OMTM, Volume *12*

Supplemental Information

Preclinical Development of an AAV8-hUGT1A1 Vector for the Treatment of Crigler-Najjar Syndrome

Fanny Collaud, Giulia Bortolussi, Laurence Guianvarc'h, Sem J. Aronson, Thierry Bordet, Philippe Veron, Severine Charles, Patrice Vidal, Marcelo Simon Sola, Stephanie Rundwasser, Delphine G. Dufour, Florence Lacoste, Cyril Luc, Laetitia v. Wittenberghe, Samia Martin, Christine Le Bec, Piter J. Bosma, Andres F. Muro, Giuseppe Ronzitti, Matthias Hebben, and Federico Mingozzi

SUPLLEMENTAL DATA ITEMS

Table S1: Biodistribution and mRNA expression of hUGT1A1 in rats from GLP-toxicology study

day 8 post-AAV injection				
tissue	Vector genome copies /µG DNA		hUGT1A1 RNA copies /µG RNA	
	average	sd	average	se
BR	1.3E+04	5.24E+03	1.9E+03	3.74E+02
CO	1.9E+05	3.38E+05	3.3E+03	5.73E+02
EP	6.2E+04	2.03E+04	1.5E+04	4.26E+03
HE	1.4E+05	4.87E+04	1.6E+04	1.68E+03
KI	1.8E+05	1.06E+05	4.9E+03	2.82E+03
LI	2.3E+06	8.76E+05	2.2E+06	5.86E+05
LLN	8.2E+05	1.98E+05	2.5E+04	1.07E+04
LU	1.0E+05	3.58E+04	5.4E+04	1.03E+04
MLN	1.4E+06	8.58E+05	1.7E+04	4.46E+03
SK	2.1E+05	9.63E+04	1.4E+04	2.71E+03
SM	8.4E+04	3.52E+04	9.7E+03	2.86E+03
SP	7.3E+05	3.48E+05	9.8E+04	3.29E+04
TA	1.2E+06	5.04E+05	5.5E+04	1.92E+04
TE	3.6E+04	2.33E+04	3.8E+03	1.21E+03
UT	2.4E+05	1.10E+05	4.8E+03	3.39E+03

(ss)AAV8-UGT1A1 biodistribution and expression were analyzed by qPCR and RT-qPCR respectively. Results are reported in the table. The number of rats (n) analyzed per each time point was n= 10 (5 males+5 females). Abbreviations : BR brain; CO colon; EP Epididymis; HE Heart; KI Kidney; LI Liver; LLN Lumbar lymph node; LU Lung ; MLN Mesenteric lymph node; SK Skin; SM Skeletal Muscle; SP Spleen; TA Tail (administration site); TE Testis; UT Uterus.

