Supplementary material to

Carbohydrate antigens Lewis a and Lewisb act as tumor markers cooperating with CA19.9 in the management of PDAC patients

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PDAC	age	sex	TNM	Stage	Grade
1	75	female	yT3N2M0	III	G3
2	43	male	T3N0M0	IIA	G3
3	73	female	T3N1M0	IIB	G3
4	68	male	yT3N1M0	IIB	NA
5	71	male	T3N0M0	IIA	G2
6	82	female	T3N0M0	IIA	G2
7	74	male	T4N1M0	IIB	G3
8	62	female	yT3N0M0	IIA	G2
9	77	male	yT3N1M0	IIB	G3
10	61	male	yT3N1M0	IIB	NA
11	60	male	T3N1M0	IIB	G2
12	61	female	T3N1M0	IIB	G3
13	65	male	yT3N0M1	IV	NA
14	71	female	yT3N0M0	IIA	NA
15	76	male	T3N1M0	IIB	G3
16	79	female	T3N0M0	IIA	G3
17	40	male	T3N1M0	IIB	G3
18	69	female	T3N1M0	IIB	G3
19	78	female	T3N1M0	IIB	G3
20	68	male	T3N1M0	IIB	G3
21	72	male	T3N1M0	IIB	G3
22	66	male	yT3N0M1	IV	NA
23	63	female	T3N1M0	IIB	G3
24	71	male	T3N1M0	IIB	G3
25	72	female	T3N1M0	IIB	G2
26	46	female	T3N1M0	IIB	G3
27	64	female	T3N1M0	IIB	G3
28	63	female	T3N1M0	IIB	G3
29	69	male	T3N1M0	IIB	G4
30	73	male	T3N1M0	IIB	G3
31	80	male	T3N1M0	IIB	G3
32	79	male	T3N1M0	IIB	G3
33	78	female	T3N1M0	IIB	G3
34	61	female	T3N1M0	IIB	G2
35	75	female	T3N1M0	IIB	G3

36	74	male	T3N1M0	IIB	G2
37	81	female	T3N1M0	IIB	G3
38	70	male	T3N1M0	IIB	NA
39	74	male	T3mN1M0	IIB	G3
40	72	male	yT3N1M0	IIB	NA
41	66	male	yT3N1M0	IIB	NA
42	82	male	T3N1M0	IIB	G3
43	68	female	T3mN1M0	IIB	G3
44	76	female	T3N1M0	IIB	G3
45	58	female	T3N1M0	IIB	G3
46	72	male	T3N1M0	IIB	G2
47	59	male	T3N1M0	IIB	G3
48	72	female	T3N1M0	IIB	G3
49	76	male	T3N1M0	IIB	G3
50	73	male	T3N1M0	IIB	G3
51	76	female	T3N1M1	IV	G3
52	66	female	T3N1M0	IIB	G3
53	73	male	T3N1M0	IIB	G3
54	70	female	T3N1M0	IIB	G3
55	69	male	T3N0M0	IIA	G3
56	72	female	T3N0M0	IIB	G3
57	51	female	T3N1M0	IIB	G3
58	64	male	T3N0M0	IIA	G3
59	80	female	T3N1M1	IV	G3
60	57	male	T3N1M0	IIB	G3
61	70	male	T3N0M0	IIB	G2
62	80	male	T3N0M0	IIA	G2
63	58	female	T3N1M0	IIB	G3
64	74	female	T3N1M0	IIB	G3
65	76	female	T3N1M0	IIB	G3
66	80	female	T3N1M0	IIB	G3
67	62	female	T3N1M0	IIB	G3
68	77	female	T3N1M0	IIB	G3
69	53	male	yT3N1M0	IIB	G3
70	67	female	T3N1M0	IIB	G3
71	65	male	T3N1M0	IIB	G4
72	73	female	T3N1M0	IIB	G3
73	55	female	T3N1M0	IIB	G3
74	73	male	T3N1M0	IIB	G3
75	79	male	T3N0M0	IIA	G2
76	79	male	T3N1M0	IIB	G3
77	69	female	yT3N0M0	IIA	G2
78	70	male	T3N1M0	IIB	G3
79	57	female	T3N1M0	IIB	G3
80	81	male	T3N0M0	IIA	G3
81	76	female	T3N1M0	IIB	G2
82	63	female	yT3N1M0	IIB	NA

