

Supplemental Materials

1. **Supplemental Figure 1**
2. **Supplemental Figure 2**
3. **Supplemental Figure 3**
4. **Supplemental Figure 4**
5. **Supplemental Figure 5**

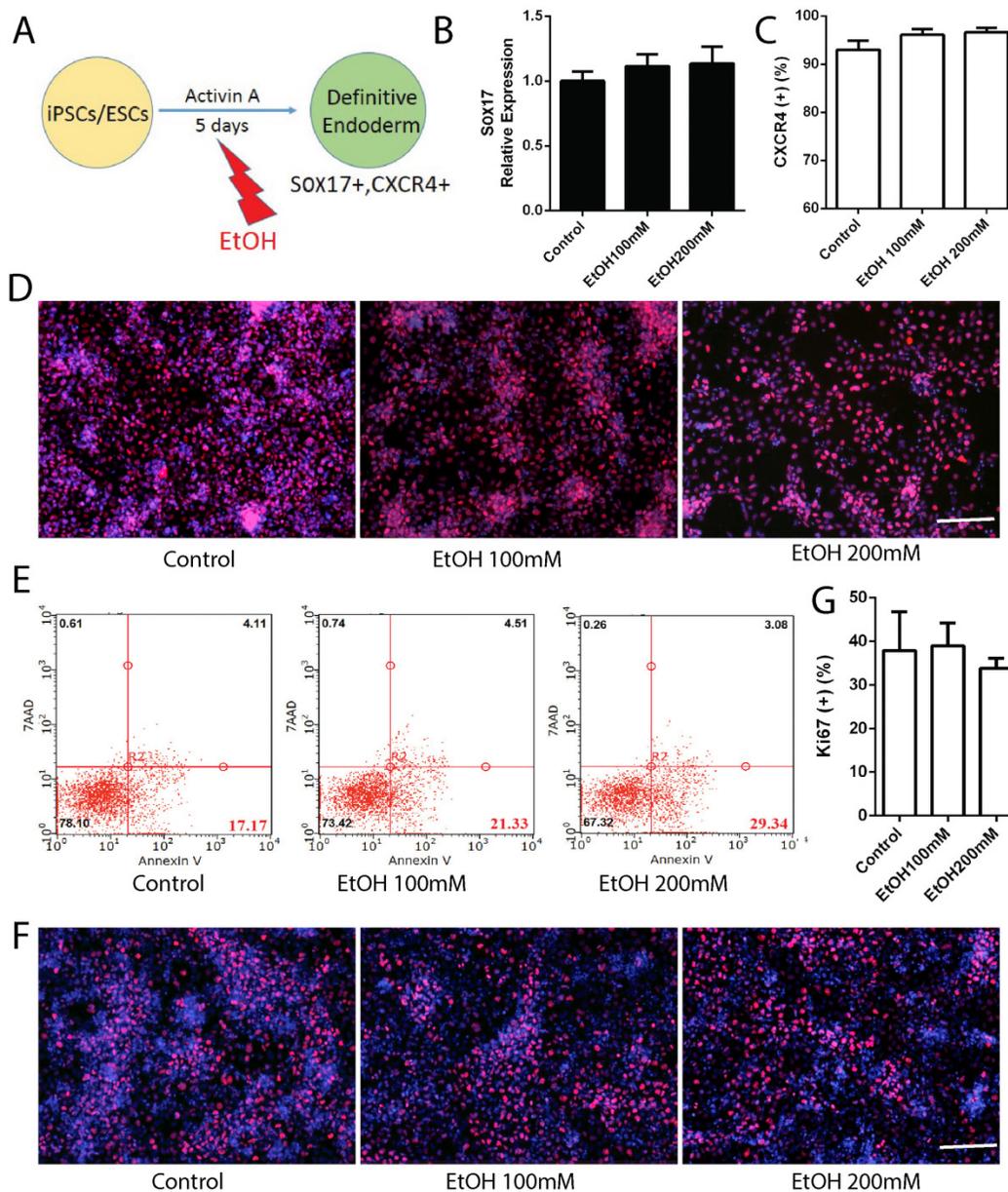


Figure S1. Effects of alcohol on differentiation of human iPSCs into endoderm cells. (A) Diagram of definitive endoderm (DE) differentiation and alcohol treatment. (B) SOX17 mRNA levels were examined by Real-time PCR at day 5, after 4 days alcohol treatment. (C) CXCR4 positive cells were detected by flow cytometric analysis at day 5, after 4 days alcohol treatment. (D) Immunostaining of SOX17 at day 5 in control and alcohol treated groups of iHep14 line. (E) Cells apoptosis was analyzed by flow cytometry with Annexin V/7-AAD staining at day 5 after alcohol treatment. (F) Representative images of Ki67 staining of DE cells derived from human iPSCs line. (G) The DAPI and Ki67 positive cells were counted with ImageJ. The percent of Ki67 positive cells is expressed as the mean of three independent experiments \pm SEM. Scale Bar, 100 μ m.