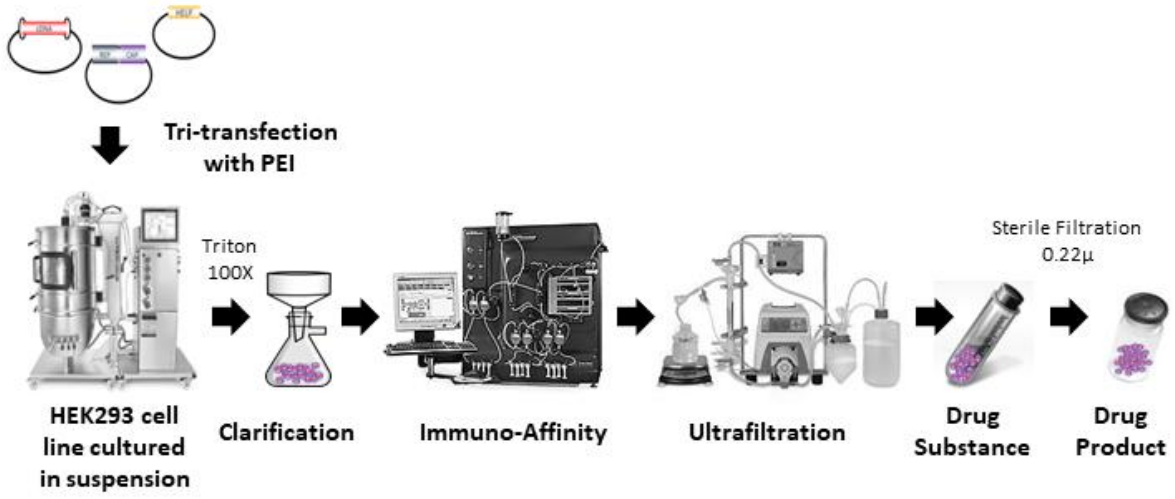

Figure S1. AAV large-scale manufacturing process scheme

HEK293 cells which were previously adapted to suspension culture are expanded to the desired volume in stirred tank bioreactors (2L, 10L or 200L). The cells are transiently transfected with 3 plasmids complexed to polyethylenimine (PEI) to generate AAV8-hUGT1A1 vectors. After the production phase, the cells are disrupted using Triton detergent treatment and the crude material is then clarified by filtration. The AAV capsids are purified by capture chromatography based on the immune-affinity matrix AVB Sepharose (GE Healthcare). The eluted vectors are subsequently concentrated by tangential flow filtration in hollow fibers and diafiltered with the formulation buffer (Ringer Lactate + 0.001% Pluronic F68). After sterile filtration on a 0.2µm filter, the final product is vialed and stored at -80°C.

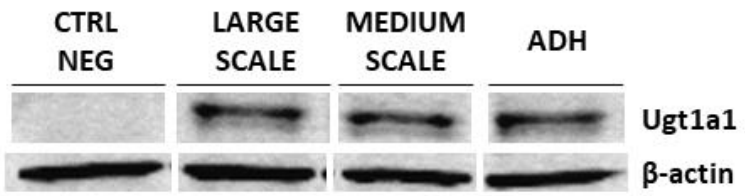

Figure S2. *In vitro* potency of (ss)AAV8-hUGT1A1 produced in HEK293 cells growing in suspension at large scale.

Huh7 cells were transduced with AAV8-GFP (CTRL NEG), (ss)AAV8-hUGT1A1 produced in HEK293 cells cultured in adhesion (ADH) or in suspension at 50L (MEDIUM SCALE) or 200L scale (LARGE SCALE). Multiplicity of infection of 25000 was used. 72 hours post-transduction, microsomal extracts were obtained, separated by SDS-PAGE and analyzed by Western Blot with UGT1A and actin antibodies.

