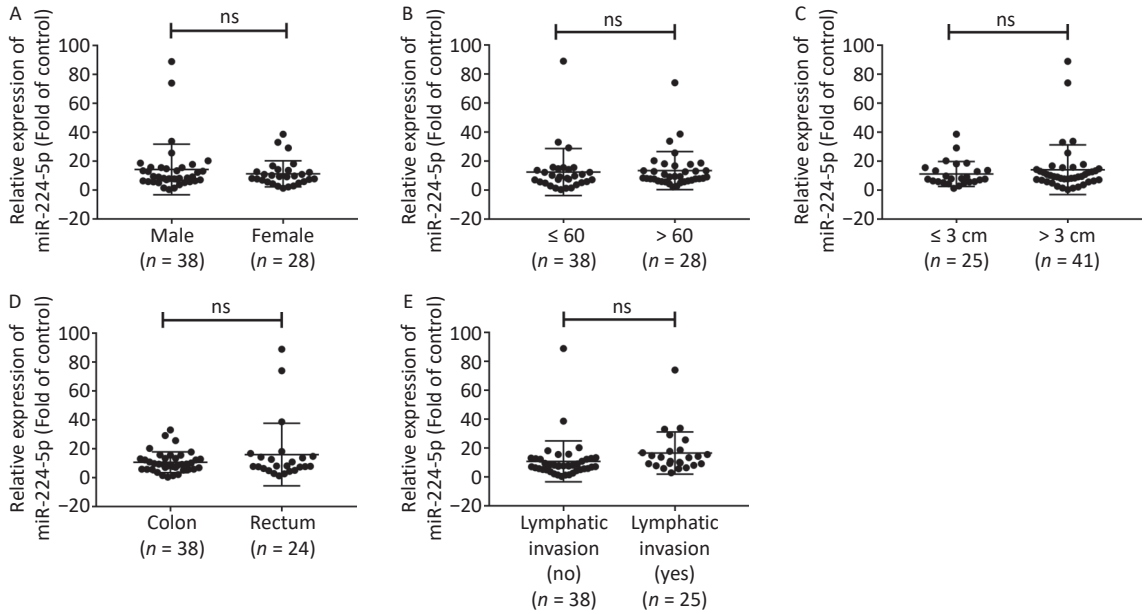
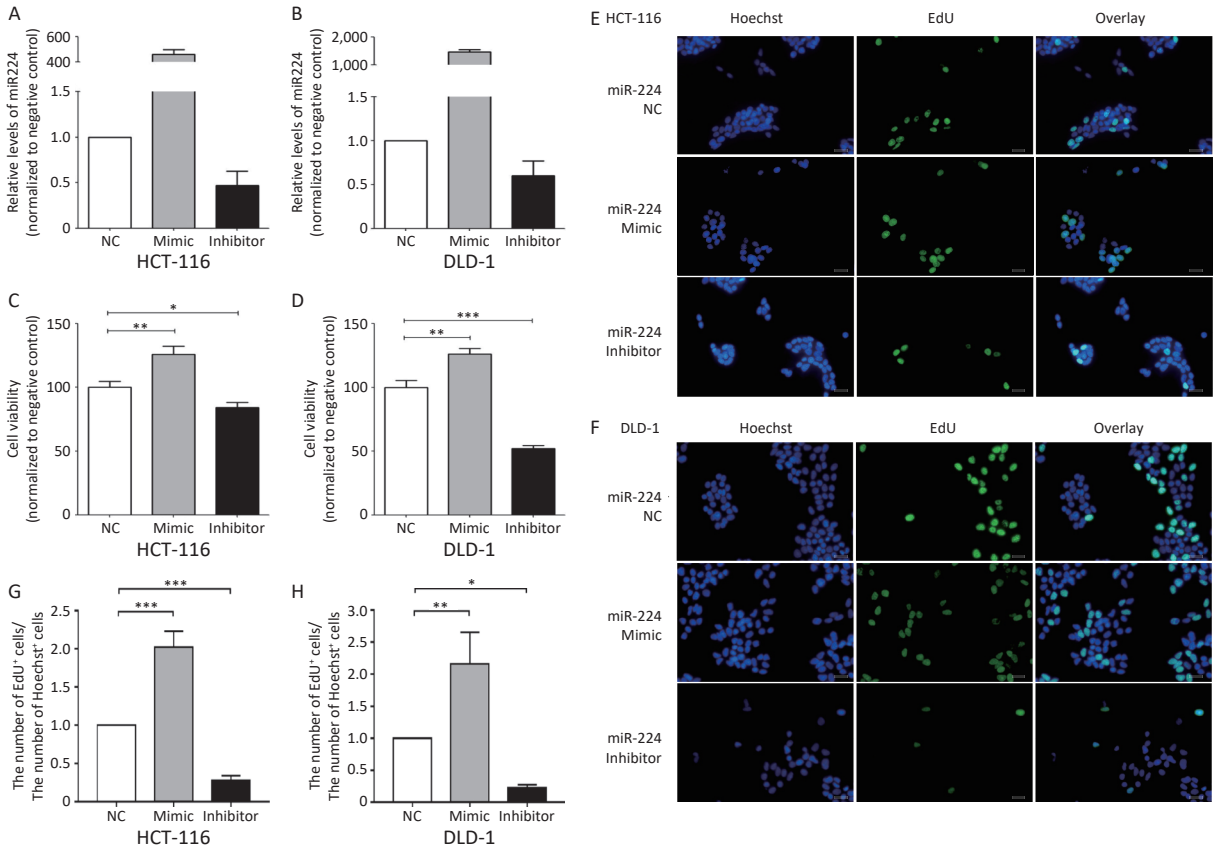
