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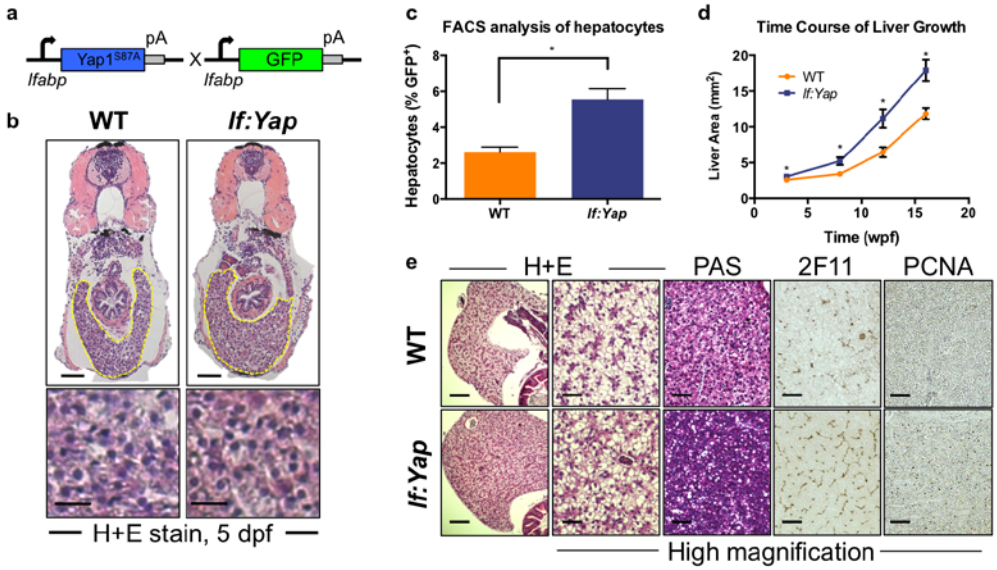

