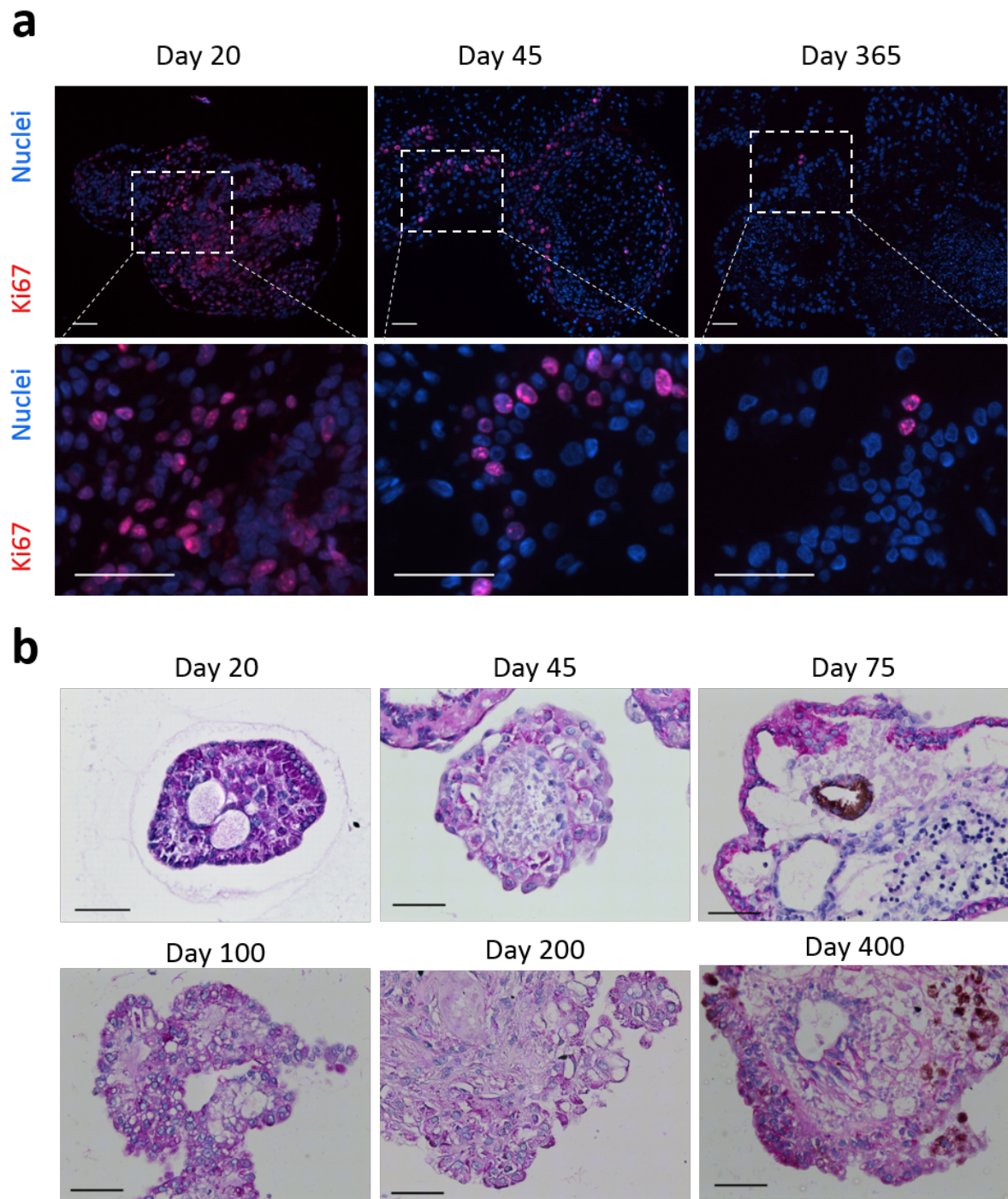
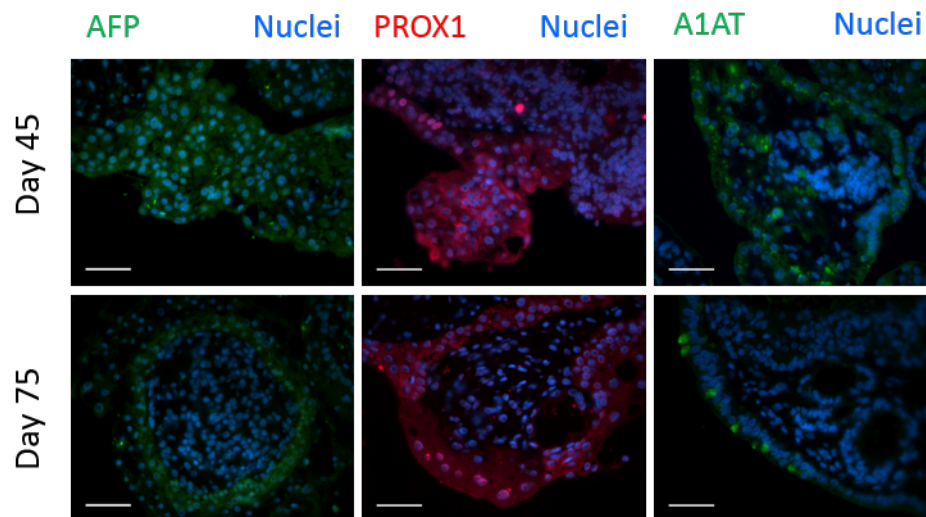


