# Supplementary Information

# IL6 Supports Long-Term Expansion of Hepatocytes *in vitro*

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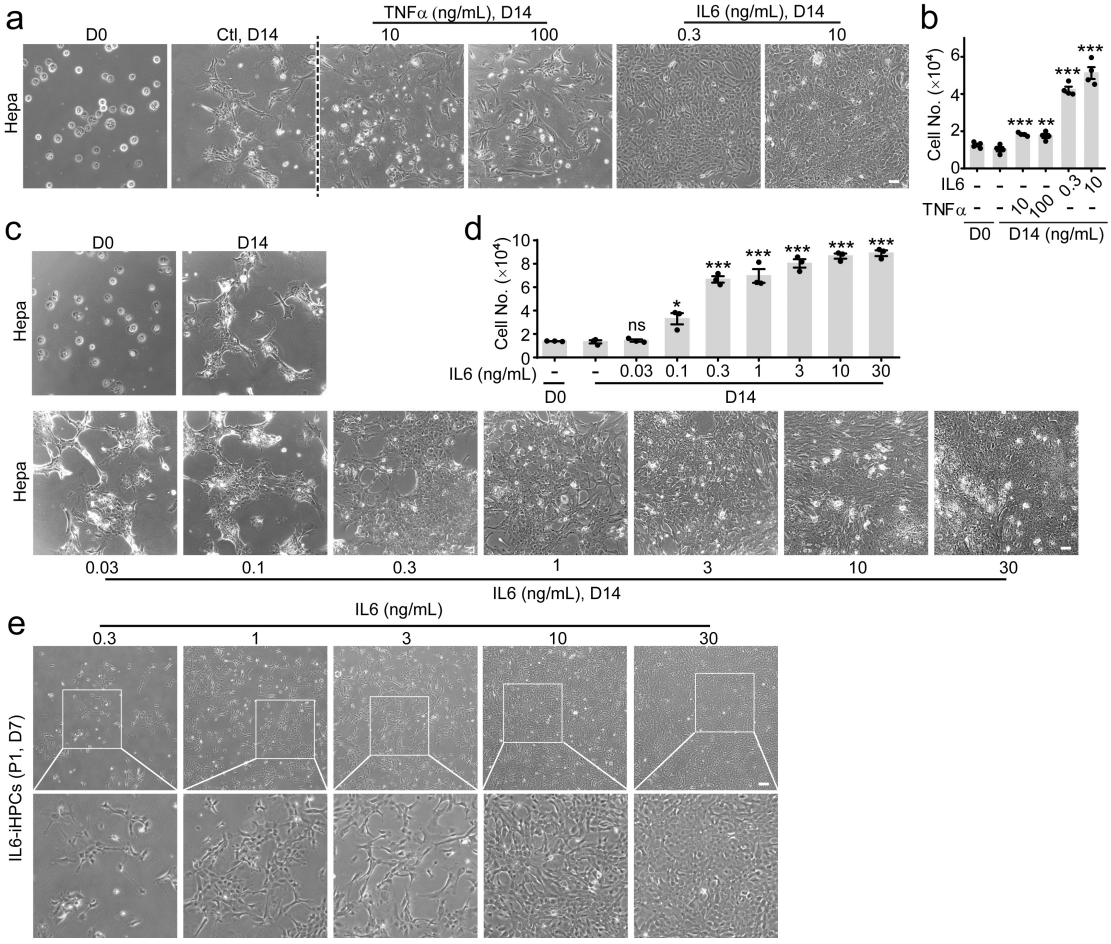
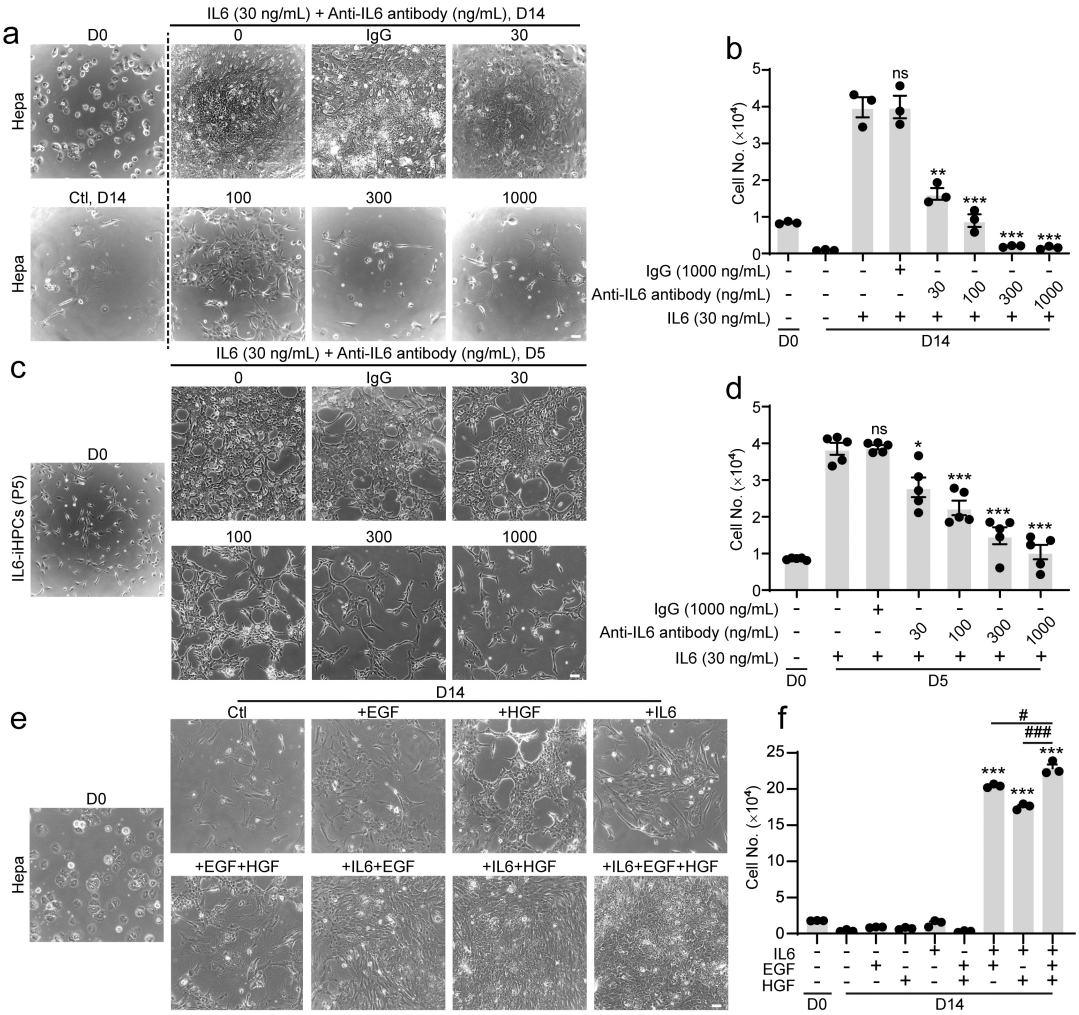
**Running title:** IL6 promotes hepatocyte growth *in vitro*

**Supplementary Fig. 1. Effects of TNFα and IL6 in hepatocyte proliferation**



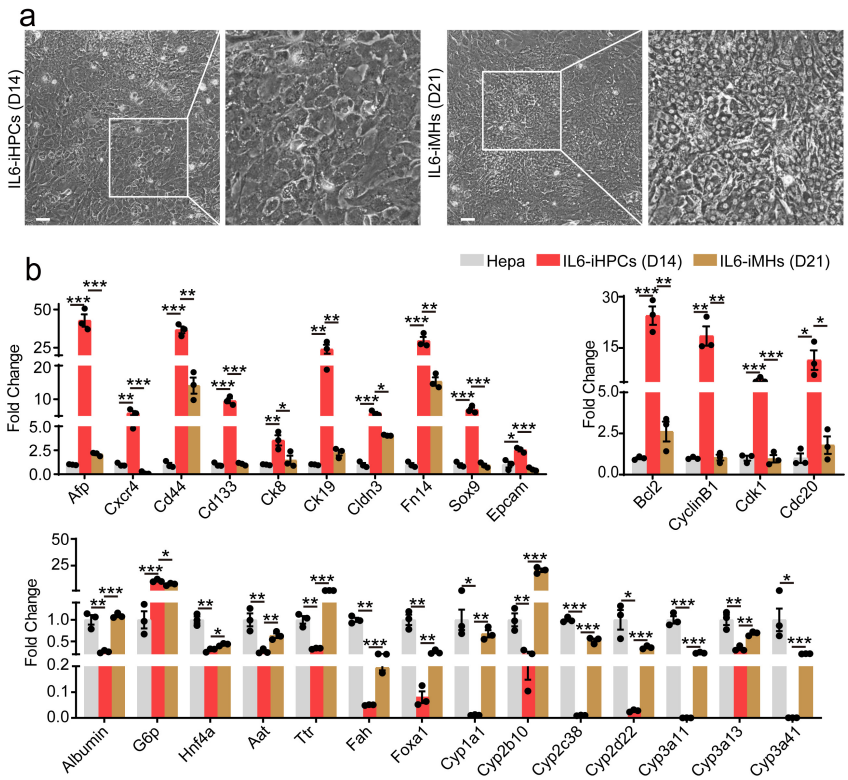

(**a**, **b**) Representative phase contrast images and cell numbers of primary hepatocytes (Hepa) cultured in media supplemented with TNFα (10, 100 ng/mL) or IL6 (0.3, 10 ng/mL) for 14 days (n=4 independent experiments). (**c**, **d**) Representative images (**c**) and cell numbers (**d**) of primary hepatocytes cultured in different concentration of IL6 for 14 days (n=3 independent experiments). (**e**) Representative images of IL6-iHPCs (P1) cultured in different concentration of IL6 for 7 days (repeated for 3 times). All data are shown as Means ± SEM. ns, not significant, *P < 0.05, **P< 0.01, ***P< 0.001 versus ctl group in D14 (two-tailed unpaired Student's t test). Source data and exact P values are provided in a Source data file. Scale bars represent 100 μm.

