The presence of senescent, transformed or damaged cells can impair tissue function or lead to tumorigenesis; therefore, organisms have evolved quality control mechanisms to eliminate them. Here, we show that YAP activation induced by inactivation of the Hippo pathway specifically in damaged hepatocytes promotes their selective elimination by using in vivo mosaic analysis in mouse liver. These damaged hepatocytes migrate into the hepatic sinusoids, undergo apoptosis and are engulfed by Kupffer cells. In contrast, YAP activation in undamaged hepatocytes leads to proliferation. Cellular stresses such as ethanol that damage both liver sinusoidal endothelial cells and hepatocytes switch cell fate from proliferation to migration/apoptosis in the presence of activated YAP. This involves the activation of CDC42 and Rac that regulate cell migration. Thus, we suggest that YAP acts as a stress sensor that induces elimination of injured cells to maintain tissue and organ homeostasis.
Cellular stress in tissues and organs leads to senescent, transformed or damaged cells\(^1\text{-}^4\). These cells can impair tissue function or lead to tumorigenesis and therefore need to be eliminated and their loss compensated for through cell proliferation to maintain tissue and organ size\(^5\text{-}^9\). However, the molecular mechanisms that act to maintain tissue and organ homeostasis during cellular stress are largely unknown.

The liver plays a central role in metabolic homeostasis due to its role in metabolism, and the synthesis, storage and redistribution of nutrients\(^10\text{-}11\). The liver is also one of the main detoxifying organs, removing waste and xenobiotics through metabolic conversion and biliary excretion. The waste and xenobiotics come from the gastrointestinal tract via the portal vein, and diffuse into small blood vessels known as hepatic sinusoids. Thus, the liver is constantly exposed to various stresses. The liver consists of several different cell types including hepatocytes, which have metabolizing and detoxifying abilities, liver sinusoidal endothelial cells (LSECs), which form the sinusoidal wall and cover the hepatocytes, and Kupffer cells, which are sinusoid-resident macrophages.

The Hippo pathway regulates organ size and cancer formation by modulating cell proliferation and death via regulation of YAP activation\(^12\text{-}16\). Central to the Hippo pathway is a kinase cascade wherein Mst (the mammalian orthologue of the Drosophila Hippo) phosphorylates and activates the adaptor protein Mob and the protein kinase LATS. Activated LATS then phosphorylates the transcription coactivator YAP, and inhibits its activation by cytoplasmic retention. Unphosphorylated YAP translocates into the nucleus, interacts with the transcription factor TEAD and induces target gene expression. Gene knockout of Hippo pathway components induces hepatomegaly and liver cancer in mice. Recently, we reported that loss of Mob causes YAP activation and cancer formation in mouse liver\(^17,18\). Depletion of the YAP gene suppressed liver cancer formation in Mob-knockout mice. Thus, the liver phenotypes caused by an impaired Hippo pathway are strongly dependent on YAP.

In this study, we examine the dynamics of YAP-activating hepatocytes by in vivo mosaic analysis in mouse and discover that the fate of YAP-expressing hepatocytes changes from proliferation to migration/apoptosis depending on the status (healthy or damaged) of the LSECs.

**Results**

**YAP-activating hepatocytes are lost in mouse liver.** To examine how the Hippo pathway affects the fate of individual hepatocytes, we first established mosaic conditions by using hydrodynamic tail vein injection (HTVi) to introduce Myc-tagged YAP-wild type (WT), or one of three active YAP mutants (YAP (1SA), YAP (2SA) or YAP (SSA)), into mouse liver *in vivo*\(^19,20\). Under such conditions, HTVi achieves exogenous gene expression in ~30% of hepatocytes (Supplementary Fig. 1a).

