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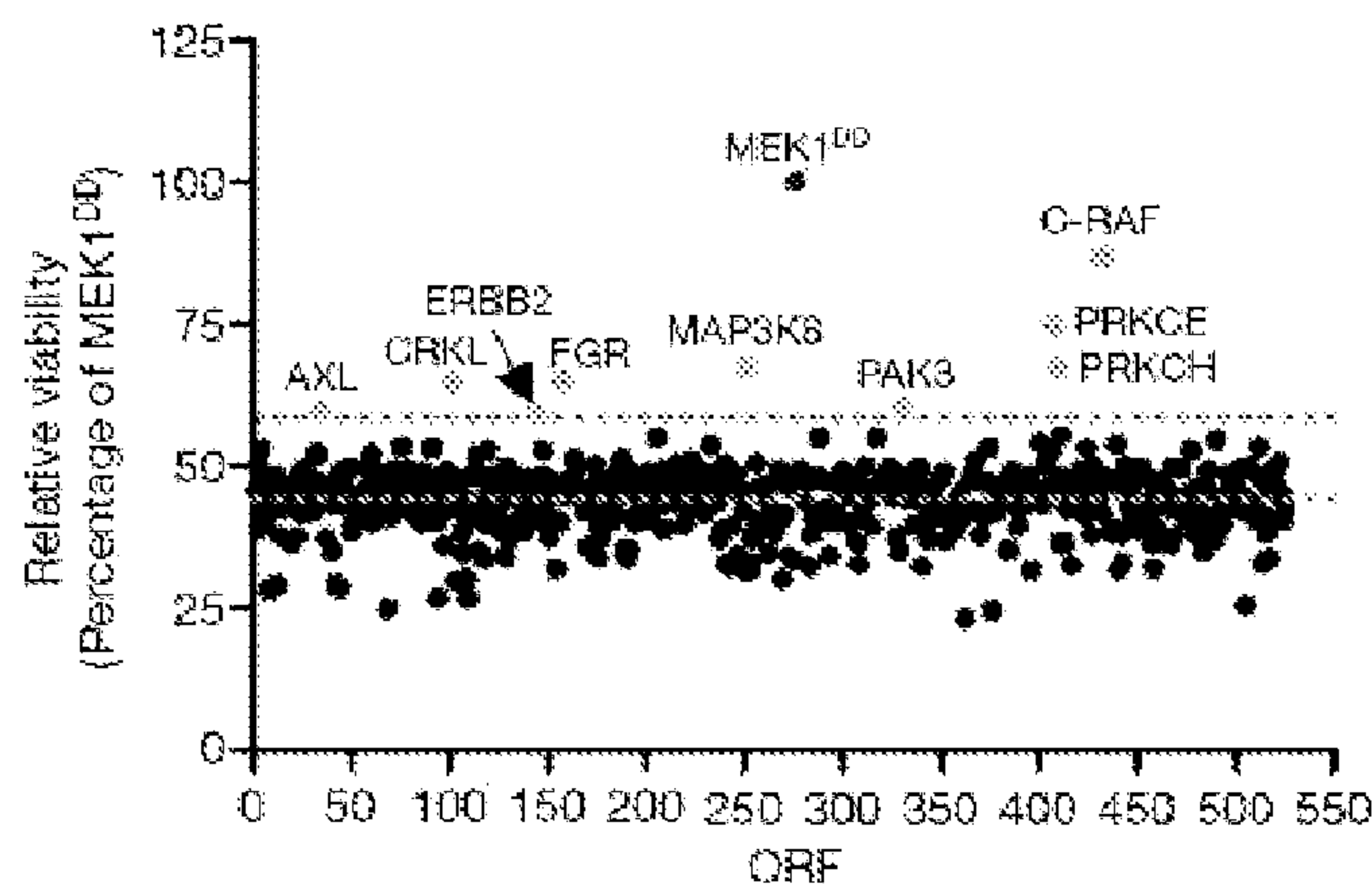
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(57) Abrégé/Abstract:

A method of identifying a subject having cancer who is likely to benefit from treatment with a combination therapy with a RAF inhibitor and a second inhibitor is provided. A method of treating cancer in a subject in need thereof is also provided and includes administering to the subject an effective amount of a RAF inhibitor and an effective amount of a second inhibitor, wherein the second inhibitor is a MEK inhibitor, a CRAF inhibitor, a CrkL inhibitor or a TPL2/COT inhibitor. A method of identifying a kinase target that confers resistance to a first inhibitor is also provided.

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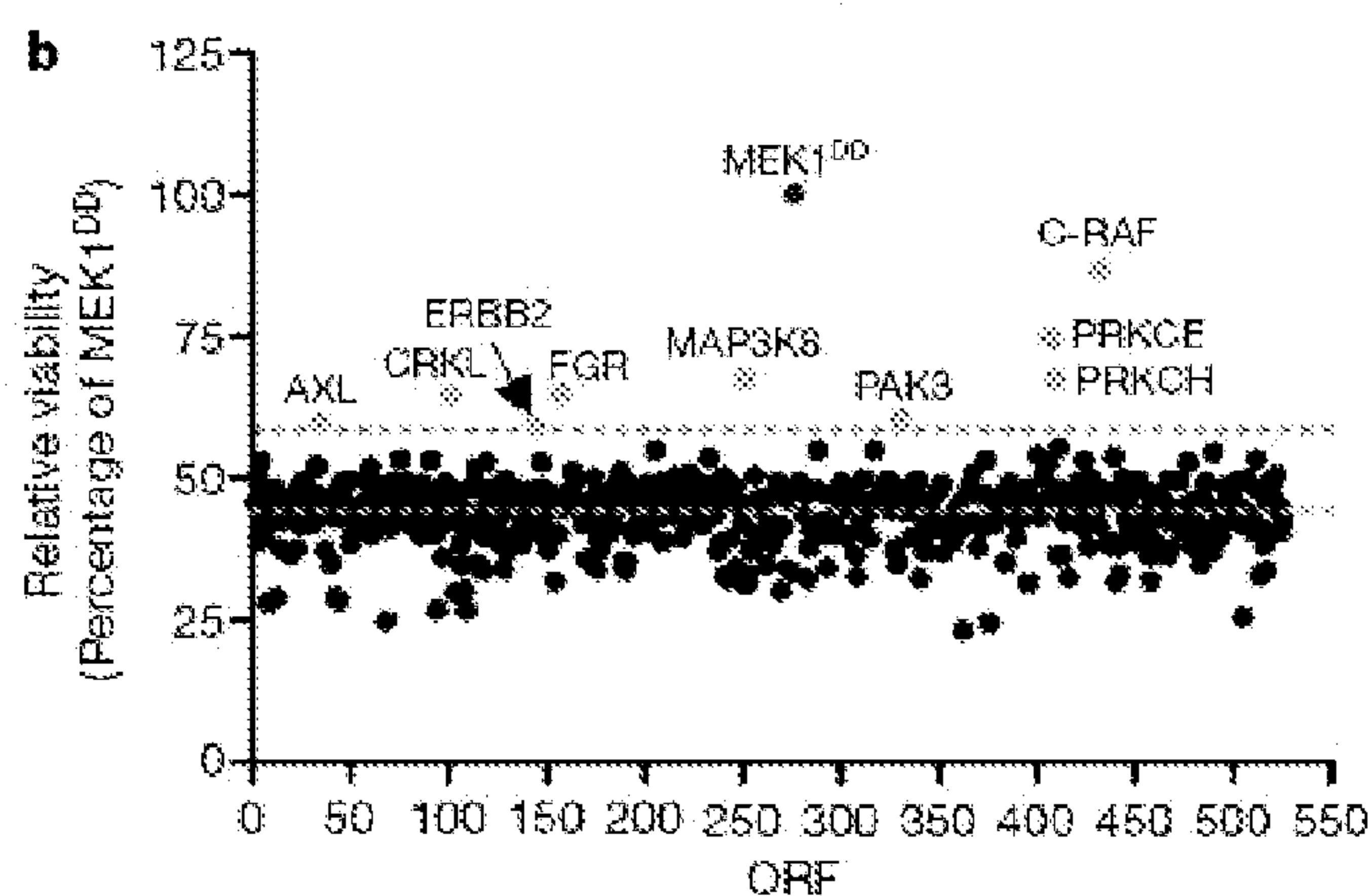


Figure 1

(57) Abstract: A method of identifying a subject having cancer who is likely to benefit from treatment with a combination therapy with a RAF inhibitor and a second inhibitor is provided. A method of treating cancer in a subject in need thereof is also provided and includes administering to the subject an effective amount of a RAF inhibitor and an effective amount of a second inhibitor, wherein the second inhibitor is a MEK inhibitor, a CRAF inhibitor, a CrkL inhibitor or a TPL2/COT inhibitor. A method of identifying a kinase target that confers resistance to a first inhibitor is also provided.

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METHODS OF DIAGNOSING AND TREATING CANCER IN PATIENTS HAVING OR DEVELOPING RESISTANCE TO A FIRST CANCER THERAPY

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BACKGROUND

[0003] Oncogenic mutations in the serine/threonine kinase B-RAF (also known as BRAF) are found in 50-70% of malignant melanomas. (Davies, H. et al., Nature **417**, 949-954 (2002).) Pre-clinical studies have demonstrated that the B-RAF(V600E) mutation predicts a dependency on the mitogen-activated protein kinase (MAPK) signalling cascade in melanoma (Hoeflich, K. P. et al., Cancer Res. 69, 3042-3051(2009) ; McDermott, U. et al., Proc. Natl Acad. Sci. USA 104, 19936-19941 (2007); Solit, D. B. et al. BRAF mutation predicts sensitivity to MEK inhibition. Nature 439, 358-362 (2006); Wan, P. T. et al., Cell 116, 855-867 (2004); Wellbrock, C. et al., Cancer Res. 64, 2338-2342 (2004))— an observation that has been validated by the success of RAF or MEK inhibitors in clinical trials (Flaherty, K. T. et al., N. Engl. J. Med. 363, 809-819 (2010) ; Infante, J. R. et al., J. Clin. Oncol. 28 (suppl.), 2503 (2010); Schwartz, G. K. et al., J. Clin. Oncol. 27 (suppl.), 3513 (2009).) However, clinical responses to targeted anticancer therapeutics are frequently confounded by de novo or acquired resistance. (Engelman, J. A. et al., Science 316, 1039-1043 (2007); Gorre, M. E. et al., Science 293, 876-880 (2001) ; Heinrich, M. C. et al., J. Clin. Oncol. 24, 4764-4774 (2006);

Daub, H., Specht, K. & Ullrich, A. *Nature Rev. Drug Discov.* 3, 1001–1010 (2004).) Accordingly, there remains a need for new methods for identification of resistance mechanisms in a manner that elucidates “druggable” targets for effective long-term treatment strategies, for new methods of identifying patients that are likely to benefit from the treatment strategies, and for methods of treating patients with the effective long-term treatment strategies.

BRIEF SUMMARY

[0004] The present invention relates to the development of resistance to therapeutic agents in the treatment of cancer and identification of targets that confer resistance to treatment of cancer. The present invention also relates to identification of parallel drug targets for facilitating an effective long-term treatment strategy and to identifying patients that would benefit from such treatment.

[0005] Accordingly, in one aspect, a method of identifying a subject having cancer who is likely to benefit from treatment with a combination therapy with a RAF inhibitor and a second inhibitor is provided. The method includes assaying a gene copy number, a mRNA or a protein level or phosphorylation of one or more kinase targets selected from the group consisting of *MAP3K8* (TPL2/COT), *RAF1* (CRAF), *CRKL* (CrkL), *FGR* (Fgr), *PRKCE* (Prkce), *PRKCH* (Prkch), *ERBB2* (ErbB2), *AXL* (Axl), or *PAK3* (Pak3) in cancer cells obtained from the subject. The method further includes comparing the gene copy number, the mRNA or the protein level or the phosphorylation with a gene copy number, a mRNA or a protein level or phosphorylation of the target kinase in cells obtained from a subject without the cancer and correlating increased gene copy number or an alteration in mRNA expression or protein overexpression or phosphorylation of the target kinase in the cancer cells relative to the cells from the subject without the cancer with the subject having the cancer who is likely to benefit from treatment with the combination therapy.

[0006] In another aspect, a method of treating cancer in a subject in need thereof is provided. The method includes administering to the subject an effective amount of a RAF inhibitor and an effective amount of a second inhibitor, wherein the second inhibitor is a MEK inhibitor or a TPL2/COT inhibitor.

[0007] In another aspect, a method of identifying a kinase target that confers resistance to a first inhibitor is provided. The method includes culturing cells having sensitivity to the first inhibitor and expressing a plurality of kinase ORF clones in the cell cultures, each cell culture expressing a different kinase ORF clone. The method further includes exposing each cell culture to the inhibitor and identifying cell cultures having greater viability than a control cell culture after exposure to the inhibitor to identify the kinase ORF clone that confers resistance to the first inhibitor.

BRIEF DESCRIPTION OF THE DRAWINGS

[0008] **Figure 1** illustrates an ORF-based functional screen which identified COT and C-RAF kinases as drivers of resistance to B-RAF inhibition. (a) Schematic overview of the CCSB/Broad Institute Kinase ORF collection. Kinase classification and number of kinases per classification are noted; (b) A375 cells expressing the CCSB/Broad Institute Kinase ORF collection were assayed for relative viability in 1 μ M PLX4720 and normalized to constitutively active MEK1 (MEK1DD). Nine ORFs (circles) scored 2 standard deviations (dashed line, 58.64%) from the mean of all ORFs (dashed line, 44.26%); (c) Indicated ORFs were expressed in 5 B-RAF^{V600E} cell lines and treated with DMSO or 1 μ M PLX4720. Viability (relative to DMSO) was quantified after 4 days. Error bars represent standard deviation between replicates (n=6); (d) Secondary screen in A375 and SKMEL28 prioritizes the top 9 candidate ORFs across a multipoint PLX4720 concentration scale.

[0009] **Figure 2** illustrates resistance to B-RAF inhibition via MAPK pathway activation. (a) Indicated ORFs were expressed in A375. Levels of phosphorylated MEK and ERK were assayed following 18 h. treatment with DMSO (-) or PLX4720 (concentration noted); (b) Proliferation of A375 expressing indicated ORFs. Error bars represent standard deviation between replicates (n=6). (c) C-RAF (S338) and ERK phosphorylation in lysates from A375 expressing indicated ORFs. (d) COT expression in lysates from immortalized primary melanocytes expressing BRAFV600E or empty vector. COT mRNA has an internal start codon (30M) resulting in two protein products of different lengths; amino acids 1-467 or 30-467, noted with arrows. (e) COT and ERK phosphorylation in lysates from A375 expressing indicated ORFs following

shRNA-mediated B-RAF depletion (shBRAF) relative to control shRNA (shLuc). (f) ERK phosphorylation in lysates from A375 expressing indicated ORFs following shRNA-mediated C-RAF depletion (shCRAF) or control shRNA (shLuc), following 18 h. treatment with DMSO (-) or 1 μ M PLX4720 (+).

[0010] **Figure 3** illustrates COT expression predicts resistance to B-RAF inhibition in cancer cell lines. (a) *MAP3K8*/COT copy number; red bars: COT amplification, blue bars: non-amplified COT; (b) COT expression in B-RAF^{V600E} cell lines and (c) short-term cultures; (d) PLX4720 GI50 in B-RAF^{V600E} cell lines. Colors as in (a); (e) MEK and ERK phosphorylation following treatment with DMSO or PLX4720 (concentration indicated); (f) ERK phosphorylation in M307 lysates (AZD-R; AZD6244-resistant) treated with DMSO or 1 μ M PLX4720 (PLX) or CI-1040 (CI); (g) COT mRNA expression (QRT/PCR) in patient/lesion-matched PLX4032-treated metastatic melanoma tissue samples. Pts. 1 and 3 had multiple biopsies from the same lesion. Error bars represent SEM (n=3) U; undetermined/undetectable; (h) ERK and MEK phosphorylation in RPMI-7951 following shRNA-mediated COT depletion (shCOT) versus control (shLuc) and treatment with DMSO (-) or 1 μ M PLX4720 (+). ERK and MEK phosphorylation are quantified; (i) ERK and MEK phosphorylation in RPMI-7951 following 1 h. treatment with a small molecule COT kinase inhibitor. ERK and MEK phosphorylation are quantified. (j) PLX4720 sensitivity curves in a panel of BRAF^{V600E} cell lines. OUMS-23 and M307 represent cell lines with COT expression/amplification and all others represent cell lines with undetectable/unaltered COT; (k) Selective expression of COT and corresponding MAPK pathway activation in a metastatic subcutaneous malignant melanoma with acquired resistance to PLX4032 (* denotes a background band, MET-MM (PLX-R); metastatic malignant melanoma, PLX4032-resistant).

[0011] **Figure 4** illustrates COT-expressing B-RAFFV600E cell lines exhibit resistance to allosteric MEK inhibitors. (a) CI-1040 GI50 in a panel of B-RAFFV600E cell lines; (b) MEK and ERK phosphorylation in lysates from indicated cell lines treated with DMSO or CI-1040 (concentration noted); (c) Fold change (relative to MEK1) GI50 of A375 ectopically expressing the indicated ORFs for PLX4720, RAF265, CI-1040 and AZD6244; (d) ERK phosphorylation in A375 expressing indicated ORFs following treatment with DMSO or 1 μ M of PLX4720, RAF265, CI-1040 or AZD6244; (e) Viability

of A375 expressing the indicated ORFs and treated with DMSO, PLX4720 (concentration indicated) and PLX4720 in combination with CI-1040 or AZD6244 (all 1 μ M). Error bars represent the standard deviation ($n=6$); (f) ERK phosphorylation in A375 expressing indicated ORFs following treatment with DMSO, PLX4720 (1 μ M) or PLX4720 in combination with CI-1040 or AZD6244 (all 1 μ M); (g) Cell lines with aberrant MAP3K8/COT copy number/expression are insensitive to the allosteric MEK inhibitor CI-1040 or (h) AZD6244; (i) A schematic outlining the formation of MAP3K complexes in response to B-RAF inhibition in B-RAF^{V600E}-mutant cell lines. PLX4720 positions C-RAF in a signaling-competent complex (upper right panel) that is activated by oncogenic events upstream of C-RAF (lower right panel), subsequently driving resistance. In the context of COT expression, COT/RAF-containing complexes are sufficient to activate the MAPK pathway and mediate resistance (lower left panel).

