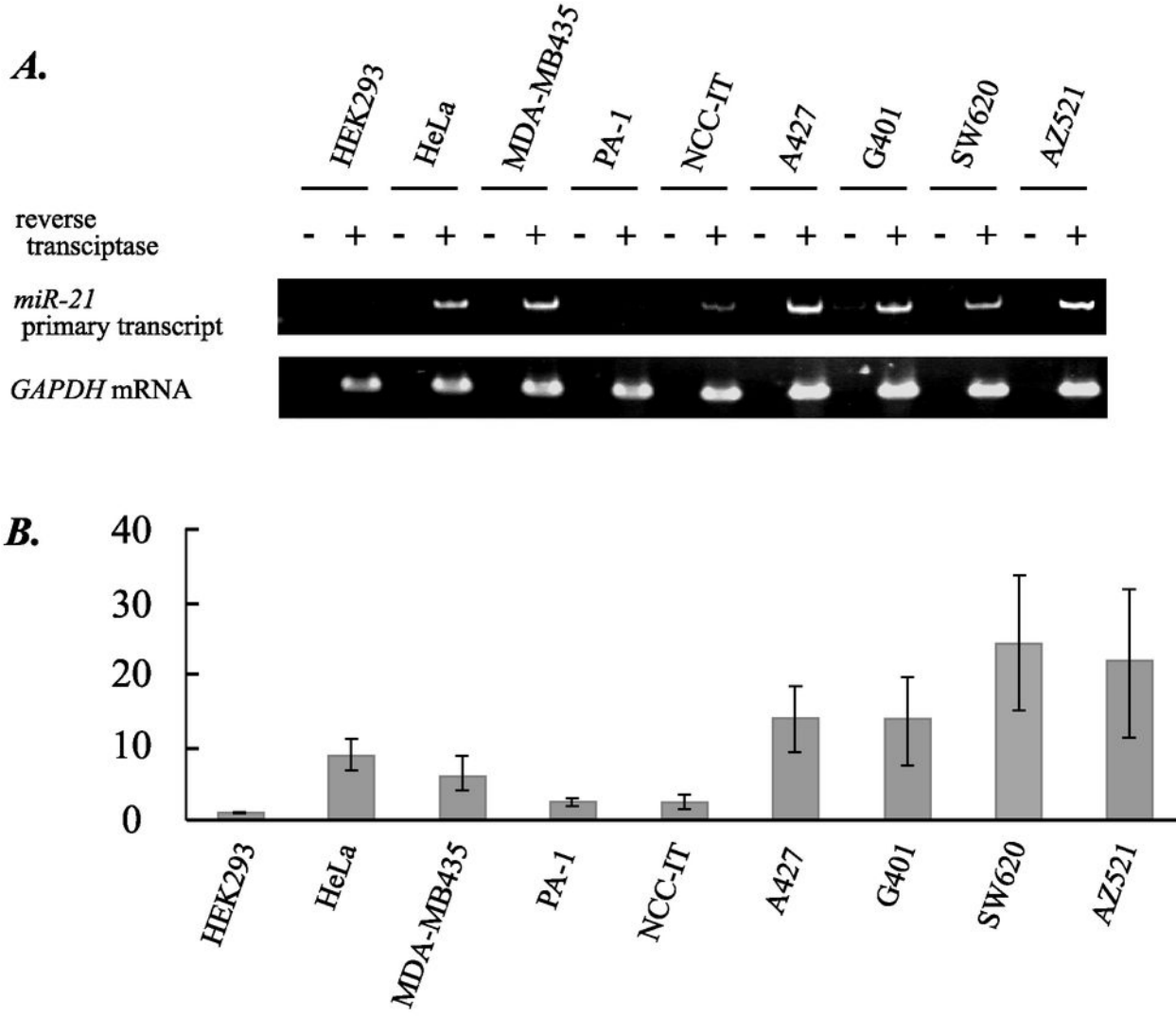

Figure S1. Yamamichi et al.

