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Supplemental information

Artificial antigen-presenting cell system reveals CD28's role in modulating T cell functions during human immunodeficiency virus infection

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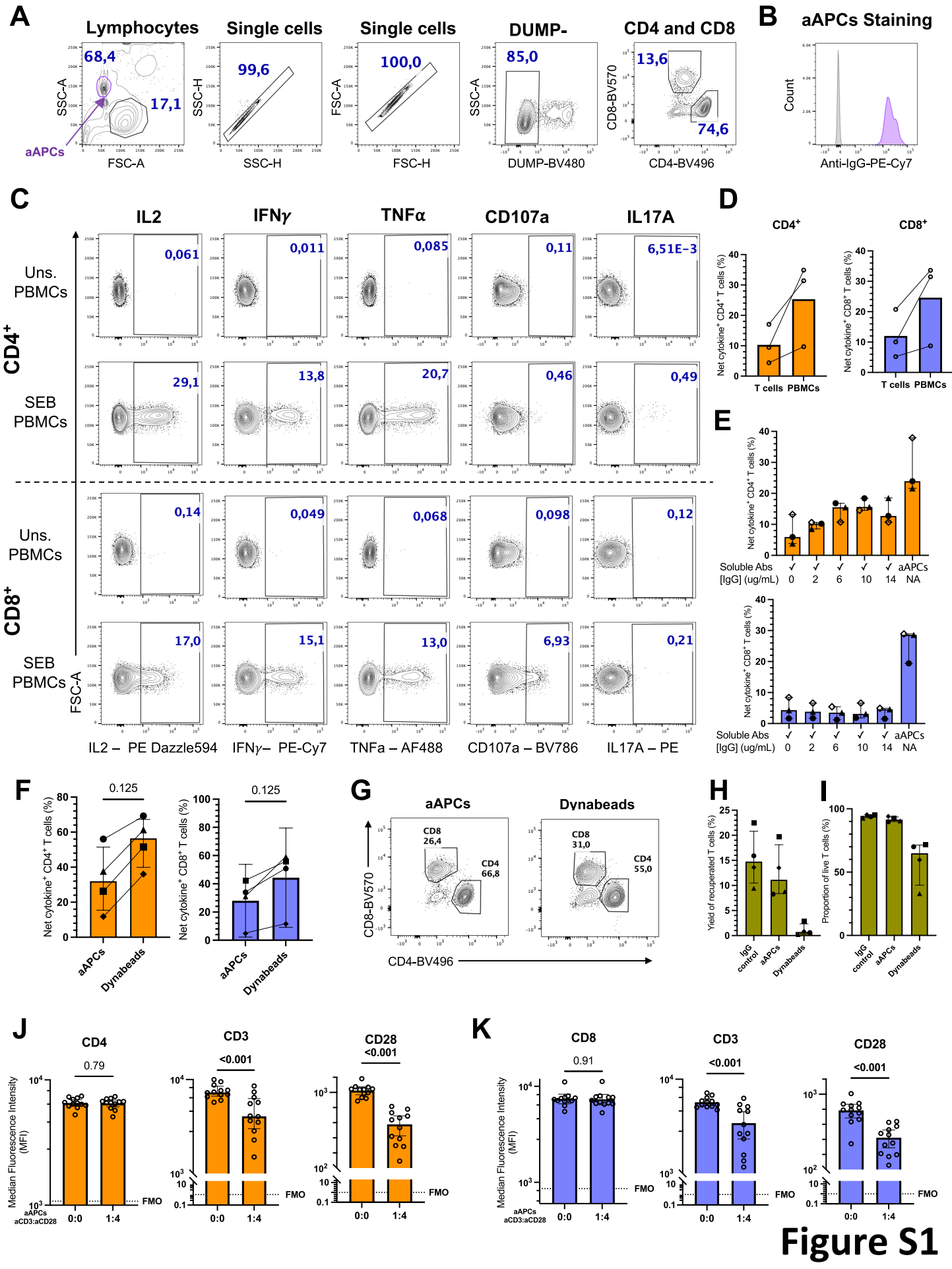
A
Lymphocytes
Single cells
Single cells
DUMP-
CD4 and CD8
68,4
17,1
aAPCs
SSC-A
FSC-A
99,6
SSC-H
SSC-H
100,0
FSC-A
FSC-H
85,0
SSC-A
DUMP-BV480
13,6
74,6
CD8-BV570
CD4-BV496
B
aAPCs Staining
Count
Anti-IgG-PE-Cy7
C
IL2
IFNγ
TNFα
CD107a
IL17A
Uns. PBMCs
SEB PBMCs
CD4+
CD8+
0,061
0,011
0,085
0,11
6,51E-3
29,1
13,8
20,7
0,46
0,49
0,14
0,049
0,068
0,098
0,12
17,0
15,1
13,0
6,93
0,21
FSC-A
IL2 – PE Dazzle594
IFNγ– PE-Cy7
TNFa – AF488
CD107a – BV786
IL17A – PE
D
CD4+
CD8+
Net cytokine+ CD4+ T cells (%)
Net cytokine+ CD8+ T cells (%)
T cells
PBMCs
E
Net cytokine+ CD4+ T cells (%)
Net cytokine+ CD8+ T cells (%)
Soluble Abs
[IgG] (ug/mL)
0
2
6
10
14
aAPCs
NA
F
0.125
Net cytokine+ CD4+ T cells (%)
Net cytokine+ CD8+ T cells (%)
aAPCs
Dynabeads
G
aAPCs
Dynabeads
CD8
26,4
CD4
66,8
CD8
31,0
CD4
55,0
CD8-BV570
CD4-BV496
H
Yield of recuperated T cells (%)
IgG control
aAPCs
Dynabeads
I
Proportion of live T cells (%)
IgG control
aAPCs
Dynabeads
J
CD4
CD3
CD28
0.79
<0.001
<0.001
Median Fluorescence Intensity (MFI)
FMO
aAPCs aCD3:aCD28
0:0
1:4
K
CD8
CD3
CD28
0.91
<0.001
<0.001
Median Fluorescence Intensity (MFI)
FMO
aAPCs aCD3:aCD28
0:0
1:4

