Primary immune responses are impacted by persistent herpesvirus infections in older people: results from an observational study on healthy subjects and a vaccination trial on subjects aged more than 70 years old

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Supplemental figures

Figure S1

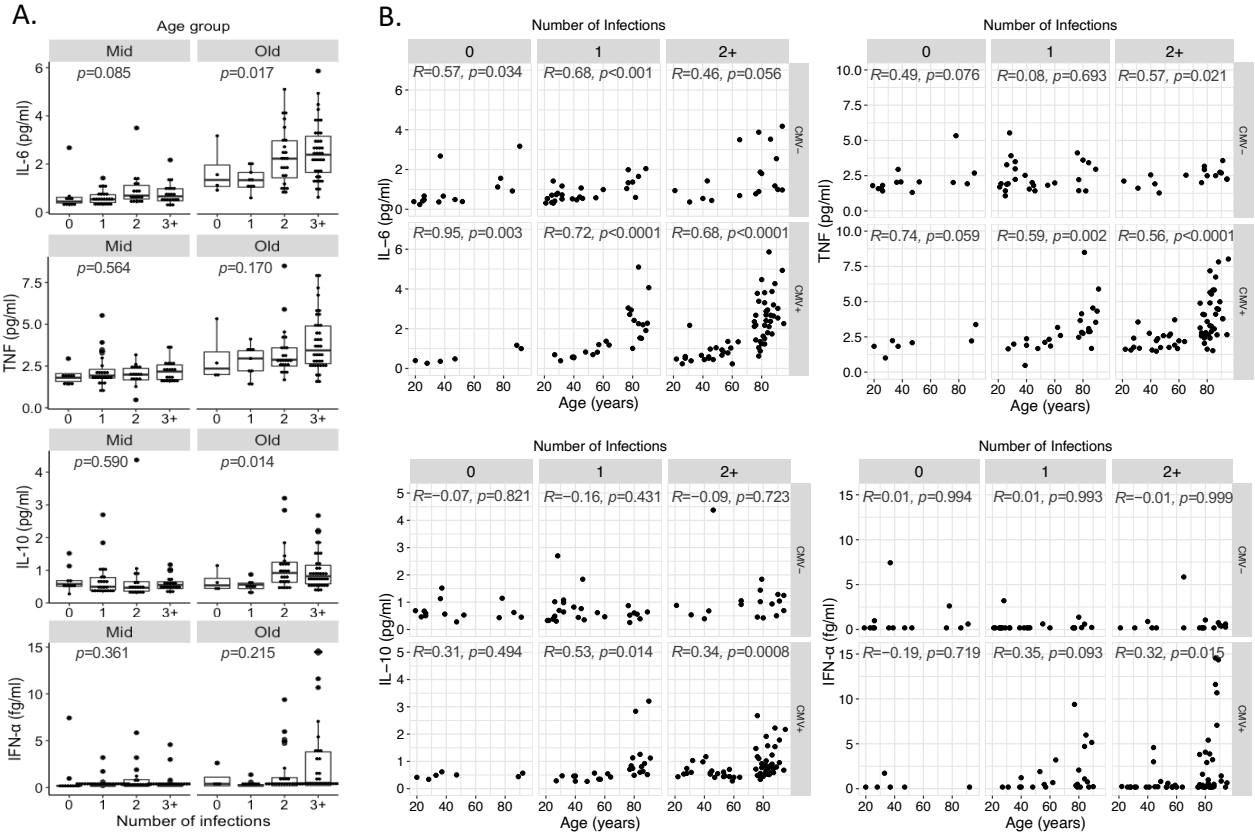

Figure S1. Impact of age and herpesvirus infections on inflammation levels

(A) Inflammatory cytokine levels according to the number of infections with herpesviruses in subjects grouped according to age and shown as box and whiskers plot (n=73 for Mid, n=76 for Old). **(B)** Correlation between inflammatory cytokine levels and age according to number of infections with herpesviruses (excluding CMV) and CMV seropositivity (n= 149). Spearman's R and p values are shown for each panel. Statistical significance was determined by Kruskal-Wallis test (A) or Spearman's rank correlation (B).

