Teacher-student collaborated multiple instance learning for pan-cancer PDL1 expression prediction from histopathology slides

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ABSTRACT

In many cancers programmed cell death ligand 1 (PDL1) expression serves as a biomarker

for immunotherapies, but its quantification relies on immunohistochemistry (IHC) and few underlying histopathological patterns are established. Digital pathology, combined with deep learning, can augment histopathological assessment and reveal patterns associated with molecular changes, but the presence of heterogeneity within histopathological images, the scale of billions of pixels and the difficulty in acquiring spatially resolved annotations pose challenges for accurate analysis. Here, we present a weakly supervised learning approach using only slide-level supervision for PDL1 expression prediction based on hematoxylin and eosin (H&E) slides. Our methods, MILTS, extends multiple instance learning paradigm (MIL) with the teacher-student framework (TS), which takes the intra-slide heterogeneity into account by dynamically assigning pseudo-labels to different slide regions and retrieves large amounts of unlabeled instances by distillation of the temporal ensemble model. The approach is evaluated on 9,744 tissue slide images across 20 types of solid tumors from TCGA and CPTAC. Among 9 tumors for PDL1 expression serves as an established biomarker, MILTS achieved a weighted average area under curve of 0.83. Predicted patterns within each slide provide insights into the heterogeneity and help identify morphotypes relevant with PDL1 expression, which include mixed inflammatory stroma with relatively high abundance of eosinophils and a cribriform growth pattern of tumor cells. This study provides a new algorithm for predicting molecular changes from H&E images and provides histological links of PDL1 expression, and thus demonstrates the potential of deep learning in discovering diverse histological patterns.

Introduction

Inhibitors for the PD1-PDL1 checkpoint have revolutionized cancer therapy in the past decade. In addition to seven anti-PD1/PDL1 monoclonal antibodies (mAbs) currently approved by US Food and Drug Administration (FDA), there are still approximately six thousand mAbs undergoing clinical trials [1–3]. Blockade of PD1-PDL1 interaction notably contributes to activating antitumor immunity and is proven to benefit the treatment of various types of tumors [4–6]. PDL1 expression serves as a biomarker associated with patients' response to anti-PD1/PDL1 mAbs such as pembrolizumab and nivolumab, which acts as the most widely adopted standard for identifying patient cohorts that are appropriate candidates for PD1-PDL1 immunotherapy [7]. From 2011 to 2021, there were totally 15 immune checkpoint inhibitor FDA approvals linked with companion PDL1 testing including non-small cell lung cancer (NSCLC) (N=7), bladder cancer (N=3), triple-negative breast cancer (N=2), cervical cancer (N=2) and gastric cancer (N=1). And the outcomes of patients with renal cell cancer, colon cancer and melanoma are also reported linked with PDL1 expression by many researchers [8–12].

Currently, PDL1 expression is predominantly quantified by immunohistochemistry (IHC) assays, and some recent research indicates that mRNA expression level also shares a strong correlation with IHC quantification[13]. Whilst IHC qualification has been successfully used in clinical practice for decades and is considered to be a gold standard for this task, recent analysis show that the interpretation of staining and decision threshold differs for different commercially available platforms and even differs within the same platform [14]. Along with the subjectivity of

pathologists, these factors introduce undesired inter- and intraobserver variance to the evaluation of staining and thus limited reproducibility. Besides, quantification by mRNA expression utilizing techniques like real-time reverse transcription polymerase chain reaction (RT-qPCR) can be costly and time-consuming [15], which is also the case with IHC tests. H&E stained slides are one of the most widely used and effective carriers of pathological information, offering a cost-effective and expedient alternative, and are employed in routine pathological assessment of clinical specimens. Developing an efficient and reliable method for estimating PDL1 expression on H&E stained slides, which does not require complex sample preparations or exhaustive manual labor, may yield a faster and cheaper diagnostic readout. Further, this process is capable of unraveling histopathological characteristics of PDL1 expression on slides, thereby assisting pathologists in comprehending the gene's expression mechanism and facilitating a more precise interpretation of immune evasion patterns.

The expanding field of computational histopathology may not only automate existing workflows, but also proposes to explore the molecular information based on morphological features via deep learning [16]. It has exhibited great potential in assisting pathologists on many routine tasks [17] including applications such as mitosis detection [18–21], tissues segmentation [22–25], tumor subtyping and grading [26–29] and biomarker assessment [30–33]. Some studies also reveal that morphotypes are closely associated with genetic alterations in tumors and thus indicative of clinical features and prognosis, which has a chance to redefine the clinical workflows [34–37]. However, the analysis of pathological images is often challenged by the limited availability of reference data, with clinical reports and patient-level diagnoses being the main sources of information[16]. And

it is also tricky to accurately capture and spatially resolve transcriptome profiles at the pixel or cell level in whole slide images [38]. The presence of such heterogeneity poses challenges in training deep learning based algorithms, which usually requires accurate reference in the form of labeled data[39]. Some existing approaches have opted to overlook the intra-tumor heterogeneity by assigning slide-level annotations to all content within a slide [31, 34, 35, 40, 41]. This strategy works well if the slides exhibit good homogeneity concerning specific properties of interest. However, it can also lead to overfitting and undesired generalization with heterogeneous composition, where inaccurate instance-level labels will either hinder the convergence of the model or result in erroneous recognition of relevant patterns [42]. More recent approaches tend to directly utilize ImageNet [43] pretrained features and incorporate specially designed attention or embedding-based MIL strategies [19, 24, 29, 36, 44–47], which significantly accelerates the training but is also more data-hungry. In addition, dimensionality reduction with pretrained features inevitably leads to information loss. These pre-trained models based on the ImageNet database, are designed to capture general visual patterns without any specific bias towards histopathology-related features or priors. Consequently, in situations where certain details in histopathology images are completely lost or unavailable, reweighting or attention mechanisms have limited effectiveness.

Therefore, to leverage the benefit of both massive amount of tile information and slide-level annotation, we propose our weakly supervised learning based methodology named MILTS (Teacher-Student collaborated Multiple Instance Learning framework), which is powered by whole slide images in H&E staining from The Cancer Genome Atlas (TCGA) and The National Cancer Institute's Clinical Proteomic Tumor Analysis Consortium (CPTAC) involving 9,744 slides from

6,715 patients across 20 kinds of tumors. MILTS utilizes an iterative, self-refining process to assign labels automatically supporting tile feature extractor training, and combines the statistical summary of tile-level predictions with tokens fused by the transformer to obtain the slide-level embedding for the final prediction. Results demonstrate there exists a salient morpho-transcriptomic link across certain cancer types, whose treatment landscape and prognosis are associated with PDL1 expression, with a weighted average AUC of 0.83. Heterogeneous tile-level predictions further provide insights into morphotypes associated with PDL1 hot regions in colon cancer, which include mixed inflammatory stroma with relatively high abundance of eosinophils and a cribriform growth pattern of tumor cells with hyperchromatic nuclei. Building on the foundation of PDL1-relevant tumors, we undertook an analysis of the other 11 cancer entities on the morpho-transcriptomic correlation that have not been verified or approved yet. Varying degrees of morpho-transcriptomic correlation was observed among these tumors. Further examination may be warranted based on the difference between predictability of these tumors and known PDL1-relevant ones. It also reveals that the molecular basis of tumors could be depicted from the view of cellular morphology via advanced deep learning techniques, which could provide a new perspective on studies of tumorigenesis and treatment.

