Supporting Information

Lu et al. 10.1073/pnas.0911427107

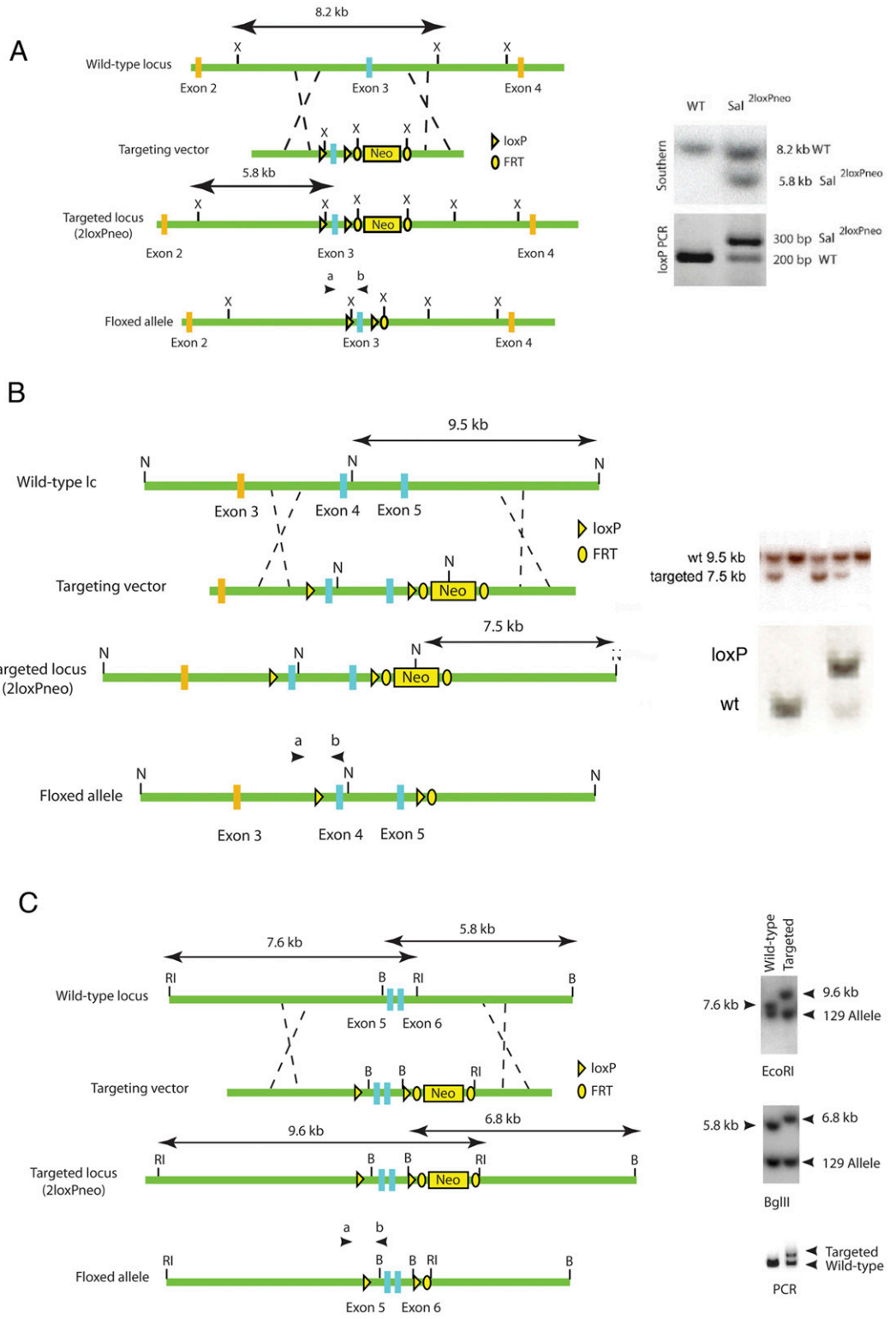

Fig. S1. (*A*) Generation of a *Salvador* conditional allele. A targeting vector was designed to flank exon 3 of *sav1* with loxP sites. Exon 3 contains the WW domain and is essential for interaction of *sav1* with *mst1* and *mst2*. Deletion of exon 3 results in premature termination and a predicted truncated non-

