Supplemental Figures and Movies

(Gross and Rotwein JOCES/2015/168773)

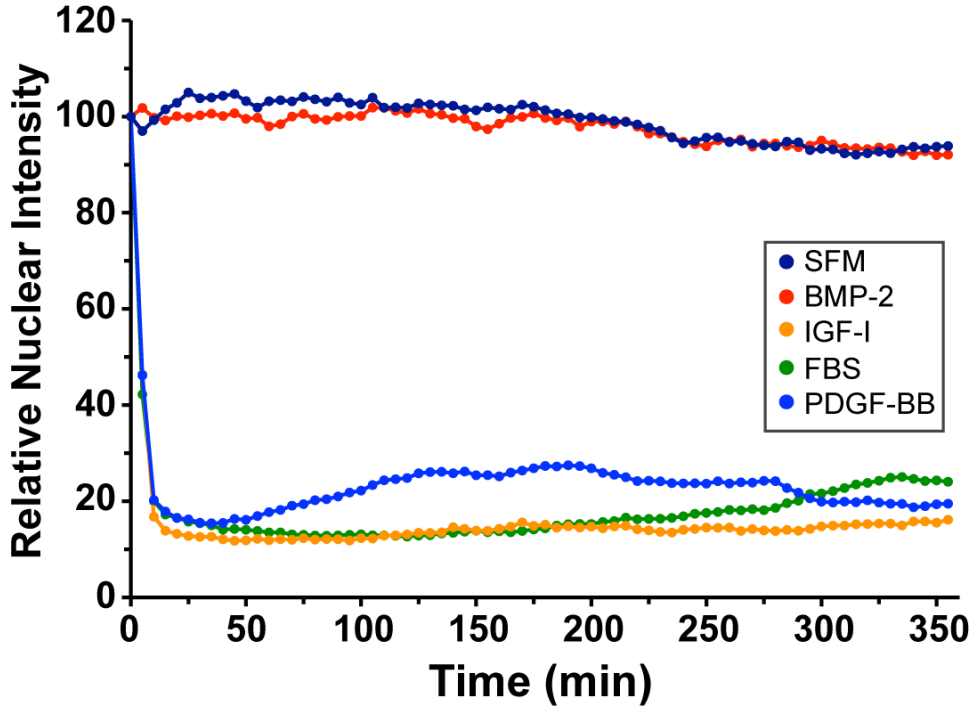


Figure S1 (connects to Figure 2). Reporter dynamics after exposure of 10T1/2 cells to different growth factors. Time course of relative nuclear intensity of the FoxO1-clover reporter in cells incubated in SFM and then exposed to SFM, BMP-2 [15 nM], R3-IGF-I [1 nM], 10% FBS, or PDGF-BB [206 pM] for 300 min. Population averages are presented (n = 50 cells per incubation). The nuclear intensity of the reporter in each cell was normalized to its value at the start of imaging during incubation in SFM.

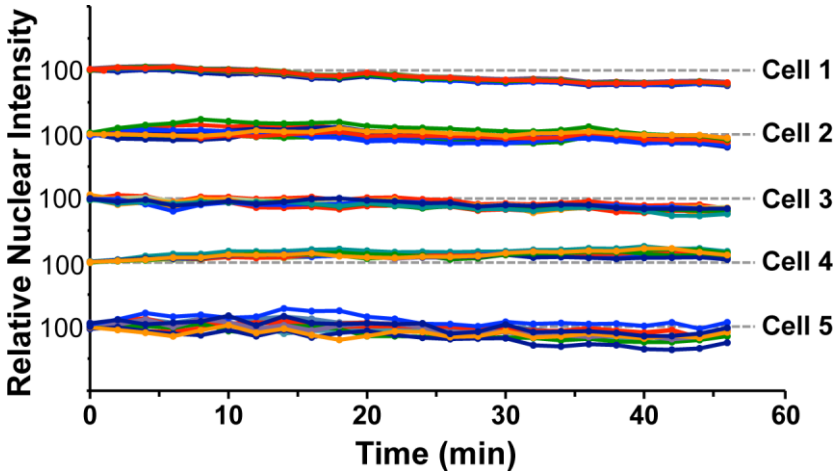


Figure S2 (connects to Figure 3). Repeated cell quantification shows minimal variation. Tracings are depicted for each of 5 individual cells that were incubated in serum-free medium for 60 min and each tracked 10 times. The average deviation from the mean for all tracings was ± 3%.

