Science Advances
AAAS

Supplementary Materials for

Remodeling of metabolism and inflammation by exercise ameliorates tumor-associated anemia

Regula Furrer, Annaïse J. Jauch, Tata Nageswara Rao, Sedat Dilbaz, Peter Rhein, Stefan A. Steurer, Mike Recher, Radek C. Skoda, Christoph Handschin*

*Corresponding author. Email: christoph.handschin@unibas.ch

Published 8 September 2021, *Sci. Adv.* **7**, eabi4852 (2021)
DOI: 10.1126/sciadv.abi4852

This PDF file includes:

SUPPLEMENTARY MATERIALS

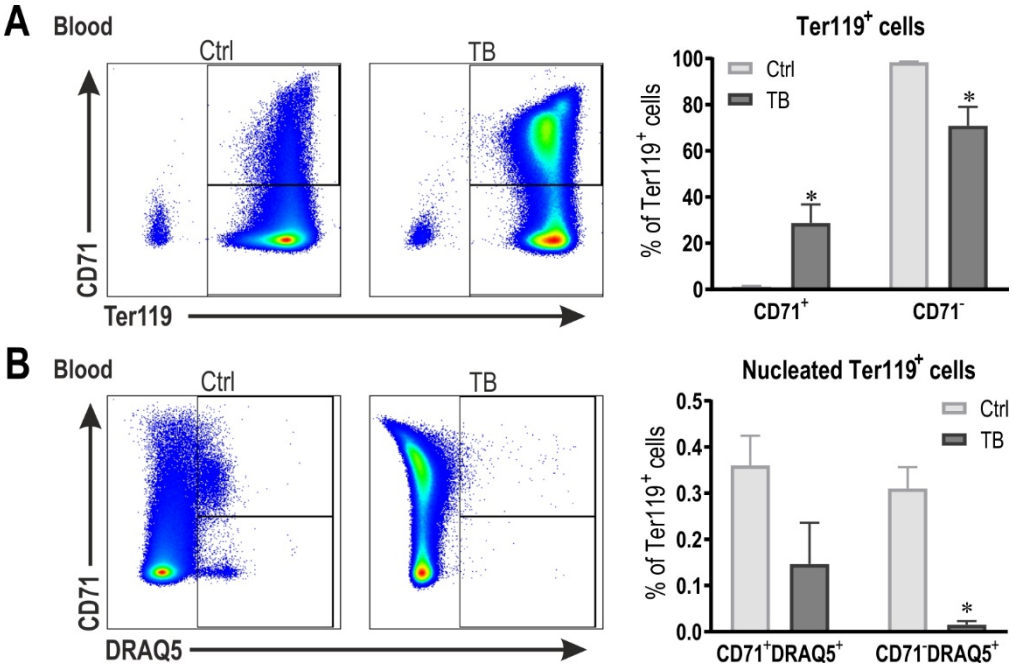

Figure S1

Fig. S1 Reticulocytosis in tumor-bearing (TB) mice. (**A,B**) Representative flow cytometry plots and quantification of blood of control (Ctrl) and TB mice 3.5 weeks after tumor cell inoculation that was stained for CD71, Ter119 and DRAQ5 to assess maturation of RBCs. Maturation was determined by the relative number of $CD71^+$ cells (A) and those containing DNA measured by DRAQ5 (B). Cells in the plots in panel (B) were gated for Ter119. Groups (n = 6 per group) were compared by two-tailed unpaired t-tests with Welch's correction and data are represented as mean +s.e.m. Asterisks indicate differences between Ctrl and TB group; * = p<0.05.

