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Supplemental Information

Nuclear-Encoded lncRNA *MALAT1* Epigenetically Controls Metabolic Reprogramming in HCC Cells through the Mitophagy Pathway

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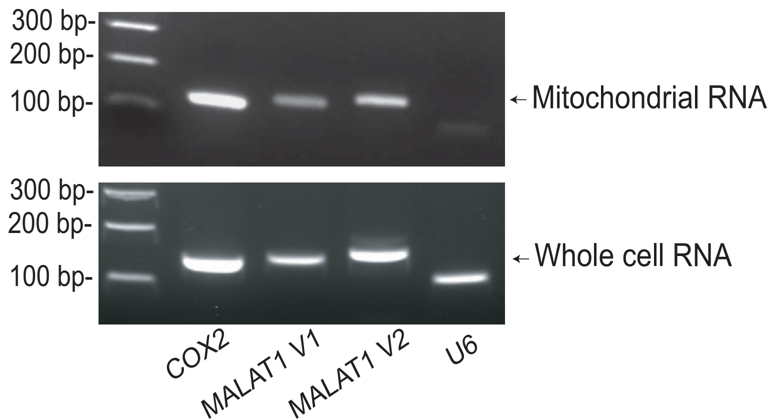


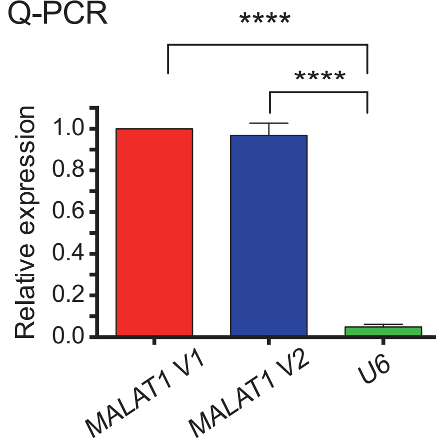
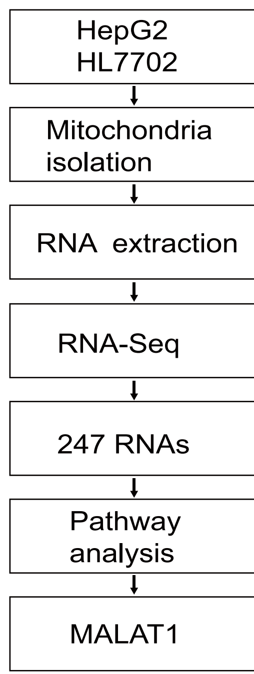
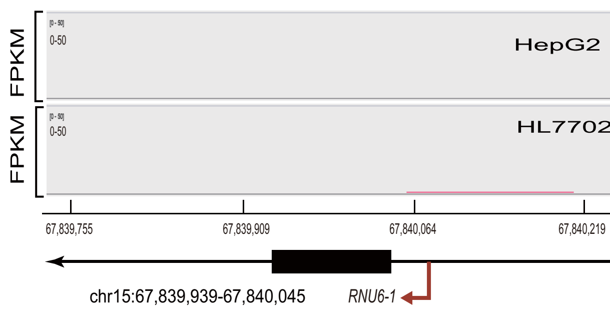
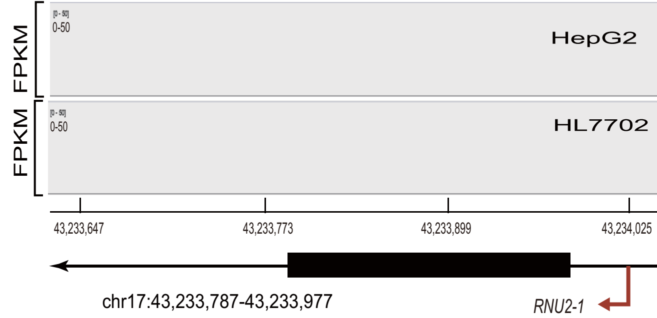


