Science Advances
AAAS

Supplementary Materials for

Comprehensive single-cell analysis demonstrates radiotherapy-induced infiltration of macrophages expressing immunosuppressive genes into tumor in esophageal squamous cell carcinoma

Hidekazu Oyoshi *et al.*

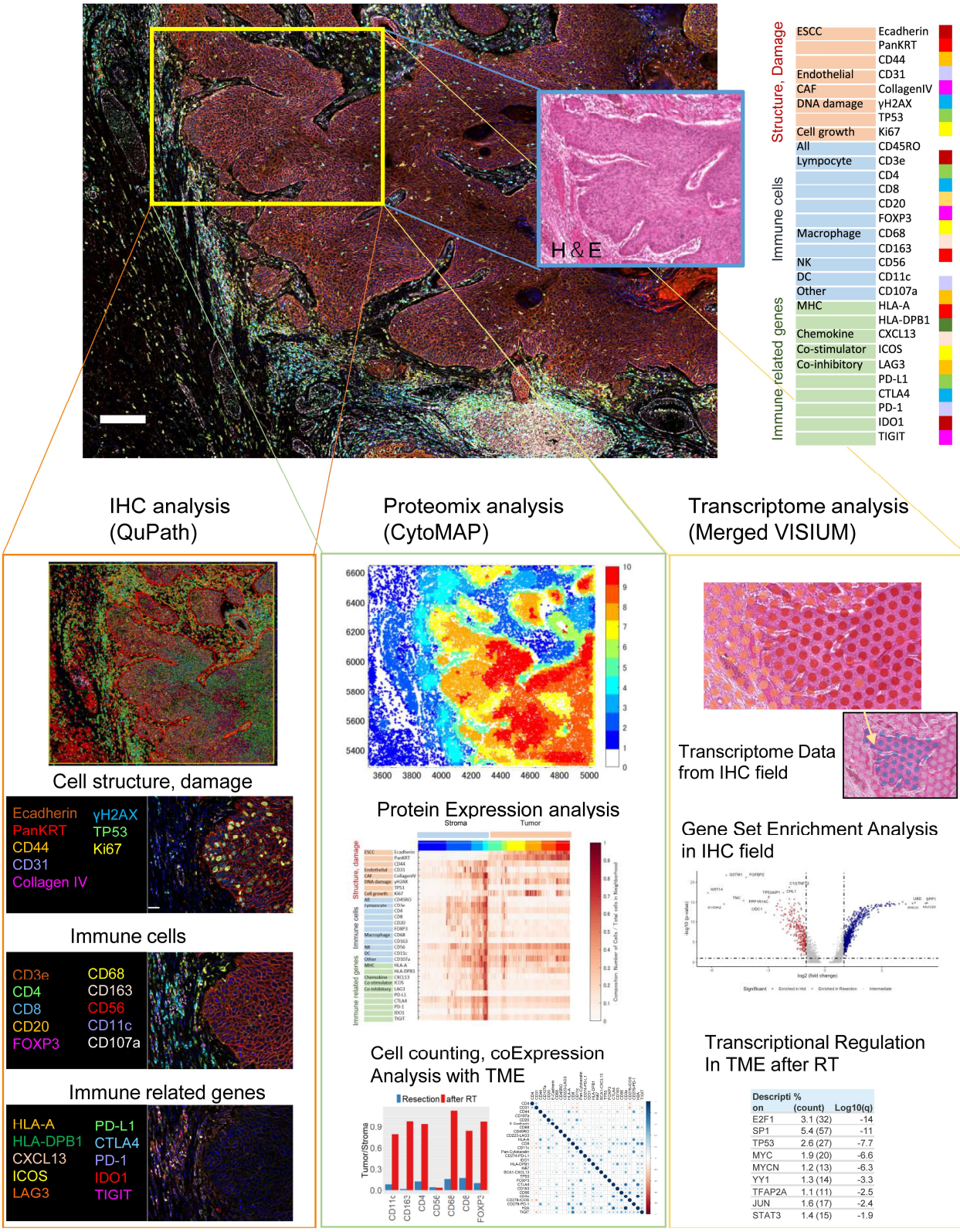
Corresponding author: Shun-Ichiro Kageyama, skageyam@east.ncc.go.jp;
Yutaka Suzuki, ysuzuki@edu.k.u-tokyo.ac.jp; Atsushi Shibata, shibata.at@keio.jp

Sci. Adv. **9**, eadh9069 (2023)
DOI: 10.1126/sciadv.adh9069

The PDF file includes:

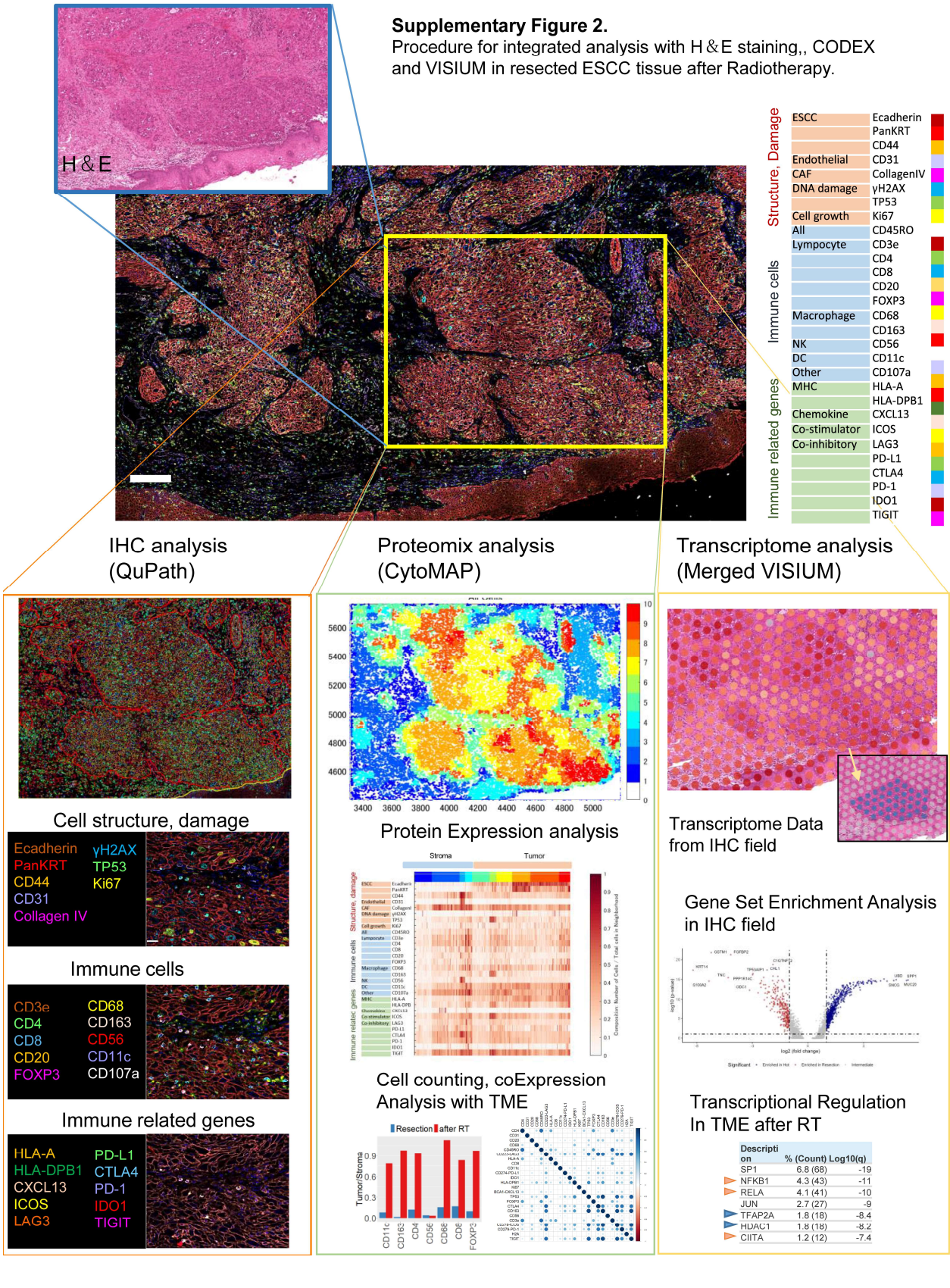
Other Supplementary Material for this manuscript includes the following:

