Figure S1

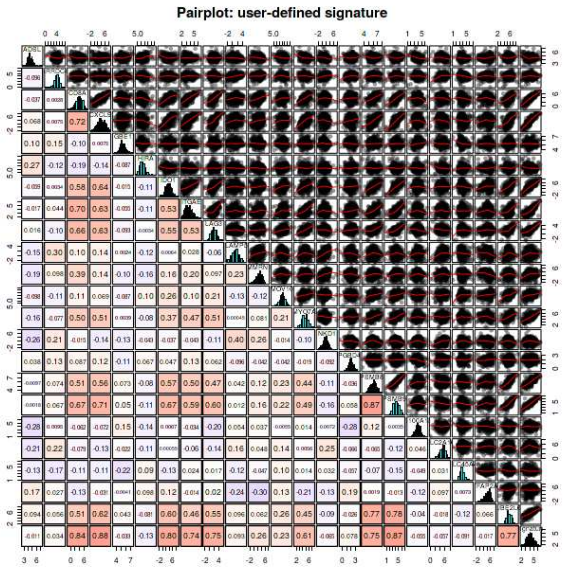



**Figure S1** Pairwise correlation between all (92) classification-relevant genes across the training dataset.

Figure S2

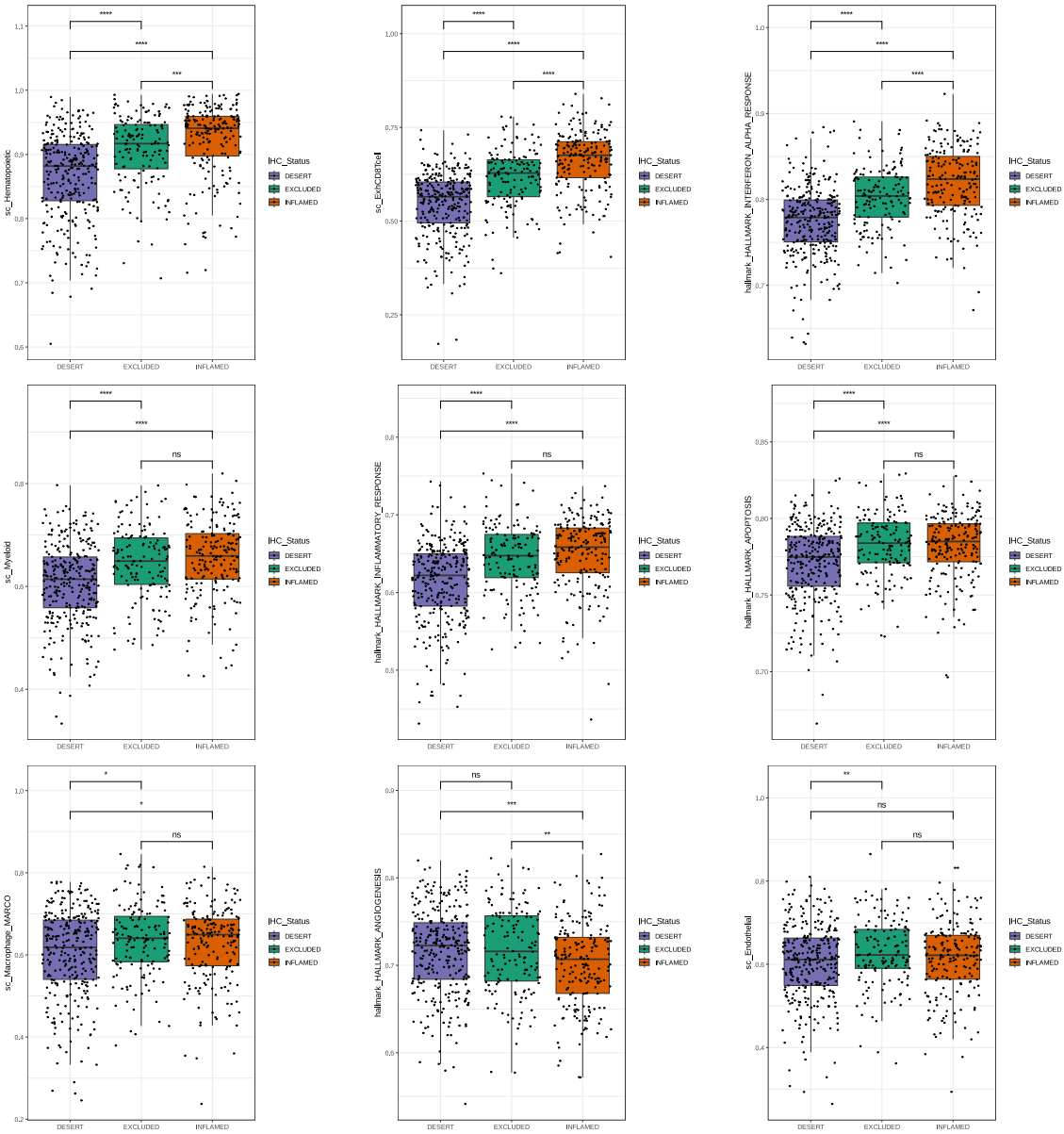



**Figure S2** Gene expression related to CD8 immunophenotypes. IHC, immunohistochemistry.

Figure S3

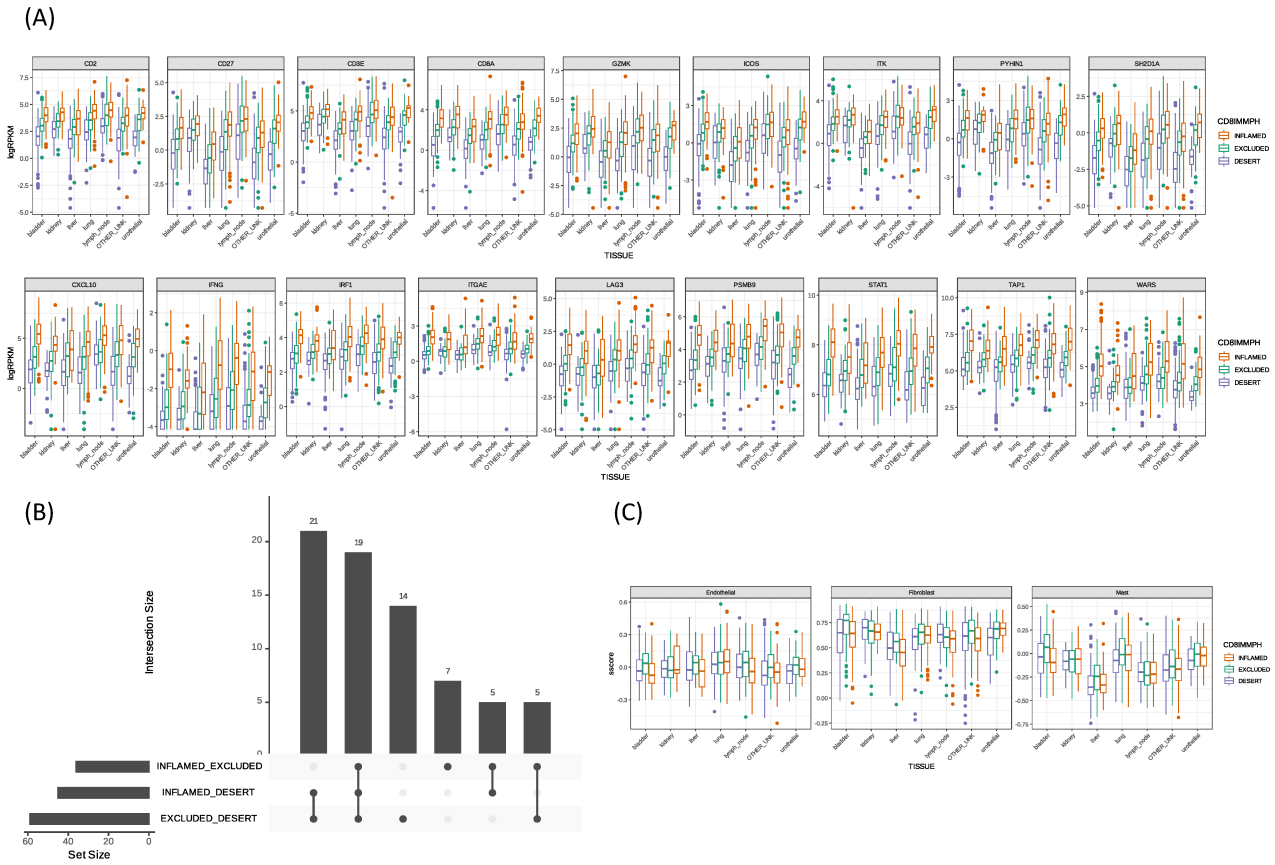



**Figure S3** Systematic characterization of gene expression differences across CD8 immunophenotype classes. (A) Overview of expression distribution for top differentially expressed genes across pairwise comparisons, stratified by excision site. (B) Overlaps between the signatures significantly differentially enriched across all pairwise comparisons. (C) Enrichment (rank biserial correlation) of CD8-excluded associated signatures across CD8 immunophenotype classes and excision sites. CD8IMMPH, CD8 immunophenotype; logRPKM, log Reads Per Kilobase of transcript, per Million mapped reads.

