



Supplementary

ImmunoAlzer: A Deep Learning-Based Computational Framework to Characterize Cell Distribution and Gene Mutation in Tumor Microenvironment

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Table S1. Scanned image sizes of the four H&E Whole Slide Images (WSIs) for cell quantification experiment.

| Image Information | WSI1 | WSI2 | WSI3 | WSI4 |
|----------------------|----------------------|----------------------|-----------------------|-----------------------|
| Image Size | 13.43mm × | 15.83mm × | 19.18mm × | 19.66mm × |
| | 19.42mm | 18.34mm | 25.54mm | 26.26mm |
| Resolution | 0.25µm/pixel | 0.25µm/pixel | 0.25µm/pixel | 0.25µm/pixel |
| Size in Pixels | 53760×77760 | 63363×73440 | 76800×102240 | 78720×105120 |

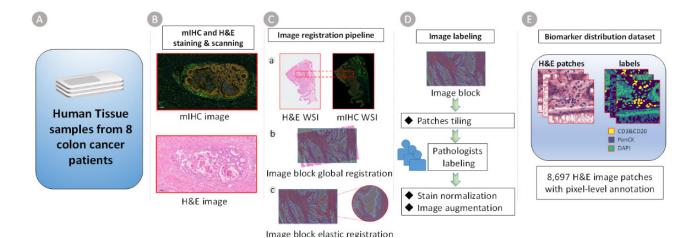


Figure S1. Construction pipeline of the dataset with H&E images and corresponding pixel-level labels. (**A**) Human tissue samples were acquired from eight colon cancer patients. (**B**) Tissue samples were stained and scanned to acquire H&E and mIHC images. (**C**) A series of registration procedures, including image block matching, global registration, and elastic registration were conducted to ensure pixel-wise registration of the mIHC images and H&E images for annotation. (**D**) Registered image blocks were tiled into 512 × 512-pixel patches and sent to pathologists for annotation, and then we conducted stain normalization and image augmentation. (**E**) A cellular biomarker distribution dataset, including H&E image patches and corresponding biomarker distribution label masks, was constructed.

Distribution of Most Frequently Mutated Genes

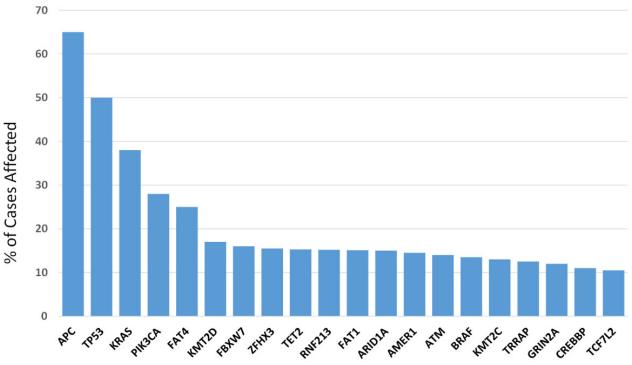


Figure S2. Distribution of most frequently mutated genes of the 446 cases from TCGA COAD project.

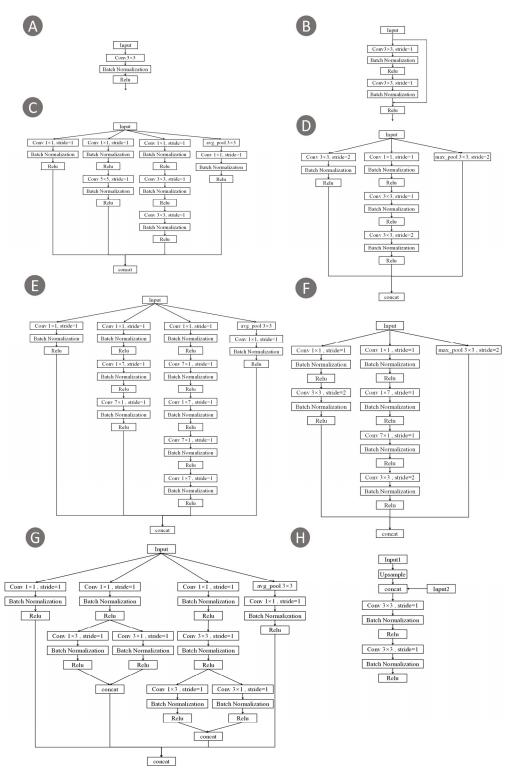


Figure S3. Internal structure of CBPDN blocks. **(A)** Convolutional block structure. **(B)** Residual block structure. **(C)** Inception A structure. **(D)** Inception B structure. **(E)** Inception C structure. **(F)** Inception D structure. **(G)** Inception E structure. **(H)** Upsample block structure.

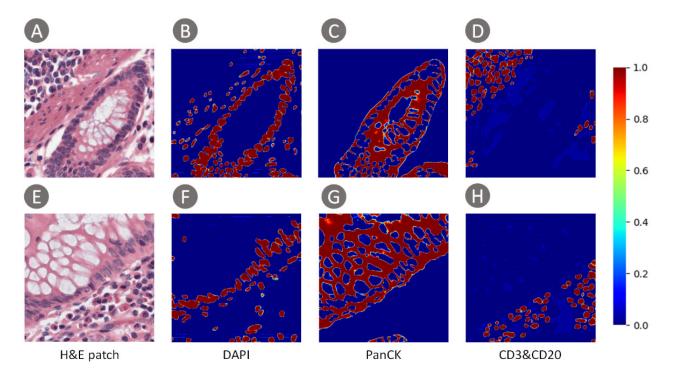


Figure S4. Feature maps obtained after softmax operation of CBDPN. The intensity of the color bar indicates the probability of the corresponding pixel being predicted. (**A**)&(**E**) H&E image patch, (**B**)&(**F**) DAPI, (**C**)&(**G**) PanCK, (**D**)&(**H**) CD3 and CD20.