

Clonally expanded, stem cell-like melanoma-antigen specific CD8 memory cells can be detected in healthy humans

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ABSTRACT: We used Four-color ImmunoSpot® assays, in conjunction with peptide pools that cover the sequence of Tyrosinase (Tyr), MAGE-3, Melan/MART-1, gp100, and NY-ESO-1 to characterize the melanoma antigen (MA)-specific CD8 cell repertoire in PBMC of 40 healthy human donors (HD). Tyr triggered IFN- γ -secreting CD8 cells in 25% HD within 24 h of antigen stimulation ex vivo. MAGE-3, Melan/MART-1, and gp100 also induced recall responses in 10%, 7.5%, and 2.5% of HD, respectively. At this time point, these CD8 cells did not yet produce GzB. However, they engaged in GzB production 72 h after antigen stimulation. By this 72 h time point ex vivo, 57.5% of the HD responded to at least one, and typically several, of the MA. A closer characterization of the Tyr-specific CD8 cell repertoire showed it to be of low affinity, and to entail primarily the stem cell-like subpopulation.

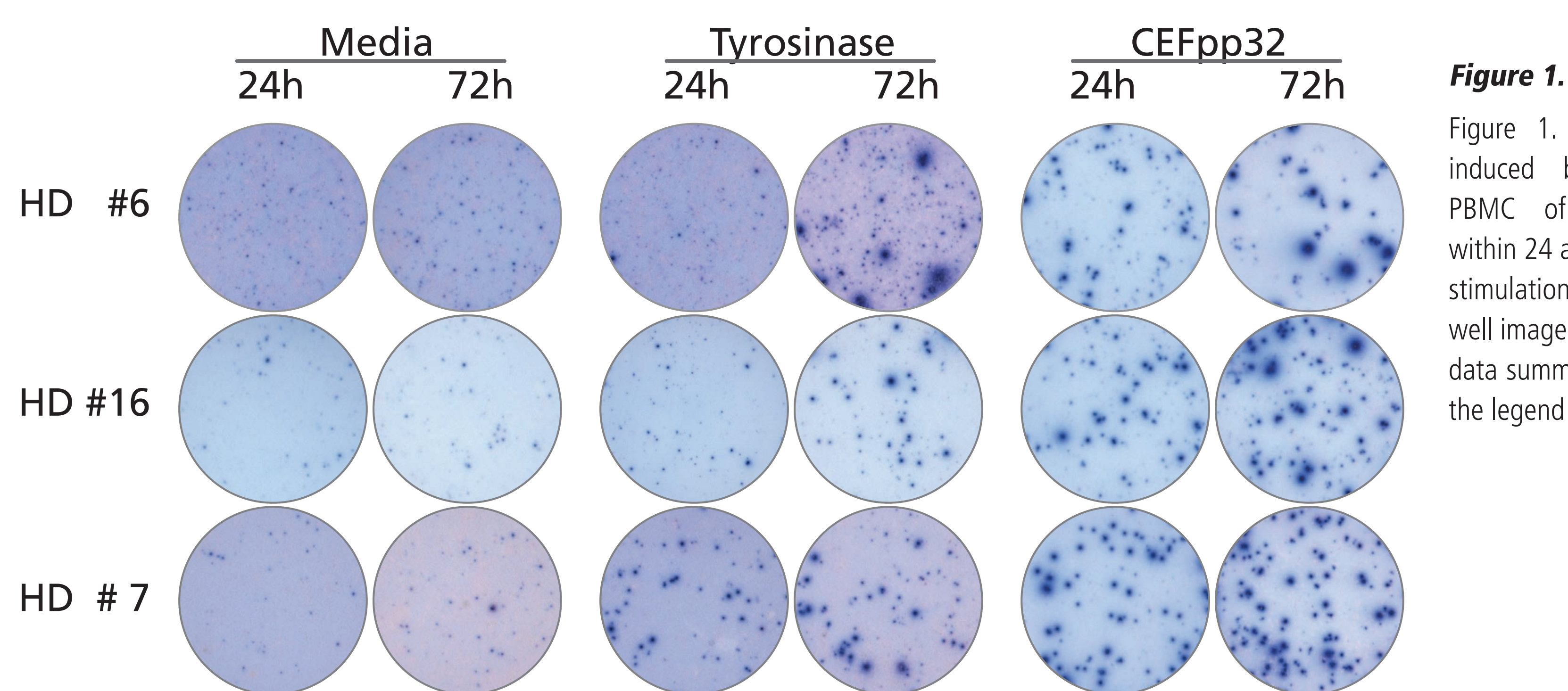


Figure 1.

