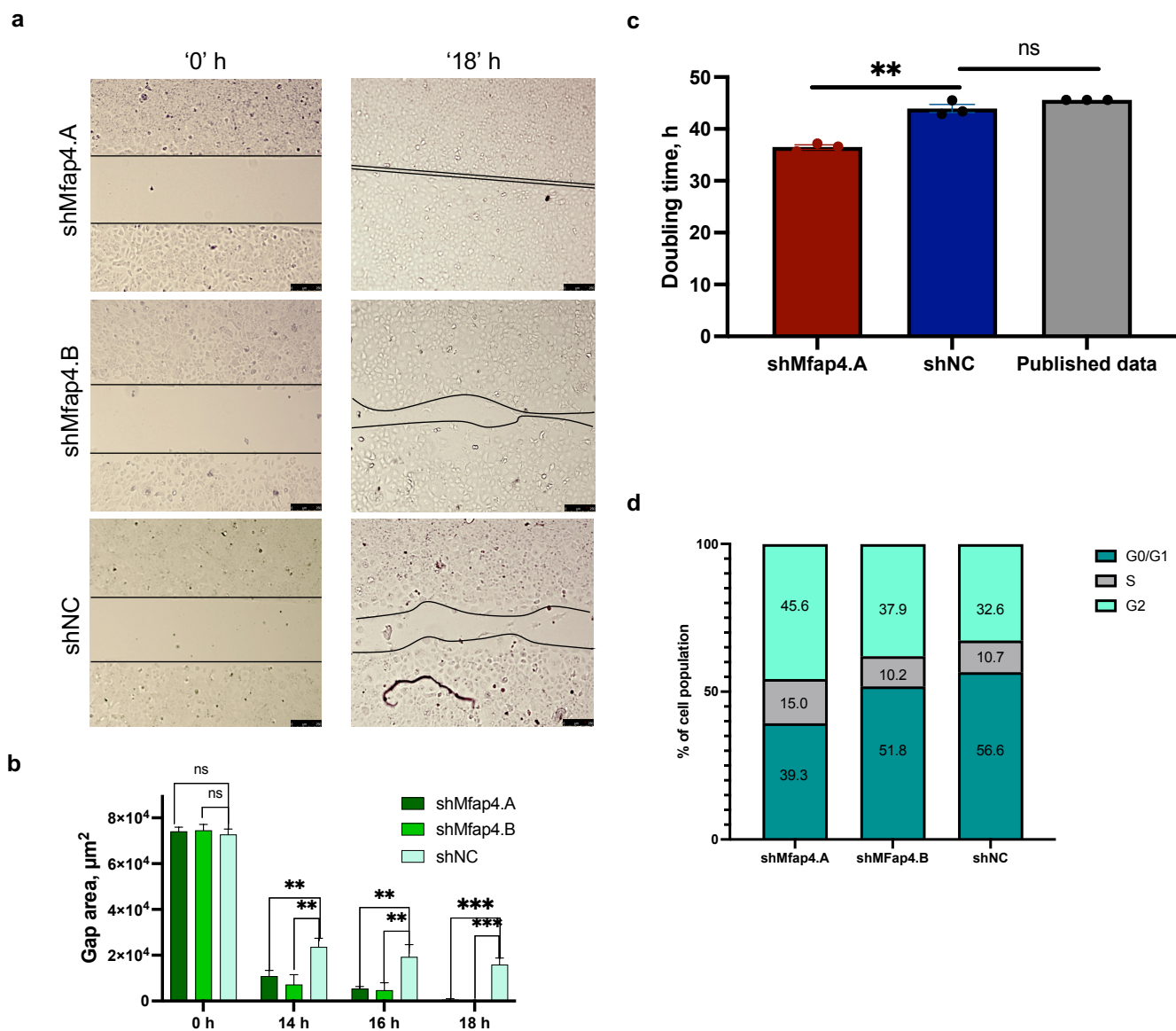
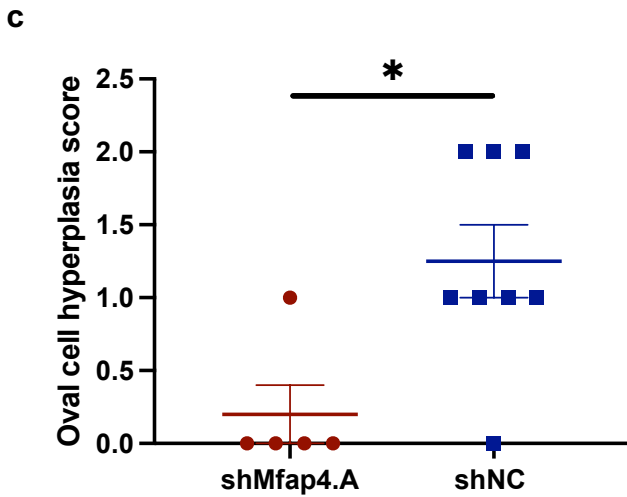
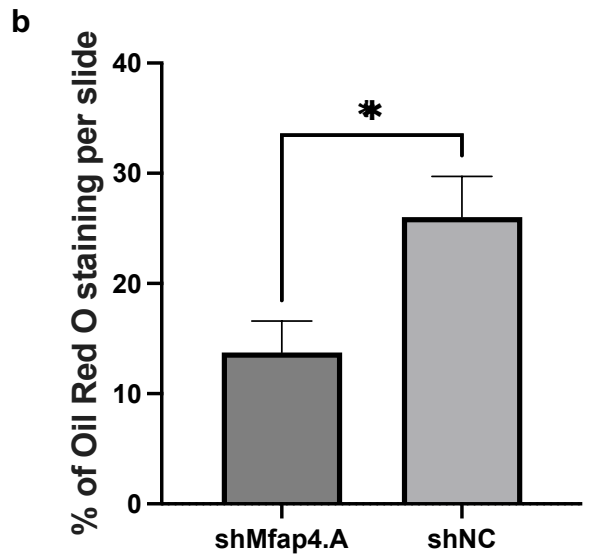
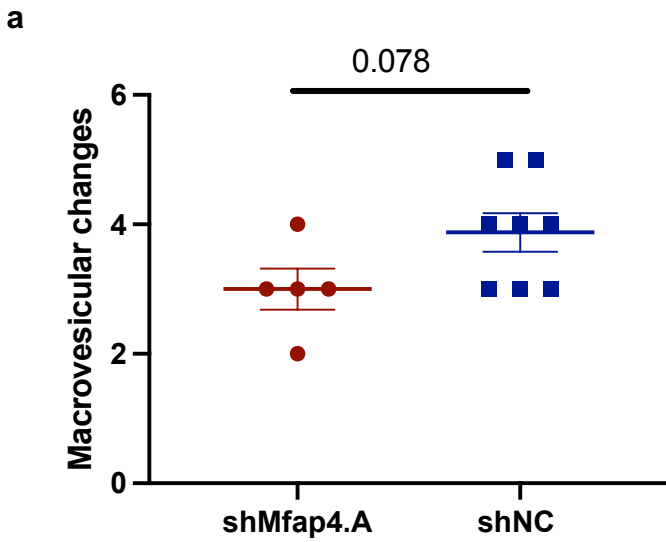


