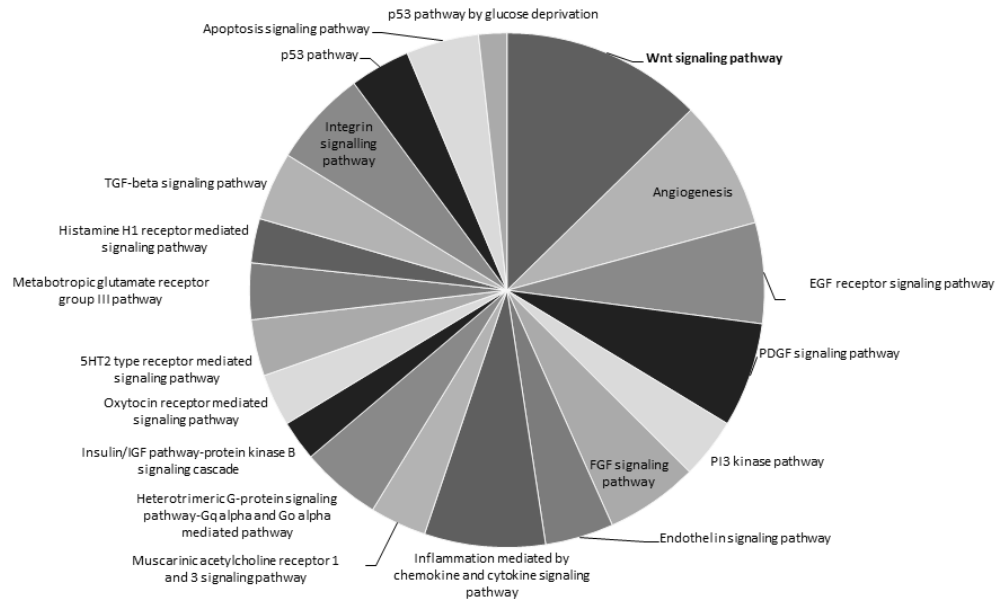


# Supplementary Figures and Tables

Figure S1

A



B

Pathway name	Genes	Hyp	Hyp_c
Wnt signaling pathway	PRKCI SMAD4 PCDH18 DKK1 CDH13 SIAH1 PCDH19 CTBP2 GNB4 PCDH7 PRKCE CDHR1 PCDH11Y NFATC4 TBL1XR1 CELSR3 AXIN2 ADSS CCND1 PCDHA9 LRP6 PRKCQ GNB1 PCDH11X CDH5 CDH2 FAT3 FZD3 PPP3CA FZD7 PPP2R5C EP400 PPP2CA SMARCD1 ITPR2 CDH1 DKK2 PRKCA PYGO2 GNG2 TGFBR1 TBL1X CTNNA2 GNG12 SMARCC1 WNT5A HDAC2 ACVR1C PLCB4 PLCB1	6,5E-11	6,6E-09
Angiogenesis	PRKCI FGFR1 PDGFB PIK3R1 NOTCH2 AKT3 PDGFRB PRKCE KRAS AXIN2 PAK2 SOS1 PIK3C2A STAT3 NOTCH1 PDGFRA PRKCQ PTPRB FZD3 JAG1 MAPK1 CRKL DLL1 F3 PRKCA WNT5A HIF1A FGF2 PIK3R3 FRS2 BIRC5 NR1I2	3,3E-09	1,7E-07
EGF receptor signaling pathway	TGFA RASAL2 PRKCI RRAS2 YWHAB AKT3 NF1 SPRY4 PRKCE KRAS SOS1 PIK3C2A STAT3 RAC1 PRKCQ ERBB4 CBL MAP3K3 PPP2R5C MAPK1 PPP2CA MAP3K2 PRKCA RHOQ EREG	6,2E-08	2,1E-06
PDGF signaling pathway	PDGFB RPS6KC1 PIK3R1 ARHGAP5 EHF RPS6KB1 KRAS SOS1 ERG STAT3 DLC1 ARHGAP26 SRGAP1 PDPK1 VAV3 MAPK1 MAP3K2 ITPR2 PRKCA RPS6KA3 NIN GABPA STARD13 PKN2 PIK3R3 SRGAP3	1,7E-07	4,4E-06
PI3 kinase pathway	INSR PIK3R1 FOXO3 AKT3 GNB4 RPS6KB1 KRAS CCND1 SOS1 CCND2 GNB1 PDPK1 FOXO1 FOXO4 PIK3R3 FGF9 PRKCI FGFR1 FGF5 YWHAB AKT3 SPRY4 PRKCE KRAS SOS1 PIK3C2A FGF7 RAC1 PRKCQ MAP3K3 PPP2R5C MAPK1 PPP2CA MAP3K2 FGF12 PRKCA FGF2 FRS2	5E-07	8,4E-06
FGF signaling pathway	PRKCI PIK3R1 ADCY9 AKT3 PRKCE PIK3C2A PRKCQ ADCY1 MAPK1 ITPR2 PRKCA PRKACB EDNRA ADCY2 PIK3R3 PLCB4 PLCB1	2,7E-06	3,8E-05
Endothelin signaling pathway	SIRT1 SIAH1 PIK3R1 AKT3 PIK3C2A HMGB1 TP63 TNFRSF10B SUMO1 PDPK1 ATM PPP2CA PML PIK3R3 SERPINE1	0,00013	0,00073

