Scholz et al.**:**

MODULATION OF mTOR SIGNALLING

TRIGGERS THE FORMATION OF STEM CELL-LIKE MEMORY T CELLS

SUPPLEMENTAL INFORMATION

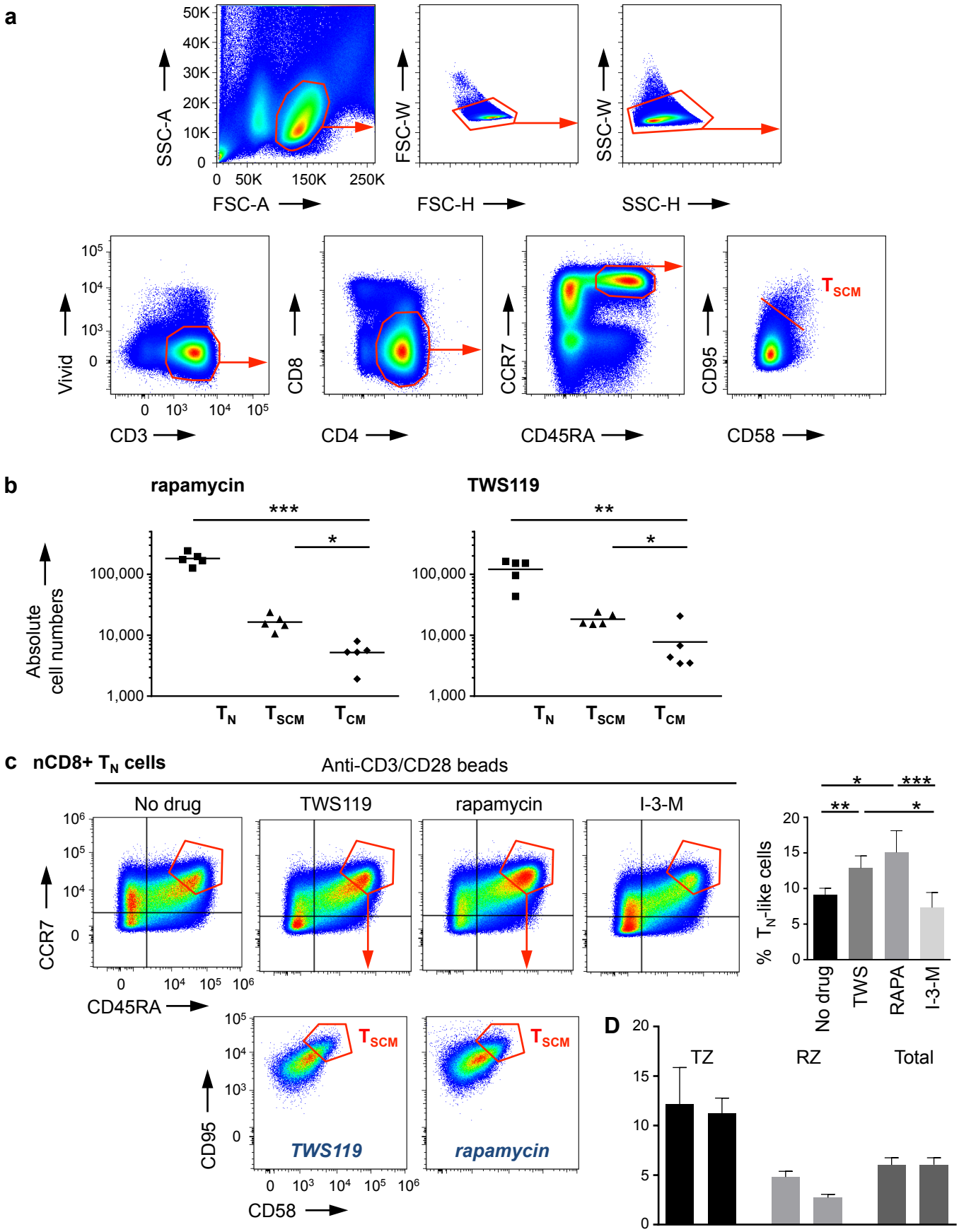
a
SSC-A
FSC-A
FSC-W
FSC-H
SSC-W
SSC-H
Vivid
CD3
CD8
CD4
CCR7
CD45RA
CD95
CD58
T_SCM
b
rapamycin
TWS119
Absolute cell numbers
T_N
T_SCM
T_CM
c
nCD8+ T_N cells
Anti-CD3/CD28 beads
No drug
TWS119
rapamycin
I-3-M
CCR7
CD45RA
CD95
CD58
% T_N-like cells
No drug
TWS
RAPA
I-3-M
D
TZ
RZ
Total
PP
KU
100 nM

Figure S1

Figure S1

(a) Gating strategy for the phenotypic detection of human T_{SCM} cells (here shown for CD4+ T_{SCM} cells): Lymphocytes → Doublet exclusion (FSC and SSC) → Live CD3+ T cells → CD4+, CD8- T cells → CCR7+, CD45RA+ T cells → CD95-, CD58- T_N cells (when sorted, termed highly purified) and CD95+, CD58+ T_{SCM} cells. FSC = forward scatter. SSC = side scatter. A = area. H = height. W = width. T_{SCM} = T_{SCM} cells.

(b) Absolute cell numbers in the small-sized lymphocyte population. 14 day-long priming of 1,000,000 nCD4+ T_N cells in the presence of rapamycin or TWS119 specifically induces T_{SCM} cells, verified by assessment of the absolute cell numbers in the cell culture. Rapamycin (left) and TWS119 (right) significantly increase the numbers of T_{SCM} cells in comparison to T_{CM} cells. T_N = T_N-like cells. T_{SCM} = pharmacologically induced T_{SCM} cells. T_{CM} = T_{CM}-like cells. n = 5.

(c) TWS119 and rapamycin induce phenotypic CD8+ T_{SCM} cells. Highly purified phenotypic nCD8+ T_{SCM} cells, activated for 14 days in the presence of TWS119 (5 μM) or rapamycin (100 nM) exhibit increased CCR7+, CD45RA+ T_N-like cell frequencies (gated on live CD3+, CD8+ T cells), containing phenotypic CD95+, CD58+ T_{SCM} cells. When nCD8+ T_N cells were cultured in the absence of TWS119 or rapamycin or on the presence of Indirubin-3-monoxime (I-3-M, 4 μM) they did not display increased T_N-like cell frequencies. T_N-like cell frequencies are depicted as percentage of live CD3+, CD8+ T cells. T_{SCM} = pharmacologically induced T_{SCM} cells. TWS = TWS119. RAPA = rapamycin. n = 4.

