Figure S1. Effects of SB203580 and U0126 on untransfected RD cells. (A) RD cells pre-treated or not with 5 μM SB203580 for 10 min were treated with 10 μM U0126 for 1 day. Western blots of ERK-PO4, ERK, Myc, p38-PO4 and p38 are shown. Tubulin was used as a loading control. The numbers on the left of the blots indicate the protein size (kDa). These blots are the results of the grouping of different parts of the same gel, specifically, the first three lanes and the last one. The right panel shows quantitative evaluations of the different western blots expressed as the mean ± SD. Statistical analyses were performed using one-way ANOVA with Dunnett's post hoc test: ***P<0.001; **P<0.01 vs. negative control. (B) Western blots of MHC and myogenin in RD cells treated or not with 10 μM U0126. GAPDH was used as a loading control. The numbers on the left of the blots indicate the protein size (kDa). In the right panel, evaluations of the different western blots performed are shown as the mean ± SD. Statistical analyses were performed using a Student's t-test: ***P<0.001 vs. negative control. C, negative control; MHC, myosin heavy chain.

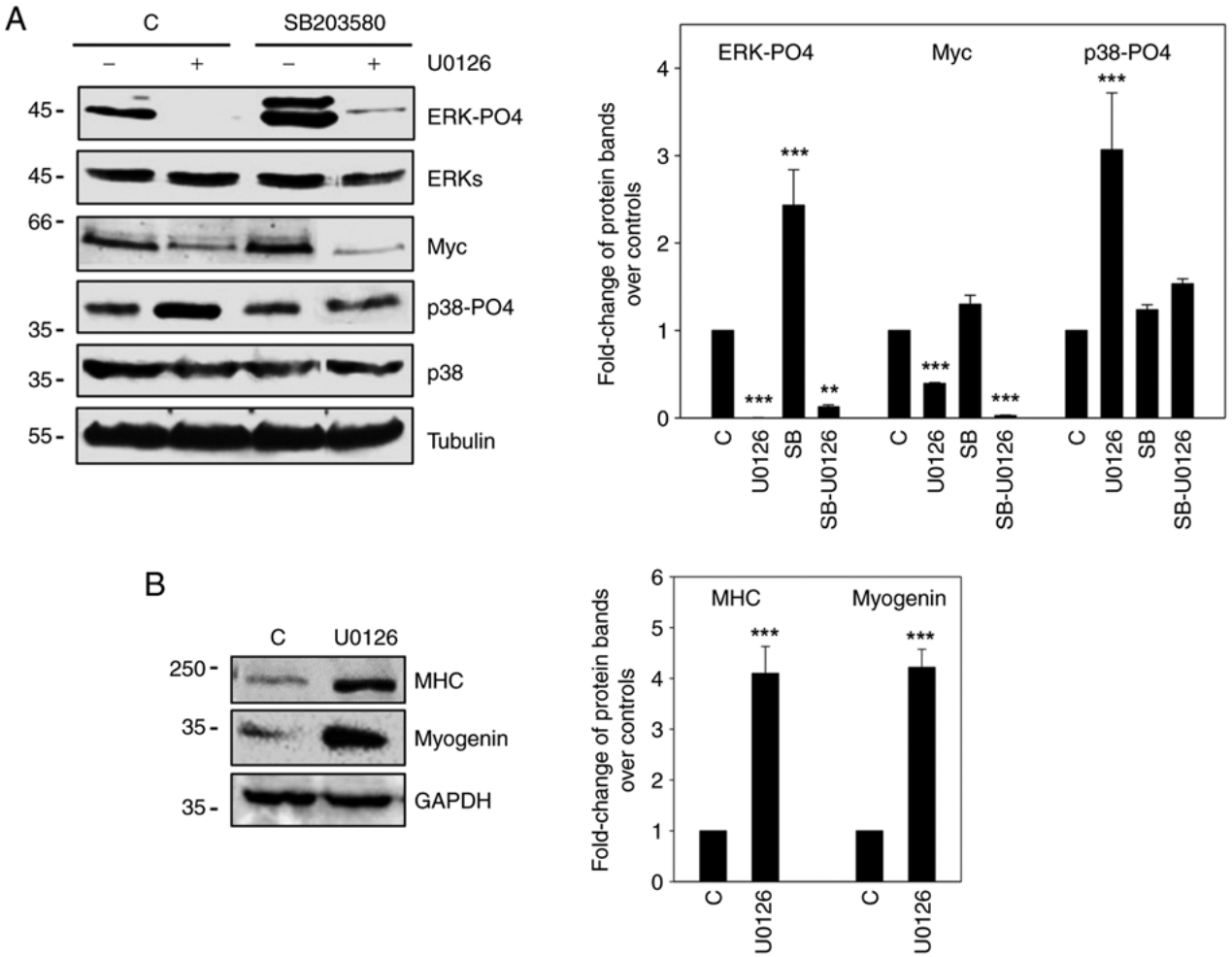
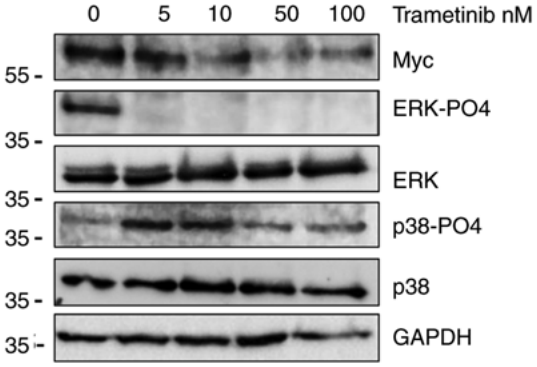


