

SUPPLEMENTARY INFORMATION

1. SUPPLEMENTAL DATA

Fig. S1 miR-574-5p MREs on human *Qki6/7/7b* mRNAs (related to Fig. 1). **A**, Human *Qki* gene structure, major transcripts, protein isoforms and 3'UTRs. Located at 6q26-q27, the human *Qki* gene contains eight exons. Due to alternative splicing, at least 4 major transcripts, namely *Qki5/6/7/7b*, can be produced. Each of these transcript isoforms has its own unique 3'UTR derived from the alternatively-spliced exons. However, a long stretch of overlapping sequences is found in the 3'UTRs for *Qki6/7/7b* respectively. Furthermore, a putative MRE for miR-574-5p was found in the 3'UTRs of *Qki6/7/7b* but not that of *Qki5* mRNA. Four major QKI proteins (QKI5/6/7/7b) can be produced from the four mRNA isoforms, which differ in their carboxyl termini encoded for by the alternatively spliced exons. A common N-terminal 311 amino acid peptide fragment encoded by the first six exons is shared by all four protein isoforms. This region contains a KH domain flanked by the QUA1 and the QUA2 motifs respectively. QUA1 is involved in protein homodimerization or heterodimerization while the KH and QUA2 domains might be responsible for RNA binding. The Y-motif is a tyrosine-rich region that serves as a site for tyrosine phosphorylation. In addition, there is a P-motif for the proline-rich region. The C-terminal of QKI5 (but not of the other three isoforms) contains a nuclear localization signal (NLS) that directs its entry into the nucleus. The figure was redrawn based on previous reports¹⁻³. The putative MREs for miR-574-5p were indicated on the 3'UTRs for *Qki6/7/7b* respectively. **B**, Alignment of putative miR-574-5p seed-binding sites of mouse *Qki6/7-3'UTR* and human *Qki6/7/7b-3'UTR*. Mature human miR-574-5p (hsa-miR-574-5p) and mouse miR-574-5p (mmu-miR-574-5p) share 100% sequence similarity, with the sequence being 5'-ugagugugugugugagugugu-3'. **C**, MREs for miR-17, miR-20a and miR-200b on mouse *Qki5-3'UTR* as predicted by the miRanda algorithm (www.microrna.org). Data sets on www.microrna.org so far contain more complete 3'UTRs for specific genes and the miRanda algorithm distinguishes the 3'UTRs. **D** & **E**, MREs for miR-17, miR-20a, miR-200b, miR-466g, miR-574-5p and miR-717 on mouse *Qki6/7-3'UTRs* as predicted by the miRanda algorithm. Surprisingly, no MRE for miR-574-3p was predicted in the 3'UTRs for mouse or human *Qki5* or *Qki6/7/7b* mRNA.

Fig. S2 Effects of miR-574-5p on *Qki* expression in human HCT116, SW480 and SW620 cells (related to Fig. 2). Human CRC cells were transfected with a miR-574 overexpressing plasmid, a miR-574-5p inhibitor. Cells were harvested for qPCR or western blot assays 24 hours after the transfections ($n = 3-4$). Statistical comparisons were made between a control (pFlag-CMV or inhibitor control)-transfected cells and a particular treatment. NS, not significant; **, $p < 0.01$; ***, $p < 0.001$. **A**, Inhibition of miR-574-5p significantly increased the levels of *Qki5/6/7/7b* mRNA isoforms in SW480 and SW620 cells, but only *Qki6/7* were increased in HCT116 cells. **B**, Overexpression of miR-574-5p significantly reduced the level of total QKI protein in SW480 cells. **C**, Inhibition of miR-574-5p significantly increased the levels of total QKI and QKI5/6/7 proteins in SW480 cells.

Fig. S3 Putative QREs on β -catenin mRNAs from a few mammals (related to Fig. 3). hsa, *Homo sapiens*; pab, *Pongo abelii*; mmu, *Mus, musculus*; bta, *Bos taurus*; ssc, *Sus scrofa*. Two putative QREs were indicated by QRE1 and QRE2 respectively. The distal QRE1 for β -catenin mRNAs from orangutans, mice, cows and pigs completely satisfies the bipartite consensus sequence of NACUAAAY-N₁₋₂₀-UAAY (Galarneau and Richard, 2005; Hafner et al., 2010). With human β -catenin mRNAs, however, the number of the intervening nucleotides in the QRE1 between the core site NACUAAAY and the half site UAAY is 22 rather than smaller than or equal to 20. Similarly, the proximal QRE2 might also be functional⁴. However, this putative QRE is also slightly different from the consensus sequence, with the half-site being “CAAY” rather than “TAAY” and 22 intervening nucleotides between the putative core site and the putative half site.

Fig. S4 Co-overexpression of *Qki5/6/7* significantly attenuated the oncogenic effects of miR-574-5p. CT26 cells were grown and transfected with pmiR-574 or pFlag-CMV in the presence or absence of pFlag-*Qki5/6/7* for 24 hours. Cells were subsequently harvested for mRNA or MTT analyses. For invasion analysis, similar transfection was performed with CT26 cells for 12 hours. Subsequently, 5×10^4 transfected cells were seeded per upper chambers in serum-free RPMI 1640 medium whereas the lower chambers were loaded with RPMI 1640 medium containing 5% FBS. After 48 hours of incubation, cells were harvested for analysis. *, $p < 0.05$; **, $p < 0.01$; ****, $p < 0.001$. **A**, Re-introduction of *Qki5/6/7* significantly decreased β -catenin and increased *p27^{kip1}* mRNA expression in miR-574-5p-overexpressing cells ($n = 3-4$). **B** and **C**, Re-introduction of *Qki5/6/7* significantly reduced cell viability and cell invasion in miR-574-5p-overexpressing cells ($n = 3-4$).

Fig. S5 Expression levels of *Qki5/6/7/7b* mRNA and protein isoforms in the human CRC tissues and the adjacent normal epithelial tissues. T, tumor tissue; N, normal adjacent epithelial tissue. **A**, *Qki5* expression from ten pairs of clinical samples (#11-20) as determined by qPCR. **B**, *Qki6* expression from ten pairs of clinical samples (#11-20) as determined by qPCR. **C**, *Qki7* expression from ten pairs of clinical samples (#11-20) as determined by qPCR. **D**, *Qki7b* expression from ten pairs of clinical samples (#11-20) as determined by qPCR. **E**, QKI5/6/7/7b protein isoform expression in five pairs of clinical samples (#16-20) as determined by western blotting. $n = 5$, ***, $p < 0.001$.

Fig. S6 Subcellular localization of QKI5/6/7 proteins in human SW620 or mouse CT26 cells and the relative expression of *Qki5/6/7/7b* mRNAs in CRC cells, clinical CRC tissue samples and normal adjacent epithelial tissue samples. **A**, Subcellular localization of QKI5/6/7 proteins in SW620 or CT26 cells as determined by western blotting. **B**, Subcellular localization of QKI5/6/7 proteins in CT26 cells as determined by immunofluorescence assays. Original magnification, $\times 1,000$. **C**, Relative expression of *Qki* mRNA isoforms in CT26, HCT116, SW480 and SW620 cells. Total RNA samples were isolated and qPCR was performed ($n = 3-4$). All values were normalized with *Qki5* expression. **D**, Relative expression of *Qki* mRNA isoforms in clinical CRC and normal adjacent epithelial samples from 10 patients. Both the values for tumor tissues (T, black-colored) and corresponding adjacent noncancerous epithelial tissues from a particular patient (N, grey-colored) were normalized with the expression of *Qki5* mRNA in normal adjacent epithelial tissue from the same patient.

Fig. S7 Effects of miR-574-5p overexpression on β -catenin subcellular localization as determined by western blots and immunofluorescent staining. For western blots, CT26 cells were transfected with a miR-574-5p overexpressing plasmid (pmiR-574) or its control (pFlag-CMV) for 24 hours and harvested for western blot analyses. For immunofluorescent staining, CT26 cells were seed on the coverslips and transfected with pmiR-574 or pFlag-CMV for 24 hours. Cells were then fixed for immunofluorescence analyses. **A**, Subcellular distribution of β -catenin protein as determined by western blots; **B**, Subcellular distribution of β -catenin protein as determined by immunofluorescent staining. Original magnification, $\times 1000$.

