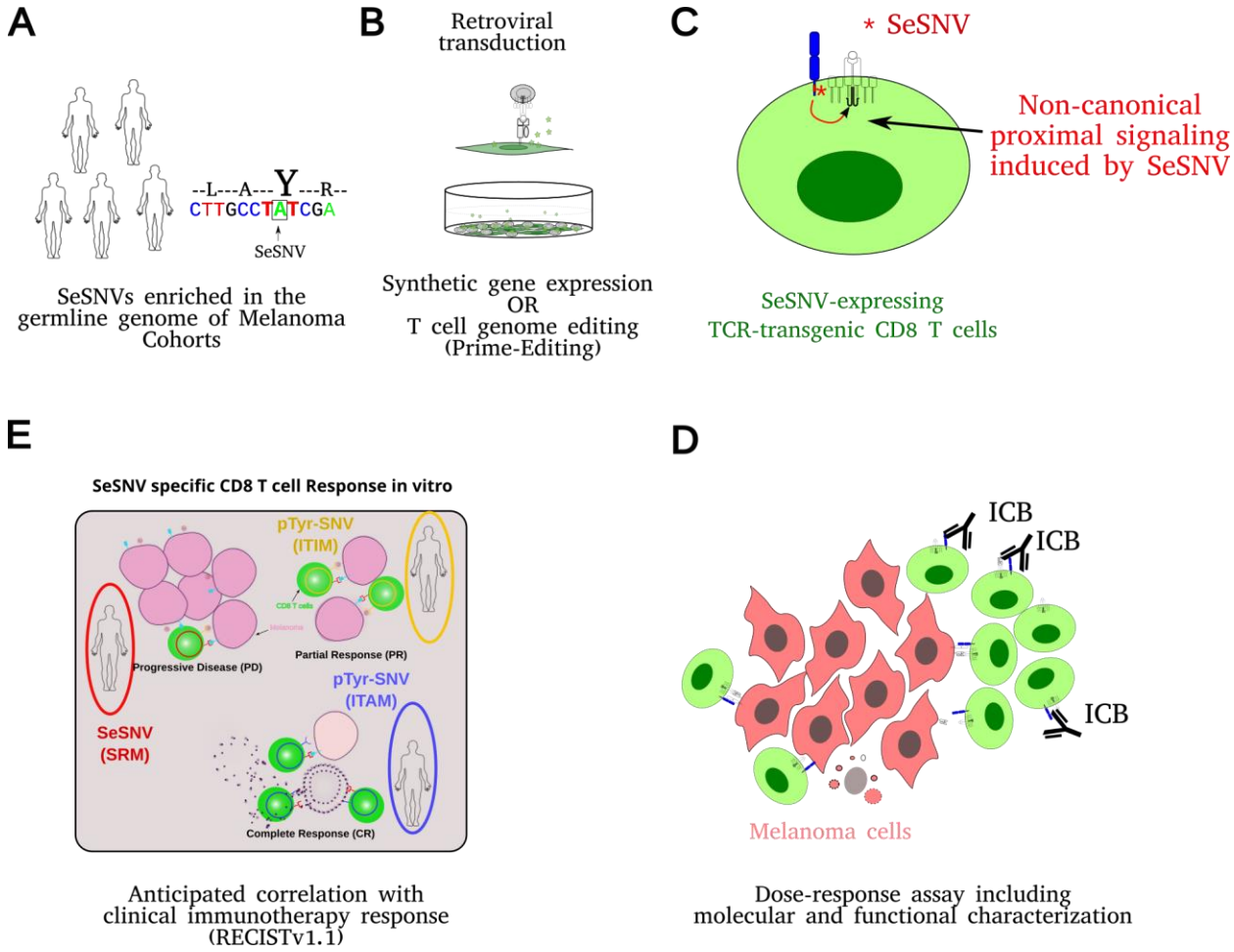

Supplementary Figure 2. Elucidation of the mechanistic basis of SeSNV-associated immune checkpoint blockade (ICB) therapy response. (A) Candidate SeSNVs are chosen using IMGAG whole exome sequencing data of blood from melanoma patients, as described in section 1.7.1.3. (B) Selected SeSNVs will be expressed in TCR-transgenic CD8 T cells (OT-I and/or PMEL-1) either by synthetic gene expression constructs or by CRISPR editing using prime editing methodologies, as described in Ulaganathan VK et al., J Genet Genomics, 2023. (C) Illustration depicting the working hypothesis described in section 1.4, which will be tested using genetically engineered TCR-transgenic CD8 T cells expressing SeSNVs. We posit SeSNVs modify the outcomes of TCR signalling and, therefore, the functional properties of tumor-reactive CD8 T cells. (D) Illustration depicting the in vitro co-cultivation setup comprising syngeneic SeSNV-expressing TCR-transgenic CD8 T cells (Clover+ve) and tumor antigen-expressing melanoma cells (Ruby+ve), which will be subjected to varying concentrations of mouse mAbs, viz., Anti-CTLA-4 (clone 9H10), Anti-PD-1 (clone RMP1-14), and Anti-PD-L1 (clone 10F.9G2). The cultivation will be performed in 96- and 48-well culture plates, allowing biological replicates and duplicates for various molecular and functional studies. The dose-dependent therapeutic response will be assessed by various flow cytometry-based assays. (E) Graphical summary of SeSNV genotype-to-phenotype investigation conducted in this research project. The molecular mechanistic basis of immunotherapy-linked SeSNVs and pTyr-SNVs (not investigated in this project) are anticipated to directly impact the functional properties of tumor-reactive T cells, potentially manifesting as alterations in cytotoxicity or interstitial migration properties, resulting in decreased tumor infiltration. SeSNVs are pTyr-SNVs that can create STAT3 recruiting motifs (SRM). Also shown are pTyr-SNVs that create ITIM and ITAM motifs, which were not investigated in this project but are being studied in other ongoing projects within Ulaganathan's working group.