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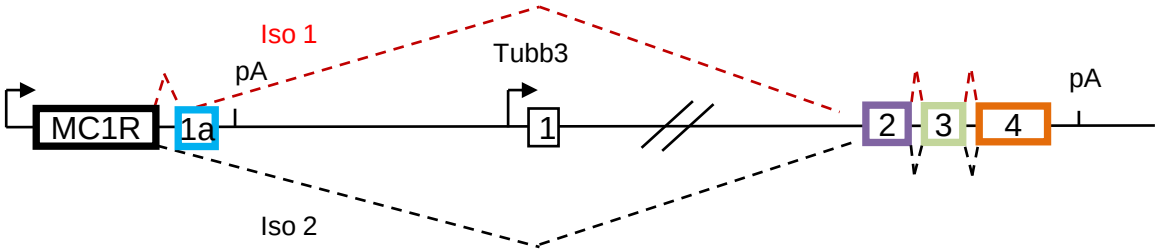

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C

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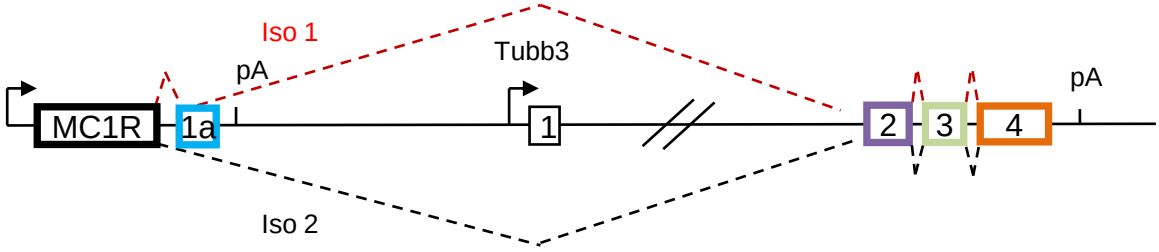
797 aa, MW: 88.4kDa

A



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C

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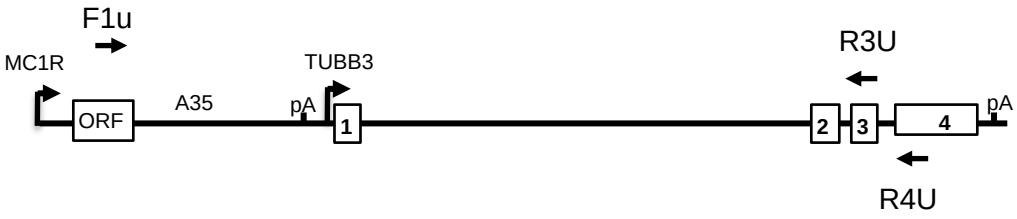
432 aa, MW:47.1kDa

