SPICE-Met: Profiling and imaging energy metabolism at the single-cell level using a fluorescent reporter mouse

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Appendix Figures

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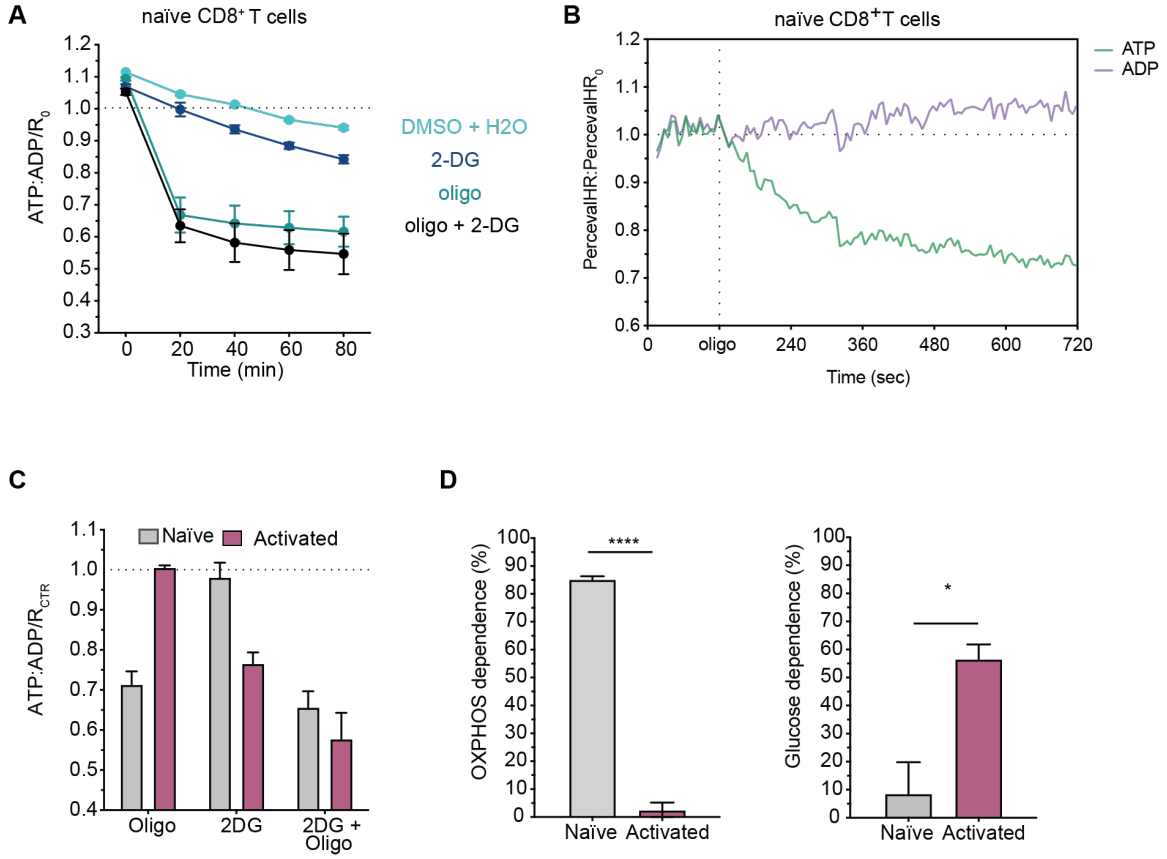
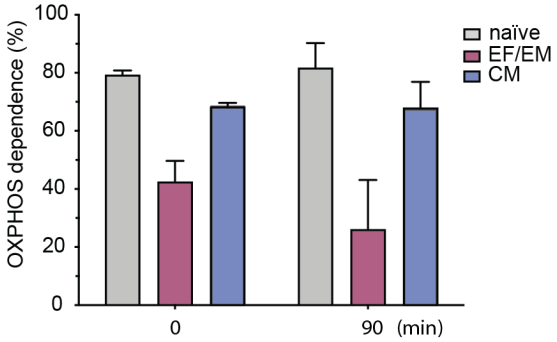


Appendix Figure S1: Kinetics of ATP:ADP changes in response to metabolic inhibitors

