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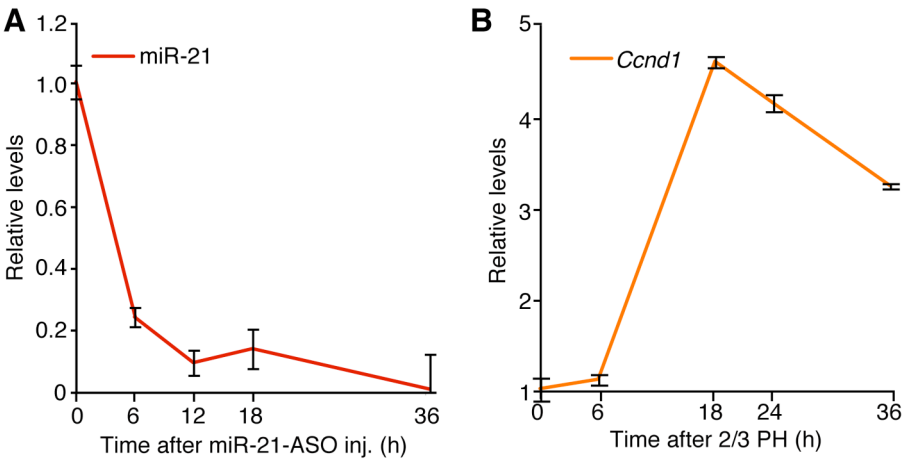
Supplemental Methods

BrdU labeling. 50 μg/g body weight BrdU (Roche) dissolved in PBS were injected intraperitoneally 2 hours before the mice were killed for analysis.

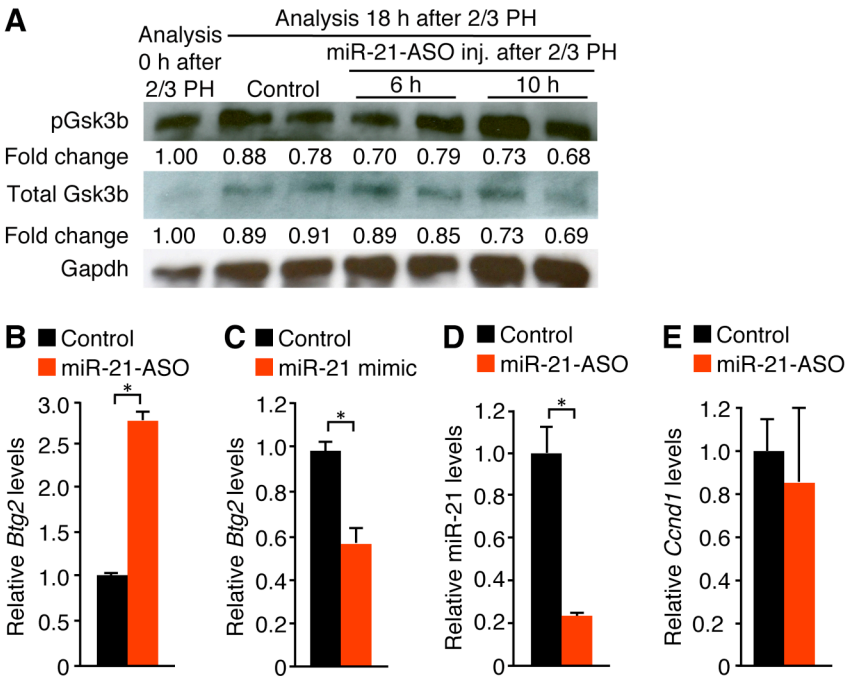
Immunoblotting. The primary antibodies rabbit anti-Gsk3b, rabbit anti-Gsk3b phosphorylated at Ser9, rabbit anti-PTEN, rabbit anti-Pdcd4, rabbit anti-S6K1 and rabbit anti-S6K1 phosphorylated at Thr389 (all Cell Signaling) were used at 1:1,000 dilutions. The secondary antibody goat anti-rabbit-HRP (Jackson ImmunoResearch) was used at 1:10,000 dilutions.

Immunostaining. The primary antibody rat anti-BrdU (Abcam) was used at 1:100 dilutions. For fluorescence microscopy, the secondary antibody goat anti-rat conjugated with Alexa Fluor 594 (Invitrogen) was used at 1:500 dilutions.

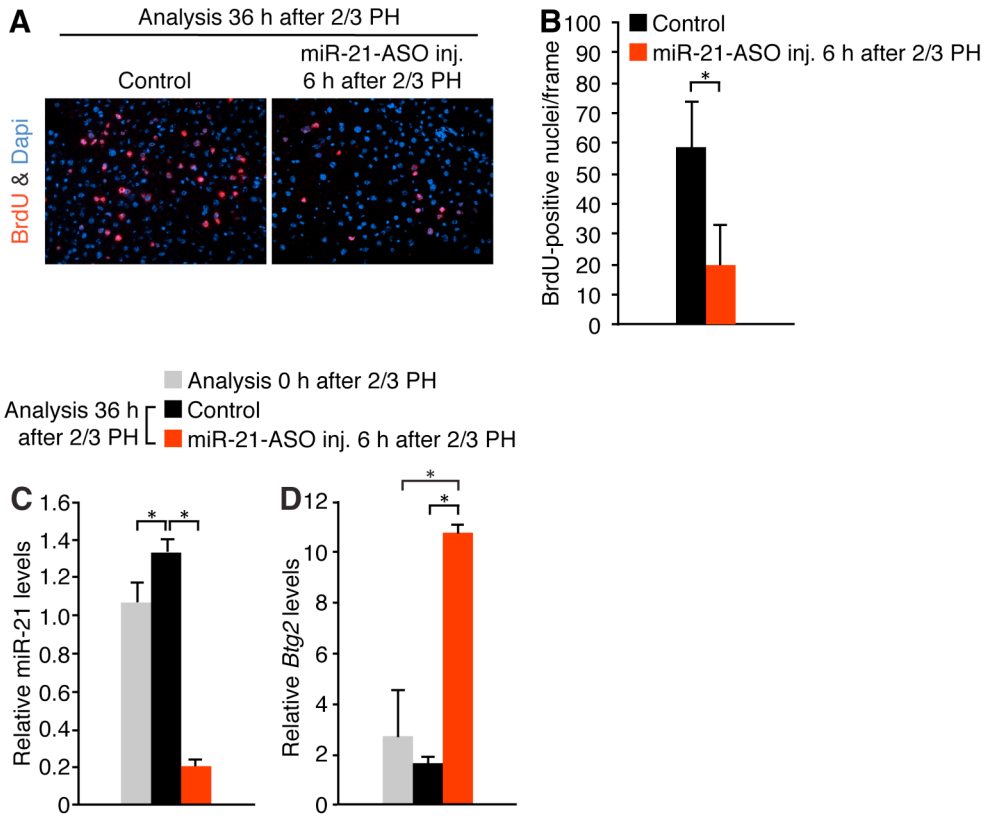
Ccnd1-ASO generation and intravenous injection. The *Ccnd1-ASO* was produced by Isis Pharmaceuticals. The 20mer was stabilized by replacing oxygen with sulphur throughout the backbone and making methoxyethyl modifications on the 2' position of the sugar of the first 5 nucleotides at the 5' and 3' end. Lyophilized *Ccnd1*-ASO and a non-targeting control ASO (NT-ASO) were resuspended in NaCl 0.9% to a final concentration of 20 μg/μl. A dose of 50 μg/g body weight ASO in a total volume of 100 μl NaCl 0.9% was injected via the tail vein into 8- to 12-week-old male C57BL/6 mice (Jackson Laboratory).



