Science Translational Medicine

Supplementary Materials for

Therapeutic liver repopulation by transient acetaminophen selection of gene-modified hepatocytes

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The PDF file includes:

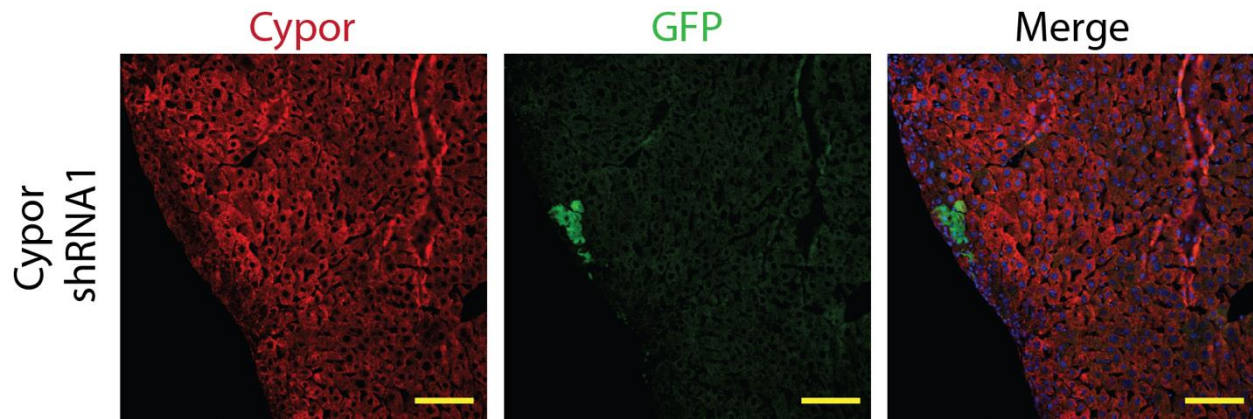**Other Supplementary Material for this manuscript includes the following:**

