Supplemental Data

Supplemental Materials and Methods

Plasmids. pcDNA3/FLAG/FOXO3a-WT and pcDNA3/FLAG/FOXO3a-TM (triple mutant in which AKT phosphorylation sites T32, S253, and S315 were converted to alanine) were provided by William Sellers (Dana Farber Cancer Institute, Harvard Medical School) (Ramaswamy et al., 2002). pcDNA3/FLAG-HA and pcDNA3/FLAG-HA/FOXO3a were used to generate stable cell lines for proteomic screening. pcDNA3/FLAG/FOXO3a-P34A was generated by standard PCR-based site-directed mutagenesis using pcDNA3/FLAG/FOXO3a as a template. pEGFP-N1/FOXO3a-WT and pEGFP-N1/FOXO3a-S207A encoding green fluorescent protein (GFP)-tagged FOXO3a were provided by Azad Bonni (Department of Pathology, Harvard Medical School) (Lehtinen et al., 2006). pEGFP-C3/FOXO3a-TM was provided by James Griffin (Dana Farber Cancer Institute) (Scheijen et al., 2004). pcDNA3/FLAG/FOXO1 and pcDNA3/FLAG/FOXO1-TM (triple mutant in which AKT phosphorylation sites T24, S256, and S319 were converted to alanine) was provided by Kun-Liang Guan (Moores Cancer Center, University of California at San Diego) (Tang et al., 1999). GLOFLAG3/FLAG/FOXO4 was provided by Boudewijn Burgering (University Medical Center Utrecht, Utrecht, Netherlands) (van der Horst et al., 2004). pCMV5/small t antigen (ST) and pCMV5/STmut3 (aa 1-110) were provided by Marc Mumby (University of Texas Southwestern Medical Center) (Sontag et al., 1993). The retroviral vectors, p-Murine stem-cell virus-Internal ribosomal entry site-GFP (pMIG) and pMIG/ST have been described (Hahn et al., 2002). pcDNA3/FLAG/SKP2 has been described (Wei et al., 2004). pCMV5/FLAG/Mst-1 was kindly provided by Joseph Avruch (Massachusetts General Hospital) (Lin et al., 2002).

Cell lines and transfection. HEK293T and HeLa cells were obtained from American Type Culture Collection (ATCC) and maintained in Dulbecco's modified Eagle's Medium (DMEM) (Mediatech) supplemented with 10% fetal bovine serum (FBS) and L-glutamine (complete DMEM). HeLa cells with stably integrated pcDNA3/FLAG-HA and pcDNA3/FLAG-HA/FOXO3a were maintained in complete DMEM supplemented with 800μg/ml G418 (Mediatech). BaF3 cells were maintained in RPMI 1640 supplemented with 10%FBS and 10% WEHI cell conditioned media as a source of interleukin-3 (IL-3). All cell lines were cultured in an atmosphere of 37°C and 5%CO_2. Transient transfection of plasmid DNA was performed using Lipofectamine 2000 (Invitrogen) according to the manufacturer's specifications. For experiments involving cotransfection, total transfected DNA was held constant by the addition of an empty control plasmid (pCMV6-XL5). Transfected cells were maintained in complete DMEM for 24h prior to harvesting. Cotransfection of pcDNA3/FLAG/FOXO3a and either 100nM of an ON-TARGETplus non-targeting pool of small interfering RNA (siRNA) or 100nM of an ON-TARGETplus SMARTpool of siRNA for the α isoform of the human PP2A catalytic subunit (*PPP2CA*) (Dharmacon) was performed using Lipofectamine 2000. Cells transfected with siRNAs were maintained in complete DMEM for 72h prior to harvesting.

Antibodies. The following antibodies were purchased from Cell Signaling Technology: Pan-Calcineurin A, PP5, PP2A B Subunit (#4953), pan-Akt (C67E7), Akt1 (C73H10), Akt2 (D6G4), pAkt(T308) (C31E5), pAkt (S473) (193H12), p70 S6 Kinase (#9202), phospho-p70S6K (T389) (#9205), Mst1, IκBα, PP2A A subunit (#2039), FoxO3a (75D8), and Bim (#2819). All phospho-FOXO antibodies were also purchased from Cell Signaling Technology. The following antibodies were purchased from Santa Cruz Biotechnology: GFP (B-2), PP1 (E-9), PPX (C-18), pan 14-3-3 (K-19), pan 14-3-3 (H-8), Sp1 (PEP2), pSGK(S422) (sc-16745-R), FOXO3a (H-144), PP2A/B56δ (H5D12), and PP2A/Aα (6F9). The following antibodies were purchased from BD Transduction Laboratories: PP2A catalytic α (clone 46) and SV40 large T and small t antigens (pAb108). The following antibodies were purchased from Sigma: FLAG (M2)-horseradish peroxidase (HRP) conjugate, β-actin (clone AC-15), and FOXO3a (clone FR1). The following antibody was purchased from Stressgen: SGK-1 (CT). The following antibody was purchased from Biosource: FOXO3a (pS207)/FOXO1 (pS212). The anti-B56ε antibody was described previously (Chen et al., 2005). Rabbit polyclonal anti-AKT-1 was produced by immunizing rabbits with a synthetic peptide (VDSERRPHFPQFSYSASGTA) and was generously provided by Alex Toker (Beth Israel Deaconess Medical Center, Harvard Medical School). All antibodies were used at a 1:1,000 dilution for immunobloting except for anti-FLAG (M2)-HRP (1:10,000), anti-β-Actin (clone AC-15) (1:10,000), anti-PP2A catalytic α (clone 46) (1:2,000), anti-PP1 (1:2,000), anti-pan 14-3-3 (1:5,000), and anti-FOXO3a (clone FR1) (1:5000).