Figure S2

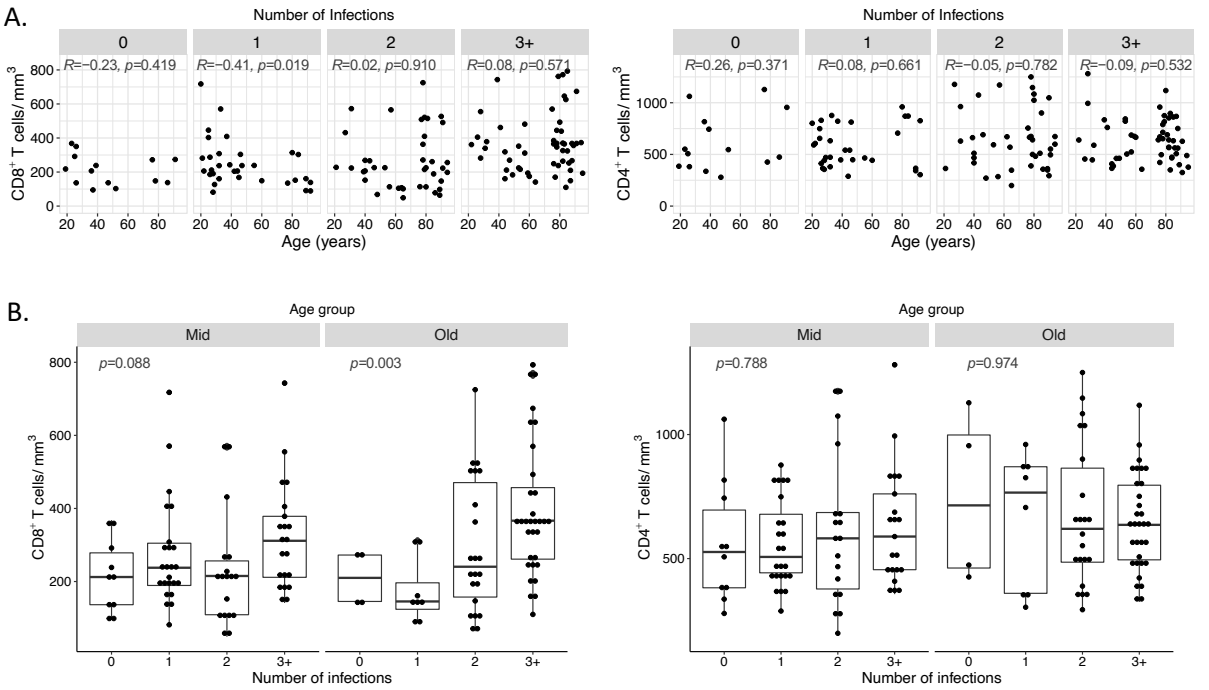

Figure S2. Impact of age and herpesvirus infections on total CD8$^+$ and CD4$^+$ T cell counts

(A) Correlation between total CD8$^+$ and CD4$^+$ T cell absolute counts and age according to the number of infections with herpesviruses (n=13, 32, 39 and 49 for 0, 1, 2, and 3+ infections). Statistical significance was determined by Spearman's rank correlation. Spearman's R and p values are shown for each panel. **(B)** Absolute counts of total CD8$^+$ and CD4$^+$ T cells according to the number of infections with herpesviruses in subjects grouped according to age and shown as box and whiskers plot (n=73 for Mid, n=60 for Old). Statistical significance was determined by Kruskal-Wallis test.

