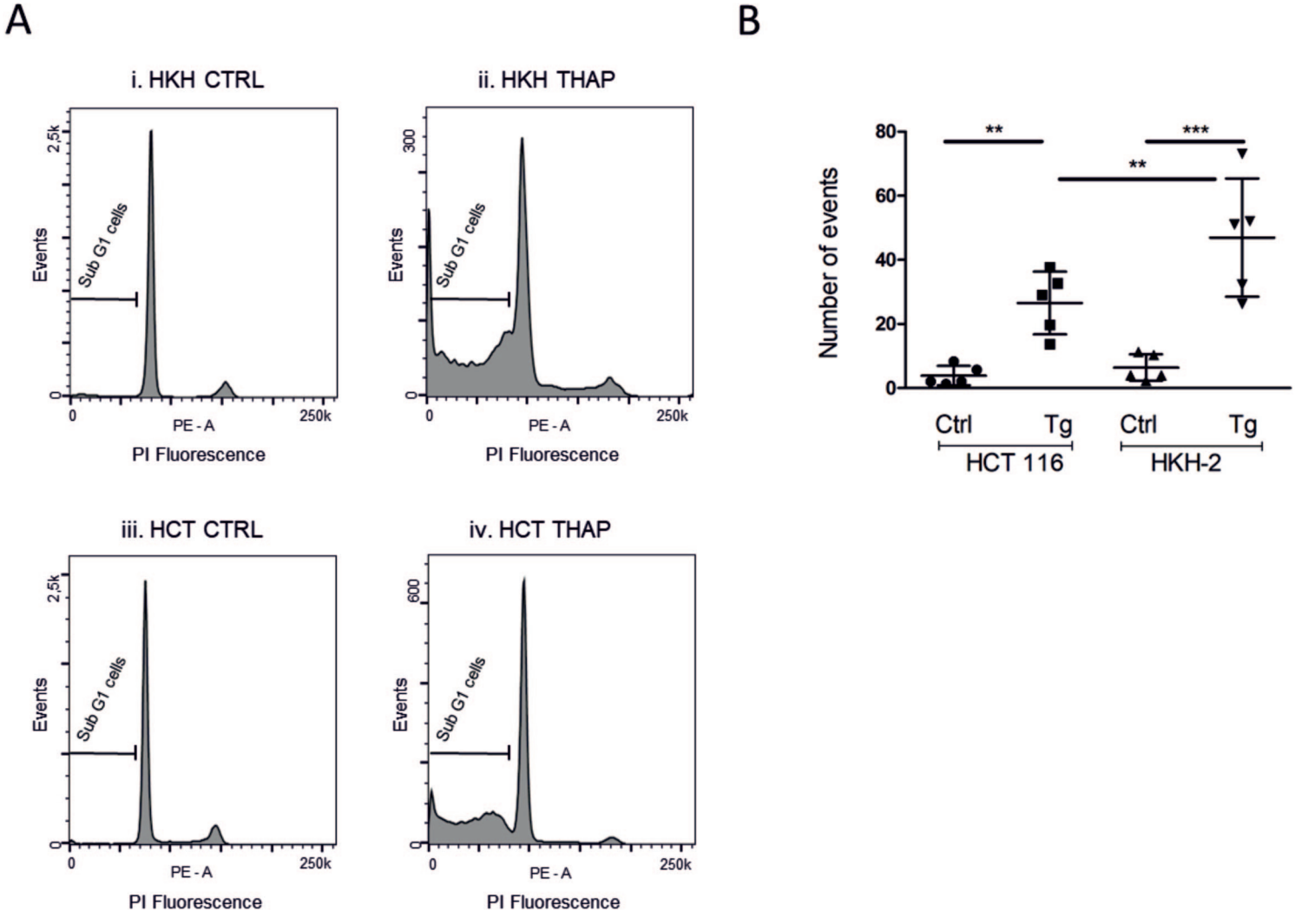
