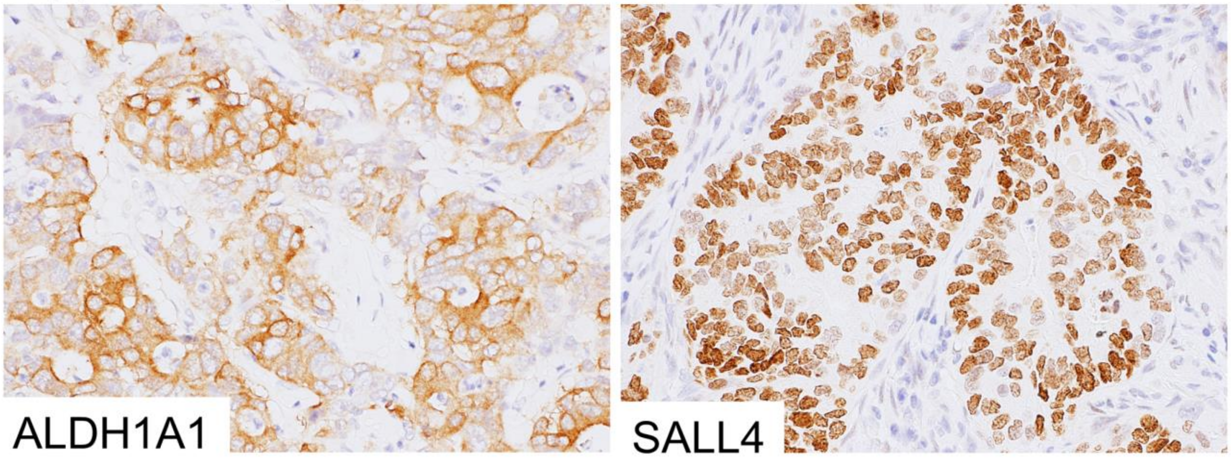
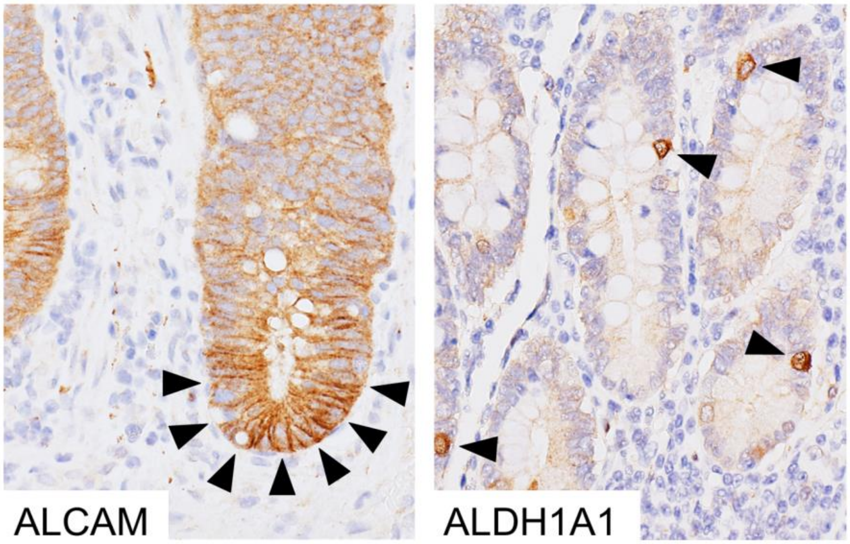
Supplementary Figure S1



Supplementary Figure S1. ALDH1A1 and SALL4 expression in colorectal adenocarcinoma. Diffuse cytoplasmic and nuclear expression of ALDH1A1 (left) and SALL4 (right).

Supplementary Figure S2



Supplementary Figure S2. ALCAM and ALDH1A1 expression in normal intestinal epithelium.
Membranous-ALCAM expression in normal colonic mucosa (left) and ALDH1A1 expression in terminal ileum (right).

Supplementary Table S1. Antibodies and Conditions for Immunohistochemistry

Genes	Retrieval	Dilution	Antibodies
ALCAM	H2	1,000	Leica Biosystems, Inc. (San Diego, CA)
ALDH1A1	H1	64,000	Clone 44, BD Biosciences (San Jose, CA)
CD274	H2	200	Clone E1L3N, Cell Signaling Technology, Inc. (Danvers, MA)
CDX2	H2	250	Clone D11D10, Cell Signaling Technology, Inc. (Danvers, MA)
SALL4	H2	200	Clone 6E3, Biocare Medical (Concord, CA)

Antigen retrieval was performed with heat activation in Bond low (H1) or high (H2) pH buffer.

