Supplemental Information (SI)

Degradation of splicing factor SRSF3 contributes to progressive liver disease

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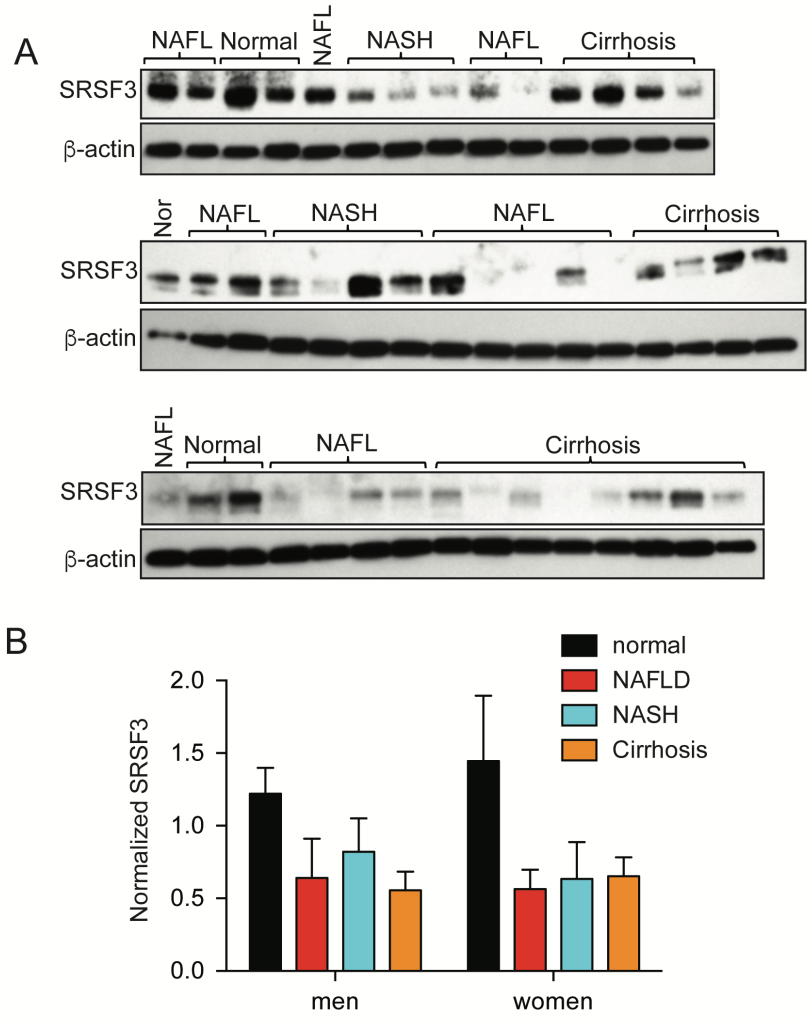
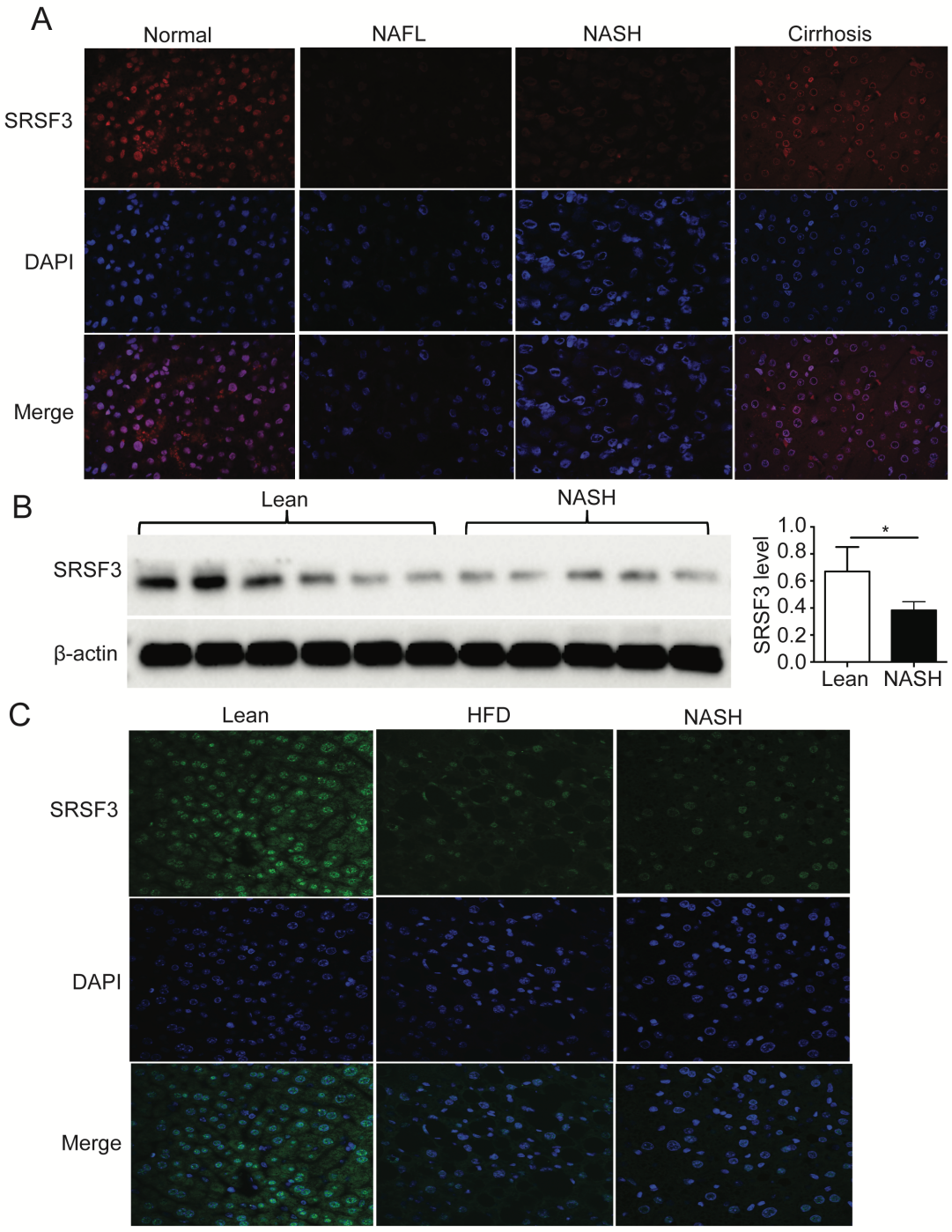
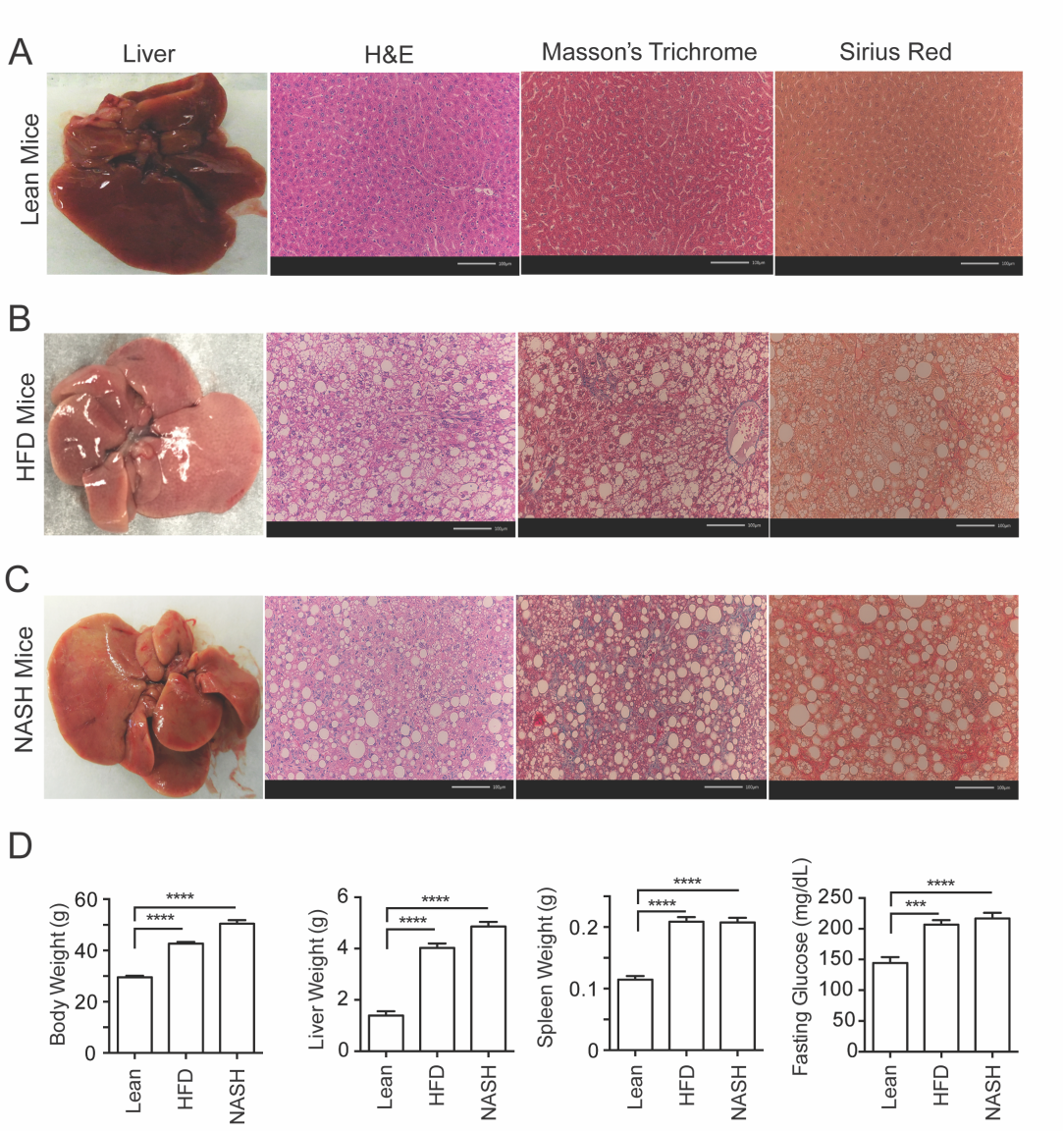


