SUPPLEMENTARY INFORMATION

Manuscript title: Inherited Salt-Losing Tubulopathies are associated with immunodeficiency due to impaired IL-17 responses

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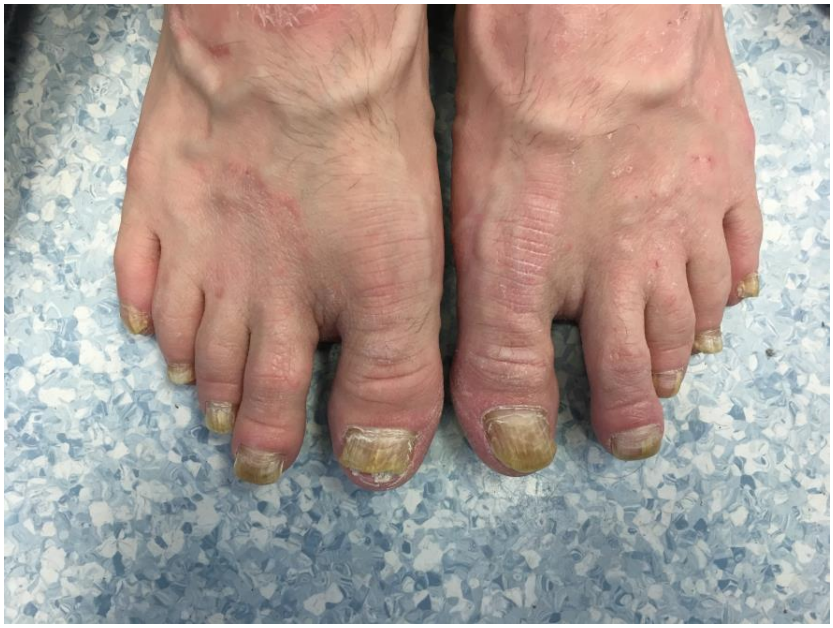
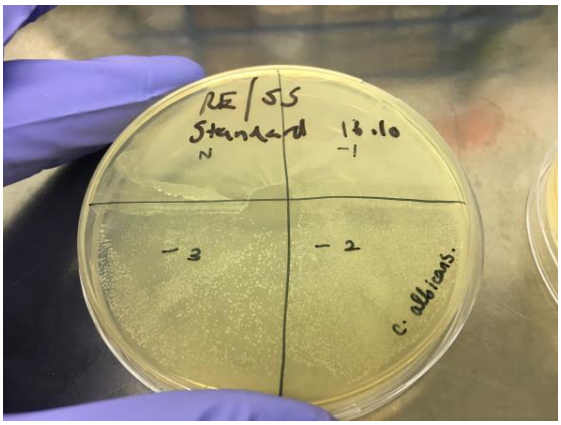


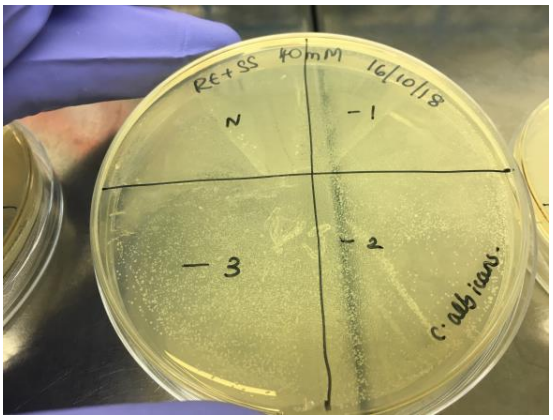
Supplemental Figure 1: Recurrent fungal toenail affecting all toes in a patient with Bartter syndrome type 3 (reported during routine clinic review)

i. *Candida albicans*. Reduction in colony size with additional NaCl

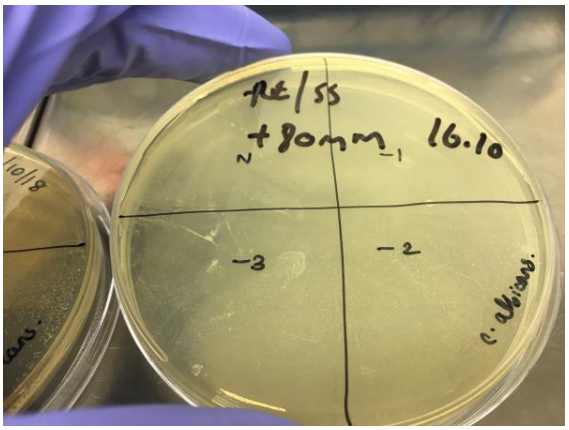
A. No additional NaCl



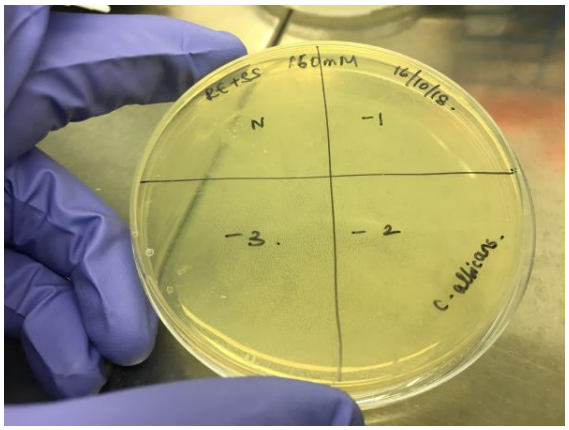
B. +40mM NaCl



C. +80mM NaCl

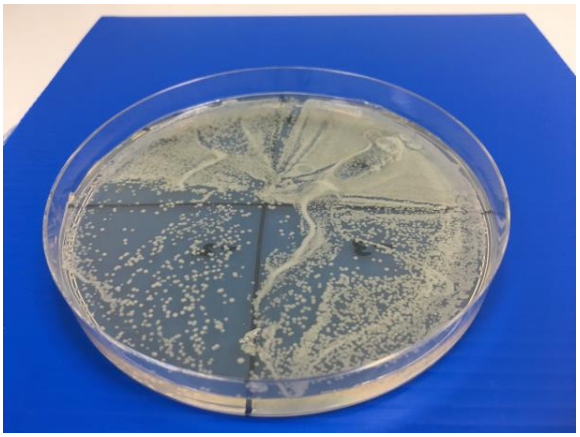


D. +160mM NaCl

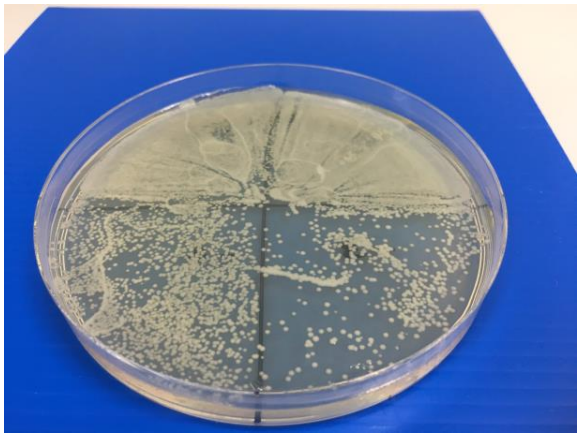


ii. *Corynebacterium amycolatum*. No effect of NaCl on growth

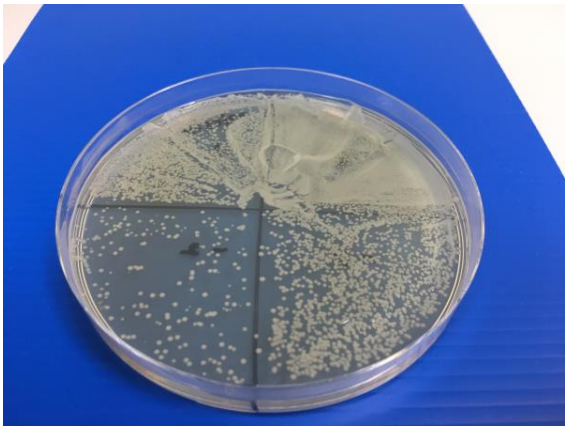
A. No additional NaCl



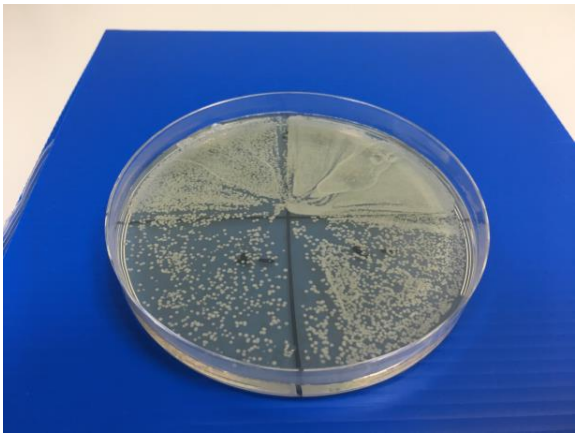
B. +40mM NaCl



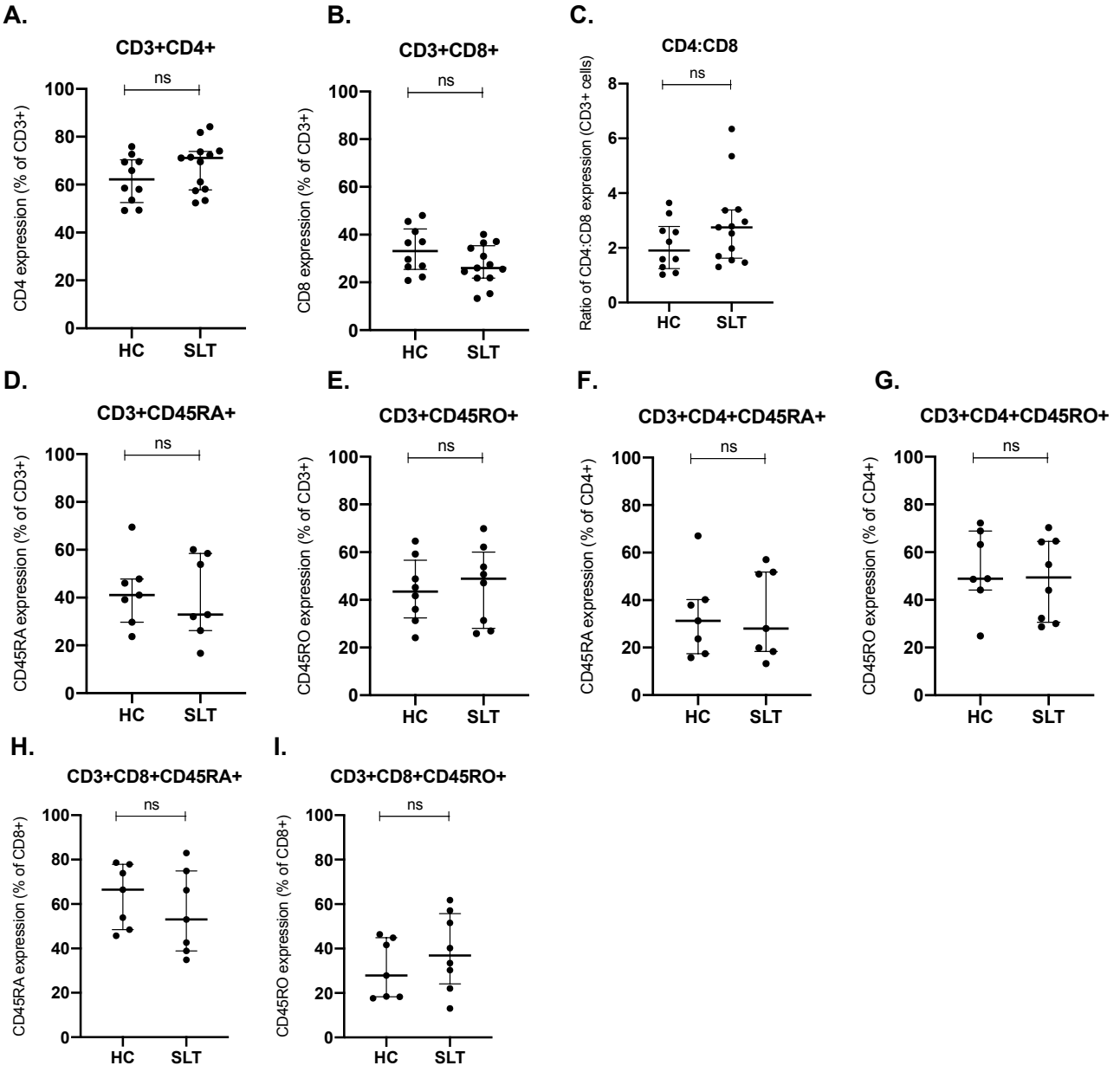
C. +80mM NaCl



D. +160mM NaCl



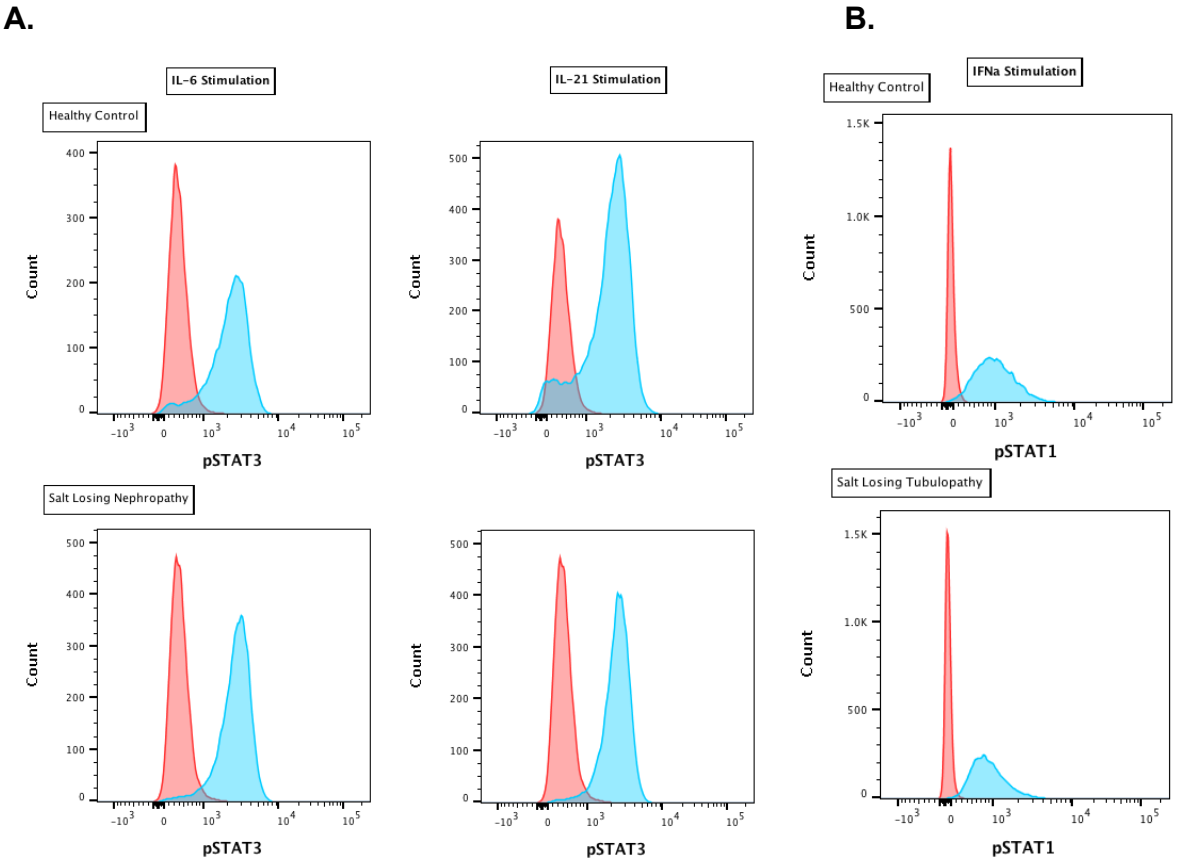
Supplemental Figure 2: Representative images of *Candida albicans* and *Corynebacterium amycolatum* culture (serial dilutions neat, 10^{-1}, 10^{-2}, 10^{-3}) in tryptone soya agar media with and without additional NaCl (+0-160mM).

