Supplementary figures and tables

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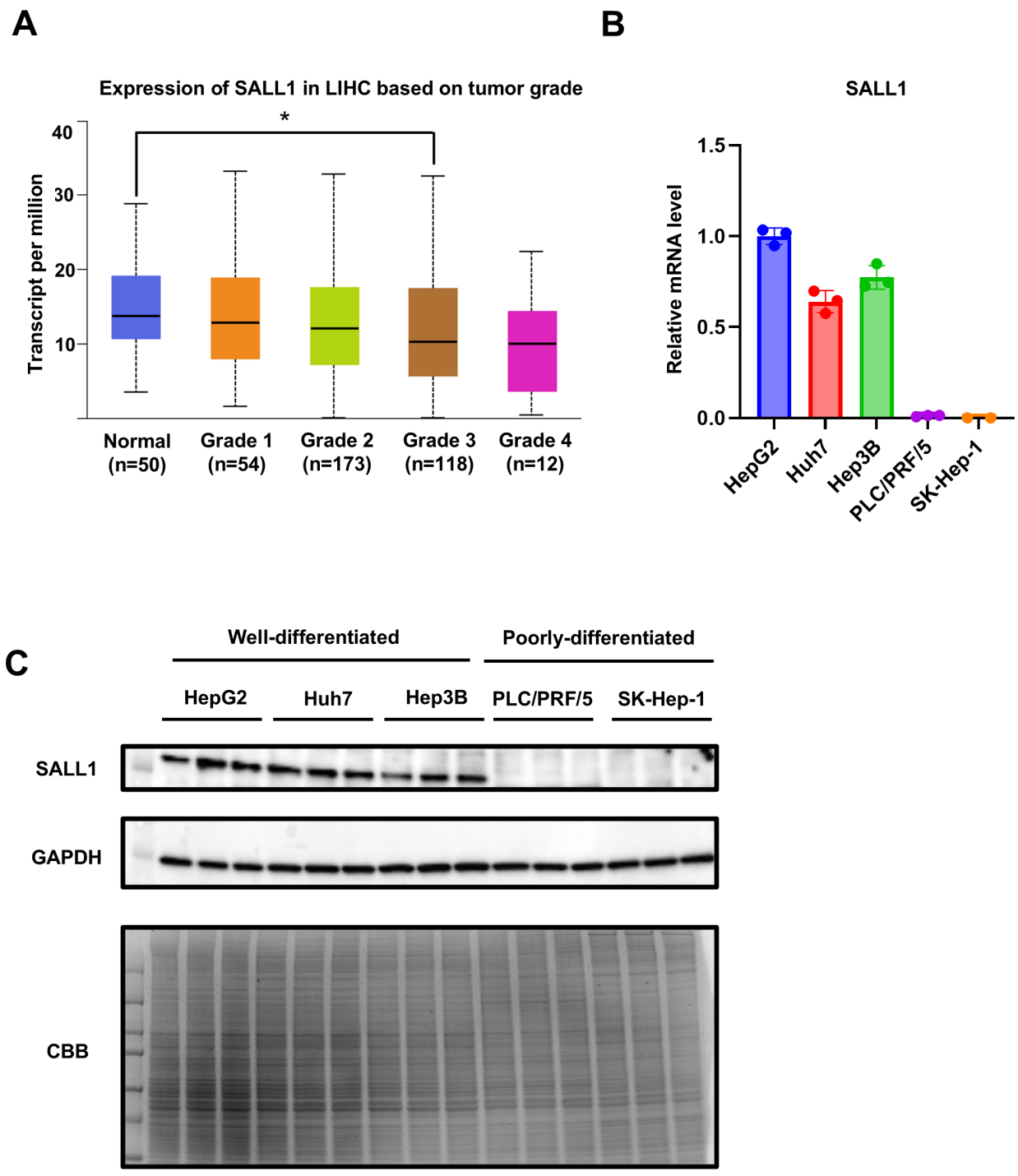


Figure S1. Expression analysis of SALL1 in tumor grades and HCC cell lines
(A) SALL1 expression across tumor histological grades (grades 1-4). **(B, C)** Comparison of SALL1 expression across five HCC cell lines. **(B)** RT-qPCR analysis of *SALL1* mRNA levels. **(C)** Western blot analysis of SALL1 protein levels. **P*<0.05 compared with Normal.

