

Figure S1. Cripto expression in patients with end-stage heart failure. Cardiac tissue samples of patients with end-stage heart failure (n=5) were stained for CRIPTO (A) α SMA (B) and collagen type I (C). Scale bar 100 μ m.

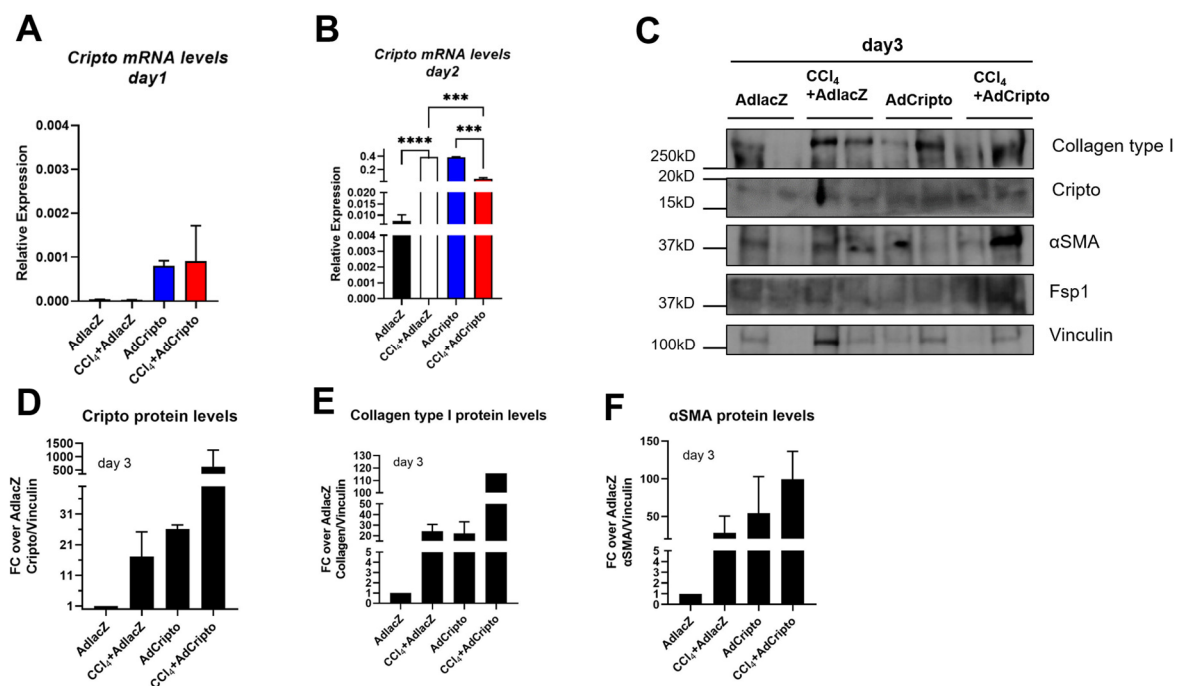


Figure S2. Cripto overexpression in liver tissues increases protein levels of fibrotic markers. (A-B) mRNA expression levels of *Cripto* normalized to β -actin in the AdCripto+/-CCl₄, AdlacZ+/-CCl₄ liver tissues at day 1 (A) and day 2 post CCl₄ administration. (B) Relative expression \pm SEM among n=2 biological replicates per time point and per group. Three technical measurements were obtained per replicate. Ordinary two-way ANOVA, ***p \leq 0.001, ****p \leq 0.0001. (C) Protein levels of Collagen type I, CRIPTO, α SMA and Fibroblast-specific protein-1 (FSP1) measured by Western blotting in liver tissue FFPE extracts of the AdCripto+/-CCl₄ and AdlacZ+/-CCl₄ groups. N=2 biological replicates per group. Time point: day 3 post toxin administration. Vinculin was used as protein loading control. Representative of two independent experiments. (D-F) Quantification of protein levels of CRIPTO (D), COLLAGEN Type I (E) and α SMA (F) based on the Western blotting of panel (C). Data were obtained after normalization to the mean intensity of the n=2 biological replicates of the control group (AdlacZ), and to the intensity of the protein loading control (vinculin) for every sample. Mean fold change over AdlacZ \pm SEM (n=2).

