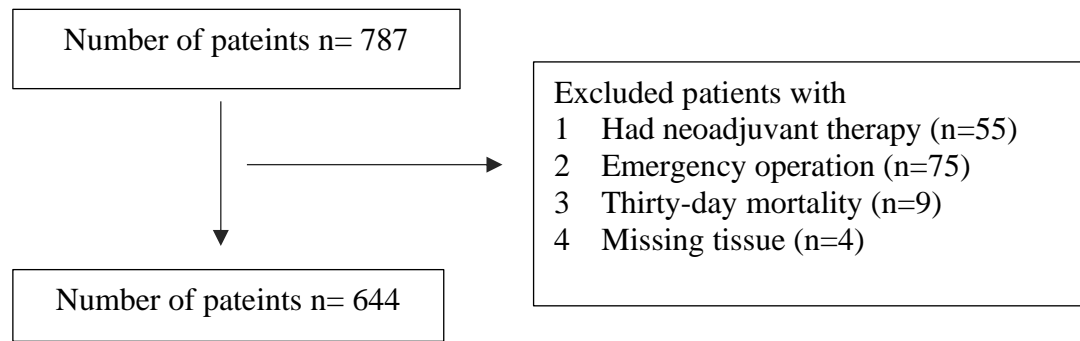


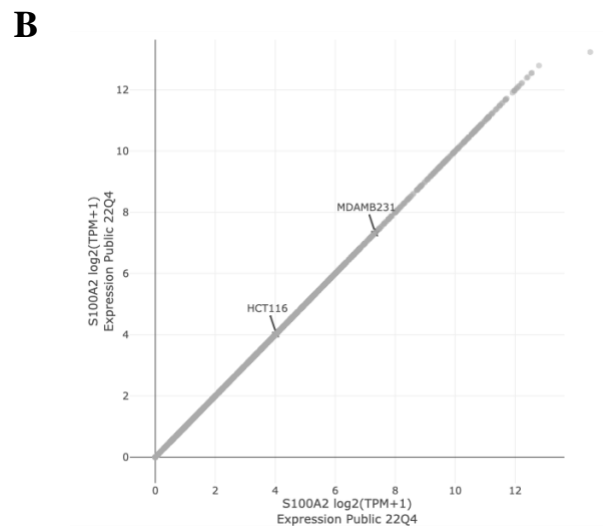
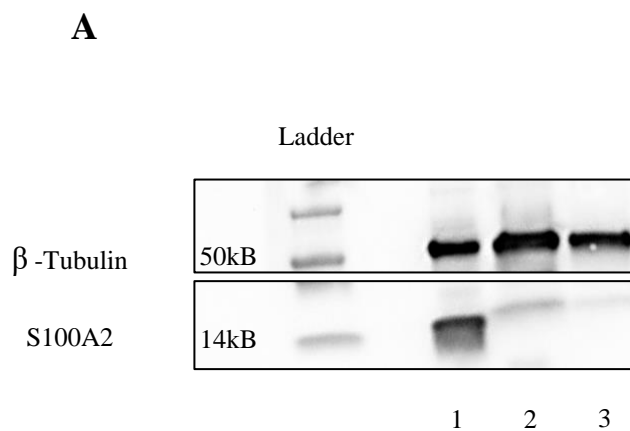
Supplementary figures

$$\frac{\text{Total number of cells count in stroma}}{\text{Total cells count in stroma}} \times 100 = \text{Percentage of cells on stroma area}$$

FigureS1 To calculate the percentage of positive cells in the stromal area, 3 replicates TMAs were constructed and obtained the average value of the staining. After that, the averaged value of positive cells was divided by the total number of cells to acquire the percent proportion of positive cells in each TMA core either in tumour or stromal area.



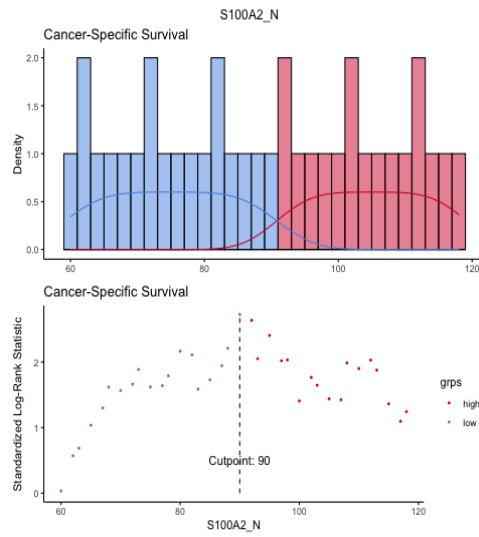
FigureS2 Consort diagram demonstrating how patients have been selected for the study



