

00SUPPLEMENTARY INFORMATIONS

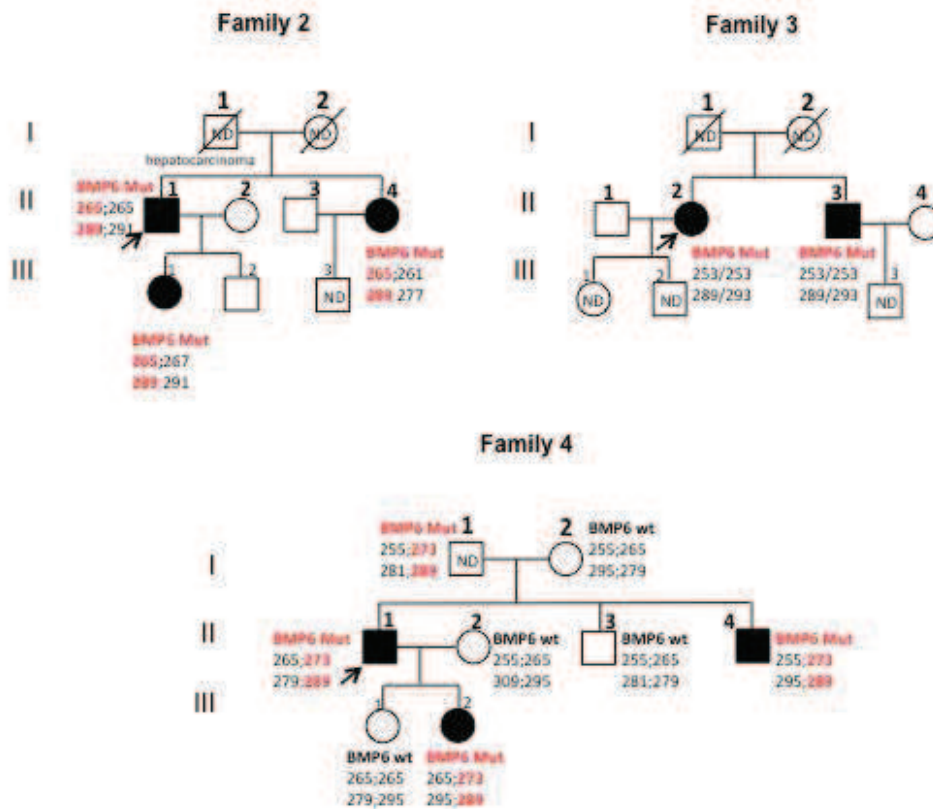


Figure S1. BMP6 p.Leu96Pro is not a founder mutation. In families 2, 3, and 4, eight patients who carried the BMP6 p.Leu96Pro mutation did not share a unique haplotype, consistent with the hypothesis of multiple independent mutational events.

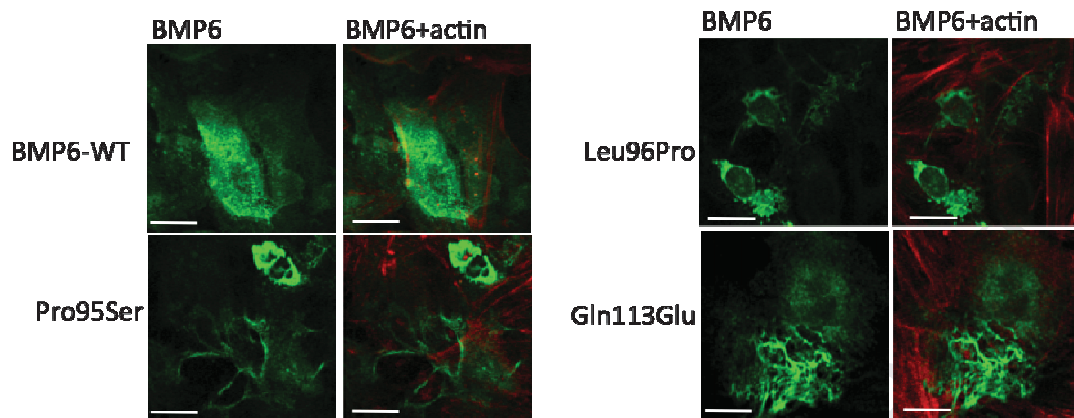


Figure S2. Impaired subcellular location of mutated BMP6 proteins.

Panel A: OK cells were transfected with DDK-tagged WT and mutated BMP6 plasmids. Proteins' staining was performed with anti-BMP6. The actin was stained with Texas Red phalloidin-rhodamine (red). Images were obtained by confocal microscopy. Scale bar: 30 μ m.