Figure S3

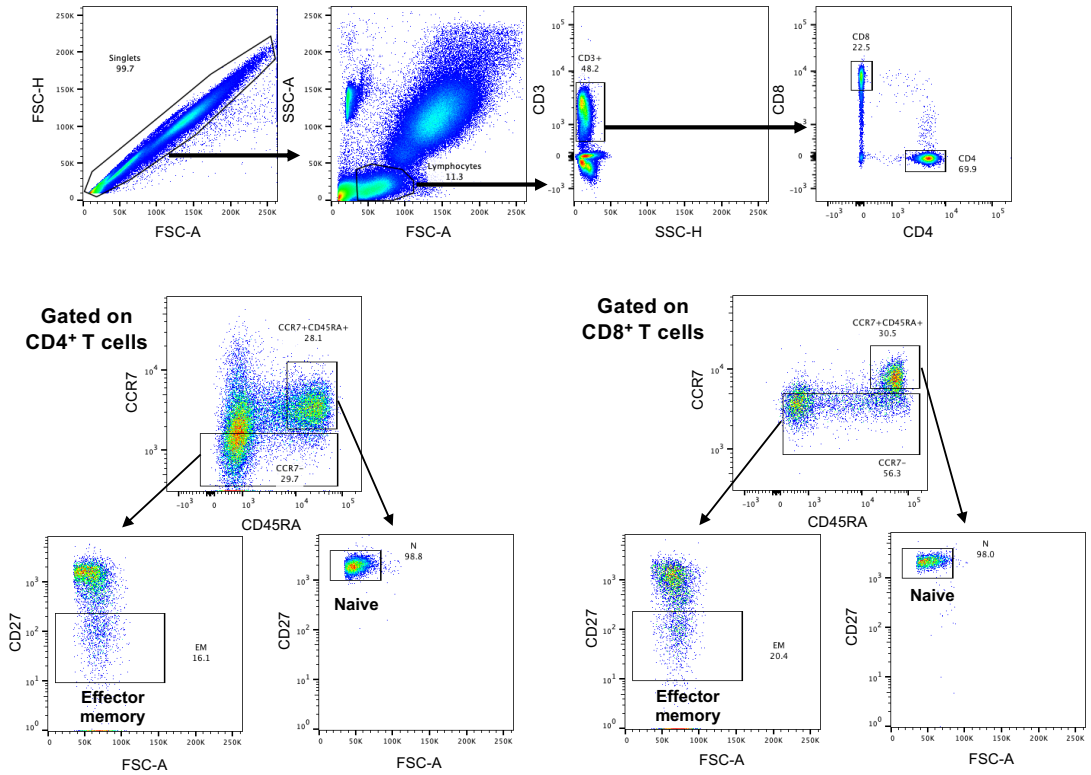

Figure S3. Gating strategy for the immunophenotyping for flow cytometry

Figure S4

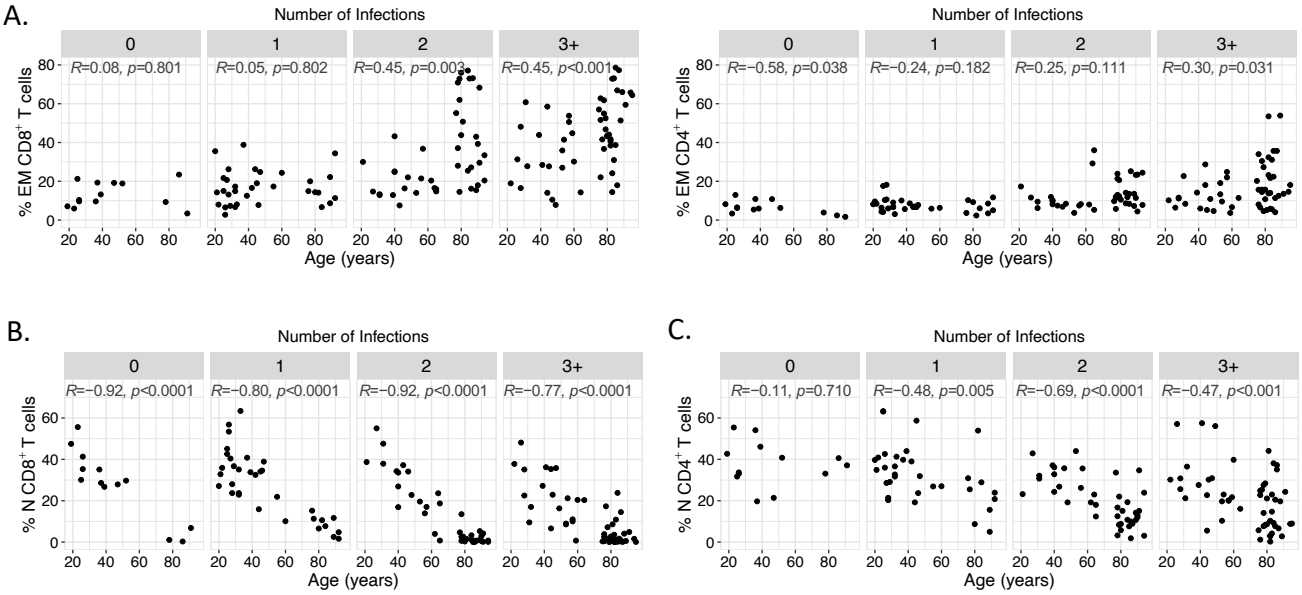

Figure S4. Impact of age and herpesvirus infections on effector memory and naive T-cell percentages

Correlation between effector memory $CD8^+$ and $CD4^+$ **(A)**, and naive $CD8^+$ **(B)** and $CD4^+$ T-cell **(C)** percentages and age according to the number of infections with herpesviruses (n=13, 32, 39 and 49 for 0, 1, 2, and 3+ infections). Statistical significance was determined by Spearman's rank correlation. Spearman's R and p values are shown for each panel.