Supplementary Figure 1. Heat map of selected top-enriched and depleted shRNAs. Shown is the log2 fold change including non-targeting control shRNAs. Red are enriched candidates, and green are depleted. Each column represents one individual animal.

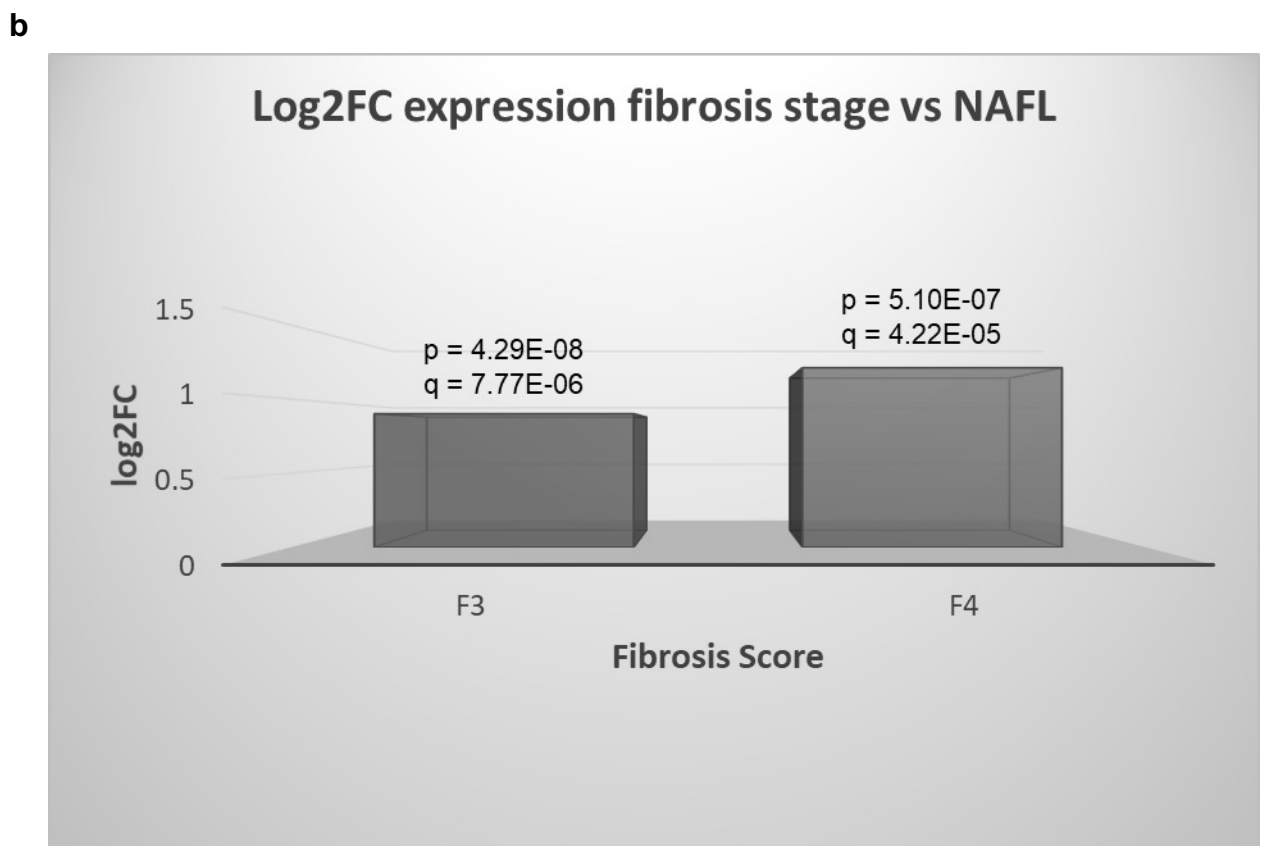
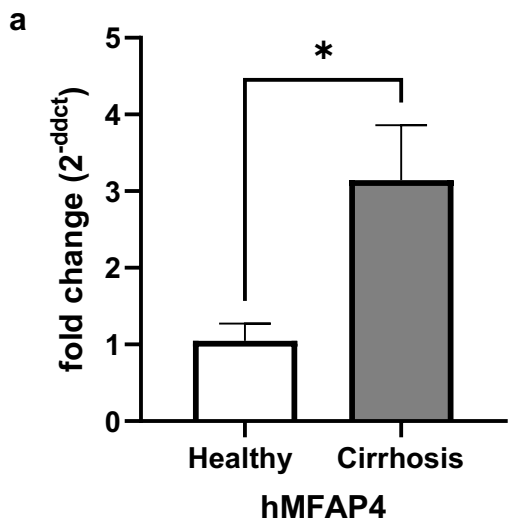