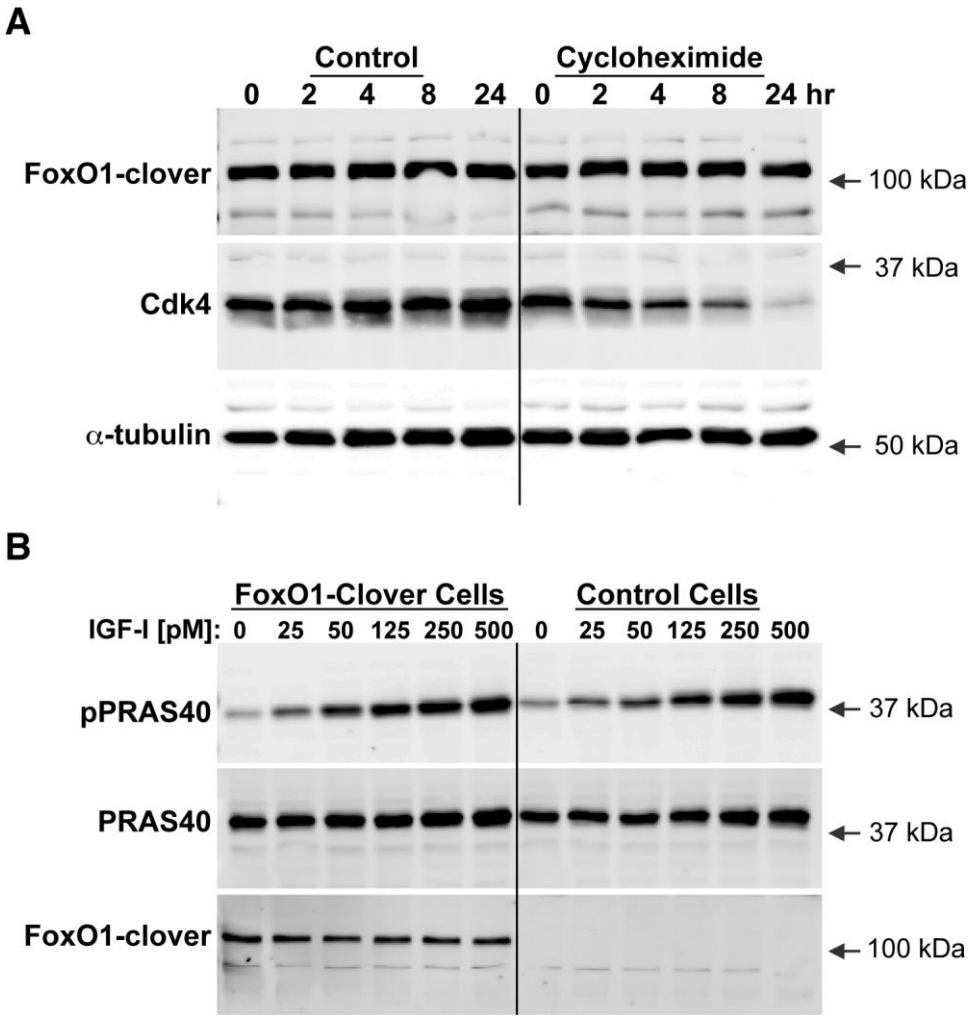


Figure S3. Stability of the FoxO1-clover reporter and lack of inhibition by the reporter of other Akt signaling pathways. **A**. Expression of FoxO1-clover, Cdk4, and α-tubulin by immunoblotting using whole cell protein lysates isolated after incubation of cells with vehicle or the protein synthesis inhibitor, cycloheximide, for 0 to 24 hr. Molecular mass markers are indicated to the right of each immunoblot. **B**. Changes in levels of phosphorylation of the Akt substrate, PRAS40, after exposure of cells stably expressing FoxO1-clover or controls to different concentrations of R3-IGF-I for 60 min. Results are presented as immunoblots for phosphorylated (p) PRAS40, total PRAS40, or FoxO1-clover using whole cell lysates. Molecular mass markers are indicated to the right of each immunoblot.

