Figure S1

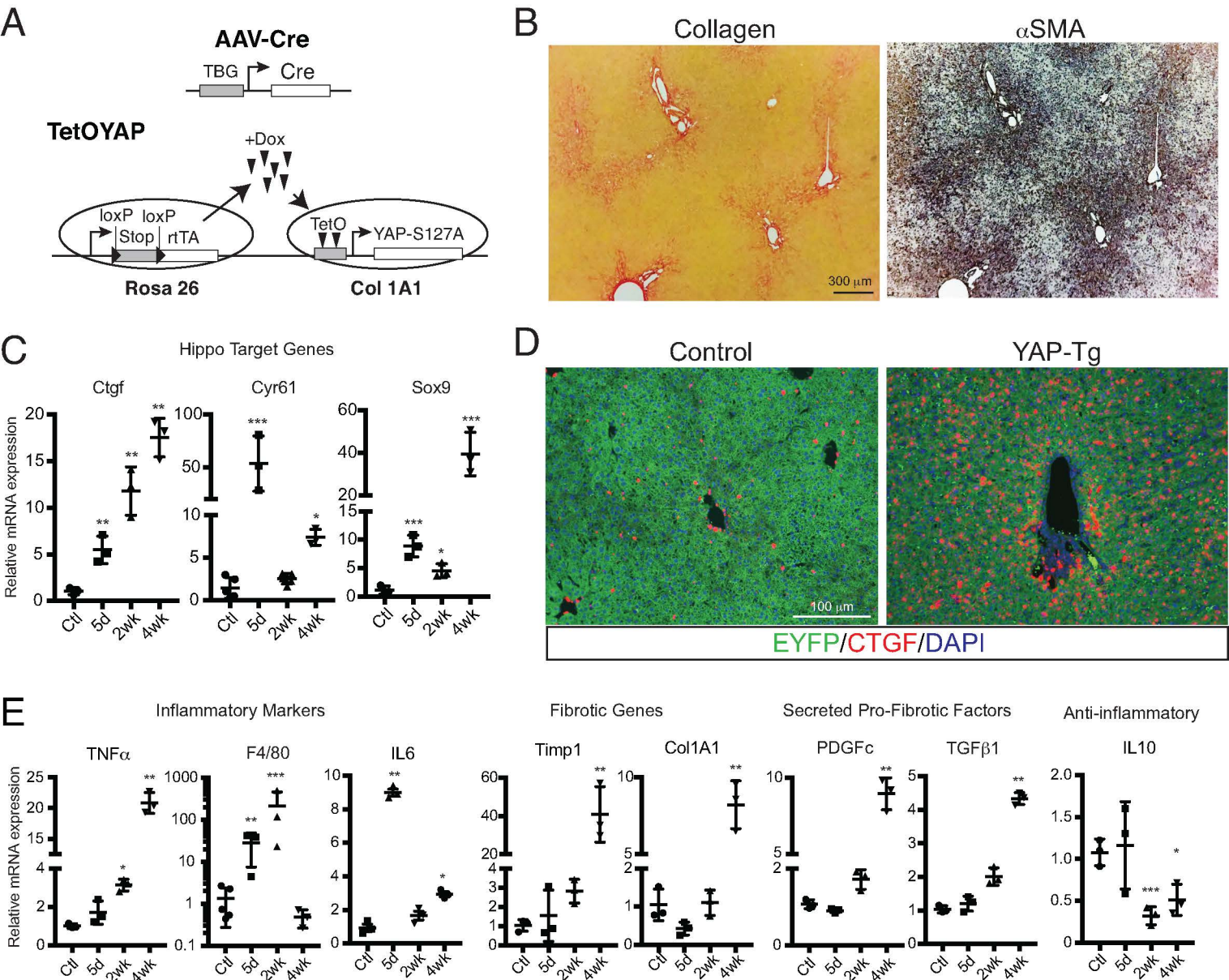

A
AAV-Cre
TBG
Cre
TetOYAP
+Dox
loxP
loxP
Stop
rtTA
TetO
YAP-S127A
Rosa 26
Col 1A1
B
Collagen
αSMA
300 μm
C
Hippo Target Genes
Ctgf
Cyr61
Sox9
Relative mRNA expression
Ctl
5d
2wk
4wk
D
Control
YAP-Tg
100 μm
EYFP/CTGF/DAPI
E
Inflammatory Markers
Fibrotic Genes
Secreted Pro-Fibrotic Factors
Anti-inflammatory
TNFα
F4/80
IL6
Timp1
Col1A1
PDGFc
TGFβ1
IL10
Relative mRNA expression