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functional Sal1 protein. Targeting was confirmed by Southern and PCR analysis in ES cells and in tail DNA from mice. The neo cassette was removed in vivo by the action of Flpe. (*B*) Generation of a *mst1* conditional allele. A targeting vector was designed to flank exons 4 and 5 of *mst1* with loxP sites. Exons 4 and 5 contain the ATP-binding site of *mst1* and are essential for kinase activity. Deletion of exons 4 and 5 results in premature termination and a predicted truncated nonfunctional *Mst1* protein. Targeting was confirmed by Southern and PCR analysis in ES cells and in tail DNA from mice. The neo cassette was removed in vivo by the action of Flpe. (*C*) Generation of a *mst2* conditional allele. A targeting vector was designed to flank exons 5 and 6 of *mst2* with loxP sites. Exons 5 and 6 contain the ATP-binding site of *mst2* and are essential for kinase activity. Deletion of exons 4 and 5 results in premature termination and a predicted truncated nonfunctional *Mst2* protein. Targeting was confirmed by Southern and PCR analysis in ES cells and in tail DNA from mice. The neo cassette was removed in vivo by the action of Flpe.

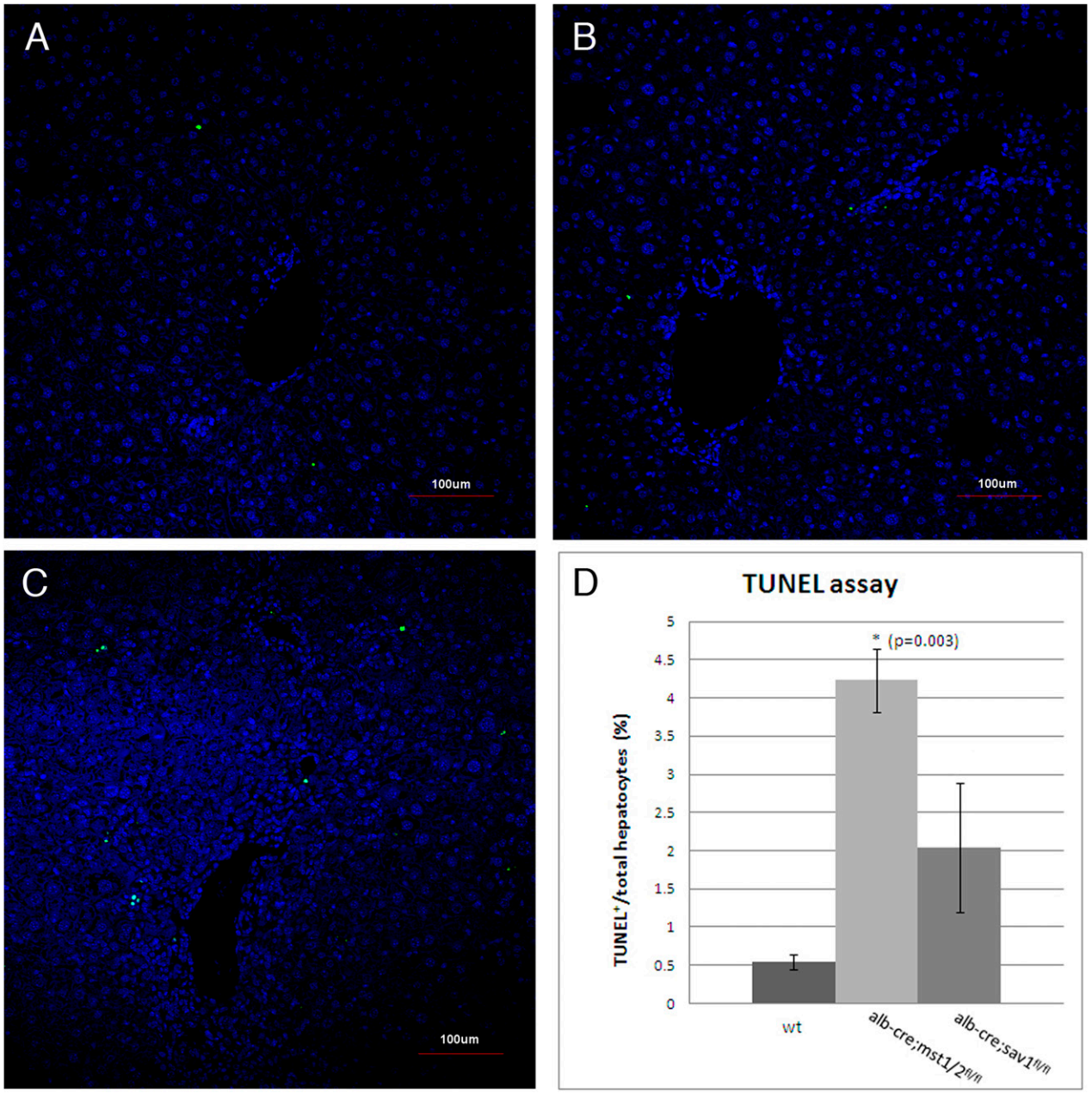
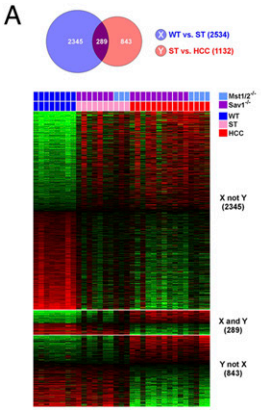


