Exclusion of previous SARS-CoV2-infection and detection and characterization of SARS-CoV-2 spike RBD specific B cells. (A) Detection of viral nucleocapsid-protein specific antibodies by ELISA with the cut-off for positivity at an O.D. ratio of 1.1 (dotted line). (B) Antigen-specific live single CD14-CD56-CD3+ (‘dump’ negative) CD19+ B cells were identified by flow cytometry based on co-staining with recombinant spike RBD-FITC and recombinant full spike-APC. Specific cells were further analyzed for memory differentiation (CD27, IgD) and IgG expression.
FACS-based identification and characterization of SARS-CoV2 vaccine specific CD4⁺ T cells. PBMC were stimulated or not for 16 h with SARS-CoV2 spike protein peptide mix. Antigen-specific live single CD14⁻CD19⁻CD3⁺ ("dump" negative) CD4⁺ T cells were identified by flow cytometry based on co-expression of CD154 and CD137. Specific cells were further analyzed for expression of cytokines (IFNγ, TNFα, IL-2 and IL-4), for the in vivo induced activation related marker PD1, or for their memory differentiation phenotype based on CD45RO/CD62L expression (T_CM: central memory-, T_EM: effector memory-, T_eff: effector T cells). Gates were set according to the respective unstimulated or unstained ("fluorescence minus one" - FMO) controls.
Characterization of CEF-specific CD4⁺ T cells. PBMC were stimulated or not for 16 h with CEF peptide mix. (A) depicts overall responder rates (left) and frequencies after background substraction (right). (B) frequencies of cytokine positive and (C) polyfunctional (IFNγ⁺TNFα⁺IL-2⁺, left) or cytokine negative (right) cells. (D) quantifies memory or effector subsets, while (E) depicts frequencies of PD-1⁺ CEF-specific T cells.