Figure S2

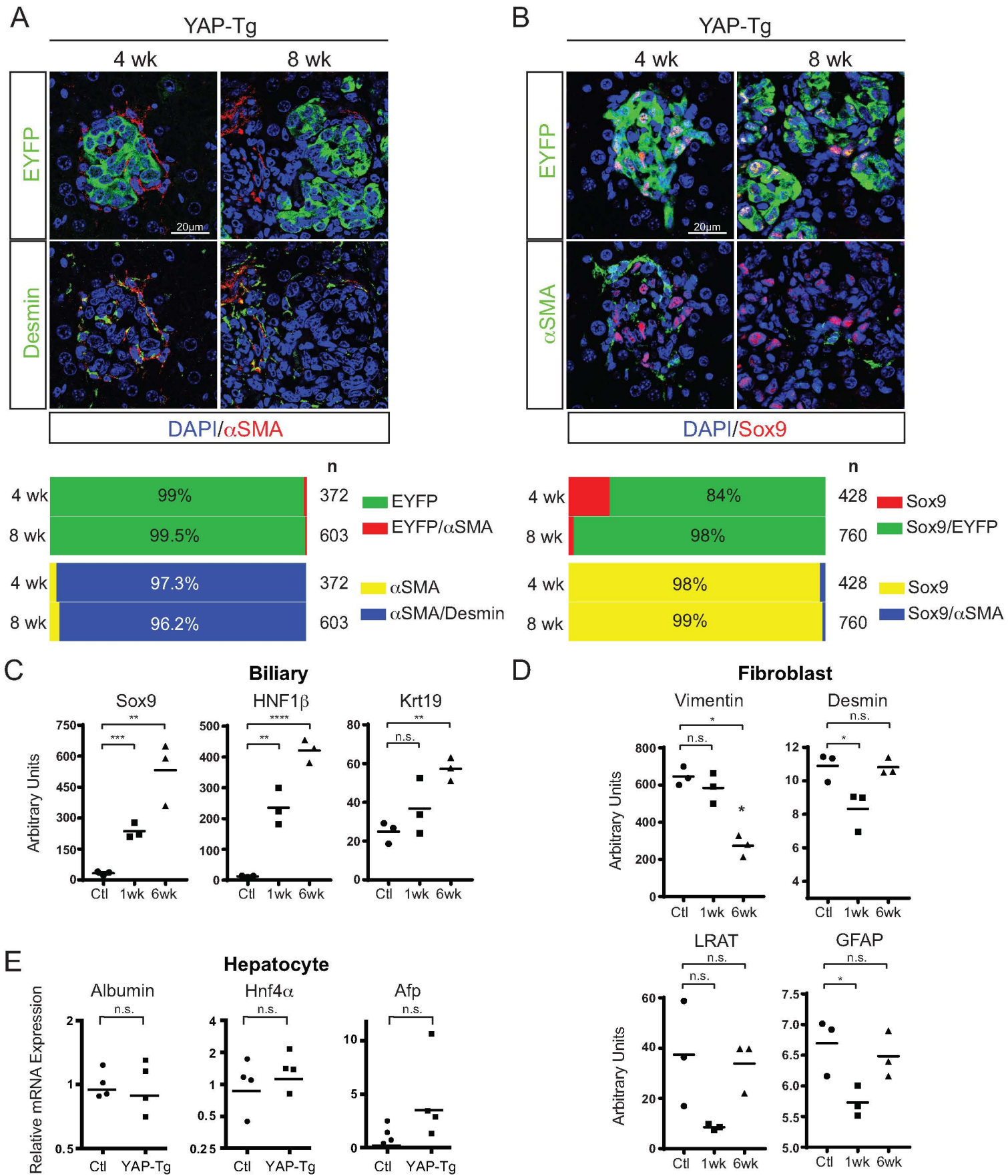

A
YAP-Tg
4 wk
8 wk
EYFP
Desmin
20μm
DAPI/αSMA
B
YAP-Tg
4 wk
8 wk
EYFP
αSMA
20μm
DAPI/Sox9
n
4 wk
99%
372
EYFP
8 wk
99.5%
603
EYFP/αSMA
4 wk
97.3%
372
αSMA
8 wk
96.2%
603
αSMA/Desmin
n
4 wk
84%
428
Sox9
8 wk
98%
760
Sox9/EYFP
4 wk
98%
428
Sox9
8 wk
99%
760
Sox9/αSMA
C
Biliary
Sox9
HNF1β
Krt19
Arbitrary Units
Ctl
1wk
6wk
D
Fibroblast
Vimentin
Desmin
LRAT
GFAP
Arbitrary Units
n.s.
E
Hepatocyte
Albumin
Hnf4α
Afp
Relative mRNA Expression
Ctl
YAP-Tg

Figure S3

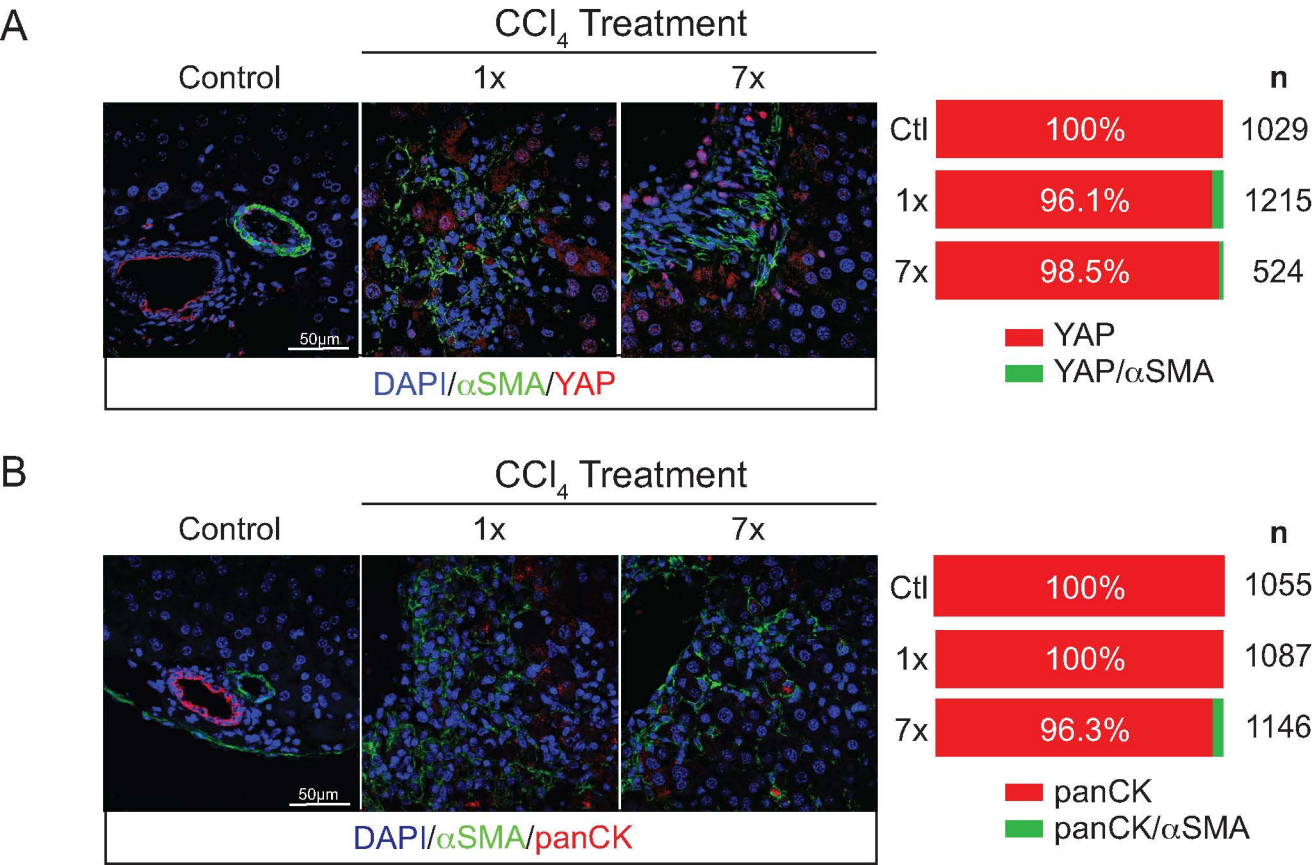

A
CCl4 Treatment
Control
1x
7x
50μm
DAPI/αSMA/YAP
n
Ctl
100%
1029
1x
96.1%
1215
7x
98.5%
524
YAP
YAP/αSMA
B
CCl4 Treatment
Control
1x
7x
50μm
DAPI/αSMA/panCK
n
Ctl
100%
1055
1x
100%
1087
7x
96.3%
1146
panCK
panCK/αSMA

