

Appendix for “METTL14 is guarded by METTL3 to evade STUB1-mediated degradation to maintain m⁶A homeostasis”

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Appendix Table S1. Clinicopathological characteristics of CCA patients.

| Code | Age (year) | Gender | Tumor size (cm) | Location | Hepatitis (HBV/HCV) | CA19-9 (U/ml) | Differentiation | Metastasis |
|-------------|-------------------|---------------|----------------------------|-----------------|--------------------------------|----------------------|------------------------|---|
| 1 | 33 | Female | 4 | Extra-hepatic | HBV-;HCV- | 3.2 | moderately poorly | Peritoneal metastasis, lymph node metastasis |
| 2 | 62 | Male | 10 | Intra-hepatic | HBV+;HCV- | 14047.5 | moderately poorly | Lymph node metastasis |
| 3 | 56 | Male | 5 | Intra-hepatic | HBV+;HCV- | 145.9 | moderately | Nerve fiber and duorectum metastasis |
| 4 | 63 | Female | 3 | Extra-hepatic | HBV-;HCV- | 2041 | poorly | Lymph node metastasis |
| 5 | 59 | Male | 7 | Intra-hepatic | HBV+;HCV- | 18706 | moderately poorly | Lymph node metastasis |
| 6 | 46 | Male | 1.2 | Extra-hepatic | HBV-;HCV- | NA | well | None |
| 7 | 66 | Male | 5 | Intra-hepatic | HBV-;HCV- | 0 | well | Lymph node metastasis |
| 8 | 67 | Male | 2.5 | Extra-hepatic | HBV-;HCV- | 606.4 | moderately poorly | Lymph node metastasis |

Abbreviations: CCA, cholangiocarcinoma; NA, not available; HBV: Hepatitis B Virus; HCV: Hepatitis C Virus.

Appendix Table S2. Sequences of primers used in this study.

| Name | Sequence (5'-3') | Research purpose |
|---|--|---|
| Primers for PCR of determined genes and truncations. | | |
| METTL3_FLAG_XbaI_F | TCTAGAATGGATTACAAGGATGACG ATGACAAGTCGGACACGTGGAGCTC TATCCAGGCC | Construction of METTL3 ORF with N-terminal FLAG tag |
| METTL3_R_NotI_R | GCGGCCGCGCTATAAATTCTTAGGTT TAGAGATG | Construction of METTL3 ORF |
| METTL14_FLAG_XbaI_F | TCTAGAATGGATTACAAGGATGACG ATGACAAGGATAGCCGCTTGCAGGA GATC | Construction of METTL14 ORF with N-terminal FLAG tag |
| METTL14_R_BamHI_R | GGATCCCTATCGAGGTGGAAAGCCA CCTCTGTG | Construction of METTL14 ORF or METTL14 truncation 286-456aa, METTL14 truncation 111-456aa, METTL14 truncation Δ 111-285aa |
| METTL14_HA_XbaI_F | TCTAGAATGTACCCATACGACGTCCC AGACTACGCGATAGCCGCTTGCAGG AGATC | Construction of METTL14 ORF or METTL14 truncation 1-110aa, METTL14 truncation Δ 111-285aa, METTL14 truncation 1-285aa |
| METTL14_1_BamHI_R | GGATCCTTATGTTCCCTTAAGAAAAG TACTAGA | Construction of METTL14 truncation 1-110aa |
| METTL14_1_NTC-BamHI_R | GGATCCTGTTCCCTTAAGAAAAGTAC TAGA | Construction of METTL14 truncation 1-110aa with C-terminal GFP tag |
| METTL14_2_HA_XbaI_F | TCTAGAATGTACCCATACGACGTCCC AGACTACGCCAGAGCTTAAATCCCCA TAATG | Construction of METTL14 truncation 111-285aa, or METTL14 truncation 111-456aa |
| METTL14_2_BamHI_R | GGATCCTTACTTTGTTCTCTGAAAGA CAGCCTT | Construction of METTL14 truncation 111-285aa, METTL14 truncation 1- 285aa |
| METTL14_2_NTC-BamHI_R | GGATCCCTTTGTTCTCTGAAAGACAG CCTT | Construction of METTL14 truncation 111-285aa with C-terminal GFP tag |
| METTL14_3_HA_XbaI_F | TCTAGAATGTACCCATACGACGTCCC AGACTACGCGAACACTGCCTCATGG GGATC | Construction of METTL14 truncation 286-456aa with N-terminal HA tag |
| METTL14_NTC_BamHI_R | GGATCCTCGAGGTGGAAAGCCACCT CTGTG | Construction of METTL14 ORF with C-terminal GFP tag, METTL14 truncation 286-456aa with C-terminal GFP tag |
| METTL14_5_fusion-F | ATTTGCCATATGAAGAAGAGAACAC TGCCTCATGGGGATC | Construction of METTL14 truncation Δ 111-285aa |
| METTL14_5_fusion-R | GATCCCCATGAGGCAGTGTCTCTTC TTCATATGGCAAAT | Construction of METTL14 truncation Δ 111-285aa |