Results

Workflow of MILTS. The corresponding workflow of MILTS is presented in Fig. 1a. In the context of MILTS, a slide-level label initialized from dichotomised mRNA expression levels is employed to supervise the representation learning for the histopathological image. Because the

slide-level labels constitute an aggregate summary, which is expected to differ across the tiles of the tumor section, the teacher-student framework combines dynamic labels assignment for individual tiles with knowledge distillation from a temporal ensemble model representing the exponentially decaying average of previous learning iterations. Specifically, tiles would be fed into both the teacher and student model with random augmentation including rotation, crop, flip and color transformation. The teach model continuously yields tile-level pseudo-labels for typical positive/negative tiles in each epoch, based on which the student model would be updated following the MIL constraint as well as the distribution generated by the teacher model on unlabeled instances. As the teacher model is continuously updated via the moving average of the student model, this collaborative procedure automatically learns optimal tile level labels. These features are further fused by a transformer to obtain a slide-level token and combined with statistical summary of patch-level outcomes in multi-layer perceptron (MLP) to infer per patient results. More details about the methodology are provided in the Methods section.

Predictability of PDL1 expression across 9 cancer types. The ability to classify gene expression was evaluated on 9 cancers for which PDL1 expression serves as an established biomarker for checkpoint inhibitors, whose distribution is shown in Fig. 1b (involving 3,121 cases with 4,215 slides). Using the upper tertile of PDL1 expression as a threshold, MILTS achieved a performance with a weighted average average area under receiver operating characteristic curve (AUC) of 0.83 (range: 0.64 to 0.91), accuracy of 0.75 (range: 0.58 to 0.87), sensitivity of 0.83 (range: 0.74 to 0.90) and specificity of 0.71 (range: 0.47 to 0.89). We also evaluated the using other two thresholds: the upper quartile (top 75%) and median point (50%). The results showed a promising predictability

with a mean AUC of 0.81 and 0.75, respectively (Supplementary Fig. S1 and Supplementary Tables S1-S2). In the following the upper tertile is used, unless stated otherwise.

Specifically, the dataset comprises bladder urothelial carcinoma (BLCA), cervical squamous cell and endocervical adenocarcinoma (CESC), colon adenocarcinoma (COAD), kidney renal papillary cell carcinoma (KIRP), lung adenocarcinoma (LUAD), lung squamous cell carcinoma (LUSC), skin cutaneous melanoma (SKCM), stomach adenocarcinoma (STAD) and triple-negative breast carcinoma (TNBC). The results are shown in Fig. 1c. Here, the threshold to determine the accuracy, sensitivity and specificity is selected by Youden's J statistic. 95% confidence interval (CI) is also computed with bootstrapping strategy (2,000 random resamples), where the performances on AUC are respectively 0.87 for bladder urothelial carcinoma (95% CI: 0.84-0.90), 0.91 for cervical squamous cell and endocervical adenocarcinoma (95% CI: 0.88-0.93), 0.92 for colon adenocarcinoma (95% CI: 0.90-0.93), 0.77 for kidney renal papillary cell carcinoma (95% CI: 0.73-0.80), 0.74 for lung adenocarcinoma (95% CI: 0.72-0.77), 0.73 for lung squamous cell carcinoma (95% CI: 0.70-0.76), 0.87 for skin cutaneous melanoma (95% CI: 0.86-0.89), 0.85 for stomach adenocarcinoma (95% CI: 0.83-0.87) and 0.64 for triple-negative breast carcinoma (95% CI: 0.60-0.69). Overall, a significant morpho-transcriptomic link is evident regarding PDL1 expression across nine PDL1-relevant cancer types, which demonstrates a good predictability independent of the specific threshold.

MILTS outperforms other methods in PDL1 expression prediction. Comparison results clearly demonstrate that MILTS outperforms the comparison methods on aforementioned tumors, exhibit-

ing a notable advantage of 9% or more in terms of average AUC. Several methods were evaluated including the method by Campanella et al. [36], TransMIL [45] and CLAM [46]. All comparison methods are deep learning based MIL algorithms, among which Campanella's adopts instance-level strategy while TransMIL and CLAM are embedding-level ones. The related hyperparameter settings are listed in Supplementary Table S3. Quantitative results are provided in Fig. 2a. Overall, MILTS gains superior performance to the comparison methods when making predictions about the expression level. The weighted average AUC of MILTS on these 9 kinds of tumors is 0.83, while those of the method by Campanella et al., TransMIL and CLAM are respectively 0.63 (range: 0.51 to 0.75), 0.71 (range: 0.53 to 0.84) and 0.74 (range: 0.56 to 0.83). The results of F1 score and Matthews correlation coefficient (MCC) further reveal that MILTS exhibits more balanced sensitivity to both positive and negative samples, with an average performance of 0.69 and 0.50, respectively. In comparison, the second-best model achieves only 0.60 and 0.39 for the same metrics.

An ablation study was conducted to evaluate the individual modules of the teacher-student MIL learning and feature aggregation using COAD, SKCM and STAD data. The results are presented in Fig. 2c. Results reported that teacher-student MIL module brought an improvement of 4.4%, 8.5%, 5% and 8.8% with respect to AUC, accuracy, F1 score and MCC. The improvements of the feature aggregation module are respectively 3.5%, 1.5%, 2.9% and 4.2%, respectively. Besides, we also implemented external validation on the CPTAC dataset [48] whose results are shown in Fig. 2b. The proposed method still demonstrated superior overall performance when compared to the other methods. However, it is noticed that the results of all the algorithms exhibit a de-

crease in performance when applied to the CPTAC dataset. This decline in accuracy could be attributed to several factors, including variations in mRNA quantification and differences in the slide image modalities used in the CPTAC dataset which is a mixture of fresh frozen slides and formalin-fixed paraffin-embedded (FFPE) slides. Further investigation and refinement of the algorithms may be necessary to address these issues and improve their performance on the CPTAC dataset. Nonetheless, the quantitative analysis of comparison confirms that the proposed model appear to better decipher morphological features associated with PDL1 expression from pathological patterns compared to other methods.

Spatially heterogeneous patterns of high PDL1 expression. Like most other MIL models, MILTS calculates predictions for each individual tile as well as for the entire slide. Strikingly, tiles from samples within upper tertile of bulk PDL1 expression are predicted to exhibit a wide range of PDL1 expression, whereas tiles from the remaining samples were uniformly low (Fig. 3a). These distributions of tile-level probability accord with the assumption that there exists a substantial part of instances in positive slides which may not exhibit the same characteristics as the overall slide-level label suggests. Similar results were observed for upper quartile and median thresholds, where it appears that the range of positive probabilities for the PDL1-low class tends to shrink as the threshold value increases (Fig. S2). Conversely the range of positive probabilities became wider when the median was chosen as the threshold, indicating that the model begins to unravel nuances of very low PDL1 expression. The distinct distributions between PDL1 high and low groups explain the observed differences of AUCs (Fig. S3 and S4). Noting that the distributions show greater overlap of other thresholds reflects the lower AUCs for these cutoffs and indicates

that the histopathological differences are less pronounced for median and upper quartile threshold.