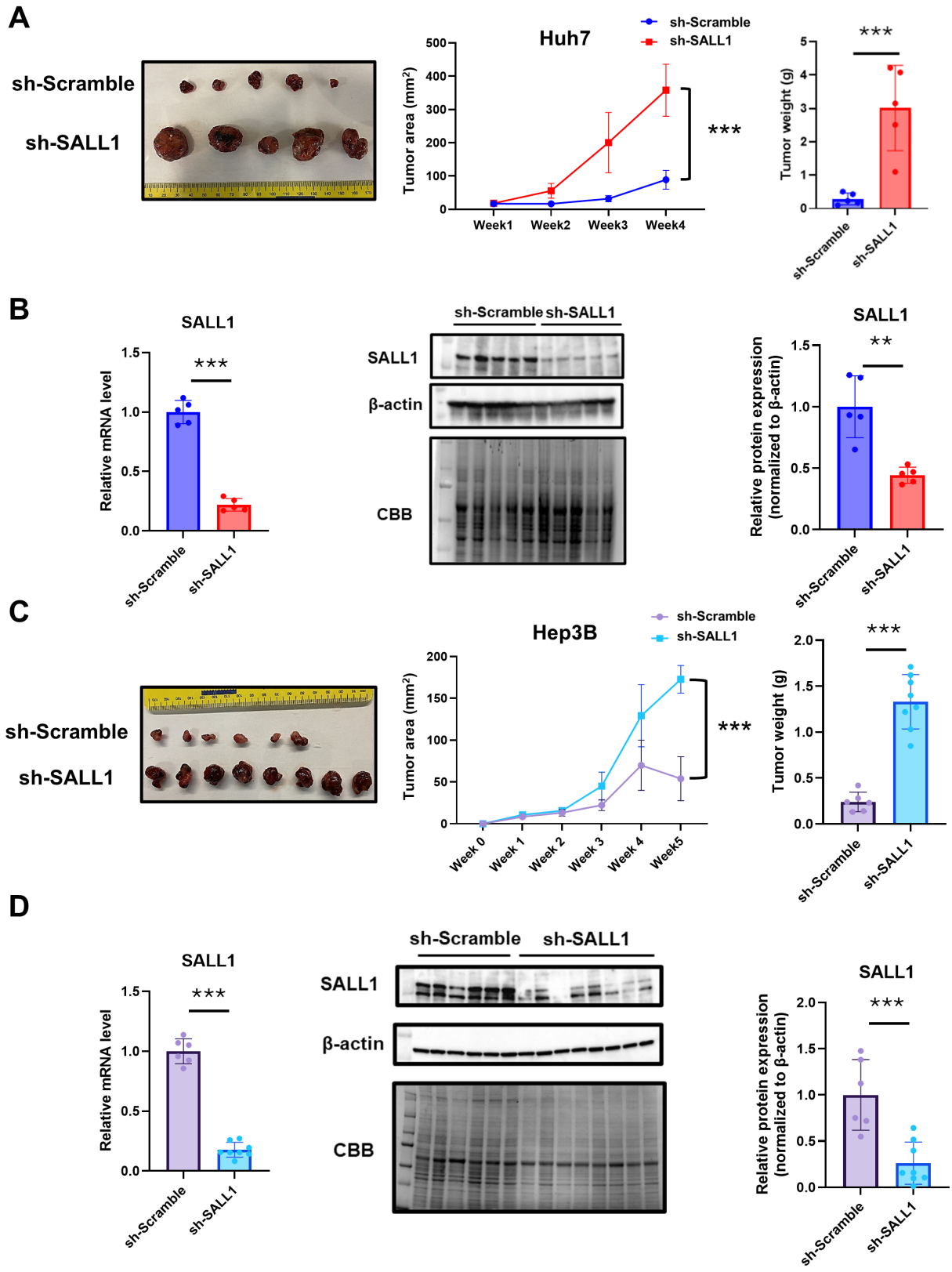

A
sh-Scramble
sh-SALL1
Huh7
sh-Scramble
sh-SALL1
Tumor area (mm²)
Week1
Week2
Week3
Week4
***
Tumor weight (g)
***
sh-Scramble
sh-SALL1
B
SALL1
Relative mRNA level
***
sh-Scramble
sh-SALL1
sh-Scramble sh-SALL1
SALL1
β-actin
CBB
SALL1
Relative protein expression (normalized to β-actin)
**
sh-Scramble
sh-SALL1
C
sh-Scramble
sh-SALL1
Hep3B
sh-Scramble
sh-SALL1
Tumor area (mm²)
Week 0
Week 1
Week 2
Week 3
Week 4
Week5
***
Tumor weight (g)
***
sh-Scramble
sh-SALL1
D
SALL1
Relative mRNA level
***
sh-Scramble
sh-SALL1
sh-Scramble sh-SALL1
SALL1
β-actin
CBB
SALL1
Relative protein expression (normalized to β-actin)
***
sh-Scramble
sh-SALL1

Figure S2. Xenograft mouse model using female immunodeficient mice
(A, C) Xenograft mouse models using immunodeficient mice (female) implanted with Huh7 **(A)** or Hep3B **(C)** cells. Images of excised tumors (left), tumor growth curves (middle), and tumor weights (right). **(B, D)** Detection of SALL1 knockdown in excised xenograft tumors. Expression analysis in tumors derived from Huh7 **(B)** and Hep3B **(D)** cells. RT-qPCR analysis of *SALL1* mRNA levels (left) and Western blotting of SALL1 protein levels (middle and right). Westen blot band intensities of SALL1 were quantified and normalized to β-actin as an internal control. $^{**}P<0.01$, $^{***}P < 0.001$ compared with sh-Scramble group (Control).