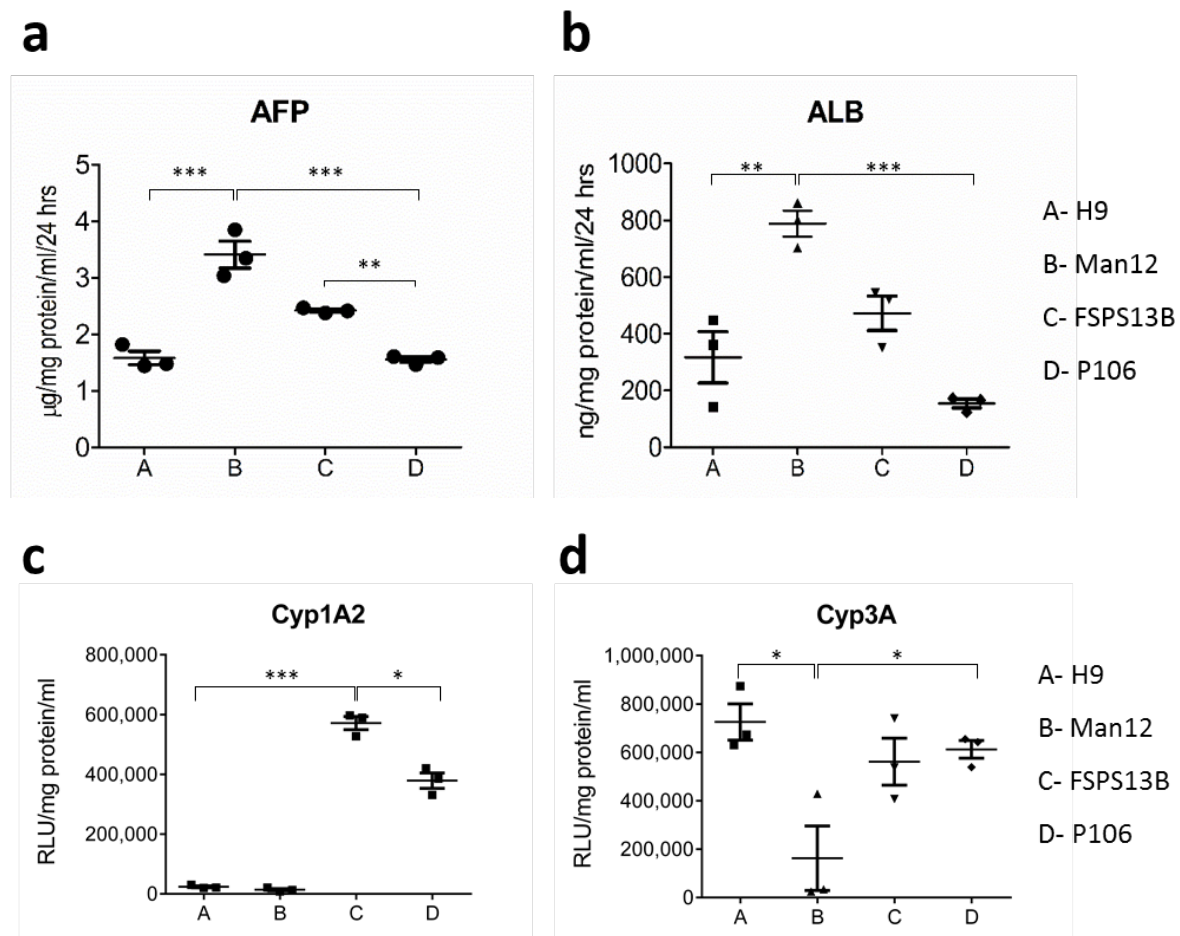
Supplementary figure 1: Generation of 3D Heps. Self-aggregation in suspension culture resulted in spheroids heterogeneous in size while the initial size of spheroids can be controlled by alteration of seeding density in agarose microplate platform.