Fig. S8 The miR-574-5p-QKI- β -catenin/p27^{Kip1} axis of signal transduction in the development of CRC. miR-574-5p derived from the first intron of either *Col3a1* or *Noxp20* regulates the expression of *Qki6/7/7b*

directly and negatively. *Qki5* may also be regulated by miR-574-5p indirectly. Down-regulation of QKIs will cause the overactivation of β -catenin and the suppression of p27^{Kip1}. As a result, activities in colorectal epithelial cell proliferation, migration and invasion will be enhanced, whereas cell cycle arrest and differentiation will be suppressed. Additionally, miR-574-5p might also repress the expression of tumor suppressor ceramide synthase-1 isoform 2 (*CerS1-2*) posttranscriptionally.⁵ QKIs, on the other hand, might also control the expression of macroH1A1.1 to impact tumorigenesis.⁶ miR-574-5p thus appears to be oncogenic and may contribute to the dysregulation of colorectal epithelial cell differentiation and tumorigenesis or cancer progression through the suppression of tumor-suppressive QKIs. miR-574-5p expression might be co-regulated with its hosting-genes (either *Noxp20* or *Col3a1*), although this needs to be verified with further study. Additionally, Sox2 is a transcriptional factor implicated in the maintenance of the pluripotency of stem cells⁷⁻⁹, neurogenesis¹⁰⁻¹³ and development of cancers.¹⁴⁻¹⁷ In glioblastoma multiforme cells, the knockdown of *Sox2* caused significant down-regulation of miR-574-5p.¹⁸ Coincidentally or not, aberrant Sox2 up-regulation and miR-574-5p up-regulation are often co-detected in diseases such as CRC (¹⁵ and current study), glioblastoma,¹⁸ lung cancer,¹⁹⁻²¹ esophageal squamous carcinoma^{16,22} etc. Together these observations suggest that Sox2 might regulate miR-574-5p positively. The possible regulation of *Noxp20*, *Col3a1* and miR-574-5p and *Qkis* by *Apc*, however, is not clear and warrant further research. *Apc*, adenomatous polyposis coli; *Col3a1*, procollagen Type III, alpha-1; *Noxp20*, nervous system overexpressed protein-20; *Sox2*, SRY-like HMG box-2.

Fig. S9 Schematic graphs for twelve plasmids used in the current study. See the Materials and Methods section for more details. hGH-pA, poly-A for human growth hormone gene; Luc, luciferase; SV40-pA, SV40 poly-A.

Table S1 The expression of miR-574-5p and pan-*Qki* mRNA and protein and the clinicopathological features of 60 CRC patients.

Table S2 Alterations of miR-574-5p expression in diseases.

Fig. S1

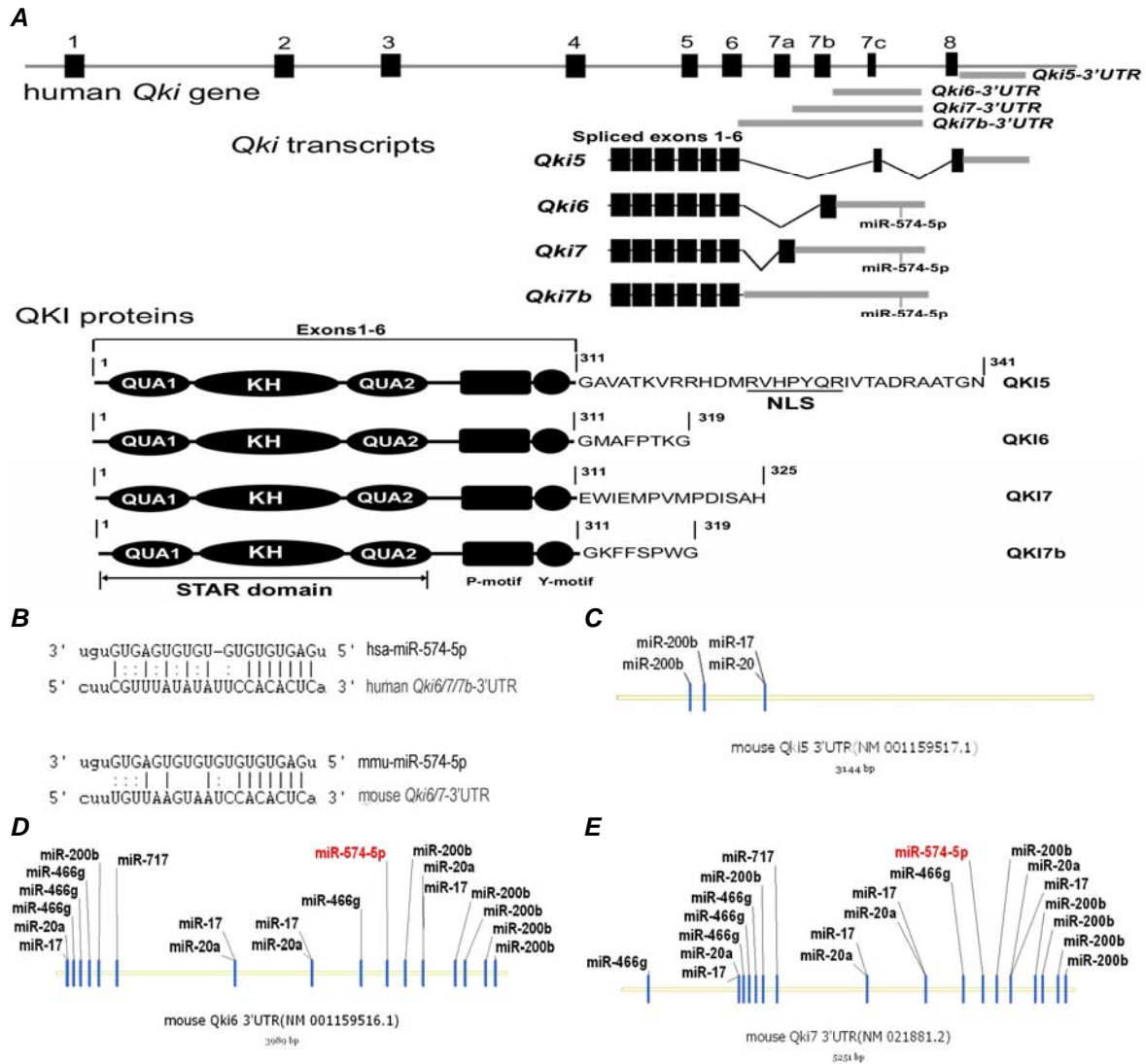
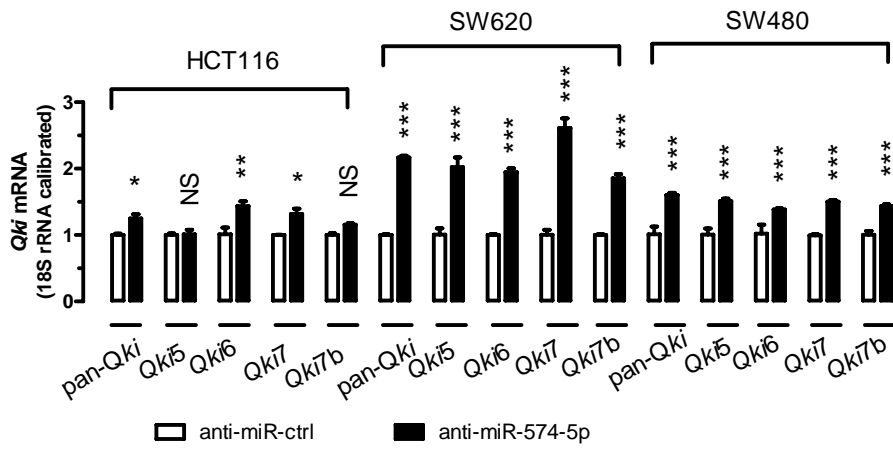
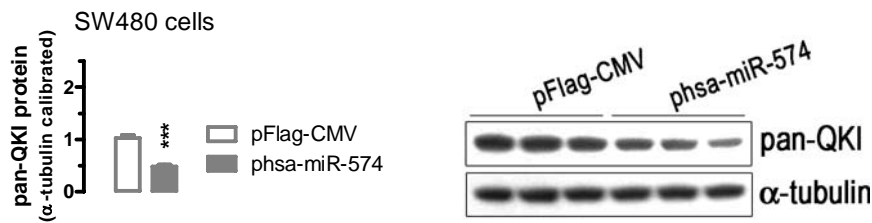


