

A) Flow cytometry data from one representative experiment showing the sorting strategy for Treg (CD8⁻CD25^{hi}CD127^{lo} cells), naïve (CD8⁻CD25^{lo}CD127^{hi}CD45RA⁺) and memory Tcon (CD8⁻CD25^{low}CD127^{hi}CD45RA⁻) population. B) Histograms show the expression of CD25, CD127, FOXP3 and CD45RA in purified naïve or memory Tcon and Treg after sorting.



A) Dot line graphs represent the absolute number of Treg after 24h of culture in absence (medium) or presence of 75, 150, 300 and 600 μ g/ml of HDL. Cells from two individuals were analyzed. B) to D): Absolute number of freshly separated, never cryopreserved, Treg (B), naïve (C) and memory (D) CD4⁺ T-cells cultured for 24h in absence or presence of HDL (300 μ g/ml). E) Representative example of flow cytometry analysis of Annexin V and 7AAD. Annexin V⁻ 7AAD⁻ cells were considered as non-apoptotic.



A) Representative histogram of HDL-Dil uptake after 4h in Treg (CD25⁺CD127^{Low}) naïve (CD25⁻CD127⁺CD45RA⁺) and memory (CD25⁻CD127⁺CD45RA⁻). Samples were analyzed by imaging flow cytometry. B) Representative confocal microscopy images showing HDL cellular localization. HDL-Dil (red), CD4 (green) and nuclear staining (blue). C) Representative flow cytometry staining of total cholesterol (Filipin III) in Treg cultured for 24h in presence or absence of HDL. D) Fold increase of filipin III MFI in Treg, naïve and memory Tcon cells cultured with HDL.



Bar figures show median (range) A-B) of HDL receptor expression, measured by qPCR (n=4) and C) LDL receptor expression, measured by flow cytometry in each subset (n=5).





Oxygen consumption rate (OCR) and extracellular acidification rate (ECAR) were measured in Treg (beadseparated CD4⁺CD25⁺ T cells) using a 96w-Seahorse in glucose-free medium (A and B) or in medium supplemented with glucose (C to F). A) ATP-coupled mitochondrial respiration in absence or presence of HDL alone or with ETX (ETX; 100 μ M). B) ECAR of Treg in presence of absence of HDL. C-F) Treg metabolism in response to HDL in presence of glucose: C) Basal respiration; D) ATP-coupled mitochondrial respiration; E) Maximal respiration; F) Spare respiratory capacity (c/a). Bar graphs show median and range of 4-5 independent experiments. Comparisons between groups were done using Wilcoxon tests.



Basal levels of ATP were determined in each subset by luminescence assay. Bar graphs show median and range of 4-5 independent experiments.