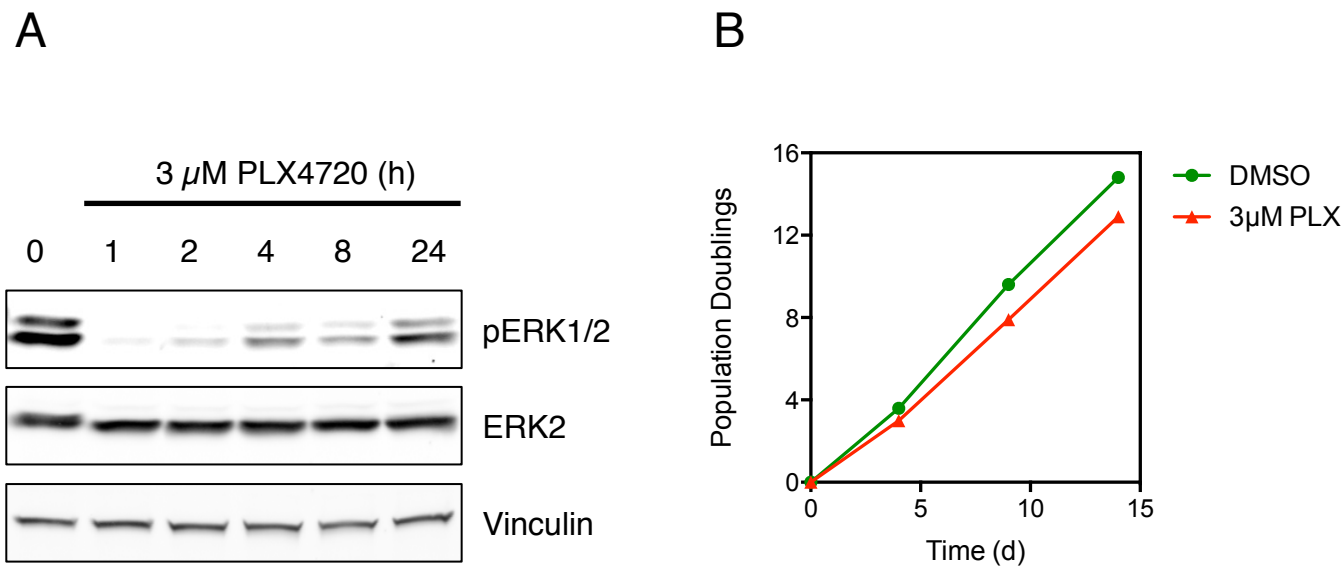


Figure S1. RKO cells are resistant to BRAF inhibition.
(A) RKO cells were treated with 3 μM PLX4720 for the indicated times, cell lysates were analyzed for the indicated proteins.
(B) RKO cells were cultured in the presence of 3 μM PLX4720 and the number of population doublings over time was determined by cell counting.

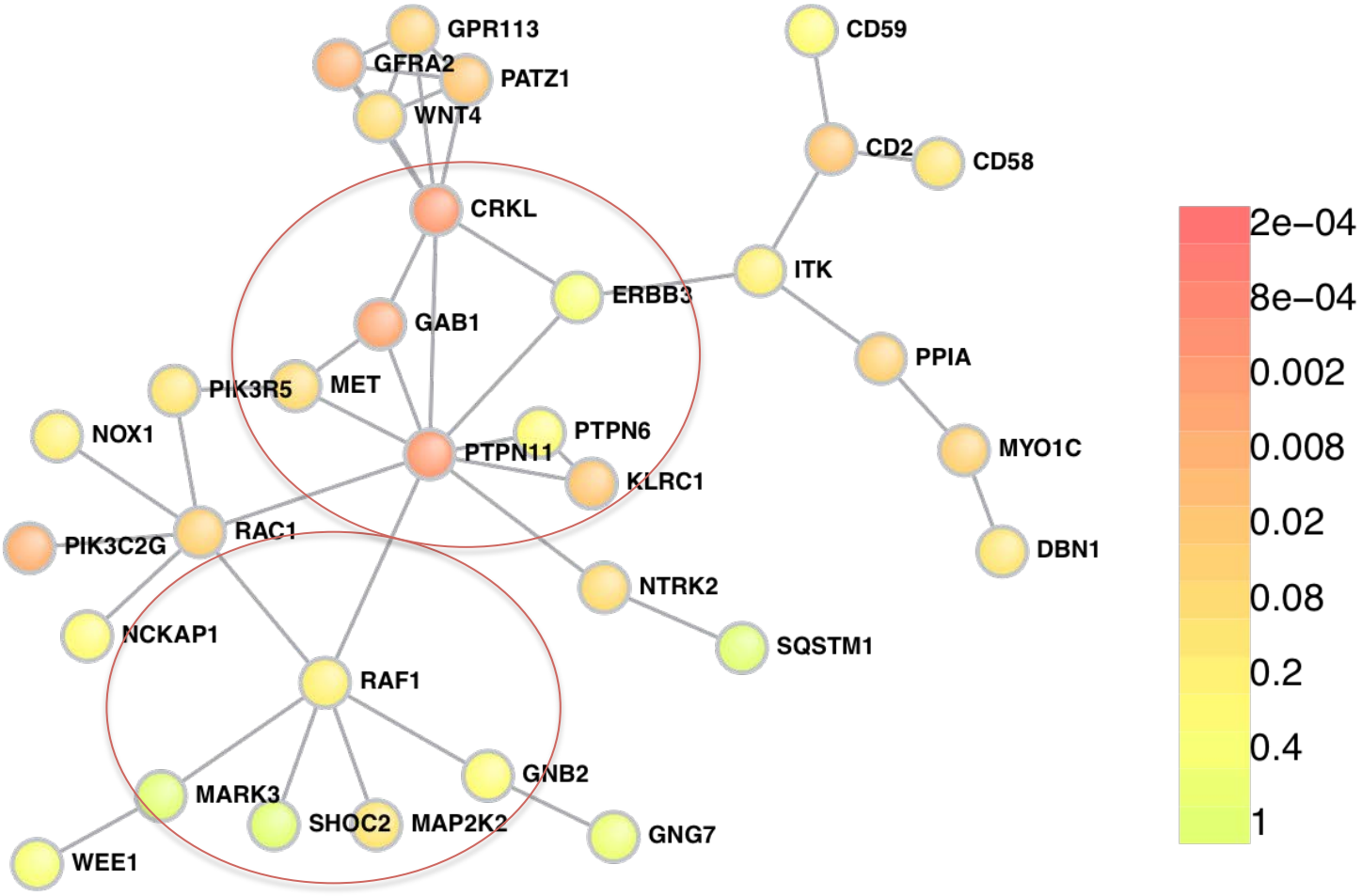


Figure S2. Identification of a potential protein interaction network from candidate synthetic lethal genes. The primary shRNA screening data was analyzed for protein-protein and functional interactions using DAPPLE [16]. A number of genes related to RTK, MAPK and PI3K signaling were enriched in the top 300 candidate synthetic lethal genes.

