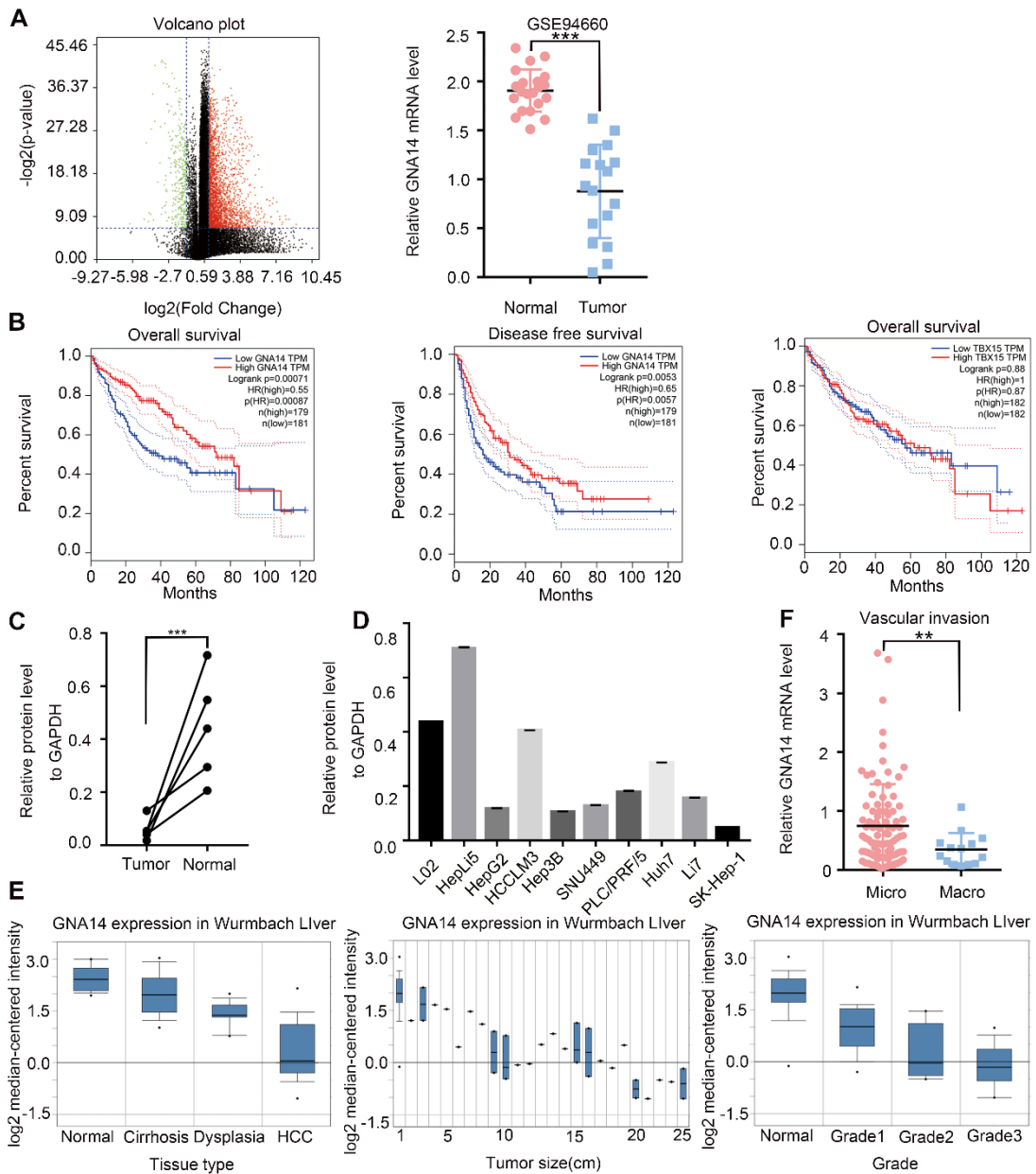
