

Figure S1. Lactic acid inhibits bulk CD4 T cell effector functions by metabolic blockade. (A – G) CD4 T cells were stimulated with anti-CD3/CD28 coated beads (bead-to-cell ratio 1:1) and treated with the indicated concentrations lactic acid in standard RPMI. (A) Viability was determined after 72 h by annexin V/7-AAD staining. Shown are median values and single data points. Statistical significance was calculated using one-way ANOVA and Bonferroni multiple comparison test (* $p < 0.05$). (B) Cells were counted at indicated timepoints using the CASY Cell Counter (mean + SEM, $n = 4$). Statistical significance was calculated with two-way ANOVA and Dunnett's multiple comparison test (* $p < 0.05$; **** $p < 0.001$). (C, D) Cytokine concentrations in supernatants were determined after 48 h using ELISA and normalized to the respective controls. Shown are median values and single data points. Statistical significance was calculated using one-way ANOVA and Bonferroni multiple comparison test (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$). (E) Oxygen consumption of the cells was measured using the PreSens technology (mean values, $n = 6$). (F) Glucose uptake was calculated by subtracting remaining glucose in culture supernatants after 48 h from basal glucose concentration in culture medium. Shown are median values and single data points. Statistical significance was calculated using one-way ANOVA and Bonferroni multiple comparison test (* $p < 0.05$, ** $p < 0.01$). (G) LDH isoenzyme distribution in unstimulated (0 h) and 48 h stimulated and lactic acid treated cells was assessed using LHD zymography analysis. Depicted is one representative example ($n = 3$).

