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Supplemental Information

RNA m^6A methylation regulates dissemination of cancer cells by modulating expression and membrane localization of β-catenin

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Supplementary information for

RNA m^{6}A methylation regulates the dissemination of cancer cells via modulating expression and membrane localization of β-catenin

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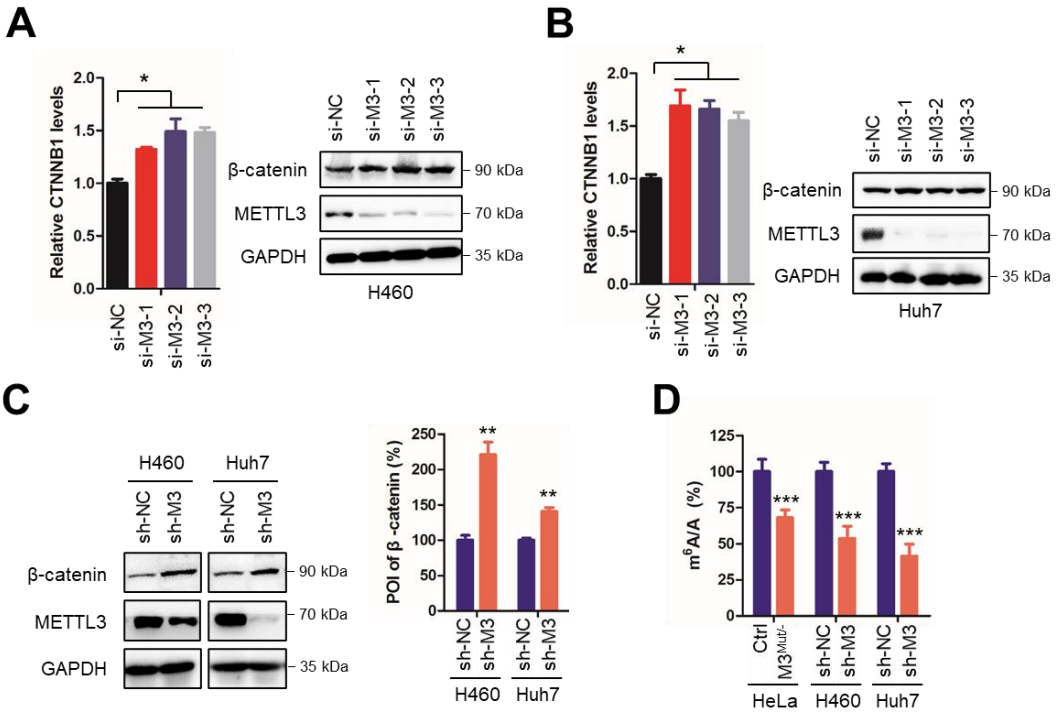

Fig.S1 Expression of *CTNNB1* is associated to METTL3 in lung cancer and liver cancer.

(A-B) Expression levels of *CTNNB1* mRNA (*left*) and β-catenin protein (*right*) in H460 cells (A) and Huh7 cells (B) silencing METTL3 were detected by RT-qPCR and Western blot, respectively;

(C) Protein levels of β-catenin in H460 sh-NC, H460 sh-METTL3, Huh7 sh-NC and Huh7 sh-METTL3 cells were detected by Western blot (*left*) and quantitatively analyzed (*right*);

(D) m^6A methylation levels of HeLa, HeLa $METTL3^{Mut/-}$, H460 sh-NC, H460 sh-METTL3, Huh7 sh-NC and Huh7 sh-METTL3 cells were detected by LC-MS/MS.