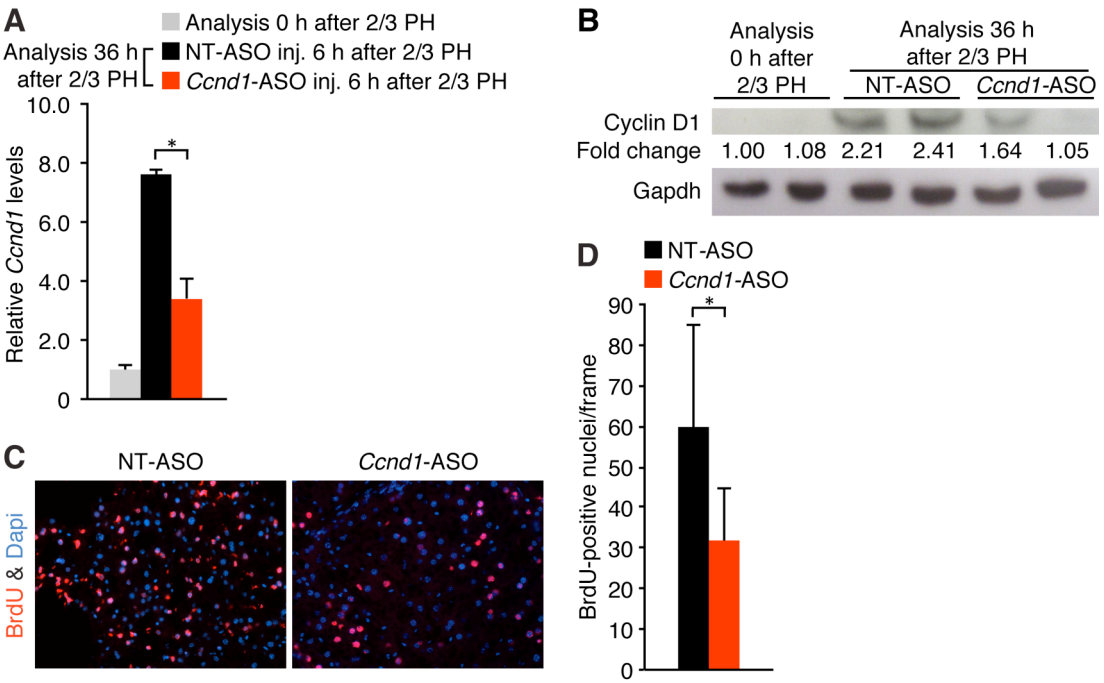
Supplemental Figure 1 Time course of miR-21 and *Ccnd1* levels in livers of mice after miR-21-ASO injection or 2/3 PH. (**A**) Time course of miR-21 levels after a single tail vein injection of miR-21-ASO. (**B**) Time course of *Ccnd1* mRNA levels after 2/3 PH. At least 3 mice were analyzed for each time point and treatment. Data represent mean ± SEM.



