Supplementary

Table S1 Sequences of primers and siRNAs

Names	Sequences (5'-3')
Primers for RT-qPCR	
FEM1C-qF	GACAAAGCCCGAGTGACC
FEM1C-qR	CGTCCTACTGCTTTCCAACA
GAPDH-qF	GAAGGTGAAGGTCGGAGTC
GAPDH-qR	GAAGATGGTGATGGGATTTC
FEM1A-qF	GCTACACATAGCAGCCCAGA
FEM1A-qR	TCTTCTTGAAGGCATTGGTG
FEM1B-qF	AAAGGTGGTACGCTTGCTCT
FEM1B-qR	TCAATGACATACCCGTCGAA
Cullin2-qF	ACGACAATAAAAGCCGTGGTC
Cullin2-qR	GGATAGGCCACACATAAAGCAT
Plasmid construction	
shFEM1C#1f	CCGGAACAACACGACTTTAACCAATCTCGAG ATTGGTTAAAGTCGTGTTGTTTTTTTG
shFEM1C#1r	AATTCAAAAAAACAACACGACTTTAACCAATCT CGAGATTGGTTAAAGTCGTGTTGTT
siRNA	
siGFP	GCAGAAGAACGGCAUCAAG dTdT
siFEM1C#1	CAACACGACUUUAACCAAUdTdT
siFEM1C#2	GGAGCUACAUUUGUAGACAdTdT
siFAM1A	AACGCCUCCAGAUCUUCCGdTdT
siFAM1B	GCCUAAUGAUUGCGGCAUAdTdT
sicullin#1	GAACTGCTTGCTAAGTACTGTdTdT
sicullin#2	GAGCTAGCATTGGATATGTGGdTdT

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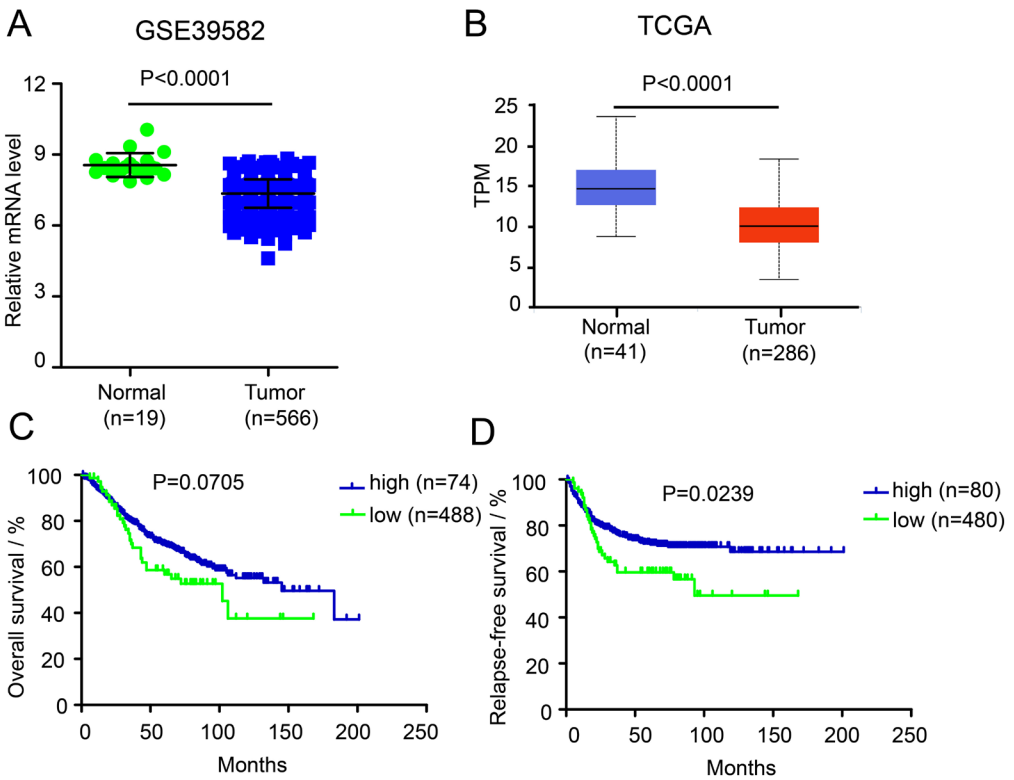

Figure S1 FEM1C is down-regulated in CRC tissue, and its expression level is correlated with prognosis in CRC patients. (A,B) The GEO (A) and TCGA (B) data showed FEM1C mRNA levels in CRC tissues and normal tissues. The TCGA data were downloaded from UALCAN (http://ualcan.path.uab.edu/index.html) (C,D). The relationship between FME1C expression level and survival times (C) or relapse-free survival times (D) in COAD patients was analyzed by the Kaplan-Meier method with a Log-rank test. The data was from GEO (GSE39582). CRC, colorectal cancer. TPM, transcript per million.

