ACCSCIENCE PUBLISHING
Advanced Neurology

ORIGINAL RESEARCH ARTICLE

In vitro suppression of glioblastoma cell functions by TG100-115, a TRPM7 kinase inhibitor

Supplementary File

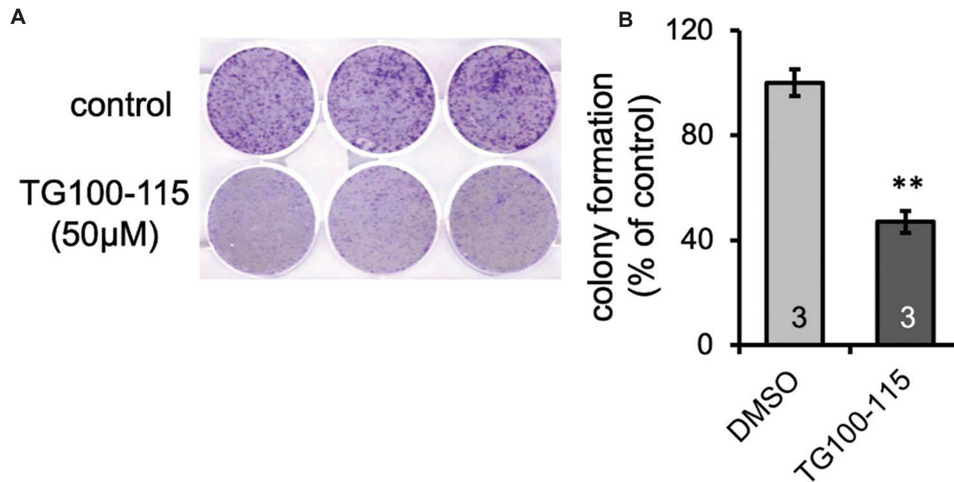

Figure S1. TG100-115 inhibited the colony formation of U251 cells. U251 Cells were treated with TG100-115 (50 μM) for 1 or 2 weeks. Colony formation was assayed by crystal violet staining.
Note: Statistical significance determined at $^{**}p<0.01$, Student's *t*-test, $n = 3$.
Abbreviation: DMSO: Dimethyl sulfoxide.

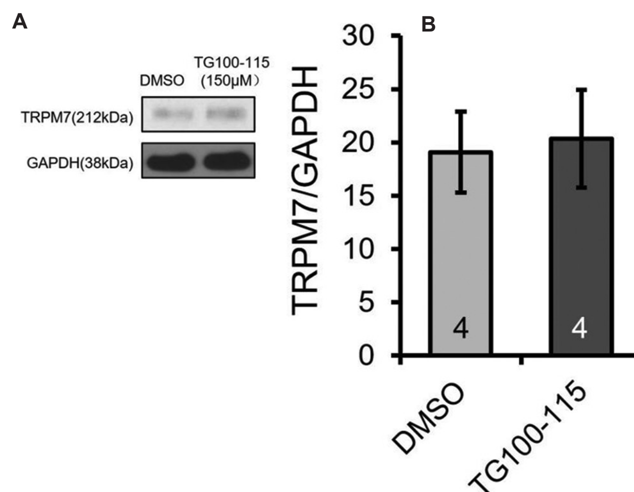

Figure S2. TG100-115 did not change the protein expression of TRPM7. U251 cells were treated with TG100-115 (150 μM) for 24 h, and then the protein expression of TRPM7 was detected using western blot. (A) Representative images of TRPM7, with GAPDH as a control. (B) The quantification of the expression of TRPM7. TG100-115 did not affect the protein expression of TRPM7 ($p>0.05$, Student's *t*-test, $n = 4$). Abbreviations: DMSO: Dimethyl sulfoxide; GAPDH: Glyceraldehyde-3-phosphate dehydrogenase; TRPM7: Transient receptor potential melastatin 7.