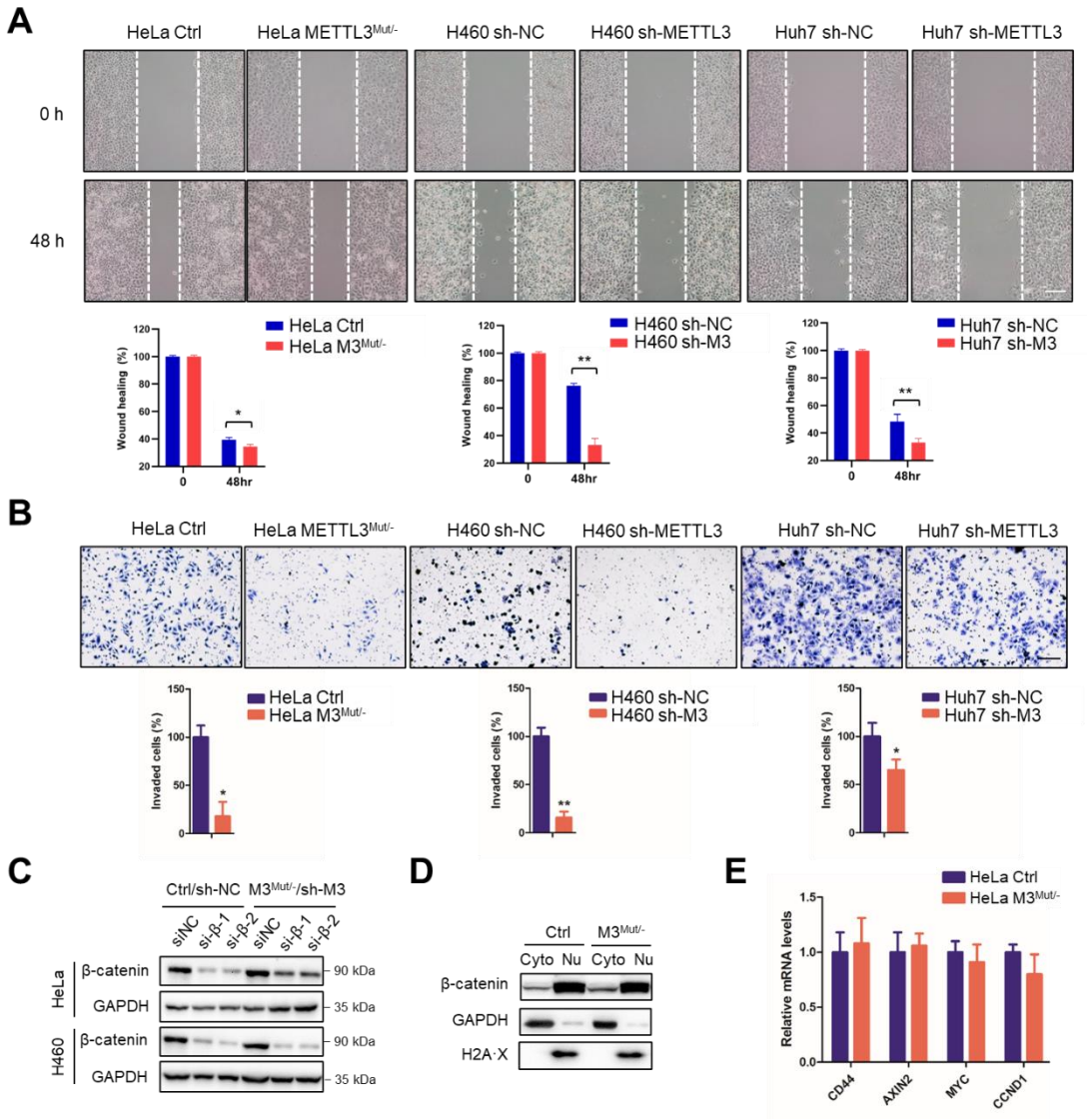

Fig.S2 METTL3-mediated EMT is independent to Wnt/β-catenin activation in cancer cells.

(A) Wound healing of HeLa, HeLa METTL3$^{Mut/-}$, H460 sh-NC, H460 sh-METTL3, Huh7 sh-NC, Huh7 sh-METTL3 cells were recorded and quantitatively analyzed; scale bar, 100μm;

(B) Cell invasion assays of HeLa, HeLa METTL3$^{Mut/-}$, H460 sh-NC, H460 sh-METTL3, Huh7 sh-NC, Huh7 sh-METTL3 cells were recorded and quantitatively analyzed; scale bar, 100μm;

(C) Protein levels of β-catenin in HeLa, HeLa METTL3$^{Mut/-}$, H460 sh-NC, H460 sh-METTL3 cells transfected with si-NC or si-β-catenin were detected by Western blot;

(D) Protein levels of β-catenin in cytoplasmic and nuclear fractions in HeLa and HeLa METTL3$^{Mut/-}$ cells;

(E) Expression levels of downstream genes Wnt signaling in HeLa and HeLa METTL3$^{Mut/-}$ cells were detected by RT-qPCR.

Data are presented as means ± SD from three independent experiments. Student's *t test*, *, $p<0.05$; **, $p< 0.01$ compared with control.