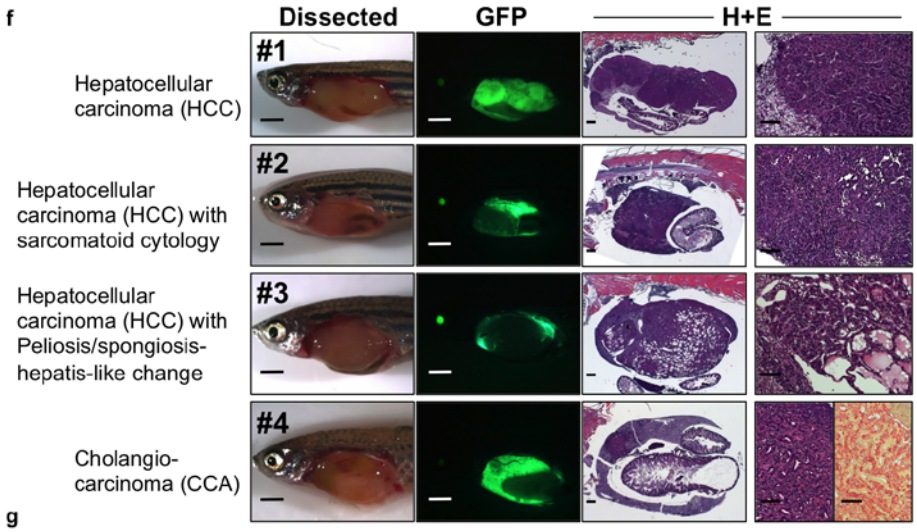

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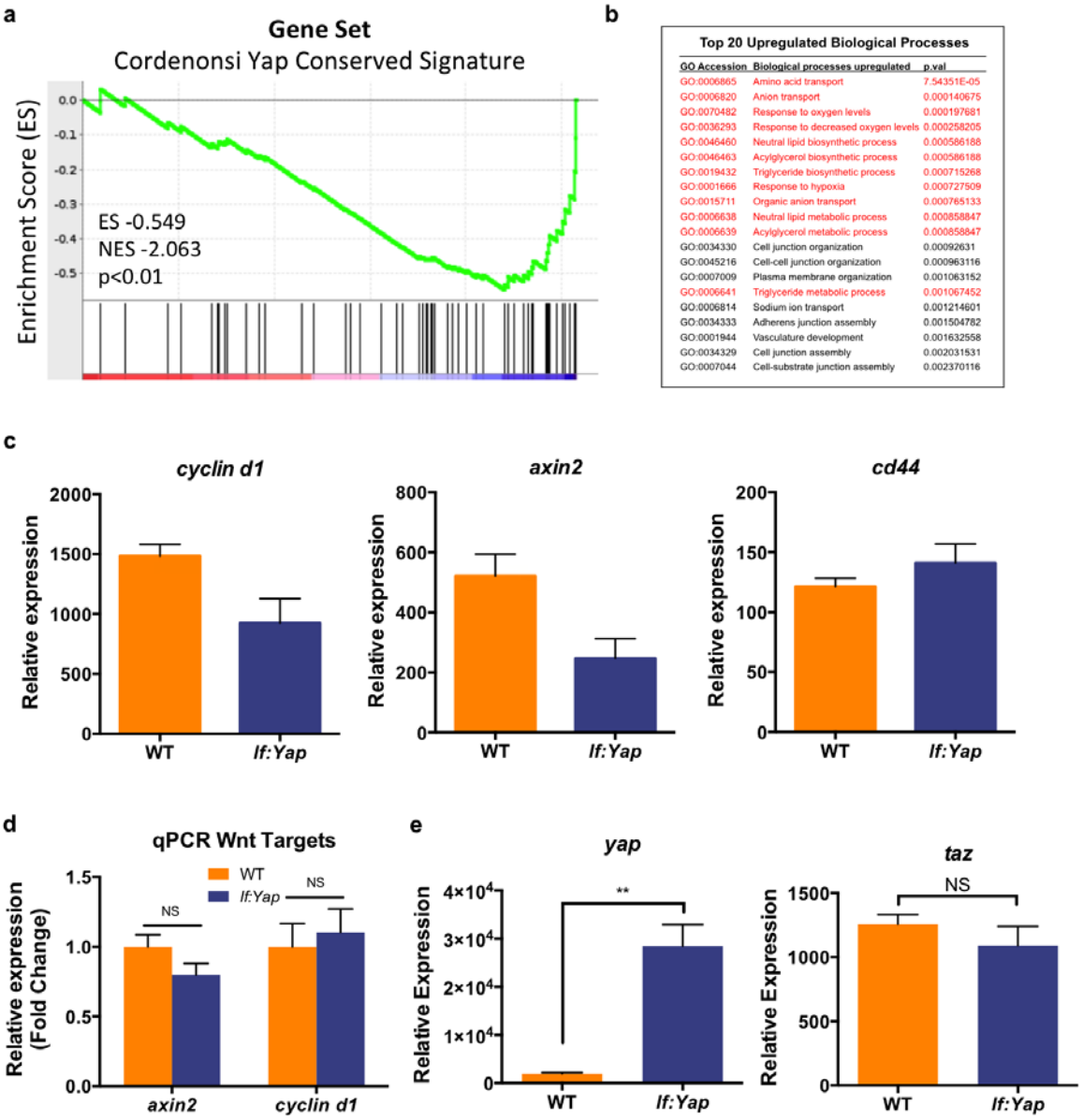
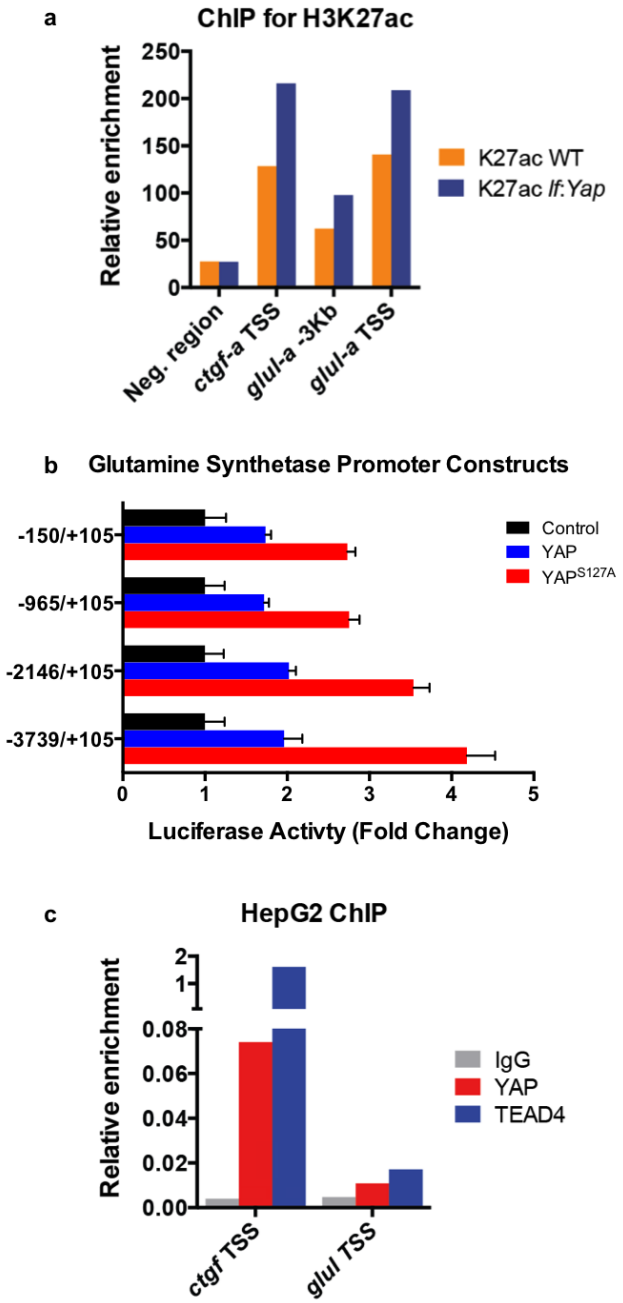
	Tumor subtype	Fibrosis	Glycogen poor	Sarcomatoid cytology	Peliosis/ spongiosis hepatis	Ascites
WT	HCC	50% (2/4)	100% (4/4)	25% (1/4)	25% (1/4)	0% (0/4)
	CCA	100% (1/1)	100% (1/1)	N/A	N/A	0% (0/1)
Yap^{S87A}	HCC	47% (9/19)	84% (16/19)	21% (4/19)	58% (11/19)	32% (6/19)
	CCA	100% (4/4)	100% (4/4)	N/A	N/A	25% (1/4)

