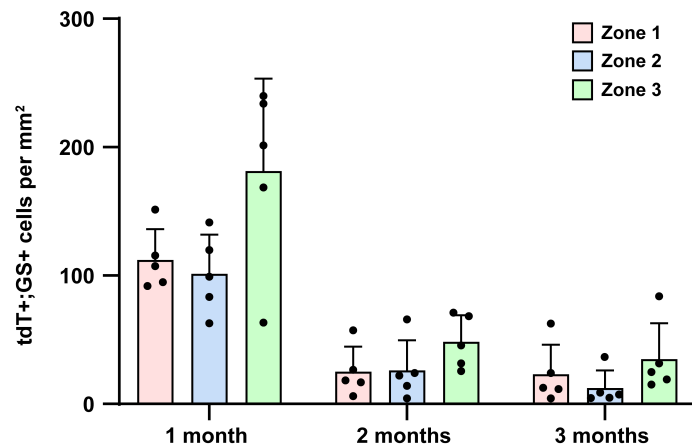


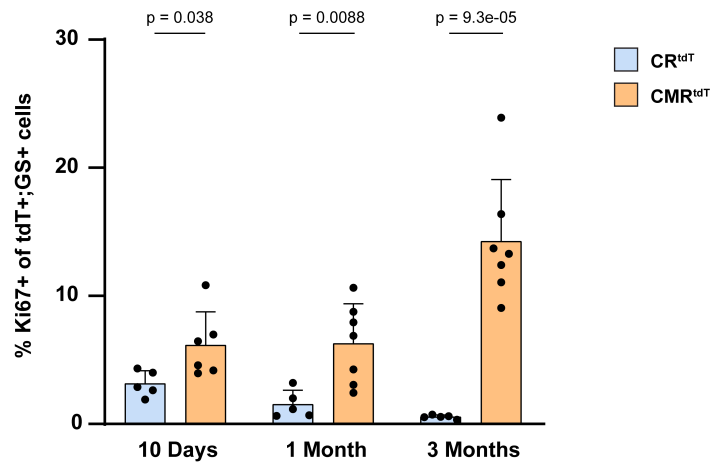
Antibody	Vendor	Catalog Number	Dilution
Goat anti-mCherry (tdTomato)	Sicgen	AB0040-200	1:1500 (R ^{tdT} Samples) 1:500 (Transposons)
Mouse anti-GS	BD	610517	1:200
Rabbit anti-GFP (YFP)	Invitrogen	A-11122	1:100
Chicken anti-GFP (YFP)	Novus	NB100-1614	1:100
Mouse anti-CHOP	CST	2895	1:100 with TSA kit
Rabbit anti-BiP	CST	3177	1:100
Rabbit anti-NQO1	Sigma	HPA007308	1:100
Goat anti-E-Cadherin	R&D Systems	AF748	1:100
Rabbit anti-P-Akt (Ser473)	CST	4060S	1:100
Rabbit anti-P-Akt (Thr308)	CST	4056S	1:100
Rabbit anti-Phospho-PRAS40 (Thr246)	CST	2997S	1:100
Rabbit anti-Fatty Acid Synthase	CST	3180S	1:100
Rabbit anti-SCD1	CST	2794S	1:100
Rabbit anti-HMGCS1	Proteintech	17643-1-AP	1:100
Rabbit anti-Ki67	Abcam	ab15580	1:500
Donkey anti-mouse 488	Invitrogen	A21202	1:600
Donkey anti-goat 555	Invitrogen	A21432	1:600
Donkey anti-goat 647	Invitrogen	A21447	1:600
Donkey anti-goat 755	Invitrogen	SA5-10091	1:600
Donkey anti-rabbit 555	Invitrogen	A31572	1:600
Donkey anti-rabbit 647	Invitrogen	A31573	1:600
Donkey anti-chicken 555	Jackson ImmunoResearch	703-165-155	1:600
Goat Anti-Mouse IgG (Biotin conjugated)	Vector Laboratories	BA-9200	1:200 with TSA kit
Rabbit anti-NRF2	GeneTex	GTX103322	1:1000
Rabbit anti-GAPDH	CST	2118	1:5000
Anti-rabbit IgG (HRP-linked)	CST	7074	1:2500 (NRF2) 1:5000 (GAPDH)

Supplementary Table 1. Antibodies Used for Experiments



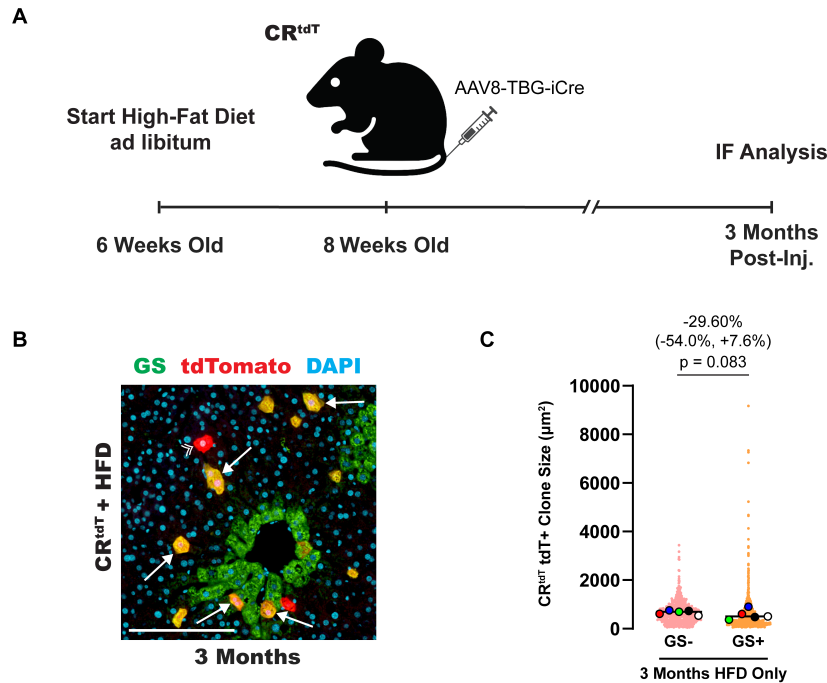
Supplementary Figure 1. β -catenin–mutant hepatocyte attrition is not affected by liver zonation.

Quantification of the number of tdT⁺;GS⁺ hepatocytes per mm² within either Zone 1 (periportal; E-Cadherin⁺), Zone 2 (E-Cadherin⁺;GS⁺), or Zone 3 (pericentral; GS⁺) at the indicated timepoints post-AAV injection. Each point represents the mean count from a mouse. Each bar represents the average of the mice means +SD. n=5 for each timepoint. Source data are provided as a Source Data file.



