Supplementary Data: A Validation Study on Immunophenotypic Differences in T-lymphocyte Chromosomal Radiosensitivity between Newborns and Adults in South Africa

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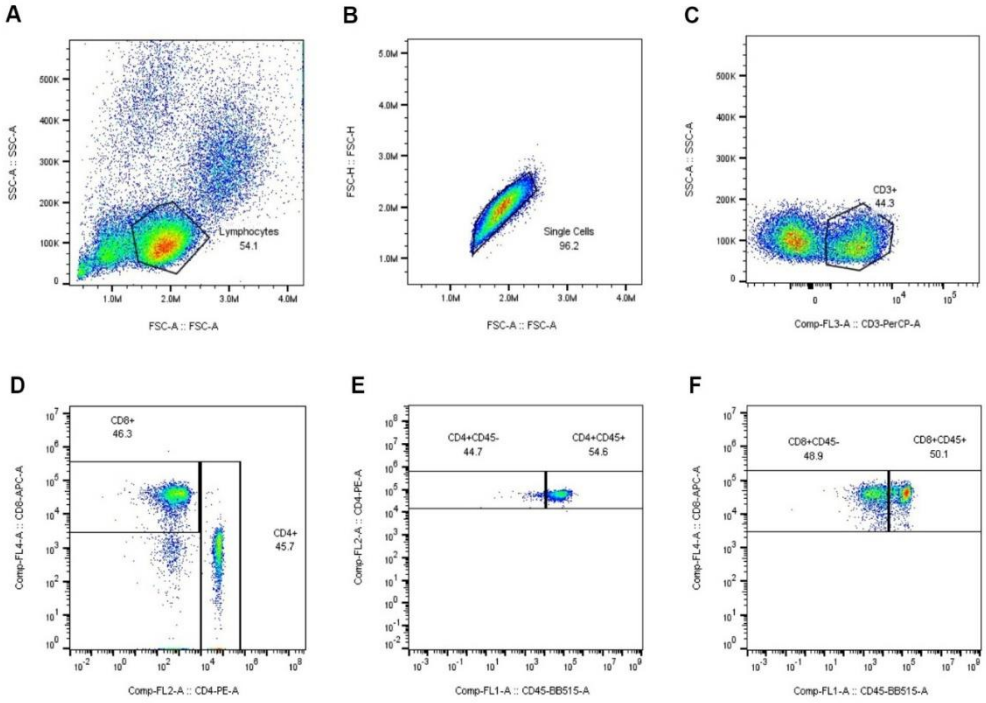

Figure S1. The gating strategy for the expression of **naïve (CD45RA) markers** on peripheral blood $CD4^+$ and $CD8^+$ T-lymphocytes in **adults (APB)**. The peripheral blood T-lymphocytes were gated on forward (FSC) versus side scatter (SSC) to select the lymphocyte cell population **(A)**. The cells were gated on FSC-Height (FSC-H) vs FSC-Area (FSC-A) to exclude all the doublets and to generate the singlets gate **(B)**. Subsequently, the cells were gated on CD3-PerCP vs SSC-A to include all $CD3^+$ cells **(C)**, then gated on the $CD4^+$ or $CD8^+$ population **(D)**. All the subpopulations were analysed on the CD4-PE-A **(E)** or CD8-APC-A **(F)** versus CD45-BB515-A. Data were analysed using FlowJo v10.6.1 software, and population frequencies expressed as percent of the $CD4^+$ or $CD8^+$ parent population.

