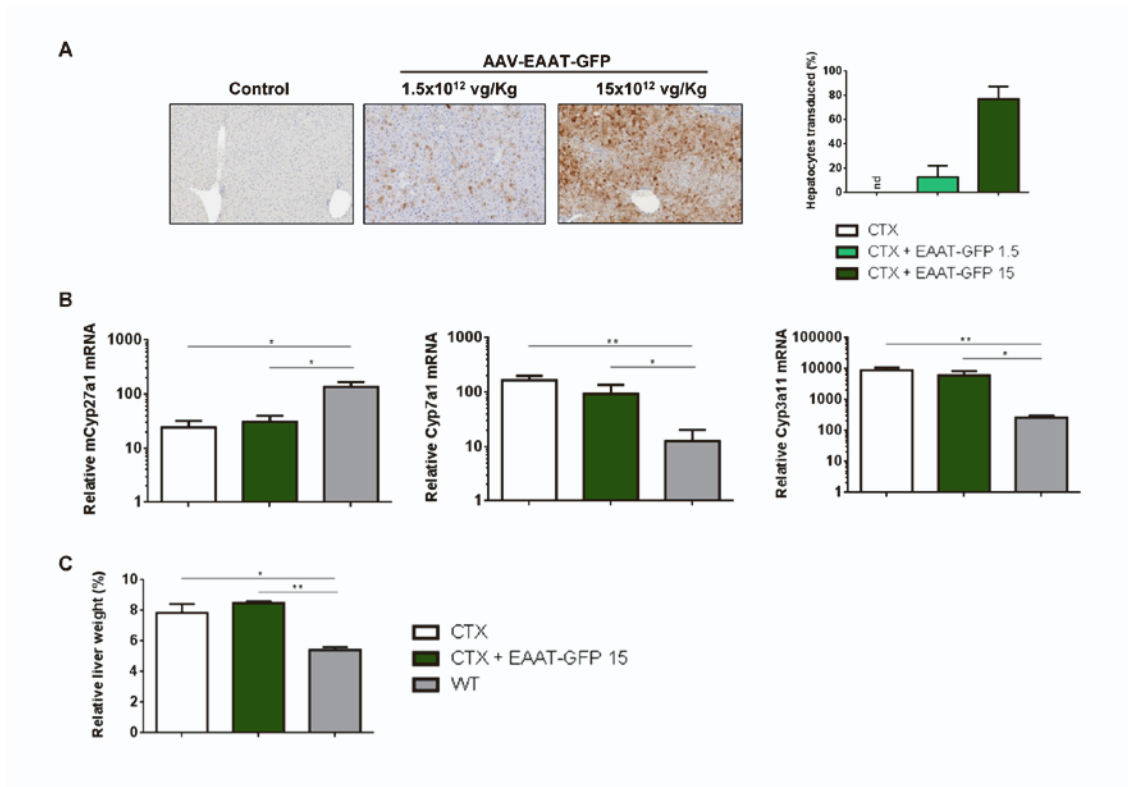


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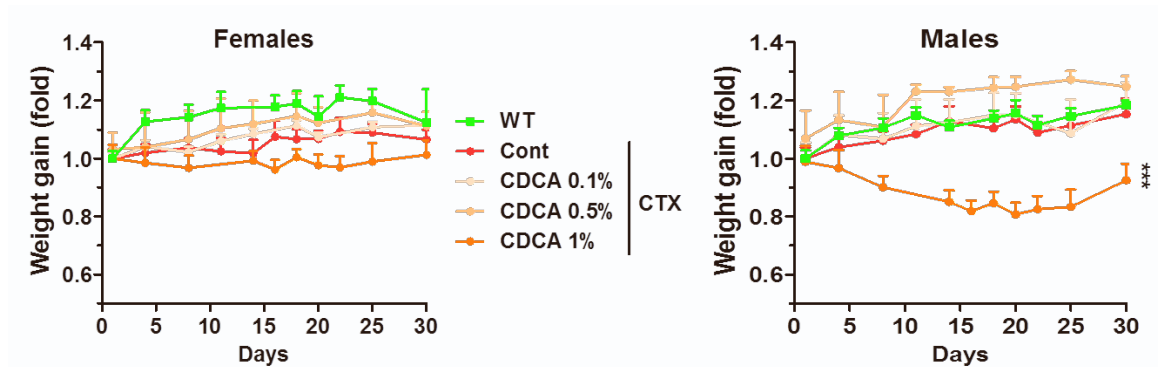
## **Supplemental information**