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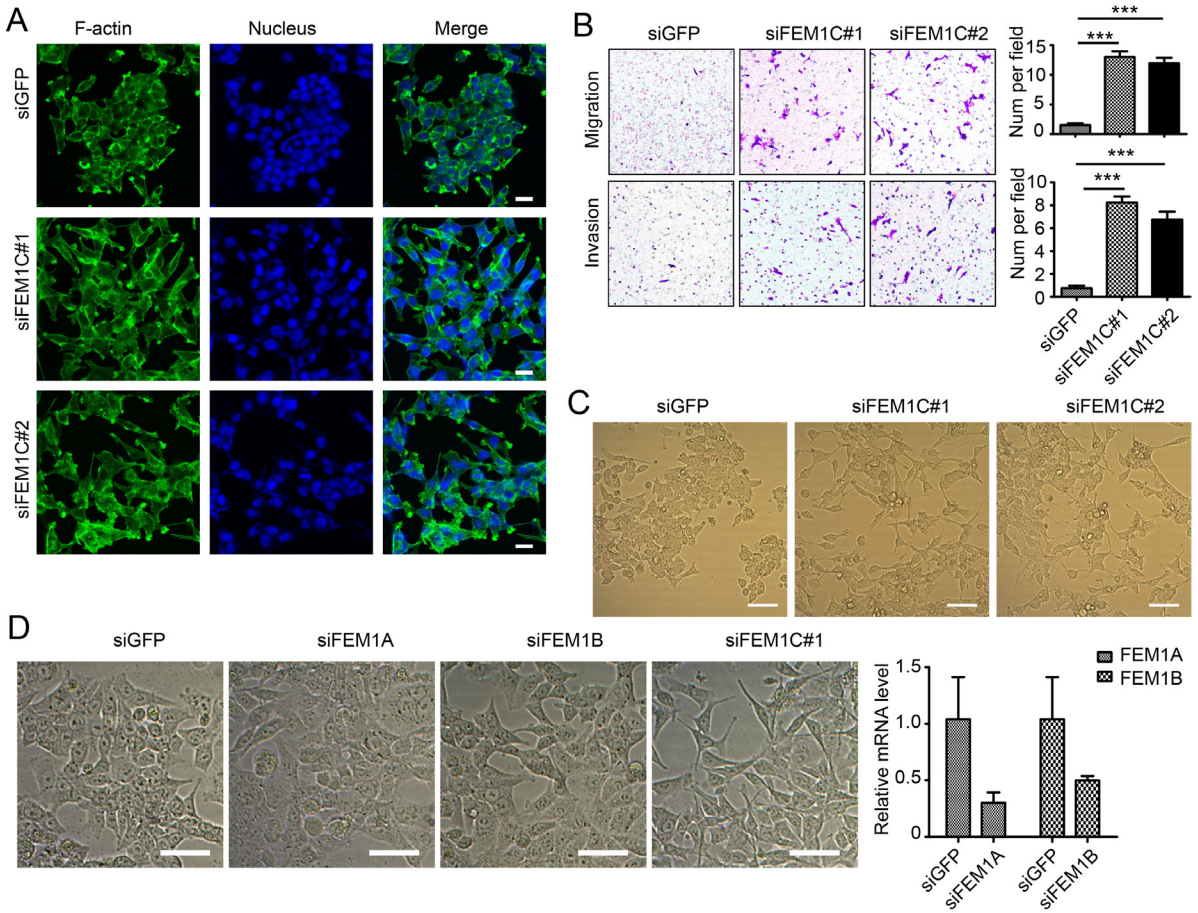

Figure S2 Knockdown of FEM1C resulted in the change of morphology and increased mobility in CRC cells. (A) HCT116 cells were transfected with the indicated siRNAs, and 48 hrs later, F-actin was stained with phalloidin-FITC. Scale bar =25 μm. (B) LoVo cells were transfected with indicated siRNAs, and 48 hrs later, the cells were harvested to perform the transwell assay (magnification times, 100×; ***, P<0.001). (C) LoVo cells were transfected with indicated siRNAs, and 48 hrs later, the cells were observed on microscopy and photographed. Scale bar =50 μm. (D) HCT116 cells were transfected with the indicated siRNAs, and 48 hrs later, the cells were observed in microscopy and photographed (left). The right panel showed the knockdown efficiency of siRNAs. Scale bar =50 μm.