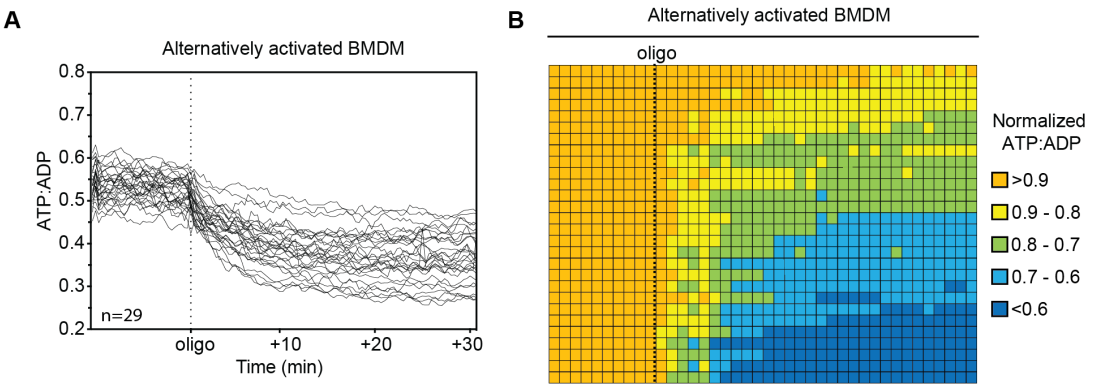
(A) Lymph node $CD8^+$ T cells collected from Lck-Cre x $Perceval^{fl/fl}$ mice were subjected to SPICE-Met. The graph shows the ATP:ADP changes over time upon treatment with oligo, 2-DG, oligo+2-DG and H_2O + DMSO (as a control). Note that most changes are detected within 40 minutes, a time point used for subsequent analyses. Data are representative of 2 independent experiments. **(B)** Naïve $CD8^+$ T cells from Lck-Cre x $Perceval^{fl/fl}$ mice were subjected to time-resolved flow cytometry. After 2 minutes, oligomycin was added and acquisition was continued for an additional period of 10 minutes. Relative ATP and ADP values were quantified using PercevalHR fluorescence excited using a blue (488 nm) and violet (405 nm) lasers respectively. The graph shows the geometric mean of the cellular ATP and ADP signals normalized to the average value measured during the first two minutes of acquisition time ($PercevalHR_0$). Three pooled experiments are shown. **(C-E)** $CD4^+$ T cells from Lck-Cre x

Perceval$^{fl/fl}$ mice were subjected to SPICE-Met **(C)** ATP:ADP ratio values were normalized to the DMSO+H_2O treatment ratio (R_{CTR}) to visualize the relative contribution of the different metabolic inhibitors. **(D-E)** OXPHOS and glucose dependencies were calculated as (($R_{DMSO+H2O}$ - R_{oligo}) / ($R_{DMSO+H2O}$ - $R_{oligo+2\text{-}DG}$)) x 100 and (($R_{DMSO+H2O}$ - $R_{2\text{-}DG}$) / ($R_{DMSO+H2O}$ -$R_{oligo+2\text{-}DG}$)) x 100, respectively. Results were evaluated using an unpaired Student's t-test. Results in B-D are representative 2-3 independent experiments.



