

Supplementary Materials for

**CXCR1⁺ neutrophil infiltration orchestrates response to third-generation
EGFR-TKI in EGFR mutant NSCLC**

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Other Supplementary Materials for this manuscript include the following:

Movies S1 to S2

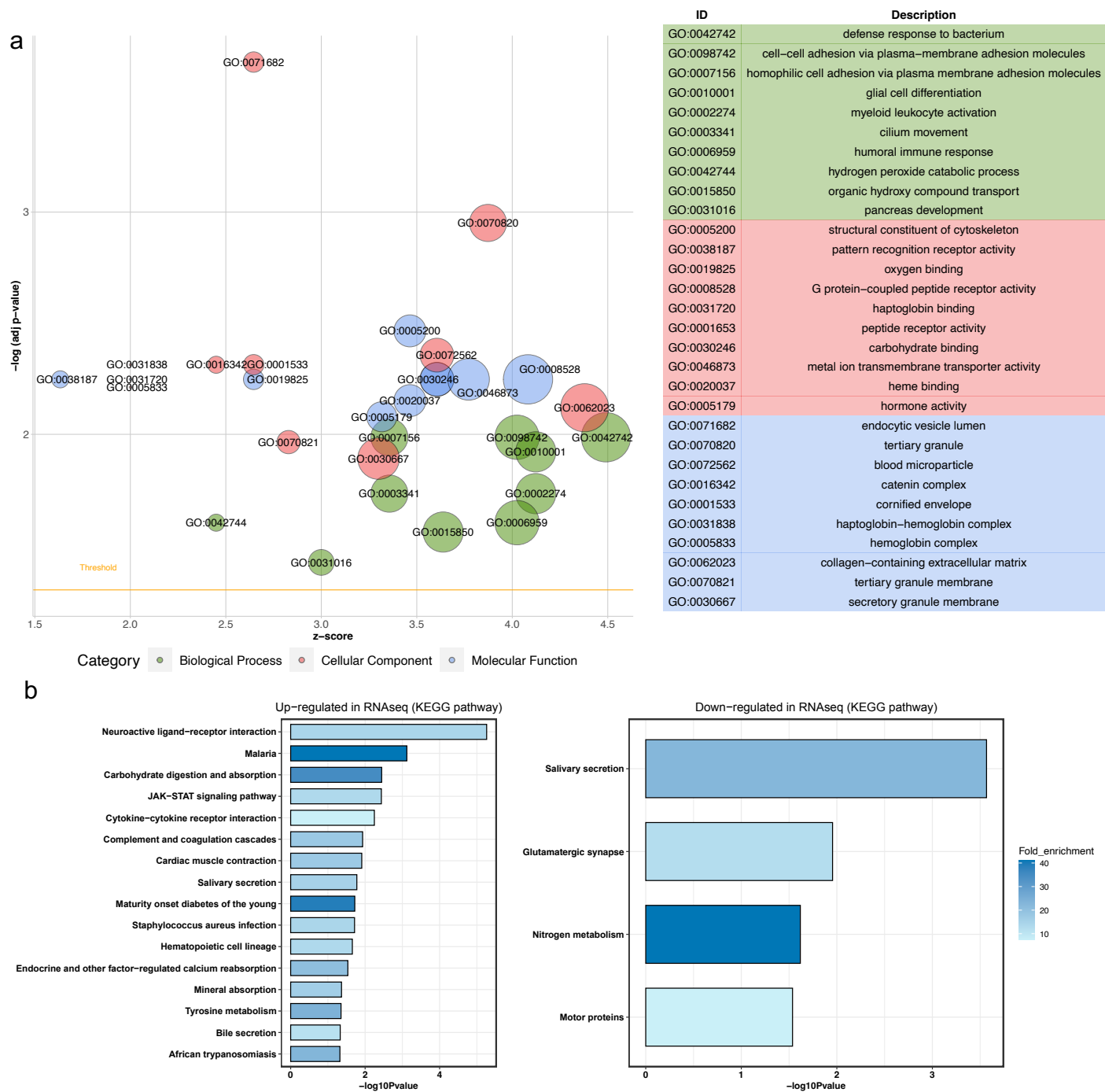


Figure. S1.

GO (a) and KEGG (b) enrichment analysis of different expression genes between pretreatment and resistant samples.

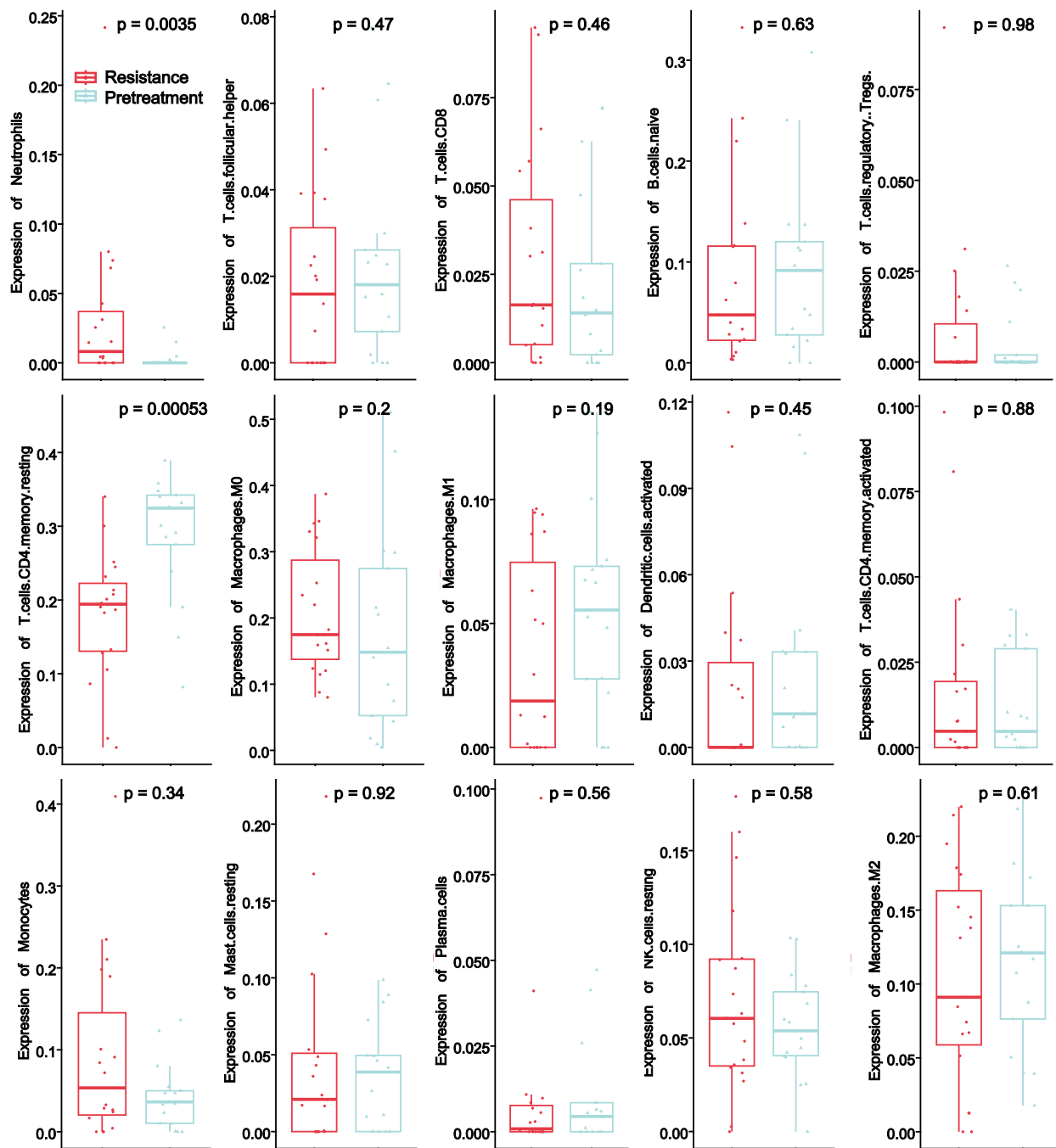


Figure. S2.