Supplementary Figure 1.
Procedure for integrated analysis with H&E staining, CODEX and VISIUM in resected ESCC tissue (Non-RT).



Description	% (count)	Log10(q)
E2F1	3.1 (32)	-14
SP1	5.4 (57)	-11
TP53	2.6 (27)	-7.7
MYC	1.9 (20)	-6.6
MYCN	1.2 (13)	-6.3
YY1	1.3 (14)	-3.3
TFAP2A	1.1 (11)	-2.5
JUN	1.6 (17)	-2.4
STAT3	1.4 (15)	-1.9

Fig. S1. Procedure for integrated analysis with H&E staining, CODEX and VISIUM of resected ESCC tissue (Non-RT). IHC observation, proteomics analysis and transcriptome analysis were performed in merged fields with QuPath, CytoMAP and Loupe browser, respectively.



Supplementary Figure 2.
Procedure for integrated analysis with H&E staining,, CODEX and VISIUM in resected ESCC tissue after Radiotherapy.

Fig. S2. Procedure for integrated analysis with H&E staining, CODEX and VISIUM in resected ESCC tissue after radiotherapy. IHC observation, proteomics analysis and transcriptome analysis were performed in merged fields by QuPath, CytoMAP and Loupe browser, respectively.

Supplementary Figure 3.
H&E observation and merged total field with VISIUM and CODEX.

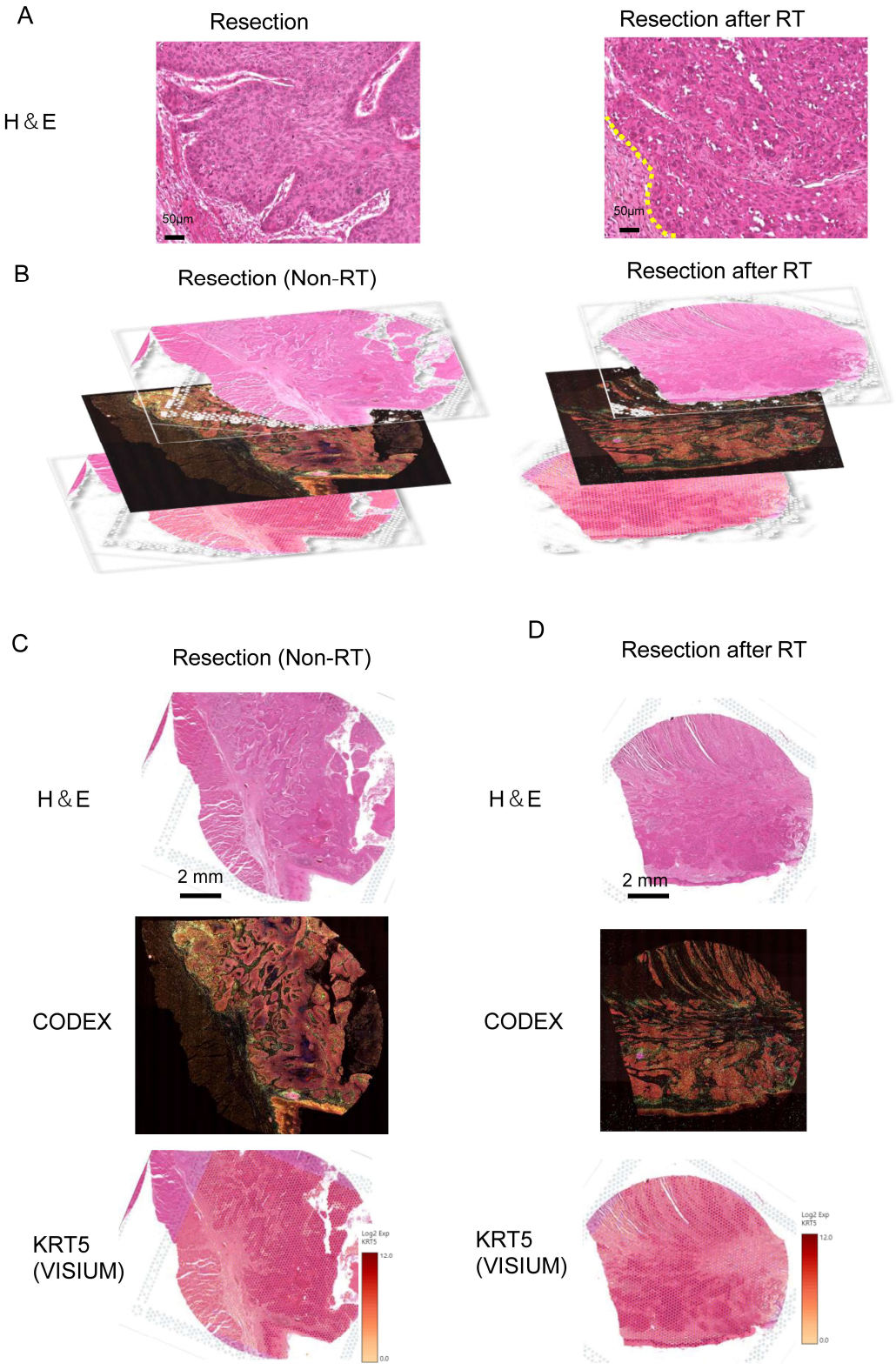


Fig. S3. H&E observation and merged total field with VISIUM and CODEX. (A) ESCC region was confirmed with morphology in each tissue by H&E field. **(B** to **D)** Merged image of H&E, CODEX and VISIUM staining in total field. ESCC cells were identified based on morphology and KRT5 expression. **(E)** The number of cells analyzed by CODEX.

