#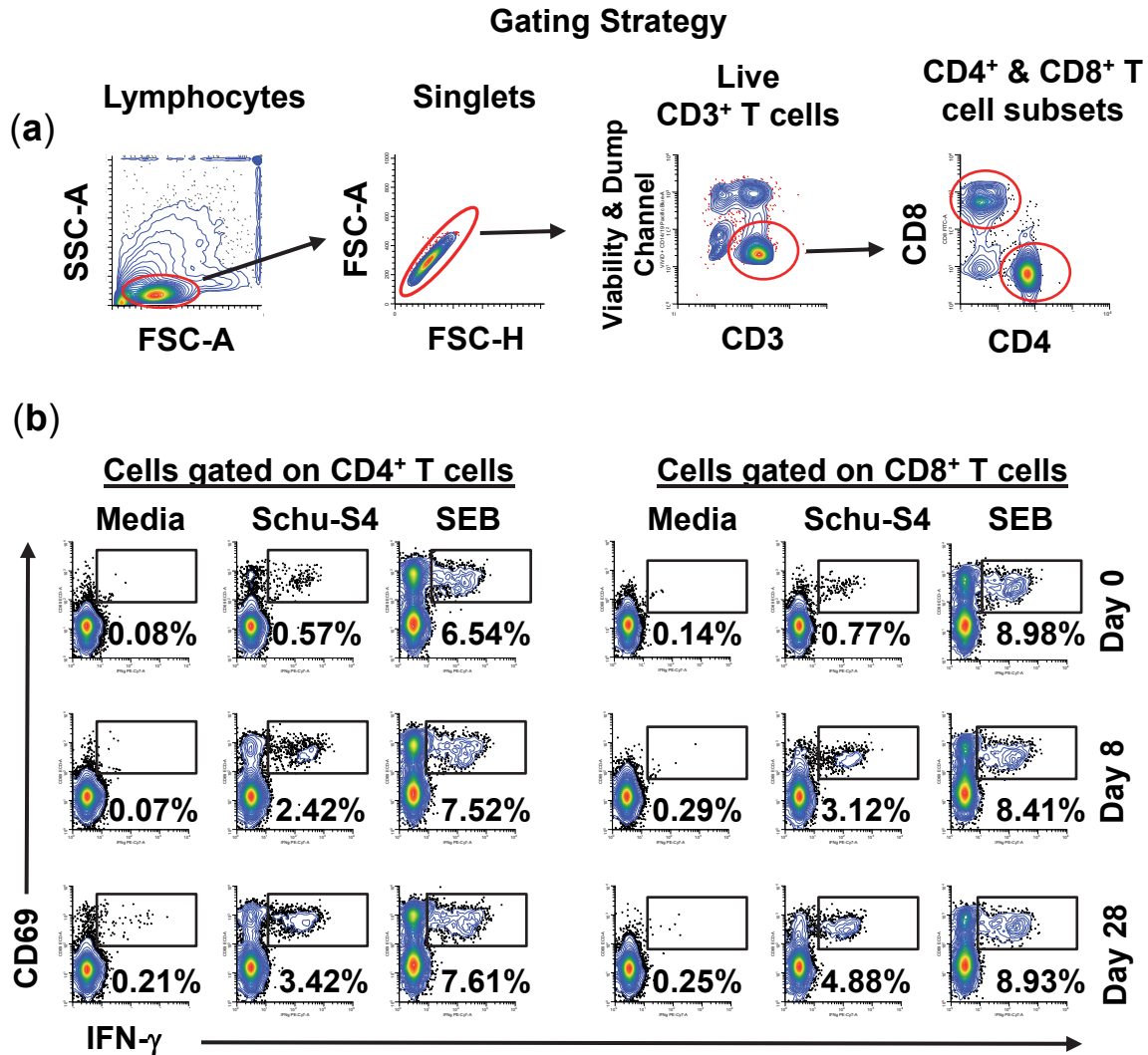 *Vaccine-related major cutaneous reaction -take - size correlates with cellular-mediated immune responses after tularemia immunization*

Rosangela Salerno-Gonçalves, Wilbur H Chen, Mark J Mulligan, Sharon E Frey, Jack T Stapleton, Wendy A Keitel, Jason Bailey, Eli Sendra, Heather Hill, Robert A Johnson and Marcelo Sztein

