

Figure S1 A flowchart diagram showing the process excluding ineligible patients in each step. HE, hematoxylin and eosin.

Table S1 Comparison of clinical features between groups with and without adjuvant chemotherapy

Clinical factors	Subgroup without adjuvant chemotherapy		Subgroup with adjuvant chemotherapy		Chi-squared	P value
	N	%	N	%		
Gender					0.505	0.47
Female	73	38.83	51	34.46		
Male	115	61.17	97	65.54		
Age (years)					45.366	<0.001
≥70	78	41.49	12	8.11		
<70	110	58.51	136	91.89		
Tumor size (cm)					0.000	>0.99
<5	115	61.17	90	60.81		
≥5	73	38.83	58	39.19		
Location					6.178	0.04
Distal colon	83	44.15	74	50.00		
Proximal colon	64	34.04	57	38.51		
Rectum	41	21.81	17	11.49		
T4					1.855	0.17
No	124	65.96	86	58.11		
Yes	64	34.04	62	41.89		
Morphological type					2.951	0.22
Lump	26	13.83	12	8.11		
Raised	31	16.49	23	15.54		
Ulcerative	131	69.68	113	76.35		
Differentiation					0.544	0.76
Middle	171	90.96	131	88.51		
Poor	7	3.72	7	4.73		
Well	10	5.32	10	6.76		
Vascular invasion					1.138	0.28
No	175	93.09	132	89.19		
Yes	13	6.91	16	10.81		
Perineural invasion					5.536	0.01
No	157	83.51	107	72.30		
Yes	31	16.49	41	27.70		
Mucinous adenocarcinoma					0.054	0.81
No	180	95.74	140	94.59		
Yes	8	4.26	8	5.41		
Intestinal obstruction					0.017	0.89
No	131	69.68	105	70.95		
Yes	57	30.32	43	29.05		
Perforation					3.623	0.05
No	185	98.40	139	93.92		
Yes	3	1.60	9	6.08		
CEA (ng/mL)					0.734	0.39
≥5	64	34.04	43	29.05		
<5	124	65.96	105	70.95		
CA199 (U/mL)					0.263	0.60
≥37	22	11.70	21	14.19		
<37	166	88.30	127	85.81		
TB					1.143	0.56
BD1	96	51.06	77	52.03		
BD2	53	28.19	35	23.65		
BD3	39	20.74	36	24.32		
TB construction					0.405	0.52
Cluster	139	89.10	116	92.06		
Single-cell	17	10.90	10	7.94		
TB location					–	0.08
Muscularis propria	57	36.54	37	29.37		
Peritoneum	4	2.56	3	2.38		
Submucosa	26	16.67	12	9.52		
Subserosa	69	44.23	74	58.73		
TB stromal reaction					0.868	0.64
Fibrotic	44	28.21	42	33.33		
Inflammatory	95	60.90	71	56.35		
Myxoid	17	10.90	13	10.32		

CEA, carcinoembryonic antigen; CA199, carbohydrate antigen; TB, tumor budding; BD, budding grade.

Table S2 Univariate and multivariate Cox regression for RFS and OS in the whole population