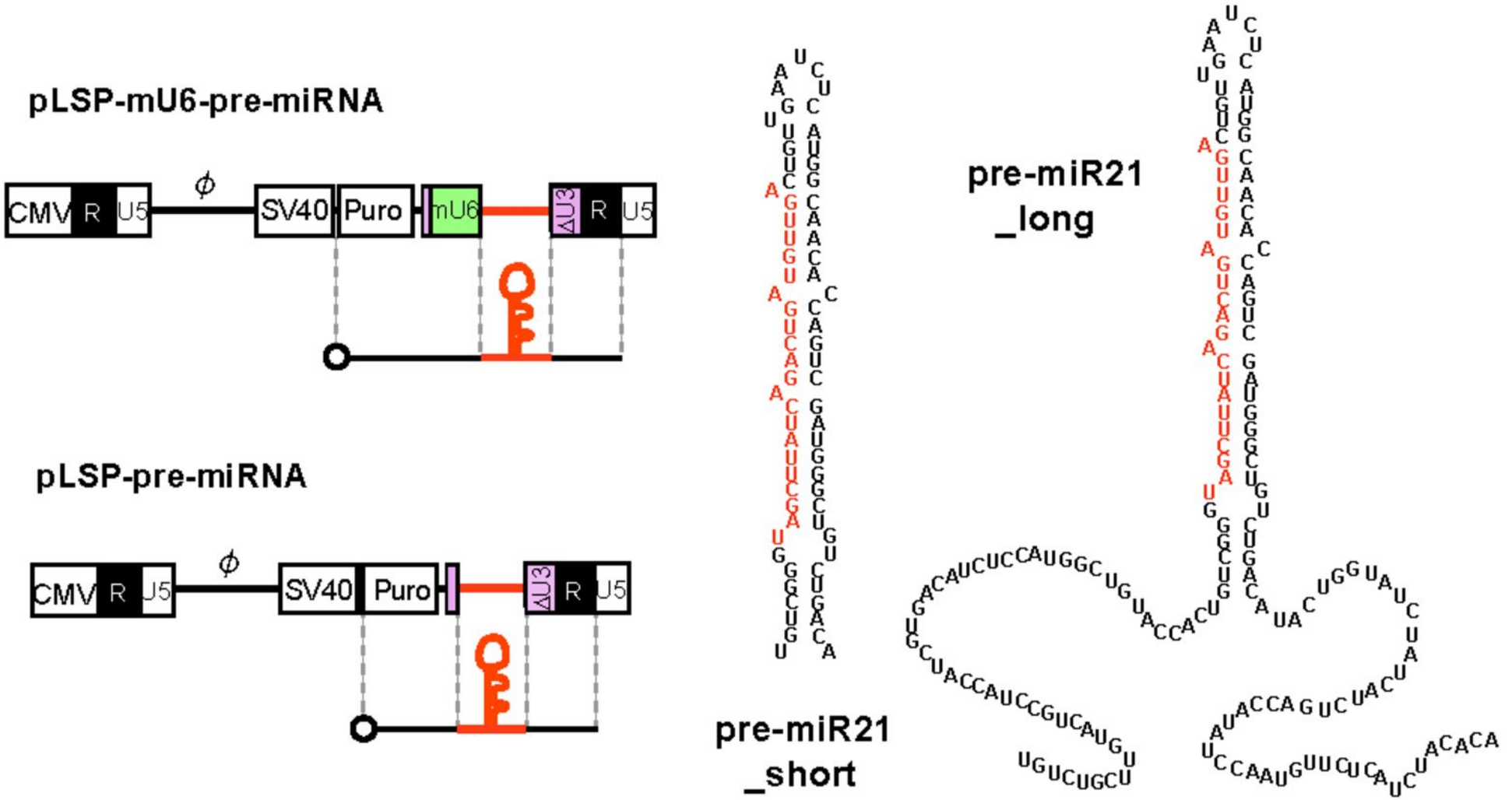

Figure S2. Yamamichi et al.

