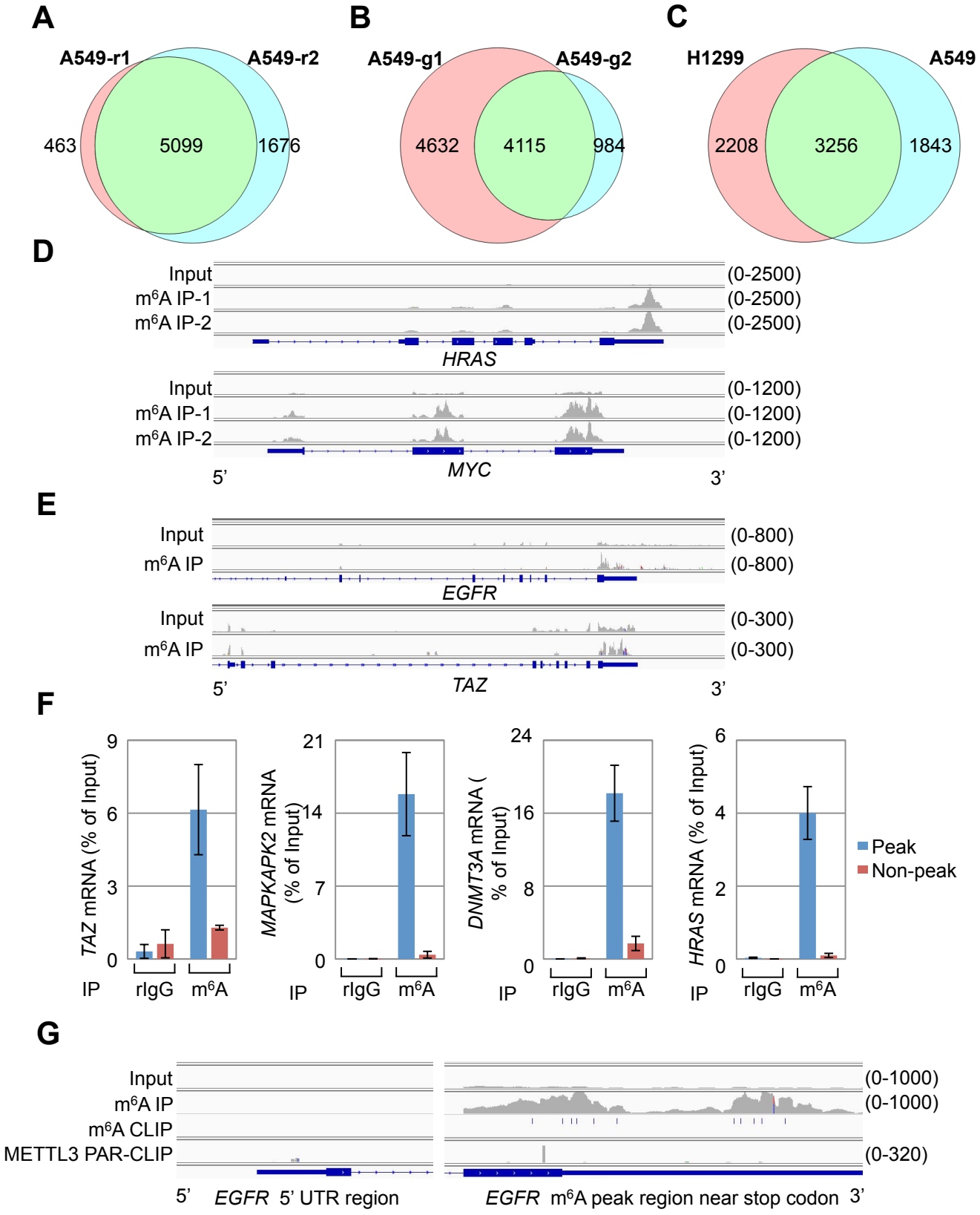
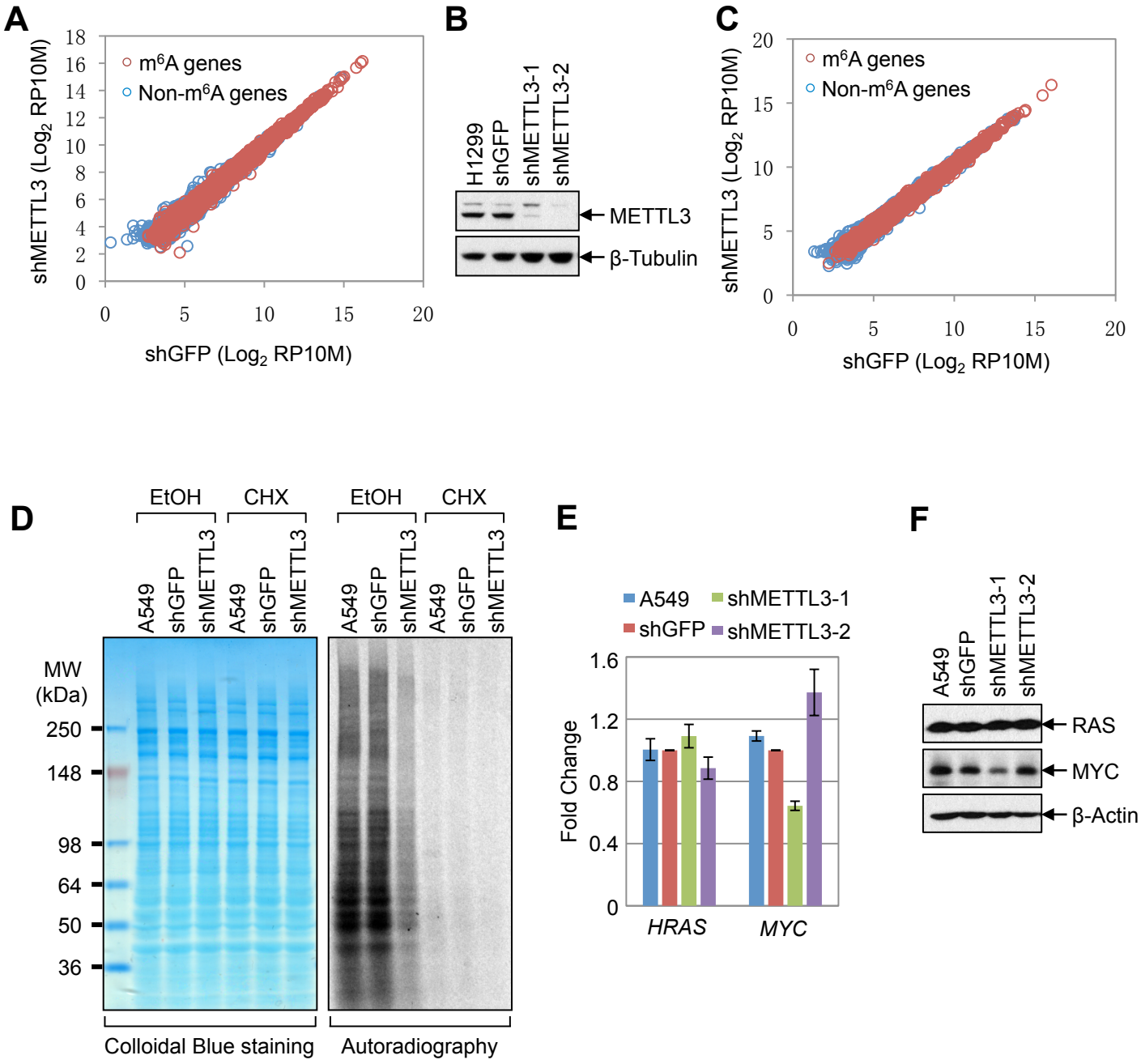
