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Supplemental information

Preclinical study and phase 2 trial of neoadjuvant pyrotinib combined with chemotherapy in luminal/HER2-low breast cancer: PILHLE-001 study

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Supplemental Information

Supplementary Figures

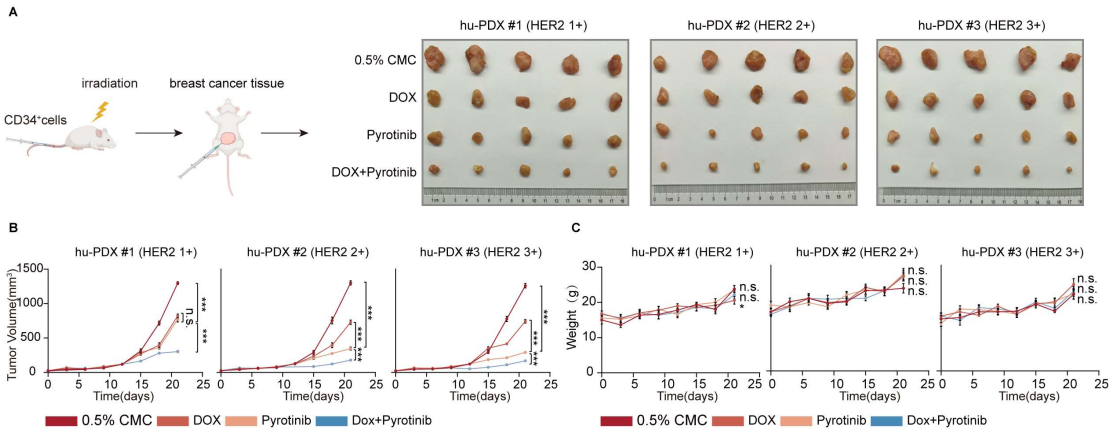

Figure S1. Pyrotinib combined with chemotherapy exhibited promising anti-tumor efficacy in humanized Luminal/HER2-low (IHC 2+/FISH-negative) breast cancer patient-derived xenograft models. Related to Figure 1.

(A) Schematic diagram and representative image of tumors of humanized Luminal/HER2-low (IHC 2+/FISH-negative) breast cancer patient-derived xenograft models. Tumor-bearing mice were treated with CMC, Dox, Pyrotinib or Dox + Pyrotinib, n = 5. CMC=carboxymethyl cellulose, Dox=doxorubicin, 5mg/kg, i.v., Pyrotinib, 10mg/kg, i.g.

(B) Tumor volume was measured at the indicated days, n=5.

(C) Body weight changes in different treatment groups during the whole testing period, n=5.

The comparative analysis was conducted between the pyrotinib monotherapy group and each treatment group. All bar values are represented as mean±SEM. **P*<0.05, ***P*<0.01, and ****P*<0.001.

HER2=Human epidermal growth factor receptor 2. IHC=immunohistochemistry. FISH= fluorescent in situ hybridization. SEM=standard error of the mean.