Supplementary Figure 2. shRNA mediated knockdown of *Mfap4* accelerates proliferation of liver cells. **a** Wound healing assay using AML 12 cell line with stable expression of shMfap4.A, shMfap4.B, and shNC. Cell lines were grown to full confluence, then the silicon gasket was removed leaving a defined cell free area (0h). Filling of this “wound” gap was monitored (18h) (3 technical replicates, bar = 250 µm). **b** Size of the wound area (in µm²) ± SEM (n = 3; *p<0.05, **p<0.01, ***p<0.005, ns=non - significant). **c** Shown is the doubling time for BNL.CL2 cells with stable expression of shMfap4.A, shNC, or without any shRNA. Cells were seeded at the same seeding densities. Doubling time was calculated based on the exponential phase of the growth curve. Three technical replicates were performed (published data refers to Ou, YH et al.). **d** Cell cycle analysis by flow cytometry using the Guava Muse Cell Analyzer of BNL.CL2 cells with stable expression of shMfap4.A, shMfap4.B, or shNC. Shown is the percentage of cells in the indicated cell cycle phase.

Ou, Y.H., et al. Butachlor, a suspected carcinogen, alters growth and transformation characteristics of mouse liver cells. *Chem Res Toxicol* **13**, 1321-1325 (2000).

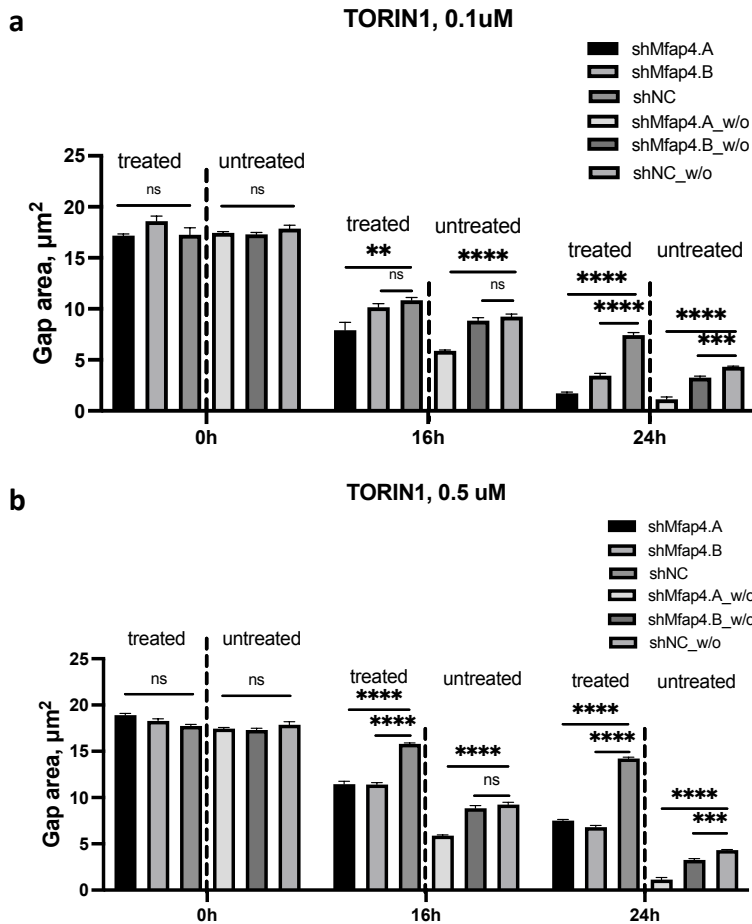


Supplementary Figure 3. shRNA mediated knockdown of *Mfap4* reduces steatosis and oval cell hyperplasia in mice exposed to the “Western” diet. **a** Reduction of macrovesicular changes upon *Mfap4* knockdown is detected by a certified pathologist compared to the control group (each dot represents one animal). **b** Quantification of Oil Red O stained samples. The percentage of red stained areas to the whole picture area was calculated per slide (5 areas per sample). A significant reduction of steatosis is observed (* $p < 0.05$). **c** Significant reduction of oval cell hyperplasia in the case of *Mfap4* knockdown compared to the non-targeting control. Oval cell hyperplasia score is determined by a certified pathologist, who was blinded regarding the experimental group (each point represents one animal; * $p < 0.05$).