Virus production and transduction. To generate lentiviral particles for short hairpin RNA (shRNA)-mediated gene silencing, HEK293T cells seeded in 100mm dishes were transiently cotransfected with lentiviral packaging mix (Sigma) and pLKO.1-based lentiviral shRNA plasmids (Sigma) using Fugene 6 (Roche Applied Science) according to the manufacturer's specifications. Two independent shRNA target sequences were used to silence *PPP2R5E/B56ε* expression: TRCN0000002560 and TRCN0000002558. TRCN0000040173 was used to silence *SGK-1* and TRCN0000071617 was used to silence *foxo3a*. The previously validated oligonucleotide sequences for silencing *AKT-1* and *AKT-2* (Irie et al., 2005) were cloned into pLKO.1 and provided by Alex Toker. pLKO.1/shTurboGFP™ (Sigma) was used as a negative control to monitor off-target effects. Supernatants containing lentiviral particles were collected 48h post-transfection and filtered using a 0.45μm filter (Milipore). For transduction, HeLa and BaF3 cells were plated on 60mm dishes in their respective complete growth medium. 24h postsplit, growth medium was removed and cells were cultured with lentiviral particles in the presence of 8μg/ml Polybrene (American Bioanalytical) for 24h in an atmosphere of 37°C and 5%CO_2. Lentiviral particles were then removed and cells were cultured in the presence of complete growth medium containing 1μg/ml puromycin (Sigma). Puromycin resistant clones were selected, pooled, and analyzed for target gene knockdown by immunoblotting with appropriate antibodies. pMIG and pMIG/ST were transiently transfected into the BOSC23 retroviral packaging cell line using

calcium phosphate coprecipitation to produce retroviral particles. Retroviral supernatants were harvested 72h after transfection, filtered, and added to BaF3 cells for 72h prior to selection of GFP-expressing cells by fluorescence-activated cell sorting (FACS).

Immunoprecipitation/Coimmunoprecipitation. Cells in 100mm dishes were washed twice with phosphate-buffered saline (PBS) then scraped on ice in EBC lysis buffer (50mm Tris-HCL [pH 8.0], 120mM NaCl, 0.5% [v/v] Nonidet P-40 (NP-40), and 5mM EDTA) supplemented with protease inhibitors (COMPLETE, Roche Applied Science) and phosphatase inhibitors (Halt Phosphatase Inhibitor Cocktail, Pierce Biotechnology). Protein concentrations were measured using the BCA protein assay reagent (Pierce). Equal amounts of soluble protein (0.5-1.0mg) were diluted with EBC lysis buffer to a final concentration of 1mg/ml. Whole cell lysates were then pre-cleared for 1h at 4°C with protein A/protein G plus agarose (Calbiochem) and then incubated for 2h at 4°C while rotating with anti-FLAG (M2) affinity gel (Sigma) (20μL packed beads). FLAG immunocomplexes were washed three times with EBC buffer supplemented with protease and phosphatase inhibitors before being boiled in 2X Laemmli reducing sample buffer. Immunocomplexes and 5% of the immunoprecipitation input from the initial whole cell lysate were analyzed by immunoblotting as indicated in the respective figures. To detect an interaction between endogenous FOXO3a, PP2A subunits, and 14-3-3, HeLa whole cell lysate (2-3mg) was pre-cleared for 1h at 4°C with protein A/protein G plus agarose and then incubated overnight at 4°C while rotating with either normal rabbit immunoglobulin G (IgG) (Santa Cruz Biotechnology) or anti-FOXO3a antibody (H-144; Santa Cruz Biotechnology) (1μg IgG/mg lysate). Immunocomplexes were then captured with protein A/protein G plus agarose (20μL packed beads) for 1h at 4°C and processed as described above. Endogenous immunoprecipitated FOXO3a was detected by immunoblotting with an anti-FOXO3a mouse monoclonal antibody (FR1; Sigma). 14-3-3 that coprecipitated with endogenous FOXO3a was detected by immunoblotting with an anti-pan 14-3-3 antibody (H-8) whereas 14-3-3 present in anti-FLAG immunocomplexes was detected with the K-19 anti-pan 14-3-3 antibody.