Parameters	Univariate		Multivariate	
	HR (95% CI)	P	HR (95% CI)	P
RFS				
Gender (male vs. female)	1.055 (0.646–1.725)	0.83	1.070 (0.608–1.883)	0.81
Age (≥ 70 vs. < 70 years)	2.435 (1.514–3.915)	< 0.001	4.501 (2.205–9.189)	< 0.001
Location (proximal colon vs. distal colon)	0.804 (0.474–1.362)	0.41	0.865 (0.456–1.642)	0.65
Location (rectum vs. distal colon)	0.831 (0.421–1.642)	0.59	0.980 (0.445–2.158)	0.95
T4 (yes vs. no)	1.203 (0.745–1.943)	0.44	0.853 (0.481–1.511)	0.58
Morphological type (raised vs. ulcerative)	0.804 (0.397–1.631)	0.54	1.585 (0.711–3.534)	0.26
Morphological type (lump vs. ulcerative)	0.773 (0.351–1.701)	0.52	0.891 (0.349–2.275)	0.80
Differentiation (poor vs. well/middle)	0.701 (0.172–2.864)	0.62	0.995 (0.215–4.604)	0.99
Vascular invasion (yes vs. no)	1.239 (0.567–2.710)	0.59	0.548 (0.212–1.419)	0.21
Perineural invasion (yes vs. no)	2.775 (1.695–4.544)	< 0.001	2.612 (1.397–4.882)	0.003
Mucinous adenocarcinoma (yes vs. no)	1.848 (0.742–4.607)	0.18	2.771 (0.864–8.887)	0.08
Intestinal obstruction (yes vs. no)	2.046 (1.270–3.296)	0.003	1.765 (0.964–3.231)	0.06
Perforation (yes vs. no)	2.712 (1.085–6.775)	0.03	1.761 (0.533–5.819)	0.35
Adjuvant chemotherapy (yes vs. no)	0.962 (0.598–1.549)	0.87	1.806 (0.894–3.650)	0.10
Tumor size (≥ 5 vs. < 5 cm)	0.709 (0.427–1.176)	0.18	0.617 (0.327–1.163)	0.13
CEA (≥ 5 vs. < 5 ng/mL)	1.783 (1.107–2.871)	0.01	1.520 (0.811–2.850)	0.19
CA199 (≥ 37 vs. < 37 U/mL)	1.639 (0.879–3.056)	0.12	1.319 (0.617–2.821)	0.47
TB construction (single-cell vs. cluster)	1.105 (0.475–2.570)	0.81	1.292 (0.507–3.291)	0.59
TB location (subserosa/peritoneum vs. submucosa/muscularis propria)	1.394 (0.831–2.337)	0.20	1.396 (0.746–2.610)	0.29
TB stromal reaction (fibrotic vs. inflammatory)	1.068 (0.610–1.868)	0.81	0.786 (0.396–1.560)	0.49
TB stromal reaction (myxoid vs. inflammatory)	0.933 (0.392–2.219)	0.87	0.612 (0.225–1.662)	0.33
TB grade (BD2 vs. BD1)	1.318 (0.725–2.393)	0.36	1.468 (0.703–3.063)	0.30
TB grade (BD3 vs. BD1)	2.256 (1.300–3.913)	0.004	2.310 (1.154–4.625)	0.01
