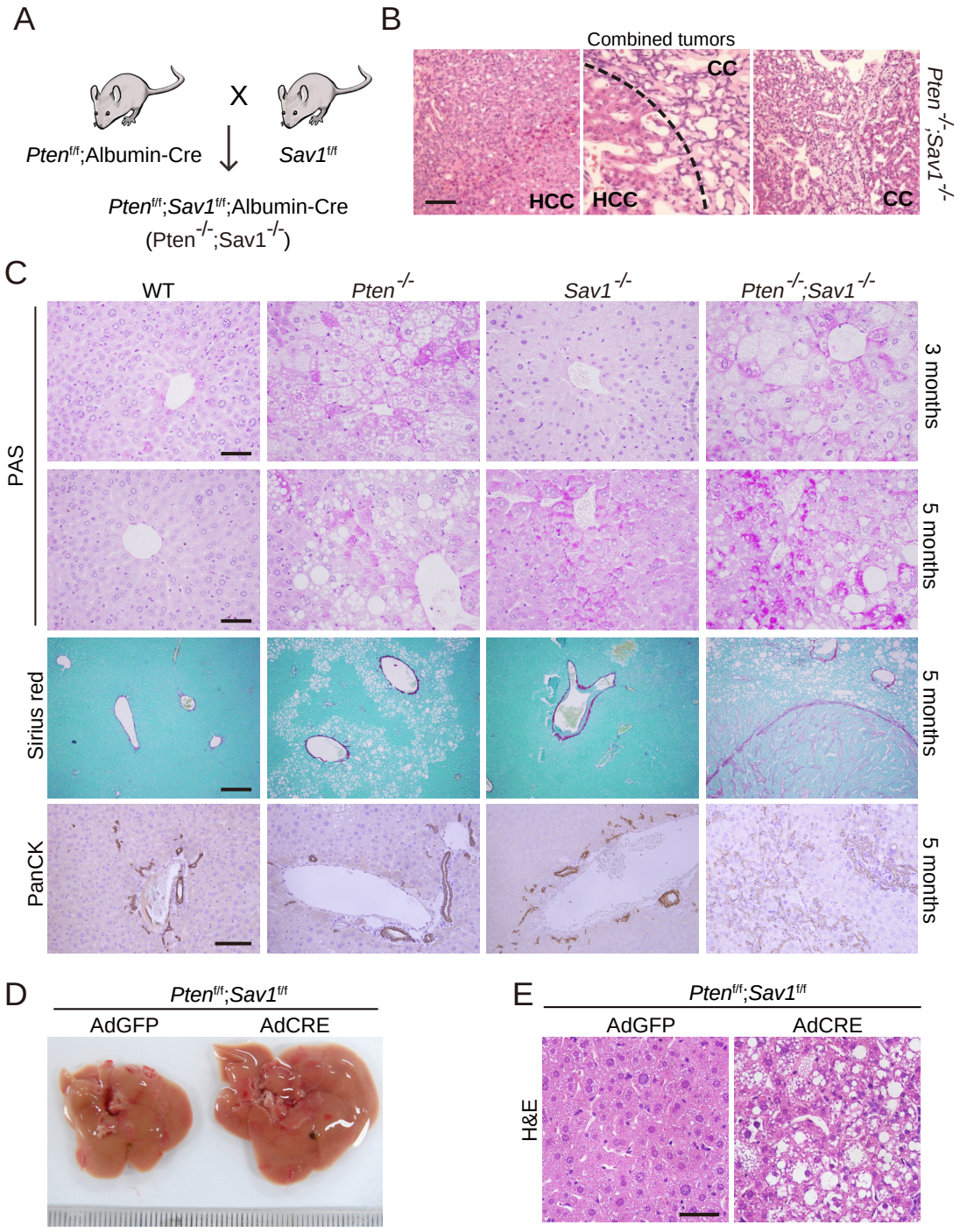
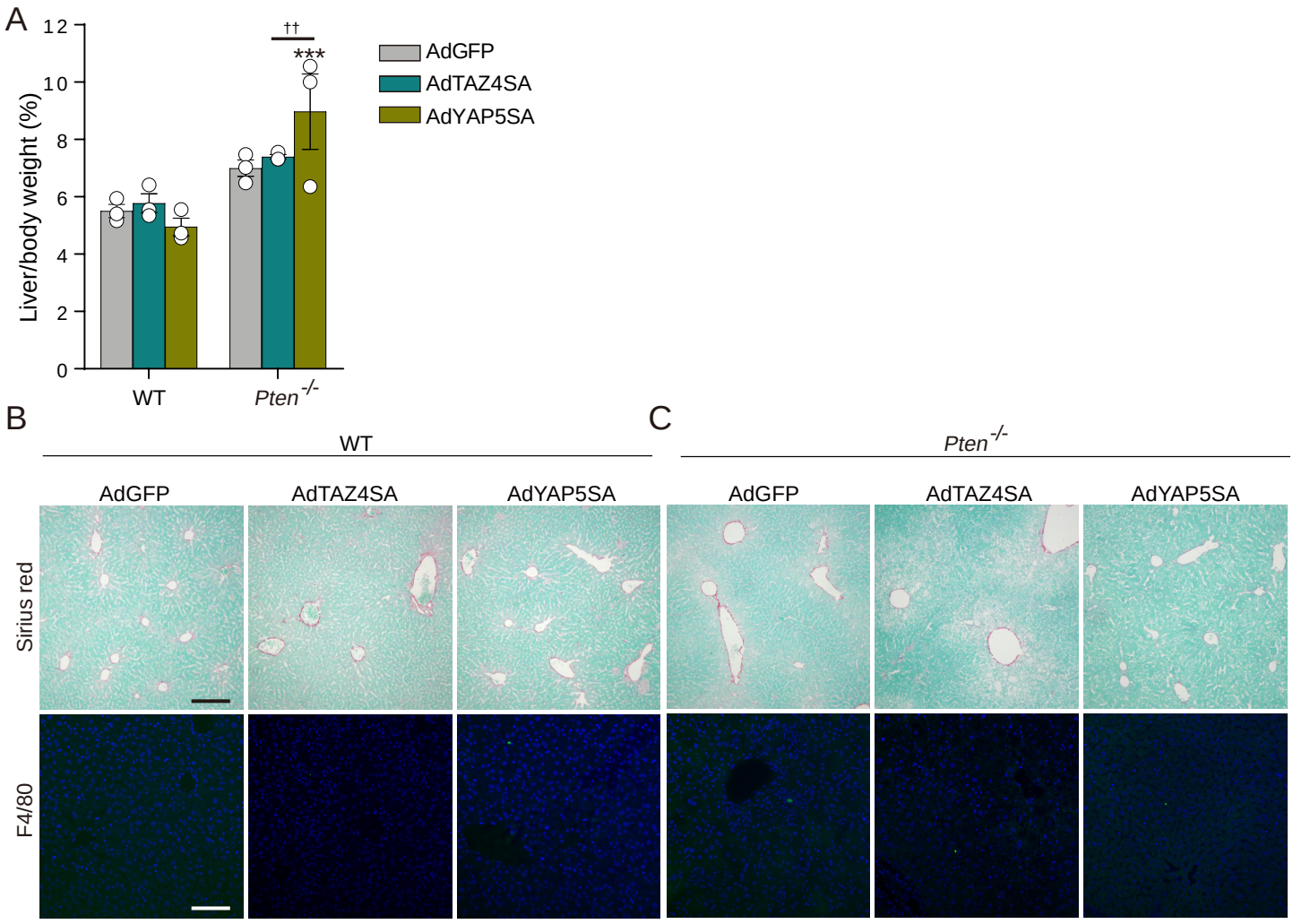
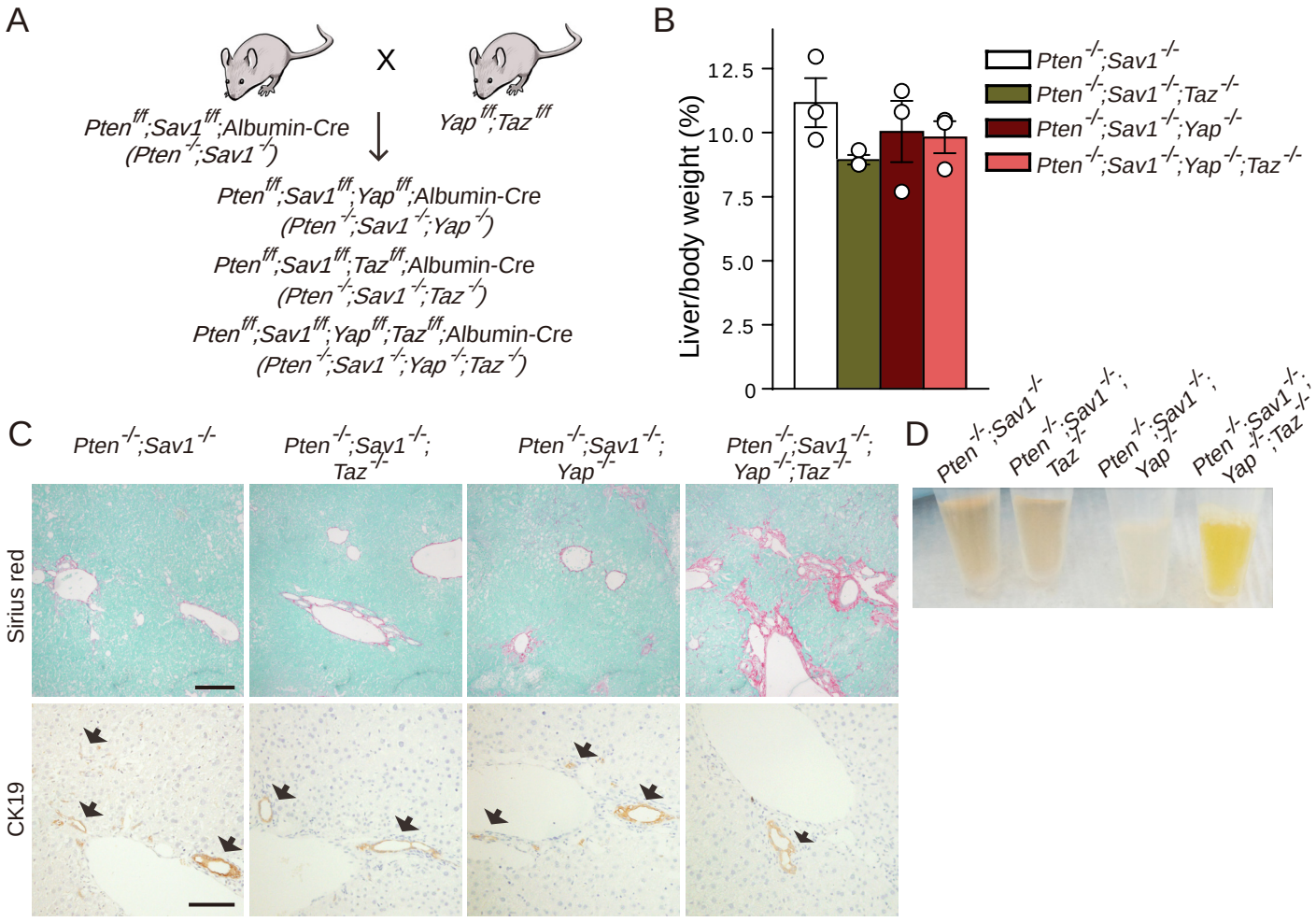


