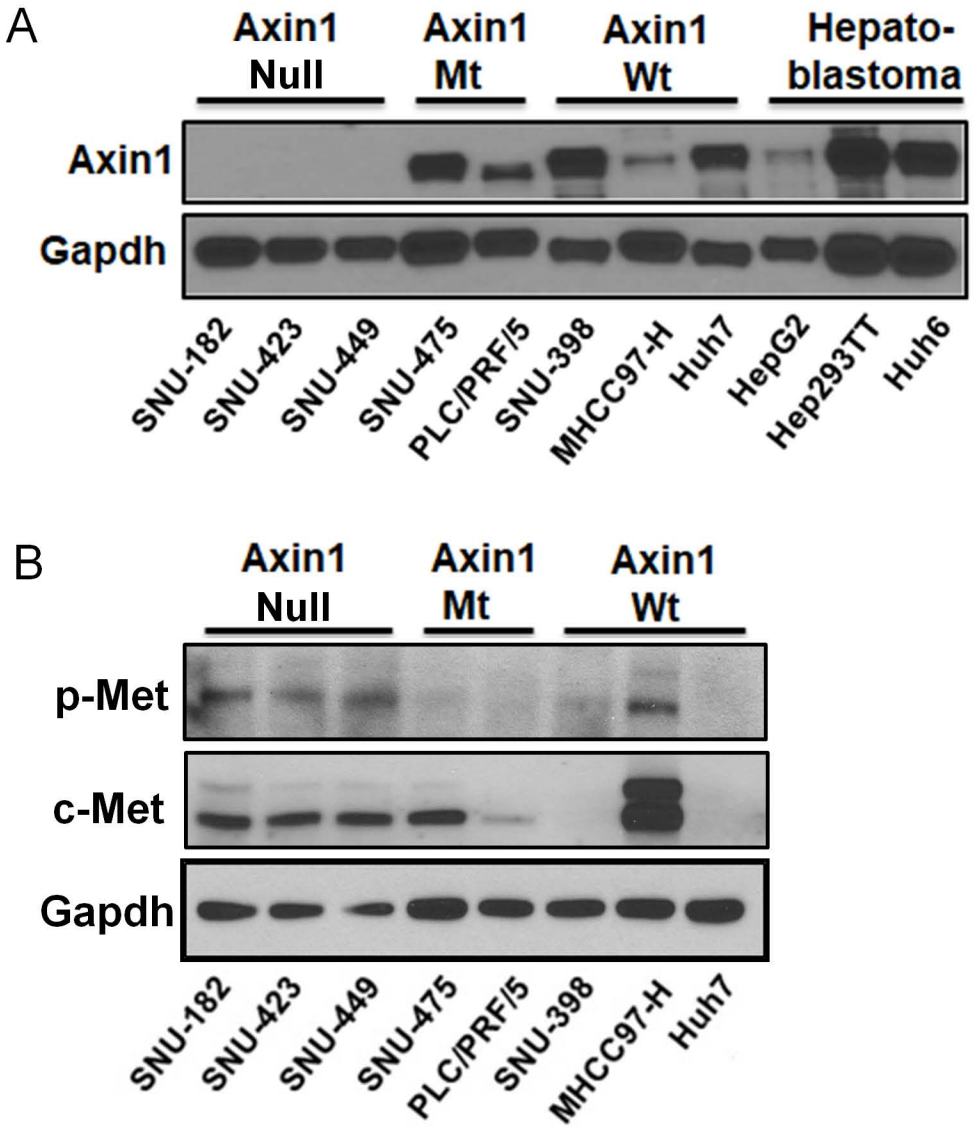
Sup. Figure 1


A
Axin1 Null
Axin1 Mt
Axin1 Wt
Hepato-blastoma
Axin1
Gapdh
SNU-182
SNU-423
SNU-449
SNU-475
PLC/PRF/5
SNU-398
MHCC97-H
Huh7
HepG2
Hep293TT
Huh6
B
Axin1 Null
Axin1 Mt
Axin1 Wt
p-Met
c-Met
Gapdh
SNU-182
SNU-423
SNU-449
SNU-475
PLC/PRF/5
SNU-398
MHCC97-H
Huh7

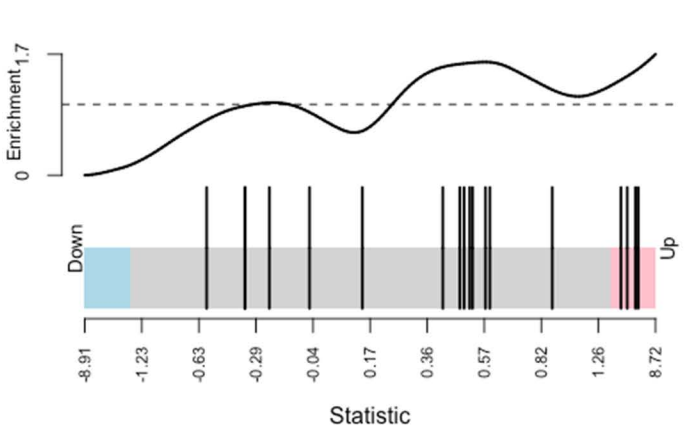
Sup. Figure 2

A

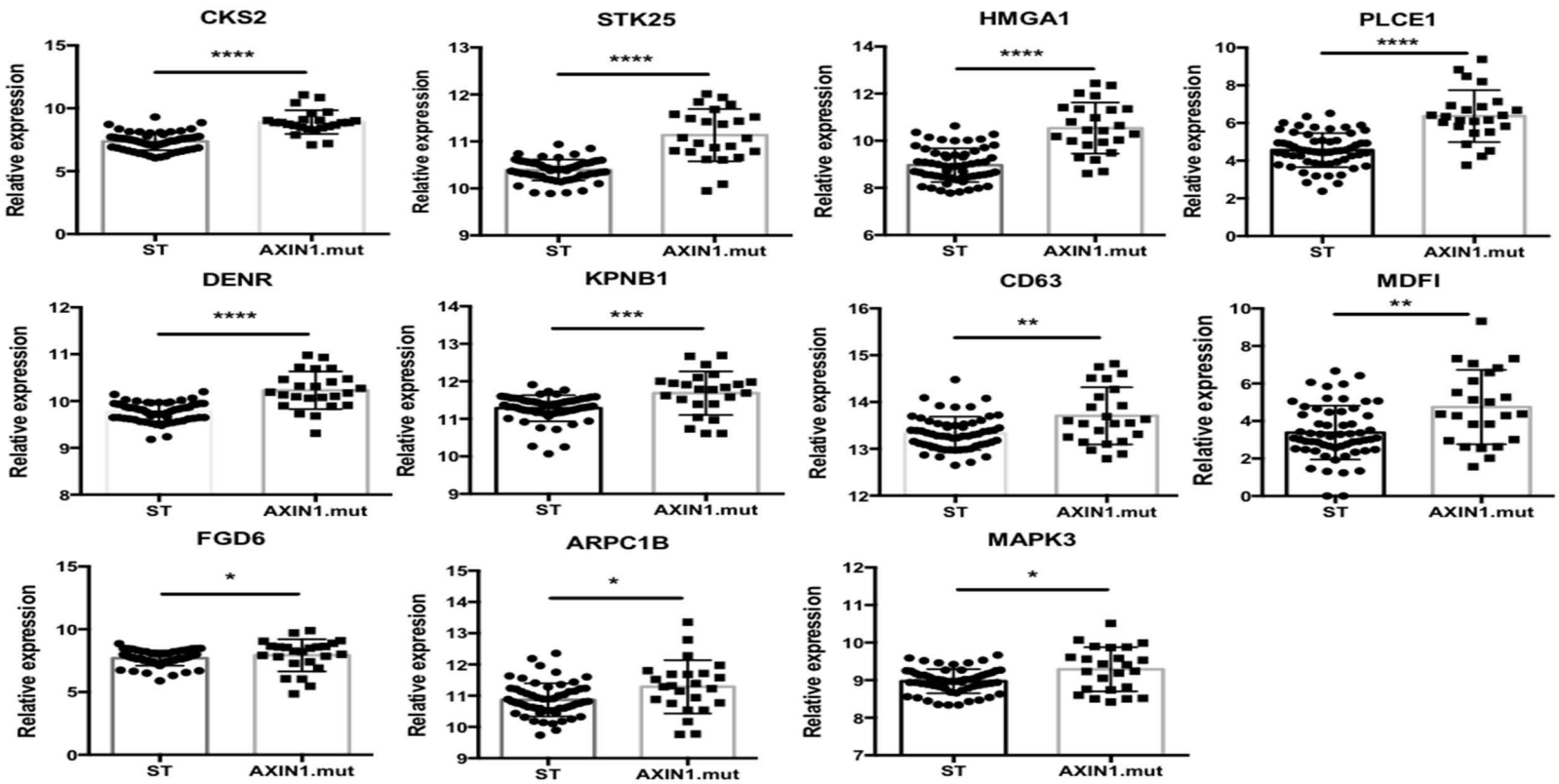
	NGenes	Direction	PValue	PValue.Mixed
Met-up	18	Up	3.90450610249243e-09	3.91018721625765e-09

