Supplementary information

Title

Opening Large-Conductance Potassium Channels Selectively Induced Cell Death of Triple-Negative Breast Cancer

Authors

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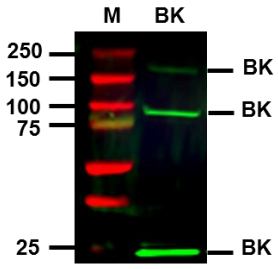
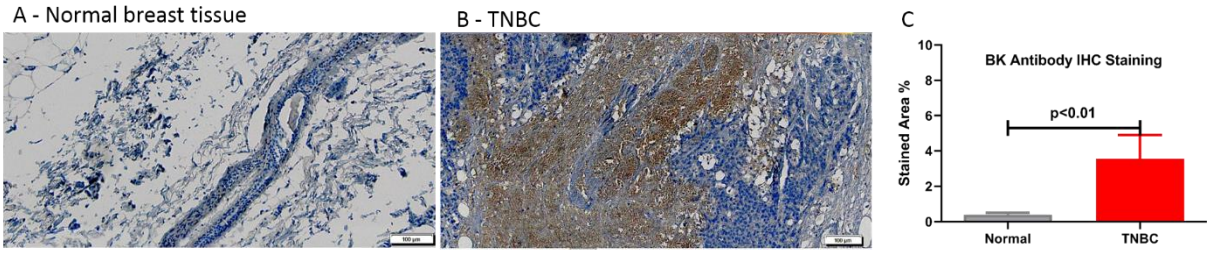
Supplemental Figures and Legends

Supplemental Fig.1



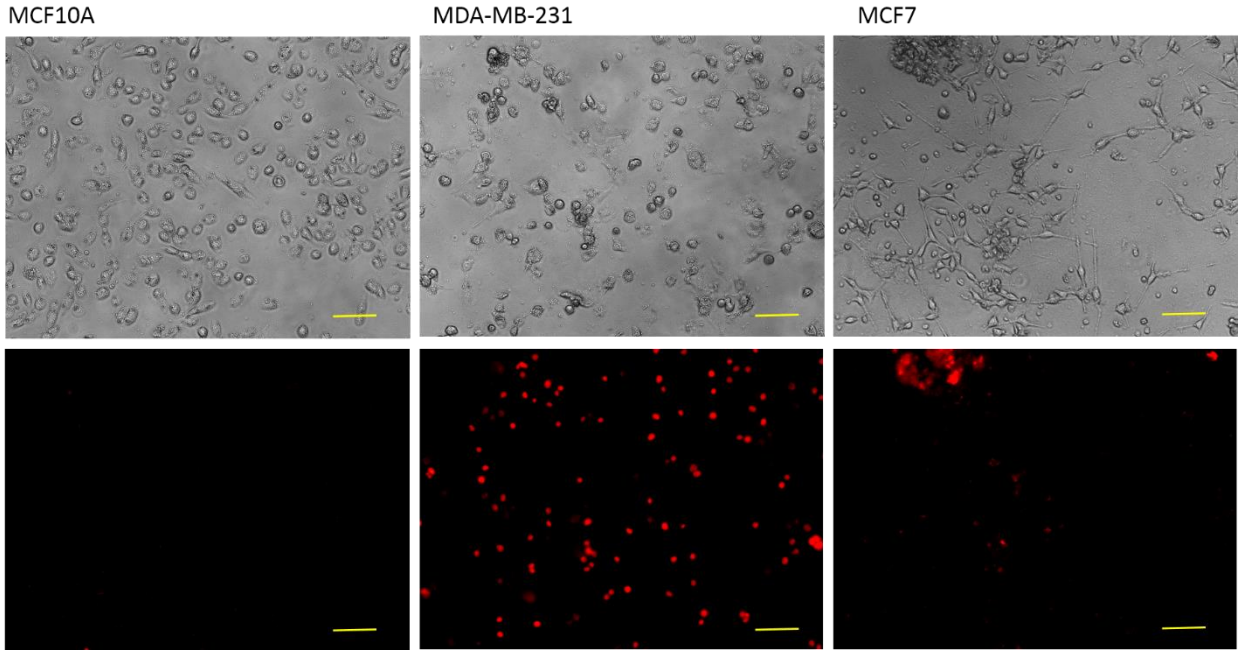
Supplemental Figure 1: A BK specific antibody recognized three bands of the channel alpha subunit – the pore forming subunit.

Supplemental Fig.2



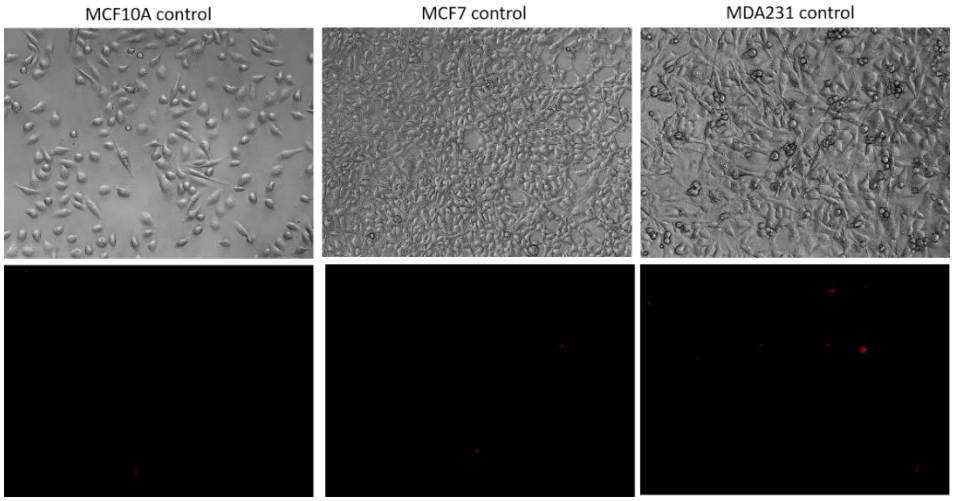
Supplemental Figure 2: Immunohistochemistry of BK channels in TNBC patient tissues (IHC). (a) normal breast tissue, (b) TNBC tissue (BK channel expression indicated by brown color, see Methods), (c) Percentage of staining area averaged from seven TNBC and three normal breast tissues. Unpaired t-test was performed, two-tailed p=0.0042, t=3.95.