Supplementary Material

Supplementary figure 1. Production of IFN-γ by CD4⁺ and CD8⁺ T cells after *in vitro* exposure to Schu-S4. *Ex vivo* PBMC from a volunteer collected before (day 0) and 8, and 28 days after immunization were left unstimulated (media, negative control) or stimulated with Schu-S4 or SEB (positive control). After an overnight incubation, PBMC were stained with ViViD, followed by surface staining with mAbs to CD3, CD4, CD8, CD14, and CD19. After fixation and permeabilization, cells were stained intracellularly for CD69 and IFN-γ, and analyzed by flow cytometry. (**a**) Representative gating strategy for identification of CD4⁺ and CD8⁺ T cells. (**b**) Shown are the CD4⁺ and CD8⁺ T cell responses from a representative volunteer. Numbers represent the percentage of positive cells..
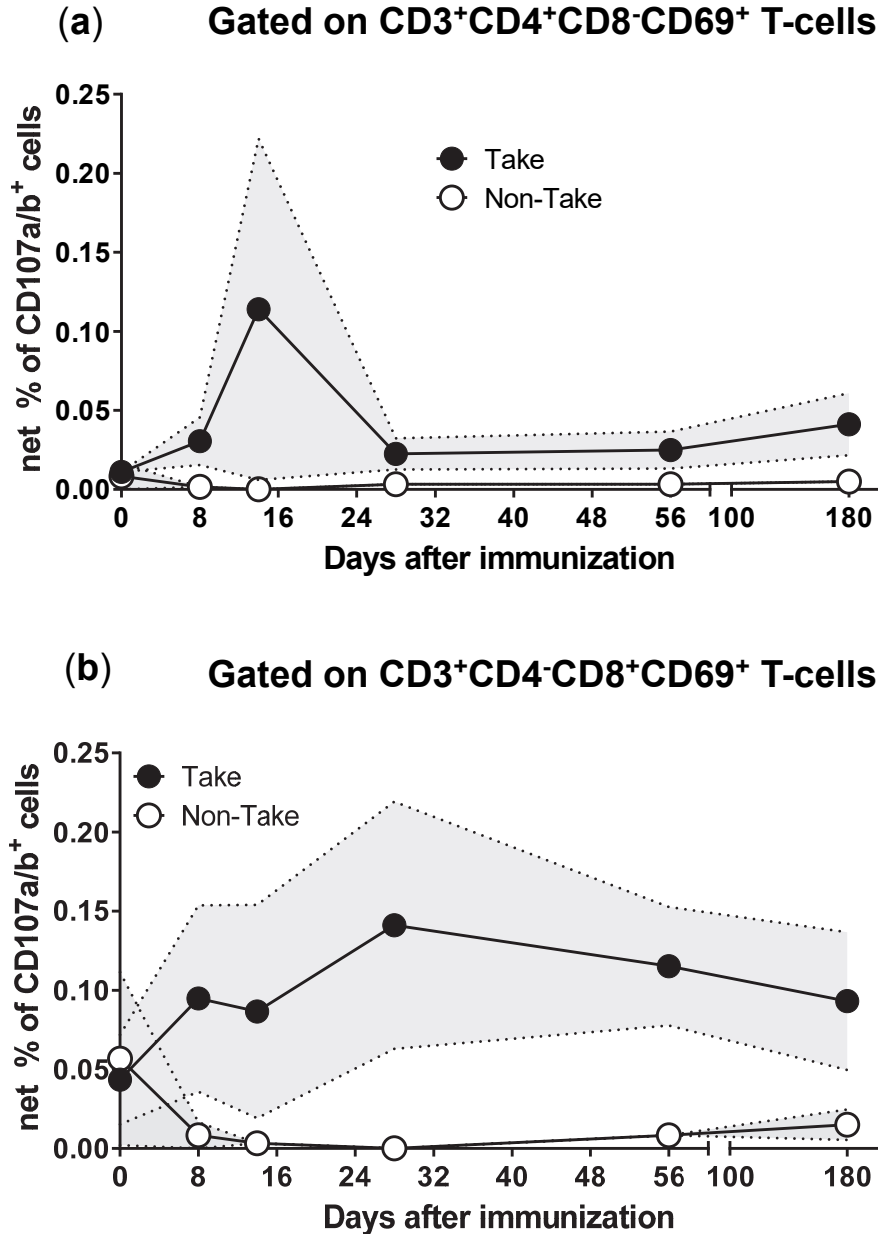
Supplementary table 1. Comparison between areas under the curves (AUC) of CD4^{+} and CD8^{+} T cell subsets and their expression of INF-γ, IL-2, CD107a & b, and TNF-α