Fig. S2

A



B



C

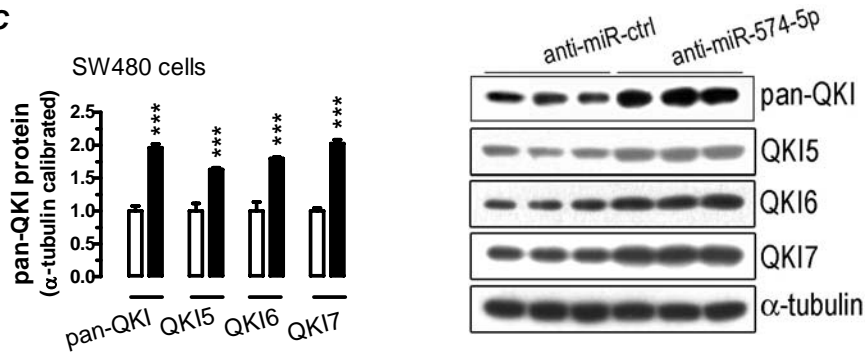


Fig. S3

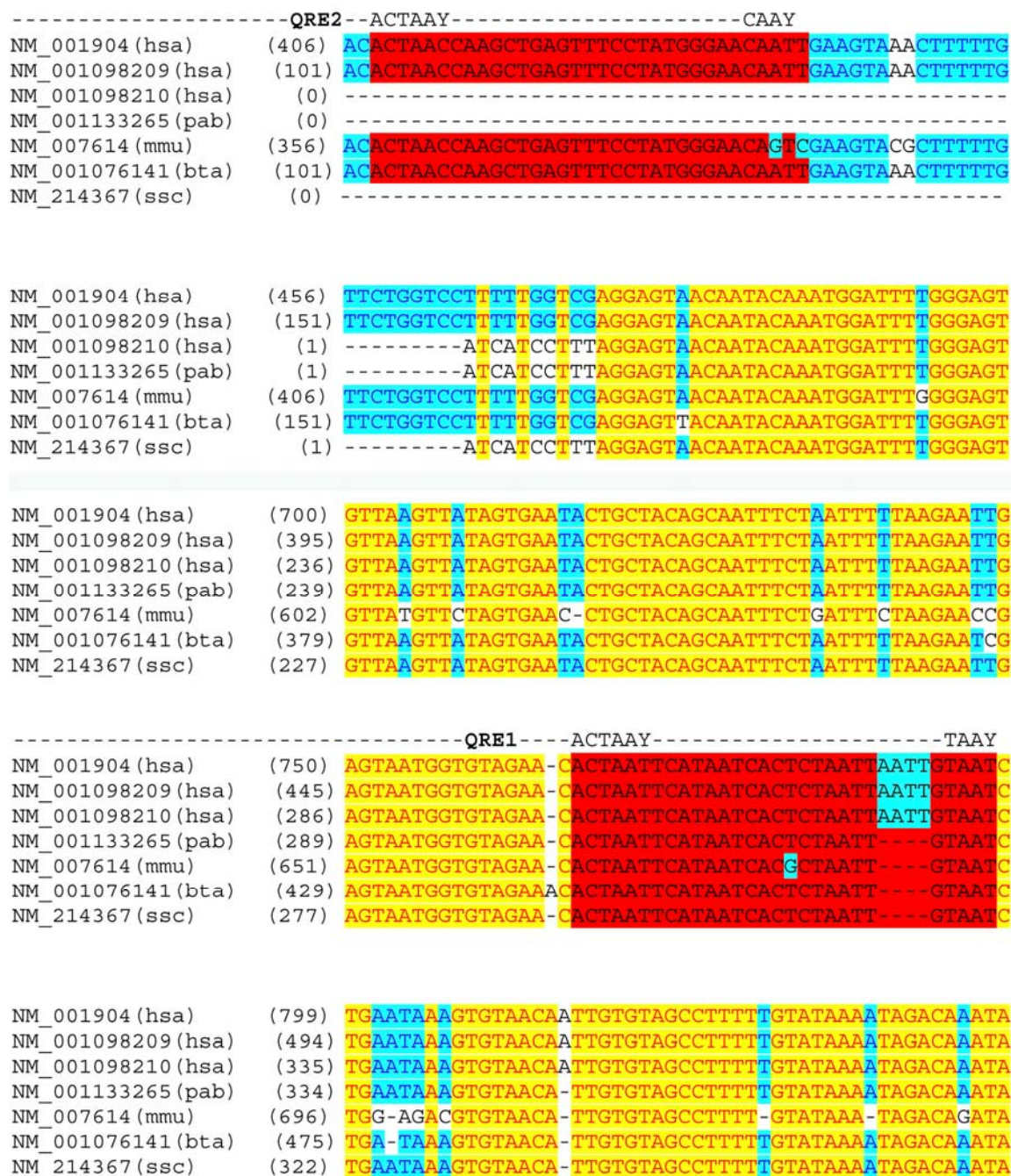
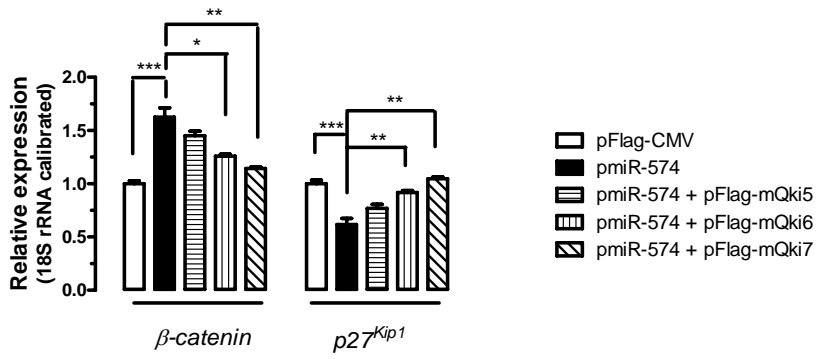
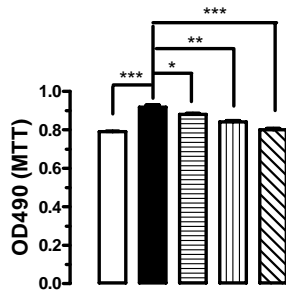


