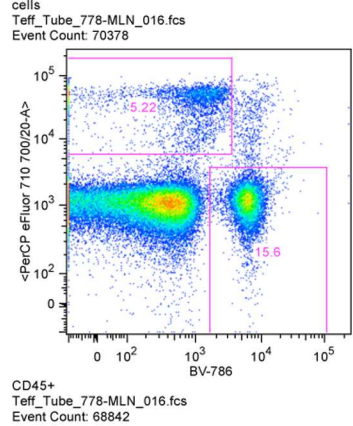
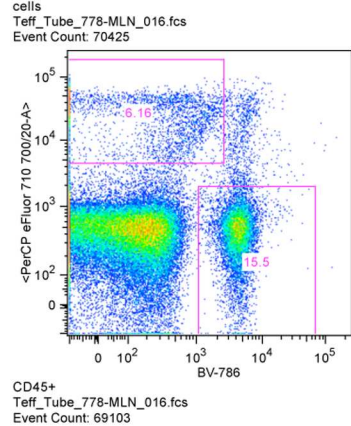
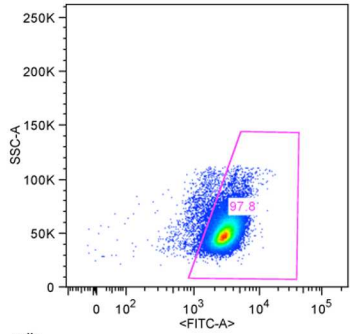
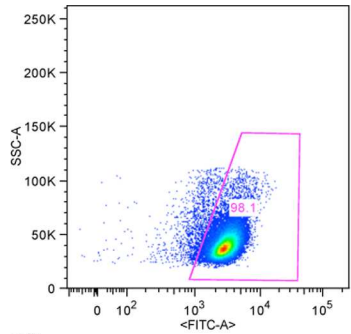
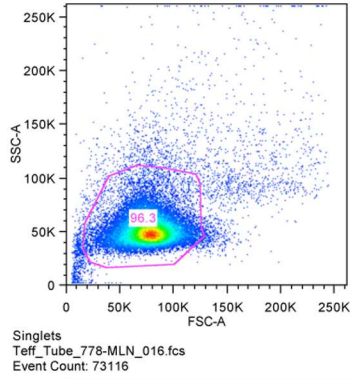
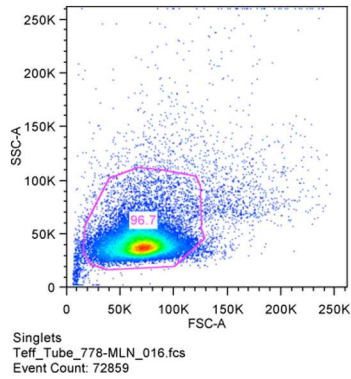
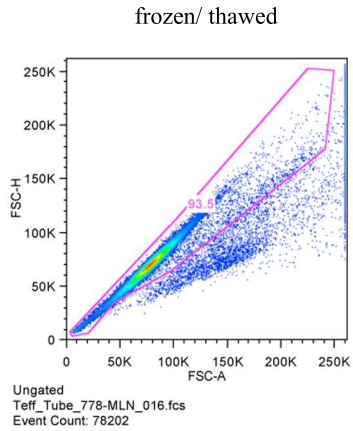
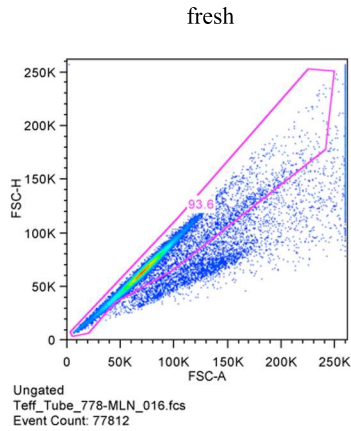


