Expanded View Figures

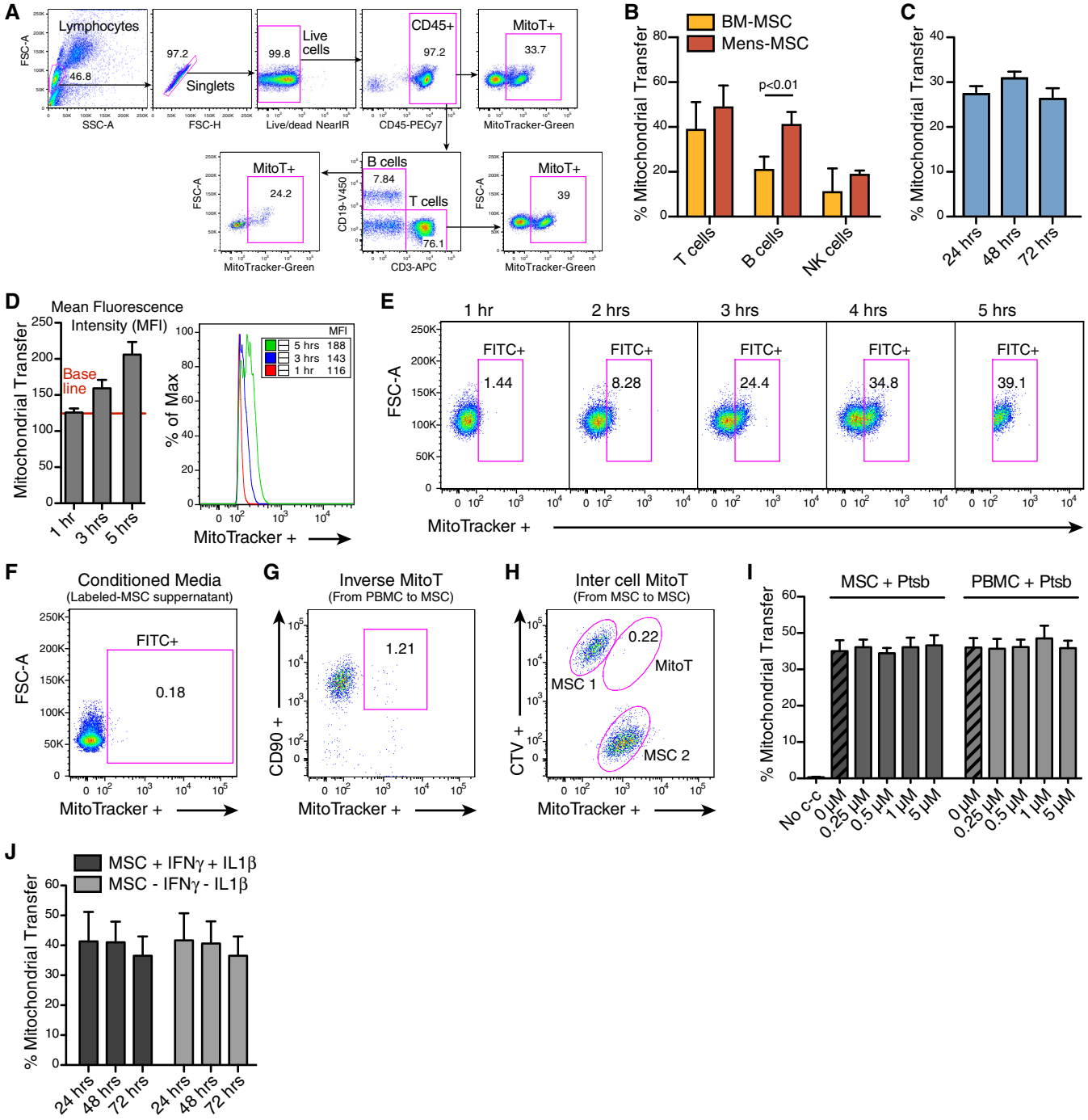

Figure EV1.

Figure EV1. Extended characterization of MitoT from MSC to lymphocytes.

