Supplemental Materials and Methods

Generation and measurement of ROS

Cellular ROS levels were ascertained using the cell permeable probe CM-H2DCFDA (5-(and-6)-chloromethyl-2'7'-dichlorodihydrofluorescein diacetate acetyl ester) from Invitrogen (Grand Island, NY). CM-H2DCFDA is non-fluorescent until cleavage of the acetyl groups by intracellular esterases and oxidation that transpires within the cell. Cells were pretreated for 3 hr with antioxidant glutathione (GSH, 5 mM) or N-acetyl cysteine (NAC, 5 mM), then subsequently treated with respective doses of penfluridol for 3, 6 or 24 hr. The antioxidant at the time of treatment of penfluridol was not washed away and penfluridol treatment was with antioxidant cotreatment. Following treatment, cells plated on a 6-well culture plate were trypsinized, neutralized, then loaded with 10 μM of probe for 20 min, washed once with serum free medium, and then ROS was measured by flow cytometry using Accuri's C6 Flow Cytometer (Accuri, Ann Arbor, MI).

Plasmids

pCDH-puro-cMyc was a gift from Jialiang Wang (Addgene plasmid #46970) and pCDH-CMV-MCS-EF1-Puro was obtained from System Biosciences (Palo Alto, CA). MDA-MB-231 and SKBR3 breast cancer cells were seeded (1.2 x 10^5 per well) in 6-well plates in Dulbecco's modified Eagle's medium (DMEM) supplemented with 2.5% charcoal-stripped fetal bovine serum and left to attach for 24 hr. Plasmid transfection was performed using Lipofectamine 2000 reagent according to the manufacturer's protocol. Cells were transfected with c-Myc-overexpressing (pCDH-puro-cMyc) or

empty control vector (pCDH-CMV-MCS-EF1-Puro) for 6 hr in fresh 2.5% charcoal-stripped fetal bovine serum DMEM. After 6 hr, medium was then replaced with fresh 2.5% charcoal-stripped fetal bovine serum DMEM and treated with either DMSO or 5 μM penfluridol. Cells were then harvested for western blot analysis.