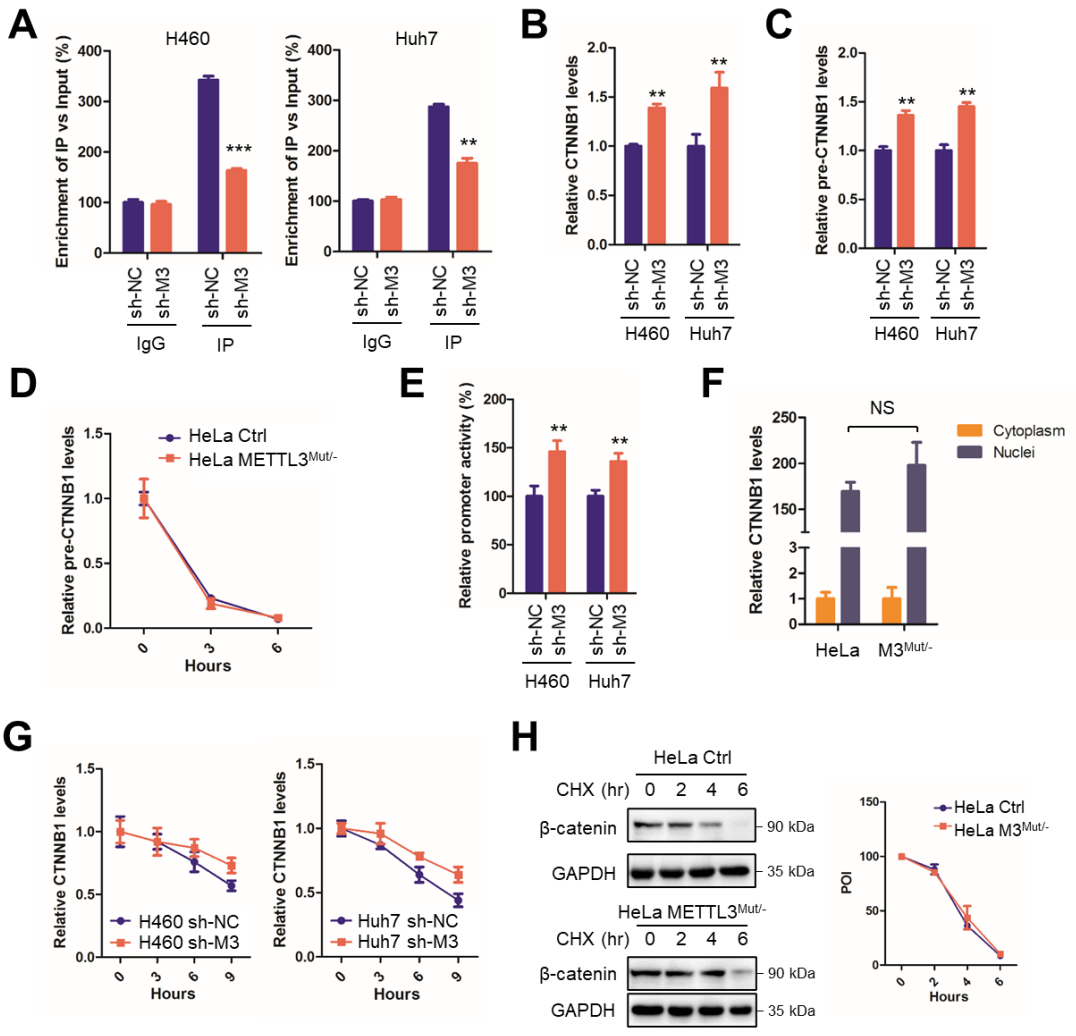

Fig.S3 METTL3 regulates transcription, stability and translation of *CTNNB1* mRNA in H460 and Huh7 cells.

(A) m^{6}A RIP-qPCR analysis of *CTNNB1* mRNA in H460 sh-NC and H460 sh-METTL3 cells (*left*), Huh7 sh-NC and Huh7 sh-METTL3 cells (*right*);

(B-C) Expression level of *CTNNB1* mRNA (B) and precursor mRNA (pre-CTNNB1; C) in H460 sh-NC, H460 sh-METTL3, Huh7 sh-NC and Huh7 sh-METTL3 cells were detected by RT-qPCR;

(D) Expression levels of *CTNNB1* precursor mRNA (pre-CTNNB1) in HeLa and HeLa METTL3$^{Mut/-}$ cells treated with Act-D were detected by RT-qPCR;

(E) H460 sh-NC, H460 sh-METTL3, Huh7 sh-NC and Huh7 sh-METTL3 cells were co-transfected with pGL3-Basic-CTNNB1-Fluc reporter and pRL-TK plasmids for 24 h. Results were presented as the ratios between the activity of the reporter plasmid and pRL-

TK;

(F) Expression of cytoplasmic or nuclear *CTNNB1* mRNA levels in HeLa and HeLa METTL3$^{Mut/-}$ cells were detected by RT-qPCR;

(G) Half-lives of *CTNNB1* mRNA in H460 sh-NC, H460 sh-METTL3, Huh7 sh-NC and Huh7 sh-METTL3 cells were detected by RT-qPCR;

(H) Protein levels of β-catenin in HeLa and HeLa METTL3$^{Mut/-}$ cells treated with CHX for indicated priors (*left*). Protein of interest (POI) are quantitatively analyzed (*right*).

Data are presented as means ± SD from three independent experiments. Student's *t test*, **, $p< 0.01$; ***, $p< 0.001$ compared with control.