Figure S1. Cypor immunohistochemistry and GFP expression in mice receiving a *Cypor*-shRNA1 containing transposon. Cypor immunofluorescent staining and GFP expression in liver sections from mice treated with *Cypor* shRNA1 GFP-expression transposon vector and APAP selection (n = 3). Scale bars, 100 μm.
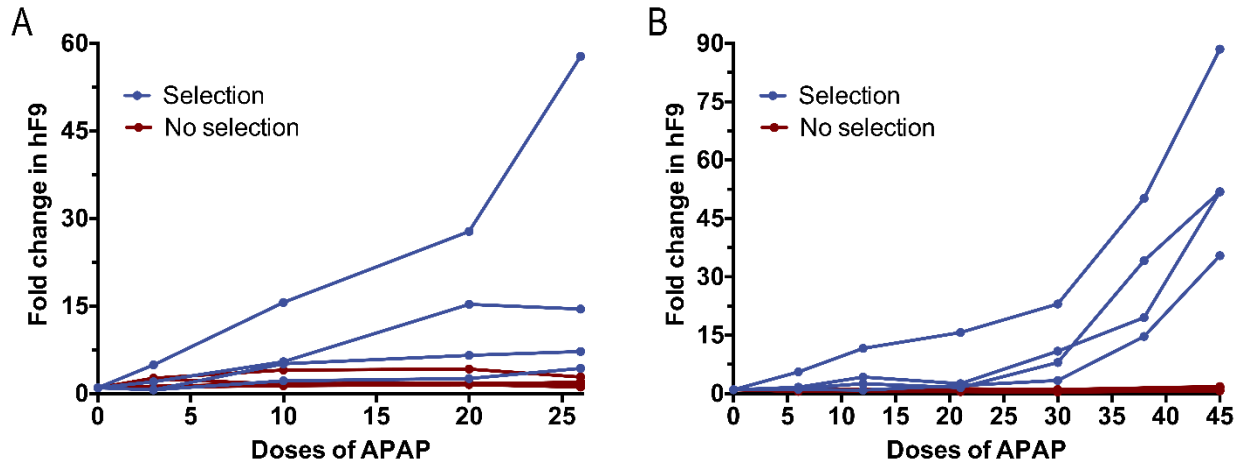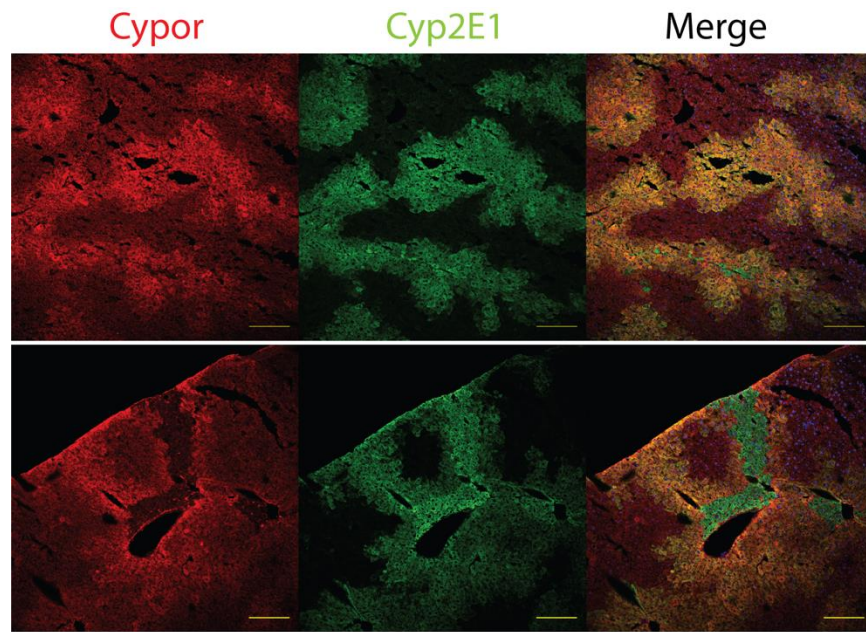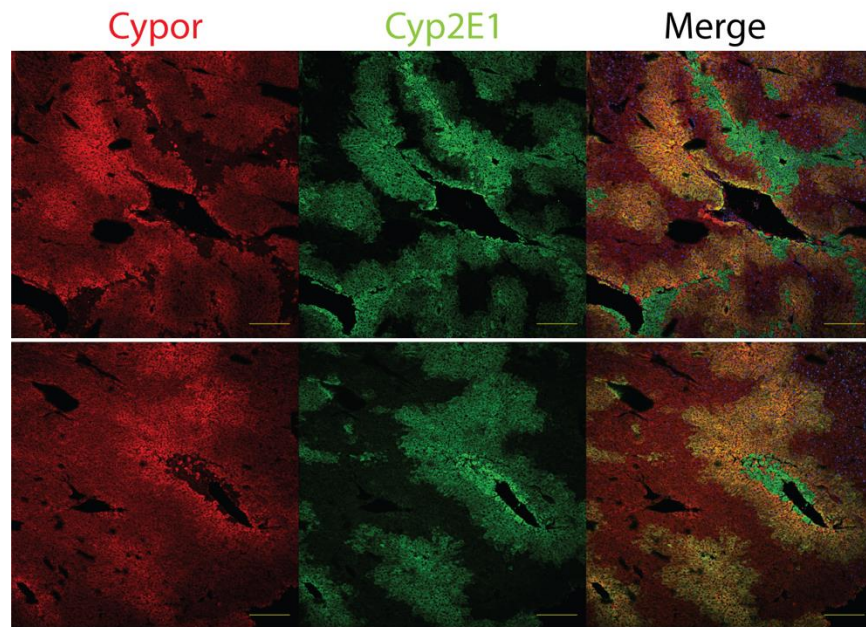
Figure S2. Fold change in hF9 in mice treated with lentiviral vectors. Fold change over baseline in blood hF9 concentration with repeated APAP injection in mice treated with LV-*hF9-Cypor* gRNA (A) or LV-*hF9-Cypor* shRNA (B). n = 4 for all groups.

