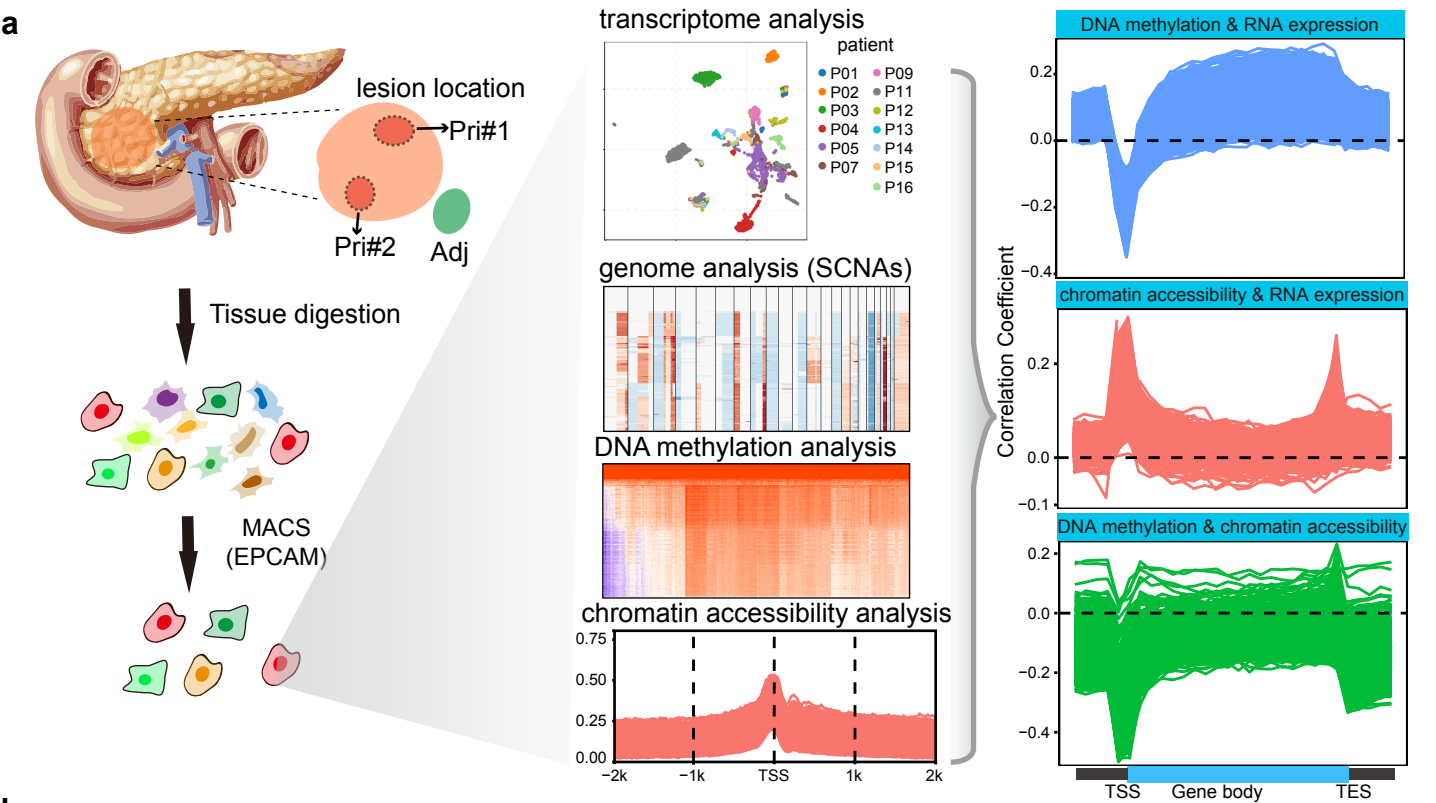


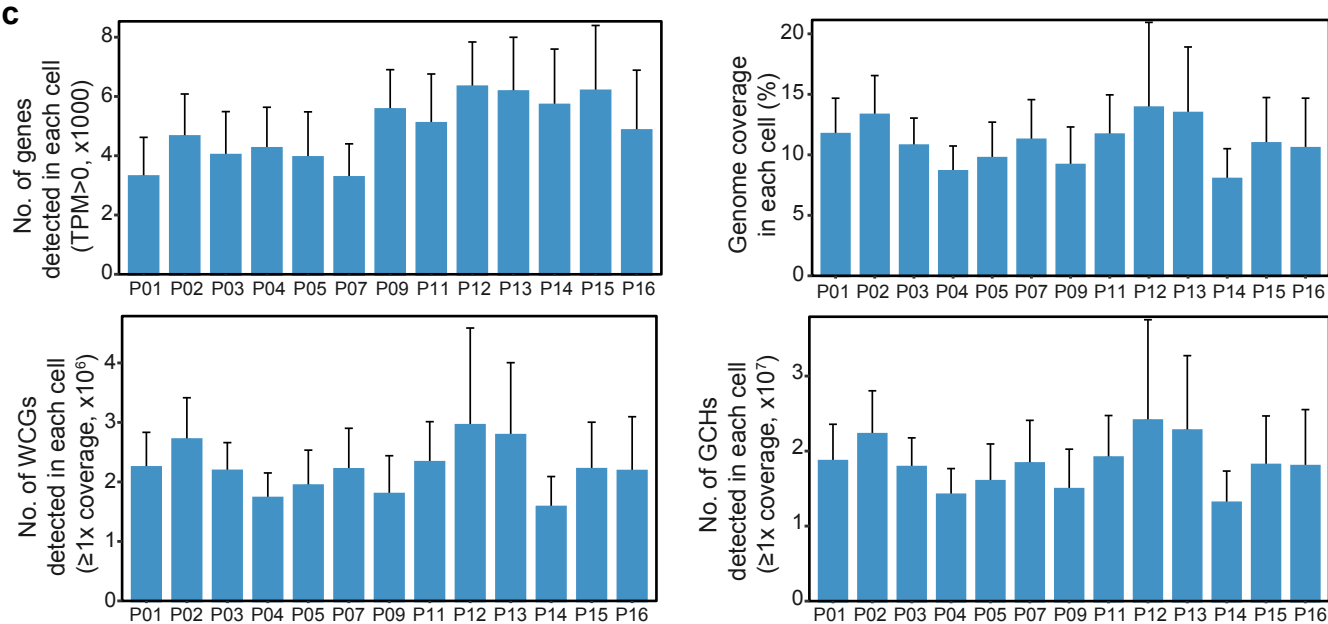
Figure S1



b

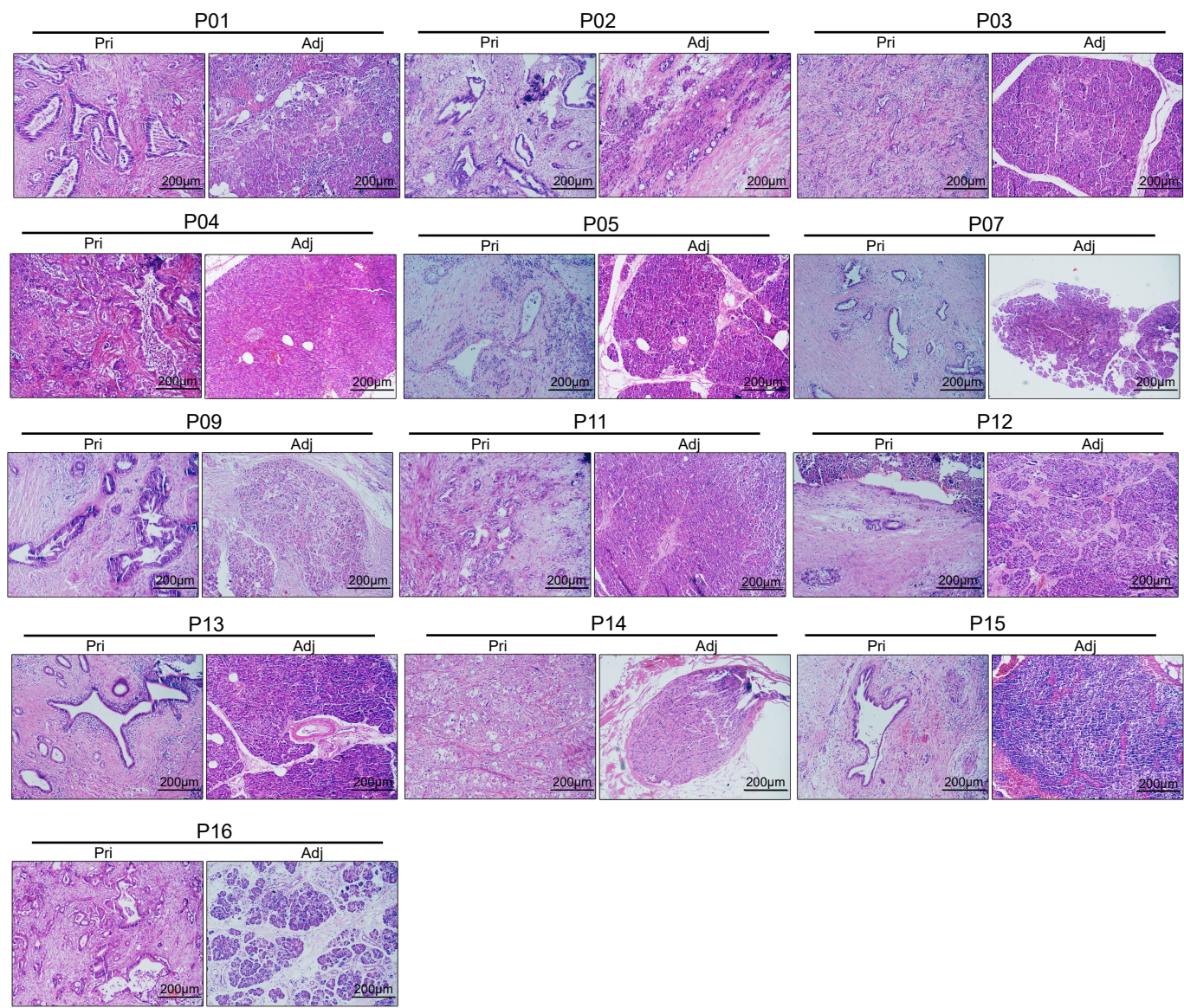
Tissue region	Pri #1				Pri #2				Pri #3				Adj		
	No. of cells with RNA	No. of cells with quality passed RNA	No. of cancer cells	No. of Norm_epi cells	No. of cells with RNA	No. of cells with quality passed RNA	No. of cancer cells	No. of Norm_epi cells	No. of cells with RNA	No. of cells with quality passed RNA	No. of cancer cells	No. of Norm_epi cells	No. of cells with RNA	No. of cells with quality passed RNA	No. of Norm_epi cells
P01	96	82	12	12	0	0	0	0	0	0	0	0	0	0	0
P02	16	16	12	0	80	64	37	0	96	93	61	1	0	0	0
P03	192	184	27	0	192	183	23	0	0	0	0	0	0	0	0
P04	192	169	21	16	192	166	33	3	0	0	0	0	0	0	0
P05	480	402	104	45	480	413	176	1	0	0	0	0	0	0	0
P07	48	43	16	16	48	42	17	8	0	0	0	0	96	79	49
P09	96	90	13	0	80	76	10	1	0	0	0	0	32	27	0
P11	192	174	56	21	192	182	132	6	192	182	101	10	96	82	23