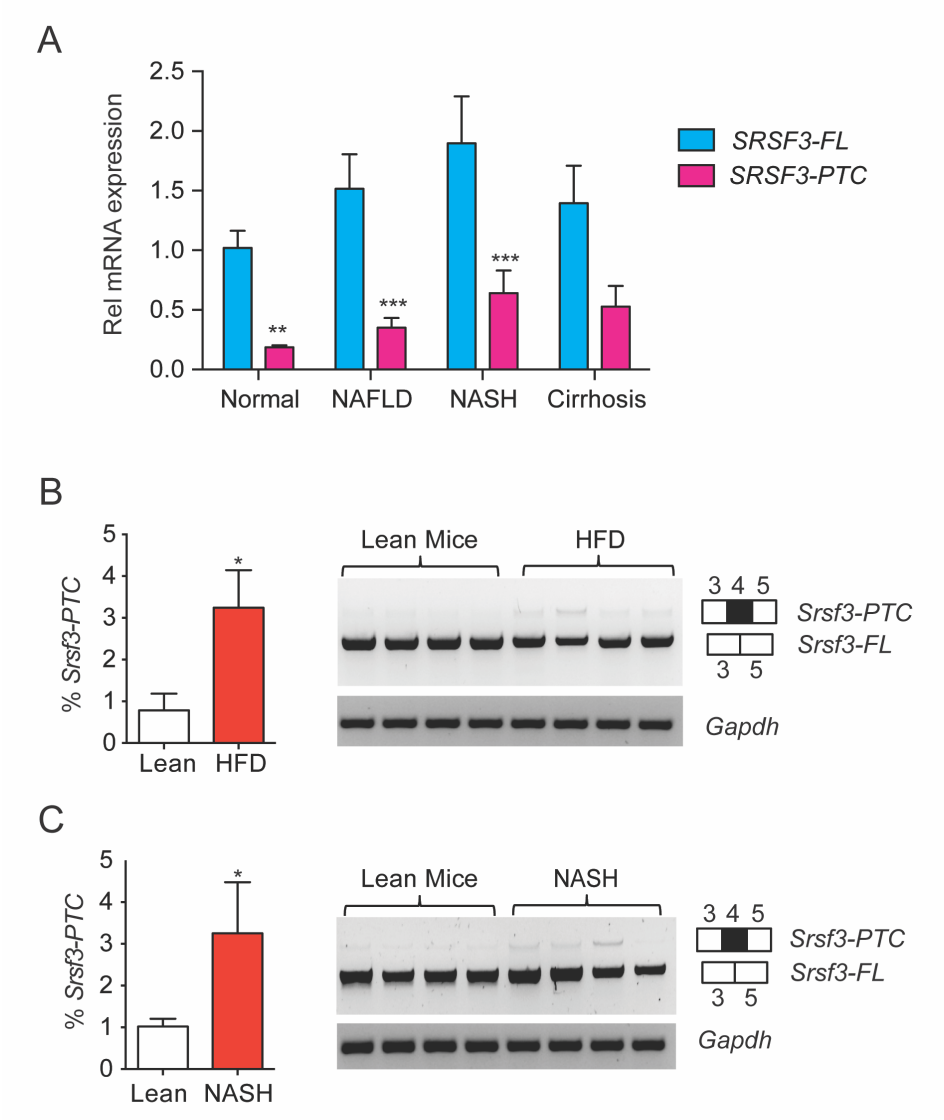
Supplemental Figure 1. (A) Immunoblotting for SRSF3 in liver extracts from deceased normal subjects or subjects with NAFL, NASH or Cirrhosis. **(B)** Quantification of SRSF3 proteins levels in the four groups separated by sex. Results are shown as mean ± SEM.