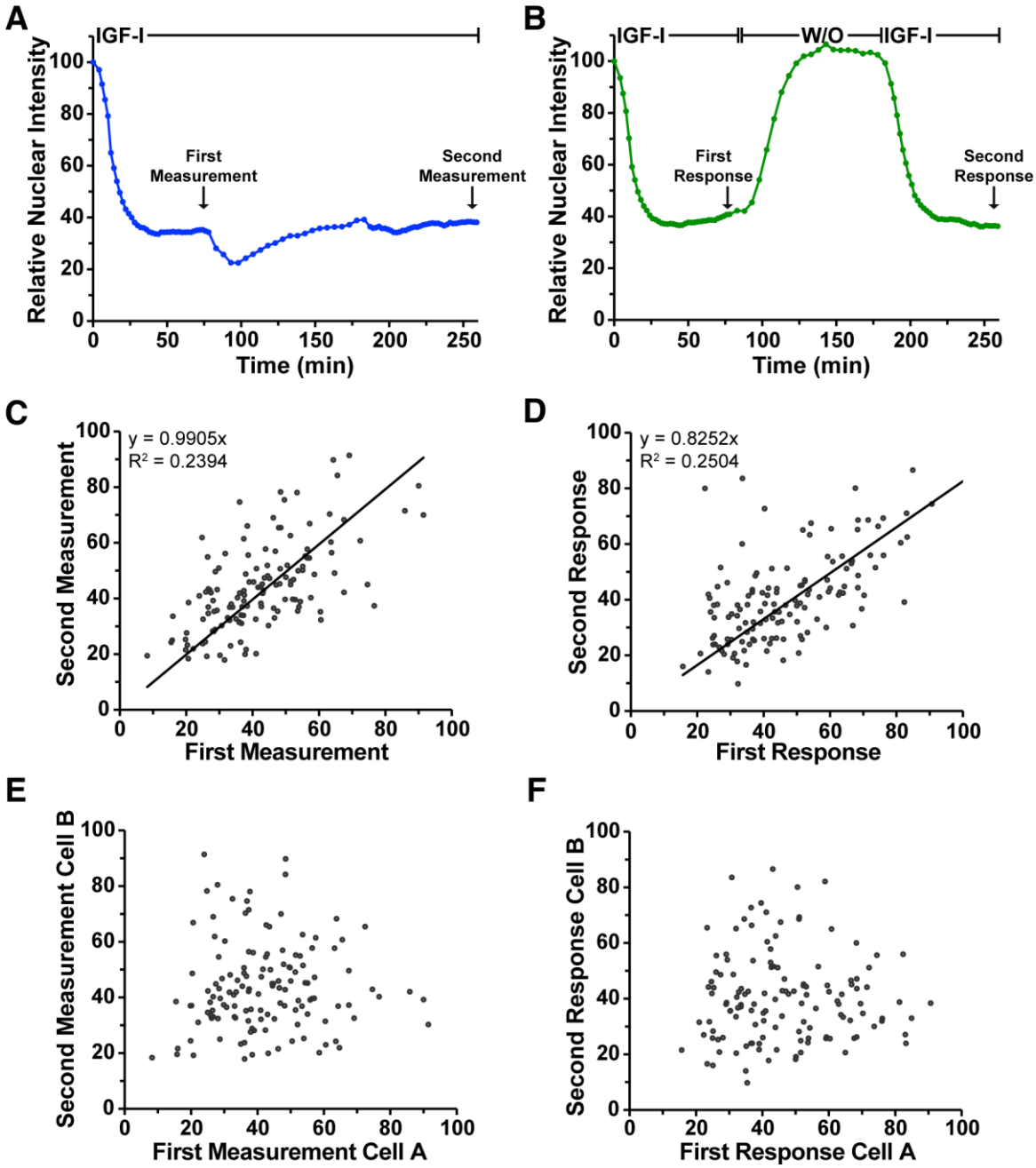
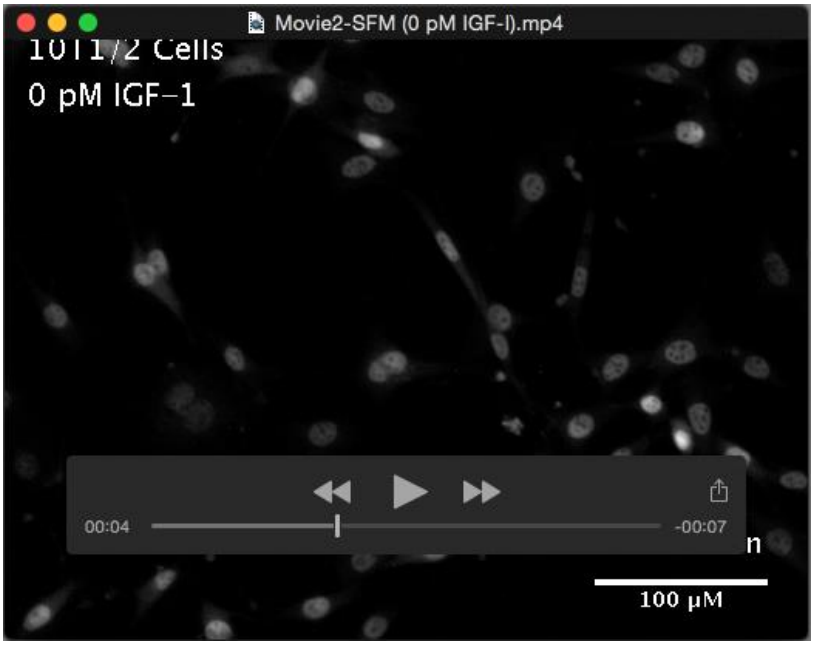


