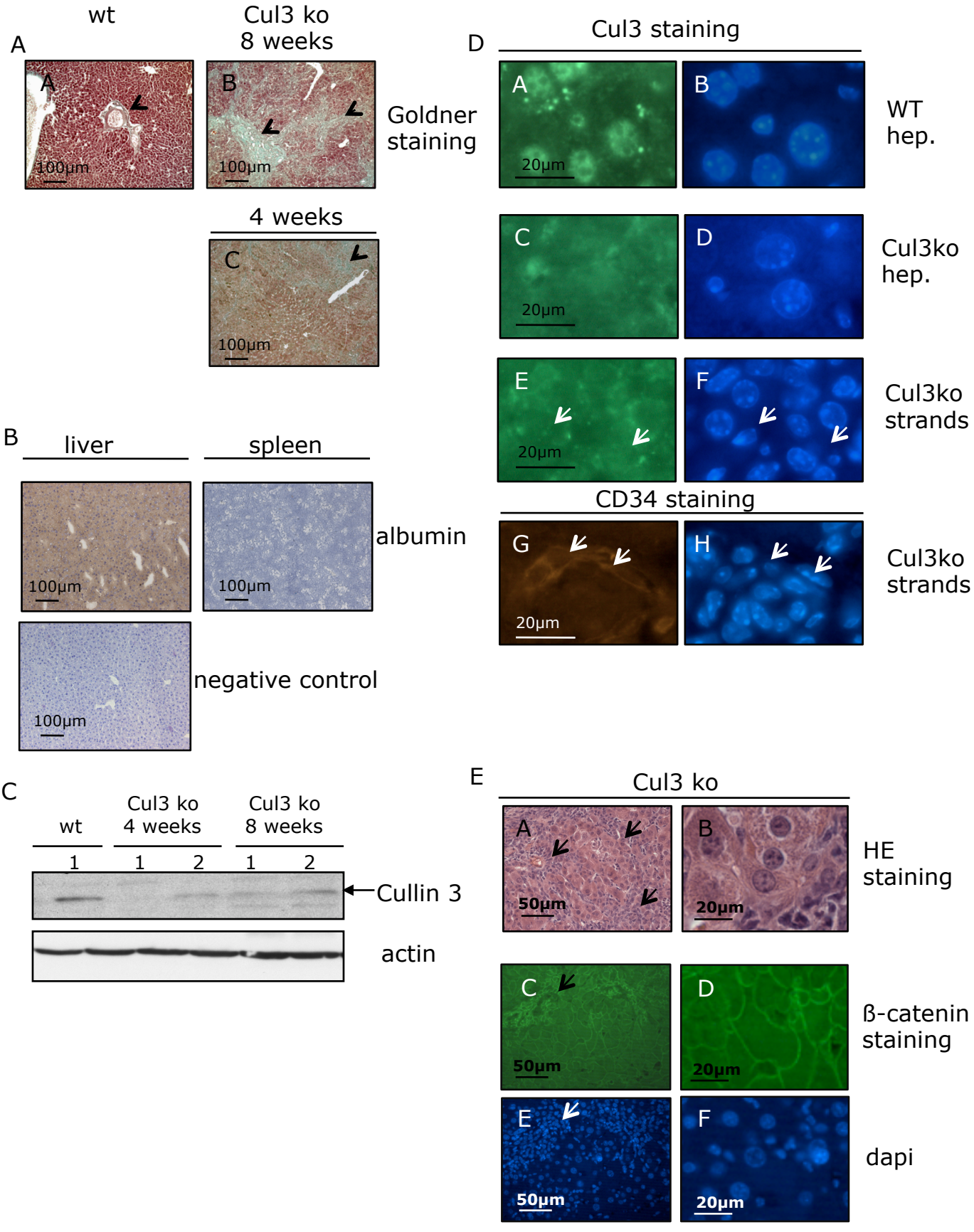