Supplementary Table S2. Concordance Rate of Initial Immunostaining Evaluation

Immunohistochemistry	Concordance Rate (%)	κ coefficient	95% CI	
			min	max
ALCAM	90.5	0.75	0.68	0.82
ALDH1A1	87.2	0.66	0.58	0.74
CD274	89.9	0.60	0.47	0.73
CDX2	97.8	0.89	0.82	0.96
SALL4	97.8	0.78	0.63	0.93

κ coefficient was calculated by EZR version 1.32. software. [27]

Supplementary Table S3. Primer Sequences and PCR Conditions Used to Amplify Targets for Sanger Sequencing

Gene	PCR Target	Forward primer (5'_3')	Reverse primer (5'_3')	A_temp (oC)	Amplicon (bp)
BRAF	Exon 15	TCTTCATGAAGACCTCACAG	AGCCTCAATTCTTACCATCC	50	125
GNAS	Exon 8	CCAAACTACTCCAGACCTTT	GGGTGAATGTCAAGAAACCA	48	221
KRAS	Exon 2	GTGTGACATGTTCTAATATAGTCA	AGAATGGTCCTGCACCAGAATTAT	48	215
KRAS	Exon 3	CCAGACTGTGTTTCTCCCTT	TACACAAAGAAAGCCCTCCC	50	157
KRAS	Exon 4	AGAGTTAAGGACTCTGAAGA	CAGTGTTACTTACCTGTCTT	45	220
NRAS	Exon 2	GACTGAGTACAAACTGGTGG	CACCTCTATGGTGGGATCAT	48	112
NRAS	Exon 3	CCCCCAGGATTCTTACAGAA	ATACACAGAGGAAGCCTTCG	49	140
PIK3CA	Exon20-1	AGGAGATGTGTTACAAGGCT	TTGTGTGGAAGATCCAATCC	48	243
PIK3CA	Exon20-2	TGCATACATTCGAAAGACCC	TTGTGTGGAAGATCCAATCC	48	128

Supplementary Table S4. Expression of the Stem Cell Marker Genes in 454 Colorectal Adenocarcinomas

			CDX2 expression					
	Total No.		Negative/weak		Strong		*P*-value	
	454 [100%]	(100%)	53 [100%]	(12%)	401 [100%]	(88%)		
Gene Mutation								
ALDH1A1							0.21	a
Positive	132 [29%]	(100%)	11 [21%]	(8%)	121 [30%]	(92%)		
Negative	322 [71%]	(100%)	42 [79%]	(13%)	280 [70%]	(87%)		
ALCAM								
Positive	107 [24%]	(100%)	24 [45%]	(22%)	83 [21%]	(78%)	*P*<0.001	a
Negative	347 [76%]	(100%)	29 [55%]	(8%)	318 [79%]	(92%)		
SALL4								
Positive	22 [5%]	(100%)	4 [8%]	(18%)	18 [4%]	(82%)	0.31	b
Negative	432 [95%]	(100%)	49 [92%]	(11%)	383 [96%]	(89%)		

[a] *P*-values were calculated by the Chi-square test for CDX2 expression. [b] *P*-values were calculated by the Fisher's exact test for CDX2 expression.
The Bonferroni-corrected *P*-value for significance was *P*=0.017 (0.05/3).

Supplementary Table S5. Characterization of 50 CD274-negative Colorectal Carcinomas