The notion that positive tiles reflect common histological features is also supported by a UMAP [49] visualization of the embeddings learned by the patch-level classifier in (Fig. S5). Here the representations of instances in slides with high PDL1 expression exhibited varying degrees of overlap with instances from negative slides, and those with high expression also demonstrated their own distinct distribution, independent from the distribution of negative samples, which were also reflected respectively by the cold region and highlighted region in heatmaps of PDL1 positive slides (Fig. 3b).

Deep morphological features help discovering morphotypes for PDL1 expression. The aforementioned dispersion of high PDL1 predictions across tiles from the same slide manifest in a spatially organized fashion as evidenced by heatmaps of the predicted PDL1 expression (Fig. 3b). In the case of negative slides, their distribution tends to have a relatively lower mean value, resulting in a preponderance of blue regions dominating the corresponding heatmaps. In contrast, the heatmaps of positive slides exhibit different contiguous areas of predicted high and low expression values. This spatial heterogeneity also coincides with a range of histopathological patterns, which we illustrate at the example of colon adenocarcinoma.

Presented in the first row of Fig. 4a, it is common to observe a mixed inflammatory stroma characterized by a relatively high abundance of eosinophils in areas with high PDL1 expression, which can be either intact or degranulated. This suggests an immune response or inflammatory process occurring in these areas. Another distinctive feature observed in areas with high PDL1

expression is the presence of a cribriform (sieve-like) growth pattern of tumor cells in the second row of Fig. 4a. This growth pattern is accompanied by tumor cells exhibiting hyperchromatic nuclei, which appear darker and more intensely stained compared to surrounding cells. The presence of cribriform growth has been identified as an independent prognostic factor in various types of cancers, indicating a higher risk of tumor progression, metastasis, and decreased overall survival. In contrast, typical negative patterns usually included areas of non-invasive adenomatous parts of the lesion as illustrated in the first row of Fig. 4b. The non-invasive adenomatous areas appear more uniform and well-differentiated glandular architecture, indicating a lower likelihood of malignancy or aggressive behavior. Other patterns also include normal colonic crypts adjacent to the invasive carcinomas, tumor necrosis, abundant tumor-associated mucin in case of mucinous carcinomas and coagulation necrosis at the sample margin in Fig. 4b. Additional examples could be found in Fig. S6.

Correlations between MILTS predictions with TME and clinical features. In order to better understand the observed histopathological associations, correlations with the immune microenvironment, as estimated by CIBERSORT [50] from RNA-seq data, and other clinical parameters were performed. The analysis of immune infiltrates revealed an overall positive correlation between the presence of cell types, such as M1 macrophages and CD8+ cytotoxic T cells, and predicted PDL1 expression across most cancer types (Fig. 5a). This finding aligns with the observed patterns of high PDL1 expression in COAD which was found to co-occur with a mixed inflammatory infiltrate. The analysis also revealed a correlation between the presence of CD4 memory-activated T cells and elevated PDL1 expression, which may indicate a previous immune response

against tumor antigens, leading to the upregulation of PD-L1 as a countermeasure by tumor cells to suppress T cell activity and evade immune attack. This correlation may imply an intricate interplay between PDL1 expression, immune cell infiltration, and the inflammatory response within the tumor microenvironment.

In addition, we conducted an analysis to assess the correlation between the predictions generated by MILTS and various clinical features specific to different cancer types which is shown in Fig. 5b. Across cancer types, only few consistent trends were observed, with tumor mutation burden and TIL Regional Fraction exhibiting generally weak positive correlations with PDL1 prediction patterns. The finding of PDL1 expression coinciding with high inflammation, which is often found in tumors with high mutation burden, agrees with the observations of the previous section. The lack of other strong correlations indicates that the features extracted by the model are mostly either independent of, or lowly correlated with, standard clinical features. Consequently, it suggests that the features captured by the model are distinct and do not overlap with or duplicate the information provided by the clinical features. This highlights the complementary nature of the model's predictive capabilities in relation to the clinical characteristics of the cancers under investigation.

Weaker histopathological associations in other cancer types. In order to provide broader context, we also conducted analysis of tumor types, for which immunotherapies have not been approved with PDL1 companion tests, or for which the prognostic significance of PDL1 expression levels has not been significantly verified. The experiments involved a total of 11 types of tu-

mors, which were respectively adrenocortical carcinoma (ACC), esophageal carcinoma (ESCA), head&neck squamous cell carcinoma (HNSC), liver hepatocellular carcinoma (LIHC), mesothelioma (MESO), ovarian serous cystadenocarcinoma (OV), prostate adenocarcinoma (PRAD), rectum adenocarcinoma (READ), testicular germ cell tumors (TCGT), thyroid carcinoma (THCA) and uterine corpus endometrial carcinoma (UCEC). The statistical performance of MILTS on these tumors is presented in Fig. 6a. The average AUC value is measured at 0.67 and the average accuracy stands at 64%, indicating a moderate level of discriminatory power in distinguishing between different mRNA expression levels. See supplementary Tables S4-S6 for the details.

Among the 11 cancer entities analyzed, it is important to note that not all tumors display a distinct morphological pattern of PDL1 expression that can be directly comparable to PDL1 relevant tumors. The AUCs for HNSC, READ, TGCT and THCA exceeded 0.7, which were comparable to the performance on cancers with PDL1 as an established biomarker where TNBC is the only one with the AUC lower than 0.7. Besides, we also devised a similarity measure based on the distribution of average classification performance of all PDL1 relevant tumors (see Similarity Measure in the supplementary material). The corresponding results are exhibited in Fig. S8, and the four aforementioned tumor types (HNSC, READ, TGCT, and THCA) still demonstrate good similarity. This implies that the intermediate processes connecting macroscopic changes in tumor morphology to microscopic gene expressions may share certain common features for these tumors with good predictability of PDL1 expression, which contribute to their consistent and accurate prediction of PDL1 expression.

The overall distribution of PDL1 expression level of these tumors is shown in Fig. 6b along with that of the PDL1 relevant tumors, where the mean values for two groups are respectively 1.71 and 3.27. From the perspective of classification metrics, it is observed that the mean AUC of these tumors demonstrate a weaker correlation between morphology and mRNA expression level compared to the PDL1-relevant tumors, where a subtle positive correlation exists between the average level of PDL1 expression and corresponding classification performance (Fig. 6b). This implies that the distinction in the predictability based on histopathology may be attributed to multiple factors in addition to PDL1 expression level, which may also have implications for therapeutic outcomes. The observed differentiation of these tumors in morpho-transcriptomic links suggests that additional investigation is warranted for tumors with better morphological correlation.

