Supplementary Figures:

Figure S1:

Genotyping of *Rassf1a* and *Sav1* mutant mice.

A. *Rassf1a* knockout mice lack exon 1 of the *Rassf1* gene. A diagram and typical PCR genotyping of wildtype and *Rassf1a*$^{-/-}$ mice is shown.

B. *Sav1* knockout mice lack exons 1 and 2 of the *Sav1* gene. A diagram and typical PCR genotyping of wildtype and *Sav1*$^{+/-}$ mice is shown.

Figure S2:

Liver from a *Sav1*$^{+/+}$ *Rassf1a*$^{-/-}$ mouse.

The image shows a typical hepatocellular carcinoma with characteristic trabecular growth pattern. H&E.

Figure S3:

Liver from a *Sav1*$^{+/-}$ *Rassf1a*$^{-/-}$ mouse.

The image shows hyperplasia of oval cells around a vein thought to be a central vein (c). These cells are sometimes arranged to form small, duct-like structures (arrows). H&E.

Figure S4:

Liver size and weight of gene-targeted mice.

A. Representative photographs of livers of 6 month-old mice of the different genotypes. a, *Rassf1a*$^{+/+}$ *Sav1*$^{+/+}$, b, *Rassf1a*$^{-/-}$ *Sav1*$^{+/+}$, c, *Rassf1a*$^{+/+}$ *Sav1*$^{+/-}$, and d, *Rassf1a*$^{-/-}$ *Sav1*$^{+/-}$ mice.

B. Liver to body weight ratios are shown as bar graphs (+/- S.D.). We analyzed four livers of each genotype at six months of age.

Figure S5:

Gene ontology analysis of differentially expressed genes using the DAVID tool.

A. Genes differentially expressed in *Rassf1a*$^{-/-}$ *Sav1*$^{+/+}$ mice relative to wildtype mice.

B. Genes differentially expressed in *Rassf1a*$^{-/-}$ *Sav1*$^{+/-}$ mice relative to wildtype mice.

Figure S6:

DNA methylation analysis of the *RASSF1A* and *SAV1* genes in human liver cancer specimens.

A. *RASSF1A* promoter. DNA from human liver cancer samples and adjacent normal tissue was treated with sodium bisulfite. The target gene promoter CpG island was amplified by PCR and the PCR products were digested with BstUI, which cleaves only unconverted, methylated DNA at 5'CGCG sequences. Cleavage indicates methylation. The samples 20y M, 83y F, and 53y M were DNAs from male and female liver samples of individuals without liver tumor. The positive control is an in vitro methylated DNA sample.

B. *SAV1* promoter. The sequences were cleaved with TaqI, which cleaves only methylated DNA at 5'TCGA sequences.

Figure S7:

DNA methylation analysis of the *Mst1* and *Mst2* genes in human liver cancer specimens.

The DNA methylation status was analyzed by COBRA assays. BstUI was used as the cleavage enzyme to indicate methylation at 5'CGCG sequences in the promoter CpG islands of the *MST1* and *MST2* genes.