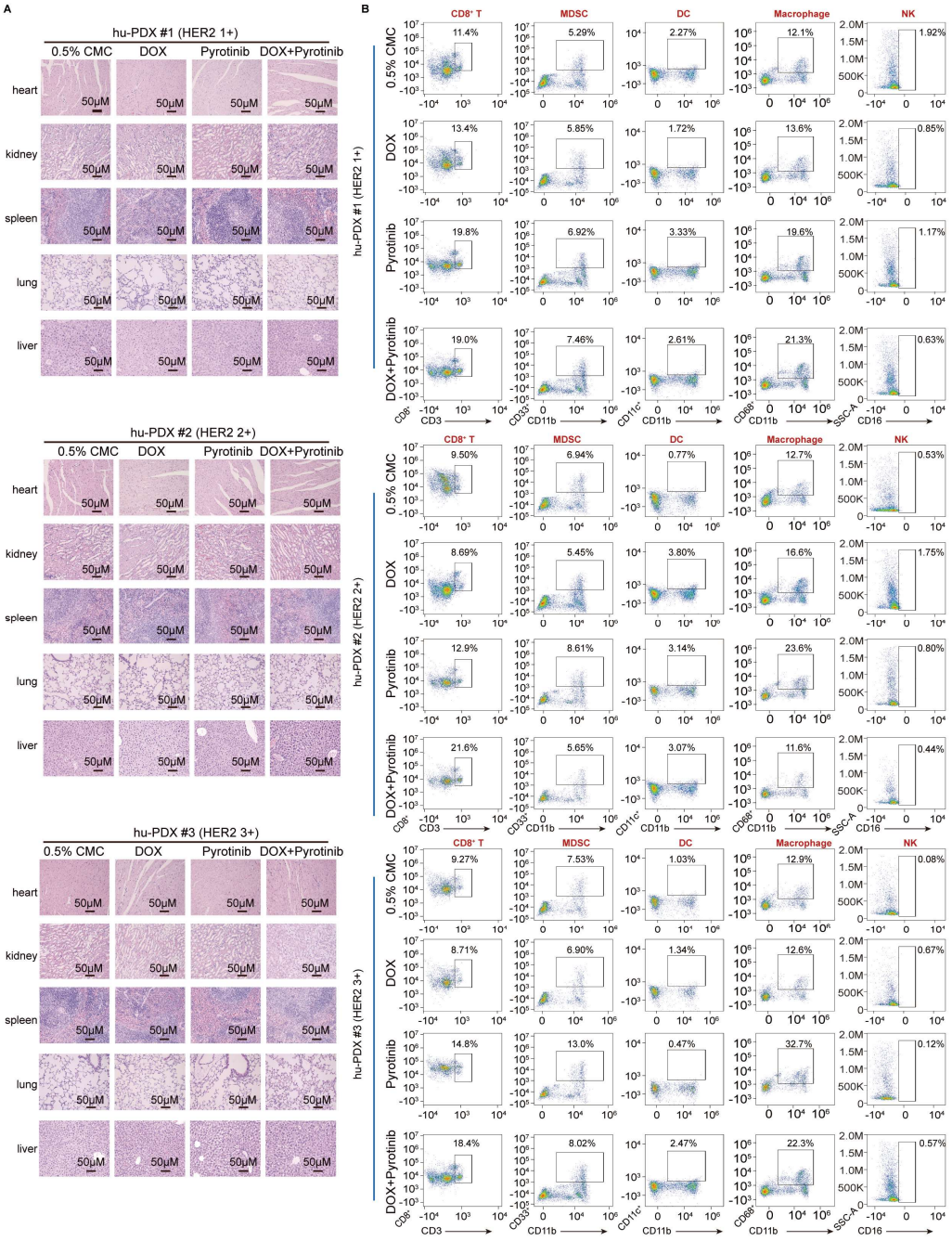

Figure S2. Hematoxylin and eosin staining and flow cytometry analysis of humanized breast cancer patient-derived xenograft models. Related to Figure 1.

(A) Hematoxylin and eosin staining is performed on the paraffin sections from organs including heart, liver, spleen, lung and kidney, scale bar, 50μm, n=5.

(B) Flow cytometry analysis of predominant immune cell infiltration, n=5.

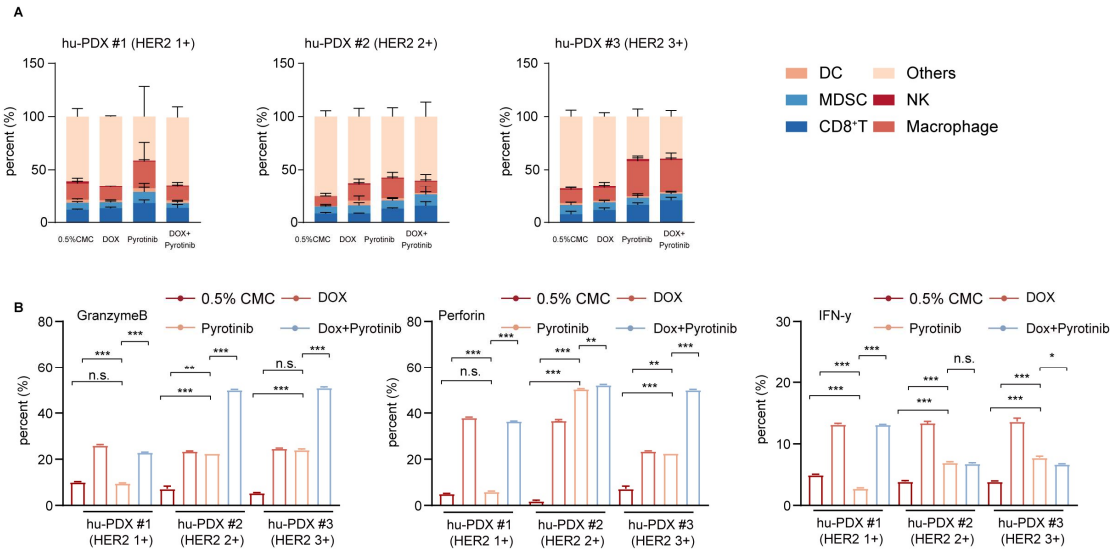

Figure S3. Pyrotinib combined with chemotherapy upregulated the proportion and cytotoxicity of immune-promoting cells in humanized Luminal/HER2-low (IHC 2+/FISH-negative) breast cancer patient-derived xenograft models. Related to Figure 1.

(A) Flow cytometry analysis of predominant immune cell infiltration. Marked immune cells including DC, MDSC, $CD8^+$T, NK, Macrophage, n=5.

(B) Flow cytometry analysis of granzyme B, perforin and IFN-γ in $CD8^+$ T cell, n=5.

The comparative analysis was conducted between the pyrotinib monotherapy group and each treatment group. All bar values are represented as mean±SEM. **P*<0.05, ***P*<0.01, and ****P*<0.001.

HER2=Human epidermal growth factor receptor 2. IHC=immunohistochemistry. FISH= fluorescent in situ hybridization. DC=dendritic cell. MDSC=myeloid-derived suppressor cell. NK=natural killer cell. SEM=standard error of the mean.

