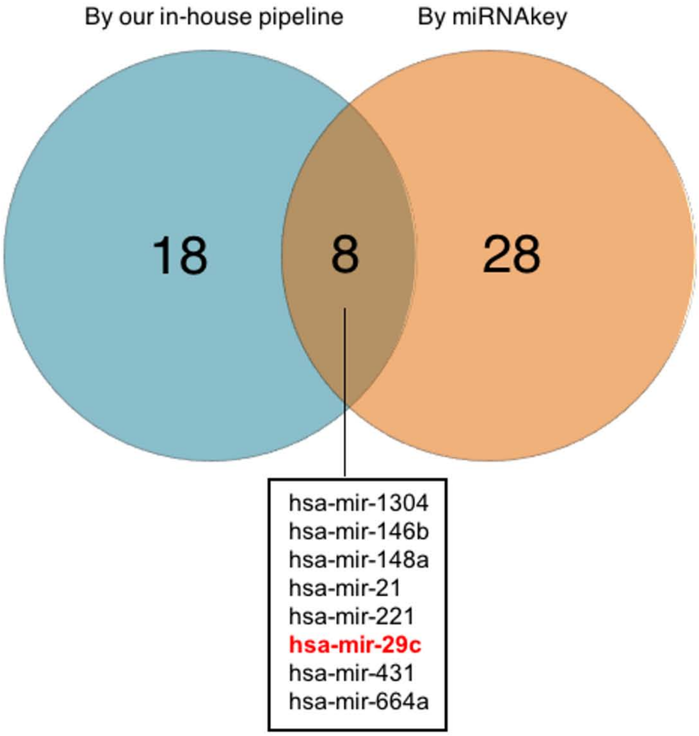
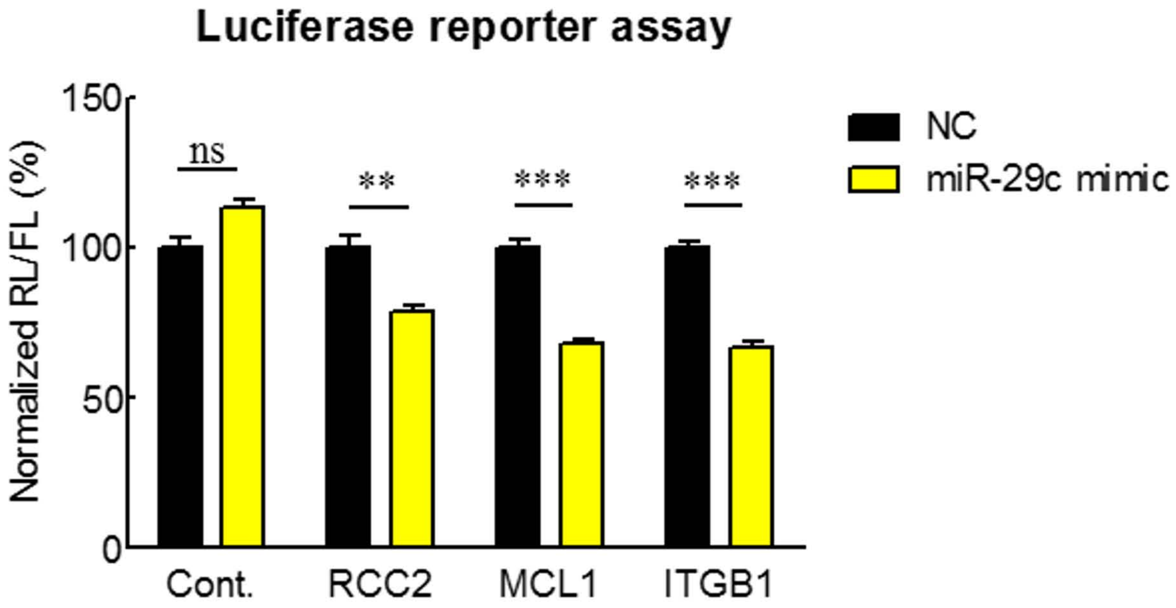
Supplementary figure 1



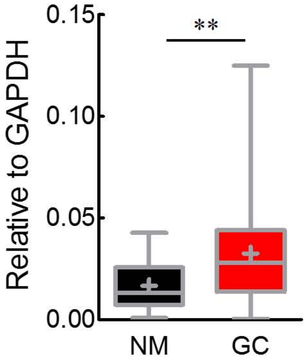
NGS data analysis using a miRNAkey and comparison with the data from our in-house pipeline. We performed one-way ANOVA tests using the read counts table generated by miRNAkey and obtained 36 miRNAs with 100 fold and raw P-vaule < 0.03. In the results by our in-house pipeline, we obtained 26 miRNAs with 10 fold and raw P-value < 0.05. The miR-29c was detectable in both NGS analysis tools.