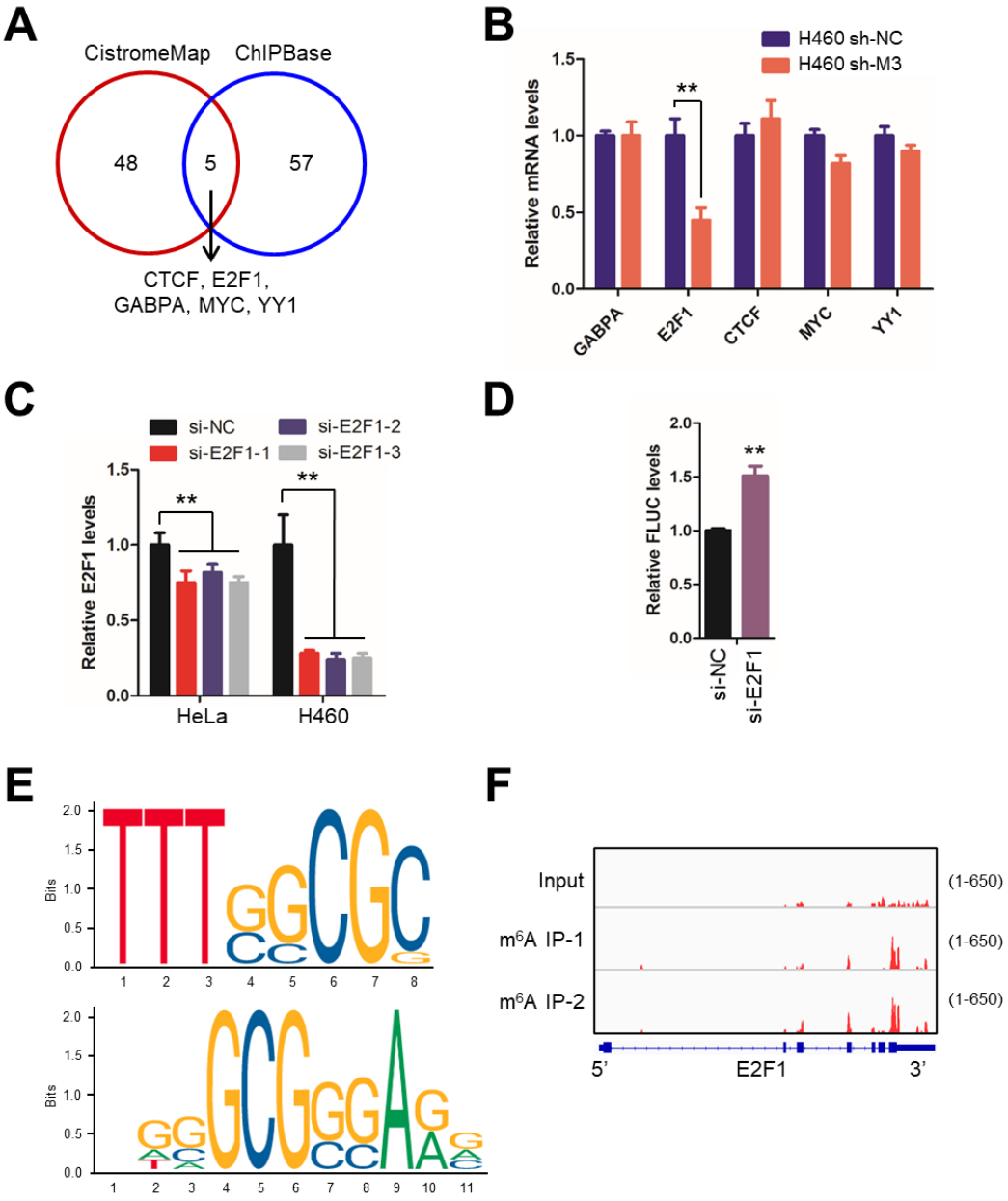

Fig.S4 E2F1 is identified as transcription factor of *CTNNB1*.

(A) Venn diagram shows the overlap of transcription factors of *CTNNB1* predicted by CistromeMap and ChIPBase, respectively;

(B) Expression levels of predicted transcription factors of *CTNNB1* in H460 sh-NC and H460 sh-METTL3 cells were detected by RT-qPCR;

(C) Expression levels of *E2F1* mRNA in HeLa and H460 cells silencing *E2F1* were detected by RT-qPCR;

(D) HeLa cells with or without *E2F1* silencing were transfected with pGL3 reporter containing

CTNNB1 promoter before *F-LUC* gene for 48 h. Expression levels of *FLUC* mRNA were detected by RT-qPCR, which was normalized to the resistant gene *AmpR* of pGL3 reporter;

(E) The binding motifs of E2F1 analyzed by ChIPBase database;

(F) m^6A peaks located in *E2F1* mRNA from m^6A RIP-seq data (Accession code GSE112795).

Data of Fig.4B, 4C, 4E are presented as means ± SD from three independent experiments. Student's *t test*, **, $p < 0.01$ compared with control.

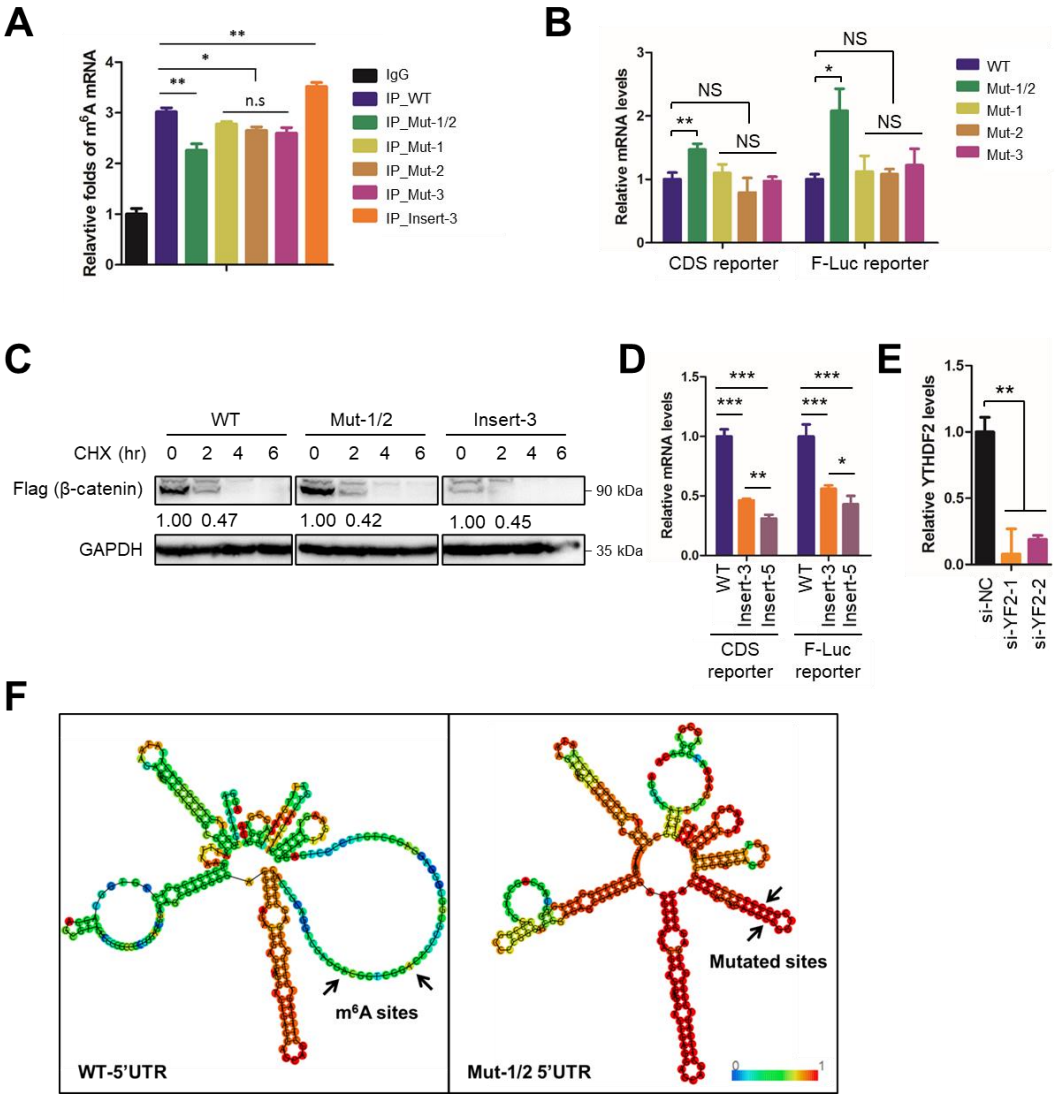

Fig.S5 5'UTR m^{6}A methylation destabilizes *CTNNB1* mRNA.