[0012] Figure 5 illustrates a schematic outline of an ORF-based functional screen for kinases that drive resistance to B-RAF inhibition. The B-RAF^{V600E} cell line A375 was lentivirally transduced with the 597 kinases in the CCSB/Broad Institute Kinase ORF Collection. ORFs having a positive or negative effect on proliferation in control-treated A375 were identified and removed from final analysis. Resistance-promoting ORFs were identified by generating a differential viability ratio between B-RAF inhibited (PLX4720-treated) and control-treated cells. Differential viability was normalized to a constitutively active MEK1 allele, MEK1^{DD}; an assay specific positive control.

[0013] Figure 6 illustrates that the CCSB/Broad Institute Kinase ORF Collection is well expressed via high titer lentivirus. (a), schematic of the pLX-BLAST-V5 lentiviral expression vector used for all ORF-screens and subsequent validation. (b) GFP-tagged ORFs representing a broad size range were lentivirally expressed in Jurkat cells and the percentage of GFP-expressing cells/ORF (e.g., infected cells) quantified, demonstrating high viral titer across a range of ORF sizes. (c), expression of 96 random ORFs detected via LiCor with antibodies against the V5-epitope tag, relative to cellular DNA. Expression was detectable in 83% of the wells.

[0014] Figure 7 illustrates the expression of candidate resistance ORFs. 293T were transiently transfected with pLX-BLAST-V5-ORF (indicated) and expression detected using an anti-V5-HRP antibody. The AXL clone is 'closed' and has a stop codon

preceding the V5 tag. **See Figure 12** for verification of expression; (*) on dark-exposure indicate the expression of three ORFs not visible in the lighter exposure.

[0015] **Figure 8** illustrates that a secondary screen prioritizes the top 9 candidate B-RAF inhibitor resistance ORFs. The top nine ORFs scoring in the primary screen were expressed in A375 or SKMEL28 and a GI_{50} from an 8-point PLX4720 concentration range.

[0016] **Figure 9** illustrates the effects of ORF expression on proliferation in B-RAF^{V600E} cell lines. Proliferation, relative to MEK1, in (a) A375 or (b) SKMEL28 expressing indicated ORFs after 7 days of growth.

[0017] **Figure 10** illustrates that ectopic expression of constitutively active MEK1 (MEK1^{DD}) and COT lead to increased pMEK/pERK in A375, whereas C-RAF reduces pMEK/pERK levels. Lysates from A375 ectopically expressing GFP, MEK1, MEK^{DD}, COT or C-RAF were analyzed via immunoblot for levels of pERK and pMEK. GFP and MEK1 (lanes 1-3) were separated from COT/C-RAF (lanes 4-5) to prevent residual V5-MEK1 signal from overwhelming that of COT and C-RAF, which are expressed at much lower levels.

[0018] **Figure 11** illustrates that the kinase activity of COT and C-RAF is required for sustained ERK phosphorylation in the context of PLX4720 treatment. Immunoblot analysis of A375 expressing ectopic (a) MEK1, wild type COT or kinase inactive COT (COT^{K167R}) or (b) MEK1, wild type C-RAF or kinase inactive C-RAF (C-RAF^{K375M}) treated with 1 μ M PLX4720 for 18 h.

[0019] **Figure 12** illustrates the effects of ORF expression on MAPK signaling in the context of the B-RAF inhibitor PLX4720. MAPK pathway activation was assessed by immunoblot analysis of pERK and pMEK in A375 expressing the indicated ORFs in the presence of PLX4720 (18 h., concentration indicated). (*) indicates the use of an antibody directed against the expressed ORF, not the V5 epitope. AXL was cloned without the V5 tag.

[0020] **Figure 13** illustrates that B-RAF associates with immunoprecipitated C-RAF in A375 following 18 hr. treatment with 1 μ M PLX4720 (+) or DMSO (-), (a). WCE; whole cell extract controls. Ectopically expressed C-RAF constitutively associates with B-RAF

and is phosphorylated at S338, consistent with membrane localization and activation. MEK1, MEK^{DD} and COT-expressing A375 show no evidence of C-RAF activation, (b).

[0021] **Figure 14** illustrates that Retroviral expression of a wild-type C-RAF or a high-activity truncation mutant of C-RAF (C-RAF(22W)) renders A375 resistant to the B-inhibitor PLX4720 (a) and leads to sustained pERK levels in the context of PLX4720 treatment (1 μ M, 18 h.), (b). C-RAF expression levels achieved with retroviruses are significantly lower than with lentiviral-based systems, resulting in a lower GI₅₀ than that achieved with lentiviral C-RAF.

[0022] **Figure 15** illustrates the effects of B-RAF^{V600E} on COT mRNA (a) Quantitative RT/PCR of COT mRNA expression relative to GAPDH mRNA expression in transformed primary melanocytes expressing wild-type B-RAF (vector) or B-RAF^{V600E}. COT expression was normalized to that of vector-expressing primary melanocytes. (**) Significant, $p < 0.05$ (Student's two-tailed, paired T-Test). Endogenous COT mRNA is undetectable in PLX4720-sensitive A375 and ectopically expressed COT mRNA levels are unaffected by 1 μ M PLX4720 treatment. A375 expressing GFP or COT were treated for 18 h. with 1 μ M PLX4720. Reverse-transcribed mRNA was analyzed for GAPDH-normalized COT expression, relative to GFP-expressing, DMSO treated, A375. (*) Not significant, $p > 0.05$ (Student's two-tailed, paired T-Test). Error bars represent SEM.

[0023] **Figure 16** illustrates that B- and C-RAF protein levels are not required for COT-mediated ERK phosphorylation. A375 expressing ectopic MEK1 (control) or COT were sequentially infected with lentivirus expressing shRNAs targeting B-RAF, C-RAF or control shRNA (shLuc) and assayed for expression of the indicated proteins in the presence (+) or absence (-) of 1 μ M PLX4720, 18 h.

[0024] **Figure 17** illustrates SNP analysis of 752 cell lines reveals copy number alterations in *MAP3K8/COT*. Of the 752 cell lines that had undergone copy number analysis, 534 had also undergone mutation profiling. Thirty-eight (7.1%) of mutation-profiled cells harbor the B-RAF^{V600E} mutation. Two cell lines (OUMS-23, RPMI-7951, indicated) harbor the B-RAF^{V600E} mutation along with copy number gain in *MAP3K8/COT*.

[0025] **Figure 18** illustrates *MAP3K8/COT* alterations in the cancer cell line OUMS-23. (a) RMA signal of a *MAP3K8 /COT* probe (noted) from mRNA microarray analysis.

OUMS-23 is one of the top 2% (of 765 cell lines) expressing COT mRNA. (b) COT mRNA expression in a panel of B-RAF^{V600E}-mutant cell lines. (c) Endogenous COT protein expression in OUMS-23 relative to ectopically expressed COT in A375 and SKMEL28 cell lines, as determined via immunoblot analysis of the indicated cells.

[0026] **Figure 19** illustrates that COT mRNA and protein are expressed in B RAF-inhibitor resistant cell lines and tissue. (a) RT/PCR analysis of GAPDH normalized COT mRNA expression in a panel of cell lines, short term cultures and tissue from relapsed, PLX4032-treated, malignant melanoma (MM-R). Corresponding protein expression for cell lines and short term cultures are shown in **Figures 3b and 3c**, respectively. (b) Western blot analysis of lysates from primary melanocytes (1° Mel(B-RAF WT)), patient matched normal skin (Skin) and metastatic malignant melanoma (MM-R; COT mRNA shown in panel a), A375 cells and primary melanocytes expressing B-RAF^{V600E} (1° Mel (B-RAFV600E)).

[0027] **Figure 20** illustrates that depletion of COT affects viability in the COT amplified cell line RPMI-7951. (a) Quantification of RPMI-7951 viability following lentiviral shRNA-mediated COT depletion (shCOT), relative to control shRNA (shLuc). Error bars represent standard deviation between replicates. (b) Immunoblot analysis showing relative COT protein expression in shLuc and shCOT-expressing RPMI-7951.

[0028] **Figure 21** illustrates effects of ORF expression on the GI₅₀ of a panel of MAPK pathway inhibitors in SKMEL28. The half-maximal growth-inhibitory concentration (GI₅₀) of SKMEL28 ectopically expressing MEK1, MEK1^{DD} or COT was determined for the RAF inhibitors PLX4720 and RAF265 and the MEK1/2 inhibitors CI-1040 and AZD6244. The change in GI₅₀ for MEK1^{DD} and COT (relative to MEK1) was determined for each compound.

[0029] **Figure 22** illustrates that COT can activate ERK via MEK-independent and MEK-dependent mechanisms. (a) Immunoblot analysis of ERK phosphorylation in lysates from A375 following expression of GFP or COT and subsequent lentiviral shRNA-mediated MEK1, MEK2 or MEK1 and MEK2 (MEK1+2) depletion, relative to control shRNA (shLuc). Left and right panels represent two different pairs of MEK1 and MEK2 shRNA constructs. (b) Immunoblot blot analysis of recombinant, inactive ERK phosphorylation (Thr202/Tyr204) by recombinant COT in an *in vitro* kinase assay.

[0030] **Figure 23** illustrates that combinatorial MAPK pathway inhibition effectively suppresses proliferation in SKMEL28. Viability (relative to DMSO) of SKMEL28 ectopically expressing MEK1, MEK1^{DD} or COT and treated with DMSO, PLX4720 (concentration indicated), PLX4720 (1 μ M) and CI-1040 (1 μ M) or PLX4720 (1 μ M) and AZD6244 (1 μ M). Error bars represent the standard deviation between replicates.

[0031] **Figure 24** illustrates that COT over expression is sufficient to render melanoma cancer cells with the B-RAF^{V600E} mutation resistant to B-RAF inhibition.

[0032] **Figure 25** illustrates the top nine ORFs scoring in the primary screen were expressed in (a) SKEL28 of (b) A375 and aGI50 is shown for 4 MAPK pathway inhibitors (PLX4720, RAF265, CI-1040, AZD-6244).

[0033] **Figure 26** illustrates that CRKL expression modifies sensitivity to the selective B-RAF inhibitor PLX4720 in a panel of B-RAFv600E cell lines.

[0034] **Figure 27** illustrates the *MAP3K8*/COT-amplified / B-RAF^{V600E}-mutant cancer cell line OUMS-23 shows constitutive phosphorylation of ERK/MEK across a dose range of PLX4720.

[0035] **Figure 28** illustrates the insensitivity to MAPK pathway inhibition corresponds with *MAP3K8*/COT copy number gains in a subset of skin cancer cell lines. A panel of 20 B-RAF^{V600E}-mutant cell lines and their sensitivity to (a) the B-RAF inhibitor PLX4720 and (b) the MEK inhibitor AZD6244 is shown.

DETAILED DESCRIPTION OF THE INVENTION

[0036] The present invention relates to the development of resistance to therapeutic agents in the treatment of cancer and identification of targets that confer resistance to treatment of cancer. The present invention also relates to identification of parallel drug targets for facilitating an effective long-term treatment strategy and to identifying patients that would benefit from such treatment. In some embodiments, the present invention relates to kinases and in particular to MAP kinase pathway components.

[0037] The practice of the present invention will employ, unless otherwise indicated, conventional techniques of molecular biology, immunology, microbiology, cell biology and recombinant DNA, which are within the skill of the art. See e.g., Sambrook, Fritsch and Maniatis, *MOLECULAR CLONING: A LABORATORY MANUAL*, (Current Edition);

CURRENT PROTOCOLS IN MOLECULAR BIOLOGY (F. M. Ausubel et al. eds., (Current Edition)); the series METHODS IN ENZYMOLOGY (Academic Press, Inc.): PCR 2: A PRACTICAL APPROACH (Current Edition) ANTIBODIES, A LABORATORY MANUAL and ANIMAL CELL CULTURE (R. I. Freshney, ed. (1987)). DNA Cloning: A Practical Approach, vol. I & II (D. Glover, ed.); Oligonucleotide Synthesis (N. Gait, ed., Current Edition); Nucleic Acid Hybridization (B. Hames & S. Higgins, eds., Current Edition); Transcription and Translation (B. Hames & S. Higgins, eds., Current Edition); Fundamental Virology, 2nd Edition, vol. I & II (B. N. Fields and D. M. Knipe, eds.)

[0038] The mitogen- activated protein kinase (MAPK) cascade is a critical intracellular signaling pathway that regulates signal transduction in response to diverse extracellular stimuli, including growth factors, cytokines, and proto-oncogenes. Activation of this pathway results in transcription factor activation and alterations in gene expression, which ultimately lead to changes in cellular functions including cell proliferation, cell cycle regulation, cell survival, angiogenesis and cell migration. Classical MAPK signaling is initiated by receptor tyrosine kinases at the cell surface, however many other cell surface molecules are capable of activating the MAPK cascade, including integrins, heterotrimeric G-proteins, and cytokine receptors.

[0039] Ligand binding to a cell surface receptor, e.g., a receptor tyrosine kinase, typically results in phosphorylation of the receptor. The adaptor protein Grb2 associates with the phosphorylated intracellular domain of the activated receptor, and this association recruits guanine nucleotide exchange factors including SOS-I and CDC25 to the cell membrane. These guanine nucleotide exchange factors interact with and activate the GTPase Ras. Common Ras isoforms include K-Ras, N-Ras, H-Ras and others. Following Ras activation, the serine/threonine kinase Raf (e.g., A-Raf, B-Raf or Raf-1) is recruited to the cell membrane through interaction with Ras. Raf is then phosphorylated. Raf directly activates MEK1 and MEK2 by phosphorylation of two serine residues at positions 217 and 221. Following activation, MEK1 and MEK2 phosphorylate tyrosine (Tyr-185) and threonine (Thr-183) residues in serine/threonine kinases Erk1 and Erk2, resulting in Erk activation. Activated Erk regulates many targets in the cytosol and also translocates to the nucleus, where it phosphorylates a number of transcription factors regulating gene expression. Erk kinase has numerous targets,

including Elk-1, c-Ets1, c-Ets2, p90RSK1, MNK1, MNK2, MSK1, MSK2 and TOB. While the foregoing pathway is a classical representation of MAPK signaling, there is considerable cross talk between the MAPK pathway and other signaling cascades.

[0040] Aberrations in MAPK signaling have a significant role in cancer biology. Altered expression of Ras is common in many cancers, and activating mutations in Ras have also been identified. Such mutations are found in up to 30% of all cancers, and are especially common in pancreatic (90%) and colon (50%) carcinomas. In addition, activating Raf mutations have been identified in melanoma and ovarian cancer. The most common mutation, BRAF^{V600E}, results in constitutive activation of the downstream MAP kinase pathway and is required for melanoma cell proliferation, soft agar growth, and tumor xenograft formation. Based on the defined role of MAPK over-activation in human cancers, targeting components of the MAPK pathway with specific inhibitors is a promising approach to cancer therapy. However, patients may have innate resistance or acquire resistance to these promising therapies. Identification of target kinases, diagnostic and/or prognostic markers and treatment therapies for these patients with innate or acquired resistance are described below.

[0041] High Throughput Functional Screening Assay

[0042] In some aspects, the present invention relates to methods of identifying targets capable of driving resistance to clinically efficacious therapies using a high throughput screening assay. In some embodiments, the method may include an open reading frame (ORF)-based functional screen for kinases that drive resistance to therapeutic agents. The method may include providing a cell line with a kinase known to have an oncogenic mutation. A library of kinase ORFs may be individually expressed in the cell line so that a plurality of clones each expressing a different ORF from the library may be further evaluated. Each clone may be (1) exposed to an inhibitor of the known kinase in the cell line and (2) monitored for growth changes based on the expression of the ORF in the cell line without the inhibitor. Any clones having a growth effect from the ORF expression alone, either positive or negative growth, are eliminated. The remaining clones each expressing a different kinase are then compared for viability between a control and a treated clone and normalized to a positive control. Increased cell viability after treatment with the inhibitor identifies ORFs that confer resistance and

therefore identifies kinase targets for treatment with an additional inhibitor. In some embodiments, clones scoring above two standard deviations from the normalized mean may be target kinases indicating treatment with an additional inhibitor is beneficial to the subject.

