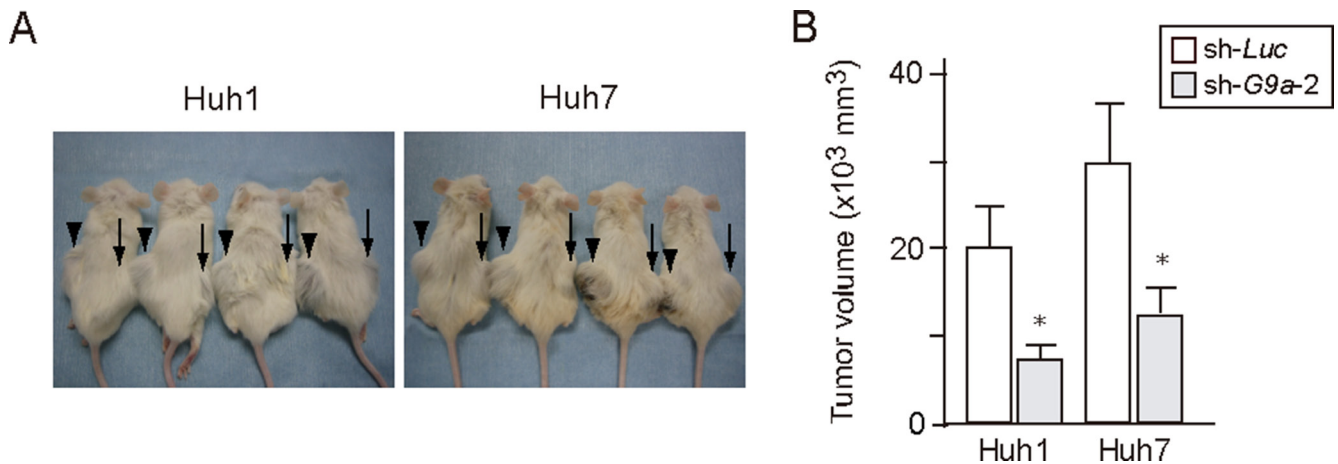
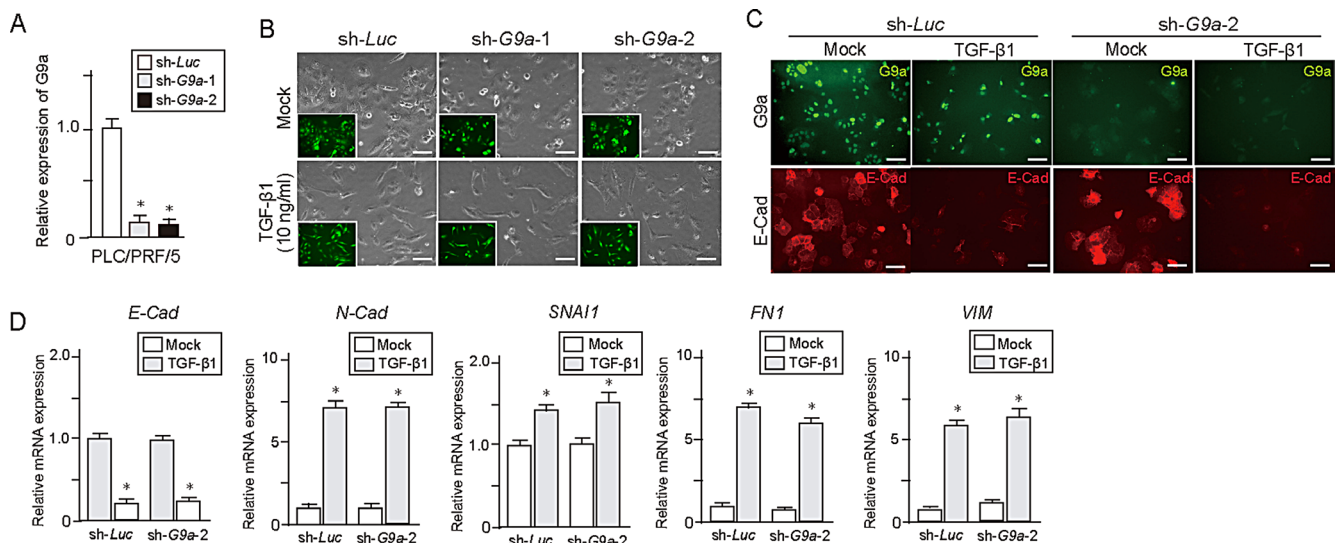