OS				
Gender (male vs. female)	0.997 (0.563–1.766)	0.99	0.966 (0.504–1.851)	0.91
Age (≥ 70 vs. < 70 years)	4.969 (2.804–8.807)	< 0.001	9.262 (3.748–22.887)	< 0.001
Location (proximal colon vs. distal colon)	0.779 (0.413–1.471)	0.44	0.673 (0.305–1.484)	0.32
Location (rectum vs. distal colon)	1.186 (0.562–2.500)	0.65	1.758 (0.739–4.179)	0.20
T4 (yes vs. no)	1.725 (0.989–3.008)	0.05	1.159 (0.606–2.217)	0.65
Morphological type (raised vs. ulcerative)	0.912 (0.406–2.047)	0.82	1.605 (0.620–4.158)	0.33
Morphological type (lump vs. ulcerative)	0.948 (0.400–2.247)	0.90	0.868 (0.303–2.484)	0.79
Differentiation (poor vs. well/middle)	0.467 (0.064–3.381)	0.45	0.963 (0.114–8.165)	0.97
Vascular invasion (yes vs. no)	1.240 (0.492–3.130)	0.64	0.482 (0.150–1.550)	0.22
Perineural invasion (yes vs. no)	2.624 (1.456–4.731)	0.001	2.833 (1.306–6.145)	0.008
Mucinous adenocarcinoma (yes vs. no)	2.125 (0.761–5.939)	0.15	2.807 (0.667–11.819)	0.15
Intestinal obstruction (yes vs. no)	2.122 (1.213–3.711)	0.008	2.461 (1.183–5.119)	0.01
Perforation (yes vs. no)	2.122 (0.656–6.859)	0.20	2.670 (0.503–14.182)	0.24
Adjuvant chemotherapy (yes vs. no)	0.510 (0.278–0.935)	0.02	1.313 (0.551–3.126)	0.53
Tumor size (≥ 5 vs. < 5 cm)	0.786 (0.437–1.411)	0.41	0.585 (0.272–1.259)	0.17
CEA (≥ 5 vs. < 5 ng/mL)	1.997 (1.144–3.487)	0.01	1.295 (0.615–2.727)	0.49
CA199 (≥ 37 vs. < 37 U/mL)	2.274 (1.163–4.446)	0.01	2.230 (0.954–5.210)	0.06
TB construction (single-cell vs. cluster)	0.970 (0.347–2.710)	0.95	0.942 (0.295–3.013)	0.92
TB location (subserosa/peritoneum vs. submucosa/muscularis propria)	1.253 (0.694–2.265)	0.45	1.867 (0.867–4.021)	0.11
TB stromal reaction (fibrotic vs. inflammatory)	0.815 (0.413–1.607)	0.55	0.565 (0.241–1.326)	0.19
TB stromal reaction (myxoid vs. inflammatory)	0.990 (0.382–2.565)	0.98	0.654 (0.202–2.117)	0.47
TB grade (BD2 vs. BD1)	1.994 (0.997–3.989)	0.05	1.962 (0.804–4.788)	0.13
TB grade (BD3 vs. BD1)	2.807 (1.430–5.510)	0.003	2.674 (1.115–6.414)	0.02

