SUPPLEMENTARY INFORMATION

to the manuscript of "Overexpression of transient receptor potential canonical type1 (TRPC1) alters both store operated calcium entry and depolarization-evoked calcium signals in C2C12 cells" by Tamás Oláh *et al.*

Supplementary Figure 1.

Examination of the effect on SOCE of the extent of TRPC1 overexpression. **(A)** Western-blot analysis of TRPC1 in the transfected clones. The 87 kDa human isoform was expressed from the plasmid used for stable transfection. Clone 8 was showing the most significant overexpression, so this clone was used in most of the other experiments (referred to as TRPC1 on the other figures). Clone 10 and 11 were also used in some experiments. Changes in calcium concentration demonstrating SOCE were recorded following the re-establishing of the normal (1.8 mM) external calcium concentration ($[Ca^{2+}]_o$) in TRPC1 overexpressing C2C12 clones (**(B)** clone 11, **(C)** clone 10). The internal calcium-stores were emptied by 4 μM thapsigargin (TG) in the absence of $[Ca^{2+}]_o$. Representative records of 2 independent cultures. **(D)** Maximal rate of rise and **(E)** amplitude of SOCE in myotubes overexpressing TRPC1 in different extents. Asterisks (*) indicate significant ($p < 0.05$) difference if compared to parental cells; paragraph signs (§) indicate significant ($p < 0.05$) difference if compared to mock-transfected, while "and" signs (&) indicate significant ($p < 0.05$) difference if compared to TRPC1 overexpressing (clone 8) myotubes. Numbers in parentheses indicate the number of cells measured.

Supplementary Figure 2.

Immunocytochemical staining of 5-day-old C2C12 myotubes demonstrating the presence of proteins associated with calcium signaling. **(A)** Expression of STIM1 protein in parental (left panel) and in TRPC1 overexpressing (right panel) C2C12 myotubes. **(B)** Expression of Orai1 protein in parental (left panel) and in TRPC1 overexpressing (right panel) C2C12 myotubes. Nuclei were stained with DAPI. Calibration is the same for all images. Original magnification was 40×.

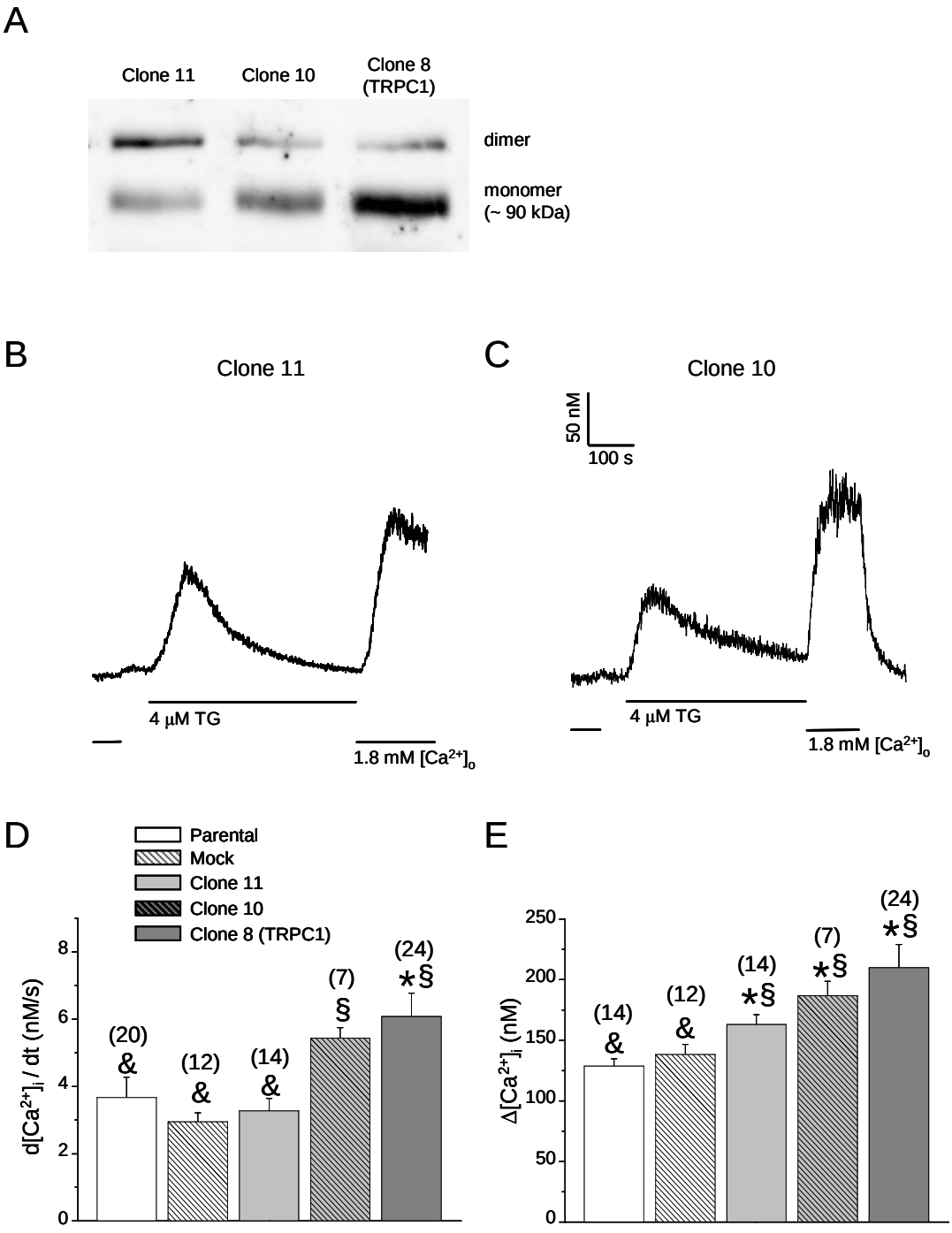
Supplementary Figure 3.