Figure 1. IFN- γ ELISPOTS induced by Tyrosinase in PBMC of healthy donors within 24 and 72 h of antigen stimulation. Representative well images are shown for the data summarized in Table 1 – the legend to Table 1 applies.

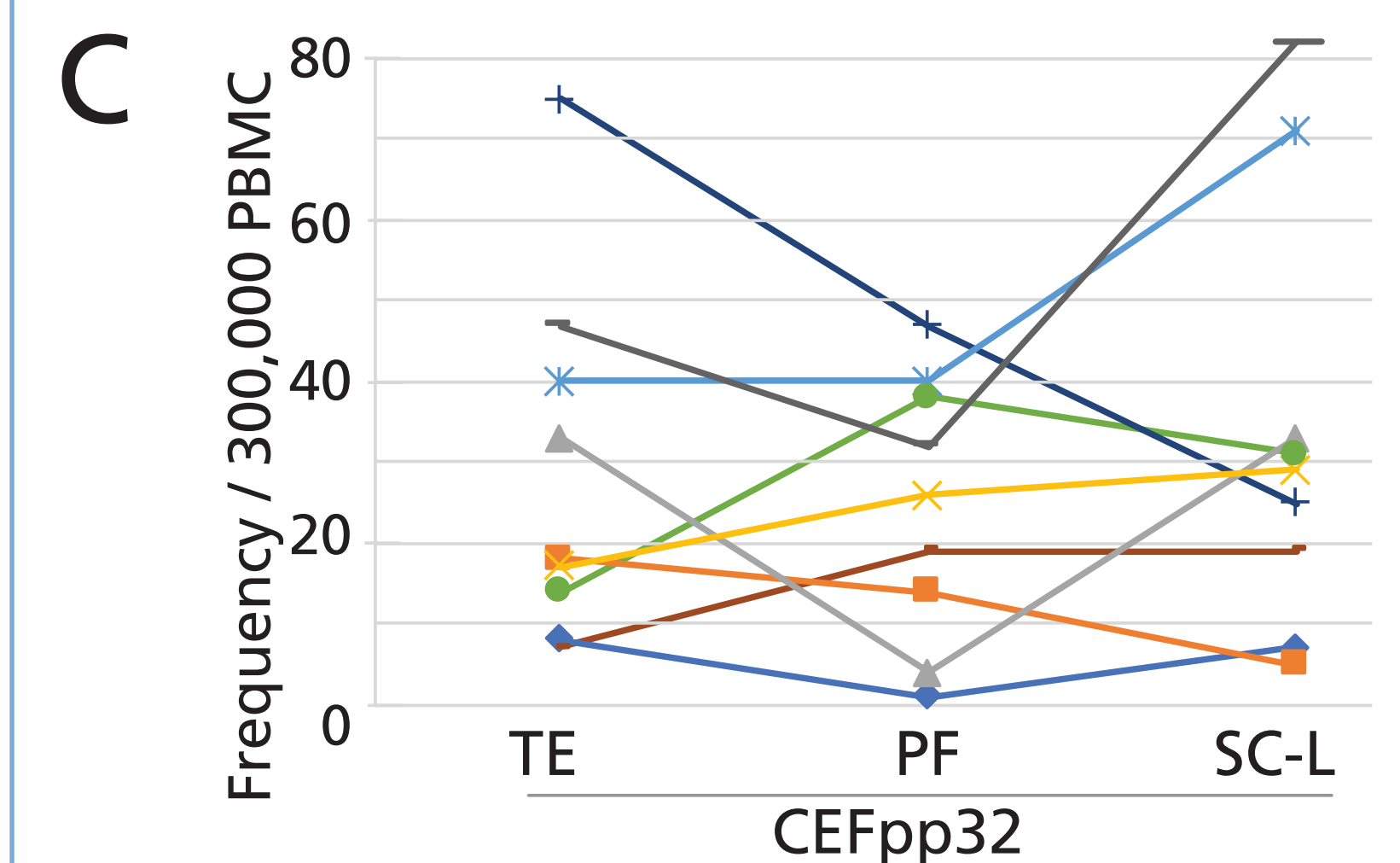
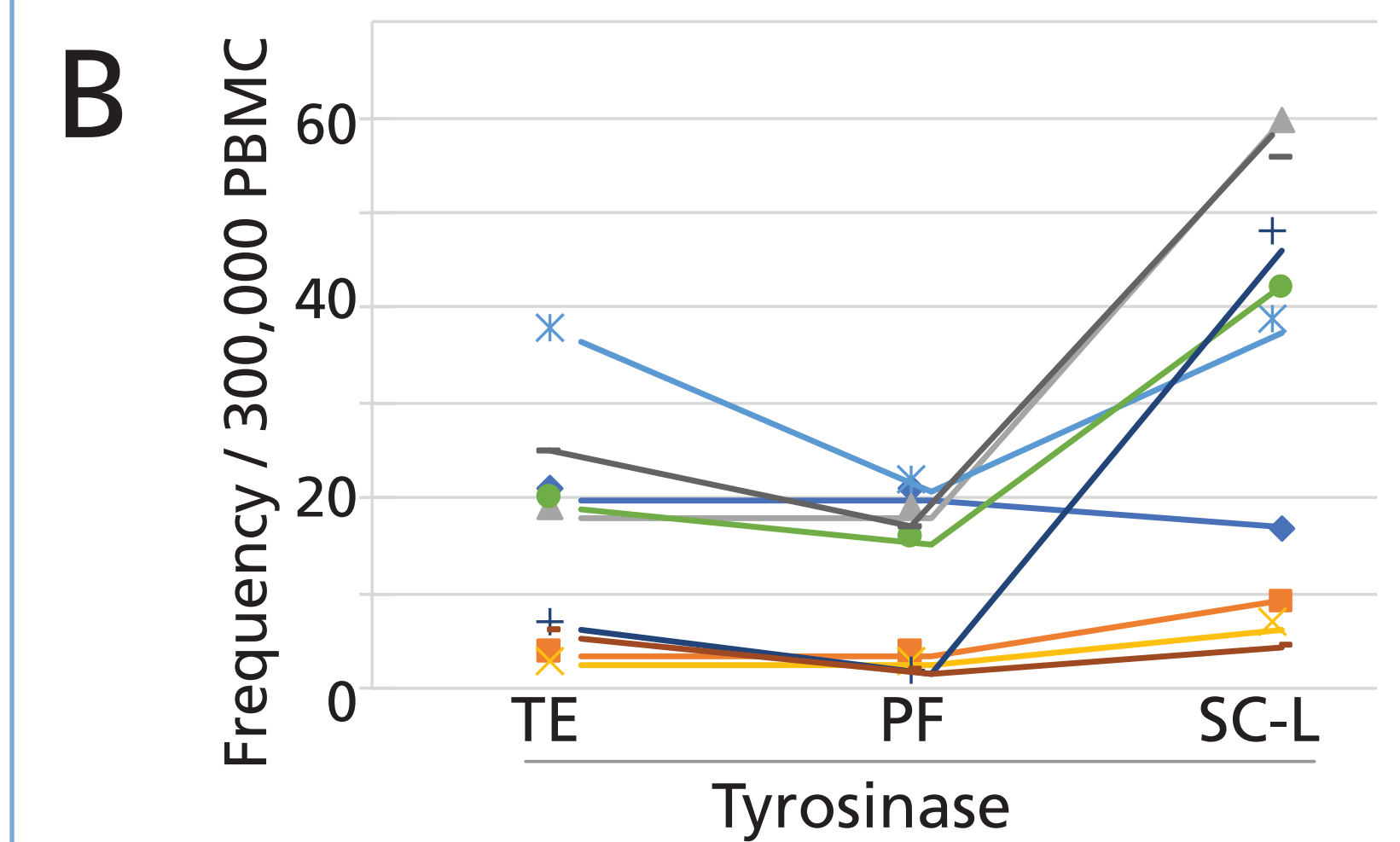
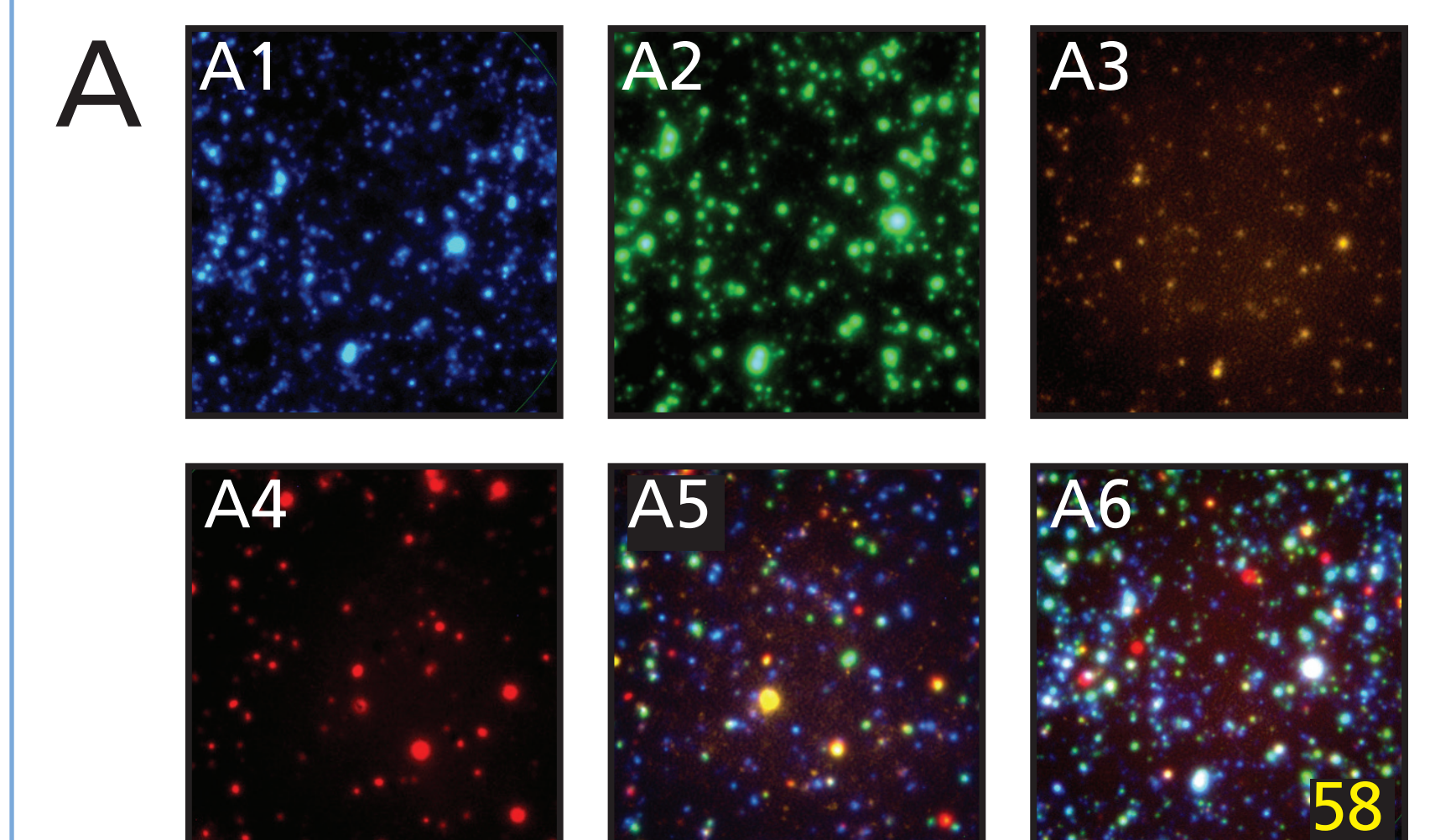