Immunofluorescence analysis revealed that YAP (WT) localized in the cytoplasm and active YAP (1SA, 2SA and SSA mutants), as expected, localized in the nucleus (Fig. 1a, insets). Real-time PCR showed that expression of one of the YAP target genes, connective tissue growth factor (*ctgf*), was increased in YAP (1SA, 2SA and SSA) but not YAP (WT) during days 1 and 2 (Supplementary Fig. 1b), consistent with functional YAP activation. Interestingly, the number of hepatocytes expressing active YAP markedly decreased during days 3–7 post-HTVi, whereas no such reduction occurred in YAP (WT) livers (Fig. 1a, Supplementary Fig. 1c). To verify that loss of active YAP (SSA) hepatocytes caused this decrease, ROSA26-LacZ reporter mice were injected with Myc-YAP (SSA)-IRES-Cre plasmid. YAP- and Cre-expressing cells were detected by immunostaining with anti-Myc and anti-β-galactosidase (β-gal) or 5-bromo-4-chloro-3-indolyl-β-D-galactoside (X-gal) staining, respectively. Both cell populations decreased in parallel within 7 days of HTVi (Fig. 1d and Supplementary Fig. 2), confirming the loss of YAP (SSA)\(^\ast\) hepatocytes. Nevertheless, normal liver size was maintained in YAP (SSA) mice (Supplementary Fig. 3a), prompting us to investigate the effect on hepatocyte proliferation. In YAP (SSA), but not YAP (WT), liver at days 3–7 post-HTVi, the proliferation of mainly Myc-negative hepatocytes was increased (Supplementary Fig. 3b–d).

We next investigated whether the above effects could be replicated by activating endogenous YAP via inactivation of the Hippo pathway. We injected LacZ-IRES-Cre plasmid into *Mst1<sup>flox/flox</sup>*; *Mst2<sup>flox/flox</sup>* mice and *Mob1<sup>a<sub>flu</sub>/Mob1<sub>btr</sub></sup>* mice, both of which lack Hippo signalling\(^17,18,21\). Under such conditions, consistent with activated YAP, *ctgf* expression was upregulated in these mice (Supplementary Fig. 4). Immunofluorescence analysis demonstrated that LacZ-expressing hepatocytes were reduced in both mutant strains within 7 days post-HTVi (Fig. 1e), consistent with our results using exogenous active YAP mutants.

YAP-activating hepatocytes are engulfed by Kupffer cells. A previous study reported that hepatocytes expressing activated Ras undergo cellular senescence and are lost by elimination dependent on CD4<sup>+</sup> T cells (termed ‘senescence surveillance’\(^22\). To determine whether senescence surveillance also played a role in the loss of YAP (SSA) hepatocytes in our system, we first examined the mouse livers for senescence-associated (SA)-β-gal<sup>+</sup> hepatocytes. Forced expression of activated K-Ras (G12V) induced hepatocyte senescence as expected. In contrast, YAP (SSA) hepatocytes were SA-β-gal<sup>−</sup> and thus not senescent (Fig. 2a). We also explored more directly whether adaptive immunity was involved in the loss of YAP (SSA) hepatocytes by introducing Myc-tagged YAP (WT)- or YAP (SSA)-expressing plasmids into immunodeficient NOD/Shi-scid, IL-2Rγ-null (NOG) mice by HTVi\(^23\). Numbers of YAP (SSA) hepatocytes steadily decreased also in NOG livers over 7 days post-HTVi (Fig. 2b). Thus, the elimination of YAP-activated hepatocytes is regulated by a mechanism distinct from senescence surveillance.

To identify this mechanism, we stained mouse liver sections to detect markers of various cell populations. Unlike YAP (WT) hepatocytes, YAP (SSA) hepatocytes migrated to hepatic venules and were engulfed by LSECs and Kupffer cells (liver-resident macrophages) (Fig. 2c,d and Supplementary Movie 1). Of note, we found that Kupffer cells also exist in the immunodeficient NOG mice (Supplementary Fig. 5e). Thus, we depleted Kupffer cells from the YAP-expressing mice using clodronate liposomes\(^24\), and found that the loss of YAP (SSA) cells from the liver was suppressed, and instead TUNEL<sup>+</sup> apoptotic cells coexpressing Myc appeared (Fig. 2e,f and Supplementary Fig. 6). These data demonstrate that Kupffer cells play a direct role in the elimination of YAP-activated hepatocytes.

