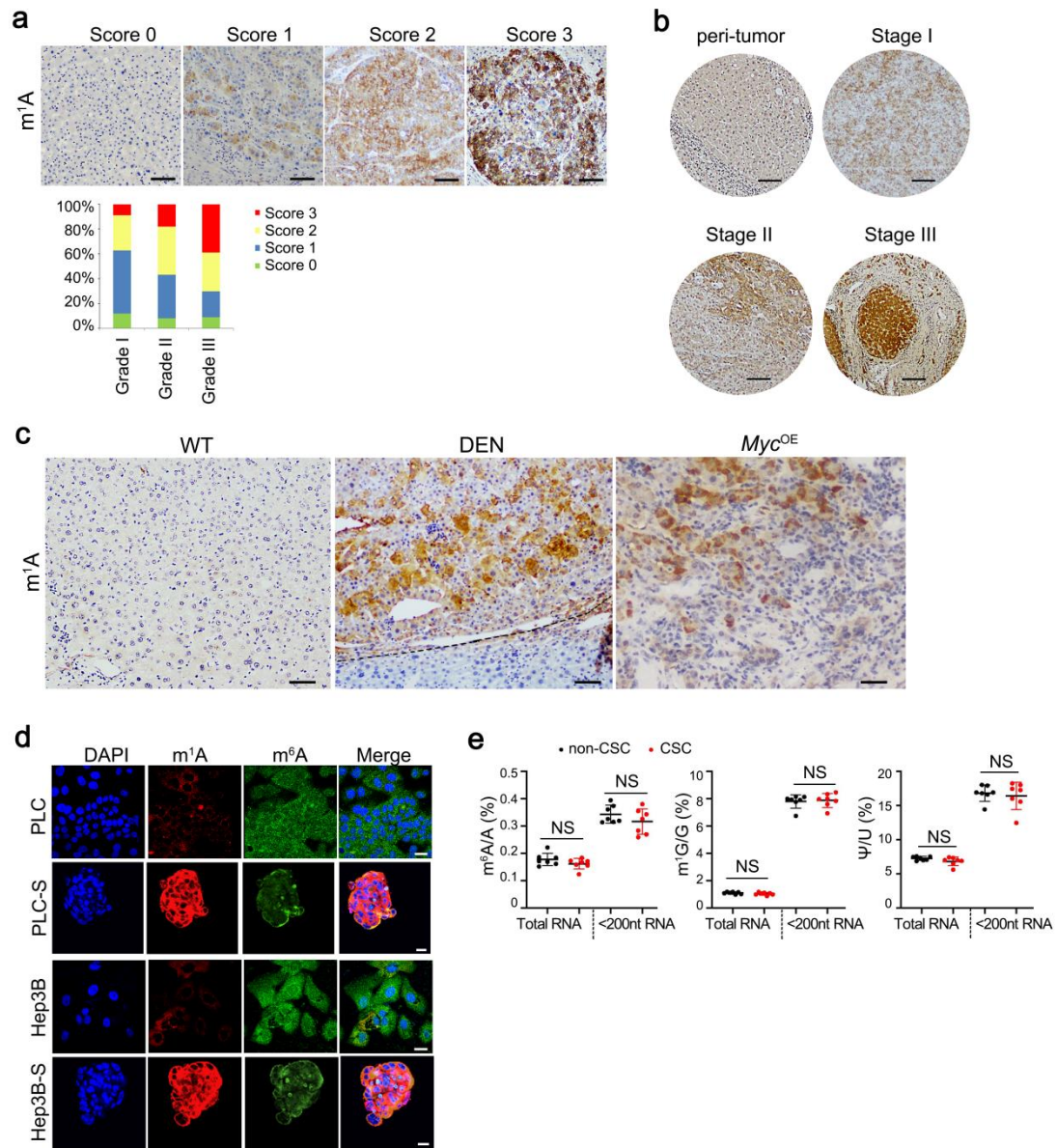


Supplementary Information

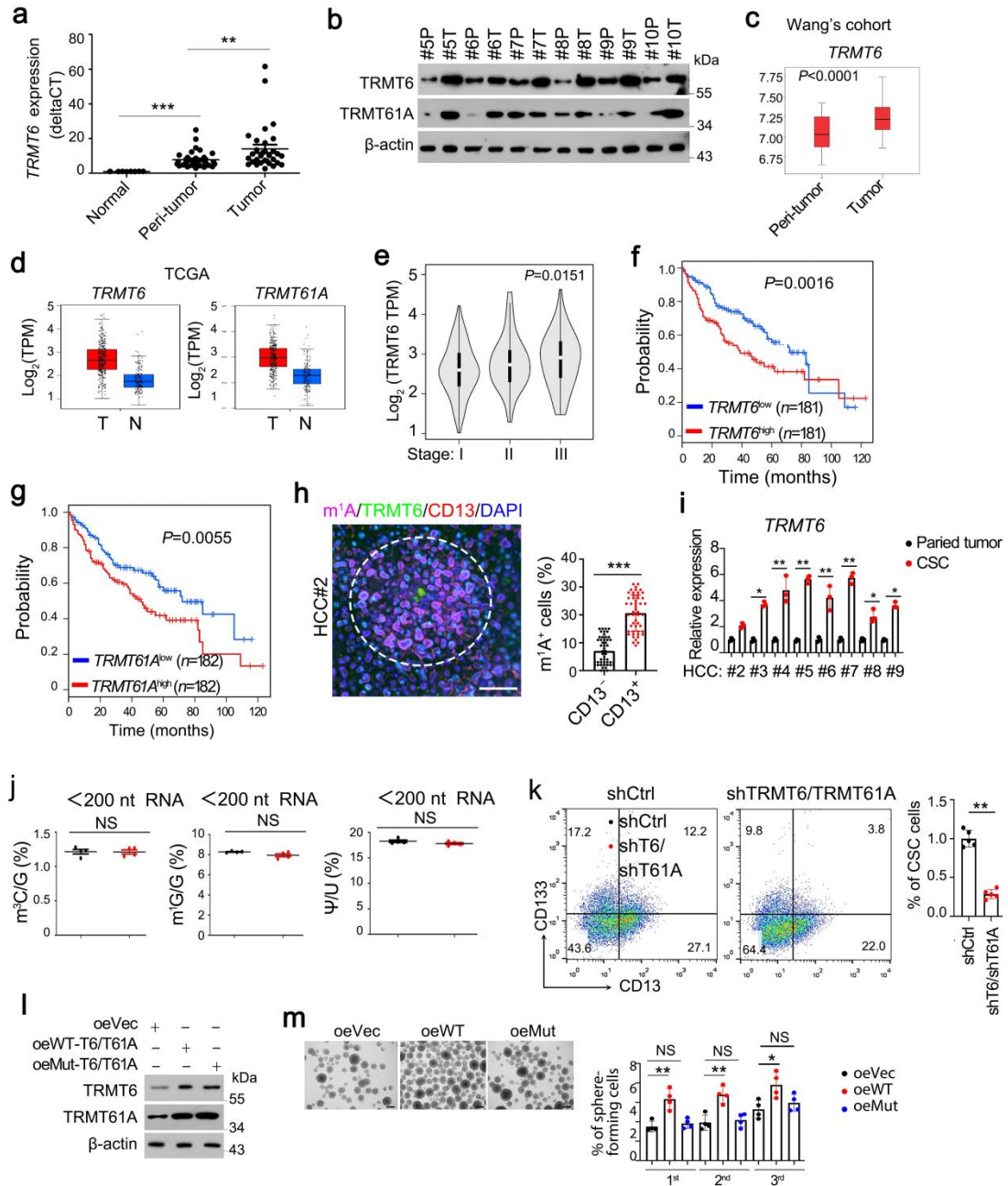
Supplementary Figure 1-7 and figure legends

