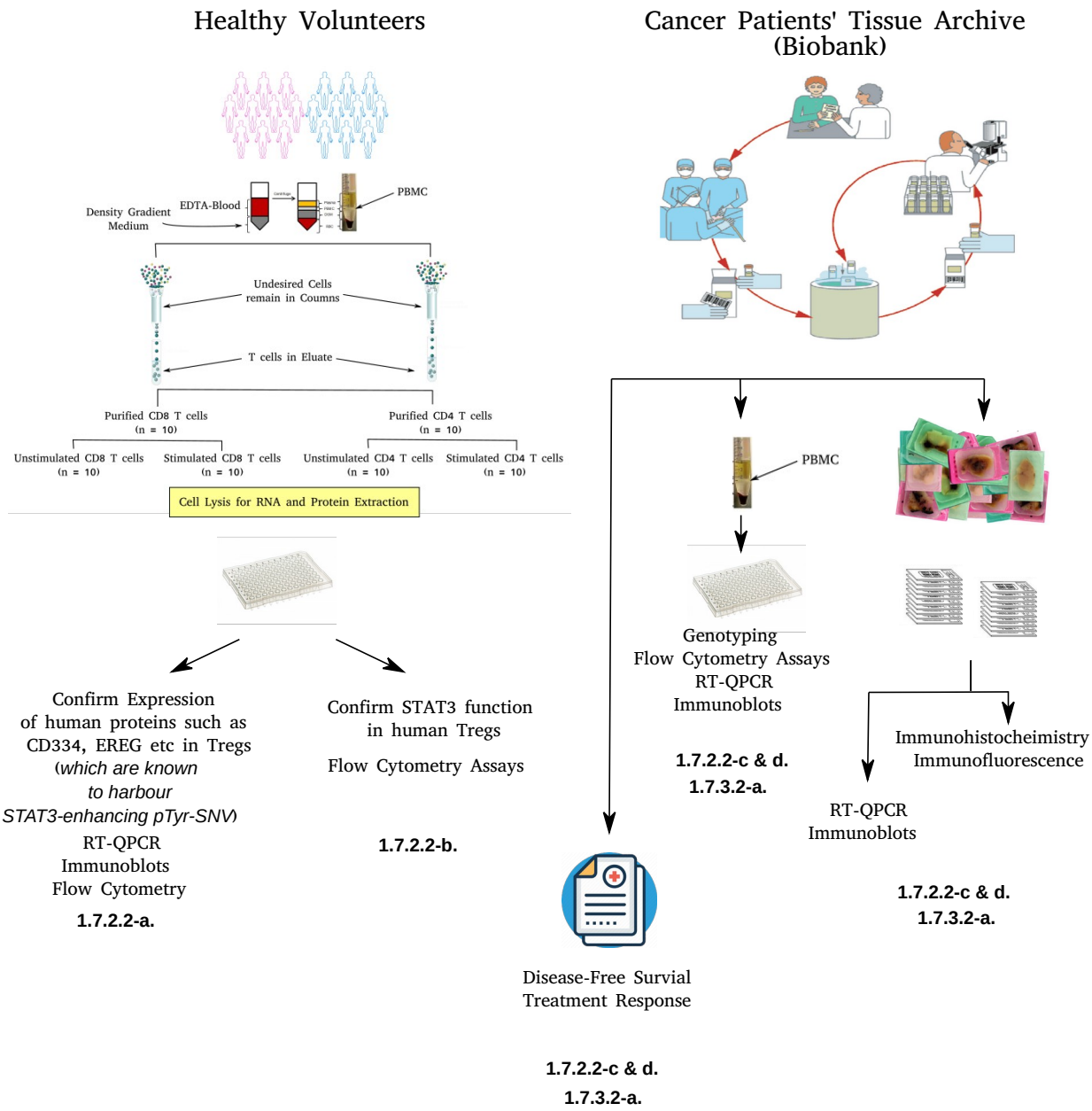

Online Supplementary Figure-1. Flow chart showing the methodology described in Project Description Section 1.7.2.2 to study melanoma-associated SeSNVs using healthy PBMCs, archived tumour materials, and patient treatment response data.

To determine the expression of receptors known to harbour SeSNVs in human T cells, we will sort Tregs, CD8, and CD4 T cells from the blood gender-matched consenting human volunteers, with a minimum cohort size of n=10. The purified immune cells from each individual will be split into several 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 blood-borne immune cells, we will evaluate the survival and proliferation potential of monocytes, macrophages, Tregs, CD4, and CD8 T cells within human peripheral blood mononuclear cells (PBMCs) using flow cytometry-based assays following the 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 signalling pathway.

Using archived melanoma tumour tissue sections from University BioBanks (UMG & UKT), and the associated patient metadata, we will deploy RT-QPCR, immunoblots, immunohistochemistry, and immunofluorescence to quantify the relative number of tumour-infiltrating Tregs, CD4, and CD8 T cells. To determine the correlation between CD8 T cells in the TME and melanoma progression, two 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 of tumour area and adjacent normal tissues. Furthermore, RT-QPCR, immunoblot, and double immunofluorescence will be performed to detect and quantify the expression of cytotoxic molecules, cytokines, and interleukins. To determine the association of SeSNVs with the anti-CD8 T-cell response, we will perform statistical correlation analyses between genotyping data and the quantification of CD8 T cells in the tumour and blood compartments.