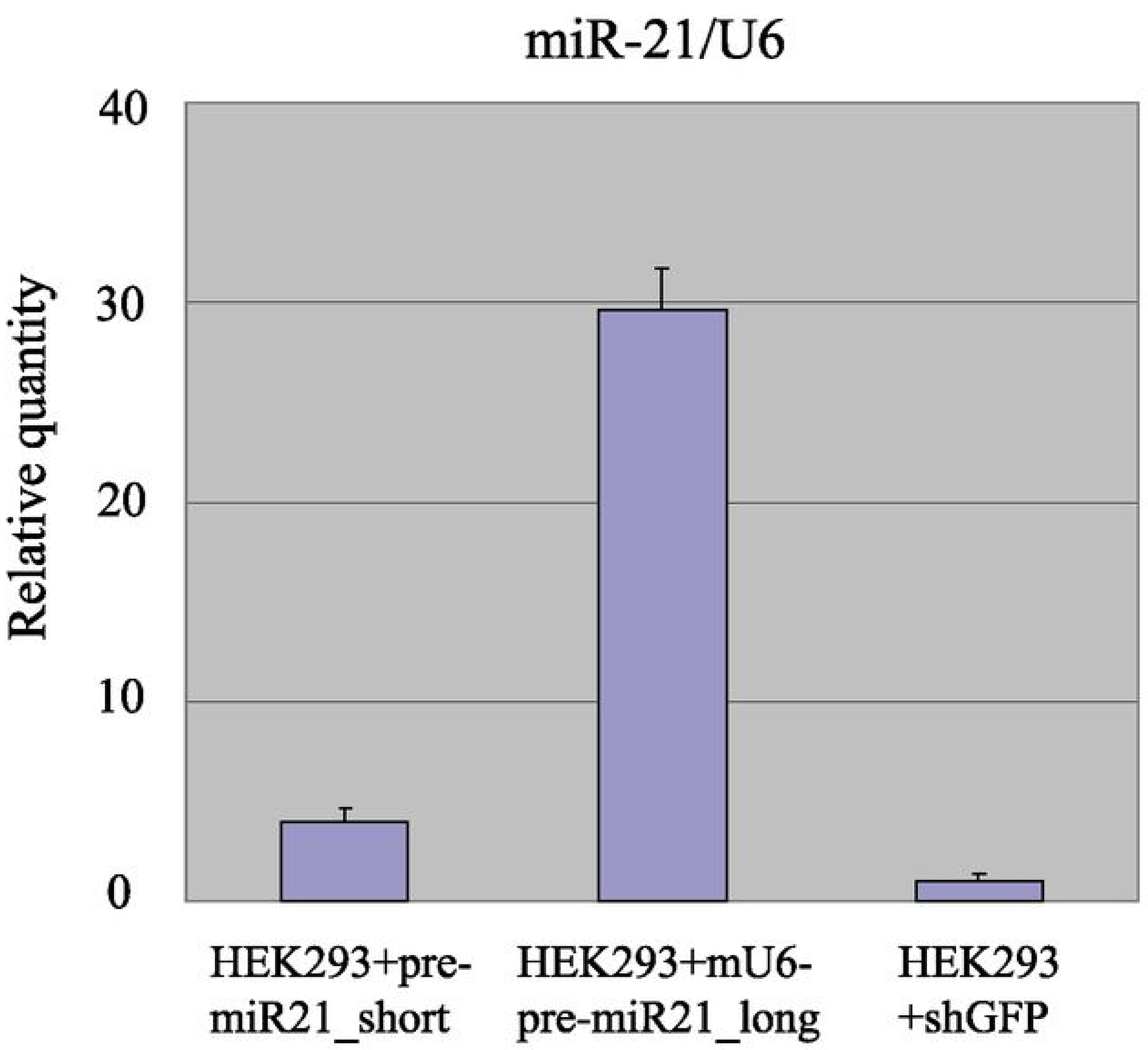

Figure S3. Yamamichi et al.