(A) Relative enrichment of m^{6}A in wild-type (WT) or mutated CDS reporters were analyzed by m^{6}A RIP-qPCR;

(B) Expression levels of *CTNNB1*-CDS and F-Luc reporter mRNAs in HeLa cells transfected with wild-type (WT) or mutated reporters;

(C) CDS reporters with mutations were transfected in HeLa cells for 48hr and then treated with CHX for indicated times. Expression levels of exogenous β-catenin (Flag) were detected by Western blot. Band intensities of Flag-β-catenin were analyzed by ImageJ and listed at the bottom of target bands;

(D) Expression levels of *CTNNB1*-CDS and F-Luc reporter mRNAs in HeLa cells transfected

with wild-type (WT) or m^{6}A-inserted reporters;

(E) Expression levels of *YTHDF2* mRNA in HeLa cells silencing *YTHDF2* were detected by RT-qPCR;

(F) The secondary structure of wild type (WT) or Mut-1/2 (GG**A**C to GG**C**C in site 1 and site 2 of m^{6}A motifs) in *CTNNB1* 5'UTR was predicted by RNAfold WebServer (http://rna.tbi.univie.ac.at/). The red color indicates strong confidence for the prediction of each base.

Data of Fig.5A-5E are presented as means ± SD from three independent experiments. Student's *t test*, NS, no significant; *, $p< 0.05$; **, $p< 0.01$; ***, $p< 0.001$ compared with control.

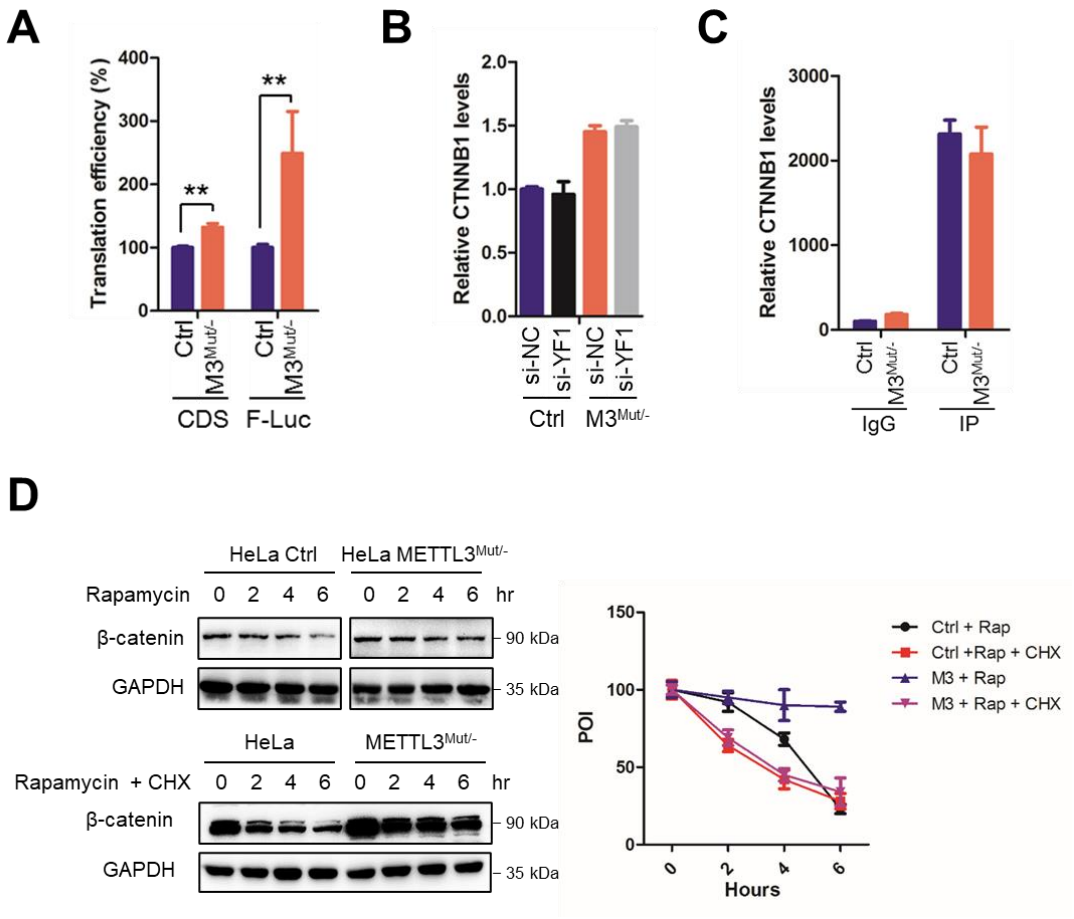

Fig.S6 METTL3 modulates translation of *CTNNB1*.

(A) Translation efficiency of CDS and F-Luc reporters in control and HeLa METTL3$^{Mut/-}$ cells were analyzed;

(B) Expression levels of *CTNNB1* mRNA in HeLa cells silencing *YTHDF1*;

(C) Control and HeLa METTL3$^{Mut/-}$ cells overexpressing β-MS2bs were pull-down by HA-MS2. Expression levels of *CTNNB1* mRNA in IgG control and HA-IP samples were detected by RT-qPCR;

(D) Expression levels of β-catenin in HeLa cells treated with 50nM rapamycin with or without 100ug/ml cycloheximide (CHX) for indicated time.

