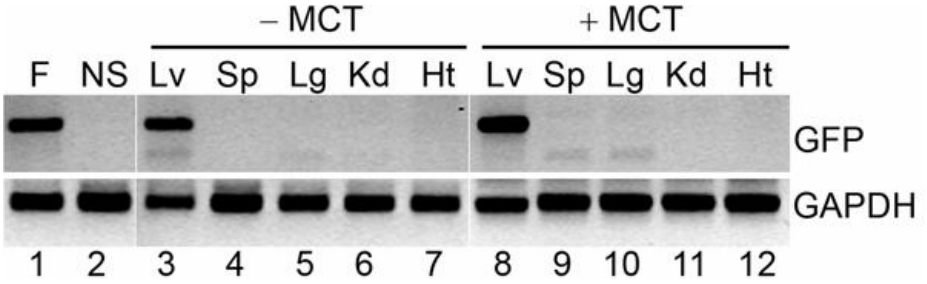
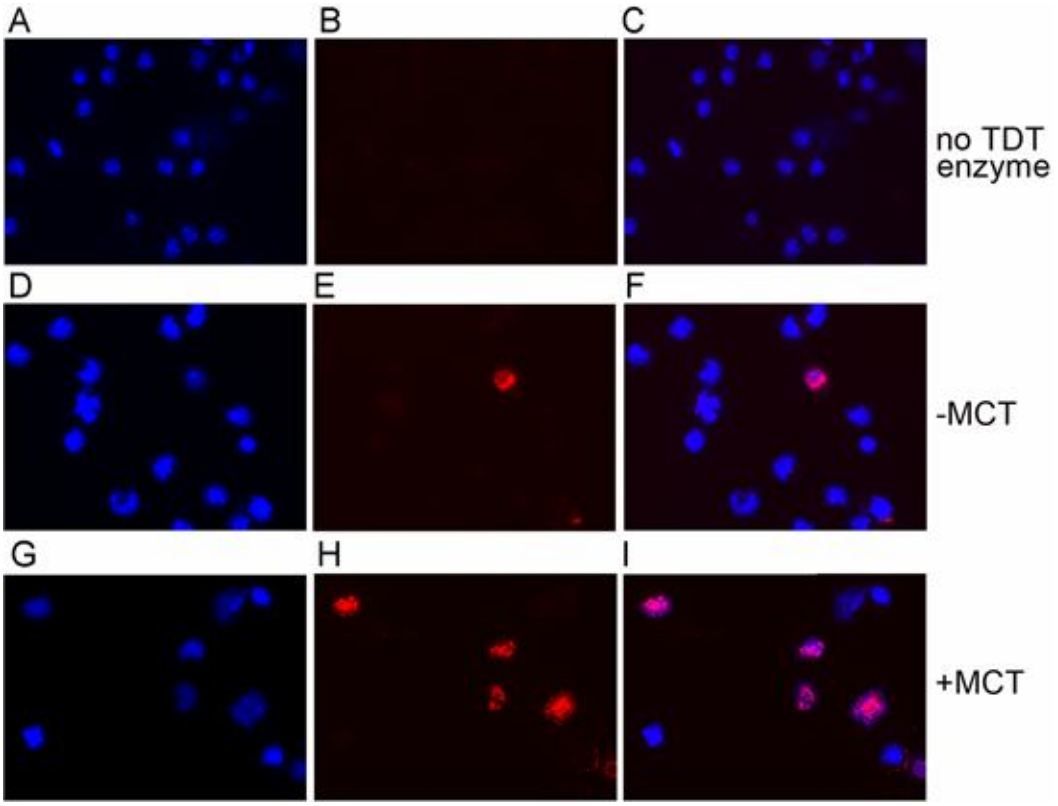
Supplementary Figure 1