83	67	female	yT3N1M0	IIB	G4
84	81	female	T3N1M0	IIB	G3
85	77	female	T3N1M0	IIB	G2
86	76	male	T3N0M0	IIA	G3
87	76	female	T3N1M0	IIB	G2
88	77	female	T3N1M0	IIB	G3
89	53	male	T3N1M1	IV	G3
90	70	male	T3N1M0	IIB	G3
91	72	male	T3N0M0	IIA	G3
92	64	female	yT1N0M0	IA	NA
93	75	male	T3N1M0	IIB	G3
94	66	female	yT3N1M0	IIB	G3
95	70	female	T3N1M0	IIB	G2
96	81	male	T3N1M1	IV	G3
97	67	male	yT3N1M1	IV	G3
98	59	female	T2N0M0	IB	G2
99	64	male	yT3N1M0	IIB	G3
100	68	female	T3N1M0	IIB	G3
101	64	female	T2N0M0	IB	G2
102	64	female	yT3N1M0	IIB	G3
103	65	female	yT2N1M0	IIB	G2
104	72	male	T(IS)N0M0		
105	80	female	T2N2M0	III	G3
106	67	female	T2N2M0	III	G3
107	73	male	T3N1M0	IIB	G3
108	59	male	T4N2M0	III	G3
109	73	male	T2N1M0	IIB	G2
110	68	female	T2N1M0	IIB	G3
111	68	female	T2N1M0	IIB	G3
112	73	male	T3N2M0	III	G3
113	77	female	T1N1M0	IIB	G3
114	65	male	T1N0M0	IA	G3
115	78	male	T3N0M0	IIA	G3

NA: not available

Table S1. List of PDAC patients and their main clinical features.

FUT1	
Forward	AGC AGC TCG GCC ATG TGG
Reverse	TCT GAT TAC CAA ACC GGC CA
FUT2	
Forward	TGA GGT GCC TGC CCA ACC
Reverse	CTG CTG AAC GTG AAA TAT AGT GG
FUT3	
Forward	GGA GCT TTG GTA AGC AGG AG
Reverse	CAC AGC CAC CAG CAG CTG
FUT5	
Forward	GGTGTGACCTCGGCGTGA
Reverse	CCAGCCTGCACCATCGCC
B3GALT5	
Forward	CTC TTA CCC AGC AAA AAA TGG
Reverse	GGA AGG GAG GTG TCT GCC
ST3GAL3	
Forward	CTC TGG GGT CAC GAA TTG AC
Reverse	TGC TCA GGC CGC TGC ATG
ST3GAL4	
Forward	CTC TAA CGT CTT TGG CAA CTA C
Reverse	CGG CAC CTG AGG CTC TG
ST3GAL6	
Forward	GGA ACG AAT GTC TAT TGG GTG
Reverse	AAG TCG AAA ATA TTC CGC TGA TG
ST6GALNAC6	
Forward	TGA GTA GCA ACA AAG AGC AGC
Reverse	GAG AGG GCA GTG TCT TGT TG
GAPDH	
Forward	GGA GAA GGC TGG GGC TC
Reverse	GGC ATG GAC TGT GGT CAT G

Table S2. Oligonucleotide primers for quantification of specific transcripts by RT-qPCR.

Actin	
Forward	TGA ACC CCA AGG CCA ACC G
Reverse	CTG CTT GCT GAT CCA CAT CTG
FUT2 WT for A385T mutation	
Forward	AGG AGG AAT ACC GCC ACA
FUT2 mutated for A385T	
Forward	GAG GAG GAA TAC CGC CAC
FUT2 WT for G428A mutation	
Forward	GCT ACC CCT GCT CCT GG
FUT2 mutated for G428A	
Forward	CGG CTA CCC CTG CTC CTA
FUT2 reverse common	
Reverse	GGC TGC CTC TGG CTT AAA G
FUT3 WT for T59G mutation	
Forward	CGC TGT CTG GCC GCA CT
FUT3 mutated for T59G	
Forward	CGC TGT CTG GCC GCA CG
FUT3 reverse common for WT and mutated T59G	
Reverse	GGA GTC GCT GCG GTA GG
FUT3 WT for T1067A mutation	
Reverse	CAG GTG AAC CAA GCC GCT
FUT3 mutated for T1067A	
Reverse	CAG GTG AAC CAA GCC GCT T
FUT3 forward common for WT and mutated T1067A	
Forward	GGT GGA CGT GTA CGG ACG

Table S3. PCR primers for the detection of specific mutations in *FUT2* and *FUT3* genes.