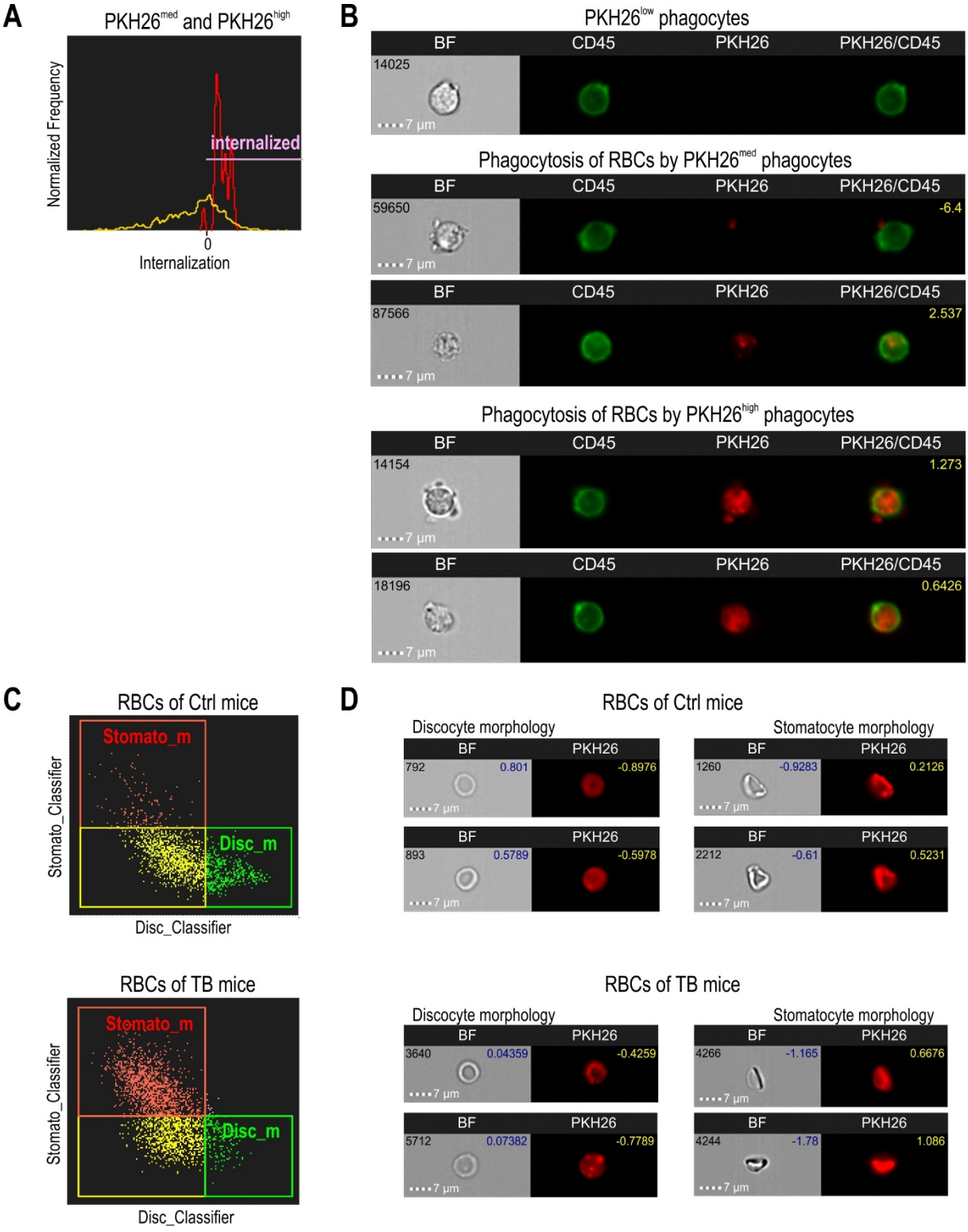

Figure S2

Fig. S2 Erythrophagocytosis by splenocytes of tumor-bearing (TB) mice and abnormal RBC morphology of TB mice. (**A**) The rate of internalization of PKH26^{med} (yellow) and PKH26^{high} (red) cells was determined (a score higher than 0 is considered internalized) and (**B**) the three different populations visualized. Only PKH26^{high} phagocytes show complete

internalization and engulfment of entire RBCs. These plots and images are from the erythrophagocytosis experiment in which RBCs from healthy Ctrl mice were co-incubated with splenocytes from TB mice. (**C**) Differences in RBC morphology were quantified using ImageStream and classified into discocyte-like morphology (Disc_m) and stomatocyte-like morphology (Stomato_m). (**D**) Representative images of the different RBC morphologies in Ctrl and TB mice.