## Histone lysine methyltransferase G9a is a novel epigenetic target for the treatment of hepatocellular carcinoma

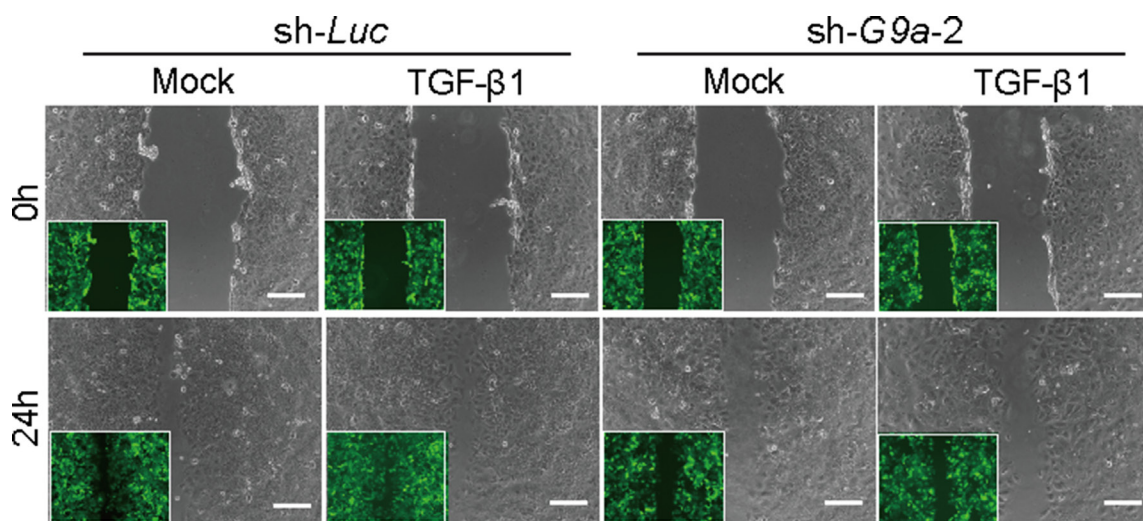
### Supplementary Materials



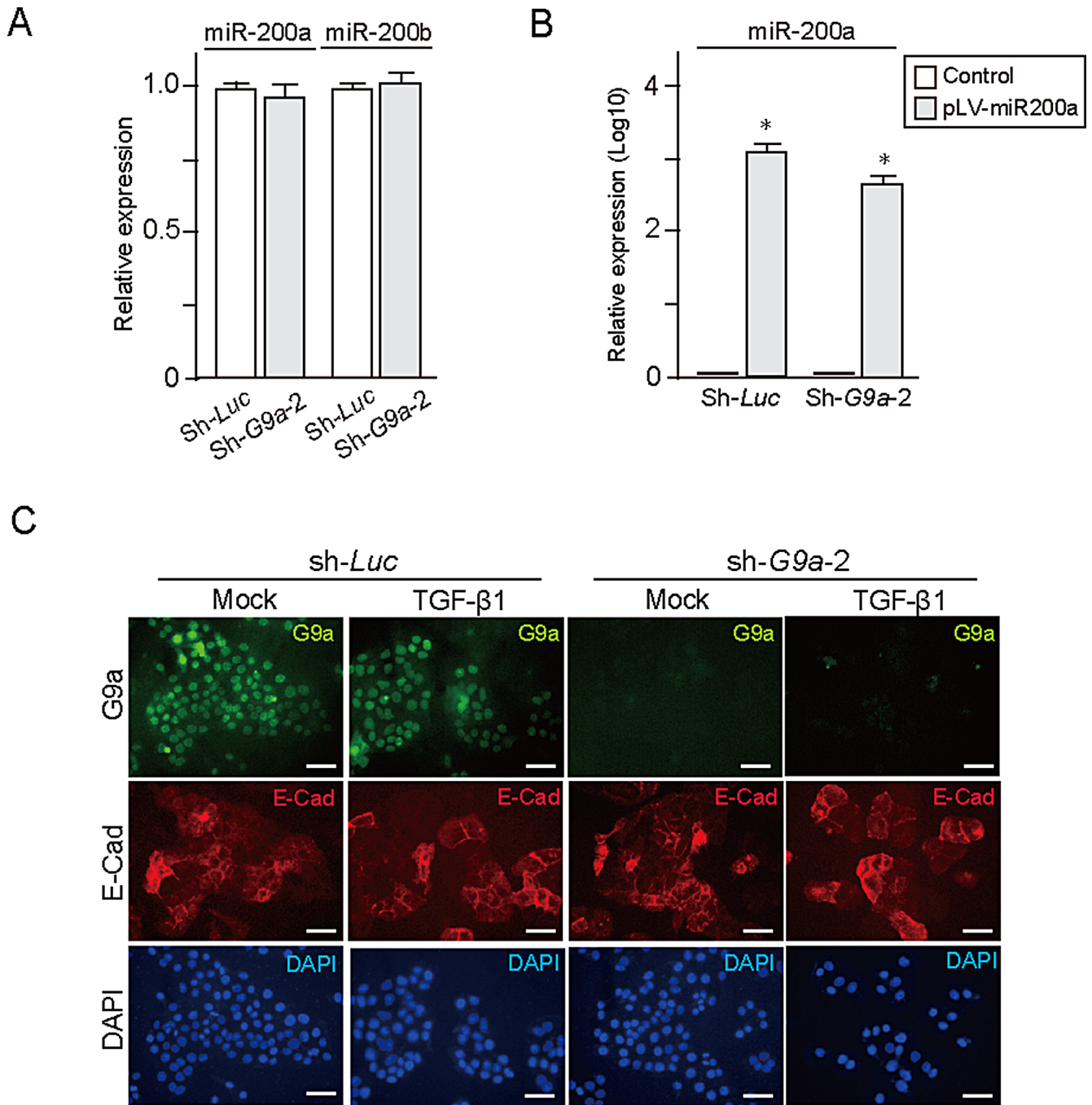
**Supplementary Figure 1: Xenograft transplantation in NOD/SCID mice.** (A) In total,  $2 \times 10^6$  *G9a* knockdown Huh1 and Huh7 cells were separately implanted into the subcutaneous space of NOD/SCID mice. *G9a* knockdown cells developed apparently smaller tumors in the right subcutaneous space (arrows) than sh-*Luc*-expressing control cells in the left space (arrowheads) 6 weeks after the transplantation ( $N = 5$  per group). (B) Tumor volume was estimated 6 weeks after transplantation. \*Statistically significant ( $p < 0.05$ ).