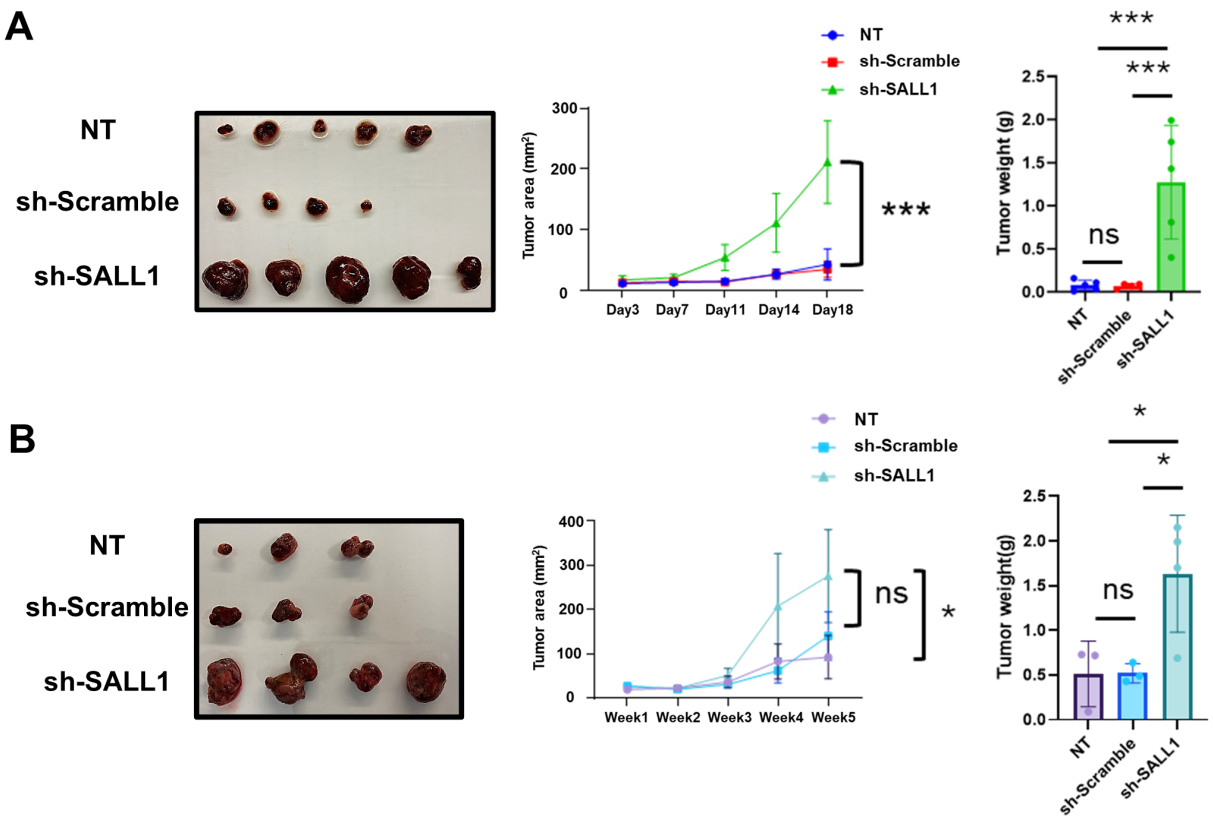


Figure S3. Xenograft mouse model including untreated cells
Xenograft mouse model including untreated (NT) cells. **(A)** Results from mice implanted with Huh7 cells and **(B)** results from mice implanted with Hep3B cells. Images of excised tumors (left), tumor growth curves (middle), and tumor weights (right). **P* < 0.05, ****P* < 0.001 compared with untreated (NT) group or sh-Scramble group.

