A novel validated enzyme-linked immunosorbent assay to quantify soluble hemojuvelin in mouse serum

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 doi:10.3324/haematol.2012.070136

Online Supplementary Design and Methods

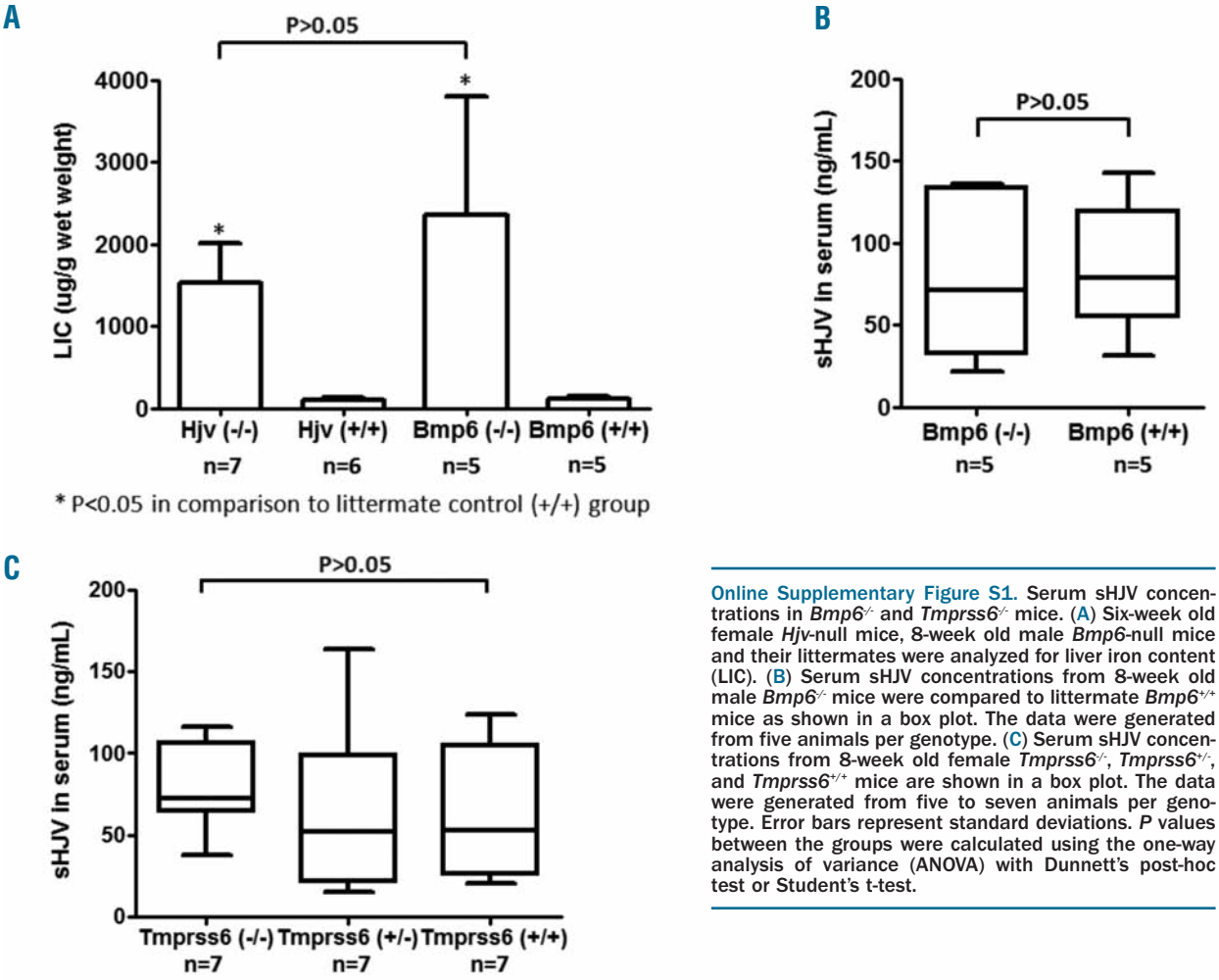
Animals

Serum from 8-week old male *Bmp6*-null mice on a mixed 129Sv/C57 background[1] was kindly provided by Dr. E.J. Robertson. The mice were housed at the University of Zagreb School of Medicine and maintained on a standard GLP diet (4RF21, Mucedola).

