Matriptase-2 Suppresses Hepcidin Expression by Cleaving Multiple Components of the Hepcidin Induction Pathway

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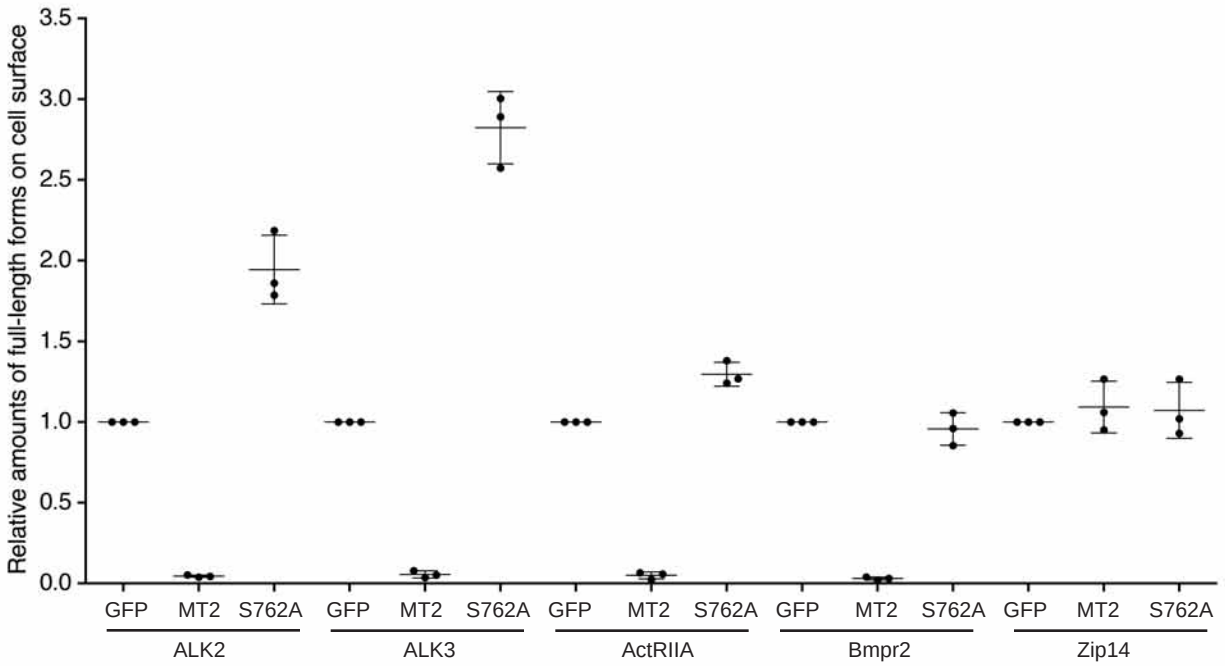
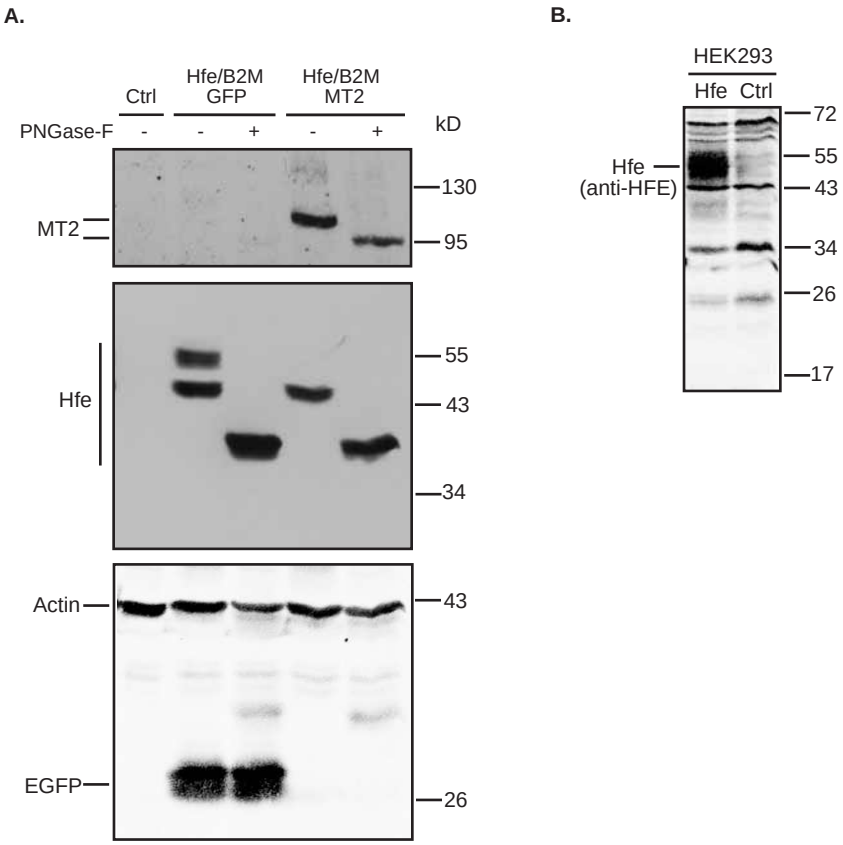
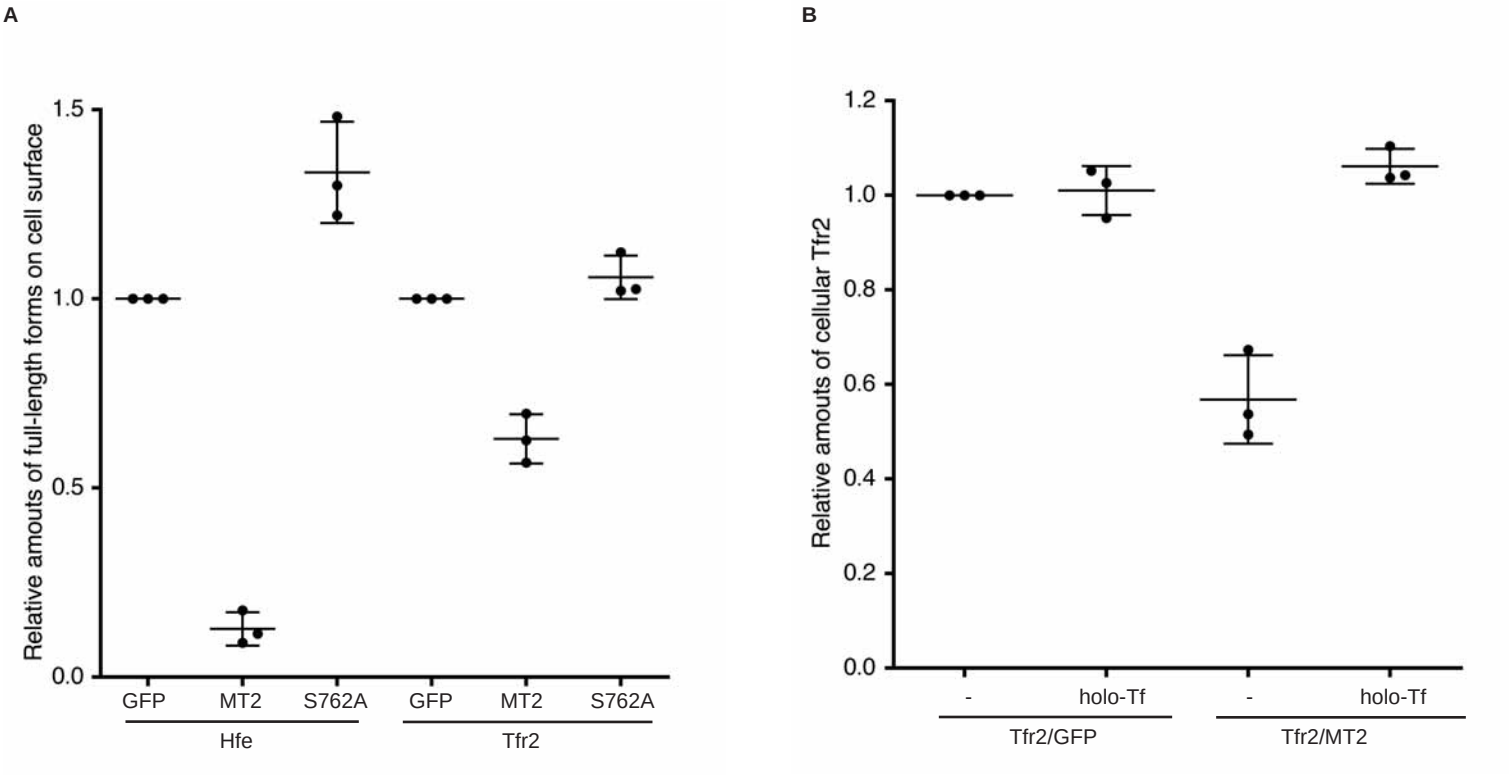


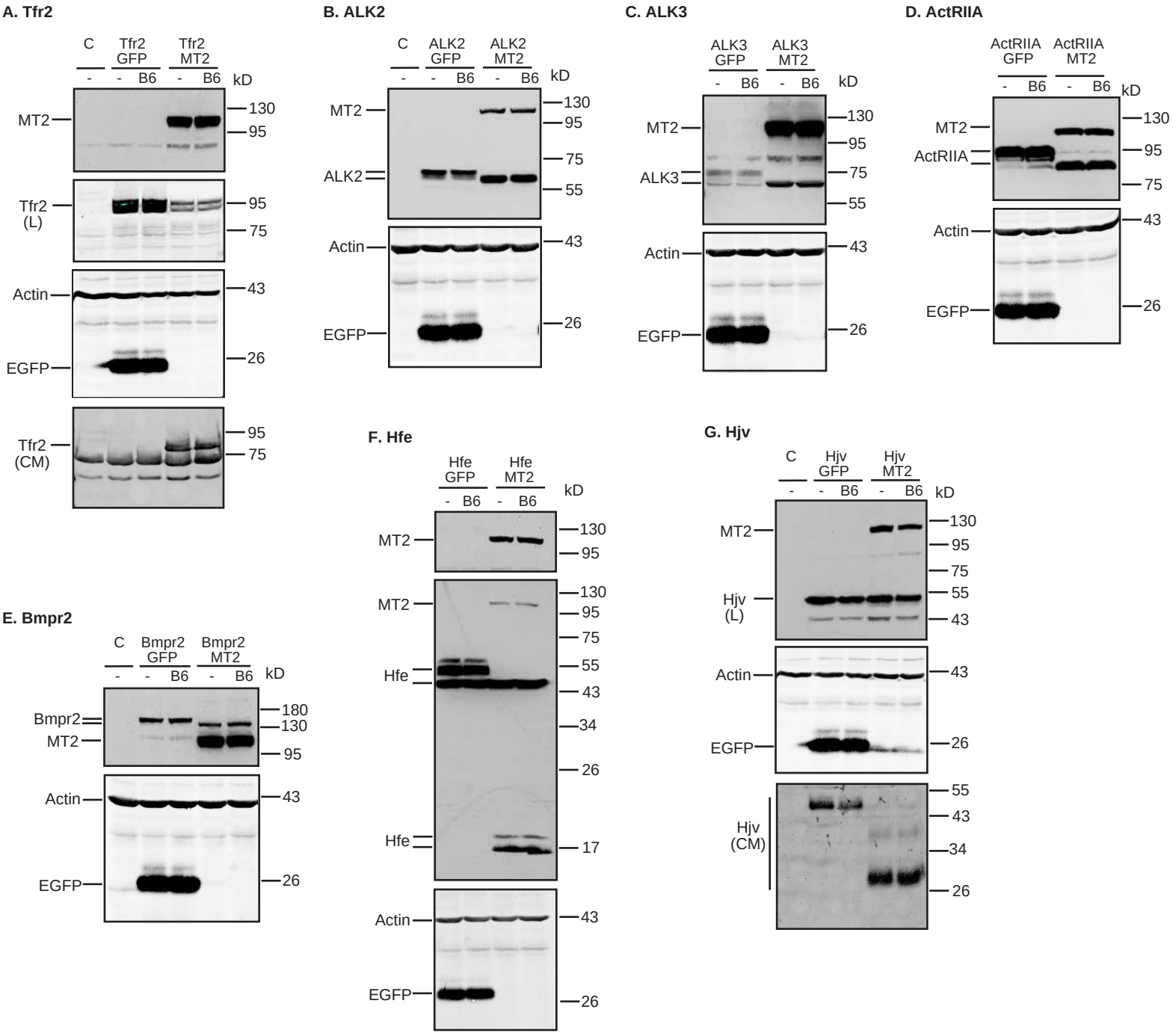
Supplemental Figure 1. Quantification for the full-length forms of biotinylated cell surface ALK2, ALK3, ActRIIA, Bmpr2, and Zip14 in western blot images of Fig. 6C, 6E, 7B, 7D, and 7F, respectively, from three independent biological replicates for each by using ImageJ software. For the convenience of analysis, the band intensities of ALK2, ALK3, ActRIIA, Bmpr2, and Zip14 with co-transfection of EGFP (GFP) were arbitrarily set to 1. The relative levels with co-transfection of MT2 and S762A-MT2 (S762A) were presented.