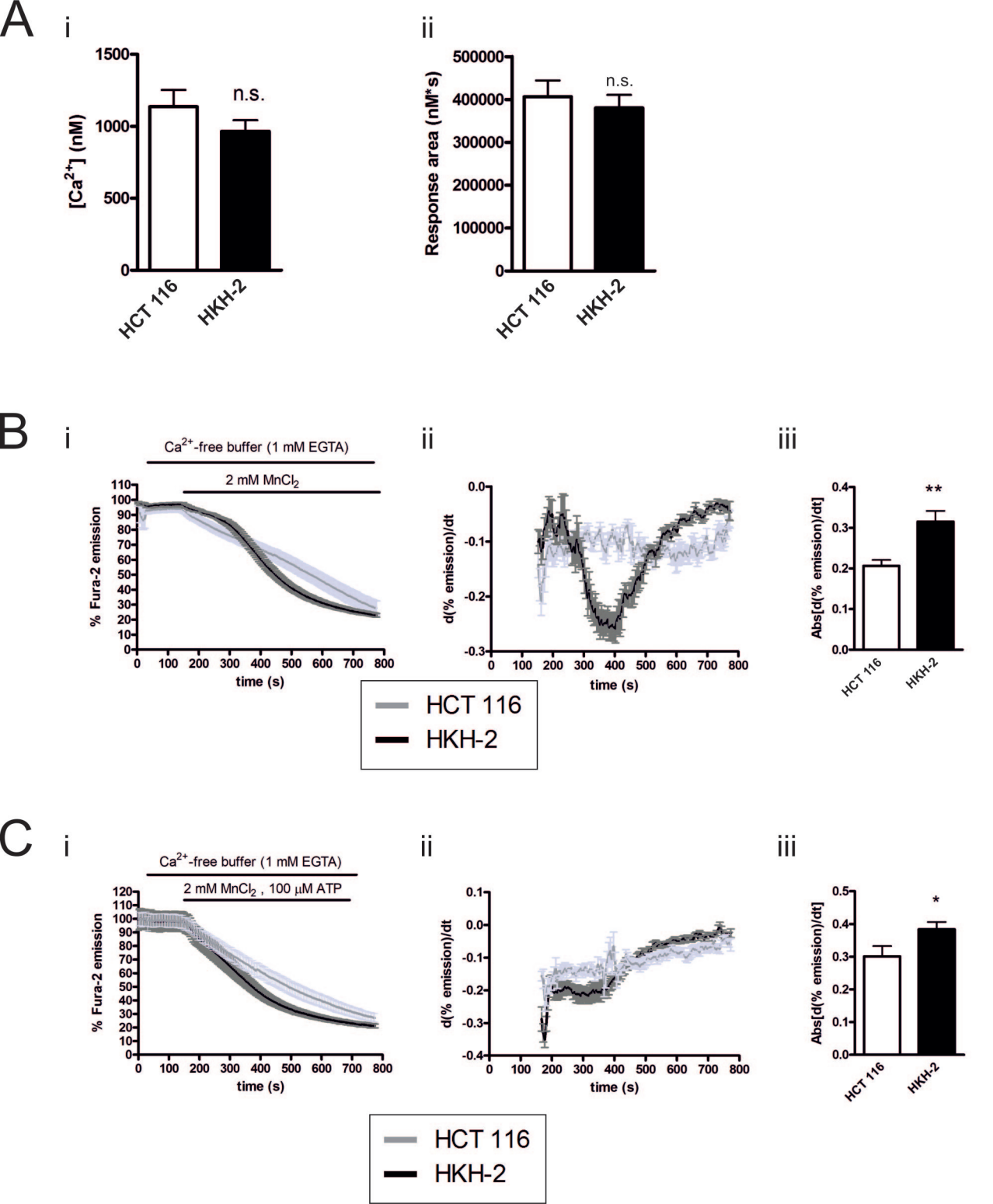
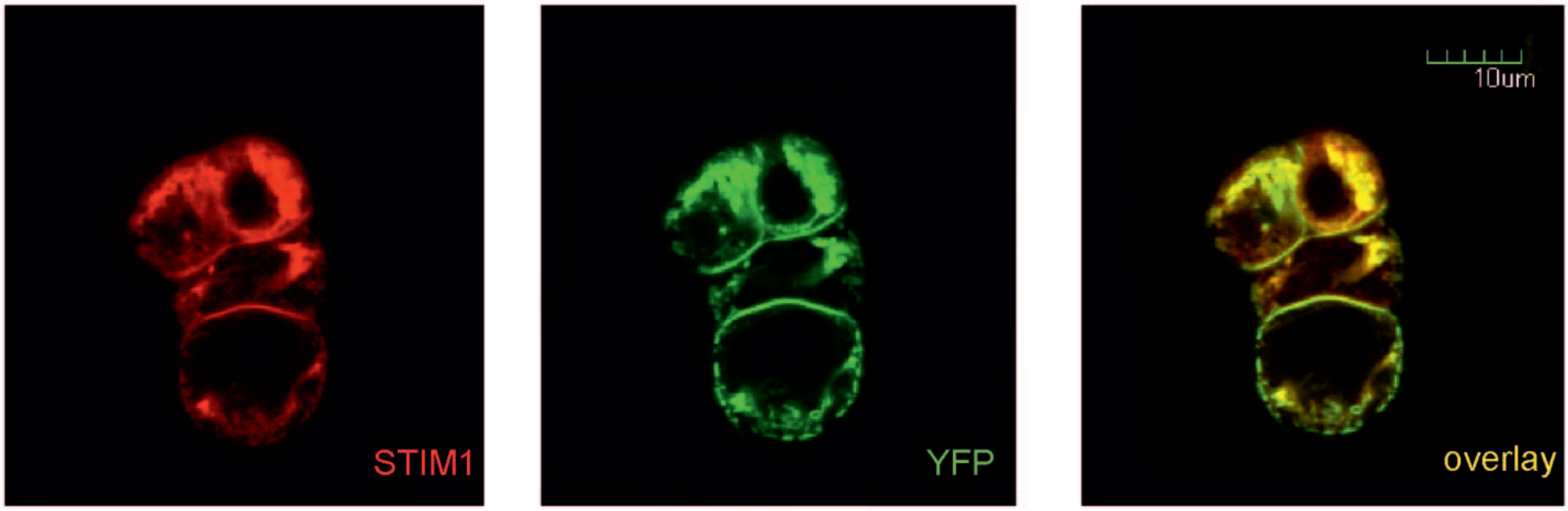