P12	48	33	13	0	48	40	23	0	0	0	0	0	48	47	0
P13	48	31	16	0	48	45	26	0	0	0	0	0	0	0	0
P14	48	33	19	0	48	45	29	0	0	0	0	0	0	0	0
P15	48	35	18	2	48	32	25	0	0	0	0	0	24	18	1
P16	48	35	22	3	48	46	35	0	0	0	0	0	48	36	0
Total	1,552	1,327	349	115	1,504	1,334	566	19	288	275	162	11	344	289	73

*A total of 3,225 single cells passed the RNA quality control, among which 1,295 single cells were also passed the DNA methylome and SCNA quality control.



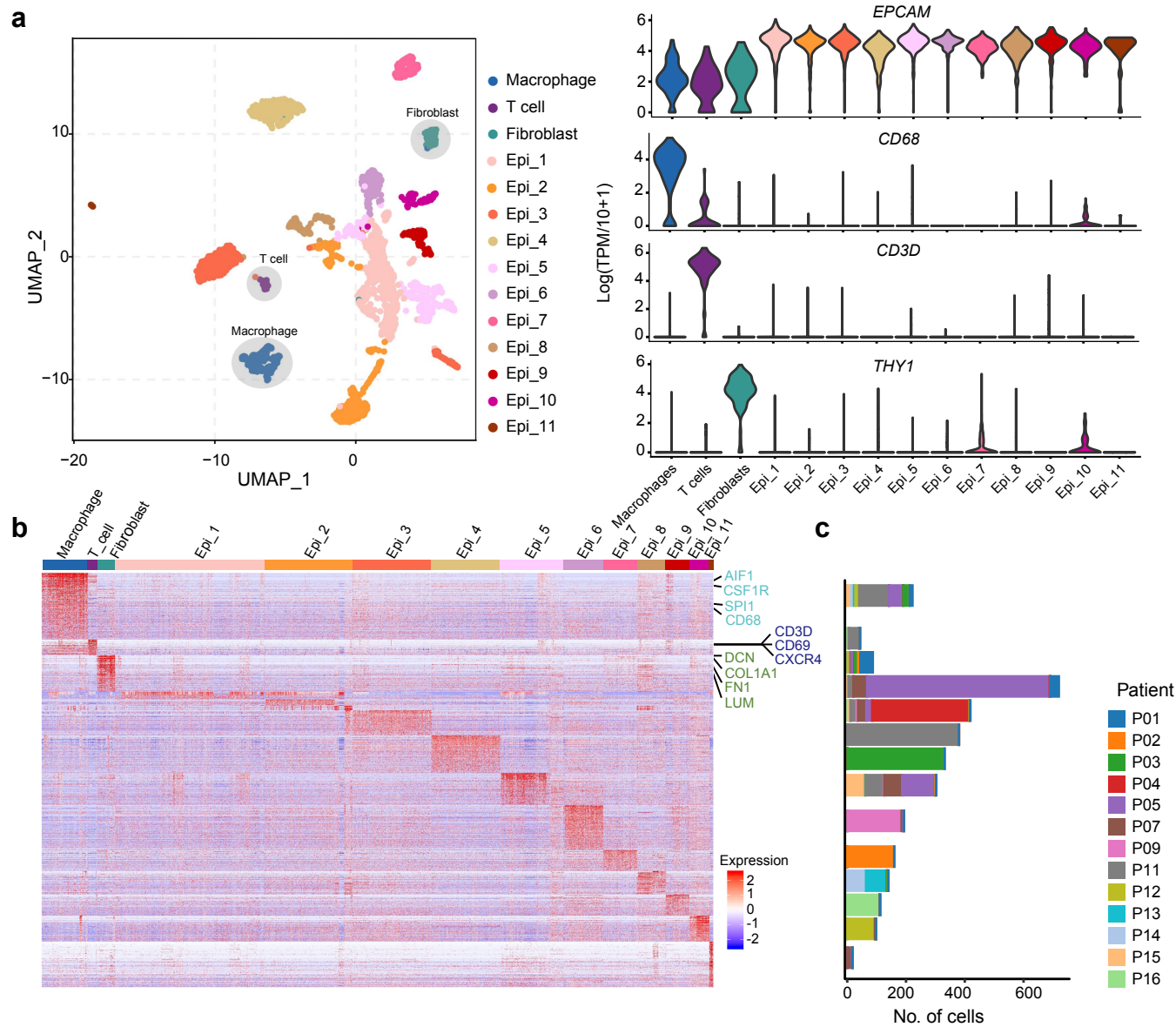
Supplementary Fig. S1 Overview of sampling and data analyses. **a** Schematics of experimental and bioinformatic approaches to obtain epithelial cells and further perform integrated multiomics analysis. **b** Details of cell numbers from each region in each patient with profiling data of different omics layers. **c** Histogram showing the number of detected genes, WCG sites and GCH sites in each individual cell. The DNA methylome data in each individual cell cover ~10% of the whole genome.

