Supplemental Figure 1

A

AICDA
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Log2 (TPM+1)
LIHC
(num(T)=369; num(N)=160)

APOBEC1
1.5
1.0
0.5
0.0
LIHC
(num(T)=369; num(N)=160)

APOBEC2
3
2
1
0
LIHC
(num(T)=369; num(N)=160)

APOBEC3A
3
2
1
0
LIHC
(num(T)=369; num(N)=160)

APOBEC3B
*
6
5
4
3
2
1
0
LIHC
(num(T)=369; num(N)=160)

APOBEC3C
6
5
4
3
2
1
0
Log2 (TPM+1)
LIHC
(num(T)=369; num(N)=160)

APOBEC3D
4
3
2
1
0
LIHC
(num(T)=369; num(N)=160)

APOBEC3F
4
3
2
1
0
LIHC
(num(T)=369; num(N)=160)

APOBEC3G
6
5
4
3
2
1
LIHC
(num(T)=369; num(N)=160)

APOBEC3H
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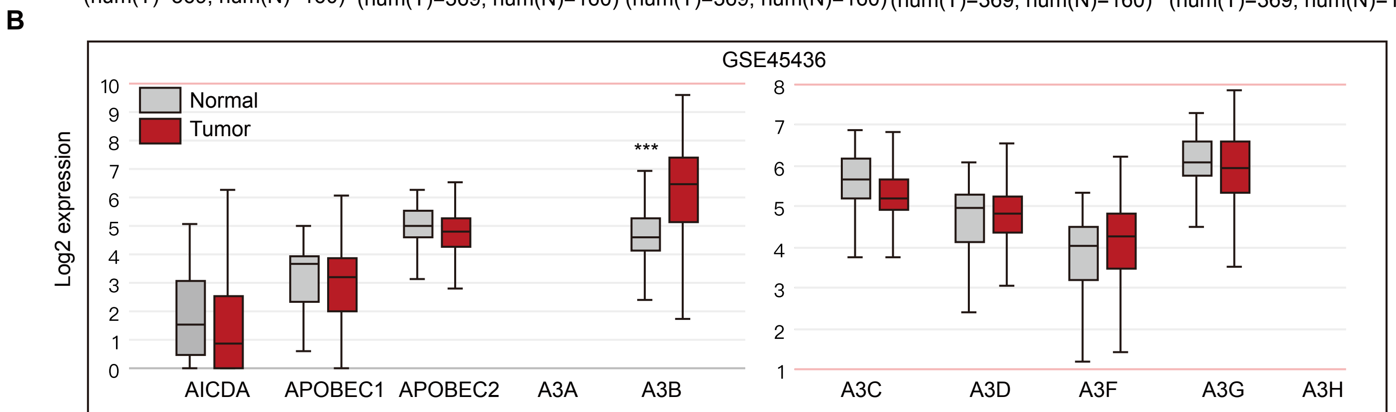
2.0
1.5
1.0
0.5
0.0
LIHC
(num(T)=369; num(N)=160)

B



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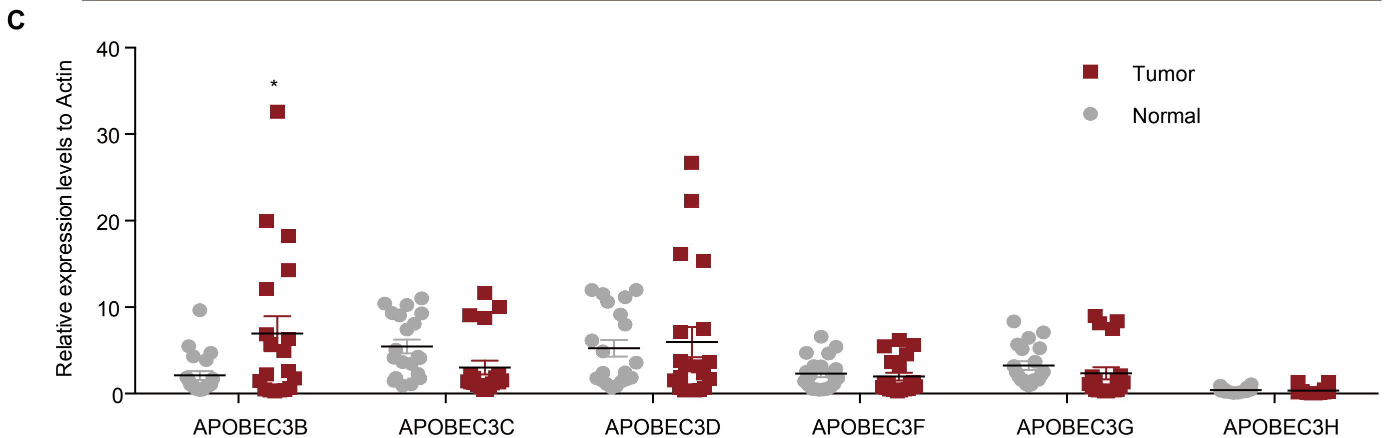
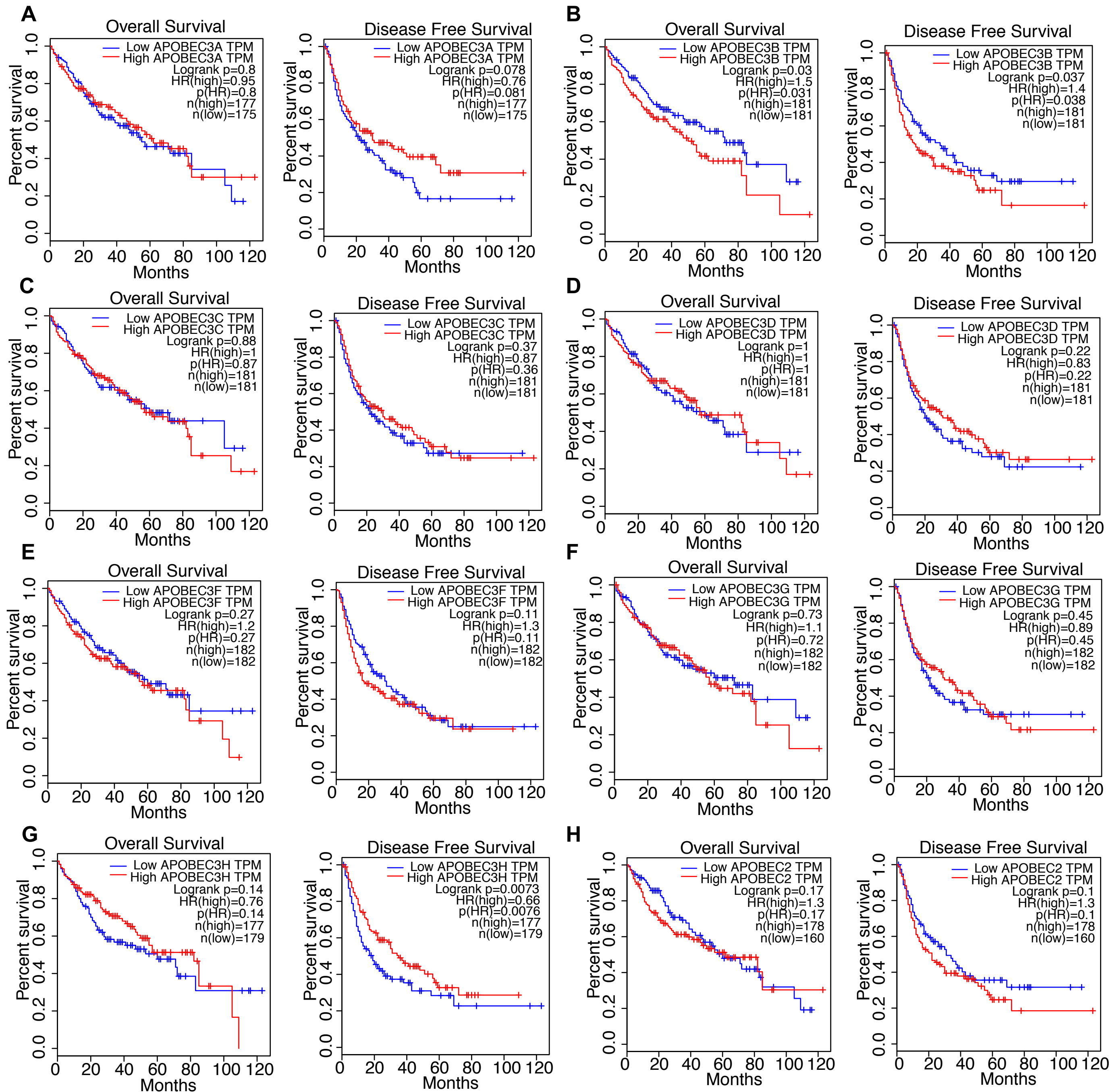


