Cancer Cell, Volume *31*

Supplemental Information

FTO Plays an Oncogenic Role in Acute Myeloid Leukemia as a N^6-Methyladenosine RNA Demethylase

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SUPPLEMENTAL DATA

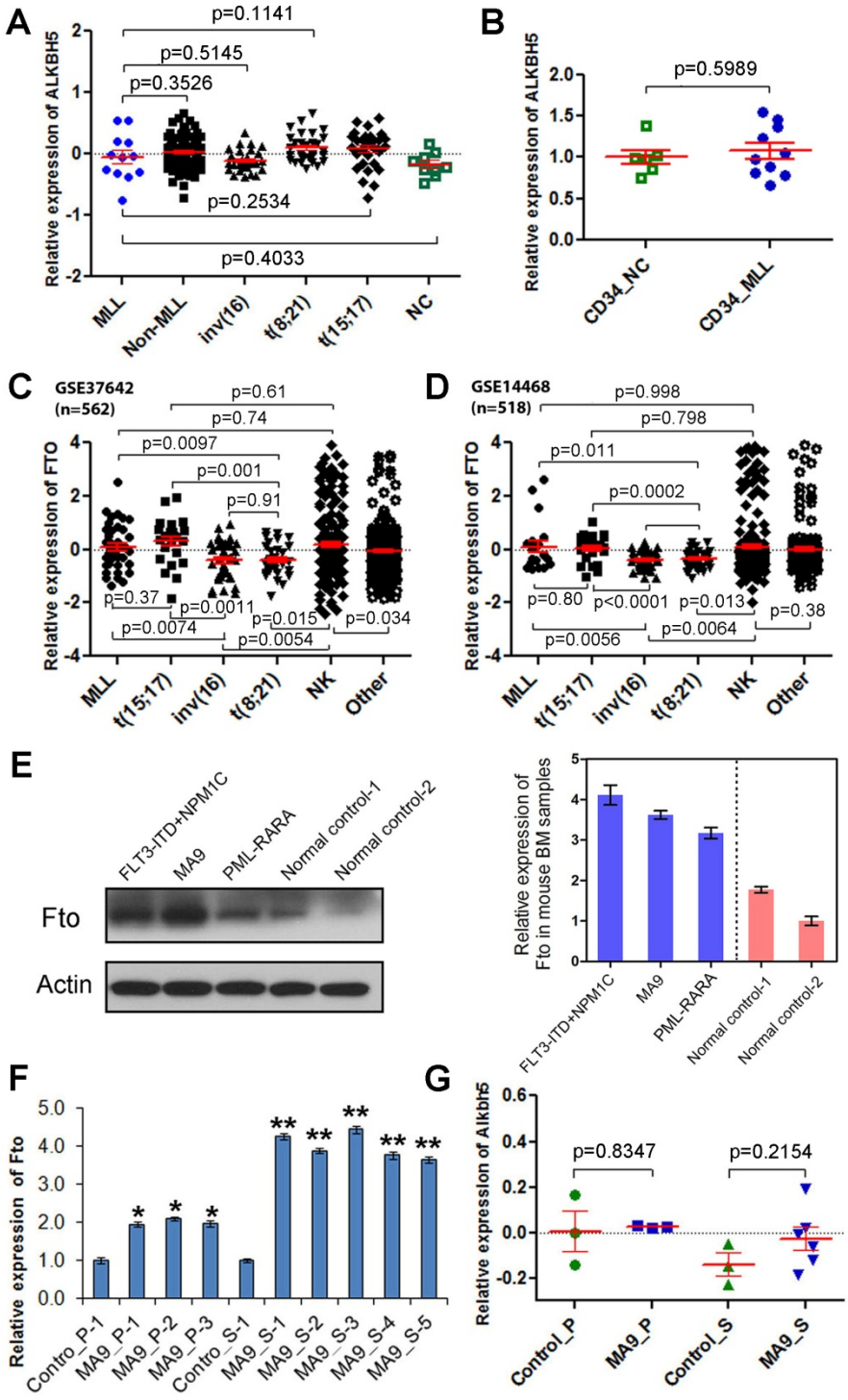

Figure S1 (related to Figure 1). Relative expression of *ALKBH5/Alkbh5* or FTO/*Fto*. (A) Comparison of *ALKBH5* expression between human primary AML cases with *MLL* rearrangements/t(11q23) (MLL) and those without *MLL* rearrangements (non-MLL), or AML

cases with inv(16), t(8;21) or t(15;17), or normal controls (NC) based Affymetrix exon array data. The expression values were detected by microarrays (Huang et al., 2013). The expression values of *ALKBH5* of the individual samples were log2-transformed and then normalized. The mean value of *ALKBH5* expression across the entire set of 109 samples was set as 0. **(B)** qPCR analysis of *ALKBH5* expression in human CD34+ AML BM cells isolated from 10 primary *MLL*-rearranged AML patients (CD34_MLL) and normal CD34+ BM cells isolated from 6 healthy donors (CD34_NC). The average expression level of *ALKBH5* in the CD34_NC samples was set as 1. **(C,D)** Relative expression of *FTO* in different AML subtypes in GSE37642 set (n=562) (**C**) and GSE14468 set (n=518) (**D**). **(E)** The protein (left panel) and RNA (right panel) levels of Fto in mouse BM progenitor cells transduced with different oncogenes or control constructs were detected by Western blot and qPCR assays, respectively. *NPM1c, NPM1* mutation, which leads to cytoplasmic delocalization of Nucleophosmin. MLL, t(11q23)/*MLL*-rearranged; MA9, *MLL-AF9*/t(9;11) fusion gene. **(F)** Relative expression of *Fto* in mouse BM cells of the control group or *MLL-AF9* (MA9) leukemic group collected from primary (_P) or secondary (_S) BMT recipient mice as detected by qPCR. **(G)** Comparison of *Alkbh5* expression between MA9 leukemic BM cells and normal control BM cells collected from the aforementioned primary or secondary BMT recipients. The expression values were detected by a microarray assay (Li et al., 2012a). Expression values of *Alkbh5* were log2-transformed and then normalized. The mean value of *Alkbh5* across the 3 normal control samples from primary BMT was set as 0**.** Error bar, mean±SEM.

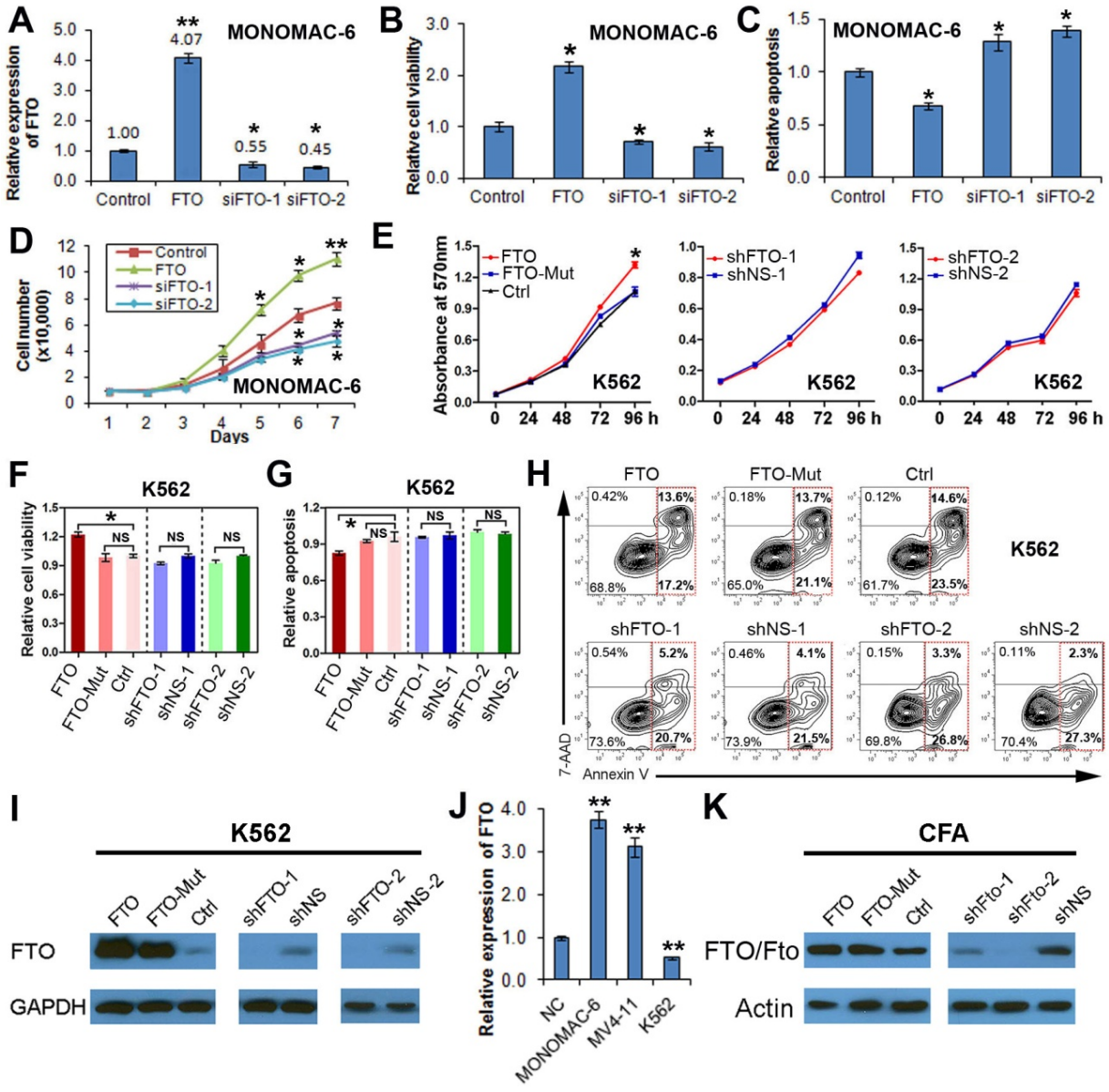

Figure S2 (related to Figure 2). Effects of forced expression and knockdown of *FTO* expression on cell apoptosis and global m^{6}A abundance in leukemia cells. (A-D) Effects of forced expression (with MSCV-PIG-FTO) or knockdown (with siRNAs) of *FTO* expression (**A**) on cell viability (**B**), apoptosis (**C**) and growth (**D**) of human MONOMAC-6/t(9;11) AML cells. **(E-I)** Effects of forced expression or knockdown of *FTO* on cell growth/proliferation (**E**), viability (**F**), apoptosis (**G** and **H**), and protein level of FTO (**I**) in K562 AML cells. FTO, pMIRNA1-FTO; FTO-Mut, pMIRNA1-FTO-Mut; Ctrl, control vector (empty pMIRNA1); shFTO-1, pLKO.1-shFTO-1; shNS-1, pLKO.1-shNS-1; shFTO-2, pGFP-C-shLenti-shFTO-2; shNS-2, pGFP-C-shLenti-shNS-2**. (J)** Relative expression of endogenous *FTO* in MONOMAC-6,

