

Supplementary Figures

HiTIMED: Hierarchical Tumor Immune Microenvironment Epigenetic Deconvolution for accurate cell type resolution in the tumor microenvironment using tumor-type-specific DNA methylation data

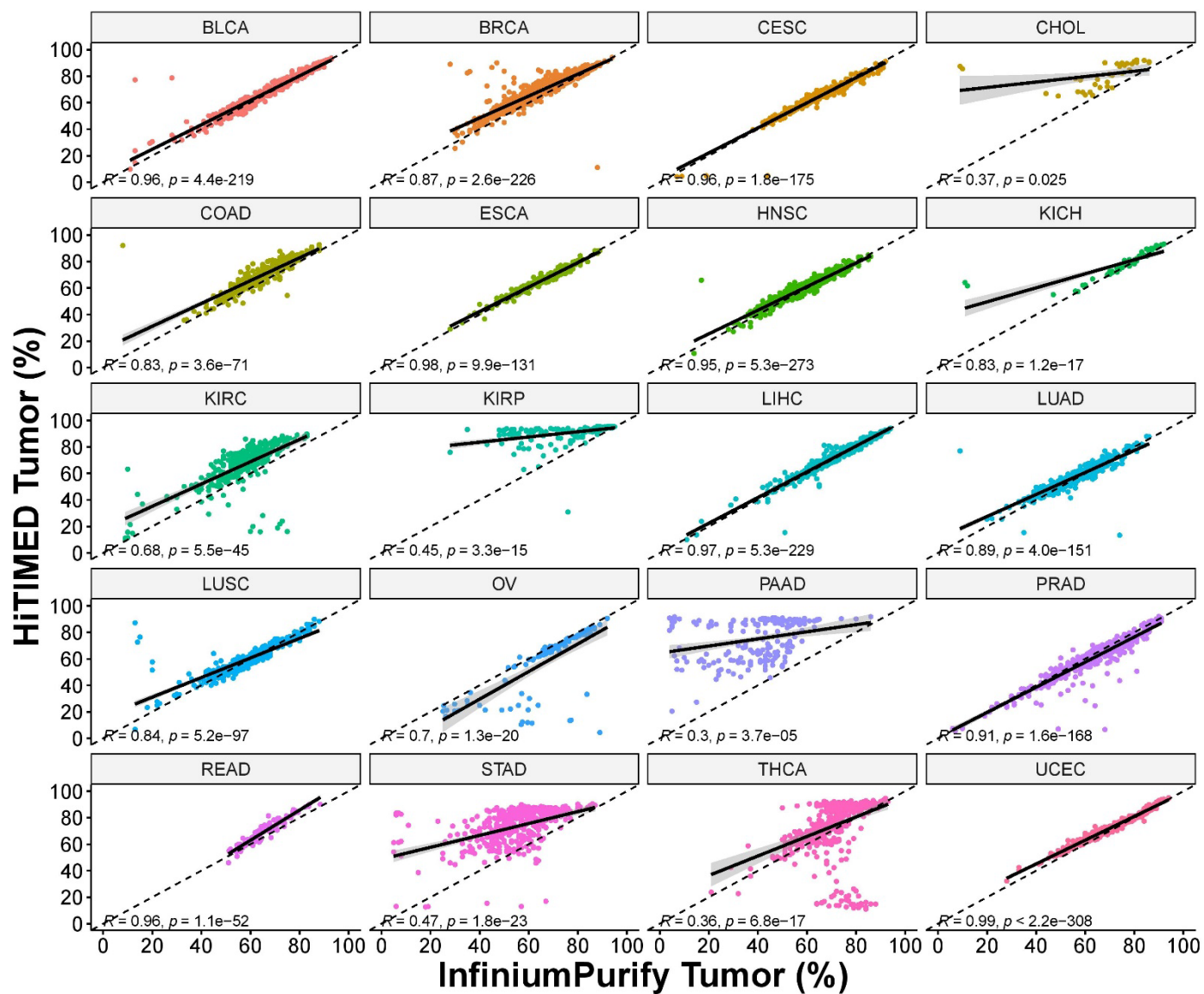
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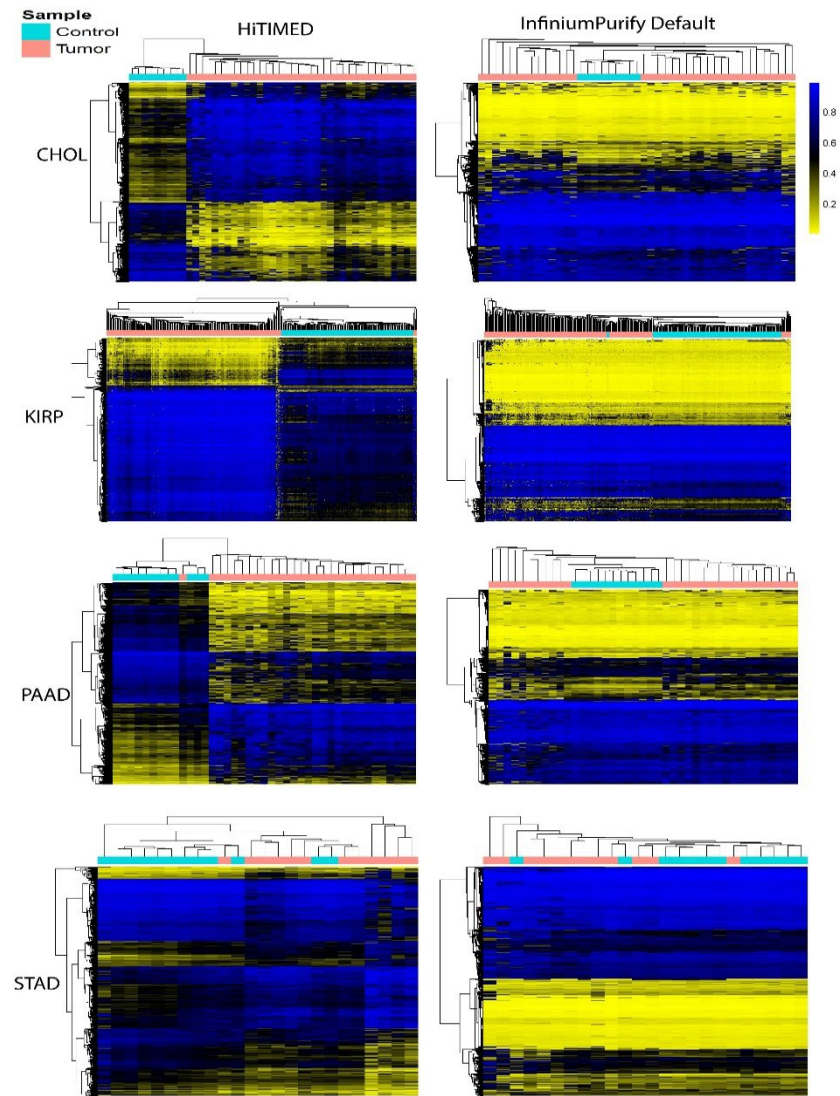
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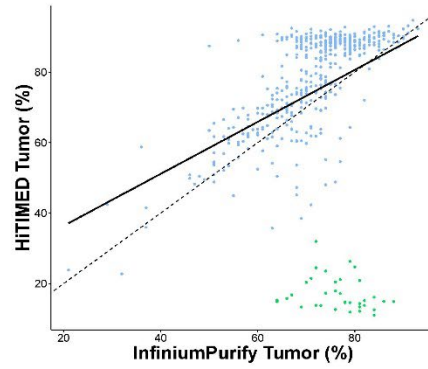
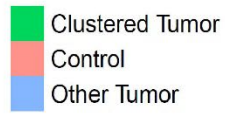


Supplementary Figure 1. Correlation between *HiTIMED* tumor and *InfiniumPurify* tumor by tumor type. *HiTIMED* projected tumor proportions are highly significantly correlated with the *InfiniumPurify* predicted tumor purities across tumor types.

