Supplementary Materials and Methods

Detection of insulin-stimulated AKT phosphorylation

Dermal fibroblasts in exponential phase were serum starved overnight followed by insulin treatment (100 nM, Actrapid, Novo Nordisk) and then harvested at the indicated times. Cells were washed with ice-cold PBS and harvested in M-PER Mammalian Protein Extraction reagent (Thermo Scientific) containing protease inhibitor mini complete cocktail (Roche) at a 1:7 ratio. Proteins were mixed with an equal volume of Laemmli sample buffer (#161-0737, Bio-Rad), denatured at 95^{o}C and resolved by SDS-PAGE before transfer to polyvinylidene-fluoride membranes using the iBlot system (Invitrogen). Blots were blocked with 5% BSA in TBST (50 mM Tris-HCl, pH7.6, 150 mM NaCl, 0.1% Tween-20) and probed overnight at 4^{o}C with anti-phospho-AKT(Thr308) (9275, Cell Signaling) or anti-AKT (9272, Cell Signaling) antibody. Bound primary antibody was detected by horseradish peroxidase conjugated secondary antibodies, followed by Immobilon^{TM} Western chemiluminescent HRP substrate (WBKLS0500, Millipore).

Metaphase spread

Cells were grown to approximately 40% confluence and then treated for 4 hours with colcemid (D1925, Sigma) to a final concentration of 1 μg/ml to induce a metaphase arrest. Medium was removed and cells washed with PBS, trypsinized (Trypsin/EDTA, T3924, Sigma) and then pelleted (5 min at 1000 rpm). Cell pellets were re-suspended in 75 mM KCl (pre-warmed to 37^{o}C) and hypotonically swollen at 37^{o}C for 30 min with intermittent inversion of tubes. Cells were pelleted at 1000 rpm for 5 min and re-suspended in residual KCl and then fixed by dropwise adding of fixative (1:3 glacial acetic acid and methanol) with gentle agitation. After adding another 500ul of fixative cells were dropped onto slides to obtain metaphase spreads. Slides were air-dried overnight, stained with Leishman's stain (L6254-25G, Sigma) and photographed and analysed using CytoVision^{TM} software (CytoVision Genus Version 4.5.4, Genetix).

Flow cytometry

To determine DNA content, asynchronously growing cells were harvested, pelleted by centrifugation (5 min at 1000rpm) and washed in PBS before re-suspension in hypotonic lysis buffer (0.05mg/ml propidium iodide, 1 mg/ml sodium citrate and 0.1% Triton X-100, 100 μg/ml RNase A). Cells were incubated in the dark at 4^{o}C for a minimum of 30 min. Cells were then analysed with flow cytometry (BD FACSCalibur), using BD CellQuest^{TM} Pro (Version 6.0, BD Biosciences).

BrdU incorporation assay

For BrdU labelling, cells on coverslips were incubated in culture medium containing 10 μM BrdU (B5002, Sigma) for 7 hours. Cells on coverslips were then washed with PBS, fixed in 4% paraformaldehyde in PBS, washed twice in PBS, permeabilized in 0.2% Triton X-100 for 5 min, and washed with PBS. DNA was denatured by incubation in 2 M HCl for 1 h followed by three washes with PBS. Coverslips were incubated for 1 h with fluorescein-conjugated mouse anti-BrdU monoclonal antibody (1:20 dilution; MAB3262F, Millipore) and mounted using the ProLong Gold Antifade Reagent with DAPI.