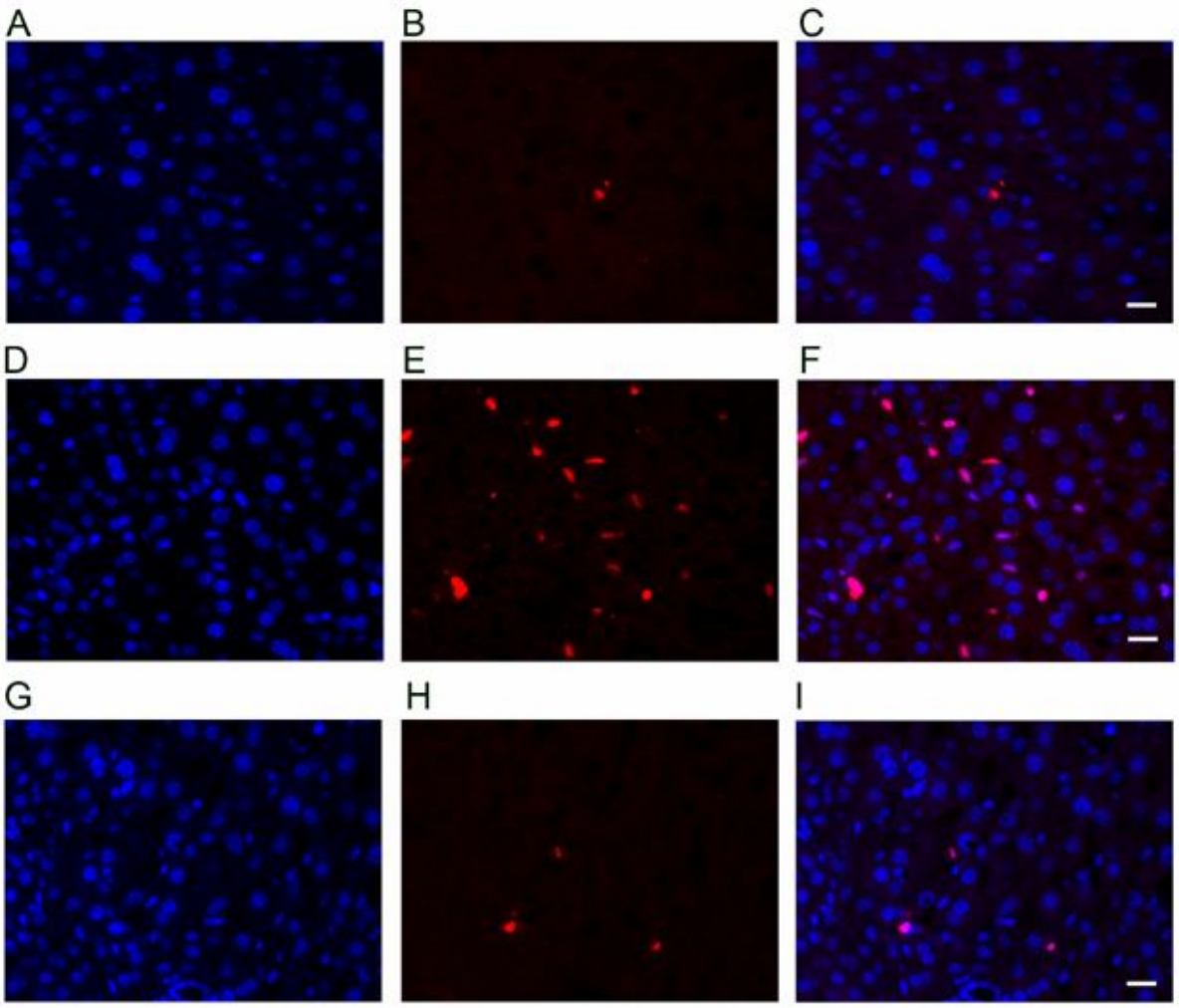
Supplementary Figure 1. Tissue biodistribution of transplanted LSEC in mice without MCT (-) or with MCT treatment (+). Tissues were surveyed for GFP transgene by PCR of genomic DNA 1-week after LSEC transplantation. Lane 1, liver from FVB/N-Tie2 GFP mouse; lane 2, liver from NOD-SCID hemophilia A mouse; lanes 3-7, organs from MCT-untreated mouse; and lanes 8-12, organs from MCT-treated mouse as follows. Lv = liver, Sp = spleen, Lg = lungs, Kd = kidney, and Ht = heart. GAPDH amplification shows that equivalent amounts of genomic DNAs were analyzed.

