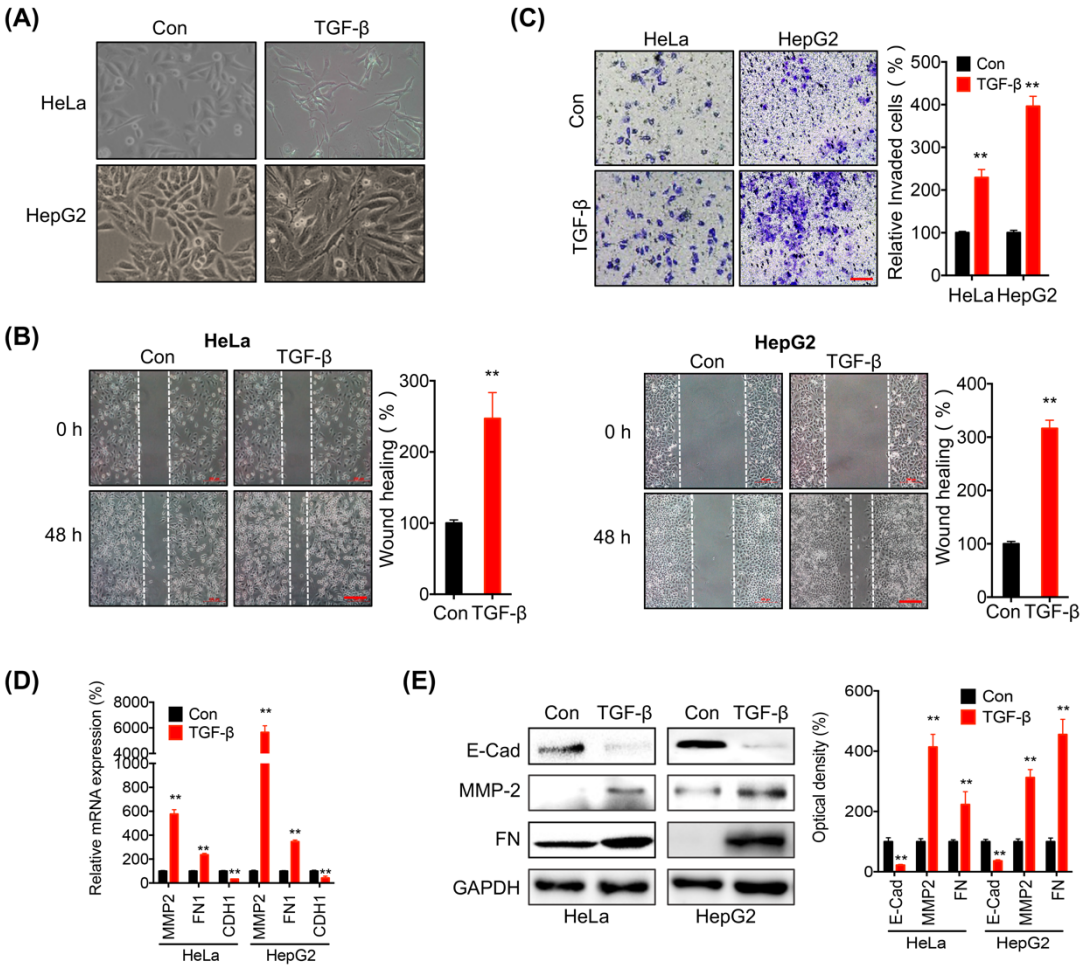
Supplementary data for

RNA m^{6}A methylation regulates the epithelial mesenchymal transition of cancer cells and translation of Snail

Lin et al

Supplementary Figures

Supplementary Figure 1



Supplementary Figure 1 Establishment of EMT models of cancer cells by TGF-β treatment.

(A) HeLa and HepG2 cells were treated with or without 10 ng/ml TGF-β for 3 days, the phenotypic changes of cells were recorded by phase contrast microscope;

(B) HeLa or HepG2 cells were treated with or without 10 ng/ml TGF-β for 48 h, wound healing was recorded (*left*) and quantitatively analyzed (*right*);

(C) The *in vitro* invasion of HeLa or HepG2 cells treated with or without 10 ng/ml TGF-β for 24 h were tested by use of CytoSelect™ 24-well Cell Invasion assay kits (8 μm, colorimetric format);

(D & E) HeLa or HepG2 cells treated with or without 10 ng/ml TGF-β for 24h (D) or 48h (E), the mRNA and protein levels of MMP-2, FN (*FN1*) and E-Cad (CDH1) were measured by

22 qRT-PCR and western blot analysis, respectively.