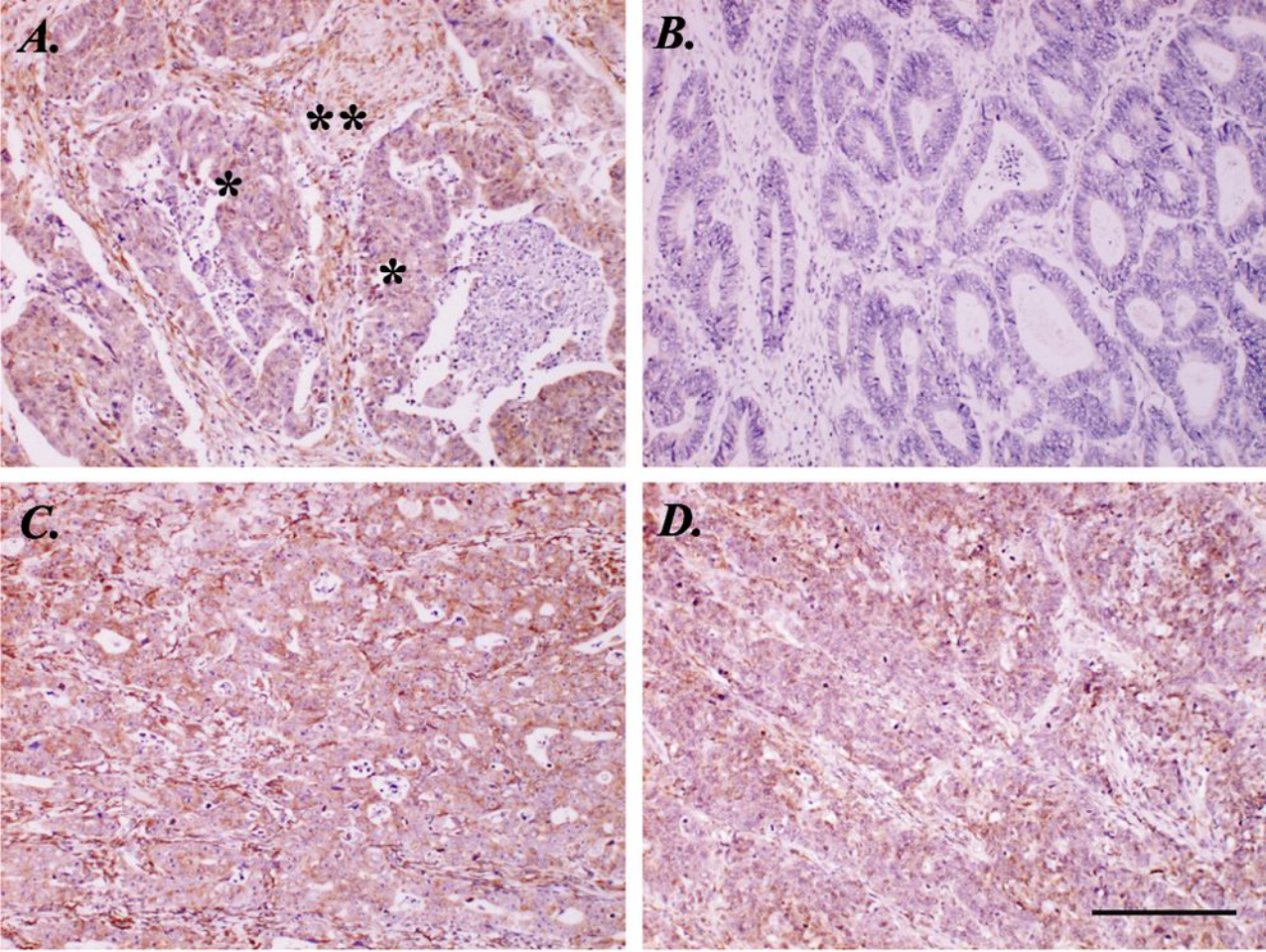

Figure S4. Yamamichi et al.