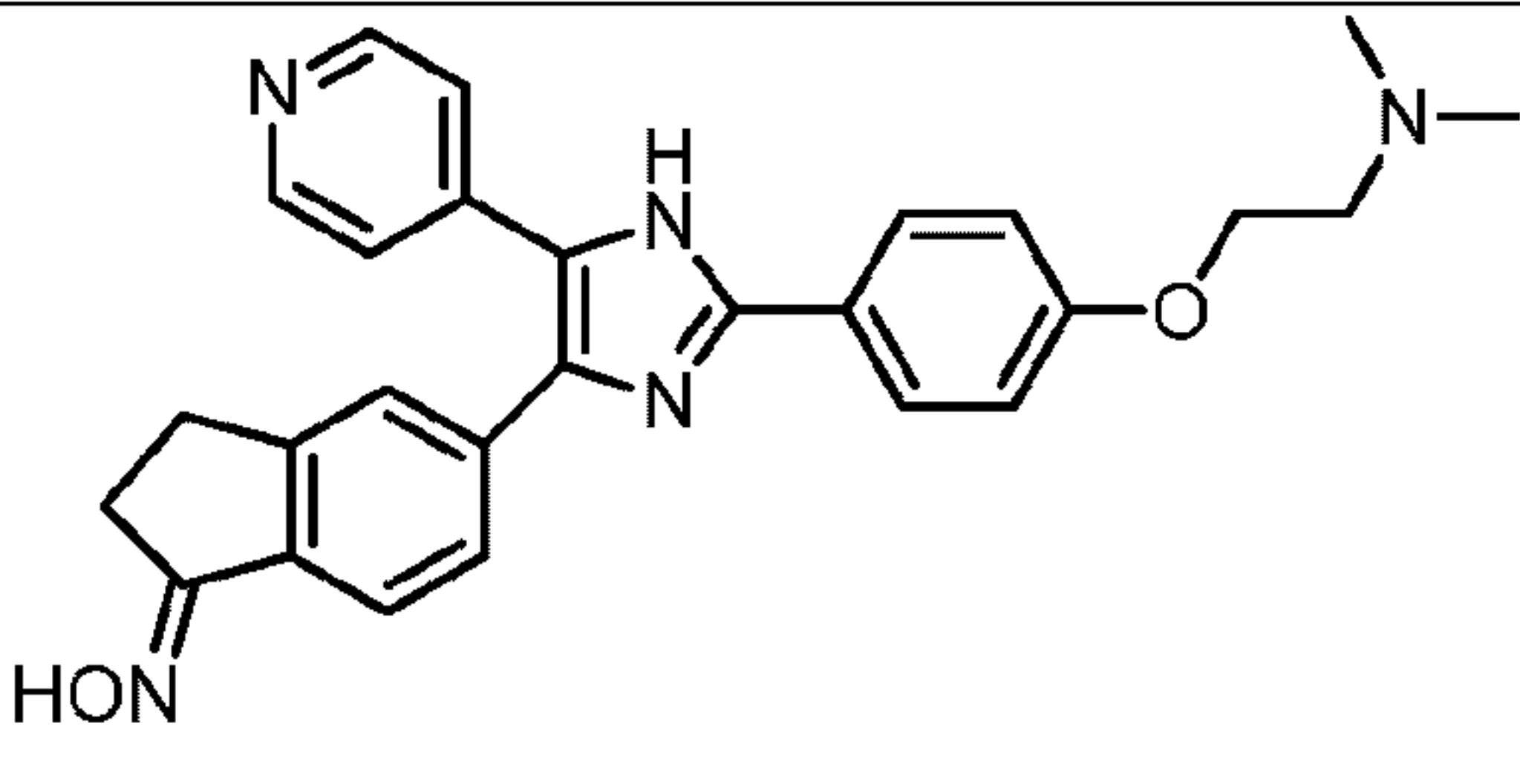
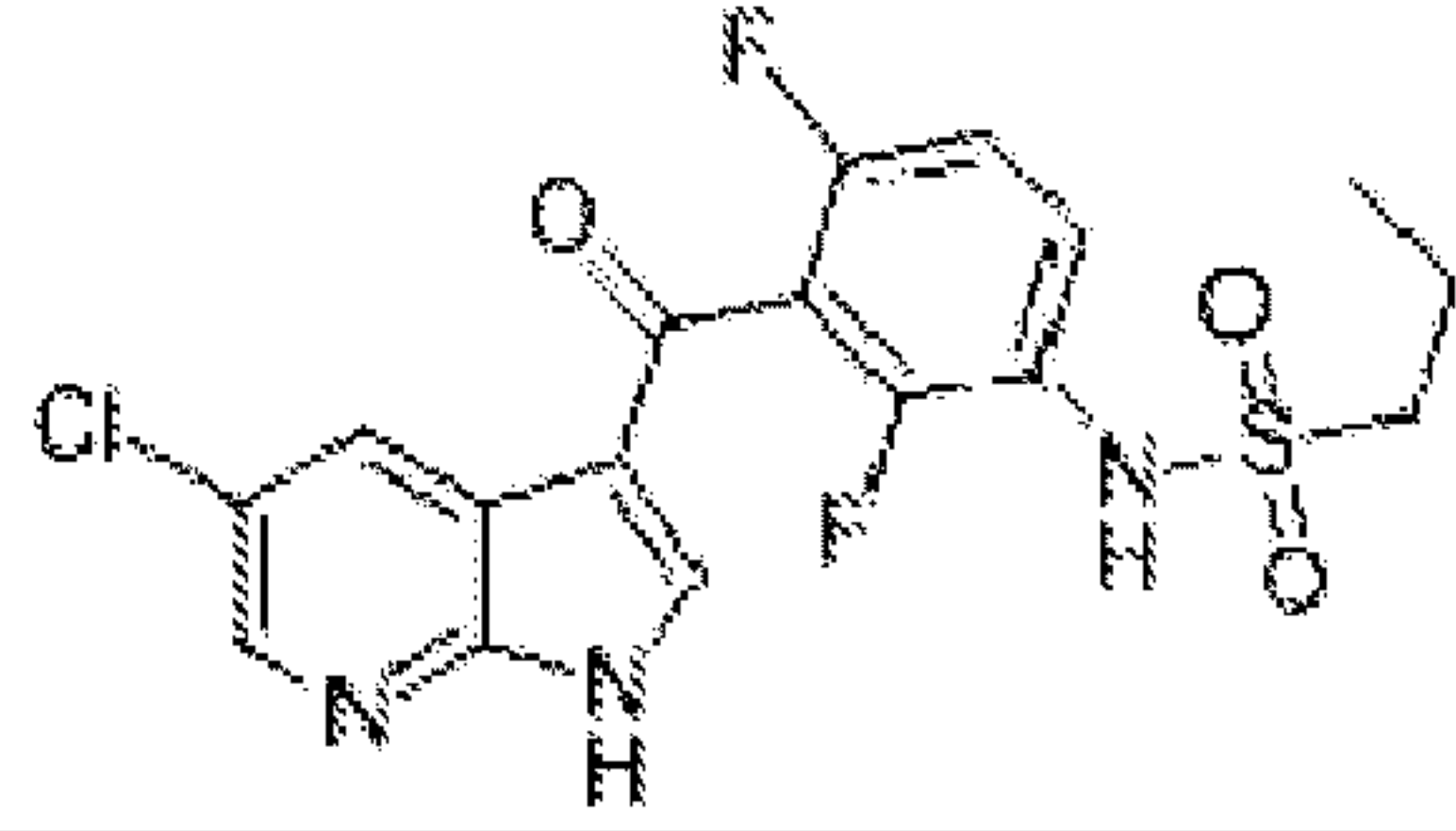
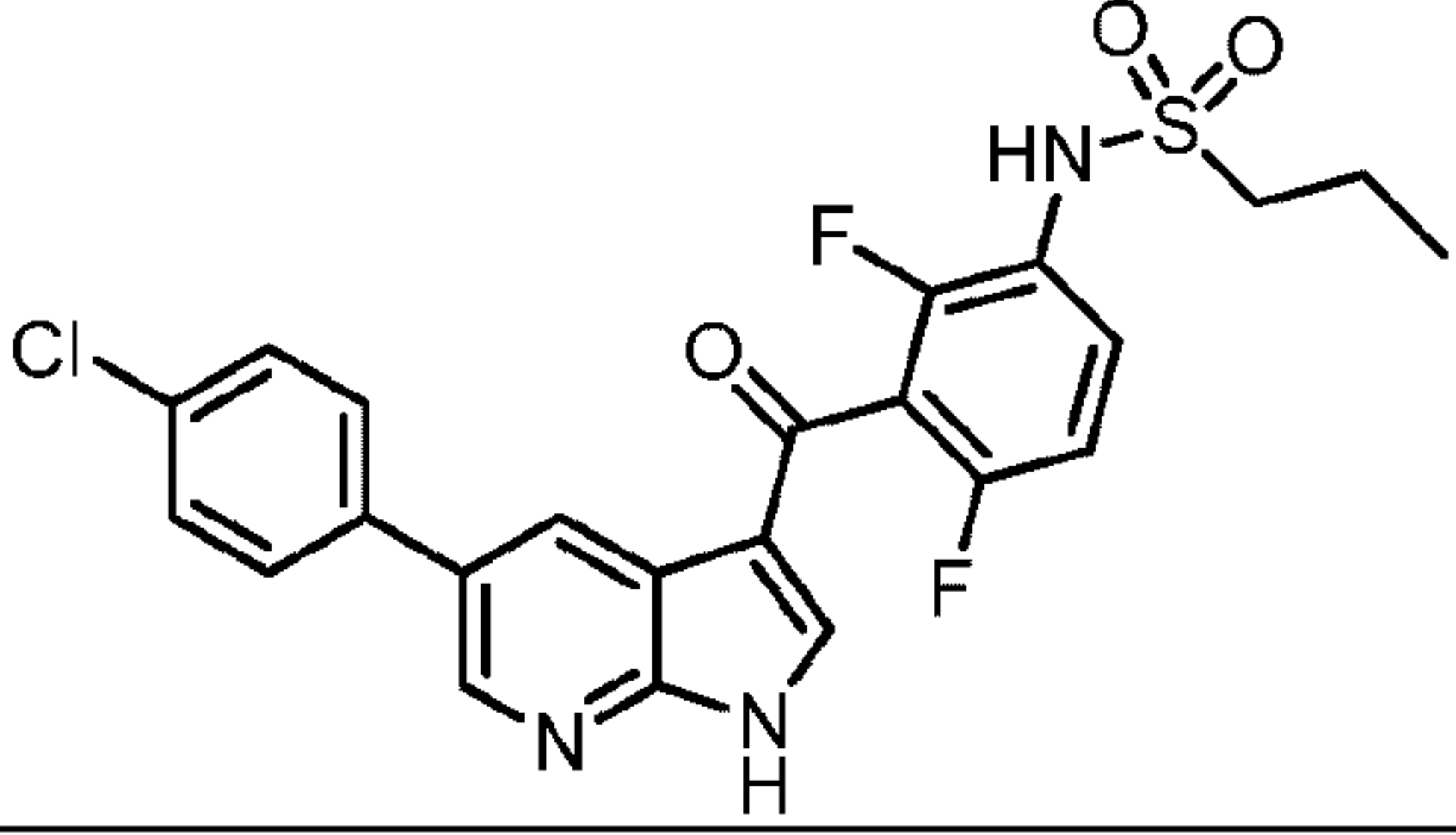
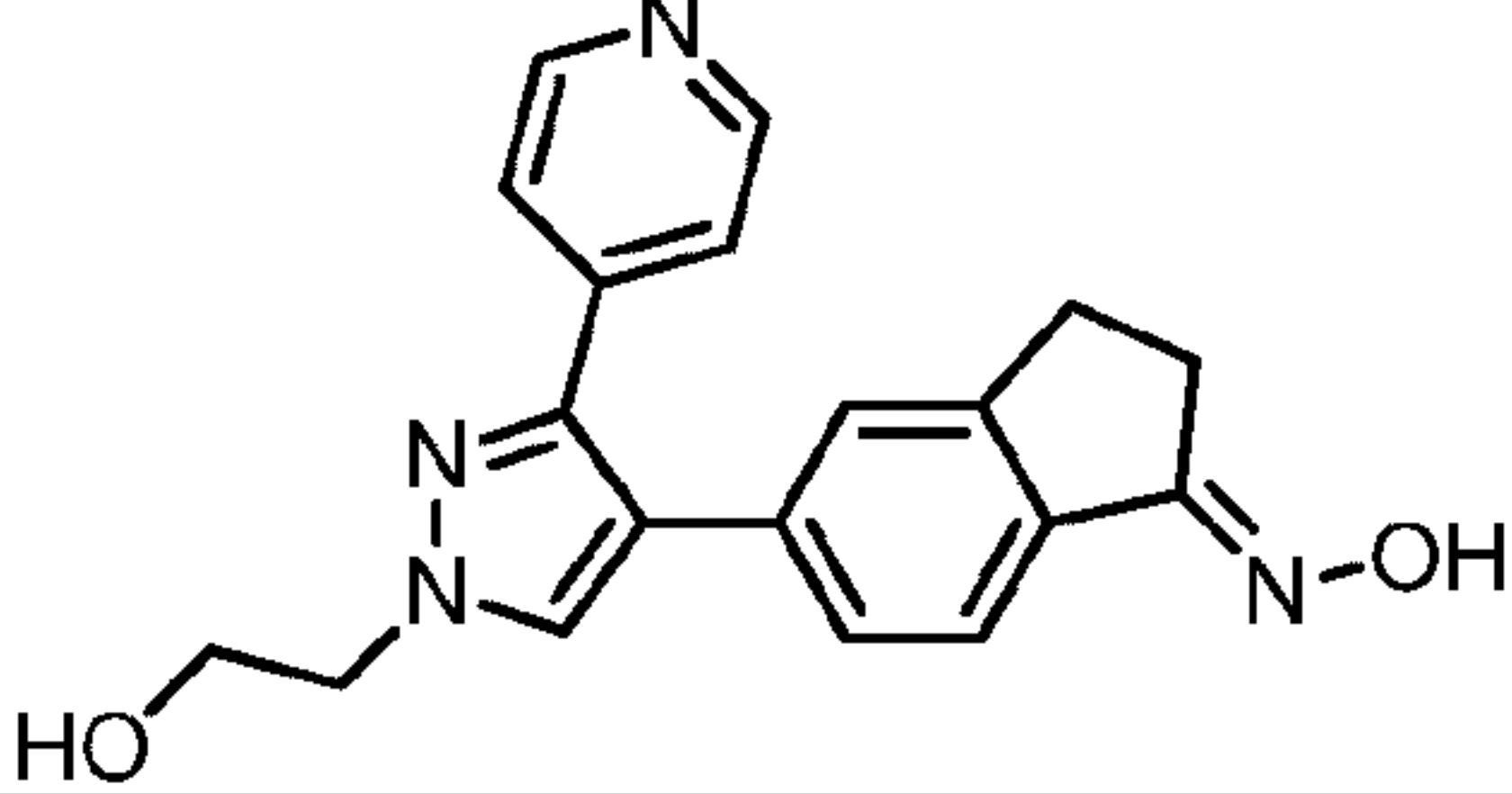
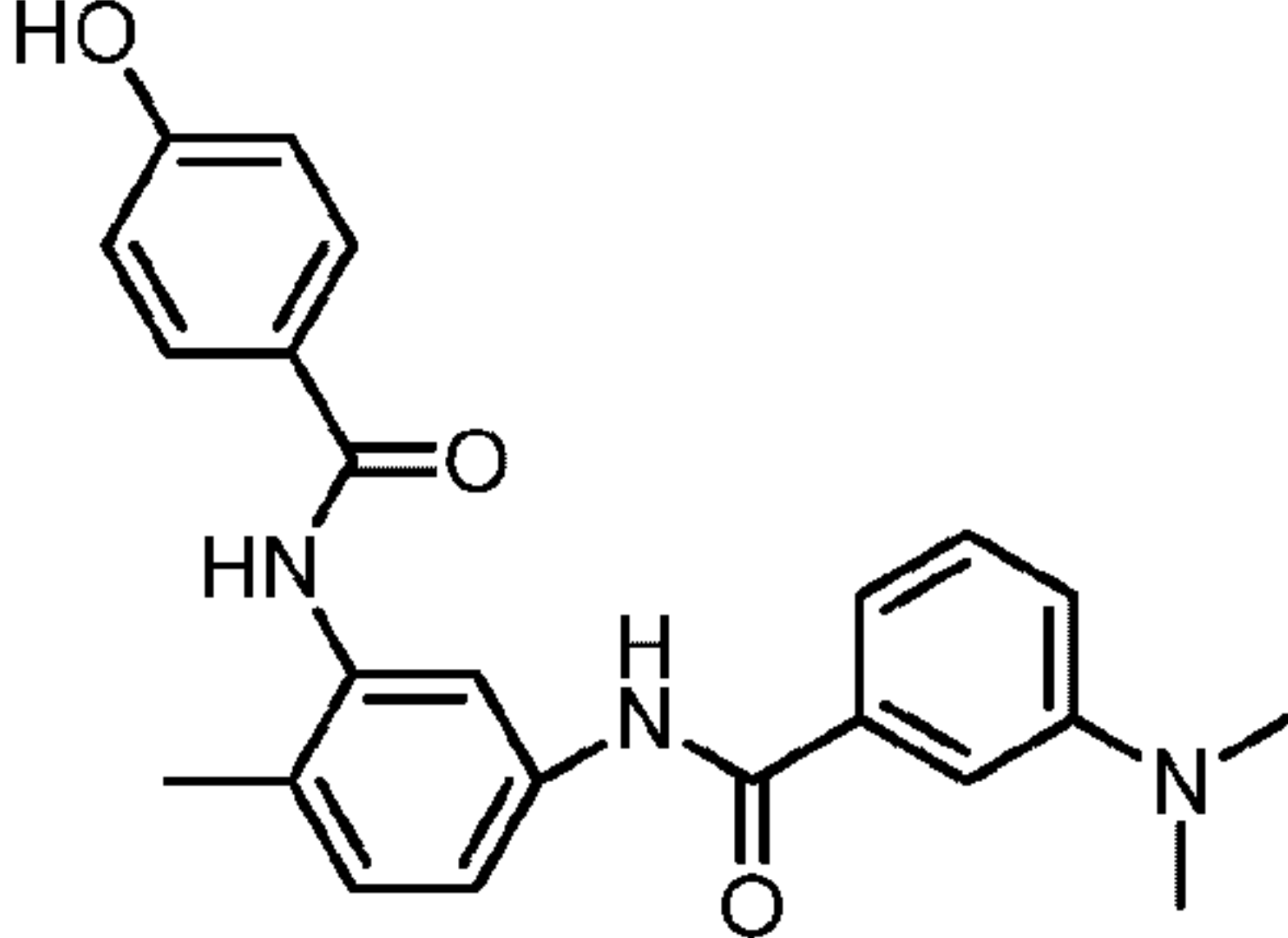
[0043] By way of non-limiting example, a schematic of a high throughput functional screening assay for kinases that drive resistance to B-RAF inhibition is shown in Figure 5. A collection of ~600 cloned and sequence validated ORFs were assembled, accounting for ~75% of all annotated kinases (Center for Cancer Systems Biology (CCSB)/Broad Institute Kinase ORF Collection, Figs. 1a, 1b, Table 3). This publically available collection can be rapidly transferred into a variety of expression vectors for various end-applications. Any type of expression vector known to one skilled in the art may be used to express the Kinase ORF collection. By way of non-limiting example, a selectable, epitope-tagged, lentiviral expression vector capable of producing high titer virus and robust ORF expression in mammalian cells may be created to express the kinase collection, (pLX-BLAST-V5, Figure 6a).

[0044] To identify kinases capable of circumventing RAF inhibition, the arrayed kinase ORF collection may be stably expressed in A375, a B-RAF^{V600E} malignant melanoma cell line that is sensitive to the RAF kinase inhibitor PLX4720 (Figs 1a, 1b and 6c, Table 3). Clones of ORF expressing cells treated with 1 μ M PLX4720 are screened for viability relative to untreated cells and normalized to an assay-specific positive control, MEK1^{S218/222D} (MEK1^{DD}) (Table 4). ORFs that affected baseline viability or proliferation are removed from the analysis. Clones scoring above two standard deviations from the normalized mean may be further evaluated to identify a resistance conferring target kinase for a second inhibitor. In some embodiments, the gene encoding the target kinase may be *MAP3K8* (TPL2/COT), *RAF1* (CRAF), *CRKL* (CrkL), *FGR* (Fgr), *PRKCE* (Prkce), *PRKCH* (Prkch), *ERBB2* (ErbB2), *AXL* (Axl), or *PAK3* (Pak3). In some embodiments, the gene encoding the target kinase may be a MAPK pathway activator. In some embodiments, the gene encoding the target kinase may be a MAP3 kinase that directly phosphorylates and activates MEK. In some embodiments, the gene encoding the target kinase may encode an adapter protein that is amplified and phosphorylated in melanoma.