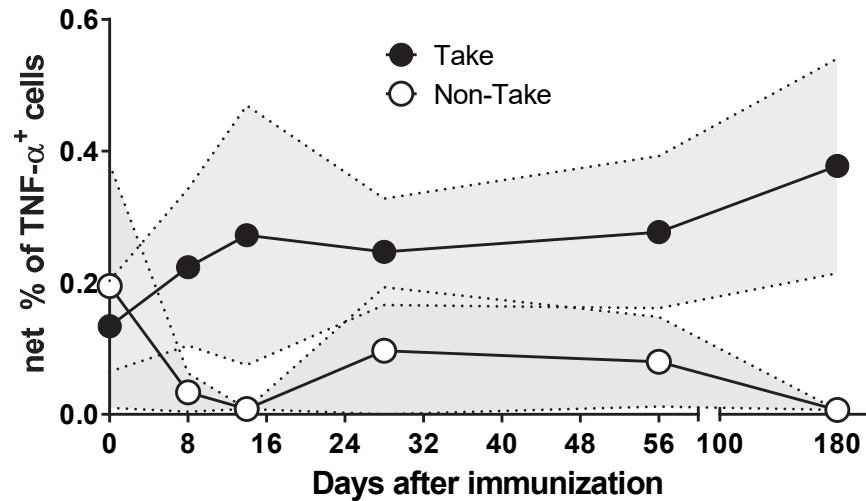| Performance parameters [‡]	Group [†]							
	IFN-γ		IL-2		CD107a & b		TNF-α	
	CD4+ cells	CD8+ cells	CD4+ cells	CD8+ cells	CD4+ cells	CD8+ cells	CD4+ cells	CD8+ cells
Total Area [§]	21.65	21.25	22.61	7.10	6.31	19.19	54.44	27.28
Std. Error	29.27	19.43	33.15	12.72	9.862	23.07	75.7	29.86
95% Confidence Interval	0 to 79.01	0 to 59.34	0 to 87.57	0 to 32.03	0 to 25.64	0 to 64.41	0 to 202.8	0 to 85.8

[†]Per Protocol Population

[‡] Pooled data from the 37 different subjects within take positive group

[§] AUC values are expressed in units (u)

(a) **Gated on CD3$^+$CD4$^+$CD8$^-$CD69$^+$ T-cells**

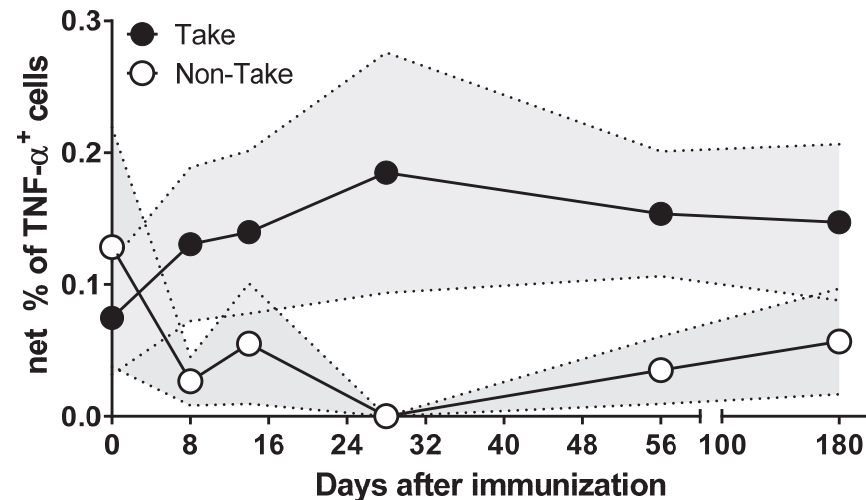**(b)** **Gated on CD3$^+$CD4$^-$CD8$^+$CD69$^+$ T-cells**

Supplementary figure 2. Kinetics of *F. tularensis*-specific T cells over 180-day after immunization. Cells from 43 subjects at six timepoints, days 0, 8, 14, 28, 56, and 180 were stimulated with Schu-S4 in the presence of CD107 "a" and "b" monoclonal antibodies . After an overnight incubation, PBMC were stained with ViViD, followed by surface staining with mAbs to CD3, CD4, CD8, CD14, and CD19. After fixation and permeabilization, cells were stained intracellularly for CD69, as well as to IL-2, IFN- γ and TNF- α cytokines and analyzed by flow cytometry. CD107a/b expressing CD4 (**a**) and CD8 (**b**) cells are shown. Symbols represent the means, and the filled area denotes the standard error bands. Subjects were divided into two groups based on their take results: (1) take positive (Take) and (2) take negative (non-Take). Per Protocol Population. Data are representative of 21 independent experiments with one replicate.