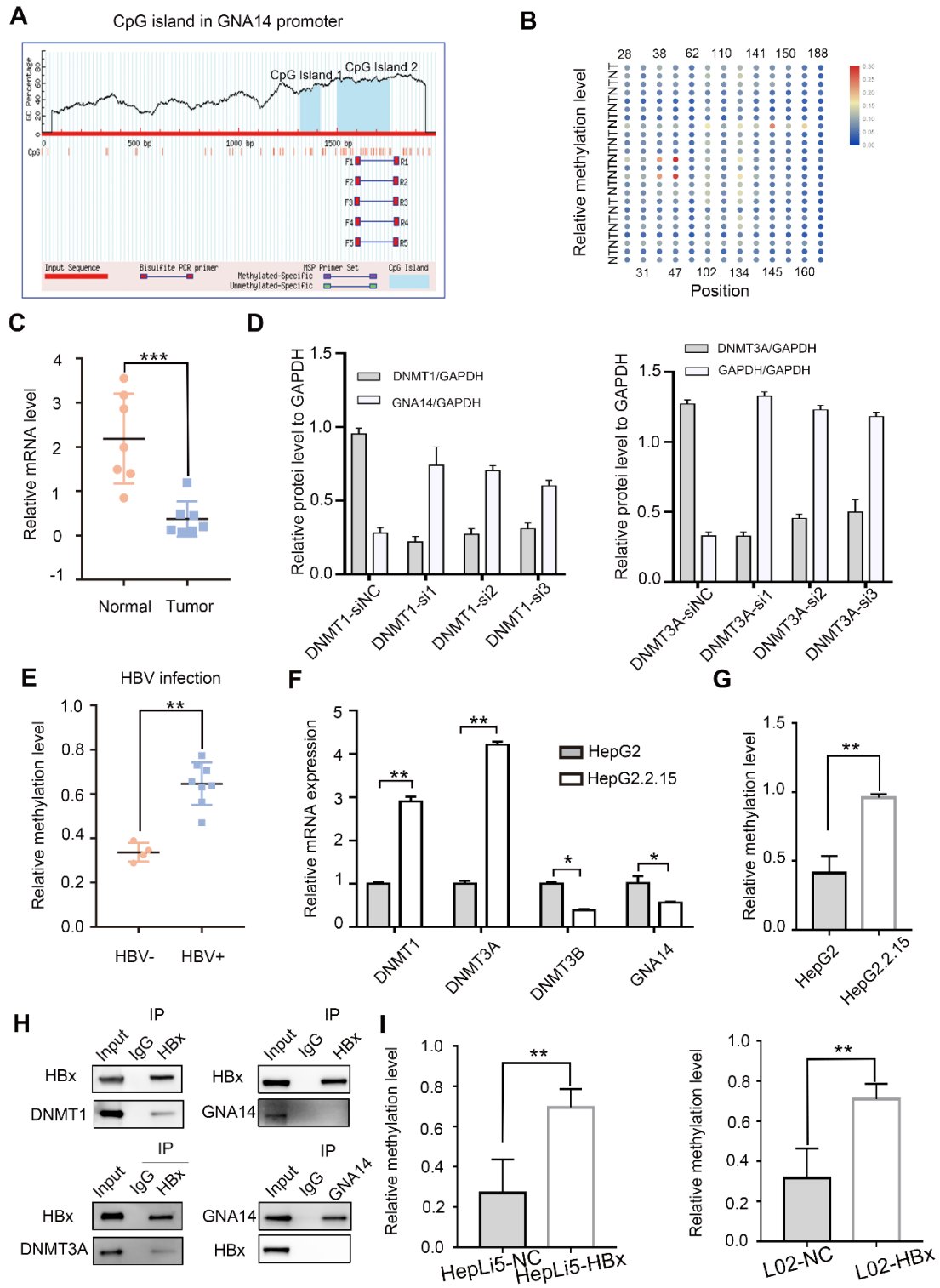