Tandem affinity purification (TAP) and mass spectrometry. Briefly, HeLa cells stably expressing FOXO3a fused to tandem FLAG and HA epitopes (TAP/FOXO3a) were lysed in EBC buffer as described above. Cell lysates were first immunoprecipitated with anti-FLAG (M2) affinity gel (Sigma) for 2h at 4°C. FLAG immunocomplexes were washed three times with EBC buffer and TAP/FOXO3a was eluted with 150ng/μl 3X FLAG peptide (Sigma). FLAG peptide eluates were then immunoprecipitated with anti-HA (HA7) agarose affinity resin (Sigma) for 2h at 4°C, washed three times with EBC buffer, and eluted with 1μg/μl HA peptide (Sigma). HA elutes were fractionated by 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and then silver stained. The marked protein bands were excised from the gel by the Taplin Mass Spectrometry Core Facility (Harvard Medical School) and analyzed

by microcapillary LC/MS/MS techniques using a LTQ FT Ultra Hybrid Mass Spectrometer (Thermo Electron).

Immune complex kinase assays. The enzymatic activity of endogenous AKT was analyzed using a non-radioactive AKT kinase assay kit according to the manafacturer's specficiations (Cell Signaling Technology).

Cellular fractionation. For nuclear translocation studies, the NE-PER kit (Pierce) was used to obtain cytoplasmic and nuclear protein from 100mm dishes in accordance with the manafacturer's instructions. Buffers were supplemented with COMPLETE (Roche Applied Science) and Halt phosphatase inhibitors (Pierce). For experiments involving localization of ectopic FOXO, 200ng of the relevant FOXO expression plasmid was transfected/100mm dish to prevent mislocalization of FOXO protein.

Immunoblotting. Whole cell lysates (20-40μg) and immunoprecipitates were resolved by either 10% or 12.5% SDS-PAGE, and transferred to 0.45μm Immobilon-P membranes (Millipore). The membranes were then blocked for 1h at room temperature in blocking buffer (5% [w/v] non-fat dry milk/Tris-buffered saline-Tween-20) (TBST; 50mM Tris-HCl [pH7.4], 150mM NaCl, .05% [v/v] Tween-20). Rabbit primary antibodies were diluted in 5% [w/v] bovine serum albumin (BSA)/TBST while mouse primary antibodies were diluted in blocking buffer then incubated with membranes for 2h at room temperature. Membranes were then washed three times with TBST, incubated for 1h at room temperature with either HRP-conjugated goat anti-mouse or goat anti-rabbit IgG secondary antibodies (sc-2055 and sc-2054, respectively, Santa Cruz Biotechnology) diluted 1:10,000 in blocking buffer, and then washed again with TBST prior to visualization using an enhanced chemiluminescent substrate (Super Signal West Pico, Pierce). To detect coimmunoprecipitated proteins that co-migrated with either IgG heavy chain (HC) or IgG light chain (LC), we utilized HRP-conjugated mouse anti-rabbit LC-specific or goat anti-rabbit F_c-specific secondary antibodies respectively (Jackson Immunoresearch Laboratories), diluted 1:10,000 in blocking buffer.

Fluorescence microscopy. For localization studies, HeLa cells grown on coverslips were transiently transfected with 1μg of the indicated GFP/FOXO3a expression plasmids and then subjected to an *in vivo* dephosphorylation assay. Cells were fixed in 2% paraformaldehyde for 15 min at room temperature, and then washed three times with PBS prior to permeabilization with 0.5% Triton X-100/PBS for 1 min at room temperature. Cells were then rinsed again in PBS prior to applying a mounting solution containing 4'6-diamido-2-phenylindole (DAPI) (Vector Laboratories). Coverslips were mounted on glass slides and images of GFP-tagged FOXO3a and DAPI-stained nuclei were captured using a 60X oil immersion objective lens on a Nikon Eclipse inverted epifluorescent microscope (Eclipse TE300; Nikon) fitted with a Retiga 2000RV camera. For quantitative analysis, nuclear fluorescence was calculated as a percentage of the

total cellular fluorescence [N/(N+C)]. All fluorescence measurements from 12 randomly chosen cells expressing GFP/FOXO3a were corrected for background fluorescence levels using IP Lab digital image analysis software (Scanalytics).