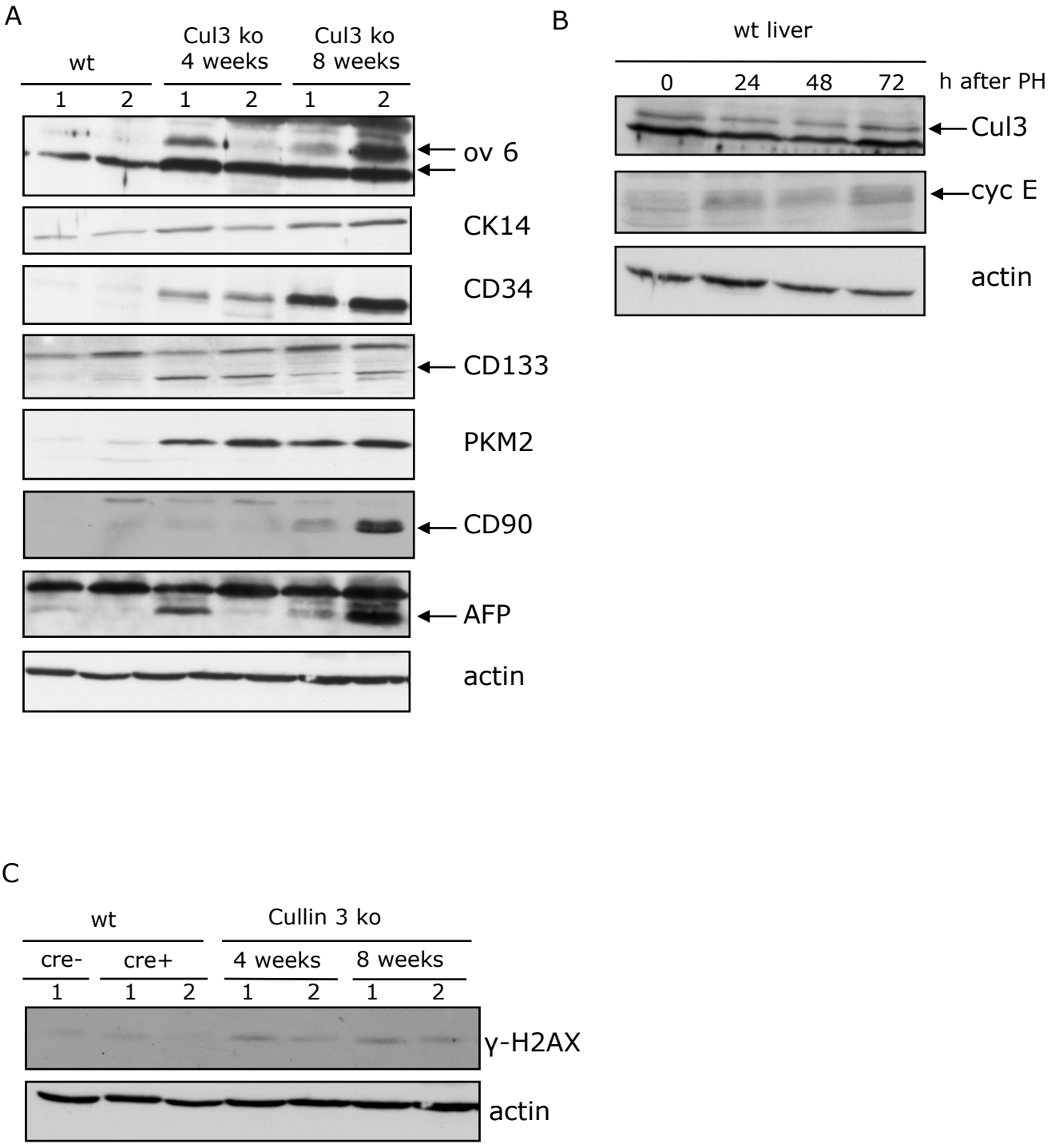
Kossatz et al. Supplemental Figure 1