Figure S4

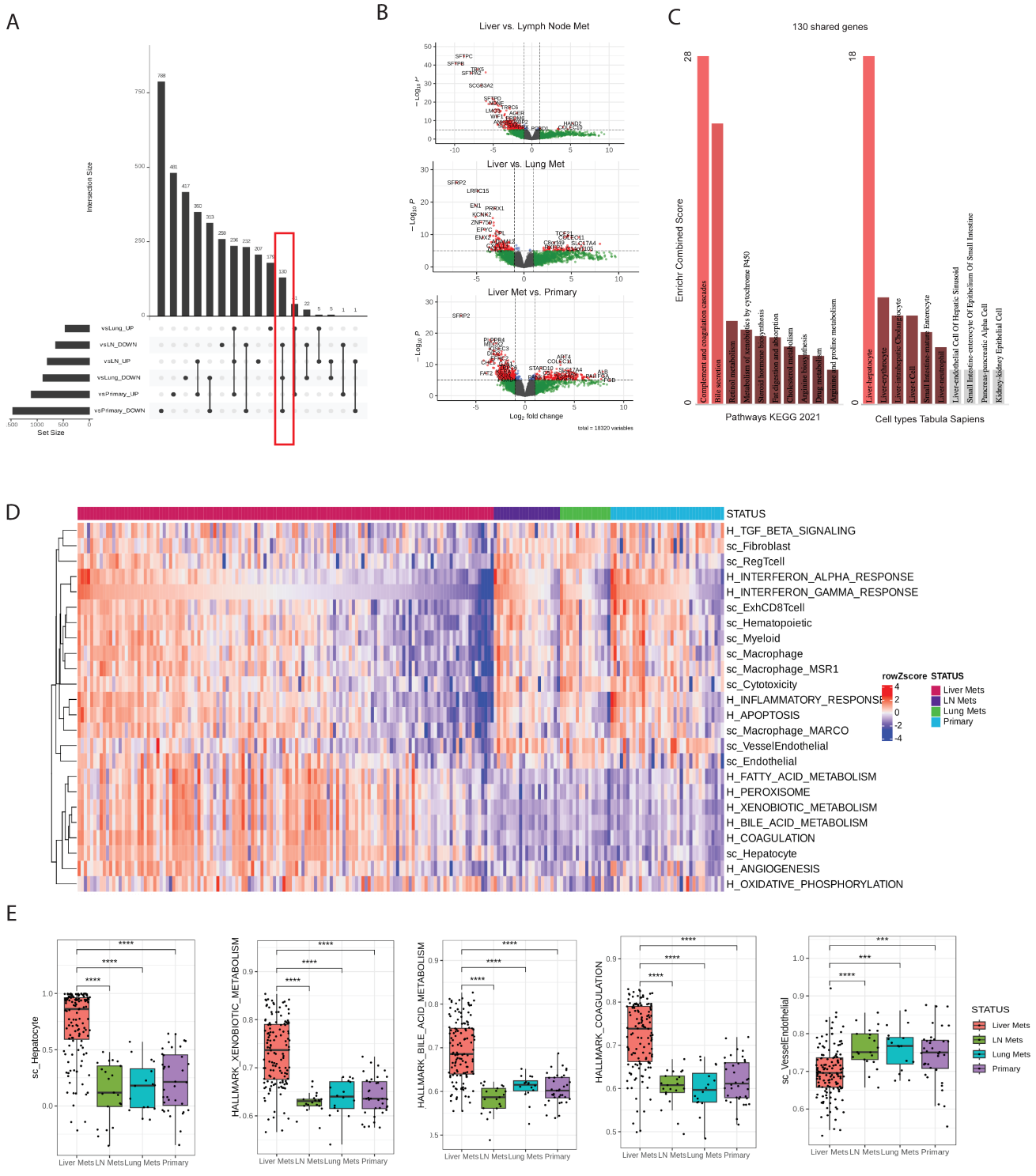



**Figure S4** Systematic characterization of gene expression differences for the CD8-desert phenotype. (A) Overlaps between the genes significantly differentially expressed across all pairwise comparisons. (B) Volcano plots highlighting the most strongly differentially expressed genes for: liver metastases versus lymph node metastases; liver metastases versus lung metastases; and liver metastases versus primary samples. (C) KEGG pathway and “Tabula Sabiens” cell type enrichment analysis results for the genes significantly up-regulated in liver metastases across all three comparisons visualized in (B). (D) Heatmap showing relative signature scores per sample, sorted according to liver metastases, lymph node metastases, lung metastases and primary samples as well as “Hallmark_IFNG” signature scores. (E) Selected liver metastases-enriched signatures scores across liver metastases, lymph node metastases, lung metastases and primary samples. “sc_” represents cell-type specific signatures.