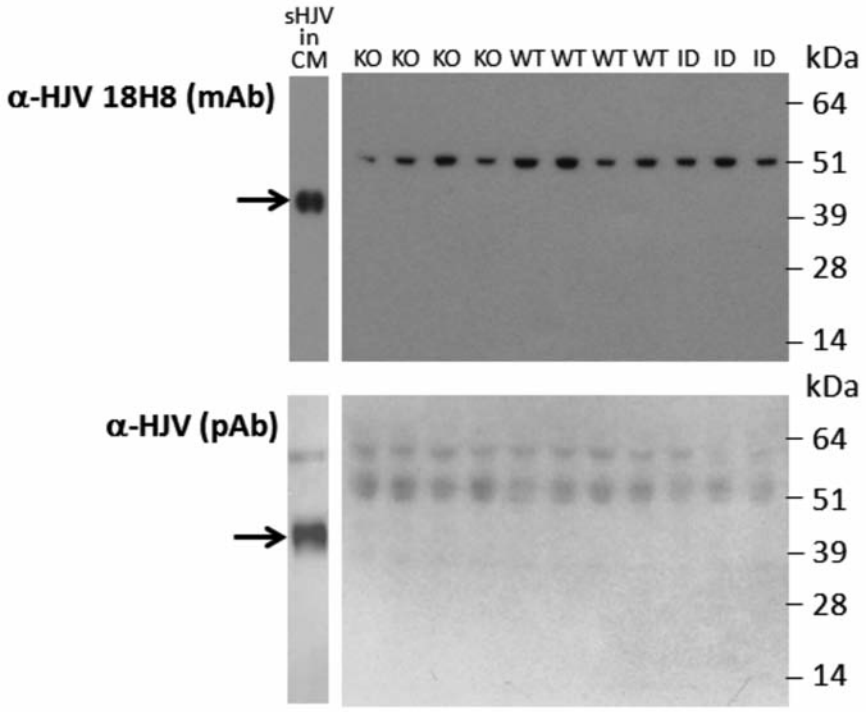
Tmprss6$^{-/-}$, *Tmprss6*$^{+/-}$, and *Tmprss6*$^{+/+}$ mice were generated by breeding *Tmprss6*$^{+/-}$ to *Tmprss6*$^{+/-}$ mice on a mixed 129/C57 background[2] (animals kindly provided by Dr. Carlos Lopez-Otin). These mice were fed on a standard rodent diet (380 ppm iron). Serum was collected from 8-week old female mice.

References

1. Solloway MJ, Dudley AT, Bikoff EK, Lyons KM, Hogan BL, Robertson EJ. Mice lacking Bmp6 function. Dev Genet. 1998;22(4):321-39.
2. Folgueras AR, de Lara FM, Pendas AM, Garabaya C, Rodriguez F, Astudillo A, et al. Membrane-bound serine protease matriptase-2 (Tmprss6) is an essential regulator of iron homeostasis. Blood. 2008;112(6):2539-45.