c-Met up signature genes

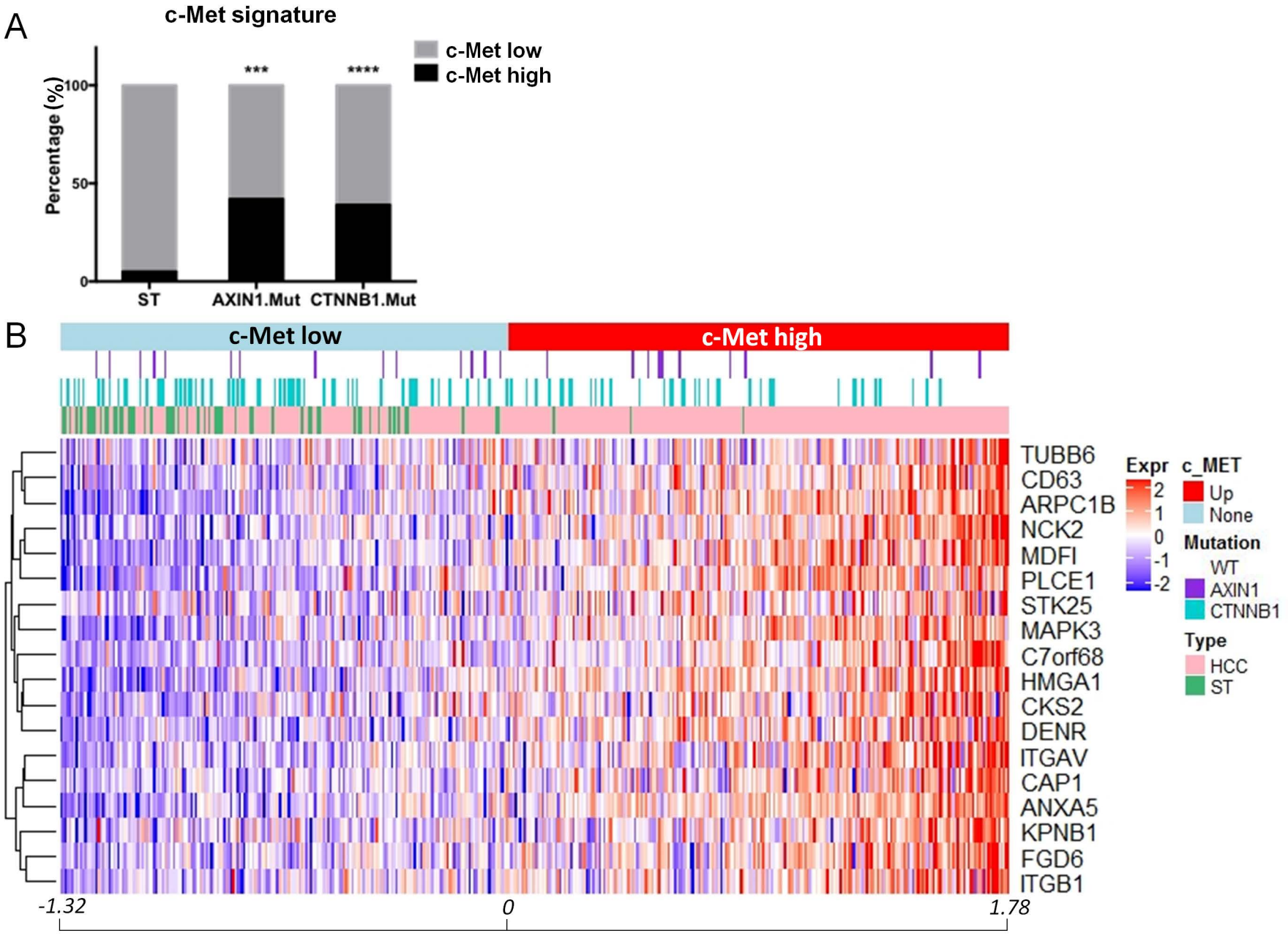
B



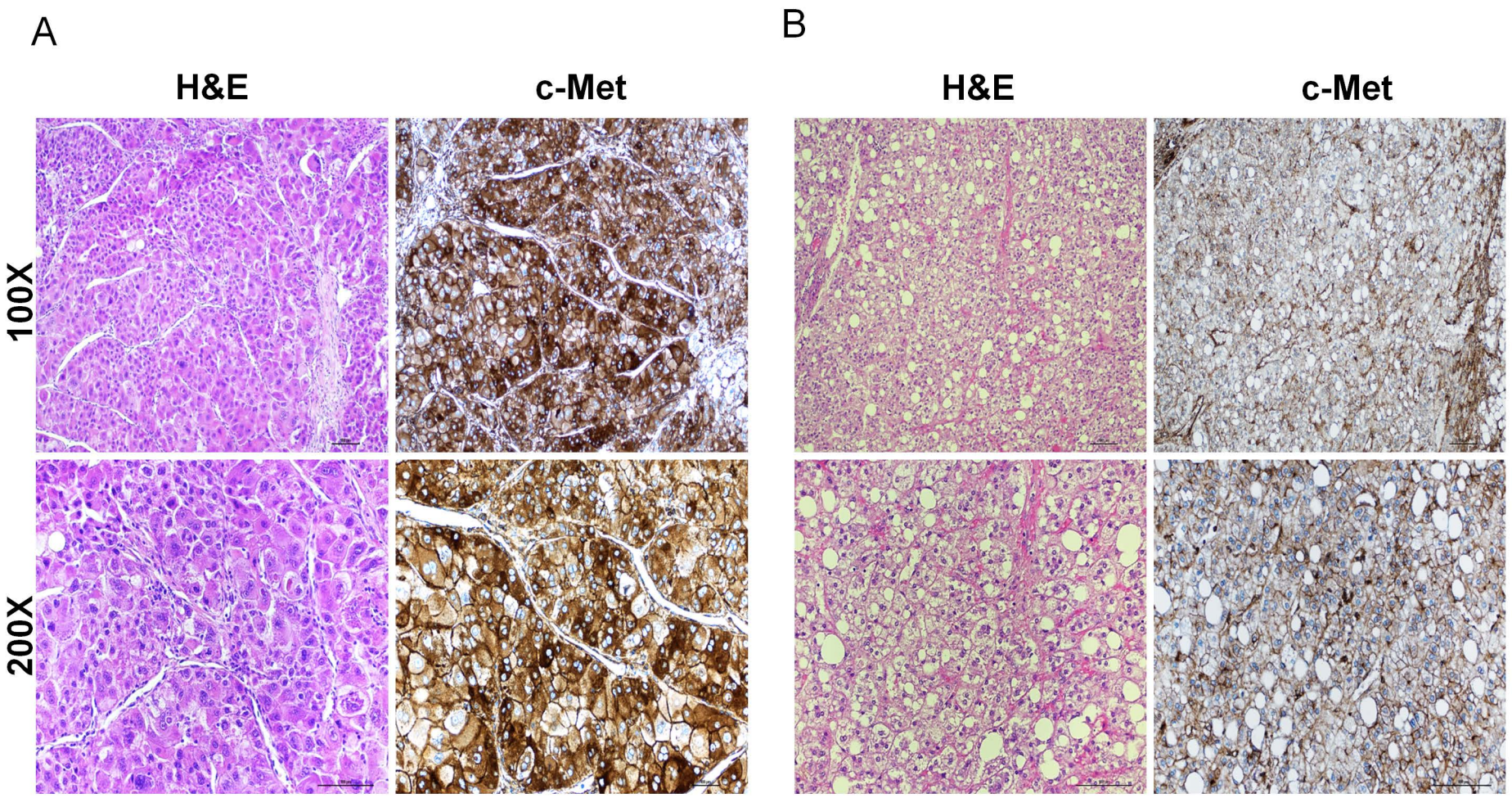
C



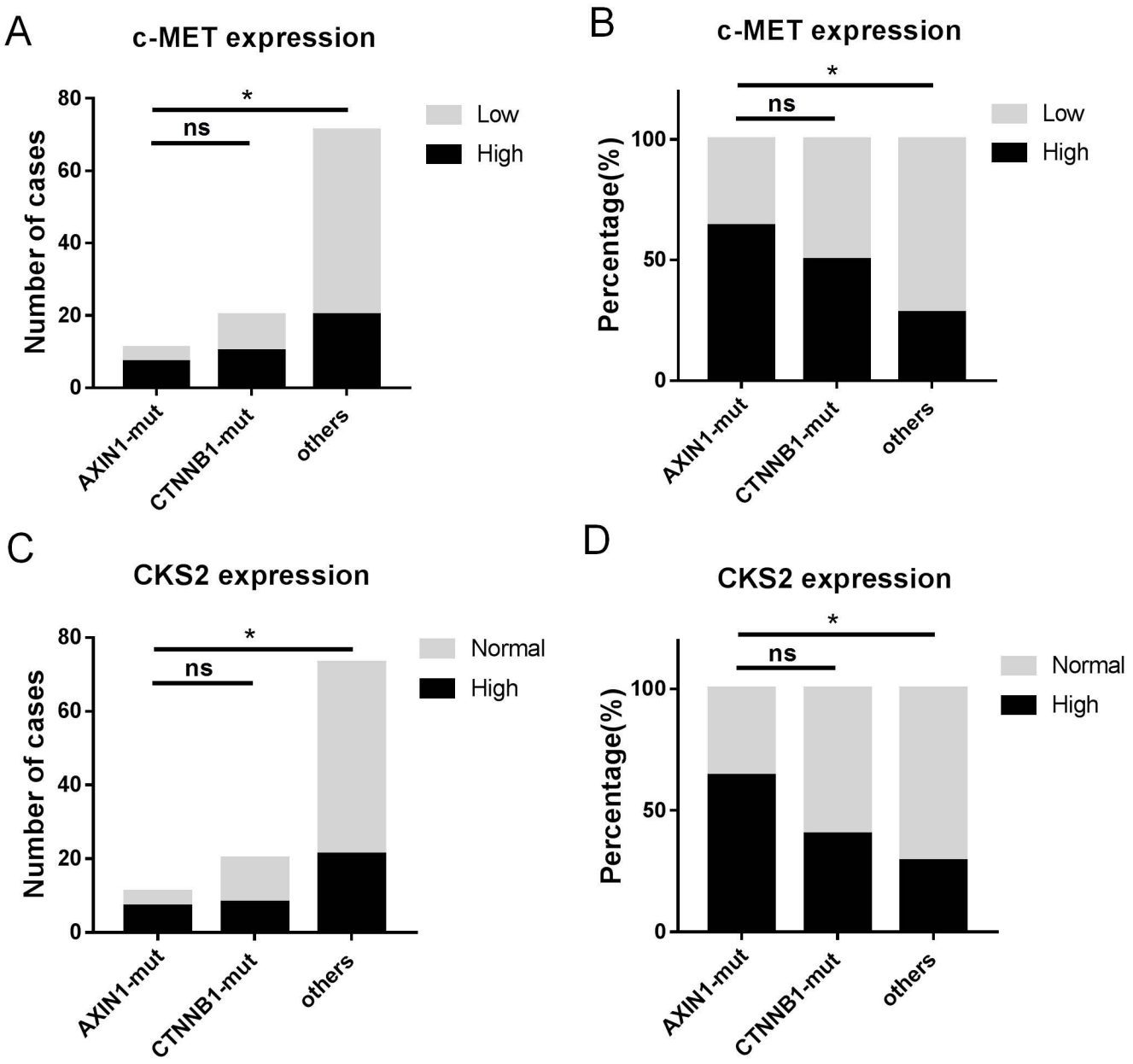
Sup. Figure 3


A
c-Met signature
c-Met low
c-Met high
***
****
Percentage (%)
100
50
0
ST
AXIN1.Mut
CTNNB1.Mut
B
c-Met low
c-Met high
TUBB6
CD63
ARPC1B
NCK2
MDFI
PLCE1
STK25
MAPK3
C7orf68
HMGA1
CKS2
DENR
ITGAV
CAP1
ANXA5
KPNB1
FGD6
ITGB1
-1.32
0
1.78
Expr
2
1
0
-1
-2
c_MET
Up
None
Mutation
WT
AXIN1
CTNNB1
Type
HCC
ST

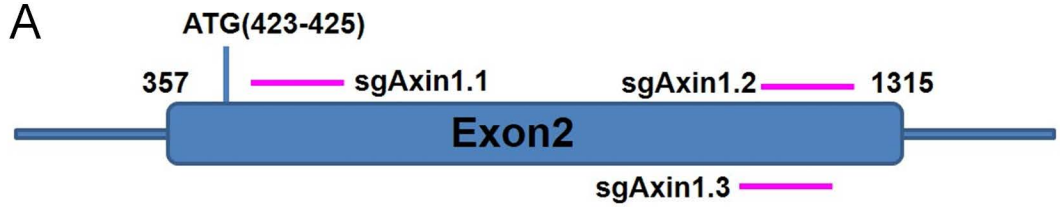
Sup. Figure 4



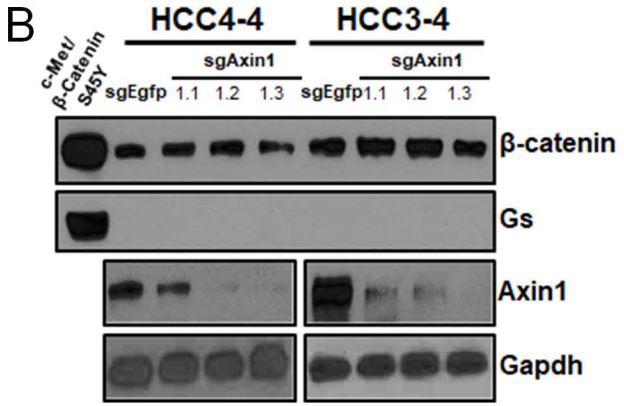
Sup. Figure 5

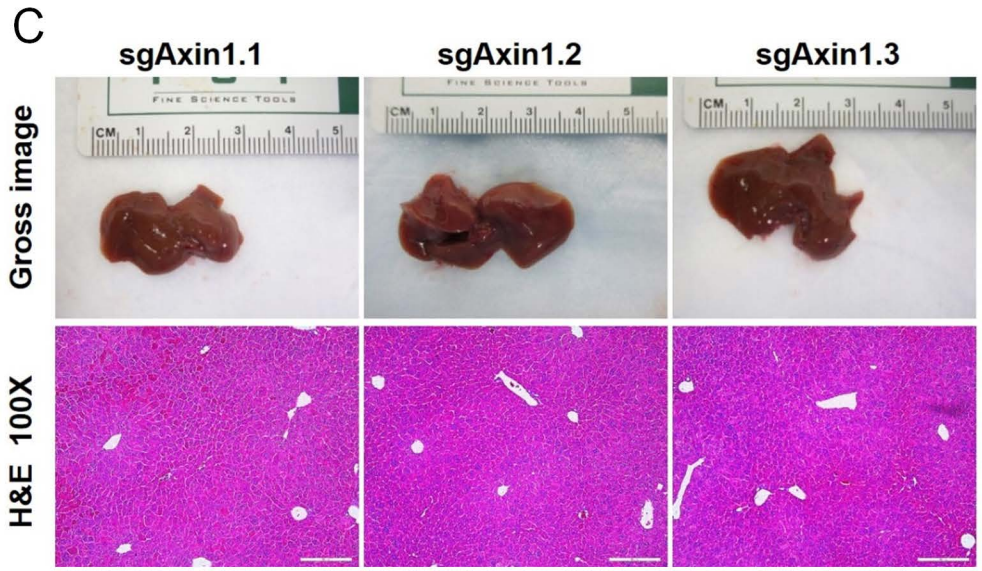

A
c-MET expression
Number of cases
80
60
40
20
0
*
ns
Low
High
AXIN1-mut
CTNNB1-mut
others
B
c-MET expression
Percentage(%)
100
50
0
*
ns
Low
High
AXIN1-mut
CTNNB1-mut
others
C
CKS2 expression
Number of cases
80
60
40
20
0
*
ns
Normal
High
AXIN1-mut
CTNNB1-mut
others
D
CKS2 expression
Percentage(%)
100
50
0
*
ns
Normal
High
AXIN1-mut
CTNNB1-mut
others

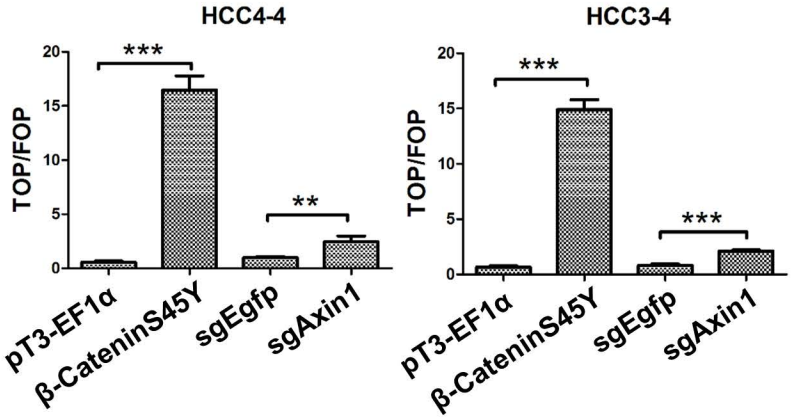
Sup. Figure 6



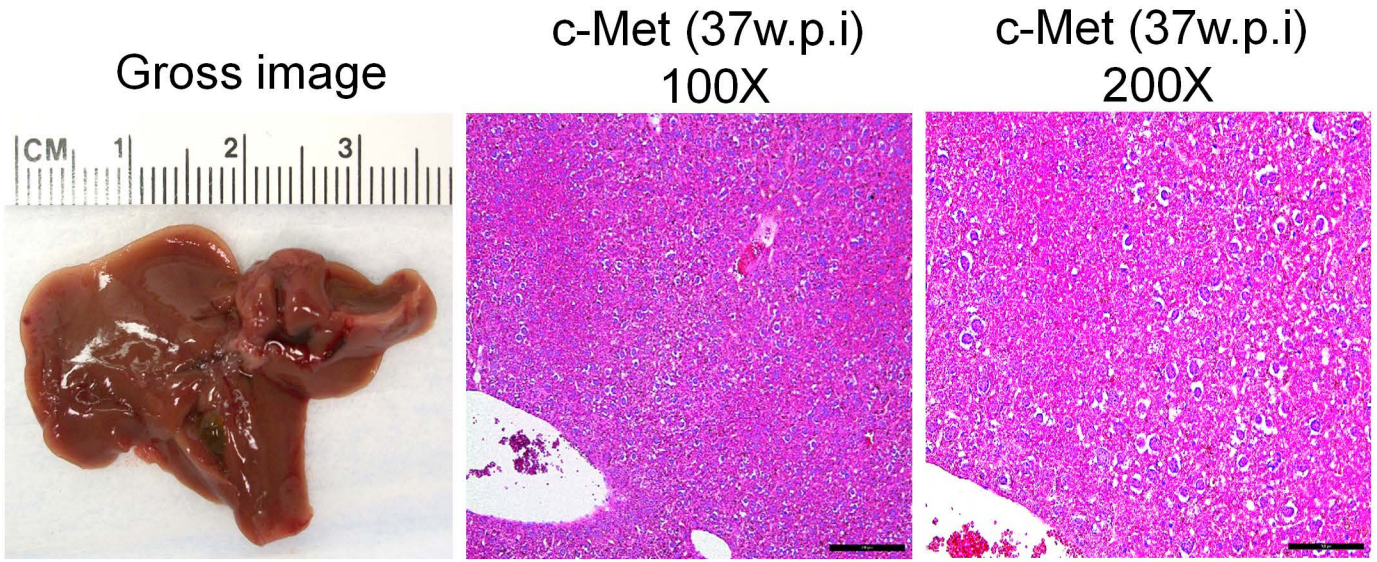
sgAxin1.1 (614-633) CACCCCAAGACGTTCAGATCTGG(sense oligo)
sgAxin1.2 (1281-1300) TCAAGTAGACGGTACAACGAAGG(sense oligo)
sgAxin1.3 (1270-1289) TCTACTTGAGGGGGCCCTCGGGG(anti-sense oligo)