**LSEC and hepatocyte injury switch YAP-activated cell fate.** To determine whether the mosaic ratio affected YAP (SSA) hepatocyte elimination, we introduced a YAP (SSA)-expressing adenovirus vector into mouse liver. This vector induces exogenous gene expression with up to 80% efficiency. We infected mouse livers with sufficient PFU of this vector to induce YAP...
(5SA) expression in 10, 25, 60 or 80% of hepatocytes (Supplementary Fig. 7a,b). No matter the degree of mosaicism, and in direct contrast to the previous results using HTVi, all YAP (5SA)-expressing adenovirus-infected livers were enlarged (Fig. 3a). Staining with the proliferation marker Ki-67 revealed that this enlargement was due to increased hepatocyte proliferation on expression of activated YAP using adenoviral infection (Fig. 3b and Supplementary Fig. 7c).

Compared to adenovirus infection, the HTVi procedure causes physical injury to liver cells. We hypothesized that this may promote the elimination of YAP-expressing cells. To test this, we injected ROSA26-LacZ mice with both Cre-1 day and 7 days. Injections of Cre and LacZ-IRES-Cre caused an increase in the percentage of β-gal-positive cells in the liver sections on the indicated days post-HTVi (Fig. 1c). Immunoblot labelled with anti-YAP, anti-Myc, anti-P-YAP or anti-GAPDH to detect Myc-tagged YAP in livers of control mice (C) or mice expressing YAP (WT) or YAP (5SA) assayed on the indicated days post-HTVi. Exo, exogenous; Endo, endogenous. GAPDH, loading control (n = 3). Uncropped images are shown in Supplementary Fig. 12. (d) Quantification of percentages of β-gal + cells in confocal immunofluorescence images of liver sections from ROSA26-LacZ mice expressing YAP (5SA)-IRES-Cre assayed on the indicated days post-HTVi. Data are the mean ± s.d. (n = 3) of three independent experiments. (e) Quantification of percentages of β-gal + cells in confocal immunofluorescence images of liver sections from Mst1f/f;Mst2f/f or Mob1af/f;Mob1btr/tr mice expressing LacZ-IRES-Cre assayed on the indicated days post-HTVi. Data are the mean ± s.d. (n = 3).

Figure 1 | The effect of YAP activation on hepatocyte fate in mouse liver. (a) Representative confocal immunofluorescence images of liver sections isolated from mice expressing Myc-tagged YAP (WT), YAP (1SA), YAP (2SA) or YAP (5SA) and stained with anti-Myc at 1 or 7 days post-HTVi (× 20 objective lens). Scale bar, 100 μm (n = 4). Insets: High magnification images of the liver sections stained with anti-Myc (× 40 objective lens). (b) Quantification of percentages of Myc + cells in the liver sections in (a) on the indicated days post-HTVi. Data are the mean ± s.d. (n = 4). (c) Immunoblot labelled with anti-YAP, anti-Myc, anti-P-YAP or anti-GAPDH to detect Myc-tagged YAP in livers of control mice (C) or mice expressing YAP (WT) or YAP (5SA) assayed on the indicated days post-HTVi. Exo, exogenous; Endo, endogenous. GAPDH, loading control (n = 3). Uncropped images are shown in Supplementary Fig. 12. (d) Quantification of Myc + and β-gal + cells in confocal immunofluorescence images of liver sections from ROSA26-LacZ mice expressing YAP (5SA)-IRES-Cre assayed on the indicated days post-HTVi. Data are the mean ± s.d. (n = 3) of three independent experiments. (e) Quantification of percentages of β-gal + cells in confocal immunofluorescence images of liver sections from Mst1f/f;Mst2f/f or Mob1af/f;Mob1btr/tr mice expressing LacZ-IRES-Cre assayed on the indicated days post-HTVi. Data are the mean ± s.d. (n = 3).
and YAP (5SA)-expressing adenovirus and subjected the animals to HTVi (Fig. 3c). HTVi indeed decreased β-gal<sup>+</sup>-infected hepatocyte numbers from 50% of all cells present to 20% within 7 days. This is in contrast to YAP (5SA)-infected hepatocytes without HTVi, which increased to 70% of all cells (Fig. 3d,e and Supplementary Fig. 8). Thus, specifically in the presence of both activated YAP and cellular damage induced by HTVi, hepatocyte fate changes from proliferation to migration/apoptosis.