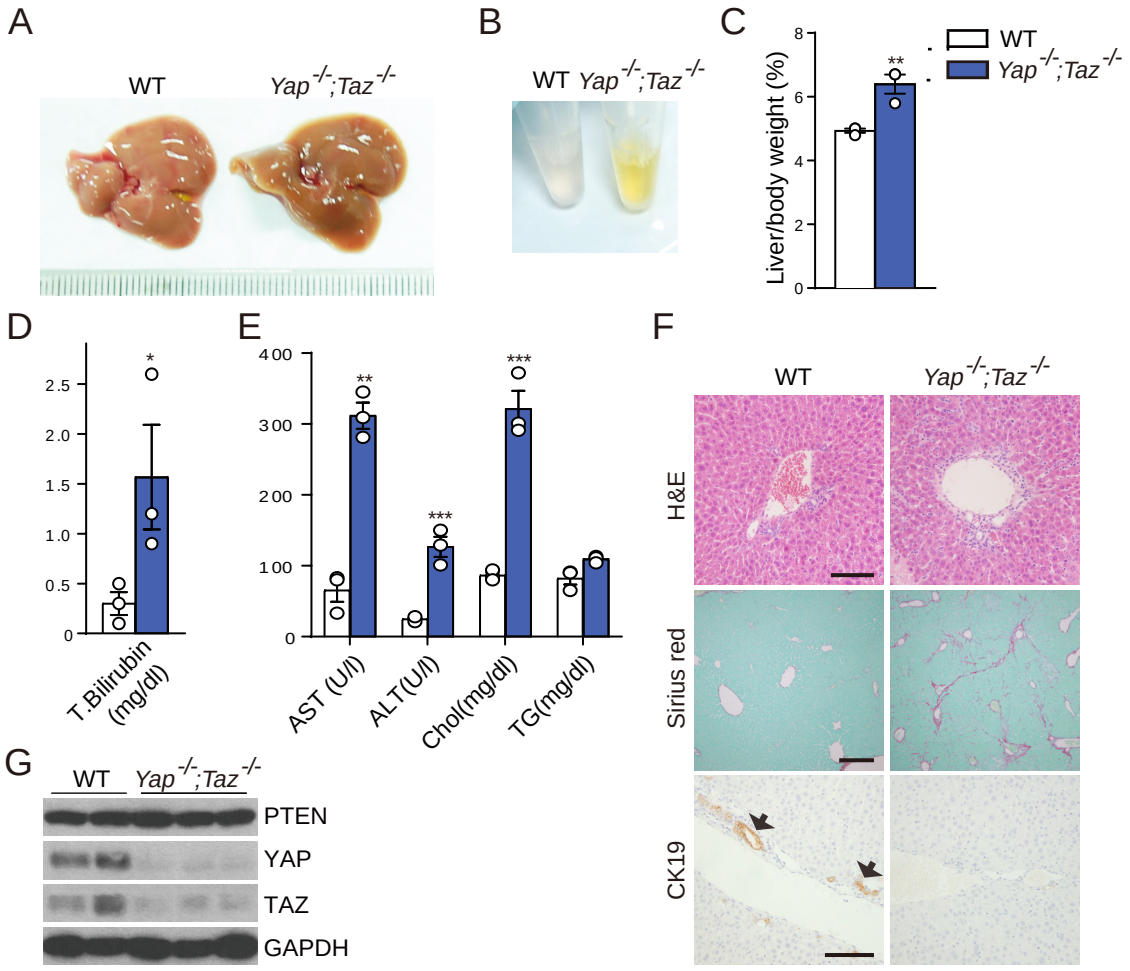
Supplemental Figure 1. Liver-specific *Pten* and *Sav1* double-knockout (DKO) mice show accelerated development of liver dysfunction. (**A**) Schematic for the generation of liver-specific DKO mice. (**B**) Representative H&E staining of combined hepatocellular carcinoma (HCC) and cholangiocarcinoma (CC) tumor types in DKO mouse livers. Scale bar, 100 μm. (**C**) PAS, Sirius red, and panCK immunohistochemical staining of the livers of mice at the indicated ages. Scale bars: 50 μm (PAS), 200 μm (Sirius red), and 100 μm (panCK). n = 5 for each group in all analyses. (**D**-**E**) Acute adult-stage deletion of *Pten* and *Sav1* induces fatty liver. Microscopic appearance (**D**) and H&E staining (**E**) of livers from 6-week-old *Pten*$^{f/f}$;*Sav1*$^{f/f}$ mice injected with adenovirus encoding CRE and GFP (control). Scale bars, 50 μm.