A

T-effectors



B

T-reg

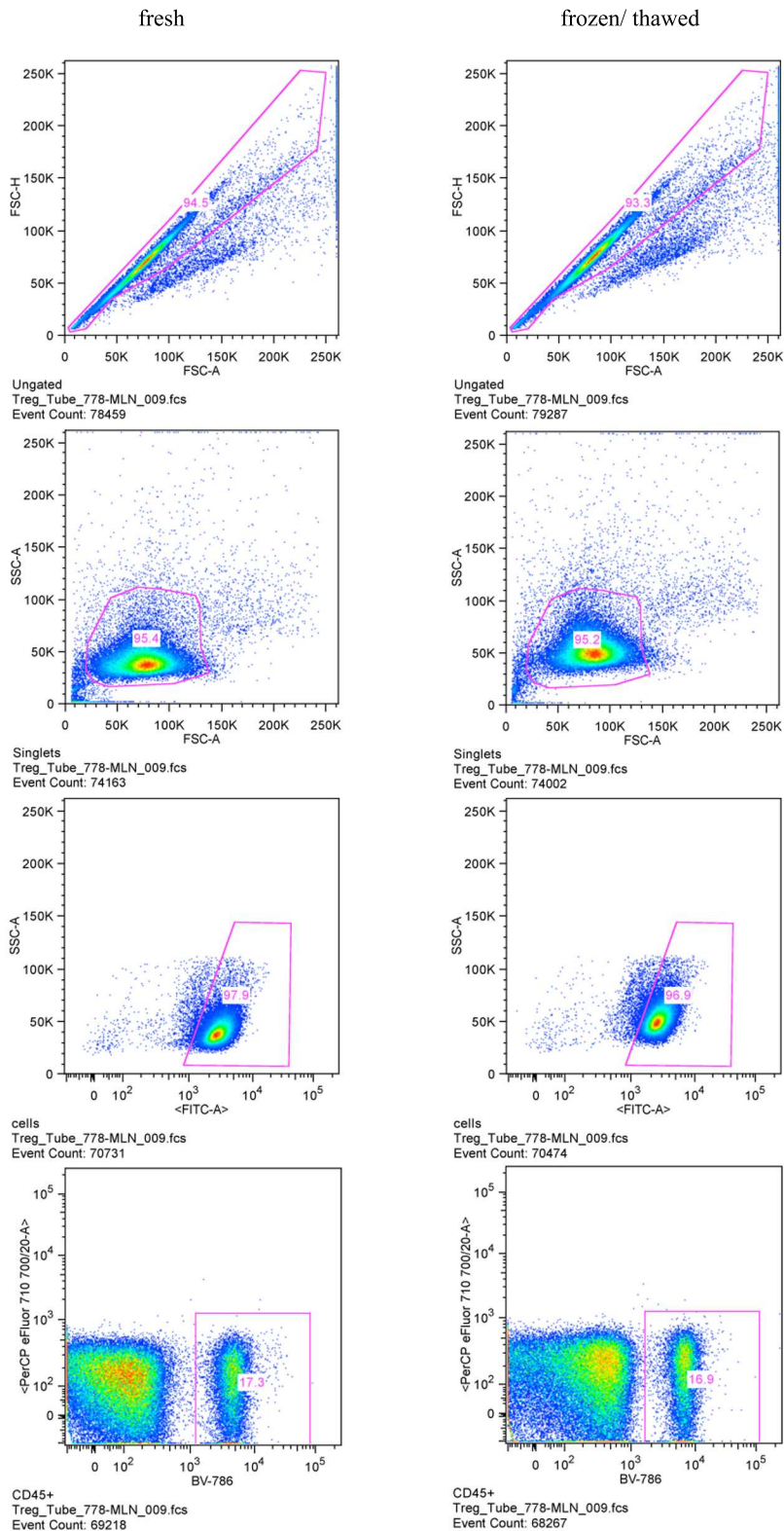


Figure S1: The effect of the one freezing/ thawing cycle on the lymphocyte antigens expression in effector (A) and regulatory (B) T cells.