Supplemental Figure 2



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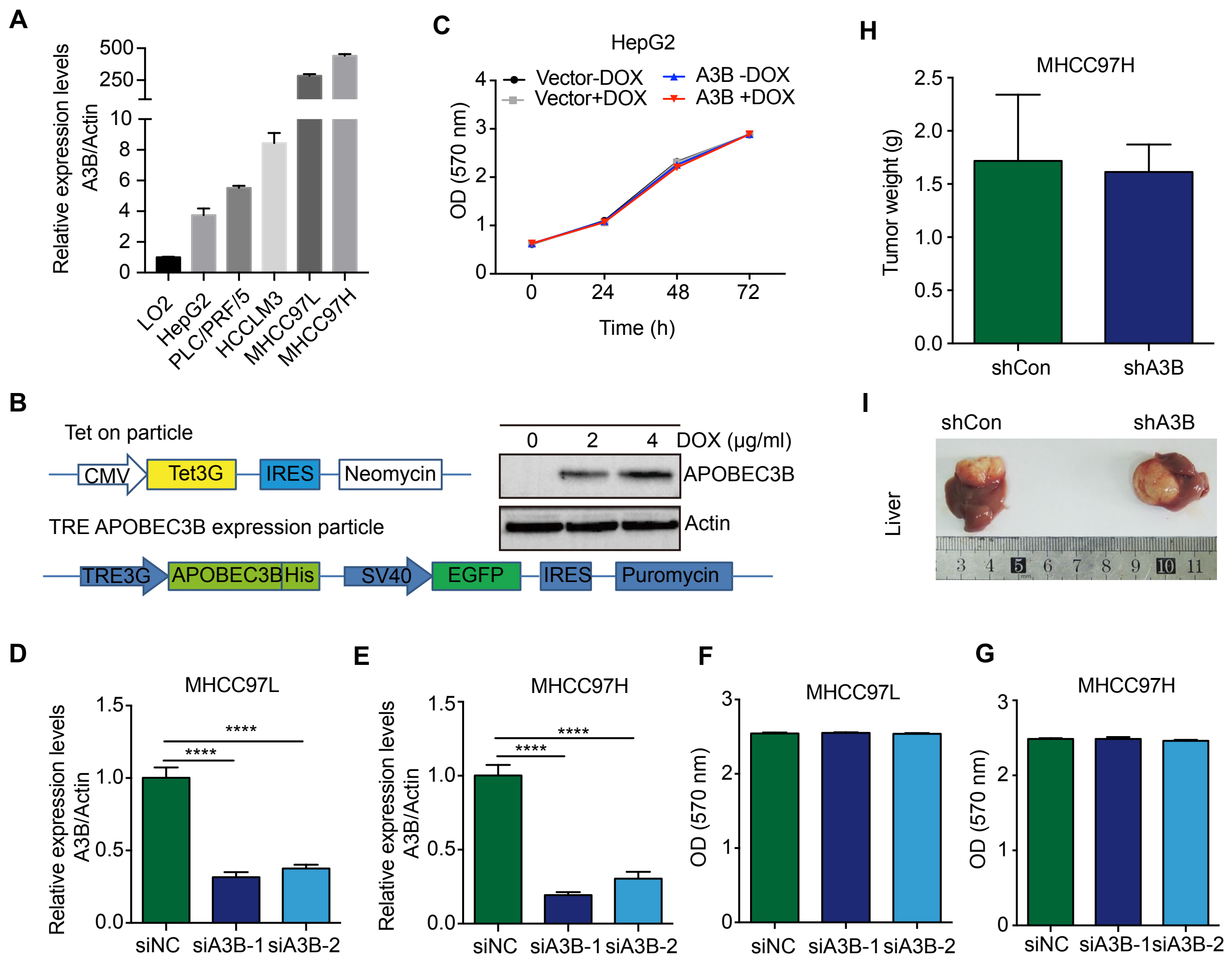

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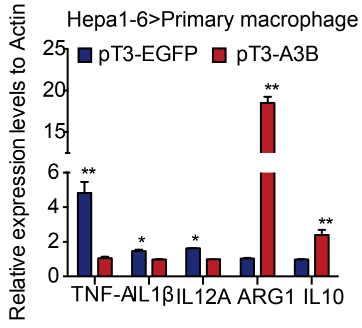

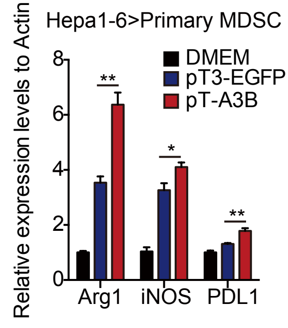

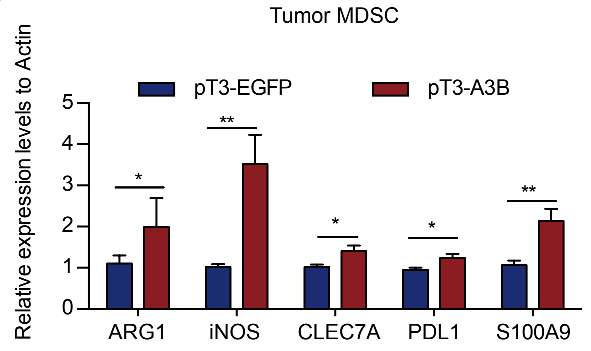

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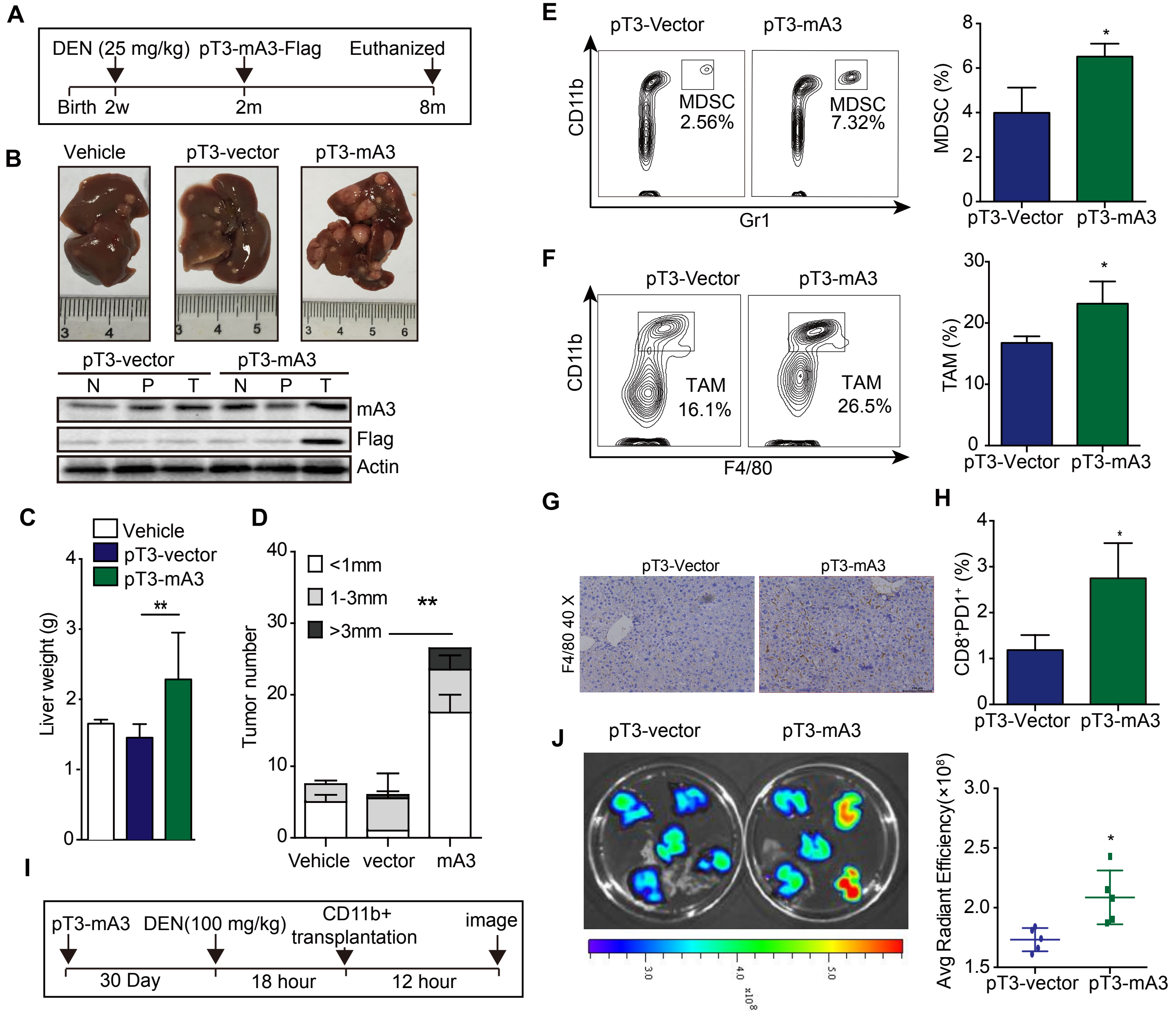