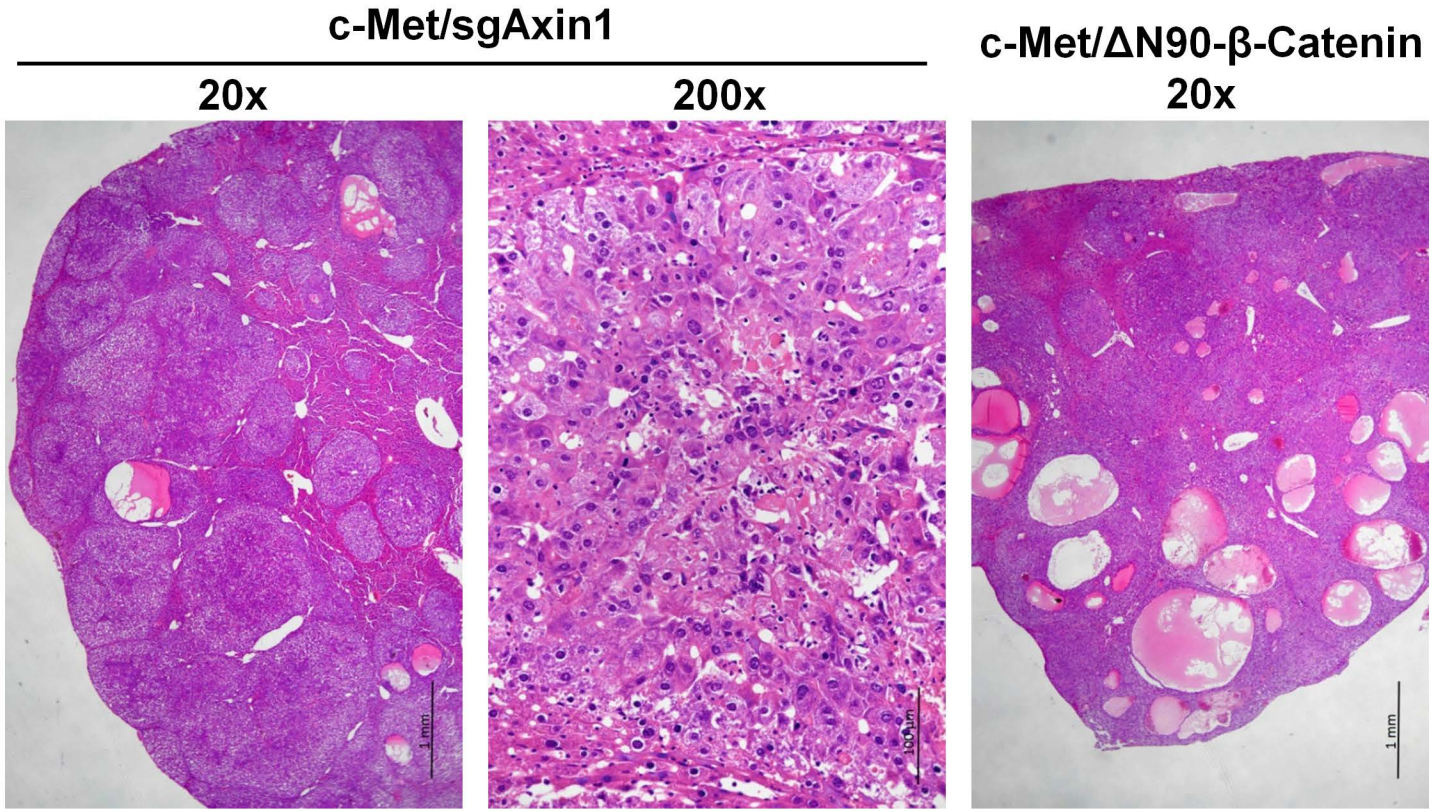




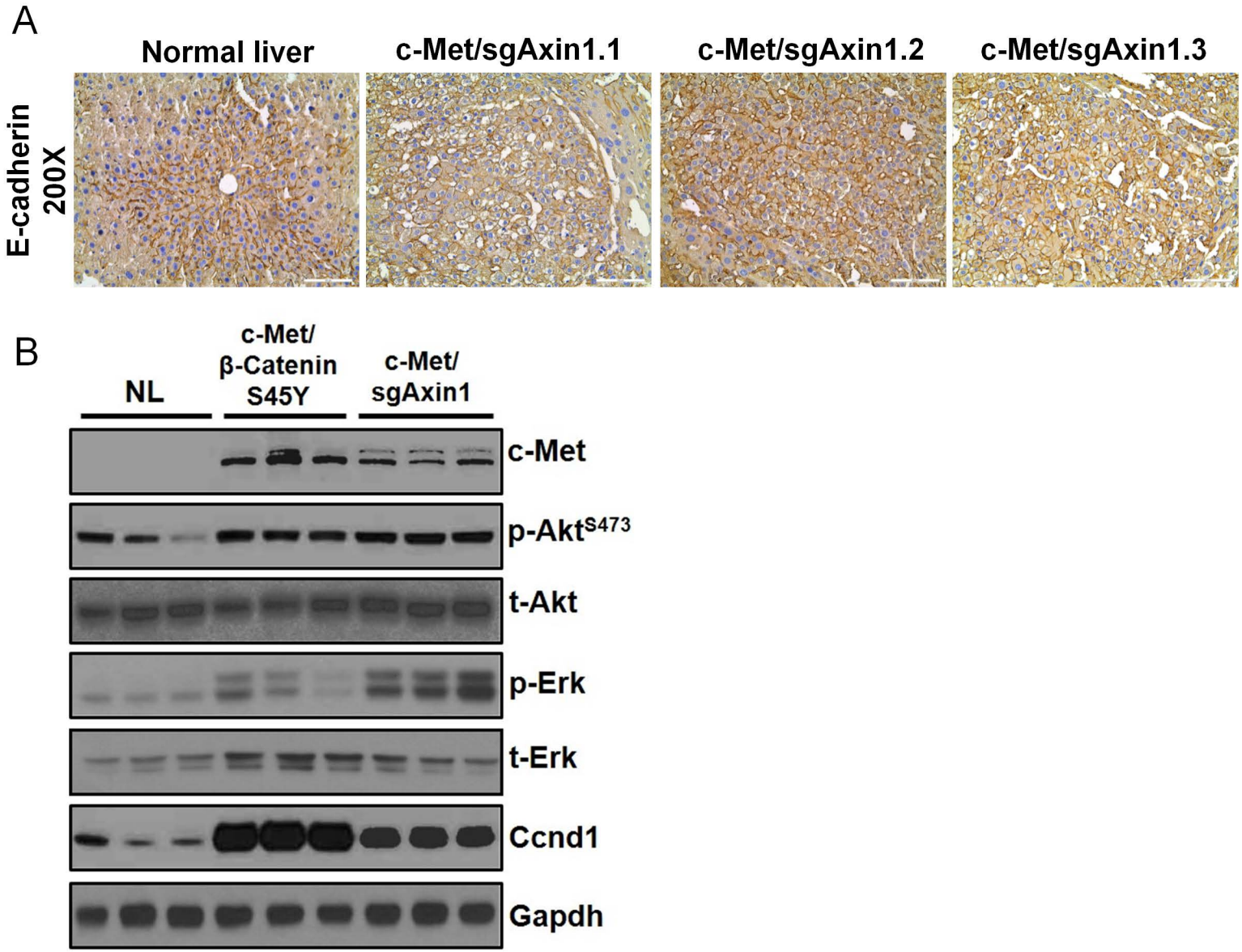
Sup. Figure 7



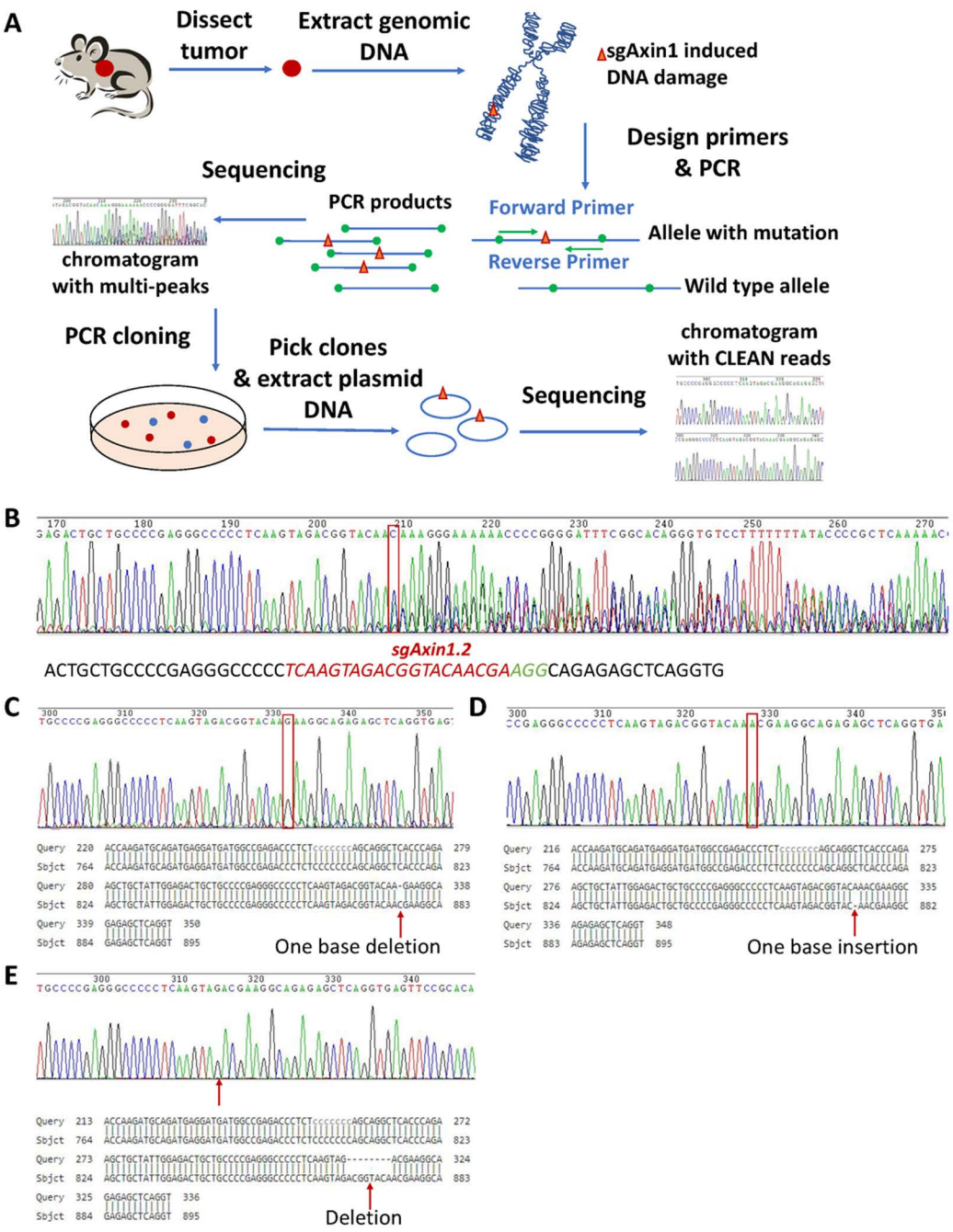
Sup. Figure 8



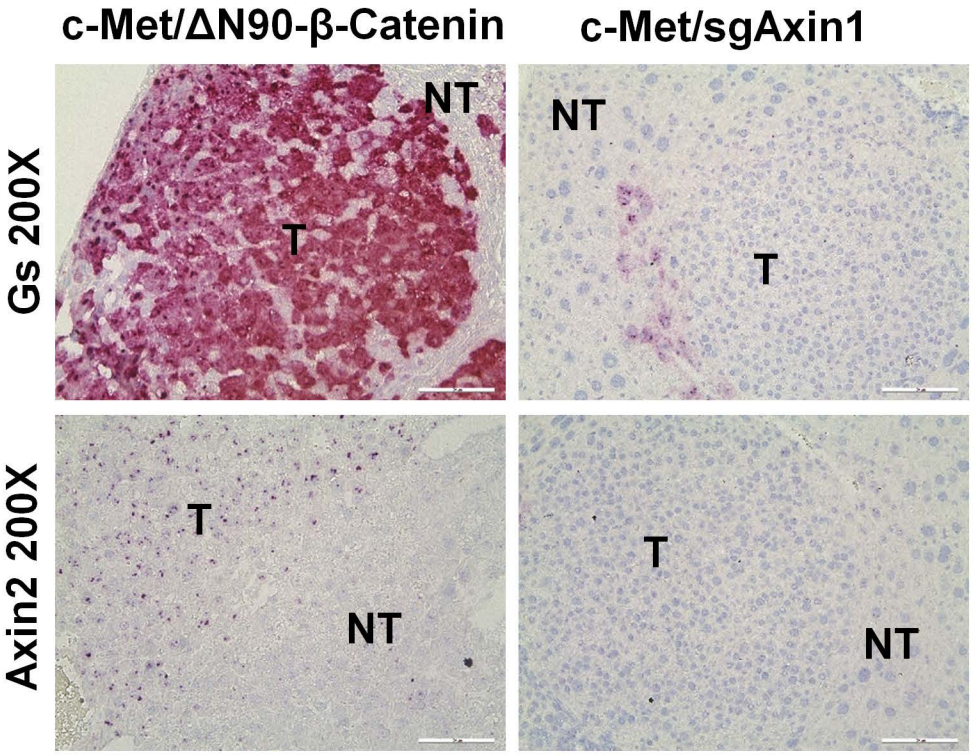
Sup. Figure 9


A
Normal liver
c-Met/sgAxin1.1
c-Met/sgAxin1.2
c-Met/sgAxin1.3
E-cadherin
200X
B
NL
c-Met/
β-Catenin
S45Y
c-Met/
sgAxin1
c-Met
p-Akt S473
t-Akt
p-Erk
t-Erk
Ccnd1
Gapdh

