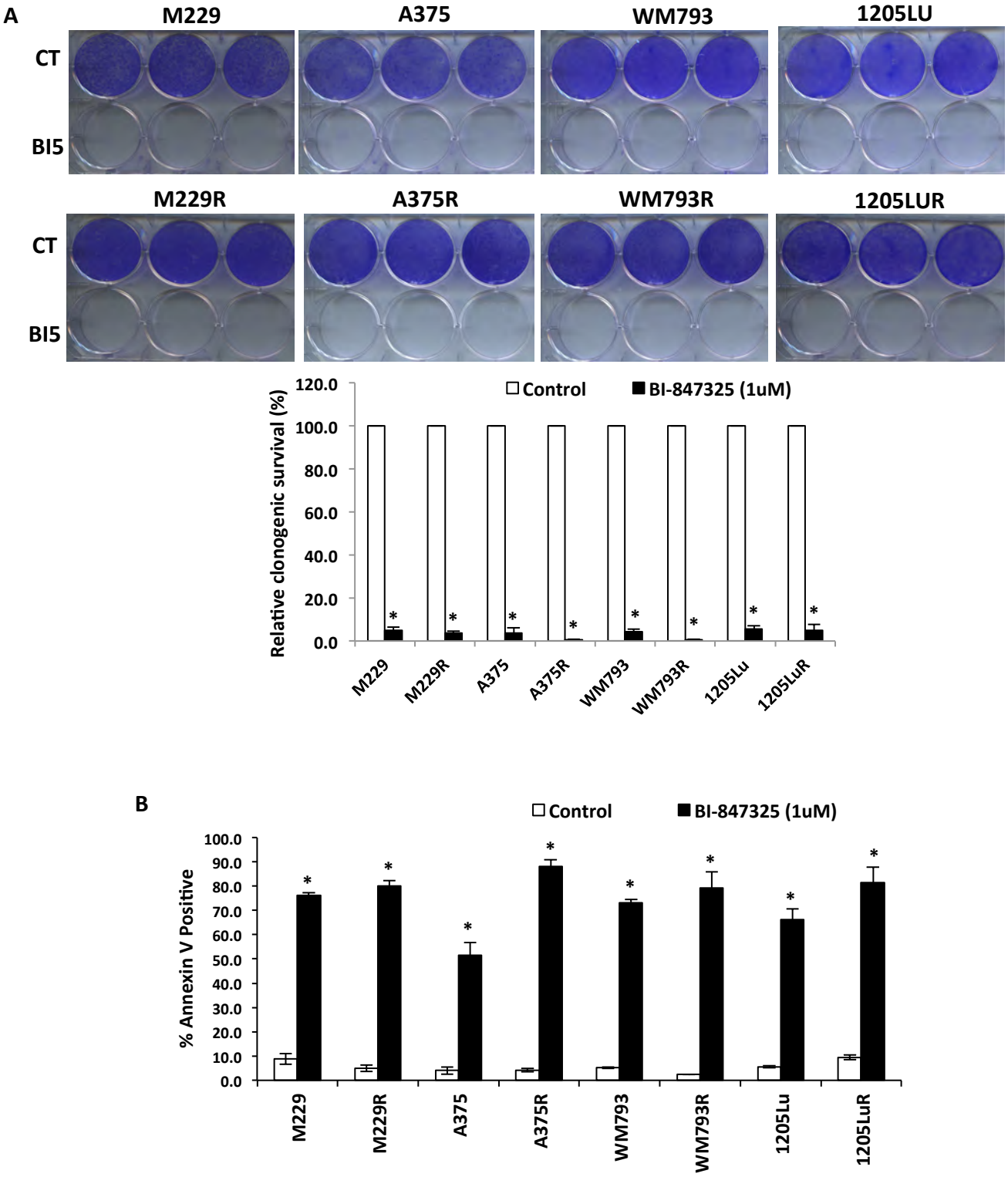
Cell line	Resistance mechanism
M229R	PDGFR
A375R	KRAS
WM793R	Unknown
1205LuR	Unknown
M249R	NRAS
WM164R	Unknown
WM39	Cyclin D1
RPMI7951	COT