A. *MST1* promoter.

B. *MST2* promoter.

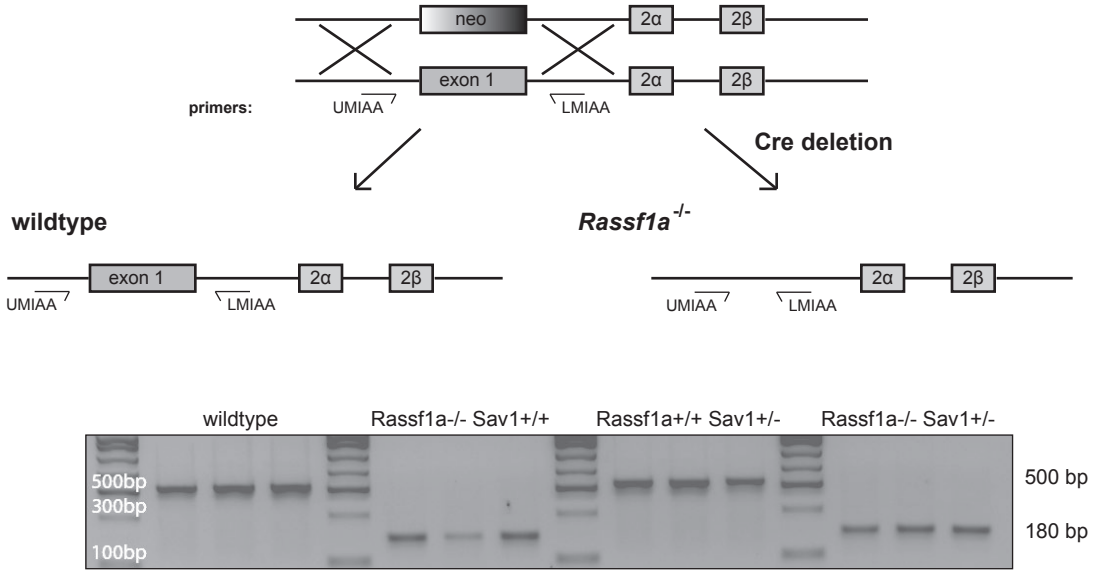
Figure S8:

Methylation and expression of *MST1* and *MST2* in human HCC.

The data were extracted from TCGA data portal (http://cancergenome.nih.gov/). Expression (RNA-seq) and DNA methylation (Illumina 450k array) data were displayed for individual specimens (each column). The methylation data are plotted for individual CpG sites along the locus (each row). White bars show samples lacking expression data. The positions of the promoter-associated CpG islands are indicated by vertical bars. **A.** *MST1* locus. **B.** *MST2/STK3* locus.

A

Rassf1a



B

Sav1

Rassf1a primers:
UMIAA: 5' TTG TGC CGT GCC CCG CCC A 3'
LMIAA: 5' TGA CCA GCC CTC CAC TGC CGC 3' locates at intron 1
PCR products: wt 500 bp; knockout 180 bp.

Sav1 primers:
LP: 5'-ACT ACC AGC AAG CAG GAG GA-3' locates at intron 2
SCRU1: 5'-CAA TAT GCA GCA TCC ATT CG-3' locates at intron 1
CreU2: 5'-CTC TGG TGC TCA AAG GCT TC-3' locates outside of exon 1
PCR products: wt 847 bp; knockout 650 bp.

