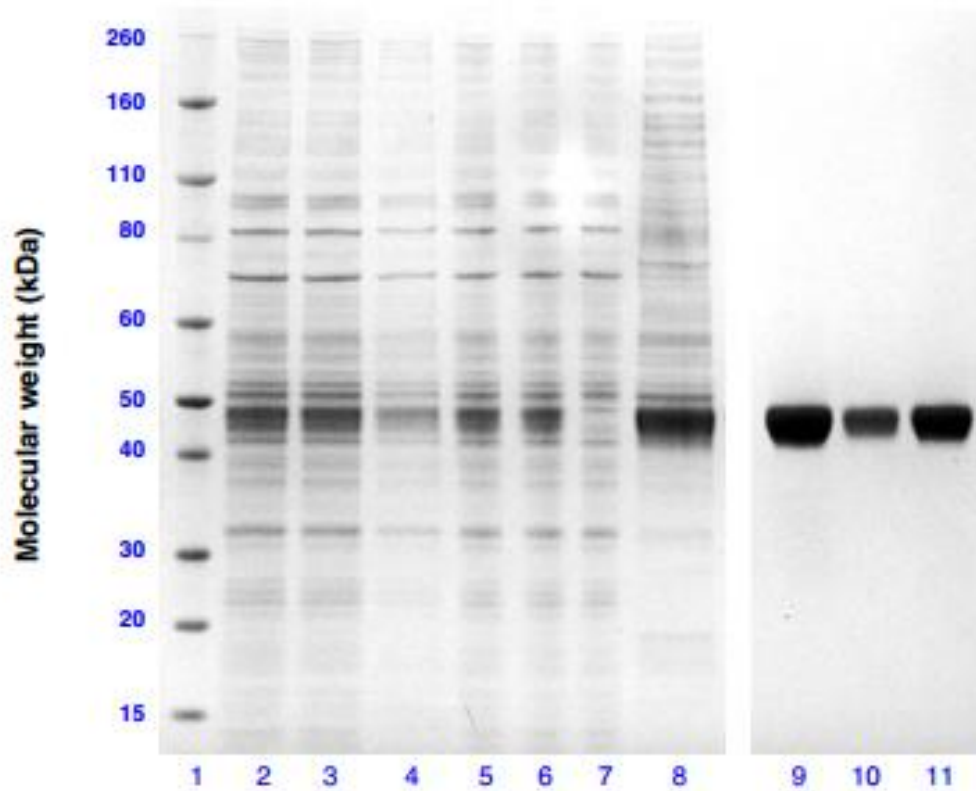


SUPPLEMENTAL MATERIALS – LEUNG ET AL.



Supplementary Figure 1. SDS-PAGE purity quantification for recombinant human sRAGE.

Lane 1: Novex Sharp Pre-Stained Molecular Weight Ladder.

Lanes 2-3: Cell culture supernatant (Lane 3 has been filtered).

Lanes 4-5: Crossflow permeate (Lane 5 is 5x concentrated).

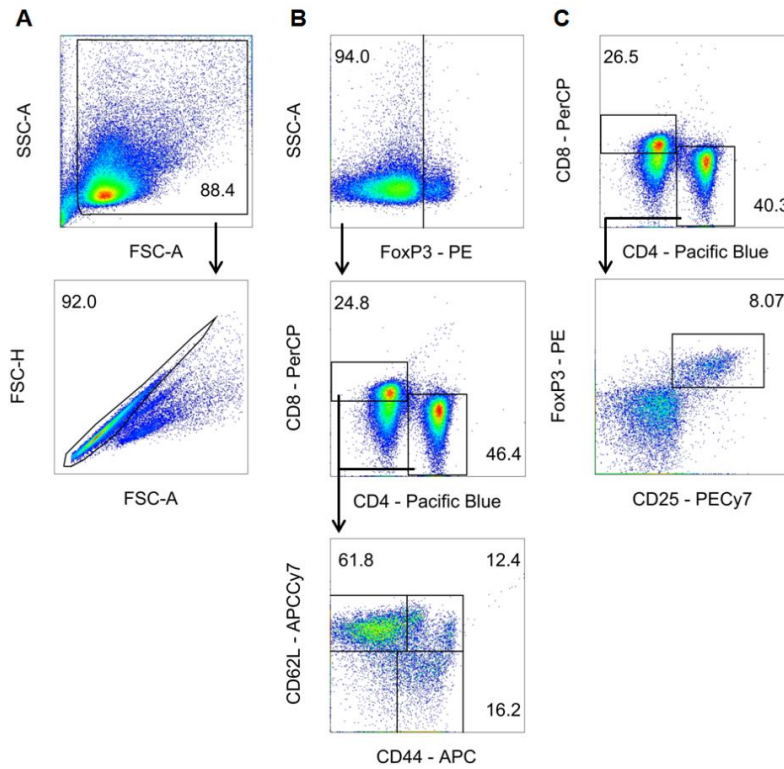
Lanes 6: Affinity chromatography load.

Lane 7: Affinity chromatography flow-through.

Lane 8: Affinity chromatography wash.

Lanes 9-10: Size exclusion chromatography pools (run twice).

Lane 11: Final production volume of recombinant human sRAGE (filtered).