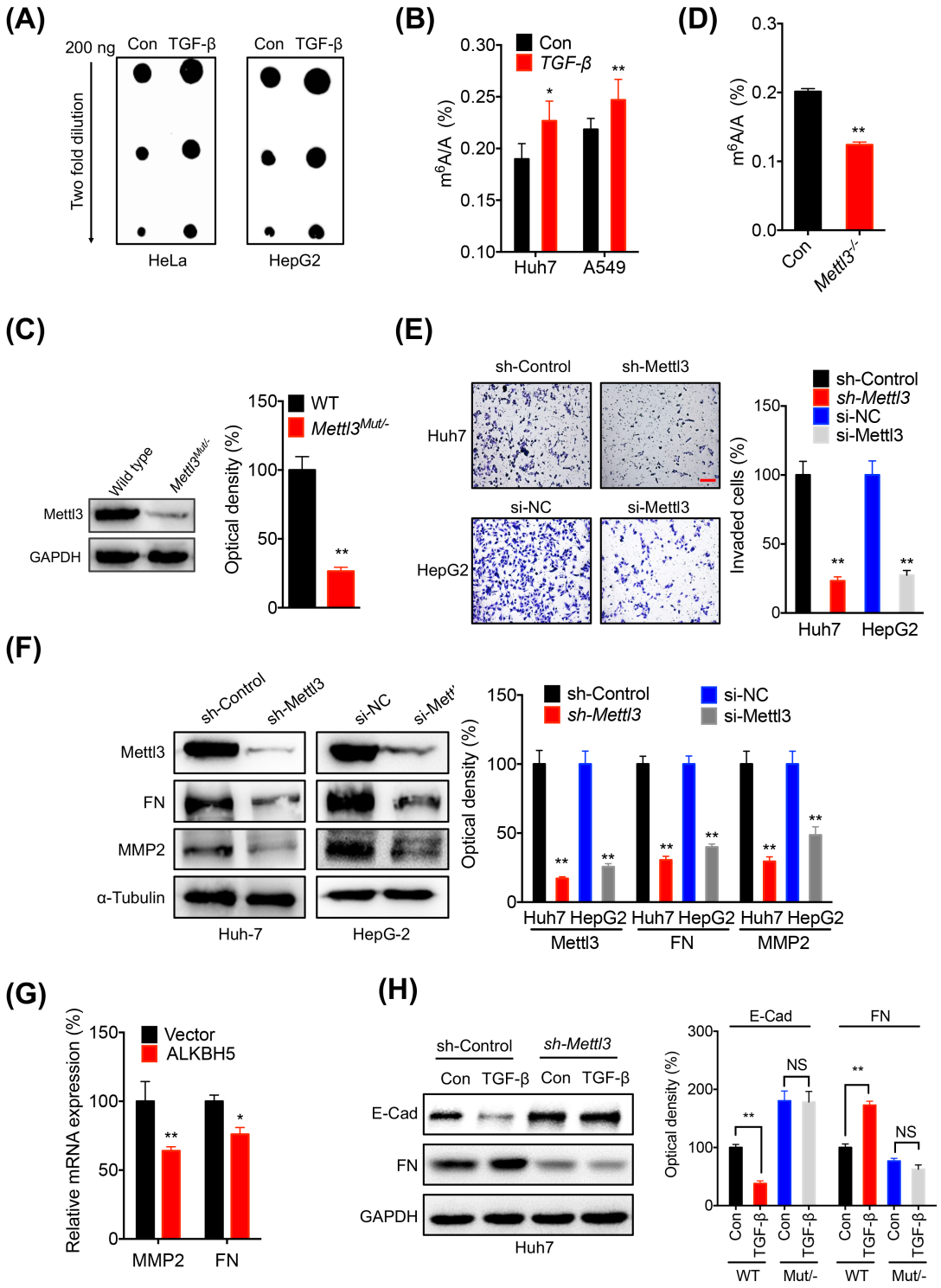
23 Data are presented as means ± SD from three independent experiments. *p<0.05, **p< 0.01

24 compared with control, by Student's t test.

25 Related to Figure 1

26

Supplementary Figure 2



Supplementary Figure 2 EMT of cancer cells is regulated by m^{6}A levels of mRNAs.

