



Supplementary Data 6. Flow cytometry gating strategy for measurement of BAX activation. (a-d) A375 cells were treated with 1 μ M selumetinib for 24 hours and then in combination with 1 μ M AZD5991 for a further 1 hour. Cells were then harvested, fixed and incubated with an anti-active BAX antibody followed by an Alexa Fluor 488 labelled secondary antibody. Antibody staining was 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. A histogram of 530/30 488 nm area (530/30 488 nm-A) signal, which corresponds to levels of active BAX, was then used to quantify the fraction of cells with active BAX (d).