Supplementary Figure 2



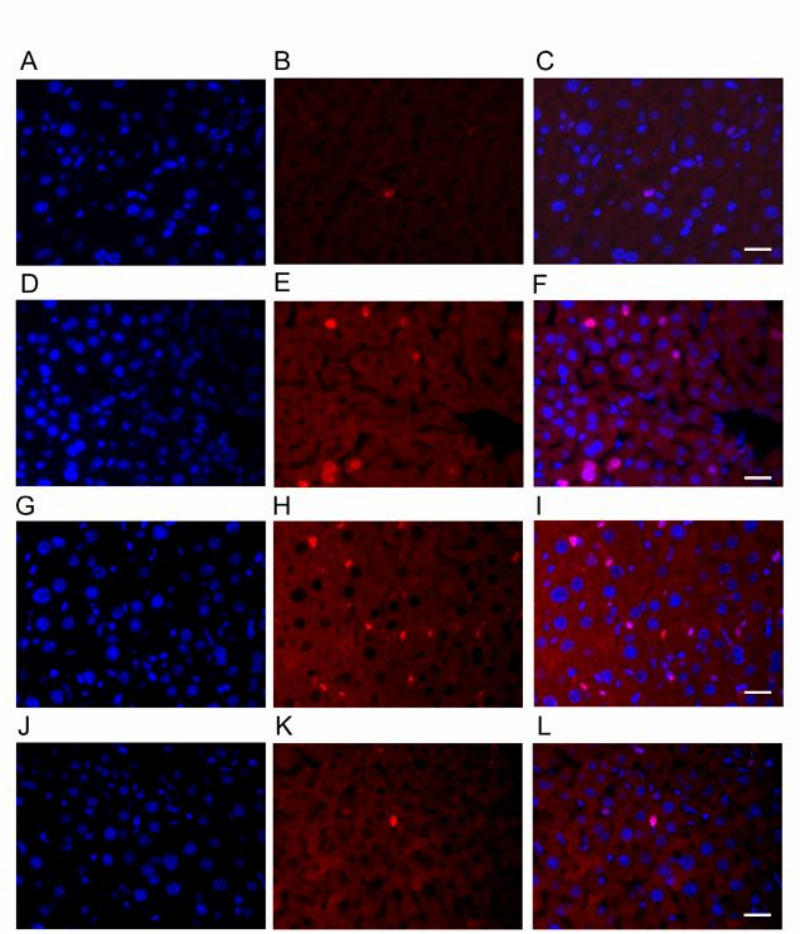
Supplementary Figure 2. TUNEL assay showing extent of apoptosis in cultured LSEC. Cells were freshly isolated from mice either without prior MCT (-) and following treatment (+) with MCT. Cells in Panels A-C were not treated with TDT enzyme and served as negative controls. LSEC from mouse without MCT treatment (D-F) showed occasional apoptotic cells (red nuclei). LSEC from MCT-treated mouse (G-I) showed far greater apoptosis (red nuclei). All nuclei were counterstained with DAPI. Merged images are shown in C, F, I. Orig. mag., x400.

