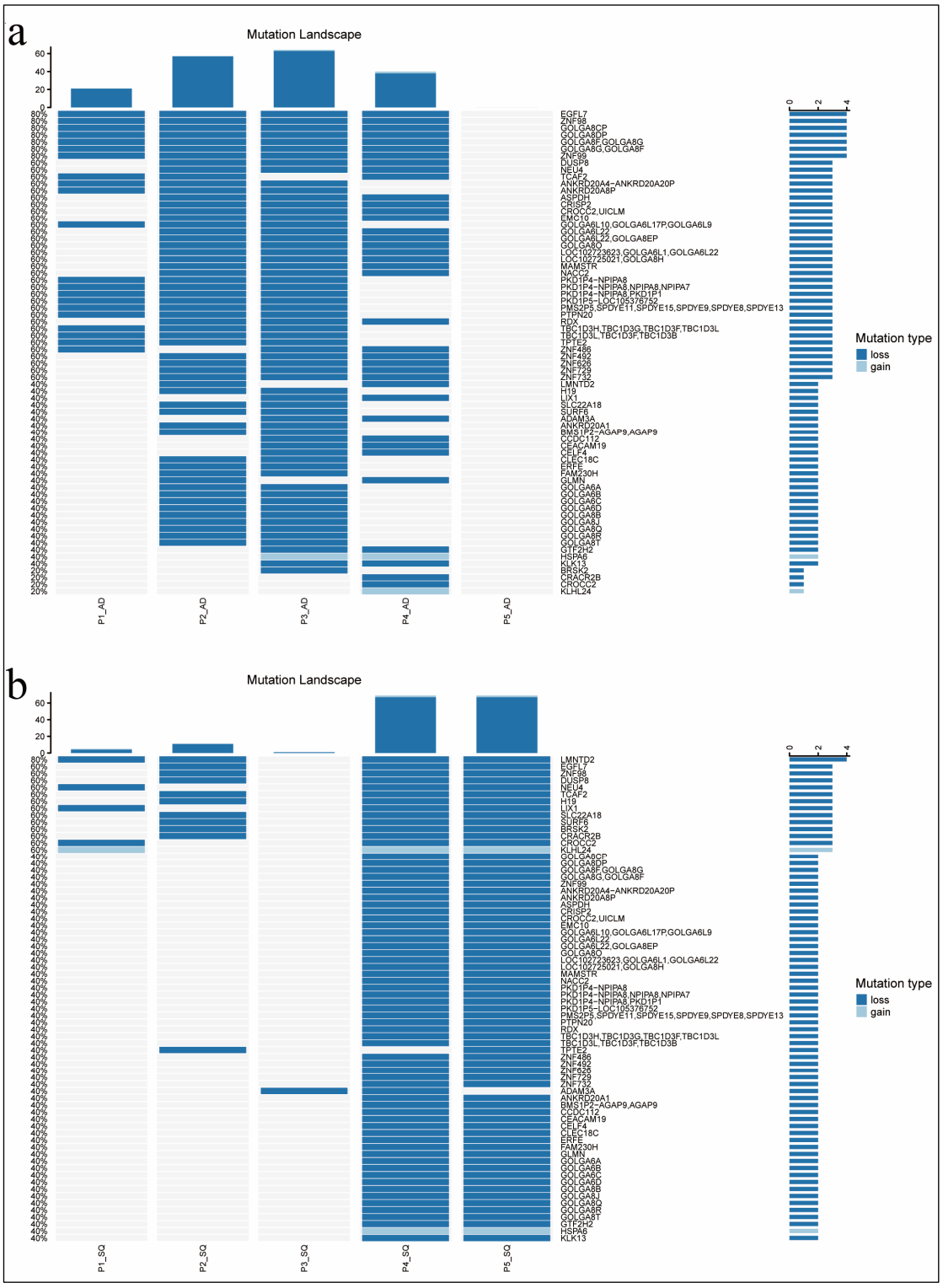
CNV landscape of adenocarcinoma and squamous cell carcinoma lesions

CNVs (copy number variants) refer to changes in the number of copies of a region on a chromosome. Supplementary Figure 1 shows the CNV landscape of ADC and SQCC patients. The genes with the most significant copy number changes in the ADC group were *EGFL7*, *ZNF98*, *GOLGA8CP*, *GOLGA8DP*, *GOLGA8F*, *GOLGA8G* and *ZNF99* (Supplementary Figure 1A, mutation frequency ≥ 80%), and *LMNTD2* (Supplementary Figure 1B, mutation frequency ≥ 80%) in SQCC group, respectively.



