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Simplifying Progress

Dynamic live-cell visualization and quantification of Akt activity using a genetically-encoded, fluorescent kinase translocation reporter

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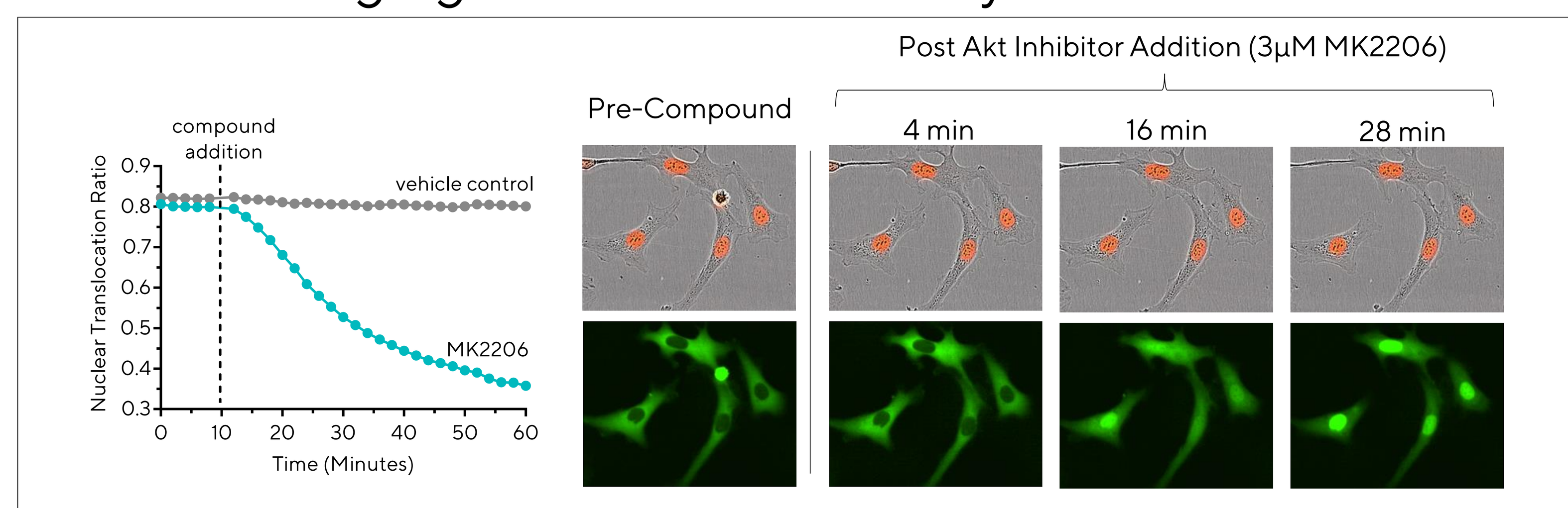
Summary and Impact