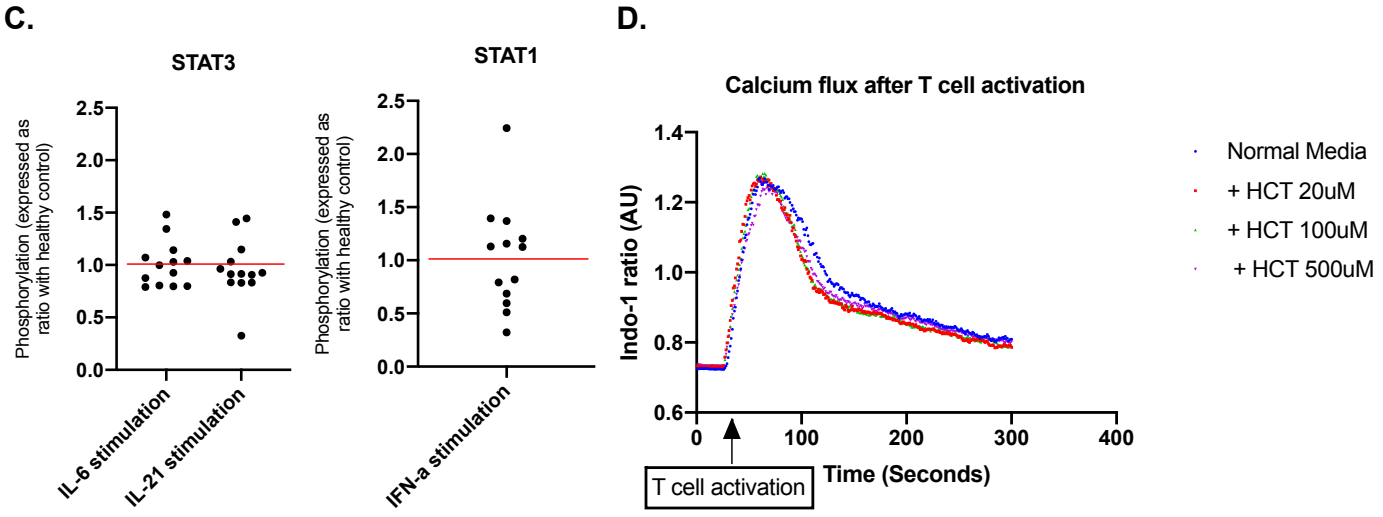


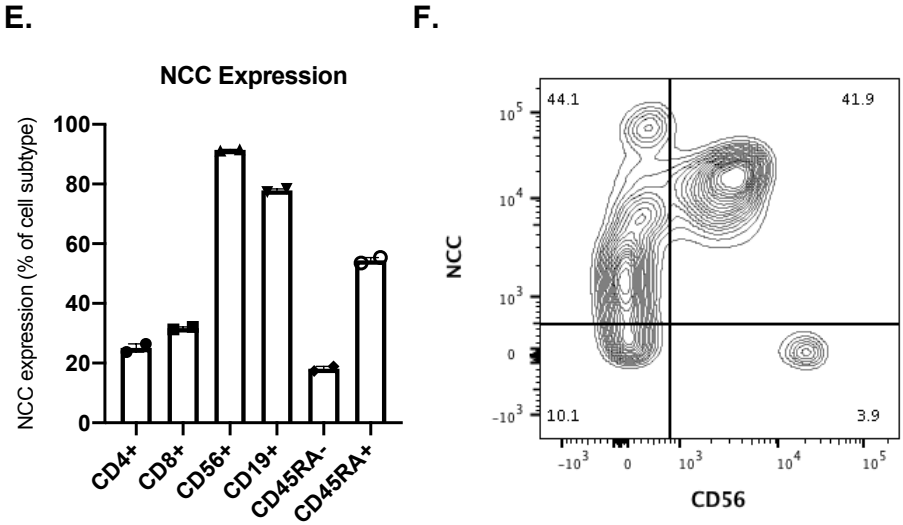
Supplemental Figure 3: <u>T cell subsets in SLT patients and healthy controls</u>
A. CD3+CD4+ cells (% of CD3+ cells): HC [n=13] 62.2% (52.5-70.4), SLT [n=10] 71.2% (57.8-73.9), p=0.23. Compared with a two-sided Mann-Whitney test. Error bars represent interquartile range around the median.
B. CD3+CD8+ cells (% of CD3+ cells): HC [n=13] 33.1% (25.5-42.4), SLT [n=10] 26.0% (21.8-35.4), p=0.15. Compared with a two-sided Mann-Whitney test. Error bars represent interquartile range around the median.
C. CD4:CD8 ratio: HC 1.9 [n=13] (1.2-2.8), SLT [n=10] 2.7 (1.6-3.4), p=0.19. Compared with a two-sided Mann-Whitney test. Error bars represent interquartile range around the median.
D. CD3+CD45RA+ cells (% of CD3+ cells): HC [n=13] 41.0% (29.7-47.8), SLT [n=10] 32.9% (26.2-58.4), p=0.90. Compared with a two-sided Mann-Whitney test. Error bars represent interquartile range around the median.
E. CD3+CD45RO+ cells (% of CD3+ cells): HC [n=13] 43.5% (32.5-56.6), SLT [n=10] 48.9% (28.1-60.0), p=0.72. Compared with a two-sided Mann-Whitney test. Error bars represent interquartile range around the median.
F. CD3+CD4+CD45RA+ cells (% of CD3+CD4+ cells): HC [n=13] 31.3% (17.5-40.2), SLT [n=10] 28.0% (18.4-51.8), p=0.99. Compared with a two-sided Mann-Whitney test. Error bars represent interquartile range around the median.
G. CD3+CD4+CD45RO+ cells (% of CD3+CD4+ cells): HC [n=13] 48.9% (44.1-68.9), SLT [n=10] 49.4% (30.6-64.6), p=0.69. Compared with a two-sided Mann-Whitney test. Error bars represent interquartile range around the median.

H. CD3+CD8+CD45RA+ cells (% of CD3+CD8+ cells): HC [n=13] 66.5% (48.5-77.8), SLT [n=10] 53.0% (38.9-74.9), p=0.38. Compared with a two-sided Mann-Whitney test. Error bars represent interquartile range around the median.
I. CD3+CD8+CD45RO+ cells (% of CD3+CD8+ cells): HC [n=13] 27.9% (18.3-44.9), SLT [n=10] 36.8% (24.1-55.7), p=0.40. Compared with a two-sided Mann-Whitney test. Error bars represent interquartile range around the median.

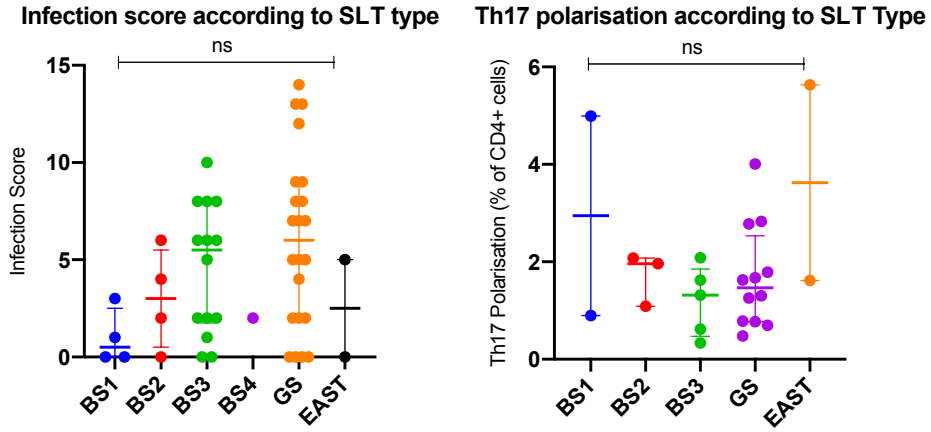
ns – not significant (p>0.05), *p≤0.05, **p≤0.01, ***p≤0.001, ****p≤0.0001. SLT – Salt-Losing Tubulopathy; HC – healthy control. Source data are provided as a **Source data file.**


A.
B.
IL-6 Stimulation
IL-21 Stimulation
IFNa Stimulation
Healthy Control
Salt Losing Nephropathy
Salt Losing Tubulopathy
Count
pSTAT3
pSTAT1


C.
D.
STAT3
STAT1
Phosphorylation (expressed as ratio with healthy control)
IL-6 stimulation
IL-21 stimulation
IFN-a stimulation
Calcium flux after T cell activation
Indo-1 ratio (AU)
Time (Seconds)
T cell activation
Normal Media
+ HCT 20uM
+ HCT 100uM
+ HCT 500uM


E.
F.
NCC Expression
NCC expression (% of cell subtype)
CD4+
CD8+
CD56+
CD19+
CD45RA-
CD45RA+
NCC
CD56
44.1
41.9
10.1
3.9

G.



Supplemental Figure 4: Investigation of impaired IL-17 responses - STAT1 and STAT3 phosphorylation in SLT patients, calcium flux after T cell activation in the presence of hydrochlorothiazide, NCC expression on lymphocytes, and infection score and Th17 polarisation according to SLT type

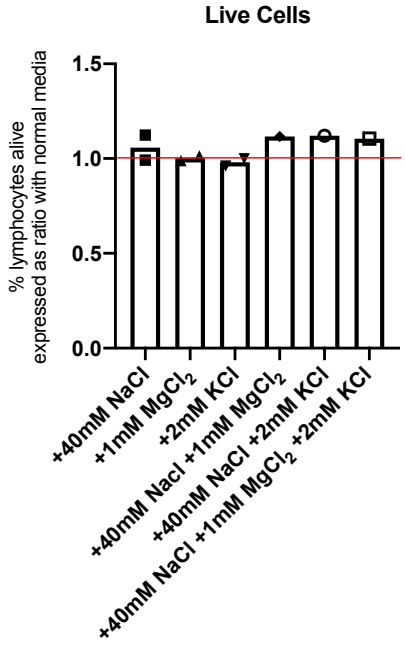
A. Representative histograms of up-regulation of pSTAT3 in CD4+ cells after stimulation with IL-6 and IL-21 in a SLT patient and a HC (red – unstimulated; blue – stimulated).
B. Representative histograms of up-regulation of pSTAT1 in CD4+ cells after stimulation with IFNα in a SLT patient and a HC (red – unstimulated; blue – stimulated).
C. Phosphorylation of STAT3 in CD4- cells after stimulation with IL-6 and IL-21, and phosphorylation of STAT1 in CD4- cells after stimulation with IFNα in SLT patients (n=13). Expressed as ratio of up-regulation in SLT compared to HC (n=4). Red line drawn at ratio of 1 represents no difference between SLT and HC.
D. Calcium flux curves (as determined using the ratiometric calcium dye Indo-1 gated on CD4+ cells) after T cell activation in normal media and in the presence of hydrochlorothiazide (HCT) 20-500uM.
E. Sodium chloride cotransporter (NCC) expression on CD4+, CD8+, CD45+CD3-CD56+, CD19+, CD45RA-, and CD45RA+ cells. Demonstrated as percentage of cell type expressing NCC in a healthy control (the median for technical duplicates is plotted); highest expression was on CD45+CD3-CD56+ (NK) cells.
F. Representative FACS contour plot of NCC expression on NK cells (gated on CD45+CD3- cells). NCC expression is predominantly on CD56 dim as opposed to CD56 bright NK cell subsets. Cell proportions as percentages are documented within each quadrant.
G. IL-17 related infection score and Th17 polarisation according to SLT type (each individual type reflects different defective ion transporter). Compared with a Kruskal-Wallis test. Infection score according to SLT type: BS 1 n=4; BS2 n=4; BS3 n= 14; BS4 n=1; GS n=22; EAST n=2. Th17 polarisation according to SLT type: BS1 n=2; BS2 n=3; BS3 n=5; GS n=12; EAST n=2. Error bars represent interquartile range around the median.

Source data are provided as a **Source data file.**

A.

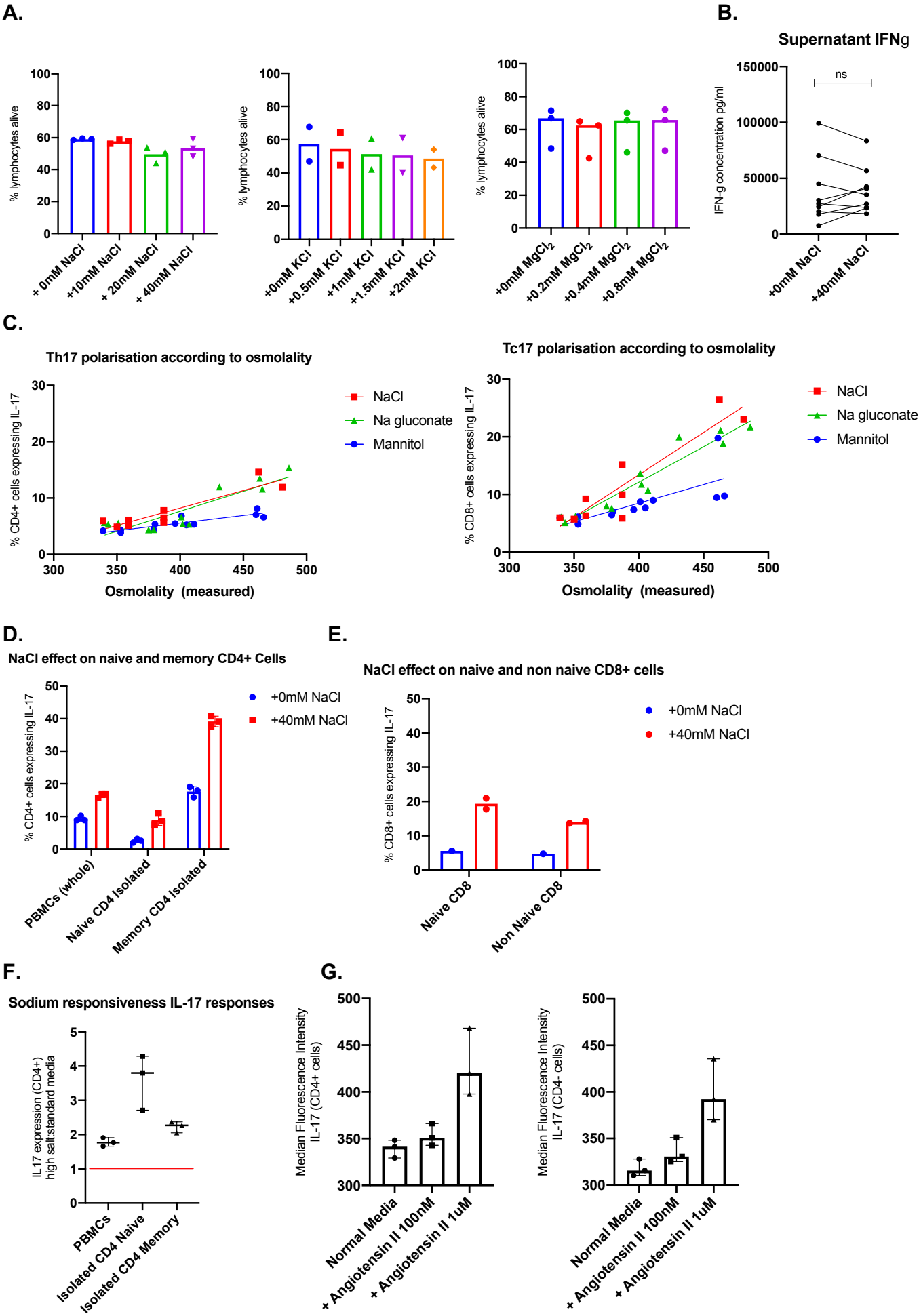
Electrolyte	Concentration in XVIVO15 (measured; mmol per l)
Sodium	131
Chloride	98
Potassium	4.4
Magnesium	0.75
Calcium	1.29
Phosphate	0.92

B.



Supplemental Figure 5: Ionic concentrations in unadjusted media, and effect of changing extracellular ionic concentrations on cell viability during T cell activation
A. Measured ionic concentrations in XVIVO15 media.
B. Effect of changing extracellular ionic concentration during T cell activation on cell viability in healthy control (n=2) cells. Expressed as a ratio to cell viability in normal media. Red line drawn at ratio of 1 represents no difference to normal media.

Source data are provided as a **Source data file.**


A.
% lymphocytes alive
+ 0mM NaCl
+10mM NaCl
+ 20mM NaCl
+ 40mM NaCl
+0mM KCl
+0.5mM KCl
+1mM KCl
+1.5mM KCl
+2mM KCl
+0mM MgCl2
+0.2mM MgCl2
+0.4mM MgCl2
+0.8mM MgCl2
B.
Supernatant IFNg
IFN-g concentration pg/ml
ns
+0mM NaCl
+40mM NaCl
C.
Th17 polarisation according to osmolality
% CD4+ cells expressing IL-17
Osmolality (measured)
NaCl
Na gluconate
Mannitol
Tc17 polarisation according to osmolality
% CD8+ cells expressing IL-17
D.
NaCl effect on naive and memory CD4+ Cells
+0mM NaCl
+40mM NaCl
PBMCs (whole)
Naive CD4 Isolated
Memory CD4 Isolated
E.
NaCl effect on naive and non naive CD8+ cells
Naive CD8
Non Naive CD8
F.
Sodium responsiveness IL-17 responses
IL17 expression (CD4+) high salt:standard media
PBMCs
Isolated CD4 Naive
Isolated CD4 Memory
G.
Median Fluorescence Intensity IL-17 (CD4+ cells)
Median Fluorescence Intensity IL-17 (CD4- cells)
Normal Media
+ Angiotensin II 100nM
+ Angiotensin II 1uM

Supplemental Figure 6: Data from 7-day Th17 polarisation experiments with altered extracellular ionic and angiotensin II concentrations

A. Effect of NaCl (0-40mM; n=1 healthy control), KCl (0-2mM; n=2 healthy controls), and $MgCl_2$ (0-0.8mM; n=3 healthy controls) on cell viability after PBMC activation under optimal Th17 polarising conditions. Mean of technical triplicates is plotted for NaCl effect. In other graphs the median for controls is plotted.

B. Effect of +40mM NaCl on supernatant IFNγ concentrations during 7-day culture experiments in healthy control cells (n=8). Conditions are compared with a two-sided Wilcoxon test.

C. Measured supernatant osmolality (mOsm per kg) created by different osmoles in the final supernatant of 7-day cultures plotted against Th17 and Tc17 polarisation.

D. Effect of +40mM NaCl on Th17 polarisation in stimulated whole PBMCs, stimulated isolated naïve (CD45RA+) CD4+ T cells, and stimulated isolated memory (CD45RO+) CD4+ T cells. The mean of technical replicates in a single experiment in cells form a healthy control is plotted.

E. Effect of +40mM NaCl on Tc17 polarisation in stimulated isolated naïve (CD45RA+) CD8+ T cells, and non-naïve CD8+ T cells. The mean of technical replicates in a single experiment in cells form a healthy control is plotted.