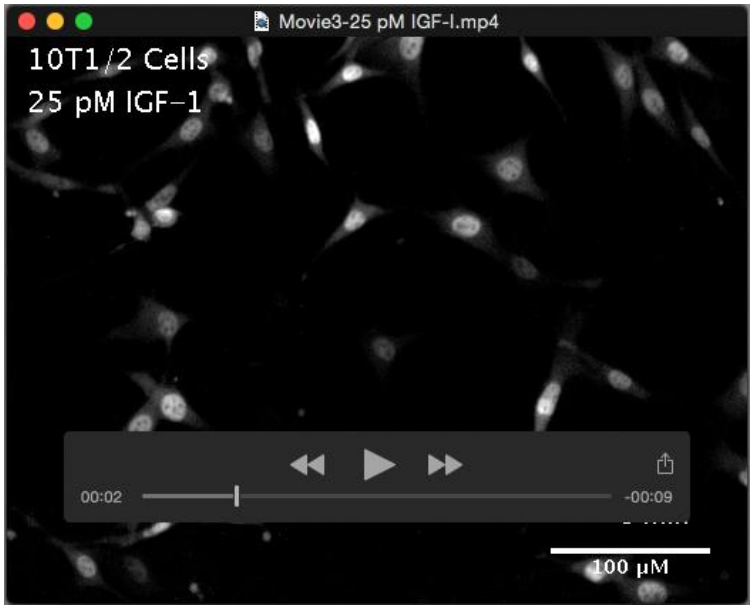
Figure S4 (connects to Figure 6). Repeated exposure to IGF-I reveals heterogeneous effects on individual cells. **A**. Time course of relative nuclear intensity of the FoxO1-clover reporter in 10T1/2 cells incubated from time 0 with R3-IGF-I [250 pM]. Population averages are presented (n = 50 cells per incubation), and the measurement times for the data in the graphs in **C** and **E** are indicated by the arrows. **B**. Time course of relative nuclear intensity of the FoxO1-clover reporter incubated sequentially with R3-IGF-I [500 pM], SFM, and R3-IGF-I. Population averages are presented (n = 50 cells per incubation). Data collection times for the graphs in **D** and **F** are labeled by arrows. **C**. Plot showing the relationship of reporter activity for the same cell at different times during sustained incubation with R3-IGF-I. The

line of best fit is depicted, and the slope and correlation coefficient are indicated (n = 150 cells). **D**. Graph showing the relationship of reporter activity for the same cell at different times during sequential incubation with R3-IGF-I. The line of best fit is depicted, and the slope and correlation coefficient are indicated (n = 150 cells). **E**. Plot showing the lack of relationship of reporter activity in different cells at different times during sustained incubation with R3-IGF-I (n = 150 cells). **F**. Graph showing the lack of relationship of reporter activity in different cells at different times during sequential incubation with R3-IGF-I (n = 150 cells).