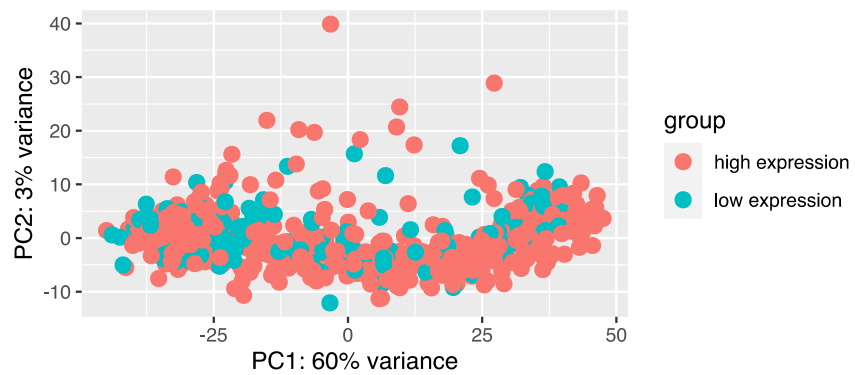
FigureS3 Antibody specificity test (A) Protein expression of S100A2 using western blot analysis of (1) 293T overexpressed S100A2, (2) MDA-MB-231 and (3) HCT116 protein lysates. Precision Plus Protein™ Dual Xtra Prestained Protein Standards (Bio-Rad) was used to visualised molecular weight. Samples were load onto for antibody specificity (n=3). S100A2 was 14 kDa and β -tubulin were used for internal housekeeping control (50 kDa) (B) S100A2 expression in tumour cell lines from DepMap

CSS Cut Offs

	cutpoint	statistic
S100A2_N	90	2.726030589344

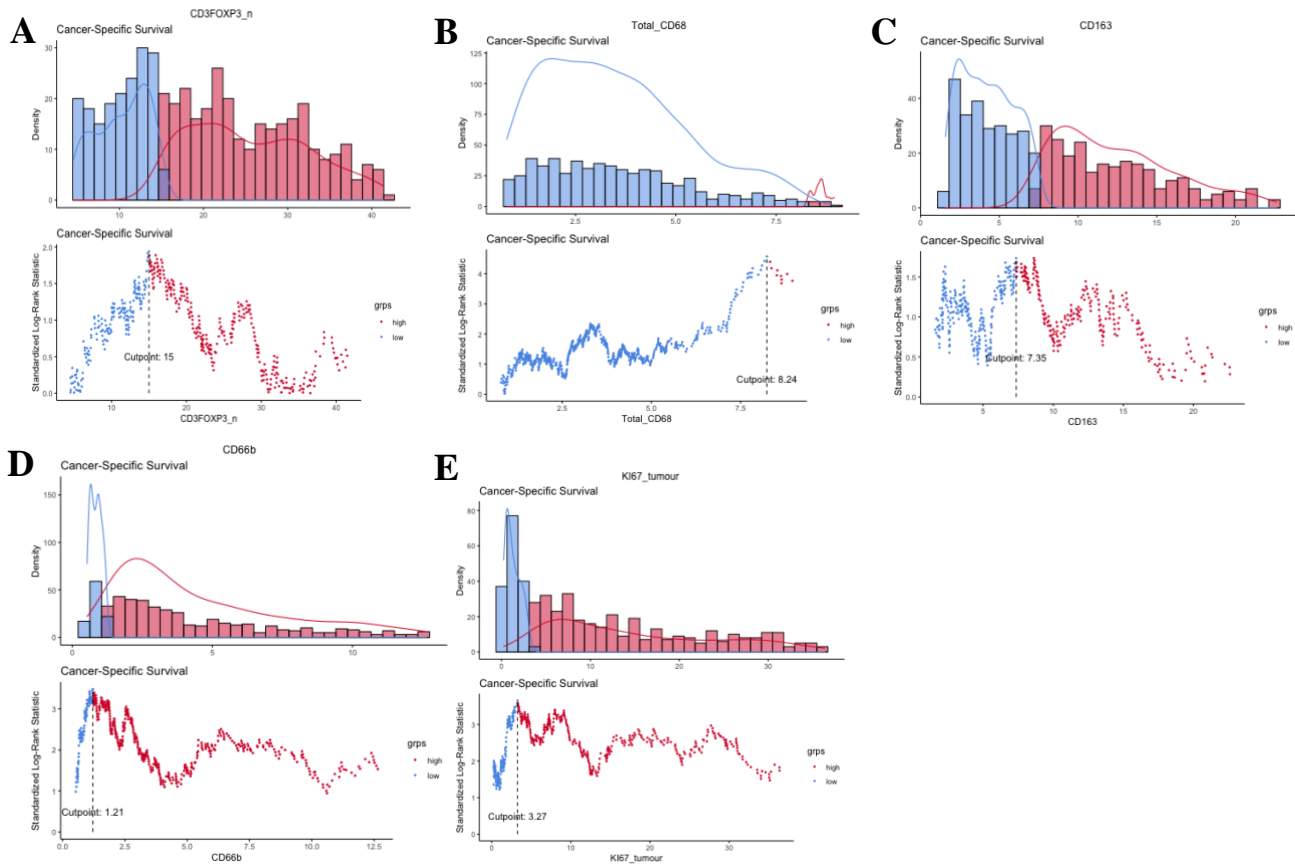


FigureS4 Cut point generated from R program defined low and high expression of cytoplasmic S100A2 with cancer specific survival



FigureS5 PCA plot showed the clustering between high (red) and low (blue) cytoplasmic S100A2

