

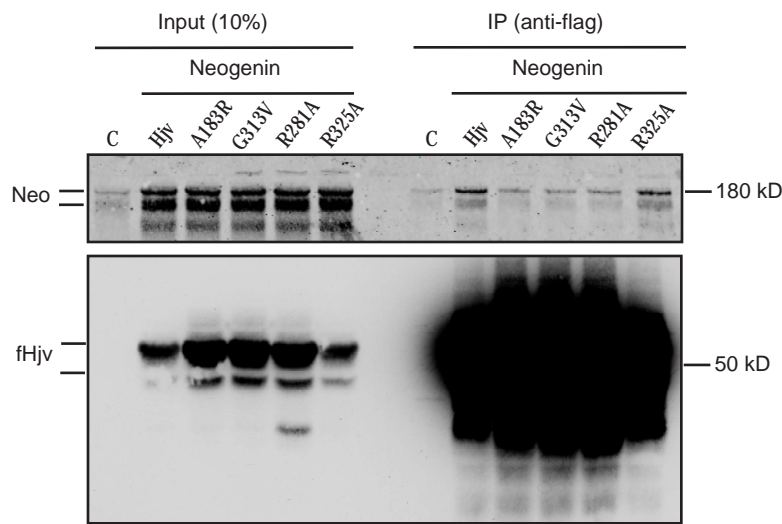
## Neogenin Facilitates the Induction of Heparin Expression by Hemojuvelin in the Liver

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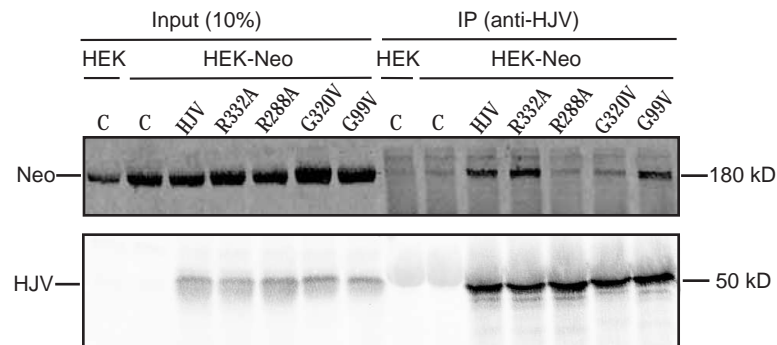
**Supplemental Table 1. List of primers used for qRT-PCR analysis**

| Gene           | Forward primer                  | Reverse primer                |
|----------------|---------------------------------|-------------------------------|
| $\beta$ -actin | 5'-CTGCCTGACGGCCAGGT-3'         | 5'-TGGATGCCACAGGATTCCAT-3'    |
| Activin-B      | 5'-TCAGCTTTGCAGAGACAG-3'        | 5'-GAAGAAGTACAGGCGGAC-3'      |
| Bmp6           | 5'-AGCACAGAGACTCTGACCTATTTTG-3' | 5'-CCACAGATTGCTAGTTGCTGTGA-3' |
| hepcidin       | 5'-CACCAACTTCCCCATCTGCATCTT-3'  | 5'-GAGGGGCTGCAGGGGTGTAGAG-3'  |
| Id1            | 5'-ACCCTGAACGGCGAGATCA-3'       | 5'-TCGTCGGCTGGAACACATG-3'     |
| IL-6           | 5'-TTCCATCCAGTTGCCTTCTTG-3'     | 5'-TTGGGAGTGGTATCCTCTGTGA-3'  |

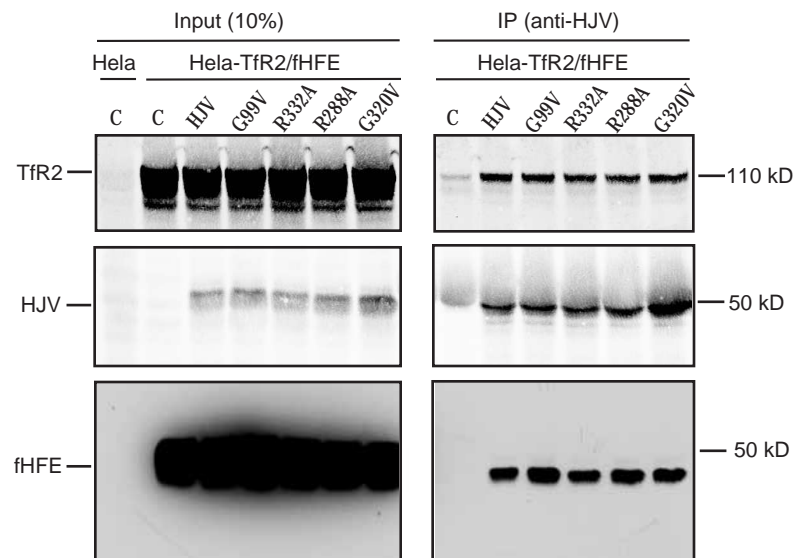
**A. IP: mouse fhjv and human neogenin**