(A) Dot-blot analysis of m^{6}A levels in total mRNA of HeLa and HepG2 cells treated with or without 10 ng/ml TGF-β for 3 days;

(B) Cells were treated with or without 10 ng/ml TGF-β for 3 days, the m^6A/A ratio of total mRNA were determined by LC-MS/MS;

(C) The protein expression of METTL3 in wide type and *Mettl3*$^{Mut/-}$ HeLa cells;

(D) The m^6A/A ratio of total mRNA in wide type and *Mettl3*$^{Mut/-}$ HeLa cells;

(E) Cells were allowed to invade for 24 h and tested by CytoSelect™ 24-well Cell Invasion assay kits (8 μm, colorimetric format);

(F) The protein expression in sh-Control and sh-Mettl3 Huh7 cells or si-NC and si-Mettl3 transfected HepG2 cells was determined by western blot analysis (*left*) and quantitatively analyzed (*right*);

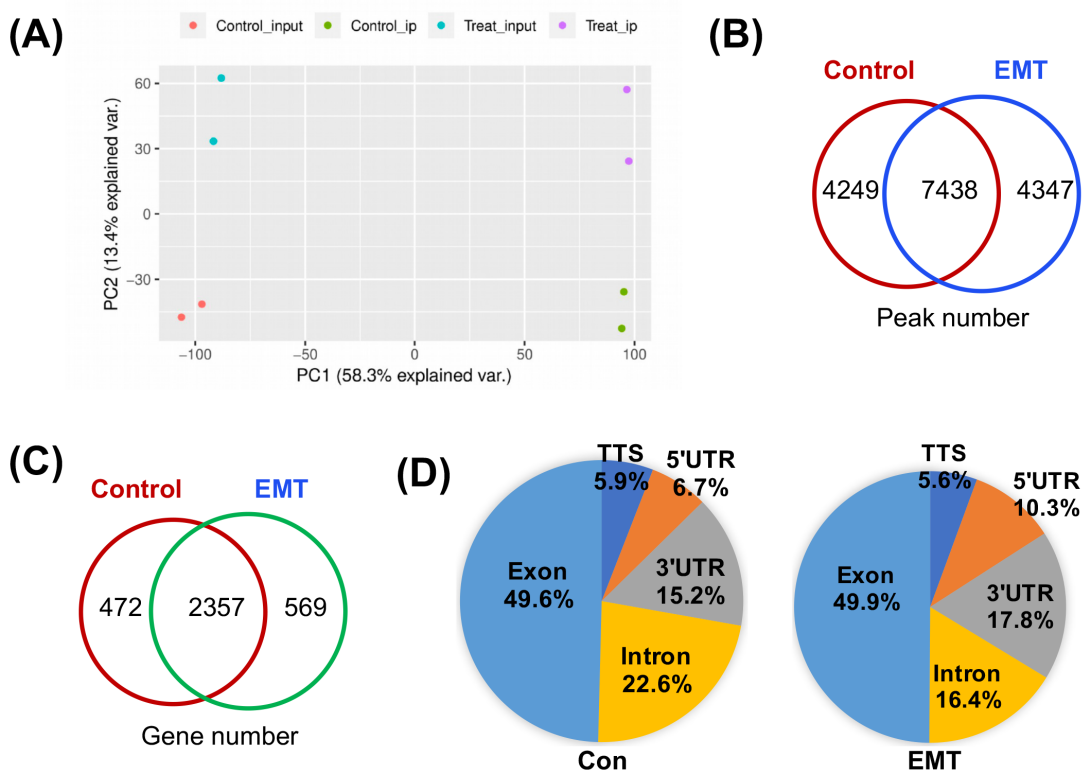
(G) HeLa cells were transfected with pcDNA (vector) or pcDNA/ALKBH5 for 24 h, the mRNA levels of MMP-2 and FN were measured by qRT-PCR;

(H) The sh-Control and sh-Mettl3 Huh7 cells were treated with or without 10 ng/ml TGF-β for 3 days, protein expression was determined by western blot analysis (*left*) and quantitatively analyzed (*right*).

Data are presented as means ± SD from three independent experiments. **p*<0.05, ***p*< 0.01; NS, no significant, by Student's *t* test.

Related to Figure 1

Supplementary Figure 3



Supplementary Figure 3 Characterization of m^{6}A-regulated genes in cancer cells undergoing EMT.

