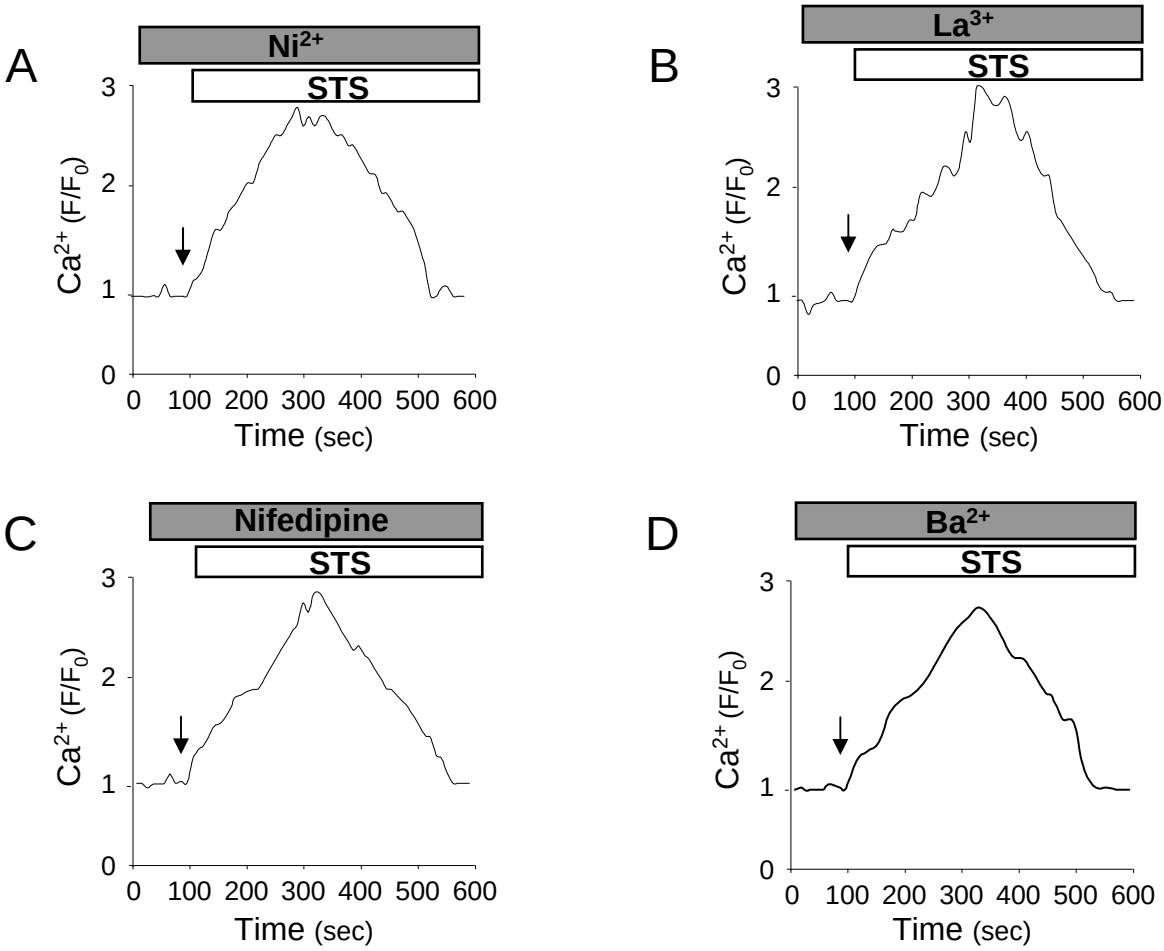

Figure S1. Lack of effect of known inhibitors of voltage-dependent Ca^{2+} channels on PKC-inhibitor-induced Ca^{2+} influx. Representative single-cell Ca^{2+} recordings of Fluo-4/AM- loaded U1810 cells pre-exposed to **(A)** 3 mM $NiCl_2$, **(B)** 3 mM $LaCl_3$, **(C)** 50 µM Nifedipine or **(D)** 3 mM $BaCl_2$ prior to administration of 0.2-1 µM STS. Ratio F/F_0 represents fluorescence intensity over baseline.