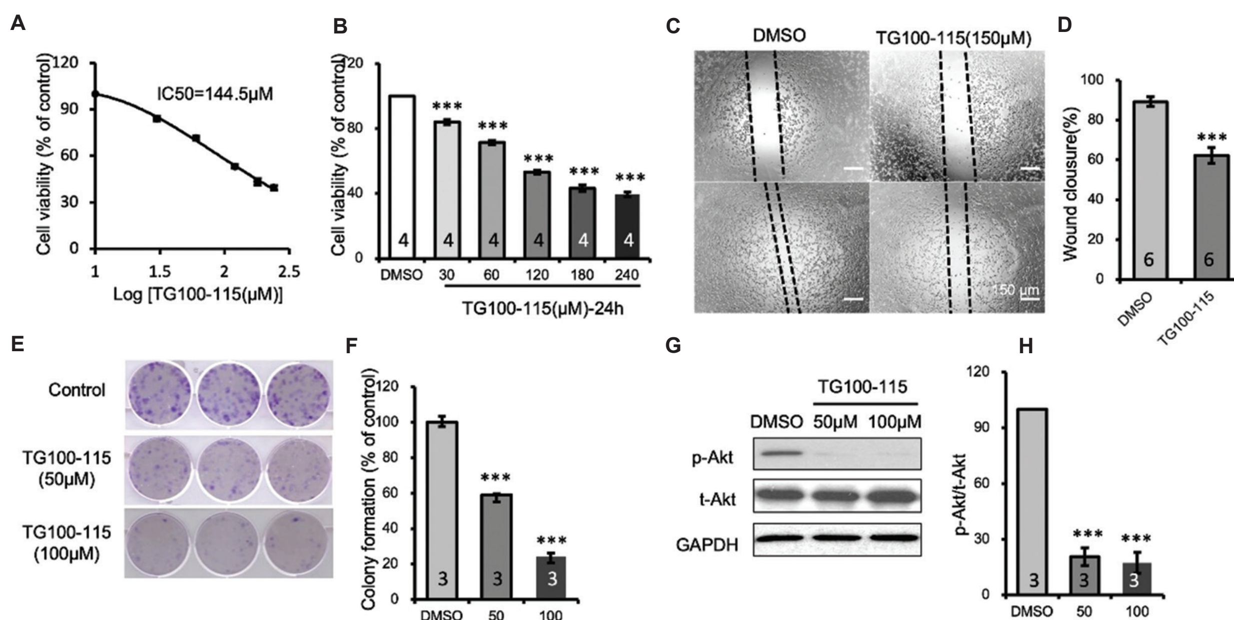

Figure S3. TG100-115 exerts anti-glioblastoma effects by suppressing U87 cell viability, migration, colony formation, and Akt phosphorylation. (A and B) TG100-115 reduced the viability of U87 cells. U87 cells were treated with TG100-115 at concentrations ranging from 30 μM to 240 μM for 24 h. Cell viability was assessed using the MTT assay, and the IC_{50} value was calculated as 144.5 μM (*n* = 4). TG100-115 significantly decreased U87 cell viability at all tested concentrations (****p*<0.001 versus DMSO, one-way analysis of variance followed by the Newman–Keuls test, *n* = 4). (C and D) TG100-115 inhibited U87 cell migration. Following a scratch wound created with a 200 μL pipette tip, U87 cells were treated with 150 μM TG100-115 or 0.1% DMSO. Wound closure was monitored and imaged after 24 h. TG100-115 significantly suppressed cell migration compared to the DMSO control (****p*<0.001 vs. DMSO, Student's *t*-test, *n* = 6). Scale bar: 150 μm, magnification: ×10. (E and F) TG100-115 suppressed the colony formation of U87 cells. Cells were treated with TG100-115 (50 μM or 100 μM) for 1 or 2 weeks. Colonies were visualized using crystal violet staining. TG100-115 significantly inhibited colony formation at both concentrations (****p*<0.001 versus DMSO, Student's *t*-test, *n* = 3). (G and H) TG100-115 reduced Akt phosphorylation in U87 cells. Treatment with TG100-115 (50 μM and 100 μM) significantly decreased the p-Akt/t-Akt ratio (****p*<0.001 versus DMSO, one-way analysis of variance followed by Newman–Keuls test, *n* = 3). Abbreviations: Akt: Protein kinase B; DMSO: Dimethyl sulfoxide; GAPDH: Glyceraldehyde-3-phosphate dehydrogenase; MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; p: Phosphorylated; t: Total.

