Supplemental Figures

Figure S1. Polyfunctional CD4+ and CD8+ T-cell subpopulations defined by single-cell analysis. These functional subsets were determined by principal component analysis, within CD4+ and CD8+ product T-cell subsets. The representation is based on clustering utilizing cytokines produced by individual cells as a 2-dimensional representation of objects in multidimensional space. The major polyfunctional subsets defined by similarity in the cytokines produced are represented as clusters, with individual dots corresponding to cells. The intensity of dots reflects the cytokine production level. The polyfunctionality of the T-cell population not stimulated with CD19+ cells is overlaid (blue color, NGFR) as a control. The major cytokines that are most commonly represented within each major population, CD4 and CD8 T cells, respectively, have been used to organize this principal component analysis (indicated on x and y axis), and the cytokines defining each cluster are indicated. The frequency of polyfunctional cells, comprising only about 20%-25% of all immunologically relevant cells, is also represented. IFN, interferon; IL, interleukin; MIP, macrophage inflammatory protein; NGFR, nerve growth factor receptor; TNF-a, tumor necrosis factor-a.

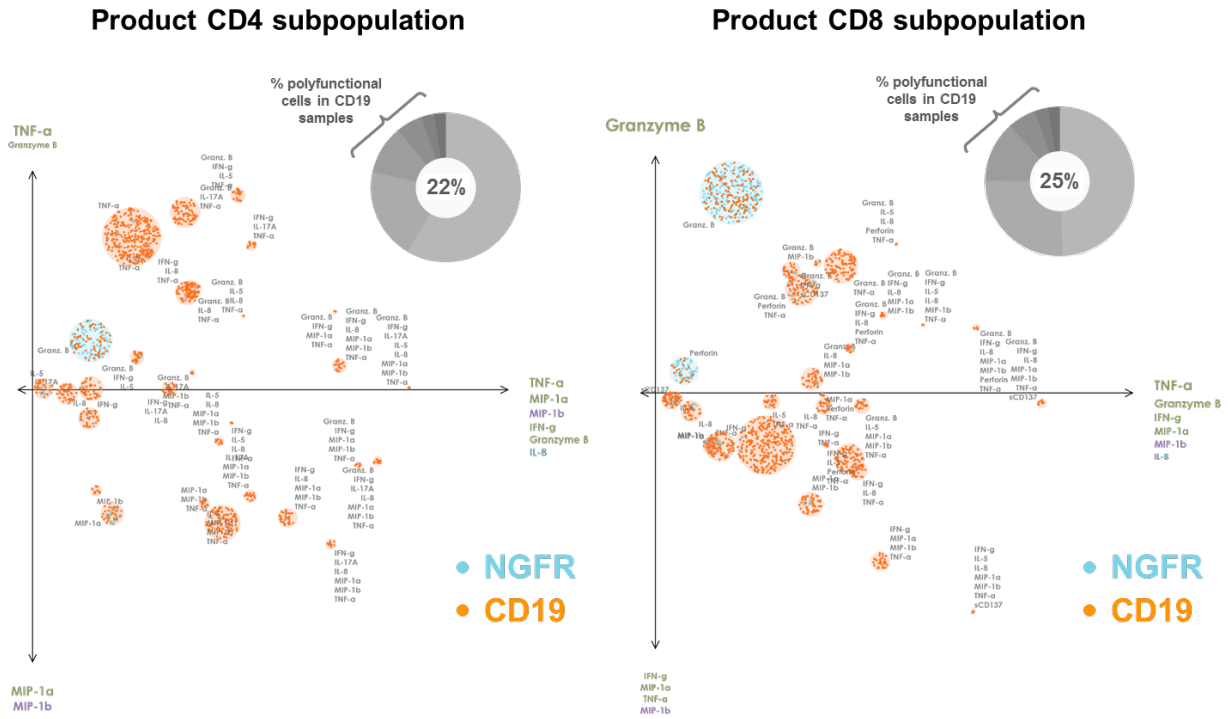


Figure S2. Association between PSI in conjunction with CAR T-cell levels in blood or day 0 IL-15 levels in serum and OR. CAR T-cell levels in blood were measured by qPCR. A composite index integrating PSI and CAR T-cell expansion in vivo was developed as detailed in Methods and associated with response outcome (R = response; N = no response). Whole-product PSI, CD4+ PSI, and IL-17A PSI indexes were all evaluated in conjunction with CAR peak levels. The 2 metrics were combined into a joint metric to test their association with a patient outcome. The metrics were added to each other after first standardizing each of them to have unit variance. This standardization was achieved by dividing the metric by their respective standard deviation to bring them to a common magnitude/scale. Joint PSI and day 0 IL-15 level metrics were calculated similarly. Statistical values were computed using the Mann Whitney *U* test. *P* values were not adjusted for multiplicity. CAR, chimeric antigen receptor; OR, objective response; PSI, polyfunctionality strength index; qPCR, quantitative polymerase chain reaction.

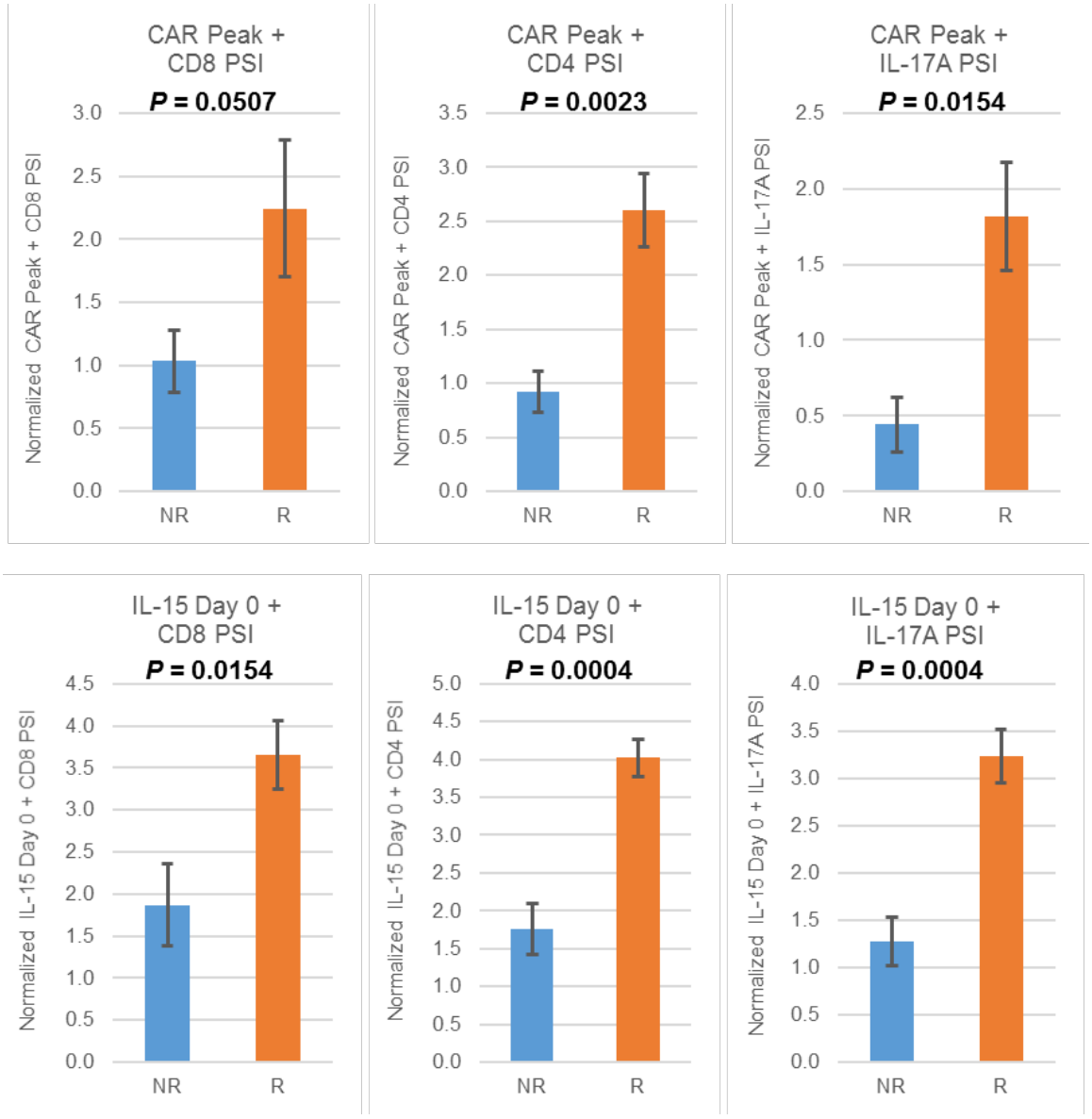
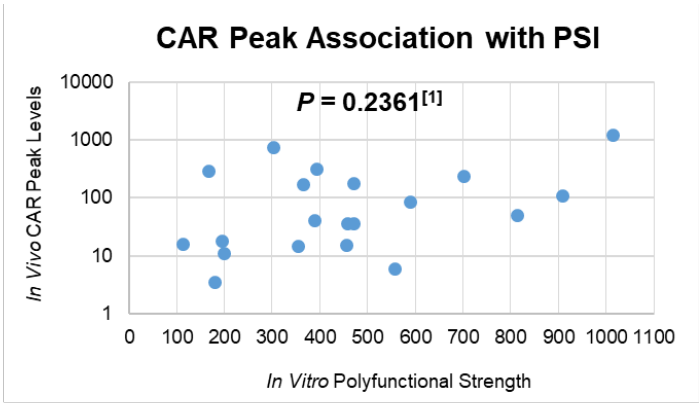


