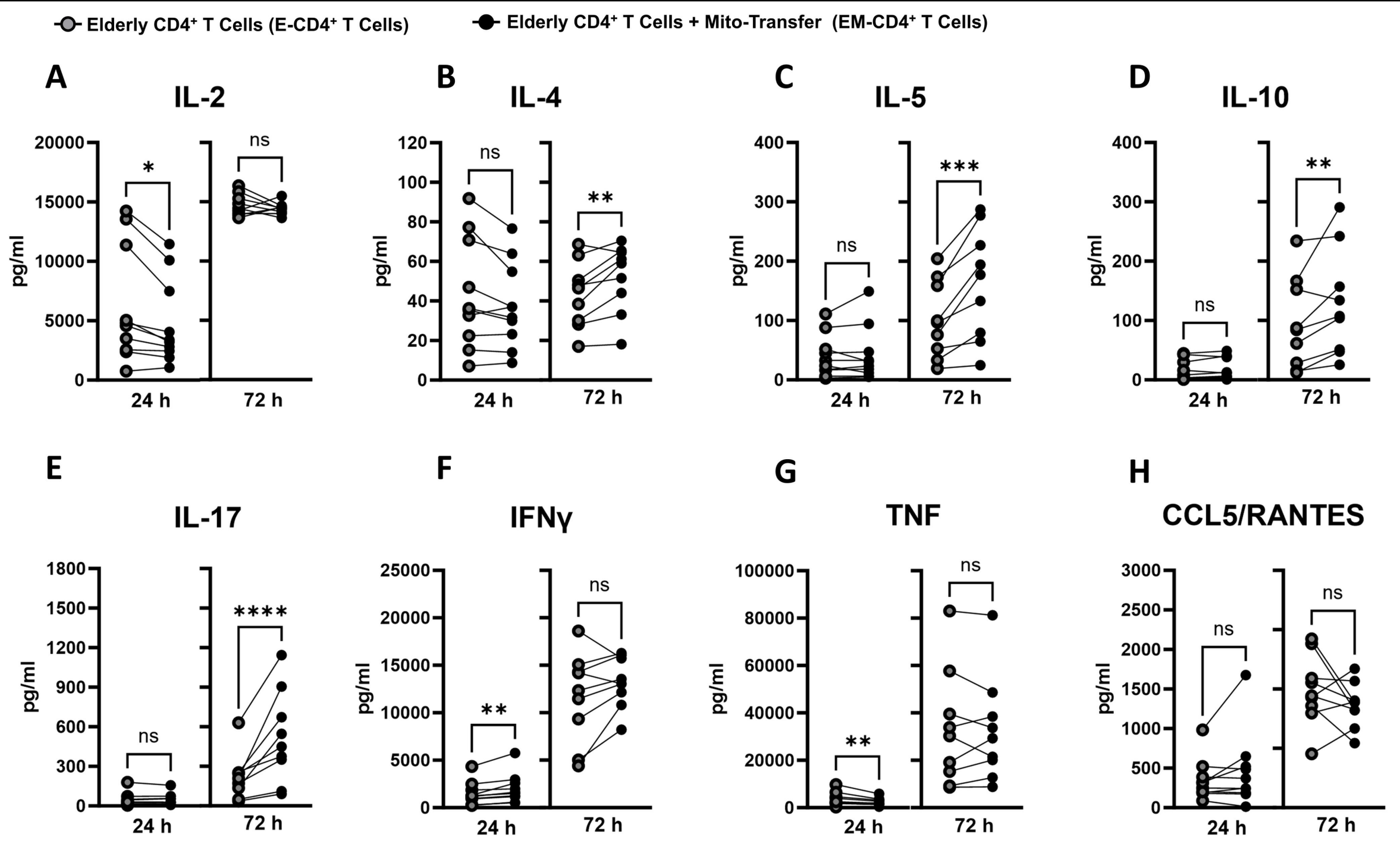

Elderly CD4+ T Cells (E-CD4+ T Cells)
Elderly CD4+ T Cells + Mito-Transfer (EM-CD4+ T Cells)
A
IL-2
pg/ml
20000
15000
10000
5000
0
*
ns
24 h
72 h
B
IL-4
pg/ml
120
100
80
60
40
20
0
ns
**
24 h
72 h
C
IL-5
pg/ml
400
300
200
100
0
ns
***
24 h
72 h
D
IL-10
pg/ml
400
300
200
100
0
ns
**
24 h
72 h
E
IL-17
pg/ml
1800
1500
1200
900
600
300
0
ns
****
24 h
72 h
F
IFNγ
pg/ml
25000
20000
15000
10000
5000
0
**
ns
24 h
72 h
G
TNF
pg/ml
100000
80000
60000
40000
20000
0
**
ns
24 h
72 h
H
CCL5/RANTES
pg/ml