Figure S2



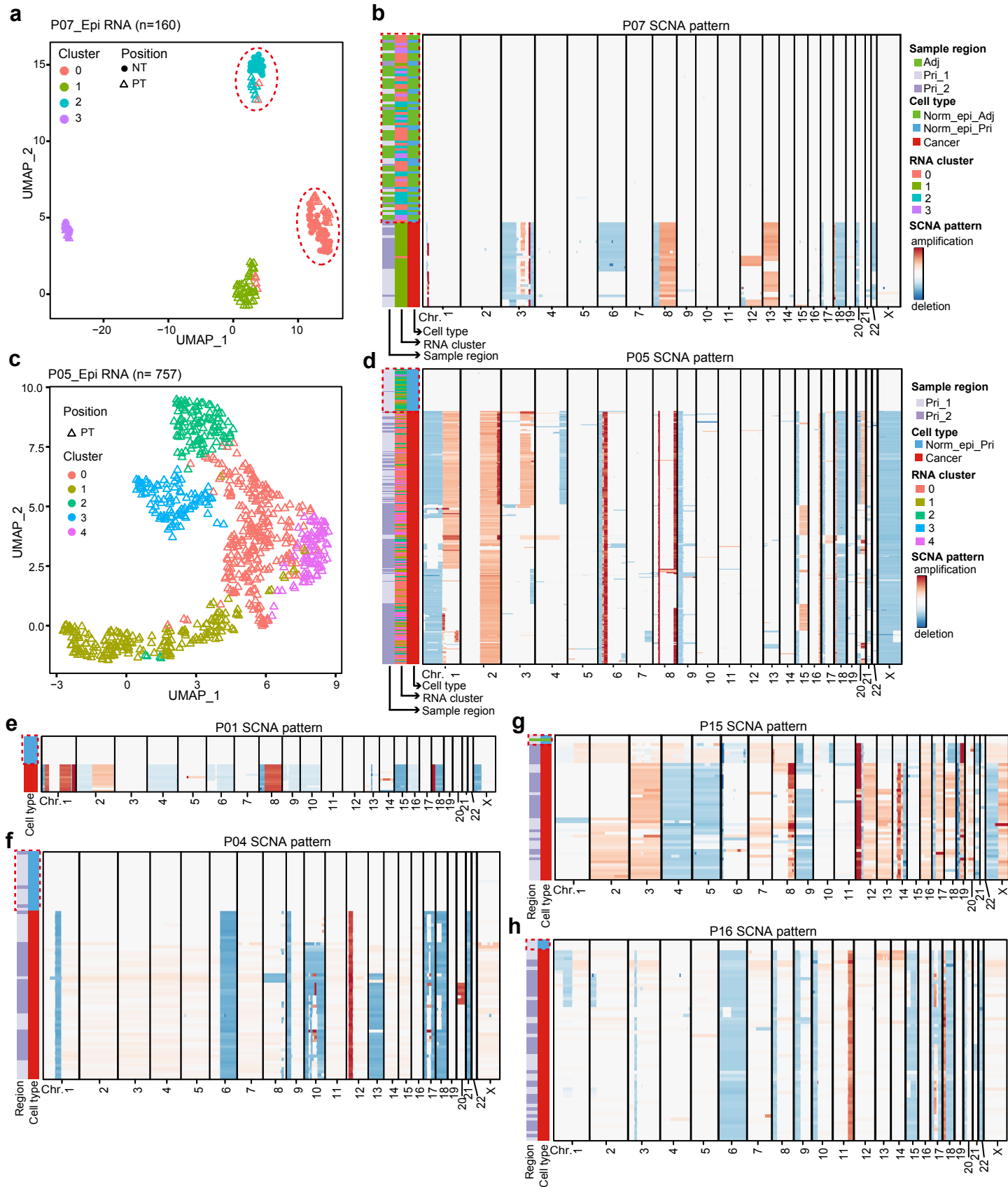
Supplementary Fig. S2 PDAC tumor tissue configuration. Hematoxylin and eosin (H&E) staining of primary tissue and adjacent tissue for each patient we analyzed in this study. The adjacent tissue of P09 shows an abnormal structure with abundant stromal cells, similar to that in tumor tissues.

Figure S3



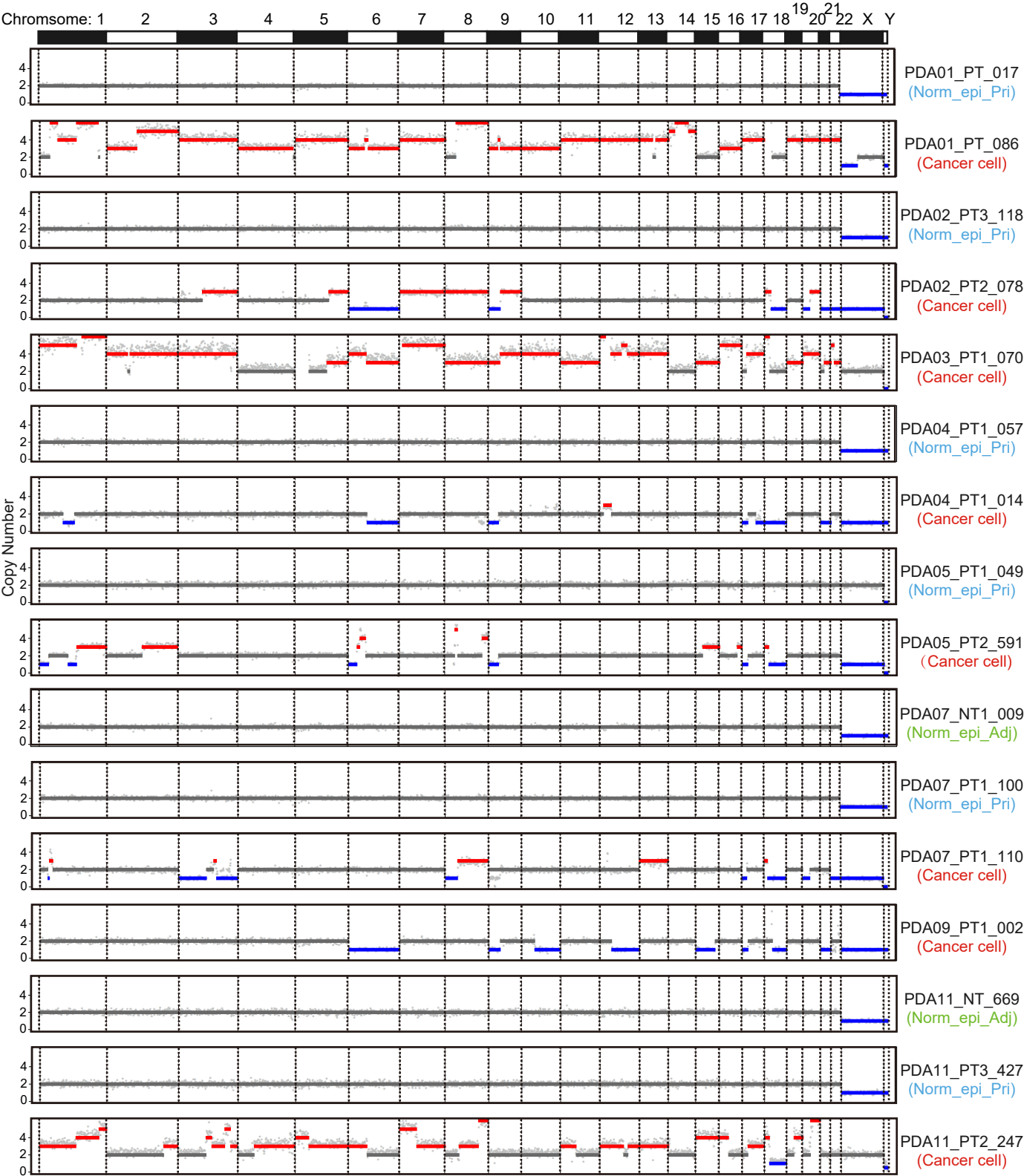
Supplementary Fig. S3 Cell type identification by the single-cell transcriptome aspects of the single-cell multiomics data. a Unsupervised clustering of all cells from the 13 patients using gene expression profiles. The expression levels of the representative cell type marker genes (*EPCAM*-high: epithelial cell; *CD68*-high: macrophage; *CD3D*-high: T cell; *THY1*-high: fibroblast) in each cluster are shown in the violin plot on the right. **b** Heatmap of cluster-specific gene expression further confirming the identification of each cell type. **c** Statistics of cell originations in each cluster corresponding to **b**.