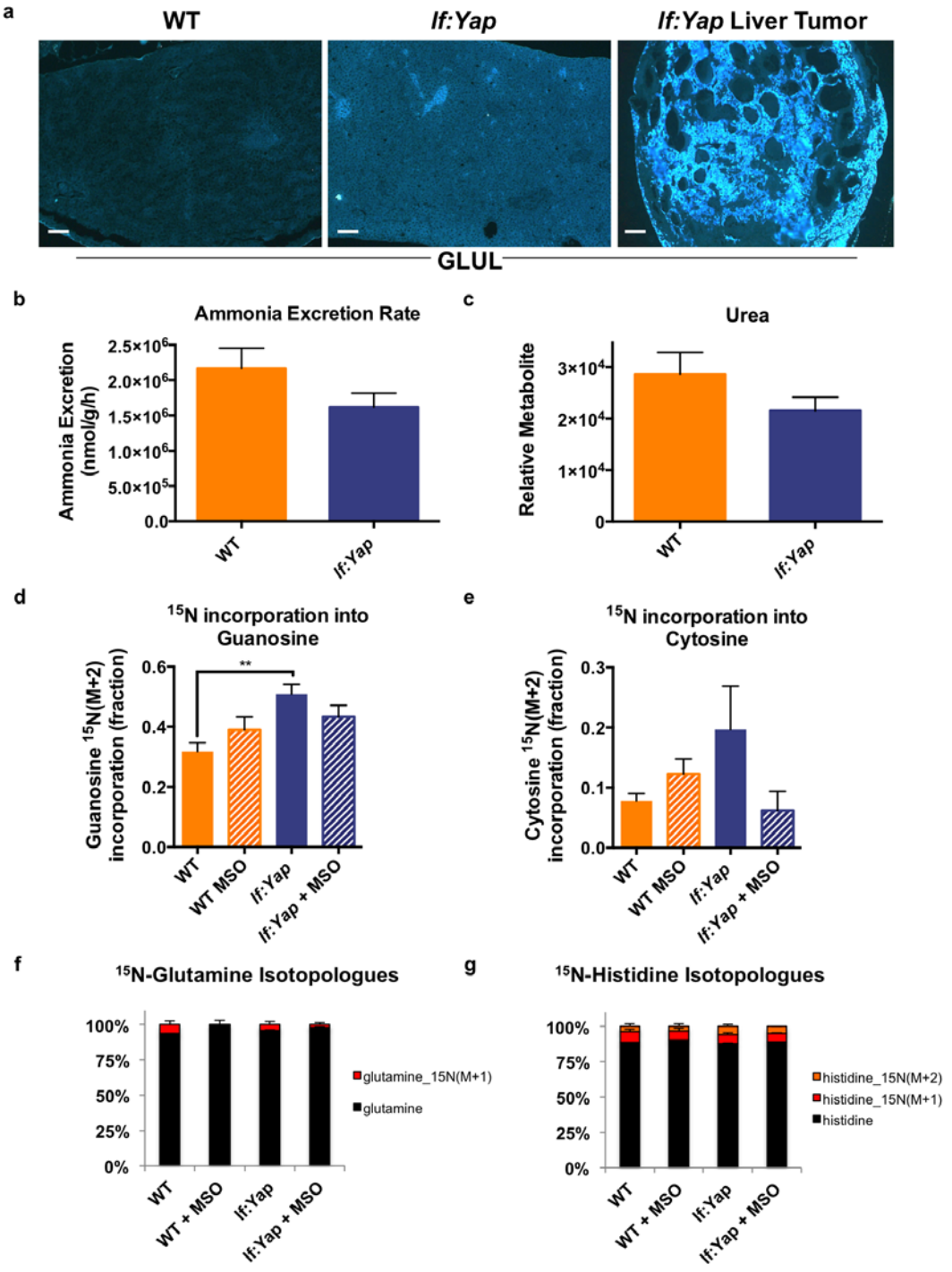
Supplementary Figure 1 Hepatocyte-specific overexpression of activated Yap causes hepatomegaly and accelerates DMBA-induced liver tumor formation. (a) Schematic diagram of the construct used to generate transgμμμenic fish with hepatocyte-specific (*fapb10a*) expression of *yap1*^{S87A}, *abbreviated to If:Yap*. (b) Liver histology (H&E stain) in transverse sections of WT and *If:Yap* larvae at 5 dpf. Scale bars: 50μm (upper) and 20μm (lower) for zoomed images. (c) Quantification of differentiated hepatocyte frequency in WT and *If:Yap* larvae at 10 dpf, as determined by FACS. n=5 biologically independent WT and *If:Yap* single cell suspensions, each of which was derived from 10 larvae; see Supplementary Table 4. **p<0.01, two-sided Student's *t*-test, values represent the mean±SEM. (d) Time course of hepatomegaly during early adulthood as determined by quantification of fluorescent liver area. n=10, 6, 6, 4, 10, 8, 6 and 4 WT 3 wpf, WT 8 wpf, WT 12 wpf, WT 16 wpf, *If:Yap* 3 wpf, *If:Yap* 8 wpf, *If:Yap* 12 wpf and *If:Yap* 16 wpf zebrafish respectively; see Supplementary Table 4. *p<0.05, two-sided Student's *t*-test, values represent the mean±SEM. (e) Histological assessment of transverse sections from liver of WT and *If:Yap* adults. H&E staining at low (10x) and high magnification (25x). Periodic-acid Schiff (PAS) stain for hepatic glycogen (pink inclusions). Bile duct morphogenesis determined by 2F11 staining. Cell proliferation as determined by PCNA staining. Scale bars: 200μm and 50μm for zoomed images. (f) Tumor heterogeneity in *If:Yap* transgenics exposed to DMBA. Tumors include hepatocellular carcinoma (HCC), HCC with sarcomatoid features, HCC with peliosis/spongiosis-hepatis-like change, and cholangiocarcinoma (CCA). Scale bars: 2mm for dissected images, 200μm for histology and 50μm for zoomed images. (g) Table illustrating the incidence of fibrosis, as determined by Sirius Red staining, glycogen, sarcomatoid cytology, peliosis/spongiosis-hepatis-like change and ascites in DMBA-induced liver tumors. n=4 and 19 WT and *If:Yap* liver tumors respectively.