Kossatz et al. Supplemental Figure 2



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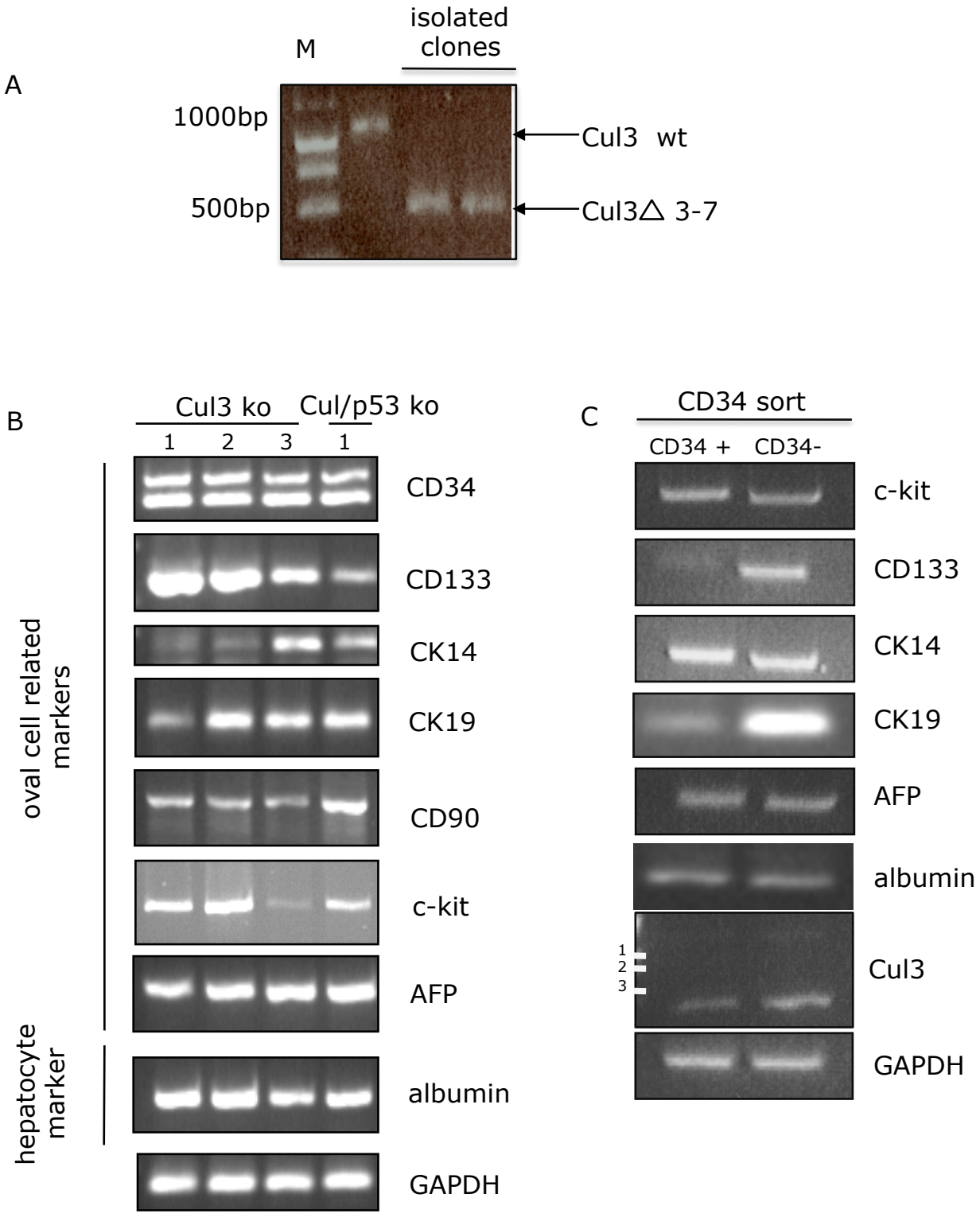

