cancers

Supplemental materials: Integrin α3β1 promotes invasive and metastatic properties of breast cancer cells through induction of the Brn-2 transcription factor.

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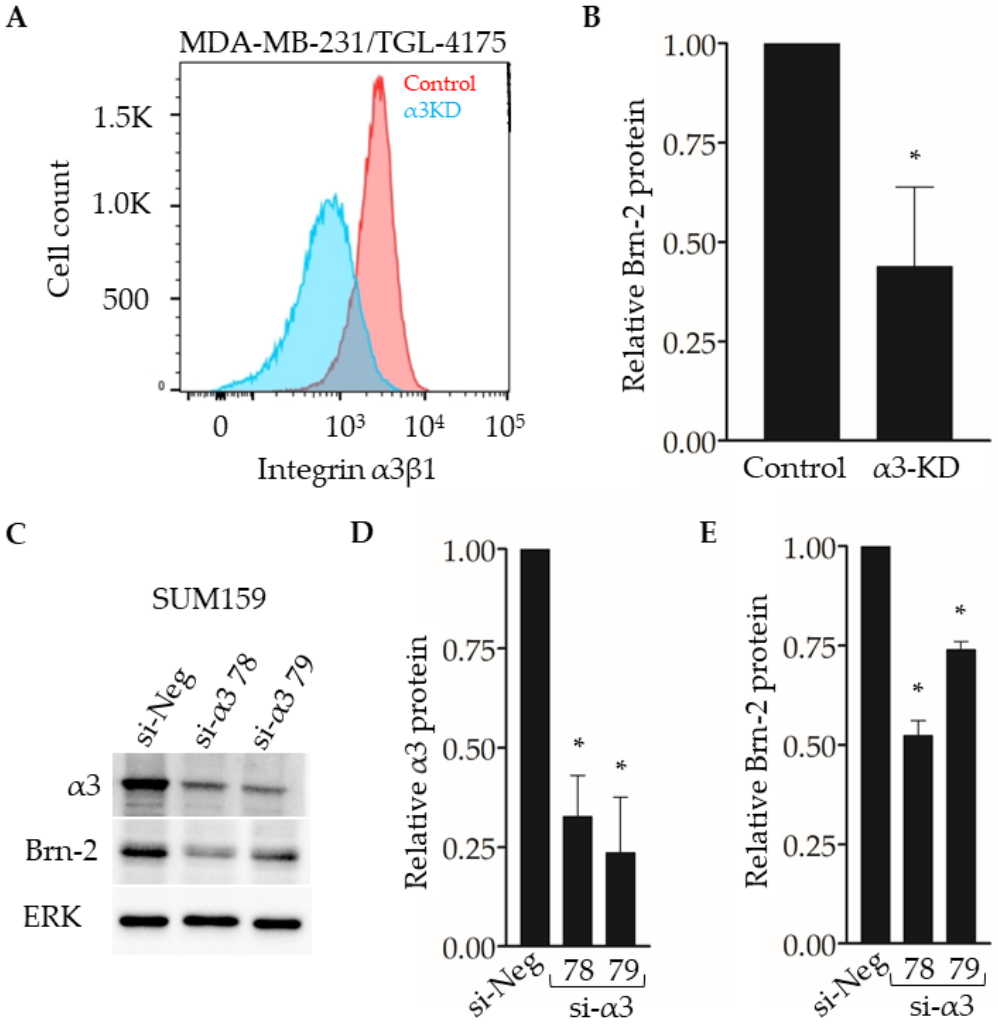

Figure S1. Suppression of integrin α3β1 decreases Brn-2 protein in the TGL-4175 variant of MDA-MB-231 line, and in SUM159 cells. (A) Flow cytometry using monoclonal antibody, P1B5, to compare cell surface levels of integrin α3 in TGL-4175 cells that stably express either non-targeting shRNA (Control) or α3-targeting shRNA (α3KD). (B) Western blot quantification of relative Brn-2 protein in α3KD TGL-4175 cells relative to control. (C) Representative western blots for integrin α3,

Brn-2 or ERK (loading control) in SUM159 cells transiently transfected with negative control siRNA (si-Neg) or with two distinct siRNAs that target integrin α3 (si-α3 78, si-α3 79). Data are quantified in (D) and (E), respectively, relative to control; n=3; mean +/- SEM; *p<0.05; two-tailed T-test (B) or two-tailed T-test with Bonferroni correction (D, E).