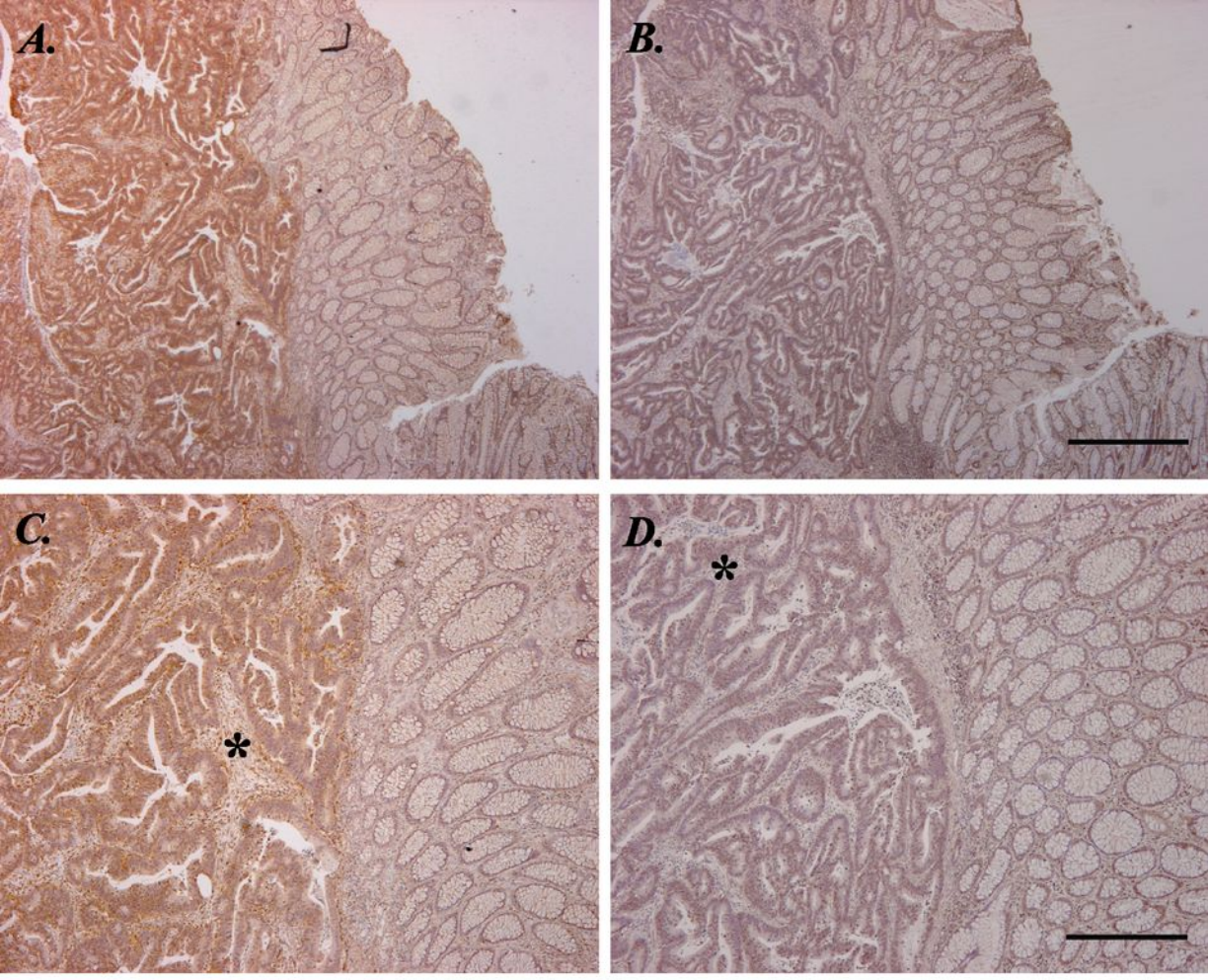

Figure S5. Yamamichi et al.