Figure S4

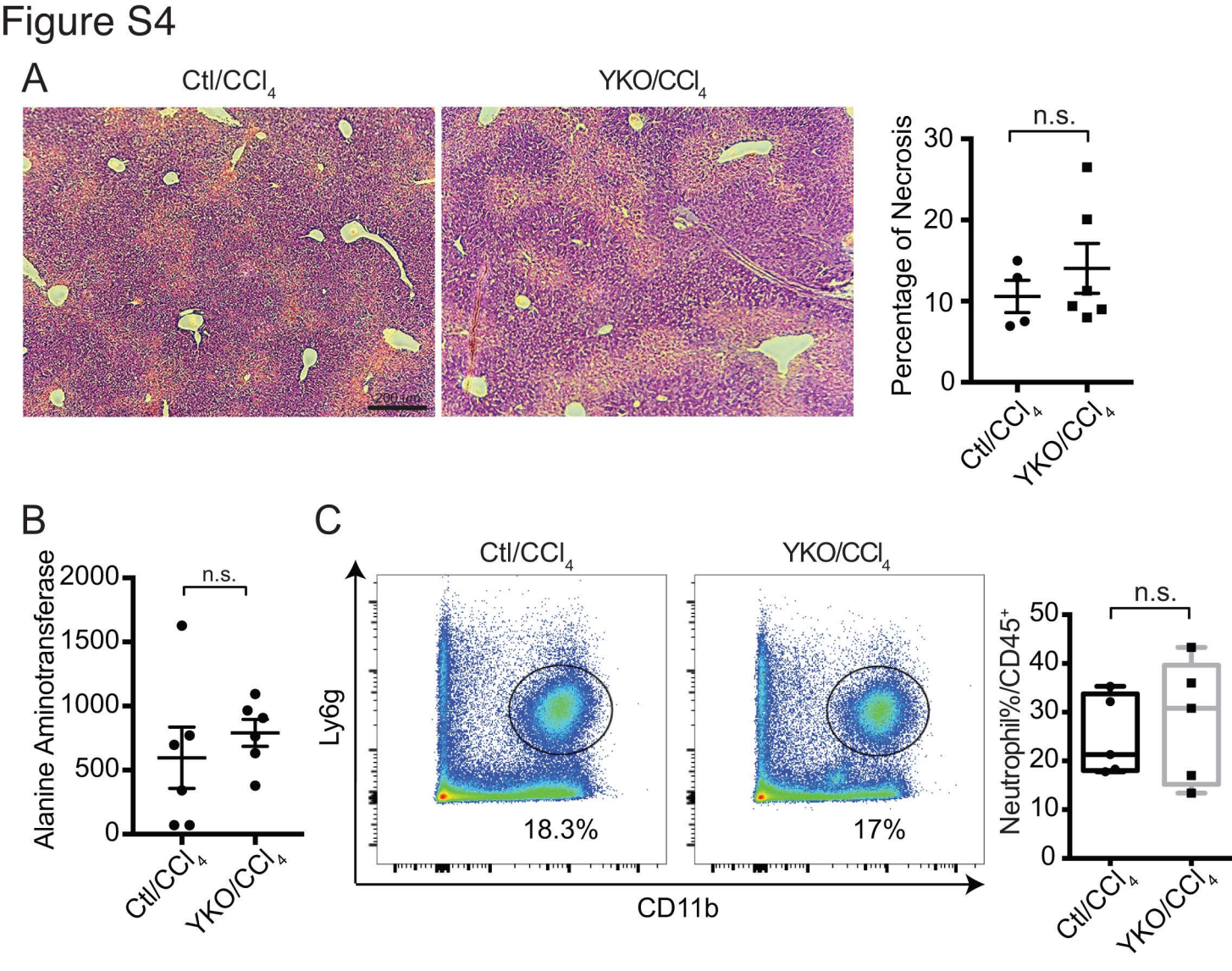
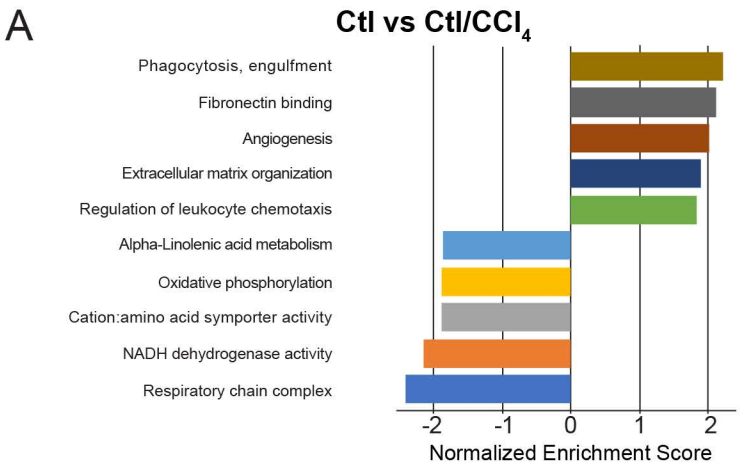
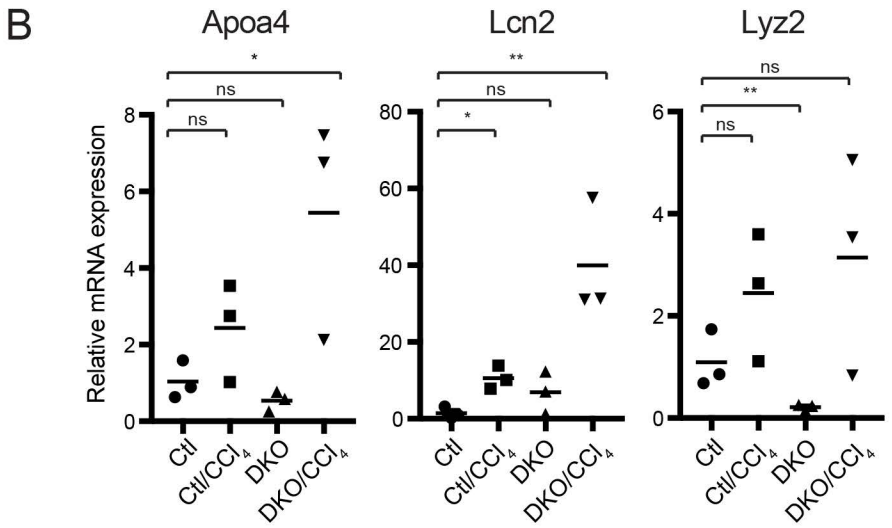

A
Ctl/CCl4
YKO/CCl4
200 μm
Percentage of Necrosis
n.s.
30
20
10
0
Ctl/CCl4
YKO/CCl4
B
Alanine Aminotransferase
n.s.
2000
1500
1000
500
0
Ctl/CCl4
YKO/CCl4
C
Ctl/CCl4
YKO/CCl4
Ly6g
CD11b
18.3%
17%
Neutrophil%/CD45+
n.s.
50
40
30
20
10
0
Ctl/CCl4
YKO/CCl4