Figure S1

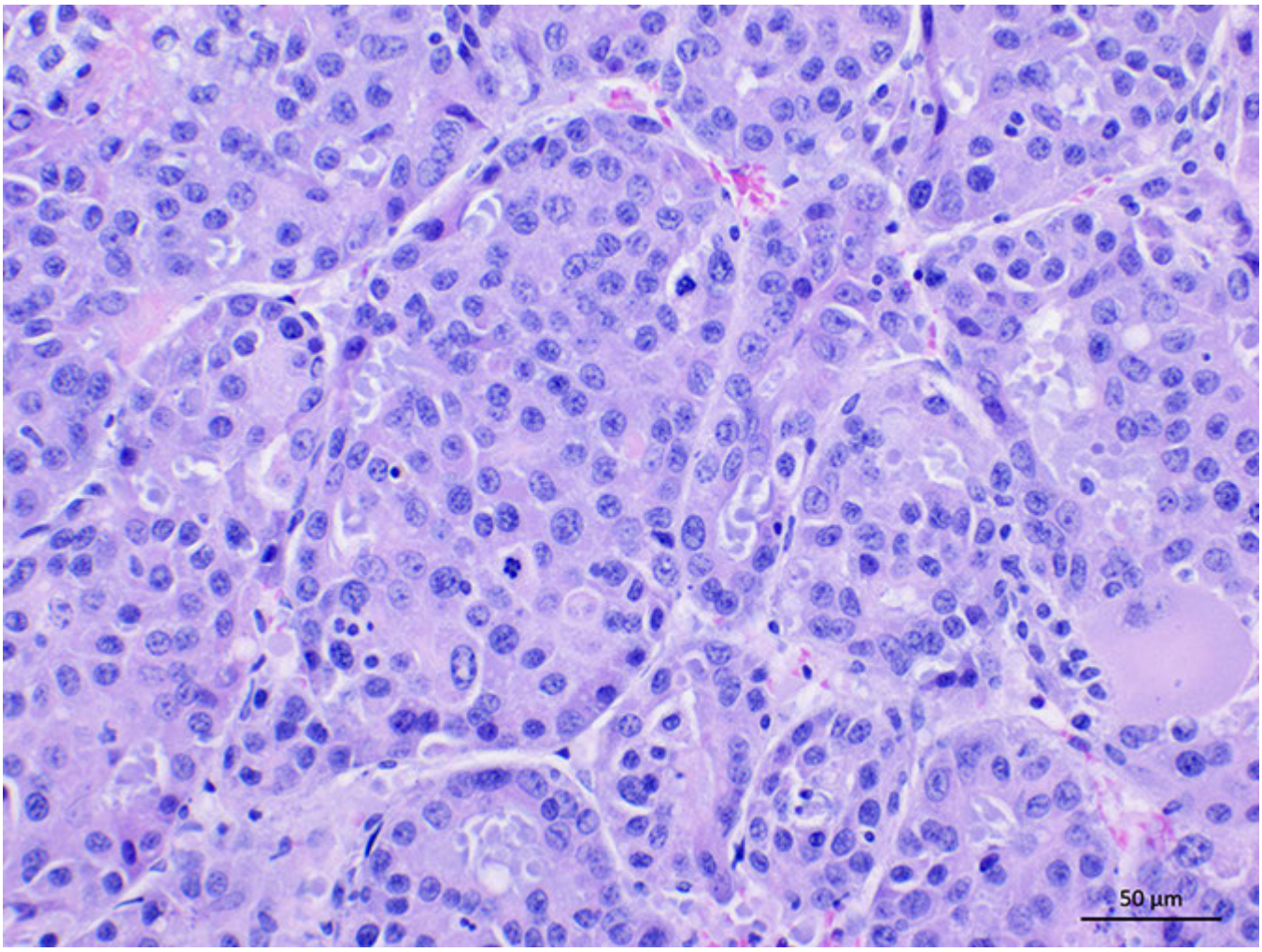


Figure S2