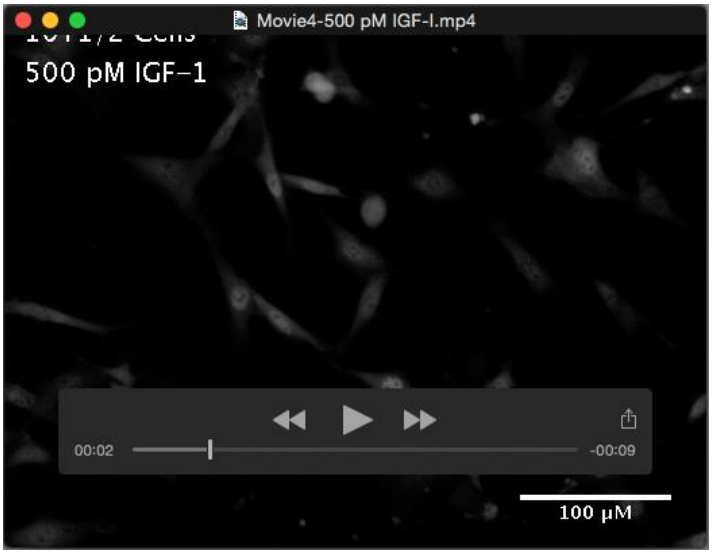
Movie 1 (connects to Figure 2). Subcellular localization of the FoxoO1-clover reporter in 10T1/2 cells during incubation in 10% FBS followed by serum-free-medium. Cells were incubated in 10% FBS for 1040 min after which the medium was replaced with serum-free medium for 80 min. Images were collected every 10 min by time-lapse epi-fluorescence microscopy (Evos FL Auto), and were registered and the background subtracted as described in Materials and Methods. The video playback rate is 6 frames per second.



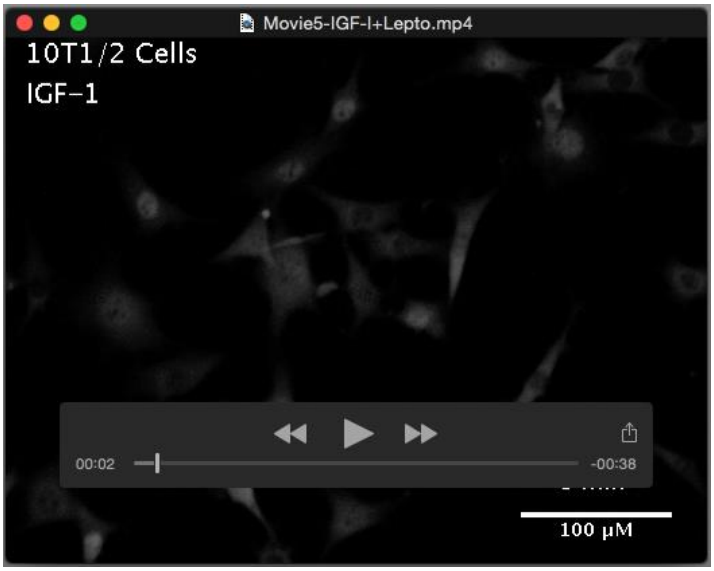
Movie 2 (connects to Figure 4). Subcellular localization of the FoxoO1-clover reporter in 10T1/2 cells during incubation in serum-free medium. Cells were incubated in serum-free medium, and images were collected every 2 min for 60 min by time-lapse epi-fluorescence microscopy (Evos FL Auto). Images were registered and the background was subtracted as described in Materials and Methods. The video playback rate is 3 frames per second.



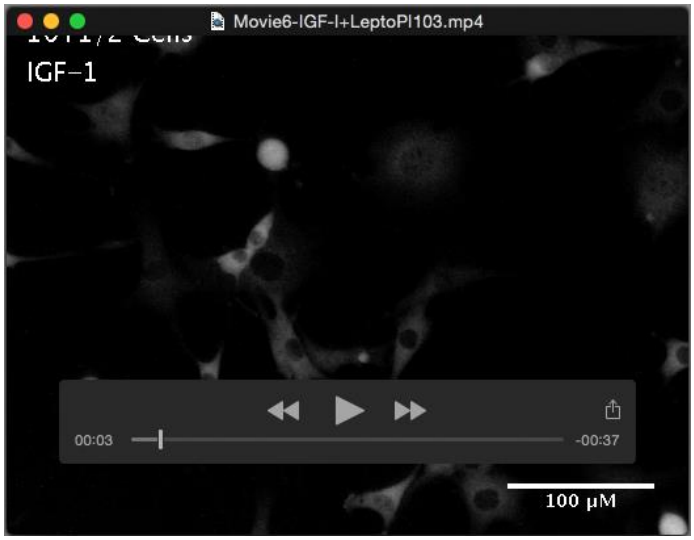
Movie 3 (connects to Figure 4). Subcellular localization of the FoxoO1-clover reporter in 10T1/2 cells during exposure to R3-IGF-I [25 pM]. Cells were incubated in serum-free medium with R3-IGF-I, and images were collected every 2 min for 60 min by time-lapse epi-fluorescence microscopy (Evos FL Auto). Images were registered and the background was subtracted as described in Materials and Methods. The video playback rate is 3 frames per second.