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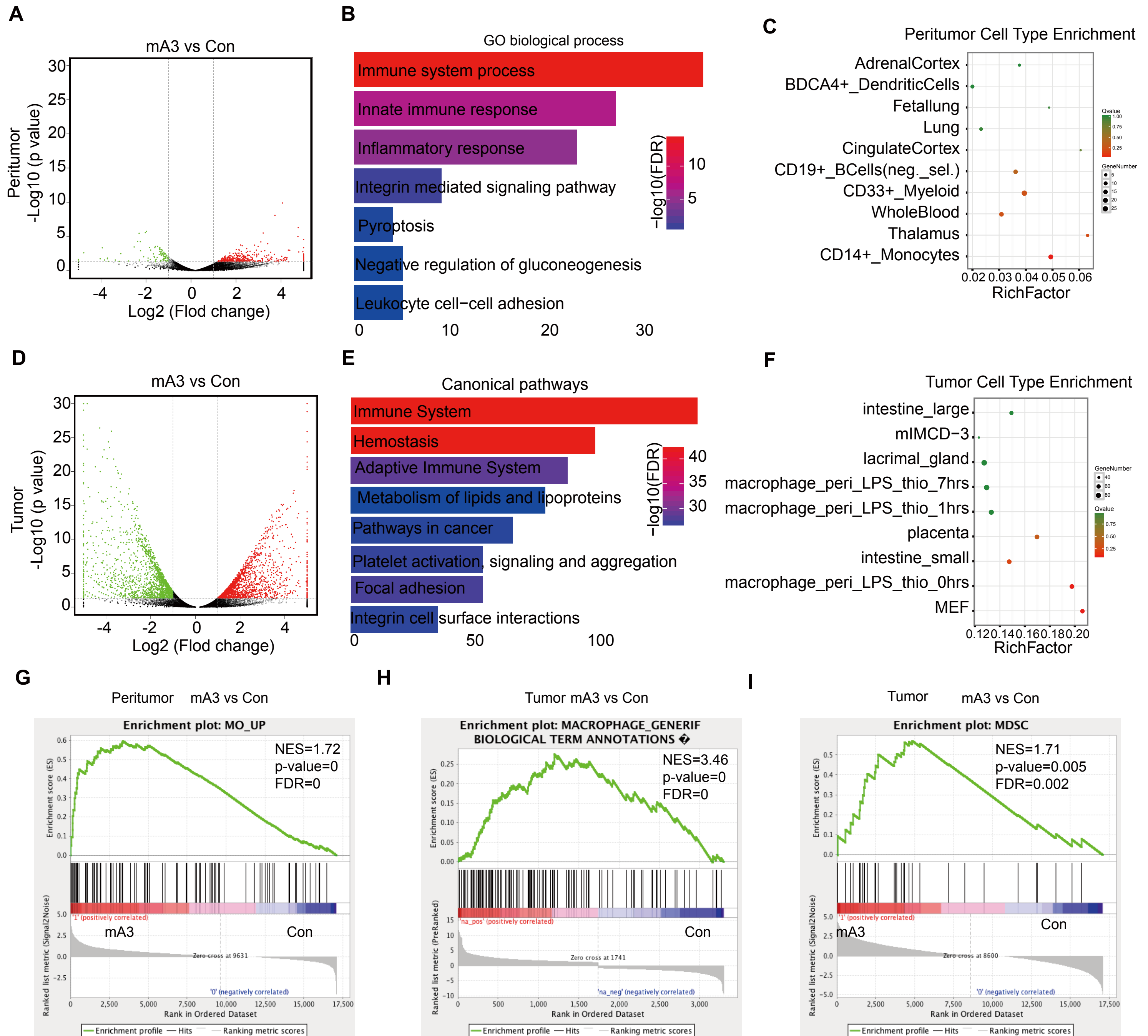
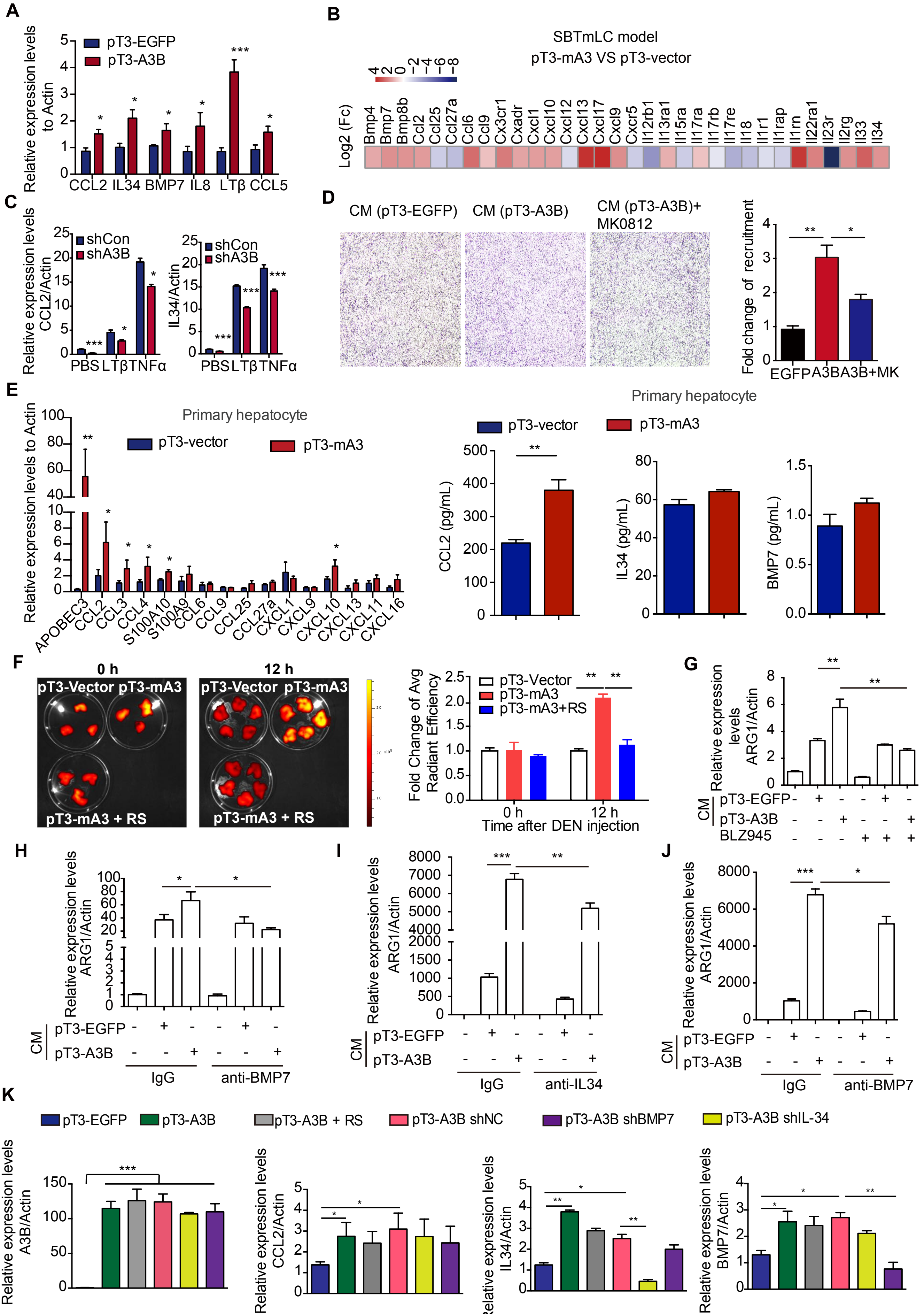
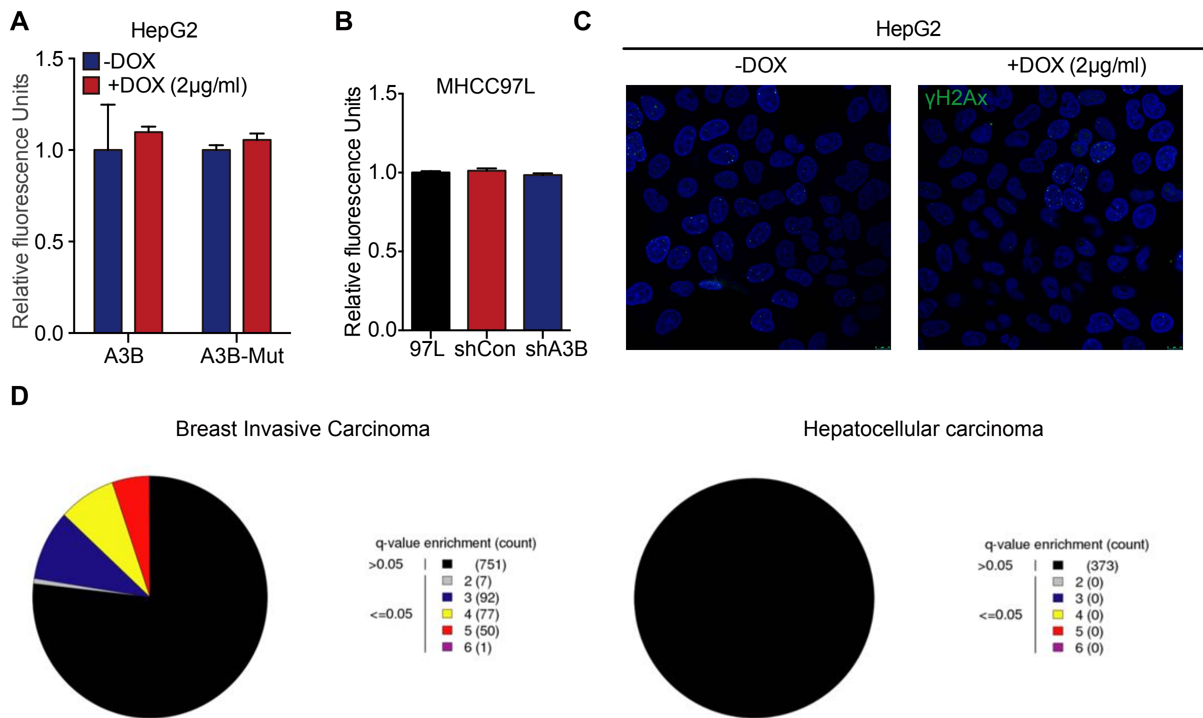