Figure S1

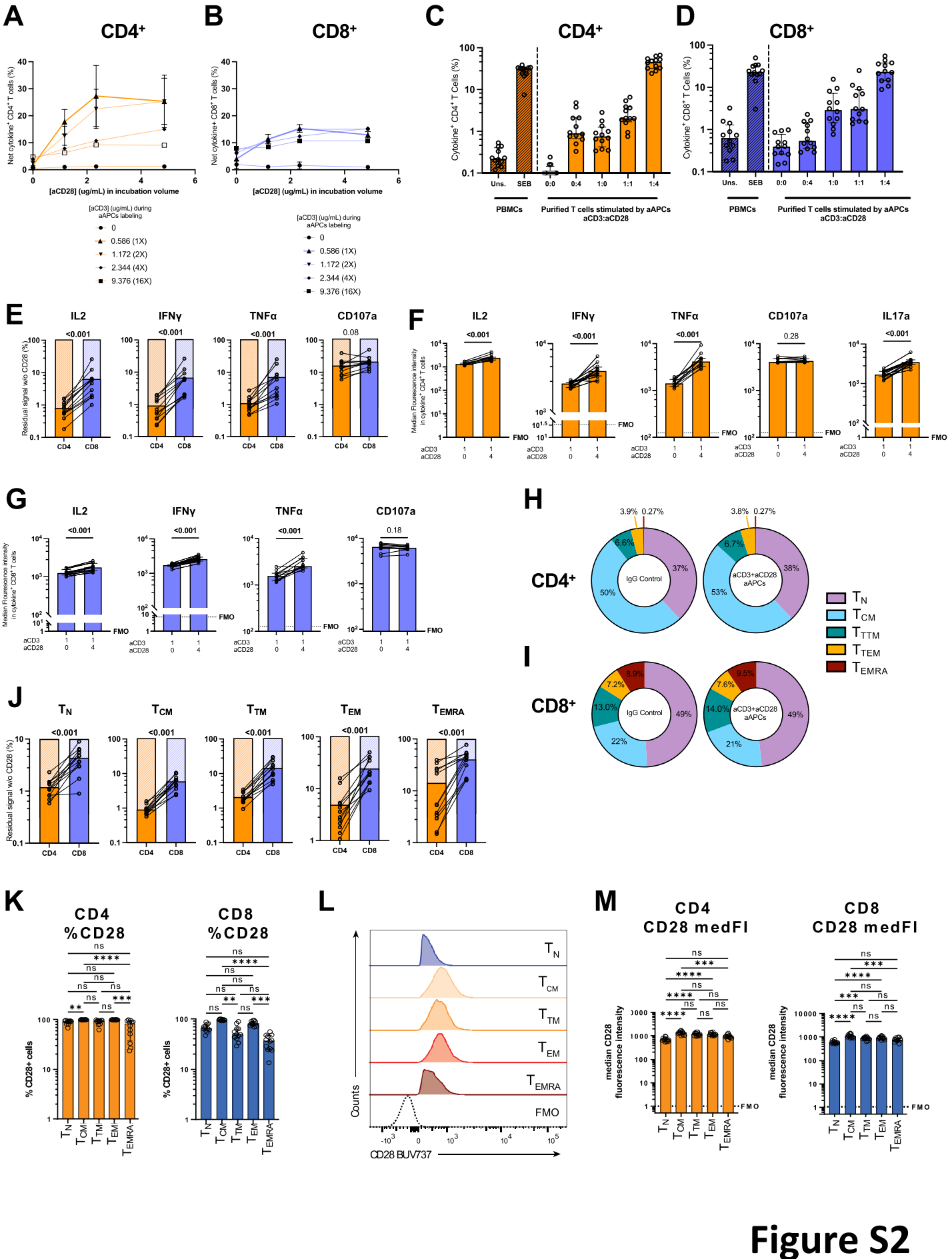
A
CD4+
Net cytokine+ CD4+ T cells (%)
[aCD28] (ug/mL) in incubation volume
[aCD3] (ug/mL) during aAPCs labeling
0
0.586 (1X)
1.172 (2X)
2.344 (4X)
9.376 (16X)
B
CD8+
Net cytokine+ CD8+ T cells (%)
[aCD28] (ug/mL) in incubation volume
[aCD3] (ug/mL) during aAPCs labeling
C
CD4+
Cytokine+ CD4+ T Cells (%)
Uns.
SEB
0:0
0:4
1:0
1:1
1:4
PBMCs
Purified T cells stimulated by aAPCs aCD3:aCD28
D
CD8+
Cytokine+ CD8+ T Cells (%)
E
IL2
IFNγ
TNFα
CD107a
Residual signal w/o CD28 (%)
<0.001
0.08
CD4
CD8
F
IL2
IFNγ
TNFα
CD107a
IL17a
Median Flourescence intensity in cytokine+ CD4+ T cells
<0.001
0.28
FMO
aCD3
aCD28
G
IL2
IFNγ
TNFα
CD107a
Median Flourescence intensity in cytokine+ CD8+ T cells
<0.001
0.18
H
CD4+
3.9%
0.27%
6.6%
37%
50%
IgG Control
3.8%
0.27%
6.7%
38%
53%
aCD3+aCD28 aAPCs
I
CD8+
7.2%
8.9%
13.0%
49%
22%
IgG Control
7.6%
9.5%
14.0%
49%
21%
aCD3+aCD28 aAPCs
TN
TCM
TTTM
TTEM
TEMRA
J
TN
TCM
TTM
TEM
TEMRA
<0.001
Residual signal w/o CD28 (%)
K
CD4 %CD28
CD8 %CD28
% CD28+ cells
ns
****
***
**
L
Counts
CD28 BUV737
FMO
M
CD4 CD28 medFI
CD8 CD28 medFI
median CD28 fluorescence intensity