[0045] In other embodiments, the ORF collection may be stably expressed in a cell line having a different mutation in B-RAF, for example, another mutation at about amino acid position 600 such as V600K, V600D, and V600R. Additional B-RAF mutations include the mutations described in Davies et al. Nature, 417, 949-954, 2002, Table 1. Cell lines may be used that are sensitive to other RAF kinase inhibitors including, but not limited to, PLX4032; GDC-0879; RAF265; sorafenib; SB590855 and/or ZM 336372. In some embodiments, the ORF collection may be stably expressed in a cell line having a sensitivity to a MEK inhibitor. Non-limiting examples of MEK inhibitors include, AZD6244; CI-1040; PD184352; PD318088, PD98059, PD334581, RDEA119, 6-Methoxy-7-(3-morpholin-4-yl-propoxy)-4-(4-phenoxy-phenylamino)-quinoline-3-carbonitrile and 4-[3-Chloro-4-(1-methyl-1H-imidazol-2-ylsulfanyl)-phenylamino]-6-methoxy-7-(3-morpholin-4-yl-propoxy)-quinoline-3-carbonitrile. Additional RAF and MEK inhibitors are described below. By way of non-limiting example, exemplary RAF inhibitors are shown in Table 1 and exemplary MEK inhibitors are shown in Table 2.

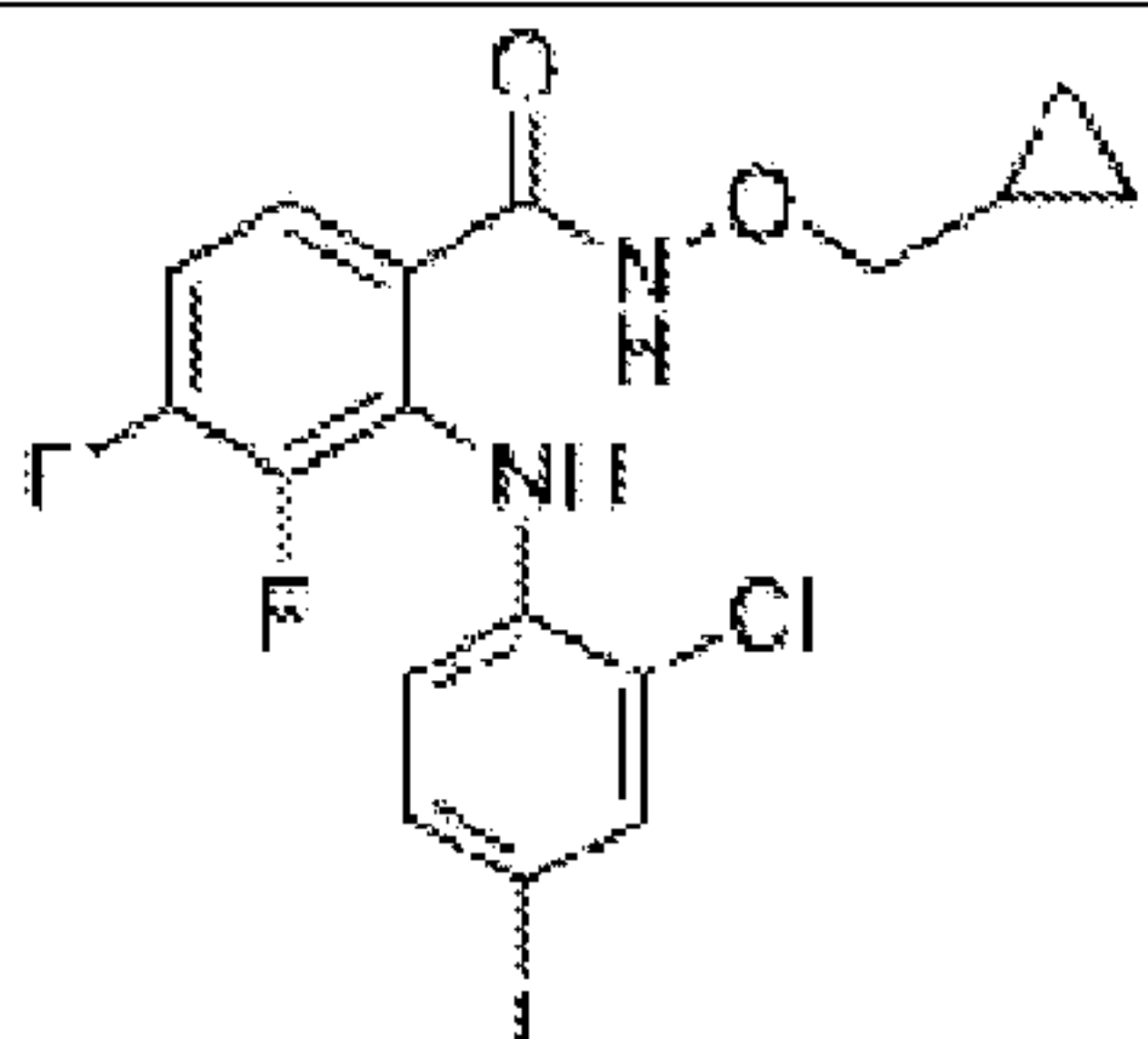
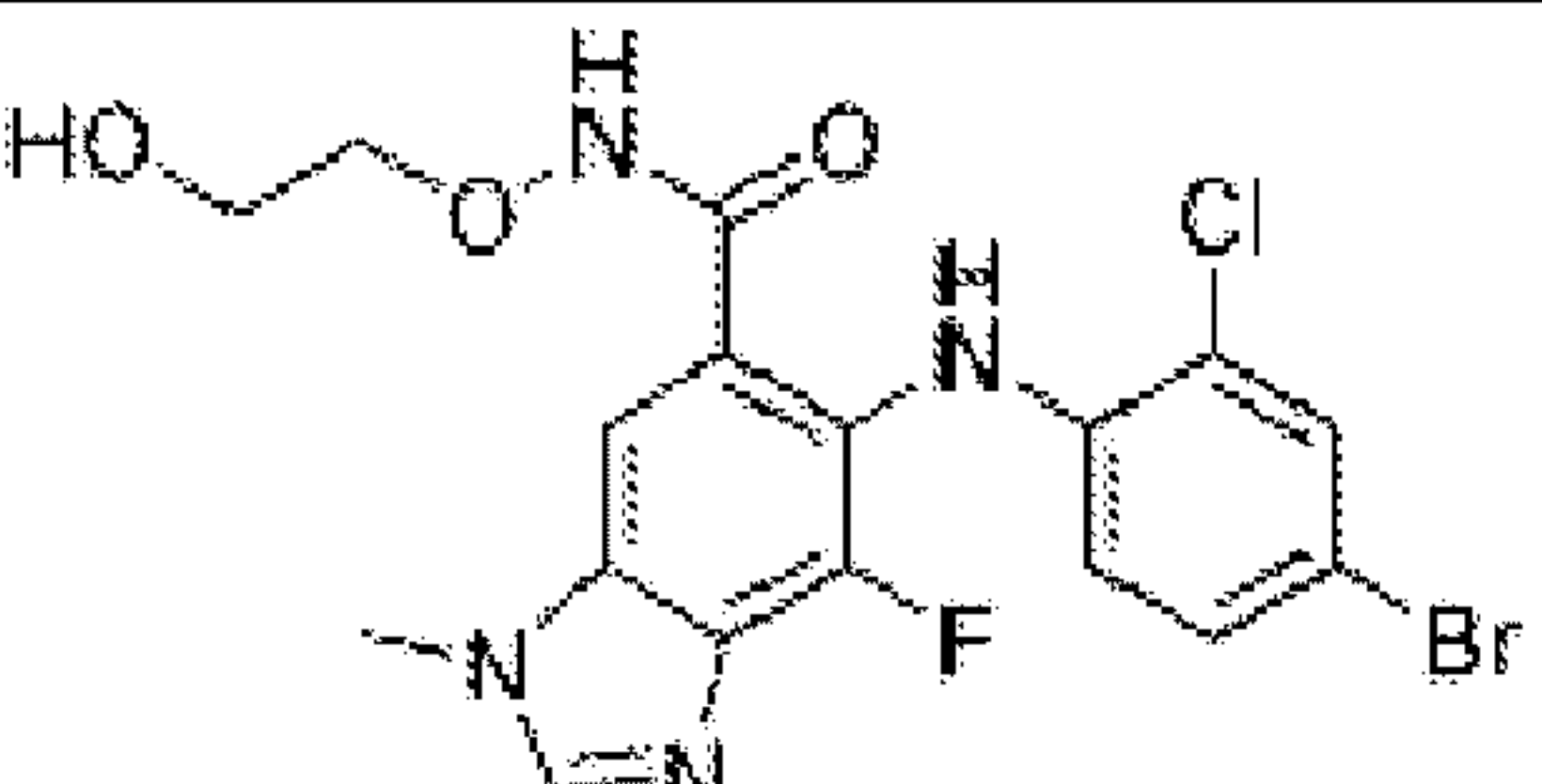
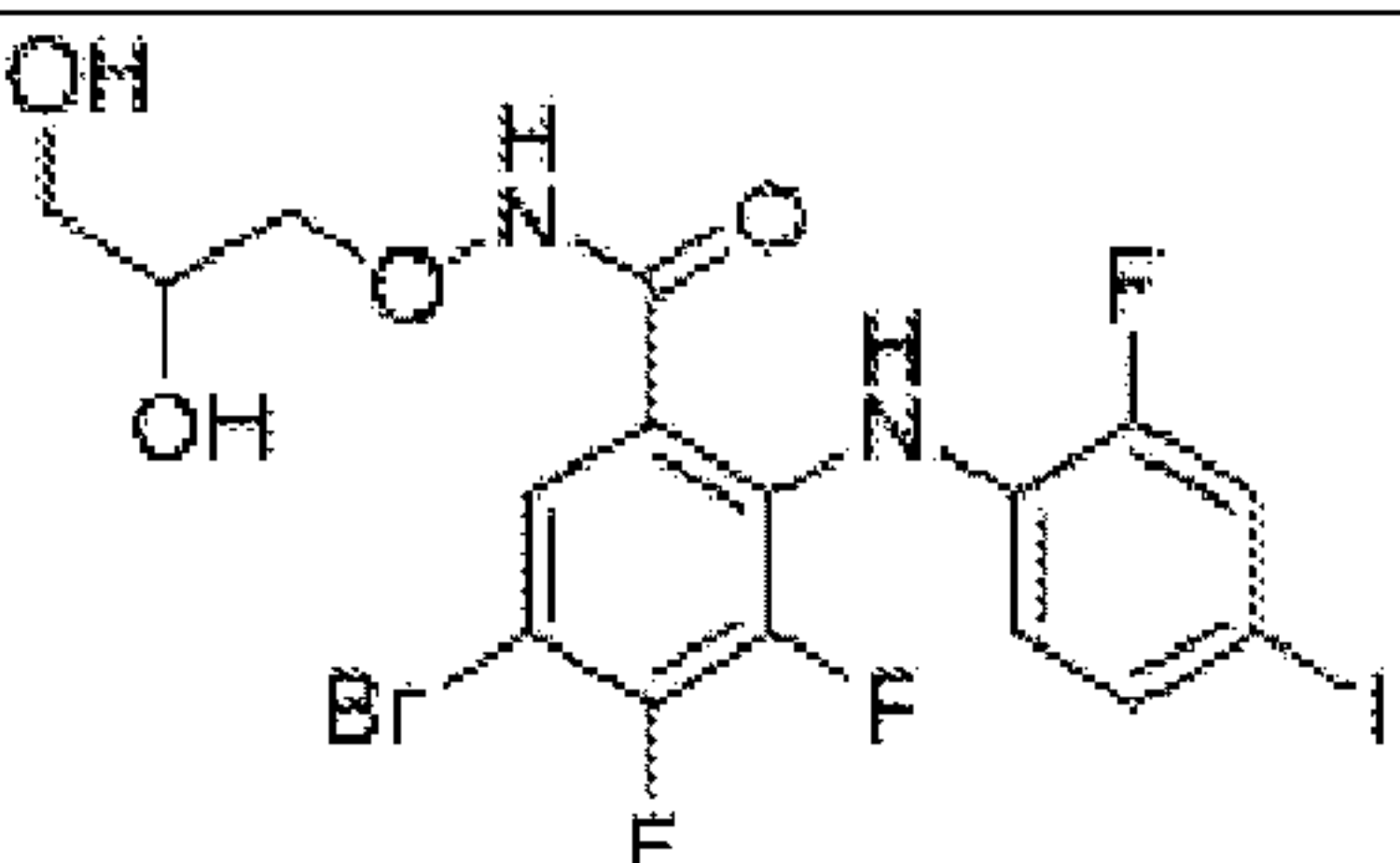
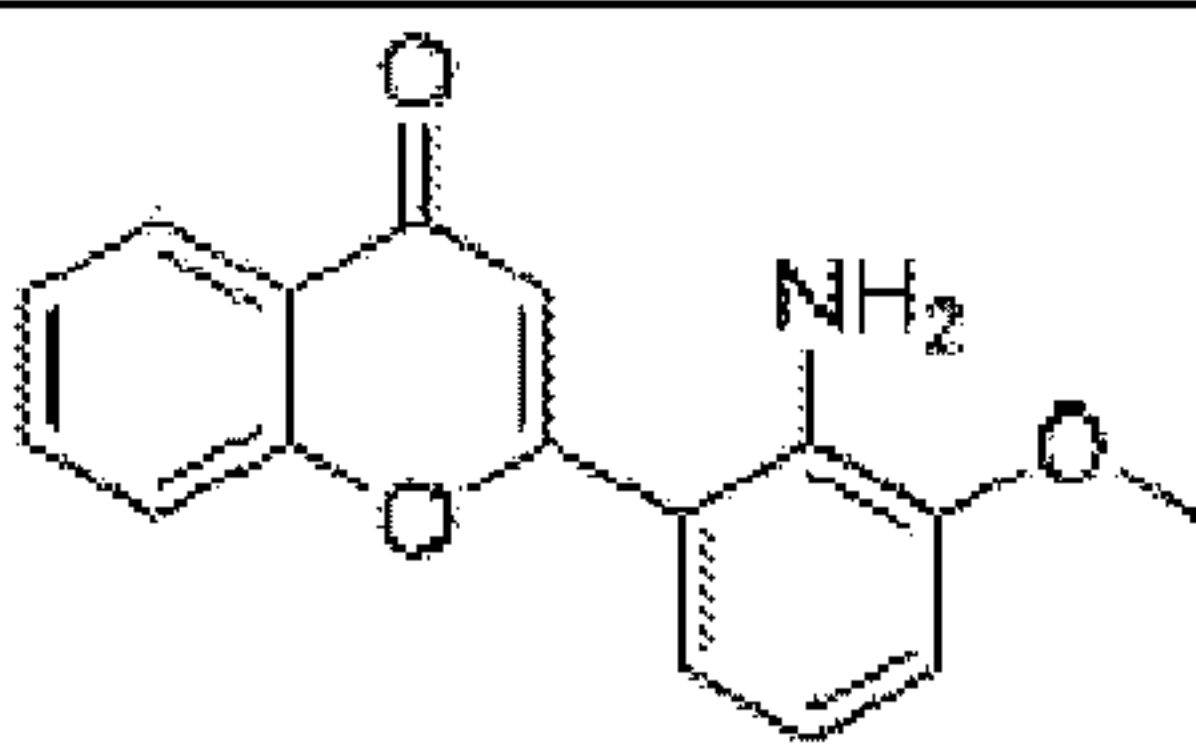
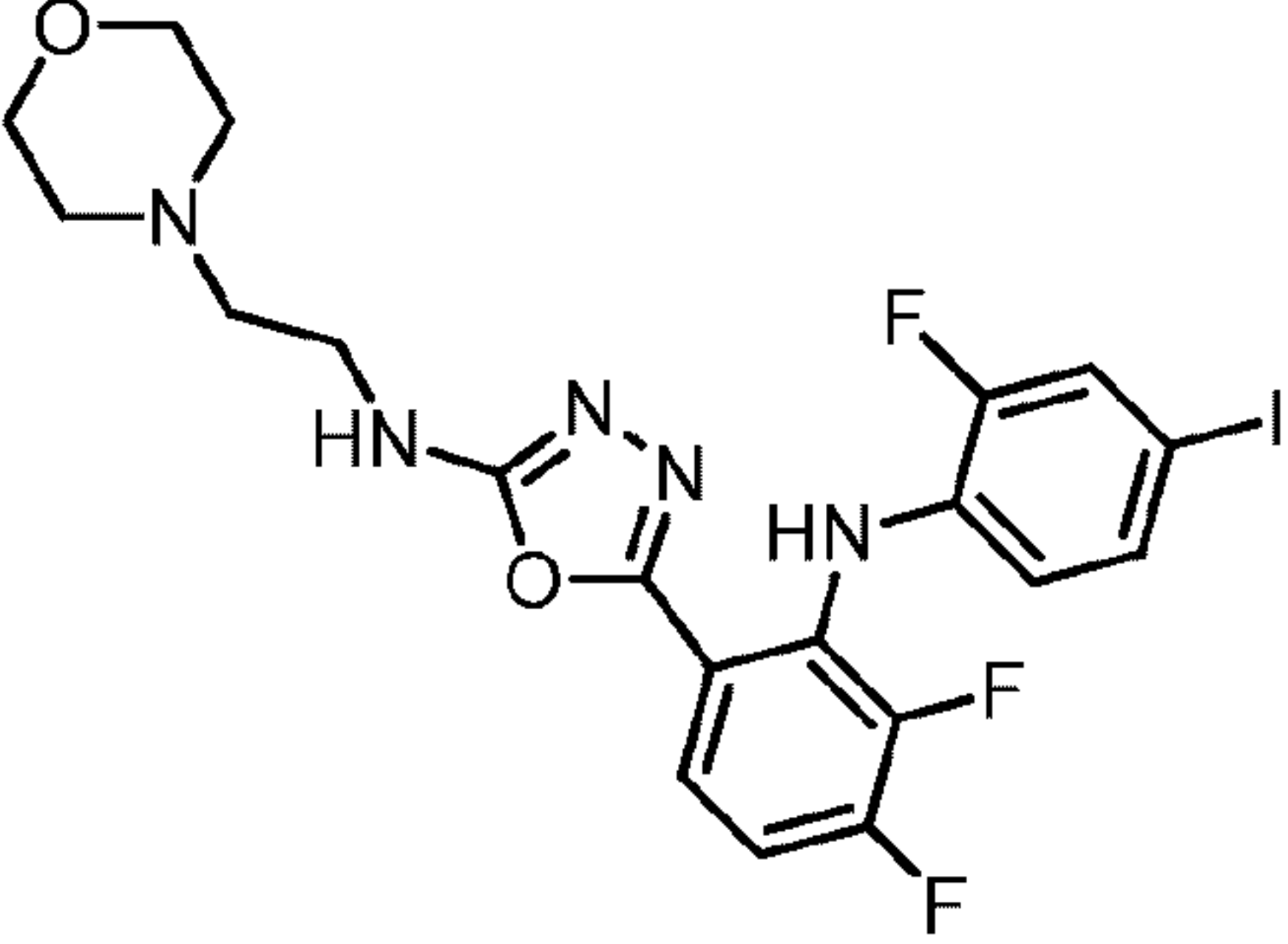
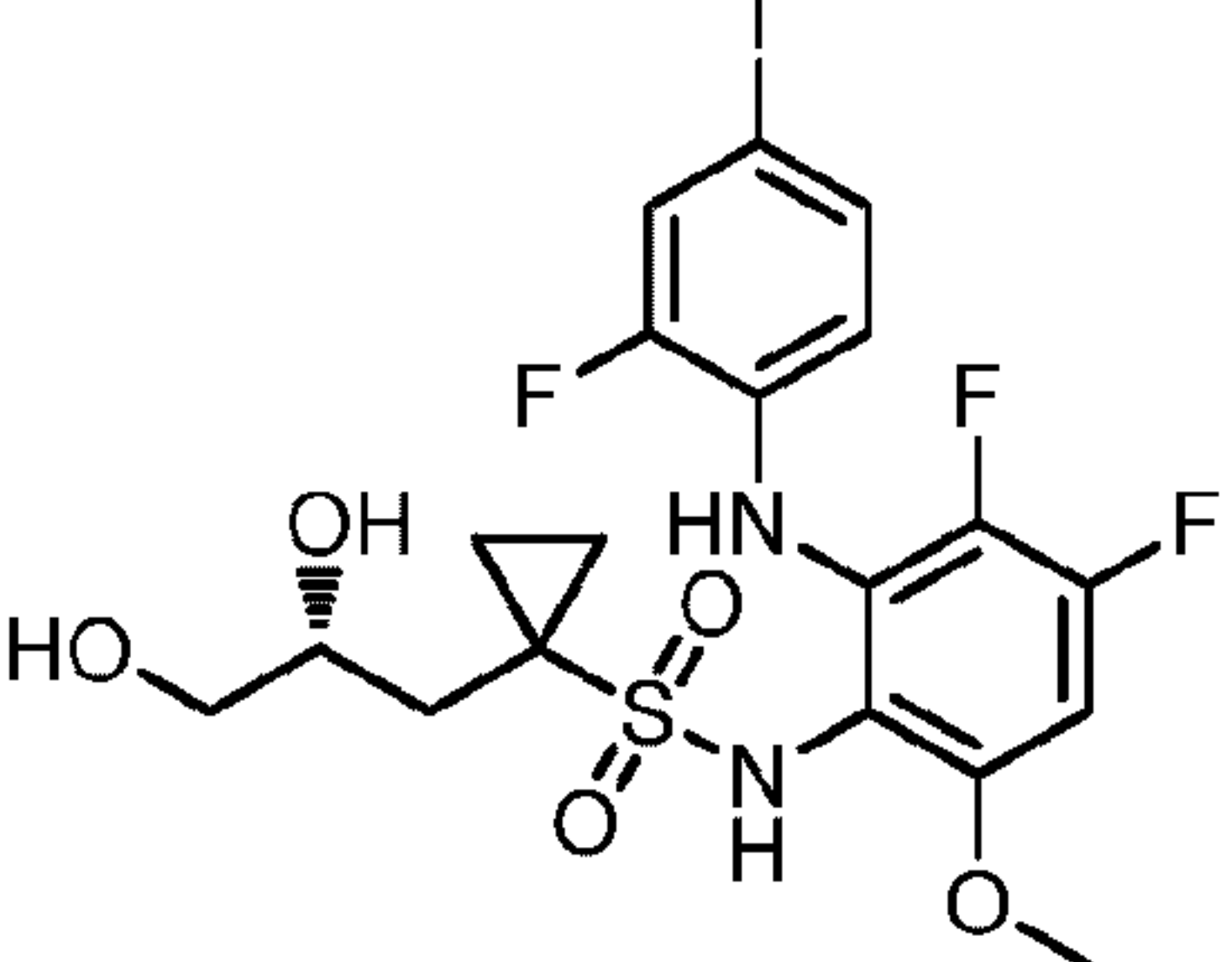
[0046] Table 1: Exemplary RAF Inhibitors