Figure 4. Lineage characterization of Tyrosinase- and CEF peptide-reactive CD8 cells of healthy donors. PBMC of Tyrosinase-reactive HD (Donor # 2, 7, 9, 16, 17, 21, 31 and 36) were cultured with the Tyrosinase (B) or the CEF peptide pool (C) for 72 h, after which they were plated into a Four-Color ImmunoSpot® assay for 24 h, detecting cells secreting IL-2, IFN- γ , TNF- α , and GzB. (A) Each analyte was detected with an excitation/emission filter combination that permits to detect the respective analyte without cross-bleeding of color, and hence allowed for multi-color analysis by the overlay of centers of spot masses without the need for compensation. For a representative well the four color planes are shown in A1-A4, for IL-2, IFN- γ , TNF- α , and GzB; respectively. A5 shows the overlay of all four colors, and in A6 cells are highlighted that qualify as polyfunctional cells being positive for all four analytes. Terminal effector cells (TE) have been defined as IL-2⁺, IFN- γ ⁺, TNF- α ⁺, and GzB⁺; polyfunctional cells (PF) as IL-2⁺, IFN- γ ⁺, TNF- α ⁺, and GzB⁺, and stem cell-like cells (S.C.L.) as IL-2⁺, IFN- γ ⁺, TNF- α ⁺, and GzB⁻.