Supplemental Fig.3

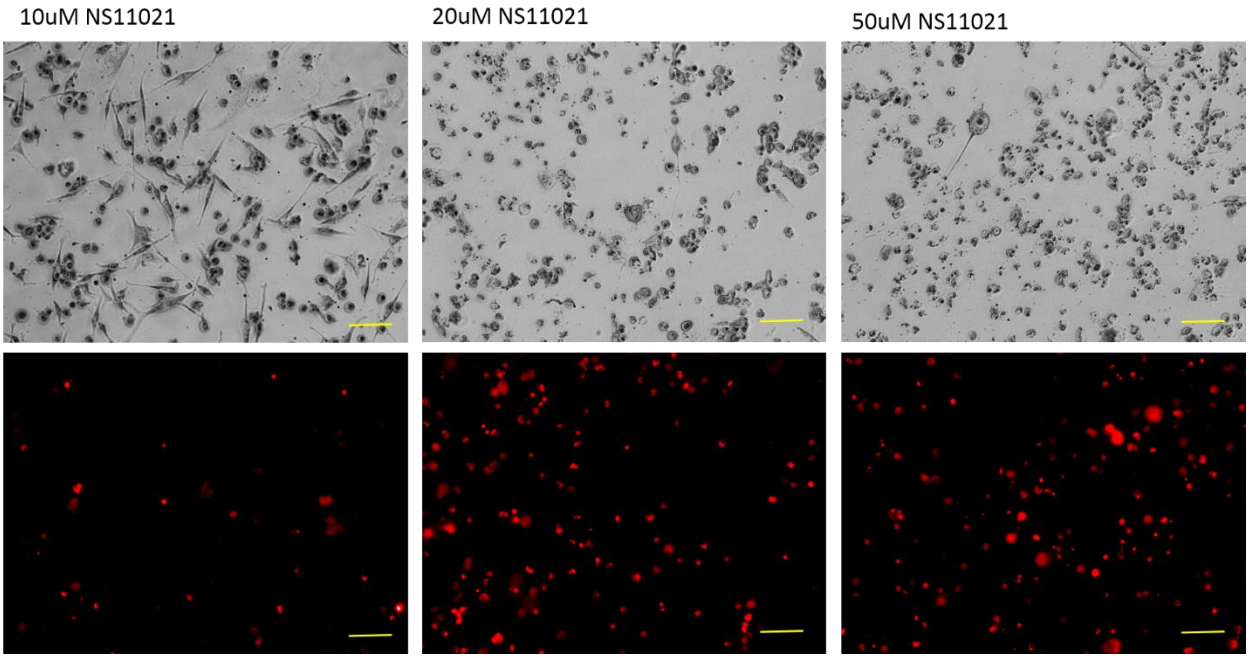


Supplemental Figure 3: BMS-191011 on human breast cancer cell lines. Upper panels show the bright-field images, lower panels show the dead cells labelled by EthD-1 dye (red). BMS-191011 (20μM) treatment of MCF10A (left), MDA-MB-231 (middle), and MCF7 (right) after 2 days. Scale bar: 20μm.

Controls for SFig3: day 0, bright field (upper) and fluorescent data (lower). Red dots indicate dead cells.