Figure S2. Effects of various concentrations of trametinib in RD cells. RD cells were treated with increasing concentrations of trametinib (5-10-50-100 nM) for 1 day. Cell lysates were examined using western blot analysis for Myc, ERK-PO4, ERK, p38-PO4 and p38. GAPDH was used as a loading control. The numbers on the left of the blots indicate the protein size (kDa). In the right panel quantitative evaluations of the different western blots performed are shown as the mean ± SD. Statistical analyses were performed using one-way ANOVA with Dunnett's post hoc test: $^{***}P<0.001$; $^{**}P<0.01$ vs. 0 nM trametinib.



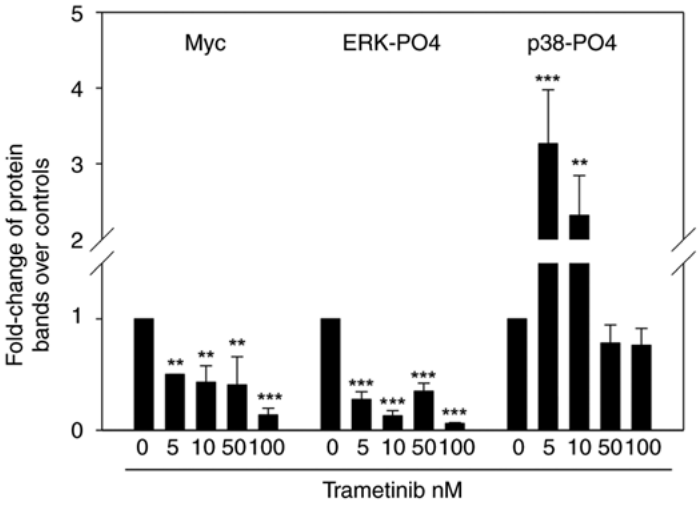


Figure S3. MKK6 is induced by MEK/ERK inhibitors in TE cells. TE cells were treated with 10 μM U0126 or 10 nM trametinib and the expression levels of MKK6-PO4, p38-PO4, MHC and myogenin were examined using western blot analysis. GAPDH and unphosphorylated kinases were used to normalise MKK6 and p38 (3 h and O/N panel); tubulin and GAPDH were used to normalise MHC and myogenin, respectively (3 days panel). The numbers on the left of the blots indicate the protein size (kDa). Lower panels represent histograms of the quantitative evaluations of the western blots, expressed as the mean ± SD. Statistical analyses were performed using one-way ANOVA with Dunnett's post hoc test: $^{***}P<0.001$; $^{**}P<0.01$; $^{*}P<0.05$ vs. negative control. C, negative control; MHC, myosin heavy chain; TE cells, TE671 cells; O/N, overnight.