Comparison of each immune cell infiltration abundance between pretreatment and resistant samples.

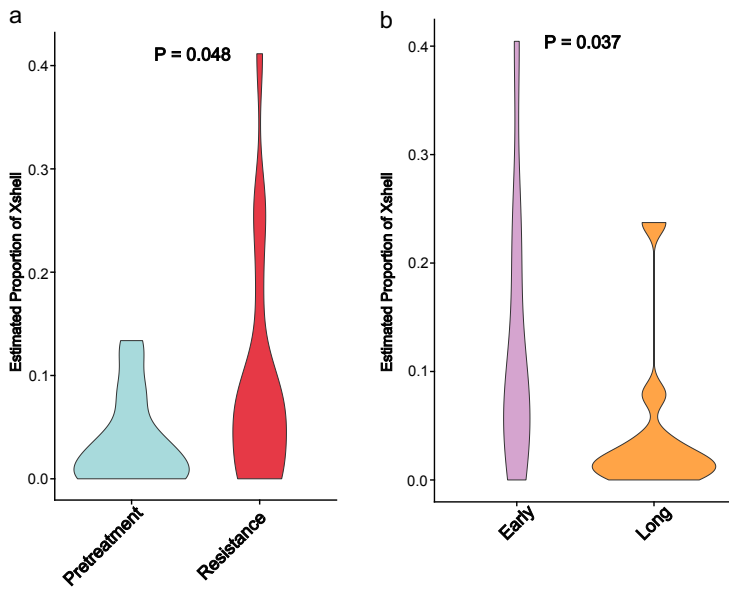


Figure. S3.

Comparison of the infiltration levels of neutrophils between pretreatment and resistant groups (**a**), and early progression and long benefit groups (**b**) by Xcell.

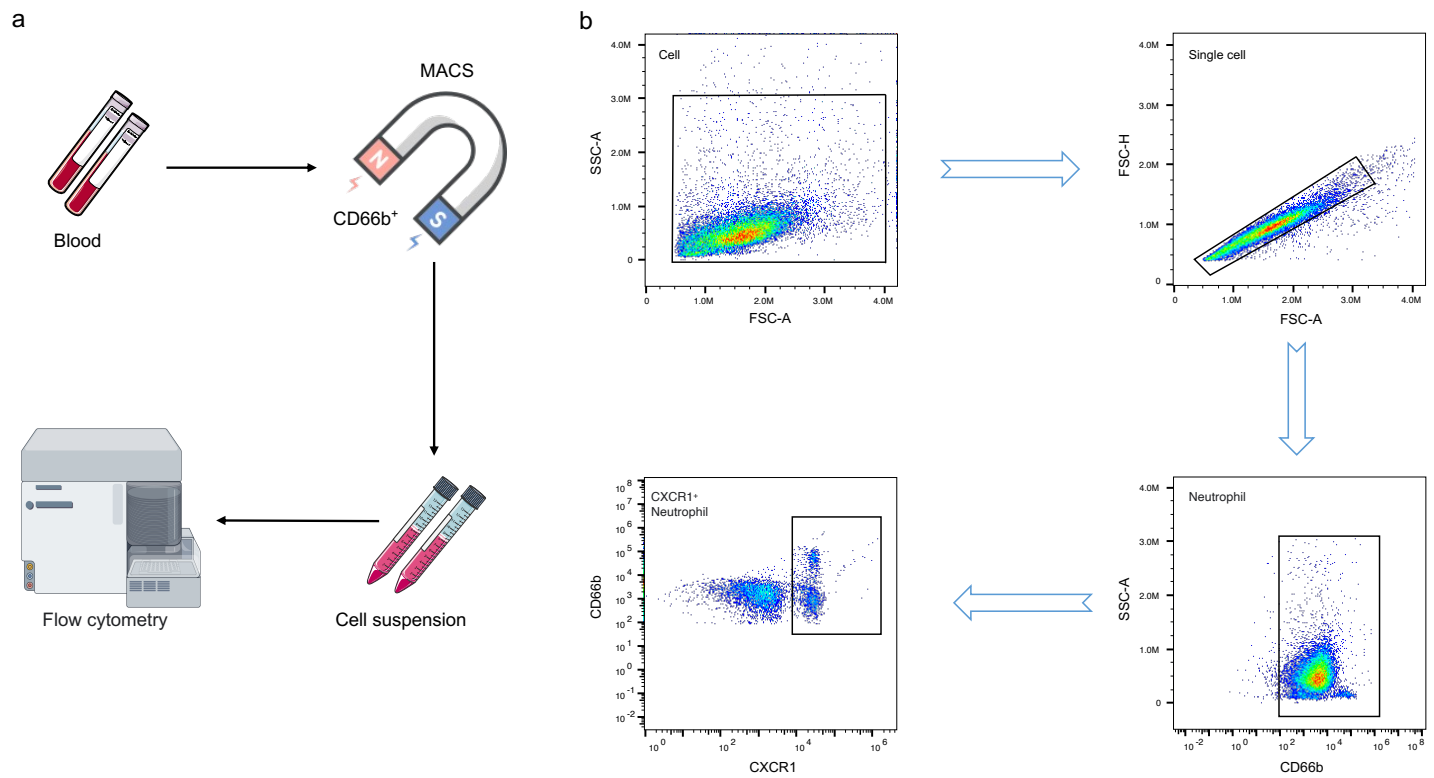


Figure. S4.

(a) Neutrophils were sorted with CD66b microbeads (the graphical model figure was created using Figdraw). (b) Flow cytometry gating strategy diagram.