Supplementary Table 1-3



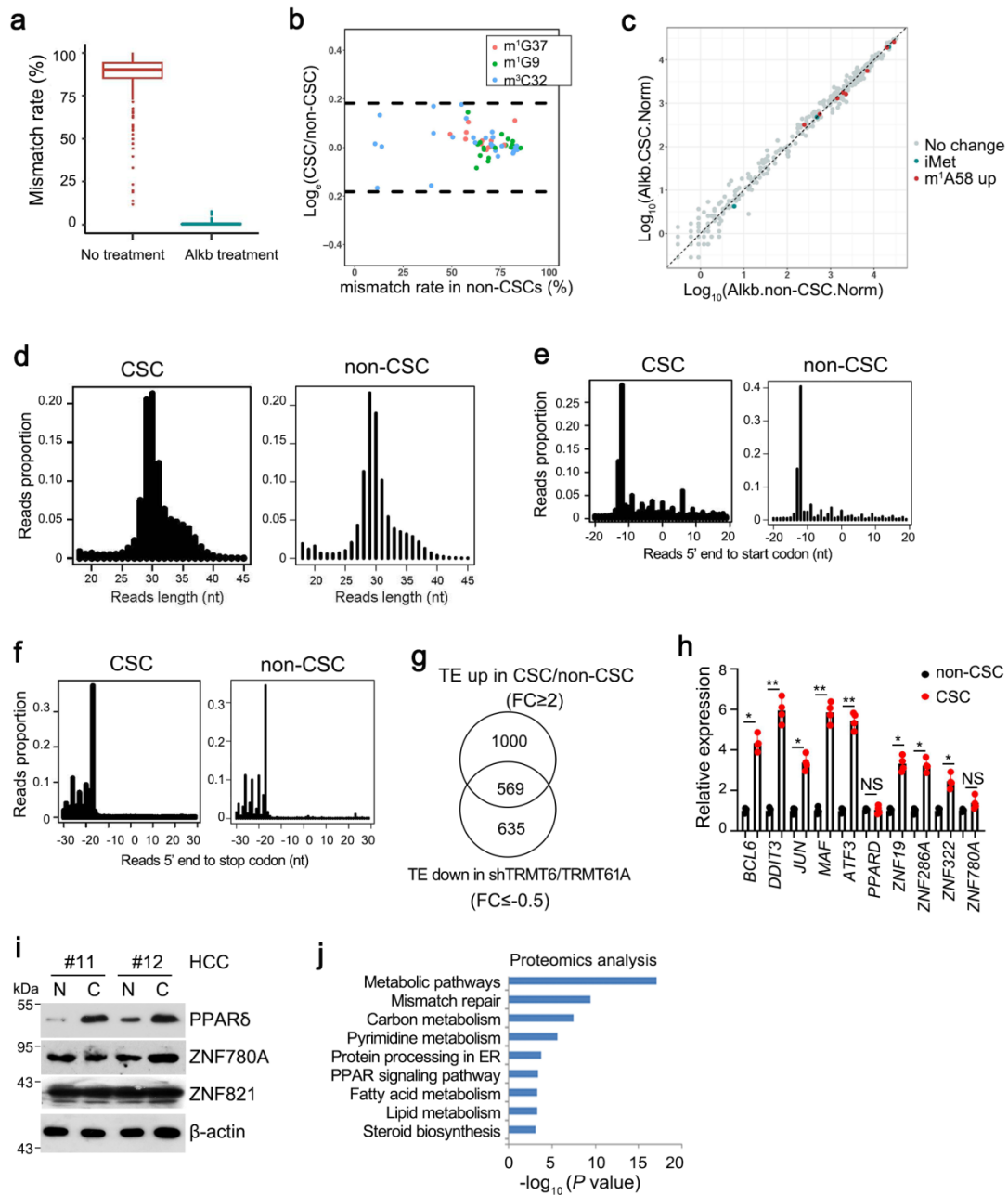
Supplementary Figure 1. m¹A modifications in RNA is high in liver CSCs and HCC tissues. (a) Representative immunohistochemistry images of m¹A levels (brown) in a panel of 191 clinically defined HCC samples. n=3 independent samples. Score 0 (0~5% positive cells/tissue section) represents m¹A staining that was considered negative, score 1 (5~15% positive cells/tissue section) represents m¹A staining that was considered weak positive, whereas scores 2 (15~35% positive cells/tissue section) and 3 (>35% positive cells/tissue section) represent m¹A staining that was considered positive. Grade I (n = 56), Grade II (n = 87), Grade III (n = 48). Scale bar, 100 μm. (b) Representative immunohistochemistry images of m¹A signals in human HCC tissue microarray (TMA). Scale bar, 100 μm. n=2 independent samples. (c) Representative immunohistochemistry