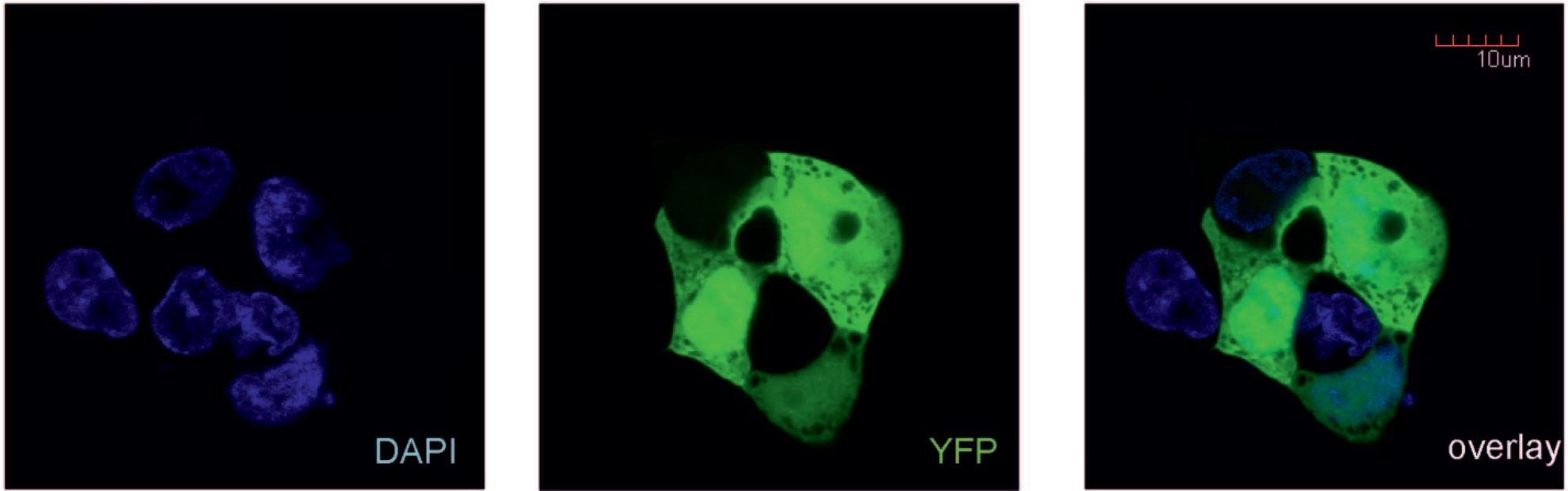