MV4-11 and K562 cells by qPCR. NC, human normal $CD34^+$ BM cells, which was used as normal control for expression level normalization and statistical analysis. **(K)** Relative expression of FTO/Fto (the antibody recognizes both FTO and Fto) in secondary passage of colony-forming cells shown in Figure 2G as detected by Western Blotting. *, $p<0.05$; **, $p<0.01$; NS, not significant ($p>0.05$); *t*-test. Error bar, mean±SD.

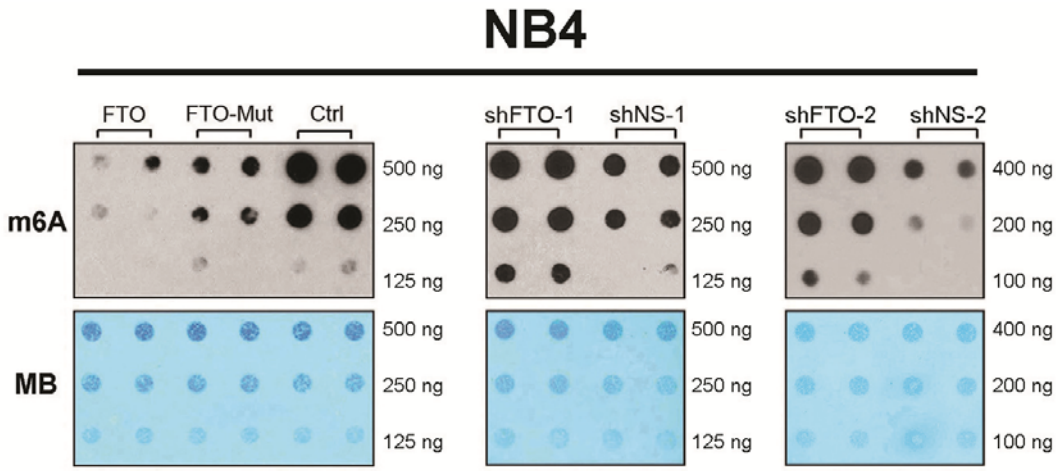

Figure S3 (related to Figure 3). Forced expression of FTO, but not FTO mutant, significantly decreased global m^6A levels, while knockdown of *FTO* by two different shRNAs increased global m^6A levels in NB4 AML cells. FTO, pMIRNA1-FTO; FTO-Mut, pMIRNA1-FTO-Mut; Ctrl, control vector (empty pMIRNA1); shFTO-1, pLKO.1-shFTO-1; shNS-1, pLKO.1-shNS-1; shFTO-2, pGFP-C-shLenti-shFTO-2; shNS-2, pGFP-C-shLenti-shNS-2; m6A, m6A dot blot staining; MB, methylene blue staining (as loading control).

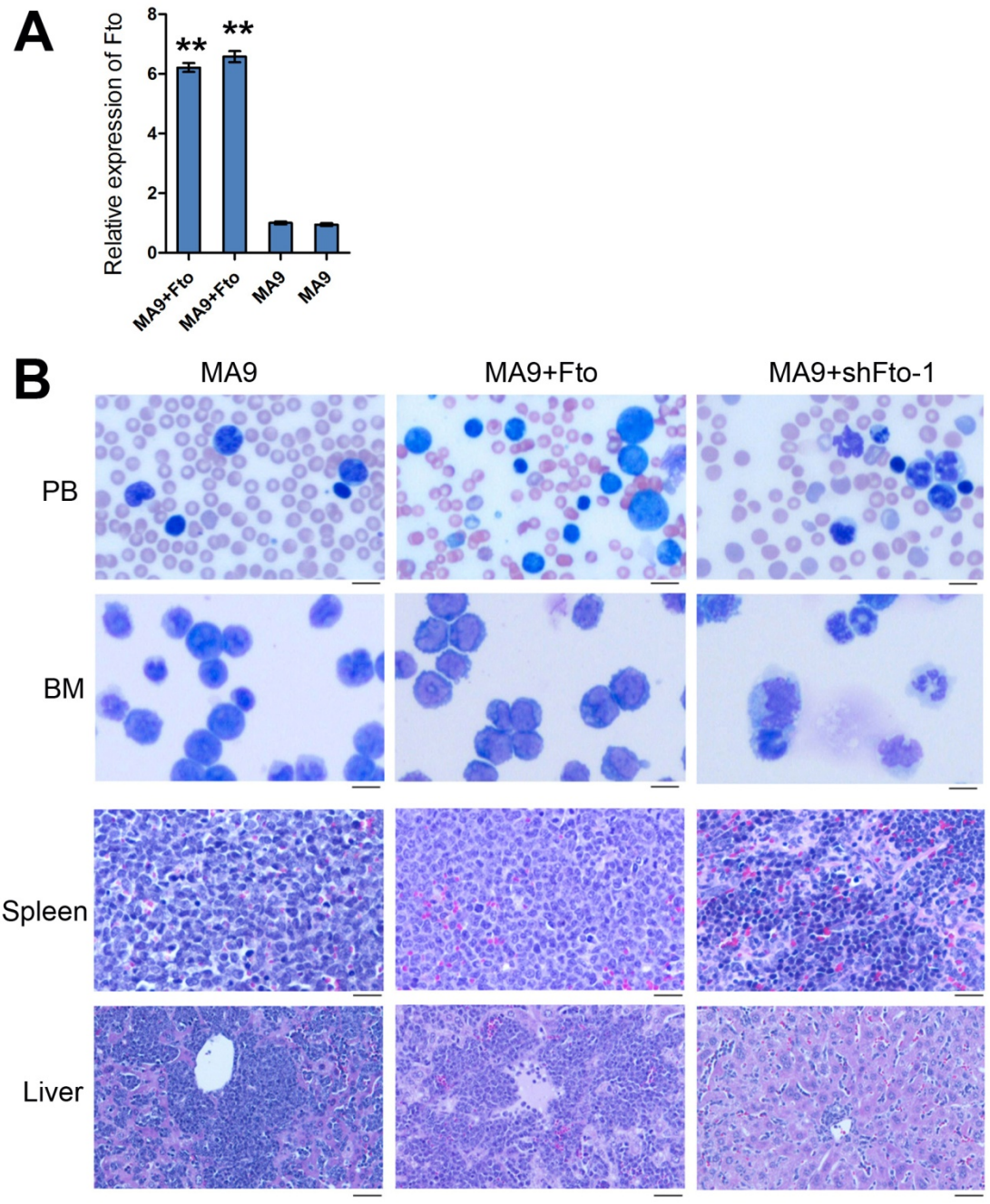

Figure S4 (related to Figure 4). Relative expression of *Fto* in mouse leukemic BM cells and histopathology analysis of representative bone marrow transplantation (BMT) mice shown in Figure 4A. (A) Relative expression of *Fto* in leukemic BM cells from representative BMT recipient mice as detected by qPCR. **, $p<0.01$; *t*-test. Error bar, mean±SD. **(B)** Wright-Giemsa stained peripheral blood (PB) and BM, and hematoxylin and eosin (H&E) stained spleen and liver of the BMT recipient mice at the end point. The length of bars (showing under the right bottom of each individual image) represents 25 μm for PB and BM, and 100 μm for spleen and liver.