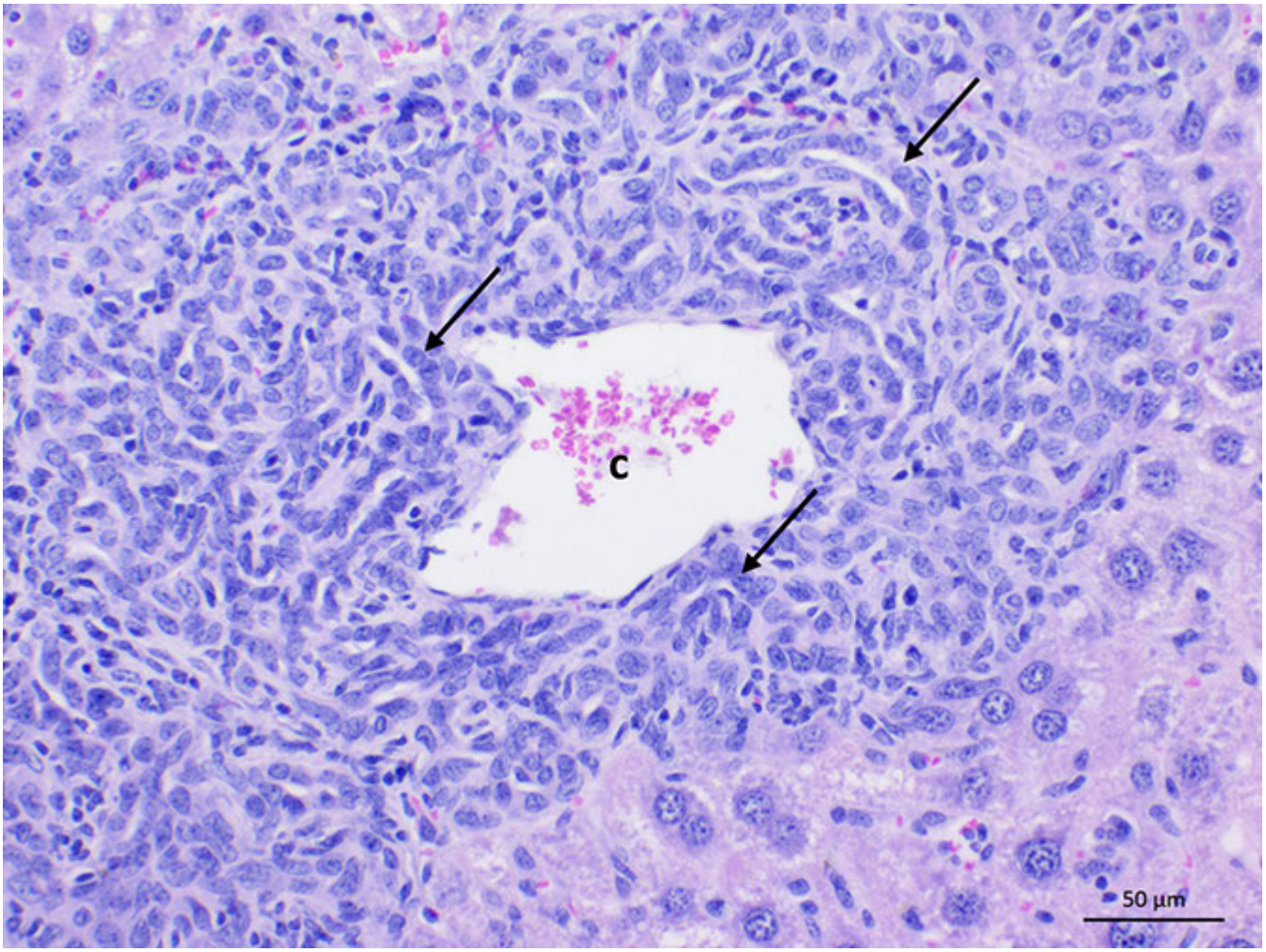
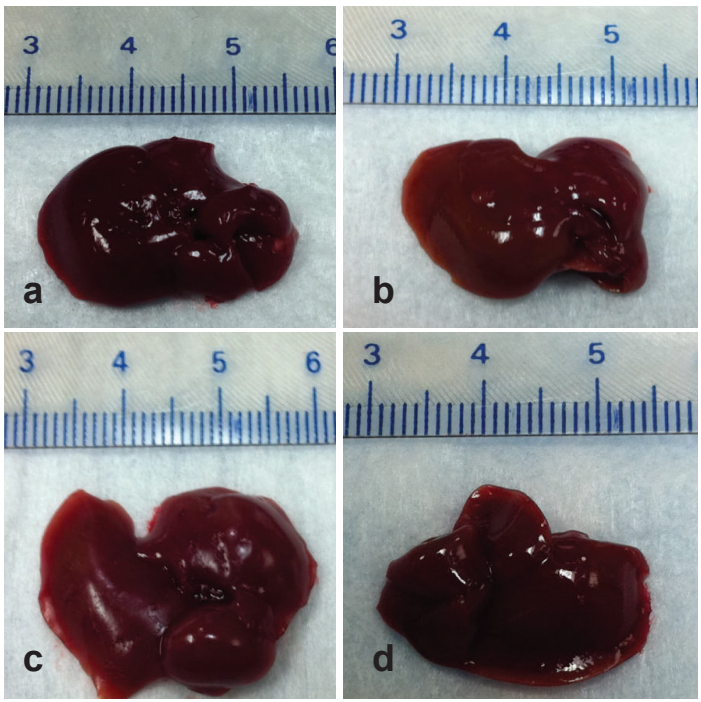


