Materials and methods

Cell migration and invasion analysis

The cells were maintained in a serum-containing growth medium for 48–72 h and then maintained in a serum-free medium for 24 h. The cells (1×105 cells/100 µL) were added to 8-µm pore transwell and matrigel chamber plates (Corning Star, Cambridge, Massachusetts). The bottom chamber was prepared using 10% fetal bovine serum as the chemoattractant. Cells were maintained at 37 ℃ and allowed to migrate through the porous membrane or the matrigel for 48 and 72 h, respectively. Fixation/staining solution (0.1% crystal violet, 1% formalin, and 20% ethanol) were used to fix and stain those cells that stuck to the lower surface of the membrane. The cells were counted under a microscope in five randomly selected fields with scale bar of 200 µm. At least five chambers from experiments in triplicate were analyzed.

Cell adhesion assay

Twenty-four-well plates coated with Fibronectin (FN, BD Biosciences, USA) were incubated for 1 h at 37 ℃. After blocking with 1% BSA, 3×10^4 cells were seeded in each well and incubated at 37 ℃ for 30 min. Then cells were fixed with 4% paraformaldehyde and stained with 0.1% crystal violet, and randomly select 5 visual fields in microscope and count the cells.

Cell motility assay

Confluent monolayers of cells were cultured in a serum-containing medium for 48–72 h and followed by 24-hour culture in serum-free medium. The monolayers were scratched by 1,000-µL plastic pipette tip followed by 48-hour culture in a serum-free medium and wounded cells photographed under an inverted phase-contrast microscope. Three different points were marked on the plate, and the distance between each point and the edge of the scratch wound was measured before and after cell migration. The mean migration distance (µm) was calculated by subtracting the length after 48 h from that at 0 h. The result was expressed as a migration index, i.e., the distance migrated by the treated cells compared with the distance migrated by the control cells. Experiments were carried out in triplicate and repeated at least five times.

Immunofluorescence assay

Cells were cultured on coverslips for 12 h, and then 4% paraformaldehyde was used to fix cells for 30 min, followed by 15-min treatment of 0.5% Triton X-100. The reaction was blocked with 10% normal blocking serum at room temperature for 15 min, the slides were incubated with rabbit antibodies to phosphorylated-LIMK2 and phosphorylated-cofilin (1:150) (Cell Signaling Technology; Danvers, Massachusetts) and rabbit antibodies to paxillin (1:100) (BD Transduction Laboratories, USA) at 4 ℃ overnight. This was followed by thrice PBS rinsing. The coverslips were then incubated with a fluorescein isothiocyanate (FITC)-conjugated anti-rabbit or mouse stain and Texas Red-conjugated anti-mouse or –rabbit antibodies (1:200) (SantaCruz Biotech) for 30 min at room temperature, then stained with 6-diamidino-2-phenylindole (DAPI, Invitrogen).

Co-immunoprecipitation assay

Cells were harvested and washed three times with chilled PBS, then lysed in IP Lysis Buffer (Pierce, USA) with a protease and phosphatase inhibitor cocktail (Sigma, USA). The protein was quantified by using a BCA Protein Assay Kit (KeyGen, Biotechnology, China), then diluted into 1 mg/mL with chilled wash buffer (with protease and phosphatase inhibitor), and divided into two equal parts, to which were added mouse anti-MYO1B or mouse anti-RhoA antibody (3–4 µg) (Santa Cruz, USA) and the same amount of rabbit IgG (Beyotime, China), respectively, rotated slowly and incubated overnight at 4 ℃. Protein A agarose beads were washed twice with cold wash buffer and added to the two parts of protein based on 20 µL for every 1 mL of total protein, then incubated for 4–6 h at 4 ℃ with low-speed rotation. Afterward, the protein mixture was washed three times with 500 µL chilled wash buffer (containing protease and phosphatase inhibitor). The protein mixtures were diluted with wash buffer. After heat denaturation in 10% SDS-PAGE sample loading buffer, the protein samples were subjected to western blot assay.

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https://dx.doi.org/10.21037/atm-21-4702

Animals

Mice were 6-week-old and purchased from Experimental Animal Center of Southern Medical University (GuangZhou, China). The body weight, feeding behavior, and motor activity of animals were monitored as indicators of general health. Mice were kept under specific pathogen-free conditions in the Experimental Animal Center of Southern Medical University. Experiments were performed under a project license granted by Ethics Committee of Zhujiang Hospital, Southern Medical University, in compliance with the Experimental Animal Center of Southern Medical University for the care and use of animals.