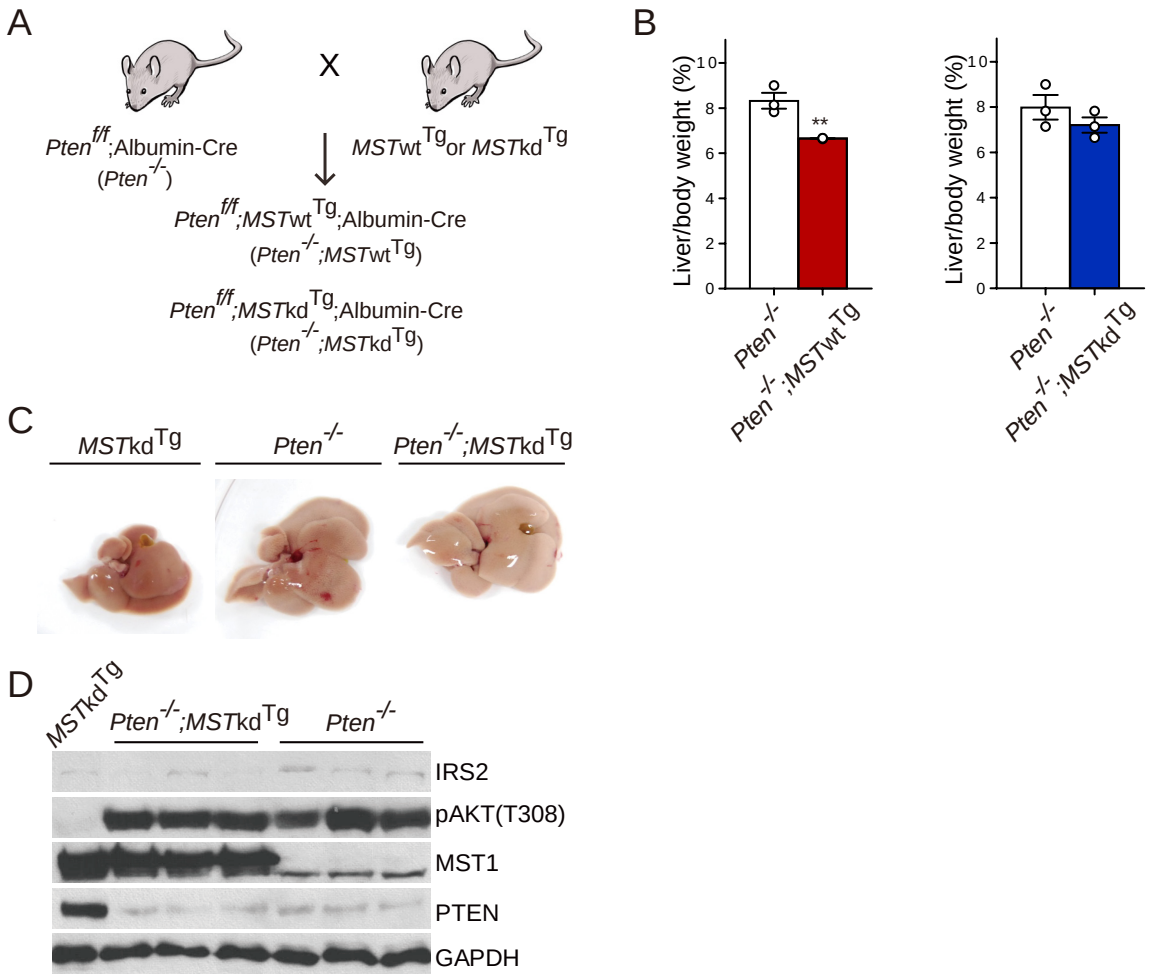
Supplemental Figure 2. A short-term increase of YAP or TAZ activity promotes fatty liver development without fibrosis or inflammation. The liver-to-body weight ratio (**A**) as well as representative Sirius red staining and F4/80 immunofluorescence staining (green fluorescence) of the liver (**B**, **C**) are shown for 6-week-old WT (**B**) or *Pten*$^{-/-}$ (**C**) mice (n = 3) 4 days after injection with adenoviruses encoding GFP, TAZ4SA, or YAP5SA. Nuclei in (**B**) and (**C**) were stained with 4',6-diamidino-2-phenylindole (blue fluorescence). Scale bars, 200 μm (top panels) or 100 μm (bottom panels). Quantitative data are means ± s.e.m. ***$P < 0.001$ versus the corresponding value for AdGFP, †† $P < 0.01$ for the indicated comparison (one-way ANOVA).



Supplemental Figure 3. *Pten*$^{-/-}$;*Sav1*$^{-/-}$;*Yap*$^{-/-}$;*Taz*$^{-/-}$ livers develop pronounced fibrosis.
(**A**) Schematic for the generation of *Pten*$^{-/-}$;*Sav1*$^{-/-}$;*Yap*$^{-/-}$;*Taz*$^{-/-}$ mice. (**B-D**) Liver-to-body weight ratio (**B**), Sirius red and CK19 immunohistochemical staining of the liver (**C**), and isolated serum color (**D**) for 1-month-old mice (n = 3) of the indicated genotypes. Scale bars (**C**), 200 µm (top) or 100 µm (bottom); arrows indicate ducts.

