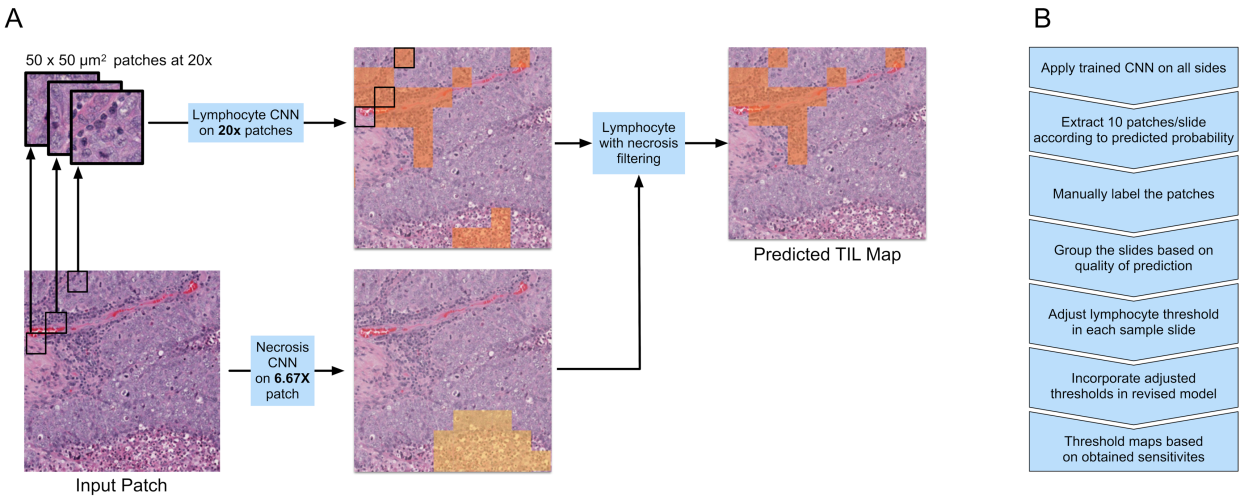
Cell Reports, Volume *23*

Supplemental Information

Spatial Organization and Molecular Correlation of Tumor-Infiltrating Lymphocytes Using Deep Learning on Pathology Images

Joel Saltz, Rajarsi Gupta, Le Hou, Tahsin Kurc, Pankaj Singh, Vu Nguyen, Dimitris Samaras, Kenneth R. Shroyer, Tianhao Zhao, Rebecca Batiste, John Van Arnam, The Cancer Genome Atlas Research Network, Ilya Shmulevich, Arvind U.K. Rao, Alexander J. Lazar, Ashish Sharma, and Vésteinn Thorsson


A
50 x 50 μm² patches at 20x
Lymphocyte CNN on 20x patches
Lymphocyte with necrosis filtering
Predicted TIL Map
Necrosis CNN on 6.67X patch
Input Patch
B
Apply trained CNN on all sides
Extract 10 patches/slide according to predicted probability
Manually label the patches
Group the slides based on quality of prediction
Adjust lymphocyte threshold in each sample slide
Incorporate adjusted thresholds in revised model
Threshold maps based on obtained sensitivites

C

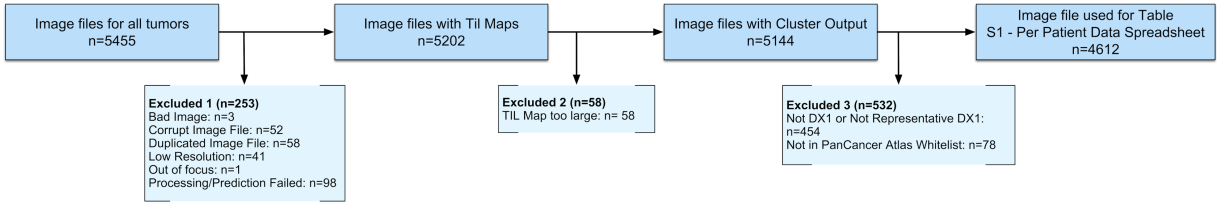


Image files for all tumors n=5455
Image files with Til Maps n=5202
Image files with Cluster Output n=5144
Image file used for Table S1 - Per Patient Data Spreadsheet n=4612
Excluded 1 (n=253)
Bad Image: n=3
Corrupt Image File: n=52
Duplicated Image File: n=58
Low Resolution: n=41
Out of focus: n=1
Processing/Prediction Failed: n=98
Excluded 2 (n=58)
TIL Map too large: n= 58
Excluded 3 (n=532)
Not DX1 or Not Representative DX1: n=454
Not in PanCancer Atlas Whitelist: n=78

