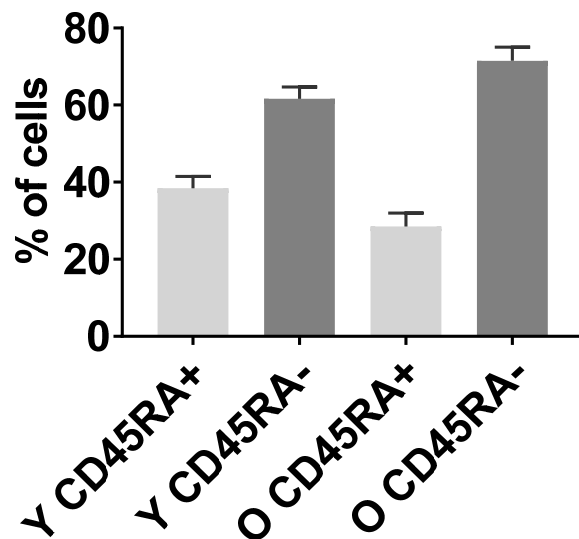
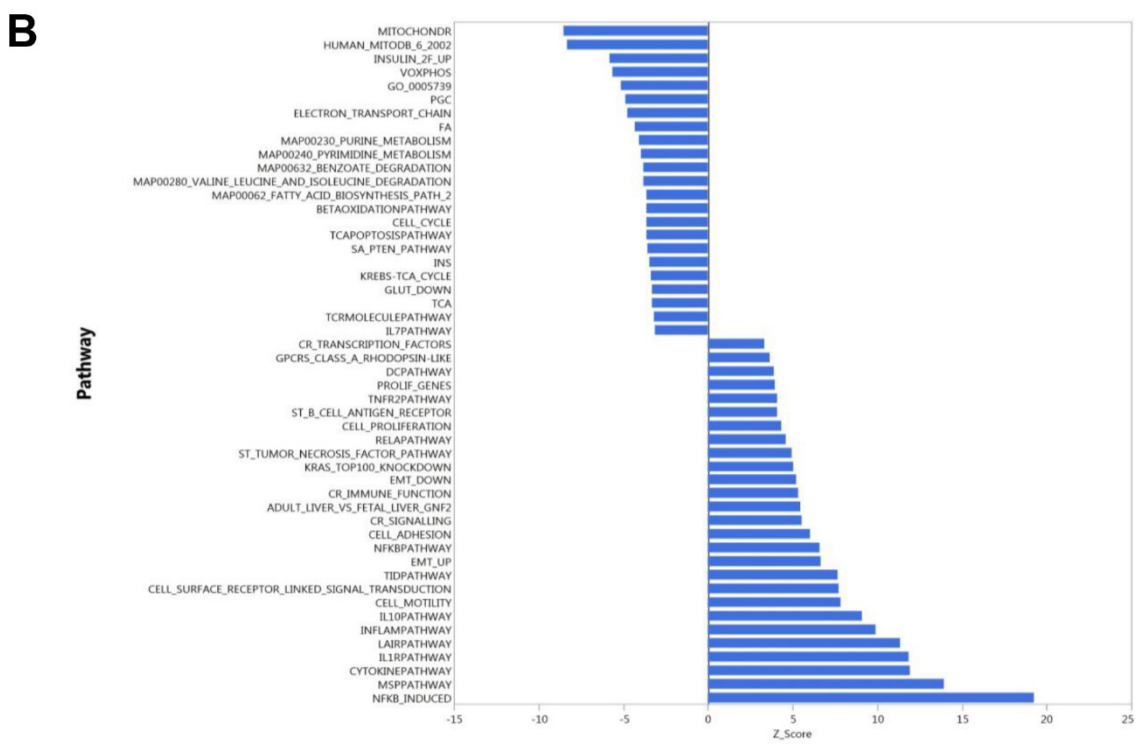
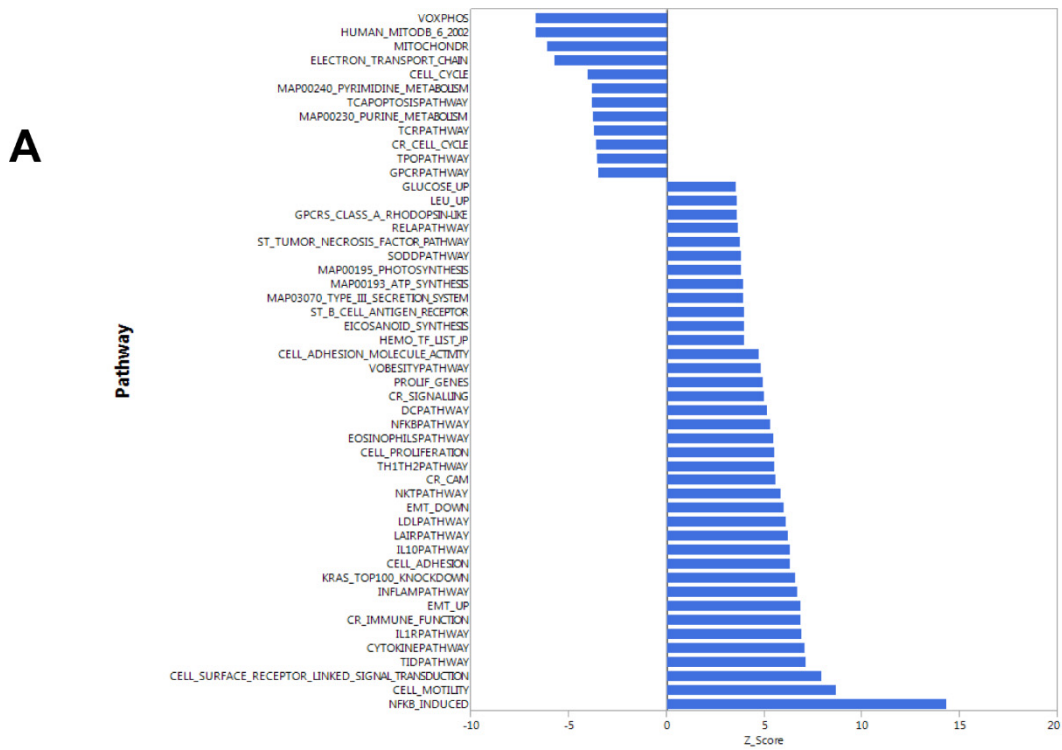


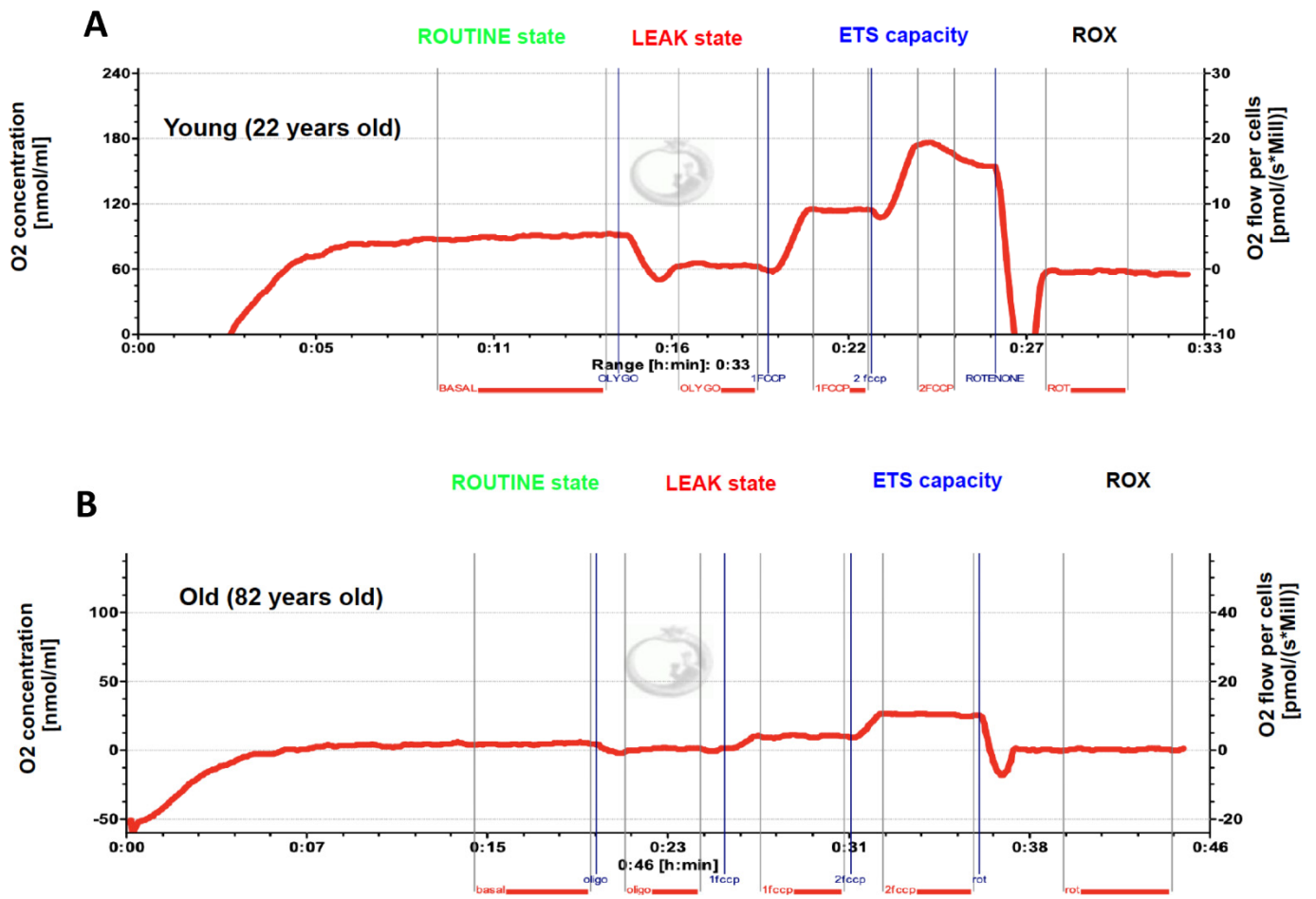
SUPPLEMENTARY FIGURES



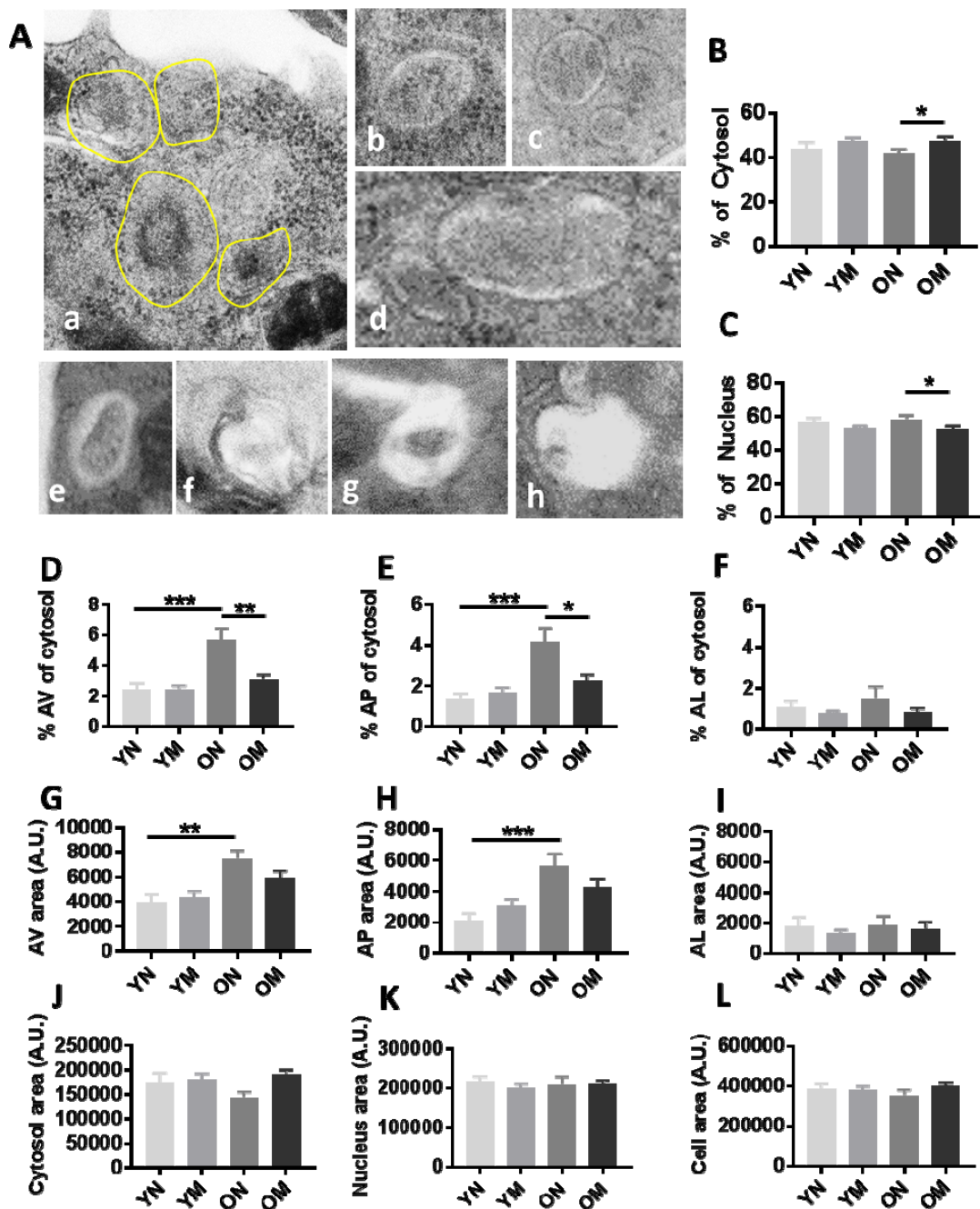
Supplementary Figure 1. FACS profile of CD4⁺T cells in iTRAQ samples. The percentage of naïve CD4⁺T cells analyzed based on CD45RA⁺ expression by flow cytometry. The expression of CD45RA⁺ in older (O) donors 70-83 years-old versus younger (Y) donors 21-34 years-old was comparable ($p=0.1540$) in the iTRAQ experiment sample groups. *P*-value was calculated by Student's t-test using GraphPad PRISM 7 software. Error bars reflect the standard error of the mean (\pm SEM). N = 4 young, 18 old donors..