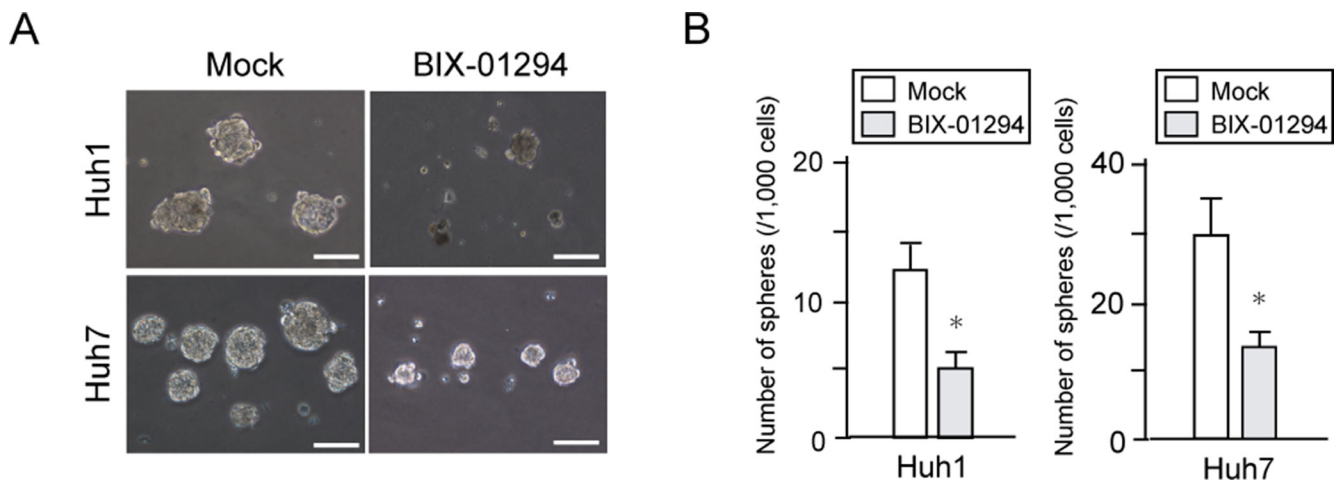
**Supplementary Figure 2: TGF- $\beta 1$ -induced EMT in *G9a* knockdown PLC/PRF/5 cells.** (A) Real-time RT-PCR analysis of *G9a* expression in stable *G9a* knockdown cells. Data sets were obtained from three independent experiments. \*Statistically significant ( $p < 0.05$ ). (B) Bright-field images of PLC/PRF/5 cells treated with TGF- $\beta 1$  for 48 hours. Fluorescence images are shown in insets. Scale bar = 100  $\mu\text{m}$ . (C) Immunocytochemical analyses for *G9a* (green) and E-cadherin (red) expression in PLC/PRF/5 cells. Scale bar = 100  $\mu\text{m}$ . (D) Real-time RT-PCR analysis of the expression of EMT markers in PLC/PRF/5 cells after TGF- $\beta 1$  treatment for 48 hours. Data sets were obtained from three independent experiments. \*Statistically significant ( $p < 0.05$ ).



