

## MiR-650 represses high-risk non-metastatic colorectal cancer progression via inhibition of AKT2/GSK3 $\beta$ /E-cadherin pathway

### Supplementary Materials

**Supplementary Table 1: Candidate miRNA biomarkers in CRC tissues among patients with different prognosis by miRNA microarray**

microRNA	P. Value	Fold Change (Poor/Good)
hsa-miR-650	0.050	0.3
hsa-miR-146a	0.027	0.4
hsa-miR-365	0.030	0.5
hsa-miR-125a-5p	0.029	2.6
hsa-let-7e	0.033	2.7

**Supplementary Table 2: The potential targets of miR-650**

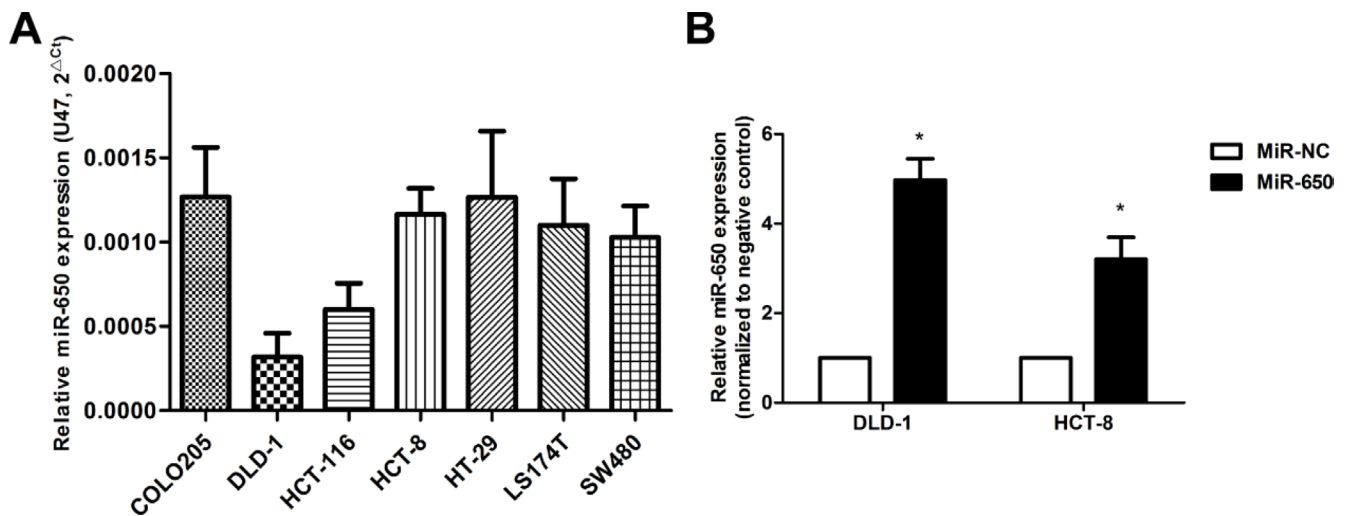
Position 174-181 of AKT2 3' UTR	5'...GGCCCCUGCAGCCCCUGCCUCCA...
hsa-miR-650	3' CAGGACUCUCGCGACGGAGGA 
Position 182-188 of AKT2 3' UTR	5'...CAGCCCCUGCCUCCAGCCUCCAG...
hsa-miR-650	3' CAGGACUCUCGCGACGGAGGA 
Position 358-364 of AKT2 3' UTR	5'...UGUCGUGCUGGUGUCUGCCUCCG...
hsa-miR-650	3' CAGGACUCUCGCGACGGAGGA 
Position 732-738 of AKT2 3' UTR	5' ... GGGGCCAAACCUGCCUGCCUCCC...
hsa-miR-650	3' CAGGACUCUCGCGACGGAGGA 