Figure S3. PSI does not associate with CAR T-cell levels in blood. CAR T-cell levels in blood were measured by qPCR and correlated with PSI or clinical outcome (OR, grade 3+ NT or CRS). Whole-product PSI, CD4+ PSI, and IL-17A PSI are displayed. Statistical analysis was performed using Spearman's correlation and Mann Whitney *U* tests. CRS, cytokine release syndrome; NT, neurologic toxicity.



[1] *P* value for Spearman correlation test, Spearman's ρ = 0.3272

CAR Peak vs. PSI

Metric	CAR Peak
PSI	0.1591[1]
CD8 PSI	0.4182
CD4 PSI	0.3440
IL-17A PSI	0.0740

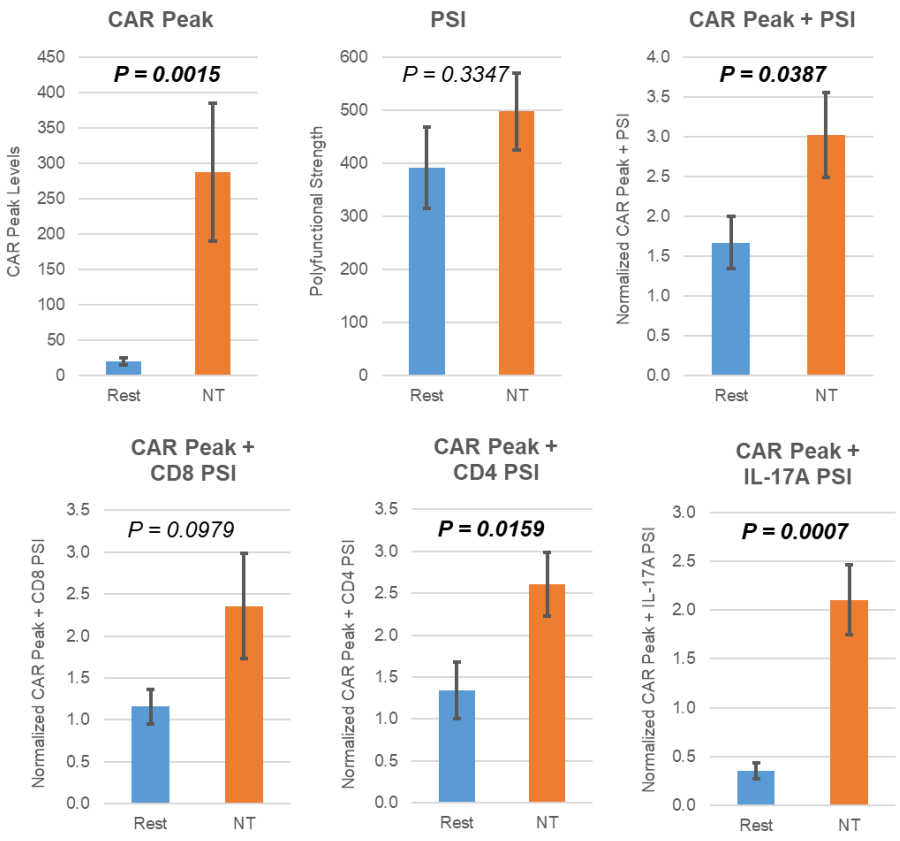
CAR Peak vs. Outcome

Outcome	CAR Peak
OR	**0.0326***
G3+ CRS	0.1574
G3+ NT	**0.0015****

* ***P* < 0.05**
** ***P* < 0.005**
*** ***P* < 0.0005**

Figure S4. Association between PSI in conjunction with CAR T-cell levels in blood, and grade 3+ NT; A) or CRS; B). CAR T-cell levels in blood were measured