(A) PCA results for the expression levels of all genes from 8 samples;

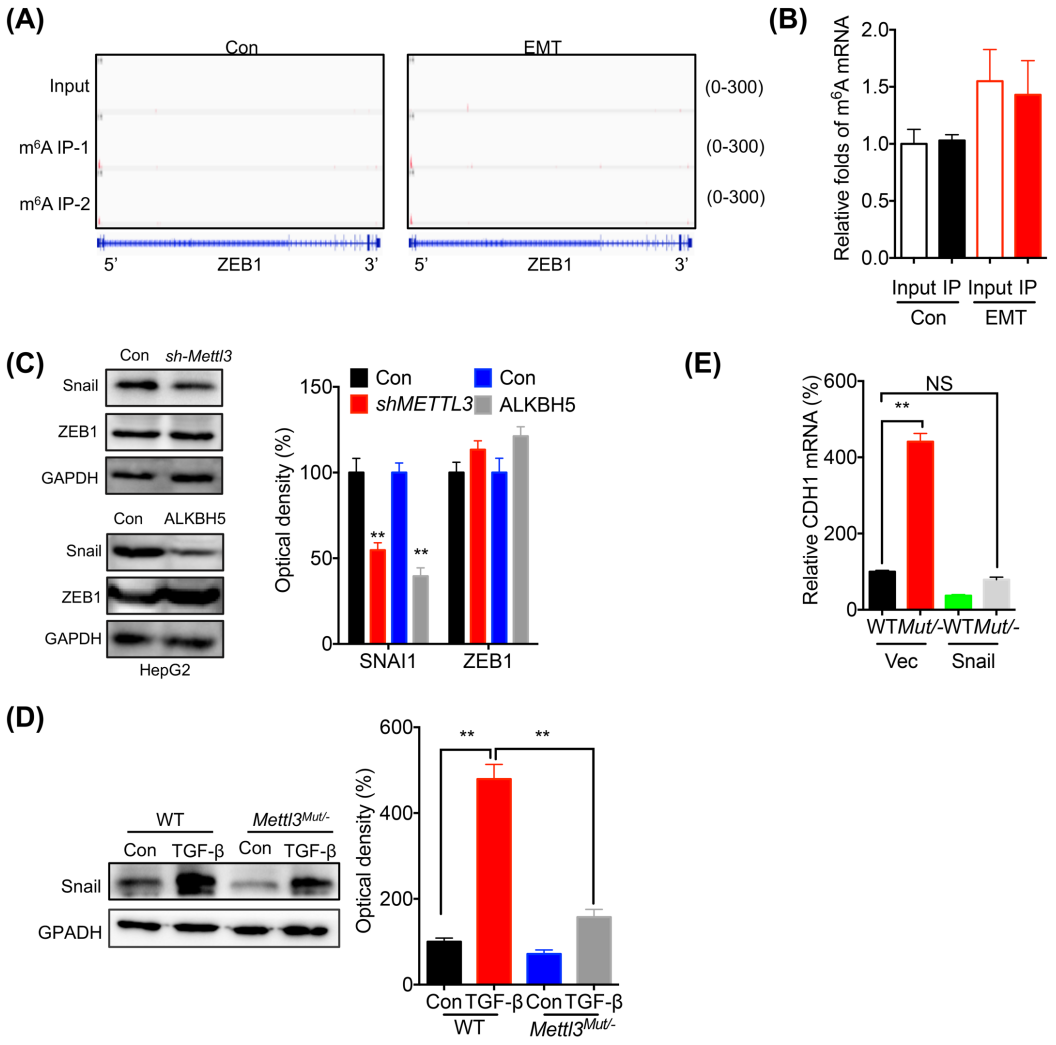
(B) Number of m^{6}A peaks identified in m^{6}A-seq from control and EMT cells;

(C) Number of m^{6}A modified genes identified in m^{6}A-seq. Common m^{6}A genes contain at least one common m^{6}A peak, while unique m^{6}A genes contain no common m^{6}A peaks;

(D) The proportion of m^{6}A peak distributions in the 5'UTR, start codon, CDS, stop codon or 3'UTR region across the entire set of mRNA transcripts;

Related to Figure 2

Supplementary Figure 4



Supplementary Figure 4 SNAI1 is involved in m^{6}A-regulated EMT in cancer cells.

(A) ZEB1 mRNA acted as negative control, without m^{6}A peak in its mRNA neither in control nor HeLa cells undergoing EMT. Data was obtained from m^{6}A RIP-seq;

(B) The m^{6}A RIP-qPCR analysis of ZEB1 mRNA in control and EMT undergoing HeLa cells;

(C) The protein expression of Snail in *si-METTL3* or AKLBH5 transfected (24 h) HepG2 and control cells was determined by western blot analysis (*left*) and quantitatively analyzed (*right*);

(D) Wild type or *Mettl3*$^{Mut/-}$ HeLa cells were treated with or without 10 ng/ml TGF-β for 48 h, the expression of Snail was detected by western blot analysis (*left*) and quantitatively

78 analyzed (*right*);

79 (E) The mRNA expression of CDH1 (E-Cad) in wild type or *Mettl3*$^{Mut/-}$ HeLa cells transfected

80 with or without pcDNA/Snail for 48 h.