**Supplementary Table 3: Expression of miR-650 in colorectal epithelial cells on microarray**

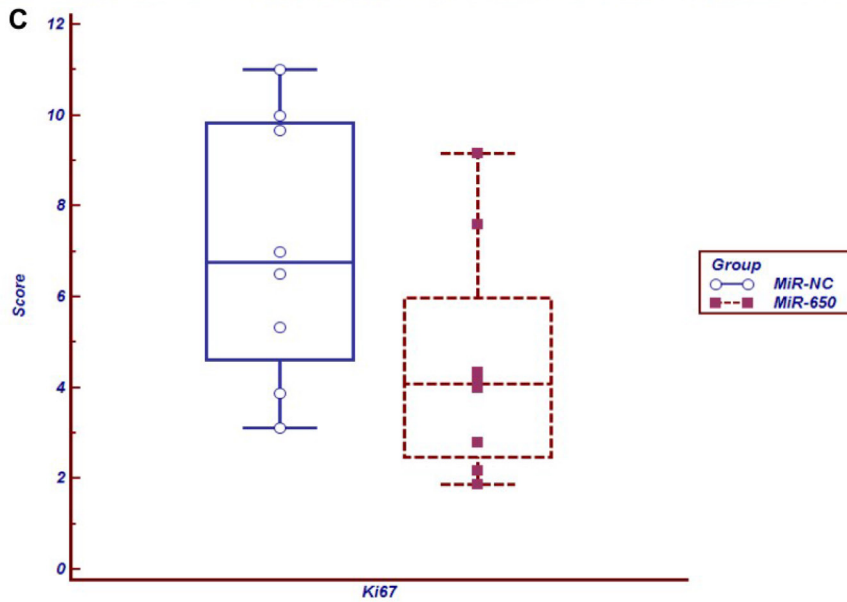
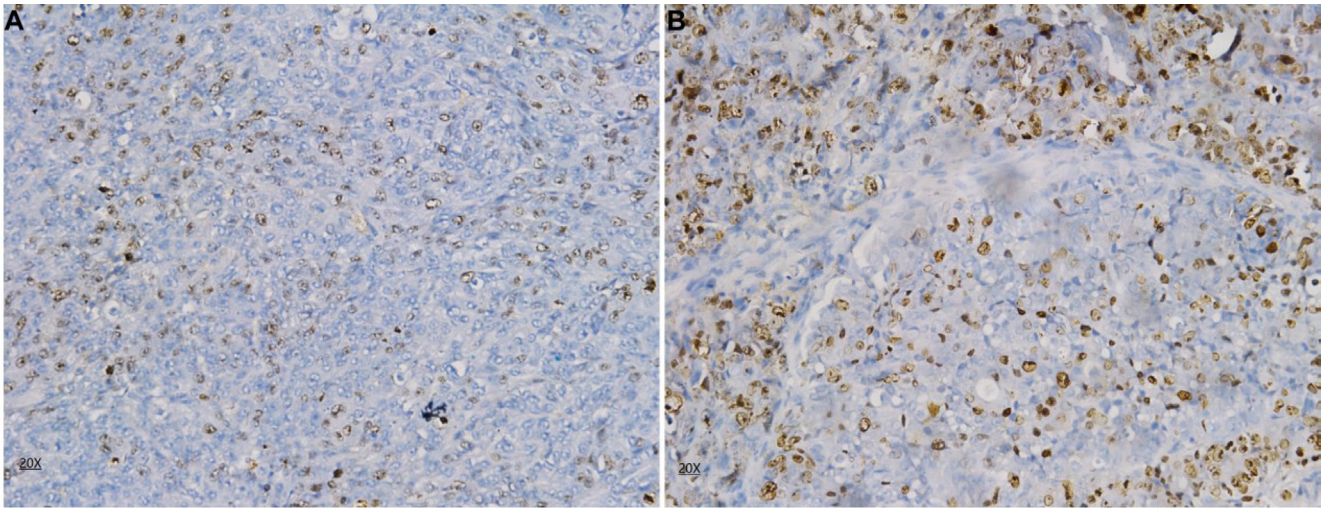
	Fold (vs Normal ( <i>n</i> = 11))	P.Value (vs Normal ( <i>n</i> = 11))
Adenoma ( <i>n</i> = 23)	0.60	0.038
Dukes' A ( <i>n</i> = 14)	0.18	0.000
Dukes' B ( <i>n</i> = 20)	0.14	0.000
Dukes' C ( <i>n</i> = 23)	0.19	0.000
Dukes' D ( <i>n</i> = 10)	0.30	0.022

