Supplemental Data

Supplemental Materials and Methods

Ratio-Fluorescence Resonance Energy Transfer (FRET)

Prior to FRET experiments, cells on coverslips were mounted in bicarbonate-buffered saline (140 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$, 1 mM $CaCl_2$, 23 mM $NaHCO_3$, 10 mM glucose and 10 mM HEPES at pH 7.3) in a heated tissue culture chamber at 37°C under 5% CO_2. Cells were analyzed on an inverted Zeiss Axiovert 135 microscope equipped with a dry Achroplan 63× objective. FRET equipment was as described previously (44). CFP was excited at 432 ± 5 nm and emission of YFP was detected with a 527 nm longpass filter and CFP using a 478-527 nm bandpass filter. FRET was expressed as the ratio of YFP to CFP signals. The ratio was arbitrarily set as 1.0 at the onset of the experiment. Changes are expressed as percent deviation from this initial value of 1.0. For data acquisition, Felix software (PTI Inc.) was used. Data were plotted using proFit (QuantumSoft).

Fluorescence Recovery After Photobleaching (FRAP) and Fluorecence Loss in Photobleaching (FLIP)

U2OS cells were cultured on coverslips in CTS containing medium. 24 hours prior to imaging, cells were transfected with ERα-CFP and/ or SRC-1 using PEI. Where indicated, cells were additionally cotransfected with exogenous cyclin D1. Prior to analysis, coverslips were placed in 2 ml bicarbonate-buffered saline and analyzed in a heated tissue culture chamber at 37°C under 5% CO_2. Cells were treated with 100 nM arzoxifene or left untreated. Images were acquired on a TCS-SP2 confocal microscope (Leica, Mannheim, Germany), using a 63x oil immersion objective. Fluorescence intensities were measured at the bleach spot (FRAP) and at the far end of the nucleus (FLIP). Zoom factor was set at 4x for all experiments. $t_{1/2}$ recovery value was calculated by $t_{1/2} = F_{FRAP\infty} - F_{BLEACH} /2$, where ∞ is the asymptotic plateau value of the corresponding curve. Immobile fraction (IF) was calculated as $IF = ((F_{FLIP\infty} - F_{BLEACH}) - (F_{FRAP\infty} - F_{BLEACH}))/ (F_{FLIP\infty} - F_{BLEACH}) * 100\%$, where $F_{FLIP\infty}$ and $F_{FRAP\infty}$ are the fluorescence plateau levels of the corresponding curves and F_{BLEACH} the fluorescence level immediately after bleach.

Immunoprecipitation

MCF-7 cells were transfected with YFP-empty vector, cyclin D1 wildtype, cyclin D1 LALA or cyclin D1 KE using electroporation. 24 hours after transfections, cells were lysed in 125mM NaCl, 50mM HEPES (pH 7.5), 0,1% Nonidet P-40, 10mM $MgCl_2$ and protease inhibitor cocktail (Roche) on ice, sonificated and debris was removed by centrifugation. The supernatant was used in immunoprecipitation using anti-cyclin

D1 (sc-753, Santa Cruz) antibody immobilized on protein A Sepharose beads (Invitrogen) during incubation overnight. Samples were taken from the supernatant for analysis of the total lysate. Beads were extensively washed, boiled and samples were analyzed by Western blotting. For detection, antibodies identifying cyclin D1 (sc-753, Santa Cruz), p21 (Calbiochem) and p27 (Santa Cruz) were used and the signal was detected using an ECL detection kit (Amersham).