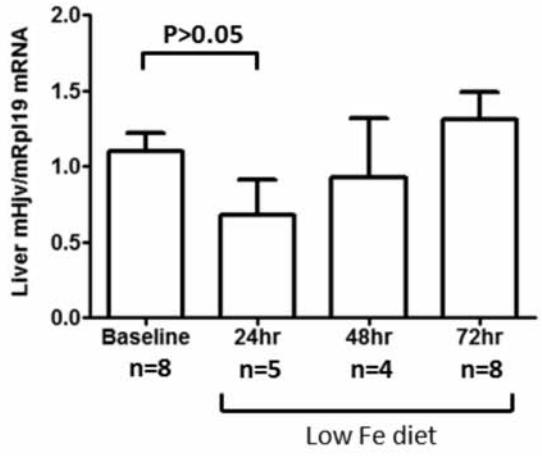


Online Supplementary Figure S1. Serum sHJV concentrations in *Bmp6*$^{-/-}$ and *Tmprss6*$^{-/-}$ mice. (A) Six-week old female *Hjv*-null mice, 8-week old male *Bmp6*-null mice and their littermates were analyzed for liver iron content (LIC). (B) Serum sHJV concentrations from 8-week old male *Bmp6*$^{-/-}$ mice were compared to littermate *Bmp6*$^{+/+}$ mice as shown in a box plot. The data were generated from five animals per genotype. (C) Serum sHJV concentrations from 8-week old female *Tmprss6*$^{-/-}$, *Tmprss6*$^{+/-}$, and *Tmprss6*$^{+/+}$ mice are shown in a box plot. The data were generated from five to seven animals per genotype. Error bars represent standard deviations. *P* values between the groups were calculated using the one-way analysis of variance (ANOVA) with Dunnett's post-hoc test or Student's t-test.

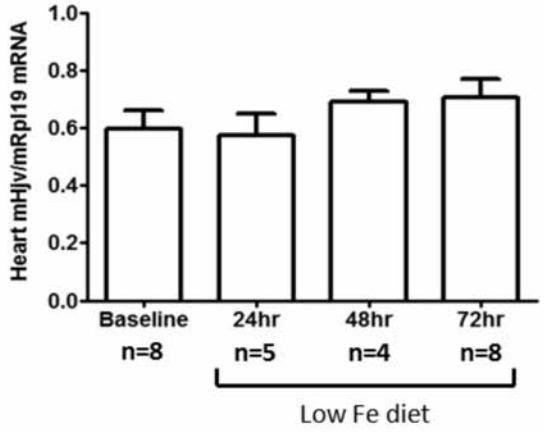


Online Supplementary Figure S2. Western blot analysis of mouse serum sHJV. Twenty micrograms of cell-conditioned media and albumin/IgG-depleted mouse serum protein were separated on a reducing SDS-PAGE and immunoblotted with monoclonal anti-HJV 18H8 (mAb) and polyclonal anti-HJV (pAb) to detect sHJV fragments in the serum. the sHJV in conditioned media from Hep3B cells transfected with pcDNA3.mHjv (sHJV in CM) was used as a positive control. A 42-kDa band indicates sHJV band in conditioned media from Hep3B cells transfected with pcDNA3.mHjv (arrow). Samples from 6-week old female *Hjv-null* mice (KO); 6-week old female wild-type mice (WT); and 9-week old female wild-type mice fed on low iron diet for 72 h (ID) showed non-specific bands and the 42-kDa band was not detected.