Figure S3

A


a
b
c
d

B

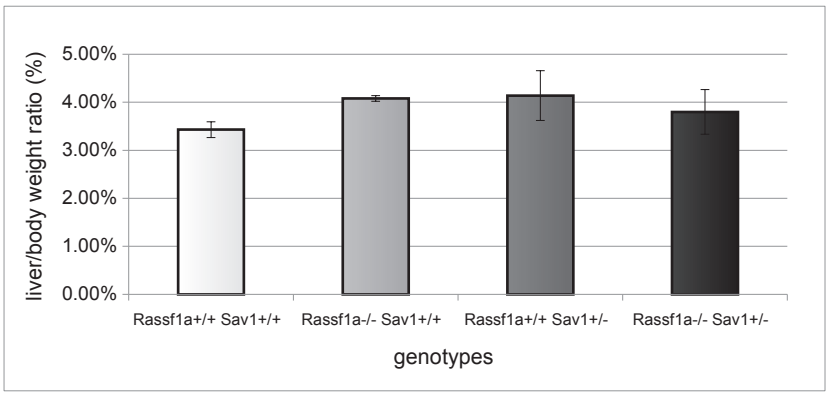

liver/body weight ratio (%)
5.00%
4.00%
3.00%
2.00%
1.00%
0.00%
Rassf1a+/+ Sav1+/+
Rassf1a-/- Sav1+/+
Rassf1a+/+ Sav1+/-
Rassf1a-/- Sav1+/-
genotypes

Figure S4

A

upregulated

Category	Term	RT	Genes	Count	%	P-Value	Benjamini
INTERPRO	Beta tubulin	RT		4	3.1	1.4E-5	4.1E-3
INTERPRO	Tubulin/FtsZ, 2-layer sandwich domain	RT		4	3.1	2.3E-4	3.3E-2
INTERPRO	Tubulin	RT		4	3.1	2.7E-4	2.6E-2
INTERPRO	Tubulin/FtsZ, GTPase domain	RT		4	3.1	2.7E-4	2.6E-2

Category	Term	RT	Genes	Count	%	P-Value	Benjamini
KEGG_PATHWAY	Circadian rhythm	RT		4	3.1	1.4E-4	9.0E-3

downregulated

Category	Term	RT	Genes	Count	%	P-Value	Benjamini
KEGG_PATHWAY	Circadian rhythm	RT		4	4.2	2.9E-5	9.8E-4

B

upregulated

Category	Term	RT	Genes	Count	%	P-Value	Benjamini
INTERPRO	Beta tubulin	RT		4	0.2	1.3E-5	3.6E-3
INTERPRO	Tubulin/FtsZ, 2-layer sandwich domain	RT		4	0.2	2.1E-4	2.9E-2
INTERPRO	Tubulin	RT		4	0.2	2.4E-4	2.3E-2
INTERPRO	Tubulin/FtsZ, GTPase domain	RT		4	0.2	2.4E-4	2.3E-2

Category	Term	RT	Genes	Count	%	P-Value	Benjamini
KEGG_PATHWAY	Circadian rhythm	RT		4	0.2	1.3E-4	7.7E-3

downregulated

Category	Term	RT	Genes	Count	%	P-Value	Benjamini
KEGG_PATHWAY	Circadian rhythm	RT		4	2.3	1.6E-4	9.9E-3