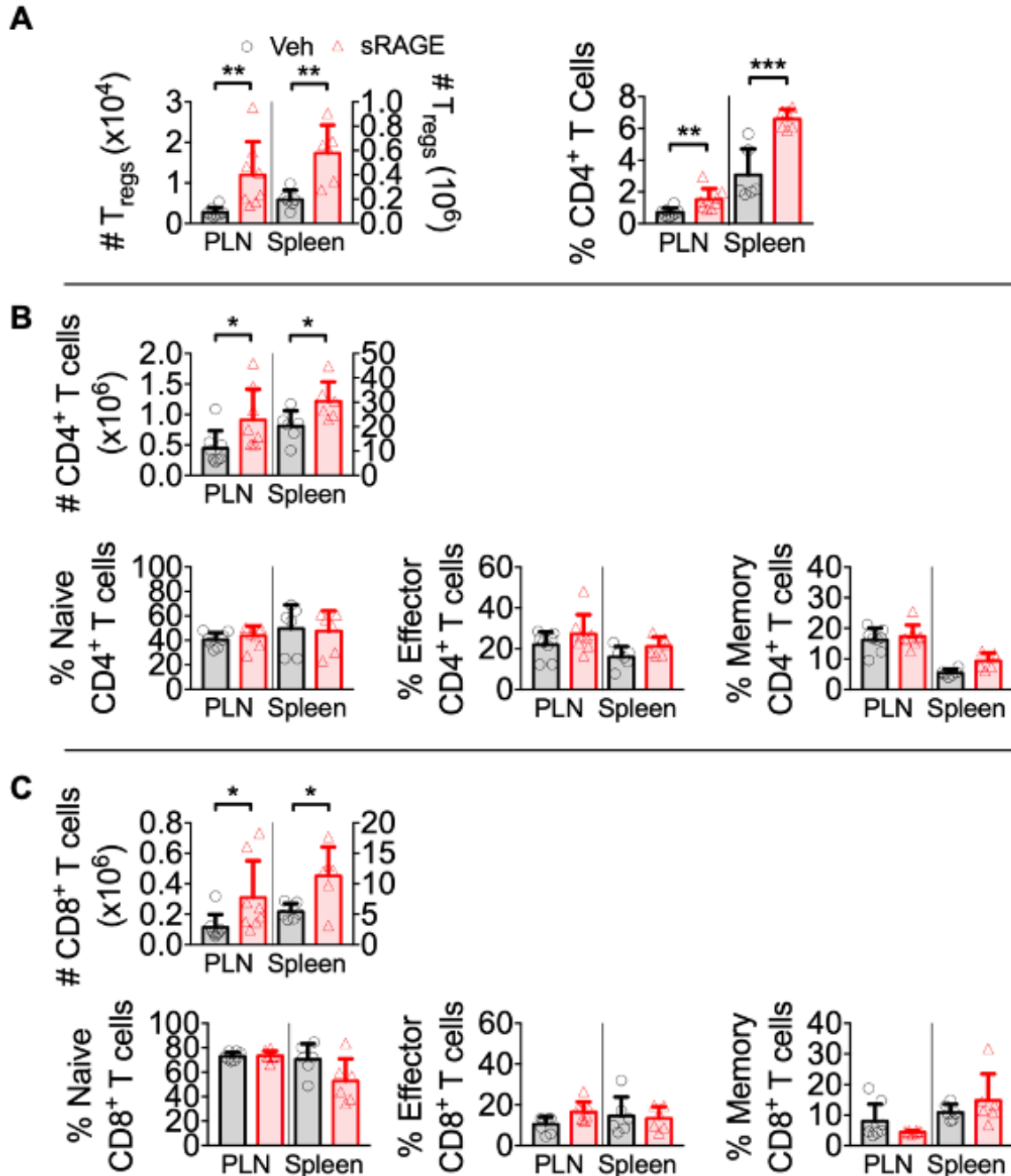


Supplementary Figure 2. Flow cytometry gating strategy for mouse T cells.

A: Debris were excluded and single cells gated.

B: Conventional T cells (T_{convs}): FoxP3⁺ T_{regs} were excluded, then CD4⁺CD8⁻ and CD8⁺CD4⁻ T cells were gated. Naïve, effector (T_{eff}) and memory cells were defined as CD62L⁺CD44⁻, CD62L⁻CD44⁺ and CD62L⁺CD44⁺, respectively.

C: T_{regs} : CD4⁺CD8⁻ T cells were gated, then CD4⁺CD8⁻CD25⁺Foxp3⁺ T_{regs} were gated.