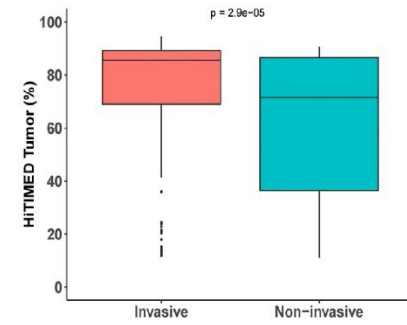
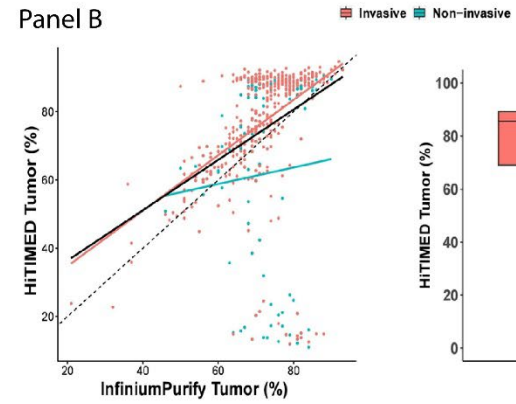


Supplementary Figure 2. Methylation state of CpGs in the *HITIMED* tumor specific library (L1) and *InfiniumPurify* default library between tumor and normal samples across cholangiocarcinoma, kidney papillary cell carcinoma, pancreatic adenocarcinoma, and stomach adenocarcinoma.