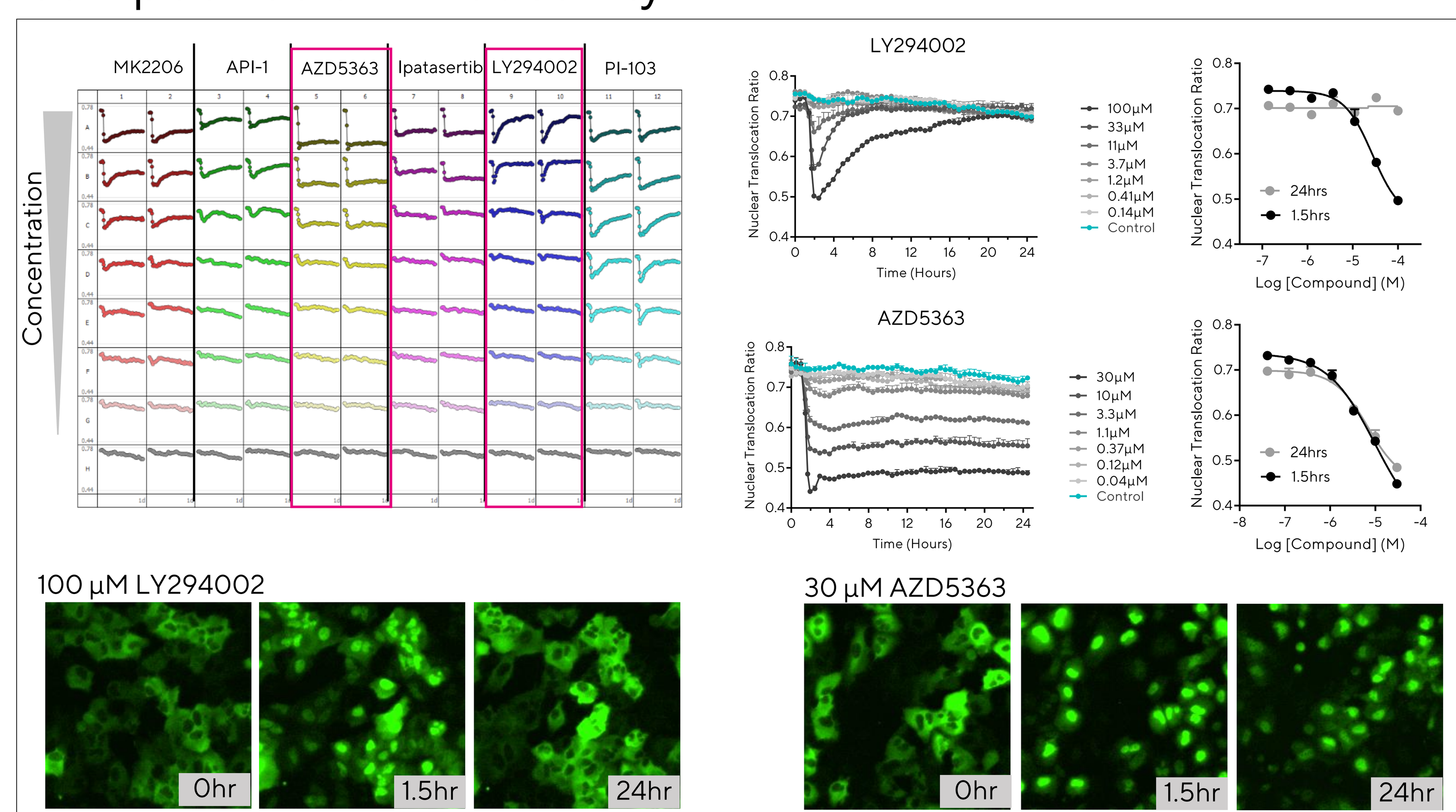
- The Incucyte® Kinase Akt Green/Red Lentivirus encodes a kinase translocation reporter based on a green fluorescent protein-tagged Akt substrate whose subcellular localization is phosphorylation-dependent, and a red fluorescent nuclear protein to denote the nuclear/cytoplasmic boundary. This enables analysis of dynamic changes in Akt kinase activity via a nuclear translocation ratio metric.
- Using this live-cell analysis approach to monitor Akt activity, we observed variable time- and concentration-dependent responses to treatment with inhibitors targeting the PI3K/Akt pathway.
- To study activation of Akt, cells were first cultured in the absence of serum to reduce Akt activity. After 4 hours of incubation in serum-free conditions, cells were treated with growth factors and monitored for variable Akt activation profiles over time.
- HeLa cells with a PTEN knockout mutation maintained high levels of Akt activity in serum free conditions, in contrast to wild type HeLa cells which exhibit a decrease in Akt activation upon removal of serum.
- Concurrent measurements of Akt activity and cell proliferation were performed in MDA-MB-231 and T-47D breast cancer cell lines. While both cell lines exhibited Akt inhibition by MK2206, differential effects on proliferation were observed.
- The Incucyte® Kinase Akt Lentivirus Reagent provides valuable kinetic measurements of Akt activity using live cells within a physiologically relevant environment.