Figure S1. Quality check of isolated mitochondria.
A.Quality check of isolated mitochondria RNA. RT-PCR was used to examine the quality of isolated mitochondrial RNAs for the contamination of nuclear U6 RNA. V1, V2: PCR primers for two MALAT1 variants. The whole cell RNAs were used as the control. MALAT1 and mitochondrial control COX2 were abundantly detected in isolated HepG2 mitochondria RNA. No nuclear U6 was detected in mitochondrial RNA samples. B.Q-PCR quantitation of MALAT1 in isolated mitochondria RNA. The nuclear MALAT1 were highly enriched in HepG2 mitochondria.







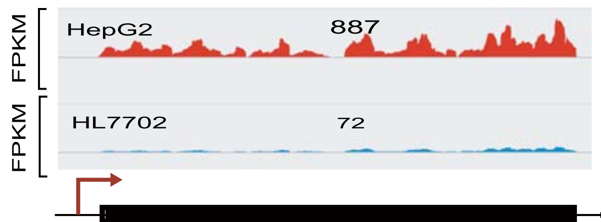
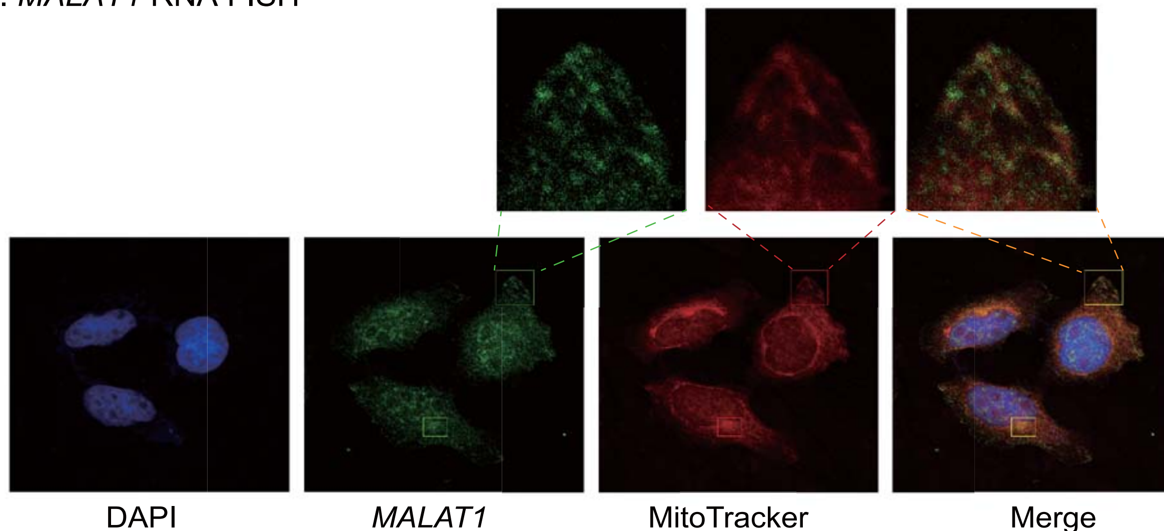
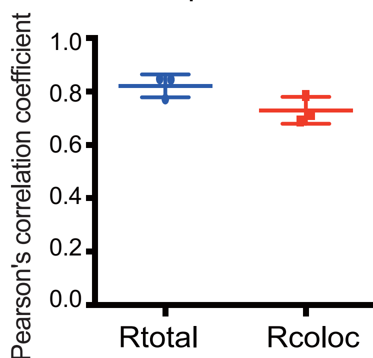