**B. IP: human HJV and neogenin**

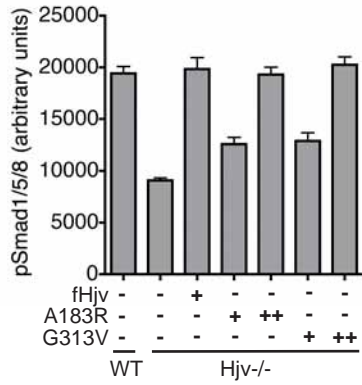


**C. IP: human HJV, TfR2 and HFE**

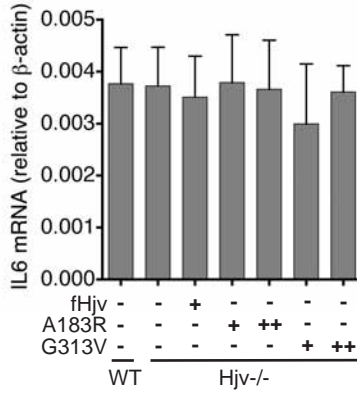


**Figure 1. Interaction of mutant hemojuvelin with neogenin.** A) Co-immunoprecipitation of WT and mutant fhjv with neogenin. HEK293 cells were co-transfected by pcDNA3-neogenin with pCMV9-fHjv, A183R-fHjv, G313V-fHjv, R281A-fHjv, or R325A-fHjv, immunoprecipitated by using anti-FLAG M2 beads, and immunodetected for neogenin (Neo) and fhjv. HEK293 cells transfected with pCMV9 alone (C) were included as a control. B) Co-immunoprecipitation of WT and mutant HJV with neogenin. HEK293-neogenin (HEK-Neo) cells were transfected by pcDNA3 (C), pcDNA3-HJV, R332A-HJV, R288A-HJV, G320V-HJV, and G99V-HJV, immunoprecipitated by using anti-HJV antibody, and immunodetected for neogenin (Neo) and HJV. HEK293 cells (HEK) were included as a control. C) Co-immunoprecipitation of WT and mutant HJV with HFE and TfR2. Hela-TfR2/fHFE cells were transfected by pcDNA3 (C), pcDNA3-HJV, G99V-HJV, R332A-HJV, R288A-HJV, and G320V-HJV, immunoprecipitated by using anti-HJV antibody, and immunodetected for TfR2, HJV, and fHFE. All experiments were repeated at least three times with consistent results.

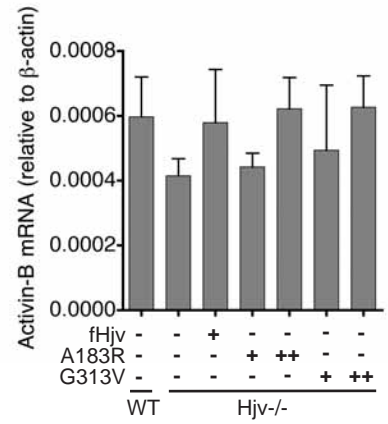
**A. Hepatic pSmad1/5/8**



**B. Hepatic IL-6 mRNA**

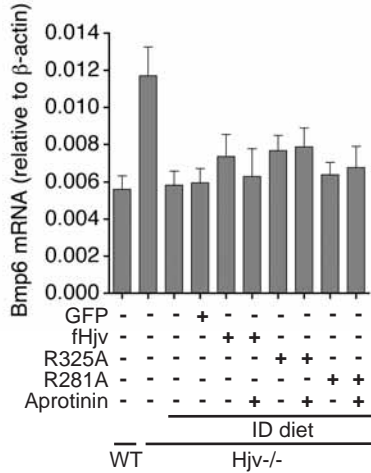


**C. Hepatic Activin-B mRNA**

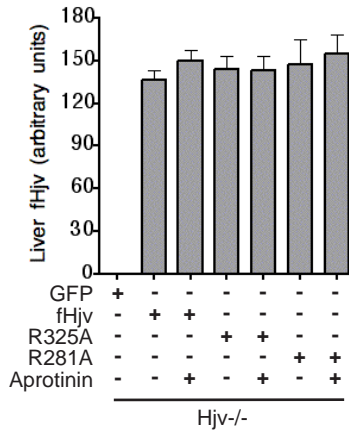


**Figure 2.** A) Quantification of pSmad1/5/8 bands in the western blot images of Figure 2B by Licor. B & C) qRT-PCR analysis of IL-6 mRNA and Activin-B mRNA in the mouse liver tissues from the studies described in Figure 2. N=5. Results for qRT-PCR analysis are expressed as the amount relative to that of  $\beta$ -actin for each sample. The mean values and the standard deviation (SD) are presented. WT, wild-type mice. HJV<sup>-/-</sup>, HJV<sup>-/-</sup> mice.