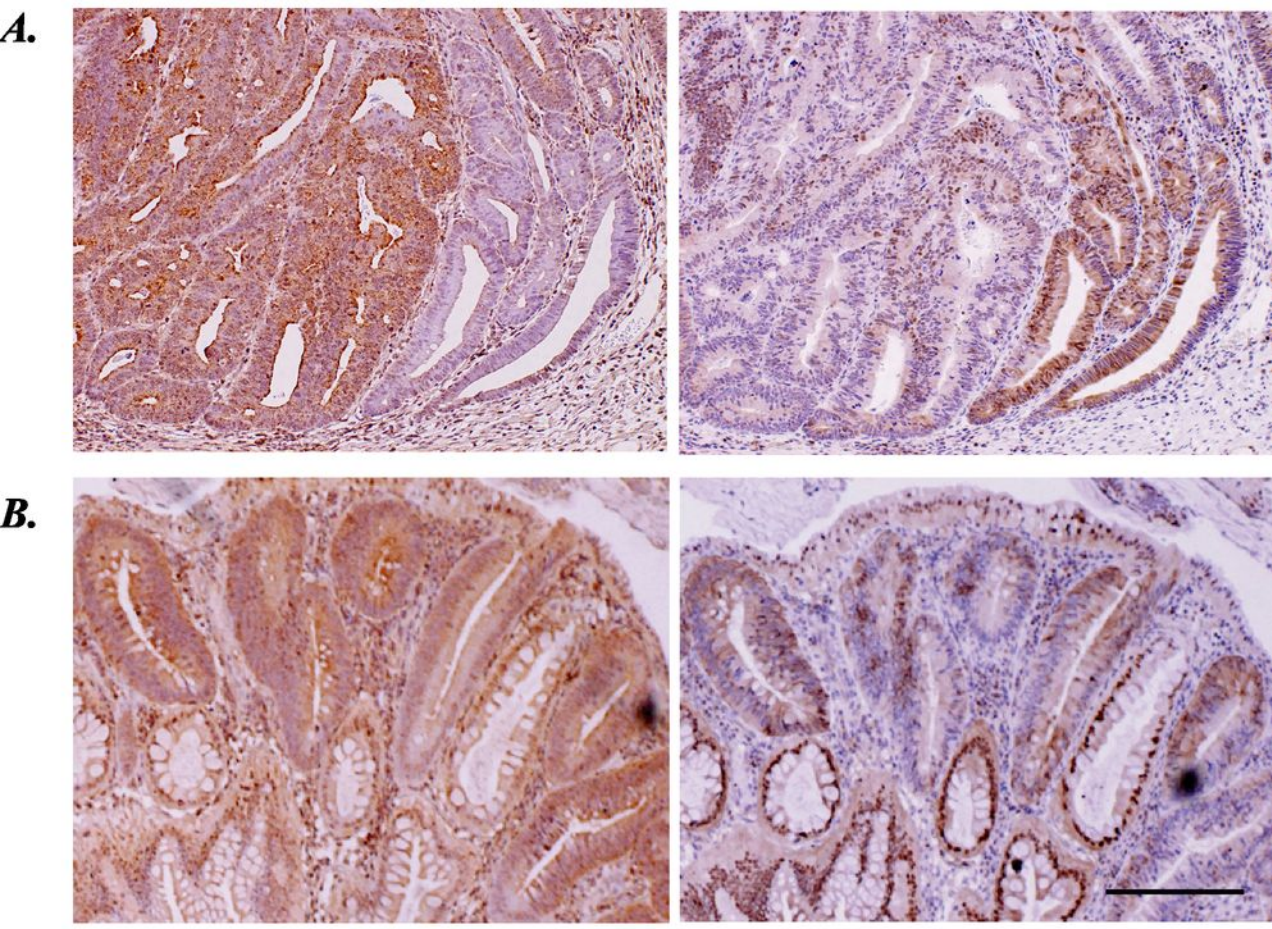

Figure S6. Yamamichi et al.

SUPPLEMENTARY MATERIALS AND METHODS

Reverse transcription-polymerase chain reaction (RT-PCR). Total cellular RNAs were prepared using the mirVana miRNA Isolation Kit (Ambion, Austin, TX) according to the manufacturer's protocol. To avoid detection of the genomic DNA sequence, these RNAs were treated with abundant DNase I (RQ1 RNase-Free DNase from Promega, Madison, WI) at 37°C for 30 min. Semi-quantitative RT-PCR was performed using the Superscript One-Step RT-PCR with Platinum Taq Kit (Invitrogen). PCR without reverse transcription was simultaneously performed to confirm the absence of DNA contamination. The primer pairs used to detect *pri-miR-21* were 5'-caaatcctgcctgactgtctg-3' and 5'-gtgttgccatgagattcaacag-3'. RNAs were reverse-transcribed for 30 min at 50°C, and after an initial denaturation at 94°C for 3 min, cDNA amplification procedures were performed which consisted of 36 cycles of 94°C for 30 sec, 58°C for 1 min, and 72°C for 1 min. RT-PCR reactions for detection of *GAPDH* were performed as described previously (1, 2).

TaqMan real-time PCR microRNA assays. RNAs were reverse transcribed using the TaqMan MicroRNA RT kit (Applied Biosystems, Foster City, CA) for 30 min at 16°C, 30 min at 42°C, 5 min at 85°C, and 10 min at 4°C. Real-time quantitative PCR amplification of the cDNA was performed using the TaqMan Universal PCR Master Mix (Applied Biosystems) and TaqMan MicroRNA Assays for miR-21 and U6 small nuclear RNA (P/N:4373090 and P/N:4373381 respectively, Applied Biosystems) in an ABI PRISM 7300 system (Applied Biosystems). PCR conditions were 95°C for 10 min, followed by 40 cycles of 95°C for 15 sec and 60°C for 1 min. Expression of miR-21 was always evaluated with comparison to U6 small nuclear RNA expression (an internal control). All reactions were run in duplicate, and the

error bars in all figures indicate standard deviations.

REFERENCE OF SUPPLEMENTARY MATERIALS AND METHODS

1. Yamamichi N, Yamamichi-Nishina M, Mizutani T, et al. The *Brm* gene suppressed at the post-transcriptional level in various human cell lines is inducible by transient HDAC inhibitor treatment, which exhibits antioncogenic potential. Oncogene 2005;24:5471-81.
2. Yamamichi-Nishina M, Ito T, Mizutani T, Yamamichi N, Watanabe H, Iba H. SW13 cells can transition between two distinct subtypes by switching expression of BRG1 and Brm genes at the post-transcriptional level. J Biol Chem 2003;278:7422-30.

SUPPLEMENTARY FIGURE LEGENDS

Figure S1. (A) Expression levels of pri-miR-21 determined by RT-PCR using nine various human cell lines. Levels of *GAPDH* mRNA were also determined as internal controls. (B) Expression levels of mature miR-21 determined by TaqMan real-time PCR microRNA assays. All reactions were run in duplicate, and the error bars indicate the standard deviations.

Figure S2. Schematic representations of two miRNA expression lentivirus vectors driven by polymerase II (pLSP) or polymerase III (pLSP-mU6). A short sequence almost corresponding to miR-21 (pre-mir21_short) directed by SV40 promoter and a longer sequence from pri-miR-21 (pre-mir21_long) directed by mouse U6 promoter were inserted respectively.