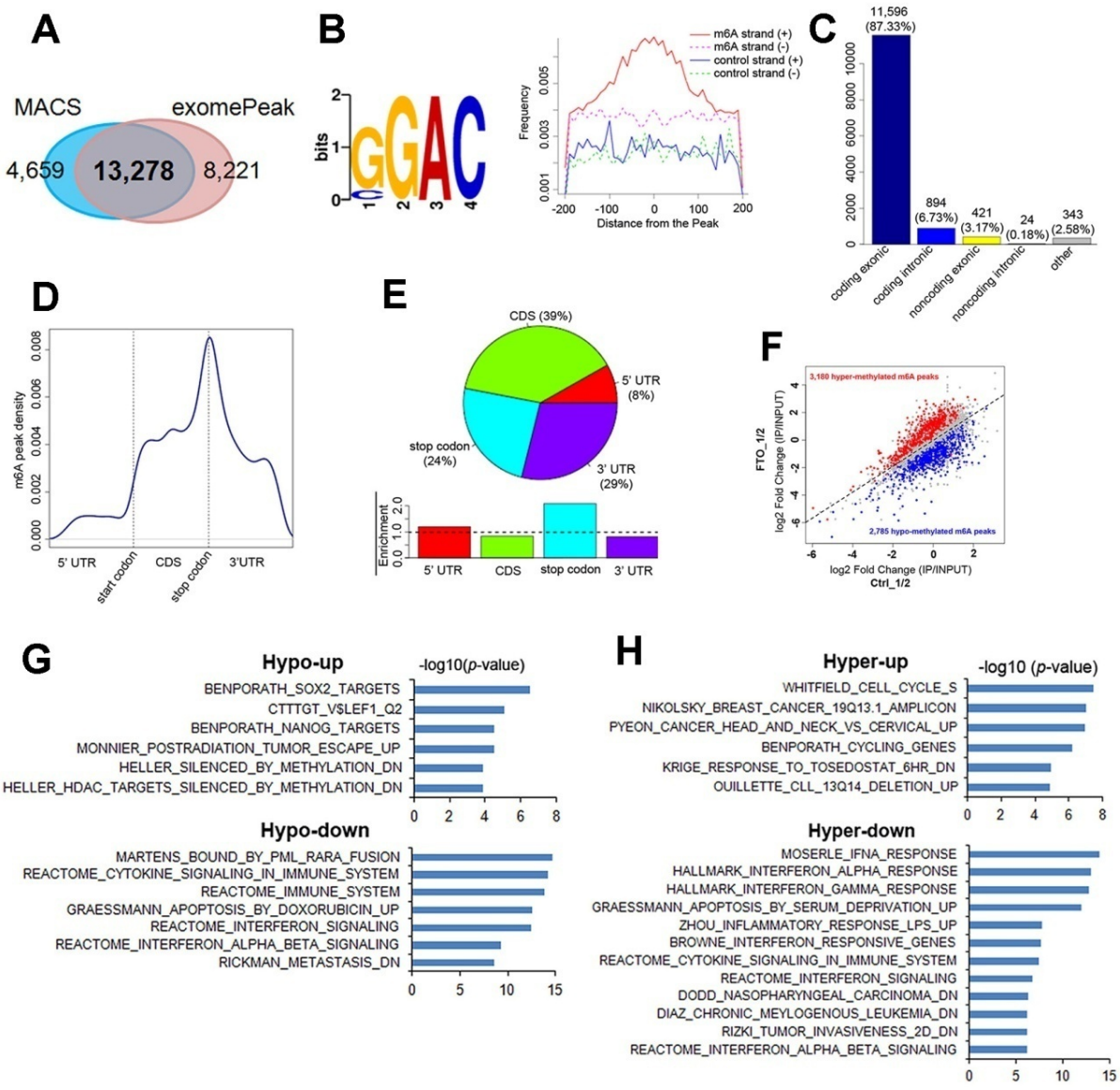

Figure S5 (Related to Figure 5). Transcriptome-wide m^{6}A-seq and analysis of m^{6}A peaks. **(A)** Identification of m^{6}A peaks by MACS and exomePeak algorithms. The numbers of m^{6}A peaks identified by each or both of these two algorithms from at least two of the four m^{6}A-seq libraries (i.e., FTO_1/2 and Ctrl_1/2) after normalized by input are shown. **(B)** A top m^{6}A motif identified from the 13,278 mRNA fragments with the reliable m^{6}A peaks (8,902/13,278 in the m^{6}A fragments *vs.* 754/2,309 in the control fragments, p=1.5e-210) is shown in the left panel, while the location and frequency of this motif in the positive strands of the mRNA fragments with the reliable m^{6}A peaks, or in the negative strands of these fragments, or in the positive or

negative strands of control mRNA fragments (i.e., those with no m^6A peaks) are shown in the right panel. **(C)** Distribution of m^6A peaks in exonic or intronic regions of protein-coding genes or non-coding genes, or in other regions. **(D)** Distribution of m^6A peaks across the length of mRNA. Each region of 5'untranslated region (5' UTR), coding region (CDS), and 3'untranslated region (3' UTR) were binned into 50 segments, and the percentage of m^6A peaks that fall within each bin was determined. **(E)** The proportion (upper panel) and enrichment (lower panel) of m^6A peak distribution in the 5'UTR, CDS, stop codon or 3' UTR region across the entire set of mRNA transcripts. The enrichment was determined by the proportion of m^6A peaks normalized by the length of the region. (**F**) Comparison of the abundance of m^6A peaks and the expression level of their associated entire RNA transcripts between human MONOMAC-6 cells with forced expression of *FTO* (i.e., FTO_1 and _2) and the control cells (i.e., Ctrl_1 and _2). Identification of 2,785 hypo- and 3,180 hyper-methylated m^6A peaks that showed a significant decrease and increase ($p<0.005$; fold change ≥ 1.2), respectively, in abundance in FTO_1/2 cells relative to Ctrl_1/2 cells**.** The remaining m^6A peaks in the list of the 13,278 m^6A peaks were termed as unchanged m^6A peaks**.** IP/INPUT, immunoprecipitation/input**,** which refers to m^6A peak abundance detected in m^6A-seq assay (IP) normalized by that detected in RNA-seq assay (INPUT). **(G)** Gene set enrichment analysis (GSEA) of genes with a significant decrease in m^6A levels as well as a significant increase (Hypo-up) or decrease (Hypo-down) in overall transcript levels in *FTO*-overexpressing AML cells. **(H)** GSEA analysis of genes with a significant increase in m^6A levels as well as a significant increase (Hyper-up) or decrease (Hyper-down) in overall transcript levels in *FTO*-overexpressing MonoMac-6 AML cells.

Table S1 (Related to Figure 5). Summary of the m^{6}A-seq and RNA-seq of four cell lines

Sample	Seq Type	Total Reads	Aligned Reads
Ctrl_1	**RNA-seq**	45,887,508	41,424,514 (90.3%)
	m^{6}A-seq	30,829,176	27,747,658 (90%)
Ctrl_2	**RNA-seq**	29,936,502	27,386,859 (91.5%)
	m^{6}A-seq	34,024,847	30,946,880 (91.0%)
FTO_1	**RNA-seq**	83,147,818	77,079,571 (92.7%)
	m^{6}A-seq	20,466,641	19,036,992 (93%)
FTO_2	**RNA-seq**	17,714,000	16,361,563 (92.4%)
	m^{6}A-seq	24,903,917	23,322,084 (93.6%)

Table S2 (Related to Figure 5). List of 25 genes that exhibit a significant change between *FTO*-overexpressing and control MONOMAC-6 AML cells in m^{6}A peak levels, and abundance of the corresponding mRNA transcript, and are also significantly positively or negatively correlated with *FTO* in expression in four datasets of large cohorts of AML[a]

Gene	Pattern[b]	m^{6}A level change[c]						mRNA transcript abundance change		Correlation between the given gene and *FTO* in expression ind							
		Peak 1			Peak 2					In-house set (n=109)		TCGA set (n=177)		GSE37642 set (n=562)		GSE14468 set (n=518)	
		Fold	*p*	Loc	Fold	*p*	Loc	Fold	*p*	r	*p*	r	*p*	r	*p*	r	*p*
ASB2	Hypo-down	0.58	6.9E-08	3'UTR				0.46	1.8E-09	-0.28	0.0030	-0.22	0	-0.21	3.4E-07	-0.18	3.1E-05
KCNG1	Hypo-down	0.21	2.3E-07	CDS				0.40	4.5E-07	-0.31	0.0012	-0.15	0.05	-0.34	5.5E-17	-0.1	0.02
PPARD	Hypo-down	0.56	0.0015	3'UTR	0.74	9E-15	StopC	0.62	1.4E-13	-0.36	0.0001	-0.28	0	-0.34	2.1E-16	-0.19	1.0E-05
RAB17	Hypo-down	0.47	9.8E-15	3'UTR				0.30	0.0002	-0.47	2.3E-07	-0.17	0.02	-0.25	3.6E-09	-0.09	0.03
RARA	Hypo-down	0.68	4.3E-15	3'UTR	0.80	3E-05	5'UTR	0.66	2.9E-07	-0.37	5.9E-05	-0.26	0.0006	-0.35	5.7E-18	-0.21	2.3E-06
SLC11A1	Hypo-down	0.63	1.0E-08	StopC	0.79	0.001	3'UTR	0.20	0	-0.32	0.0007	-0.34	4.3E-06	-0.37	1.9E-19	-0.28	5.7E-11
SLCO4A1	Hypo-down	0.29	1.2E-14	CDS	0.69	0.004	StopC	0.50	0.0002	-0.47	3.2E-07	-0.28	0	-0.16	0.0001	-0.1	0.03
TBC1D9	Hypo-down	0.34	4.2E-06	CDS				0.02	0	NA	NA	-0.2	0.01	-0.24	1.3E-08	-0.24	3.2E-08
C21orf59	Hypo-up	0.70	0.0019	CDS				1.27	0.0039	0.64	6.0E-14	0.33	8.2E-06	0.5	1.4E-36	0.25	4.0E-09
MZF1	Hypo-up	0.50	1.9E-52	CDS				1.31	0.0027	0.19	0.0446	0.24	0	0.44	3.3E-28	0.32	9.8E-14
TXLNA	Hypo-up	0.74	5.5E-06	3'UTR				1.21	5.6E-16	0.40	1.9E-05	0.21	0.01	0.25	1.9E-09	0.21	1.9E-06
AIM1	Hyper-down	1.49	5.0E-05	CDS				0.43	0	NA	NA	-0.2	0.01	-0.18	1.6E-05	-0.3	1.6E-12
HLA-F	Hyper-down	2.16	0.0007	Intron				0.27	2.8E-11	-0.21	0.0256	-0.32	1.3E-05	-0.37	2.6E-19	-0.33	1.3E-14
RHBDF2	Hyper-down	1.26	0.0046	StopC	1.19	0.028	3'UTR	0.62	0	-0.24	0.0106	-0.35	2.3E-06	-0.23	2.0E-08	-0.35	9.5E-17
SLFN5	Hyper-down	1.23	0.0017	CDS				0.30	0	-0.32	0.0008	-0.17	0.02	-0.39	2.7E-22	-0.12	0.01
CEP78	Hyper-up	1.86	5.9E-07	3'UTR	1.16	0.012	StopC	1.41	0.0015	NA	NA	0.36	6.3E-07	0.21	3.0E-07	0.14	0
HIBADH	Hyper-up	1.77	0.0012	3'UTR				1.39	9.3E-05	0.54	1.4E-09	0.15	0.04	0.42	9.6E-26	0.13	0
MLF1IP	Hyper-up	1.58	0.0028	3'UTR				1.27	0.0022	0.30	0.0018	0.45	4.4E-10	0.3	4.1E-13	0.29	1.7E-11
PPAPDC1B	Hyper-up	1.23	1.9E-04	3'UTR				1.69	0	-0.03	0.7421	0.19	0.0136	0.39	1.2E-21	0.28	1.8E-10
RANBP6	Hyper-up	1.77	8.9E-97	CDS				1.43	0	NA	NA	0.36	7.7E-07	0.3	2.6E-13	0.32	6.3E-14
S100PBP	Hyper-up	1.26	1.9E-07	CDS				1.30	0	0.37	9.3E-05	0.21	0	0.41	1.2E-23	0.24	5.0E-08
ZFP62	Hyper-up	1.37	0.0002	3'UTR	1.14	8E-06	CDS	1.42	0.0003	NA	NA	0.37	5.3E-07	0.51	1.2E-38	0.37	7.9E-18
ZNF320	Hyper-up	2.28	0.0014	StopCd	2.33	1E-10	CDS	1.43	6.0E-11	NA	NA	0.15	0.05	0.32	3.3E-15	0.27	8.1E-10
ZNF445	Hyper-up	1.21	0.0005	3'UTR	1.32	7E-09	CDS	1.23	0	0.20	0.0373	0.22	0	0.5	3.3E-36	0.26	2.1E-09
ZNF605	Hyper-up	2.33	0.0227	CDS				1.55	1.1E-09	0.31	0.0009	0.16	0.04	0.46	3.8E-31	0.29	3.3E-11

