



Supplemental Figure 4. Drug Resistant POS Cells Emerge Following Chronic Trametinib Treatment. (A) Cell cycle analysis shows POS R2 proliferate in 30 nM trametinib. Naïve cells treated with DMSO (grey) or 30 nM trametinib (blue) for 24 hours were stained with propidium iodide and compared to resistant cells cultured in 30 nM trametinib, then stained with propidium iodide (dark blue). (B) Quantification of cell cycle analysis in Fig. 4B and Fig. S4A. Percent G1 (light grey), S (medium grey), G2 (black) calculated for POS N treated with DMSO or 30 nM trametinib and compared to POS R1 and POS R2. (C) NEG N and NEG R cells do not establish tumors. 2 million NEG naïve cells were injected into the mammary fat pad of nod/scid mice (n=6) and treated with control chow (grey). Nod/scid mice were pre-treated with trametinib chow (0.2 mpk) and then injected with 2 million NEG resistant cells (maroon) each (n=6). (D) Heatmap of row-normalized expression of the intersection upregulated genes identified in Fig. 4H. 156 of 202 genes upregulated in both POS R1 and POS R2 are continuously upregulated during chronic treatment with 10 nM trametinib.