Figure S5


A
Ctl vs Ctl/CCl4
Phagocytosis, engulfment
Fibronectin binding
Angiogenesis
Extracellular matrix organization
Regulation of leukocyte chemotaxis
Alpha-Linolenic acid metabolism
Oxidative phosphorylation
Cation:amino acid symporter activity
NADH dehydrogenase activity
Respiratory chain complex
-2
-1
0
1
2
Normalized Enrichment Score


B
Apoa4
Lcn2
Lyz2
Relative mRNA expression
*
**
ns
Ctl
Ctl/CCl4
DKO
DKO/CCl4

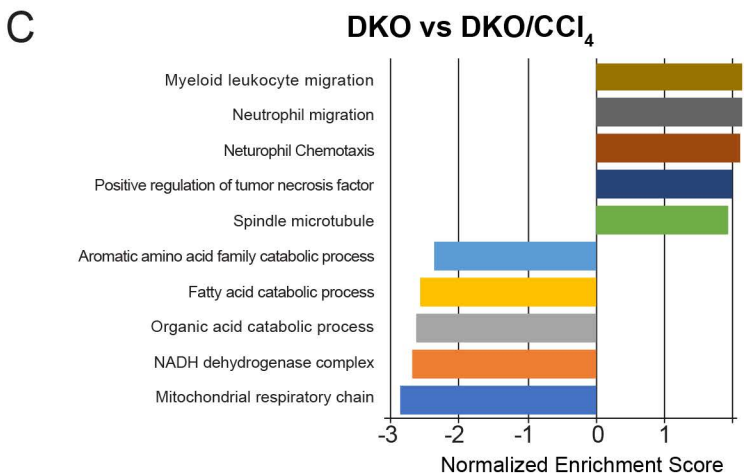

C
DKO vs DKO/CCl4
Myeloid leukocyte migration
Neutrophil migration
Neturophil Chemotaxis
Positive regulation of tumor necrosis factor
Spindle microtubule
Aromatic amino acid family catabolic process
Fatty acid catabolic process
Organic acid catabolic process
NADH dehydrogenase complex
Mitochondrial respiratory chain
-3
-2
-1
0
1
Normalized Enrichment Score

Figure S6

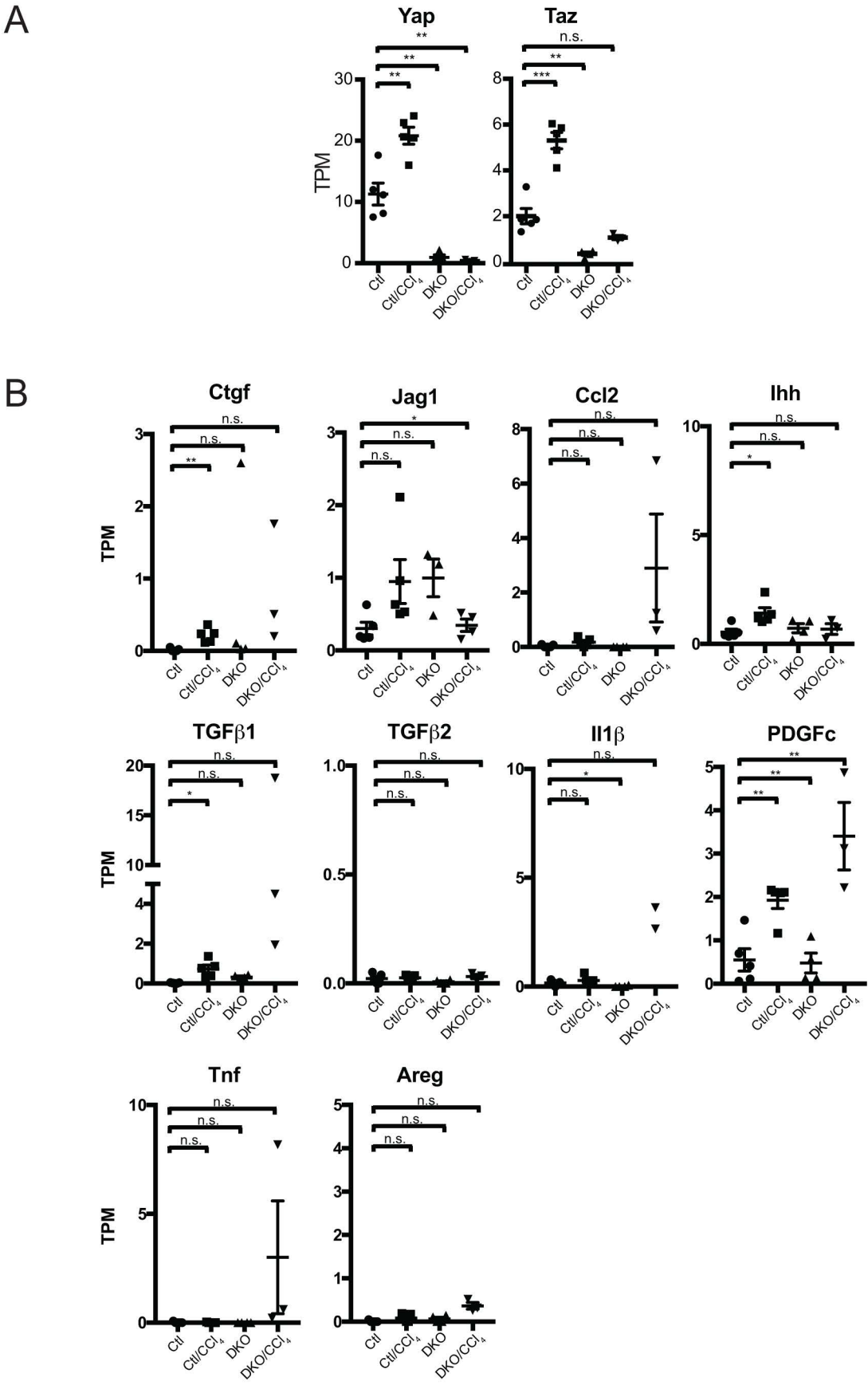

A
Yap
Taz
TPM
n.s.
Ctl
Ctl/CCl4
DKO
DKO/CCl4
B
Ctgf
Jag1
Ccl2
Ihh
TGFβ1
TGFβ2
Il1β
PDGFc
Tnf
Areg