Figure S4



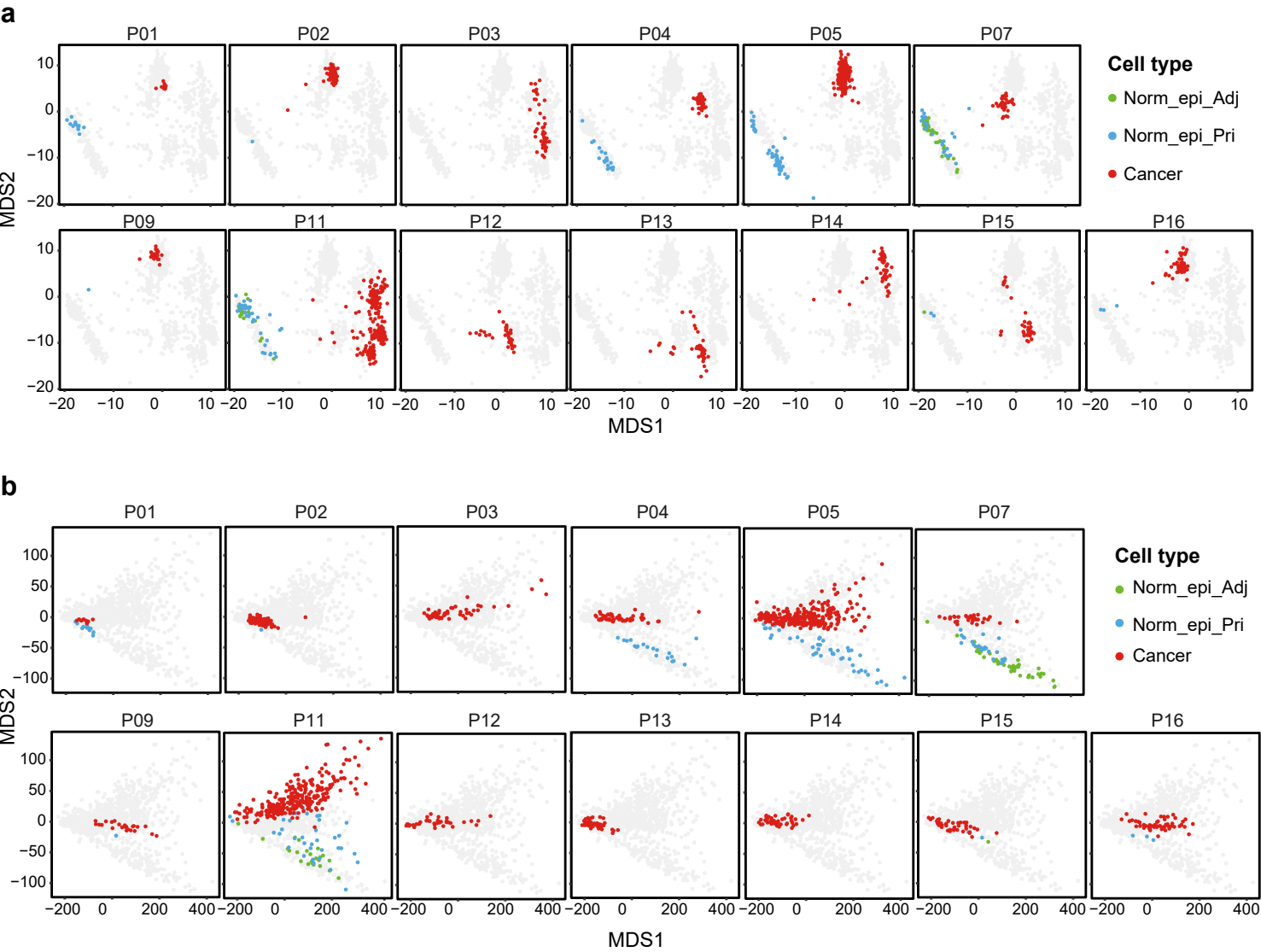
Supplementary Fig. S4 Epithelial cells with euploid genomes identified in the primary tumor tissue of each patient. a, c Unsupervised clustering of epithelial cells in P07 and P05 using gene expression profiles. The red dashed line indicates that epithelial cells in the primary tumor show similar expression patterns by clustering with those in adjacent tissue. **b, d–h** SCNA maps showing a high frequency of subchromosome-scale SCNAs in each PDAC patient. The dashed line and the red arrow indicate epithelial cells in the primary tissue regions.

Figure S5



Supplementary Fig. S5 Representative SCNA results for each cell type in the patients. Single Norm_epi cells have euploid genomes, and cancer cells show diversified subchromosome-scale SCNAs across different chromosomes in different patients.

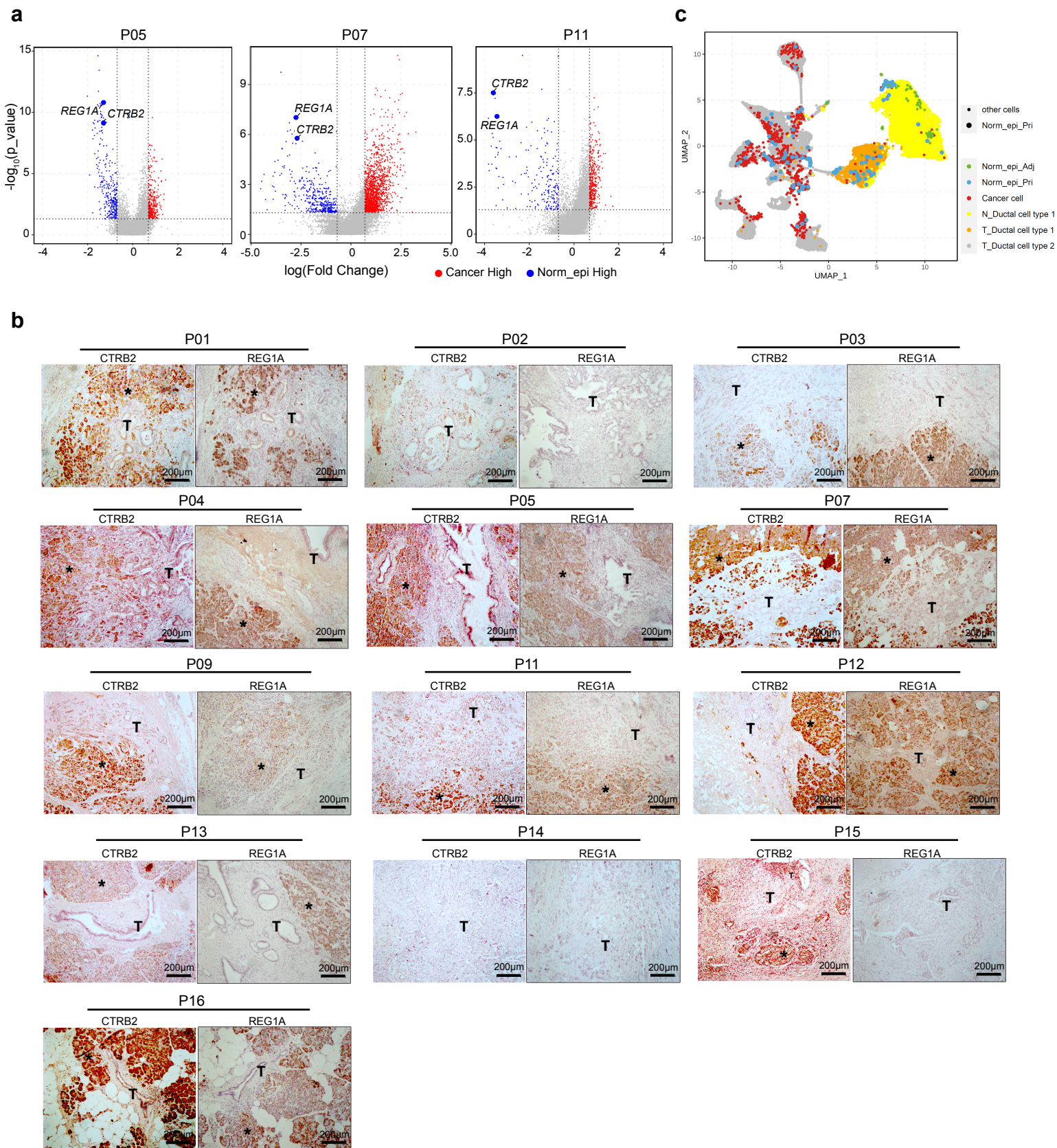
Figure S6



Supplementary Fig. S6 Norm_epi_Pri cells can be separated from cancer cells according to the GCH and WCG methylation levels in promoter region. a, b