Real-time quantitative RT-PCR analysis

Briefly, quantification of mRNA was done in a two-step procedure using TaqMan Gene Expression Cells-to-Ct® Kit (Applied Biosystems). 10^5 cells were lysed in 50μl Lysis Solution with DNAase I, and 10 μl of the terminated reaction containing XenoRNA Control was used for reverse transcription. Real-time PCR cocktail reactions of 20μl volume were aliquoted in a 384-well plate. The individual gene expression assays (FAM reporter), including mouse *trail* (Mm01283606m1) and *bim* (Mm00437796m1) were from Applied Biosystems, with all amplicons designed to span exons and include all splicing variants. Real-time PCR analysis was performed on the 7900 HT Real-Time PCR System (Applied Biosystems) by ΔΔCt assay of SDS2.3 program, with cycling parameters as described in the manual. Results were processed and normalized to endogenous β-actin levels (Taqman Gene Cells-to-Ct Control Kit) using RQ Manager1.2 program.

Luciferase reporter assays

Briefly, HeLa cells were cotransfected with the pTK81 luciferase reporter plasmid containing six tandem FOXO response elements from the human *TRAIL* promoter together with a construct containing a neomycin resistance cassette. Stable clones responsive to transfected FOXO3a were expanded and maintained in complete DMEM supplemented with 800μg/ml G418 (Mediatech). A stable clone was transiently transfected as indicated in the figure legends. Lysis and determination of firefly luciferase activity was carried out 24h after transfection using the Luciferase Assay System, according the manufacturers instructions (Promega) on a multilabel plate reader (Victor-V, Perkin-Elmer). For experiments involving siRNA, cells were transfected for 48h with siRNAs before transfection with FOXO3a expression plasmids for an additional 24h. Cells were then harvested for measurement of luciferase activity.

Supplemental References

Chen, W., Arroyo, J.D., Timmons, J.C., Possemato, R., and Hahn, W.C. (2005). Cancer-associated PP2A Aalpha subunits induce functional haploinsufficiency and tumorigenicity. Cancer research *65*, 8183-8192.

Hahn, W.C., Dessain, S.K., Brooks, M.W., King, J.E., Elenbaas, B., Sabatini, D.M., DeCaprio, J.A., and Weinberg, R.A. (2002). Enumeration of the simian virus 40 early region elements necessary for human cell transformation. Molecular and cellular biology *22*, 2111-2123.

Irie, H.Y., Pearline, R.V., Grueneberg, D., Hsia, M., Ravichandran, P., Kothari, N., Natesan, S., and Brugge, J.S. (2005). Distinct roles of Akt1 and Akt2 in

regulating cell migration and epithelial-mesenchymal transition. The Journal of cell biology *171*, 1023-1034.

Lehtinen, M.K., Yuan, Z., Boag, P.R., Yang, Y., Villen, J., Becker, E.B., DiBacco, S., de la Iglesia, N., Gygi, S., Blackwell, T.K., and Bonni, A. (2006). A conserved MST-FOXO signaling pathway mediates oxidative-stress responses and extends life span. Cell *125*, 987-1001.

Lin, Y., Khokhlatchev, A., Figeys, D., and Avruch, J. (2002). Death-associated protein 4 binds MST1 and augments MST1-induced apoptosis. The Journal of biological chemistry *277*, 47991-48001.

Ramaswamy, S., Nakamura, N., Sansal, I., Bergeron, L., and Sellers, W.R. (2002). A novel mechanism of gene regulation and tumor suppression by the transcription factor FKHR. Cancer cell *2*, 81-91.

Scheijen, B., Ngo, H.T., Kang, H., and Griffin, J.D. (2004). FLT3 receptors with internal tandem duplications promote cell viability and proliferation by signaling through Foxo proteins. Oncogene *23*, 3338-3349.

Sontag, E., Fedorov, S., Kamibayashi, C., Robbins, D., Cobb, M., and Mumby, M. (1993). The interaction of SV40 small tumor antigen with protein phosphatase 2A stimulates the map kinase pathway and induces cell proliferation. Cell *75*, 887-897.

Tang, E.D., Nunez, G., Barr, F.G., and Guan, K.L. (1999). Negative regulation of the forkhead transcription factor FKHR by Akt. The Journal of biological chemistry *274*, 16741-16746.

van der Horst, A., Tertoolen, L.G., de Vries-Smits, L.M., Frye, R.A., Medema, R.H., and Burgering, B.M. (2004). FOXO4 is acetylated upon peroxide stress and deacetylated by the longevity protein hSir2(SIRT1). The Journal of biological chemistry *279*, 28873-28879.

Wei, W., Ayad, N.G., Wan, Y., Zhang, G.J., Kirschner, M.W., and Kaelin, W.G., Jr. (2004). Degradation of the SCF component Skp2 in cell-cycle phase G1 by the anaphase-promoting complex. Nature *428*, 194-198.