To investigate in more detail the type of liver injury that can change hepatocyte fate in the presence of activated YAP, we treated Ad-Cre/Ad-YAP (5SA)-infected ROSA26-LacZ mice with either carbon tetrachloride (CCL<sub>4</sub>), which causes specific injury to hepatocytes<sup>25,26</sup>; monocrotaline, which mainly causes LSEC injury<sup>27</sup>; or ethanol, which injures both LSECs and hepatocytes<sup>28,29</sup>. No loss of β-gal<sup>+</sup> hepatocytes was detected in livers of mice treated with CCL<sub>4</sub> or monocrotaline, but a decrease in β-gal<sup>+</sup> hepatocytes from 30% to 5% occurred in ethanol-treated livers by 7 days postinfection (Fig. 3f). Next, we administered ethanol to control mice and mice expressing YAP (WT) or YAP (5SA) via adenovirus infection. Overexpression specifically of YAP (5SA) induced substantially more hepatocyte death in the

**Figure 2 | YAP activation leads to hepatocyte apoptosis and engulfment by Kupffer cells in mouse liver.** (a) Light microscopy to detect SA-β-gal-stained cells in liver sections of mice expressing the indicated molecules on day 2 post-HTVi. Scale bar, 10 μm (n = 3). (b) Quantification of percentages of Myc<sup>+</sup> cells in confocal immunofluorescence images of NOG mice expressing Myc-tagged YAP (WT) or YAP (5SA) assayed on the indicated days post-HTVi. Data are the mean ± s.d. (n = 3). (c) Representative confocal immunofluorescence images of the liver sections stained with anti-Myc, anti-F4/80 or 4',6-diamidino-2-phenylindole (DAPI) on day 3 post-HTVi. Control, without HTVi. Scale bar, 20 μm (n = 3). (d) High magnification image of the liver sections stained with anti-Myc or anti-F4/80 on day 3 post-HTVi (× 60 objective lens). Scale bar, 10 μm (n = 3). (e) Representative confocal immunofluorescence images of livers expressing Myc-tagged YAP (5SA) and treated with clodronate liposomes for the indicated days post-HTVi to deplete Kupffer cells. Sections were stained with anti-Myc, anti-F4/80 or TUNEL as indicated. Scale bar, 10 μm (n = 4). (f) Quantification of percentages of cells positive for F4/80, Myc or TUNEL in the liver sections in (e). Data are the mean ± s.d. (n = 4).
presence of ethanol compared to YAP (WT) or control infected cells (Fig. 3g). Thus, injury to both LSECs and hepatocytes is required for significant hepatocyte elimination.

**CDC42 and Rac are required for the hepatocyte elimination.**

To determine the functional domain of YAP that mediates cell elimination in injured hepatocytes, we performed a domain analysis
using the following mutants: YAP (5SA/WW1,2*), defective in binding to transcription factors such as p73; YAP (1SA/ΔC) and YAP (5SA/ΔC), deletion mutants lacking the YAP activation domain; YAP (5SA/APDZb), a deletion mutant lacking the PDZ-binding motif and defective in YAP nuclear localization; and YAP (5SA/TEAD*), defective in binding to TEAD[3,30,31]. YAP (5SA/WW1,2*)-expressing hepatocytes underwent the same elimination process as YAP (1SA), YAP (2SA) and YAP (5SA) hepatocytes (Fig. 4a). In contrast, hepatocytes expressing YAP (1SA/ΔC), YAP (5SA/ΔC), YAP (5SA/APDZb) or YAP (5SA/TEAD*) were not lost. Thus, YAP nuclear localization, as well as TEAD binding and transcriptional activation, are indispensable for hepatic cell elimination.