Figure S2

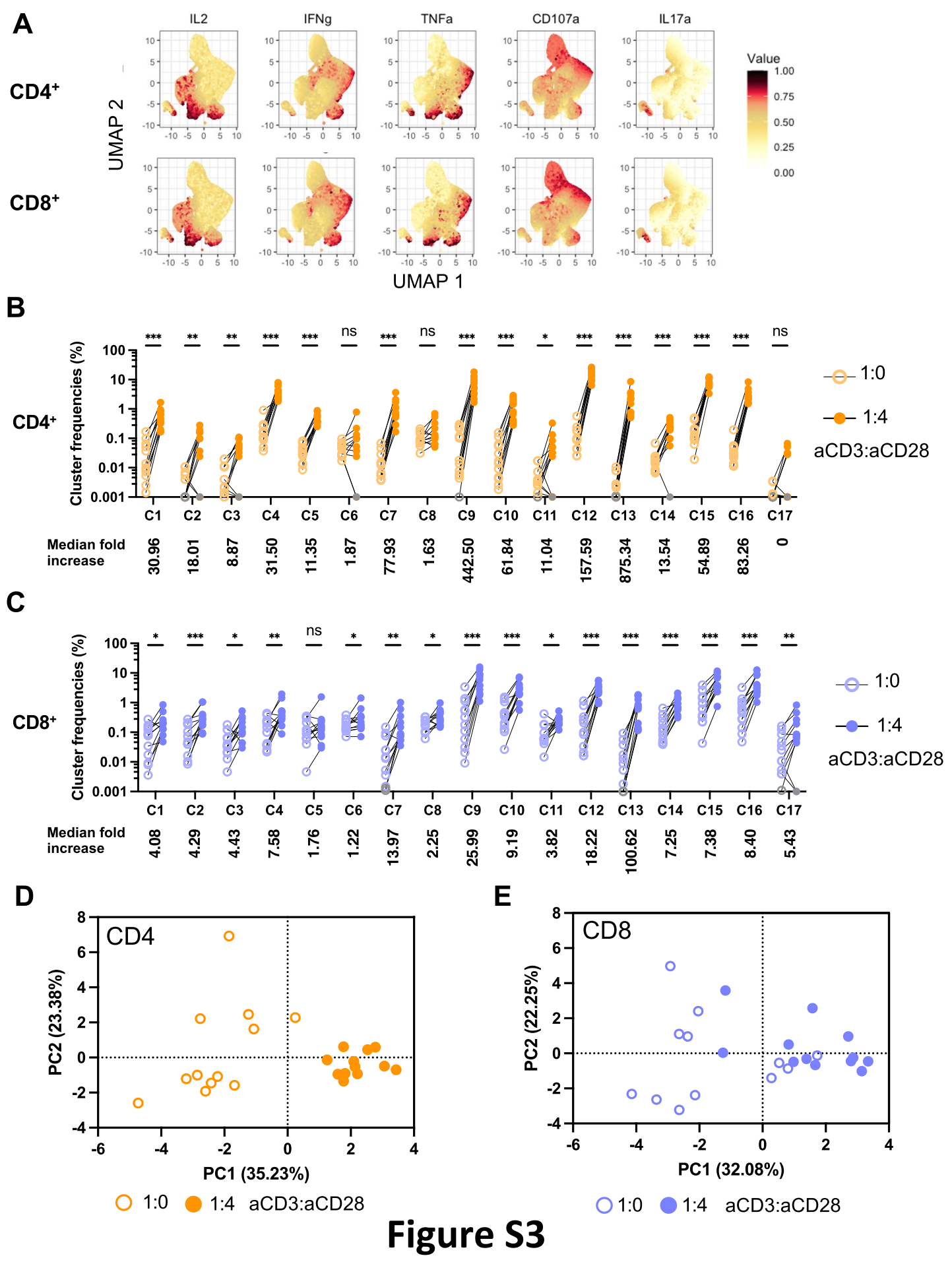
A
IL2
IFNg
TNFa
CD107a
IL17a
CD4+
CD8+
UMAP 2
UMAP 1
Value
1.00
0.75
0.50
0.25
0.00
B
CD4+
Cluster frequencies (%)
1:0
1:4
aCD3:aCD28
C1 C2 C3 C4 C5 C6 C7 C8 C9 C10 C11 C12 C13 C14 C15 C16 C17
Median fold increase
30.96 18.01 8.87 31.50 11.35 1.87 77.93 1.63 442.50 61.84 11.04 157.59 875.34 13.54 54.89 83.26 0
C
CD8+
Cluster frequencies (%)
1:0
1:4
aCD3:aCD28
C1 C2 C3 C4 C5 C6 C7 C8 C9 C10 C11 C12 C13 C14 C15 C16 C17
Median fold increase
4.08 4.29 4.43 7.58 1.76 1.22 13.97 2.25 25.99 9.19 3.82 18.22 100.62 7.25 7.38 8.40 5.43
D
CD4
PC2 (23.38%)
PC1 (35.23%)
1:0 1:4 aCD3:aCD28
E
CD8
PC2 (22.25%)
PC1 (32.08%)
1:0 1:4 aCD3:aCD28

Figure S3

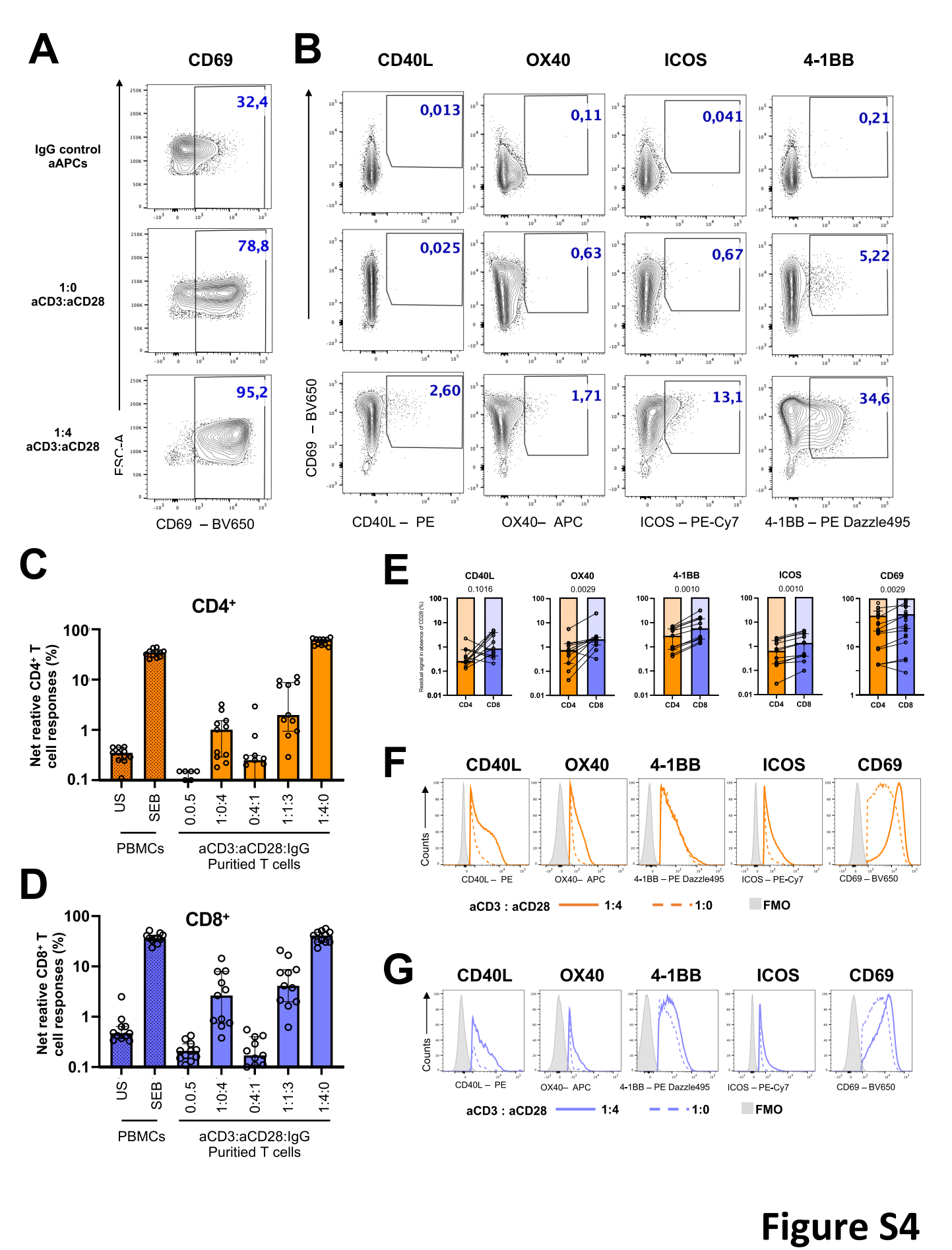
A
CD69
IgG control aAPCs
1:0 aCD3:aCD28
1:4 aCD3:aCD28
32,4
78,8
95,2
FSC-A
CD69 – BV650
B
CD40L
OX40
ICOS
4-1BB
0,013
0,11
0,041
0,21
0,025
0,63
0,67
5,22
2,60
1,71
13,1
34,6
CD69 – BV650
CD40L – PE
OX40– APC
ICOS – PE-Cy7
4-1BB – PE Dazzle495
C
CD4+
Net reative CD4+ T cell responses (%)
US
SEB
0:0.5
1:0:4
0:4:1
1:1:3
1:4:0
PBMCs
aCD3:aCD28:IgG Puritied T cells
D
CD8+
Net reative CD8+ T cell responses (%)
E
CD40L
0.1016
OX40
0.0029
4-1BB
0.0010
ICOS
0.0010
CD69
0.0029
Residual signal in absence of CD28 (%)
CD4
CD8
F
CD40L
OX40
4-1BB
ICOS
CD69
Counts
CD40L – PE
OX40– APC
4-1BB – PE Dazzle495
ICOS – PE-Cy7
CD69 – BV650
aCD3 : aCD28
1:4
1:0
FMO
G
CD40L
OX40
4-1BB
ICOS
CD69
Counts
CD40L – PE
OX40– APC
4-1BB – PE Dazzle495
ICOS – PE-Cy7
CD69 – BV650
aCD3 : aCD28
1:4
1:0
FMO