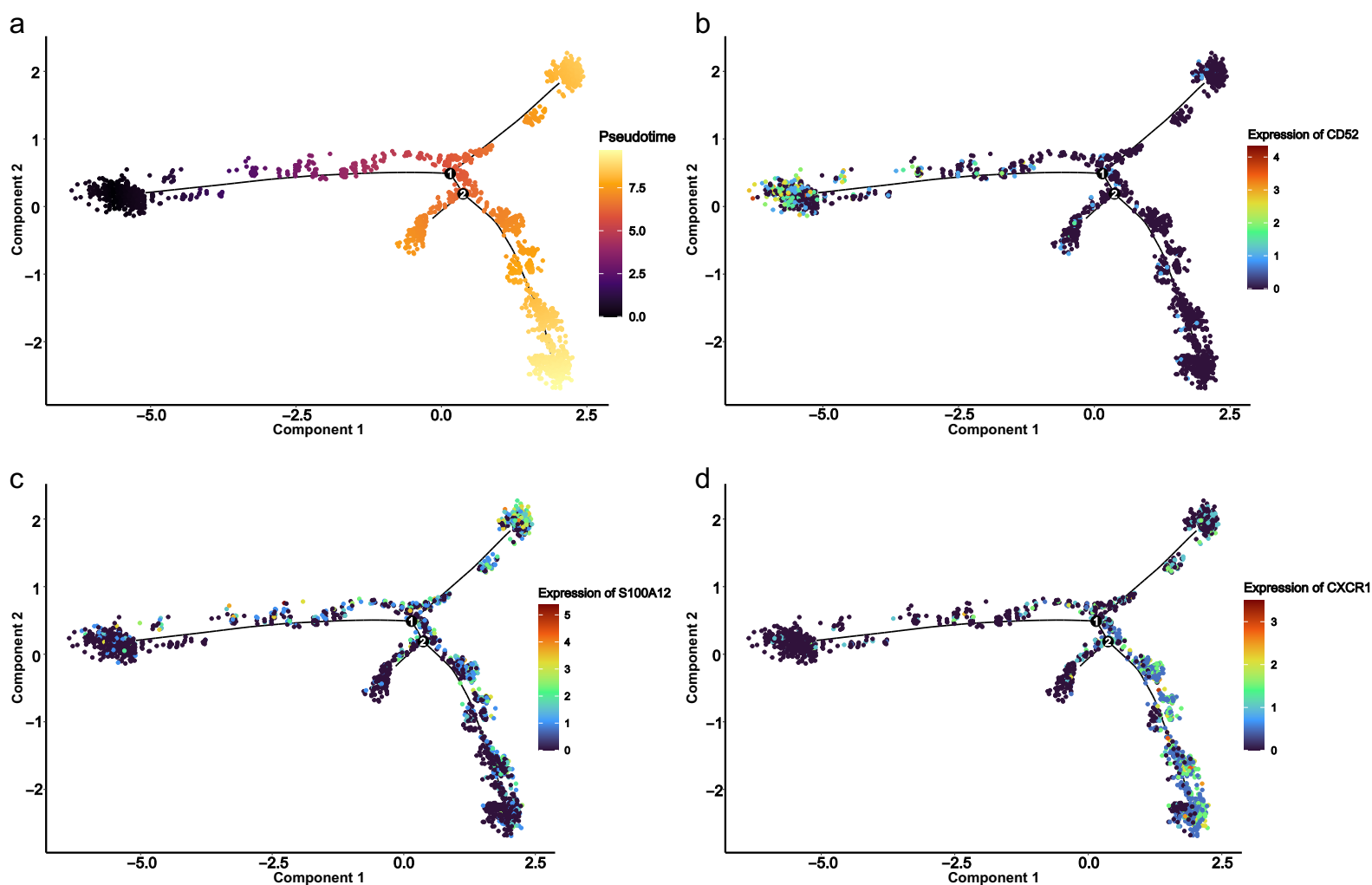


Figure. S5.

(a) The pseudotime value according neutrophil trajectories detected by Monocle2. The gene expression value of CD52 (b), S100A12 (c) and CXCR1 (d) mapped in the trajectory.

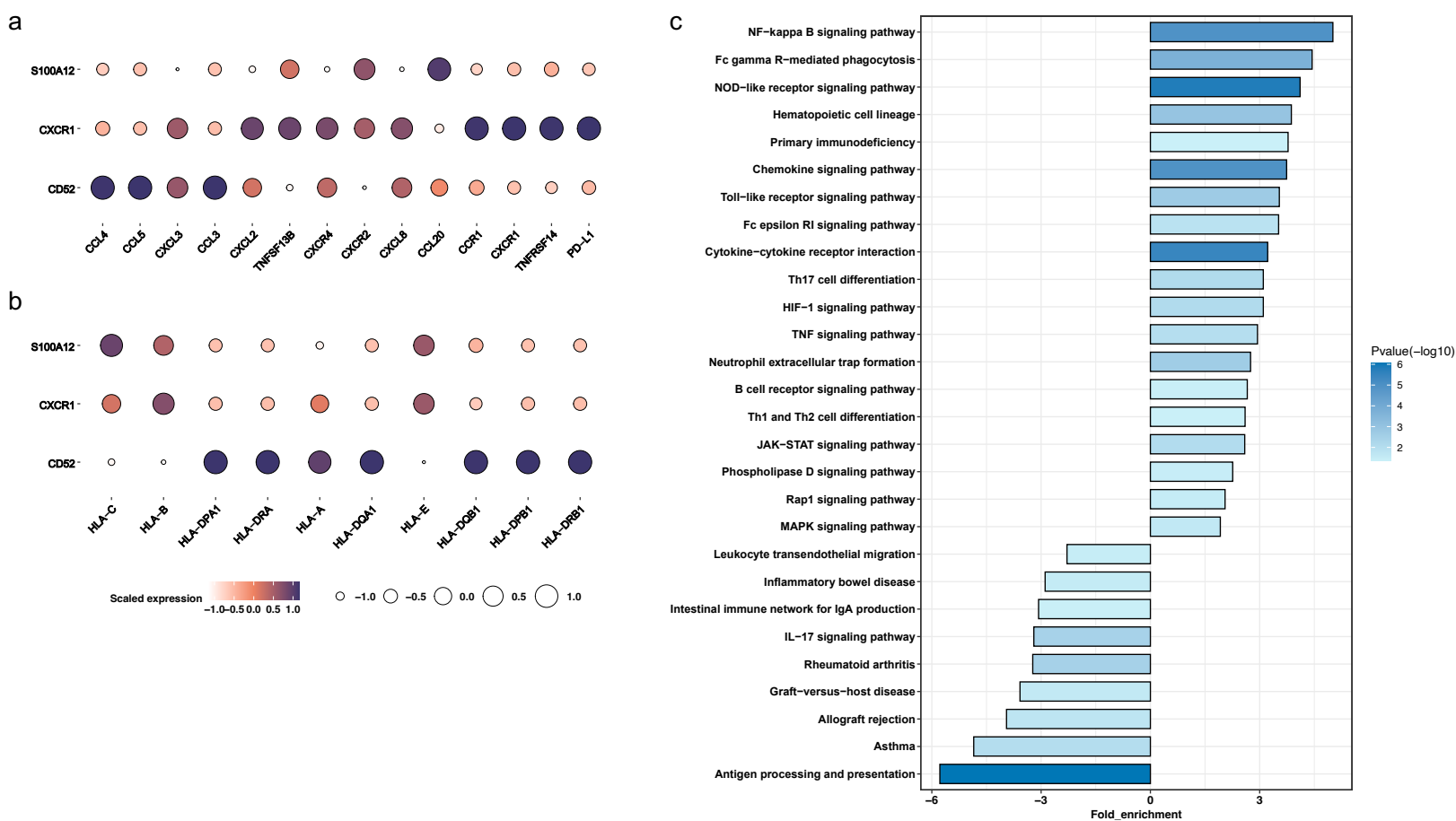


Figure. S6.

(a) Expression profiles of differentially expressed cytokines. **(b)** Expression profiles of differentially expressed MHC molecules. **(c)** KEGG enrichment analysis in CXCR1⁺ neutrophils.