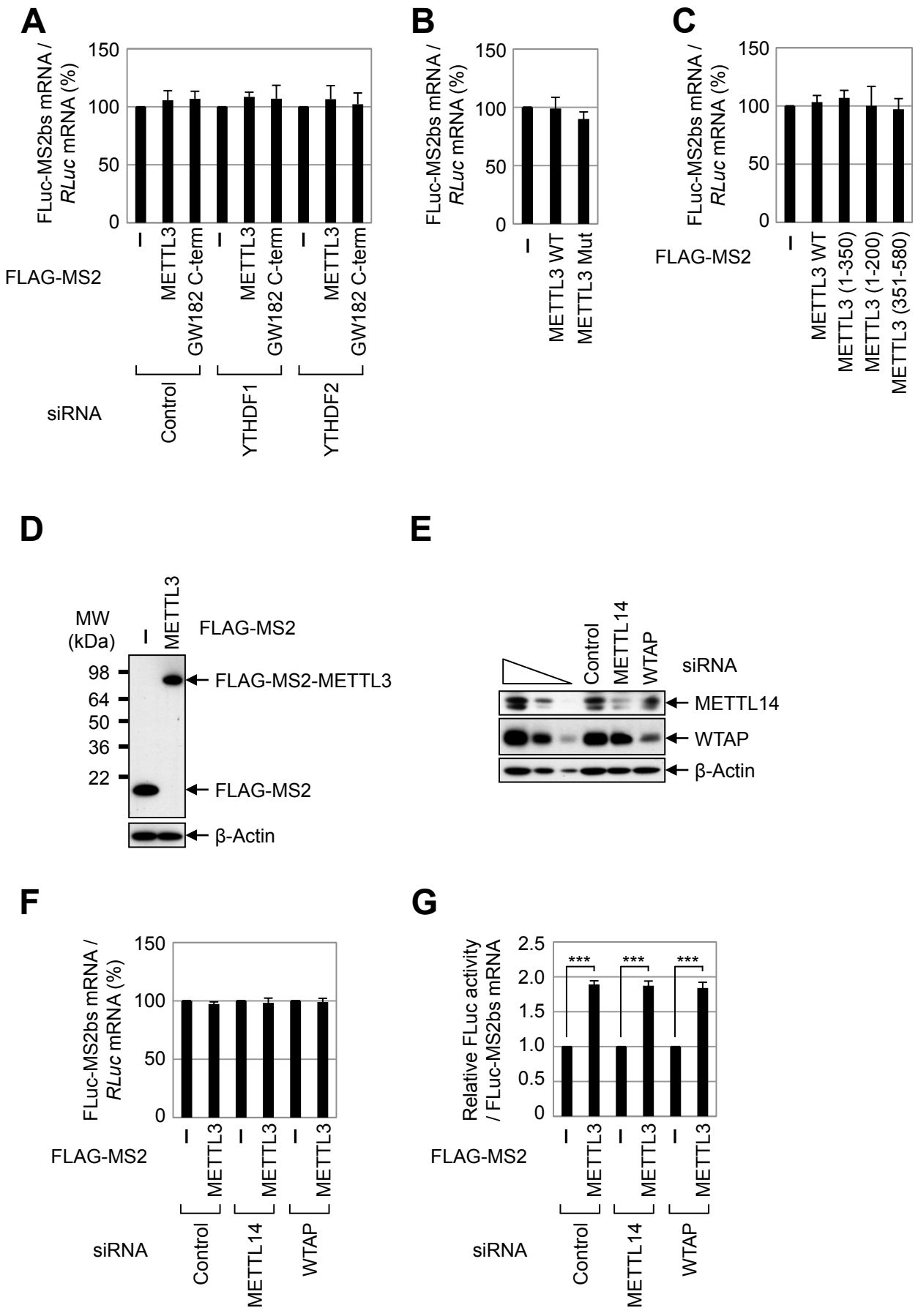
