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Supplementary Materials for

BRAF Gene Amplification Can Promote Acquired Resistance to MEK Inhibitors in Cancer Cells Harboring the BRAF V600E Mutation

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The PDF file includes:

Corcoran, et al. Figure S1

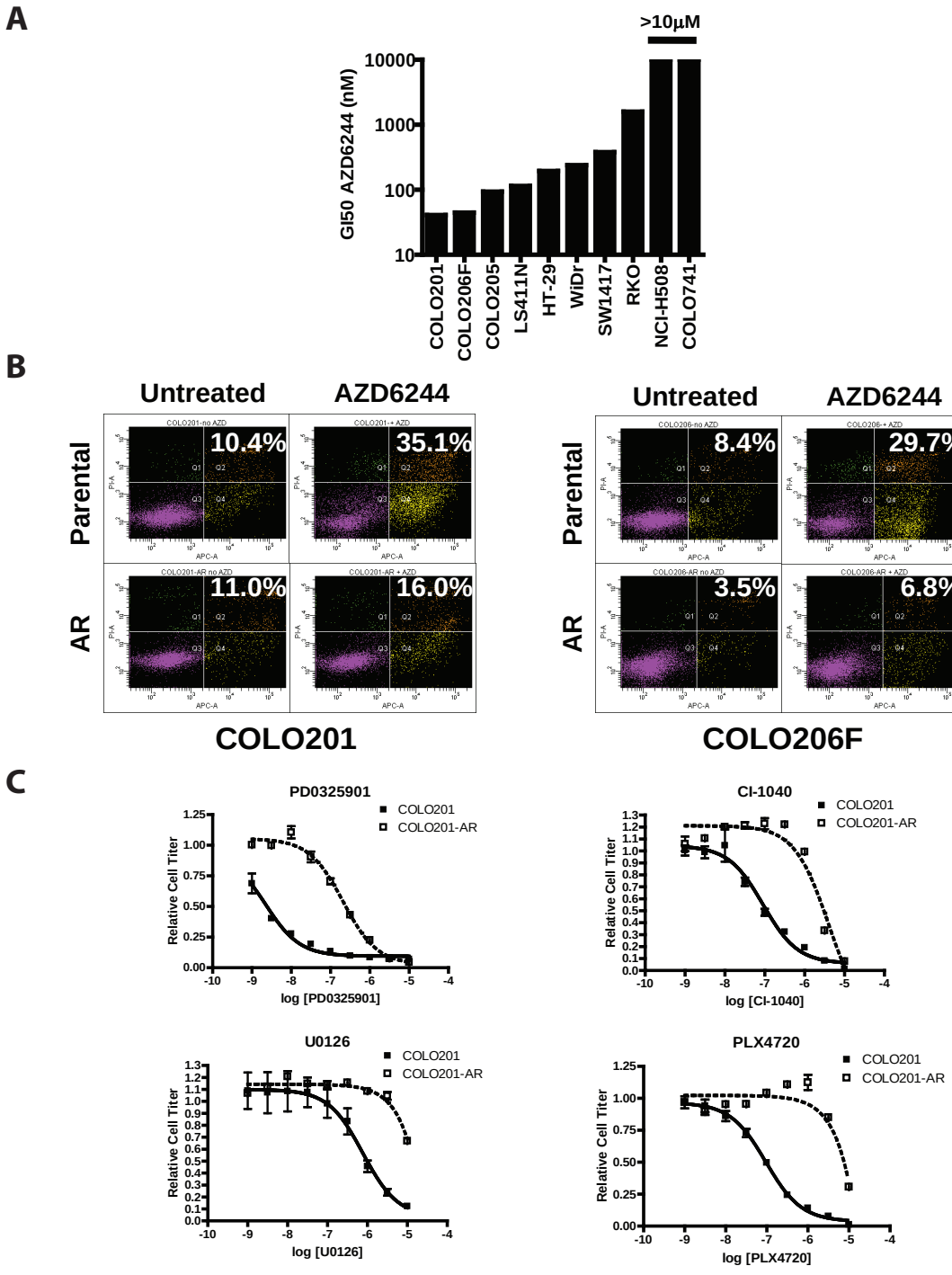

Fig. S1: AR cells are resistant to multiple MEK and BRAF inhibitors. (A.) GI50s (defined as the concentration required to inhibit viable cell titer to 50% of untreated control) of a panel of BRAF-mutant colorectal cancer cell lines treated with AZD6244 revealed that COLO201 and COLO206F were the two most sensitive cell lines. (B.) AR cells show reduced apoptotic response to AZD6244. Cells were treated for 72h in the presence or absence of 1 μM AZD6244. Apoptosis was assessed by propidium iodide (PI) and Annexin V staining with PI shown on the Y-axis and Annexin V on the X-axis. Percent apoptotic cells (shown in the upper right-hand corner of each frame) were calculated as the percent of Annexin-positive cells (total of right-upper and right lower quadrants). (C.) AR cells exhibit resistance to MEK inhibitors and the selective BRAF inhibitor PLX4720. Parental (solid lines) and AR (dashed lines) COLO201 cells were treated with the indicated concentrations of MEK inhibitors (PD0325901, CI-1040, and U0126) or the selective BRAF inhibitor PLX4720 for 72h. Viable cell titer was determined as described in Fig. 1A.

