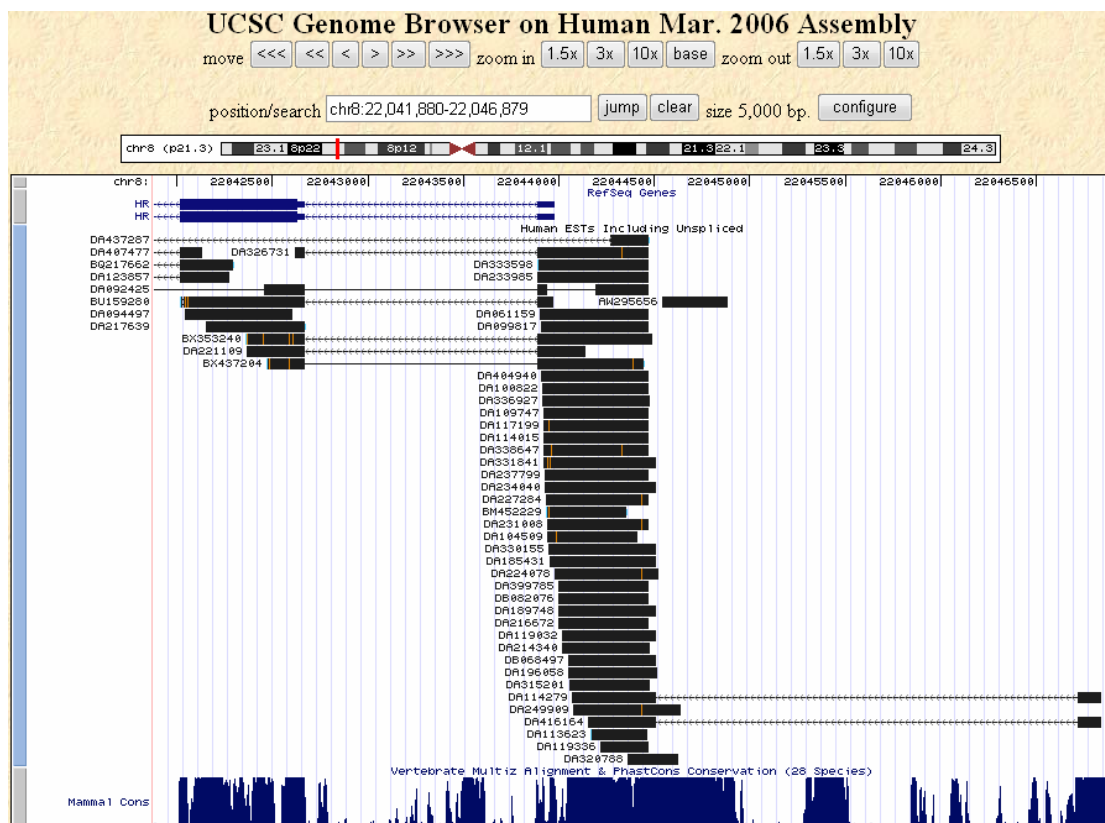


Loss-of-function mutations of an inhibitory upstream ORF in the human hairless transcript cause Marie Unna hereditary hypotrichosis

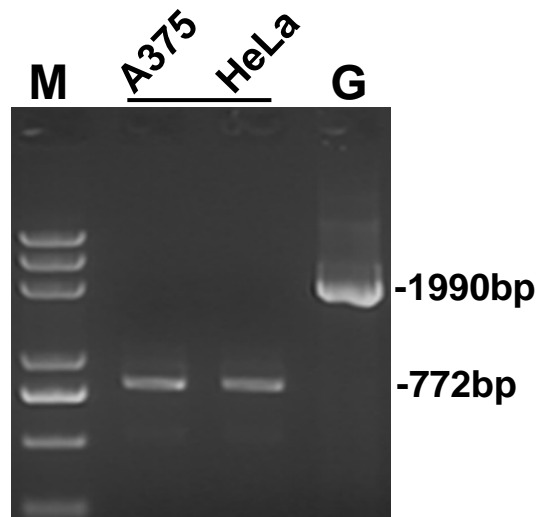
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Supplementary Figures



Supplementary Figure 1 The UCSC human genome browser shows multiple longer expressed sequence tags (ESTs) to *HR* exon 1, suggesting that the RefSeq *HR* mRNA is incomplete.



Supplementary Figure 2 RT-PCR analysis in A375 and HeLa cells. Total RNA was isolated using Trizol reagent and first-strand cDNA synthesis was performed using PrimeScript RTase (TaKaRa). The PCR primers were HR0+F and HR1R2 (**Supplementary Table 1f**). PCR was also performed using human genomic and the same primer pair. The size of the RT-PCR and genomic PCR products are indicated on the right. M: DL2000 DNA Marker (TaKaRa); G: genomic PCR control.

