

Supplementary Table 1. Clinicopathological factors in the locally advanced rectal cancer cells treated with neoadjuvant chemoradiotherapy group and untreated with neoadjuvant chemoradiotherapy group

Variable	No.	LARC-nCRT	LARC-no nCRT	<i>p</i> *
<b>Gender</b>		<b>61</b>	<b>60</b>	<b>0.406</b>
Male	79	42	37	
Female	42	19	23	
<b>Age (years)</b>				<b>0.019</b>
≤65	74	31	43	
>65	47	30	17	
<b>(y)pT</b>				<b>0.010</b>
2	44	29	15	
3	77	32	45	
<b>(y)pN</b>				<b>0.762</b>
0	75	37	38	
1-2	46	24	22	
<b>(y)p TNM stage</b>				<b>0.626</b>
II	74	36	38	
III	47	25	22	
<b>Post-Chemotherapy</b>				<b>0.636</b>
No	30	14	16	
Yes	91	47	44	

\* Pearson's chi-square test; nCRT, neoadjuvant chemoradiotherapy; pStage, pathologic stage; LARC-nCRT, locally advanced rectal cancer cells treated with neoadjuvant chemoradiotherapy group; LARC-no nCRT, locally advanced rectal cancer cells untreated with neoadjuvant chemoradiotherapy group; ypTNM stage, neoadjuvant pathologic stage in American Joint Committee on Cancer (AJCC) cancer staging system (8th edition); and post-chemotherapy, post-operative adjuvant chemotherapy.

Supplementary Table 2. Correlations between the levels of CXCL12, CXCR4, and FAP $\alpha$  in the locally advanced rectal cancer cells treated with neoadjuvant chemoradiotherapy group using immunohistochemical staining.

Spearman's rho		CXCL12	CXCR4	FAP $\alpha$
CXCL12	Correlation coefficient	1.000	0.402**	0.372**
	Sig. (2-tailed)	.	0.001	0.003
	No.	61	61	61
CXCR4	Correlation coefficient	0.402**	1.000	0.402**
	Sig. (2-tailed)	0.001	.	0.001
	No.	61	61	61
FAP $\alpha$	Correlation coefficient	0.372**	0.402**	1.000
	Sig. (2-tailed)	0.003	0.001	.
	No.	61	61	61

\*\* , *p* < 0.01

Supplementary **Table 3.** Univariate analysis of overall survival and freedom from recurrence in 60 patients with locally advanced rectal cancers untreated with neoadjuvant chemoradiotherapy.

Prognostic factor	Overall survival		Freedom From Recurrence	
	HR (95% CI)	<i>p</i> *	HR (95% CI)	<i>p</i> *
<b>CXCL12 expression</b>	0.521 (0.210-1.292)	0.160	1.069 (0.408-2.797)	0.892
<b>CXCR4 expression</b>	N/A	N/A	N/A	N/A
<b>FAP<math>\alpha</math> expression</b>	0.859 (0.556-1.327)	0.493	1.301 (0.782-2.164)	0.311
<b>Age at operation</b>		0.007		0.963
≤65 (n=43)	1 (reference)		1 (reference)	
>65 (n=17)	3.286 (1.391-7.764)		1.032 (0.271-3.935)	
<b>Sex</b>		0.326		0.597
Male (n=37)	1 (reference)		1 (reference)	
Female (n=23)	1.537 (0.652-3.621)		1.380 (0.418-4.558)	
<b>pTNM stage</b>		0.424		0.224
II (n=38)	1 (reference)		1 (reference)	
III (n=22)	0.679 (0.264-1.752)		2.090 (0.638-6.850)	
<b>Post-Chemotherapy</b>		0.207		0.525
No (n=16)	1 (reference)		1 (reference)	
Yes (n=44)	0.567 (0.235-1.370)		1.645 (0.355-7.621)	
<b>Post-radiotherapy</b>		0.555		0.716
No (n=25)	1 (reference)		1 (reference)	
Yes (n=35)	0.773 (0.328-1.820)		1.256 (0.368-4.294)	