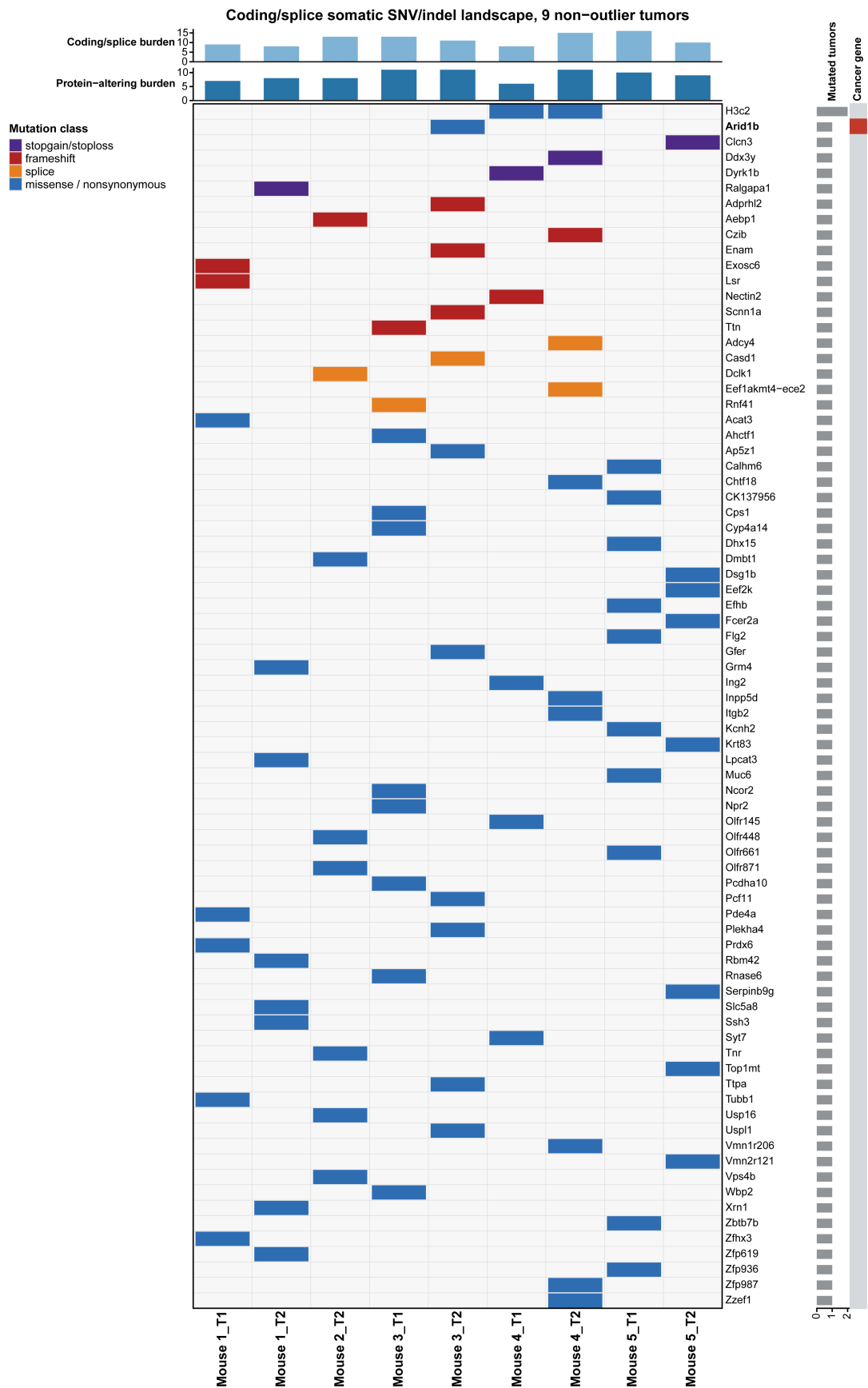
Supplementary Figure 2. *Ctnnb1*^{ex3} hepatocytes proliferate in the damaged liver but not in the normal liver.

%tdT⁺;GS⁺ cells that are also Ki67⁺ from same samples described in (Fig. 1C and 2C) at the indicated timepoints. Each point represents the mean count from a mouse. Each bar represents the average of the mice means +SD. Statistical significance was determined between CR^{tdT} and CMR^{tdT} groups using an unpaired, two-tailed t-test using the mice means. n=5 for CR^{tdT} at each timepoint. n=6, 7, 7 for 10 days, 1 month, and 3 months CMR^{tdT}, respectively. Source data are provided as a Source Data file.



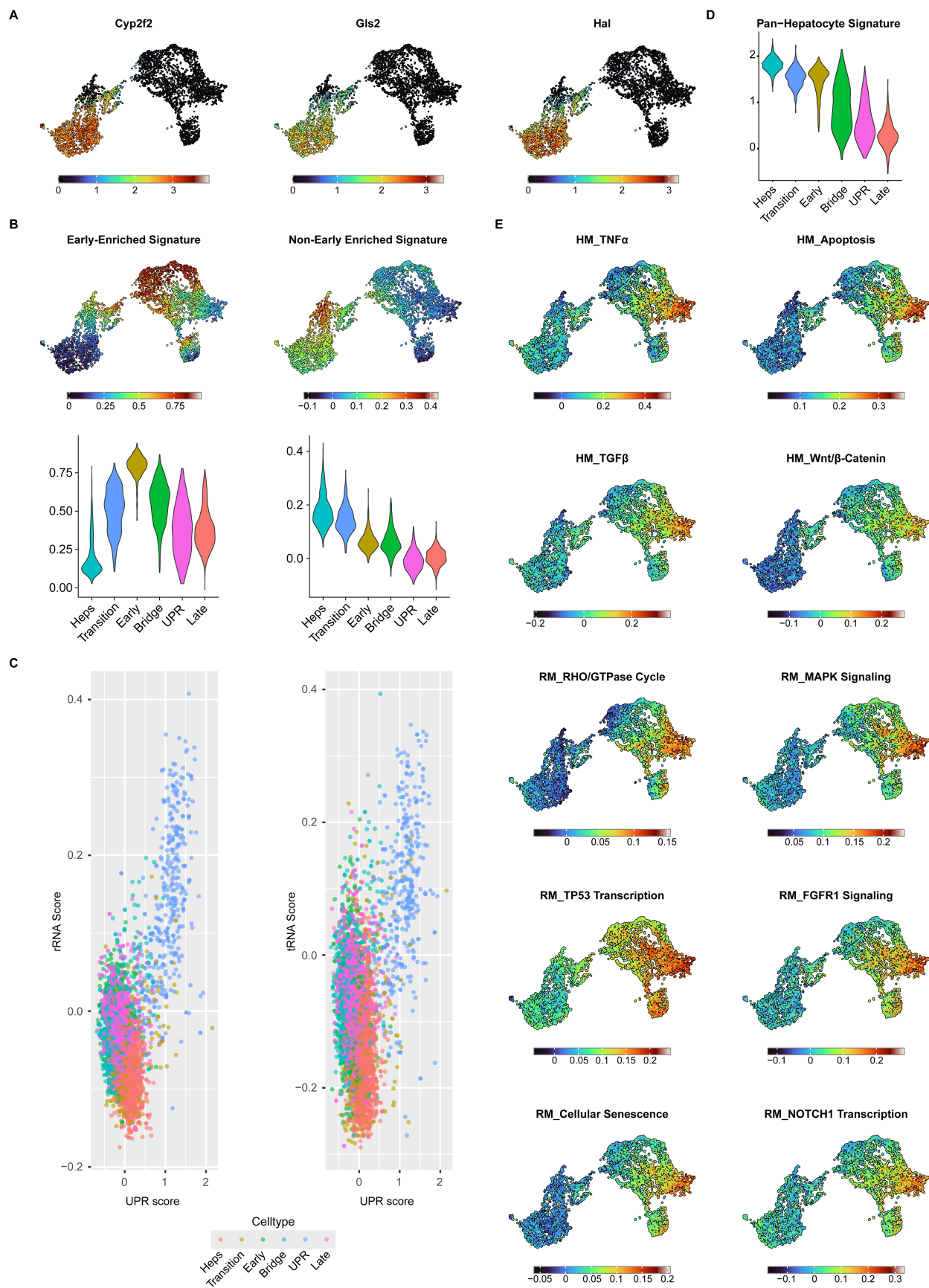
Supplementary Figure 3. *Ctnnb1*^{ex3} hepatocytes do not clonally expand with High-Fat Diet alone at 3 months.