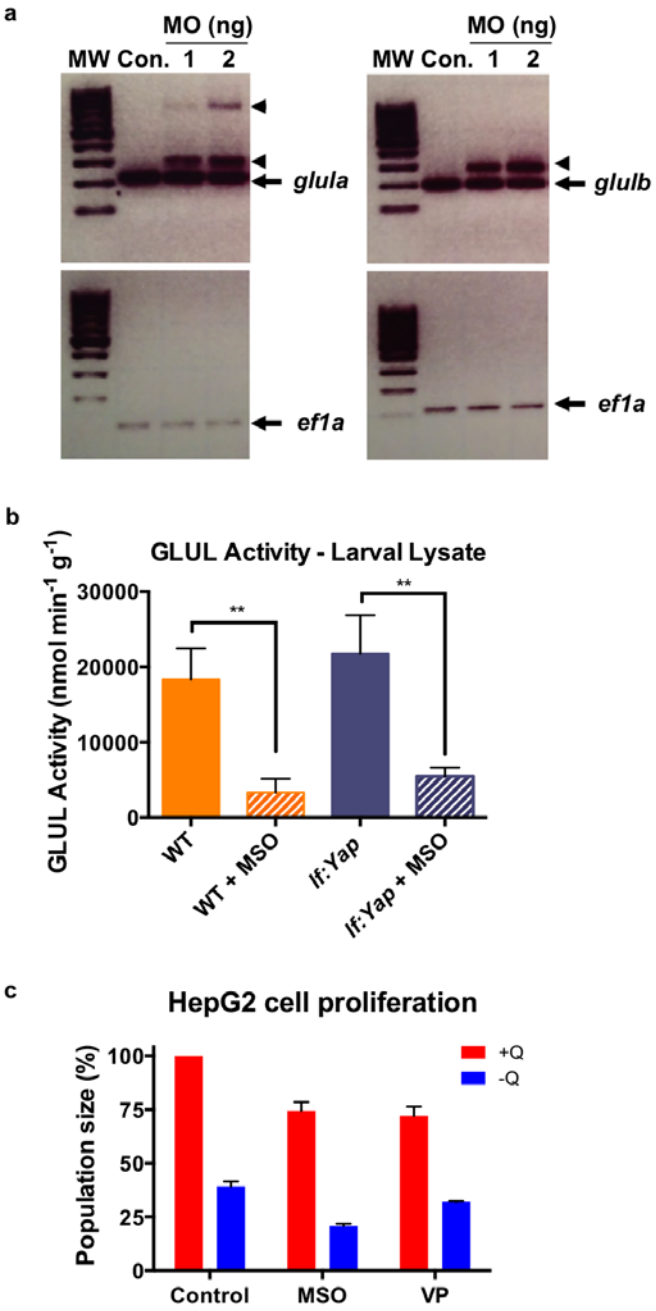
Supplementary Figure 2 Yap alters expression of metabolism-related genes and enhances *glul* expression. (a) Gene set enrichment (GSEA) derived from RNAseq of WT and *lf:Yap* adult livers identifies a conserved Yap target gene signature. (b) Gene ontology (GO) analysis of the top 20 biological processes upregulated by Yap. Biological processes related to metabolism are highlighted in red. (c) RNAseq analysis of the Wnt target genes *cyclind1*, *axin2*, *cd44* in dissected WT and *lf:Yap* livers. n=3 WT and *lf:Yap* adult livers; see Supplementary Table 4. Values represent the mean±SEM. (d) qPCR validation of Wnt target genes *axin2* and *cyclind1* in adult zebrafish livers. n=3 WT and *lf:Yap* adult livers; see Supplementary Table 4. Values represent the mean±SEM. (e) RNAseq analysis of *yap* and *taz* expression in dissected WT and *lf:Yap* livers. n=3 WT and *lf:Yap* adult livers; see Supplementary Table 4. **p<0.01, two-sided Student's *t*-test, values represent the mean±SEM.