**Supplementary Table 4: The seed nucleotides and mutated nucleotides of AKT2**

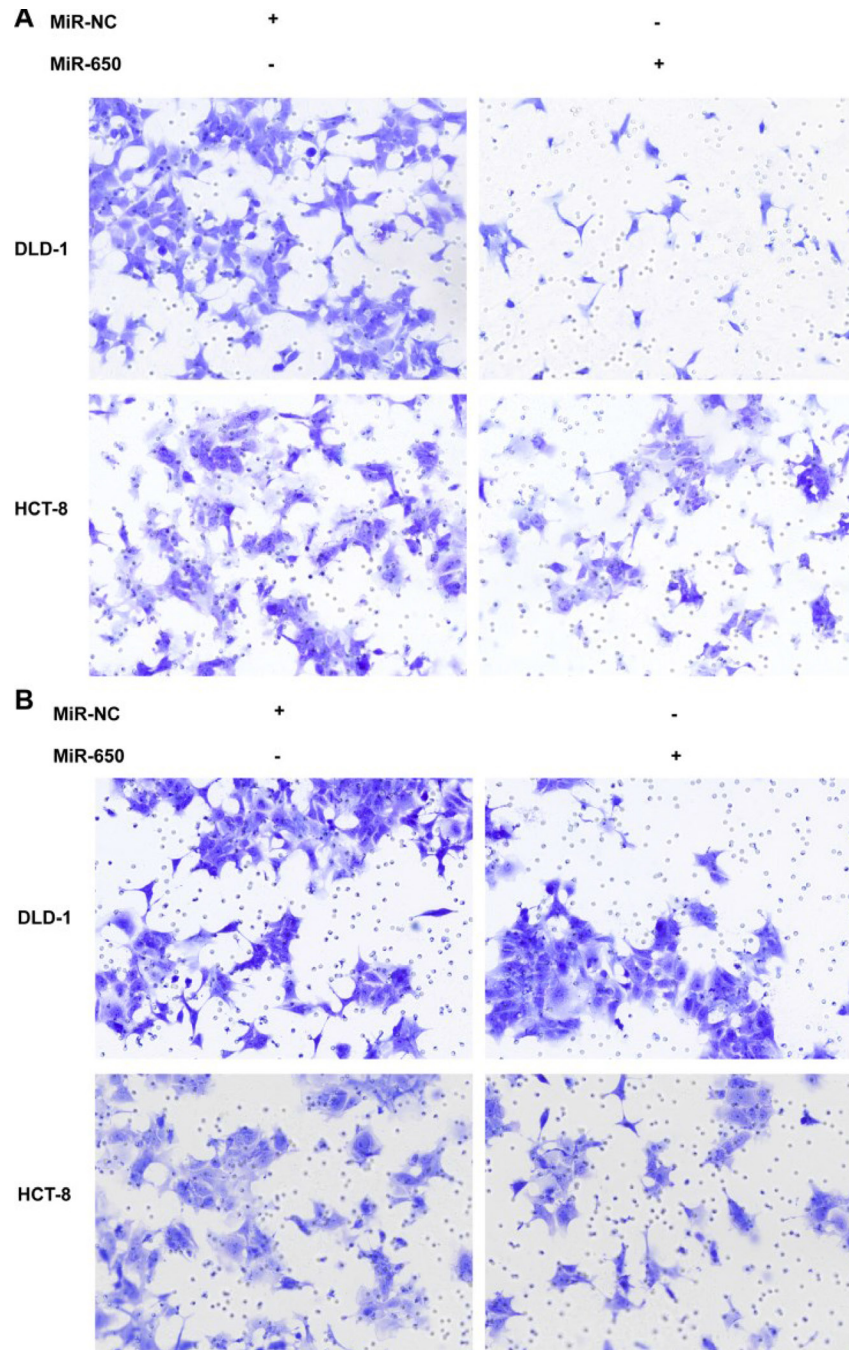
1st, 2nd Seed Wild	5'-CCAGCGGCCCTGCAGCCCCTGCCTCCAGCCTCCAGCCTCACCTTTGTGCCAGT-3'
1st Seed Mutant	5'-CCAGCGGCCCTGCAGCCCCTTCATCTAGCCTCCAGCCTCACCTTTGTGCCAGT-3'
2nd Seed Mutant	5'-CCAGCGGCCCTGCAGCCCCTGCCTCCAATTTCTAGCCTCACCTTTGTGCCAGT-3'
3rd Seed Wild	5'-CCTGGGTGTCGTGCTGGTGTCTGCCTCCGCGCTGCTGCATCTGGACGAAT-3'
4th Seed Wild	5'-CACATTTGGGGCCAAACCTGCCTGCCTCCCAGCCCCGTGCCTTACTAGT-3'