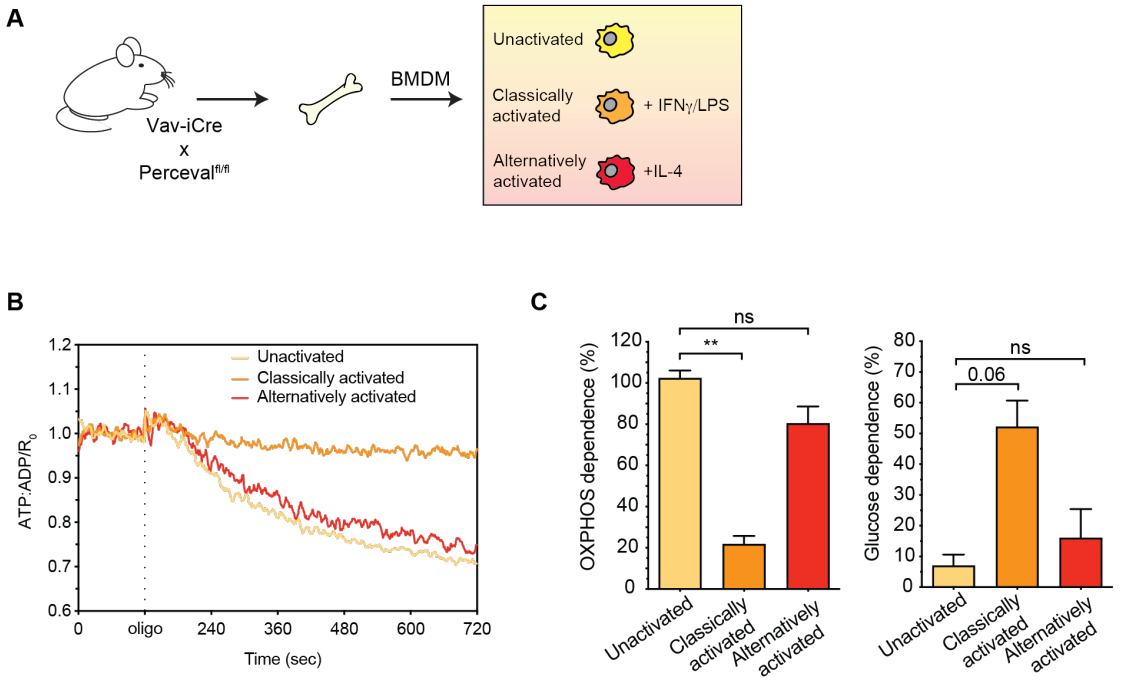
Appendix Figure S2. Assessing the stability of energy metabolic profiles after short-term culture

Lck-Cre x Perceval$^{fl/fl}$ mice were immunized with 2x10^{6} PFU of MVA-HIVB. (A) OXPHOS dependence was calculated in phenotypic defined CD8^{+} T cells populations either immediately or after 90 min of culture at 37°C to control for the stability of the assay.



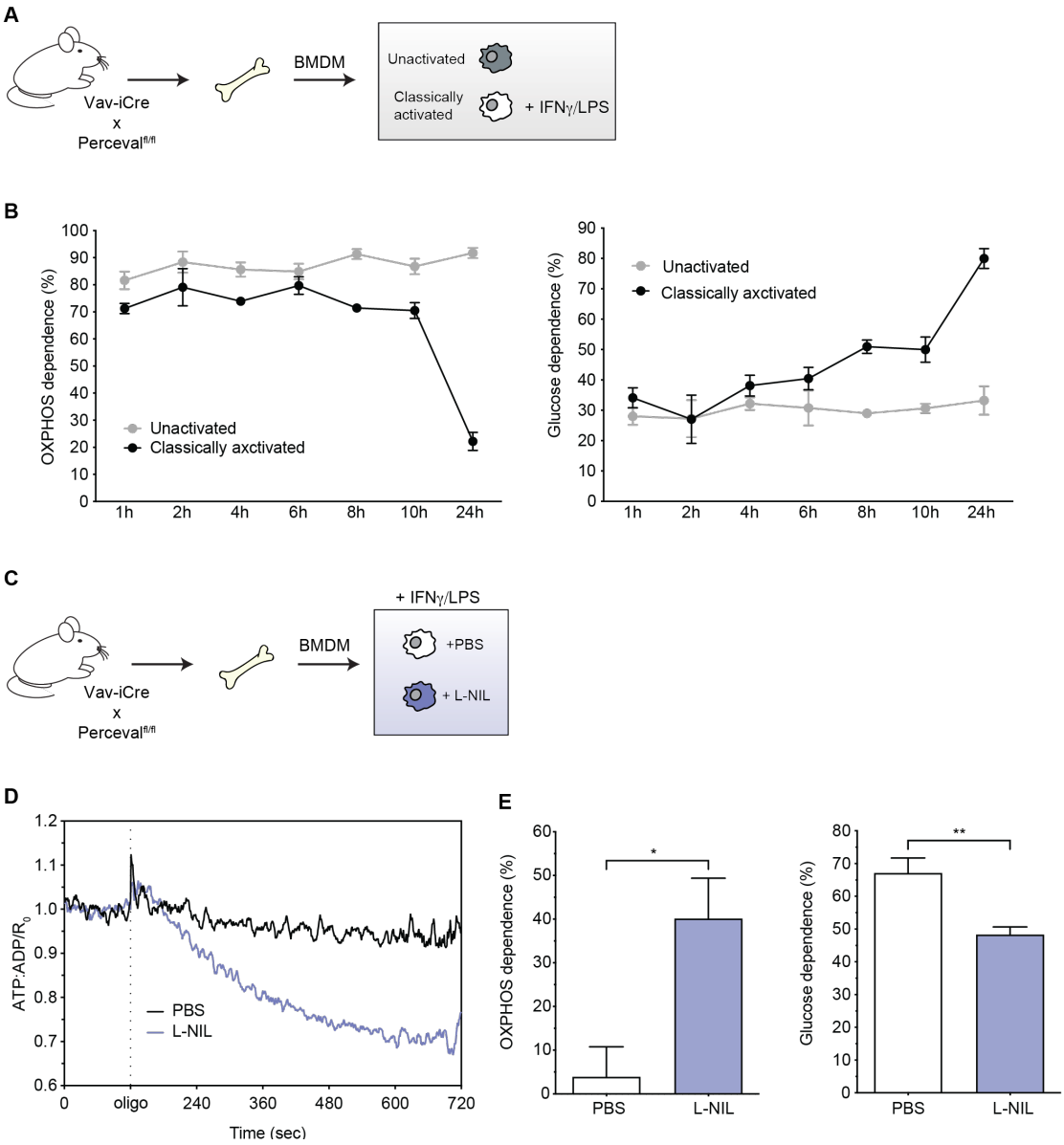
Appendix Figure S3: SPICE-Met imaging uncovers energy metabolism of single alternatively activated macrophages *in vitro*