F. Sodium responsiveness of PMBCs (whole), isolated naïve CD4+ cells, and memory CD4+ cells. IL-17 expression (% of CD4+ cells) plotted in high salt conditions (+40mM NaCl) as a ratio to standard conditions. Red line drawn at ratio = 1 (i.e. no difference in IL-17 expression between conditions). Error bars represent range around the median of technical triplicates in a single experiment in cells form a healthy control.

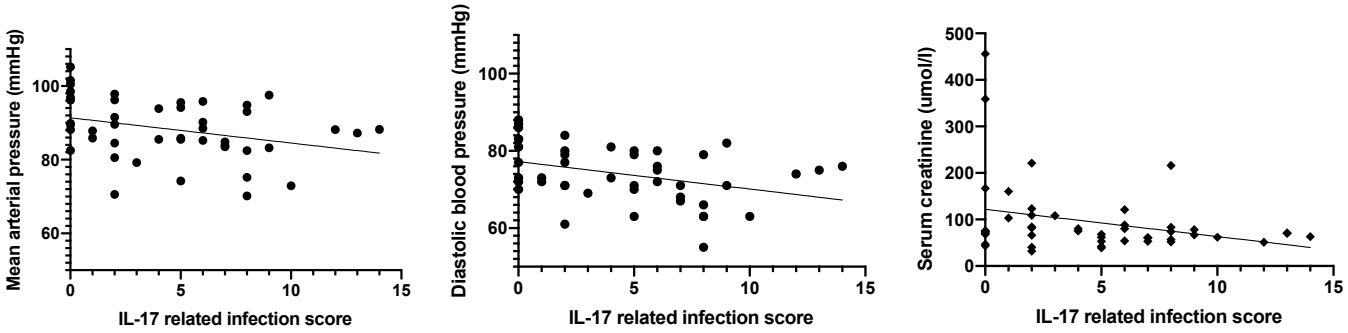
G. IL-17 expression in CD4+ and CD4- cells (expressed as median fluorescence intensity) in PBMCs stimulated in optimal Th17 polarising conditions with and with angiotensin II (0.1-1uM). Error bars represent range around the median of technical triplicates in a single experiment in cells form a healthy control.

ns – not significant ($p>0.05$), *$p\leq0.05$, **$p\leq0.01$, ***$p\leq0.001$, ****$p\leq0.0001$. Source data are provided as a **Source data file.**

A.

	Pearson r	95% Confidence Interval	R squared	P value
Infection Score vs. Na (mmol per l)	-0.1654	-0.4321 to 0.1278	0.02737	0.2664
Infection Score vs. K (mmol per l)	-0.09249	-0.3728 to 0.2033	0.008554	0.541
Infection Score vs. Cl (mmol per l)	-0.2305	-0.4908 to 0.06760	0.05313	0.1277
Infection Score vs. HCO3 (mmol per l)	0.0489	-0.2449 to 0.3345	0.002391	0.7469
Infection Score vs. Creatinine (µmol per l)	-0.2718	-0.5185 to 0.01666	0.07388	0.0646
Infection Score vs. cCa (mmol per l)	-0.1446	-0.4203 to 0.1556	0.0209	0.3434
Infection Score vs. PO4 (mmol per l)	-0.239	-0.4950 to 0.05510	0.05713	0.1096
Infection Score vs. Mg (mmol per l)	-0.04587	-0.3450 to 0.2617	0.002104	0.773
Infection Score vs. Mean arterial pressure (mmHg)	-0.3233	-0.5634 to -0.03290	0.1045	0.0303
Infection Score vs. Systolic blood pressure (mmHg)	-0.1988	-0.4652 to 0.1006	0.03951	0.1905
Infection Score vs. Diastolic blood pressure (mmHg)	-0.3695	-0.5982 to -0.08526	0.1366	0.0125

B.

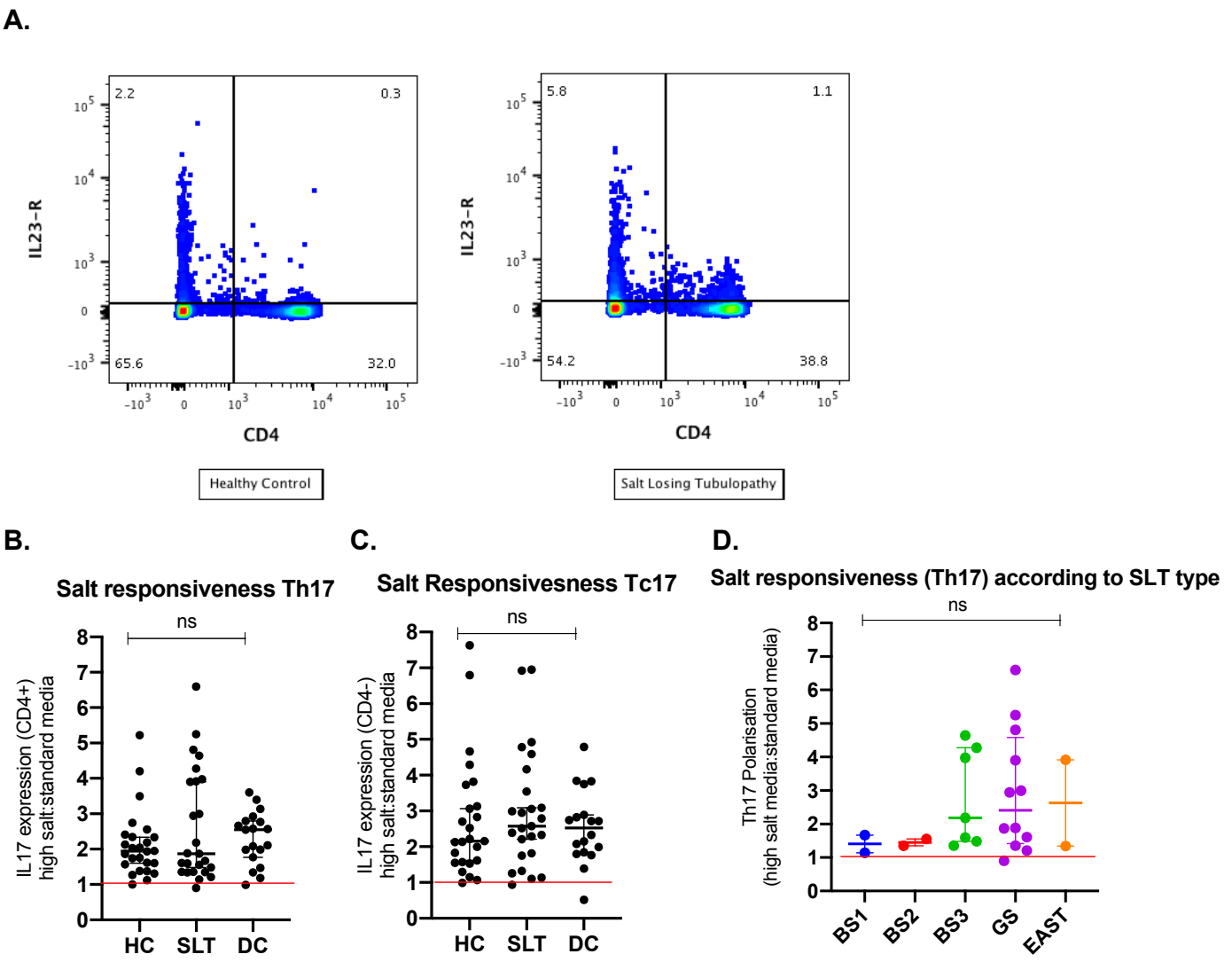


Supplemental Figure 7: IL-17 related infection score according to serum biochemical parameters and disease subtypes in Salt-Losing Tubulopathy patients

A. Correlation of serum biochemical parameters to IL-17 related infection score in SLT patients (n=45). Analysed with a Pearson correlation; a two-sided p value is recorded.

B. Mean arterial pressure, diastolic blood pressure, and serum creatinine plotted against IL-17 related infection score in SLT patients (n=45).

Source data are provided as a **Source data file.**



Supplemental Figure 8: IL23-receptor expression and salt responsiveness of IL-17 responses in Salt-Losing Tubulopathy

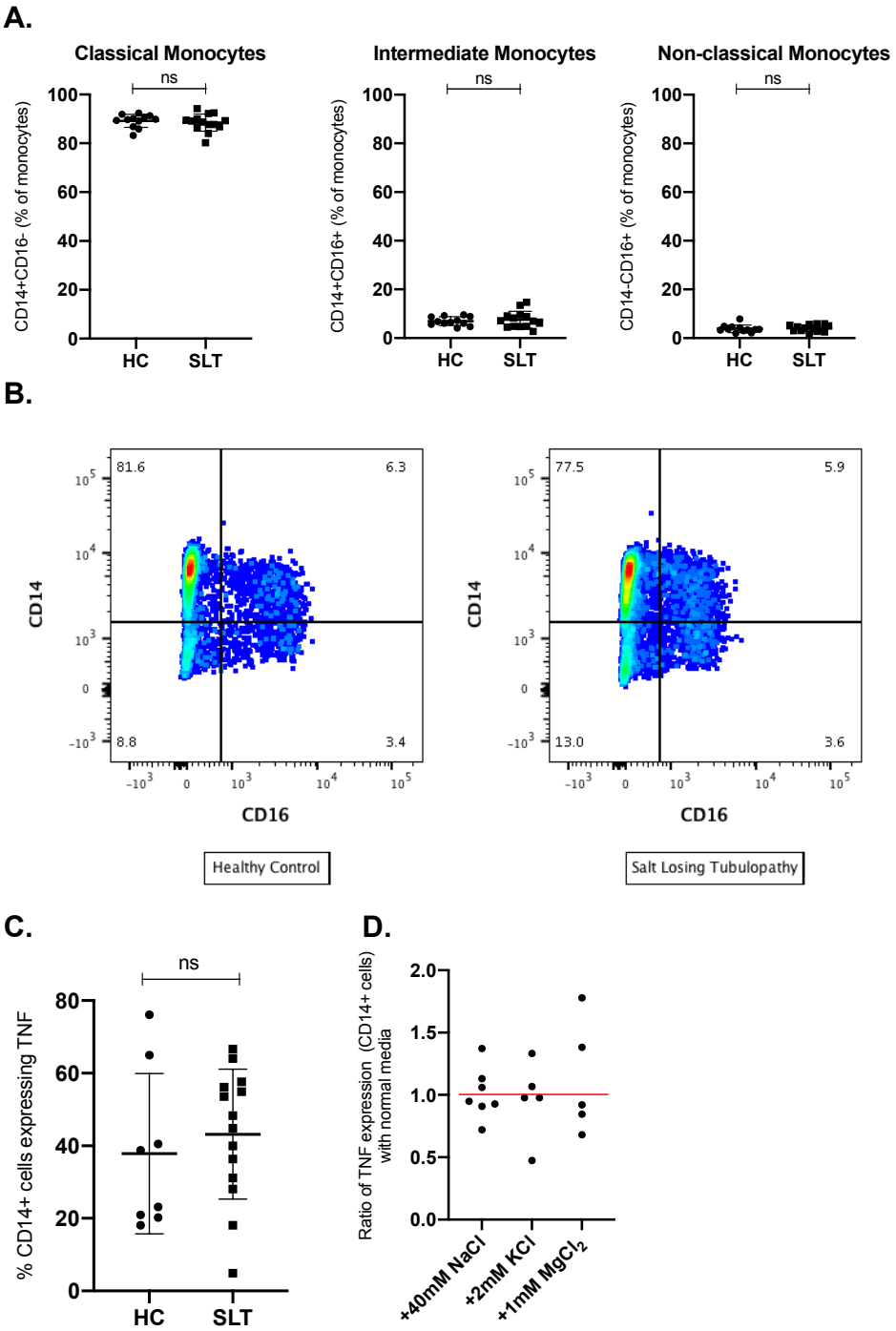
A. Representative FACS dot plots of IL23 receptor (IL23-R) expression on CD4+ cells in a SLT patient and HC. Cell proportions as percentages are documented within each quadrant.

B. Salt responsiveness (readout in media supplemented with NaCl 40mM expressed as ratio to readout in normal media) of Th17 cells in SLT patients (n=25), healthy controls (n=26), and disease controls (n=19). Red line drawn at ratio of 1 demonstrates no difference between high salt media and normal media. Groups are compared with a Kruskal-Wallis test. Error bars represent the interquartile range around the median.

C. Salt responsiveness (readout in media supplemented with NaCl 40mM expressed as ratio to readout in normal media) of Tc17 cells in SLT patients (n=25), healthy controls (n=27), and disease controls (n=19). Red line drawn at ratio of 1 demonstrates no difference between high salt media and normal media. Groups are compared with a Kruskal-Wallis test. Error bars represent the interquartile range around the median.

D. Salt responsiveness (readout in media supplemented with NaCl 40mM expressed as ratio to readout in normal media) of Th17 cells according to SLT type (BS1 = 2, BS2 = 2, BS3 = 7, GS = 12, and EAST = 2). Red line drawn at ratio of 1 demonstrates no difference between high salt media and normal media. Groups are compared with a Kruskal-Wallis test. Error bars represent the interquartile range around the median.

SLT – Salt-Losing Tubulopathy; HC – healthy control; DC – disease control; BS – Bartter syndrome; GS – Gitelman syndrome; ns – not significant (p>0.05), *p≤0.05, **p≤0.01, ***p≤0.001, ****p≤0.0001. Source data are provided as a **Source data file.**



Supplemental Figure 9: Monocyte analysis in Salt-Losing Tubulopathy patients.

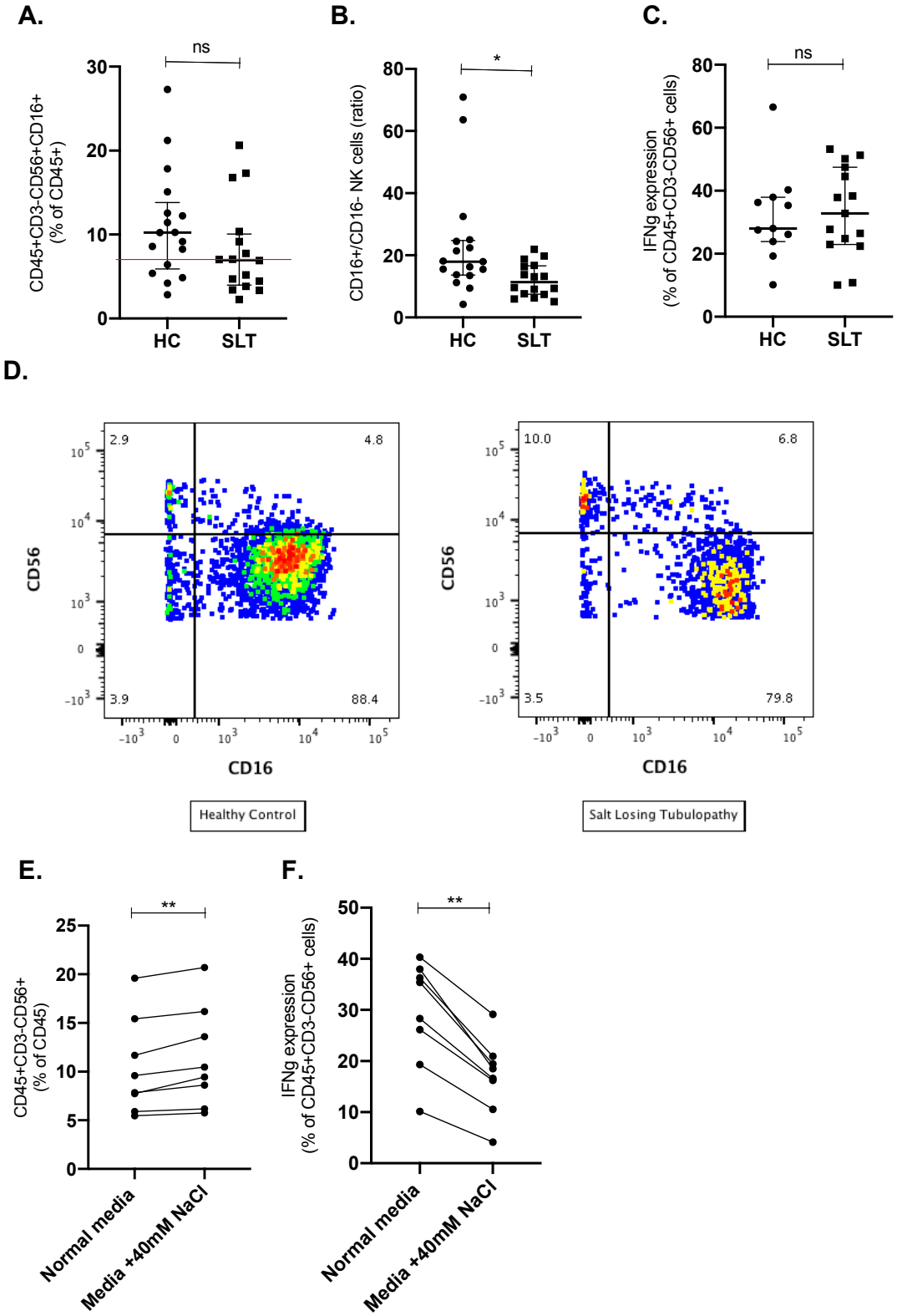
A. Classical (CD14+CD16-), intermediate (CD14+CD16+), and non-classical (CD14-CD16+) monocyte subsets in SLT patients (n=14) and HCs (n=12). Groups are compared with a two-sided Mann-Whitney test. Error bars represent interquartile range around the median.

