



**Figure S3. Assessment of T cell phenotype and metabolism following pharmacological or genetic enhancement of mitochondrial fusion (Related to Figure 3).**

(A-E) IL-2 T<sub>E</sub> and IL-15 T<sub>M</sub> cells generated from OT-I mice were treated with DMSO control or M1+Mdivi-1. (A) ECAR of indicated cells at baseline and after PMA and ionomycin (PMA+iono) stimulation, oligomycin (Oligo), FCCP, and rotenone plus antimycin A (R+A). (B) qPCR analysis of relative mitochondrial DNA (mtDNA) to nuclear DNA (nDNA) ratios of indicated cells. (C) ECAR (left) and OCR/ECAR ratios (right) of indicated cells under basal conditions. (D) Histograms of membrane potential (CMxROS, TMRM) and mitochondrial ROS (MitoSOX) using indicated fluorescent dyes and (E) KLRG1, CD127, CCR7, and CD25 surface marker expression of indicated cells analyzed by flow cytometry. (F-H) OT-I IL-2 T<sub>E</sub> cells were activated and transduced with empty vector (Control), Mfn1, Mfn2, or Opa1 expressing retrovirus. (F) ECAR, OCR/ECAR, and SRC analyzed by Seahorse EFA, (G) KLRG1, CD127, CCR7, CD25 and PD-1 surface marker expression assessed by flow cytometry, and (H) gene expression analysis by qPCR. (A-G) Data are shown as mean ± SEM and are representative or (B-C, F) combined from 2-3 experiments, not significant (ns), \*\*p<0.001, \*\*\*p<0.0001.