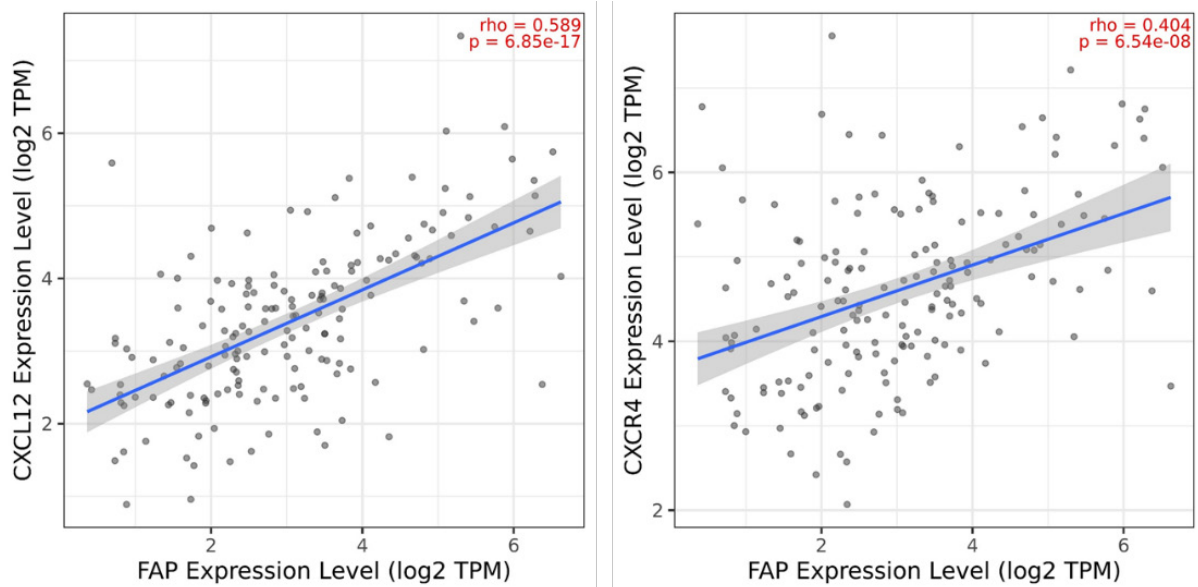
\* Univariate Cox regression analysis; HR, hazard ratio; CI, confidence interval; CXCL12 expression, plasma membrane expression in cancer cells, scored as 0,1,2, or 3; CXCR4 expression, plasma membrane expression in cancer cells, scored as 0,1,2, or 3, All 60 cases are score 1.; FAP $\alpha$  expression, cytoplasmic expression in cancer cells, scored as 0,1,2, or 3; pTNM stage, pathologic stage in American Joint Committee on Cancer (AJCC) cancer staging system (8<sup>th</sup> edition); post-chemotherapy, post-operative adjuvant chemotherapy; and post-radiotherapy, post-operative adjuvant radiotherapy; and N/A, not applicable.

Supplementary **Table 4.** Multivariate analysis of overall survival and freedom from recurrence in 60 patients with locally advanced rectal cancers untreated with neoadjuvant chemoradiotherapy.