Figure S2. MALAT1 is enriched in the mitochondria of hepatoma cells.
A. Flow chart of RNA-sequencing to identify RNAs in isolated mitochondria; B. Quality check of RNA-seq data for nuclear U6 RNA. IGV Sashimi blot shows the absence of U6 RNA in the HepG2 mitochondria RNA-seq dataset; C. Quality check of RNA-seq data for nuclear U2 RNA. U2 RNA is absent in HepG2 mitochondria RNA-seq data; D. IGV Sashimi blot of MALAT1. MALAT1 is highly enriched in hepatoma HepG2 mitochondria compared with normal hepatic HL7702 mitochondria; E. RT-PCR analyses of MALAT1 and mitochondrial gene transcripts in isolated mitochondrial RNAs. MALAT1-1, MALAT1-2: RT-PCR for two different MALAT1 variants. MT-ND5, MT-ND6, MT-CYB: mitochondrial gene transcripts as the PCR controls.





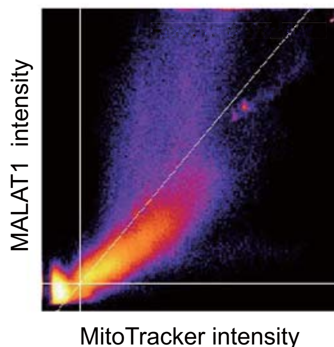


Figure S3. MALAT1 RNA FISH using antisense single stranded DNA probes.
A. RNA-FISH of mitochondria-associated MALAT1 in HepG2 cells. Dig-labeled single stranded MALAT1 probes were synthesized by asymmetric PCR and were used for hybridization with FITC-coupled anti-dig antibody (green). Mitochondria were labeled with MitoTracker (red).B. Quantitation of MALAT1 and MitoTracker. Pearson's correlation coefficient was measured for the entire image (Rtotal) and the pixels above thresholds (Rcoloc) from 3 tested field of views. C. Scatter plot of channel 1 (MALAT1, green) and channel 2 (MitoTracker, red). The regression line is plotted along with the threshold level for channel 1 (vertical line) and channel 2 (horizontal line). Mean +/- SEM are indicated in the right upper corner of the image.