A) Schematic of High-Fat Diet treatment in CR^{tdT} mice experimental design. B) Representative immunofluorescence images of CR^{tdT} High-Fat Diet treated mice 3 months after injection stained for tdT and GS. White arrows: tdT⁺;GS⁺ clones, double arrowheads: tdT⁺ only cells. Scale bar=200 μm . C) Quantification of tdT⁺;GS⁻ (GS⁻) and tdT⁺;GS⁺ (GS⁺) clone sizes in CR^{tdT} High-Fat Diet treated liver samples. Points are individual clones; colored dots are mouse means; black lines indicate the mean of mouse means. Same color indicates same mouse. Statistical significance between GS⁻ and GS⁺ clone size in each class was determined by fitting a linear mixed effects model with a likelihood-ratio test. Significance of pairwise comparisons was determined using post-hoc comparisons from the fitted mixed-effects model, with two-sided tests. Percentage change (top), 95% CI (middle), and p-value (bottom) of GS⁺ vs. GS⁻ cell size is listed above. n=5. Panel A was created in BioRender. Hewett, C. (2026) <https://BioRender.com/t3qttns>. Source data are provided as a Source Data file.



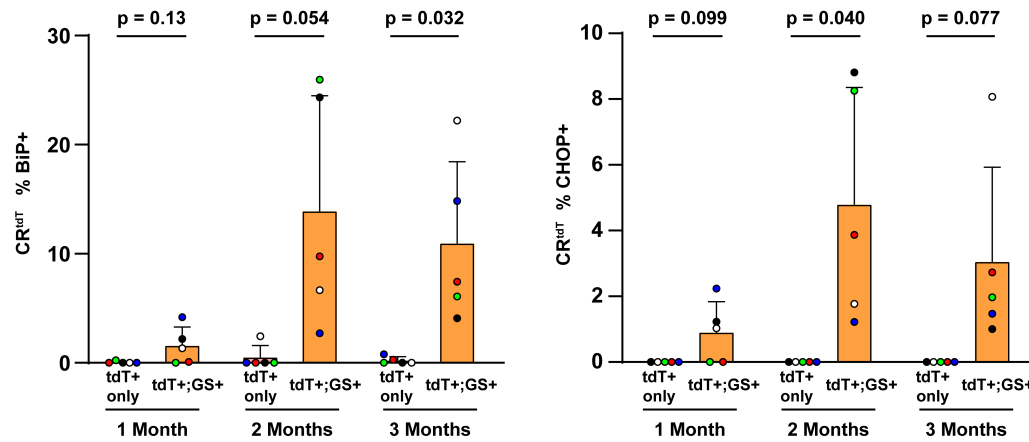
Supplementary Figure 4. No recurrent cooperation with additional oncogenic mutations on CMR^{tdT} tumors.

Whole-exome sequencing was performed on tumors isolated from 6.5-month CMR^{tdT} mice. Two tumors (T1 and T2) were sequenced in five independent mice, and variants were identified by comparing with matched surrounding non-tumor liver tissue from the same mouse. Mouse 2_T1 showed a marked outlier profile and was excluded from the primary cohort-level interpretation, further described in Methods.



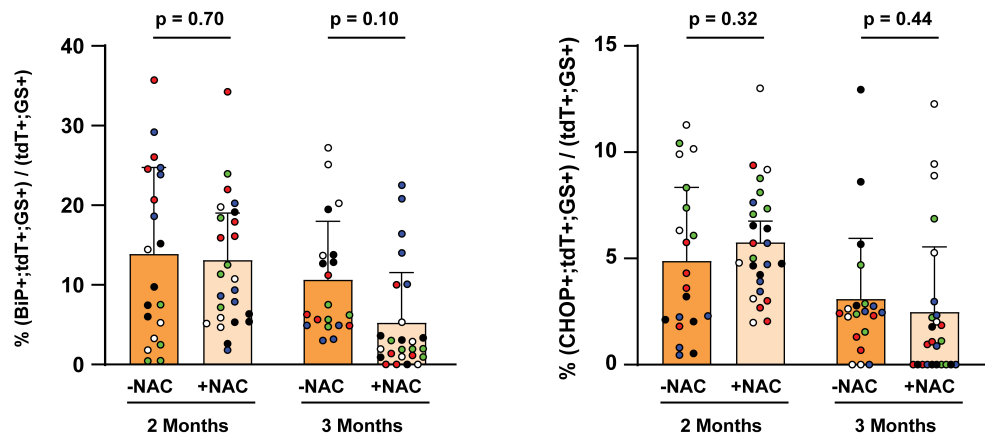
Supplementary Figure 6. Additional scRNA-seq characterization of β -catenin–mutant hepatocyte clusters in the normal liver.

A) UMAP plots showing expression of *Cyp2f2*, *Gls2*, and *Hal* in the dataset in Fig. 3. B) UMAP plots showing expression of the zone 3 signature (Fig. 3D) split in two sub-signatures based on whether the genes are enriched in the Early cluster. Relative enrichment levels for each cluster identified in Fig. 3C are depicted in a violin plot (below). C) Scatterplots showing rRNA and tRNA versus UPR gene set scores for all hepatocytes in the dataset in Fig. 3, with each cluster highlighted with different colors. D) Violin plots of relative pan-hepatocyte gene signature expression for each cluster identified in Fig. 3C. E) UMAP plots showing expression levels of the signatures built from the Hallmark and Reactome pathways identified in Fig. 3G.