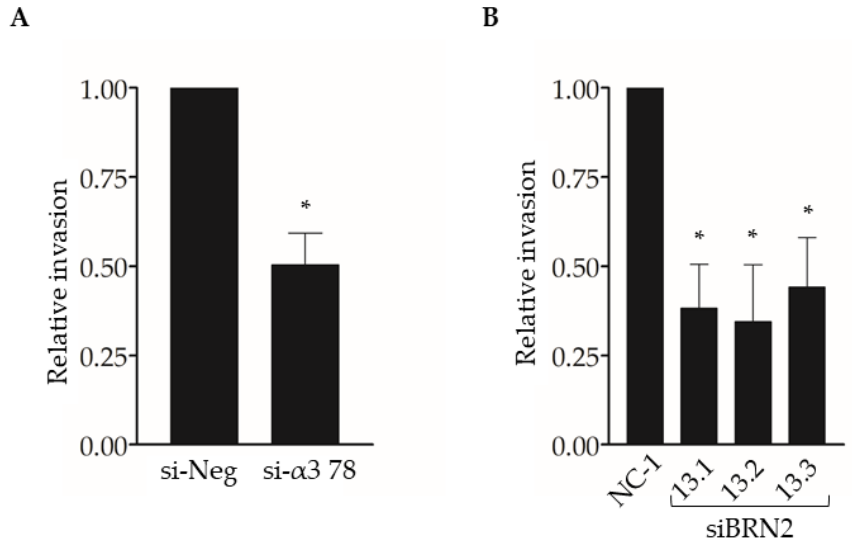

Figure S2. RNAi-targeting of integrin α3 or *BRN2* reduces SUM159 cell invasion. Matrigel transwell invasion assays were performed using SUM159 cells transfected with (A) non-targeting siRNA (si-Neg) or integrin α3-targeting siRNA (si-α3 78) and (B) non-targeting dicer-substrate siRNA (NC-1) or *BRN2*-targeting dicer-substrate siRNA (si*BRN2* 13.1, 13.2, or 13.3). Graphs show invasion of α3-knockdown cells (A) or Brn-2 knockdown cells (B) relative to control cells; n=3; mean +/- SEM; *p<0.05; two-tailed T-test (A) or two-tailed T-test with Bonferroni correction (B).

