Expanded View Figures

Figure EV1. Assessment of cellular localization of FOXO1 and phospho-mimicking FOXO1 peptide HA-FOXO1-IQBP S319E and its wild-type (S319) and non-phosphorylatable (S319A) counterparts in PCa cells.

A, B Immunofluorescence cytochemistry using antibodies for anti-Flag and IQGAP1 in LNCaP cells transfected with control vector (pcDNA3.1) and plasmids for Flag-FOXO1-WT, Flag-FOXO1-NESm, and Flag-FOXO1-NLSm. Cellular localization (nuclear, cytoplasm, or both) was photographed (A) and analyzed in > 100 cells in each experimental condition (B). Scale bars, 20 μm. The experiment was repeated at least once and similar results were obtained.

C Immunofluorescence cytochemistry using antibodies for anti-HA and IQGAP1 in LNCaP cells transfected with empty vector (E.V.) pcDNA3.1 and plasmids for phospho-mimicking FOXO1 peptide HA-FOXO1-IQBP S319E and its wild-type (S319) and non-phosphorylatable (S319A) counterparts. > 100 cells in each experimental condition were analyzed. Scale bars, 20 μm. The experiment was repeated at least once and similar results were obtained.

D LNCaP cells were transfected with SFB-tagged empty vector (SFB-EV) and SFB-tagged plasmids for phospho-mimicking FOXO1 peptide SFB-FOXO1-IQBP S319E and its wild-type (S319) and non-phosphorylatable (S319A) counterparts. 24 h after transfection, cells were harvested and lysed for co-IP with IgG or anti-Flag antibodies and immunoprecipitated proteins were analyzed by Western blotting. SFB tags are S, Flag, and biotin-binding protein (streptavidin) binding peptide tags. These three tags encode an ~12-kDa peptide (119 amino acids).

E LNCaP cells were transfected with SFB-tagged plasmids for phospho-mimicking FOXO1 peptides SFB-FOXO1-IQBP S319E and S319D and the non-phosphorylatable counterpart S319A. 24 h after transfection, cells were harvested and lysed for co-IP with IgG or anti-Flag antibodies and immunoprecipitated proteins were analyzed by Western blotting.

F LNCaP cells were transfected with SFB-tagged empty vector (EV) and SFB-tagged plasmids for SFB-FOXO1-IQBP S319E and its wild-type (S319) and non-phosphorylatable (S319A) counterparts. 24 h after transfection, cells were harvested and lysed for co-IP with IgG or anti-IQGAP1 antibodies and immunoprecipitated proteins were analyzed by Western blotting using indicated antibodies.

G Limited proteolysis assay. Limited proteolysis of GST-IQGAP1 incubated with GST-FOXO1-IQBP S319E or GST alone using trypsin. Proteolysis was carried out on ice for the times indicated under each lane. The products of proteolysis were resolved by 13.5% acrylamide SDS–PAGE and visualized by staining with Coomassie blue. The experiments were repeated once, and similar results were obtained.