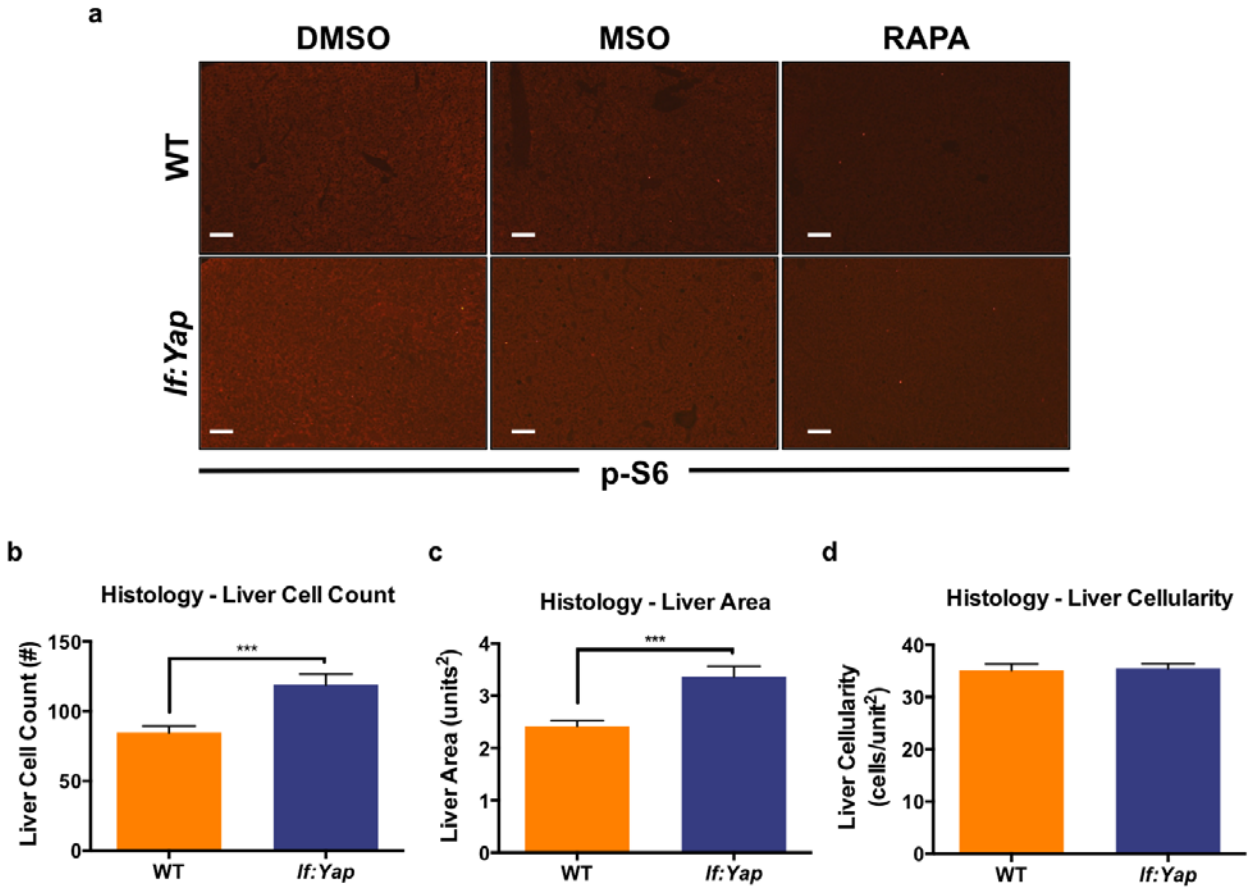
Supplementary Figure 3 Yap transcriptionally upregulates GLUL in an evolutionarily conserved fashion. (a) ChIP qPCR analysis of transcriptional activity (H3K27ac) at the *glula* promoter in dissected WT and *lf:Yap* livers. Shown is the average of 2 biologically independent WT and *lf:Yap* adult liver chromatin preps, each of which was derived from a pooled sample of 2; see Supplementary Table 4. (b) Luciferase GLUL reporter assay using truncated promoter constructs in Hek293 cells expressing GFP, *YAP* or *YAP1*^{S127A}. n=3 biologically independent replicates; see Supplementary Table 4. Values represent the mean±SEM. (c) ChIP qPCR analysis of YAP and TEAD4 enrichment at the *GLUL* promoter in Hep3B and HepG2 cells. Shown is the average of 2 biologically independent replicates; see Supplementary Table 4.



