Pathways for the regulation of hepcidin expression in anemia of chronic disease and iron deficiency anemia *in vivo*

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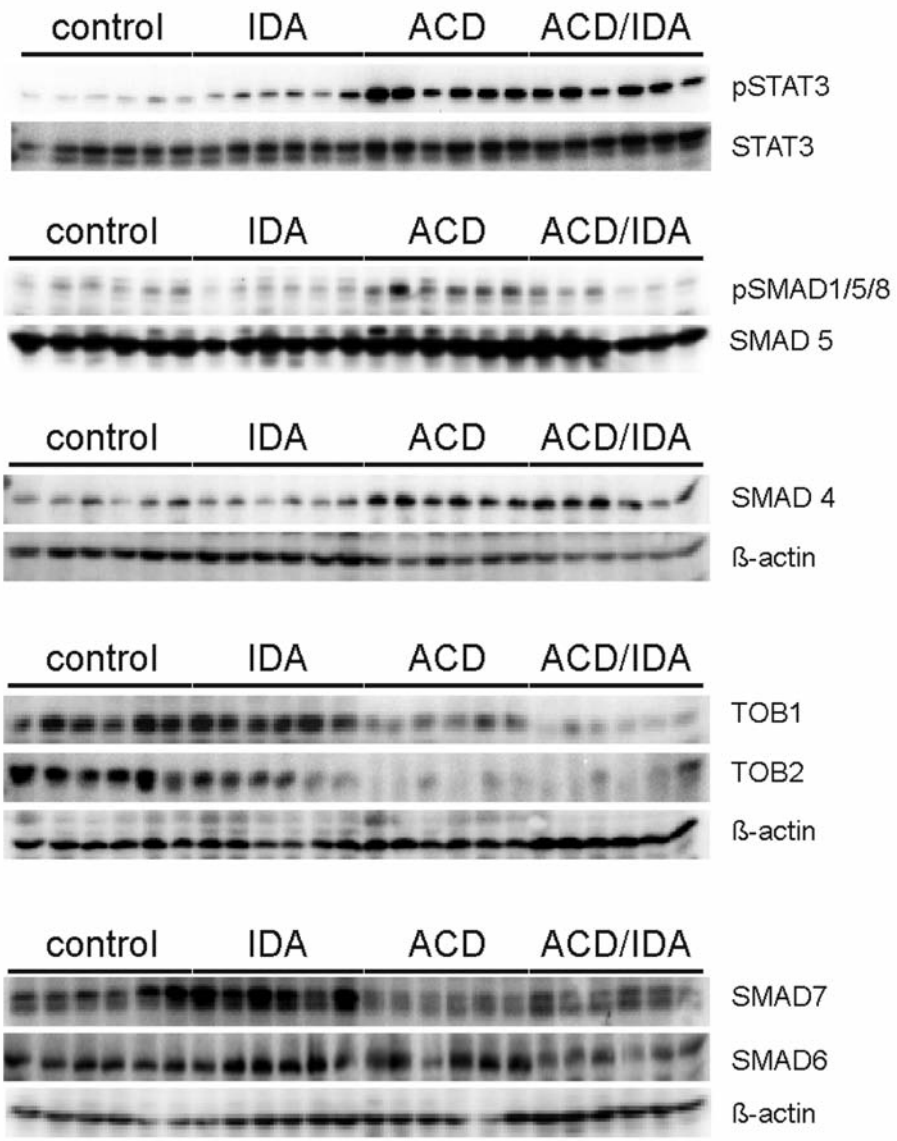
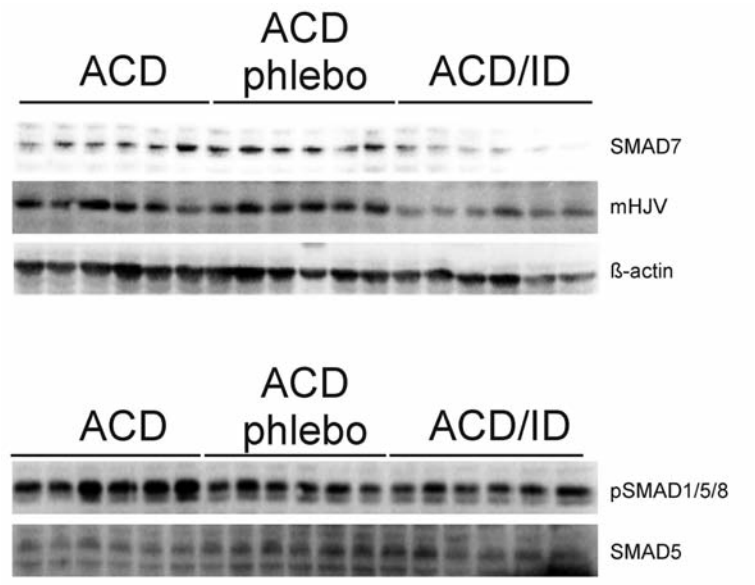
Online Supplementary Figure S1. Western blots for densitometric quantification.



ACD was induced by i.p. injection of PG-APS (n=6) as detailed in the Design and Methods *section or left untreated (control) (n=6). One group of PG-APS treated (n=6) and one of solvent treated rats (n=6) were phlebotomized, starting one week before sacrifice, to create a combination of ACD and iron deficiency anemia (ACD/IDA) or IDA alone, respectively. Liver tissue samples were subjected to immunoblot analysis using antibodies against pSTAT3, pSMAD1/5/8 SMAD1, SMAD4, SMAD6, SMAD7, TOB1, TOB2 and β-actin. Protein levels were quantified by densitometry using Quantity One Basic software (Bio-Rad, CA) See Figures 1 and 2.*

Online Supplementary Figure S2. Western blots for densitometric quantification.



ACD was induced by i.p. injection of PG-APS as detailed in the Design and Methods *section or left untreated (control) (n=6). One group of PG-APS treated rats was phlebotomized (ACD phlebo; n=6), starting one week before sacrifice, whereas another group of rats was put on an iron deficient diet one week before PG-APS administration (ACD/ID; n=6). Liver tissue samples from the three groups of animals were subjected to immunoblot analysis using antibodies against pSMAD1/5/8, SMAD5, SMAD7, mHJV and β-actin. Protein levels were quantified by densitometry using Quantity One Basic software (Bio-Rad, CA). See Figure 4.*

Online Supplementary Table S1.

	Control	ACD	ACD/ phlebotomy	ACD/iron def.diet
Rel. abundance Hepcidin/Gus-b mRNA	0.69(±0.24)	1.79(±0.80)*	0.25(±0.10)[%]	0.37(±0.10)[%]
Epo serum levels [pg/ml]	n.d.	358(±380)	2821(±1181)[%]	858(±763)[-]

*All parameters were tested for normality by Kolmogorov-Smirnov test. All parameters followed a Gaussian distribution. Calculations for statistical differences between the various groups were carried out by ANOVA with Bonferroni's correction for multiple tests. Data are shown as mean (±SD) for each group. *P<0.001 or when comparing control with ACD, ACD/phlebotomy or ACD/iron def.diet, respectively. [%]P<0.001 when comparing ACD with ACD/phlebotomy or ACD/iron def.diet, respectively. [-]P<0.001 when comparing ACD/phlebotomy with ACD/iron def.diet. n.d.: not detectable*