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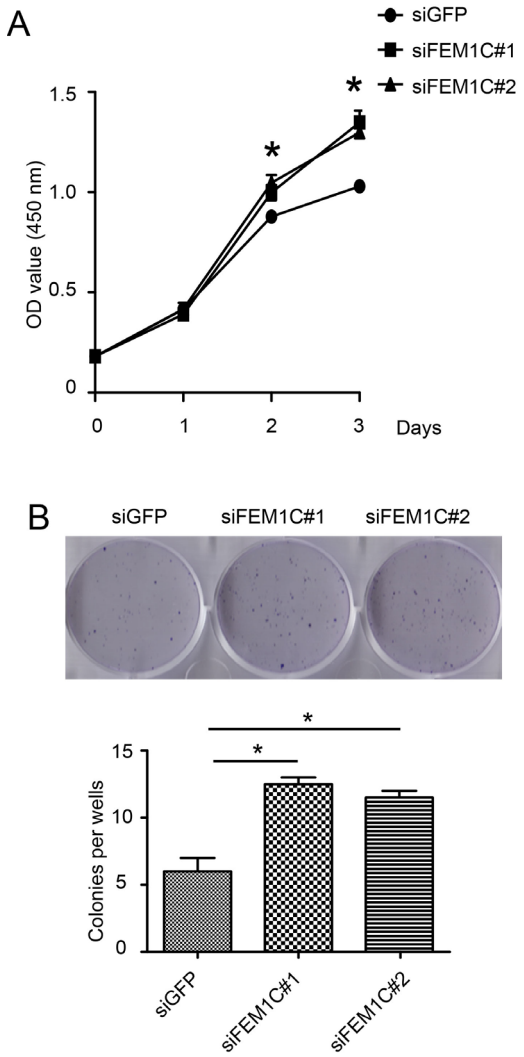

Figure S3 FEM1C knockdown promoted cell proliferation. LoVo cells were transfected with indicated siRNAs, and cell proliferation was determined by the CCK-8 assay (A) and the colony formation assay (B). *, P<0.05. For colony formation assay, five hundred cells were seeded in 6-well culture plates and cultured for 10 days. Cells were fixed with 4% paraformaldehyde for 10 min, followed by staining with 1% crystal violet for 15 min. The plates were photographed, and the colonies were counted.

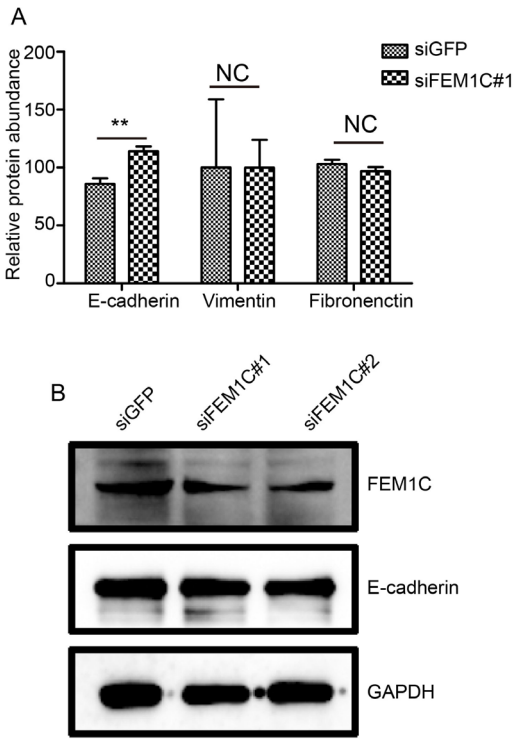

Figure S4 Knockdown of FEM1C did not change the protein level of conventional EMT markers, including vimentin, fibronectin and E-cadherin. (A) Change in the protein level of EMT markers (E-cadherin, vimentin, and fibronectin) after FEM1C knockdown in HCT116 cells. Data were from TMT proteomics analysis between FEM1C knockdown groups and negative control groups (**, P<0.01). (B) HCT116 cells were transfected with indicated siRNAs, and 48 hrs later, Western Blotting determined the level of the E-cadherin protein. EMT, epithelial-mesenchymal transition.

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