by qPCR and correlated with grade 3+ AEs. A composite index integrating PSI and CAR T-cell expansion in vivo was developed as detailed in Methods and associated with NT or CRS, respectively. Whole-product PSI, CD4+ PSI, and IL-17A PSI indexes were all evaluated in conjunction with CAR peak levels. The 2 metrics were combined into a joint metric to test their association with a patient outcome. The metrics were added to each other after first standardizing each of them to have unit variance. This standardization was achieved by dividing the metric by their respective standard deviation to bring them to a common magnitude/scale. Statistical values were computed using the Mann Whitney *U* test. *P* values were not adjusted for multiplicity. CRS, cytokine release syndrome; NT, neurologic toxicity.

A.



B.

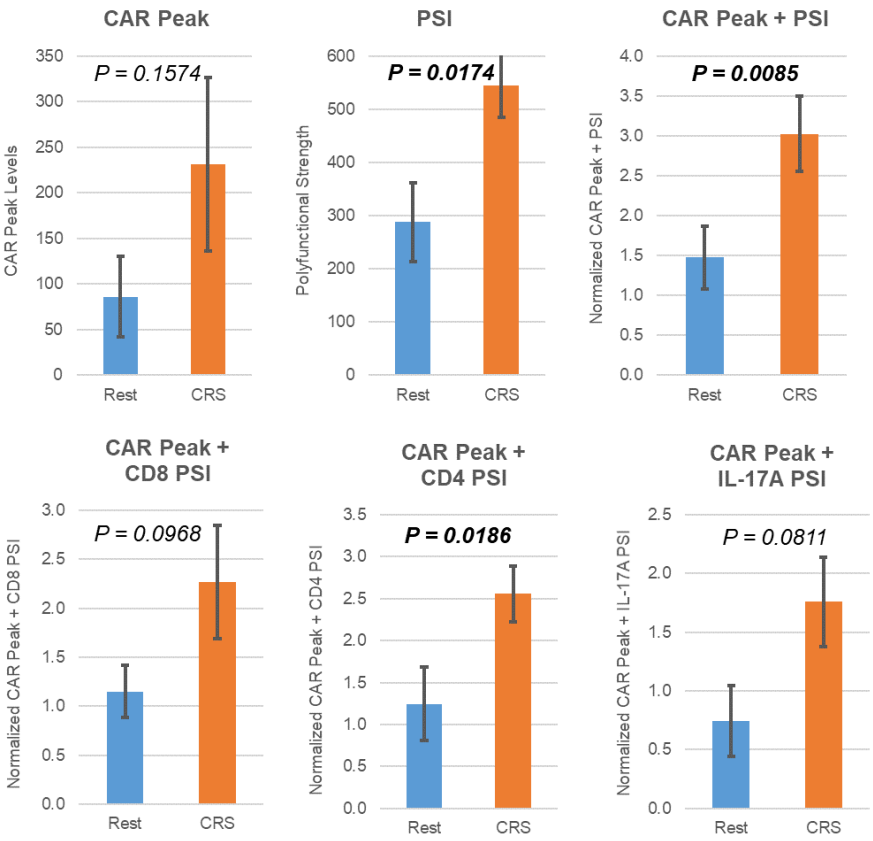
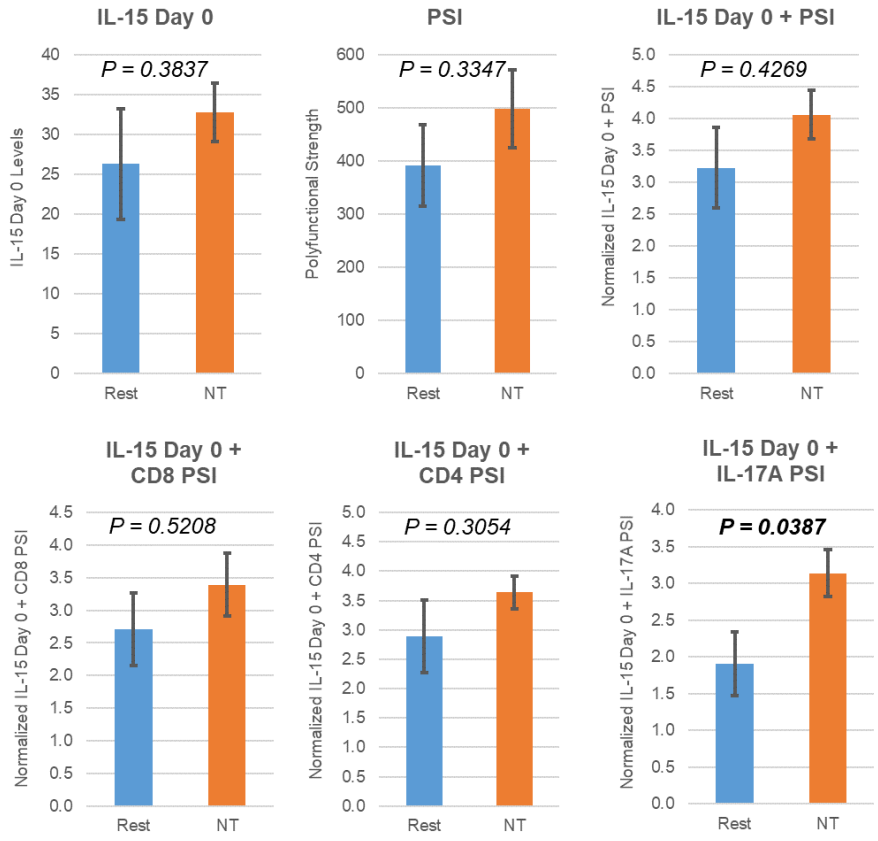