| | | |
|------------------------|--|---|
| METTL14-GST-F | TAAGGGATCCAAGCTTGATAGCCGCT TGCAGGAGATCC | Construction of METTL14 ORF with N-terminal GST tag |
| METTL14-GST-R | TAGGGGACATAAGCTTTCGAGGTGG AAAGCCACCTCTG | Construction of METTL14 ORF with N-terminal GST tag |
| STUB1-6×His-R | GCGAAGCTTGTAGTCCTCCACCCAGC CATTC | Construction of STUB1 ORF with C- terminal His tag |
| METTL3_APPA_F | GTTTGCAGTTGTGATGGCTGCACCAC CCGCAGATATTCACATGGAACCTG | Construction of METTL3 APPA mutant isoform |
| METTL3_APPA_R | CAGTTCCATGTGAATATCTGCGGGTG GTGCAGCCATCACAACCTGCAAAC | Construction of METTL3 APPA mutant isoform |
| METTL3_ (Δ450_454aa)_F | CTAAACCTCTGGGGGTATATTATTG GGTGAAGACAAAT | Construction of METT3 truncation Δ450-454aa |
| METTL3_ (Δ450_454aa)_R | ATTTGTCTTCACCCAAATAATATACC CCCAGAGGTTTAG | Construction of METT3 truncation Δ450-454aa |
| METTL3_ (Δ464_480aa)_F | GGGTGAAGACAAATCAACTGGAACA CTGCTTGGTTGGTGT | Construction of METT3 truncation Δ464-480aa |
| METTL3_ (Δ464_480aa)_R | ACACCAACCAAGCAGTGTTCAGTTG ATTTGTCTTCACCC | Construction of METT3 truncation Δ464-480aa |
| STUB1_F | AGCGAATTCGAAGGATCCATGAAGG GCAAGGAGGAGAAGG | Construction of STUB1 ORF or STUB1 ORF with C-terminal His tag |
| STUB1_R | ATGATCTTTGTAATCGGATCCGTAGT CCTCCACCCAGCCATTC | Construction of STUB1 ORF |
| STUB1_ΔU-box_R | ATGATCTTTGTAATCGGATCCGGCCC GGACGTGGCTGTCGTCCTCA | Construction of STUB1 truncation 1- 195aa |
| STUB1_ΔTPR_F | AGCGAATTCGAAGGATCCATGAAGA AGAAGCGCTGGAACAGCATTG | Construction of STUB1 truncation 143-312aa |
| STUB1-H260Q-F | CGAGGAGCAACTGCAGCGTGTGGGT CATTTTGAC | Construction of STUB1 ^{H260Q} mutant |
| STUB1-H260Q-R | ACGCTGCAGTTGCTCCTCGATGTCCT TGCGGTCGTA | Construction of STUB1 ^{H260Q} mutant |

Primers for transcription quantitative polymerase chain reaction(RT-qPCR).

| | |
|-------------|--------------------------|
| q-METTL3-F | TTGTCTCCAACCTTCCGTAGTG |
| q-METTL3-R | CCAGATCAGAGAGGTGGTGTAG |
| q-METTL14-F | AGAAACTTGCAGGGCTTCCTAT |
| q-METTL14-R | TCTTCTTCATATGGCAAATTTTCT |
| q-WTAP-F | CCTCTTCCCAAGAAGGTTTCGATT |
| q-WTAP-R | TGCAGACTCCTGCTGTTGTTG |
| q-ACTIN-F | TTGTTACAGGAAGTCCCTTGCC |

| | |
|------------|-------------------------|
| q-ACTIN-R | ATGCTATCACCTCCCCTGTGTG |
| q-STUB1-F | AGGCCAAGCACGACAAGTACAT |
| q-STUB1-R | CTGATCTTGCCACACAGGTAGT |
| q-TRIM33-F | CCAATTCACTTTCTAGATGCAGG |
| q-TRIM33-R | GGAGTGCTTGCATGTTGAG |
| q-UBR5-F | GAAGAGGTTGAGGTGGTGGGA |
| q-UBR5-R | CAGCTCCATATCACTCCCGT |
| q-UBR1-F | AGTAGACACAGGCCTACCCC |
| q-UBR1-R | ACCCACAAATACCAGCCAGG |

