

## Supplementary Information

**Title:** Identification of Triptonide as a Therapeutic Agent for Triple Negative Breast Cancer Treatment

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## Supplementary Figure Legends

### **Figure S1. Dose-response studies of the triptonide effect on cell proliferation.**

(a) HCC1806 cells were treated 24 hours with triptonide at different concentrations (0.01  $\mu$ M, 0.1  $\mu$ M, 0.2  $\mu$ M, 0.5  $\mu$ M, and 1  $\mu$ M) using the Cell Titer-Glo luminescence assay. Each experiment was repeated twice. Each bar is presented as mean  $\pm$  SD (n = 3). (b) representative images showing the effects of 0.2  $\mu$ M triptonide treatment (24 hours) in ER+ MCF-7 and HER2+ SKBR3 cells. Magnification, x 200. Right, the effect of 0.2  $\mu$ M triptonide on cell growth measured by CellTiter-Glo assays. MCF-7 and SKBR3 cells were treated for 24 (D1), 48 (D2), and 72 hours (D3). Each bar is presented as mean  $\pm$  SD (n = 3). (c) Examination of cell apoptosis in fixed xenograft tumor tissues by TUNEL assays ( $\times$ 400 magnification). Images show induced apoptosis in triptonide-treated tumor tissues.

### **Figure S2. Triptonide attenuates stem-like properties of TNBC cells.**

(a) List of genes with 4-fold or more changes in expression levels detected by RT<sup>2</sup> profiler PCR analysis in HCC1806 cells. (b) Examination of ITGA6, Myc, KLF17, and SNAI1 expression by qRT-PCR in MDA-MB-231 TNBC cells. Each bar is presented as mean  $\pm$  SD (n = 3) (\*, p < 0.05. \*\*, p < 0.01. \*\*\*, p < 0.001).

### **Figure S3. SNAI1 expression is regulated by JNK activation in TNBC cells.**

The effects of ERK inhibition by 10  $\mu$ M U0126 and JNK inhibition by 10  $\mu$ M SP600126 on SNAI1 expression in HCC1806 cells were examined by immunoblotting.