3000
2500
2000
1500
1000
500
0
ns
ns
24 h
72 h

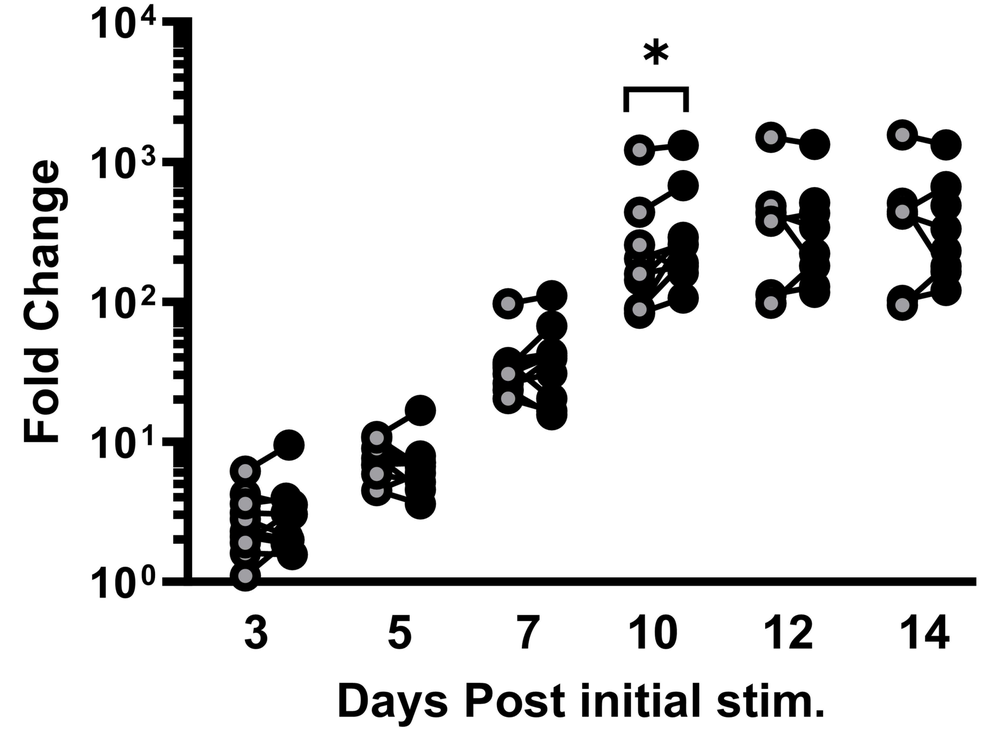
Supp. Fig. 1. Mito-transfer alters cytokine production of CD4+ T cells in aged humans. CD4+ T cells from aged humans with or without mito-transfer were stimulated with PMA/Ionomycin. After 24h and 72h stimulation with PMA/Ionomycin, the supernatants were examined by Luminex array for cytokines produced. 9-10 biological replicates per group with $p \leq 0.05$ = *, $p \leq 0.01$ = **, or $p \leq 0.001$ = *** using paired Student's *t*-test.