### **Gene supplementation of *CYP27A1* in the liver restores bile acid metabolism in a mouse model of cerebrotendinous xanthomatosis**

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**Supplemental figure 1. The control vector AAV-EAAT-GFP shows dose-dependent transduction of mouse liver but has no effect on the expression of bile acid biosynthesis enzymes.** The AAV-EAAT-GFP vector was administered intravenously to 7 week-old CTX mice at the indicated doses ( $\times 10^{12}$  vg/Kg). Mice were sacrificed 2 weeks later, and livers samples were processed for anti-GFP immunohistochemistry (**A**) and quantification of mRNA expression of the indicated genes by qRT-PCR (**B**). Cyp27a1 was amplified using primers targeting exons 1/2. **C**. Liver weight relative to body weight (%). nd, not detected. \* $p < 0.05$ . Bars represent means  $\pm$  SEM for each group. \*\* $p < 0.01$ . Kruskal-Wallis with Dunn's post-test.



**Supplemental figure 2. A diet based on 1% CDCA causes weight loss in CTX mice.** Seven week-old female and male CTX mice were fed a diet supplemented with the indicated CDCA percentages, and body weight was monitored for one month. Untreated CTX and WT littermates were included as a reference. Symbols represent means  $\pm$  SEM for each group. \*\*\* $p < 0.001$  linear regression, control vs CDCA 1%.

**Supplemental table 1.** List of antibodies. B, Bovine; H, Human; M, Mouse; Mk, Monkey; Pg, Pig; R, Rat; Rb, Rabbit.

<b>Primary antibodies</b>	<b>Species reactivity</b>	<b>Source</b>	<b>Catalog number</b>
CYP27A1	H M R	Abcam	ab126785
GAPDH	H M R Mk B Pg	Cell Signaling Technology	3683
$\beta$ -catenine	H M R Mk	Cell Signaling Technology	8480
Ki67	H M	Neomarkers	RM9106
<b>Secondary antibodies</b>	<b>Species reactivity</b>	<b>Source</b>	<b>Catalog number</b>
IgG HRP conjugated	Rb	GE Healthcare	NA934V
IgG HRP conjugated	Rb	DAKO	K4003

**Supplementary table 2.** Concentration ( $\mu\text{M}$ ) of the main bile acids in plasma of CTX mice treated with AAV8-EAAT-CYP27A1 or 0.5% CDCA. The AAV8-EAAT-CYP27A1 vector was administered intravenously to 7 week-old CTX mice at the indicated doses ( $\times 10^{12}$  vg/Kg). CDCA was mixed in mouse chow at 0.5%. Untreated CTX and WT littermates were included as a reference. Blood was collected 5 months after the initiation of treatment, and the concentration of the main bile acids (free, tauroconjugated and glycoconjugated) was analyzed in plasma (expressed in  $\mu\text{M}$ ). See abbreviation list for full names.