Figure S1; Related to Figure 1. Details of Slide Processing (A) Fusion of Lymphocyte and Necrosis CNN. A large input H&E patch (lower left in figure) is processed to yield a TIL map, shown in upper right. The lymphocyte CNN (upper left) first takes smaller patches of 50x50 microns within the large patch at 20X magnification and predicts if those patches are lymphocyte-infiltrated and displays predictions as a "heatmap", superimposed on the H&E image (upper middle, TIL positive patches shown in dark orange). The necrosis CNN (lower left and lower middle) takes the larger region with more contextual information to predict if patches are mostly necrotic (shown in light orange). The two results are combined (upper right) for the final tumor infiltration lymphocyte prediction (TIL-positive patches in dark orange). **(B) Determination of Lymphocyte Selection Thresholds**. In this phase, our quality control step picks out slides that have significantly over- or under-predicted lymphocytes and adjusts the lymphocyte sensitivities and necrosis specificities for these slides.**(C) Flowchart of slide image processing in the project.** A summary of three main steps in processing of image files. At each step, a set of slides is excluded from further processing, as follows *Excluded 1: Reasons no TIL Map was generated* Corrupt Image File: either the image file is corrupted, unable to be read, or the image only contains a small portion of the whole slide; Low Resolution: The image does not have enough high resolution (of at least 20X) to be processed by the CNN model; Out of focus: The image is out of focus; Bad Image File: The image is either captured with bad quality, or marked by markers; Processing/Prediction Failed: Either the pipeline failed processing those slides because of malfunctions such as process being killed in the middle of the process, or the lymphocyte predictions are not good (i.e., a visual inspection of the images showed too many incorrectly labeled patches -- results for some of the images, for example, had a high false positive rate due to the cytology of the tumor cells that closely resembled lymphocytes); Duplicated Image File: there is another image file corresponding to the same diagnostic slide barcode *Excluded 2 , Reasons no Cluster File was Generated* In clustering indices process, some of the slides have too many TIL patches. As a result, either the clustering indices algorithm cannot fit them into memory to process or it may take too long to finish clustering those slides. Those slides do not yield cluster file results. *Excluded 3, Exclusions to Create Final List of Single Slide per Participant* For each participant we choose a single slide where multiple slides are available, as follows we choose only the slide containing label DX1 (not DX2,DX3,..). In 15 cases there were two DX1 slides for each patient, and one was slide chosen by random sampling. Finally, only slides from TCGA participants with data included in the PanCancer Atlas cohort (the PanCancer Atlas whitelist) are retained for final integrative analysis work.