(d) Phenotypic CD95+, CD58+ T_{SCM} cells in the TZ (transition zone), RZ (rand zone) and total T_N-like cell population after activation of highly purified human nCD4+ T_N cells for 14 days in the presence of PP242 (100 nM) or KU-0063794 (100 nM). T_{SCM} cell frequencies are depicted as percentages of the defined zones. Data are represented as mean ± SEM. n = 4. PP = PP242. KU = KU-0063794.

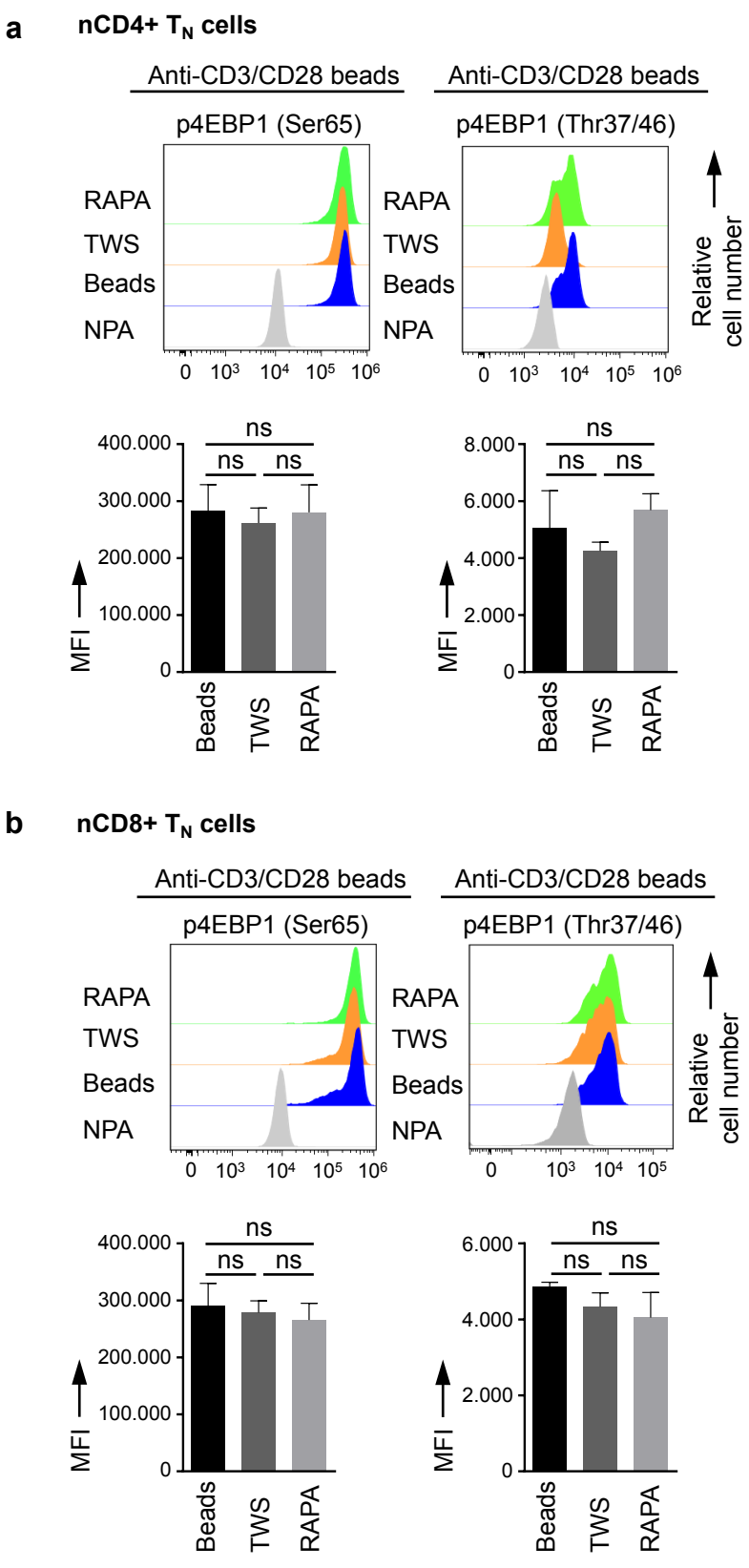
a
nCD4+ T$_N$ cells
Anti-CD3/CD28 beads
Anti-CD3/CD28 beads
p4EBP1 (Ser65)
p4EBP1 (Thr37/46)
RAPA
TWS
Beads
NPA
Relative cell number
0 10^3 10^4 10^5 10^6
ns
400.000
300.000
200.000
100.000
0
MFI
Beads
TWS
RAPA
8.000
6.000
4.000
2.000
b
nCD8+ T$_N$ cells

Figure S2

Figure S2

Assessment of 4EBP1 phosphorylation by flow cytometry.

TWS119 (5 μM) and rapamycin (100 nM) have no significant effects on the phosphorylation of 4EBP1 (Ser65 and Thr37/46) in (a) highly purified (T_{SCM} cell-freed), activated human nCD4+ T_N cells and in (b) highly purified, activated human nCD8+ T_N cells. NPA = no primary antibody. Beads = anti-CD3/CD28 beads. TWS = TWS119. RAPA = rapamycin. MFI = Median Fluorescence Intensity. n = 3.