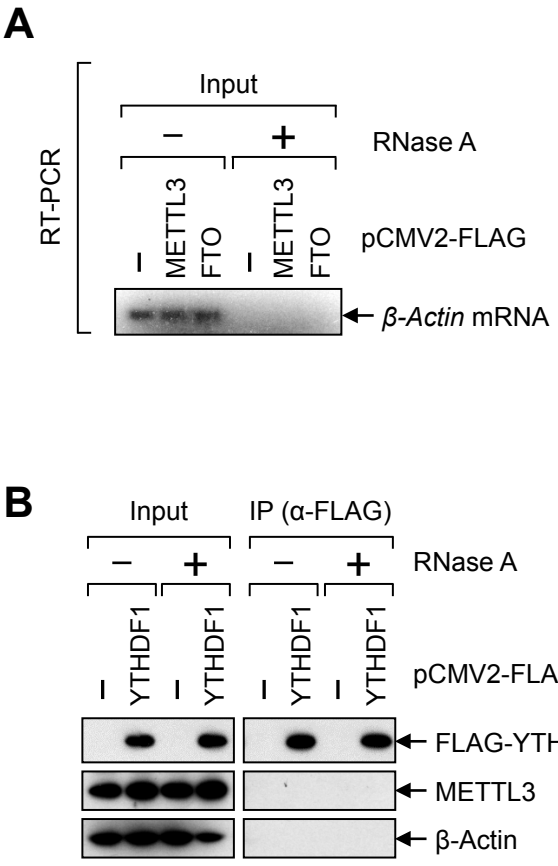
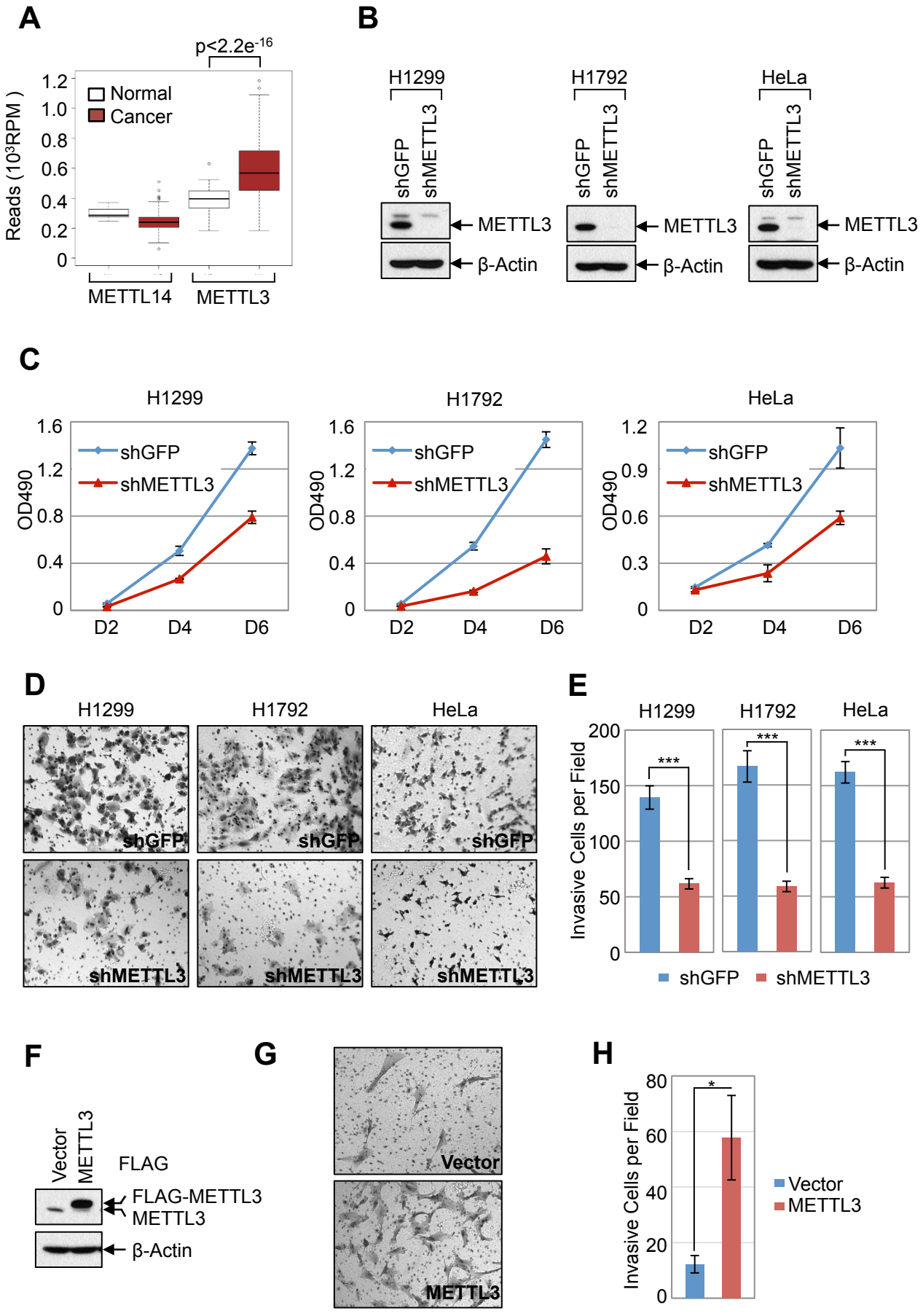