Supplemental Figure 7



Supplemental Figure 8



Supplementary Figure Legends:

Figure S1 A3B was the only upregulated gene of the APOBEC family in HCC

(A) Expression levels of APOBEC family members in GEPIA database. The differential analysis is based on the selected datasets with TCGA tumors (n=369) vs TCGA normal + GTEx normal (n=160). *$p < 0.01$ (one-way ANOVA). (B) Expression levels of APOBEC family members in human liver cancer (n=95) and non-tumor tissues (n=35) in the GSE45436 dataset. ***$p < 0.001$ (one-way ANOVA). (C) RT-qPCR analysis of APOBEC family member mRNA levels in human peripheral and central liver cancer tissues (n=18). *$p < 0.05$ (Student's t test). Error bars represent mean ± SEM.

Figure S2. A3B is the only member of the APOBEC family which correlates with poor prognosis of HCC patients

(A-H) Kaplan-Meier overall survival and disease-free survival curves of patients in the GEPIA database with HCC with high and low expressions (stratified by median) of APOBEC family member mRNAs using TCGA data (Log-rank test).

Figure S3. A3B did not stimulate tumor growth in immunodeficient nude mice

(A) RT-qPCR assays of A3B mRNA levels in normal cells L02 and indicated HCC cell lines. (B) Schematic map of construction for tet-on system of induced A3B expression and immunoblot assay analysis of A3B-His protein in HepG2-Teton-A3B cells.(C) Proliferation assays of HepG2-Teton-A3B exposed to doxycycline (2 µg/ml) for the indicated times. (D and E) RT-qPCR assays of A3B mRNA in MHCC97L and MHCC97H cells with A3B knockdown. After 24h from the transfection of SiA3B,

RNA of the cells were extracted for further measurement. (F and G) Proliferation assays of MHCC97L and MHCC97H cells was performed after 72h from the transfection of SiA3B. (H) Weight of the MHCC97H orthotopic tumors in indicated groups 30 days after implantation (n=6 mice per group). (I) Representative images of the MHCC97H orthotopic tumors in the indicated groups 30 days after implantation. Error bars represent mean ± SEM. *p < 0.05, **p < 0.01, ***p < 0.001 (Student's t test).

Figure S4. Hepatoma-intrinsic A3B induce expression of immunosuppression-associated genes in TAMs and MDSCs.

(A) Graph showing expression of immunosuppressive-associated genes in BMDMs with exposure to conditioned medium. (B) Graph showing expression of immunosuppression-associated genes in primary MDSCs exposed to conditioned medium. (C) Graph showing expression of immunosuppressive-associated genes in MDSCs isolated from Hepa1-6-A3B transplantation tumors (n=5). Error bars represent mean ± SEM. *p < 0.05, **p < 0.01, ***p < 0.001 (Student's t test).

Figure S5. mA3 activates HCC initiation in DEN-induced mouse HCC model

(A) Schematic map of construction for the SBTmLC model. (B) Photomicrographs of representative liver in SBTmLC model at the end time point showed in A. (C-D) Enumeration of liver weight (C) and total tumor burden (D) of tumors following classification according to their size in the SBTmLC model (n=9 mice per group). (E-F) The fractions of $CD11b^{+}F4/80^{-}Gr1^{+}$ MDSCs (E) and $CD11b^{+}Gr1^{-}F4/80^{+}$ TAMs (F) were determined by flow cytometry in SBTmLC model (n=3 mice per group). (G)

IHC of F4/80 showing the recruitment of macrophages in SBTmLC model. (H) The fractions of $CD3^{+}CD8^{+}PD1^{+}$ T cells were determined by flow cytometry in SBTmLC model (n=3 mice per group). (I) Schematic map of construction for chemotaxis assay *in vivo*. (J) Representative *ex vivo* images of the livers of mice given DiR-labelled $CD11b^{+}$ monocytes intravenously, showing monocytes tracking to livers of DEN-induced acute hepatitis model in mA3 overexpression and parallel control mice (n=5 mice per group). Error bars represent mean ± SEM. *p < 0.05, **p < 0.01, ***p < 0.001 (Student's t test).

Figure S6. Immune landscapes of SBTmLC model.

(A and D) A scatterplot showing the results of the RNA-seq in pT3-mA3 versus pT3-vector in peri-tumor tissues (A) and tumor tissues (D) of the SBTmLC model. (B and E) A summary of the results of the RNA-seq data analyzed by gene ontology in peri-tumor tissues (B) and tumor tissues (E) of the SBTmLC model. (C and F) A summary of the results of the RNA-seq data analyzed by cell type enrichment in peri-tumor tissues (C) and tumor tissues (F) of the SBTmLC model. (G-I) GSEA analysis of the RNA-seq data analyzed by cell type enrichment in peritumor and tumor tissues of the SBTmLC model.

Figure S7. A3B mediates TAMs and MDSCs accumulation triggered by CCL2 to promote HCC progression

(A) RT-qPCR analysis of cytokine mRNA to validation of RNA-seq data in Hepa1-6-A3B versus Hepa1-6-EGFP. (B) A heatmap showing the cytokine mRNA of RNA-seq data in the SBTmLC model (n=2 mice per group). (C) RT-qPCR analysis of

CCL2 and IL34 mRNA levels in MHCC97H cells with A3B knockdown exposed to TNF-α or $LT\alpha_1\beta_2$ for 24 hours. (D) Transwell migration assay of iBMDM attracted by Hepa1-6-A3B. The CCR2 antagonist MK0812 (400 nM) was added. (E) RT-qPCR and ELISA analysis of cytokine mRNA and protein of primary hepatocyte in acute DEN liver injected model (n=5 mice per group). (F) Representative *ex vivo* images of the livers of mice given DiR-labelled $CD11b^+$ monocytes intravenously. Mice were treated by i.p. injection with vehicle or RS102895 (5mg/kg). (G-H) RT-qPCR analysis of ARG1 mRNA in iBMDM cells with Hepa1-6-A3B conditioned medium. A antagonist (G) and neutralizing antibodies (H) were added as indicated. (I-J) RT-qPCR analysis of ARG1 mRNA in BMDMs with Hepa1-6-A3B conditioned medium. Different neutralizing antibodies were added as indicated. (K) RT-qPCR analysis of CCL2, IL34 and BMP7 mRNA levels in Hepa1-6 tumor with indicated treatment. Error bars represent mean ± SEM. $*p < 0.05$, $**p < 0.01$, $***p < 0.001$ (Student's t test).

Figure S8. A3B does not exhibit C>T deaminase activity in HCC cells

(A) Nuclear DNA C-to-U activity in extracts from HepG2-Teton-A3B/$A3B^{E68Q/E255Q}$ cells exposed to doxycycline (2 μg/ml) for 48 hours. (B) Nuclear DNA C-to-U activity in extracts from MHCC97L cells with A3B stable knockdown. (C) Representative fields of cells imaged for γ-H2AX in HepG2-Teton-A3B cells exposed to doxycycline (2 μg/ml) for 48 hours. (D) Fold-enrichment of APOBEC mutagenesis signature over the expected occurrence of random mutagenesis from TCGA

integrated data in the FireBrowse database. Left: BRCA,[1] right: HCC.[2] Error bars represent mean ± SEM. *p < 0.05, **p < 0.01, ***p < 0.001 (Student's t test).

Supplementary Materials and Methods

Animals

The C57BL/6J mice and Balb/C Nude mice were purchased from Shanghai SLAC Laboratory Animal Co., Ltd. All mice used in the experiments were 6-8 week-old male mice (weighing 20-22 g), and maintained in a 12-hour light and 12-hour dark turnover environment in the Center for New Drug Safety Evaluation and Research in China Pharmaceutical University. All animal experiments were conducted with the approval of the Center for New Drug Evaluation and Research, China Pharmaceutical University (Nanjing, China) and in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Human samples

Frozen human liver tumor tissues and peritumor tissues were obtained from The Affiliated Drum Tower Hospital. This study was approved by The Affiliated Drum Tower Hospital Research Ethics Committee, and patients provided their informed consents.