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GAATCACGGGCTCCTGTTTCCCGCAGGGTGCTGGAGGAGGAAACCGGCGGAGCAGCT

uORF1

TCCCCACTCTCAGTTGCGCTTCTGGCG**ATGGCGATCAGAGGTCTGCTGCGCTCTCC**

M A I R G P A A L S

GCCGCGCTCTACCTCCATTAGCCGCGCTGCGCGGTGCTGCGCCCTCGCCGGTGCCTC

A A L Y L H

TCTCCTGGGTCCCAGGATCGGCCCCACCATCCAGGCACGACCCCTTCCCCGGCCC

CTCGGCCTTTCCCCAACTCGGCCATCTCCGACCCGGGGCGCGTGTTCCCCCGGCC

CGGCGCCTTCTCTCCCTCCGGGGCACCCTCCCTAGCCCCGGCCCGGCCCTCCCC

uORF2

GCGGCGCAGCACGGAGTCTCGGCGTCCC**ATGGCGCAACCTACGGCCTCGGCCAGAA**

M A Q P T A S A Q K

GCTGGTTCGGCCGATCCGCGCCGTGTGCCGCATCCTGCAGATCCCGGAGTCCGACCC

L V R P I R A V C R I L Q I P E S D P

CTCCAACCTGCGGCCCTAGAGCGCCCCCGCCGCCCGGGGAAGGAGAGCGCGAGCG

S N L R P

CGCTGAGCAGACAGAGCGGGAGAACGCGTCTCGCCCGCCGGCCGGGAGGCCCCGGA

uORF3

GCTGGCCC**ATGGGGAGCAGGCGCCCGGTGCCGGCCACGACGACCGCCACCGCCCGCG**

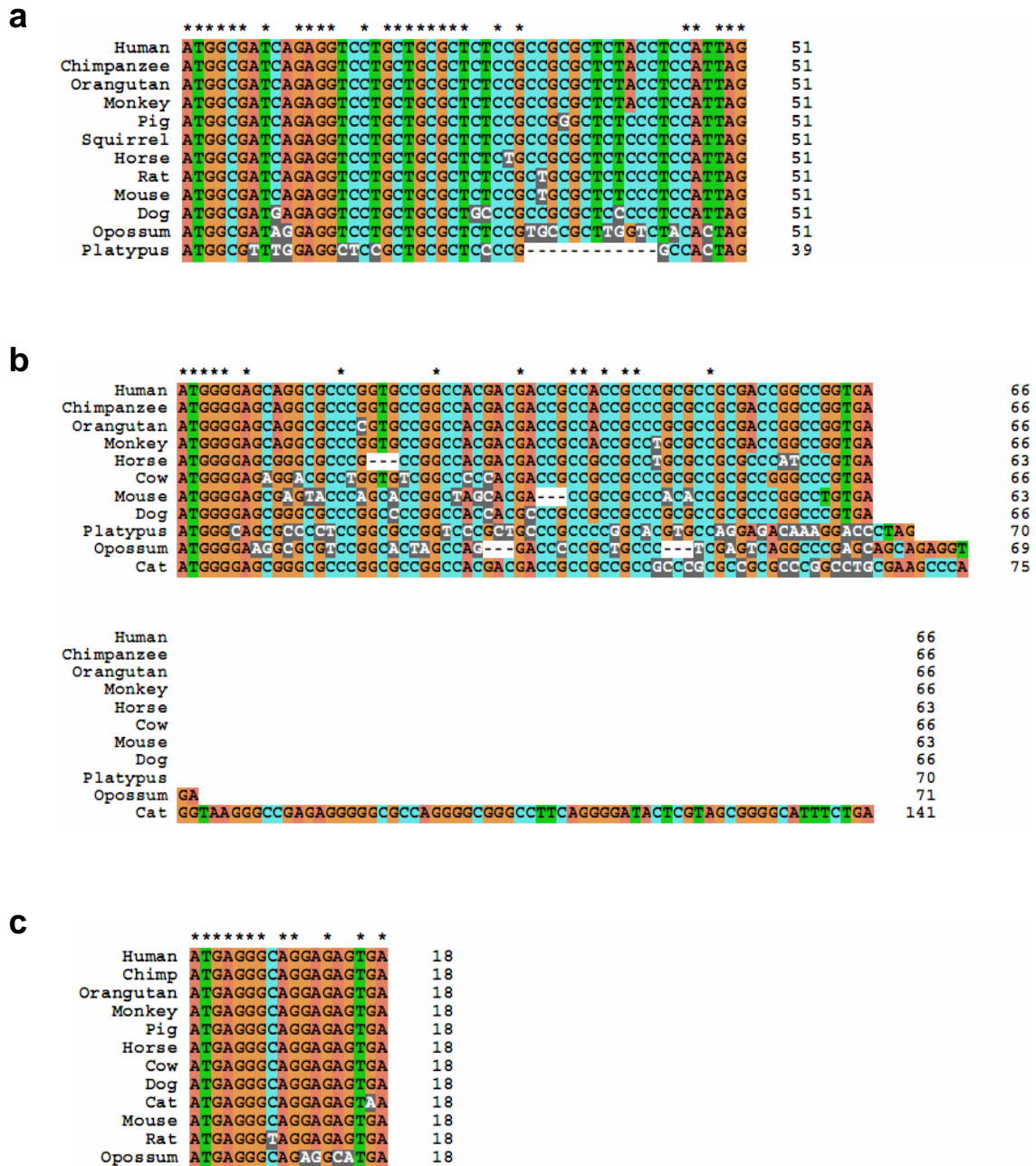
uORF4

CCGCGACCGGCCGGTGAAGCCCAGGGACCCCCCTCTGGGAGAGCCCC**ATGAGGGCAG**

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GAGAGTGA**ATGGAG** **main ORF** **AAATAG**

Supplementary Figure 3 Sequence of the extended *HR* 5'UTR. The four uORFs are highlighted: U1HR in green, U2HR in red, and U3HR and U4HR in blue. The predicted amino acids of U1HR and U2HR are given in single letters. The main HR ORF is indicated as a box, showing the first and the last six nucleotides.



Supplementary Figure 4 Nucleic acid multiple sequence alignment of mammalian U1HRs, U3HRs and U4HRs. Identical nucleotide is indicated by an asterisk. The size of the uORFs and the species are given on the right and the left, respectively.

Supplementary Table 1 Primers used in the study

a) Sequences and positions of the primers used in linkage analysis

Marker	Forward (5'-3')	Reverser (5'-3')	Start	End
ATAAC	TCTAGAAACCCGCTGAGAGG	CTAGGGTGCATGTGGGTTTC	21829927	21830078
D8S405	GGCTGTGGGATATCACTAGAAGG	GGTGGGAAAACCTGAGGGATTAC	22024661	22024894
D8S1786	CGAAAGATTGAGACCCCAT	GTTTCCACACCGAAGCC	22489214	22489578
D8S1733	CACAGTCTCAAACCTCTGGG	ACGAAAAACCATGAACAAGA	22576582	22576836