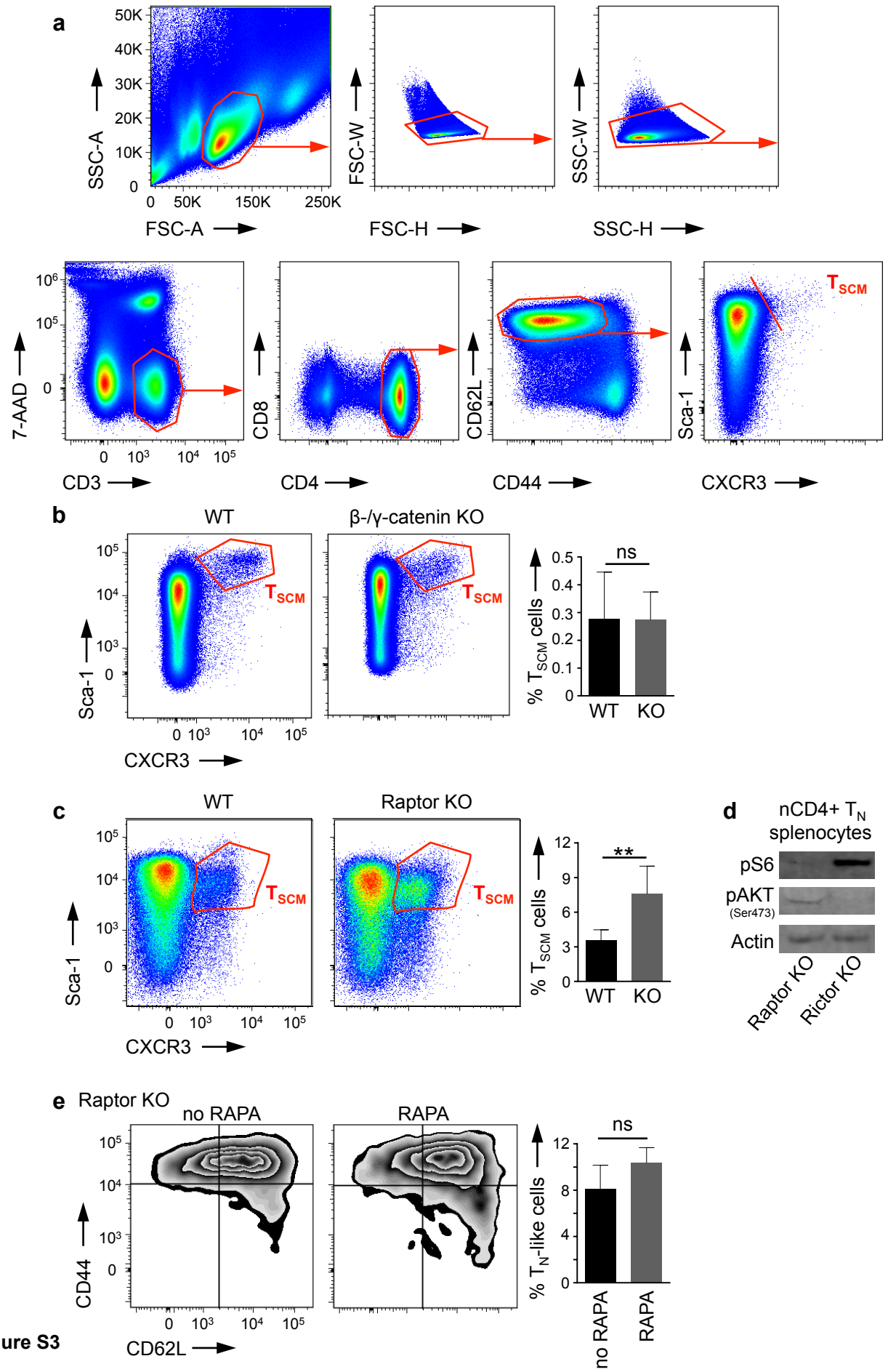
a
SSC-A
FSC-A
FSC-W
FSC-H
SSC-W
SSC-H
7-AAD
CD3
CD8
CD4
CD62L
CD44
Sca-1
CXCR3
TSCM
b
WT
β-/γ-catenin KO
% TSCM cells
ns
WT
KO
c
WT
Raptor KO
**
d
nCD4+ TN splenocytes
pS6
pAKT (Ser473)
Actin
Raptor KO
Rictor KO
e
Raptor KO
no RAPA
RAPA
% TN-like cells
ns

Figure S3

Figure S3

(a) Gating strategy for the phenotypic detection of mouse T_{SCM} cells (here shown for CD4+ T cells): Lymphocytes → Doublet exclusion (FSC and SSC) → Live CD3+ T cells → CD4+, CD8- T cells → CD62L+, CD44- T cells → CXCR3-, Sca-1- T_N cells (when sorted, termed highly purified) and CXCR3+, Sca-1+ T_{SCM} cells. FSC = forward scatter. SSC = side scatter. A = area. H = height. W = width. T_{SCM} = T_{SCM} cells.

(b) Mice with haematopoietic deletion of β- and γ-catenin display naturally occurring T_{SCM} cells.

Similar frequencies of Sca-1+, CXCR3+, CD44-, CD62L+ naturally occurring T_{SCM} cells in mice with haematopoietic knockout (KO) of β- and γ-catenin as in their wild-type (WT) counterparts (gated on live CD3+, CD4+, CD44-, CD62L+ T cells). T_{SCM} cell frequencies are depicted as percentage of live CD3+, CD4+, CD44-, CD62L+ T cells. Data are represented as mean ± SEM. T_{SCM} = naturally occurring T_{SCM} cells. n = 3.

(c) Mice with T cell-specific deletion of Raptor display increased T_{SCM} cell frequencies.

Increased frequencies of naturally occurring Sca-1+, CXCR3+, CD44-, CD62L+ T_{SCM} cells in mice with T cell-specific knockout (KO) of Raptor compared to their wild-type (WT) counterparts (gated on live CD3+, CD44-, CD62L+ T cells). T_{SCM} cell frequencies are depicted as percentage of live CD3+, CD44-, CD62L+ T cells. Data are represented as mean ± SEM. T_{SCM} = naturally occurring T_{SCM} cells. n = 9.

(d) Assessment of S6 and AKT (Ser473) phosphorylation in mice with either a T cell-specific knockout (KO) of Raptor or of Rictor by Western blot technique.

Mice with T cell-specific KO of Raptor display decreased phosphorylation of S6, but phosphorylation of AKT (Ser473) in highly purified nCD4+ T_N cells, indicating inhibition of mTORC1 and simultaneous activity of mTORC2. Highly purified nCD4+ T_N cells from mice with T cell-specific KO of Rictor show the opposite. n = 5.

(e) Treatment of Raptor KO nCD4+ T_N cells with rapamycin.

Treatment of Raptor KO nCD4+ T_N cells during 4-day priming with rapamycin (100 nM) does not result in an increased fraction of T_N-like phenotype cells. RAPA = rapamycin. n = 3.