Live-cell imaging of Akt kinase activity



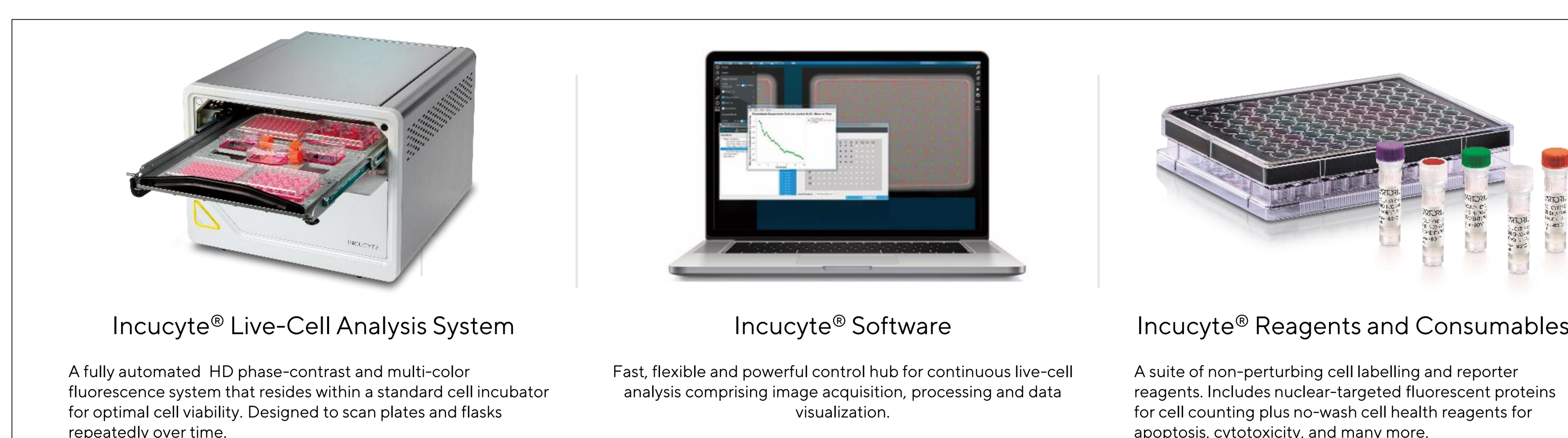
SK-OV-3 cells stably expressing the Incucyte® Kinase Akt Green/Red Indicator were treated with Akt selective inhibitor MK2206, resulting in translocation of the green fluorescent sensor from the cytoplasm to the nucleus. The kinetic graph on the left shows a decrease in the Nuclear Translocation Ratio (NTR) over time, indicating Akt inhibition. The image panel shows the phase and red fluorescence image channels on the top and the green fluorescence channel on the bottom. Movement of the green fluorescent sensor from the cytoplasm to the nucleus can be seen over 28 minutes, while localization of the red fluorescent nuclear marker does not change.

Concentration and time- dependent effects of compounds on Akt activity



A549 cells stably expressing the Incucyte® Kinase Akt Green/Red Indicator were treated with inhibitors targeting the PI3K/Akt kinase pathway, including allosteric Akt inhibitors MK2206 and API-1, competitive Akt inhibitors AZD5363 and Ipatasertib, and upstream PI3K kinase inhibitors LY294002 and PI-103. The 96-well microplate graph (left) shows time- and concentration-dependent decreases in NTR for all compounds tested, indicating Akt inhibition. Evaluation of the NTR over 24 hr for AZD5363 and LY294002 (middle) revealed varying kinetic profiles. Addition of AZD5363 caused sustained inhibition of Akt over 24 hr, while PI3K inhibitor LY294002 caused a transient decrease in NTR followed by a recovery to baseline, indicating reactivation of Akt. IC_{50} curves (right) depict 1.5 h and 24 h time point data. Green fluorescent images are shown for 100 μM LY294002 and 30 μM AZD5363 at 0, 1.5, and 24 h timepoints (bottom).