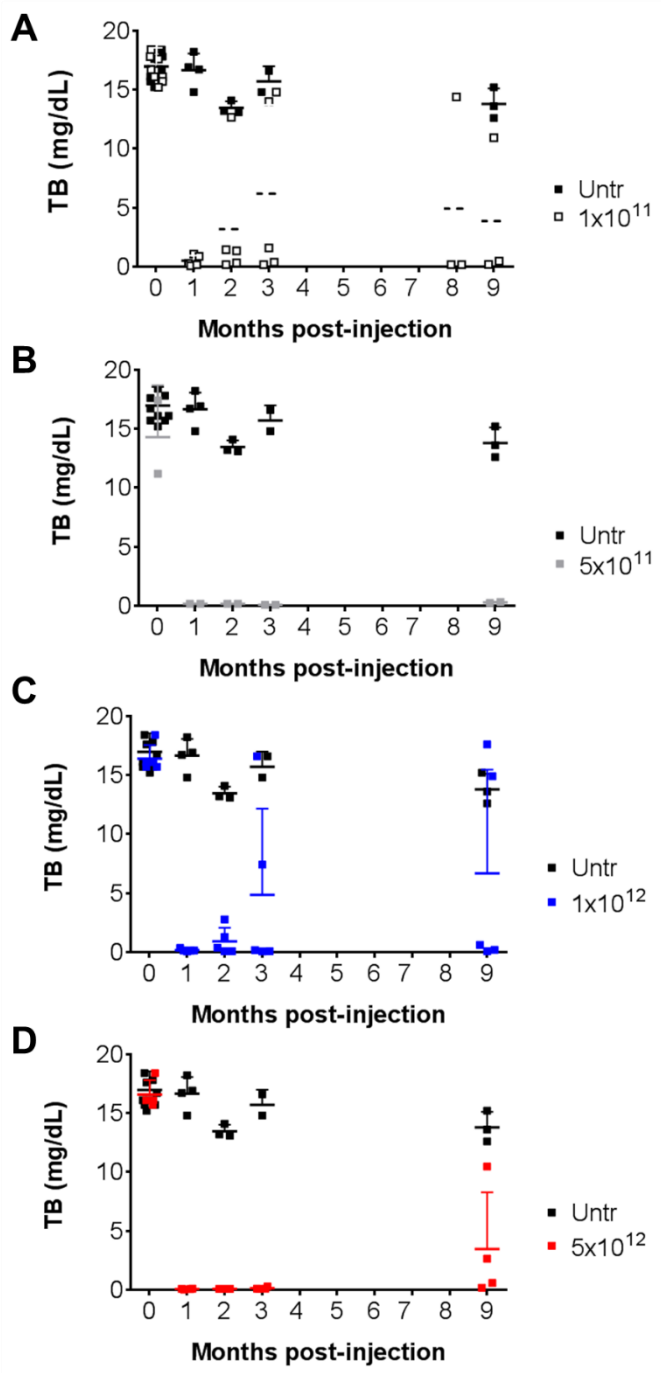

Figure S3. Plasma bilirubin in female Ugt1-/- mice.
Plasma bilirubin determination in female Ugt1-/- mice treated with $1x10^{11}$ vg/kg (A), $5x10^{11}$ vg/kg (B), $1x10^{12}$ vg/kg (C) and $5x10^{12}$ vg/kg (D). Each dot represents a single animal. Untr, untreated Ugt1-/- mice.

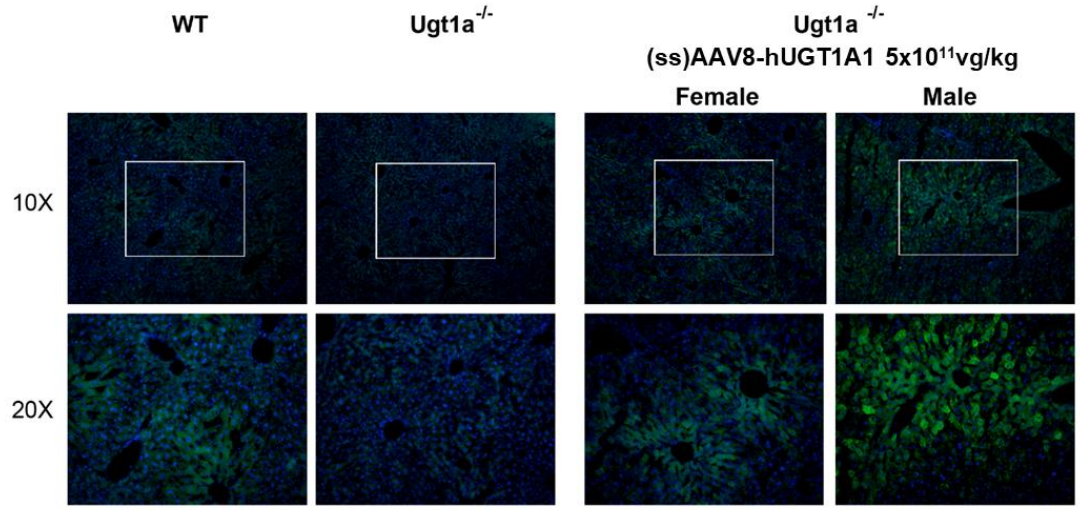

Figure S4. UGT1A1 expression in mice liver
Immunofluorescence analysis of liver sections from WT, Ugt1$^{-/-}$ untreated and treated with 5x10^{11} vg/kg vector dose. Rectangles show magnification images shown below. Results are expressed as mean ± SD.