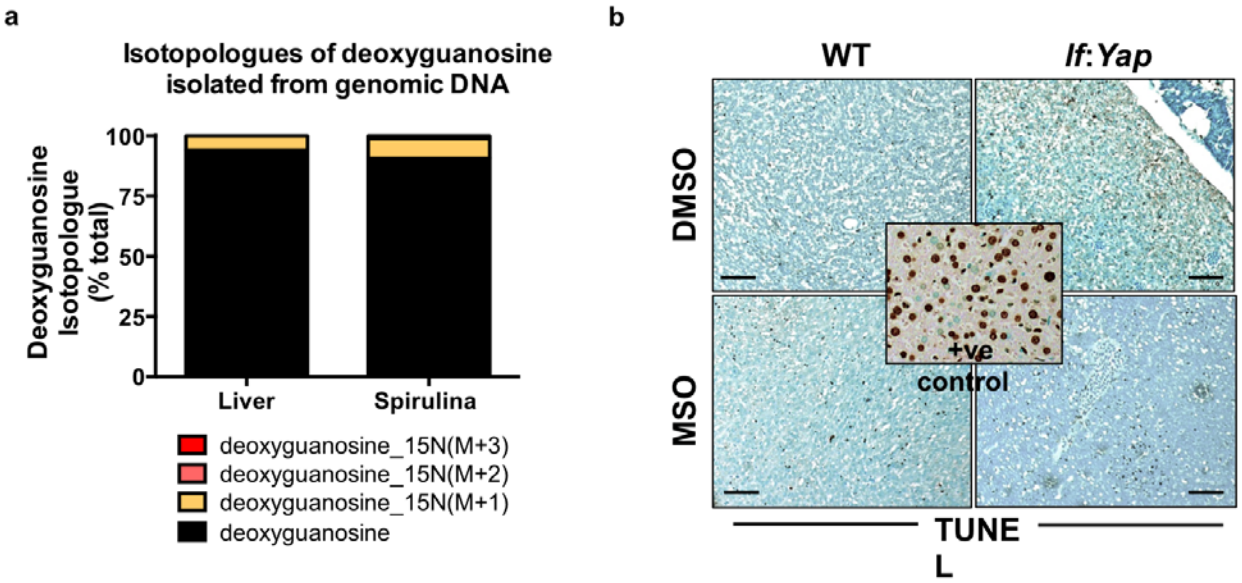
Supplementary Figure 4 Yap reprograms nitrogen metabolism by enhancing GLUL-dependent anabolic assimilation of ammonia for *de novo* nucleotide biosynthesis. (a) Immunohistochemical detection of GLUL in WT, *If:Yap* transgenic livers and DMBA-induced *If:Yap* liver tumors. Scale bar: 50μm. (b) Ammonia excretion rates in individual WT and *If:Yap* adult fish. n=16 and 12 WT and *If:Yap* adult zebrafish respectively; see Supplementary Table 4. Values represent the mean±SEM. (c) Steady-state abundance of urea in WT and *If:Yap* livers as determined by selected reaction monitoring (SRM) analysis. n=5 WT and *If:Yap* adult livers; see Supplementary Table 4. Values represent the mean±SEM. (d) Abundance of ^{15}N-labelled Guanosine (M+2 fraction) from methanol extracted WT and *If:Yap* liver lysates, as determined by LC-MS/MS via SRM. n=5 biologically independent WT and *If:Yap* adult liver lysates; see Supplementary Table 4. *p<0.05, two-sided Student's *t*-test, values represent the mean±SEM. (e) Abundance of ^{15}N-labelled Cytosine (M+2 fraction) from methanol extracted WT and *If:Yap* liver lysates, as determined by LC-MS/MS via SRM. n=5 biologically independent WT and *If:Yap* adult liver lysates; see Supplementary Table 4. Values represent the mean±SEM. (f) Percentage of ^{15}N-labelled Glutamine isotopologues in WT and *If:Yap* transgenic liver lysates following ammonia assimilation in the presence or absence or MSO. n=5 biologically independent WT and *If:Yap* adult liver lysates; see Supplementary Table 4. Values represent the mean±SEM. (g) Percentage of ^{15}N-labelled Histidine isotopologues in WT and *If:Yap* transgenic liver lysates following ammonia assimilation in the presence or absence or MSO. n=5 biologically independent WT and *If:Yap* adult liver lysates; see Supplementary Table 4. Values represent the mean±SEM.