A Representative gating strategy showing MitoT to different cell type populations analyzed.
B FACS analysis of MitoT on T $CD3^+$ (n = 3), B $CD19^+$ (n = 3) and NK $CD56^+$ (n = 2) cells after 24 h co-culture with MitoGreen labeled bone marrow (BM) or menstrual (Mens) MSCs (ratio 1:25).
C Percentage of MitoT on $CD45^+$ $CD3^+$ human cells after 1–3 days of co-culture with UC-MSCs (ratio 1:25) (n = 3 biological replicates).
D Mean fluorescence intensity (MFI) of T $CD3^+$ $MitoT^{pos}$ cells after short times of co-culture with UC-MSCs. Average bar graph (n = 3, left panel) and representative FACS plot (right panel).
E Representative FACS plots of MitoT on $CD45^+$ $CD3^+$ cells after short times of co-culture with MitoTracker Green labeled UC-MSCs.
F Representative FACS plot, out of five independent experiments, on human $CD3^+$ cells cultured for 24 h with MitoGreen labeled UC-MSC supernatant.
G Representative FACS analysis of MitoT from MitoTracker stained PBMC to $CD90^+$ UC-MSC, 24 h following the co-culture (ratio 1:25).
H Representative FACS analysis of MitoT from MitoGreen stained MSC to CellTrace Violet (CTV) labeled MSC, after 24 h co-culture (ratio 1:1).
I Percentage of MitoT on $CD3^+$ T cells from UC-MSCs or PBMCs pre-treated with increasing concentrations of the anti-oxidant Pterostilbene (Ptsb), after 4 h co-culture (n = 2 biological replicates).
J Percentage of MitoT on $CD45^+$ $CD3^+$ human cells after 1–3 days of co-culture with UC-MSCs (ratio 1:25) with or without pro-inflammatory licensing cytokines (n = 3 biological replicates).

Data information: All graphs show mean ± SEM and statistical analysis by Student's t-test.