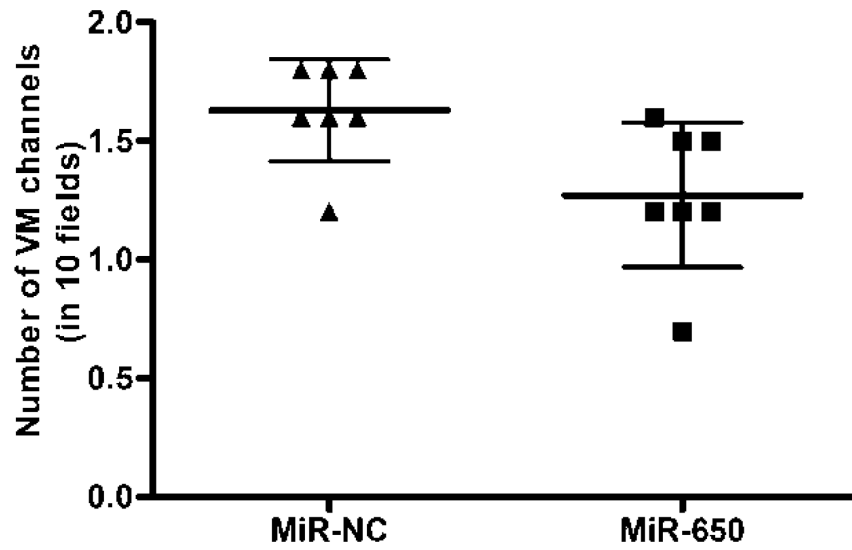
**Supplementary Figure 1: Expression levels of miR-650 in colon cancer cells.** (A) The basal expression levels in seven colon cancer cell lines. (B) Expression levels of miR-650 in DLD-1 and HCT-8 transfectants. MiR-650 precursor was transduced to DLD-1 and HCT-8 cells by lentiviral vectors. \*  $P < 0.05$ .



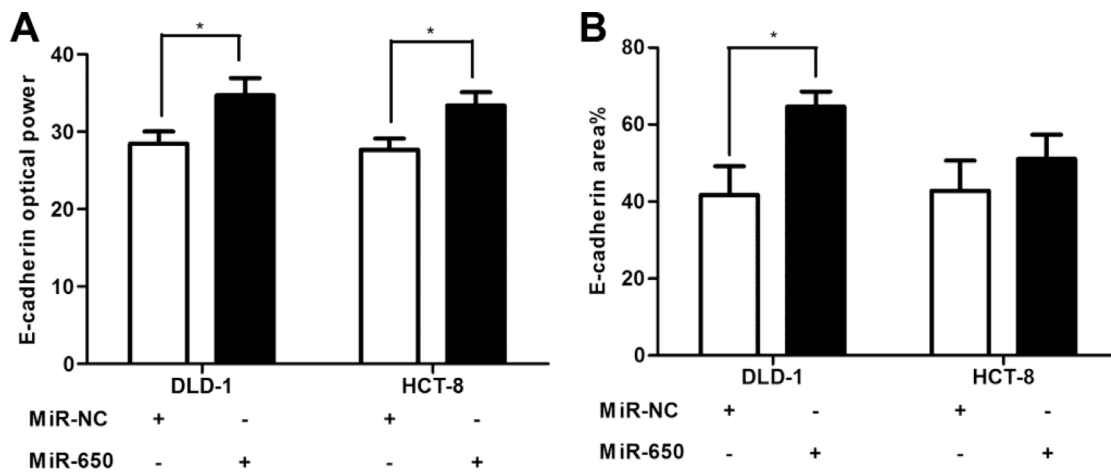
**Supplementary Figure 2: Ki-67 expression in tumor xenograft mice model.** Scores were given by two pathologists. (A) Low score of Ki-67. (B) High score of Ki-67. (C) Box-and-whisker.



