ACCSCIENCE PUBLISHING

Advanced Neurology

ORIGINAL RESEARCH ARTICLE

Transient receptor potential melastatin 7 signaling in U251 cell migration and invasion involves calcineurin

Supplementary Files

(A) Supporting information

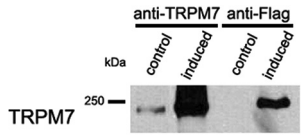

Figure S1. Validation of the tetracycline-inducible system in HEK293 cells for Flag-TRPM7 overexpression. HEK293 cell proteins were harvested 24 h after administration of 1 μg/mL tetracycline or ddH_2O of the same volume. Flag-TRPM7 (212 kDa) was observed to be overexpressed by tetracycline induction and the Flag-tag was only observed in the induced sample.
Abbreviation: TRPM7: Transient receptor potential melastatin 7.

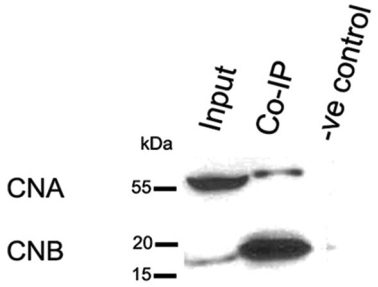

Figure S2. Precipitation of calcineurin B-subunit along with calcineurin A by Flag-TRPM7 in HEK293 cells. Flag-tagged TRPM7 protein was immunoprecipitated using anti-Flag antibody and protein A/G agarose beads. The immunoprecipitated samples were analyzed using Western blot. Both proteins (calcineurin A: 61 kDa, calcineurin B: 19 kDa) were shown in the co-IP sample along with Flag-TRPM7 (not shown here) and the input (HEK293 cell total lysate), whereas neither was found in the negative control (antibody-free co-IP mixture).
Abbreviation: TRPM7: Transient receptor potential melastatin 7.

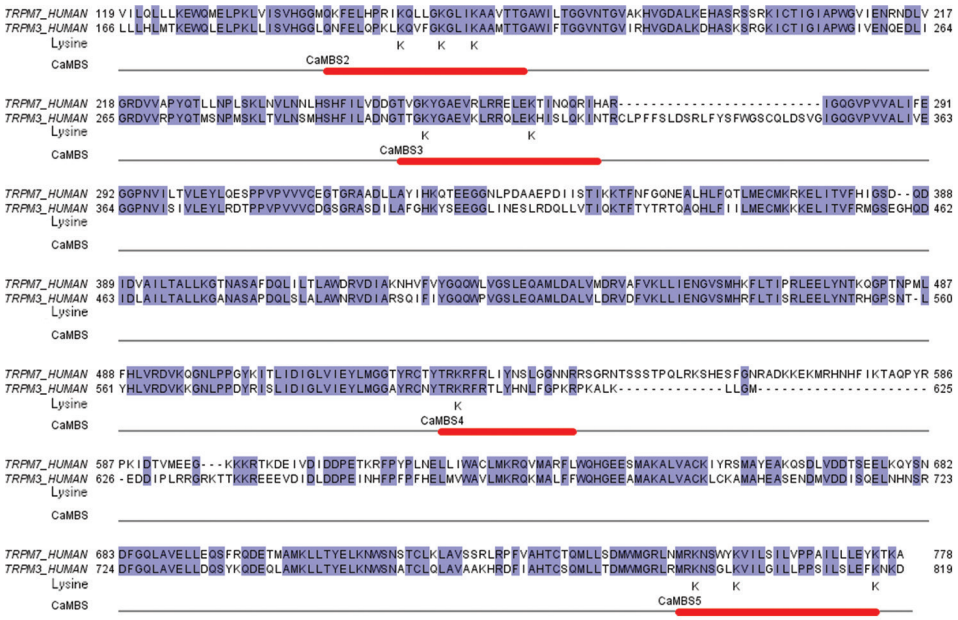

Figure S3. Alignment of four calmodulin-binding sequences in TRPM3 with the TRPM7 channel sequence. The human TRPM7 sequence (top) is aligned with the human TRPM3 sequence (bottom). The calmodulin-binding sequences[1] are highlighted in red and lysine residues that are reported to be crucial for calmodulin binding are displayed below the TRPM3 sequence. Lysine residues within calmodulin-binding sites are conserved between TRPM7 and TRPM3, and calmodulin-binding sites show a large degree of alignment between TRPM7 and TRPM3 in general. Three out of these four binding sites align 100% with the mouse TRPM7 protein used in this study, while the other one aligns 24 out of 25 amino acids. Alignment was generated using T-Coffee multiple sequence aligner[2] and the figure was generated using Jalview[3].
Abbreviation: TRPM7: Transient receptor potential melastatin 7.