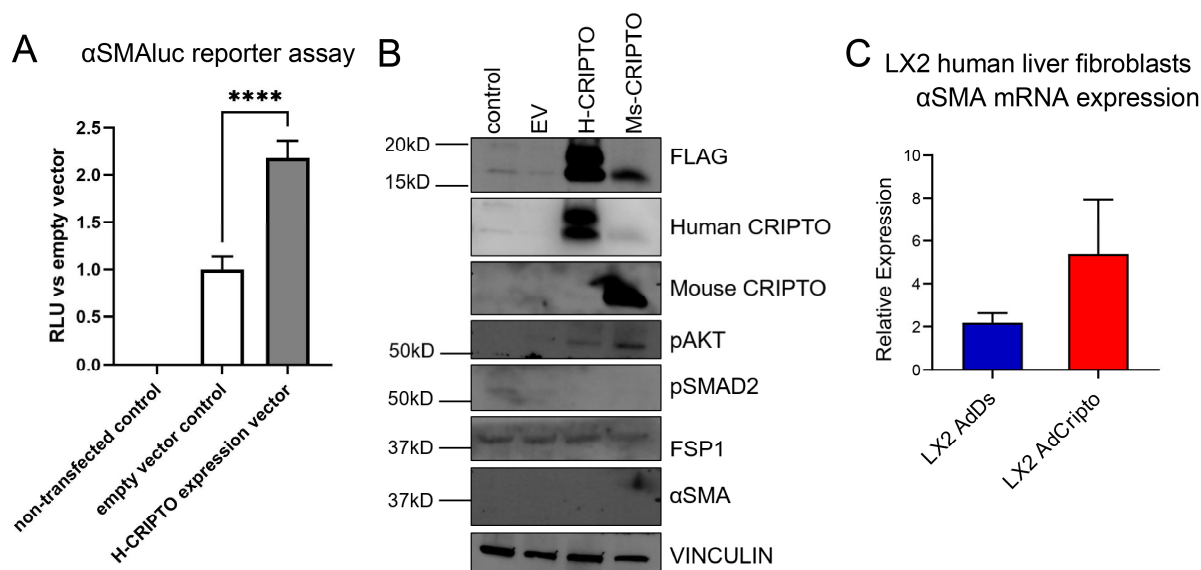


Figure S3. CRIPTO is an upstream regulator of αSMA expression. (A) *In vitro* αSMA luciferase reporter assay on 293T cells, transfected with empty vector or full length human CRIPTO-expressing plasmid (H-CRIPTO). Relative Luciferase (RLU) measurements were obtained 48 hours after transfection and normalized to the empty vector control. Mean±SD from quadruplicate technical replicate measurements. Unpaired t-test. ****p≤0.0001. (B) Protein levels measured by Western blotting in 293T cells transfected with empty vector, mouse CRIPTO-FLAG (Ms-CRIPTO) plasmid, human CRIPTO-FLAG (H-CRIPTO) plasmid. Proteins detected: human/mouse CRIPTO, FLAG (to detect CRIPTO-FLAG protein of the mouse and human expression vectors), phosphorylated AKT (pAKT), phosphorylated SMAD2 (pSMAD2), Fibroblast-specific protein-1 (FSP1) and αSMA. Time point: 48 hours after transfection. Vinculin was used as protein loading control. Representative of two independent experiments. (C) αSMA mRNA expression in LX2 human liver fibroblast cell line after adenoviral-mediated CRIPTO (AdCRIPTO) overexpression *in vitro*. AdDs; control adenovirus containing dsRED fluorescent protein.

Table S1. Patient characteristics. Data presented as median (range) for continuous variables and percentage (number) for categorized variables.

Variable	Healthy controls (N=16)	Pre-LT (N=45)	Post-LT (N=45)
Gender (male), % (n)			78% (35)
Age (median, range)	50% (8) 29 (23-65)		54 (42-69)
Aetiology			
- Alcoholic liver disease			25
- Viral Hepatitis			20
Blood (median, range)			
- AST (U/L)		72 (24-517)	27 (11-240)
- ALT (U/L)		37 (15-360)	25 (8-401)
- INR		1.2 (1-2.4)	1.0 (0.9-2.4)
- ALP (U/L)		130 (50-555)	88 (47-487)
- Creatinin ($\mu\text{mol/L}$)		92 (34-171)	111 (68-204)
- γGT (U/L)		43 (7-374)	39 (9-1395)
- Sodium (mmol/L)		138 (124-156)	142 (134-148)
- Bilirubin ($\mu\text{mol/L}$)		46 (5-593)	12 (5-29)
- Platelet count ($10^9/\text{L}$)		72 (30-142)	144 (93-243)
Cripto plasma (pg/ml)		1381 (0-12108)	357 (0-5314)
Clinical scores	0 (0-818)		
- MELD		15 (8-33)	10 (6-18)

LT = Liver Transplantation, ALD = Alcoholic Liver Disease, AST = Aspartate aminotransferase, ALT = Alanine aminotransferase, INR = International Normalized Ratio, ALP = Alkaline phosphatase

Table S2. Primer sequences.

Gene	Forward	Reversed
<u>Human</u>		
α -smooth muscle actin	TTGCCTGATGGGCAAGTGAT	GTGGTTTCATGGATGCCAGC
Collagen-1 α 1	GGAACCTGGGGCAAGACAGT	GAGGGAACCAGATTGGGGTG
Cripto (human)	CACGATGTGCGCAAAGAGAA	TGACCGTGCCAGCATTTACA
β -actin (human)	AATGTCGCGGAGGACTTTGATTGC	GGATGGCAAGGGACTTCCTGTAAA
<u>Mouse</u>		
α -smooth muscle actin	GTCCCAGACATCAGGGAGTAA	TCGGATACTTCAGCGTCAGGA
Collagen-1 α 1	GTGGAAACCCGAGCCCTGCC	TCCCTTGGGTCCCTCGACGC
Cripto (mouse)	CGCCAGCTAGCATAAAAGTG	CCCAAGAAGTGTTCCCTGTG
β -actin (mouse)	GGGGTGTTGAAGGTCTCAAA	AGAAAATCTGGCACCCC