Supplementary Figure 4.
Proteomics analysis by CODEX with CytoMAP

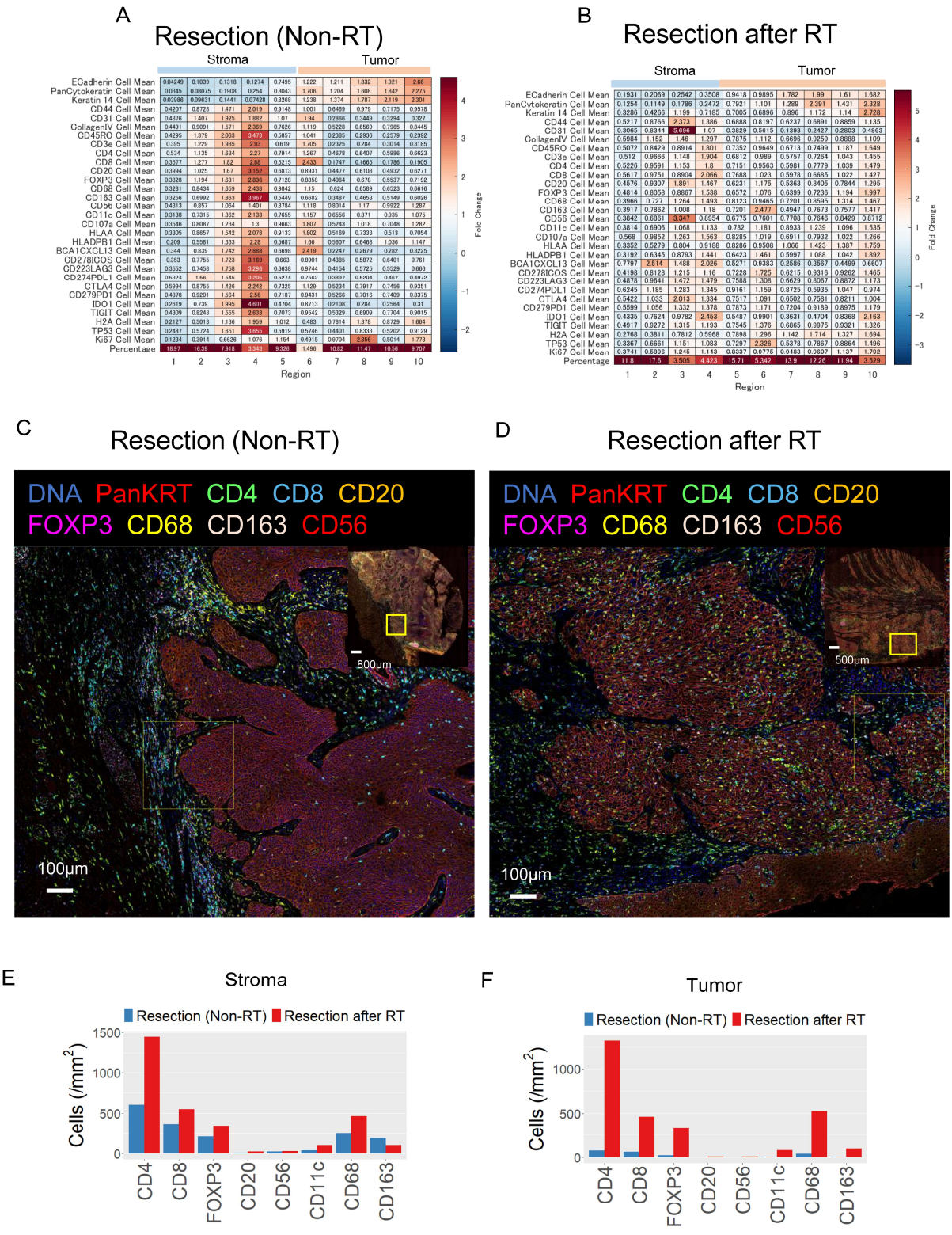
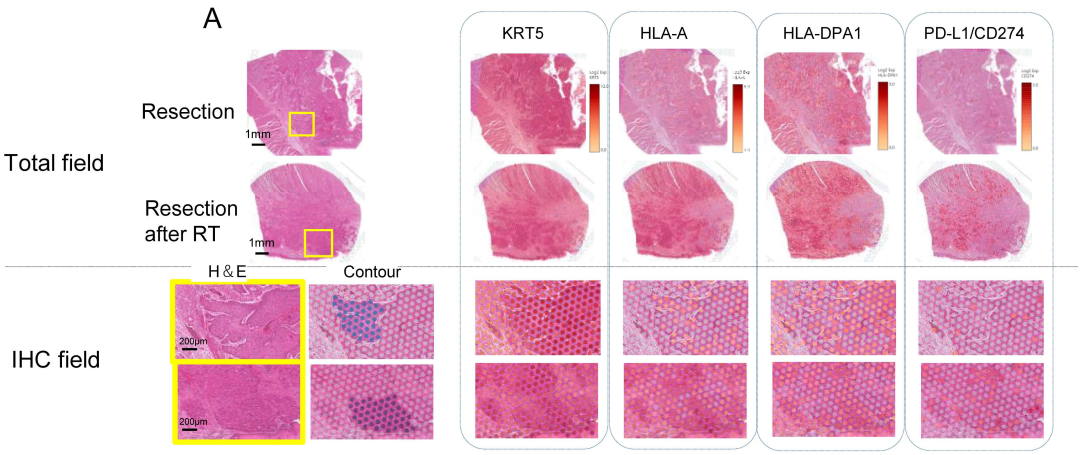
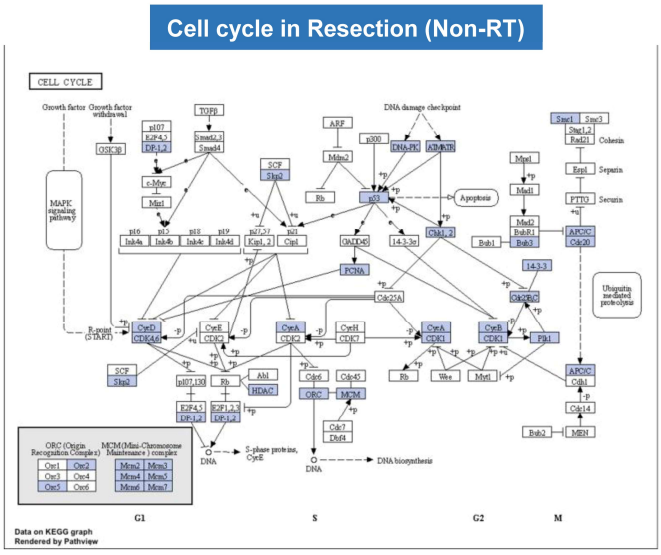