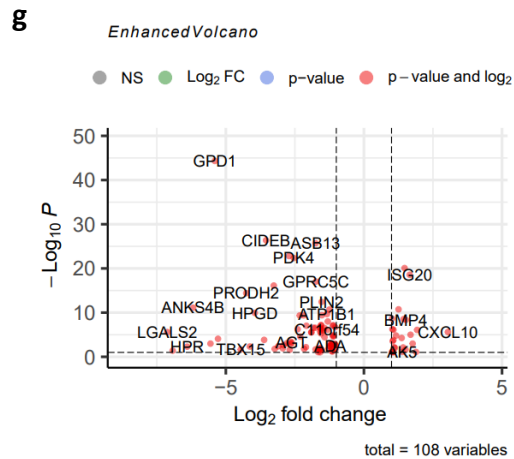
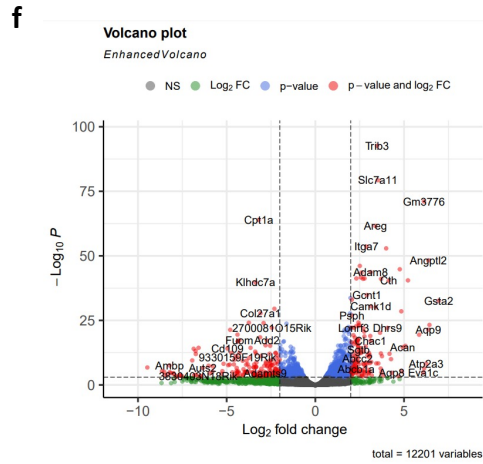
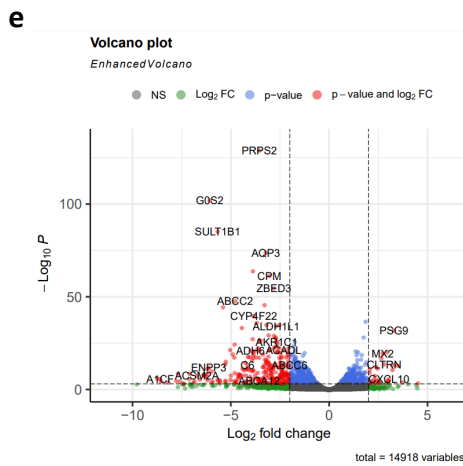
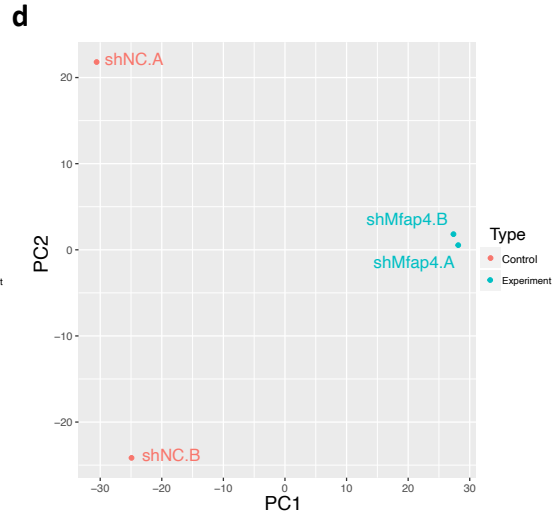
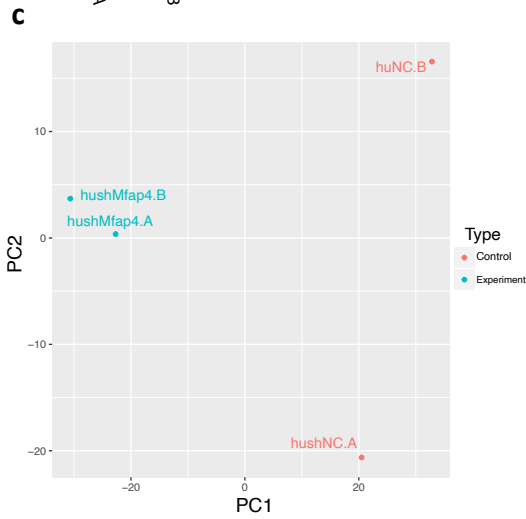
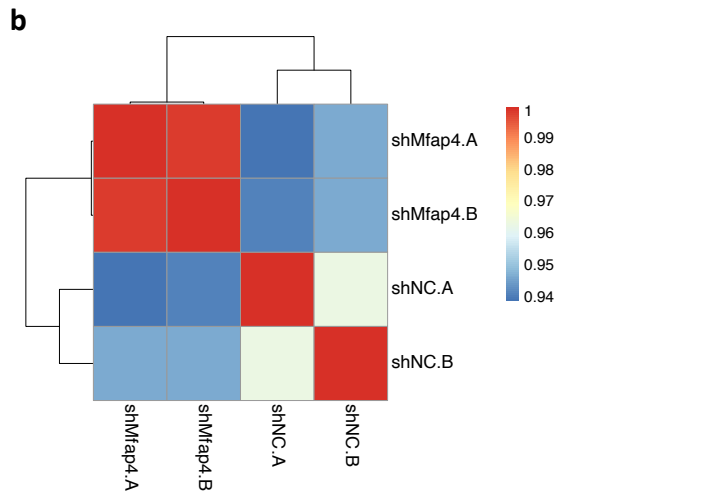
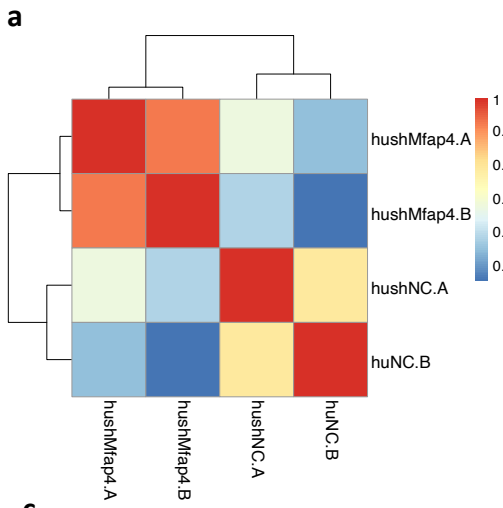


Supplementary Figure 4. *MFAP4* shows fibrosis stage dependent significant increased expression. **a** Higher expression of *MFAP4* is detected in cirrhotic livers compared to healthy donors ($p < 0.005$). **b** Based on the supplementary table provided in the Govaere et al. study of a nonalcoholic fatty liver disease patient cohort, *MFAP4* shows a highly significant upregulation at fibrosis score 3 and 4 vs simple steatosis (p-value of 4.29E-08 / q-value of 7.77E-06 and p-value of 5.10E-07 / q-value of 4.22E-05, respectively; based on the analysis of 206 patients).