Figure S7

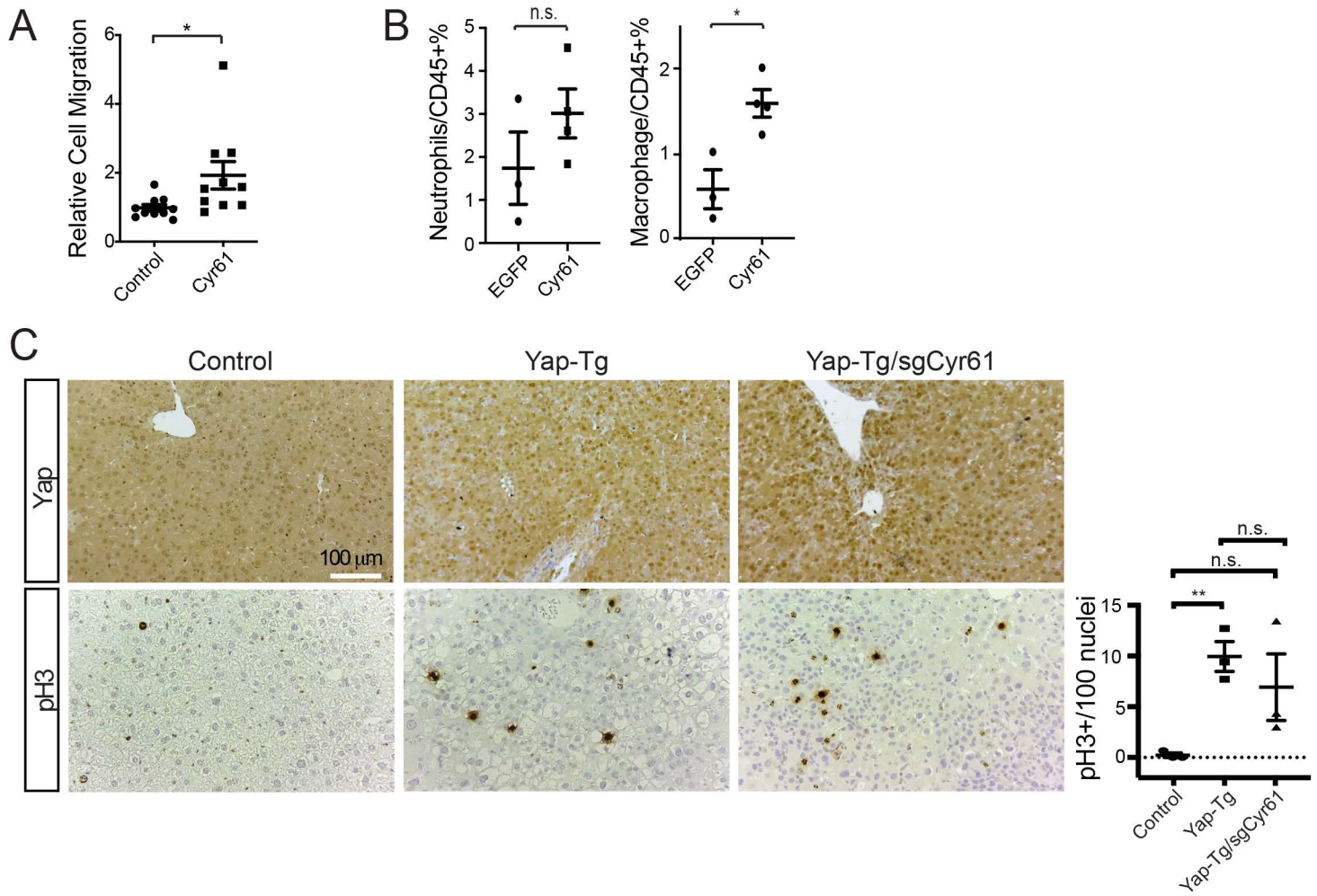
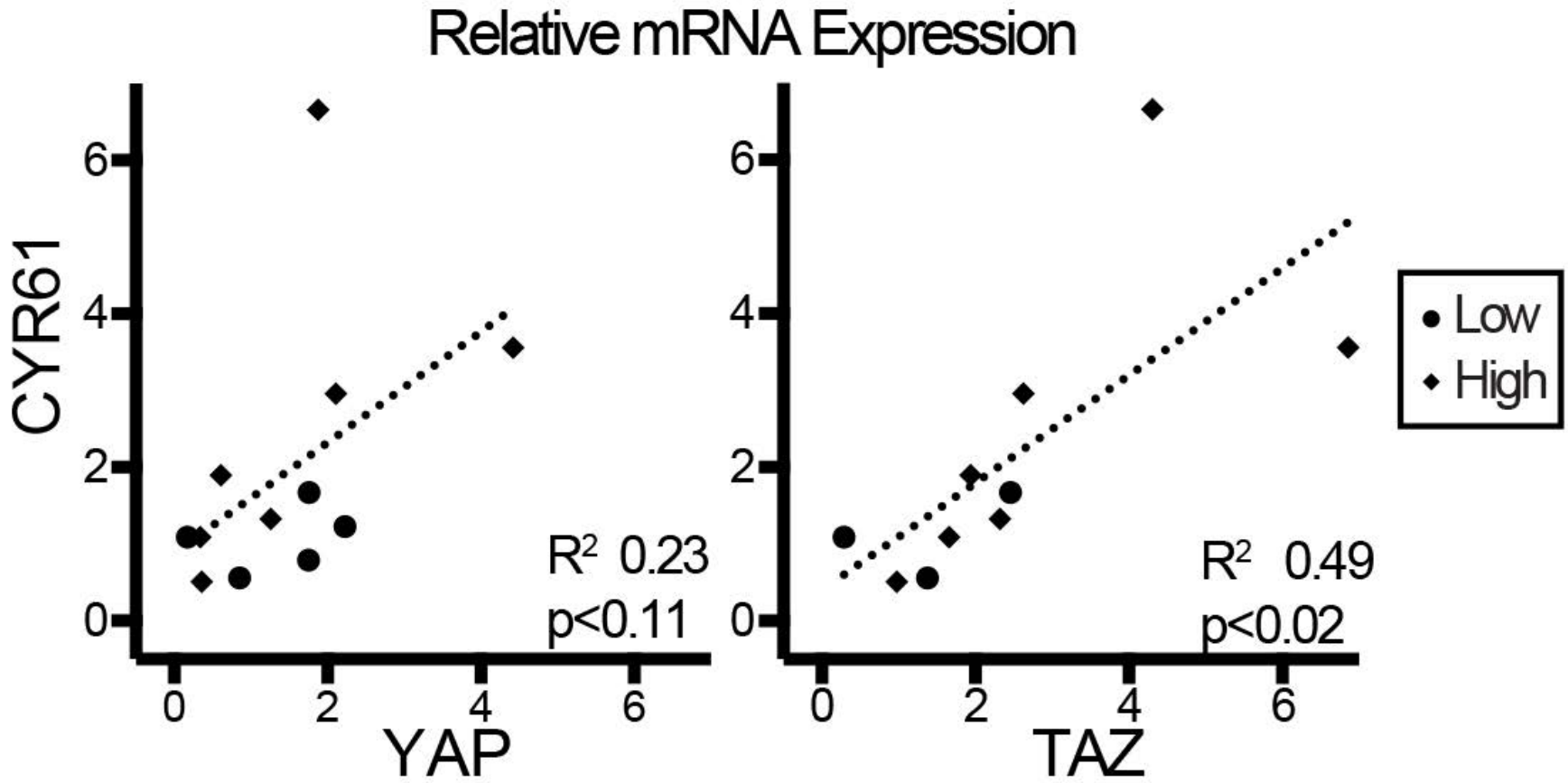

