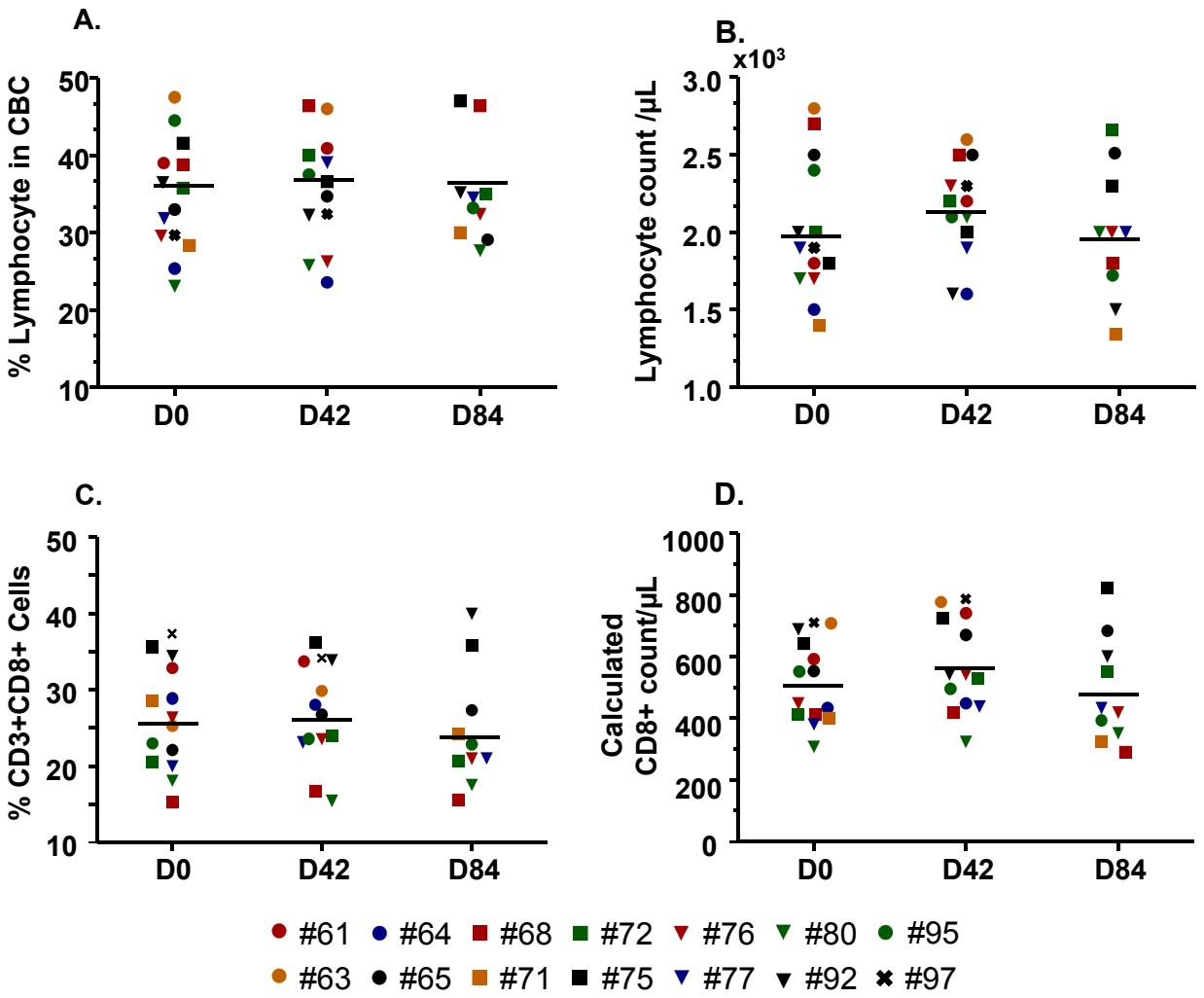

Figure S1. Blood lymphocyte counts and absolute numbers of CD3+CD8+ cells before and following Ty21a vaccination. Percentages (**panel A**) and absolute lymphocyte counts/µL (**panel B**) were obtained from available Complete Blood Cell counts (CBC) performed in blood specimens collected at Days 0 (n=14), 42 (n=13) and 84 (n=10) from Ty21a vaccinated volunteers. The % of CD3+CD8+ cells (**panel C**) were determined by flow cytometry. Absolute numbers of CD8+ cells/µL (**panel D**) were calculated as: [(Absolute counts of lymphocyte/µL) X (% of CD3+CD8+ cells; by flow cytometry)/100]]

Horizontal bars represent the mean values for each group.

