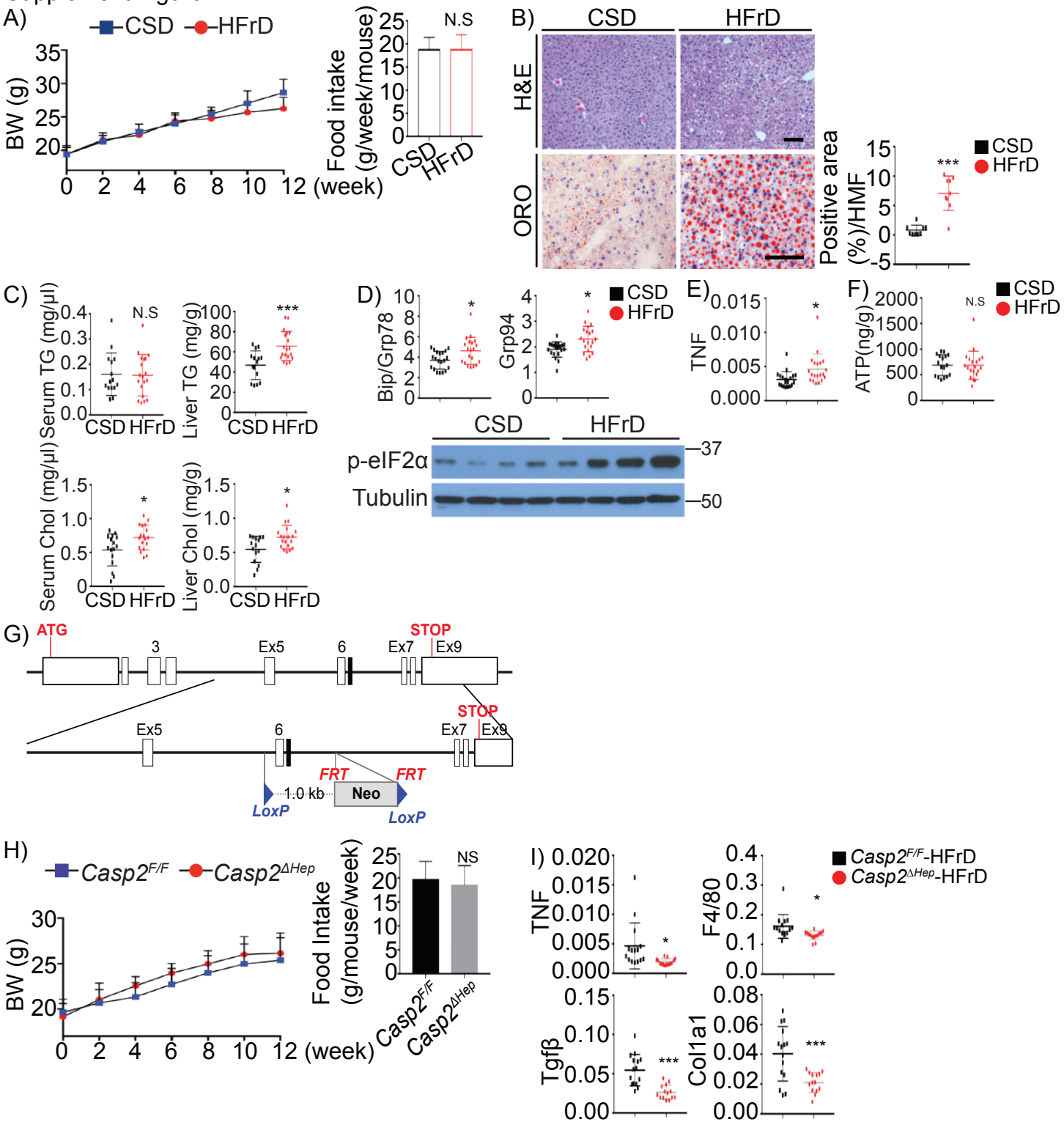