Norberg *et al* Figure S1

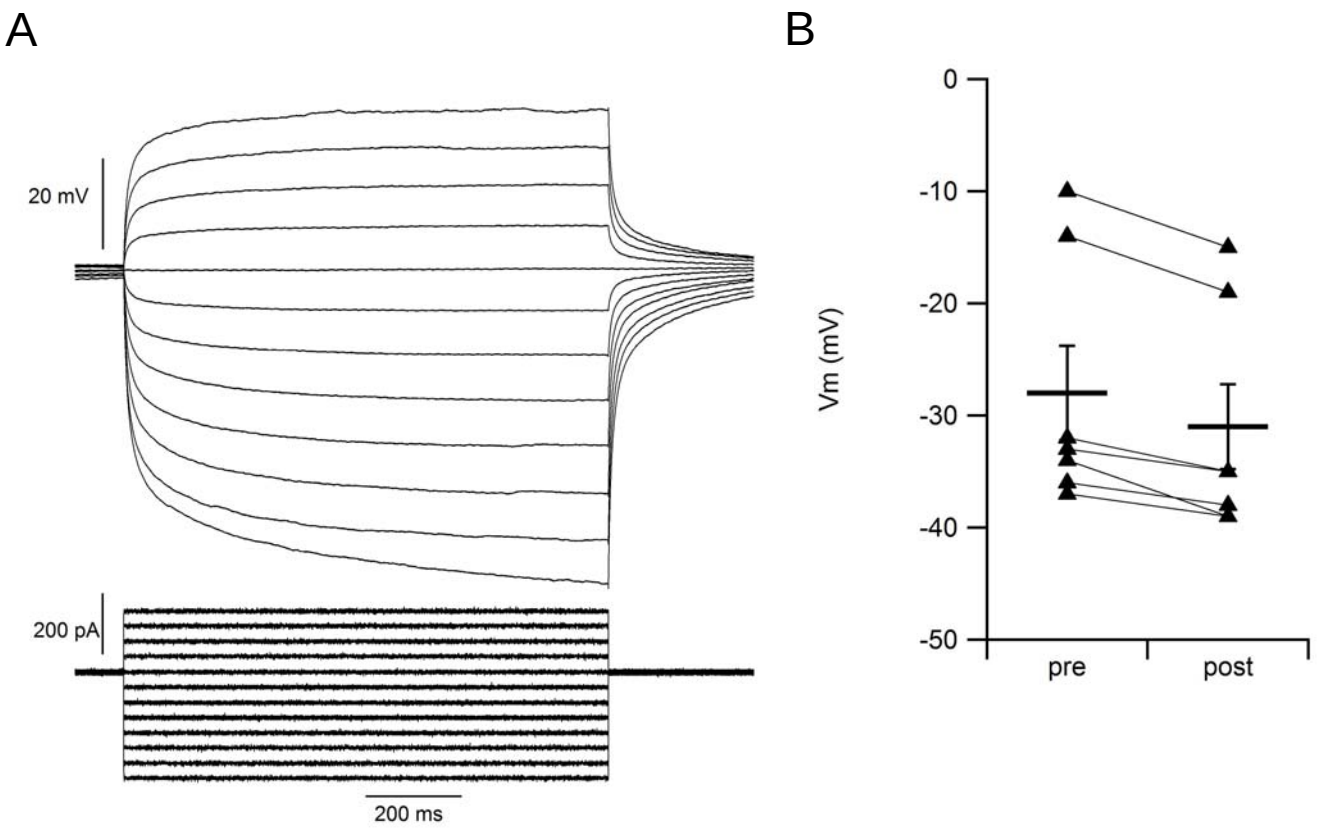

Figure S2. Electrophysiology recording of U1810 cells. (A) Injected current steps and their corresponding voltage responses. **(B)** All recorded cells (7/7) hyperpolarized following application of 0.5 μM STS. The black triangles show the membrane potential of the individual cells before and after STS application and the horizontal bars denote the average membrane potential.