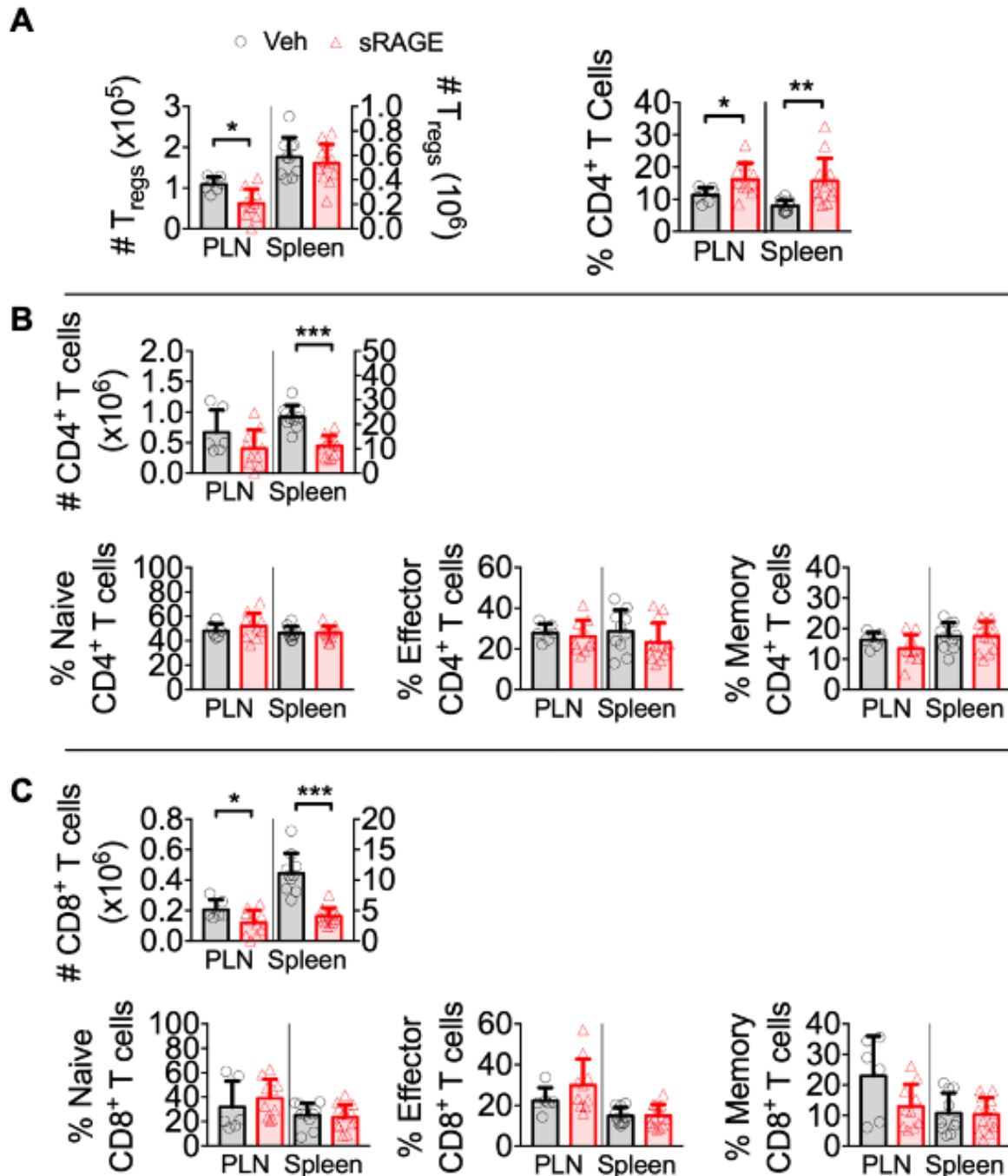
Supplementary Figure 3. sRAGE increases the numbers of $\text{CD4}^+\text{CD8}^-\text{CD25}^+\text{Foxp3}^+$ T_{regs} and T_{convs} in the pancreatic lymph nodes (PLN) and spleen on day 64.

A: $\text{CD4}^+\text{CD8}^-\text{CD25}^+\text{Foxp3}^+$ T_{regs} , presented as absolute numbers and percentage of CD4^+ T cells;

B: $\text{CD4}^+\text{FoxP3}^-$ T_{convs} ; and

C: $\text{CD8}^+\text{FoxP3}^-$ T_{convs} , accompanied with their $\text{CD62L}^+\text{CD44}^-$ naïve, $\text{CD62L}^-\text{CD44}^+$ effector (T_{eff}) and $\text{CD62L}^+\text{CD44}^+$ memory subsets.

Two-tailed Mann-Whitney U-test. $n = 4-13/\text{group}$. * $P < 0.05$; ** $P < 0.01$.



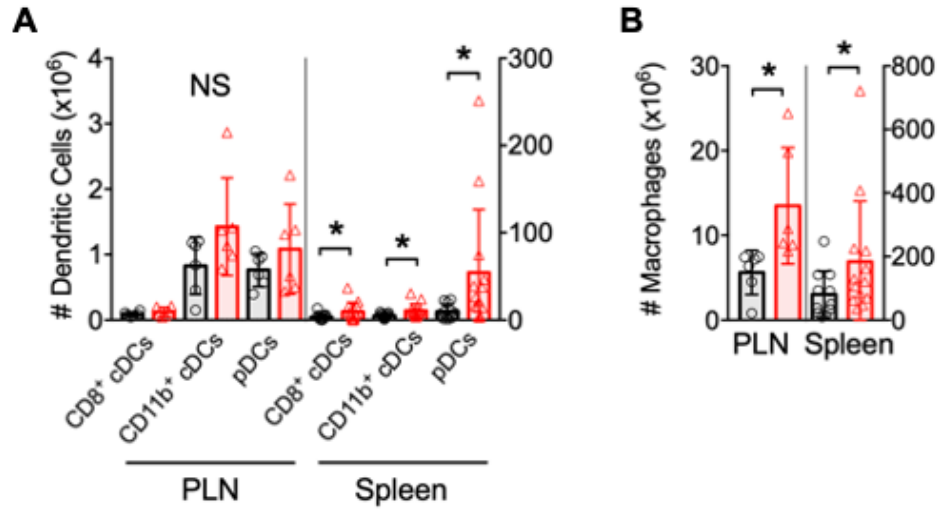
Supplementary Figure 4. sRAGE decreases the numbers of CD4⁺CD8⁻CD25⁺Foxp3⁺ T_{regs} and T_{convs} in the PLN and spleen on day 225.