Cell Lines

All cell lines were cultured under an atmosphere of 5% CO_2 at 37℃. HepG2, HCCLM3, Hepa1-6, 293T, iBMDM, bone marrow-derived macrophages (BMDMs) and myeloid-derived suppressor cells (MDSCs) were cultured in DMEM (BI) supplemented with 10% fetal bovine serum (FBS, BI), penicillin (100 U/ml), and

streptomycin (100 mg/ml). PLC/PRF/5, MHCC97L and MHCC97H cells were cultured in RPMI 1640 (Bioind) supplemented with 10% FBS (Bioind), penicillin (100 U/ml), and streptomycin (100 mg/ml).

Bacterial strains

DH5α and Stbl3 were grown in LB medium at 37℃ and used to propagate plasmids. BL21 were grown in LB medium at 30℃ and used to express recombinant proteins.

Transfections and RNA Interference

Transient transfections and siRNA interference were performed using jetPRIME (PolyPlus) according to manufacturer's protocol.

Lentiviral infection

HepG2 cells stably expressing the tetracycline inducible A3B, $A3B^{E68Q/E255Q}$, and HCCLM3, MHCC97L and MHCC97H cells with stable knockdown of A3B were generated by lentiviral infection. 293 T packaging cells were used to generate lentivirus according to manufacturer's protocol (Lenti-Pac HIV Expression Packaging Kit, GeneCopoeia). lentivirus was supplemented with 5 μg/ml polybrene and used for infection. 72 hours after infection, HepG2 cells were grown in medium containing G418 (500 μg/ml) to select for the Tet-repressor, puromycin (4 μg/ml) to select for the stably expressing A3B, A3B (E68Q/E255Q). HCCLM3, MHCC97L and MHCC97H cells were selected with 4 μg/ml puromycin in culture medium. Hepa1-6 cells stably expressing A3B and $A3B^{E68Q/E255Q}$ were generated by sleeping beauty transposon. Hepa1-6 cells were transfected with SB100 and pT3-A3B-EGFP

constructs, 14 days after transfection, fluorescence-activated cell sorting was used to isolate Hepa1-6-A3B and Hepa1-6-A3B$^{E68Q/E255Q}$ cell lines.

Protein extraction and Immunoblotting

For nuclear extract preparation: cells were lysed in buffer A (10 mM HEPES, 10 mM KCl, 0.1 mM EDTA, 0.1 mM EGTA, 2 mM NaF, 2 mM Na_3VO_4, 0.1 mM PMSF and protease inhibitor cocktail). After 15 min on ice with occasional shaking, the cell lysate was centrifuged for 10 min at 12000g. The nuclear pellet was suspended in Buffer B (20 mM HEPES, 0.4 M NaCl, 1 mM EDTA, 1 mM EGTA, 2 mM NaF, 2 mM Na_3VO_4, 0.1 mM PMSF and protease inhibitor cocktail) and occasionally shaken for 60 min on ice. After 12000g centrifugation for 10 min, the supernatant was collected as the nuclear extract. For total protein extraction, cells were incubated in buffer containing 20 mM Tris-HCl (pH 8.0), 150 mM NaCl, 1% NP-40, 2 mM EDTA, 0.1% SDS, 20 mM NaF, 1 mM Na_3VO_4, 0.1 mM PMSF and protease inhibitor cocktail and occasionally shaken for 60 min on ice. The cell lysate was centrifuged for 10 min at 12000g, the supernatant was collected as total protein sample.

The extracted proteins (20 μg) were electrophoresed by SDS-PAGE, transferred to a polyvinylidene difluoride (PVDF) membrane (Millipore), and probed with primary antibodies. Primary antibodies were detected by incubation with horseradish peroxidase (HRP)-conjugated secondary antibodies and imaging on an ChemiDoc™ XRS+ imaging device (BIO-RAD).

RNA extraction and Real time-PCR

RNA extraction was conducted according to the manufacture instructions of RNAiso Plus (Takara, 9108). 1.5μg total RNA was converted to complementary DNA (cDNA) by reverse transcription according to the instructions of PrimeScript™ RT Reagent Kit with gDNA Eraser (Takara, RR047A). Realtime-PCR was conducted using SYBR® Premix Ex Taq™ II (Tli RNase H Plus) (Takara, RR820A) and the signal was detected using Applied Biosystems detector. All primers are listed in table S2.

DNA pull-down assay

The DNA pull-down assay was performed as described previously.[3] Briefly, a biotinylated DNA probe corresponding to −456/+107 from the A3B promoter was prepared by PCR. A DNA probe with mutations was prepared with DNA oligonucleotide synthesis and annealing. The biotinylated DNA probe (1 μg) was bound to 150 μg of Dynabeads M-280 Streptavidin (Thermo Fisher) according to manufacturer's protocol. Nuclear extracts were prepared from HepG2 cells stimulated by $LT\alpha_1\beta_2$, and 65 μg of the extracted protein was incubated with 150 μg of the DNA-coupled magnetic beads at 4 °C for 90 min. The beads were washed three times with buffer containing 5 mM Tris-HCl pH7.5, 0.5 mM EDTA, 1 M NaCl, and the bound proteins were eluted in 20 µL of SDS sample buffer and were analyzed with immunoblotting using anti-RelA (8242, CST) and anti-RelB antibody (10544, SCT).

Chromatin immunoprecipitation (ChIP) assay

The ChIP assay was performed using the EZ-Magna ChIP™ A/G Chromatin Immunoprecipitation Kit (Millipore) according to manufacturer's protocol. Anti-RelB,

anti-RelA, anti-H3K27me3, anti-EZH2 antibodies and normal IgG were used to precipitate DNA. The precipitated DNA was subjected to PCR to amplify the A3B, CCL2, IL34 and BMP7 promoter regions with the primers listed in table S2, and was quantified with real-time PCR using SYBR® Green (TAKARA).

Luciferase reporter assay

293T (1×10^5 cells/well) were grown in 24-well plates for 24 h and transfected with 250 ng of luciferase reporter plasmids of A3B promoter (HPRM21800-LvPG04, GeneCopoeia), together with 250 ng of pOTENT-1-RelB plasmids or pOTENT-1-vector plasmids. Gaussia luciferase and secreted alkaline phosphatase activities were measured 24 h after transfection, using the Secrete-Pair™ Dual Luminescence Assay Kit (GeneCopoeia) and a multimode plate reader (PerkinElmer). The Gaussia luciferase activities were normalized to the secreted alkaline phosphatase activities.

Cell proliferation assays

Cells were plated into multiple 96-well plates (5000 cells per well) and measured after 96 hours. For the tetracycline inducible A3B, cells were induced with 2 μg/ml Dox (Sigma). The WST-8 reagents were used as directed (Beyotime). Absorbance was measured at 450 nm (PerkinElmer).

Tumor transplantation

For the orthotopic hepatocellular carcinoma mouse model, the mice were anesthetized. After performing a median laparotomy, 5×10^6 cells (MHCC97H and Hepa1-6) in 25 μl PBS/Matrigel (1:1, Corning) were injected into the anterior hepatic lobe in nude

Balb/C mice and C57BL/6, respectively. The abdominal incision was closed with staples. 30 days later, mice were sacrificed and tumors weights were determined. Alternatively, Hepa1-6 tumors were processed for isolation of tumor infiltrating immune cells and subsequent analysis by flow cytometry.

For the subcutaneous hepatocellular carcinoma mouse model, Hepa1-6 cells (5×10^5) were subcutaneously transplanted into back flanks of C57BL/6 mice. Tumor volume was measured with a caliper every 2-3 days, and calculated tumor volume (= $width^2\times height\times0.5$). 5 days after injection, RS102895 hydrochloride (5 mg/kg) were administered twice a day by i.p. injection. 15 days after cell injection, the tumors were dissected, photographed, tumors weights were determined, and tumors were processed for isolation of tumor infiltrating immune cells and subsequent analysis by flow cytometry.

Sleeping beauty transposon-based mouse liver cancer model

Two-week old male C57BL/6 mice were treated with one single i.p injection of diethylnitrosamine (DEN, Sigma, 25 mg/kg in PBS) and hydrodynamic tail vein injection of DNA 6 weeks later . 5 μg of transposase (CMV-SB100) and 25 μg of the transposon plasmids (pT3 plasmid) were diluted in saline solution at a final volume of 10% of body weight. Mice were injected with the 0.9% NaCl solution/plasmid mix into the lateral tail vein in 5-7 s. 8 months after DEN injection, the tumors were dissected, photographed, tumors weights were determined, and tumors were processed for isolation of tumor infiltrating immune cells and subsequent analysis by flow cytometry.