Fig. S4

A



B



C

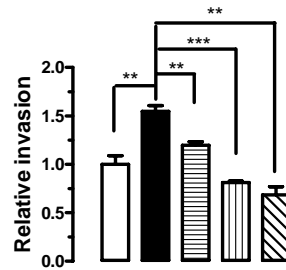


Fig. S5

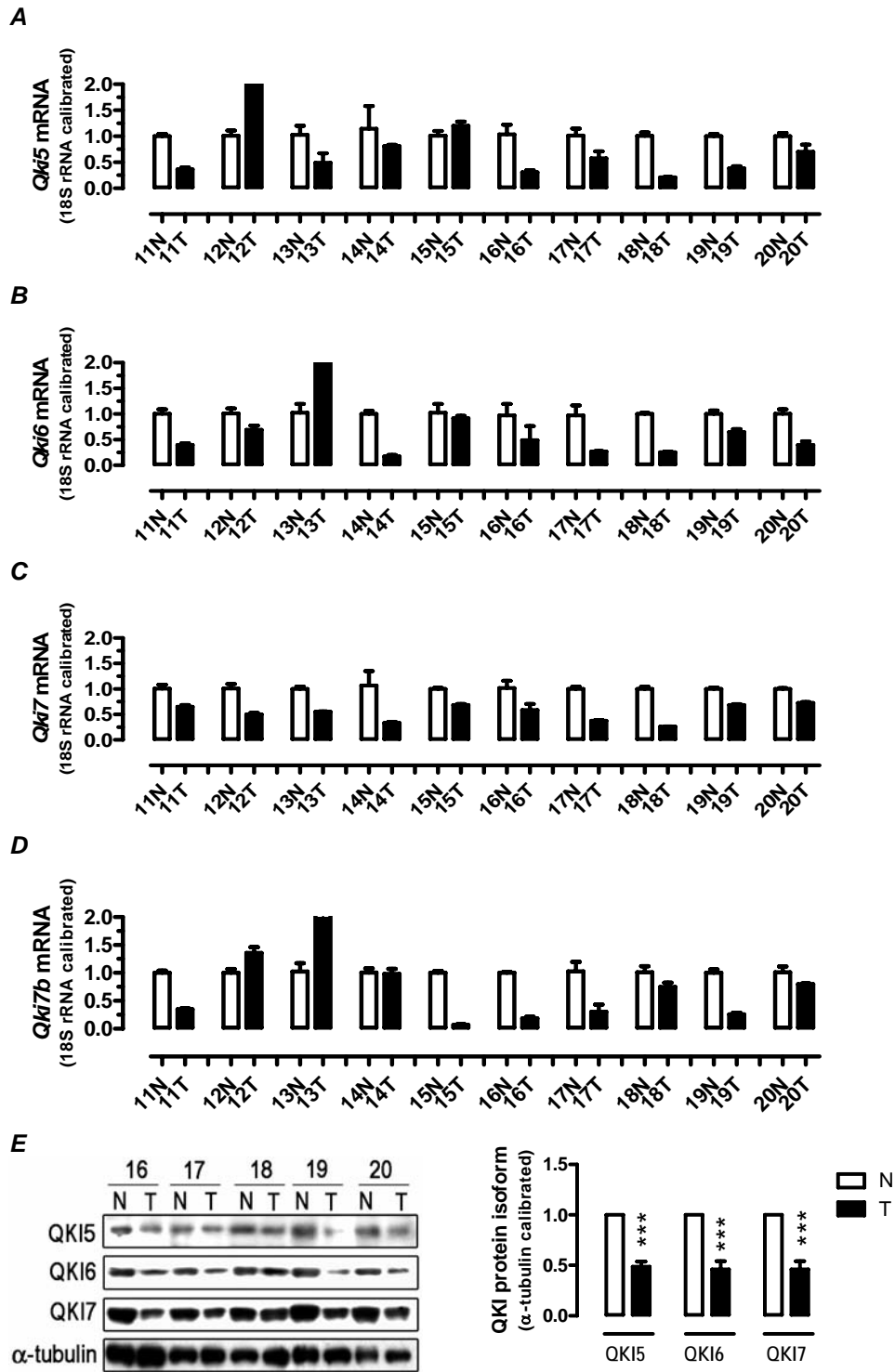


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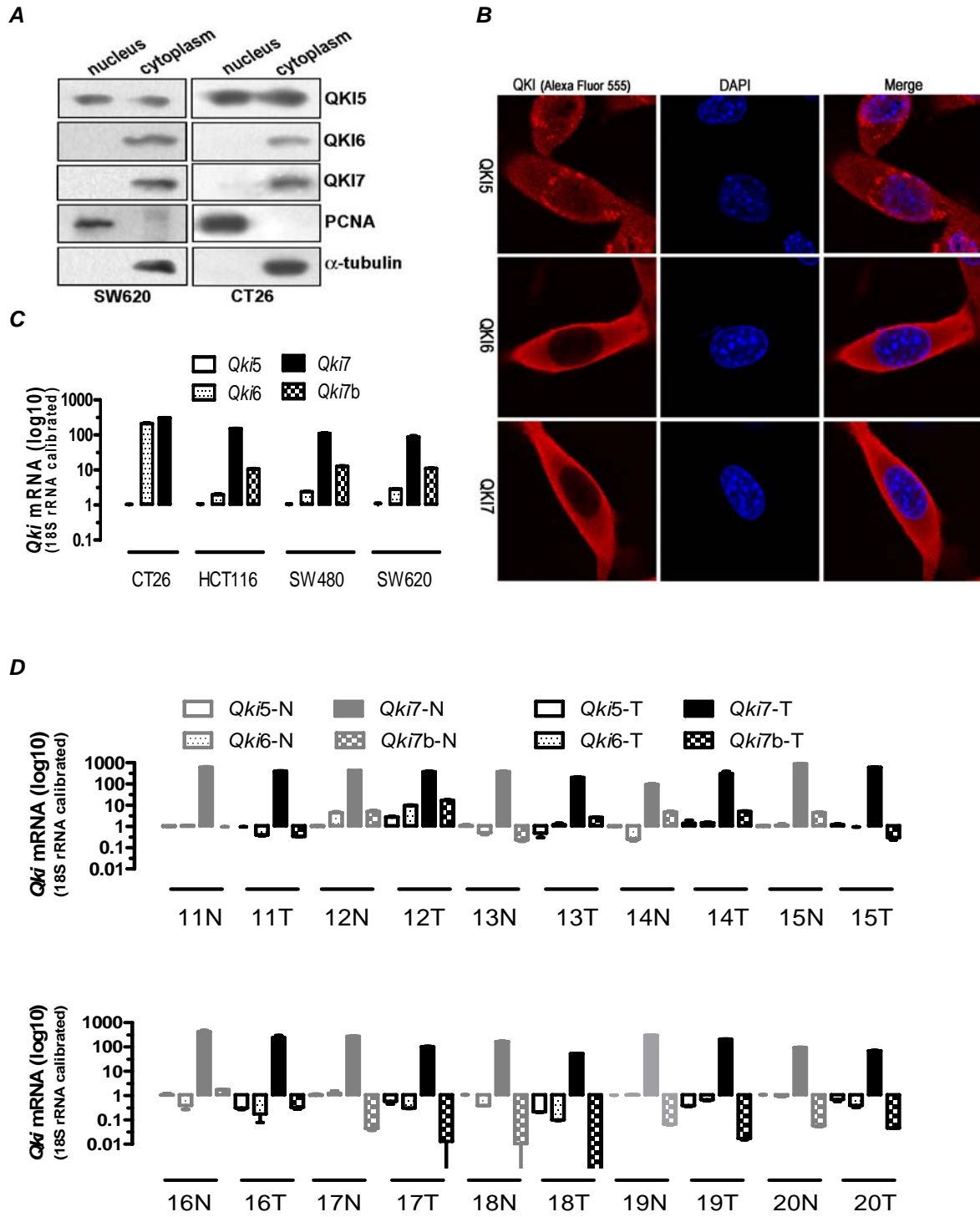