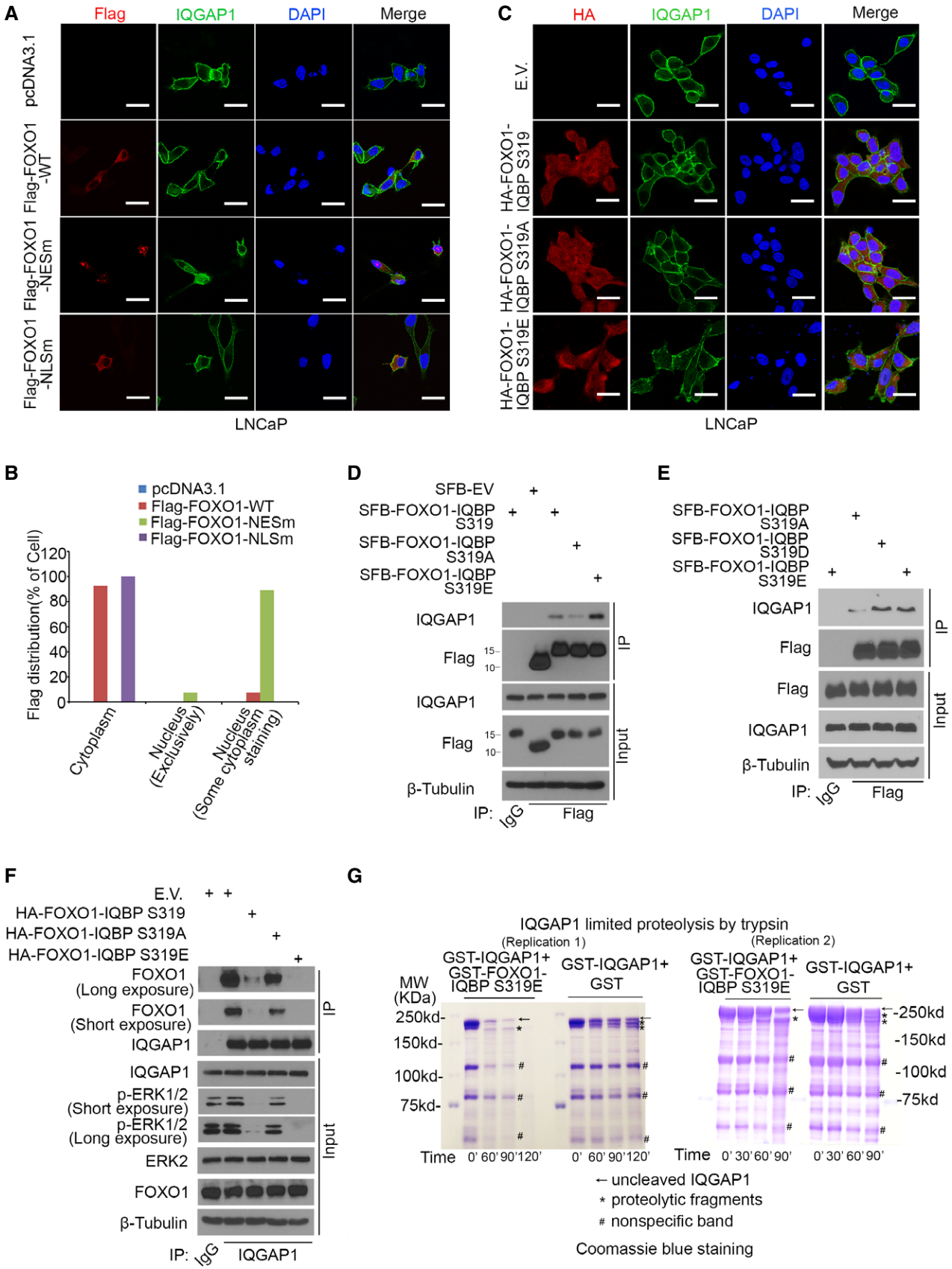

Figure EV1.