Fig. S4. Proteomics analysis by CODEX with CytoMAP.
(A and **B)** Gene expression signatures corresponding to the heatmap (Fig. 1C, E). CytoMAP analysis showed the fold change relative to the total expression average. **(C** and **D)** Multicolour IHC staining in ESCC tissue after resection and resection after RT. Scale bar, 100 μm. The area shown in each figure corresponds to the solid yellow square of the total field in the upper right, and the field Figure 1F shows with a thin dotted frame. **(E** and **F)** Number of immune cells analysed in Fig. 1.

Supplementary Figure 5.
Transcriptome analysis by VISIUM in IHC field.



B



C

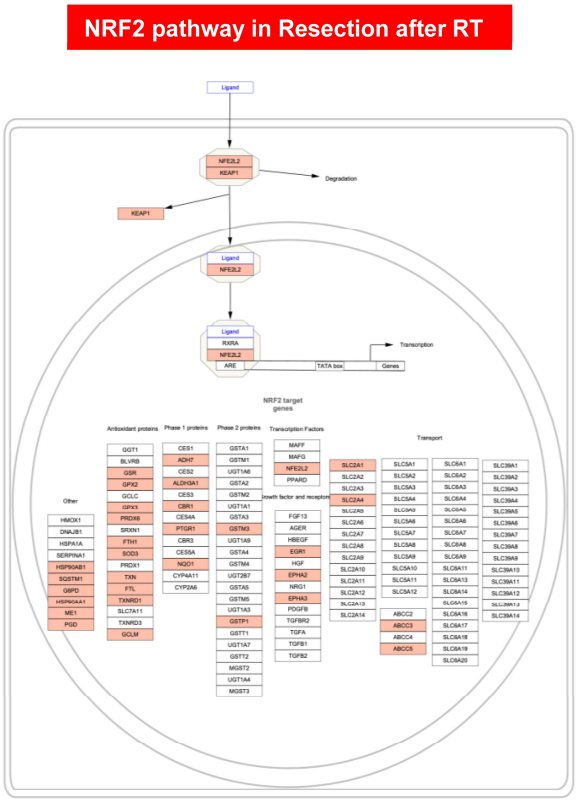
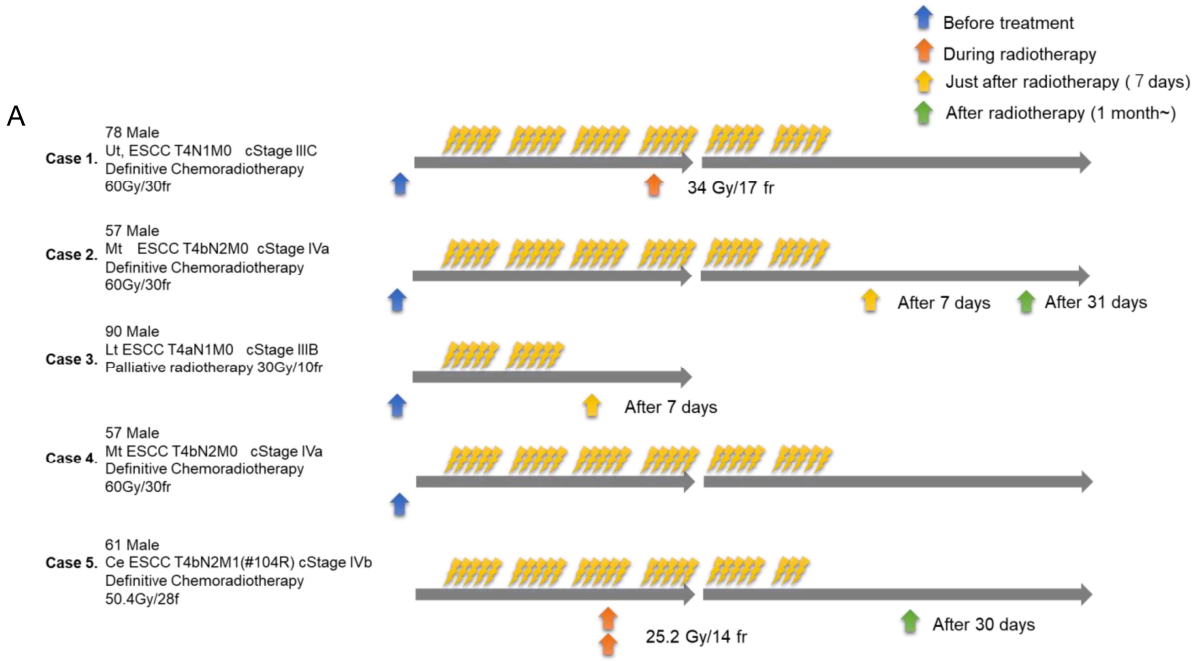


Fig. S5. Transcriptome analysis by VISIUM in IHC field. (A) Gene Expression of MHC class I (HLA-A), MHC class II (HLA-DPB1), and PD-L1 (CD274) according to 10x VISIUM. **(B** and **C)** Typical pathway map for non-RT and after RT tissues. Cell cycle pathway in resection (non-RT) samples and NRF2 pathway in resection after RT samples. In B and C, gene names with color (blue or red) represent genes whose expression was observed in the pathway analysis.