**Figure S1. Biological processes predictably elicited by selected miRNAs: 142-3p, 544a, 19b-1**

A) Panther pathways analysis showing biological processes predictably elicited by 142-3p, 544a, 19b-1 miRNAs jointly. The chart is ordered according to the increasing number of genes involved in each process (Hyp\_c <0,001). B) Table showing predicted genes involved in each biological predicted process elicited by 142-3p, 544a, 19b-1 miRNAs jointly and ordered by corrected hypergeometric test (Hyp c), the corrected *P-value* obtained from the hypergeometric test.

Gene name	Forward primer	Reverse primer
<b>ACSL1</b>	ACATTATGTTCTGGGCCCA	AGTCAGAAGGCCATTGTCTGA
<b>ACSL4</b>	GGCACAACAGAAAGGGGTAG	GGTTCCTCAGCTCCTTCCTT
<b>SCD-1</b>	TGCCACCACAAGTTTTCAG	CATCAGCAAGCCAGGTTTGT
<b>GAPDH</b>	TGGTATCGTGAAGGACTCATGAC	ATGCCAGTGAGCTTCCCGTTCAGC

**Supplementary Table 1:** Primers used for quantitative real-time PCR

<b>miRBase ID</b>	<b>Cat No</b>
<b>hsa-miR-19b-1-5p</b>	4427975
<b>hsa-miR-19b-3p</b>	4427975
<b>hsa-miR-142-3p</b>	4427975
<b>hsa-miR-142-5p</b>	4427975
<b>hsa-miR-544a</b>	4427975
<b>U6 snRNA</b>	4427975

**Supplementary Table 2:** Probes from TaqMan® MicroRNA Assays (ThermoFisher) used for quantitative real-time PCR

<b>Name</b>	<b>Origin</b>	<b>Working dilution</b>
<b>ACSL1 Rabbit Polyclonal Antibody</b>	Cell Signaling (Cat No 4047)	1:500
<b>ACSL4 Rabbit Polyclonal Antibody</b>	Dr. Stephen Prescott and Dr. Diana Stafforini [Supplementary methods]	1:2000
<b>SCD Rabbit Polyclonal Antibody</b>	Dr. Jean-Baptiste Demoulin [Supplementary methods]	1:2000
<b>Vinculin Mouse Monoclonal Antibody</b>	Sigma (Cat No V9131)	1:5000
<b>Goat Anti-Rabbit IgG Antibody, (H+L) HRP conjugate</b>	Millipore (Cat No AP307P)	1:20000
<b>Goat Anti-Mouse IgG &amp; IgM Antibody, HRP conjugate</b>	Millipore (Cat No AP130P)	1:40000