images of m¹A signals in two kinds of mouse liver cancer models including DEN-induced liver cancer and *Myc*^{OE} liver cancer tissues. Scale bar, 100 μ m. n=5 biologically independent mice. (d) Immunofluorescence staining of m¹A and m⁶A signals in HCC cell lines and oncospheres. Scale bar, 10 μ m. n=4 biologically independent samples. (e) LC-MS/MS quantification of m⁶A/A, m¹G/G and Ψ /U ratios in total RNAs and small RNA fraction (<200 nt) purified from liver cancer stem cells (CSCs). Data are means $\pm$ SD. n=7 biologically independent samples. Exact *P* values from left to right: 0.12, 0.15, 0.078, 0.11, 0.058, 0.062. **P* < 0.05; ***P* < 0.01; ****P* < 0.001, and NS, not significant (*P* > 0.05) by two-tailed Student's *t* test.



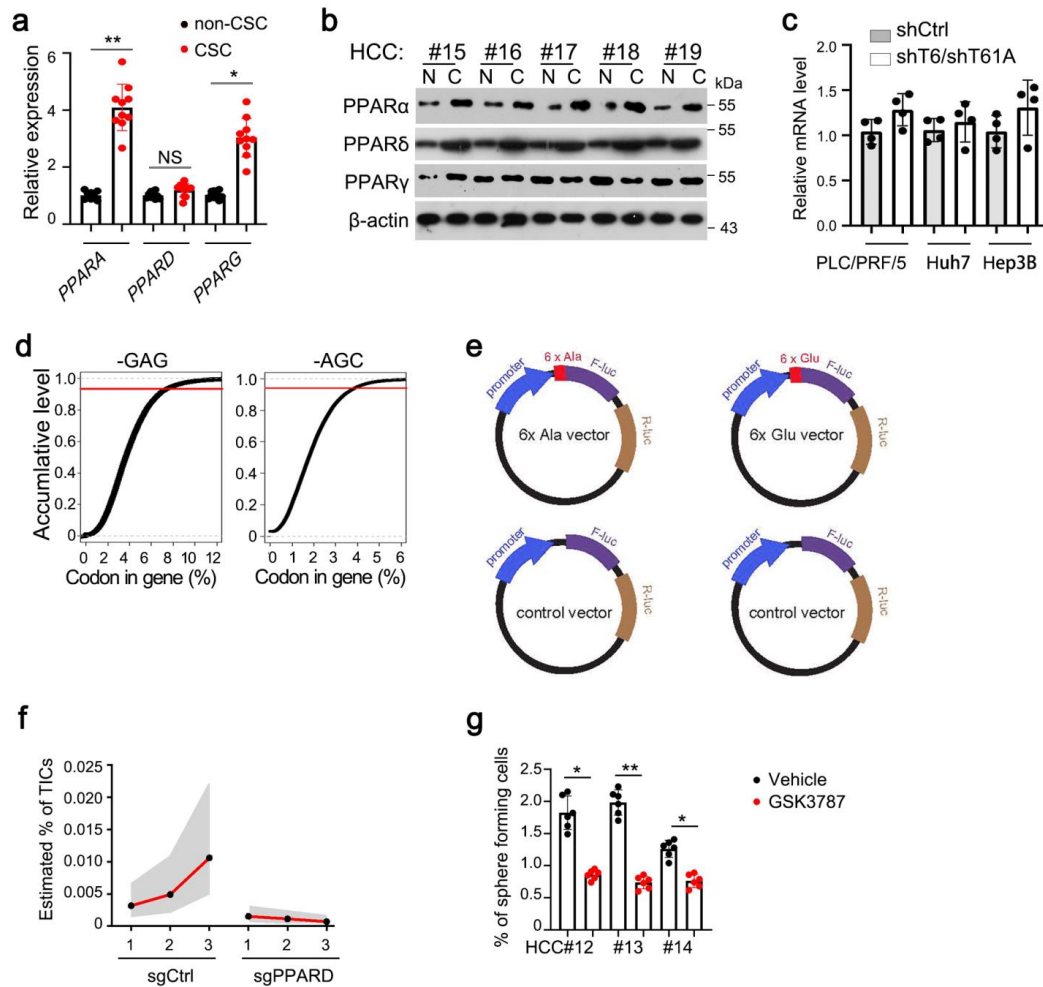
Supplementary Figure 2. TRMT6/TRMT61A components are highly expressed in liver CSCs and HCC tissues. (a) *TRMT6* mRNA was highly expressed in our liver cancer tissue cohort including tumors (n=30) and paired per-tumors (n=30) by quantitative PCR with reverse transcription (qRT-PCR) analyses. Data were normalized to endogenous 18S rRNA expression and 8 normal liver tissues were assigned with a value of 1. Data are means $\pm$ SD. Exact *P* values from left to right: 0.00013, 0.0055. (b) Western blot of *TRMT6* protein levels in a panel of peri-tumor (P) and tumor (T) tissues. n=3 biologically independent samples. (c-e) Expression analyses of *TRMT6* and *TRMT61A* in Wang's and