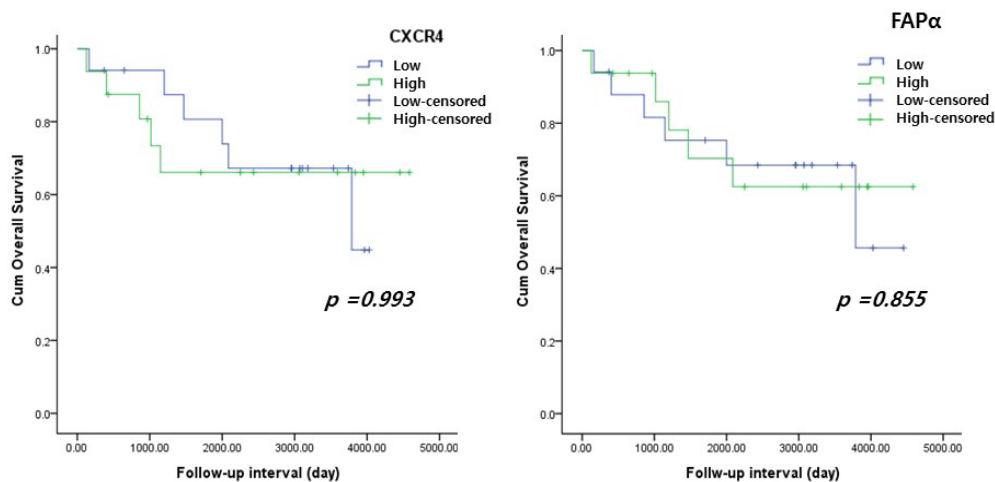
Prognostic factor	Overall survival		Freedom From Recurrence	
	HR (95% CI)	<i>p</i> *	HR (95% CI)	<i>p</i> *
<b>CXCL12 expression</b>	0.499 (0.184-1.351)	0.171	0.764 (0.271-2.152)	0.610
<b>FAP<math>\alpha</math> expression</b>	0.837 (0.541-1.295)	0.424	1.267 (0.738-2.174)	0.391
<b>Age at operation</b>		0.012		0.881
≤65 (n=43)	1 (reference)		1 (reference)	
>65 (n=17)	3.518 (1.313-9.424)		1.123 (0.246-5.117)	
<b>Sex</b>		0.508		0.734
Male (n=37)	1 (reference)		1 (reference)	
Female (n=23)	1.373 (0.537-3.505)		1.253 (0.341-4.600)	
<b>pTNM stage</b>		0.865		0.350
II (n=38)	1 (reference)		1 (reference)	
III (n=22)	0.909 (0.303-2.729)		1.990 (0.470-8.422)	
<b>Post-Chemotherapy</b>		0.928		0.785
No (n=16)	1 (reference)		1 (reference)	
Yes (n=44)	1.084 (0.190-6.201)		1.385 (0.133-14.461)	
<b>Post-radiotherapy</b>		0.803		0.883
No (n=25)	1 (reference)		1 (reference)	
Yes (n=35)	1.218 (0.259-5.722)		0.883 (0.168-4.641)	

\* Multivariate Cox regression analysis; HR, hazard ratio; CI, confidence interval; CXCL12 expression, plasma membrane expression in cancer cells, scored as 0,1,2, or 3; FAP $\alpha$  expression, cytoplasmic expression in cancer cells, scored as 0,1,2, or 3; pTNM stage, pathologic stage in American Joint Committee on Cancer (AJCC) cancer staging system (8<sup>th</sup> edition); post-chemotherapy, post-operative adjuvant chemotherapy; and post-radiotherapy, post-operative adjuvant radiotherapy.

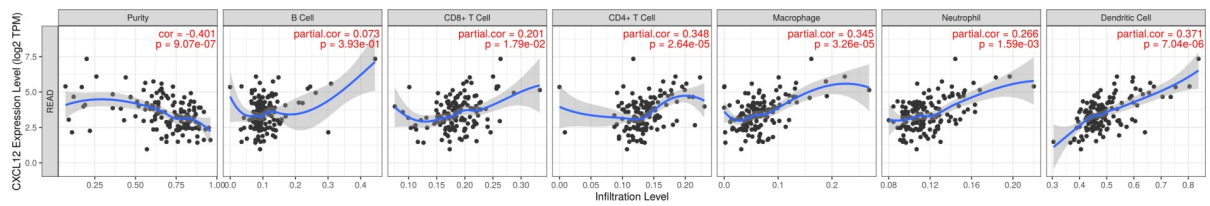
**Supplementary Figure 1.** Correlation between CXCL12/CXCR4 expression and FAP $\alpha$  in locally advanced rectal cancer (LARC). A significant positive correlation between CXCL12/CXCR4 and FAP $\alpha$  expression was observed in LARC.