b) Sequences and positions of the primers used in DNA sequencing

Amplicon	Forward (5'-3')	Reverse (5'-3')	Start	End
FGF17-1	AATTTCCAACCTCCAGGCCAG	GAGACAAAACCTCAGTCAGC	21955834	21956834
FGF17-2	CCATATGCCCACTTCACTCT	TGAGAGTAGGATGGGAAGTC	21959434	21960219
FGF17-3	AAGCCTGCAACTCAGATCCA	GGTTTTCTTCCCAACAGGT	21961204	21962513
FAM160B2-1	TTCGAAAACACCTCCCTTAG	AGGACGCACAGACAGGAAGC	22002156	22003017
FAM160B2-2	GGAGAAGTGCCTGTCAGGAG	CTTGACAGATCAGGCCAGTG	22007689	22008257
FAM160B2-3	CATGCGTTCTCAGAGGACTG	GCCACTTTCAGTGCAGGAC	22009570	22010188
FAM160B2-4	CAACGTGGCAAGGGAAGCAG	AGGGTGAGATGAGGCCAGAC	22010640	22011516
FAM160B2-5	AAGCAGAGATCAGGTACCCG	CTCCTGTGGAACCTCTGTGG	22011376	22012315
FAM160B2-6	AGCTCCTTCTCCACTCTCTG	GCAGTTCAGTGTCTCAAAGG	22012359	22013468
FAM160B2-7	CCAGCCAAGGGAGGTGACTC	GAACCGAAGCAGAGGCAACC	22013931	22015403
FAM160B2-8	CTGGGGGAGTGCAAAAATAC	TGCCCTGGAGTTGAGTCAC	22015565	22016742
FAM160B2-9	CTCATGTGCTGTGTGCCTGG	GAAGATGGGCCCGAATTAGC	22016664	22017910
HR-U1	AGGTGCAGACTGAGAAGAGG	CCTGAGGACCTATCGCTGAC	22046180	22047209
HR-U2	GACAAAGCAGACATGGCAAC	CCTGCCTCATCTCACCTGG	22045022	22046279
HR-U3	GGAAGATGGCAAAGAGCAGC	GGGATTAAGAAGCCTCAGC	22043412	22045065
HR-1	GCAGACAGAGCGGGAGAACG	AGCTTCTGGGGCACATGTCC	22042693	22044017
HR-2	GCCTTACTGGTTTGAGCTGC	CCTAAATGGATGGGCAGAAC	22041337	22042783
HR-3	AGAGACCTAGGGCAGTGCTG	ACAAGCAATCCCCAACAGG	22039972	22041431
HR-4	GAGTTGCTTCGCCTCTCTGG	ACAGAGACCCACGCAGACC	22038732	22040180
HR-5	GTTTGGGGGACCGACTGTGC	CCTCTCCAGCAAAGCCTTCC	22037478	22038837
HR-6	TAATAATTCAGGGCCAGTGG	GGGACAATCAGACGGGAAGC	22036174	22037583
HR-7	CTGCCTGTTCCCTGATGCTG	AGAAGGTGTTTGAGGCATG	22034909	22036289
HR-8	GACGATGACCTTAATGCTACC	GAGTTTGACCGCGGTAGAAG	22034583	22035417
HR-9	ACCATCTTCTCCCTGACAG	GGAAGCCAGGTTGAGTTTTTC	22033276	22034706
HR-10	GAGAACCTAGCTGCCAGTCTG	GCAGTCTCCCCTAACAAGATG	22031889	22033344
HR-11	GAGAACACCGAAGCTCAGTG	GAATGAGCGAGATGGGGAG	22030516	22032053
HR-12	CCAAACAAACTCAGCCATGC	GAACACAGCCCAGTCCATCTAG	22029203	22030653
HR-13	GCATCTGGCCAGAGTATTCC	ACTGGGAGGGAATATGCC	22027779	22029316