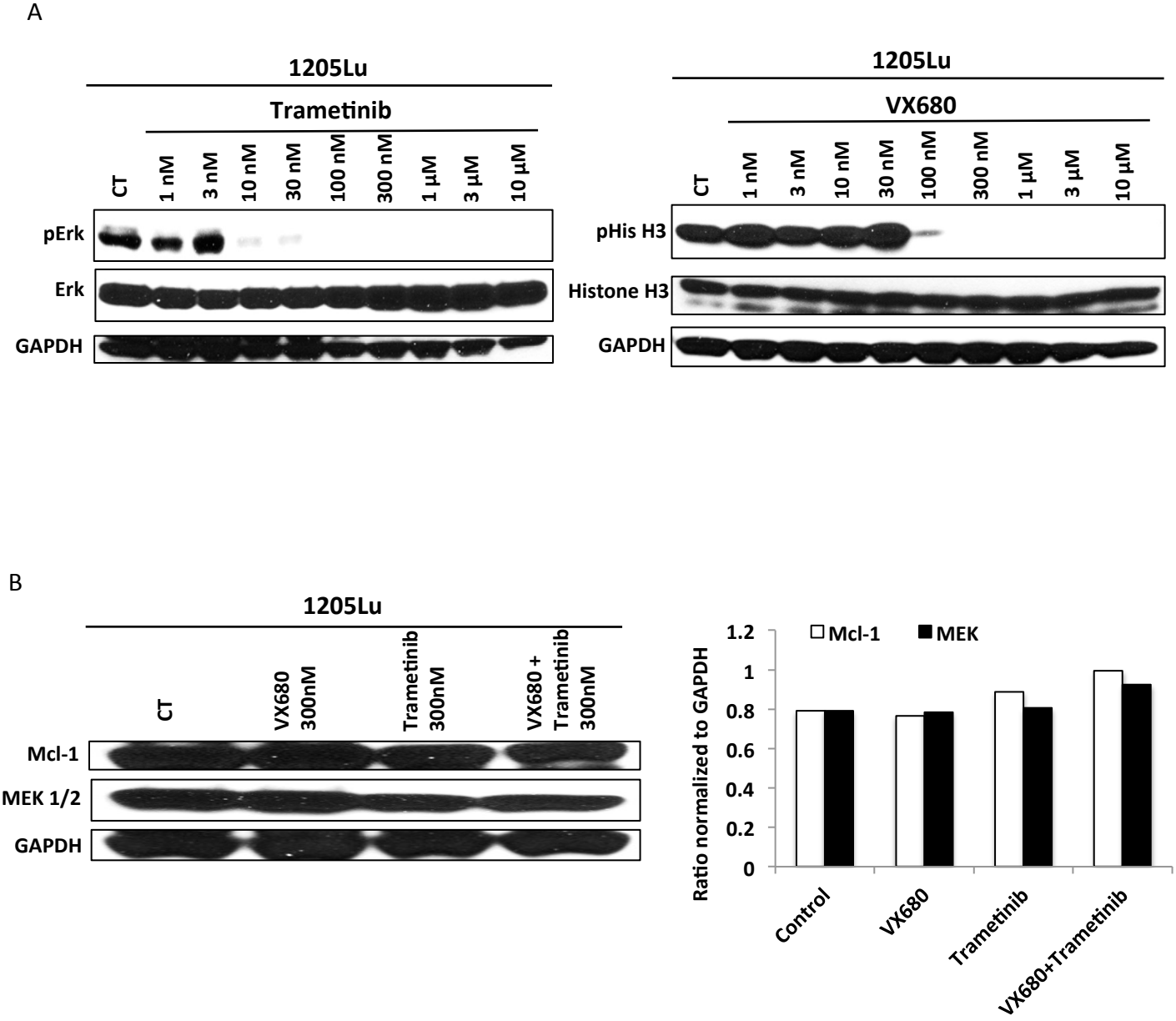
Supplemental Table 1: Resistance mechanisms for cell lines under evaluation.

Cell line	IC_{50} values (μM)
M229	0.0007
M229R	0.004
A375	0.0003
A375R	0.0005
WM793	2
WM793R	0.23
1205Lu	0.14
1205LuR	0.32
M249	0.0003
M249R	0.001
WM164	0.005
WM164R	0.08
WM39	0.0006
RPMI17951	0.0006