TCGA cohorts. Expression values were log₂-transformed and mean centered. Data were shown as box plots. Centre, lower and upper lines correspond to median, first and third quartiles, respectively; whiskers extend to 1.5 × interquartile range. Exact *P* value for (c): 0.000025. (f, g) Kaplan–Meier plots of overall survival for *TRMT6* and *TRMT61A* in TCGA cohort. *P* values for Kaplan-Meier curve were determined using a two-sided log-rank test. (h) m¹A signals were highly expressed in CD13⁺ cells in liver tissues by immunofluorescence staining. Scale bar, 100 μm. Bar graph data are means ± SD. *n*=50 samples. Exact *P* value: 0.00039. (i) *TRMT6* was highly expressed in CSCs sorted from HCC tissues compared with paired tumor cells by qRT-PCR analyses. Data were normalized to endogenous 18S rRNA expression and paired tumor tissues were assigned with a value of 1. Data are means ± SD. *n*=8. Exact *P* values from left to right: 0.038, 0.0087, 0.0062, 0.0095, 0.0084, 0.023, 0.035. (j) LC-MS/MS quantification of m³C, m¹G and pseudouridine (Ψ) levels in tRNA (<200 nt) purified from indicated liver CSCs, presented as percentage of unmodified G and U, respectively. Data are means ± SD. *n*=4. (k) CD13⁺CD133⁺ (CSC) subpopulations were detected in *TRMT6/61A* -depleted Huh7 cells by FACS analysis. Graph results are shown as means ± SD. Exact *P* value: 0.0056. *n*=5 biologically independent samples. (l, m) Effect of overexpression of wild type (WT)- or mutant (Mut)-*TRMT6/61A* cells on oncosphere formation. Scale bars, 100 μm. oe, overexpression. Exact significant *P* values from left to right: 0.0031, 0.0027, 0.019. *n*=4 biologically independent samples. **P* < 0.05; ***P* < 0.01, ****P* < 0.001 by two-tailed Student's *t* test.



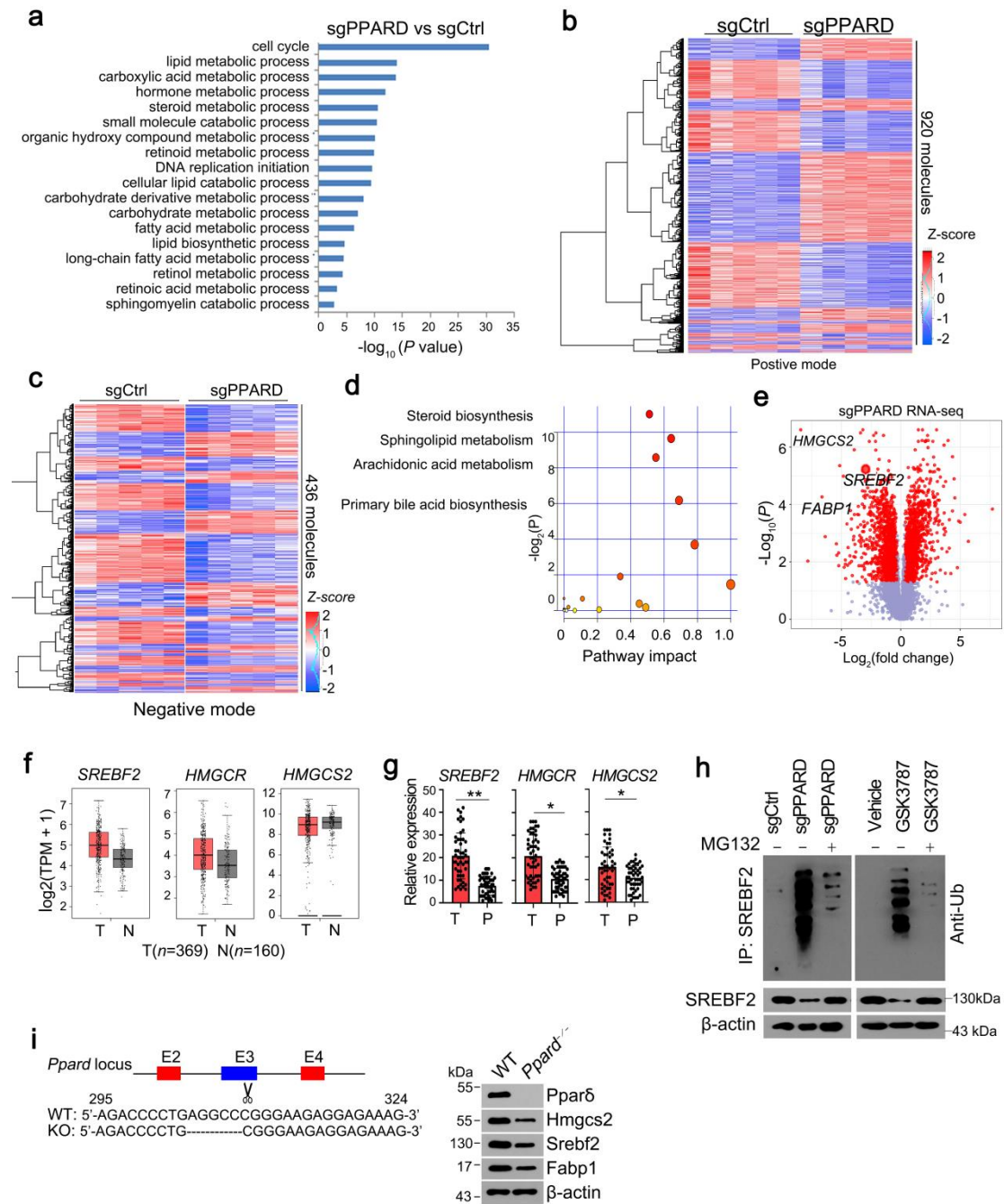
Supplementary Figure 3. m¹A-seq and ribosome profiling sequencing (ribo-seq) analyses in liver CSCs. (a) Misincorporation signals caused by m¹A58 in tRNAs dramatically decreased upon AlkB treatment. Data were shown as box plots. Centre, lower and upper lines correspond to median, first and third quartiles, respectively; whiskers extend to 1.5 $\times$ interquartile range. Points show outliers. (b) Other tRNA modifications including m¹G37, m¹G9, and m³C32 between CSCs and non-CSCs. (c) The expression level of each tRNA between CSCs and non-CSCs. (d) Distance between 5' of ribosome footprint to the start codon in PLC/PRF/5 CSCs. (e) Distance between 5' of