A
A549-r1
A549-r2
463
5099
1676
B
A549-g1
A549-g2
4632
4115
984
C
H1299
A549
2208
3256
1843
D
Input
m6A IP-1
m6A IP-2
(0-2500)
HRAS
(0-1200)
MYC
5'
3'
E
m6A IP
(0-800)
EGFR
(0-300)
TAZ
F
TAZ mRNA (% of Input)
MAPKAPK2 mRNA (% of Input)
DNMT3A mRNA (% of Input)
HRAS mRNA (% of Input)
IP
rIgG
m6A
Peak
Non-peak
G
m6A CLIP
METTL3 PAR-CLIP
(0-1000)
(0-320)
EGFR 5' UTR region
EGFR m6A peak region near stop codon


A
shMETTL3 (Log2 RP10M)
shGFP (Log2 RP10M)
m6A genes
Non-m6A genes
B
H1299
shGFP
shMETTL3-1
shMETTL3-2
METTL3
β-Tubulin
C
shMETTL3 (Log2 RP10M)
shGFP (Log2 RP10M)
m6A genes
Non-m6A genes
D
EtOH
CHX
A549
shGFP
shMETTL3
MW (kDa)
250
148
98
64
50
36
Colloidal Blue staining
Autoradiography
E
A549
shGFP
shMETTL3-1
shMETTL3-2
Fold Change
HRAS
MYC
F
A549
shGFP
shMETTL3-1
shMETTL3-2
RAS
MYC
β-Actin