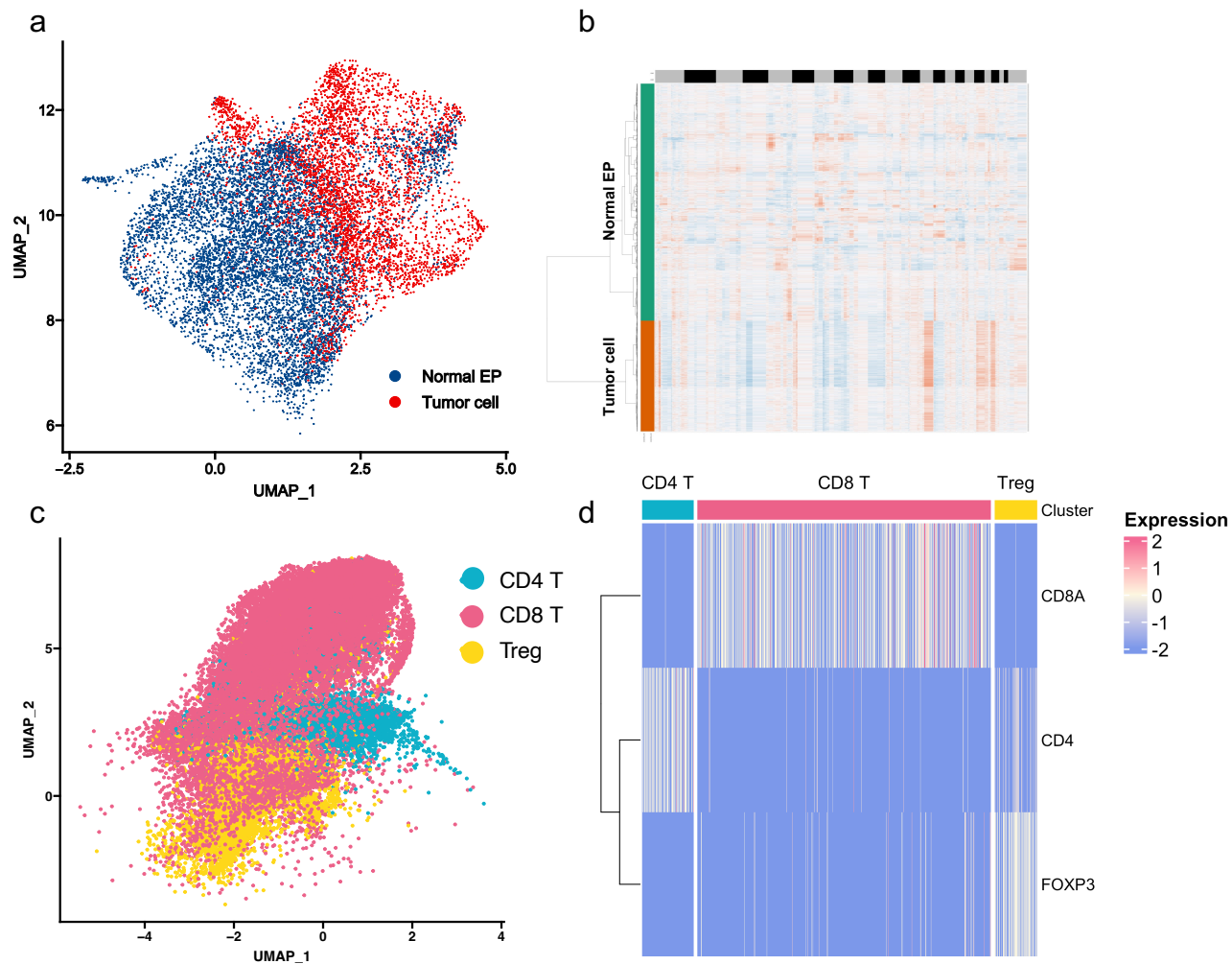


Figure. S7.

(a) The UMAP plot of normal epithelial cell and tumor cell. **(b)** Copykat algorithm was performed to distinguish normal epithelial cell and tumor cell. **(c)** The UMAP plot of CD8 T cell, CD4 T cell and Treg. **(d)** Heatmap of marker genes.

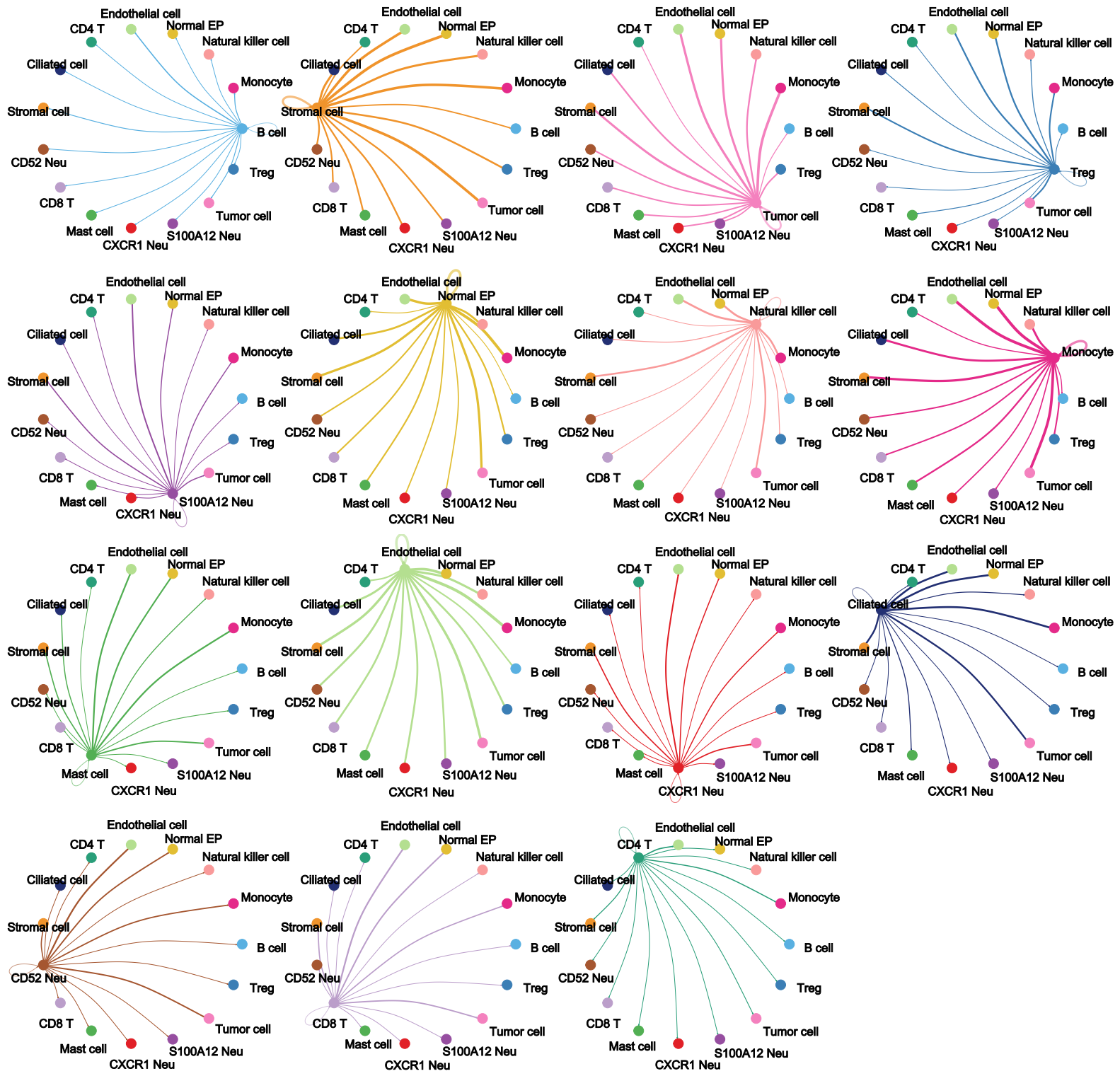


Figure. S8.
Communication patterns among different cell types.