ribosome footprint to the stop codon in PLC/PRF/5 CSCs. (f) Length distribution of mRNA-mapped reads in PLC/PRF/5 CSCs. (g) Differential genes of TE upregulated in CSCs and TE downregulated in TRMT6/TRMT61A depleted CSCs. (h) qRT-PCR analysis of 10 TFs in liver CSCs and non-CSCs from HCC patient samples. Data were normalized to endogenous 18S rRNA expression and non-CSCs were assigned with a value of 1. Data are means $\pm$ SD. $n=4$. $*P < 0.05$, $***P < 0.001$, and NS, not significant ($P > 0.05$) by two-tailed Student's t test. Exact P values from left to right: 0.015, 0.0033, 0.032, 0.0051, 0.0069, 0.12, 0.011, 0.026, 0.038, 0.067, 0.031. (i) Immunoblotting analysis of candidate TFs in liver CSCs (C) and non-CSCs (N) from HCC samples. $n=3$ biologically independent samples. (j) Unbiased pathway analysis of proteins downregulated in TRMT6/TRMT61A depleted CSCs compared with control CSCs identified by proteomics analyses.



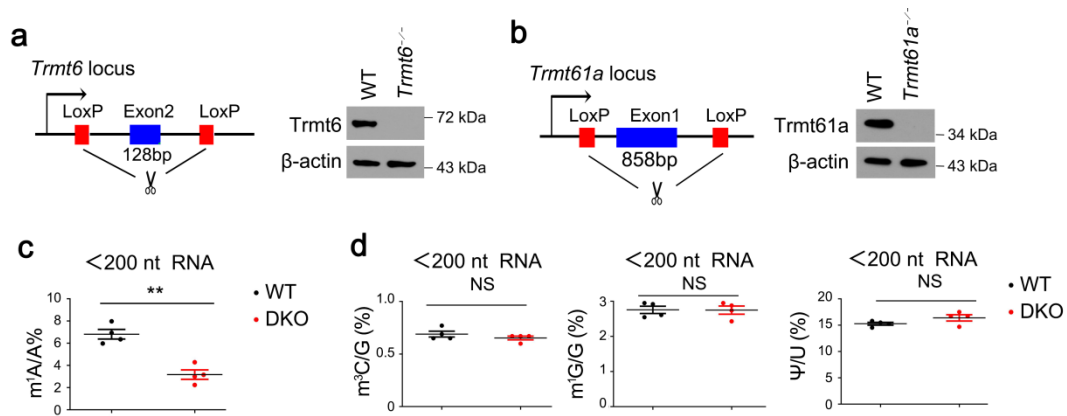
Supplementary Figure 4. PPAR δ promotes self-renewal of liver CSCs. (a) *PPARA*, *PPARD* and *PPARG* mRNA expression in liver CSCs and non-CSCs. Data were normalized to endogenous 18S rRNA expression and non-CSCs were assigned with a value of 1. Data are means $\pm$ SD. n=10. Exact *P* values from left to right: 0.0067, 0.055, 0.011. (b) Western blotting confirmation of *PPARA*, *PPARD*, *PPARG* in liver CSCs. N: non-CSC. C: CSC. n=3 biologically independent samples. (c) *TRMT6/61A* depletion did not alter *PPARD* mRNA levels in liver CSCs. Data were normalized to endogenous 18S rRNA expression and shCtrls were assigned with a value of 1. Data are means $\pm$ SD. n=4. (d) Codon frequency analysis of *PPARD* mRNA among all genes. (e) Illustration of luciferase reporter plasmids. 6xGCU (Ala)-coding sequences (recognized by tRNA^{Ala(AGC)}) or 6xGAG (Glu)-coding sequences (recognized by tRNA^{Glu(CTC)}) were inserted before Firefly luciferase, respectively and Renilla luciferase was used as a transfection control. Control reporter without any insertion was used to normalize translation differences

among different cell lines. (f) Estimated frequency of TICs in PPAR δ depleted and control HCC cells during serial transplantations. (g) Oncosphere formation capacity of HCC primary cells treated with PPAR δ antagonist GSK3787 (20 μ M) and DMSO (vehicle). Data are means $\pm$ SD. n=6. Exact *P* values from left to right: 0.032, 0.0066, 0.045. **P* < 0.05; ***P* < 0.01 and NS, not significant (*P* > 0.05) by two-tailed Student's *t* test.