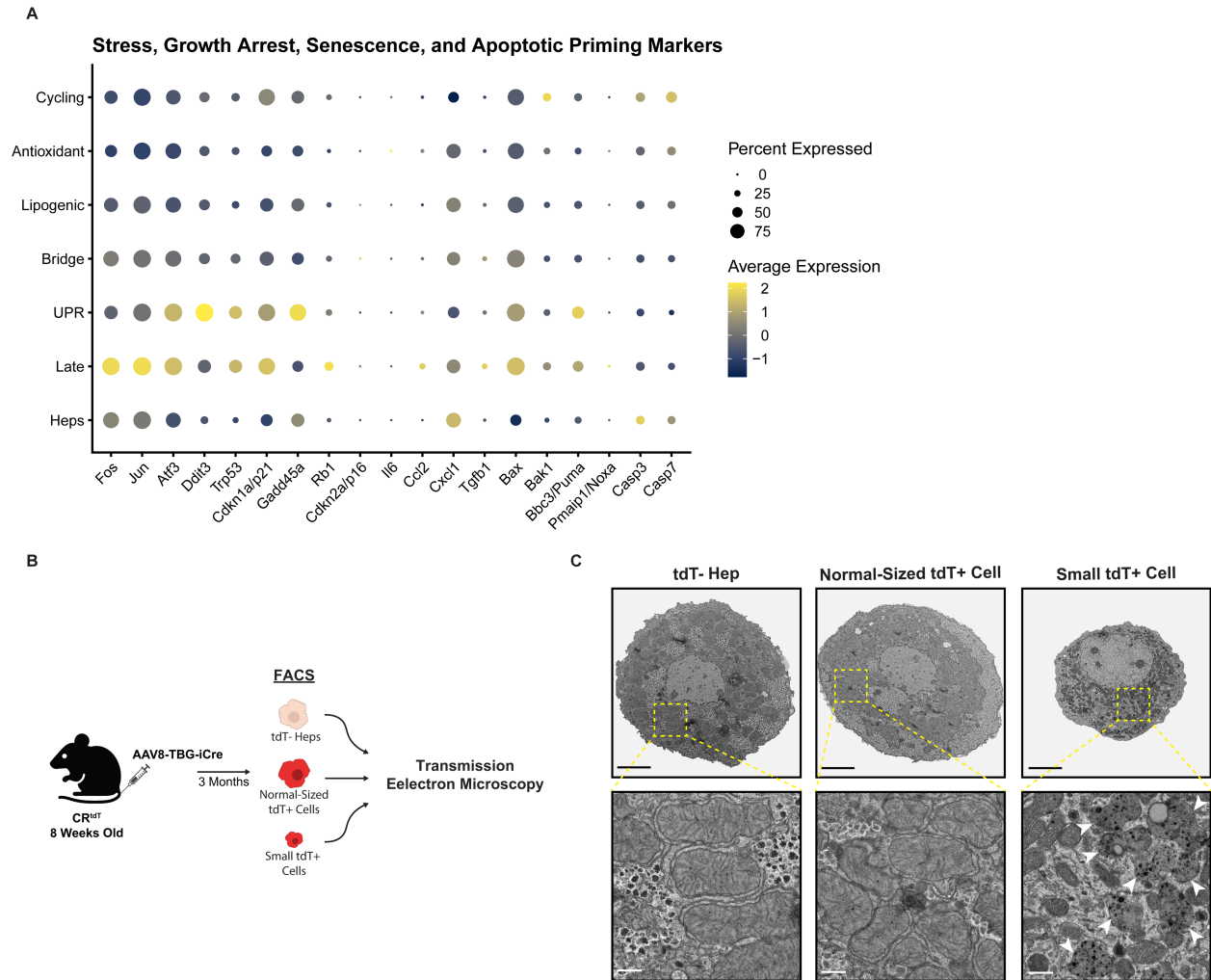
Supplementary Figure 7. CHOP and BiP are selectively elevated in *Ctnnb1*^{ex3} hepatocytes.

%tdT⁺ and tdT⁺;GS⁺ cells that are also CHOP⁺ or BiP⁺ from CR^{tdT} samples in Fig. 1C. Each point represents the mean count for each mouse, and each pair of colored points at each timepoint corresponds to the same mouse. Each bar represents the average of the mice means ±SD. Statistical significance was determined between tdT⁺ and tdT⁺;GS⁺ groups at each timepoint using a paired, two-tailed t-test using the mean of the areas counted from each mouse. n=5. Source data are provided as a Source Data file.



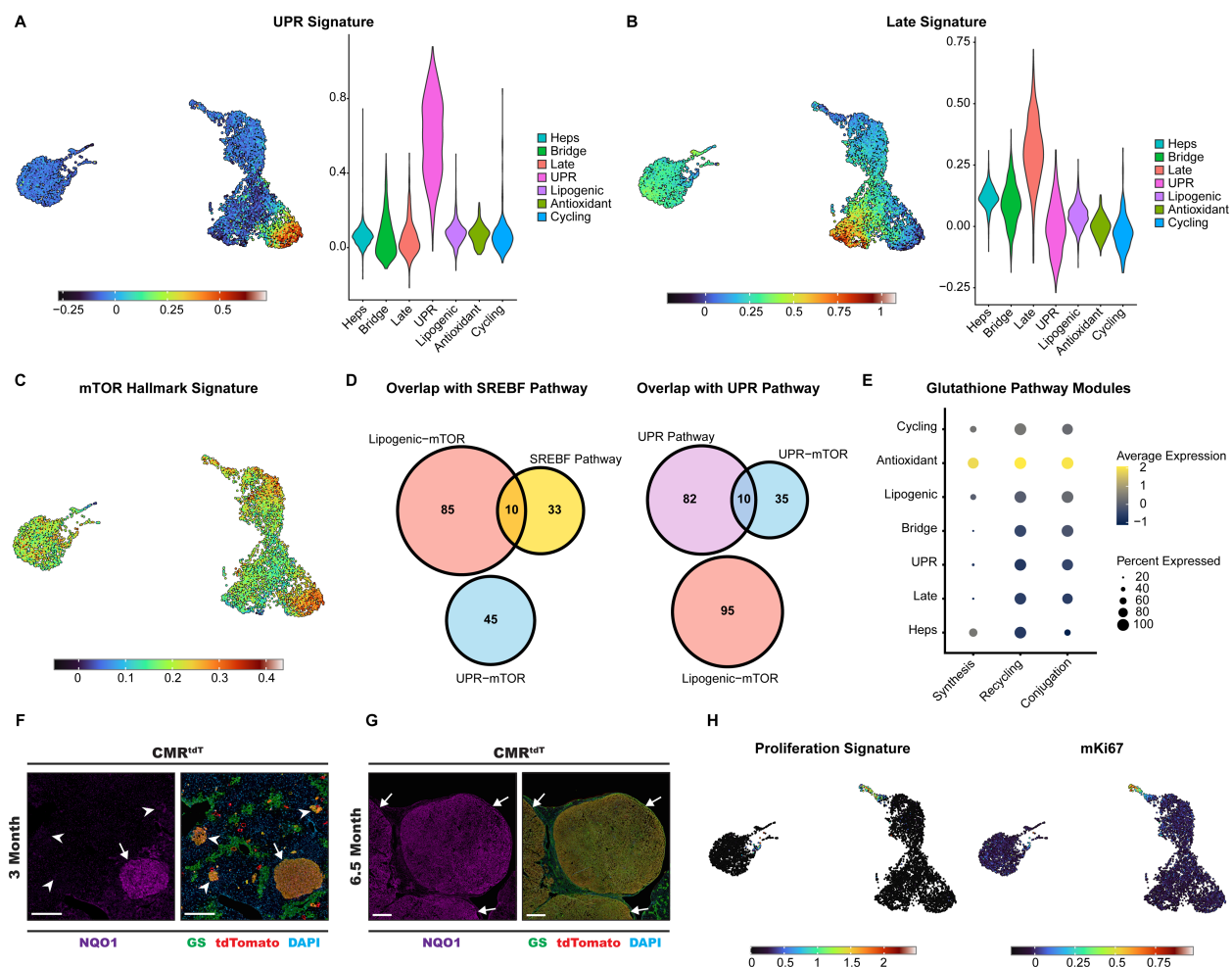
Supplementary Figure 8. NAC treatment does not significantly reduce UPR markers in *Ctnnb1^{ex3}* hepatocytes.

%tdT⁺;GS⁺ cells that are also CHOP⁺ or BiP⁺ from NAC treated CR^{tdT} samples in Fig. 4E, and CR^{tdT} samples from Fig. 1C-D. Each point represents an area counted within a lobe, and each set of colored points within a treatment group corresponds to the same mouse. Four or five areas were counted in each mouse. Each bar represents the average of the mice means +SD. Statistical significance was determined between -NAC and +NAC groups at each timepoint using an unpaired, two-tailed t-test using the mean of the areas counted from each mouse. n=5. Source data are provided as a Source Data file.