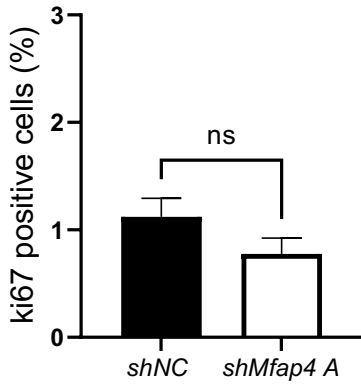
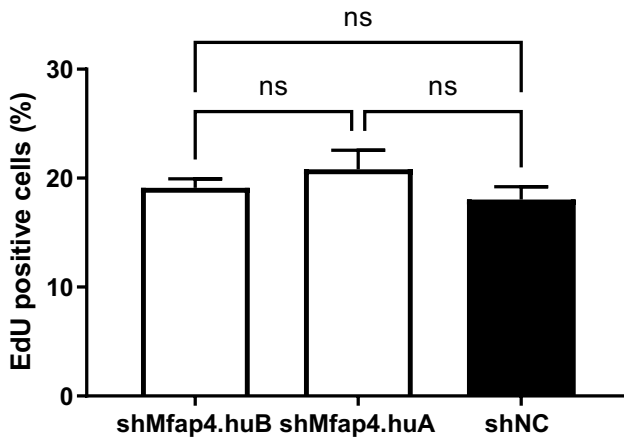
Govaere, O., et al. Transcriptomic profiling across the nonalcoholic fatty liver disease spectrum reveals gene signatures for steatohepatitis and fibrosis. *Sci Transl Med* 12(2020).



Supplementary Figure 5. Inhibition of mTOR strongly attenuates *Mfap4* knockdown driven faster wound healing. Quantification of Wound healing assay is shown. Cell lines with stable *Mfap4* knockdown were treated with the mTOR inhibitor Torin or carrier. Wound gap closure was monitored **a** 0.1 μ M Torin1 was administered **b** 0.5 μ M Torin1 was administered. Data were analyzed by ImageJ software and 2-way ANOVA statistical test was performed to determine significance.



Supplementary Figure 6. Interspecies transcriptomic profiling of immortalized human and mouse hepatocytes with *Mfap4* knockdown. **a** Correlation heat map for immortalized human hepatocytes with *MFAP4* knockdown and respective control. **b** Correlation map for immortalized mouse hepatocytes with *Mfap4* knockdown and respective control. **c** PCA plot for immortalized human hepatocytes with *MFAP4* knockdown and respective control. **d** PCA plot for immortalized mouse hepatocytes with *Mfap4* knockdown and respective control. **e** Volcano plot of expressed genes comparing immortalized human hepatocytes with *MFAP4* knockdown and respective control. **f** Volcano plot of expressed genes comparing immortalized mouse hepatocytes with *Mfap4* knockdown and respective control. **g** Integration of the interspecies transcriptome analysis. Volcano plot of significantly differentially expressed genes conserved between both species mouse and man.

a**b**

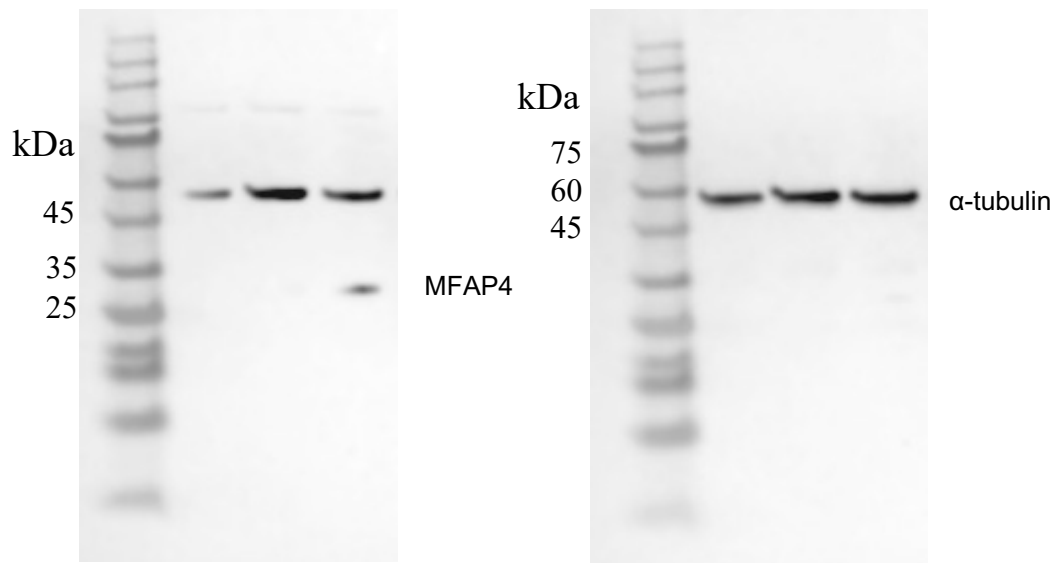
Supplementary Figure 7 Mfap4 knockdown does not drive liver cancer or unregulated proliferation. **a** Quantification of Ki67 positive hepatocytes. Shown are % Ki67 positive cells in mouse livers after 1 year repopulation. Data shows average \pm SEM (ns=non-significant). **b** DNA synthesis of HepG2 cells with stable expression of *shMfap4.A*, *shMfap4.B* or *shNC* was assessed by EdU incorporation. Shown is the value of % EdU positive cells \pm SEM (ns=non-significant, 3 independent replicates).