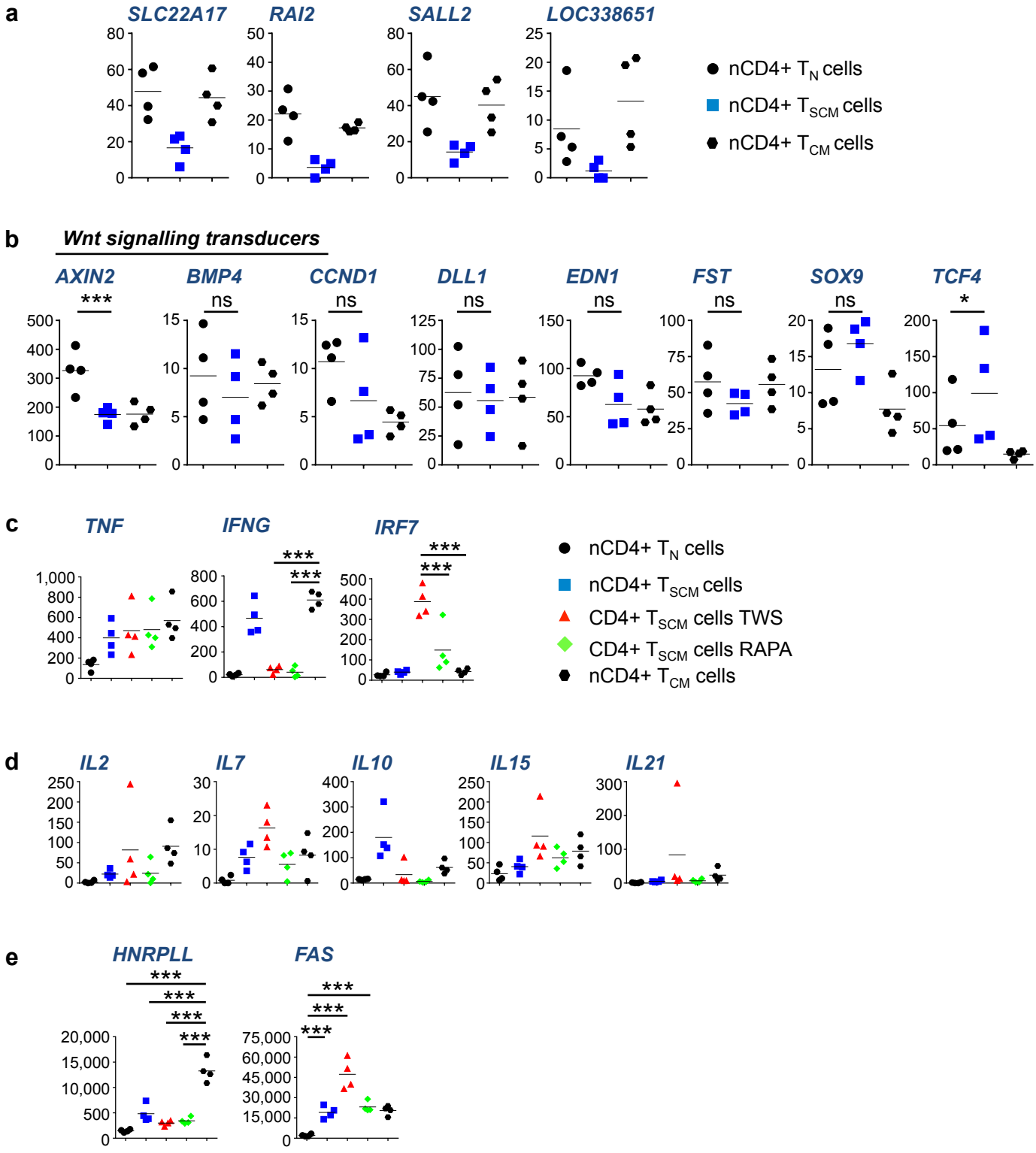
a
SLC22A17
RAI2
SALL2
LOC338651
nCD4+ T_N cells
nCD4+ T_SCM cells
nCD4+ T_CM cells
b
Wnt signalling transducers
AXIN2
BMP4
CCND1
DLL1
EDN1
FST
SOX9
TCF4
***
ns
*
c
TNF
IFNG
IRF7
nCD4+ T_N cells
nCD4+ T_SCM cells
CD4+ T_SCM cells TWS
CD4+ T_SCM cells RAPA
nCD4+ T_CM cells
d
IL2
IL7
IL10
IL15
IL21
e
HNRPLL
FAS

Figure S4

Figure S4

(a) Gene expression profiles of *SLC22A17*, *RAI2*, *SALL2* and *LOC338651* in nCD4+ T_{SCM} cells.

From the set of 56 genes which show a unique expression profile in nCD4+ T_{SCM} cells (Table 1), only 4, namely *SLC22A17*, *RAI2*, *SALL2* and *LOC338651*, are found to be significantly down-regulated in nCD4+ T_{SCM} cells in comparison to nCD4+ T_N cells and nCD4+ T_{CM} cells.

(b) Gene expression profiles of Wnt signalling transducers in nCD4+ T_N cells and nCD4+ T_{SCM} cells.

Widespread similar expression of genes encoding transducers of Wnt signalling in nCD4+ T_N cells and nCD4+ T_{SCM} cells.

(c) Gene expression profiles of the genes encoding *TNF*, *IFNG* and *IRF7*.

(d) Gene expression profiles of the genes encoding *IL2*, *IL7*, *IL10*, *IL15* and *IL21*.

(e) Gene expression profile of the genes of the key regulator of the alternative splicing of the CD45 pre-mRNA, *HNRPLL*, and of *FAS*.

Data are based on transcriptome analysis in 4 healthy human individuals. The y-axes show normalized counts. TWS = TWS119-induced T_{SCM} cells. RAPA = rapamycin-induced T_{SCM} cells.