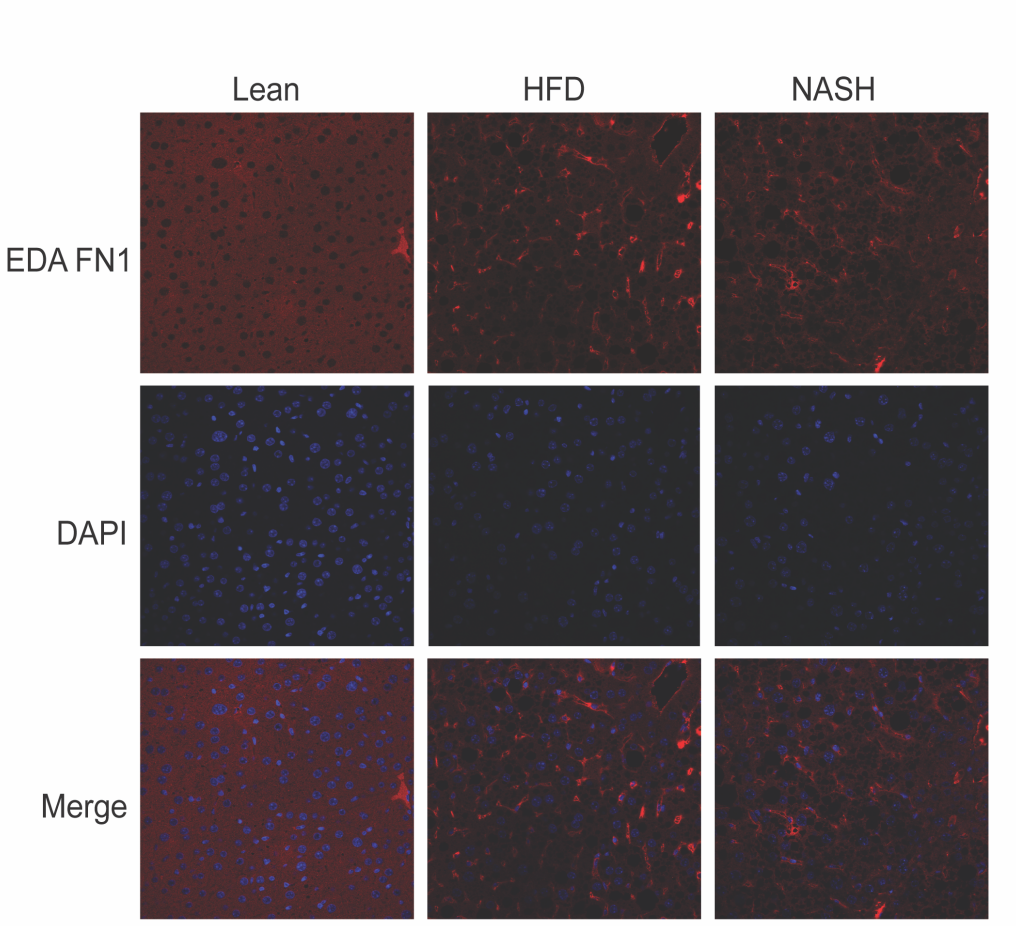
Supplemental Figure 2. (A) Immunofluorescence staining for SRSF3 on FFPE sections from human normal, NAFL, NASH and cirrhosis**. (B)** Immunoblotting for SRSF3 in liver extracts from lean and obese (NASH) mice. Graph shows quantification of SRSF3 protein levels normalized to β-actin. Results are presented as mean±SEM. **(C)** Immunofluorescent staining for SRSF3 on FFPE liver section from lean, obese (HFD), and obese NASH diet mice. Scale bar, 50 µM.



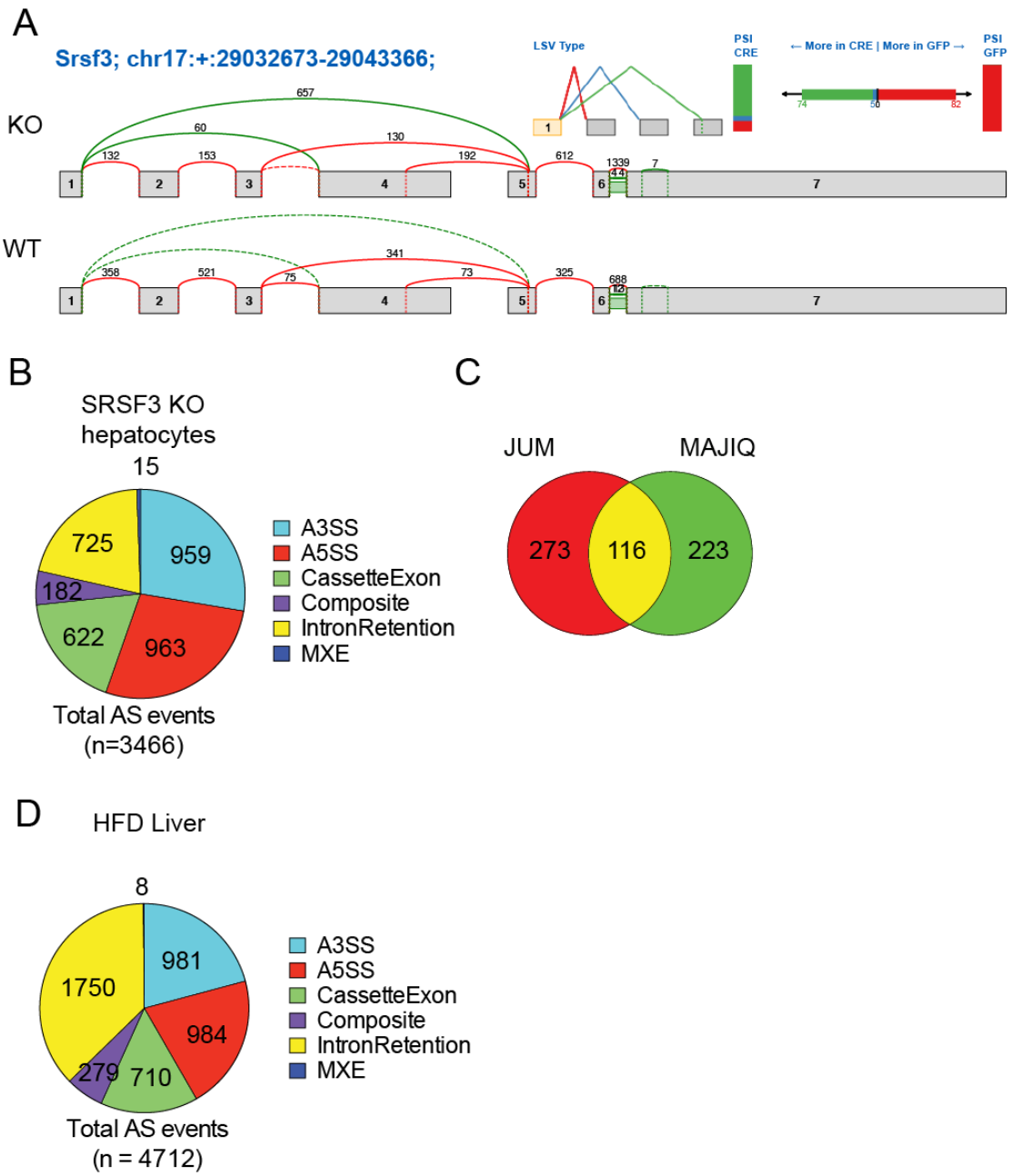
Supplemental Figure 3. Representative images of liver and liver sections from lean mice **(A)**, obese HFD **(B)** and obese NASH **(C)** mice. Liver section stained with H&E, Masson's trichrome and Sirius red for fibrosis. (**D**) Body weight, liver weight, spleen weight and fasting glucose from HFD and NASH mice. Asterisks indicate statistical significance by ANOVA, *p<0.05, **p<0.01, ****p<0.0001. Scale bar, 100 μM.