Supplementary Figure 6.
Information of scRNA seq analysis in ESCC patients



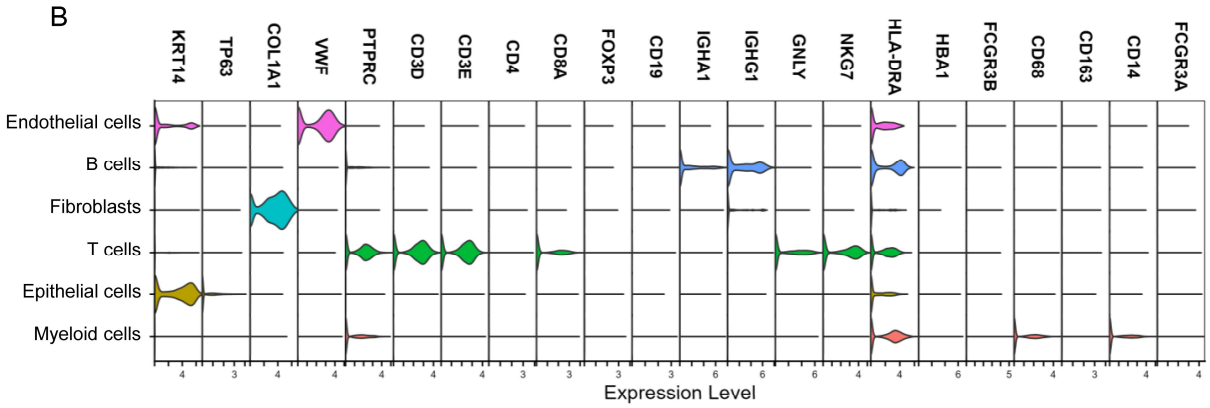
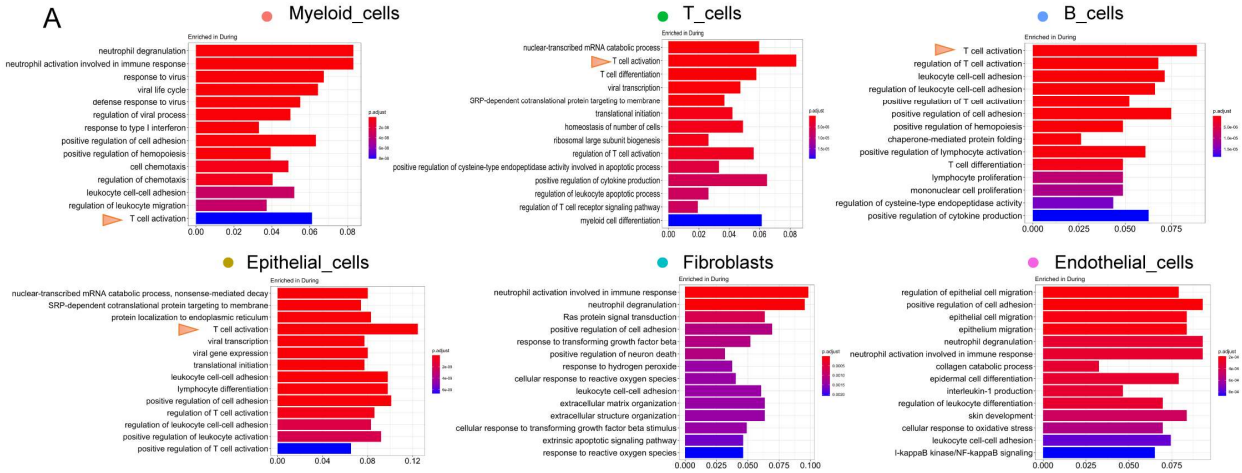


Fig. S6. Information on scRNA-seq analysis in ESCC patients. (A) Sampling schedule for the cohort from which scRNA-seq samples were obtained for this research. **(B)** Violin plot of the expression of representative marker genes in each cell cluster annotated in Fig. 3B.

Supplementary Figure 7.
Analysis of the microenvironment and biological pathways after radiation therapy with scRNA-seq.



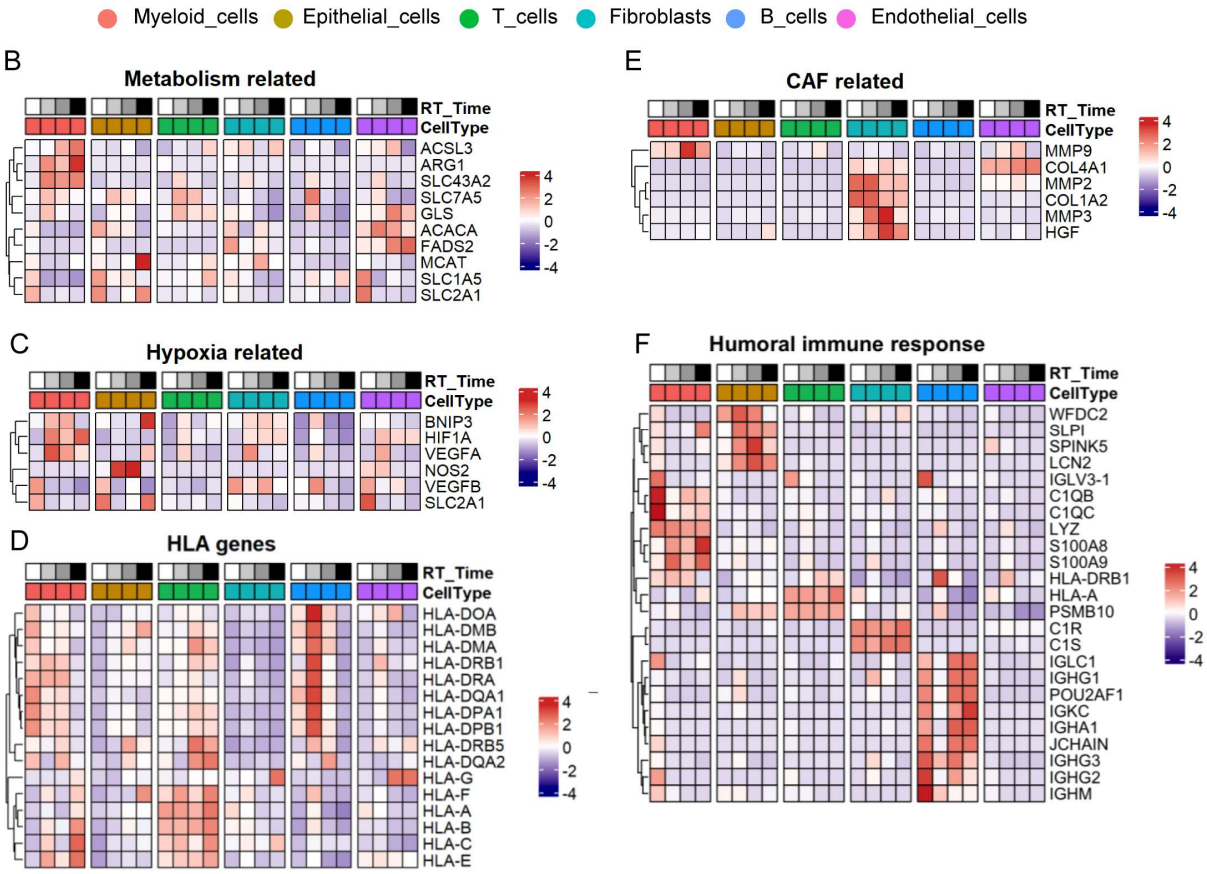
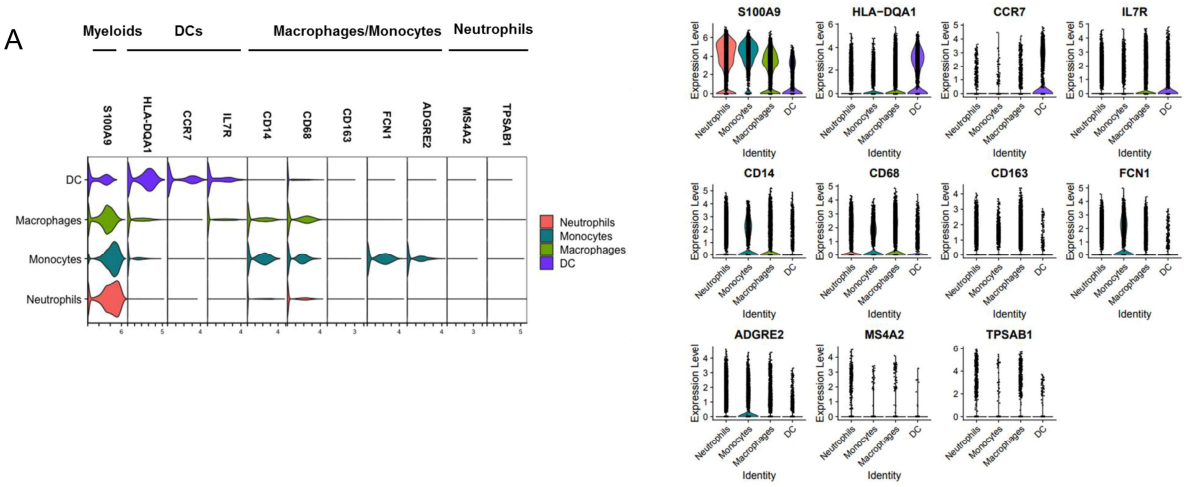