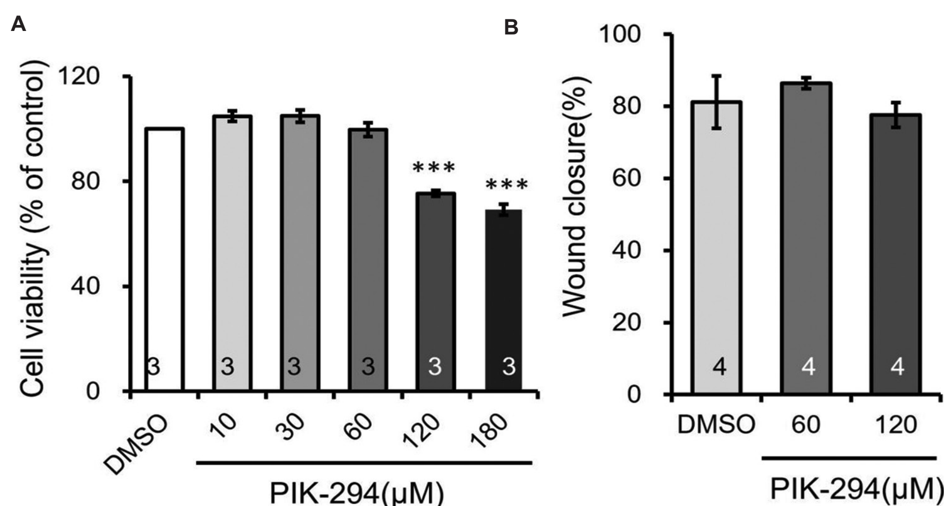

Figure S4. The PI3K p110δ-selective inhibitor PIK-294 significantly decreases U251 cell viability at high doses but does not alter cell migration. (A) PI3K p110δ-selective inhibitor, PIK-294, reduced U251 cell viability. PIK-294 reduced the cell viability of U251 at higher doses. U251 cells were treated with PIK-294 at concentrations from 10 to 180 μM for 24 h. An MTT assay was used to evaluate cell viability. At higher concentrations of 120 and 180 μM, PIK-294 significantly inhibited U251 cell viability after 24 h (****p*<0.001 vs. DMSO, one-way analysis of variance with subsequent Newman–Keuls test, *n* = 12). (B) PIK-294 had no effect on the migration of U251 cells at different doses for 24 h. The wound closure rate of PIK-294 treatment at different doses was not significantly changed compared with DMSO (One-way analysis of variance with subsequent Newman–Keuls test, *n* = 4). Abbreviations: DMSO: Dimethyl sulfoxide; MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; PI3K: Phosphoinositide 3-kinase.

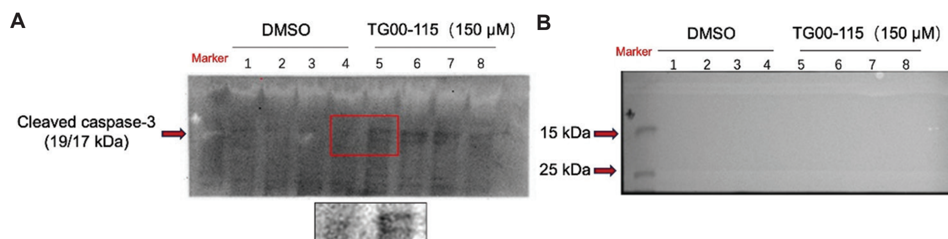

Figure S5. Original images for Western blotting for cleaved caspase-3 in Figure 2. (A) Western blot of cleaved caspase-3 (19/17 kDa), with the marker located on the left. Lanes 1 – 4 were treated with DMSO, whereas lanes 5 – 8 were treated with 150 µM TG100-115. The lower band shown in the main manuscript was cropped from the red-boxed area and presented as the representative image. Each lane represents an independent experiment (*n* = 4 per group). (B) The membrane from image (A), with clear molecular weight markers visible at 15 and 25 kDa. Protein detection was performed using a chemiluminescence reagent system with a multichannel imaging setup. Due to differences in exposure time and signal intensity between the channels, the molecular weight marker and the target protein were not captured simultaneously in a single merged image. Therefore, the marker is shown separately in Panel (B) to indicate the relevant molecular weight range. A duplicate blot for cleaved caspase-3 using the same experimental samples was not performed. While the cleaved caspase-3 signal is detectable, it is relatively low, and the blot background is high, which may affect signal clarity. Abbreviation: DMSO: Dimethyl sulfoxide.

