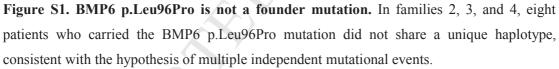


00SUPPLEMENTARY INFORMATIONS





ACCEPTED MANUSCRIPT

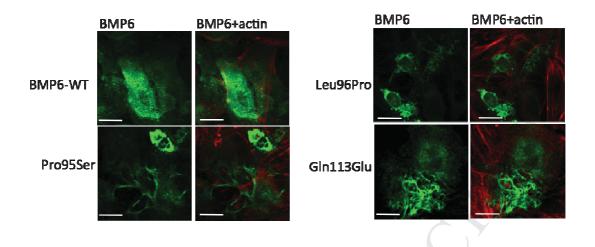


Figure S2. Impaired subcellular location of mutated BMP6 proteins.

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 µm.

CER AN

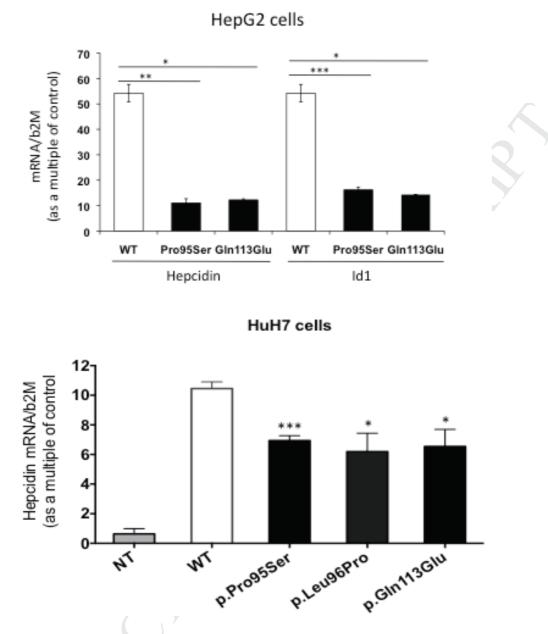


Figure S3. Defective induction of *HAMP* and *Id1* mRNA expression by mutated BMP6 in cultured liver cells.

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.

ACCEPTED MANUSCRIPT

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

4

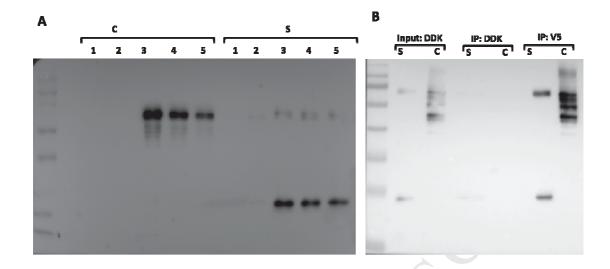


Figure S4. Co-immunoprecipitation controls.