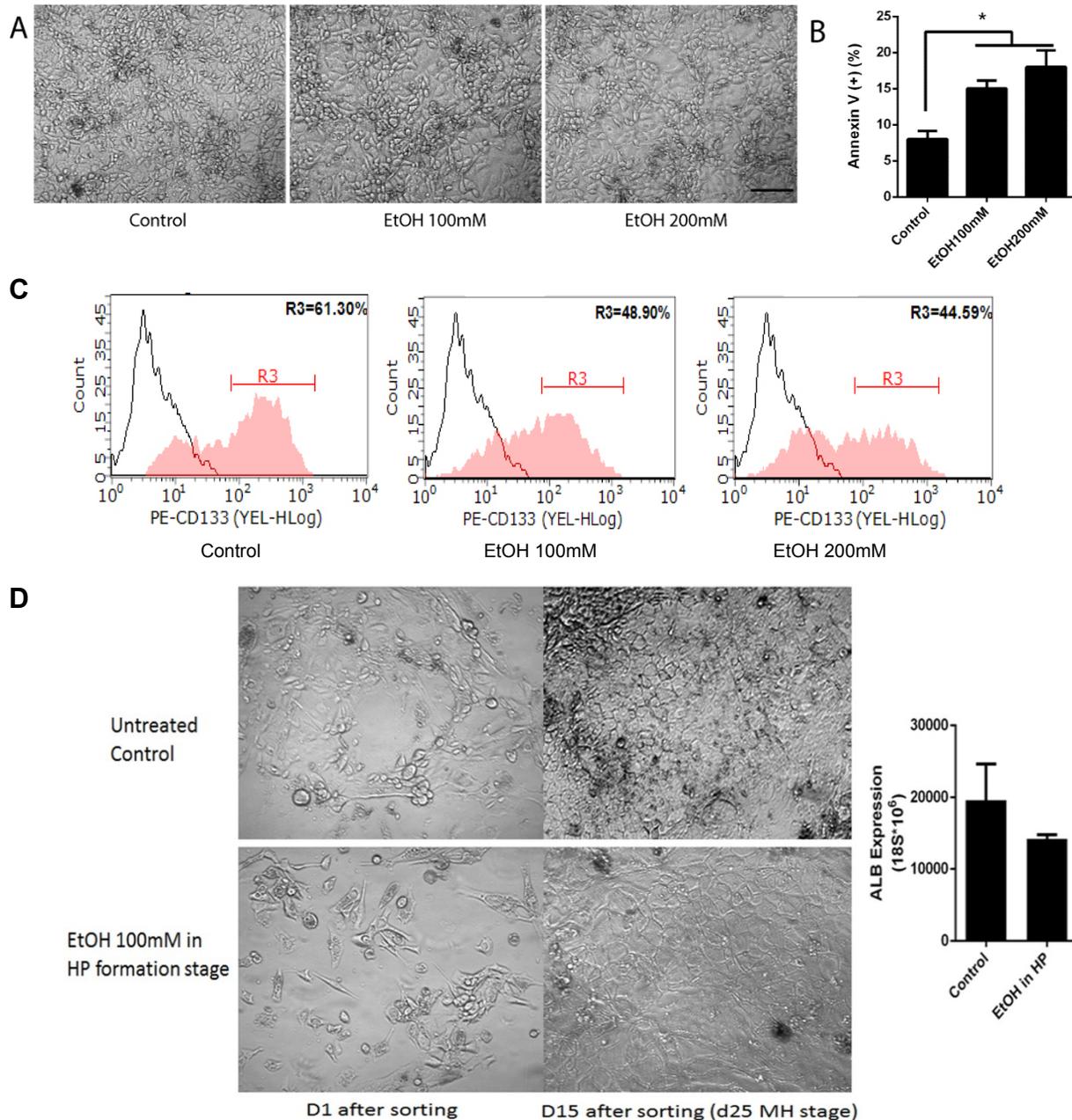


Figure S2. Effects of alcohol on differentiation of DE cells into hepatic progenitor (HP) cells.

(A) Cell morphology at HP stage cells after alcohol treatment. (B) Percent of Annexin V positive cells and (C) Percentage of CD133 positive cells by flow cytometric analysis at day 10 after alcohol treatment. *: $p < 0.05$ Scale Bar, 100 μ m. (D) To determine whether the alcohol treated HP cells maintain their progenitor potential, we purified CD133+ HP cells from alcohol exposed culture plates at day 10 by FACS and cultured them under normal hepatic differentiation condition. Of note, cell sorting cannot be performed by another HP marker AFP because it is an intracellular protein. Compared to the untreated control (upper right panel), alcohol treated HP cells differentiated into less mature hepatocyte-like cells (lower right panel- flat morphology, no/few intracellular granules/globules) with lower albumin expression level at MH stage.