CAR Peak
P = 0.1574
CAR Peak Levels
350
300
250
200
150
100
50
0
Rest
CRS
PSI
P = 0.0174
Polyfunctional Strength
600
500
400
300
200
100
0
Rest
CRS
CAR Peak + PSI
P = 0.0085
Normalized CAR Peak + PSI
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
Rest
CRS
CAR Peak + CD8 PSI
P = 0.0968
Normalized CAR Peak + CD8 PSI
3.0
2.5
2.0
1.5
1.0
0.5
0.0
Rest
CRS
CAR Peak + CD4 PSI
P = 0.0186
Normalized CAR Peak + CD4 PSI
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
Rest
CRS
CAR Peak + IL-17A PSI
P = 0.0811
Normalized CAR Peak + IL-17A PSI
2.5
2.0
1.5
1.0
0.5
0.0
Rest
CRS

Figure S5. Association between PSI in conjunction with pretreatment IL-15 levels in blood, and grade 3+ NT (A) or CRS (B). IL-15 levels in blood were measured by ELISA and correlated with grade 3+ AEs. A composite index integrating PSI and IL-15 levels was developed as detailed in Methods and associated with grade 3+ NT or CRS, respectively. Whole-product PSI, CD4+ PSI, and IL-17A PSI were all evaluated in conjunction with IL-15 levels. Statistical values were computed using the Mann Whitney *U* test. *P* values were not adjusted for multiplicity. ELISA, enzyme-linked immunosorbent assay. CRS, cytokine release syndrome; NT, neurologic toxicity.

A.



B.