Results

We obtained stable whole-cell patch clamp recordings. All cells had a negative membrane potential (-28 ± 4.2 mV). Following perfusion of 0.5 μM STS to the bath solution, all cells hyperpolarized (3.4 ± 1.5 mV, n = 7/7, p=0.0103 Wilcoxon rank sum test), and most cells (6/7) showed a slight increase in their input resistance.

Material and methods

Patch –clamp recordings

Whole-cell patch clamp recordings were obtained from U1810 cells, visualized using IR-DIC microscopy (BX51WI, Olympus, Germany) in KREBS medium. Recordings were amplified using axoclamp 2B (Molecular Devices, CA, USA), filtered at 2 KHz, digitized (5-20 KHz) using ITC-18 (Instrutech, NY, USA), and acquired using Igor Pro (Wavemetrics, OR, USA). Patch pipettes were pulled with a Flamming/Brown micropipette puller P-97 (Sutter Instruments Co, Novato, CA) and had an initial resistance of 5-10 MΩ, containing 110 mM K-gluconate, 10 mM KCl, 10 mM HEPES, 4 mM Mg-ATP, 0.3 mM GTP, 10 mM phosphocreatine.

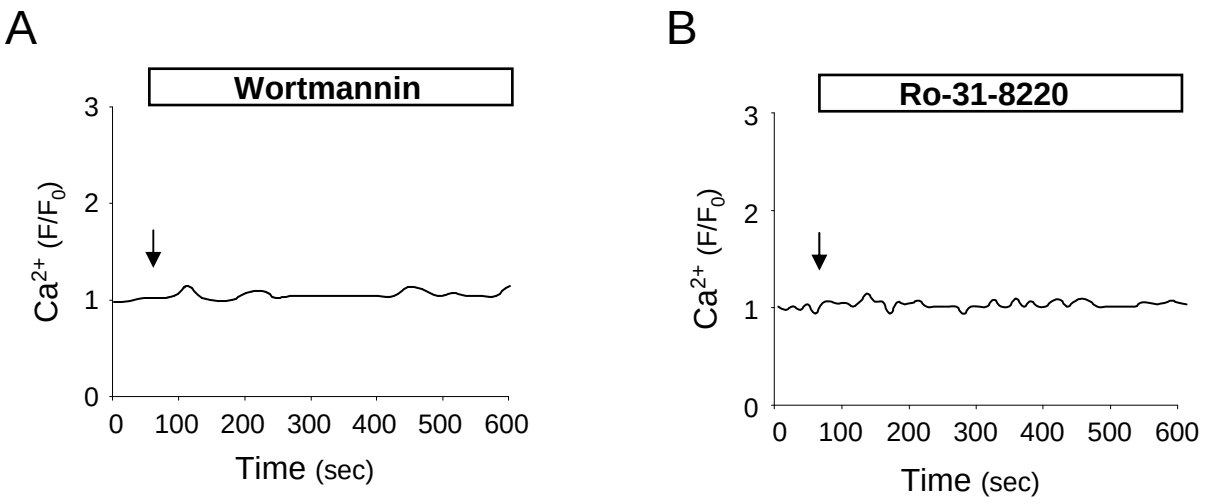

Figure S3. Lack of effect of other kinase inhibitors on Ca^{2+} import by U1810 cells. Representative single-cell Ca^{2+} recordings of Fluo-4/AM- loaded U1810 cells exposed to **(A)** 0.1 µM Wortmannin and **(B)** 10 µM Ro-31-8220. Ratio F/F_0 represents fluorescence intensity over baseline.

Norberg *et al* Figure S3

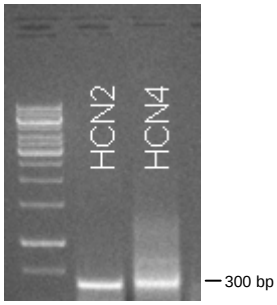

Figure S4. Expression analysis of HCN2 and HCN4 in NSCLC H661 cells.
mRNA levels were analyzed using RT-PCR as described in Material and Methods section.