Discussion

PDL1 has been proven to be an effective biomarker for determining the response to the immunotherapies in a number of cancer types. Here, we developed MILTS, a novel weakly-supervised multiple instance learning algorithm for predicting slide-level labels from H&E slide scans. We demonstrated its efficacy in predicting elevated PDL1 expression for a broad range of cancers including 9 cancers where PDL1 expression served as an established biomarker. Given the absence of a clinically optimal threshold, different cutoffs thresholds of mRNA quantification were employed in this study to verify the correlation. Compared to existing tools MILTS displayed competitive performance. Our analysis revealed that salient links between histopathology and the level of PDL1 expression for these tumors across various thresholds. Of note, tumor types for

which PDL1 is not considered to be a relevant biomarker, also exhibited lower histopathological predictability, likely because of overall lower PDL1 expression.

In addition to the predictability of PDL1 based solely on histology, which could potentially help reduce the need for additional tests, this study also highlighted a diversity of histopathological patterns associated with high and low PDL1 expression. Utilizing the deep histopathological features and differentiated patch-level predictions, some typical patterns for high PDL1 expression in colon adenocarcinomas were demonstrated which include a mixed inflammatory stroma characterized by a relatively high abundance of eosinophils and a cribriform growth pattern of tumor cells. Furthermore, low expression patterns include tumor necrosis, coagulation necrosis at the sample margin, tumor-associated mucin in case of mucinous carcinomas, normal colonic crypts adjacent to the invasive carcinomas, and, most interestingly, non-invasive adenomatous parts of the lesion. It reflected that this weakly-supervised learning manner was capable of pinpointing the potential morphological patterns or cytomorphology of different PDL1 expression levels, which due to their diverse nature and heterogeneous occurrence across and within a slide are difficult to establish by conventional means.

Despite these insights gained from this study, there were still certain limitations. The first was that our experiments were performed based on fresh-frozen tissue sections which were believed to preserve a better structure of molecular content [26]. The reliance on fresh-frozen tissue sections limited the generalizability of the findings to other sample types, such as formalin-fixed paraffin-embedded (FFPE) samples, which were more commonly available in routine clinical set-

tings. Therefore, future studies should aim to validate the findings using diverse tissue types, including FFPE samples. Another important limitation was the consistency of the quantification scale used for supervised information. The accuracy and reliability of the results heavily depended on the quantification method employed to generate supervised information, such as mRNA expression levels in this study. The potential mismatch between the bulk RNA sequences and the tissue presented in the histopathology slide can introduce challenges in integrating the molecular information from bulk RNA sequencing with the spatial information provided by histopathology slides. And variations in the protocols and techniques used for RNA expression quantification can also impact the knowledge transferability between different datasets. And it was believed that these are plausible factors that could contribute to the observed performance differences in the external validation on the CPTAC dataset in this study.

Overall, our study revealed that it is possible to predict high and low PDL1 mRNA expression based on H&E slides with the proposed weakly supervised method MILTS. Furthermore, MILTS captures and identifies meaningful histopathological patterns that are associated with molecular changes, which may not be readily discernible due to their heterogeneous occurrence across and within slides. These findings underscore the utility of AI to augment the capabilities of pathologists by leveraging large amounts of digitized histopathological slides with slide-level annotations, and thereby providing valuable insights into the complex interactions between histology and molecular biology.

Methods

Datasets. Whole slide images and transcriptome profiling data utilized in the experiments comes from TCGA project via National Cancer Institute (NCI) Genomic Data Commons Portal [51] and CPTAC project via the Cancer Imaging Archive (TCIA) Pathology Portal, which comprises 9,744 H&E stained whole slide images of frozen tissue sections and corresponding mRNA quantification data obtained from 6,715 patients diagnosed across 20 different types of cancer. Data from CPTAC dataset is employed for external validation purposes. The sample types comprise mainly primary solid tumor as well as metastatic tissues. We collect all available whole slide images scanned at a magnification of 20$\times$ or higher, along with their CD274 mRNA expression level read counts normalized by the upper quartile fragments per kilobase of transcript per million mapped reads (FPKM-UQ). A commonly used split for data, in which 60% is reserved for training, 15% for validation, and 25% for testing, was employed for the majority of the tumors analyzed. This resulted in 5,373 samples for training, 1,189 samples for validation, and 3,182 samples for testing.

Data preprocessing. Due to the tremendously large size, whole slide images should be decomposed into smaller elements so that deep learning techniques like convolutional neural network could handle them at an acceptable computational cost. Hysteresis thresholding is applied to exclude the invalid background by finding contours of stained tissues. The valid regions within the contours or partly overlapped are cropped into tiles of 256 pixels$\times$256 pixels at resolution of 20$\times$, which correspond to the physical size of 128 μm$\times$128 μm respectively. In total, 52,587,256 tiles are extracted for the establishment and verification of the model. To mitigate the bias introduced by variations in staining protocols and laboratory conditions, we applied random adjustments to

the brightness, contrast, saturation, and hue of generated tiles. In addition, random rotation and crop of the tiles were also applied to increase the variability of scales and orientations.

Teacher-student collaborated multiple instance learning (MILTS) for patch feature extraction. A two-stage strategy is adopted in the diagnosis of a patient's slide, starting with patch-level assessment, followed by slide-level feature aggregation and prediction. This framework enables not only higher-level diagnosis of the patient, but also interpretation of the slide's inner structure based on the patch-level predictions. Specifically, prediction of PDL1 expression is formulated as a classification problem using a specific mRNA quantification value as the threshold. This threshold was set based on the median point, upper tertile, and upper quartile of the mRNA quantification distribution. Classification based on the patch-level classifier is usually devised under multiple instance learning framework when only the slide-level property is known and potential heterogeneity is implied. During the patch-level feature extraction stage, MILTS made two important improvements. Firstly, we modified the optimization of multiple instance learning framework to a class-targeted way considering the heterogeneous composition of a slide image. Secondly, inspired by the human-decision making process, the single model of MIL is decomposed into a teacher and a student model, in which the teacher model dynamically assigns probabilities to tiles, and the student model subsequently learns to predict. These two components work collaboratively to boost the performance on patch-level pathological feature extraction.

In the classic MIL approaches, the target label Y_k is predicted based on the distribution of the instances (or patches) $\{x_{1,k}, x_{2,k}, ..., x_{n,k}\}$ from a bag (a slide image in this case) X_k, where the

hypothetical distribution should follow the principle below [52]:

$$Y_k = \begin{cases} 0, & iff \sum y_{i,k} = 0 \\ 1, & otherwise \end{cases}$$

where $y_{i,k}$ is the label for instance $x_{i,k}$ and the principle applies to the binary classification. This assumption implies that positive instances should only be present in positive bags, which accurately reflects certain scenarios in pathological diagnosis, such as distinguishing between benign and malignant lesions.

