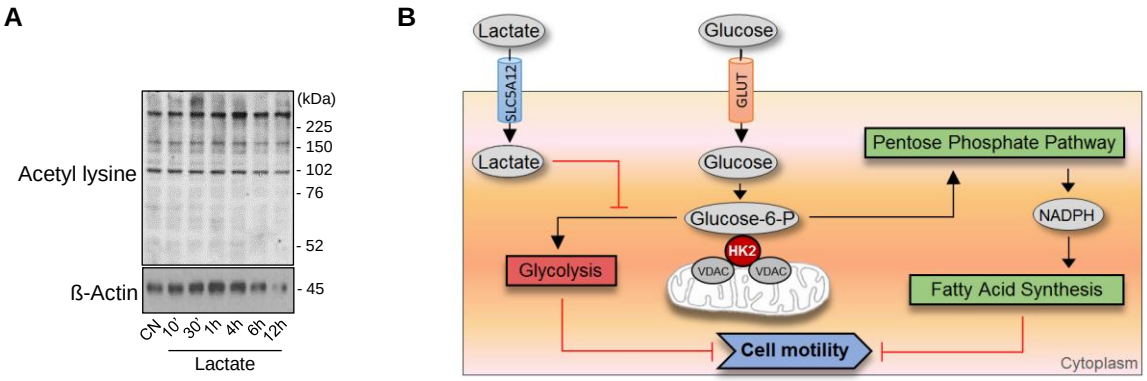
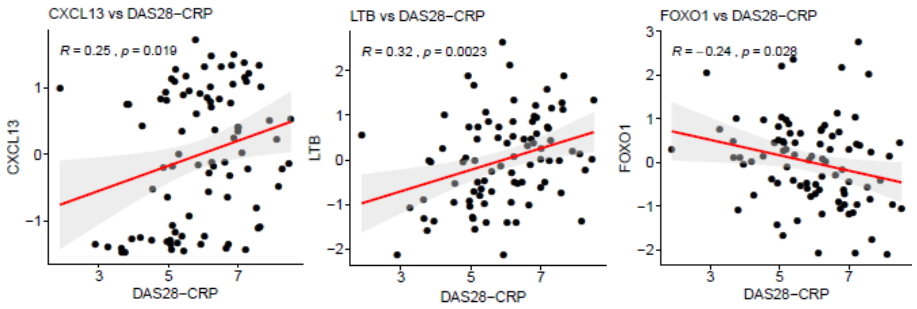


Figure S6. Related to Figure 6. Acetylation is not required for lactate induced metabolic reprogramming in CD4⁺ T cells.

(A) Representative western blots of acetyl lysine-conjugated cytosolic proteins in activated CD4⁺ T cells treated with sodium lactate for the indicated time points. (B) Schematic depicting the described findings: lactate-induced inhibition of CD4⁺ T cell response to migratory stimuli is due to a metabolic adaptation to inflamed tissue levels of lactate that results in reduced glycolysis and translocation of HK2 to the outer membrane of mitochondria, which in turn supports NADPH-dependent de novo fatty acid synthesis (FAS).

A



B

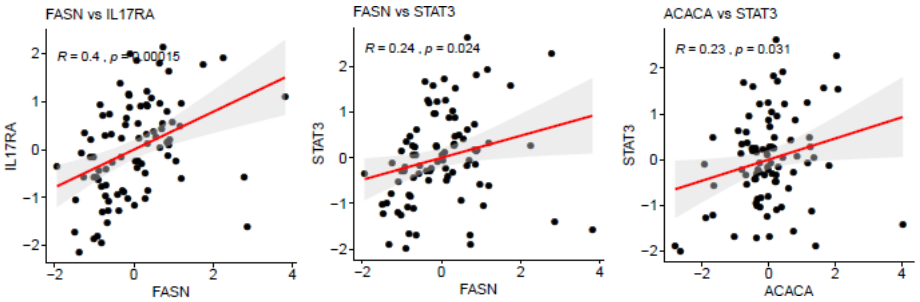


Figure S7. Related to Figure 7. Analysis of key transcripts related to disease activity and Th17 signature in RA patients.

(A) Correlations between the inflammatory score DAS28-CRP with CXCL13, LTB and FOXO1 transcripts (n=87). (B) Correlations between the following transcripts: FASN vs IL17RA, FASN vs STAT3 and ACACA vs STAT3 (n=87). Correlation analyses performed using Spearman's correlation coefficients.

Study cohort		**Treatment**	
Age	35-76		
Gender	Female (n=6) Male (n=2)	DMARDs	87%
Parameters		Steroids	12%
ESR	2-50		
CRP	5-26	Biologics	62%
DAS28 <2.1	75%	RF+ and/or CCP+ (%)	65%
DAS28 >5.2	25%		
Erosive	63%		

Table S1. Related to Patients section in STAR METHODS. Demographical patient data. ESR, Erythrocyte Sedimentation Rate; CRP, C-Reactive Protein; DAS28, Disease Activity Score; DMARDs, Disease-Modifying Antirheumatic Drugs; RF, Rheumatoid Factor; CCP, anti-Cyclic Citrullinated Peptide.