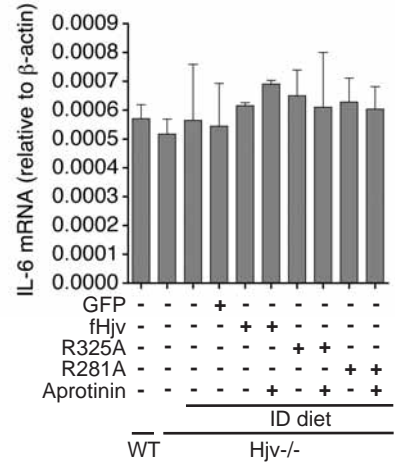
**A. Bmp6 mRNA**



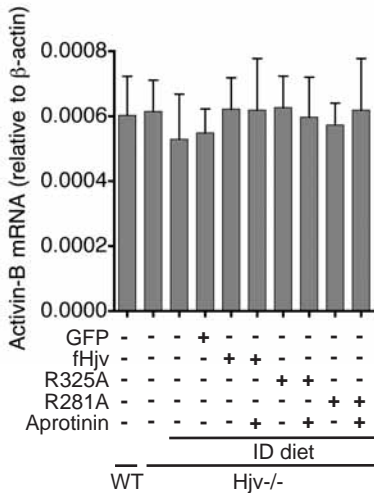
**B. Liver fHjv quantification**



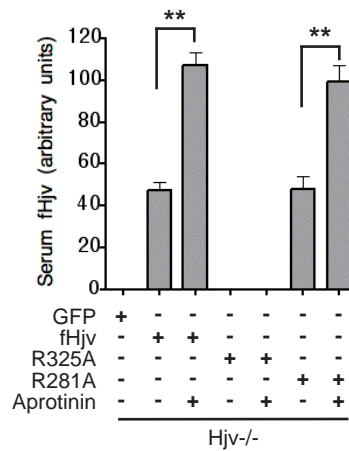
**C. IL-6 mRNA**



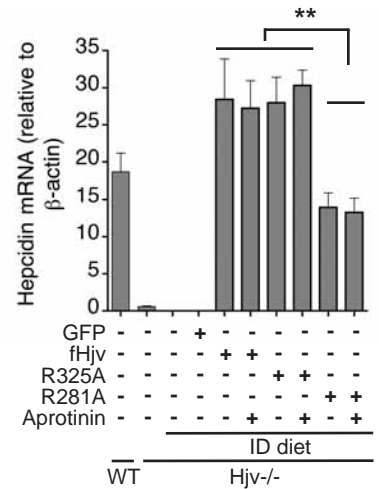
**D. Activin-B mRNA**



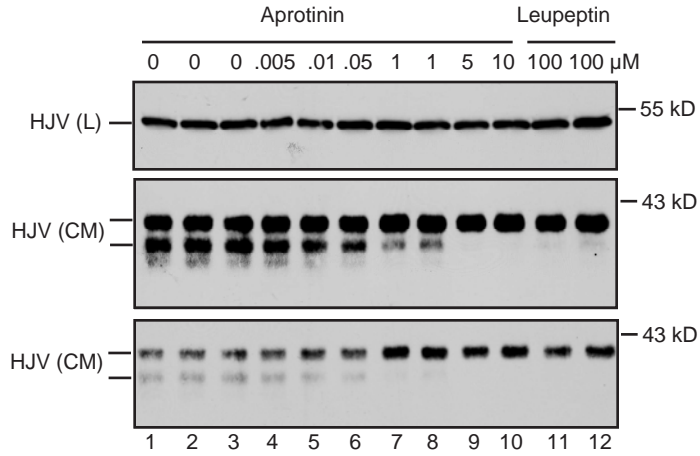
**E. Serum fHjv quantification**



**F. Hepcidin mRNA**



**sFigure 3.** qRT-PCR analysis of *Bmp6* mRNA (A), *IL-6* mRNA (C), *Activin-B* mRNA (D), and *hepcidin* mRNA (F) in the mouse liver tissues from the studies described in Figure 3, and quantification of the liver *fHjv* (B) and serum *fHjv* (E) in Figure 3E. Results from qRT-PCR analysis are expressed as the amount relative to the corresponding  $\beta$ -actin mRNA. The mean values and the standard deviation (SD) for all analysis are presented. One-way ANOVA and Tukey's post-test were used to compare the difference between the groups, and only the results for the groups of interest were presented. N=5 for each group. \*\* P<0.01. WT, wild-type mice. Hjv<sup>-/-</sup>, Hjv<sup>-/-</sup> mice.



**Figure 4.** *Aprotinin inhibits MT2 cleavage of HJV from HepG2 cells.* HepG2-HJV cells in 12-plates were incubated with MEM/10% FCS containing different concentrations of aprotinin for 16 hr. About 50% of cell lysate (L) and 10% of conditioned medium (CM) were subjected to immunodetection for HJV. Two images for HJV in CM with different exposure times were presented. All experiments were repeated at least three times with consistent results.