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Supplemental Information

Improved Lentiviral Gene Delivery to Mouse Liver by Hydrodynamic Vector Injection through Tail Vein

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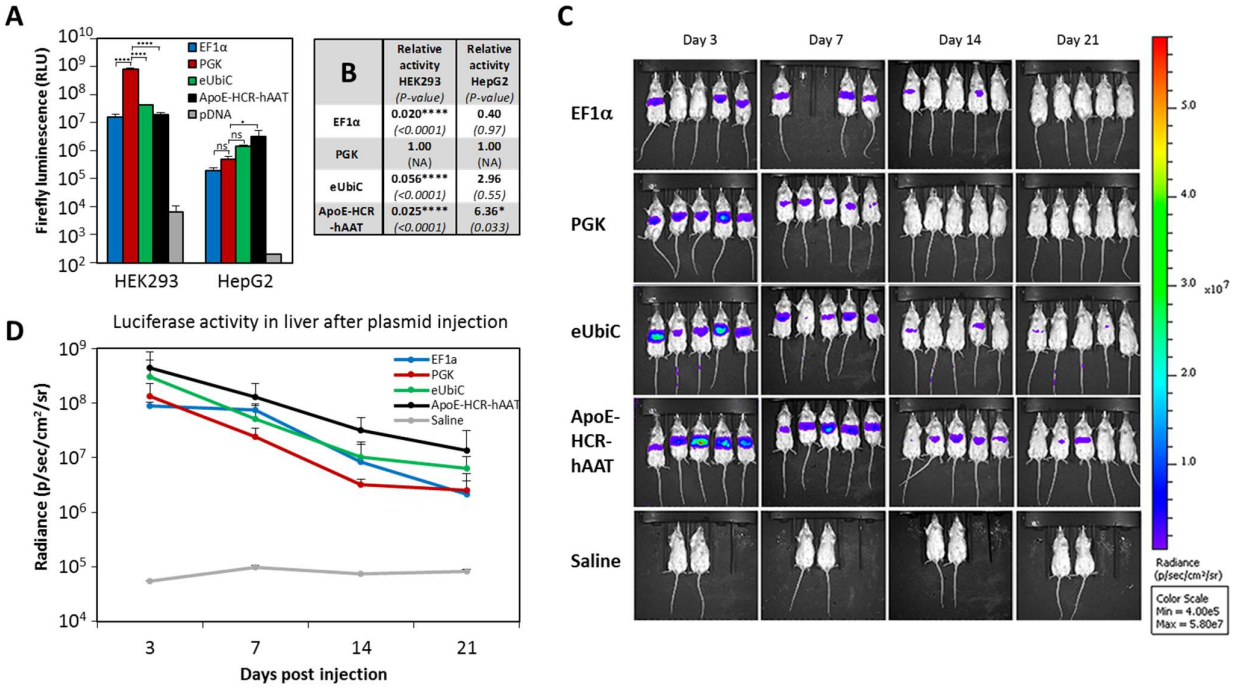

Figure S1: Quantification of promoter activity *in vitro* after DNA transfection and *in vivo* after hydrodynamic delivery of plasmid DNA. A) Quantification of luciferase activity in HEK293 and HepG2 cells after transient transfection of plasmid DNA. Firefly luciferase expression was measured in relative units of light (RLU), and plotted as the mean three replicates plus standard deviations. Control cells were transfected with plasmid DNA devoid of luciferase activity. **B)** Table showing reporter activity in HEK293 and HepG2 cells normalized to the pCCL-PGK-fLuc group and P-value of test statistics in brackets. One (*) or four stars (****) indicates significance levels below 0.05 or 0.0001, respectively. **C)** Bioluminescence imaging of the mice visualize the localization and expression level of the firefly luciferase reporter gene. The colored scale indicates Radiance (photons/sec/cm^2/steradian). 30 µg of the various LV vector plasmids pCCL-EF1α-fLuc (first row, n=3), pCCL-PGK-fLuc (second row, n=5), pCCL-eUbiC-fLuc (third row, n=5), and pCCL-ApoE-HCR-hAAT-fLuc (forth row, n=5) were delivered to the liver by hydrodynamic tail vein injections. Saline injected animals served as controls (fifth row, n=2). Note, the second and third mouse in the EF1α group was omitted from the analysis due to technical injection difficulties. The mice were analyzed in an IVIS bioluminescence scanner after subcutaneous injections of luciferin for quantification of reporter gene activity. **D)** A ROI surrounding the liver was defined and Radiance was quantified within each ROI. The mean and standard deviation were calculated for each group and plotted at weekly time point (3, 7, 14, and 21 days post injection). Abbreviations: ns, non-significant.

