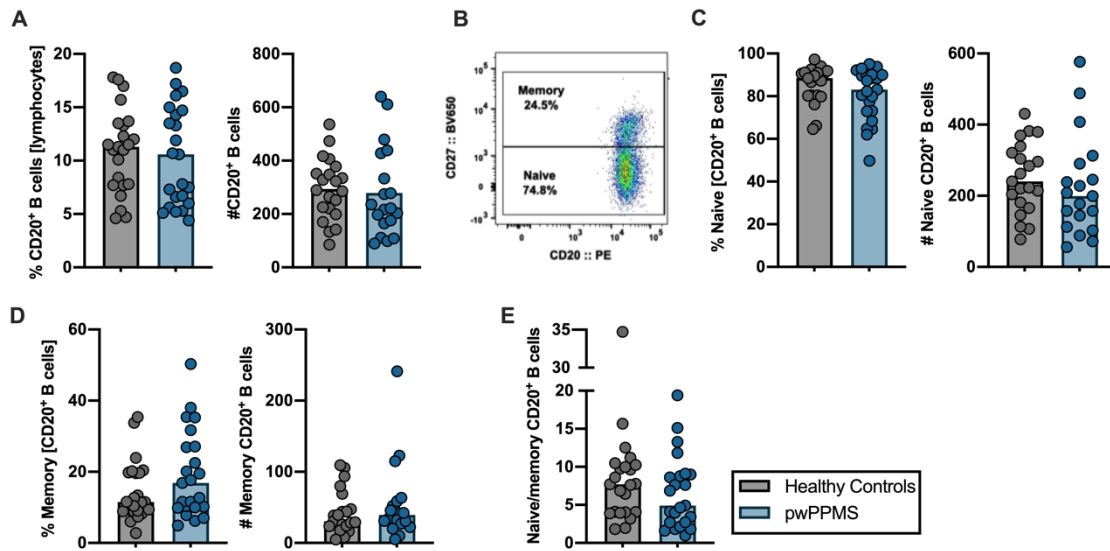
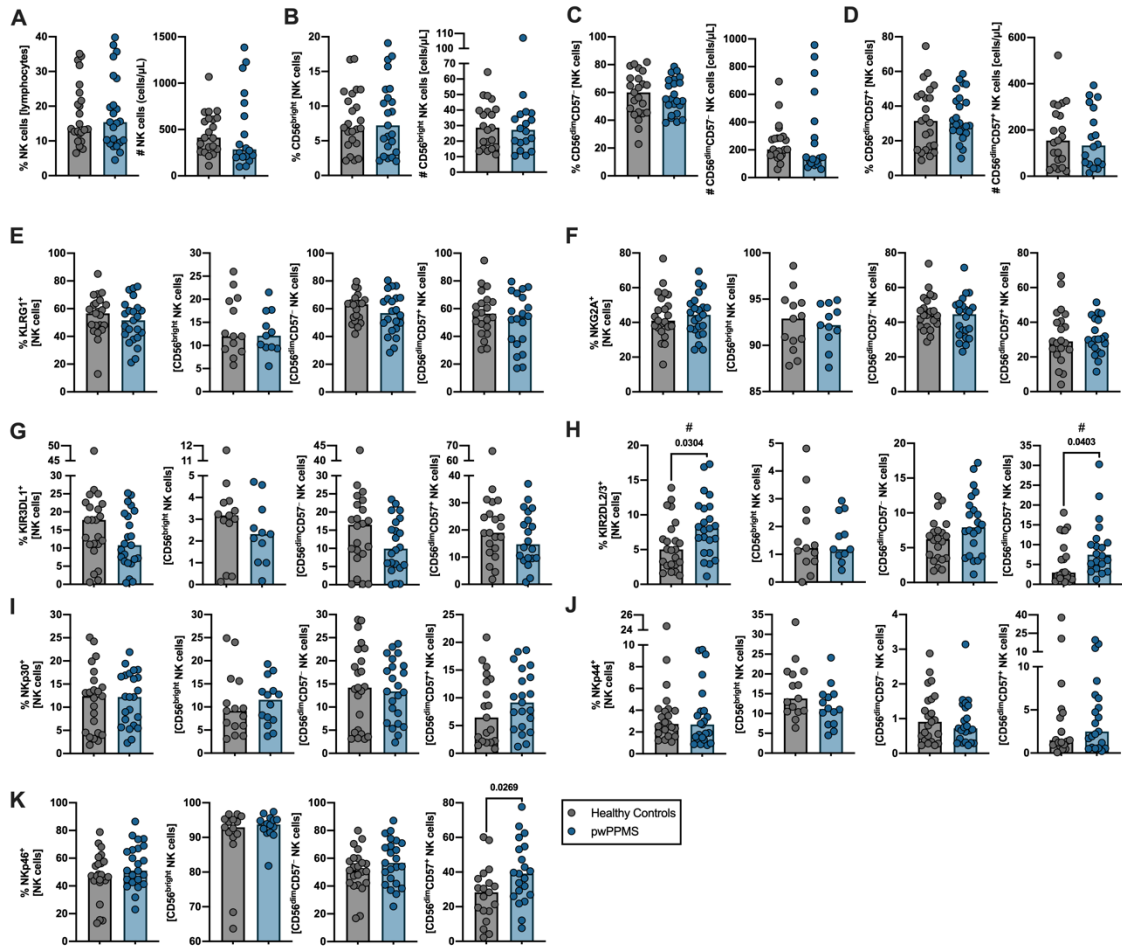


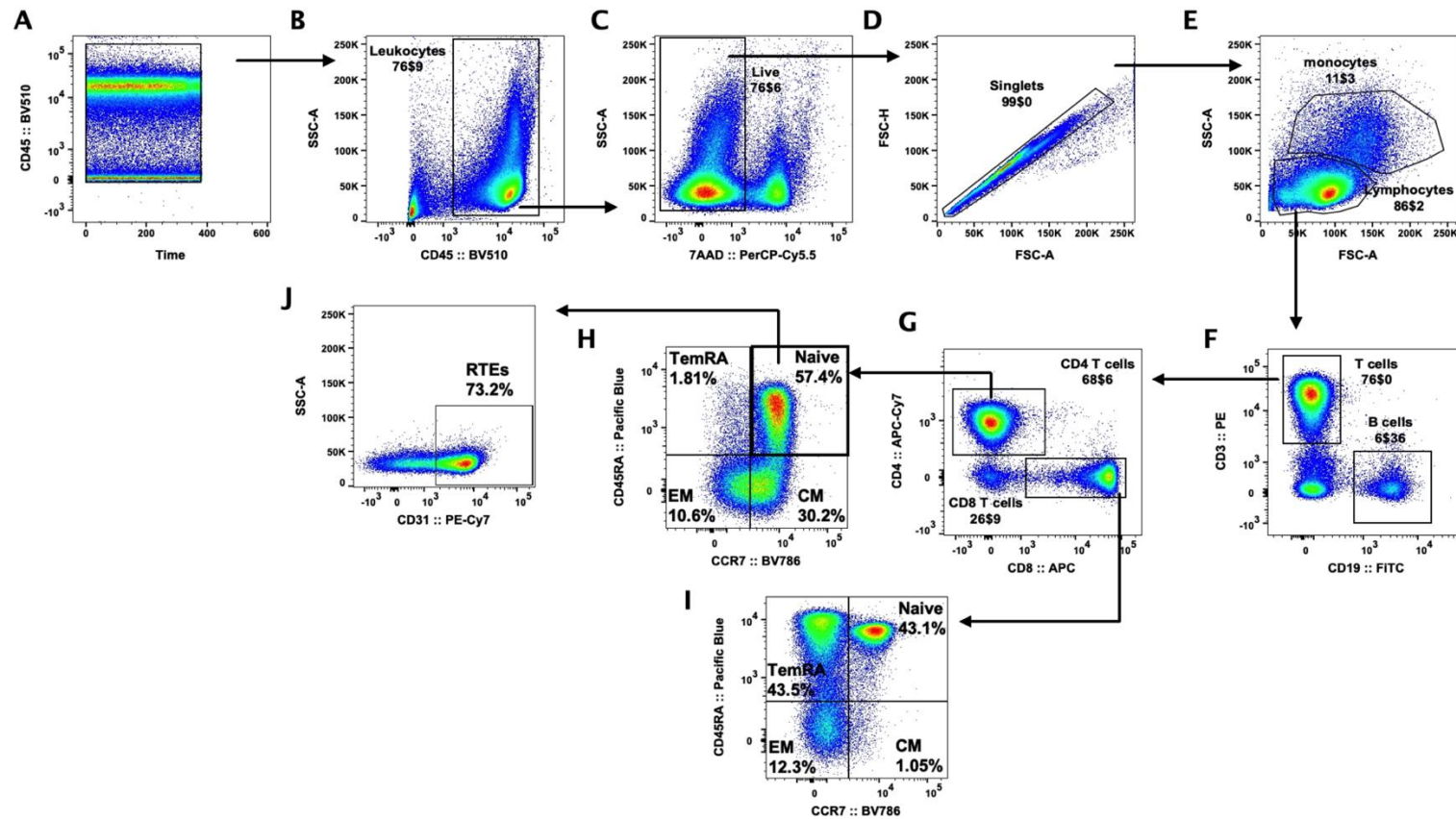
Supplementary Figure S1. pwPPMS display lower central memory CD8⁺ T cells number. Number and percentage of CD8⁺ T cells are represented for healthy controls (gray circles) and pwPPMS [blue circles; (A)]. Representative dot plot defining CD8⁺ T cell subpopulations according to the expression of CD45RA and CCR7 (B): naïve (CD45RA⁺CCR7⁺); central memory (CM; CD45RA⁺CCR7⁺), effector memory (EM; CD45RA⁺CCR7⁺); and terminally differentiated CD45RA-expressing memory T cells (TemRA; CD45RA⁺CCR7⁺). Ratio of naïve to memory CD8⁺ T cells