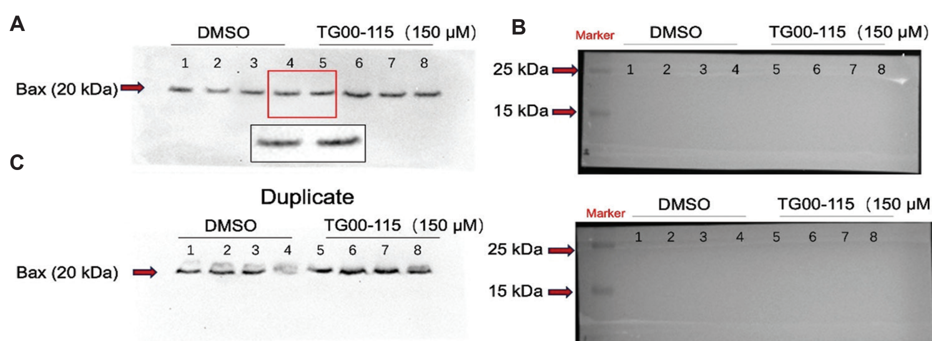

Figure S6. Original images for western blotting for Bax in Figure 2. (A) Western blot of Bax (20 kDa). Lanes 1 – 4 were treated with DMSO, whereas lanes 5 – 8 were treated with 150 µM TG100-115. The lower band shown in the main manuscript was cropped from the red-boxed area and presented as the representative image. Each lane represents an independent experiment (*n* = 4 per group). (B) The membrane from image (A), with clear molecular weight markers visible at 15 and 25 kDa. Protein detection was performed using a chemiluminescence reagent system with a multichannel imaging setup. Due to differences in exposure time and signal intensity between the channels, the molecular weight marker and the target protein were not captured simultaneously in a single merged image. Therefore, the marker is shown separately in Panel (B) to indicate the relevant molecular weight range. (C) A duplicate western blot of Bax using the same set of samples. Abbreviations: Bax: B-cell lymphoma 2-associated X protein; DMSO: Dimethyl sulfoxide.

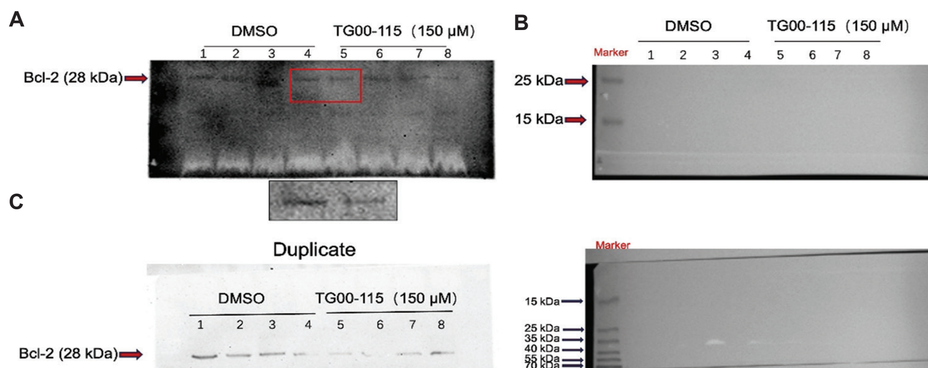

Figure S7. Original images for western blotting for Bcl-2 in Figure 2. (A) Western blot of Bcl-2 (28 kDa). Lanes 1 – 4 were treated with DMSO, whereas lanes 5 – 8 were treated with 150 µM TG100-115. The lower band shown in the main manuscript was cropped from the red-boxed area and presented as the representative image. Each lane represents an independent experiment (*n* = 4 per group). (B) The membrane from image (A), with clear molecular weight markers visible at 15 and 25 kDa. Protein detection was performed using a chemiluminescence reagent system with a multichannel imaging setup. Due to differences in exposure time and signal intensity between the channels, the molecular weight marker and the target protein were not captured simultaneously in a single merged image. Therefore, the marker is shown separately in Panel (B) to indicate the relevant molecular weight range. (C) A duplicate western blot of Bcl-2 using the same set of samples. Abbreviations: Bcl-2: B-cell lymphoma 2; DMSO: Dimethyl sulfoxide.

