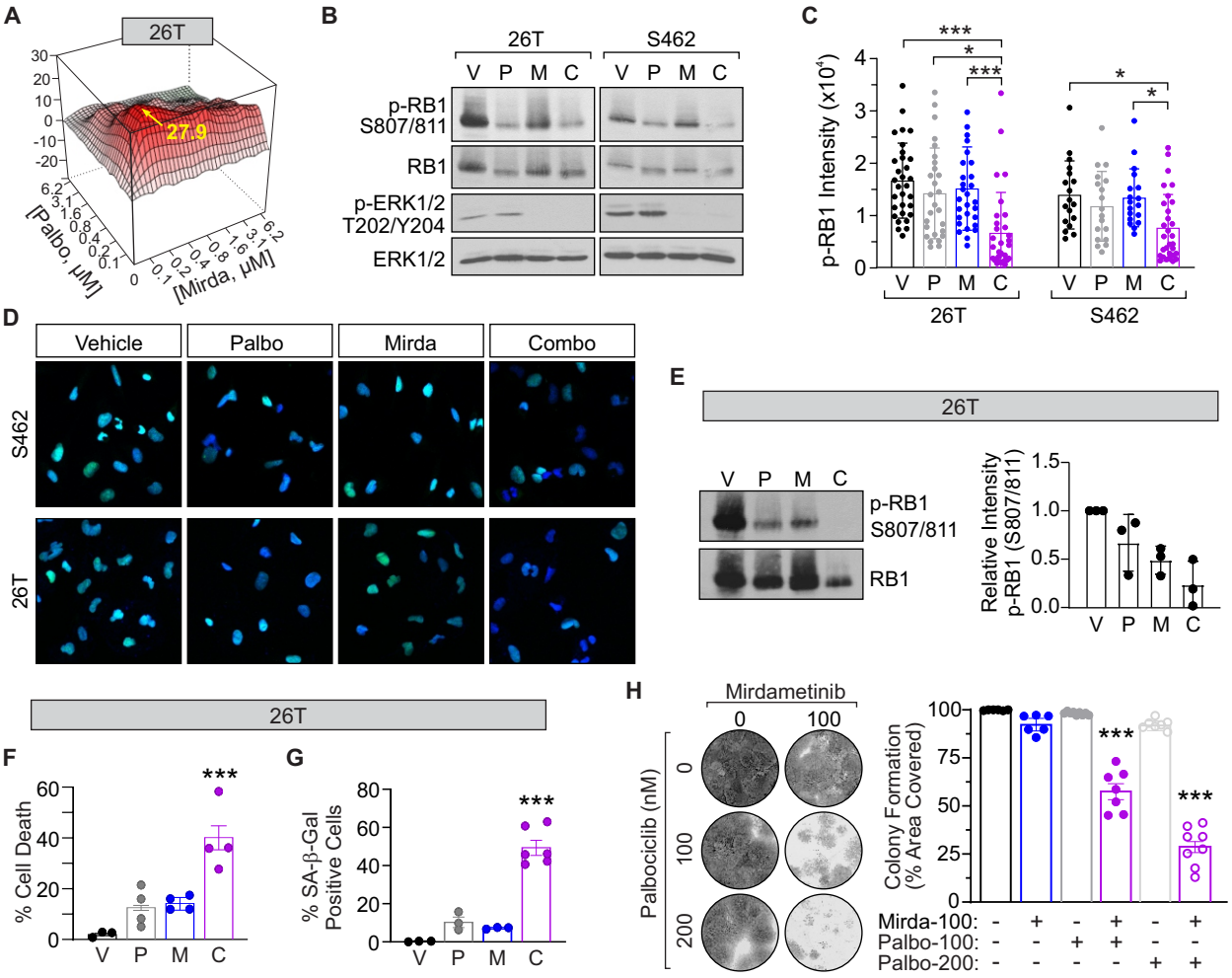
Supplementary Figure S1



Supplementary Figure S1. **Combination therapy co-targeting CDK4/6 and MEK acts synergistically against MPNST cells in vitro**. (**A**) Contour plot of interaction index (Bliss independence model) for the combination of low doses of palbociclib plus mirdametinib in 26T cells. Red, synergy; green, antagonism. Peak synergy score was 27.9. (**B**) Representative westerns of 26T and S462 cell lysates following 4hr of cell treatment with vehicle (V), palbociclib (P, 200nM), mirdametinib (M, 200 nM) or the combination (C). P-RB1 (S807/811) is a readout of CDK4/6 inhibition by palbociclib, phosphorylation S807/811 in 26T cells. Right, Imagep-ERK1/2 (T202/Y204) is a readout of MEK inhibition by mirdametinib. (**C**) Quantification using ImageJ of IF staining intensity for p-RB1 (S807/811) in 26T and S462 cells following treatment as in (B). Results from 3 independent experiments with each dot representing the mean fluorescent value of 10 cells. (**D**) Representative IF merged images from C. Green, p-RB1 positive; Blue, DAPI stained nuclei. (**E**) Representative westerns after 24hr of drug treatment in 26T cells show the combination of palbociclib and mirdametinib reduced RB1 J quantification of p-RB1 detection from 3 independent experiments. Mean p-RB1 levels were lowest in combination treated cells although values were not statistically significant. **Panels F,G:** 26T cell viability assayed by Trypan blue exclusion (**F**) and senescence measured by senescence associated (SA)-β-galactosidase positivity (**G**) following treatment for 3 days with the indicated drugs. mirdametinib, 500 nM; palbociclib, 1000 nM. Data were quantified from 3 or more biological repeats. (**H**) Colony formation assays in 26T cells show synergism between low concentrations of mirdametinib and palbociclib. Left, representative images of the colonies. Right, quantification of percentage area covered by cells from 3 or more experiments. C,F,G,H: Error bars, SEM. *P* value, One-way ANOVA with Tukey's correction (*, $P < 0.05$; ***, $P < 0.001$).