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

ForwardReverseDNA ANALYSISEX1Atgtaaaacgacggccagtacctctagcctgggcaac EX1Btgtaaaacgacggccagtctgcacggcctcaacaag caggaaacagctatgaccacggggggggggggggcgcatgt caggaaacagctatgacctgggggggggggggggggggg						
EX1AtgtaaaacgacggccagtacctctagcctgggcaaccaggaaacagctatgaccacagatccagatgaagaggEX1BtgtaaaacgacggccagtctgcacggcctccaacagcaggaaacagctatgaccacggggggagggcgcagtEX2tgtaaaacgacggccagtctcctcatgggccagccagtcaggaaacagctatgaccggggggagggggggggggggg		Forward	Reverse			
EX1BIgtaaaacgacggccagtctgcacggcctcaacagcaggaaacagctatgaccgcggggggcgcagtgEX2tgtaaaacgacggccagtctgcacggcctcaacagcaggaaacagctatgaccgggggggcgcgagcgagagEX3tgtaaaacgacggccagttggtaccacggccagtcagggcagggcaggaaacagctatgacctcaagggEX4tgtaaaacgacggccagttggtaccacggcaaggtggcaggaaacagctatgacctgtgccaaggggggggggggg		DNA ANALYSIS				
EX1BtgtaaaacgacggccagtctgcacggcctccaacagcaggaaacagctatgaccgcggggggggcgcatgtEX2tgtaaaacgacggccagtctctctatgggccagccagtcaggaaacagctatgacctccaggtcacctgacagaEX3tgtaaaacgacggccagttggtaccacgccttcagggcaggaaacagctatgacctccaagtgccaagaEX4tgtaaaacgacggccagttggtaccaggcaggtggccagctggccagctggccagctggccagctggccagctggccagctggcagccgggggagcgcagctgagtctcaggaaacagctatgacctccaagtgccaagtgccaagaEX5tgtaaaacgacggccagttgtactgggcaagtcggccagcca	EX1A	tgtaaaacgacggccagtacctctctagcctgggcaac	caggaaacagctatgaccacagatccagcatgaagagg			
EX2tgtaaaacgacggccagtctcctcatgggccagccagtcaggaaacagctatgacctccaggtcacctgcacagaEX3tgtaaaacgacggccagttggtaccacgcctttcagggcaggaaacagctatgacctccaggtcacctgagtccagtcEX4tgtaaaacgacggccagttgtactgggcaagtcggcacaggaaacagctatgaccatgcgggcagcctgagtcttEX5tgtaaaacgacggccagttgtactgggcaagtcggcccaggaaacagctatgaccctcccaggtgcagcctgagtctEX6-7tgtaaaacgacggccagtgcatgctcatcttttgttgccaggaaacagctatgaccccaactgcggcagccggggccBMP-22ACgcagggaggaaccccaaatgcttcctaccttcagccagggBMP-16GTccacttgtcaagctgccattcagggtagttgcaaggggggggccggtgcggBMP6 CONSTRUCTIONS AND MUTAGENESISPro95SerccgcaccggccccggtccctgcacggcctccggaggccgtgcagggggggccggggccggggccggGln113GluggcagcaggaggaggagcagcagcagcagcagcgctgctgctgctcctcctcctctgcccBMP6StopgcttgtggatgccattgacggggccgggtggcatccaaagcV5-BMP6gaccatgaggatagggagcagcagggggtggcatccaaagcttacRT-qPCRtgacagcagcggcagcagcagaaHepcidinggccagctggatgcccatgt	EX1B	tgtaaaacgacggccagtctgcacggcctccaacag	caggaaacagctatgaccgcgggggggggcgcatgt			
EX4tgtaaaacgacggccagtagggtgccaggcaggtgccaggtgcaggtgccagggccaggtgccagggccaggtgccagggccagggccaggggccaggggccagggggggagaaccccaaatgcaggaaacagctatgaccatgcgggcagcctgagtcttEX6-7tgtaaaacgacggccagtgcatgctcatcttttgttgccaggaaacagctatgaccccaactgtgcttccagggagccEX6-7tgtaaaacgacggccagtgcatgctcatcttttgttgccaggaaacagctatgaccccaactgtgcttccgtgtttHAPLOTYPE ANALYSISBMP-22ACgcaggggaggaggagcccaaatgcttcctaccttcagccagggBMP-16GTccacttgtcaagctgccattcagggtagttgcaaagggtgtBMP6 CONSTRUCTIONS AND MUTAGENESISPro95SerccgcaccggccccgggcccgggcccgCuey6ProcggccagcaggaggaggagcagcagcagcagcagcgctgttggaggccgtgggggccggggccgGln113GluggcagcaggaggaggagcagcagcagcagcagcgctgctgctgctgctcctcctcctctgctgccBMP6StopgcttgtggatgccactgacggcgggccgggcggccggtacgggtcagtgggagcagcagcagcV5-BMP6gaccatgaggataaggactgagggtggcatccacaagcttacHepcidinggccagctggatgcccatgttgacagcagcagcagcagaa	EX2					