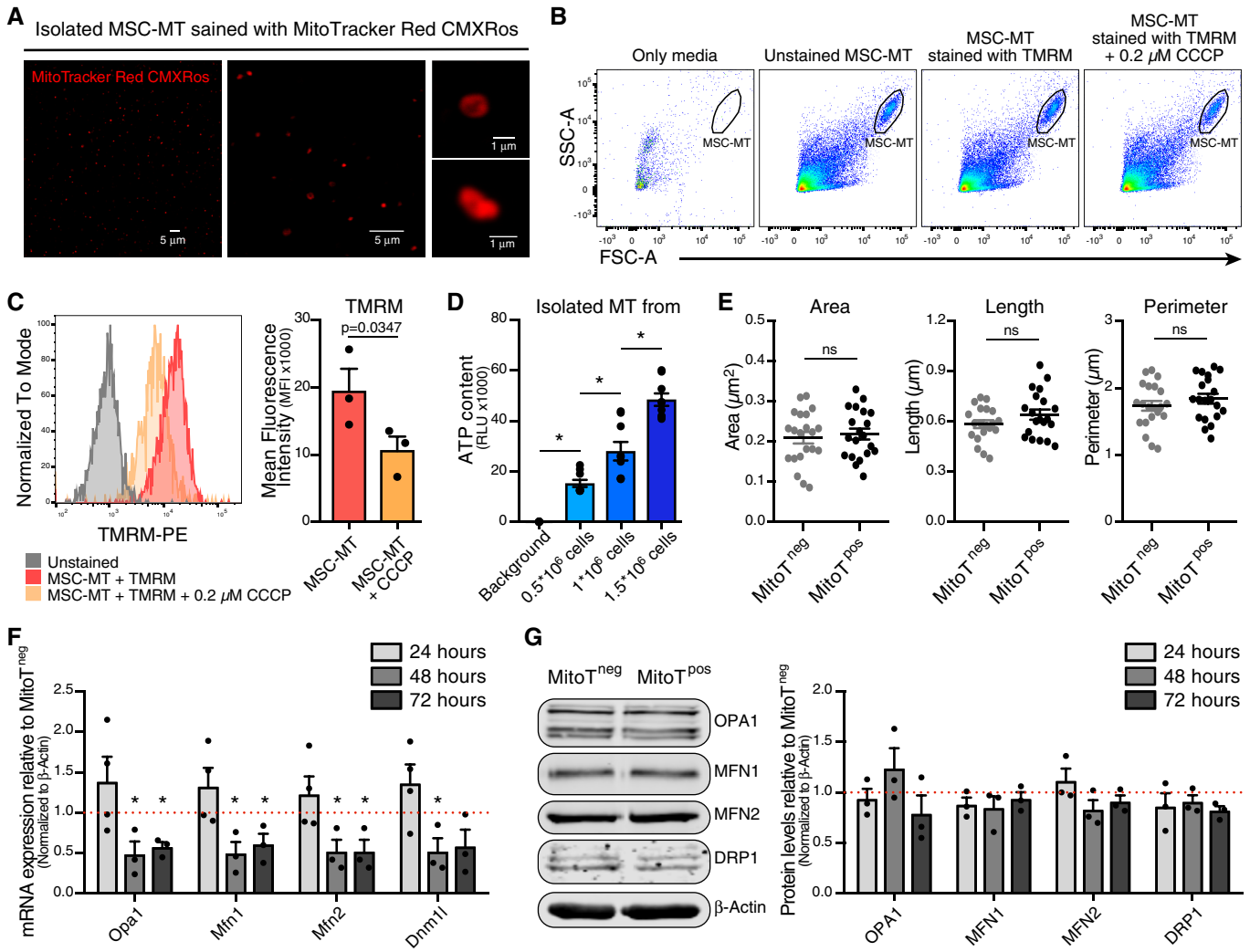

Figure EV2. Characterization of MSC-derived mitochondria activity and fate. Functional assays on isolated MT.

A Representative confocal microscopy of isolated MSC-MT stained with MitoTracker Red CMXRos at different magnifications (40× left and middle panel, and 63× right panels).

B Representative gating strategy showing isolated mitochondria from 1 × 10^6 MSCs per condition by FACS.

C Mean fluorescence intensity (MFI) of TMRM dye on unstained MSC-MT, MSC-MT stained with TMRM or MSC-MT stained with TMRM previously treated with 0.2 µM of CCCP (carbonyl cyanide 3-chlorophenylhydrazone). Representative FACS plot (left panel) and average of MFI (n = 3 biological replicates, with three different UC-MSCs) (right panel).

D ATP production of isolated MSC-MT from increasing amounts of MSC cells (n = 3 biological replicates run in triplicates). Graph shows mean ± SEM and statistical analysis by one-way ANOVA with Tukey's post-test.

E Analysis of mitochondrial morphology parameters of human MT in sorted $CD3^+$ $MitoT^{neg}$ and $MitoT^{pos}$ cells after Mitoception with UC-MSCs, by transmission electron microscopy (n = 20 independent cells analyzed per group).

F qRT-PCR analysis of genes related to mitochondrial fusion/fission in FACS-sorted $CD3^+$ $MitoT^{neg}$ and $CD3^+$ $MitoT^{pos}$ cells from healthy human PBMCs (n = 3 biological replicates, with three different donors of PBMCs), after 24, 48 and 72 h post Mitoception. The graph depicts fold expression in $CD3^+$ $MitoT^{pos}$ cells relative to $CD3^+$ $MitoT^{neg}$ cells, which are set at 1 (red dotted line).

G Western Blot analysis of protein levels related to mitochondrial fusion/fission in FACS-sorted $CD3^+$ $MitoT^{neg}$ and $CD3^+$ $MitoT^{pos}$ cells from healthy human PBMCs. Representative blots at 24 h post Mitoception (left panel) and bar graph showing 24, 48 and 72 h post Mitoception (right panel) (n = 3 biological replicates, with three different donors of PBMCs). The graph depicts fold expression in $CD3^+$ $MitoT^{pos}$ cells relative to $CD3^+$ $MitoT^{neg}$ cells, which are set at 1.

Data information: Graphs show mean ± SEM and statistical analysis by Student's t-test.