siRNA for target gene transient knockdown

| | |
|------------|---------------------------|
| siMETTL3-1 | CUGCAAGUAUGUUCACUAUGA |
| siMETTL3-2 | AGGAGCCAGCCAAGAAAUCA |
| siSTUB1-1 | GGCAAUCGUCUGUUCGUGGGCCGAA |
| siSTUB1-2 | CCAGCGCUCUUCGAAUCGCGAAGAA |
| siUBR1-1 | AAGCAGGAGGAAAGUGUACAA |
| siUBR1-2 | AUGGAAAUCAGCGCGGAGUUA |
| siUBR5-1 | GCGACUCUCCAUGGUUUCU |
| siUBR5-2 | GCGACUCUCCAUGGUUUCU |
| siTRIM33-1 | CCUGCAUCUAGAAAGUGAA |
| siTRIM33-2 | GCGACUGAUUACUUCCA |

shRNA for target gene stable knockdown.

| | |
|--------------|---|
| shMETTL3-1-F | GATCCCTGCAAGTATGTTCACTATGACTTCCTGTCAGATCATAGTGAACATA CTTGACAG TTTTTG |
| shMETTL3-1-R | AATTCAAAAACACTGCAAGTATGTTCACTATGATCTGACAGGAAGTCATAGTG AACATACTTGCAGG |
| shMETTL3-2-F | GATCCAGGAGCCAGCCAAGAAATCAACTTCCTGTCAGATTGATTTCTTGGCT GGCTCCTTTTTTG |

| | |
|--------------|--|
| shMETTL3-2-R | AATTCAAAAAAGGAGCCAGCCAAGAAATCAATCTGACAGGAAGTTGATTTCTTGGCTGGCTCCTG |
| shSTUB1-1-F | GATCCGGCAATCGTCTGTTTCGTGGGCCGAACCTCCTGTCAGATTCGGCCACGAACAGACGATTGCCTTTTTG |
| shSTUB1-1-R | AATTCAAAAAAGGCAATCGTCTGTTTCGTGGGCCGAATCTGACAGGAAGTTCCGCCACGAACAGACGATTGCCG |
| shSTUB1-2-F | GATCCCAGCGCTCTTCGAATCGCGAAGAACTCCTGTCAGATTCTTCGCGATTTCGAAGAGCGCTGGTTTTG |
| shSTUB1-2-R | AATTCAAAAACCAGCGCTCTTCGAATCGCGAAGAATCTGACAGGAAGTTCTTCGCGATTTCGAAGAGCGCTGGG |
| shUBR1-1-F | GATCCAAGCAGGAGGAAAGTGTACAACCTCCTGTCAGATTGTACACTTTCTCCTGCTTTTTTTG |
| shUBR1-1-R | AATTCAAAAAAGCAGGAGGAAAGTGTACAATCTGACAGGAAGTTGTACACTTTCTCCTGCTT |
| shUBR1-2-F | GATCCATGGAATCAGCGCGGAGTTACTTCTGTCAGATAACTCCGCGCTGATTTCCATTTTTT |
| shUBR1-2-R | AATTCAAAAAATGGAATCAGCGCGGAGTTATCTGACAGGAAGTAACTCCGCGCTGATTTCCATG |
| shUBR5-1-F | GATCCGCGACTCTCCATGGTTTCTTCTCCTGTCAGAAGAAACCATGGAGAGTCGCTTTTTG |
| shUBR5-1-R | AATTCAAAAAGCGACTCTCCATGGTTTCTTCTGACAGGAAGAGAAACCATGGAGAGTCGCG |
| shUBR5-2-F | GATCCCAACTTAGATCTCCTGAAACTTCTGTCAGATTTTCAGGAGATCTAAGTTGTTTTG |
| shUBR5-2-R | AATTCAAAAACAACCTTAGATCTCCTGAAATCTGACAGGAAGTTTCAGGAGATCTAAGTTG |
| shTRIM33-1-F | GATCCCCTGCATCTAGAAAGTGAACCTCCTGTCAGATTTCTAGATGCAGGTTTTG |
| shTRIM33-1-R | AATTCAAAAACCTGCATCTAGAAAGTGAACCTCTGACAGGAAGTTCACTTTCTAGATGCAGG |
| shTRIM33-2-F | GATCCGCGACTGATTACTTTCCACTTCTGTCAGATGGAAAGTAATCAGTCGCTTTTTG |
| shTRIM33-2-R | AATTCAAAAAGCGACTGATTACTTTCCATCTGACAGGAAGTGGAAAGTAATCAGTCGCG |