Fig. S7 Analysis of the microenvironment and the related biological pathways during radiotherapy
（**A**） DEG analysis between the pre and during groups in scRNA-seq. Gene enrichment analysis showed upregulated biological pathways from pre to during radiotherapy in myeloid cells, T cells, B cells, Epithelial cells, Fibroblast, and Endothelial cells. The red arrow indicates the T cell activation pathway. **(B** to **F)** Expression of representative markers of the indicated biological class by scRNA-seq. The greyscale bar indicates the time point during RT: white, pre-RT; light grey, during RT; dark grey, immediately post-RT; black, post-RT.

Supplementary Figure 8.
Analysis of Myeloid cells with scRNA-seq



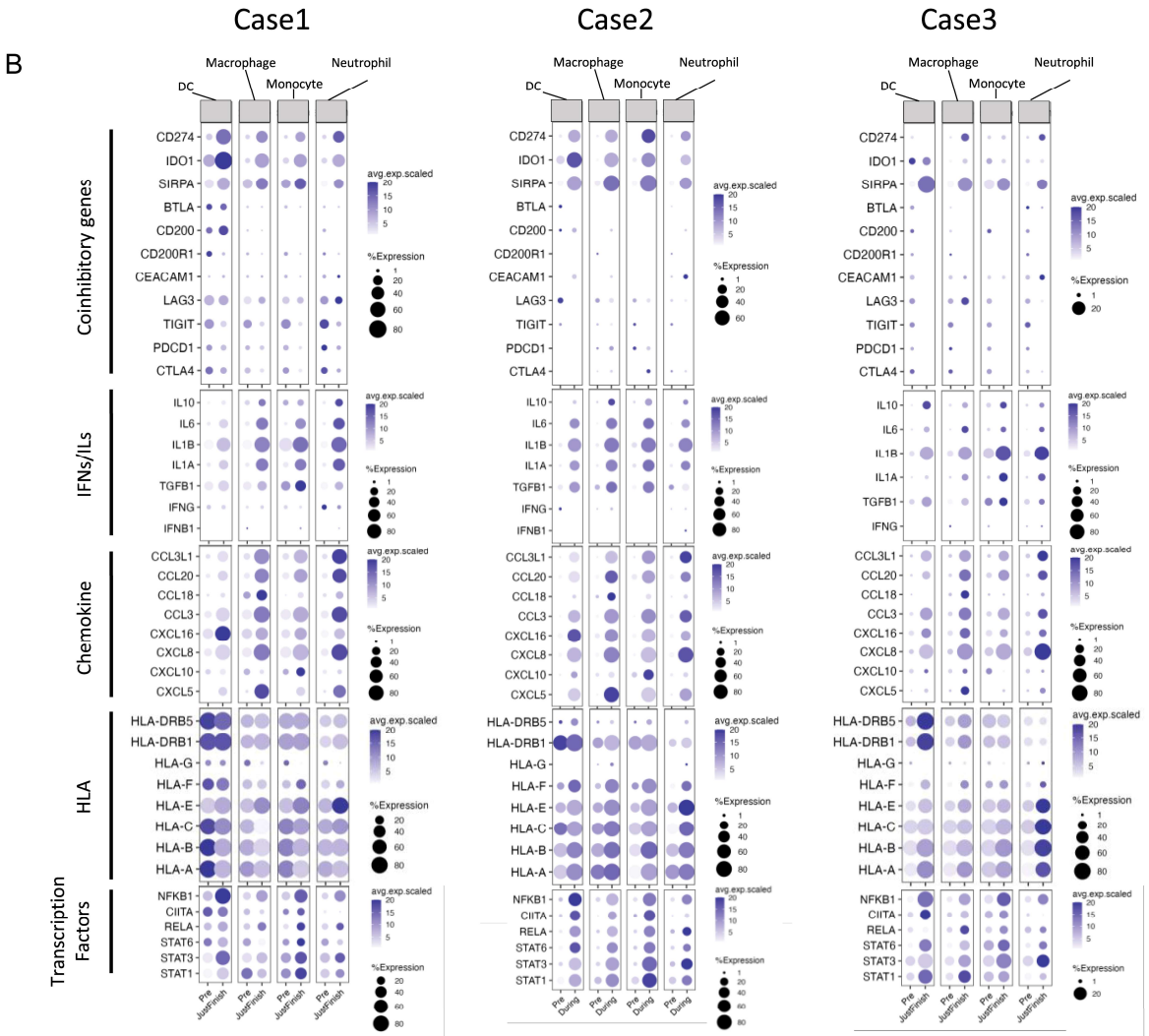


Fig. S8. Information on scRNA-seq analysis in Myeloid cells. (A) Information on scRNA-seq analysis in myeloid cells. Violin plot of the expression of representative marker genes in each cell cluster annotated in Fig. 4B is shown in the left panel. Violin plot with jitter exhibiting the expression of representative marker genes is shown in the right panel. **(B)** Gene expression analysis for each patient in Case1, Case2, and Case3.Tthe upregulation of PD-L1, IDO1, and SARPA as well as ILs and Chemokines was similarly observed between patients. The responsiveness of Case 3 seems to be weaker than other Cases. This may be dependent on the total dose, i.e., the sample of Case 1 and Case 2 were collected after 60 Gy, whereas the samples of Case 3 were collected after 30 Gy.