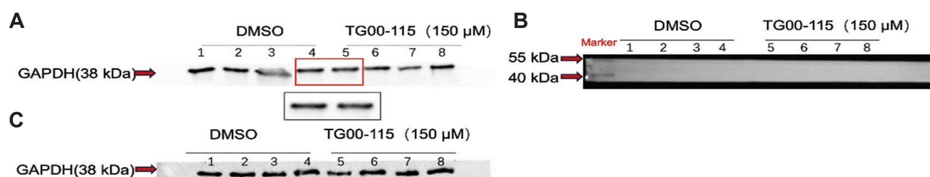

Figure S8. Original images for western blotting for GAPDH in Figure 2. (A) Western blot of GAPDH (38 kDa). Lanes 1 – 4 were treated with DMSO, whereas lanes 5 – 8 were treated with 150 μM TG100-115. The lower band shown in the main manuscript was cropped from the red-boxed area and presented as the representative image. Each lane represents an independent experiment (*n* = 4 per group). (B) The membrane from image (A), with clear molecular weight markers visible at 40 and 55 kDa. (C) An additional GAPDH blot was obtained using the same set of samples. Abbreviations: DMSO: Dimethyl sulfoxide; GAPDH: Glyceraldehyde-3-phosphate dehydrogenase.

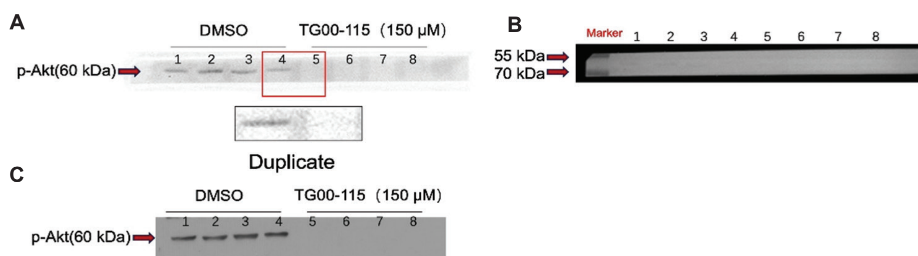

Figure S9. Original images for western blotting for p-Akt in Figure 6. (A) Western blot of p-Akt (60 kDa). Lanes 1 – 4 were treated with DMSO, whereas lanes 5 – 8 were treated with 150 μM TG100-115. The lower band shown in the main manuscript was cropped from the red-boxed area and presented as the representative image. Each lane represents an independent experiment (*n* = 4 per group). (B) The membrane from image (A), with clear molecular weight markers visible at 55 and 75 kDa. (C) A duplicate western blot of p-Akt using the same set of samples. Abbreviations: DMSO: Dimethyl sulfoxide; p-Akt: Phosphorylated protein kinase B.

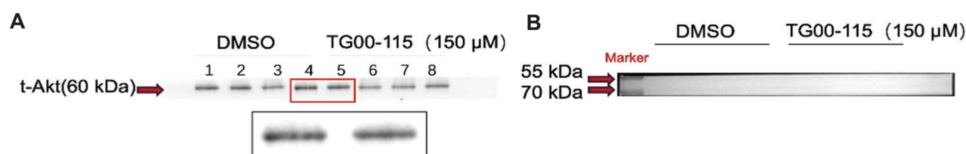

Figure S10. Original images for western blotting for t-Akt in Figure 6. (A) Western blot of t-Akt (60 kDa). Lanes 1 – 4 were treated with DMSO, whereas lanes 5 – 8 were treated with 150 μM TG100-115. The lower band shown in the main manuscript was cropped from the red-boxed area and presented as the representative image. Each lane represents an independent experiment (*n* = 4 per group). (B) The membrane from image (A), with clear molecular weight markers visible at 55 and 70 kDa.
Abbreviations: DMSO: Dimethyl sulfoxide; t-Akt: Total protein kinase B.

