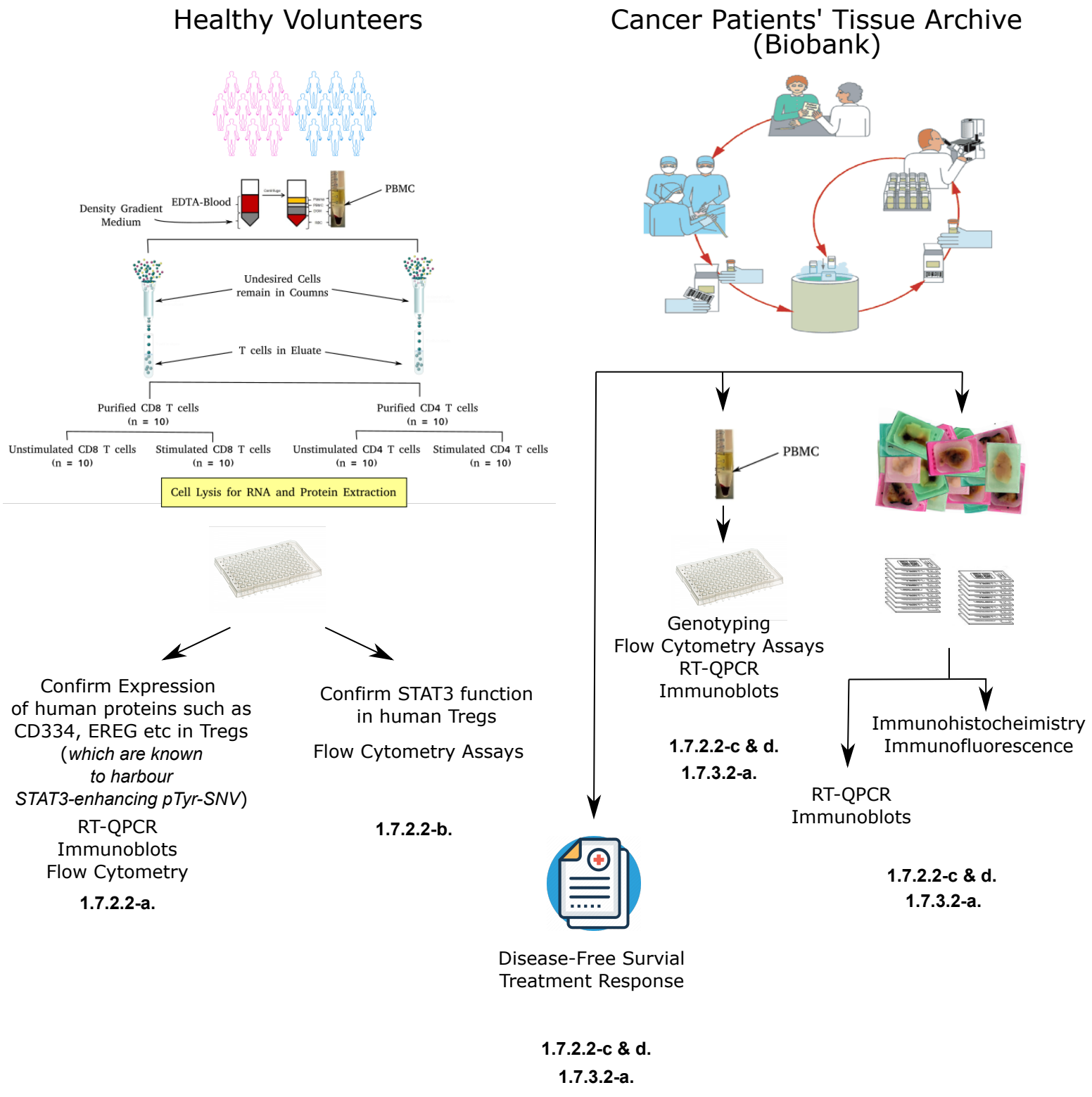

Online Supplementary Figure-1. Methodology applied to determine whether SeSNVs can directly modify anti-tumour CD8 T-cell responses and, consequently, influence the therapeutic response to ICB therapy. To determine the expression of SeSNVs harbouring proteins in CD8 T-cells, we will sort out Tregs, CD8 and CD4 T-cells from the bloods of ten gender-matched consenting human volunteers with a minimum cohort size of n=10.The purified immune cells from each individual will be split into two portions. One portion will be used for transcript analysis via RT-qPCR, while the other will be used for protein analysis through immunoblotting. The data obtained from RT-qPCR and immunoblotting will be validated and complemented by conducting intracellular and surface staining experiments with flow cytometry and immunofluorescence-compatible antibodies. To assess the preferential expression of STAT3 by functional analysis, we will assess the survival and proliferation potential of APCs, Tregs, CD4, and CD8 T-cells within human peripheral blood mononuclear cells (PBMCs) using flow cytometry-based assays following pharmacological inhibition of STAT3. The specific cell types that are preferentially depleted as a result of STAT3 inhibition are expected to be highly reliant on the STAT3 signaling pathway. To determine the correlation between CD8 T-cells in the TME and Melanoma progression ,2 methods will be employed: IHC, IF, RT-QPCR and IB analyses will be performed to deduce a reliable and reproducible quantitative estimation of CD8 T-cell infiltration per unit tumor area and adjacent normal tissues. Furthermore, QPCR, immunoblot, double immunofluorescence will be performed to detect and quantify the expression of cytotoxic molecules, cytokines, and interleukins. To Determine the association of SeSNVs to anti-CD8 T-cell response, we will be correlating genotyping data with the CD8 T-cell immune contexture data in tumour and blood conclusions. We will also generate retroviral packaging cell lines and SeSNV-expressing TCR transgenic T-cells. Finally, the co-cultivation of B16-F10 cell line and TCR transgenic T-cells will take place.