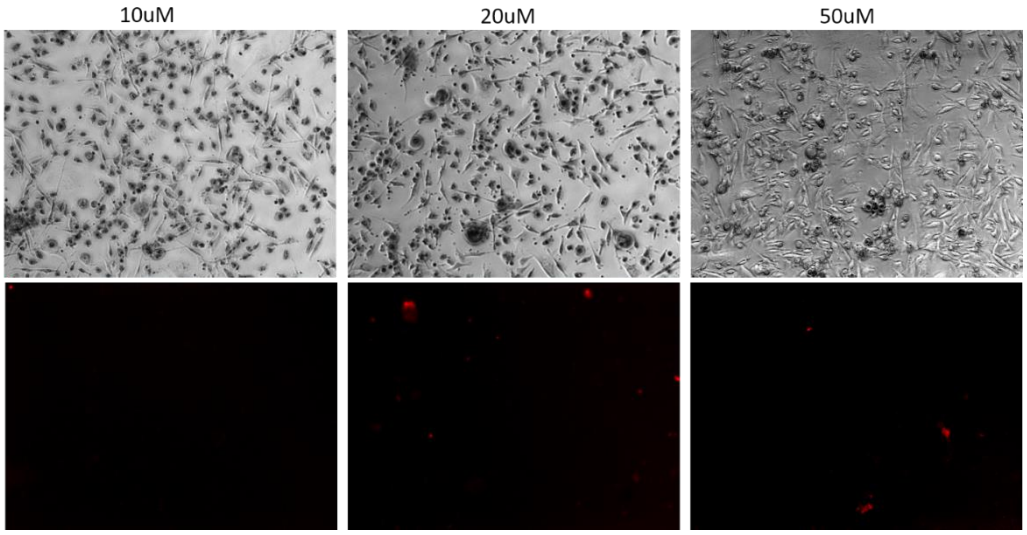


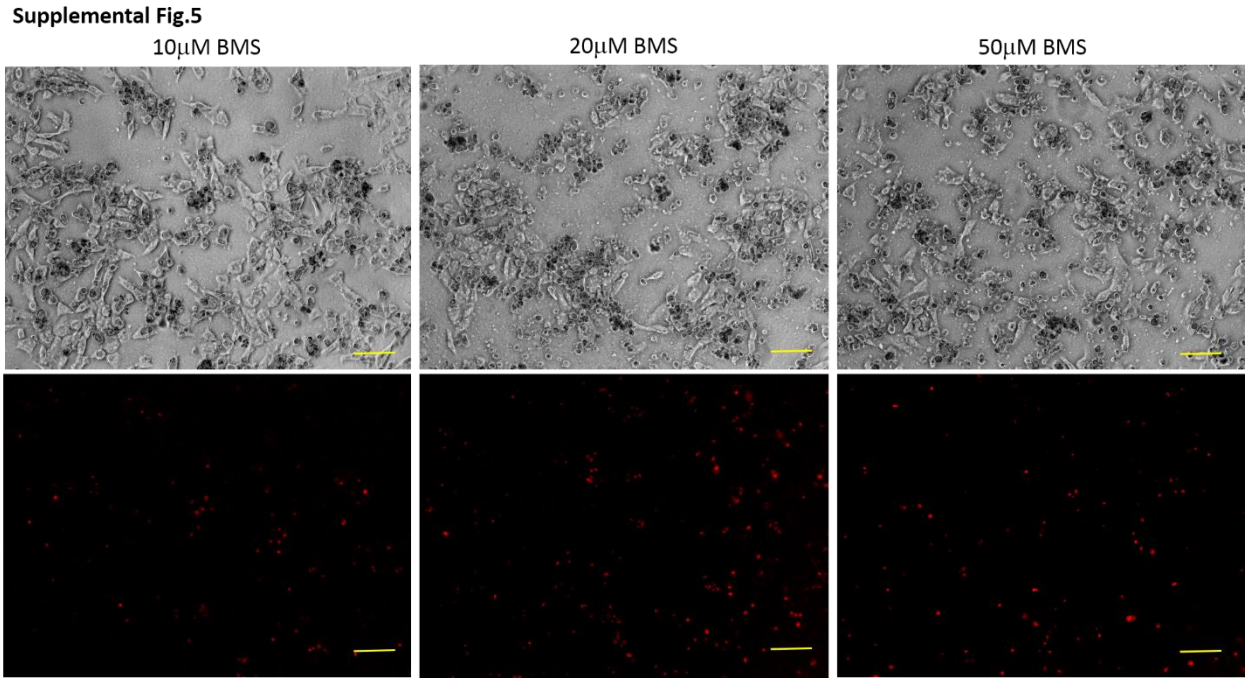
Supplemental Fig.4



Supplemental Figure 4: Concentration-dependent effects of NS11021 on MDA-MB-231 after 5 days. Upper panels show the effects of NS11021 in bright field, lower panels show the dead cells labelled by EthD-1 dye (red). Scale bar: 20μm.

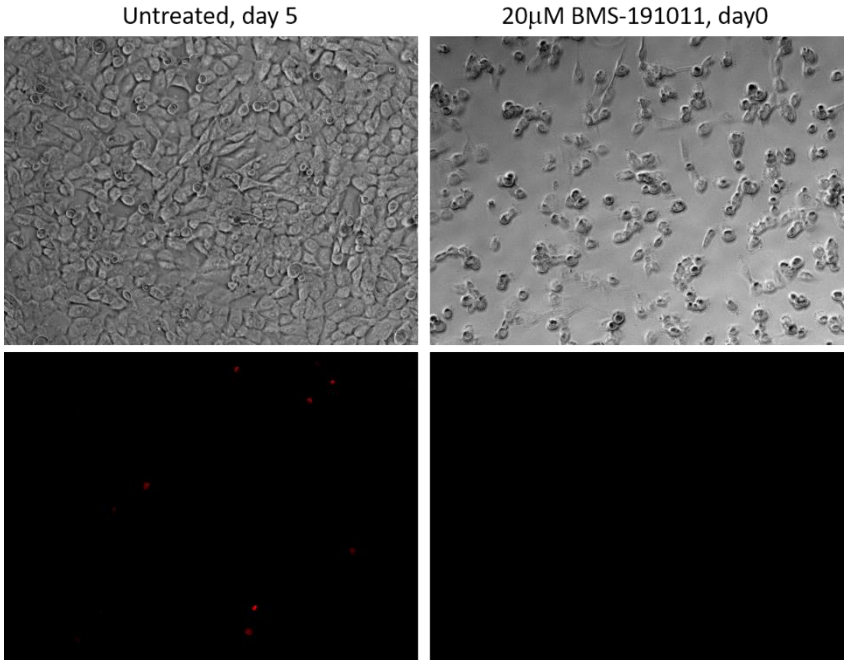
Controls for SFig4: day 0, bright field (upper) and fluorescent data (lower). Red dots indicate dead cells.