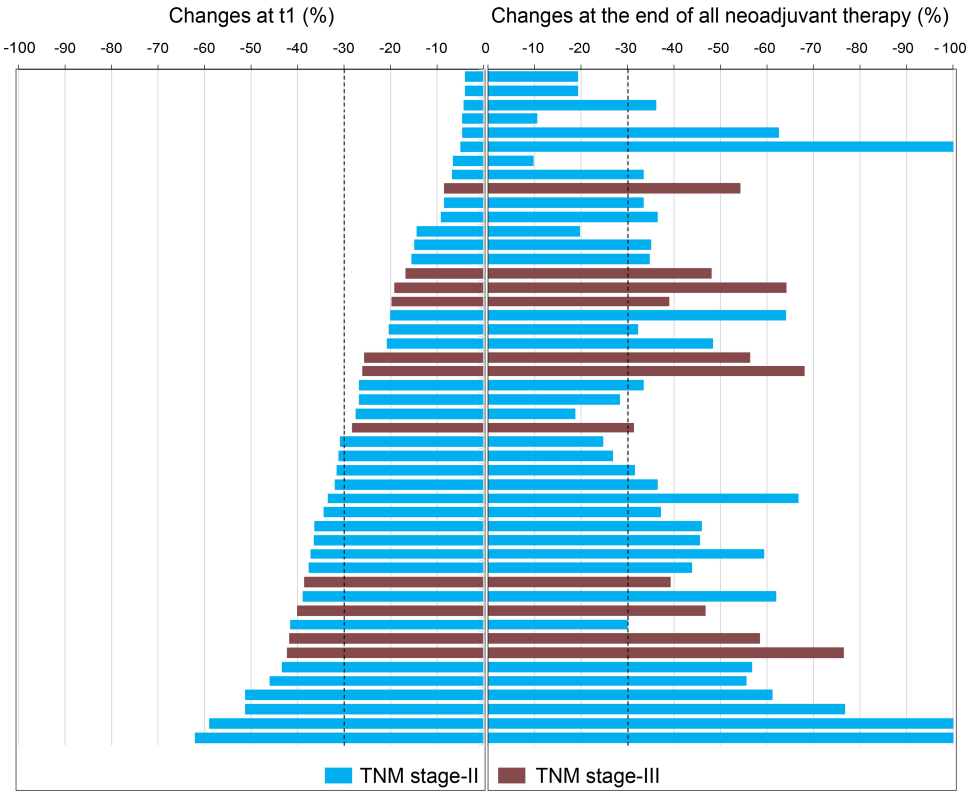

Figure S4. Changes in tumor size from baseline to the end of cycle 2 (t1) and the end of all neoadjuvant therapy. Related to Table 2.

According to Response Evaluation Criteria in Solid Tumors (version 1.1) based on magnetic resonance imaging. Each row represents a patient. The dotted line at -30% represents partial response.

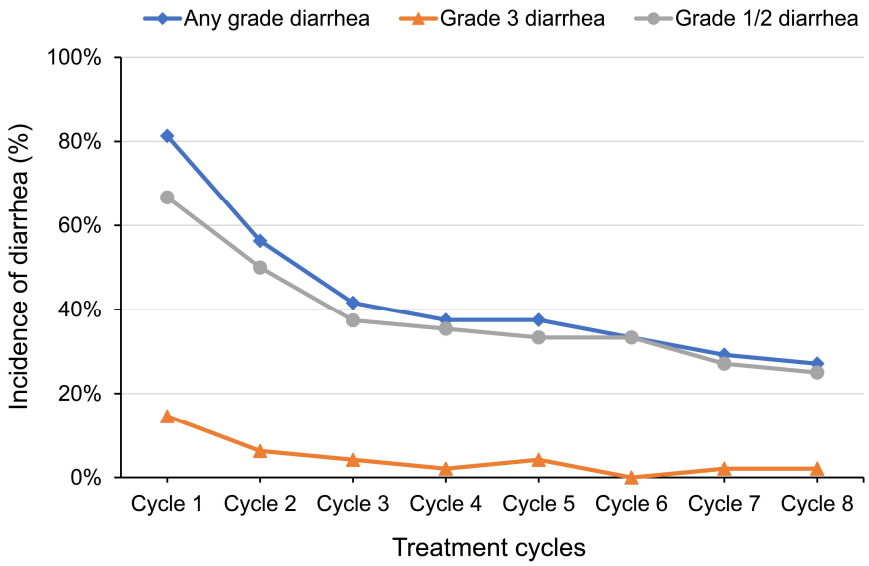

Figure S5. Incidence of diarrhea during neoadjuvant treatment. Related to Table 3.