A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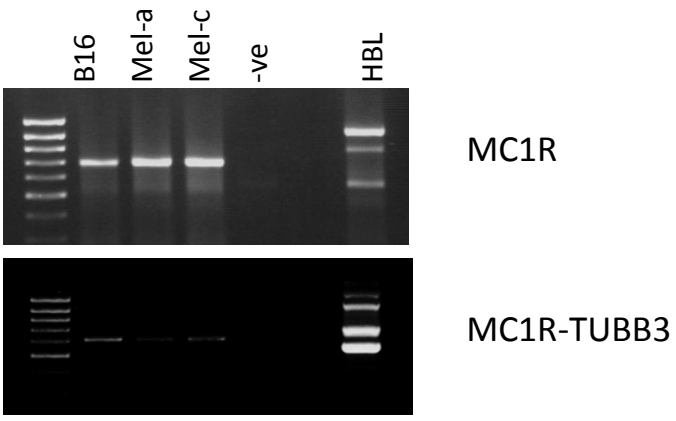
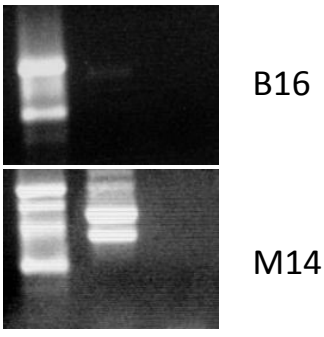
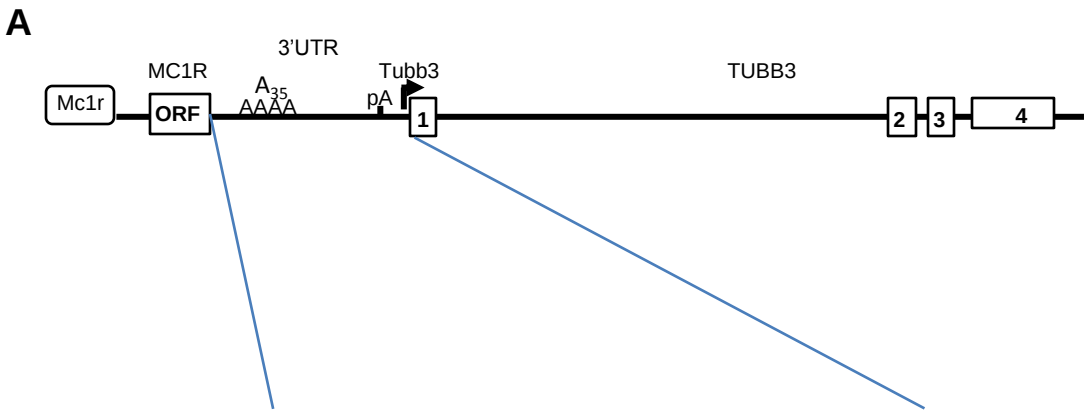
TUBB3
R3U
ORF
A35
pA
1
2
3
4
pA
R4U

B


B16
Mel-a
Mel-c
-ve
HBL
MC1R
MC1R-TUBB3

C


MC1R
MC1R-TUBB3
-ve
B16
M14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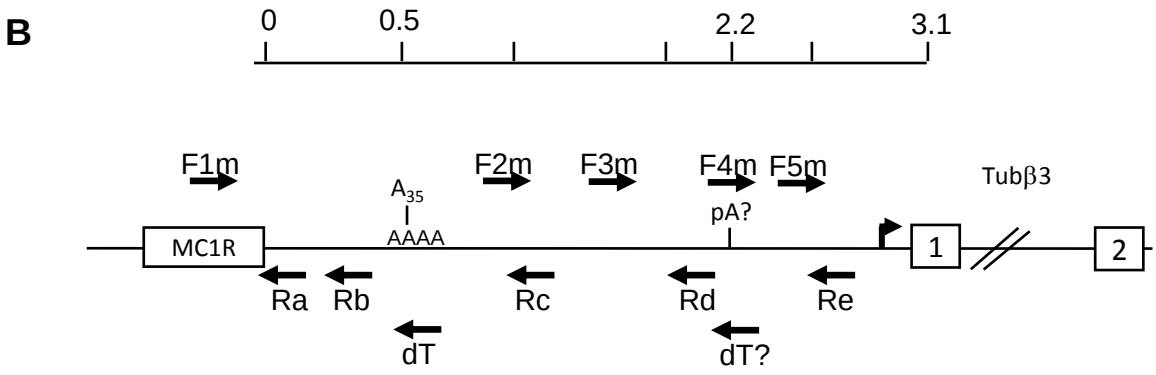
Tubb3
TUBB3
Mc1r
ORF
A35
AAAA
pA
1
2
3
4