A one way ANOVA with Bonferroni's Multiple Comparison Test showed no significant differences ($p>0.15$) in any of the parameters shown for D0, D42 or D84 samples.

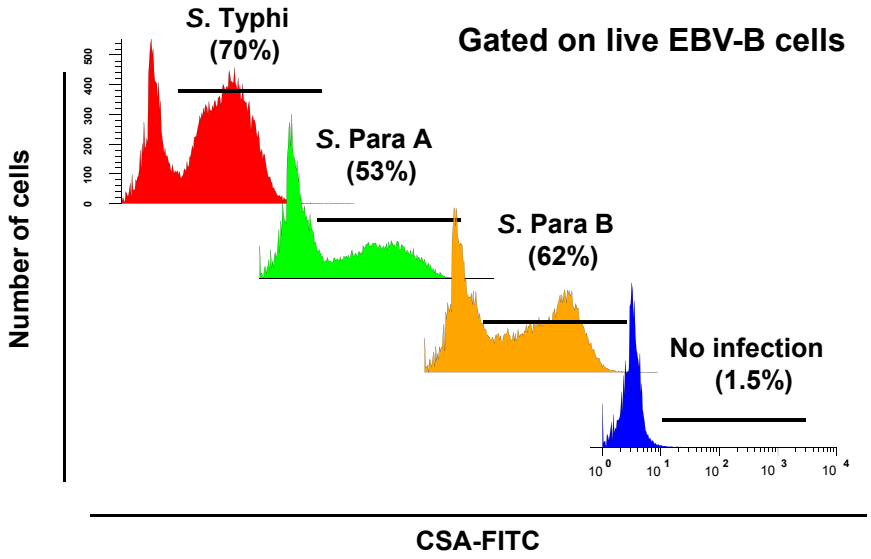

Figure S2. Preparation of EBV-infected targets. EBV transformed B Cell lines were infected with *S*. Typhi, *S.* Paratyphi A and *S.* Paratyphi A (MOI: 10:1, Bacteria:Cell) and stained 18 hours post-infection. Percentages of *Salmonella* antigen expressing cells (CSA-1^{+}: solid bar) are shown in parenthesis.