Figure S5

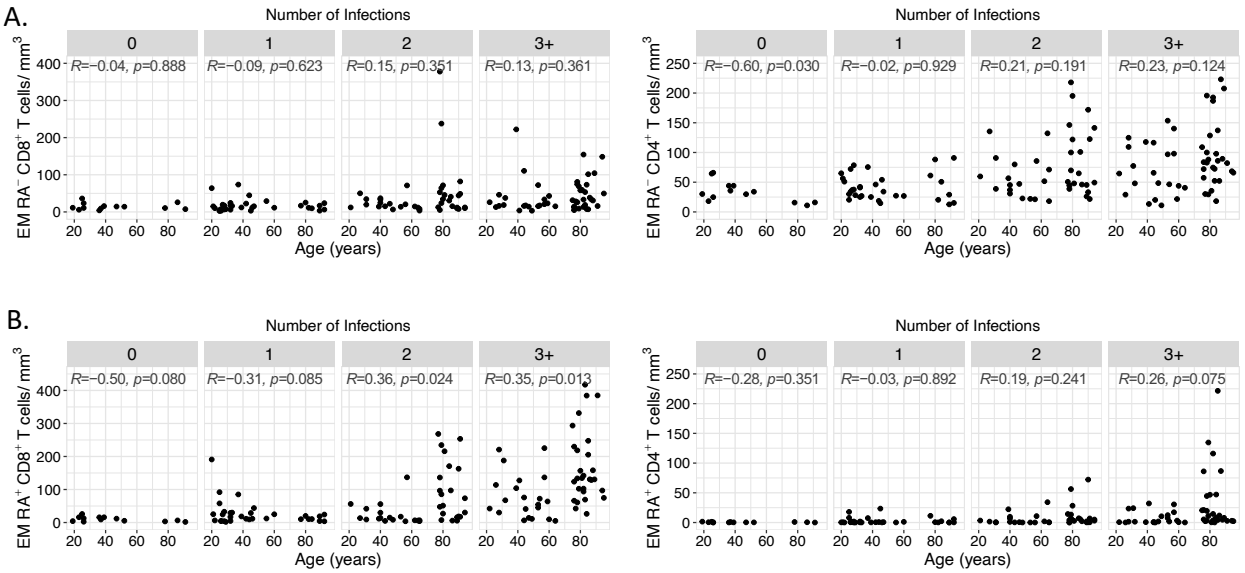

Figure S5. Impact of age and herpesvirus infections on effector memory T-cell subsets

Correlation between effector memory CD45RA$^-$ CD8$^+$ and CD4$^+$ **(A)**, and effector memory CD45RA$^+$ CD8$^+$ and CD4$^+$ T-cell **(B)** absolute counts and age according to the number of infections with herpesviruses (n=13, 32, 39 and 49 for 0, 1, 2, and 3+ infections). Statistical significance was determined by Spearman's rank correlation. Spearman's R and p values are shown for each panel.

