

eFigure 4 Age-dependent changes in the CD4 T cell compartment in the peripheral blood of MS patients and HD. Flow cytometric analysis of frozen PBMC from young ( $\leq 50$  years) and old (> 50 years) patients with multiple sclerosis (MS) (MS: young: n=40, old: n=38; relapsing-remitting MS (RRMS): young: n=20, old: n=18; primary progressive (PPMS): young: n=20, old: n=20) and healthy donors (HD) (young: n=20, old: n=20). Demographic data of study subjects are depicted in eTable1. (A) Frequencies of naïve, memory, effector memory (EM) and central memory (CM) CD4 T cells in HD and MS patients. (B) Correlation analysis of data depicted in A with age. (C) Proportions of naïve, memory, EM and CM CD4 T cells in HD and patients with RRMS and PPMS. (D) Frequencies of CD45RO CD27 CD4 T cells in HD and MS patients (*left*) or in HD and patients with RRMS and PPMS (*right*). Data are displayed as boxplots of the median and the  $25^{th}$  and  $75^{th}$  percentile  $\pm$  IQR. Statistical analysis was conducted by two-tailed Mann-Whitney test. For correlation analysis, the Pearson product-moment correlation coefficients (Pearson's R) were computed. Differences were considered statistically significant with the following *P*-values: \*P < 0.05, \*\*P < 0.01, \*\*\*P < 0.001 and \*\*\*\*P < 0.0001