Data are presented as means ± SD from three independent experiments. Student's *t test*, **, $p<$ 0.01, NS, no significant compared with control.

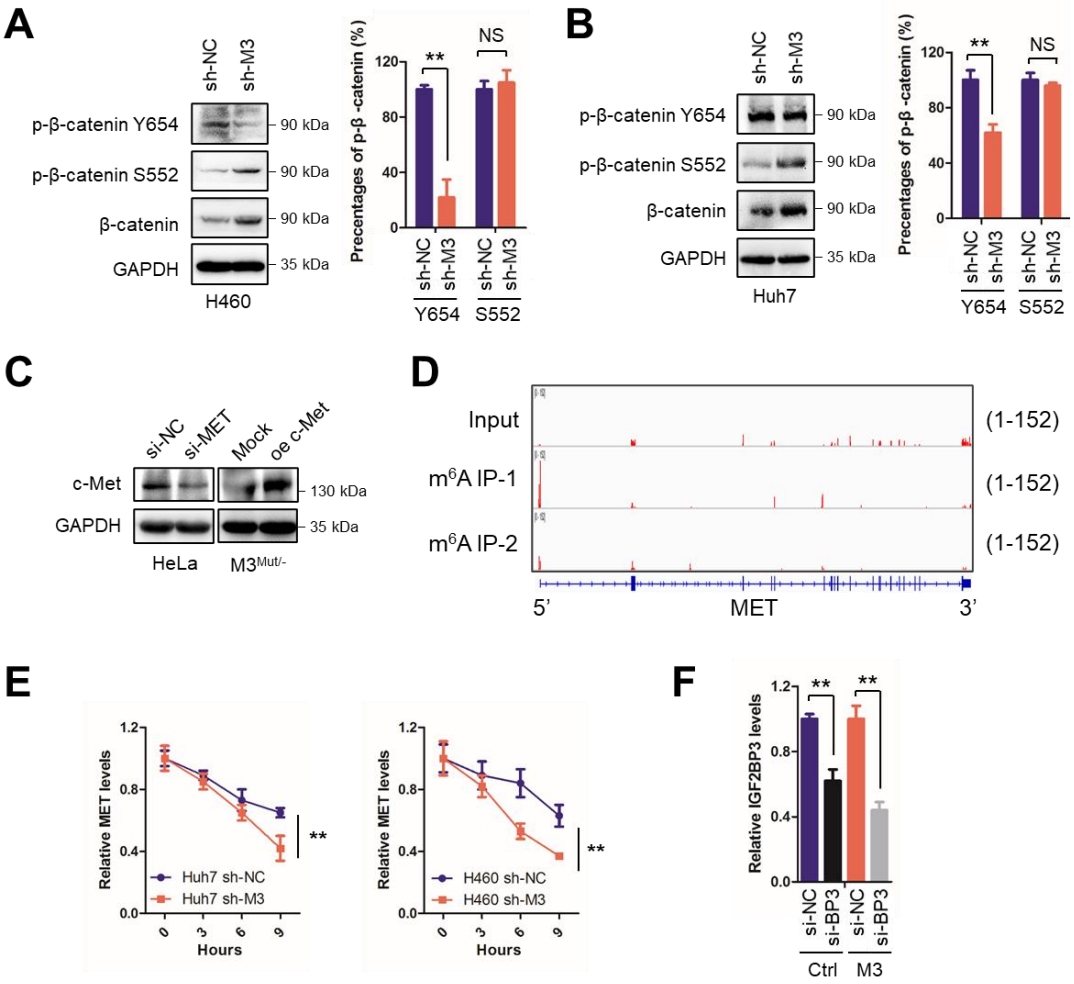

Fig.S7 METTL3 regulates membrane localization of β-catenin via Met kinase.

(A) Protein (*left*) and mRNA (*right*) levels of β-catenin and METTL3 in H460 sh-NC and H460 sh-METTL3 cells were detected by Western blot analysis and RT-qPCR, respectively

(B) Protein (*left*) and mRNA (*right*) levels of β-catenin and METTL3 in Huh7 sh-NC and Huh7 sh-METTL3 cells were detected by Western blot analysis and RT-qPCR, respectively;

(C) Expression of c-Met in HeLa cells silencing c-Met and HeLa METTL3$^{Mut/-}$ overexpressing c-Met were detected by Western blot analysis;

(D) m^{6}A peaks located in *MET* mRNA from m^{6}A RIP-seq data (Accession code GSE112795);

(E) Half-lives of *MET* in H460 sh-NC and H460 sh-METTL3 (*left*), Huh7 sh-NC and Huh7 sh-METTL3 (*right*) cells;

(F) Expression levels of *IGF2BP3* mRNA in HeLa (Ctrl) and HeLa METTL3$^{Mut/-}$ (M3) cells silencing *IGF2BP3* were detected by RT-qPCR.

Data are presented as means ± SD from three independent experiments. Student's *t test*, **, *p*<

0.01, NS, no significant compared with control.