81 Data are presented as means ± SD from three independent experiments. **p< 0.01, NS, no

82 significant, by one-way ANOVA with Bonferroni test.

83 Related to Figure 3

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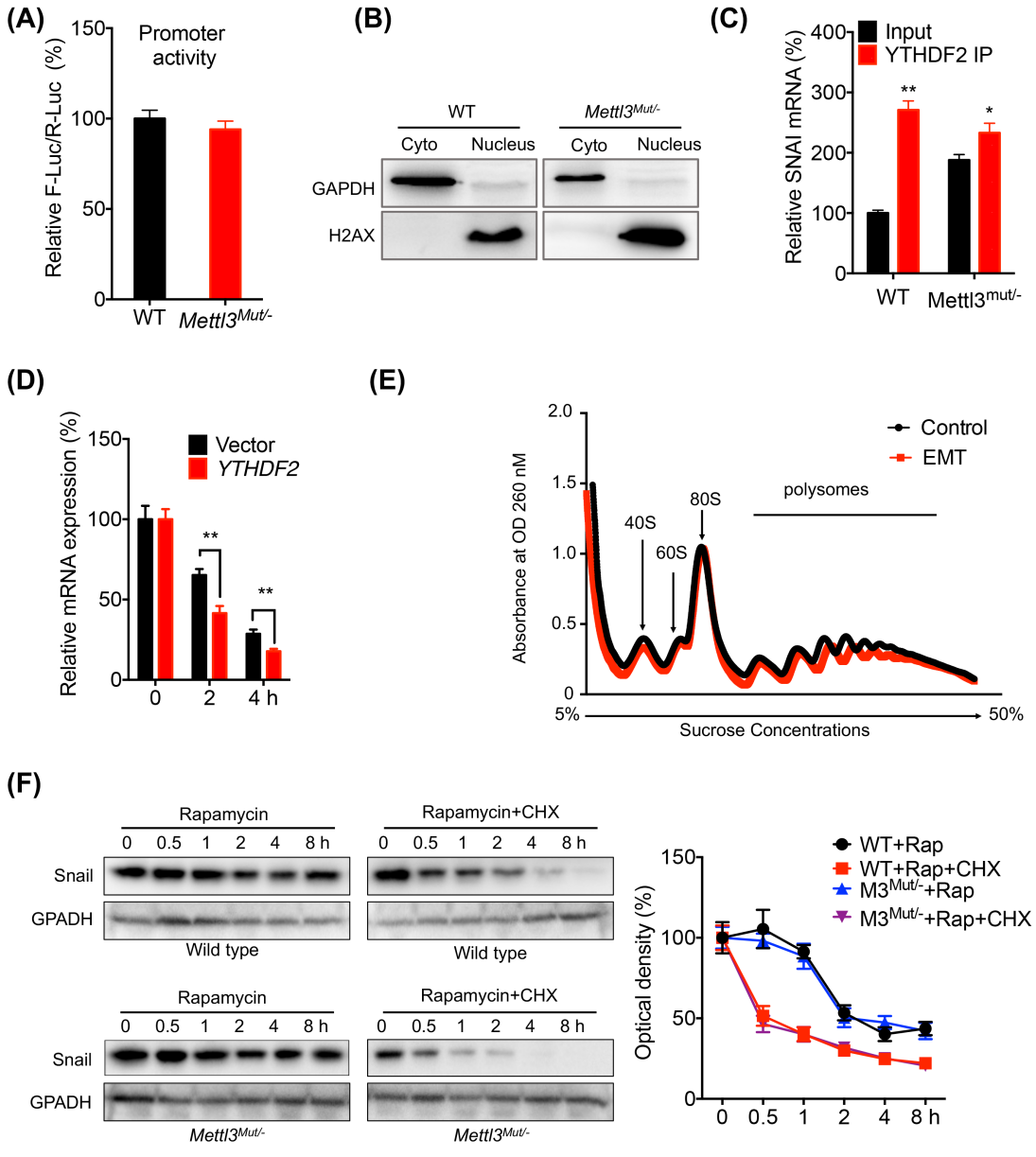
Supplementary Figure 5



Supplementary Figure 5 m^{6}A triggers the translation of Snail mRNA in cancer cells.