Norberg *et al* Figure S4

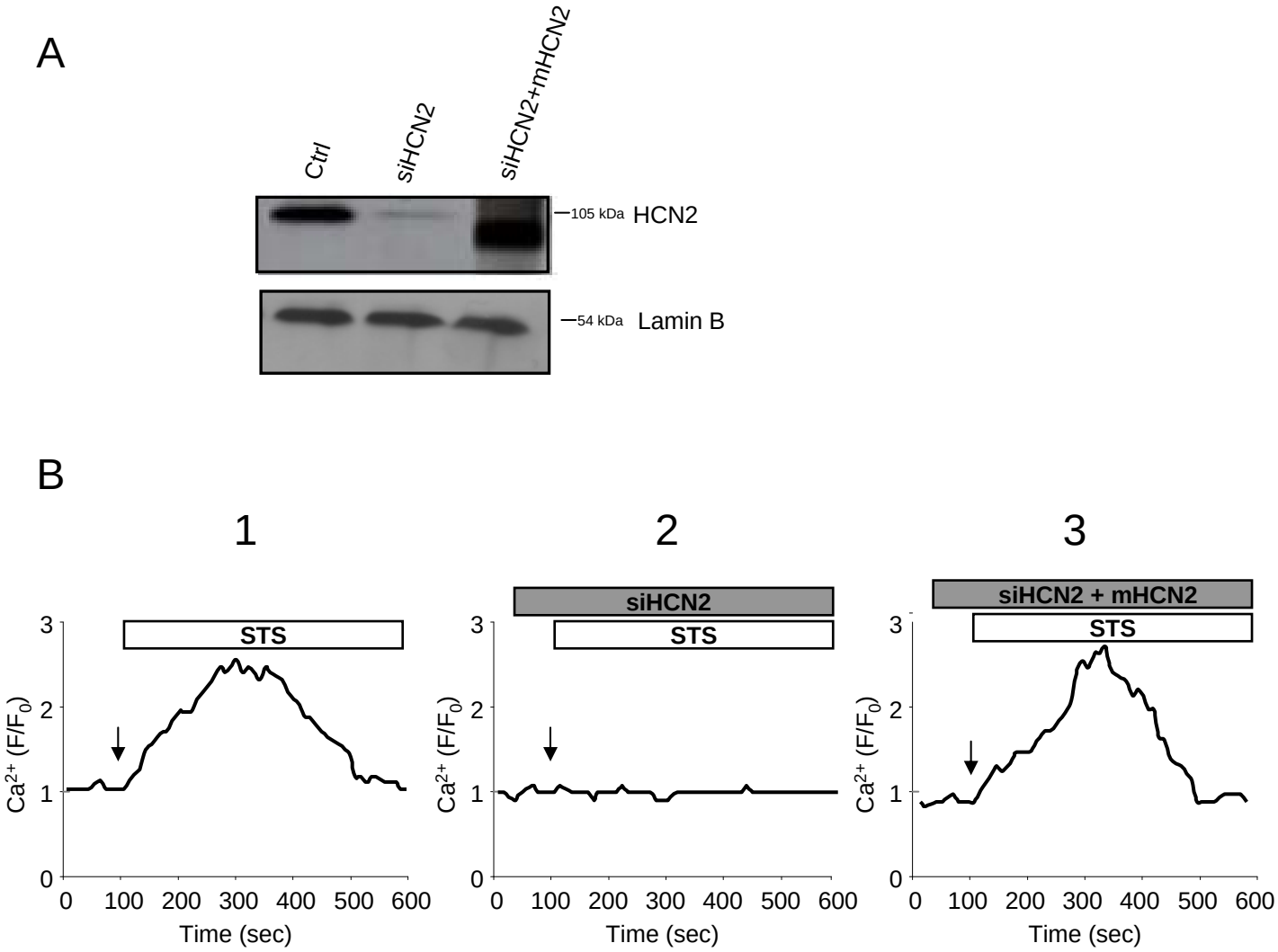

Figure S5. Rescue experiment revealed that the STS-induced Ca^{2+} responses could be restored by re-transfection of HCN2-depleted U1810 cells with mHCN2.
(A) Western Blot analysis of the HCN2 protein expression level. 1. wild-type, 2. siHCN2, and 3. siHCN2 followed by expression of mHCN2. The membrane was re/probed for Lamin B to confirm equal loading of samples. **(B)** Representative single-cell Ca^{2+} recordings of Fluo-4/AM-loaded U1810 cells, treated as in (A), which were exposed to 0.2 µM STS. Ratio F/F_0 represents fluorescence intensity over baseline.