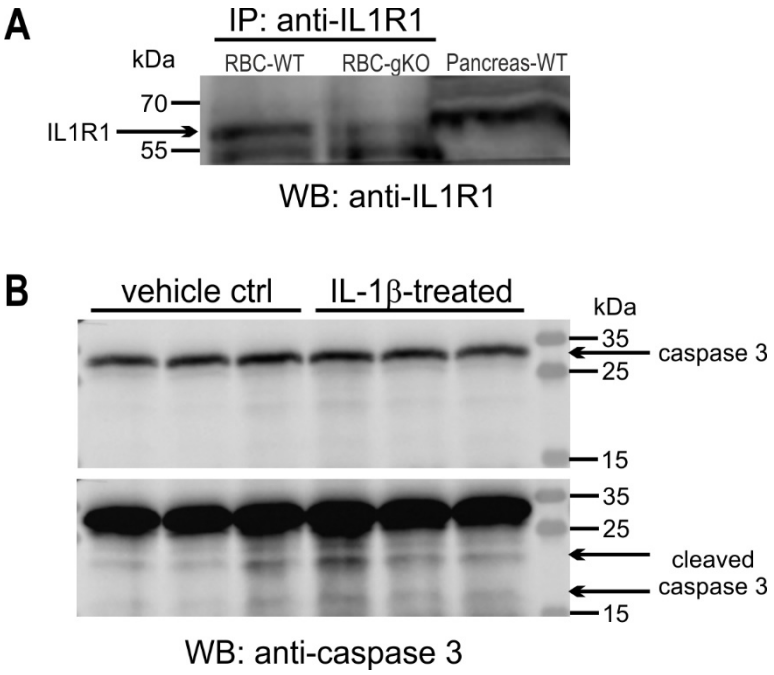

Figure S3

Fig. S3 IL-1β signaling in RBCs. (**A**) Immunoprecipitation (IP) of the interleukin 1 receptor 1 (IL1R1) in RBCs of wild type (WT) and IL1R1 global knock-out (gKO) mice followed by Western blot (WB) analysis of IL1R1. WT pancreas tissue was used as a positive control. (**B**) To assess whether IL-1β induces apoptotic signaling, RBCs were treated with pathophysiological concentrations of IL-1β (1 pg/mL) for 24 hours, and caspase 3 activation was assessed by WB. Cleaved caspase 3 shows the activation of caspase 3 and thereby an eryptotic signal.

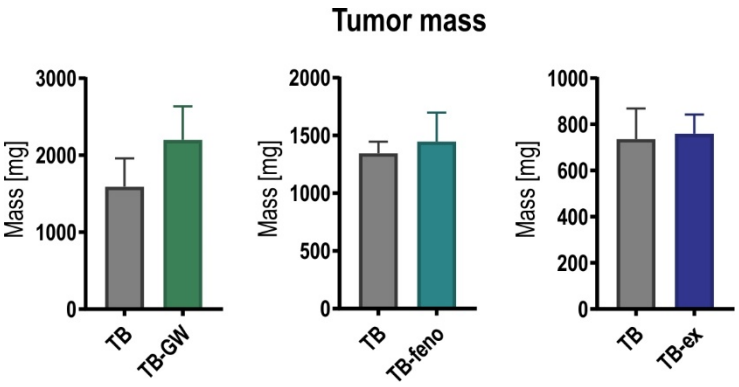

Figure S4

Fig. S4 Pharmacological treatments or exercise do not affect tumor growth. Tumor mass was assessed in the different intervention studies during which tumor-bearing (TB) mice were treated with the peroxisome proliferator-activated receptor β/δ (PPARδ) agonist GW501516 (TB-GW), PPARα agonist fenofibrate (TB-feno) or performed endurance training (TB-ex). Groups (n = 5-7 per group) were compared by two-tailed unpaired t-tests with Welch's correction and data are represented as mean +s.e.m. There was no difference between the groups.