(B) Raw images

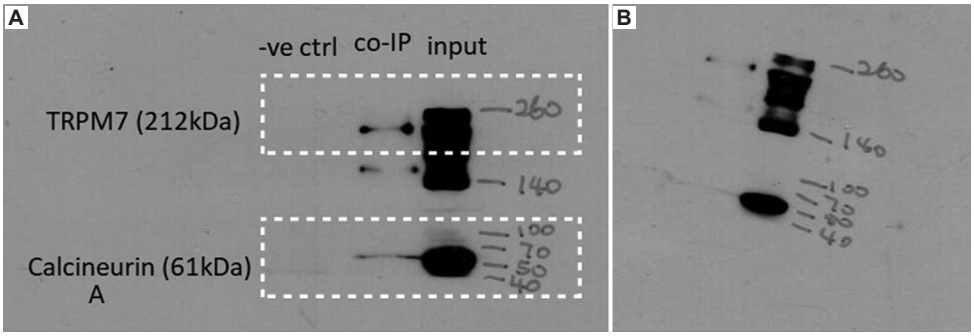

Figure S4. Original images of Figure 1A of main article: Western blotting of HEK cell lysate for TRPM7 and Calcineurin A. (A) The image is flipped horizontally for the manuscript. The sections enclosed in the dotted line are included in the manuscript. (B) This image is the same blot as (A) but with low exposure.
Abbreviation: TRPM7: Transient receptor potential melastatin 7.

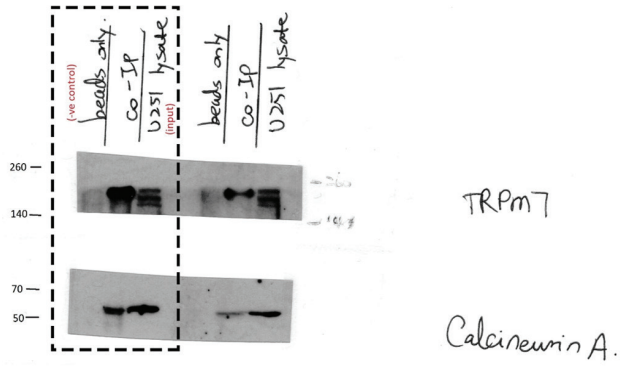

Figure S5. Original images of Figure 1B of main article: Western blotting of U251 cell lysate for TRPM7 (212 kDa) and Calcineurin A (61 kDa). The image is flipped horizontally for the manuscript. The section enclosed in the dotted line is included in the manuscript. This figure shows two experimental groups of the same experiment.
Abbreviation: TRPM7: Transient receptor potential melastatin 7.

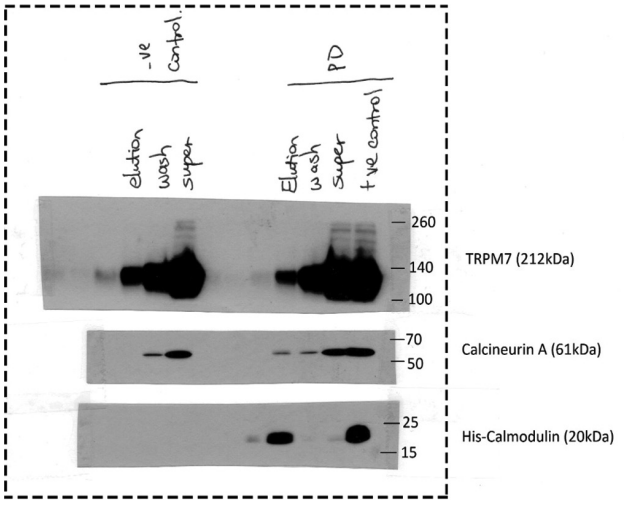

Figure S6. Original images of Figure 2 of main article: Western blotting for TRPM7 (212 kDa), Calcineurin A (61 kDa), and His-calmodulin (20 kDa). The image is flipped horizontally for the manuscript. The section enclosed in the dotted line is included in the manuscript.
Abbreviation: TRPM7: Transient receptor potential melastatin 7.