Corcoran, et al. Figure S2

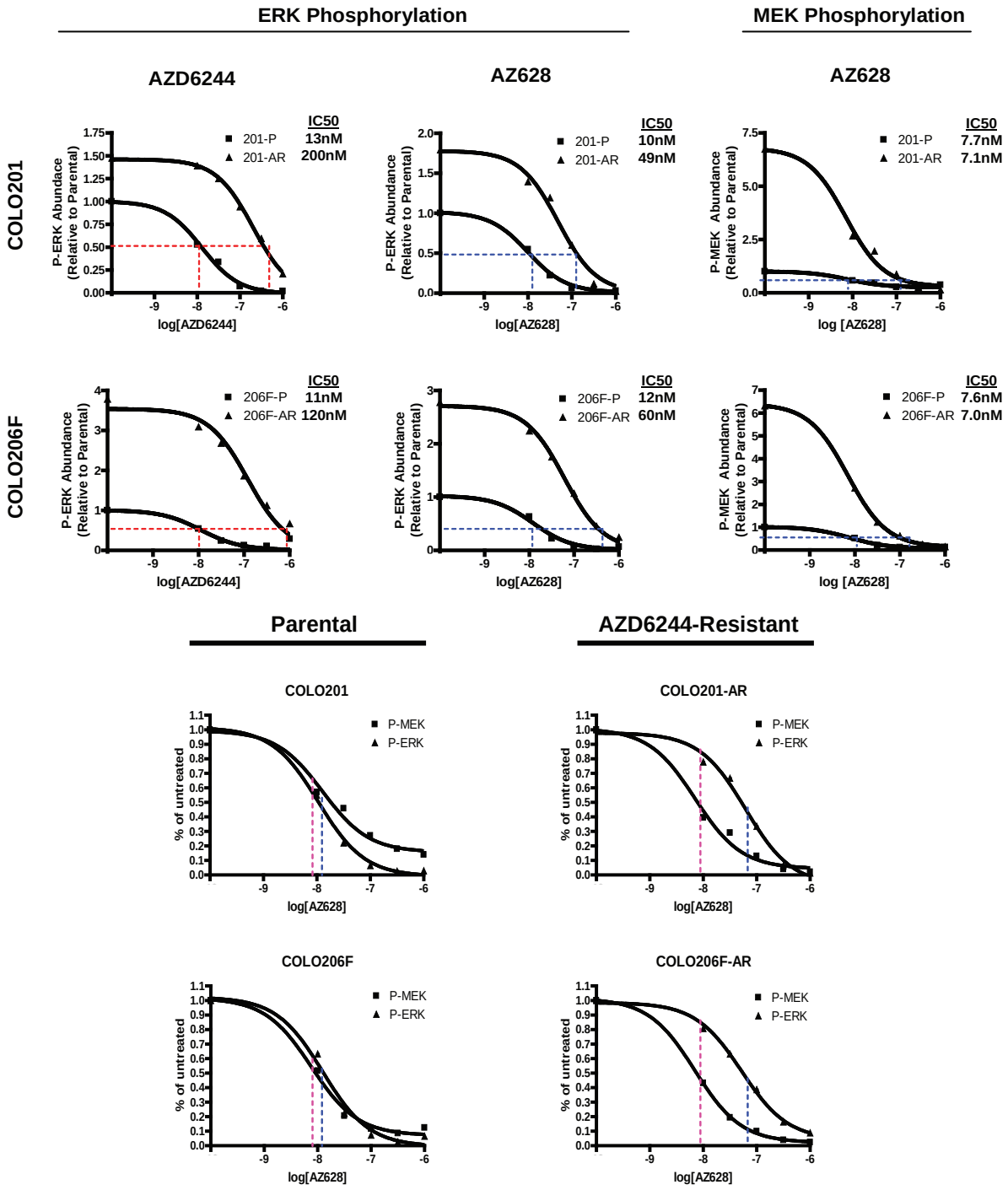

Fig. S2: The ability of AZD6244 and AZ628 to inhibit ERK phosphorylation is attenuated in AR cells. (A.) Dose-response relationships for AZD6244 and AZ628 with respect to inhibition of ERK or MEK phosphorylation were generated by plotting phospho-ERK (P-ERK) or phospho-MEK (P-MEK) abundance versus inhibitor concentration. Phospho-ERK and phospho-MEK abundance was determined by chemiluminescence signal intensity measurements from the Western blots shown in Figs. 1B and 1C. Values are shown relative to untreated parental cells. Approximately 1 μM AZD6244 or 100 to 300 nM AZ628 was required to reduce P-ERK to 50% of the basal P-ERK found in the parental cells (indicated by dashed lines). The IC50s for inhibition of ERK phosphorylation, shown for each graph, are increased in AR cells. The IC50 of AZ628 for MEK phosphorylation is unchanged in AR relative to parental cells. However, because of the marked increase in P-MEK abundance in AR cells, ~100-300 nM AZ628 is required to reduce P-MEK to 50% of the basal abundance observed in the parental cells (indicated by dashed lines). (B.) Dose-response relationships for P-MEK and P-ERK in parental and AR cells treated with AZ628. Chemiluminescence signal intensity measurements for P-MEK and P-ERK obtained as in (A.) are shown relative to untreated control for each cell line. The relationship between P-MEK and P-ERK is shifted in the AR cells relative to the parental cells. Dashed lines indicate the concentration of AZ628 required to reduce P-MEK abundance (pink) or P-ERK abundance (blue) by 50% relative to untreated control for each cell line.

