

Supplementary Fig. 2 (A) Freshly isolated PBMC derived from 3 healthy blood donors were fixed, permeabilized and stained with fluorophore-conjugated anti-CD8 mab and anti-CD56 mab. 10.000 cells were acquired with an ImageStream Mark II imaging cytometer. Only focused, single, CD8⁺ cells were considered for further analyses. Data are displayed as mean values of experiments with cells derived from three healthy blood donors +/- SEM. Statistical significance between different subpopulations is displayed as * for p < 0.1 (Mann-Whitney U test). (B, C) MACS-purified CD8⁺ T cells were expanded for 14 days and stimulated with anti-CD3 mab OKT3 (1 µg/mL) and cross-linking rabbit antimouse IgG (1.2 µg/mL) for up to 3 h. (B) Cells were stained with a FITC-conjugated anti-CD26/DPP4 mab (clone BA5b) and surface expression was determined by flow cytometry. Geometric mean fluorescence intensities of unstimulated cells were set to 100%. Data are displayed as mean values of 3 single experiments +/- SD. Statistical significance between stimulated and unstimulated cells is displayed as * for p < 0.1 (Mann-Whitney U test). (C) The amount of sCD26/DPP4 in cellular supernatants was determined by ELISA. The experiment is representative of two independent experiments. (D) Zoledronate-expanded yδ T cells were stimulated with phorbol ester (TPA, 20 ng/mL) and calcium ionophore (ionomycin, 500 ng/mL) for up to 2 h in serum-free medium or were left untreated. DPP4 activity in cell-free supernatants as well as medium (Med) and serum from a healthy donor as a controls was assessed employing a commercial DPP4 Activity Assay Kit (Sigma-Aldrich) following manufacturer's instructions. The experiment is representative of two independent experiments