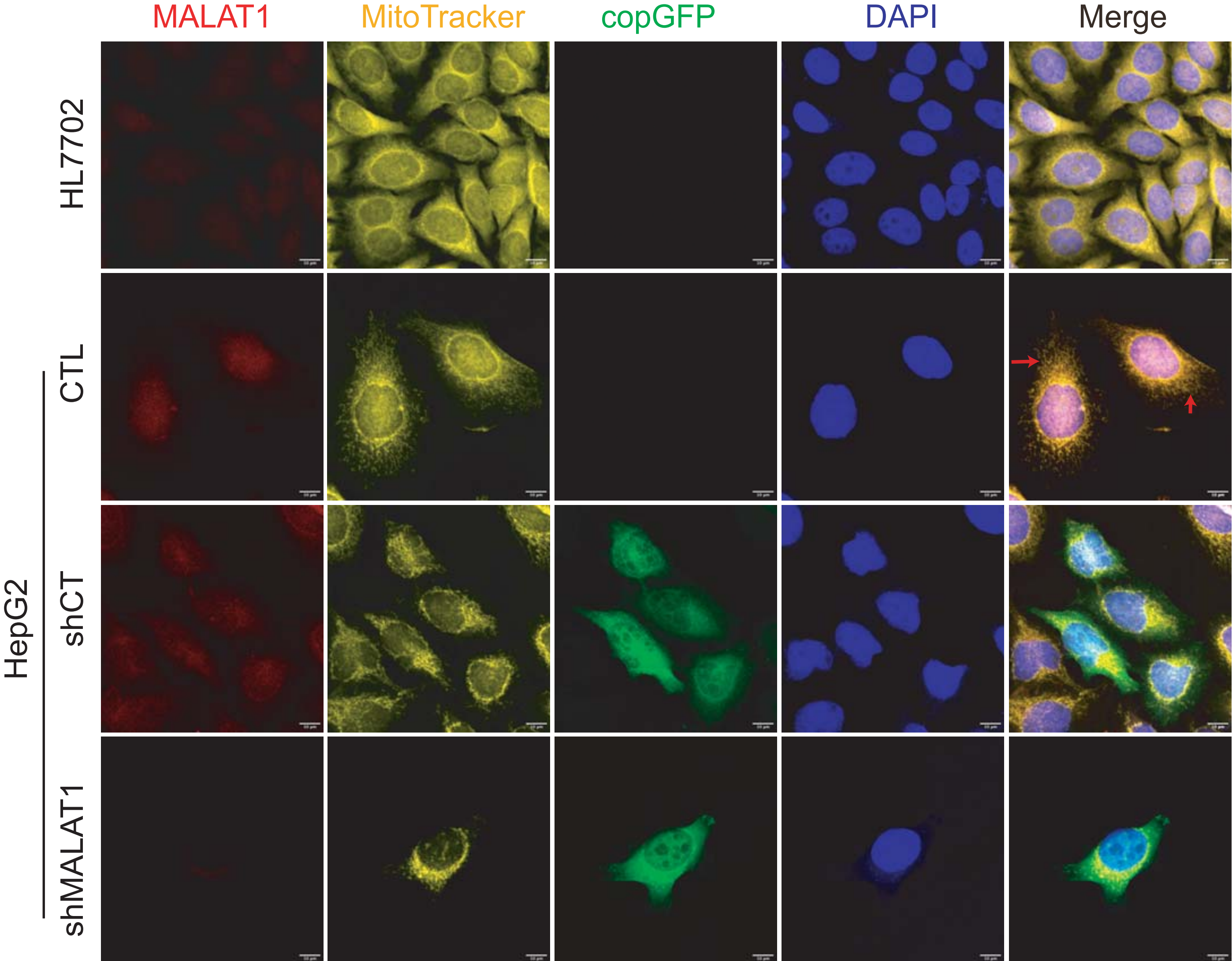
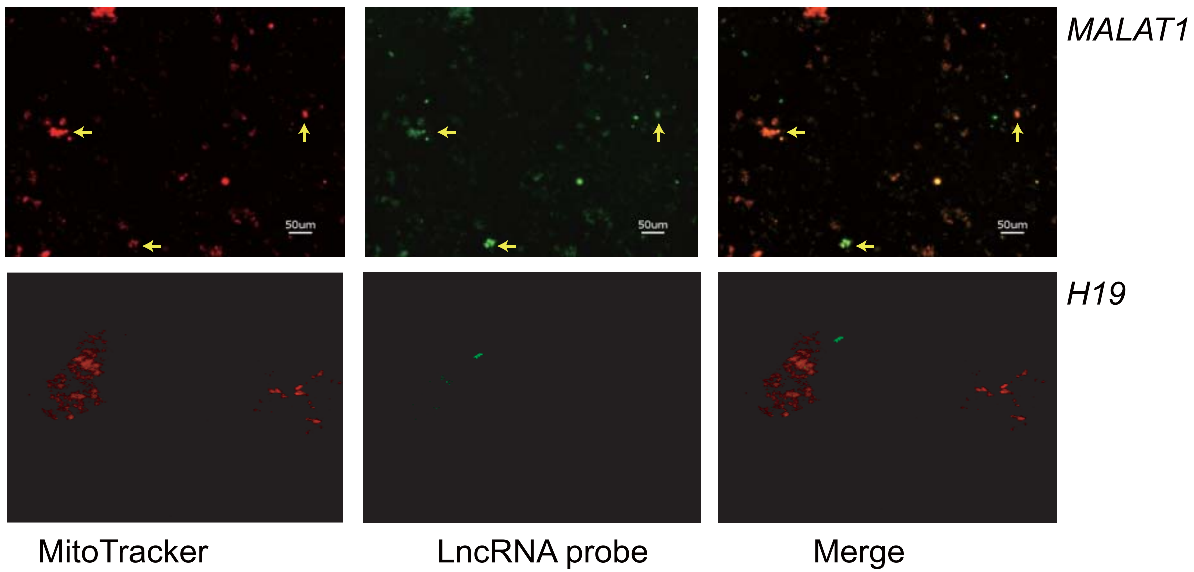
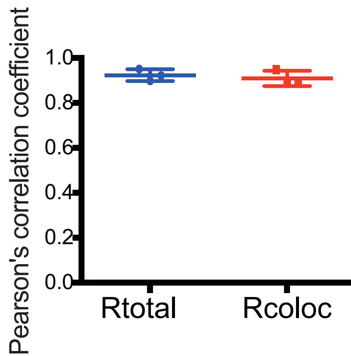