Corcoran, et al. Figure S3

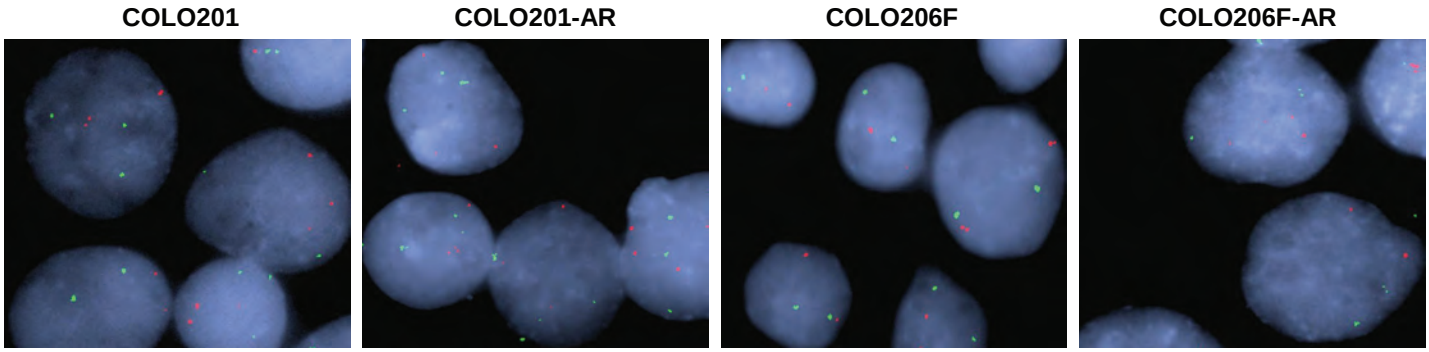

Fig. S3: *CRAF* is not amplified in AR cells. FISH images demonstrating that CRAF is not amplified in AR cells. *CRAF* is shown in orange, centromere control in green, and nuclear stain in blue.

Corcoran, et al. Figure S4

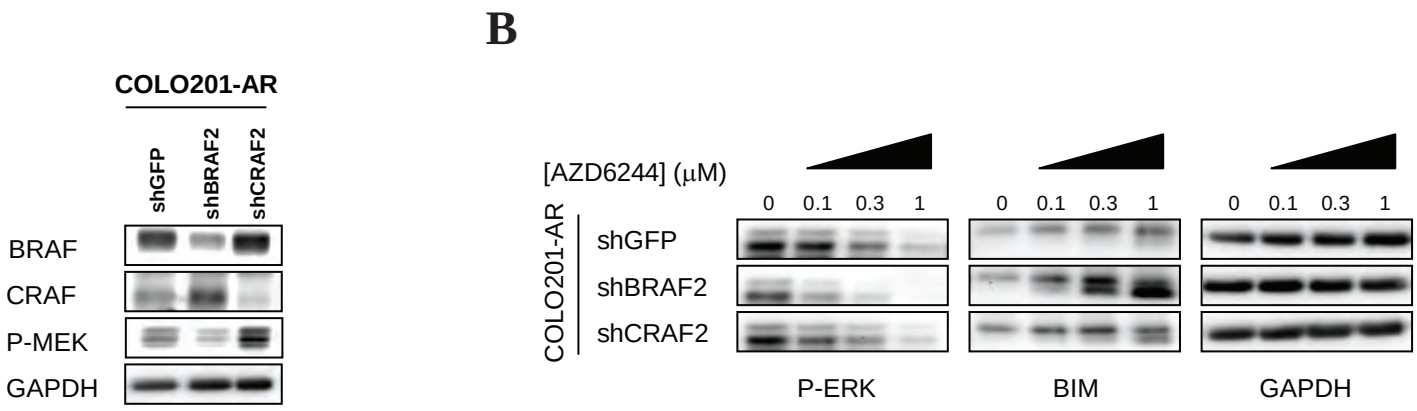

Fig. S4: Knockdown of BRAF, but not CRAF, restores the ability of AZD6244 to inhibit ERK phosphorylation. (A.) Western blot of COLO201 cells infected with lentiviruses encoding control shRNA (shGFP) or shRNAs directed against BRAF or CRAF. Cell lysates were made following 5d of puromycin selection and were probed with the indicated antibodies. Experiments were repeated at least three times. (B.) COLO201-AR cells infected with the indicated shRNAs were selected in puromycin for 96h and treated with the indicated concentrations of AZD6244 for an additional 24h. Cell lysates were probed with the indicated antibodies.