Figure S3. Expression of miR-21 in HEK293 cells stably transduced with two kinds of miR-21 expression lentiviral vectors analyzed by TaqMan real-time PCR microRNA assays. HEK293 cells stably transduced with *shGFP* virus were used as control.

Figure S4. LNA-ISH detecting miR-21 with FFPE clinical tissues obtained from surgical operations. Scale bar indicates 200 μm. (A) LNA-ISH of miR-21 using the colorectal adenocarcinoma sample, in which ISH staining of stromal fibroblasts around the cancer (**) show the stronger intensity than colorectal cancer cells (*). (B) ISH staining without the LNA/DNA probe, using the same samples shown in Figure 2B. (C) LNA-ISH detection of miR-21 in gastric cancer tissue. (D) LNA-ISH detection of miR-21 in lung cancer tissue.

Figure S5. (A, B) LNA-ISH analyses with miR-21 probe (A) or the scramble probe (negative control) (B) performed using sequential tissue sections derived from

the colorectal adenocarcinoma case. Scale bar indicates 400 μm. The border of the colorectal cancer (left half) and normal colorectal mucosa (right half) is apparent in the two photos. (C, D) Enlarged images of parts of (A) and (B), respectively. Stromal fibroblasts around the cancer were shown by (*). Scale bar indicates 200 μm.

Figure S6. Enlarged images of the colorectal polyp cases shown in Figure 5. LNA-ISH for detection of miR-21 RNA (left) and immunostaining of PDCD4 protein (right) were performed using sequential tissue sections. Scale bar indicates 200 μm. (A) A colorectal polyp containing adenocarcinoma and adenoma shown in Figure 5B, which is obtained from endoscopic mucosal resection (EMR). (B) A non-malignant adenomatous colorectal polyp shown in Figure 5C, which is obtained from endoscopic mucosal resection (EMR).

Table S1

Number	Pathohistological Diagnosis	miR-21 in tumor	Age	Gender	Depth	Stage	miR-21 in adjacent fibroblast
1	moderately differentiated adenocarcinoma	3	58	M	T3	III	+
2	moderately differentiated adenocarcinoma	5	76	M	T4	II	+
3	moderately differentiated adenocarcinoma	4	68	M	T4	III	+
4	moderately differentiated adenocarcinoma	3	83	F	T4	IV	+
5	moderately differentiated adenocarcinoma	2	58	M	T4	III	+
6	poorly differentiated adenocarcinoma	3	57	M	T4	IV	+
7	moderately differentiated adenocarcinoma	4	69	F	T4	III	+
8	moderately differentiated adenocarcinoma	4	59	M	T4	III	+
9	well differentiated adenocarcinoma	1	54	F	T4	II	+/-
10	moderately differentiated adenocarcinoma	4	68	F	T4	II	+
11	moderately differentiated adenocarcinoma	2	65	F	T4	II	+
12	moderately differentiated adenocarcinoma	5	64	M	T4	III	+
13	moderately differentiated adenocarcinoma	5	41	F	T4	II	+
14	well differentiated adenocarcinoma	1	60	F	T4	II	+
15	moderately differentiated adenocarcinoma	3	52	M	T3	II	+
16	moderately differentiated adenocarcinoma	4	69	M	T4	III	+
17	moderately differentiated adenocarcinoma	2	71	M	T2	I	+
18	well differentiated adenocarcinoma	5	79	F	T4	II	+
19	poorly differentiated adenocarcinoma	5	79	F	T4	II	+
20	well differentiated adenocarcinoma	4	53	F	T4	II	+
21	moderately differentiated adenocarcinoma	2	76	M	T4	III	+/-
22	moderately differentiated adenocarcinoma	3	56	M	T4	III	+
23	moderately differentiated adenocarcinoma	3	77	M	T4	II	+

24	moderately differentiated adenocarcinoma	3	74	M	T4	II	+
25	moderately differentiated adenocarcinoma	1	63	F	T4	II	-
26	moderately differentiated adenocarcinoma	4	71	F	T4	III	+
27	moderately differentiated adenocarcinoma	1	82	M	T4	III	-
28	moderately differentiated adenocarcinoma	4	60	M	T4	II	+
29	moderately differentiated adenocarcinoma	2	63	M	T4	III	+
30	well differentiated adenocarcinoma	5	68	M	T3	II	+
31	poorly differentiated adenocarcinoma	2	64	F	T4	II	+
32	moderately differentiated adenocarcinoma	3	58	M	T4	III	+
33	well differentiated adenocarcinoma	4	72	M	T3	III	+
34	moderately differentiated adenocarcinoma	4	60	M	T4	III	+
35	low grade adenoma (tubulovillous)	1	58	M			-
36	low grade adenoma (tubular and serrated)	1	59	M			-
37	low grade adenoma (tubulovillous)	2	68	F			+/-
38	low grade adenoma (tubular, in adenocarcinoma)	1	79	F			-
39	low grade adenoma (tubular, in adenocarcinoma)	1	71	F			-