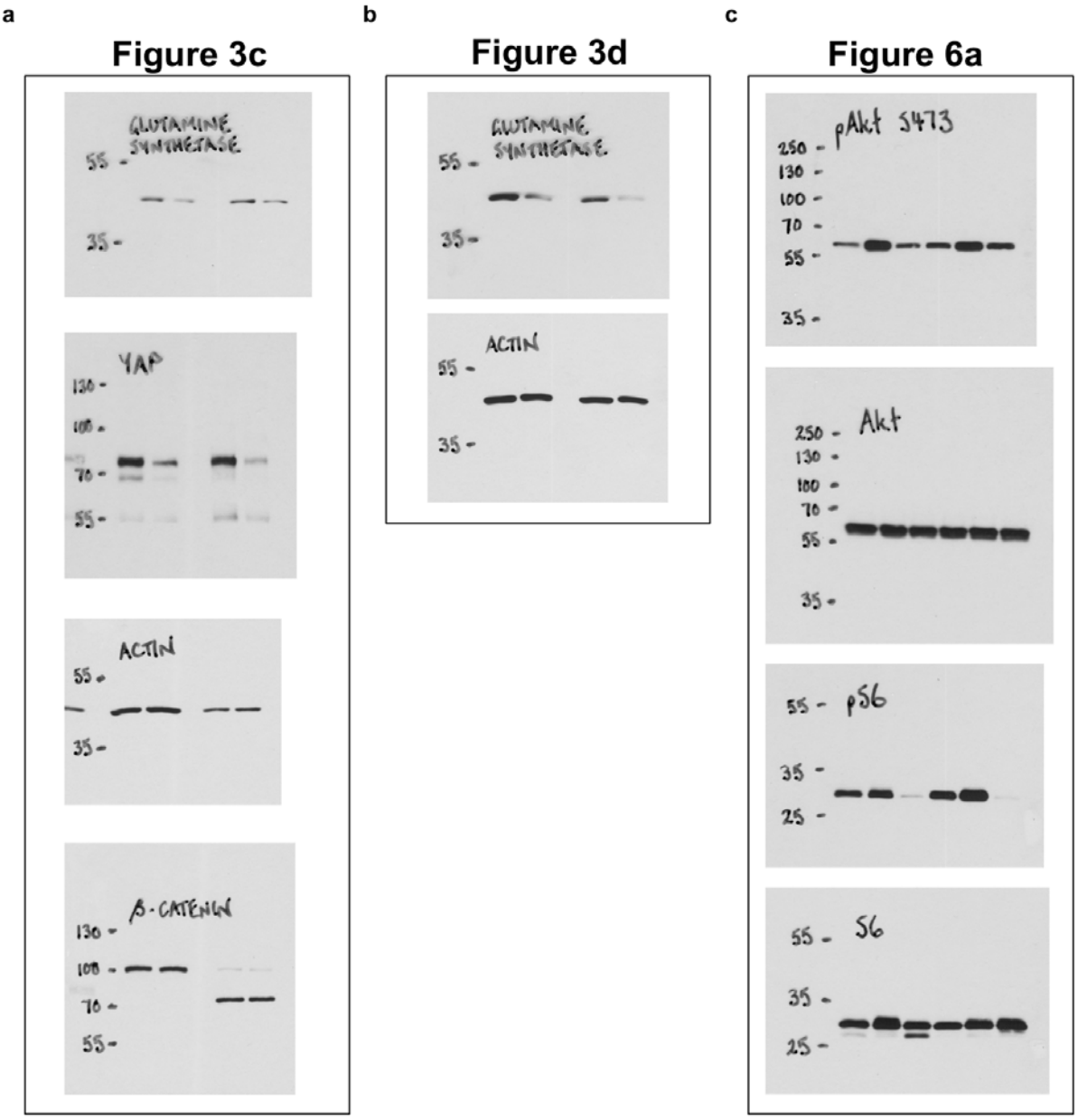
Supplementary Figure 5 GLUL activity and nucleotide biosynthesis contribute to Yap-induced hepatomegaly and the growth of liver cancer cells. (a) RT-PCR validation of morpholinos targeting splice sites in *glula* and *glulb,* resulting in alternative transcripts (indicated by arrow heads). (b) Glul activity in WT and *lf:Yap* larval extracts derived from larvae exposed to MSO from 3-5 dpf. n=8, 7, 9 and 9 biologically independent WT, WT+MSO, *lf:Yap* and *lf:Yap*+MSO larval lysates respectively, each of which was derived from a pool of 20 larvae; see Supplementary Table 4. **p<0.01, two-sided Student's *t*-test, values represent the mean±SEM. (c) Proliferation of HepG2 liver cancer cells over 4 days in the presence or absence of glutamine (Q), MSO or VP. n=3 biologically independent replicates; see Supplementary Table 4. Values represent the mean±SEM.