c) Primers for U2HR mutation confirmation and screening

Amplicon	Primer (5'-3')	
U2HR	HRUP3F1	AATCACGGGCTCCTGTTTCC
	HRUP3R1	CGTTCTCCCGCTCTGTCTGC

d) Oligonucleotides used in modification of the pGL3-Promoter plasmid

	Sequence (5'-3')
A	AGCTGGCATTCCGGTACTGTTGGTAAAGCCAGAATTCGTCTGCAGGACCGCGGCA
B	CATGTGCCGCGGTCCTGCAGACGAATTCTGGCTTTACCAACAGTACCGGAATGCC

e) Primers for PCR-mediated mutagenesis

Mutation	Primer (5'-3')	
2T>C, 104A>G	Forward	GCGAATTCGAGCAGCTTCCCCACTCTCAGTTGC
	Reverse	CTTTCAGGTGTCAGAATGTGTGG
U1HRm	Forward	GTTGCGCTTCTGGCGACGGCGATCAGAG
	Reverse	CTCTGATCGCCGTCGCCAGAAGCGCAAC
U3HRm	Forward	CCCGGAGCTGGCCACGGGGAGCA
	Reverse	TGCTCCCCGTGGGCCAGCTCCGGG
U4HRm	Forward	GGGAGAGCCCCACGAGGGCAGGAGA
	Reverse	TCTCCTGCCCTCGTGGGGCTCTCCC
7C>T	Forward	GCGTCCCATGGCGTAACTACGGCCTC
	Reverse	GAGGCCGTAGGTTACGCCATGGGACGC
71T>A	Forward	GTGCCGCATCCTGCAGAACCCGGAGTC
	Reverse	GACTCCGGGTTCTGCAGGATGCGGCAC
73C>G	Forward	CATCCTGCAGATCGCGGAGTCCGACCC
	Reverse	GGGTCGGACTCCGCGATCTGCAGGATG
82G>C	Forward	ATCCCGGAGTCCCACCCCTCCAACC
	Reverse	GGTTGGAGGGGTGGGACTCCGGGAT
76G>A	Forward	CCTGCAGATCCCGAAGTCCGACCCCTC
	Reverse	GAGGGGTCGGACTTCGGGATCTGCAGG

f) Primers for RT-PCR

Amplicon	Primer (5'-3')	
HR	HR0+F	GAATCACGGGCTCCTGTTTCCCGCAG
	HR1R2	CTCCTGTCTCACGATGCCGTTCTCTG
HR-EGFP	GFP-F	TAAACGGCCACAAGTTCAGC
	GFP-R	GGTGGTGCAGATGAACTTCAGG
FLAG-Neo ^r	Neo-F	CTTGCCGAATATCATGGTGG
	Neo-R	AAGCTCTCAGCAATATCACGG