Gene name	Primer sequence Forward	Primer sequence Reverse
MFAP4	AGGAGATGCTCTGGAGAGG	AAAACCGTCCACTTCCCGCC
LRP8	GCCAAGGATTGCGAAAAGGAC	GTGGTCTAAGCAGTCATCGTC
DUSP4	AGTCTCAAGGCGGCTATGAG	AGGACCCCCCTGGTCGTG
DUSP5	TGTCGTCCTCACCTCGCTA	GGGCTCTCTCACTCTCAATCTTC
PLAT	AGCGAGCCAAGGTGTTTCAA	CTTCCCAGCAAATCCTTCGGG
SLC7A5	AGATCGGGAAGGGTGATGTG	AATTCAAGTAATTCCATCCTCC
GAPDH	TCAAGATCATCAGCAATGCC	ACTGTGGTCATGAGTCCTTC

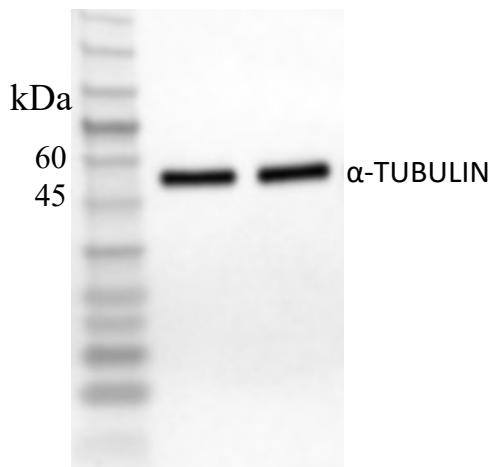
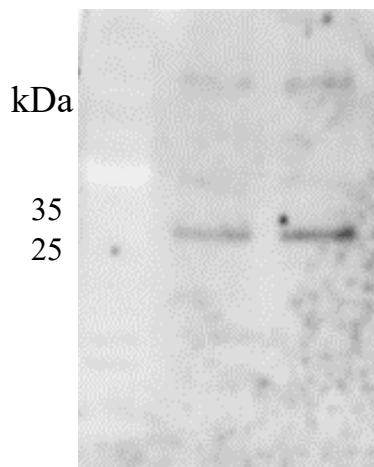
Supplementary table 1: Primers sequences are provided.

siRNA / shRNA	Sequence
shMfap4.1356	TGCTGTTGACAGTGAGCGCCGGCTACTGCTCAACTCTGAATAGTGAAGCCACA GATGTATTAGAGTTGAGCAGTAGCCGTTGCCTACTGCCTCGGA
shMfap4.760	TGCTGTTGACAGTGAGCGCAGGCTTCTATTACTCCCTCAATAGTGAAGCCACAG ATGTATTGAGGGAGTAATAGAAGCCTTTCCTACTGCCTCGGA
shNC	AAGGTATATTGCTGTTGACAGTGAGCGCGCAGTTCGATATCTACTGAAATAGT GAAGCCACAGATGTATTCAGTAGATATCGAACTGCTTGCCTACTGCCTCG
siP70S6K	Dharmacon (Horizon) SMARTpool; 72508, 5nmol
sihuMfap4	Dharmacon (Horizon) SMARTpool; 4239, 5nmol
siNC	Dharmacon (Horizon) Non-targeting SMARTpool, 5nmol
shhuMfap4.1602	TGCTGTTGACAGTGAGCGATAGGGACTGAAGGTCTCAATATAGTGAAGCCACA GATGTATATTGAGACCTTCAGTCCCTACTGCCTACTGCCTCGGA
shNC (shRenila)	TGCTGTTGACAGTGAGCGCAGGAATTATAATGCTTATCTATAGTGAAGCCACA GATGTATAGATAAGCATTATAATTCCTATGCCTACTGCCTCGGA