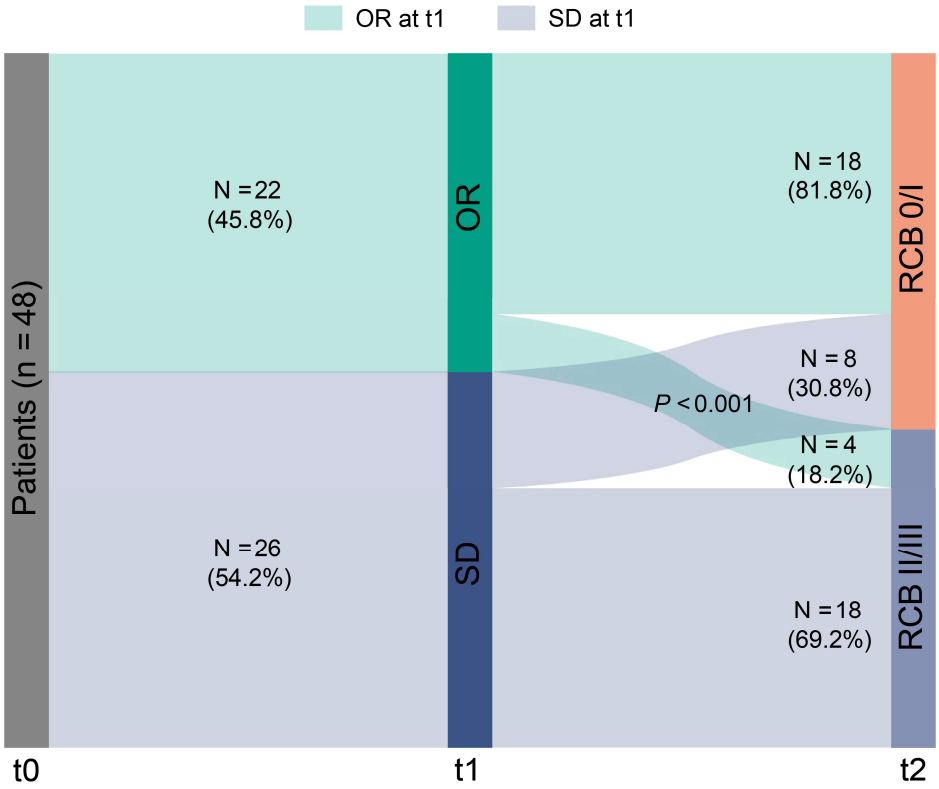

Figure S6. Tumor early response status evaluated by MRI at the end of cycle 2 (t1) versus RCB status at surgery (t2). Related to Table 2.

According to Response Evaluation Criteria in Solid Tumors (version 1.1). *P*-value is from the χ^2 test. MRI=magnetic resonance imaging. OR=objective response. SD=stable disease. RCB=residual cancer burden.