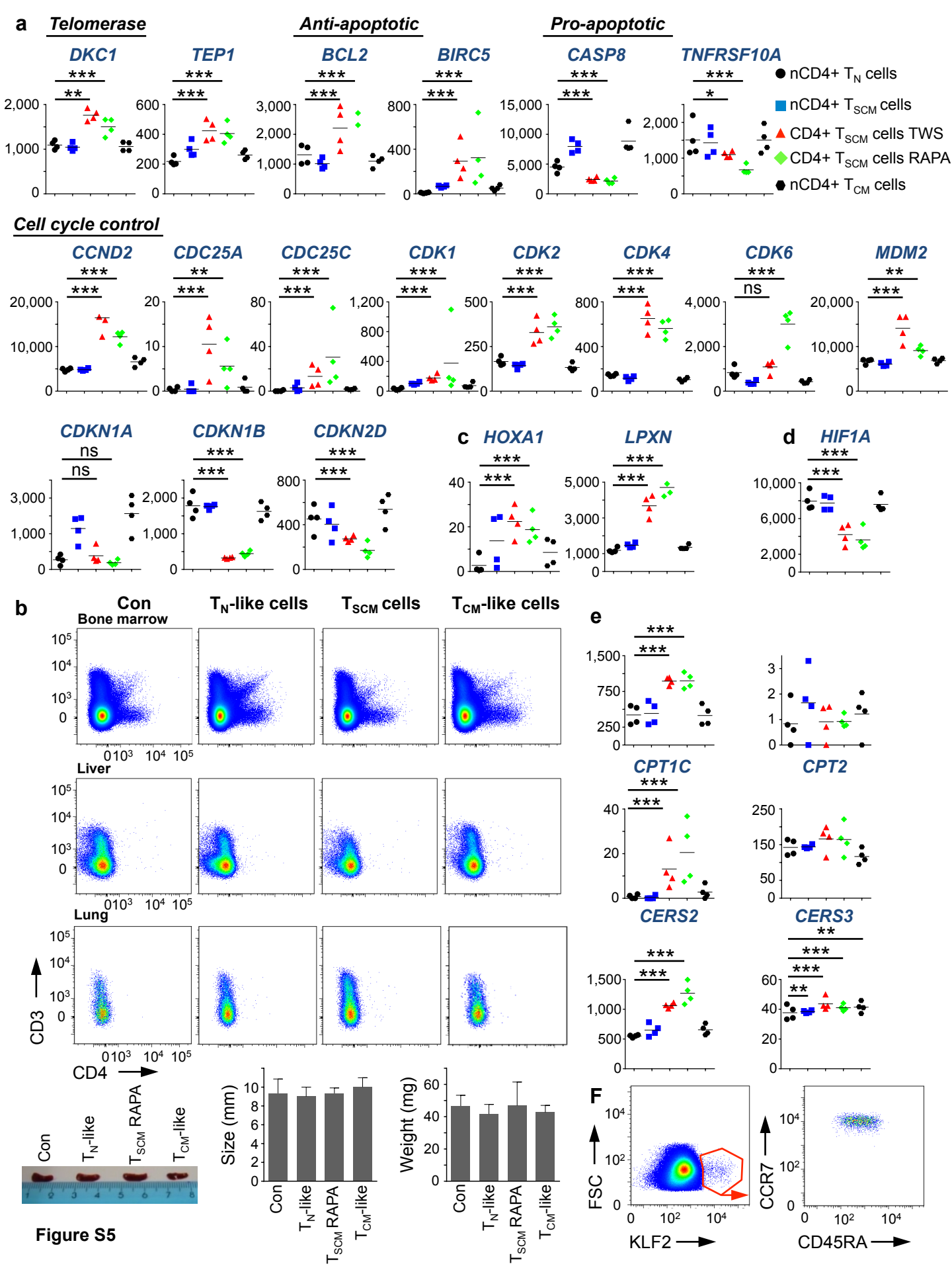
a
Telomerase
Anti-apoptotic
Pro-apoptotic
DKC1
TEP1
BCL2
BIRC5
CASP8
TNFRSF10A
nCD4+ T_N cells
nCD4+ T_SCM cells
CD4+ T_SCM cells TWS
CD4+ T_SCM cells RAPA
nCD4+ T_CM cells
Cell cycle control
CCND2
CDC25A
CDC25C
CDK1
CDK2
CDK4
CDK6
MDM2
CDKN1A
CDKN1B
CDKN2D
c
HOXA1
LPXN
d
HIF1A
b
Con
T_N-like cells
T_SCM cells
T_CM-like cells
Bone marrow
Liver
Lung
CD3
CD4
Con
T_N-like
T_SCM RAPA
T_CM-like
Size (mm)
Weight (mg)
e
CPT1C
CPT2
CERS2
CERS3
F
FSC
KLF2
CCR7
CD45RA

Figure S5

Figure S5

(a) Gene expression profiles of genes involved in cell cycle control.

Rapamycin-induced CD4+ T_{SCM} cells exhibit an expression profile of genes involved in the regulation of cell death, longevity and cell cycle control, suggesting that they are equipped with a superior long-term survival capacity.

(b) *In vivo* long-term repopulation capacity of rapamycin-induced CD4+ T_{SCM} cells.

No live CD3+, CD4+ T cells could be detected in the bone marrow, liver and lung of NSG mice after adoptive transfer. Con = control: injection of 200 µl cell culture medium. The spleens of the adoptively transferred NSG mice did not display differences in size or weight with respect to the adoptively transferred T cell subset. n = 3.

(c) Gene expression profiles of engraftment genes in *in vitro* induced T_{SCM} cells.

Up-regulation of *HOXA1* and *LPXN* in rapamycin-induced CD4+ T_{SCM} cells and of *HOXA1* in naturally occurring CD4+ T_{SCM} cells.

(d) Glucose uptake in CD4+ T cell subsets.

Low expression levels of *HIFA* pharmacologically induced CD4+T_{SCM} cells.

(e) Gene expression profiles of carnitine palmitoyl transferases and ceramide synthases.

Naturally occurring and pharmacologically induced CD4+ T_{SCM} cells display a characteristic gene expression profile of the genes encoding carnitine palmitoyl transferases (CPT) and ceramide synthases (CERS). Also view the expression of *CERS6* in Figure 5D.

(f) Expression of KLF2 in nCD4+ T_N cells.

Only a small fraction of naturally occurring live CD3+, CD4+, CCR7+, CD45RA+ T_N cells expresses KLF2 (left). KLF2 expressing nCD4+ T_N cells are homogeneously CCR7+, CD45RA+ double positive T cells (right). n = 3.

(a), (c), (d) and (e): Data are based on transcriptome analysis in 4 healthy human individuals. The y-axes show normalized counts. TWS = TWS119-induced CD4+ T_{SCM} cells. RAPA = rapamycin-induced CD4+ T_{SCM} cells.

Table S1: Relative percentage and absolute cell number of live CD4+ T_N, T_{SCM}, T_{CM} cells and T_{EM} cells in the large-sized lymphocyte population. Numbers are presented as mean ± standard error of the mean (SEM). n = 5.

	Beads		***rapamycin***		***TWS119***	
	%	absolute	%	absolute	%	absolute
T_N	30.79 ± 5.31	576.015 ± 95.234	24.53 ± 3.93	751.889 ± 273.219	16.33 ± 3.06	324.197 ± 118.263
T_{SCM}	0.15 ± 0.05	3.627 ± 1.892	0.28 ± 0.11	10.198 ± 5.772	0.14 ± 0.05	4.160 ± 2210
T_{CM}	31.88 ± 6.15	781.579 ± 296.358	37.82 ± 4.58	1.103.000 ± 351.570	39.32 ± 2.65	956.886 ± 380.725
T_{EM}	10.20 ± 3.56	223.432 ± 123.831	14.92 ± 2.51	461.691 ± 188.073	21.61 ± 6.31	543.628 ± 344.107

Table S2: The 25 top-ranked (by adjusted p-value) significantly differentially expressed genes between nCD4+ T_N cells and nCD4+ T_{SCM} cells.