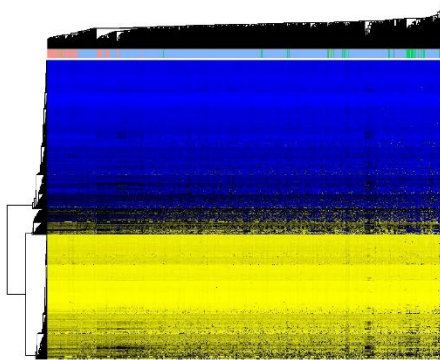
Panel A



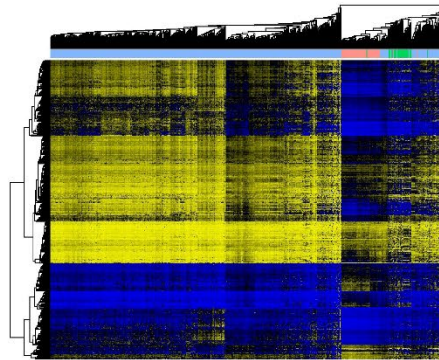
Panel B



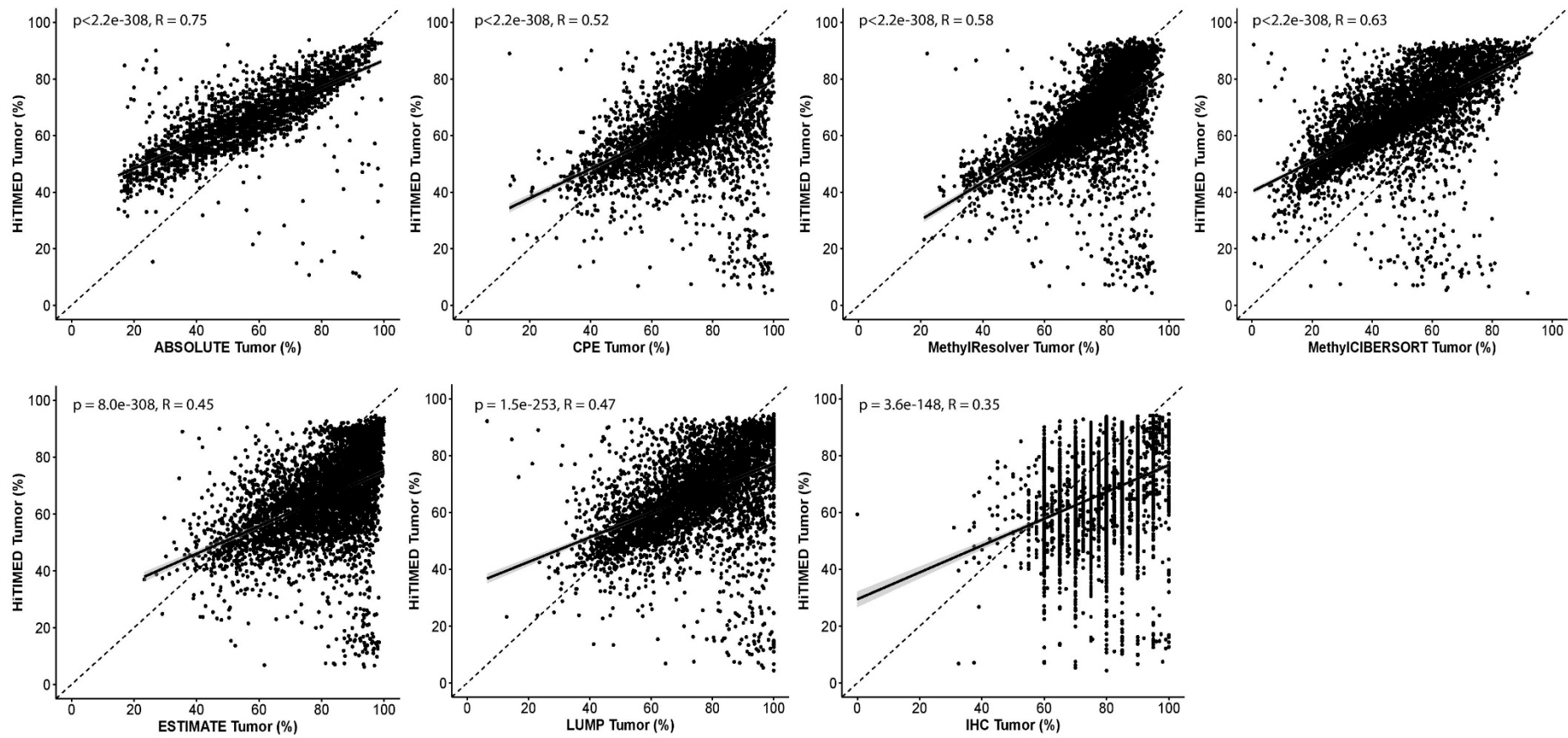
iDMC Default Library



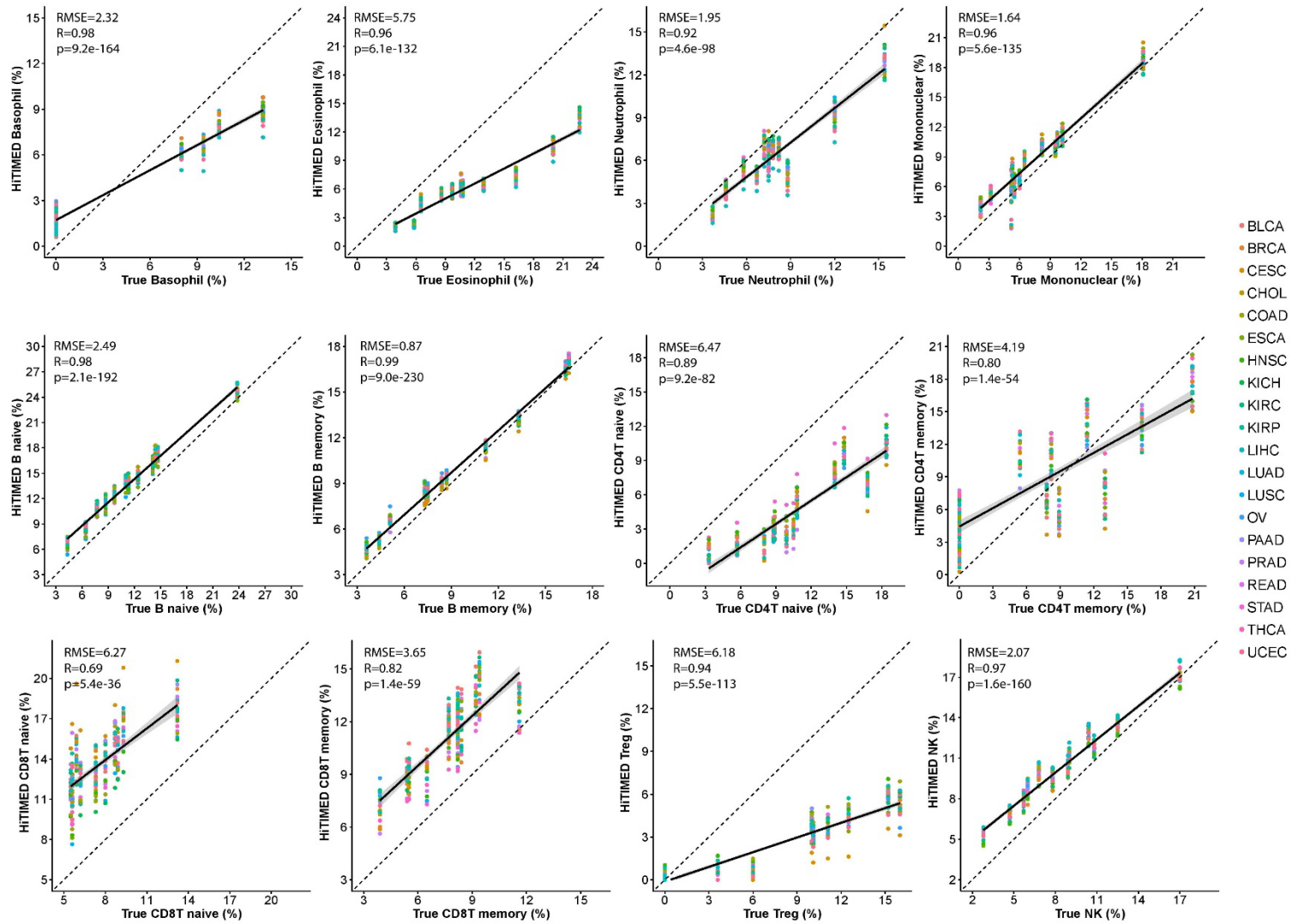
HiTIMED THCA Library



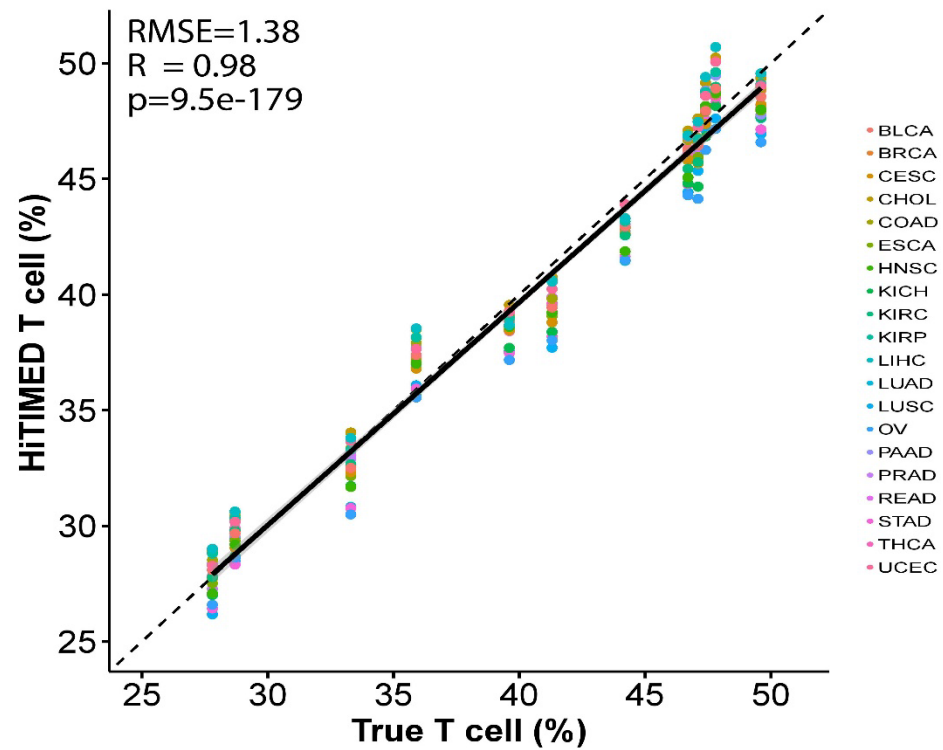
Supplementary Figure 3. *HiTIMED* tumor purity vs *InfiniumPurify* tumor purity in thyroid carcinoma. **Panel A.** A cluster of *HiTIMED*-predicted tumor purity low but *InfiniumPurify*-predicted high tumor was identified and colored in heatmaps. **Panel B.** *HiTIMED* tumor proportion in thyroid carcinoma colored by invasive and non-invasive tumor type.



Supplementary Figure 4. *HiTIMED* tumor proportion vs other method predicted tumor proportion.