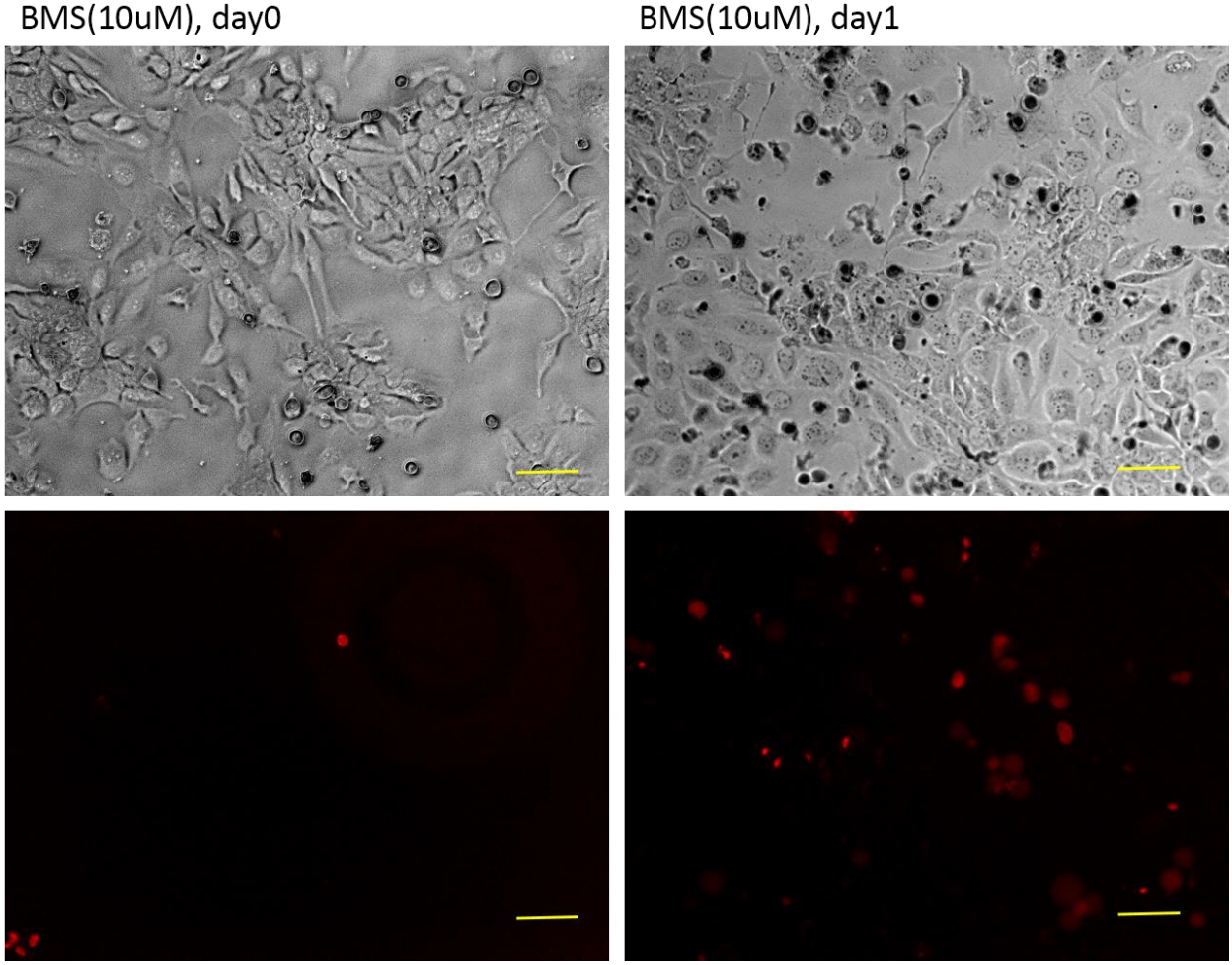


Supplemental Figure 5: Concentration-dependent effects of BMS-191011 on SUM159 after 5 days. Upper panels show the bright-field images, lower panels show the dead cells labelled by EthD-1 dye (red). Scale bar: 20μm.

Controls for SFig5: bright field (upper) and fluorescent data (lower). Red dots indicate dead cells.



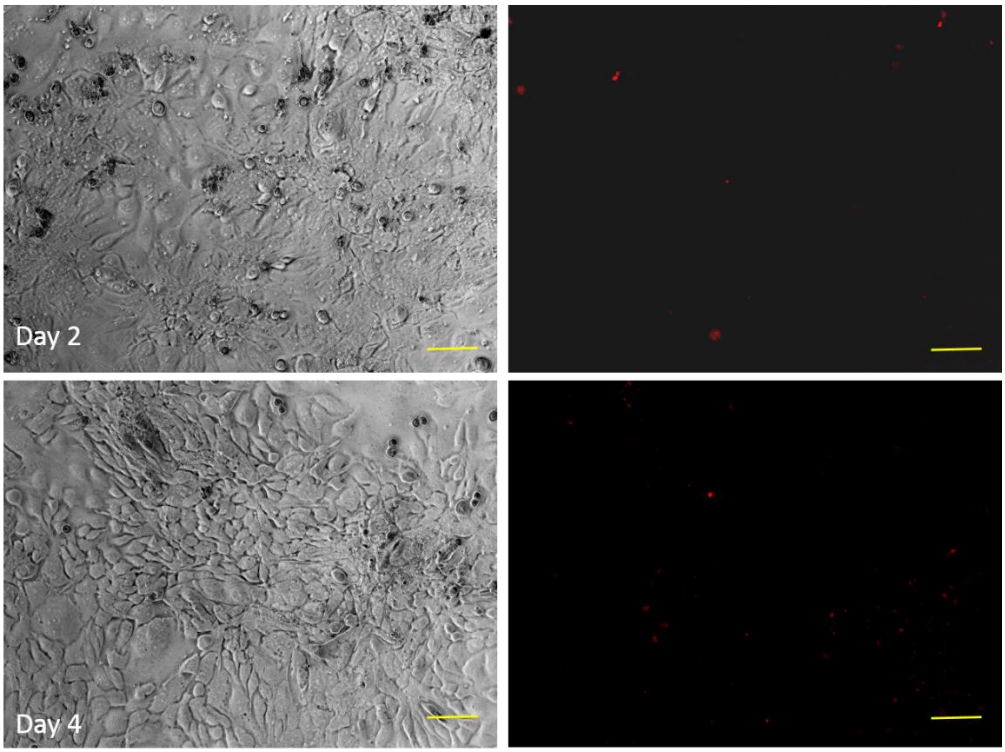
Supplemental Fig.6



Supplemental Figure 6: BMS-191011 on HCC1143. Upper panels show the effects of BMS-191011, lower panels show the dead cells labelled by EthD-1 dye (red). Left: control; Right: one day after 10μM BMS-191011 treatment. Scale bar: 20μm.