**S1 Table Composition of parenteral nutrition mixture**

Ingredients	kcal . kg <sup>-1</sup> . day <sup>-1</sup>	% of non-amino acid energy
amino acid solution (Aminoplasmal 15%)	5	
glucose	140	70
lipid emulsion (Intralipid 20%)	60	30
total energy	205	

**Crude elements****Amino acid composition**

	mg . kg <sup>-1</sup> . day <sup>-1</sup>
Isoleucine	47
Leucine	91
Lysine	64
Methionine	46
Phenylalanine	46
Threonine	43
Tryptophan	17
Valine	58
Arginine	128
Histidine	42
Glycine	154
Alanine	179
Proline	59
Aspartic acid	64
Cysteine	3
Glutamic acid	130
Serine	24
Tyrosine	4

**Vitamins**

	µg . kg <sup>-1</sup> . day <sup>-1</sup>
Thiamin (B <sub>1</sub> )	124
Riboflavin B <sub>2</sub> )	140
Pyridoxine (B <sub>6</sub> )	155
Cyancobalamine (B <sub>12</sub> )	0.2
Nicotinamide	1552
Panthenic acid	582
Ascorbic acid (C)	3880
Biotin	2.3
Folic acid	15.5

### Electrolytes

	mmol . kg <sup>-1</sup> . day <sup>-1</sup>
Na <sup>+</sup>	15.6
K <sup>+</sup>	1.05
Mg <sup>2+</sup>	0.45
Ca <sup>2+</sup>	0.18
Cl <sup>-</sup>	16.1
SO <sub>4</sub> <sup>2-</sup>	0.45
HPO <sub>4</sub> <sup>2-</sup>	0.21
H <sub>2</sub> PO <sub>4</sub> <sup>-</sup>	0.11

### Trace elements

	μmol . kg <sup>-1</sup> . day <sup>-1</sup>
Cr <sup>3+</sup>	0.008
Cu <sup>2+</sup>	0.233
Fe <sup>3+</sup>	0.776
Mn <sup>2+</sup>	0.039
Mo <sup>6+</sup>	0.008
Se <sup>4+</sup>	0.039
Zn <sup>2+</sup>	2.988
I <sup>-</sup>	0.039
F <sup>-</sup>	1.940

**Supplementary table S2** Statistical analysis of sequencing data: genus level

Bacteroidetes;D_2_Bacteroidia;D_3_Bacteroidales;D_4_Bacteroidaceae;D_5_Bacteroides
Bacteroidetes;D_2_Bacteroidia;D_3_Bacteroidales;D_4_Bacteroidales S24-7 uncultured bacterium
Bacteroidetes;D_2_Bacteroidia;D_3_Bacteroidales;D_4_Porphyrromonadaceae;D_5_Parabacteroides
Bacteroidetes;D_2_Bacteroidia;D_3_Bacteroidales;D_4_Prevotellaceae;D_5_Alloprevotella
Bacteroidetes;D_2_Bacteroidia;D_3_Bacteroidales;D_4_Prevotellaceae;D_5_Prevotellaceae Ga6A1 group
Bacteroidetes;D_2_Bacteroidia;D_3_Bacteroidales;D_4_Prevotellaceae;D_5_Prevotellaceae NK3B31
Bacteroidetes;D_2_Bacteroidia;D_3_Bacteroidales;D_4_Prevotellaceae;D_5_Prevotellaceae UCG-001
Bacteroidetes;D_2_Bacteroidia;D_3_Bacteroidales;D_4_Rikenellaceae;D_5_Alistipes
Bacteroidetes;D_2_Bacteroidia;D_3_Bacteroidales;D_4_Rikenellaceae;D_5_Rikenellaceae RC9 gut group
Cyanobacteria;D_2_Melainabacteria;D_3_Gastranaerophilales;D_4_uncultured bacterium
D_1_Firmicutes;D_2_Bacilli;D_3_Lactobacillales;D_4_Lactobacillaceae;D_5_Lactobacillus
Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Christensenellaceae;D_5_Christensenellaceae R-7 group
Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Lachnospiraceae;D_5_Anaerostipes
Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Lachnospiraceae;D_5_Blautia
Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Lachnospiraceae;D_5_Coproccoccus
Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Lachnospiraceae;D_5_Coproccoccus
Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Lachnospiraceae;D_5_Incertae sedis
Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Lachnospiraceae;D_5_Lachnoclostridium
Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Lachnospiraceae;D_5_Lachnospiraceae AC2044
Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Lachnospiraceae;D_5_Lachnospiraceae NC2004
Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Lachnospiraceae;D_5_Lachnospiraceae NK4A136
Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Lachnospiraceae;D_5_Lachnospiraceae UCG-001
Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Lachnospiraceae;D_5_Roseburia
Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Lachnospiraceae;D_5_uncultured
Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Lachnospiraceae;Other
Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Peptococcaceae;D_5_uncultured
Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Peptostreptococcaceae;D_5_Peptoclostridium
Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Ruminococcaceae;D_5_Oscillibacter
Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Ruminococcaceae;D_5_Ruminiclostridium
Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Ruminococcaceae;D_5_Ruminococcaceae NK4A214
Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Ruminococcaceae;D_5_Ruminococcaceae UCG-005
Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Ruminococcaceae;D_5_Ruminococcaceae UCG-014
Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Ruminococcaceae;D_5_Ruminococcus
Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Ruminococcaceae;D_5_[Eubacterium] coprostan group
Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Ruminococcaceae;D_5_uncultured
Firmicutes;D_2_Erysipelotrichia;D_3_Erysipelotrichales;D_4_Erysipelotrichaceae;D_5_Allobaculum
Firmicutes;D_2_Erysipelotrichia;D_3_Erysipelotrichales;D_4_Erysipelotrichaceae;D_5_Candidatus Stoquefic
Firmicutes;D_2_Erysipelotrichia;D_3_Erysipelotrichales;D_4_Erysipelotrichaceae;D_5_Turicibacter
Firmicutes;D_2_Erysipelotrichia;D_3_Erysipelotrichales;D_4_Erysipelotrichaceae;Other
Proteobacteria;D_2_Alphaproteobacteria;D_3_Rhodospirillales;D_4_Rhodospirillaceae;D_5_Thalassospira