Supplementary figure 2

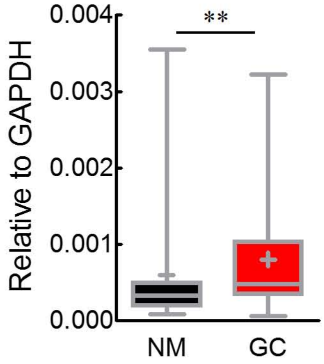


**$p<0.01$, ***$p<0.001$, t test.

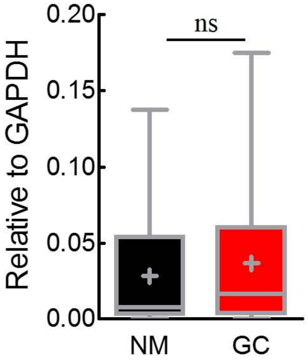
Supplementary figure 3



ITGB1 expression
in 24 pairs of GC and NM tissues

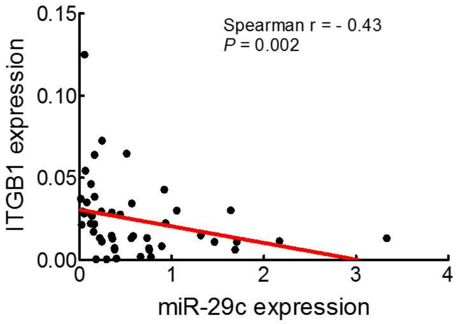


RCC2 expression
in 24 pairs of GC and NM tissues

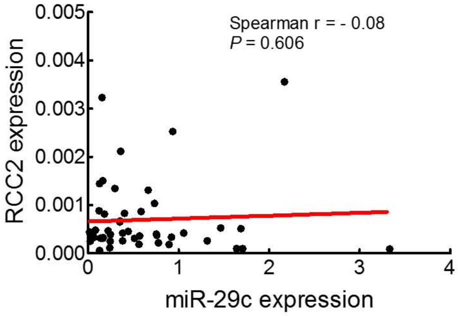


MCL1 expression
in 24 pairs of GC and NM tissues

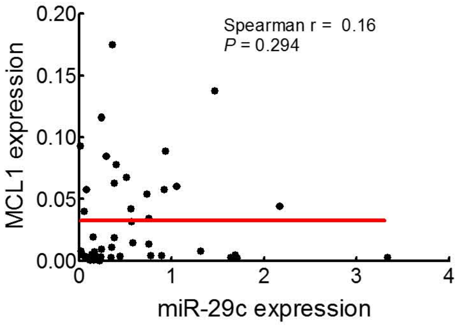
**p<0.01, t test.



Correlation between
ITGB1 and **miR-29c** expression



Correlation between
RCC2 and **miR-29c** expression



Correlation between
MCL1 and **miR-29c** expression

Supplementary Materials and Methods

Transgenic mice models

The K19 promoter with a synthetic intron and SV40 poly(A) cassette were cloned into pBluescript vector (Stratagene, La Jolla, CA, USA) to construct pK19 as described previously.[1] For *K19-C2mE* mice, Full-length cDNAs for COX-2 and mPGES-1 were amplified by RT–PCR. After sequence confirmation, the cDNA fragments were subcloned into pK19 to construct pK19-COX-2 and pK19-mPGES, respectively. Two expression vectors were co-microinjected into fertilized eggs of the F_1 (C3H and C57BL/6) hybrid females crossed with C57BL/6 male as described previously.[1]

For *Gan (K19-Wnt1/C2mE)* mice, *Wnt1* cDNA was excised from pUSEamp-Wnt1 (Upstate, Charlottesville, VA). These fragments were cloned into pBluescript (Stratagene, La Jolla, CA) to construct the pK19-Wnt1 transgenic vector as described previously.[2] The expression vector was microinjected into the fertilized eggs of F1 (C3H and C57BL/6) hybrid females crossed with C57BL/6 males to generate *K19-Wnt1*transgenic mice. To minimize any genetic background differences, littermate mice for the experiments from the mating of N2-backcrossed *K19-Wnt1* with N6-backcrossed *K19-C2mE* mice were used as described previously.[2] Backcrossing was performed using wild-type C57BL/6 mice.