**Supplementary Figure 3: Wound healing assay of Huh7 cells.** No remarkable differences in migration ability were observed between control and *G9a* knockdown cells after *TGF-β1* treatment.



**Supplementary Figure 4: Role of miR-200 in *TGF-β1*-induced EMT in Huh7 cells.** (A) Expression of miR-200a and 200b in stable *G9a* knockdown cells was analyzed using real-time RT-PCR and normalized to U6. Data sets were obtained from three independent experiments. (B) Real-time RT-PCR analysis of miR-200a expression in *G9a* knockdown cells stably expressing miR-200a. Data sets were obtained from three independent experiments. (C) Immunocytochemical analyses of G9a (green) and E-cadherin (red) expression in *G9a* knockdown cells with miR-200a overexpression. Nuclei stained with DAPI (blue) are also shown. Scale bar = 100  $\mu$ m.



**Supplementary Figure 5: Non-adherent sphere formation assay of xenograft tumor-derived cells.** (A) Bright-field images of tumor cells in non-adherent spheres formed after 14 days of culture. (B) Number of large spheres (> 100  $\mu\text{m}$  in diameter) generated from 1,000 tumor-derived cells. Data sets were obtained from three independent experiments. \*Statistically significant ( $p < 0.05$ ).

**Supplementary Table 1: Potential target genes for G9a**

RefSeq	Definition	RefSeq	Definition
C7orf43	chromosome 7 open reading frame 43	HES4	hairly and enhancer of split 4 (drosophila)
FBX02	F-box protein 2	CLIC3	chloride intracellular channel 3
TMEM82	transmembrane protein 82	MTRNR2L9	MT-RNR2-like 9
CADM1	cell adhesion molecule 1	SLC51A	solute carrier family 51 alpha subunit
ISM2	isthmin 2 homolog (zebrafish)	TP53INP1	tumor protein p53 inducible nuclear protein 1
BMP1	bone morphogenetic protein 1	CD55	CD55 molecule
SERINC2	serine incorporator 2	AKR1C2	aldo-keto reductase family 1, member C2
SEC14L2	SEC14-like 2 (s.cerevisiae)	YPEL3	yippee-like 3 (drosophila)
IN080B	IN080 complex subunit B	CYP1B1	cytochrome P450, family 1, subfamily B, member 1
VMA7	von Willebrand factor A domain containing 7	TMEM151A	transmembrane protein 151A
C2orf82	chromosome 2 open reading frame 82	CDKN1A	cyclin-dependent kinase inhibitor 1A (p21, Cip1)
ALDH3B1	aldehyde dehydrogenase 3 family, member B1	ACY1	aminoacylase 1
MOGAT3	monoacylglycerol O-acyltransferase 3	SPOCK2	sparc/osteonectin, cwcv and kazal-like domains proteoglycan 2
PPP1R3B	protein phosphatase 1, regulatory subunit 3B	RTN4RL2	reticulin 4 receptor-like 2
LAMB2	laminin, beta 2 (laminin S)	RRAS	related RAS viral (r-ras) oncogene homolog
PLAU	plasminogen activator, urokinase	C19orf33	chromosome 19 open reading frame 33
SYT12	synaptotagmin XII	SOCS2	suppressor of cytokine signaling 2
TFF1	trefoil factor 1	TRIB1	tribbles homolog 1 (drosophila)
TIMP3	TIMP metalloproteinase inhibitor 3	ITPR3	inositol 1,4,5-trisphosphate receptor, type 3