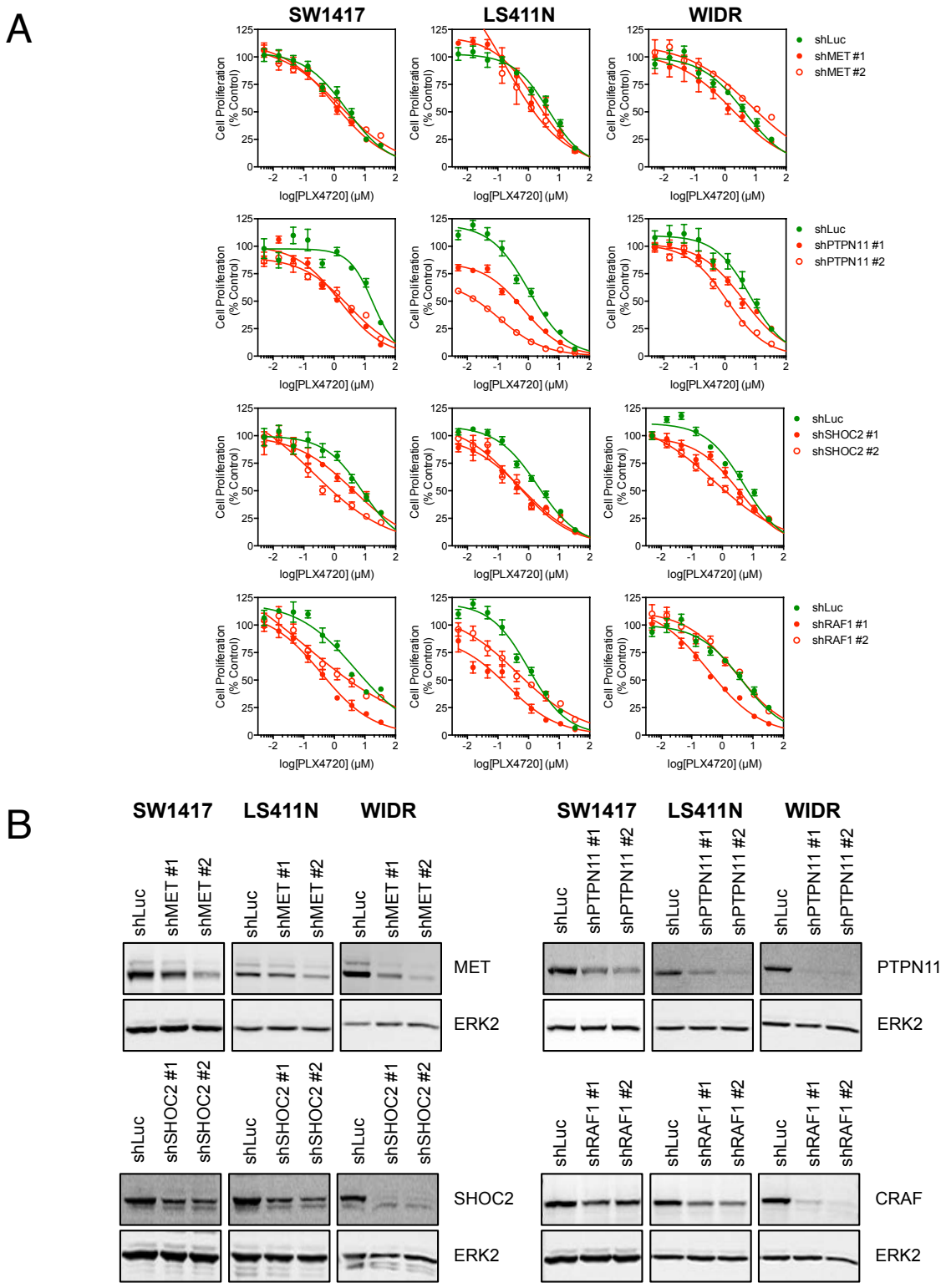


Figure S3. Validation of candidate synthetic lethal genes.
(A) A panel of colorectal cancer cell lines were infected with individual shRNAs targeting MET, PTPN11, SHOC2 and RAF1, relative to a control shRNA targeting luciferase. After 72 h, cells were treated with a titration of PLX4720 for 96 h, after which cell proliferation was assessed by CellTiter-Glo assay.
(B) Cells were infected as in (A) and cell lysates were analyzed for the indicated proteins.