RFS, recurrence-free survival; OS, overall survival; HR, hazard ratio; CI, confidence interval; CEA, carcinoembryonic antigen; CA199, carbohydrate antigen; TB, tumor budding; BD, budding grade.

Table S3 Subgroup analysis for RFS

Parameters	RFS rate at 3 years (95% CI)	RFS rate at 5 years (95% CI)	Chi-squared	P value
Gender			0.046	0.83
Female	0.8622 (0.8035–0.9252)	0.7989 (0.7295–0.8748)		
Male	0.8818 (0.8394–0.9264)	0.7993 (0.7447–0.8579)		
Age (years)			14.397	<0.001
<70	0.9063 (0.8706–0.9435)	0.8350 (0.7877–0.8852)		
≥70	0.7875 (0.7071–0.8771)	0.6998 (0.6087–0.8046)		
Location			0.751	0.68
Distal colon	0.8790 (0.8294–0.9315)	0.7704 (0.7024–0.8450)		
Proximal colon	0.8756 (0.8186–0.9365)	0.8267 (0.7596–0.8997)		
Rectum	0.8602 (0.7748–0.9549)	0.8075 (0.7115–0.9165)		
T4			0.574	0.44
No	0.8806 (0.8377–0.9256)	0.8164 (0.7628–0.8737)		
Yes	0.8647 (0.8069–0.9266)	0.7716 (0.7004–0.8500)		
Morphological type			0.690	0.70
Ulcerative	0.8765 (0.8360–0.9189)	0.7810 (0.7282–0.8375)		
Raised	0.8519 (0.7622–0.9521)	0.8519 (0.7622–0.9521)		
Lump	0.8947 (0.8023–0.9978)	0.8421 (0.7338–0.9664)		
Differentiation			0.247	0.61
Middle/well	0.8722 (0.8365–0.9095)	0.7971 (0.7527–0.8441)		
Poor	0.9286 (0.8030–1.0000)	0.8571 (0.6921–1.0000)		
Vascular invasion			0.290	0.59
No	0.8693 (0.8323–0.9079)	0.8056 (0.7611–0.8528)		
Yes	0.9310 (0.8432–1.0000)	0.7162 (0.5539–0.9262)		
Perineural invasion			17.895	<0.001
No	0.8937 (0.8572–0.9317)	0.8487 (0.8051–0.8947)		
Yes	0.8042 (0.7173–0.9017)	0.6069 (0.4959–0.7428)		
Mucinous adenocarcinoma			1.793	0.18
No	0.8746 (0.8390–0.9117)	0.8024 (0.7584–0.8491)		
Yes	0.8750 (0.7271–1.0000)	0.7448 (0.5569–0.9961)		
Intestinal obstruction			9.027	0.003
No	0.8981 (0.8603–0.9376)	0.8390 (0.7917–0.8892)		
Yes	0.8188 (0.7464–0.8982)	0.7016 (0.6127–0.8033)		
Perforation			4.949	0.02
No	0.8792 (0.8444–0.9155)	0.8083 (0.7650–0.8541)		
Yes	0.7500 (0.5410–1.0000)	0.7500 (0.5410–1.0000)		
Adjuvant chemotherapy			0.025	0.87
No	0.8719 (0.8253–0.9211)	0.8066 (0.7506–0.8668)		
Yes	0.8779 (0.8267–0.9324)	0.7890 (0.7224–0.8617)		
Tumor size (cm)			1.792	0.18
<5	0.8578 (0.8111–0.9071)	0.7765 (0.7200–0.8374)		
≥5	0.9008 (0.8510–0.9534)	0.8331 (0.7670–0.9050)		
CEA (ng/mL)			5.818	0.01
<5	0.8952 (0.8564–0.9358)	0.8365 (0.7886–0.8874)		
≥5	0.8301 (0.7616–0.9048)	0.7191 (0.6346–0.8149)		
CA199 (U/mL)			2.463	0.11
<37	0.8872 (0.8517–0.9242)	0.8116 (0.7667–0.8590)		
≥37	0.7882 (0.6743–0.9213)	0.7089 (0.5720–0.8786)		
TB construction			0.054	0.81
Cluster	0.8741 (0.8342–0.9158)	0.7921 (0.7417–0.8459)		
Single-cell	0.7778 (0.6358–0.9515)	0.7778 (0.6358–0.9515)		
TB location			1.600	0.20
Submucosa/muscularis propria	0.8939 (0.8429–0.9480)	0.8356 (0.7733–0.9029)		
Subserosa/peritoneum	0.8389 (0.7819–0.9001)	0.7500 (0.6802–0.8269)		
TB stromal reaction			0.099	0.95
Inflammatory	0.8614 (0.8105–0.9156)	0.7968 (0.7354–0.8633)		
Fibrotic	0.8592 (0.7885–0.9364)	0.7794 (0.6935–0.8758)		
Myxoid	0.9000 (0.7988–1.0000)	0.7895 (0.6516–0.9565)		
TB			8.916	0.01
BD1	0.9188 (0.8789–0.9605)	0.8461 (0.7915–0.9045)		
BD2	0.8514 (0.7800–0.9293)	0.8117 (0.7321–0.8999)		
BD3	0.8000 (0.7144–0.8958)	0.6803 (0.5795–0.7987)		