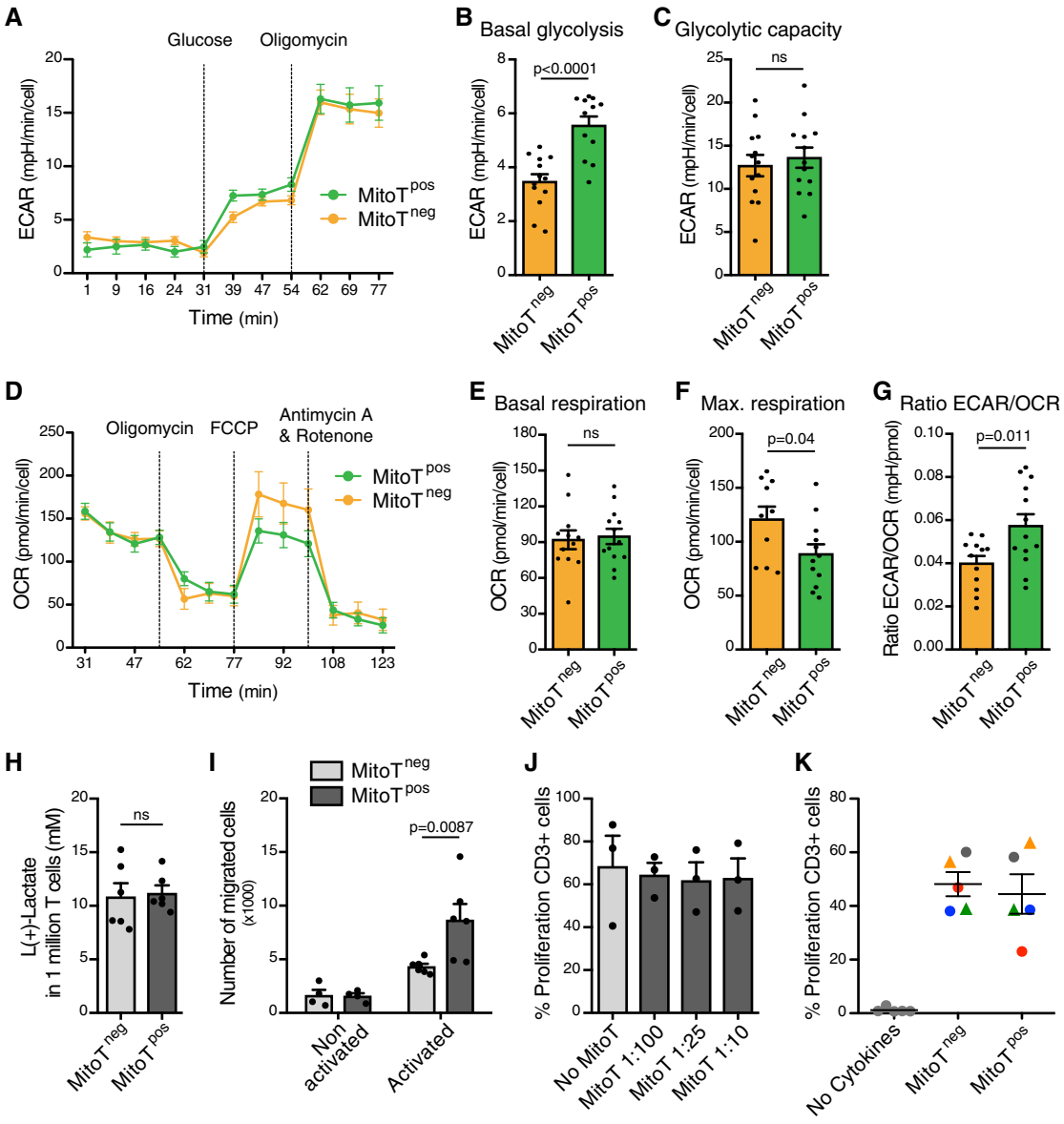

Figure EV3. Functional analysis of MitoT on CD3⁺ T cells. Metabolic changes, transmigration capacity and proliferation analysis on MitoT^{pos} cells.

A–C Extracellular Acidification Rate (ECAR) analysis measured in a Seahorse XFp extracellular flux analyzer in FACS-sorted MitoT^{pos} and MitoT^{neg} CD3$^+$ T cells mitocepted with UC-MSC mitochondria (n = 3 biological replicates, ran in quadruplicates).