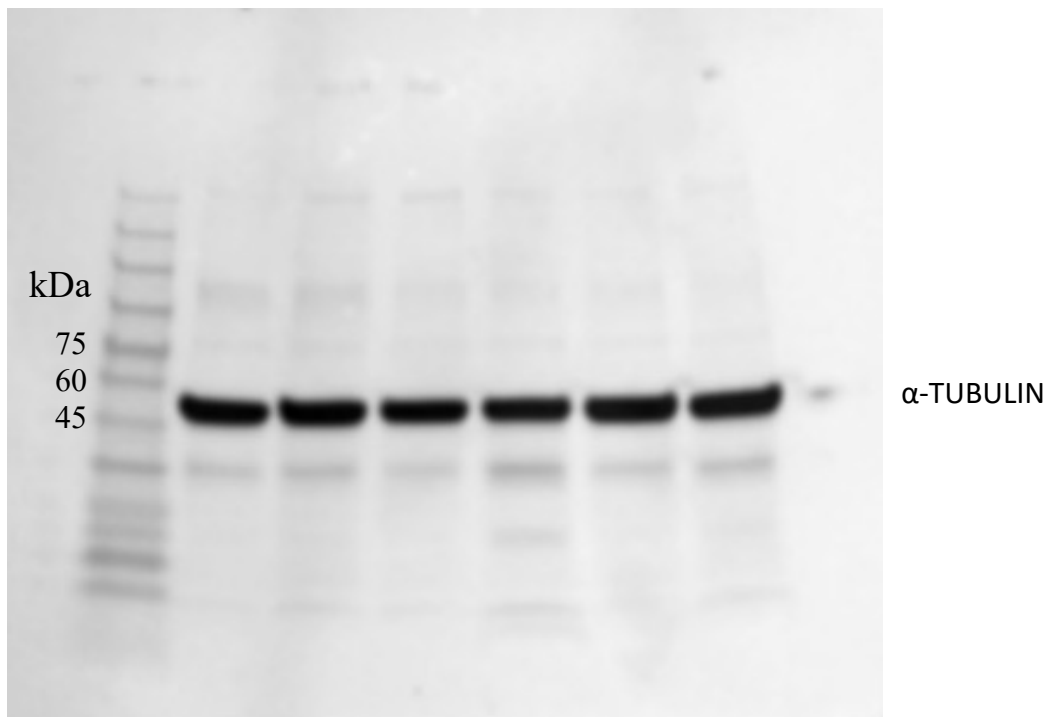
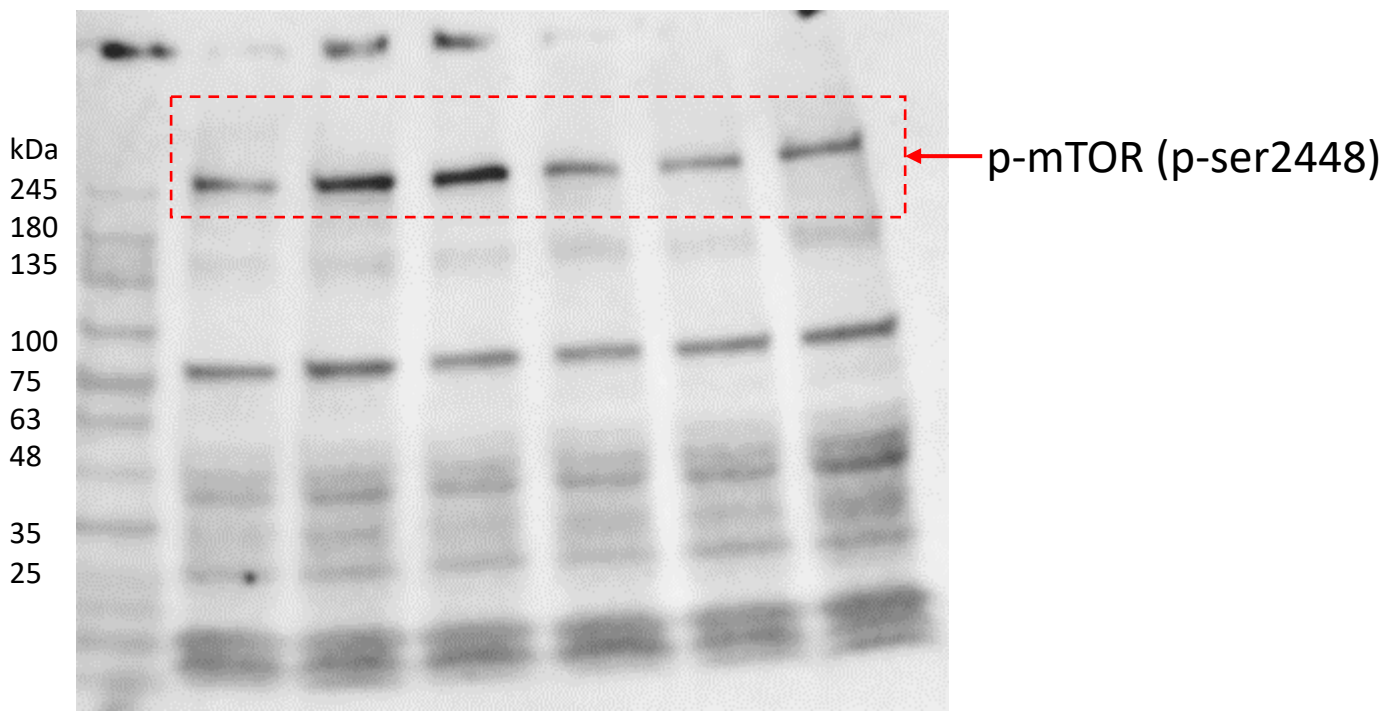
Supplementary table 2: siRNA/shRNA sequences are provided.



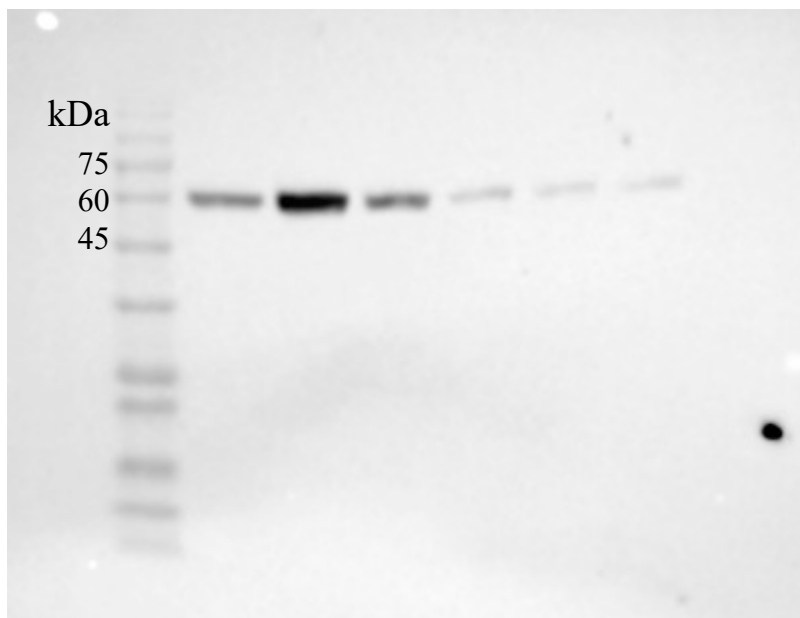
Uncropped Western Blot for Figure 2b for Mfap4.