**Supplementary Fig. 2 IL6 promotes primary hepatocytes proliferation *in vitro***



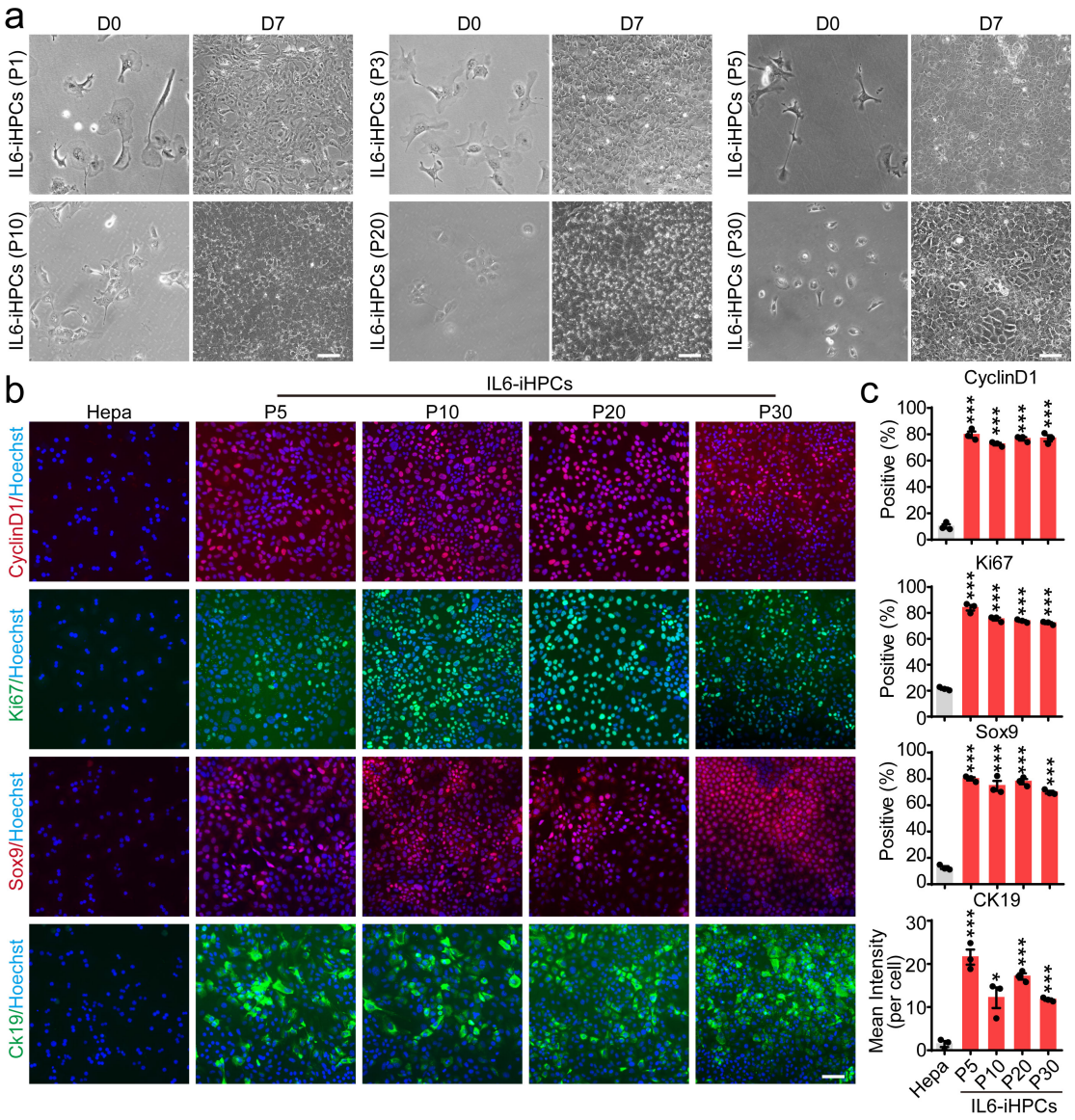

(**a**, **b**) Representative images (**a**) and cell numbers (**b**) of primary hepatocytes cultured in IL6 and various concentration of anti-IL6 antibody or control IgG for 14 days (n=3 independent experiments). ns, not significant, **P < 0.01, ***P < 0.001 versus IL6 (30 ng/mL, D14) alone. (**c**, **d**) Representative images (**c**) and cell numbers (**d**) of IL6-iHPCs (P5) cultured in IL6 and various concentration of anti-IL6 antibody or control IgG for 5 days (n=5 independent experiments). ns, not significant, *P < 0.05, ***P< 0.001 versus IL6 (30 ng/mL, D5) alone. (**e**, **f**) Representative images (**e**) and cell numbers (**f**) of primary hepatocytes cultured in EGF (20 ng/mL), HGF (20 ng/mL)，IL6 (30 ng/mL), or various combinations, for 14 days (n=3 independent experiments). ***P< 0.001 versus first bar in D14 group, $^{\#}$P< 0.05, $^{\#\#\#}$P< 0.001. All data are shown as Means ± SEM. P-values were calculated by two-tailed unpaired Student's t test. Source data and exact P values are provided in a Source data file. Scale bars represent 100 μm.

**Supplementary Fig. 3 IL6-iHPCs could be differentiated into mature hepatocytes**



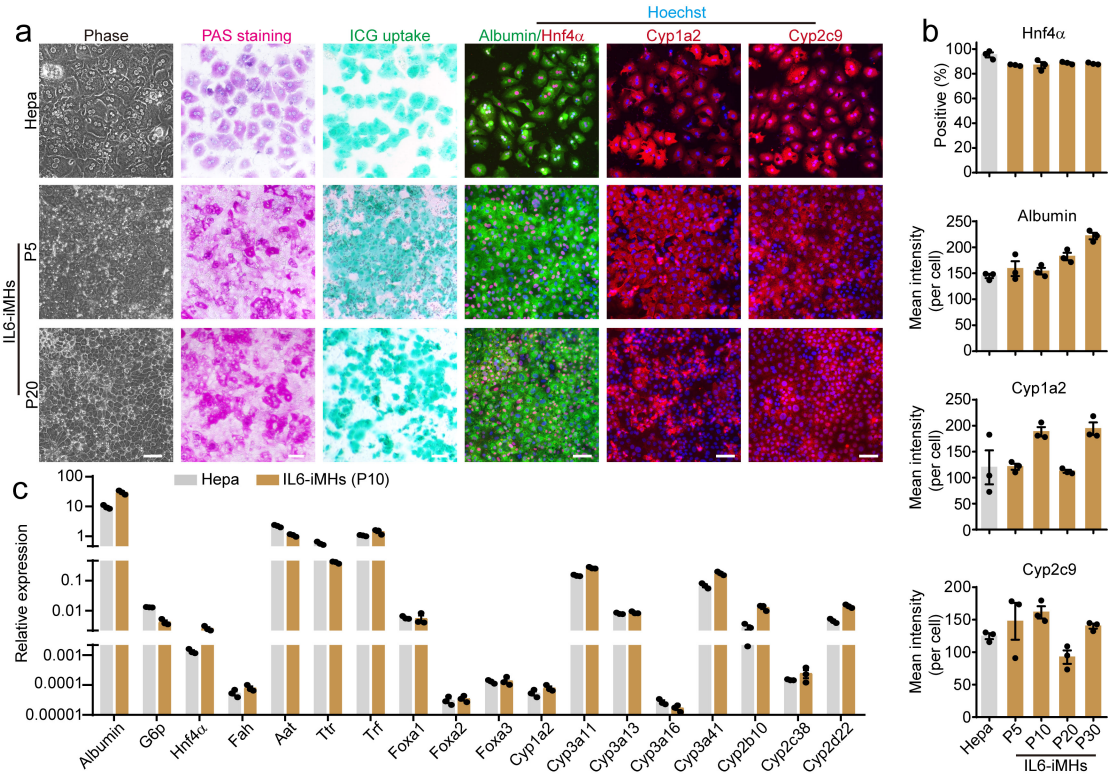

(**a**) Representative images of IL6-iHPCs (D14) and IL6-iMHs (D21). (**b**) Quantitative RT-PCR analysis of hepatic progenitor genes including *Afp, Cxcr4, Cd44, Cd133, Ck8, Ck19, Cldn3, Fn14, Sox9* and *Epcam,* cell cycle genes including *Bcl2, CyclinB1, Cdk1,* and *Cdc20,* and hepatic genes including *Albumin, G6p, Hnf4a, Aat, Ttr, Fah, Foxa1, Cyp1a1, Cyp2b10, Cyp2c38, Cyp2d22, Cyp3a11, Cyp3a13, and Cyp3a41* in primary hepatocytes (Hepa), IL6-iHPCs (D14) and IL6-iMHs (D21) (n=3 independent experiments). All data are shown as Means ± SEM, *P < 0.05, **P< 0.01, ***P< 0.001 (two-tailed unpaired Student's t test). Source data and exact P values are provided in a Source data file. Scale bars represent 100 μm.