Incucyte® Live-Cell Imaging and Analysis Solutions



Incucyte® Live-Cell Analysis System

A fully automated HD phase-contrast and multi-color fluorescence system that resides within a standard cell incubator for optimal cell viability. Designed to scan plates and flasks repeatedly over time.

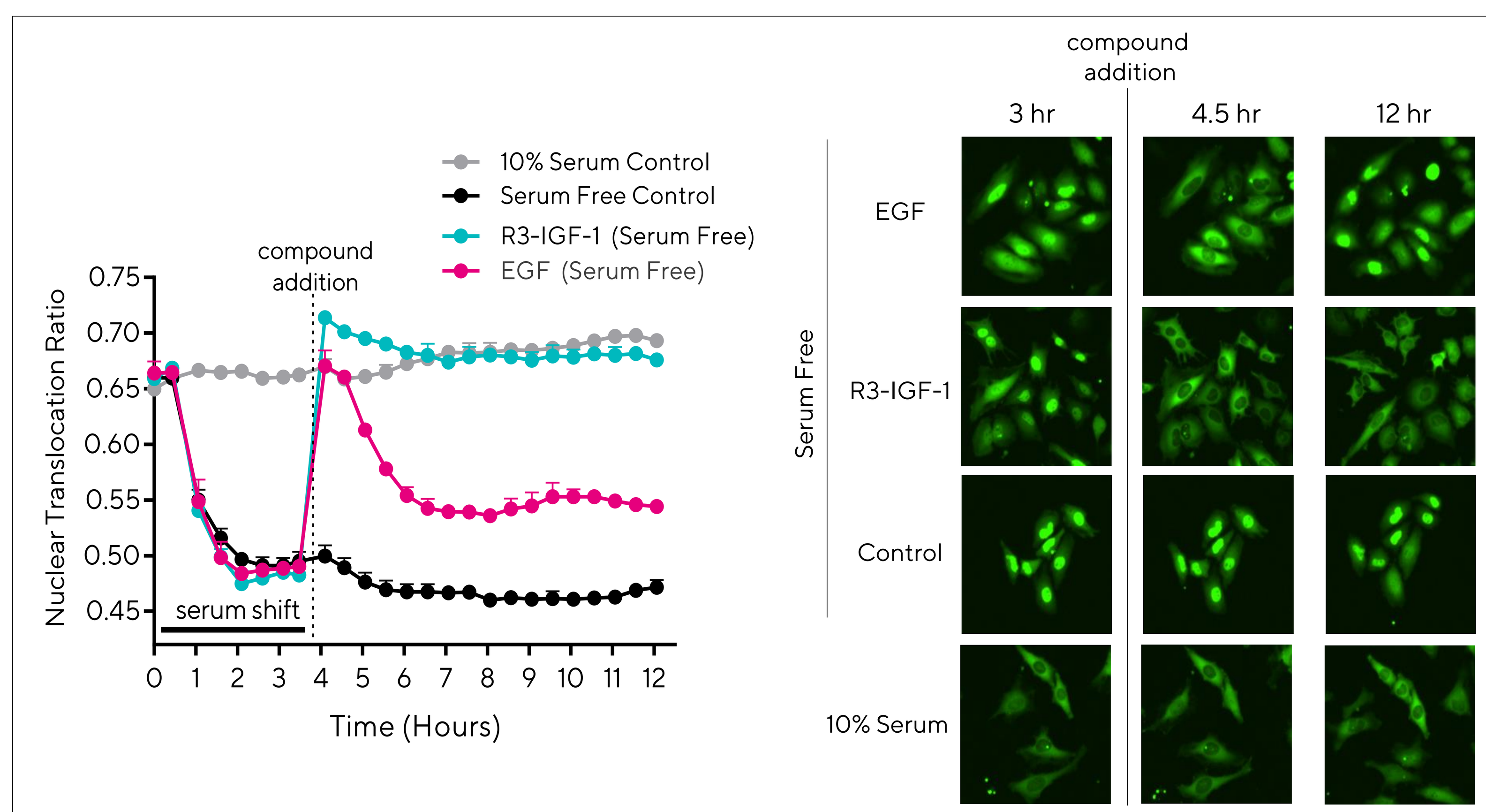
Incucyte® Software