Supplemental Fig.7

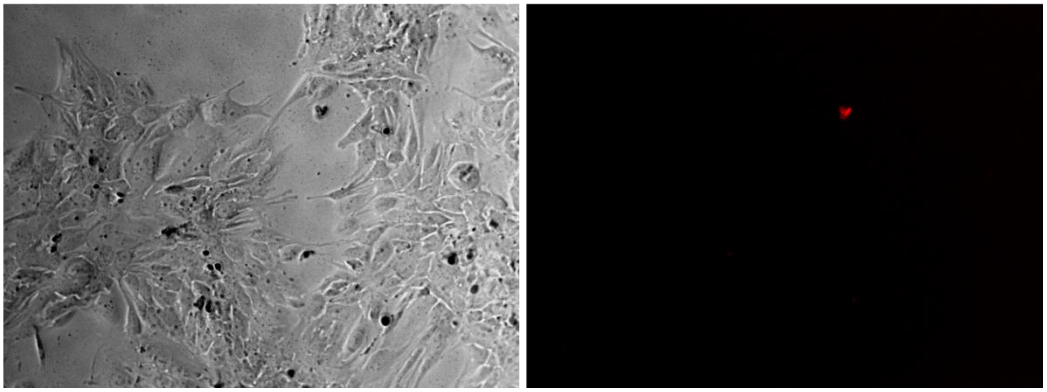
0.5% DMSO



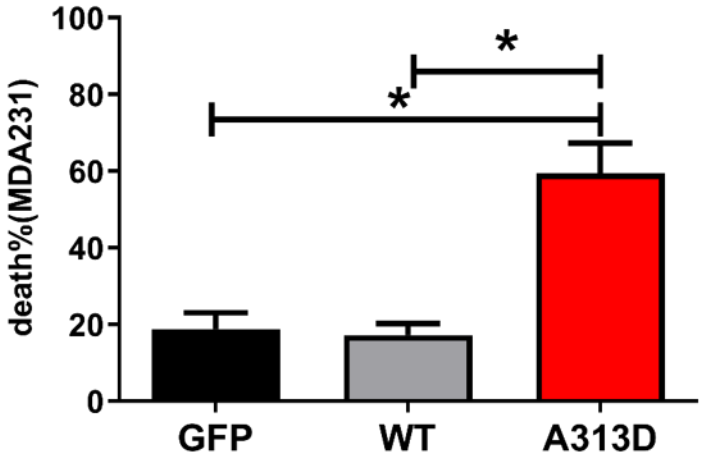
Supplemental Figure 7: DMSO on HCC1143. DMSO (5μl, corresponding to the volume used for 50μM BMS-191011) on HCC1143. Upper: after 48hr; Lower: after 96hr. Left: light image, Right: dead cells labelled by EthD-1 dye (red). Scale bar: 20μm.

Controls for SFig7: day0. bright field (upper) and fluorescent data (lower). Red dots indicate dead cells. 5μL in 1ml medium makes 0.5%

5ul DMSO

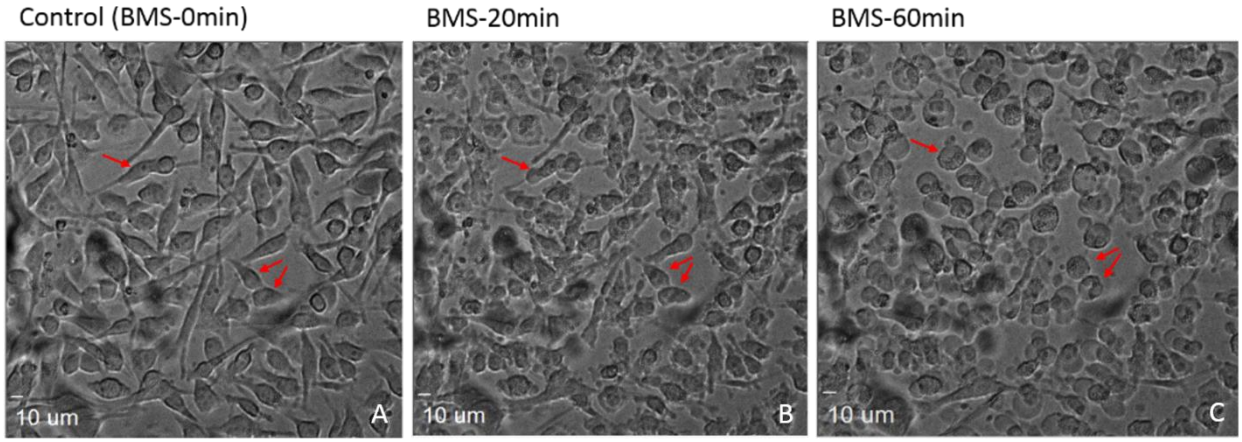


Supplemental Fig.8



Supplemental Figure 8: MDA-MB-231 cell death induced by a constitutively open BK channel mutant, A313D. WT: wild-type BK channel, A313D: mutant channel, GFP: GFP plasmid, *: $p<0.05$.