**Supplementary Fig. 4 Characterization of long-term expanded IL6-iHPCs**



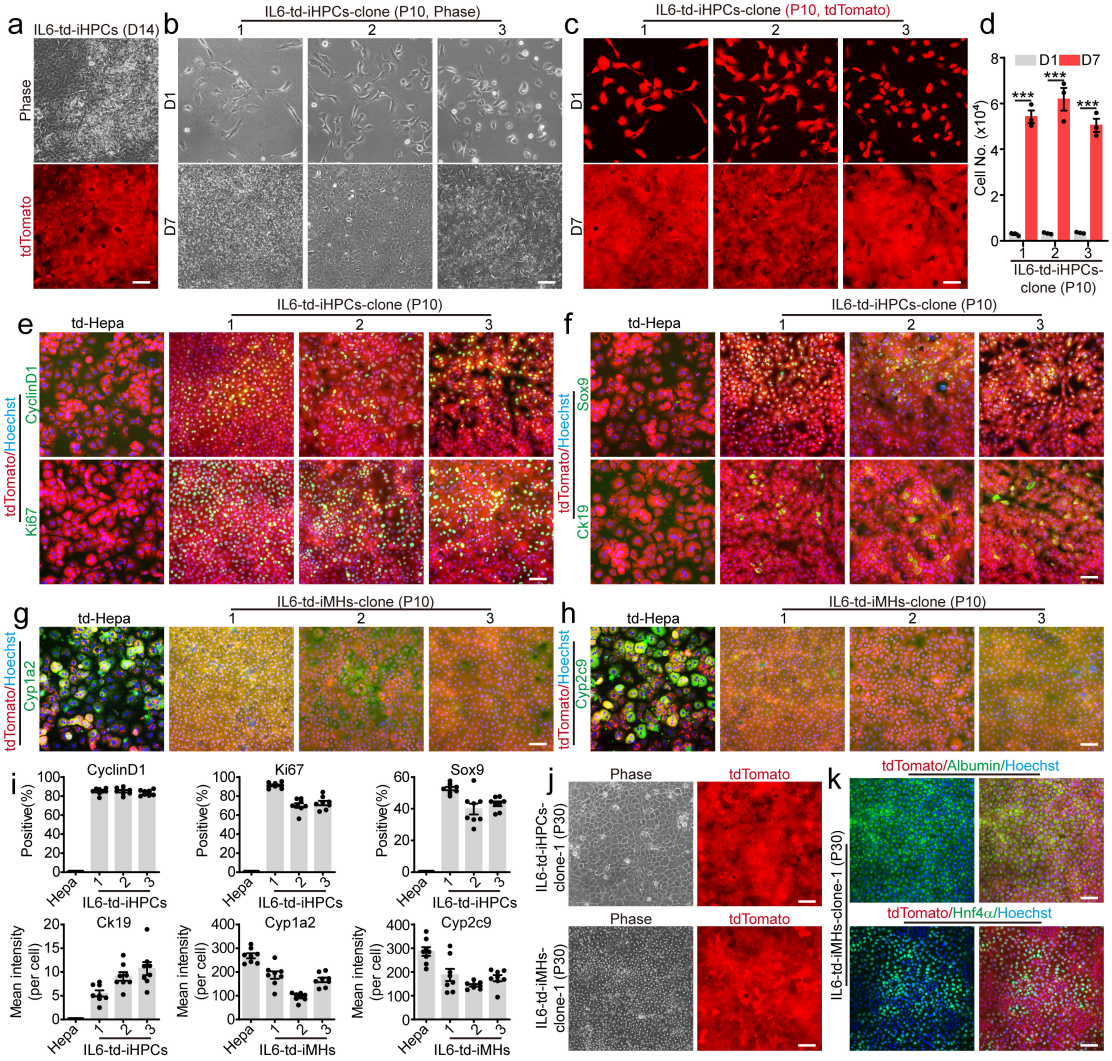

(**a**) Representative images showing the proliferation ability of IL6-iHPCs passaged for 1, 3, 5, 10, 20 and 30 times (repeated for 3 times). For readers' convenience, the images of IL6-iHPCs at P10 and P30 are also presented in Fig. 2b. (**b**, **c**) Immunofluorescence staining (**b**) and statistical data (**c**) of cell cycle markers CyclinD1 (red) and Ki67 (green) and hepatic progenitor cell markers Sox9 (red) and Ck19 (green) in IL6-iHPCs at different passage (n=3 independent experiments). Nuclei were stained with Hoechst 33342 (blue). All data are shown as Means ± SEM, *P < 0.05, ***P< 0.001 versus primary hepatocytes (two-tailed unpaired Student's t test). Source data and exact P values are provided in a Source data file. Scale bars represent 100 μm.

**Supplementary Fig. 5 Characterization of IL6-iMHs differentiated from IL6-iHPCs**




(**a**) Representative phase contrast images, PAS staining, ICG uptake and immunofluorescence staining of hepatic markers Albumin (green), Hnf4α (red), Cyp1a2 (red), and Cyp2c9 (red) in IL6-iMHs (P5 and P20). Primary hepatocytes (Hepa) were used as control. Nuclei were stained with Hoechst 33342 (blue). (**b**) Statistical data of the immunofluorescence staining data in (**a**) and Figure 2D (n=3 independent experiments). (**c**) Quantitative RT-PCR analysis hepatic genes including *Albumin, G6p, Hnf4α, Fah, Aat, Ttr, Trf, Foxa1, Foxa2, Foxa3, Cyp1a2, Cyp3a11, Cyp3a13, Cyp3a16, Cyp3a41, Cyp2b10, Cyp2c38,* and *Cyp2d22* in IL6-iMHs (P10) and Hepa (n=3 independent experiments). All data are shown as Means ± SEM. Source data are provided in a Source data file. Scale bars represent 100 μm.

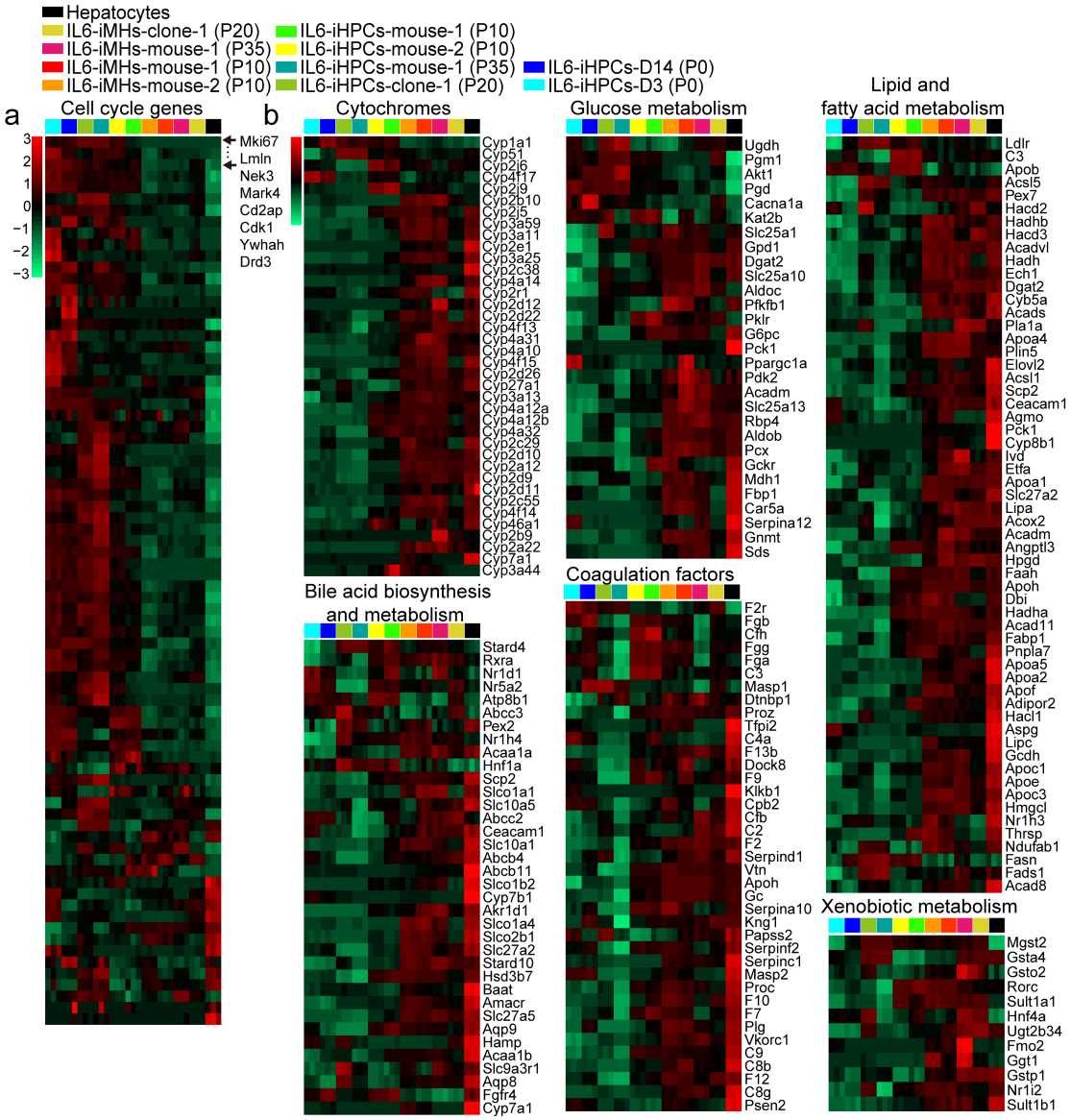
**Supplementary Fig. 6 Characterization of IL6-td-iHPC clones generated from single cells**