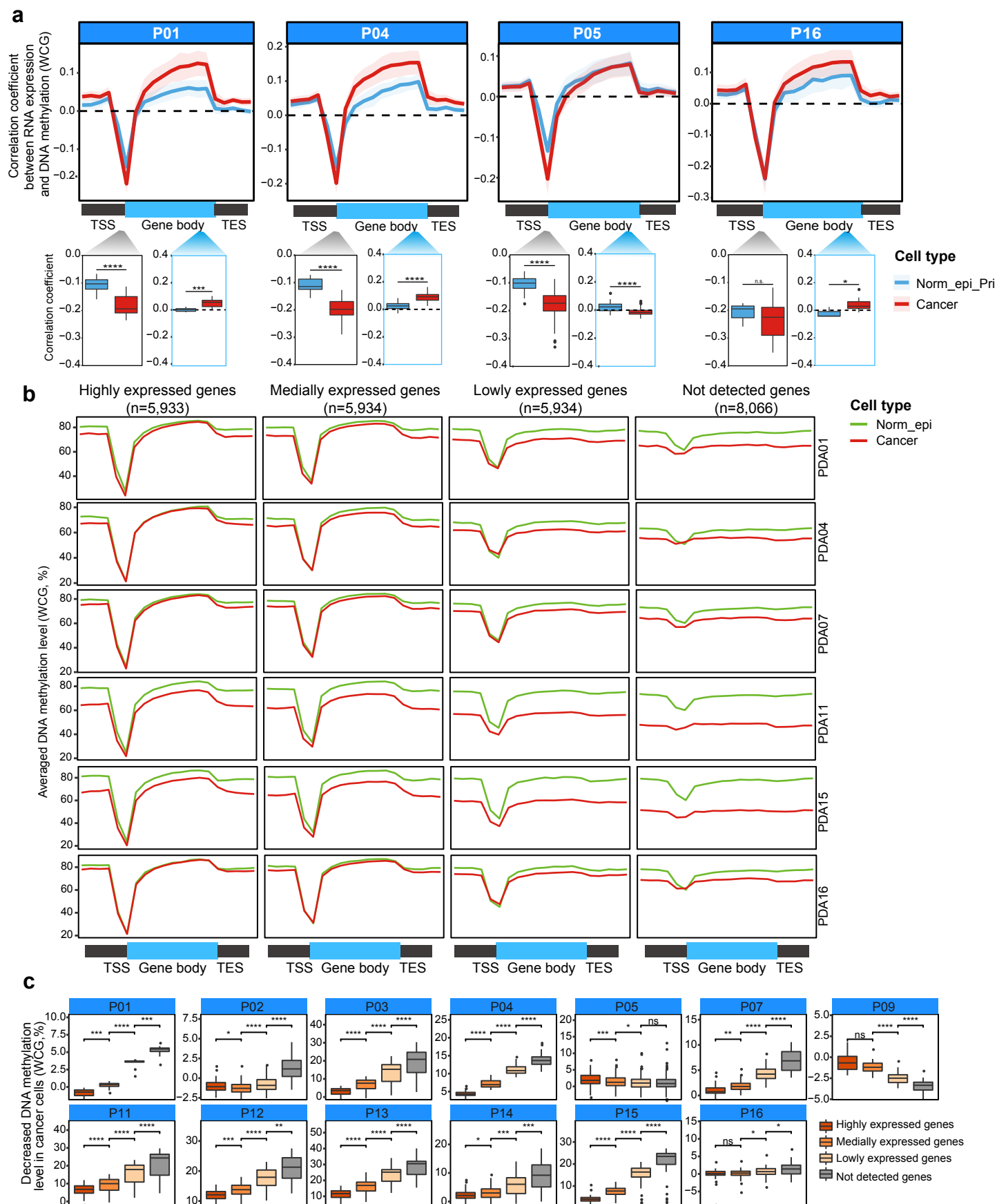
Multidimensional scaling analysis of promoter DNA methylation levels **(a)** and normalized chromatin accessibility levels **(b)** for all epithelial cells in each patient.

Figure S7



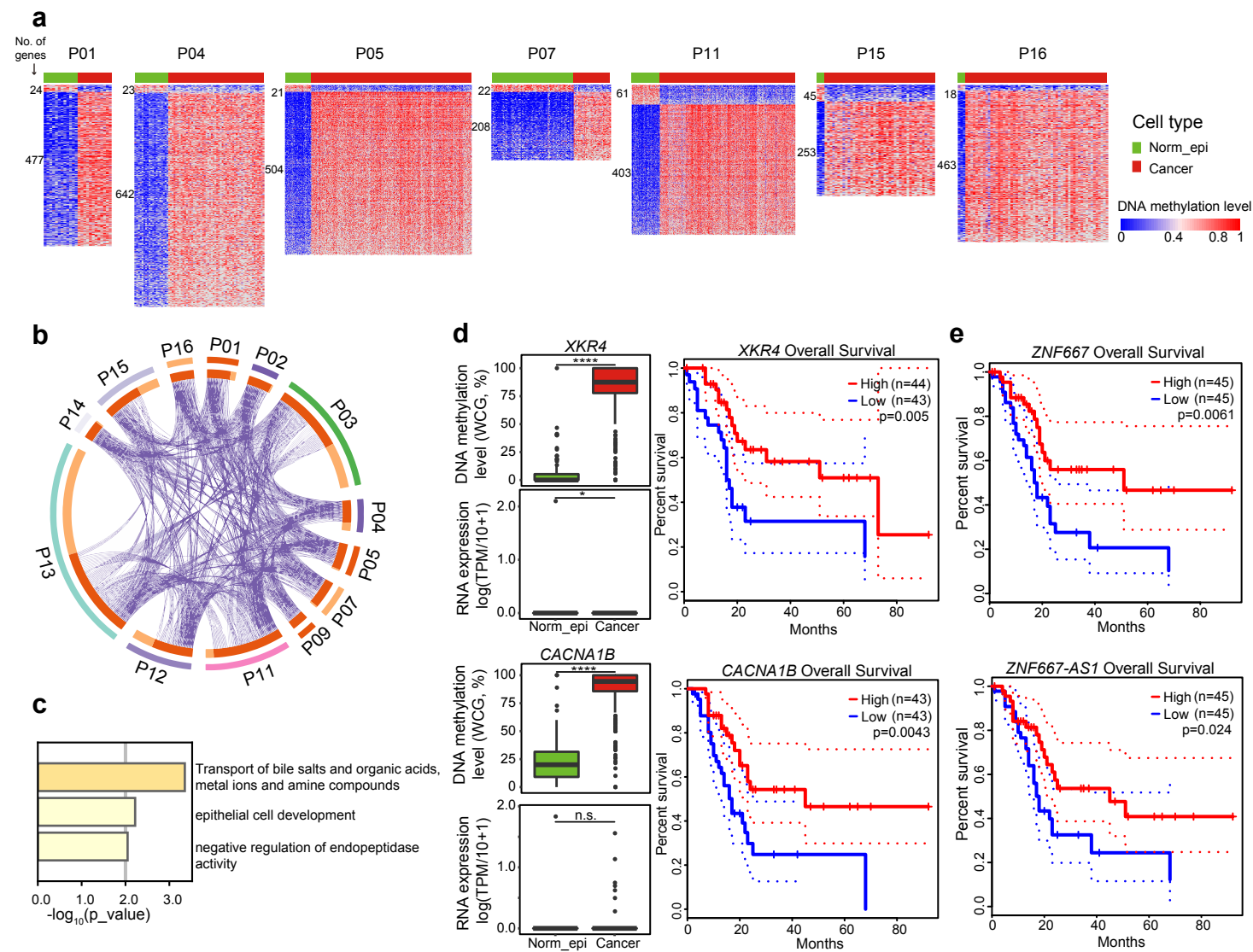
Supplementary Fig. S7 Characteristics of Norm_epi_Pri cells. **a** The volcano plots of DEGs (P -value < 0.05 , Fold Change > 2 or < 0.5) between cancer cells and Norm_epi cells in P05, P07 and P11. *REG1A* and *CTRB2* were highly detected in the Norm_epi cells of each patient. **b** The UMAP plot of epithelium data integration between this study and Peng *et al.* (2019). **c** Immunostaining of *REG1A* and *CTRB2* in the tumor tissue of each patient. Only P02 and P14 were not detected with positive signals.