B. Representative FACS dot plots of CD14 and CD16 staining in a HC and SLT patient. Cell proportions as percentages are documented within each quadrant.

C. Proportion of Lipopolysaccharide (LPS) stimulated CD14+ cells expressing TNF in SLT patients (n=14) and HCs (n=8). Groups are compared with a two-sided Mann-Whitney test. Error bars represent interquartile range around the median.

D. Effect of 40mM NaCl (n=7 healthy controls), 2mM KCl (n=5 healthy controls), and 1mM $MgCl_2$ (n=5 healthy controls) on TNF expression in LPS stimulated CD14+ cells. Expressed as a ratio of TNF expression in ionic supplemented media compared to standard conditions. Red line drawn at ratio of 1 represents no difference with addition of ions.

SLT – Salt-Losing Tubulopathy; HC – healthy control. ns – not significant ($p>0.05$), *$p\leq0.05$, **$p\leq0.01$, ***$p\leq0.001$, ****$p\leq0.0001$. Source data are provided as a **Source data file.**

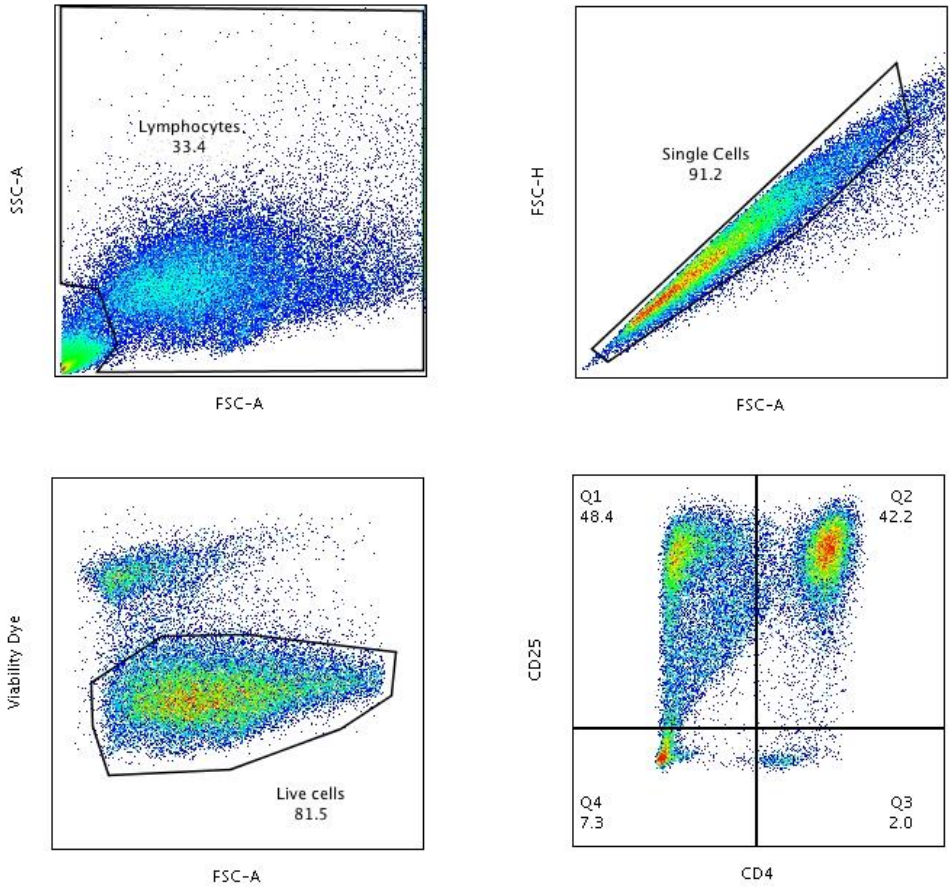


Supplemental Figure 10: Analysis of Natural Killer (NK) cells in Salt-Losing Tubulopathy patients. **A**. NK cells (CD45+CD3-CD56+CD16+ cells) expressed as a proportion of lymphocytes (CD45+ cells) in SLT patients (n=16) and HCs (n=17): HC 10.2% (5.9-13.8), SLT 7.0% (4.0-10.1), p=0.11. Groups are compared with a two-sided Mann-Whitney test. Error bars represent interquartile range around the median. Red line drawn at 7% represents clinical laboratory lower limit of normal range. **B.** Ratio of CD56+CD16+ to CD56+CD16- cells in SLT patients (n=16) and HCs (n=17): HC 18.0 (13.7-24.8), SLT 11.4 (7.5-16.7), p=0.01. Groups are compared with a two-sided Mann-Whitney test. Error bars represent interquartile range around the median.

C. IFNγ expression by CD45+CD3-CD56+ cells after 4 hours IL-12 and IL-18 stimulation in SLT patients (n=15) and healthy controls (n=12): HC 28.0% (23.9-38.0), SLT 32.8% (23.0-47.5), p=0.72. Groups are compared with a two-sided Mann-Whitney test. Error bars represent interquartile range around the median.
D. Representative FACS dot plots of CD56 and CD16 staining in a SLT patient and HC (gated on single CD45+CD3-CD56+ cells, therefore CD56 gate distinguishes CD56 bright from dim). SLT patients had reduced CD16 staining on CD56+ cells, as shown. Cell proportions as percentages are documented within each quadrant.
E. NK cells in healthy controls (n=8), expressed as a proportion of lymphocytes (CD45+ cells) after stimulation (IL-12 and IL-18) with and without additional 40mM NaCl: normal media 8.7% (6.4-14.5), +40mM NaCl 10.0% (6.8-15.5), p=0.008. Change within individual subjects is plotted. Conditions compared with a two-sided Wilcoxon test.
F. IFNγ expression by healthy control (n=8) NK cells after stimulation (IL-12 and IL-18) with and without additional 40mM NaCl: normal media 31.8% (21.0-37.6), +40mM NaCl 17.6% (12.0-20.6), p=0.008. Change in individual subjects is plotted. Conditions compared with a two-sided Wilcoxon test.

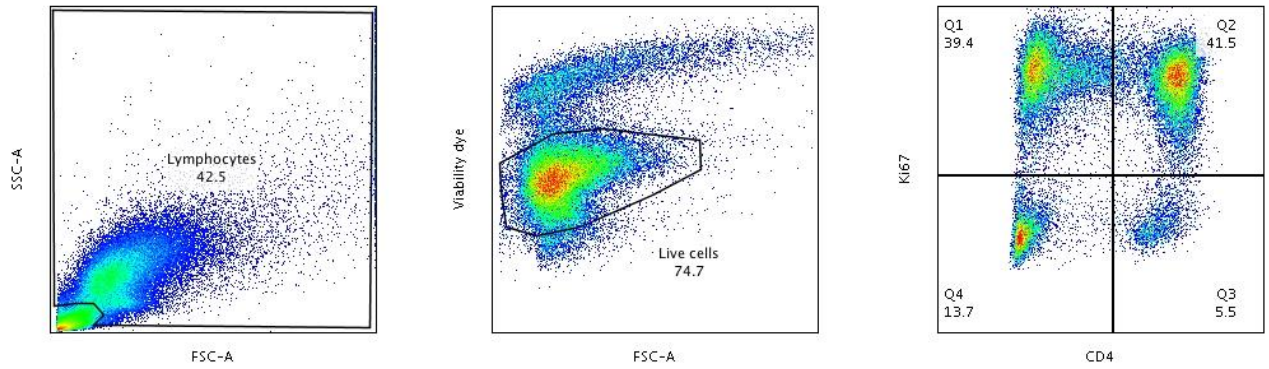
ns – not significant (p>0.05), *p≤0.05, **p≤0.01, ***p≤0.001, ****p≤0.0001; SLT – Salt-Losing Tubulopathy; HC – healthy control. Source data are provided as a **Source data file.**

Supplemental Figure 11: FACS gating strategies. Legends underneath each set of plots describe the gating strategy and outline the relevant figures in which the FACS analysis has been used.



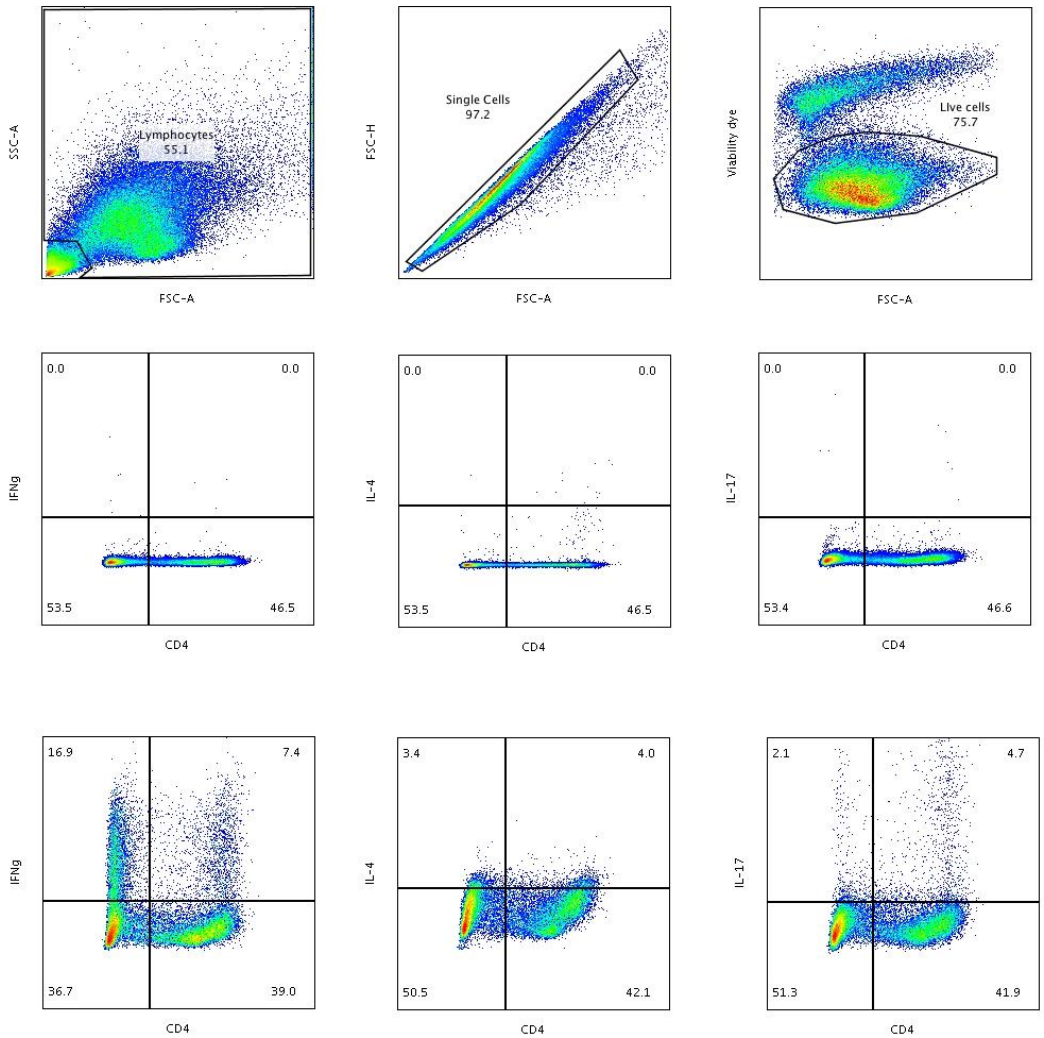
i. Gating used during FACS analysis of T cell activation (Figure 2)

Top panel: Lymphocytes were gated on a FSC-SSC plot; single cells were gated on a FCS-A-FSC-H plot.
Middle Panel: Live cells were gated on a FSC-Viability dye plot; CD25 expression in CD4+ cells was determined by CD4-CD25 gating.



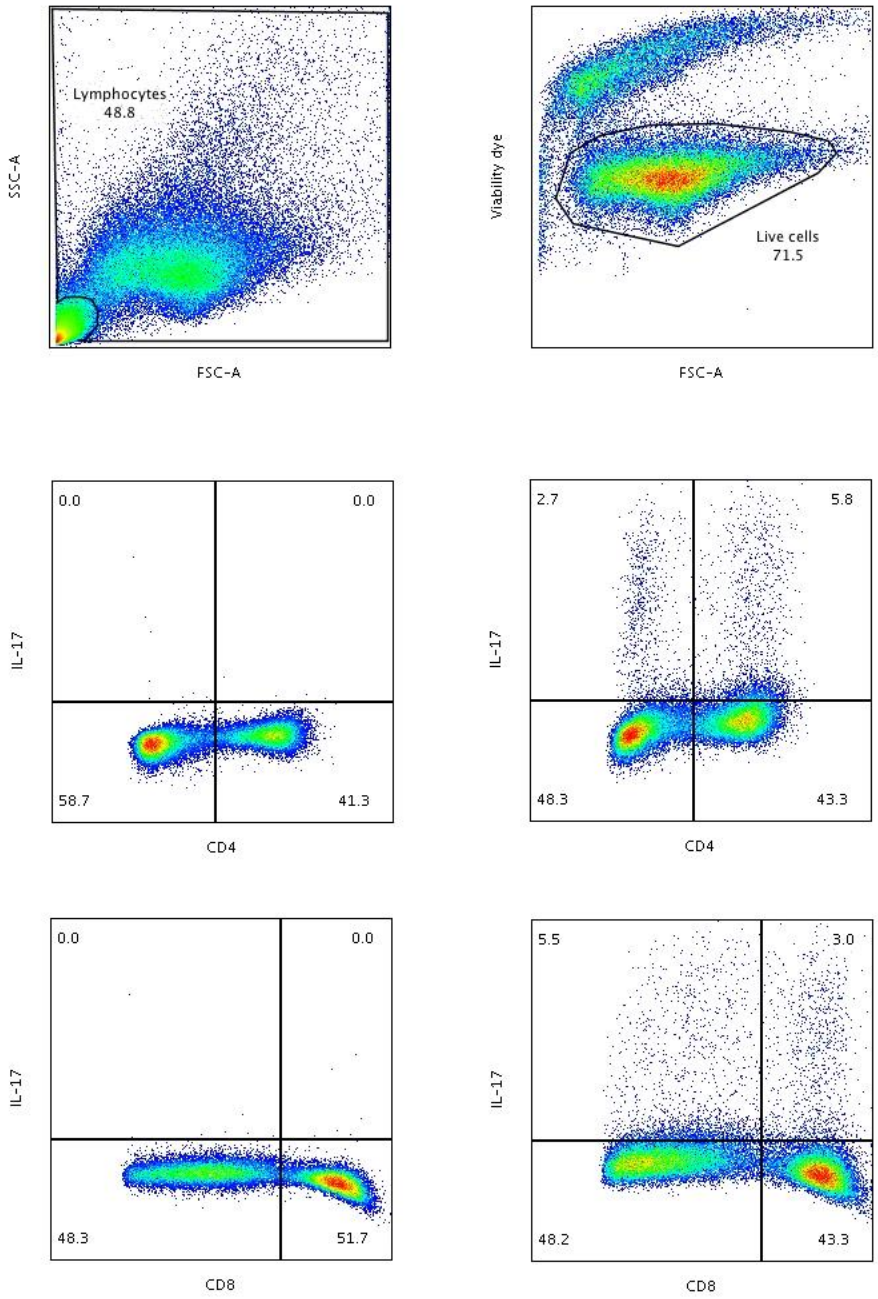
ii. Gating used during FACS analysis of T cell proliferation (Figure 2)

Lymphocytes were gated on a FSC-SSC plot; live cells were gated on a FSC-Viability dye plot; Ki67 expression in CD4+ cells was determined by CD4-Ki67 gating.



