Supp. Figure S1: Membrane localization of WT MT2 and IRIDA variants in Huh7 cells.

Huh7 cells were transiently transfected with an empty vector (mock), WT MT2-FLAG, and MT2-FLAG mutants: Y418C, L235P, P765A, E114K, A605fs. Immunofluorescence detection of the expressed protein was performed using rabbit polyclonal anti-FLAG antibody and anti-rabbit secondary antibody labeled with FITC (green) in non-permeabilized cells, as described in the method section.
Supp. Figure S2: Repression of the HAMP promoter by MT2 mutants in basal conditions, and after stimulation by BMP2.

Huh7 cells were transfected with the HAMP promoter/Photinus luciferase construct, with TK Renilla luciferase as control. They were also co-transfected with empty vector (mock), or MT2 either WT or mutant expressing vectors: WT, Y418C, L235R, E114K, P765A, or A605fs. Each transfection was done in triplicate.
A. transfected cells not treated with BMP2
B. transfected cells treated with BMP2 (40ng/μL) for 24 hours.
Relative hepcidin expression was determined as the ratio of Photinus luciferase signal/Renilla luciferase activity. Values indicate mean activity and error bars indicate the SD.
Supp. Figure S3: The four missense IRIDA mutants Co-Immunoprecipitate with WT MT2

HeLa cells were co-transfected with a wild type MT2 expressing plasmid tagged with a epitope V5 tag (MT2-V5) and the empty vector (mock), or a construct expressing either wild type MT2 or mutants with a FLAG epitope (WT-FLAG, Y418C-FLAG, L235P-FLAG, E114K-FLAG, P765A-FLAG). Whole cell extracts were immunoprecipitated with anti-V5 or anti-FLAG antibodies as indicated and revealed with anti-FLAG and anti-V5 antibodies, respectively.