BMDMs of Vav-iCre x Perceval$^{fl/fl}$ mice were activated 24 hours with IL-4. (**A-B**) Live-imaging of ATP:ADP ratio in alternatively activated BMDMs. Macrophages were exposed to oligomycin (1 µM) during image acquisition. **(A)** Kinetic analysis of ATP:ADP ratios for single macrophages (n=29 cells for each condition). (**B**) Table representing the ATP:ADP variations in individual macrophages treated with oligomycin. Ratios were normalized to the steady-state (initial) values and were color-coded as indicated. Each line represents one cell and each square corresponds to 1 minute. Results are representative of three independent experiments (n=29 cells for each condition).



Appendix Figure S4: Probing energy metabolism in BMDM using flow cytometry-based SPICE-Met

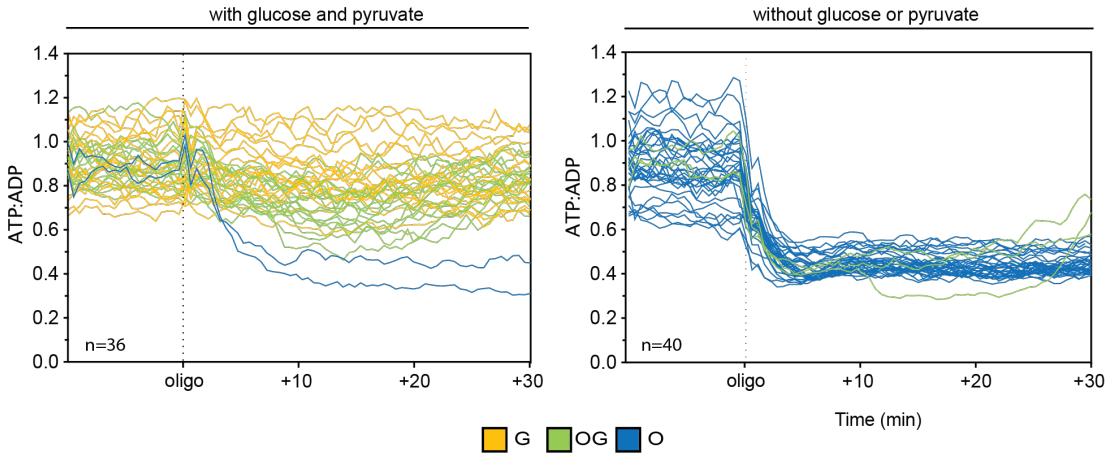
BMDMs generated from Vav-iCre x Perceval$^{fl/fl}$ mice were activated 24 hours with LPS+IFNγ (classically activated), IL-4 (alternatively activated) or left untreated (unactivated). **(A)** Experimental setup. **(B)** Alternatively activated, classically activated or unactivated BMDM were differently labeled and mixed together to perform time-resolved SPICE-Met simultaneously. After 2 minutes, oligomycin was added and acquisition was continued for an additional 10 minutes. Relative ATP:ADP ratio values were calculated using PercevalHR fluorescence excited using a blue (488 nm) and violet (405 nm) laser to quantify ATP and ADP, respectively. The graph shows the geometric mean of the cellular ATP:ADP ratio normalized to the average value measured during the first two minutes of acquisition time (R_0). One representative experiment out of three is shown. **(C)** OXPHOS and glucose dependencies are shown. Results were evaluated using an unpaired Student's t-test. Results are pooled from 3 independent experiments.