Fast, flexible and powerful control hub for continuous live-cell analysis comprising image acquisition, processing and data visualization.

Incucyte® Reagents and Consumables

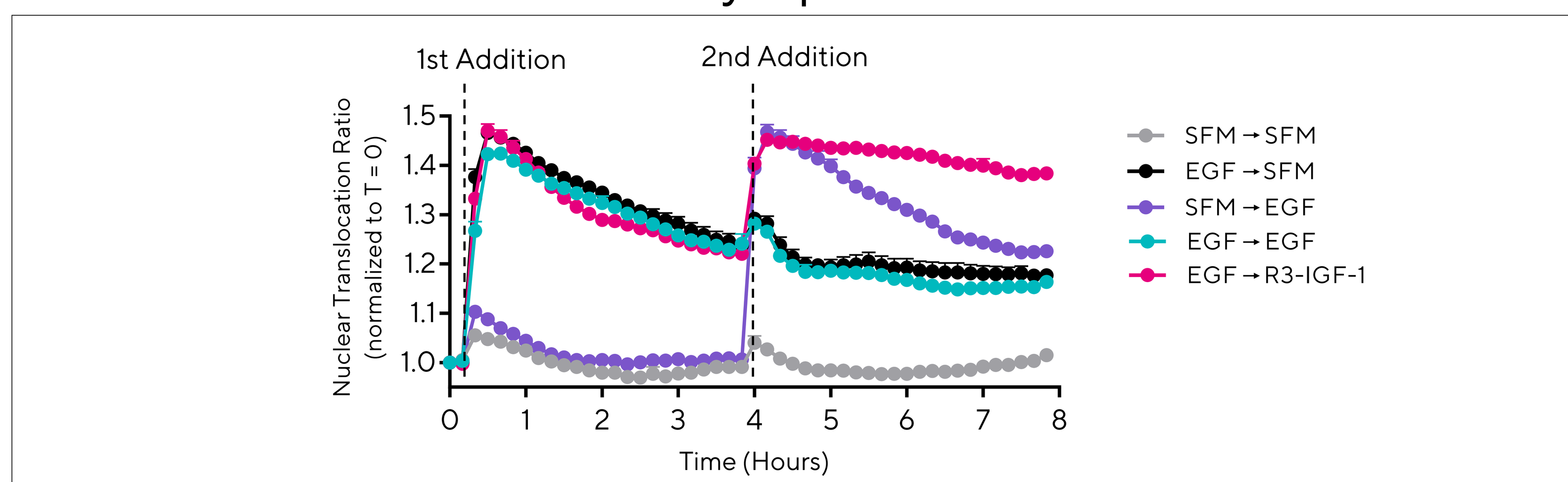
A suite of non-perturbing cell labelling and reporter reagents. Includes nuclear-targeted fluorescent proteins for cell counting plus no-wash cell health reagents for apoptosis, cytotoxicity, and many more.