aFold, fold change; Loc, location; 3'UTR, 3' untranslated region; 5'UTR, 5' untranslated region; CDS, coding region; StopC, stop codon; *p*, *p* value; r, correlation coefficient.

bHypo-down and Hypo-up refer to the gene has a significant decrease in at least one m^{6}A peak and a significant decrease and increase, respectively, in the overall abundance of the mRNA transcript in *FTO*-overexpressing MonoMac-6 AML cells compared to the control AML cells; Hyper-down and Hyper-up refer to the gene has a significant increase in at least one m^{6}A peak and a significant decrease and increase, respectively, in the overall abundance of the mRNA transcript in *FTO*-overexpressing MonoMac-6 AML cells compared to the control AML cells.

cSix genes have two m^{6}A peaks exhibiting a significant change between the *FTO*-overexpressing and control MonoMac-6 AML cells.

dThe in-house set (n=109) (Huang et al., 2013; Li et al., 2013a; Li et al., 2012b), TCGA set (n=177) (Ley et al., 2013), and GSE14468 set (n=518) (Li et al., 2013a; Taskesen et al., 2011; Wouters et al., 2009) were generated Affymetrix human exon arrays, RNA-seq, Affymetrix U133Plus2.0 arrays, respectively. GSE37642 set (n=562) (Herold et al., 2014; Janke et al., 2014; Li et al., 2013a) were generated by Affymetrix U133Plus2.0 (n=140) and U133A and B (U133A+B) (n=422) arrays. Pearson correlation was used to analyze the correlation between the given gene and FTO in expression in each dataset. NA, expression data is not available in that dataset (the gene was not included in the microarrays).

Table S3 (Related to Figure 5). The patterns of the m^{6}A level and RNA transcript abundance changes of the 11 m^{6}A-Hypo genes (shown in Table S2) in human AML cells with *FTO* overexpression or knockdown

Gene	In *FTO*-overexpressing MONOMAC-6 cells relative to the control MONOMAC-6 cells	In *FTO*-knockdown *MA9/FLT3*-ITD cells relative to the control *MA9/FLT3*-ITD cells (with expected patterns?)
ASB2	Hypo-down	Hyper-Up (Yes)
KCNG1	Hypo-down	Hyper-Up (Yes)
PPARD	Hypo-down	Hyper-Up (Yes)
RAB17	Hypo-down	Hyper-Up (Yes)
RARA	Hypo-down	Hyper-Up (Yes)
SLC11A1	Hypo-down	Hyper-down (No)
SLCO4A1	Hypo-down	Hyper-up (Yes)
TBC1D9	Hypo-down	ND[a] (-)
C21orf59	Hypo-up	Hyper-down (Yes)
MZF1	Hypo-up	Hyper-down (Yes)
TXLNA	Hypo-up	Hyper-up (No)

[a]ND, with no detectable m^{6}A peaks.

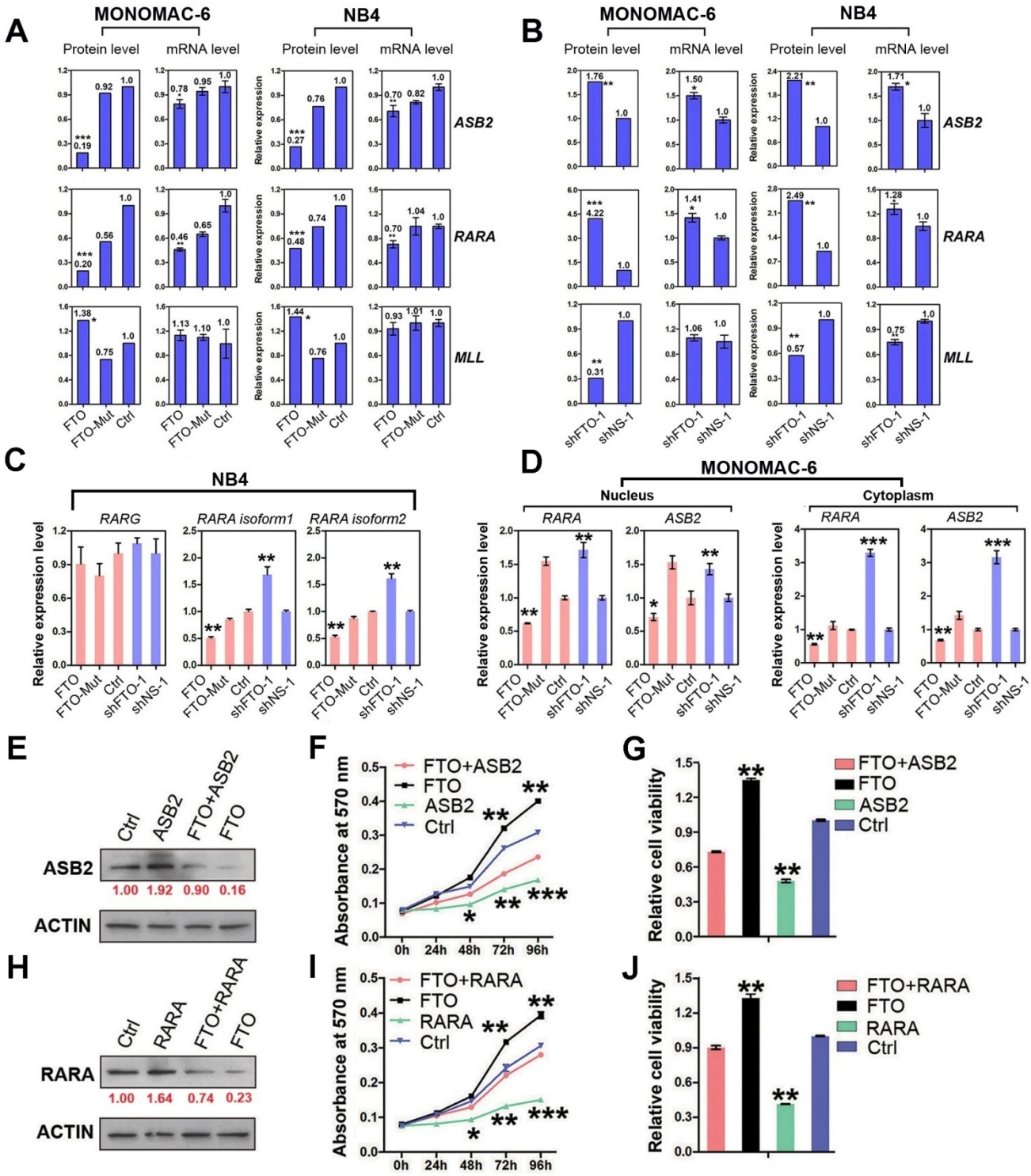

Figure S6 (related to Figure 6). FTO functioned as an oncogene via targeting *ASB2* and *RARA* in AMLs. **(A,B)** Quantitative changes of ASB2, RARA and MLL at the protein and mRNA levels in MONOMAC-6 or NB4 AML cells with forced expression of *FTO* (or *FTO* mutant) (**A**) or *FTO* knockdown (**B**), compared to the corresponding control cells. The relative quantitative information about the protein level changes was derived from Western blot assays of ASB2, RARA and MLL in MONOMAC-6 or NB4 AML cells with lentivirally

transduced *FTO*, *FTO* mutant (H231A and D233A) or control (Ctrl) construct, or with *FTO* shRNA (shFTO) or non-specific scrambled shRNA (shNS); the original images of the Western blot were shown in Figure 6A and B. The mRNA level changes were detected by qPCR. ACTIN was used as the endogenous control for both Western blot and qPCR assays. **(C)** Relative expression of *RARG* and two *RARA* isoforms in NB4 cells with forced expression of *FTO* or *FTO*-Mut or without (Ctrl), or with knockdown of *FTO* (shFTO-1) or without (shNS-1). **(D)** Relative level of *ASB2* and *RARA* in nucleus and cytoplasm of MONOMAC-6 cell with forced expression of *FTO*, *FTO*-Mut or knockdown of *FTO*. **(E)** Western blot assays of ASB2 in MONOMAC-6 AML cells lentivirally transduced with *ASB2* (pmiRNA1+ pLJM1-*ASB2*), *FTO*+*ASB2* (pmiRNA1-*FTO*+ pLJM1-*ASB2*), *FTO* (pmiRNA1-*FTO*+ pLJM1), or control vector (Ctrl; pmiRNA1+ pLJM1). **(F, G)** Effects of forced expression of *FTO* and/or *ASB2* on cell growth/proliferation (**F**) and viability (**G**) in MONOMAC-6 AML cells. **(H)** Western blot assays of RARA in MONOMAC-6 AML cells lentivirally transduced with *RARA* (pmiRNA1 + pCDH-*RARA*), *FTO*+*RARA* (pmiRNA1-*FTO* + pCDH-*RARA*), *FTO* (pmiRNAs-*FTO* + pCDH) or control vector (Ctrl; pmiRNA1 + pCDH). **(I, J)** Function of forced expression of *FTO* and/or *RARA* on cell growth/proliferation (**I**) and viability (**J**) in MONOMAC-6 cells. *, $p<0.05$; **, $p<0.01$; ***, $p< 0.001$; *t*-test. Error bar, mean±SD.