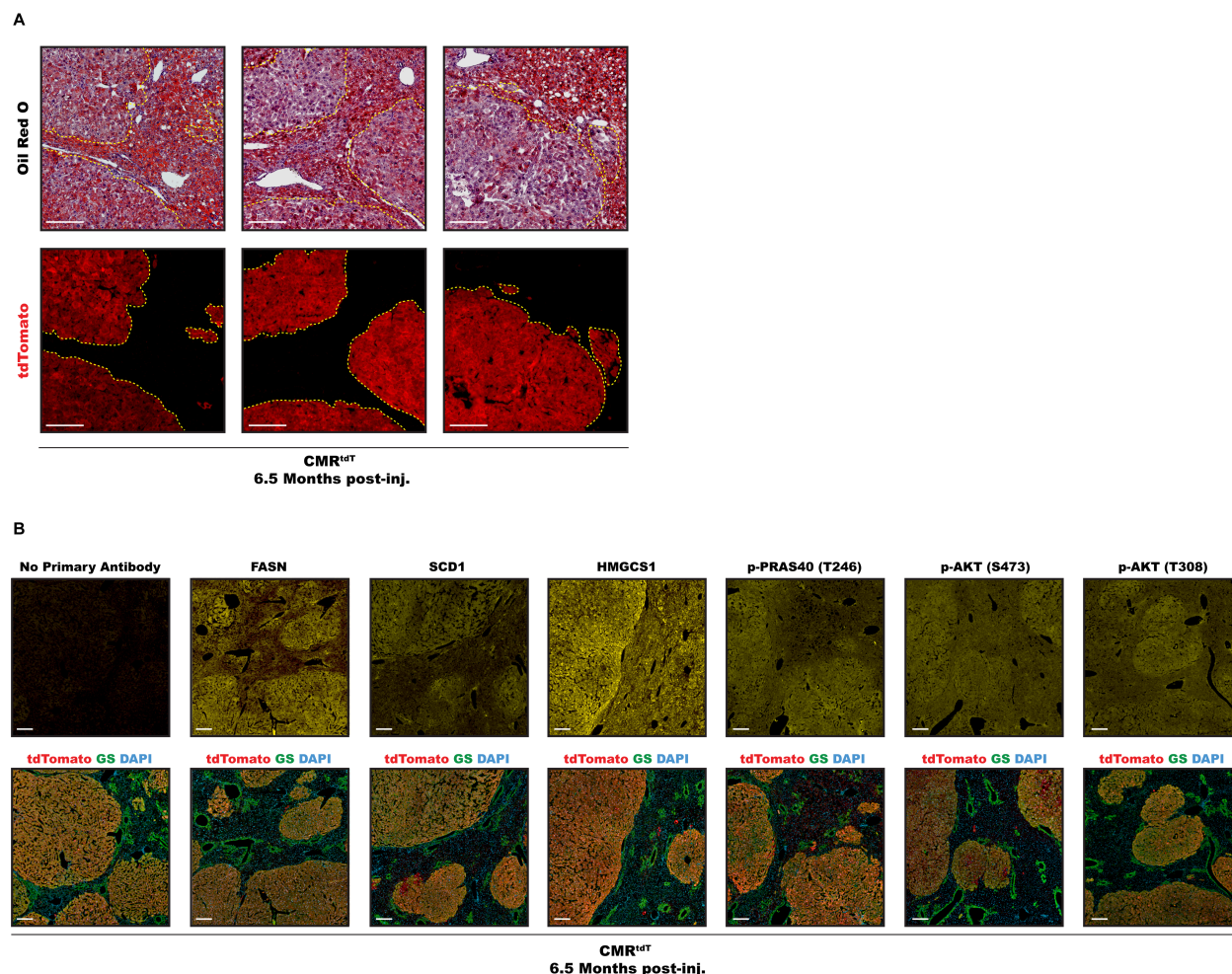
Supplementary Figure 9. Cellular stress pathways in *Ctnnb1*^{ex3} hepatocytes in the normal liver.

A) Dot plot of markers for cellular stress, growth arrest, senescence, and apoptotic priming in *Ctnnb1*^{ex3} hepatocytes from CMR^{tdT} mice in the dataset in Fig. 5. B) Schematic of experimental approach to image hepatocytes by electron microscopy. 3 months post-AAV injection in a CR^{tdT} mouse, hepatocytes were isolated and FACS sorted to separate tdT⁻ hepatocytes, Normal-Sized tdT⁺, and Small tdT⁺ cells. Each cell population was fixed and imaged by transmission electron microscopy. C) Electron microscopy images of representative cells from the three populations isolated. Yellow-dashed boxes indicate the area displayed in higher magnification. Arrowheads highlight autolysosome structures. Findings were confirmed by imaging two independent mouse samples. Black scale bars=4μm, White scale bars=0.5μm. Panel B was created in BioRender. Hewett, C. (2026) <https://BioRender.com/5pwn7dp>.



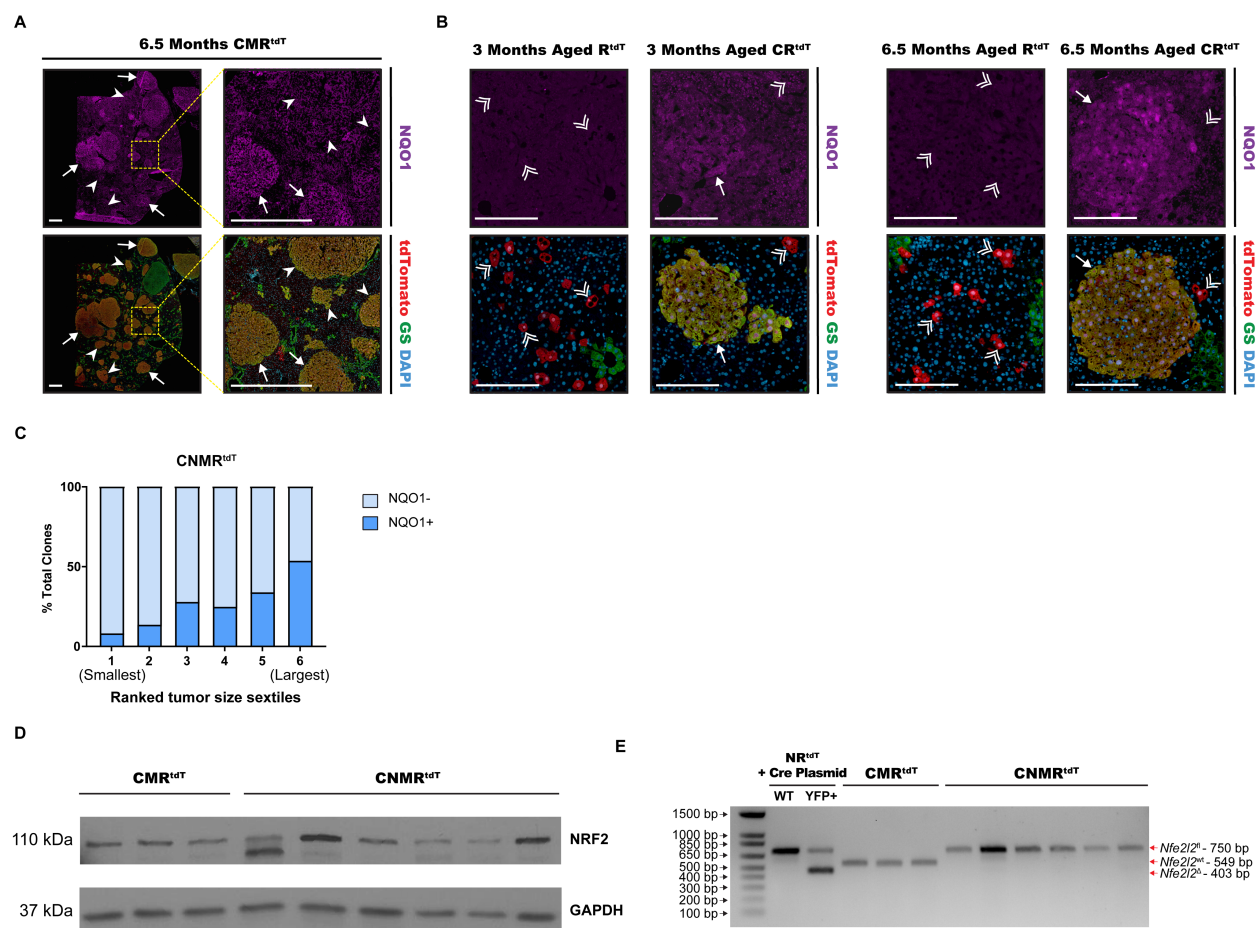
Supplementary Figure 10. Extended scRNA-seq and phenotypic characterization of β -catenin-mutant hepatocytes in chronic liver disease.