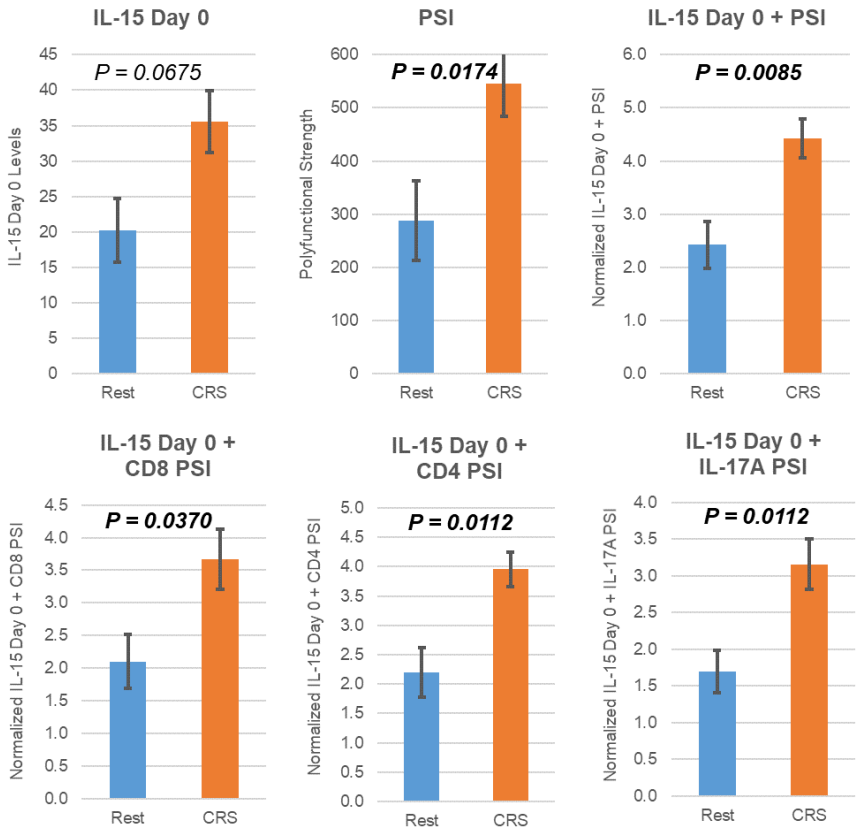

IL-15 Day 0
P = 0.0675
IL-15 Day 0 Levels
Rest
CRS
PSI
P = 0.0174
Polyfunctional Strength
IL-15 Day 0 + PSI
P = 0.0085
Normalized IL-15 Day 0 + PSI
IL-15 Day 0 + CD8 PSI
P = 0.0370
Normalized IL-15 Day 0 + CD8 PSI
IL-15 Day 0 + CD4 PSI
P = 0.0112
Normalized IL-15 Day 0 + CD4 PSI
IL-15 Day 0 + IL-17A PSI
P = 0.0112
Normalized IL-15 Day 0 + IL-17A PSI

Table S1. Product gene expression correlations with PSI. The table shows associations between PSI and ratio of CAR gene expression /T-cell–related molecules, all measured quantitatively by NanoString. CAR gene expression was measured utilizing probes for CD28 CD3zeta junction and scFv, respectively. T-cell–related mRNA was measured utilizing probes for CD3D, CD3E, CD3G, and CD6. CD3E was also measured at protein level using anti-CD3 mAb OKT3, by NanoString. PSI was also analyzed against T-cell molecules alone. The analysis was done by linear regression. mAb, monoclonal antibody; * $P < .05$. ** $P < .005$.

PSI versus	Alone	Ratio versus CD28_CD3z	Ratio versus scFv
CD3D	0.9925	**0.0077***	**0.0141***
CD3E	0.7122	**0.0029****	**0.0052***
CD3G	0.7051	**0.0096***	**0.0141***
CD6	0.7502	**0.0019****	**0.0075***
CD3E (OKT3)	0.8849	**0.0043****	**0.0032****

Table S2. Associations between product IL-17 PSI and product T-cell characteristics. Association between major product characteristics and IL-17A PSI was performed by linear regression. CD4:CD8 ratio was calculated based on flow cytometry measurements; % T-helper and % Th17 cells were measured based on epigenetic analysis as described in Methods; IL-17A and IL-6 in co-culture of product and target cells were measured by ELISA; and IL-17A PSI was computed as described in Methods. * $P < .05$. ** $P < .005$. *** $P < .0005$.

Product characteristics	PSI	IL-17A PSI
CD4:CD8 ratio	0.2008	**0.0008****
% T Helper (epigenetic)	0.7074	**0.0038****
% Th17 (epigenetic)	0.7407	**< 0.0001*****
IL-17A in co-culture	0.3129	**<0.0001*****
IL-6 in co-culture	0.0864	**0.0006****