Fig.S4 RNA-FISH of MALAT1 in HL7702 and shRNA-treated HepG2 cells.
MALAT1 was probed with antisense oligonucleotide probes (red) provided in the Ribo Fluorescent In Situ Hybridization Kit (C10910, RiboBio). Mitochondria were labeled with MitoTracker (yellow). Cells were counterstained with DAPI and imaged under a confocal laser-scanning microscope (Carl Zeiss). HL7702: normal hepatic cells; shMALAT1, shCT: HepG2 cells transfected with lentiviruses carrying shMALAT1, shRNA control (shCT); CTL: untreated HepG2 cells; copGFP: the track marker in the lentiviral vector (green). Red arrow: Co-localization of lncRNA MALAT1 and MitoTracker.

A. *MALAT1* in isolated mitochondria



B. RNA-FISH quantitation



C. Colocalization

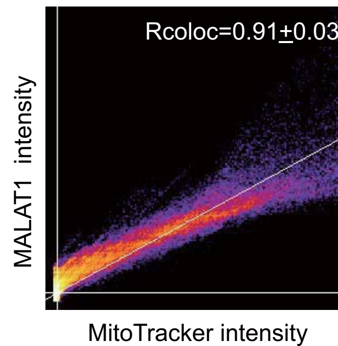
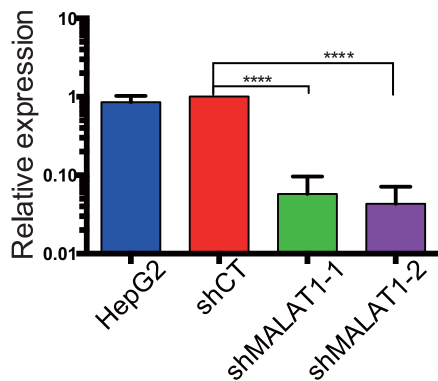
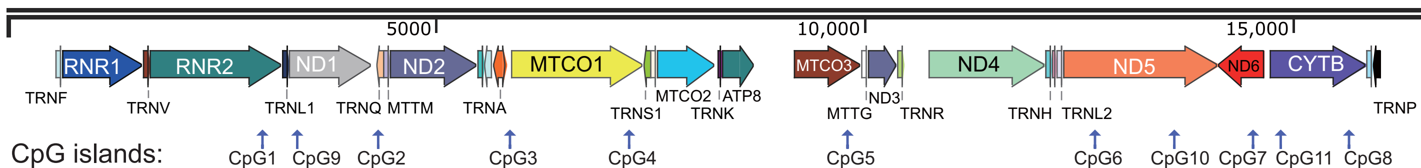


Figure S5. ***MALAT1* and *H19* RNA FISH in isolated mitochondria.**
A. Co-localization of MALAT1 and MitoTracker in isolated mitochondria smear. Mitochondria were isolated and smeared on slides for RNA FISH detection of MALAT1 (green). Mitochondria were tracked by MitoTracker staining (red). B. Quantitation of MALAT1 and MitoTracker in isolated mitochondria smear. Pearson's correlation coefficient was calculated for the entire image (Rtotal) and the pixels above thresholds (Rcoloc) from 3 tested field of views. C. Scatter plot of channel 1 (MALAT1, green) vs. channel 2 (MitoTracker, red). The regression line is plotted along with the threshold level for channel 1 (vertical line) and channel 2 (horizontal line). Mean +/- SEM are indicated in the right upper corner of the image.