Supplementary Figure 3



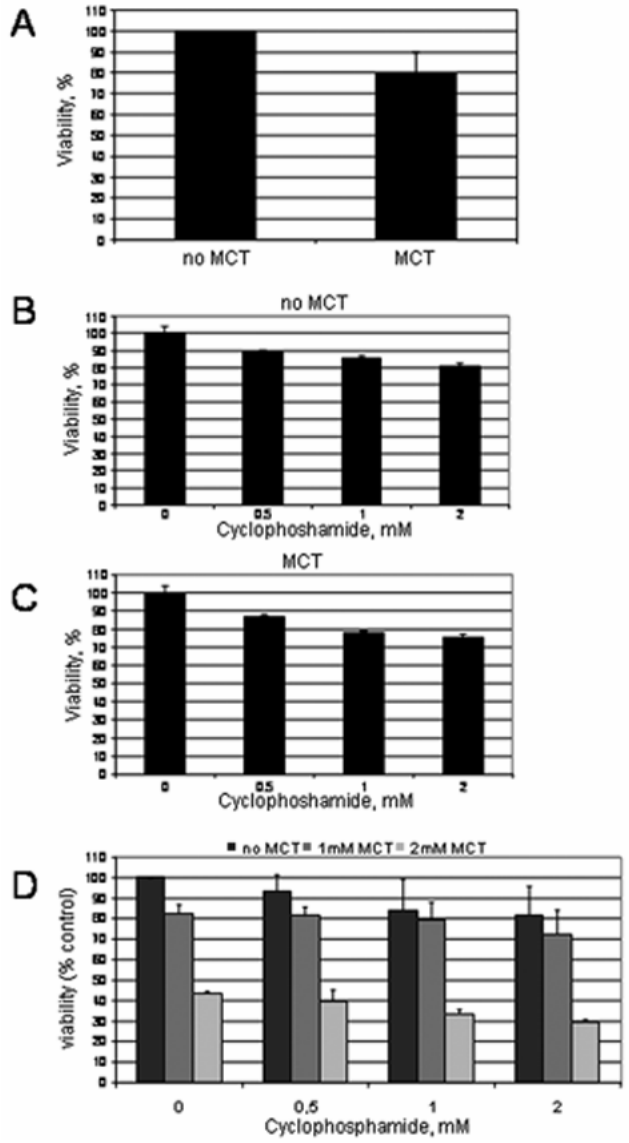
Supplementary Figure 3. Analysis of apoptosis with TUNEL assay in liver tissue. Shown are mice without prior MCT treatment (-) and following treatment (+) with MCT. A-C show liver from MCT-untreated mouse. D-F show mouse liver 3 d after MCT treatment. G-I show liver of MCT-treated mouse 1-month after LSEC transplantation. Apoptotic cells are shown in red in B, E, H. Nuclei were counterstained by DAPI in A,D and G. Merged images are shown in C, F, I. The data demonstrated that apoptosis was rare in sinusoidal cells in the MCT-untreated mouse liver, whereas after MCT-treatment, extensive apoptosis was occurring after 3-days. By contrast, after one month when transplanted cells were proliferating extensively, apoptosis was far less pronounced. Orig. mag., for all pictures is x400, bar= 20 μm.

Supplementary Figure 4



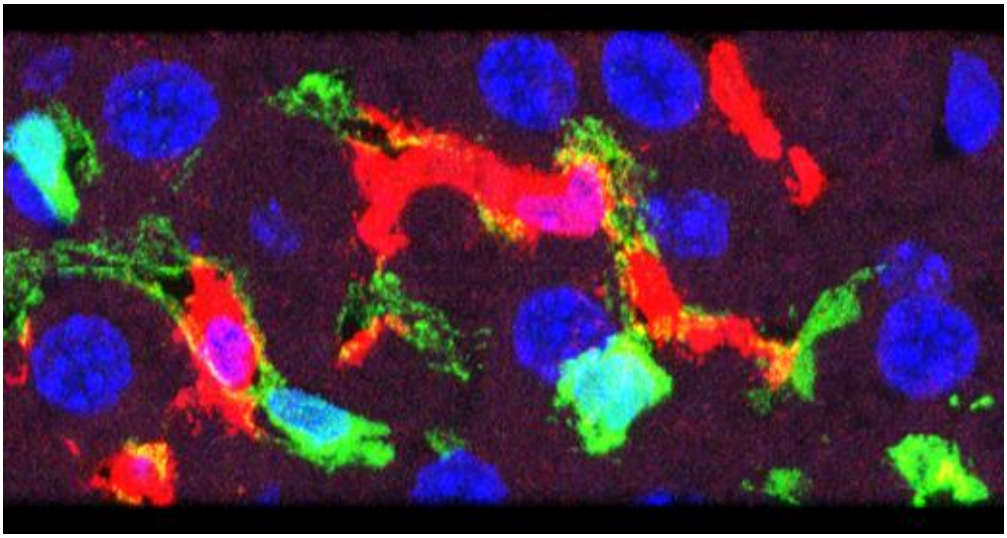
Supplementary Figure 4. Cell proliferation analysis with Ki67 expression in mouse liver 1 and 3 months after LSEC transplantation. Panels A-C show mouse liver without MCT, where only rare parenchymal cells showed Ki67 immunostaining. Panels D-F show mouse liver 3 d after MCT with activation of Ki67 expression following early hepatic necrosis induced by MCT. Panels G-I show liver of MCT-treated mouse after one month following LSEC transplantation. In this situation, significant proliferation is evident in nonparenchymal sinusoidal cells with Ki67 staining. Panels J-L show mouse liver 3 months following LSEC transplantation with subsidence of sinusoidal cell proliferation. Nuclei were counterstained by DAPI in panels A, D, G, and J. Ki67-positive cells are shown in red in B, E, H, and K. Merged images are shown in C, F, I and L. The data were in agreement with lack of apoptosis in MCT-treated livers 1-month after LSEC transplantation (supplementary Fig. 3, panel H) and indicated that transplanted cells were proliferating as also further verified by progressive increases in the number of GFP-positive transplanted cells in MCT-treated recipients. Orig. mag., x400; bar = 20 μm.