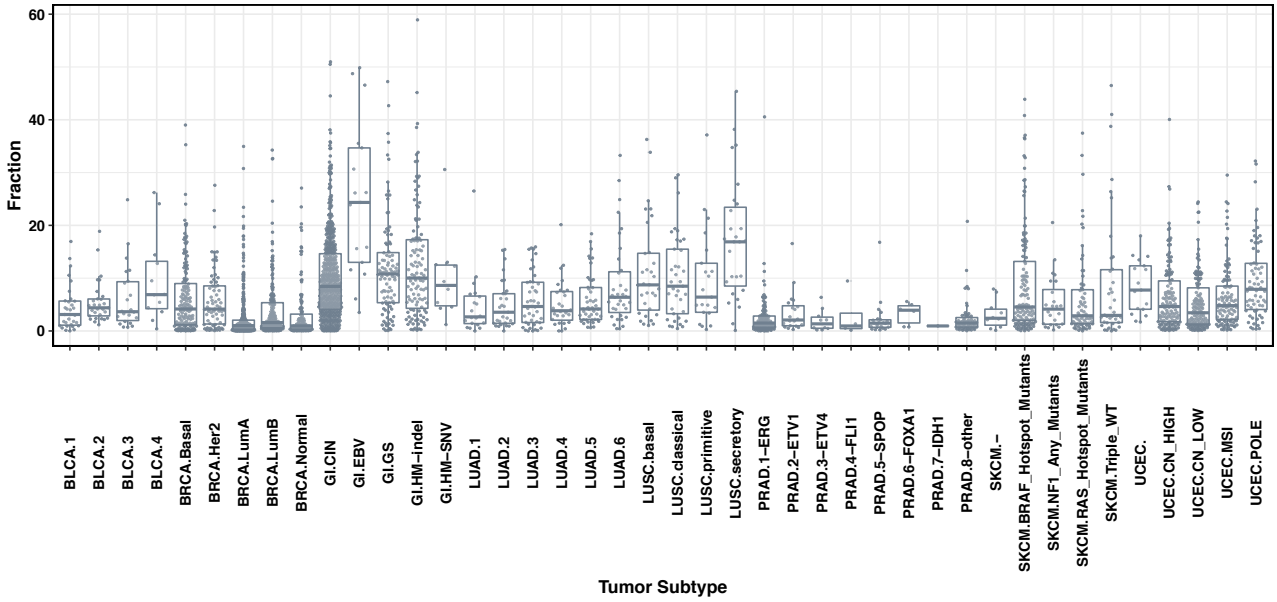

Fraction
60
40
20
0
BLCA.1
BLCA.2
BLCA.3
BLCA.4
BRCA.Basal
BRCA.Her2
BRCA.LumA
BRCA.LumB
BRCA.Normal
GI.CIN
GI.EBV
GI.GS
GI.HM−indel
GI.HM−SNV
LUAD.1
LUAD.2
LUAD.3
LUAD.4
LUAD.5
LUAD.6
LUSC.basal
LUSC.classical
LUSC.primitive
LUSC.secretory
PRAD.1−ERG
PRAD.2−ETV1
PRAD.3−ETV4
PRAD.4−FLI1
PRAD.5−SPOP
PRAD.6−FOXA1
PRAD.7−IDH1
PRAD.8−other
SKCM.−
SKCM.BRAF_Hotspot_Mutants
SKCM.NF1_Any_Mutants
SKCM.RAS_Hotspot_Mutants
SKCM.Triple_WT
UCEC.
UCEC.CN_HIGH
UCEC.CN_LOW
UCEC.MSI
UCEC.POLE
Tumor Subtype

Figure S2; Related to Figure 3. TIL Fraction by Tumor Category (A). Percent TIL fraction, the proportion of TIL-positive patches within a TIL map, is shown for TCGA molecular subtypes.