A. *MALAT1* knockdown



B. MT-DNA CpG island



C. MT-DNA methylation

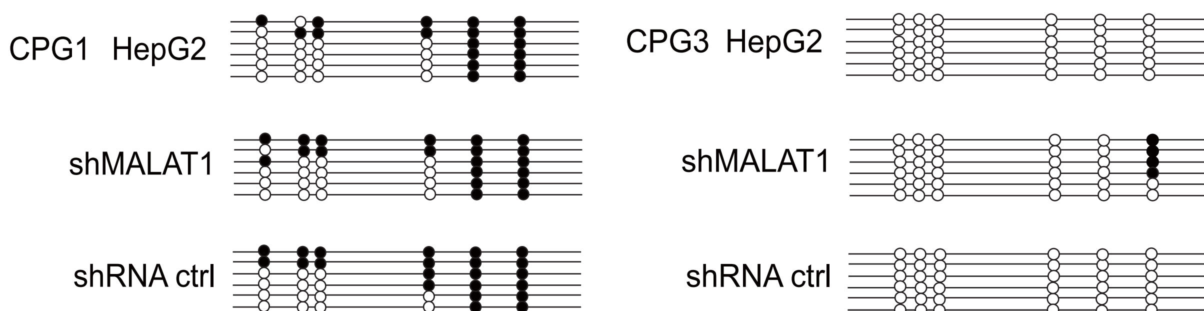


Figure S6. ***MALAT1*** **knockdown and mitochodnrial DNA methylation**

A. MALAT1 knockdown by shRNAs in HepG2 cells. shMALAT1-1, shMALAT1-2: MALAT1 shRNAs; shCT: random shRNA control. MALAT1 was quantitated by Q-PCR. The Ct value was normalized over that of β-ACTIN (housekeeping gene) and then standardized by setting shCT as 1 for comparison. **** $P<0.0001$ compared with the shCT group, one-way ANOVA, followed by student t test.

B. Location of CpG islands in the mitochondrial DNA genome. C. The status of CpG methylation. Sodium bisulfite pre-treated mitochondria DNA used EZ DNA methylation kit to detect the MALAT1 binding region methylation. Black dots, methylated CpG sites; white dots, unmethylated CpG sites.

A.Mitochondria number under TEM

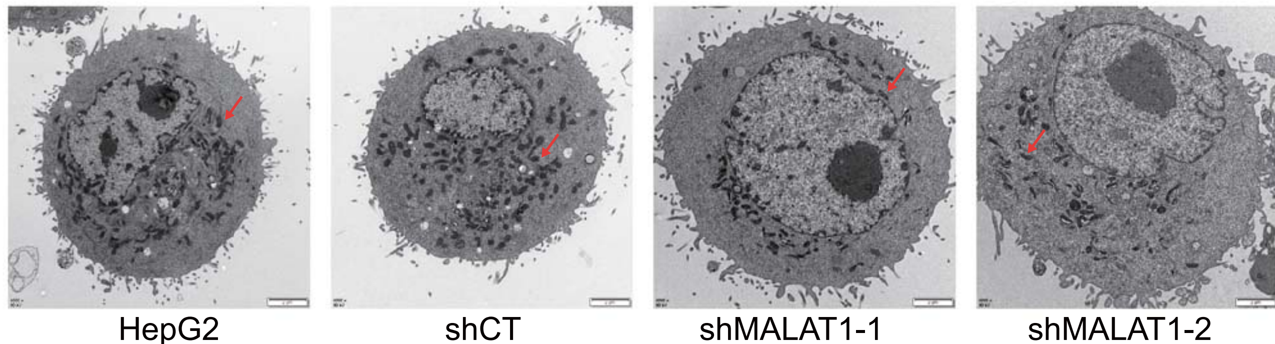
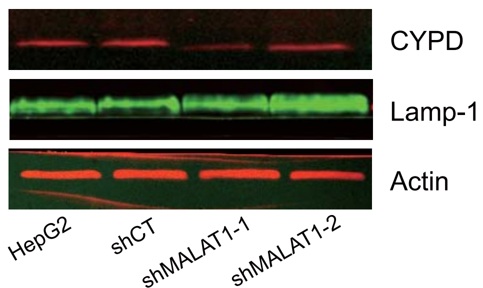
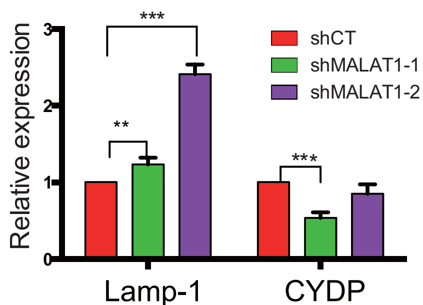