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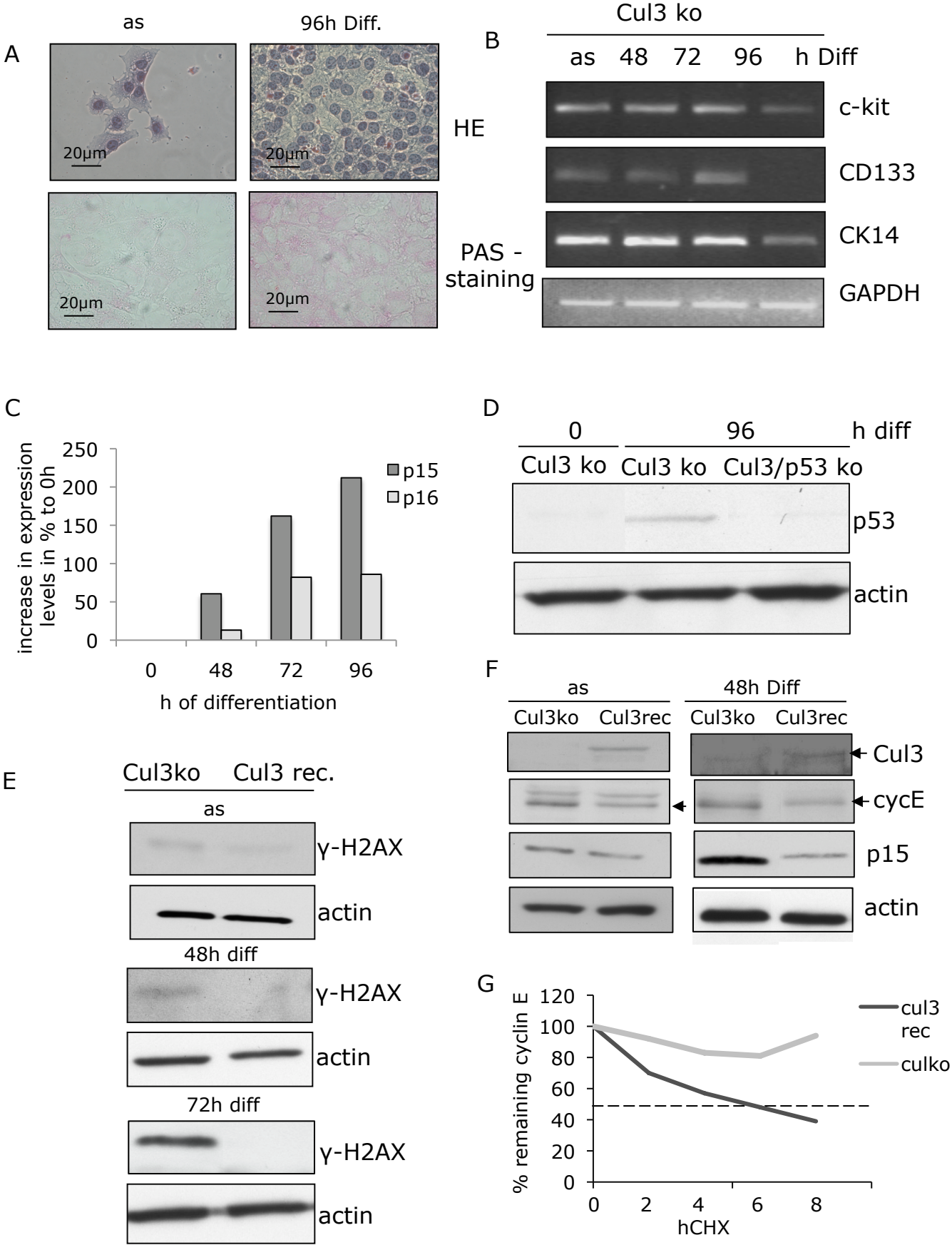

Kossatz et al. Supplemental 5

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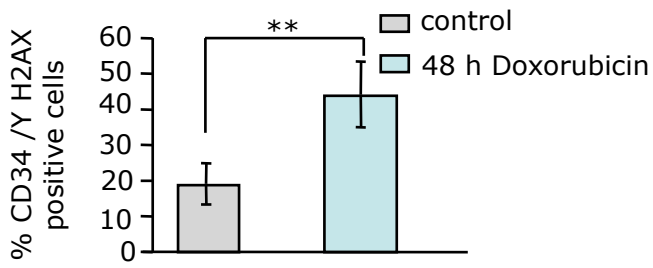

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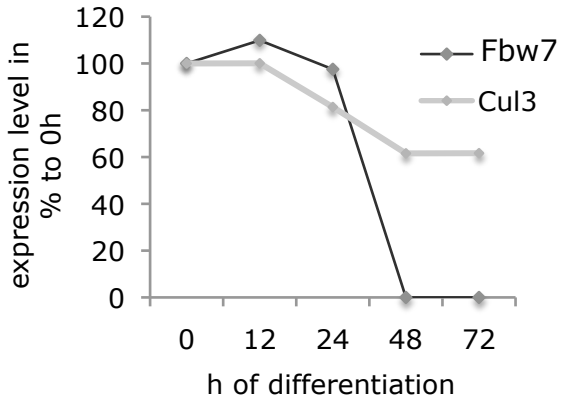