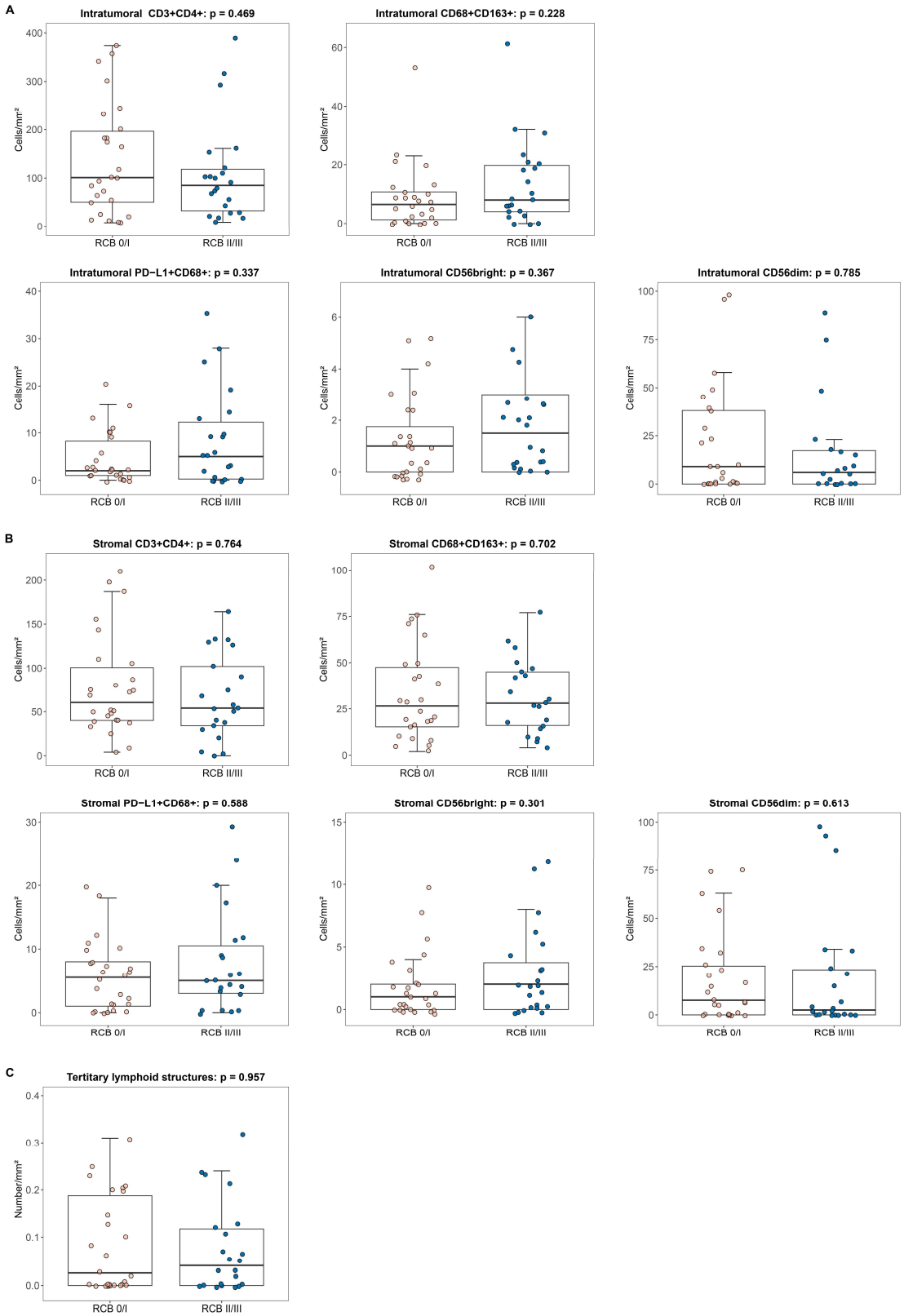

Figure S7. Baseline (t0) immune cell populations and tertiary lymphoid structure by multiplex immunofluorescence in patients with RCB 0/I versus RCB II/III. Related to Table 2.

(A) Intratumoral immune cell populations[a]. (B) Stromal immune cell populations[a]. (C) Tertiary lymphoid structure. [a]Some points outside the range are not shown in the box plots. *P*-values are from the Wilcoxon rank sum test. RCB=residual cancer burden.

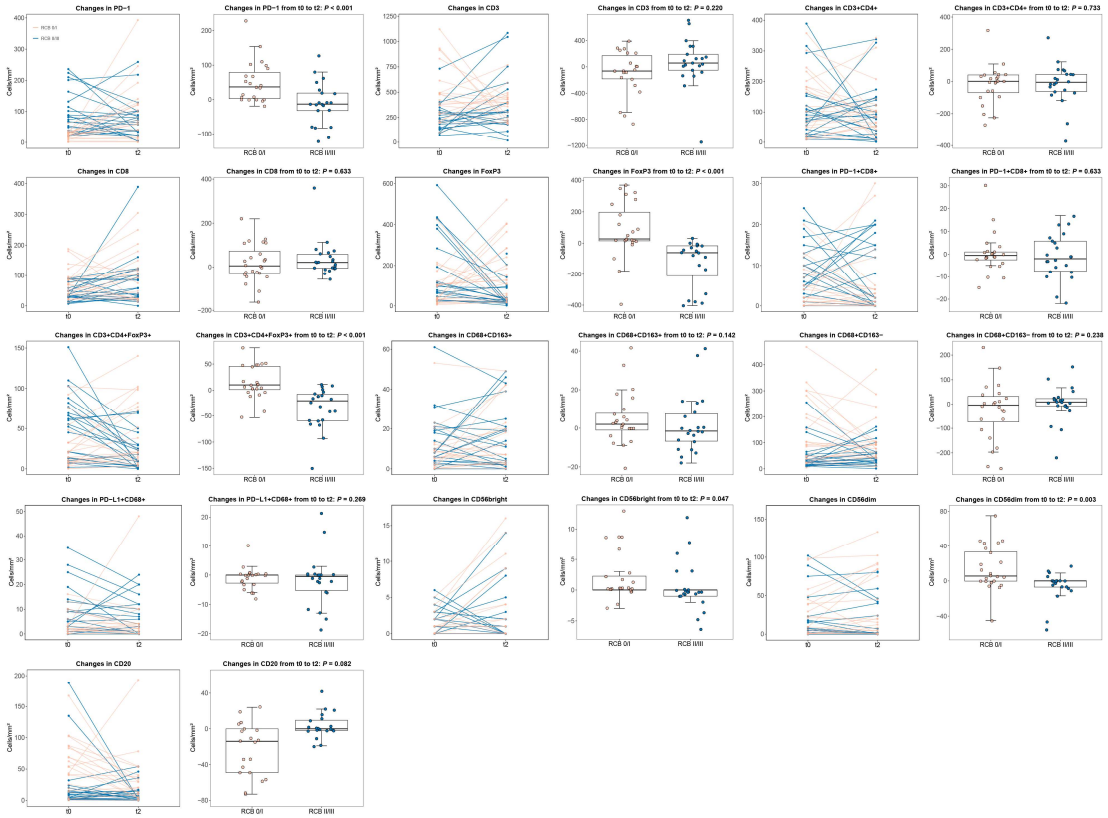

Figure S8. Changes in intratumoral immune cell populations by multiplex immunofluorescence from baseline (t0) to surgery (t2) in patients with RCB 0/I *vs.* RCB II/III. Related to Table 2. Three (6%) of 48 patients were not assessable for intratumoral immune cell populations at surgery because they achieved a RCB 0 at t2. Some points outside the range are not shown in the box plots. *P*-values are from the Wilcoxon rank sum test. RCB=residual cancer burden.