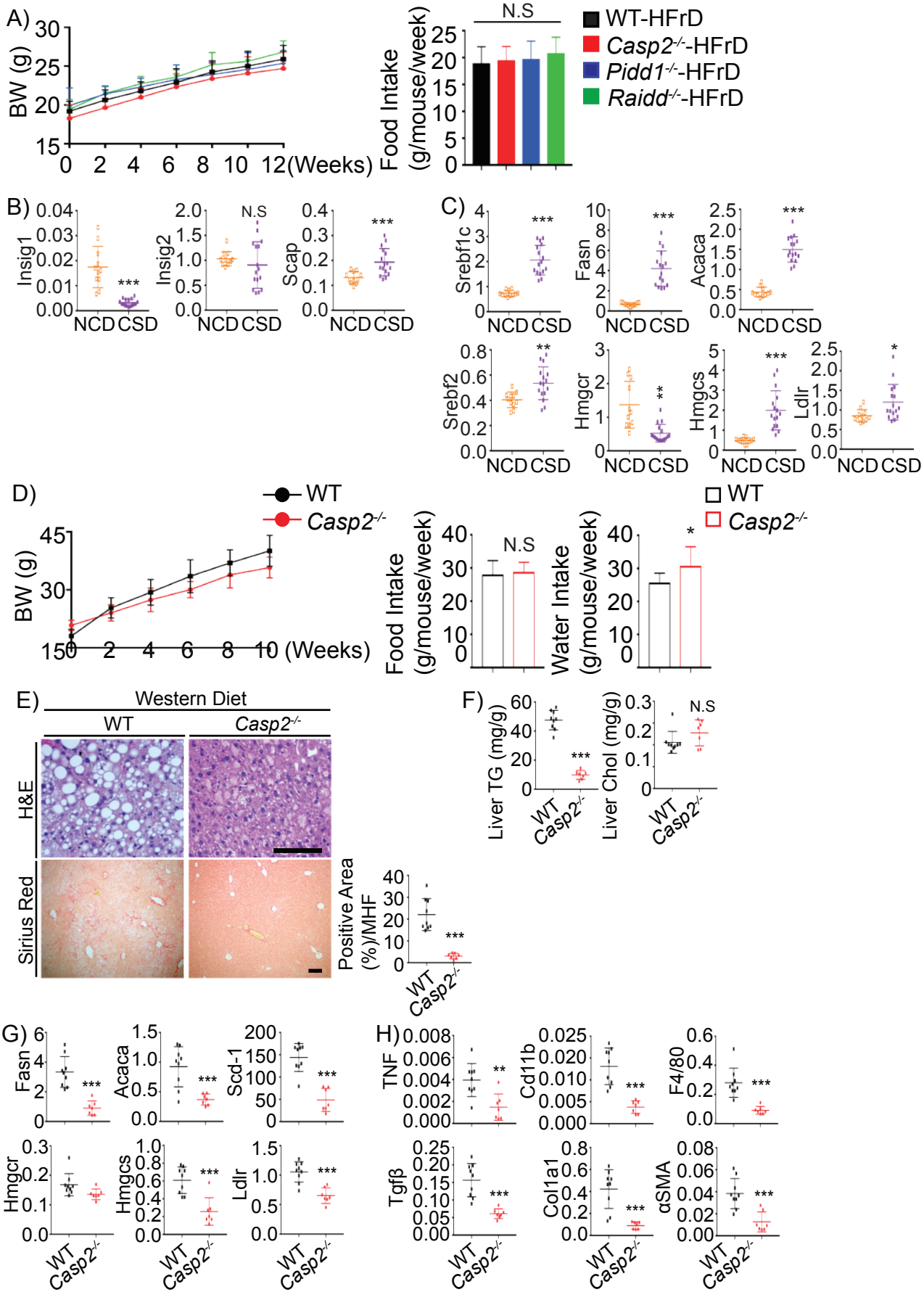
Supplement Figure 1