A-B) UMAP visualizations of UPR (A) and Late (B) gene signatures identified in Fig. 3 applied to the chronic liver damage dataset and corresponding violin plots. C) UMAP plot of the Hallmark mTOR signature corresponding to the Lipogenic and UPR clusters. D) Venn Diagram visualization of bivalent mTOR response overlap with SREBF or UPR pathways. Numbers within each colored area represent genes upregulated by the indicated pathway, with numbers in the overlapping areas indicating the number of upregulated genes shared by both pathways. E) Dot plot of glutathione metabolism in *Ctnnb1*^{ex3} hepatocytes from the dataset in Fig. 5. F-G) Representative immunofluorescence images of NQO1 staining in CMR^{tdT} mice at 3 (F) or 6.5 (G) months stained for tdT, GS, and NQO1. White arrows: tdT⁺;GS⁺ clones positive for NQO1. Arrowheads: small tdT⁺;GS⁺ clones negative for NQO1. Scale bars= 400 μ m (F) and 1mm (G). n=7 (F) and 6 (G). H) UMAP plots visualizing expression of a general proliferation signature and *mKi67*.



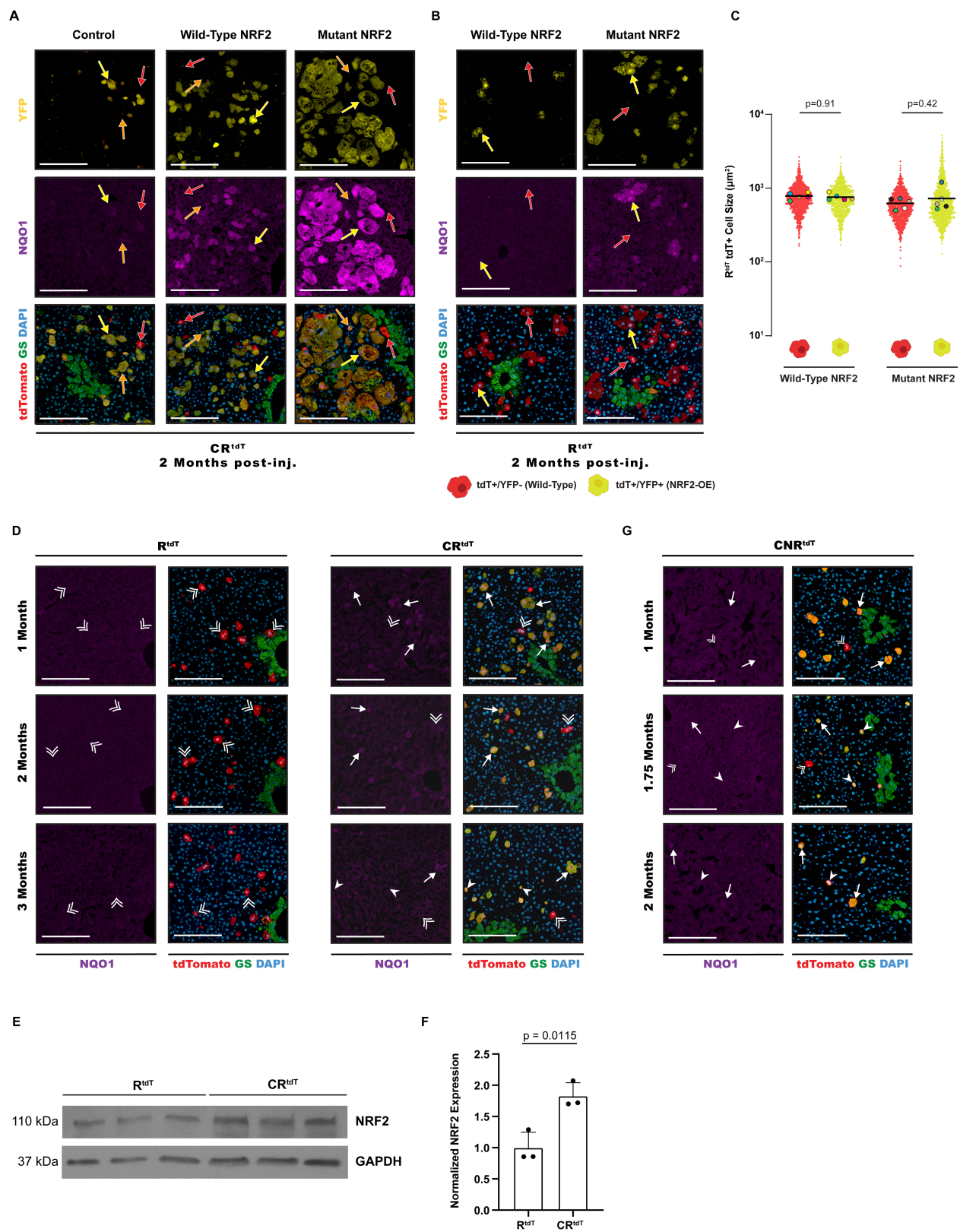
Supplementary Figure 11. Lipogenic markers are maintained in CMR^{tdT} tumors without neutral lipid accumulation.

A) Representative Oil Red O staining and endogenous tdTomato fluorescence in three independent CMR^{tdT} liver tissue areas 6.5 months post-AAV injection. Yellow-dashed lines circumscribe tdT⁺ tumors. Scale bars=200μm. n=6. B) Representative immunofluorescence images of CMR^{tdT} liver 6.5 months post-AAV injection stained with tdT, GS, and either No Primary Antibody, FASN, SCD1, HMGCS1, p-PRAS40 (T246), p-AKT (S473), or p-AKT (T308). Scale bars=200μm. n=6.