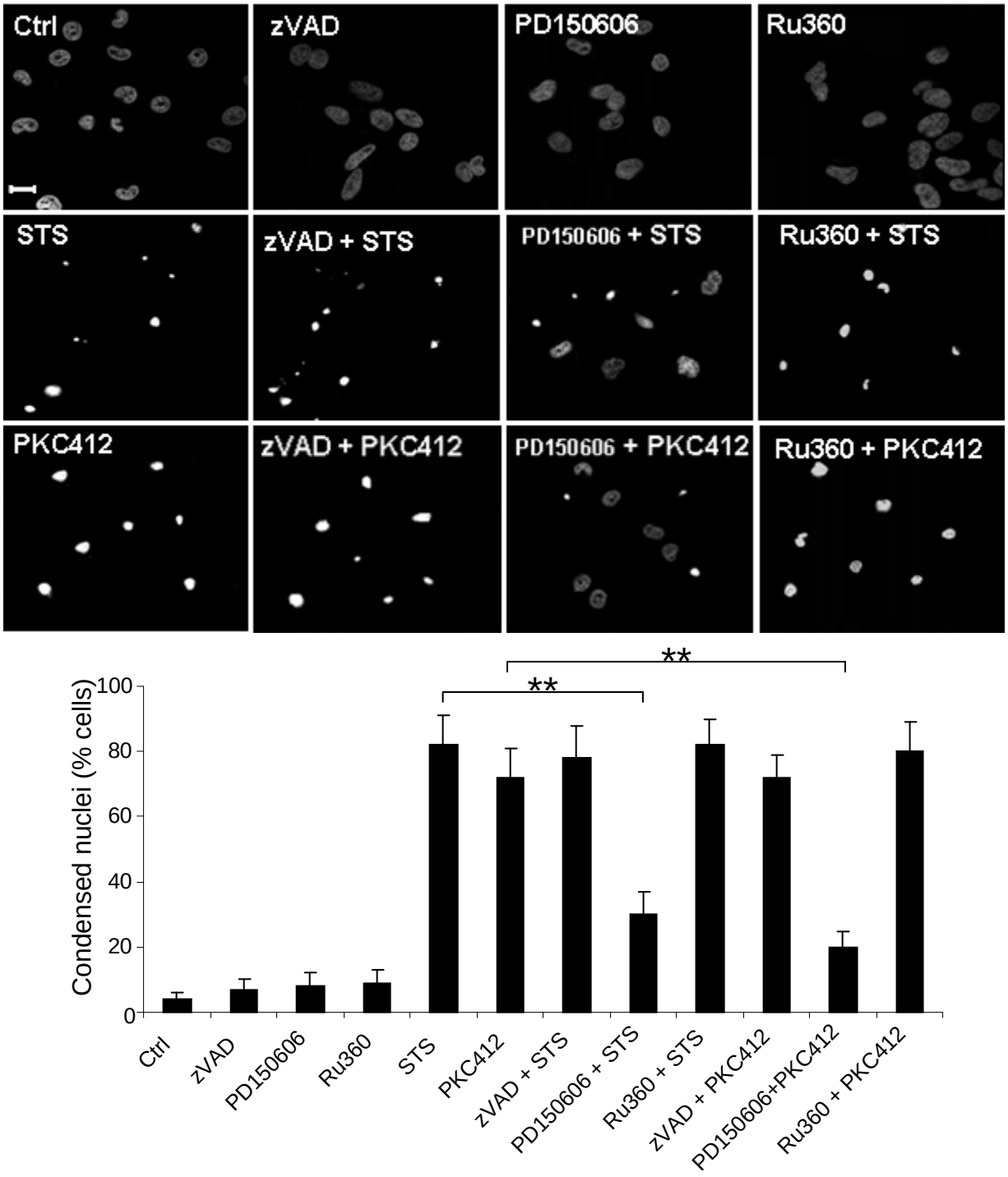

Figure S6. Calpain-AIF-mediated nuclear condensation in U1810 cells. Cells were pre-treated with various inhibitors (indicated in the figure), exposed to STS or PKC412, fixed and the nuclei were stained with DAPI. Condensed nuclei were counted and presented
in the graph as mean±SD (n=3). **, $P < 0.01$.