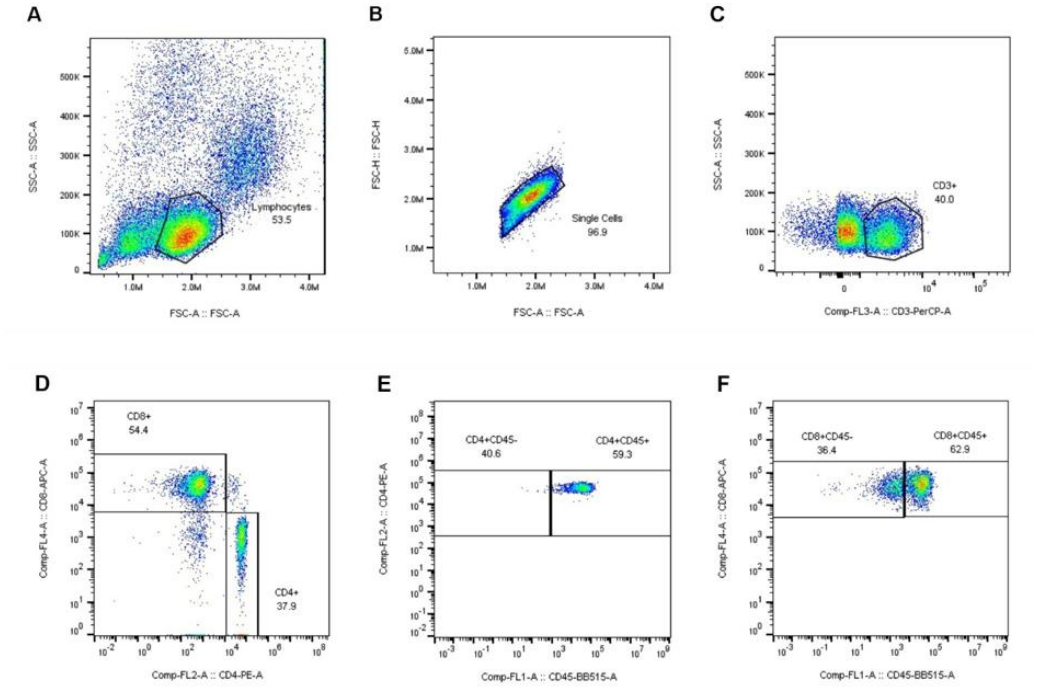

Figure S2. The gating strategy for the expression of memory (CD45RO) markers on peripheral blood $CD4^+$ and $CD8^+$ T-lymphocytes in adults (APB). The peripheral blood T-lymphocytes were gated on forward (FSC) versus side scatter (SSC) to select the lymphocyte cell population (A). The cells were gated on FSC-Height (FSC-H) vs FSC-Area (FSC-A) to exclude all the doublets and to generate the singlets gate (B). Subsequently, the cells were gated on CD3-PerCP vs SSC-A to include all $CD3^+$ cells (C), then gated on the $CD4^+$ or $CD8^+$ population (D). All the subpopulations were analysed on the CD4-PE-A (E) or CD8-APC-A (F) versus CD45-BB515-A. Data were analysed using FlowJo v10.6.1 software, and population frequencies expressed as percent of the $CD4^+$ or $CD8^+$ parent population.