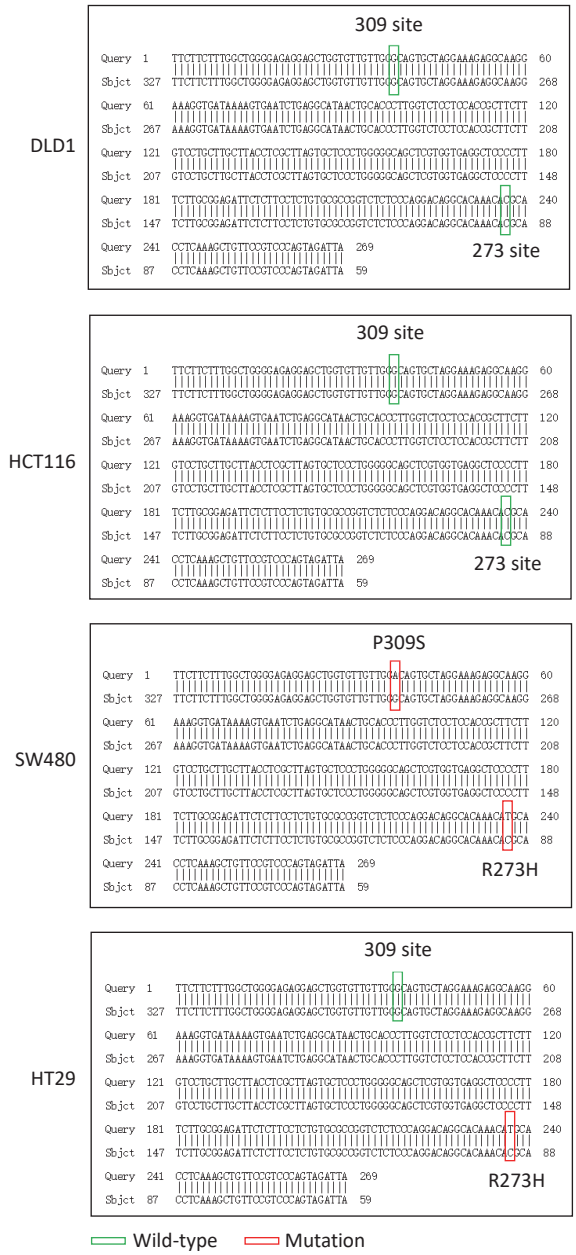