Figure S5

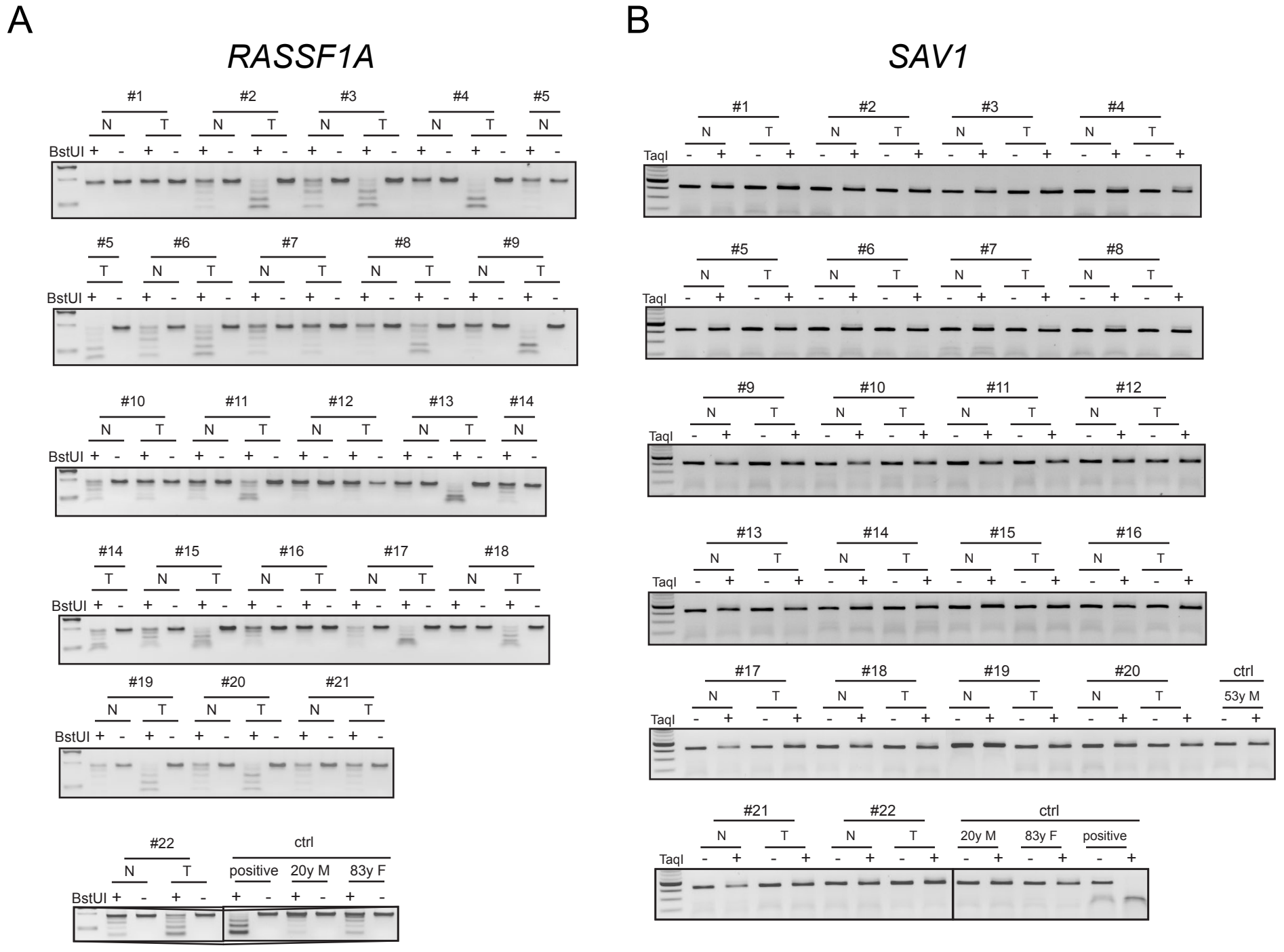

A
RASSF1A
#1
#2
#3
#4
#5
N
T
BstUI
#5
#6
#7
#8
#9
#10
#11
#12
#13
#14
#15
#16
#17
#18
#19
#20
#21
#22
ctrl
positive
20y M
83y F
B
SAV1
TaqI
53y M