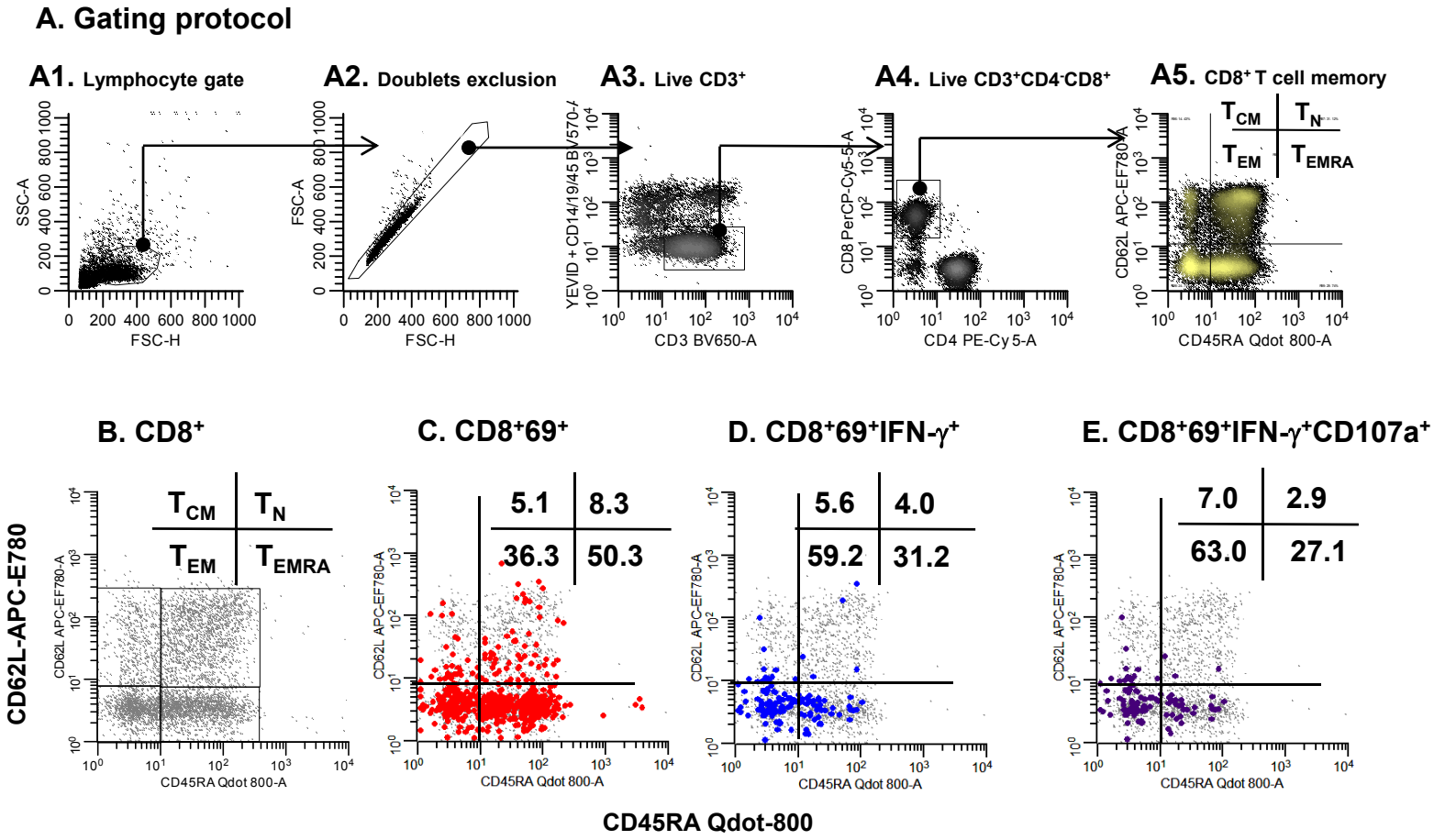

Figure S3. Gating protocol and characteristics of Ty21a-induced effector CD8⁺ T cells. A sequential gating protocol (**Panels A1-A5**) was used to define memory subsets of CD8+ T cells, i.e., T central memory (T_{CM}; CD62L+CD45RA−), T naive (T_N; CD62L+CD45RA+), T effector memory (T_{EM}; CD62L− CD45RA−), and CD45RA positive T effector (T_{EMRA}; CD62L−CD45RA+). PBMC collected from a representative volunteer 42 days following immunization with Ty21a was stimulated with *S.* Typhi-infected targets. Representative histograms show memory T subpopulations (gated on live CD14-CD3+CD4-CD8- depicted in gray dots; **panel B**) on which back-gated cells were superimposed as follows: **panel C** (CD69+ activated cells in red), **panel D** (CD69+ IFN-γ+ cells in blue) and **panel E** (CD69+IFN-γ+CD107a+ cells in purple). The numbers shown in the boxes within histograms (**panels C, D, E**) are the % of the superimposed gated populations into the different T effector and memory subsets (as shown in **panel B**).