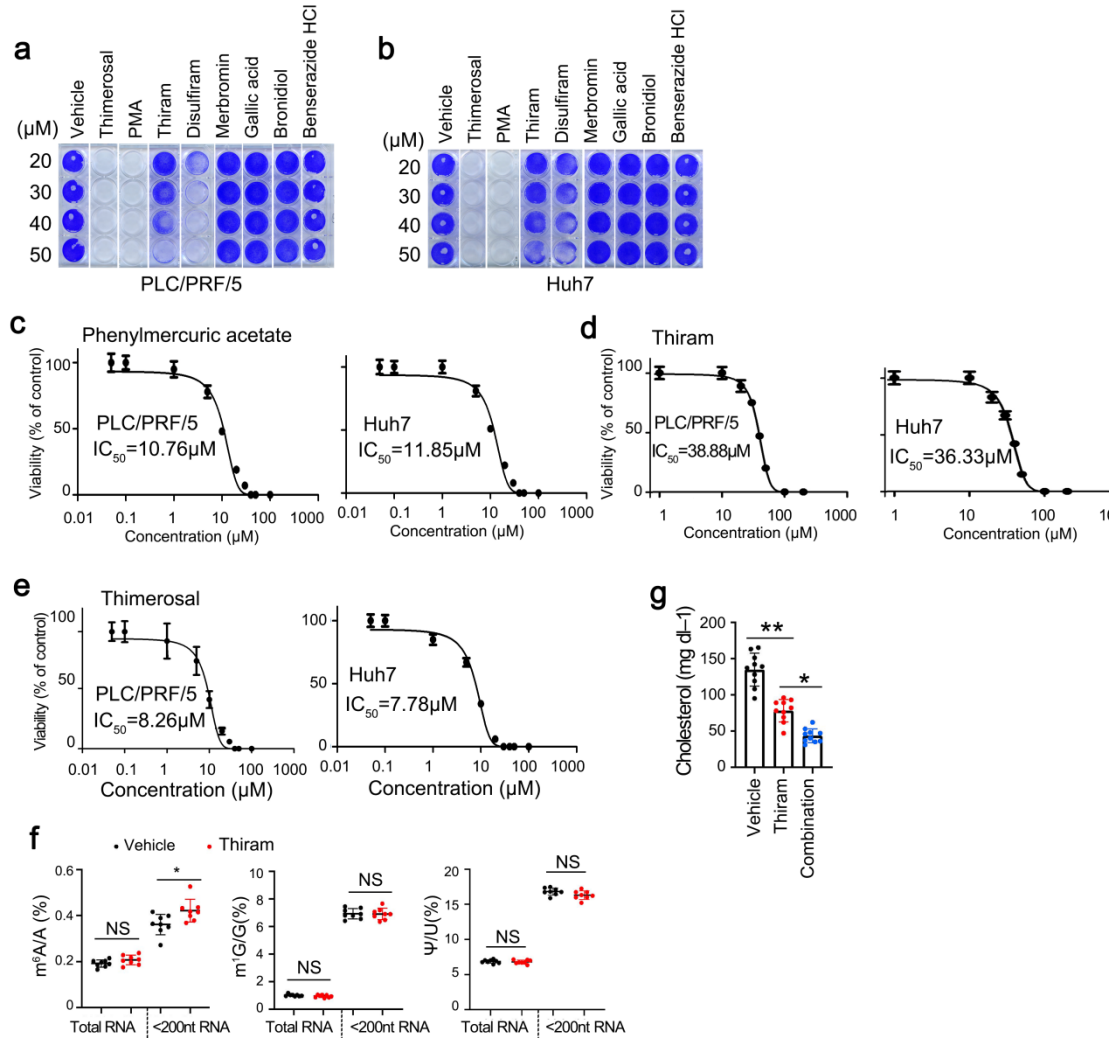


Supplementary Figure 5. *PPARD* depletion reduces cholesterol synthesis in liver CSCs. (a) Gene ontology (GO) analysis of genes downregulated in *PPAR* δ depleted compared with control CSCs. (b) Heatmap analysis of lipidomics profiling under positive ion mode in *PPAR* δ depletion compared with control CSCs. (c) Heatmap analysis of lipidomics profiling under negative ion mode in *PPAR* δ depleted liver CSCs compared with control CSCs. (d) Pathway analysis of differential lipids regulated by *PPAR* δ . (e) Volcano map analysis of *PPAR* δ depleted compared with control CSCs. (f) Levels of cholesterol

synthesis related genes in HCC tumor and normal tissues from TCGA dataset. Data were shown as box plots. Centre, lower and upper lines correspond to median, first and third quartiles, respectively; whiskers extend to $1.5 \times$ interquartile range. (g) qRT-PCR analysis of cholesterol synthesis related genes in our HCC dataset including tumor tissues paired with adjacent tumor tissues ($n=50$). Data were normalized to endogenous 18S rRNA expression and 7 normal liver tissues were assigned with a value of 1. Data are means $\pm$ SD. Exact P values from left to right: 0.0028, 0.019, 0.017. T, tumor. P, peri-tumor. (h) Western blots for SREBF2 ubiquitination. Samples are immunoprecipitated SREBF2 from PPAR δ -silenced and GSK3787 treated CSCs and control CSCs. $n=3$ biologically independent samples. (i) Schematic diagram of *Ppard* KO mice using CRISPR/Cas9 knockin strategy. Frameshift mutations were identified by DNA sequencing. Right panel: Western blotting detection of Ppar δ and cholesterol homeostasis-related proteins in *Ppard* knockout mouse livers. $n=4$ biologically independent mice. * $P < 0.05$; ** $P < 0.01$ by two-tailed Student's t test.



Supplementary Figure 6. *Trmt6*/*Trmt61a* knockout strategy in mice. (a, b) Diagram of generation strategies for *Trmt6*^{fllox/fllox} and *Trmt61a*^{fllox/fllox} using CRISPR-Cas9 technology. Gene knockout deficiencies in hepatocytes were confirmed by immunoblotting. n=5 biologically independent mice. (c, d) LC-MS/MS quantification of m¹A, m³C, m¹G and Ψ levels in tRNA (<200 nt) purified from indicated liver tissues, presented as percentage of unmodified A, G, G and U, respectively. Data are means ± SD. n=4. Exact *P* value for (c): 0.0081. ***P* < 0.01, and NS, not significant (*P* > 0.05) by two-tailed Student's *t* test.



Supplementary Figure 7. Inhibition of TRMT6/TRMT61A complex reduces liver CSC self-renewal and liver cancer development. (a-b) Long-term colony formation assay was performed over 7–10 days on HCC cell lines PLC/PRF/5 and Huh7, treated with vehicle (DMSO) or indicated drugs of different concentrations. Cells were stained with crystal violet solution. (c-e) Dose–response curves of HCC cell lines to other candidate treatment, with an endpoint measurement at 120 h (means $\pm$ s.e.m., $n = 5$ biological repeats). (f) LC-MS/MS quantification of m^6A/A , m^1G/G and Ψ/U ratios in total RNAs and small RNA fraction (<200 nt) purified from liver CSCs treated with vehicle (DMSO) and thiram for 96 hr. Data are means $\pm$ SD. $n=8$. (g) Total serum cholesterol levels in PDC orthotopic engraft mouse models treated with vehicle, thiram, or combination. Exact P values from left to right: 0.0059, 0.023. * $P < 0.05$, ** $P < 0.01$; NS, not significant ($P > 0.05$) by two-tailed Student's t test.