Supplemental Figure Legends

Figure S1. Interaction with PP2A is required for ST to impair FOXO3a dephosphorylation independent of SGK-1 activation. ***A.*** A FLAG-FOXO3a expression plasmid was cotransfected into stable shSGK-1/HeLa cells with either an empty vector (EV) or ST expression plasmid. Cells were grown in serum and then either lysed (0 min) or treated with 20μM each of LY and AKT-I in the absence of serum for the indicated time points prior to lysis. Whole cell lysates were immunoblotted (IB) with the indicated antibodies. ***B.*** Whole cell lysates

derived from HeLa cells transfected with either ST or ST/m3 expression plasmids were immunoprecipitated (IP) with either an IgG isotype control (IgG) or an anti-PP2A/Aα IgG (Aα) as indicated. Immunocomplexes were immublotted (IB) with the indicated antibodies. ***C.*** A FLAG-FOXO3a expression plasmid was cotransfected into HeLa cells with either an empty vector (EV) or ST and ST/m3 expression plasmids. Cells were grown in serum and then either lysed (-) or treated with 20μM each of LY and AKT-I in the absence of serum for 30 min (+) prior to lysis. Whole cell lysates were immunoblotted (IB) with the indicated antibodies.

Figure S2. ST does not promote an interaction between AKT isoforms and FOXO3a. HeLa cells were transfected with the indicated expression plasmids followed by immunoprecipitation (IP) of either FLAG-FOXO3a or FLAG-SKP2 from cell lysates using anti-FLAG(M2). Immunocomplexes were immunoblotted (IB) with the indicated antibodies. IB for 14-3-3 served as a positive control for FOXO3a Co-IP.

Figure S3. B56ε is not essential for dephosphorylation of FOXO3a at either AKT or CK1 sites. ***A.*** HeLa cells transfected with a FLAG-FOXO3a expression plasmid were grown in serum and either lysed (0 min) or treated with 20μM each of LY and AKT-I in the absence of serum for the indicated time points prior to lysis. FLAG-FOXO3a was immunoprecipitated (IP) from lysates and immunocomplexes were immunoblotted (IB) with the indicated antibodies. ***B.*** HeLa cells stably expressing either shGFP or shB56ε were transiently transfected with the indicated expression plasmids. FLAG-FOXO3a was immunoprecipitated (IP) from cell lysates and immunocomplexes were immunoblotted (IB) with the indicated antibodies. ***C.*** HeLa cells stably expressing either shGFP or shB56ε were transfected with a FLAG-FOXO3a expression plasmid and either lysed (-) or treated with 20μM each of LY and AKT-I in the absence of serum for 30 min (+) prior to lysis. Whole cell lysates were immunoblotted (IB) with the indicated antibodies. The IB for B56δ served as control for the specificity of B56ε knockdown.

Figure S4. ST is unable to prevent nuclear translocation of either FOXO1 or FOXO4 following inhibition of PI3K/AKT signaling. ***A.*** HeLa cells were cotransfected with a FLAG-FOXO1-TM expression plasmid and either an empty vector (EV) or ST expression plasmid. Cells grown in serum were fractionated and cytoplasmic (CE) and nuclear (NE) extracts were immunoblotted (IB) with the indicated antibodies. IB with anti-IκBα and -Sp1 antibodies was performed to evaluate the purity of the extracts. ***B.*** HeLa cells were cotransfected with a wild-type FLAG-FOXO1 expression plasmid and either an empty vector (EV) or ST expression plasmid. Cells grown in serum were either fractionated (-) or treated with 20μM each of LY and AKT-I for 30 min in the absence of serum (+) prior to fractionation. Cytoplasmic (CE) and nuclear (NE) extracts were immunoblotted (IB) with the anti-IκBα and -Sp1 antibodies to evaluate the purity of the extracts. ***C.*** Nuclear translocation of FOXO4 was analyzed as described in (***B***).

Figure S5. Foxo3a is essential for Bim expression in BaF3 cells following inhibition of PI3K/AKT signaling. BaF3 cells stably expressing either shGFP or shFoxo3a were cultured in the presence of IL-3 and then either lysed (-) or treated with 20μM each of LY and AKT-I in the absence of IL-3 for 7h (+) prior to lysis. Whole cell lysates were immunoblotted (IB) with the indicated antibodies.