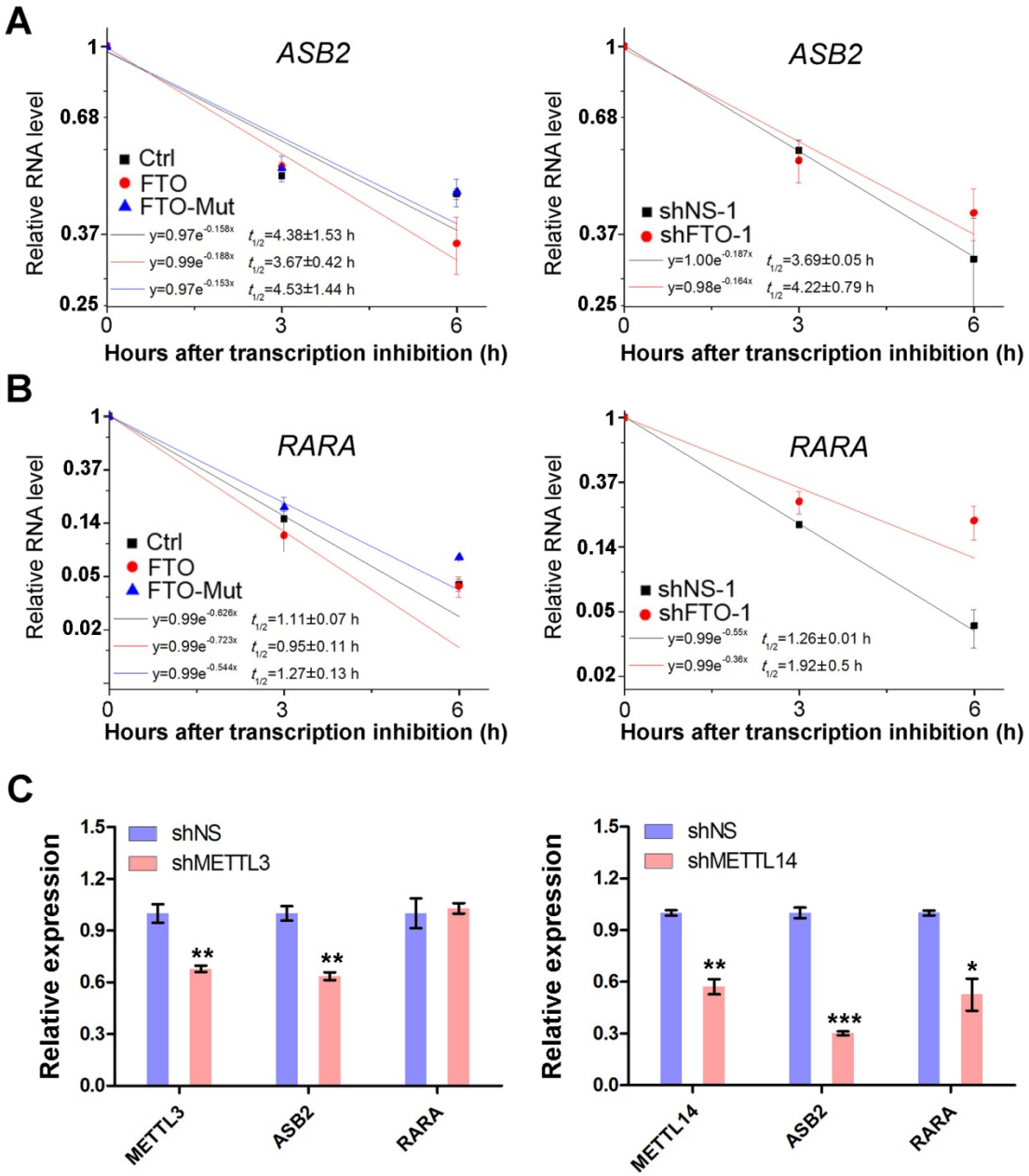

Figure S7 (related to Figure 7). The effects of forced or depleted expression of *FTO* on the half-life ($t_{1/2}$) of *ASB2* and *RARA* mRNA transcripts and the effects of knockdown of m^{6}A writers on the expression levels of *ASB2* and *RARA* in AML cells. (A,B) Forced and depleted expression of *FTO* decreased and increased, respectively, the half-life of *ASB2* (**A**) and RARA (**B**) mRNA transcripts in MONOMAC-6 cells. **(C)** Knockdown of m^{6}A writers, *METTL3* or *METTL14*, caused down-regulated expression of FTO targets, *ASB2* and *RARA* in AML cells. *, p<0.05; **, p<0.01; ***, p< 0.001; *t*-test. Error bar, mean±SD.

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Leukemic patient samples, cell lines and normal hematopoietic cell samples

The leukemic samples were stored in liquid nitrogen until used. Blasts and mononuclear cells were purified by use of NycoPrep 1.077A (Axis-Shield, Oslo, Norway). All cell lines were maintained in the laboratory. **N**ormal CD34+ hematopoietic stem/progenitor cell (HSPC), CD33+ myeloid cell, and mononuclear cell (MNC) samples were purchased from AllCells, LLC (Emeryville, CA).

Microarray assays of the 109 human samples.

As described previously (Huang et al., 2013; Li et al., 2013a; Li et al., 2012a), genome-wide gene expression profiles of a total of 100 human AML with t(11q23)/*MLL*-rearranged, t(8;21), inv(16) or t(15;17) and 9 normal control samples (with 3 each of CD34+, CD33+ and MNC cell samples) were analyzed by use of Affymetrix GeneChip Human Exon 1.0 ST arrays (Affymetirx, Santa Clara, CA). 1 μg total RNA was used for each sample. After hybridization and background correction according to the standard protocols, the quantified signals were then log2-transformed and normalized using Robust Multi-array Average (RMA) (Irizarry et al., 2003). The data have been deposited in the Gene expression Ominibus (GEO) repository with the accession numbers GSE34184 and GSE30285.

Cell Culture and transfection

K 562 and NB4 cells were grown in RPMI medium 1640 (Invitrogen, Carlsbad, CA) containing 10% FBS (Invitrogen), 1% HEPES and 1% penicillin-streptomycin. MONOMAC-6 cells were maintained in RPMI 1640 supplemented with 10% FBS, 1% HEPES, 2 mM L-Glutamine, 100×Non-Essential Amino Acid (Invitrogen), 1 mM sodium pyruvate, 9 μg/ml insulin (Invitrogen) and 1% penicillin-streptomycin. siRNAs (Thermo Scientific, BufferRockford, IL) and/or plasmids were transfected into MONOMAC-6 cells with Cell Line Nucleofector Kit V following program T-027 using the Amaxa® Nucleofector® Technology (Amaxa Biosystems, Berlin, Germany). Experiments were performed 48 hours after transfection.

Apoptosis, cell viability and cell proliferation/growth assays

For apoptosis and cell viability assays, 48 hours after transfection, cells were collected and seeded with requested concentration, and then apoptosis and viability were assessed using ApoLive-Glo Multiplex Assay Kit (Promega, Madison, WI) and PE Annexin V apoptosis Detection Kit 1 (BD Biosciences, San Diego, CA) following the corresponding manufacturer's manuals. For cell growth/proliferation assays, 24 hours after transfection, cells were seeded into 96-well plates at the concentration of 10000 cells/ well in triplicates. The cell proliferation was assessed by MTT (G4000, Promega, Madison, WI) following the manufacturer's instructions. Briefly, the cells were seeded on 96-well plate at the density of 5000-10000 cells/100 μL, and then dye solution was added at indicated time points. After incubation at 37°C for 2-4 hours, the reaction was stopped by adding solubilization/stop. The absorbance at 570nm was read on the next day.

Chromatin immunoprecipitation (ChIP)-qPCR assays

The procedure for ChIP assays was based on the description in the ChampionChIP One-Day Kit (Qiagen, Valencia, CA). Briefly, MONOMAC-6 or K-562 cells were crosslinked with formaldehyde 1% at 37°C for 10 min. The reaction was stopped by adding stop buffer at room temperature for 5 min. After cell lysis, cross-linked chromatin was sheared using a sonicator. The 5 μg of anti-FTO (Santa cruze biotechnology, Santa Cruz, CA), anti-MLL N-terminal, anti-MLL C-terminal, and H3K79me2/3, or control IgG (Abcam, Cambridge, MA) antibodies were used for immunoprecipitation. After eluting DNA from the precipitated immune complex, DNA gel electrophoresis or quantitative real-time PCR (qPCR) analysis was performed. For the input control, 1% of the sonicated DNA was directly purified before immunoprecipitation and subjected to PCR with the same primers.