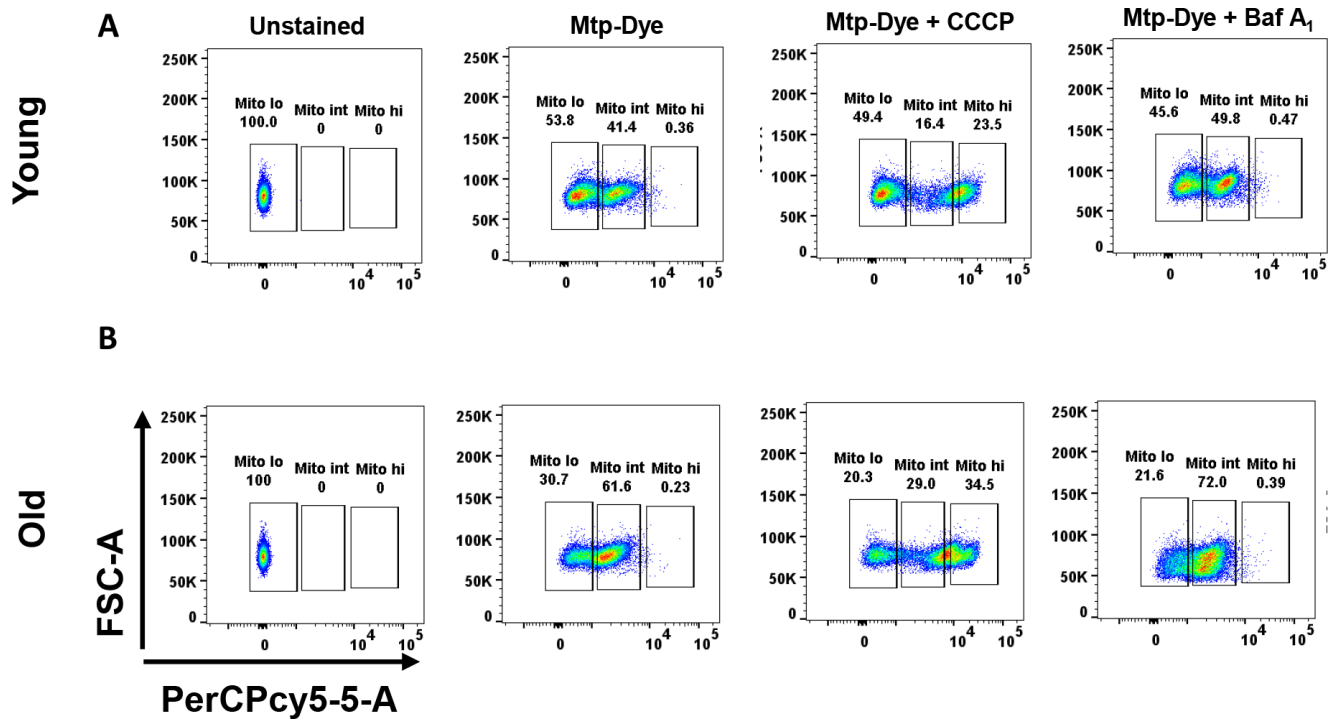
Supplementary Figure 2. (A) Gene expression analysis showing top 50 up-and down-regulated biological pathways in CD4⁺T cells of older (70 to 80 years old) as compared to younger (20 to 30 years-old) women. N = 3 young, 6 old donors. (B) Gene expression analysis showing top 50 up-and down-regulated biological pathways in CD4⁺T cells of older (70 to 80 years-old) compared to younger (20 to 30 years-old) women and men. N = 8 young, 25 old donors.



Supplementary Figure 3. Representative mitochondrial respiration measurements using OROBOROS from a young (A) and an old (B) donor.