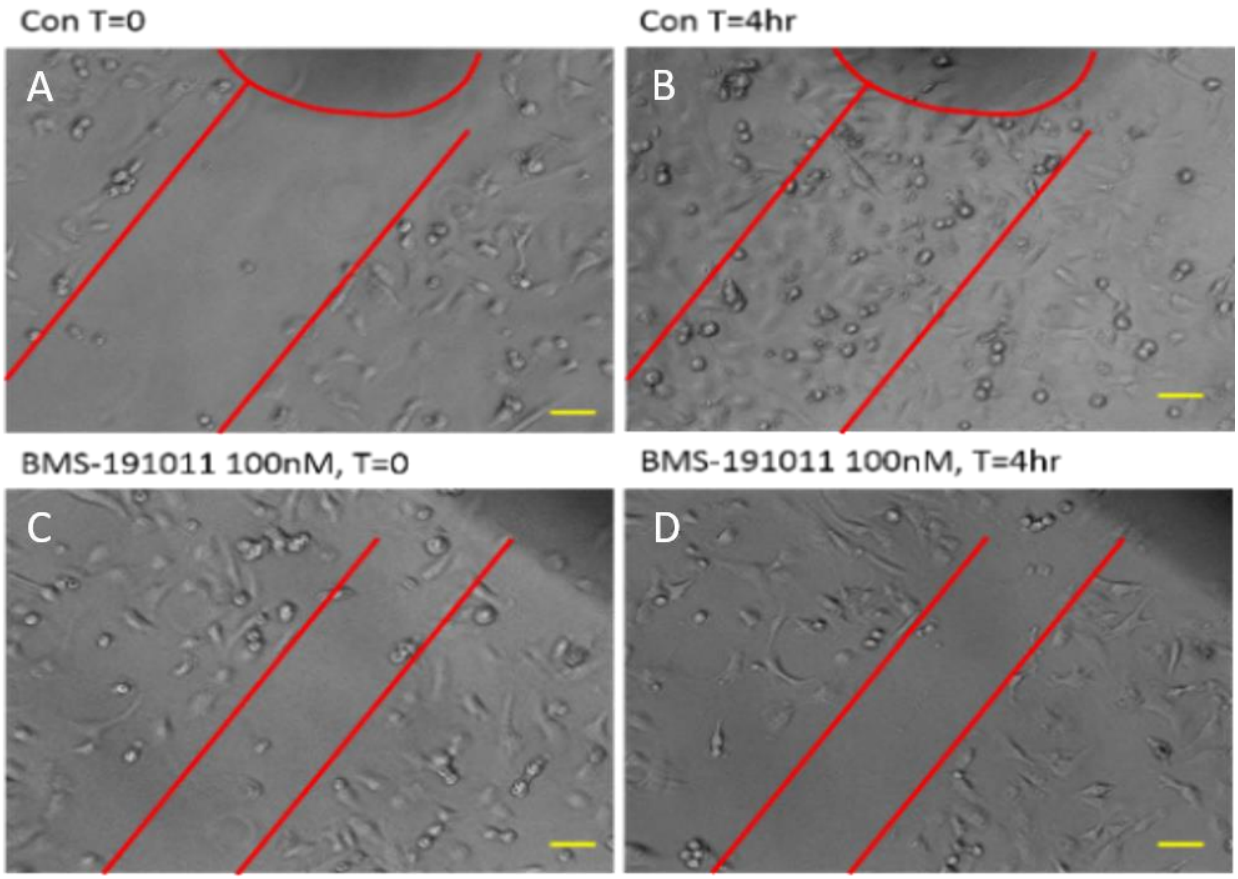
Supplemental Fig.9



Supplemental Figure 9: Time-lapse imaging of early phase of apoptosis induced by BMS-191011. Three images corresponding to starting time point (control, t=0) (A), 20min (B), and 60min (C) after BMS-191011 (1μM) application are shown. Red arrows illustrate

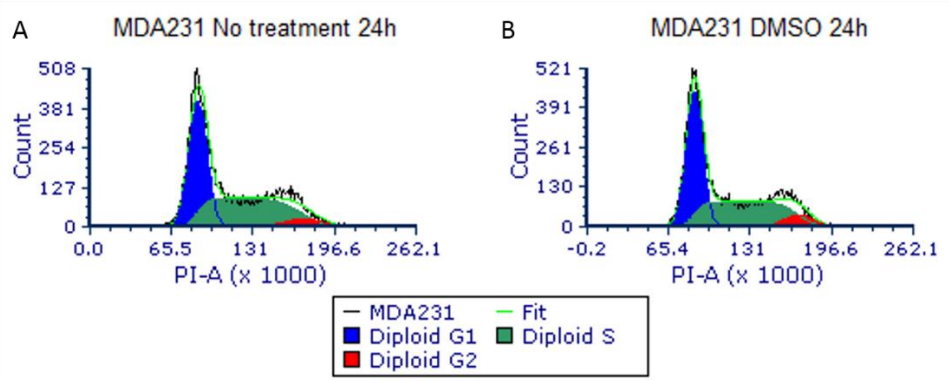
the representative cells undergoing morphological changes during the time course of BMS-191011.