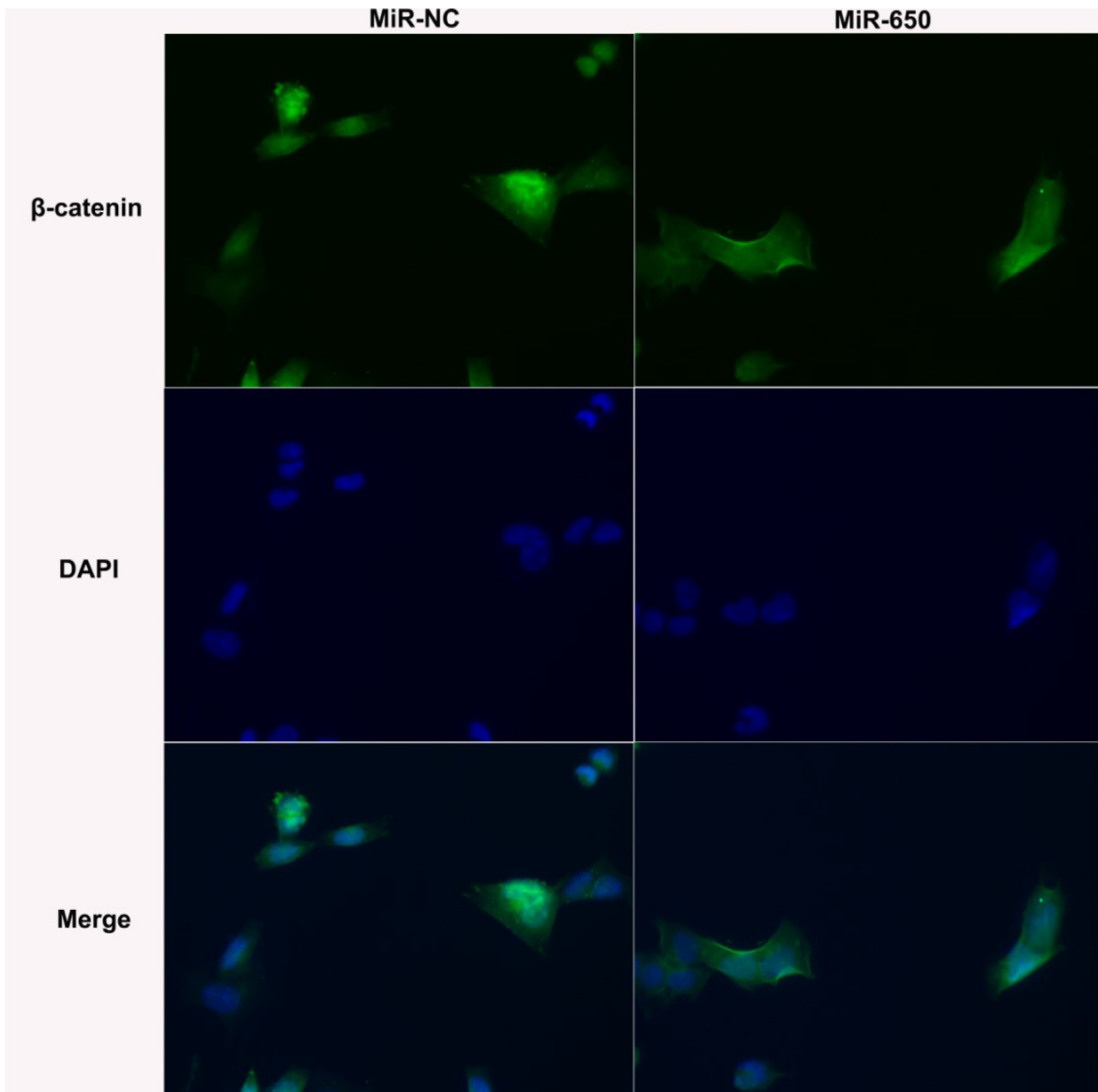
**Supplementary Figure 3: Effects of miR-650 on regulating migratory and invasive abilities in DLD-1 and HCT-8 cells (200×).** (A) Cell migratory abilities. (B) Cell invasive abilities.