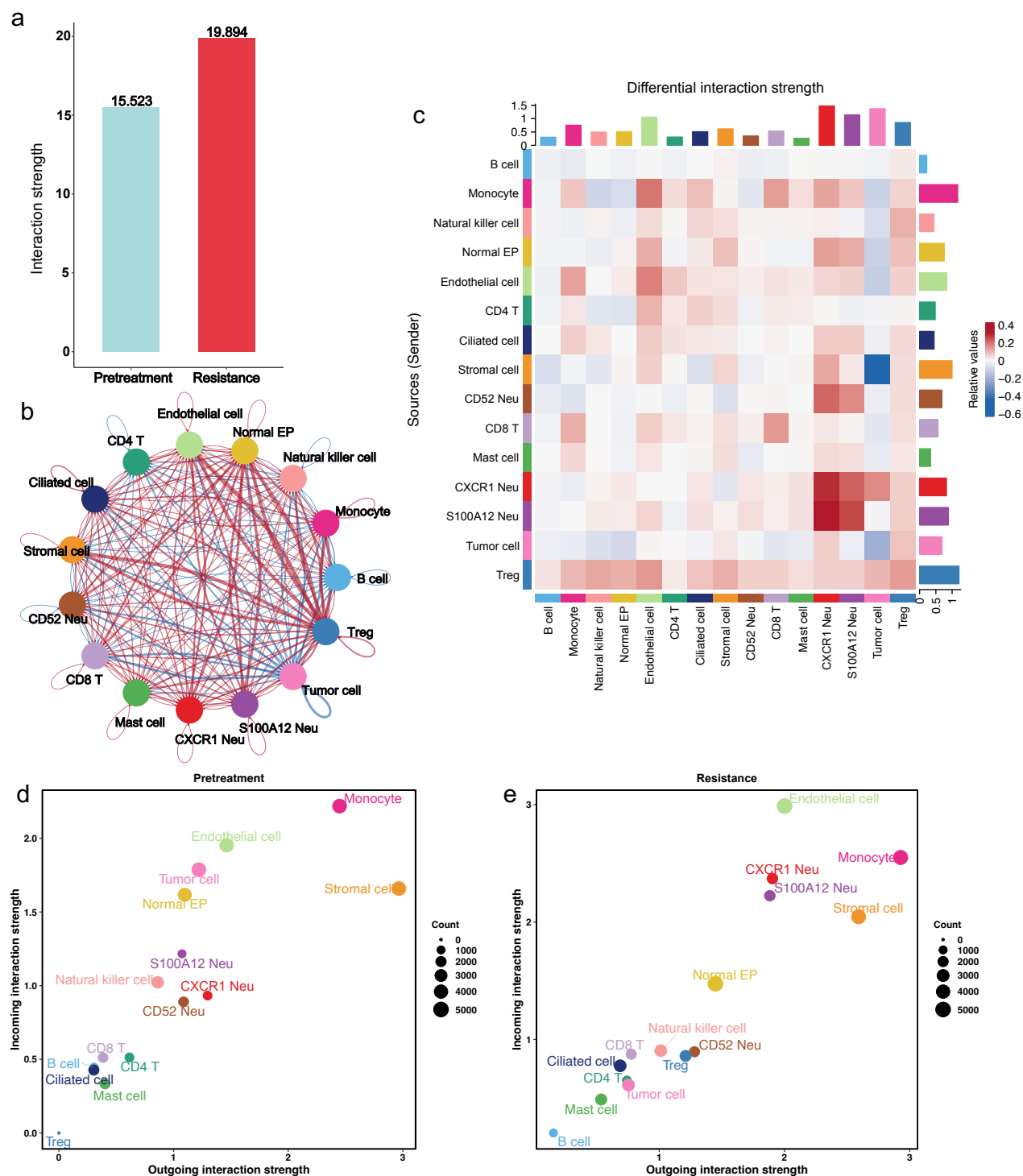


Figure. S9.

(a) Cell interaction strength in pretreatment and resistance. The comparison of interaction strength between pretreatment and resistance among various cells (Red: upregulation; Blue: downregulation) in circle chart (b) and heatmap (c). Dotplot of outgoing and incoming interaction strength in pretreatment (d) and resistance (e).

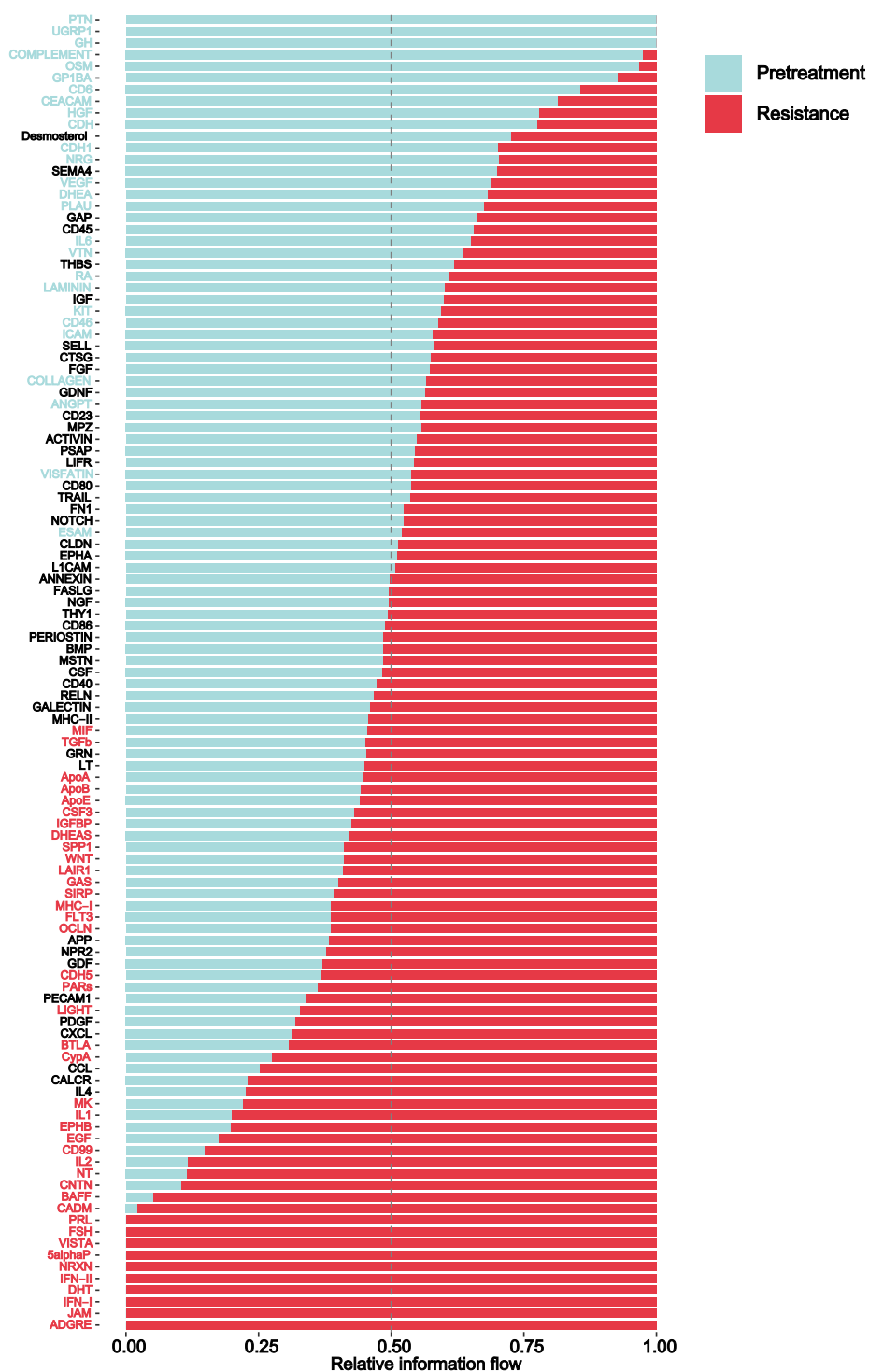


Figure. S10.

The enriched pathways in pretreatment and resistance in CellChat.