(A) Wild-type (WT) or *Mettl3*$^{Mut/-}$ cells were transfected with pGL3-Basic-Snail-luc reporter and pRL-TK plasmid for 24 h. Results were expressed as the ratios between the activity of the reporter plasmid and pRL-TK;

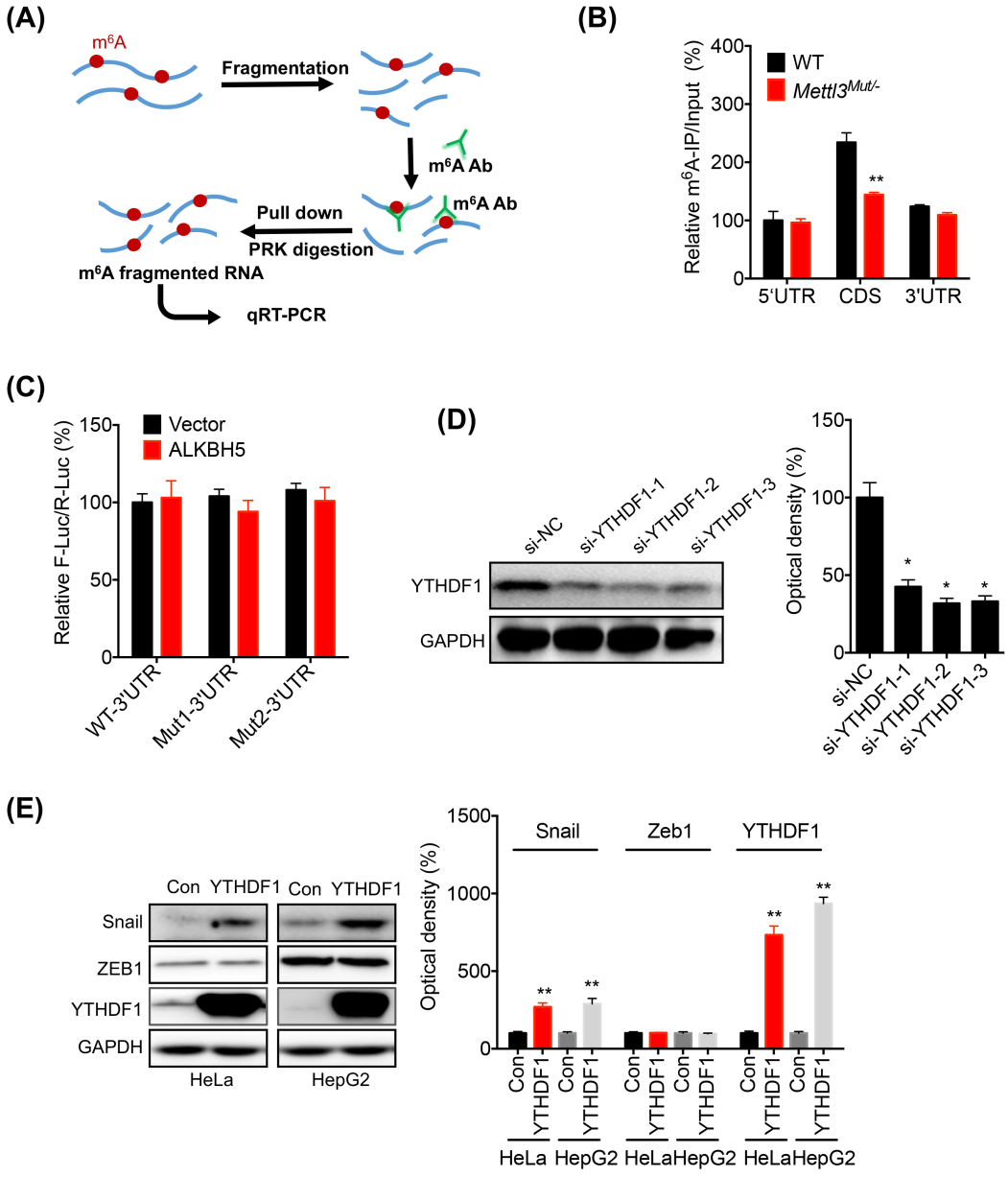
(B) The cytoplasmic and nuclear fractions of wild-type (WT) or *Mettl3*$^{Mut/-}$ cells were separated, respectively,

(C) YTHDF2 RIP-qPCR analysis of SNAI1 mRNA in wild type or *Mettl3*$^{Mut/-}$ HeLa cells;

(D) HeLa cells were transfected with vector control or pcDNA/YTHDF2 for 24 and then further

96 treated with Act-D for 90 min, then then mature mRNA of Snail was analyzed at the
97 indicated time periods;
98 (E) The polysome profiling of HeLa cells treated with or without 10 ng/ml TGF-β for 3 days;
99 (F) Immunoblot of Snail1 expression in HeLa cells treated with 50 nM rapamycin ±
100 cycloheximide (CHX, 100 μg/ml).
101 Data are presented as means ± SD from three independent experiments. *p<0.05, NS, no
102 significant, by Student's *t* test.
103 Related to Figure 4
104

Supplementary Figure 6



Supplementary Figure 6 m^{6}A methylation in CDS binding with YTHDF1 regulates the expression of Snail.