Annealing 62°C	Annealing 64°C	Annealing 62°C
1. 94°C 3 min	1. 94°C 0,1 sec	1. 94°C 0,1 sec
2. 94°C 1 min	2. 94°C 50 sec	2. 94°C 1 min
3. 66°C 1 min	3. 64°C 1 min	3. 62°C 1 min
4. 72°C 1 min 30 sec	4. 72°C 1 min 30 sec	4. 72°C 1 min 30 sec
5. repeat twice from step 2 to step 4	5. repeat twice from step 2 to step 4	5. repeat 26 times from step 2 to step 4
6. 94°C 2 min	6. 94°C 2 min	6. 72°C 8 min
7. Go to annealing 64°C program	7. Go to annealing 62°C final program	7. 10°C infinite

Table S4. PCR amplification programs for the detection of specific mutations in *FUT2* and *FUT3* genes. They consist in 3 different steps that have to be performed sequentially.
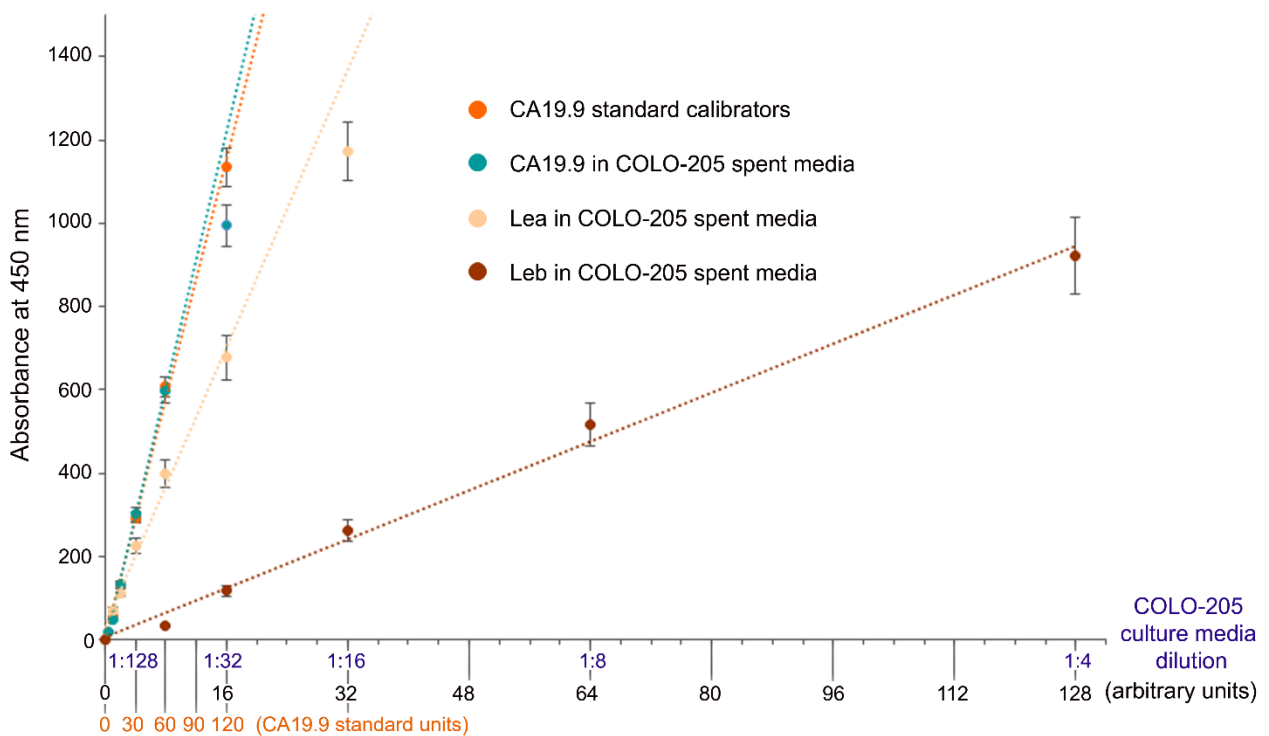
Supplementary Figures