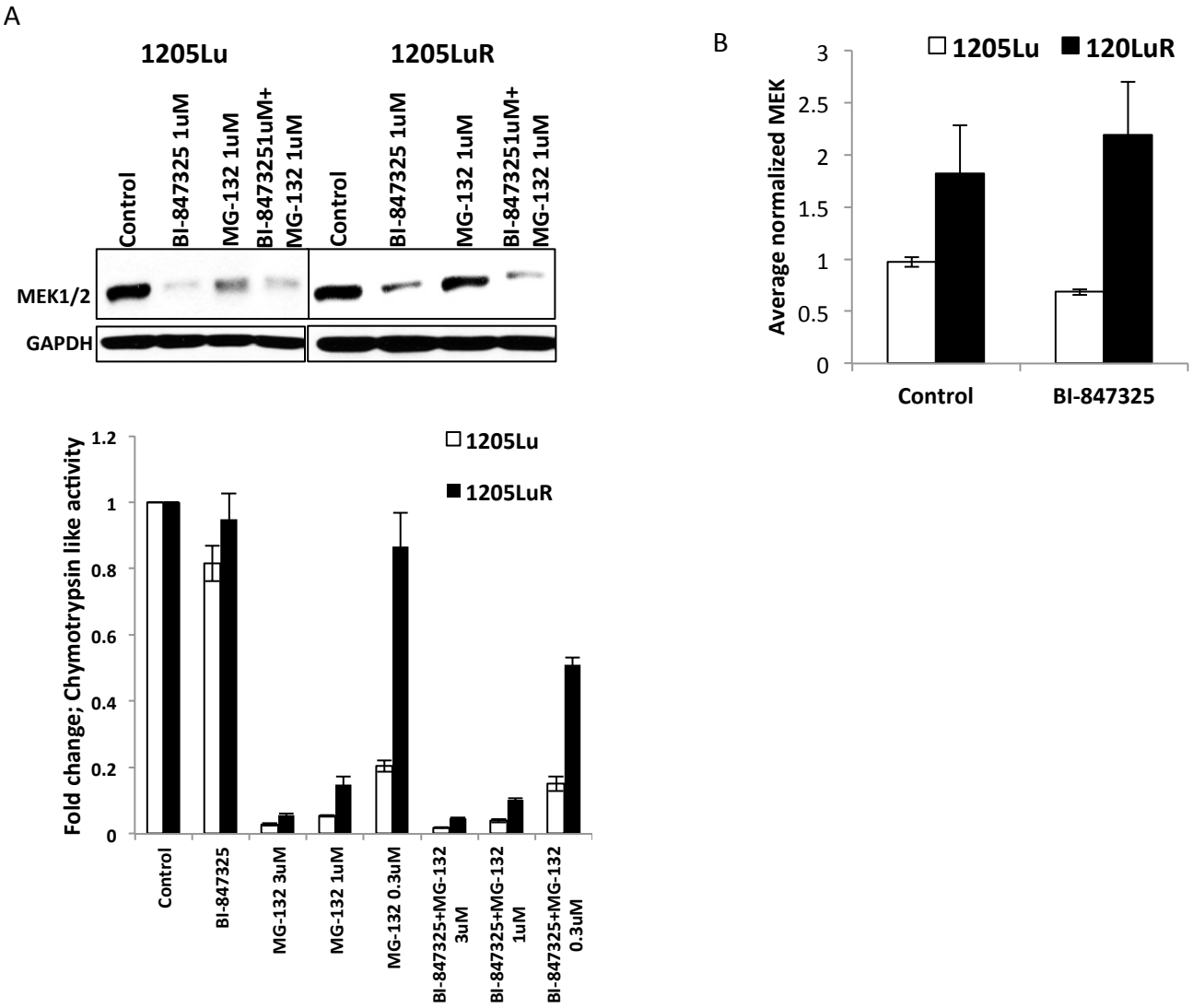
Supplemental Table 2: IC_{50} values for cell lines under evaluation on treatment with BI-847325 for 72 hours.



Supplemental Figure 1: BI-847325 blocks the proliferation and induces apoptosis in *BRAF*-mutant melanoma. A, colony formation assay showing growth inhibition in melanoma cell lines following treatment with BI-847325. Top; *BRAF*-mutant melanoma cell lines M229, A375, WM793, 1205Lu naïve and vemurafenib-resistant were treated with 1 µM BI-847325 for 4 weeks and then fixed and stained with crystal violet. Bottom; quantitative analysis of percent relative clonogenic survival after 4 weeks of treatment. B, *BRAF*-mutant melanoma cell lines M229, A375, WM793, 1205Lu naïve and vemurafenib-resistant were treated with 1 µM BI-847325 for 48 h and stained with Annexin V. Apoptosis induction was measured using flow cytometry.



Supplemental Figure 2: MEK and Aurora kinase inhibitors do not decrease expression of Mcl-1 and MEK. A, A MEK inhibitor (trametinib) and an Aurora kinase inhibitor (VX680) inhibit the phosphorylation of Erk and Histone H3, respectively, in a dose-dependent manner. 1205Lu melanoma cells were treated with trametinib and VX680 (1 nM-10 µM: 48hr) followed by Western Blotting for pErk and pHistone H3, respectively. B, Western Blot showing that inhibition of MEK and Aurora kinase alone and in combination do not decrease Mcl-1 or MEK expression. 1205Lu cells were treated with VX680 (300nM), trametinib (300nM) or the combination for 72 h. Western blotting was performed for Mcl-1 and total MEK. Right; the bar graphs represent quantitative analysis of Mcl-1 and MEK levels normalized to GAPDH.



Supplemental Figure 3: Inhibition of the proteasome does not reverse the effects of BI-847325 upon MEK expression. A, MEK suppression in 1205Lu and 1205LuR cell lines is independent of proteasome activity. Top; Western blot analysis show decreased MEK expression on treating both the cell lines with 1 µM BI-847325 in combination with 1 µM proteasome inhibitor MG-132 for 48 h. Treatment with 1 µM MG-132 for 48 h, demonstrate elevated levels of MEK in both the cell lines. Bottom; Proteasome Glo-Cell based assay revealed the chymotrypsin-like activity in both the cell lines on treatment with 300 nM, 1 µM and 3 µM of MG-132 for 48 h and in combination with 1 µM BI-847325. The bar graphs represent fold change in chymotrypsin–like activity normalized to untreated control cells. B, qRT-PCR results show no alteration in MEK mRNA levelsin 1205Lu or 1205LuR cells following treatment with 1 µM BI-847325 for 48 h.