A
i. HKH CTRL
ii. HKH THAP
iii. HCT CTRL
iv. HCT THAP
Events
Sub G1 cells
PE - A
PI Fluorescence
0
250k
2.5k
300
600
B
Number of events
80
60
40
20
0
**
***
**
Ctrl
Tg
Ctrl
Tg
HCT 116
HKH-2


A
i
[Ca2+] (nM)
1500
1000
500
0
n.s.
HCT 116
HKH-2
ii
Response area (nM*s)
500000
400000
300000
200000
100000
0
n.s.
HCT 116
HKH-2
B
i
Ca2+-free buffer (1 mM EGTA)
2 mM MnCl2
% Fura-2 emission
time (s)
ii
d(% emission)/dt
time (s)
iii
Abs[d(% emission)/dt]
**
HCT 116
HKH-2
HCT 116
HKH-2
C
i
Ca2+-free buffer (1 mM EGTA)
2 mM MnCl2 , 100 μM ATP
% Fura-2 emission
time (s)
ii
d(% emission)/dt
time (s)
iii
Abs[d(% emission)/dt]
*
HCT 116
HKH-2
HCT 116
HKH-2


YFP-STIM1
STIM1
YFP
10um
overlay


YFP
DAPI
YFP
10um
overlay

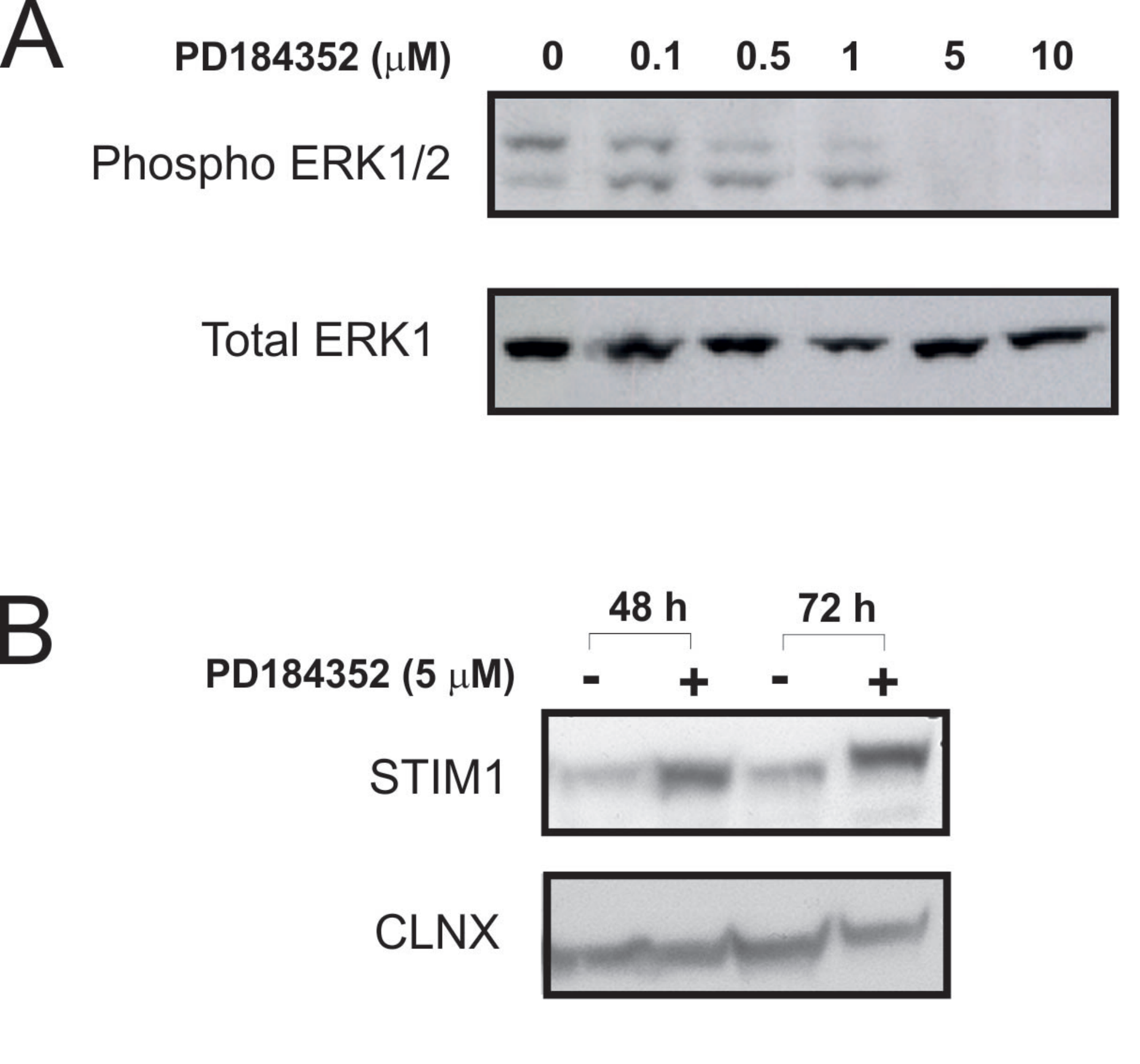

A
PD184352 (μM)
0
0.1
0.5
1
5
10
Phospho ERK1/2
Total ERK1
B
48 h
72 h
PD184352 (5 μM)
-
+
-
+
STIM1
CLNX

Fig. S1. Analysis of sub G-1 DNA content of HCT 116 and HKH-2 cells following treatment with Tg for 24 h. A. Frequency histograms of PI-stained control and Tg-treated HCT 116 and HKH 2 cells. The x axis indicates intensity of PI staining (DNA content) and the y axis, the number of events (cells). Cells with Sub-G1 DNA content are indicated. **B.** Histogram showing summary data of % of cells with sub-G1 DNA content. The experiment was performed on 6 occasions in triplicate. Statistical significance was determined by 2 way ANOVA and post-hoc test and accepted when $p<0.05$.