A
FLuc-MS2bs mRNA / RLuc mRNA (%)
150
100
50
0
–
METTL3
METTL3 C-term
GW182 C-term
FLAG-MS2
siRNA
Control
YTHDF1
YTHDF2
B
METTL3 WT
METTL3 Mut
C
METTL3 WT
METTL3 (1-350)
METTL3 (1-200)
METTL3 (351-580)
D
MW (kDa)
98
64
50
36
22
FLAG-MS2-METTL3
FLAG-MS2
β-Actin
E
Control
METTL14
WTAP
siRNA
METTL14
WTAP
β-Actin
F
Relative FLuc activity / FLuc-MS2bs mRNA
2.5
2.0
1.5
1.0
0.5
0
***
G


A
Input
RT-PCR
–
+
RNase A
–
METTL3
FTO
–
METTL3
FTO
pCMV2-FLAG
β-Actin mRNA
B
Input
IP (α-FLAG)
–
+
–
+
RNase A
–
YTHDF1
–
YTHDF1
–
YTHDF1
–
YTHDF1
pCMV2-FLAG
FLAG-YTHDF1
METTL3
β-Actin


A
p<2.2e-16
Reads (10³RPM)
Normal
Cancer
METTL14
METTL3
B
H1299
H1792
HeLa
shGFP
shMETTL3
METTL3
β-Actin
C
H1299
H1792
HeLa
OD490
shGFP
shMETTL3
D2
D4
D6
D
H1299
H1792
HeLa
shGFP
shMETTL3
E
H1299
H1792
HeLa
Invasive Cells per Field
***
shGFP
shMETTL3
F
Vector
METTL3
FLAG
FLAG-METTL3
METTL3
β-Actin
G
Vector
METTL3
H
Invasive Cells per Field
*
Vector
METTL3

Supplemental Figure 1. (Related to Figure 1) (A) Overlap of m^6A peaks between individual repeats (r1: repeat 1; r2: repeat 2). **(B)** Overlap of m^6A peaks between our m6A MeRIP-Seq data (g2, this study) with a recently published A546 m6A MeRIP-Seq data (g1, GSM1828596) (Ke et al., 2015). **(C)** Comparison of m6A peaks between H1299 and A549 cells. **(D)** m^6A peaks in A549 cells. **(E)** m^6A peaks in H1299 cells. **(F)** q.RT-PCR analysis of α-m^6A IP in A549 cells using indicated PCR primers. **(G)** Analysis of EGFR 5' UTR and m^6A peak region near stop codon using published m^6A MeRIP-Seq (GSM1135033), m^6A CLIP (GSM1828596) and METTL3 PAR-CLIP (GSM1135006) data, the bars in the m^6A CLIP lane indicate methylated A sites (Ke et al., 2015; Liu et al., 2014).