A
Relative Cell Migration
*
Control
Cyr61
B
Neutrophils/CD45+%
n.s.
EGFP
Cyr61
Macrophage/CD45+%
*
EGFP
Cyr61
C
Control
Yap-Tg
Yap-Tg/sgCyr61
Yap
pH3
100 μm
pH3+/100 nuclei
n.s.
n.s.
**
Control
Yap-Tg
Yap-Tg/sgCyr61

Figure S8


Relative mRNA Expression
CYR61
YAP
TAZ
R² 0.23
p<0.11
R² 0.49
p<0.02
Low
High
0
2
4
6

SUPPLEMENTAL FIGURE LEGENDS

FIGURE S1. Hepatocyte-specific YAP expression Drives Canonical Hippo Gene Expression

A. Schematic diagramming strategy of hepatocyte-specific YAP S127A expression. Tetracycline-inducible YAP S127A mice contain the Rosa26 locus, a floxed STOP cassette inhibiting a reverse tetracycline transactivator (rtTA) (TetOYAP). TetOYAP mice are intravenously injected with an adeno-associated virus, serotype 8 with Cre recombinase expressed under the control of the thyroxine-binding globulin promoter (AAV-Cre). Doxycycline is administered to induce YAP S127A expression in 8- to 12-week-old mice (YAP-Tg).
B. Representative image of serial section stained YAP-Tg (2 weeks) expressing livers showing Collagen and αSMA in the portal area.
C. Dot plots of whole liver RT-qPCR of common Hippo Targets in YAP-Tg livers at the indicated timepoints.
D. Representative image of Control and YAP-Tg (2 weeks) expressing livers showing CTGF in EYFP-labeled hepatocytes by RISH.
E. Dot plots of whole liver RT-qPCR of the noted genes after YAP-Tg at the indicated timepoints. Ctl – Control, 5d – 5 day, 2wk – 2 week, 4wk – 4 week. *$p<0.05$, **$p<0.01$, ***$p<0.001$.

FIGURE S2. YAP-Tg cells Potently Upregulate Ductal Genes and Demonstrate Minimal Expression of Fibroblast Genes

A. Immunofluorescent staining of titrated AAV-Cre YAP-Tg livers for EYFP (YAP-Tg hepatocytes), SMA, and Desmin. Parts of a whole analysis shown below.

B. Immunofluorescent staining of titrated AAV-Cre YAP-Tg livers for EYFP (YAP-Tg hepatocytes), αSMA, and Sox9. Parts of a whole analysis shown below.

C. Expression of ductal genes in sorted hepatocytes in control liver (Ctl) or after 1 week YAP-Tg (1wk) or 6 weeks YAP-Tg (6wk).

D. Expression of fibroblast genes in sorted hepatocytes in control liver (Ctl) or after 1 week YAP-Tg (1wk) or 6 weeks YAP-Tg (6wk).

E. Relative mRNA expression of common hepatocyte-associated genes from control (Ctl) and YAP-Tg cells after 2 weeks of expression. Horizontal line represents the mean, each dot represents an experiment. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001, n.s.= not significant.

FIGURE S3. A Minimal Number of Cells Undergo Epithelial to Mesenchymal Transition During Acute and Chronic CCl_4 Injury

A. Representative YAP/SMA immunostaining of murine livers in control, 1 or 7 treatments of CCl_4. Parts of a whole analysis shown to the right.

B. Representative SMA/panCK immunostaining of murine livers in control, 1 or 7 treatments of CCl_4. Parts of a whole analysis shown to the right.

FIGURE S4. Liver Neutrophil Proportion are Unaffected by Hepatocyte Yap Status During Chronic CCl_4 injury

A. Representative H&E stain of the indicated genotypes after a single dose of CCl_4 showing areas of necrosis. Right, Dot plot of quantified necrotic areas by ImageJ.

B. Dot plot of serum Alanine Aminotransferase activity 24 hours after CCl_4 treatment in the indicated groups.

C. Representative flow cytometry plot of liver neutrophils one day after chronic CCl_4 treatment. Percentages in the graph are of the gated population. Right, dot plot of all

performed experiments (n=5, each). Horizontal line represents the mean, each dot represents a sample.

FIGURE S5. Liver mRNA Expression is Increased in the Presence of DKO/CCl_4 Injury over CCl_4 Injury Alone

A. The top and bottom differentially expressed gene programs identified by GSEA for Ctl vs Ctl/CCl_4
B. Dot plots of whole liver RT-qPCR of genes identified by hepatocyte RNA-seq to be increased in DKO/CCl_4 mice to a higher degree than from control mice exposed to CCl_4. n.s. – not significant, *p<0.05, **p<0.01. Horizontal line represents the mean, each dot represents an experiment.
C. The top and bottom differentially expressed gene programs identified by GSEA for DKO vs DKO/CCl_4

FIGURE S6. In Hepatocytes, Canonical Hippo Pathway Targets and Inflammatory Genes Are Not Yap/Taz Responsive Genes

A. Yap and Taz mRNA hepatocyte expression levels as assessed by RNA-seq under the indicated conditions.
B. Hippo pathway or inflammatory gene mRNA hepatocyte expression levels as assessed by RNA-seq under the indicated conditions. Transcripts per million base pairs (TPM). n.s. – not significant, *p<0.05, **p<0.01. Horizontal line represents the mean, each dot represents an experiment. Upper and lower bounds represent SEM.

FIGURE S7. Cyr61 is the Primary Macrophage Chemoattractant of the Hippo Pathway

A. Dot plot of transwell migration assay of the macrophage cell line RAW264.7 to control or Cyr61 supplemented media.

B. Dot plot of flow cytometry analysis for liver neutrophils ($CD45^+CD11b^+Ly6G^+$) or macrophages ($CD45^+CD11b^+Ly6G^-F4/80^+$) in AAV-EGFP or AAV-Cyr61 mice, one week after treatment.