Supplementary Figure 6 The mTOR pathway is not deregulated by Yap expression or GLUL inhibition, but it is required for Yap-induced hepatomegaly. (a) Immunohistochemical analysis of phospho-S6 (pS6) levels in liver sections from WT and *lf:Yap* transgenic fish 24 hr after exposure to DMSO, MSO or Rapamycin (RAPA). Scale bars: 50μm. (b) Analysis of the number of liver cells in H+E stained transverse liver sections from WT and *lf:Yap* transgenic larvae at 5dpf. n=16 WT and *lf:Yap* larvae; see Supplementary Table 4. ***p<0.001, two-sided Student's *t*-test, values represent the mean±SEM. (c) Analysis of liver area in H+E stained transverse liver sections from WT and *lf:Yap* transgenic larvae at 5dpf. n=16 WT and *lf:Yap* larvae; see Supplementary Table 4. ***p<0.001, two-sided Student's *t*-test, values represent the mean±SEM. (d) Analysis of cellularity (cells/unit^2) in H+E stained transverse liver sections from WT and *lf:Yap* transgenic larvae at 5dpf. n=16 WT and *lf:Yap* larvae; see Supplementary Table 4. Values represent the mean±SEM.



Supplementary Figure 7 Yap reprograms the relative isotopic enrichment of nutritional nitrogen into nucleotide biosynthesis in a GLUL-dependent manner to support liver growth. (a) Relative isotopic enrichment of ^{15}N into deoxyguanosine isotopologues derived from hydrolyzed genomic DNA of liver and ^{15}N-spirulina as determined by LC-MS/MS. One experiment is shown. (b) Histological analysis of cell death (TUNEL) from WT and *If:Yap* adults derived from the long-term MSO intervention studies. The positive control showing DAB-stained nuclei is derived from a murine liver section. Scale bar: 50μm.



Supplementary Figure 8 Unprocessed scans of immunoblots accompanied by size markers. Images were obtained by enhanced chemiluminescence.

Supplementary Table Legends

Supplementary Table 1 List of primers, morpholinos and shRNA constructs used in the study.

Supplementary Table 2 List of antibodies used in the study.

Supplementary Table 3 List of LC-MS/MS SRM Q1 and Q3 masses used for ^{15}N isotopic enrichment.

Supplementary Table 4 Statistics Source Data.