	WT	CTX	CTX EAAT 1.5	CTX EAAT 15	CTX 0.5% CDCA
<b>CA</b>	0,353 $\pm$ 0,267	0,165 $\pm$ 0,094	0,872 $\pm$ 1,250	1,101 $\pm$ 1,112	0,150 $\pm$ 0,201
<b>TCA</b>	0,316 $\pm$ 0,265	0,245 $\pm$ 0,429	1,267 $\pm$ 0,888	1,817 $\pm$ 0,939	0,003 $\pm$ 0,004
<b>GCA</b>	0,003 $\pm$ 0,003	0,001 $\pm$ 0,002	0,006 $\pm$ 0,004	0,028 $\pm$ 0,013	0,001 $\pm$ 0,001
<b>DCA</b>	0,244 $\pm$ 0,135	0,034 $\pm$ 0,014	0,431 $\pm$ 0,364	0,392 $\pm$ 0,149	0,005 $\pm$ 0,006
<b>TDCA</b>	0,102 $\pm$ 0,090	0,004 $\pm$ 0,004	0,149 $\pm$ 0,096	0,199 $\pm$ 0,079	0,009 $\pm$ 0,013
<b>GDCA</b>	0,000 $\pm$ 0,000	0,000 $\pm$ 0,000	0,000 $\pm$ 0,000	0,000 $\pm$ 0,000	0,000 $\pm$ 0,000
<b>CDCA</b>	0,055 $\pm$ 0,038	0,026 $\pm$ 0,010	0,053 $\pm$ 0,037	0,081 $\pm$ 0,070	58,620 $\pm$ 38,732
<b>TCDCa</b>	0,035 $\pm$ 0,021	0,021 $\pm$ 0,033	0,083 $\pm$ 0,054	0,088 $\pm$ 0,054	1,755 $\pm$ 2,418
<b>GCDCA</b>	0,000 $\pm$ 0,000	0,000 $\pm$ 0,000	0,000 $\pm$ 0,000	0,000 $\pm$ 0,000	0,000 $\pm$ 0,000
<b>UDCA</b>	0,109 $\pm$ 0,111	0,023 $\pm$ 0,031	0,173 $\pm$ 0,217	0,154 $\pm$ 0,133	30,160 $\pm$ 31,113
<b>TUDCA</b>	0,079 $\pm$ 0,061	0,006 $\pm$ 0,007	0,112 $\pm$ 0,087	0,150 $\pm$ 0,102	0,551 $\pm$ 0,828
<b>GUDCA</b>	0,000 $\pm$ 0,000	0,000 $\pm$ 0,000	0,000 $\pm$ 0,000	0,000 $\pm$ 0,000	0,000 $\pm$ 0,000
<b>LCA</b>	0,025 $\pm$ 0,015	0,020 $\pm$ 0,018	0,023 $\pm$ 0,059	0,019 $\pm$ 0,009	5,017 $\pm$ 3,131
<b>TLCA</b>	0,002 $\pm$ 0,001	0,002 $\pm$ 0,002	0,002 $\pm$ 0,002	0,002 $\pm$ 0,002	0,081 $\pm$ 0,030
<b>TSLCA</b>	0,000 $\pm$ 0,000	0,000 $\pm$ 0,000	0,000 $\pm$ 0,000	0,000 $\pm$ 0,000	0,445 $\pm$ 0,036
<b>GLCA</b>	0,000 $\pm$ 0,000	0,000 $\pm$ 0,000	0,000 $\pm$ 0,000	0,000 $\pm$ 0,000	0,000 $\pm$ 0,000
<b><math>\alpha</math>MCA</b>	0,503 $\pm$ 0,357	0,132 $\pm$ 0,113	0,617 $\pm$ 0,744	0,608 $\pm$ 0,387	8,236 $\pm$ 5,699
<b><math>\beta</math>MCA</b>	0,373 $\pm$ 0,294	0,114 $\pm$ 0,066	0,593 $\pm$ 0,730	1,074 $\pm$ 0,928	7,923 $\pm$ 7,072
<b>TMCAs</b>	0,339 $\pm$ 0,221	0,055 $\pm$ 0,026	0,565 $\pm$ 0,530	1,345 $\pm$ 0,962	0,532 $\pm$ 0,586
<b>HDCA</b>	0,025 $\pm$ 0,023	0,010 $\pm$ 0,006	0,050 $\pm$ 0,066	0,019 $\pm$ 0,011	1,112 $\pm$ 1,525
<b>THDCA</b>	0,020 $\pm$ 0,019	0,001 $\pm$ 0,001	0,018 $\pm$ 0,012	0,040 $\pm$ 0,028	0,030 $\pm$ 0,020
<b>THCA</b>	0,000 $\pm$ 0,000	0,000 $\pm$ 0,000	0,000 $\pm$ 0,000	0,000 $\pm$ 0,000	0,000 $\pm$ 0,000
<b>Total BAs</b>	2,583	0,860	5,013	7,119	114,629