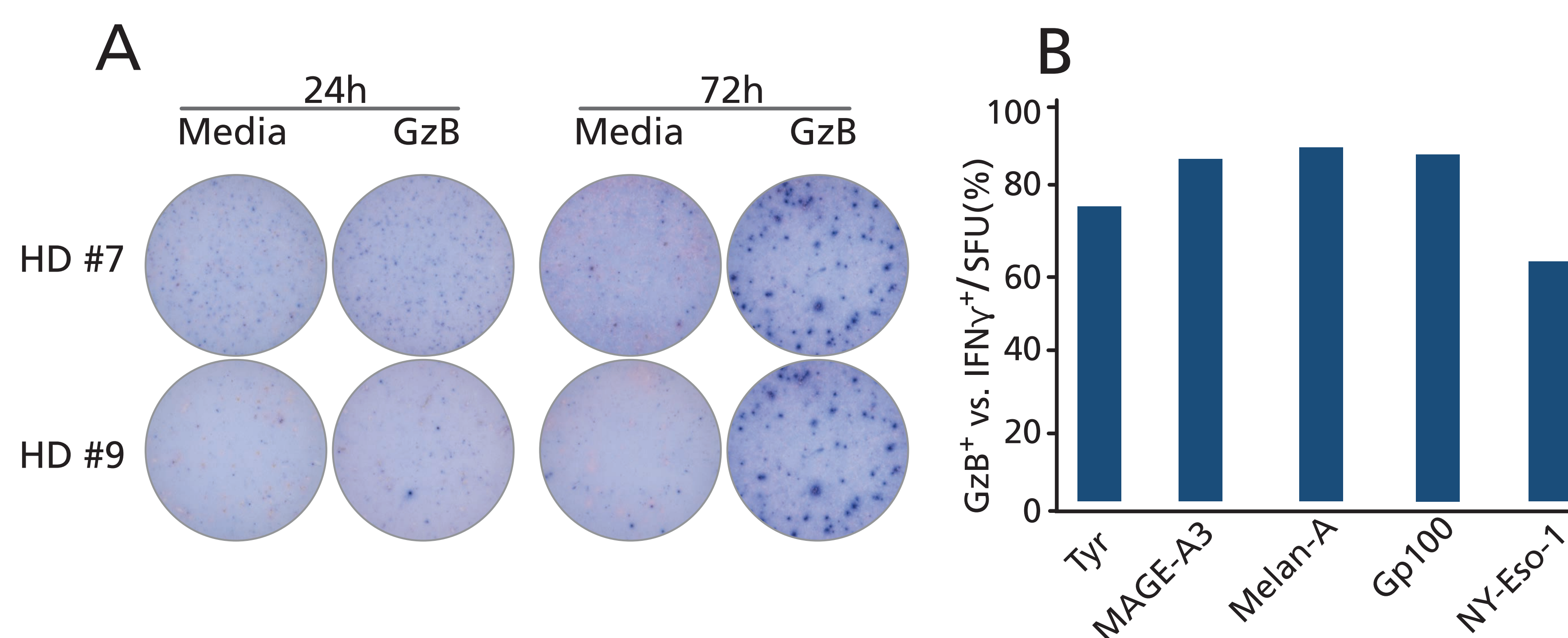


Figure 2. Granzyme B production by PBMC of healthy donors after melanoma antigen exposure. The experiments were performed as detailed in the legend to Table 1 and in the text, except that instead of IFN- γ , GzB-secreting cells were detected. (A) Representative images are shown GzB secretion for two HD who responded with IFN- γ production to Tyrosinase at the 24 h time point. (B) GzB positivity of IFN- γ positive cultures after 72h of MA stimulation: setting IFN- γ -positive MA-responses in each HD as 100% (see the 72 h IFN- γ data in Table 1), the graph shows what percentage of these cultures were also positive for GzB at the 72 h time point.