Fig. S2. A. Peak amplitude (**i**) and magnitude (**ii**) of SOCE (Ca^{2+} re-addition phase) following Tg-induced Ca^{2+} leak in HCT 116 and HKH-2 cells. **B. i**: Mn^{2+} influx in unstimulated HCT 116 and HKH-2 cells normalised to point of addition revealed by fluorescence quench of fura-2 (excitation at 360 nm). **ii**: first derivative of the normalised Mn^{2+} quench. **iii**: summary data of maximum absolute derivative obtained from the data shown in the centre panel. **C. i**: Mn^{2+} influx in ATP-stimulated HCT 116 and HKH-2 cells normalised to point of addition revealed by quench of fura-2 fluorescence (excitation at 360 nm). **ii**: first derivative of the normalised Mn^{2+} quench. **iii**: summary data of maximum absolute derivative obtained from the data shown in the centre panel. All graphs represent the mean ± SEM of three days of experiments, where 3 coverslips per cell type were imaged on each day. Each coverslip contained at least 100 cells. ** and *** indicate $P < 0.01$ and $P < 0.001$ (Student's *t*-test).

Fig. S3. Confocal images of HCT 116 cells transfected with YFP or YFP-STIM1 plasmids. HCT 116 cells transfected with YFP or YFP-STIM1 plasmids were visualised using confocal imaging. Cells were labelled with a primary antibody directed against STIM1, and detected with an ALEXA 568-coupled secondary antibody. YFP and YFP-STIM1 were detected based on the fluorescence of YFP. Nuclei are stained in blue with DAPI. Confocal images of HCT 116 cells transfected with YFP-STIM1 (top row) or with YFP alone (bottom row) are shown. Scale bar = 10 µm.

