

Table S1. Primer sequences for quantitative real-time RT-PCR

Gene	Sequence(5'-3')
DNMT1	F: GGCTGAGATGAGGCAAAAG R: ACCAACCTCGGTACAGGATGC
miR-34a-5p	F: TGGCAGTGTCTTAGCTGGTTGT R: GCGAGCACAGAATTAAATACGAC
CD133	F: ATGCTCTCAGCTCTCCCGC R: TTCTGTCTGAGGCTGGCTTG
CD44	F: GCAAACACAACCTCTGGTCC R: CCCACACCTTCTTCGACTGT
ALDH1	F: GCACGCCAGACTTACCTGTC R: CCTCCTCAGTTGCAGGATTAAAG
Bmi1	F: CTGGTTGCCATTGACAGCG R: AAATCCCGGAAAGAGAGCAGCC
Sox2	F: GCCCTGCAGTACAACCTCCAT R: CTGATCATGTCCCGTAGGT
Oct4	F: GTGAGAGGCAACCTGGAGAG R: GAATGGGACCGAGGAGTACA
FOXM1	F: ATACGTGGATTGAGGACCACT R: TCCAATGTCAAGTAGCGGTTG
GAPDH	F: ACACTCACTCTTCTACCTTG R: CAAATTCAATTGTCGTACCAAG
U6	F: CTCGCTTCGGCAGCACA R: AACGCTTCACGAATTGCGT

Table S2. Primer sequences for methylation specific PCR

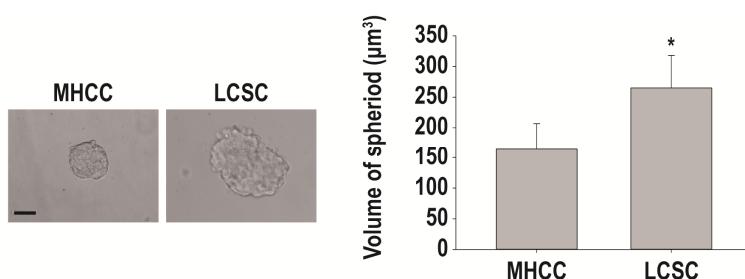
Gene	Sequence(5'-3')
miR-34a-5p-M	F: ATGAGGATTAGGATTCGGAG R: AACGCATAAAAACGACGACAA
miR-34a-5p-U	F: GGGGATGAGGATTAGGATTCTT R: ATAAAAACTAAAACCTCTACCTTCGCT

Table S3. Comparison of tumor formation ability of LCSCs and parental MHCC97H

cells in Balb/c-*nu* mice (n = 6)

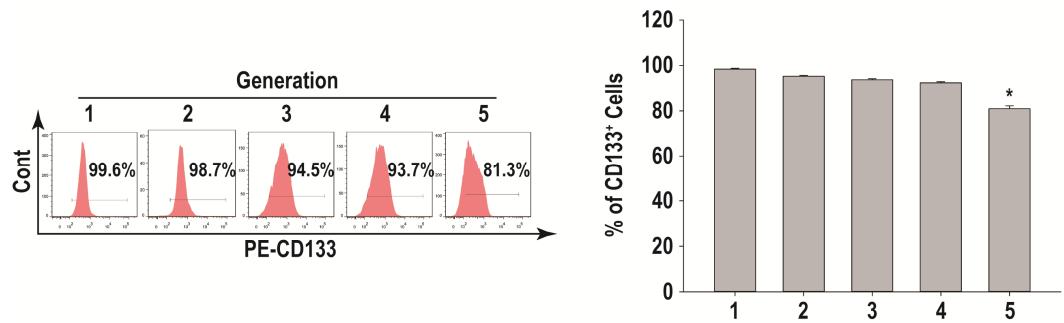
Cell Type	Cell (n)	Inoculated	Tumor Incidence (n/4)	Latency (d)	Tumor volume (mm ³)	Tumor weight (g)
MHCC	10 ⁵		6/6	23~48	659±76	0.54±0.15
HCSLC	10 ⁵		6/6	6~12	1968±92*	1.75±0.25*

The control samples included the tumor volume and tumor weight of LCSCs xenografts. Comparisons were carried out for LCSCs derived from MHCC97H cells with control cells, *P< 0.05.



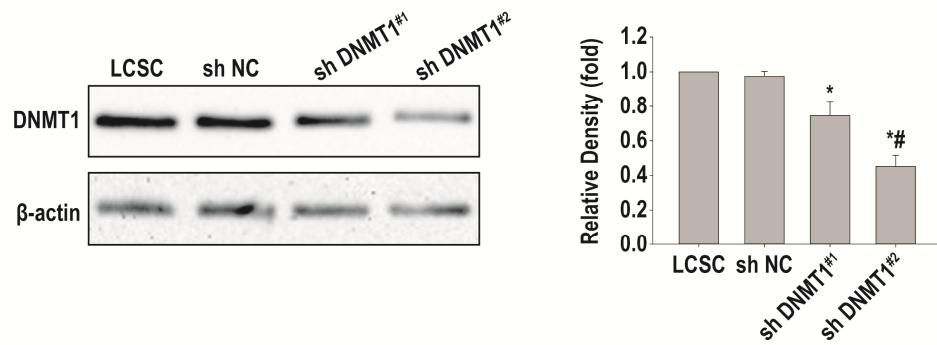
Supplementary Figure S1 Comparing the volume of spheroids between MHCC97H cells and LCSCs.

*P<0.05 vs MHCC97H cells (n=3).



Supplementary Figure S2 CD133⁺ cell percentages of LCSC during serial passage in sphere culture.

*P<0.05 vs First Generation of LCSC (n=3).



Supplementary Figure S3 Effects of different DNMT1 shRNA on DNMT1 protein expression of MHCC97H derived LCSCs

*P<0.05 vs LCSC or sh NC (n=3); # P<0.05 vs sh DNMT1#1 (n=3).