B
0
0.5
2.2
3.1
F1m
F2m
F3m
F4m
F5m
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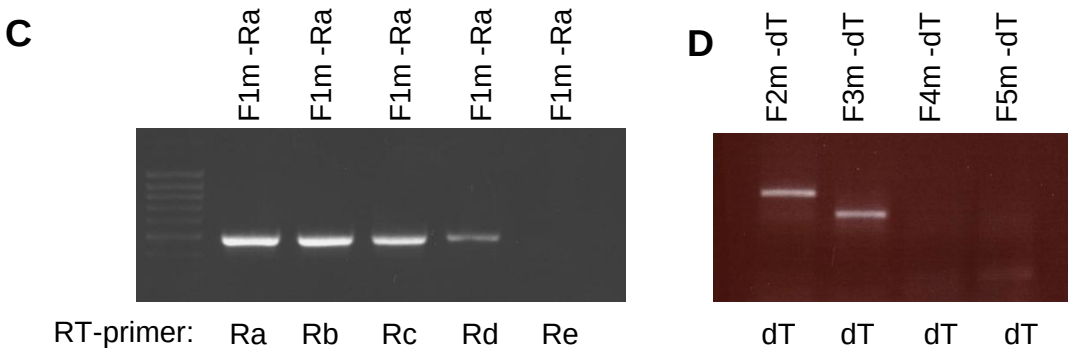
AAAA
pA?
MC1R
1
2
Ra
Rb
Rc
Rd
Re
dT
dT?


C
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F1m -Ra
F1m -Ra
F1m -Ra
RT-primer:
Ra
Rb
Rc
Rd
Re
D
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F4m -dT
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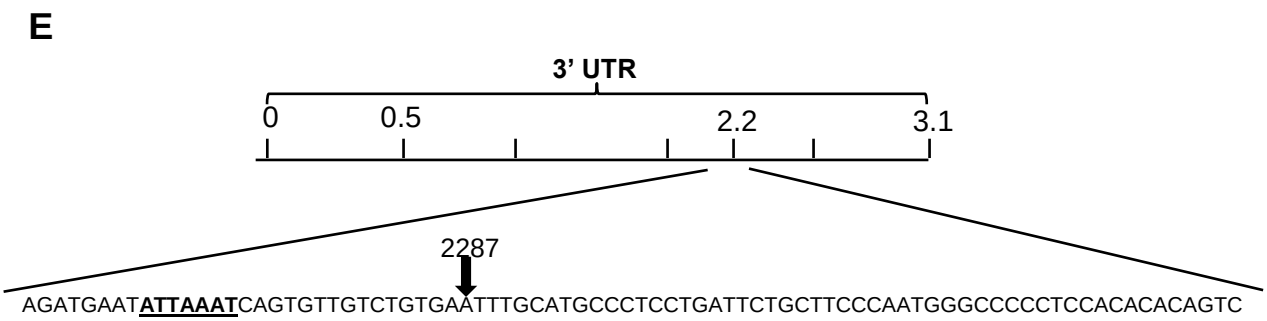
dT
dT
dT
dT