Elderly CD4+ T Cells (E-CD4+ T Cells)

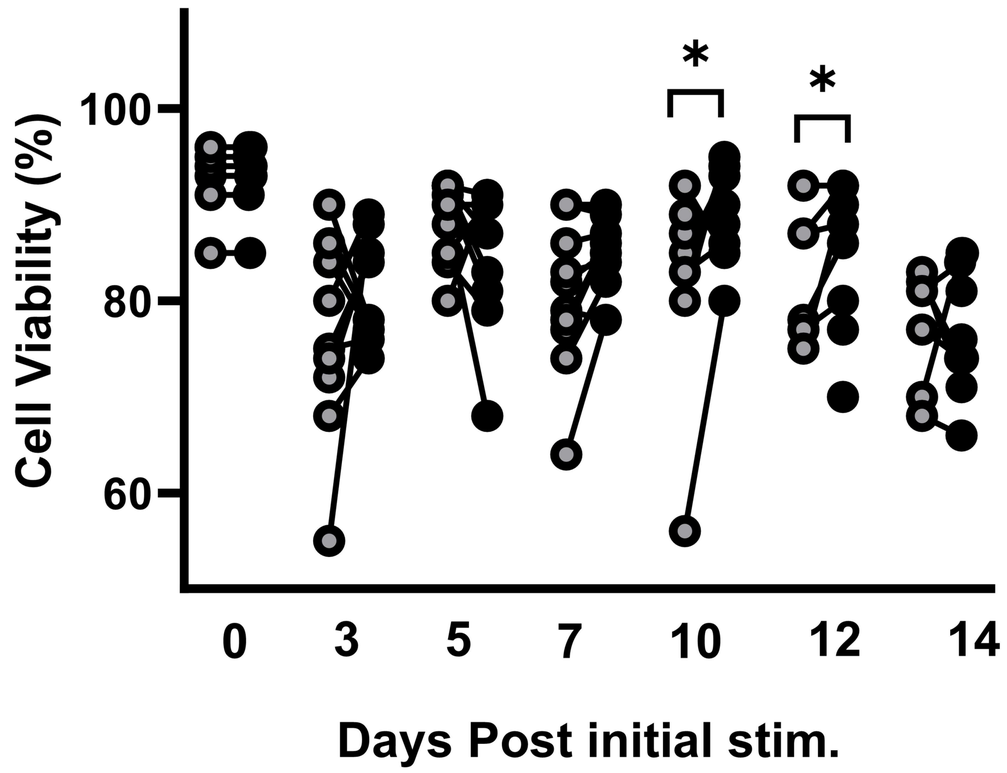
Elderly CD4+ T Cells + Mito-Transfer (EM-CD4+ T Cells)

Single Stim.