Figure S8



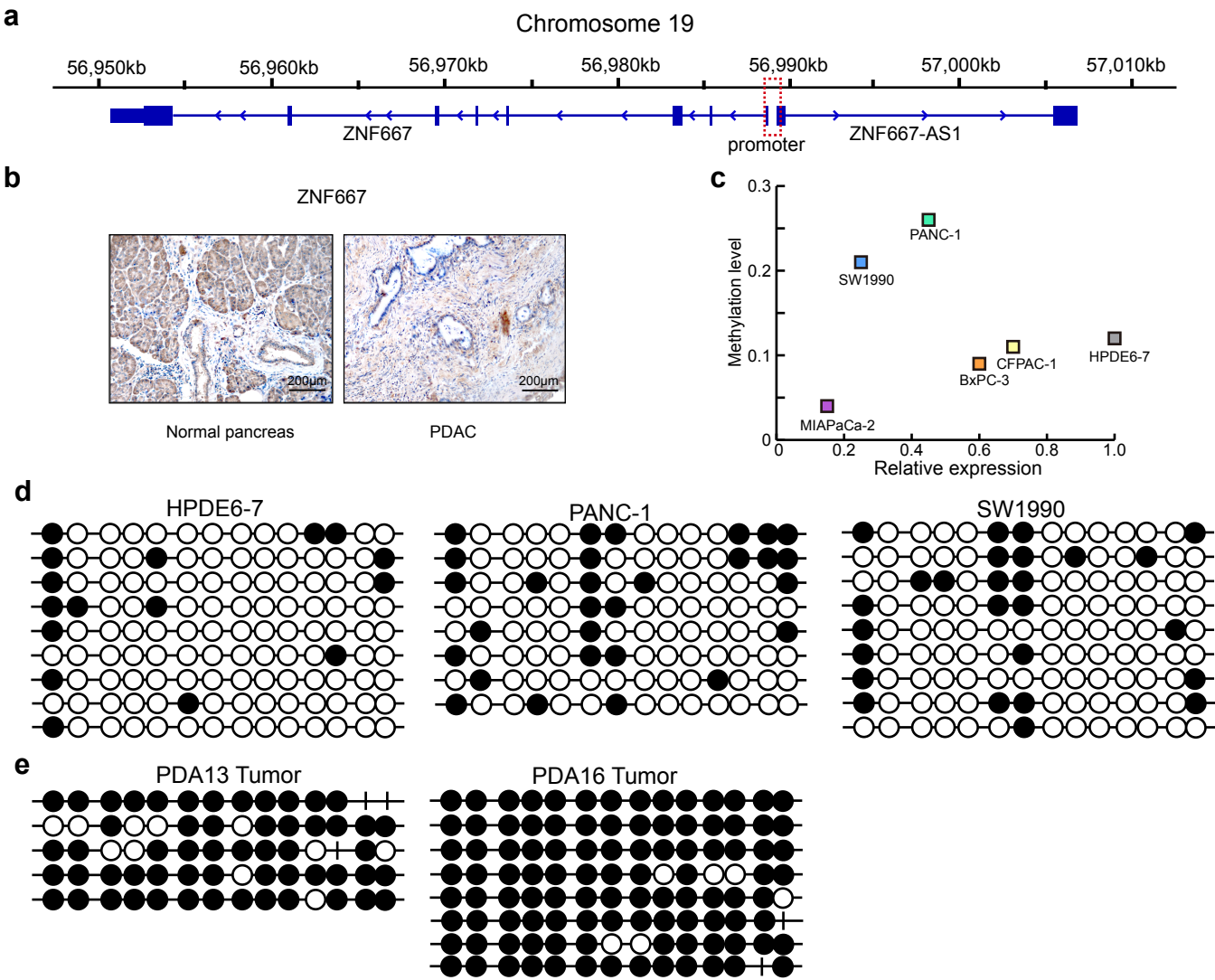
Supplementary Fig. S8 DNA methylation shows a stronger correlation with RNA expression in cancer cells. **a** Spearman correlations between DNA methylation levels across gene bodies (including 15 kb flanking regions) and corresponding RNA expression levels in each cell type in representative patients. The boxplots at the bottom show the statistical test results between cancer cells and Norm_epi cells using the Wilcoxon rank sum test. The promoter regions are from -1 kb to +0.5 kb around the TSS, and the gene body regions are +2 kb from the TSS to the TES. n.s., no significance; $*P < 0.05$; $**P < 0.01$; $***P < 0.001$; $****P < 0.0001$. **b** Averaged DNA methylation levels across gene bodies in different groups of genes. The genes are grouped according to their expression levels in Norm_epi cells. **c** Boxplots showing the decreased DNA methylation levels in cancer cells compared to Norm_epi cells in each group of genes. All 13 patients showed significantly stronger DNA demethylation in lowly and not expressed genes. P09 showed a reversed pattern.

Figure S9



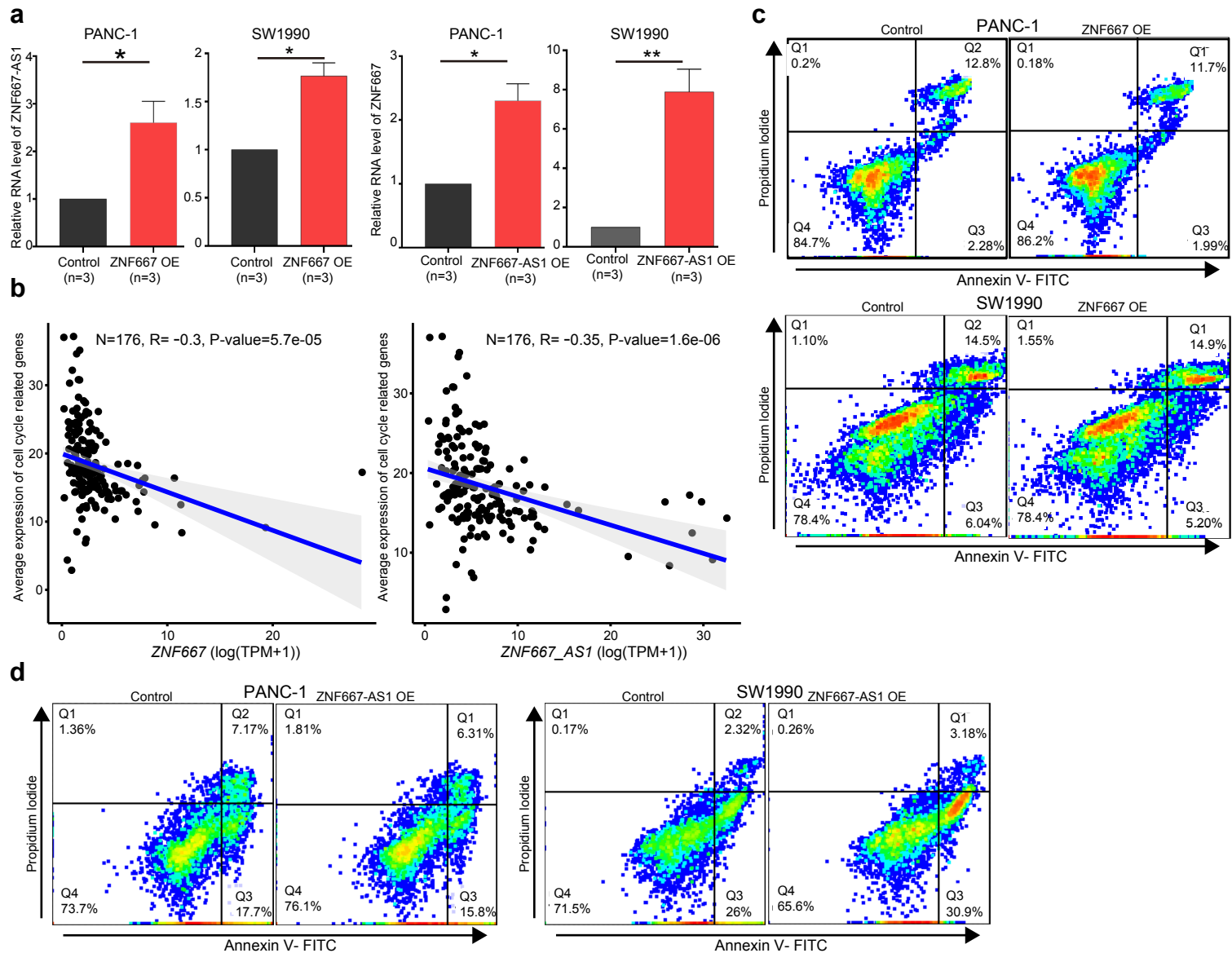
Supplementary Fig. S9 Promoter regions in cancer cells show global DNA hypermethylation. **a** Heatmaps showing the DNA methylation levels of DMPs (differentially methylated promoters) between cancer cells in each patient and all Norm_epi cells. Patients with over 3 Norm_epi cells are shown. **b** Overlap of hypomethylated promoters across all patients. The purple lines indicate shared genes between different patients. **c** Enriched biological processes for hypomethylated genes in at least 3 patients. **d** The representative genes, *XKR4* and *CACNA1B*, showing increased promoter DNA methylation levels, were significantly correlated with overall survival of PDAC patients recorded in the TCGA database. **e** The two novel candidate genes *ZNF667* and *ZNF667-AS1* are significantly correlated with overall survival of the PDAC patients in the TCGA database.