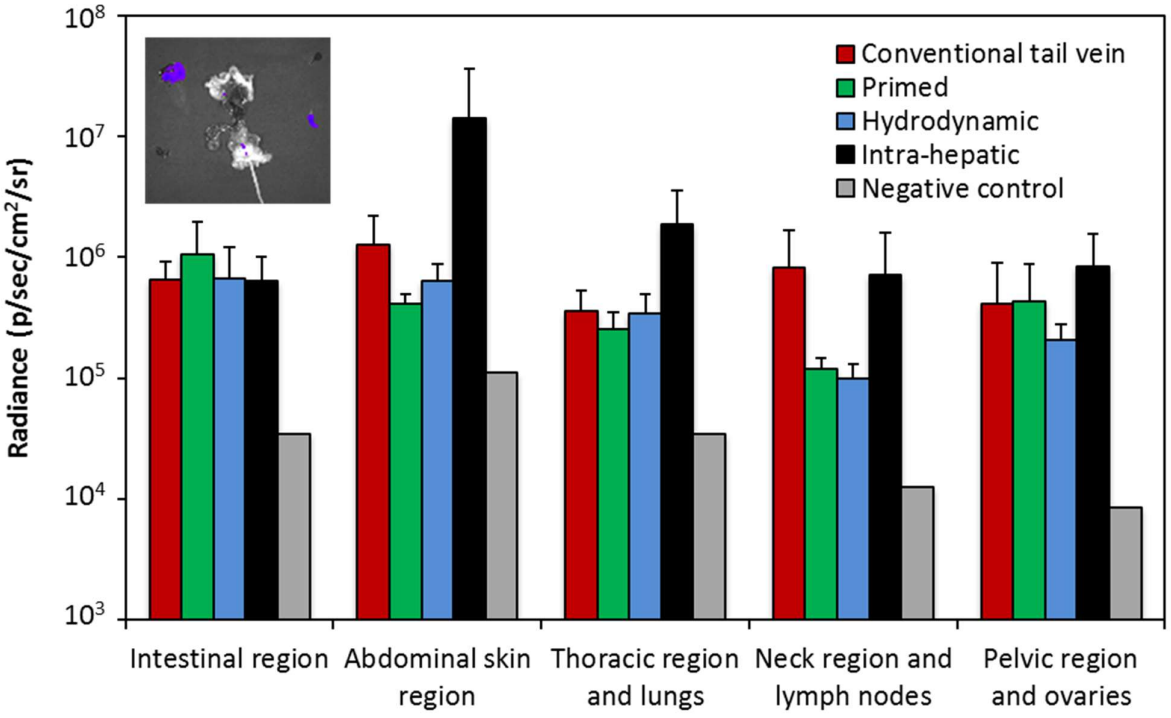

Figure S2: Vector dissemination to off-target organs vary according to injection strategy. Quantification of bioluminescence images of additional regions and body segments after dissection of organs from mice shown in Figure 2. This end-point data was collected 29 days after treatment. Mice were administered a vector dose of 4 µg p24 of pCCL-PGK-fLuc vector-encoding particles at day 0 as follows: Conventional (0.4 ml) tail vein injections (red bars), 'Primed' injections (green bars), Hydrodynamic injections (blue bars), Intrahepatic injections (black bars), or an Untreated control mouse (grey bars). At day 29 live mice were subcutaneously injected with luciferin to allow quantification of reporter gene activity of the whole body. Next the mice were sacrificed, and the liver, spleen, heart and kidneys were isolated for separate analysis (see Figure 3). Finally, intestines, abdominal skin, lungs, and ovaries were isolated and analyzed in an IVIS bioluminescence scanner along with the remaining carcass (see inset in upper left corner). ROIs outlining organs or regions of the body were defined and Radiance (photons/sec/cm^2/steradian) was quantified within each ROI. The mean and standard deviation were calculated for each group (n=6) and plotted.