Figure S4

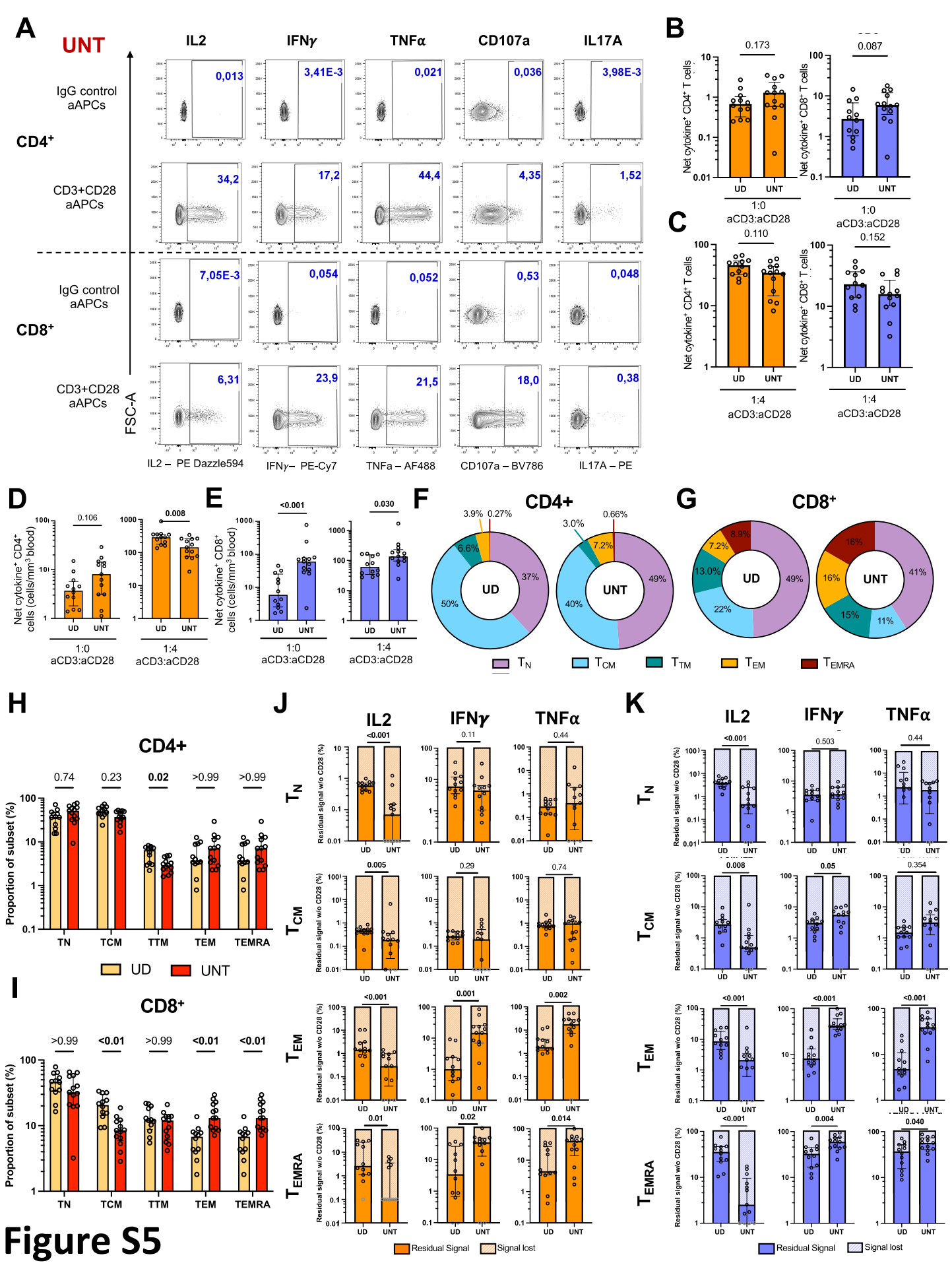
A
UNT
IL2
IFNγ
TNFα
CD107a
IL17A
IgG control aAPCs
CD4+
CD3+CD28 aAPCs
IgG control aAPCs
CD8+
CD3+CD28 aAPCs
FSC-A
IL2 – PE Dazzle594
IFNγ– PE-Cy7
TNFa – AF488
CD107a – BV786
IL17A – PE
B
Net cytokine+ CD4+ T cells
Net cytokine+ CD8+ T cells
UD
UNT
1:0 aCD3:aCD28
C
1:4 aCD3:aCD28
D
Net cytokine+ CD4+ cells (cells/mm3 blood)
E
Net cytokine+ CD8+ cells (cells/mm3 blood)
F
CD4+
UD
UNT
TN
TCM
TTM
TEM
TEMRA
G
CD8+
H
CD4+
Proportion of subset (%)
TN
TCM
TTM
TEM
TEMRA
UD
UNT
I
CD8+
J
IL2
IFNγ
TNFα
Residual signal w/o CD28 (%)
Residual Signal
Signal lost
K
IL2
IFNγ
TNFα
Residual signal w/o CD28 (%)
Residual Signal
Signal lost