Fig. S7

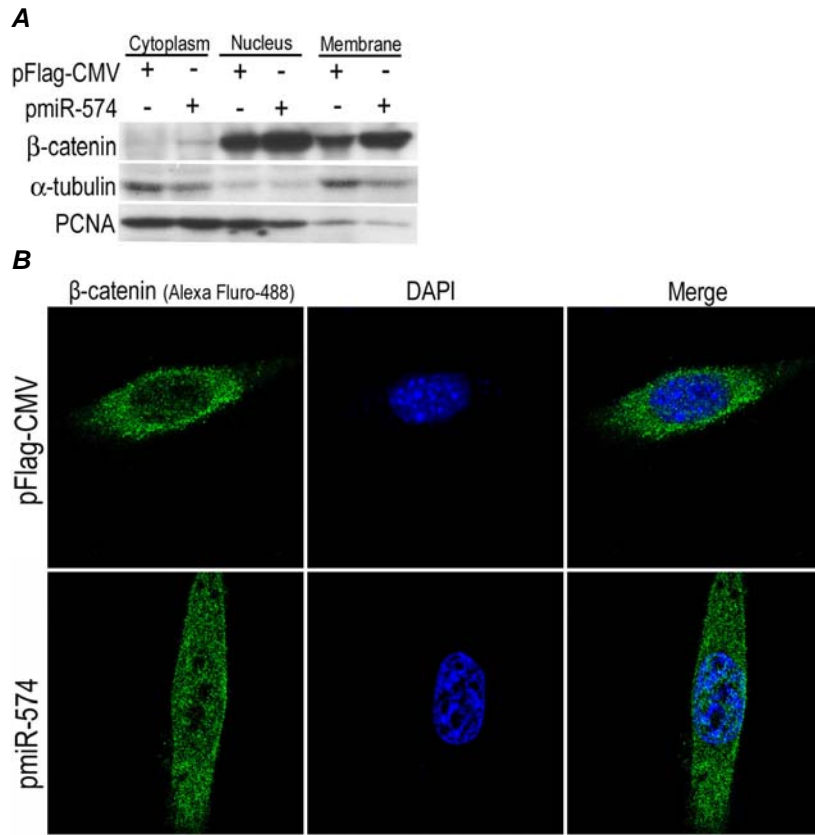


Fig. S8

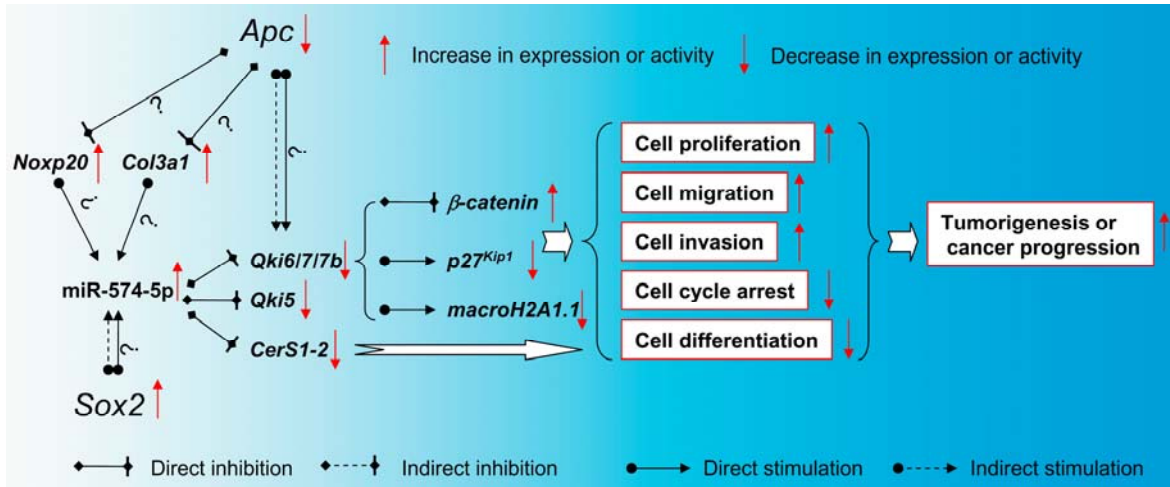


Fig. S9

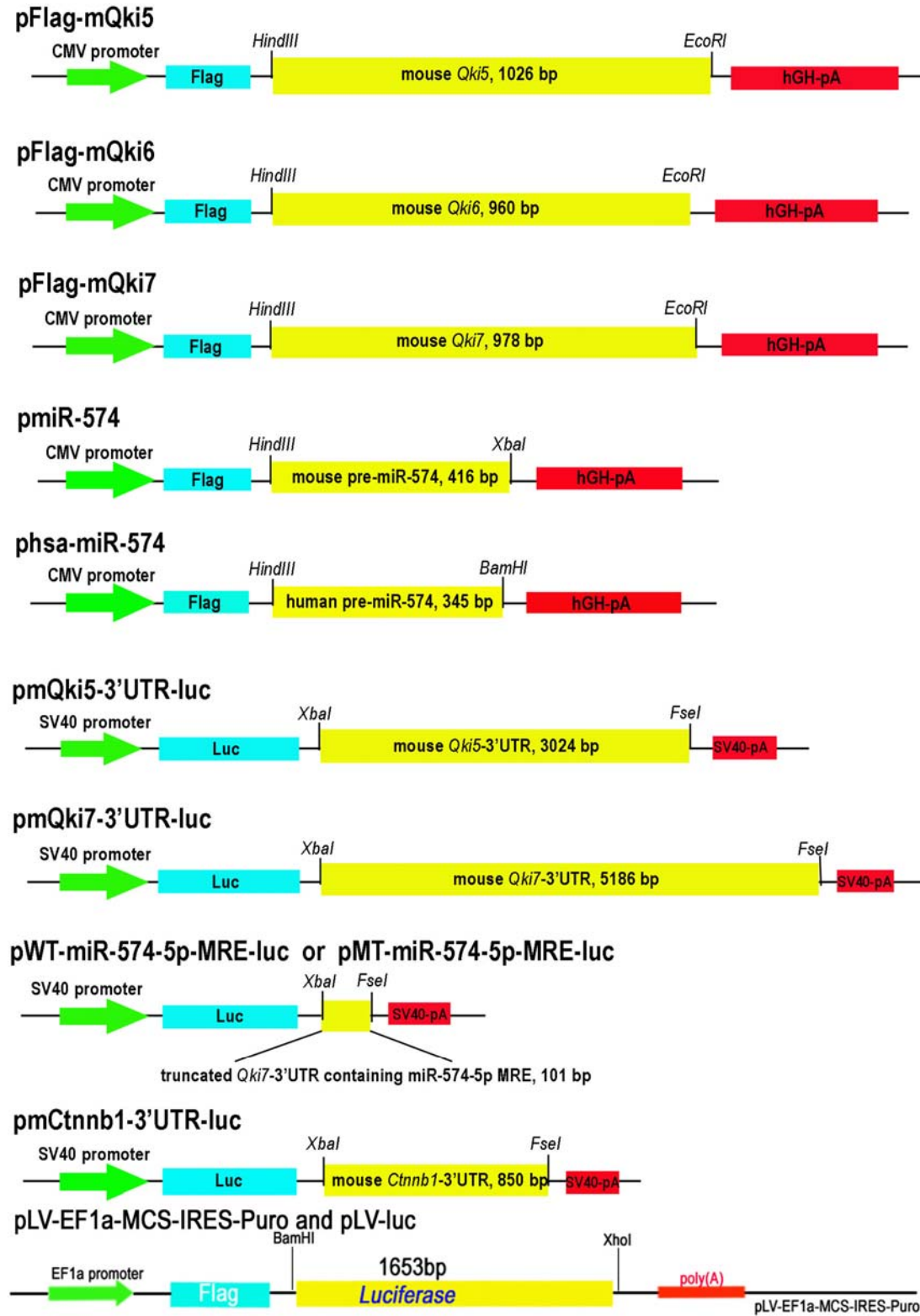


Table S1

		<i>n</i>	%	miR-574-5p (Normalized)	Pan- <i>Qki</i> mRNA (Normalized)	Pan-QKI protein (Normalized)
Gender	M	40	67.7	3.136 ± 0.5718	0.8483 ± 0.1774	0.7554 ± 0.0977
	F	20	33.3	2.793 ± 0.532	0.7278 ± 0.1335	0.7882 ± 0.0929
Age	≤60	28	46.7	3.238 ± 0.8269	0.9206 ± 0.2304	0.7509 ± 0.094
	>60	32	53.3	2.843 ± 0.3409	0.7134 ± 0.1315	0.7789 ± 0.1097
TNM stage	I	8	13.3	5.737 ± 2.546	0.9503 ± 0.4494	0.8613 ± 0.3316
	II	15	25	2.666 ± 0.5652	1.115 ± 0.412	0.8046 ± 0.1773
	III	36	60	2.593 ± 0.3291	0.6608 ± 0.082	0.7292 ± 0.0674
	IV	1	1.7	2.415	0.4825	0.74
Lymph node status	N0	23	38.4	3.734 ± 0.9734	1.058 ± 0.3051	0.8243 ± 0.1589
	N1	17	28.3	2.549 ± 0.5684	0.7131 ± 0.1462	0.7564 ± 0.0935
	N2	20	33.3	2.621 ± 0.3574	0.6075 ± 0.0828	0.7066 ± 0.0935
	N3	0				