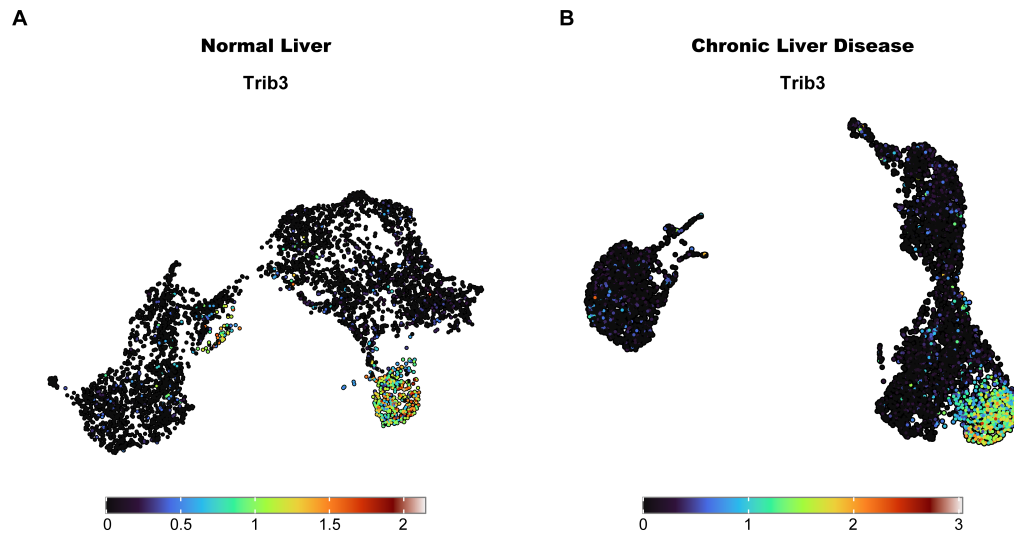
Supplementary Figure 12. NRF2-active (NQO1⁺) β -catenin–mutant clones are enriched during clonal expansion and tumorigenesis.

A) Representative immunofluorescence images of CMR^{tdT} liver at the indicated timepoints post-AAV injection stained for tdT, GS, and NQO1. White arrows: tdT⁺;GS⁺ clones positive for NQO1. Arrowheads: tdT⁺;GS⁺ clones negative for NQO1. Yellow-dashed boxes: area displayed in higher magnification. Scale bars=1mm. B) Representative immunofluorescence pictures of Aged CR^{tdT} mice described in Fig. 2E at the indicated timepoints post-AAV injection stained for tdT, GS, and NQO1. White arrows: tdT⁺;GS⁺ clones. Double-arrowheads: tdT⁺ only clones. Scale bars=200µm. C) %tdT⁺;GS⁺ clones in 6.5-month CNMR^{tdT} mice containing 5 cells or more that are either NQO1 positive or negative. Clones were divided into sextiles based on area. n=3. D) Western blot of excised liver tumors from CMR^{tdT} and CNMR^{tdT} mice 6.5 months post-AAV injection with the indicated antibodies. Each lane represents one tumor. E) Gel electrophoresis of *Nfe2l2* gene from hepatocytes isolated from NR^{tdT} mice injected with the Cre-containing plasmid described in Fig. 4C one-week post-HDTV and the same tumors as in (D). 549 bp band: wild-type *Nfe2l2* (*Nfe2l2*^{wt}); 750 base-pair (bp) band: non-recombined, floxed *Nfe2l2* (*Nfe2l2*^{fl}); 403 bp band: recombined *Nfe2l2* (*Nfe2l2*^Δ). Source data are provided as a Source Data file.



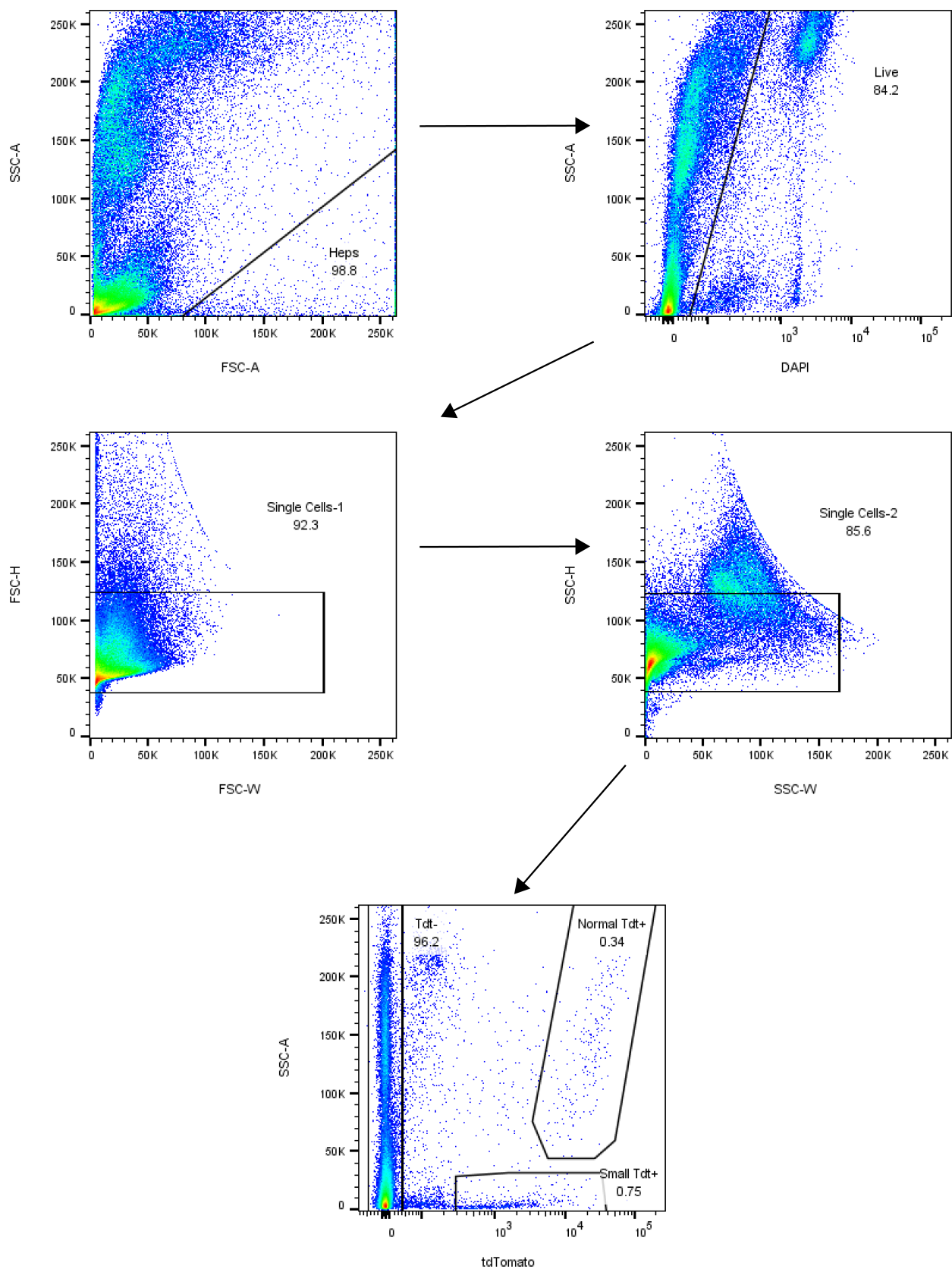
Supplementary Figure 13. NRF2 activity in *Ctnnb1*^{ex3} hepatocytes across experimental contexts in the normal liver.