Figure S5

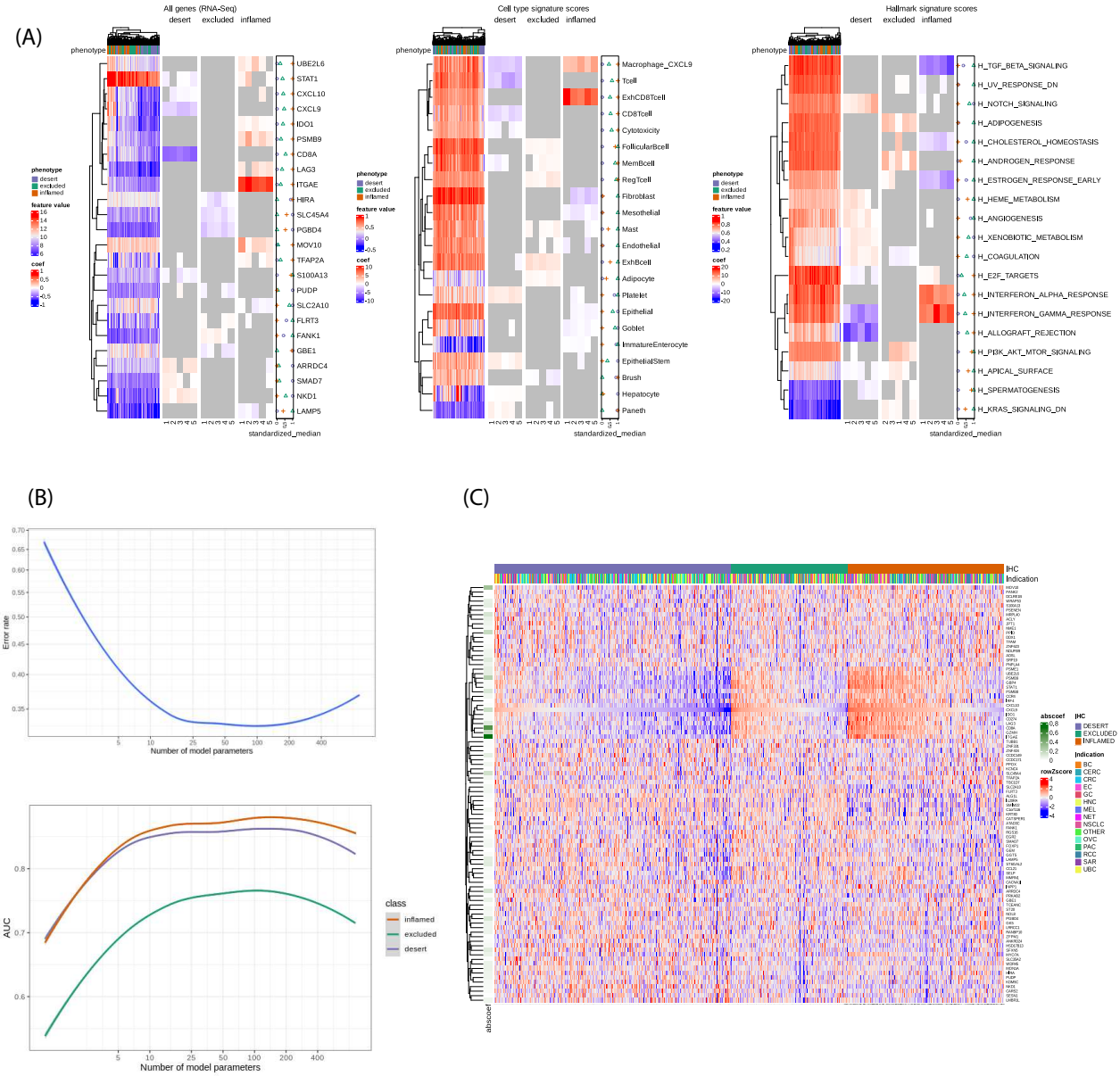
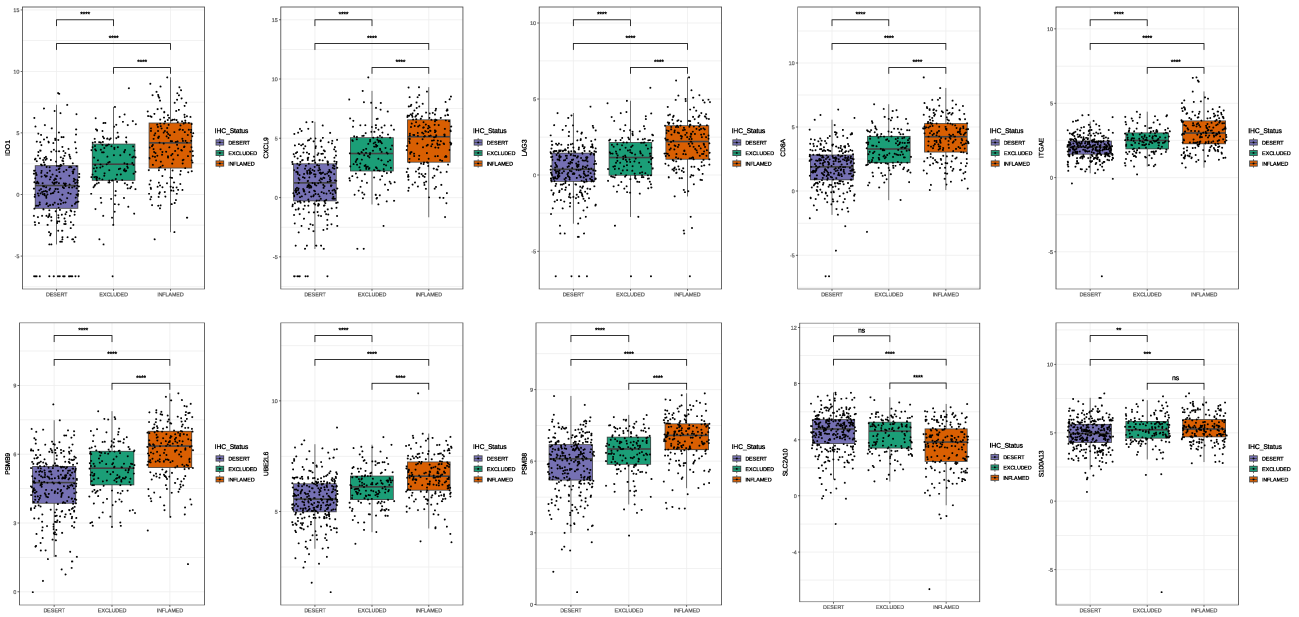