Table S2

Organism	Condition	miR-574-5p expression	Affected cells or tissues	Fold change	References
Human	CRC	Up	Colorectal cells	Very significant	Current study
Human	Pituitary adenomas	Up	Pituitary tissue	2.89	²³
Human	Systemic lupus erythematosus	Up	T lymphocyte	> 2	²⁴
Human	Pancreatic cancer	Up	Pancreatic tissue	51	²⁵
Human	Lymphoma	Altered	Lymphocyte	?	²⁶
Human	Myocardial infarction	Up	Heart	Very significant	²⁷
Human	Non-small cell lung cancer	Up	Lung	2.17	²⁰
Human	Small cell lung cancer	Altered	Lung	?	²¹
Human	Eosophageal squamous cell carcinoma	Up	Eosophageal squamous cells	1.72	²²
Human	Alzheimer's	Up	Gray matter	?	²⁸
Human	Schizophrenia	Altered	Brain tissue	?	²⁹
Human	Mitral stenosis	Up	Right atrial appendage	1.96	³⁰
Human	Ovarian cancer	Up	Ovary tissue	1.24	³¹
Mouse	CRC	Up	Colorectal cells	3.7	Current study
Mouse	Vesicular stomatitis virus infection	Up	Macrophage	?	³²
Mouse	Asthma	Up	Lung cells	13	³³
Mouse	SARS infection	Altered	Bronchoalveolar stem cells	?	³⁴
Mouse	Liver injury	Up	Liver/plasma	3.4/1.49	³⁵

2. SUPPLEMENTAL MATERIALS AND METHODS

Plasmids. TOP/FOP-Flash plasmids and the Wnt1-overexpressing (Wnt1) plasmid were kind gifts from Prof. Qiao Wu, Xiamen University.

For *Qki* overexpression plasmids, primers were designed based on the genomic sequences from the NCBI databases, with the forward primer carrying a *HindIII* site and the reverse primer carrying an *EcoRI* respectively. Mouse *Qki5*, *Qki6*, *Qki7* were individually PCR-amplified using mouse brain tissue cDNA and appropriate primers (see List of primers used for miRNA or *Qki* overexpression plasmids), with the LA Taq DNA Polymerase (TaKaRa, Dalian). The resultant DNA fragments were subcloned into pFlag-CMV (Sigma Aldrich, St. Louis, MO, USA) using the *HindIII* and *EcoRI* sites.

For mouse miRNA overexpressing plasmids, primers were designed based on the genomic sequences from the miRBase (microna.sanger.ac.uk), with the forward primers carrying a *HindIII* site and the reverse primers carrying a *XbaI* site respectively. Pre-miRNA gene fragments were individually PCR-amplified using genomic DNA prepared from mouse inner medullary collecting duct epithelial mIMCD3 cells (mmu-miR-574) or human CRC SW480 cells (hsa-miR-574) and appropriate primers (see List of primers used for miRNA or *Qki* overexpression plasmids), with Pyrobest DNA Polymerase (TaKaRa). The resultant DNA fragments were subcloned into pFlag-CMV, using the *HindIII* and *XbaI* (mmu-miR-574) or *HindIII* and *BamHI* (hsa-miR-574) sites. The insertion sequences of the resultant plasmids were confirmed by sequencing.

For the construction of mouse *Qki5*-3'UTR, wildtype *Qki7*-3'UTR miR-574-5p MRE, mutant *Qki7*-3'UTR miR-574-5p MRE and *Ctnnb1* luciferase reporters, mouse 3'UTR regions were amplified from mouse brain tissue cDNA through PCR amplification with the LA Taq DNA Polymerase (TaKaRa) and appropriate primers (see List of primers used for the construction of five luciferase reporters). The resultant PCR fragments carrying a *NheI* site and a *FseI* site was subcloned into the pGL3-control vector (Promega), using the *XbaI* and *FseI* sites immediately downstream of the stop codon of the luciferase cDNA, generating pmQki5-3'UTR-luc, pmQki7-3'UTR-luc, pWT-miR574-5p-MRE-luc, pMT-miR574-5p-MRE-luc and pmCtnnb1-3'UTR-luc respectively.

For the construction of lentiviral plasmid carrying a luciferase gene, the luciferase reporter gene on plasmid pGL3 (Promega, USA) was PCR amplified and a *BamHI-XhoI* insert gene cassette was inserted

into a lentiviral vector pLV-EF1a-MCS-IRES-Puro (A gift from Prof. Jiahuai Han) to obtain a plasmid pLV-luc.

The resulting twelve plasmids are shown in Fig. S9 in the previous section.

Cell culture and transient transfections. Mouse CT26, human SW480 and SW620 CRC cells were obtained from ATCC (Manassas, VA, USA), human HCT116 CRC cells was obtained from the Chinese Academy of Sciences (Shanghai, China). CT26 cells and HCT116 cells were normally cultured in RPMI 1640 with 10% fetal bovine serum (FBS). SW480 and SW620 cells were cultured in DMEM supplemented with 10% FBS. Plasmids, miRNA mimics and inhibitor transfections were performed with Lipofectamine 2000 reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocols.

Western blot, immunohistochemistry, immunofluorescence and in situ hybridization analyses.

Western blots were performed according to standard protocols. The detection was achieved using the Immobilon Western Chemiluminescent HRP Substrate kit (Millipore, Billerica, MA, USA). Antibodies and the dilutions are anti- α -tubulin (sc-5286, Santa Cruz, 1:10000); anti- β -actin (sc-47778, Santa Cruz, 1:20000); anti-pan-QKI (sc-103851, Santa Cruz, 1:2000); anti-QKI5 (AB9904, Millipore, 1:2000); anti-QKI6 (AB9906, , Millipore, 1:2000); anti-QKI7 (AB9908, Millipore, 1:2000); anti-p27^{Kip1} (sc-528, Santa Cruz, 1:2000); anti- β -catenin (sc-7199, Santa Cruz, 1:2000); anti-PCNA (sc-7907, Santa Cruz, 1:2000); Alexa Fluor 555-labeled goat Anti-Rabbit IgG (H+L) (A0452, Beyotime Institute of Biotechnology, 1:500) respectively.

Immunohistochemistry was performed with an instant-type SABC immunohistochemistry kit purchased from the Boster Bioengineering Company (Wuhan, China) as instructed. Briefly, 5 μ m fixed tissue sections were microwave-heated for 10 min for antigen retrieval. Slides were washed and incubated with primary antibody for 1 hour followed by incubation with instant-type secondary antibody for 30 minutes at room temperature. After being thoroughly washed with PBS (pH 7.5), the tissues were incubated with SABC (Strept-Avidin-Biotin Complex) for 20 minutes at room temperature. After washing thoroughly, 100-200 μ l freshly-made diaminobenzidine (DAB) solution was added to each slide. After incubation for ~10 minutes, sections were lightly counter-stained with hematoxylin.

For immunofluorescence analyses, cells seeded on cover glass overnight were fixed in 4% paraformaldehyde. Fixed cells were incubated with anti-QKI5, QKI6 or QKI7 primary antibody

respectively, followed by incubation with Alexa Fluor® 555-conjugated secondary antibody or Alexa Fluor® 488-conjugated secondary antibody (Beyotime Institute of Biotechnology, Jiangsu, China). Cell nuclei were stained by DAPI (Beyotime Institute of Biotechnology). Stained cells were visualized and photographed with a Leica-TCS-SP2-SE confocal microscope.

In situ hybridization of miRNAs was performed as described³⁶. miRCURY™ LNA miRNA probes (hsa-miR-574-5p, 5'-ACACACTCA-CACACACACTCA-3'; hsa-miR-200b, 5'-TCATCATTACCAGGCAGTATTA-3'; U6, 5'-CACGAATTTGCGTGTCATCCTT-3'; Scramble-miR, 5'-GTGTAACACGTCTATACGCCCA-3') were purchased from Exiqon (Copenhagen, Denmark).

qPCR analyses of mRNAs and miRNAs. For qPCR mRNA quantification, reverse transcription was performed with TRIzol (Invitrogen)-extracted total RNAs using a ReverTra Ace-α-® Kit as instructed (TOYOBO, Shanghai). qPCR was performed by using the SYBR Green qPCR Master Mix (TOYOBO) and the StepOne Plus qPCR system (Applied Biosystems Inc., Foster City, CA) according to the manufacturer's protocols and with the primer pairs listed below.