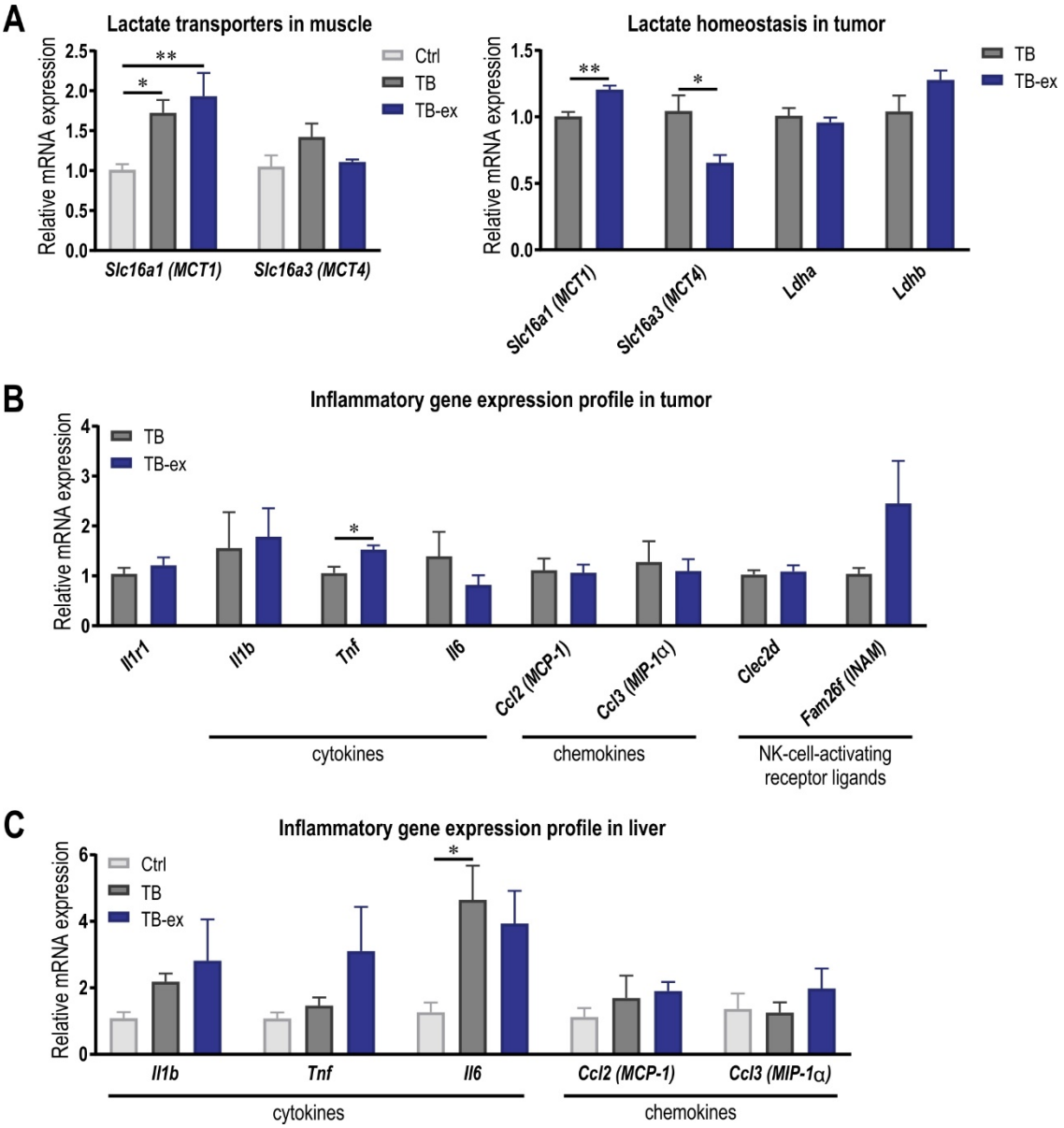

Figure S5

Fig. S5 Exercise affects non-malignant as well as malignant tissue. (**A**) Gene expression of lactate transporters MCT1 and MCT4 as well as lactate dehydrogenase (*Ldh*) were assessed in quadriceps muscle and tumor tissue 3.5 weeks after tumor cell inoculation. (**B,C**) mRNA levels of different cytokines, chemokines and NK-cell-activating receptor ligands were measured in tumor (B) as well as liver (C) tissue by qPCR. Differences in mRNA levels in tumor tissue (n = 5-7 per group) were tested by two-tailed unpaired t-tests with Welch's correction and differences in gene expression in liver and muscle tissue (n = 5-7 per group) were tested with one-way ANOVAs followed by Tukey's post-hoc analysis. Data are represented as mean +s.e.m. * = p<0.05; ** = p<0.01. Slc16a = Solute carrier family 16 member; MCT = Monocarboxylate transporter; IL = Interleukin; Tnf = Tumor necrosis factor α; Ccl = chemokine

(C-C motif) ligand; MCP-1 = Monocyte chemoattractant protein 1; MIP-1α = Macrophage inflammatory protein 1α; Clec2d = C-type lectin domain family 2 member D; INAM = Interferon regulatory factor 3-dependent NK-activating molecule.