Figure S1

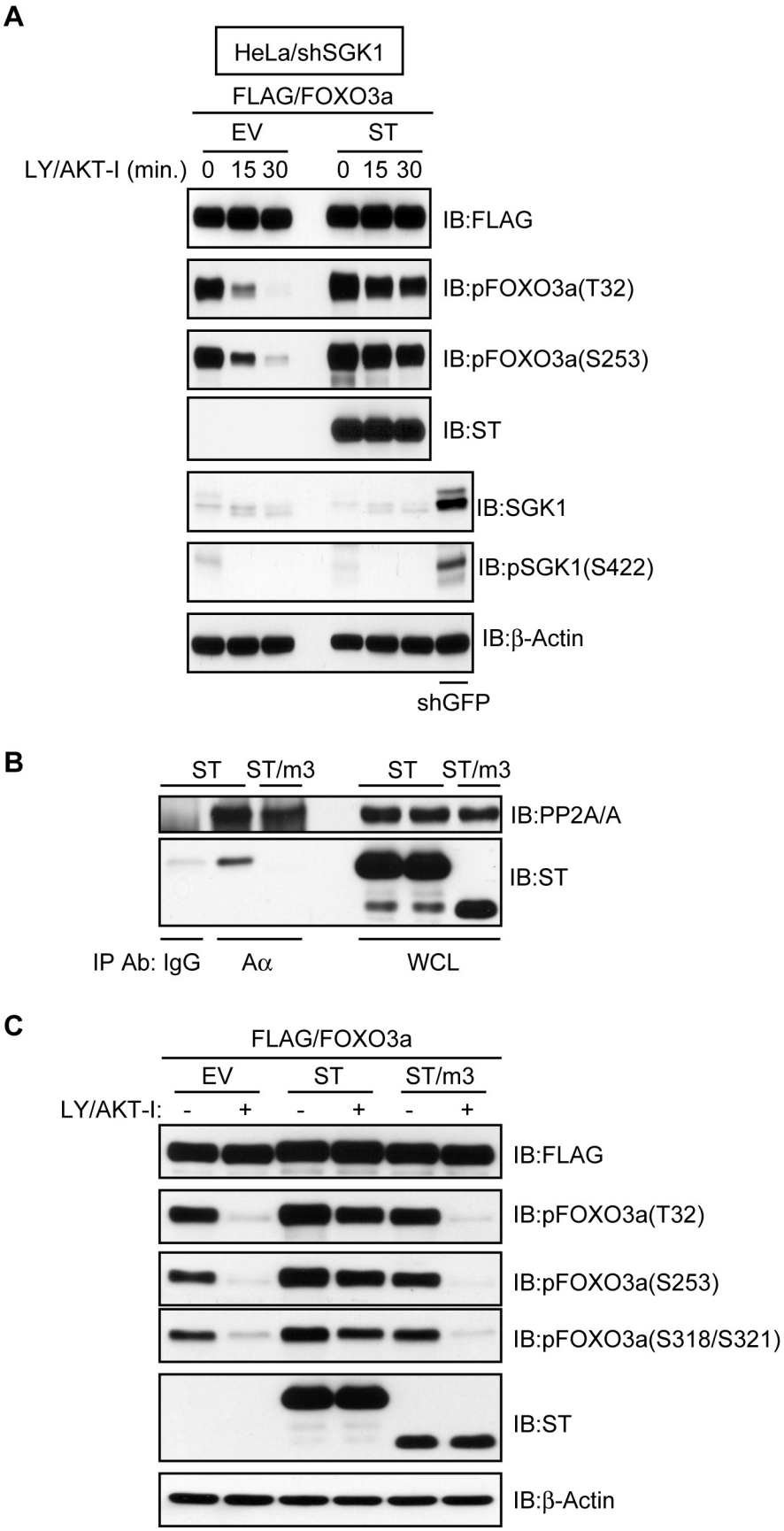
A
HeLa/shSGK1
FLAG/FOXO3a
EV
ST
LY/AKT-I (min.) 0 15 30 0 15 30
IB:FLAG
IB:pFOXO3a(T32)
IB:pFOXO3a(S253)
IB:ST
IB:SGK1
IB:pSGK1(S422)
IB:β-Actin
shGFP
B
ST ST/m3 ST ST/m3
IB:PP2A/A
IB:ST
IP Ab: IgG Aα WCL
C
FLAG/FOXO3a
EV ST ST/m3
LY/AKT-I: - + - + - +
IB:FLAG
IB:pFOXO3a(T32)
IB:pFOXO3a(S253)
IB:pFOXO3a(S318/S321)
IB:ST
IB:β-Actin