Cypor   Cyp2E1   Merge

9.3% Cypor-deficient hepatocytes, hF9= 3136 ng/mL

Cypor   Cyp2E1   Merge

8.5% Cypor-deficient hepatocytes, hF9= 3144 ng/mL

Figure S3. Additional Cypor/Cyp2E1 immunohistochemistry in partially selected mice.
Cypor and Cyp2E1 immunohistochemistry in two mice which were partially selected. Scale bars, 200 µm. The percentage of Cypor deficient hepatocytes determined by in-del analysis in hepatocytes and blood levels of hF9 at harvest are indicated below each set of images.
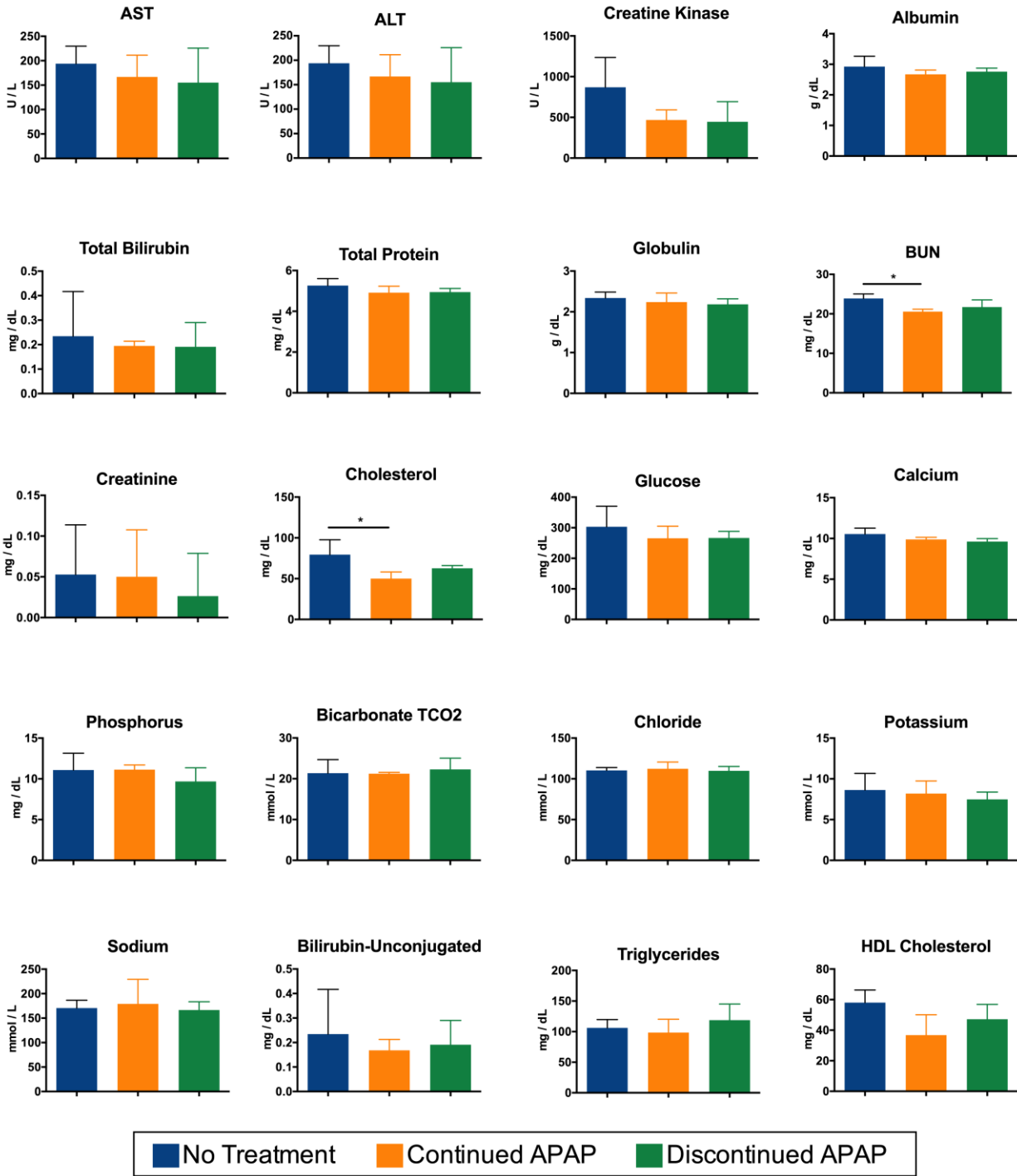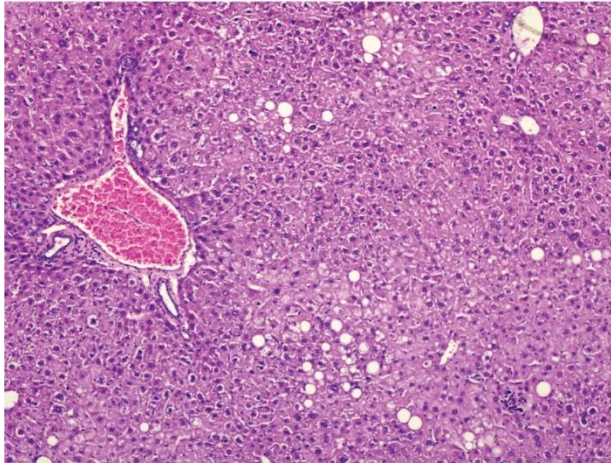
Figure S4. Liver and lipid panel in long-term selected mice. Results of a full liver function test and lipid panel in mice receiving continued weekly APAP or no further APAP for 42 weeks after selection and untreated controls. n = 4 for all groups. Differences are not significant unless indicated. Data are means ± SD. *P<0.05 by Mann-Whitney U test.
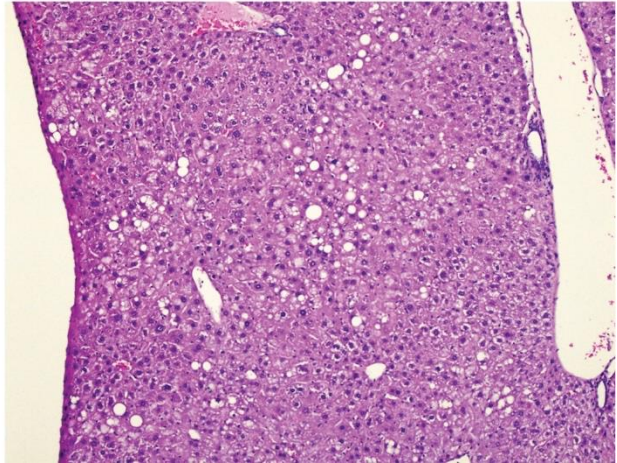
Figure S5. Histology in long term selected mice. H&E staining of liver sections in APAP selected mice receiving either continued weekly APAP or no further APAP for 42 weeks.