**Supplementary Table 3:** Commercial antibodies used in this study

Characteristics	Stage II CRC		Stage III CRC	
	n <sup>o</sup> of Patients	(%)	n <sup>o</sup> of Patients	(%)
<b>Total sample size (n)</b>	80	(100)	46	(100)
<b>Age at Diagnosis (years)</b>				
Mean	73.55		63.26	
Median	75		62.5	
<70	24	30	33	71.74
≥70	56	70	13	28.26
<b>Gender</b>				
Female	28	35	26	56.52
Male	52	65	20	43.48
<b>Stage</b>				
IIA (T3 N0 M0)	68	85	26	56.52
IIB (T4 N0 M0)	12	15	20	43.48
<b>Total Lymph Nodes Resected</b>				
Mean Lymph nodes resected	15.75		17.04	
≤12	30	37.5	20	43.48
>12	50	62.5	26	56.52
<b>Location of Primary</b>				
Cecum and Ileocecal Valve	7	8.75	4	8.7
Ascending colon and Hepatic flexure	15	18.75	9	19.57
Transverse colon	3	3.75	3	6.52
Splenic flexure and Descending colon	15	18.75	6	13.04
Sigmoid colon and rectosigmoid junction	40	50	24	52.17
<b>Grade/Differentiation</b>				
Well	5	6.41	9	19.57
Moderately	72	92.31	29	63.04
Poor	1	1.28	8	17.39
<b>Bowel Obstruction/Perforation</b>				
Yes	17	21.25	32	69.57
No	63	78.75	14	30.43
<b>Other Histological Features</b>				
Perineural invasion	15	18.75	19	41.3
Vascular invasion	22	27.5	15	32.61
<b>Adjuvant treatment</b>				
Xelox/Folox	32	40	46	100
No treatment	48	60	0	0
<b>Disease-free survival</b>				
Patients with recurrence	14	17.5	18	39.13
<b>Overall survival</b>				
n <sup>o</sup> of Exitus	18	22.5	8	17.39

**Inclusion criteria:** Age ≥18, completely resected rectal cancer or colon adenocarcinoma located at ≥15 cm of the anal verge as determined by endoscopy or above the peritoneal reflection in the surgical resection, confirmed Stage II AJCC/UICC primary CRC and follow-up of at least 36 months. **Exclusion criteria:** death within 30 days after surgery, other cancers in previous 5 years and inflammatory bowel disease or specific gene-related cancer.