RFS, recurrence-free survival; CI, confidence interval; CEA, carcinoembryonic antigen; CA199, carbohydrate antigen; TB, tumor budding; BD, budding grade.

Table S4 Subgroup analysis for OS

Parameters	OS rate at 3 years (95% CI)	OS rate at 5 years (95% CI)	Chi-squared	P value
Gender			0.000	0.99
Female	0.9189 (0.8719–0.9684)	0.8461 (0.7826–0.9148)		
Male	0.9243 (0.8893–0.9607)	0.8635 (0.8164–0.9132)		
Age (years)			37.090	<0.001
<70	0.9633 (0.9400–0.9871)	0.9158 (0.8799–0.9533)		
≥70	0.8096 (0.7322–0.8953)	0.6965 (0.6045–0.8024)		
Location			1.155	0.56
Distal colon	0.9236 (0.8829–0.9661)	0.8514 (0.7954–0.9113)		
Proximal colon	0.9419 (0.9010–0.9846)	0.8799 (0.8196–0.9446)		
Rectum	0.8777 (0.7968–0.9669)	0.8251 (0.7322–0.9297)		
T4			3.782	0.05
No	0.9379 (0.9057–0.9712)	0.8935 (0.8511–0.9380)		
Yes	0.8964 (0.8447–0.9514)	0.8011 (0.7325–0.8761)		
Morphological type			0.058	0.97
Ulcerative	0.9217 (0.8885–0.9561)	0.8472 (0.8005–0.8967)		
Raised	0.9074 (0.8333–0.9881)	0.8889 (0.8089–0.9768)		
Lump	0.9474 (0.8790–1.0000)	0.8684 (0.7673–0.9828)		
Differentiation			0.597	0.44
Middle/well	0.9189 (0.8895–0.9493)	0.8537 (0.8142–0.8951)		
Poor	1.0000 (1.0000–1.0000)	0.9286 (0.8030–1.0000)		
Vascular invasion			0.209	0.64
No	0.9149 (0.8842–0.9468)	0.8624 (0.8233–0.9034)		
Yes	1.0000 (1.0000–1.0000)	0.7685 (0.6061–0.9743)		
Perineural invasion			11.093	0.001
No	0.9316 (0.9016–0.9626)	0.8931 (0.8555–0.9323)		
Yes	0.8879 (0.8176–0.9642)	0.7033 (0.5919–0.8357)		
Mucinous adenocarcinoma			2.167	0.14
No	0.9216 (0.8925–0.9516)	0.8591 (0.8200–0.9000)		
Yes	0.9375 (0.8261–1.0000)	0.8077 (0.6336–1.0000)		
Intestinal obstruction			7.284	0.007
No	0.9490 (0.9213–0.9775)	0.8882 (0.8475–0.9308)		
Yes	0.8590 (0.7933–0.9303)	0.7740 (0.6886–0.8700)		
Perforation			1.654	0.19
No	0.9256 (0.8974–0.9547)	0.8606 (0.8219–0.9012)		
Yes	0.8333 (0.6470–1.0000)	0.8333 (0.6470–1.0000)		
Adjuvant chemotherapy			4.928	0.02
No	0.9039 (0.8626–0.9471)	0.8254 (0.7708–0.8838)		
Yes	0.9457 (0.9098–0.9830)	0.8965 (0.8458–0.9501)		
Tumor size (cm)			0.656	0.41
<5	0.9166 (0.8793–0.9553)	0.8441 (0.7940–0.8975)		
≥5	0.9313 (0.8890–0.9756)	0.8743 (0.8151–0.9378)		
CEA (ng/mL)			6.164	0.01
<5	0.9432 (0.9137–0.9737)	0.8906 (0.8501–0.9331)		
≥5	0.8770 (0.8166–0.9419)	0.7831 (0.7035–0.8718)		
CA199 (U/mL)			6.094	0.01
<37	0.9350 (0.9072–0.9637)	0.8740 (0.8356–0.9141)		
≥37	0.8348 (0.7301–0.9545)	0.7311 (0.5955–0.8975)		
TB construction			0.003	0.95
Cluster	0.9133 (0.8794–0.9486)	0.8445 (0.7990–0.8925)		
Single-cell	0.8519 (0.7279–0.9970)	0.8519 (0.7279–0.9970)		
TB location			0.562	0.45
Submucosa/muscularis propria	0.9242 (0.8802–0.9705)	0.8728 (0.8162–0.9334)		
Subserosa/peritoneum	0.8923 (0.8438–0.9436)	0.8195 (0.7567–0.8876)		
TB stromal reaction			0.362	0.83
Inflammatory	0.9036 (0.8598–0.9496)	0.8421 (0.7865–0.9016)		
Fibrotic	0.9062 (0.8464–0.9703)	0.8604 (0.7862–0.9417)		
Myxoid	0.9333 (0.8482–1.0000)	0.8233 (0.6928–0.9785)		
TB			9.883	0.007
BD1	0.9652 (0.9382–0.9929)	0.9102 (0.8659–0.9568)		
BD2	0.8740 (0.8071–0.9465)	0.8333 (0.7567–0.9176)		
BD3	0.8800 (0.8094–0.9567)	0.7624 (0.6689–0.8691)		

OS, overall survival; CI, confidence interval; CEA, carcinoembryonic antigen; CA199, carbohydrate antigen; TB, tumor budding; BD, budding grade.