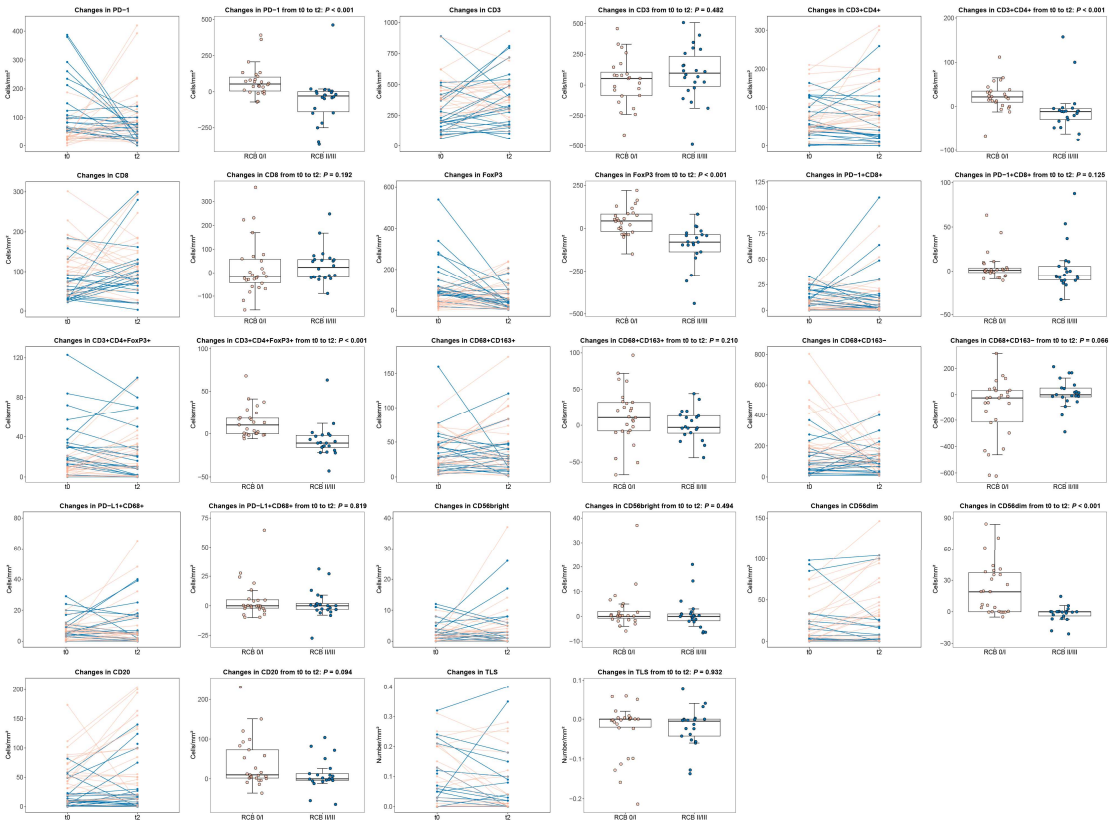

Figure S9. Changes in stromal immune cell populations and tertiary lymphoid structure (TLS) by multiplex immunofluorescence from baseline (t0) to surgery (t2) in patients with RCB 0/I *vs.* RCB II/III. Related to Table 2.

Some points outside the range are not shown in the box plots. *P*-values are from the Wilcoxon rank sum test. RCB=residual cancer burden. TLS=tertiary lymphoid structure.