A

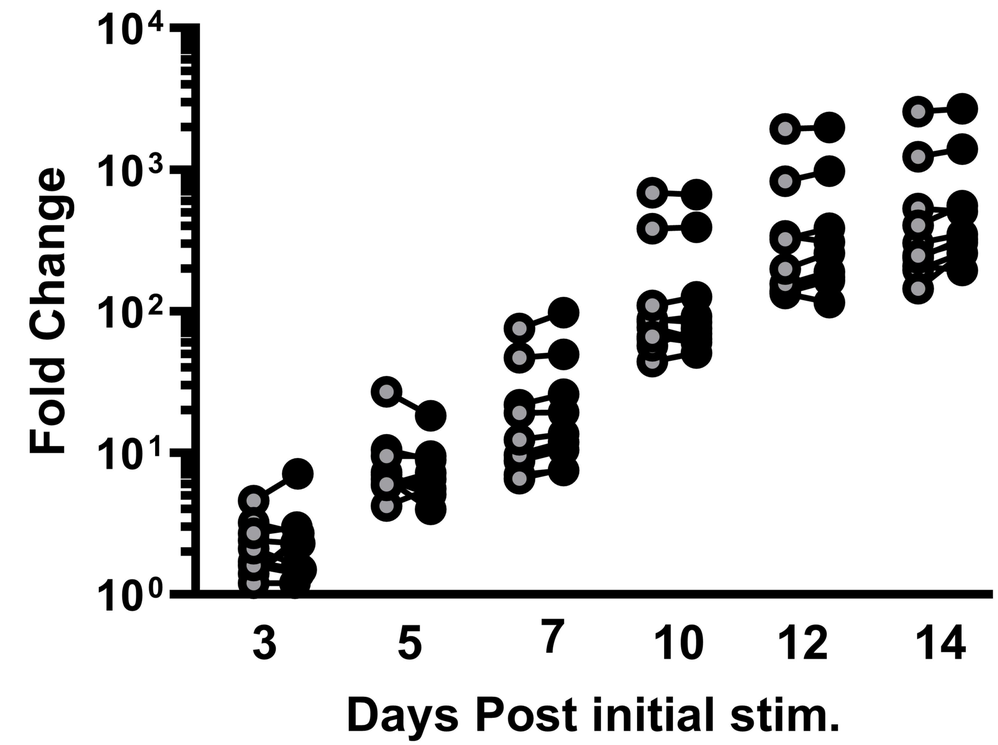


B

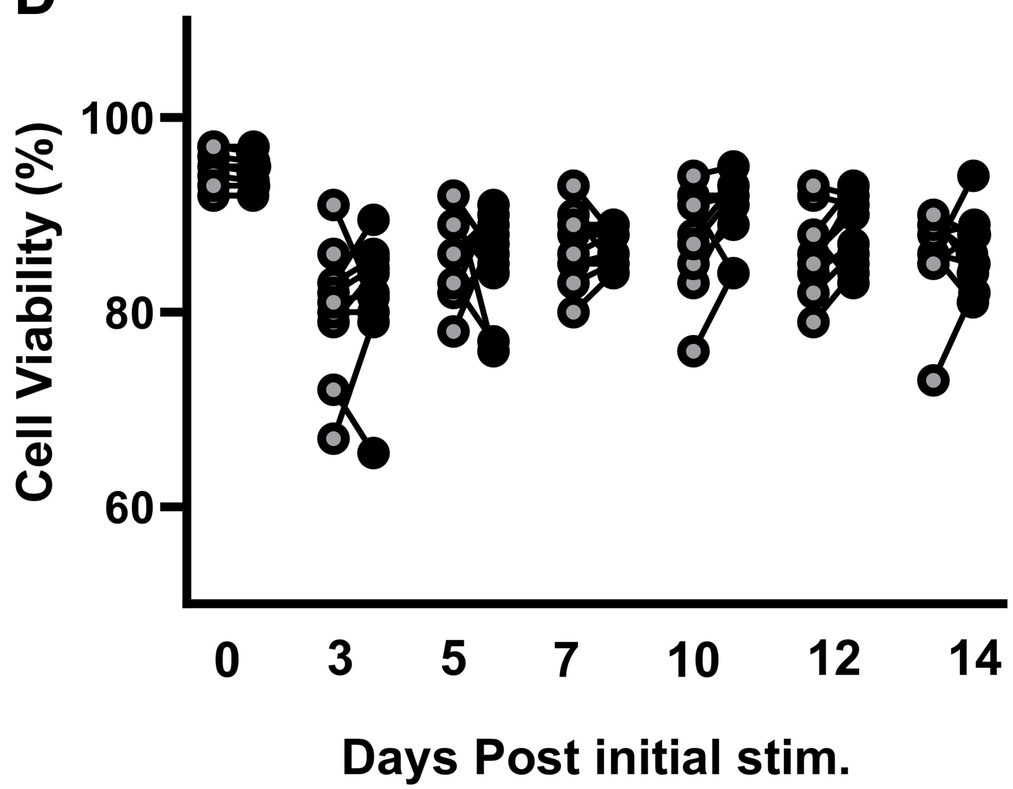


Cont. Stim.