A



B



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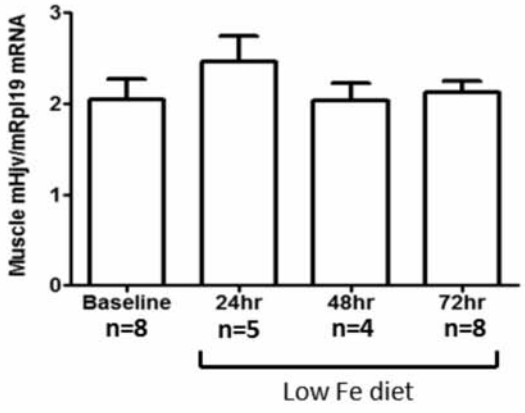
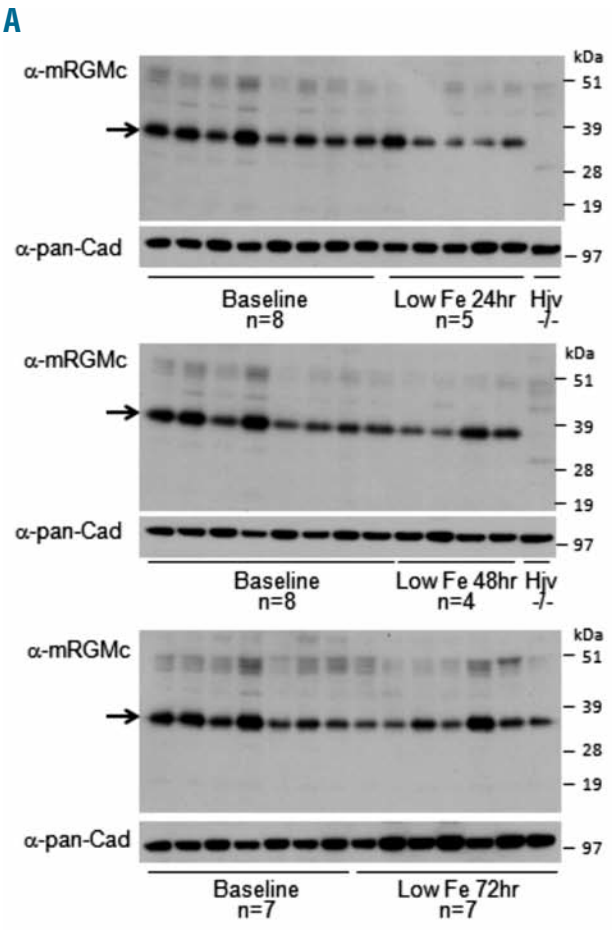
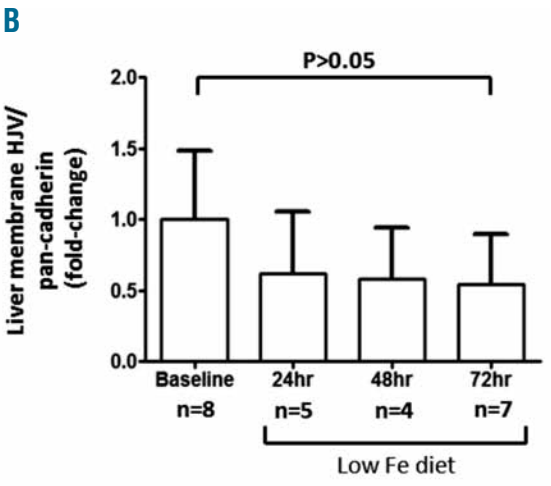


Online Supplementary Figure S3. *HJV* mRNA expression in liver, heart and muscle tissues in mice after acute low iron treatment. Nine-week old C57BL/6J female mice receiving a low iron diet at 0, 24, 48, and 72 h (N=4-8 per group) were analyzed for *Hjv* normalized to *Rpl19* mRNA expression in the liver (A), heart (B) and muscle (C). Error bars represent standard deviations. One-way analysis of variance (ANOVA) with Dunnett's post-hoc test was used to calculate *P* values between multiple groups.





Online Supplementary Figure S4. Liver membrane HJV expression in mice after acute low iron treatment. Nine-week old C57BL/6J female mice receiving a low iron diet at 0, 24, 48, and 72 h (N=4-8 per group) were analyzed for liver membrane HJV by western blot analysis. (A) Western blot images of liver membrane HJV at each time point are shown. A 37-kDa band indicates the presence of membrane HJV in the liver at all time points (arrow). Liver membrane protein from an *Hjv*-null mouse was used as the negative control. α-pan-Cadherin antibody was used as a loading control. (B) Liver membrane HJV was quantified by densitometry analysis of western blots. One-way ANOVA with Dunnett's post-hoc test was used to calculate the *P*-values between multiple groups.