Plasmid construction

The wild type *FTO*-CDS and mutant *FTO*-CDS (coding region sequence) were amplified from pcDNA3.1_FTO and pcDNA3.1_mutFTO (the two plasmids were kindly provided by Dr. Chuan He) by PCR using the following primers: forward 5'-AGAGCTCTAGAACCACCATGGATTACAAAGATGAC-3' and reverse 5'-CTAAGATTGCGGCCGCCTAGGGTTTTGCTTCCAGAAGC-3', and then subsequently cloned into lentivector-based pMIRNA1 (SBI, Mountain View, CA). The *RARA*-CDS was

transferred from pcDNA Flag-RARalpha, purchased from Addgene (Cambridge, MA), to lentivector-based pCDH (SBI) with the following primers: forward 5'-TTTTCTAGACCACC ATGGCCAGCAACAGCAGCT-3' and reverse 5'-TTATGCGGCCGCTCAGGGGA GTGGGTGG-3'. The *ASB2*-CDS was amplified from human genomic cDNA with the primers, forward 5'-TTTTGTCGACCCACCATGGCCACGCAGATCAG-3' and reverse 5'-CCCTCTAGATTACTGGGTGTTCTCGTATTTCAGGT-3', and directly inserted into pLJM1-EGFP vector (#19319, Addgene). The pGFP-C-shFTO and pGFP-C-shNS were purchased from Origene technologies (Rockville, MD). The pTRIPZ shRNA against *ASB2* was purchased from Dharmacon (clone ID: V2THS_97800) and *RARA* shRNA plasmid was purchased from Santa Cruz Biotechnology (sc-29465-SH), which have been shown by others to be able to specifically and effectively targeting *ASB2* and *RARA*, respectively (Marchwicka et al., 2016; Wang et al., 2012) .

Retrovirus preparation and *in vitro* colony-forming and replating assay

These assays were conducted as described previously (Huang et al., 2013; Jiang et al., 2012; Li et al., 2012a; Li et al., 2012c; Li et al., 2013b) with some modifications. Briefly, retroviruses were produced in 293T cells by co-transfection of individual retroviral construct and the pCL-Eco packaging vector (IMGENEX, San Diego, CA) with Effectene Transfection Reagent (Qiagen, Valencia, CA). Bone marrow (BM) cells were collected from 4- to 6-week-old B6.SJL (CD45.1) mice five days after 5-fluorouracil (5-FU) treatment (150 mg/kg), and BM progenitor (i.e., lineage negative, Lin-) cells were enriched with the Mouse Lineage Cell Depletion Kit (Miltenyi Biotec Inc., Auburn, CA). BM progenitor cells were then co-transduced with different combinations of MSCVneo-based and/or MSCV-PIG-based retroviruses, through two rounds of "spinoculation". Thereafter, the retrovirally transduced cells were plated into methylcellulose medium dishes (with 15,000 cells per dish), supplied with murine recombinant IL-3, IL-6, GM-CSF and SCF (10 ng/ml for the first three and 30 ng/ml for SCF; R&D Systems, Minneapolis, MN), along with 1.0 mg/ml of G418 (Gibco BRL, Gaithersburg, MD) and 2.5 μg/ml of puromycin (Sigma, St. Louis, MO). Cultures were incubated at 37°C in a humidified atmosphere of 5% CO2 in air for 7 days. Then, colony cells were collected and replated in methylcellulose dishes every 7 days with 15,000 cells/dish for 3 passages. Colony numbers were counted and compared for each passage. In some experiments BM from wildtype or *Fto* heterozygous

knockout (Fto+/-) (Gao et al., 2010) C57BL/6 (CD45.2) mice was used. Cells were transduced with MSCVneo-*MLL-AF9* through spinoculation and seeded in Methocult M3230 methylcellulose medium as described above with selection of 1.0 mg/mL of G418.

Mouse bone marrow transplantation (BMT; *in vivo* reconstitution) assays

These assays were conducted as described previously (Huang et al., 2013; Jiang et al., 2012; Li et al., 2012a; Li et al., 2012c; Li et al., 2013b) with some modifications. Briefly, colony cells were collected from the colony-forming assays, and after PBS washing twice, the cells were transplanted via tail vein injection into lethally irradiated (960 cGy, 96 cGy/min, γ-rays) 8- to 10-week-old C57BL/6 (CD45.2) or B6.SJL (CD45.1) recipient mice. For each recipient mouse, 0.2-0.3× 106 donor cells from CFA assays and a radioprotective dose of whole bone marrow cells (1 × 106) freshly harvested from a C57BL/6 or B6.SJL (CD45.1) mouse were transplanted. Leukemic mice were euthanized by CO2 inhalation if they showed signs of systemic illness.

The care and maintenance of animals

C57BL/6 (CD45.2) and B6.SJL (CD45.1) were purchased from the Jackson Lab (Bar Harbor, ME, USA) or Harlan Laboratories, Inc (Indianapolis, IN, USA). Both male and female mice were used for the experiments. All laboratory mice were maintained in the animal facility at the University of Chicago or University of Cincinnati. All experiments on mice in our research protocol were approved by Institutional Animal Care and Use Committee (IACUC) of the University of Chicago or University of Cincinnati.

Histolopathology

Leukemic mice were euthanized by CO2 inhalation if they showed signs of systemic illness, and some negative control recipient mice were also sacrified at same time points (though they did not develop leukemia) to collect specimens as controls for further analyses. Portions of the spleen and liver were collected and fixed in formalin, embedded in paraffin, sectioned and stained with haematoxylin and eosin (H&E) or anti-5hmC antibody (Active Motif, Carlsbad, CA). The staining was performed by the Tissue Resource Center (TRC) Core Facility at The University of Chicago. BM Cells were isolated from the tibia and femur. 50,000 cells were washed twice and were diluted in 200 μl of cold MACS Buffer. Each sample was loaded into the appropriate wells

of the cytospin, and then spun at 2000 rpm for 2 min. Blood smear and BM cytospin slides were stained with Wright-Giemsa.

Lentivirus production, precipitation and infection

Lentivirus for pmiRNA1-*FTO*, pmiRNA1-*FTO*-Mut, sh*FTO*-1, sh*FTO*-2, sh*RARA*, pCDH-*RARA*, pLJM1-*ASB2* as well as their controls were packaged with pMD2.G, pMDLg/pRRE and pRSV-Rev (purchased from Addgene). Briefly, 0.5 μg pMD2.G, 0.3μg pMDLg/pRRE, 0.7 μgpRSV-Rev and 1.5 μg construct for overexpression or knockdown of specific genes were co-transfected into HEK-293T cells in 60mm cell culture dish with Effectene Transfection Reagent (301427, QIAGEN, Valencia, CA). The pTRIPZ-shASB2 lentivirus was packaged with psPAX2 and pMD2.G vectors. Briefly, 2.24μg psPAX2, 0.76μg pMD2.G and 1.5μg pTRIPZ-shASB2 were co-transfected into HEK-293T cells in 60mm dish with Effectene Transfection Reagent. The lentivirus particles were harvested at 48 and 72 hours after transfection and concentrated with PEG-it^{TM} Virus Precipitation Solution (# LV810A-1, SBI).Finally, the concentrated lentivirus particles were directly added into leukemic cells and incubated at 37°C for 24-48 hours before they were washed out with PBS. For pTRIPZ-shASB2, 1ug/mL Doxycycline (D9891, Sigma, St. Louis, MO) was added into medium to induce its expression after puromycin selection.

RNA extraction and quantitative RT-PCR analysis

Total RNAs were isolated using the miRNeasy kit (Qiagen, Valencia, CA). For mRNA expression, 200 ng RNA was reverse-transcribed into cDNA in a total reaction volume of 10 μl with Qiagen's RT kit according to the manufacturer's instructions. Quantitative real-time PCR analysis was performed with 0.5 μl cDNA using SYBR green PCR master mix (Qiagen, Valencia, CA) in an AB 7900HT real-time PCR instrument (Applied Biosystems, Foster City, CA). *Gapdh* or *Actin* was used as endogenous control. Each sample was run in triplicate. Nuclear and cytoplasmic RNA were purified with SurePrep™ Nuclear or Cytoplasmic RNA Purification Kit (Fisher BioReagents, Fair Lawn, NJ). For quantitative RT-PCR, GAPDH were used as the endogenous control for cytoplasmic RNA, while 18S RNA was selected as endogenous control for Nuclear RNA.

Analysis of m^6A/A ratio using HPLC-MS/MS

As described previously (Fu et al., 2013), total RNA was isolated from cells using TRIzol® reagent according to the manufacture procedure. mRNA was then isolated with biotin-oligo(dT) and streptavidin beads from Promega, following a modified procedure, which eliminated all heating steps. 8 ug of mRNA was digested with RNase T1 in 20 uL for 15 min at 37 °C, followed by nuclease P1 (1 U) for 30 min at 37 °C. The solution was diluted 5 times, and 20 µl of the solution was analyzed by HPLC-QQQ-MS/MS. The nucleosides were quantified using the nucleoside to base ion mass transitions of 282.1 to 150.1 in MRM positive ESI mode.

m^6A dot blot assay

Total RNA was isolated from different cells with miRNeasy Mini Kit (QIAGEN, 217004) according to the manufacturer's instructions and quantified by UV spectrophotometry. The m^6A dot blot assay was performed following a published protocol (Rapley and Manning, 1998) with some modifications (Jia et al., 2011). Briefly, the RNA samples were loaded to the Amersham Hybond-N+ membrane (RPN119B, GE Healthcare,) with a Bio-Dot Apparatus (#170-6545, Bio-Rad) and UV crosslinked to the membrane. Then the membrane was blocked with 5% nonfat dry milk (in 1X PBST) for 1-2 hours and incubated with a specific anti-m^6A antibody (1:2000 dilution, Synaptic Systems, 202003) overnight at 4°C. Then the HRP-conjugated goat anti-rabbit IgG (sc-2030, Santa Cruz Biotechnology) was added to the blots for 1 hour at room temperature and the membrane was developed with Amersham ECL Prime Western Blotting Detection Reagent (RPN2232, GE Healthcare). The relative signal density of each dot was quantified by Gel-Pro analyzer software (Media Cybernetics) in all experiments.