	Name	CAS No.	Structure
1	RAF265	927880-90-	
2	Sorafenib Tosylate Nexavar Bay 43-9006	475207-59-1	
	Sorafenib 4-[4-[[4-chloro-3-(trifluoromethyl)phenyl]carbamoyl]phenoxy]-N-methyl-pyridine-2-carboxamide	284461-73-0	

3	SB590885		
4	PLX4720	918505-84-7	
5	PLX4032	1029872-54-5	
6	GDC-0879	905281-76-7	
7	ZM 336372	208260-29-1	

[0047] Table 2: Exemplary MEK Inhibitors

	Name	CAS No.	Structure
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1	CI-1040/PD184352	212631-79-3	
2	AZD6244	606143-52-6	
3	PD318088	391210-00-7	
4	PD98059	167869-21-8	
5	PD334581		
6	RDEA119 N-[3,4-difluoro-2-[(2-fluoro-4-iodophenyl)amino]-6-methoxyphenyl]-1-[(2R)-2,3-dihydroxypropyl]-Cyclopropanesulfonamide	923032-38-6	

7	6-Methoxy-7-(3-morpholin-4-yl-propoxy)-4-(4-phenoxy-phenylamino)-quinoline-3-carbonitrile		
8	4-[3-Chloro-4-(1-methyl-1H-imidazol-2-ylsulfanyl)-phenylamino]-6-methoxy-7-(3-morpholin-4-yl-propoxy)-quinoline-3-carbonitrile		

[0048] Diagnostic/Prognostic Markers for Innate and Acquired Resistance to Targeted Therapies

[0049] In some aspects, the present invention relates to methods of detecting the presence of one or more diagnostic or prognostic markers in a sample (e.g. a biological sample from a cancer patient). A variety of screening methods known to one of skill in the art may be used to detect the presence of the marker in the sample including DNA, RNA and protein detection. The techniques described below can be used to determine the presence or absence of a kinase target in a sample obtained from a patient. In some embodiments, the patient may have innate or acquired resistance to kinase targeted therapies, including B-RAF inhibitors or MEK inhibitors. For example, the patient may have an innate or acquired resistance to B-RAF inhibitors PLX4720 and/or PLX4032. In some embodiments, the patient may have innate or acquired resistance to MEK inhibitor AZD6244. Identification of one or more kinase targets markers in a patient assists the physician in determining a treatment protocol for the patient. For example, in a patient having one or more kinase target markers, the physician may treat the patient with a combination therapy as described in more detail below.

[0050] In some embodiments, the kinase target may include, but is not limited to, *MAP3K8* (TPL2/COT), *RAF1* (CRAF), *CRKL* (CrkL), *FGR* (Fgr), *PRKCE* (Prkce), *PRKCH* (Prkch), *ERBB2* (ErbB2), *AXL* (Axl), or *PAK3* (Pak3). The marker may be an

increase in the gene copy number, an increase in protein expression, phosphorylation of one or more MAP kinase pathway members, a change in mRNA expression and the like, for the kinase target.

[0051] By way of non-limiting example, in a patient having an oncogenic mutation in B-RAF, identification of an activated target kinase can be useful for characterizing a treatment protocol for the patient. For example, in a patient having a B-RAF^{V600E} mutation, treatment with a RAF inhibitor alone may indicate that the patient is at relatively high risk of acquiring resistance to the treatment after a period of time. In a patient having an oncogenic mutation, identification of an activated kinase target in that patient may indicate inclusion of a second inhibitor in the treatment protocol.

[0052] Identification of an activated kinase target may include an analysis of a gene copy number and identification of an increase in copy number of a target kinase. For example, a copy number gain in MAP3K8 is indicative of a patient having innate resistance or developing acquired resistance, in particular if the patient also has a B-RAF^{V600E} mutation.

[0053] In some embodiments, identification of an activated kinase target may include an analysis of phosphorylation of a kinase target and/or a member of the MAP kinase pathway. For example, phosphorylation of C-RAF at S338 is indicative of a patient having innate resistance or developing acquired resistance, in particular if the patient also has a B-RAF^{V600E} mutation. In some embodiments, identification of an increase in MEK/ERK phosphorylation may be indicative of a patient having innate resistance or developing acquired resistance. Increased COT protein expression in patients having a B-RAF^{V600E} mutation may predict resistance to RAF inhibition and MEK inhibition.

[0054] Identification of an activated kinase target may include an analysis of mRNA expression of a kinase target. For example, an increase in COT mRNA expression following initial treatment with a first kinase inhibitor is indicative of a patient having or developing resistance. In some embodiments, the first kinase inhibitor may be a RAF inhibitor or a MEK inhibitor.

[0055] Methods of treatment

[0056] In various embodiments, the invention provides methods for treatment of a patient having cancer. The methods generally comprise administration of a first inhibitor

and a second inhibitor. One inhibitor may be a RAF inhibitor. The RAF inhibitor may be a pan-RAF inhibitor or a selective RAF inhibitor. Pan-RAF inhibitors include but are not limited to RAF265, sorafenib, or SB590885. In some embodiments, the RAF inhibitor is a B-RAF inhibitor. In some embodiments, the selective RAF inhibitor is PLX4720, PLX4032, or GDC-0879-A. One inhibitor may be a MEK inhibitor (see Table 2 illustrating exemplary MEK inhibitors). One inhibitor may be a COT inhibitor. By way of non-limiting example, the COT inhibitor may be a shRNA inhibitor as described below or a small molecule COT inhibitor, 4-(3-chloro-4-fluorophenylamino)-6-(pyridin-3-yl-methylamino)-3-cyano-[1,7]-naphthyridine (EMD;TPL2 inhibitor I; catalogue number 616373, PubChem ID: 9549300) Inhibitors of the present invention inhibit one or more of the kinase targets including *MAP3K8* (TPL2/COT), *RAF1* (CRAF), *CRKL*(CrkL), *FGR* (Fgr), *PRKCE* (Prkce), *PRKCH* (Prkch), *ERBB2* (ErbB2), *AXL* (Axl), or *PAK3* (Pak3) or other MAP kinase pathway targets.

[0057] In some embodiments, a combination therapy for cancer is provided, comprising an effective amount of a RAF inhibitor and an effective amount of a *MAP3K8* (TPL2/COT) inhibitor. Also provided herein is a combination therapy for cancer, comprising an effective amount of a RAF inhibitor and an effective amount of a MEK inhibitor. Other combination therapies include an effective amount of a RAF inhibitor and an effective amount of a second inhibitor targeting the gene, mRNA or protein encoded by one or more of the following: *MAP3K8* (TPL2/COT), *RAF1* (CRAF), *CRKL*(CrkL), *FGR* (Fgr), *PRKCE* (Prkce), *PRKCH* (Prkch) , *ERBB2* (ErbB2), *AXL* (Axl), or *PAK3* (Pak3). The combination therapy is suitable for treatment of a patient wherein the cancer contains B-RAF mutant cells and in particular, B-RAF^{V600E} mutant cells. The present invention further provides a combination therapy for cancer, comprising an effective amount of a RAF inhibitor and an effective amount of a MEK inhibitor, wherein the subject with the cancer contains cells with altered *MAP3K8* (TPL2/COT) expression or gene copy number. In some embodiments, the MEK inhibitor is CI-1040/PD184352 or AZD6244.

[0058] As a non-limiting example, the MEK inhibitor provided herein can be CI-1040, AZD6244, PD318088, PD98059, PD334581, RDEA119, 6-Methoxy-7-(3-morpholin-4-yl-propoxy)-4-(4-phenoxy-phenylamino)-quinoline-3-carbonitrile or 4-[3-

Chloro-4-(1 -methyl-1 H-imidazol-2-ylsulfanyl)-phenylamino]-6-methoxy-7-(3-morpholin-4-yl-propoxy)-quinoline-3-carbonitrile, Roche compound RG7420, or combinations thereof. Additional MEK inhibitors known in the art may also be used.

[0059] In exemplary embodiments of the foregoing aspects, the RAF inhibitor provided herein is PLX4720, PLX4032, BAY 43-9006 (Sorafenib), ZM 336372, RAF 265, AAL-881 , LBT-613, or CJS352 (NVP-AAL881 -NX (hereafter referred to as AAL881) and NVP-LBT613-AG-8 (LBT613) are isoquinoline compounds (Novartis, Cambridge, MA). Additional exemplary RAF inhibitors useful for combination therapy include pan-RAF inhibitors, inhibitors of B-RAF, inhibitors of A-RAF, and inhibitors of RAF-1 . In exemplary embodiments RAF inhibitors useful for combination therapy include PLX4720, PLX4032, BAY 43-9006 (Sorafenib), ZM 336372, RAF 265, AAL-881 , LBT-613, and CJS352. Exemplary RAF inhibitors further include the compounds set forth in PCT Publication No. WO/2008/028141. Exemplary RAF inhibitors additionally include the quinazolinone derivatives described in PCT Publication No. WO/2006/024836, and the pyridinylquinazolinamine derivatives described in PCT Publication No. WO/2008/020203.

[0060] Administration of the combination includes administration of the combination in a single formulation or unit dosage form, administration of the individual agents of the combination concurrently but separately, or administration of the individual agents of the combination sequentially by any suitable route. The dosage of the individual agents of the combination may require more frequent administration of one of the agents as compared to the other agent in the combination. Therefore, to permit appropriate dosing, packaged pharmaceutical products may contain one or more dosage forms that contain the combination of agents, and one or more dosage forms that contain one of the combinations of agents, but not the other agent(s) of the combination.

[0061] Agents may contain one or more asymmetric elements such as stereogenic centers or stereogenic axes, e.g., asymmetric carbon atoms, so that the compounds can exist in different stereoisomeric forms. These compounds can be, for example, racemates or optically active forms. For compounds with two or more asymmetric

elements, these compounds can additionally be mixtures of diastereomers. For compounds having asymmetric centers, it should be understood that all of the optical isomers and mixtures thereof are encompassed. In addition, compounds with carbon-carbon double bonds may occur in Z- and E-forms; all isomeric forms of the compounds are included in the present invention. In these situations, the single enantiomers (optically active forms) can be obtained by asymmetric synthesis, synthesis from optically pure precursors, or by resolution of the racemates. Resolution of the racemates can also be accomplished, for example, by conventional methods such as crystallization in the presence of a resolving agent, or chromatography, using, for example a chiral HPLC column.

[0062] Unless otherwise specified, or clearly indicated by the text, reference to compounds useful in the combination therapy of the invention includes both the free base of the compounds, and all pharmaceutically acceptable salts of the compounds. A preferred salt is the hydrochloride salt.

[0063] The term “pharmaceutically acceptable salts” includes derivatives of the disclosed compounds, wherein the parent compound is modified by making non-toxic acid or base addition salts thereof, and further refers to pharmaceutically acceptable solvates, including hydrates, of such compounds and such salts. Examples of pharmaceutically acceptable salts include, but are not limited to, mineral or organic acid addition salts of basic residues such as amines; alkali or organic addition salts of acidic residues such as carboxylic acids; and the like, and combinations comprising one or more of the foregoing salts. The pharmaceutically acceptable salts include non-toxic salts and the quaternary ammonium salts of the parent compound formed, for example, from non-toxic inorganic or organic acids. For example, non-toxic acid salts include those derived from inorganic acids such as hydrochloric, hydrobromic, sulfuric, sulfamic, phosphoric, and nitric; other acceptable inorganic salts include metal salts such as sodium salt, potassium salt, and cesium salt; and alkaline earth metal salts, such as calcium salt and magnesium salt; and combinations comprising one or more of the foregoing salts.

[0064] Pharmaceutically acceptable organic salts include salts prepared from organic acids such as acetic, trifluoroacetic, propionic, succinic, glycolic, stearic, lactic,

malic, tartaric, citric, ascorbic, pantoic, maleic, hydroxymaleic, phenylacetic, glutamic, benzoic, salicylic, mesylic, esylic, besylic, sulfanilic, 2-acetoxybenzoic, fumaric, toluenesulfonic, methanesulfonic, ethane disulfonic, oxalic, isethionic, $\text{HOOC}(\text{CH}_2)_n\text{COOH}$ where n is 0-4; organic amine salts such as triethylamine salt, pyridine salt, picoline salt, ethanolamine salt, triethanolamine salt, dicyclohexylamine salt, N,N' -dibenzylethylenediamine salt; and amino acid salts such as arginate, asparaginate, and glutamate, and combinations comprising one or more of the foregoing salts.

[0065] An “effective amount” of a combination of agents (e.g., MEK and RAF inhibitors, or RAF and COT inhibitors, or RAF and an inhibitor targeting *MAP3K8* (TPL2/COT), *RAF1* (CRAF), *CRKL* (CrkL), *FGR* (Fgr), *PRKCE* (Prkce), *PRKCH* (Prkch), *ERBB2* (ErbB2), *AXL* (Axl), or *PAK3* (Pak3)) is an amount sufficient to provide an observable improvement over the baseline clinically observable signs and symptoms of the disorder treated with the combination.

