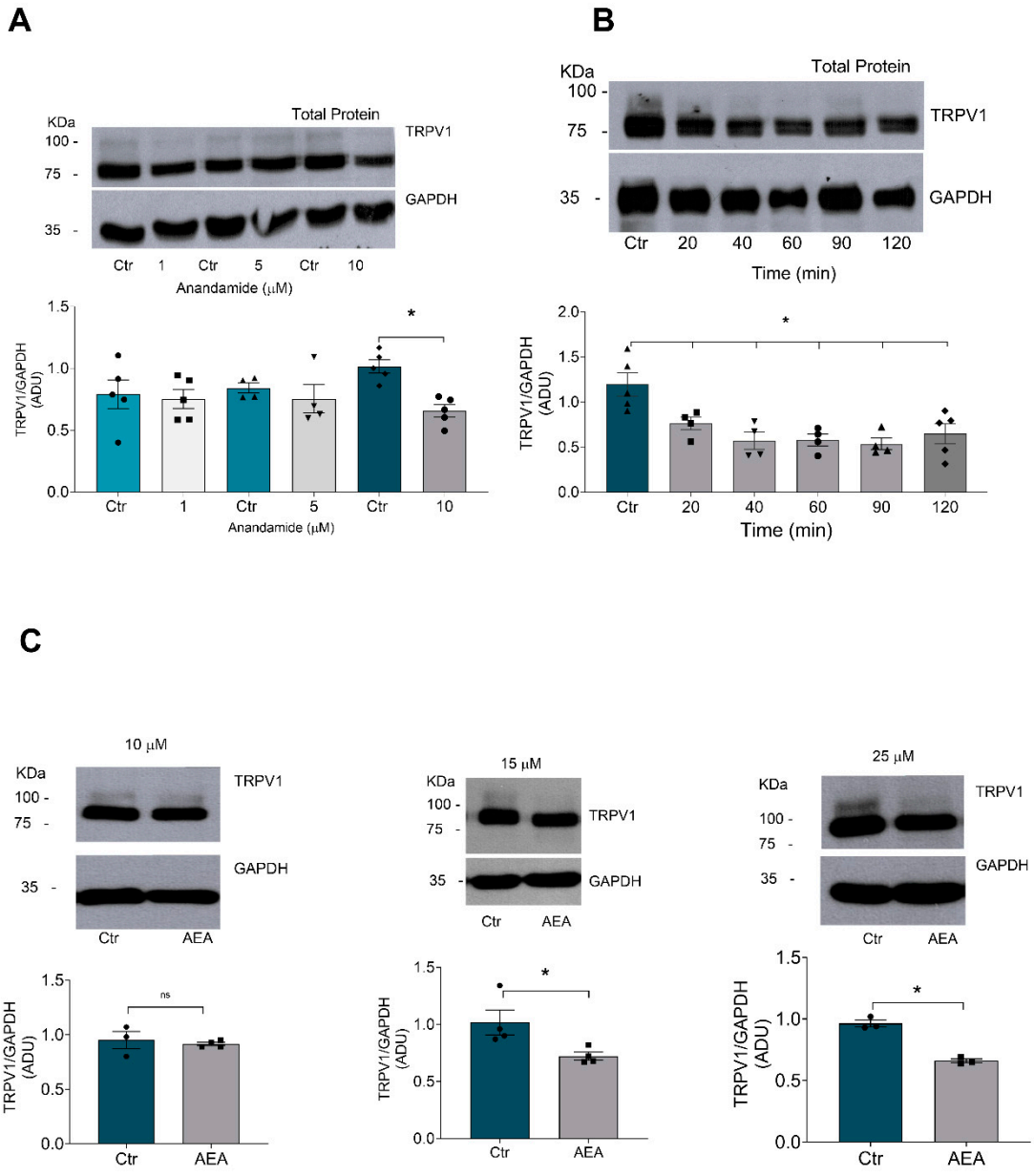
pharmaceutics

Supplementary Materials:

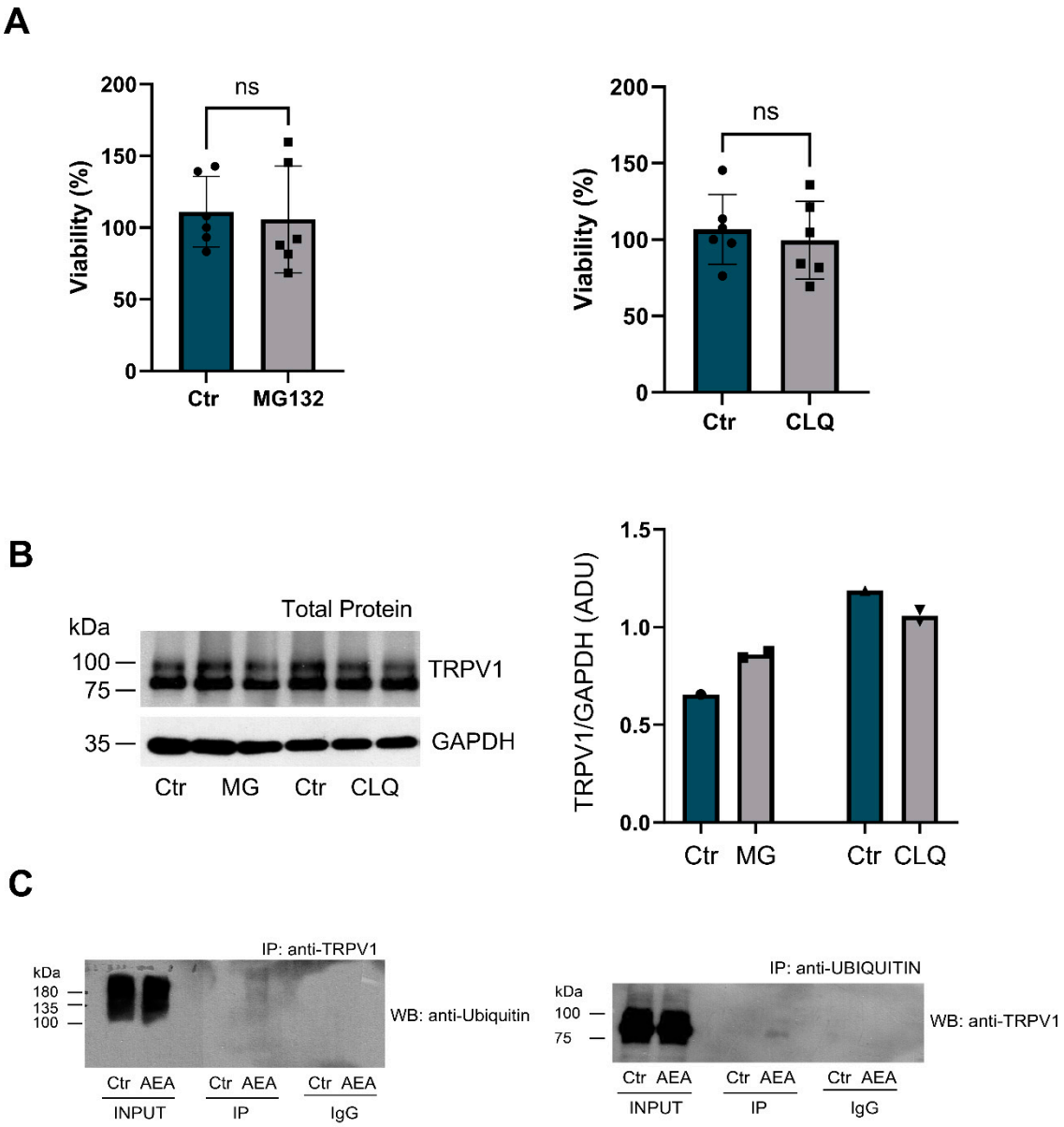
Anandamide Reduces the Capsaicin-Evoked Pain by Regulating TRPV1 Protein Levels