RNA-seq and m^6A-seq assays and data analysis

The m^6A-seq procedure was performed as described previously (Dominissini et al., 2013). Polyadenylated RNA was extracted using FastTrack MAG Maxi mRNA isolation kit (Life technology). RNA fragmentation Reagents (Ambion) was used to randomly fragment RNA. The specific anti-m^6A antibody (Synaptic Systems) was applied for m^6A pull down (i.e., m^6A IP). Both input and m^6A IP samples were prepared for next-generation sequencing (NGS). The NGS library preparation was constructed by TruSeq Stranded mRNA Sample Prep Kit (Illumina) and was quantified by BioAnalyzer High Sensitivity DNA chip, and then was deeply sequenced on

the Illumina HiSeq 2500. The data have been deposited in the GEO repository with the accession numbers GSE76414 (http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?token=avcxukwiztyntud&acc=GSE76414), GSE84944 (http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?token=mforsiggdtetdah&acc=GSE84944), and GSE85008 (http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?token=kbiduyasxlstrib&acc=GSE85008).

For the data analysis, the following pipeline was used to identify m^6A peaks. The reads from mRNA input (RNA-seq) and m^6A IP (m^6A-seq) sequencing libraries were aligned to hg19/hg38 reference genome using Tophat (Kim et al., 2013). Both MACS/MACS2 (Zhang et al., 2008) and exomePeak (Meng et al., 2013) were used to call m^6A peaks based on the RNA-seq and m^6A-seq bam files. To achieve high specificity, only the m^6A peaks called by both MACS/MACS2 and exomePeak were retained for the further analysis. The m^6A peaks were annotated using an *ad hoc* perl script. The differentially methylated m^6A peaks were identified according to the procedure described by Schwartz et al (Schwartz et al., 2013). Sequence motifs enriched in m^6A peak regions compared to control regions were identified using DREME (Bailey, 2011). The RNA-seq reads were normalized using RSEM method (Li and Dewey, 2011) and Cufflinks (Trapnell et al., 2010). EBSeq (Leng et al., 2013) and Cuffdiff (Trapnell et al., 2013) were employed to find differentially expressed genes between *FTO*-overexpressing and control AML cell lines. DAVID tool (Rapley and Manning, 1998) was used to perform pathway enrichment analysis.

Gene-specific m^6A qPCR

Real-time quantitative PCR (qPCR) was performed to assess the relative abundance of the selected mRNA in m^6A antibody IP samples and input samples between the FTO-overexpressing, shFTO cell lines and their control cell lines. Here the house-keeping gene *HPRT1* was chose as internal control according to the reason that *HPRT1* mRNA did not have m^6A peaks from the m^6A profiling data (Wang et al., 2014). Total RNA was isolated by RNeasy kit (QIAGEN) with additional DNase I on-column digestion then followed polyadenylated RNA extraction using Dynabeads® mRNA Purification Kit (Life technology). While 50 ng mRNA was saved as input sample, the rest mRNA was used for m^6A-immunoprecipitation as described above in m^6A-seq procedures to obtain m^6A pull down portion (m^6A IP portion). Briefly, nearly 2 ug mRNA was

incubated with m^6A antibody (Synaptic Systems) and diluted into 500 uL IP buffer (150 mM NaCl, 0.1% NP-40, 10 mM Tris, pH 7.4, 100 U RNase inhibitor). The mixture was rotated at 4 °C for 2 hours, following Dynabeads® Protein A (ThermoFisher Scientific) was added into the solution and rotated for another 2 hours at 4 °C. After four times washing by IP buffer, the m^6A IP portion was eluted twice by 50 uL m^6A-elute buffer (IP buffer, 6.7mM m^6A, 30 U RNase inhibitor) with incubating and shaking at 4 °C for 1 hour. Finally, m^6A IP mRNA was recovered by ethanol precipitation and the RNA concentration was measured with Qubit® RNA HS Assay Kit (ThermoFisher Scientific). RT-qPCR was performed in Roche LightCycle® 96 system (Roche) by using the Verso SYBR Green 1-Step qRT-PCR Low ROX Kit (ThermoFisher Scientific) from 1 ng m^6A IP mRNA or input mRNA as the template. The mRNA expression was determined by the number of amplification cycle (Cq). The relative mRNA expression was calculated by the value of Cq in m^6A IP portion divide by the value of Cq in input portion (CqIP/Cqinput). And the gene-specific m^6A level was determined by the target mRNA relative expression level between the FTO-overexpressing cell lines or shFTO cell lines with their control cell lines.

RT-PCR primer used:

ASB2: 5'-CGTGGTGCAGTTCTGTGAGT-3'; 5'-GTGAGCCAGAGGTCTTGGAG-3'
RARA: 5'-CCAGCTCCAACAGAAGCAG-3'; 5'-AGGCCTCTGTCCAAGGAGTC-3'
PPARD: 5'-ACGACATCGAGACATTGTGG-3'; 5'-GGTAACCTGGTCGTTGAGGA-3'
SLC11A1: 5'-GCGAGGTCTGCCATCTCTAC-3'; 5'-GTGTCCACGATGGTGATGAG-3'
TXLNA: 5'-TCCAAAAGCAGCGAGGTATT-3'; 5'-TCCAGCCGTTGGATTTTTAC-3'

RNA stability assays

The mRNA stability *in vivo* is normally reported as the time for degrading half of the existing mRNA molecules. *FTO*-overexpressing cell lines, shFTO cell lines and their control cell lines were cultured in 6-well plates. Then actinomycin D was added to 5 μg/mL at 6 hours, 3 hours and 0 hour before cell scraping collection. Total RNA was isolated by RNeasy kit (QIAGEN) with additional DNase I on-column digestion. RT-PCR was conducted to quantify the relative levels of target mRNA in Roche LightCycle® 96 system (Roche) by using the Verso SYBR Green 1-Step qRT-PCR Low ROX Kit (ThermoFisher Scientific) from 50 ng total RNA. The

degradation rate of target mRNA was estimated according with the previous published paper (Liu et al., 2014). With the transcriptional inhibitor actinomycin D, the mRNA transcription was turned off and the degradation rate of RNA (K_{decay}) was estimated by following equation:

$$In(C/C_0)=-K_{\text{decay}}t$$

Where C_0 is the concentration of mRNA at time 0 hour, which means the mRNA concentration before decay starts. And t is the transcription inhibition time, meanwhile C is the mRNA concentration at the time t. Thus the K_{decay} can be derived by the exponential decay fitting of C/C_0 versus time t. The half-time ($t_{1/2}$), which means C/C_0=50%/100%=1/2, can be calculated by the following equation:

$$In(1/2)= -K_{decay}t_{1/2}$$

Rearrangement of the above equation leads to the mRNA half-life time value, $t_{1/2}= In2/K_{decay}$.

Immunoblotting (Western blot)

Cells were washed twice with ice-cold phosphate-buffered saline (PBS) and ruptured with RIPA buffer (Pierce, Rockford, IL) containing 5 mM EDTA, PMSF, cocktail inhibitor, and phosphatase inhibitor cocktail. Cell extracts were microcentrifuged for 20 min at 10000 x g and supernatants were collected. Cell lysates (20 μl) were resolved by SDS-PAGE and transferred onto PVDF membranes. Membranes were blocked for 1 hour with 5% skim milk in Tris-buffered saline containing 0.1% Tween 20 and incubated overnight at 4 °C with anti-Fto antibody (Millipore, Billerica, MA), anti-Actb antibody (Santa Cruz Biotechnology Inc., Santa Cruz, CA), anti-FTO (#14386, Cell Signal Technology), anti-β-Actin (3700S, Cell Signal Technology), anti-GAPDH (Santa Cruz), anti-ASB2 (ab13710, Abcam), anti-RARA (616801, BioLegend) or anti-MLL1 (#14689, Cell Signaling Technology). Membranes were washed 30 min with Tris-buffered saline containing 0.1% Tween 20, incubated for 1 h with appropriate secondary antibodies conjugated to horseradish peroxidase, and developed using chemiluminescent substrates.

Flow cytomety analysis

Flow cytometry analysis of mouse BM cells were conducted as described previously (Huang et al., 2013; Jiang et al., 2012; Li et al., 2012a; Li et al., 2012c; Li et al., 2008) with some modifications. For in vitro ATRA induction experiments, MONOMAC-6 or NB4 cells were

induced into myeloid differentiation with 100nM or 500nM ATRA (223018, Sigma-Aldrich), harvested at indicated time points, washed with chilled PBS and stained with PE-labeled anti-CD11b (101208, BioLegend) and APC-labeled anti-CD14 (17-0149-41, eBioscience) for flow cytometry analysis. Cells from BM of transplanted mice were harvested for analysis of immunophenotypes. After washing with PBS, blocking nonspecific binding with affinity-purified anti–mouse CD16/32 (eBioscience), cells were stained at 4°C with various antibodies diluted in Flow Cytometry Staining Buffer (eBioscience) for 30 minutes. Subsequently, cells were washed with PBS and resuspended in IC Fixation Buffer (eBioscience) for flow cytometry analysis. Antibodies (eBioscience) used for our flow cytometric analysis -include anti–mouse PE-CD11b (Mac-1; 12-0112), anti–mouse APC-CD117 (17-1172; c-kit), PE anti–mouse CD45.1 (12-0453), and anti–mouse APC-CD45.2 (17-0454).