Effects of serum starvation and Akt activation over time



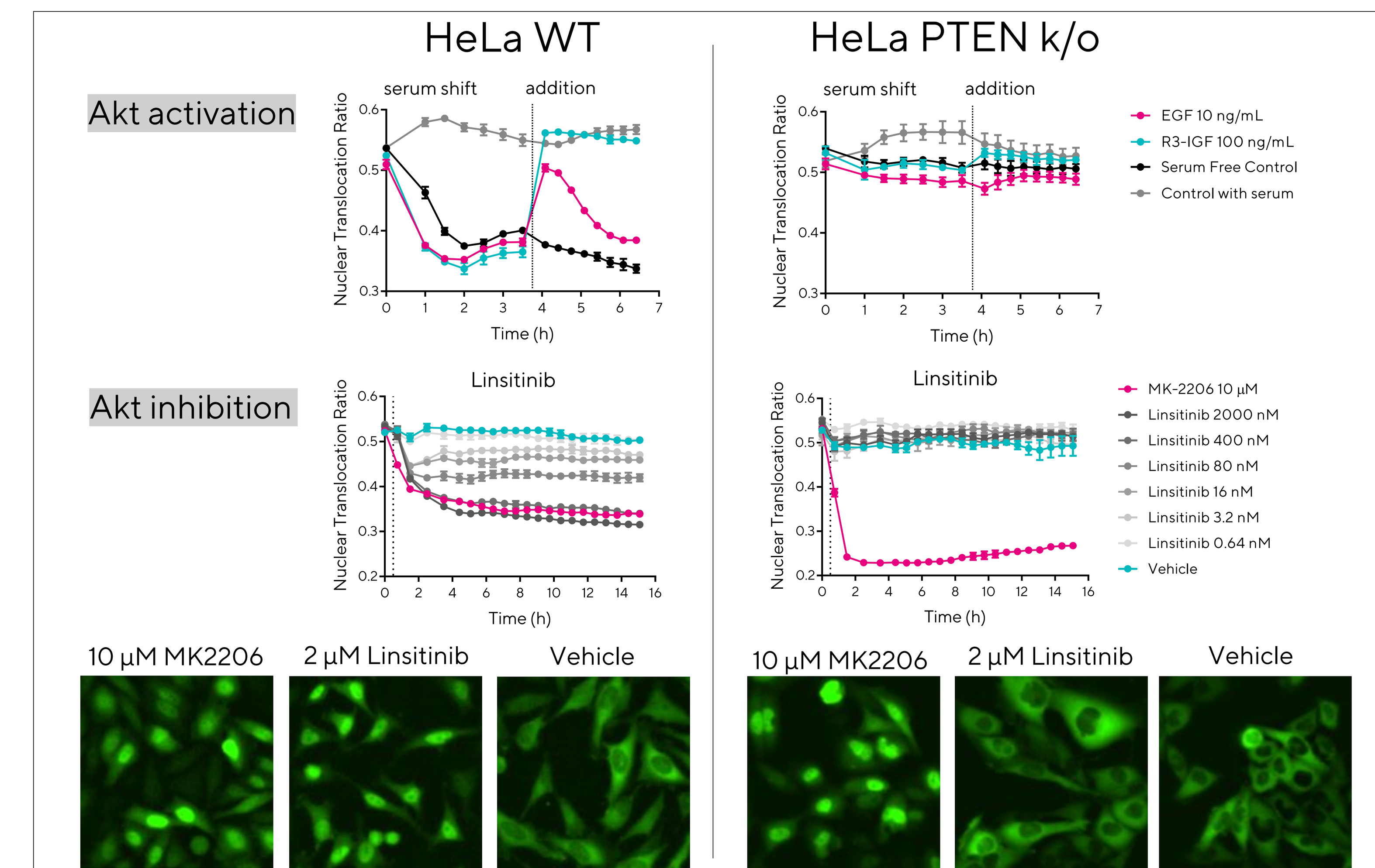
HeLa cells stably expressing the Incucyte® Kinase Akt Green/Red Indicator were shifted into media containing no serum to remove various growth factors known to activate the PI3K/Akt kinase pathway. The serum shift caused a decrease in the NTR, indicating a decrease in Akt activity, while no change was observed in control wells maintained in 10% serum. After 4 hr, cells were treated with either 1.1 ng/mL epidermal growth factor (EGF) or 3.3 nM recombinant insulin-like growth factor (R3-IGF-1). Both compounds induced activation of Akt, as demonstrated by a rapid increase in the NTR. R3-IGF-1 addition resulted in sustained activation over the 12-hour time course, while activation by EGF diminished over time. Representative images from all conditions are shown. Prior to compound addition, the sensor is localized to the nucleus of cells in serum free media and the cytoplasm of control cells in 10% serum (3 hr). Upon activation by EGF or R3-IGF-1, the sensor translocates from the nucleus to the cytoplasm (4.5 hr). The sensor returns to the nucleus as Akt activity decreases over time in EGF-treated cells, while it remains in the cytoplasm of R3-IGF-1 treated cells (12 hr).