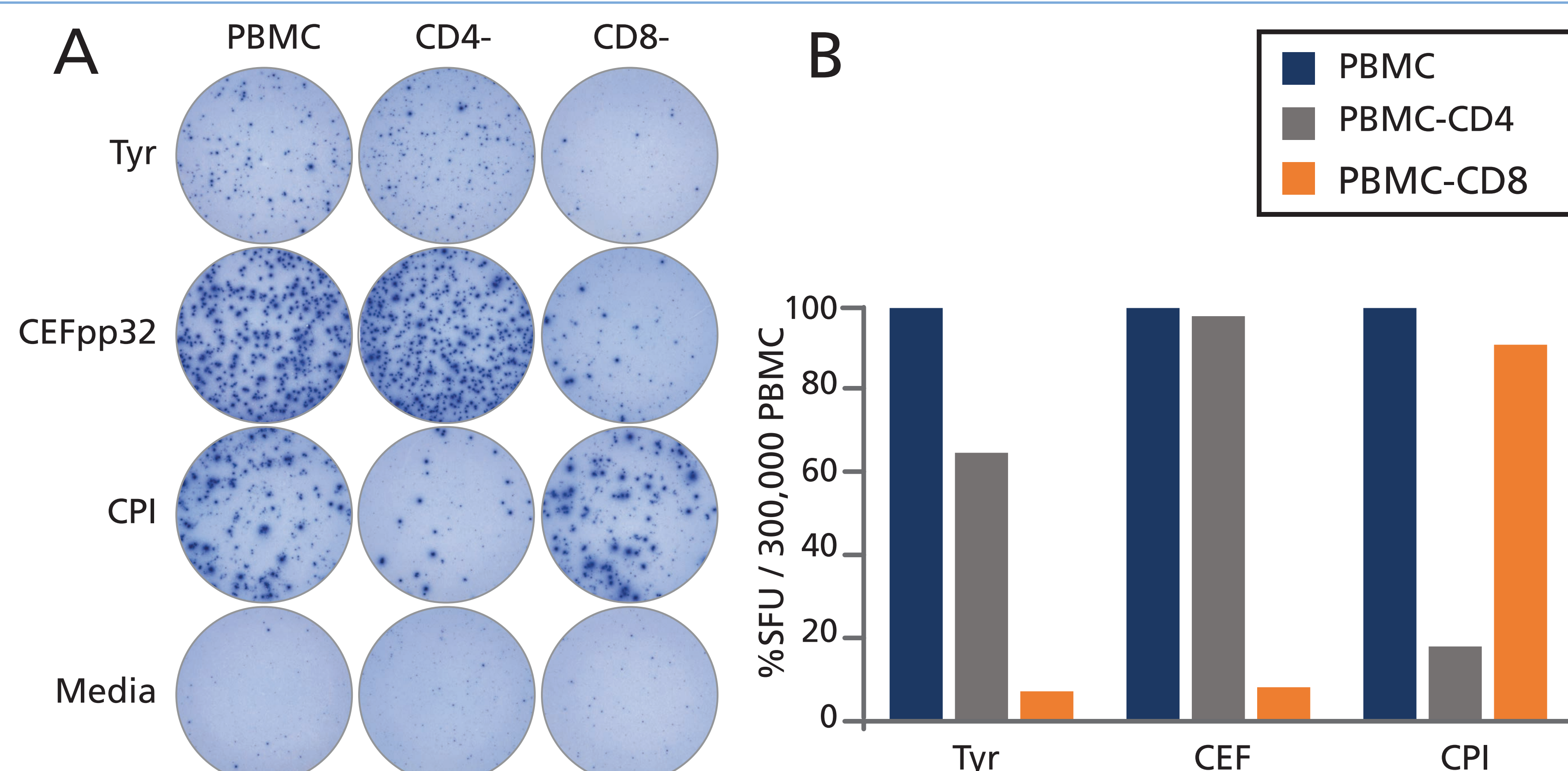


Figure 3. Tyrosinase-triggered IFN- γ spots are produced primarily by CD8 cells. PBMC of a HD#36 were tested “as is” (whole PBMC), after depletion of CD4 cells (CD4-) or CD8 cells (CD8-) via magnetic beads, as specified. These cells were cultured either with Tyrosinase (Tyr), CEF peptides (CEFpp32), CPI, or left unstimulated (Media). IFN- γ was detected after a 24 h culture period. (A) The original wells are shown. (B) The % reduction of SFU counts is shown after CD4 or CD8 cell depletion taking the response by the unseparated PBMC as 100%.