Figure S6

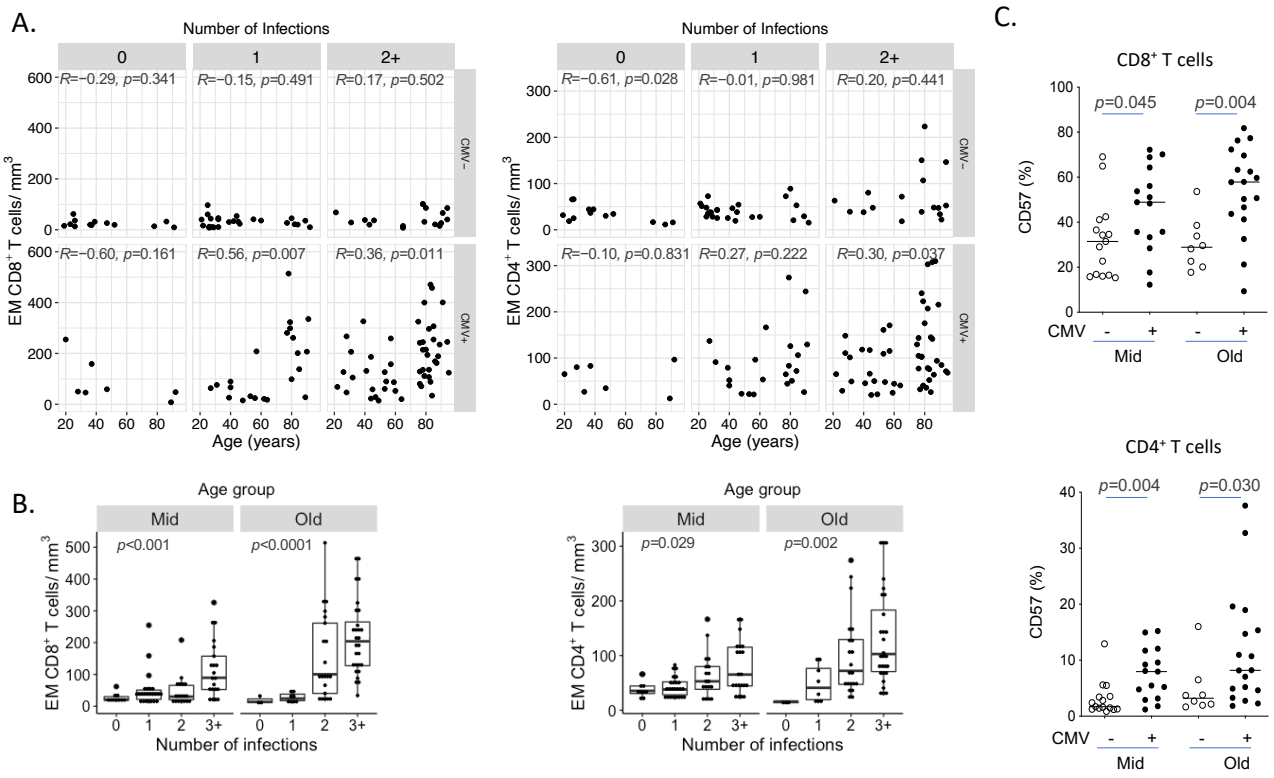

Figure S6. Impact of age and herpesvirus infections on effector memory T-cell levels

(A) Correlation between absolute counts of effector memory $CD8^+$ and $CD4^+$ T cells and age according to number of infections with herpesviruses (excluding CMV) and CMV seropositivity (n=133). **(B)** Absolute counts of effector memory $CD8^+$ and $CD4^+$ T cells according to the number of infections with herpesviruses in subjects grouped according to age and shown as box and whiskers plot (n=73 for Mid, n=60 for Old). **(C)** Percentages of CD57+ memory $CD8^+$ and $CD4^+$ T lymphocytes in different age groups (n=31 for Mid, n=26 for Old) according CMV serostatus. Statistical significance was determined by Spearman's rank correlation (A), Kruskal-Wallis test (B) or Mann-Whitney test (C).