A: CD4⁺CD8⁻CD25⁺Foxp3⁺ T_{regs}, presented as absolute numbers and percentage of CD4⁺ T cells;

B: CD4⁺FoxP3⁻ T_{convs}; and

C: CD8⁺FoxP3⁻ T_{convs}, accompanied with their CD62L⁺CD44⁻ naïve, CD62L⁻CD44⁺ effector (T_{eff}) and CD62L⁺CD44⁺ memory subsets.

Two-tailed Mann-Whitney U-test. *n* = 4-13/group. * *P* < 0.05; *** *P* < 0.001.

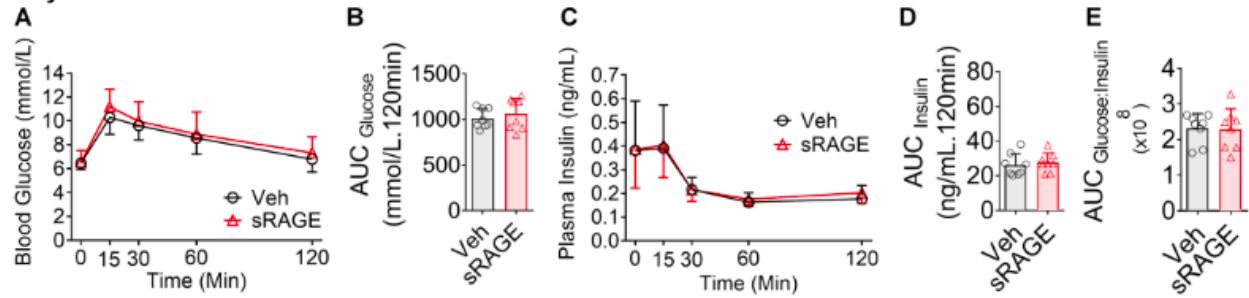


Supplementary Figure 5. sRAGE increases the numbers of dendritic cells and macrophages on day 64.

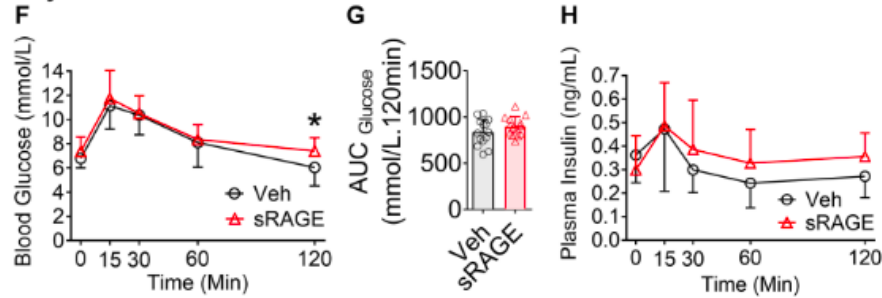
A: Conventional dendritic cells (cDCs) including CD8⁺ cDCs (CD11c⁺CD11b⁻B220⁻CD8⁺) and CD11b⁺ cDCs (CD11c⁺CD11b⁺B220⁻CD8⁻), as well as plasmacytoid dendritic cells (pDCs; CD11c⁺CD11b⁻B220⁺).

B: Macrophages (F4/80⁺CD11c⁻CD11b⁺B220^{int/hi}).

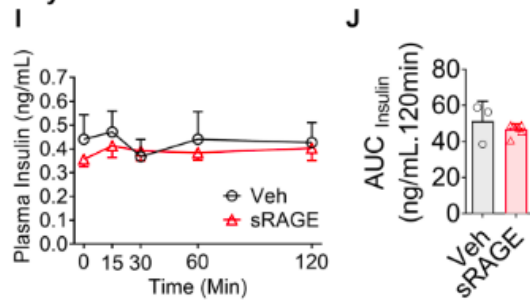
Day 64:



Day 80:



Day 225:



Supplementary Figure 6. Oral glucose tolerance tests (OGTTs).