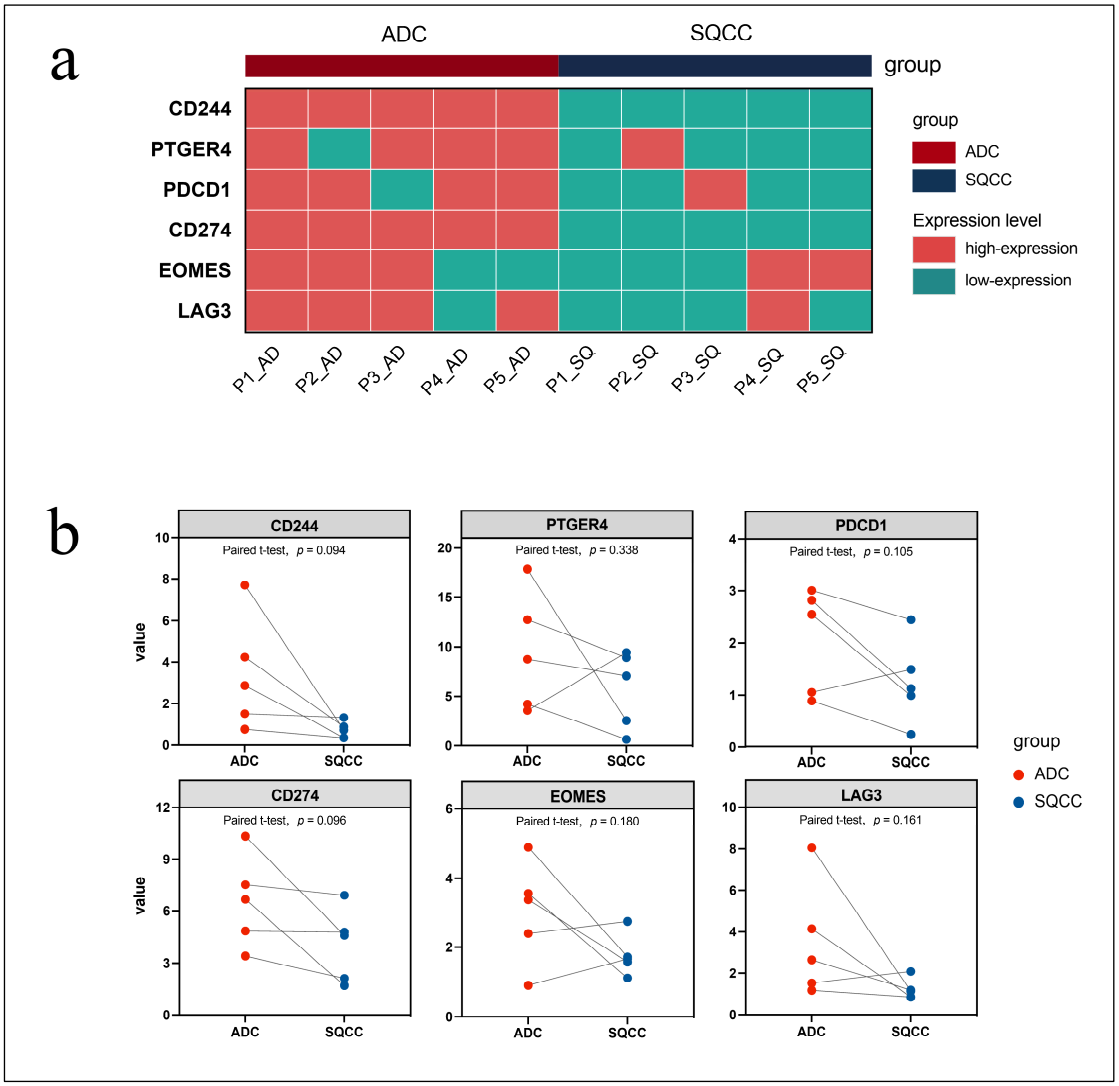
Supplementary Figure 1. CNV landscape of adenocarcinoma and squamous cell carcinoma lesions.

A, CNV landscape of 5 lesions in adenocarcinoma group; B CNV landscape of 5 lesions in squamous cell carcinoma group.