Figure S10



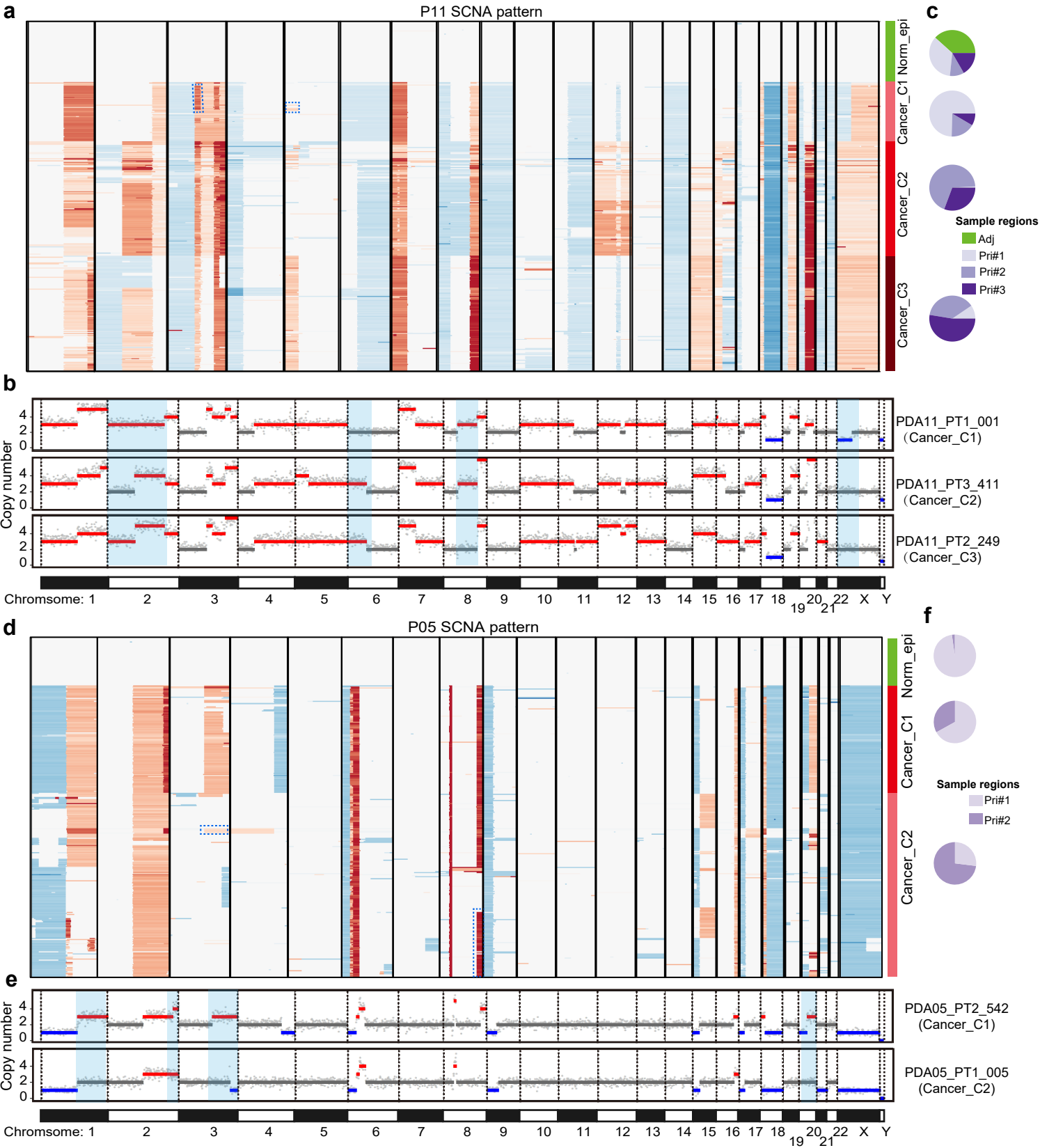
Supplementary Fig. S10 Abundance of *ZNF667* and *ZNF667-AS1* in pancreas cancer cell lines. **a** The genome locations of *ZNF667* and *ZNF667-AS1*. **b** Immunohistochemistry of *ZNF667* showing variations of abundance in normal pancreas and PDAC using the tissue microarray analysis. Scale bar, 200 μm . **c** The methylation level and relative expression of *ZNF667-AS1* in each cell line. All *in vitro* cell types showed lower methylation level than that we detected in *in vivo* PDAC cancer cells. **d, e** Plots showing the methylation pattern of a CpG island containing 14 CpG sites in the *ZNF667-AS1* promoter region in the normal cell line HPDE6-7, the two pancreas cancer cell lines PANC-1 and SW1990 (**d**), and two tumor samples (**e**).

Figure S11



Supplementary Fig. S11 Functional validations of *ZNF667* and *ZNF667-AS1* in the pancreas cancer cell lines. **a** Boxplot showing the relative changes in the expression levels of *ZNF667* when overexpressing *ZNF667-AS1*, and the other way around, in the two cancer cell lines. **b** Correlation of the expression levels between *ZNF667*, *ZNF667-AS1* and the cell cycle genes according to the transcriptome data in TCGA PAAD patients. **c, d** FACS record of apoptosis detection in the two pancreas cell lines after overexpression *ZNF667* and *ZNF667-AS1*.

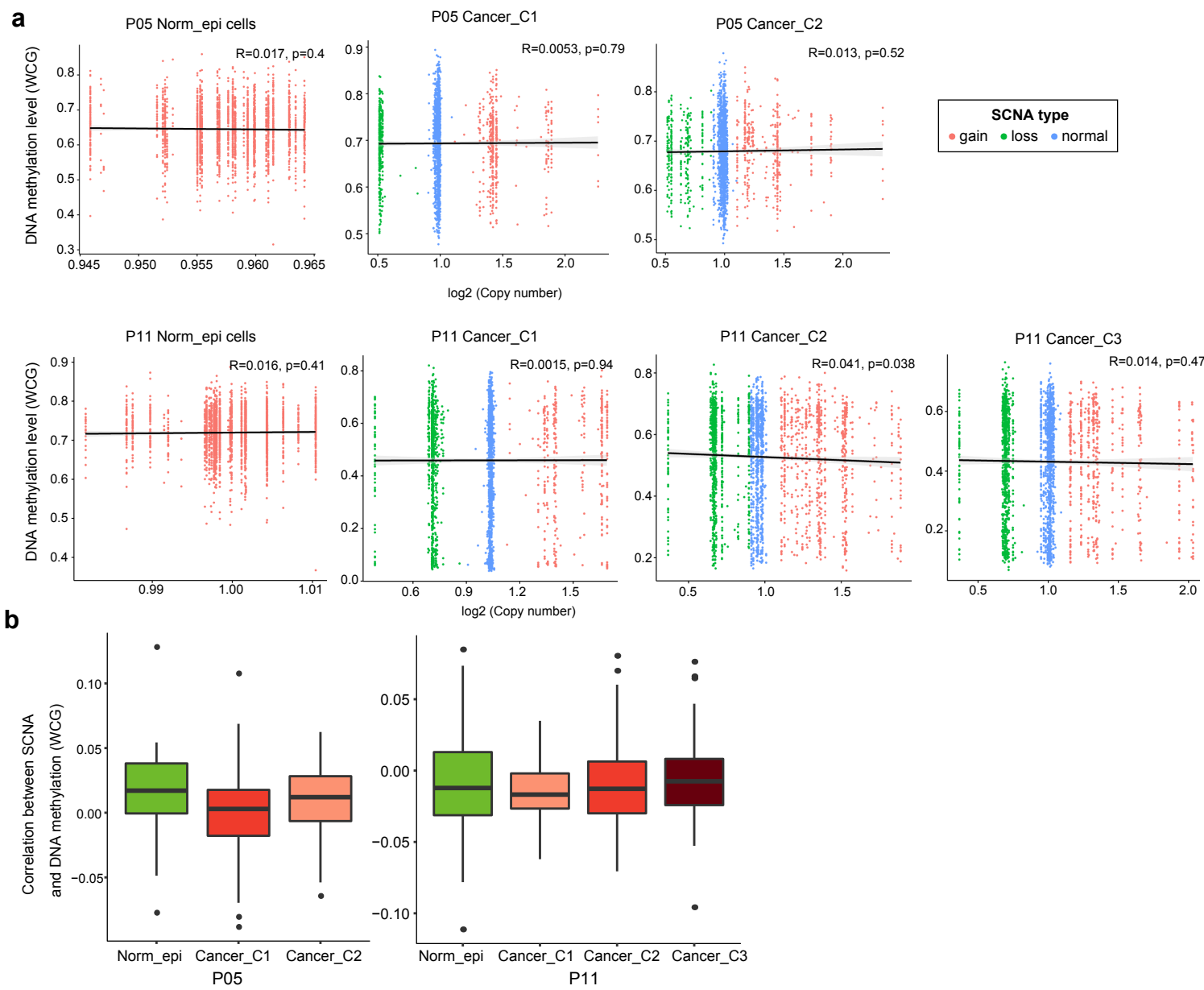
Figure S12



Supplementary Fig. S12 Global SCNA patterns indicate subclones in P05 and P11.

a, d The global SCNA patterns of P05 and P11 (each with > 200 cancer cells) and subclones indicated on the right. **b, e** Examples of single-cell SCNA patterns in each subclone. **c, f** Pie charts showing the regional compositions in each subclone of cells.