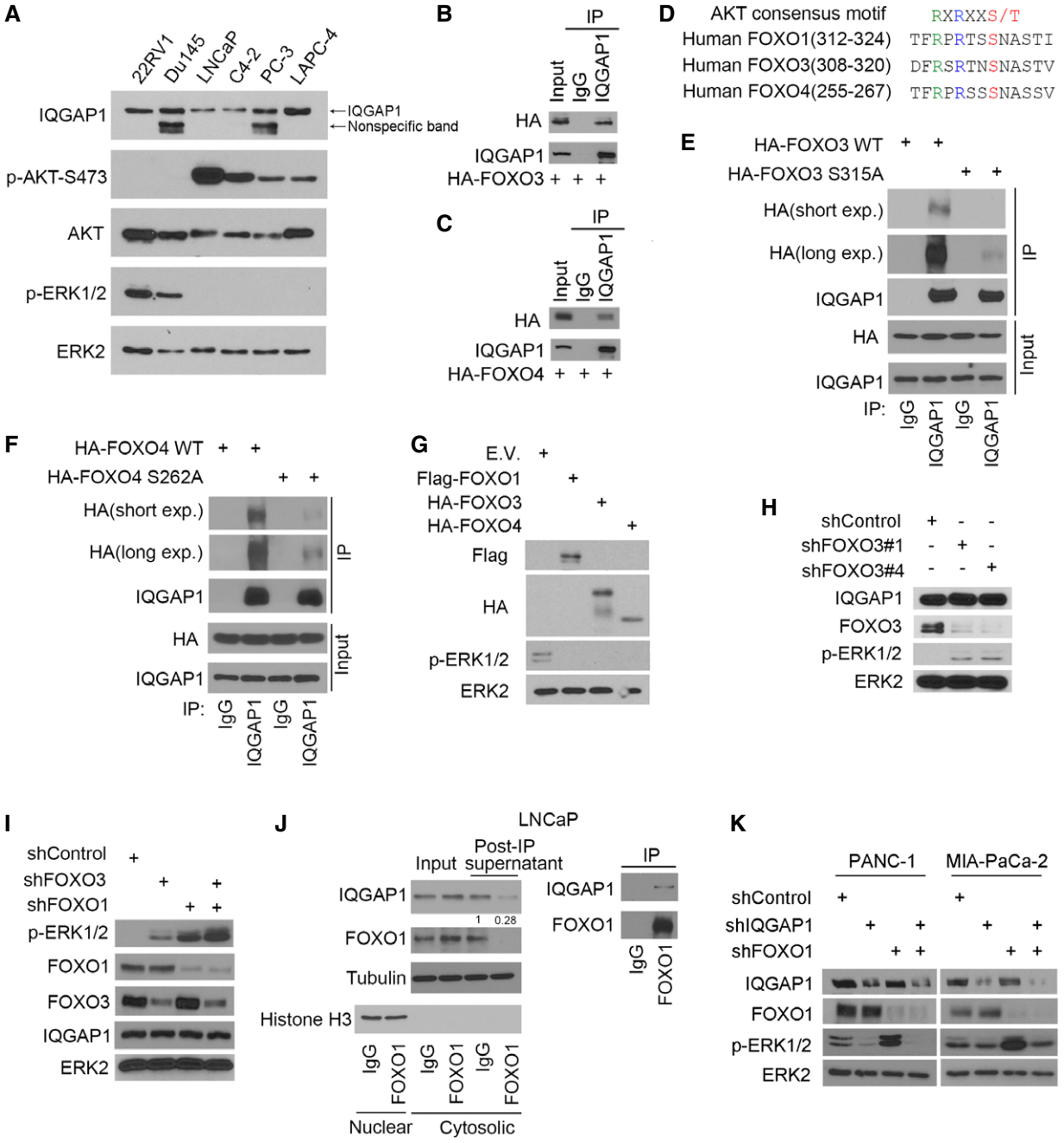

Figure EV2. Assessment of the interaction of IQGAP1 with FOXO3 and FOXO4, their effect on ERK1/2 phosphorylation and effect of FOXO1 on ERK1/2 phosphorylation in non-prostate cancer cells.

A Western blot analysis of expression of phospho-AKT (serine 473) and ERK1/2 in prostate cancer cell lines indicated. Arrow indicates non-specific reaction protein bands.

B, C Western blot analysis of whole-cell lysate (WCL) and co-immunoprecipitation (co-IP) samples of IgG or anti-IQGAP1 antibody from LNCaP cells 24 h after transfection with HA-FOXO3 (B) or HA-FOXO4 (C).

D Comparison of S319, an AKT phosphorylation site in FOXO1 with the homologous site in FOXO3 (S315) and FOXO4 (S262) by multiple sequence alignments.

E, F Western blot analysis of WCL and co-IP samples from LNCaP cells 24 h after transfection with indicated plasmids.

G Western blot analysis of WCL from DU145 cells 24 h after transfection with indicated plasmids. E.V., empty vector.

H, I Western blot analysis of WCL from LNCaP cells 48 h after infection with lentivirus expressing indicated shRNAs.

J Western blot analysis of indicated proteins in cytosolic fractionation (input), the supernatant post IP and co-IP samples from LNCaP cells. Nuclear fractionation was used to indicate the effectiveness of the fractionation.

K Western blot analysis of WCL from PANC-1 and MIA-PaCa-2 cells 48 h after infection with lentivirus expressing indicated shRNAs.