(a) **Gated on CD3$^+$CD4$^+$CD8$^-$CD69$^+$ T-cells**

(b) **Gated on CD3$^+$CD4$^-$CD8$^+$CD69$^+$ T-cells**

Supplementary figure 3. Kinetics of *F. tularensis*-specific T cells over 180-day after immunization. Cells from 43 subjects at six timepoints, days 0, 8, 14, 28, 56, and 180 were stimulated with Schu-S4 in the presence of CD107 "a" and "b" monoclonal antibodies . After an overnight incubation, PBMC were stained with ViViD, followed by surface staining with mAbs to CD3, CD4, CD8, CD14, and CD19. After fixation and permeabilization, cells were stained intracellularly for CD69, as well as to IL-2, IFN- γ and TNF- α cytokines and analyzed by flow cytometry. TNF- α expressing CD4 (**a**) and CD8 (**b**) cells are shown. Symbols represent the means, and the filled area denotes the standard error bands. Subjects were divided into two groups based on their take results: (1) take positive (Take) and (2) take negative (non-Take). Per Protocol Population. Data are representative of 21 independent experiments with one replicate.
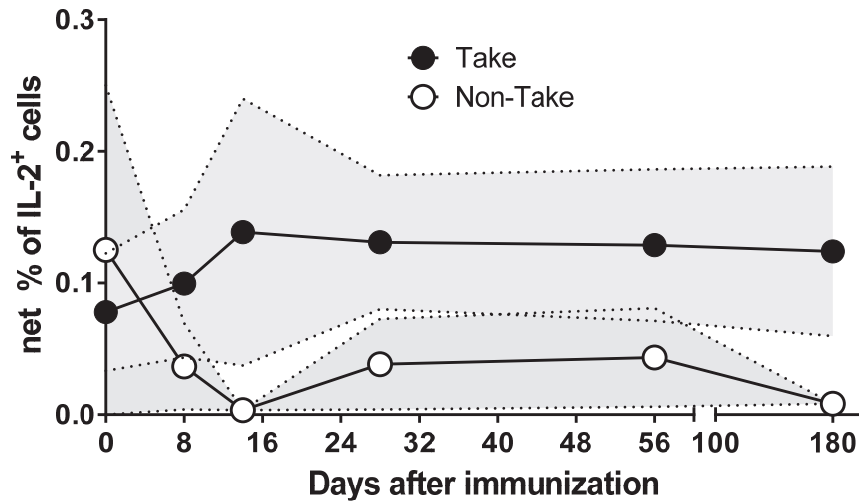
(a) **Gated on CD3$^+$CD4$^+$CD8$^-$CD69$^+$ T-cells**

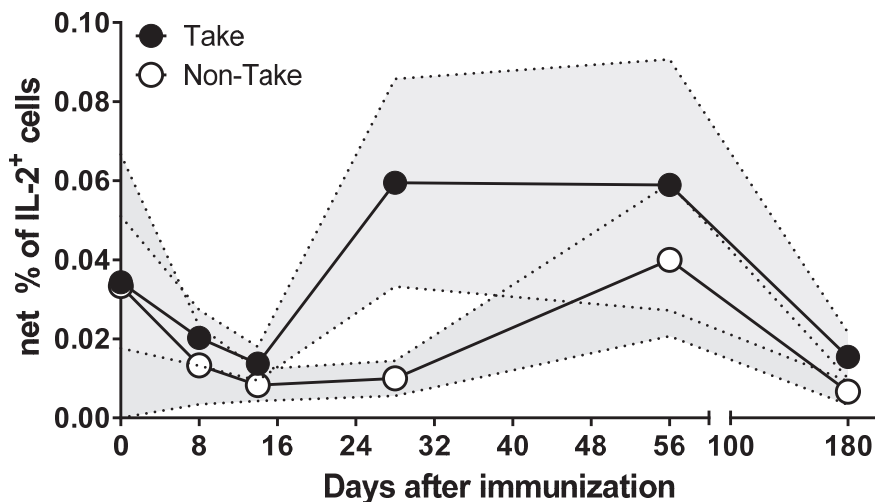**(b)** **Gated on CD3$^+$CD4$^-$CD8$^+$CD69$^+$ T-cells**