Supplemental Figure 2. *PNGase F sensitivity of cellular Hfe, and immunodetection of Hfe by a rabbit anti-HFE antibody.* **A)** PNGase F sensitity of cellular Hfe. Cell lysate from HEK293 cells cotransfected with Hfe/B2M and GFP or MT2 were subjected to PNGase F digestion. Immunoblots were performed using anti-FLAG antibody for MT2 and Hfe, anti-beta-actin, and anti-GFP antibody. **B)** Immunodetection of Hfe by a rabbit anti-HFE antibody. HEK293 cells in 12-well plate were transfected with pCMV6-Hfe/B2M (Hfe) or pcDNA3 (Ctrl). After 48 hr of transfection, cell lysates were collected for immunodetection of Hfe by using a rabbit anti-HFE antibody (ab176123; Abcam). All experiments were repeated at three times (technical replicate = 1; independent biological replicates = 3) with consistent results.



Supplemental Figure 3. A) Quantification of biotinylated total cell surface Hfe and Tfr2 in western blat images of Fig. 8B and 8D by using ImageJ software and Licor, respectively, from three independent biological replicates for each. For the convenience of analysis, the band intensities of Hfe and Tfr2 with co-transfection of EGFP (GFP) were arbitrarily set to 1. The relative levels with co-transfection of MT2 and S762A-MT2 (S762A) were presented. **B)** Quantification of Tfr2 band intensities in Fig. 9A by Licor from three independent biological replicates. For the convenience of analysis, the Tfr2 band intensities with co-transfection of EGFP (GFP) and no pre-incubation with holo-Tf were arbitrarily set to 1.



Supplemental Figure 4. *Lack of BMP6 effect on MT2 cleavage of Tfr2 (***A***), ALK2 (***B***), ALK3 (***C***), ActRIIA (***D***), Bmpr2 (***E***), Hfe (***F***), or Hjv (***G***).* pcDNA3-Tfr2, pCMV6-ALK2, ALK3, ActRIIA, Bmpr2, or Hfe/B2m, or pCMV9-Hjv was co-transfected with an equal amount of pEGFP-N1 or pCMV6-MT2. At 24 hr post-transfection, medium was changed to Opti-MEM with or without BMP6 at 25 ng/ml. After another 24 hr of incubation, cell lysate was collected for immunodetection of MT2, ALK2, ALK3, ActRIIA, Bmpr2, Hfe, and Hjv by using an anti-FLAG antibody, and Tfr2 and beta-actin by using specific antibodies. Additionally, Tfr2 and Hjv in the conditioned medium (CM) were also immunodetected by using anti-Tfr2 and HJV antibodies. All experiments were repeated three times (technical replicate = 1; independent biological replicates = 3) with consistent results .