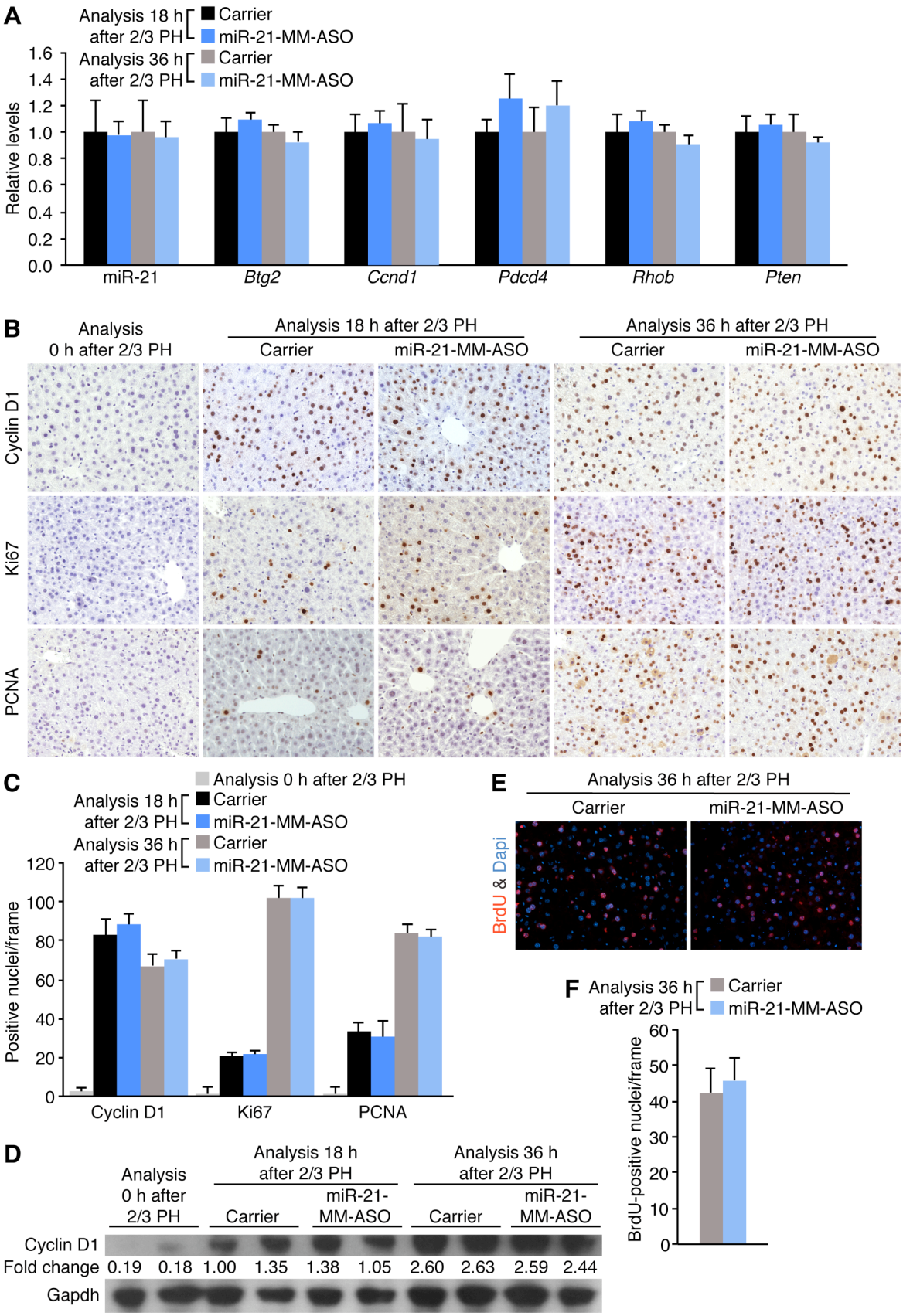
Supplemental Figure 2 miR-21 promotes expression of cyclin D1 by facilitating its translation, not by preventing its degradation. (**A**) Phosphorylation at Ser9 prevents Gsk3b from triggering proteosomal degradation of cyclin D1. Immunoblotting shows that, 18 hours after 2/3 PH, the levels of Gsk3b phosphorylated at Ser9 (pGsk3b) are not increased in livers of mice injected with miR-21-ASO at 6 or 10 hours after 2/3 PH as compared to controls. Total Gsk3b protein levels are indistinguishable between controls and mice injected with miR-21-ASO at 6 hours after 2/3 PH and slightly decreased in mice injected with miR-21-ASO at 10 hours after 2/3 PH. Numbers indicate protein levels relative to time point 0 hours after 2/3 PH. At least 3 mice were analyzed for each time point and treatment. Control mice were injected with carrier. Gapdh was analyzed as a loading control. (**B** and **C**) qRT-PCR shows that transfection of miR-21-ASO or miR-21 mimic into Hepa1,6 cells increases or decreases *Btg2* mRNA levels, respectively. (**D**) qRT-PCR shows 5-fold suppression of miR-21 48 hours after transfection with miR-21-ASO in Hepa1,6 cells used for polysome analysis. (**E**) qRT-PCR shows *Ccnd1* mRNA levels are unaltered in Hepa1,6 cells 48 hours after transfection with miR-21-ASO. Data represent mean ± SEM. **P* < 0.05.



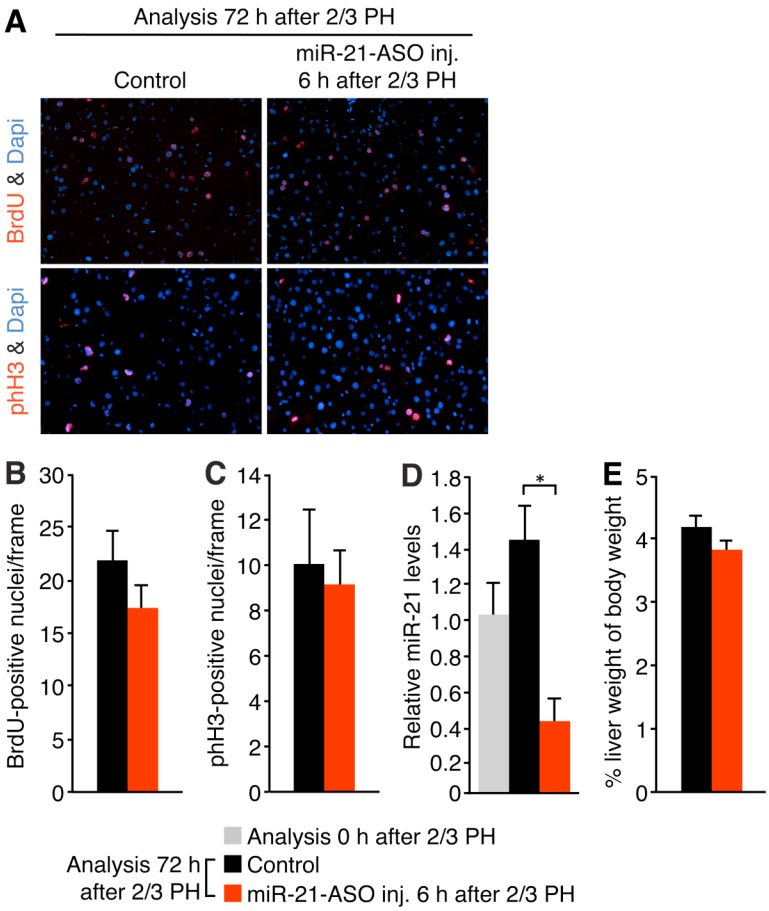
Supplemental Figure 3 Impaired S phase entry and miR-21 depletion 36 hours after 2/3 PH in hepatocytes of miR-21-ASO-injected mice. (**A** and **B**) BrdU immunostaining shows that many hepatocytes synthesize DNA 36 hours after 2/3 PH in control mice. Significantly fewer hepatocytes are BrdU positive in mice injected with miR-21-ASO. Approximately 1,500 hepatocytes (250 per frame) were analyzed for each treatment. Original magnification, x200. (**C**) qRT-PCR shows strong inhibition of miR-21 36 hours after 2/3 PH in livers of miR-21-ASO-injected mice. (**D**) The repression of *Btg2* mRNA levels in livers of control mice 36 hours after 2/3 PH is reversed in mice injected with miR-21-ASO. At least 3 mice were analyzed for each time point and treatment. Control mice were injected with carrier at 6 hours after 2/3 PH. Data represent mean ± SEM. **P* < 0.05.