E
3' UTR
0
0.5
2.2
3.1
2287
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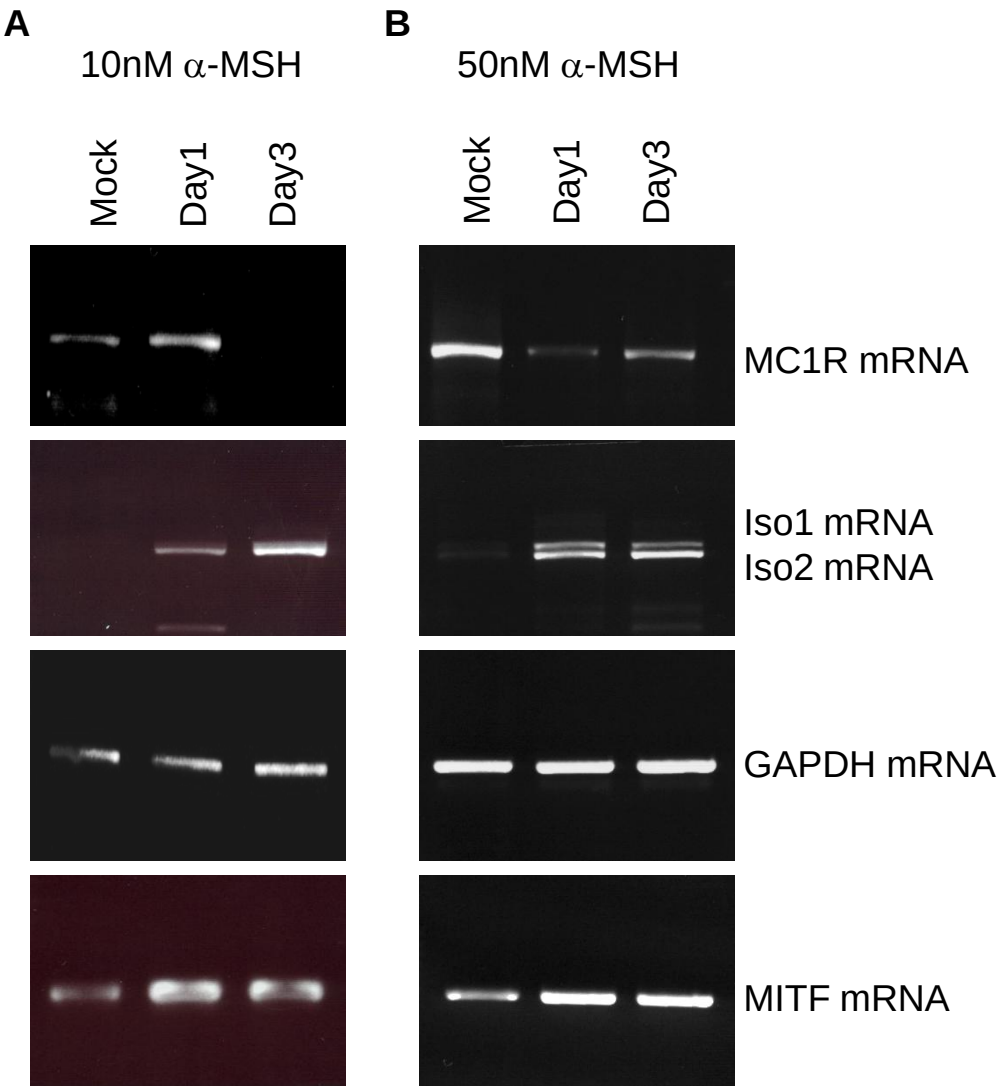
A
10nM α-MSH
Mock
Day1
Day3
B
50nM α-MSH
Mock
Day1
Day3
MC1R mRNA
Iso1 mRNA
Iso2 mRNA
GAPDH mRNA
MITF mRNA

Supplementary Information

Sup_1. MC1R-TUBB3 chimera splicing pattern, mRNA sequence and amino acid composition of isoform 1. (A) The genomic architecture of the MC1R TUBB3 locus is shown. The splicing pattern of chimera transcripts Iso1 and Iso2 is indicated by the red dotted line. Exons of all MC1R derived transcripts are depicted in coloured boxes. **(B)** Nucleotide sequence of the MC1R-TUBB3 chimera transcript Iso1. The colouring of letters refers to the colouring scheme shown in the graph: black = MC1R, blue = exon 1a, purple = TUBB3 exon 2, green = TUBB3 exon 3 and orange = TUBB3 exon 4. Underlined di-nucleotide TG indicates the 5'splice site in the MC1R ORF. **(C)** Amino acid sequence of the uninterrupted open reading frame present in the Iso1 transcript. The first amino acid differing from the MC1R ORF is indicated in red and is underlined.

Sup_2. MC1R-TUBB3 chimera splicing pattern, mRNA sequence and amino acid composition of isoform 2. (A) The genomic architecture of the MC1R TUBB3 locus is shown. The splicing pattern of chimera transcripts Iso1 and Iso2 is represented by the black dotted line. Exons of all MC1R derived transcripts are depicted in coloured boxes. **(B)** Nucleotide sequence of the MC1R-TUBB3 chimera transcript Iso2. The colouring of letters refers to the colouring scheme shown in the graph: black = MC1R, purple = TUBB3 exon 2, green = TUBB3 exon 3 and orange = TUBB3 exon 4. Underlined di-nucleotide TG indicates the 5'splice site in the MC1R ORF. **(C)** Amino acid sequence of the uninterrupted open reading frame present

in Iso2 transcript. The first amino acid differing from the MC1R ORF is indicated in red and is underlined.

