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Supplementary Materials for

TRPM1 Forms Ion Channels Associated with Melanin Content in Melanocytes

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Other Supplementary Material for this manuscript includes the following:
(available at www.sciencesignaling.org/cgi/content/full/2/70/ra21/DC1)

Movie S1 (mov format)

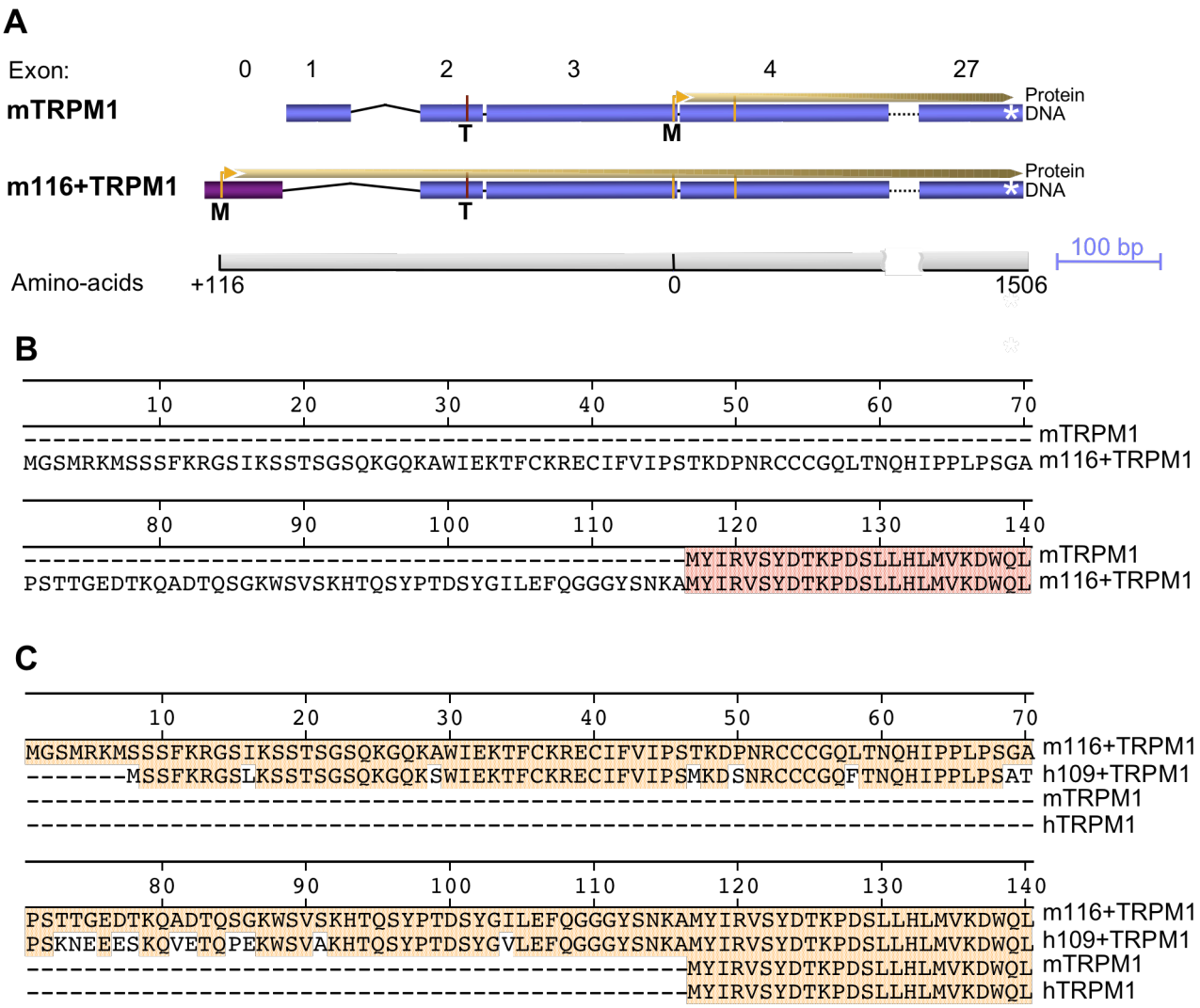

***Fig. S1*. Mouse TRPM1 isoforms.**

A. Exon composition and open reading frame (ORF) of TRPM1 ion channel mouse isoforms identified from database sequences. mTRPM1 contains exons 1 and 2 in its 5'UTR; its start site is in exon 3. M(116+TRPM1) starts in exon 0 and is missing exon1, resulting in a protein with an additional 116 additional N-terminal amino acids compared to mTRPM1.

B. Protein alignments of the N-termini of the two mouse TRPM1 splice variants shown in A. The sequence of the two isoforms is identical, with the exception of the first 116 amino acids that are missing from mTRPM1. Red shading represents conserved amino acids.