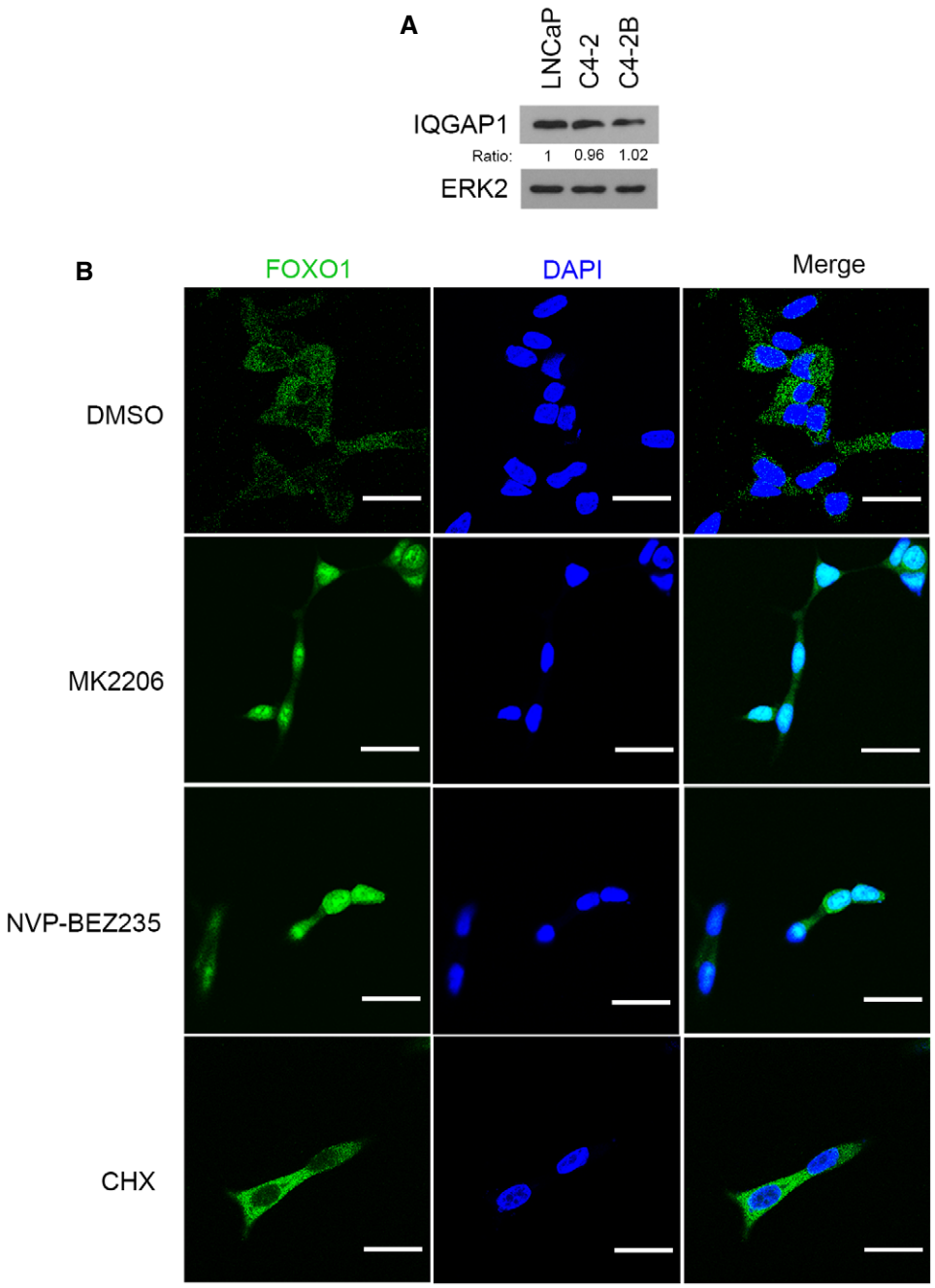

Figure EV3. Assessment of IQGAP1 protein expression in PCa cell lines and effect of PI3K/AKT inhibitors and CHX on cellular localization of FOXO1.

A Western blot analysis of IQGAP1 protein expression in prostate cancer cell lines LNCaP, C4-2, and C4-2B. ERK2 was used as a loading control. The density of IQGAP1 was determined by normalizing to ERK2 first and then to the normalized value in LNCaP cells.

B Immunofluorescence cytochemistry using anti-FOXO1 antibody in LNCaP (PTEN-negative and FOXO1 in the cytoplasm) cells treated with DMSO, MK2206 (0.5 μM), NVP-BEZ235 (50 nM), and CHX (20 μg/ml) for 24 h. > 100 cells in each experimental condition were analyzed. Scale bars, 20 μm. The experiment was repeated at least once and similar results were obtained.