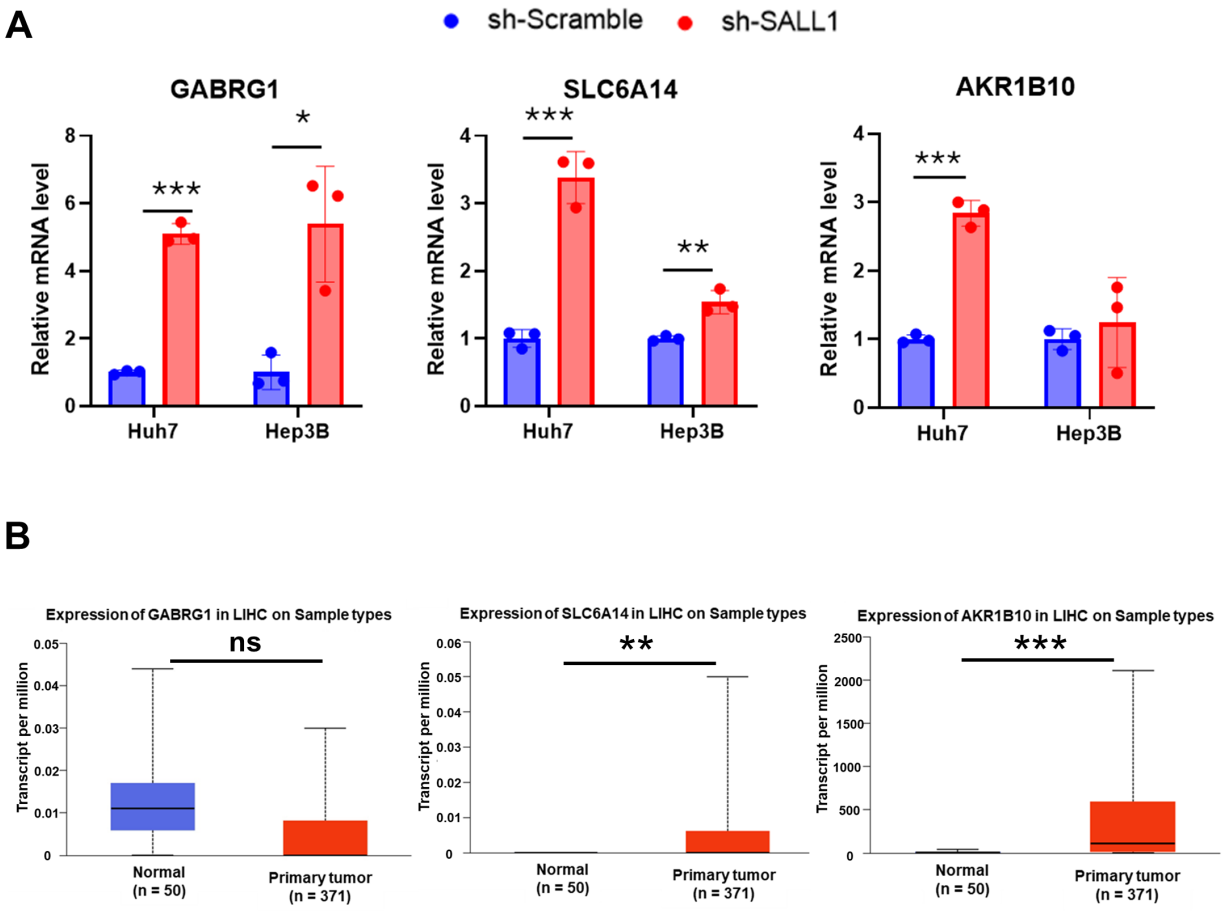


Figure S4. Expression analysis of novel candidate SALL1 target genes
(A) RT-qPCR analysis of candidate SALL1 target gene expression in SALL1-knockdown cells. **(B)** Expression analysis of candidate target genes in HCC based on the TCGA database. **P*< 0.05, ***P* < 0.01, ****P* < 0.001 compared with sh-Scramble group (Control) or normal.

Supplementary Table 1. Patient information

Category	Characteristic	Value
Clinical characteristics	Total case	19
	Age: Average (Range)	62.1 (23-78)
	Gender: n (%)	Male: 14 (73.7), Female: 5 (26.3)
	Virus	0
	Alcohol: n (%)	1 (5.2)
	Fatty liver: n (%)	6 (31.5)
	NASH: n (%)	1 (5.2)
	Diabetes: n (%)	2 (10.5)
	Hyperlipidemia: n (%)	2 (10.5)
	Cirrhosis: n (%)	2 (10.5)
Pathology	Well-differentiated: n (%)	1 (5.2)
	Well to moderately differentiated: n (%)	2 (10.5)
	Moderately differentiated: n (%)	5 (26.3)
	Others: n (%)	11 (57.9)
Primary Clinical Diagnosis	Space-occupying lesion of the liver: n (%)	12 (63.1)
	Secondary malignant neoplasm of the liver: n (%)	1 (5.2)
	HCC: n (%)	1 (5.2)
	Malignant neoplasm of the liver: n (%)	4 (21.0)
	Liver mass: n (%)	1 (5.2)
Etiology	Metabolic liver disease: n (%)	10 (52.6)
	Immune-mediated liver disease: n (%)	9 (47.3)