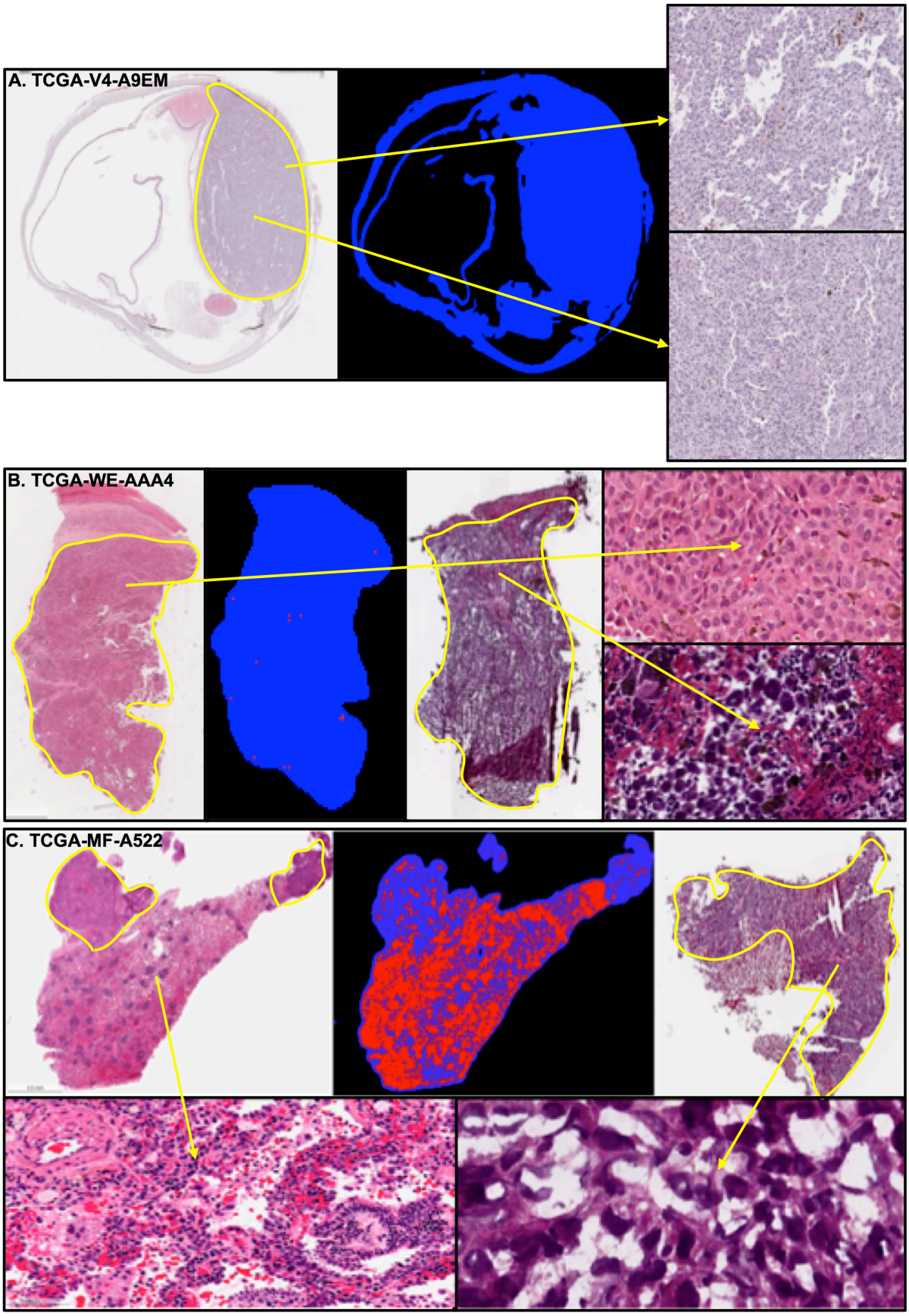

A. TCGA-V4-A9EM
B. TCGA-WE-AAA4
C. TCGA-MF-A522

Figure S3; Related to Figure 4. Examples of the Negative Control and Discordant Results between Molecular and Image-derived Analyses for TIL estimates (A) The uveal melanoma cases, exemplified here by TCGA-V4-A9EM, served as our group of negative controls for the lymphocyte-detection algorithm, as this tumor type is not generally associated with notable immune infiltrates. After the initial assessment of the H&E image (left) and corresponding TIL map (center), we performed additional histopathologic evaluation to confirm the absence of lymphocytes (right), showing the representative sections at higher magnification. The uveal melanoma cases show how the lymphocyte-detection algorithm correctly distinguishes tumor cells and aggregates of melanotic pigment by not labelling them as lymphocytes in a very challenging and complex scenario.**(B)** An example representing the discordant results between the high molecular and low image-derived TIL estimates where the H&E of the FFPE is virtually devoid of lymphocytes, whereas the frozen section has mild to patchy moderate level of lymphocytes. Images are presented in the following sequence from left to right in the left panel: H&E diagnostic image at low-magnification, TIL map, and the fresh frozen section for molecular genomics assays at low magnification. For TIL maps: red represents a positive TIL patch; blue represents a tissue region with no TIL patch; black represents no tissue. Right panels: Representative sections at higher magnification. **(C)** An example of the discordance of low molecular and high image-derived TIL estimates where the H&E of the FFPE contains numerous lymphocytes in the peritumoral regions and distant stroma, whereas the frozen section consists of relatively pure tumor with minimal lymphocytes. Images are presented in the following sequence from left to right in the top panel: H&E diagnostic image at low-magnification, TIL map, and the fresh frozen section for molecular genomics assays at low magnification and the bottom panels show representative sections at higher magnification.

