

# Supplemental material

## Hemojuvelin mediated hepcidin induction requires both bone morphogenetic protein type I receptors ALK2 and ALK3

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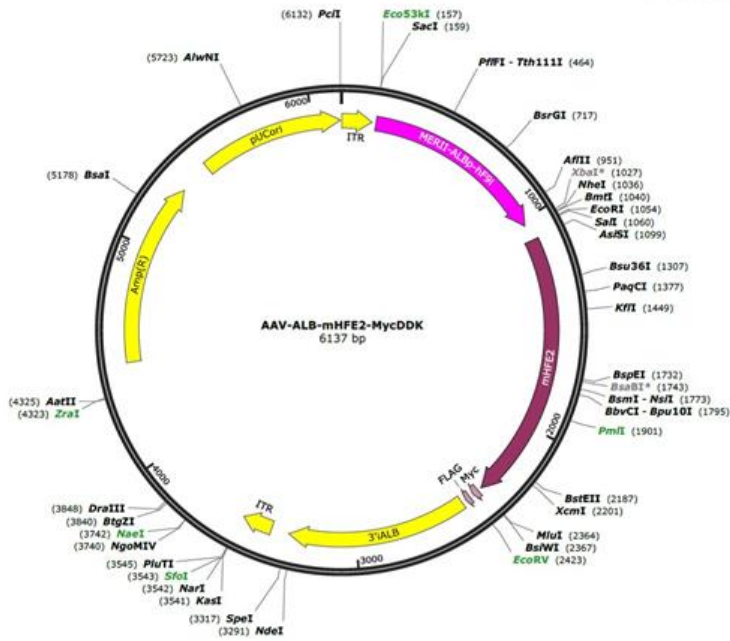
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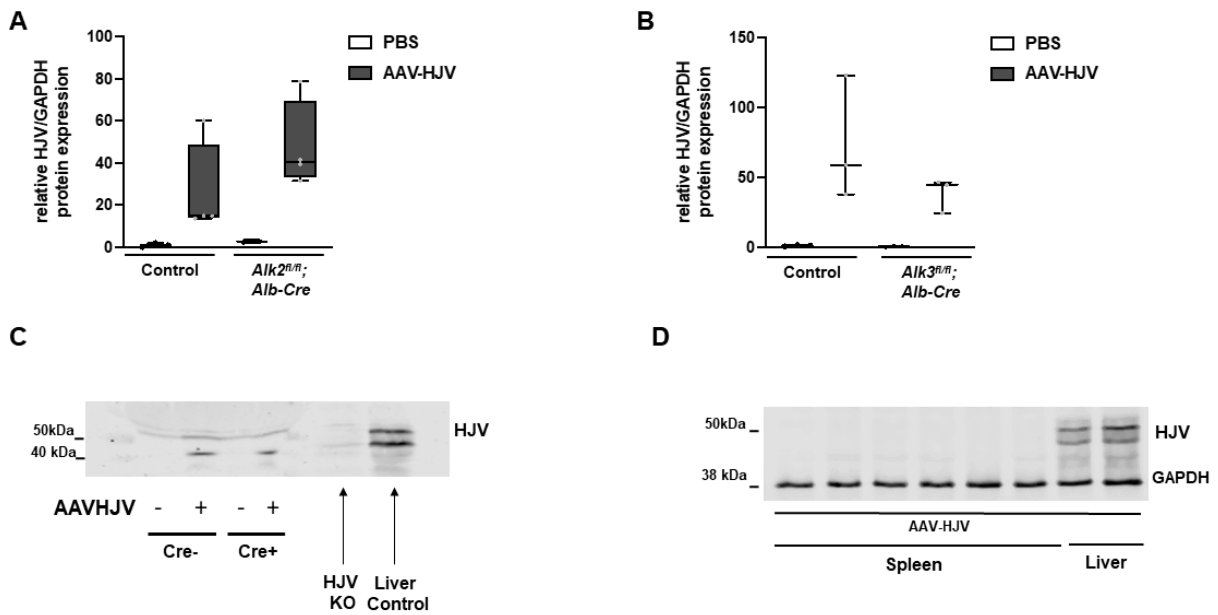
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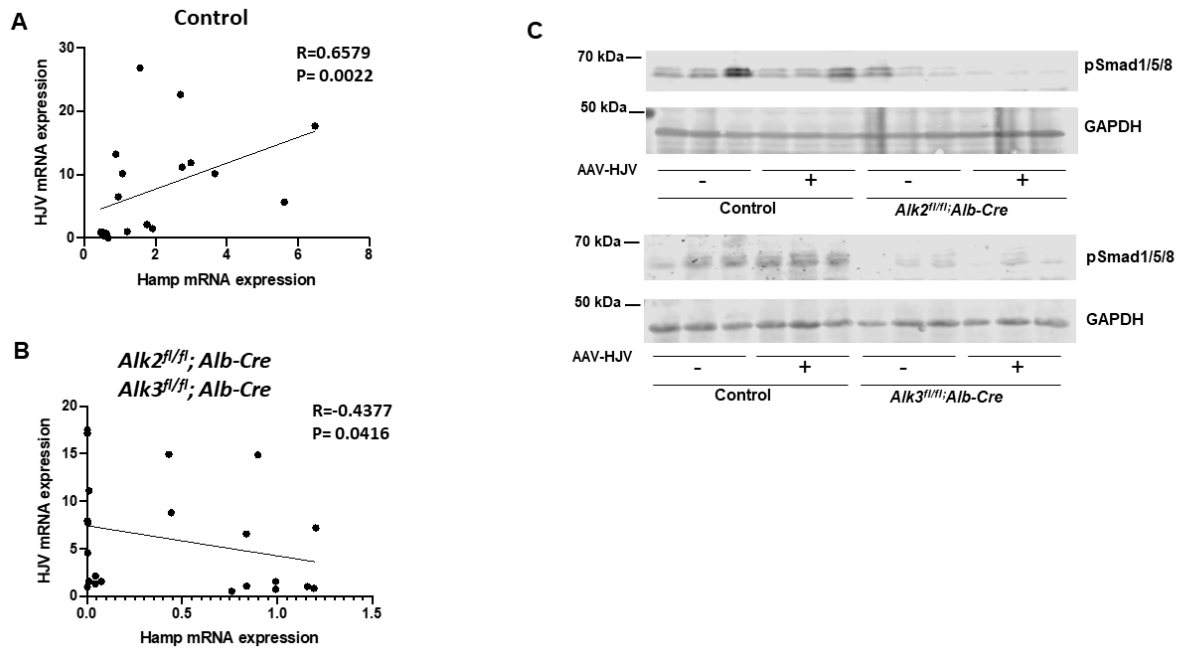
### Supplemental Figure 1