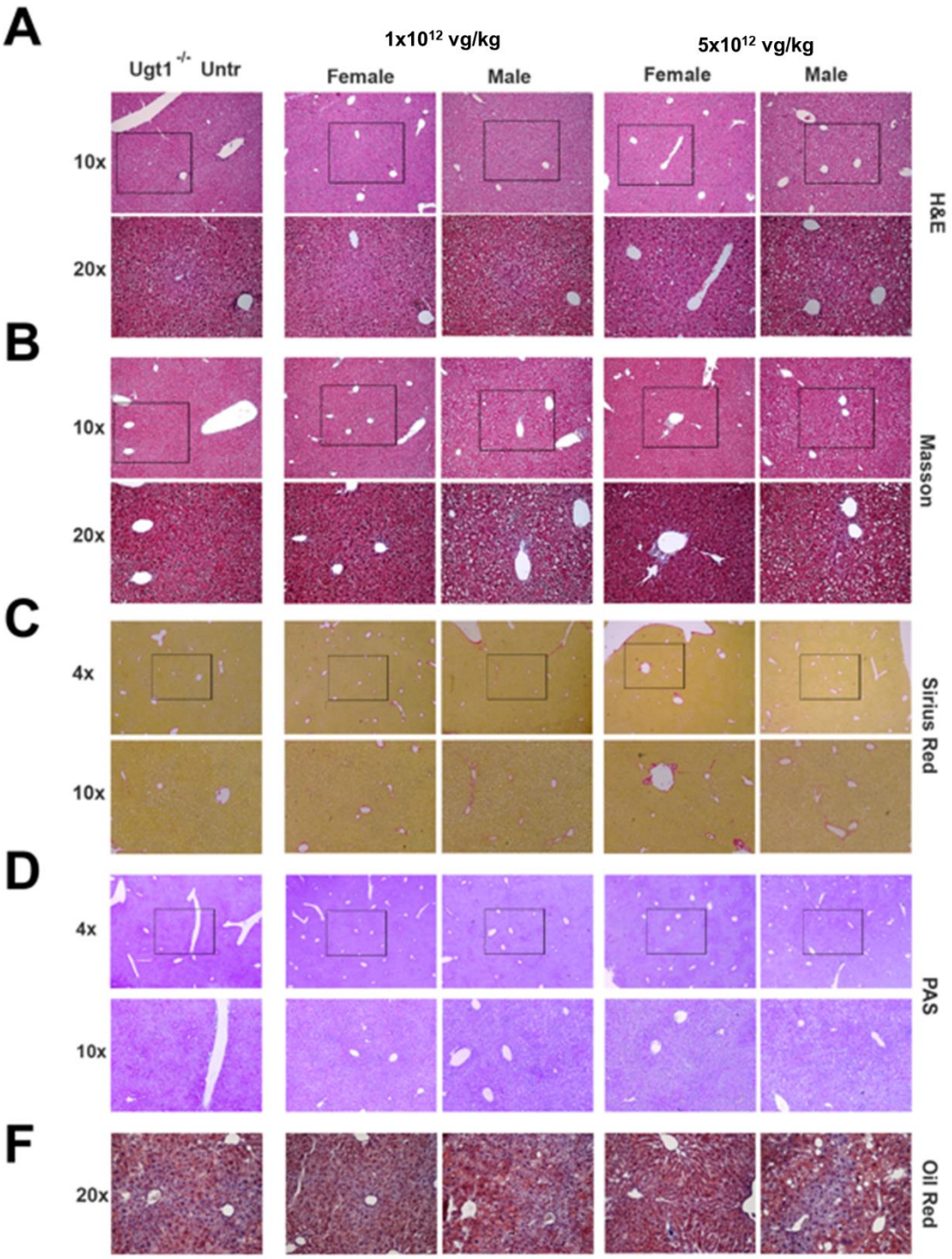

Figure S5. Histological analysis in Ugt1-/- treated animals.
(A) Representative images of H&E staining; (B) Masson trichrome; (C) Sirius red; (D) PAS staining and (F) Oil red. Ugt1-/- untreated animals where used as control. Rectangles show magnification images shown below.