[0066] The pharmaceutical products can be administered by oral or other forms, e.g., rectally or by parenteral injection. “Oral dosage form” is meant to include a unit dosage form prescribed or intended for oral administration. An oral dosage form may or may not comprise a plurality of subunits such as, for example, microcapsules or microtablets, packaged for administration in a single dose.

[0067] The pharmaceutical products can be released in various forms. “Releasable form” is meant to include instant release, immediate-release, controlled-release, and sustained-release forms.

[0068] “Instant-release” is meant to include a dosage form designed to ensure rapid dissolution of the active agent by modifying the normal crystal form of the active agent to obtain a more rapid dissolution.

[0069] “Immediate-release” is meant to include a conventional or non-modified release form in which greater than or equal to about 50% or more preferably about 75% of the active agents is released within two hours of administration, preferably within one hour of administration.

[0070] “Sustained-release” or “extended-release” includes the release of active agents at such a rate that blood (e.g., plasma) levels are maintained within a

therapeutic range but below toxic levels for at least about 8 hours, preferably at least about 12 hours, more preferably about 24 hours after administration at steady-state. The term “steady-state” means that a plasma level for a given active agent or combination of active agents, has been achieved and which is maintained with subsequent doses of the active agent(s) at a level which is at or above the minimum effective therapeutic level and is below the minimum toxic plasma level for a given active agent(s).

[0071] The term “treat”, “treated,” “treating” or “treatment” is used herein to mean to relieve, reduce or alleviate at least one symptom of a disease in a subject. For example, treatment can be diminishment of one or several symptoms of a disorder or complete eradication of a disorder, such as cancer. Within the meaning of the present invention, the term “treat” also denote to arrest, delay the onset (i.e., the period prior to clinical manifestation of a disease) and/or reduce the risk of developing or worsening a disease. The term “protect” is used herein to mean prevent delay or treat, or all, as appropriate, development or continuance or aggravation of a disease in a subject. Within the meaning of the present invention, the disease is associated with a cancer.

[0072] The term “subject” or “patient” is intended to include animals, which are capable of suffering from or afflicted with a cancer or any disorder involving, directly or indirectly, a cancer. Examples of subjects include mammals, e.g., humans, dogs, cows, horses, pigs, sheep, goats, cats, mice, rabbits, rats, and transgenic non-human animals. In certain embodiments, the subject is a human, e.g., a human suffering from, at risk of suffering from, or potentially capable of suffering from cancers.

[0073] The term “about” or “approximately” usually means within 20%, more preferably within 10%, and most preferably still within 5% of a given value or range. Alternatively, especially in biological systems, the term “about” means within about a log (i.e., an order of magnitude) preferably within a factor of two of a given value.

[0074] The use of the terms “a” and “an” and “the” and similar referents in the context of describing the invention (especially in the context of the following claims) are to be construed to cover both the singular and the plural, unless otherwise indicated herein or clearly contradicted by context. The terms “comprising,” “having,” “including,” and “containing” are to be construed as open-ended terms (i.e., meaning “including, but

not limited to”) unless otherwise noted. Recitation of ranges of values herein are merely intended to serve as a shorthand method of referring individually to each separate value falling within the range, unless otherwise indicated herein, and each separate value is incorporated into the specification as if it were individually recited herein.

[0075] As specified above, in one aspect, the instant invention provides a drug combination useful for treating, preventing, arresting, delaying the onset of and/or reducing the risk of developing, or reversing at least one symptom of cancer, in a subject comprising administering to the subject a combination therapy, comprising an effective amount of a RAF inhibitor and an effective amount of a *MAP3K8* (TPL2/COT) inhibitor, or an effective amount of a RAF inhibitor and an effective amount of MEK inhibitor or an effective amount of a RAF inhibitor and an effective amount of a second inhibitor targeting *MAP3K8* (TPL2/COT), *RAF1* (CRAF), *CRKL* (CrkL), *FGR* (Fgr), *PRKCE* (Prkce), *PRKCH* (Prkch), *ERBB2* (ErbB2), *AXL* (Axl), or *PAK3* (Pak3). Preferably, these inhibitors are administered at therapeutically effective dosages which, when combined, provide a beneficial effect. The administration may be simultaneous or sequential.

[0076] The term “cancer” is used herein to mean a broad spectrum of tumors, including all solid tumors and hematological malignancies. Examples of such tumors include but are not limited to leukemias, lymphomas, myelomas, carcinomas, metastatic carcinomas, sarcomas, adenomas, nervous system cancers and geritourinary cancers. In exemplary embodiments, the foregoing methods are useful in treating adult and pediatric acute lymphoblastic leukemia, acute myeloid leukemia, adrenocortical carcinoma, AIDS-related cancers, anal cancer, cancer of the appendix, astrocytoma, basal cell carcinoma, bile duct cancer, bladder cancer, bone cancer, osteosarcoma, fibrous histiocytoma, brain cancer, brain stem glioma, cerebellar astrocytoma, malignant glioma, ependymoma, medulloblastoma, supratentorial primitive neuroectodermal tumors, hypothalamic glioma, breast cancer, male breast cancer, bronchial adenomas, Burkitt lymphoma, carcinoid tumor, carcinoma of unknown origin, central nervous system lymphoma, cerebellar astrocytoma, malignant glioma, cervical cancer, childhood cancers, chronic lymphocytic leukemia, chronic myelogenous leukemia, chronic myeloproliferative disorders, colorectal cancer, cutaneous T-cell lymphoma, endometrial

cancer, ependymoma, esophageal cancer, Ewing family tumors, extracranial germ cell tumor, extragonadal germ cell tumor, extrahepatic bile duct cancer, intraocular melanoma, retinoblastoma, gallbladder cancer, gastric cancer, gastrointestinal stromal tumor, extracranial germ cell tumor, extragonadal germ cell tumor, ovarian germ cell tumor, gestational trophoblastic tumor, glioma, hairy cell leukemia, head and neck cancer, hepatocellular cancer, Hodgkin lymphoma, non-Hodgkin lymphoma, hypopharyngeal cancer, hypothalamic and visual pathway glioma, intraocular melanoma, islet cell tumors, Kaposi sarcoma, kidney cancer, renal cell cancer, laryngeal cancer, lip and oral cavity cancer, small cell lung cancer, non-small cell lung cancer, primary central nervous system lymphoma, Waldenstrom macroglobulinemia, malignant fibrous histiocytoma, medulloblastoma, melanoma, Merkel cell carcinoma, malignant mesothelioma, squamous neck cancer, multiple endocrine neoplasia syndrome, multiple myeloma, mycosis fungoides, myelodysplastic syndromes, myeloproliferative disorders, chronic myeloproliferative disorders, nasal cavity and paranasal sinus cancer, nasopharyngeal cancer, neuroblastoma, oropharyngeal cancer, ovarian cancer, pancreatic cancer, parathyroid cancer, penile cancer, pharyngeal cancer, pheochromocytoma, pineoblastoma and supratentorial primitive neuroectodermal tumors, pituitary cancer, plasma cell neoplasms, pleuropulmonary blastoma, prostate cancer, rectal cancer, rhabdomyosarcoma, salivary gland cancer, soft tissue sarcoma, uterine sarcoma, Sezary syndrome, non-melanoma skin cancer, small intestine cancer, squamous cell carcinoma, squamous neck cancer, supratentorial primitive neuroectodermal tumors, testicular cancer, throat cancer, thymoma and thymic carcinoma, thyroid cancer, transitional cell cancer, trophoblastic tumors, urethral cancer, uterine cancer, uterine sarcoma, vaginal cancer, vulvar cancer, and Wilms tumor.

[0077] In particular, the cancer may be associated with a mutation in the B-RAF gene. These cancers include melanoma, breast cancer, colorectal cancers, glioma, lung cancer, ovarian cancer, sarcoma and thyroid cancer.

[0078] In a particular embodiment, the therapeutic combination provided herein is effective for the treatment of moderate to severe cancer in a subject.

[0079] Dosages

[0080] The optimal dose of the combination of agents for treatment of cancer can be determined empirically for each subject using known methods and will depend upon a variety of factors, including the activity of the agents; the age, body weight, general health, gender and diet of the subject; the time and route of administration; and other medications the subject is taking. Optimal dosages may be established using routine testing and procedures that are well known in the art.

[0081] The amount of combination of agents that may be combined with the carrier materials to produce a single dosage form will vary depending upon the individual treated and the particular mode of administration. In some embodiments the unit dosage forms containing the combination of agents as described herein will contain the amounts of each agent of the combination that are typically administered when the agents are administered alone.

[0082] A physician or veterinarian having ordinary skill in the art can readily determine and prescribe the effective amount of the pharmaceutical composition required. For example, the physician or veterinarian could start doses of the compounds of the invention employed in the pharmaceutical composition at levels lower than that required in order to achieve the desired therapeutic effect and gradually increase the dosage until the desired effect is achieved.

[0083] In general, a suitable daily dose of a compound of the invention will be that amount of the compound that is the lowest dose effective to produce a therapeutic effect. Such an effective dose will generally depend upon the factors described above and is readily determined by one having skill in the art.

[0084] Generally, therapeutically effective doses of the compounds of this invention for a patient, when used for the indicated analgesic effects, will range from about 0.0001 to about 1000 mg per kilogram of body weight per day, more preferably from about 0.01 to about 50 mg per kg per day.

[0085] If desired, the effective daily dose of the active compound may be administered as two, three, four, five, six or more sub-doses administered separately at appropriate intervals throughout the day, optionally, in unit dosage forms.

[0086] Pharmaceutical Formulations and Routes of Administration

[0087] Provided herein are pharmaceutical formulations comprising a combination of agents for the treatment of cancer, e.g., melanoma. The pharmaceutical formulations may additionally comprise a carrier or excipient, stabilizer, flavoring agent, and/or coloring agent.

[0088] Provided herein are pharmaceutical formulations comprising combination of agents which can be, for example, a combination of two types of agents: (1) a RAF inhibitor and/or pharmacologically active metabolites, salts, solvates and racemates of the inhibitor and (2) a *MAP3K8* (TPL2/COT) inhibitor and/or pharmacologically active metabolites, salts, solvates and racemates of the COT inhibitor. In another embodiment the combination of agents may be provided for a subject comprising BRAF mutant cells or comprising cells over expressing *MAP3K8* (TPL2/COT) and include: (1) a RAF inhibitor and/or pharmacologically active metabolites, salts, solvates and racemates of the inhibitor and (2) a MEK inhibitor and/or pharmacologically active metabolites, salts, solvates and racemates of the MEK inhibitor.

[0089] The combination of agents may be administered using a variety of routes of administration known to those skilled in the art. The combination of agents may be administered to humans and other animals orally, parenterally, sublingually, by aerosolization or inhalation spray, rectally, intracisternally, intravaginally, intraperitoneally, buccally, or topically in dosage unit formulations containing conventional nontoxic pharmaceutically acceptable carriers, adjuvants, and vehicles as desired. Topical administration may also involve the use of transdermal administration such as transdermal patches or ionophoresis devices. The term parenteral as used herein includes subcutaneous injections, intravenous, intramuscular, intrasternal injection, or infusion techniques.

[0090] Methods of formulation are well known in the art and are disclosed, for example, in Remington: The Science and Practice of Pharmacy, Mack Publishing Company, Easton, Pa., 19th Edition (1995). Pharmaceutical compositions for use in the present invention can be in the form of sterile, non-pyrogenic liquid solutions or suspensions, coated capsules, suppositories, lyophilized powders, transdermal patches or other forms known in the art.

[0091] Injectable preparations, for example, sterile injectable aqueous or oleaginous suspensions may be formulated according to the known art using suitable dispersing or wetting agents and suspending agents. The sterile injectable preparation may also be a sterile injectable solution, suspension or emulsion in a nontoxic parenterally acceptable diluent or solvent, for example, as a solution in 1,3 propanediol or 1,3 butanediol. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution, U.S.P. and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose any bland fixed oil may be employed including synthetic mono or di glycerides. In addition, fatty acids such as oleic acid find use in the preparation of injectables. The injectable formulations can be sterilized, for example, by filtration through a bacterial-retaining filter, or by incorporating sterilizing agents in the form of sterile solid compositions which can be dissolved or dispersed in sterile water or other sterile injectable medium prior to use.

[0092] In order to prolong the effect of a drug, it is often desirable to slow the absorption of the drug from subcutaneous or intramuscular injection. This may be accomplished by the use of a liquid suspension of crystalline or amorphous material with poor water solubility. The rate of absorption of the drug then depends upon its rate of dissolution which, in turn, may depend upon crystal size and crystalline form. Alternatively, delayed absorption of a parenterally administered drug form may be accomplished by dissolving or suspending the drug in an oil vehicle. Injectable depot forms are made by forming microencapsule matrices of the drug in biodegradable polymers such as polylactide polyglycolide. Depending upon the ratio of drug to polymer and the nature of the particular polymer employed, the rate of drug release can be controlled. Examples of other biodegradable polymers include poly(orthoesters) and poly(anhydrides). Depot injectable formulations may also be prepared by entrapping the drug in liposomes or microemulsions, which are compatible with body tissues.

[0093] Compositions for rectal or vaginal administration are preferably suppositories which can be prepared by mixing the compounds of this invention with suitable non irritating excipients or carriers such as cocoa butter, polyethylene glycol or a suppository

wax which are solid at ambient temperature but liquid at body temperature and therefore melt in the rectum or vaginal cavity and release the active compound.

[0094] Solid dosage forms for oral administration include capsules, tablets, pills, powders, and granules. In such solid dosage forms, the active compound is mixed with at least one inert, pharmaceutically acceptable excipient or carrier such as sodium citrate or dicalcium phosphate and/or a) fillers or extenders such as starches, lactose, sucrose, glucose, mannitol, and silicic acid, b) binders such as, for example, carboxymethylcellulose, alginates, gelatin, polyvinylpyrrolidinone, sucrose, and acacia, c) humectants such as glycerol, d) disintegrating agents such as agar-agar, calcium carbonate, potato or tapioca starch, alginic acid, certain silicates, and sodium carbonate, e) solution retarding agents such as paraffin, f) absorption accelerators such as quaternary ammonium compounds, g) wetting agents such as, for example, acetyl alcohol and glycerol monostearate, h) absorbents such as kaolin and bentonite clay, and i) lubricants such as talc, calcium stearate, magnesium stearate, solid polyethylene glycols, sodium lauryl sulfate, and mixtures thereof. In the case of capsules, tablets and pills, the dosage form may also comprise buffering agents.

[0095] Solid compositions of a similar type may also be employed as fillers in soft and hard-filled gelatin capsules using such excipients as lactose or milk sugar as well as high molecular weight polyethylene glycols and the like.

[0096] The solid dosage forms of tablets, dragees, capsules, pills, and granules can be prepared with coatings and shells such as enteric coatings and other coatings well known in the pharmaceutical formulating art. They may optionally contain opacifying agents and can also be of a composition that they release the active ingredient(s) only, or preferentially, in a certain part of the intestinal tract, optionally, in a delayed manner. Examples of embedding compositions that can be used include polymeric substances and waxes.

[0097] The active compounds can also be in micro-encapsulated form with one or more excipients as noted above. The solid dosage forms of tablets, dragees, capsules, pills, and granules can be prepared with coatings and shells such as enteric coatings, release controlling coatings and other coatings well known in the pharmaceutical formulating art. In such solid dosage forms the active compound may be admixed with

at least one inert diluent such as sucrose, lactose or starch. Such dosage forms may also comprise, as is normal practice, additional substances other than inert diluents, e.g., tableting lubricants and other tableting aids such as magnesium stearate and microcrystalline cellulose. In the case of capsules, tablets and pills, the dosage forms may also comprise buffering agents. They may optionally contain opacifying agents and can also be of a composition that they release the active ingredient(s) only, or preferentially, in a certain part of the intestinal tract, optionally, in a delayed manner. Examples of embedding compositions that can be used include polymeric substances and waxes.

[0098] Liquid dosage forms for oral administration include pharmaceutically acceptable emulsions, microemulsions, solutions, suspensions, syrups and elixirs. In addition to the active compounds, the liquid dosage forms may contain inert diluents commonly used in the art such as, for example, water or other solvents, solubilizing agents and emulsifiers such as ethyl alcohol, isopropyl alcohol, ethyl carbonate, EtOAc, benzyl alcohol, benzyl benzoate, propylene glycol, 1,3 butylene glycol, dimethylformamide, oils (in particular, cottonseed, groundnut, corn, germ, olive, castor, and sesame oils), glycerol, tetrahydrofurfuryl alcohol, polyethylene glycols and fatty acid esters of sorbitan, and mixtures thereof. Besides inert diluents, the oral compositions can also include adjuvants such as wetting agents, emulsifying and suspending agents, sweetening, flavoring, and perfuming agents.

[0099] Dosage forms for topical or transdermal administration of a compound of this invention include ointments, pastes, creams, lotions, gels, powders, solutions, sprays, inhalants or patches. The active component is admixed under sterile conditions with a pharmaceutically acceptable carrier and any needed preservatives or buffers as may be required. Ophthalmic formulations, ear drops, and the like are also contemplated as being within the scope of this invention.

[00100] The ointments, pastes, creams and gels may contain, in addition to an active compound of this invention, excipients such as animal and vegetable fats, oils, waxes, paraffins, starch, tragacanth, cellulose derivatives, polyethylene glycols, silicones, bentonites, silicic acid, talc and zinc oxide, or mixtures thereof.

[00101] Compositions of the invention may also be formulated for delivery as a liquid aerosol or inhalable dry powder. Liquid aerosol formulations may be nebulized predominantly into particle sizes that can be delivered to the terminal and respiratory bronchioles.

[00102] Aerosolized formulations of the invention may be delivered using an aerosol forming device, such as a jet, vibrating porous plate or ultrasonic nebulizer, preferably selected to allow the formation of an aerosol particles having with a mass medium average diameter predominantly between 1 to 5 μ m. Further, the formulation preferably has balanced osmolarity ionic strength and chloride concentration, and the smallest aerosolizable volume able to deliver effective dose of the compounds of the invention to the site of the infection. Additionally, the aerosolized formulation preferably does not impair negatively the functionality of the airways and does not cause undesirable side effects.