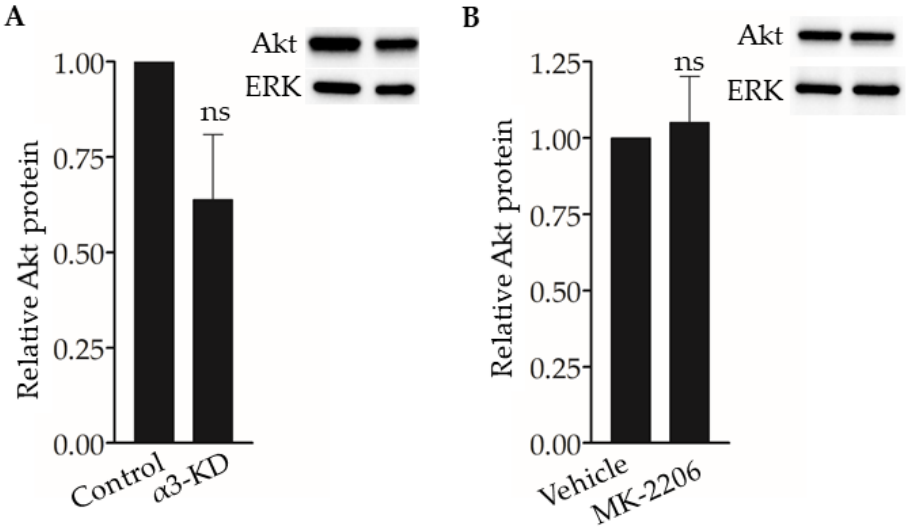

Figure S3. Suppression of integrin α3β1 or treatment with MK-2206 does not significantly alter total Akt protein levels in MDA-MB-231 cells. (A, B) Representative western blots and corresponding graphs show Akt levels in (A) α3KD cells relative to control cells, and (B) MDA-MB-231 cells treated with MK-2206 relative to vehicle-treated cells; n=3; mean +/- SEM; *p<0.05; two-tailed T-test.