Supplementary Figure 9.
Analysis of Myeloid cells with scRNA-seq

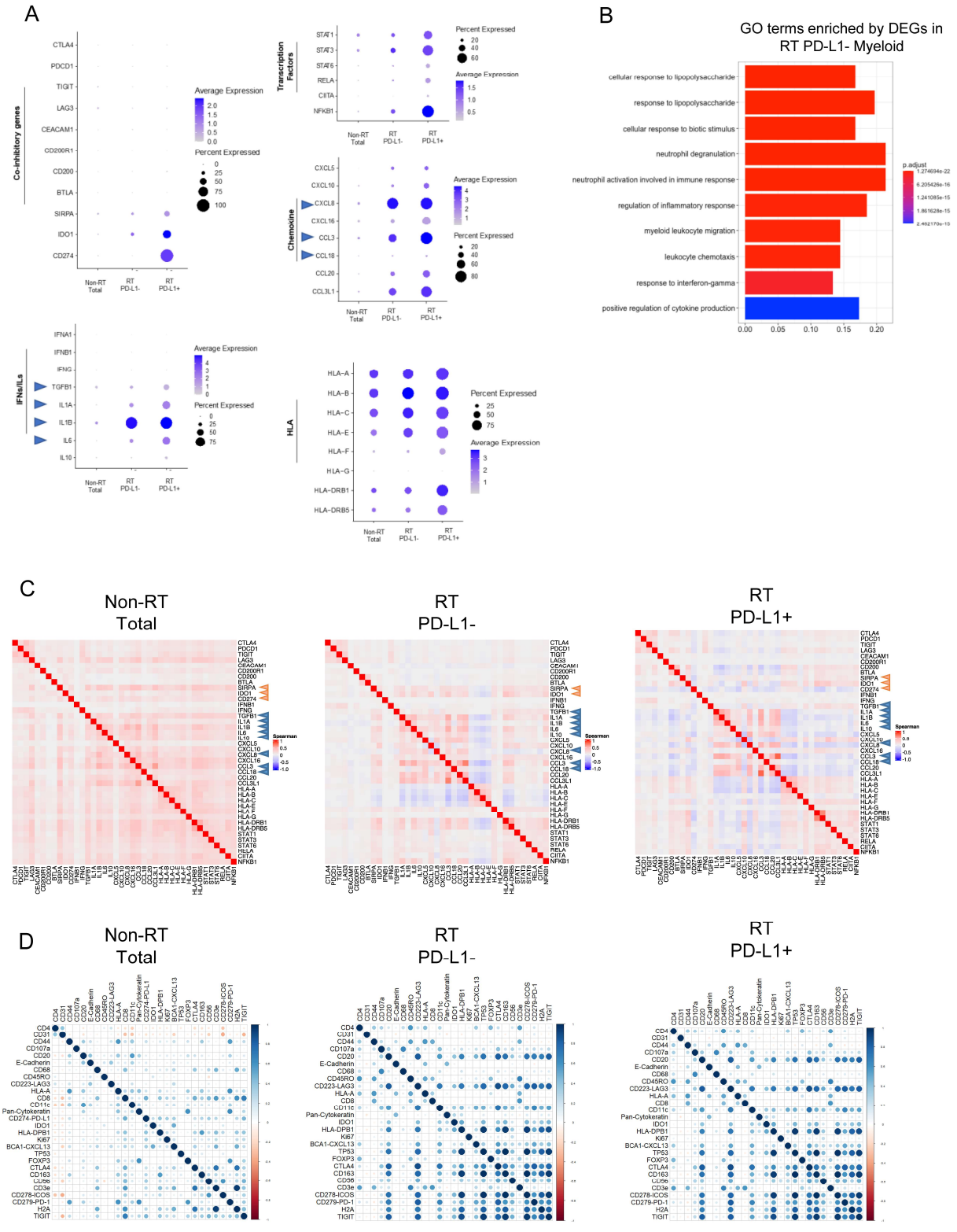


Fig. S9. Detailed gene expression analysis in the myeloid cluster. (A) Detailed gene expression analysis in Non-RT, RT PD-L1-, RT PD-L1+. **(B)** Biological signaling analysis with RT PD-L1-. **(C)** Gene correlation analysis with Non-RT, RT PD-L1-, RT PD-L1+. **(D)** Gene correlation analysis from CODEX results.

Supplementary Figure 10.
Characterization of RT-TAM by multicolor IHC staining.