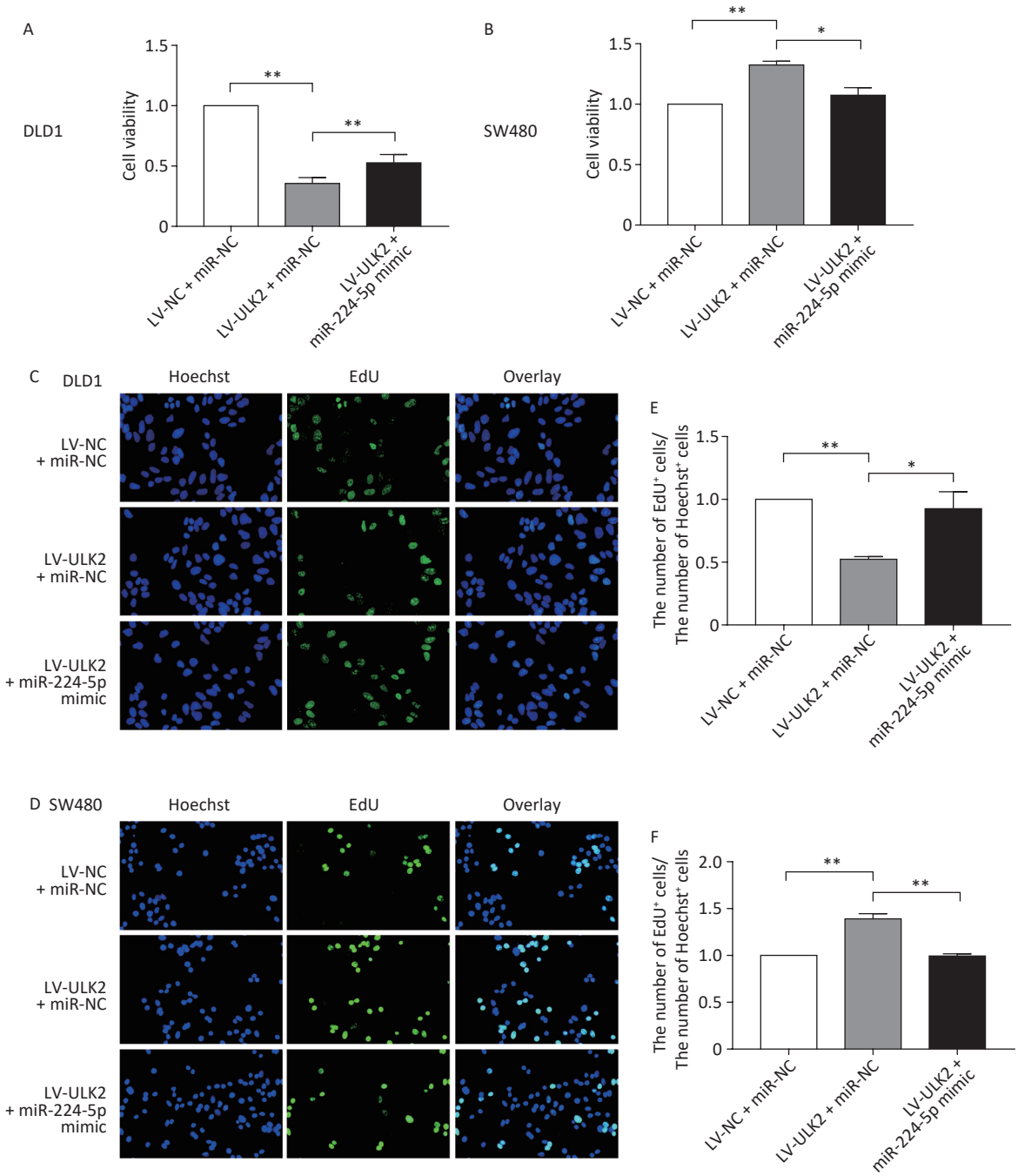
Supplementary Figure S1. A–E microarray analysis of miR-224-5p expression related to 66 carcinomas based on sex (A), age (y, B), size (C), position (D) and lymphatic invasion (E). Data are shown as the ratio of signal of miR-224-5p of colorectal cancer tissues normalized with the signal value of miR-224-5p of normal tissues. ns means no significance (Student's *t*-test).