Dual-Luciferase reporter and mutagenesis assays

The DNA fragments of ASB2-3'UTR, RARA-3'UTR and RARA-5'UTR containing the wild type m^6A motifs as well as mutant motifs (m^6A was replaced by T) were directly synthesized from Integrated DNA Technologies (Coralville, Iowa). Wild type and mutant ASB2-3'UTR and RARA-3'UTR were inserted into downstream of firefly luciferase of pMIR-REPORT vector (Luciferase miRNA Expression Reporter Vector, Ambion); while the wild type and mutant RARA-5'UTR were subcloned into upstream of firefly luciferase of pGL3-basic vector (Promega, Madison, WI). For dual-luciferase reporter assay, 100ng wild-type or mutant ASB2-3'UTR (or RARA-3'UTR, or RARA-5'UTR), 200ng pMIRNA1-FTO (or pMIRNA1-FTO-Mut or pMIRNA1), and 20ng pRL-TK (renilla luciferase control reporter vector) were co-transfected into HEK-293T cells in 24-well plate. The relative luciferase activities were accessed 48 hours post transfection by Dual-Luciferase Reporter Assay System (Promega). Each group was repeated in triplicate.

(1) *ASB2*-3'UTR with wild-type m^6A sites:

ATGACTAGACTAGTGGCCACGGGGAGAGAGGAGTAGCCCCTC**AGACT**CTTCTTACTAAGTCTCAGGACGTCGG
TGTTCCCAACTCCAAGGGGACCTGGTGACAGACGAGGCTGCAGGCTGCCTCCCTCTCAGCCT**GGACA**GCTACC
AGGATCTCACTGGGTCTCAGGGCCCAGAGCTTTGGCCAGAGCAGAGAACAGAATGTGTCAAGGAGAAGAATCA
TTTGTTTAC**AAACT**GATGAGCAGATCCCAGACCTTCTCTACCTTCAGGAATGGCAGAAACCTCTATTCCTGGG
GCCAGGGCAGAGCTTGAGGTGTTCTGGGGAAGGTGGTGCTCAGAGCCTTCCCTGTGCCCCTCCACTTGTTCTG
GAAAACTCACCACTTGACTTCAGAGCTTTCTCTCCAA**AGACT**AAGATGAAGACGTGGCCCAAGGTAGGGGGTA
GGGGGAGCCTGGAAGCTTGGGATCTA

***ASB2*-3'UTR with mutant m^{6}A sites:**

ATGACTAGACTAGTGGCCACGGGGAGAGAGGAGTAGCCCCTC**AGTCT**CTTCTTACTAAGTCTCAGGACGTCGG
TGTTCCCAACTCCAAGGGGACCTGGTGACAGACGAGGCTGCAGGCTGCCTCCCTCTCAGCCT**GGTCA**GCTACC
AGGATCTCACTGGGTCTCAGGGCCCAGAGCTTTGGCCAGAGCAGAGAACAGAATGTGTCAAGGAGAAGAATCA
TTTGTTTAC**AATCT**GATGAGCAGATCCCAGACCTTCTCTACCTTCAGGAATGGCAGAAACCTCTATTCCTGGG
GCCAGGGCAGAGCTTGAGGTGTTCTGGGGAAGGTGGTGCTCAGAGCCTTCCCTGTGCCCCTCCACTTGTTCTG
GAAAACTCACCACTTGACTTCAGAGCTTTCTCTCCAA**AGTCT**AAGATGAAGACGTGGCCCAAGGTAGGGGGTA
GGGGGAGCCTGGAAGCTTGGGATCTA

(2) ***RARA*-3'UTR with wild-type m^{6}A sites:**

ATGACTAGACTAGTCGACCATGTGACCCCGCACCAGCCCTGCCCCCACCTGCCCTCCCGGGCAGTACTGGGGA
CCTTCCCTGGGGGACGGGGAGGGAGGAGGCAGCGACTCCTT**GGACA**GAGGCCTGGGCCCTCAGT**GGACT**GCCT
GCTCCCACAGCCTGGGCTGACGTCAGAGGCCGAGGCCAG**GAACT**GAGTGAGGCCCCTGGTCCTGGGTCTCAGG
ATGGGTCCTGGGGGCCTCGTGTTCATCAAGACACCCCTCTGCCCAGCTCACCACATCTTCATCACCAGCAAAC
GCCA**GGACT**TGGCTCCCCCATCCTCA**GAACT**CACAAGCCATTGCTCCCCAGCTGGGGAACCTCAACCTCCCCC
CTGCCTCGGTTGGTGACAGAGGGGGTGGGACAGGGGCGGGGGGTTCCCCCTGTACATACCCTGCCATACCAAC
CCCAGGTATTAATTCTCGCTGGAAGCTTGGGATCTA

***RARA*-3'UTR with mutant m^{6}A sites:**

ATGACTAGACTAGTCGACCATGTGACCCCGCACCAGCCCTGCCCCCACCTGCCCTCCCGGGCAGTACTGGGGA
CCTTCCCTGGGGGACGGGGAGGGAGGAGGCAGCGACTCCTT**GGTCA**GAGGCCTGGGCCCTCAGT**GGTCT**GCCT
GCTCCCACAGCCTGGGCTGACGTCAGAGGCCGAGGCCAG**GATCT**GAGTGAGGCCCCTGGTCCTGGGTCTCAGG
ATGGGTCCTGGGGGCCTCGTGTTCATCAAGACACCCCTCTGCCCAGCTCACCACATCTTCATCACCAGCAAAC
GCCA**GGTCT**TGGCTCCCCCATCCTCA**GATCT**CACAAGCCATTGCTCCCCAGCTGGGGAACCTCAACCTCCCCC
CTGCCTCGGTTGGTGACAGAGGGGGTGGGACAGGGGCGGGGGGTTCCCCCTGTACATACCCTGCCATACCAAC
CCCAGGTATTAATTCTCGCTGGAAGCTTGGGATCTA

(3) ***RARA*-5'UTR with wild-type m^{6}A sites:**

ATGACCGGGGTACCGCCAGCACACACCTGAGCAGCATCACAGGACATGGCCCCCTCAGCCACCTAGCTGGGGC
CCATCTAGGAGTGGCATCTTTTTTGGTGCCCTGAAGGCCAGCTCT**GGACC**TTCCCAGGAAAAGTGCCAGCTCA
CA**GAACT**GCTTGACCAAA**GGACC**GGCTCTTGAGACATCCCCCAACCCACCTGGCCCCCAGCTAGGGTGGGGGC
TCCAGGAGACTGAGATTAGCCTGCCCTCTTT**GGACA**GCAGCTCCA**GGACA**GGGCGGGTGGGCTGACCACCCAA
ACCCCATCTGGGCCCAGGCCCCATGCCCCGAGGAGGGGTGGTCTGAAGCCCACCAGAGCCCCCTGCC**AGACT**G
TCTGCCTCCCTTCTGACTCGAGCGGATCTA

***RARA*-5'UTR with mutant m^{6}A sites:**

ATGACCGGGGTACCGCCAGCACACACCTGAGCAGCATCACAGGACATGGCCCCCTCAGCCACCTAGCTGGGGC
CCATCTAGGAGTGGCATCTTTTTTGGTGCCCTGAAGGCCAGCTCT**GGTCC**TTCCCAGGAAAAGTGCCAGCTCA
CA**GATCT**GCTTGACCAAA**GGTCC**GGCTCTTGAGACATCCCCCAACCCACCTGGCCCCCAGCTAGGGTGGGGGC
TCCAGGAGACTGAGATTAGCCTGCCCTCTTT**GGTCA**GCAGCTCCA**GGTCA**GGGCGGGTGGGCTGACCACCCAA
ACCCCATCTGGGCCCAGGCCCCATGCCCCGAGGAGGGGTGGTCTGAAGCCCACCAGAGCCCCCTGCC**AGTCT**G
TCTGCCTCCCTTCTGACTCGAGCGGATCTA

Luciferase reporter-related gene specific m^{6}A qPCR

To access whether m6A presents on the luciferase reporter constructs and further confirm whether FTO participates in regulating m6A modification of the constructs bearing ASB2 and RARA 3'UTR, we conducted luciferase reporter-related gene specific m6A qPCR. Firstly, 1.5ug luciferase reporter constructs, including *ASB2*-3'UTR-WT, *ASB2*-3'UTR-Mut, *RARA*-3'UTR-WT

and *RARA*-3'UTR-Mut, were transfected into HEK-293T cells with pmiRNA1-FTO, pmiRNA1-FTO-Mut or pmiRNA1 in 100mm cell culture dishes. And 48 hours after transfection, samples were collected, RNAs were extracted, m6A RNA immunoprecipitation was performed with Magna MeRIP m^6A kit (17-10499, Millipore Sigma, Temecula, CA) and qPCR was conducted to assess m6A modification levels with primers covering the joints between inserted ASB2 and RARA 3'UTR and pMIR-REPORT vector.

qPCR primers used:

ASB2_WT and Mut_Primer 1_Forward: 5'-GCGGAAAGTCCAAATTGCTC-3'

ASB2_WT_Primer_1_Reverse: 5'-GTAAGAAGAGTCTGAGGGGCTA-3'

ASB2_Mut_Primer_1_Reverse: 5'- GTAAGAAGAGACTGAGGGGCTA-3'

ASB2_WT_Primer 2_Forward: 5'- TCCAAAGACTAAGATGAAGACGTGG-3'

ASB2_Mut_Primer 2_Forward: 5'- TCCAAAGTCTAAGATGAAGACGTGG-3'

ASB2_WT and Mut_Primer_2_Reverse: 5'-CGCATACAAAAAACCAACACACA-3'

RARA_WT and Mut_Primer 1_Forward: 5'- AAAGTCCAAATTGCTCGAGTGAT-3'

RARA_WT_Primer_1_Reverse: 5'-TGGGAGCAGGCAGTCCACTG-3'

RARA_Mut_Primer_1_Reverse: 5'- TGGGAGCAGGCAGACCACTG-3'

RARA_WT_Primer 2_Forward: 5'- CCCCCATCCTCAGAACTCACAA-3'

RARA_Mut_Primer 2_Forward: 5'- CCCCCATCCTCAGATCTCACAA-3'

RARA_WT and Mut_Primer_2_Reverse: 5'- TAAGCTTCCAGCGAGAATTAATACC-3'

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