Supplemental Fig.10



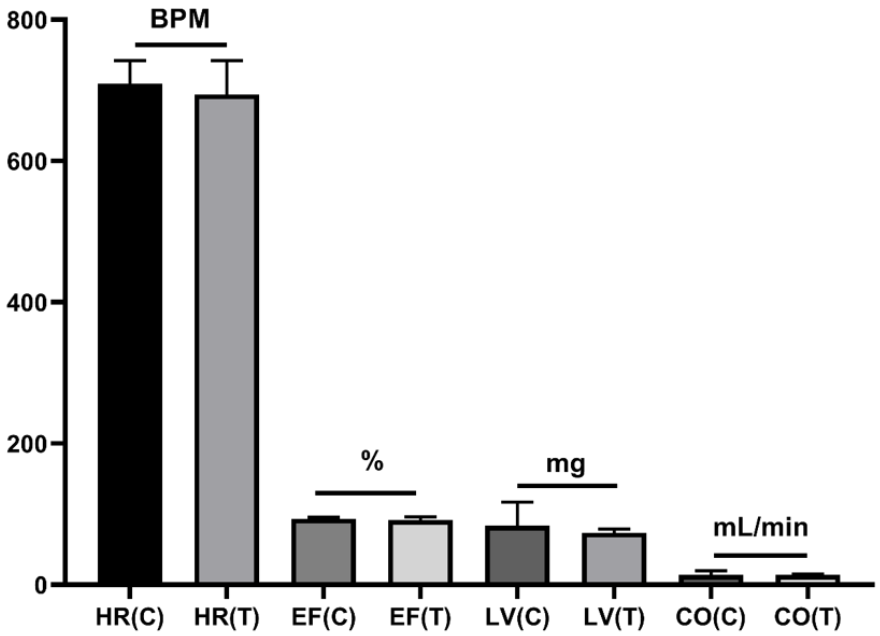
Supplemental Figure 10: Prevention of MDA-MB-231 cell migration by BMS-191011. In the absence of BK channel opener (Con) (A), cells migrated to fill the gap ("wound") after 4hrs (B). Low concentration (100nM) of BK-191011 (C) prevented the "heal" (cells filling the gap) after 4hrs (D). Scratch is defined by the space within two red lines. Curved red line indicates the marker (shadowed area) used to identify the location of the scratch. Scale bar: 20μm.

Supplemental Fig.11



Supplemental Figure 11: DMSO does not affect cell cycle in MDA-MB-231. a: no DMSO treatment, b: DMSO treatment after 24h. Blue: G1 phase; Green: S phase; Red: G2 phase. Count: amount of cells, PI-A: fluorescence intensity.

Supplemental Fig.12

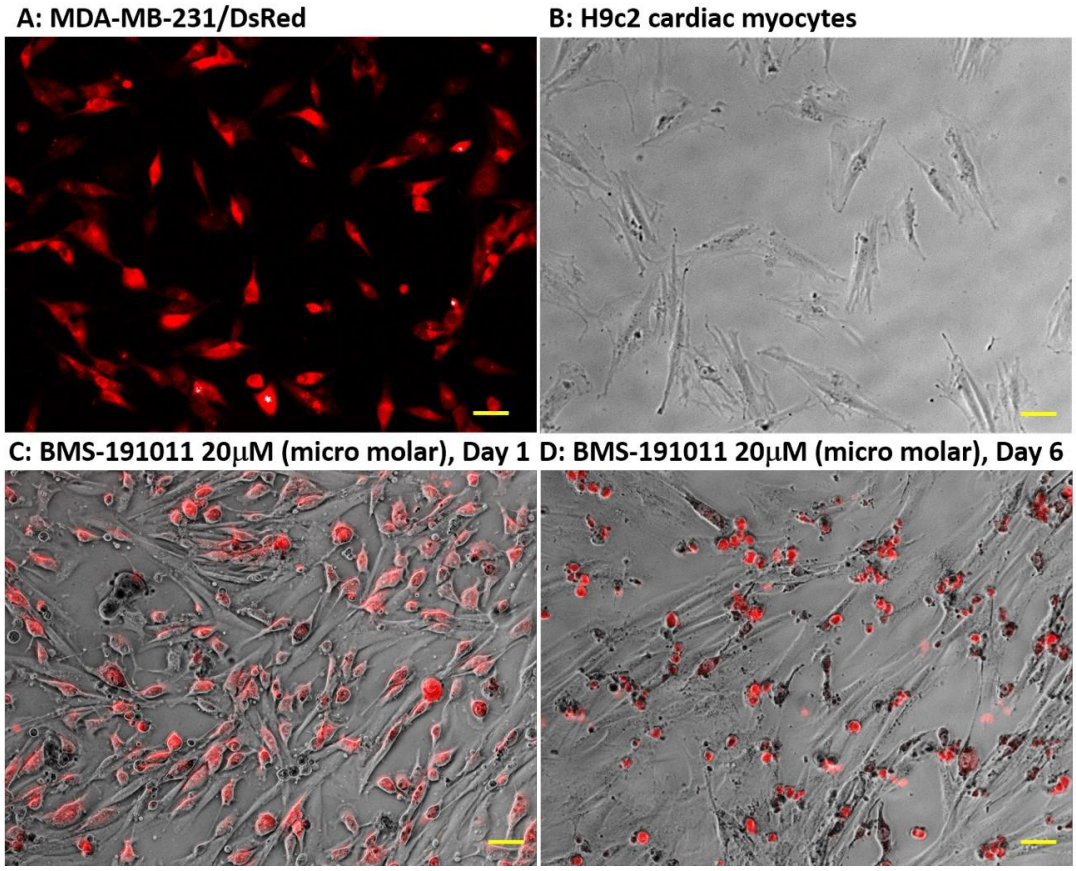


Supplemental Figure 12: BK channel opener does not alter cardiac functions in NSG xenograft model. The Y axis label is described in the figure. Beats per minute (BPM)

is for heart rate, % for ejection fraction, mg for left ventricular mass, and mL/min for cardiac output.

Results of three control and four treated mice were compared. C: control, T: BMS-191011 treated. HR: heart rate (beat per minute, BMP), EF: ejection fraction (%), LV: corrected left ventricular mass (mg), CO: cardiac output (mL/min). Unpaired t-Test was performed. For HR, p=0.6621, t=0.4595; For EF, p=0.5281, t=0.6695; For LV, p=0.5209, t=0.6816; For CO, p=0.9443, t=0.07285.