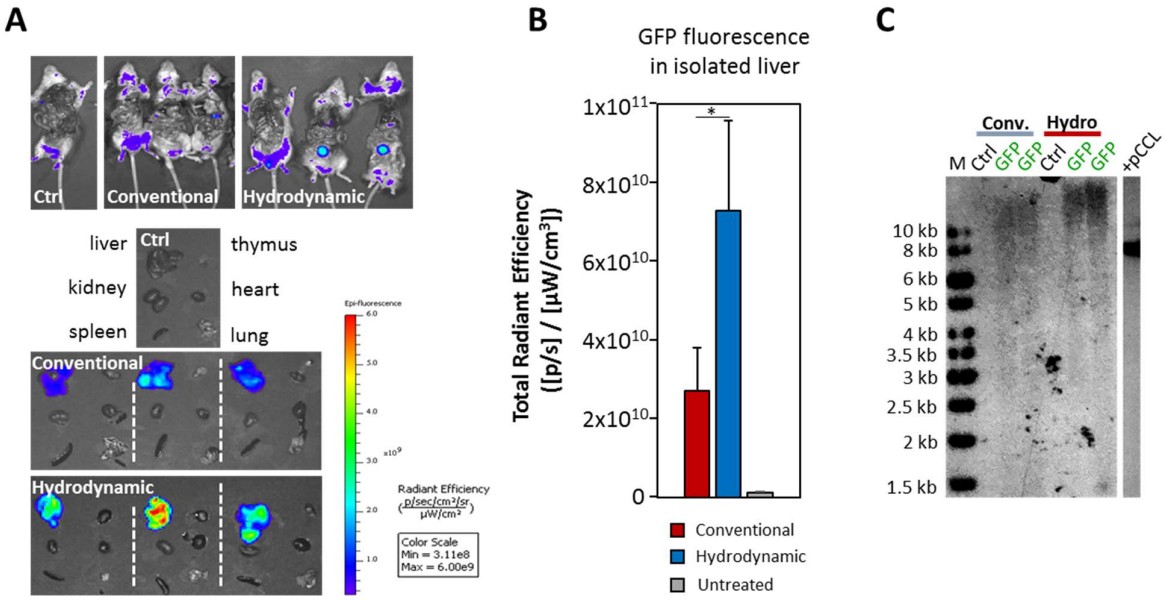

Figure S3: Enhanced GFP expression in liver by hydrodynamic vector injection through tail vein. A) Fluorescence imaging of dissected organs and carcasses from mice administered with a single dose of lentiviral vectors encoding GFP. A vector dose of 15 µg p24 of pCCL-ApoE-HCR-hAAT-GFP vector encoding particles was administered to all animals as follows: Untreated mouse (Ctrl), Conventional tail vein injections, or Hydrodynamic injections. The mice were sacrificed and analyzed using an IVIS bioluminescence scanner 93 days post injection. The colored scale indicates total Radiant Efficiency (photons/sec per µW/cm^3). **B)** A ROI outlining the liver was defined and the Radiant Efficiency was quantified within each ROI. The mean and standard deviation were calculated for each group (n=3) and plotted. The asterisk (*) indicates significance levels below 0.05. **C)** Southern blot analysis for presence of a GFP-specific sequence in genomic DNA from liver samples digested with Sal*I* indicate that the vector mainly associates with high molecular weight DNA larger than approximately 8-10 kb consistent with integration into chromosomal DNA. Sal*I* is not present in the GFP vector. Lane 1 (M) was loaded with a 1 kb DNA ladder (GeneRuler™), lane 2-7 were loaded with 25 µg Sal*I*-digested gDNA from either untreated mice (Ctrl) or animals conventional (conv) or hydrodynamic (hydro) injected with the GFP vector, as indicated above each lane. Lane 8 (+ pCCL) was loaded with 25 µg Sal*I*-digested gDNA from the untreated group spiked with 100 pg Not*I* linearized plasmid DNA of our pCCL-based vector as a ~8,4 kb GFP positive size marker (pCCL-ApoE-HCR-hAAT-GFP).