Figure S7

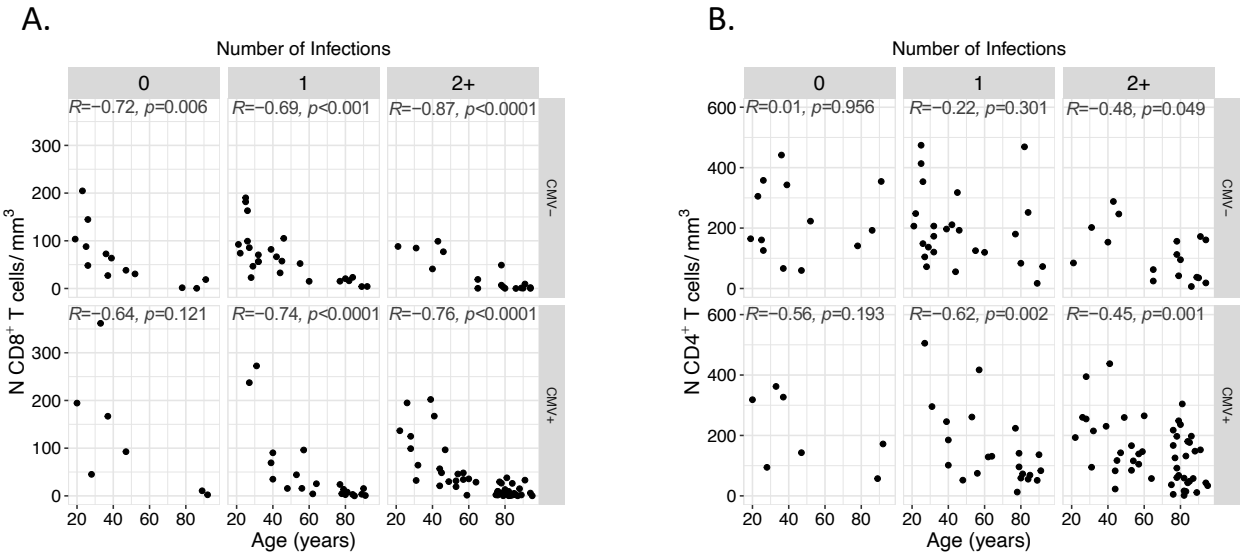

Figure S7. Impact of age and herpesvirus infections on $CD8^+$ and $CD4^+$ naive T-cell levels
Correlation between absolute counts of naive $CD8^+$ **(A)** and $CD4^+$ **(B)** T cells and age according to number of infections with herpesviruses (excluding CMV) and CMV seropositivity (n=133). Statistical significance was determined by Spearman's rank correlation, Spearman's R and p values are shown for each panel.

Figure S8

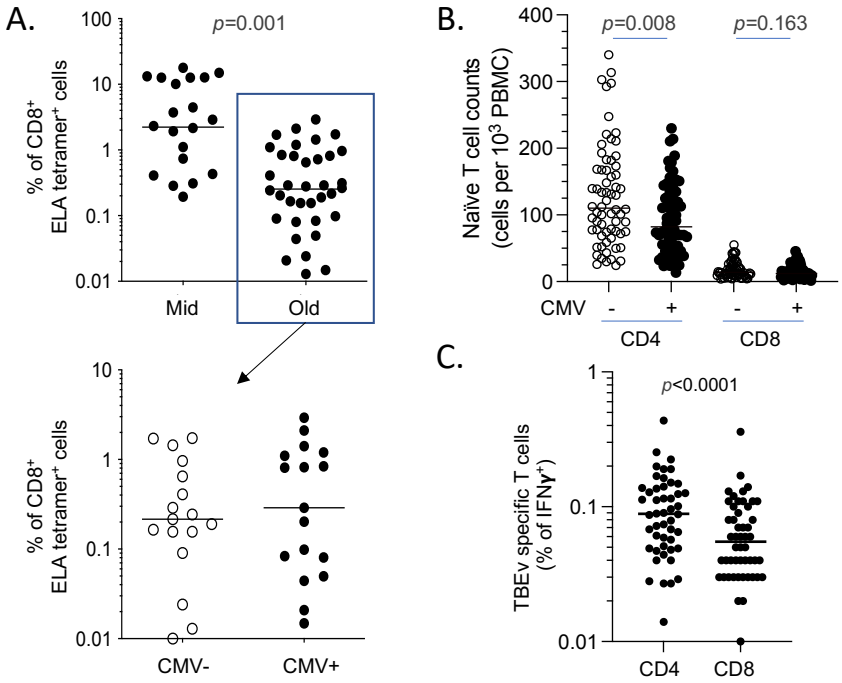

Figure S8. Impact of CMV on CD8$^+$ T-cell priming efficacy in older subjects

(A) Efficacy of antigen specific naive CD8$^+$ T-cell priming determined *in vitro* as frequency of tetramer$^+$CD8$^+$ T cells at day 10 after stimulation with the ELA peptide in middle aged or older subjects (top panel) or older subjects grouped according to CMV seropositivity (bottom panel) (n=20 for Mid, n=34 for Old)**. (B)** Absolute counts of naive CD4$^+$ or CD8$^+$ T cells according to the CMV serostatus of subjects (n=66 for CMV- and n=66 CMV+)**. (C)** Frequency of TBEv-specific CD4$^+$ or CD8$^+$ T cells determined by intracellular IFNγ staining upon stimulation with TBEv overlapping peptides at week 26 after vaccination in older subjects (>70y) (n=49). Statistical significance was determined by Mann-Whitney test. Each dot represents one donor and line median values.