# 1 Supplementary Figure legend

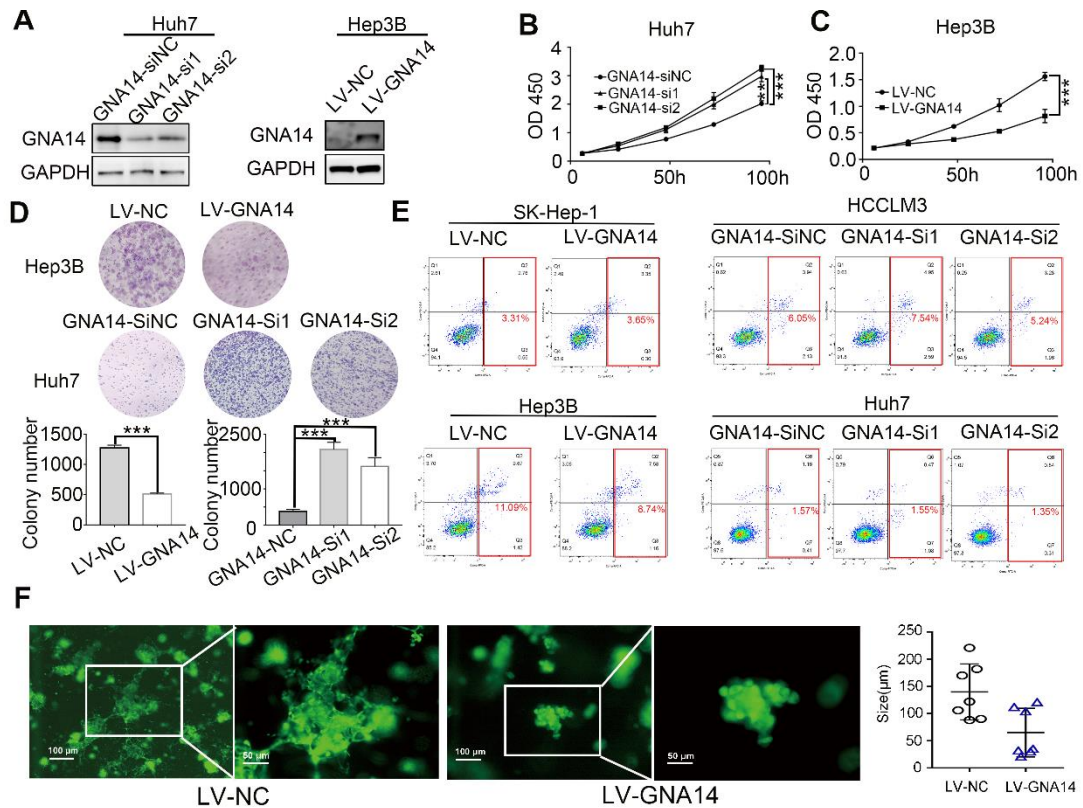


2  
 3 **Supplement 1.** GNA14 is downregulated in HCC. (A) Volcano plot of genome-  
 4 wide gene expression profiles and GNA14 mRNA expression profiles in HCC  
 5 and adjacent normal tissues from GSE94660. (B) Kaplan–Meier survival  
 6 analysis of GNA14 and TBX15 in TCGA database. (C) Quantitative analysis of  
 7 Western blot results of GNA14 expression in HCC tissues and matched normal  
 8 tissues. (D) Quantitative analysis of Western blot results of GNA14 expression  
 9 in HCC cell lines. (E) GNA14 mRNA levels in HCC patients with different tissue  
 10 type, different tumor size and different tumor grade in Wurmbach Liver cohort

11 from omcomine database. (F) GNA14 mRNA levels in HCC patients with  
 12 different vascular invasion in TCGA database. Statistical significance was  
 13 determined by unpaired t test. Kaplan–Meier survival analysis was performed  
 14 to analyze the survival percentage.