Isolation of primary hepatocytes and immune cells from mouse liver

Briefly, 6- to 8-week-old male C57BL/6 mice were injected with 5 μg of transposase (CMV-SB100) and 25 μg of the transposon plasmids (pT3 plasmid) as described above. 30 days after injection, mice were given 100 mg/kg DEN by i.p. injection to induce liver damage. 18 hours after DEN injection, mice were anesthetized and the inferior vena cava was cannulated. The liver was perfused with EGTA solution and digested with 0.075% collagenase solution. After passage through nylon filter and washing, centrifuging for 5 min at 50g, 4℃, the pellet was hepatocytes and immune cells were in the supernatant. Hepatocytes were cultured in serum-free Williams' E medium with thyroxine (1 μM), dexamethasone (0.1 μM) and penicillin (100 units/ml). Hepatocytes were cultured for 48 hours and the supernatant were collected for the further detection of CCL2, BMP7 and IL34. RNA extraction from primary hepatocytes and real time PCR were conducted as described above. The supernatant was collected and centrifuged for 5 min at 1500rpm, 4℃. The cell pellet was resuspended in 4mL 20% Percoll and overlaid on 4mL 50% Percoll, centrifuged for 20 min at 2000 rpm, 20°C. The middle layer cells, which were the mononuclear cells, were collected and used for flow cytometry analysis.

Flow cytometry

Single cell suspensions form liver-infiltrating immune cells were prepared as described above. Tumor infiltrating immune cells were prepared using mouse tumor dissociation kit (Miltenyi Biotec) and gentleMACS dissociator device (Miltenyi Biotec), according to manufacturer instructions. Spleens from tumor bearing mice

were triturated grinded with the flat end of a syringe in a 100 mm culture dish, passed through a 100-μm cell strainer and centrifuged at 300g for 5 min to get single cell suspensions. RBCs were lysed. After blocking FcγRIII/II with an anti-CD16/CD32 mAb (eBioscience, San Diego, CA), cell labelling was performed by fluorescently conjugated mAbs directed against mouse CD45, CD11b, F4/80, Gr1, Ly6C, Ly6G, CD3, CD4, CD8, and PD1. Flow cytometry was performed on Attune NxT (Thermo Fisher Scientific). Data were analysed using FlowJo v.10.4.2 (Treestar, Ashland, OR).

Chemotaxis Assay

For in vivo chemotaxis assay, bone marrow cells were extracted from the 6- to 8-week-old male C57BL/6 mice. $CD11b^+$ monocytes were isolated by flow cytometry (BD FACScantoII). Monocytes were labelled using the DiR fluorescent kit (Keygentec), according to manufacturer instructions, then 5×10^6 cells were injected intravenously via the tail vein after 18 h of DEN treatment in control and mA3 overexpression mice as described above. Twelve hours after cell injection, the IVIS spectrum system was used to track monocytes migration.

For in vitro chemotaxis assay, 30% conditioned medium (CM) from Hepa1-6-EGFP and Hepa1-6-A3B cells was added to the lower wells in a transwell (Corning) using 5-μm polycarbonate membranes, iBMDM cells suspended in DMEM containing 2% FBS (2×10^5 cells/100 μL) were added to the upper wells and incubated for 24 hours. iBMDM that migrated to the lower surface of the transwell membrane were fixed with 4% paraformaldehyde, followed with 0.5% crystal violet staining and then subjected to microscopy imaging. To evaluate the CCL2 induced macrophage

chemotaxis, the iBMDM cells were pretreated with CCR2 selective inhibitors MK0812 (400nM) for 1 h, then MK0812 was added to the upper and lower wells; the chemotaxis assay was performed as described above.

Primary BMDMs and MDSCs generation

Tibias and femurs from C57BL/6 mice were removed in sterile condition and bone marrow was flushed. To obtain bone marrow-derived macrophage, $1x10^7$ cells were plated into dishes with 100 mm diameter in 10 ml of medium supplemented with M-CSF (10 ng/ml) for 7 days.

To obtain BM-derived MDSCs, $1x10^7$ cells were plated into dishes with 100 mm diameter in 10 ml of medium supplemented with GM-CSF (40 ng/ml) and IL-6 (40 ng/ml) for 5 days. For both cultures medium was DMEM (Bioind) supplemented with 10 mM HEPES, 1 mM sodium pyruvate, 100 U/mL penicillin, 100 mg/mL streptomycin, and 2 mM L-glutamine plus 10% fetal calf serum (Bioind).

BMDMs and MDSCs stimulation experiments

$4x10^6$ Hepa1-6-EGFP or Hepa1-6-A3B cells were plated into dishes with 100 mm diameter in 10 ml 10% serum-free DMEM. After 24 h, conditioned medium was generated by incubating cells in 10 ml serum-free DMEM for 24 h. Collected conditioned medium (CM) was centrifuged to remove cellular debris. 30% CM was used to stimulate BMDMs and MDSCs for 24 h without further addition of M-CSF, GM-CSF or other media. Control macrophages and MDSCs received serum-free fresh DMEM. To evaluate the involvement of IL34 and BMP7 on the CM induced change of gene expression, the BMDMs and MDSCs were cultivated in 30% CM containing

either DMSO as vehicle, 500 nM BLZ945 or 1 μg/ml of the IL34 neutralizing antibody (R&D Systems) or 4 μg/ml of the BMP7 neutralizing antibody (R&D Systems) for 24 h before experimental analysis.

Immunoprecipitation

For nuclear extract immunoprecipitation, nuclear extracts were prepared using Buffer A and Buffer B as described above. For total protein extract immunoprecipitation, total protein extracts were prepared using buffer C containing 25 mM Tris-HCl pH 7.4, 150 mM NaCl, 1% NP-40, 1 mM EDTA, 5% glycerol, 20 mM NaF, 1 mM Na_3VO_4, 0.1 mM PMSF and protease inhibitor cocktail. 1 mg of nuclear extract protein from HepG2-Tet on A3B cells was precipitated with anti-His-tag magnetic beads (MBL), or for HCCLM3 cells, with normal rabbit IgG (Millipore) and anti-EZH2/EED/SUZ12 antibody (Cell Signaling Technology) or for 293T cells with anti-flag agarose beads (Bimake). For in vitro binding assays, purified recombinant A3B protein was incubated with recombinant Flag-tagged Ezh2, Suz12 and Eed proteins, and anti-flag agarose beads (Bimake) in Buffer C. Bound immunocomplexes were washed three times with buffer B for nuclear extracts or buffer C-400 mM NaCl for total protein extracts immunoprecipitation and in vitro binding assays. The immunocomplexes were eluted by heat in SDS sample buffer and subjected to Western blot analysis.

DNA cytidine deaminase activity assays

The nuclear extracts were prepared using Buffer A and Buffer B as described above. Lysates were tested with a fluorescence-based deaminase activity assay as described.[4]

[5] This assay uses fluorescently tagged oligonucleotides in table S2. Briefly, 10 pmol of oligonucleotide and 20 μg nuclear extracts were incubated with a master mix containing 0.4 units uracil DNA glycosylase (NEB), 50 mM Tris (pH 7.4), and 10 mM EDTA. The plates were incubated at 37°C for 2 h followed by addition of 100 mM NaOH and incubation for 30 min at 37°C. 3 μL of 4 N HCl and 37 μL of 2 M Tris–Cl (pH 7.9) was then added to neutralize reactions. The plates were cooled to 4°C and fluorescence was measured using a spectrophotoflourometer with excitation at 490 nm and emission at 520 nm.

HMT assays

For histone methyltransferase (HMT) assays, purified recombinant A3B protein was incubated with the reconstituted PRC2 complex (EZH2/EED/SUZ12/RbAp46/48, Active Motif) in histone assay buffer using the Histone H3 (K27) Methyltransferase Activity Quantification Assay Kit (Abcam) according to manufacturer's protocol. Anti-H3K27me3 (CST) antibodies were used to test histone methylation. The plates was measured using a microplate reader at 450 nm.

Immunohistochemistry and Immunofluorescence

Mouse liver tissue was fixed in neutral buffered formalin, paraffin-embedded and sectioned using standard techniques. The tissue sections were incubated with a peroxidase block and then a primary antibody: F4/80. Bound primary antibody was detected using the DAB kit. The staining was visualized using an Olympus microscope.

As for cell immunofluorescence, cells were grown in plates on a cover glass. The glass was fixed with 4% paraformaldehyde, washed with PBS three times and blocked with goat serum, treated with anti-His tag (abcam), anti-EZH2 (CST), anti-EED (CST) primary antibodies, subsequently reacted with Alexa Fluor 488 or 647 secondary antibodies (Invitrogen). Naive IgGs were used as negative controls. DAPI staining was used to stain all cells in the sections. Images were taken using a confocal microscope TCS SPE Ⅱ (Leica).