where the total memory CD8⁺ T cells were considered the sum of CM, EM, and TemRA subsets (C). Percentage and number of CD8⁺ T cell subsets: naïve (D), total memory (E), CM (F), EM (G), and TemRA (H). Representative dot plot and percentage of proliferating (Ki67⁺) CD8⁺ T cells (I). All graphs display a dot per individual, with the horizontal lines representing the groups' median. The parametric Student's *t*-test was performed in (A), (C), (D; percentage), (E) and (H; percentage); the non-parametric Mann-Whitney *U*-test in (D; number), (F), (G), (H; number) and (I). *P*-values were represented in the graph whenever <0.100, and differences were considered statistically significant for *p*-value <0.050. Statistical outputs and effect size calculations in **Supplementary Table S1**. §, stands for situations when significance is gained upon controlling for age and sex on the multiple linear regression models (**Supplementary Table S3**).



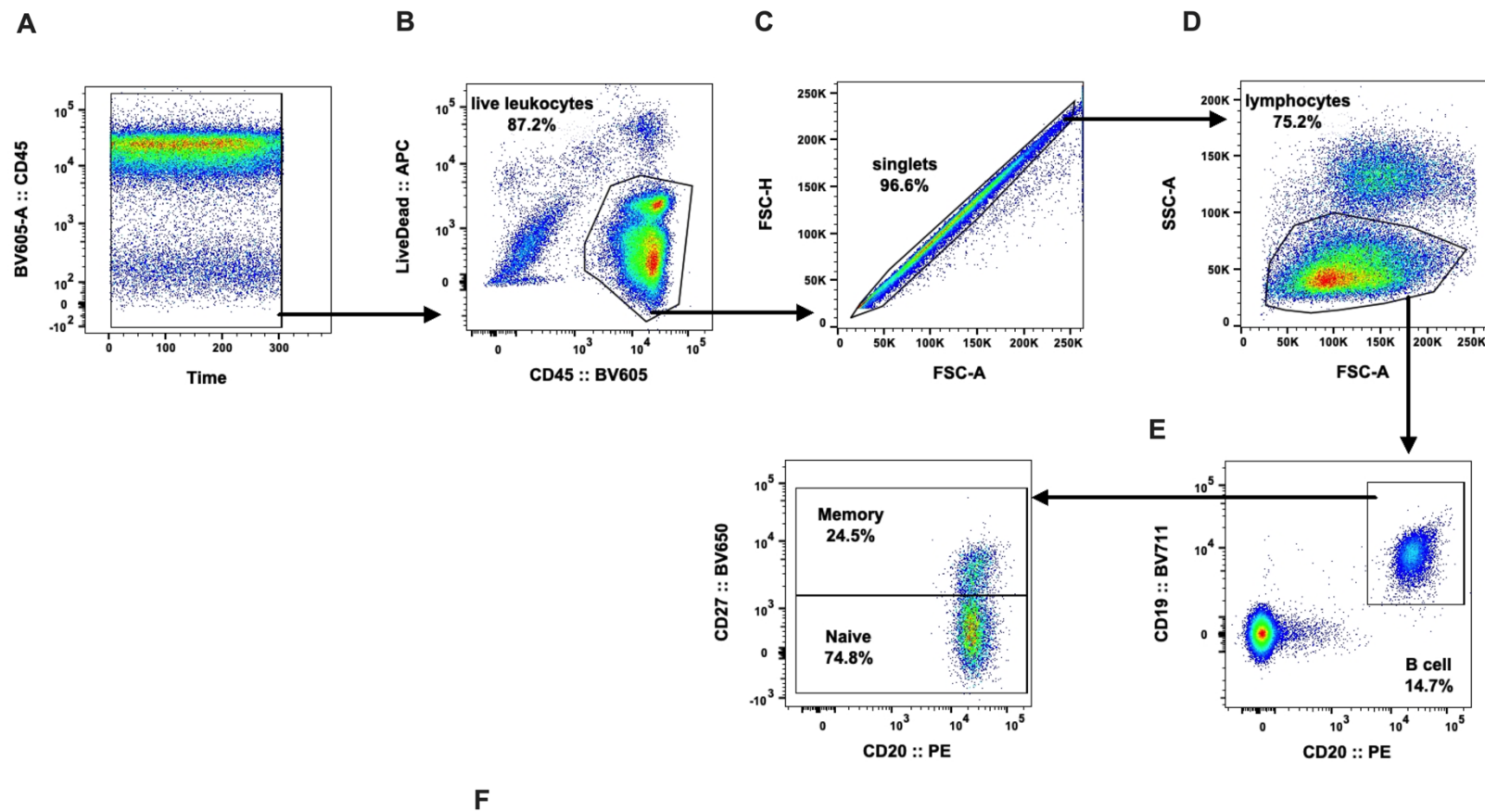
Supplementary Figure S2. pwPPMS present no alterations on B cell subsets. Number and percentage of B cells (CD19⁺ CD20⁺) are represented for healthy controls (gray circles) and pwPPMS [blue circles; (**A**)]. Representative dot plot defining B cell subpopulations according to the expression of CD27 (**B**): naïve (CD19⁺CD20⁺CD27⁻) and memory (CD19⁺CD20⁺CD27⁺). Percentage and number of B cell subsets: naïve (**C**), memory (**D**) and ratio of naïve to memory B cells (**E**). All graphs display a dot per individual, with the horizontal lines representing the groups' median. The parametric Student's *t*-test was performed in (**A**, number); the non-parametric Mann-Whitney *U*-test in (**A**; percentage), (**C**), (**D**) and (**E**). *P*-values were represented in the graph whenever <0.100, and differences were considered statistically significant for *p*-value <0.050. Statistical outputs and effect size calculations in **Supplementary Table S1** and **S4**.