(A) Schematic representation of m^{6}A RIP-PCR with fragmented RNA from cells;

(B) m^{6}A RIP-qPCR analysis of SNAI1 mRNA in wild type or *Mettl3*$^{Mut/-}$ HeLa cells by using fragmented RNA;

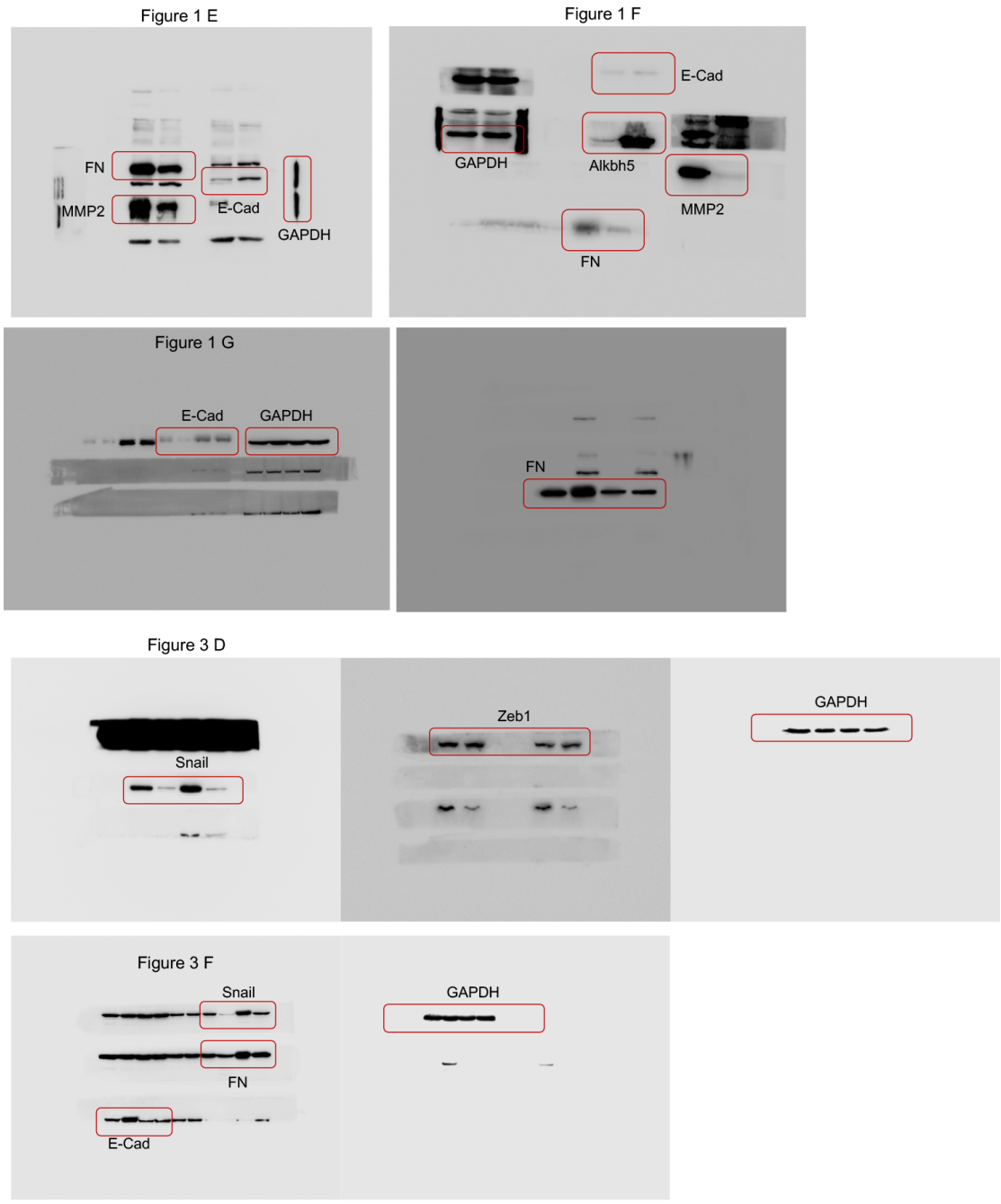
(C) HeLa cells were transfected with or without pmir-GLO-3'UTR or pmir-GLO-3'UTR_Mut1/2 and pcDNA/ALKBH5 for 24 h;

(D) HeLa cells were transfected with si-NC or si-YTHDF1-1~3 for 24 h. The si-YTHDF1-2 was chosen for further studies because it showed the highest knockdown efficiency;

(E) HeLa or HepG2 cells were transfected with empty vector (Con) or pcDNA/YTHDF1 for 24 h. Protein expression was detected by western blot analysis (*left*) and quantitatively analyzed (*right*).