For miRNAs, qPCR was performed with the stem-loop primers as described previously³⁷ using the miRNA-specific reverse transcription primers, the universal primer and the miRNA-specific reverse locked nucleic acid (LNA)-primers as described below.

Cell cycle, proliferation, migration, invasion and colony formation assays. For cell cycle analysis, 5×10^5 cells were synchronized by serum starvation for 24 hours and induced to re-enter the cell cycle by replacing old media with a fresh media containing 10% fetal bovine serum (FBS) for 24 hours. Cells were harvested and fixed in 75% ethanol at 4 °C overnight. Cells were incubated with RNase A at 37 °C for 30 min, and then stained with propidium iodide. The cell cycle phases were measured by flow cytometry.

Cell proliferation was analyzed by the MTT assay. A total of 4×10^3 cells was seeded in 96-well plates and MTT was added to each well every 24 hours. The plates were incubated for 4 hours before the addition of 10% SDS (in 0.01M HCl) (Sigma Aldrich, St. Louis, MO, USA). The absorbance was measured at 490 nm using a microplate reader.

Cell migration was analyzed by the wound-healing assays. SW480 cells were seeded onto 6-well plates. After transfection, a wound was incised in the center of the confluent culture, followed by careful washing to remove detached cells and the addition of fresh medium. Phase contrast images of the wounded

area were recorded using an inverted microscope at indicated time points.

Matrigel invasion assays were performed using Millicell inserts coated with matrigel (BD Biosciences, Sparks, MD, USA). 5×10^4 CT26 cells were seeded per upper chambers in serum-free RPMI1640 whereas the lower chambers were loaded with RPMI1640 containing 5% FBS. After 48 hours, the non-migrating cells on the upper chambers were removed by a cotton swab, and cells invaded through the matrigel layer to the underside of the membrane were stained with a 0.1% crystal violet solution and counted manually in eight random microscopic fields.

For colony formation assays, control and miRNA inhibitor or LV-miR-shRNA (Supplemental Materials and Methods section, Supplementary Information) transfected SW480 cells were seeded on six-well plates and maintained in DMEM containing 10% FBS for 2 weeks. Cells were fixed with methanol and stained with 0.5% crystal violet in 50% methanol for 1 hour and colonies larger than 100 μm in diameter were counted.

Luciferase reporter assays and IAP enzyme activity assays. Luciferase reporter activities were determined using a Luciferase Reporter Gene Assay System (Promega, Madison, WI, USA) as instructed. For all luciferase assays, β -galactosidase activities were determined to calibrate the transfection efficiency. The calibrated value for a proper control was used to normalize all other values to obtain the normalized relative luciferase units (RLU) representing the activities of 3'UTRs.

The enzyme activity of IAP was measured by an IAP kit (Jiancheng, Nanjing, China) according to the manufacturer's instructions. Protein concentration was measured by the Pierce BCA kit (Pierce Biotechnology, Rockford, USA).

Bioluminescence imaging of tumor growth in live animals. Briefly, CT26 cells were transfected with a luciferase-overexpressing plasmid or its control vector (pLV-luc or pLV-EF1a-MCS-IRES-Puro). Stable clones of cells overexpressing luciferase were selected with the addition of 8-10 $\mu\text{g}/\text{ml}$ puromycin in the media for 3 weeks (Invitrogen, Carlsbad, CA, USA). Luciferase-overexpressing cells or its controls were subsequently injected into the nude mice intraperitoneally (5×10^5 cells/mouse). Starting from the third day after CT26 cell inoculation, each mouse was injected with 2×10^7 transducing units of control lentiviruses or lentiviruses carrying shRNA for miR-574-5p once a week for 2 weeks. Three days after lentiviral injection, the mice were subjected to bioluminescence imaging under an IVIS Lunina II *in vivo* imaging system

(Xenogen, Hopkinton, MA, USA) as described by Lan et al.³⁸ every other three days. Ten minutes prior to imaging, each mouse was injected with 100 μ l D-luciferin solution (15 mg/ml in PBS, Promega #E1602) by i.p. injection. Immediately before imaging taking, the experimental mice were anesthetized using an XGI-8 Gas Anesthesia System (Xenogen). Images and amount of bioluminescent signals were analyzed using Living Image software (Xenogen).

Serum miRNA preparations and analyses. Total RNA from serum was extracted using a miRVana miRNA isolation kit (Ambion, Austin, TX, USA) according to the manufacturer's protocol. RNA was eluted in nuclease free water at 95°C. Five microliters of total RNA was reverse transcribed using the ReverTra Ace- α -[®] Kit as instructed (TOYOBO, Shanghai) and miRNA-specific stem-loop primers as shown in the Materials and Methods section. has-miR-16-5p served as an internal control. Visible and dissectable peritoneal tumors were dissected and weighted.

Bioinformatics, data acquisition, image processing and statistical analyses. Mature and pre-miRNA sequences were based on miRBase (microrna.sanger.ac.uk). miRNA target predictions were performed with the miRanda (www.microrna.org) algorithm. Western blot images were captured by Biosense SC8108 Gel Documentation System with GeneScope V1.73 software (Shanghai BioTech, Shanghai, China). Gel images were imported into Photoshop for orientation and cropping. The digital density values were acquired by Image-Pro Plus software (Media Cybernetics) and analyzed by Graphpad Prism 5.0. Data are the means \pm SEM. One-way ANOVA with Bonferonni's post-test was used for multiple comparisons and the Student's *t*-test (two-tailed) for pair-wise comparisons. The correlation analyses were performed with Pearson's test.

List of primers used for the construction of miRNA or Qki overexpression plasmids

Plasmid	Primer sequence (from 5'→3')	Gene ID	Amplicon (bp)
pFlag-mQki5 (mouse)	Forward:CCCAAGCTTATGGTCGGGGAAAT GGAAAC Reverse:CCGGAATTCCTAGTTGCCGGTGGC GGCTC	NM_001159517 (Genbank)	1026
pFlag-mQki6 (mouse)	Forward:CCCAAGCTTATGGTCGGGGAAAT GGAAAC Reverse:CCGGAATTCCTAGCCTTTCGTTGG GAAAG	NM_001159516 (Genbank)	960
pFlag-mQki7 (mouse)	Forward:CCCAAGCTTATGGTCGGGGAAAT GGAAAC Reverse:CCGGAATTCCTCAATGGGCTGAAAT ATCAG	NM_021881 (Genbank)	978
pmiR-574 (mouse)	Forward: CCCAAGCTTTGTCCGCTGTAGGGTGTGAG AA Reverse: TGCTCTAGAATCAGGATGGAGGTCAAGGC CT	MI0005518 (miRBase)	416
phsa-miR-574 (human)	Forward: CCCAAGCTTCCTCTGCGTTAGTGAGAAGC AG Reverse: GCGGATCCTCTGTCTTACAGGGACCTGC TC	MI0003581 (miRBase)	345

List of primers used for the construction of six luciferase reporters.