C. The N-terminus of m116+TRPM1 shares a high degree of homology with h109+TRPM1 (94.3% identity), whereas mTRPM1 is 96% identical to hTRPM1. Orange shading represents conserved amino acids.

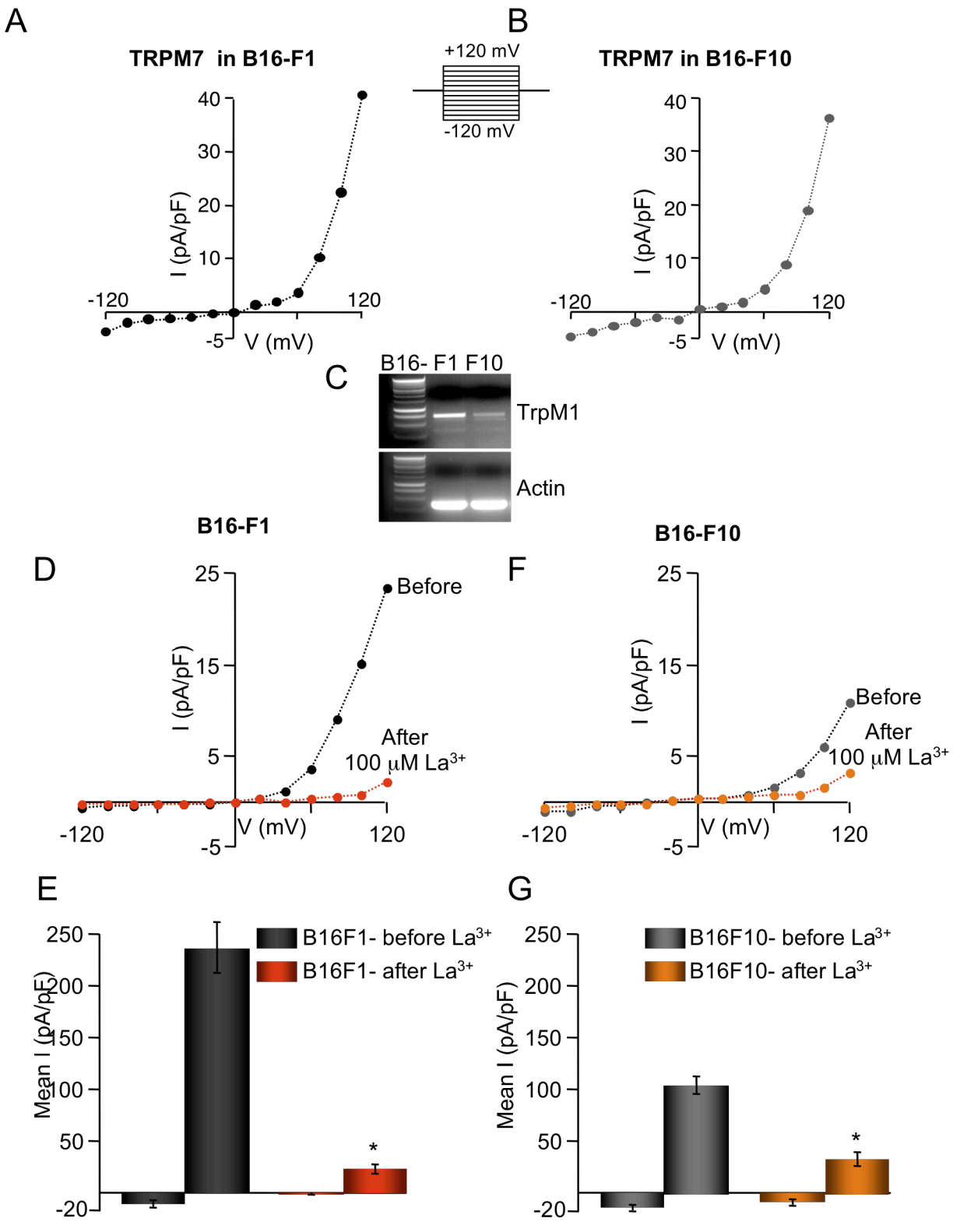

Fig. S2. **Lanthanum blocks endogenous TRPM1 current in B16-F1 and B16-F10 mouse melanocytes**