Up-regulated in T_{SCM} cells			**Down-regulated in T_{SCM} cells**		
Gene name	***log2FC***	***adj. p***	***Gene name***	***log2FC***	***adj. p***
1. **SAMSN1**	-1,8720285	2,74E-179	1. **IGF1R**	1,427881915	2,31E-106
2. **LINC00152**	-4,0839451	9,51E-122	2. **ITGA6**	1,231727997	2,92E-74
3. **CCR6**	-5,3922914	6,95E-119	3. **EEA1**	1,230984185	9,09E-56
4. **RNF19A**	-1,6260319	4,17E-117	4. **FAM46A**	1,066686583	4,69E-34
5. **S100A4**	-1,7347476	8,23E-108	5. **ACPL2**	1,381638941	2,89E-29
6. **ADAM19**	-5,3566214	2,86E-101	6. **NR3C2**	1,018553372	1,23E-26
7. **STAM**	-1,8600099	3,13E-92	7. **SORCS3**	1,780891951	2,97E-26
8. **FAM129A**	-4,8305544	1,73E-90	8. **SFMBT2**	1,107115196	1,60E-25
9. **SYT11**	-3,4862738	1,12E-87	9. **ANKRD55**	1,268831037	1,76E-23
10. **TIGIT**	-5,0768887	1,39E-85	10. **KRT73**	1,802570768	1,60E-20
11. **MAF**	-2,9002404	1,75E-81	11. **LRRC16A**	1,046216571	3,19E-20
12. **ANXA2**	-3,437778	4,21E-75	12. **SDK2**	1,598688716	3,31E-20
13. **KLRB1**	-4,3849081	2,92E-74	13. **ACVR2B**	1,256880619	4,58E-20
14. **DUSP4**	-4,4591383	3,83E-73	14. **LCLAT1**	1,102964862	6,65E-20
15. **FAS**	-3,1098147	2,74E-72	15. **PLAG1**	1,483654292	2,56E-19
16. **ARAP2**	-1,4998584	2,17E-68	16. **PCSK5**	1,395119208	5,54E-19
17. **RUNX3**	-1,6754918	6,76E-68	17. **TRABD2A**	1,314541925	7,60E-19
18. **COTL1**	-1,4702624	1,21E-66	18. **ZNF516**	1,632620207	1,03E-18
19. **CD74**	-1,6687414	3,74E-65	19. **EGR1**	1,159124848	2,25E-18
20. **CXCR5**	-2,905313	6,71E-60	20. **EMR4P**	1,404969196	9,00E-18
21. **PDIA6**	-2,1070971	2,60E-59	21. **TSEN2**	1,330122989	9,55E-18
22. **ITGB1**	-2,9217569	3,56E-53	22. **RBM11**	1,344951381	1,08E-17
23. **IL18R1**	-2,1791794	1,03E-52	23. **DEPDC7**	1,975226806	2,32E-17
24. **TOP2A**	-2,9015196	1,27E-52	24. **PLEKHH2**	1,533851363	2,57E-17
25. **MIAT**	-2,7683017	6,62E-52	25. **KRT72**	1,976578231	8,22E-17

Table S3: The 25 top-ranked (by adjusted p-value) significantly differentially expressed genes between nCD4+ T_{SCM} and T_{CM} cells.

Up-regulated in T_{SCM} cells			**Down-regulated in T_{SCM} cells**		
Gene name	***log2FC***	***adj. p***	***Gene name***	***log2FC***	***adj. p***
1. **HBB**	-6,0411627	4,65E-129	1. **DST**	1,05616476	1,93E-22
2. **GZMA**	-2,2912417	1,79E-46	2. **NAB1**	1,25297626	3,21E-19
3. **RTKN2**	-1,2929428	1,91E-25	3. **CXCR5**	1,02118417	2,16E-12
4. **RBMS3**	-2,0342402	1,30E-17	4. **HNRPLL**	1,37267746	7,85E-09
5. **PECAM1**	-1,9106633	1,79E-17	5. **IL2**	1,74024059	9,33E-09
6. **BANK1**	-3,4515431	4,20E-17	6. **NETO2**	1,41658235	6,98E-08
7. **IKZF2**	-1,7893613	3,57E-15	7. **LOC100128420**	1,49411931	1,15E-06
8. **HBA2**	-3,100931	4,00E-13	8. **KLRB1**	1,14802413	2,85E-06
9. **CD38**	-1,4179443	6,65E-13	9. **SMAD5**	1,02779121	6,39E-05
10. **NKG7**	-2,6249869	1,55E-11	10. **CCR6**	1,21155421	9,57E-05
11. **ENC1**	-1,2340584	3,52E-11	11. **CYP24A1**	1,51420011	9,78E-05
12. **LAYN**	-2,1747497	3,53E-11	12. **LOC100505676**	1,46485435	0,00019599
13. **CNR2**	-2,8348965	6,63E-11	13. **SLC22A17**	1,19257275	0,00213168
14. **HLA-DMA**	-1,3576176	1,13E-10	14. **KCNQ3**	1,55002485	0,00254751
15. **FCRL2**	-2,7184507	5,98E-10	15. **H1F0**	1,00909404	0,00261769
16. **CRTAM**	-2,1784364	1,78E-09	16. **CDR1**	1,47957064	0,0051696
17. **GZMK**	-1,5935992	1,78E-09	17. **LOC338651**	1,47185677	0,00535925
18. **IGLL5**	-2,6436633	2,34E-09	18. **ELOVL4**	1,25441341	0,00553021
19. **MYB**	-2,0440295	4,55E-09	19. **NPDC1**	1,0812657	0,00589619
20. **SLAMF7**	-2,137982	1,63E-08	20. **SALL2**	1,13254525	0,00921469
21. **KIAA0101**	-1,4027786	1,95E-08	21. **LOC339894**	1,17366326	0,01070789
22. **AFF3**	-1,0652742	2,44E-08	22. **CDS1**	1,00968714	0,01255335
23. **HLA-DMB**	-1,9413391	4,75E-08	23. **TBC1D8B**	1,13488974	0,01538873
24. **DTHD1**	-1,8741905	6,89E-08	24. **RAI2**	1,29395937	0,01762307
25. **HBA1**	-2,2914809	6,23E-07	25. **KIAA1598**	1,08462698	0,02564904

Table S4: Genes found to be significantly differentially expressed between nCD4+ T_{SCM} and T_{CM} cells (ranked by adjusted p-value), but not in any other comparison among the naturally occurring T cell subsets.