Figure S1

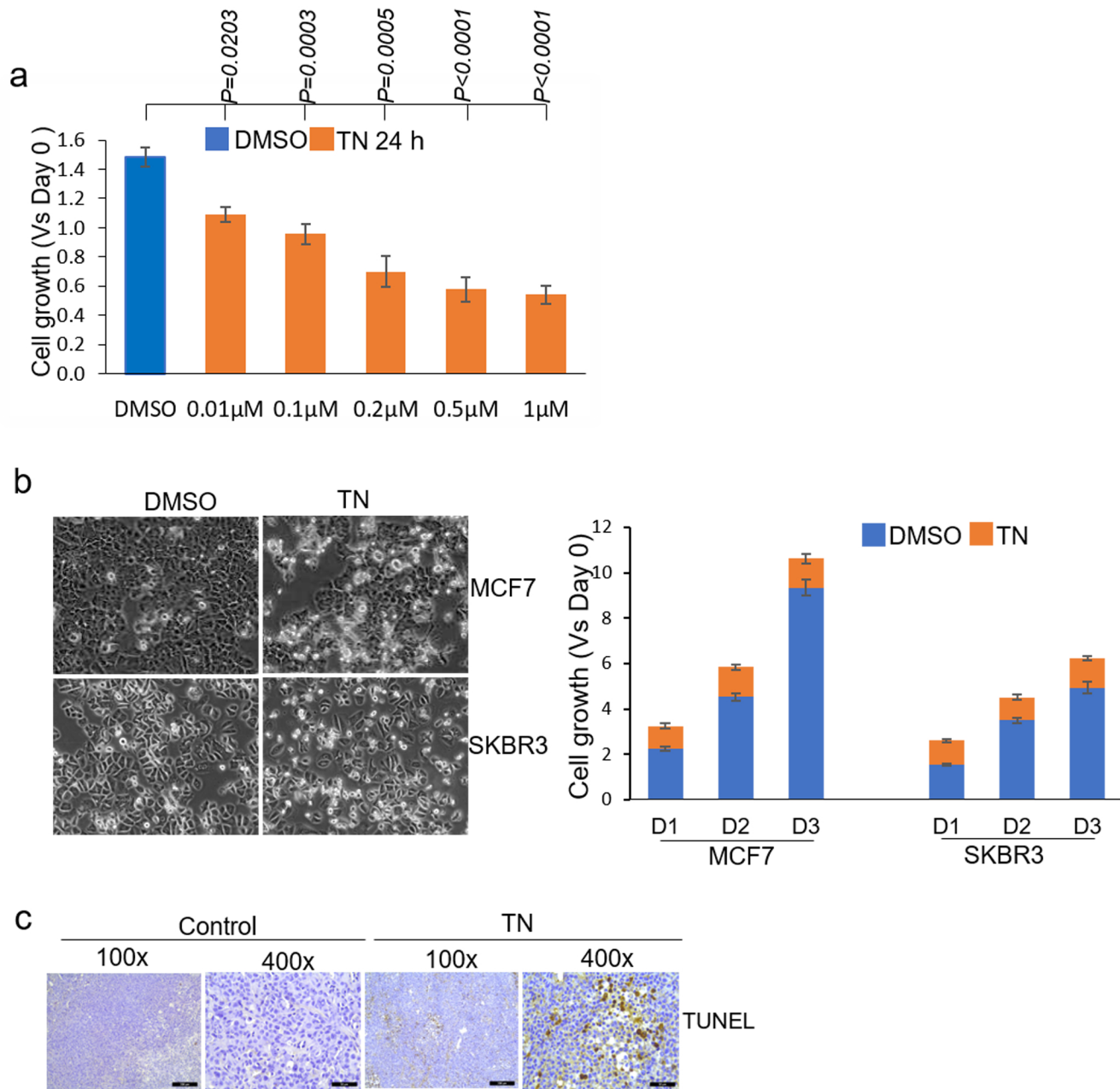


Figure S2

a

Position	Gene Symbol	Fold Regulation	p-Value
G06	<b>TWIST2</b>	398.51	N/A
F11	<b>SNAI1</b>	29.36	N/A
D09	<b>KLF17</b>	13.05	N/A
E07	MYCN	9.66	N/A
E10	NOS2	4.71	N/A
A11	CD34	4.30	N/A

Position	Gene Symbol	Fold Regulation	p-Value
D03	<b>ITGA6</b>	-1992.17	N/A
C10	ID1	-22.12	N/A
B05	DKK1	-14.90	N/A
E06	<b>MYC</b>	-14.69	N/A
D07	KIT	-11.96	N/A
C12	CXCL8	-9.23	N/A
D08	KITLG	-7.25	N/A
A06	ATXN1	-5.33	N/A
D06	JAK2	-4.99	N/A
H06	HGDC	-4.84	N/A
E11	NOTCH1	-4.71	N/A
G12	ZEB2	-4.69	N/A
G07	WEE1	-4.25	N/A
C02	FGFR2	-4.25	N/A