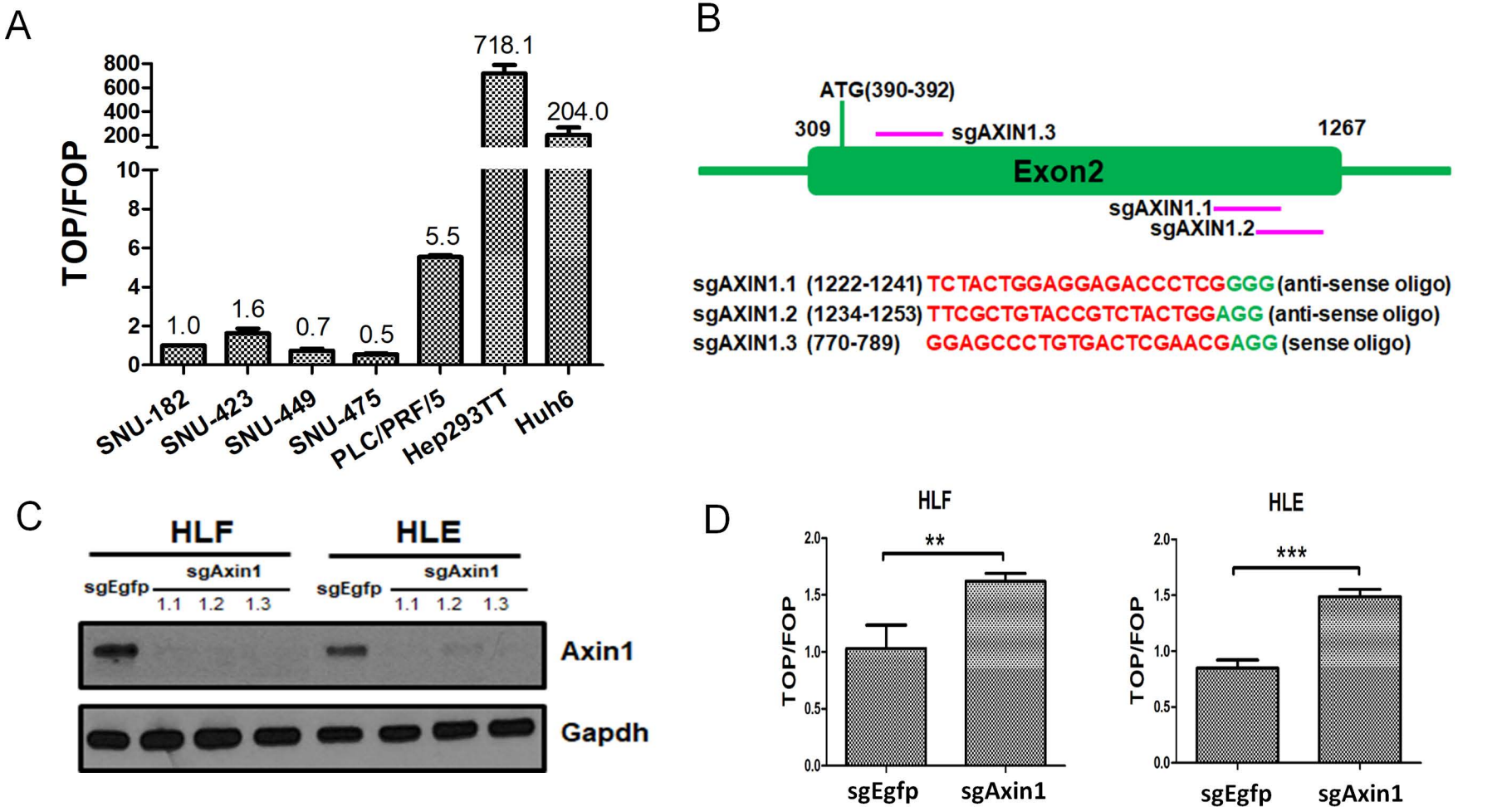
Sup. Figure 10



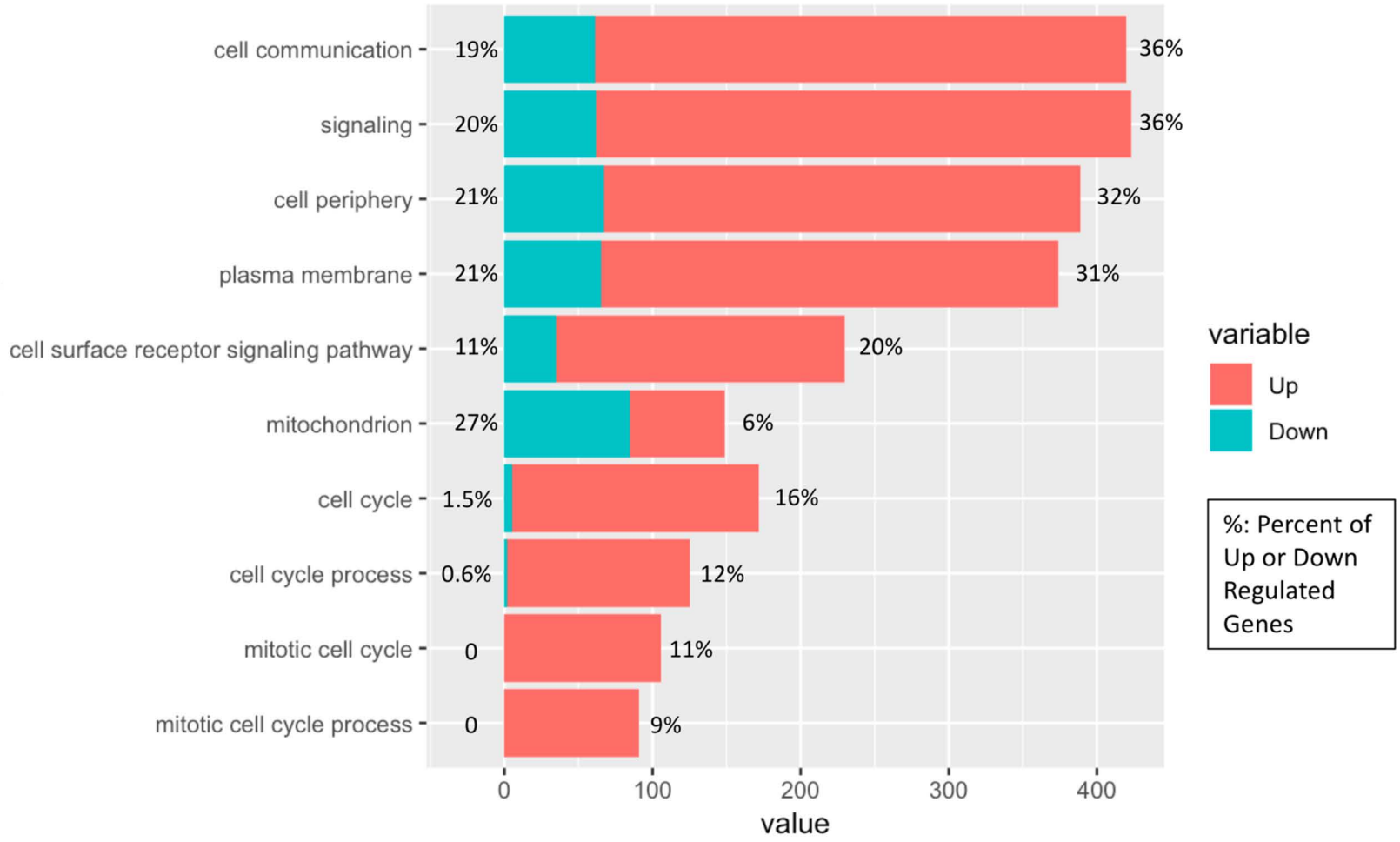
Sup. Figure 11



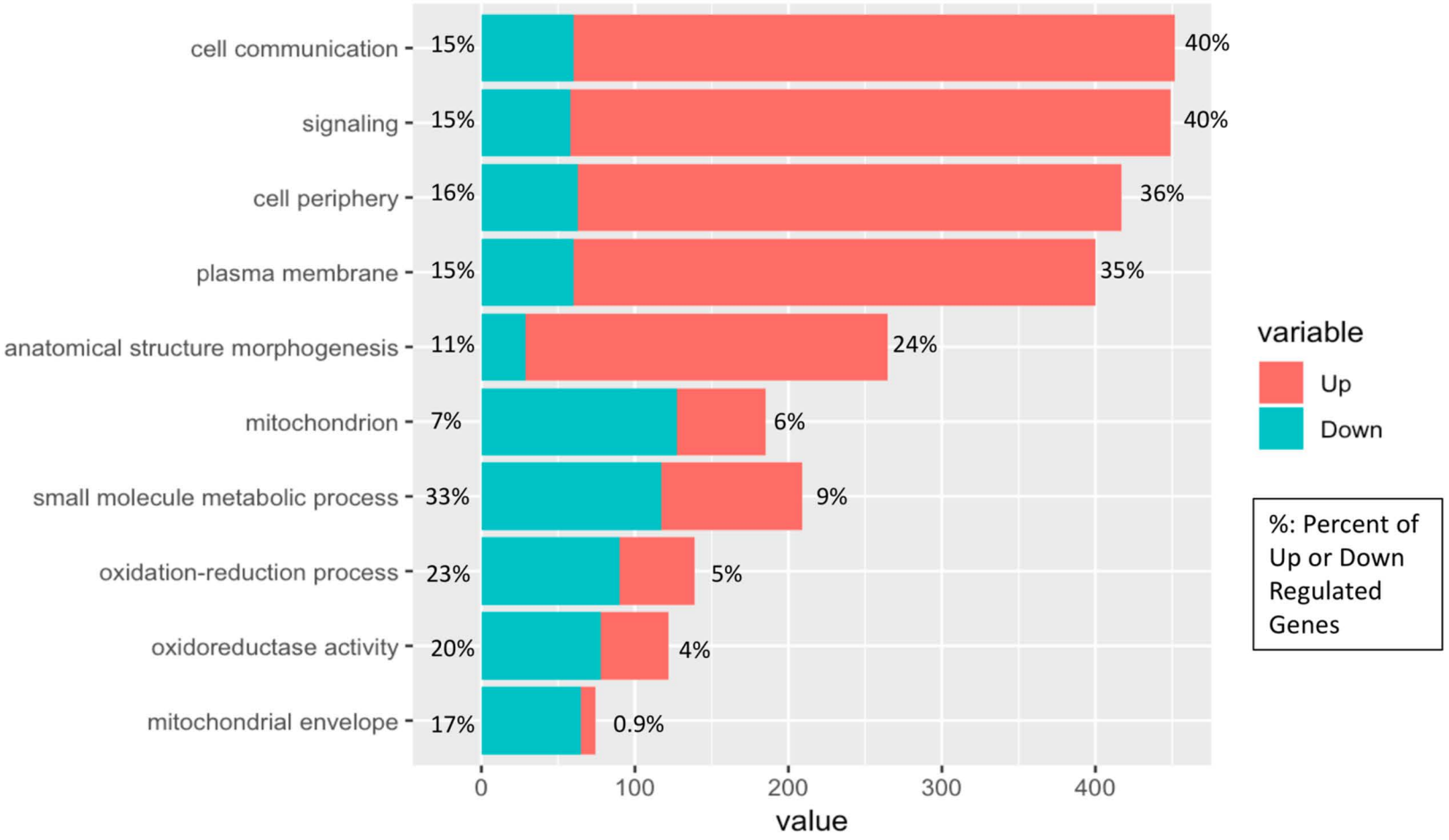
Sup. Figure 12



Sup. Figure 13



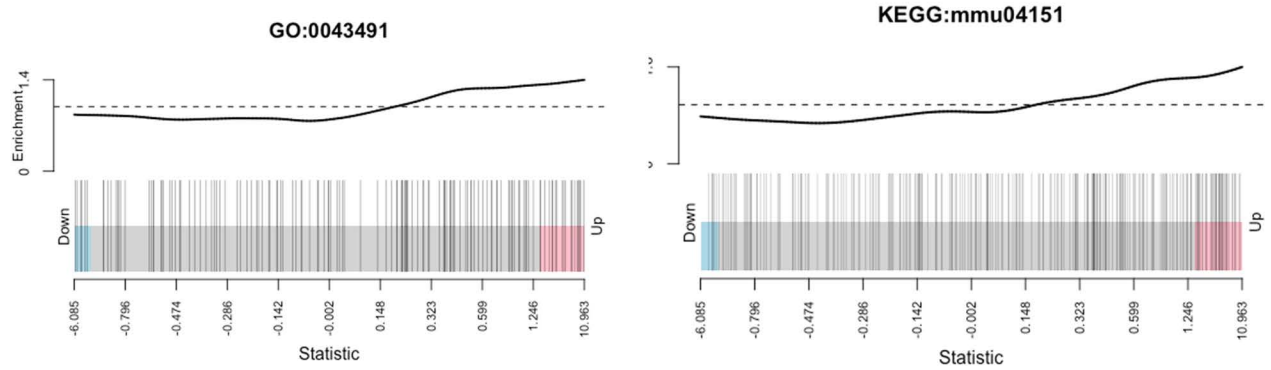
Sup. Figure 14



Sup. Figure 15

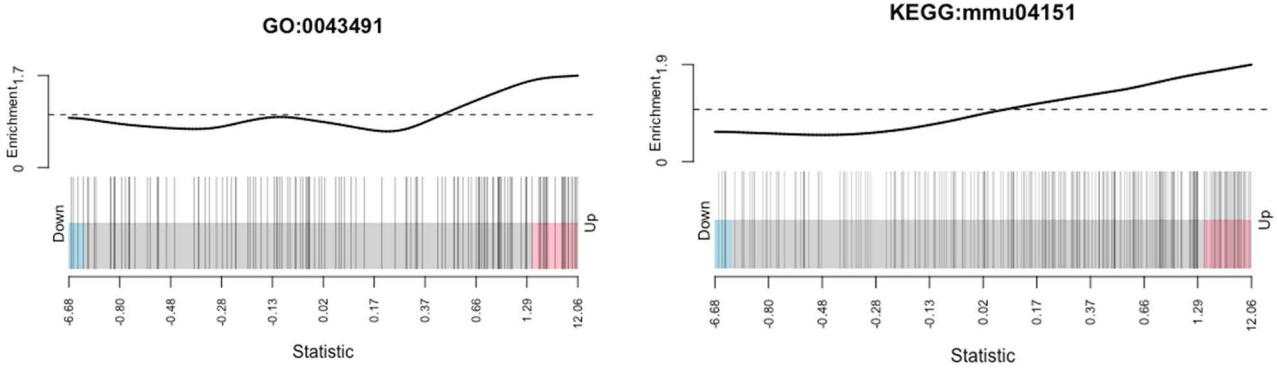
A

Bcat vs NL					
GO ID	Term	NGenes	Direction	PValue	PValue.Mixed
GO:0043491	AKT Signaling Pathway	139	Up	5.92E-06	8.82E-07
KEGG ID	Term	NGenes	Direction	PValue	PValue.Mixed
mmu04151	AKT Signaling Pathway	271	Up	4.65E-05	7.42E-07



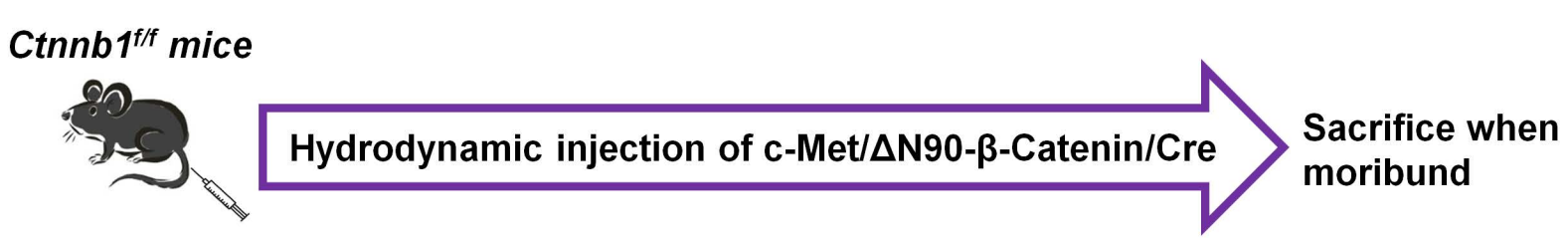
B

sgAxin vs NL					
GO ID	Term	NGenes	Direction	PValue	PValue.Mixed
GO:0043491	AKT Signaling Pathway	139	Up	5.10E-07	2.72E-07
KEGG ID	Term	NGenes	Direction	PValue	PValue.Mixed
mmu04151	AKT Signaling Pathway	271	Up	7.28E-06	6.18E-07