Figure S4: Uncropped Western blotting figures

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Figure 3A

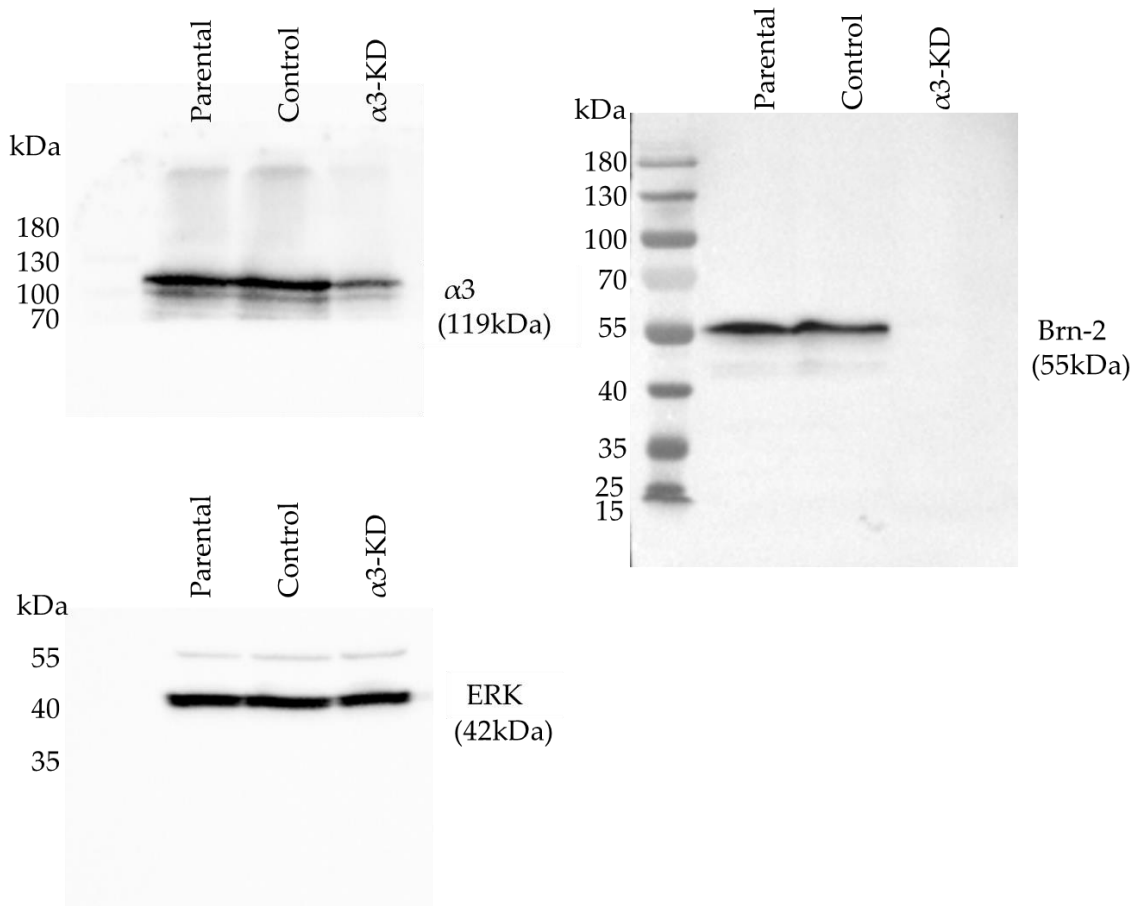


Figure 3C

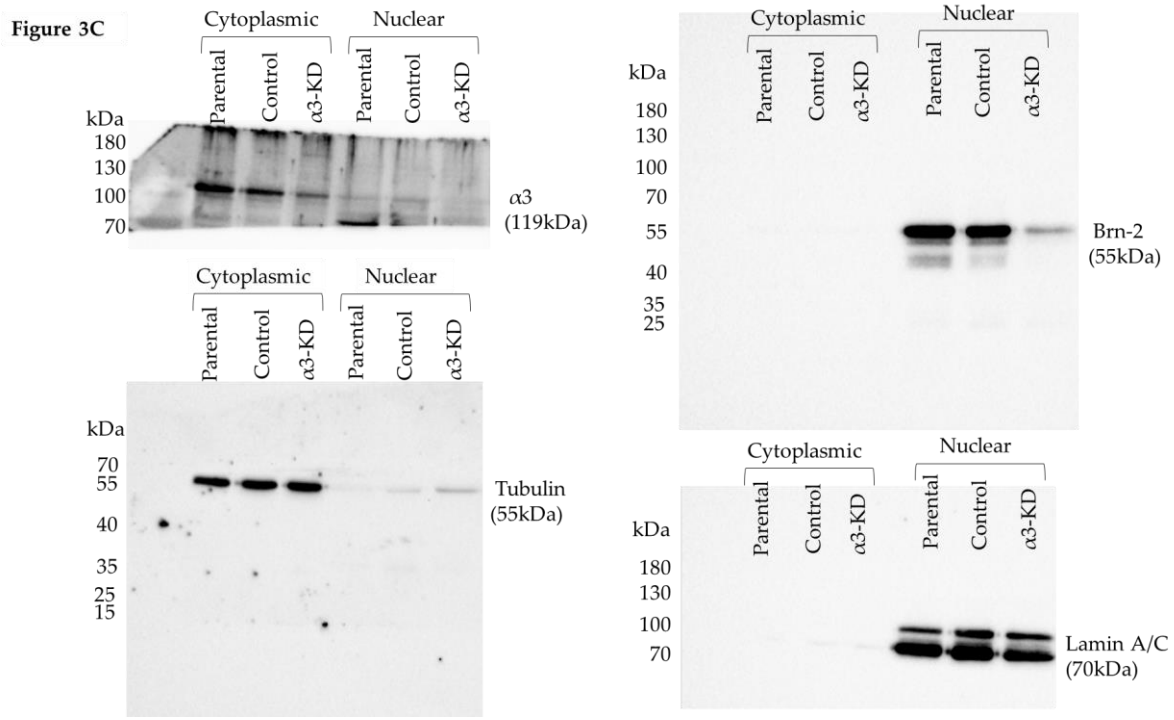


Figure S1C