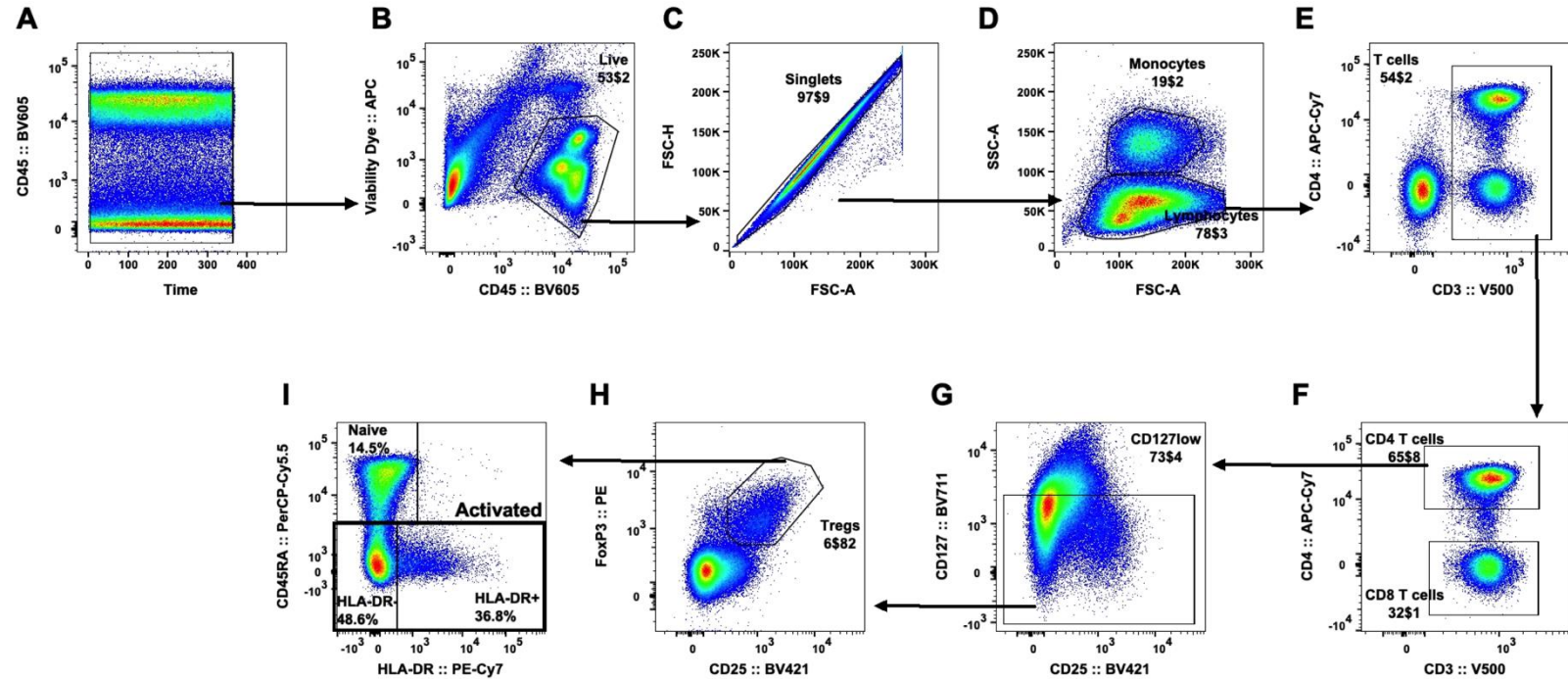
Supplementary Figure S3. pwPPMS display a higher percentage of the most mature NK cells expressing NKG48. Percentage and number of NK cells (CD3⁺CD56⁺) are represented for healthy controls (gray circles) and pwPPMS [blue circles; (A)]. Representation of percentage and number of NK cells subpopulations: CD56^{bright} [most immature; (B)]; CD56^{dim}CD57⁻ (C); and CD56^{dim}CD57⁺ [most differentiated; (D)]. Percentage of cells expressing inhibitory receptors in total NK cells and on its three subsets: KLRG1 (E), NKG2A (F), KIR3DL1 (G) and KIR2DL2/3 (H). Percentage of cells expressing activating receptors in total NK cells and in its subsets: NKG30 (I), NKG44 (J), and NKG48 (K). All graphs display a dot per individual, with the horizontal lines representing the groups' median. The parametric Student's *t*-test was performed in (A), (B-C; percentage), (D), (E; NK cells, CD56^{bright}, CD56^{dim}CD57⁻ [percentage] and CD56^{dim}CD57⁺ [percentage]), (F; percentage), (G; CD56^{dim}CD57⁻ [percentage] and CD56^{dim}CD57⁺ [number]), (H; NK cells [percentage], CD56^{bright} [percentage] and CD56^{dim}CD57⁻ [percentage]), (I; percentage), (J; CD56^{bright} [percentage]), (K; NK cells [percentage], CD56^{dim}CD57⁻ [percentage] and CD56^{dim}CD57⁺); the non-parametric Mann-Whitney *U*-test in (B-C; number), (E; CD56^{dim}CD57⁻ [number] and CD56^{dim}CD57⁺ [number]), (F; number), (G; NK cells, CD56^{bright}, CD56^{dim}CD57⁻ [number] and CD56^{dim}CD57⁺ [percentage]), (H; NK cells [number], CD56^{bright} [number], CD56^{dim}CD57⁻ [number] and CD56^{dim}CD57⁺), (I; number), (J; NK cells, CD56^{bright} [number], CD56^{dim}CD57⁻ and CD56^{dim}CD57⁺), (K; NK cells [number], CD56^{bright} and CD56^{dim}CD57⁻ [number]). *P*-values were represented in the graph whenever <0.100, and differences were considered statistically significant for *p*-value <0.050. Statistical outputs and effect size calculations in **Supplementary Table S1**. #, stands for situations when significance is lost upon controlling for age and sex on the multiple linear regression models (Supplementary Table S6).