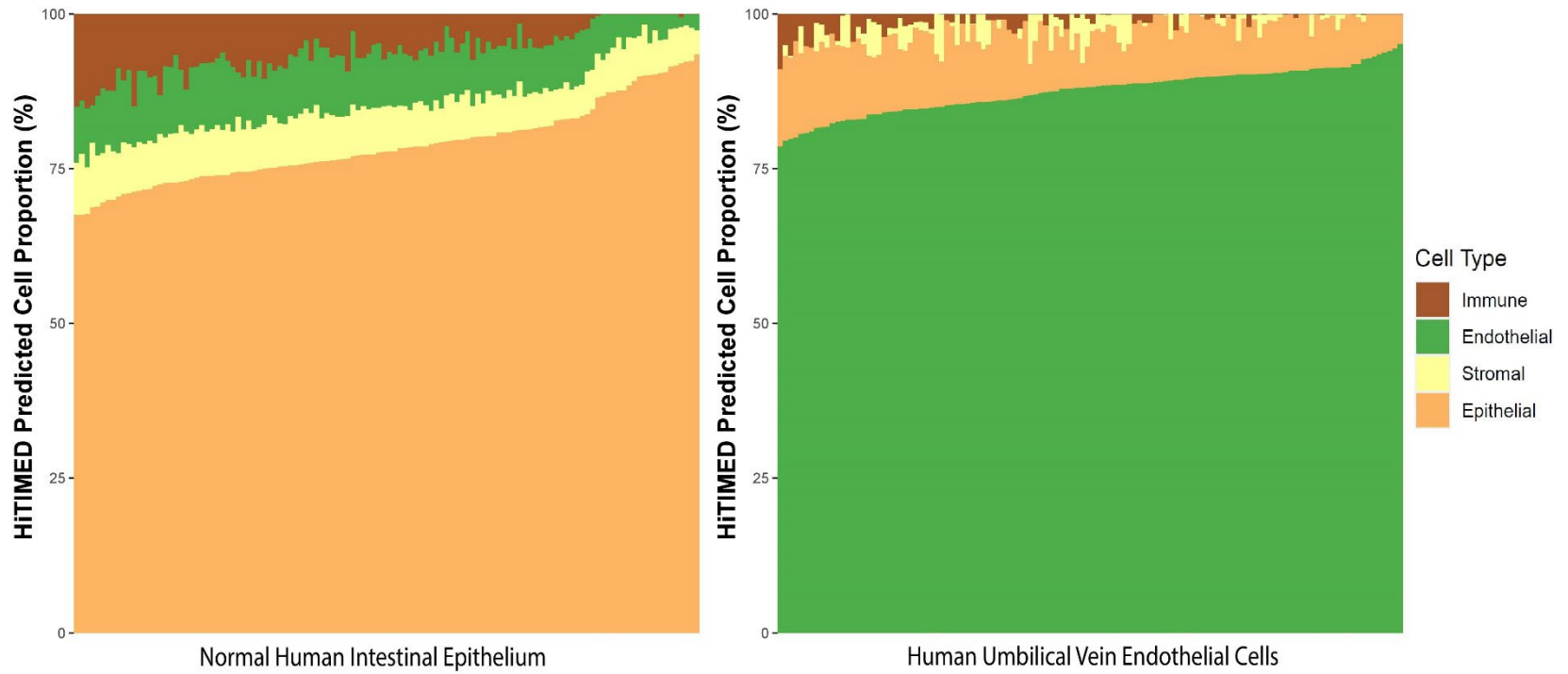


Supplementary Figure 5. *HiTImED* immune cell proportions vs true immune cell proportions in artificial mixtures.



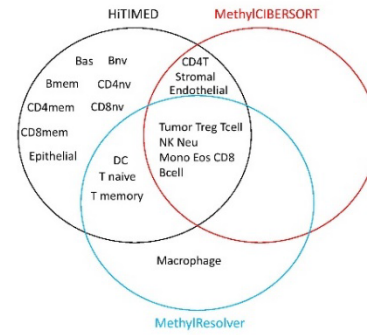
Supplementary Figure 6. *HiTIMED* T cell proportion vs true T cell proportion in artificial mixtures.

Note: T cell proportions correspond to the sum of CD4T naive and memory, CD8 naive and memory and T regulatory cells.

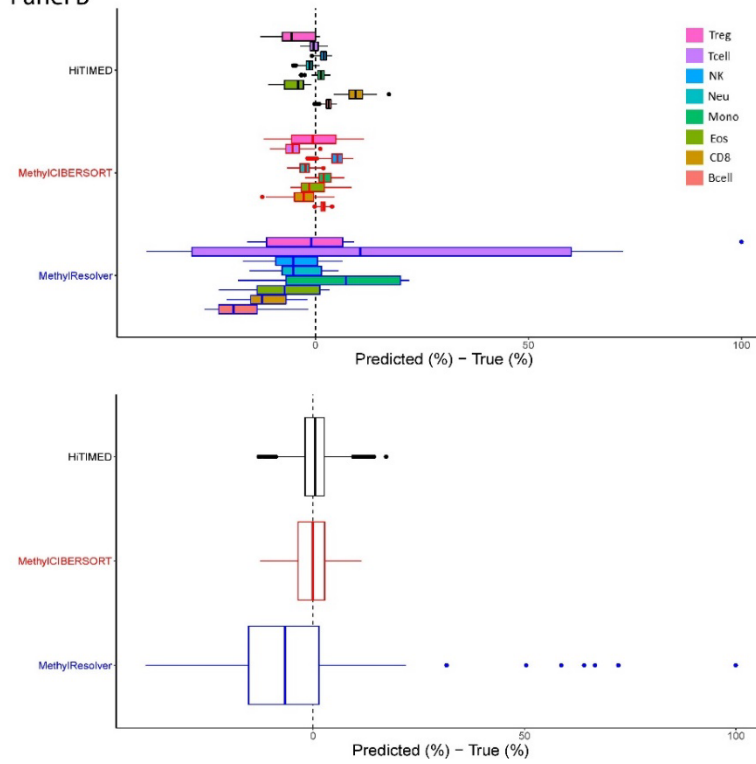


Supplementary Figure 7. *HiTIMED* cell composition in human normal intestinal epithelium and umbilical vein endothelial cells.