Gene name
1. **CD38**
2. **ENC1**
3. **AFF3**
4. **AKR1C3**
5. **FGFBP2**
6. **SMAD5**
7. **KIAA0125**
8. **IRF8**
9. **H1F0**
10. **CA1**
11. **CCRL2**
12. **CTBP2**
13. **CDS1**
14. **TBC1D8B**
15. **FGFBP3**
16. **VWDE**
17. **ABCB4**
18. **TCEAL2**

Table S5: The 25 top-ranked (by adjusted p-value) significantly differentially expressed genes between rapamycin- and TWS119-induced CD4+ T_{SCM} cells.

Up-regulated in rapamycin-induced T_{SCM} cells			Up-regulated in TWS119-induced T_{SCM} cells		
Gene name	***log2FC***	***adj. p***	***Gene name***	***log2FC***	***adj. p***
1. **NQO1**	-2,8730812	1,10E-121	1. **LAMP3**	3,56551112	1,04E-130
2. **CDK6**	-1,4605378	2,00E-67	2. **IFIT3**	4,92954195	1,19E-118
3. **SPIN1**	-1,2016612	2,45E-47	3. **USP18**	3,0647312	7,79E-93
4. **KLHL24**	-1,4391299	3,28E-46	4. **IFI27**	6,20694105	8,40E-88
5. **TXNRD1**	-1,1725384	1,11E-43	5. **IFI6**	2,89648652	8,70E-86
6. **AHI1**	-1,7503645	6,14E-42	6. **TLR7**	4,21238707	1,53E-79
7. **TPCN1**	-1,3928224	4,63E-40	7. **RASGRP3**	2,29468472	3,11E-72
8. **IRF2BP2**	-1,2786665	3,24E-36	8. **HERC5**	2,01162857	6,16E-72
9. **LOC100128420**	-2,4644944	1,77E-30	9. **PARP12**	1,46461934	6,17E-67
10. **LAYN**	-2,2649444	2,24E-29	10. **JAK2**	1,69870158	1,30E-62
11. **B3GALNT1**	-2,2620449	7,36E-29	11. **IFIT2**	3,41258634	6,20E-52
12. **CSF2RB**	-4,0303569	7,69E-29	12. **OAS3**	2,54400792	1,47E-51
13. **IL2RA**	-1,3568667	1,18E-27	13. **EIF2AK2**	2,05518913	3,67E-51
14. **ARNTL2**	-1,6887385	1,93E-25	14. **EPB41L5**	1,6763618	6,20E-50
15. **P2RY14**	-2,2532221	2,78E-24	15. **SELL**	1,73757504	1,17E-48
16. **CCDC28A**	-1,2184364	5,37E-24	16. **MX1**	2,84169448	2,73E-47
17. **LDLRAD4**	-1,0044388	6,27E-23	17. **ISG15**	2,53343526	4,53E-47
18. **RDH10**	-2,2193282	1,40E-22	18. **SAMD9L**	1,83900334	6,85E-47
19. **CXCR6**	-2,9912327	1,46E-22	19. **DTX3L**	1,63542121	3,04E-46
20. **ITGAE**	-1,0243546	3,10E-22	20. **BST2**	1,43044376	4,14E-45
21. **GSTP1**	-1,1368922	7,78E-22	21. **PYHIN1**	1,63198905	6,47E-45
22. **SRXN1**	-1,6506528	1,29E-21	22. **DHX58**	1,67590179	1,09E-44
23. **TALDO1**	-1,6267585	3,36E-21	23. **TRIM25**	1,17836377	1,34E-44
24. **CREM**	-1,3582741	9,38E-21	24. **IFIT1**	3,95532666	5,36E-43
25. **GCLM**	-1,1804538	1,94E-19	25. **NEXN**	1,98919938	1,81E-42

Table S6: The 1,000 genes with the highest average normalized count across all samples, used to construct the heat map in **Figure 4A** (excel file).

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Statistical analysis for RNA Sequencing: Reads shorter than 20 bp after the trimming were removed. The remaining reads were aligned to the human genome (hg19) with TopHat2 (Version 2.0.9) (Kim et al., 2013). A gtf file from the Illumina iGenomes project (https://support.illumina.com/sequencing/sequencing_software/igenome.ilmn), defining the gene models, was supplied to TopHat2. htseq-count was used to quantify the number of reads mapping to each gene in the gtf file, using the 'union' counting mode to handle reads overlapping several genes (Anders et al., 2014). All genes with zero counts in all samples were excluded, leaving in total 21,481 genes for further interrogation. For exploratory analysis (heat map, hierarchical clustering and principal components analysis), we transformed the counts by use of the regularized log transformation in the DESeq2 R package (Version 1.2.10) (Love et al., 2014). The transformed values for each gene were further standardized by subtraction of the mean value and division by the standard deviation across all samples. Differential expression analysis was performed on the count data by DESeq2 with default settings. To visually compare the expression levels of genes across different conditions, we used the normalized counts calculated with DESeq2.

The “stem cell genes” for GSEA can be found under the following link (04/2015):
http://www.sabiosciences.com/Stem_Cell.php
Tested genes were: *APC*, *AXIN1*, *CCNA2*, *CCND1*, *CCND2*, *CCNE1*, *CDK1*, *CDC42*, *EP300*, *FGF2*, *MYC*, *NOTCH2*, *PARD6A*, *RB1*, *KAT2A*, *HDAC2*, *KAT8*, *KAT7*, *TERT*, *NOTCH1*, *NUMB*, *HSPA9*, *SOX2*, *BMP1*, *BMP2*, *BMP3*, *IGF1*, *JAG1*, *DLL1*, *GJA1*, *CD4*, *CD44*, *CDH1*, *CDH2*, *COL9A1*, *CTNNA1*, *NCAM1*, *ABCG2*, *ALDH2*, *FGFR1*, *FOXA2*, *MSX1*, *CD3D*, *CD8A*, *CD8B*, *MME*, *ACAN*, *COL1A1*, *COL2A1*, *PPARG*, *SIGMAR1*, *S100B*, *TUBB3*, *DTX1*, *DTX2*, *DVL1*, *ADAR*, *BTRC*, *FRAT1*, *FZD1*, *PPARD*, *WNT1*

Staining panels

Staining panel for T_N cell sorting and T_{SCM} cell assessment in humans.