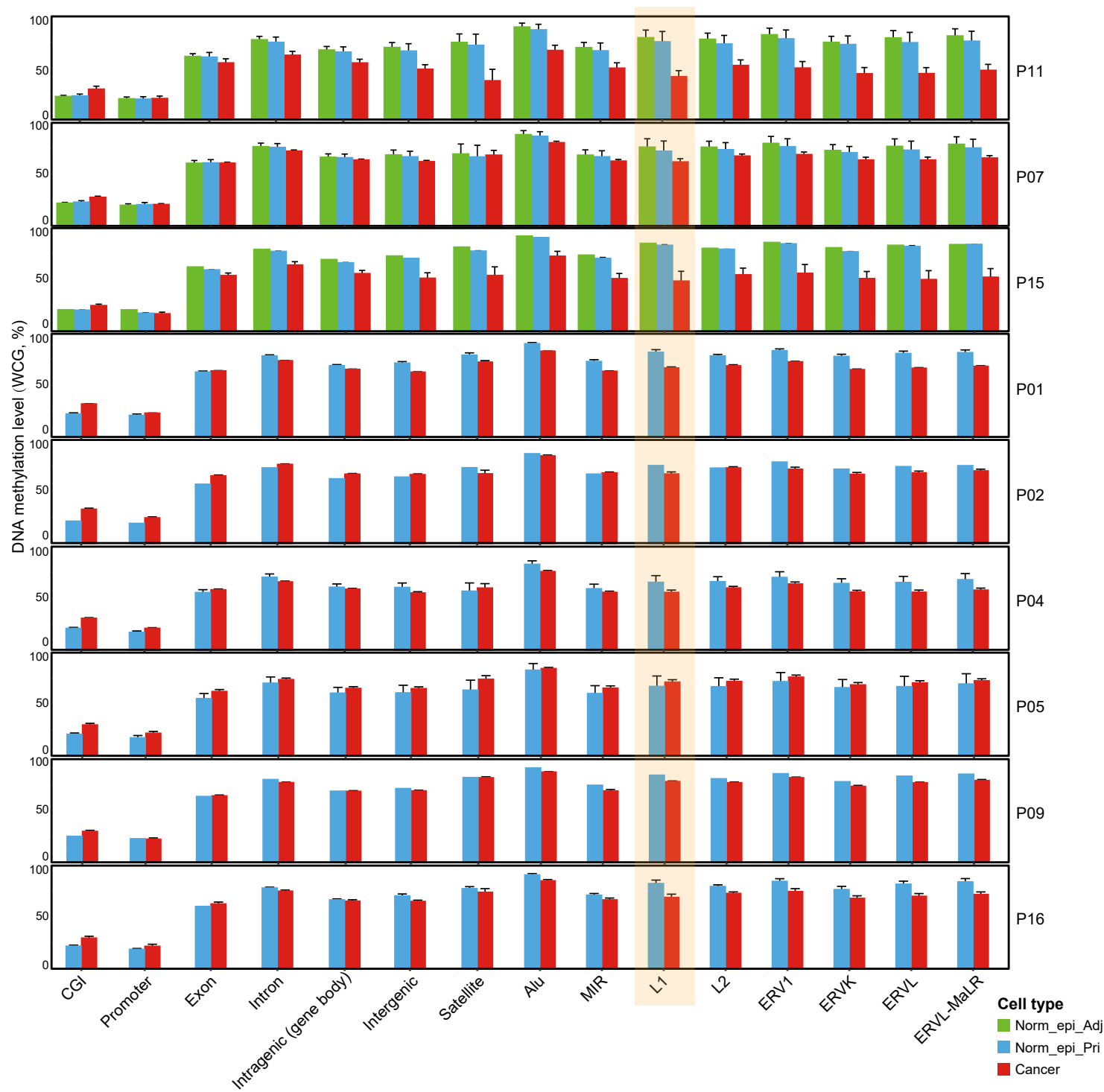
Figure S13



Supplementary Fig. S13 Correlations between SCNA and DNA methylation level.

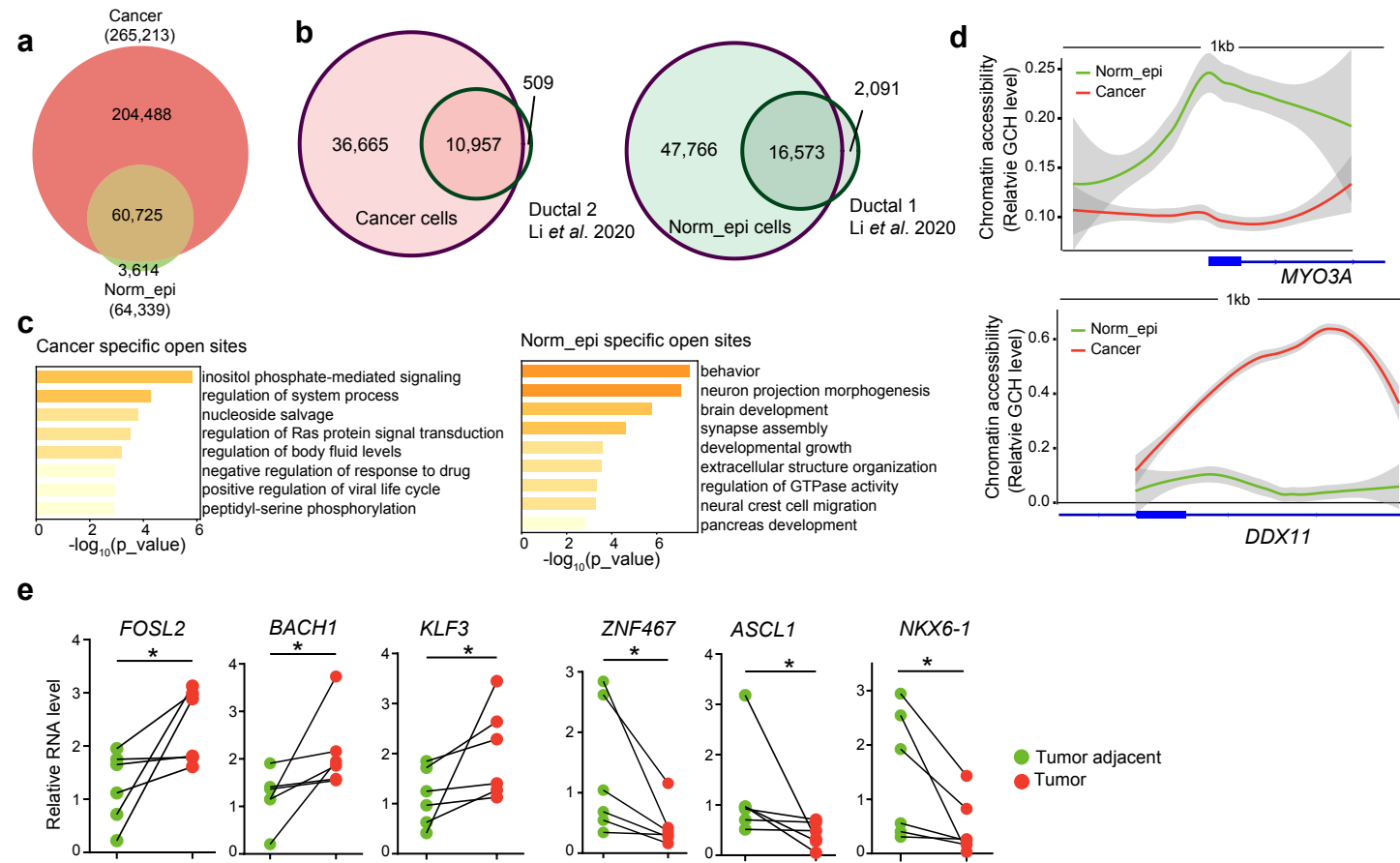
a The DNA methylation level in each 1 Mb bin belonging to different SCNA types in every subclones of cells in P05 and P11 patients. **b** Boxplot showing the distribution of correlation values between the genome copy number and the DNA methylation level in each cell subclones.

Figure S14



Supplementary Fig. S14 Genome-wide DNA demethylation in cancer cells is strongly enriched in heterochromatin regions. Histogram showing the absolute DNA methylation levels of repeat elements and other genomic elements of different cell types in each patient (each with over 3 Norm_epi cells).

Figure S15



Supplementary Fig. S15 Chromatin accessibility features in PDAC cells. **a** Venn diagram indicating the merged NDR numbers in cancer cells and Norm_epi cells. Merged NDRs in two types of cells were defined with two standards: i) NDRs were merged when they had at least a 100-bp overlap; ii) Each merged NDR was detected in at least 11 cells in each cell group. **b** Venn diagram showing the overlap of chromatin open sites identified in our dataset and the previous study by Li *et al.* (2021), respectively, in cancer cells and normal cells. **c** The enriched biological processes for the top 200 genes detected with cell type-specific chromatin open status. **d** Representative NDRs for the cancer cells and Norm_epi cells. **e** RT-qPCR results using the pairwise tumor and tumor-adjacent samples ($n = 6$) showing the candidate TFs higher expressed in the suspected cell types. $*P < 0.05$, Wilcoxon matched-pairs signed rank test.