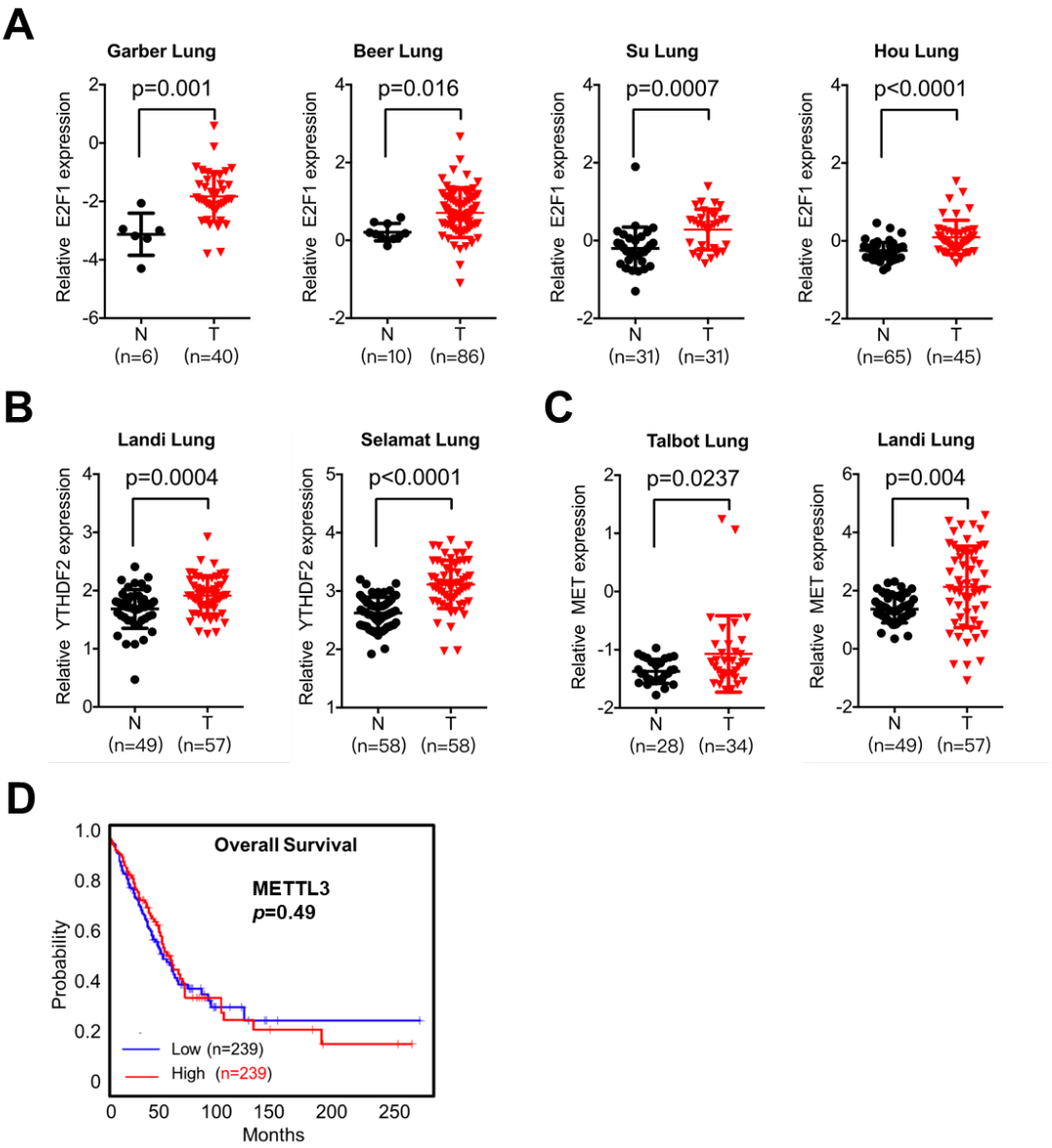

Fig.S8 Regulators of β-catenin expression are involved in the development of cancer cells.

(A) The relative mRNA expression of *E2F1* in lung cancers from Oncomine datasets;

(B) The relative mRNA expression of *YTHDF2* in lung cancers from Oncomine datasets;

(C) The relative mRNA expression of *MET* in lung cancers from Oncomine datasets;

(D) The Kaplan–Meier survival curves of OS based on METTL3 expression in lung cancer patients from TCGA data base.

Table S1 Sequences for siRNA

Name	Sequences (5’ – 3’)
si-CTNNB1-1	CCAGACACGCUAUCAUGCGdTdT
si-CTNNB1-2	UUGGAUUGAUUCGAAAUCUdTdT
si-METTL3-1	GCACUUGGAUCUACGGAAUdTdT
si-METTL3-2	CGACUACAGUAGCUGCCUUdTdT
si-METTL3-3	CAGUGGAUCUGUUGUGAUAdTdT
si-E2F1-1	GCAUCUAUGACAUCACCAAdTdT
si-E2F1-2	GGACUCUUCGGAGAACUUUdTdT
si-E2F1-3	CCUCUUCGACUGUGACUUUdTdT
si-YTHDF1-1	CCGCGUCUAGUUGUUCAUGAAdTdT
si-YTHDF1-2	CCUCCACCCAUAAAGCAUAdTdT
si-YTHDF2-1	GCGGGUCCAUUACUAGUAAdTdT
si-YTHDF2-2	GGGCUGAUAUUGCUAGCAAdTdT
si-IGF2BP3-1	AAUUCCUGCAAUGGAGAUAUCdTdT
si-IGF2BP3-2	UUCAUGAUCUCCUCCUCAGCUdTdT
si-MET	AACATGGCTCTAGTTGTCGACdTdT