Figure S7. MALAT1 knockdown rduced mitochondria DNA copy number in HepG2 cells. Mitochondria quantity and modality were observed under Transmission electronic microscope (TEM). Red arrow: mitochondria under TEM. Mitochondria per cell was quantitated in Figure 2E.

A. WB: Mitochondria and lysosome marker



B. Mitochondria and Lysosome marker



C.Lysotracker

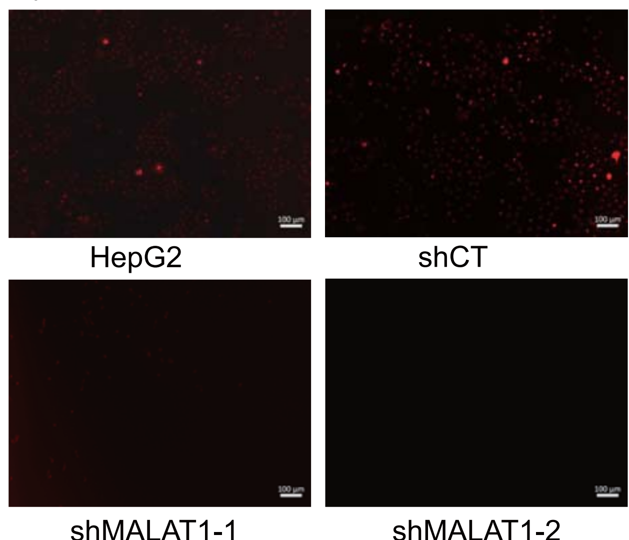
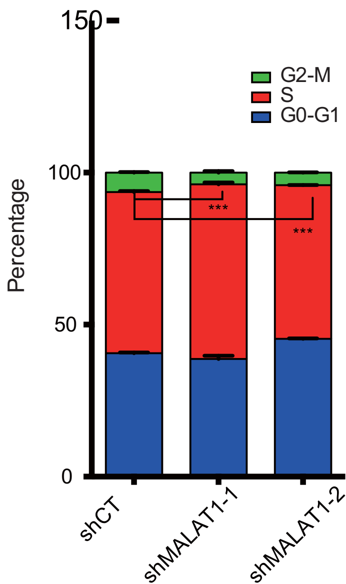


Figure S8. MALAT1 knockdown impaired the mitophagy pathway.
A. Mitochondria and lysosome by Western blot. The quantity of mitochondria was assessed by mitochondria membrane marker CYPD and lysosome by lysosome marker Lamp-1. MALAT1 knockdown cells (shMALAT1-1 and shMALAT1-2) show decreased mitochondria membrane marker CYPD and increased lysosome marker Lamp-1; B. Quantitation of CYPD and Lamp-1 Western blot results. ** $P<0.01$, *** $P<0.001$compared with controls; C.Acidic lysosome staining by Lysotracker. Reduced acidic lysosomes were observed in shMALAT1-treated HepG2 cells.