However, it is crucial to note that PDL1 expression is quantified by the accumulation of cancer cell surface PD-L1, rather than simply by its presence or absence. This implies that elements defined as highly or lowly expressed could exist in cohorts with contrary macro statistical properties. Based on this observation, we propose our class-targeted optimization way, which regularizes the instance-level learning by filtrating the representative instances for slides with varying labels. Instances from different slides are selected based on class-representative criteria defined by their respective slide labels. Specifically, a maximum-minimum selection criteria is established to determine the groups of instances involved in model training for binary classification. In the scenario where patients should be classified as high or low PDL1 expression, instances from positive samples with maximum response and negative samples with minimum response tend to be selected and share the same label with bags they belong to. The criteria could be summarized as:

$$\tilde{i}_k = \begin{cases} \underset{i}{argmax}(f_\theta(x_{i,k})), & Y_k = 1 \\ \underset{i}{argmin}(f_\theta(x_{i,k})), & Y_k = 0 \end{cases}$$

where $\tilde{i}_k$ is the index of the selected instance and f_θ is the function of instance-level classifier which

maps the input patch $x_{i,k}$ into a normalized hidden variable indicative of its typicality for the slide. To incorporate additional histological pattern references, the criteria can be further relaxed by allowing $M > 1$ representative instances in the slide.

Building on our improved class-representative MIL approach, we further consider data composition in this task as a mixture of labeled and unlabeled instances and introduce the teacher-student collaborative learning into MIL according to the characteristics of whole slide images and weakly annotations. Typically, the representative instances selected from a slide are considered as labeled data and assigned the same label as the slide. Nevertheless, these instances only represent a small portion of the entire slide image. Relying solely on these instances for training can result in overfitting and suboptimal performance due to their limited representation of the entire slide image. There still exist a considerable number of instances within whole slide images that also contain pathological information and can be utilized for representation learning. Usually to address the challenge of interpreting slides containing both clear and ambiguous regions, a pathologist typically relies on their own expertise to interpret the clear parts of the slide (labeled) and consults with more experienced or senior pathologists for guidance on the ambiguous regions (unlabeled). And this process also benefits senior pathologists by exposing them to difficult cases. Taking inspiration from this process, we propose to decompose the single model in the MIL framework into a temporal ensemble model consisting of a teacher model, acting as the senior pathologist retrieving regularization of unlabeled data on feature representation, and a student model, learning from the teacher model. The mean teacher method [53] is adopted to constructed the teacher model and ResNet34 [54] as baseline architecture for teacher&student model. Specifically,

labeled instances $X_{labeled} = \{x_{1,1}, x_{1,2}, ..., x_{K,1}, ..., x_{K,n}\}$ selected by the teacher model, where K instances of each slide are assigned with the label consistent with the sample, and unlabeled instances $X_{unlabeled} = \complement_X X_{labeled}$ are both inputted into student model and compute a probability of high PDL1 expression in every iteration of training. The weighted cross-entropy loss function, which is used as the classification cost, penalizes the difference between the output and pseudo label for the clear regions of the slide represented by $X_{labeled}$. Meanwhile, the consistency cost is applied to the ambiguous regions represented by $X_{unlabeled}$ and constrains the distance to the distribution given by the teacher model. The loss function was formulated as:

$$
\begin{aligned}
L &= L_{WCE}(X_{labeled}, \theta) + \lambda L_{cons}(X_{unlabeled}, \theta, \theta^{'}) \\
&= -\sum_{x_i \in X_{labeled}} (\omega_0 y_i log(f_\theta(x_i)) + \omega_1 (1 - y_i)(1 - log f_\theta(x_i))) \\
&\quad + \lambda \sum_{x_j \in X_{unlabeled}} \|f_{\theta'}(T(x_j)) - f_\theta(T(x_j))\|^2
\end{aligned}
$$

where ω_i is the weight to balance the class frequency and $f_{\theta'}$ is the function of the teacher model generated by exponential moving average (EMA) of previous student models. $T()$ is a combination of transforms including random rotation and color jitter. The parameters of the teacher model would be updated after the student model is optimized in each iteration.

In other words, the proposed approach constructs a teacher model to assess the reliability of knowledge and pairing the reliable parts with corresponding answers for the student model to learn. For data with greater uncertainty judged by the teacher model, the student model will attempt to imitate the teacher model's response even if an exact answer is not available. The teacher model will then use feedback from the student model to update itself and improve its own

knowledge. Among different epochs of training, labels are dynamically assigned for instances according to the predicted probability of the teacher model via the above mentioned MIL manner, where meaningful histopathological patterns are expected to be uncovered through aggregated information distilled from mean teacher. And the student model optimized by gradient descent with regard to objective function L would promote the prediction of the teacher model in turn by a weighted average behavior over training steps. This iterative process allows for continuous learning and refinement of both the teacher and student models to capture the effective histopathological features associated with PDL1 expression. Usually both models could be utilized to output the positive probability at the end of the training which share similar performance for patch-level prediction.

Enhancing patient-level diagnosis by fused slide tokens with statistical summary of patch-level predictions. In the slide-level feature aggregation stage, statistical summary of patch-level predictions are merged with slide tokens by leveraging the local details extracted by the patch-level CNN and the global perspective of a transformer model. Given the trained model $f_{\theta'}$ or f_{θ} at patch level, the typicality of all effective patches in a slide would be computed, which could be further interpreted as positive or negative probability according to the slide-level property. Based on the patch-level prediction, several statistical features of the slide were extracted including percentage of positive patches, histogram of probability distribution, median value and mean value of positive probability. Specifically, patches with typicality over 0.8 and below 0.2 are removed when computing the above features because a trimmed estimator is supposed to better reflect the central tendency of data. Further, features $e = [e_1, e_2, ..., e_m]$ extracted by the patch-level backbone (i.e.

feature vectors output by the adaptive pooling layer of ResNet34 in the end) are aggregated to form the global representation of the slide through the attention mechanism. Transformer is utilized to fuse these features into a class token $H_k^{(0)}$ which could be formulated as [45]:

$$H_k = LN(MSA(e))$$

where $LN(\cdot)$ is layer normalization and $MSA(\cdot)$ indicates multi-head self attention operation. The first row vector of correlation matrix $H_k^{(0)}$ i.e. class token is taken as the aggregation of all local features in a slide. Training details follow the settings in [45]. Along with the statistical features mentioned above, the representation of the slide $E \in \mathbb{R}^{1\times 523}$ was constructed by concatenation and conducted the diagnosis by using MLP to classify the slide representation as PD-L1 high expression or low expression. The MLP model is trained with cross-entropy loss and SGD optimizer, where the learning rate is set as 2×10^{-4}.

Implementation details. Data preprocessing mainly including segmentation and patching of whole slide images was implemented on multiple workstations considering massive amount of data, whose central processing units are respectively AMD Ryzen 5950X, Intel i9-12900K and Intel i9-10900K. Our weakly supervised framework MILTS and patient-level feature aggregation&diagnosis models were then trained and evaluated on a platform of GIGABYTE GeForce RTX 3090Ti with 24 GB of graphics memory. Algorithms were mostly programmed with Python (version 3.8.10) and libraries mainly involved were OpenCV (version 4.5.3), OpenSlide (version 1.1.2), Pillow (version 8.4.0), scikit-image (version 0.18.3) and Numpy (version 1.19.5). We utilized Pytorch [55] as the basic machine learning framework for data loading, enhancement, training and inference pipeline. Before the start of MILTS training, we initialized ResNet34 with ImageNet [43]

pretrained parameters for both teacher and student model. A mini-batch size of 512 was adopted to accelerate computation and we used a stochastic gradient descent (SGD) optimizer with an initial learning rate of 1×10^{-2} to optimize weights of the models. The strategy of cosine annealing was applied to schedule the learning rate and the minimum value was set as 1×10^{-4}. All the models were trained for 30 epochs, during which two circles of learning rate change were completed. On our platform using a single GPU, the typical inference time for a $20\times$ whole slide image with non-overlap patches was 6.96s, which indicated a high efficiency and may be further promoted by large-scale parallel computing.