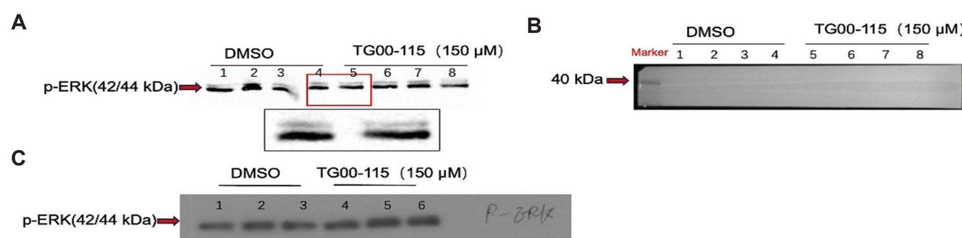

Figure S11. Original images for western blotting for p-ERK in Figure 6. (A) Western blot of p-ERK (42/44 kDa). Lanes 1 – 4 were treated with DMSO, whereas lanes 5 – 8 were treated with 150 μM TG100-115. The lower band shown in the main manuscript was cropped from the red-boxed area and presented as the representative image. Each lane represents an independent experiment (*n* = 4 per group). (B) The membrane from image (A), with clear molecular weight markers visible at 40 kDa. (C) A duplicate western blot for p-ERK using the same batch of samples was not performed. However, preliminary western blots for p-Erk from an earlier set of experiments using the same treatment conditions are available and have been included as supplementary data to support the findings shown here. These preliminary blots were developed using traditional darkroom exposure to X-ray film rather than the chemiluminescence reagent system. Unfortunately, the protein marker bands did not transfer clearly onto the film, likely due to exposure settings at the time. We acknowledge the absence of visible left-side markers and have annotated the blot accordingly based on known protein sizes.
Abbreviations: DMSO: Dimethyl sulfoxide; p-ERK: Phosphorylated extracellular signal-regulated kinase.

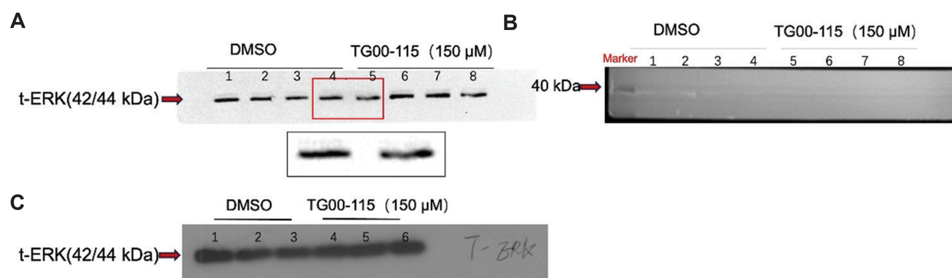

Figure S12. Original images for western blotting for t-ERK in Figure 6. (A) Western blot of t-ERK (42/44 kDa). Lanes 1 – 4 were treated with DMSO, whereas lanes 5 – 8 were treated with 150 μM TG100-115. The lower band shown in the main manuscript was cropped from the red-boxed area and presented as the representative image. Each lane represents an independent experiment (n = 4 per group). (B) The membrane from image (A), with clear molecular weight markers visible at 40 kDa. (C) A duplicate western blot for t-ERK using the same batch of samples was not performed. However, preliminary western blots for t-Erk from an earlier set of experiments using the same treatment conditions are available and have been included as supplementary data to support the findings shown here.
Abbreviations: DMSO: Dimethyl sulfoxide; t-ERK: Total extracellular signal-regulated kinase.