Supplementary Figure 2. (Related to Figure 1) (A) Scatter plot of RNA-Seq data in METTL3 knockdown (shMETTL3) and control (shGFP) A549 cells. Two individual shRNAs against METTL3 were used for the METTL3 knockdown and the average read number from the two METTL3 knockdown samples is plotted on the y-axis. **(B)** Western blot of knockdown of METTL3 in H1299 cells. **(C)** Scatter plot of RNA-Seq data in METTL3 knockdown (shMETTL3) and control (shGFP) H1299 cells. (**D**) Metabolic labeling A549 cells with [^{35}S]-methionine. Labeled proteins were cell lysates were subjected to 4-12% Tris-Glycine SDS-PAGE and analyzed by autoradiography. Colloidal blue staining was performed to verify equal amounts of loaded protein. **(E)** q.RT-PCR analysis of mRNA levels in A549 cells using indicated primers. **(F)** Western blotting of proteins with indicated antibodies in A549 cells.

Supplemental Figure 3. (Related to Figure 3) (A-C) q.RT-PCR analysis of reporter mRNAs. FLuc-MS2bs mRNA levels were normalized to RLuc mRNAs. The FLuc:RLuc ratio obtained in MS2 (control) was set to 100%. Error bars = mean ±s.d., n=3. **(D)** α-FLAG western blot of indicated proteins. **(E)** Western blot analysis of METTL14 and WTAP expression in control and siRNA knockdown cells. **(F)** q.RT-PCR analysis of reporter mRNAs. Error bars = mean ±s.d.,

n=3. **(G)** Tethering assay to measure translation efficiency of reporter mRNAs as in Figure 3 (D). ***P<0.001. Error bars = mean ±s.d., n=3.

Supplemental Figure 4. (Related to Figure 4) (A) Effectiveness of RNase A treatment demonstrated using RT-PCR analysis of β-Actin mRNA. **(B)** Co-IPs of FLAG-YTHDF1.

Supplemental Figure 5. (Related to Figure 6) (A) Expression of METTL3 and METTL14 in normal tissue (n=41) and colon andenocarcinoma (n=228) from TCGA-COAD dataset. **(B-E)** Depletion of METTL3 regulates cellular proliferation, survival and invasion of H1299, H1792 and HeLa cells. **(B)** Western blot. **(C)** MTS assay of cellular proliferation. **(D)** *In vitro* cell invasion assay. **(E)** Quantification of invasive cells. **(F-H)** METTL3 overexpression regulate cell invasion in BJ cells. **(F)** Western blot. **(G)** *In vitro* cell invasion assay. **(H)** Quantification of invasive BJ cells. Data are presented as ±SEM. **p<0.01, ***p<0.001.

Supplemental Table 1 (Related to Figure 1): List and annotation of m6A peaks and target genes in A549 and H1299 cells

Supplemental Table 2 (Related to Experimental Procedures): List of primers used for cloning and RT-PCR

Cloning Primers	
NotI-hMETTL3-F	AACAAGCGGCCGCGTCGGACACGTGGAGCTCTATCC
BglII-hMETTL3-R	AACAAAGATCTTAGGTTTAGAGATGATACCATCTGGG
NotI-YTHDF1-F	AACAAGCGGCCGCGTCGGCCACCAGCGTGG
BglII-YTHDF1-R	AACAAAGATCTCATTGTTTGTTTCGACTCTGCCG
HindIII-MS-F	CATAAAGCTTATGGCTTCTAACTTTACTCAGTTCGTTC
HindIII-MS-R	CGATAAGCTTGTAGATGCCGGAGTTTGCTGCGATTGC
NotI-2xMS2site-F	ATAAGAATGCGGCCGCCGCGTACACGATCACGGTAC
NotI-2xMS2site-R	ATAAGAATGCGGCCGCCCCCGGGAGCATGGGTGAT
NheI-Kozak-FLAG-F	GTCATGCTAGCGCCACCATGGACTACAAAGACGATG
SwaI-METTL3-R	TCAGATTTAAATTTAGGTTTAGAGATGATACCATCTGGG