Supplementary Table 1. Characteristics of All Patients from each GC Cohort

Characteristic	**NGS discovery cohort** Patients *n*=4	**NGS validation cohort** Patients *n*=24	**Clinical validation cohort** Patients *n*=113
Age (years)			
Mean (SD)	65.5 (12.9)	60.8 (11.5)	65.5 (13.2)
Median (Min-Max)	67 (50-78)	61 (34-76)	68 (18-90)
Gender			
Male	1	16	71
Female	3	8	42
WHO Classification			
WD	1	2	19
MD	0	10	27
PD	2	11	51
Mucinous	1	1	5
SRC	0	0	11
Lauren Classification			
Intestinal	1	9	46
Diffuse	3	10	64
Mixed	0	5	3
TNM stage			
I	0	5	38
II	2	3	19
III	1	11	30
IV	1	5	26

WD, well differentiated adenocarcinoma; MD, moderately differentiated adenocarcinoma; PD, poorly differentiated adenocarcinoma; MUC, mucinous; SRC, signet ring cell

Supplementary Table 2. Primers used in gene expression analyses, stable miR-29c expressing vectors, and luciferase assays

Gene symbol		**Sequence**
Human *ITGB1*	Forward	5'-ATCCCAGAGGCTCCAAAGAT-3'
	Reverse	5'-CCCCTGATCTTAATCGCAAA-3'
Human *RCC2*	Forward	5'-AAGGAGCGCGTCAAACTTGAA-3'
	Reverse	5'-GCTTGCTGTTTAGGCACTTCTT-3'
Human *MCL1*	Forward	5'-CGGTAATCGGACTCAACCTC-3'
	Reverse	5'-CCTCCTTCTCCGTAGCCAA-3'
Mouse *itgb1*	Forward	5'-TGTGACCCATTGCAAGGAGAAGGA-3'
	Reverse	5'-AATTGGGATGATGTCGGGACCAGT-3'
hsa-miR-29c cloning	Forward	5'-AAAgagctcACAATGTATTGGGTGTCGATTG-3' (SacI)
	Reverse	5'-AAggtaccATCCATTAAACTATCTCTTACCTCTGC-3' (KpnI)
ITGB1 3'UTR reporter construct	Forward	5'-AATCTAGATATTTGCTGAATGGGG-3'
	Reverse	5'-AAGATATCAAGATGGGCAACTCA-3'
ITGB1 Mut 3'UTR reporter construct	Forward	5'-TCACCTCTTCTAATCTTTTA-3'
	Reverse	5'-CAGAAATTATTAGACATTAAACAA-3'
RCC2 3'UTR reporter construct	Forward	5'-AAAgagctcTGAGTTATGAGGTGCCTTTCC-3' (SacI)
	Reverse	5'-AAAActcgagACTTTCCCATCCACCCA-3' (XhoI)
MCL1 3'UTR reporter construct	Forward	5'-AAAgagctcTGGAACTCATTAGCTGTGTGC-3' (SacI)
	Reverse	5'-AAAActcgagAAATCCAAAGATGCCAATG-3' (XhoI)

Mut, mutated

Supplementary Table 3. Summary of read/sequence counts for miRNA analysis from Illumina HiSeq sequencing the *NGS Profile*.

Description	Total count	Gastric cancer (GC)	Normal gastric mucosa (NM)
Raw Illumina HiSeq reads	22,051,844	9,580,826	12,471,018
Size selection ≥ 12 and ≤ 27	-1,769	-854	-915
Unmapped reads	-12,354,267	-6,001,243	-6,353,024
Allow ≤ 1 internal mismatch	-51,486	-17,933	-33,553
Undefined mapped reads	-12,142	-4,958	-7,184
Total miRNA abundance	9,625,084	3,555,838	6,076,342

Supplementary Table 4. Sequencing counts for 26 miRNAs distinctly different between GCs and NMs from the *NGS Profile*.