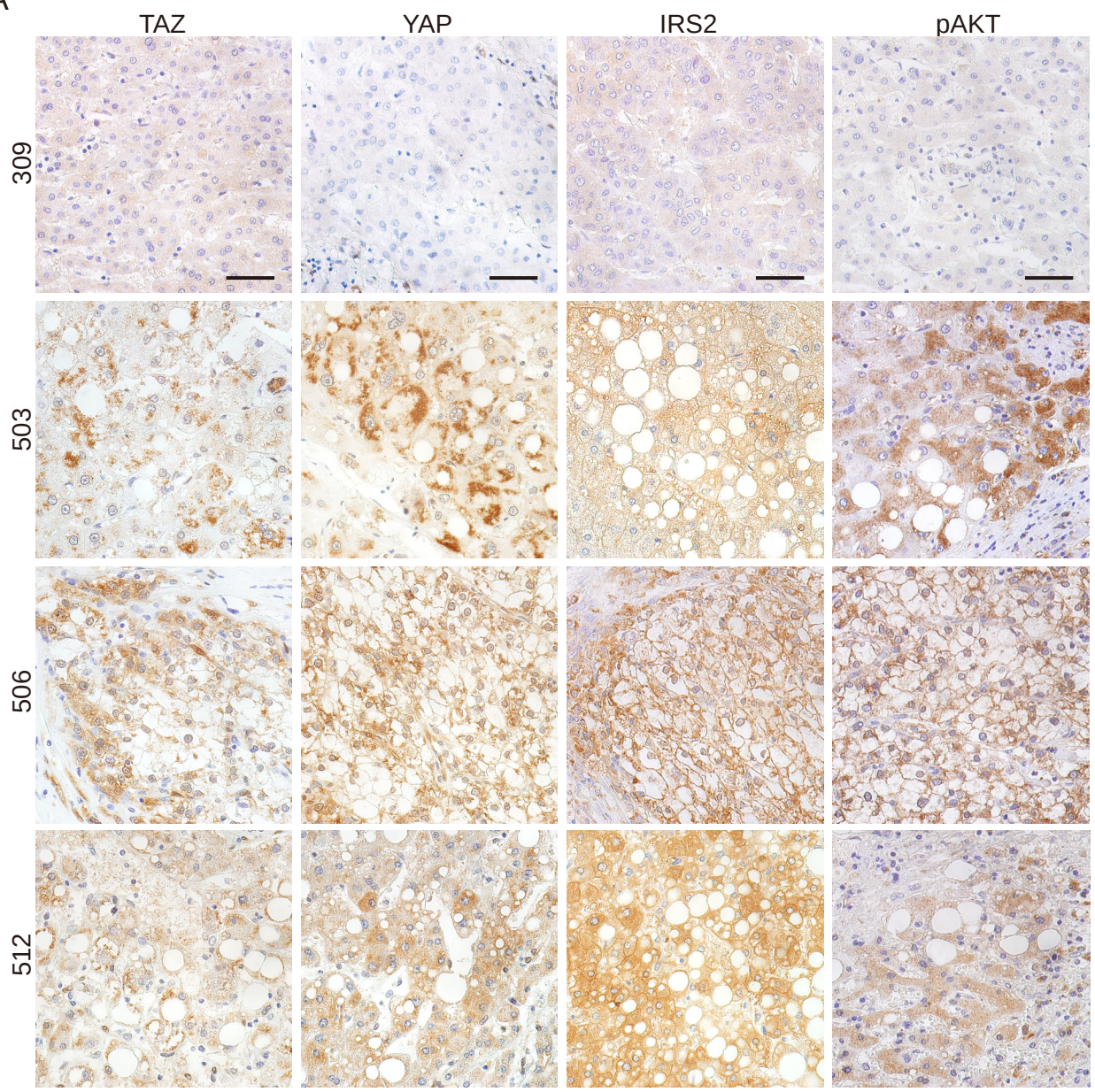


Supplemental Figure 4. The ablation of both *Yap* and *Taz* leads to ductal malformation and severe fibrosis. (**A**-**G**) Macroscopic appearance of the liver (**A**), isolated serum color (**B**), liver-to-body weight ratio (**C**), serum analysis (**D**, **E**), H&E, Sirius red, and CK19 immunohistochemical staining of the liver (**F**), and immunoblot analysis of the liver (**G**) for 1-month-old WT or liver-specific *Yap*$^{-/-}$;*Taz*$^{-/-}$ mice (each group, n = 3). Scale bars (**F**), 100 μm (top and bottom) or 200 μm (middle); arrows indicate ducts. T.Bilirubin, total bilirubin; Chol, cholesterol; TG, triglycerides. All quantitative data are means ± s.e.m. **P* < 0.05, ***P* < 0.01, ****P* < 0.001 versus the corresponding WT value (Student's t test).



Supplemental Figure 5. Expression of a kinase-dead form of MST1 does not rescue the fatty liver phenotype of *Pten*$^{-/-}$ mice. (**A**) Schematic for the generation of *Pten*$^{-/-}$;*MST*wt^{Tg} or *Pten*$^{-/-}$;*MST*kd^{Tg} mice. (**B**) Liver-to-body weight ratio for 2-month-old mice (each group, n = 3) of the indicated genotypes. Data are means ± s.e.m. (**C**-**D**) Macroscopic appearance (**C**), and immunoblot analysis (**D**) of the livers from 2-month-old mice (n = 3) of the indicated genotypes.

A



B

Sample Number	Sex	Age	TAZ	YAP	IRS2	pAKT
501	M	78	+++	+	++	+++
503	M	83	+++	+++	++	+++
504	F	58	+++	++	+++	+++
506	M	76	+++	+++	+++	+++
507	M	62	++	++	+++	+++
509	F	72	++	+	++	+++
510	M	75	++	+++	++	+
512	F	69	+++	++	+++	+++
515	M	72	+++	++	+++	+

C

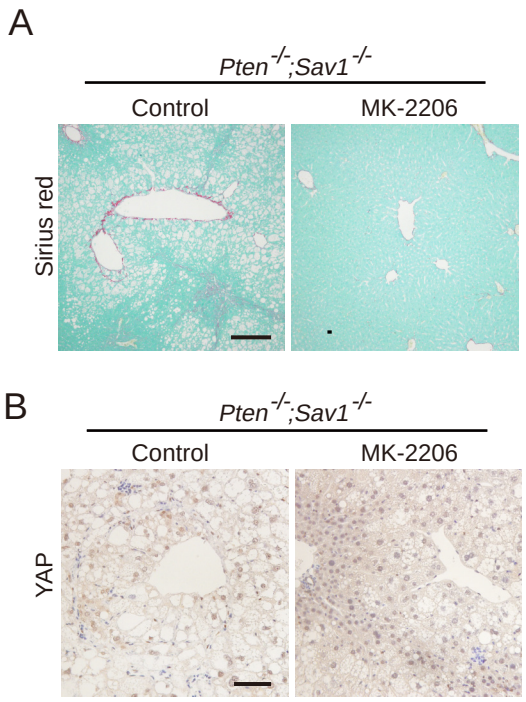
	non-NAFLD	NAFLD
TAZ (Low)	31	0
TAZ (High)	10	15
total	41	15

	non-NAFLD	NAFLD
IRS2 (Low)	34	0
IRS2 (High)	7	15
total	41	15

	non-NAFLD	NAFLD
YAP (Low)	31	3
YAP (High)	10	12
total	41	15

	non-NAFLD	NAFLD
pAKT (Low)	37	6
pAKT (High)	4	9
total	41	15

Supplemental Figure 6. Comparison of IHC intensities of TAZ, YAP, IRS2, and pAKT(S473) protein levels between NAFLD-associated HCC and non-NAFLD HCC.
(**A**) Representative immunohistochemical staining of TAZ, YAP, IRS2, and pAKT(S473) in human HCC not associated with NAFLD (sample No. 309) or associated with NAFLD (sample No. 503, 506, 512). Scale bars, 50 μm. (**B**) Immunohistochemistry signal intensity scores for TAZ, YAP, IRS2, and pAKT in human HCC associated with NAFLD. -, no expression; +, low expression; ++, moderate expression; +++, high expression.
(**C**) The number of specimens with intense staining of TAZ, YAP, IRS2, and pAKT in the human HCC associated with NAFLD compared to HCC not associated with NAFLD (all $P < 0.001$, by chi-square test).



Supplemental Figure 7. Effects of MK-2206 treatment on the liver of DKO mice. (**A**) Representative Sirius red staining of the liver of DKO mice treated for 2 weeks with MK-2206 or vehicle beginning at 3 weeks of age. Scale bar, 200 μm. (**B**) Representative YAP staining of the liver of DKO mice treated for 2 weeks with MK-2206 or vehicle beginning at 12 weeks of age. Scale bar, 100 μm.

Supplementary Table 1 Primer sequences for RT-qPCR analysis, luciferase reporter construction, ChIP-qPCR analysis, and mouse genotyping.

RT-qPCR		
Gene	Sense	Antisense
Srebp1a	5'-GGCCGAGATGTGCGAACT-3'	5'-TTGTTGATGAGCTGGAGCATGT-3'
Srebp1c	5'-GGAGCCATGGATTGCACATT-3'	5'-CAGGAAGGCTTCCAGAGAGG-3'
Srebp2	5'-GCGTTCTGGAGACCATGGA-3'	5'-ACAAAGTTGCTCTGAAAACAAATCA-3'
Fasn	5'-GCCTACACCCAGAGCTACCG -3'	5'-GCCATGGTACTTGGCCTTG -3'
Acc1	5'-CAACGAGATTTCACTGTGGCT-3'	5'-TTCTGCATTGGCTTTAAGGTCT-3'
Scd1	5'-CCGGAGACCCCTTAGATCGA-3'	5'-TAGCCTGTAAAGATTTCTGCAAACC-3'
Tnfa	5'-CATCTTCTCAAAATTCGAGT-3'	5'-TGGGAGTGACAAGGTACAA-3'
Il6	5'-TCCACGATTTCCCAGAGAAC-3'	5'-AGTTGCCTTCTTGGGACTGA-3'
Irs2	5'-CAAGAGCCCTGGCGAGTACA-3'	5'-CCGCGGATGCCAGTAGTG-3'
Acta2	5'-ATGCTCCCAGGGCTGTTTTCCCAT-3'	5'-GTGGTGCCAGATCTTTTCCATGTCG-3'
Desmin	5'-CTAAAGGATGAGATGGCCCG-3'	5'-GAAGGTCTGGATAGGAAGGTTG -3'
Tgfb	5'-CTCCCGTGGCTTCTAGTGC-3'	5'-GCCTTAGTTTGGACAGGATCTG-3'
Hmox1	5'-GCTCGAATGAACACTCTGG-3'	5'-GTTCCTCTGTCAGCATCAC-3'
Gadd 153	5'-CTGGAAGCCTGGTATGAGGAT-3'	5'-CAGGGTCAAGAGTAGTGAAGGT-3'