Computational Analysis of Human Liver TCGA Data

We used the web tool GAPIA[6] (http://gepia.cancer-pku.cn/) to analyze TCGA data. For comparison gene expression in tumor and non-tumor tissues, we selected "TCGA tumors vs TCGA normal + GTEx normal" for differential analysis and plotting. The expression data were first log2(TPM+1) transformed for differential analysis. The log2FC is defined as median(Tumor) - median(Normal). Genes with |log2FC|>1values and q value <0.01 were considered differentially expressed genes. We performed overall survival (OS) or disease free survival (DFS) analysis based on gene expression stratified by median. Log-rank test was used for hypothesis test. The cox proportional hazard ratio and the 95% confidence interval information can also be included in the survival plot. In Figure 1E and 1F, we took the median of APOBEC3B TPM as a control. If the APOBEC3B TPM in a patient was lower than the median, we classified him into "Low APOBEC3B TPM" group. If the APOBEC3B TPM in a patient was higher than the median, we classified him into "High APOBEC3B TPM " group. In Figure 3M, we defined the sum of APOBEC3B TPM, CD11b TPM and

CD33 TPM as “A3B/CD11b/CD33” signatures. We took the median of “A3B/CD11b/CD33” signatures as a control. If the “A3B/CD11b/CD33” signatures in a patient was lower than the median, we classified him into “Low A3B/CD11b/CD33” group. If the “A3B/CD11b/CD33” signatures in a patient was higher than the median, we classified him into “High A3B/CD11b/CD33” group.

RNA-sequencing

Total RNA was extracted using TRIZOL Reagent (Life technologies) following the manufacturer’s instructions and checked for a RIN number to inspect RNA integrity using an Agilent Bioanalyzer 2100 (Agilent technologies). Qualified total RNA was further purified by RNAClean XP Kit (Beckman) and RNase-Free DNase Set (QIAGEN). 1 µg-3 µg of total RNA was subsequently used to prepare RNA-seq libraries using TruSeq RNA Sample Pre Kit v2 (Illumina), Agencourt ® AMPure XP Beads (Beckman), SuperScript II Reverse Transcriptase (Invitrogen), Qubit™ dsDNA HS Assay Kit (Invitrogen) and Qubit™ dsDNA HS Assay Kit (Invitrogen) following the manufacturers’ protocols. The libraries were paired-end sequenced with HiSeq 2500 (Illumina).

Gene ontology analysis

We used the web tool (http://software.broadinstitute.org/gsea/msigdb/annotate.jsp) to determine GO terms enriched with down and upregulated genes in RNA-Seq data. Biological Process terms in Gene Ontology were analyzed, and the most significant Gene Ontology terms were reported with their respective p values.

Gene set enrichment analysis

Gene set enrichment analysis was performed using GSEA software (v 3.0). The mouse RNA-Seq data were converted to human gene identifiers using the Biomart R package. The hallmark gene sets were obtained from MSigDB in chemical and genetic perturbations and employed to evaluate the gene sets enriched in RNA-seq data.

Cell type enrichment analysis

We used the Enrichr web tool to determine cell type enriched with down and upregulated genes in mouse tumor RNA-Seq data. Most significant cell types are reported with their respective p values. In addition, GSEA was then used to identify cell type using well-established immune expression signatures and the 39 MDSC genes.

Statistical Analysis

Statistical analyses were performed using GraphPad Prism 6 software (GraphPad Software, Inc, La Jolla, CA, USA). Statistical parameters and methods are reported in the Figures and the Figure Legends. A value of $p < 0.05$ was considered statistically significant. Association of gene expression with the survival of patients was evaluated using log-rank test and a value of $p < 0.05$ was considered statistically significant.

Supplementary References

1 Broad Institute TCGA Genome Data Analysis Center. Analysis of mutagenesis by APOBEC cytidine deaminases (P-MACD). Broad Institute of MIT and Harvard 2016. doi:10.7908/C1JS9PSZ

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3 Mori S, Takeuchi T, Ishii Y, *et al.* Identification of APOBEC3B promoter elements responsible for activation by human papillomavirus type 16 E6. *Biochem Biophys Res Commun* 2015;**460**:555–60. doi:10.1016/j.bbrc.2015.03.068

4 Burns MB, Lackey L, Carpenter MA, *et al.* APOBEC3B is an enzymatic source of mutation in breast cancer. *Nature* 2013;**494**:366–70. doi:10.1038/nature11881

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Supplementary Table S1. Key resources table

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
anti-RelA	Cell Signaling Technology	8242
anti-RelB	Cell Signaling Technology	10544
anti-APOBEC3B	Abcam	ab184990
anti-APOBEC3B	Santa Cruz Biotechnology	SC86289
anti-EZH2	Cell Signaling Technology	5246
anti-EED	Abcam	ab4469
anti-SUZ12	Cell Signaling Technology	3737
anti-His Tag	Cell Signaling Technology	12698
anti-Flag Tag	Cell Signaling Technology	2368
anti-H3K27me3	Cell Signaling Technology	9733
anti-H3	Santa Cruz Biotechnology	SC8654
anti-His tag	Cell Signaling Technology	12698
anti-His tag	Abcam	Ab18184
anti-Flag tag	Cell Signaling Technology	2368
Normal mouse IgG	merck	12-371
Normal rabbit	merck	12-370
anti-Pol II	merck	05-623B
anti-CCL2	R&D Systems	AF-479-NA
anti-IL34	R&D Systems	AF5195
anti-BMP7	R&D Systems	MAB3541
anti-CD45-Apc-cy7	biolegend	103115
anti-CD11B-BV510	biolegend	101245
anti-Gr1-FITC	biolegend	108405
anti-F4/80-Apc	ebioscience	17-4801-80
anti-Ly6G-PEcy7	biolegend	127618
anti-Ly6C-PE	biolegend	128007
anti-CD3-FITC	ebioscience	11-0031-82
anti-CD4-Apc-cy7	biolegend	100414

anti-CD8-PE	invitrogen	12-0081-82
anti-PD1-Apc	ebioscience	17-9981-80
Anti-FLAG Affinity Gel	Bimake	B23101
Protein A/G Magnetic Beads for IP	Bimake	B23201
HisPur Ni-NTA Resin	Thermo Fisher Scientific	88221
Anti-His-tag mAb-Magnetic Beads	MBL	D291-11
Bacterial and Virus Strains		
DH5α Competent Cells	Thermofisher	18265017
Stbl3 E. coli	Thermofisher	C737303
Biological Samples		
The tumor and corresponding non-tumor tissues	Gulou Hospital in Nanjing	N/A
Chemicals, Peptides, and Recombinant Proteins		
Lipopolysaccharide	Sigma	L6529
Diethylnitrosamine	Sigma	N0756
DiR	Keygentec	KGMP0026
Recombinant Human Lymphotoxin α1/β2 Protein	R&D systems	8884-LY/CF
Mouse GM-CSF Recombinant Protein	peprotech	cat.#315-03-20
Mouse M-CSF Recombinant Protein	peprotech	cat.#315-02-50
Mouse IL-6 Recombinant Protein	peprotech	cat.#216-16-10
Recombinant EZH2 protein complex	Activemotif	31337
Recombinant PCR2 complex	Activemotif	31387
Recombinant APOBEC3B protein	This paper	N/A
RS102895 hydrochloride	MedChemExpress	HY-18611
GSK126	Selleck Chemicals	S7061
Doxycycline hyclate	Sigma	D9891
MK-0812	MedChemExpress	HY-50669
Critical Commercial Assays		
PrimeScript™ RT reagent Kit with gDNA Eraser	TAKARA	RR047

Secrete-Pair Gaussia Luciferase Assay Kit	Genecopoeia	SPGA-G010
TB Green™ Premix Ex Taq™ II	TAKARA	RR820
Lenti-Pac™ Lentiviral Packaging Kits	Genecopoeia	LT001
Tumor Dissociation Kit, mous	Miltenyi Biotec	130-096-730
EZ-Magna ChIP™ A/G Chromatin Immunoprecipitation Kit	Millipore	17-10086
Histone H3 (K27) Methyltransferase Activity Quantification Assay Kit	Abcam	ab113454
Deposited Data		
RNA-seq	This paper	GEO: GSE117546
Experimental Models: Cell Lines		
Human cell line: HepG2	ATCC	HB-8065
Human cell line: HCCLM3	National Infrastructure of Cell line Resource, China	3131C0001000700094
Human cell line: MHCC97H	Liver Cancer Institute, Zhongshan Hospital, Shanghai, China	N/A
Human cell line: MHCC97L	Liver Cancer Institute, Zhongshan Hospital, Shanghai, China	N/A
Human cell line: PLC/PRF/5	ATCC	CRL-8024
Human cell line: 293T	ATCC	CRL-3216
Mouse primary cell: bone marrow-derived macrophage cells	This paper	N/A
Mouse primary cell: myeloid-derived suppressor cell	This paper	N/A
Mouse cell line: Hepa1-6	ATCC	CRL-1830
Experimental Models: Organisms/Strains		
Mouse: C57BL/6	SLAC Laboratory Animal Co., Ltd	N/A