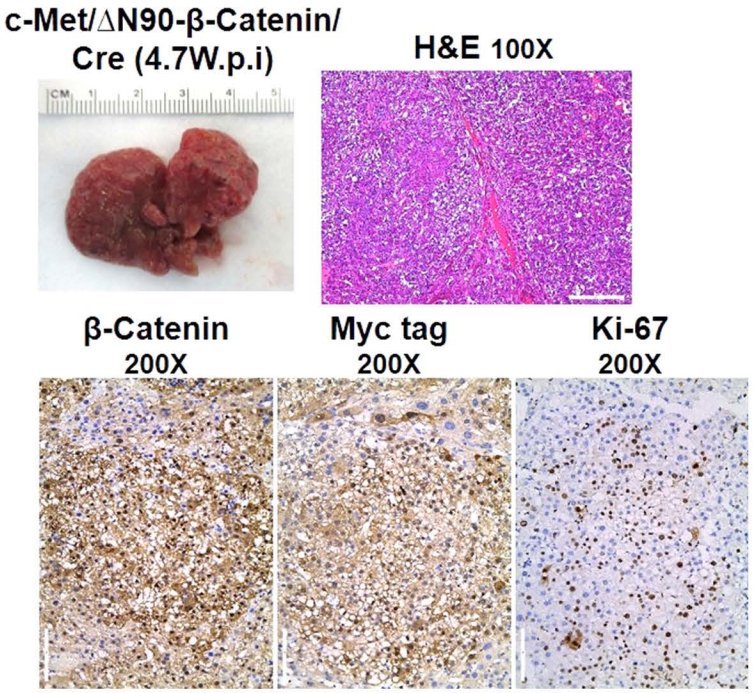


Sup. Figure 16

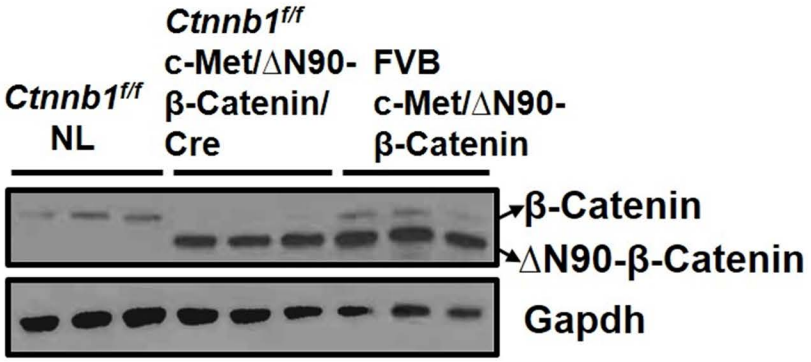
A



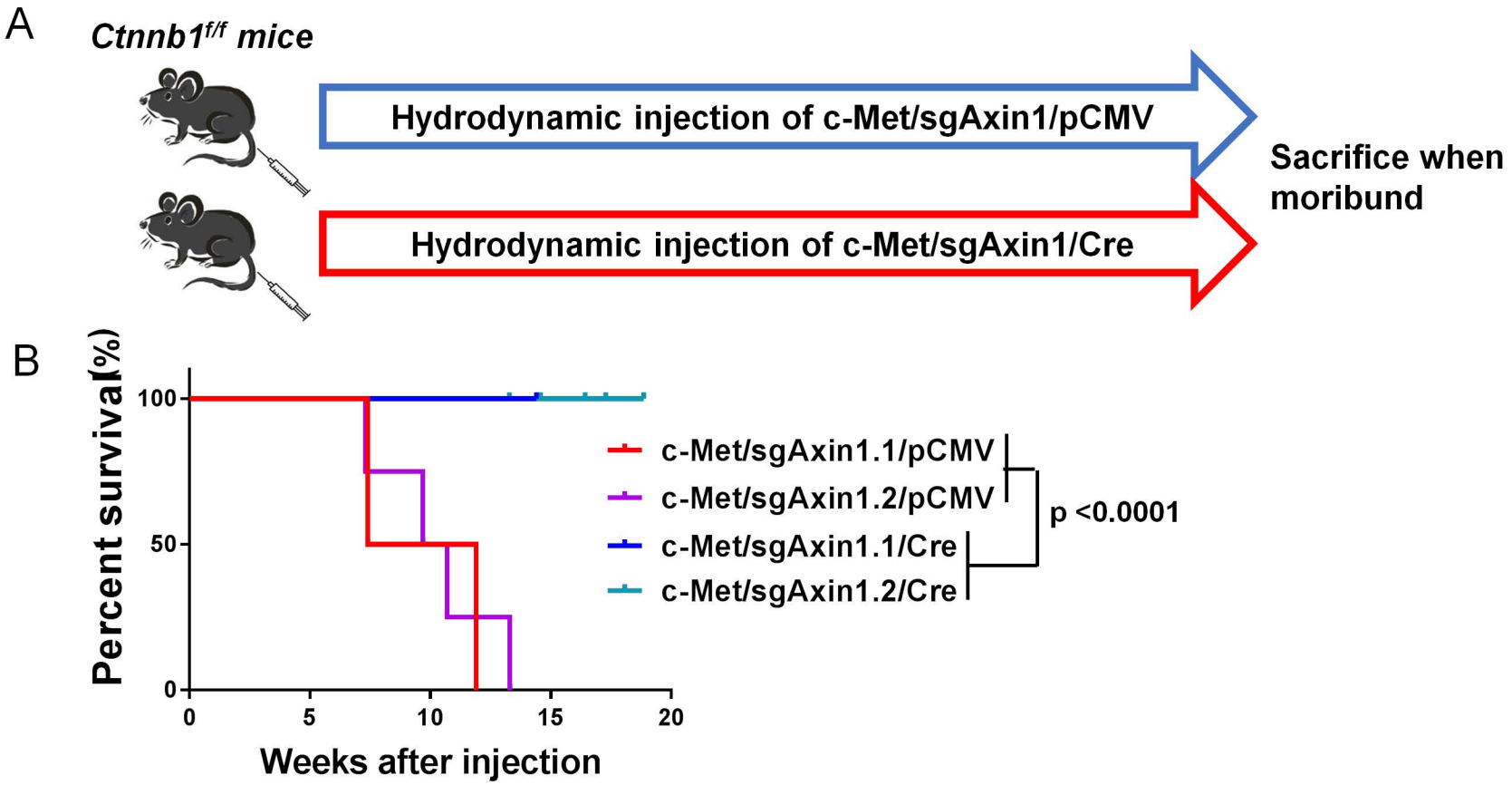
B



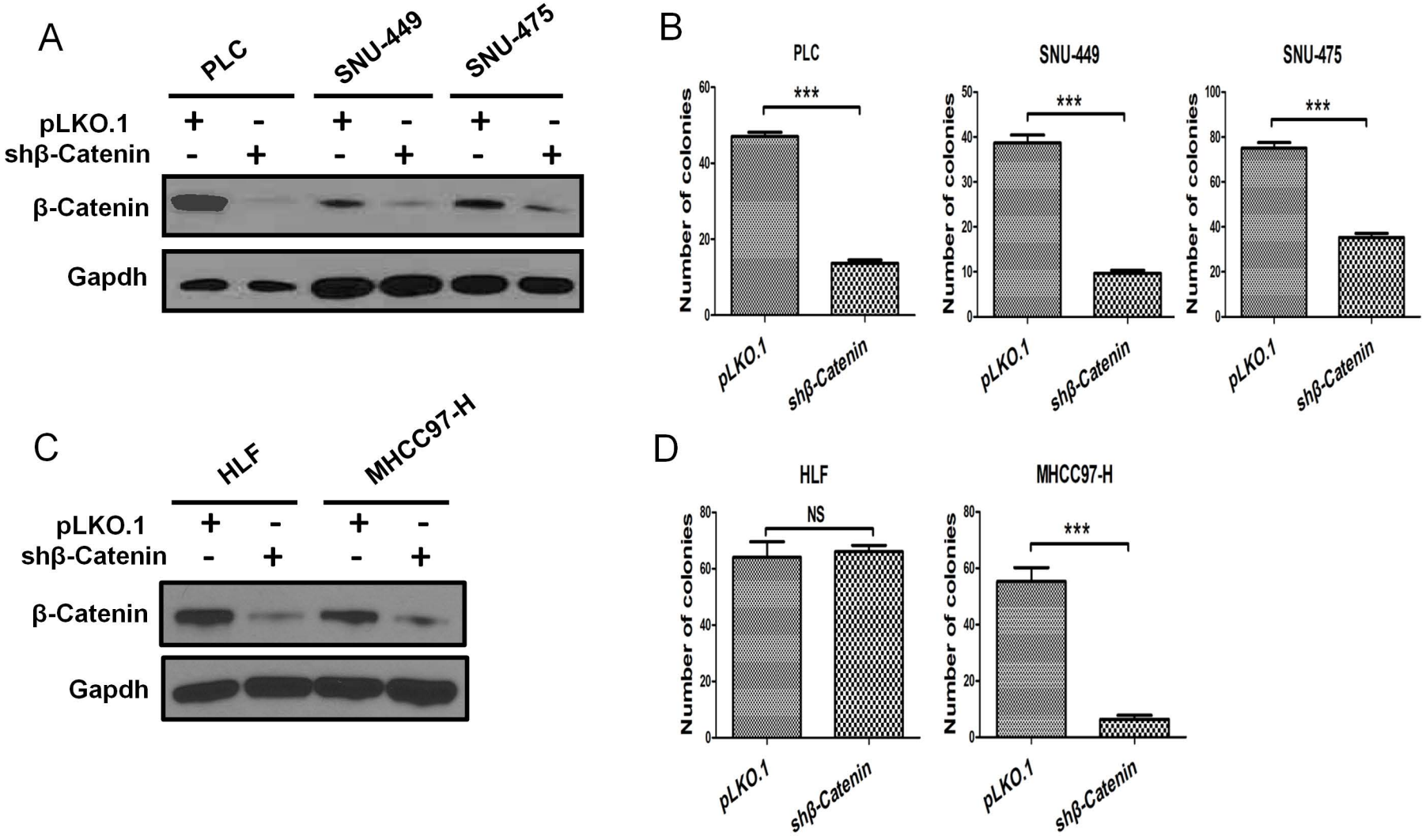
C



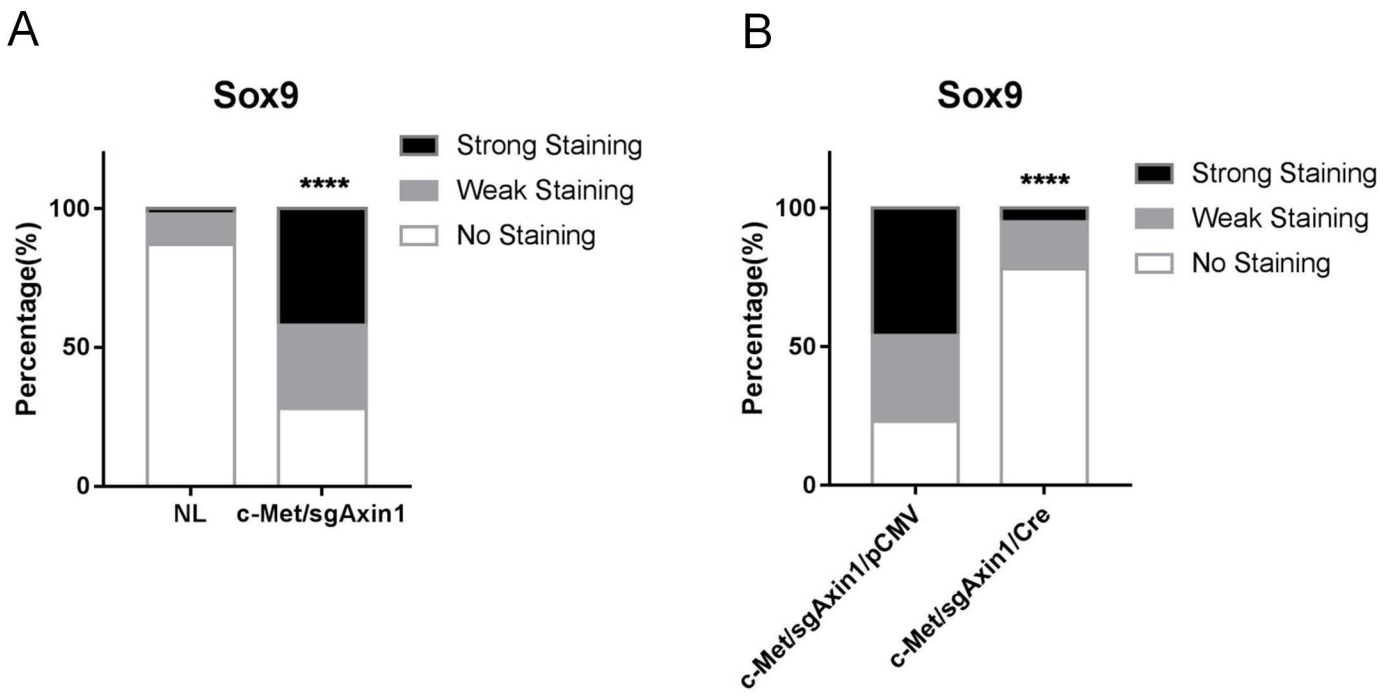
Sup. Figure 17



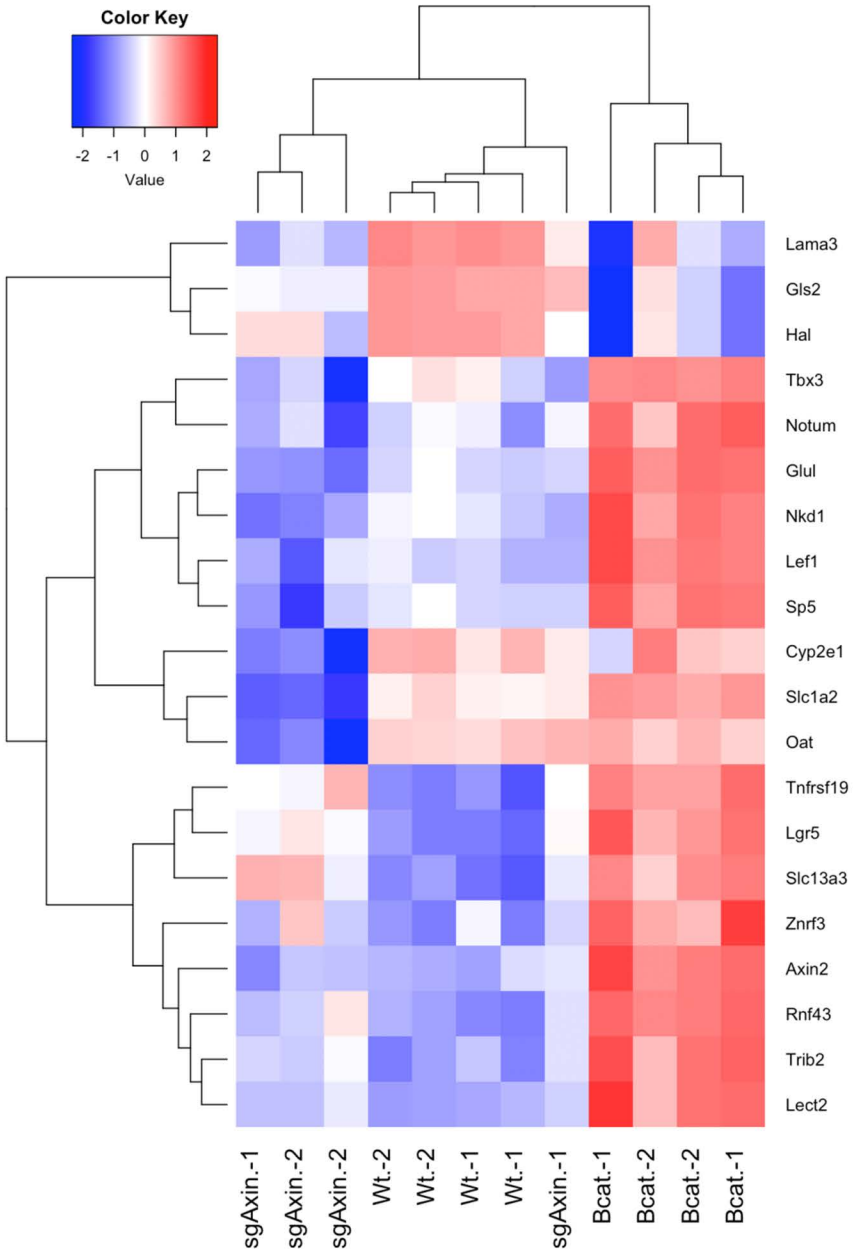
Sup. Figure 18



Sup. Figure 19



Sup. Figure 20



Supplementary Figure Legends

Supplementary Figure 1. *AXIN1* status and c-MET expression in human HCC cell lines. (A) Western blot analysis of AXIN1 protein levels in a panel of 8 human HCC cell lines (SNU-182, SNU-423, SNU-449, SNU-475, PLC/PRF/5, SNU-398, MHCC97-H, and Huh7) and 3 human hepatoblastoma cell lines (HepG2, Hep293TT, and Huh6) with *CTNNB1* mutations. **(B)** Western blot analysis of p-MET and c-MET protein levels in the 8 human HCC cell lines. GAPDH was used as a loading control.