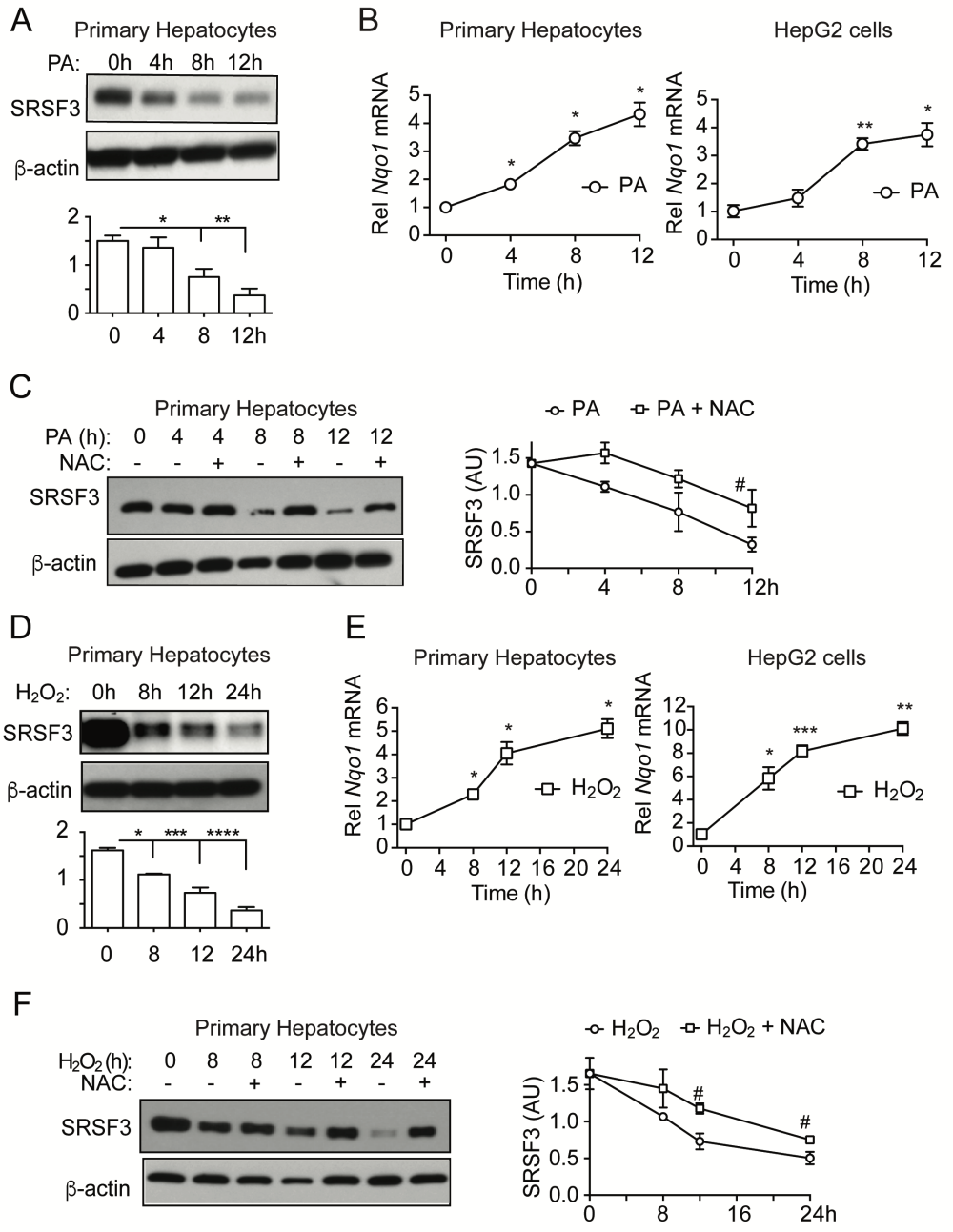
Supplemental Figure 4. (A) Relative expression of *SRSF3-FL* and *SRSF3-PTC* transcripts in liver RNA from normal subjects, and subjects with NAFLD, NASH and cirrhosis. 2-way ANOVA indicates a significant isoform effect ($p<0.0001$) and a weaker group effect ($p=0.033$) but no significant interaction. **(B and C)** Graphs on left show QPCR for *Srsf3-PTC* expressed as % *Srsf3* transcript expression. Gel images show RT-PCR on RNA from hepatocyte cells from lean, HFD and NASH mice using *Srsf3* primers spanning exon 3 to 5. Results are shown as mean ± SEM. Asterisks indicate statistical significance by ANOVA, *$p<0.05$, **$p<0.01$, ***$p<0.001$.



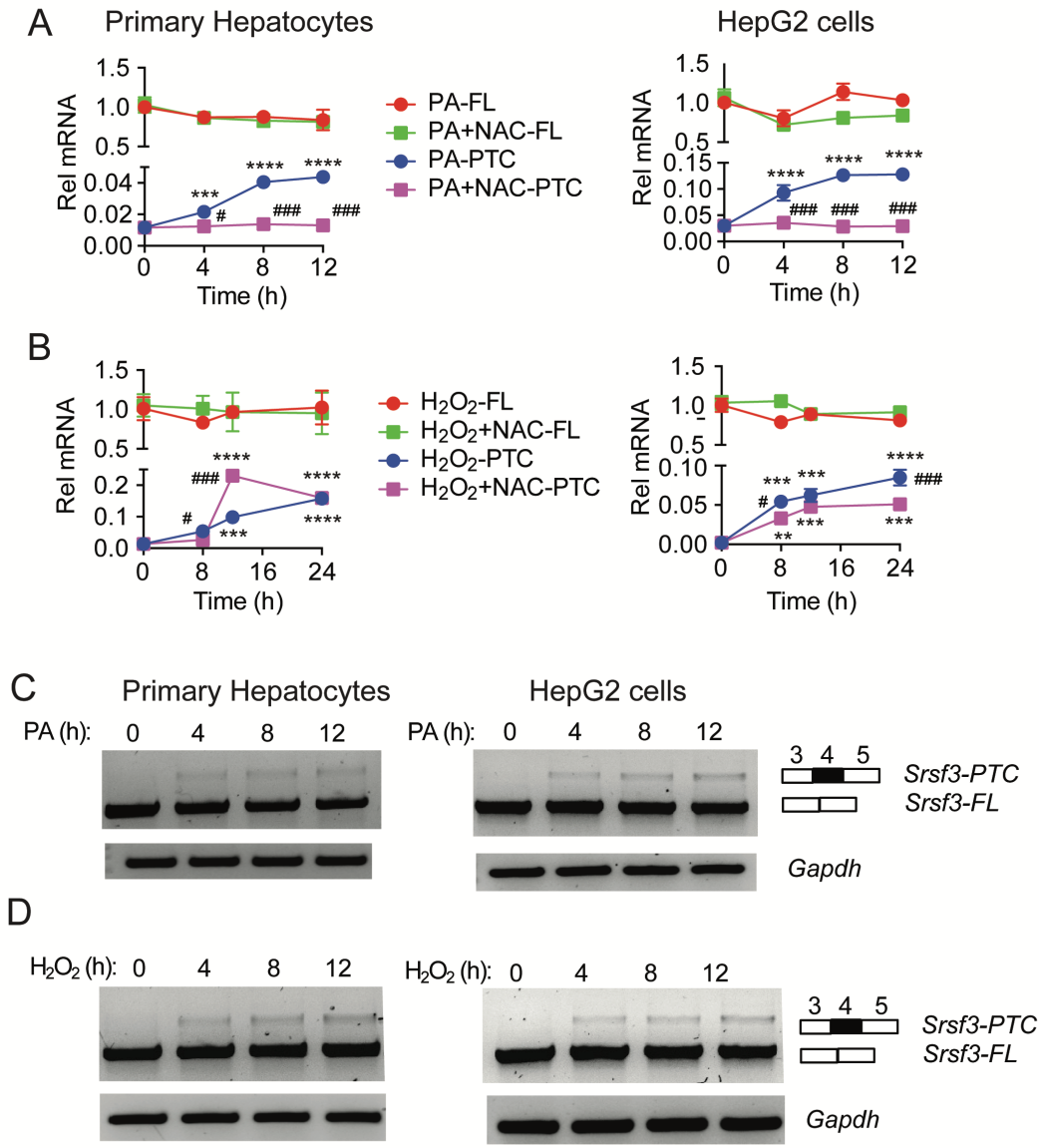
Supplemental Figure 5. Immunofluorescent staining for EDA-FN1 on FFPE section from normal, HFD, and NASH diet mice. Scale bar, 100 μM.