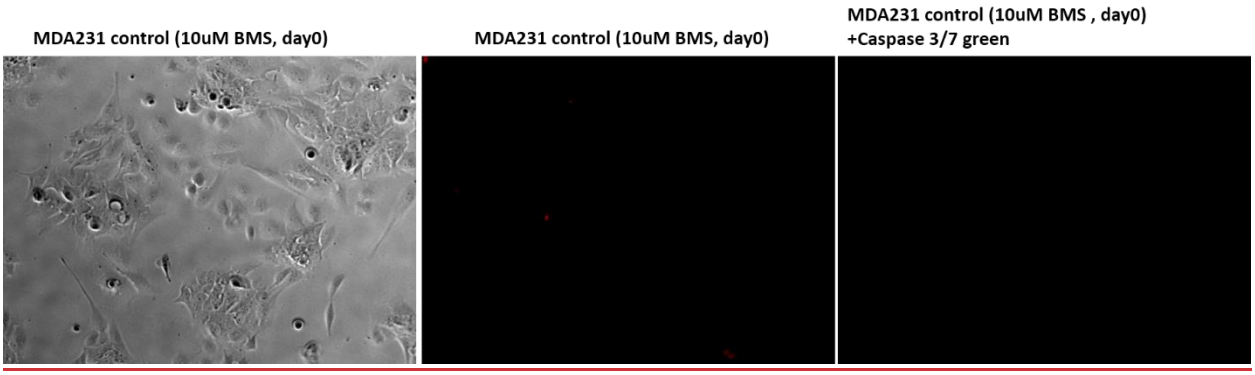
Supplemental Fig.13



Supplemental Figure 13: BK channel opener induced cell death in MDA-MB-231, but not in cardiac myocytes. A: MDA-MB-231 stable cell line with DsRed inserted. B:

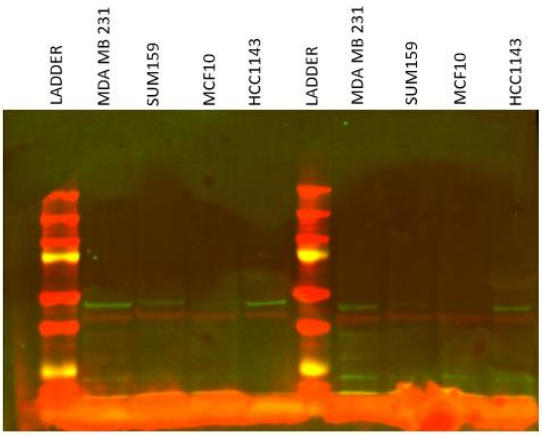
H9c2 cardiac myocytes. C: co-culture of MDA-MB-231/DsRed cells (red) with H9c2 cardiac myocytes (gray) after one-day treatment of BMS-191011 (20μM). D: co-culture of MDA-MB-231/DsRed cells (red) with H9c2 cardiac myocytes (gray) after six-day treatment of BMS-191011 (20μM). Scale bar: 30μm.

Supplemental Figure 14: control data for Figure 5. At day0, bright field, fluorescent red (ETHD-1), and fluorescent green (caspase 3/7 dye)



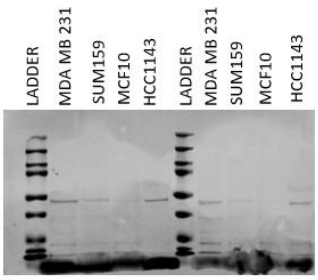
Full WB gel blot for Figure 2d (BK protein expression in TNBC cell lines)

June 13, 2019 Western: MDA, SUM, MCF10, and HCC1143
with antibody for epitope 199-213 external

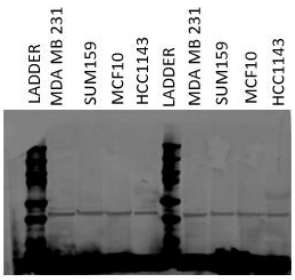


GREEN = BK protein of Interest

RED = Beta Actin



BK protein of Interest

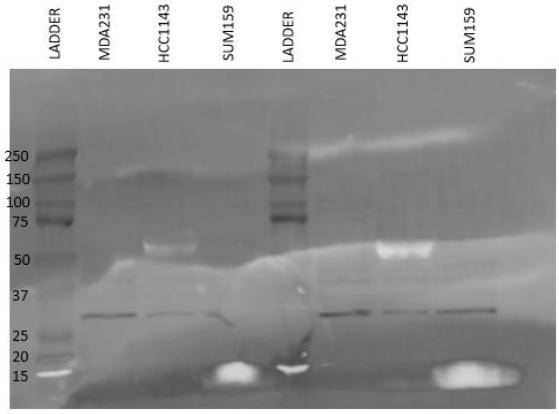


Beta Actin

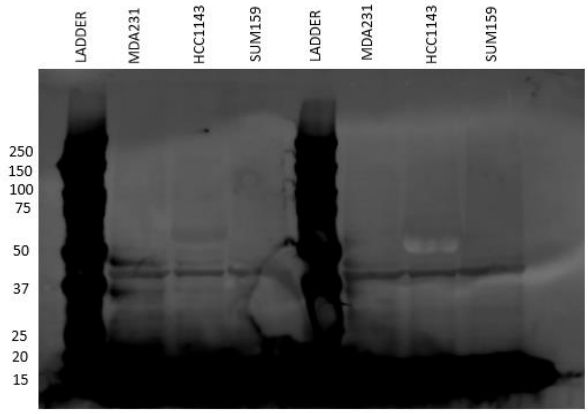
Full WB gel blots for Figure 5c and 5d (caspase-3 protein expression)

CASPASE 3 UNTREATED CELLS MDA231, HCC1143, SUM159
AND Beta Actin

Western in Duplicate
Dilution of 1:500 seems to work best
Air bubble at SUM159 first panel of samples



CASPASE 3 at 33 kD



Beta Actin at 45 kD