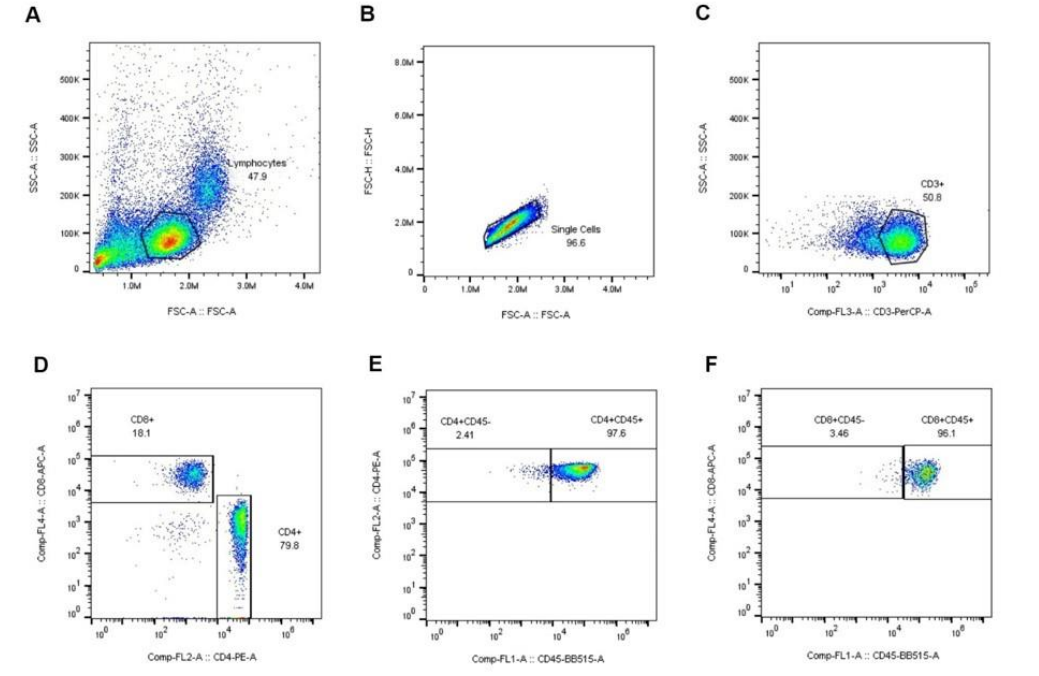

Figure S3. The gating strategy for the expression of naïve (CD45RA) markers on peripheral blood $CD4^+$ and $CD8^+$ T-lymphocytes in newborns (UCB). The peripheral blood T-lymphocytes were gated on forward (FSC) versus side scatter (SSC) to select the lymphocyte cell population (A). The cells were gated on FSC-Height (FSC-H) vs FSC-Area (FSC-A) to exclude all the doublets and to generate

the singlets gate (B). Subsequently, the cells were gated on CD3-PerCP vs SSC-A to include all $CD3^+$ cells (C), then gated on the $CD4^+$ or $CD8^+$ population (D). All the subpopulations were analysed on the CD4-PE-A (E) or CD8-APC-A (F) versus CD45-BB515-A. Data were analysed using FlowJo v10.6.1 software, and population frequencies expressed as percent of the $CD4^+$ or $CD8^+$ parent population.

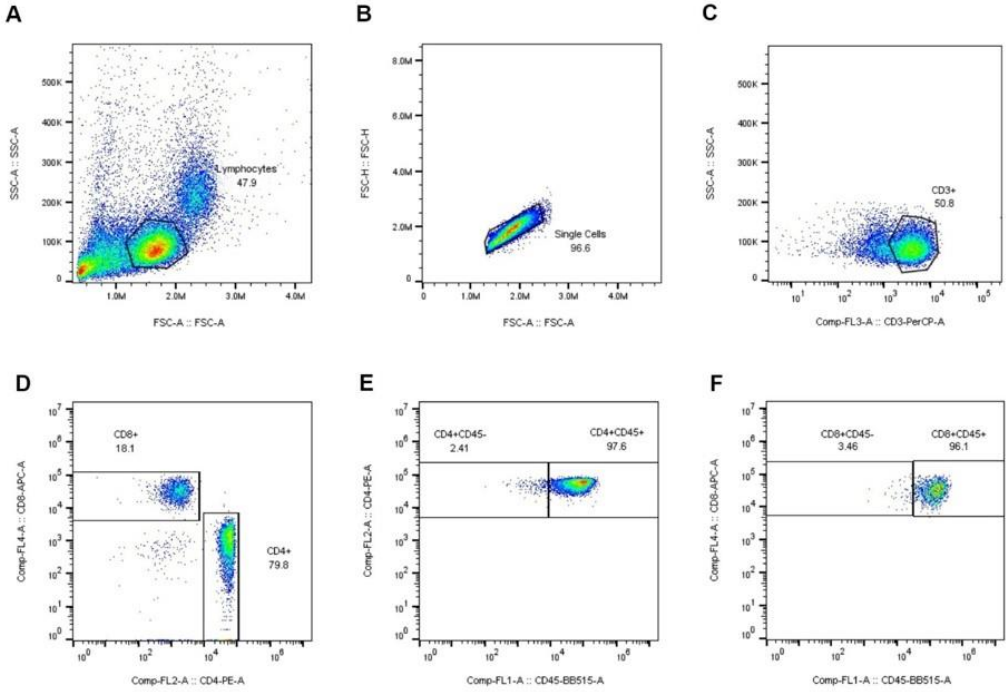

Figure S4. The gating strategy for the expression of memory (CD45RO) markers on peripheral blood $CD4^+$ and $CD8^+$ T-lymphocytes in newborns (UCB). The peripheral blood T-lymphocytes were gated on forward (FSC) versus side scatter (SSC) to select the lymphocyte cell population (A). The cells were gated on FSC-Height (FSC-H) vs FSC-Area (FSC-A) to exclude all the doublets and to generate the singlets gate (B). Subsequently, the cells were gated on CD3-PerCP vs SSC-A to include all $CD3^+$ cells (C), then gated on the $CD4^+$ or $CD8^+$ population (D). All the subpopulations were analysed on the CD4-PE-A (E) or CD8-APC-A (F) versus CD45-BB515-A. Data were analysed using FlowJo v10.6.1 software, and population frequencies expressed as percent of the $CD4^+$ or $CD8^+$ parent population.