Proteobacteria;D_2__Betaproteobacteria;D_3__Burkholderiales;D_4__Alcaligenaceae;D_5__Parasutterella
Proteobacteria;D_2__Deltaproteobacteria;D_3__Desulfovibrionales;D_4__Desulfovibrionaceae;D_5__Desulfovib
Proteobacteria;D_2__Gammaproteobacteria;D_3__Enterobacteriales;D_4__Enterobacteriaceae;D_5__Escherichi
Proteobacteria;D_2__Gammaproteobacteria;D_3__Enterobacteriales;D_4__Enterobacteriaceae;Other
Verrucomicrobia;D_2__Verrucomicrobiae;D_3__Verrucomicrobiales;D_4__Verrucomicrobiaceae;D_5__Akkerma
Unassigned;Other;Other;Other;Other;Other

Fitted ANOVA model					Tukey's HSD pairwise comparis			
Coefficients					Effect size: fold change (95% CI)			
Intercept (control)	PN+But	PN	p-value	adjusted p-value	PN+But to control	PN to control	PN+But to PN	PN+But to control
-0.214	4.574	2.944	0.001	0.010	4.574	2.944	-1.629	0.001
3.842	-1.003	-0.884	0.222	0.387	-1.003	-0.884	0.119	0.257
-2.571	3.730	3.973	0.000	0.005	3.730	3.973	0.243	0.000
-1.459	0.945	-0.992	0.568	0.653	0.945	-0.992	-1.937	0.846
2.814	-3.390	-0.869	0.004	0.029	-3.390	-0.869	2.521	0.003
2.203	-2.312	-2.944	0.002	0.020	-2.312	-2.944	-0.632	0.014
2.105	-0.146	-0.979	0.579	0.653	-0.146	-0.979	-0.832	0.988
-0.371	2.164	1.679	0.008	0.046	2.164	1.679	-0.485	0.009
-0.731	0.731	0.727	0.681	0.727	0.731	0.727	-0.004	0.732
0.840	-1.486	1.607	0.015	0.069	-1.486	1.607	3.093	0.231
0.648	-3.128	-2.088	0.028	0.082	-3.128	-2.088	1.041	0.026
-1.203	0.847	0.135	0.485	0.633	0.847	0.135	-0.712	0.488
0.148	-1.658	-4.118	0.001	0.008	-1.658	-4.118	-2.459	0.118
0.182	1.194	1.214	0.280	0.425	1.194	1.214	0.020	0.361
-0.713	-0.212	0.486	0.562	0.653	-0.212	0.486	0.699	0.939
-1.083	1.083	0.217	0.597	0.653	1.083	0.217	-0.866	0.592
0.221	-1.398	-1.591	0.338	0.497	-1.398	-1.591	-0.193	0.463
0.016	0.326	-1.068	0.087	0.194	0.326	-1.068	-1.394	0.841
-1.212	1.445	-0.785	0.038	0.099	1.445	-0.785	-2.230	0.166
0.039	-1.342	-1.252	0.370	0.499	-1.342	-1.252	0.091	0.425
3.480	-2.164	-2.043	0.055	0.136	-2.164	-2.043	0.121	0.081