Table S1. Quantification of Cypor-negative hepatocytes by image analysis. Approximate quantification of Cypor-negative liver area by measurement of representative IF images for mice treated with the *Cypor* shRNA containing vectors and APAP selection. No APAP control animals were not quantifiable by this method.

Cypor shRNA transposon	
Mouse ID	% Cypor-negative hepatocytes
A	62% (Fig. 2E)
B	38%
C	50%

LV-*hF9-Cypor* shRNA	
Mouse ID	% Cypor-negative hepatocytes
A	14%
B	6%
C	11%
D	11%

LV-*PAH-Cypor* shRNA - Females	
Mouse ID	% Cypor-negative hepatocytes
A	45%
B	40%
C	51%

LV-*PAH-Cypor* shRNA - Males	
Mouse ID	% Cypor-negative hepatocytes
A	56%
B	43% (Fig. 4E)
C	45%
D	50%

Table S2. hF9 concentrations and proportion Cypor-deficient hepatocytes in partially selected mice. Transgenic cas9 mice that received a hydrodynamic injection of pX330-*Cypor* gRNA and underwent partial APAP selection. Cypor deficient hepatocytes were quantified by TIDE analysis. Mice numbered 10 to 13 underwent partial hepatectomy and analysis was based on the liver tissue that was removed.

Mouse	Baseline hF9 (ng/mL)	Final hF9 (ng/mL)	hF9 fold increase	% Cypor-deficient hepatocytes
1	56	3307	59	16.7
2	32	3820	119	14.7
3	61	3136	51	9.3
4	82	3662	45	10.2
5	36	3181	88	15.3
6	9	3144	349	8.5
7	30	4224	141	9.8
8	12	3009	251	6.7
9	33	3159	96	10.8
10 (PH)		1579		2.2
11 (PH)		3555		5.8
12 (PH)		2057		3
13 (PH)		995		1.5