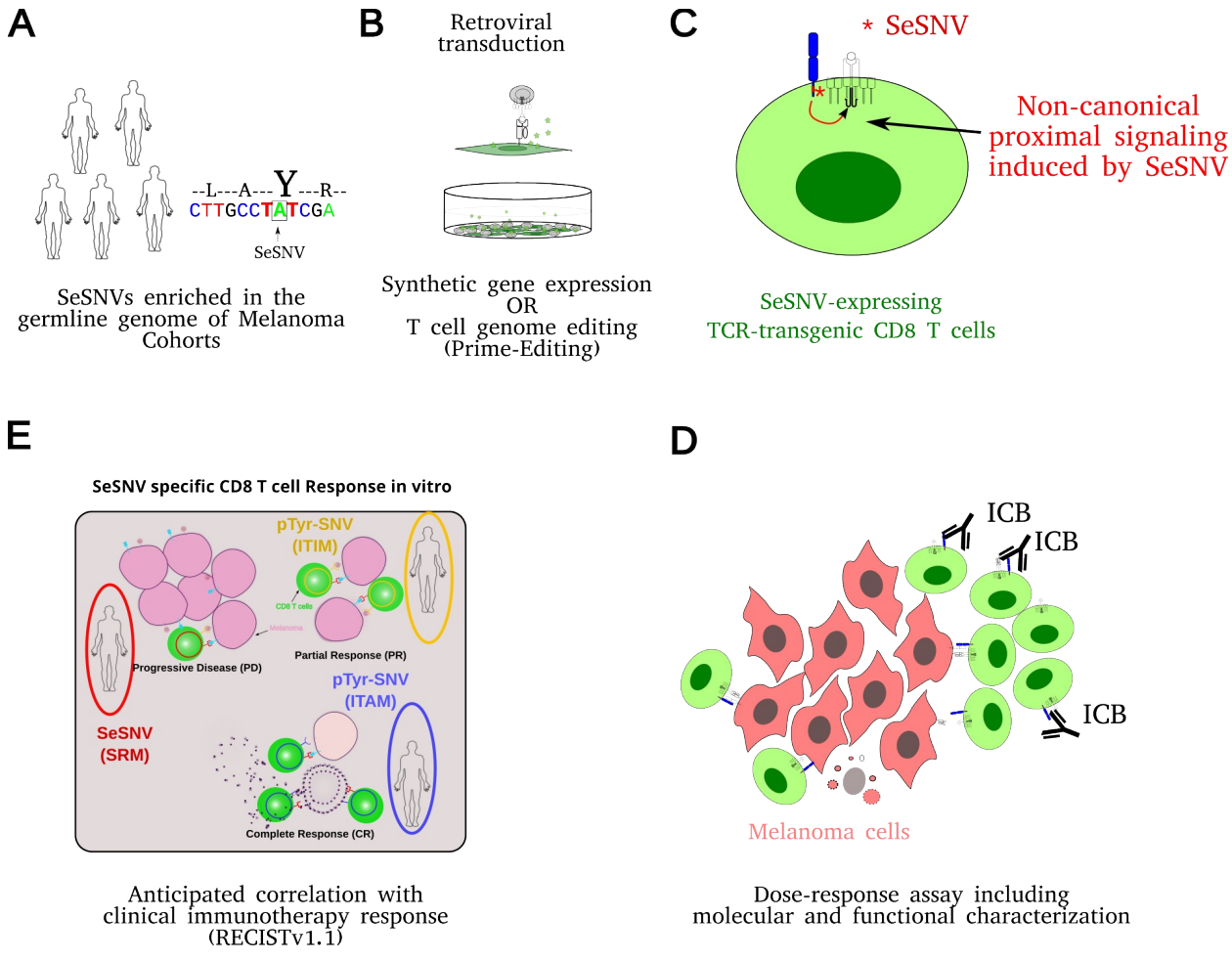


**Online Supplementary Figure 2. Elucidation of the mechanistic basis of SeSNV-associated immune checkpoint blockade (ICB) therapy response.** (A) Candidate SeSNVs are chosen using IMGAG whole exome sequencing data of blood from melanoma patients, as described in section 1.7.1.3. (B) Selected SeSNVs 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 tumour-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 tumour 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 tumour-reactive T cells, potentially manifesting as alterations in cytotoxicity or interstitial migration properties, resulting in decreased tumour 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.