Supplemental Information

MicroRNA-140 Inhibits the Epithelial-Mesenchymal Transition and Metastasis in Colorectal Cancer

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Supplementary Figure 1: Real time qRT-PCR analysis of ectopic expressed miR-140. A. and B. HCT116 and RKO cells were transfected with miR-140 mimic (miR-140) by Oligofectamine. CRC cells alone (Con) and negative miRNA (NC) were the negative controls. The value of miR-140 in the NC group was set at 1, and the relative amounts in the groups of CRC cells or miR-140 transfected cells were indicated as relative fold (RQ). RNU6B RNA was used to normalize the expression. Each experiment was repeated 3 times and the error bars represent standard deviations (SD). n=3, **P < 0.01, two-tailed Student’s t test.

Supplementary Figure 2: The protein levels of Smad2 and Smad4 by Western blot analysis. Ectopic expression of miR-140 in HCT116 and RKO cells decreased Smad2 and Smad4 protein levels. HCT116 and RKO cells were transfected with miR-140 mimic (miR-140) using Oligofectamine. CRC cells alone (Con) and
negative miRNA (NC) were the negative controls. The quantitative results of Western blot were analyzed by Gel-Pro Analyzer 4.0 software, and GAPDH was used as an endogenous control. n=3, *P < 0.05.

Supplementary Figure 3

Supplementary Figure 3: Lentiviral vector transduced HCT116 cells. HCT116 cells were transduced with lentiviral vectors of miR-140, negative control and shRNA against Smad3 respectively. 

A. The green fluorescence was viewed under the fluorescence microscope at 72 h after the transduction. Scale bar, 100 μm. 

B. The levels of miR-140 was measured by real time qRT-PCR. n=3, **P < 0.01. 

C. The expression of Smad3 mRNA was determined by real time qRT-PCR. Each experiment was repeated 3 times and the error bars represent standard deviations. n=3, **P < 0.01, two-tailed Student’s t test.
| 3’UTR | Location  | Sequences of oligonucleotides |
|-------|-----------|-----------------------------|
| Smad3 mRNA | 421-600bp | wild type: 5’-CCGCAGGGCCATGCAGACCTCATGCCCAGCTCTCTGACGCTTGTGACAGTGCCTCTTCCAGTGAACATTCCCAGCCCAGCCCCGCCCCGCCCACACCTCCAGCAGACCTTGCCCCTTGTGAGCTGGATAGACTTGGGATGGGGAGGGAGGGAGTTTTGTCTGTCTCCCTCCCCT-3’ |
|        |           | mutant: 5’-CCGCAGGGCCATGCAGACCTCATGCCCAGCTCTCTGACGCTTGTGACAGTGCTCTTCCAGTGAACATTCCCAGCCCAGCCCCGCCCCGCCCACACCTCCAGCAGACCTTGCCCCTTGTGAGCTGGATAGACTTGGGATGGGGAGGGAGGGAGTTTTGTCTGTCTCCCTCCCCT-3’ |

Underlined bases are the predicted binding sites; italic base pairs are mutant.