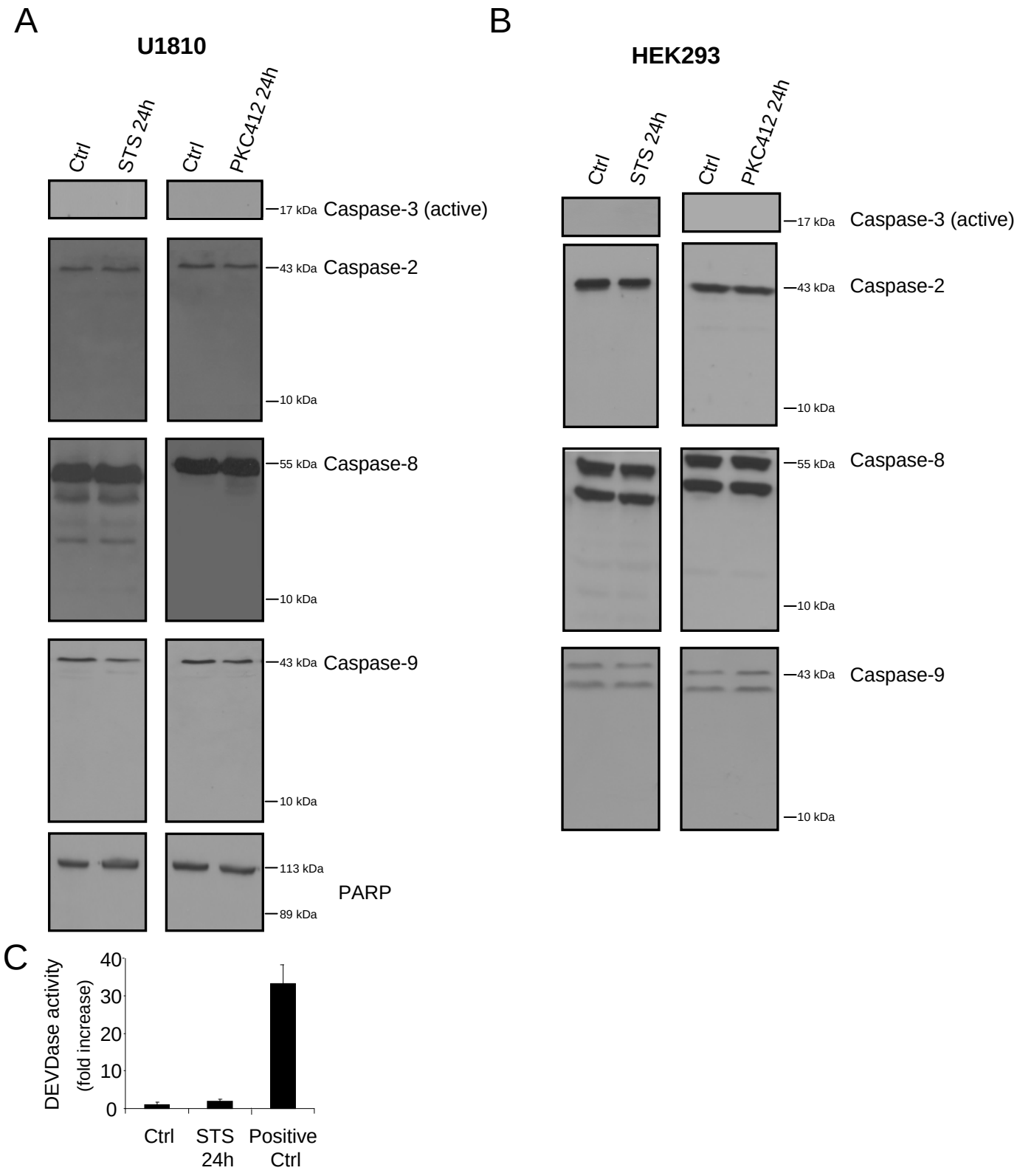

Figure S7. No caspase processing or activity is induced upon treatment of U1810 or HEK293 cells with STS or PKC412. (A) U1810 and **(B)** HEK293 cells were exposed to either 0.2µM STS or 1µM PKC412 for 24h and processing of caspases was analyzed using Western Blotting. Caspase-3/-7-like activity was measured by cleavage of DEVD-AMC substrate in control U1810 cells and cells exposed to STS for 24h. As positive control, HeLa cells were treated with STS for 8h. Results are presented as Mean±SD (n=3).