Data are presented as means ± SD from three independent experiments. **p*<0.05, ***p*< 0.01, NS, no significant, by Student's *t* test.

Related to Figure 5



Supplementary Figure 7 Uncropped images of western blot analysis.

Supplementary Figure 8 Uncropped images of western blot analysis.

Supplementary Table

Supplementary Table 1 The EMT-related 84 key genes in cancer cells

Catalog	Genes
Upregulated gene during EMT	AHNAK, BMP1, CALD1, CAMK2N1, CDH2 (N-cadherin), COL1A2, COL3A1, COL5A2, FN1, FOXC2, GNG11, GSC, IGFBP4, ITGA5, ITGAV, MMP2, MMP3, MMP9, MSN, SERPINE1 (PAI-1), SNAI1, SNAI2, SNAI3, SOX10, SPARC, TCF4, TIMP1, TMEFF1, TMEM132A, TWIST1, VCAN, VIM, VPS13A, WNT5A, WNT5B.
Upregulated gene during EMT	CAV2, CDH1, DSP, IL1RN, KRT19, MITF, MST1R, NUDT13, OCLN, RGS2, SPP1, TFPI2, TSPAN13.
Differentiation & Development	AKT1, BMP1, BMP7, COL3A1, COL5A2, CTNNB1, DSP, ERBB3, F11R, FGFR2, FOXC2, FZD7, GSC, JAG1, KRT14, MST1R, NODAL, NOTCH1, PPP3R1, PTP4A1, SMAD2, SNAI1, SNAI2, SOX10, TGFB2, TGFB3, TMEFF1, TWIST1, VCAN, WNT11, WNT5A, WNT5B.
Cell Morphogenesis	CTNNB1, FOXC2, JAG1, RAC1, SMAD2, SNAI1, SOX10, TGFB1, TGFB2, TGFB3, TWIST1, WNT11, WNT5A.
Cell Growth & Proliferation	AKT1, BMP1, BMP7, CAV2, CTNNB1, EGFR, ERBB3, FOXC2, IGFBP4, ILK, JAG1, MST1R, NODAL, PDGFRB, TGFB1, TGFB2, TGFB3, TIMP1, VCAN, ZEB1.
Cell Migration & Motility	CALD1, CAV2, EGFR, FN1, ITGB1, JAG1, MSN, MST1R, NODAL, PDGFRB, RAC1, STAT3, TGFB1, VIM.
Cytoskeleton Regulators	CAV2, KRT7, MAP1B, PLEK2, RAC1, VIM.
Extracellular Matrix (ECM) & Cell Adhesion Molecules	BMP1, BMP7, CDH1, CDH2 (N-cadherin), COL1A2, COL3A1, COL5A2, CTGF, CTNNB1, DSC2, EGFR, ERBB3, F11R, FGFR2, FN1, FOXC2, ILK, ITGA5, ITGAV, ITGB1, MMP2, MMP3, MMP9, PTK2, RAC1, SERPINE1 (PAI-1), SPP1, TGFB1, TGFB2, TIMP1, VCAN.
Signal Transduction	Estrogen Receptors: CAV2. Estrogen Receptor Signaling: ESR1 (ERα), KRT19, TGFB3. G-Protein Coupled Receptor Signaling: AKT1, FZD7, GNG11, RAC1, RGS2. Integrin-Mediated Signaling: COL3A1, CTGF, ILK, ITGA5, ITGAV, ITGB1, PTK2. Notch Signaling: FOXC2, JAG1, NOTCH1. Receptor Tyrosine Kinase Signaling: EGFR, ERBB3, FGFR2, PDGFRB, RGS2, SPARC. TGFβ / BMP Signaling: BMP1, BMP7, COL3A1, SMAD2, TGFB1, TGFB2, TGFB3. WNT Signaling: CTNNB1, FZD7, GSK3B, WNT11, WNT5A, WNT5B.
Transcription Factors	CTNNB1, ESR1 (ERα), FOXC2, GSC, SMAD2, SNAI2, SNAI3, SOX10, STAT3, TCF3, TCF4, TCF7L1, TWIST1, ZEB1, ZEB2.

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