(**a**) Representative morphology and fluorescence images of td-Hepa (red) cultured in IL6-HCM for 14 days. (**b-d**) Representative morphology (**b**), fluorescence images (**c**) and cell numbers (**d**) of 3 single hepatocyte-generated IL6-td-iHPC clones (P10) cultured in IL6-HCM for 7 days (n=3 independent experiments). ***P< 0.001 (two-tailed unpaired Student's t test). (**e**, **f**) Immunofluorescence staining of cell cycle markers CyclinD1 (green) and Ki67 (green) (**e**) and hepatic progenitor cell marker Sox9 (green) and Ck19 (green) (**f**) in IL6-td-iHPC clones (P10). (**g**, **h**) Immunofluorescence staining of hepatic markers Cyp1a2 (green) (**g**) and Cyp2c9 (green) (**h**) in IL6-td-iMH clones (P10). Nuclei were stained with Hoechst 33342 (blue). (**i**) The statistical analysis of **e-h** (eight random fields each group, 3 independently generated IL6-td-iMH clones

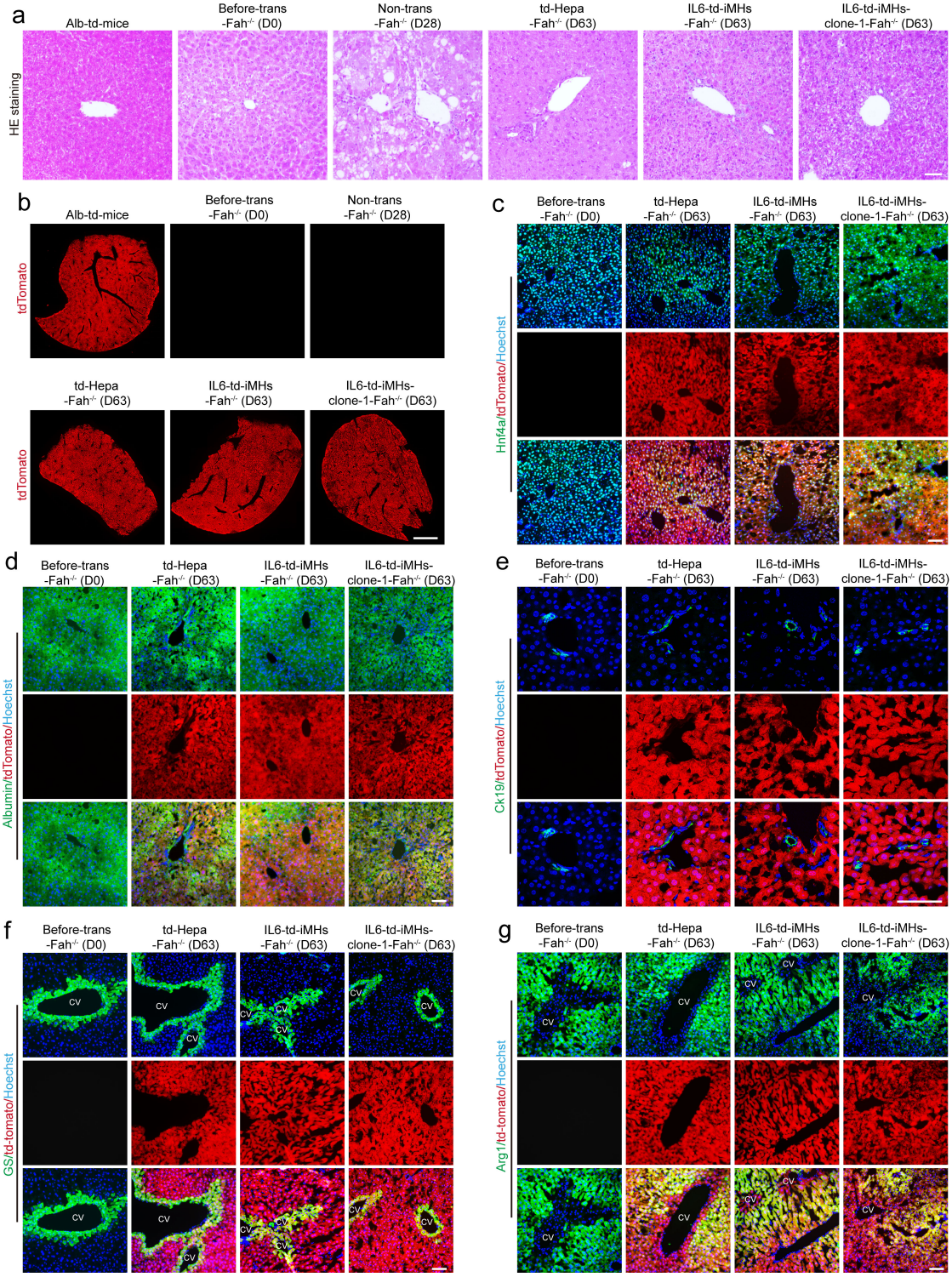
were analyzed). (**j**) Representative morphology and fluorescence images (red) of IL6-td-iHPC-clone-1 and IL6-td-iMHs-clone-1 (P30). (**k**) Immunofluorescence staining of hepatic markers Albumin (green) and Hnf4α (green) in IL6-td-iMHs-clone-1 (P30). Nuclei were stained with Hoechst 33342 (blue). All data are shown as Means ± SEM. All images are representative images from at least 3 independent experiments. Source data and exact P values are provided in a Source data file. Scale bars represent 100 μm.

**Supplementary Fig. 7 Transcriptome analysis of IL6-iHPCs and IL6-iMHs**




(**a**, **b**) Heatmaps for the expression of cell cycle genes (**a**) and representative hepatic function-related genes: Cytochromes, Bile acid biosynthesis and metabolism, Glucose metabolism, Coagulation factors, Lipid and fatty acid metabolism and Xenobiotic metabolism (**b**) in primary hepatocytes, IL6-iHPCs-D3 (P0), IL6-iHPCs-D14 (P0), IL6-iHPCs from different mouse and different passages and the IL6-iMHs differentated from them. Gene expression is presented in log 2 scale as defined by the corresponding color bars in the left. Red and green represent higher and lower gene expression levels respectively. Three technical replicates for all groups.

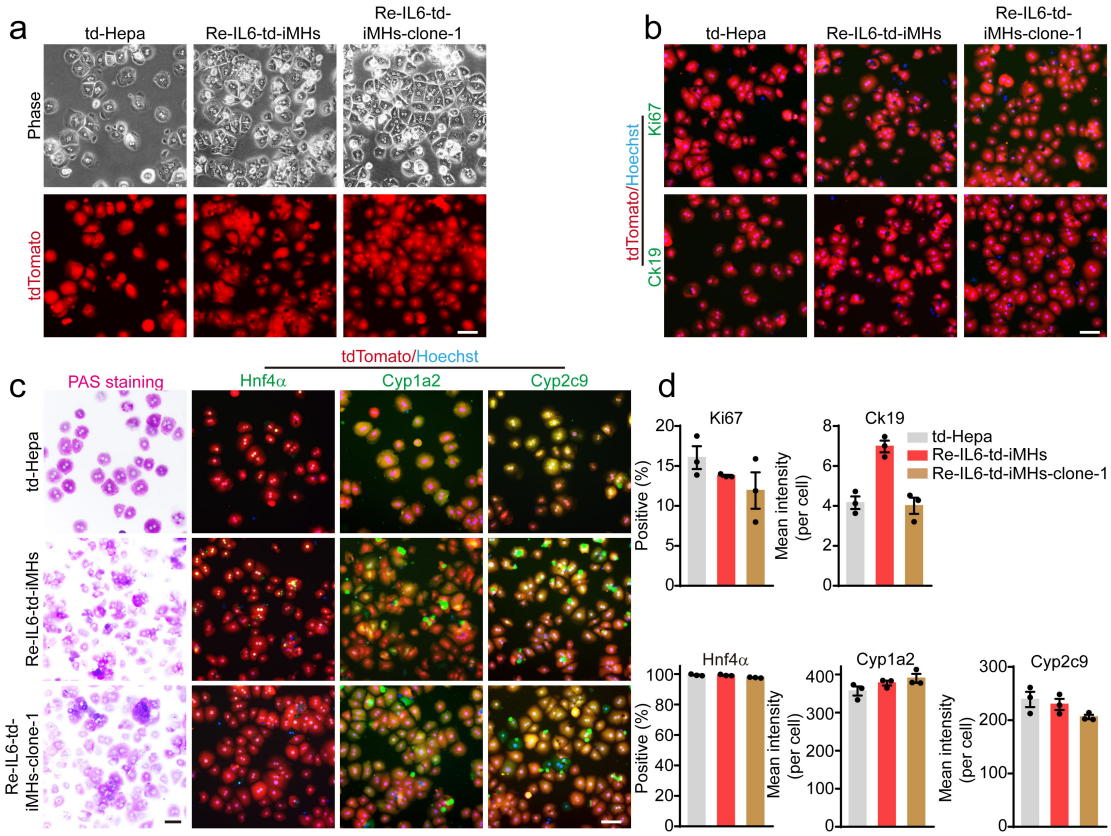
**Supplementary Fig. 8 Repopulation of *Fah*$^{-/-}$ liver by IL6-iMHs**