To identify the molecular mechanisms involved in YAP-mediated elimination of injured hepatocytes, we analysed gene expression profiles in livers of mice expressing YAP (WT), YAP (1SA), YAP (2SA), YAP (5SA) or YAP (5SA/TEAD*) via HTVi. Using hierarchical cluster analysis, we identified gene clusters that were upregulated only in YAP (5SA) livers and not in YAP (WT) or YAP (5SA/TEAD*) livers (Supplementary Fig. 9). Examination of the gene ontology annotations of genes upregulated in YAP (1SA), YAP (2SA) and YAP (5SA) livers revealed the involvement of CDC42, which are small Rho family GTP proteins that regulate cytoskeleton dynamics. The expression of activated YAP matches the phenotype observed in normal liver. The Hippo pathway is constitutively activated and specifically in damaged hepatocytes triggers their elimination in normal liver. The Hippo pathway is constitutively activated and rapidly inactivates YAP by phosphorylation. Conversely, when the Hippo pathway is inactivated by stress, YAP immediately becomes unphosphorylated, translocates into the nucleus and induces target gene expression. Based on this, it is considered that YAP plays a role in an emergency stress response to maintain tissue homeostasis due to the elimination of injured cells. These findings demonstrate the complexity of cell fate determination mechanisms in vivo, and highlight a new role for YAP in tissue dynamics.

Methods

Mice. C57BL/6J mice were purchased from CREA Japan and Sankyo Labo Service Corporation (Japan). ROSA26-LacZ reporter, Mob1a[1] and Mob1b[1] mice were purchased from the Jackson Laboratory. To generate Mob1a[1] and Mob1b[1] mice, Mob1a[1] mice were mated to Mob1b[1] mice[17]. NOG mice were purchased from the Central Institute for Experimental Animals (Kanagawa, Japan). Male mice aged 8 weeks were mainly used for the experiments. All experiments were reviewed and approved by the Institutional Animal Care and Use Committee in Tokyo Medical and Dental University, and were conducted according to the committees’ guidelines.

Hydrodynamic tail vein injection. Vectors expressing cDNAs (20 μg) were diluted in TransIT-EE Hydrodynamic Delivery Solution (Mirus Bio) to a volume equivalent to ~10% of a mouse’s body weight (that is, 2 ml for a 20 g mouse). Eight-week-old male mice were placed in a restrainer, and the tail of the mice were placed in a 45–50°C water bath for 10–20 s. Using a 2.5 ml syringe with a 27-gauge needle, the diluted vectors were injected within 7–8 s into the mouse tail veins[41–43].

Plasmids. The full-length human YAP cDNA was PCR-amplified and ligated to Xbal restriction sites of the expression vectors used. The YAP (1SA), YAP (2SA), YAP (5SA), YAP (5SA/ΔC), YAP (5SA/ΔC), YAP (5SA/APDZb), YAP (5SA/TEAD*) and YAP (5SA/WW1,2*) mutants were constructed by site-directed mutagenesis or deletion by PCR. The Xbal fragments of each YAP’s cDNA were cloned into the pLIVE vector (Mirus Bio), in which expression of the cloned gene is driven by the hepatocyte-specific mouse AFP enhancer II and mouse minimal albumin promoter. K-Ras-active form (G12V) cDNA was cloned into pLIVE vector.

Discussion

The hepatocyte proliferation induced by adenovirus-mediated expression of activated YAP matches the phenotype observed in mice deficient for a Hippo pathway component and in mice transgenically expressing YAP[12–16]. In contrast, hepatocyte migration/apoptosis induced by HTVi/ethanol shown in this study is a novel cellular response. The observed apoptosis may occur through anoikis in a p73-independent manner because YAP (5SA/WW1,2*) induced hepatocyte loss. We therefore consider this hepatocyte elimination mechanism to be a primitive cellular response that is independent of adaptive immunity.