Corcoran, et al. Figure S5

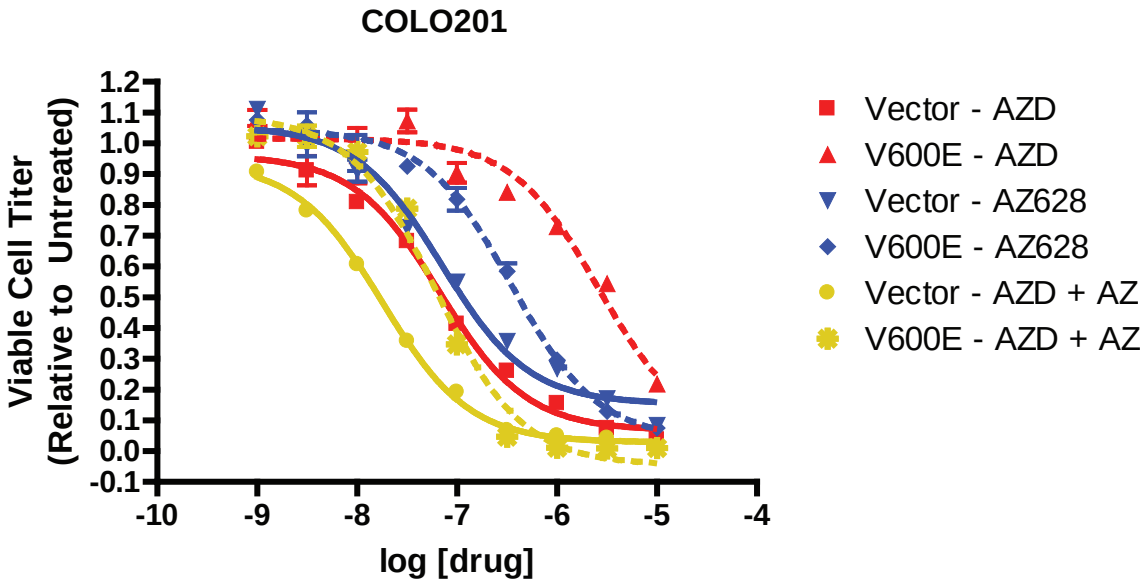

Fig. S5: Resistance caused by overexpression of V600E BRAF can be overcome by combined MEK and BRAF inhibition. COLO201 cells expressing vector control (Vector) or V600E BRAF (V600E) as in Fig. 3A were treated with the indicated concentrations of AZD6244 (AZD), AZ628, or with a combination of each inhibitor at the indicated concentrations (AZD + AZ), and cell viability was determined as in Fig. 1A.