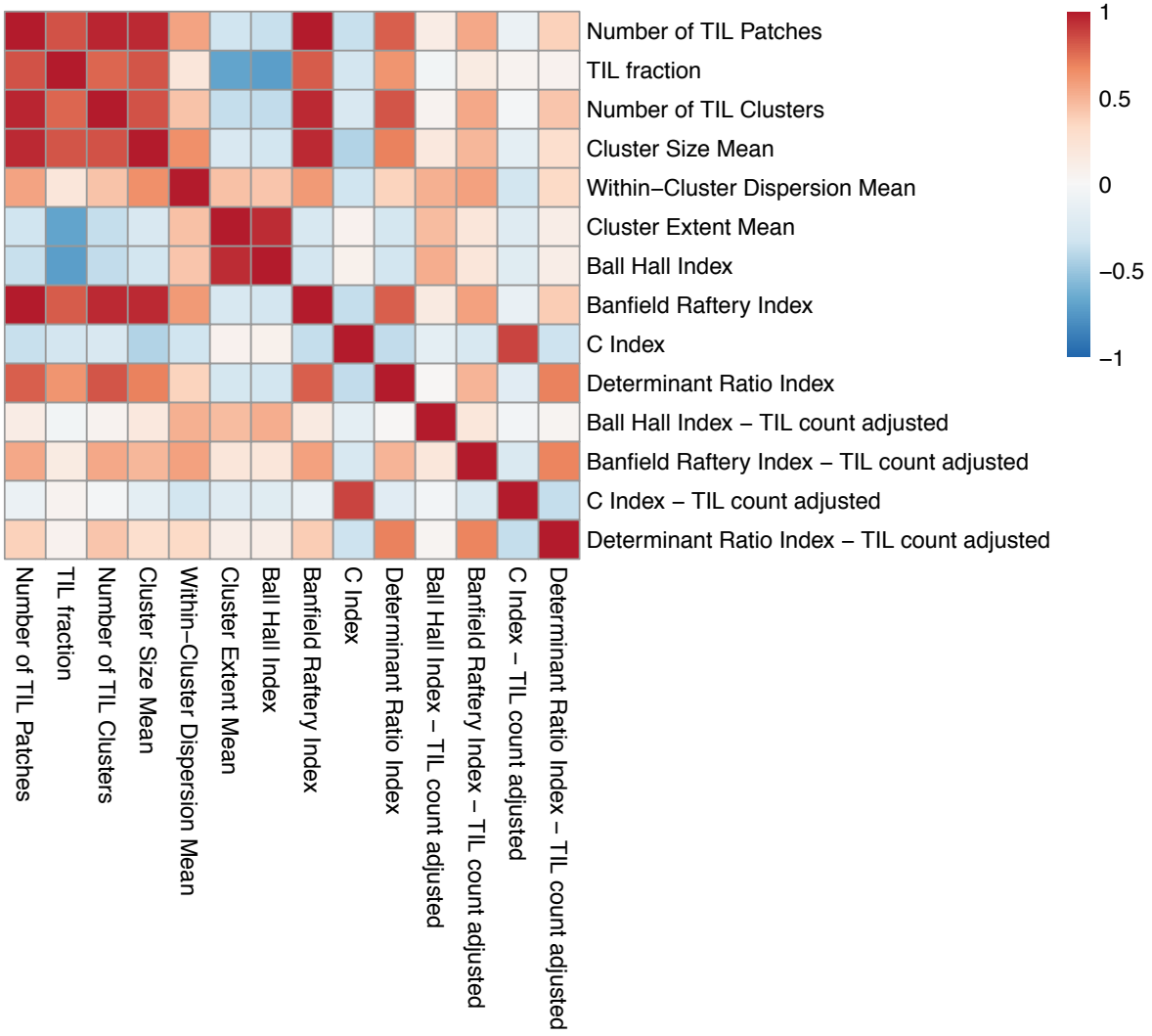

Number of TIL Patches
TIL fraction
Number of TIL Clusters
Cluster Size Mean
Within−Cluster Dispersion Mean
Cluster Extent Mean
Ball Hall Index
Banfield Raftery Index
C Index
Determinant Ratio Index
Ball Hall Index − TIL count adjusted
Banfield Raftery Index − TIL count adjusted
C Index − TIL count adjusted
Determinant Ratio Index − TIL count adjusted
1
0.5
0
−0.5
−1

Figure S4; Related to Figure 6. Relation Among Scores of Local Spatial Structure of the Tumor Immune
Infiltrate Pearson correlation coefficients relating each cluster characterization to all others. The colorbar shows the correlation coefficient value.