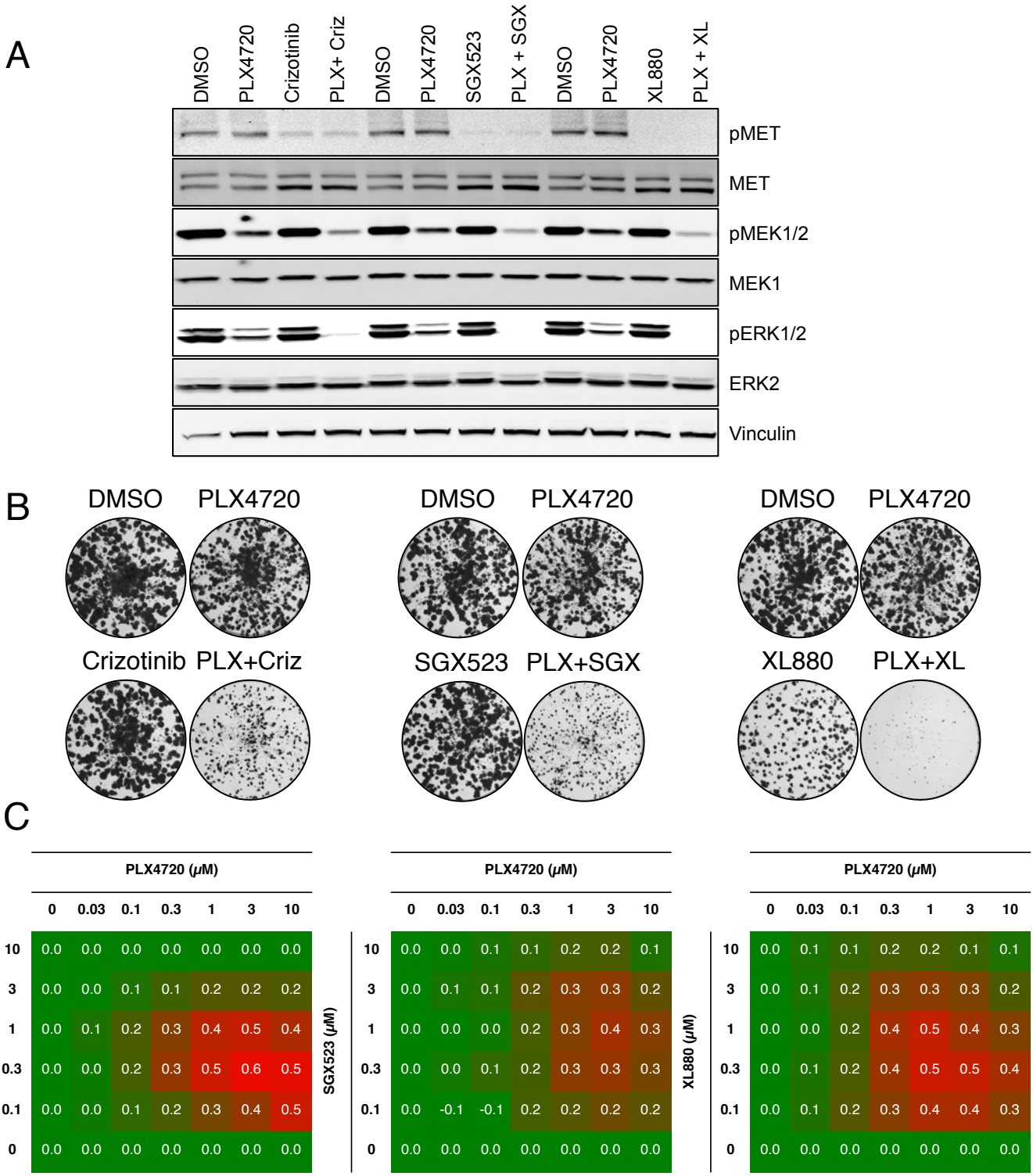


Figure S4. Pharmacologic inhibition of MET synergizes with PLX4720.
(A) RKO cells were treated with PLX4720 (3 μM) in combination with the MET inhibitors crizotinib (0.3 μM), SGX523 (2 μM) or XL880 (0.1 μM) for 4 h and cell lysates analyzed by Western blotting.
(B) RKO cells were treated as in (A) and cell proliferation was assessed after 14 d in culture by crystal violet staining.
(C) RKO cells were treated with a titration of PLX4720 versus either crizotinib, SGX523 or XL880 for 96 h and cell proliferation was assessed by CellTiter-Glo assay. Synergy was determined using the Bliss Independence model. Synergy is indicated by positive values, colored red.

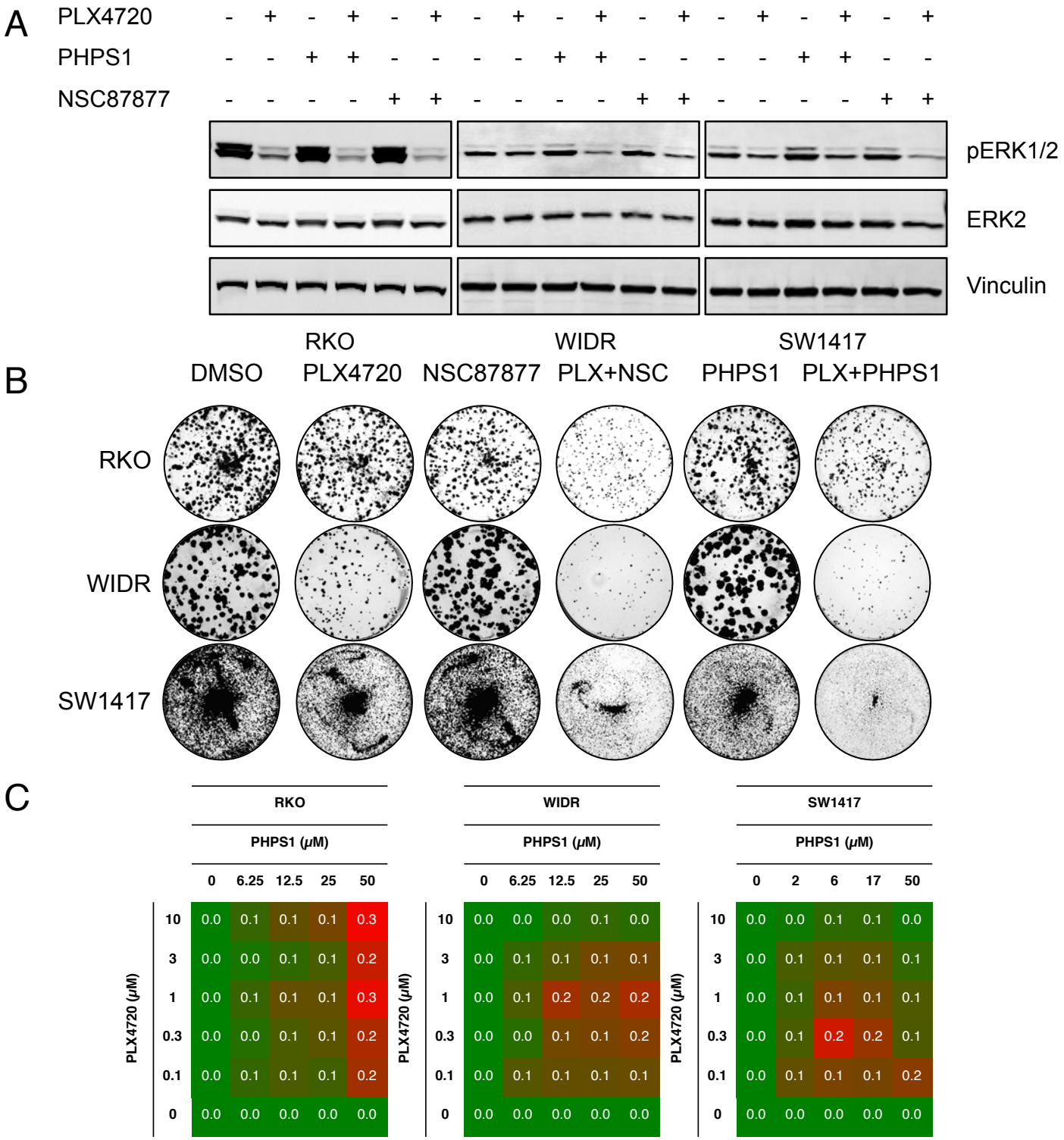


Figure S5. SHP2 inhibition enhances sensitivity to PLX4720.