Luciferase reporter construction		
Region	Sense	Antisense
TBS1	5'-CTTATCTGGCAGCAGGAAGGAGAG-3'	5'-CACACCCTCGCACACATATCCCTC-3'
TBS2	5'- CACCCTTGCACACGTAGAGACGCT-3'	5'- ACCGTGTTCACCCAGCACCCGGG-3'
TBS3	5'-CCTGGCAGTGTCCCATAGTTGA -3'	5'- CAGCTGCTGCTTCTTTAGGGG-3'
TBS4	5'- GCAGCAGCTGAAGTGCTAAAGA-3'	5'-GCTGCTTTCCTCTCATTGCTC -3'
TBS5	5'- GCCTCTGAGCCAACATCTCTCT-3'	5'-TATGACCTCCCACCCACTTCA -3'
TBS6	5'- CATGGCTCGTTTCTCCTTCTGG-3'	5'-GAGTACTCAGGCCCAGGATGC -3'

ChIP-qPCR		
Region	Sense	Antisense
TBS1	5'-ATCTATGGTCTTCAGAATCACAC-3'	5'-CCAGAGTTTATCTTACAATTTAACCT-3'
TBS2	5'-CACAGTTTACACAAAGGGTAAAGCA-3'	5'-GCTCTGGATGCGTAAACAAAACA-3'
TBS4	5'-GCAGCAGTTGATTCCCATCCT-3'	5'-ACAATGGGGCAGGGAAGGTA-3'
TBS5	5'-GTAAAACCCAGAAACCCCACTTTC-3'	5'-TGCTCTGCTTCTCTCACTAGGA-3'

Mouse genotyping		
Gene	Sense	Antisense
Sav1	5'-TGGTTTGCTTTTTAGTGGCC-3'	5'-TGCTGGTTTTGTCTCACTAA-3'
Pten	5'-CTC CTC TAC TCC ATT CTT CCC-3'	5'-ACT CCC ACC AAT GAA CAA AC-3'
Yap1	5'-ACATGTAGGTCTGCATGCCAGAGGAGG-3'	5'-AGGCTGAGACAGGAGGATCTCTGTGAG-3'
Taz	5'-GGCTTGTGACAAAGAACCTGGGGCTATCTGAG-3'	5'-CCCACAGTTAAATGCTTCTCCCAAGACTGGG-3'
Cre	5'-GTGTTGCCGCGCCATCTGC-3'	5'-CACCATTGCCCCTGTTTCACTATC-3'
MST^{Tg}	5'-GCTCTAGAGCCTCTGCTAACCA-3'	5'-CCAGGGACCAGATGTCTGC-3'

AAV-sg*Irs2*		
Region	Sense	Antisense
Ex1-1	5'- CACC GATCGCCCTCTACACCAAGG-3'	5'- AAAC CCTTGGTGTAGAGGGCGATC-3'
Ex1-2	5'- CACC GCCGCCGCAGCCTCCGCGGC-3'	5'- AAAC GCCGCGGAGGCTGCGGCGGC-3'

Full unedited blot

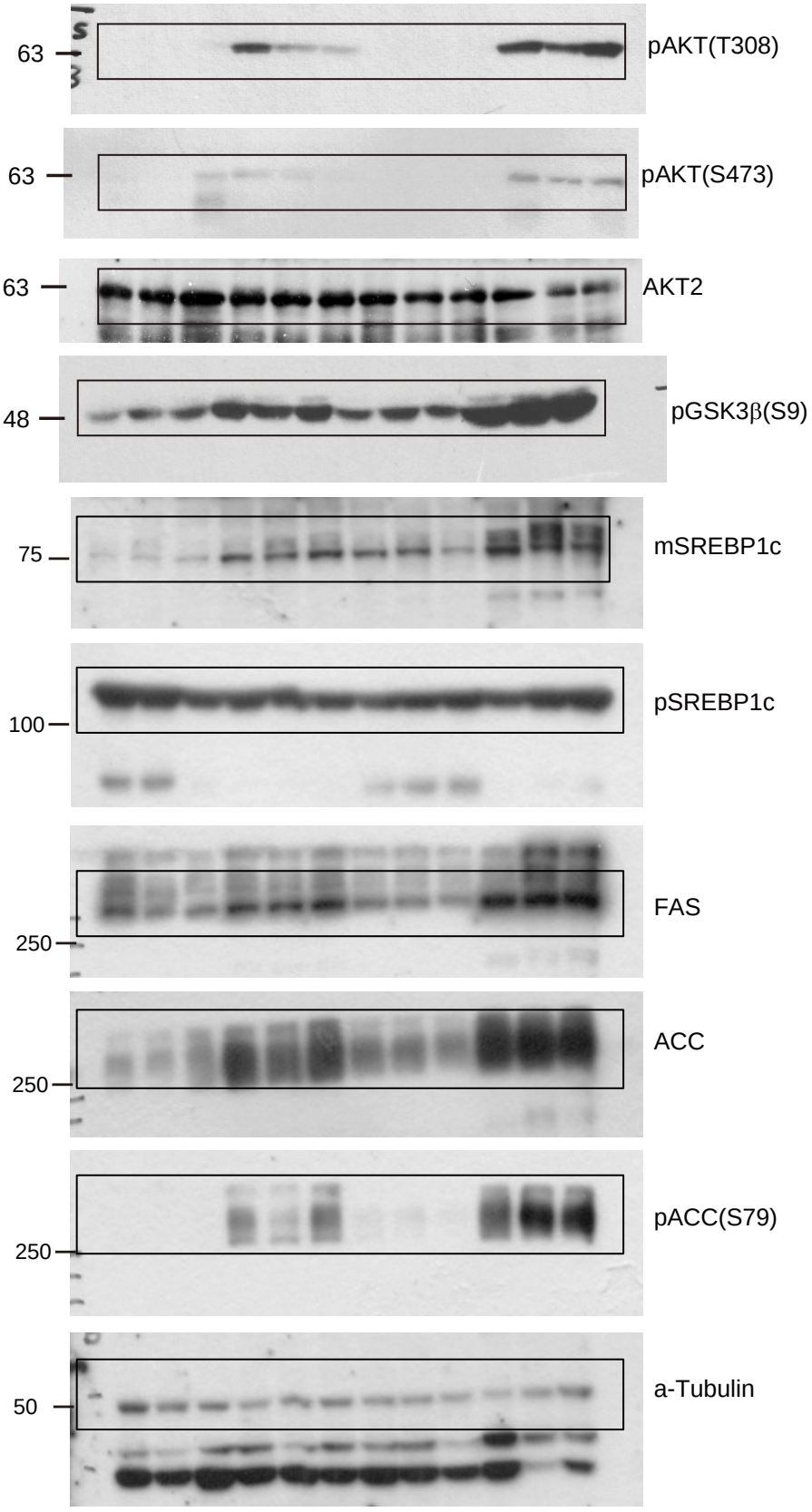
The Hippo-YAP/TAZ Pathway Suppresses IRS2-AKT Signaling to Prevent Fatty Liver and Liver Cancer

It includes;