C. Immunostaining for Yap and pH3 in Control, Yap-Tg and Yap-Tg/sgCyr61 livers at 2 weeks of induction. Right, Dot plot of $pH3^+$/100 nuclei for the indicated conditions.

FIGURE S8. Relationship of YAP and TAZ to CYR61 mRNA expression in NASH patients

X-Y plots $\Delta\Delta C_t$ of Yap-Cyr61 and Taz-Cyr61 expression from NASH patients.

SUPPLEMENTAL METHODS

CCl_4 and CDE Injury

20% CCl_4 in corn oil was administered through intraperitoneal(IP) injections at a dose of 1 μg/g body weight every 72 hour intervals. Injury was either acute(1 injection) or chronic(7 injections). Unless otherwise indicated, animals were analyzed three days after the last dose of CCl_4. The CDE diet was purchased from MP Biomedicals(Solon, OH, Cat# 02960214) and provided to mice *ad libitum* at the start of the experiment.

Tissue Preparation, Staining, Immunohistochemistry and RNA in situ Hybridization(RISH)

Tissue was fixed overnight in 10% formalin(Sigma-Aldrich, St. Louis, MO) and embedded in paraffin for sectioning. 5 μm tissue sections were rehydrated followed by antigen retrieval using low pH Antigen Unmasking Solution(Vector Labs, Burlingame, CA). Antibodies used in this study are noted in **Supplementary Table 2**. For immunohistochemistry, Vectastain Elite ABC kit and secondary antibody (Vector Labs) were used to detect primary antibody expression. For immunofluorescence, the Alexa series of fluorescently labeled antibodies (Thermo-Fisher Scientific, Waltham, MA) were used as secondary antibodies at a 1:1000 dilution. Confocal imaging was performed on a Zeiss LSM 710.

Collagen staining was accomplished by incubating rehydrated liver sections in 0.04% Direct Red 80(Cat# 365548, Sigma-Aldrich)/0.01% Fast Green(Cat# F7258, Sigma-Aldrich) in picric acid solution. Collagen quantification is performed by examining a minimum of five random fields in at least three mice using the density quantification function in ImageJ.

For studies of *in vivo* cell proliferation, experimental mice were given a single 100 mg/kg IP injection of EdU in PBS, 24 hours prior to sacrifice. Rehydrated liver sections were incubated at room temperature with Click-iT cocktail(Thermo-Scientific) according to the manufacturer's

directions followed by mounting and fluorescent imaging on a Zeiss Axiovert 200(Carl Zeiss Microscopy, Jena, Germany).

RISH is performed with RNAScope 2.5 HD Duplex Reagent Kit(Advanced Cell Diagnostics, Cat# 322430). Probes for mCTGF(Cat# 314541), mCyr61(Cat# 429001) and hCyr61(Cat# 452081) were used.

Cell Migration Assay

24 hours prior to seeding migration experiments, TIB-71 RAW 264.7(ATCC) mouse macrophages were serum-starved: media was changed from DMEM(Gibco #11995-065) + 10% FBS(22)(Gibco #104370-028), to DMEM + 1% FBS. At seeding, 1x10^5 cells in DMEM + 1% FBS were placed into Corning Fluoroblok 24-well format cell culture inserts(#351152). Cells were allowed to migrate for 19 hours toward either fresh media(DMEM/F12 + 10% FBS), fresh media + 2ug/ml rhCyr61/Fc Chimera(R&D Systems #4055-CR), or fresh media + 4ug/ml BSA (Sigma #A2153). Inserts were then placed into 4 μM Calcein-AM(Corning #354217) and imaged on an epifluorescent microscope at 10x magnification. Composite images showing cells on the trans side along the entire length of each insert were quantified using ImageJ.

Assessment of YAP, TAZ, CYR61 Expression in NASH Patient Samples
Patient liver explants were obtained at the time of surgery after informed consent. These were evaluated as having a low or high NASH score (22) by an independent clinical pathologist with a total of 10 patients per group. These were either immunostained for YAP or TAZ or probed for CYR61 (ACDBio, Newark, CA, Cat# 452081). Two independent blinded investigators scored the staining intensity of each slide. Staining was scored as 0 – none, 1 - minimal, 2 – moderate, 3 – moderate/severe, 4 – severe. RNA was extracted from this cohort from paraffin samples using Qiagen RNeasy FFPE Kit (Cat# 73504) and qPCR performed as noted above.

Statistical Analysis

A minimum of three separate mice was combined for all displayed graphs unless otherwise indicated. Student's t-test was used to determine significance unless otherwise indicated. Significance is defined as a p value of 0.05 or less. Error bars on all graphs are standard error of the mean.

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Tissue was fixed overnight in 10% formalin (Sigma-Aldrich, St. Louis, MO) and embedded in paraffin for sectioning. 5 μm tissue sections were rehydrated followed by antigen retrieval using low pH Antigen Unmasking Solution (Vector Labs, Burlingame, CA). Antibodies used in this study are noted in **Supplementary Table 2**. For immunohistochemistry, Vectastain Elite ABC kit and secondary antibody (Vector Labs) were used to detect primary antibody expression. For immunofluorescence, the Alexa series of fluorescently labeled antibodies (Thermo-Fisher Scientific, Waltham, MA) were used as secondary antibodies at a 1:1000 dilution. Confocal imaging was performed on a Zeiss LSM 710.

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Liver qRT-PCR and Hepatocyte Isolation for qRT-PCR and RNA-Seq Analysis

Whole liver total RNA was isolated using Trizol Reagent (Thermo-Fisher) according to the manufacturer's directions. This RNA was reversed transcribed using iScript (Bio-Rad, Hercules, CA) and was used as material to perform reverse-transcription qPCR on a StepOnePlus using Fast Taqman reagents (Thermo-Fisher). mRNA expression levels were estimated using the $2\Delta\Delta C_T$ method. A full inventory of probes is available in **Supplementary Table 5**.

For hepatocyte isolations, mice containing either TetOYap or Yap/Taz floxed alleles and the inducible tdTomato reporter allele were administered AAV-TBG-Cre one week prior to experimentation. Control hepatocytes were isolated from tdTomato reporter mice after the administration of AAV-TBG-Cre. After collagenase perfusion of the liver, the livers were mechanically disassociated and resuspended for fluorescent-activated cell sorting on a BD FACSAria II (BD Biosciences, San Jose, CA). Hepatocytes were purified using a strategy gaiting for $DAPI^{-}tdTomato^{+}$ cells. RNA from these cells were isolated using the RNeasy Micro Kit (Qiagen, Germantown, MD) according to the manufacturer's directions. Illumina sequencing libraries were prepared with the TruSeq Stranded mRNA Library Preparation Kit (Illumina Inc., San Diego, CA) and run on a HiSeq 2500.