b

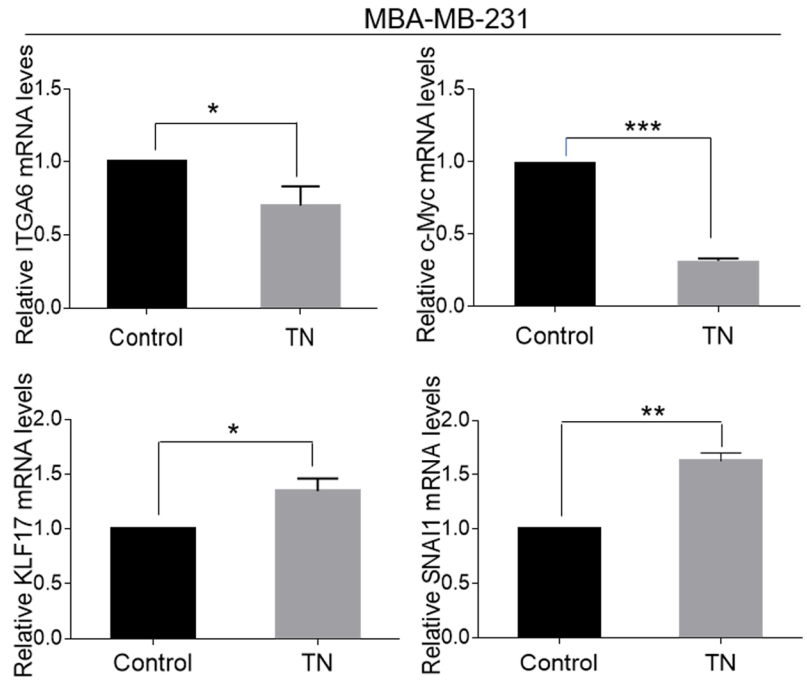




Figure S3

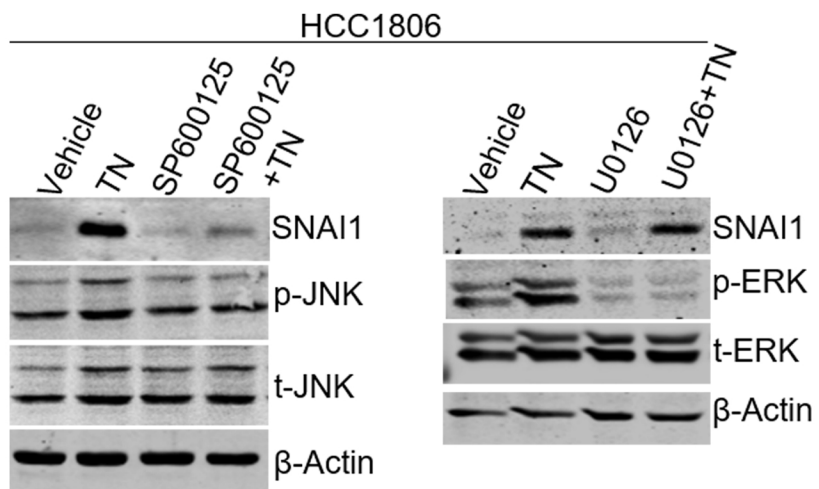


Figure S4, full-length images

Figure 1c

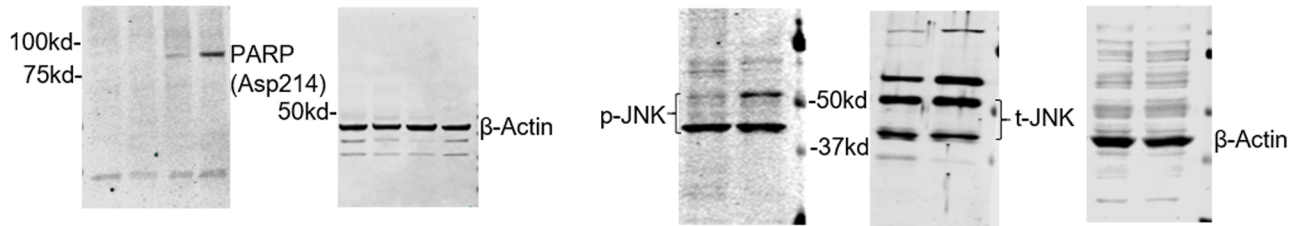


Figure 2e

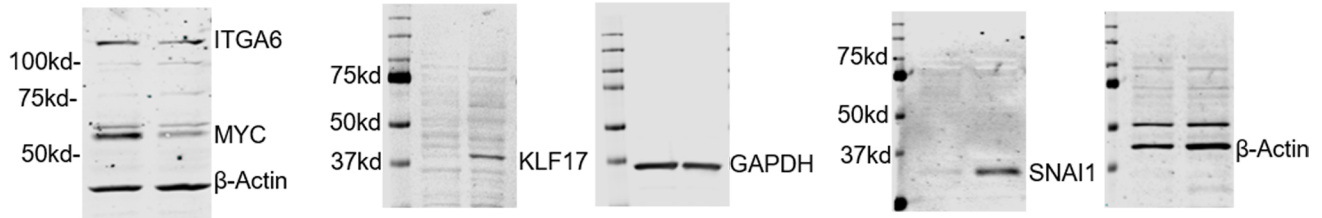


Figure 3c

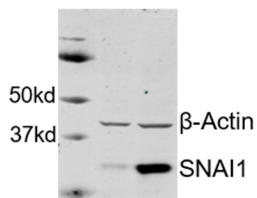


Figure 3d

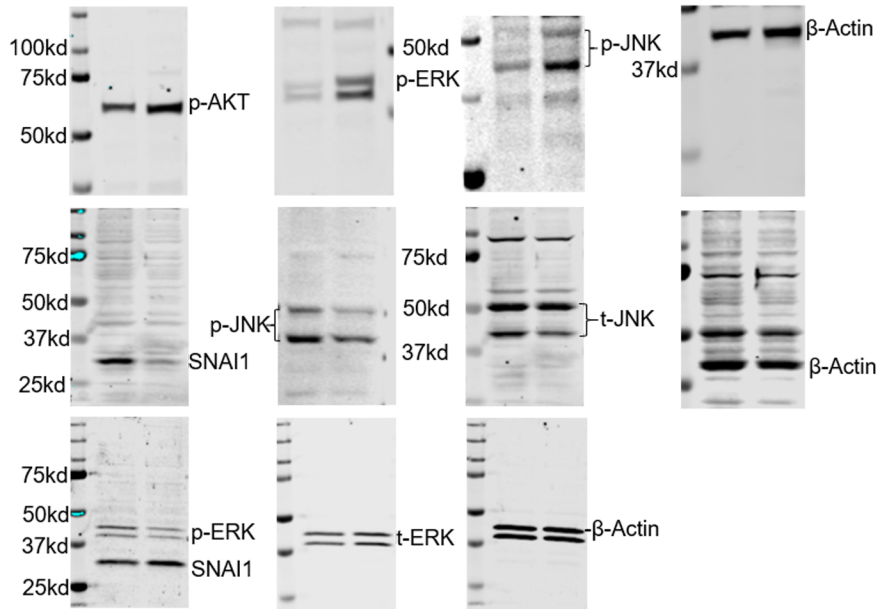


Figure 4a

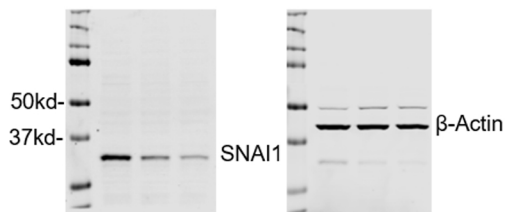


Figure 4b

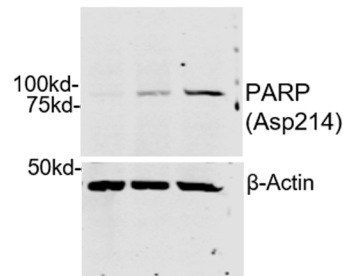


Figure S3