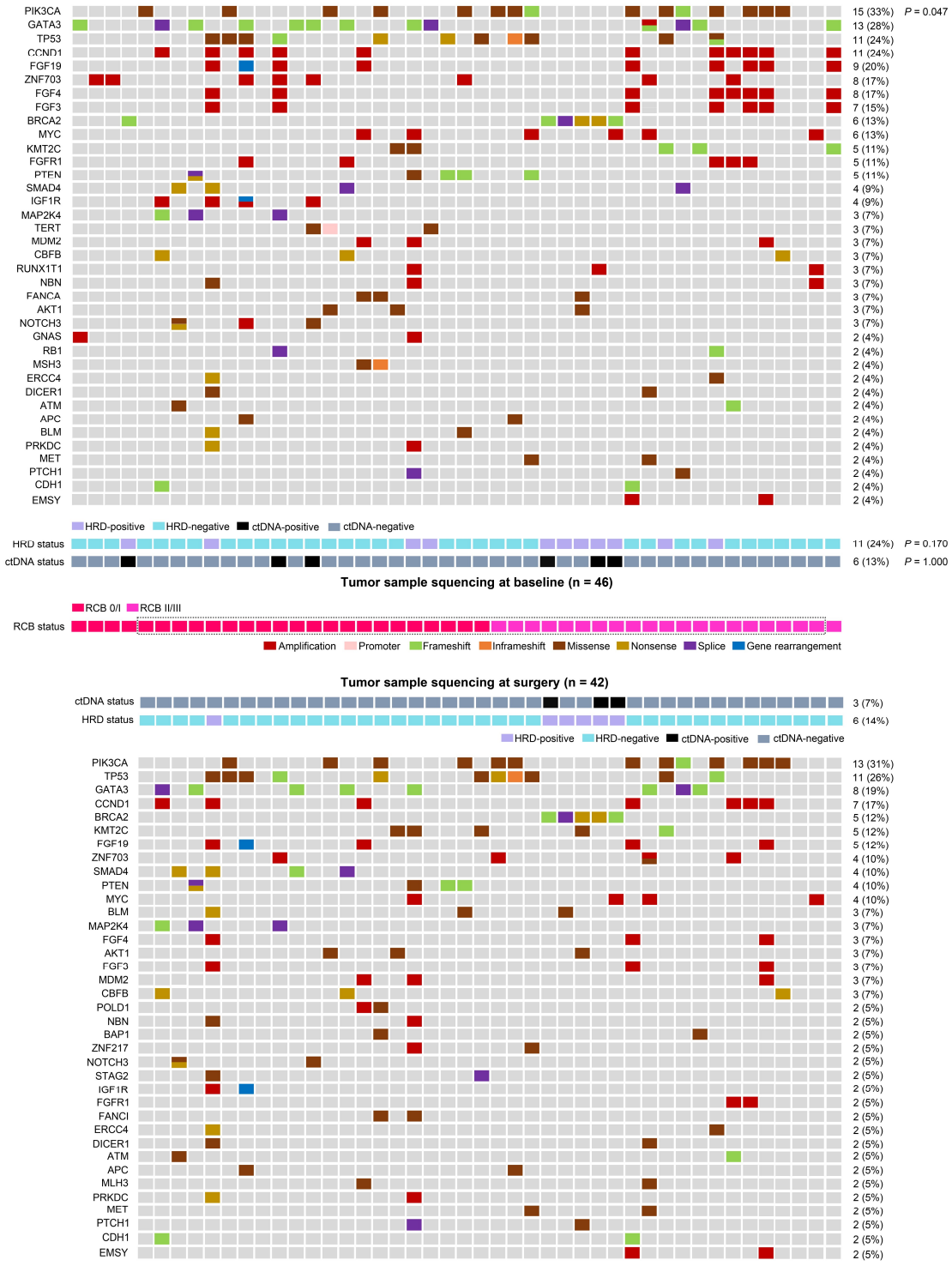

Figure S10. Gene sequencing of patients with available tumor tissue at baseline or surgery in the study cohort. Related to Table 2.

Each column represents a patient. Patients in the dotted box had matched tumor samples before and after treatment (n = 41). *P*-values (the association between gene mutation and RCB status) are from the χ^2 test. RCB=residual cancer burden. HRD=homologous recombination deficiency. ctDNA=circulating tumor DNA.

Supplementary Tables

Table S1. The detailed high-risk characteristics for each patient in the PILHLE-001 trial. Related to Table 1.

No.	Genomic high-risk	T3-4	N+	TNM stage-IIB/III	Histologic grade III	Ki67 ≥ 20%
1	√	√	√	√	x	√
2	√	√	√	√	x	√
3	√	x	√	√	√	√
4	√	x	√	√	√	√
5	√	√	√	√	x	x
6	√	x	√	√	x	√
7	√	x	√	√	x	√
8	√	x	√	√	x	√
9	√	x	√	√	x	√
10	√	x	√	√	x	√
11	√	x	√	√	x	√
12	√	x	√	√	x	√
13	√	x	√	√	x	√
14	√	x	√	√	x	x
15	√	x	√	√	x	x
16	√	x	x	x	√	√
17	√	x	x	x	√	√
18	√	x	x	x	√	√
19	√	x	x	x	√	√
20	√	x	x	x	√	x
21	√	x	x	x	x	√
22	√	x	x	x	x	√
23	√	x	x	x	x	√
24	√	x	x	x	x	√
25	√	x	x	x	x	√
26	√	x	x	x	x	√
27	√	x	x	x	x	√
28	√	x	x	x	x	√
29	x	√	x	√	√	√
30	x	√	x	√	x	x
31	x	x	√	√	x	√
32	x	x	√	√	x	x
33	x	x	√	√	x	x
34	x	x	x	x	√	√
35	x	x	x	x	√	√
36	x	x	x	x	√	√
37	x	x	x	x	√	√

38	x	x	x	x	√	√
39	x	x	x	x	√	√
40	x	x	x	x	√	√
41	x	x	x	x	√	x
42	x	x	x	x	√	x
43	x	x	x	x	x	√
44	x	x	x	x	x	√
45	x	x	x	x	x	√
46	x	x	x	x	x	√
47	-	x	x	x	x	√
48	-	x	x	x	x	√
For these six patients (No.43-48) with TNM stage-IIA tumors and Ki67 ≥ 20%, their respective Ki67 expression levels were 40%, 50%, 30%, 50%, 30%, and 40%.						

Table S2. Treatment-related diarrhea of pyrotinib plus chemotherapy. Related to Table 3.

	Pyrotinib plus chemotherapy
Diarrhea incidence, n (%)	
All grade	43 (89.6%)
Grade 1	15 (31.3%)
Grade 2	18 (37.5%)
Grade 3	10 (20.8%)
Time to onset, median (IQR), days	
Grade 1/2	2 (2-6)
Grade 3	7 (5-24)
Duration per event, median (IQR), days	
Grade 1/2	2 (2-10)
Grade 3	2 (1-3)

IQR=interquartile range.

Table S3. Treatment adjustment in the PILHLE-001 trial. Related to Table 3.