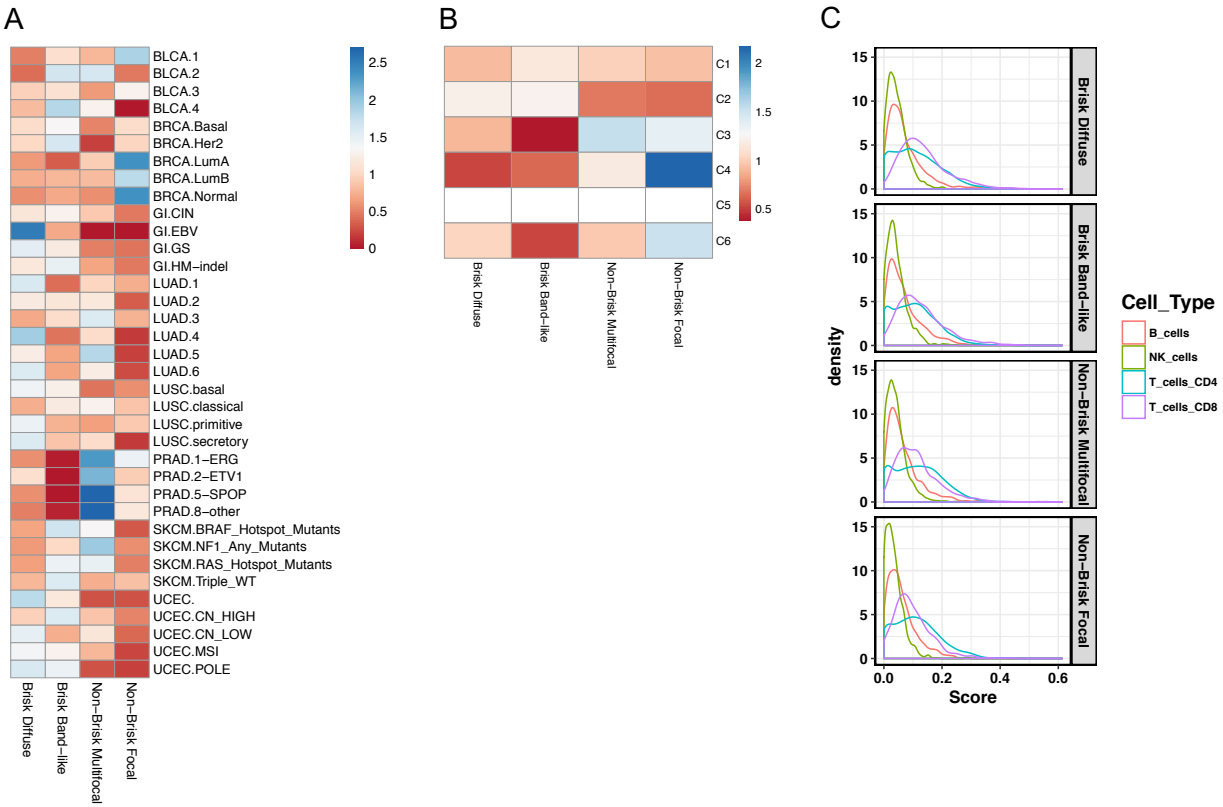

A
BLCA.1
BLCA.2
BLCA.3
BLCA.4
BRCA.Basal
BRCA.Her2
BRCA.LumA
BRCA.LumB
BRCA.Normal
GI.CIN
GI.EBV
GI.GS
GI.HM−indel
LUAD.1
LUAD.2
LUAD.3
LUAD.4
LUAD.5
LUAD.6
LUSC.basal
LUSC.classical
LUSC.primitive
LUSC.secretory
PRAD.1−ERG
PRAD.2−ETV1
PRAD.5−SPOP
PRAD.8−other
SKCM.BRAF_Hotspot_Mutants
SKCM.NF1_Any_Mutants
SKCM.RAS_Hotspot_Mutants
SKCM.Triple_WT
UCEC.
UCEC.CN_HIGH
UCEC.CN_LOW
UCEC.MSI
UCEC.POLE
2.5
2
1.5
1
0.5
0
Brisk Diffuse
Brisk Band−like
Non−Brisk Multifocal
Non−Brisk Focal
B
C1
C2
C3
C4
C5
C6
2
1.5
1
0.5
Brisk Diffuse
Brisk Band−like
Non−Brisk Multifocal
Non−Brisk Focal
C
density
Score
0.0
0.2
0.4
0.6
Brisk Diffuse
Brisk Band−like
Non−Brisk Multifocal
Non−Brisk Focal
Cell_Type
B_cells
NK_cells
T_cells_CD4
T_cells_CD8

Figure S5; Related to Figure 7. Enrichment of TIL Structural Patterns Enrichment of structural patterns among TCGA tumor molecular subtypes **(A)** and among immune subtypes **(B)**. The ratio of observed to expected values is shown on a color scale, where the largest ratios are in red, values near unity as yellow and lower than expected in blue. **(C)** Distribution of scores for cellular fraction of TIL from molecular estimates, segregated by TIL structural pattern.