Supplement Figure 2



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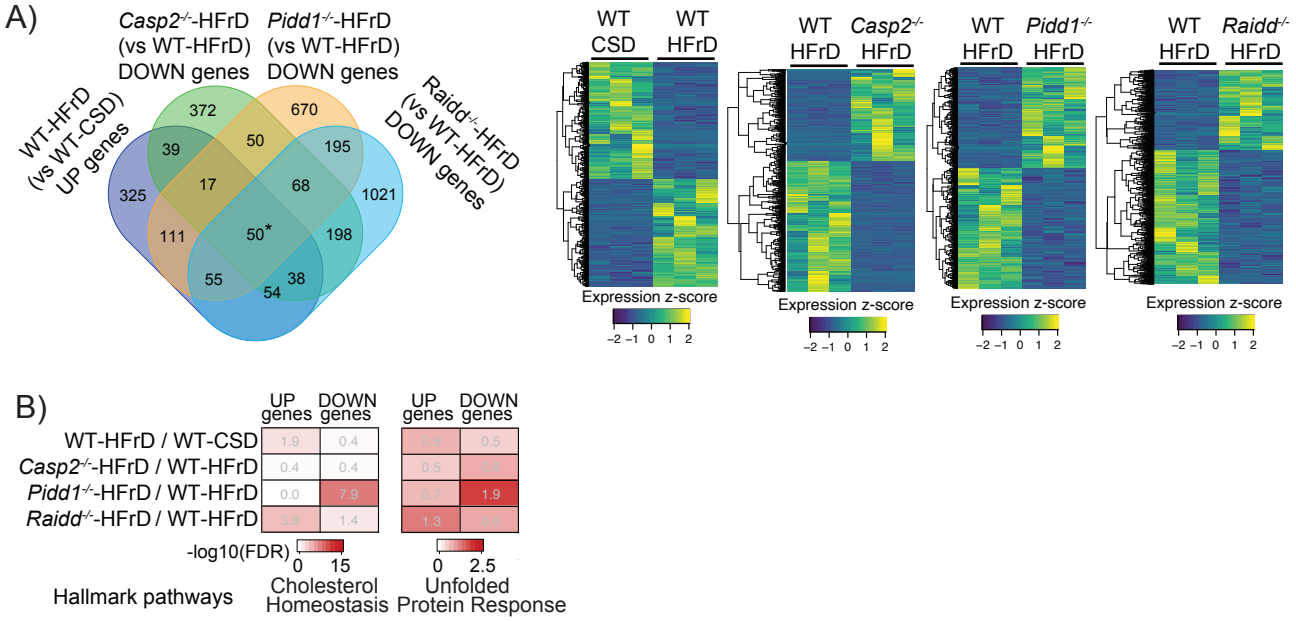

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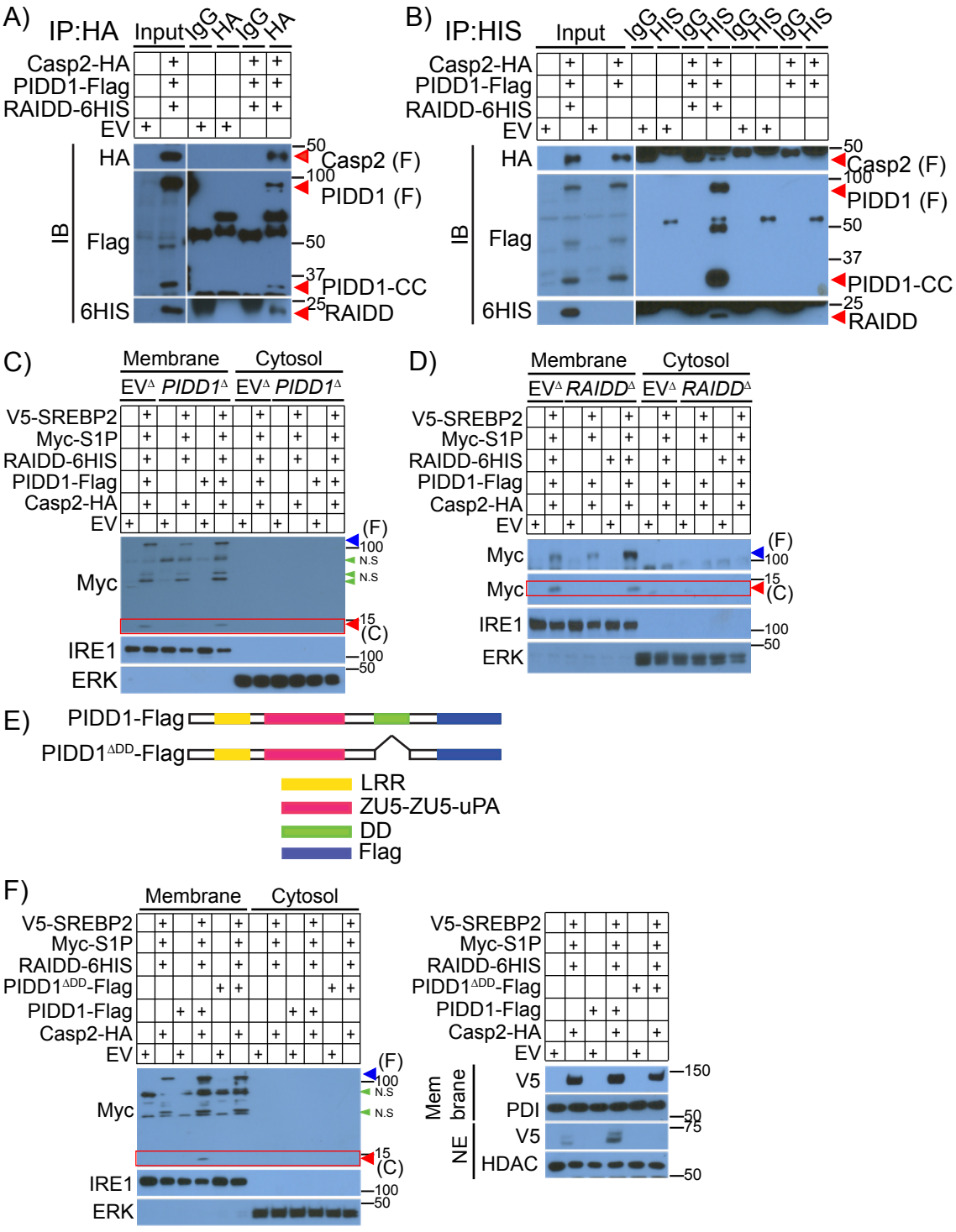