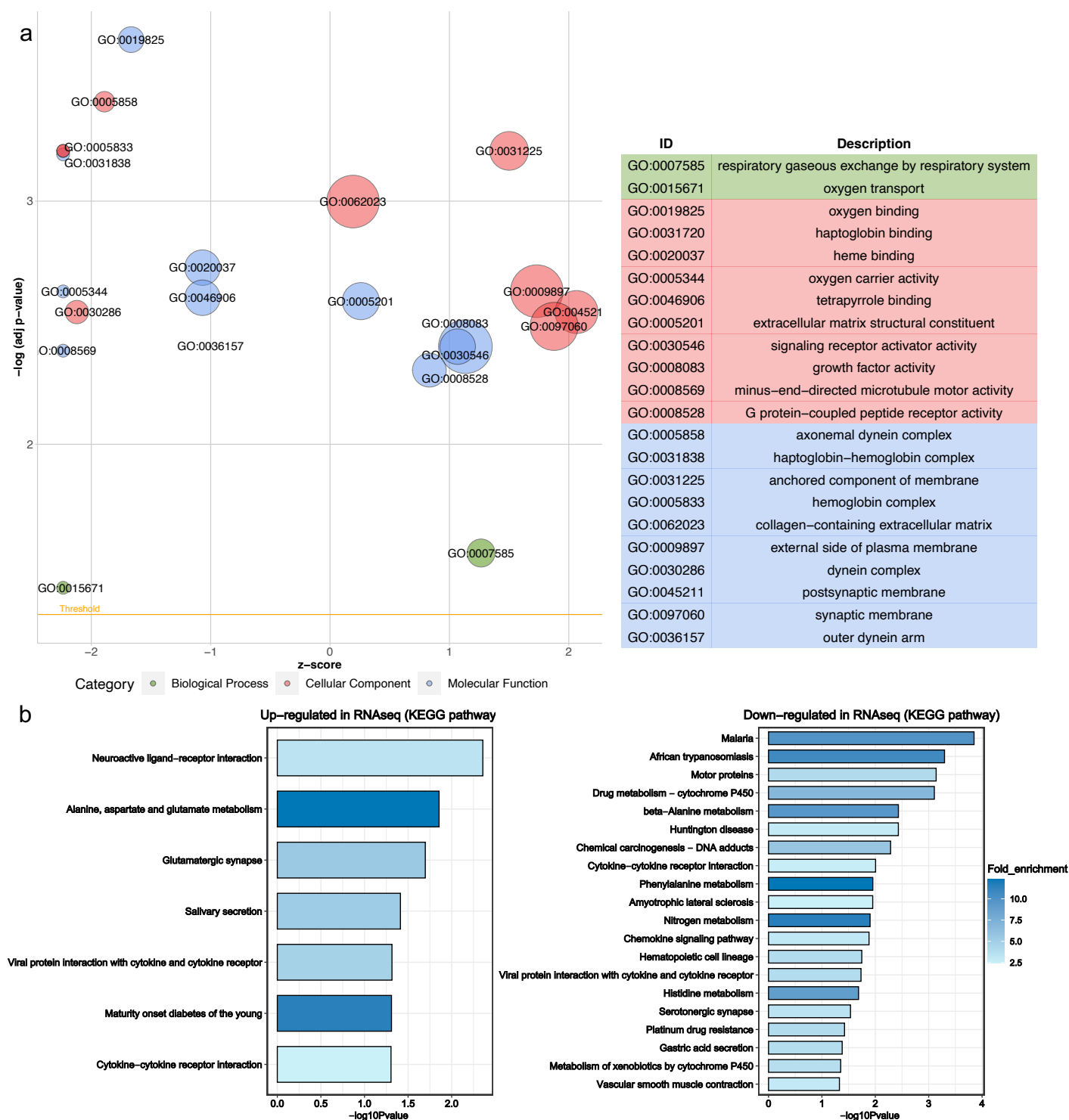


Figure. S12.

GO (a) and KEGG (b) enrichment analysis of different expression genes between long benefit and early disease progression groups.

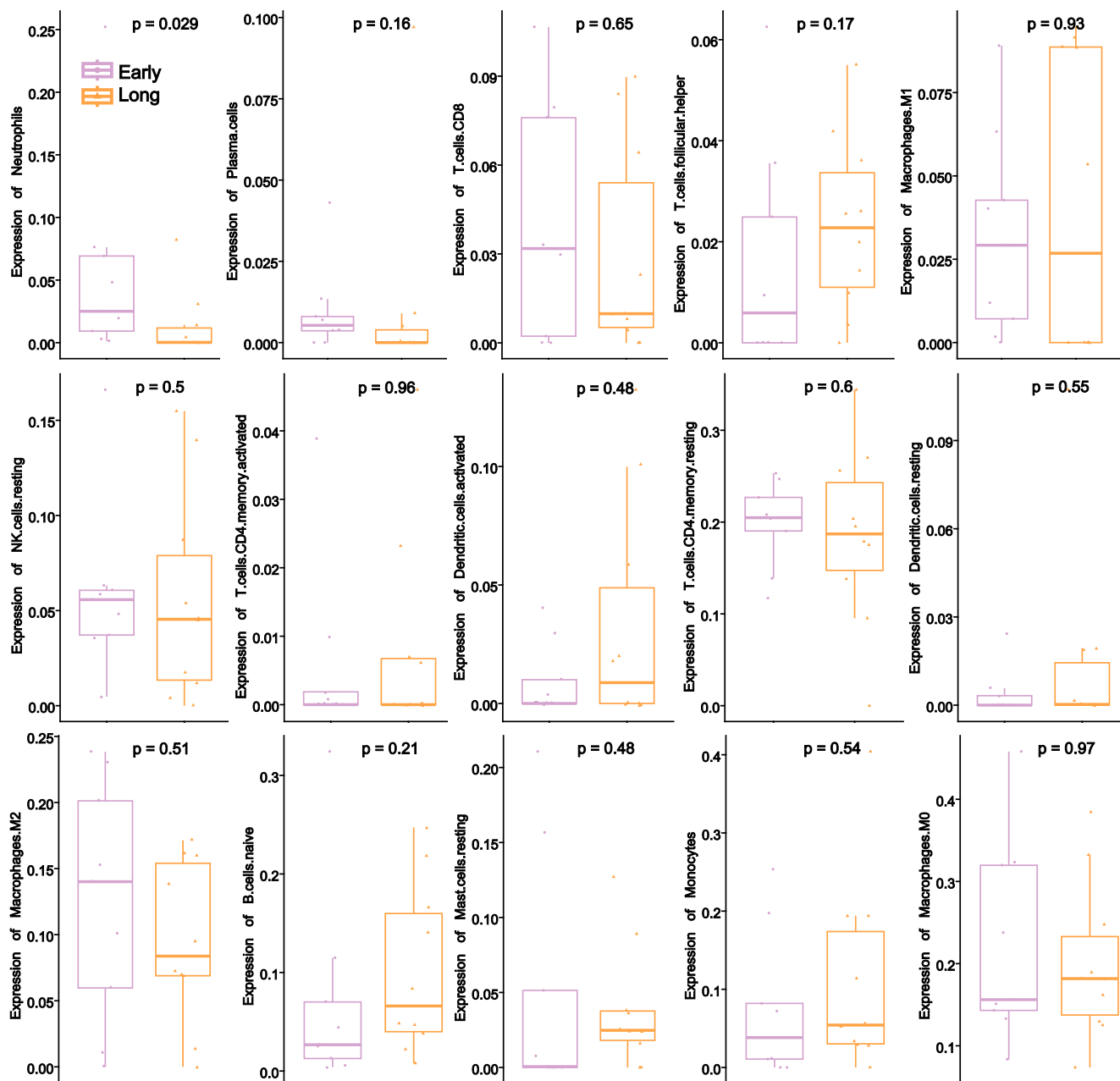


Figure. S13.

Comparison of each immune cell infiltration abundance between early progression and long benefit groups.

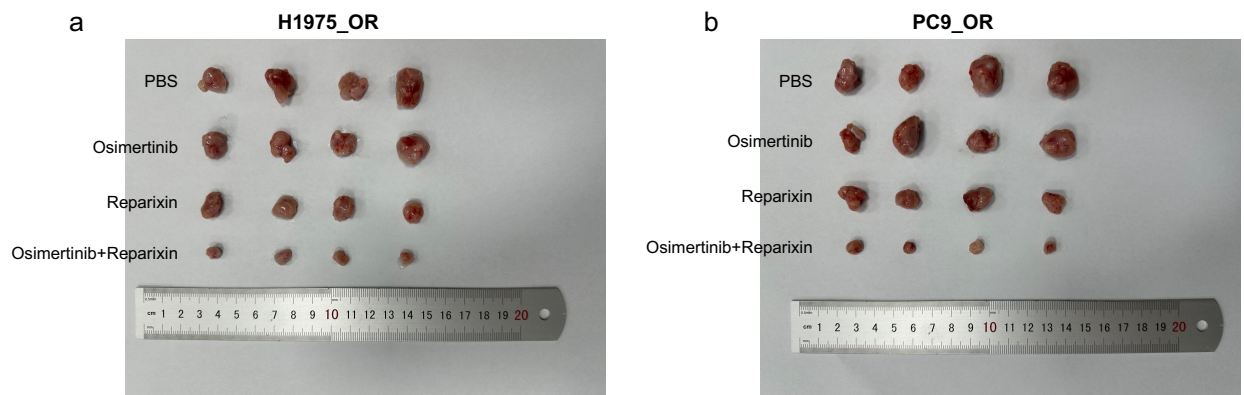


Figure. S14.

Original figures of tumor mass in different treatment groups. (a) H1975_OR cell line derived xenografts. (b) PC9_OR cell line derived xenografts.