	Gastric cancer				Normal mucosa					
	GC1	GC2	GC3	GC4	NM1	NM2	NM3	NM4	NM5	NM6
hsa-mir-1286	6.3431	1.6059	1.2831	3.9922	0.1727	0.1888	0.0928	1.1592	0.9758	1.6617
hsa-mir-129-2	0	0.5353	1.4664	1.8426	7.9445	9.8156	7.5193	10.819	0.0976	6.093
hsa-mir-1304	61.3169	50.5851	25.6613	21.4967	10.0169	15.4785	10.4898	8.1143	37.9602	12.74
hsa-mir-135a-1	0	7.7617	1.2831	3.378	7.599	11.7033	9.283	28.9795	2.5372	19.9408
hsa-mir-135b	62.7265	53.7968	62.8702	56.8126	5.1812	13.7797	7.9834	4.6367	121.8825	6.6469
hsa-mir-146b	877.4662	1025.6188	2226.4851	1081.5897	722.0823	585.7297	881.4238	174.6501	504.5097	280.2794
hsa-mir-148a	13853.3945	33873.2578	7621.043	37923.8164	35232.1211	45085.7148	38935.0781	122218.7344	29455.5566	94633.6406
hsa-mir-196a-1	395.3884	314.7515	668.6605	149.5554	10.535	74.9387	15.7812	3.4775	889.3813	4.9852
hsa-mir-204	1.4096	50.0498	8.6149	63.2616	492.9019	351.4756	558.8385	1560.2589	355.2061	3295.2224
hsa-mir-2114	0.7048	0.2676	0.7332	0	5.0085	1.8876	2.3208	2.3184	0.4879	3.3235
hsa-mir-2116	12.6863	5.3529	6.4153	8.2916	4.3176	2.2651	3.5276	0.7728	3.2203	1.1078
hsa-mir-21	74148.3672	77557.6094	66978.0313	69479.7031	29565.6504	57241.0664	32994.8633	31861.2734	45368.7891	28403.498
hsa-mir-218-1	2.8192	14.1852	28.4107	11.9767	46.1124	23.2178	51.428	31.6843	16.0038	49.2982
hsa-mir-221	1652.0328	1642.0071	2166.9143	1870.2106	1610.6527	1809.8539	1531.1432	660.3473	840.7844	925.5869
hsa-mir-222	3193.4133	2183.4546	2564.6646	2284.7891	1255.3973	1483.1063	1467.3688	626.731	2225.5024	1008.1198
hsa-mir-223	333.3667	577.8473	416.6297	802.7472	127.4568	184.0434	207.2901	81.1427	274.9919	182.791
hsa-mir-29c	260.7731	1000.7277	564.3655	1282.7372	1357.4663	1329.8312	1659.9917	3078.0144	405.0715	2340.2783
hsa-mir-3195	5.6383	5.0853	5.4989	15.0477	17.2706	23.4065	73.9858	43.6625	2.0493	21.6026
hsa-mir-33b	66.2505	53.7968	31.71	76.4667	23.8334	50.9659	21.0725	38.253	12.8811	27.6956
hsa-mir-431	29.6013	14.4529	19.0627	23.6463	10.1896	14.9122	11.6038	2.3184	17.7603	4.9852
hsa-mir-485	4.9335	8.297	8.9815	14.1264	17.4433	14.1572	20.237	11.9782	9.4657	7.7548
hsa-mir-549a	8.4575	8.8323	15.0302	1.2284	0.1727	0.755	0.4642	0	5.7575	0.5539
hsa-mir-551a	3.524	2.6765	0.1833	1.2284	0	0	0	0	0.0976	0
hsa-mir-664a	6.3431	22.2146	24.0117	22.418	31.6051	45.6805	50.2212	46.3673	19.5168	83.0868
hsa-mir-874	9.1623	33.7234	23.4618	25.796	51.2936	56.44	58.7616	66.0734	15.1255	58.1608
hsa-mir-888	0	0	0	0	0.6908	2.6427	0.8355	3.0912	0	1.1078

Supplementary Table 5. Summary of read/sequence counts for miRNA analysis from AB SOLiD sequencing of *Profile#1*