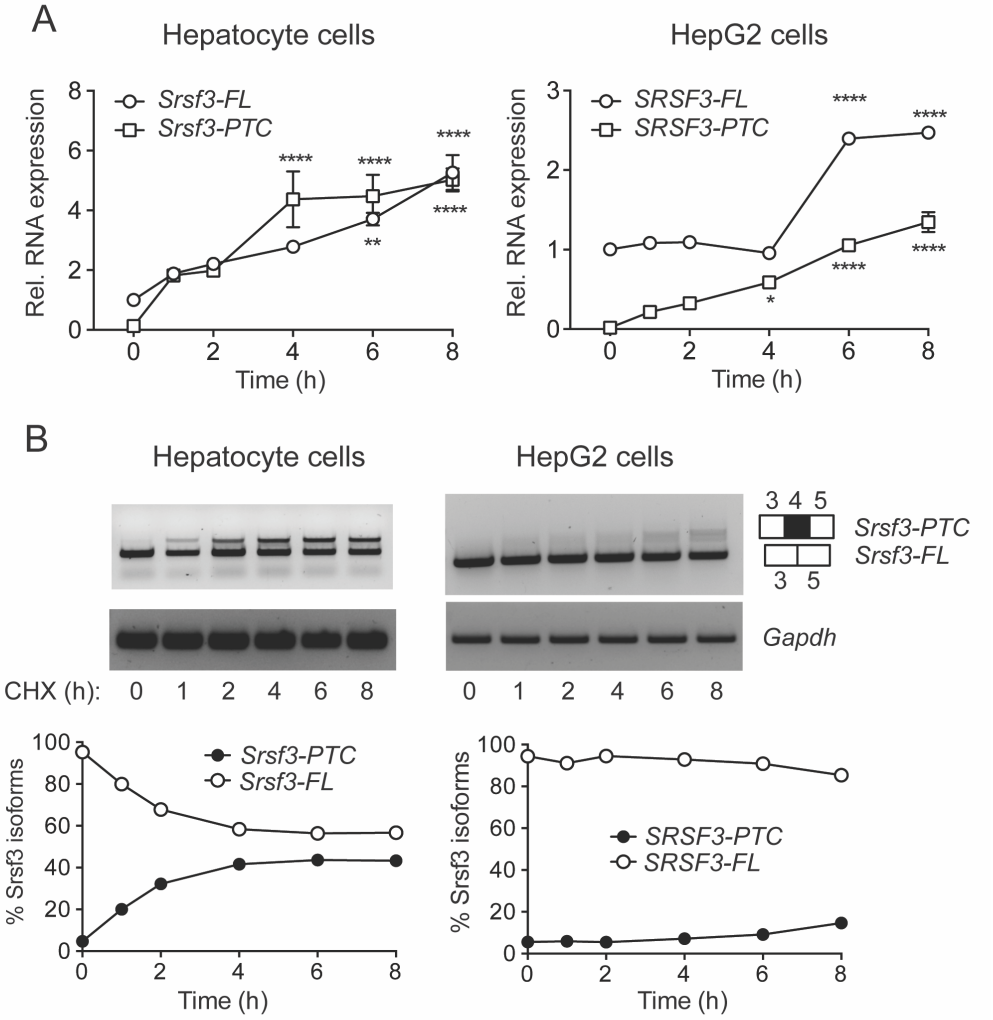
Supplemental Figure 6: (A) Exon junction reads in mRNA extracted from WT or KO hepatocytes in which SRSF3 exons 2 and 3 has been deleted by adenoviral expression of Cre recombinase. Exon gene structure is indicated by grey blocks. Red lines indicate known exon junctions, green indicate novel exon junctions. Data were analyzed by MAJIQ. Alternative splicing is shown in inset. **(B)** Total (n = 3466) alternative splicing events from hepatocytes with acute loss of SRSF3 by adenoviral expression of CRE vs. adenoviral expression of GFP analyzed using JUM. **(C)** Venn diagram showing number of genes altered in SRSFKO hepatocytes analyzed by JUM and MAJIQ. **(D)** Total (n = 4712) alternative splicing events from liver from lean mice on normal chow and obese mice on HFD analyzed using JUM. For **B** and **D** colors show different classes of events. A3SS indicates alternative 3' splice sites, A5SS alternative 5' splice sites, CassetteExon, Composite complex splicing events that do not fit a single category, IntronRetention retained introns, and MXE mutually-exclusive exons.



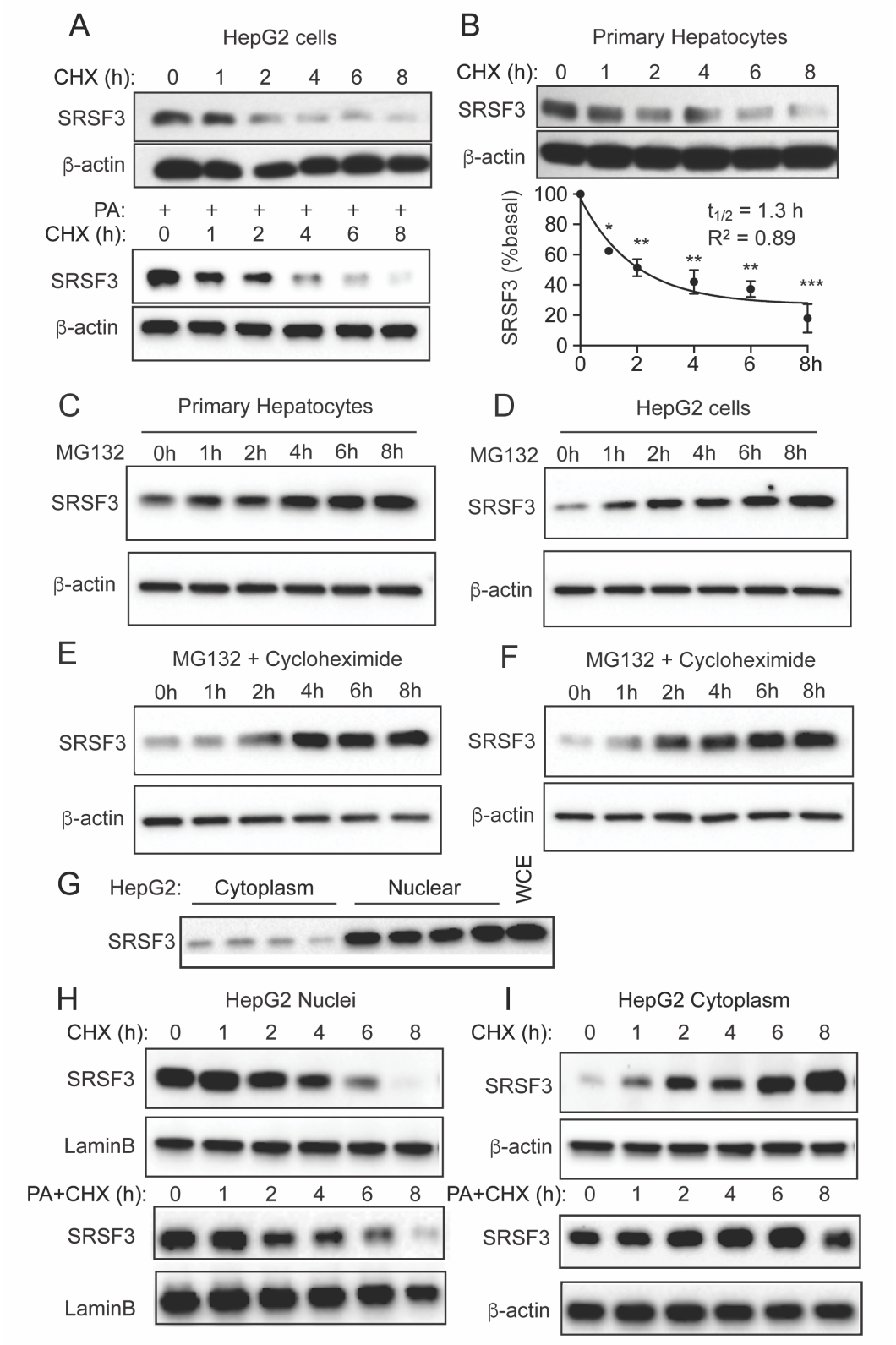
Supplemental Figure 7. (A) Mouse primary hepatocytes were exposed to 250μM palmitic acid (PA) for 4, 8, and 12 h. Extracts were immunoblotted for SRSF3 and β-actin. **(B)** Time course of *Nqo1* gene expression in mouse primary hepatocyte and human HepG2 cells treated with PA. Expression of *Nqo1* was measured by QPCR. **(C)** Mouse primary hepatocytes were exposed to 250 μM PA for 4, 8, and 12 h in the presence of the anti-oxidant N-acetyl-cysteine (NAC) to scavenge ROS. Cell extracts were immunoblotted for SRSF3. **(D)** Mouse primary hepatocytes were exposed to 500 μM H_2O_2 for 8, 12, and 24 h in the presence of NAC. Cell extracts were immunoblotted for SRSF3. Graphs show quantification of the SRSF3 protein normalized to β-actin (mean±SEM, n=3/group). **(E)** Time course of *Nqo1* gene expression in mouse primary hepatocyte and human HepG2 cells treated with 500 μM H_2O_2 for 8, 12, and 24 h. **(F)** Mouse primary hepatocytes were exposed to 500 μM H_2O_2 for 8, 12, and 24 h in the presence of NAC. Asterisks indicate statistical significance by ANOVA vs. time 0, # indicates significance v.s. untreated control, *p<0.05, **p<0.01, ***p<0.001.