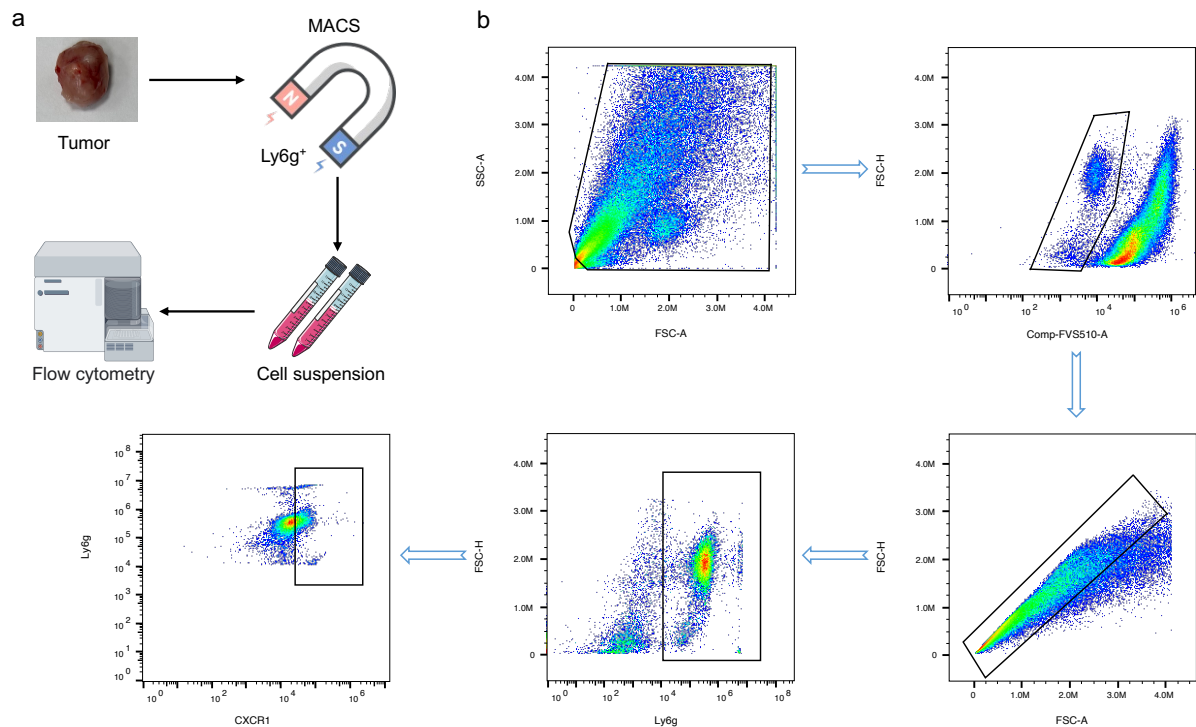


Figure. S15.

(a) Neutrophils in tumor were sorted with microbeads (the graphical model figure was created using Figdraw). (b) Flow cytometry gating strategy diagram.

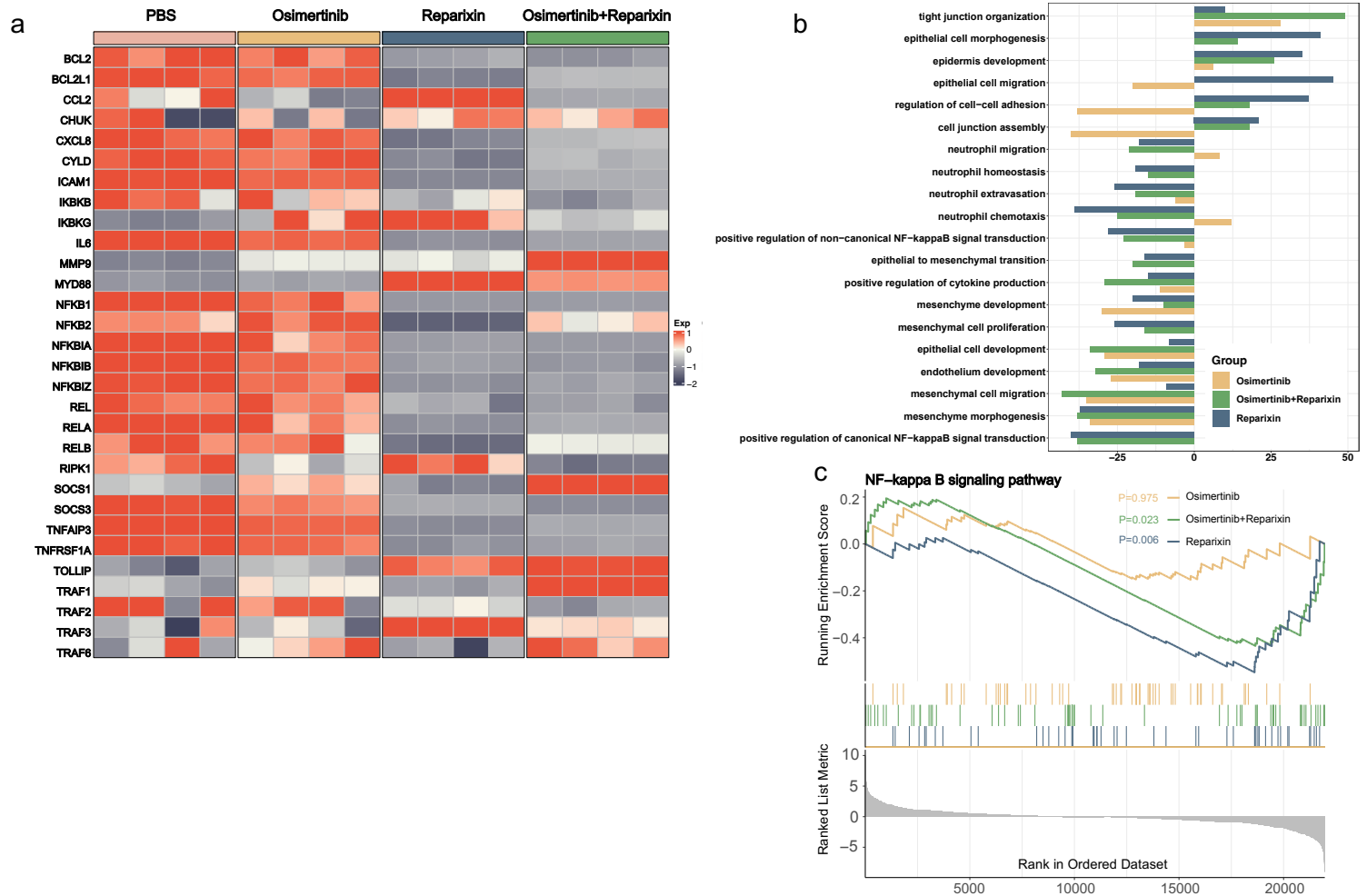


Figure. S16.

(a) Heatmap showing the expression profiles of NF- κ B related genes. (b) GO enrichment analysis of differently expressed genes. (c) GSEA enrichment analysis of NF- κ B signaling pathway in different treated groups.

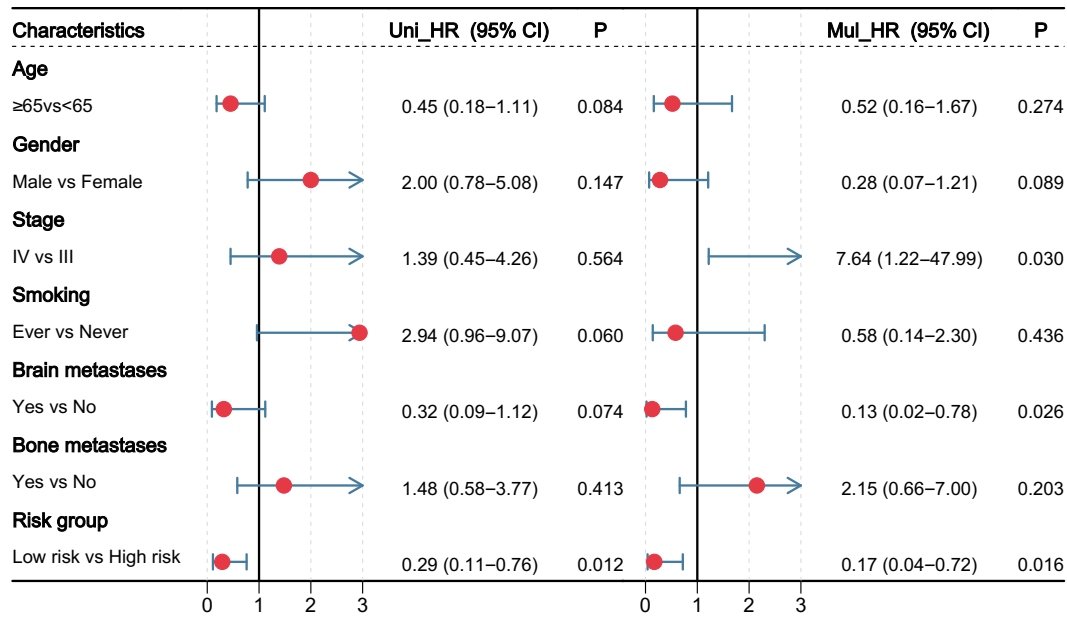


Figure. S17.