D–F Oxygen Consumption Rate (OCR) analysis measured in a Seahorse XFp extracellular flux analyzer in FACS-sorted MitoT^{pos} and MitoT^{neg} CD3$^+$ T cells mitocepted with UC-MSC mitochondria (n = 3 biological replicates, ran in quadruplicates).

G Glycolysis/OXPHOS ratio on FACS-sorted MitoT^{pos} and MitoT^{neg} CD3$^+$ T cells mitocepted with UC-MSC-MT (n = 3 biological replicates, ran in quadruplicates).

H L(+)-Lactate concentrations from supernatants of sorted CD3$^+$ MitoT^{neg} and MitoT^{pos} T cells, previously mitocepted with UC-MSC-MT (n = 2 biological replicates, each one in triplicate).

I Number of migrated CD4$^+$ MitoT^{neg} and MitoT^{pos} sorted T cells from 100.000 cells seeded on media non-activated or activated with anti-CD3 and IL-2 for 3 days (n = 3 biological replicates, in duplicates).

J Percentage of CD3$^+$ T cell proliferation after increasing Mitoception ratios, quantified by measuring the corresponding decrease in CellTrace Violet (CTV) proliferation dye intensity by flow cytometry (n = 3 biological replicates with three different PBMC donors).

K Percentage of proliferation of FACS-sorted MitoT^{neg} and MitoT^{pos} CD3$^+$ cells, after 5 days in culture with anti-CD3/CD28 beads (1/20) and IL-2 (50 U/ml) in the presence or absence of PHA (15 µg/ml) (round or triangle dots respectively) (n = 5 biological replicates with five different PBMC donors).

Data information: All graphs show mean ± SEM and statistical analysis by Student's t-test.