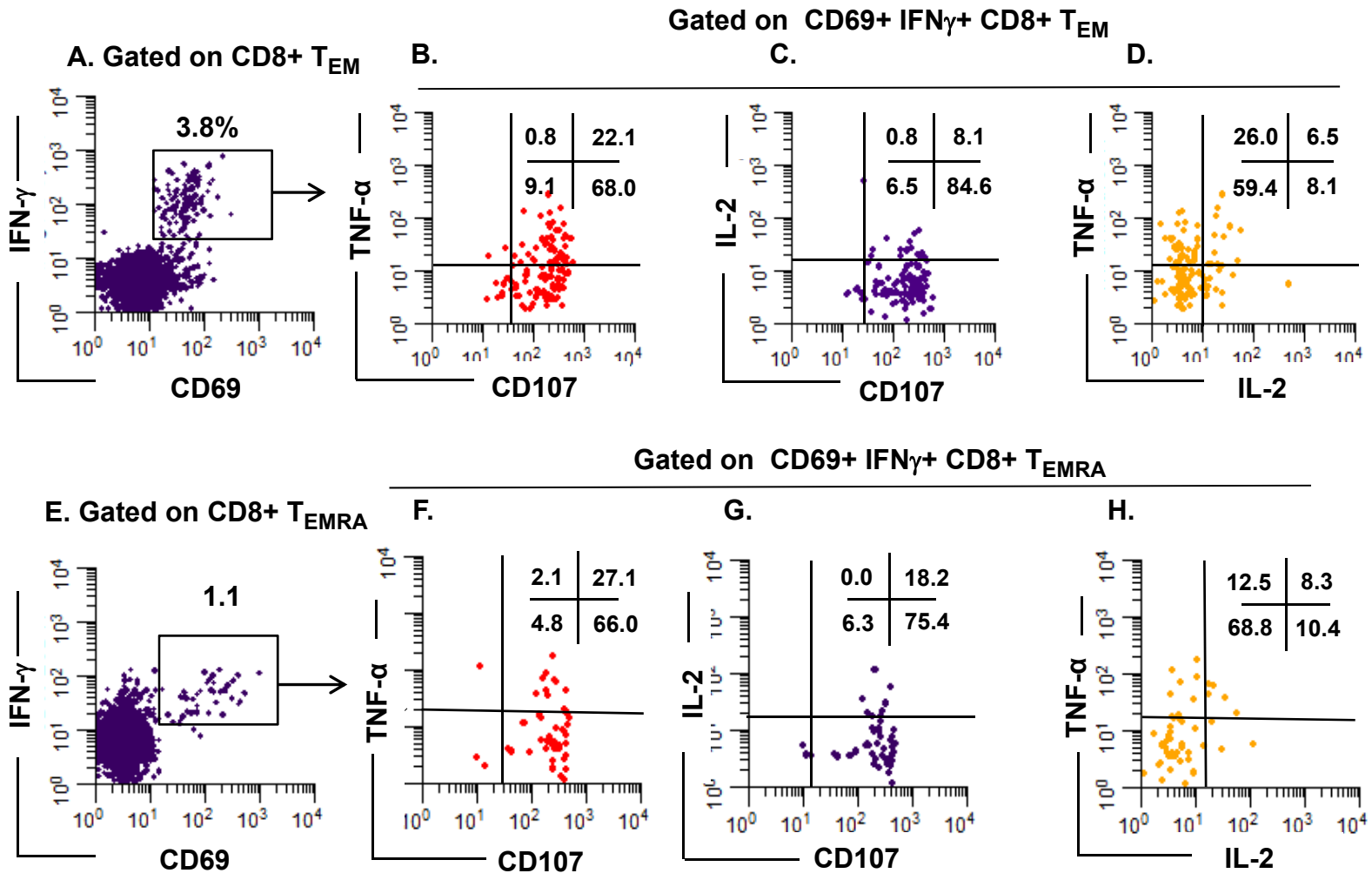

Figure S4. Representative histograms of multifunctional cells. PBMC collected from a representative volunteer (#92) 42 days following immunization with Ty21a were stimulated with *S.* Typhi-infected targets. Shown are two parameter histograms of activated (CD69+) IFN-γ+ cells corresponding to T_{EM} (**panel A**) and T_{EMRA} (**panel E**) subsets of CD8+ cells (gating protocol is shown in **Fig. S3**). CD8+CD69+IFNγ+ cells in T_{EM} (**panels B,C,D**) and T_{EMRA} subsets (**panels F,G,H**) were further analyzed regarding their co-production of TNF-α, CD107, or IL-2.
The numbers shown within histograms represent the % of gated subsets in the corresponding quadrant.