**Figure S5** Development of a transcriptome-based CD8 immunophenotype classifier. (A) Genes and signatures used by distinct transcriptome-based classifiers, trained on (left) all genes, (middle) cell type-specific signature scores and (right) hallmark pathway scores. A 5-fold cross-validation across the training data was used and only features present at least in four iterations are shown. The left section of every tile shows expression among the training samples (columns). The middle section shows the classifier coefficient in every iteration. Gray color indicates that a feature was not used in that iteration. The right section shows relative median expression value among the CD8 immunophenotypes. (B) Performance of the classifier depending on the number of features used, assessed using inner cross-validation. (C) Heatmap showing the relative expression of all (92) classification-relevant genes across CD8 immunophenotype classes, tumor indications and excision sites.

Figure S6A & B

(A)




(B)

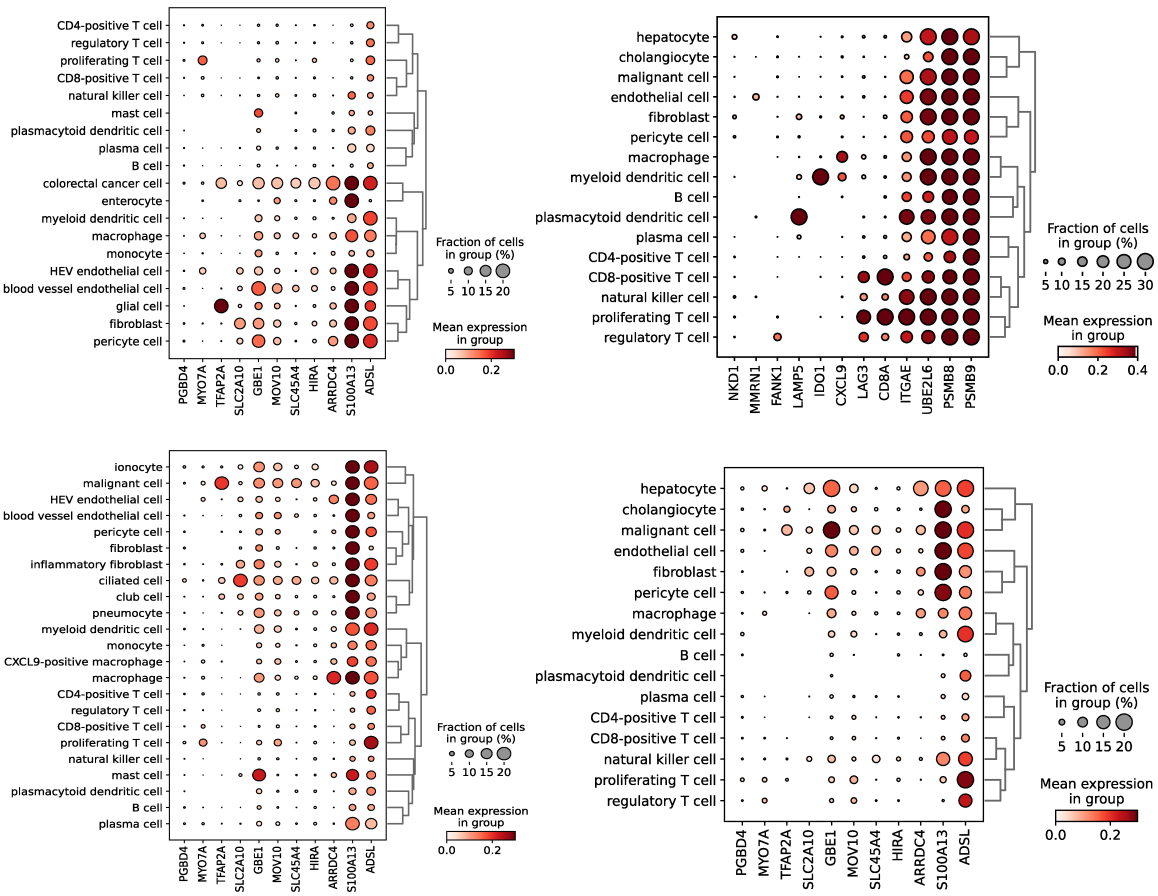
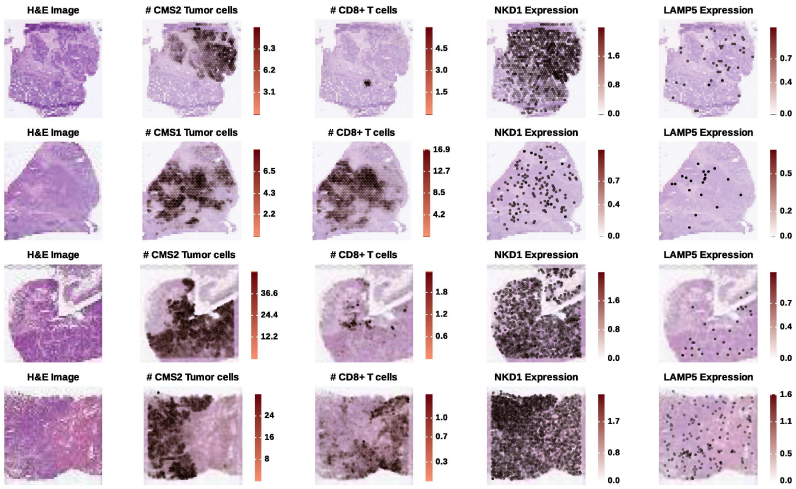


Figure S6C & D

(C)




(D)

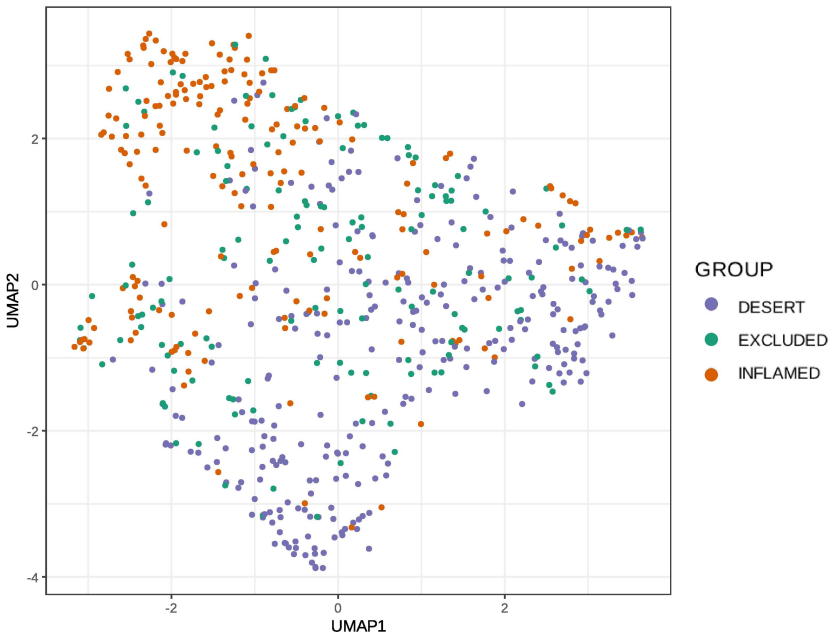


Figure S6E

(E)