[00103] Aerosolization devices suitable for administration of aerosol formulations of the invention include, for example, jet, vibrating porous plate, ultrasonic nebulizers and energized dry powder inhalers, that are able to nebulize the formulation of the invention into aerosol particle size predominantly in the size range from 1 to 5 μ m. Predominantly in this application means that at least 70% but preferably more than 90% of all generated aerosol particles are within 1 to 5 μ m range. A jet nebulizer works by air pressure to break a liquid solution into aerosol droplets. Vibrating porous plate nebulizers work by using a sonic vacuum produced by a rapidly vibrating porous plate to extrude a solvent droplet through a porous plate. An ultrasonic nebulizer works by a piezoelectric crystal that shears a liquid into small aerosol droplets. A variety of suitable devices are available, including, for example, AERONEB and AERODOSE vibrating porous plate nebulizers (AeroGen, Inc., Sunnyvale, California), SIDESTREAM nebulizers (Medic Aid Ltd., West Sussex, England), PARI LC and PARI LC STAR jet nebulizers (Pari Respiratory Equipment, Inc., Richmond, Virginia), and AEROSONIC (DeVilbiss Medizinische Produkte (Deutschland) GmbH, Heiden, Germany) and ULTRAIRE (Omron Healthcare, Inc., Vernon Hills, Illinois) ultrasonic nebulizers.

[00104] Compounds of the invention may also be formulated for use as topical powders and sprays that can contain, in addition to the compounds of this invention,

excipients such as lactose, talc, silicic acid, aluminum hydroxide, calcium silicates and polyamide powder, or mixtures of these substances. Sprays can additionally contain customary propellants such as chlorofluorohydrocarbons.

[00105] Transdermal patches have the added advantage of providing controlled delivery of a compound to the body. Such dosage forms can be made by dissolving or dispensing the compound in the proper medium. Absorption enhancers can also be used to increase the flux of the compound across the skin. The rate can be controlled by either providing a rate controlling membrane or by dispersing the compound in a polymer matrix or gel. The compounds of the present invention can also be administered in the form of liposomes. As is known in the art, liposomes are generally derived from phospholipids or other lipid substances. Liposomes are formed by mono or multi lamellar hydrated liquid crystals that are dispersed in an aqueous medium. Any non toxic, physiologically acceptable and metabolizable lipid capable of forming liposomes can be used. The present compositions in liposome form can contain, in addition to a compound of the present invention, stabilizers, preservatives, excipients, and the like. The preferred lipids are the phospholipids and phosphatidyl cholines (lecithins), both natural and synthetic. Methods to form liposomes are known in the art. See, for example, Prescott (ed.), "Methods in Cell Biology," Volume XIV, Academic Press, New York, 1976, p. 33 et seq.

[00106] Examples

[00107] Example 1: An ORF-based functional screen identifies specific kinases as drivers of resistance to B-RAF inhibition.

[00108] To **identify** kinases capable of circumventing RAF inhibition, 597 sequence-validated kinase ORF clones representing ~75% of annotated kinases (Center for Cancer Systems Biology (CCSB)/Broad Institute Kinase ORF Collection) were assembled and stably expressed in A375, a B-RAF^{V600E} malignant melanoma cell line that is sensitive to the RAF kinase inhibitor PLX4720 (Tsai, J. *et al. Proc. Natl Acad. Sci. USA* 105, 3041–3046 (2008)) (Fig. 1a, 1b, Table 3, Fig. 6c). ORF expressing cells treated with 1 μ M PLX4720 were screened for viability relative to untreated cells and normalized to an assay-specific positive control, MEK1^{S218/222D} (MEK1^{DD}) (Emery, C. M. *et al. Proc. Natl Acad. Sci. USA* 106, 20411–20416 (2009).)

(Table 4 and summarized in Fig. 5). Nine ORFs conferred resistance at levels exceeding two standard deviations from the mean (Fig. 1b and Table 4) and were selected for follow-up analysis (Fig. 7). Three of nine candidate ORFs were receptor tyrosine kinases, underscoring the potential of this class of kinases to engage resistance pathways. Resistance effects were validated and prioritized across a multi-point PLX4720 drug concentration scale in the B-RAF^{V600E} cell lines A375 and SKMEL28. The Ser/Thr MAP kinase kinase kinases (MAP3Ks) *MAP3K8* (COT/Tpl2) and *RAF1* (C-RAF) emerged as top candidates from both cell lines; these ORFs shifted the PLX4720 GI₅₀ by 10-600 fold without affecting viability (Table 5 and Figs. 8 and 9). *CRKL*, an ORF that shifted the PLX4720 GI₅₀ to a lesser extent (9.7 fold in SKMEL28 cells; Fig. 8), encodes an adapter protein phosphorylated by tyrosine kinases such as BCR-ABL (Birge, R.B. *et al.*, *Cell Commun Signal* 7, 13 (2009)), but lacks intrinsic kinase activity. COT and C-RAF reduced sensitivity to PLX4720 in multiple B-RAF^{V600E} cell lines (Fig. 1c) confirming the ability of these kinases to mediate resistance to RAF inhibition. A secondary screen in A375 and SKMEL28 prioritizes the top 9 candidate ORFs across a multipoint PLX4720 concentration scale (Fig. 1d).

[00109] Interestingly, the top two validated kinases are both Ser/Thr MAP kinase kinase kinases (MAP3Ks) known to activate MEK/ERK signaling in several contexts. Like B-RAF, C-RAF is a MAP3K in the canonical MAPK cascade (McKay, M.M. and Morrison, D.K. *Oncogene* 26, 3113-3121 (2007)) that was previously implicated in resistance associated with stepwise selection in vitro using a pan-RAF inhibitor (Montagut, C. *et al.* *Cancer Res* 68, 4853-4861 (2008)). COT (the protein product of the human MAP3K8 gene) is best characterized as the MAP3K (Salmeron, A. *et al.* *EMBO J* 15, 817-826 (1996)) downstream of NFκB signaling in inflammatory cells (Banerjee, A. *et al.*, *Proc Natl Acad Sci U.S.A.* 103, 3274-3279 (2006)); however, its functional importance in human cancer has not previously been elucidated.

[00110] Example 2: Resistance to B-RAF inhibition via MAPK pathway activation.

[00111] Whether the overexpression of these genes was sufficient to activate the MAPK pathway was also tested. At baseline, COT expression increased ERK

phosphorylation in a manner comparable to MEK1^{DD}, consistent with MAP kinase pathway activation (Figs. 2a and 10). Overexpression of wild-type COT or C-RAF resulted in constitutive phosphorylation of ERK and MEK in the presence of PLX4720, whereas kinase-dead derivatives had no effect (Figs. 2a and 11). Thus, COT and C-RAF drive resistance to RAF inhibition predominantly through re-activation of MAPK signaling. Notably, of the nine candidate ORFs from the initial screen, a subset (3) did not show persistent ERK/MEK phosphorylation following RAF inhibition, suggesting MAPK pathway-independent alteration of drug sensitivity (Figure 12).

[00112] Example 3: C-RAF activation and heterodimerization with B-RAF.

[00113] C-RAF activation and heterodimerization with B-RAF constitute critical components of the cellular response to B-RAF inhibition. In A375 cells, endogenous C-RAF: B-RAF heterodimers were measurable and inducible following treatment with PLX4720 (Fig. 13). However, endogenous C-RAF phosphorylation at S338—an event required for C-RAF activation—remained low (Fig. 13). In contrast, ectopically expressed C-RAF was phosphorylated on S338 (Fig. 13) and its PLX4720 resistance phenotype was associated with sustained MEK/ERK activation (Figs. 2a and 13). Moreover, ectopic expression of a high-activity C-RAF truncation mutant (C-RAF(W22)) was more effective than wild-type C-RAF in mediating PLX4720 resistance and ERK activation (Fig. 14), further indicating that elevated C-RAF activity directs resistance to this agent. Consistent with this model, oncogenic alleles of *NRAS* and *KRAS* conferred PLX4720 resistance in A375 cells (Fig. 2b) and yielded sustained C-RAF(S338) and ERK phosphorylation in the context of drug treatment (Fig. 2c). Thus, although genetic alterations that engender C-RAF activation (e.g., oncogenic RAS mutations) tend to show mutual exclusivity with B-RAF^{V600E} mutation, such co-occurring events are favored in the context of acquired resistance to B-RAF inhibition.

[00114] Example 4: Investigation of COT expression in Melanoma

[00115] While C-RAF has been linked previously to melanoma and MAPK pathway dependencies (Montagut, C. *et al.* 2008; Karreth, F.A., DeNicola, G.M. *et al.*, 2009; Dumaz, N. *et al.* *Cancer Res* 66, 9483-9491 (2006); Hatzivassiliou, G. *et al.*

Nature (2010); Heidorn, S.J. *et al.*, *Cell* 140, 209-221 (2010); Poulikakos, P.I. *et al.*, *Nature* (2010)), COT has not been described as a melanoma-associated kinase.

[00116] The role of COT in melanoma was investigated, and its expression in human melanocytes was examined. Primary immortalized melanocytes (B-RAF wild-type) expressed COT (Fig. 2d), although ectopic B-RAF^{V600E} expression reduced COT mRNA levels (Fig. 15) and rendered COT protein undetectable (Fig. 2d). Conversely, whereas ectopically expressed COT was only weakly detectable in A375 cells (Figs. 2a, 2e), shRNA-mediated depletion of endogenous B-RAF^{V600E} caused an increase in COT protein levels that correlated with the extent of B-RAF knockdown (Fig. 2e). Moreover, treatment of COT-expressing A375 cells with PLX4720 led to a dose-dependent increase in COT protein (Fig. 2a) without affecting ectopic COT mRNA levels (Fig. 15). Oncogenic B-RAF antagonizes COT expression largely through altered protein stability (Figs. 2a, d, e, and 15), and B-RAF inhibition potentiates the outgrowth of COT-expressing cells during the course of treatment. Notably, neither C-RAF nor B-RAF alone or in combination was required for ERK phosphorylation in the context of COT expression, even in the presence of PLX4720 (Figs. 2e, 2f and Fig. 16). As shown, COT expression is sufficient to induce MAP kinase pathway activation in a RAF-independent manner.

[00117] Example 5: COT expression predicts resistance to B-RAF inhibition in cancer cell lines.

[00118] Whether cell lines expressing elevated COT in a B-RAF^{V600E} background exhibit *de novo* resistance to PLX4720 treatment was tested. To identify such instances, a panel of cell lines was screened for evidence of *MAP3K8/COT* copy number gains coincident with the B-RAF^{V600E} mutation. Of 534 cell lines that had undergone copy number analysis and mutation profiling, 38 cell lines (7.1%) contained the B-RAF^{V600E} mutation. Within this subgroup, two cell lines—OUMS-23 (colon cancer) and RPMI-7951 (melanoma)—also showed evidence of chromosomal copy gains spanning the *MAP3K8/COT* locus (Figs. 3a and 17) and robust COT protein expression (Figs. 3b and 18). A panel of melanoma short-term cultures was also screened for COT protein expression. One of these lines expressed COT: M307, a short-term culture derived from a B-RAF^{V600E} tumor that developed resistance to allosteric MEK inhibition

following initial disease stabilization (Fig. 3c). All three cell lines were refractory to PLX4720 treatment, exhibiting GI_{50} values in the range of 8-10 μ M (Fig. 3d) and showing sustained ERK phosphorylation in the context of B-RAF inhibition (Figs. 3e, 3f). OUMS-23 and RPMI-7951 are MAPK pathway inhibitor-naïve cell lines; thus, these results demonstrate that COT confers *de novo* resistance to RAF inhibition (a phenomenon observed in ~10% of B-RAF^{V600E} melanomas).

[00119] Example 6: COT expression in patients treated with a RAF inhibitor.

[00120] COT expression in the context of resistance to the clinical RAF inhibitor PLX4032 was examined by obtaining biopsy material from 3 patients with metastatic, B-RAF^{V600E} melanoma. Each case consisted of frozen, lesion-matched biopsy material obtained prior to and during treatment (“pre-treatment” and “on-treatment”; Fig. 3g, Table 6); additionally, one sample contained two independent biopsy specimens from the same relapsing tumor site (“post-relapse”; Fig. 3g). Consistent with the experimental models presented above, quantitative real-time RT-PCR (qRT/PCR) analysis revealed increased COT mRNA expression concurrent with PLX4032 treatment in 2 of 3 cases. COT mRNA levels were further increased in a relapsing specimen relative to its pre-treatment and on-treatment counterparts (Fig. 3g, Patient #1). An additional, unmatched relapsed malignant melanoma biopsy showed elevated COT mRNA expression comparable to levels observed in RAF inhibitor-resistant, COT-amplified cell lines (Fig. 19). This specimen also exhibited robust MAPK pathway activation and elevated expression of B-RAF, C-RAF and COT relative to matched normal skin or B-RAF^{V600E} cell lines (Fig. 19). Sequencing studies of this tumor revealed no additional mutations in *BRAF*, *NRAS* or *KRAS* (data not shown). These analyses provided clinical evidence that COT-dependent mechanisms are operant in PLX4032-resistant malignant melanomas.

[00121] Example 7: COT regulation of MEK/ERK phosphorylation.

[00122] Whether COT actively regulates MEK/ERK phosphorylation in B-RAF^{V600E} cells that harbor naturally elevated COT expression was tested by introducing shRNA constructs targeting COT into RPMI-7951 cells. Depletion of COT suppressed RPMI-7951 viability (Fig. 20) and decreased ERK phosphorylation (Fig. 3h); thus, targeting COT kinase activity suppresses MEK/ERK phosphorylation in cancer cells with

COT overexpression or amplification. Additionally, the targeting COT kinase activity in the presence of a B-RAF inhibitor (PLX4720) suppresses MEK/ERK phosphorylation (Fig. 3h). Treatment of RPMI-7951 cells with a small molecule COT kinase inhibitor (Wyeth, Abbot compound ID 9549300) (George, D. *et al.*, *Bioorg. Med. Chem. Lett.* 18, 4952–4955 (2008); Hirata, K. *et al.*, *Biol. Pharm. Bull.* 33, 133–137 (2010); Lee, K. M. *et al.*, *Cancer Res.* 69, 8043–8049 (2009)) resulted in dose-dependent suppression of MEK and ERK phosphorylation, providing additional evidence that COT contributes to MEK/ERK activation in these cells (Fig. 3i).

[00123] Example 8: COT-expressing B-RAF^{V600E} cell lines exhibit resistance to allosteric MEK inhibitors.

[00124] Whether COT-expressing cancer cells remain sensitive to MAPK pathway inhibition at a target downstream of COT or RAF was analyzed. The OUMS-23 and RPMI-7951 cell lines were queried for sensitivity to the MEK1/2 inhibitor CI-1040. Both cell lines were refractory to MEK inhibition (Fig. 4a) and displayed sustained ERK phosphorylation even at 1 μ M CI-1040 (Fig. 4b). Ectopic COT expression in A375 and SKMEL28 cells also conferred decreased sensitivity to the MEK inhibitors CI-1040 and AZD6244, suggesting that COT expression alone was sufficient to induce this phenotype (Figs. 4c, 4d and 21). Similar to results observed with pharmacological MEK inhibitors, MEK1/2 knockdown only modestly suppressed COT-mediated ERK phosphorylation in A375 cells (Fig. 22). These data demonstrate that COT activates ERK through MEK-independent and MEK-dependent mechanisms. Furthermore, an *in vitro* kinase assay using recombinant COT and ERK1 was performed, and it was demonstrated that recombinant COT induced pThr202/Tyr204 phosphorylation of ERK1 *in vitro* (Fig. 22). Thus, COT expression potentiates ERK activation in a MEK-independent manner.

[00125] Example 9: Combinatorial MAPK pathway inhibition to suppress cell proliferation.

[00126] The use of RAF and MEK inhibitors in combination can override resistance to single-agents as shown in Fig 23. It was tested whether the combined RAF/MEK inhibition might circumvent COT-driven resistance. In the setting of ectopic COT expression, exposure to AZD6244 or CI-1040 in combination with PLX470 (1 μ M

each) reduced cell growth and pERK expression more effectively than did single-agent PLX4720, even at concentrations of 10 μ M (Figs. 4e, 4f and 23). These data underscore the importance of this pathway in B-RAF^{V600E} tumor cells and demonstrate that dual B-RAF/MEK inhibition helps circumvent resistance to RAF inhibitors.

[00127] Methods

[00128] Center for Cancer Systems Biology (CCSB)/Broad Institute Kinase Open Reading Frame Collection

[00129] A library of 597 kinase ORFs in pDONR-223 Entry vectors (Invitrogen) was assembled. Individual clones were end-sequenced using vector-specific primers in both directions. Clones with substantial deviations from reported sequences were discarded. Entry clones and sequences are available via Addgene (http://www.addgene.org/human_kinases). Kinase ORFs were assembled from multiple sources; 337 kinases were isolated as single clones from the ORFeome 5.1 collection (<http://horfdb.dfci.harvard.edu>), 183 kinases were cloned from normal human tissue RNA (Ambion) by reverse transcription and subsequent PCR amplification to add Gateway sequences (Invitrogen), 64 kinases were cloned from templates provided by the Harvard Institute of Proteomics (HIP), and 13 kinases were cloned into the Gateway system from templates obtained from collaborating laboratories. The Gateway-compatible lentiviral vector pLX-Blast-V5 was created from the pLKO.1 backbone. LR Clonase enzymatic recombination reactions were performed to introduce the 597 kinases into pLX-Blast-V5 according to the manufacturer's protocol (Invitrogen).

[00130] High throughput ORF screening

[00131] A375 melanoma cells were plated in 384-well microtiter plates (500 cells per well). The following day, cells were spin-infected with the lentivirally-packaged kinase ORF library in the presence of 8 μ g/ml polybrene. 48 hours post-infection, media was replaced with standard growth media (2 replicates), media containing 1 μ M PLX4720 (2 replicates, 2 time points) or media containing 10 μ g/ml blasticidin (2 replicates). After four days and 6 days, cell growth was assayed using Cell Titer-Glo (Promega) according to manufacturer instructions. The entire experiment was performed twice.