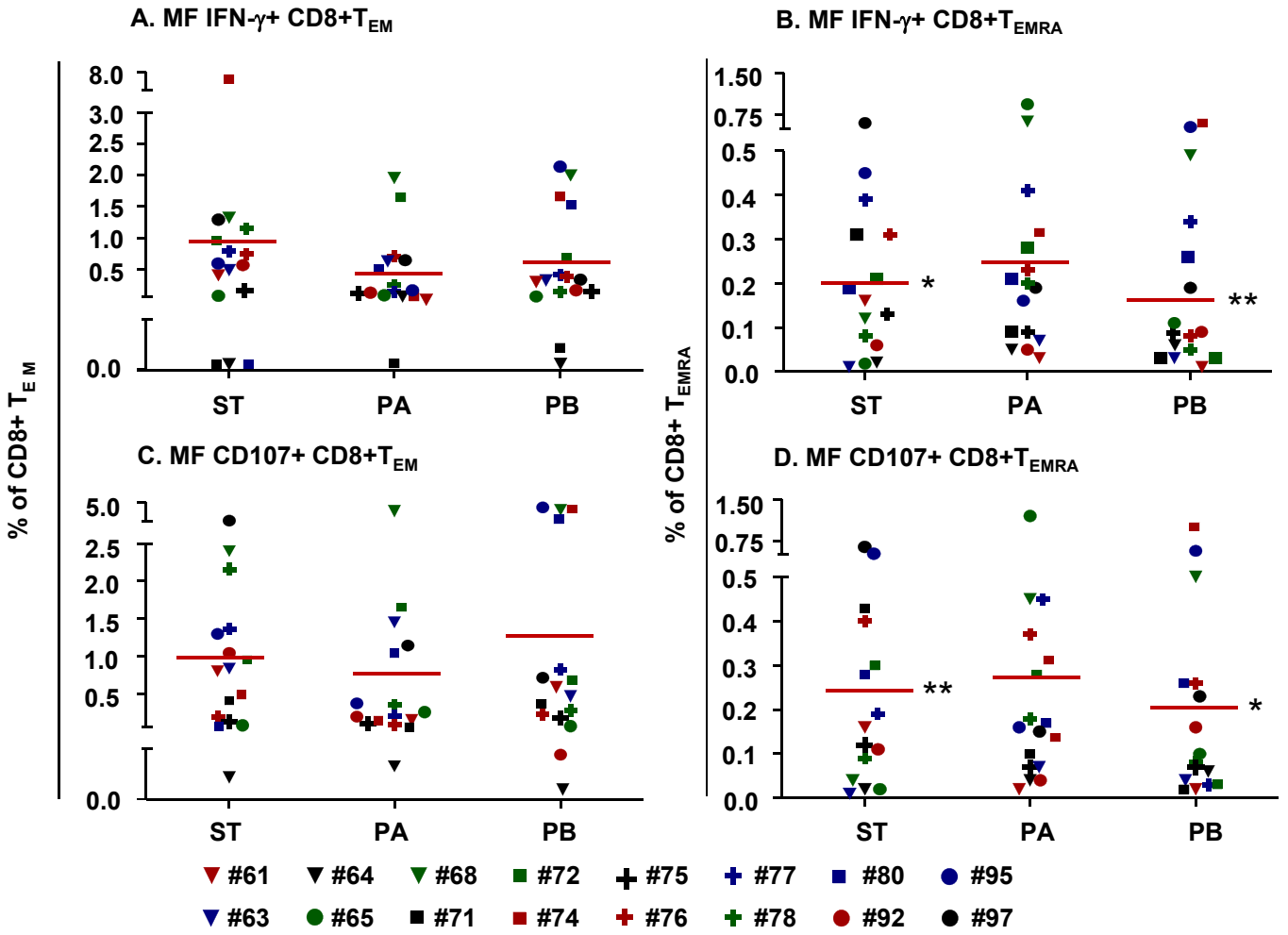

Figure S5. Post-vaccination peak increases in *Salmonella*-specific cross-reactive multifunctional cells in individual Ty21a vaccinees: Shown are the peak post-vaccination increases of *S*. Typhi (ST)-, *S*. Paratyphi A (PA)- or *S*. Paratyphi B (PB)-specific IFN-γ+ (**Panels A,B**) and CD107+ (**Panels C,D**) of total multifunctional (MF, the sum of all multifunctional subsets) cells in CD8+T_{EM} (n=16, **panels A, C**) and CD8+T_{EMRA}(n=15, **panels B,D**) subsets.
Post-vaccination peaks: Peak responses at days 42 or 84 minus pre-vaccination [day 0] levels
Horizontal bars represent Mean **p<0.01. *p<0.05 compared to the corresponding T_{EM} (in **panels A and C**) by Wilcoxon signed rank test, 2-tail.
ST specific increases in CD8+ T_{EMRA} subsets in volunteer #74 were outliers (above mean+3SD) and thus were excluded for this comparative analysis