Kinetics of Akt activation by Epidermal Growth Factor



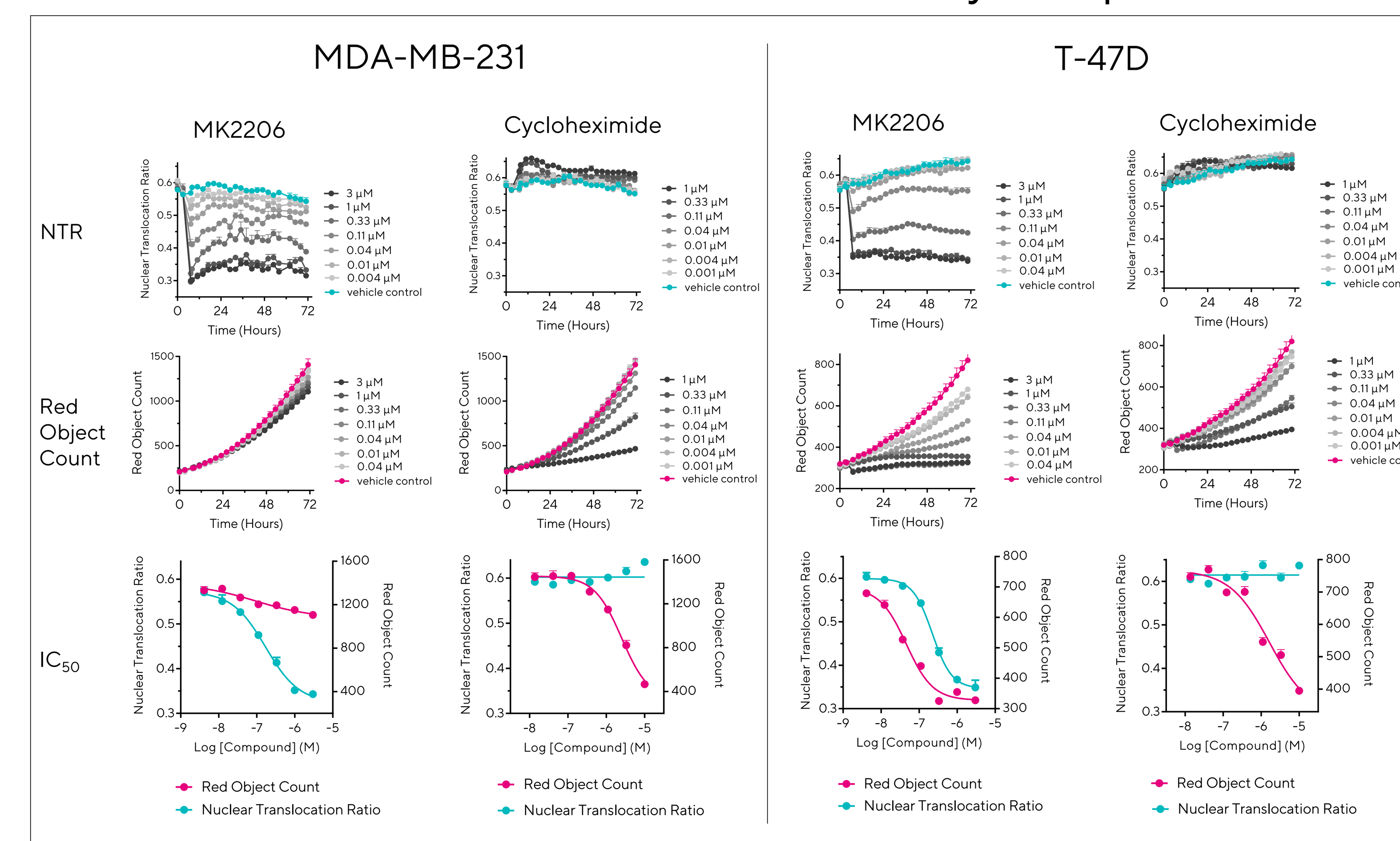
MDA-MB-231 cells stably expressing the Incucyte® Kinase Akt Green/Red Indicator were serum starved for 3 hr to inactivate Akt. Cells were then treated with 10 ng/mL EGF or serum free medium control (SFM, 1st addition). Four hours later, the same cells received a second addition of 10 ng/mL EGF, 30 nM R3-IGF-1, or SFM control (2nd addition). EGF treatment caused a rapid increase in Akt activity followed by a slow decrease over 4 h. The response to a second addition of EGF (EGF – EGF) was indistinguishable from cells receiving EGF followed by SFM control (EGF – SFM). However, in cells previously treated with EGF, addition of R3-IGF-1 (EGF – R3-IGF-1) induced rapid and sustained activation of Akt. As a control, addition of EGF at 4 hr to cells initially treated with SFM (SFM - EGF) produced a rapid but transient activation of Akt equivalent to cells treated with EGF at 0 hr. These data indicate the transient activation of EGF is due to a biological feedback process and not degradation of EGF.