Supplemental Figure 8. (A,B) Time course of *Srsf3* transcript isoform expression in cells treated with PA or H_2O_2 in the absence or presence of N-acetylcysteine (NAC). *Srsf3-FL* and *Srsf3-PTC* expression were measured by QPCR. Results are shown as mean ± SEM. 2-way ANOVA indicates a significant time and isoform effect ($p<0.0010$ and significant interaction ($p=0.002$) for PA on hepatocytes, significant time, isoform and interaction effects ($p<0.0001$) for PA on human HepG2 cells, a significant isoform effect only ($p<0.001$) for H2O2 on hepatocytes, and significant time an isoform effects ($p<0.0001$, 0.01) for H2O2 on HepG2 cells. Asterisks indicate statistical significance v.s. time 0 by post-hoc testing, *$p<0.05$, **$p<0.01$, ****$p<0.0001$. # Indicates statistical significance v.s. untreated control. **(C,D)** RT-PCR splicing analysis using RNA from primary hepatocyte and human HepG2 cells treated with PA and H_2O_2 using SRSF3 primers spanning exon 3 to 5.

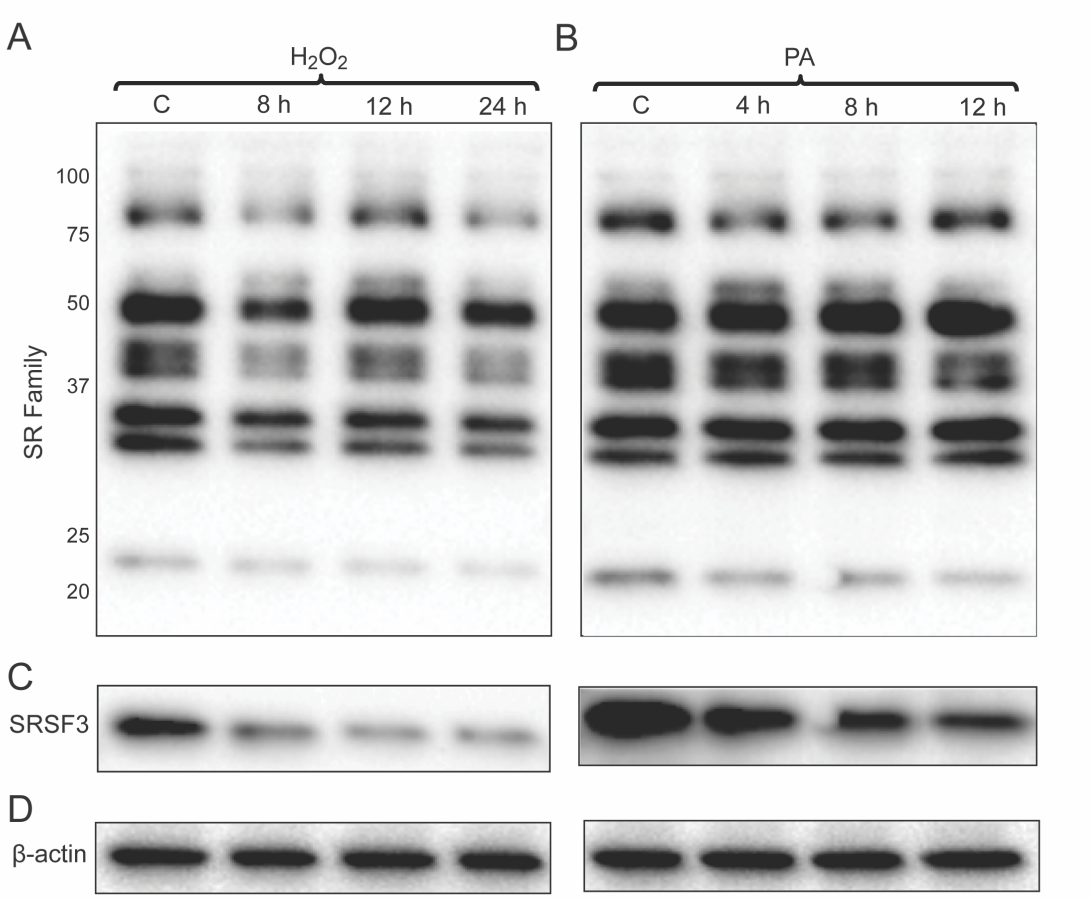


Supplemental Figure 9. (A) Time course of *Srsf3* expression in mouse primary hepatocyte and human HepG2 cells treated with cycloheximide (CHX). *Srsf3-FL* and *Srsf3-PTC* expression were measured by QPCR. Results are shown as mean ± SEM. 2-way ANOVA indicated a significant time effect ($p<0.0001$) in primary hepatocytes, and significant time and isoform effects ($p<0.0001$), and a significant interaction ($p=0.003$). Asterisks indicate statistical significance by post-hoc testing, *$p<0.05$, **$p<0.01$, ****$p<0.0001$. **(B)** RT-PCR splicing analysis using RNA from primary hepatocyte and human HepG2 cells treated with CHX using SRSF3 primers spanning exon 3 to 5. Isoform expression is quantified below.