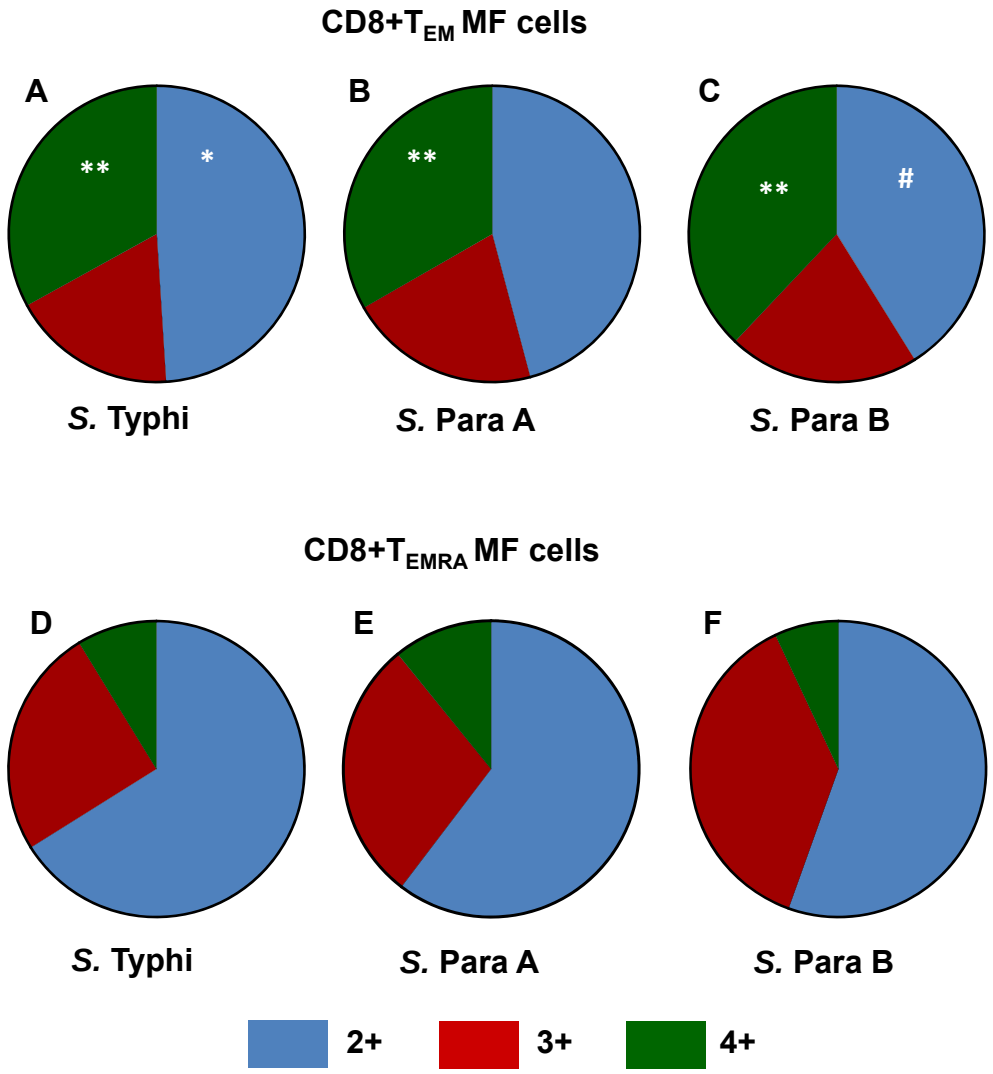

Figure S6. Characterization of post-vaccination increases in multifunctional responses by CD8+ T_{EM} and T_{EMRA} subsets. Post-vaccination peak increases (peak level at days 42 or 84 post-vaccination minus the corresponding pre-vaccination levels) in IFN-γ, TNF-α and IL-2 producing and/or CD107a expressing MF subpopulations were determined by FCOM analysis in 16 subjects. CD8+ T_{EM} (**panels A,B,C**) and CD8+T_{EMRA} (**panels D,E,F**) cells concomitantly producing double (2+), triple (3+) or all (quadruple, 4+) cytokines or expressing CD107 cells are shown as percentages of the corresponding total *Salmonella*-specific