Data availability. TCGA data (images, as well as transcriptomic and clinical data) are publically available from `http://gdc.cancer.gov`. CPTAC image data are publically available from `https://wiki.cancerimagingarchive.net/display/Public/CPTAC+Imaging+Proteomics` and transcriptomic data from `http://gdc.cancer.gov`.

Code availability. The code in this paper would be available through a Code Ocean compute capsule.

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Author Contributions Statement

D. J., M. G. and X. B. conceived the study. D. J. developed the deep learning algorithms. D. J., S. L. and A. S. performed the experiment analysis. T. G. P. G. and D. J. conducted the histopathological analysis. D. J., M. G. and X. B. wrote the manuscript with help of all other authors. M. G. and X. B. supervised the project.

Competing Interests Statement

The authors declare no competing interests.

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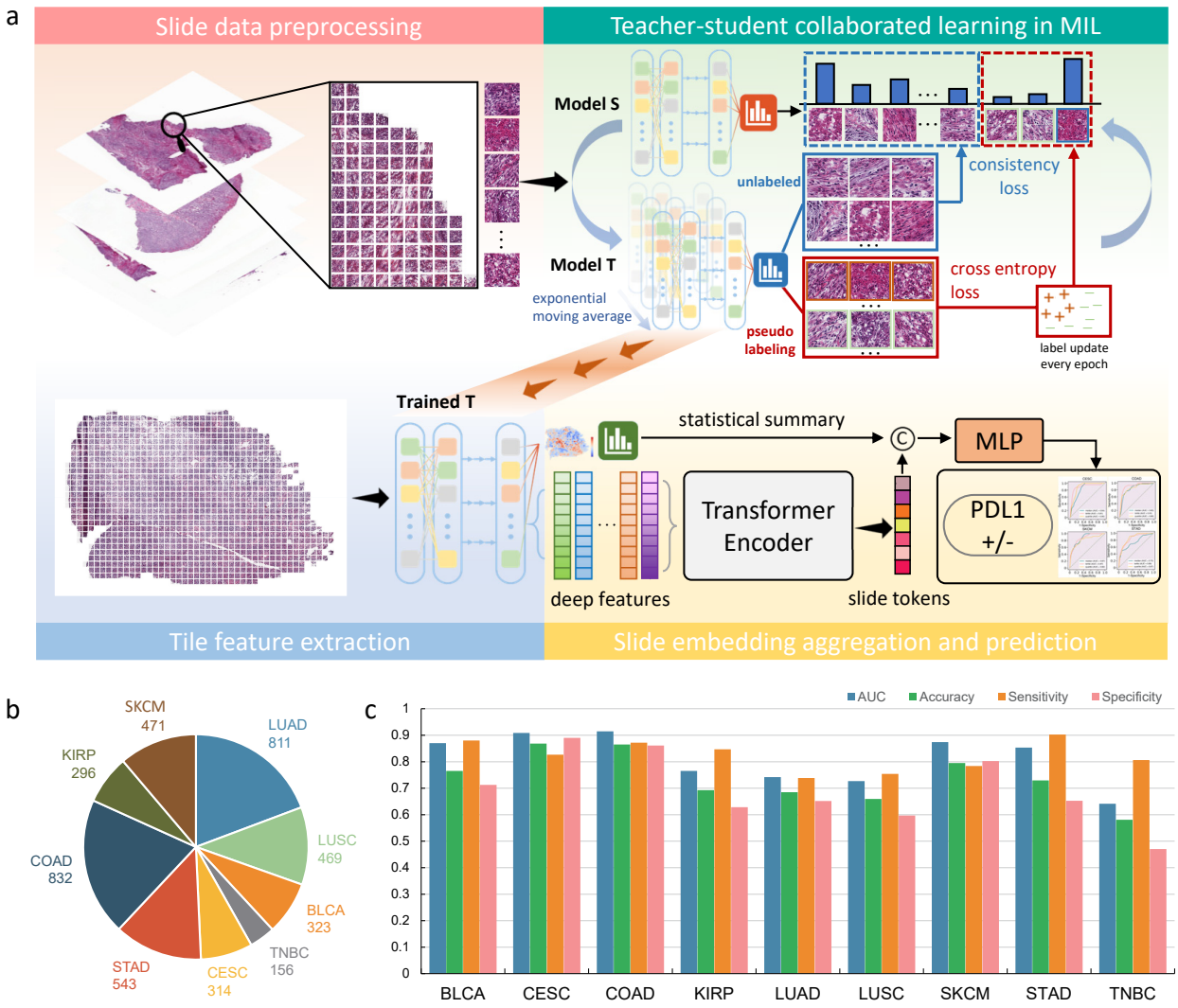