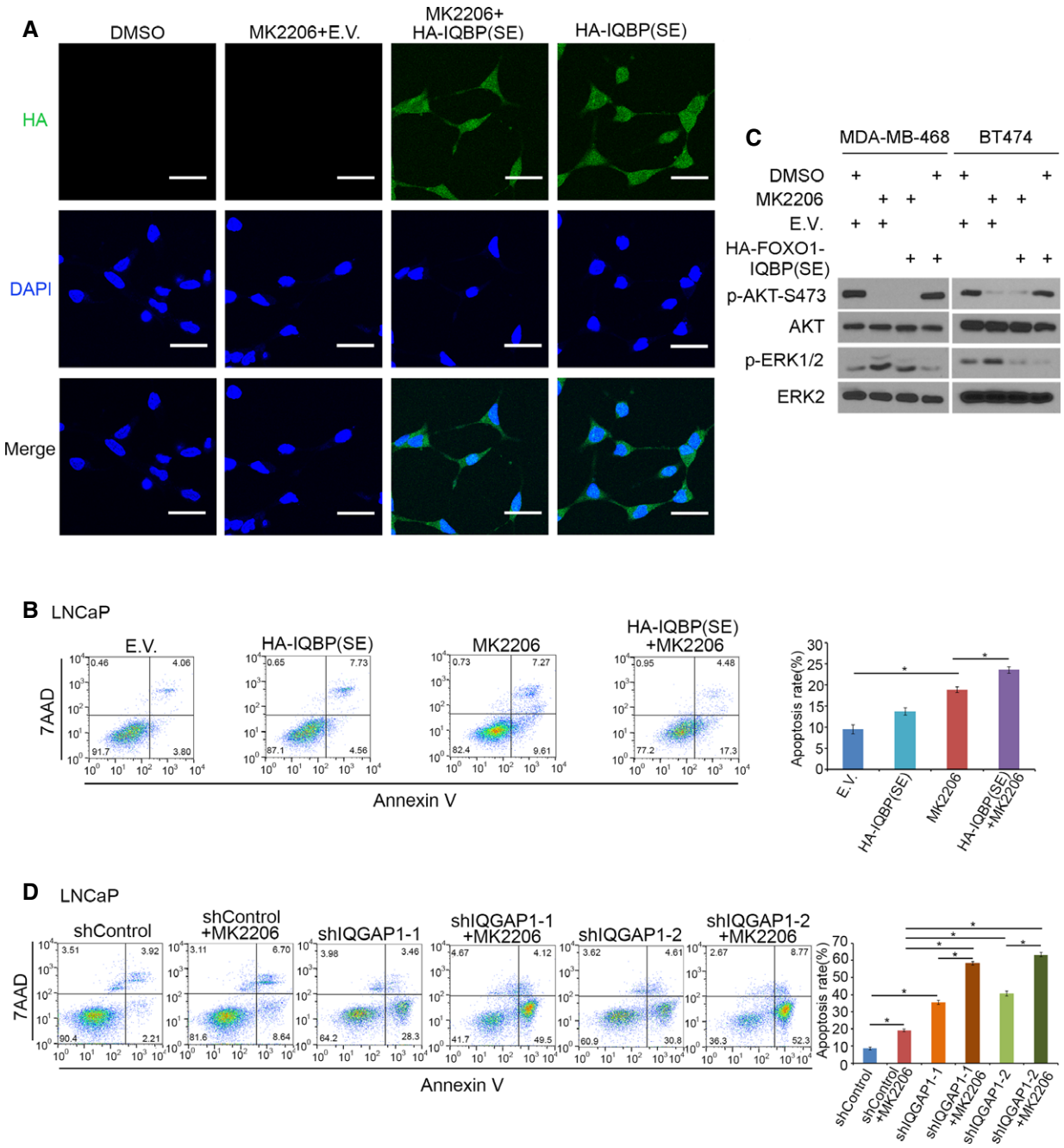

Figure EV4. Assessment of effect of FOXO1 phospho-mimicking peptide on AKT inhibitor-induced cell death and pERK1/2.

A, B LNCaP cells were transfected with empty vector (E.V.) or the FOXO1 phospho-mimicking peptide HA-FOXO1-IQBP(SE) for 24 h and then treated with or without MK2206 (0.5 μM) for 24 h followed by immunofluorescence cytochemistry using anti-HA antibody (A) and Annexin V assay and flow cytometry analysis (B). For panel (A), > 100 cells in each experimental condition were analyzed. Scale bars, 20 μm. The experiment was repeated at least once and similar results were obtained. For panel (B), data from two biological replicates were quantified (means ± s.d., n = 2). *P = 0.001687442 comparing MK2206 versus EV; *P = 0.014293987 comparing MK2206 + EV versus MK2206 + IQBP(SE) (two-sided Student's t-test at the 48-h time point).

