

Figure S4. Exogenous ligand-dependence of PLX4032-induced HER3 activation in melanoma cell lines. A, lysates of melanoma cell lines treated with 2 μ M PLX4032 for the indicated times were immunoblotted for tHER3. B, response of SK-MEL-28 cells to ligand induced activation of HER2/HER3 after exposure to PLX4032. Cells were treated with PLX4032 for 48 h, and then with or without 50 ng/ml of NRG1 for 5 minutes. RAS-GTP was detected by IP with RAS binding domain of CRAF (RBD) followed by Western blotting with anti-RAS. Input lysates were immunoblotted with the indicated antibodies. C, SK-MEL-28 cells were treated with increasing concentrations of PLX4032 alone (dark grey) or in combination with NRG1 (red). Cells were collected and counted after 4 days of treatment. Bars represent percent change (mean +/- SD) in cell counts of triplicate wells compared to untreated cells. *p < 0.05.