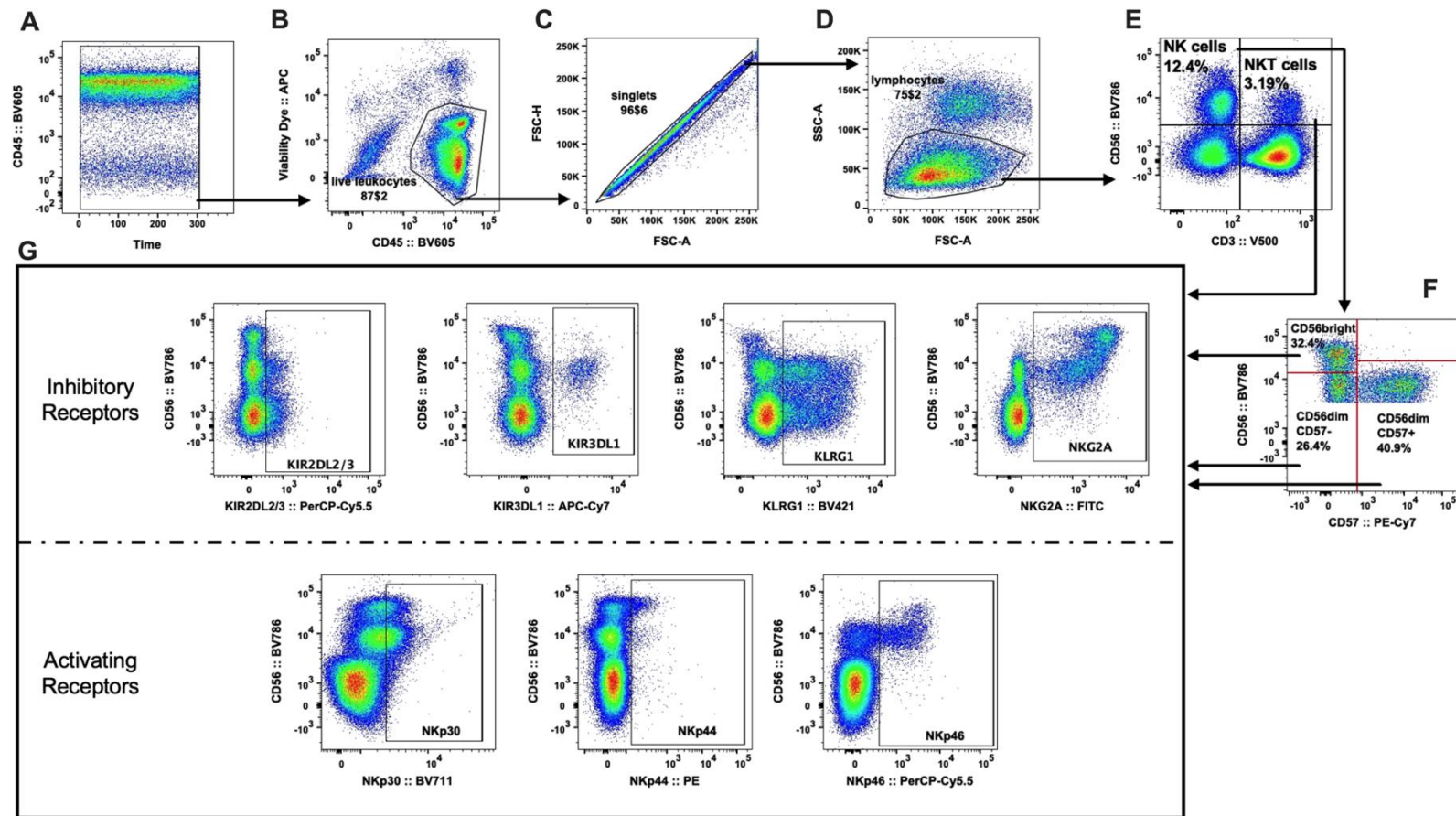
Supplementary Figure S4. Gating strategy for phenotypic analysis of the main T cell subsets. Issues during acquisition were verified by accompanying acquisition throughout time (**A**). Leukocytes were selected by their positive expression of CD45 (**B**), and viable cells selected by excluding 7AAD positive cells (**C**). After isolating the singlets (**D**), the monocytes and lymphocytes were gated based on their size (FSC-A) and complexity (SSC-A; **E**). Within the lymphocytes population, B cells were distinguished from T cells, through the differential expression of CD19 and CD3, respectively (**F**). CD4⁺ and CD8⁺ cell subsets were then discriminated among T cells (**G**). CD45RA and CCR7 were used both for CD4⁺ (**H**) and CD8⁺ (**I**) T cells to discriminate the Naïve (CD45RA⁺CCR7⁺), Central Memory (CM; CD45RA⁻CCR7⁺), Effector Memory (EM; CD45RA⁻CCR7⁻) and CD45RA-expressing memory cells (TemRA; CD45RA⁺CCR7⁻) subsets [**H** and **I**]. Total memory CD4⁺ or CD8⁺ T cells were the sum of CM, EM and TemRA cells. Finally, recent thymic emigrants (RTEs) were defined by the expression of the CD31 among naïve CD4⁺ T cells (CD45RA⁺CCR7⁺CD31⁺; **J**).



Supplementary Figure S5. Gating strategy for analysis of B cell subsets. Any acquisition issues were controlled by checking acquisition throughout time (A). Live leukocytes were defined based on negative staining for the viability dye and CD45 positive expression (B). Following singlet isolation (C), size (FSC-A) and complexity (SSC-A) gating was used to define lymphocytes (D). Within the lymphocyte population, B cells were defined as CD19⁺CD20⁺ (E). According to CD27 expression the naïve (CD27⁻) and memory (CD27⁺) CD20⁺ B cell subsets were defined.