Supplementary Figure 2. Upregulation of c-MET_UP gene signature in AXIN1 mutant human HCC samples based on the TCGA data set. (A) and (B) c-MET_UP signature was evident in human HCC samples with *AXIN1* mutations using FRY analysis. **(C)** Eleven of eighteen c-MET_UP genes were expressed at higher levels in *AXIN1* mutant human HCC when compared with surrounding liver tissues (ST).

Supplementary Figure 3. A subset of AXIN1 mutant HCC samples shows c-MET activation based on the TCGA data set. (A) c-MET signature is enriched in *AXIN1* mutant as well as *CTNNB1* mutant human HCC samples; (B) Heatmap of the human TCGA samples depicting

CTNNB1 and *AXIN1* mutations and inferred c-MET activation. ***, p<0.001.

Supplementary Figure 4. Immunohistochemical patterns of human HCC harboring *AXIN1* mutations. (A) Moderately-differentiated trabecular HCC harboring a GAG→TAG mutation at codon 443 displaying strong membranous and cytoplasmic (a sign of its activation) immunoreactivity for c-Met. (B) A solid, clear-cell HCC with a TGG→TGA mutation at codon 284 exhibiting low membranous c-Met immunolabeling. Scale bar: 100μm. Abbreviation: H&E, hematoxylin and eosin staining.

Supplementary Figure 5. Expression pattern of c-MET and its downstream target, CKS2, in human HCC samples. (A) (B) Number of cases and percentage of samples with low and high expression of c-MET. **(C) (D)** Number of cases and percentage of samples with normal and high expression of CKS2. For CKS2, “high expression” indicates at least 2-fold increase in HCC when compared with corresponding non-tumorous livers, whereas “low expression” indicates lower or equal expression of CKS2 in tumors and non-tumorous tissues.

Supplementary Figure 6. Construction and validation of CRISPR/Cas9 plasmids for Axin1 in mouse. (A) Schematic representation of

pX330-sgAxin1. Three different target sites (red) followed by the PAM sequence (green) in mouse *Axin1* gene. Short horizontal line (pink) indicates the three sgRNA target sites used here. **(B)** Western blot analysis of three kinds of lentiCRISPRv2-Axin1 (mouse) and lentiCRISPRv2-EGFP plasmid transfected into HCC3-4 and HCC4-4 cell line. **(C)** Gross and H&E images of mice livers injected with three sgAxin1 constructs individually. Magnifications: 100, scale bar: 200μm. **(D)** TCF/LEF reporter activity in HCC3-4 and HCC4-4 mouse HCC cell lines following transfection of pT3-EF1α, pT3-EF1α-β-CateninS45Y, lentiCRISPRv2-sgEgfp, and lentiCRISPRv2-sgAxin1. Data were analyzed using TopFlash/FopFlash reporter assays and are presented as mean ± SD. **, $p<0.01$; ***, $p<0.001$.

Supplementary Figure 7. Gross and H&E images of mouse livers injected with c-Met. Samples were harvested 37 weeks post injection of c-Met. Magnifications: 100, scale bar: 200μm; magnification: 200, scale bar: 100μm.

Supplementary Figure 8. Representative H&E staining. Sections of c-Met/sgAxin1 and c-Met/β-catenin tumors. Magnification: 20, scale bar: 1mm; magnification: 200, scale bar: 100μm.

Supplementary Figure 9. Molecular and biochemical analyses of c-Met/sgAxin1 mouse HCC. (A) Immunohistochemical staining of E-Cadherin in normal liver, c-Met/sgAxin1.1, c-Met/sgAxin1.2, and c-Met/sgAxin1.3 HCCs. Magnification: 200, scale bar: 100μm. **(B)** Western blot analysis of proteins downstream of c-Met. Proteins were extracted from normal liver (NL), c-Met/β-CateninS45Y and sgAxin1/c-Met tumor tissues. Gapdh was used as a loading control.

Supplementary Figure 10. Genomic sequencing and validation of Axin1 deletion in mouse HCCs. (A) Illustration of the genomic sequencing. Tumors were isolated carefully from normal liver tissue and genomic DNA was extracted. Specific primers were designed to amplify the sequences with/without sgAxin1.2 induced mutation using PCR. Subsequently, chromatograms of sequencing of the PCR products showed multi-colored peaks indicating different reads starting within the sgAxin1 locus **(B)**. Then, we employed PCR cloning to obtain different clones harboring Axin1 wild-type or mutated sequences. Through the sequencing of the plasmids collected from each individual clone, we identified several mutations caused by sgAxin1.2. **(C) (D) (E)** Representative mutations caused by sgAxin1.2.

Supplementary Figure 11. *In Situ* Hybridization (ISH) of Gs and Axin2 in c-Met/∆N90-β-Catenin, and c-Met/sgAxin1 tumors. Samples

were harvested 9 weeks p.i. Magnification: 200, scale bar: 100μm.

Supplementary Figure 12. CRISPR/Cas9 plasmids for *AXIN1* in human HCC cell lines. (A) TCF/LEF reporter activity in a panel of 5 human HCC cell lines (SNU-182, SNU-423, SNU-449, SNU-475, PLC/PRF/5) and 2 strong Wnt/β-Catenin activated human hepatoblastoma cell lines (Hep293TT and Huh6) analyzed using the TopFlash/FopFlash reporter assays. Data are presented as mean ± SD. **(B)** Schematic representation of LentiCRISPRv2-sgAXIN1. Three different target sites (red) followed by the PAM sequence (green) in human *AXIN1* gene. Short horizontal lines (pink) indicate the three sgRNA target sites used here. **(C)** Western blot analysis confirmed diminished expression of AXIN1 after transfection in HLE and HLF human HCC cell lines. GAPDH was used as a loading control. **(D)** TCF/LEF reporter activity in HLE and HLF cell lines following the transfection of lentiCRISPRv2-sgEGFP and lentiCRISPRv2-sgAxin1 analyzed using the TopFlash/FopFlash reporter assays. Data are presented as mean ± SD. **, $p<0.01$; ***, $p<0.001$.

Supplementary Figure 13. c-Met/ΔN90β-Catenin HCC vs Wild-Type: 10 most statistically significant differentially expressed gene ontologies

Supplementary Figure 14. c-Met/sgAxin1 HCC vs Wild-Type: 10 most statistically significant differentially expressed gene ontologies

Supplementary Figure 15. Akt signaling pathway is upregulated in c-Met/ΔN90β-Catenin HCC and c-Met/sgAxin1 mouse HCC samples using RNA-Seq data. (A) Akt signaling pathway is upregulated in c-Met/ΔN90β-Catenin HCC samples; **(B)** Akt signaling pathway is upregulated in c-Met/sgAxin1 HCC samples.

Supplementary Figure 16. c-Met/ΔN90β-Catenin/Cre induced HCC formation in *Ctnnb1$^{f/f}$* mouse. (A) Study design. **(B)** Gross images of livers, H&E, and immunohistochemistry (IHC) of c-Met/ΔN90-β-Catenin/Cre *Ctnnb1$^{f/f}$* mouse livers. Magnifications: 100, scale bar: 200μm; 200, scale bar: 100μm. **(C)** Western blot analysis of normal liver and c-Met/ΔN90-β-Catenin/Cre tissues in *Ctnnb1$^{f/f}$* mice as well as c-Met/ΔN90-β-Catenin tumors in FVB mice showing the absence of endogenous β-Catenin in c-Met/ΔN90-β-Catenin/Cre lesions. Gapdh was used as a loading control.

Supplementary Figure 17. Injection of c-Met/sgAxin1/pCMV and c-Met/sgAxin1/Cre in *Ctnnb1*$^{f/f}$ mice using sgAxin1.1 and sgAxin1.2. **(A)** Study design. **(B)** Survival curve of c-Met/sgAxin1.1/pCMV (n=2), c-Met/sgAxin1.1/Cre (n=3), c-Met/sgAxin1.2/pCMV (n=4), and c-Met/sgAxin1.2/Cre (n=10).

Supplementary Figure 18. Proliferation in human HCC cell lines after silencing of β-CATENIN. (A) Western blot analysis revealing the reduced expression of β- CATENIN after transfection of pLKO.1-sh-β-CATENIN in *AXIN1* mutant or null human HCC cell lines (SNU-449, SNU-475 and PLC/PRF/5). **(B)** Colony formation assays confirming the strong inhibition of proliferation following the silencing of β-CATENIN in SNU-449, SNU-475, and PLC/PRF/5 cells lines. **(C)** Western blot analysis revealing the diminished expression of β-CATENIN after transfection of pLKO.1-sh-β-CATENIN in human HCC cell lines with wild-type *AXIN1* alleles (HLF and MHCC97-H). **(D)** Colony formation assays confirming the robust suppression of proliferation due to β-CATENIN silencing in the MHCC97-H cell line, but not in HLF cells. Data are presented as mean ± SD. ***, p<0.001.

Supplementary Figure 19. Quantification of Sox9 immunohistochemistry. (A) The expression of Sox9 was significantly increased in c-

Met/sgAxin1 HCC when compared to normal liver. **(B)** The expression of Sox9 in c-Met/sgAxin1/Cre injected *Notch2*$^{fl/fl}$ mice was significantly decreased when compared to that in c-Met/sgAxin1/pCMV injected *Notch2*$^{fl/fl}$ mice. ***, Chi-square test p<0.001.

Supplementary Figure 20. Heatmap of a gene list for β-Catenin activation signature in wild-type, c-Met/sgAxin1 and c-Met/ΔN90β-Catenin HCC samples. Red, upregulated; blue, downregulated.

Supplementary Table 1. Main characteristics of the plasmids used in the experiments

Plasmid name	Abbreviation	Promoter	Gene name	Function	Tag	Reference	Vector type
pT3-EF1 α	pT3	EF1 α	N/A	Control			human
pT3-EF1 α -c-Met	c-Met	EF1 α	c-Met	Overexpression			human
pT3-EF1 α - β -CateninS45Y	β -CateninS45Y	EF1 α	β -Catenin	Overexpression	Myc		human
pT3-EF1 α - Δ N90- β -Catenin	Δ N90- β -Catenin	EF1 α	β -Catenin	Overexpression	Myc		human
pT3-EF1 α -dnRBPJ	dnRBPJ	EF1 α	RBPJ	Loss of function	V5		human
pCMV	pCMV	CMV	N/A	Control			human
pCMV-Cre	Cre	CMV	Cre recombinase	Induce expression			Universal
pCMV/SB	SB	CMV	Sleeping Beauty transposase	Induce expression			Universal
pX330						Addgene #42230	
pX330-sgAxin1.1	sgAxin1.1		Axin1	Loss of function			mouse

pX330-sgAxin1.2	sgAxin1.2		Axin1	Loss of function		mouse
pX330-sgAxin1.3	sgAxin1.3		Axin1	Loss of function		mouse
pX330-sgAXIN1.1	sgAXIN1.1		AXIN1	Loss of function		human
pX330-sgAXIN1.2	sgAXIN1.2		AXIN1	Loss of function		human
pX330-sgAXIN1.3	sgAXIN1.3		AXIN1	Loss of function		human
pRL-CMV		CMV	Renilla Luciferase	Induce expression	Promega E2231	
lentiCRISPRv2			Cas9		Addgene #98290	Lentiviral
lentiCRISPRv2-sgAxin1			Axin1	Loss of function		Lentiviral
shEgfp/pLKO.1			EGFP	Control		
shβ-Catenin/pLKO.1			β-Catenin	Loss of function	Addgene #18803	
Super 8x TopFlash					Addgene #12456	
Super 8x FopFlash					Addgene #12457	

Supplementary Table 2. Clinicopathological features of HCC Patients

Variables	Features	
	HCCB[a]	HCCP[b]
No. of patients	49	54
Male	45	48
Female	4	6
Age (Mean ± SD)	55.8 ± 12.4	54.2 ± 14.5
Etiology		
HBV	26	25
HCV	13	14
Ethanol	7	9
Wilson disease	2	2
Hemochromatosis	0	1
NA	1	2
Cirrhosis		
+	40	46
-	9	8
Tumor size		
> 5 cm	24	36
< 5 cm	25	18
Edmondson and Steiner grade		
I	6	4
II	15	12
III	14	18
IV	14	20
Serum alpha-fetoprotein level (ng/ml)		
> 300	19	20
< 300	28	33
NA	1	1
Survival after partial liver resection (months)		
Means ± SD[c]	66.8 ± 26.4	14.4 ± 8.7
β-Catenin (*CTNNB1*) mutation		
Present	11	9
Absent	38	50

Axin1 mutation		
Present	2	9
Absent	47	50
C-Met expression		
High	16	21
Low	33	38
CKS2 mRNA levels (compared to SL)		
High (2 fold higher than SL)	14	24
Low (equal or lower than SL)	35	35

[a]HCCB, HCC with better prognosis (survival longer than 3 years)

[b]HCCP, HCC with poorer prognosis (survival shorter than 3 years)

[c]$P < 0.0001$

Abbreviations: NA, not available; SL, surrounding non-tumorous tissue.