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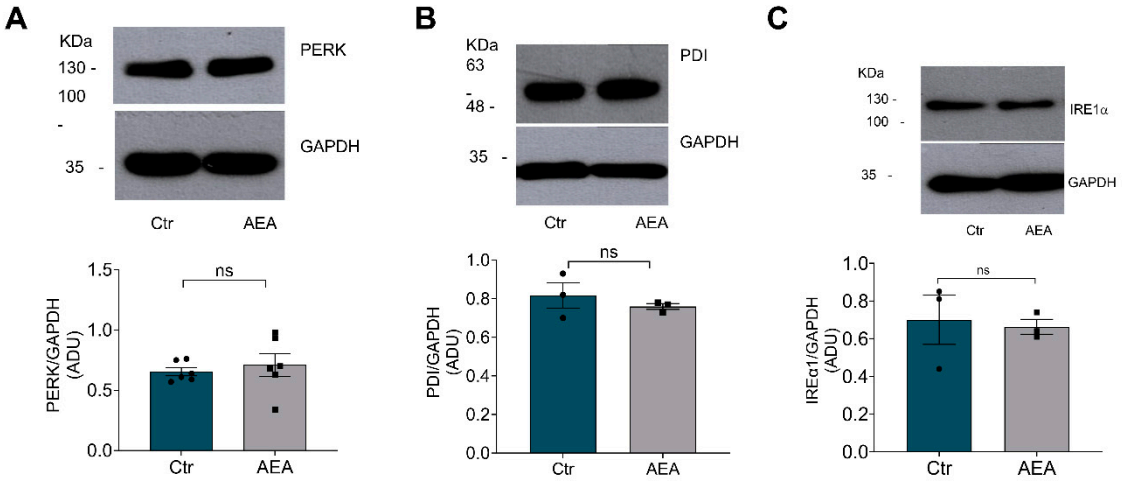
Supplementary Figure S1. Dose- and time-response curves for AEA treatment. (A) WB representative of TRPV1 total protein from HEK293 cells transiently transfected and treated with 1, 5, and 10 μM for 20 min. Treatment with 10 μM significantly reduced TRPV1 expression to 0.65 ± 0.03 ADU compared with controls (1.00 ± 0.04, $n = 8$). AEA at 10 μM consistently produced midrange effects and was therefore selected for subsequent experiments. (B) WB representative of TRPV1

protein levels in HEK293 transiently transfected cells treated with 10 μM during different periods of time: 20, 40, 60, 90, and 120 min. Treatment for 20 min reduced TRPV1 protein levels to 0.76 ± 0.07 ADU, compared with the control (1.20 ± 0.13 ADU; n = 4). We performed ANOVA followed by Tukey´s multiple comparisons test. *p < 0.001; ns: not statistically significant. (C) Immunodetection of TRPV1 and GAPDH total proteins from TRPV1-expressing HEK293 cells treated for 10 min at three different AEA concentrations (10, 15, and 25 μM). The graphs show that 10-min AEA treatment at three concentrations decreases TRPV1 protein levels compared with control cultures. The bars represent the TRPV1 densitometric value normalized to GAPDH signal: Ctr= 0.95 ± 0.07 vs 10 μM AEA= 0.91 ± 0.015, n = 3; Ctr= 1.02 ± 0.1 vs 15 μM AEA= 0.72 ± 0.03, n = 3 and Ctr= 0.96 ± 0.03 vs 25 μM AEA= 0.66 ± 0.02, n = 3. The bars represent the TRPV1 values normalized to GAPDH (mean± SEM): Student´s t-test was performed *p < 0.01; ADU= arbitrary densitometric units.



Supplementary Figure S2. Pretreatment with MG132 and chloroquine does not affect cell viability or TRPV1 protein levels. (A) HEK293 cells were seeded in 96 well dishes and treated with 25 μM MG132 and 100 μM Chloroquine (CLQ) for 8 and 4 h, respectively. Cells were then stained with

crystal violet, and absorbances at 570 nm were measured on a spectrophotometer. The graphs show that neither compound affected HEK293 cell viability; the bars represent absorbances at 570 nm (mean± SD) (n = 6). We performed a Student´s t-test; ns, statistically non-significant. (B) Immunoblots of TRPV1 and GAPDH from protein extracts of control cultures and cultures treated with MG132 or Chloroquine. The graph shows that the treatments do not affect the TRPV1 protein levels (n = 2). (C) Immunoblot of ubiquitinated proteins co-immunoprecipitated with anti-TRPV1 antibody from total protein of TRPV1-expressing HEK293 cells under control and AEA treatment conditions (left) and immunoblot of TRPV1 co-immunoprecipitated with anti-ubiquitin antibody. The data show that ubiquitinated proteins co-immunoprecipitate under AEA treatment. (n = 2).



Supplementary Figure S3. Evaluation of ER stress markers after AEA treatment. (A) WB representative of stress protein from HEK293-transiently transfected cells treated with 10 μM AEA for 20 min. (A) PERK expression was 0.65 ± 0.03 in control and 0.71 ± 0.09 in treated; (B) PDI expression was 0.8±0.06 in control and 0.76 ± 0.01 in treated; (C) IRE1α expression was 0.71 ± 0.13 in control and 0.66 ± 0.03 in AEA-treated cells. Student´s t-test was performed; ns = not significant; ADU = arbitrary densitometric units.