Figure S6

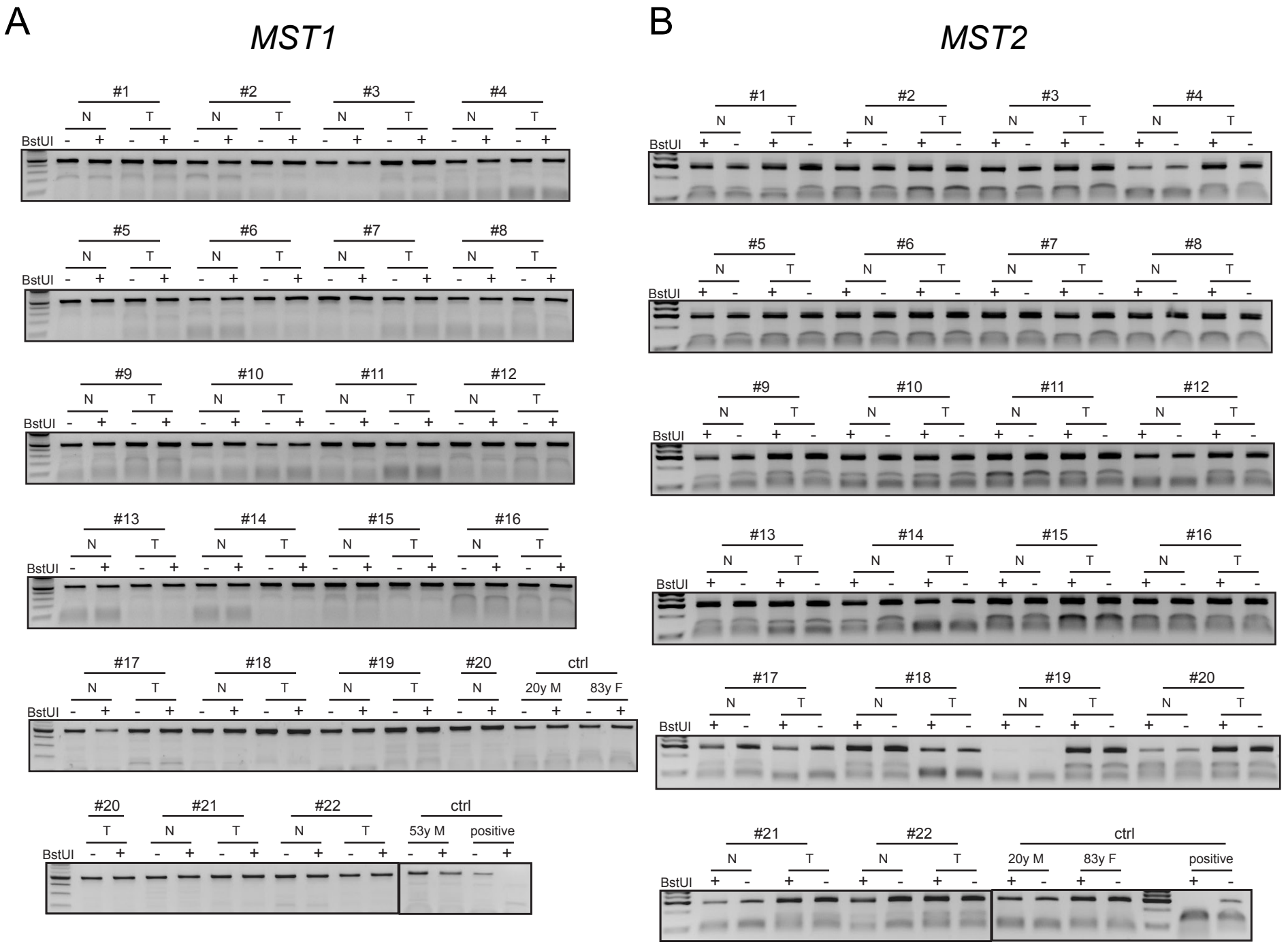

A
MST1
#1
#2
#3
#4
N
T
BstUI
- + - + - + - + - + - + - + - +
#5
#6
#7
#8
#9
#10
#11
#12
#13
#14
#15
#16
#17
#18
#19
#20
ctrl
20y M
83y F
#20
#21
#22
ctrl
T
53y M
positive
B
MST2
+ - + - + - + - + - + - + - +
20y M
83y F
positive