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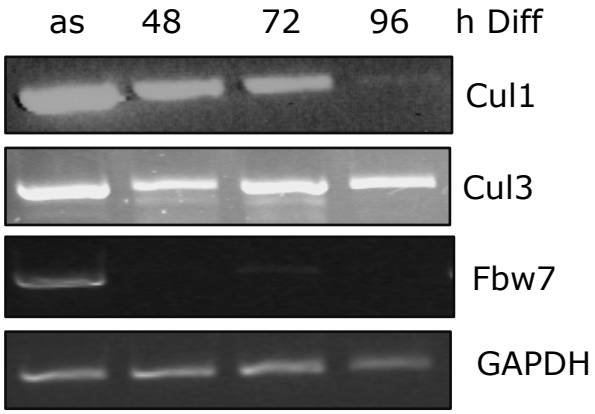

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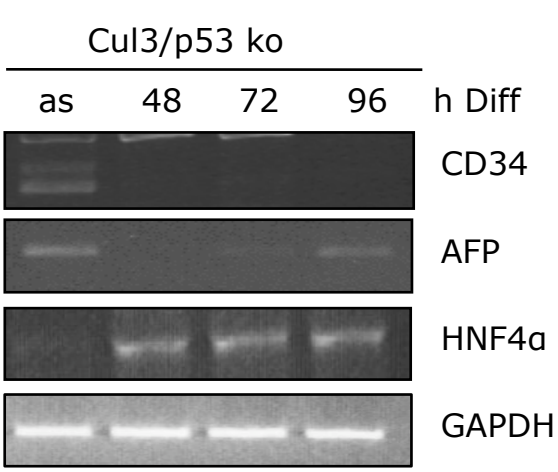
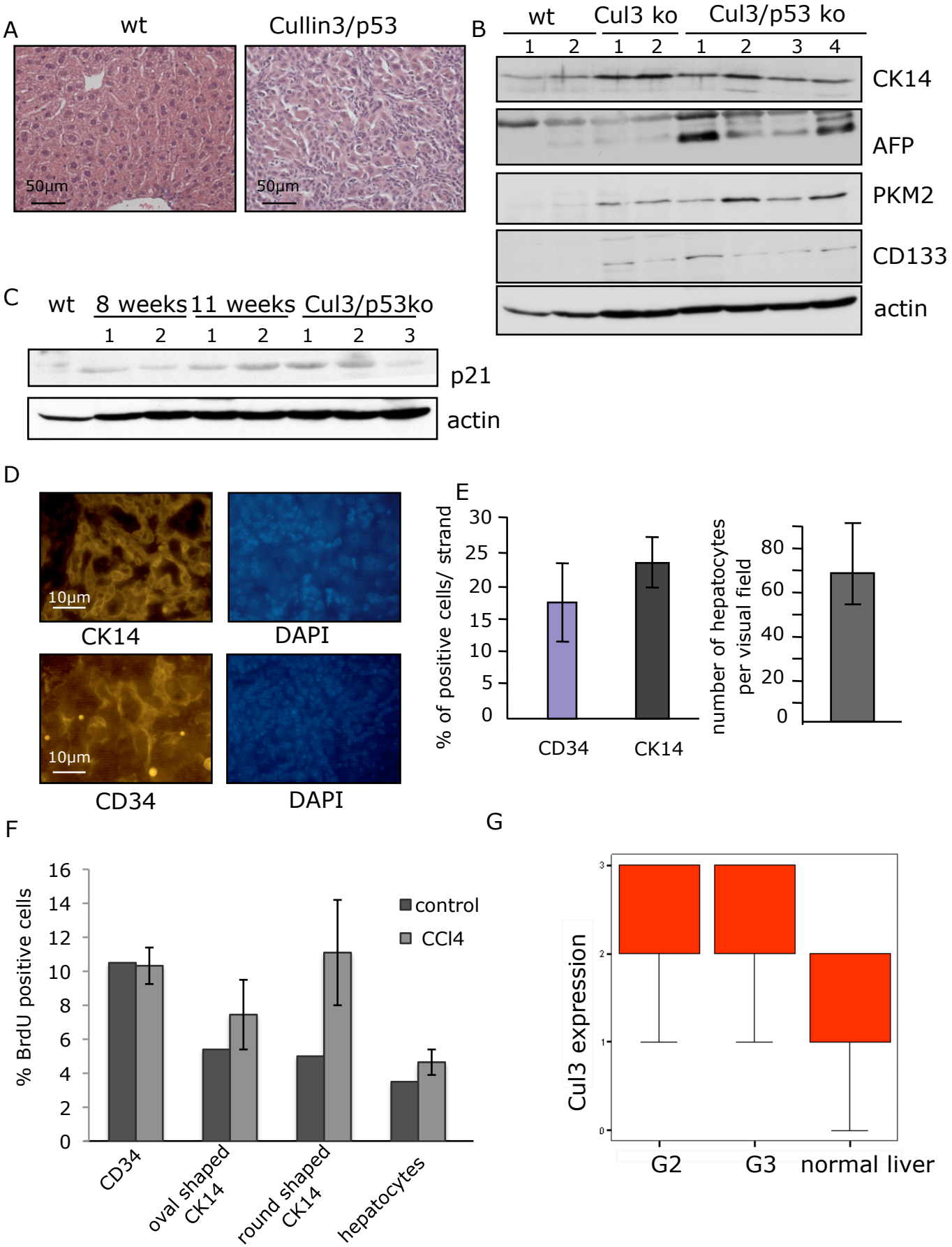