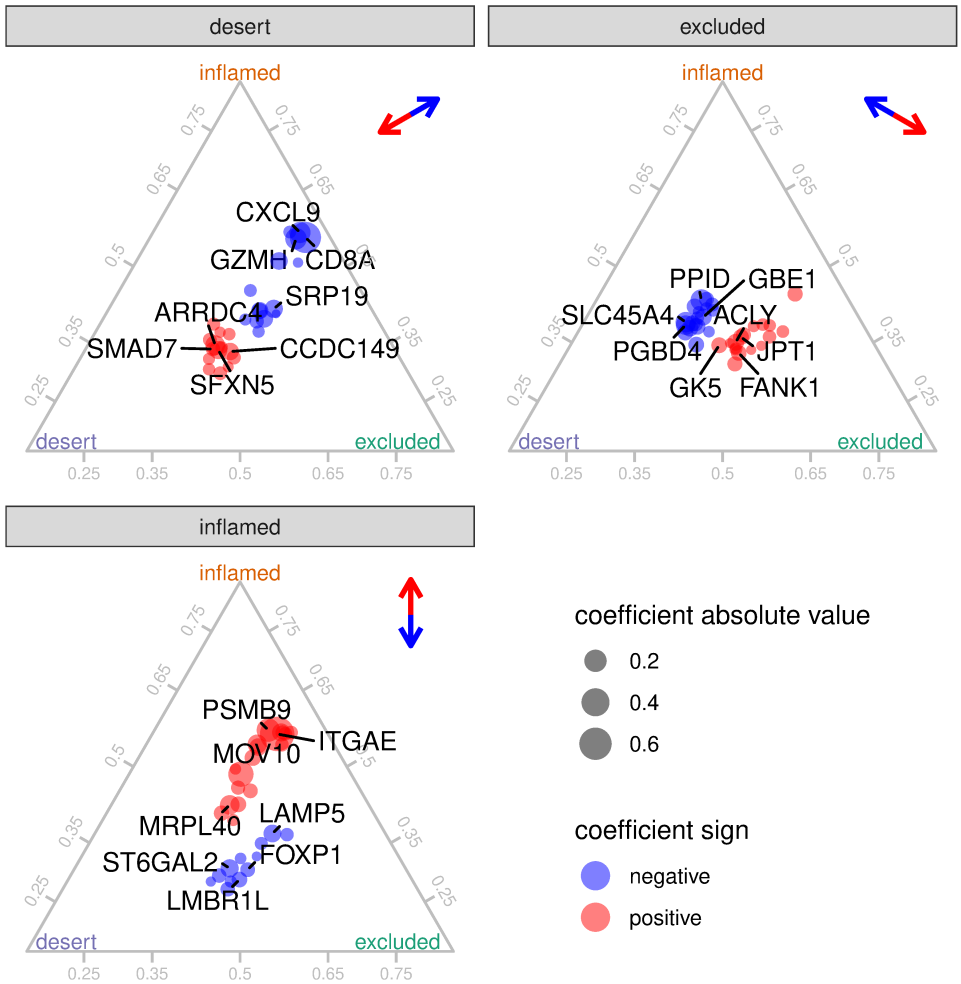
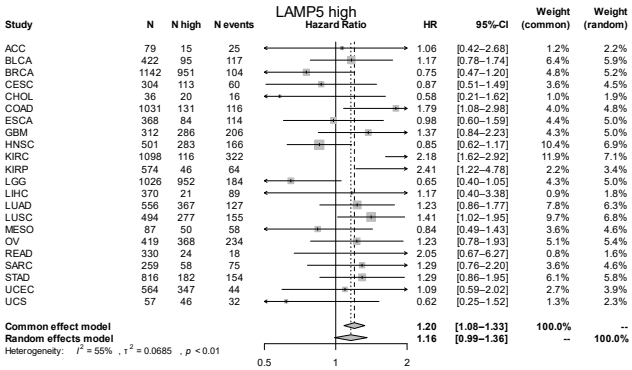