F-actin formation promotes YAP activation[37], and YAP regulates actin remodelling through the Rho GTPase-activating protein[38]. Here we found that YAP induces the Rho guanine nucleotide exchange factors Ect2 and Fgd3 in a manner dependent on an additional signal from LSECs. Thus, F-actin formation and YAP activation regulate each other through a feedback mechanism.

Cultured Madin–Darby canine kidney epithelial cells overexpressing YAP (5SA) are removed by apical extrusion when surrounded by normal Madin–Darby canine kidney cells[39]. This apical extrusion in vitro corresponds to the elimination of YAP (5SA) hepatocytes in vivo observed here. However, in vivo, hepatocyte elimination requires not only YAP activation but also liver injury.

Recently, Su et al[40] reported interesting findings that YAP activation is insufficient to promote cellular proliferation in normal livers. They found YAP-expressing hepatocytes proliferate specifically in the presence of hepatocyte damage or inflammation. In contrast, we found a novel cell response whereby YAP-expressing hepatocytes migrate into sinusoids on both hepatocyte and LSEC injury. These data indicated that YAP-expressing hepatocytes have altered cellular dynamics depending on the status of LSECs.

In mice, impairment of the Hippo pathway or activation of YAP in intact hepatocytes leads to their proliferation and eventually hepatocellular carcinoma (Supplementary Fig. 11)[12–16]. In humans, YAP activation is observed in cases of liver fibrosis and/or liver cancer. However, our study has shown that YAP activation specifically in damaged hepatocytes triggers their elimination in normal liver. The Hippo pathway is constitutively activated and rapidly inactivates YAP by phosphorylation. Conversely, when the Hippo pathway is inactivated by stress, YAP immediately becomes unphosphorylated, translocates into the nucleus and induces target gene expression. Based on this, it is considered that YAP plays a role in an emergency stress response to maintain tissue homeostasis due to the elimination of injured cells. These findings demonstrate the complexity of cell fate determination mechanisms in vivo, and highlight a new role for YAP in tissue dynamics.
Figure 4 | Molecular mechanism by which activated YAP induces hepatocyte migration. (a) Structure and domains of human YAP WT and YAP mutants (please see main text) in plasmids that were introduced into mice by HTVi. Elimination of hepatocytes in livers was monitored as indicated (n=3). (b) Immunoblot to detect Flag-tagged WT and DN forms of CDC42 and Rac1 in livers of mice expressing these proteins via HTVi (n=3). Uncropped images are shown in Supplementary Fig. 14. (c) Quantification of percentages of Myc⁺ cells in liver sections from mice expressing YAP (1SA) plus WT or DN CDC42 or Rac1. Livers were assayed on the indicated days post-HTVi. Data are the mean ± s.d. (n=3). (d) RT–PCR analysis of Ect2 and Fgd3 mRNA levels in livers of mice expressing YAP (WT), YAP (5SA) or YAP (5SA/TEAD*) at 2 days post-HTVi. Control, no HTVi (n=3). (e) Quantitation of mRNA levels of Ect2 and Fgd3 in livers of mice that were infected with YAP (5SA)-expressing adenovirus vector and left untreated (–) or treated with CCl₄, ethanol (EtOH) or HTVi. Real-time RT–PCR was performed on day 2 postinfection. Data are the mean ± s.d. (n=3). (f) Schematic model of the change in mouse hepatocyte fate from proliferation to migration/apoptosis when YAP is activated in these cells by adenoviral vector infection vs inactivation of Hippo pathway by a stress such as EtOH. Statistical analyses were carried out using a paired two-tailed t-test and *P<0.05 was considered significant.
Clodronate liposome treatment according to the manufacturer’s instructions. Antibodies certified by sequencing. Plasmid DNAs were purified using an EndoFree Plasmid Maxi Kit (Qiagen). The purity and quantity of plasmid DNAs were analysed by electrophoresis and absorption spectrophotometry.