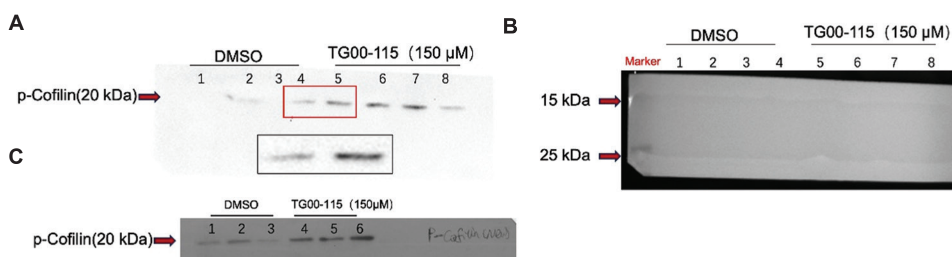

Figure S13. Original images for western blotting for p-Cofilin in Figure 6. (A) Western blot of p-Cofilin (20 kDa). Lanes 1 – 4 were treated with DMSO, whereas lanes 5 – 8 were treated with 150 μM TG100-115. (B) The membrane from image (A), with clear molecular weight markers visible at 15 and 25 kDa. Each lane represents an independent experiment (n = 4 per group). (C) A duplicate Western blot for p-Cofilin using the same batch of samples was not performed. However, preliminary western blots for p-Cofilin from an earlier set of experiments using the same treatment conditions are available and have been included as supplementary data to support the findings shown here.
Abbreviations: DMSO: Dimethyl sulfoxide; p: Phosphorylated.

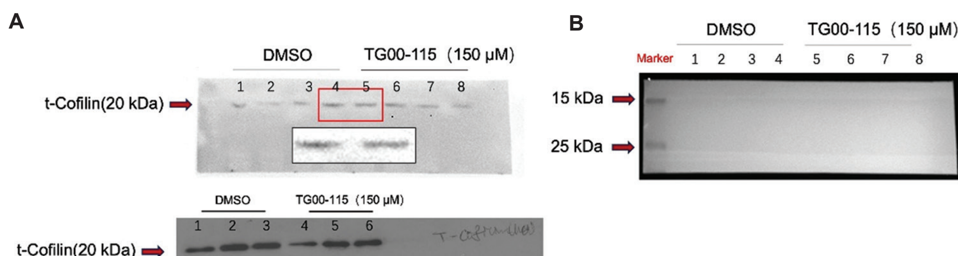

Figure S14. Original images for western blotting for t-Cofilin in Figure 6. (A) Western blot of t-Cofilin (20 kDa). Lanes 1 – 4 were treated with DMSO, whereas lanes 5 – 8 were treated with 150 μM TG100-115. (B) The membrane from image (A), with clear molecular weight markers visible at 15 and 25 kDa. Each lane represents an independent experiment (n = 4 per group). (C) A duplicate western blot for t-Cofilin using the same batch of samples was not performed. However, preliminary Western blots for t-Cofilin from an earlier set of experiments using the same treatment conditions are available and have been included as supplementary data to support the findings shown here.
Abbreviations: DMSO: Dimethyl sulfoxide; t: Total.

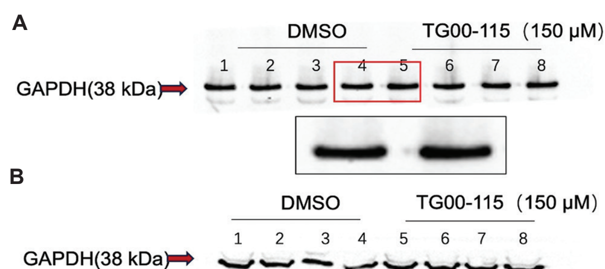

Figure S15. Original images for western blotting for GAPDH in Figure 6. (A) Western blot of GAPDH is shown (kDa). Lanes 1 – 4 were treated with DMSO, whereas lanes 5 – 8 were treated with 150 μM TG100-115. The lower band shown in the main manuscript was cropped from the red-boxed area and presented as the representative image. Each lane represents an independent experiment (*n* = 4 per group). (B) An additional GAPDH blot was obtained using the same set of samples.

Abbreviations: DMSO: Dimethyl sulfoxide; GAPDH: Glyceraldehyde-3-phosphate dehydrogenase.