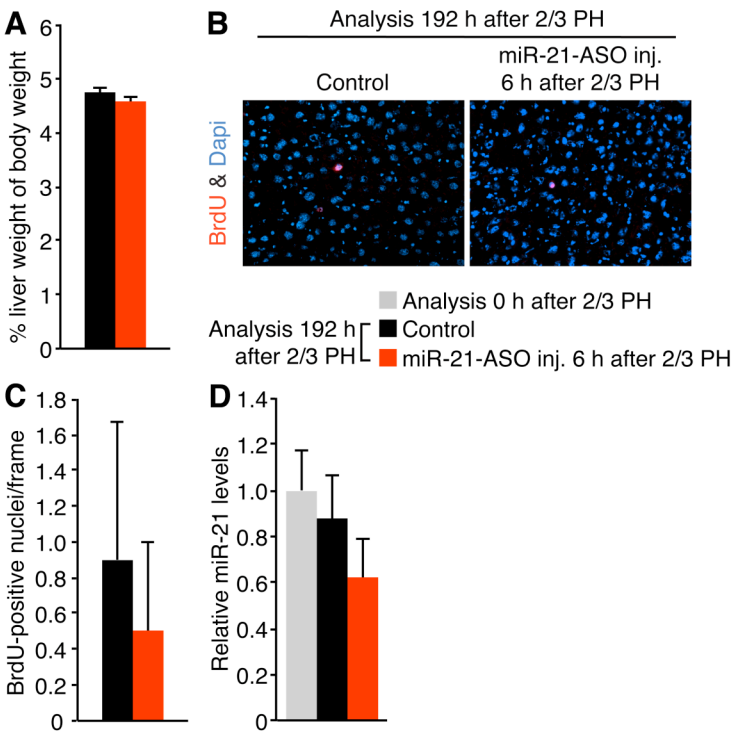
Supplemental Figure 4 Knockdown of cyclin D1 in hepatocytes during the early phase of liver regeneration impairs S phase entry. (**A**-**D**) Comparison of liver cyclin D1 mRNA and protein levels and number of BrdU-positive hepatocytes at 36 hours after 2/3 PH between mice injected with *Ccnd1*-ASO and mice injected with control NT-ASO at 6 hours after 2/3 PH. qRT-PCR shows that *Ccnd1* mRNA levels are 2-fold lower in *Ccnd1*-ASO-injected mice than in NT-ASO-injected mice (**A**). Immunoblotting shows that cyclin D1 protein levels are also lower in *Ccnd1*-ASO-injected mice than in NT-ASO-injected mice. Numbers indicate protein levels relative to time point 0 hours after 2/3 PH. Gapdh was analyzed as a loading control (**B**). BrdU immunostaining shows that fewer hepatocytes are synthesizing DNA in *Ccnd1*-ASO-injected mice than in NT-ASO-injected mice (**C**). For quantification, approximately 1,500 hepatocytes (250 per frame) were analyzed for each treatment (**D**). At least 3 mice were analyzed for each time point and treatment. Original magnification, x200. Data represent mean ± SEM. *$P < 0.05$.


A
Analysis 18 h after 2/3 PH
Carrier
miR-21-MM-ASO
Analysis 36 h after 2/3 PH
Carrier
miR-21-MM-ASO
Relative levels
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
miR-21
Btg2
Ccnd1
Pdcd4
Rhob
Pten
B
Analysis 0 h after 2/3 PH
Analysis 18 h after 2/3 PH
Carrier
miR-21-MM-ASO
Analysis 36 h after 2/3 PH
Carrier
miR-21-MM-ASO
Cyclin D1
Ki67
PCNA
C
Analysis 0 h after 2/3 PH
Analysis 18 h after 2/3 PH
Carrier
miR-21-MM-ASO
Analysis 36 h after 2/3 PH
Carrier
miR-21-MM-ASO
Positive nuclei/frame
120
100
80
60
40
20
0
Cyclin D1
Ki67
PCNA
D
Analysis 0 h after 2/3 PH
Analysis 18 h after 2/3 PH
Carrier
miR-21-MM-ASO
Analysis 36 h after 2/3 PH
Carrier
miR-21-MM-ASO
Cyclin D1
Fold change 0.19 0.18 1.00 1.35 1.38 1.05 2.60 2.63 2.59 2.44
Gapdh
E
Analysis 36 h after 2/3 PH
Carrier
miR-21-MM-ASO
BrdU & Dapi
F
Analysis 36 h after 2/3 PH
Carrier
miR-21-MM-ASO
BrdU-positive nuclei/frame
60
50
40
30
20
10
0