Supplementary Figure 5



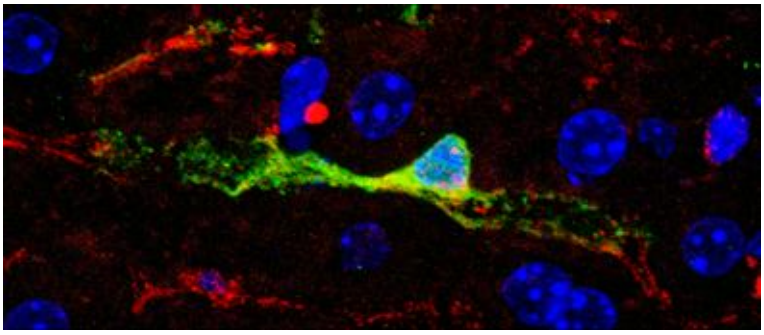
Supplementary Figure 5. MCT-induced perturbations in LSEC. Primary LSEC were isolated from mice without MCT treatment or 1-month after MCT-treatment and subjected to cell culture. This was followed by overnight exposure to MCT or cyclophosphamide under various concentrations as indicated. Cell viability was measured by timed MTT incorporation and data are expressed as percent control in individual cell preparations for comparing experimental groups. (A) Showing viability of LSEC from MCT-treated donors was inferior to LSEC from healthy donors, indicating survival disadvantages for the former. (B and C) Showing limited effects of cyclophosphamide on cell viability irrespective of their origin from healthy donors (B) or from MCT-treated animals (C). (D) Showing dose-dependent toxicity of MCT in cultured LSEC from healthy donors. Addition of cyclophosphamide did not alter cell viability, indicating that MCT and cyclophosphamide did not exert synergistic endothelial toxicity under these conditions in vitro.

Supplementary Video Clip 1



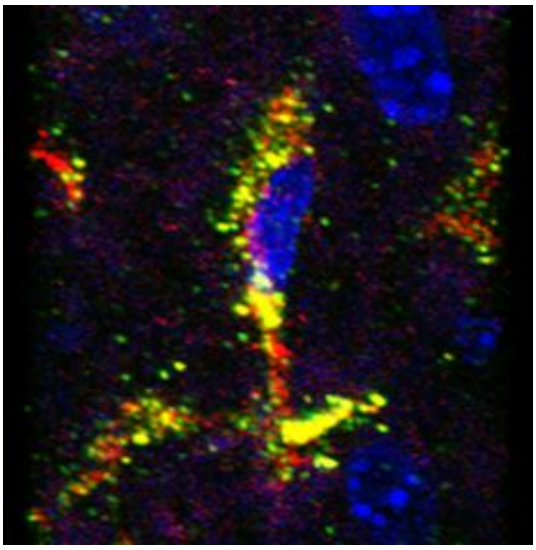
Supplementary Video Clip 1. Transplanted cells in mouse liver sinusoids 1 month after transplantation of FVB/N-Tie2-GFP LSEC. Confocal microscopy showing transplanted LSEC in the liver of hemophilia A mouse. Immunostaining was performed to identify Kupffer cells reacting with F4/80 antibody (red color) and LSEC expressing GFP (green). Cell nuclei were counterstained with DAPI (blue color). The images show 360 degree rotation views to indicate that transplanted LSEC were distinct from Kupffer cells.

Supplementary Video Clip 2



Supplementary Video Clip 2. CD31 and GFP staining of sinusoidal liver cells in hemophilia A mouse 1 month after transplantation of FVB/N-Tie2-GFP LSEC. Shown are video images of confocal microscopy with transplanted LSEC expressing GFP (green) and CD31 endothelial marker (red). The data verified that transplanted cells expressed both markers. Cell nuclei were counterstained with DAPI (blue color).

Supplementary Video Clip 3



Supplementary Video Clip 3. Immunostaining of vWF in LSEC. Confocal microscopy of liver from hemophilia A mouse showing coexpression of CD31 endothelial marker (red) and vWF (green) in LSEC. The overlay of CD31 and vWF appears yellow.