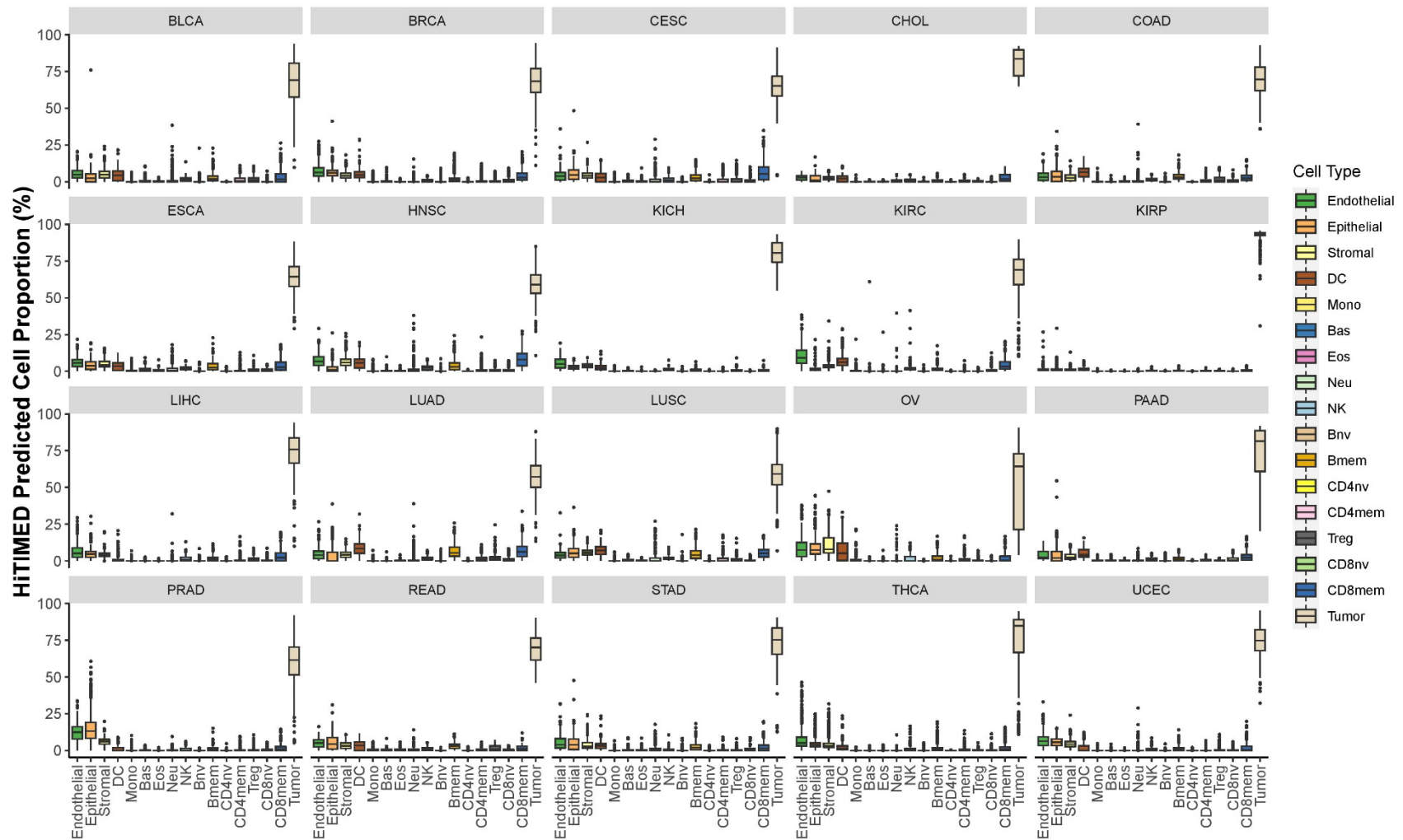
Panel A



Panel B

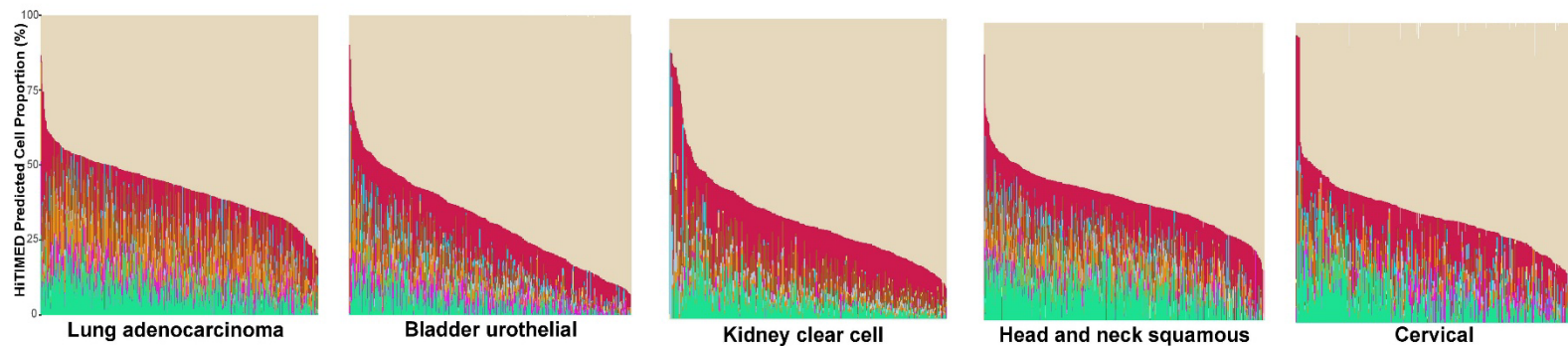


Supplementary Figure 8. Performance comparison across *HiTIMED*, *MethylCIBERSORT*, and *MethylResolver* using artificial mixtures.



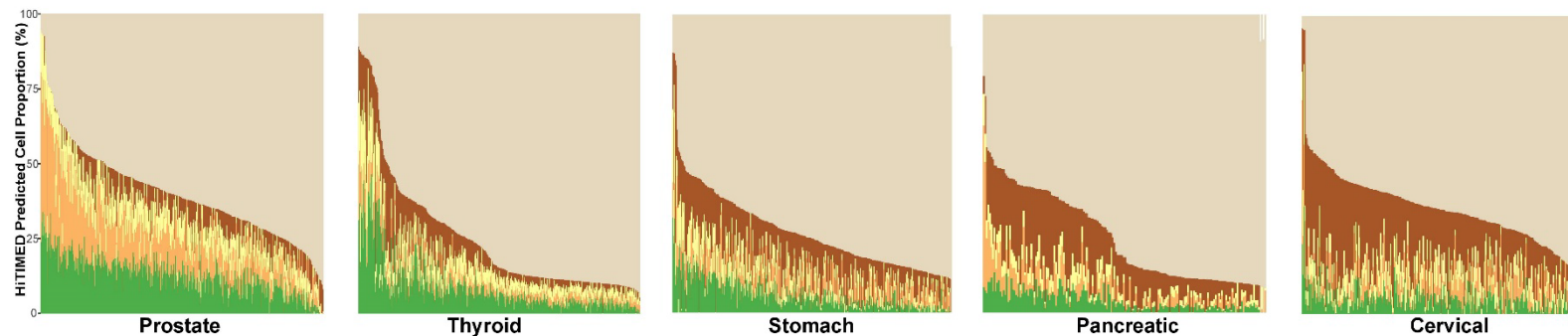
Supplementary Figure 9. The distribution of the *HiTIMED* cell composition in TCGA tumors.

Panel A



Tumor type	HITMED-projected immune variance
Lung adenocarcinoma	156.83
Bladder urothelial	152.33
Kidney clear cell	147.83
Head and neck squamous	134.17
Cervical	118.69
Lung squamous	96.94
Thyroid	93.45
Colon	91.15
Liver hepatocellular	91.07
Breast invasive	90.86

Panel B

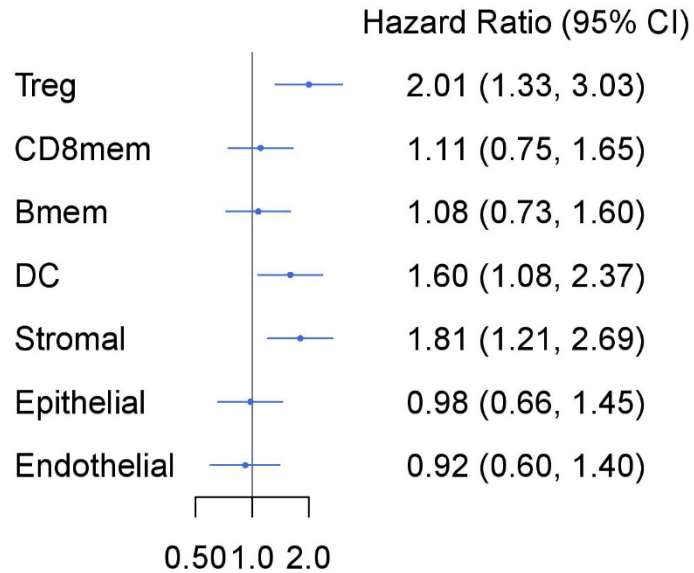


Tumor type	HITMED-projected angiogenic variance
Prostate	284.73
Thyroid	208.77
Stomach	112.98
Pancreatic	105.84
Cervical	89.01
Lung adenocarcinoma	88.33
Liver hepatocellular	79.43
Kidney clear cell	79.15
Breast invasive	73.19
Endometrial	66.68

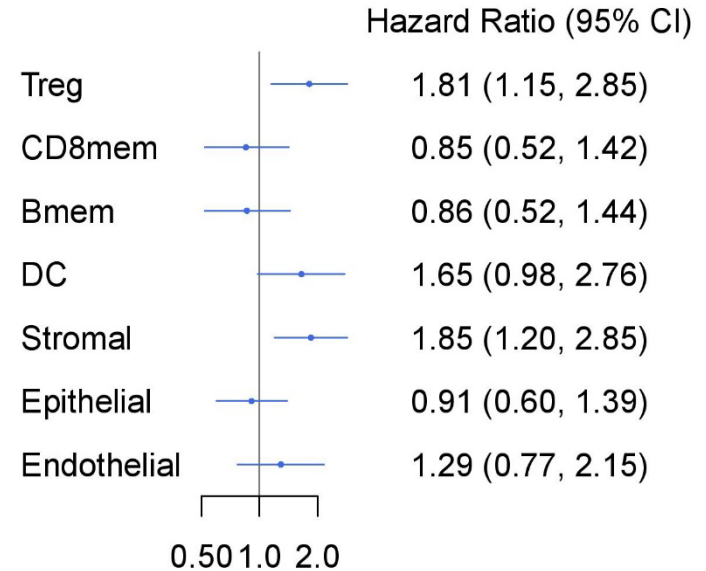
Supplementary Figure 10. Cell composition differs substantially and captures sample heterogeneity using *HiTIMED*-projected proportions. Seventeen cell types were captured for each sample by tumor type.