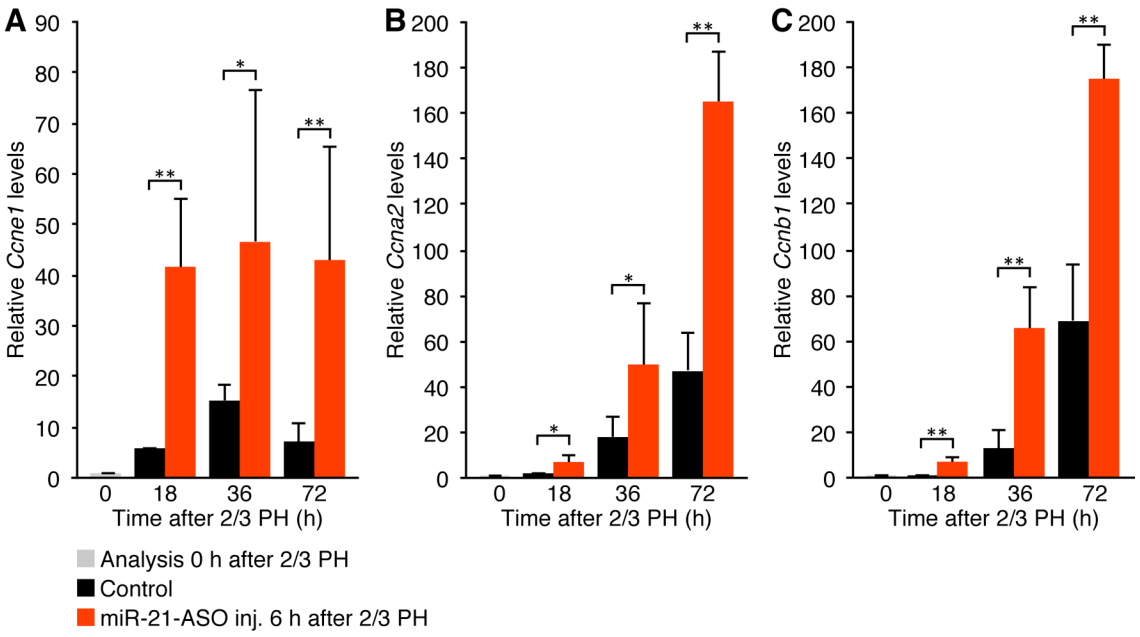
Supplemental Figure 5 Impaired cyclin D1 translation and cell cycle progression of hepatocytes of miR-21-ASO-injected mice after 2/3 PH are not due to unspecific effects or toxicity caused by the ASO. (**A**-**D**) Mice received tail vein injection of carrier or miR-21-MM-ASO at 6 hours after 2/3 PH; livers were analyzed at 18 or 36 hours after 2/3 PH. qRT-PCR shows that carrier and miR-21-MM-ASO injection have indistinguishable effects on expression of miR-21 and its target genes. Average carrier levels were set to 1 (**A**). Immunostainings show that the number of hepatocytes expressing markers of progression through G1 (cyclin D1 and Ki67, brown) and into S (Ki67 and PCNA, brown) phase of the cell cycle is indistinguishable between mice injected with carrier and mice injected with miR-21-MM-ASO (**B**). Quantification of hepatocytes expressing markers of cell cycle progression (**C**). Immunoblotting shows that cyclin D1 protein levels increase equally in mice injected with carrier and mice injected with miR-21-MM-ASO. Numbers indicate protein levels relative to carrier at time point 18 hours after 2/3 PH. Gapdh was analyzed as a loading control (**D**). (**E** and **F**) BrdU immunostaining shows a similar number of hepatocytes synthesizing DNA 36 hours after 2/3 PH in mice injected with carrier and mice injected with miR-21-MM-ASO at 6 hours after 2/3 PH. For each immunostaining, approximately 1,500 hepatocytes (250 per frame) were analyzed per time point and treatment. At least 3 mice were analyzed for each time point and treatment. Original magnification, x200. Data represent mean ± SEM.



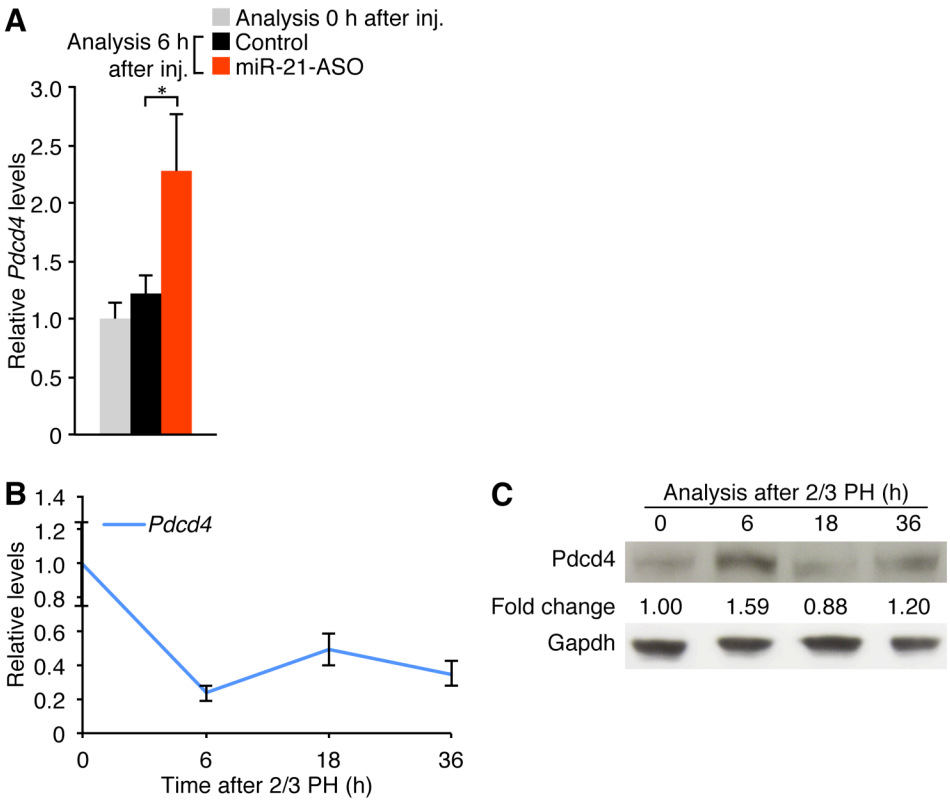
Supplemental Figure 6 Normal mitosis and liver mass restoration despite miR-21 depletion 72 hours after 2/3 PH in hepatocytes of miR-21-ASO-injected mice. (**A-C**) BrdU and phosphorylated histone H3 (phH3) immunostainings. BrdU immunostaining shows a similar number of hepatocytes synthesizing DNA in miR-21-ASO-injected and control mice 72 hours after 2/3 PH (**A** and **B**). phH3 immunostaining shows a similar number of hepatocytes in mitosis in miR-21-ASO-injected and control mice 72 hours after 2/3 PH (**A** and **C**). For each immunostaining, approximately 1,500 hepatocytes (250 per frame) were analyzed per treatment. Original magnification, x200. (**D**) qRT-PCR shows that miR-21 is still inhibited in livers of miR-21-ASO-injected mice 72 hours after 2/3 PH. (**E**) The extent of liver mass restoration 72 hours after 2/3 PH is similar in mice injected with miR-21-ASO and control mice. At least 3 mice were analyzed for each time point and treatment. Control mice were injected with carrier at 6 hours after 2/3 PH. Data represent mean ± SEM. *$P < 0.05$.