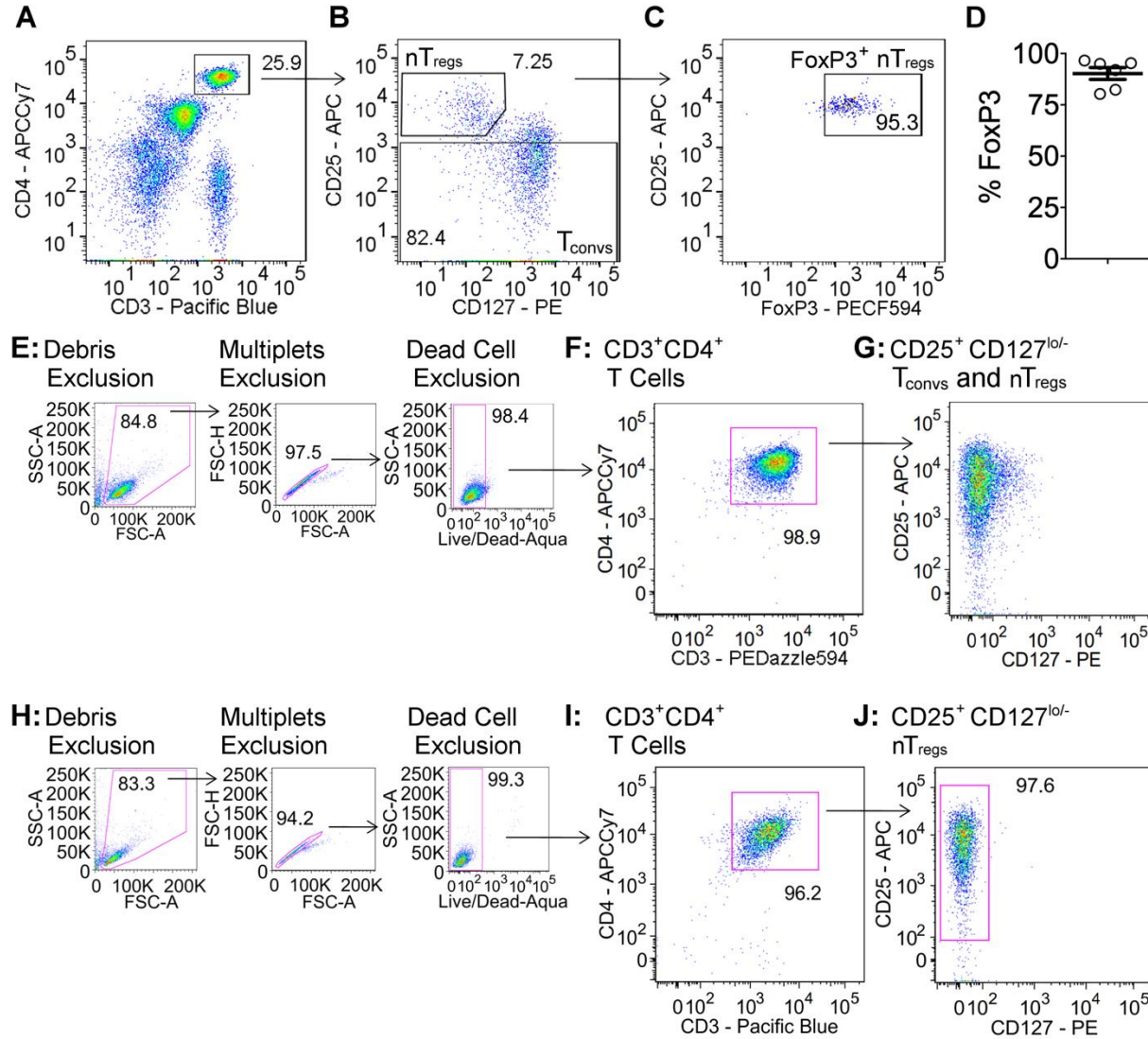
A-E: OGTTs on day 64 ($n = 8/\text{group}$).

F-H: OGTTs on day 80 ($n = 15/\text{group}$).

I-J: OGTTs on day 225 ($n = 3-6/\text{group}$).

A, F: Blood glucose concentrations; B, G: Area under the curve for blood glucose ($\text{AUC}_{\text{glucose}}$); C, H, I: Plasma insulin concentrations; D, J: $\text{AUC}_{\text{insulin}}$; E: $\text{AUC}_{\text{glucose:insulin}}$ ratio.

Data shown as mean $\pm$ SD and analyzed by two-tailed unpaired Student t-tests. * $P < 0.05$ between groups.



Supplementary Figure 7. Gating strategies for human T cell proliferation experiments.