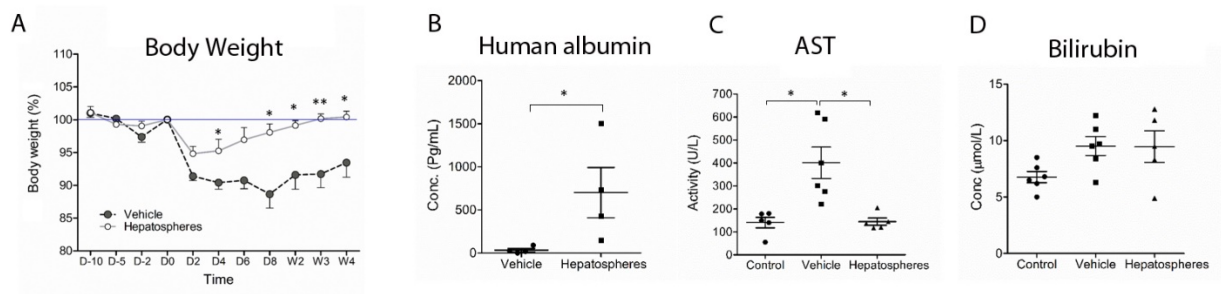
Supplementary figure 2: 3D HepS characterisation. (a) The number of proliferative cells reduced by elongation of differentiation. **(b)** Glycogen storage in 3D HepS.



Supplementary figure 3: Expression of hepatic markers. (a) AFP expression at d45 and d75. (b) Expression of PROX1 at d45 and d75. (b) Expression of A1AT at d45 and d75. Scale bar 50 μ m.



Supplementary Figure 4: Functional analysis of 3D Heps generated from two ESC lines (H9 & Man12) and two integration-free iPSC lines (FSPS13B & P106). (a) Secretion of AFP and (b) ALB by PSC derived 3D Heps in 24 hrs. (c) Cyp1A2 and (d) Cyp3A activity detected in PSC derived 3D Heps. n= 3 per group, significance was determined One-way ANOVA Tukey post hoc test. Data are represented as mean \pm SEM, * = $p < 0.05$, ** = $p < 0.01$, * = $p < 0.001$.**



Supplementary Figure 5: Stem cell derived hepatocytes provide liver support following 50% partial hepatectomy in immunocompromised mice.

(a) Percentage of body weight change 4 weeks after 50% PHx. Mice weights were measured 10 days before cell transplantation up to 4 weeks post transplantation. **(b)** Human albumin levels in mouse serum after 4 weeks of receiving vehicle or cells, assayed by MSD System. **(c)** Hepatocellular damage markers, aspartate transaminase activity in serum, and **(d)** Serum bilirubin levels after 4 week of 50% PHx. $n = 3-7$ per group, significance was determined One-way ANOVA Tukey post hoc or by Mann Whitney test, as suitable. Data are represented as mean \pm SEM, * = $p < 0.05$, ** = $p < 0.01$.