Supplementary Table 3. List of mice used for the experiments

Directory	Mice breed	Plasmid injected	Sample size (n)
Figure 1	FVB/N	sgAxin1.1(40μg)	10
		sgAxin1.2(40μg)	7
		sgAxin1.3(40μg)	6
		c-Met (20μg)	10
		sgAxin1.1(40μg)+c-Met(20μg)+SB(0.8μg)	10
		sgAxin1.2(40μg)+c-Met(20μg)+SB(0.8μg)	7
		sgAxin1.3(40μg)+c-Met(20μg)+SB(0.8μg)	6
		β-cateninS45Y(20μg)+c-Met(20μg)+SB(1.6μg)	4
Figure 2	FVB/N	sgAxin1.2(40μg)+c-Met(20μg)+SB(0.8μg)	As Figure 1
		β-cateninS45Y(20μg)+c-Met(20μg)+SB(1.6μg)	As Figure 1
		ΔN90-β-catenin(20μg)+c-Met(20μg)+SB(1.6μg)	4
Figure 3	FVB/N	sgAxin1.2(40μg)+c-Met(20μg)+SB(0.8μg)	As Figure 1
		ΔN90-β-catenin(20μg)+c-Met(20μg)+SB(1.6μg)	As Figure 2
Figure 4	*Ctnnb1*$^{+/+}$	sgAxin1.2(40μg)+c-Met(20μg)+Cre(60)+SB(3.2μg)	10
	Ctnnb1$^{flox/flox}$	sgAxin1.2(40μg)+c-Met(20μg)+Cre(60)+SB(3.2μg)	10
Figure 5	FVB/N	sgAxin1.2(40μg)+c-Met(20μg)+SB(0.8μg)	As Figure 1
Figure 6	*Notch2*$^{flox/flox}$	sgAxin1.2(40μg)+c-Met(20μg)+pCMV(60)+SB(3.2μg)	6
		sgAxin1.2(40μg)+c-Met(20μg)+Cre(60)+SB(3.2μg)	9

Figure 7	FVB/N	sgAxin1.2(40μg)+c-Met(20μg)+pT3(60)+SB(3.2μg)	6
		sgAxin1.2(40μg)+c-Met(20μg)+dnRBPJ(60)+SB(3.2μg)	4
Sup Figure 6	FVB/N	sgAxin1.1(40μg)	As Figure 1
		sgAxin1.2(40μg)	As Figure 1
		sgAxin1.3(40μg)	As Figure 1
Sup Figure 8	FVB/N		As Figure 1
		sgAxin1.2(40μg)+c-Met(20μg)+SB(0.8μg)	
		ΔN90-β-catenin(20μg)+c-Met(20μg)+SB(1.6μg)	As Figure 2
Sup Figure 13	*Ctnnb1*$^{flox/flox}$	ΔN90-β-catenin(20μg)+c-Met(20μg)+Cre(60)+SB(4μg)	5
Sup Figure 14	*Ctnnb1*$^{flox/flox}$	sgAxin1.1(40μg)+c-Met(20μg)+pCMV(60)+SB(3.2μg)	2
		sgAxin1.1(40μg)+c-Met(20μg)+Cre(60)+SB(3.2μg)	3
		sgAxin1.2(40μg)+c-Met(20μg)+pCMV(60)+SB(3.2μg)	4
		sgAxin1.2(40μg)+c-Met(20μg)+Cre(60)+SB(3.2μg)	As Figure 4

Supplementary Table 4. List of antibodies used for immunohistochemistry

Antibody	Catalogue Number	Company	Species	Dilution
β -Catenin	Bd 610153	BD Biosciences	Mouse	1:200
Glutamine Synthetase	Bd 610518	BD Biosciences	Mouse	1:500
V5 TAG	R96025	Thermo Fisher scientific	Mouse	1:400
Ki-67	MA5-14520	Thermo Fisher scientific	Rabbit	1:150
SOX9	Ab185230	Abcam	Rabbit	1:2000
c-Met	Ab227637	Abcam	Rabbit	1:400
E-cadherin	02660	BD Transduction Laboratories	Mouse	1:200

Supplementary Table 5. List of antibodies used for Western blot analysis

Antibody	Catalogue Number	Company	Species	Dilution
β-Catenin	Bd 610153	BD Biosciences	Mouse	1:2000
Glutamine Synthetase	Bd 610518	BD Biosciences	Mouse	1:5000
GAPDH	AB2302	EMD Millipore	Mouse	1:10000
β-Actin	A5441	Sigma-Aldrich	Mouse	1:5000
V5 TAG	R96025	Thermo Fisher scientific	Mouse	1:5000
c-Met	71-8000	Invitrogen	Rabbit	1:400
Phospho-Met (Y1234/1235)	3077	Cell Signaling Technology	Rabbit	1:400
Cyclin D1	ab134175	Abcam	Rabbit	1:20000
c-Myc	ab32072	Abcam	Rabbit	1:10000
β-Tubulin	ab6046	Abcam	Rabbit	1:10000
Jagged1	Ab109536	Abcam	Rabbit	1:1000
SOX9	Ab185230	Abcam	Rabbit	1:5000

Axin1	2087	Cell Signaling Technology	Rabbit	1:1000
Phospho-β-Catenin (Ser552)	9566	Cell Signaling Technology	Rabbit	1:1000
Phospho-β-Catenin (Ser675)	9567	Cell Signaling Technology	Rabbit	1:1000
Phospho-β-Catenin (Ser33/37/Thr41)	9561	Cell Signaling Technology	Rabbit	1:1000
Phospho-β-Catenin (Ser45)	9564	Cell Signaling Technology	Rabbit	1:1000
Phospho-β-Catenin (Thr41/Ser45)	9565	Cell Signaling Technology	Rabbit	1:1000
Non-phospho-β-Catenin (Ser33/37/Thr41)	4270	Cell Signaling Technology	Rabbit	1:1000
Akt	9272	Cell Signaling	Rabbit	1:1000

		Technology		
Phospho-Akt (Ser473)	4060	Cell Signaling Technology	Rabbit	1:1000
Phospho-Akt (Thr308)	9275	Cell Signaling Technology	Rabbit	1:1000
p44/42 MAPK (Erk1/2)	9102	Cell Signaling Technology	Rabbit	1:1000
Phospho-p44/42 MAPK (Erk1/2) (Thr202/Tyr204)	4370	Cell Signaling Technology	Rabbit	1:1000
Flag Tag	14793	Cell Signaling Technology	Rabbit	1:1000
Notch2	5732	Cell Signaling Technology	Rabbit	1:10000
Histone H3	4499	Cell Signaling Technology	Rabbit	1:2000

Supplementary Table 6. List of primer pairs used in qRT-PCR analysis

Gene name	Forward	Reverse
18s rRNA	CGGCTACCACATCCAAGGAA	GCTGGAATTACCGCGGCT
Mouse-AXIN2	GCTCCAGAAGATCACAAAGAGC	AGCTTTGAGCCTTCAGCATC
Mouse-LGR5	ACCGAGCCTTACAGAGCCT	GCCGTCGTCTTTATTCCATTGG
Mouse-c-Myc	TGTACCTCGTCCGATTCC	CATCTTCTTGCTCTTCTTCAG
Mouse-CyclinD1	CGTGGCCTCTAAGATGAAGGA	CCTCGGGCCGGATAGAGTAG
Mouse-GS	CAGGCTGCCATACCAACTTCA	TCCTCAATGCACTTCAGACCAT
Mouse-LECT2	CCCACAACAATCCTCATTTCAGC	ACACCTGGGTGATGCCTTTG
Mouse-TBX3	CAGGCAGCCTTCAACTGCTT	GGACACAGATCTTTGAGGTTGGA
Mouse-OAT	GGAGTCCACACCTCAGTCG	CCACATCCCACATATAAATGCCT
Mouse-AFP	TCTGCTGGCACGCAAGAAG	TCGGCAGGTTCTGGAAACTG
Mouse-GPC3	CAGCCCGGACTCAAATGGG	CAGCCGTGCTGTTAGTTGGTA
Mouse-Hey1	GCGCGGACGAGAATGGAAA	TCAGGTGATCCACAGTCATCTG
Mouse-Axin1	AGTCCACCATGGAGGAGAAT	CCATAACTCAACAGCAGCTG

Supplementary Table 7. List of the cell lines used for the experiments

Cell lines	Cell line type	Order Information	Catalog Number	Culture Condition
HCC3-4	Mouse HCC	Dr. Dean Felsher (1)	N/A	DMEM+10%FBS+1%Pen/Strep
HCC4-4	Mouse HCC	Dr. Dean Felsher (1)	N/A	DMEM+10%FBS+1%Pen/Strep
Hep40	Human HCC	Dr. Brian Carr (2)	N/A	DMEM+10%FBS+1%Pen/Strep
HLE	Human HCC	JCRB Cell Bank	JCRB0405	DMEM+10%FBS+1%Pen/Strep
HLF	Human HCC	JCRB Cell Bank	JCRB0404	DMEM+10%FBS+1%Pen/Strep
MHCC97H	Human HCC	Dr. Binbin Liu (3)	N/A	DMEM+10%FBS+1%Pen/Strep
Huh7	Human HCC	JCRB Cell Bank	JCRB0403	DMEM+10%FBS+1%Pen/Strep
PLC/PRF/5	Human HCC	ATCC	CRL-8024	DMEM+10%FBS+1%Pen/Strep
SNU-398	Human HCC	ATCC	CRL-2233	DMEM+10%FBS+1%Pen/Strep
SNU-449	Human HCC	ATCC	CRL-2234	DMEM+10%FBS+1%Pen/Strep
SNU-475	Human HCC	ATCC	CRL-2236	DMEM+10%FBS+1%Pen/Strep
SNU-423	Human HCC	ATCC	CRL-2238	RPMI 1640+10%FBS+1%Pen/Strep
SNU-182	Human HCC	ATCC	CRL-2235	RPMI 1640+10%FBS+1%Pen/Strep
HepG2	Human hepatoblastoma	ATCC	HB-8065	DMEM+10%FBS+1%Pen/Strep
Hep293TT	Human hepatoblastoma	Dr. Gail Tomlinson	N/A	RPMI 1640+25mM HEPES+10%FBS+1%Pen/Strep

(4)

Huh6	Human hepatoblastoma	JCRB Cell Bank	JCRB0401	DMEM+10%FBS+1%Pen/Strep

1: PubMed ID: 21262914
2: PubMed ID: 7590653
3: PubMed ID: 10555751
4: PubMed ID: 19637320

Materials and methods

Constructs and reagents

The plasmids used for mouse injection are reported in Supplementary Table 1. pT3-EF1α, pT3-EF1α-c-Met, pT3-EF1α-β-cateninS45Y, pT3-EF1α-Δ90-β-catenin, pT3-EF5α-dnRBPJ (with N-terminal V5 tag), pT3-EF5α-dnTCF4(with N-terminal Flag tag), pCMV, pCMV-Cre and pCMV/sleeping beauty transposase (SB) have been described in our previous publications (1-3). dnTCF4, dnRBPJ were cloned into pLenti-puro via the Gateway PCR cloning strategy (Invitrogen, Carlsbad, CA). To delete Axin1 while co-expressing c-Met into the mouse liver and delete Axin1 in human HCC cell line, we constructed pX330 plasmids expressing mouse Cas9 and single-guide RNAs (sgRNAs) against the mouse Axin1(NM_001159598.1). We constructed lentiCRISPRv2 plasmids expressing human or mouse Cas9 and sgRNAs against the mouse Axin1, human Axin1(NM_003502.3), EGFP(YP_009062989.1). pX330-U6-Chimeric_BB-CBh-hSpCas9 (pX330) and lentiCRISPRv2 puro were obtained from Addgene (Addgene plasmid #42230, #98290). pX330 and lentiCRISPRv2-puro were digested with BbsI and BsmBI, respectively, and ligated with annealed oligos. Twenty-nucleotide sequences followed by the PAM sequence were used as seed sequences for sgRNA. An extra G is added for sgRNAs lacking a 5' G for U6 transcriptional initiation (4, 5). For silencing of β-catenin in human HCC cell line, pLKO.1 puro shRNA β-catenin was obtained from Addgene (Addgene plasmid #18803), empty vector pLKO.1 puro as control was also from Addgene (Addgene plasmid #8453). Super 8x TopFlash and Super 8x FopFlash plasmids were obtained from Addgene (plasmid #12456, plasmid #12457), and pRL-CMV Renilla luciferase plasmid was purchased from Promega (Madison, WI). Before use, all the plasmids were purified using the Endotoxin free Maxi prep kit (Sigma-Aldrich, St. Louis, MO).

Human liver tissue specimens

A collection of frozen HCC samples (n=103) was used in the present study. Tumors were divided in HCC with shorter survival/poorer prognosis (HCCP; n=54) and longer survival/better prognosis

(HCCB; n=49), characterized by <3 and >3 years' survival following partial liver resection, respectively. The clinicopathological features of liver cancer patients are summarized in Supplementary Table 2. HCC specimens were generously provided by Dr. Snorri S. Thorgeirsson (National Institutes of Health, National Cancer Institute, Bethesda, MD). Institutional Review Board approval was obtained at the National Institutes of Health and the local Ethical Committee of the Medical University of Greifswald. Informed consent was obtained from all individuals.

Mice and hydrodynamic tail injection

We obtained wild-type (WT) FVB/N, *Ctnnb1*$^{+/+}$, *Ctnnb1*$^{fl/fl}$ and *Notch2*$^{fl/fl}$ mice from The Jackson Laboratory (Sacramento, CA). To establish the sgAxin1/c-Met-induced HCC model, we performed hydrodynamic injection of plasmid DNAs into the tail vein of 6- to 8-week-old mice following a published protocol (6). Sleeping beauty(SB) transposase hydrodynamic tail vein injection was performed as described (6). For the tumorigenesis models, mice received 40μg pX330-Axin1.1 or pX330-Axin1.2 or pX330-Axin1.3 mixed with 20μg pT3-EF1α-c-Met (human) along with 0.8μg pCVM/SB in 2ml of normal saline (0.9%NaCl). To determine whether or not sgAxin1/c-Met induced HCC development depended on intact β-catenin signaling and Notch2 cascade in mice, *Ctnnb1*$^{fl/fl}$ mice and *Ctnnb1*$^{+/+}$ mice were injected with 40μg pX330-Axin1.2, 20μg pT3-EF1α-c-Met, 60μg pCMV-Cre together with pCMV-SB (3.2 μg). *Notch2*$^{fl/fl}$ mice were given same dose of plasmid as mentioned above，while 60μg pCMV as vector control was delivered into mice. For FVB/N mice, we injected 40μg pX330-Axin1.2, 20μg pT3-EF1α-c-Met, 60μg dominant negative TCF4(dnTCF4)-pT3-EF5α or 60μg dominant negative RBPJ(dnRBPJ)-pT3-EF5α, pT3-EF1α as vector control together with pCMV-SB (3.2 μg). The solution was filtered through 0.22μm filter (EMD Millipore, Burlington, MA). Mice were housed, fed, and monitored in accord with protocols approved by the Committee for Animal Research at the University of

California San Francisco (San Francisco, CA). All mice were monitored for signs of morbidity or discomfort. Pay close attention to the abdominal girth. Mice were sacrificed at indicated time points or when they became moribund. The body weight, liver weight and liver tumor size were measured for each mouse. A complete list of the mice used in this study is provided in Supplementary Table 3.