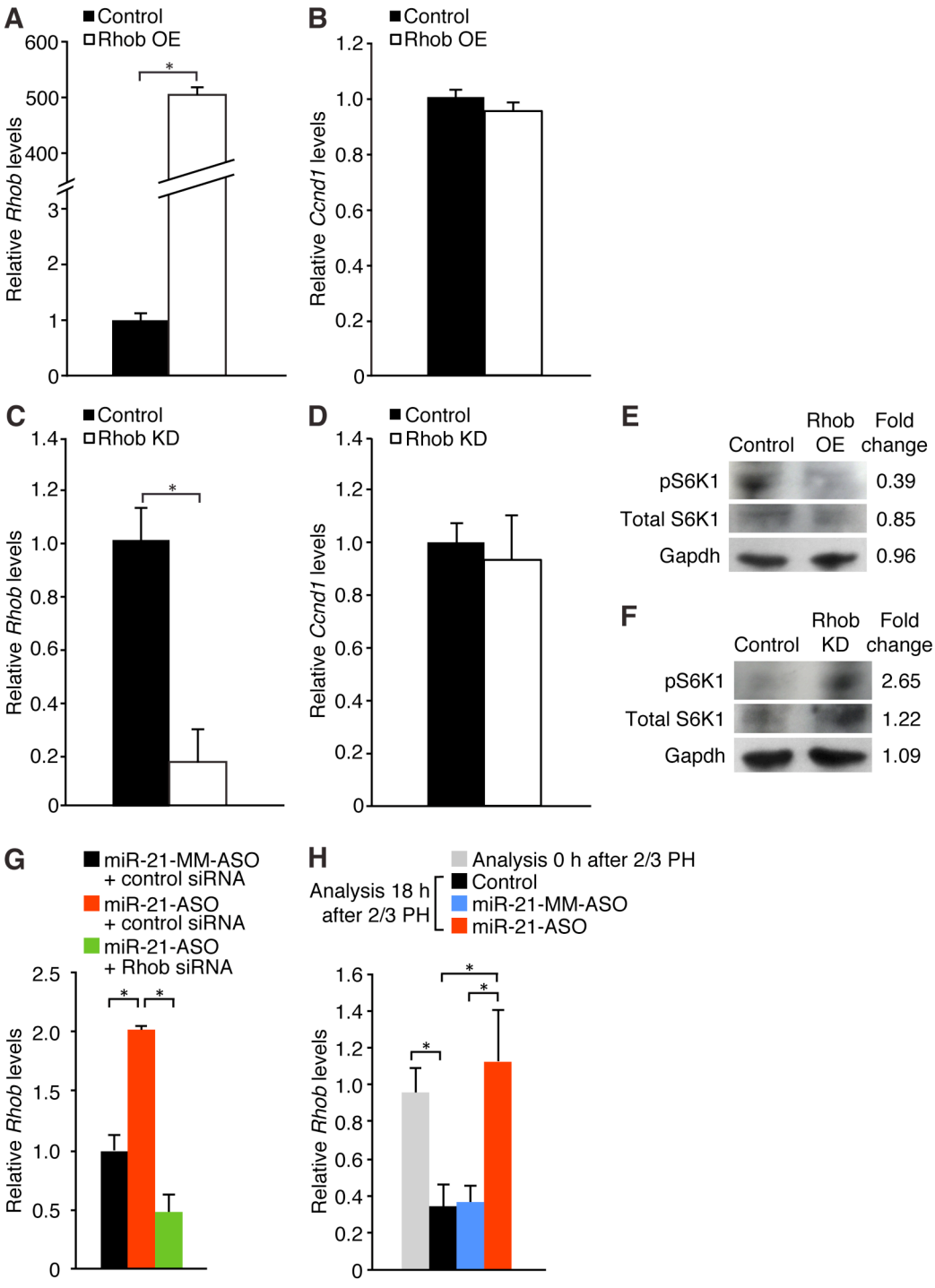
Supplemental Figure 7 Complete liver mass restoration 192 hours after 2/3 PH in miR-21-ASO-injected mice. (**A**) The liver to body weight ratio is indistinguishable between miR-21-ASO-injected mice and control mice 192 hours after 2/3 PH. (**B** and **C**) BrdU immunostaining shows a similarly small number of hepatocytes synthesizing DNA in miR-21-ASO-injected mice and control mice 192 hours after 2/3 PH. Approximately 1,500 hepatocytes (250 per frame) were analyzed for each treatment. Original magnification, x200. (**D**) qRT-PCR shows near-complete normalization of miR-21 levels in miR-21-ASO-injected mice 192 hours after 2/3 PH. At least 3 mice were analyzed for each time point and treatment. Control mice were injected with carrier at 6 hours after 2/3 PH. Data represent mean ± SEM.