Figure S1. Detection of Lewis antigens in the spent media of COLO-205 cells. Sandwich ELISAs were performed using various dilutions of COLO-205 media and quantified through the absorbance at 450 nm. One arbitrary unit was defined as the absorbance obtained for each antigen at a dilution of 1:512. Comparison with CA19.9 calibrators (Fujrebio) was also reported (orange values). Values are the mean ± standard deviation for triplicate assays performed in three independent assays.
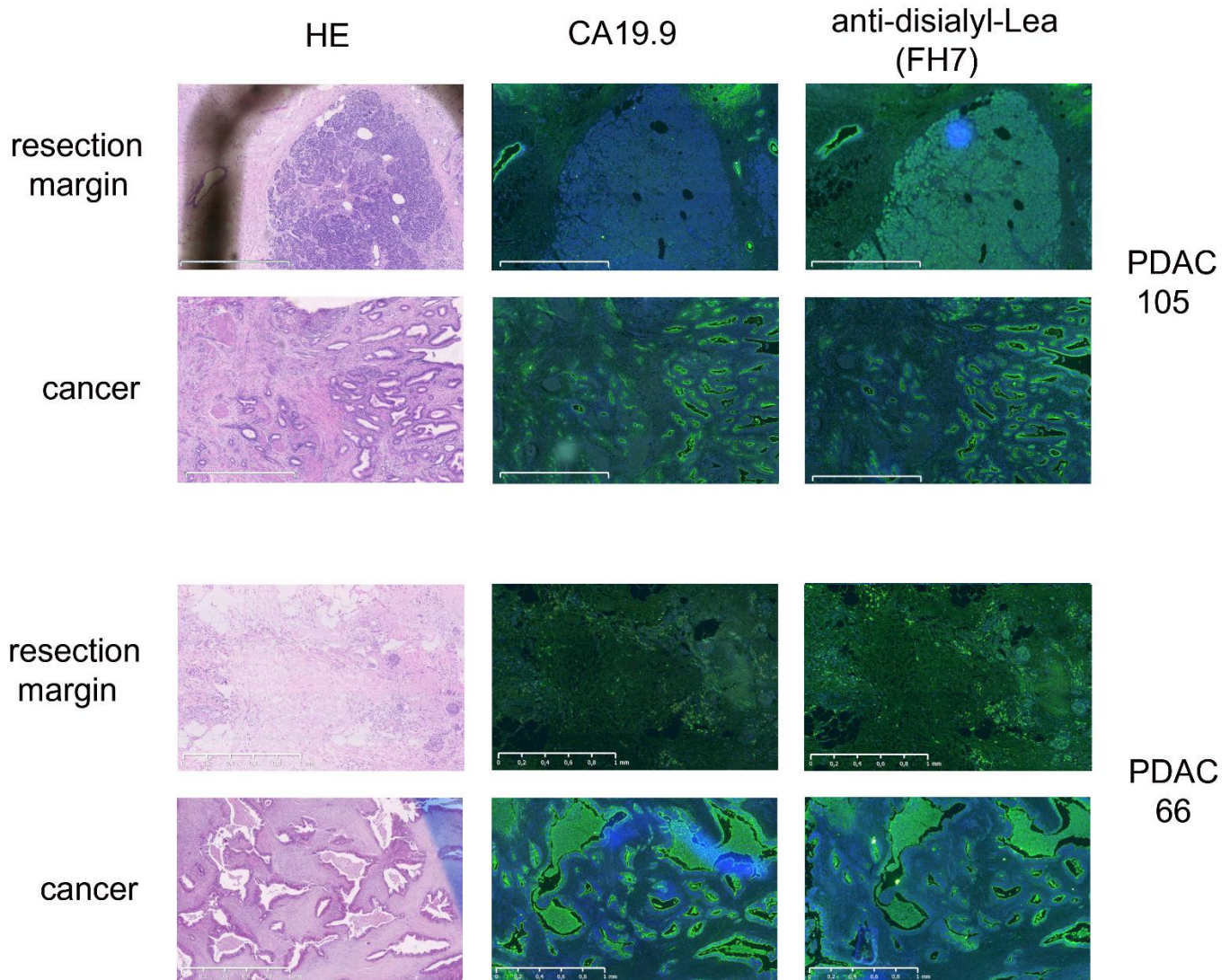

Figure S2. Detection of CA19.9 and disialyl-Lea on PDAC resections by immunofluorescence microscopy. Paraffin embedded serial slices of PDAC resections were processed as in Figure 2 of the main text. Nuclei were counterstained with Hoechst 33342 as a reference. Two representative cases were shown where the resection margins included adjacent normal tissue. Scale bars: 1 mm; magnification: 2.5x.