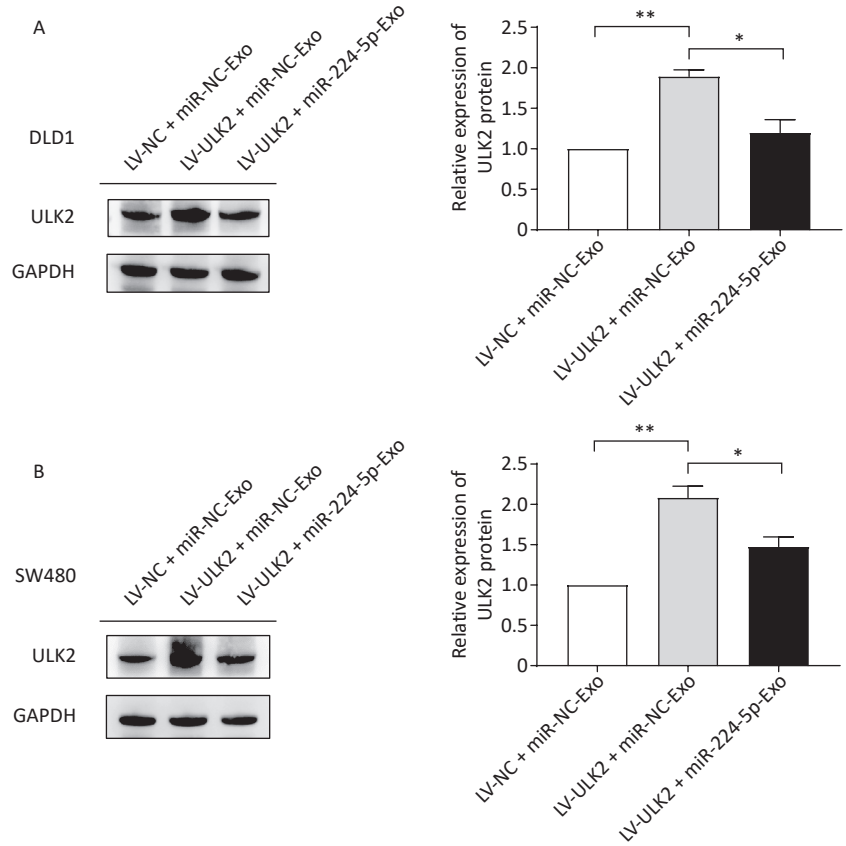
Supplementary Figure S2. miR-224-5p promotes CRC cell proliferation. A and B Expression of miR-224-5p in HCT116 (A) and DLD1 (B) cells 48h after transfection with miR-224-5p mimic/inhibitor and miR-NC by qRT-PCR. Data are shown as mean ± SD (n = 3). C and D CCK-8 assays were conducted to detect cell viability 48 h after transfection with miR-224-5p mimic/inhibitor and miR-NC in HCT116 (C) and DLD1 (D) cells. Data are shown as mean ± SD (n = 3). $^{*}P < 0.05$, $^{**}P < 0.01$ & $^{***}P < 0.001$ *vs*. NC (Student's *t*-test). E and F Representative images of Hoechest^{+} cells, EdU^{+} cells, and overlay 48 h after transfection with miR-224-5p mimic/inhibitor and miR-NC in HCT116 (E) and DLD1 (F) cells, using 40× objective lens. G and H The ratio of the number of EdU^{+} cells to the number of Hoechst^{+} cells 48 h after transfection with miR-224-5p mimic/inhibitor and miR-NC in HCT116 (G) and DLD1 (H) cells. Data are shown as mean ± SD (n = 3). $^{*}P < 0.05$, $^{**}P < 0.01$ & $^{***}P < 0.001$ *vs*. NC (Student's *t*-test).