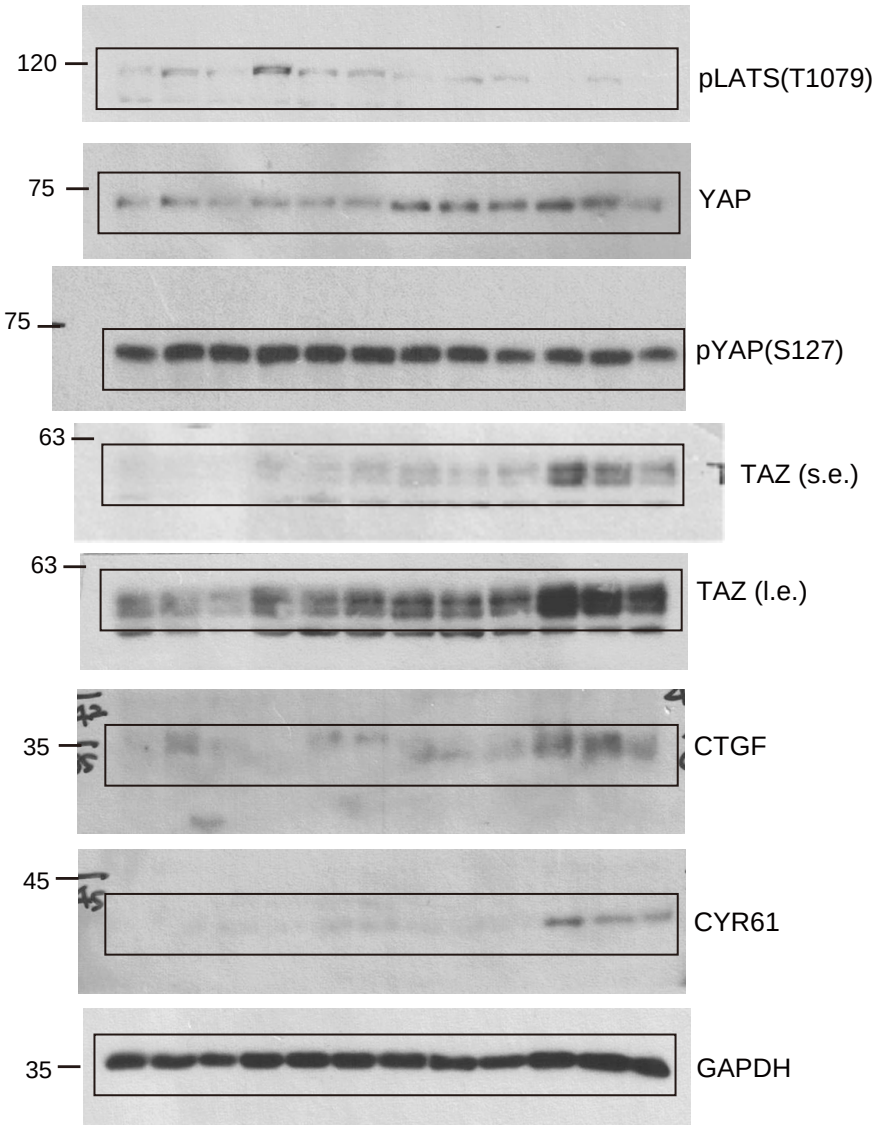
1. Full unedited blot for Figure 2D, 3A, 3D, 3G, 3H, 4A, 4C, 4D, 4E, 4F, 4G, 5B, 5F and 7M.