(**a**) Representative images of H&E staining in liver sections from *Fah*$^{-/-}$ mice before NTBC withdrawal (D0, n=2 mice), 28 days after NTBC withdrawal without transplantation (n=3 mice), or 63 days after NTBC withdrawal but receiving td-Hepa (n=3 mice), IL6-td-iMHs (n=6 mice), or IL6-td-iMHs-clone-1 (n=7 mice). (**b**) Frozen section of the left lateral lobe of the liver from *Fah*$^{-/-}$ mice before NTBC withdrawal

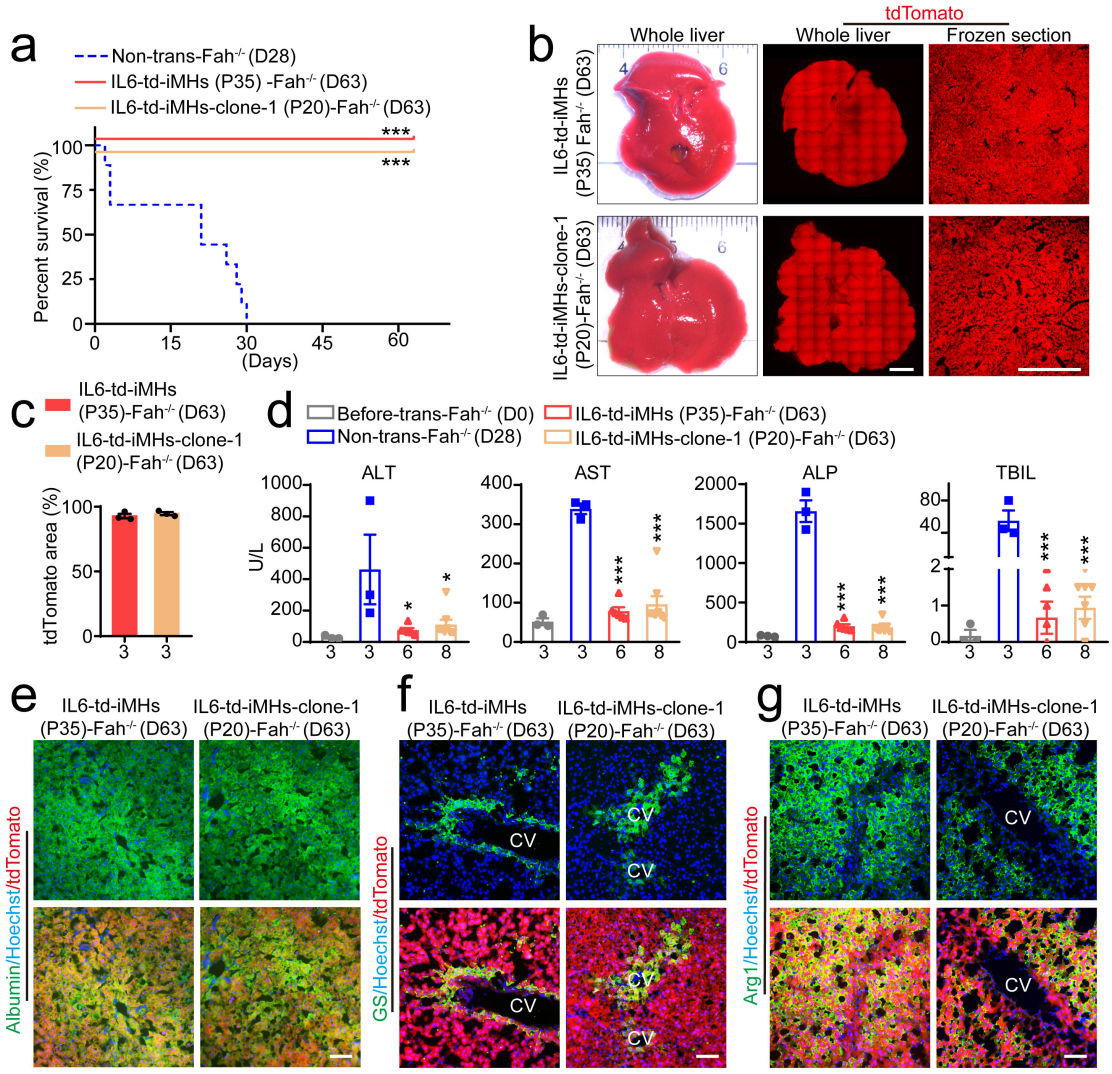
(D0), 28 days after NTBC withdrawal without transplantation, or 63 days after NTBC withdrawal but receiving td-Hepa, IL6-td-iMHs, or IL6-td-iMHs-clone-1. The liver of Alb-td-mice was used as control. Scale bars represent 2 mm. (**c-g**) Immunofluorescence staining of hepatic markers Hnf4α (green), Albumin (green), GS (green), Arg1 (green), and cholangiocytes maker Ck19 (green) in frozen liver sections of $Fah^{-/-}$ mice before or 63 days after transplantation. Nuclei were stained with Hoechst 33342 (blue). Scale bars represent 100 μm.

**Supplementary Fig. 9 Characterization of hepatocytes isolated from the *Fah*$^{-/-}$ mice transplanted with IL6-td-iMHs for 2 months**




(**a**) Representative morphology and fluorescence images of hepatocytes isolated from *Fah*$^{-/-}$ mice receiving IL6-td-iMHs and IL6-td-iMHs-clone-1 for 63 days after NTBC withdrawal (n=3 mice in each group). Td-Hepa isolated from Alb-td mice were used as control. (**b**) Immunofluorescence staining of cell cycle marker Ki67 (green) and hepatic progenitor cell marker Ck19 (green) in (**a**). (**c**) Representative images of PAS staining and immunofluorescence staining of hepatic markers Hnf4α (green), Cyp1a2 (green) and Cyp2c9 (green) in cells in (**a**). Nuclei were stained with Hoechst 33342 (blue). (**d**) Statistical data of the intensity of Ck19, Cyp1a2 and Cyp2c9, and percent of Ki67 and Hnf4α staining in (**b** and **c**) (n=3 independent experiments). All data are shown as Means ± SEM. Source data are provided in a Source data file. Scale bars represent 100 μm.

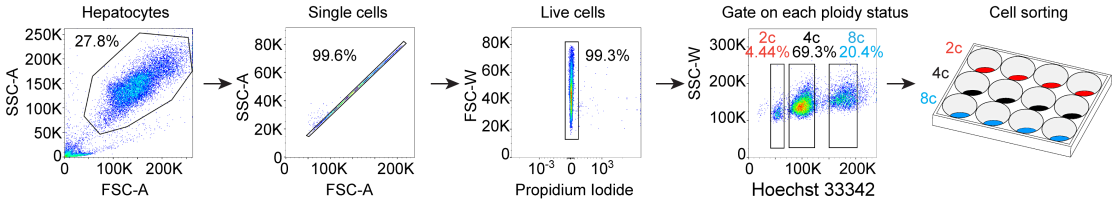
**Supplementary Fig. 10 Repopulation of Fah$^{-/-}$ liver by long-term cultured IL6-iMHs**




(**a**) Survival curves of *Fah*$^{-/-}$ mice (no NTBC) receiving IL6-td-iMHs (P35, n=6) and IL6-td-iMHs-clone-1 (P20, n=8) or vehicle (Non-trans, n=9, cited from Fig. 4c), ***P< 0.001 (Log-rank test). (**b**) Images of the whole livers (left), fluorescent images of the whole livers (middle, scale bars represent 5 mm) and frozen sections of liver (right, scale bars represent 1 mm) 63 days after IL6-td-iMHs (P35, n=2 mice), or IL6-td-iMHs-clone-1 (P20, n=2 mice) transplantation (no NTBC). (**c**) Quantitative analysis of tdTomato positive areas in liver (n=3 mice per group). (**d**) Serum levels of ALT, AST, ALP and TBIL in the *Fah*$^{-/-}$ mice after transplantation (n=3~8 mice per group, the numbers of the animals were listed under the bars). Data are shown as Means ± SEM. *P < 0.05, ***P< 0.001, vs non-trans (two-tailed unpaired Student's t test). Exact P