Table S2 Primers for RT-qPCR

Gene	Forward (5’ - 3’)	Reverse (5’- 3’)
CTNNB1	TGGATTGATTCGAAATCTTGCC	GAACAAGCAACTGAACTAGTCG
pre-CTNNB1	GCCTTACTGAAAGTCAGAATGC	AACACTCACTATCCACAGTTCA
CTNNB1 5’UTR	TGGATTGATTCGAAATCTTGCC	GAACAAGCAACTGAACTAGTCG
CTNNB1 3’UTR	TGGGTAGGGTAAATCAGTAAGAGGT	AGCATCGTATCACAGCAGGT
CDH1	GCCTCCTGAAAAGAGAGTGGAAG	TGGCAGTGTCTCTCCAAATCCG
FN1	ACAACACCGAGGTGACTGAGAC	GGACACAACGATGCTTCCTGAG
METTL3	CTATCTCCTGGCACTCGCAAGA	GCTTGAACCGTGCAACCACATC
GABPA	GTGCACAGAAGAAAGCATTGTA	ACACTGTAGTCTTGGTTCTAGC
E2F1	ATAGTGTCACCACCACCATCAT	GAAAGGCTGATGAACTCCTCAG
CTCF	TTAAAACGTCACATTCGCTCTC	TCATAAGGCTTTTCCCCTGAAT
MYC	CGACGAGACCTTCATCAAAAAC	CTTCTCTGAGACGAGCTTGG
YY1	CCCACGGTCCCAGAGTCCAC	AAAGCGTTTCCCACAGCCTTCG
CTNNB1 promoter	GCTATGGTGACAGACGGCAGTTG	TCATGTTTCAGGGCCAGCGTATTC
FLUC	GGCCTGACAGAAACAACCAG	AAGTCCACCACCTTAGCCTC
RLUC	CGCTATTGTCGAGGGAGCTA	GCTCCACGAAGCTCTTGATG
AmpR	CCGGCGTCAATACGGGATAA	TCCTTGAGAGTTTTCGCCCC
GAPDH	GTCTCCTCTGACTTCAACAGCG	ACCACCCTGTTGCTGTAGCCAA
18S	CGGACAGGATTGACAGATTGATAGC	TGCCAGAGTCTCGTTCGTTATCG
HPRT	TGACACTGGCAAAACAATGCA-	GGTCCTTTTCACCAGCAAGCT
EIF4E1	AGGATGGTATTGAGCCTATGTG	GTCTCTGCTGTTTGTTCAATGT
EIF4E3	CCAAGGACAGCACGTCCACAG	CATCTGCTGCGGCACAGTCTG
FOXP3	AAGAACGCCATCCGCCACAAC	TCCAGCTCATCCACGGTCCAC
ZNF191	AGCGATTCAGGCAGTTTGGA	CTTTTGTGTGCGTCTCTGGC
ZBP-89	GAAACAACGTTCTCCCGCAAA	TTGCTCCAGTGGCTGTTTCA
PWWP2A	ACCCTTCTCCTGTCCCGCATC	GGAGGTGGCTGAGGTATTGATTGG
YTHDF1	TGGATGATGCTGCTGGTTGT	CAACGGTCCCTCATTCCACA
YTHDF2	AGCCTCTTGGAGCAGTACAAA	GCATTATTGGGCCTTGCCTG
MET	ATCAGGAGGTGTTTGGAAAGAT	CGACTGTATGTCAGCAGTATGA
CD44	CACACCCTCCCCTCATTCAC	AGGTCCTGCTTTCCTTCGTG
AXIN2	CTCCGAGCTCACACTCAATTC	GACAGGTGATCGTCCAGTATC
CCND1	TACCGCCTCACACGCTTCCTC	ACCTCCTCCTCCTCCTCTTCCTC

Table S3 Primers for PCR

Name	Forward (5' – 3')	Reverse (5' – 3')	Remarks
pcDNA3_5'UTRctnnb1	GGGGTACCAGGATACAGCGGCTTC	CGGGATCCTGTCCACGCTGGATT	Cloning CTNNB1 5'UTR to pcDNA3
pGL3-PROMctnnb1	GGGGTACCTTGCGGCCGCGGATCTCACTGAACATCATC	CCAAGCTTTTCTCGAGGCCGCCACCGGCGCAGC	Cloning CTNNB1 promoter to pGL3-basic
pGL3_5UTRctnnb1	GGCTCGAGAGGATACAGCGGCTTC	CGAAGCTTTGTCCACGCTGGATTTTCAAAAC	Cloning CTNNB1 5'UTR to pGL3-basic
5UTR mut3_pGL	AATCCAGCGTGGCCAAAGCTT GGC	GCCAAGCTTTGGCCACGCTGGATT	β-catenin mutant 1 in pGL3-basic
5UTR mut3_pcD	AATCCAGCGTGGCCAGGATCCCCA	TGGGGATCCTGGCCACGCTGGATT	β-catenin mutant 1 in pcDNA3
5UTR mut2	AGGACGGTCGGCCTCCCGCGGC	GCCGCGGGAGGCCGACCGTCCT	β-catenin mutant 2
5UTR mut3	CGCAGGTCGAGGCCGGTCGGACTC	GAGTCCGACCGGCCTCGACCTGCG	β-catenin mutant 3
5UTR mut2/3	CAGGTCGAGGCCGGTCGGCCTCCCGCGG	CCGCGGGAGGCCGACCGGCCTCGACCTG	β-catenin mutant 2/3
5UTR ctrl	CGGCGGGAGGGGCCTGTTCCCCTG	CAGGGGAACAGGCCCCTCCCGCCG	β-catenin mutant control
5UTR insert-3	CAGGTCGAGGACGGACGGACTCCCGCGG	CCGCGGGAGTCCGTCCGTCCTCGACCTG	β-catenin mutant Insert-3
5UTR insert-5	AGCGGAGAGCGAGGGGACGCGGACACGGACGAAGGACTGAGGACCAGCTTCAGTCCCC	GGGGACTGAAGCTGGTCCTCAGTCCTTCGTCCGTGTCCGCGTCCCCTCGCTCTCCGCT	β-catenin mutant Insert-5