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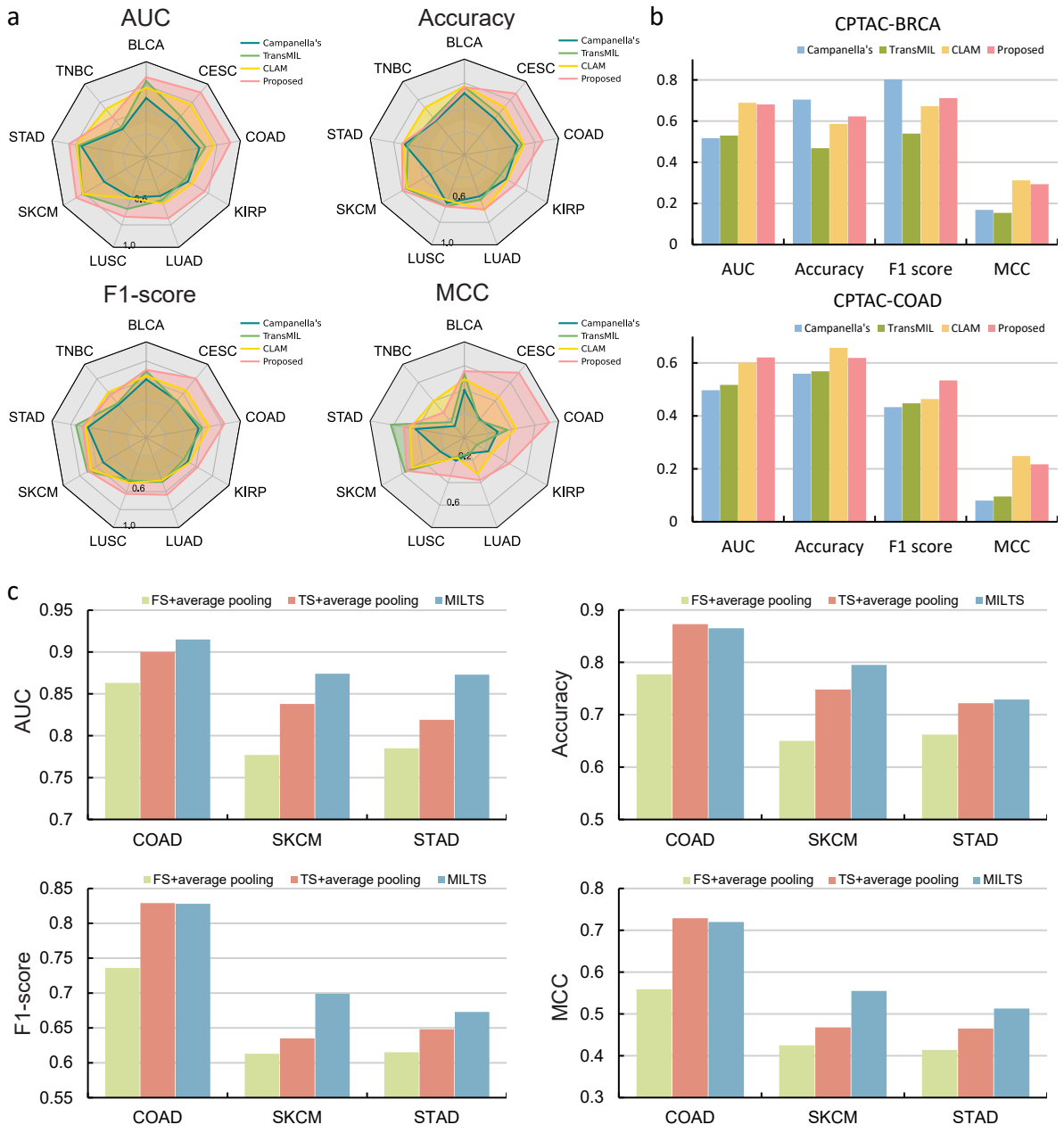

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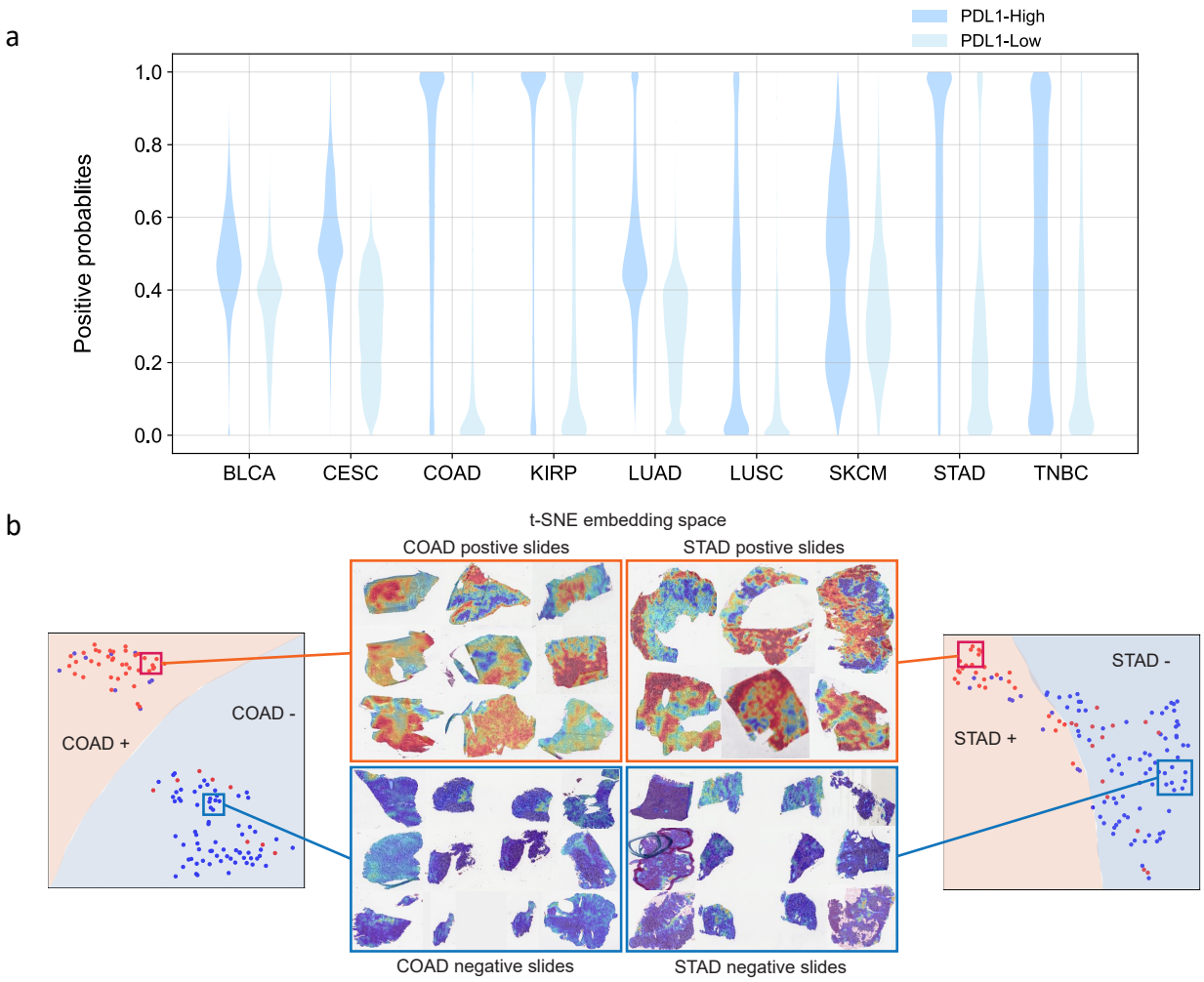