**Antibodies** The antibodies used in this study were as follows: anti-Myc (c956; western blotting (WB):1/1,000, immunofluorescent (IF) staining:1/100) and anti-Flag (M2, F1804; WB:1/1,000) were purchased from Sigma; anti-β-actin (55976; IF:1/100) was from MP Cappi; anti-Ki-67 (Sp6: IF:1/150) was from Thermo Fisher Scientific Inc.; anti-My (9E10; IF:1/100) and control mouse IgG (sc-2025; IF:1/100) were from Santa Cruz; anti-LYVE1 (103-P5A9; IF:1/100) was from Relatehunt; anti-GAPDH (MAB374; WB:1/5,000) was from Millipore Bioscience Research Reagents; and anti-F4/80 (MCA497GA; IF:1/100) was from Serotec. Anti-P-YAP (no. 4911; WB:1/1,000) was purchased from Cell Signaling Technology. Anti-YAP (8G5; WB: 1:500; IF:1/50) and anti-Stab1 (IF:1/100) antibodies were established in our laboratory48. Actin in liver sections was stained using Alexa Fluor 488 phalloidin (A12379; IF:1/3000, Thermo Fisher Scientific).

**IF staining.** Mouse livers were dissected, embedded in O.C.T. compound and sectioned at 10 μm thickness. For Ki-67 and Myc or β-gal and Myc double staining, sections were heated in 10 mmol l⁻¹ sodium citrate buffer (pH 6.0) at 95°C for 10 min to facilitate antigen retrieval. Sections were precipitated with 5% bovine serum albumin and 0.1% Triton-X in PBS, followed by incubation with primary antibodies at 4°C overnight. Sections were washed three times in PBS. Primary antibodies were visualized by incubation with secondary antibodies conjugated to Alexa Fluor 546 (mouse: A11030; rabbit: A11035; rat: A11081; 1:1,000; Thermo Fisher Scientific) or Alexa Fluor 488 (mouse: A11029; rabbit: A11033; rat: A11006; 1:1,000; Thermo Fisher Scientific) and Hoechst 33342 (H3750; 1:5,000; Thermo Fisher Scientific) for 2 h at room temperature (RT). Sections were washed three times in PBS. Sections were mounted with Mowiol 4-48 solution and images were acquired by LSM 510 Meta or LSM 710 confocal microscopy (Carl Zeiss).

**Immunoblotting.** Liver extracts were fractionated by SDS–PAGE and transferred electrophoretically onto a polyvinylidene difluoride membrane. The membrane was blocked with 2 or 5% non-fat milk and incubated with each of the antibodies described in the figures for 10 h at 4°C. Blots were incubated with the appropriate secondary antibody, peroxidase-conjugated anti-mouse, anti-rabbit or anti-rat IgG antibody (Santa Cruz), and developed with the ECL Western Blotting Detection Secondary Antibody, peroxidase-conjugated anti-mouse, anti-rabbit or anti-rat IgG antibody (Santa Cruz), and developed with the ECL Western Blotting Detection System (Amersham Biosciences).

**Microarray analysis.** Total RNA was extracted from mouse livers using Trizol Reagent (Invitrogen) and further purified using RNaseasy Mini Kits (Qiagen). The quality of RNA was initially assessed by electrophoresis on a 1.5% agarose gel and further by absorption spectrophotometry (Agilent Bioanalyzer 2100; Agilent, Palo Alto, CA, USA). cDNAs were synthesized by the Low Input Quick Amp Labelling Kit. Cy3-labelled cRNA was synthesized by in vitro transcription with T7 RNA polymerase. Following fragmentation, 0.6 μg cRNA was hybridized for 17 h at 65°C on the SurePrint G2 Mouse GE 8 × 60K Microarray using the Gene Expression Hybridization Kit4. GeneChips were washed using the Gene Expression Wash Buffers Pack and scanned using an Agilent DNA Microarray Scanner (G2565CA). Microarray data were processed using GeneChip Operating Software (Feature Extraction) and GeneSpring GX (Institute of Medicinal Model Design Inc.). Gene expression microarray data have been deposited in the GEO database, with accession number GSE98231.