Figure S7

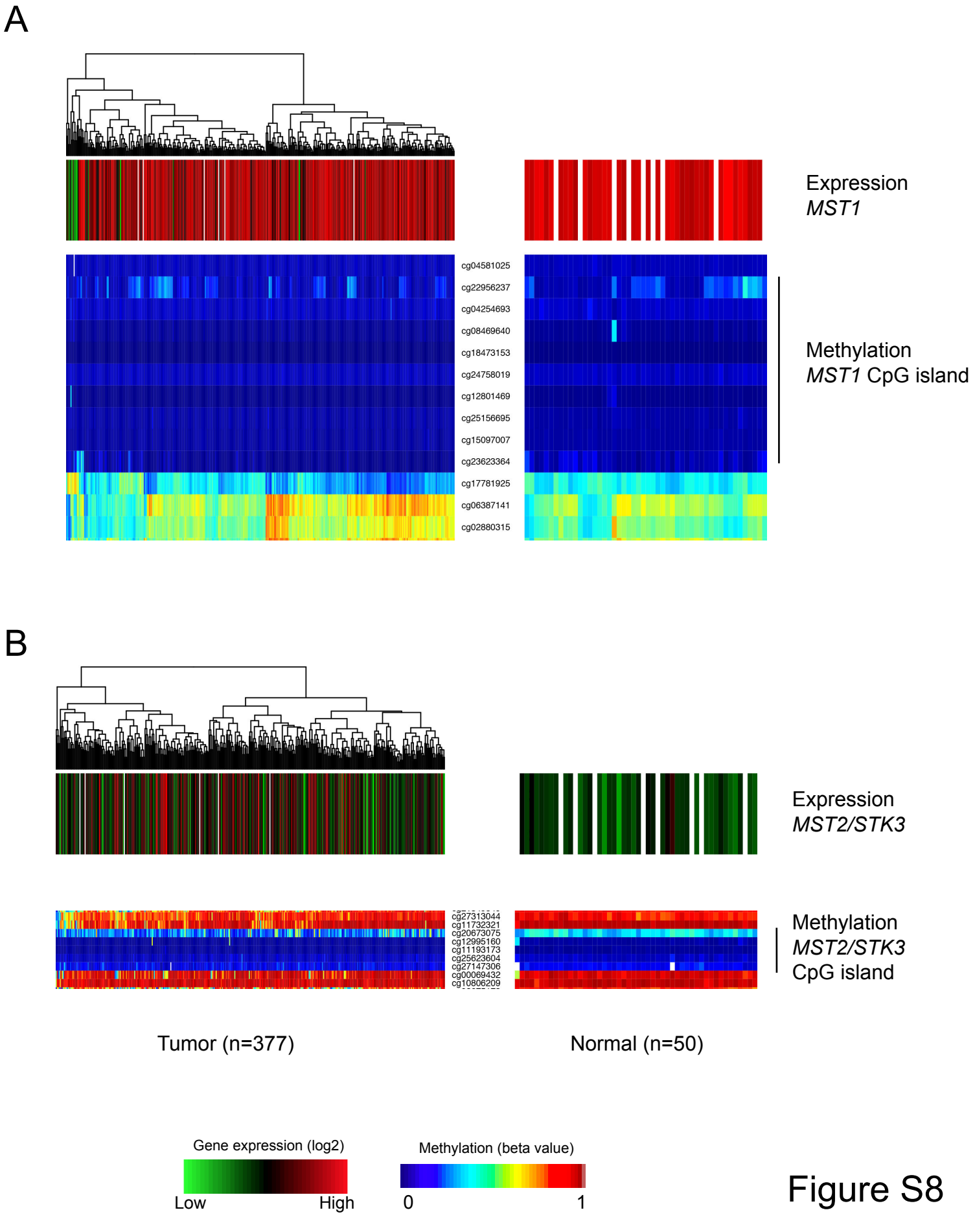

A
Expression
MST1
cg04581025
cg22956237
cg04254693
cg08469640
cg18473153
cg24758019
cg12801469
cg25156695
cg15097007
cg23623364
cg17781925
cg06387141
cg02880315
Methylation
MST1 CpG island
B
Expression
MST2/STK3
cg27313044
cg11732321
cg20673075
cg12995160
cg11193173
cg25623604
cg27147306
cg00069432
cg10806209
Methylation
MST2/STK3
CpG island
Tumor (n=377)
Normal (n=50)
Gene expression (log2)
Low
High
Methylation (beta value)
0
1

Figure S8