Intrahepatic Lymphocyte (IHL) Purification

Intrahepatic lymphocytes were purified according to the procedure outlined by Blom et al.[20] Briefly, livers are isolated and flushed with PBS/Collagenase. These are mechanically disassociated and forced through a 100 μm sieve. Lymphocytes are recovered through a Percoll gradient (Sigma-Aldrich, Cat# P4937) and resuspended for immunostaining. IHLs were

stained either one week after Yap induction or 24 hours after the last dose of CCl_4. See **Supplemental Table 3** for antibody details.

Immunoblotting

Whole liver lysates of the indicated treatments were run on SDS-PAGE gels and immunoblotted by standard methods. See **Supplemental Table 4** for antibody details.

Bioinformatic Analysis

Samples were processed through the bcbio-nextgen RNA-seq pipeline, version 1.0.0a0-8aa8f0c. Samples were aligned to mm10 augmented with the Ensembl gene models using the STAR aligner. Quality control information from FastQC version 0.11.5, samtools 1.3.1, qualimap 2.2 and custom tools was calculated from the STAR alignments. Transcript level quantifications were calculated with Sailfish version 0.10.1 and imported for gene-level differential expression using tximport. Samples were quality controlled and differential expression was calculated with DESeq2, fitting a model looking at the effect of genotype, treatment and their interaction. Transcript-level differential expression was performed with sleuth, fitting a similar model. GSEA was performed for each contrast by ranking genes based on signed signal-to-noise ratio with clusterProfiler.

Cell Migration Assay

24 hours prior to seeding migration experiments, TIB-71 RAW 264.7 (ATCC) mouse macrophages were serum-starved: media was changed from DMEM (Gibco #11995-065) + 10% FBS (Gibco #104370-028), to DMEM + 1% FBS. At seeding, $1x10^5$ cells in DMEM + 1% FBS were placed into Corning Fluoroblok 24-well format cell culture inserts (#351152). Cells were allowed to migrate for 19 hours toward either fresh media (DMEM/F12 + 10% FBS), fresh media + 2ug/ml rhCyr61/Fc Chimera (R&D Systems #4055-CR), or fresh media + 4ug/ml BSA (Sigma #A2153). Inserts were then placed into 4 μM Calcein-AM (Corning #354217) and

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Assessment of Yap, Taz, Cyr61 Expression in NASH Patient Samples

Patient liver explants were obtained at the time of surgery after informed consent. These were evaluated as low or high grade NASH by an independent clinical pathologist with a total of 10 patients per group. These were either immunostained for Yap or Taz or probed for Cyr61 (ACDBio, Newark, CA, Cat# 452081). Two independent blinded investigators scored the staining intensity of each slide. Staining was scored as 0 – none, 1 - minimal, 2 – moderate, 3 – moderate/severe, 4 – severe. RNA was extracted from this cohort from paraffin samples using Qiagen RNeasy FFPE Kit (Cat# 73504) and qPCR performed as noted above.

Statistical Analysis

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SUPPLEMENTAL REFERENCES

1. Camargo FD, Gokhale S, Johnnidis JB, et al. YAP1 increases organ size and expands undifferentiated progenitor cells. Current Biology 2007;17:2054-60.

SUPPLEMENTAL TABLE 1. NASH Patient Scoring

ID	Steatosis Score	Lobular Inflammation	Hepatocyte Ballooning	Fibrosis
Control				
C1	0	0	0	0
C2	0	0	0	0
C3	1	0	0	0
C4	0	0	0	0
C5	0	0	0	0
C6	0	0	0	0
C7	0	0	0	0
C8	1	0	0	0
C9	0	0	0	0
C10	0	0	0	0
NASH				
H1	1	1	2	4
H2	3	2	2	4
H3	3	2	2	4
H4	2	1	1	3
H5	2	2	1	3
H6	3	2	2	4
H7	3	2	1	4
H8	3	1	2	3
H9	3	1	1	3
H10	2	1	2	3

SUPPLEMENTAL TABLE 2 – List of Antibodies for immunocytochemistry

Antibody	Species	Catalog #	Dilution	Manufacturer	Notes
Yap	Rabbit	D8H1X	1:400	Cell Signaling	Tyramide amplification
DsRed	Mouse	632392	1:500	Clontech	
αSMA	Mouse	1A4	1:400	Dako	
GFP	Chicken	GFP-1020	1:500	Aves Labs	
panCK	Rabbit	Z0622	1:1000	DakoCytomation	
F4/80	Rat	123106	1:200	BioLegend	
pH3	Rabbit	9701	1:200	Cell Signaling	
Hnf4α	Mouse	Ab41898	1:200	Abcam	
Rabbit IgG	Goat	BA-1000	1:200	Vector Labs	Biotinylated
Mouse IgG	Mouse	MKB-2225	1:250	Vector Labs	Biotinylated

SUPPLEMENTAL TABLE 3 – List of Antibodies for flow cytometry

Antibody	Species	Catalog #	Dilution	Manufacturer	Clone#
CD45	Rat	103126	1:200	BioLegend	30-F11
CD11b	Rat	101212	1:200	BioLegend	M1/70
F4/80	Rat	123106	1:200	BioLegend	BM8
Ly6G	Rat	127608	1:200	BioLegend	1A8

SUPPLEMENTAL TABLE 4 – List of Antibodies for Immunoblotting

Antibody	Species	Catalog #	Dilution	Manufacturer
F4/80	Rat	123106	1:200	BioLegend
Yap	Rabbit	4912	1:1000	Cell Signaling
Taz	Rabbit	48835	1:1000	Cell Signaling
αSMA	Mouse	1A4	1:300	Dako
Cyr61	Rabbit	ab24448	1:1000	Abcam
Gapdh	Rabbit	4970	1:1000	Cell Signaling

β-Actin	Mouse	3700	1:1000	Cell Signaling

SUPPLEMENTAL TABLE 5 – List of probes/primers for qRT-PCR

All Taqman probes were purchased from Thermo-Scientific.

Gene	**Catalog number**
IHH	Mm00439613_m1
Col1A1	Mm00801666_g1
Ctgf	Mm01192933_g1
F4/80	Mm00802529_m1
18S	4319413E
Pdgfc	Mm00480205_m1
Timp1	Mm00441818_m1
TNF	Mm00443258_m1
Taz	Mm01289583_m1
Yap1	Mm01143263_m1

TGFb1	Mm01178820_m1
TGFb2	Mm00436955_m1
IL-10	Mm01288386_m1
IL-1β	Mm00434228_m1
Sox9	Mm00448840_m1
IL-6	Mm00446190_m1
Jag1	Mm00496902_m1
Notch2	Mm00803077_m1
Yap	Hs00902712_g1
Taz	Hs00210007_m1
Cyr61	Hs00155479_m1