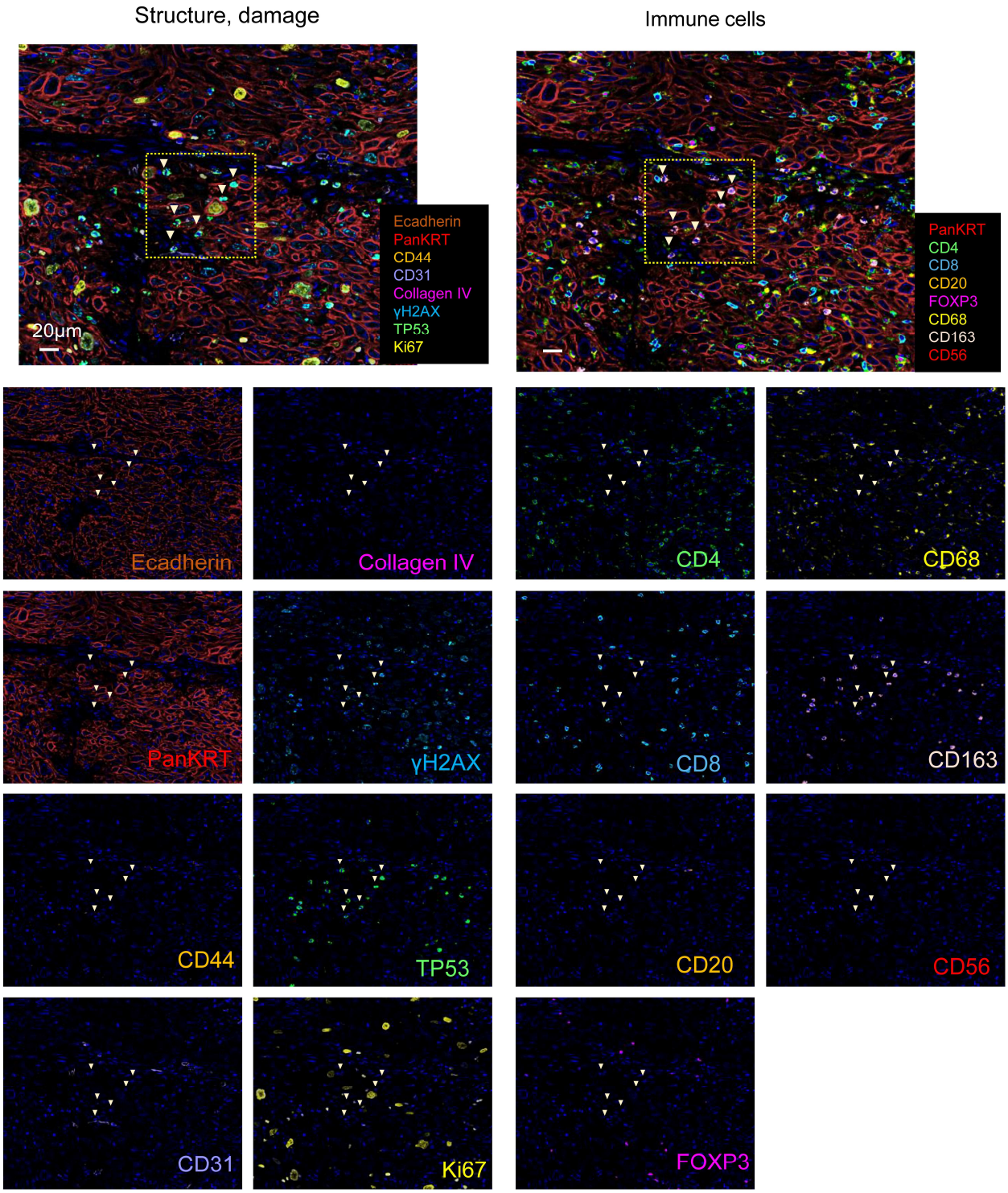


Fig. S10. Multicolour IHC staining and characterization of PD-L1+ myeloid cells. Scale bar, 100 μm. The arrow matches Fig. 5E, and the field in Fig. 5E is shown with a thin dotted frame.

Supplementary Figure 11.
Characterization of RT-TAM by multicolor IHC staining.

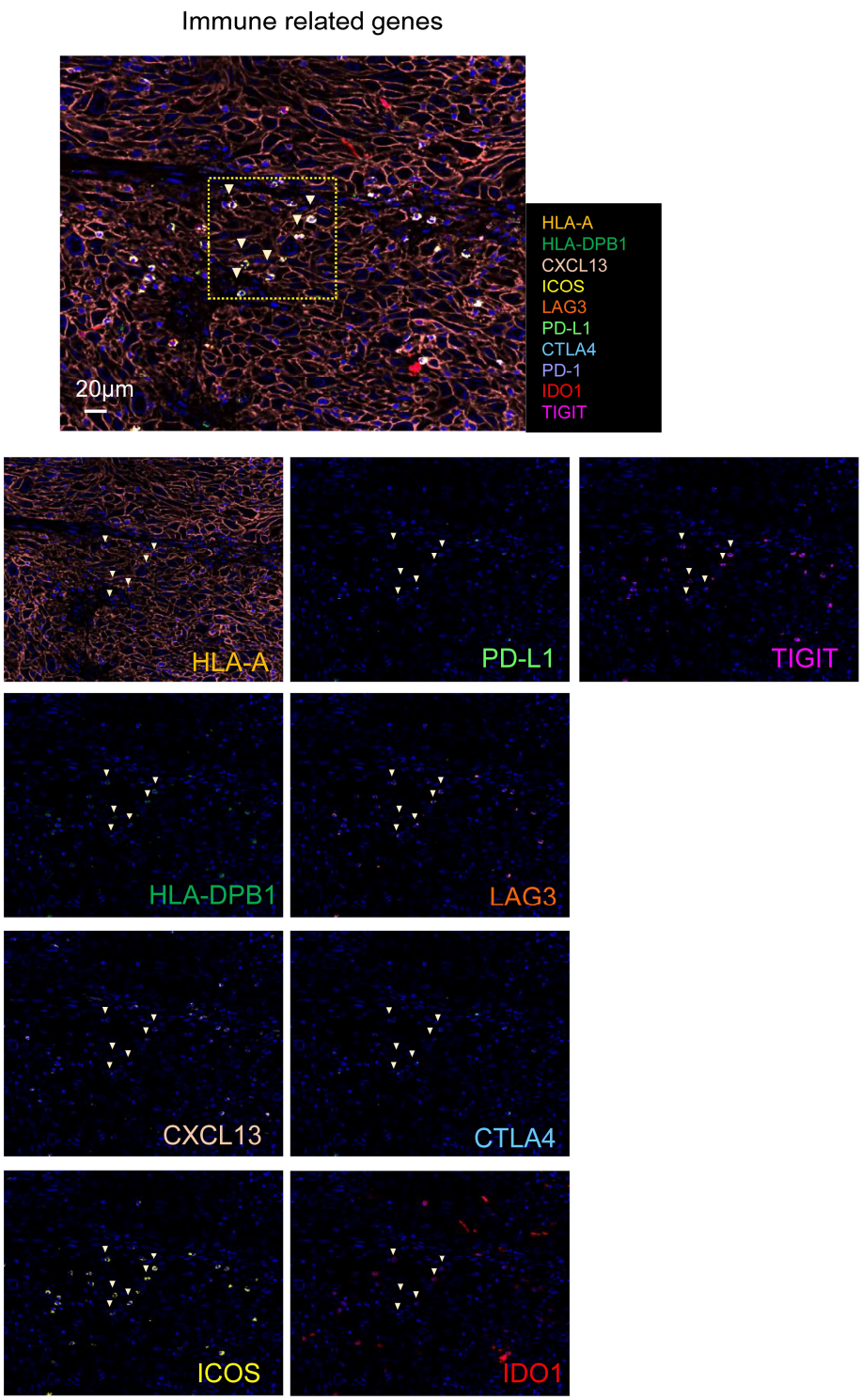


Fig. S11. Multicolour IHC staining and characterization of PD-L1+ myeloid cells. Scale bar, 100 μm. The arrow matches Fig. 5E, and the field in Fig. 5E is shown with a thin dotted frame.

Table S1. Gene list for Gene set enrichment analysis.

Table S2. Antibodies for CODEX staining.

Table S3. Data of Typical pathways and genes from each result are shown in Fig. 2F and Fig. S5B-C.

Table S4. The information on gene selection for the analysis of scRNA-seq.

Table S5. The number of cells analyzed by CODEX.

Table S6. The number of cells analyzed by scRNA-seq.