Evaluation of miR-21 expression in 34 colorectal adenocarcinomas and 5 colorectal adenomas, all of which were surgically excised. Pathohistological diagnoses were made using the WHO classificatio criteria for tumours of the colon and rectum and by the Vienna classification of gastrointestinal epithelial neoplasia for adenoma. Patient gender and age, tumor depth, and tumor clinical stage are also shown. Compared with LNA-ISH staining of normal colorectal mucosa on individual slides, values assigned to the staining of tumors (from 1 to 5) were based on the following criteria:

5: most tumor cells (more than 80%) show much stronger staining than normal epithelial cells.

4: most tumor cells (more than 80%) show stronger staining than normal epithelial cells.

3: a part of tumor cells (about 20-80%) show stronger staining than normal epithelial cells.

2: some tumor cells (more than 20%) show slightly stronger staining than normal epithelial cells.

1: almost all tumor cells show a silmilar staining intensity to that observed in normal epithelial cells.

As for the adjacent stromal fibroblasts around the tumor (cancer-associated fibroblasts, i.e., CAFs), evaluations of the intensity of miR-21 staining by LNA-ISH were decided as follows:

+: CAFs show much stronger staining compared with normal epithelial cells and fibroblasts on the same slide.

+/-: CAFs show a little stronger staining compared with normal epithelial cells and fibroblasts on the same slide.

-: CAFs show a staining intensity that is similar to normal epithelial cells and fibroblasts on the same slide.

Table S2

Number	Pathohistological Diagnosis	miR-21 in polyp	Age	Gender
1	non-invasive carcinoma	++	61	M
2	non-invasive carcinoma	++	69	M
3	non-invasive carcinoma	-	68	M
4	non-invasive carcinoma	++	57	F
5	non-invasive carcinoma	++	54	M
6	non-invasive carcinoma	++	65	M
7	non-invasive carcinoma	++	53	M
8	non-invasive carcinoma	++	69	F
9	low grade adenoma (serrated)	-	64	F
10	low grade adenoma (serrated)	-	60	M
11	low grade adenoma (tubular)	-	60	M
12	low and high grade adenoma (tubular)	-	39	M
13	low and high grade adenoma (tubular)	+	73	M
14	low grade adenoma (tubular)	-	63	M
15	high grade adenoma (tubular)	++	54	M
16	low and high grade adenoma (tubular)	++	55	M
17	low grade adenoma (tubular)	++	72	M
18	low and high grade adenoma (tubulovillous)	++	58	F
19	low grade adenoma (tubular and serrated)	-	65	M
20	low and high grade adenoma (tubulovillous)	+	41	M
21	low and high grade adenoma (tubular)	++	64	F
22	low grade adenoma (tubular)	-	59	F
23	low grade adenoma (tubular)	++	59	F
24	low grade adenoma (tubulovillous)	++	58	M

25	low and high grade adenoma (tubulovillous)	++	83	F
26	low grade adenoma (tubular)	-	56	M
27	low grade adenoma (tubular)	-	67	F
28	low grade adenoma (tubular)	-	76	M
29	hyperplastic polyp	-	59	F
30	juvenile polyp	-	35	F
31	hyperplastic polyp	-	72	M
32	Peutz-Jagher's hamartomatous polyp	-	37	M
33	Peutz-Jagher's hamartomatous polyp	-	37	M
34	hyperplastic polyp	-	38	M

Expression of miR-21 in and pathohistological diagnosis of 34 endoscopically resected colorectal polyps. Pathohistological diagnoses were determined using the Vienna classification of gastrointestinal epithelial neoplasia. Patient gender and age in each case are also presented. Evaluations of the intensity of miR-21 staining by LNA-ISH were decided based on the following criteria;

++: cells in the polyp show much stronger staining compared with normal epithelial cells on the same slide.

+: cells in the polyp show stronger staining compared with normal epithelial cells on the same slide.

-: cells in the polyp show a staining intensity that is similar to normal epithelial cells on the same slide.