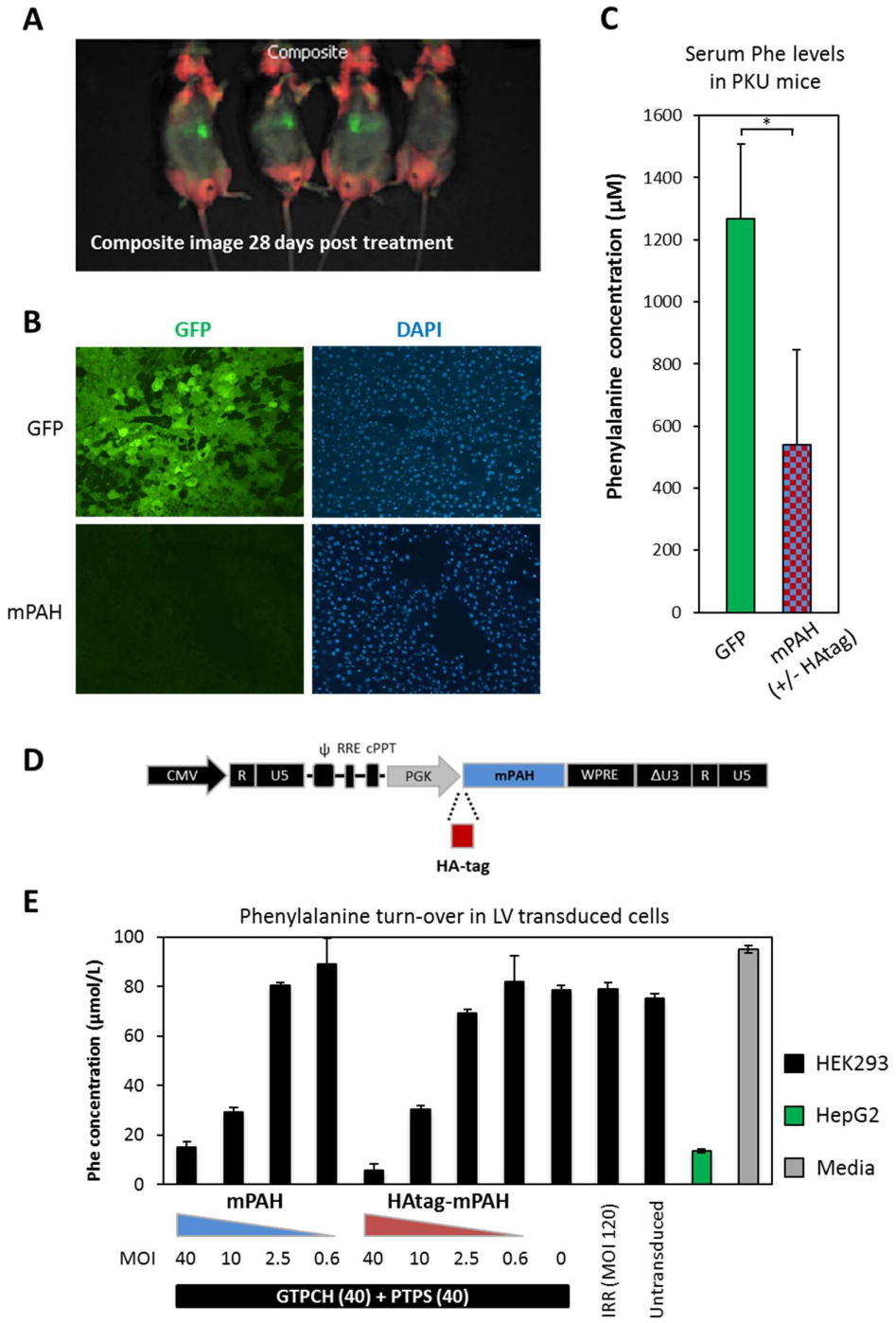

Figure S4: Live detection of GFP and reduction in serum phenylalanine levels in PKU mice hydrodynamic injected with LV. B6:BTBR phenylketonuria (PKU) mice, homozygous for the *Pah*^{enu2} mutation, were treated with a single dose of GFP (n=4), mPAH (n=3), or HA-tagged mPAH (n=4) expressing lentiviral vectors. Mice were administered a vector dose of 15 µg p24 of pCCL-ApoE-HCR-hAAT-GFP, pCCL-ApoE-HCR-hAAT-mPAH, or pCCL-ApoE-HCR-hAAT-HAtag-mPAH particles at day 0 using hydrodynamic injections (transgene underlined). At day 14 (data not shown) and day 28 live mice were scanned, and next the mice were sacrificed, and the liver was isolated for fluorescence microscopy. **A)** Epifluorescence imaging by the IVIS Spectrum imaging system and utilizing the spectral unmixing tool in the Living

image 4.3 software allowed detection of green fluorescent livers above background in living animals 28 days post injection. Three GFP treated animals to the left, one mPAH treated animal to the right. **B)** Representative images of DAPI stained liver cryosections from mice shown in A analyzed by fluorescence microscopy. Green colour indicates GFP signal (left panel), blue colour indicates DAPI stain (right panel). **C)** Phenylalanine levels in the blood of treated mice at day 28 post injection. The mean and standard deviation were calculated and plotted for the GFP group and the two mPAH groups combined (+/- HAtag). The asterisk (*) indicates significance levels below 0.05. **D)** Schematic diagram of the HIV-1-based 3rd generation lentiviral vector (denoted pCCL) expressing murine Phenylalanine Hydrolase (mPAH) with or without an N-terminal HA-tag