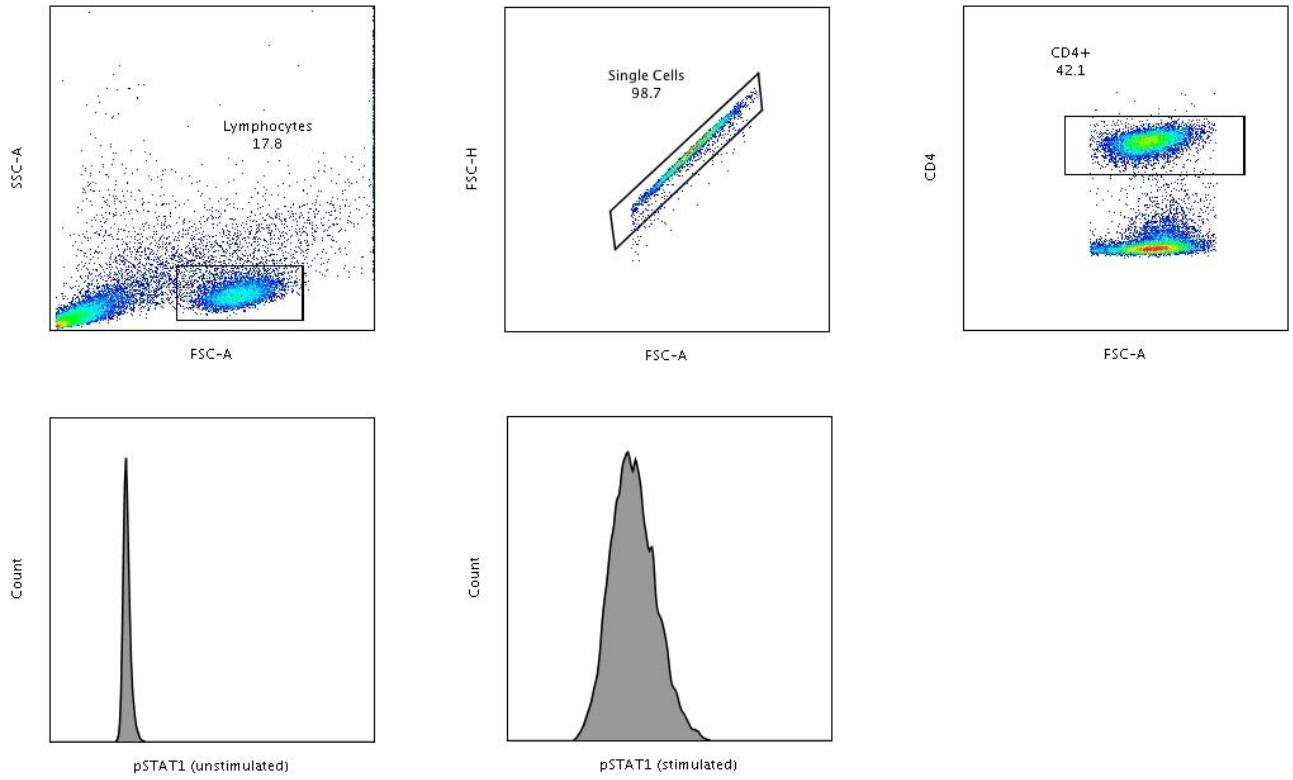
iii. Gating used during FACS analysis of CD4 subsets (Figures 3 and 6)

Cytokine (IFNγ, IL-4, and IL-17) gates in CD4+ cells were determined by FMO
Top panel: Lymphocytes were gated on a FSC-SSC plot; Single cells were gated on a FCS-A-FSC-H plot; live cells were gated on a FSC-Viability dye plot.
Middle Panel: Cytokine expression was determined by CD4-cytokine gating; middle panel demonstrates FMO for each cytokine.
Bottom panel: Representative staining of IFNγ, IL-4, and IL-17 against CD4 in a healthy control



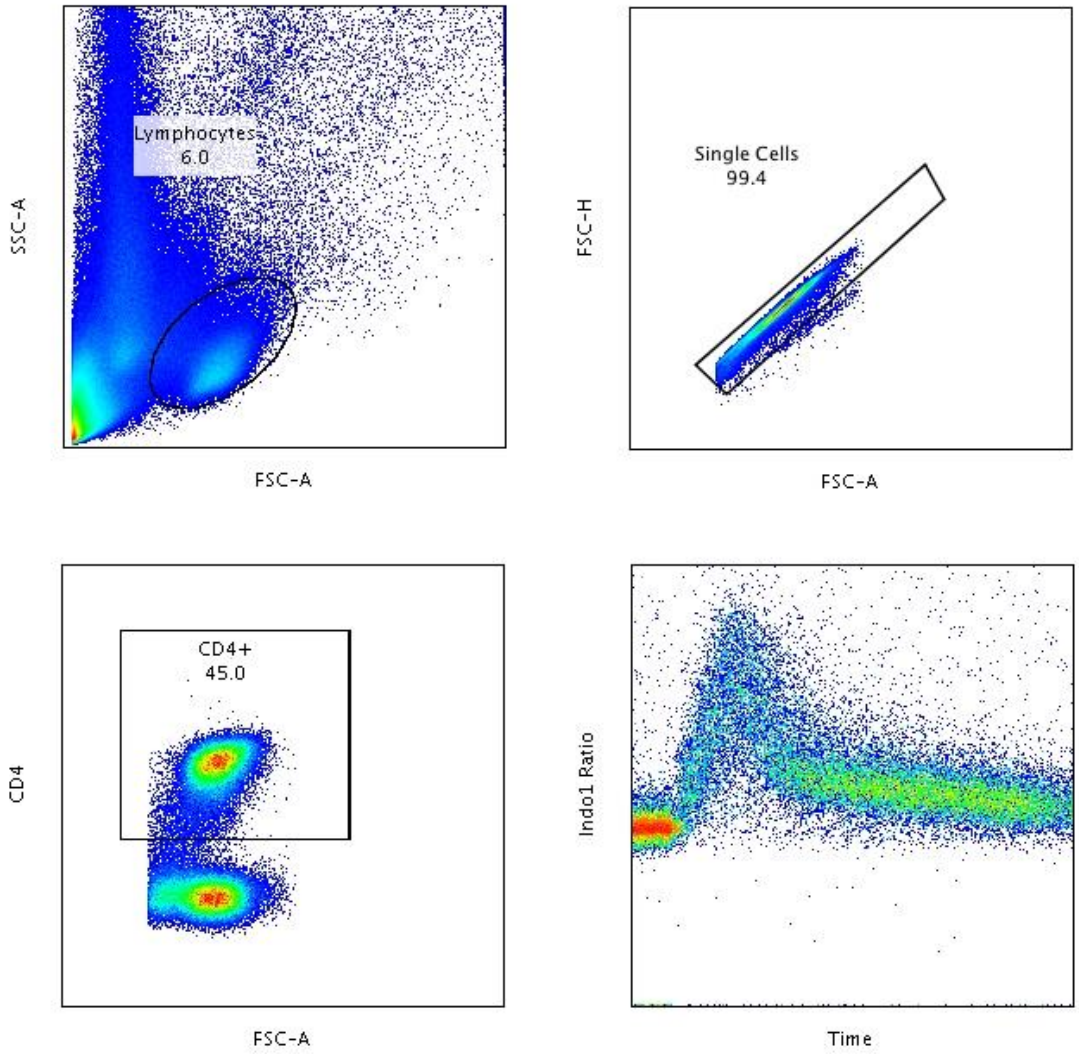
iv. Gating used during FACS analysis of Th17 and Tc17 polarisation (Figures 4, 5, 7, and 8, and Supplemental Figures 6 and 8)

Gates for IL-17 were determined by fluorescence minus one (FMO)
Top panel: Lymphocytes were gated on a FSC-SSC plot; live cells were gated on a FSC-Viability dye plot.
Middle Panel: IL-17 expression was determined by CD4-IL-17 gating; left panel demonstrates FMO.
Bottom panel: IL-17 expression was determined by CD8-IL-17 gating; left panel demonstrates FMO.



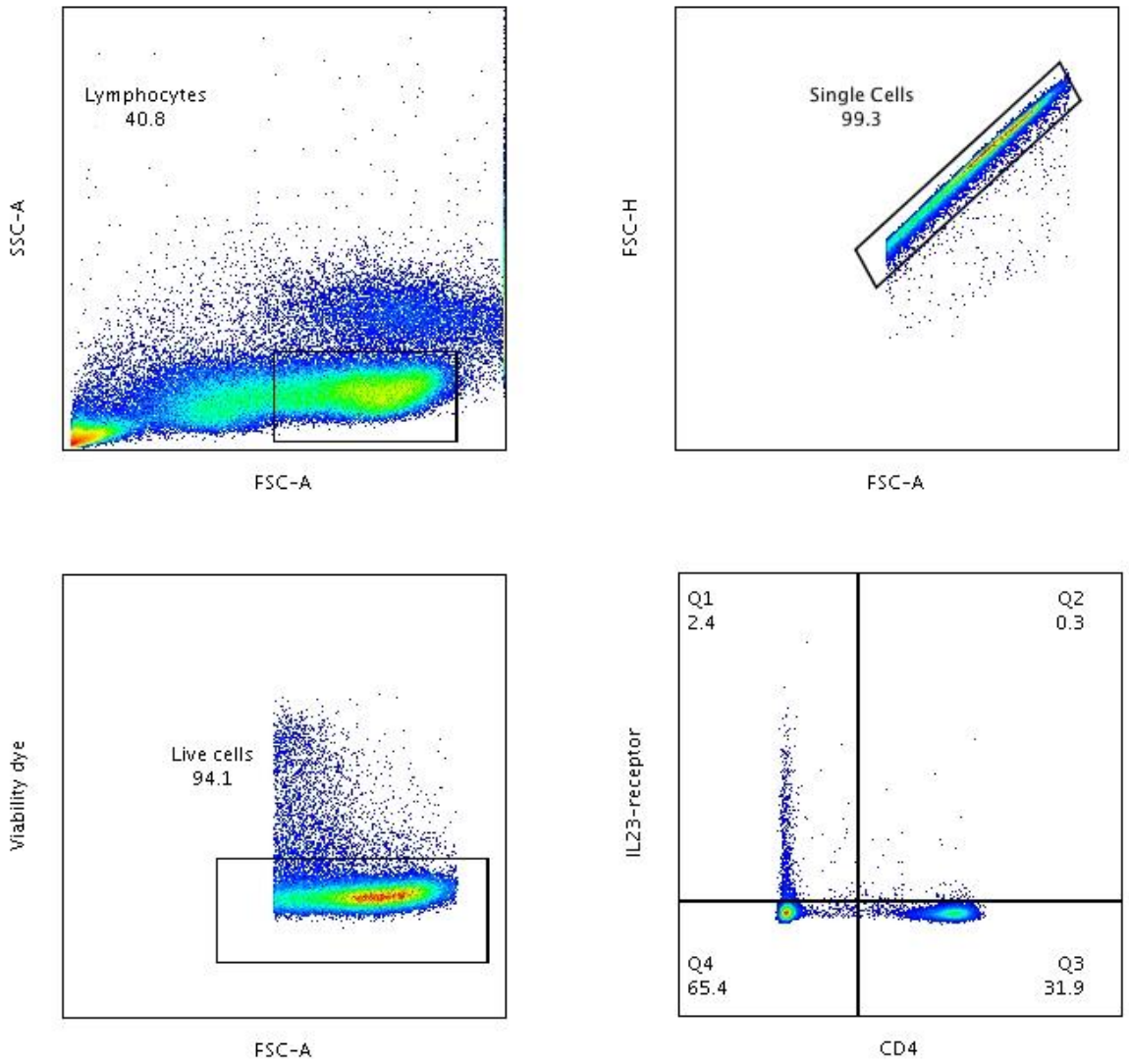
v. Gating used during FACS analysis of STAT1 and STAT3 phosphorylation (Figure 5 and Supplemental Figure 4)

Top panel: Lymphocytes were gated on a FSC-SSC plot; single cells were gated on a FSC-A-FSC-H plot; CD4 cells were then gated on a FSC-CD4 plot.
Median fluorescence staining intensity (MFI) of pSTAT1 and pSTAT3 were determined in the CD4+ population.
Bottom panel: Histograms represent pSTAT1 staining in unstimulated (left panel) and stimulated (right panel) cells. Increase in MFI was determined for each subject



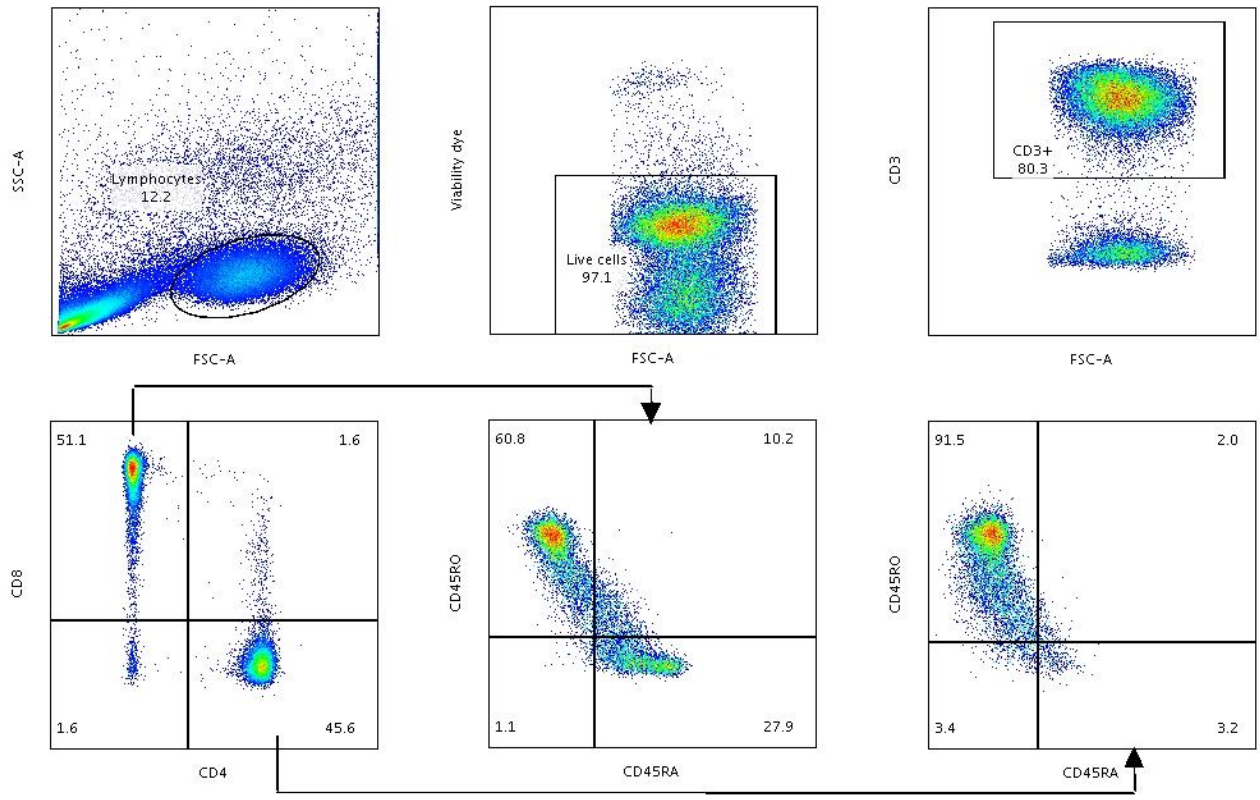
vi. Gating used for FACS analysis of Calcium Flux during T cell activation (Figure 5 and Supplemental Figure 4)

Top panel: Lymphocytes were gated on a FSC-SSC plot; single cells were gated on a FSC-A-FSC-H plot.
Bottom panel: CD4+ cells were gated on a FSC-CD4 plot; calcium flux was determined by plotting Indo1 ratio (fluorescence at 400nM compared to 475nm) over time



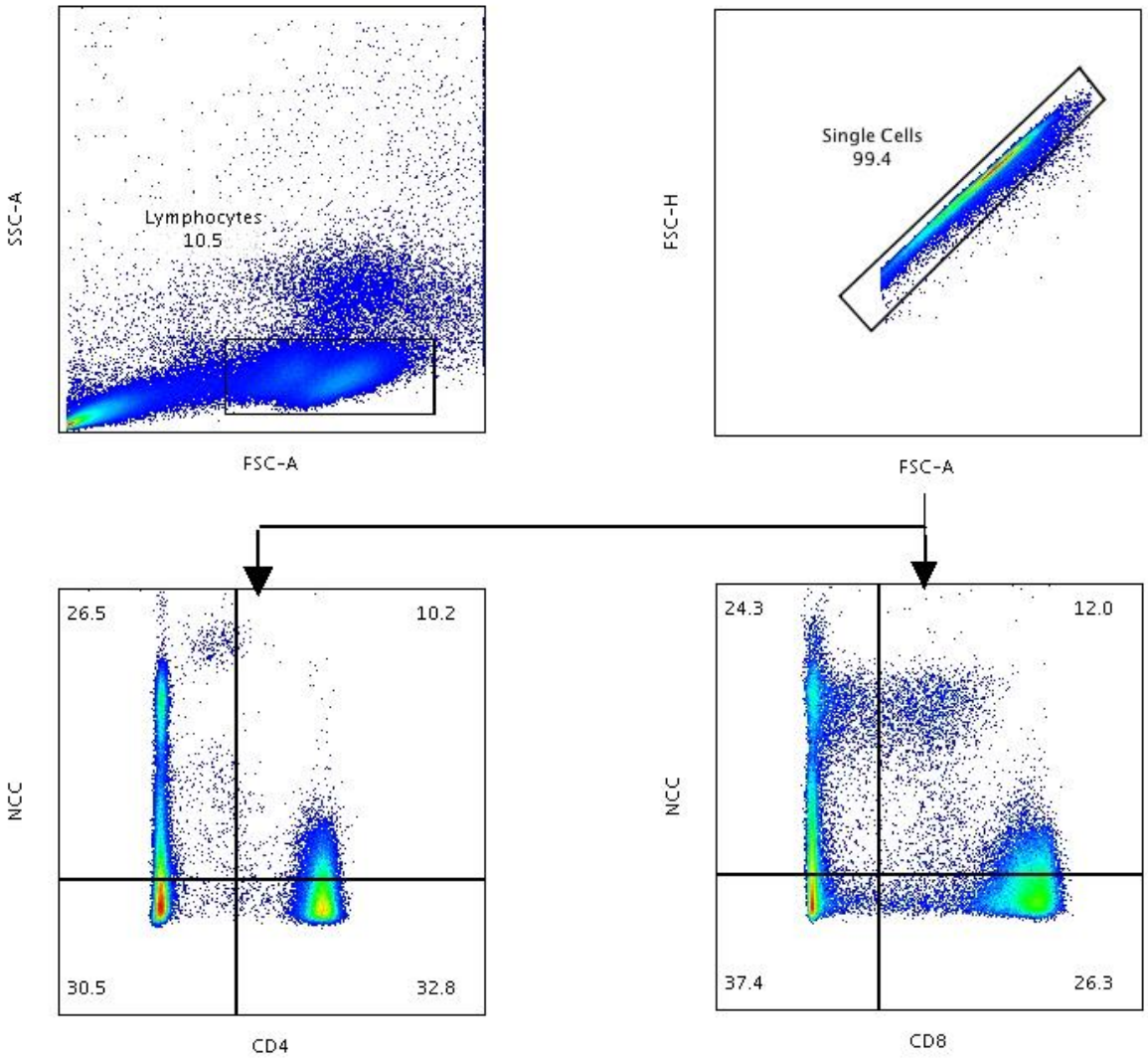
vii. Gating used for FACS analysis of T cell expression of the IL23 receptor (Figure 8 and Supplemental Figure 8)