Sup_3. Detection of MC1R-TUBB3 intergenic splicing using gene specific reverse transcription primers. (A) Schematic of the mouse genomic MC1R-TUBB3 locus. Locations of gene specific primer (R4U) used in the RT reaction is shown. **(B)** RT-PCR analysis comparing MC1R and MC1R-TUBB3 chimeric expression in total RNA derived from mouse (B16, MELa, MELc) and human (HBL) melanocytes. The RT step was carried out using the TUBB3 exon 4 reverse R4U primer instead of dT and the PCR was performed using the TUBB3 exon 3 reverse R3U in combination with a forward MC1R primer (F1U, again shared by both species). Both primer sequences are conserved in human and mouse TUBB3 (confirmed by genomic sequencing). –ve control represents a RT-PCR reaction using B16 and M14 total RNA without reverse transcriptase. **(C)** RT-PCR analysis comparing MC1R and MC1R-TUBB3 chimeric expression in total RNA derived from mouse (B16) and human (M14) melanocytes. Primers and conditions as (B).

Sup_4. Mouse MC1R has a long 3'UTR. **(A)** Genomic organisation of the mouse MC1R-TUBB3 locus. The Mouse MC1R 3'UTR contains a stretch of 35 adenosines (A35) at approximately the same position as the human pA site, and an annotated pA site (1 AceView accession) located immediately upstream of the TUBB3 promoter (pA). Initial B16 MC1R 3'RACE data were consistent with priming at the A35 site. However, northern blot analysis (data not shown) indicated that this is likely to be mis-priming and that the MC1R 3'UTR

extends beyond the A35 stretch. (**B).** Magnified representation of the mouse MC1R 3'UTR. The location of RT-PCR primers used to confirm that transcription of the MC1R gene extends far beyond the A35 stretch are indicated. **(C,D)** RT-PCR using total RNA isolated from B16 cells confirming that mouse MC1R mRNAs contain a long 3'UTR extending into the TUBB3 promoter region. **(E)** Confirmation by 3' RACE that the poly(A) site verified by 1 accession in Aceview is located immediately upstream of the TUBB3 promoter. Therefore, the mouse MC1R 3'UTR is 2,287 nucleotides long compared to 385 nucleotides in the human MC1R. The 3'UTR is depicted and the sequence immediately surrounding the poly(A) cleavage site, indicated by the vertical arrow, is shown. The hexamer sequence is in bold letters and underlined.

Sup_5. Exposure of a human transformed primary cell line to α-MSH results in a loss of MC1R mRNA coupled with elevated levels of MC1R-TUBB3 chimera transcripts. (A) α-MSH treatment (10nM) of the transformed primary melanocyte cell line HERMES-1 results in a progressive accumulation of MC1R-TUBB3 chimera and a concomitant loss of intronless MC1R transcripts over time. Lanes 1-3; MC1R specific RT-PCR, MC1R-TUBB3 specific RT-PCR, GADPH specific RT-PCR and MITF specific RT-PCR of total RNA isolated from HERMES-1 cells exposed to 10 nM α-MSH for 0 (mock), 1 and 3 days respectively as indicated above each image. The identity of the PCR amplified transcripts are indicated to the right of each panel. **(B)**. α-MSH treatment (50nM) of the same cell line results in a faster accumulation of MC1R-TUBB3 chimeras and a similar parallel loss of intronless MC1R transcripts over time, details as in (A). These results indicate that the time-point when a decrease in MC1R message is observed, may depend on both the cell type and the amount of α-MSH they are exposed to.