Supplementary figure 4. Kinetics of _F. tularensis_-specific T cells over 180-day after immunization. Cells from 43 subjects at six timepoints, days 0, 8, 14, 28, 56, and 180 were stimulated with Schu-S4 in the presence of CD107 "a" and "b" monoclonal antibodies . After an overnight incubation, PBMC were stained with ViViD, followed by surface staining with mAbs to CD3, CD4, CD8, CD14, and CD19. After fixation and permeabilization, cells were stained intracellularly for CD69, as well as to IL-2, IFN-γ and TNF-αcytokines and analyzed by flow cytometry. IL-2 expressing CD4 (**a**) and CD8 (**b**) cells are shown. Symbols represent the means, and the filled area denotes the standard error bands. Subjects were divided into two groups based on their take results: (1) take positive (Take) and (2) take negative (non-Take). Per Protocol Population. Data are representative of 21 independent experiments with one replicate.
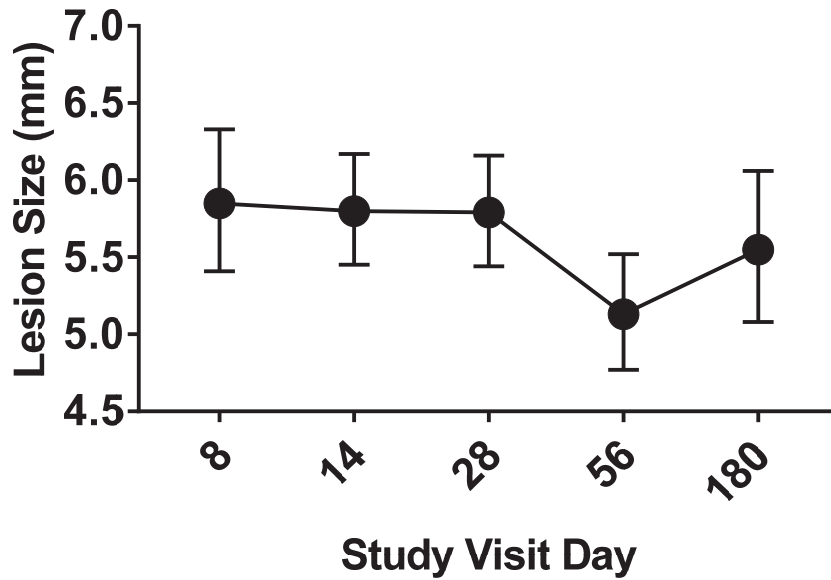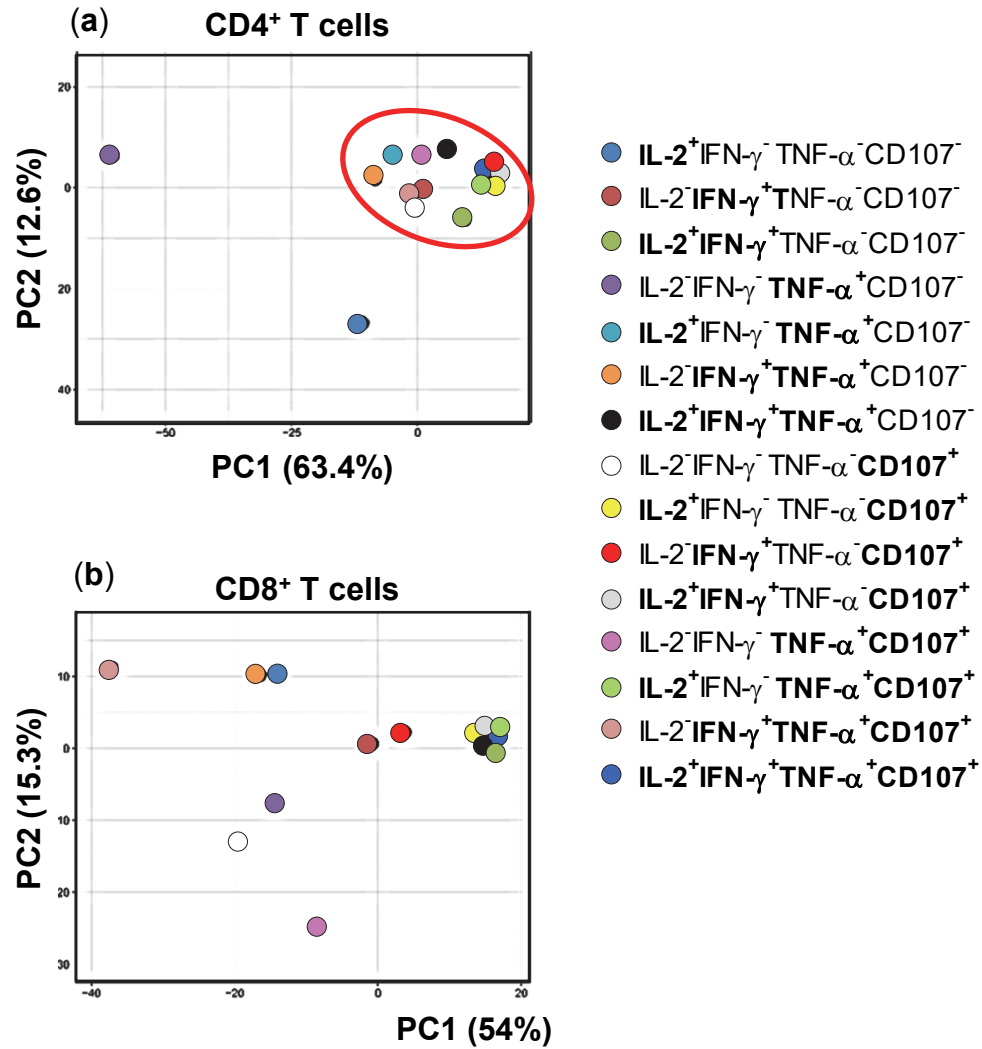
Supplementary figure 5. Lesion size by study timepoint per protocol population. Lesion size of 164 subjects who developed a positive "take" response, as assessed by the clinical site, defined as the development of an erythematous papule, vesicle, and/or eschar with or without underlying induration, by study Visit 5 (7-9 days post vaccination). Symbols and error bars represent the geometric mean of the lesion size (mm) with 95% confidence interval.

