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following each treatment, the cells were incubated with 105 /ml calcein-am (keygen calcein-am staining kit, china) for 30 min at 37, then washed three times with pbs. the cells were then viewed and photographed under a laser scanning confocal microscope (fv1000, olympus, japan). the cells were incubated with annexin v (keygen annexin v-fluorescein isothiocyanate apoptosis detection kit, china) and propidium iodide (keygen pi/annexin v-fluorescein isothiocyanate apoptosis detection kit, china) for 30 min at room temperature in the dark. then cells were washed three times with pbs and the samples were analyzed using a facscan flow cytometer (becton dickinson, franklin lakes, nj). cellquest version 3.1 software (becton dickinson) was used to analyze the data. the total percentage of apoptotic cells in the single cell assay was expressed as the sum of the numbers of early and late apoptotic cells. apoptosis was assayed by immunofluorescence staining with anti-annexin v and anti-caspase 3 antibodies. apoptotic cell death was measured by direct counting the green-stained cells (excitation at 488 nm and emission at 505 nm). green fluorescence intensity was measured using a fluorescence microscope (leica). the cells were fixed in 4% paraformaldehyde solution in pbs for 30 min, permeabilized with 0.2% triton x-100 and blocked with 10% goat serum. rabbit polyclonal antibodies to phospho-p38 (ser202/thr204), p38, phospho-extracellular signal-regulated kinase (erk)1/2 (thr202/tyr204) and total erk1/2, and mouse monoclonal antibodies to caspase-3 and β -actin (cell signaling, usa) were applied in dilution 1 : 500 at 4 $\text{\textcircled{C}}$ for 16 h. the membrane was incubated with diluted (1 : 500) secondary antibodies for 1 h at room temperature. finally, after three washes in pbst, the membrane was treated with enhanced chemiluminescence (amersham biosciences, uk). images of the blots were acquired by a high-resolution scanner. the blots were quantified by measuring the optical density of individual bands of the same width on the blots using the quantity one version 4.5.2 image analysis software (bio-rad, usa). relative levels of phosphorylated proteins were calculated as the ratio of phosphorylated protein to the total protein in the same lane. the membrane was stripped and then reprobred with anti-actin monoclonal antibody (1 : 5,000) to verify the total amount of protein.

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cell culture and immunocytochemistry {#s9e} ----- mcf10a cells were kindly provided by dr. colette e. markham (mcmaster university). monolayer cells were maintained in dmem-f12 medium supplemented with 10% fetal bovine serum, 10 µg/ml insulin, 5 µg/ml egf, 20 µg/ml gentamycin. cells were cultured at 37°c in 5% co2. for immunocytochemistry, cells were grown on glass coverslips placed in 12-well plates. the following antibodies were used: rabbit anti-ki-67 (boster bio. engineering co., china, product no: ba0312), mouse anti-e-cadherin (bd biosciences, china, product no: 610182), mouse anti-n-cadherin (bd biosciences, china, product no: 610920), rabbit anti-slug (abcam, usa, product no: ab50581), and rabbit anti-snail (abcam, usa, product no: ab156877). species-specific conjugated secondary antibody was goat anti-rabbit igg conjugated with alexa flour 488 or texas red (invitrogen) or anti-mouse igg conjugated with alexa flour 594 (invitrogen). cell nuclei were stained with hoechst 33258. the cells were washed in pbs and processed for immunocytochemistry immediately after fixation in 4% formaldehyde solution for 30 min at room temperature. the cells were then washed three times in pbs and mounted on slides using glycerol mounting medium. slides were observed using a leica dm5000 b fluorescence microscope. a novel optical fiber tip for simultaneous multiphoton microscopy and fluorescence microscopy {#s9f} ----- within

a few years many new techniques have been developed for tracking signals. most of these techniques are focused on generating thermal or acoustic vibrations and detecting them by various sensing means. these techniques include a) imaging the surface temperature elevation or acoustic emission, b) imaging the reflected light through a change in absorption, c) tracking the fluorescence signals of the ca²⁺ or rhodamine crystals, and d) tracking the evanescent waves refractive index. these above techniques to tracking signals are usually specific in nature and are limited to specific samples such as salt crystals, fluorescent dyes, or specific cells of interest. usually, the tracking signal is a specific interaction such as changing the intensity of an absorption, exciting a fluorescence, or reflecting the light and this signal is generated from the cells. 5ec8ef588b

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