Top panel: Lymphocytes were gated on a FSC-SSC plot; single cells were gated on a FSC-A-FSC-H plot.
Bottom panel: Live cells were gated on a FSC-Viability dye plot; IL23 receptor (IL23R) expression was determined by CD4-IL23R gating



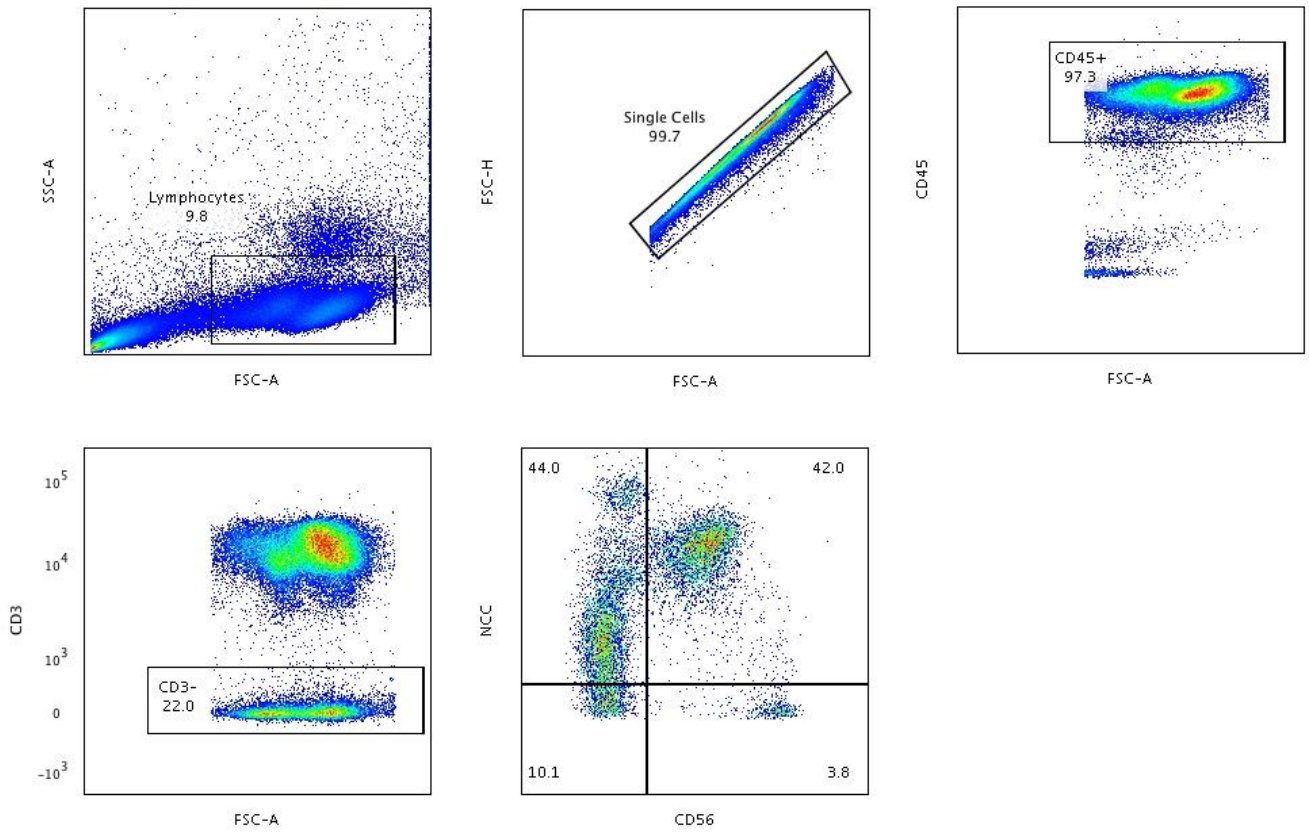
viii. Gating used for FACS analysis of T cell populations (Supplemental Figure 3)

Top panel: Lymphocytes were gated on a FSC-SSC plot; live cells were gated on a FSC-Viability dye plot; CD3+ cells were gated on a FSC-CD3 plot.
Bottom panel: CD4 and CD8 expression on CD3 cells were determined by a CD4-CD8 plot; CD45RA-CD45RO plots were then used to determine expression of CD3+CD8+CD4- (bottom middle panel) and CD3+CD8-CD4+ (bottom right panel) cells.



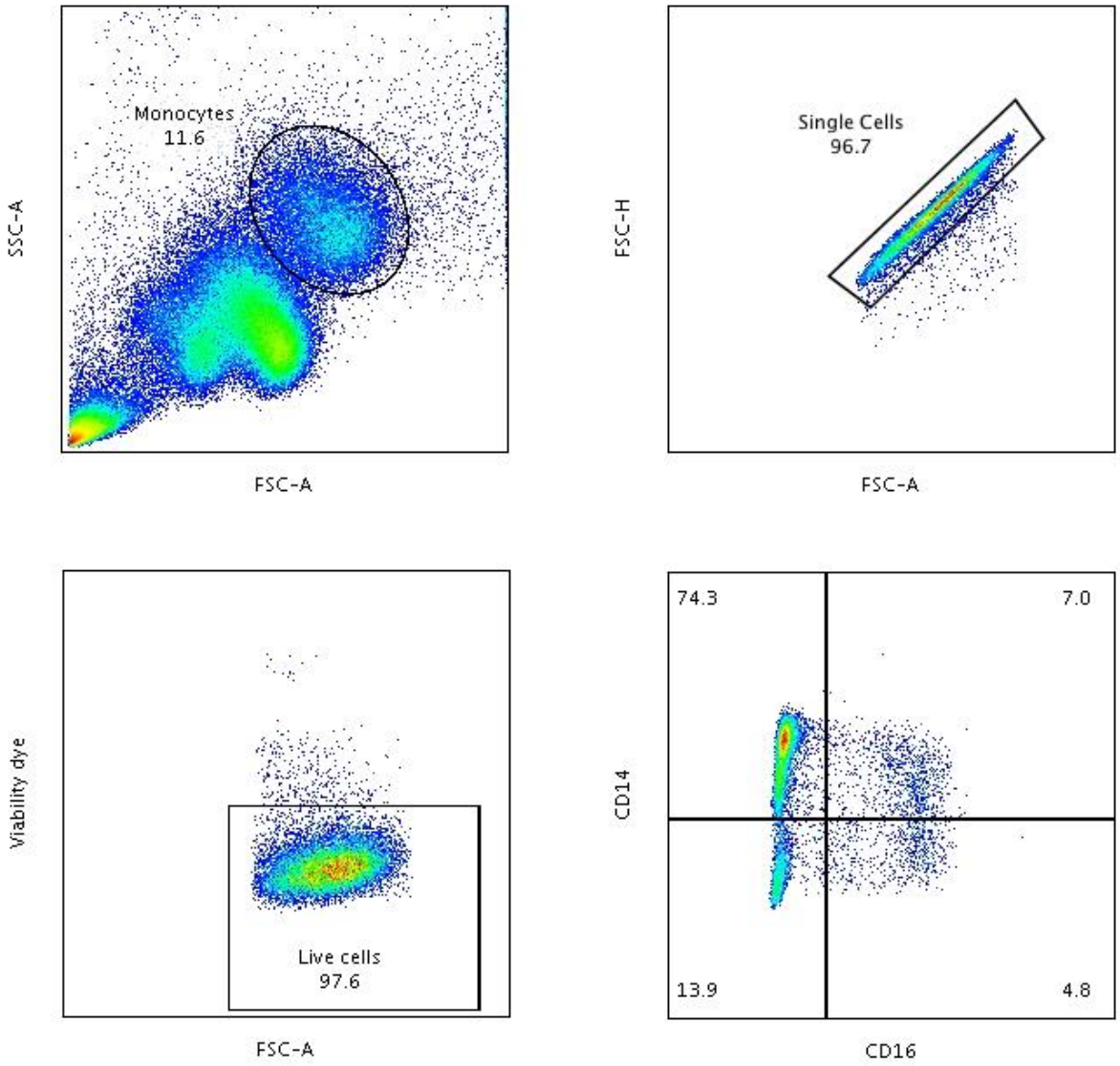
ix. Gating used during FACS analysis of T cell NCC expression (Supplemental Figure 4)

Top panel: Lymphocytes were gated on a FSC-SSC plot; single cells were gated on a FCS-A-FSC-H plot.
Bottom Panel: NCC expression on CD4 cells was determined by a CD4-NCC plot; and NCC expression on CD8 cells was determined by a CD8-NCC plot with these gates determined by FMO.



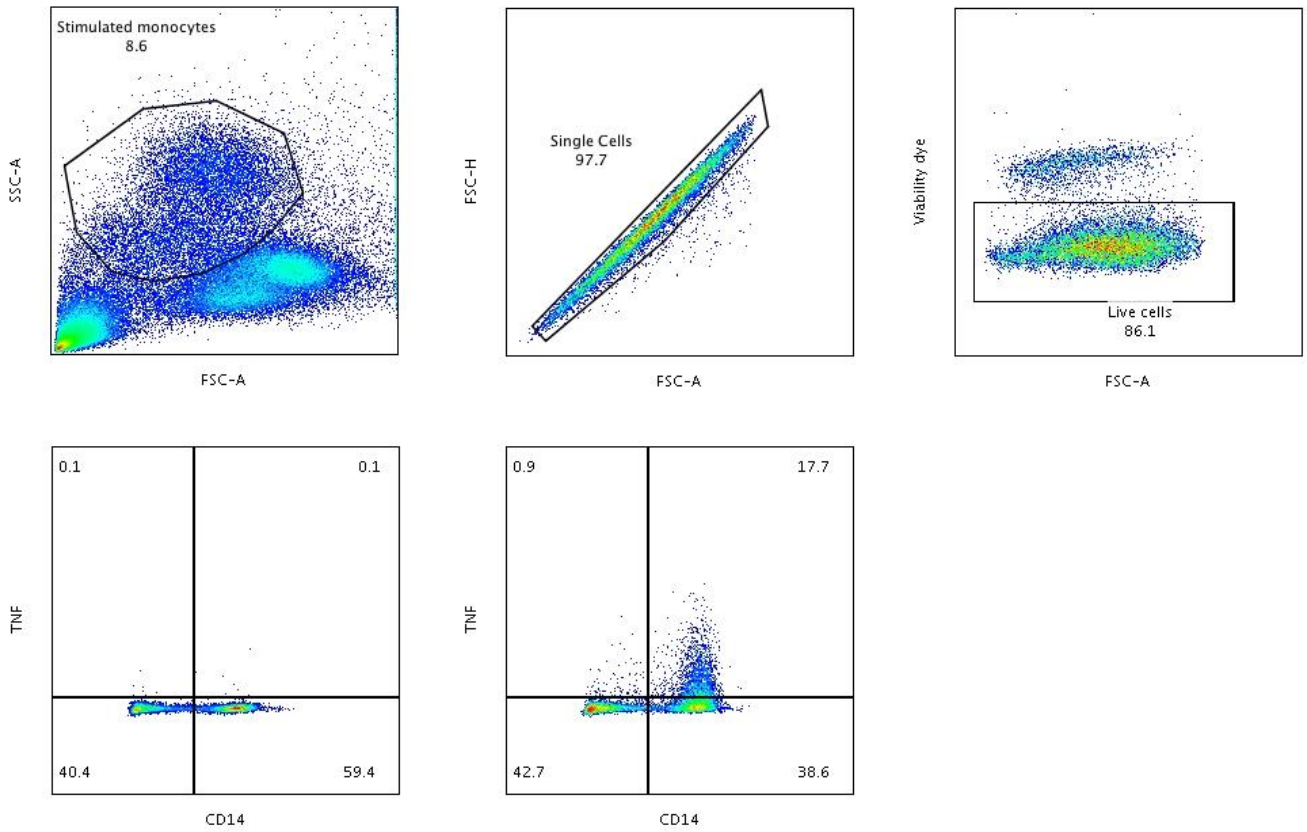
x. Gating used during FACS analysis of NK cell NCC expression (Supplemental Figure 4)

Top panel: Lymphocytes were gated on a FSC-SSC plot; single cells were gated on a FCS-A-FSC-H plot; CD45+ cells were gated on a FSC-CD45 plot.
Bottom Panel: CD45+CD3- cells were gated on a FSC-CD3 plot; NCC expression on CD45+CD3- CD56+ cells was determined by a CD56-NCC plot with these gates determined by FMO.



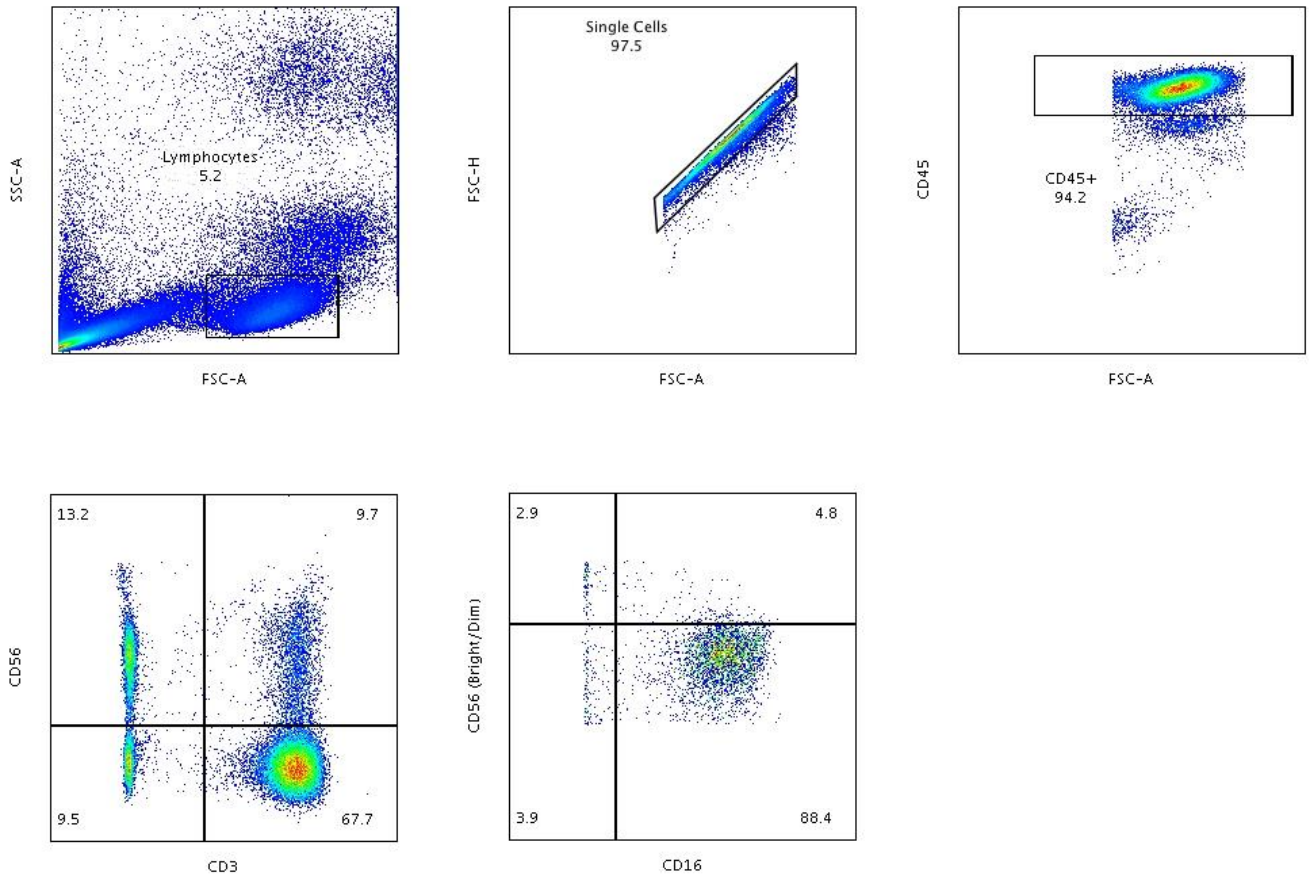
xi. Gating used for FACS analysis of monocyte subsets (Supplemental Figure 9)

Top Panel: Monocytes were gated on a FSC-SSC plot, and single cells on a FSC-A-FSC-H plot
Bottom Panel: Live cells were gated on a FSC-viability dye plot; CD14-CD16 gates then determined classical (CD14+CD16-), intermediate (CD14+CD16+) and non-classical (CD14-CD16+) monocyte populations.