Figure S5

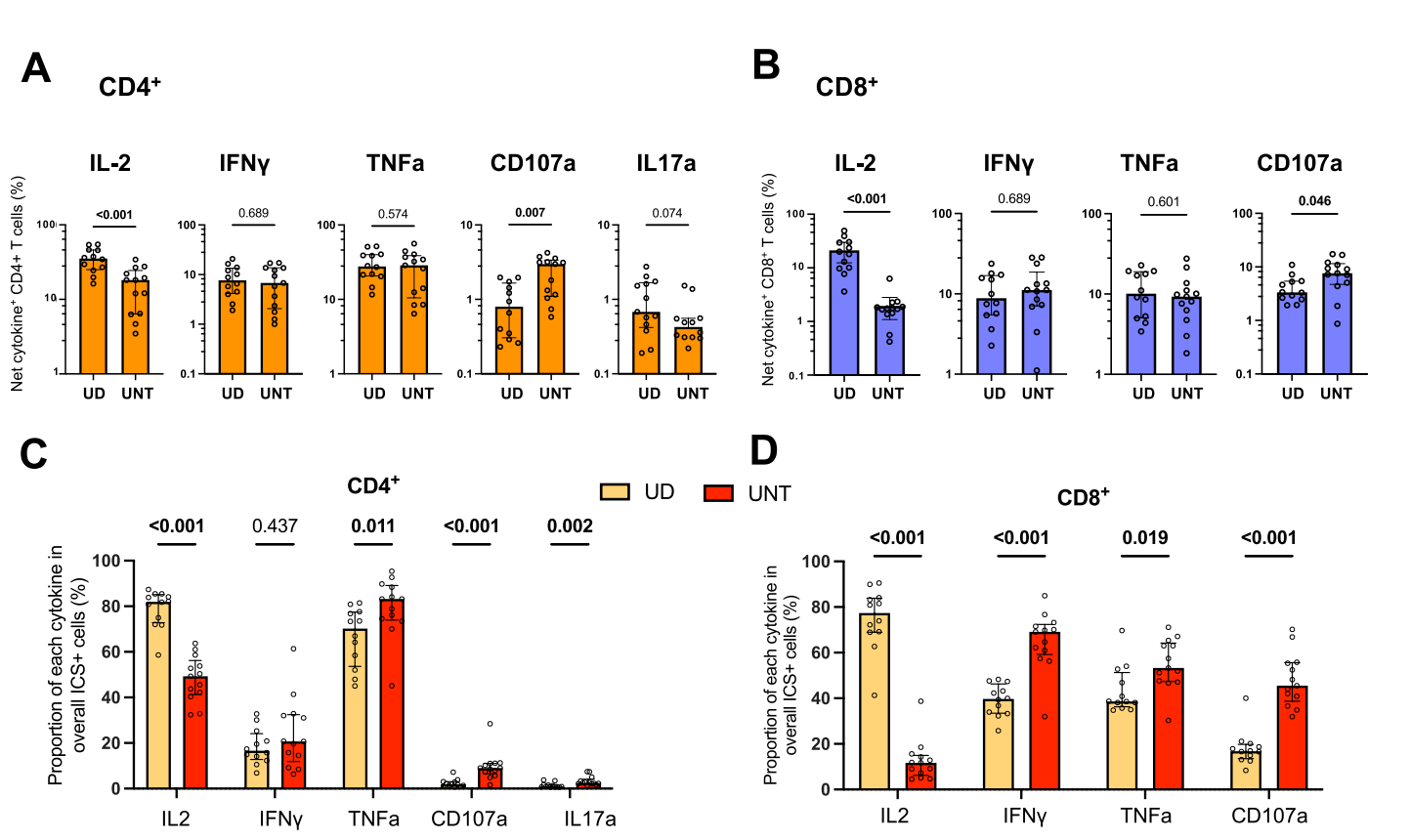
A
CD4+
IL-2
IFNγ
TNFa
CD107a
IL17a
Net cytokine+ CD4+ T cells (%)
<0.001
0.689
0.574
0.007
0.074
UD
UNT
B
CD8+
IL-2
IFNγ
TNFa
CD107a
Net cytokine+ CD8+ T cells (%)
<0.001
0.689
0.601
0.046
C
CD4+
UD
UNT
Proportion of each cytokine in overall ICS+ cells (%)
<0.001
0.437
0.011
<0.001
0.002
IL2
IFNγ
TNFa
CD107a
IL17a
D
CD8+
<0.001
<0.001
0.019
<0.001

Figure S6

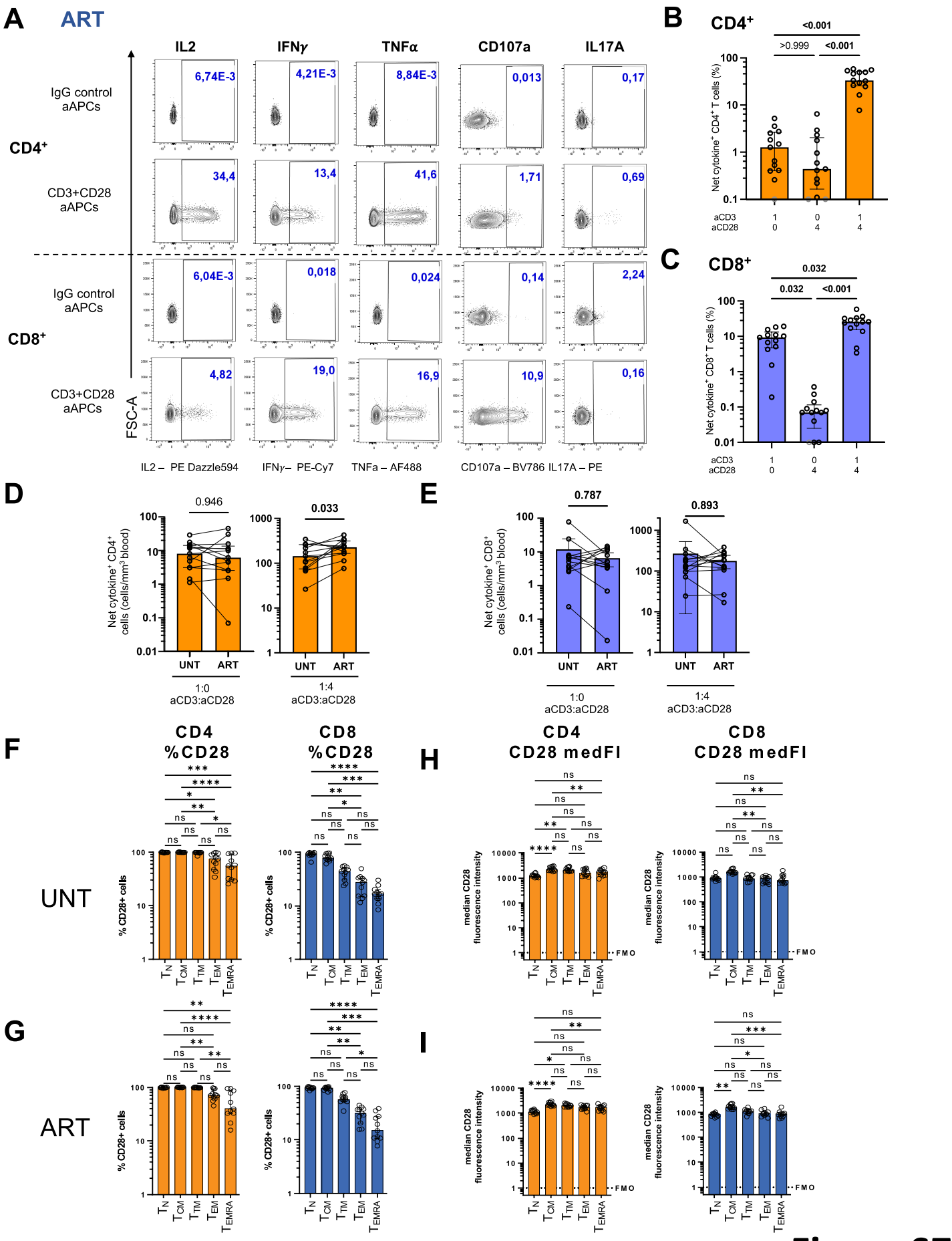
A
ART
IL2
IFNγ
TNFα
CD107a
IL17A
IgG control aAPCs
CD4+
CD3+CD28 aAPCs
6,74E-3
4,21E-3
8,84E-3
0,013
0,17
34,4
13,4
41,6
1,71
0,69
IgG control aAPCs
CD8+
CD3+CD28 aAPCs
6,04E-3
0,018
0,024
0,14
2,24
4,82
19,0
16,9
10,9
0,16
FSC-A
IL2 – PE Dazzle594
IFNγ – PE-Cy7
TNFa – AF488
CD107a – BV786
IL17A – PE
B
CD4+
<0.001
>0.999
<0.001
Net cytokine+ CD4+ T cells (%)
aCD3
aCD28
C
CD8+
0.032
0.032
<0.001
Net cytokine+ CD8+ T cells (%)
D
0.946
0.033
Net cytokine+ CD4+ cells (cells/mm3 blood)
UNT
ART
1:0 aCD3:aCD28
1:4 aCD3:aCD28
E
0.787
0.893
Net cytokine+ CD8+ cells (cells/mm3 blood)
F
CD4 %CD28
CD8 %CD28
UNT
% CD28+ cells
TN
TCM
TTM
TEM
TEMRA
G
ART
H
CD4 CD28 medFI
CD8 CD28 medFI
median CD28 fluorescence intensity
FMO
I