***Fig. S2*. Lanthanum blocks endogenous TRPM1 current in B16-F1 and B16-F10 mouse melanocytes.**

A. Endogenous whole-cell TRPM7 (MIC) current recorded from B16-F1 cells in response to the voltage step protocol shown.
B. Endogenous whole-cell TRPM7 (MIC) current recorded from B16-F10 cells in response to the voltage step protocol shown. The amplitude of the TRPM7 (MIC) current is similar in B16F1 and B16F10 cells.
C. TRPM1 mRNA is more abundant in B16-F1 cells compared to B16-F10 cells. RT-PCR of TRPM1 trans-membrane fragment (top gel) and actin (bottom gel) using the same amount of mRNA extracted from B16-F1 (left lanes on both gels) or B16-F10 (right lane on both gels) and a sub-saturating number of PCR cycles.
D. I-V curve of the current recorded from a B16-F1 cell before (black) and after (red) addition of 100 μM extracellular La^{3+}.
E. Mean inward and outward current in B16-F1 cells before (black, n = 7) and after (red, n = 8) addition of 100 μM extracellular La^{3+}. In the presence of La^{3+} the outward current is reduced by ~ 90%. *P < 0.001; t test. Error bar, ± S.E.M.
F. I-V curve of the current recorded from a B16-F10 cell before (gray) and after (orange) addition of 100 μM extracellular La^{3+}.
G. Mean endogenous inward (-120 mV) and outward (+120 mV) current from B16-F10 cells before (gray, n=7) or after (orange, n = 5) addition of 100 μM extracellular La^{3+}. In the presence of La^{3+} the outward current is reduced by ~ 75%. *P < 0.05; t test. Error bar, ± S.E.M.