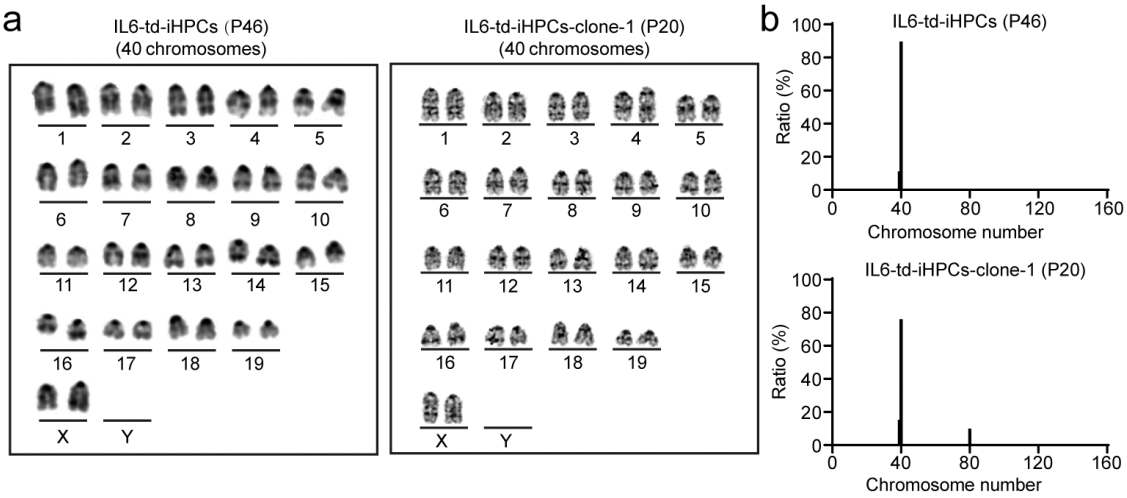
values are provided in a Source data file. The data of *Fah*$^{-/-}$ mice before NTBC withdrawal (D0), 28 days after vehicle transplantation (no NTBC) were cited from Fig. 4f. (**e-g**) Immunofluorescence staining of Albumin (green), GS (green) and Arg1 (green) in frozen liver sections of *Fah*$^{-/-}$ mice 63 days after transplantation (n=3 mice). Nuclei were stained with Hoechst 33342 (blue). Source data and exact P values are provided in a Source data file. Scale bars represent 100 μm.

**Supplementary Fig. 11 Sorting of hepatocytes with different ploidy**



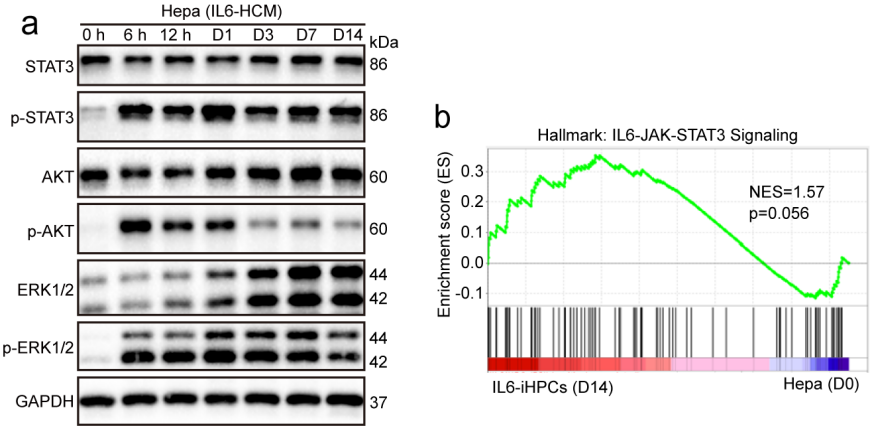

Schematic of the experimental strategy for the FACS sorting of diploid (2c), tetraploid (4c) and octaploid (8c) hepatocytes.

**Supplementary Fig. 12 Karyotypes analysis of IL6-iHPCs**



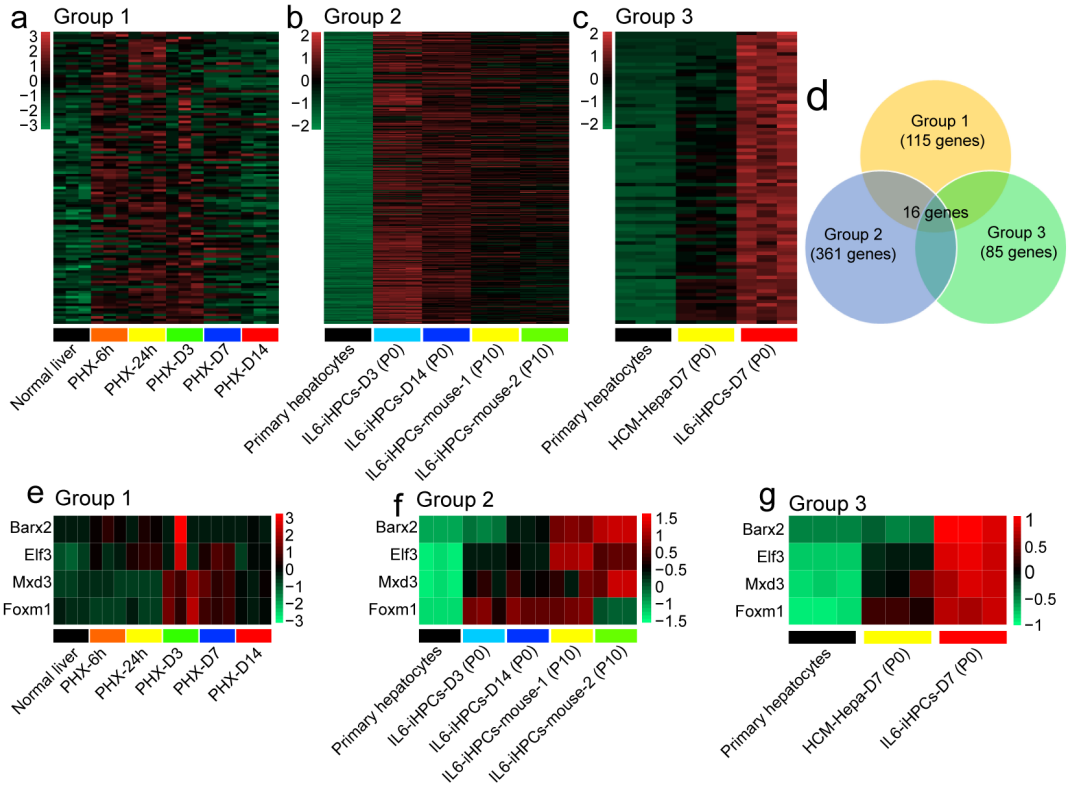

(**a**, **b**) Representative karyotype images (**a**) and statistical analysis of chromosome numbers (**b**) of IL6-td-iHPCs (P46) (n=120 cells) and IL6-td-iHPCs-clone-1 (P20) (n=115 cells).

**Supplementary Fig. 13 Involvement of Stat3 signaling in IL6 mediated hepatocyte growth**




(**a**) Western blotting analysis of STAT3, p-STAT3, AKT, p-AKT, ERK1/2 and p-ERK1/2 in hepatocytes cultured in IL6-HCM for 6 hours to 14 days. GAPDH was used as loading control. Representative blot from 3 independent experiments is shown. (**b**) Gene Set Enrichment Analysis (GSEA) was performed to assess the difference of IL6-iHPCs (D14) and primary hepatocytes using IL6-JAK-STAT3 signature. Normalized enrichment score (NES) reflects the degree of over-representation for each group at the peak of the entire set. P value is calculated using GSEA empirical phenotype-based permutation test, two-sided, and no adjustments were made for multiple comparisons.