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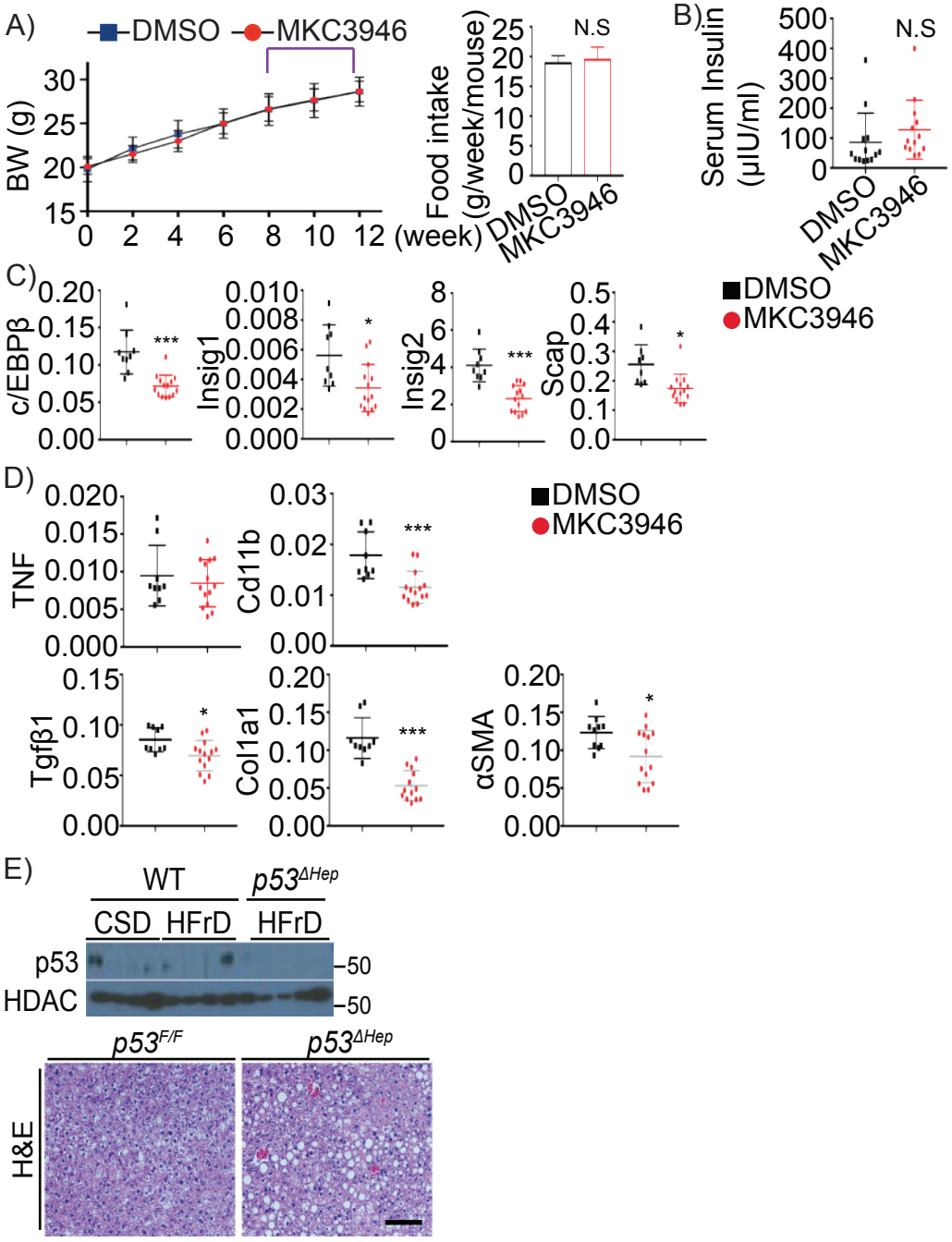
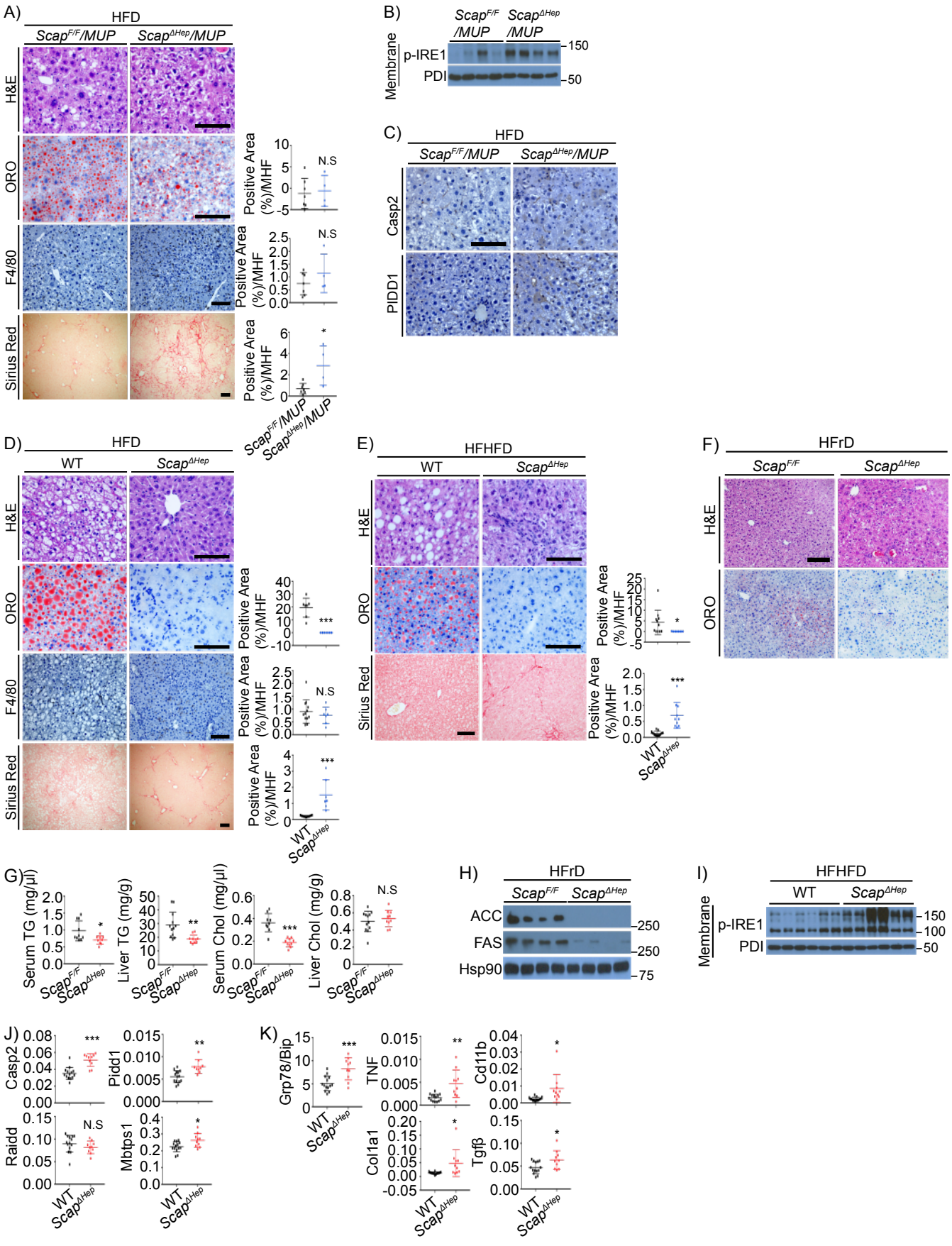