Appendix Figure S5: Kinetics and role of iNOS in macrophage metabolic switch during classical activation

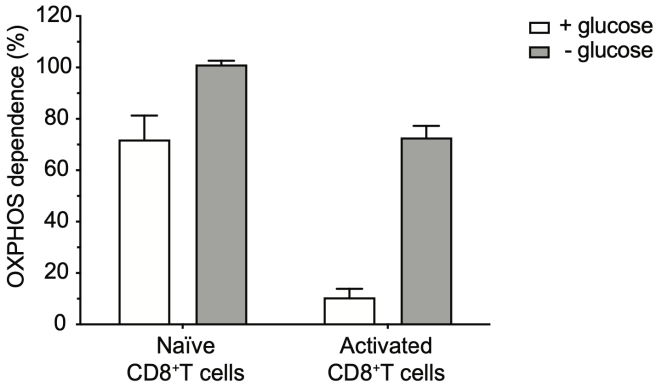
(A-B) BMDMs of Vav-iCre x Perceval$^{fl/fl}$ mice were activated for different periods with LPS+IFNγ (classically activated) or left untreated (unactivated). **(A)** Experimental setup. **(B)** OXPHOS and glucose dependencies were calculated at different time points. Results are representative 2 independent experiments. **(C-E)** BMDMs of Vav-iCre x Perceval$^{fl/fl}$ mice were activated 24 hours with LPS+IFNγ in the presence or absence of the iNOS inhibitor L-NIL. **(C)** Experimental setup. **(D)** Activated BMDM in the presence or absence of L-NIL were subjected

to time-resolved flow cytometry. After 2 minutes, oligomycin was added and acquisition was continued for an additional 10 minutes. The graph shows the geometric mean of the cellular ATP:ADP ratio normalized to the average value measured during the first two minutes of acquisition time (R_0). One representative experiment of two is shown. **(E)** OXPHOS and glucose dependencies are shown. Results were evaluated using an unpaired Student's t-test. Results are representative 2 independent experiments.



Appendix Figure S6: Role of glucose in metabolic adaptation of peritoneal macrophages upon OXPHOS blockade

Vav-iCre x Perceval$^{fl/fl}$ mice were injected i.p. with thioglycolate. Peritoneal macrophages were isolated 3 days later and immediately subjected to SPICE-Met imaging in the presence of Alexa 594-conjugated F4/80 Ab. Peritoneal macrophages were cultured with RPMI medium supplemented with or without glucose and pyruvate. Kinetic analysis of ATP:ADP ratios for single macrophages. Each line represents one cell.



Appendix Figure S7: Impact of culture media composition in energy metabolic profiling

Naïve or *in vitro* activated $CD8^+$ T cells from Lck-Cre x $Perceval^{fl/fl}$ mice were cultured for 1 hour in RPMI medium supplemented or not with glucose and subjected to SPICE-Met. OXPHOS dependence was calculated as $((R_{DMSO+H2O} - R_{oligo}) / (R_{DMSO+H2O} - R_{oligo+2\text{-}DG})) \times 100$.