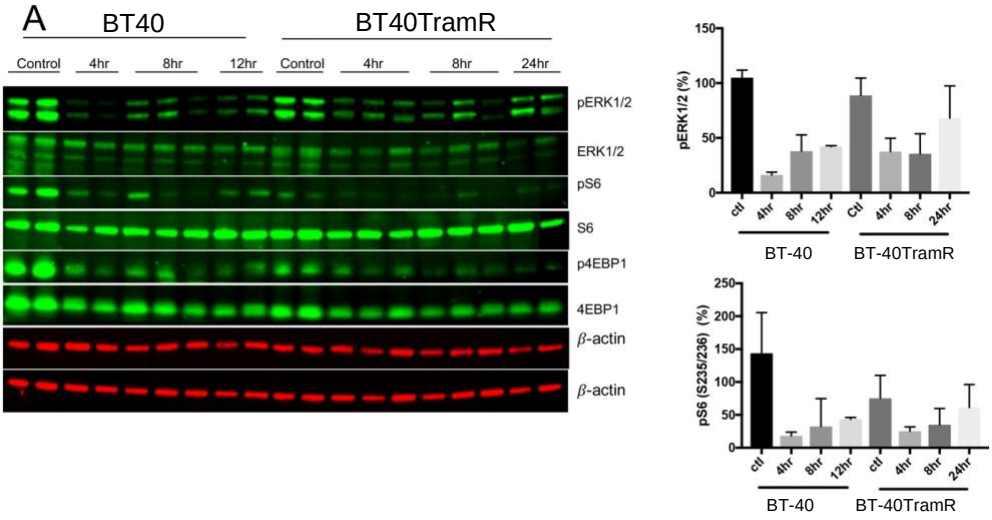
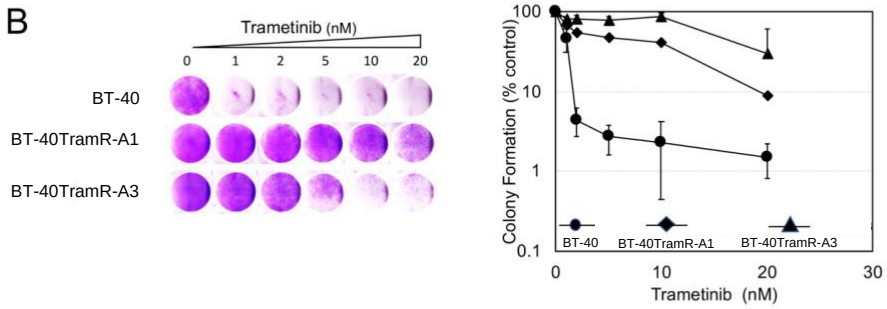

A
BT40
BT40TramR
Control
4hr
8hr
12hr
Control
4hr
8hr
24hr
pERK1/2
ERK1/2
pS6
S6
p4EBP1
4EBP1
β-actin
β-actin
pERK1/2 (%)
150
100
50
0
ctl
4hr
8hr
12hr
Ctl
4hr
8hr
24hr
BT-40
BT-40TramR
pS6 (S235/236) (%)
250
200
150
100
50
0
ctl
4hr
8hr
12hr
ctl
4hr
8hr
24hr
BT-40
BT-40TramR


B
Trametinib (nM)
0
1
2
5
10
20
BT-40
BT-40TramR-A1
BT-40TramR-A3
Colony Formation (% control)
100
10
1
0.1
BT-40
BT-40TramR-A1
BT-40TramR-A3
0
10
20
30
Trametinib (nM)

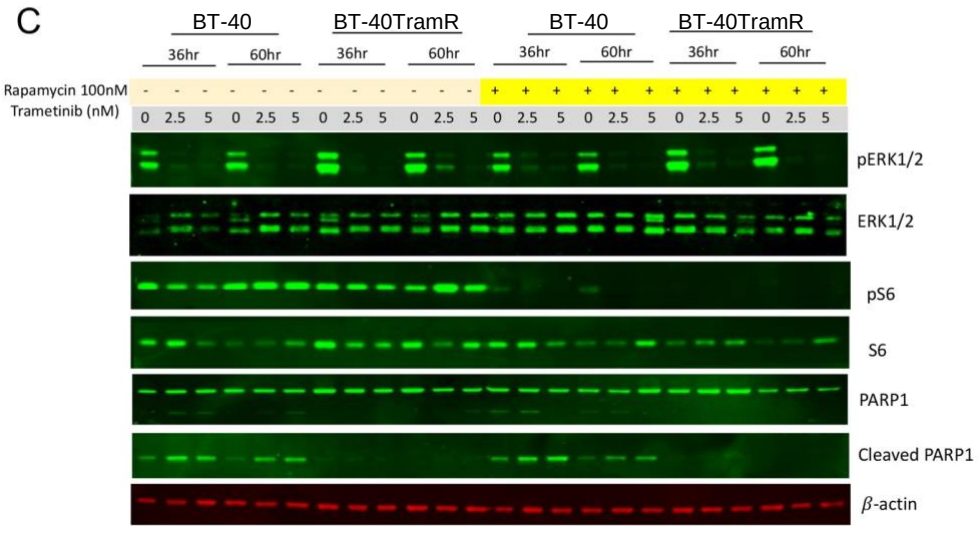

C
BT-40
BT-40TramR
BT-40
BT-40TramR
36hr
60hr
36hr
60hr
36hr
60hr
36hr
60hr
Rapamycin 100nM
Trametinib (nM)
0 2.5 5 0 2.5 5 0 2.5 5 0 2.5 5 0 2.5 5 0 2.5 5 0 2.5 5 0 2.5 5
pERK1/2
ERK1/2
pS6
S6
PARP1
Cleaved PARP1
β-actin

Fig. S9. Characteristics of trametinib resistant PDX models and in cells isolated from PDX models. **A**. Mice bearing subcutaneous BT-40 or trametinib resistant BT-40TramR xenografts were treated for 5 days with trametinib (1 mg/kg/day). BT-40 parental and BT-40TramR-A3 trametinib xenografts were harvested at the times shown after the final treatment with trametinib. *Left panel:* Tumors were processed for western blotting as described in Materials and Methods. β-actin from two gels used to run the immunoblots are shown as loading controls. *Right panel:* quantitation of pERK1/2 and pS6 in naïve (BT-40) and trametinib resistant (BT-40TramR-A3) tumors.

B. Sensitivity of cells freshly isolated from BT-40, and two independently derived tumors resistant to trametinib (BT-40TramR-A1 or BT-40TramR-A3) isolated after 3 cycles of trametinib therapy in mice. Cells were seeded in serum-free neuronal stem cell culture medium and allowed to grow overnight. Trametinib was added for 48 Hr, then replaced with fresh drug-free medium. Cells were cultured for an additional 3 weeks and colony formation assessed.

C. Cells freshly isolated from BT-40 or BT-40TramR xenografts were incubated with trametinib (2.5nM, 5nM) without or with rapamycin (100nM). Samples were harvested at 36 or 60 Hr drug exposure, processed for western blotting and probed for total and phosphorylated ERK1/2, S6 and PARP1 and cleaved PARP1. β-actin is shown as a loading control.