	Pyrotinib	Reason	Chemotherapy	Reason
Dose reduction	3 (6.3%)	Diarrhea	7 (14.6%)	Neutrophil count decreased: 4 (8.3%)
				ALT/AST increased: 2 (4.2%)
				Hand-foot syndrome: 1 (2.1%)
Treatment interruption	13 (27.1%)	Diarrhea: 10 (20.8%)	4 (8.3%)	ALT/AST increased: 2 (4.2%)
		ALT/AST increased: 3 (6.3%)		COVID-19 prevention: 2 (4.2%)
Treatment discontinued	5 (10.4%)	Diarrhea: 1 (2.1%)	2 (4.2%)	Individual decision
		Acute gastroenteritis: 1 (2.1%)		
		Individual decision: 3 (6.3%)		

Data are n (%). ALT=alanine aminotransferase. AST=aspartate aminotransferase

Table S4. Association between MRI parameters and RCB status. Related to Table 2.

	Baseline			The end of cycle 2			Change, %		
	RCB 0/I	**RCB II/III**	***P***	**RCB 0/I**	**RCB II/III**	***P***	**RCB 0/I**	**RCB II/III**	***P***
ADC									
Mean	0.96	1.02	0.222	1.11	1.09	0.901	12.2	7.6	0.605
(SD)	(0.14)	(0.16)		(0.29)	(0.22)		(21.0)	(18.5)	
Median	0.94	1.00		1.10	1.03		7.2	8.4	
(range)	(0.73 to 1.24)	(0.79 to 1.53)		(0.73 to 2.08)	(0.75 to 1.65)		(-22.2 to 78.3)	(-27.0 to 58.3)	
K^{trans}									
Mean	0.40	0.52	0.214	0.17	0.45	< 0.001	-48.8	-3.9	0.003
(SD)	(0.24)	(0.36)		(0.12)	(0.33)		(35.7)	(63.1)	
Median	0.28	0.41		0.18	0.33		-50.1	-16.8	
(range)	(0.18 to 1.10)	(0.15 to 1.48)		(0.01 to 0.49)	(0.13 to 1.48)		(-94.3 to 17.4)	(-62.2 to 236.9)	
K_{ep}									
Mean	0.54	0.67	0.301	0.29	0.62	0.002	-36.3	-2.3	0.012
(SD)	(0.33)	(0.44)		(0.24)	(0.50)		(40.3)	(44.2)	
Median	0.50	0.55		0.23	0.46		-42.4	-13.8	
(range)	(0.20 to 1.21)	(0.20 to 1.44)		(0.01 to 1.22)	(0.13 to 2.22)		(-94.3 to 63.4)	(-71.3 to 93.8)	
V_e									
Mean	0.81	0.83	0.967	0.77	0.84	0.694	4.6	21.1	0.242
(SD)	(0.23)	(0.21)		(0.31)	(0.21)		(61.1)	(93.9)	
Median	0.97	0.90		1.00	0.97		0	0.4	
(range)	(0.31 to 1.00)	(0.20 to 1.00)		(0.16 to 1.00)	(0.32 to 1.00)		(-76.6 to 186.0)	(-66.7 to 401.4)	
iAUC									
Mean	0.55	0.58	0.591*	0.51	0.50	0.918	9.8	1.6	0.983
(SD)	(0.22)	(0.23)		(0.34)	(0.29)		(97.7)	(76.8)	
Median	0.54	0.53		0.43	0.45		-4.6	-34.4	
(range)	(0.13 to 0.97)	(0.14 to 0.99)		(0.01 to 1.15)	(0.14 to 1.36)		(-98.4 to 246.9)	(-77.6 to 190.1)	
Wash-in									
Mean	0.84	0.83	0.634	0.59	0.57	0.869	-24.1	-32.2	0.918
(SD)	(0.39)	(0.51)		(0.60)	(0.53)		(56.2)	(41.4)	
Median	0.92	0.75		0.42	0.52		-30.6	-34.4	
(range)	(0.18 to 1.67)	(0.11 to 2.50)		(0.03 to 2.44)	(0.04 to 2.46)		(-96.8 to 193.6)	(-91.4 to 86.7)	
Wash-out									
Mean	-0.011	-0.011	0.548	-0.016	-0.010	0.264	23.0	-71.3	0.321
(SD)	(0.029)	(0.027)		(0.055)	(0.063)		(291.1)	(133.1)	
Median	-0.008	-0.003		-0.005	0.009		-33.8	-40.5	
(range)	(-0.06 to 0.08)	(-0.08 to 0.02)		(-0.23 to 0.01)	(-0.19 to 0.10)		(-475.5 to 1095.0)	(-2914.3 to 4948.6)	
TTP									
Mean	0.89	0.90	0.812	1.23	1.11	0.983	44.9	34.6	0.909
(SD)	(0.33)	(0.38)		(0.67)	(0.46)		(82.0)	(67.8)	
Median	0.85	0.77		0.92	1.06		20.8	15.0	
(range)	(0.40 to 2.11)	(0.28 to 1.66)		(0.40 to 2.67)	(0.35 to 2.07)		(-47.8 to 268.0)	(-39.3 to 268.0)	

Data are mean (SD) or median (range), unless otherwise stated. *P*-values are come from the Student's t-test or Wilcoxon rank sum test. RCB=residual cancer burden. ADC=apparent diffusion coefficient.

*Student's t-test.