Immunocytochemical staining of 5-day-old C2C12 myotubes demonstrating the aggregation of STIM1 protein into puncta after store depletion caused by 4 μM thapsigargin for 30 minutes. On the right panel the zoom was 4×, on the same visual field as shown in the left panel. **(A)** Expression of STIM1 protein in parental C2C12 myotubes. **(B)** Expression of STIM1 protein in mock-transfected C2C12 myotubes. **(C)** Expression of STIM1 protein in TRPC1 overexpressing C2C12 myotubes. Nuclei were stained with DAPI. Original magnification was 40×.

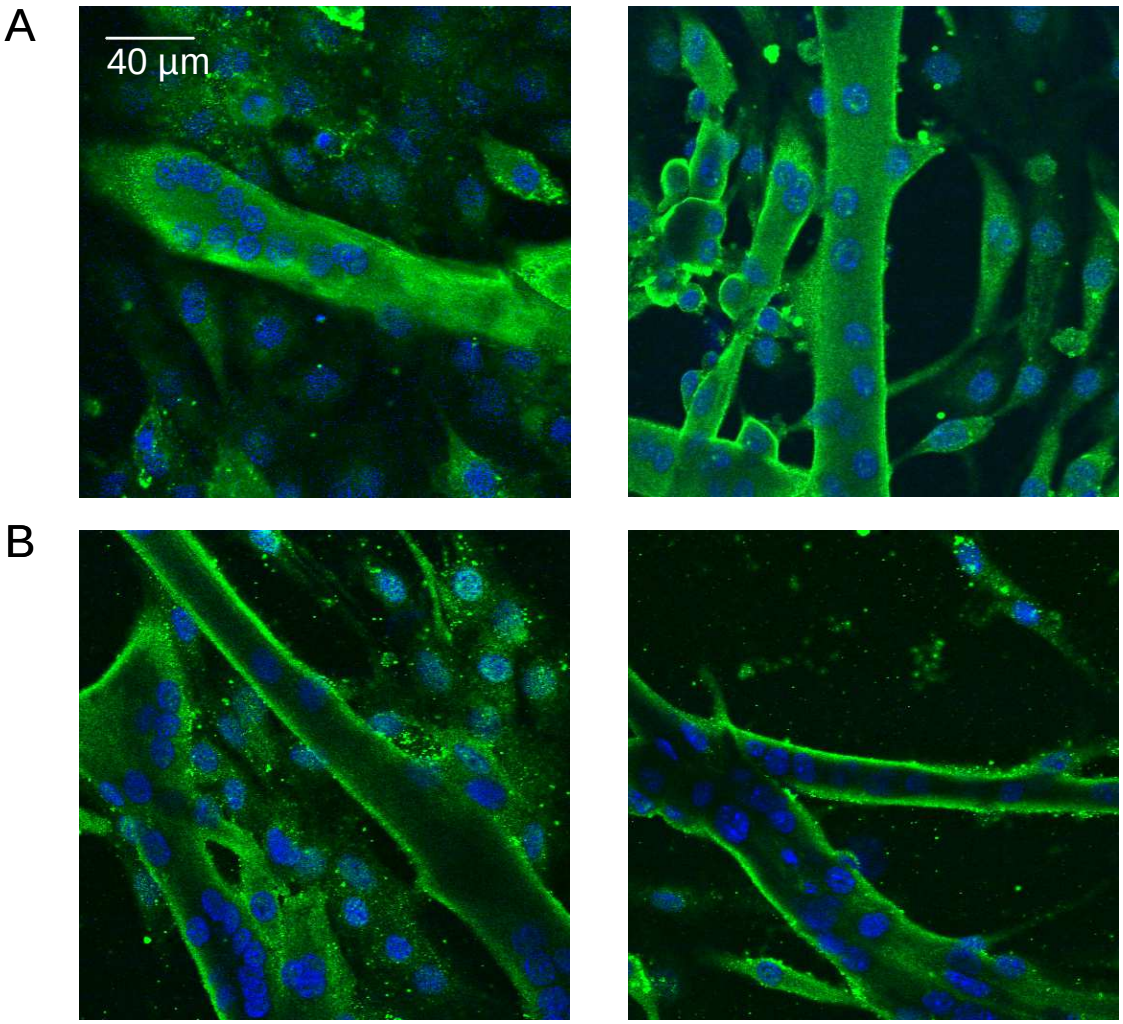
Supplementary Figure 4.

Effects of NFAT activity in parental and TRPC1 overexpressing myotubes. Cells were transfected with a HSP-NRE lacZ plasmid as described by Rosenberg and co-workers [36] (a kind gift from E. Zádor). The transfected cells expressed β-galactosidase from the plasmid in function of NFAT activity. The cells were fixed with 4 ºC 0.5% glutaraldehyde (diluted in PBS) for 10 min, and then washed three times with PBS. Then 1 mg/ml X-Gal (5-bromo-4-chloro-3-indoyl-B-D-galactoside; from Invitrogen) was dissolved in reaction buffer (20 mM $K_4Fe(CN)_6$, 20 mM $K_3Fe(CN)_6$, 2 mM $MgCl_2$ in PBS, pH 7.8) and placed on the cells overnight. In the dependence of β-galactosidase activity, the cells became blue. The stained cells were examined by a fluorescent microscope. 5-5 ROIs were selected on each myotube. Quantitative analysis was performed with ImageJ on 15 parental and 9 TRPC1 overexpressing myotubes ($p<0.05$; 57.5±1.4 AU, n=75 in parental and 46.1±2.6 AU, n=45 in TRPC1 overexpressing myotubes). **(A)** Parental myotubes. **(B)** TRPC1 overexpressing myotubes. Original magnification was 10×. **(C)** Representative images of differentiating C2C12 cells on different days of culturing. Parental, mock-transfected and TRPC1 overexpressing C2C12 myotubes were cultured under the same conditions. The proliferating solution was exchanged to differentiating solution on the 2nd day of culturing. Note the thinner diameter of the terminally differentiated myotubes in the TRPC1 overexpressing cultures compared to parental and mock-transfected cultures.