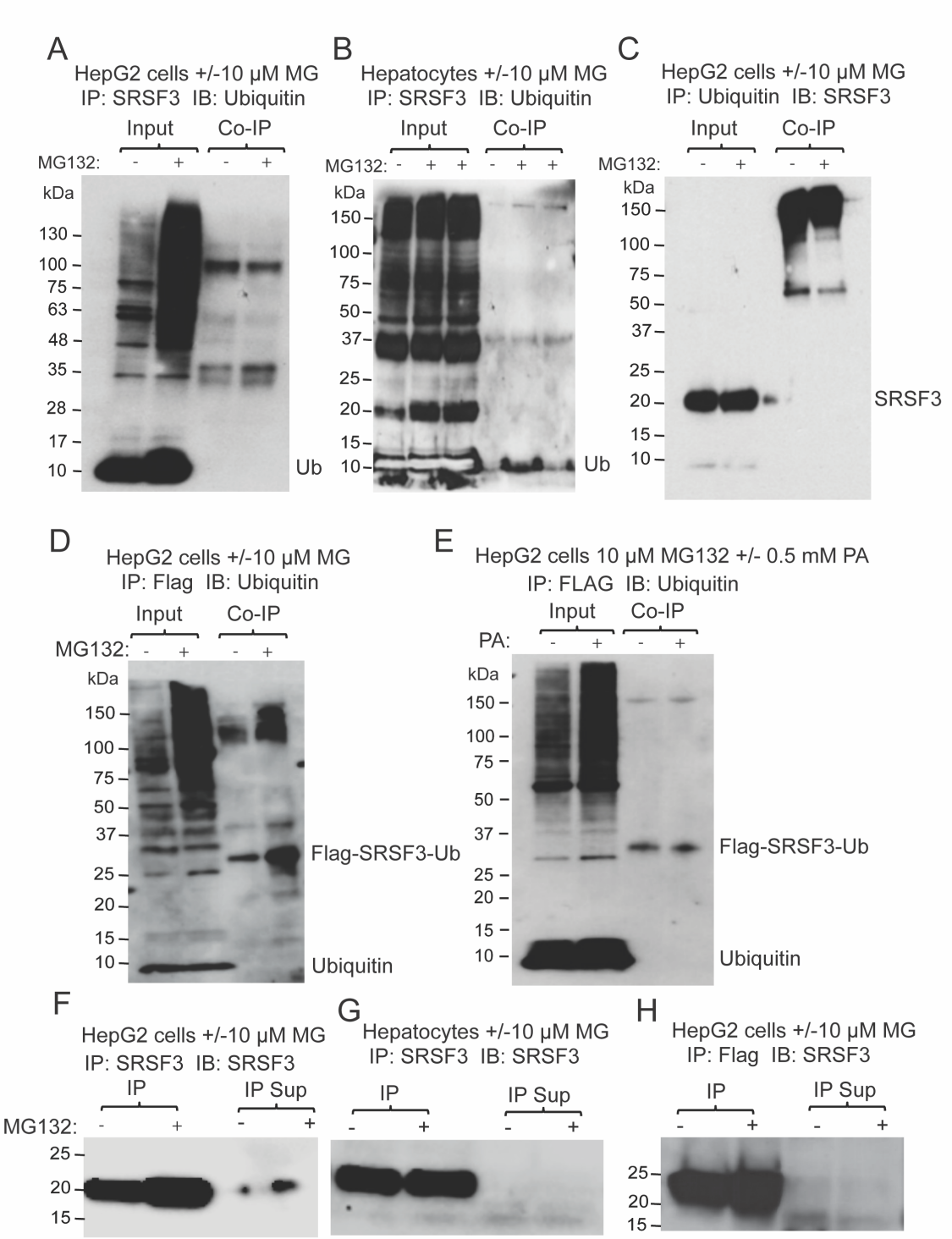


Supplemental Figure 10. (A) Human HepG2 cells were exposed to 50 µg/ml cycloheximide for 0-8 h in the absence or presence of 500 µM PA. Cell extracts were immunoblotted for SRSF3 and β-actin. Quantification is shown in Figure 3E. (B) Mouse primary hepatocytes were exposed to 50 µg/ml cycloheximide for 0-8 h. Cell extracts were immunoblotted for SRSF3. Graphs show curve fit analysis of SRSF3 levels using exponential decay kinetics. The half-life of SRSF3 is

given ($t_{1/2}$) as is the R^2 value for the curve fit. Results are presented as mean ±SEM (n=3/group). Asterisks indicate statistical significance by ANOVA v.s. time 0, *p<0.05, **p<0.01, ***p<0.001. **(C and D)** Mouse primary hepatocytes and human HepG2 cells exposed to 10 μM MG132 for 0-8 h. Cell extracts were Immunoblotted for SRSF3. **(E and F)** Expression of SRSF3 in mouse primary hepatocytes and human HepG2 cells exposed to 10 μM MG132 plus 50 μg/ml cycloheximide for 0-8 h. Cell extracts were Immunoblotted for SRSF3. **(G)** Human HepG2 cells were fractionated into cytoplasmic or nuclear extracts and equal amounts of protein immunoblotted for SRSF3. WCE indicates a whole cell lysate run as a control lane. **(H and I)** Human HepG2 cells were exposed to 50 μg/ml cycloheximide for 0-8 h. Cells were fractionated into nuclear versus cytoplasmic extracts that were immunoblotted for SRSF3. Nuclear extracts were immunoblotted for laminB to control for loading whereas cytoplasmic extracts were blotted to β-actin.

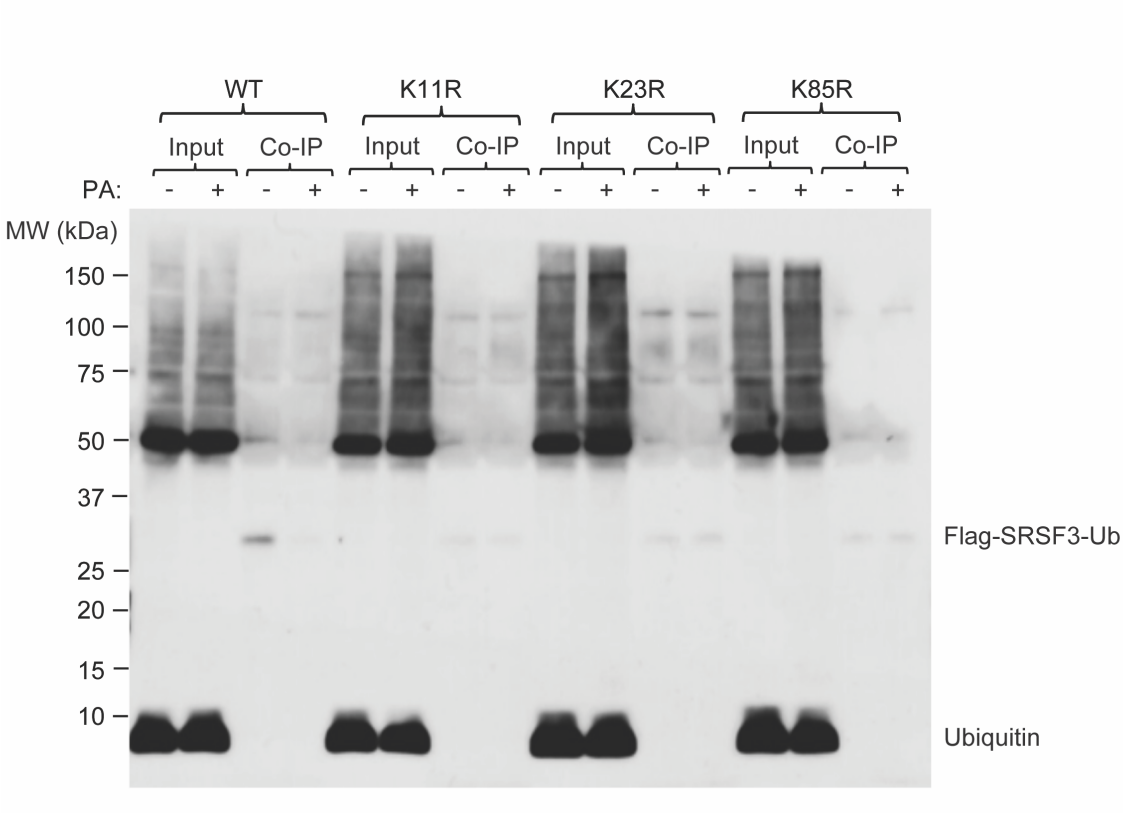


Supplemental Figure 11. Expression of SR family in HepG2 cells exposed to **(A)** 500 μM of H_2O_2 for 8-24 hrs and **(B)** 500 μM of PA for 4-12 hrs. Cells extracts were immunoblotted of anti-phosphoepitope SR proteins antibody (MABE50). **(C)** Blots are stripped and immunoblotted for SRSF3. **(D)** Blots are stripped and immunoblotted for β−actin.