Figure S7

SUPPLEMENTAL FIGURE LEGENDS

Figure S1. T cell activation by aAPCs. Related to Figure 1. 15h stimulation of purified T cells from UD participants with aAPCs (aCD3:aCD28:IgG 1:4:0), unless otherwise indicated. **(A)** Gating strategy for identifying $CD4^+$ and $CD8^+$ T cells. **(B)** Staining of aCD3 and aCD28 antibodies on aAPCs with anti-IgG. **(C)** Representative flow cytometry plots showing cytokine expression in $CD4^+$ and $CD8^+$ T cells following 15h of SEB stimulation in total PBMCs. **(D)** Background-subtracted net $cytokine^+$ $CD4^+$ or $CD8^+$ T cells after 15h of SEB stimulation in total PBMCs or purified T cells. Wilcoxon test is shown. **(E)** Background-subtracted net ICS^+ $CD4^+$ and $CD8^+$ T cells responses to aAPCs aCD3:aCD28:IgG at a ratio of 1:4:0 or with equivalent amounts of soluble aCD3 and aCD28 crosslinked with different anti-IgG concentrations in purified T cells, N=3. **(F)** Background-subtracted net cytokine+ $CD4^+$ or $CD8^+$ T cells, when activated for 15h with aCD3+aCD28 aAPCs (cells-to-beads ratio of 1-4) or with Dynabeads (cells-to-beads ratio of 1-1), N=3, Wilcoxon test is shown. **(G)** Dot plots of CD4+ and CD8+ T cell populations after stimulation with aAPCs (aCD3+aCD28) or Dynabeads. **(H)** The proportion of live T cells ($DUMP^-$) within the lymphocyte population. **(I)** Live T cells recuperation yields from the intial 2 million purified T cells (number of (DUMP-) T cells/ number of input T cells (2 million)). **(J-K)** Median fluorescence intensity (MFI) of different surface markers' expression on $CD4^+$ T cells **(J)** or $CD8^+$ T cells **(K)** after stimulation with different conditions of aAPCs for 15h. N=12, Wilcoxon test results are shown. In **E,F, H-K**, error bars represent interquartile.

Figure S2. CD28 co-stimulation enhances effector functions in $CD4^+$ and $CD8^+$ T cells to varying degrees. Related to Figure 2. Univariate analyses of cytokine expression in $CD4^+$ and $CD8^+$ T cells after 15h of aAPCs stimulation of purified T cells from UD participants. Unless specified otherwise, the 1:4:0 (aCD3:aCD28:IgG; aCD3 aCD28 aAPCs) stimulation is presented. N=2, except for [aCD3]=2.344 and 9.376 µg/mL, N=1. **(C-D)** Raw $cytokine^+$ $CD4^+$ **(C)** or $CD8^+$ **(D)** T cell responses to SEB stimulation in total PBMCs (left) and to aAPCs stimulation with different aCD3:aCD28 ratios in purified T cells (right). Uns. Unstimulated. **(E)** Percentage of residual $cytokine^+$ signal when activating with aCD3 aAPCs compared to aCD3+aCD28 in $CD4^+$ vs $CD8^+$ T cells. **(F-G)** Median fluorescence intensity (MFI) of each gated $cytokine^+$ in $CD4^+$ **(F)** or $CD8^+$ **(G)** T cells when stimulated with aCD3 aAPCs compared to aCD3+aCD28 aAPCs. **(H-I)** Median proportions of each memory subset in $CD4^+$ **(H)** or $CD8^+$ **(I)** T cells. **(J)** Percentage of residual $cytokine^+$ signal when activating with aCD3 aAPCs compared to aCD3+aCD28 in different $CD4^+$ vs $CD8^+$ T cells memory subsets. **K**) Proportion of CD28-expressing cells in memory subsets. (**L**) Representative overlay depicting the level of CD28 expression on indicated memory CD4+ T cell subsets. The FMO is presented as a negative control. (**M**) Median fluorescence intensity of CD28 expression on CD28+ naïve and indicated memory subsets in unstimulated CD4+ (orange) and CD8+ (blue) T cells. **(C-M)** N=12. In **(E-G,J)**, Wilcoxon test was used for paired comparisons**. (K,M)** Freedman test are shown. Ns: non-significant, $p > 0.05$. In **(C-D)**,error bars represent interquartile range

