Supplemental Information

METTL14 Suppresses CRC Progression via Regulating N6-Methyladenosine-Dependent Primary miR-375 Processing

Xiaoxiang Chen, Mu Xu, Xueni Xu, Kaixuan Zeng, Xiangxiang Liu, Li Sun, Bei Pan, Bangshun He, Yuqin Pan, Huiling Sun, Xinyi Xia, and Shukui Wang
Table S1 Correlation between METTL14 expression and different clinical characteristics

| Characteristics          | n=112 | METTL14 expression | P value |
|--------------------------|-------|--------------------|---------|
|                          |       | high(%)(n= 56)     | low(%)(n=56) |
| Gender                   | 0.842 |                    |         |
| Male                     | 74(66.1%) | 36(64.3%) | 38(67.9%) |
| Female                   | 38(33.9%) | 20(35.7%) | 18(32.1%) |
| Age(years)               | 0.840 |                    |         |
| <60                      | 36(32.1%) | 19(33.9%) | 17(30.4%) |
| ≥60                      | 76(67.9%) | 37(66.1%) | 39(69.6%) |
| Tumor location           | 0.449 |                    |         |
| colon                    | 59(52.3%) | 27(48.2%) | 32(57.1%) |
| rectal                   | 53(47.7%) | 29(51.8%) | 24(42.9%) |
| TNM stage                | 0.002 |                    |         |
| I - II                   | 65(58.0%) | 41(73.2%) | 24(42.9%) |
| III-IV                   | 47(42.0%) | 15(26.8%) | 32(57.1%) |
| Differentiation          | 0.016 |                    |         |
| Low                      | 27(24.1%) | 7(12.5%)  | 20(35.7%) |
| Moderate                 | 70(62.5%) | 41(73.2%) | 29(51.8%) |
| High                     | 15(13.4%) | 8(14.3%)  | 7(12.5%)  |
| Tumor size               | 0.179 |                    |         |
| <5cm                     | 66(58.9%) | 37(66.1%) | 29(51.8%) |
| ≥5cm                     | 46(41.1%) | 19(33.9%) | 27(48.2%) |
| CEA                      | 0.844 |                    |         |
| <10                      | 72(64.3%) | 35(62.5%) | 37(66.1%) |
| ≥10                      | 40(35.7%) | 21(37.5%) | 19(33.9%) |
| CA199                    | 0.705 |                    |         |
| <10                      | 51(45.5%) | 24(42.9%) | 27(48.2%) |
| ≥10                      | 61(54.5%) | 32(57.1%) | 29(51.8%) |

Table S2 univariate and multivariate analysis for OS in patients with CRC

| Characteristics          | Multivariate analysis for OS | Univariate analysis for OS |
|--------------------------|------------------------------|-----------------------------|
|                          | HR(95% CI)       | P     | HR(95% CI)       | P     |
| Gender(male/female)      | —                | —     | 1.158(0.739-1.812) | 0.522 |
| Age(<60/≥60)             | —                | —     | 0.928(0.618-1.551) | 0.928 |
| Tumor location(colon/rectal) | —            | —     | 1.160(0.754-1.785) | 0.500 |
| TNM stage( I - II/III-IV) | 2.216(1.652-3.721) | 0.016 | 2.073(1.339-3.209) | 0.001 |
| Differentiation(low/moderate/high) | —         | —     | 1.322(0.935-1.871) | 0.114 |
| Tumor size(<5cm/≥5cm)    | —                | —     | 0.654(0.420-1.018) | 0.060 |
| CEA(<10/≥10)             | —                | —     | 0.775(0.479-1.192) | 0.228 |
| CA199(<10/≥10)           | —                | —     | 0.751(0.488-1.156) | 0.193 |
Table S3. The sequence of mimic, mimic-NC inhibitor, inhibitor-NC, shNC, shMETTL14-1, shMETTL14-2

|                | Sequence                                                                 |
|----------------|---------------------------------------------------------------------------|
| mimic          | 5’- UCACCGAGCCGAACGAACAA-3’                                              |
| Mimic-NC       | 5’- CAGUACUUUUGUGUAGUACAA - 3’                                          |
| inhibitor      | 5’-UUUGUUCGUUCGCUCGCUGUA-3’                                              |
| Inhibitor-NC   | 5’-CAGUACUUUUGUGUAGUACAA-3’                                              |
| shNC           | 5’-ACT CAA AAG GAA GTG ACA AGA-3’                                        |
| shMETTL14-1    | 5’-GCT AAA GGA TGA GTT AAT-3’                                            |
| shMETTL14-2    | 5’-GGA CTT GGG ATG ATA TTA T- 3’                                         |

Table S4. The sequence of primers

|          | Sequence                                                                 |
|----------|---------------------------------------------------------------------------|
| METTL14  | Forward: 5’-GTT GGA ACA TGG ATA GCC GC-3’                                  |
|          | Reverse: 5’-CAA TGC TGT CGG CAC TTT CA-3’                                  |
| GAPDH    | Forward: 5’-GGTGGTCTCTCTGACTTCAA-3’                                       |
|          | Reverse: 5’-GTTGCTGTAGCCAAAATTCGTTGT-3’                                   |

Figure S1. M6A motifs in the primiR-375
Figure S2. miR-375 is a downstream target of METTL14. A. Representative images of EdU in the NC group, METTL14+ inhibitor-NC group, METTL14+ inhibitor group. B. Representative images of wound healing in the NC group, METTL14+inhibitor-NC group, METTL14+inhibitor group. C. Representative images of transwell migration and invasion in the NC group, METTL14+ inhibitor-NC group, METTL14+ inhibitor group.
Figure S3. miR-375 is a downstream target of METTL14. A. Representative images of EdU in the NC group, shMETTL14+ mimic-NC group, shMETTL14+ mimic group. B. Representative images of wound healing in the NC group, shMETTL14+ mimic-NC group, shMETTL14+ mimic group. C. Representative images of transwell migration and invasion in the NC group, shMETTL14+ mimic-NC group, shMETTL14+ mimic group.
**Figure S4.** METTL14 regulates CRC progression through the miR-375/YAP1 and miR-375/SP1 pathways. A. Representative images of EdU in the NC group, METTL14+vector group and METTL14+YAP1 group. B. Representative images of wound healing in the NC group, METTL14+vector group and METTL14+SP1 group. C. Representative images of transwell migration and invasion in the NC group, METTL14+vector group and METTL14+SP1 group.