Marker	Fluorochrome	Clone	Isotype	Vendor
CD3	A700	HIT3a	Mouse IgG2a, κ	BioLegend
CD4	Pacific Blue	13B8.2	Mouse IgG1	Beckman Coulter
(CD4)	(PE)	(13B8.2)	(Mouse IgG1)	(Beckman Coulter)
CD8	APC-A750	B9.11	Mouse IgG1	Beckman Coulter
CCR7	PerCP-Cy5.5	TG8/CCR7	Mouse IgG2a, κ	BioLegend
(CCR7)	(Brilliant Violet)	(G043H7)	(Mouse IgG2a, κ)	(BioLegend)
CD45RA	ECD	2H4LDH11LDB9	Mouse IgG1	Beckman Coulter
CD95	APC	DX2	Mouse IgG1, κ	BioLegend
CD58	FITC	1C3	Mouse IgG2a, κ	BD Biosciences
Dead cells	Vivid Aqua	Not specified	Not specified	Life Technologies

Staining panel for T_N cell sorting and T_{SCM} cell assessment in mice.

Marker	Fluorochrome	Clone	Isotype	Vendor
CD3e	A700	17A2	Rat IgG2b, κ	eBioscience
CD4	FITC	GK1.5	Not specified	In house
CD8a	Pe-AlexaFluor610	5H10	RatIgG2b	Invitrogen
CD62L	PE	Mel14	Rat IgG2a, κ	eBioscience
CD44	APC-eFluor780	1M7	Rat IgG2b, κ	eBioscience
CD122	eFluor 450	TM-b1	Rat IgG2b	eBioscience
CXCR3	APC	CXCR3-173	Armenian Hamster IgG	BioLegend
Sca-1	Pe-Cy7	D7	Rat IgG2a, κ	eBioscience
Dead cells	7-AAD	-	-	BioLegend

Staining panel for T_N cell sorting for phospho-specific flow cytometry.

Marker	Fluorochrome	Clone	Isotype	Vendor
CD3	A700	HIT3a	Mouse IgG2a, κ	BioLegend
CD4	Pacific Blue	13B8.2	Mouse IgG1	Beckman Coulter
CD8	APC-A750	B9.11	Mouse IgG1	Beckman Coulter
CCR7	PerCp-Cy5.5	TG8/CCR7	Mouse IgG2a, κ	BioLegend
CD45RA	ECD	2H4LDH11LDB9	Mouse IgG1	Beckman Coulter
CD95	APC	DX2	Mouse IgG1, κ	BioLegend
CD58	FITC	1C3	Mouse IgG2a, κ	BD Biosciences
Dead cells	Vivid Aqua	Not specified	Not specified	Life Technologies

Staining panel for CFSE dilution assay.

Marker	Fluorochrome	Clone	Isotype	Vendor
Cell proliferation	Cell Trace Violet	Not specified	Not specified	LifeTechnologies
CD3	A700	HIT3a	Mouse IgG2a, κ	BioLegend
CD4	PE	13B8.2	Mouse IgG1	Beckman Coulter
CD8	PE-Cy7	SK1	Mouse IgG1, κ	BD Biosciences
CCR7	PerCp-Cy5.5	TG8/CCR7	Mouse IgG2a, κ	BioLegend
CD45RA	ECD	2H4LDH11LDB9	Mouse IgG1	Beckman Coulter
CD95	APC	DX2	Mouse IgG1, κ	BioLegend
CD58	FITC	1C3	Mouse IgG2a, κ	BD Biosciences
Dead cells	Vivid Near	Not specified	Not specified	LifeTechnologies

Staining panel for TMRE assessment.

Marker	Fluorochrome	Clone	Isotype	Vendor
MMP	TMRE	Not specified	Not specified	MitoScience
CD3	PerCP-Cy5.5	UCHT1	Mouse IgG1, κ	BioLegend
CD4	PE-Cy7	SK3	Mouse IgG1, κ	BD Biosciences
CD8	APC-A750	B9.11	Mouse IgG1	Beckman Coulter
CCR7	Brilliant Violet	G043H7	Mouse IgG2a, κ	BioLegend
CD45RA	A700	HI100	Mouse IgG2b, κ	BD Biosciences
CD95	APC	DX2	Mouse IgG1, κ	BioLegend
CD58	FITC	1C3	Mouse IgG2a, κ	BD Biosciences
Dead cells	Vivid Aqua	Not specified	Not specified	Life Technologies

Staining panel for assessment of 2-NBDG uptake.

Marker	Fluorochrome	Clone	Isotype	Vendor
2-NBDG	Fluorescein	Not specified	Not specified	LifeTechnologies
CD3	A700	HIT3a	Mouse IgG2a, κ	BioLegend
CD4	PE-Cy7	SK3	Mouse IgG1, κ	BD Biosciences
CD8	APC-A750	B9.11	Mouse IgG1	Beckman Coulter
CCR7	Brilliant Violet 421	G043H7	Mouse IgG2a, κ	BioLegend
CD45RA	ECD	2H4LDH11LDB9	Mouse IgG1	Beckman Coulter
CD95	PE-Cy7	DX2	Mouse IgG1, κ	BioLegend
CD58	APC	TS2/9	Mouse IgG1	eBioscience
Dead cells	Vivid Aqua	Not specified	Not specified	Life Technologies

Staining panel for KLF2 assessment.

Marker	Fluorochrome	Clone	Isotype	Vendor
KLF2	Alexa488	Polyclonal	Rabbit IgG	BIOSS
CD3	A700	HIT3a	Mouse IgG2a, κ	BioLegend
CD4	PE-Cy7	SK3	Mouse IgG1, κ	BD Biosciences
CD8	APC-A750	B9.11	Mouse IgG1	Beckman Coulter
CCR7	Brilliant Violet 421	G043H7	Mouse IgG2a, κ	BioLegend
CD45RA	ECD	2H4LDH11LDB9	Mouse IgG1	Beckman Coulter
CD95	PE-Cy7	DX2	Mouse IgG1, κ	BioLegend
CD58	APC	TS2/9	Mouse IgG1	eBioscience
Dead cells	Vivid Aqua	Not specified	Not specified	Life Technologies

SUPPLEMENTAL REFERENCES

Anders, S., Pyl, P.T., and Huber, W. (2014). HTSeq A Python framework to work with high-throughput sequencing data.

Kim, D., Pertea, G., Trapnell, C., Pimentel, H., Kelley, R., and Salzberg, S.L. (2013). TopHat2: accurate alignment of transcriptomes in the presence of insertions, deletions and gene fusions. Genome Biol. *14*, R36.

Love, M.I., Huber, W., and Anders, S. (2014). Moderated estimation of fold change and dispersion for RNA-Seq data with DESeq2.