Age	Sex	Location	Size (cm)	Tumor differentiation	Mucinous histology	Solid/medullary histology	CDX2	ALCAM	ALDH1A1	SALL4	MLH1	PMS2	MSH2	MSH6	*BRAF*	*KRAS*	*PIK3CA*
81	F	Colon NOS		Well	+	-	+	+	-	-	+	+	+	+	WT	Codon12 GGT>TGT	WT
54	F	S	7.0	Well	-	-	+	-	-	-	+	+	+	+	WT	Codon12 GGT>GAT	WT
83	F	C	6.0	Well	-	-	+	-	-	-	+	+	+	+	WT	Codon12 GGT>GAT	WT
78	F	C	6.0	Well	-	-	+	-	-	-	+	+	+	+	WT	Codon12 GGT>GTT	WT
52	F	C	9.0	Well	-	-	+	-	-	-	+	+	+	+	WT	Codon12 GGT>TGT	WT
87	M	S	7.0	Moderate	-	-	+	+	-	-	+	+	+	+	WT	Codon12 GGT>AGT	WT
58	F	C		Moderate	-	-	+	+	-	-	+	+	+	+	WT	Codon12 GGT>GAT	WT
77	M	S	3.0	Moderate	-	-	+	-	-	-	+	+	+	+	WT	Codon12 GGT>GAT	WT
75	M	C		Moderate	-	-	+	+	+	-	+	+	+	+	WT	Codon12 GGT>GAT	WT
64	F	S	10.0	Moderate	-	-	+	-	-	-	+	+	+	-	WT	Codon12 GGT>GCT	WT
63	F	S	6.0	Moderate	-	-	+	-	+	-	+	+	+	+	WT	Codon12 GGT>GTT	WT
49	F	C		Moderate	-	-	+	+	+	-	+	+	+	+	WT	Codon12 GGT>GTT	WT_rs17849079
77	F	Colon NOS	4.0	Moderate	-	-	+	-	+	-	+	+	+	+	WT	Codon12 GGT>TGT	WT
71	F	Colon NOS	4.5	Moderate	-	-	+	-	-	-	+	+	+	+	WT	Codon12 GGT>TGT	WT
71	M	Colon NOS	6.5	Moderate	-	-	+	+	-	-	+	+	+	+	WT	Codon12 GGT>TGT	WT
63	F	T		Poor	-	-	+	-	-	-	+	+	+	+	WT	Codon12 GGT>TGT	WT
43	M	D	10.0	Well	-	-	+	-	-	-	-	-	+	+	WT	Codon13 GGC>GAC	c.3140A>G
68	M	C	5.5	Moderate	-	-	+	-	-	-	+	+	+	+	WT	Codon13 GGC>GAC	WT
74	M	C	2.3	Well	-	-	+	-	-	-	+	+	+	+	WT	Codon13 GGC>GAC	WT
68	M	C		Moderate	-	-	+	-	-	-	+	+	+	+	WT	Codon13 GGC>GAC	WT
66	M	S	5.0	Moderate	-	-	+	-	-	-	+	+	+	+	WT	Codon13 GGC>GAC	WT
70	F	C	7.0	Moderate	-	-	+	+	-	-	+	+	+	+	WT	Codon61 CAA>CAT	WT
74	F	C	6.0	Well	-	-	+	+	+	-	+	+	+	+	WT	Codon146 GCA>ACA	WT
65	F	S	5.0	Moderate	-	-	+	+	+	-	+	+	+	+	WT	Codon146 GCA>ACA	WT
40	M	C	6.5	Moderate	-	-	+	+	+	-	+	+	+	+	WT	Codon146 GCA>ACA	WT
67	F	C	4.0	Well	-	-	+	-	+	-	+	+	+	+	WT	Codon146 GCA>GTA	c.3140A>T
77	F	C	5.0	Moderate	-	-	+	+	+	-	-	-	+	+	MUT	WT	WT
64	M	D	6.0	Moderate	-	-	+	+	-	+	+	+	+	+	MUT	WT	WT
65	F	A	3.7	Moderate	+	-	+	-	-	-	+	+	+	+	MUT	WT	ND
72	M	Colon NOS	9.0	Poor	+	-	+	-	-	-	-	-	+	+	MUT	WT	WT
67	M	C	9.0	Poor	+	-	+	-	-	-	-	-	+	+	MUT	WT	WT
81	M	SF		Well	-	-	+	-	-	-	+	+	+	+	WT	WT	WT
52	M	HF	6.0	Well	-	-	+	-	-	-	+	+	+	+	WT	WT	WT
48	M	T	5.0	Well	-	-	+	+	-	-	+	+	+	+	WT	WT	WT
49	F	T	7.5	Well	-	-	+	-	-	-	-	-	+	+	WT	WT	WT
65	F	S	5.0	Well	-	-	+	-	-	-	+	+	+	+	WT	WT	WT
74	F	HF	2.0	Well	-	-	+	-	-	-	+	+	+	+	WT	WT	WT
74	M	S	5.5	Well	-	-	+	+	+	-	+	+	+	+	WT	WT	WT

Continued

Supplementary Table S5. Continued

Age	Sex	Location	Size (cm)	Tumor differentiation	Mucinous histology	Solid/medullary histology	CDX2	ALCAM	ALDH1A1	SALL4	MLH1	PMS2	MSH2	MSH6	*BRAF*	*KRAS*	*PIK3CA*
52	F	T	3.5	Well	-	-	-	-	+	+	+	+	+	+	WT	WT	c.3129G>A
87	M	T	7.0	Moderate	-	-	+	-	+	-	+	+	+	+	WT	WT	WT
66	M	T		Moderate	-	-	+	+	+	-	+	+	+	+	WT	WT	WT
51	F	S	4.0	Moderate	-	-	+	-	+	-	+	+	+	+	WT	WT	WT
53	M	S	10.0	Moderate	-	-	+	-	-	-	+	+	+	+	WT	WT	WT
57	F	S	2.5	Moderate	-	-	+	-	-	-	+	+	+	+	WT	WT	WT
54	M	SF	4.0	Moderate	-	-	+	-	-	-	+	+	+	+	WT	WT	WT
78	F	C	6.0	Moderate	-	-	+	-	-	-	+	+	+	+	WT	WT	WT
55	F	S	4.0	Moderate	-	-	+	-	-	-	+	+	+	+	WT	WT	WT
80	M	Colon NOS	4.0	Moderate	-	-	+	-	-	-	+	+	+	+	WT	WT	WT
43	F	S	7.0	Moderate	-	-	+	-	-	-	+	+	+	+	WT	WT	WT
59	F	Colon NOS	4.0	Moderate	-	-	+	-	+	-	+	+	+	+	WT	WT	WT

C, cecum; D, disending; HF, hepatic flexure; S, sigmoid; SF, splenic flexure; T, transverse. WT, wild type. MUT, V600E mutant. ND, not determined. No mutations were found in *NRAS* and *GNAS*.