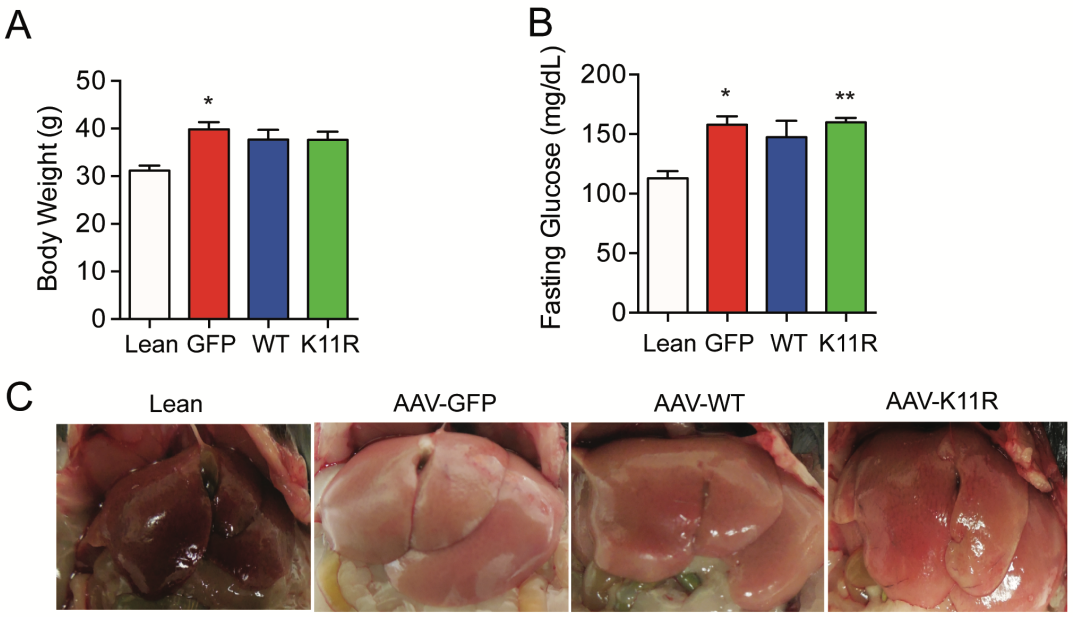


Supplemental Figure 12. Ubiquitination of SRSF3 in response to MG132. Human HepG2 cells **(A)** and primary hepatocytes **(B)** were treated with 10 μM MG132 for 2 h. Cell lysates were immunoprecipitated with SRSF3 antibodies then immunoblotted for ubiquitin. **(C)** Human HepG2 cells were treated with 10 μM MG132 for 2 h. Cell lysates were immunoprecipitated with ubiquitin antibodies then immunoblotted for SRSF3. **(D)** Human HepG2 cells were transfected with Flag-tagged SRSF3 then treated with 10 μM MG132, and cell lysates immunoprecipitated with an anti-Flag antibody then immunoblotted for ubiquitin. **(E)** HepG2 cells were transfected with Flag-tagged SRSF3 then treated with 10 μM MG132 plus 500 μM PA, and cell lysates

immunoprecipitated with an anti-Flag antibody then immunoblotted for ubiquitin. Input indicates input control, Co-IP indicates the co-immunoprecipitated proteins. Human HepG2 cells **(F)** and primary hepatocytes **(G)** were treated with 10 μM MG132 for 2 h. Cell lysates were immunoprecipitated with SRSF3 antibodies then immunoblotted for SRSF3. **(H)** Human HepG2 cells were transfected with Flag-tagged SRSF3 then treated with 10 μM MG132, and cell lysates immunoprecipitated with an anti-Flag antibody then immunoblotted for SRSF3. IP indicates the immunoprecipitated proteins; IP Sup indicates the immunoprecipitated proteins in supernatant.



Supplemental Figure 13. HepG2 cells were transfected with series of mutant SRSF3s, then cells were treated with PA or vehicle for 8 h. Cell lysates immunoprecipitated with an anti-Flag antibody then immunoblotted for ubiquitin antibody. Input indicates input control, Co-IP indicates the co-immunoprecipitated proteins.



Supplemental Figure 14. Body weight **(A)** and fasting glucose **(B)** from GFP, WT and K11R infected mice on NASH diet. **(C)** Representative images of liver from lean, GFP, WT and K11R mice**.** Asterisks indicate statistical significance by ANOVA, *p<0.05, **p<0.01, ****p<0.0001.

Supplemental Table 2. List of oligonucleotides

Gene	Forward Primer (5'-3')	Reverse Primer (5'-3')	Use in this study
FN1 EDA	TGACTATTGAAGGCTTGCAGCC	CTGATACAACCACGGATGAGCT	RT-PCR (Human)
INSRA	AGGACCTGGTCTCCACCATTC	ATGGTCCTCGCACTGACGTA	RT-PCR (Human)
Slk	ATCGAACGCCTGGAACAAGAG	CTCTCATGAGCTGTTGCTTG	RT-PCR (Human)
Myo1b	AGAGGTACCAGCAGACAAAGA	GATCCAAGCCAGTAAGCCCA	RT-PCR (Human)
FN1 EDA	GAAGGTTTGCAACCCACTGT	GCAGTAAAGCTGGTGGGTGT	RT-PCR (Mouse)
INSRA	CTGAAGGAGCTGGAGGAGTC	CACATTCCCAACATCGCCAA	RT-PCR (Mouse)
Slk	ACTAACCGCCTGAGAGACGA	TCTTCAAGCTCCCAAATTGC	RT-PCR (Mouse)
Myo1b	GTGATTCAGTCGTACATCCG	GTACCTTCAGTCCAAGCCAGTA	RT-PCR (Mouse)
SRSF3-FL-PTC	ATGCATCGTGATTCCTGTCCATTG	CTATTTCCTTTCATTTGACCTAGA	RT-PCR
NQO1	CGATCCTCCCTCAACATCTG	TCCCATCCTCTCTTCTTCAGAG	qRT-PCR
SRSF3-FL	TCGTCGTCCTCGAGATGATT	CCTATCTCTAGAAAGTGACCTGCTC	qRT-PCR
SRSF3-PTC	GCAACATCTGGCAAAACCTT	GCTTCTCCTTCTTGGGGATTA	qRT-PCR
Cidea	TGACATTCATGGGATTGCAGAC	GGCCAGTTGTGATGACTAAGAC	qRT-PCR
Cidec	GATGGACTACGCCATGAAGTC	GTGCTCACTGCCACATGC	qRT-PCR
Cd36	GGACATTGAGATTCTTTTCCTCTG	GCAAAGGCATTGGCTGGAAGAAC	qRT-PCR
Emr1 (F4/80)	CTTTGGCTATGGGCTTCCAGTC	GCAAGGAGGACAGAGTTTATCGTG	qRT-PCR
Clec4f	GAGGCCGAGCTGAACAGAG	TGTGAAGCCACCACAAAAAGAG	qRT-PCR
M36B4	ACCCTGAAGTGCTCGACATCACAG	GCAGGGGCAGCAGCCGCAAATGC	qRT-PCR

IL6	ATCCAGTTGCCTTCTTGGGACTGA	TAAGCCTCCGACTTGTGAAGTGGT	qRT-PCR
TNFα	TGAGACCAGCCTGTGCTATG	AAGCCAAAGCTGGTCAGTCTA	qRT-PCR
Acta2	CTGAGCGTGGCTATTCCTTC	CTTCTGCATCCTGTCAGCAA	qRT-PCR
Col1a1	CACCCTCAAGAGCCTGAGTC	GTTCGGGCTGATGTACCAGT	qRT-PCR
Fn1	TGGTGGCCACTAAATACGAA	GGAGGGCTAACATTCTCCAG	qRT-PCR
Timp1	CCAGAGCCGTCACTTTGCTT	AGGAAAAGTAGACAGTGTTCAGGCTT	qRT-PCR