Figure S3. Markers of T cell exhaustion are increased in CD8^{+} T cells later in pseudotime, corresponding to cells from advanced/metastatic disease, related to **Figure 3**. **(A)** Volcano plot of differentially expressed genes (edgeR) between CD8^{+} T cells from metastatic disease vs those from early and locally advanced disease (thresholds: at least 1.5-fold change, and q value less than 10^{-10}. TradeSeq plots showing both the average expression pattern and individual cell expression values for **(B)** T cell inhibitory checkpoint genes, **(C)** transcription factor genes related to progenitor-like and terminally exhausted states, and **(D)** signatures of T cell exhaustion and terminal differentiation. The expression of genes and a signature associated with cell stress are different between the trajectory branches, as shown in the **(E)** feature plots and **(F)** TradeSeq plots. **(G)** Gene set enrichment analysis showing top Reactome pathways enriched in the different CD8^{+} T cell trajectory branches, demonstrating that the differences are mostly driven by differential expression of genes relating to cell stress, likely as a technical artifact. **(H)** Example gene set enrichment analysis of two top pathways that were different in the two trajectory branches.