Table S1 RT-PCR primer sequences for human genes

Gene	Forward primer	Reverse primer	Product length
MYO1B	GCTGGAGACCATGGCCAAAA	TCCTCCTAGTGGATCGCCTT	559 bp
GAPDH	GGAGCGAGATCCCTCCAAAAT	GGCTGTTGTCATACTTCTCATGG	197 bp

Table S2 Correlation of demographic characteristics and MYO1B transcription among 50 CRC patients

Characteristics	Total (n)	MYO1B		P value[1]
		Low [n (%)]	High [n (%)]	
Sex				0.007
Female	19	9 (47.4)	10 (52.6)	
Male	31	4 (12.9)	27 (87.1)	
Age at diagnosis (year)				0.050
≤50	9	0 (0.0)	9 (100.0)	
>50	41	13 (31.7)	28 (68.3)	
Tumor location				0.509
Right colon	27	6 (22.2)	21 (77.8)	
Left colon	23	7 (30.4)	16 (69.6)	
T classification				0.007
Tis + T0	0	0 (0.0)	0 (0.0)	
T1 + T2	19	9 (47.4)	10 (52.6)	
T3 + T4	31	4 (12.9)	27 (87.1)	
N classification				0.004
N0	18	9 (50.0)	9 (50.0)	
N1 + N2	32	4 (12.5)	17 (87.5)	
M classification				0.148
M0	39	12 (30.8)	27 (69.2)	
M1	11	1 (9.1)	10 (90.9)	
Stage				0.026
S1 + S2	18	8 (44.4)	10 (55.6)	
S3 + S4	32	5 (15.6)	27 (84.4)	

[1], Pearson χ^2 test of independence between covariables and MYO1B transcription.

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Table S3 Correlation of demographic characteristics and MYO1B expression among 101 CRC patients

Characteristics	Total (n)	MYO1B		P value[1]
		Low [n (%)]	High [n (%)]	
Sex				0.386
Female	41	11 (26.8)	30 (73.2)	
Male	60	21 (35.0)	39 (65.0)	
Age at diagnosis (year)				0.453
≤50	27	7 (25.9)	20 (74.1)	
>50	74	25 (33.8)	49 (66.2)	
Stage				0.053
S1 + S2	31	14 (45.2)	17 (54.8)	
S3 + S4	70	18 (25.7)	52 (74.3)	
T classification				0.739
Tis + T0	0	0 (0.0)	0 (0.0)	
T1 + T2	11	3 (27.3)	8 (72.7)	
T3 + T4	90	29 (32.2)	61 (67.8)	
N classification				0.029
N0	41	18 (43.9)	23 (56.1)	
N1 + N2	60	14 (23.3)	46 (76.7)	
M classification				0.006
M0	82	31 (37.8)	51 (62.2)	
M1	19	1 (5.3)	18 (94.3)	

[1], Pearson χ^2 test of independence between covariables and MYO1B expression

Table S4 Univariate cox regression analysis of factors associated with overall survival in colorectal cancer patients

	Exp(B)	95% CI for Exp(B)		P value
		Lower	Upper	
Sex	1.464	0.689	3.109	0.322
Age	1.009	0.979	1.040	0.556
T	2.072	0.493	8.704	0.320
N	1.663	0.799	3.457	0.174
M	1.862	0.793	4.373	0.153
Chemotherapy	0.775	0.382	1.575	0.482
MYO1B	1.205	1.054	1.377	0.006

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Table S5 Multivariate cox regression analysis of factors associated with overall survival in colorectal cancer patients

	Exp(B)	95% CI for Exp(B)		P value
		Lower	Upper	
Sex	1.449	0.651	3.228	0.364
Age	1.014	0.983	1.046	0.385
T	1.250	0.659	2.368	0.495
N	1.372	0.619	3.041	0.436
M	0.983	0.381	2.532	0.971
Chemotherapy	0.884	0.415	1.883	0.749
MYO1B	1.218	1.054	1.407	0.008

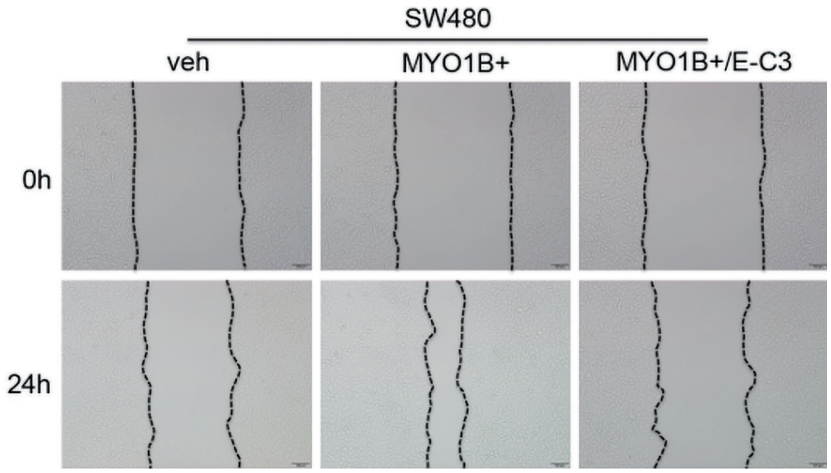

Figure S1 Representative figures for would healing assay.

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