

Supplementary Data 5. Flow cytometry gating strategy for cell death and apoptosis assessment using Annexin V-DAPI. (a-d) A375 cells were treated with 1 μ M selumetinib for 48 hours in combination with 1 μ M AZD5991 for the last 24 hours. Cells were then harvested, stained with Annexin V and DAPI and staining assessed by flow cytometry. Cells were first gated on forward scatter area (FSC-A) versus side scatter area (SSC-A) to give population 1 (P1) that eliminated very small debris events (a), followed by SSC-A versus side scatter width (SSC-W) (P2) (b) and then FSC-A versus forward scatter width (FSC-W) (P3) (c) to isolate single cells only. Annexin V-FITC- and/or DAPI-positive single cells were then quantified using the lasers and filter sets indicated (d). Quadrant gate positions were determined by comparing samples of viable cells to samples exhibiting cell death, which were therefore either negative or positive for Annexin V and/or DAPI staining.