Figure S2

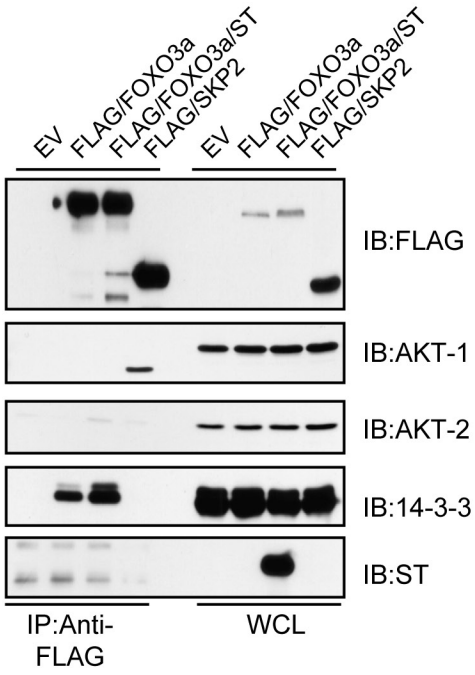
EV
FLAG/FOXO3a
FLAG/FOXO3a/ST
FLAG/SKP2
EV
FLAG/FOXO3a
FLAG/FOXO3a/ST
FLAG/SKP2
IB:FLAG
IB:AKT-1
IB:AKT-2
IB:14-3-3
IB:ST
IP:Anti-FLAG
WCL

Figure S3

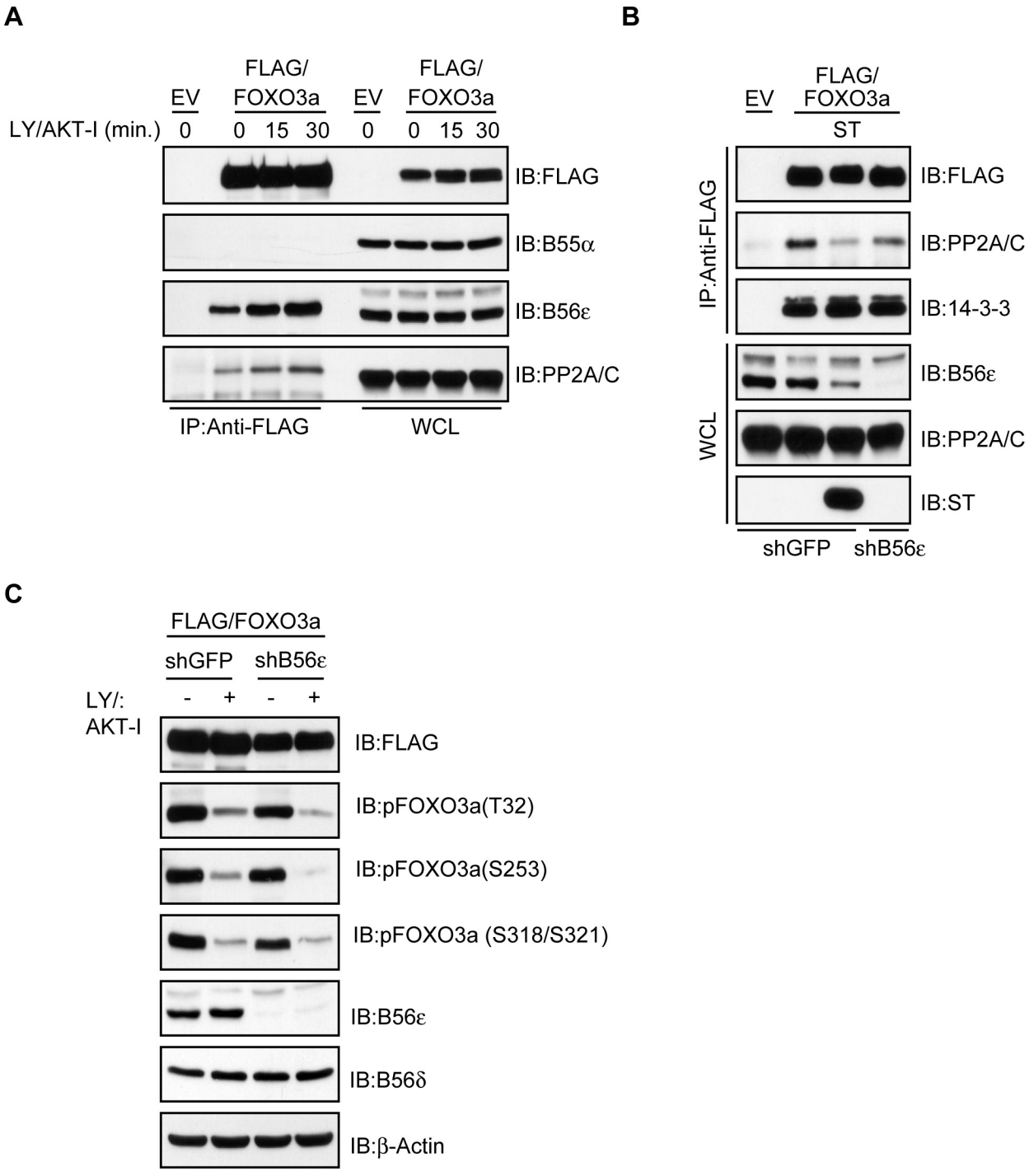
A
EV
FLAG/ FOXO3a
EV
FLAG/ FOXO3a
LY/AKT-I (min.)
0
0 15 30
0
0 15 30
IB:FLAG
IB:B55α
IB:B56ε
IB:PP2A/C
IP:Anti-FLAG
WCL
B
EV
FLAG/ FOXO3a
ST
IP:Anti-FLAG
IB:FLAG
IB:PP2A/C
IB:14-3-3
WCL
IB:B56ε
IB:PP2A/C
IB:ST
shGFP
shB56ε
C
FLAG/FOXO3a
shGFP
shB56ε
LY/: AKT-I
- + - +
IB:FLAG
IB:pFOXO3a(T32)
IB:pFOXO3a(S253)
IB:pFOXO3a (S318/S321)
IB:B56ε
IB:B56δ
IB:β-Actin