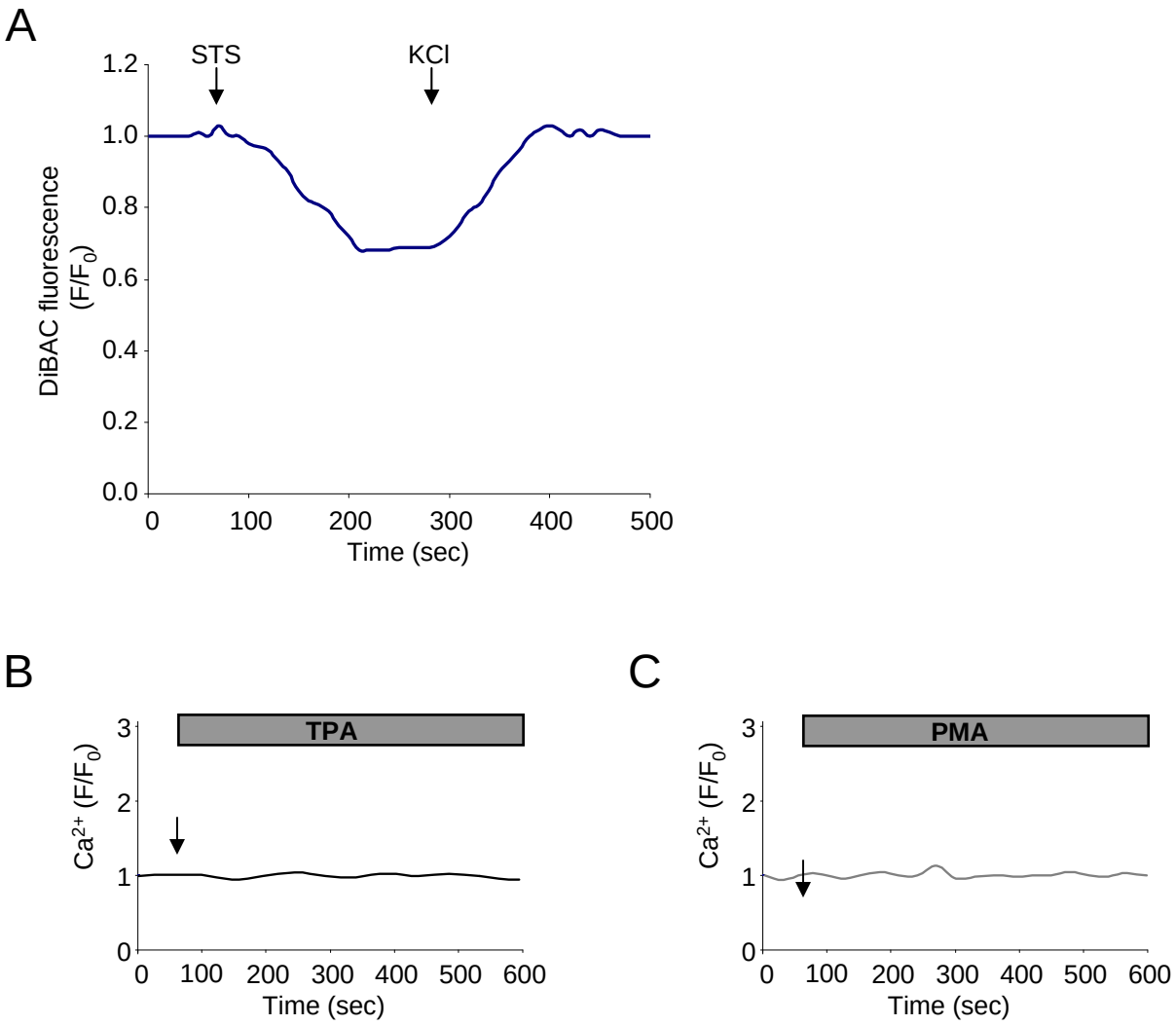

Figure S8. Hyperpolarization by STS treatment and effects of PKC activators on the intracellular Ca^{2+} level. (A) Representative single-cell polarization recordings of cell s exposed to STS followed by depolarization with KCl. Representative single-cell Ca^{2+} recordings of Fluo-4/AM-loaded U1810 cells exposed to **(B)** TPA, **(C)** 50 µM PMA. Ratio F/F_0 represents fluorescence intensity over baseline. Arrow indicates time of addition of drugs.

A