Fig. S2. Cell death is moderately increased in *sav1* and *mst1/2* mutant livers relative to wild-type controls. (*A*) Tunel staining of wild-type sections reveals few cells undergoing apoptosis. (*B*) *Sav1* and (*C*) *mst1/2* mutants show increased numbers of tunel-positive cells. (*D*) Quantitation of tunel staining. *Mst1/2* mutants have ~8-fold higher numbers of tunel-positive cells. *Sav1* mutants have also have increased tunel-positive hepatocytes, but the difference is not statistically significant.



B

X not Y

SYMBOL	NL	ST	HCC	ST/NL
Spink3	6.72925	12.459	13.9018	5.72975
Cib3	6.792375	11.4942	11.4799333	4.701825
H2-Aa	8.7205	12.74	13.474	4.0195
Lcn2	9.61325	13.483	14.3233333	3.86975
Sftpd	6.9725	10.681	11.9248667	3.7085
H2-Eb1	8.069625	11.718	12.5573333	3.648375
Igh-VJ558	9.028	12.6343	12.399	3.6063
Spp1	10.514875	13.978	14.6573333	3.463125
Slpi	7.260375	10.673	12.0126667	3.412625
LOC10004762	9.697625	13.0446	12.3978	3.346975
Igk-C	10.084	13.392	12.8268667	3.308
Igh-VJ558	7.49	10.7202	10.3536667	3.2302
S100a8	7.763375	10.8751	10.4960667	3.111725
Mgp	7.667	10.7748	11.0896667	3.1078
Ifi27	7.827375	10.8817	10.1092	3.054325
4930533K18R	7.37275	10.4052	11.6752667	3.03245
Bicc1	8.318125	11.277	12.54	2.958875
Esm1	6.456625	9.3901	11.2428667	2.933475
Cxcl14	6.46925	9.3899	9.63533333	2.92065
S100a9	7.092125	9.9527	9.6158	2.860575
LOC10004716	7.447125	10.2936	9.64313333	2.846475
LOC669053	7.1095	9.9162	9.2104	2.8067
Cxcl14	6.770875	9.5464	9.91246667	2.775525
Emp1	8.07525	10.8239	11.4702667	2.74865