Description	**Total count**	**GC stage I**	**GC stage II**	**GC stage III**	**GC stage IV**	**NM**
Raw AB SOLiD reads	183,332,039	35,941,161	29,014,565	57,596,038	33,334,536	27,445,739
Size selection ≥ 12 and ≤ 27	-135,426	-102,059	-5,301	-12,887	-8,320	-6,859
Unmapped reads	-144,179,145	-27,329,247	-23,927,086	-45,769,608	-26,456,209	-20,696,995
Allow ≤ 1 internal mismatch	-4,779,951	-1,144,552	-580,301	-1,324,163	-816,462	-914,473
Undefined mapped reads	-390,848	-351,432	-7,990	-14,387	-9,236	-7,803
Total miRNA abundance	33,846,669	7,013,871	4,493,887	10,474,993	6,044,309	5,819,609

GC, gastric cancer; NM, normal mucosa

Supplementary Table 6. List of miRNA significantly different between GC and NM for each profile

Profile name	***NGS profile***	***Profile#1***	***Profile#2***
Specimen	4 pairs of GC and NM, and 2 NMs	19 GCs and 6 NMs	22 pairs of GC and NM
Experiment type	Small RNA-seq (Illumina HiSeq 2000)	Small RNA-seq (AB SOLiD System 3.0)	miRNA microarray
	hsa-miR-29c	hsa-let-7i	ebv-miR-BART12
	hsa-miR-146b	hsa-miR-192	hsa-miR-100
	hsa-miR-135b	hsa-miR-106a	ebv-miR-BART2
	hsa-miR-148a	hsa-miR-126	hsa-let-7b
	hsa-miR-21	hsa-miR-155	hsa-miR-1
	hsa-miR-221	hsa-miR-20a	hsa-miR-101
	hsa-miR-222	hsa-miR-20b	hsa-miR-125b
	hsa-miR-223	hsa-miR-211	hsa-miR-127
	hsa-miR-204	hsa-miR-140	hsa-let-7c
	hsa-miR-33b	hsa-miR-214	hsa-miR-129
	hsa-miR-431	hsa-miR-215	hsa-miR-133a
	hsa-miR-551a	hsa-miR-30d	hsa-miR-139
		hsa-miR-125a	hsa-miR-368
		hsa-miR-130a	hsa-miR-375
		hsa-miR-133b	hsa-miR-376a
		hsa-miR-135b	hsa-miR-520d
		hsa-miR-136	hsa-miR-377
		hsa-miR-145	hsa-miR-378
		hsa-miR-148a	hsa-miR-550
		hsa-miR-193a	hsa-miR-379
		hsa-miR-193b	hsa-miR-381
		hsa-miR-195	hsa-miR-422b
		hsa-miR-204	hsa-miR-552
		hsa-miR-21	hsa-miR-497
		hsa-miR-29c	hsa-miR-603
		hsa-miR-335	hsa-miR-143
		hsa-miR-339	hsa-miR-146a
		hsa-miR-340	hsa-miR-149
		hsa-miR-363	hsa-miR-452*
		hsa-miR-423	hsa-miR-154
		hsa-miR-424	hsa-miR-18a
		hsa-miR-484	hsa-miR-18a*
		hsa-miR-490	hsa-miR-18b
		hsa-miR-491	hsa-miR-196a
		hsa-miR-496	hsa-miR-196b
		hsa-miR-518c	hsa-miR-218
		hsa-miR-548c	hsa-miR-26a
		hsa-miR-551b	hsa-miR-492
		hsa-miR-559	hsa-miR-299-3p
		hsa-miR-574	hsa-miR-299-5p
		hsa-miR-592	hsa-miR-30c
		hsa-miR-93	hsa-miR-30e-3p
		hsa-miR-95	hsa-miR-30e-5p
		hsa-miR-98	hsa-miR-365
			hsa-miR-125a
			hsa-miR-130a
			hsa-miR-133b
			hsa-miR-135b
			hsa-miR-136
			hsa-miR-145
			hsa-miR-148a
			hsa-miR-193a
			hsa-miR-193b
			hsa-miR-195
			hsa-miR-204
			hsa-miR-29c
			hsa-miR-363
			hsa-miR-551b
			hsa-miR-622
			hsa-miR-633
			hsa-miR-645
			hsa-miR-655
			hsa-miR-768-3p
			hsa-miR-768-5p
			hsa-miR-99a

Bold represents overlapped miRNAs in all 3 miRNA profiles.

Supplementary references

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2. Oshima H, Matsunaga A, Fujimura T, et al. Carcinogenesis in mouse stomach by simultaneous activation of the Wnt signaling and prostaglandin E2 pathway. *Gastroenterology* 2006;**131**:1086-95.