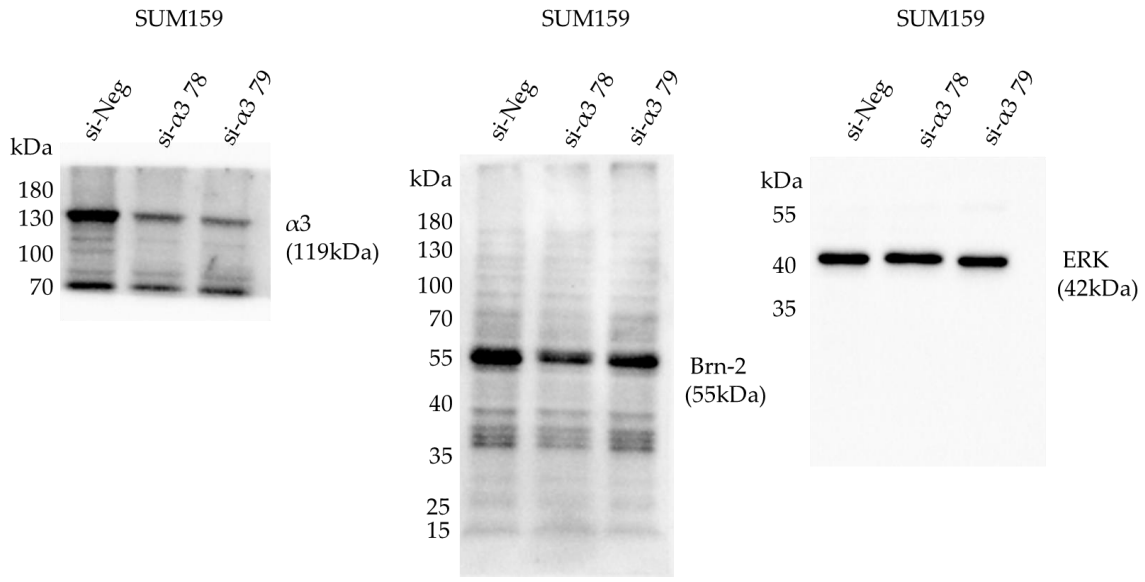


Figure 5A

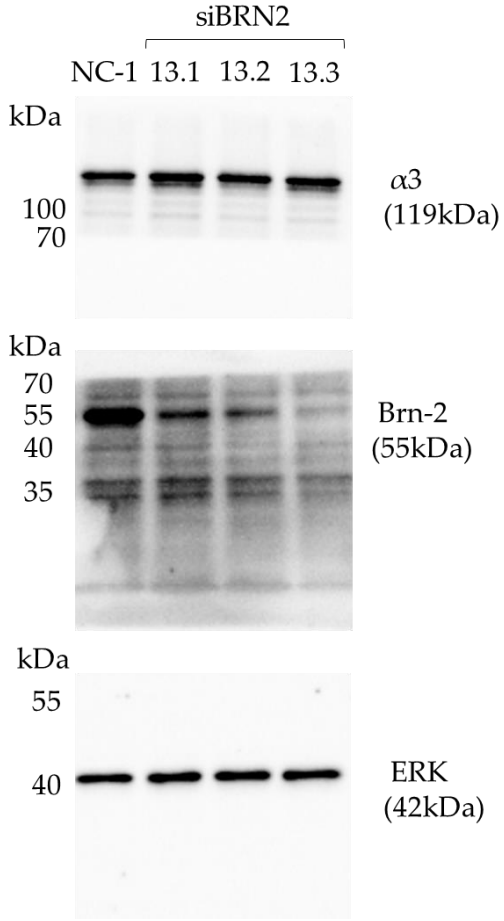
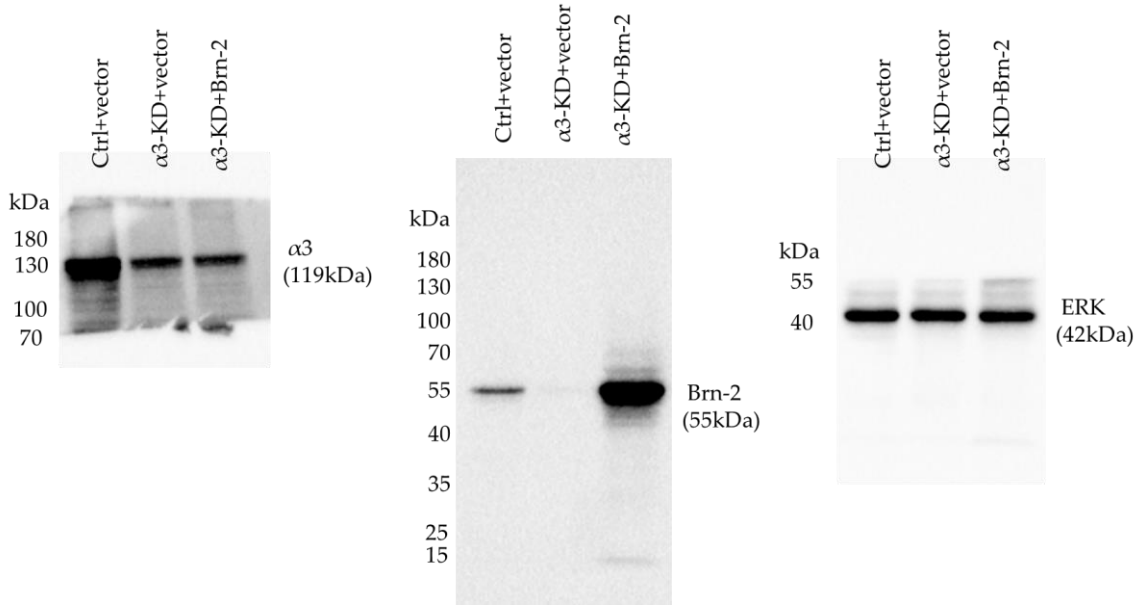