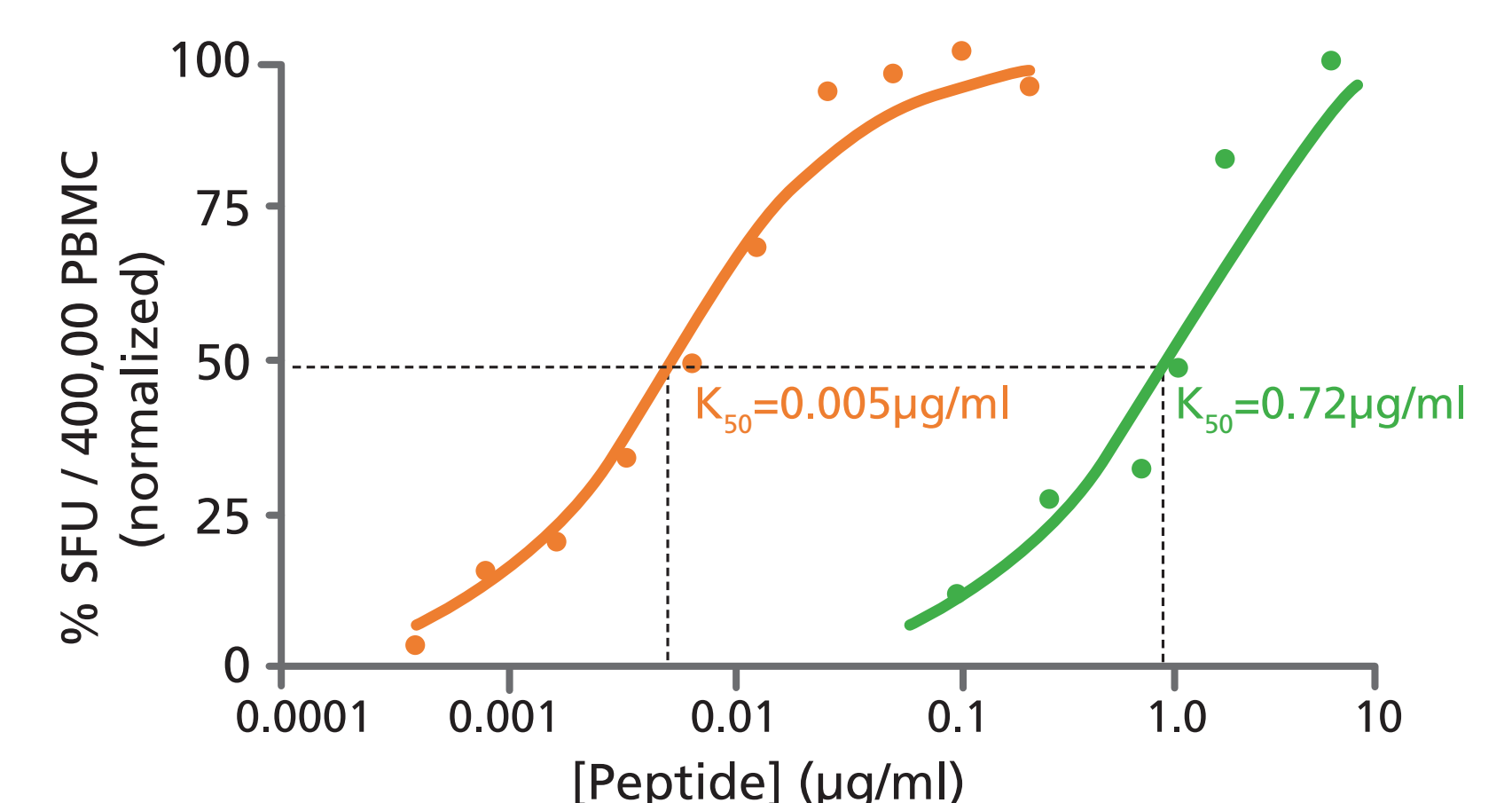


Figure 5. Tyrosinase-specific T cells in healthy donors are of low affinity compared to CEF-specific T cells. Tyrosinase- (green) and CEF (orange) peptides were serially diluted and tested at the specified concentrations stimulating PBMC of a HD#17 who was shown to respond to both (see Table 1). Taking the SFU counts elicited at 10 μ g/ml of peptide as 100%, the % response induced by the specified peptide concentrations is shown - the peptide concentration that elicits 50% maximal stimulation was established and is specified as the K₅₀ value.