0.135	-1.665	-1.872	0.130	0.266	-1.665	-1.872	-0.207	0.225
0.278	0.919	-0.737	0.078	0.183	0.919	-0.737	-1.657	0.348
2.377	-1.190	-0.753	0.243	0.394	-1.190	-0.753	0.437	0.227
0.364	-1.417	-1.075	0.165	0.310	-1.417	-1.075	0.342	0.170
-0.377	1.273	0.087	0.110	0.235	1.273	0.087	-1.186	0.128
0.238	-0.252	0.413	0.573	0.653	-0.252	0.413	0.665	0.907
0.095	-0.765	-0.057	0.366	0.499	-0.765	-0.057	0.708	0.391
1.251	-0.287	0.068	0.862	0.881	-0.287	0.068	0.355	0.903
-1.050	0.748	1.381	0.014	0.069	0.748	1.381	0.632	0.185
-0.340	-0.184	1.353	0.007	0.044	-0.184	1.353	1.537	0.899
0.240	-0.473	0.076	0.553	0.653	-0.473	0.076	0.549	0.640
0.801	-2.283	-1.355	0.021	0.081	-2.283	-1.355	0.929	0.017
-0.665	1.018	1.300	0.028	0.082	1.018	1.300	0.282	0.096
1.395	-0.142	0.662	0.372	0.499	-0.142	0.662	0.804	0.966
-1.543	1.232	2.809	0.030	0.082	1.232	2.809	1.577	0.399
-1.749	0.686	-1.816	0.233	0.392	0.686	-1.816	-2.502	0.873
-0.897	-1.089	0.333	0.274	0.425	-1.089	0.333	1.422	0.427
-0.789	-0.556	-1.730	0.202	0.365	-0.556	-1.730	-1.174	0.820
-1.498	0.634	2.258	0.138	0.270	0.634	2.258	1.624	0.826

-1.135	2.046	2.618	0.000	0.005	2.046	2.618	0.572	0.002
-0.825	0.373	3.375	0.021	0.081	0.373	3.375	3.002	0.940
-2.896	3.979	3.590	0.024	0.082	3.979	3.590	-0.389	0.035
-0.716	-0.609	-1.496	0.591	0.653	-0.609	-1.496	-0.886	0.906
1.648	-1.717	-3.521	0.026	0.082	-1.717	-3.521	1.804	0.313
0.200	0.320	0.420	0.724	0.756	0.320	0.420	0.100	0.830



ion	
p-values	
PN to control	PN+But to PN
0.019	0.250
0.340	0.981
0.000	0.944
0.832	0.538
0.558	0.030
0.003	0.666
0.584	0.696
0.039	0.729
0.735	1.000
0.186	0.011
0.150	0.615
0.981	0.622
0.000	0.023
0.349	1.000
0.724	0.550
0.978	0.732
0.376	0.986
0.191	0.089
0.557	0.033
0.472	0.996
0.102	0.991
0.160	0.977
0.496	0.065
0.529	0.816
0.339	0.897
0.989	0.185
0.772	0.550
0.994	0.473
0.994	0.867
0.011	0.316
0.016	0.010
0.988	0.579
0.176	0.445
0.031	0.821
0.491	0.389
0.023	0.264
0.410	0.228
0.919	0.277
0.182	0.460
0.126	0.343

0.000	0.503
0.024	0.052
0.058	0.962
0.563	0.826
0.021	0.062
0.728	0.983