EX5tgtaaaacgacggccagttgtactgggcaagtcggccc tgtaaaacgacggccagtgcatgctatgtactgggcaagtcggccc caggaaacagctatgacccctctgccttactccaggagcc caggaaacagctatgaccccaactgtgcttccgtgtttEX6-7tgtaaaacgacggccagtgcatgctatgttactgggcaagtcggccc caggaaacagctatgaccccaactgtgcttccgtgtttHAPLOTYPE HAPLOTYPEANALYSISBMP-22AC cacttgtcaagctgccattcgcagggaggaaccccaaatg agggtagttgcaaagggtgtBMP-16GT ccacttgtcaagctgccattccttcctaccttcagccaggg agggtagttgcaaagggtgtPro95Ser Leu96Pro cggccccggccccggccccggccccgacggcgccg gcagcaggaggaggaggaggagcagcagcagcagc gctgttggaggccgtgcggggccggggccggggccg gctgttggaggccgtgcagtgcactcacaagcgaaggccgtggaggccggggccggggccggggccg gcggccgcgtacgcgtacggggccg gcggccggtacgcgtacggggccgBMP6Stop V5-BMP6 gaccatgagagtaaggagtagcagcaggggtggcatccacaagctettacRT-qPCR Hepcidinggccagctggatgcccatgttgacagcagcggaggagcagcagttgacagcagcagcagcagaa	EX 3	tgtaaaacgacggccagttggtaccacgcctttcaggg	caggaaacagctatgacctgtctccaagttgcccaagcc			
EX6-7tgtaaaacgacggccagtgcatgctcatcttttgttgccaggaaacagctatgaccccaactgtgcttccgtgtttHAPLOTYPE ANALYSISBMP-22ACgcagggaggaaccccaaatgcttcctaccttcagccagggBMP-16GTccacttgtcaagctgccattcagggtagttgcaaagggtgtBMP6 CONSTRUCTIONS AND MUTAGENESISPro95SerccgcaccggccccggtccctgcacggcctccggaggccgtgcagggagccggggccggLeu96ProcggccccggccccgacggcctccaacagcgctgttggaggccgtgcgggccggggccgGln113GluggcagcaggaggagcagcagcagcagcagcgctgctgctgctgctgccccgccccBMP6StopgcttgtggatgccactgacgcgtacgcggccgcgcggccgcgtacgcgtcagtggcatccacaagcV5-BMP6gaccatgagagataaggactgagggtggcatccacaagctcttacRT-qPCRHepcidinggccagctggatgcccatgttuttgacagcagcagcagaa	EX4	tgtaaaacgacggccagtatgggtgccaggcaaggttt	caggaaacagctatgaccatgcgggcagcctgagtctt			
HAPLOTYPE ANALYSISBMP-22ACgcagggaggaaccccaaatgcttcctaccttcagccagggBMP-16GTccacttgtcaagctgccattcagggtagttgcaaagggtgtBMP6 CONSTRUCTIONS AND MUTAGENESISPro95SerccgcaccggccccggtccctgcacggcctccggaggccgtgcagggagcggggccggggccgLeu96ProcggccccggacgcgcaggaggaggagcagcagcagcagcgctgttggaggccgtgcgggccggggccggGln113GluggcagcaggaggaggagcagcagcagcagcgctgctgctgctgctgccccacaagcBMP6Stopgcttgtggatgccactgacgcgtacgcggccgcgcgggccgcgtacgcgtcactgaggagagagagagagaga	EX5	tgtaaaacgacggccagttgtactgggcaagtcggccc	caggaaacagctatgacccctctgccttactccaggagcc			
BMP-22AC BMP-16GTgcagggaggaaccccaaatg ccacttgtcaagctgccattccttcctaccttcagccaggg aggtagttgcaaagggtgtBMP-16GTBMP6 CONSTRUCTIONS AND MUTAGENESISPro95Serccgcaccggccccggtcctgcacggcctcc ggacccggccccggccccggccccggccccgacggccccg gcgcccgggcccggggccggggccggggccggggccgggg	EX6-7	tgtaaaacgacggccagtgcatgctcatcttttgttgc	caggaaacagctatgaccccaactgtgcttccgtgttt			