C Western blot analysis of whole-cell lysate from indicated cell lines 24 h after transfection with empty vector (E.V.) and the FOXO1 phospho-mimicking peptide HA-FOXO1-IQBP(SE) and then treated with or without MK2206 (0.5 μM) for 24 h.

D Annexin V assay and flow cytometry analysis. 72 h after infection with lentivirus expressing indicated shRNAs, LNCaP cells treated with or without MK2206 (0.5 μM) for 24 h. Quantitative data were obtained from two biological replicates (means ± s.d., n = 2). *P = 0.004378751 comparing shControl versus shControl + MK2206; *P = 0.001300305 comparing shIQGAP1-1 versus shIQGAP1-1 + MK2206; *P = 0.000554 comparing shIQGAP1-2 versus shIQGAP1-2 + MK2206; *P = 0.000967413 comparing shIQGAP1-1 versus shControl; *P = 0.000363332 comparing shIQGAP1-2 versus shControl; *P = 0.000232676 comparing shIQGAP1-1 + MK2206 versus shControl + MK2206; *P = 0.000180198 comparing shIQGAP1-2 + MK2206 versus shControl + MK2206 (two-sided Student's t-test at the 48-h time point).

Figure EV5. The FOXO1 small peptide inhibits taxol-induced ERK activation and chemoresistance.

A Immunofluorescence cytochemistry using indicated antibodies in LNCaP and BT474 cells 24 h after transfection with empty vector (EV) or FOXO1-IQBP(SE) and then treated with or without paclitaxel (PTX) (10 nM) for 24 h. E-cadherin was used to mark the plasma membrane (cell boundary). > 100 cells were analyzed in each experimental condition. Scale bars, 20 μm. The experiment was repeated at least once and similar results were obtained.

B Western blot analysis of whole-cell lysate (WCL) from LNCaP cells treated with or without paclitaxel (10 nM) for 24 h before harvest.

C Western blot analysis of WCL from LNCaP cells 24 h after transfection with indicated plasmids and then treated with or without docetaxel (10 nM) for 24 h.

D Western blot analysis of WCL from PC-3-Luc cells 72 h after infection with lentivirus expressing empty vector (pTsin) or FOXO1-IQBP(SE). Cells were then treated with or without docetaxel (10 nM) for 24 h prior to harvest.

E Immunofluorescence cytochemistry using anti-HA antibody in PC-3-Luc cells 72 h after infection with lentivirus expressing indicated empty vector or HA-FOXO1-IQBP (SE). Cells were then treated with or without docetaxel (10 nM) for 24 h. > 100 cells were analyzed in each experimental condition. Scale bars, 50 μm. The experiment was repeated at least once and similar results were obtained.

F Immunofluorescence histochemistry using anti-phospho-ERK (p-ERK) and anti-HA antibodies in frozen sections acquired from indicated mouse xenografts harvested at the end of treatment. > 100 cells were analyzed in each experimental condition. Scale bars, 50 μm. Similar experiments were performed in samples obtained from at least three animals (n = 3).

G MTS assay performed in PC-3-Luc cells. 72 h after infection with lentivirus expressing empty vector (EV) or HA-IQBP(SE), cells were treated with or without docetaxel (10 nM) for 24 h (means ± s.d., n = 6). *P = 1.24674E-07 comparing EV+DMSO versus EV+DTX; *P = 3.29127E-07 comparing IQBP(SE)+DTX versus EV+DTX (two-sided Student's t-test at the 4-day time point).