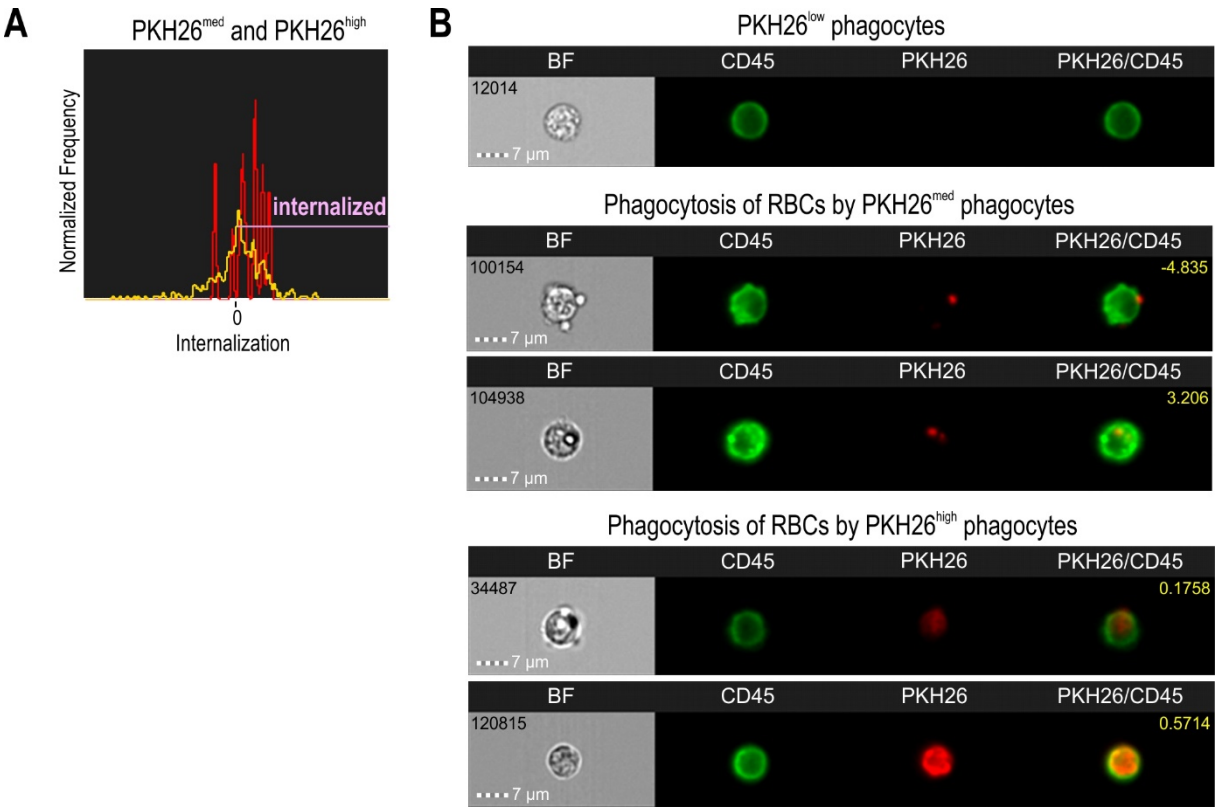

Figure S6

Fig. S6 Erythrophagocytosis by splenocytes that were pre-treated with IL-1β. (**A**) The rate of internalization of PKH26^{med} (yellow) and PKH26^{high} (red) cells was determined (a score higher than 0 is considered internalized) and (**B**) the three different populations visualized. Only PKH26^{high} phagocytes show nearly complete internalization and engulfment of entire RBCs. These plots and images are from the erythrophagocytosis experiment in which RBCs from healthy Ctrl mice were co-incubated with splenocytes that were pre-treated with IL-1β (1 pg/mL).