Supplementary Figure 4. (A) Examples of autophagic vacuolar compartments in CD4⁺T cells from older individuals. (a) Accumulation of autophagosomes, (b–e) autophagosomes containing sequestered mitochondria, and (f–h) examples of autolysosomes at different stages of maturation. (B) In older (O) donors, cytosol percentage was higher in memory (M) CD4⁺T cells than naive (N) CD4⁺T cells (**p*=0.0458). There was no difference in younger (Y) donors. (C) Accordingly in older donors, the nucleus percentage was higher in naive CD4⁺T cells compared to memory cells (**p*=0.0458). (D) The autophagic vacuoles (AV) and also (E) autophagosomes (AP) percentage in the cytosol were higher in naive CD4⁺T cells from older compared to younger donors (***p*=0.0004, ****p*=0.0002, respectively). Also the AV and AP percentage in the cytosol were higher in naive CD4⁺T cells compared to memory CD4⁺T cells in older donors (***p*=0.0027, **p*=0.0116, respectively). There was no difference in younger donors. (F) There were no significant differences in AL percentage in either cell type or between young and older donors. (G) AV area and (H) AP area were higher in naive CD4⁺T cells from older donors than naive CD4⁺T cells from younger donors (***p*=0.0014, ****p*=0.0005, respectively). (I–L) AL, cytosol, nucleus, and cell area were similar in naive and memory CD4⁺T cells from older and younger participants. All *p*-values were calculated by Student's *t*-test using GraphPad PRISM 7 software. Error bars reflect the standard error of the mean (±SEM). (G–L) Arbitrary Unit (A.U.). N = 5 young, 4 old donors.



Supplementary Figure 5. Dot plot examples from (A) one young and (B) one old donor showing the percentage of gated cells for four treatment conditions (Column 1: Unstained, Column 2: Mtp-Dye, Column 3: Mtp-Dye with CCCP treatment, Column 4: Mtp-Dye with bafilomycin A₁(Baf A₁) treatment. Percentages are shown for gates corresponding to low ('lo'), intermediate ('int'), and high ('hi') fluorescence. N = 12 young, 12 old donors.