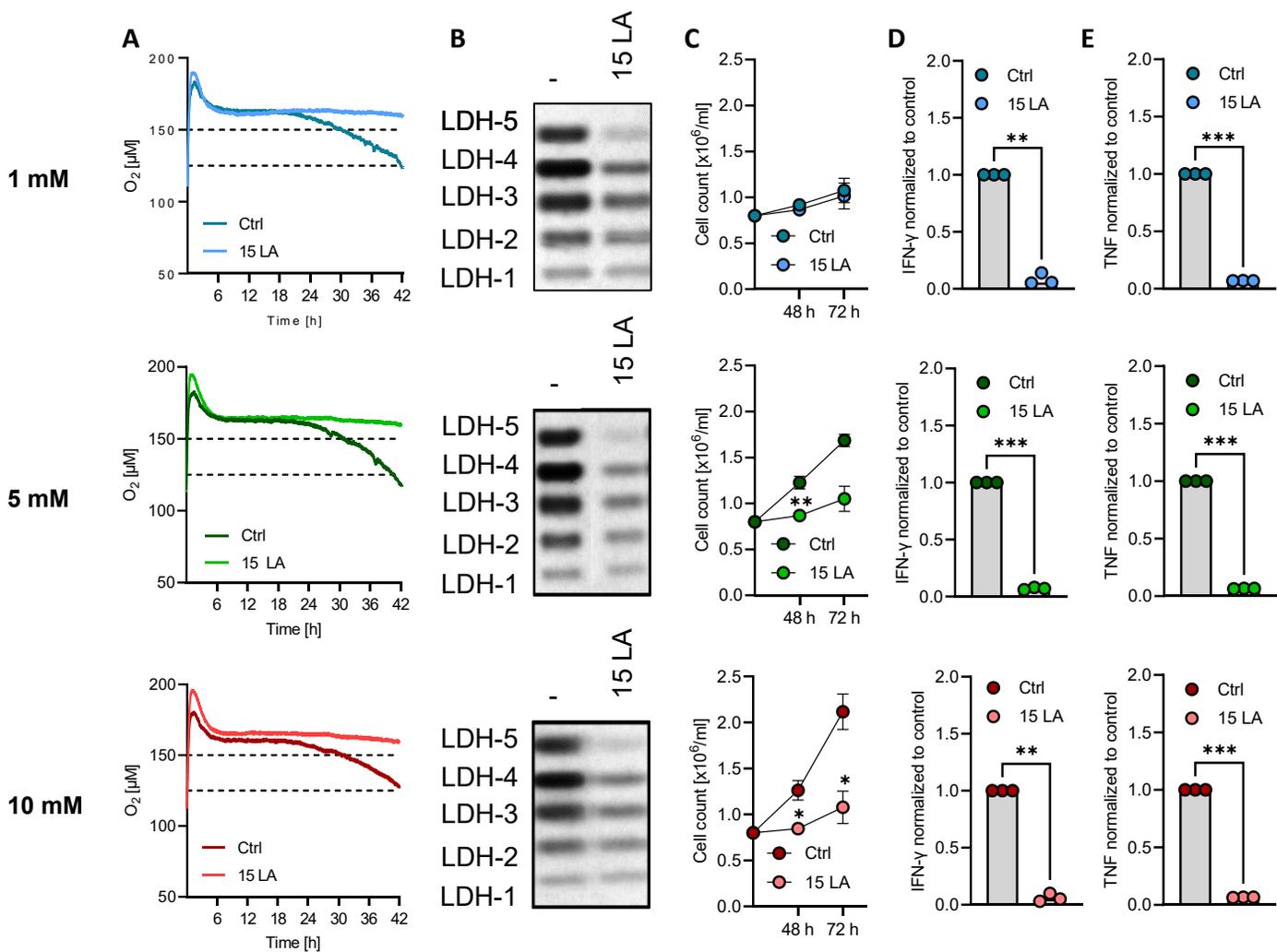


Figure S2. Lactic acid inhibits bulk CD4 T cell effector functions by metabolic blockade independent of glucose supplementation. (A – E) CD4 T cells were stimulated with anti-CD3/CD28 coated beads (bead-to-cell ratio 1:1) and treated with the indicated concentrations lactic acid in RPMI supplemented with given glucose concentrations. (A) Oxygen consumption of the cells was measured using the PreSens technology (mean values, $n = 6$). (B) LDH isoenzyme distribution in unstimulated 48 h stimulated and lactic acid treated cells was assessed using LHD zymography analysis. Depicted is one representative example ($n = 3$). (C) Cells were counted at indicated timepoints using the CASY Cell Counter (mean + SEM, $n = 4$). Statistical significance was calculated with two-way ANOVA and Dunnet's multiple comparison test (* $p < 0.05$; ** $p < 0.01$). (D, E) Cytokine concentrations in supernatants were determined after 48 h using ELISA and normalized to the respective controls. Shown are median values and single data points. Statistical significance was calculated using one-way ANOVA and Bonferroni multiple comparison test (** $p < 0.01$, *** $p < 0.001$).