Figure S3. High responsiveness of IL-2 to CD28 co-stimulation shapes effector functions. Related to Figure 3. Multivariate analyses of cytokine expression in $CD4^+$ and $CD8^+$ T cells after 15h of aAPCs stimulation of purified T cells from UD participants. The 1:4:0 (aCD3:aCD28:IgG; aCD3 aCD28 aAPCs) and 1:0:4 (aCD3:aCD28:IgG; aCD3 aAPCs) are herein qualitatively compared. $Cytokine^+$ CD4 and CD8 T cells are identified by Boolean ORgate gating, downsampled to 1000 cell per participant per condition, concatenated then analyzed as follows. **(A)** Heat map overlaid on the $cytokine^+$ UMAP showing the expression gradient for each cytokine**. (B-C)** Frequencies of $cytokine^+$ clusters among $cytokine^+$ $CD4^+$ **(B)** and $CD8^+$ **(C)** T cells in UD. ($*p < 0.05$, $**p < 0.01$, $***p < 0.001$). **(D-E)** Global PCA analysis using the proportions of clusters in $cytokine^+$ $CD4^+$ **(D)** and $CD8^+$ **(E)** activated T cells. The percentage on the x and y axes presents the variance attributed to PC1 and PC2, respectively.

Figure S4. CD28 co-stimulation induces expression of other co-stimulatory molecules. Related to Figure 4. Univariate analyses of co-stimulatory marker expression in $CD4^+$ and $CD8^+$ T cells after 15h of aAPCs stimulation of purified T cells from UD participants. The stimulation conditions are indicated in each panel. **(A-B)** Representative flow cytometry plots of CD69 **(A)** or co-stimulatory molecules **(B)** expression in $CD8^+$ T cells following 15h aAPCs (aCD3:aCD28:IgG) stimulation of 1:0:4 or 1:4:0 in purified T cells. **(C-D)** Raw $CD4^+$ **(C)** or $CD8^+$ **(D)** T cells responses to SEB in total PBMCs and to aAPCs stimulation with different aCD3:aCD28 ratios in purified T cells. Uns. Unstimulated. **(E)** Percentage of residual signal in absence of CD28 costimulation in $CD4^+$ vs $CD8^+$ T cells. Wilcoxon test result is shown. **(F-G)** Representative examples of median fluorescence intensity (MFI) of each cytokine expression in $CD4^+$ and $CD8^+$ T cells when stimulated with aCD3 aAPCs (aCD3:aCD28:IgG 1:0:4) compared to aCD3+aCD28 aAPCs (aCD3:aCD28:IgG 1:4:0). In **(C-E)**, n=11. In **(C-E)**, error bars represent interquartile range.

Figure S5. Altered Dependency of IL-2, IFNγ, and TNFα on CD28 co-stimulation in HIV infection. Related to Figure 6. Univariate analyses of cytokine expression in $CD4^+$ and $CD8^+$ T cells after 15h of aAPCs stimulation of purified T cells from UD versus UNT participants. Unless, specified, the ratios presented are 1:4:0 (aCD3:aCD28:IgG; aCD3 aCD28 aAPCs). **(A)** Representative flow cytometry plots of cytokine expression in $CD4^+$ and $CD8^+$ T cells following 15h aAPCs (aCD3:aCD28:IgG 1:4:0) stimulation in purified T cells from UNT PWH. Unstimulated conditions represent T cells incubated with control IgG aAPCs (aCD3:aCD28:IgG 0:0:5). **(B)** Net $cytokine^+$ $CD4^+$ and $CD8^+$ T cells responses to aCD3 aAPCs (aCD3:aCD28:IgG 1:0:4) stimulation of purified T cells from UD vs UNT. **(C)** Net $cytokine^+$ $CD4^+$ and $CD8^+$ T cells responses to aAPCs (aCD3:aCD28:IgG 1:4:0) stimulation of purified T cells from UD vs UNT. **(D)** Number of net cytokine+ CD4 T cells per mm3 after aAPCs (aCD3:aCD28:IgG 1:0:4) or (aCD3:aCD28:IgG 1:4:0) stimulation of purified T cells from UD vs UNT. **(E)** Number of net cytokine+ CD8 T cells per mm3 after aAPCs (aCD3:aCD28:IgG 1:0:4) or (aCD3:aCD28:IgG 1:4:0) stimulation of purified T cells from UD vs UNT. **(F-I)** Proportions of memory subsets in $CD4^+$ **(F and H)** or $CD8^+$ **(G and I)** T cells from UD or UNT cohorts. **(J-K)** Comparison of residual signal in the absence of CD28 co-stimulation in each memory subset of $CD4^+$ **(H)** or $CD8^+$ **(I)** T cells between UD and UNT for each individual cytokine. In **(B-E, H,I)**, Mann-Whitney test results are shown. In **(B-I)**, N=12. In (**B-E, H,I**), error bars represent interquartile range.

Figure S6. T cells from untreated PWH exhibit imbalanced effector functions favoring IFNγ, TNFα, and CD107a, with reduced IL-2 production. Related to Figure 6. Multivariate analyses of cytokine expression in $CD4^+$ and $CD8^+$ T cells after 15h of aAPCs stimulation of purified T cells at the 1:4:0 (aCD3:aCD28:IgG; aCD3 aCD28 aAPCs) ratio. UD and UNT participants are herein qualitatively compared. $Cytokine^+$ CD4 and CD8 cells are identified by Boolean ORgate gating, downsampled to 1000 cells per participant per condition, concatenated, then analyzed as follows. **(A-B)** Net $CD4^+$ **(A)** or $CD8^+$ **(B)** T cell cytokine responses to aAPCs stimulation (aCD3:aCD28:IgG 1:4:0) of purified T cells. **(C-D)** Proportion of $cytokine^+$ cells in activated $CD4^+$ **(C)** or $CD8^+$ **(D)** T cells. In **(A-D)**, Mann-Whitney test results are shown, UD=12, UNT=13. In (**A-D**), error bars represent interquartile range.

Figure S7. Partial normalization of cytokine profile in CD4$^+$ T cells by ART, with limited effects on CD8$^+$ T cell functions. Related to Figure 7. Univariate analyses of cytokine expression in CD4$^+$ and CD8$^+$ T cells after 15h of aAPCs stimulation of purified T cells from UNT versus ART participants. **(A)** Representative flow cytometry plots of cytokines expression in CD4$^+$ and CD8$^+$ T cells following 15h aAPCs (aCD3:aCD28:IgG 1:4:0) stimulation of purified T cells from ART PWH. Unstimulated conditions correspond to T cells incubated with control IgG aAPCs (aCD3:aCD28:IgG 0:0:5). **(B-C)** Net CD4$^+$ **(B)** or CD8$^+$ **(C)** T cells responses to aAPCs stimulation of purified T cells with different aCD3:aCD28 ratios. Friedman test was used. **(D)** Number of net cytokine+ CD4 $^+$ T cells per mm3 after aAPCs (aCD3:aCD28:IgG 1:0:4) or (aCD3:aCD28:IgG 1:4:0) stimulation of purified T cells from UNT vs ART. **(E)** N Number of net cytokine+ CD8$^+$ T cells per mm3 after aAPCs (aCD3:aCD28:IgG 1:0:4) or (aCD3:aCD28:IgG 1:4:0) stimulation of purified T cells from UNT vs ART. Wilcoxon test result is shown. **(F-G)** Proportion of CD28-expressing cells in memory subsets. **(F)** CD4 (orange) and CD8 (blue) in UNT, **(G)** CD4 (orange) and CD8 (blue) in ART. **(H-I)** Median fluorescence intensity of CD28 expression on CD28$^+$ naïve and indicated memory subsets in **(H)** CD4 (orange) and CD8 (blue) in UNT, **(I)** CD4 (orange) and CD8 (blue) in ART. In (**B-M)**, UNT=13, ART=13. In (**F-I**), Freeman tests are shown.