KLF2	Kruppel-like factor 2 (lung)	PPP1R13L	protein phosphatase 1, regulatory subunit 13 like
TMEM176B	transmembrane protein 176B	CYP4F3	cytochrome P450, family 4, subfamily F, member 3
CRYL1	crystallin, lambda 1	PLLP	plasmalipin
ACP5	acid phosphatase 5, tartrate resistant	PMP22	peripheral myelin protein 22
ATP2B4	ATPase, Ca <sup>2+</sup> transporting, plasma membrane 4	NDRG4	NDRG family member 4
JUNB	jun B proto-oncogene	KCNMB4	charybdotoxin receptor subunit beta-4
B4GALNT1	beta-1, 4-N-acetyl-galactosaminyltransferase 1	INHBB	inhibin, beta B
GTPBP2	GTP binding protein 2	TIMP1	TIMP metalloproteinase inhibitor 1
ZFP36	zinc finger protein 36, C3H type, homolog (mouse)	ADM	adrenomedullin
POLD4	polymerase (DNA directed), delta 4	BHLHE40	basic helix-loop-helix family, member e40
DHRS3	dehydrogenase/reductase (SDR family) member 3	OPTN	optineurin
CAB39L	calcium binding protein 39-like	TUBB3	tubulin, beta 3 class III
MAPK11	mitogen-activated protein kinase 11	SPON2	spondin 2, extracellular matrix protein
CXCL16	chemokine (C-X-C motif) ligand 16	NEU4	neuraminidase 4
OLFM2	olfactomedin 2	COL7A1	collagen, type VII, alpha 1
LOXL2	lysyl oxidase-like 2	LAMA3	laminin, alpha 3
LIF	leukemia inhibitory factor	UAP1L1	UDP-N-acetylglucosamine pyrophosphorylase 1 like 1
MTSS1	metastasis suppressor 1	SOCS3	suppressor of cytokine signaling 3
TSKU	tukushi small leucine rich proteoglycan homolog (xenopus laevis)	SAA1	serum amyloid A1
KRT19	keratin 19	DKK3	dickkopf 3 homolog (xenopus laevis)
PNRC1	proline-rich nuclear receptor coactivator 1	SHH	sonic hedgehog
ANGPTL4	angiopoietin-like 4	MXD1	MAX dimerization protein 1
SEZ6L2	seizure related 6 homolog (mouse)-like 2	FGF2	fibroblast growth factor 2 (basic)
TAPBP	TAP binding protein (tapasin)	EGLN3	egl-9 family hypoxia inducible factor 3
KLF4	Kruppel-like factor 4	ADM2	adrenomedullin 2
TRPV2	transient receptor potential cation channel, subfamily V, member 2	LYPD6B	LY6/PLAUR domain containing 6B
PDGFRA	platelet-derived growth factor receptor, alpha polypeptide	PDLIM2	PDZ and LIM domain 2
IGFBP1	insulin-like growth factor binding protein 1	C10orf91	chromosome 10 open reading frame 91
MICAL1	microtubule associated monooxygenase, calponin and LIM domain containing 1	AGRN	agrin

**Supplementary Table 2: Primer sequences designed for real-time RT-PCR**

Gene name	Sequence
<i>G9a</i>	Fw 5'- TTTCCGCATGAGTGATGATG -3'
	Rv 5'- GGGCAGAACCTAACTCCTCTG -3'
<i>E-Cad</i>	Fw 5'- AAGTTTTCCACCAAAGTCACG -3'
	Rv 5'- TGCTTGGATTCCAGAAACG -3'
<i>N-Cad</i>	Fw 5'- GGTGGAGGAGAAGAAGACCAG -3'
	Rv 5'- GGCATCAGGCTCCACAGT -3'
<i>SNAIL</i>	Fw 5'- GCTGCAGGACTCTAATCCAGA -3'
	Rv 5'- ATCTCCGGAGGTGGGATG-3'
<i>FNI</i>	Fw 5'- GCCACTGGAGTCTTTACCACA -3'
	Rv 5'- CCTCGGTGTTGTAAGGTGGA -3'
<i>VIM</i>	Fw 5'- TACAGGAAGCTGCTGGAAGG -3'
	Rv 5'- ACCAGAGGGAGTGAATCCAG -3'
<i>GAPDH</i>	Fw 5'- CTGACTTCAACAGCGACACC -3'
	Rv 5'- TAGCCAAATTCGTTGTCATACC -3'

Fw, Forward, Rv, Reverse.