(a) **CD4$^+$ T cells**

PC2 (12.6%)

20

0

20

40

−50   −25   0

PC1 (63.4%)

(b) **CD8$^+$ T cells**

PC2 (15.3%)

10

0

10

20

30

−40   −20   0   20

PC1 (54%)

Legend:

- **IL-2$^+$**IFN-γ^- TNF-α^-CD107$^-$
- IL-2$^-$**IFN-γ^+TNF-α^-**CD107$^-$
- **IL-2$^+$IFN-γ^+**TNF-α^-CD107$^-$
- IL-2$^-$IFN-γ^- **TNF-α^+**CD107$^-$
- **IL-2$^+$**IFN-γ^- **TNF-α^+**CD107$^-$
- IL-2$^-$**IFN-γ^+TNF-α^+**CD107$^-$
- **IL-2$^+$IFN-γ^+TNF-α^+**CD107$^-$
- IL-2$^-$IFN-γ^- TNF-α^-**CD107$^+$**
- **IL-2$^+$**IFN-γ^- TNF-α^-**CD107$^+$**
- IL-2$^-$**IFN-γ^+**TNF-α^-**CD107$^+$**
- **IL-2$^+$IFN-γ^+**TNF-α^-**CD107$^+$**
- IL-2$^-$IFN-γ^- **TNF-α^+CD107$^+$**
- **IL-2$^+$**IFN-γ^- **TNF-α^+CD107$^+$**
- IL-2$^-$**IFN-γ^+TNF-α^+CD107$^+$**
- **IL-2$^+$IFN-γ^+TNF-α^+CD107$^+$**

Supplementary figure 6. **Principal Component Analysis variances**. PC1 vs PC2 percent variations are plotted for (**a**) CD4$^+$ and (**b**) CD8$^+$ T cells. Each dot represents the cumulative values of 43 individuals. The PCA analyses summarizes the variance of 3870 data points. Red centroid shows the cluster formed by CD4$^+$ T cell subsets