Supplemental Figure 8 Overexpression of *Ccne1*, *Ccna2* and *Ccnb1* in miR-21-ASO-injected mice. (**A-C**) qRT-PCR shows that *Ccne1* (**A**), *Ccna2* (**B**) and *Ccnb1* (**C**) mRNA levels are induced from 18 to 72 hours after 2/3 PH in miR-21-ASO-injected mice as compared to control mice. At least 3 mice were analyzed for each time point and treatment. Control mice were injected with carrier at 6 hours after 2/3 PH. Data represent mean ± SEM. **P* < 0.05, ***P* < 0.01.

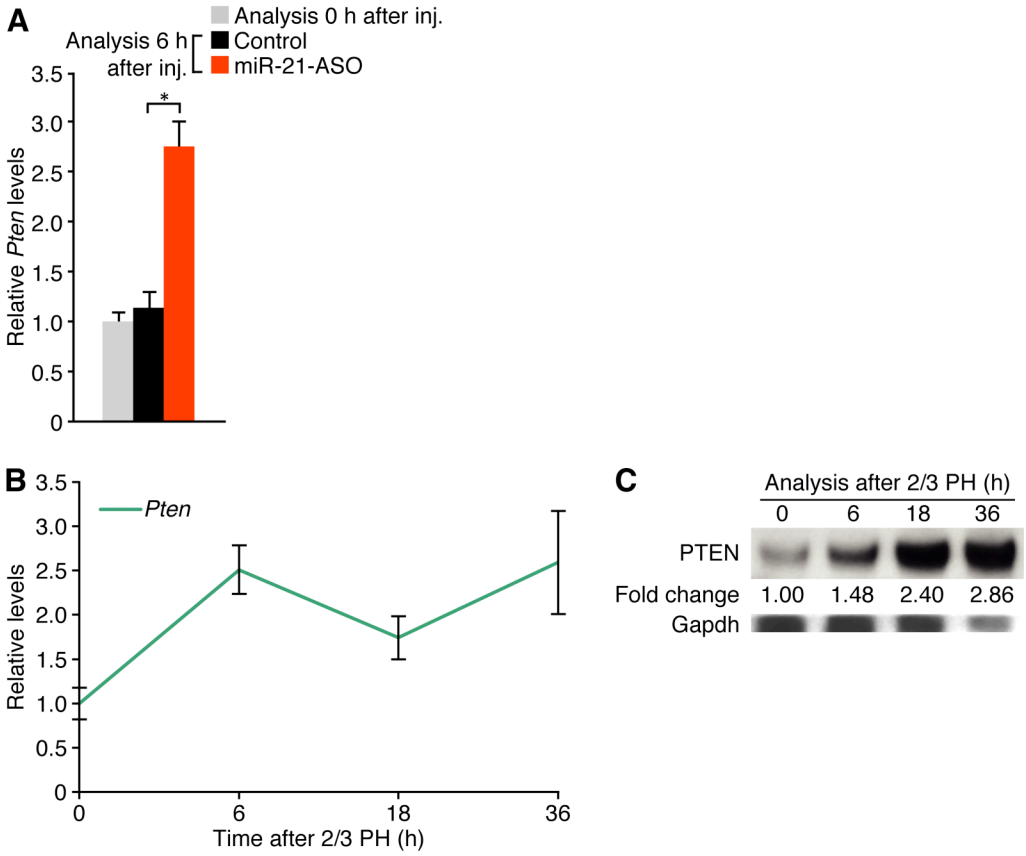


Supplemental Figure 9 Moderate suppression of the miR-21 target Pdcd4 in early liver regeneration. (**A**) qRT-PCR shows a 2-fold increase in *Pdcd4* mRNA levels in livers of mice 6 hours after a single tail vein injection of miR-21-ASO. Control mice were injected with carrier. (**B**) qRT-PCR shows a rapid and continuous decrease in *Pdcd4* mRNA levels after 2/3 PH in normal mice (not injected with miR-21-ASO). (**C**) Immunoblotting of liver samples from the same mice shows that Pdcd4 protein levels undulate after 2/3 PH and are moderately decreased at 18 hours after 2/3 PH when miR-21 expression peaks. Numbers indicate protein levels relative to time point 0 hours after 2/3 PH. Gapdh was analyzed as a loading control. At least 3 mice were analyzed for each time point and treatment. Data represent mean ± SEM. $*P < 0.05$.