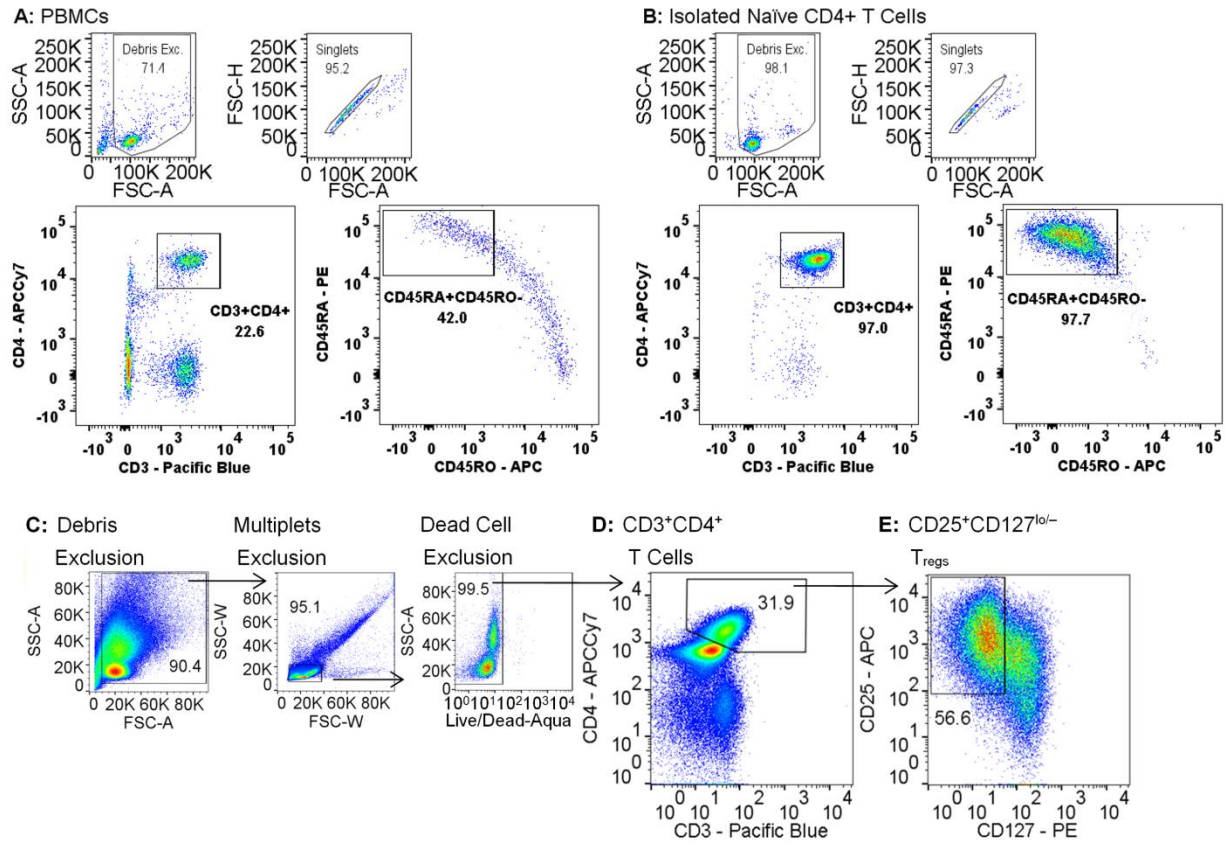
A-B: CD3⁺CD4⁺CD25⁺CD127^{lo/-} natural T_{regs} (nT_{regs}) and CD3⁺CD4⁺ CD25⁻ T_{convs} were positively isolated by FACS.

C-D: CD3⁺CD4⁺CD25⁺CD127^{lo/-} nT_{regs} were assessed for FoxP3 expression.

E-G: Gating strategy for analyzing nT_{reg} and T_{conv} proliferation when co-cultured. **E:** Debris, multiplets and dead cells were excluded, **F:** CD3⁺CD4⁺ cells were gated. CD3⁺CD4⁺ cells were then analyzed for nT_{reg} and T_{conv} proliferation by CFSE and CellTrace Violet dilution, respectively.

G: nT_{regs} and T_{convs} were both CD25⁺CD127^{lo/-} post-stimulation, so CD25 and CD127 expression was not used to delineate T_{regs} and T_{convs} prior to CFSE and CellTrace Violet dye dilution analysis

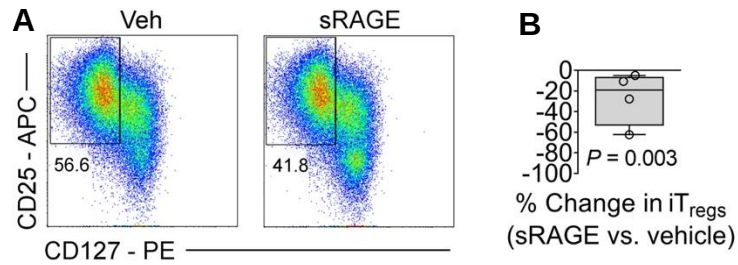
H-J: Gating strategy for analyzing nT_{reg} proliferation when cultured alone. **H:** Debris, multiplets and dead cells were excluded, **I:** CD3⁺CD4⁺ cells were gated, **J:** CD127^{lo/-} cells were gated as nT_{regs}. nT_{regs} were then analyzed for proliferation by CFSE dilution.



Supplementary Figure 8. Gating strategies for human induced T_{reg} (iT_{reg}) differentiation experiment.

A-B: Purity check for naïve CD4⁺ T cells (CD3⁺CD4⁺CD45RA⁺CD45RO⁻) *A:* before negative isolation (peripheral blood mononuclear cells, PBMCs) and *B:* after negative isolation.

C-E: Gating strategy for analyzing iT_{reg} differentiation. *C:* Debris, multiplets and dead cells were excluded, *D:* CD3⁺CD4⁺ cells were gated, *E:* CD25⁺CD127^{lo/-} cells were gated as iT_{regs}



Supplementary Figure 9. Human iT_{reg} differentiation in AGE-containing culture media is modestly decreased by sRAGE treatment.

Human naive CD3⁺CD4⁺CD45RA⁺CD45RO⁻ T cells were incubated in 3 day co-culture containing 5 ng/mL IL-2, 2 ng/mL TGF- β and 1 μ g/mL plate-bound anti-CD3 antibody.

A: Representative dot plots of CD3⁺CD4⁺CD25⁺CD127^{lo/-} iT_{regs} in AGE-supplemented media with co-administration of vehicle or sRAGE (50 μ g/day).

B: Quantification of change in iT_{reg} differentiation by sRAGE treatment. Data shown as mean $\pm$ SD; paired t-tests; $n = 4$ /group.

Supplementary Table 1. Human nT_{reg} NanoString predicted upstream regulators.