Kossatz et al. Supplemental 6



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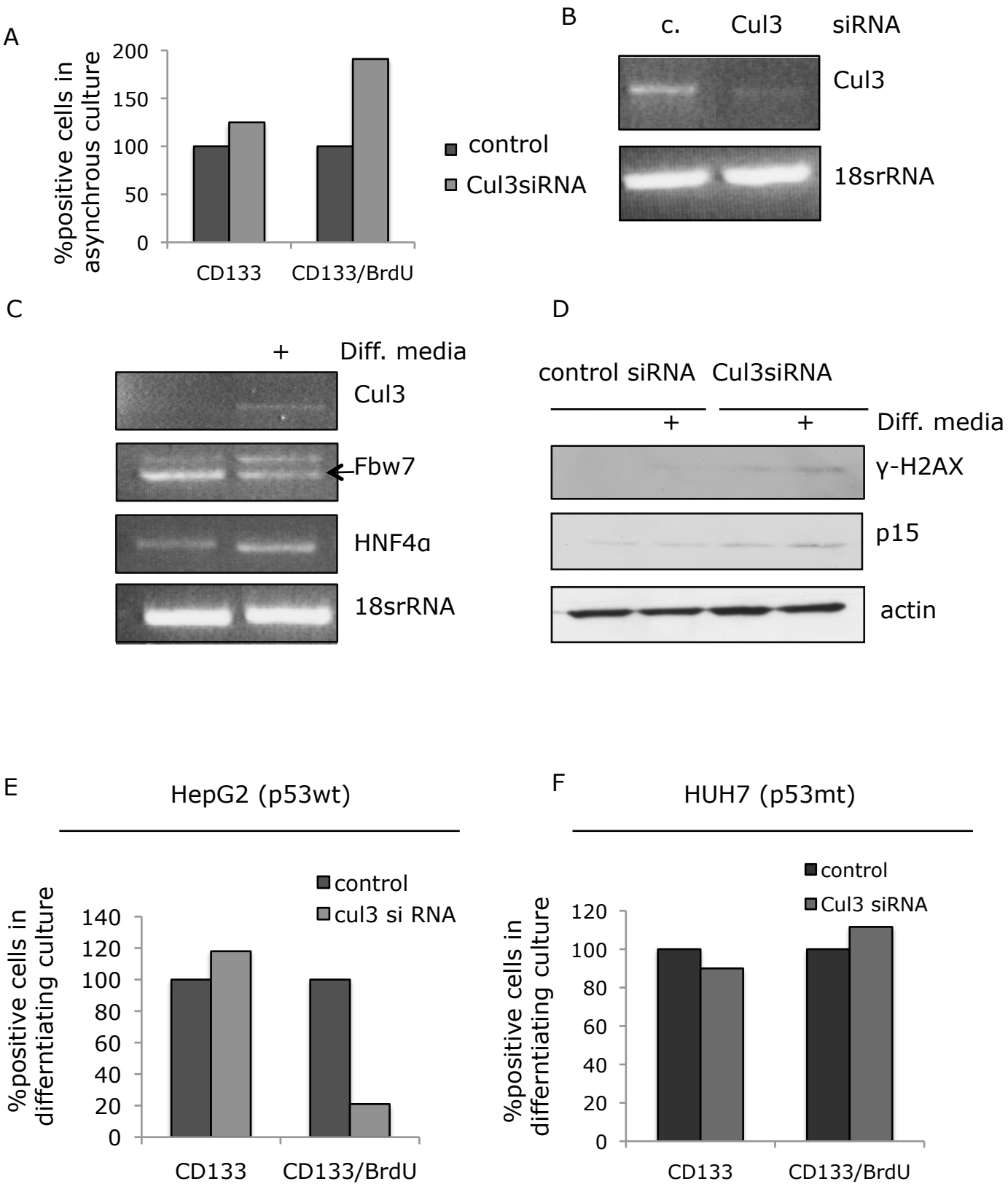