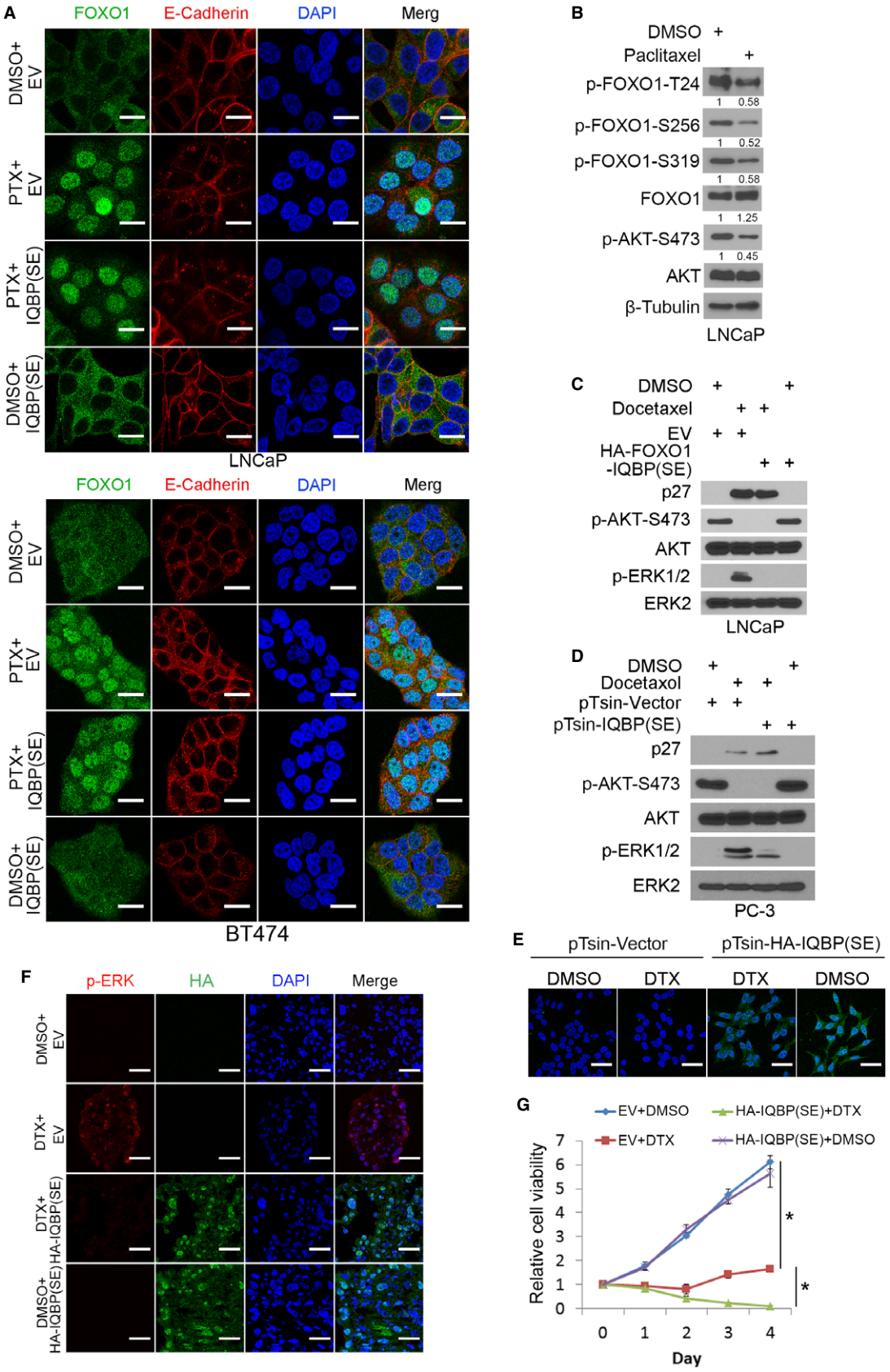
A
FOXO1
E-Cadherin
DAPI
Merg
DMSO+ EV
PTX+ EV
PTX+ IQBP(SE)
DMSO+ IQBP(SE)
LNCaP
BT474
B
DMSO
Paclitaxel
p-FOXO1-T24
1 0.58
p-FOXO1-S256
1 0.52
p-FOXO1-S319
1 0.58
FOXO1
1 1.25
p-AKT-S473
1 0.45
AKT
β-Tubulin
LNCaP
C
DMSO
Docetaxel
EV
HA-FOXO1 -IQBP(SE)
p27
p-AKT-S473
AKT
p-ERK1/2
ERK2
LNCaP
D
DMSO
Docetaxol
pTsin-Vector
pTsin-IQBP(SE)
p27
p-AKT-S473
AKT
p-ERK1/2
ERK2
PC-3
E
pTsin-Vector
pTsin-HA-IQBP(SE)
DMSO
DTX
DTX
DMSO
F
p-ERK
HA
DAPI
Merge
DMSO+ EV
DTX+ EV
DTX+ HA-IQBP(SE)
DMSO+ HA-IQBP(SE)
G
EV+DMSO
EV+DTX
HA-IQBP(SE)+DTX
HA-IQBP(SE)+DMSO
Relative cell viability
Day
*
*

Figure EV5.