BMP-16GTccacttgtcaagctgccattcaggtagttgcaaagggtgtBMP6 CONSTRUCTIONS AND MUTAGENESISPro95SerccgcaccggccccggtcctgcacggcctccggaggccgtgcagggaccggggccggtgcggcggccccggccccggtcctgcacggcctccggcagcaggaggaggaggaggagcagcagcagcagcgctgttggaggccgtgcggggccggggccggggccgBMP6StopV5-BMP6gaccatgaggagtaggagcagcaggggggccagctggaggagagagagagagaggaggagggggggggccagctggatgccatgaggggccagctggatgccatgttgacagcagcagcagaa						
BMP-16GTccacttgtcaagctgccattcaggtagttgcaaaggtgtBMP6 CONSTRUCTIONS AND MUTAGENESISPro95SerccgcaccggccccggtccctgcacggcctccggaggccgtgcagggaccggggccggggccggLeu96ProcggccccggcccccgcacggcctccaacagcgctgttggaggccgtgcggggccggggccgGln113GluggcagcaggaggaggagcagcagcagcagcagcgctgctgctgctgctgctgccccgcacggccccBMP6StopgcttgtggatgccactgacgcgtacgcggccgcgcggccgcgtacgcgtcagtggcatccacaagcV5-BMP6gaccatgagagataaggactgagggtgcatccacaagccttacRT-qPCRHepcidinggccagctggatgcccatgttuttgacagcagcagaa	BMP-22AC	gcagggaggaaccccaaatg	cttcctaccttcagccaggg			
Pro95SerccgcaccggccccggtccctgcacggcctccggaggccgtgcagggaccggggccggtgcggLeu96ProcggccccggccccgacggctccaacagcgctgttggaggccgggggccggggccgGln113GluggcagcaggaggaggaggagcagcagcagcagcagcgctgctgctgctgctgctgctgctgctgctgctgccBMP6StopgcttgtggatgccactgacgcgtacgcggggggtggcatccacaagcV5-BMP6gaccatgagagataaggactgagggtggcatccacaagcttacRT-qPCRHepcidinggccagctggatgccatgttgacagcagcagcagaa	BMP-16GT					
Leu96Pro Gln113Glucgccccggcccccgcacggcctccaacagc ggcagcaggaggaggaggagcagcagcagcagc gctgttggatgccactgacgcggccgc gaccatgaggagtaaggaggaggaggaggaggaggaggaggagcagcagc ggcgcgcgcgcgcgcgcgcgcgcgcgcgcgcgcgcgcg						
Leu96ProcggccccggcccccgacggcctcaacagcgctgttggaggccgtgcgggggccggggccgGln113GluggcagcaggaggaggaggagcagcagcagcagcgctgctgctgctgctgctgctgctgctgctgctgccgBMP6StopgctgttggatgccactgacgcgtacgcggccgcgcggccgcgtacgcgtgcggggccggggccgV5-BMP6gaccatgaggagataaggactgagggtggcatccacaagctcttacRT-qPCRHepcidinggccagctggatgcccatgttdiatgacagcagcagcagaa	Pro95Ser	ccgcaccggccccggtccctgcacggcctcc	ggaggccgtgcagggaccggggccggtgcgg			
Gln113Glu BMP6Stopggcagcaggaggaggaggaggaggaggagcagcagcagca	Leu96Pro					
V5-BMP6 gaccatgagagataaggactgagg gtggcatccacaagctcttac RT-qPCR Hepcidin ggccagctggatgcccatgt tgacagcagcagcagaa	Gln113Glu					
RT-qPCR Hepcidin ggccagctggatgcccatgt	BMP6Stop	gcttgtggatgccactgacgcgtacgcggccgc	gcggccgcgtacgcgtcagtggcatccacaagc			
Hepcidin ggccagctggatgcccatgt tgacagcagcagcagaa	V5-BMP6	gaccatgagagataaggactgagg gtggcatccacaagctcttac				
		RT-qPCR				
	Hepcidin	ggccagctggatgcccatgt	tgacagcagccgcagcagaa			
	Id1		cacgacggggggggggcgctttt			
BMP6 gccttcctcaacgacgcggac tggtgtcgctgacgagggga	BMP6					
β2M tgctgtctctccatgtttgatgtatct tctctgctccccacctctaagt	β2Μ	tgctgtctctccatgtttgatgtatct				