Hematoxylin-Eosin (H&E) staining and Immunohistochemistry (IHC)

Mouse and human liver tissues were fixed in 4% paraformaldehyde overnight at 4°C, then processed and embedded in paraffin. Tissue sections of 5 μm thickness were cut from paraffin blocks and used for Hematoxylin & Eosin (H&E) staining and immunohistochemistry. H&E staining was performed to determine the time of appearance and characteristics of neoplastic foci. For immunohistochemistry, the slides were put through de-paraffinization, antigen retrieval was performed in 10mM sodium citrate buffer (pH 6.0) by placement in a microwave oven on high for 10 min, then cooling down for 20 minutes at room temperature. After a blocking step with the 5% goat serum and Avidin-Biotin blocking kit (Vector Laboratories Inc., Burlingame, CA), the sections were incubated with the primary antibodies (see Supplementary Table 4) overnight at 4°C. Slides were then subjected to 3% hydrogen peroxide for 10 min to quench endogenous peroxidase activity and subsequently the secondary antibody was applied at a 1:500 dilution for 30 min at room temperature. Signal was detected using the Vectastain ABC Elite kit (Vector Laboratories Inc.) and developed using DAB (Vector Laboratories, Inc.). Sections were counterstained with hematoxylin solution (ThermoFisher Scientific, Pittsburgh, PA) and passed through the dehydration process and covered-slipped.

Protein isolation and Western blot analysis

Liver tissue were homogenized with a Polytron and cells were washed in PBS and lysed in M-PER™ Mammalian Protein Extraction Reagent (ThermoFisher Scientific) containing the Halt™

Protease Inhibitor Cocktail (ThermoFisher Scientific). Protein concentration was quantified using the Pierce™ Microplate BCA Protein Assay Kit (ThermoFisher Scientific). Membranes were blocked with 5% (w/v) non-fat milk in Tris-buffered saline containing 0.05% Tween-20 at room temperature for 1 hour and then incubated with the primary antibodies (see Supplementary Table 5) at 4°C overnight, followed by incubation with horseradish peroxidase-conjugated goat anti-rabbit or goat anti-mouse secondary antibody (1:5000; Jackson ImmunoResearch Laboratories Inc., West Grove, PA) for 1 hour at room temperature. After appropriate washing, membranes were developed with the Super Signal West Dura Kit (ThermoFisher Scientific). Experiments were replicated three times.

Quantitative real-time reverse-transcription polymerase chain reaction

Total RNAs were extracted from frozen mouse tissue samples using the Quick-RNA™ MiniPrep (Zymo Research, Irvine, CA), The preparation of cDNA was carried out prior to real-time PCR (q-PCR) by reverse transcription of purified RNA according to the manufacturer's instructions (Invitrogen, Carlsbad, CA). The primers used for PCR analysis were synthesized by Integrated DNA Technologies (Coralville, IA). The sequences of the primers are listed on Supplementary Table 6. Amplification was done with 100 ng of cDNA using an ABI Prism 7000 Sequence Detection System and TaqMan Universal PCR Master Mix (ThermoFisher Scientific). Cycling conditions were: 10 min of denaturation at 95°C, and 40 cycles at 95°C for 15 s and at 52°C for 1 min. Quantitative values were calculated by using the PE Biosystems Analysis software and expressed as N target (NT). NT = 2-ΔCt, the ΔCt value of each sample was calculated by subtracting the maximum Ct value of the target gene from the average Ct value of the rRNA gene. To analyze *CKS2* expression in human HCC samples, Gene Expression Assays for human *CKS2* (ID # Hs00829071_s1) and β-Actin (ID # 4333762T) genes were purchased from Applied Biosystems (Foster City, CA, USA). Quantitative values were calculated by using the PE Biosystems Analysis software and expressed as Number target (NT). NT = 2-ΔCt, wherein ΔCt

value of each sample was calculated by subtracting the average Ct value of the *CKS2* gene from the average Ct value of the *β-Actin* gene.

Mouse and human tumor genomic DNA extraction and sequencing

Mouse genomic DNA was extracted from frozen mouse tissue samples using the Mouse Direct PCR Kit, according to the manufacturer's instructions (Biotools, Jupiter, FL). The primers used for PCR amplification were synthesized by Integrated DNA Technologies (Coralville, IA). The sequences of the primers are listed in Supplementary Table 6. Amplification was carried out using Gene Amp PCR System 2700. Cycling conditions were: 5 min of denaturation at 94°C and 35 cycles at 94°C for 20 s, at 49°C for 30s, and at 72°C for 30s. PCR products were purified and sequenced. For individual clonal sequencing, we inserted the purified PCR amplification product to the pJET1.2/blunt cloning vector according to the manufacturer's instructions (ThermoFisher Scientific). Individual clones were cultured and plasmids were purified using Zyppy Plasmid Miniprep kit (Genesee Scientific). The inserted sequence was subsequently sequenced using T7 primers.

Human DNA was extracted from frozen human liver tissues using the DNeasy Blood & Tissue Kit (Qiagen Inc., Germantown, MD) following the manufacturer’s instructions. PCR amplifications were performed using genomic DNA from 103 human HCC samples using a previously described set of primers for *CTNNB1* and *AXIN1* genes(7). PCR products were electrophoresed, excised, and purified from 2% TAE-agarose gel using the QIAEX II gel extraction kit (Qiagen, Inc.), and sequenced following a previously established protocol (7).

***In situ* hybridization**

In situ hybridization was performed as described (8), using RNAscope 2-plex Detection Kit according to the manufacturer’s instructions (Advanced Cell Diagnostics, Newark, CA).

Cell lines and culture

Two mouse HCC cell lines (HCC3-4 and HCC4-4), 10 human HCC cell lines (SNU-398, SNU-423, SNU-182, SNU-475, SNU-449, PLC/PRF/5, HLE, HLF, Huh7, and MHCC-97H), and 3 human hepatoblastoma cell lines (HepG2, Hep293TT, and Huh6) were used in the study. The HCC3-4 and HCC4-4 cell lines were kindly provided by Dr. Felsher of Stanford University. Hep293TT cell line was provided to us by Dr. Gail Tomlinson from the University of Texas Southwestern Medical Center. SNU-398, SNU-423, SNU-182, SNU-475, SNU-449, HepG2, and PLC HCC cells were obtained from ATCC (Manassas, VA). HLE, HLF, Huh7, and Huh6 cells were obtained from JCRB Cell Bank (Osaka, Japan). MHCC-97H cells are a gift from Dr Binbin Liu. All cell line information is included in Supplementary Table 7. HCC3-4, HCC4-4, HLE, HLF, Huh7, Huh6, MHCC-97，HepG2, and PLC HCC cell lines were cultured in Dulbecco's modified Eagle medium (DMEM) supplemented with 10% FBS, penicillin (100 U/mL), and streptomycin (100 µg/mL). SNU-475, SNU-449, SNU-182, SNU-423, and SNU-398 HCC cells were instead cultured in Roswell Park Memorial Institute 1640 (RPMI 1640) medium supplemented with 10% FBS, penicillin (100 U/mL) and streptomycin (100 µg/mL). Hep293TT cell lines were cultured in RPMI 1640 medium, 25mM Hepes, 10% FBS, penicillin (100 U/mL) and streptomycin (100 µg/mL). All cell lines were cultured at 37°C in a humidified 5% CO2 incubator and underwent validation before being used in the experiments (Genetica DNA Laboratories, Burlington, NC).

Lentiviral transduction

HEK293T cells were obtained from the American Type Culture Collection (Manassas, VA, USA) and were seeded into 6-wells. When cells reached 50% confluence, 2µg objective plasmid and 2µg lentivirus mix were co-transfected into HEK293T cells by 8ul Lipofectamine 2000 reagents (Invitrogen) with 500µl Opti-MEM medium. After 24h, the medium was replaced with fresh

DMEM containing 30% fetal bovine serum. After incubation for additional 24-48 hour at 37°C, the viral supernatant was harvested and filtered through a 0.45-mm filter (Millipore, Bedford, MA, USA), and then HCC3-4, HCC4-4, HLE, and HLF cells were infected with lentivirus plasmid at the volume ratio 1:1. Seventy-two hours post-infection, cells were treated with different concentrations of puromycin (2μg/ml for HCC3-4, HCC4-4, HLE, SNU-475 and 5μg/ml for HLF, SNU-449, and 30μg/ml SNU-182) to select cells containing sgAixn1(mouse)-pLentiCRISPRv.2, sgAxin1(human)-pLentiCRISPRv.2, pLKO.1-sh-β-catenin, and pLenti-dnTCF4. Above cells infected with the empty sgEGFP-pLentiCRISPRv.2 or pLKO.1 or pLenti-EGFP vector were used as control.

Colony formation assay

For colony forming assay, SNU-475, SNU-449，SNU-182，and PLC/PRF/5 cells transfected with corresponding lentivirus were plated in 6-well culture plates at a density of 1×10^3 and 0.5×10^3 cells per well, respectively, in triplicate. Two weeks later, colonies were stained with crystal violet and then counted for quantification.

Dual-Luciferase Reporter Assay

HCC3-4, HCC4-4, HLE, HLF, Huh6, Hep293TT, SNU-449, SNU-182, SNU-423, SNU-475, PLC/PRF/5 cells were plated in triplicate in 24-well plate at 70-80% confluency. Cells were transfected using the Lipofectamine 2000 reagents (Invitrogen). In brief, both HCC3-4 and HCC4-4 were transfected with 600ng of pT3-EF1α (empty vector control) or pT3-EF1α-β-cateninS45Y together with 200ng of TOPFlash plasmid DNA or the negative control FOPFlash, as well as 8ng of pRL-CMV. Each of sgAxin1(mouse)-HCC3-4, sgEGFP-HCC3-4, sgAxin1(mouse)-HCC4-4, sgEGFP-HCC4-4, sgAxin1(human)-HLE, sgEGFP-HLE, sgAxin1(human)-HLF, sgEGFP-HLF and Huh6, Hep293TT, SNU-449, SNU-182, SNU-423,

SNU-475, and PLC cells were transfected with 400ng of TOPFlash plasmid DNA or the negative control FOPFlash, as well as 16ng of pRL-CMV. Cells were harvested 48 hours post transfection. Luciferase activity was measured using the Dual-Luciferase® Reporter Assay System (Promega), according to the manufacturer's protocol. Experiments were repeated at least three times in triplicate.

RNAseq experiment

Total RNA was extracted from normal and liver tumor tissues using the Quick RNA Mini-Prep Kit (Genesee Scientific, El Cajon, CA). Total RNA was submitted to Novogene (Beijing, China). RNA quantification, library preparation, and sequencing were performed by Novogene. The count data are available as Supplementary Data 1.

RNAseq analysis

All analyses were performed in R. Experimental design had 3 groups: "Bcat"(β-Catenin Activation mutation), "sgAxin"(Axin1 deletion), "WT"(Wild-Type) and each group had 4 samples. Gene read counts were in Ensembl Gene ID and converted to Entrez Gene ID. Corresponding Symbol annotations and full gene names were added using the "org.Mm.eg.db" library. NA (Not Annotated), duplicate Entrez IDs and genes without symbols were removed. Only the genes having CPM values above 0.5 in at least two libraries were kept. Normalization by TMM (Trimmed mean of M values) was performed by using *calcNormFactors* function to eliminate composition biases between libraries. Three comparison analyses were conducted between Bcat vs WT, sgAxin vs WT and sgAxin vs Bcat. For identifying differentially expressed genes, R package "edgeR" and *glmTreat* function were used in all three comparisons. Differentially expressed genes were limited by a *p* value of 0.05 and FDR (False Discovery Rate) of 0.05. Genes were mapped to Gene Ontologies using GO.db package, *goana* function which is based on Gene Ontology Consortium. Analysis of GO:0016055 (Wnt Signaling Pathway), GO:0007219 (Notch

Signaling Pathway) and GO:0035329 (Hippo Signaling Pathway) was conducted for each comparison using the *FRY* gene set test. The Fry gene set test is a fast approximation to Roast gene set test proposed by Wu *et al* (9), which performs self-contained gene set test (10). It tests whether any of the genes in the set are differentially expressed and can be used for any expression data which could be represented by a linear model. Total numbers of genes involved, Number of up-regulated genes, Number of down-regulated genes, P value, and Direction of Regulation were obtained for each Gene Ontology analysis. List of differentially expressed genes for each Gene Ontology was created for all the comparisons by *p* value of 0.05 and FDR of 0.05. Genes were mapped to KEGG Pathways using GO.db package, *kegga* function which obtains the KEGG annotations from http://rest.kegg.jp website.

Human Data HCC TCGA Retrieval and Analysis

To investigate the relationship with c-MET activation and AXIN1 or CTNNB1 mutation status in human HCC samples, TGCG data set were retrieved based on frozen on 2/25/2015. The overall sample size is 430, including 59 surrounding non-tumor liver tissues (ST) from both TCGA-LIHC and TCGA-CHOL database and 371 primary HCCs from LIHC database. *AXIN1* and *CTNNB1* mutation information was extracted. Among the 371 HCCs, 24 harbor *AXIN1* mutations and 96 possess *CTNNB1* mutations. RNA sequencing data were also extracted and analyzed in R. EdgeR package (11) was employed to read gene counts. Data were normalized using *calcNormFactors* function to eliminate composition biases between libraries. Gene symbol annotations and full gene names were added using the “org.Hs.eg.db” library (12). For c-MET activation status, we extracted genes from the “KAPOSI_LIVER_CANCER_MET_UP” gene set, which contains 18 genes which were upregulated in liver cancer samples in response to c-MET activation. *FRY* gene set test was applied to investigate the enrichment of the c-MET_UP genes in HCC samples. Mann-Whitney test was used for comparison of different gene expression between the ST and *AXIN1* mutated groups. And Fisher’s exact test was employed to compare

the difference in composition of samples with c-MET up signature in ST, *AXIN1* mutated and *CTNNB1* mutated groups. Heatmap was generated using Complex heatmap package (13) in R. We standardized the data with mean as 0 and standard deviation (SD) as 1 and ordered by ascending Average of 18-gene expression of each sample from left to right. As the weight of each gene is 1 in this gene set, samples with Averages more than the Average plus 1.5-fold SD of the ST group was considered as HCC with “c-MET activation”. Tissue types and mutation information was also included in the heatmap.

Statistical analysis

The Prism 7.0 software (GraphPad, San Diego, CA) was used to analyze the data. Statistical analysis was performed using Student’s t-test and Tukey-Kramer test. The data were expressed as the mean±SD of at least three independent experiments. $P < 0.05$ was considered significant.

References:

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