

**Figure S1. AURK, PLK, and Tubulin inhibitors do not synergize with vemurafenib in UACC62P/R cells.** UACC62P/R cells were seeded into 384-well plates at a density of 1,000 cells/well. The next day the cells were treated in a concentration response matrix with a top concentration of 10 µM for all compounds and a ½ dilution series. Viability was analyzed as described in the Materials and Methods section. Data is expressed as relative viability wherein a value of 1 (blue) indicates 100% viability and a value of 0 (red) indicates 0% viability. **A.** UACC62P/R cells were treated with a GSK461364 x Vemurafenib concentration response matrix. **B.** UACC62P/R cells were treated with a MLN8237 x Vemurafenib concentration response matrix. **C.** UACC62P/R cells were treated with a Mebendazole x Vemurafenib concentration response matrix. This experiment was repeated with n = 3 biological replicates. The data in these matrices represent the average compound response across all experimental replicates.

**Figure S2. Identification of compound classes which are selective for BRAFi-resistant cells. A)** Overall compound representation in the MIPE library. AURKi, PLKi, Tubulin inhibitors, Kinesin inhibitors, and Chk1/2 inhibitors are highlighted. Compound class enrichment for the top 25 most selective compounds in **B)** UACC62P/R cells, **C)** M238P/R cells, **D)** M229P/R cells, and **E)** M249P/R cells.



**Figure S3. ROS production is not altered in BRAFi-resistant cells.** **A)** UACC62P/R and **B)** M229P/R cells were seeded into 96-well plates. The next day the cells were treated with H2O2 and the ROS assay was performed as described in the Materials and Methods with cells treated with H2O2 serving as a positive control. Relative ROS production is normalized to ROS levels in UACC62P (panel A) or M229P (panel B). This experiment was repeated with n = 3 technical replicates and n = 3 biological replicates.



**Figure S4. p-γH2AX staining is not altered in compound-treated UACC62P/R cells. A**) UACC62P/R cells were treated with 1 µM GSK461364, MLN8237, or Mebendazole for 24 h. The cells were fixed and stained with a p-γH2AX antibody and quantified as described *Materials and Methods*. **B)** Representative immunofluorescence images. This experiment was repeated with n = 3 biological replicates.

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**Figure S5. TNFα does not alter AURK, PLK, Tubulin, or Chk1/2 inhibitor sensitivity. A)** UACC62P or **B)** M229P cells were seeded into 384-well plates at a density of 1,000 cells/well. The next day the cells were treated -/+ 10 ng/mL TNFα and a concentration gradient of the indicated compound. Viability was measured and quantified as described in the Materials and Methods section. This experiment was repeated with n = 3 technical replicates and n = 3 biological replicates.

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**Figure S6.** **Cyclin B1, Cyclin A1, and PARP levels in MLN8237-treated UACC62P/R cells.** UACC62P and UACC62R cells were engineered to express EGFP-Cyclin B1 as described in the Materials and Methods section. The cells were treated with 1 µM MLN8237 for 24 h before cellular lysates were collected. Immunoblots were performed to measure levels of **(A)** Cyclin A1, **(B)** Cyclin B1, and **(C)** PARP. GAPDH was using as a loading control.



**Figure S7. Chk1/2 inhibitors do not synergize with vemurafenib in M229P/R cells.** M229P/R cells were seeded into 384-well plates at a density of 1,000 cells/well. The next day the cells were treated in a concentration response matrix with a top concentration of 10 µM for all compounds and a ½ dilution series. Viability was analyzed as described in the Materials and Methods section. Data is expressed as relative viability wherein a value of 1 (blue) indicates 100% viability and a value of 0 (red) indicates 0% viability. **A.** M229P/R cells were treated with a AZD7762 x Vemurafenib concentration response matrix. **B.** M229P/R cells were treated with a LY2603618 x Vemurafenib concentration response matrix. **C.** M229P/R cells were treated with a SCH900776 x Vemurafenib concentration response matrix. This experiment was repeated with n = 3 biological replicates. The data in these matrices represent the average compound response across all experimental replicates.

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**Figure S8: Chk1/2 inhibitors increase γH2AX staining in M229P/R cells.** M229P/R cells were treated with 100 nM AZD7762, 1 µM LY2603618, or 1 µM SCH900776 for 24 h. The cells were subsequently fixed and stained with an antibody raised against p-γH2AX. Scale bar = 10 µM. These images are color versions of the images in Fig. 4C. Scale bar = 10 µM.