Supplement Figure 6



Supplement Figure 7

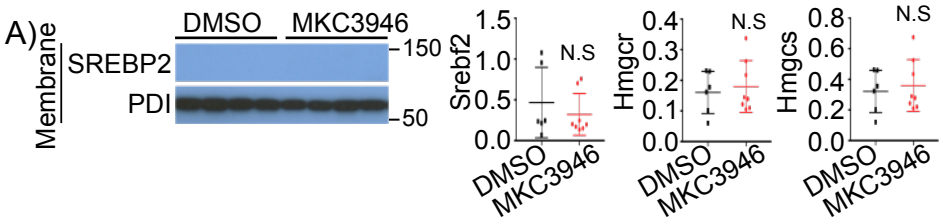


Figure S1, related to Figure 1. Hepatocyte-specific Casp2 ablation prevents fructose induced steatosis.

WT mice were fed cornstarch (CSD, n=21) or fructose diet (HFrD, n=20) for 12 weeks.

A. Body weight (BW) and food intake during the 12-week special diet feeding period.

B. Representative H&E-stained formalin fixed paraffin embedded (FFPE) liver sections and ORO-stained frozen liver sections. Four HMFs were taken from each liver and quantified by Image J software. Quantification is on the right.

C. Serum and liver TG and Chol from above livers.

D. Relative BIP/Grp78 and Grp94 mRNA amounts (top) and p-eIF2α IB (bottom) in above livers.

E. Relative TNF mRNA amounts in above livers.

F. ATP concentration in above livers.

G. A schematic illustration describing the generation of Casp2 floxed mice.

H. BW and food intake during HFrD feeding in *Casp2*$^{F/F}$ and *Casp2*$^{\Delta Hep}$ mice.

I. Relative fibrogenic gene mRNA amounts in HFrD-fed *Casp2*$^{F/F}$ or *Casp2*$^{\Delta Hep}$ livers.

Results are mean $\pm$ SEM. Scale bar, 100 μm. Statistical significance was determined by two-tailed Student's t test. *$p < 0.05$, **$p < 0.005$, ***$p < 0.001$.

Figure S2, related to Figure 2. PIDDosome components are required for fructose induced hepatosteatosis.

A. BW and food intake during HFrD feeding in the indicated mice.

B. Relative *Scap*, *Insig1*, and *Insig2* mRNA amounts in livers of WT mice fed normal chow diet (NCD, n=11) or CSD (n=16).

C. Relative lipogenic enzyme mRNA amounts in above livers.

WT (n=9) and *Casp2*$^{-/-}$ (n=7) mice were fed Western diet along with 15% fructose and glucose water for 10 weeks.

D. BW and food intake during Western diet feeding in the indicated mice.

E. Representative H&E and Sirius red stained FFPE above livers. Quantification is on the right.

F. Liver TG and Chol amounts in above livers.

G-H. Relative mRNAs of lipogenic enzymes (G) and inflammatory, fibrogenic genes (H) in above livers.

Statistical significance was evaluated by two-tailed Student's t test.

*$p < 0.05$, **$p < 0.005$, ***$p < 0.001$.

Figure S3, related to Figure 3. PIDDosome components control fructose induced lipid metabolizing genes.

A. Venn diagram showing the overlapping genes from 4 comparison analysis and Heatmap showing differentially expressed genes between the groups. Four comparative analyses were conducted. Gene expressions were visualized with z-score.

B. Relative expression of cholesterol biosynthesis and unfolded protein response (UPR) pathways in indicated livers.

Figure S4, related to Figure 4. PIDD1 and RAIDD are needed for Casp2 activation.

A-B. IP analysis of PIDDosome components using HA (A) or His (B) antibodies in lysates of cells transfected with the indicated plasmids.

C-D. Parental, *PIDD1* (*PIDD1*$^{\Delta}$)-ablated (C) or *RAIDD* (*RAIDD*$^{\Delta}$)-ablated (D) HEK293 cells were transfected with the indicated plasmids, membrane and cytoplasmic fractions were separated and the indicated proteins were detected by IB analysis.