C



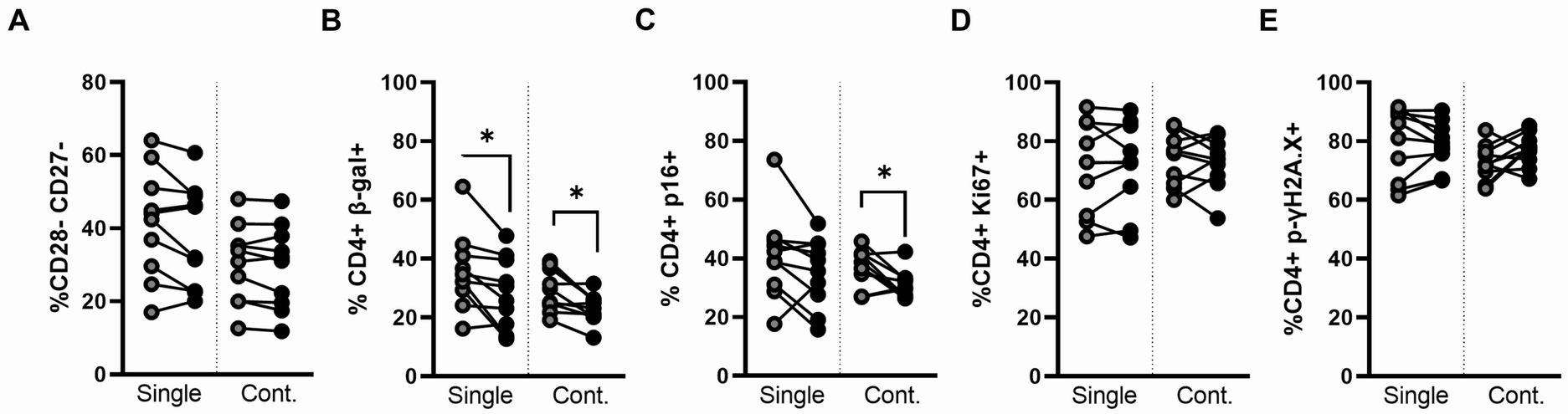
D



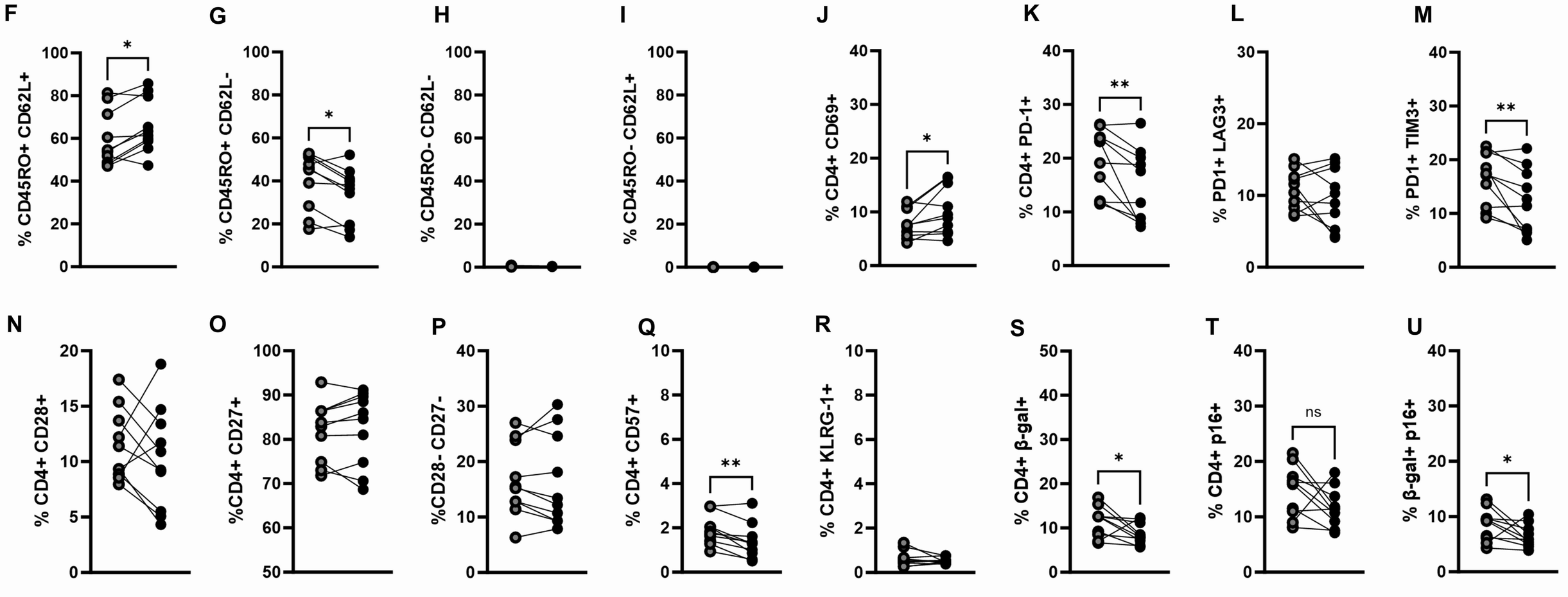
Supp. Fig 2. Impact of mito-transfer on elderly T cell proliferation and viability. Elderly CD4 T cells with or without mito-transfer were activated via surface cross-linking (CD3/CD28). CD4 T cells either received a single (single) or continuous (cont.) stimulation. **A)** Fold change and **B)** viability of CD4+ T cells with or without mito-transfer after single stimulation. **C)** Fold change and **D)** viability of CD4+ T cells with or without mito-transfer with continuous stimulation. 10 biological replicates per group, with $p \leq 0.05$ = *, $p \leq 0.01$ = **, using paired Student's *t*-test.

Elderly CD4+ T Cells (E-CD4+ T Cells) Elderly CD4+ T Cells + Mito-Transfer (EM-CD4+ T Cells)

Day 7 - Single & Continuous Stimulation



Day 14 - Continuous Stimulation