Fig. S4. Concentration and time dependence of MEK inhibitor PD184352 treatment on ERK1/2 activation (Phospho-ERK) and STIM1 expression in HCT 116 cells. A. Effect of 0 to 10 µM PD184352 for 24 h on ERK1/2 phosphorylation. 5 µM PD184352 was sufficient to suppress MEK, preventing ERK1/2 phosphorylation. Total ERK1 is used as loading control. **B.** Effect of exposure of HCT 116 cells to PD184352 (5 µM) for 48 and 72 h on STIM1 expression. A 48 h treatment was sufficient to cause an increase in STIM1 expression. Blots are representative of three experiments.

Supplemental information

Composition of buffer solutions

Widefield imaging

Ca^{2+}-containing imaging buffer, pH 7.3

121 mM NaCl (AnalaR Normapur)

5.4 mM KCl (AnalaR Normapur)

0.8 mM $MgCl_2$ (AnalaR Normapur)

1.8 mM $CaCl_2$ (Sigma)

6 mM $NaHCO_3$ (Fischer Scientific)

5.5 mM glucose (Fischer Scientific)

25 mM HEPES (N-(2-Hydroxyethyl)piperazine-N'-(2-ethanesulfonic acid) (Sigma)

5 M NaOH to adjust pH

Ca^{2+}-free imaging buffer, pH 7.3

As Ca^{2+}-containing

-1.8 mM $CaCl_2$

+ 1.8 mM EGTA (Sigma)

5 M NaOH to adjust pH

Whole-cell patch clamp electrophysiology

Store depletion-activated currents

Bath Solution, pH 7.4: 115 mM Na-methanesulfonate, 10 mM CsCl, 1.2 mM $MgSO_4$, 10 mM HEPES, 20 mM $CaCl_2$, and 10 mM glucose (pH adjusted with NaOH).

Pipette Solution, pH 7.2: 115 mM Cs-methanesulfonate, 20 mM Cs-1,2-bis-(2-aminophenoxy)ethane-N,N,N′,N′-tetraacetic acid (Cs-BAPTA), 8 mM $MgCl_2$, and 10 mM HEPES (pH adjusted with CsOH)

ATP-activated currents

Bath Solution, pH 7.4: 115 mM Na-methanesulfonate, 10 mM CsCl, 1.2 mM $MgSO_4$, 10 mM HEPES, 20 mM $CaCl_2$, and 10 mM glucose (pH adjusted with NaOH). 10µM &100µM Mg-ATP was added.

Pipette Solution, pH 7.2: 115 mM Cs-methanesulfonate, 10 mM Cs-1,2-bis-(2-aminophenoxy)ethane-N,N,N′,N′-tetraacetic acid (Cs-BAPTA), 5 mM$CaCl_2$, 8 mM $MgCl_2$, and 10 mM HEPES (pH adjusted with CsOH).

Thapsigargin-activated currents

Bath Solution, pH 7.4: 115 mM Na-methanesulfonate, 10 mM CsCl, 1.2 mM $MgSO_4$, 10 mM HEPES, 20 mM $CaCl_2$, and 10 mM glucose (pH adjusted with NaOH). 2µM Thapsigargin was added.

Pipette Solution, pH 7.2: 115 mM Cs-methanesulfonate, 10 mM Cs-1,2-bis-(2-aminophenoxy)ethane-N,N,N′,N′-tetraacetic acid (Cs-BAPTA), 5 mM$CaCl_2$, 8 mM $MgCl_2$, and 10 mM HEPES (pH adjusted with CsOH).

Divalent-free (DVF) bath solution, pH 7.4: 155 mM Na-methanesulfonate, 10 mM HEDTA, 1 mM EDTA, and 10 mM HEPES (pH adjusted with NaOH).

Declarations of interest: None

We certify that all authors have seen and approved the final version of the manuscript being submitted. They warrant that the article is the authors' original work, hasn't received prior publication and isn't under consideration for publication elsewhere.