Supplementary Figure S6. Gating strategy for phenotypical analysis of the regulatory T cells (Tregs). Issues during acquisition were verified by accompanying acquisition throughout time (**A**). Live leukocytes were selected by their positive expression of CD45 and by excluding positive cells for the viability dye (**B**). After isolating the singlets (**C**), the monocytes and lymphocytes were gated based on their size (FSC-A) and complexity (SSC-A; **D**). Within the lymphocytes the T cells were defined according to the positive expression of CD3 (**E**). The population CD4⁺ T cells was then defined by its positive expression of CD4 (**F**). Tregs were defined as the CD4⁺ T cells expressing low levels of CD127 (**G**), followed by selection of FoxP3⁺ CD25⁺ cells (**H**). Finally, the CD45RA and HLA-DR markers were used to define three Tregs subsets: the naive (CD45RA⁺HLA-DR⁻), the activated HLA-DR⁻ (CD45RA⁺HLA-DR⁻) and the activated HLA-DR⁺ (CD45RA⁺HLA-DR⁺) Tregs (**I**). Total activated Tregs was the sum of activated HLA-DR⁻ and HLA-DR⁺ cells.



Supplementary Figure S7. Gating strategy for analysis of inhibitory and activating receptor expression on NKT and NK subsets. Any acquisition issues were controlled by checking acquisition throughout time (A). Negative staining for the viability dye and CD45 positive expression were used to define live leukocytes (B). Following singlet isolation (C), size (FSC-A) and complexity (SSC-A) gating was used to define lymphocytes (D). Within the lymphocyte population, NK and NKT cells were defined as CD56⁺CD3⁻ and CD56⁺CD3⁺, respectively (E). Three main NK cell subsets were discriminated according to CD56 and CD57 expression levels: CD56^{bright} NK cells, CD56^{dim}CD57⁻ NK cells and CD56^{dim}CD57⁺ NK cells (F). Inhibitory (KIR2DL2/3, KIR3DL1, KLRG1 and NKG2A) or activating receptor (NKp30, NKp44 and NKp46) expression were assessed within all NK subsets and NKT cells, according to the staining panel (G).