Supp Fig 3. Mito-transfer improves T cell activation and reduces human T cell exhaustion and senescence. Donor mitochondria isolated from primary human neonatal dermal fibroblasts were transplanted into CD4+ T cells from elderly humans. Elderly $CD4^+$ T cells with or without mito-transfer were activated via surface cross-linking (CD3/CD28), T cells either received a single (single) or continuous (cont.) stimulation. The percentages of **A)** CD28- CD27, **B)** β-gal+, **C)** p16+, **D)** Ki67+, and **E**) γH2A.X+ human CD4+ cells at 7 days after intial activation with CD3/CD28/CD2. The percentages of **F**) CD45RO+ CD62L+, **G**) CD45RO+ CD62L , **H**) CD45RO- CD62L-, **I**) CD45RO- CD62L-, **J**) CD69+, **K**) PD-1+, **L**) PD-1+ LAG3+, **M**) PD-1+ TIM3+, **N**) CD28+, **O**) CD27+, **P**) CD28- CD27 , **Q**) CD57+, **R**) KLRG1+, and **S**) β-Gal+, **T**) p16+, **U**) β-gal+ p16+ human CD4+ T cells at 14 days after continuous stimulation with CD3/CD28/CD2. 10 biological replicates per group, with $p \leq 0.05$ = *, $p \leq 0.01$ = **, using paired Student's t-test.