Corcoran, et al. Figure S6

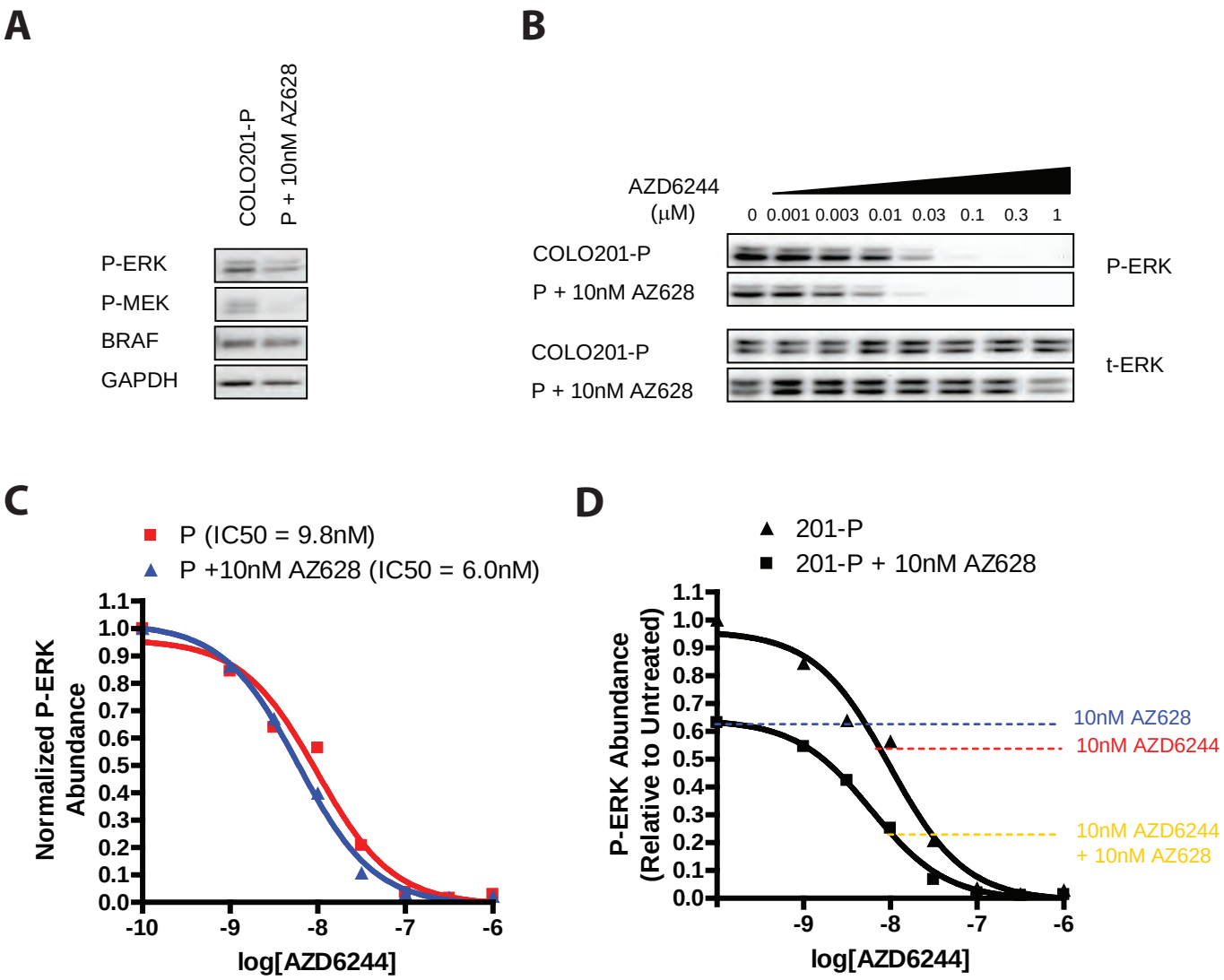

Fig. S6: Enhanced inhibition of ERK phosphorylation underlies the increased sensitivity of parental cells to combination treatment with AZD6244 and AZ628. (A.) Parental COLO201 cells (P) were treated for 24h in the presence or absence of 10 nM AZ628. Lysates were probed with the indicated antibodies. (B.) Western blot analyses of parental COLO201 cells (P) treated for 24h in the presence or absence of 10 nM AZ628 and increasing concentrations of AZD6244. (C, D.) Dose response curves for the decrease in P-ERK abundance were generated as in Fig. 4F by plotting P-ERK abundance versus AZD6244 concentration. P-ERK abundance was determined from chemiluminescence signal intensity measurements from the blots in (B). P-ERK was normalized to that in cells in the absence of AZD6244 (C) or to untreated parental cells in the absence of AZ628 (D). Absolute amounts of P-ERK (normalized to GAPDH loading control) for 10 nM AZD6244 alone (red), 10 nM AZ628 alone (blue), or 10 nM of combined AZD6244 and AZ628 (gold) are shown for example.

Corcoran, et al. Supplemental Table 1

Human *BRAF*-mutated colorectal tumors

Patient #	Average *BRAF* Copy Number	Average *BRAF*/Chr7 Ratio	% of Cells with *BRAF* Amplification
1	2.0	1.0	0
2	2.6	1.0	0
3	1.9	0.9	0
4	1.5	1.0	0
5	4.0	1.8	28
6	2.1	0.9	0
7	1.6	1.1	0
8	1.7	0.9	0
9	1.4	1.0	0
10	1.6	0.9	0
11	1.8	1.0	0

Table S1: *BRAF* amplification in *BRAF*-mutant human colorectal cancer. FISH analysis for *BRAF* was performed on 11 human colorectal tumors known to harbor the *BRAF* V600E mutation. The average *BRAF* copy number and the average *BRAF* to chromosome 7 ratio (*BRAF*/Chr7) was determined by counting and scoring 50 cells from each tumor. The percentage of cells counted in each tumor with *BRAF* amplification, as described in Materials and Methods, is also shown.