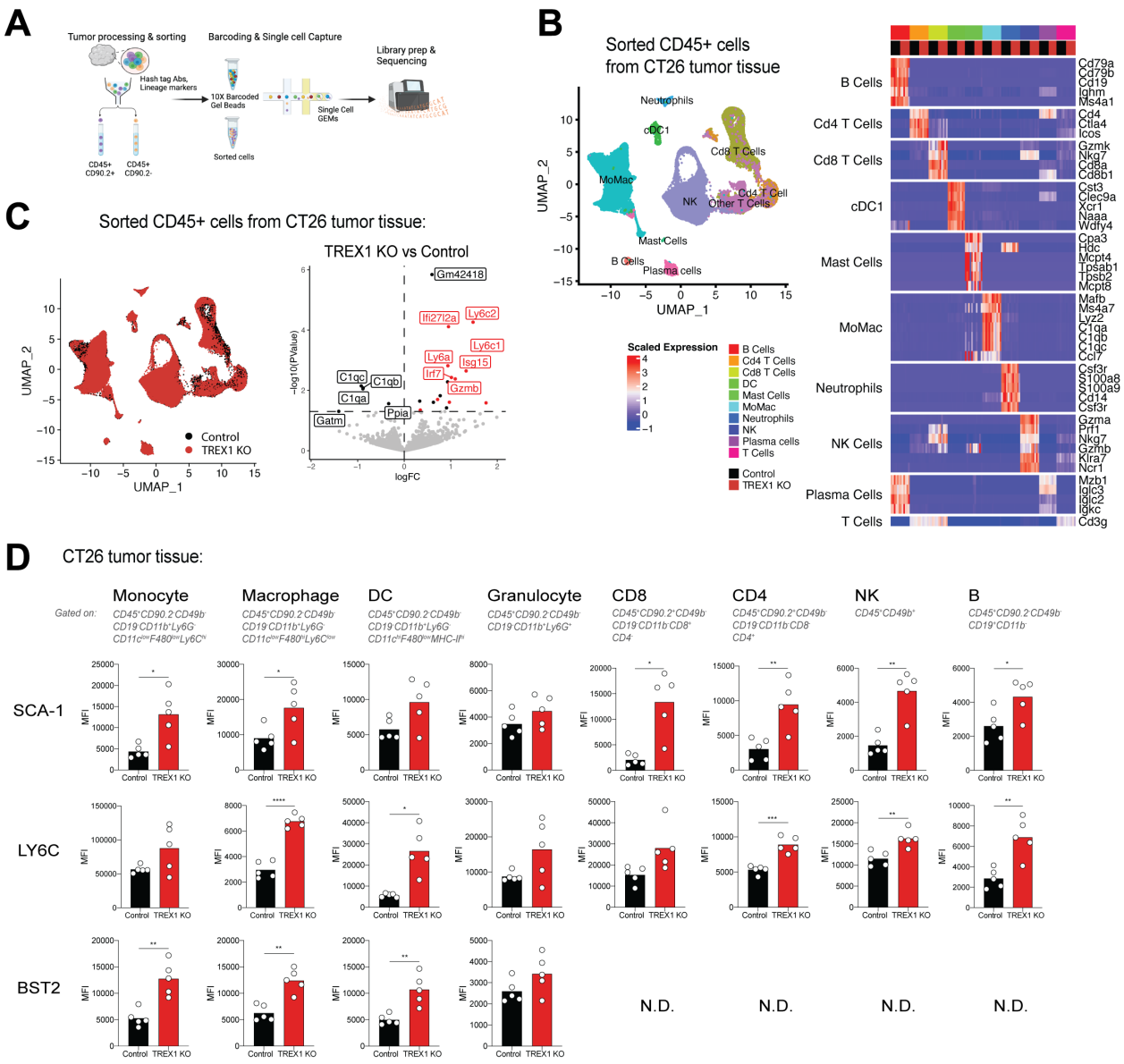

Supplementary Figure 4. Single-cell RNA sequencing workflow and ISG expression across immune cells in the tumor microenvironment

A. Workflow for single cell RNA sequencing. CT26 tumors were processed and labeled with a unique hash tag antibody per individual animal together with surface antibodies. After sorting CD90.2+ and CD90.2- immune cells, same number of cells from individual animals were pooled. **B.** UMAP of sorted immune cells from control and TREX1 KO CT26 tumors (left). Heatmap of immune cell type markers (right). Each column is the average scaled expression of the given cell type per animal. **C.** Same UMAP as in B colored by tumor

genotype (left). Volcano plot of genes differentially expressed between pseudobulk of TREX1 KO and control immune cells (right). ISGs are labelled in red. The five genes with the lowest p-value in each direction are labelled in addition to *Isg15*, *Irf7*, and *Gzmb*. Dashed horizontal line represents a p-value cutoff of 0.05. Only animals with more than 200 cells were included for comparison. **D**. Bar graphs showing SCA-1, LY6C and BST2 expression on different immune cells types in control and TREX1-deficient CT26 tumors. Circles represent individual animals. Bars represent the mean. One-way ANOVA (n=5 per group). *P < 0.05, **P < 0.01, ***P < 0.001 and ***P < 0.0001 (N.D.; not determined). Data for SCA-1 expression on CD8 T and NK cells are identical to Figure 4C and Supplementary Figure 6F, respectively.