Gene Name	Log10 FDR <i>P</i>	NFATC2	2.9393	BACH2	2.0630
		mir-155	2.8268	MYC	2.0462
CD28	9.9136	POU5F1	2.8125	MTOR	2.0205
IL2	8.5086	REL	2.8013	CYTOR	1.9830
CD3	6.0650	VAV	2.7620	TNFRSF8	1.9830
IL4	6.0022	PRELID1	2.7620	CTBP2	1.9830
TBX21	5.5784	TNFRSF4	2.7620	ALK	1.9830
NPC2	5.4168	MIR4281	2.7620	NANOG	1.9469
CEBPB	4.8601	TPO	2.7620	ETS	1.9172
STAT3	4.8097	TCF12	2.7496	PRKDC	1.9172
GATA3	4.5986	mir-21	2.7033	NR4A2	1.9172
TCR	4.3893	STAT5a/b	2.5969	STAT5B	1.9172
PDCD1	4.3449	TGFB1	2.5376	WNT3A	1.9172
IL12 (complex)	4.2197	RELA	2.4724	CAMK4	1.8601
miR-9-5p (and other miRNAs w/seed CUUUGGU)	4.2140	BTK	2.4685	TNFSF9	1.8601
		PIWIL2	2.4597	SSTR2	1.8601
NPC1	3.9830	USP21	2.4597	CDKN1B	1.8601
mir-9	3.8861	IL21	2.2993	EGFR	1.8210
TIGIT	3.8861	TLR9	2.2993	CLEC7A	1.8097
HSPD1	3.8861	IL10RA	2.2840	TPM3	1.8097
CHRNA3	3.8861	FLOT2	2.2840	mir-132	1.8097
TNF	3.8297	ICOSLG/ LOC102723996	2.2840	STK4	1.8097
CTNNB1	3.7328	PTPN22	2.2840	IL9	1.8097
PHB2	3.7190	BCR (complex)	2.2588	PTPN6	1.8097
IL24	3.7190	COL18A1	2.2328	RPS6KA3	1.8097
SAA	3.6478	HIF1A	2.2218	CYP1B1	1.8097
TLR2	3.6345	calpain	2.1599	BRD4	1.7696
CD46	3.5186	CRBN	2.1599	Wnt	1.7645
IL12B	3.5186	MAP2K4	2.1599	BCL6B	1.7645
P2RX7	3.4078	COPA	2.1599	CD5	1.7645
NR3C1	3.2457	IL4R	2.1599	FCGR2A	1.7235
Interferon alpha	3.1778	IL10	2.0926	PIM2	1.7235
FOXP3	3.1035	ELAVL1	2.0814	ENG	1.7235
NFkB (complex)	3.1024	Integrin	2.0630	IL7	1.7235
HSF1	3.1002	HIF3A	2.0630	ELF5	1.7235
miR-21-5p (and other miRNAs w/seed AGCUUAU)	2.9393	PIM3	2.0630	CEBPA	1.6968
		ZBTB7B	2.0630	mir-154	1.6861
SELPLG	2.9393	SPIB	2.0630	NR6A1	1.6861
				CFLAR	1.6861

PRKCD	1.6840	SMARCA4	1.3706
IFNG	1.6737	Hsp27	1.3546
IL6	1.6655	F7	1.3546
CRTC2	1.6517	NFKB2	1.3546
IL12 (family)	1.6517	RNASE2	1.3391
LRP6	1.6517	RARA	1.3363
SYN1	1.6517	TAL1	1.3279
ICOS	1.6517	miR-155-5p	
TMSB4	1.6198	(miRNAs w/ seed	1.3233
Calcineurin		UAAUGCU)	
protein(s)	1.6198	mir-29	1.3233
NFKBIZ	1.6198	HMGA1	1.3089
RORC	1.6198	PKM	1.3089
MIF	1.6198		
TRIB3	1.5901		
FOXP1	1.5901		
Tgf beta	1.5622		
DPH5	1.5622		
PRDM1	1.5622		
BCL11B	1.5622		
USP7	1.5361		
CAV1	1.5361		
RAC1	1.5114		
MMP2	1.5114		
TP73	1.4935		
Smad2/3	1.4881		
TNFRSF1A	1.4881		
KIT	1.4881		
Mapk	1.4461		
GFI1	1.4461		
ZBTB16	1.4260		
RAF1	1.4260		
ATF2	1.4260		
CCL2	1.4067		
ZEB1	1.4067		
NR1I2	1.4067		
USF2	1.4067		
PIM1	1.3893		
CTLA4	1.3716		
RELB	1.3716		

Supplementary Table 2. Human nT_{reg} NanoString network analysis.

Focus Molecules (Count / Total)	Log10 <i>P</i> (Score)
AHR, GZMA, JAK1, STAT4, BACH2, FOXP3, GZMB, IL23A, JAK3, TIGIT, CCR4, GATA3, IL10RA, IL4R, IRF4, SOCS5, STAT5B, TNFRSF1A, CD226, IL7R, ITK, NFATC2, STAT3, STAT6 (24/44 molecules)	57
BTLA, KLF3, LGALS1, TNF, CCR4, CCR7, EOMES, GZMK, PTGER2 (9/35 molecules)	11
CD96 (1/2 molecules)	2
GNLY, PDCD1 (2/5 molecules)	2
IFNG, KLRB1, ZBTB16 (3/8 molecules)	2
CD27, CD40LG (2/12 molecules) – <i>not significant</i>	1
ITGA1, TGFB1 (2/22 molecules) – <i>not significant</i>	1

Supplementary Table 3. Human nT_{reg} NanoString volcano plot values.