2. Full unedited blot for Supplemental Figure 4G and 5D.

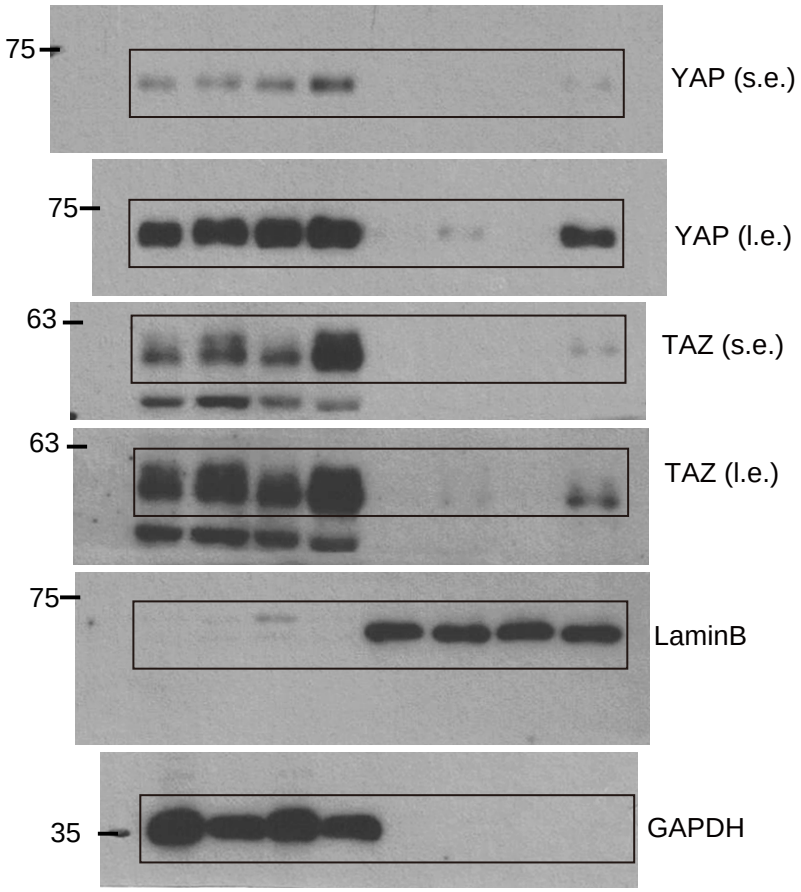
Full unedited blot for Figure 2D



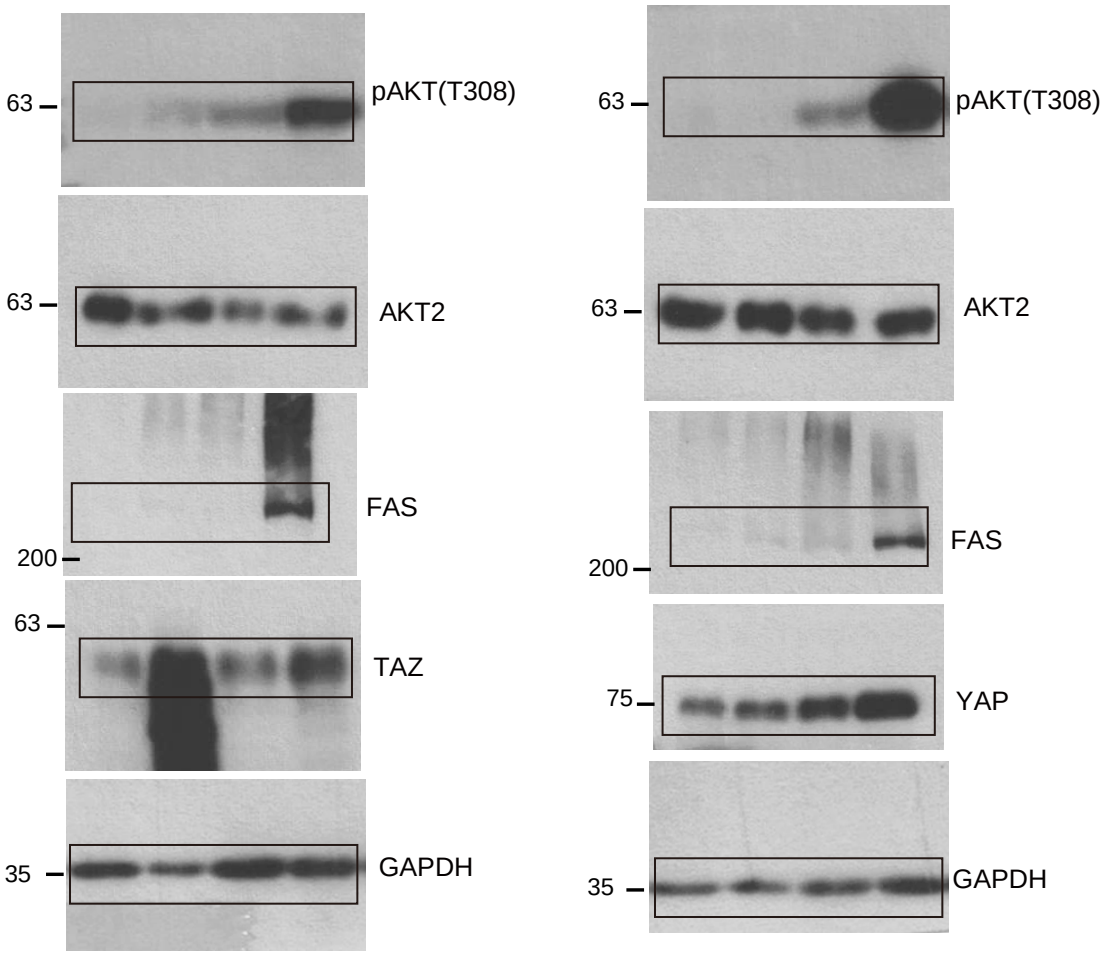
Full unedited blot for Figure 3A



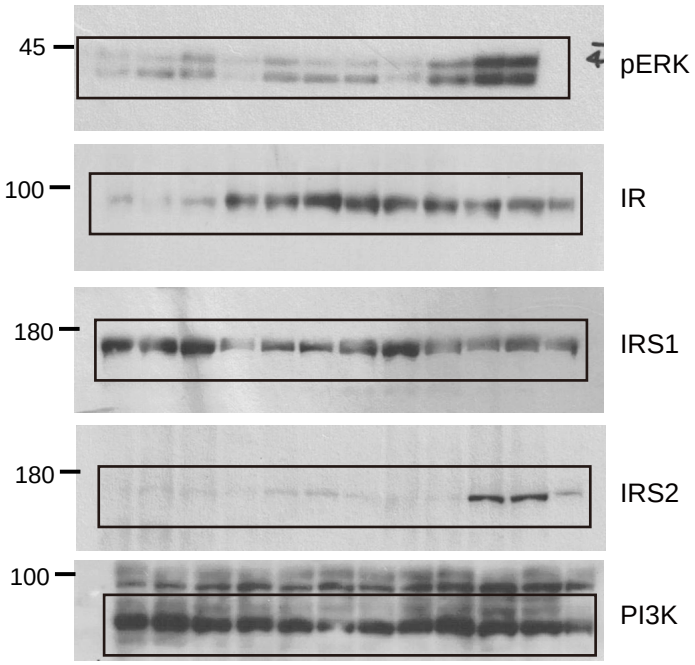
Full unedited blot for Figure 3D



Full unedited blot for Figure 3G, H



Full unedited blot for Figure 4A



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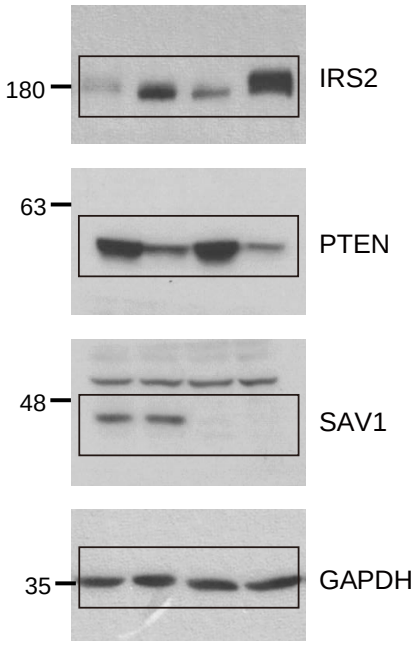
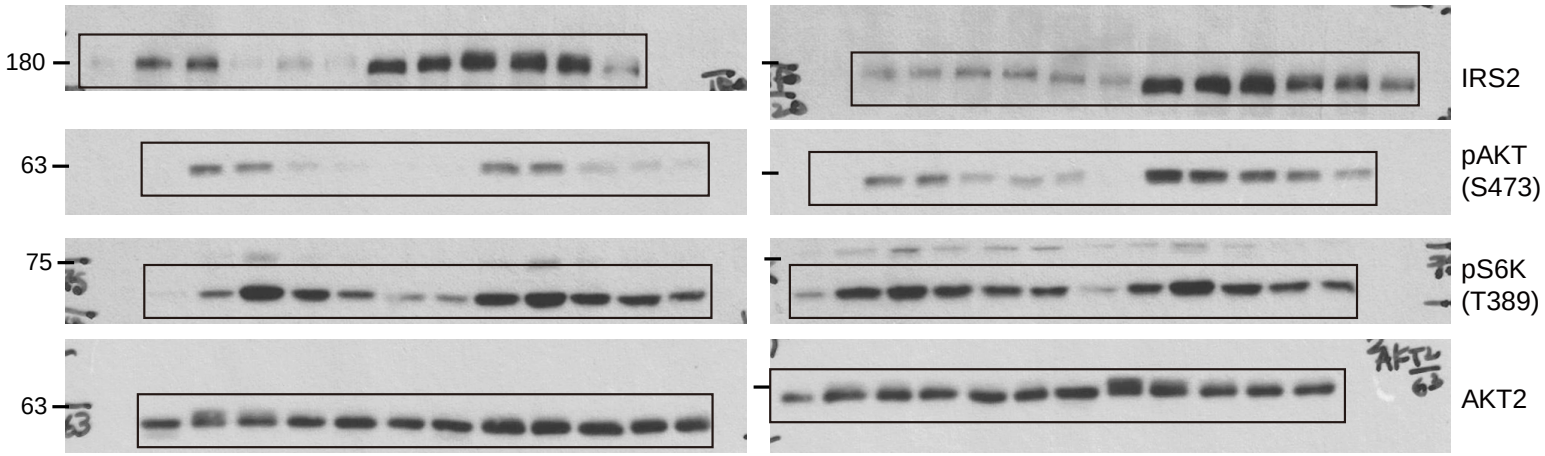


Full unedited blot for Figure 4D



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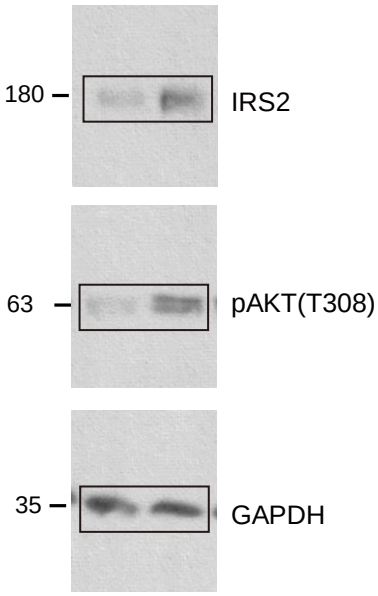

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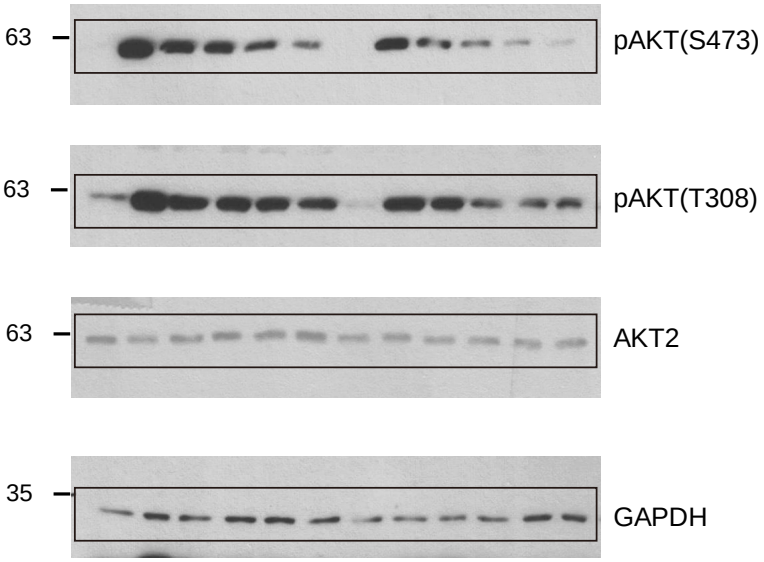

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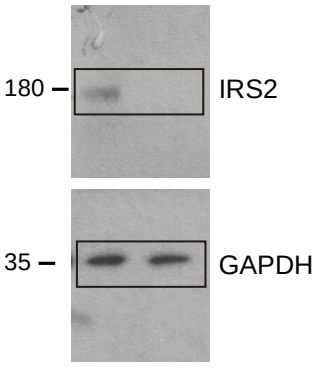