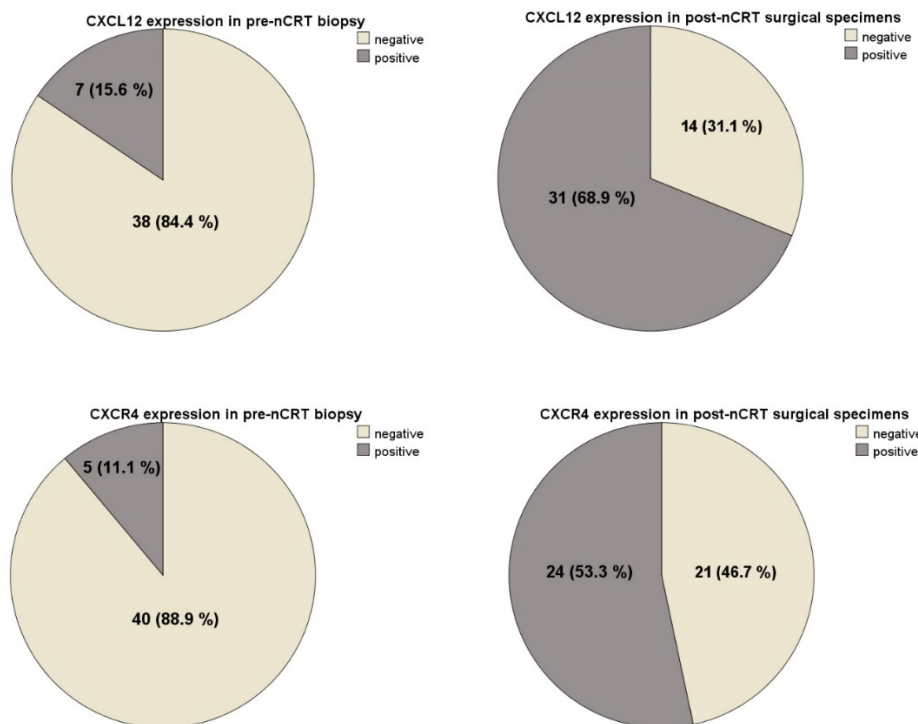
**Supplementary Figure 2.** Correlation between CXCR4 and FAP $\alpha$  expression and patient survival in locally advanced rectal cancer. Kaplan-Meier curves for low and high groups of the CXCR4 and FAP $\alpha$  gene expression in GSE133057 dataset. CXCR4 and FAP $\alpha$  mRNA expression levels are not significant prognostic factors for overall survival in GSE133057.



**Supplementary Figure 3.** Correlation of CXCL12 expression with immune infiltration level in LARC.



**Supplementary Figure 4.** Changes in CXCL12 and CXCR4 levels in pre-neoadjuvant chemoradiotherapy (nCRT) biopsy and paired post-nCRT surgical specimen tissue samples from 45 LARC patients showed higher expression in LARC cancer cells after nCRT ( $p < 0.001$  for each, Wilcoxon signed-rank test).



**Supplementary Figure 5.** Images showing immunohistochemical staining of CXCL12, CXCR4, and FAP $\alpha$  in the case of each pathways in non-neoplastic rectal glands (A, C, E) and rectal tubular adenoma (B, D, F). In both, CXCL12 and CXCR4 showed no plasma membrane expression (A, B, C, D) and FAP $\alpha$  exhibited little or no expression in the epithelial cells (E, F) (original magnification 400 $\times$ ; scale bar= 50  $\mu$ m).