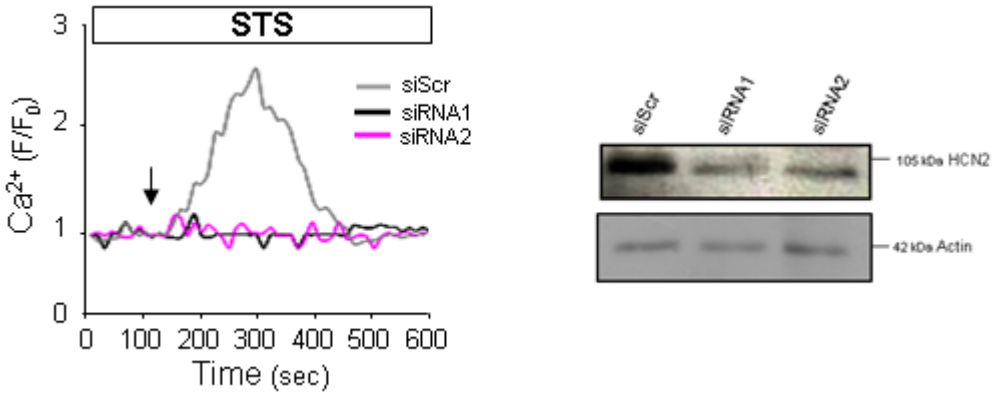

Figure S9A. Use of two different non-overlapping siRNAs to downregulate HCN2 and corresponding Ca^{2+} responses . (A) Representative single-cell Ca^{2+} recordings of Fluo-4/AM- loaded U1810 cells that have been depleted of HCN2 using two distinct non-overlapping siRNAs.

B

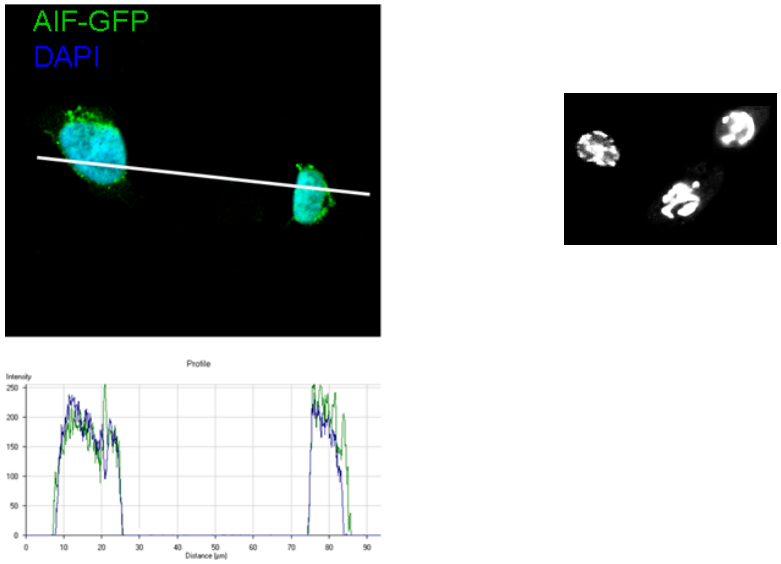

Figure S9B. Assessment of nuclear translocation of AIF-GFP upon STS exposure of cells. U1810 cells were transfected with AIF-GFP and treated with STS.