Kidney clear cell carcinoma

Cox model with age, gender, tumor stage adjusted

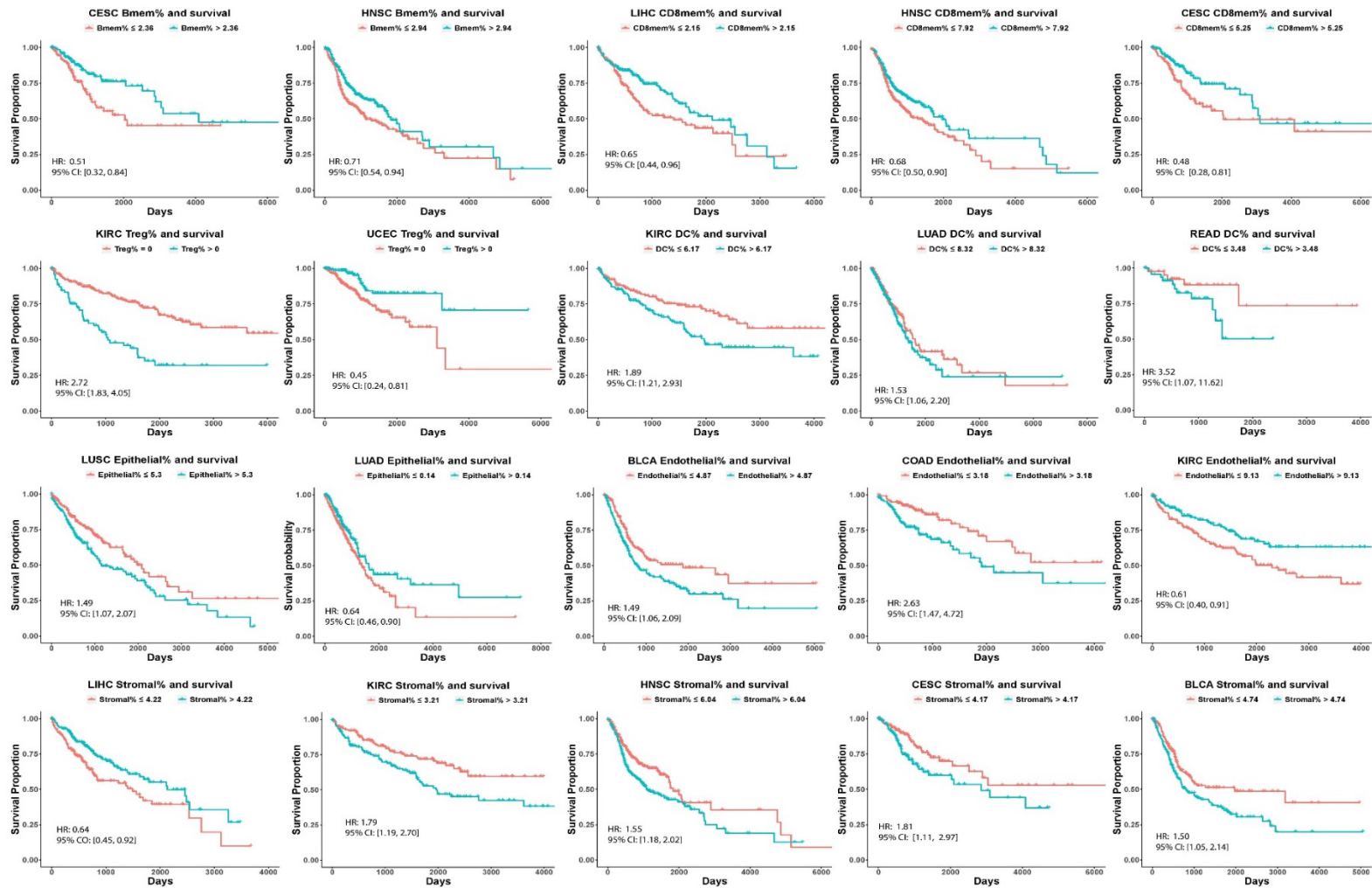


Cox model with age, gender, tumor stage, and HiTIMED-projected cell proportions¹ adjusted

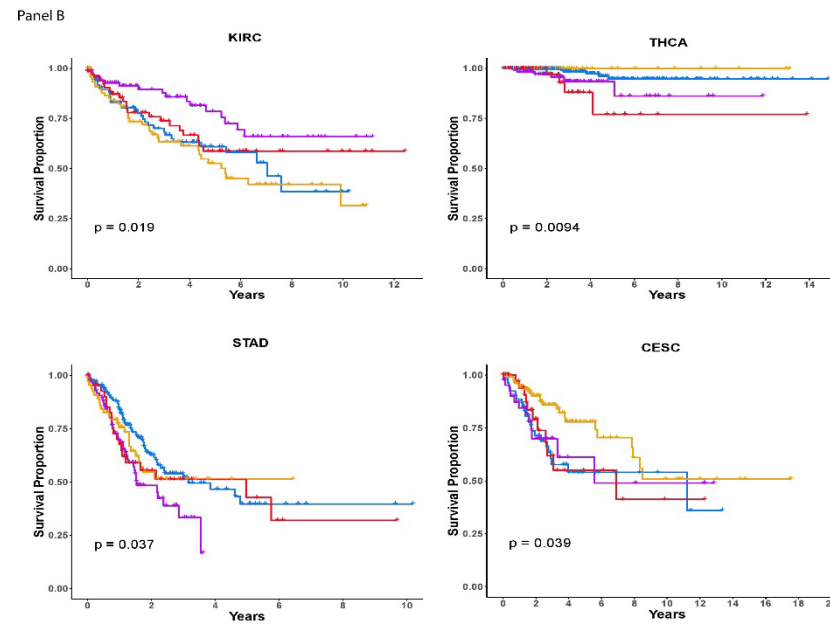
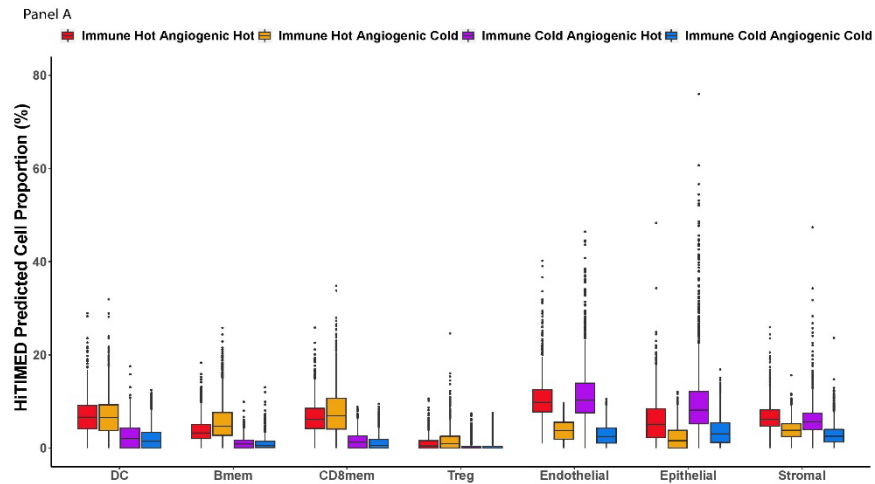


¹Cell types include tumor, Treg, CD8mem, Bmem, DC, stromal, epithelial, endothelial