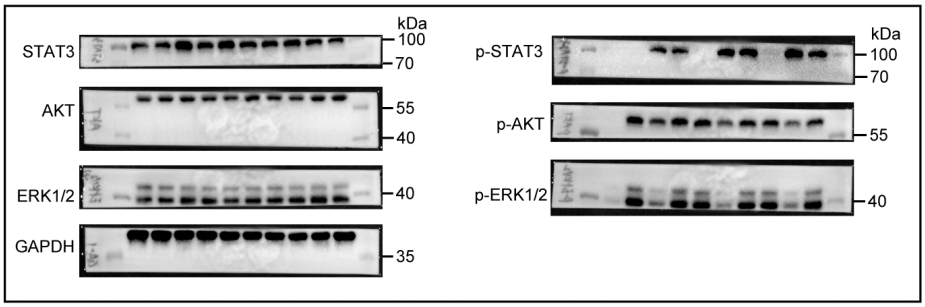
**Supplementary Fig. 14 Screening of TFs involved in IL6-mediated hepatocyte to iHPC reprogramming**



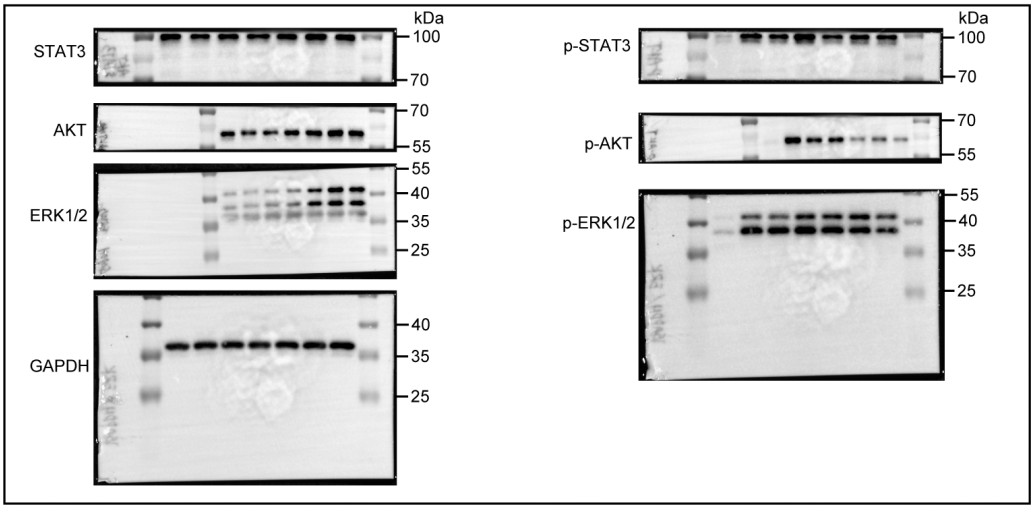

(**a**) Transcriptomic analysis of normal liver and livers post 2/3 partial hepatectomy (PHX) at different time points. Gene expression was compared between PHX-6 h and normal, PHX-24 h and normal, or PHX-D3 and normal. Genes with ⩾2-fold changes ($p < 0.05$) from any of the above comparisons were selected (Group 1, 115 genes). (**b**) Transcriptomic analysis of primary hepatocytes, IL6-iHPCs-D3 (P0), IL6-iHPCs-D14 (P0), IL6-iHPCs-mouse-1 (P10) and IL6-iHPCs-mouse-2 (P10). Gene expression was compared between IL6-iHPCs-D14 (P0) and primary hepatocytes, IL6-iHPCs-mouse-1 (P10) and primary hepatocytes, or IL6-iHPCs-mouse-2 (P10) and primary hepatocytes. Genes with ⩾2-fold changes ($p < 0.05$) in all of the above comparisons were selected (Group 2, 361 genes). (**c**) Gene expression was compared between IL6-iHPCs-D7 (P0) and HCM-Hepa-D7 (P0), IL6-iHPCs-D7 (P0) and primary hepatocytes. Genes with ⩾1.5-fold changes ($p < 0.05$) in all of the above comparisons were selected (Group 3, 85 genes). (**d**) Venn diagram for the selection of TFs. 16 TFs were identified. (**e-g**) Representative genes (*Barx2, Elf3, Mxd3 and FoxM1*) are indicated in group 1, 2, 3. Each element represents the log 2 (normalized expression), as scaled by the corresponding color legends. Three technical replicates for all groups.

**Supplementary Fig. 15 Uncropped scans of the immunoblots in Fig. 6a and Supplementary Fig. 11a**

Fig. 6a




Supplementary. Fig. 11a



**Supplementary Table 1：Reported methods of primary hepatocytes expansion**

| Cell type | Culture format | Expansion medium/Time | Expansion chemicals/cytokines | Maturation medium/Time | Passages in vitro | Latest passage used to access differentiation and repopulation capacity | Support single cell-derived clones | Ref |
|---|---|---|---|---|---|---|---|---|
| Primary hepatocytes (Mice; Rat) | 2D | DMEM/F12, 2.4 g/L NaHCO3, L-glutamine, 5 mM HEPES, 30 mg/L L-proline, 0.05% BSA, 10 ng/mL EGF, ITS-X, $10^{-7}$ M Dex, 10 mM nicotinamide, 1 mM ascorbic acid-2, antibiotic/antimycotic solution;14-16 days | 10 μM Y27632, 0.5 μM A-83-01, 3 μM CHIR99021; | DMEM/F12, 2.4 g/L NaHCO3, L-glutamine, 5 mM HEPES, 30 mg/L L-proline, 0.05% BSA, 10 ng/mL EGF, ITS, $10^{-6}$ M Dex, 10 mM nicotinamide, 1 mM ascorbic acid-2, 10 μM Y27631, 0.5 μM A-83-01, 3 μM CHIR99021, 20 ng/mL OSM; 8 days | Rat: At least 26 passages, doubling time =14.7 ± 1.1 hr; Mice: At least 20 passages | Rat: differentiation capacity was measured at P ≥15. In vivo repopulation capacity was measured for clones with passages not specified. Mice: differentiation capacity was measured at P11-12. | Rat (Yes); Mice (Not described) | 1 |
| Primary hepatocytes (Mice) | 2D | DMEM/F12, N2 or ITS, 20 ng/mL EGF, 20 ng/mL HGF; 7 days | 10 μM Y27632, 3 μM CHIR99021, 1 μM S1P, 5 μM LPA, 1 μM A83-01; | DMEM/F12, N2 or ITS, 10 μM DAPT, 20 ng/mL OSM, 10 μM Dex, 10 μM SB431542; 12-21 days | At least 30 passages, doubling time=15-20 hr | Differentiation and in vivo repopulation capacity were measured at passages not specified. | Not described | 2 |
| Primary hepatocytes (Mice) | 2D | DMEM/F12, 1% FBS, ITS, $10^{-7}$ M Dex, 10 mM nicotinamide, 50 μM β-mercaptoethanol, | 4 μM A-83-01, 3 μM CHIR99021 | DMEM/F12, 1%FBS, ITS, $10^{-5}$ M Dex, 10 mM nicotinamide, 50 μM β-mercaptoethanol, 20 ng/mL EGF, 20 ng/mL HGF, 4 μM A-83-01, 3 | At least 23 passages, doubling time=35.42± | Differentiation was measured at P23. In vivo repopulation capacity was measured with single cell- | Yes | 3 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | 20 ng/mL EGF, 20 ng/mL HGF, 1% penicillin/streptomycin; 8 days | | μM CHIR99021, 20 ng/mL OSM, 1% penicillin/streptomycin; 8 days | 0.8 hr | derived clone with passage not specified. | | |
| Primary hepatocytes (Mice) | 3D | William's E medium, 1% Glutamax, 1% Non-Essential Amino Acids, 0. 2% normocin, 2% B27, 1% N2, 10 mM nicotinamide, 1.25 mM N-acetylcysteine, 25 ng/mL EGF, 50 ng/mL HGF, 1% penicillin/streptomycin; 14 days; | 10 μM Y27632, 1 μM A-83-01, 3 μM CHIR99021, 100 ng/mL TNFa, 50 ng/mL noggin | William's E medium, 1% Glutamax, 1% Non-Essential Amino Acids, 0. 2% normocin, 2% B27, 1% N2, 10 mM nicotinamide, 1.25 mM N-acetylcysteine, 25 ng/mL EGF, 50 ng/mL HGF, 1% penicillin/streptomycin, (condition 1: 10 μM Y27632, 1 μM A-83-01, 3 μM Dex; condition 2: 3 μM CHIR99021, 3 μM Dex); 3-5 days | About 14 passages, 7 months | Functional properties are retained in 7 months cultures (P14). In vivo repopulation capacity was measured with the cells cultured for 105 days. | Not described | 4 |
| Primary hepatocytes (Human) | 2D | DMEM/F12, 1% FBS, ITS, $10^{-7}$ M Dex, 10 mM nicotinamide, 20 ng/mL EGF, 20 ng/mL HGF; 15 days | 4 μM A-83-01, 3 μM CHIR99021 | DMEM/F12, 1%FBS, ITS, $10^{-7}$ M Dex, 10 mM nicotinamide, 20 ng/mL EGF, 20 ng/mL HGF, 20 ng/mL OSM; 8 days | 10 passages, doubling time=37-47 hr | Differentiation was measured at P10. In vivo repopulation was measured at P6 | Not described | 5 |
| Primary hepatocytes (Human) | 2D | Advanced DMEM/F12, N2, B27, 1 mM sodium pyruvate, 10 μg/mL ascorbic acid, 20 ng/mL EGF, 20 ng/mL HGF; 6-12 days | 10 μM Y27632, 3 μM CHIR99021, 1 μM A-83-01, 1 μM S1P, 5 μM LPA | Advanced DMEM/F12, N2, B27, 10 μM DAPT, 20 ng/mL OSM, 10 μM Dex 10 μM SB431542; 6 days | 10 passages, doubling time=24.7± 1.4 hr | Differentiation was measured at P10. In vivo repopulation was measured at P5. | Not described | 6 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Primary hepatocytes (Human) | 2D | Advanced DMEM/F12, N2, B27 (minus vitamin A), 1mM N-acetyl-cysteine, 10 mM nicotinamide, 50 ng/mL EGF, 25 ng/mL HGF, 1% FBS, 1% penicillin/streptomycin; 5 days | 10 nM Human [Leu15]-gastrin I, 2 ng/mL FGF10, 5 μM A83-01, 10 μM Y27632, 50 ng/mL Wnt3a protein | Advanced DMEM/F12, N2, B27 (minus vitamin A), 1mM N-acetyl-cysteine, 10 mM nicotinamide, 2 ng/mL FGF10, 50 ng/mL EGF, 25 ng/l HGF, 10 nM Human [Leu15]-gastrin I, 1% FBS, 5 μM A83-01, 10 μM Y27632, 50 ng/mL Wnt3a protein, 5 μM Forskolin, 1 μM Dex, 20 ng/mL OSM, 1% FBS, 1% penicillin/streptomycin; 10 days | (Hypoxia) 8 passages | Differentiation was measured at P8. Repopulation was measured at P6. | Not described | 7 |
| Primary hepatocytes (mice) | 3D | AdDMEM/F12, 15% RSPO1 conditioned medium, B27 (minus vitamin A), 50 ng/mL EGF, 1.25 mM N-acetylcysteine, 10 mM Nicotinamide, 25 ng/mL HGF; 14 days | 10 nM gastrin, 100 ng/mL FGF7, 100 ng/mL FGF10, 3 μM CHIR99021, 2 μM A83-01, 10 μM Y27632 | None | 7 passages estimated from Figure 3B | Not described. | Not described | 8 |
| Primary hepatocytes (Human fetal, adult) | 3D | AdDMEM/F12, 15% RSPO1 conditioned medium, B27 (minus vitamin A), 50 ng/mL EGF, 1.25 mM N-acetylcysteine, 10 mM Nicotinamide, 50 ng/mL HGF; 9-10 | 10 nM gastrin, 100 ng/mL FGF7, 100 ng/mL FGF10, 2 μM A83-01, 10 μM Y27632, 20 ng/mL TGFa | For fetal organoids: AdDMEM/F12, 15% RSPO1 conditioned medium,B27 (minus vitamin A), 50 ng/mL EGF, 1.25 mM N-acetylcysteine, 10 mM Nicotinamide, 50 ng/mL HGF, 10 nM gastrin, 100 ng/mL FGF7, 100 ng/mL FGF10, 2 μM A83-01, 10 | Fetal: 28 passages; adult: 2–2.5 months | Human fetal hepatocyte organoids: Differentiation was measured at P22. Repopulation was measured at P16. Human adult hepatocyte organoids: In vivo | Not described | 8 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | days | | μM Y27632, 20 ng/mL TGFa, 1 μM Dex, 10 ng/mL OSM; | | repopulation capacity was measured (passage was not specified) | | |
| Primary hepatocytes (mice) | 2D | DMEM/F12, ITS, $10^{-7}$ M Dex, 20 ng/mL EGF, 20 ng/mL HGF; 14 days | 30 ng/mL IL6 | DMEM/F12, ITS, $10^{-6}$ M Dex, 20 ng/mL OSM; 7days | At least 46 passages, doubling time=13-20 hr | Differentiation was measured at P5, P10, P20, P30 and P35. Repopulation was measured at P10, P20 and P35. | Yes | here |