Supplementary Tables

Table S1. Clinical data for uninfected (UD), untreated (UNT), and treated (ART) participants. Related to Figures 1 to 7

	Participant ID	Sex	Age (years)	CD4/CD8 Ratio	CD4 count (cells/uL)	Viral Load (copies/mL)	Time of infection (years)	Time on ART (years)	Time between pre-post ART timepoints (years)
UD	UD1	M	64	1.78	552	NA	NA	NA	NA
	UD2	M	59	2.40	530	NA	NA	NA	NA
	UD3	M	40	1.26	461	NA	NA	NA	NA
	UD4	F	41	3.93	1273	NA	NA	NA	NA
	UD5	F	45	2.31	632	NA	NA	NA	NA
	UD6	F	38	1.65	529	NA	NA	NA	NA
	UD7	M	63	3.50	675	NA	NA	NA	NA
	UD8	M	65	2.61	310	NA	NA	NA	NA
	UD9	F	39	2.05	667	NA	NA	NA	NA
	UD10	F	60	1.46	670	NA	NA	NA	NA
	UD11	M	57	1.95	726	NA	NA	NA	NA
	UD12	M	50	4.13	1758	NA	NA	NA	NA
	MEDIAN	NA	54	2.18	650	NA	NA	NA	NA
UNT	UNT1	M	40	0.75	571	6678	0.2	NA	NA
	UNT2	M	38	0.23	320	132886	0.4	NA	NA
	UNT3	M	51	0.12	216	96873	13.4	NA	NA
	UNT4	M	42	0.45	320	105130	23.8	NA	NA
	UNT5	M	34	0.07	300	1000000	1.1	NA	NA
	UNT6	F	37	0.59	321	36715	0.2	NA	NA
	UNT7	M	47	0.96	492	39489	8.5	NA	NA
	UNT8	M	22	0.31	597	35859	0.1	NA	NA
	UNT9	M	26	1.11	597	2700	5.6	NA	NA
	UNT10	M	38	1.63	962	7000	0.1	NA	NA
	UNT11	M	24	0.80	971	16316	0.1	NA	NA
	UNT12	M	48	0.35	416	15250	6.5	NA	NA
	UNT13	M	38	0.89	1036	5362	0.4	NA	NA
	MEDIAN	NA	38	0.59	492	35859	0.45	NA	NA
ART	ART1	M	42	1.8	602	< 20	2.7	2.2	2.45
	ART2	M	40	0.49	616	< 40	1.8	1.2	1.44
	ART3	M	52	0.30	361	< 20	14.5	1.1	1.17
	ART4	M	43	0.61	482	< 40	24.9	1.0	1.13
	ART5	M	37	0.29	662	< 40	4.2	2.6	3.10
	ART6	F	37	0.99	416	44	0.9	0.5	0.61
	ART7	M	48	1.72	640	< 40	9.8	0.7	1.27
	ART8	M	26	1.34	871	< 40	3.9	3.6	3.74
	ART9	M	28	1.19	694	< 40	7.4	1.1	1.82
	ART10	M	42	2.30	509	< 40	3.3	3.1	3.18
	ART11	M	26	1.65	862	< 40	1.8	1.3	1.67
	ART12	M	50	0.80	700	< 40	9.2	1.2	2.68
	ART13	M	42	1.34	876	< 40	4.7	3.5	4.23
	MEDIAN	NA	42	1.19	640	NA	4.24	1.25	1.82

NA : Not-Applicable

Table S2. Flow cytometry antibody staining panel for intracellular detection. Related to the STAR Methods section.

Marker	Fluorophore	Clone	Vendor	Catalogue #
CD3	BUV395	UCHT1	BD Bioscience	563546
CD4	BUV496	SK3	BD Bioscience	564651
CD45RA	BUV563	HI100	BD Bioscience	612926
CD27	BUV661	L128	BD Bioscience	750167
CD57	BV421	NK-1	BD Bioscience	563896
CD14	BV480	M5E2	BD Bioscience	746304
CD19	BV480	HIB19	BD Bioscience	746457
LIVE/DEAD Fixable dead cell	Aquavivid	NA	Thermo Fisher Scientific	L34960
CD8	BV570	RPA-T8	Biolegend	301037
CD107a	BV786	H4A3	BD Bioscience	563869
TNFα	AF488	Mab11	Biolegend	502915
CD69	PerCPeF710	FN50	ebioscience	46-0699-42
IL17a	PE	REA1063	Miltenyi	130-120-551
IL-2	PE-Dazzle594	MQ1-17H12	Biolegend	500344
IFNγ	PE-Cy7	557643	BD Bioscience	557643
CCR7	APC-R700	2-L1-A	BD Bioscience	566767
CD40L	APC	TRAP1	BD Bioscience	555702

Table S3. Flow cytometry antibody staining panel for co-stimulatory molecules detection. Related to the STAR Methods section.

Marker	Fluorophore	Clone	Vendor	Catalogue #
CD3	BUV395	UCHT1	BD Bioscience	563546
CD4	BUV496	SK3	BD Bioscience	564651
CD45RA	BUV563	HI100	BD Bioscience	612926
CD27	BUV661	L128	BD Bioscience	750167
CD57	BV421	NK-1	BD Bioscience	563896
CD14	BV480	M5E2	BD Bioscience	746304
CD19	BV480	HIB19	BD Bioscience	746457
LIVE/DEAD Fixable dead cell	Aquavivid	NA	Thermo Fisher Scientific	L34960
CD8	BV570	RPA-T8	Biolegend	301037
CD28	BUV737	CD28.2	BD Bioscience	564438
PD-1	BB515	EH12.1	BD Bioscience	564494
CD69	BV650	FN50	Biolegend	310934
TIGIT	PerCPeF710	MBSA43	ebioscience	46-9500-42
4-1BB	PE-Dazzle594	4B4-1	Biolegend	309826
ICOS	PE-Cy7	ISA-3	ebioscience	25-9948
CCR7	APC-R700	2-L1-A	BD Bioscience	566767
OX40	APC	ACT35	BD Bioscience	563473
CD40L	PE	TRAP	BD Bioscience	555700