(A) RKO, WIDR and SW1417 cells were treated with PLX4720 alone or in combination with PHPS1 (50 μM) or NSC87877 (100 μM) for 18 h. Cell lysates were analyzed by Western blotting for the indicated proteins.

(B) RKO, WIDR and SW1417 cells were treated with PLX4720 (3 μM), PHPS1 (50 μM), NSC87877 (100 μM) or a combination of the RAF and SHP2 inhibitors for 14 d. Cell proliferation was assessed by crystal violet staining.

(C) RKO, WIDR and SW1417 cells were treated with a titration of PLX4720 versus a titration of PHPS1 for 96 h. Cell proliferation was determined by CellTiter-Glo assay. The degree of synergy between the two compounds was calculated using the Bliss Independence model.

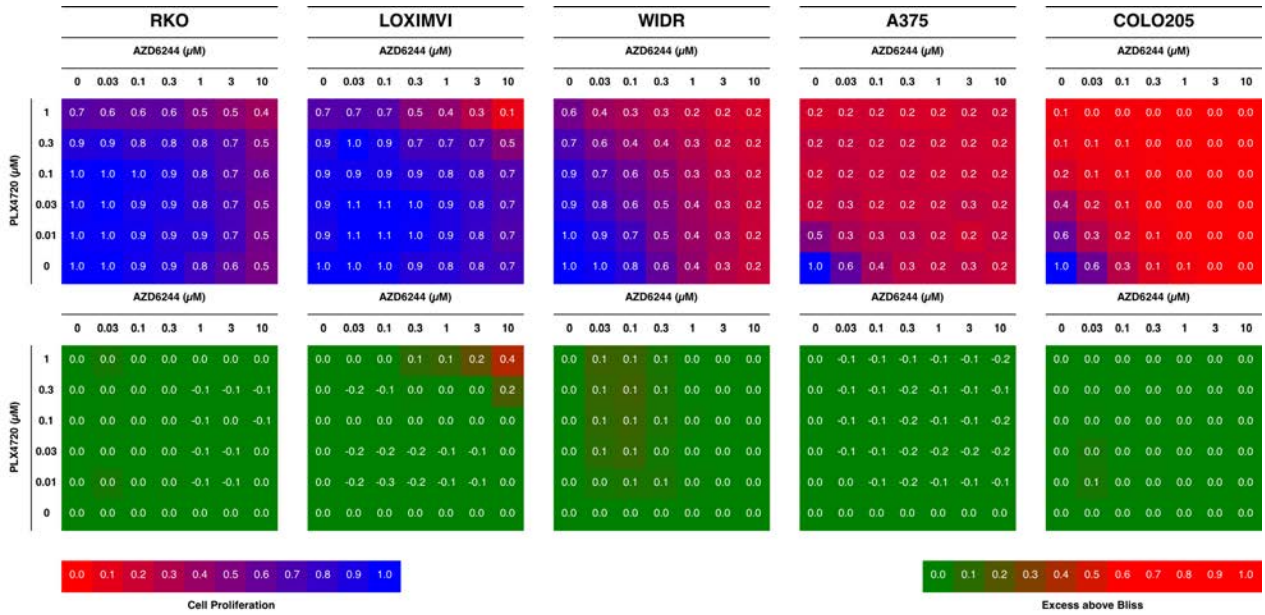


Figure S6. The combination of AZD6244 and PLX4720 is not synergistic.
The indicated cell lines were treated with a matrix of the MEK inhibitor AZD6244 and the BRAF inhibitor PLX4720 for 96 h. Cell proliferation was assessed by CellTiter-glo and synergy calculated using the Bliss independence model.