Supplemental Figure 1

1A) Cul3 knockout livers (4 and 8 weeks) compared to wildtype tissue after Goldner staining. Stroma cells (blue) and parenchymal cells (redish color) are shown. In wildtype livers stroma cells can be found around the bile ducts (arrow), in Cul3 ko livers stroma cells are expanded. Increase in stromal cells can be found from 4 to 8 week (B, C). Arrows in B and C indicate fields of stromal cells. 1B) Albumin staining of liver sections with spleen tissue and tissue that was not treated with a secondary antibody as negative controls. 1C) Western blot analysis of whole liver lysates in wildtype and Cul3 knockout livers (arrow indicates Cul3). Actin was used as a control. Numbers indicate examples of different mice being analyzed. 1D) Immunofluorescence for Cul3 in Cul3 knockout and wildtype livers. Cul3 wildtype hepatocytes show a strong nuclear staining (A). Hepatocytes in Cul3 knockout livers (Cul3ko hep.) (C) and also cells in the strands (E) do not stain positive for Cul3. CD34 positive cells (G) which are located in the strands (compare Figure 1B for albumin staining) are shown for comparison. DAPI staining is shown in B, D, F, H. 1E) Analysis of hepatocytic fields was done by morphological features, such as increased nuclear size and increased content of cytoplasm (compare B). In (A) picture of hepatocytes in Cul3 knock out livers surrounded by mononuclear cells (indicated by arrows) is shown. Typical picture of hepatocytes (B). In addition ß-catenin staining was used to identify cells with an increase in cytoplasmatic content (C, D) Arrows indicate mononucleated cells of the strands. DAPI staining is shown in E and F.

Supplemental Figure 2:

2A) Western blot analysis of liver lysates of 4 and 8 week old mice using markers expressed on hepatic stem cells or oval cells, such as OV-6, CK14, CD34, CD133, PKM2, CD90 and AFP. Numbers indicate different mice being analyzed. 2B) Expression analysis of Cul3 and cyclin E upon partial hepatectomy at 0, 24, 48 and 72 h. Cyclin E and Cul3 are indicated by arrows (n=2 for each timepoint). 2C) Western blot analysis of γH2AX in whole liver lysates of Cul3 ko livers in comparison to wt liver being negative or positive for cre-recombinase expression. Numbers indicate different mice being analyzed.

Supplemental Figure 3:

3A) Analysis of Cul3 recombination by RT-PCR analysis in isolated cell clones. Deletion of exons 3-7 by cre- recombinase leads to expression of a shortened RNA product which can be amplified. Deletion of exon 3-7 leads to a shift of approx. 500bp when compared to wildtype Cul3. 3B) RT-PCR analysis of isolated Cul3 knockout clones for markers being expressed on hepatic stem cells. Numbers indicate different clones being analysed. 3C) CD34 positive and negative cells which were obtained by flow cytometry (90% purity each) were subjected to RT-PCR analysis for different surface markers being expressed on progenitor cells as well as AFP and Cul3; marker bands are indicated in the Cul3 RT-PCR: 1000bp (1), 750bp (2) and 500bp (3).