Gene Name	Log2 fold change	Log10 FDR <i>P</i>			
Downregulated Genes			BCL6	-1.6249	-1.1240
<i>Significant & Log2 Fold Change > 1 </i>			IL9	-1.6055	-0.2823
IL7R	-2.5484	-1.7546	NT5E	-1.3630	-1.2081
CD49a	-2.0803	-2.0649	ENTPD1	-1.2501	-0.8104
PTGER2	-1.9939	-2.1195	IL1A	-1.1942	-1.2454
TIGIT	-1.8914	-2.9172	IFNAR1	-0.9949	-1.1934
IL23A	-1.5512	-2.0649	JUN	-0.9625	-0.8104
BACH2	-1.4216	-1.3429	CD137	-0.9331	-1.0007
CD96	-1.3483	-2.2805	CD45RA	-0.9205	-0.2759
IL10RA	-1.2758	-1.8328	CD134	-0.8898	-0.8104
GZMK	-1.2721	-1.5859	CXCR5	-0.8268	-0.6385
EOMES	-1.1603	-2.2805	CCR6	-0.7857	-1.1781
STAT5B	-1.0845	-2.2805	ADORA2A	-0.7784	-1.2081
STAT4	-1.0534	-2.2805	CD69	-0.7732	-1.0007
SOCS5	-1.0118	-2.2805	FOS	-0.7692	-0.4594
AHR	-1.0036	-2.2805	CCL5	-0.7610	-0.5731
KLRB1	-1.0012	-1.6611	IL18R1	-0.7587	-0.9195
<i>Significant & Log2 Fold Change < 1 </i>			CXCR6	-0.7412	-1.1861
JAK1	-0.9607	-2.2805	MAF	-0.7076	-0.8492
ITK	-0.9462	-2.2399	CCR7	-0.6972	-0.9349
CD27	-0.9205	-1.5859	JAK2	-0.6870	-1.1781
GNLY	-0.9122	-1.7023	ITGA4	-0.6773	-0.8492
CD272	-0.8973	-1.7546	CXCL8	-0.6668	-0.2232
GATA3	-0.8789	-2.2805	CX3CR1	-0.6629	-0.6085
IRF4	-0.8451	-1.7023	KLF2	-0.6381	-1.0614
CCR4	-0.7954	-2.2274	IL21R	-0.6134	-0.5089
STAT6	-0.7749	-2.0649	HLA.DRA	-0.5774	-1.2081
KLF3	-0.7526	-1.3783	IL27RA	-0.5764	-1.1781
CD226	-0.7137	-1.9163	PRDM1	-0.5591	-0.8104
FOXP3	-0.6978	-1.5387	IFNGR1	-0.5530	-1.2081
TNFR1	-0.6097	-1.4873	STAT5A	-0.5115	-1.0310
STAT3	-0.6097	-1.3095	GZMM	-0.5023	-0.1776
NFATC2	-0.6054	-1.7023	IL2RB	-0.5011	-0.9057
IL4R	-0.5634	-1.4870	SOCS1	-0.4920	-1.0009
JAK3	-0.5127	-1.3019	PTGDR2	-0.4850	-0.5087
<i>Not Significant</i>			ZBTB16	-0.4826	-0.4766
EGR2	-1.8547	-0.9349	STAT1	-0.4627	-1.1781
			CD45R0	-0.4396	-0.8966
			SELL	-0.4279	-1.1240

RUNX3	-0.4201	-0.9349
RUNX1	-0.4166	-1.2454
IL32	-0.3852	-1.2569
PVRIG	-0.3722	-0.6077
CTLA4	-0.3668	-0.5973
IL13	-0.3609	-0.1390
CCR5	-0.3567	-0.1867
IL12RB2	-0.3445	-0.7985
CCL4	-0.3087	-0.1074
CD3D	-0.3003	-1.0009
FAS	-0.2993	-0.7200
NFKB1	-0.2641	-0.3327
CD4	-0.2562	-0.8104
GFI1	-0.2466	-0.5371
PDCD1	-0.2256	-0.2111
IL2RA	-0.1373	-0.2670
TGFB1	-0.1181	-0.2759
LCK	-0.1157	-0.2823
CD28	-0.0971	-0.2681
IFNG	-0.0852	-0.0887
TNF	-0.0831	-0.0751
S1PR1	-0.0366	-0.0942
PRF1	-0.0214	-0.0208
SOCS3	0.0052	-0.0034

Upregulated Genes

Significant & Log2 Fold Change > |1|

GZMB	2.4512	-2.1857
GZMA	1.8410	-2.2805
LGALS1	1.0369	-2.1817

Not Significant

IL10	1.6348	-1.2429
IL17F	0.9889	-0.5126
IL4	0.9328	-0.9304
CXCR3	0.8543	-1.0007
IL22	0.8312	-0.4766
HAVCR2	0.4377	-1.1479
LAG3	0.3871	-0.5112
CD40LG	0.3713	-0.8358
ITGAE	0.2601	-0.6085
TBX21	0.2408	-0.4799

CD38	0.2017	-0.2113
ICOS	0.1964	-0.4859
RORC	0.1478	-0.2113
IL5	0.1379	-0.0361