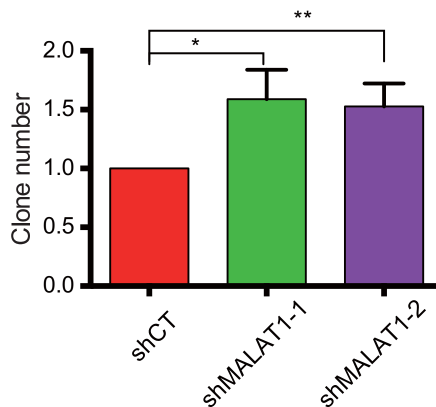
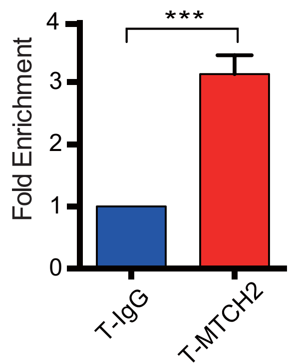
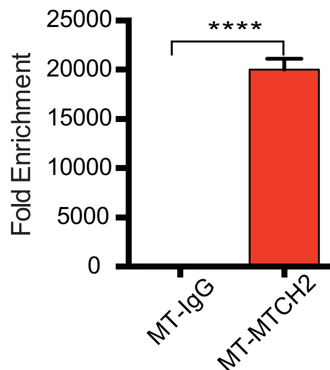


Figure S9. MALAT1 knockdown affect cell cycle and colony formation.

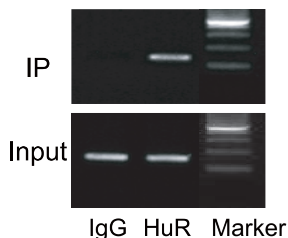
A. Cell cycle analysis. MALAT1 knockdown cells (shMALAT1-1 and shMALAT1-2) showed less on G2 stage compared to shCT. *** $P<0.001$ compared with the control group. B. Clone formation assay. MALAT1 knockdown cells (shMALAT1-1 and shMALAT1-2) showed more clone formation ability compared to the shCT group. * $P=0.0149$, ** $P<0.01$ compared with t he control group.

A. MTCH2 RIP (whole cell) B. MTCH2 RIP (mitochondria)





C.HuR-RIP-qPCR



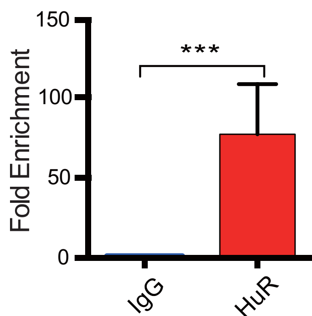


Figure S10. Putative transportation of MALAT1 in mitochondria.
A. Interaction of MALAT1 with mitochondria membrane protein MTCH2 in the whole cell lysate. The interaction was assessed by RIP. *** $P<0.001$ compared with the IgG control group. B. MALAT1 interaction with MTCH2 in mitochondria lysate. **** $P<0.0001$ compared with the IgG control group. C. Interaction of MALAT1 with RNA transporter protein HuR. The interaction was detected by RIP PCR and Q-PCR. *** $P<0.001$ compared with the IgG control group.

chr11:65,265,000 65,270,000 1kb 65,275,000

MALAT1

108bp 141bp 360bp 155bp

4632 4633 5239 5240 4630 4631 5241 5242 PCR primers

4491 4492 4633 4493 4631 4494 4495 RAT probe

360bp 155bp

4630 4631 5241 5242 FISH probe

4491 4492 4633 4493 4631 4494 4495 CHIRP biotin probe

Figure S11. Location of RAT, CHIRP, FISH probes and PCR primers in the MALAT1 locus. The daigraam shows the chr11:65,265,000-65,275,000 region, where the MALAT1 gene is located. The scale bar is 1kb. Arrows: the direction of PCR primer or probe oligonucletides. Red arrow: RAT probes; blue arrow: CHIRP probes; green arrow: FISH probe; black arrow: PCR primers. RAT and CHIRP probes are labled with biotin.