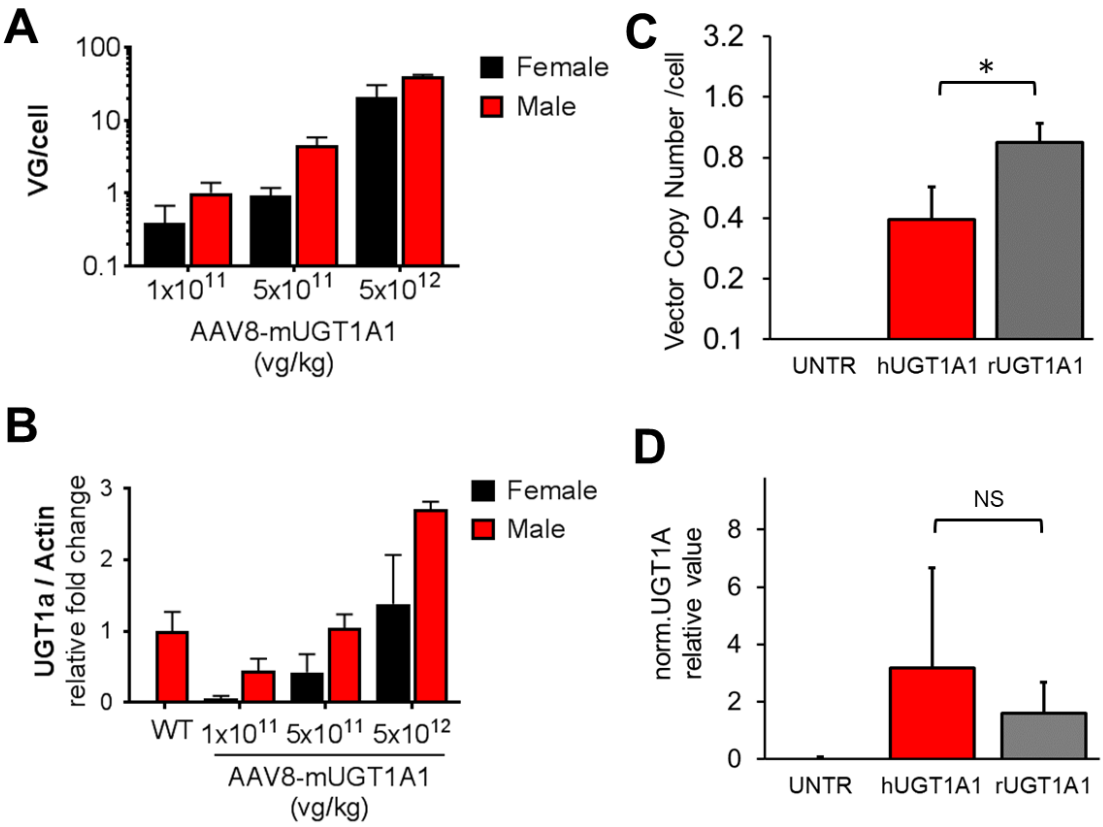

Figure S6. Expression of species-specific UGT1A1 transgene in liver of CN rodent models.
(A) Viral genome copy number (VG/cell) in male and female mice 9 months after treatment with different doses of rAAV8-mUGT1A1 and **(B)** UGT1A1 protein quantification in the liver by Western blot analysis of liver total protein extracts. Endogenous mouse UGT1 protein levels in WT mice where considered as reference. 8 week-old Gunn rats were injected i.v. with PBS (UNTR) or with $5x10^{12}$ vg/kg of (ss)AAV8-UGT1A1 vectors encoding for human UGT1A1 (hUGT1A1) or rat UGT1A1 (rUGT1A1). **(C)** Vector genome copy number, and UGT1A1 protein expression by Western blot **(D)** in livers of rats analyzed 186 days post treatment. Data are plotted as mean ± SD. Statistical analyses were performed by ANOVA (*=$p<0.05$, NS not significant).

