

SUPPLEMENTAL METHODS

Apoptosis assay and cell-cycle analysis

Cells at a density of 4×10^4 cells well⁻¹ were seeded into 12-well plates and incubated at 37°C for 24 hours. Rhodomyrtone (100-1,000 ng ml⁻¹) was added for 24 hours, after which the adherent cells were washed twice with PBS, trypsinized, and collected by centrifugation at 200xg for 4 minutes at 4°C. Cells were fixed and permeabilized with 3 ml cold 70% ethanol at -20°C overnight before centrifugation at 1500xg for 10 minutes. After washing, the cell pellets were incubated with 0.5 ml of PIPES buffer (10 mM Tris, 0.1 M NaCl, 2 mM MgCl₂, 0.1% Triton X-100, pH 6.8) and 1 µg ml⁻¹ DAPI at RT for 10 minutes. Cell cycle and sub-G₁ distributions were analyzed by measuring DNA content using an LSR2 flow cytometer (Beckton-Dickinson). A second fraction of cells was used for the quantification of apoptosis and necrosis using FITC-annexin V and propidium iodide staining, followed by flow cytometric analysis.