Plasmid	Primer sequence (from 5'→3')	Gene ID	Amplicon length and location
pmQki5-3'UTR-luc	Forward: TGCGCTAGCTATGACCTTCTGACCTCTGAACTCT Reverse: ACTGGCCGGCCTATGGGTTAATAGAAACAGCAAAGA	<i>mQki5</i> NM_001159517	(NTs 1517-4540) 3024 bp
pmQki7-3'UTR-luc	Forward: TGCGCTAGCCTTGCTGGATGAAGGACTAGA Reverse: ACTGGCCGGCCTTGGCCTCATGATACAAAGCAATAC	<i>mQki7</i> NM_021881	(NTs 1467-6652) 5186 bp
pWT-miR574-5p-MRE-luc	Forward: TGCTCTAGACTTTGTTAAGTAATCCACACTC Reverse: ACTGGCCGGCCAACGGTTGTCCCATAGTCTTAA	<i>mQki7</i> NM_021881	(NTs 5650-5750) 101bp
pMT-miR574-5p-MRE-luc	Forward: TGCTCTAGACTTTGTTAAGTAATCCGCACGC Reverse: ACTGGCCGGCCAACGGTTGTCCCATAGTCTTAA	<i>mQki7</i> NM_021881	(NTs 5650-5750) 101 bp
pmCnnb1-3'UTR-luc	Forward: TGATCTAGAAAGACTTGGTAGGGTGGGAATGG Reverse: ACTGGCCGGCCGCAGGTTACAACAACCTTTGGGAT	Mouse β -catenin NM_001904	(NTs 2707-3557) 850 bp
pLV-luc	Forward: AGAGAATTCGGATCCATGGAAGACGCCAAAAACATAA Reverse: CCATGGCTCGAGCCCTTACACGGCGATCTTTCCG	pGL3 (Promega)	1365 bp

List of miR-mimics, anti-miRs, lentiviral miR-shRNAs and LNA-probes for miRNA in situ hybridization.

Name	Sequence or target (5'→3')	Catalog #	Supplier
mimics-ctrl	Scrambled		GenePharma, Shanghai
miR-574-5p mimics	UGAGUGUGUGUGUGUGAGUGUGU ACACUCACACACACACUCAUU		GenePharma, Shanghai
anti-miR-ctrl	Scrambled		GenePharma, Shanghai
anti-miR-574-5p	UGAGUGUGUGUGUGUGAGUGUGU	AM17000	Ambion
LV-miR-shRNA- ctrl	TTCTCCGAACGTGTCACGT	pLVT4	Sunbio, Shanghai
LV-miR-574-5p- shRNA	ACACACTCACACACACACTCA	pLVT278	Sunbio, Shanghai
Scrambled miRNA	GTGTAACACGTCTATACGCCCA/3Dig	99004-05	Exiqon, Copenhagen
U6 probe	CACGAATTTGCGTGTCATCCTT/3Dig	99002-05	Exiqon, Copenhagen
hsa-miR-574-5p probe	ACACACTCACACACACACTCA//3Dig	38674-05	Exiqon, Copenhagen

List of primers used for qPCR analyses of mRNAs.

Organism	Gene	Gene ID	Primer sequence (5'→3')	Amplicon (bp)
Human	pan- <i>Qki</i>	NM_006775		
		NM_206853	Forward:CATCAGCTGCATCTTCTTCAG	121
		NM_206854	Reverse:CACTGTGGAAGATGCTCAGAA	
		NM_206855		
	<i>Qki5</i>	NM_006775	Forward:GCCCTACCATAATGCCTTTGA Reverse:AACTTTAGTAGCCACCGCAACC	
	<i>Qki6</i>	NM_206853	Forward:GCCCCAAGCTGGTTTAATCTATA Reverse:TCGTTGGGAAAGCCATACCTAAT	118
	<i>Qki7</i>	NM_206854	Forward:GCTGGTTTAATCTATACACCCTATG A	113
			Reverse:GACTGGCATTCAATCCACTCTA	
	<i>Qki7b</i>	NM_206855	Forward:AATGCCTTTGATCAGACAAATACA G Reverse:TGGGGAGAAGAAGCTTACCTAATAC A	198
	<i>β-catenin</i>	NM_001904	Forward:AGCCACAAGATTACAAGAAACGG Reverse:ATCCACCAGAGTGAAAAGAACGA	173
<i>p27^{Kip1}</i>	NM_004064	Forward:GGGGCTCCGGCTAACTCTGA Reverse:AGGCTTCTTGGGCGTCTGCT	215	
18S rRNA	NR_003286.2	Forward:CGACGACCCATTCGAACGTCT Reverse:CTCTCCGGAATCGAACCTGA	102	
Mouse	pan- <i>Qki</i>	NM_001159517	Forward:TAGAGGACTTACAGCTAAACAACCT	288
		NM_001159516	Reverse:ATTCAGAATTGCAAGCTCCATCA	
		NM_021881		
	<i>Qki5</i>	NM_001159517	Forward:GCCCTACCATAATGCCTTTGA Reverse:AACTTTAGTAGCCACCGCAACC	211
	<i>Qki6</i>	NM_001159516	Forward:GCCTGAAGCTGGGTTAATCTACA Reverse:TCGTTGGGAAAGCCATACCTAAC	118
	<i>Qki7</i>	NM_021881	Forward:GCTGGGTTAATCTACACACCCTAT GA	113
Reverse:GACTGGCATTCAATCCACTCTA				
<i>β-catenin</i>	NM_007614	Forward:TGGACCCCAAGCCTTAGTAAACA Reverse:GTCTGTGATGAAGCCCCAGTG	159	

<i>Lactase</i>	NM_001081078	Forward:GAGACCCAGAACTCAATGACACC Reverse:GGTCAGAGCGGTTCACAAAGT	165
<i>p27^{Kip1}</i>	NM_009875	Forward:GCGGTGCCTTTAATTGGGTCT Reverse:TCTTGGGCGTCTGCTCCACA	225
<i>Col3a1</i>	NM_009930	Forward:GTTTCTTCTCACCTTCTTCATCCC Reverse:GCAGTCTAGTGGCTCCTCATCACA G	196
<i>Noxp20</i>	NM_026667	Forward:AGGGAGACACCGGATCTGAAATA Reverse:GAATTGGCAGTGTGGATTCGTAG	199
<i>Sox2</i>	NM_011443	Forward: GCGGAGTGGAACTTTTGTCC Reverse: GGGAAGCGTGTACTTATCCTTCT	156

List of conventional or LNA-primers for qPCR analyses of U6 and miRNAs.

RNA or miRNA	Genbank or miRBase seq#	Primer sequence (5'→3')
Mouse and human U6	NR_004394.1	Reverse transcription: CGCTTCACGAATTTGCGTGTCAT Forward: GCTTCGGCAGCACATATACTAAAAT Reverse: CGCTTCACGAATTTGCGTGTCAT (LNA)
hsa-miR-16-5p	MI0000070	Reverse transcription: GTCGTATCCAGTGC GTGTCGTGGAGTCGGCAATTGCA CTGGATACGACTCGCCAA Forward: GGGTAGCAGCACGTAAA Reverse: TCGTGTCGTGGAGTC (LNA)
mmu-miR-574-5p	MI0005518	Reverse transcription: GTCGTATCCAGTGC GTGTCGTGGAGTCGGCAATTGC ACTGGATACGACTACACAC Forward: GGGTGAGTGTGTGTGTG Reverse: TCGTGTCGTGGAGTC (LNA)
hsa-miR-574-5p	MI0003581	Reverse transcription: GTCGTATCCAGTGC GTGTCGTGGAGTCGGCAATTGC ACTGGATACGACTACACAC Forward: GGGTGAGTGTGTGTGTG Reverse: TCGTGTCGTGGAGTC (LNA)
mmu-miR-200b	MI0000243	See Huang et al. ³⁷
mmu-miR-717	MI0004704	See Huang et al. ³⁷
mmu-miR-466g	MI0005510	Reverse transcription: GTCGTATCCAGTGC GTGTCGTGGAGTCGGCAATTGC ACTGGATACGACTGTGTGT Forward: GGGATACAGACACATGC Reverse: TCGTGTCGTGGAGTC (LNA)
mmu-miR-17	MI0000687	Reverse transcription: GTCGTATCCAGTGC GTGTCGTGGAGTCGGCAATTGC ACTGGATACGACTCTACCT Forward: GGGCAAAGTGCTTACAG Reverse: TCGTGTCGTGGAGTC (LNA)
mmu-miR-20a	MI0000568	Reverse transcription: GTCGTATCCAGTGC GTGTCGTGGAGTCGGCAATTGC ACTGGATACGACTCTACCT

Forward: GGGGTAAAGTGCTTATAG

Reverse: TGC GTGTCGTGGAGTC (LNA)

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