Exhausted CD8+ T cell-associated genes expression between MPLC

adenocarcinoma and squamous cell carcinoma lesions

In cancer, exhausted CD8+ is hyporesponsive to tumor cells[49]. We analyzed the expression of genes associated with exhausted CD8+ T cells with paired t-tests between SQCC and ADC lesions[50]. The analysis showed (Supplementary Figure 2A) that the overall trend of exhausted CD8+ T cell-related marker expression was more obvious in the ADC group; the analysis of specific group differences (Supplementary Figure 2B) showed that in the ADC group *CD244* ($p = 0.094$), *PTGER4* (Prostaglandin E 2 receptor 4) ($p = 0.338$), *PDCD1* (PD-1) ($p = 0.105$), *CD274* (PD-L1) ($p = 0.096$), *EOMES* (Eomesodermin) ($p = 0.108$), and *LAG3* (Lymphocyte activation gene 3) ($p = 0.161$) were higher than those in the SQCC group, but the differences were not significant ($p > 0.05$). This suggests that the abundance of exhausted CD8+ T cells and their depletion may be higher in ADC than in SQCC, thus ADC may be more likely to benefit from immune checkpoint inhibitor therapy.



Supplementary Figure 2. Expression analysis of exhausted CD8+ T cell-associated genes between groups of MPLC adenocarcinoma samples and squamous carcinoma samples.

A. Double heat map of exhausted CD8+ T cell-associated genes expression between two groups. High-expression, the relative expression level of gene sets in one lesion higher than the histologically paired lesion (eg. P1_AD and P1_SQ). B. Paired scatter-link plot of CD244, PTGER4 (Prostaglandin E 2 receptor 4), PDCD1 (PD-1), CD274 (PD-L1), EOMES (Eomesodermin), and LAG3 (Lymphocyte activation gene 3) expression between two groups.

Gene	Cell Type	Gene	Cell Type	Gene	Cell Type	Gene	Cell Type
BLK	B-cells	PTPRC	CD45	CCL13	DC	CSF3R	Neutrophils
CD19		CD8A	CD8 T cells	CD209		S100A12	
MS4A1		CD8B		HSD11B1		CEACAM3	
TNFRSF17		CTSW	Cytotoxic cells	CD244	Exhausted CD8	FCAR	
FCRL2		GNLY		EOMES		FCGR3B	
KIAA0125		GZMA		LAG3		FPR1	
PNOC		GZMB		PTGER4		SIGLEC5	
SPIB		GZMH		CD163	Macrophages	CD3D	T-cells
TCL1A		KLRB1		CD68		CD3E	
MS4A2	Mast cells	KLRD1		CD84		CD3G	
TPSAB1		KLRK1		MS4A4A		CD6	
CPA3		PRF1		IL21R	NK CD56dim cells	SH2D1A	
HDC		NKG7		KIR2DL3		TRAT1	
TPSB2		NCR1	NK cells	KIR3DL1		TBX21	Th1 cells
		XCL2		KIR3DL2		FOXP3	Treg
		XCL1					

Supplementary Table 1. Cell marker gene lists used in Scoring of Tumor Infiltrating Lymphocytes. DC, dendritic cells; Treg, regulatory T cells; NK cells, natural killer cells

Gene	Signature name	Gene	Signature name	Gene	Signature name	Gene	Signature name
IDO1	IFNg-signature	HLA-DPA1	MHC-Class-II	CTLA4	Immunoinhibitors	CD27	Immunocostimulators
CXCL10		HLA-DPB1		PDCD1		CD28	
CXCL9		HLA-DPB2		LAG3		CD40LG	
HLA-DRA		HLA-DQA1		BTLA		CD40	
STAT1		HLA-DQB1		CD160		CD70	
IFNG		HLA-DQB2		IDO1		CD80	
B2M	MHC-Class-I	HLA-DRB1		IL10		CD86	
TAP1		HLA-E	Non-Class	TIGIT		ICOS	
TAP2		HLA-F		TGFB1		IL6	
HLA-A		HLA-G		HAVCR2		TMEM173	
HLA-B		CCR5	Chemokines	CD274		TNFRSF13B	
HLA-C		CCL3		PDCD1LG2		TNFRSF14	
ICAM1	Adhesion-molecules	CCL4		GZMA	Cytolytic-activity	TNFRSF17	
ICAM2		CCL5		PRF1		TNFRSF18	
ICAM3		CXCL9				TNFRSF4	
ICAM4		CXCL10				TNFRSF9	
ICAM5		CXCL11				TNFSF13B	
VCAM1						TNFSF13	

Supplementary Table 2. Lists of genes used in Immune microenvironment characterization.