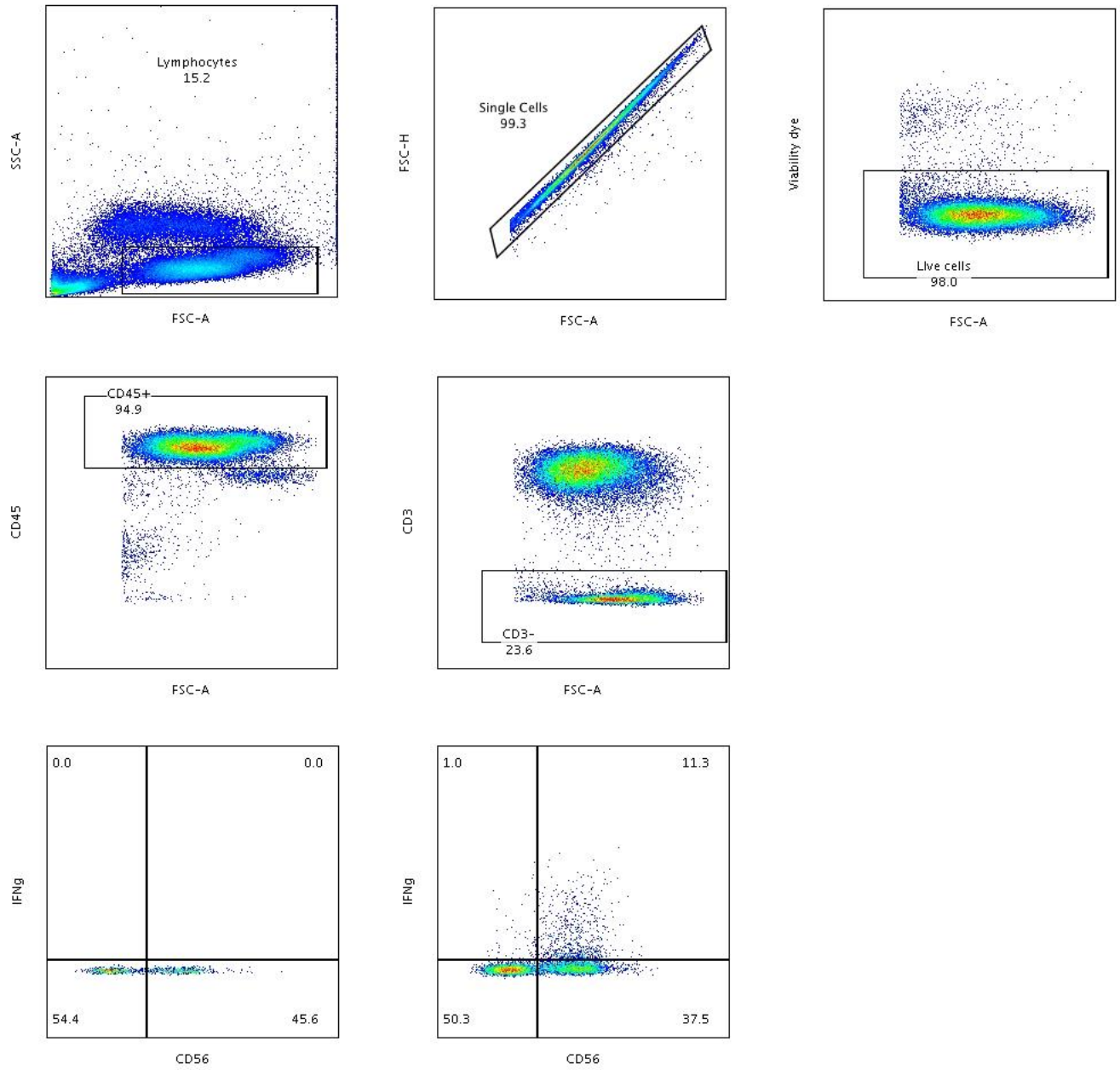
xii. Gating used for FACS analysis of monocyte expression of TNF (Supplemental Figure 9)

Top panel: Stimulated monocytes were gated on a FSC-SSC plot; single cells were gated on a FSC-A-FSC-H plot; live cells were gated on a FSC-Viability dye plot.
Bottom Panel: TNF expression was determined by CD14-TNF gating; bottom left panel demonstrates FMO for TNF and bottom right panel demonstrates TNF staining in a HC.



xiii. Gating used for analysis of NK cell subsets (Supplemental Figure 10)

Top Panel: Lymphocytes were gated on an FCS-SSC plot; single cells were gated on a FSC-A-FSC-H plot; CD45+ cells were gated on a FSC-CD45 plot.
Bottom Panel: CD3-CD56+ cells were gated on a CD3-CD56 plot (bottom left panel, top left quadrant); CD56 bright versus dim was then gated against CD16 to determine CD56brightCD16neg, CD56brightCD16pos, and CD56dimCD16pos cells.



xiv. Gating used for FACS analysis of NK cell expression of IFNγ (Supplemental Figure 10)

Top panel: Lymphocytes were gated on a FSC-SSC plot; single cells were gated on a FSC-A-FSC-H plot; live cells were gated on a FSC-Viability dye plot.
Middle panel: CD45+ cells were gated on a FSC-CD45 plot; CD3- cells were gated on a FSC-CD3 plot
Bottom Panel: IFNγ expression was determined by CD56-IFNγ gating; bottom left panel demonstrates FMO for IFNγ and bottom right panel demonstrates IFNγ staining in a HC.

Patient number	Age	Sex	Diagnosis	Gene Affected	Nucleotide Change	Protein Change	Status
1	36	M	Bartter (type 1)	*SLC12A1*	c.1215G>A	p.(=)	Homozygous
2	26	F	Bartter (type 1)	*SLC12A1*	c.1316G>A	p.Arg439Gln	Homozygous
3	18	M	Bartter (type 1)	*SLC12A1*	c.450-451del; c.967G>A	Asp150Glufs*4; Glu323Lys	Compound heterozygous
4	17	M	Bartter (type 1)	*SLC12A1*	c.1316G>A	p.Arg439Gln	Homozygous
5	19	M	Bartter (type 2)	*KCNJ1*	Not available		
6	18	F	Bartter (type 2)	*KCNJ1*	Not available		
7	33	M	Bartter (type 2)	*KCNJ1*	Not available		
8	41	F	Bartter (type 2)	*KCNJ1*	c.89G>A	p.Cys30Tyr	Homozygous
9	28	M	Bartter (type 3)	*CLCNKB*	Not available		
10	31	M	Bartter (type 3)	*CLCNKB*	Whole gene deletion		Homozygous
11	41	F	Bartter (type 3)	*CLCNKB*	Whole gene deletion		Homozygous
12	32	F	Bartter (type 3)	*CLCNKB*	c.887G>A; c.1929+1G>A	p.gly296asp	Compound heterozygous
13	34	M	Bartter (type 3)	*CLCNKB*	c.887G>A; c.1929+1G>A	p.gly296asp	Compound heterozygous
14	31	M	Bartter (type 3)	*CLCNKB*	c.1897del	p.Leu633*	Homozygous
15	30	M	Bartter (type 3)	*CLCNKB*	c.875G>T	p.Cys292Phe	Homozygous
16	23	M	Bartter (type 3)	*CLCNKB*	c.1395del	p.Tyr466Metfs*13	Homozygous
17	2	F	Bartter (type 3)	*CLCNKA; CLCNKB*	Partial Deletion both genes		Heterozygous
18	59	F	Bartter (type 3)	*CLCNKB*	Partial Deletion		Homozygous
19	39	M	Bartter (type 3)	*CLCNKB*	Whole gene deletion		
20	34	M	Bartter (type 3)	*CLCNKB*	Not available		
21	24	F	Bartter (type 3)	*CLCNKB*	Whole gene deletion		Homozygous
22	33	M	Bartter (type 3)	*CLCNKB*	c.1783C>T	p.Arg595*	Homozygous
23	18	F	Bartter (type 4a)	*BSND*	c.125G>A; c.139G>A	p.Ser42Asn; p.Gly47Arg	Compound heterozygous
24	27	M	EAST	*KCNJ10*	c.194G>C	p.Arg65Pro	Homozygous
25	25	F	EAST	*KCNJ10*	c.194G>C	p.Arg65Pro	Homozygous
26	52	F	Gitelman	*SLC12A3*	c.1000C>T; c.2221G>A	p.Arg334Trp; p.Gly741Arg	Compound heterozygous
27	37	F	Gitelman	*SLC12A3*	c.2221G>A; c.2581C>T	p. Gly741Arg; p.Arg861Cys	Compound heterozygous
28	35	M	Gitelman	*SLC12A3*	c.1825del; ex1_ex8del	p.Glu609Argfs*2	Compound heterozygous
29	85	M	Gitelman	*SLC12A3*	c.961C>T; c.2883+1G>T	p.Arg321Trp; p.?	Compound heterozygous
30	57	F	Gitelman	*SLC12A3; WNK4*	c.111T>A; c.1510C>T	p.Tyr37*; p.Gln504*	SLC12A3 heterozygous, WNK4 heterozygous
31	41	F	Gitelman	*SLC12A3*	c.1196_1202dup; c.2221G>A	p.Ser402*; p.Gly741Arg	Compound heterozygous
32	64	F	Gitelman	*SLC12A3*	c.1928C>T; ex14del	p.Pro643Leu	Compound heterozygous
33	52	M	Gitelman	*SLC12A3*	c.1664C>T; c.2882+1G>T	p.Ser555Leu	Compound heterozygous
34	36	F	Gitelman	*SLC12A3*	c.1261T>C; c.1930del	p.Cys421Arg; p.Gln644Serfs*28	Compound heterozygous
35	47	M	Gitelman	*SLC12A3*	c.1028T>A	p.Met343Lys	Homozygous

36	33	F	Gitelman	*SLC12A3*	c.237_238dup	p.Arg80Profs*35	Homozygous
37	68	F	Gitelman	*SLC12A3*	c.1046C>T; c.1055C>A	p.Pro349Leu; p.Thr352Lys	Compound heterozygous
38	39	M	Gitelman	*SLC12A3*	c.2576T>C; c.2965G>A	p.Leu859Pro; p.Gly989Arg	Compound heterozygous
39	40	F	Gitelman	*SLC12A3*	Not available		
40	62	M	Gitelman	*SLC12A3*	c.1046C>T; c.1055C>A	p.Pro349Leu; p.Thr352Lys	Compound heterozygous
41	31	F	Gitelman	*SLC12A3*	Not available		
42	35	F	Gitelman	*SLC12A3*	Not available		
43	58	F	Gitelman	*SLC12A3*	c.910A>C; c.2883+1G>T	p.Thr304Pro	Compound heterozygous
44	39	F	Gitelman	*SLC12A3*	c.2368+1del; ex6del		Compound heterozygous
45	67	F	Gitelman	*SLC12A3*	c.427A>G; c.2221G>A	p.Met143Val; p.Gly741Arg	Compound heterozygous
46	43	F	Gitelman	*SLC12A3*	c.1664C>T; c.2882+1G>T	p.Ser555Leu	Compound heterozygous
47	31	F	Gitelman	*SLC12A3*	c.1964G>A; c.2221G>A	p. Arg655His; p.Gly741Arg	Compound heterozygous

Supplemental Table 1: Demographic data and genotype of Salt-Losing Tubulopathy patients (n=47)

Number	Age	Sex	Diagnosis	Na (mmol per litre)	K (mmol per litre)	Cl (mmol per litre)	HCO3 (mmol per litre)	Creatinine (µmol per litre)	cCa (mmol per litre)	PO4 (mmol per litre)	Mg (mmol per litre)
1	35	M	Proximal tubulopathy (fumarate toxicity)	141	4.4	103	23	79	2.29	1.22	
2	38	F	Proximal tubulopathy (HNF4A mutation)	137	3.9	102	16	211	2.4	1.03	0.93
3	48	M	Proximal tubulopathy (Lowe syndrome)	142	4.4	102	22	162	2.39	0.87	0.98
4	53	M	Proximal tubulopathy (Wilson disease)	141	4.5	103	22	111	2.49	1.13	0.87
5	33	F	Proximal tubulopathy (fumarate toxicity)	143	3.9	105	23	81	2.36	0.77	1
6	46	M	Proximal tubulopathy (Wilson disease)	141	4.5	99	25	152	2.39	1.26	0.91
7	51	M	Proximal tubulopathy (inherited)	141	4	102	25	120	2.51	0.81	0.93
8	34	F	FHH	141	5	102	25	62	2.4	1.12	0.77
9	60	F	FHH	137	6.4	108	19	59	2.36	1.16	0.75
10	6	M	FHH								
11	23	F	dRTA	140	4.2	103	24	52	2.33	0.93	0.87
12	8	M	dRTA								
13	55	F	dRTA	138	4.7	104	21	51	2.23	0.85	0.85
14	20	F	TRPM6 mutation (isolated Mg wasting)	141	4.1	106	22	42	2.36	0.72	0.51
15	53	M	Primary	142	4.2		25	93	2.49	0.92	0.89

			hyperparathyroidism								
16	49	F	STX16 mutation (pseudohypoparathyroidism)	140	4.1	98	25	62	2.43	1.19	0.81
17	19	M	STX16 mutation (pseudohypoparathyroidism)	140	4.7	104	23	78	2.24	1.66	0.89
18	59	F	TIN	138	4.3	100	28	118	2.38	1.08	0.87
19	51	F	TIN	138	4.2	103	26	145	2.42	1.1	0.76
20	75	M	Medullary sponge kidney	144	4.2	103	25	94	2.39	1.11	0.79
21	15	M	Nephrogenic diabetes insipidus								
22	31	F	Hypokalaemia (resolved) – extra renal loss	142	4.4	101	27	76	2.45	1.16	0.85

Supplemental Table 2: Demographic and clinical data (diagnosis and serum biochemistry) of disease controls (n=22)
FHH – familial hyperkalaemic hypertension; dRTA – distal renal tubular acidosis; TIN – tubulointerstitial nephritis

	Whole SLT cohort n=47	Bartter syndrome types 1,2, and 4 n=9	Bartter syndrome type 3 n=14	Gitelman and EAST syndrome n=24	P-value
Na (mmol per litre)	140 (3)	141 (4)	140 (3)	140 (3)	0.68
K (mmol per litre)	3.3 (0.6)	3.7 (0.6)	2.9 (0.6)	3.4 (0.5)	**0.0045**
Cl (mmol per litre)	95 (4)	99 (2)	91 (4)	96 (3)	**<0.0001**
HCO3 (mmol per litre)	28 (4)	25 (3)	30 (4)	28 (3)	**0.014**
Creatinine (µmol per litre)	94 (79)	166 (140)	103 (63)	62 (14)	**0.0005**
cCa (mmol per litre)	2.43 (0.13)	2.41 (0.12)	2.41 (0.14)	2.45 (0.12)	0.59
PO4 (mmol per litre)	1.11 (0.20)	1.19 (0.25)	1.13 (0.21)	1.06 (0.16)	0.20
Mg (mmol per litre)	0.70 (0.17)	0.79 (0.18)	0.75 (0.16)	0.64 (0.16)	0.05

Supplemental Table 3: Serum biochemistry in the Salt-Losing Tubulopathy (SLT) Cohort (n=47). Values reported are mean and standard deviation. Variables are compared between BS types 1,2, and 4 ('antenatal BS/loop phenotype'; n=9), BS type 3 ('classical BS/mixed loop and distal tubule phenotype'; n=14), and GS/EAST syndrome ('distal tubule phenotype'; n=24) with a one-way analysis of variance. Source data are provided as a **Source data file.**

	Salt-Losing Tubulopathy n=47	Disease Control n=19	P-value
Na (mmol per litre)	140 (138-142)	141 (138-142)	0.54
K (mmol per litre)	3.3 (2.9-3.8)	4.3 (4.1-4.5)	**<0.0001**
Cl (mmol per litre)	95 (91-99)	103 (102-104)	**<0.0001**
HCO3 (mmol per litre)	29 (26-30)	24 (22-25)	**<0.0001**
Creatinine (µmol per litre)	71 (57-88)	81 (62-120)	0.21
cCa (mmol per litre)	2.42 (2.37-2.51)	2.39 (2.36-2.43)	0.09
PO4 (mmol per litre)	1.08 (1.00-1.23)	1.10 (0.87-1.16)	0.42
Mg (mmol per litre)	0.70 (0.58-0.79)	0.87 (0.79-0.92)	**0.0004**

Supplemental Table 4: Serum biochemistry in Salt-Losing Tubulopathy patients (n=47) and disease controls (n=19). Values reported are median and interquartile range, and are compared between groups with a two-sided Mann-Whitney test. Source data are provided as a **Source data file.**

Total number of skin abscesses in lifetime	None (0)	1-2 (2)	3-4 (4)	>4 (8)
Total number x-ray proven pneumonias in lifetime	None (0)	1 (2)	2 (4)	3 (6)
Other serious infections (requiring hospital admission or intravenous antibiotics)	None (0)	Severe (4)		
Number of upper respiratory tract infections (tonsillitis, sinusitis and otitis) in worst year	0-2 (0)	3 (1)	4-6 (2)	>6 (or tonsillectomy) (4)
Fungal infection?	None (0)	Oral, vaginal (*Candida*) (1)	Fingernail (2)	Systemic (4)

Supplemental Table 5: Scoring system used to assess infection related to IL-17 defects (points attributed to each answer in red parentheses). Based on infection related criteria and scores used in the Hyper-IgE syndrome diagnostic criteria.