METTL3 MUT-F	CCCGCGGATATTCACATGGAACTGCC
METTL3 MUT-R	TGGGGCAGCCATCACAACTGCAAAC
PCR Primers for m6A-IP-q.PCR	
hEGFR(peak)-F	CAGAAAGGCAGCCACCAAAT
hEGFR(peak)-R	GCTTGGCTTCCTTGGGAAAG
hEGFR(non-peak)-F	AGCAGTCCTTTGTAAACAGT
hEGFR(non-peak)-R	TCTGAACCATTTCTTCCTTGAT
hTAZ(peak)-F	CCTTCTGGATTCTTGGCCCG
hTAZ(peak)-R	GTCTAAAAGCCACGTTTGAGC
hTAZ(non-peak)-F	ATGAGTTCCGAATTCCTGCGT
hTAZ (non-peak)-R	ATTCCGACATGCCACAGGG
hMAPKAPK2(peak)-F	CCCACTGAGCCACCGC
hMAPKAPK2(peak)-R	TTCCTGTAGAGAGTTATTGCTTGT
hMAPKAPK2(non-peak)-F	ACGGATCGTGGATGTGTACG
hMAPKAPK2(non-peak)-R	AGTTCTCCACCGTCCAAACA
hDNMT3A(peak)-F	TCGCTCCGCTGAAGGAGTAT
hDNMT3A(peak)-R	CTACCTCAGTTTGCCCCCAT
hDNMT3A(non-peak)-F	AGGACATCTTATGGTGCACTGA
hDNMT3Anon-peak)-R	CCAAGCGGCTCATGTTGGAG
hHRAS(peak)-F	ACTGCAGACCCTCCCAGG
hHRAS(peak)-R	GGAGCTAAGGGCTGGGGTTC
hHRAS(non-peak)-F	TGGACGAATACGACCCCACTA
hHRAS(non-peak)-R	GATGTCCAACAGGCACGTCT
RT-PCR Primers	
hEGFR-F	TCTGAGTGCAACCAGCAACA
hEGFR-R	GTGGGGTCTGAGCTGTATCG
hMETTL3-F	CAAGCTGCACTTCAGACGAA
hMETTL3-R	GCTTGGCGTGTGGTCTTT
hTAZ-F	ATGAGTTCCGAATTCCTGCGT
hTAZ-R	ATTCCGACATGCCACAGGG
hMAPKAPK2-F	ACGGATCGTGGATGTGTACG
hMAPKAPK2-R	AGTTCTCCACCGTCCAAACA
hDNMT3A-F	TGATGCCAAAGAAGTGTCAGC
hDNMT3A-R	TTCACAGTGGATGCCAACGG
h-β-Actin-F	TTCTACAATGAGCTGCGTGTG
h-β-Actin-R	GGGGTGTTGAAGGTCTCAAA
hHRAS-F	GGCATCCCCTACATCGAGAC
hHRAS-R	TCCCGGAGCTGGAGCTAGA
hMYC-F	GGACTATCCTGCTGCCAAGA
hMYC-R	CGCCTCTTGACATTCTCCTC
hRCN2-F	AGGGCATTGCACAAGAGGAG
hRCN2-R	CATCATGGAGCTGTCTGCCA
hHPRT1-F	TGACACTGGCAAAACAATGCA
hHPRT1-R	GGTCCTTTTCACCAGCAAGCT
Firefly-luc-F	GGTACTGTTGGTAAAGCCAC
Firefly-luc-R	CTCTTCATAGCCTTATGCAG
Renilla-luc-F	CACTGGGCAGGTGTCCACTC
Renilla-luc-R	GTTCTGGATCATAAACTTTC

Supplemental Table 2 (Related to Experimental Procedures): List of primers sequences used for cloning and q.RT-PCR.