The univariate and multivariate Cox regression analysis was performed to analyze the impact of baseline neutrophil infiltration on the progression free survival. High-risk group was defined as pretreatment tumors samples with $\geq$ median CXCR1⁺ neutrophil infiltration level. Low-risk group was defined as pretreatment tumors samples with $<$ median infiltration level.

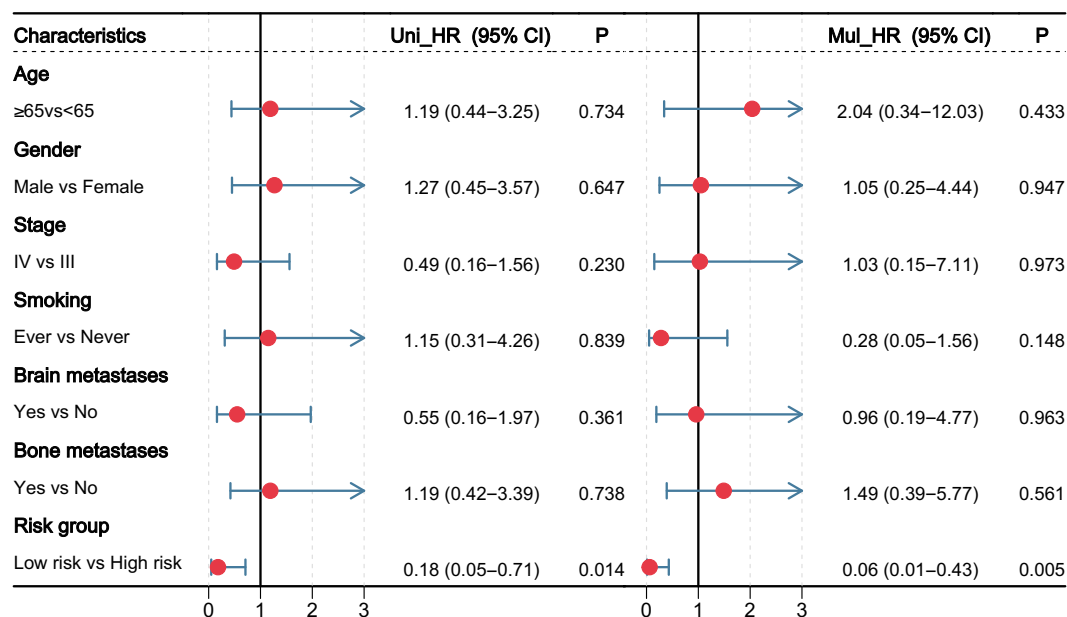


Figure. S18.

The univariate and multivariate Cox regression analysis was performed to analyze the impact of baseline neutrophil infiltration on the overall survival. High-risk group was defined as pretreatment tumors samples with $\geq$ median CXCR1⁺ neutrophil infiltration level. Low-risk group was defined as pretreatment tumors samples with $<$ median infiltration level.

Table S1.

Baseline demographic and characteristics of all included and analyzed patients.

Characteristics	Overall (n=25)	Analyzed (n=17)
Age, median (range)	64 (43-78)	63 (43-76)
<65	15 (60.0)	10 (58.8)
≥65	10 (40.0)	7 (41.2)
Gender (%)		
Female	10 (40.0)	8 (47.1)
Male	15 (60.0)	9 (52.9)
Smoking status (%)		
Never	17 (68.0)	13 (76.5)
Ever	8 (32.0)	4 (23.5)
Pathological types (%)		
Adenocarcinoma	25 (100.0)	17 (100.0)
Others	0 (0.0)	0 (0.0)
<i>EGFR</i> mutation type (%)		
T790M	25 (100.0)	17 (100.0)
Exon 19 deletion	13 (52.0)	11 (64.7)
L858R	9 (36.0)	6 (35.3)
Others	2 (8.0)	0 (0.0)
Disease stage (%)		
III	4 (16.0)	2 (11.8)
IV	21 (84.0)	15 (88.2)
Brain metastases (%)		
No	18 (72.0)	14 (82.4)
Yes	7 (28.0)	3 (17.6)
Bone metastases (%)		
No	17 (68.0)	11 (64.7)
Yes	8 (32.0)	6 (35.3)

Table S2.

Summary of clinicopathological parameters of 22 patients in scRNA sequencing cohort.

Sample ID	Tissue sample	Age	Sex	Smoking history	Histology	Gene mutation	Resistant sample	3 rd EGFR-TKI
P1	Lung	56	Male	Yes	Lung adenocarcinoma	T790M/19del	No	Osimertinib
P2	Lung	70	Female	No	Lung adenocarcinoma	T790M/L858R	No	Osimertinib
P3	Lung	68	Male	No	Lung adenocarcinoma	T790M/L858R	Yes	Osimertinib
P4	Lung	61	Female	No	Lung adenocarcinoma	T790M/19del	No	Almonertinib
P5	Lung	62	Male	No	Lung adenocarcinoma	T790M/19del	No	Osimertinib
P6	Lung	59	Male	No	Lung adenocarcinoma	T790M/L858R	Yes	Osimertinib
P7	Lung	67	Female	No	Lung adenocarcinoma	T790M/L858R	No	Furmonertinib
P8	Lung	55	Female	No	Lung adenocarcinoma	T790M/19del	Yes	Osimertinib
P9	Lung	49	Female	No	Lung adenocarcinoma	T790M/19del	No	Furmonertinib
P10	Lung	60	Male	Yes	Lung adenocarcinoma	T790M/19del	Yes	Osimertinib
P11	Lung	65	Female	No	Lung adenocarcinoma	T790M/19del	Yes	Osimertinib
P12	Lung	48	Female	No	Lung adenocarcinoma	T790M/L858R	No	Osimertinib
P13	Lung	66	Female	No	Lung adenocarcinoma	T790M/L858R	No	Almonertinib
P14	Lung	71	Male	Yes	Lung adenocarcinoma	T790M/19del	Yes	Osimertinib
P15	Lung	63	Female	No	Lung adenocarcinoma	T790M/19del	Yes	Osimertinib
P16	Lung	66	Female	No	Lung adenocarcinoma	T790M/L858R	No	Furmonertinib
P17	Lung	57	Female	No	Lung adenocarcinoma	T790M/L858R	Yes	Osimertinib
P18	Lung	58	Male	Yes	Lung adenocarcinoma	T790M/L858R	Yes	Osimertinib
P19	Lung	64	Female	No	Lung adenocarcinoma	T790M/19del	Yes	Almonertinib
P20	Lung	45	Male	No	Lung adenocarcinoma	T790M/19del	No	Osimertinib
P21	Lung	73	Male	Yes	Lung adenocarcinoma	T790M/19del	Yes	Osimertinib
P22	Lung	72	Female	Yes	Lung adenocarcinoma	T790M/L858R	Yes	Furmonertinib

Movie S1.

Intravital Imaging of CXCR1⁺ neutrophils in mouse in H1975_OR model.

Movie S2.

Intravital Imaging of CXCR1⁺ neutrophils in mouse in PC9_OR model.