## Supplementary Table 2：Primer sequences for quantitative RT-PCR Analysis

| Gene | Forward | Reverse |
|---|---|---|
| Afp | TGCAGAAACACATCGAGGAGAG | GCTTCACCAGGTTAATGAGAAGCT |
| Cxcr4 | TTGTCCACGCCACCAACAGTCA | TGAAACACCACCATCCACAGGC |
| Cd44 | CACCATTGCCTCAACTGTGC | TTGTGGGCTCCTGAGTCTGA |
| Cd133 | CTCCCATCAGTGGATAGAGAACT | ATACCCCCTTTTGACGAGGCT |
| Cldn3 | TCACGGCGCAGATCACCT | ACCAACGGGTTATAGAAATCCCT |
| Ck8 | AGATGAACCGCAACATCAACC | TCAATCTTCTTCACAACCACAGC |
| Ck19 | GTCCTACAGATTGACAATGC | CACGCTCTGGATCTGTGACAG |
| Fn14 | GTGTTGGGATTCGGCTTGGT | GTCCATGCACTTGTCGAGGTC |
| Sox9 | CACCCCGATTACAAGTACCAG | TGCTCAGTTCACCGATGTCCA |
| Epcam | CTGGGAGGAGGATAAAGC | AGAAGAATGGAACAGGGAC |
| Bcl2 | GTCGCTACCGTCGTGACTTC | CAGACATGCACCTACCCAGC |
| Cdk1 | AGAAGGTACTTACGGTGTGGT | GAGAGATTTCCCGAATTGCAGT |
| CyclinB1 | AAGGTGCCTGTGTGTGAACC | GTCAGCCCCATCATCTGCG |
| Cdc20 | TTCGTGTTCGAGAGCGATTTG | CAGACATGCACCTACCCAGC |
| Albumin | CCACTGTTGAAGAAAGCCCA | CAGATAGTCTTCCACACAAGGCA |
| G6p | GCGCAGCAGGTGTATACTATGT | ATCAACTCAACCTGGGATGG |
| Hnf4a | CAGGGGCTTGGGTGGCATCCT | CTGCAGGAGCGCGTTGATGGA |
| Fah | CACGAGACATCCAGCAAT | GGTTCCAGAAGCCAAGAG |
| Aat | AATGGAAGAAGCCATTCGAT | AAGACTGTAGCTGCTGCAGC |
| Trf | CCCACTCAAATGTGCTCCGAAC | ACCGACAGATTGCATGTACTCC |
| Ttr | CTCACCACAGATGAGAAG | GGCTGAGTCTCTCAATTC |
| Foxa1 | CACAGGGTTGGATGGTTGTGT | GTACGCCATGGGACTCATGCA |
| Foxa2 | GGAGCAGCGGCCAGCGAGTTA | TCTGCTGGATGGCCATGGTGA |
| Foxa3 | TGTAGAGAGACCGAAGCAC | AGGTCCATGATCCATTGGTA |
| Cyp1a1 | CAATGAGTTTGGGGAGGTTACTG | CCCTTCTCAAATGTCCTGTAGTG |
| Cyp1a2 | AGTACATCTCCTTAGCCCCAG | GGTCCGGGTGGATTCTTCAG |
| Cyp3a11 | TGAGGCAGAAGGCAAAGAAA | GGTATTCCATCTCCATCACA |
| Cyp3a13 | GACGATTCTTGCTTACCAGAAGG | CCGGTTTGTGAAGGTAGAGTAAC |
| Cyp3a16 | TGTCCTTGTCAGTAGCACTCT | TGTGATCTCGATTTCAGAAAGGG |
| Cyp3a41 | AAAGCCGCCTCGATTCTAAGC | ACTACATCCCGTGGTACAACC |
| Cyp2b10 | AAAGTCCCGTGGCAACTTCC | TTGGCTCAACGACAGCAACT |
| Cyp2c38 | CACGGCCCATTGTTGTATTGC | TGAGTGTGAAACGTCTTGTCTCT |
| Cyp2d22 | CAGTGGTTGTACTAAATGGGCT | GCTAGGACTATACCTTGAGAGCG |
| Barx2 | GATGGTCCTTAAAGGTGGACAG | TGGGCTCCTGGGTATCACAG |
| FoxM1 | CTGATTCTCAAAAGACGGAGGC | TTGATAATCTTGATTCCGGCTGG |
| Mxd3 | GAGGCAGAGCACGGTTATG | TGTAGTGTATCGGGTACAGTCAA |
| Elf3 | GCTGCCACCTGTGAGATCAG | GTGCCAAAGGTAGTCGGAGG |
| GAPDH | AGGTCGGTGTGAACGGATTTG | TGTAGACCATGTAGTTGAGGTCA |

**Supplementary Table 3：Short hairpin RNAs oligonucleotide used**

| Name | Forward | Reverse |
|---|---|---|
| Scramble | CCGGCCTAAGGTTAAGTCGCCCTC GCTCGAGCGAGGGCGACTTAACCT TAGGTTTTTG | GGCCGGATTCCAATTCAGCGGGAGC GAGCTCGCTCCCGCTGAATTGGAAT CCAAAAAC |
| Barx2 shRNA | CCGGGCTGCAAGTGAAGACTTGGT ACTCGAGTACCAAGTCTTCACTTG CAGCTTTTTG | AATTCAAAAAGCTGCAAGTGAAGA CTTGGTACTCGAGTACCAAGTCTTC ACTTGCAGC |
| FoxM1 shRNA | CCGGGCTCCATAGAAATGTGACCA TCTCGAGATGGTCACATTTCTATGG AGCTTTTTG | AATTCAAAAAGCTCCATAGAAATGT GACCATCTCGAGATGGTCACATTTCT ATGGAGC |
| Elf3 shRNA | CCGGCTTGGTGTTGACCCTGAACA ACTCGAGTTGTTCAGGGTCAACAC CAAGTTTTTG | AATTCAAAAACTTGGTGTTGACCCT GAACAACTCGAGTTGTTCAGGGTCA ACACCAAG |
| Mxd3 shRNA | CCGGCCACATGTTGAAGAGACTAA ACTCGAGTTTAGTCTCTTCAACATG TGGTTTTTG | AATTCAAAAACCACATGTTGAAGAG ACTAAACTCGAGTTTAGTCTCTTCA ACATGTGG |

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