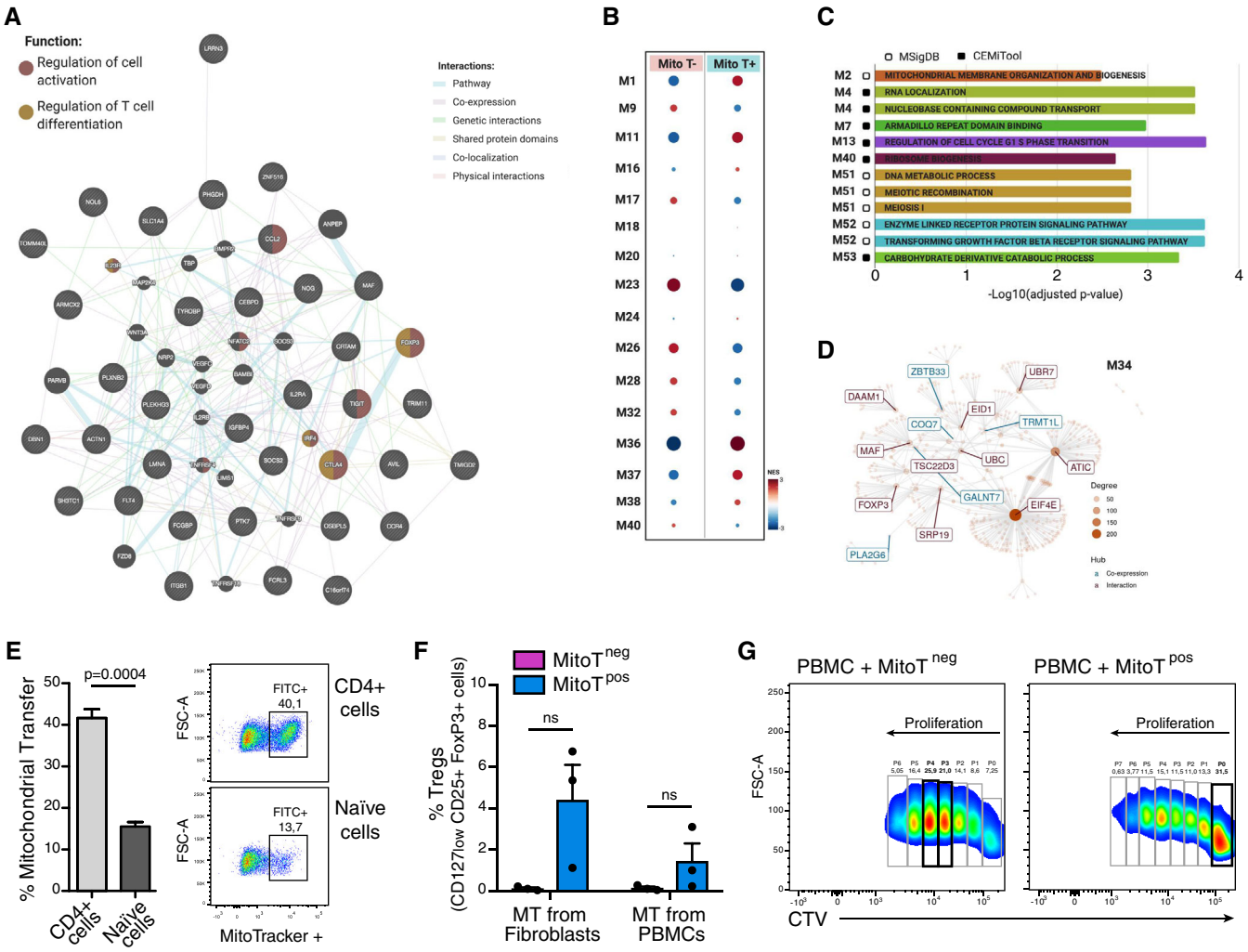

Figure EV4. MitoT-driven Treg differentiation. Analysis of gene set enrichment of RNA-Seq data.

A Interaction network from FOXP3-associated DE coding genes in sorted MitoTposCD3$^+$ compared to MitoTnegCD3$^+$ T cells (n = 4 different donors of PBMCs), using Genemania.

B Gene Set Enrichment Analysis (GSEA) of co-expressed modules identified by webCEMiTool showing, the enriched module activity between sorted MitoTpos and MitoTnegCD3$^+$ T cells (from 4 different donors of PBMCs).

C Top over-represented functional categories according to webCEMiTool and MSigDB, identified co-expressed gene modules.

D Gene co-expression/interaction networks of module 34 (M34) displaying FOXP3 as hub gene.

E FACS analysis of MitoT on CD4$^+$ and naïve (CD4$^+$ CD45RA$^+$ CD45RO$^-$) T cells after Mitoception with UC-MSC mitochondria, represented as bar graph (left panel, n = 3 biological replicates) and representative FACS plot (right panel). Graph shows mean ± SEM and statistical analysis by Student's t-test.

F FACS analysis of Treg induction after 5–7 days differentiation in plain media of FACS-sorted CD4$^+$ naïve MitoTneg and MitoTpos cells previously mitocepted with Fibroblasts or same donor PBMCs mitochondria (n = 3 biological replicates for each condition).

G Representative proliferation FACS plots of CTV-stained PBMC co-cultured with MitoTneg or MitoTpos cells (ratio 1:1) after 5–7 days of CD4$^+$ naïve cell activation with differentiation media.