Supplementary Figure 1. (Oláh *et al*.)



Supplementary Figure 2. (Oláh *et al*.)



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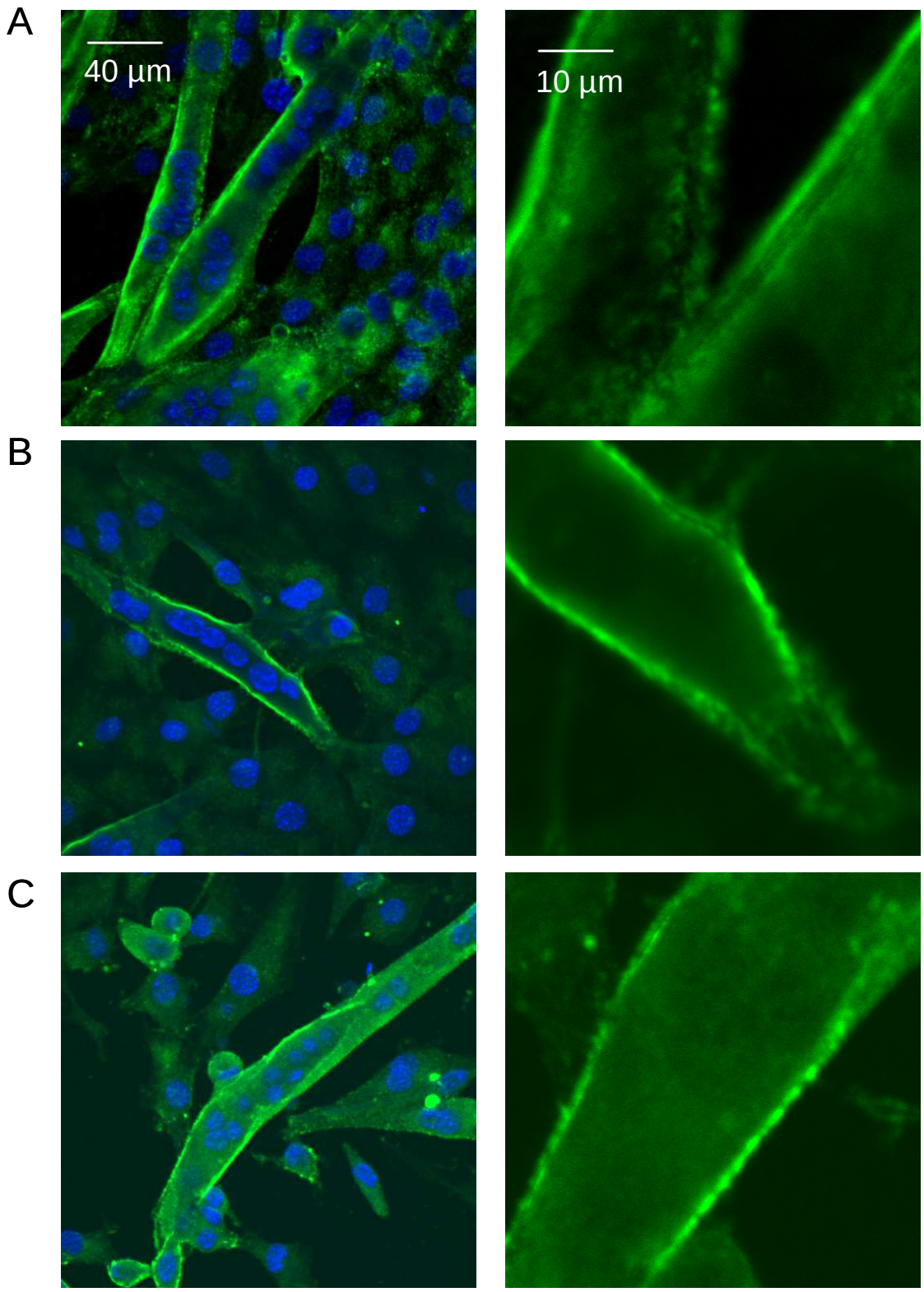

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