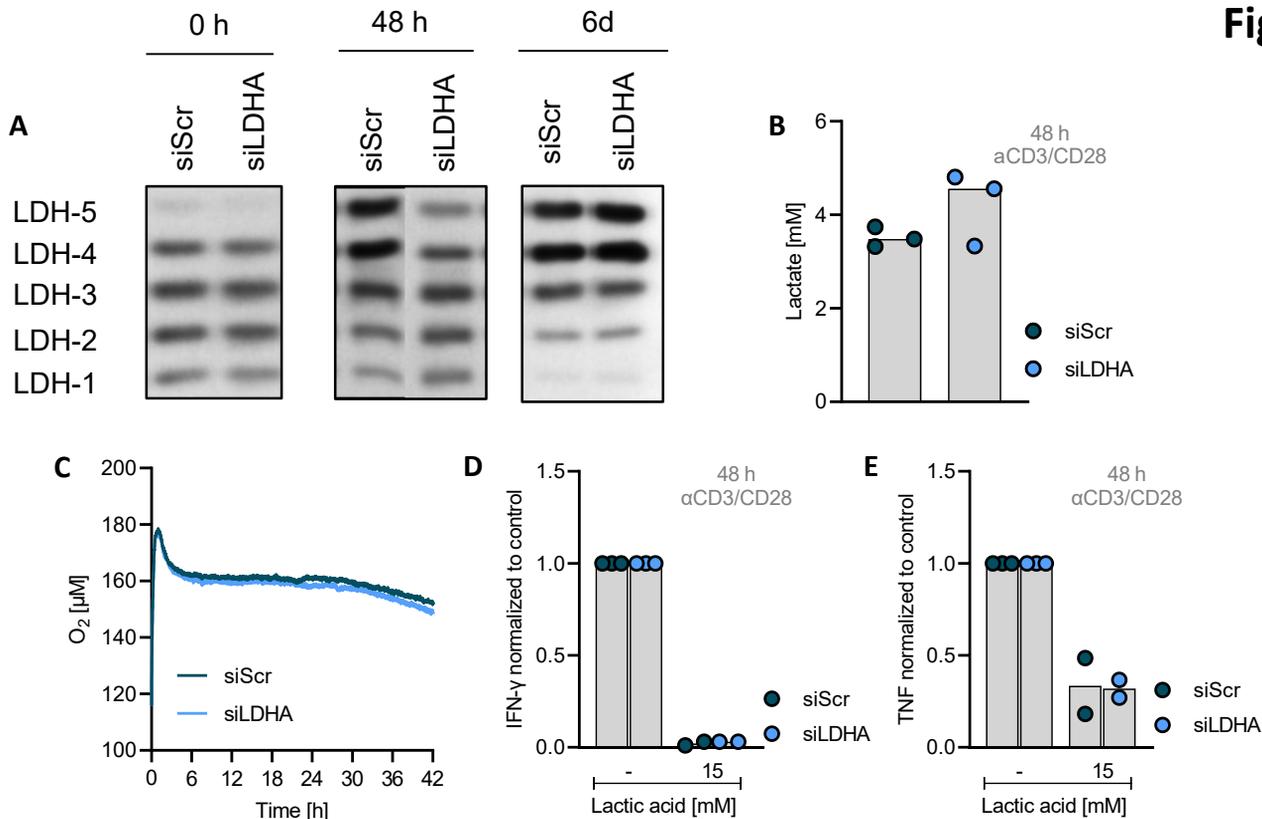


Figure S3. LDHA knockdown in T cells does not increase lactic acid resistance. CD4 T cells were electroporated with siRNA targeting LDHA (siLDHA) or a scrambled control (siScr). After resting over night, cells were stimulated with anti-CD3/CD28 coated beads (bead-to-cell ratio 1:1) and treated with 15 mM lactic acid. **(A)** LDH isoenzyme distribution in unstimulated (0 h) and 48 h stimulated and lactic acid treated cells was assessed using LHD zymography analysis. Depicted is one representative example ($n = 3$). **(B)** Lactate concentration in culture supernatants was assessed enzymatically after 48 h stimulation. **(C)** Oxygen consumption of the cells was measured using the PreSens technology (mean values, $n = 3$). **(D, E)** Cytokine concentrations in supernatants were determined after 48 h using ELISA and normalized to the respective controls. Shown are median values and single data points. **(B, D, E)** Statistical significance was calculated using one-way ANOVA and Bonferroni multiple comparison test.