Monitoring Akt activity in PTEN knockout mutant cells



HeLa PTEN knockout and wild type (WT) cell lines obtained from Abcam (knockout line ab255419; WT line ab255928) were stably transduced with the Incucyte® Kinase Akt Green/Red Lentivirus. Both cell lines were serum starved for 4 hr and then treated with 10 ng/mL EGF, 100 ng/mL R3-IGF, serum free control, or 10% serum control (top). In the wild type cells, Akt activity was reduced upon serum starvation and rapidly increased in response to EGF or R3-IGF addition, while Akt activity in the PTEN mutant cells was minimally affected by both serum starvation and the addition of growth factors. Both PTEN k/o and WT stable lines were then treated with the IGF1 receptor inhibitor Linsitinib in the presence of 10% serum which caused concentration dependent inhibition of Akt in the WT cells but no reduction of Akt activity in the PTEN mutant cells (middle). The Akt inhibitor MK-2206 (10 μM) was added as a positive control which reduced Akt activity significantly in both cell lines. Representative images from wells treated with 10 μM MK2206, 2μM Linsitinib, and vehicle control at 12 hr post addition are shown below.

Concurrent measurements of Akt activity and proliferation



NTR as a measure of Akt activity and Red Object Count as a measure of cell proliferation were monitored concurrently in MDA-MB-231 and T-47D cells expressing the Incucyte® Kinase Akt Green/Red Indicator. Kinetic graphs of NTR (top row) demonstrate concentration-dependent inhibition of Akt by the selective Akt inhibitor MK2206 in both cell lines. Measurements of Red Object Count from the same cells (middle row) reveal differential effects of MK2206 on proliferation between the two cell lines, with concentration-dependent inhibition in T-47D cells but little effect in MDA-MB-231 cells. IC_{50} graphs of Red Object Count (24 h) and Nuclear Translocation Ratio (72 h) are shown (bottom row). The IC_{50} for Akt inhibition by MK2206 was similar in both cell types, while the IC_{50} for proliferation was significantly left shifted in T-47D cells compared to MDA-MB-231 cells. The protein synthesis inhibitor Cycloheximide inhibited cell proliferation without affecting Akt activity in both cell lines.