Supplemental Figure 4:

4A) HE staining and PAS staining (detects glycogen storage) in asynchronously growing cells and 96 hours after the induction of differentiation. 4B) Quantification of RT-PCR analysis of p15 and p16 during differentiation in Cul3 knockout cells. Data is shown as percent increase to asynchronously proliferating cells. 4C) Analysis of p53 protein levels at 0h and 96h of differentiation. Actin and Cul3/p53 ko cells during differentiation were used as controls. 4D) Analyses of ϒ-H2AX in Cul3 reconstituted cells in comparison to Cul3 ko cells at 48 and 72 h of differentiation using actin as control. 4E) Analysis of Cyclin E and p15 in asynchronously proliferating cells and at 48 h of differentiation. Western blots were used to analyze Cul3 expression. Actin was used as a loading control. Differentiation of progenitor cells expressing Cul3 led to a decrease of cyclin E expression and the senescence associated marker p15 48 h after induction of differentiation. The experiment was repeated 3 times in duplicates. 4F) RT-PCR analysis of stem cell markers during differentiation. GAPDH is used as control. 4G) Determination of cyclin E turnover in Cul3 deficient (Cul3ko) and reconstituted (Cul3rec) progenitor cells 48 h after induction of differentiation.

Supplemental Figure 5:

5A) Treatment of mice with the DNA damaging agent doxorubicin and quantification of γH2AX positive CD34 positive cells 48h after injection. Control mice were injected with NaCl 0.9% (n=3/group). 5B) Quantification of RT-PCR analysis of Figure 4H. Shown is the decrease in % compared to asynchronously proliferating cells. 5C) RT-PCR analysis of Cul1 and Fbw7 at 48, 72 and 96 h of differentiation. Cul1 and Fbw7 are

downregulated during differentiation. 5D) Expression of progenitor and hepatocyte markers during differentiation of Cul3/p53 double knock out cells. GAPDH was used as a control.

Supplemental Figure 6

6A) HE staining of wildtype and Cul3/p53 double knock out livers. 6B) Western blot analysis of whole liver lysates for stem cell markers in wildtype and Cul3 knockout livers in comparison to Cul3/p53 knockout livers. Numbers indicate different mice being analyzed. 6C) Analysis of p21 in wt, Cul3ko and Cul3/p53 ko. Actin was used as a control. 6D) CK14 and CD34 staining in Cul/p53 knockout livers. 6E) Quantification of cells being positive for CK14 and CD34 and hepatocytes. These cells cannot not be detected in wildtype livers (compare 1C). The graph shows a quantification of positive cells per visual field in 3 mice. 6F) Induction of proliferation by liver damage. CCl4 was injected i.p.. 48 h later proliferation of CD34, CK14 positive cells as well as hepatocytes was analyzed. 2 mice for each group were analyzed. Graph shows quantification of proliferating cells. 6G) Box Plot for expression analysis of Cul3 in cholangiocellular carcinomas. Expression of Cul3 is not regulated upon de-differentiation of Chhoilangiocekllular carcinomas (G2/G3).

Supplemental Figure 7:

7A) HepG2 cells were transfected with siRNA against Cul3 and the CD133 positive as well as CD133/BrdU positive fraction was determined by FACS analysis. Percentage of positive cells was calculated in comparison to cells treated with control siRNA. 7B)

Knock down of Cul3 was analyzed by RT-PCR. 7C) RT PCR for Cul3, Fbw7 and HNF4α. 18srRNA was used as loading control. 7D) Accumulation of γH2AX and p15 upon differentiation of HepG2 cells after knock down of Cul3. 7E) Analysis of CD133 and CD133/BrdU positive cells upon differentiation of HepG2 (p53 wt) cells by FACS analysis. Percentage of positive cells was calculated in comparison to cells treated with control siRNA. 7F) Analysis of CD133 and CD133/BrdU positive cells upon differentiation of HUH7 (p53 mutant) cells by FACS analysis. Percentage of positive cells was calculated in comparison to cells treated with control siRNA.