**Quantitative RT-PCR.** Quantitative real-time PCR with reverse transcription (RT–PCR) was performed as below87. For a 20 μl PCR reaction, cDNA template was mixed with TaKaRa Ex Taq (TaKaRa) plus the appropriate primers to a final concentration of 200 nM each. The reaction was first incubated at 95°C for 3.5 min, followed by 15–23 cycles of 95°C for 12 s, 60°C for 13 s and 72°C for 30 s. PCR primers for the Ext2, Fgd3 and GAPDH genes were as follows: Ext2 FW, 5′-GATTAAAGGAGGTCTGCAGC-3′ and Ext2 RW, 5′-AGAAAAAGAGGGGCTGCAAAGGG-3′; Fgd3 FW, 5′-CAGCTCGTCCTATCTTGACAGG-3′ and Fgd3 RW, 5′-AGTCGTTAGAGCGACGTCTTACAG-3′; GAPDH FW, 5′-GCTACCACTGTTGCTGCA-AAGGC-3′ and GAPDH RW, 5′-TAGGCCCTCTGTGATTATAGG-3′.

**Statistical analyses.** Sample sizes were determined on the basis of pilot experiments and previous experience from similar experiments. To examine whether the data had the same variances, we analysed them by F-test. As all the data were determined to be normally distributed, parametric statistics were used throughout. Data were analysed by Student’s t-test or Welch’s t-test. All the t-tests were performed as two-tailed t-tests. The statistical test used for each experiment is stated in the figure legend.

**Data availability.** The authors declare that all data supporting the findings of this study are available within the paper and its Supplementary Information files or are available from the corresponding author on request.

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Acknowledgements

We thank numerous members of the Nishina laboratory and Helen Pickersgill of Life Science Editors for their helpful discussions and critical comments on the manuscript. This work is supported by a Japan Society for the Promotion of Science (JSPS) Grant-in-Aid for Scientific Research on Innovative Areas 26114001, a Grant-in-Aid from the Ministry of Health, Labour and Welfare of Japan and a Grant-in-Aid from the Uehara Memorial Foundation.

Author contributions

N.M. and H.N. designed experiments and wrote the manuscript. N.M. and S.H. performed experiments. M.N. and H.N. designed experiments and wrote the manuscript. N.M. and S.H. contributed to the design of the mouse gene expression experiments. M.I. and Y.O. contributed to the design and execution of the knockout mouse experiments. M.N. and A.M. contributed to the design and execution of the knockdown mouse experiments. T.I., M.T. and A.M. contributed to the design and execution of the HDAC knockdown experiments. M.N. and H.N. designed experiments and wrote the manuscript. N.M. and H.N. designed experiments and wrote the manuscript.

Additional information

Supplementary Information accompanies this paper at http://www.nature.com/naturecommunications

Competing interests: The authors declare no competing financial interests.

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How to cite this article: Miyamura, N. et al. YAP determines the cell fate of injured mouse hepatocytes in vivo. *Nat. Commun.* **8**, 16017 doi: 10.1038/ncomms16017 (2017).

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Erratum: YAP determines the cell fate of injured mouse hepatocytes in vivo

Norio Miyamura, Shoji Hata, Tohru Itoh, Minoru Tanaka, Miki Nishio, Michiko Itoh, Yoshihiro Ogawa, Shuji Terai, Isao Sakaida, Akira Suzuki, Atsushi Miyajima & Hiroshi Nishina

Nature Communications 8:16017 doi: 10.1038/ncomms16017 (2017); Published 6 Jul 2017; Updated 7 Aug 2017.

The financial support for this Article was not fully acknowledged. The Acknowledgements should have included the following:

This work was supported by a Nanken-Kyoten grant from Tokyo Medical and Dental University (TMDU).