Table S5 Multivariate Cox regression for OS in the subgroup encompassing BD1 and BD2

Parameters	Including age		Excluding age	
	HR (95% CI)	P	HR (95% CI)	P
Gender (male vs. female)	0.928 (0.406–2.118)	0.85	0.804 (0.379–1.704)	0.56
Age (≥ 70 vs. < 70 years)	15.496 (5.269–45.568)	< 0.001		
Location (proximal colon vs. distal colon)	0.500 (0.184–1.356)	0.17	0.634 (0.246–1.635)	0.34
Location (rectum vs. distal colon)	2.585 (0.950–7.035)	0.06	2.181 (0.842–5.651)	0.10
T4 (yes vs. no)	0.992 (0.453–2.172)	0.98	1.181 (0.539–2.589)	0.67
Morphological type (raised vs. ulcerative)	1.376 (0.461–4.107)	0.56	0.950 (0.323–2.794)	0.92
Morphological type (lump vs. ulcerative)	2.480 (0.766–8.026)	0.13	1.428 (0.495–4.115)	0.51
Differentiation (poor vs. well/middle)	4.960 (0.475–51.847)	0.18	1.511 (0.160–14.305)	0.71
Vascular invasion (yes vs. no)	0.313 (0.038–2.604)	0.28	0.431 (0.055–3.356)	0.42
Perineural invasion (yes vs. no)	2.975 (1.177–7.519)	0.02	2.224 (0.965–5.127)	0.06
Mucinous adenocarcinoma (yes vs. no)	1.333 (0.304–5.835)	0.70	1.506 (0.339–6.689)	0.59
Intestinal obstruction (yes vs. no)	2.792 (1.119–6.969)	0.02	2.124 (0.915–4.931)	0.08
Perforation (yes vs. no)	15.747 (2.525–98.201)	0.003	3.948 (0.758–20.575)	0.10
Adjuvant chemotherapy (yes vs. no)	0.969 (0.314–2.990)	0.95	0.302 (0.119–0.765)	0.01
Tumor size (≥ 5 vs. < 5 cm)	0.439 (0.178–1.084)	0.07	0.596 (0.257–1.380)	0.22
CEA (≥ 5 vs. < 5 ng/mL)	1.064 (0.434–2.609)	0.89	1.761 (0.791–3.921)	0.16
CA199 (≥ 37 vs. < 37 U/mL)	1.845 (0.630–5.400)	0.26	1.697 (0.623–4.622)	0.30

OS, overall survival; BD, budding grade; HR, hazard ratio; CI, confidence interval; CEA, carcinoembryonic antigen; CA199, carbohydrate antigen.

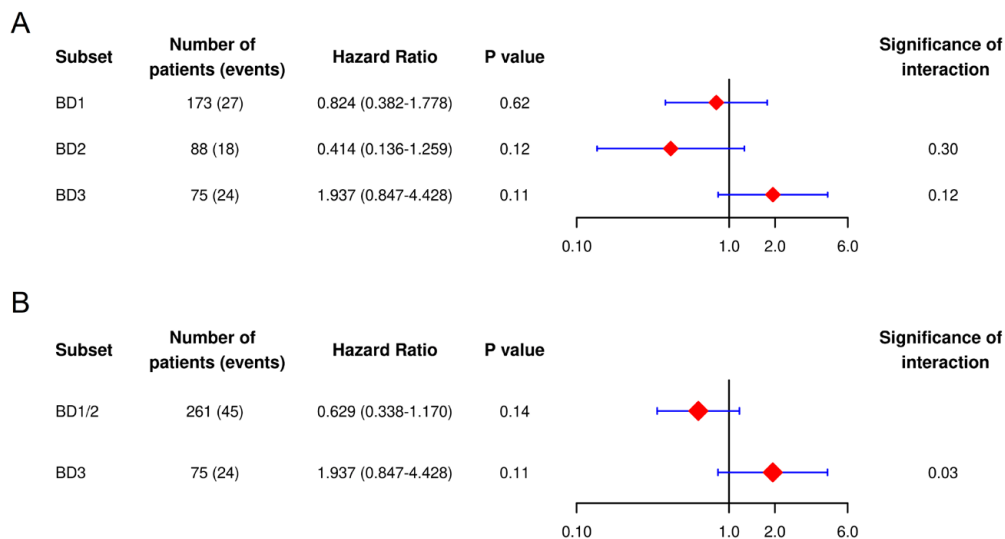


Figure S2 Forest plot to illustrate the differential effect size of benefit from adjuvant chemotherapy in different BD subgroups and their significance of interaction for RFS. (A) Forest plot for individual BD group. (B) Forest plot for BD1/2 subgroup vs. BD3. BD, budding grade; RFS, recurrence-free survival.