E. Schematic illustration of the PIDD1$^{\Delta DD}$ construct.

F. *PIDD1*$^{\Delta}$ HEK293 cells were transfected with the indicated plasmids, and membrane fractions were prepared and IB analyzed for the indicated proteins.

Cleaved S1P is shown in the red box.

F-Full length, C-Cleaved, P-precursor, N.S-non-specific.

Each experiment was triplicated, and one representative result is shown.

Figure S5, related to Figure 5. IRE1 inhibition prevents PIDDosome and SREBP activation.

WT mice were fed HFrD for 8 weeks, followed by DMSO (n=16) or MKC3946 (3 mg/kg, n=20) treatment for 4 weeks.

A) BW increase during the HFrD feeding period. DMSO or MKC injected period is indicated by purple block. Food intake during the injection period.

B) Serum insulin in above mice.

C-D. Relative amounts of c/EBPβ, INSIG1, INSIG2, SCAP (C) and inflammatory marker (D) mRNAs in above livers.

E. IB of nuclear p53 in livers of CSD or HFrD fed WT mice and HFrD fed *p53*$^{\Delta Hep}$ mice (top) and representative H&E images (bottom).

Results are mean ± SEM. Scale bar, 100 μm. Statistical significance was determined by two-tailed Student's t test. *p < 0.05, **p < 0.005, ***p < 0.001.

Figure S6, related to Figure 6. *Scap* ablation potentiates fructose induced ER stress and PIDDosome activation.

Scap-floxed (*Scap*$^{F/F}$*/MUP*, n=7) and liver specific *Scap* ablated (*Scap*$^{\Delta hep}$*/MUP*, n=4) *MUP-uPA* mice were fed with HFD for 12 weeks.

A. H&E, F4/80, Sirius red staining of FFPE liver sections and ORO staining of frozen liver sections from above mice.

B. IB analysis of p-IRE1 in livers of above mice.

C. Casp2 and PIDD1 IHC in livers of above mice.

WT (n=12) and *Scap* ablated (*Scap*$^{\Delta hep}$, n=7) mice were high fat diet (HFD)- or HFD plus 30% fructose water diet (HFHFD)-fed for 12 weeks. [HFD: (WT: n=12, *Scap*$^{\Delta hep}$: n=7)], [HFHFD: (WT: n=14, *Scap*$^{\Delta hep}$: n=10)]

D. H&E, F4/80, Sirius red staining of FFPE liver sections and ORO staining of frozen liver section from HFD-fed indicated mice.

E. H&E, Sirius red staining of FFPE liver sections and ORO staining of frozen liver section from HFHFD-fed indicated mice.

WT (*Scap*$^{F/F}$, n=13) and liver specific *Scap* ablated (*Scap*$^{\Delta hep}$, n=11) mice were fed HFrD for 12 weeks.

F. H&E of FFPE liver sections and ORO staining of frozen liver sections from above mice.

G. Serum and liver TG and Chol from above mice.

H. IB of lipogenic enzymes in livers of above mice.

I. IB of p-IRE1 in livers of HFHFD-fed indicated mice.

J-K. Relative mRNAs of lipogenic enzymes (J) and ER stress, inflammatory, fibrogenic genes (K) in livers of HFHFD-fed above mice.

Four HMFs from each liver were taken, quantified by Image J software and the results are shown on the right. Results are mean ± SEM. Scale bar, 100 μm. Statistical significance was determined by two-tailed Student's t test. *p < 0.05, **p < 0.005, ***p < 0.001.

Figure S7, related to Figure 7. IRE1 inhibition rescues ER stress and damage in the HFrD-fed *Scap*$^{\Delta hep}$ liver.

A. IB analysis of SREBP2 and relative *Srebf2*, *Hmgcr*, and *Hmgcs* mRNA amounts in livers of DMSO or MKC3946-treated HFrD-fed *Scap*$^{\Delta hep}$ mice.

Statistical significance was determined by two-tailed Student's t test. *p < 0.05, **p < 0.005, ***p < 0.001.

Table S1. RNAseq analysis in HFrD-fed PIDDosome or *Scap*-ablated liver. Related to Figure 3.

Table S2. Histologic score of HFrD- or WD-fed *Scap*-ablated liver. Related to Figure 6.

Data S1. Unprocessed data underlying the display items in the manuscript. Related to Figures 1-7 and S1-S7.