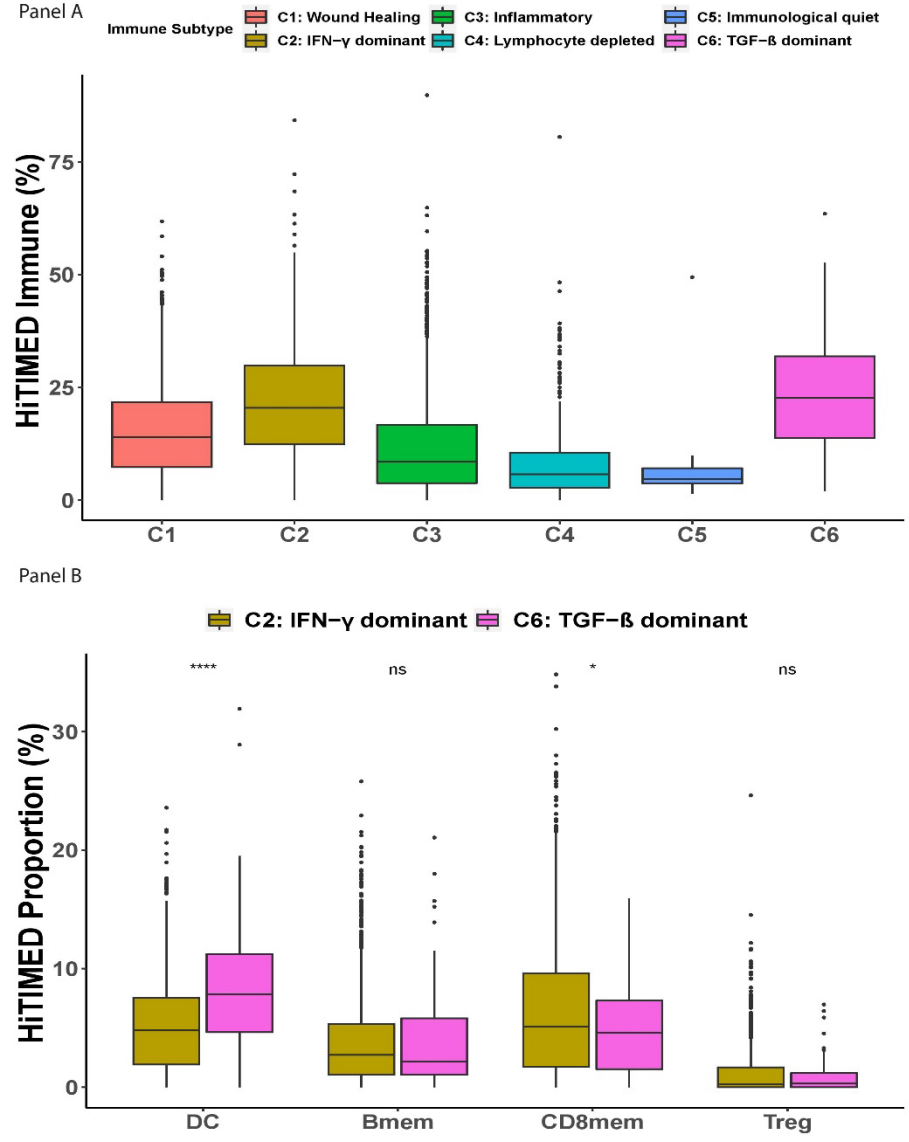
Supplementary Figure 11. Sensitive analysis comparing outputs from two Cox models with or without cell type proportions adjusted in kidney clear cell carcinoma.



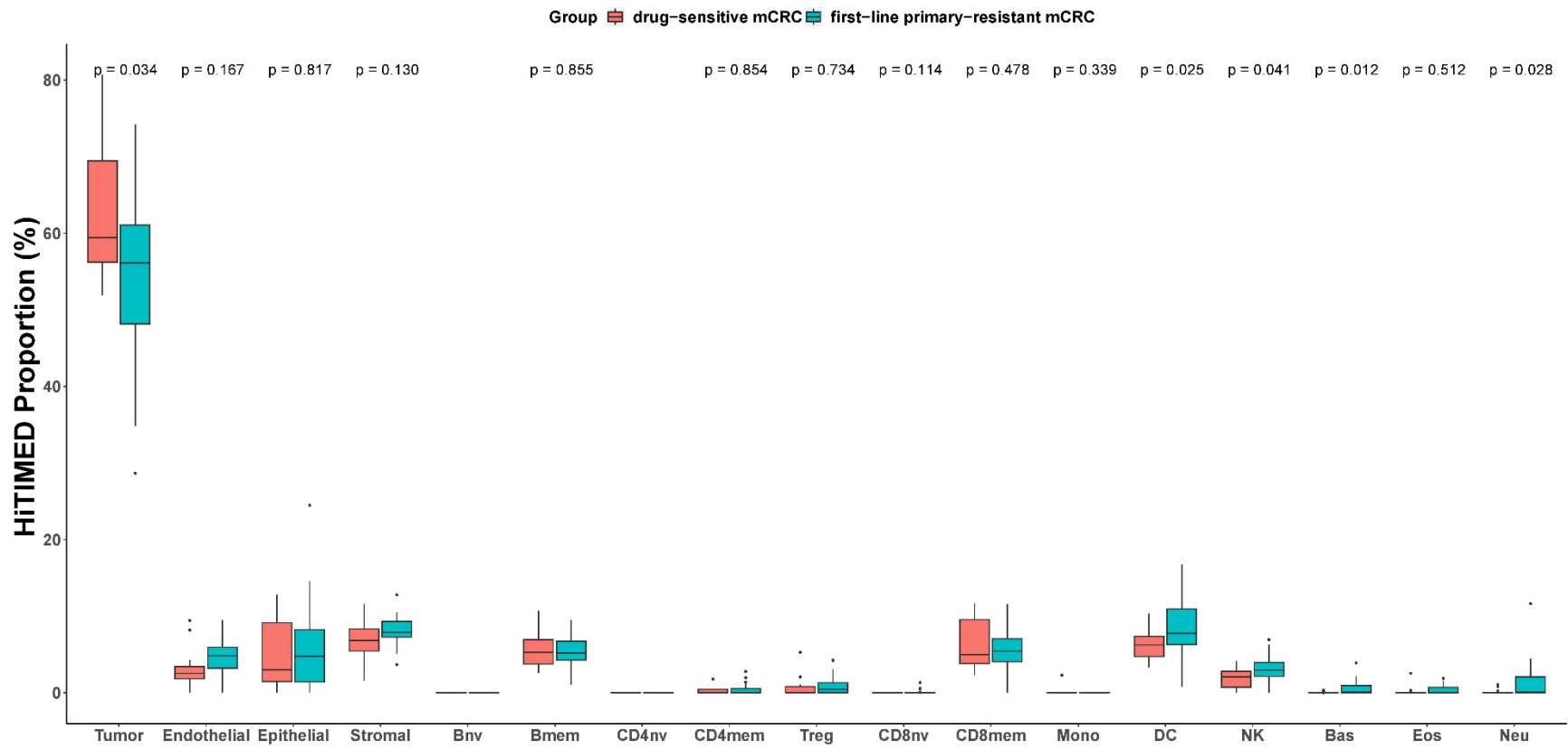
Supplementary Figure 12. Kaplan-Meier survival curves for *HITIMED* cells estimates in TCGA tumors. Hazard ratios were calculated from the Cox proportional hazard models with age, gender, and tumor proportion adjusted (gender was not adjusted for gender-specific tumors).