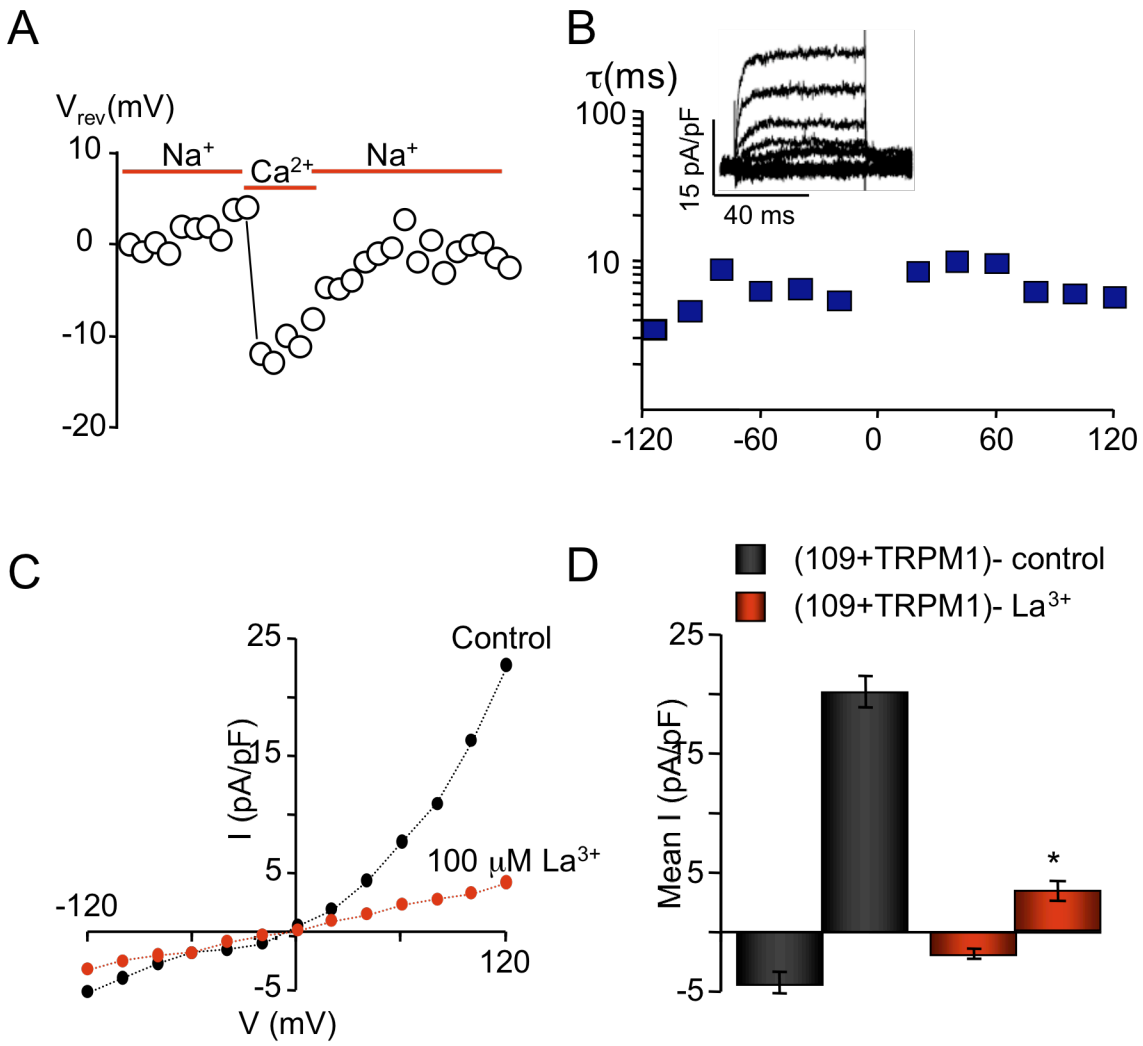

***Fig. S3*. Characterization of 92+TRPM1 and 109+TRPM1 current in SK-Mel19 human melanocytes.**

A. Shift in V_{rev} of 109+TRPM1 current in whole-cell recordings by changing extracellular solution from 150 mM NaCl to 30 mM $CaCl_2$. We observed a small drift in reversal potential during calcium application. Whole-cell current was measured in response to a voltage ramp protocol applied every 2 s.

B. Current activation time constant (t) in response to voltage steps. t was calculated from leak-subtracted single cell traces obtained in response to a step protocol (inset). For each voltage step, t was measured as the time required for the current to reach 67% of the maximal amplitude. Average t was 6.7 ± 0.6 ms.

C. I-V curve of a SK-Mel19 cell expressing 109+TRPM1 before (black) and after (red) addition of 100 μM extracellular La^{3+}.

D. Mean 109+TRPM1 current amplitude of SK-Mel19 cells before (black, n=3) or after (red, n=3) addition of 100 μM extracellular La^{3+}. The outward current amplitude is reduced by 84% from 20.0 ± 0.9 pA/pF to 3.2 ± 0.8 pA/pF upon addition of La^{3+}. *P < 0.001; t test. Error bar, ± S.E.M..

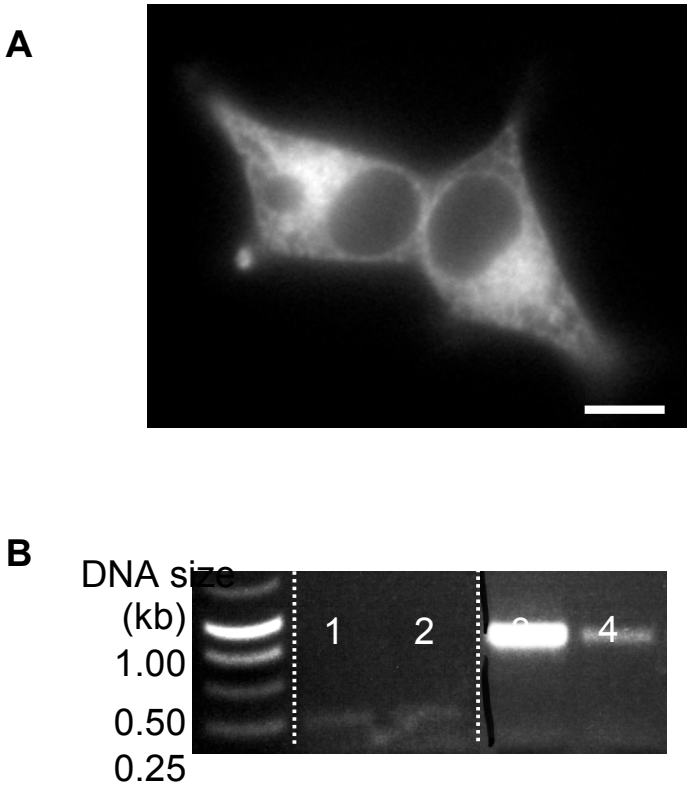

***Fig. S4*. Cellular localization of GFP-(109+TRPM1) in HEK cells and tyrosinase mRNA levels in HEM cells treated with TRPM1 miRNA.**

A. Fluorescent image of two HEK-293T cells expressing GFP-(109+TRPM1) primarily localized to intracellular structures. Calibration bar 10 μm.

B. Tyrosinase mRNA abundance is similar in HEM cells treated with TRPM1 miRNA (lane 2) and control miRNA (lane 1). RT-PCR of tyrosinase fragment using the same amount of mRNA extracted from HEM cells treated with control (lane 1) or TRPM1 (lane 2) miRNA and a sub-saturating number of PCR cycles. The amount of TRPM1 mRNA was markedly decreased in HEM cells treated with TRPM1 miRNA (lane 4) compared to control miRNA (lane 3).

***Movie S1*. Dynamic localization of GFP-(70+TRPM1) in human melanoma cells.** GFP-(70+TRPM1) expressed in SK-Mel22 human melanoma cells is localized to highly motile tubulo-vesicular structures. TIRF images of a region of a cell were recorded every 2 s and were animated at 5 fps. The field shown is ~ 7 μm wide.