**AAV2/8-ALB-mHFE2-MycDDK vector map.** The AAV vector map was provided by Vector Biolabs and was created with SnapGene. *Hjv-MycDDK* is expressed under the control of a liver-specific promoter (ALB). MycDDK-tag is located at the C-terminus of HJV.



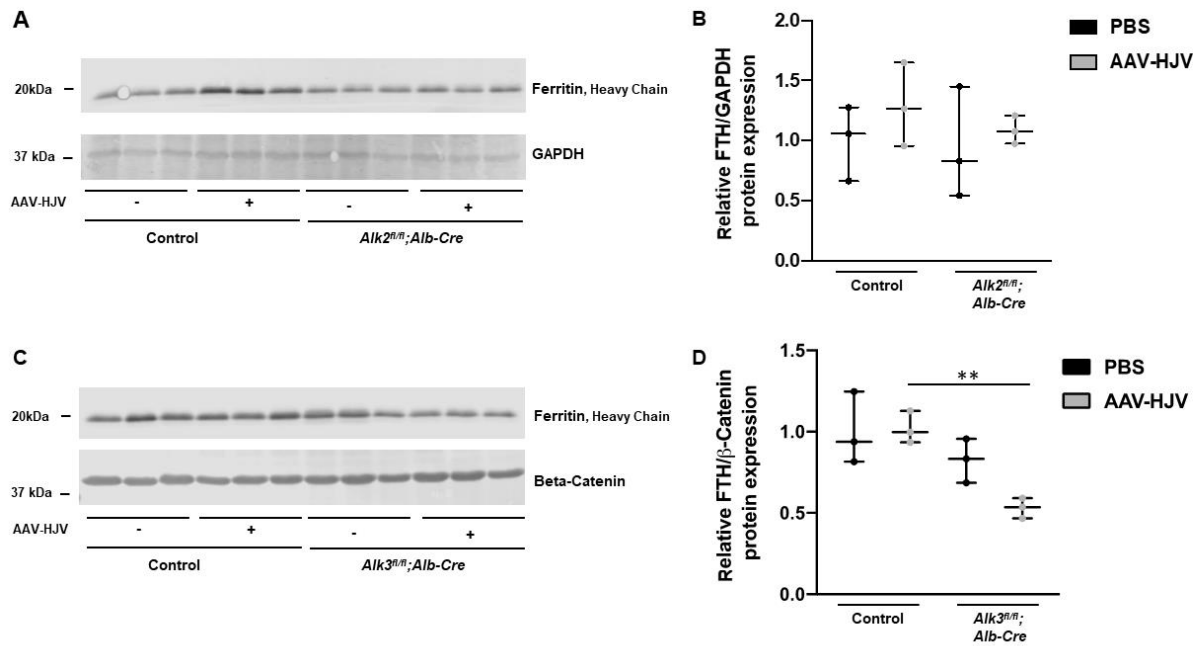
## Supplemental Figure 2

**AAV2/8-ALB-mHFE2-MycDDK induces liver specific HJV expression.** (A) HJV protein level in AAV-HJV injected hepatocyte-specific *Alk2*-deficient mice and their control littermates and (B) hepatocyte-specific *Alk3*-deficient mice and their control littermates were determined to validate the hepatic overexpression of HJV. GAPDH was used as internal control. Relative HJV protein expression was determined by densitometric analysis of immunoblots (n: *Alk2<sup>fl/fl</sup>* PBS = 3, *Alk2<sup>fl/fl</sup>; Alb-Cre* PBS= 3, *Alk2<sup>fl/fl</sup>* AAV-HJV = 4, *Alk2<sup>fl/fl</sup>; Alb-Cre* AAV-HJV= 4, *Alk3<sup>fl/fl</sup>* PBS= 3, *Alk3<sup>fl/fl</sup>; Alb-Cre* PBS=3, *Alk3<sup>fl/fl</sup>* AAV-HJV= 3, *Alk3<sup>fl/fl</sup>; Alb-Cre* AAV-HJV=3). (C) HJV protein level were detected in sera of Cre- and Cre+ mice overexpressing HJV. A liver sample of an AAV-HJV injected animal and a liver sample of *Hjv* KO mouse was used as control. (D) Splenic and hepatic HJV protein level in AAV-HJV injected mice were determined by immunoblotting to validate a liver specific HJV overexpression. GAPDH was used as internal control.



### Supplemental Figure 3

***HJV* mRNA levels correlate with *Hamp* mRNA levels in control mice. (A)** *Hjv* mRNA expression positively correlated with *Hamp* mRNA levels in control mice (R=0.6579). Every mouse represents one data point (n=23). **(B)** In contrast, there was no correlation between *Hjv* mRNA expression and *Hamp* mRNA in *Alk2<sup>fl/fl</sup>; Alb-Cre* and *Alk3<sup>fl/fl</sup>; Alb-Cre* mice (R=-0.4377). Every mouse represents one data point (n=21). **(C)** Phosphorylated SMAD1/5/8 levels were determined by immunoblotting and showed that after HJV overexpression there was little signaling activity in *Alk2<sup>fl/fl</sup>; Alb-Cre* mice, but no activity in *Alk3<sup>fl/fl</sup>; Alb-Cre* mice (n=3 per group).



#### Supplemental Figure 4

#### HJV overexpression effects Ferritin, Heavy Chain, expression in *Alk3<sup>fl/fl</sup>; Alb-Cre* mice.

**(A)** Ferritin, Heavy chain (FTH), protein expression in *Alk2<sup>fl/fl</sup>; Alb-Cre* mice was determined via immunoblotting. GAPDH was used as internal control (n =3 per group). **(B)** Relative FTH protein expression was determined by densitometric analysis of immunoblots. HJV overexpression had no effects on FTH protein levels in *Alk2<sup>fl/fl</sup>; Alb-Cre* mice. **(C)** Ferritin, Heavy chain (FTH), protein expression in *Alk3<sup>fl/fl</sup>; Alb-Cre* mice was determined via immunoblotting. Beta-Actin was used as internal control (n =3 per group). **(D)** Relative FTH protein expression was determined by densitometric analysis of immunoblots. In *Alk3<sup>fl/fl</sup>; Alb-Cre* mice, HJV overexpression further decreased FTH protein levels.

#### Supplemental Table 1. Primers used for quantitative Reverse-Transcriptase PCR

<i>mHamp</i>	F : 5'-AAGCAGGGCAGACATTGCGAT-3' R : 5'-CAGGATGTGGCTCTAGGCTATGT-3'
<i>mHJV</i>	F : 5'-ATGGGCCAGTCCCCTAGTC-3' R : 5'-GACGAGACATACTCGGCATTG-3'
<i>m18S</i>	F : 5'-CGGCTACCACTCCAAGGAA-3' R : 5'-GCTGGAATTACCGCGGCT-3'
<i>mld1</i>	F : 5'-TCTGTCCGAGCAAAGCGTGGCC-3' R : 5'-CCGGTGGTCCCGACTTCAGACT-3'
<i>mErfe</i>	F : 5'-ATGGGGCTGGAGAACAGC-3' R : 5'-TGGCATTGTCCAAGAAGACA-3'
<i>mBMP-6</i>	F : 5'-ATGGCAGGACTGGATCATTGC-3' R : 5'-CCATCACAGTAGTTGGCAGCG-3'
Alk2 (Taqman)	Mm00431646_m1 Acvr1 FAM

Alk3 (Taqman)	Bmpr1a- Mm00477650_m1 Bmpr1a
18S (Taqman)	RN 18S-Mm03928990_g1