Supplementary Figure S3. DNA sequencing validation was conducted to detect p53 gene mutation in codon 273 and 309 of CRC cells. The sequences above (Query) represent the result of sequencing and the sequences below (Sbjct) represent the p53 gene sequence in the NCBI database. R273H: a G -> A mutation in codon 273 resulting in an Arg -> His substitution; P309S: a C -> T mutation in codon 309 resulting in a Pro -> Ser substitution.



Supplementary Figure S4. Exogenous miR-224-5p partially reverses the regulation effect of ULK2 on CRC cell proliferation. A and B CCK-8 assays were conducted to detect cell viability after transfection with miR-224-5p mimic/miR-NC in ULK2-overexpressed DLD1 (A) and SW480 (B) cells. Data are shown as mean ± SD (n = 3). $^{**}P < 0.01$ *vs*. LV-NC+miR-NC; $^{*}P < 0.05$ & $^{**}P < 0.01$ *vs*. LV-ULK2 + miR-NC (Student's *t*-test). C and D Representative images of Hoechest^{+} cells, EdU^{+} cells, and overlay after transfection with miR-224-5p mimic/miR-NC in ULK2-overexpressed DLD1 (C) and SW480 (D) cells, using 20×objective lens. E and F The ratio of the number of EdU^{+} cells to the number of Hoechst^{+} cells after transfection with miR-224-5p mimic/miR-NC in ULK2-overexpressed DLD1 (E) and SW480 (F) cells. Data are shown as mean ± SD (n = 3). $^{**}P < 0.01$ *vs*. LV-NC+miR-NC; $^{*}P < 0.05$ & $^{**}P < 0.01$ *vs*. LV-ULK2+miR-NC (Student's *t*-test).



Supplementary Figure S5. Effects of exosomal miR-224-5p on ULK2 expression in CRC cells. (A) and (B) Western blot assays of ULK2 expression in LV-NC/LV-ULK2-transfected DLD1 (A) and SW480 (B) cells after being treated with miR-NC-Exo/ miR-224-5p-Exo. Data are shown as mean ± SD (n = 3). $^{**}P < 0.01$ *vs*. LV-NC + miR-NC-Exo; $^{*}P < 0.05$ *vs*. LV-ULK2+miR-NC-Exo (Student's *t*-test).

Supplementary Table S1. The clinical-pathological features of 5 cases of CRC patients for ULK2 immunohistochemistry analysis

No.	Sex	Age (y)	Histologic type	Lymphatic Invasion	TNM
1	Male	46	Adenocarcinoma	Present	T3N1bM0
2	Male	52	Adenocarcinoma	Present	T2N1aM0
3	Female	67	Adenocarcinoma	Absent	T1N0M0
4	Male	57	Adenocarcinoma	Present	T2N2aM0
5	Female	69	Adenocarcinoma	Absent	T3N0M0

Supplementary Table S2. The primer sequences used in this study

Target	Primer sequences
GAPDH (human)	Forward: 5′-CTGACTTCAACAGCGACACC-3′
	Reverse: 5′-TGCTGTAGCCAAATTCGTTGT-3′
ULK2 (human)	Forward: 5′-ACAGCAAAGGAATCATCCACAG-3′
	Reverse: 5′-TGATGCGAATACCACTGACAC-3′
p53 (human)	Forward: 5′-AATCTACTGGGACGGAACAGC-3′
	Reverse: 5′-CCAAGACTTAGTACCTGAAGGGTG-3′
U6 (human)	Forward: 5′-GGAACGATACAGAGAAGATTAGC-3′
	Reverse: 5′-TGGAACGCTTCACGAATTTGCG-3′
miR-224-5p (human)	Forward: produced by Ribobio
	Reverse: Universal reverse sequence of the kit