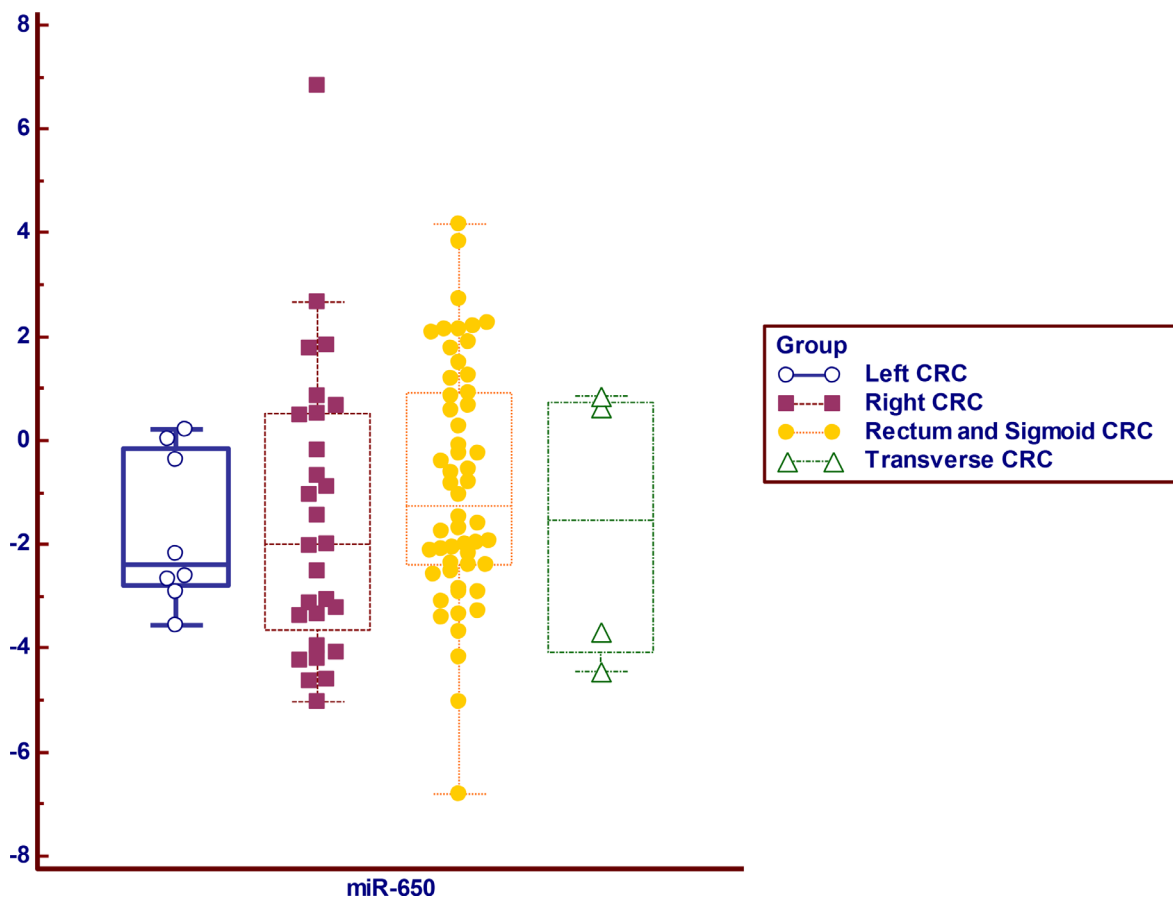
Supplementary Figure 4: Number of VM channels in xenograft mice model.



Supplementary Figure 5: Expression of E-cadherin in DLD-1 and HCT-8 cells. (A) E-cadherin optical power on cell surface. \* $P = 0.03$  in DLD-1 and \* $P = 0.02$  in HCT-8. (B) E-cadherin area% on cell surface. \* $P = 0.01$ .



Supplementary Figure 6: Expression of  $\beta$ -catenin in DLD-1 cells.



Supplementary Figure 7: MiR-650 expression in different location of CRC.