Supplementary Table 2. Primers used in mRNA quantification

Gene	Forward sequence (5′→3′)	Reverse sequence (5′→3′)
PPIA	CCCACCGTGTTCTTCGACATT	GGACCCGTATGCTTTAGGATGA
TBP	TTCGGAGAGTTCTGGGATTG	GGATTATATTCGGCGTTTCG
GAPDH	ATGACCCCTTCATTGACC	GAAGATGGTGATGGGATTTC
SALL1	CCTGCAGATTCACGAGAGAAC	GTGCAGGGGTGCTATTCC
ADAMTS16	TGCGATCACACAGAAACTCC	TGAAGGAAGTGGCCTTAGGA
AKR1B10	GGAAAAGCAACGTTCTTGGA	GCTTCTCGATCTGGAAGTGG
ANKRD1	TGAAGGCTGCTCTGGAGAAT	TCCTTCCAAGCATGCTCTATG
ANXA1	TAAGCGAAACAATGCACAGC	GGTGACCTGTAAGGGCTTTCT
ASB4	GTGGACACACAGCTTTGCAC	GCGTCTCCTCATCTTGGTTG
CRLF1	GCTGGATATCCTGGATGTGG	CGGATCTGGTATTTGGCTTG
DYDC2	GCCTCAAGGAAATGGAAATG	TGGTCTTCTTCGTGGAAACA
FLNC	AGTCCCTTCCCTGTCCATGT	CACCTTGAAGTCAGCCACCT
FSTL1	CCAGACCAGGAGAACAACAAG	GGGTTGAGGCACTTGAGAAA
GABRG1	AGTGCCTGCAAGAACATCGT	AGAGATCCATCGCAGTCACA
IGFBP5	TTTGCCTCAACGAAAAGAGC	CGGAAGATCTTGGGGGAGTA
LOXL2	CCTGGGGAGAGGACATACAA	CCATGGAGAATGGCCAGTAG
LRP12	CTGGTTCATAAGGGCAAACC	CCAAATTGCACCTTCTGGAT
NPNT	AGGACAAATACGGTGCCAGT	TGCCTAAATCTAGGGCAGGA
NPPB	CTTCTTGCATCTGGCTTTCC	AGTTTGCCCTGCAAATGGT
RGS16	AGGGCACACCAGATCTTTGA	GTCTGCAGGTTCATCCTCGT
SLC6A14	ACTCTGGAGGGTGCTTCAAA	GAGTGGCAGCATCTTTCCAT
SYTL5	TGGAAGTGCACTGTCTGTGA	GACAACATCAGTGCCGAGAA
TAGLN	AGGTGGCTCAGTTCCTGAAG	GCACTGCTGCCATGTCTTT
TMEM45B	CTGGAACTTTTCCGAACCAG	AAGGTGGGAACAGCACAAAC

Supplementary Table 3. Sequence of shRNA

shRNA	Sequence（5′→3′）
sh-Scramble	CCTAAGGTTAAGTCGCCCTCGCTCGAGCGAGGGCGACTTAACCTTAGG
sh-SALL1	ACCCTTTGCTTGCACTATTTGCTCGAGCAAATAGTGCAAGCAAAGGGT

Supplementary Table 4. Primers used in ChIP-qPCR

Gene	Forward sequence (5′→3′)	Reverse sequence (5′→3′)	Region
GABRG1-1	TCAGTGAGTTAGTGTGTGCATTC	AGTCAAGGTAGCCCATATCAAA	1464-1469
GABRG1-2	CCCAAGGAGATACCCTTCAC	CTGCATGCAACAGTAGCTGA	1810-1815
GABRG1-3	GGATCAGTTTCCCATAAAACAC	CCCGTATTCTCCAAAATCCA	2066-2087
GABRG1-4	CCTTTACCCCCAGATTGTGA	CGTTTTCAAGTGGAGGCAAC	2537-2544
GABRG1-5	TAATGGCGAAGCCCATGAAT	CCCCCAAAATTTGTTCAGG	2897-2902
SLC6A14-1	CTGTTACCTGGGCAGACTGA	GAGGAAACATCCCAGCTTGA	765-774
SLC6A14-2	CATGCACATCAGCCTTTGTT	GGGAATCTTGGCAGGTAAGG	1069-1074
SLC6A14-3	GTGTTTGCTTTCCCTTCCAC	TGAAGAATTGCTCGAGACTAGG	1489-1496
SLC6A14-4	ACCAGGTGTCCTTCCCTTTT	CCTGATGCAGAGTAGGCACT	2400-2407,2432-2437
SLC6A14-5	CCCTCTCTCTTTCACCCATTC	CTCAAAGGCTCAAGAGATCCA	2535-2542
SLC6A14-6	GCCTAGATTGCAAGGCAGAT	GGTGTGGGAAAACAGGAGAA	2701-2706
AKR1B10-1	TGGGATTACAGGTGTGAGACA	GGCTTATGACAGCTCATAGAAGG	1067-1074
AKR1B10-2	GGCCACCAGATTATGAGGAA	AGGTGGAGAATGCAGTGAGC	1364-1371
AKR1B10-3	TGCAGTGCTCTATTCCCAGA	TGGATGACATGCAGTGCTA	1543-1550
AKR1B10-4	GCGTGTCCAACCTCAGACTA	GGAATATGCATGCCACAGTAAA	1672-1679
AKR1B10-5	AGCGGCACTACTCGTTCATT	CCTGGTTTTGTACCCTTAAGAA	2652-2659