Supplementary Table S3. The sequences inserted in the pmirGLO to reconstruct ULK2 CDS-WT or ULK2 CDS-MUT plasmid

Names of inserts	Inserted sequences (5′→3′)
ULK2 CDS-WT	GTTTAAACCGTCCGCTGTGTGCCCCGGGGGCGCGGCCATGGAGGTGGTGGGTGACTTCGAGTACAGCAAGAGGGATCTCGTGGGACACGGGGCCTTCGTCTAGA
ULK2 CDS-MUT	GTTTAAACCGTCCGCTGTGTGCCCCGGGGGCGCGGCCATGGAGGTGGTGGGCATGCGCGAGTACAGCAAGAGGGATCTCGTGGGACACGGGGCCTTCGTCTAGA

***Note*.** Restriction sites are in underline; Wild-type or mutant seed regions are in red.

Supplementary Table S4. DNA reverse sequencing results of the p53 gene in CRC cells

CRC cell line	Sequencing results
DLD1	NNNNNNCNNNNNNGGNTTCTTCTTTGGCTGGGGAGAGGAGCTGGTGTTGTTGGGCAGTGCTAGGAAAGAGGCAAGGAAAGGTGATAAAAGTGAATCTGAGGCATAACTGCACCCTTGGTCTCCTCCACCGCTTCTTGTCCTGCTTGCTTACCTCGCTTAGTGCTCCCTGGGGGCAGCTCGTGGTGAGGCTCCCCTTTCTTGCGGAGATTCTCTTCCTCTGTGCGCCGGTCTCTCCCAGGACAGGCACAAACACGCACCTCAAAGCTGTTCCGTCCCAGTAGATTA
HCT116	NNNNNNNNNNCNTNNNNGGTTTCTTCTTTGGCTGGGGAGAGGAGCTGGTGTTGTTGGGCAGTGCTAGGAAAGAGGCAAGGAAAGGTGATAAAAGTGAATCTGAGGCATAACTGCACCCTTGGTCTCCTCCACCGCTTCTTGTCCTGCTTGCTTACCTCGCTTAGTGCTCCCTGGGGGCAGCTCGTGGTGAGGCTCCCCTTTCTTGCGGAGATTCTCTTCCTCTGTGCGCCGGTCTCTCCCAGGACAGGCACAAACACGCACCTCAAAGCTGTTCCGTCCCAGTAGATTA
SW480	NNNNNNNNTNNNNNGTTTCTTCTTTGGCTGGGGAGAGGAGCTGGTGTTGTTGGACAGTGCTAGGAAAGAGGCAAGGAAAGGTGATAAAAGTGAATCTGAGGCATAACTGCACCCTTGGTCTCCTCCACCGCTTCTTGTCCTGCTTGCTTACCTCGCTTAGTGCTCCCTGGGGCAGCTCGTGGTGAGGCTCCCCTTTCTTGCGGAGATTCTCTTCCTCTGTGCGCCGGTCTCTCCCAGGACAGGCACAAACATGCACCTCAAAGCTGTTCCGTCCCAGTAGATTA
HT29	NNNNNNNNNTNNGNNGNTTCTTCTTTGGCTGGGGAGAGGAGCTGGTGTTGTTGGGCAGTGCTAGGAAAGAGGCAAGGAAAGGTGATAAAAGTGAATCTGAGGCATAACTGCACCCTTGGTCTCCTCCACCGCTTCTTGTCCTGCTTGCTTACCTCGCTTAGTGCTCCCTGGGGGCAGCTCGTGGTGAGGCTCCCCTTTCTTGCGGAGATTCTCTTCCTCTGTGCGCCGGTCTCTCCCAGGACAGGCACAAACATGCACCTCAAAGCTGTTCCGTCCCAGTAGATTA