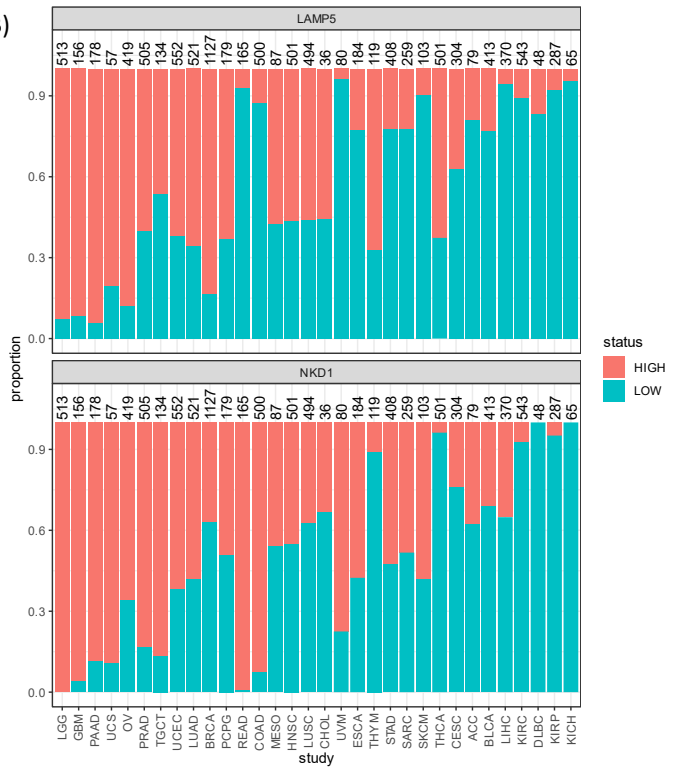

**Figure S6** Characteristics of genes highly contributing to an accurate CD8 immunophenotype classification. (A) Expression of selected CD8-inflammed associated genes in bulk RNA-seq data. (B) Expression of CD8-immunophenotype-enriched genes in colorectal cancer (CRC), lung cancer and liver cancer single-cell RNA-seq data, stratified per cell type. (C) Gene expression maps of *NKD1* and *LAMP5* in selected spatial transcriptomics samples derived from CRC patients. CMS1 tumors are known to present large infiltration of CD8+ T cells, while CMS2 tumors are considered an immune-desert. The bottom sample corresponds to liver metastases of a primary CRC tumor that is shown immediately above it. (D) Samples from the training dataset in a 2D UMAP space generated based on the expression values of all (92) genes selected by the classifier. (E) Coefficients of the final 92-gene classifier. Each panel corresponds to a multinomial model coefficient matrix; i.e., one for inflamed, one for excluded, and one for desert. Each circle corresponds to a non-zero coefficient, circle size corresponding to coefficient absolute value. Circle color corresponds to the sign of coefficient; e.g., a red circle labeled *ITGAE* on the inflamed panel pushes the model into predicting the inflamed phenotype, while *LAMP5* pushes the model against the "inflamed" prediction. Blue-red arrows indicate the direction in which positive (red) and negative (blue) coefficients push the predictions. Top four positive and negative genes are labeled per figure panel. Position on ternary plots corresponds to the quantile of median expression per phenotype in training & validation samples. IHC, immunohistochemistry.

Figure S7

(A)




(B)




(C)

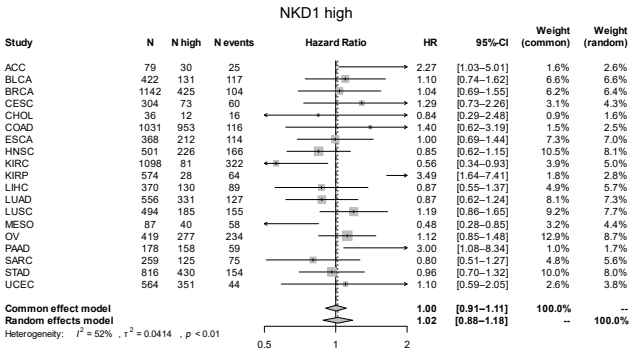



**Figure S7** Survival association of novel genes contributing to CD8 immunophenotype classification. (A) Hazard ratio for mortality in *LAMP5*-high tumors vs all tumors. (B) Status of *LAMP5* and *NKD1* expression in various TCGA indications. (C) Hazard ratio for mortality in *NKD1*-high tumors vs all tumors. All study names and codes can be found on TCGA The Cancer Genome Atlas's portal at https://gdc.cancer.gov/resources-tcga-users/tcga-code-tables.