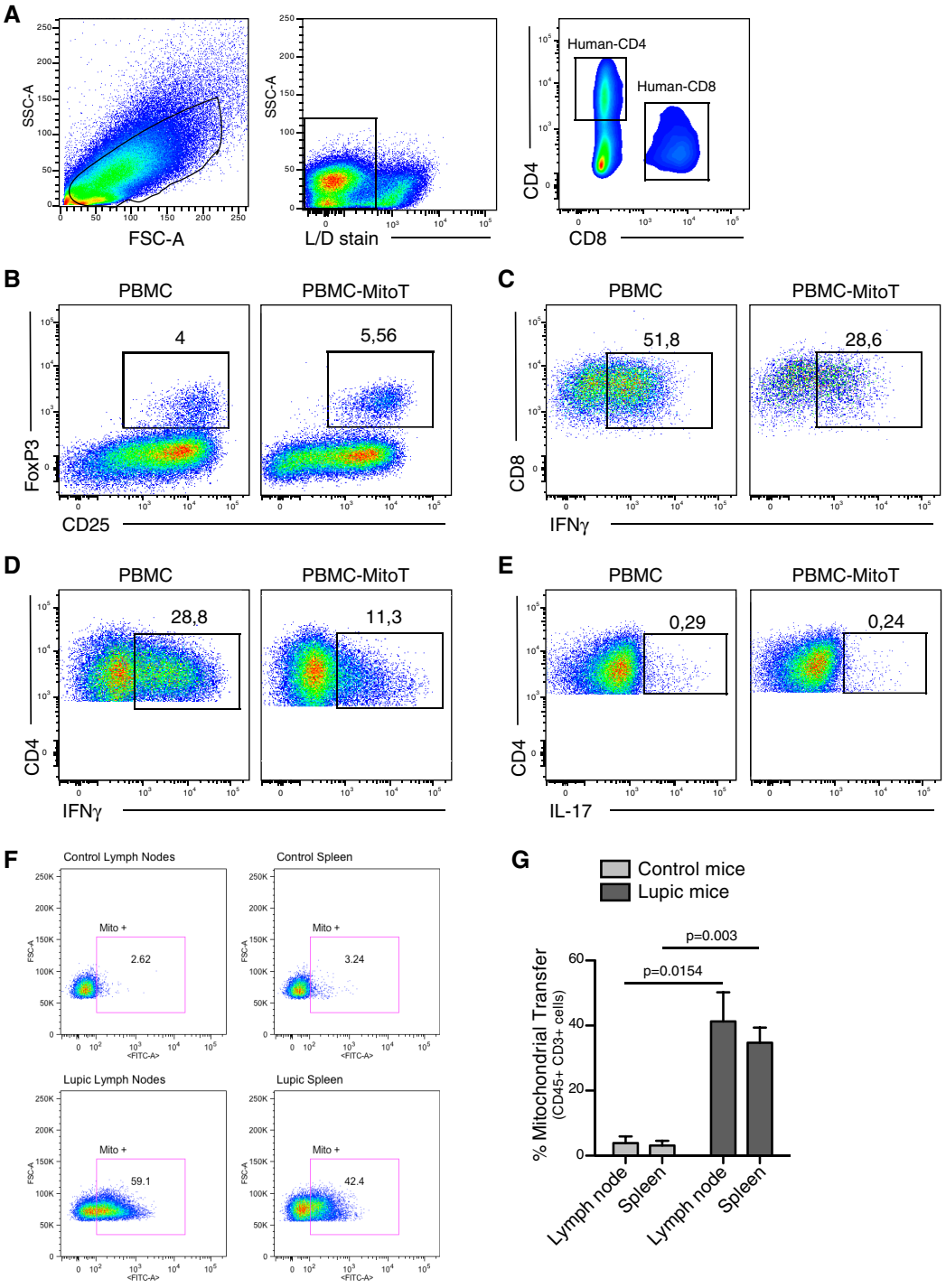

Figure EV5. Efficient *ex vivo* MitoT in inflammatory T cells isolated from a SLE mouse model.

A–E Representative gating strategy used for the *in vivo* analyses. Representative FACS plots of (B) Tregs (FoxP3$^+$ CD25$^+$), (C) T cytotoxic (CD8$^+$ IFNg$^+$), (D) Thelper 1 (CD4$^+$ IFNg$^+$) and (E) Thelper 17 (CD4$^+$ IL17$^+$) populations cell engraftment in mouse spleen following transplantation of mitocepted or control PBMC cells.

F Representative FACS plot, out of 3 independent experiments, on MRL/MpJ/Fas mouse CD45$^+$ CD3$^+$ cells from spleen or lymph nodes co-cultured for 24 h with MitoGreen labeled UC-MSCs.

G FACS analysis of *ex vivo* MitoT from labeled UC-MSCs to MRL/MpJ/Fas (lupic) or MRL/MpJ (control) mouse CD45$^+$ CD3$^+$ cells from spleen or lymph nodes (n = 3 biological replicates). Graph shows mean ± SEM and statistical analysis by Student's t-test.