C

X and Y

SYMBOL	NL	ST	HCC	HCC/ST
Gpc3	6.622	9.2965	11.8131333	2.51663333
Sdcbp2	6.573	7.7652	10.2107333	2.44553333
Gpc3	6.775	10.0478	12.384	2.3362
Cd63	8.229	10.7311	12.8936	2.1625
Chrnb1	6.808875	9.628	11.7401333	2.11213333
Rragd	6.75575	8.4944	10.6032667	2.10886667
LOC217328	6.453875	7.3845	9.47766667	2.09316667
Tubb2b	6.476375	8.8192	10.8628667	2.04366667
Cav1	8.343	10.09	11.9888667	1.89886667
Cd63	7.92225	9.7986	11.5974	1.7988
Cd63	7.069	8.7968	10.5380667	1.74126667
9130213B05R	6.66225	7.8248	9.54086667	1.71606667
Gipc2	6.362625	7.1196	8.82473333	1.70513333
EG665509	6.47525	8.9503	10.6420667	1.69176667
Tnfrsf12a	8.09425	9.9527	11.6318667	1.67916667
D17H6S56E-5	6.53625	8.8319	10.5038	1.6719
Dab2	9.75075	10.783	12.4153333	1.63233333
S100a11	8.747625	12.338	13.9653333	1.62733333
Ahnak	8.587125	10.247	11.8614	1.6144
Chrnb1	6.426875	7.9322	9.54646667	1.61426667
Osbpl3	7.527875	9.7649	11.3613333	1.59643333
Dab2	8.93375	9.7897	11.3754667	1.58576667
Psat1	6.805875	7.7406	9.32493333	1.58433333
Anxa2	10.30275	12.468	14.0506667	1.58266667

D

Y not X

SYMBOL	NL	ST	HCC	HCC/ST
Fgfbp1	6.376625	7.168	9.91793333	2.74993333
Slc23a3	6.441125	6.7537	8.9716	2.2179
Nope	6.465375	6.9331	9.10926667	2.17616667
Wisp2	6.367625	6.9477	9.0736	2.1259
C030003H22R	6.635625	7.2403	9.0856	1.8453
Slc6a20b	6.293375	7.0576	8.8622	1.8046
Lpl	10.133875	11.837	13.632	1.795
Gnai1	7.18675	8.1383	9.87793333	1.73963333
Fgf21	7.897125	8.4093	10.0823333	1.67303333
Csad	12.06625	11.712	13.3393333	1.62733333
Slc24a3	6.86075	7.2107	8.82206667	1.61136667
Adam32	7.2095	8.0961	9.68446667	1.58836667
Ctps	9.1535	9.7352	11.3146667	1.57946667
Arhgap22	6.494875	6.9851	8.47006667	1.48496667
Ctps	8.091	8.3381	9.7854	1.4473
Rab34	7.153625	8.0048	9.44553333	1.44073333
Vil1	6.36025	6.6633	8.03346667	1.37016667
AA407270	7.08575	7.6283	8.99673333	1.36843333
Lysmd2	6.623625	6.9621	8.29726667	1.33516667
Lamb2	7.066375	7.7187	9.048	1.3293
Il7	6.64525	7.2405	8.56353333	1.32303333
Abcc4	7.710625	8.1019	9.40873333	1.30683333
Fam148c	6.35475	6.8114	8.0946	1.2832
Rab34	7.8555	8.5379	9.8148	1.2769

Fig. S3. Transcriptional profiling of Hippo pathway mutant liver tissue. (*A*) Venn diagram of genes selected by univariate test (two-sample *t* test) with a multivariate permutation test (10,000 random permutations). The blue circle (gene list X) represents genes differentially expressed between wild-type (WT) liver and all *sav1* and *mst1/2* nontumor liver samples. The red circle (gene list Y) represents genes differentially expressed between all *sav1* and *mst1/2* nontumor liver tissues and all *sav1* and *mst1/2* tumor (hepatocellular carcinoma, HCC) tissues. We applied a cutoff *P* value of <0.001 to retain genes whose expression is significantly different between the two groups of tissues examined. Below, expression patterns of selected genes in the Venn diagram are shown. Colored bars at the top of the heat map represent tissues as indicated. (*B*–*D*) Tabular data from microarray analysis including the 25 most highly up-regulated genes in each category. (*B*) "X not Y" represents transcripts that are differentially expressed in mutant nontumor tissues (ST: either from *sav1* or *mst1/2* mutants) versus WT tissues. These transcripts represent cDNAs that are up- or down-regulated in response to attenuated Hippo signaling and may contribute to liver growth, oval cell response, liver injury, and predisposition to tumorigenesis. (*C*) "X and Y" represents transcripts that are differentially expressed in mutant nontumor tissues (ST: from either *sav1* or *mst1/2* mutants) and in mutant nontumor tissues (ST) versus tumor tissues (HCC). These transcripts represent cDNAs that may contribute to phenotypic differences between wild-type and mutant nontumor tissues as well as to transformation of mutant tissues to tumor tissues. (*D*) "Y not X" represents transcripts that are up- or down-regulated in tumor tissues but not in mutant nontumor tissues. These cDNAs may be involved in the transformation of mutant tissues to tumor tissues. NL, normal liver. Numbers listed are raw Illumina data. The ratios (ST/NL and HCC/ST) represent $\log_2$ differences. Full tabular data are given in Tables S1–S3. Raw excel files are available on request.