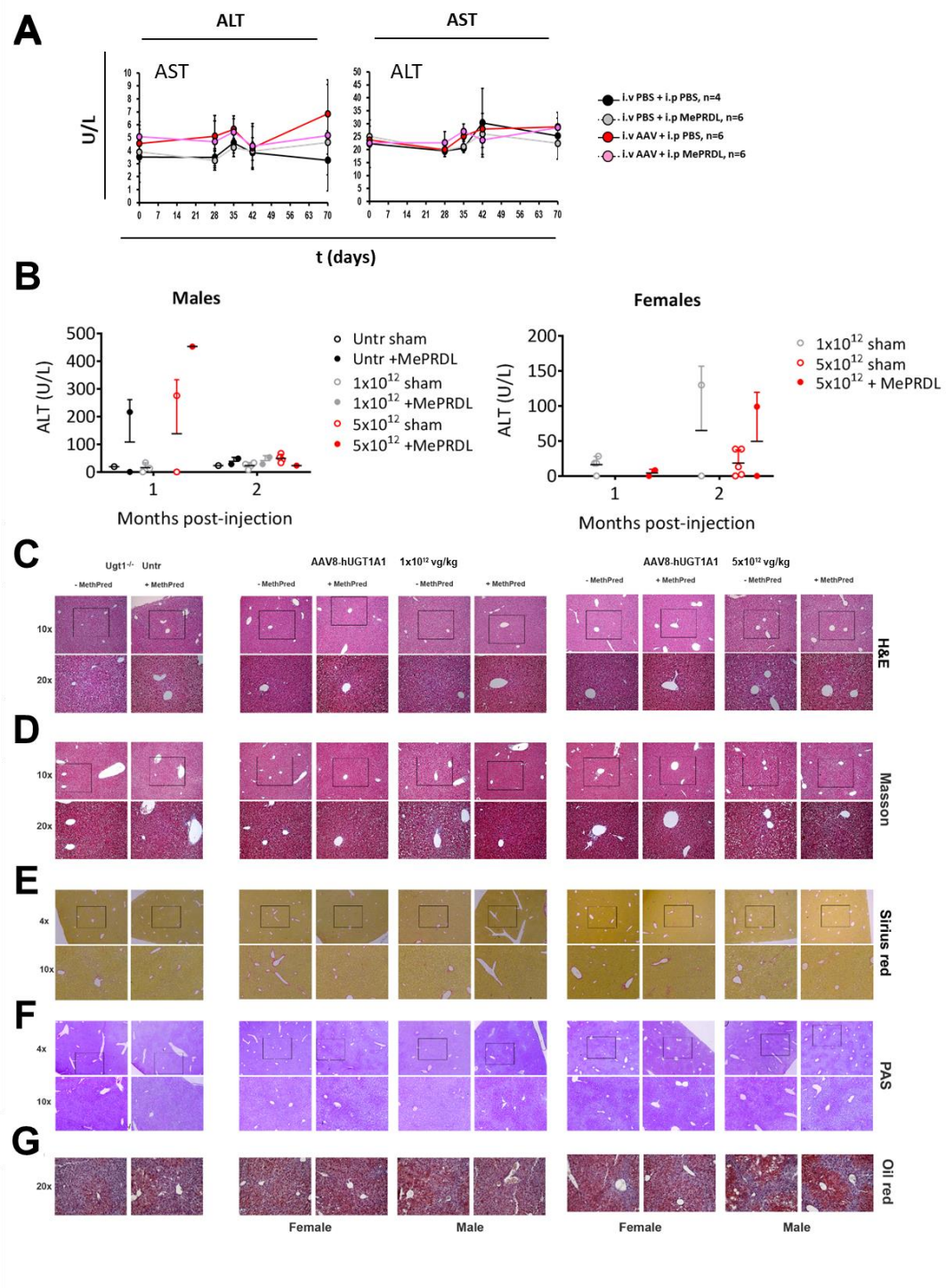

Figure S7. Corticosteroid treatment safety in CN animal models.
(A) Plasma transaminases levels in CN rat model. Gunn rats were injected with PBS (black) or $5x10^{12}$vg/kg of (ss)AAV8-UGT1A1 (red) at day 0. One month after the treatment, a 15 days tapered dose of methyl-prednisolone (MePRDL) was administered intraperitoneally in half of the rats (grey and pink). Rats were weekly bled and sacrificed 3 months after the first injection. Values of activity of AST and ALT enzymes are plotted versus time. Data are expressed as mean ± standard error. **(B-G)** $Ugt1^{-/-}$ mice were injected as adults with the indicated doses (vg/kg). Corticosteroid treatment was initiated the day before the injection of the AAV vector and lasted for 5 days at decreasing doses (5.0, 2.5, 1.3, 0.6 and 0.3 mg/kg). Mice were monthly bled and sacrificed 9 months after the first injection. **(B)** Plasma transaminases levels in CN mouse model. ALT levels (U/m) at 1 and 2 months post-injection, as mean+SD. Each dot represents a single animal. **(C)** Representative images of H&E; **(D)** Masson trichrome; **(E)** Sirius red; **(F)** PAS and **(G)** Oil red stainings. $Ugt1^{-/-}$ untreated animals where used as control. Rectangles show magnification