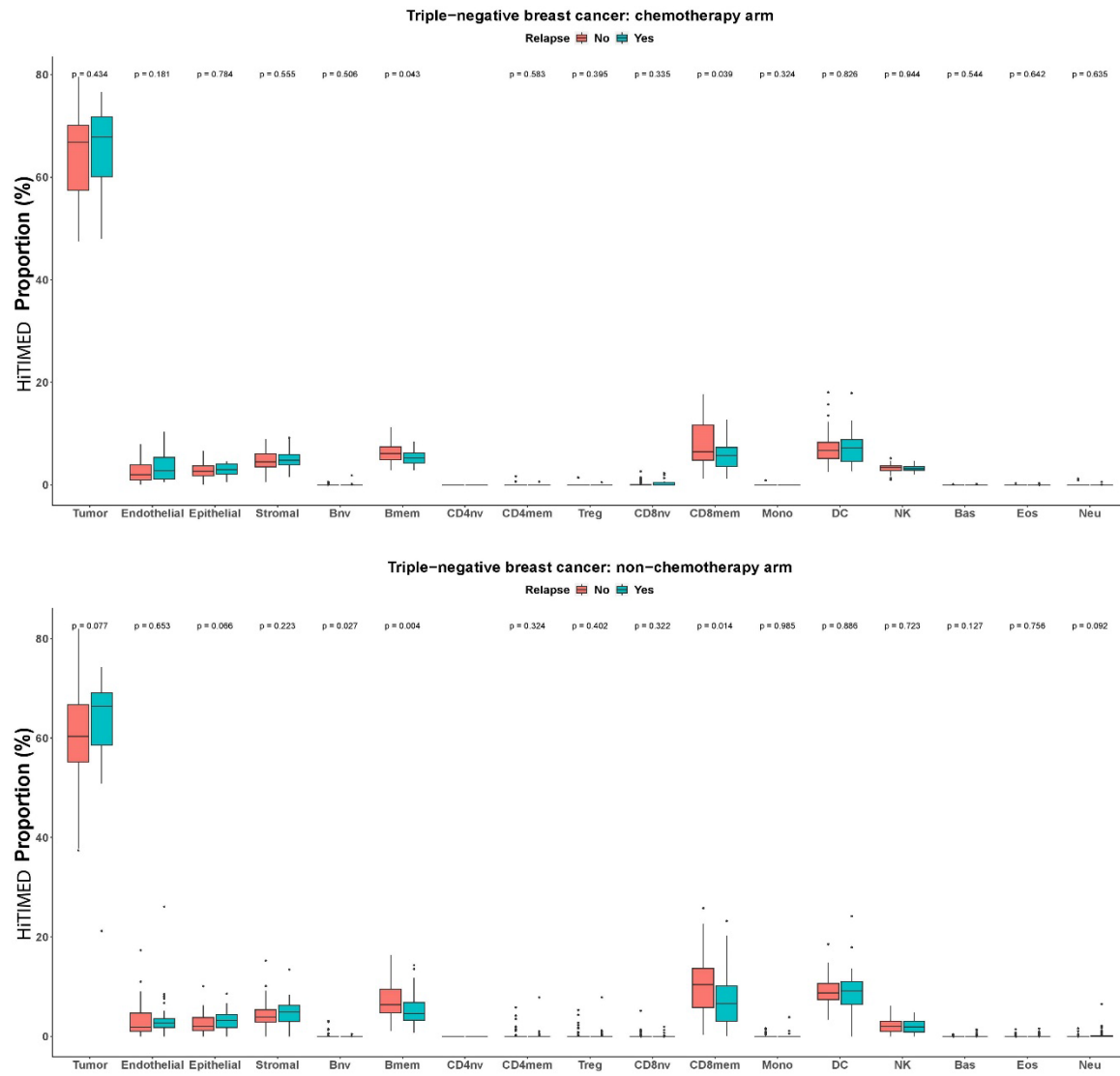
Supplementary Figure 13. Panel A. *HITMED* cell comparison and **Panel B.** Kaplan-Meier survival curves across immune/angiogenic hot and cold tumors. P-values were calculated from the log-rank tests.



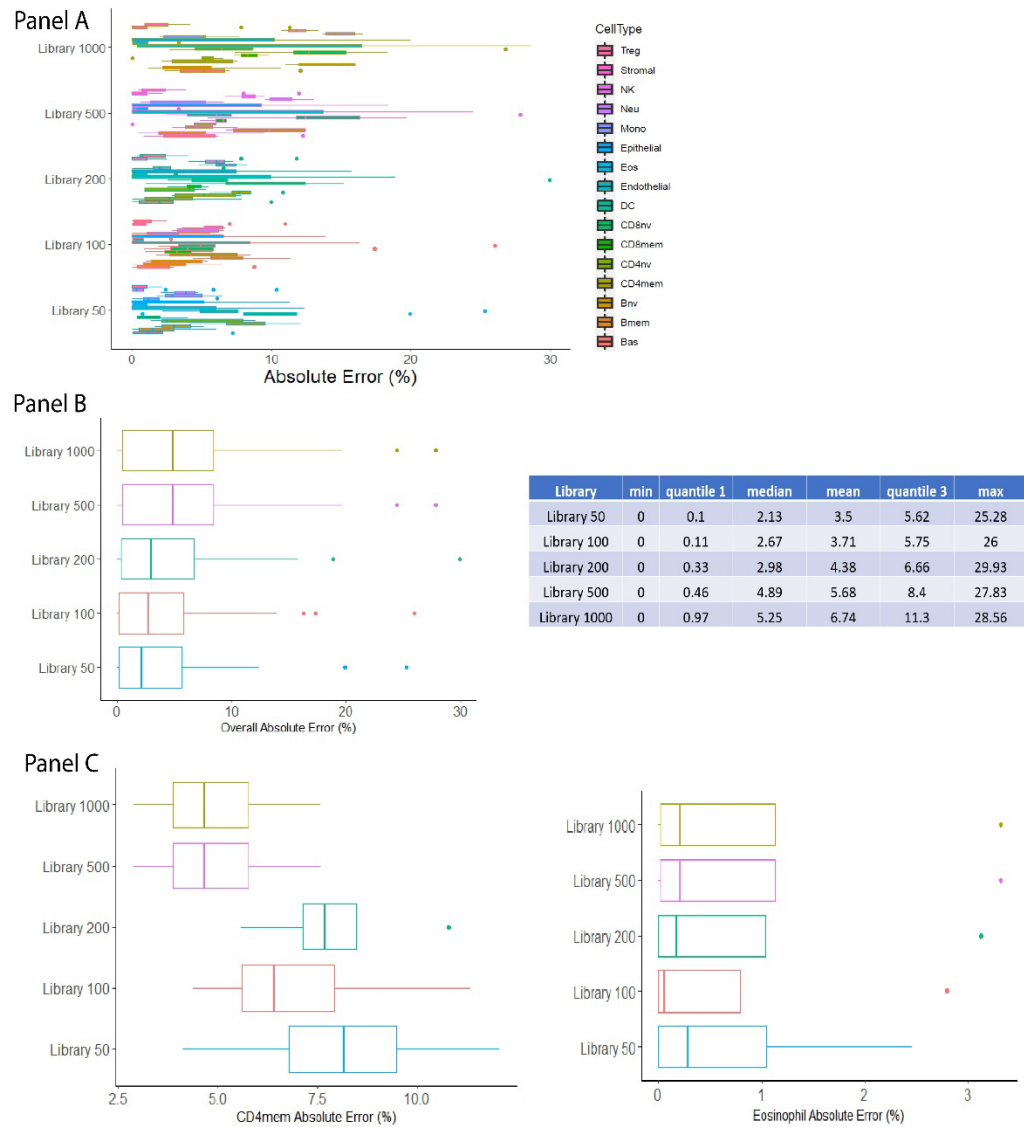
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