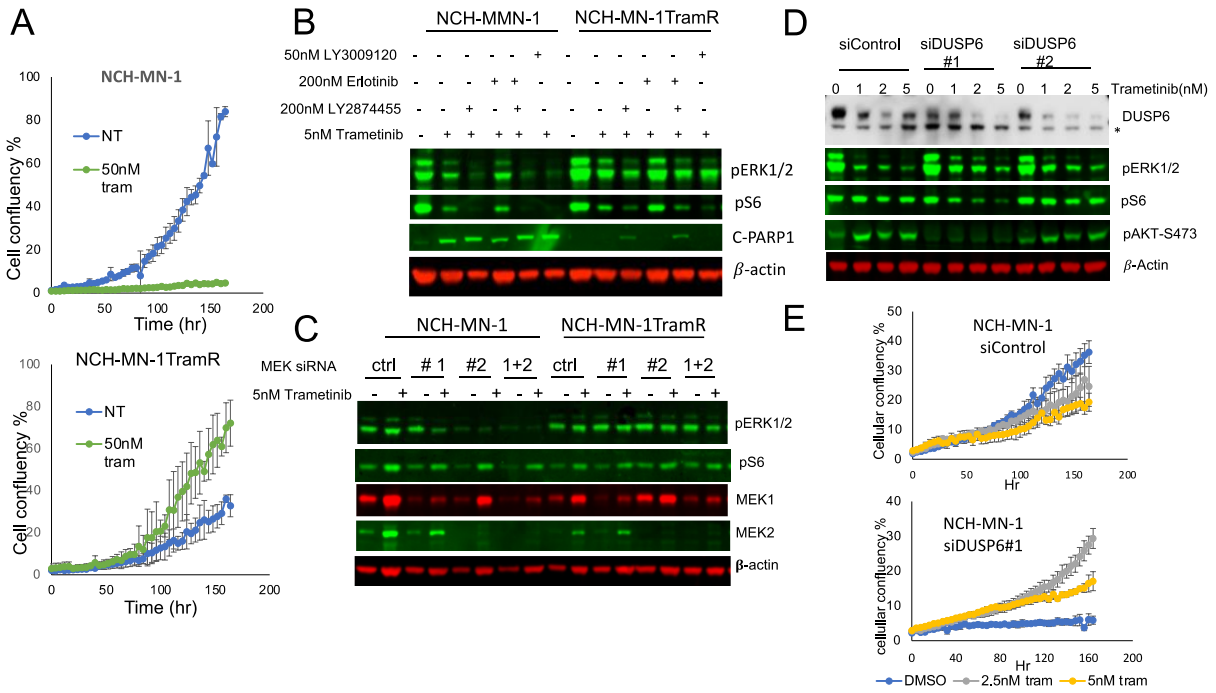

Figure S11. Characterization of NCH-MN-1 cells isolated parental or trametinib resistant xenografts.

A. Cells from NCH-MN-1 (parental) and NCH-MN-1TramR (trametinib resistant Cycle 3) were exposed in culture to trametinib (tram 50 nM) or DMSO (NT). Confluence was determined using an Incucyte Zoom over 160 Hr. Parental NCH-MN-1 cells were sensitive to trametinib. In contrast, cells derived from trametinib resistant tumors grew more slowly than parental cells, and proliferation was stimulated by trametinib.

B. Upstream inhibitors of MAPK signaling fail to inhibit pERK1/2 and induce apoptosis in trametinib resistant NCH-MN-1 cells. Parental or NCH-MN-1TramR cells were exposed to upstream signaling inhibitors, as single agents or combined, for 96 Hr and assessed for inhibition of pERK1/2 or markers of apoptosis (PARP1 cleavage).

C. Knockdown of MEK1/2 does not suppress pERK1/2 in NCH-MN-1TramR cells. Cells from parental or trametinib resistant xenografts were treated with siRNA targeting MAEK1 (#1) or MEK2 (#2) or both siRNA's for 48 Hr. MAPK and mTORC1 signaling were assessed by pERK1/2 and pS6, respectively in the absence or presence of trametinib (5 nM). ERK1/2 phosphorylation was suppressed in cells with MEK knockdown, irrespective of trametinib. In NCH-MN-1TramR cells neither MEK1/2 knockdown or trametinib suppressed pERK1/2.

D. Knockdown of DUSP6 reduces trametinib inhibition of pERK1/2 and pS6 in cells derived from NCH-MN-1 (parental) xenografts. Cells were transfected with two siRNAs against DUSP6. After 48 Hr cells were exposed to trametinib for 96 Hr, and pERK1/2 and pS6 determined by western blot.

E. Knockdown of DUSP6 reduces proliferation of NCH-MN-1 cells in vitro, and trametinib increases proliferation. Cells from NCH-MN-1 (parental) xenografts were transfected with siDUSP6, then were exposed in culture to trametinib (tram 0-5 nM nM) or DMSO for 160Hr. Confluence was determined using an Incucyte Zoom. Parental NCH-MN-1 cells were sensitive

to trametinib. In contrast, cells where DUSP6 was suppressed grew more slowly than parental cells, and proliferation was stimulated by trametinib.