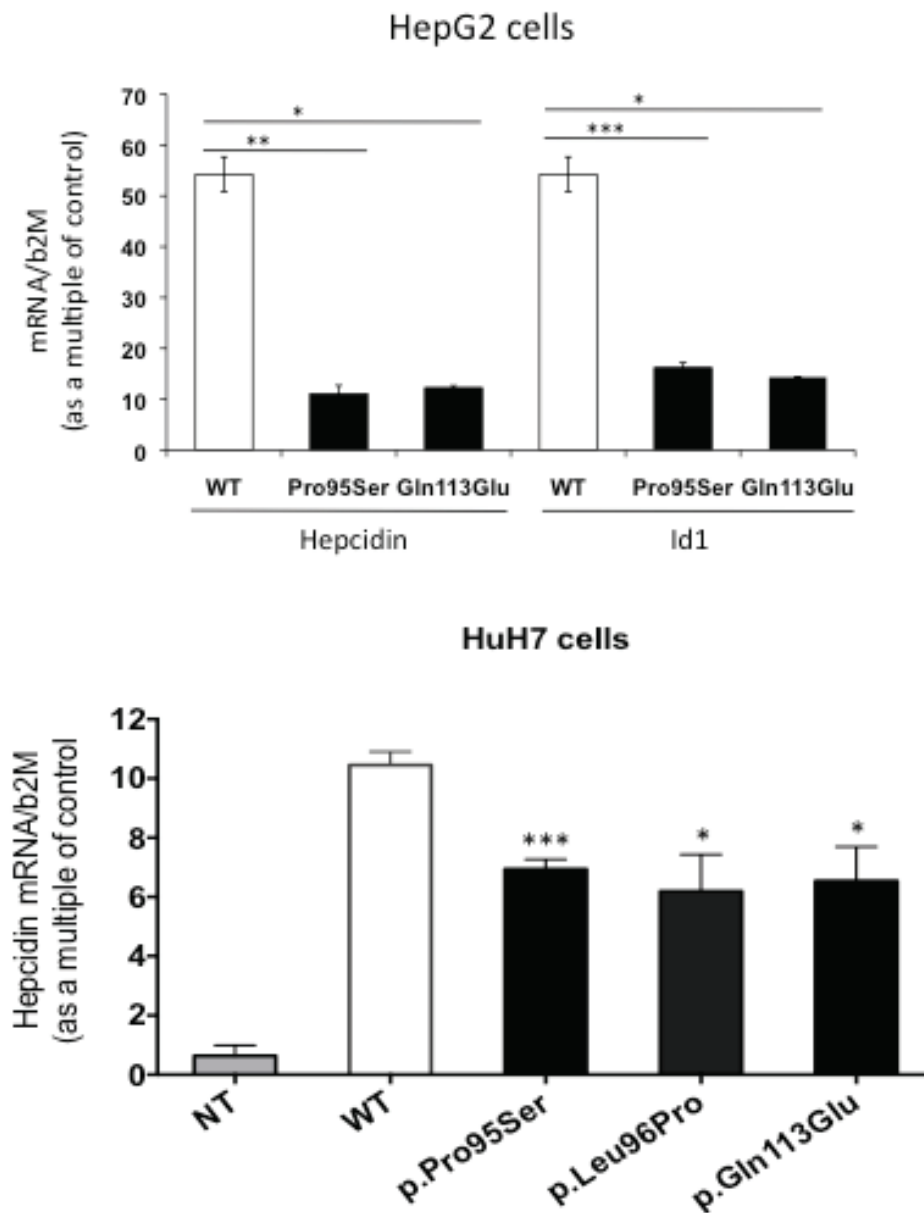


Figure S3. Defective induction of *HAMP* and *Id1* mRNA expression by mutated BMP6 in cultured liver cells.

Upper panel: Induction of *HAMP* and *Id1* mRNA-expressions in HepG2 cells. OK cells were transfected with BMP6-WT or mutants. The conditioned medium was used to treat confluent HepG2 cells for 24 h. Lower panel: Induction of *HAMP* mRNA-expressions in HuH7 cells. OK cells were transfected with BMP6-WT or mutants. The conditioned medium was used to treat confluent HuH7 cells for 24 h.

The mRNA levels were determined through RT-qPCR. The data were normalized to the levels of β 2-microglobulin (β 2M) mRNA and reported as the mean \pm sem (n=3 in each experience). *P < 0.05, **P < 0.01, ***P < 0.001