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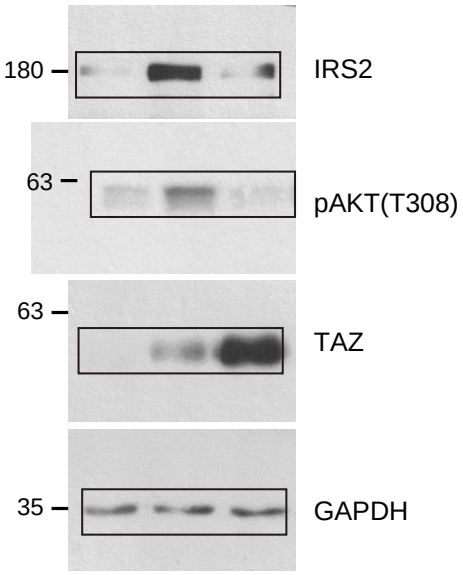
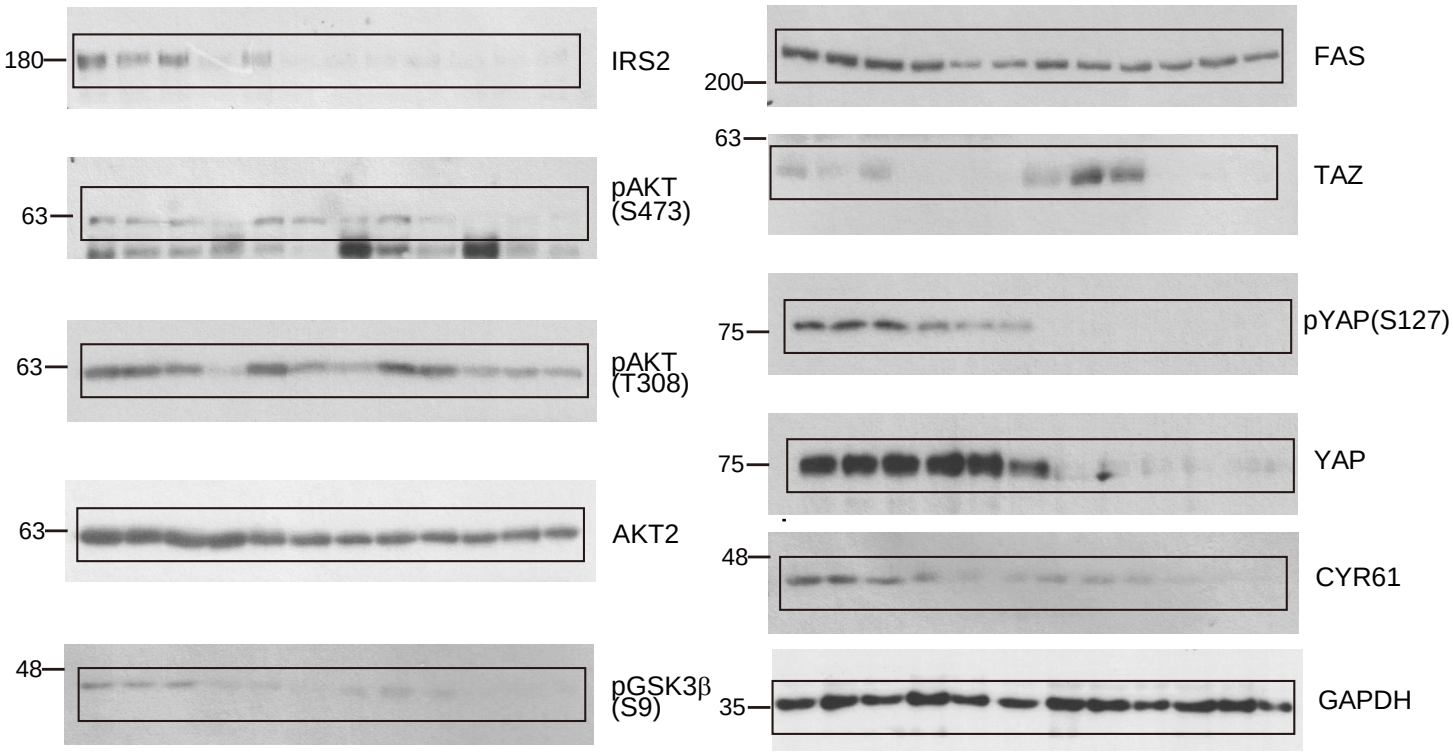
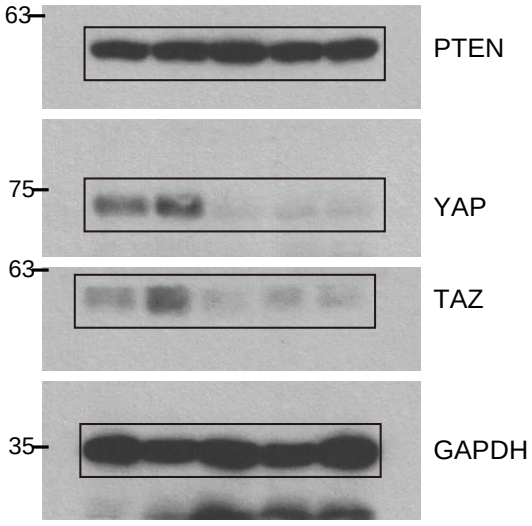


Full unedited blot for Figure 5B



Full unedited blot for Figure S4G



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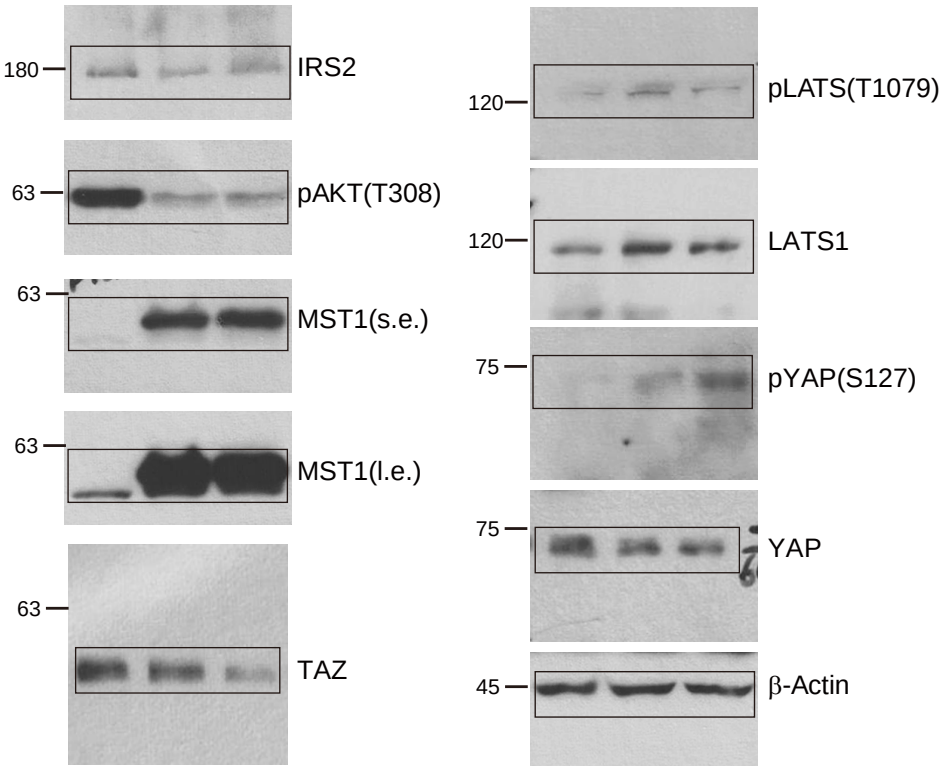

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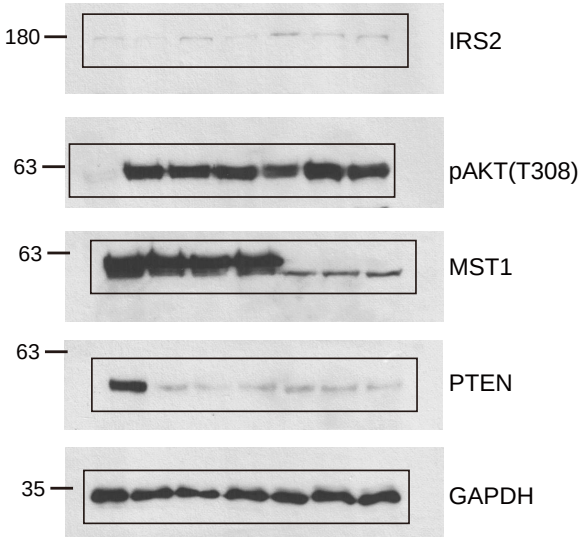

Full unedited blot for Figure 7M