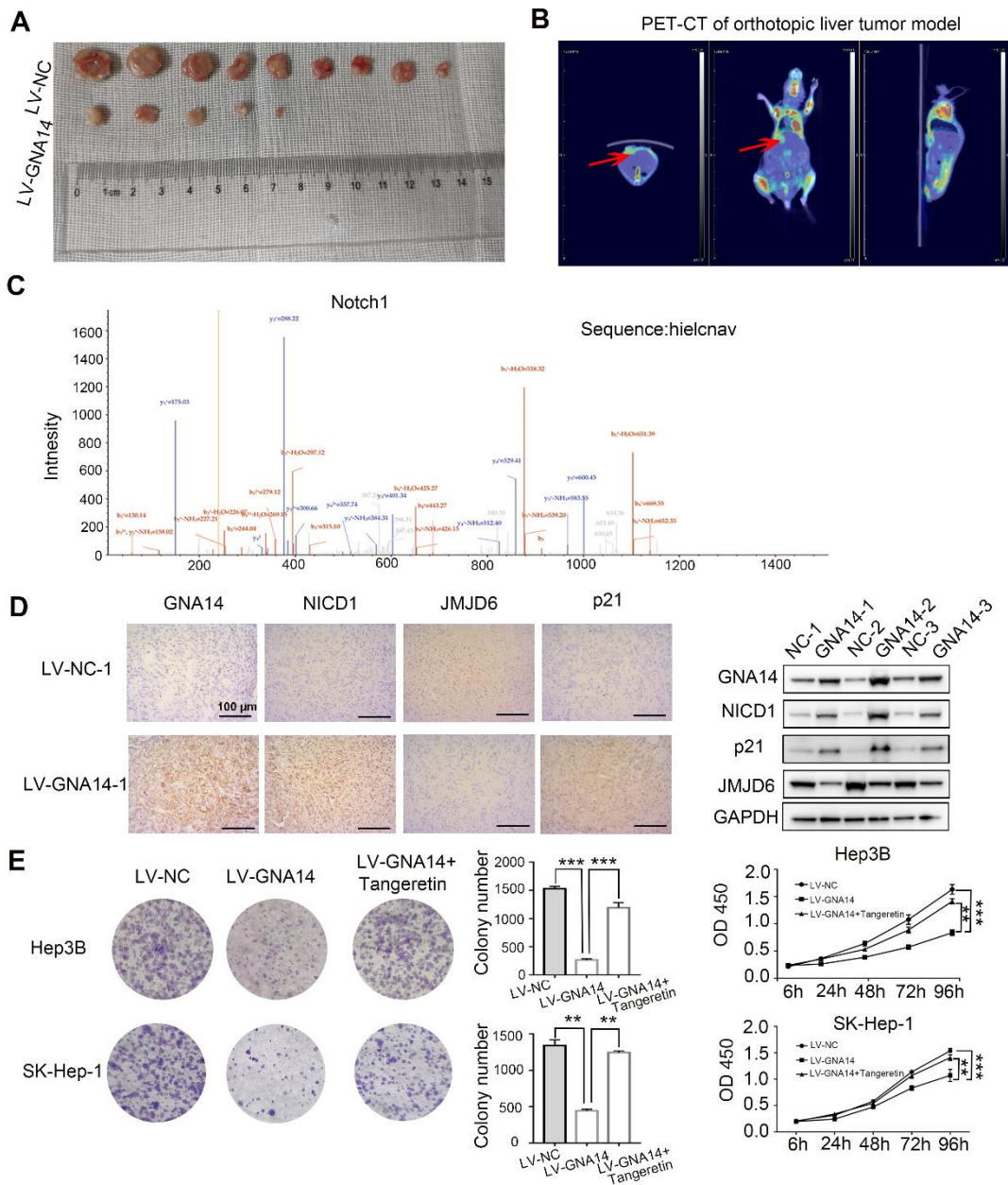


16 **Supplement 2.** GNA14 methylation is up-regulated in HCC and is regulated by  
17 HBx. (A) CpG islands in GNA14 promoter predicted by METHPRIMER. (B) The  
18 heatmap of the methyl target sequencing results indicated the methylation  
19 levels of 15 methylation sites in CpG island (Genomic position 2: 80263568-  
20 80263728) of the GNA14 promoter region in 12 pairs of matched tumor tissues  
21 and normal tissues in our hospital. “T” refers to tumor tissue and “N” refers to  
22 normal tissue. (C) Relative GNA14 mRNA level in 7 pairs of the tumor tissues  
23 and matched normal tissues used in Methyl-target DNA methylation  
24 sequencing analysis. (D) Quantitative analysis of Western blot results of SK-  
25 Hep-1 cells knocking down DNMT1 or DNMT3A. (E) Relative GNA14  
26 methylation levels in HCC patients with or without HBV infection of Zheyi  
27 Hospital. Statistical significance was determined by unpaired t test. (F) The  
28 relative mRNA levels of DNMT1, DNMT3A and GNA14 in HepG2 and  
29 HepG2.2.15 cells. (G) DNA methylation sequencing of HepG2 and HepG2.2.15  
30 cells in target CpG island. (H) The results of Co-IP were used to explore the  
31 combination of HBx and DNMT1 or DNMT3A or GNA14. (I) Results of DNA  
32 methylation sequencing after transfection of HBx overexpression plasmid in  
33 HepLi5 and L02 cells.



34

35 **Supplement 3.** GNA14 inhibits HCC proliferation. (A) Western blot was  
 36 performed to verify the effect of GNA14 knockdown in Huh7 cells and  
 37 overexpression in Hep3B cells. (B) CCK8 analysis was performed to determine  
 38 the proliferation of Huh7 cells with GNA14 knockdown. (C) CCK8 analysis was  
 39 performed to determine the proliferation of Hep3B cells infected with GNA14  
 40 lentivirus (LV-GNA14). (D) Colony assay of Hep3B cells infected with LV-  
 41 GNA14 or LV-NC. And clone assay after knockdown of GNA14 in Huh7 cells.  
 42 (E) Annexin V and propidium iodide staining experiments detected the  
 43 apoptosis of HCC cells after overexpressing GNA14 (Hep3B and SK-Hep-1  
 44 cells) and knocking down GNA14 (HCCLM3 and Huh7 cells). (F) The effect of  
 45 GNA14 overexpression on cell migration was examined by 3D cell culture.