Supplementary Table 1. Clinical characteristics of HCC patients

Characteristics	Hepatocellular carcinoma (n=191) *
Sex	
Male	117 (61)
Female	74 (39)
Age (yr)	57.2± 11.5
≤55	89 (47)
>55	102 (53)
HBV positive	187 (98)
AFP (ug/L)	
≤20	110 (58)
>20	81 (42)
Tumor size (cm)	
≤5	99 (52)
>5	92 (48)
Cirrhosis	
With	187 (98)
Without	4 (2)
Differentiation	
Low	48 (25)
Medium	87 (46)
High	56 (29)

* Data are shown as means ± standard deviation (SD) or numbers (%).

Supplementary Table 2. qRT-PCR primers used in this study

Gene	Forward	Reverse
<i>TRMT6</i>	5'- TTGGGCGATTTTGAAGTGC-3'	5'- GGTGGAGAGGACTGGTTTCG -3'
<i>TRMT61A</i>	5'-TGGCTAAAGAGCATTCTGCTAAG-3'	5'-TGTGTAGTCGAAGCATGTTGTG-3'
<i>PPARD</i>	5'-GATCCAGATTGATCGACCAGC-3'	5'-GAGCCCATAGCGTCGTAGT-3'
<i>PPARA</i>	5'-TGAAATCAGACTCCGACCAGA-3'	5'-TGGCAAAGCAATGTCCATTAGTT-3'
<i>PPARG</i>	5'-CTGCTCTACGACATGAACGG-3'	5'-GAAGGTCCCTGATGTAGTCGAT-3'
<i>18S rRNA</i>	5'-AACCCGTTGAACCCCAT-3'	5'-CCATCCAATCGGTAGTAGCG-3'
<i>ACTB</i>	5'-CATGTACGTTGCTATCCAGGC-3'	5'-CTCCTTAATGTCACGCACGAT-3'
<i>ABCA1</i>	5'-ACCCACCCTATGAACAACATGA-3'	5'-GAGTCGGGTAACGGAAACAGG-3'
<i>ABCG1</i>	5'-ATTGAGGACCTTTCCTATTTCGG-3'	5'-CTCACCATTATTGAACCTCCCG-3'
<i>APOA1</i>	5'-CCCTGGGATCGAGTGAAGGA-3'	5'-CTGGGACACATAGTCTCTGCC-3'
<i>APOA2</i>	5'-CTGTGCTACTCCTCACCATCT-3'	5'-CTCTCCACACATGGCTCCTTT-3'
<i>APOA4</i>	5'-CTCAAGGGACGCTTACGC-3'	5'-GTCCTGAGCATAGGGAGCCA-3'
<i>APOE</i>	5'-GTTGCTGGTCACATTCCTGG-3'	5'-GCAGGTAATCCCCAAAGCGAC-3'
<i>LDLR</i>	5'-TCTGCAACATGGCTAGAGACT-3'	5'-TCCAAGCATTCGTTGGTCCC-3'
<i>SCARB1</i>	5'-CCTATCCCCTTCTATCTCTCCG-3'	5'-GGATGTTGGGCATGACGATGT-3'
<i>OLR1</i>	5'-ACCTTCCCCTCACACTCCTA-3'	5'-CTGTCTGTCTGTCTGTCCGT-3'
<i>APOB</i>	5'-TGCTCCACTCACTTTACCGTC-3'	5'-TAGCGTCCAGTGTGTAAGTAC-3'
<i>MYLIP</i>	5'-GCAGGCGACTGGGAATCATAG-3'	5'-CGGTTTCTCAGGTTTAGCCAT-3'
<i>MSR1</i>	5'-GCAGTGGGATCACTTTCACAA-3'	5'-AGCTGTCATTGAGCGAGCATC-3'
<i>VLDLR</i>	5'-AGAAAAGCCAAATGTGAACCT-3'	5'-CACTGCCGTCAACACAGTCT-3'
<i>SREBF1</i>	5'-CGGAACCATCTTGGCAACAGT-3'	5'-CGCTTCTCAATGGCGTTGT-3'
<i>SREBF2</i>	5'-CCTGGGAGACATCGACGAGAT-3'	5'-TGAATGACCGTTGCACTGAAG-3'
<i>HMGCR</i>	5'-TGATTGACCTTCCAGAGCAAG-3'	5'-CTAAAATTGCCATTCCACGAGC-3'
<i>HMGCS1</i>	5'-GATGTGGGAATTGTTGCCCTT-3'	5'-ATTGTCTCTGTTCCAATTCCAG-3'
<i>HMGCS2</i>	5'-GACTCCAGTGAAGCGCATTCT-3'	5'-CTGGGAAGTAGACCTCCAGG-3'
<i>DHCR7</i>	5'-GCTGCAAAATCGCAACCCAA-3'	5'-GCTCGCCAGTGAACACCAGT-3'
<i>CYP7A1</i>	5'-GCAATTTGGTGCCAATCCTCT-3'	5'-GCACAACACCTTATGGTATGACA-3'
<i>CYP7B1</i>	5'-AAAACCAAGTTGGGACACG-3'	5'-GAAGCTCAATGGGTATGTTGGAT-3'
<i>CH25H</i>	5'-ATCACCACATACGTGGGCTTT-3'	5'-GTCAGGGTGGATCTTGTAGCG-3'
<i>CYP8B1</i>	5'-GAAGCGCATGAGGACCAAG-3'	5'-TTGCATATTGCCCAAAGTCTAGT-3'
<i>CYP27A1</i>	5'-CGGCAACGGAGCTTAGAGG-3'	5'-GGCATAGCCTTGAACGAACAG-3'
<i>CYP39A1</i>	5'-CTTCAGCGGAAGAATTTGCGT-3'	5'-AAAGTCAATCGGTTTCCCATAG-3'
<i>CYP46A1</i>	5'-TGTGTTTTTGGATTGGGCTAAGA-3'	5'-ACTCAGGACTCGTGACGATGA-3'
<i>HSD3B1</i>	5'-CACATGGCCCGCTCCATAC-3'	5'-GTGCCGCGTCTTTTTCAGATTC-3'
<i>HSD3B7</i>	5'-CTGGTAGACGTGTTTGGCAGG-3'	5'-TGTTCGGTCTGCACACAAG-3'
<i>FABP1</i>	5'-GTGTCGGAAATCGTGCAGAAT-3'	5'-GACTTTCTCCCCTGTCATTGTC-3'
<i>NR1F1</i>	5'-AGAACTATCAAAACAAGCGGA-3'	5'-AAGGCACGGCACATTCTGATA-3'
<i>NR1F3</i>	5'-CGTCTGCAAGTCTACAGGG-3'	5'-TCCACCACGTACTGAATGGC-3'
<i>NR1H2</i>	5'-AGAACTAATGATCCAGCAGTTGG-3'	5'-TTGCTTAGCGAAGTCCACGAT-3'
<i>NR1H3</i>	5'-TCTGGAGACATCTCGGAGGTA-3'	5'-GGCCCTGGAGAACTCGAAG-3'
<i>NR1H4</i>	5'-TGCAGATCAGACCGTGAATGA-3'	5'-TTGGTTGCCATTTCCGTCAA-3'
<i>GPR183</i>	5'-GGGAACTTACTAGCCTTGGTC-3'	5'-GCATCTCCGATTCTCCAGTCAA-3'