Study Reference	Salt-Losing Tubulopathy Cohort in this study	**Autosomal Dominant Hyper-IgE Syndrome in the USIDNET Registry.** Gernez et al. J Allergy Clin Immunol Pract 2018.	**Heterozygous STAT1 gain-of-function mutations underlie an unexpectedly broad clinical phenotype.** Toubiana J et al. Blood 2016.
Location	United Kingdom	North America	International (62% European)
Genetic cause of chronic mucocutaneous candiasis	NA	STAT3 deficiency (Autosomal Dominant Hyper-IgE Syndrome)	STAT1 gain-or-function
Number of patients in cohort	47	85	274
Demographics	Median age 35 (time of study); 55.3% female	59% male; 62.4% Caucasian	Median age 22 (time of study); M:F ratio 1.03
Bacterial Infections			
Any bacterial infection	79%		74%
Abscess	21%	74%	28% (recurrent skin infections including cellulitis, abscess, and paronychia)
Lower Respiratory Tract Infection	17%	72%	47%
Upper respiratory tract infection	49%	48.8% sinusitis; 42.7% otitis; 11.7% tonsillectomy	44%
Urinary Tract Infection	36%	2.4% pyelonephritis	2.9% recurrent pyelonephritis
Other severe bacterial infection	6.4% appendicitis/diverticulitis; 6.5% skin infection; 4.3% recurrent gastroenteritis; 2.1% sepsis	18.2% cellulitis, 7.3% sepsis, 6.1% meningitis, 5.8% lymphadenitis	2.9% severe gastroenteritis, 2.6% sepsis, 6% mycobacterial diseases.
Fungal Infections			
Recurrent oral/vaginal candidiasis	38%	21%	98% chronic mucocutaneous candidiasis (93% oral thrush, glossitis, and/or chelitis; 56% oesophageal/gential)
Fingernail fungal infection	13%	28%	56%
Other fungal infection	15% fungal skin infection		10% invasive fungal infections.
Viral Infections			
Any recurrent or severe viral infection	33%	7.4% herpes.	38% at least 1 systemic or atypical viral infection, or recurrent mucocutaneous viral infection
Recurrent VZV	2%		12% shingles and 7% severe chcken-pox
Recurrent HPV	21%		12% moluscum contagiosum or warts

Recurrent HSV	14%		32% recurrent mucocutaneous viral infection (herpes simplex or varicella zoster virus)
Other viral infection			8% severe systemic viral infection
Atopic disease			
Asthma	17%	39%	
Eczema	32%	57%	
Allergic disease			
Any allergic disease	60%	65%	
Contact and environmental allergy (inc wasp stings, dust, dander pollens)	36%	18%	
Food allergy	17%	37%	
Drug allergy	26%	43%	
Angioedema/hives unexplained	11%	8.5% anaphylaxis, 15.9% urticaria	
Other features			
Malignancy		7% malignancy (lymphoma 3, thyroid 1, brain 1, squamous cell carcinoma 1)	
Autoimmune	30%		37% autoimmune manifestation (largely thyroid disease, 22%)

Supplemental Table 6: Clinical features in the Salt-Losing Tubulopathy cohort in this study and in cohorts of patients with inherited defects in IL-17 mediated immunity due to mutations in STAT1 and STAT3.

	Salt-Losing Tubulopathy N =14	Healthy Controls N =12	P-value
Demographic			
Age (median; IQR)	33 (30-42)	31 (25-42)	0.64
Male (n;%)	7 (50)	6 (50)	0.99
Microbial diversity			
Number of microbial isolates per subject (median; IQR)	4 (2-4)	3 (2-4)	0.84
Microbial burden*			
All isolates CFU/ml (median; IQR)	66 (3.2-3000)	24 (5-2400)	0.59
Staphylococcus CFU/ml (median; IQR)	36 (3-800)	15 (2-42)	0.24
Streptococcus CFU/ml (median; IQR)	1450 (27-4900)	2400 (78-422400)	0.73
Enterococcus CFU/ml (median; IQR)	60 (5-2000)	20 (5-3200)	0.99
Microbial constituents**			
Citrobacter (n; %)	0 (0.0)	1 (8.3)	0.46
Corynebacterium (n; %)***	7 (50.0)	1 (8.3)	**0.0357**
Staphylococcus (n; %)	10 (71.4)	9 (75.0)	0.99
Streptococcus (n; %)	4 (28.6)	5 (41.7)	0.68
Escherichia (n; %)	2 (14.3)	3 (25.0)	0.99
Lactobacillus (n; %)	1 7.1)	1 (8.3)	0.99
Enterococcus (n; %)	7 (50.0)	4 (33.3)	0.45
Aerococcus (n; %)	1 (7.1)	0 (0.0)	0.99
Facklamia (n; %)	0 (0.0)	1 (8.3)	0.46
Klebsiella (n; %)	2 (14.3)	1 (8.3)	0.99

Supplemental Table 7: Urinary microbial community in Salt-Losing Tubulopathy patients and healthy controls: summary findings.

* Reported as median and interquartile range CFU/ml across all isolates or across isolates of a specific genus, and compared between SLT and HC with a two-sided Mann-Whitney test.

**Reported as number and % of patients with genus present in culture, and compared between SLT and HC with a two-sided Fisher's exact test.

***Corynebacterium species isolated in Salt-Losing nephropathy: *Corynebacterium amycolatum* = 1; *Corynebacterium aurimucosum* = 2; *Corynebacterium coyleae* = 1; *Corynebacterium singular* = 2; *Corynebacterium tuberculostearicum* = 1.

A. Salt-Losing Tubulopathy patients

Patient number	Age	Sex	Diagnosis	Number of isolates	Genus	Species	Colony Forming Units/ml
29	85	Male	Gitelman	5	*Klebsiella*	*Klebsiella oxytoca*	2.4
					Enterococcus	*Enterococcus faecalis*	33.6
					Staphylococcus	*Staphylococcus epidermidis*	8.4
					Corynebacterium	*Corynebacterium singulare*	38
					Staphylococcus	*Staphylococcus haemolyticus*	3.2
12	32	Female	Bartter (3)	4	*Corynebacterium*	*Corynebacterium aurimucosum*	2.8
					Staphylococcus	*Staphylococcus haemolyticus*	0.8
					No ID	No ID	1.2
					No ID	No ID	0.4
13	34	Male	Bartter (3)	1	No ID	No ID	332
6	18	Female	Bartter (2)	3	*Streptococcus*	*Streptococcus vestibularis*	100
					Staphylococcus	*Staphylococcus haemolyticus*	100
					Staphylococcus	*Staphylococcus epidermidis*	0.4
47	31	Female	Gitelman	4	*Escherichia*	*Escherichia coli*	0.4
					Staphylococcus	*Staphylococcus hominis*	800
					Staphylococcus	*Staphylococcus epidermidis*	600
					Lactobacillus	*Lactobacillus crispatus*	800000
33	52	Male	Gitelman	2	*Staphylococcus*	*Staphylococcus haemolyticus*	800
					Enterococcus	*Enterococcis faecium*	0.4
46	43	Female	Gitelman	4	*Staphylococcus*	*Staphylococcus haemolyticus*	440
					Staphylococcus	*Staphylococcus epidermidis*	800
					Corynebacterium	*Corynebacterium aurimucosum*	72
					Enterococcus	*Enterococcus faecalis*	5.2
31	41	Female	Gitelman	4	*Escherichia*	*Escherichia coli*	3600
					Enterococcus	*Enterococcus faecalis*	60
					Enterococcus	*Enterococcus faecalis*	11600
					Corynebacterium	*Corynebacterium coyleae*	56
20	34	Male	Bartter (3)	3	*Staphylococcus*	*Staphylococcus haemolyticus*	36
					Staphylococcus	*Staphylococcus haemolyticus*	20
					Corynebacterium	*Corynebacterium singulare*	52
10	31	Male	Bartter (3)	4	*Enterococcus*	*Enterococcus faecalis*	560
					Staphylococcus	*Staphylococcus capitis*	5.2

					Staphylococcus	*Staphylococcus warneri*	2
					Streptococcus	*Streptococcus agalactiae*	2800
2	26	Female	Bartter (1)	4	*Streptococcus*	*Streptococcus agalactiae*	5600
					Aerococcus	*Aerococcus urinae*	32000
					No ID	No ID	3600
					Corynebacterium	*Corynebacterium amycolatum*	3600
27	37	Female	Gitelman	5	*Staphylococcus*	*Staphylococcus haemolyticus*	44000
					Enterococcus	*Enterococcus faecalis*	2000
					No ID	No ID	10000000
					Staphylococcus	*Staphylococcus epidermidis*	44000
					Corynebacterium	*Corynebacterium tuberculostearicum*	4000000
14	31	Male	Bartter (3)	1	*Streptococcus*	*Streptococcus oralis*	3.2
5	19	Male	Bartter (2)	2	*Klebsiella*	*Klebsiella oxytoca*	4.4
					Staphylococcus	*Staphylococcus haemolyticus*	2.8

B. Healthy Controls

Age	Sex	Number isolates	Genus	Species	Colony Forming Units/ml
30	Female	3	*Staphylococcus*	*Staphylococcus epidermidis*	32000
			Staphylococcus	*Staphylococcus hominis*	3600
			No ID	No ID	8.8
30	Female	3	*Citrobacter*	*Citrobacter koseri*	640
			Escherichia	*Escherichia coli*	24
			Enterococcus	*Enterococcus raffinosus*	5.2
22	Female	6	*Escherichia*	*Escherichia coli*	16
			Klebsiella	*Klebsiella pneumoniae*	40
			Enterococcus	*Enterococcus faecalis*	20
			Enterococcus	*Enterococcus faecalis*	5.2
			Staphylococcus	*Staphylococcus hominis*	28
			Staphylcoccus	*Stapylococcus epidermidis*	8
36	Male	0	No growth	No growth	NA
28	Male	3	*Staphylococcus*	*Staphylococcus epidermidis*	2.4
			Streptococcus	*Streptococcus mitis*	44
			Staphylococcus	*Staphylococcus epidermidis*	2.8
24	Male	3	*Staphylococcus*	*Staphylococcus haemolyticus*	0.4
			Staphylococcus	*Staphylococcus haemolyticus*	2.4
			Streptococcus	*Streptococcus mitis*	112
53	Male	4	*Staphylococcus*	*Staphylococcus hominis*	1.2
			Staphylococcus	*Staphylococcus hominis*	60
			Staphylococcus	*Staphylococcus hominis*	56
			Facklamia	*Facklamia hominis*	4
24	Female	2	*Staphylococcus*	*Staphylococcus epidermidis*	14.8
			Lactobacillus	*Lactobacillus crispatus*	1800000
50	Female	4	*Streptococcus*	*Streptococcus agalactiae*	840000
			No ID	No ID	36
			Staphylococcus	*Staphylococcus lugdenensis*	5.6
			Staphylococcus	*Staphylococcus haemolyticus*	24
42	Female	5	*Escherichia*	*Escherichia coli*	11200
			Enterococcus	*Enterococcus faecalis*	3200
			Streptococcus	*Streptococcus agalactiae*	2400

			Enterococcus	*Enerococcus faecalis*	2400
			No ID	No ID	1200
40	Male	4	*Staphylococcus*	*Staphylococcus epidermidis*	20
			Staphylococcus	*Staphylococcus capitis*	16
			Corynebacterium	*Corynebacterium tuberculostearicum*	3.6
			No ID	No ID	3200
31	Male	2	*Streptococcus*	*Streptococcus mitis*	4800
			Staphylococcus	*Staphylococcus hominis*	0.4

Supplemental Table 8: Complete list of organisms isolated from urine sediment cultures in Salt-Losing Tubulopathy patients (n=14) and healthy controls (n=12). Where reliable identification could not be achieved for a cultured isolate by MALDI-TOF MS, strains are reported as “No ID”.

	Normal Range	Number of values	Salt-Losing Tubulopathy (median; IQR)
Haemoglobin (g/l)	110-150 (F); 135-170 (M)	46	140.5 (129.5-153.5)
White Cell Count (x10^9/l)	3.5-11	46	8.6 (6.8-10.5)
Neutrophil (x10^9/l)	1.7-7.5	46	5.4 (4.0-6.7)
Lymphocyte (x10^9/l)	1-4	46	2.5 (2.1-3.0)
Monocyte (x10^9/l)	0.2-1.5	46	0.6 (0.5-0.8)
Eosinophil (x10^9/l)	0-0.5	46	0.1 (0.1-0.2)
Basophil (x10^9/l)	0-0.1	46	0.1 (0.1-0.1)
Erythrocyte Sedimentation Rate (mm/hr)	0-20	32	9.0 (7.0-31.3)
C-Reactive Protein (mg/L)	0-5	36	2.0 (1.0-5.0)
Immunoglobulin A (g/l)	0.7-4	34	2.3 (1.5-3.2)
Immunoglobulin G (g/l)	7-16	34	11.3 (9.5-14.9)
Immunoglobulin M (g/l)	0.4-2.3	34	1.1 (0.8-1.8)
Total Immunoglobulin E (KUL)	0-120	30	55.0 (26.8-170.8)
Complement C3 (g/l)	0.9-1.8	32	1.4 (1.2-1.7)
Complement C4 (g/l)	0.1-0.4	32	0.3 (0.2-0.3)
Lymphocyte (absolute; g/l)	1-2.8	32	2.1 (1.6-2.6)
CD3 (absolute; g/l)	0.7-2.1	32	1.6 (1.2-1.9)
CD4 (absolute; g/l)	0.3-1.4	32	1.0 (0.8-1.2)
CD8 (absolute; g/l)	0.2-0.9	32	0.5 (0.4-0.6)
CD19 (absolute; g/l)	0.1-0.5	32	0.2 (0.2-0.4)
CD16, CD56 (absolute; g/l)	0.09-0.6	32	0.1 (0.1-0.2)
CD3 (% of lymphocytes)	55-83	32	77.0 (69.0-81.8)
CD4 (% of lymphocytes)	28-57	32	46.0 (44.0-54.8)
CD8 (% of lymphocytes)	10-39	32	24.0 (18.3-28.0)
CD19 (% of lymphocytes)	6-19	32	11.0 (9.0-15.8)
CD16, CD56 (% of lymphocytes)	7-31	32	7.5 (5.0-11.0)
CD4/CD8 (ratio)	1-3.6	32	2.0 (1.6-3.0)

Supplemental Table 9: Initial immunological analysis of Salt-Losing Tubulopathy patients. Median and interquartile range values within the SLT cohort are reported, along with the clinical laboratory reference range and the number of values for each variable recorded. Source data are provided as a **Source data file.**

Experiment	Product	Company	Catalog number	Dilution / Concentration
FACS STAINING				
T cell proliferation and activation	Fixable viability dye - efluor450	Invitrogen	65-0863-14	1 in 1000
	anti human CD4 - FITC	BioLegend	300538	1 in 25
	anti human Ki-67 - APC	BioLegend	350514	1 in 40
	anti human CD25 - PE/Cy7	BioLegend	302612	1 in 40
T cell subset analysis (stimulated cells and intracellular staining)	anti human CD4 - FITC	BioLegend	300538	1 in 20
	anti human IFNg - APC	BioLegend	502512	1 in 40
	anti-human IL17 - PE	BioLegend	512306	1 in 40
	anti-human IL4 - PE/Cy7	BioLegend	500824	1 in 40
T cell subset analysis (unstimulated cells and surface staining)	anti-human CD3 - PE/Cy5	BioLegend	300410	1 in 80
	anti human CD4 - FITC	BioLegend	300538	1 in 25
	anti-human CD8 - BV785	BioLegend	301046	1 in 40
	anti human CD45RA - AlexaFluor700	BioLegend	304120	1 in 40
	anti human CD45RO-PE	BioLegend	304206	1 in 40
Th17 and Tc17 analysis	Fixable viability dye - efluor450	Invitrogen	65-0863-14	1 in 1000
	anti human CD4 - FITC	BioLegend	300538	1 in 20
	anti-human CD8 - PE/Cy7	BioLegend	300914	1 in 40
	anti-human IL17 - PE	BioLegend	512306	1 in 40
Phosphostain (STAT1 and STAT3)	anti human CD4 - FITC	BioLegend	300538	1 in 20
	STAT3 (pY705) - PE	BD Biosciences	612569	1 in 5
	STAT1 (pY701) - Alexa 647	BD Biosciences	612597	1 in 5
NCC expression on lymphocytes	SLC12A3 Polyclonal antibody (rabbit)	Life Technologies	PA5-80004	1 in 50
	Goat anti-rabbit APC	Invitrogen	A-10931	1 in 40
	anti human CD4 - FITC	BioLegend	300538	1 in 25
	anti-human CD8 - PE/Cy7	BioLegend	300914	1 in 40
	anti human CD45RA - AlexaFluor700	BioLegend	304120	1 in 40

	anti human CD45 - FITC	BioLegend	304006	1 in 50
	anti human CD3 - BV711	BioLegend	317328	1 in 50
	anti human CD56 - BV510	BioLegend	318340	1 in 50
	anti human CD19 - PE/Cy7	Invitrogen	25-0199-42	1 in 40
Calcium flux	Indo-1, AM, cell permeant	Thermo Fisher	I1223	1 in 500
	anti-human CD4 - PerCP/Cy5.5	BioLegend	300530	1 in 40
	biotinylated anti-human CD3	BioLegend	317320	10ug per ml
IL23 receptor expression	IL-23 receptor - PE	R&D Systems	FAB14001P	1 in 25
	anti human CD4 - FITC	BioLegend	300538	1 in 20
Monocyte analysis	anti-human CD14 - APC/Cy7	BioLegend	325619	1 in 50
	anti-human CD16 - FITC	BioLegend	302006	1 in 50
	anti-human TNF - Alexaflour488	BD Biosciences	557722	1 in 40
NK cell analysis	anti human CD45 - FITC	BioLegend	304006	1 in 50
	anti human CD3 - BV711	BioLegend	317328	1 in 50
	anti human CD56 - BV510	BioLegend	318340	1 in 50
	anti human CD16 - PE/Cy7	BioLegend	302016	1 in 40
	anti human IFNg - APC	BioLegend	502512	1 in 40
WESTERN BLOT				
	Rabbit SGK1 polyclonal antibody	Abcam	ab43606	1 in 900
	Mouse GAPDH monoclonal antibody	Abcam	ab8245	1 in 10000
	anti-rabbit HRP	Abcam	ab205718	1 in 3000
	anti-mouse HRP	Abcam	ab205719	1 in 5000

Supplemental Table 10: List of antibodies used