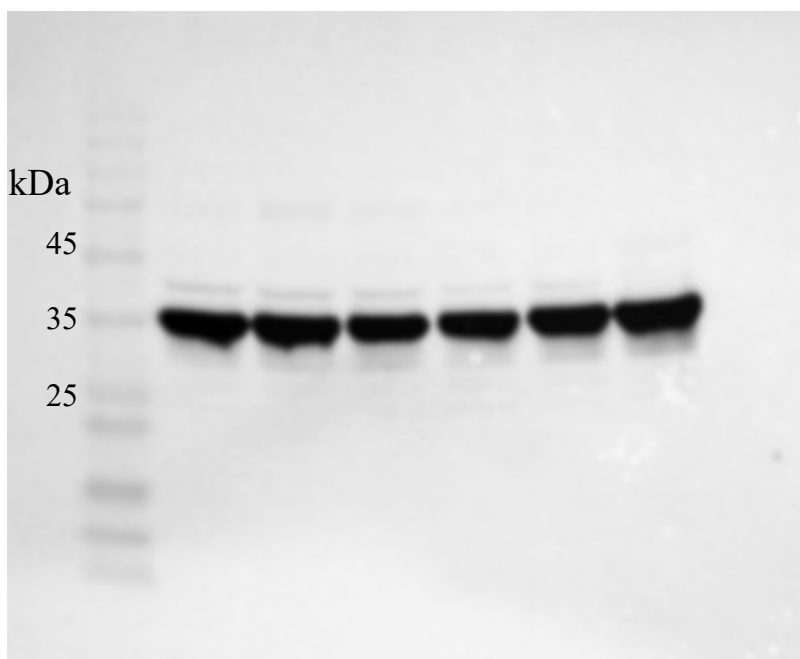
Uncropped Western Blot for Figure 5e for *si huMAFP4*.



Uncropped Western Blot for Figure 6c for p-mTOR.

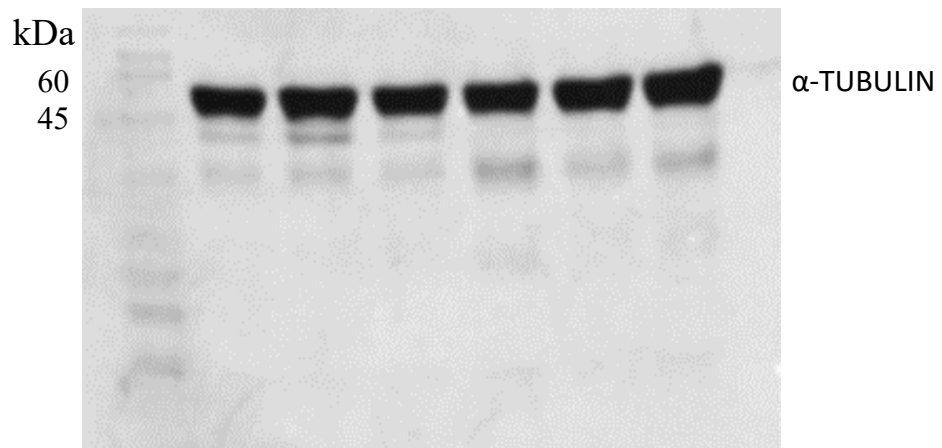
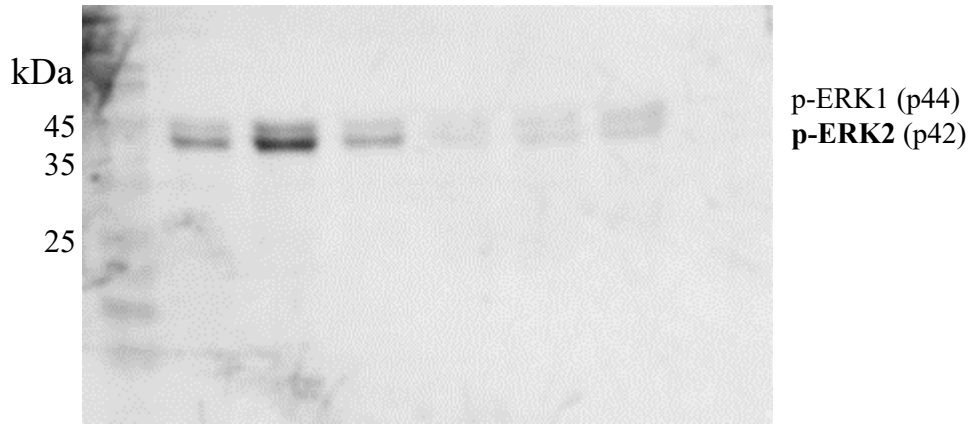


p-P70S6K
(p-T421/s424)



GAPDH

Uncropped Western Blot for Figure 6c for p-P70S6K.



Uncropped Western Blot for Figure 6c for p-ERK.