**Supplementary Table 4:** Detailed clinical and histopathological characteristics of patients included in the study.

ACSL1	$2^{(\Delta\Delta Ct)}$ replicate 1	$2^{(\Delta\Delta Ct)}$ replicate 2	ACSL4	$2^{(\Delta\Delta Ct)}$ replicate 1	$2^{(\Delta\Delta Ct)}$ replicate 2	SCD	$2^{(\Delta\Delta Ct)}$ replicate 1	$2^{(\Delta\Delta Ct)}$ replicate 2	ACSL1 replicate mean	SD	Downregulation percentage (%)	ACSL4 replicate mean	SD	Downregulation percentage (%)	SCD replicate mean	SD	Downregulation percentage (%)	ACSL1 (individual score)	ACSL4 (individual score)	SCD (individual score)	FINAL SCORE
hsa-miR-544a	0.528	0.172	hsa-miR-544a	0.232	0.090	hsa-miR-544a	0.615	0.299	0.350	0.25	64.98	0.161	0.10	83.88	0.457	0.22	54.31	-0.406	-0.530	-0.551	-0.496
hsa-miR-142	0.328	0.300	hsa-miR-142	0.414	0.414	hsa-miR-142	0.473	0.354	0.314	0.02	68.59	0.414	0.00	58.60	0.413	0.08	58.65	-0.442	-0.277	-0.594	-0.438
hsa-miR-19b-1	0.670	0.204	hsa-miR-19b-1	0.543	0.196	hsa-miR-19b-1	0.780	0.264	0.437	0.33	56.29	0.370	0.25	63.04	0.522	0.37	47.79	-0.319	-0.322	-0.486	-0.376
hsa-miR-224	0.737	0.230	hsa-miR-224	0.567	0.213	hsa-miR-224	0.834	0.405	0.483	0.36	51.68	0.390	0.25	60.97	0.620	0.30	38.05	-0.273	-0.301	-0.388	-0.321
hsa-miR-106a	0.448	0.337	hsa-miR-106a	0.277	0.220	hsa-miR-106a	0.987	0.817	0.393	0.08	60.74	0.249	0.04	75.15	0.902	0.12	9.79	-0.364	-0.443	-0.106	-0.304
hsa-miR-31	0.679	0.238	hsa-miR-31	0.645	0.275	hsa-miR-31	0.898	0.395	0.458	0.31	54.19	0.460	0.26	53.97	0.647	0.36	35.35	-0.298	-0.231	-0.361	-0.297
hsa-miR-20b	0.635	0.188	hsa-miR-20b	0.708	0.265	hsa-miR-20b	1.027	0.460	0.412	0.32	58.84	0.486	0.31	51.35	0.870	0.40	25.62	-0.345	-0.205	-0.264	-0.271
hsa-miR-20a	0.629	0.190	hsa-miR-20a	0.545	0.189	hsa-miR-20a	1.240	0.501	0.410	0.31	59.04	0.367	0.25	63.30	0.811	0.44	18.87	-0.346	-0.324	-0.137	-0.269
hsa-miR-342	0.585	0.221	hsa-miR-342	0.649	0.256	hsa-miR-342	1.125	0.498	0.403	0.26	59.69	0.452	0.28	54.79	0.811	0.76	-13.91	-0.353	-0.239	-0.196	-0.263
hsa-miR-205	0.752	0.196	hsa-miR-205	0.246	0.076	hsa-miR-205	1.674	0.604	0.474	0.39	52.62	0.161	0.12	83.92	1.139	0.76	-13.91	-0.282	-0.531	0.131	-0.227
hsa-miR-490b	0.633	0.375	hsa-miR-490b	1.020	0.757	hsa-miR-490b	0.473	0.330	0.504	0.18	49.62	0.889	0.19	11.13	0.402	0.10	59.82	0.094	-0.369	-0.379	-0.218
hsa-miR-26b	1.400	0.299	hsa-miR-26b	0.449	0.196	hsa-miR-26b	0.864	0.394	0.850	0.78	15.03	0.323	0.18	67.74	0.629	0.33	37.10	0.094	-0.369	-0.379	-0.218
hsa-miR-548b	0.596	0.176	hsa-miR-548b	0.702	0.306	hsa-miR-548b	1.349	0.544	0.386	0.30	61.39	0.504	0.28	49.63	0.946	0.57	5.36	0.094	-0.369	-0.379	-0.218
hsa-miR-199b	1.035	0.836	hsa-miR-199b	0.589	0.471	hsa-miR-199b	0.555	0.403	0.936	0.14	6.43	0.530	0.08	46.99	0.479	0.11	52.10	-0.370	-0.188	-0.061	-0.206
hsa-miR-106b	0.568	0.398	hsa-miR-106b	0.749	0.736	hsa-miR-106b	0.830	0.614	0.483	0.12	51.71	0.743	0.01	25.75	0.722	0.15	27.84	0.180	-0.161	-0.529	-0.170
hsa-miR-548a-3	0.638	0.297	hsa-miR-548a-3	0.864	0.430	hsa-miR-548a-3	1.118	0.655	0.468	0.24	53.23	0.647	0.31	35.28	0.887	0.33	11.33	-0.273	0.051	-0.286	-0.169
hsa-miR-515-1	0.709	0.281	hsa-miR-515-1	0.849	0.462	hsa-miR-515-1	1.228	0.682	0.495	0.30	50.48	0.655	0.27	34.46	0.955	0.39	4.52	-0.252	0.197	-0.606	-0.220
hsa-miR-519d	0.647	0.299	hsa-miR-519d	0.539	0.313	hsa-miR-519d	1.547	0.907	0.473	0.25	52.70	0.426	0.16	57.37	1.227	0.45	-22.69	0.094	-0.369	-0.379	-0.218
hsa-miR-19a	0.577	0.362	hsa-miR-19a	0.551	0.423	hsa-miR-19a	1.839	1.375	0.469	0.15	53.06	0.487	0.09	51.28	1.607	0.33	-60.69	0.094	-0.369	-0.379	-0.218