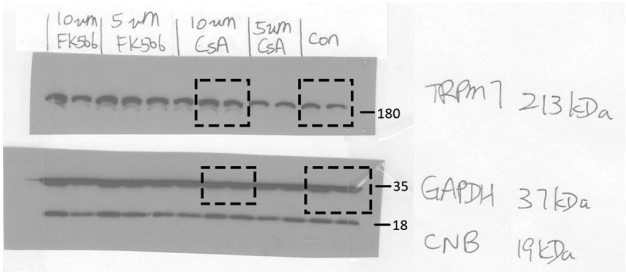

Figure S7. Original images of Figure 3B of main article: Western blotting for TRPM7 (212 kDa) and GAPDH (37 kDa). The image is flipped horizontally for the manuscript. The sections enclosed in the dotted line are included in the manuscript.
Abbreviation: TRPM7: Transient receptor potential melastatin 7.

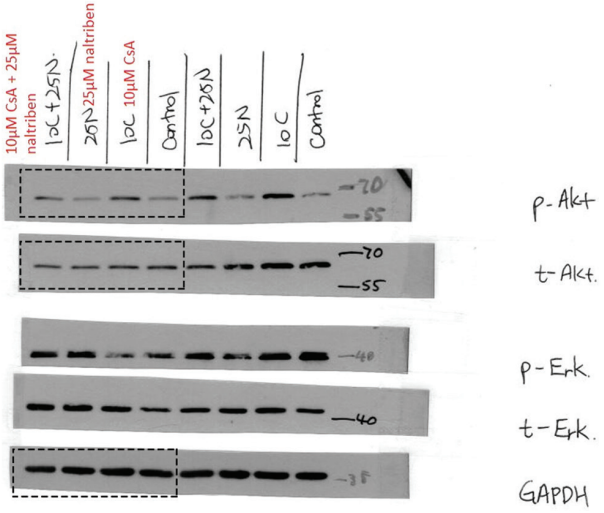

Figure S8. Original images of Figure 5A of main article: for Western blotting for p-AKT (60 kDa), t-AKT (60 kDa), and GAPDH (37 kDa). The image is flipped horizontally for the manuscript. The sections enclosed in the dotted line are included in the manuscript. This figure shows two experimental groups of the same experiment.

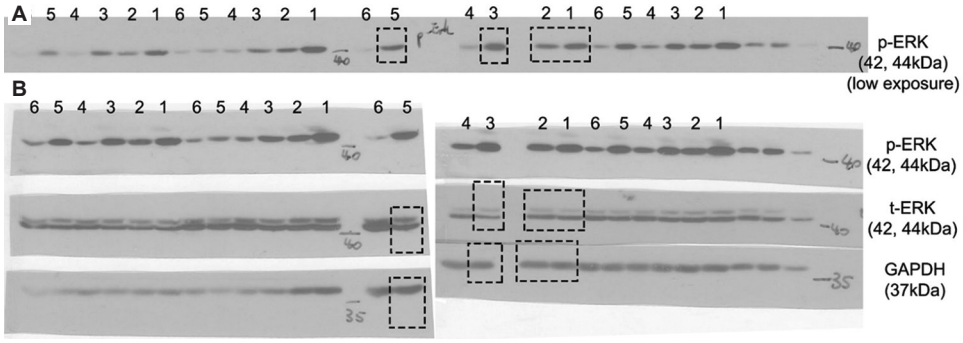

Figure S9. Original images of Figure 5D of main article: Western blotting for (A) p-ERK (42, 44 kDa), (B) t-AKT (42,44 kDa) and GAPDH (37 kDa). The numbered treatment groups are (1) control (DMSO); (2) 10 μM CsA; (3) 25 μM naltriben; (4) unrelated to this manuscript; (5) 10 μM CsA + 25 μM naltriben; and (6) unrelated to this manuscript. The bands for p-ERK in (A) and (B) are the same blots but were exposed with different lengths of time. The image is flipped horizontally for the manuscript. The sections enclosed in the dotted line are included in the manuscript. This figure shows three experimental groups of the same experiment.

References

1. Przibilla J, Dembla S, Rizun O, *et al*., 2018, $Ca2^{+}$-dependent regulation and binding of calmodulin to multiple sites of Transient Receptor Potential Melastatin 3 (TRPM3) ion channels. *Cell Calcium*, 73: 40–52.

https://doi.org/10.1016/j.ceca.2018.03.005

2. Wallace IM, 2006, M-Coffee: Combining multiple sequence alignment methods with T-coffee. *Nucleic Acids Res*, 34: 1692–1699.

https://doi.org/10.1093/nar/gkl091

3. Waterhouse AM, Procter JB, Martin DMA, *et al*., 2009, Jalview Version 2--a multiple sequence alignment editor and analysis workbench. *Bioinformatics*, 25: 1189–1191.

https://doi.org/10.1093/bioinformatics/btp033