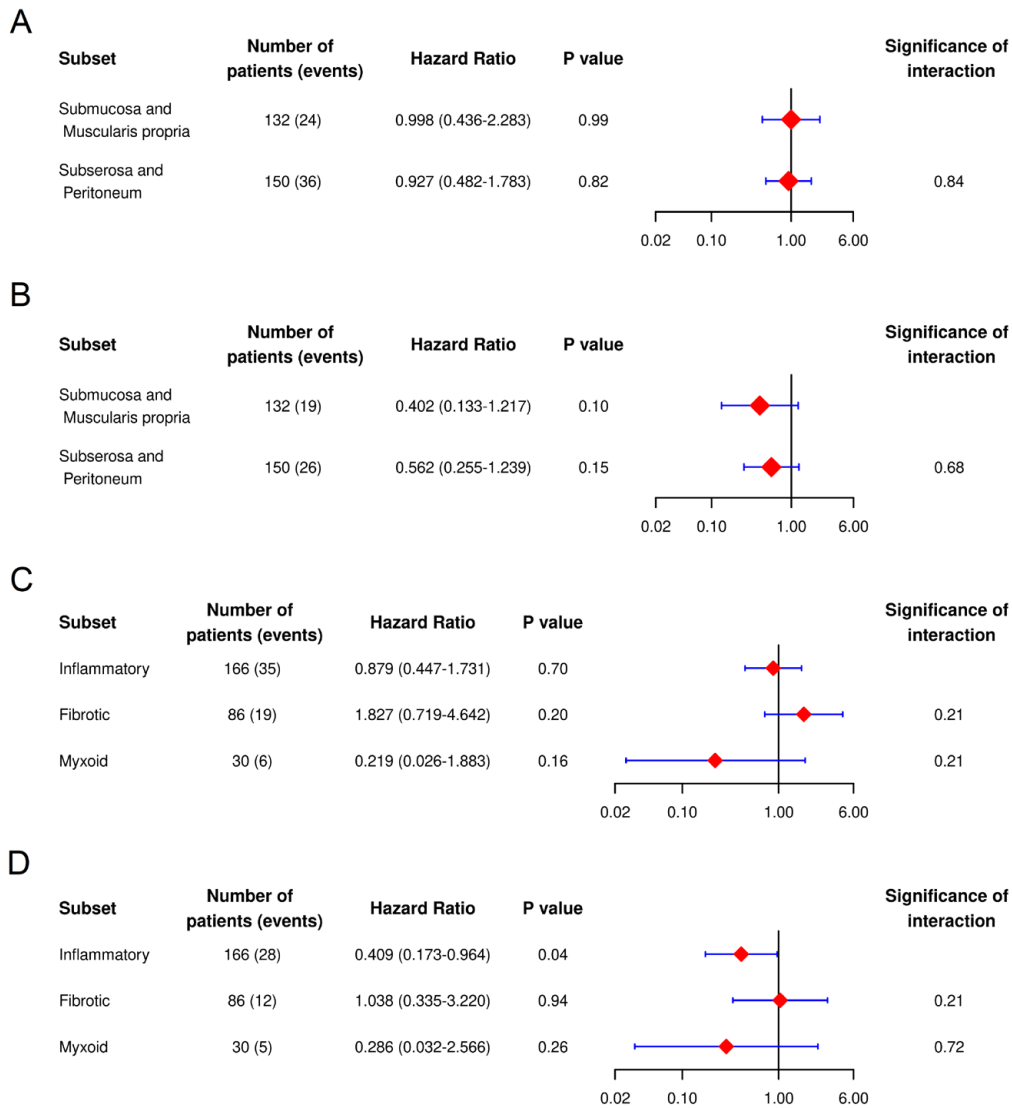


Figure S3 Forest plot to illustrate the differential effect size of benefit from adjuvant chemotherapy in different TB location subgroups (A,B) or different TB stromal reaction subgroups (C,D) and their significance of interactions. (A) Forest plot of interaction between TB location and RFS; (B) forest plot of interaction between TB location and OS (n=282); (C) forest plot of interaction between TB stromal reaction and RFS; and (D) forest plot of interaction between TB stromal reaction and OS. TB, tumor budding; RFS, recurrence-free survival; OS, overall survival.