Mouse: Balb/C Nude	SLAC Laboratory Animal Co., Ltd	N.A
Oligonucleotides		
Primers and Probe, see Supplementary Table S2	This paper	N/A
A3B siRNA-1: ccugauggauccagacaca	This paper	N/A
A3B siRNA-2: gguguauuucaagccucag	This paper	N/A
IL34 shRNA: gcuacaaauguguacccuutt	This paper	N/A
BMP7 shRNA: gcugguugguguuugauautt	This paper	N/A
Recombinant DNA		
pIRES2-ZsGreen1	Clontech	Cat. No. 632478
pIRES2-ZsGreen1-RELA	This paper	N/A
pOTENT-1	This paper	N/A
pOTENT-1-RELB	This paper	N/A
HPRM21800-LvPG04	Genecopoeia	HPRM2180
HPRM21800-LvPG04-M1	This paper	N/A
HPRM21800-LvPG04-M2	This paper	N/A
pEX-TET3G	Genecopoeia	N/A
pEX-TRE3G-A3B-his	This paper	N/A
pEX-TET3G-A3B$^{E68Q/E255Q}$-his	This paper	N/A
psi-LVRU6GP-shcon	This paper	N/A
psi-LVRU6GP-shA3B	This paper	N/A
pCMV(CAT)T7-SB100	Addgene	Plasmid #34879
pT3-EF1α-ployA	This paper	N/A
pT3- EF1α-mA3-Flag-ployA	This paper	N/A
pT3-EF1α-EGFP-ployA	This paper	N/A
pT3-EF1α-A3B-T2A-EGFP	This paper	N/A
pT3-EF1α-A3B$^{E68Q/E255Q}$-T2A-EGFP	This paper	N/A
pcDNA3.1-A3B-his	This paper	N/A
pcDNA3.1-A3B^{CDT}-his	This paper	N/A
pcDNA3.1-A3B^{NDT}-his	This paper	N/A
pcDNA3.1-EZH2-3×FLAG	This paper	N/A

pcDNA3.1-EED-3×FLAG	This paper	N/A
pcDNA3.1-SUZ12-3×FLAG	This paper	N/A
Software and Algorithms		
GEPIA	Tang, Z. et al. (2017)	http://gepia.cancer-pku.cn/index.html
GSEA	Tamayo, et al. (2005)	http://software.broadinstitute.org/gsea/index.jsp
NF-kB PBM Dataset	Siggers T, et al. (2011)	http://thebrain.bwh.harvard.edu/nfkb/
GraphPad Prism 6	GraphPad Software	N/A
FlowJo	Treestar	N/A
Enrichr	Kuleshov MV, et al. (2016)	http://amp.pharm.mssm.edu/Enrichr/

Supplementary Table S2. Sequences of DNA oligos, primers, and probe.

Assay	Gene		Sequence (5'to 3')
QPCR	APOBEC3A	F	CTACCTGTGCTACGAAGT
		R	TTCTTAGCCTGGTTGTGTA
	APOBEC3B	F	ACTTGGCTGTGCTATGAA
		R	GTGGTACTGAGGCTTGAA
	APOBEC3F	F	AGACACATTCTCCTACAACT
		R	CTACTGAGCACTTGAAATGG
	APOBEC3G	F	CCGCCTCTACTACTTCTG
		R	TACACGAACTTGCTCCAA
	APOBEC3C	F	CCTGGTACACATCTTGGA
		R	TGGTAACATGGATACTGGAA
	APOBEC3D	F	GACACATTCTACGACAACTT
		R	GCCACAGAACCAAGATAAG
	APOBEC3H	F	CCAAGTCACCTGTTACCT
		R	TGAGCCTTGATGAAGTCA
	mAPOBEC3	F	AATCACCTGCTACCTCAC

		R	GATTGCCACAGAGAACAC
	mCLEC7A	F	CCTGGTATGGAAGTAAGAGA
		R	TAATACGGTGAGACGATGTT
	mCD274	F	TGCTGCATAATCAGCTACGG
		R	TCCACGGAAATTCTCTGGTT
	mARG1	F	AGGTCTCTACATCACAGAAG
		R	GAAGCAAGCCAAGGTTAA
	mTNF-A	F	TTCTGTCTACTGAACTTC
		R	CCATAGAACTGATGAGAG
	mIL-1β	F	CAATGGACAGAATATCAAC
		R	ACAGGACAGGTATAGATT
	mIL12A	F	ACATCTGCTGCTCCACAAG
		R	GGTGCTTCACACTTCAGGAA
	mIL10	F	GGC CCA GAA ATC AAG GAG
		R	CCT TGT AGA CAC CTT GGT
	mCCL2	F	ATGAGATCAGAACCTACAACT
		R	TCCTACAGAAGTGCTTGAG
	mIL-34	F	GATTGCTGTGCCTTATGAG
		R	CTGAACCTCCTGTAGATACTT
	mBMP7	F	AACCTAGTGGAACATGACAA
		R	CTGATAGACTGTGATCTGGAA
	mCXCL15 (IL8)	F	GCTACGATGTCTGTGTATTC
		R	GTAGGAACCTGTTAGTAATTGG
	mCCL5	F	GAGTATTTCTACACCAGCAG
		R	GTATTCTTGAACCCACTTCTT
	hCCL2	F	ATAGCAGCCACCTTCATT
		R	GCTTCTTTGGGACACTTG
	hIL-34	F	TATCTTGGGATCTTCCTTGG
		R	CACTGATCTTGTAGTTGATGG
	hBMP7	F	CAAGATAGCCATTTCCTCAC
		R	CGGATGTAGTCCTTGTAGAT
	mS100A10	F	TGGCTGTGGACAAAATAATG

		R	CCCTTCTGCTTCATGTTTAC
	mS100A9	F	ACACCTTCCATCAATACTCT
		R	ATCAGCATCATACACTCCTC
	mCCL3	F	GCCAATTCATCGTTGACTATT
		R	CAGGTCAGTGATGTATTCTTG
	mCCL4	F	CTTCCTGCTGTTTCTCTTAC
		R	TTCAACTCCAAGTCACTCAT
	mCCL6	F	GTCGCTATAACCCTCCAATA
		R	CTCCTGCTGATAAAGATGATG
	mCCL9	F	CTCCTTCCTCATTCTTACAAC
		R	ACCTATAAATCTTGAACACTGAA
	mCCL25	F	GTGCTGTGAGATTCTACTTC
		R	TGGTTTGACTTCTTCCTTTC
	mCCL27a	F	GATTGAGGAGATACGAGGTG
		R	CTTCTTGCTTCTGCTTAGTC
	mCXCl1	F	GGATTCACCTCAAGAACATC
		R	CTTTTAGCATCTTTTGGACAATT
	mCXCL9	F	ACCCTAGTGATAAGGAATGC
		R	ATCTCCGTTCTTCAGTGTAG
	mCXCL11	F	CTCAAGGCTTCCTTATGTTC
		R	CTTCATAGTAACAATCACTTCAAC
	mCXCl13	F	AACATCATAGATCGGATTCAAG
		R	CTCTTCTCTTACTCACTGGA
	mCXCL16	F	AAATGAGAAACAGCAAGATGA
		R	CAGTGAGGAAGAAGACAATG
CHIP	A3B promoter	F	GGAAAACCAGAGCCAGAG
		R	ACAGCCCTCCTTAAAGTG
	CCL2 promoter-1	F	TGGTCAGTCTGGGCTTAA
		R	CAGGAAGTAGGAAAGGGA
	CCL2 promoter-2	F	AAACTGAAGCTCGCACTC
		R	TGGATGGTCCATGATAACT
	CCL2 promoter-3	F	TCCGTCTTAATGACACTT

		R	GACCCAATGACTCAGTTT
	IL-34 promoter-1	F	GGTAGTCCCTTAGAACAGTC
		R	AAGTAGGAGGGAGTCTTCAA
	IL-34 promoter-2	F	CTCAGCCTCTCCAACCTG
		R	CCAAGACTCGGTCTCCTA
	IL-34 promoter-3	F	AAGTATGCCTCAGAGAAGTATA
		R	TTGCTTTATGGTTATGTGGAT
	BMP7 promoter-1	F	AGGTCTTGGAGGTCTCTG
		R	CTCAGTCCCTGTATCCTTT
	BMP7 promoter-2	F	CAGAGGAGCGGGAAGAAG
		R	GAACGAAAAGGCGAGTGA
	BMP7 promoter-3	F	GTTCATGCTGGACCTGTA
		R	GTGAGGAAATGGCTATCTTG
	MYT-1 promoter	F	ACAAAGGCAGATACCCAACG
		R	GCAGTTTCAAAAAGCCATCC
	GAPDH	F	TACTAGCGGTTTTACGGGCG
		R	TCGAACAGGAGGAGCAGAGAGCGA
Biotin-Labeled Probe	A3B promoter PCR	F	CTCGAGGCACACCTAAGCCTTC
		R	GTTCAGCCTCTTAGATACGCTTGTC
	DAPA-A3B-promoter	F	GGAAGGGTCCGGGGAAAACCAGAGCCAGAG
		R	CTCTGGCTCTGGTTTTCCCCGGACCCTTCC
	DAPA-A3B-Promoter-mut	F	GGAAGGGTCCGCCGCCCGCCAGAGCCAGAG
		R	CTCTGGCTCTGGCGGGCGGCGGACCCTTCC
Fluorescence probe	A3B fluorescence probe	5'-6-FAM-3'-TAMRA	AAATTCTAATAGATAATGTGA
	Positive-probe-1	5'-6-FAM	AAATTC
	Positive-probe-2	3'-TAMRA	TAATAGATAATGTGA