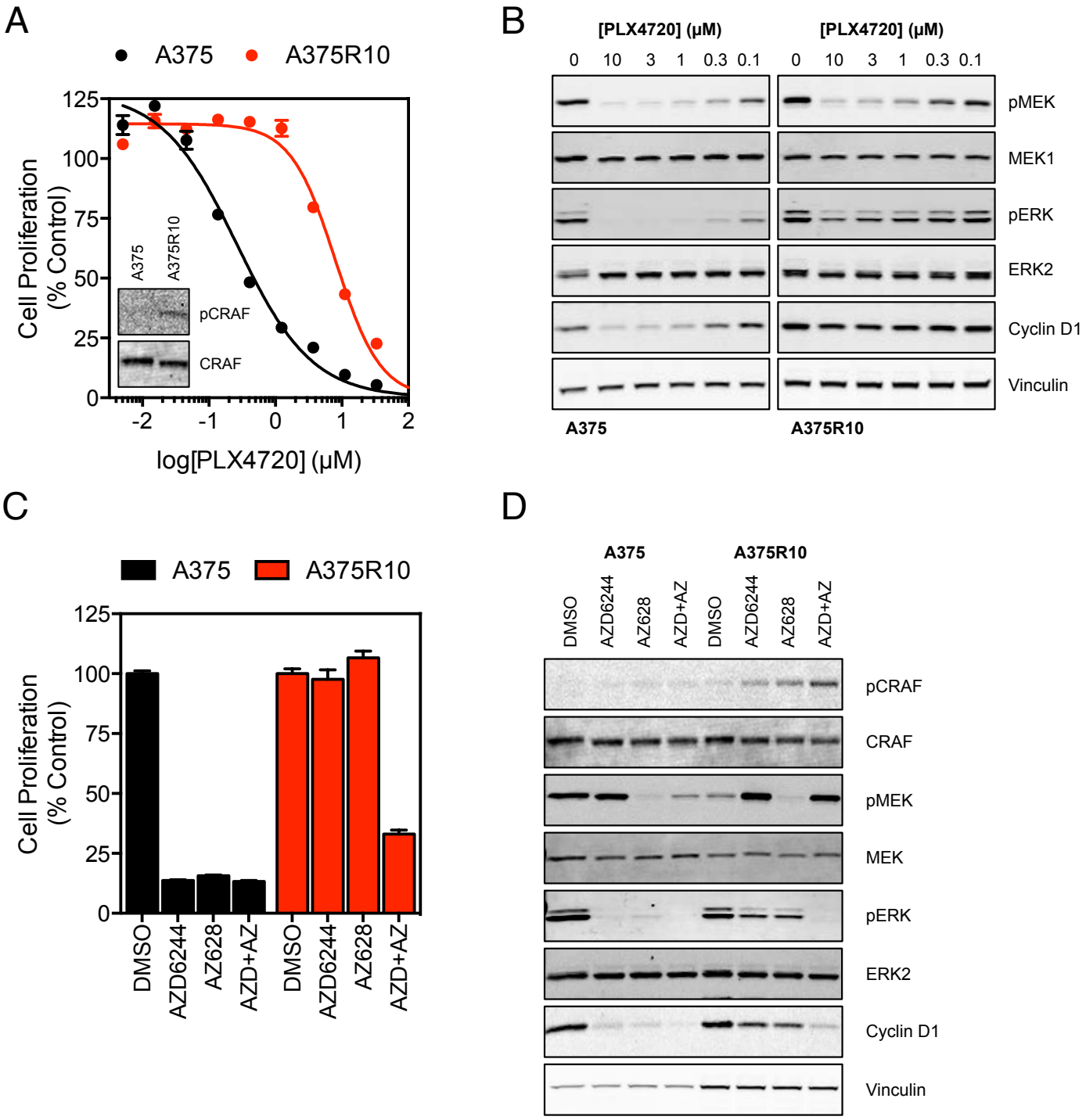


Figure S7. Pan-RAF and MEK inhibition overcomes acquired resistance to PLX4720.
(A) A375 cells were cultured in the presence of 1 μM PLX4720 for up to 4 weeks, colonies were isolated and expanded to generate a panel of PLX4720-resistant sub-clones. Sensitivity to PLX4720 was assessed following a 96 h exposure to the compound and cell proliferation was determined using CellTiter-Glo, a GI_{50} was calculated using GraphPad Prism. Cell lysates were generated from asynchronously growing cultures of the parental and R10 cell lines and analyzed by Western blotting for the expression of phospho-CRAF S338 and total CRAF (inset).
(B) A375 and A375R10 cells were treated with a titration of PLX4720 for 18 h. Cell lysates were analyzed by Western blotting for the indicated proteins.
(C) A375 and A375R10 cell lines were treated with 3 μM AZD6244, 0.3 μM AZ628 alone or in combination and cell proliferation assessed after 96 h by CellTiter-Glo.
(D) Parental and A375R10 cells were treated with 3 μM AZD6244, 0.3 μM AZ628 alone or in combination for 18 h. Cell lysates were analyzed by Western blotting for the indicated proteins.

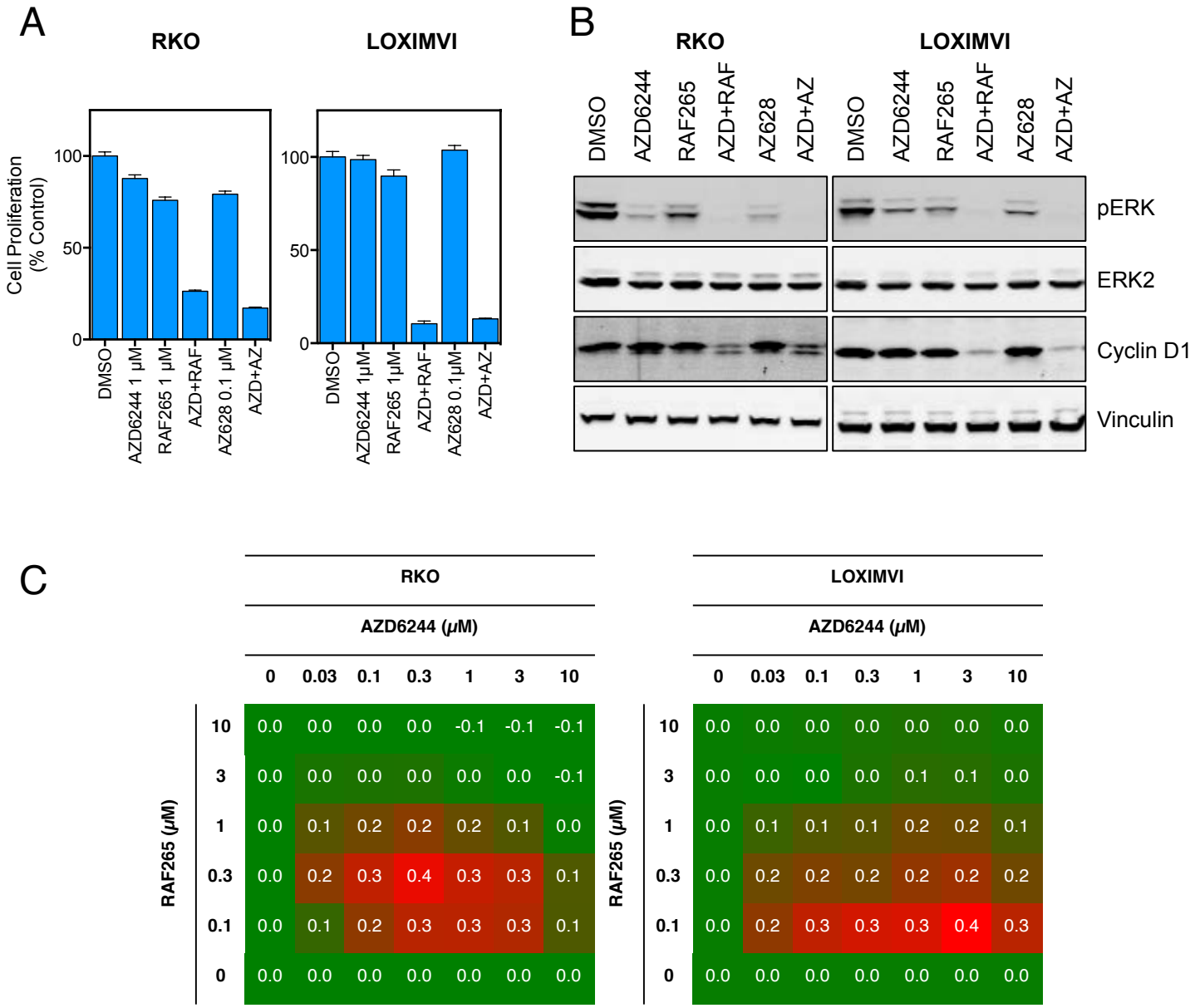


Figure S8. The combination of AZD6244 and RAF265 is synergistic.
(A) RKO and LOXIMVI cells were treated with either 1 µM AZD6244, 1 µM RAF265 or a combination of both inhibitors for 96 h. Cell proliferation was assessed by CellTiter-glo.
(B) RKO and LOXIMVI cells were treated as in (A) for 18 h. Cell lysates were analyzed by Western blotting for the indicated proteins.
(C) RKO and LOXIMVI cells were treated with a matrix of AZD6244 or RAF265 for 96 h and cell proliferation assessed by CellTiter-glo. Synergy was determined using the Bliss independence model.