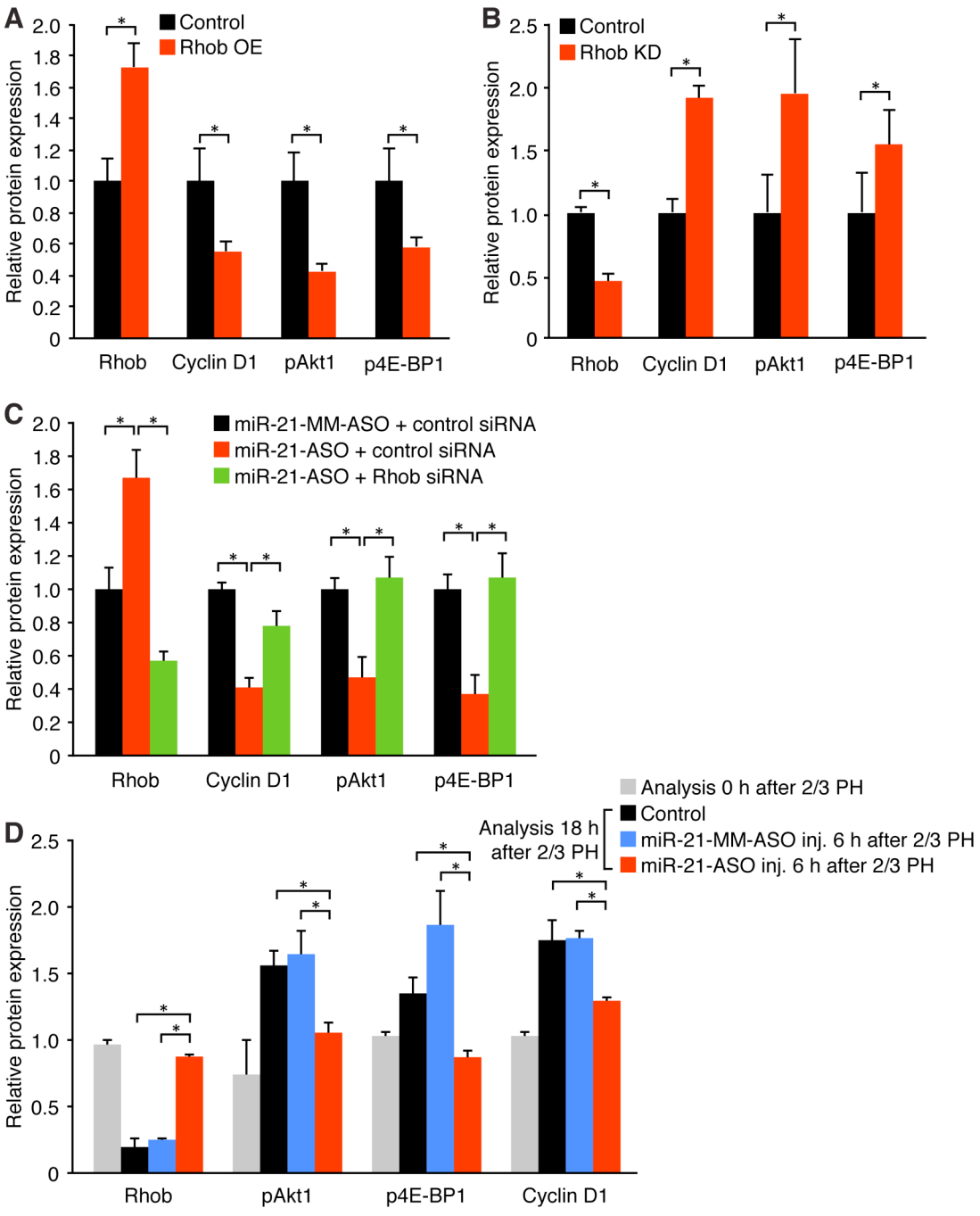


Supplemental Figure 10 Further evidence that miR-21 promotes cyclin D1 translation by relieving Akt1-mediated activation of mTORC1 from suppression by Rhob. qRT-PCR was used to determine *Rhob* and *Ccnd1* mRNA levels in Hepa1,6 cells transfected with Rhob OE or Rhob OE and KD plasmids. (**A**) Increased *Rhob* mRNA levels in Hepa1,6

cells transfected with Rhob OE plasmid as compared to cells transfected with empty OE plasmid (Control). (**B**) Despite Rhob overexpression, *Ccnd1* mRNA levels are unchanged. (**C**) Decreased *Rhob* mRNA levels in Hepa1,6 cells transfected with both Rhob OE and Rhob KD plasmids (Rhob KD) as compared to cells transfected with Rhob OE plasmid alone (Control). (**D**) Despite Rhob knockdown, *Ccnd1* mRNA levels are unchanged. (**E**) Immunoblotting shows that transfection of Rhob OE plasmid into Hepa1,6 cells decreases the level of S6 kinase 1 (S6K1) phosphorylated at Thr389 (pS6K1). Hepa1,6 cells transfected with empty OE plasmid were used as control. (**F**) Transfection of Rhob KD plasmid into Hepa1,6 cells overexpressing Rhob (Control) increases the level of pS6K1. Numbers indicate protein levels relative to control. Results representative of 3 separate experiments are shown. Gapdh was analyzed as a loading control. (**G**) qRT-PCR shows that derepressed *Rhob* mRNA levels in Hepa1,6 cells transfected with miR-21-ASO are normalized after additional transfection of Rhob siRNA. Negative control siRNA was cotransfected in cells transfected with miR-21-ASO alone. Hepa1,6 cells transfected with miR-21-MM-ASO and negative control siRNA were used as control. (**H**) qRT-PCR shows a 3-fold increase in *Rhob* mRNA levels at 18 hours after 2/3 PH in mice injected with miR-21-ASO as compared to mice injected with carrier (Control) or miR-21-MM-ASO. Carrier, miR-21-MM-ASO or miR-21-ASO was injected at 6 hours after 2/3 PH. At least 3 mice were analyzed for each time point and treatment. Data represent mean ± SEM. $*P < 0.05$.



Supplemental Figure 11 Overexpression of the miR-21 target PTEN in early liver regeneration. (**A**) qRT-PCR shows a 2.5-fold increase in *Pten* mRNA levels in livers of mice 6 hours after a single tail vein injection of miR-21-ASO. Control mice were injected with carrier. (**B**) qRT-PCR shows a rapid and continuous increase in *Pten* mRNA levels after 2/3 PH in normal mice (not injected with miR-21-ASO). (**C**) Immunoblotting of liver samples from the same mice shows that PTEN protein levels also increase after 2/3 PH, which excludes effective inhibition by miR-21. Numbers indicate protein levels relative to time point 0 hours after 2/3 PH. Gapdh was analyzed as a loading control. At least 3 mice were analyzed for each time point and treatment. Data represent mean ± SEM. **P* < 0.05.



Supplemental Figure 12 Statistical analysis of immunoblotting results. (**A**) Rhob OE results. (**B**) Rhob KD results. (**C**) miR-21-ASO and Rhob siRNA cotransfection results. Results are from 3 separate experiments. (**D**) miR-21-ASO injection results. At least 3 mice were analyzed for each time point and treatment. Control mice were injected with carrier at 6 hours after 2/3 PH. Data represent mean ± SEM. **P* < 0.05.