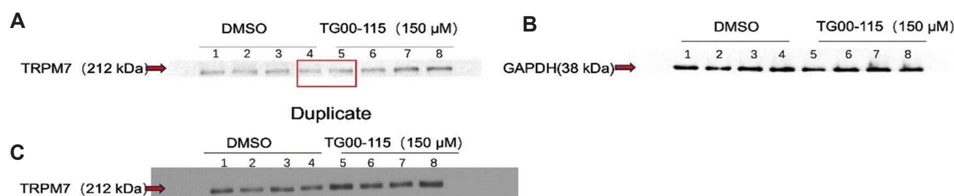

Figure S16. Original images for western blotting for TRPM7 and GAPDH in Figure S2. (A) Western blot of TRPM7 (212 kDa). Lanes 1 – 4 were treated with DMSO, whereas lanes 5 – 8 were treated with 150 μM TG100-115. Cropped images of lanes 4 and 5 represent the DMSO and TG100-115 treatment groups, respectively. Each lane represents an independent experiment (*n* = 4 per group). (B) Western blot image of GAPDH (38 kDa). (C) A duplicate western blot of TRPM7 using the same set of samples.

Abbreviations: DMSO: Dimethyl sulfoxide; GAPDH: Glyceraldehyde-3-phosphate dehydrogenase; TRPM7: Transient receptor potential melastatin 7.

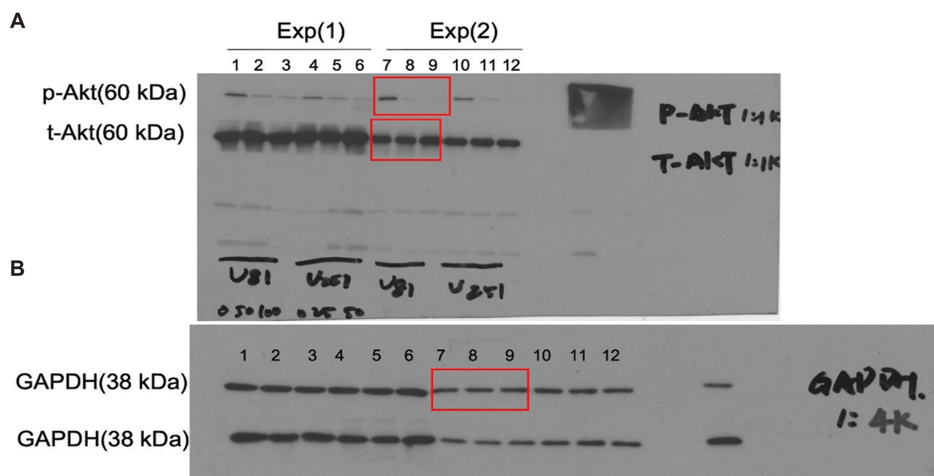

Figure S17. Original images for western blotting for p-Akt, t-Akt, and GAPDH for U87 cells in Figure S3. (A) Representative raw western blot image showing the expression of phosphorylated Akt (p-Akt) and total Akt (t-Akt) from two independent experiments. Lanes 1 – 6 correspond to Experiment 1 (Exp 1) and Lanes 7 – 12 correspond to Experiment 2 (Exp 2). Lanes 1 – 3 and 7 – 9: U87 cell lysates; Lanes 4 – 6 and 10 – 12: U251 cell lysates; Lanes 1, 4, 7, and 10: DMSO-treated controls; other lanes: Cells treated with increasing concentrations of TG100-115. The upper bands were blotted with p-Akt antibody, and the lower bands with t-Akt antibody at 60 kDa. (B) Representative raw western blot image showing the expression of GAPDH at 38 kDa. The bands outlined in red boxes were used in the final figures to represent p-Akt, t-Akt, and GAPDH in U87 cells, respectively. These preliminary blots were developed using traditional darkroom exposure to X-ray film rather than the chemiluminescence reagent system. Unfortunately, the protein marker bands did not transfer clearly onto the film, likely due to exposure settings at the time. We acknowledge the absence of visible left-side markers and have annotated the blot accordingly based on known protein sizes.

Abbreviations: Akt: Protein kinase B; DMSO: Dimethyl sulfoxide; GAPDH: Glyceraldehyde-3-phosphate dehydrogenase.