ACCEPTED MANUSCRIPT

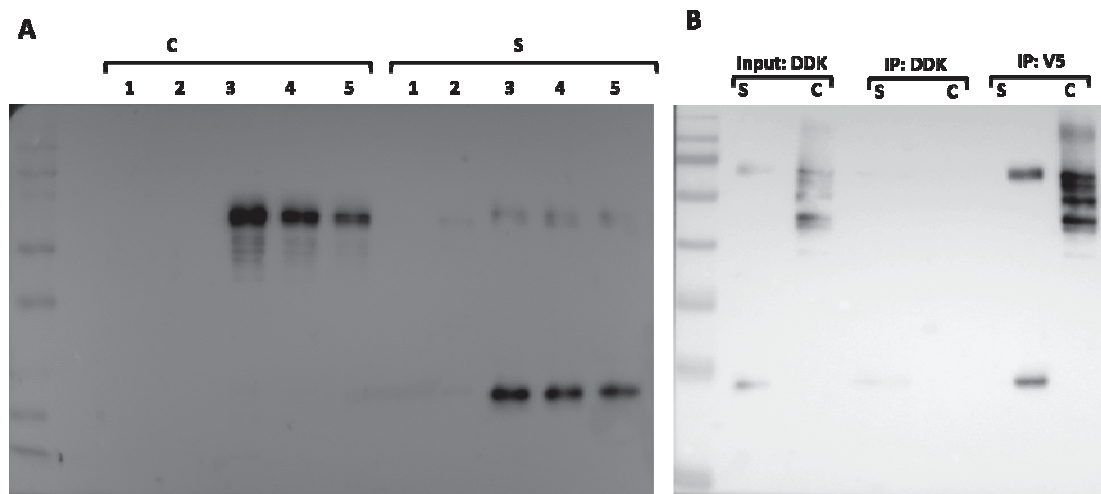


Figure S4. Co-immunoprecipitation controls.

Panel A: OK cells were transfected with 1 μ g of V5-BMP6 plasmid alone (3) or equally combined with either DDK-WT (4) or DDK-M (5). Cell extracts (C) and conditioned medium (S) were immunoprecipitated with beads alone (1), isotype IgG (2) or anti-V5 (3-5). The Western blots were revealed by anti-V5-BMP6. Panel B: OK cells were transfected with 1 μ g of V5-BMP6 plasmid alone. Cell extracts (C) and conditioned medium (S) were immunoprecipitated with anti-DDK or anti-V5. The Western blots were revealed by anti-V5-BMP6. Input blot (Input: DDK, 10% input) was also performed to ensure expression of V5-proteins in samples used for IP.

Table S1. Characteristics of consecutive non-HFE hemochromatosis patients referred to the centre (2004-2011)

n	Sex, M/F	Age, y	Ferritin, µg/L	TS, %	Liver iron content, µmol/g
70	51/19	62(37-83)	976 (333-4064)	45.5 (25-99)	160(45-300)

Values are median (range).

M-male, F-female. Normal values: serum ferritin F 5-105, M 30-300 µg/L, % Tf saturation (TS) 20-45. Liver iron concentration was determined by MRI; normal value <36 µmol/g.

Table S2. PRIMERS SEQUENCES

	Forward	Reverse
DNA ANALYSIS		
EX1A	tgtaaacgacggccagctacctctagcctgggcaac	caggaaacagctatgaccacagatccagcatgaagagg
EX1B	tgtaaacgacggccagctctgcacggcctccaacag	caggaaacagctatgaccgcgggggagcgcgatgt
EX2	tgtaaacgacggccagctctctcatgggccaagcagct	caggaaacagctatgacctccaggtcaccctgcacaga
EX 3	tgtaaacgacggccagcttggtaccacgccttcagg	caggaaacagctatgacctgtctccaagttgccaagcc
EX4	tgtaaacgacggccagctatgggtgccaggaaggttt	caggaaacagctatgaccatcgggcagcctgagcttt
EX5	tgtaaacgacggccagctgtactgggcaagtcggccc	caggaaacagctatgacctctgccttactccaggagcc
EX6-7	tgtaaacgacggccagctgcatgctcatctttgtgtgc	caggaaacagctatgacccaactgtgcttccgtgttt
HAPLOTYPE ANALYSIS		
BMP-22AC	gcaggggaggaaccccaaatg	cttctactctcagccagg
BMP-16GT	ccactgtcaagctgccattc	aggtagtgcgaaagggtgt
BMP6 CONSTRUCTIONS AND MUTAGENESIS		
Pro95Ser	ccgcaccggccccggtccctgcacggcctec	ggaggccgtgcagggaccggggccgggtgccc
Leu96Pro	cggccccggccccgcacggcctccaacagc	gctgttgaggccgtcgggggccggggccc
Gln113Glu	ggcagcaggaggaggagcagcagcagcagc	gctgctgctgctgctcctcctcctgctgcc
BMP6Stop	gcttgtgatgccactgacgcgtacggcccgc	gcggccgcgtacgcgtcagtggtccacaagc
V5-BMP6	gaccatgagagataaggactgagg	gtggcatccacaagctctac
RT-qPCR		
Hepcidin	ggccagctggatgccatgt	tgacagcagccgcagcagaa
Id1	tccgtccatccttgcgggt	cacgacgggggagcgtttt
BMP6	gccttctcaacgacgcggac	tggtgtcgtgacgagggga
β2M	tgctgtctctccatgtttgatgtatct	tctctgctccccaccttaagt