<i>CXCR2</i>	5'- TACTGGCCTGCATCAGTGTG-3'	5'- CAGGCTGGGCTAACATTGGA-3'
<i>ESR1</i>	5'-CCCACTCAACAGCGTGTCTC-3'	5'-CGTCGATTATCTGAATTTGGCCT-3'
<i>Gli1</i>	5'-AGCGTGAGCCTGAATCTGTG-3'	5'-CAGCATGTACTGGGCTTTGAA-3'
<i>Gli2</i>	5'-CTGCCTCCGAGAAGCAAGAAG-3'	5'-GCATGGAATGGTGGCAAGAG-3'
<i>Gli3</i>	5'-TGGTTACATGGAGCCCCACTA-3'	5'-GAATCGGAGATGGATCGTAATGG-3'
<i>PTCH1</i>	5'-ACCCAGAAAGCAGACTACCC-3'	5'-ATCCTGAAGTCCCTGAAGCC-3'
<i>HHIP</i>	5'-TCTCAAAGCCTGTTCCACTCA-3'	5'-GCCTCGGCAAGTGTAAGAA-3'
<i>BCL2</i>	5'-GAGCTGGTGGTTGACTTTCTC-3'	5'-TCCATCTCCGATTCACTCCCT-3'
<i>MYC</i>	5'-GGCTCCTGGCAAAAGGTCA-3'	5'-CTGCGTAGTTGTGCTGATGT-3'
<i>TCF7</i>	5'-CACGGGCAAACACTACGGT-3'	5'-TTGACCTTCGAGTGCTGATCC-3'
<i>SOX4</i>	5'-AGCGACAAGATCCCTTTCATTC-3'	5'-CGTTGCCGGACTTCACCTT-3'
<i>CCND1</i>	5'-CAATGACCCCGCACGATTTTC-3'	5'-CATGGAGGGCGGATTGGAA-3'
<i>HES1</i>	5'-TCAACACGACACCGGATAAAC-3'	5'-GCCGCGAGCTATCTTTCTTCA-3'
<i>HES6</i>	5'-AGCAGGAGCCTGACTCAGTT-3'	5'-AGCTCCTGAACCATCTGCTC-3'
<i>NRARP</i>	5'-CACGGGGTGATCACTGCTAA-3'	5'-CGCTGGGCTACAGGTCAATA-3'
<i>HEY1</i>	5'-GTTTCGGCTCTAGGTTCCATGT-3'	5'-CGTCGGCGCTTCTCAATTATTC-3'
<i>mTrmt6</i>	5'-GAGATTGCTGAACAAGCGGAT-3'	5'-AGGCTCTGAAGCTATCACAAGA-3'
<i>mTrmt61a</i>	5'-ATGAGTTTCGTGGCATAACGAG-3'	5'-CCTCTGCTGCAAATCACCTT-3'
<i>mPpard</i>	5'-TCCATCGTCAACAAAGACGGG-3'	5'-ACTTGGGCTCAATGATGTCAC-3'
<i>mActb</i>	5'-TGTTACCAACTGGGACGACA-3'	5'--ACCAGAGGCATACAGGGACA3'

m: mouse

Supplementary Table 3. ShRNAs, sgRNAs for human gene deficiency and guide RNAs for gene knockout in mice by CRISPR/Cas9 technology

Gene	Sequence (forward)	Sequence (reverse)
<i>TRMT6</i> shRNA#3	5'- GGGCACTGATAATCGAAAT -3'	
<i>TRMT6</i> shRNA#6	5'- GGTTCTACCTGGATAACGT -3'	
<i>TRMT61A</i> shRNA#1	5'- GGCACTCAGTTGACCTTAT -3'	
<i>TRMT61A</i> shRNA#2	5'- GCATACGAGGAGCTGATCAAG -3'	
<i>PPARD</i> sgRNA#1	5'- caccgGCTGCAAGATTCAGAAGAAC -3'	5'-aaacCCGACCAAAACGGATAGCTGc-3'
<i>SREBF2</i> sgRNA#3	5'-caccgGCTGCAATTTGTCAGTAAT-3'	5'-aaacAGTCAGGGAACCTCTCCCACTTc-3'
Guide RNA		
<i>Trmt6</i> ^{flox/flox} -up	5'- AACTAGAGAGAAGCAGGTTGGGG -3'	
<i>Trmt6</i> ^{flox/flox} -down	5'- ACGTCCGGCTTAGCTTCACAGGG -3'	
<i>Trmt61a</i> ^{flox/flox} -up	5'- GGAGCACGCAAAGGACAGCGCGG -3'	
<i>Trmt61a</i> ^{flox/flox} -down	5'- TGGTATGAGCTCAGATAAGACGG -3'	
<i>Ppard</i> ^{-/-}	5'- GCCACAGGAGGAGACCCCTG -3'	