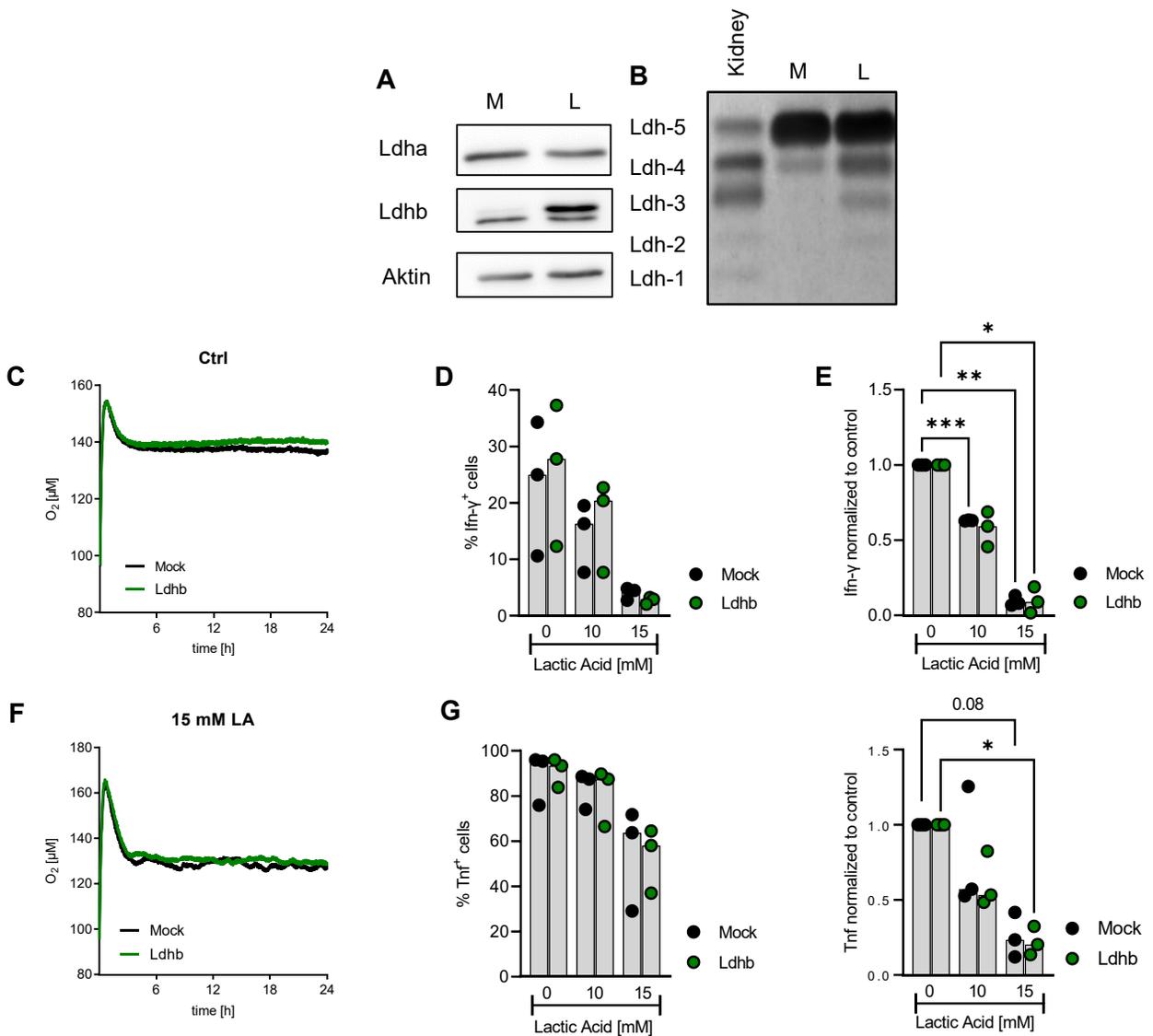


Figure S4. Overexpression of Ldhb in murine T cells fails to alter cellular respiration or increase lactic acid resistance. Ldhb was overexpressed in murine CD4 T cells. **(A)** Expression of Ldha and Ldhb was assessed by western blot analysis. Actin served as a loading control. Depicted is one representative example (M: Mock, L: Ldhb; $n = 3$). **(B)** LDH isoenzyme distribution was assessed using LHD zymography analysis. Depicted is one representative example (M: Mock, L: Ldhb; $n = 3$). **(C)** Cellular oxygen consumption under control conditions and upon 15 mM lactic acid treatment was measured using the PreSens technology (mean values, $n = 3$). **(D)** Intracellular production of Ifn- γ and Tnf was analyzed after stimulation with PMA/Ionomycin and monensin treatment. **(F)** Cytokine concentrations in supernatants were determined after PMA/Ionomycin stimulation using ELISA and normalized to the respective controls. **(D – G)** Shown are median values and single data points. Statistical significance was calculated using one-way ANOVA and Bonferroni multiple comparison test (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).