Figure 7A



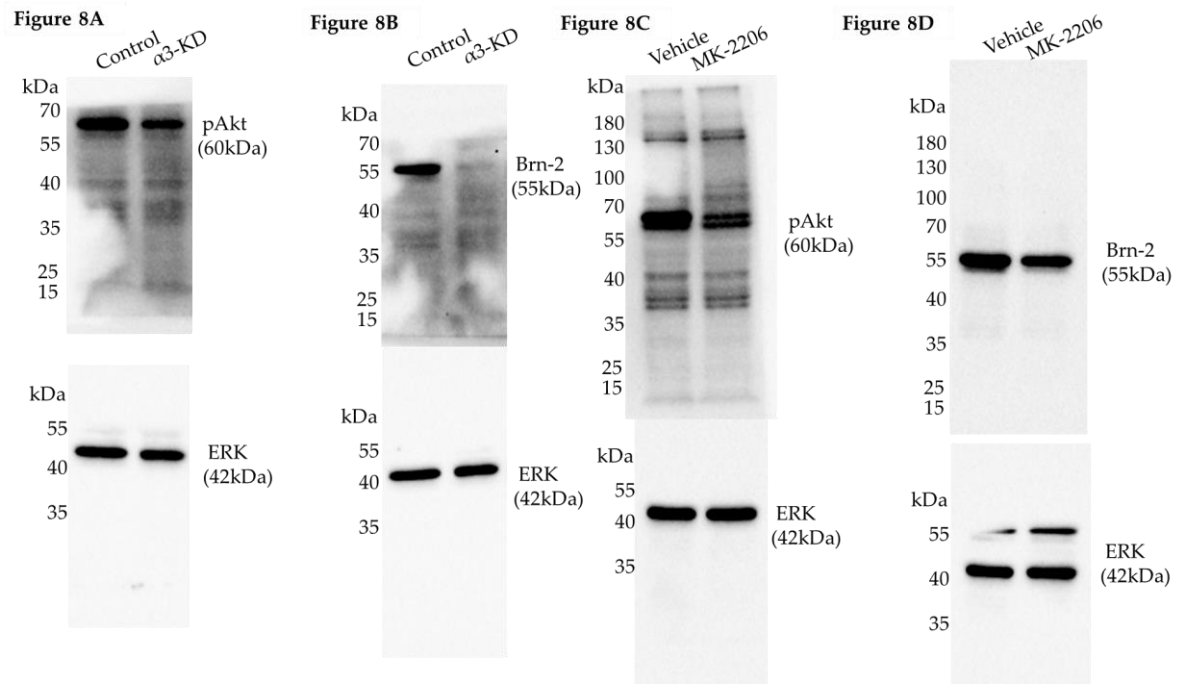


Figure 8A
Control
α3-KD
kDa
70
55
40
35
25
15
pAkt
(60kDa)
kDa
55
40
35
ERK
(42kDa)
Figure 8B
Control
α3-KD
kDa
70
55
40
35
25
15
Brn-2
(55kDa)
kDa
55
40
35
ERK
(42kDa)
Figure 8C
Vehicle
MK-2206
kDa
180
130
100
70
55
40
35
25
15
pAkt
(60kDa)
kDa
55
40
35
ERK
(42kDa)
Figure 8D
Vehicle
MK-2206
kDa
180
130
100
70
55
40
35
25
15
Brn-2
(55kDa)
kDa
55
40
35
ERK
(42kDa)

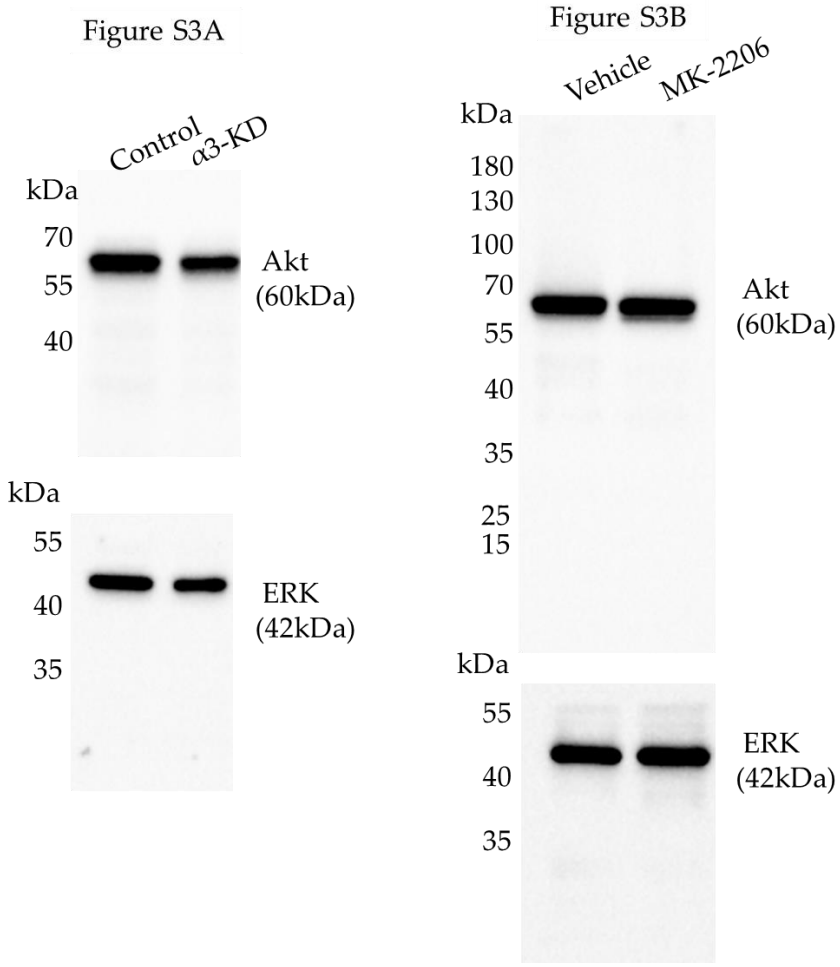


Figure S3A
Control
α3-KD
kDa
70
55
40
Akt
(60kDa)
kDa
55
40
35
ERK
(42kDa)
Figure S3B
Vehicle
MK-2206
kDa
180
130
100
70
55
40
35
25
15
Akt
(60kDa)
kDa
55
40
35
ERK
(42kDa)