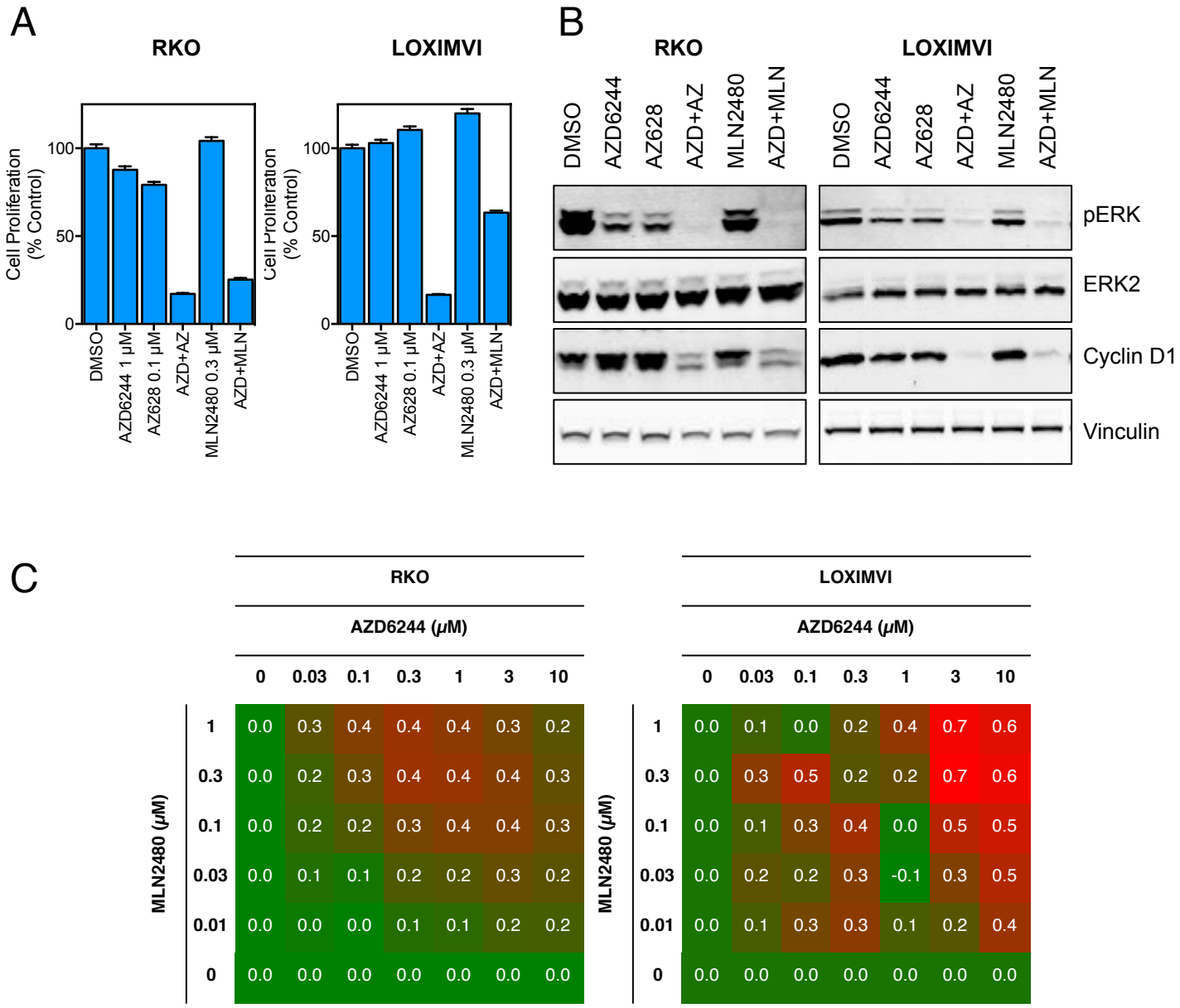


Figure S9. The combination of AZD6244 and MLN2480 is synergistic.
(A) RKO and LOXIMVI cells were treated with either 1 µM AZD6244, 0.3 µM MLN2480 or a combination of both inhibitors for 96 h. Cell proliferation was assessed by CellTiter-glo.
(B) RKO and LOXIMVI cells were treated as in (A) for 18 h. Cell lysates were analyzed by Western blotting for the indicated proteins.
(C) RKO and LOXIMVI cells were treated with a matrix of AZD6244 or MLN2480 for 96 h and cell proliferation assessed by CellTiter-glo. Synergy was determined using the Bliss independence model.