46

47 **Supplement 4.** GNA14 inhibits HCC proliferation through Notch1 pathway. (A)

48 Xenograft tumors were generated by injecting SK-Hep-1 cells overexpressing

49 GNA14 or carrying a control vector. (B) PET-CT images of nude mice orthotopic

50 liver tumor model injected SK-Hep-1 cells overexpressing GNA14 or carrying a

51 control vector. Red arrows indicated HCC. (C) Notch1 fragment detected by

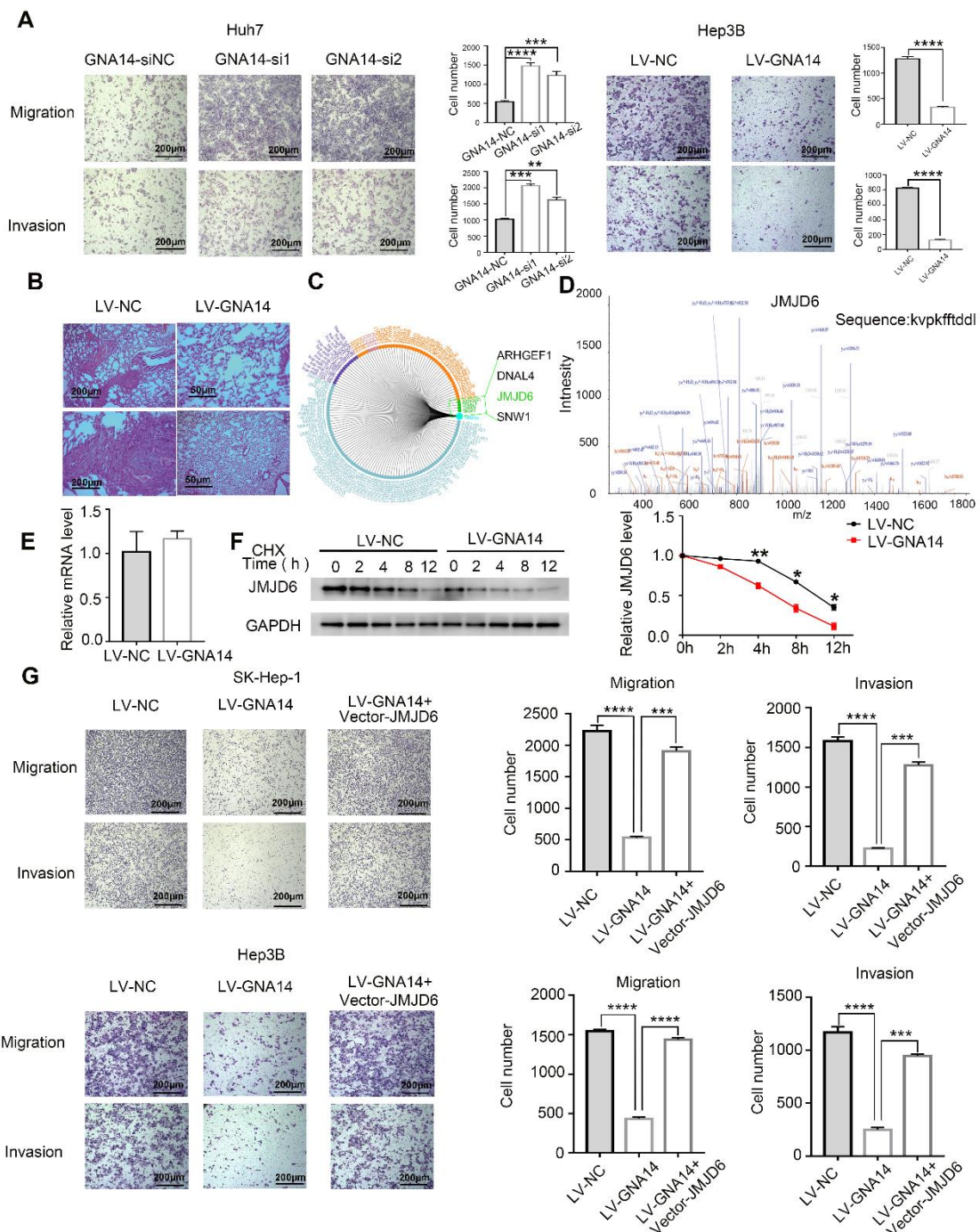
52 mass spectrometry is presented. (D) IHC staining and Western blot