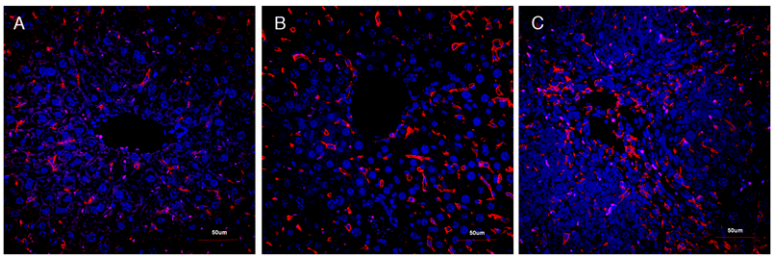


Fig. S4. Macrophage marker-positive cells accumulate in *sav1* and *mst1/2* mutant livers. The F4/80 antibody detects both resident liver macrophages (Kupffer cells) and infiltrating macrophage populations. (*A*) F4/80 staining of wild-type liver (red). Nuclei are counterstained blue with DAPI. (*B*) *Albumin-cre;sav1* mutant tissue has slightly elevated numbers of F4/80-positive cells. (*C*) *Albumin-cre;mst1,2* mutant tissue also has elevated F4/80 immunoreactivity.

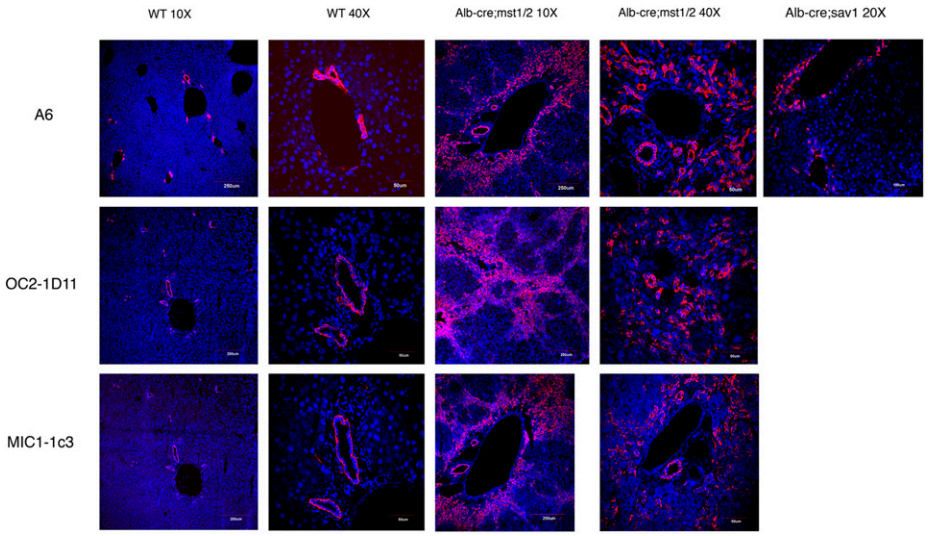


Fig. S5. Antibody characterization of oval cell response in *mst1/2* mutants. The antibodies A6, OC2-1D11, and MIC1-1c3 stain ductal cells in wild type (see WT 10× and 40×). In *albumin-cre*;*mst1/2* mutants, A6, OC2-1D11, and MIC1-1c3 stain ductal cells and in addition populations of infiltrating periductal cells. This staining pattern is consistent with positive cells being oval cells. All sections were counterstained with DAPI. *Albumin-cre*;*sav1* mutants also exhibit enhanced A6 labeling at 4 months of age. A6 WT 10× and A6 *albumin-cre*;*mst1*,*2* 10× are reproduced from Fig. 4 and are shown here for comparison.

Other Supporting Information Files

Table S1 (XLS)
Table S2 (XLS)
Table S3 (XLS)