A-B) Representative immunofluorescence pictures of (A) CR^{tdT} mice and (B) R^{tdT} mice injected with NRF2-containing transposons shown in Fig. 6E stained for tdT, GS, YFP, and NQO1. Red arrows: tdT⁺ only; orange arrows: tdT⁺;GS⁺;YFP⁻ hepatocytes (*Ctnnb1*^{ex3} hepatocytes); yellow arrows: tdT⁺;GS⁺;YFP⁺ hepatocytes (*Ctnnb1*^{ex3} hepatocytes + integrated transposon). Scale bars=200µm. C) Cell size quantification of the two hepatocyte populations in R^{tdT} mice injected with the transposons described in (B) 2 months post-HDTV. Points are individual cells; colored dots are mouse means; black lines indicate the mean of mouse means. Same color indicates same mouse. Statistical significance between the two cell populations' cell sizes was determined by fitting a linear mixed effects model with a likelihood-ratio test. Significance of pairwise comparisons was determined using post-hoc comparisons from the fitted mixed-effects model, with two-sided tests. n=5 for each group. D) Representative immunofluorescence pictures of R^{tdT} and CR^{tdT} mice shown in Fig. 1B stained for tdT, GS, and NQO1. White arrows: normal size tdT⁺;GS⁺ cells. Arrowheads: small tdT⁺;GS⁺ cells. Double-arrowheads: tdT⁺ only cells. Scale bars=200µm. n=5 for all groups. E) Western blot of purified tdT⁺ hepatocytes from R^{tdT} and CR^{tdT} mice 1 month post-AAV injection with the indicated antibodies. Each lane represents one mouse. F) Normalized NRF2 expression from the western blot in (E). Each dot represents one mouse, with black lines indicating mean +SD. Statistical significance was determined between R^{tdT} and CR^{tdT} groups using an unpaired, two-tailed t-test. n=3 for each strain. G) Representative immunofluorescence pictures of CNR^{tdT} mice shown in Fig. 7C stained for tdT, GS, and NQO1. White arrows: normal size tdT⁺;GS⁺ cells. Arrowheads: small tdT⁺;GS⁺ cells. Double-arrowheads: tdT⁺ only cells. Scale bars=200µm. n=4, 4, and 3 for 1, 1.75, and 2 months, respectively. Panels B and C were created in BioRender. Hewett, C. (2026) <https://BioRender.com/v3wb0p5>. Source data are provided as a Source Data file.



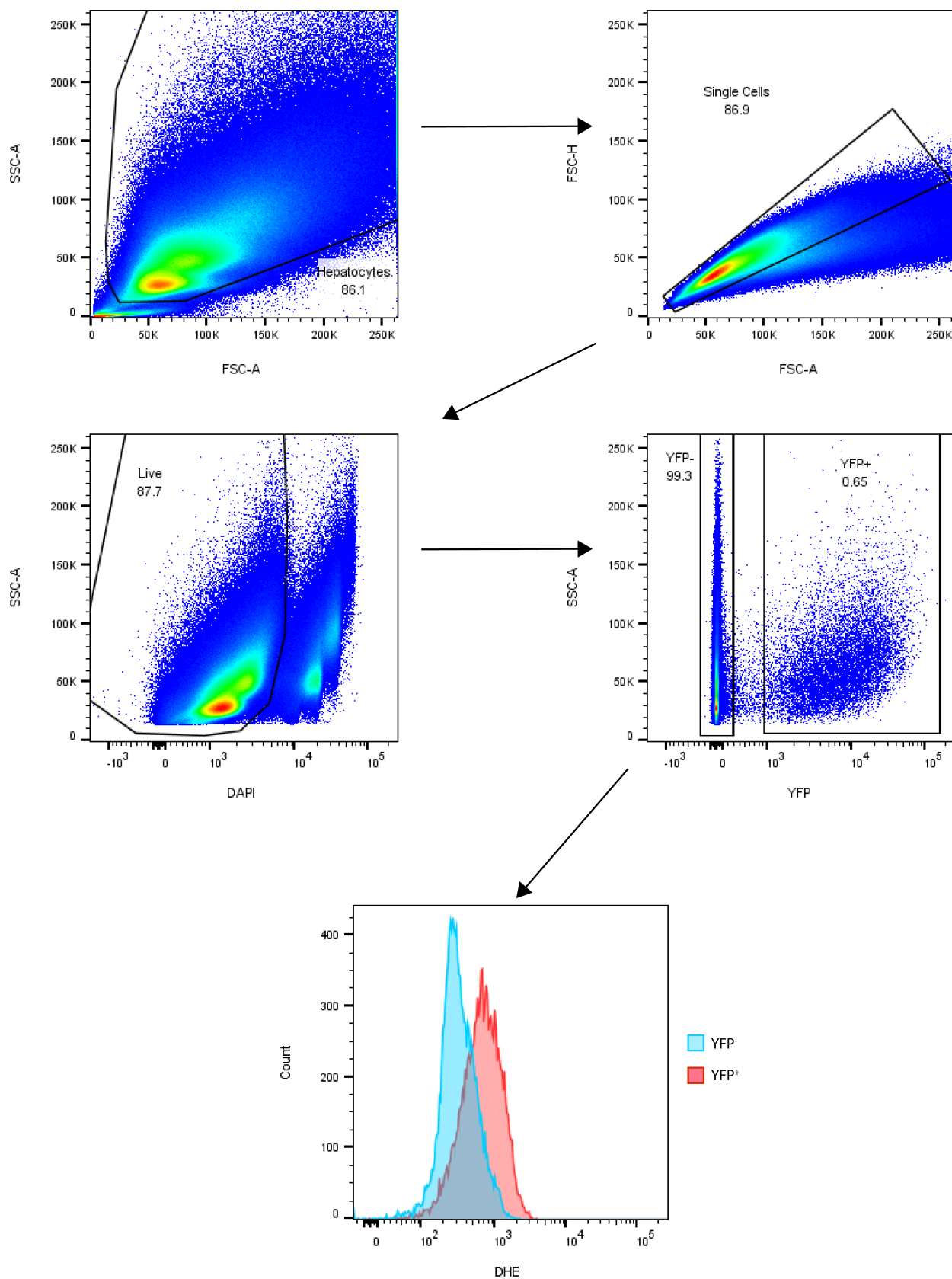
Supplementary Figure 14. *Trib3* expression is enriched in the UPR cluster in both the normal liver and in chronic liver disease.

A-B) UMAP visualizations of *Trib3* (TRB3) in the (A) normal liver (Fig. 3) and (B) chronic liver damage (Fig. 5).



Supplementary Figure 15. Flow cytometry gating strategy for sorting of tdT⁻ and tdT⁺ mouse hepatocyte populations.

Example of the gating strategy used for sorting tdT⁻, normal-sized tdT⁺, and small tdT⁺ hepatocyte populations isolated from the mouse liver.



Supplementary Figure 16. Flow cytometry gating strategy for DHE analysis of YFP⁻ and YFP⁺ mouse hepatocyte populations.

Example of the gating strategy used for DHE analysis of hepatocytes from the mouse liver.