53 experiments of subcutaneous tumor were performed to study the expression of

54 GNA14, NICD1, JMJD6 and P21. (E) Colony assay and CCK8 experiment were

55 used to detect the proliferation of Hep3B and SK-hep-1 cells after

56 overexpression of GNA14 and treated with Tangeretin (Notch1 inhibitor).



57

58 **Supplement 5.** GNA14 inhibits HCC invasion and migration through JMJD6.

59 (A) The effects of knockdown of GNA14 in Huh7 and overexpression of GNA14

60 in Hep3B on cell migration and invasion were detected by transwell assay. (B)

61 IHC image of lung in the lung colonization mouse model. (C) Potential

62 interacting proteins with GNA14 analyzed by GCBI. (D) JMJD6 fragment

63 detected by mass spectrometry is presented; b and y stand for N-terminal and

64 C-terminal collision-induced dissociation fragments. (E) The qPCR results of  
 65 JMJD6 after overexpression of GNA14 in SK-Hep-1 cells. (F) The SK-Hep-1  
 66 cell line was treated with 10ug/ml CHX to detect the changes in JMJD6 protein  
 67 expression at different treatment times. (G) The effect of JMJD6 overexpression  
 68 on cell migration and invasion in SK-Hep-1 and Hep3B cells overexpressed  
 69 GNA14.

70

71 **Table S1**

Sequences of siRNAs

| Name              | Sequence               |
|-------------------|------------------------|
| HBx siRNA1        | UCACCUCUGCACGUAGCAUTT; |
| HBx siRNA2        | CCUUGAGGCAUACUUCAATT;  |
| HBx siRNA3        | GAGGCUGUAGGCAUAAAUUTT; |
| HBx siRNA control | UUCUCCGAACGUGUCACGUTT. |
| GNA14-si1         | TCACGAAGCTGGTTTACCA    |
| GNA14-si2         | CATGTACTCTCATCTAATT    |
| DNMT1-si1         | GAAGAGACGTAGAGTTACA    |
| DNMT1-si2         | GGAAC TTTGTCTCCTTCAA   |
| DNMT1-si3         | CAATGAGACTGACATCAAA    |
| DNMT3A-si1        | CCACCAAAGCAGGCGATGA    |
| DNMT3A-si2        | CCACGACAGCGATGAGAGT    |
| DNMT3A-si3        | GCCTGGAGCCACCAGAAGA    |
| siRNA control     | TTCTCCGAACGTGTCACGT    |

72 **Table S2**

The primers for qRT-PCR

| Name          | Sequence              |
|---------------|-----------------------|
| GNA14 forward | GCTGAGTGTGACAACGAGAAT |

|                |                        |
|----------------|------------------------|
| GNA14 reverse  | TCCTGTTTCGGTCCTGTGTAT  |
| GAPDH forward  | GAGCCAAAAGGGTCATCATCT  |
| GAPDH reverse  | TTCCACGATACCAAAGTTGTCA |
| HBx forward    | GCACTTCGCTTCACCTCT     |
| HBx reverse    | TATGCCTACAGCCTCCTA     |
| DNMT1forward   | GGAAGAAGACAAAGACCAGGAT |
| DNMT1 reverse  | AGTTTCTGTTTGGGTGTTGGTT |
| DNMT3A forward | GAAAGGACGGAGAGGAGCA    |
| DNMT3A reverse | ATGGATGGGGACTTGGAGAT   |

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**Table S3**

Primary Antibodies

| Name   | Manufacturer | Catalog    |
|--------|--------------|------------|
| GAPDH  | CST          | 5174T      |
| GNA14  | Sigma        | SAB1402481 |
| HBx    | abcam        | AB157480   |
| DNMT1  | Santa Cruz   | sc-271729  |
| DNMT3A | Santa Cruz   | sc-365769  |
| Notch1 | ThermoFisher | MA5-11961  |
| NICD1  | ThermoFisher | PA5-99448  |
| P21    | abcam        | ab109520   |
| RB     | abcam        | ab181616   |
| JMJD6  | Proteintech  | 16476-1-AP |

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