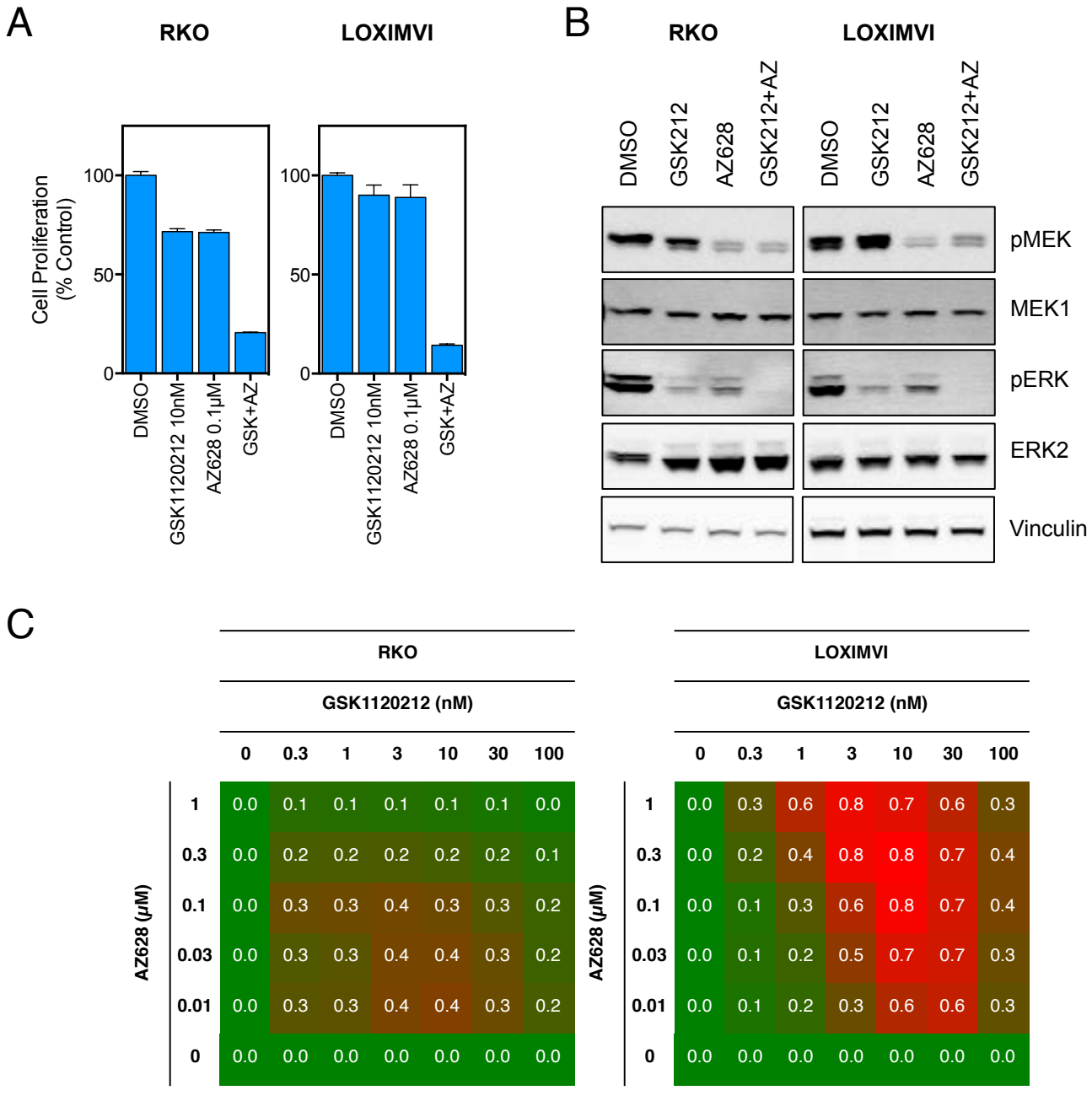
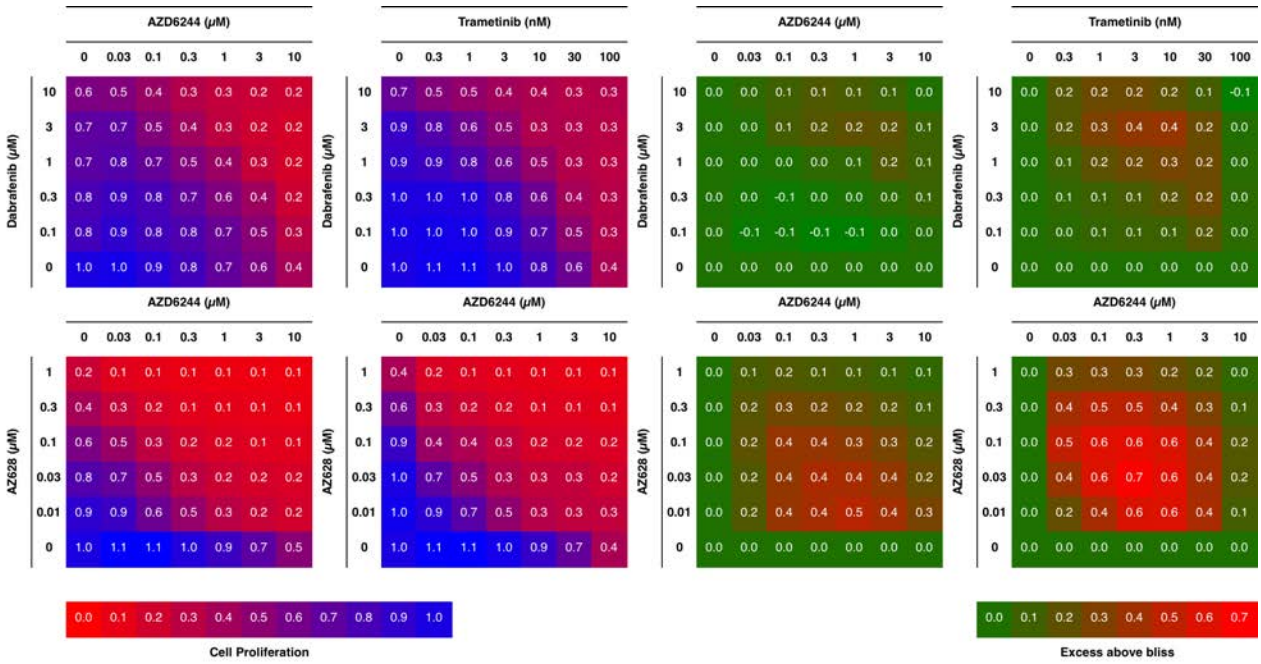


Figure S10. The combination of GSK1120212 and AZ628 is synergistic.
(A) RKO and LOXIMVI cells were treated with either 10 nM GSK1120212, 0.1 μM AZ628 or a combination of both inhibitors for 96 h. Cell proliferation was assessed by CellTiter-glo.
(B) RKO and LOXIMVI cells were treated as in (A) for 18 h. Cell lysates were analyzed by Western blotting for the indicated proteins.
(C) RKO and LOXIMVI cells were treated with a matrix of GSK1120212 or AZ628 for 96 h and cell proliferation assessed by CellTiter-glo. Synergy was determined using the Bliss independence model.