used in this study. **E)** Phenylalanine turn-over assay in transduced cells demonstrating equal functionality of the N-terminal HA-tagged Phenylalanine Hydrolase enzyme as compared to wildtype mPAH. Phenylalanine levels were quantified in the growth media from HEK293 cells transduced at a total multiplicity of infection (MOI) of 120 with a combination of lentiviral vectors and compared to untreated HEK293 cells (black bars), untreated HepG2 hepatocytes (green bar) or growth media (grey bar). HEK293 cells were transduced at an MOI of 120 using a combination of lentiviral vectors. Control cells (denoted IRR) were transduced with pCCL-PGK-GFP, pCCL-PGK-fLuc, and pCCL-PGK-Puro (each MOI 40), while treated cells were transduced with pCCL-PGK-mGTPCH (MOI 40), pCCL-PGK-mPTPS (MOI 40) and varying amounts of pCCL-PGK-mPAH (blue) or pCCL-PGK-mPAH-HA-tag (red) and pCCL-PGK-GFP (combined MOI 40). The cells were passaged for one week, before seeding an equal number of cells at high confluence. 24 hours later cell-free supernatant (media) was collected and assayed for phenylalanine concentration (μM). The assay was performed in triplicates, and mean and standard deviation were calculated and plotted for each group. Abbreviations: CMV, cytomegalovirus promoter; cPPT, central PolyPurine Tract; ΔU3, partial deletion of the viral Unique 3' region; fLuc, firefly luciferase gene; HA-tag, N-terminal fusion of a MYPYDVPDYA peptide sequence; mPAH, murine Phenylalanine Hydrolase cDNA; PGK, human phosphoglycerate kinase promoter; Ψ, Packaging signal (psi); R, Repeat region; RRE, Rev Response Element; U5, Unique 5' region; WPRE, Woodchuck Hepatitis Virus Posttranscriptional Regulatory Element.