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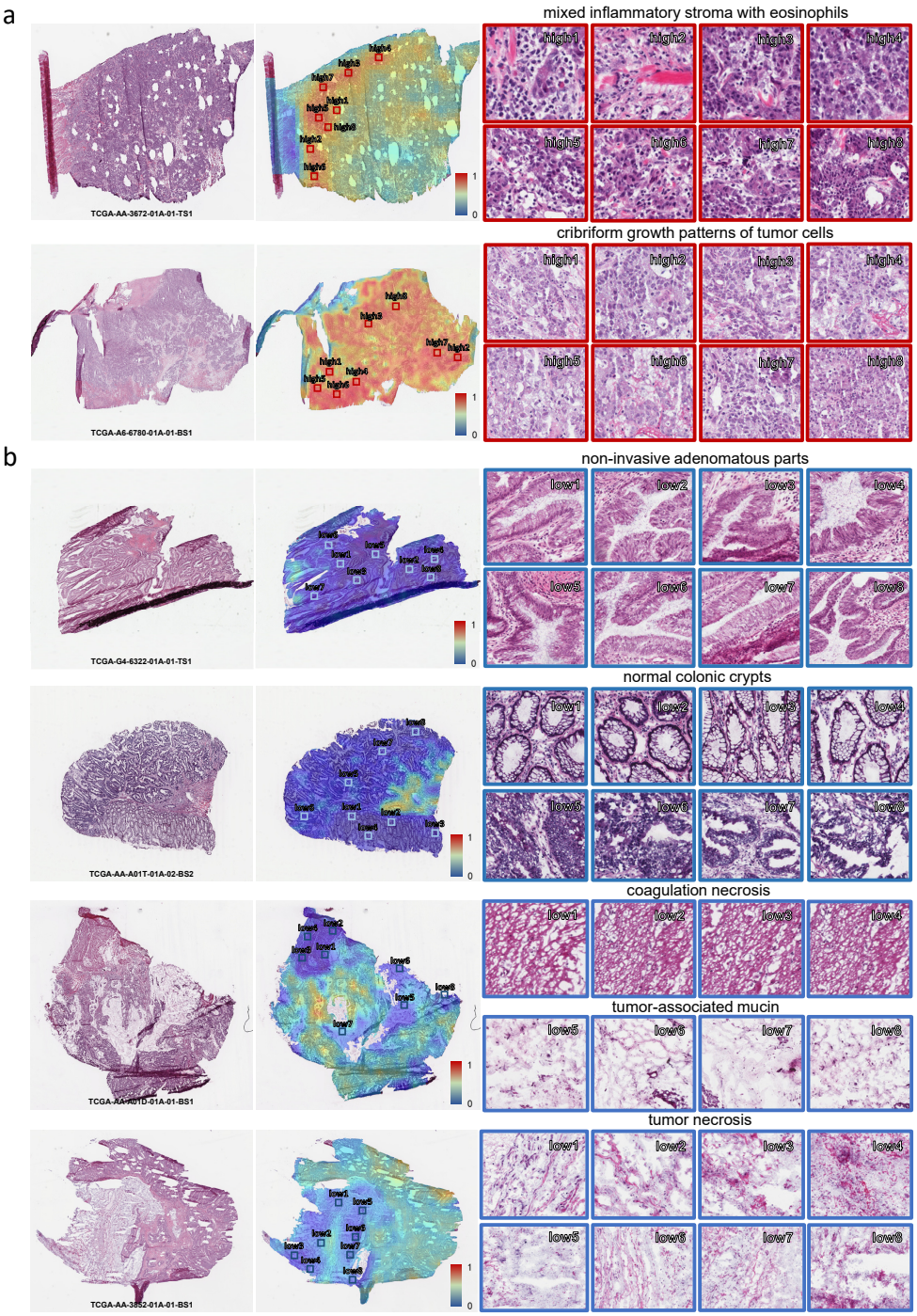

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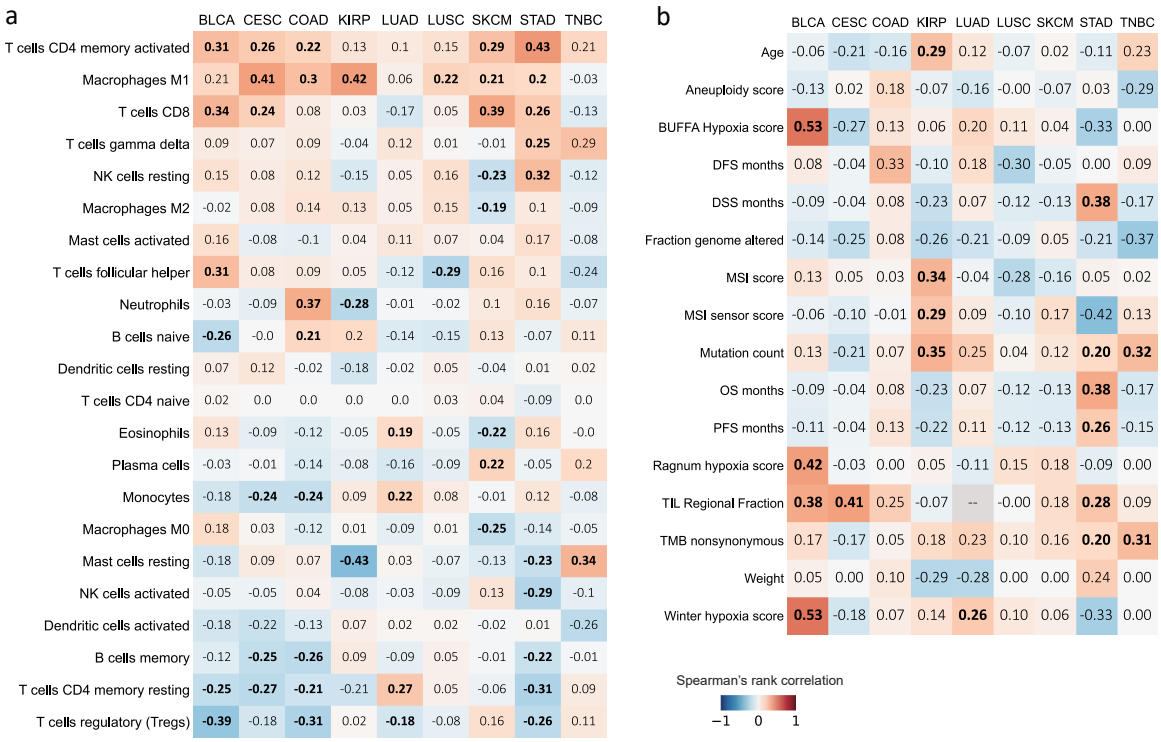

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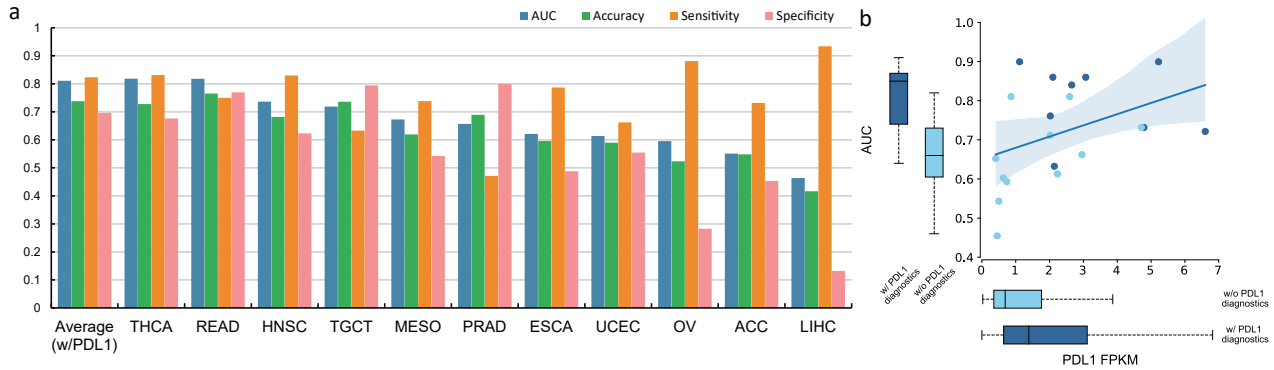

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