CSS cut offs		
	cutpoint	statistic
CD3FOXP3	15	1.94
CD68	8.24	4.57
CD163	7.35	1.73
CD66b	1.21	3.48
KI67	3.27	3.65



FigureS6 Cut point generated from R program defined low and high expression of (A) CDFOXP3 (B) CD68 (C) CD163 (D) CD66b and (E) KI67 with cancer specific survival

Supplementary tables

TableS1 Antibodies or multiplex staining

Antibody	Clone	Company	Dilution
anti-CD3 (2GV6)	Rabbit Monoclonal Primary Antibody	Roche Tissue Diagnostics (790-4341)	RTU
Pan-Cytokeratin (AE1/AE3)	Mouse Monoclonal Antibody	Leica Biosystems (AE1/AE3-601-L-CE)	1:250
FOXP3 (236A/E7)	Mouse Monoclonal Antibody	abcam (ab20034)	1:20
Actin, Smooth Muscle (1A4)	Mouse Monoclonal Antibody	Roche Tissue Diagnostics (760-2833)	RTU
CD8a (C8/144B)	Mouse Monoclonal Antibody	Cell Signaling Technology (70306)	1:100
CD68 (D4B9C) XP®	Rabbit Monoclonal Antibody	Cell Signaling Technology (76437)	1:200
CD163	Rabbit Monoclonal Antibody	Abcam (ab182422)	1:45000
CD66b	Mouse Monoclonal Antibody	Novus Biological (NB100-77808)	1:8000

RTU =Ready to use

Table S2 Patients characteristic (N=644)

Characteristic		
Sex	Female	291 (45%)
	Male	353 (55%)
Age	<65	205 (32%)
	65-74	208 (32%)
	>75	231 (36%)
Tumour site	Right	260 (40%)
	Left	384 (60%)
MMR status	dMMR	111 (17%)
	pMMR	418 (65%)
	NA	100 (16%)
	Missing data	15 (2%)
Local recurrence	No	551 (86%)
	Yes	55 (9%)
	Missing data	38 (5%)
Distant recurrence	No	480 (75%)
	Yes	127 (20%)
	Missing data	37 (5%)
TNM stage	I	87 (14%)
	II	308 (48%)
	III	249 (39%)
T stage	1	30 (5%)
	2	74 (12%)
	3	375 (58%)
	4	165 (25%)
N stage	0	395 (61%)
	1	182 (28%)
	2	67 (10%)
Margin involvement	No	608 (94%)
	Yes	36 (6%)
Peritoneal involvement	No	498 (77%)
	Yes	146 (23%)

Tumour perforation	No Yes	631 (98%) 13 (2%)
Venous invasion	No Yes	320 (50%) 324 (50%)
TB	Low High Missing data	441 (69%) 180 (28%) 23 (3%)
TSP	Low High Missing data	490 (76%) 133 (21%) 21 (3%)
KM	Low High Missing data	519 (81%) 103 (16%) 22 (3%)
GMS	0 1 2 Missing data	103 (16%) 399 (62%) 120 (19%) 22 (3%)
mGPS	0 1 2	409 (64%) 137 (21%) 98 (15%)

dMMR = deficient mismatch repair, pMMR = proficient mismatch repair, TB =Tumour budding, TSP = Tumour stroma percentage, KM = Klintrup-Mäkinen, GMS = Glasgow Microenvironment Score, mGPS = modified Glasgow prognostic score, NA= not available

Clinicopathological characteristics	Univariable analysis		Multivariate analysis	
	Hazard ratio (95% CI)	<i>P</i>	Hazard ratio (95% CI)	<i>P</i>
Sex	1.32 (0.97-1.79)	0.082		
Age	1.29 (1.06-1.55)	0.009	1.40 (1.13-1.75)	0.003
MMR status	0.67 (0.52-0.88)	0.003		
Local recurrence	6.95 (4.85-9.98)	<0.001	3.83 (2.60-5.64)	<0.001
Distant recurrence	24.39 (16.26-36.58)	<0.001	23.14 (14.76-36.27)	<0.001
TNM stage	2.49 (1.92-3.24)	<0.001		
T stage	1.88 (1.49-2.38)	<0.001		
N stage	2.09 (1.72-2.54)	<0.001	1.64 (1.28-2.12)	<0.001
Margin involvement	4.05 (2.58-6.34)	<0.001	3.67 (2.01-6.67)	<0.001
Peritoneal involvement	2.07 (1.51-2.84)	<0.001		
Tumour perforation	2.65 (1.17-5.99)	0.019		
Venous invasion	1.51 (1.11-2.04)	0.008		
TB	1.88 (1.37-2.56)	<0.001	1.68 (1.16-2.42)	0.006
S100A2	0.54 (3.94-0.74)	<0.001		
GMS	1.74 (1.35-2.25)	<0.001		
mGPS	1.42 (1.18-1.72)	<0.001		

Table S3 Univariate and Multivariate analysed for cancer specific survival (CSS)

MMR = Mismatch repair, TB = Tumour budding, GMS = Glasgow Microenvironment Scores, mGPS = modified Glasgow prognostic score