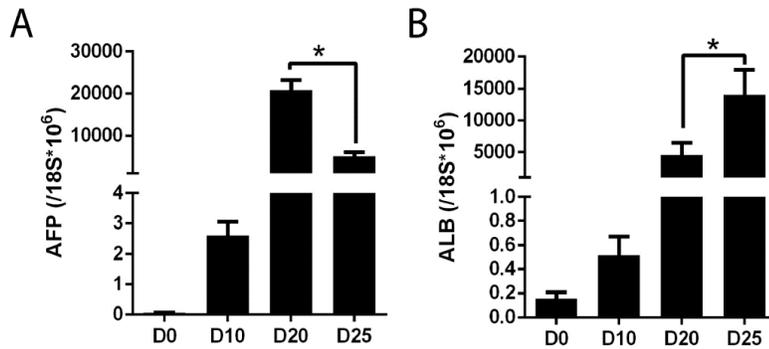


Figure S3. Expression of AFP and ALB during mature stage hepatocyte differentiation from day 0 to day 25. Compared to day 20 early hepatocytes, day 25 mature hepatocytes derived from human iPSCs have 5-10 fold lower AFP (A) and 3-5 fold higher ALB (B) expression. *: $p < 0.05$

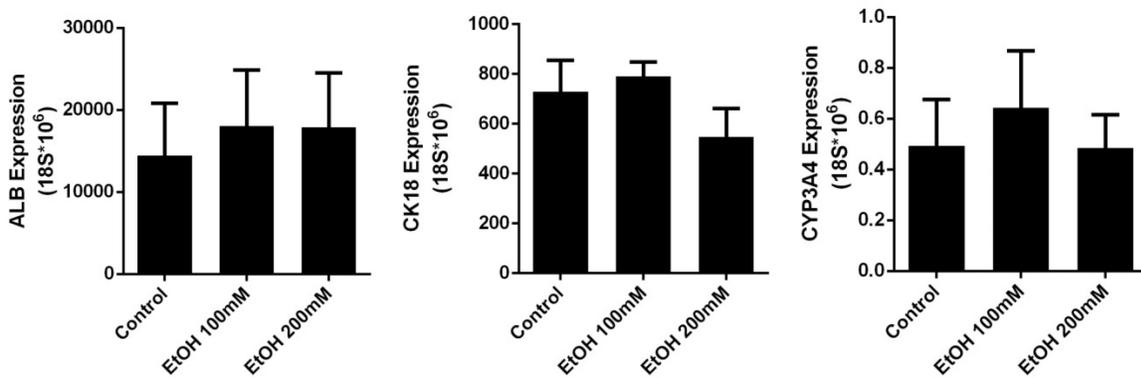


Figure S4. Expression of mature hepatocyte (MH) markers in alcohol treated human iPSC-derived mature hepatocyte-like cells. ALB, CK18 and CYP3A4 expression were examined by Real time PCR at day 25 after alcohol treatment. These MH markers were not significantly altered by ethanol treatment.

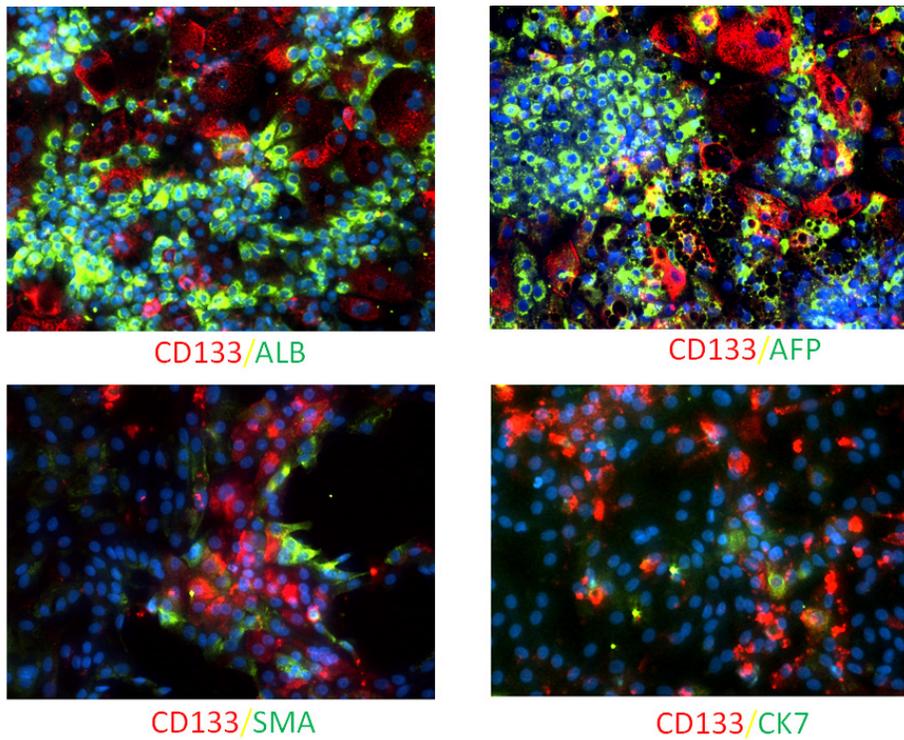


Figure S5. CD133 co-staining with other cell markers in MH stage cells derived from human iPSCs. A majority of CD133 (red) positive cells did not co-stain with either hepatocytic (ALB, AFP) or non-hepatocytic (SMA, or CK7) liver cell markers (all green). In this system there are no/few Kupffer cells (data not shown).