A



B

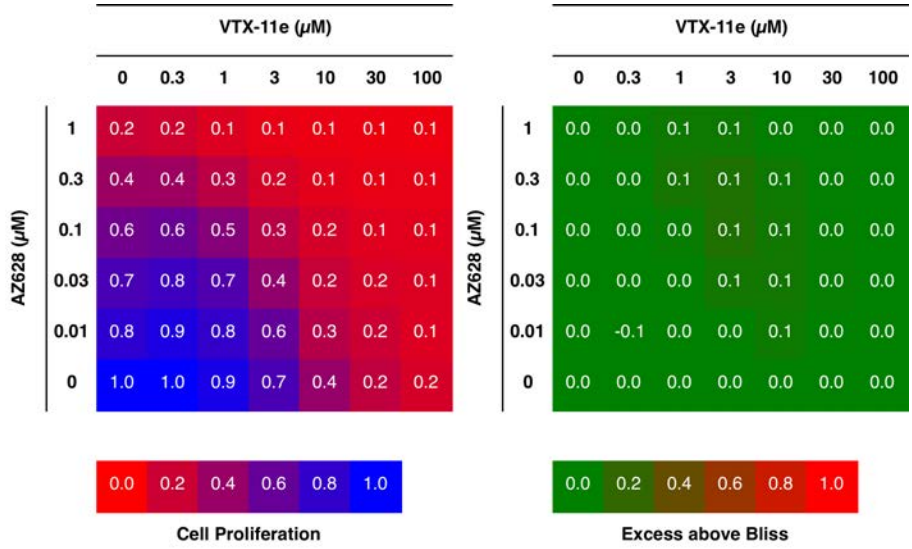
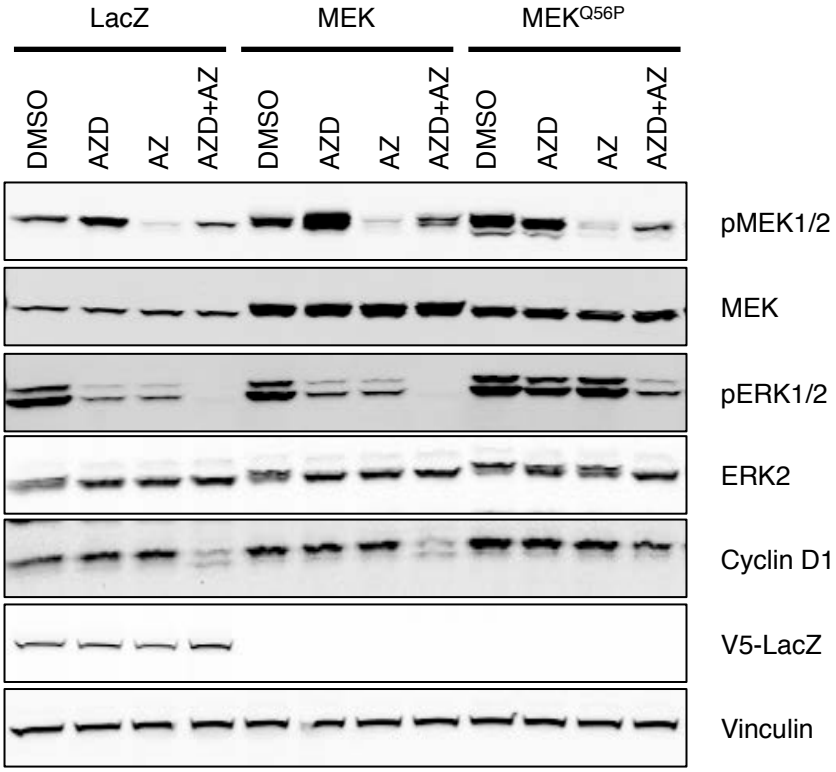


Figure S11. The pan-RAF inhibitor AZ628 drives greater synergy than GSK2118436 when in combination with a MEK inhibitor.

(A) RKO cells were treated with a matrix of the MEK inhibitors AZD6244 or GSK1120212 and the RAF inhibitors AZ628 and GSK2118436 for 96 h. Cell proliferation was assessed by CellTiter-glo and synergy calculated using the Bliss independence model.

(B) RKO cells were treated with a matrix of the ERK2 inhibitor VTX-11e and AZ628 for 96 h. Cell proliferation was assessed by CellTiter-glo and synergy calculated using the Bliss independence model.

A



B

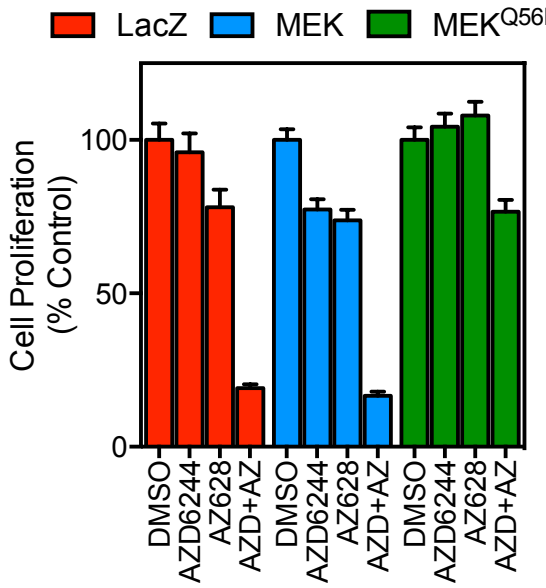


Figure S12. Synergy between pan-RAF inhibitors and MEK inhibitors is blocked by a drug-resistant allele of MEK.

(A) RKO cells were infected with lentiviral vectors encoding ORFs for LacZ, MEK and MEK^{Q56P}. 72 h after infection, cells were treated with 1 μM AZ6244, 0.1 μM AZ628 and a combination of the two inhibitors for 18 h. Cell lysates were analyzed for the indicated proteins.

(B) Cells were treated as in A and following a 96 h exposure to the inhibitors, cell proliferation was assessed by CellTiter-Glo.

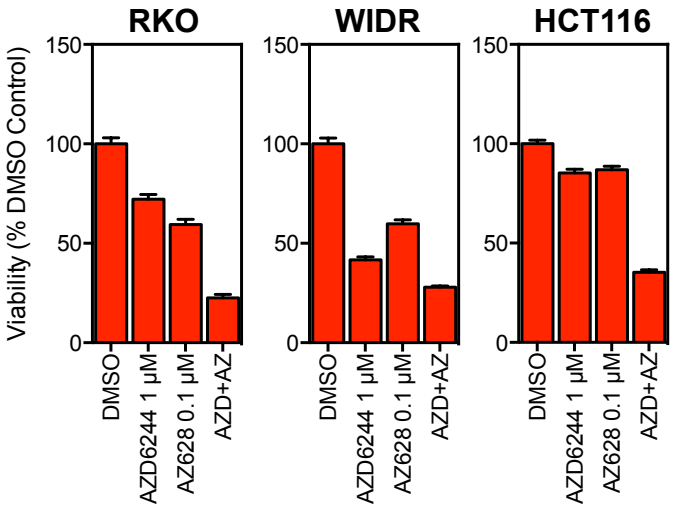


Figure S13. Cell viability is decreased by combined pan-RAF and MEK inhibition.
RKO, WIDR and HCT116 cells were treated with 1 μM AZD6244, 0.1 μM AZ628 or a combination of the two for 72 h. The relative number of viable cells was determined using the CytoTox-Glo assay.