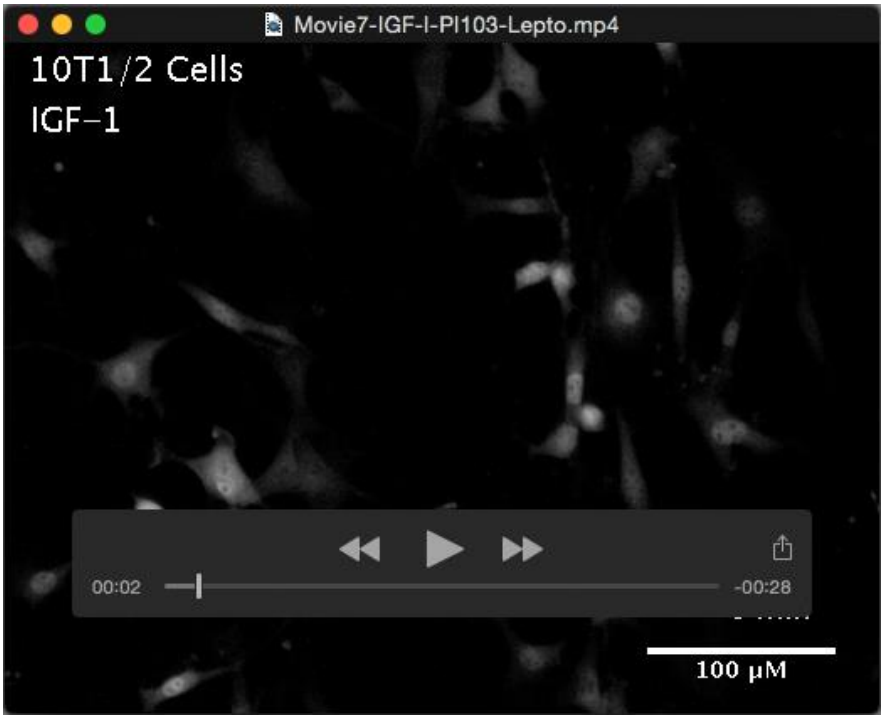
Movie 4 (connects to Figure 4). Subcellular localization of the FoxoO1-clover reporter in 10T1/2 cells during exposure to R3-IGF-I [500 pM]. Cells were incubated in serum-free medium with R3-IGF-I, and images were collected every 2 min for 60 min by time-lapse epi-fluorescence microscopy (Evos FL Auto). Images were registered and the background was subtracted as described in Materials and Methods. The video playback rate is 3 frames per second.



Movie 5 (connects to Figure 7). Subcellular localization of the FoxoO1-clover reporter in 10T1/2 cells during sequential treatment with R3-IGF-I and leptomycin B. Cells were incubated in serum-free medium with R3-IGF-I [250 pM] for 60 min and leptomycin B [100 nM] was added for 180 min. Images were collected every 2 min by time-lapse epi-fluorescence microscopy (Evos FL Auto). Images were registered and the background was subtracted as described in Materials and Methods. The video playback rate is 3 frames per second.



Movie 6 (connects to Figure 7). Subcellular localization of the FoxoO1-clover reporter in 10T1/2 cells during treatment with R3-IGF-I followed by addition of both PI103 and leptomycin B. Cells were incubated in serum-free medium with R3-IGF-I [250 pM] for 60 min and PI103 [500 nM] and leptomycin B [100 nM] were added together for 180 min. Images were collected every 2 min by time-lapse epi-fluorescence microscopy (Evos FL Auto). Images were registered and the background was subtracted as described in Materials and Methods. The video playback rate is 3 frames per second.



Movie 7 (connects to Figure 8). Subcellular localization of the FoxoO1-clover reporter in 10T1/2 cells during sequential treatment with R3-IGF-I, PI103, and leptomycin B. Cells were incubated in serum-free medium with R3-IGF-I [500 pM] for 60 min. PI103 [500 nM] was added during the next 60 min, and leptomycin B [100 nM] for the final 60 min. Images were collected every 2 min by time-lapse epi-fluorescence microscopy (Evos FL Auto) for 180 min. Images were registered and the background was subtracted as described in Materials and Methods. The video playback rate is 3 frames per second.