Figure S4

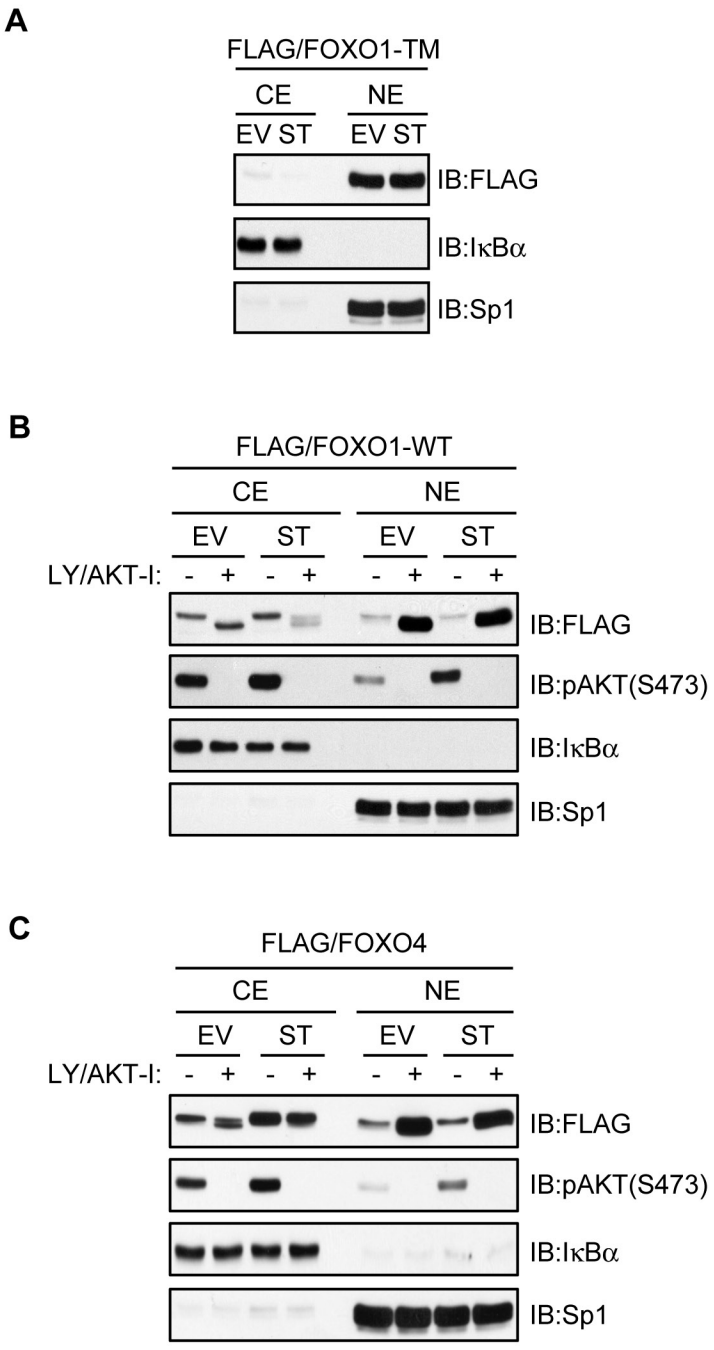
A
FLAG/FOXO1-TM
CE
NE
EV ST
EV ST
IB:FLAG
IB:IκBα
IB:Sp1
B
FLAG/FOXO1-WT
CE
NE
EV
ST
EV
ST
LY/AKT-I: - + - + - + - +
IB:FLAG
IB:pAKT(S473)
IB:IκBα
IB:Sp1
C
FLAG/FOXO4
CE
NE
EV
ST
EV
ST
LY/AKT-I: - + - + - + - +
IB:FLAG
IB:pAKT(S473)
IB:IκBα
IB:Sp1

Figure S5