hsa-miR-195	0.557	0.891	hsa-miR-195	0.640	1.203	hsa-miR-195	0.950	1.181	0.724	0.24	27.63	0.921	0.40	7.88	1.065	0.16	-6.52	-0.370	-0.188	-0.061	-0.206
hsa-miR-556	0.453	1.571	hsa-miR-556	0.380	0.918	hsa-miR-556	0.753	1.357	1.012	0.79	-1.19	0.649	0.38	35.12	1.055	0.43	-5.49	0.180	-0.161	-0.529	-0.170
hsa-miR-186	0.657	0.924	hsa-miR-186	0.514	0.909	hsa-miR-186	1.067	1.475	0.790	0.19	20.98	0.712	0.28	28.81	1.271	0.29	-27.13	0.180	-0.161	-0.529	-0.170
hsa-miR-369	1.268	0.408	hsa-miR-369	0.976	0.347	hsa-miR-369	1.869	0.742	0.838	0.61	16.20	0.662	0.44	33.84	1.305	0.80	-30.55	-0.273	0.051	-0.286	-0.169
hsa-miR-17	0.802	0.834	hsa-miR-17	0.787	1.076	hsa-miR-17	1.020	1.148	0.818	0.02	18.18	0.932	0.20	6.84	1.084	0.09	-8.40	-0.288	-0.044	-0.121	-0.151
hsa-miR-578	1.552	1.139	hsa-miR-578	0.684	0.588	hsa-miR-578	1.112	0.662	1.346	0.29	-34.56	0.636	0.07	36.42	0.887	0.32	11.30	-0.288	-0.044	-0.121	-0.151
hsa-miR-34c	1.320	1.215	hsa-miR-34c	0.682	0.967	hsa-miR-34c	1.302	1.711	1.267	0.07	-26.75	0.824	0.20	17.56	1.506	0.29	-50.62	-0.261	-0.036	-0.053	-0.117
hsa-miR-93	0.632	1.099	hsa-miR-93	1.089	2.185	hsa-miR-93	1.060	1.539	0.865	0.33	13.46	1.637	0.78	-63.69	1.300	0.34	-29.96	-0.283	-0.265	0.219	-0.110
hsa-miR-875	0.886	1.031	hsa-miR-875	1.100	2.003	hsa-miR-875	1.162	1.786	0.959	0.10	4.14	1.552	0.64	-55.15	1.474	0.44	-47.40	-0.283	-0.265	0.219	-0.110
hsa-miR-548c	1.155	1.568	hsa-miR-548c	0.604	1.336	hsa-miR-548c	1.325	2.097	1.361	0.29	-36.13	0.970	0.52	3.03	1.711	0.55	-71.07	-0.287	-0.204	0.599	0.036
hsa-miR-940	0.709	2.029	hsa-miR-940	0.530	1.692	hsa-miR-940	0.844	2.659	1.369	0.93	-36.92	1.111	0.82	-11.13	1.752	1.28	-75.16	-0.032	0.230	0.058	0.085
hsa-miR-944	1.574	2.912	hsa-miR-944	0.590	1.965	hsa-miR-944	1.054	1.561	2.243	0.95	-124.33	1.278	0.97	-27.76	1.308	0.36	-30.77	0.256	-0.043	0.047	0.087
hsa-miR-186			hsa-miR-186			hsa-miR-186			0.034			0.034			0.034			0.034	0.020	0.264	0.106
hsa-miR-369			hsa-miR-369			hsa-miR-369			0.082			0.082			0.082			0.082	-0.030	0.298	0.117
hsa-miR-17			hsa-miR-17			hsa-miR-17			0.062			0.062			0.062			0.062	0.240	0.076	0.126
hsa-miR-578			hsa-miR-578			hsa-miR-578			0.589			0.589			0.589			0.589	-0.056	-0.121	0.138
hsa-miR-34c			hsa-miR-34c			hsa-miR-34c			0.511			0.511			0.511			0.511	0.133	0.498	0.381
hsa-miR-93			hsa-miR-93			hsa-miR-93			0.109			0.109			0.109			0.109	0.945	0.292	0.449
hsa-miR-875			hsa-miR-875			hsa-miR-875			0.203			0.203			0.203			0.203	0.860	0.466	0.510
hsa-miR-548c			hsa-miR-548c			hsa-miR-548c			0.605			0.605			0.605			0.605	0.278	0.703	0.529
hsa-miR-940			hsa-miR-940			hsa-miR-940			0.613			0.613			0.613			0.613	0.420	0.744	0.592
hsa-miR-944			hsa-miR-944			hsa-miR-944			1.487			1.487			1.487			1.487	0.586	0.300	0.791

Global mean	ACSL1 total mean	ACSL4 total mean	SCD total mean
	0.7561	0.6915	1.0077
SD	0.4250	0.3569	0.3741

**Supplementary Table 5: Detailed data from RT-QPCR experiment for miRNA-gene interaction**

Left panel shows  $2^{-\Delta\Delta Ct}$  data (fold change) from 2 independent experiments for each miRNA-gene interaction as well as the mean of both replicas with their corresponding downregulation percentages and the global mean of the 31 miRNAs interactions for each gene (overall mean value of the whole interactions panel). Negative downregulation percentages mean overexpression instead of downregulation. Right panel shows the replicas mean of each interaction subtracted by the global mean (IS) ensuring only the best interactions within each gene. Final score (FS) is defined as the average of the 3 individual scores.