MF cells. Data were analyzed by Mann-Whitney tests. Comparisons represent those between CD8+ T_{EM} and T_{EMRA} subsets. *p<0.05 **p<0.01. #p=0.06

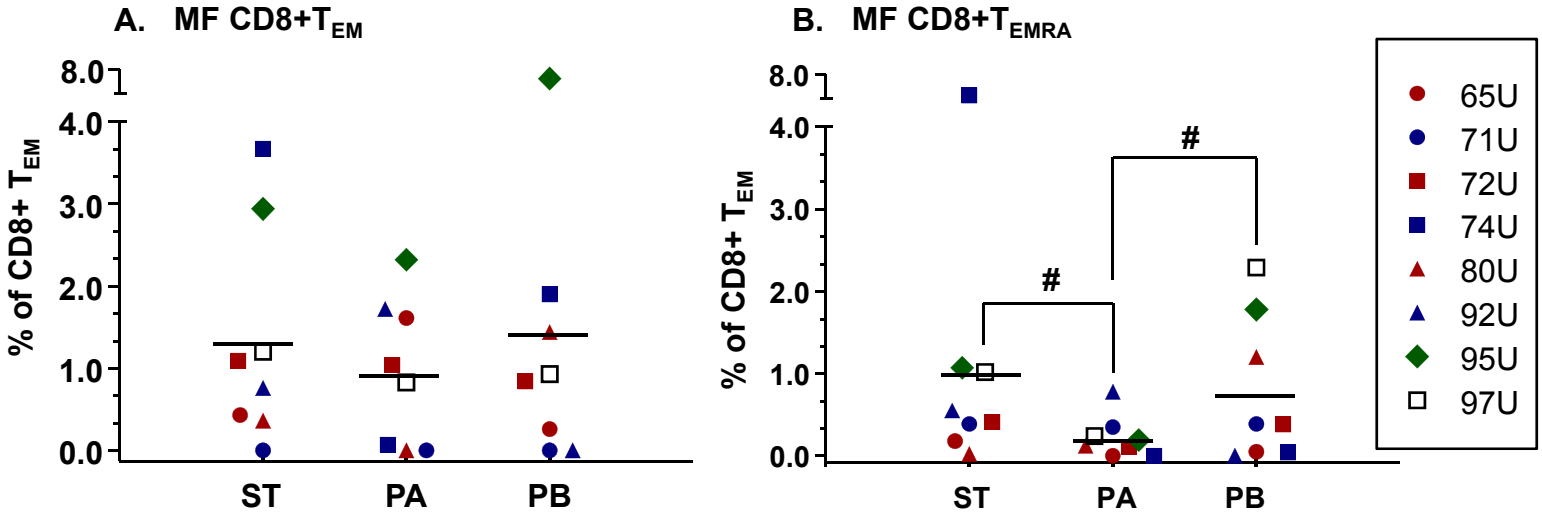

Figure S7. Post-vaccination increases in MIP-1β producing CD8⁺ cells in individual Ty21a vaccinees. Shown are the post-vaccination peak increases in total multifunctional (MF) MIP-1β+ cells in CD8+T_{EM} (**panel A**) and CD8+T_{EMRA} (**panel B**) subsets following stimulation with *S.* Typhi (ST)-, *S.* Paratyphi A (PA)- and *S.* Paratyphi B (PB)-infected targets. Horizontal Bars indicate the means of each group (n=8).
#p=0.12 by Wilcoxon signed rank test, 2-tail.