[00132] Identification of candidate resistance ORFs

[00133] Raw luminescence values were imported into Microsoft Excel. Infection efficiency was determined by the percentage of duplicate-averaged raw luminescence in blasticidin selected cells relative to non-selected cells. ORFs with an infection efficiency of less than 0.70 were excluded from further analysis along with any ORF having a standard deviation of >15,000 raw luminescence units between duplicates. To identify ORFs whose expression affects proliferation, we compared the duplicate-averaged raw luminescence of individual ORFs against the average and standard deviation of all control-treated cells via the z-score, or standard score, below,

$$Z = \frac{x - \mu}{\sigma}$$

where x = average raw luminescence of a given ORF, μ = the mean raw luminescence of all ORFs and σ = the standard deviation of the raw luminescence of all wells. Any individual ORF with a z-score >+2 or <-2 was annotated as affecting proliferation and removed from final analysis. Differential proliferation was determined by the percentage of duplicate-averaged raw luminescence values in PLX4720 (1 μ M) treated cells relative to untreated cells. Subsequently, differential proliferation was normalized to the positive control for PLX4720 resistance, MEK1^{S218/222D} (MEK1^{DD}), with MEK1^{DD} differential proliferation = 1.0. MEK1^{DD} normalized differential proliferation for each individual ORF was averaged across two duplicate experiments, with two time points for each experiment (day 4 and day 6). A z-score was then generated, as described above for average MEK1^{DD} normalized differential proliferation. ORFs with a z-score of >2 were considered hits and were followed up in the secondary screen.

[00134] ORF and shRNA expression

[00135] ORFs were expressed from pLX-Blast-V5 (lentiviral) or pWZL-Blast, pBABE-Puro or pBABE-zeocin (retroviral) expression plasmids. For lentiviral transduction, 293T cells were transfected with 1 μ g of pLX-Blast-V5-ORF or

pLKO.1-shRNA, 900 ng Δ 8.9 (*gag*, *pol*) and 100 ng VSV-G using 6 μ l Fugene6 transfection reagent (Roche). Viral supernatant was harvested 72 h post-transfection. Mammalian cells were infected at a 1:10-1:20 dilution of virus in 6-well plates in the presence of 5 μ g/ml polybrene and centrifuged at 2250 RPM for 1 h at 37°C. Twenty-four hours after infection blasticidin (pLX-Blast-V5, 10 μ g/ml) or puro (pLKO.1, 0.75 μ g/ml) was added and cells were selected for 48 hrs. For retrovirus production, 293T were transfected with 1 μ g of retroviral plasmid-ORF, 1 μ g pCL-AMPHO and 100 ng VSV-G, as described above. Cells were infected with retrovirus containing supernatant at a 1:2 dilution in 5 μ g/ml polybrene overnight, followed by media change to growth medium. Infection was repeated once more (twice total), followed by selection, above.

[00136] Secondary screen

[00137] A375 (1.5×10^3) and SKMEL28 cells (3×10^3) were seeded in 96-well plates for 18 h. ORF-expressing lentivirus was added at a 1:10 dilution in the presence of 8 μ g/ml polybrene, and centrifuged at 2250 RPM and 37° C for 1 h. Following centrifugation, virus-containing media was changed to normal growth media and allowed to incubate for 18 h. Twenty-four hours after infection, DMSO (1:1000) or 10x PLX4720 (in DMSO) was added to a final concentration of 100, 10, 1, 0.1, 0.01, 0.001, 0.0001 or 0.00001 μ M. Cell viability was assayed using WST-1 (Roche), per manufacturer recommendation, 4 days after the addition of PLX4720.

[00138] Cell lines and reagents

[00139] A375, SKMEL28, SKMEL30, COLO-679, WM451lu, SKMEL5, Malme 3M, SKMEL30, WM3627, WM1976, WM3163, WM3130, WM3629, WM3453, WM3682 and WM3702 were all grown in RPMI (Cellgro), 10% FBS and 1% penicillin/streptomycin. M307 was grown in RPMI (Cellgro), 10% FBS and 1% penicillin/streptomycin supplemented with 1 mM sodium pyruvate. 293T and OUMS-23 were grown in DMEM (Cellgro), 10% FBS and 1% penicillin/streptomycin. RPMI-7951 cells (ATCC) were grown in MEM (Cellgro), 10% FBS and 1% penicillin/streptomycin. Wild-type primary melanocytes were grown in HAM's F10 (Cellgro), 10% FBS and 1% penicillin/streptomycin. B-RAF^{V600E}-expressing primary

melanocytes were grown in TIVA media [Ham's F-10 (Cellgro), 7% FBS, 1% penicillin/streptomycin, 2mM glutamine (Cellgro), 100 μ M IBMX, 50 ng/ml TPA, 1mM dbcAMP (Sigma) and 1 μ M sodium vanadate]. CI-1040 (PubChem ID: 6918454) was purchased from Shanghai Lechen International Trading Co., AZD6244 (PubChem ID: 10127622) from Selleck Chemicals, and PLX4720 (PubChem ID: 24180719) from Symansis. RAF265 (PubChem ID: 11656518) was a generous gift from Novartis Pharma AG. Unless otherwise indicated, all drug treatments were for 16 h. Activated alleles of NRAS and KRAS have been previously described. (Boehm, J. S. *et al. Cell* 129, 1065–1079 (2007); Lundberg, A. S. *et al. Oncogene* 21, 4577–4586 (2002)).

[00140] Pharmacologic growth inhibition assays

[00141] Cultured cells were seeded into 96-well plates (3,000 cells per well) for all melanoma cell lines; 1,500 cells were seeded for A375. Twenty-four hours after seeding, serial dilutions of the relevant compound were prepared in DMSO added to cells, yielding final drug concentrations ranging from 100 μ M to 1×10^5 μ M, with the final volume of DMSO not exceeding 1%. Cells were incubated for 96 h following addition of drug. Cell viability was measured using the WST1 viability assay (Roche). Viability was calculated as a percentage of control (untreated cells) after background subtraction. A minimum of six replicates were performed for each cell line and drug combination. Data from growth-inhibition assays were modeled using a nonlinear regression curve fit with a sigmoid dose–response. These curves were displayed and GI50 generated using GraphPad Prism 5 for Windows (GraphPad). Sigmoid-response curves that crossed the 50% inhibition point at or above 10 μ M have GI50 values annotated as >10 μ M. For single-dose studies, the identical protocol was followed, using a single dose of indicated drug (1 μ M unless otherwise noted).

[00142] Immunoblots and immunoprecipitations

[00143] Cells were washed twice with ice-cold PBS and lysed with 1% NP-40 buffer [150 mM NaCl, 50 mM Tris pH 7.5, 2 mM EDTA pH 8, 25 mM NaF and 1% NP-40] containing 2x protease inhibitors (Roche) and 1x Phosphatase Inhibitor Cocktails I and II (CalBioChem). Lysates were quantified (Bradford assay),

normalized, reduced, denatured (95°C) and resolved by SDS gel electrophoresis on 10% Tris/Glycine gels (Invitrogen). Protein was transferred to PVDF membranes and probed with primary antibodies recognizing pERK1/2 (T202/Y204), pMEK1/2 (S217/221), MEK1/2, MEK1, MEK2, C-RAF (rabbit host), pC-RAF (pS338) (Cell Signaling Technology; 1:1,000), V5-HRP (Invitrogen; (1:5,000), COT (1:500), B-RAF (1:2,000), Actin (1:1,000), Actin-HRP (1:1,000; Santa Cruz)), C-RAF (mouse host; 1:1,000; BD Transduction Labs), Vinculin (Sigma; 1:20,000), AXL (1:500; R&D Systems). After incubation with the appropriate secondary antibody (anti-rabbit, anti-mouse IgG, HRP-linked; 1:1,000 dilution, Cell Signaling Technology or anti-goat IgG, HRP-linked; 1:1,000 dilution; Santa Cruz), proteins were detected using chemiluminescence (Pierce). Immunoprecipitations were performed overnight at 4° C in 1% NP-40 lysis buffer, as described above, at a concentration of 1 µg/µl total protein using an antibody recognizing C-RAF (1:50; Cell Signaling Technology). Antibody: antigen complexes were bound to Protein A agarose (25 µL, 50% slurry; Pierce) for 2 hrs. at 4° C. Beads were centrifuged and washed three times in lysis buffer and eluted and denatured (95 °C) in 2x reduced sample buffer (Invitrogen). Immunoblots were performed as above. Phospho-protein quantification was performed using NIH Image J.

[00144] Lysates from tumor and matched normal skin were generated by mechanical homogenization of tissue in RIPA [50 mM Tris (pH 7.4), 150 mM NaCl, 1mM EDTA, 0.1% SDS, 1.0% NaDOC, 1.0% Triton X-100, 25 mM NaF, 1mM NA3VO4] containing protease and phosphatase inhibitors, as above. Subsequent normalization and immunoblots were performed as above.

[00145] Biopsied melanoma tumour material

[00146] Biopsied tumor material consisted of discarded and de-identified tissue that was obtained with informed consent and characterized under protocol 02-017 (paired samples, Massachusetts General Hospital) and 07-087 (unpaired sample, Dana-Farber Cancer Institute). For paired specimens, 'on-treatment' samples were collected 10-14 days after initiation of PLX4032 treatment (Table 6).

[00147] Inhibition of COT kinase activity

[00148] Adherent RPMI-7951 cells were washed twice with 1x PBS and incubated overnight in serum-free growth media. Subsequently, 4-(3-Chloro-4-fluorophenylamino)-6-(pyridin-3-yl-methylamino)-3-cyano-[1,7]-naphthyridine (EMD;TPL2 inhibitor I; Cat#: 616373, PubChem ID: 9549300), suspended in DMSO at the indicated concentration, was added to cells for 1 hour, after which protein extracts were made as described above.

[00149] Quantitative RT-PCR

[00150] mRNA was extracted from cell lines and fresh-frozen tumors using the RNeasy kit (Qiagen). Total mRNA was used for subsequent reverse transcription using the SuperScript III First-Strand Synthesis SuperMix (Invitrogen) for cell lines and unpaired tumor samples, and the SuperScript VILO cDNA synthesis kit (Invitrogen) for paired frozen tumor samples. 5 µl of the RT reaction was used for quantitative PCR using SYBR Green PCR Master Mix and gene-specific primers, in triplicate, using an ABI 7300 Real Time PCR System. Primers used for detection are as follows:

Primer	Sequence	SEQ.ID. NO.
<i>COT</i> forward	CAAGTGAAGAGCCAGCAGTTT	SEQ. ID.NO: 1
<i>COT</i> reverse	GCAAGCAAATCCTCCACAGTTC	SEQ. ID.NO: 2
<i>TBP</i> forward	CCCGAAACGCCGAATATAATCC	SEQ. ID.NO: 3
<i>TBP</i> reverse	GACTGTTCTTCACTCTTGGCTC	SEQ. ID.NO: 4
<i>GAPDH</i> forward	CATCATCTCTGCCCCCTCT	SEQ. ID.NO: 5
<i>GAPDH</i> reverse	GGTGCTAAGCAGTTGGTGGT	SEQ. ID.NO: 6

[00151] *In vitro* kinase assay

[00152] *In vitro* kinase assays were performed as previously described using 1 µg each of COT (amino acids 30- 397, R&D Systems) and inactive ERK1 (Millipore).

[00153] Cellular viability assays

[00154] Adherent RPMI-7951 cells were infected with virus expressing shRNAs against COT or Luciferase as described above. Following selection, cells were plated (1.5×10^5 cells/well) onto a 24-well plate in quadruplicate. Viable cells were

counted via trypan blue exclusion using a VI-CELL Cell Viability Analyzer, per manufacturer's specifications. Quadruplicate cell counts were averaged and normalized relative to that of the control shRNA.

[00155] The Cancer Cell Line Encyclopedia (CCLE)

[00156] The Cancer Cell Line Encyclopedia (CCLE) project is a collaboration between the Broad Institute, the Novartis Institutes for Biomedical Research (NIBR) and the Genomics Institute of the Novartis Research Foundation (GNF) to conduct a detailed genetic and pharmacologic characterization of a large panel of human cancer models, to develop integrated computational analyses that link distinct pharmacologic vulnerabilities to genomic patterns and to translate cell line integrative genomics into cancer patient stratification. Chromosomal copy number and gene expression data used for this study are available online at <http://www.broadinstitute.org/cgi-bin/cancer/datasets.cgi>.

[00157] Expression profiling of cancer cell lines

[00158] Oligonucleotide microarray analysis was carried out using the GeneChip Human Genome U133 Plus 2.0 Affymetrix expression array (Affymetrix). Samples were converted to labeled, fragmented, cRNA following the Affymetrix protocol for use on the expression microarray.

[00159] shRNA constructs used (pLKO.1)

[00160] The DNA sequences for preparing the shRNA constructs used were as follows:

shRNA	TRC Identifier	NM No.	Sequence	SEQ. ID. NO.
shLuc	TRCN 0000072243	NA	CTTCGAAATGTCCGTTCCGGTT	SEQ. ID. NO. 7
shBRAF(1)	TRCN 0000006289	NM_004333.2- 1106s1c1	CTTCGAAATGTCCGTTCCGGTT	SEQ. ID. NO. 8
shBRAF(2)	TRCN 0000006291	NM_004333.2- 2267s1c1	GCTGGTTTCCAAACAGAGGAT	SEQ. ID. NO. 9
shCRAF(1)	TRCN	NM_002880.x-	CGGAGATGTTGCAGTAAAGAT	SEQ. ID.

	0000001066	1236s1c1		NO. 10
shCRAF(2)	TRCN 0000001068	NM_002880.x- 1529s1c1	GAGACATGAAATCCAACAATA	SEQ. ID. NO. 11
shMEK1(1)	TRCN 0000002332	NM_002755.x- 1015s1c1	GATTACATAGTCAACGAGCCT	SEQ. ID. NO. 12
shMEK1(2)	TRCN 0000002329	NM_002755.x- 455s1c1	GCTTCTATGGTGCGTTCTACA	SEQ. ID. NO. 13
shMEK2(1)	TRCN 0000007007	NM_030662.2- 1219s1c1	TGGACTATATTGTGAACGAGC	SEQ. ID. NO. 14
shMEK2(2)	TRCN 0000007005	NM_030662.2- 847s1c1	CCAACATCCTCGTGAACCTCTA	SEQ. ID. NO. 15
shCOT(1)	TRCN 0000010013	NM_005204.x- 1826s1c1	CAAGAGCCGCAGACCTACTAA	SEQ. ID. NO. 16
shCOT(2)	TRCN 0000196518	NM_005204.2- 2809s1c1	GATGAGAATGTGACCTTTAAG	SEQ. ID. NO. 17

[00161] The definitions and disclosures provided herein govern and supersede all others incorporated by reference. Although the invention herein has been described in connection with preferred embodiments thereof, it will be appreciated by those skilled in the art that additions, modifications, substitutions, and deletions not specifically described may be made without departing from the spirit and scope of the invention as defined in the appended claims. It is therefore intended that the foregoing detailed description be regarded as illustrative rather than limiting, and that it be understood that it is the following claims, including all equivalents, that are intended to define the spirit and scope of this invention.

Table 3: CCSB/Broad Institute Kinase ORF Library description and ORF classification

Abbreviations: RS/TK (Receptor Serine/Threonine Kinase); RTK (Receptor Tyrosine Kinase); NRS/TK(Non-Receptor Serine/Threonine Kinase); NRTK (Non-Receptor Tyrosine Kinase)

hGENE	GENE ID	DESCRIPTION	KINASE CLASS
<i>AAK1</i>	22848	AP2 associated kinase 1	protein kinase (NRS/TK)
<i>ABL1</i>	25	v-abl Abelson murine leukemia viral oncogene homolog 1	protein kinase (NRTK)
<i>ABL2</i>	27	v-abl Abelson murine leukemia viral oncogene homolog 2 (arg, Abelson-related gene)	protein kinase (NRTK)
<i>ACVR1</i>	90	activin A receptor, type I	protein kinase (RS/TK)
<i>ACVR1B</i>	91	activin A receptor, type IB	protein kinase (RS/TK)
<i>ACVR1C</i>	130399	activin A receptor, type IC	protein kinase (RS/TK)
<i>ACVR2A</i>	92	activin A receptor, type II	protein kinase (RS/TK)
<i>ACVR2B</i>	93	activin A receptor, type IIB	protein kinase (RS/TK)
<i>ACVRL1</i>	94	activin A receptor type II-like 1	protein kinase (RS/TK)
<i>ADCK1</i>	57143	aarF domain containing kinase 1	protein kinase
<i>ADCK2</i>	90956	aarF domain containing kinase 2	protein kinase
<i>ADCK4</i>	79934	aarF domain containing kinase 4	protein kinase
<i>ADPGK</i>	83440	ADP-dependent glucokinase	kinase related
<i>ADRBK1</i>	156	adrenergic, beta, receptor kinase 1	protein kinase (NRS/TK)
<i>ADRBK2</i>	157	adrenergic, beta, receptor kinase 2	protein kinase (NRS/TK)
<i>AGK</i>	55750	multiple substrate lipid kinase;MULK	kinase related
<i>AK1</i>	203	adenylate kinase 1	nucleotide kinase
<i>AK2</i>	204	adenylate kinase 2	nucleotide kinase
<i>AK3</i>	205	adenylate kinase 3	nucleotide kinase
<i>AK3L1</i>	50808	adenylate kinase 3 like 1	nucleotide kinase
<i>AK7</i>	122481	adenylate kinase 7	nucleotide kinase
<i>AKT1</i>	207	v-akt murine thymoma viral oncogene homolog 1	protein kinase (NRS/TK)
<i>AKT3</i>	10000	v-akt murine thymoma viral oncogene homolog 3 (protein kinase B, gamma)	protein kinase (NRS/TK)
<i>ALDH18A1</i>	5832	aldehyde dehydrogenase 18 family, member A1;ALDH18A1	kinase related
<i>ALK</i>	238	anaplastic lymphoma kinase (Ki-1)	protein kinase (RTK)
<i>ALPK1</i>	80216	alpha-kinase 1	protein kinase (NRS/TK)
<i>ALPK2</i>	115701	alpha-kinase 2	protein kinase (NRS/TK)
<i>ALS2CR7</i>	65061	amyotrophic lateral sclerosis 2 (juvenile) chromosome region, candidate 7	protein kinase (NRS/TK)
<i>AMHR2</i>	269	anti-Mullerian hormone receptor, type II	protein kinase (RS/TK)

hGENE	GENE ID	DESCRIPTION	KINASE CLASS
<i>ARAF</i>	369	v-raf murine sarcoma 3611 viral oncogene homolog 1	protein kinase (NRS/TK)
<i>ARSG</i>	22901	arylsulfatase G;ARSG	kinase related
<i>ASCIZ</i>	23300	ATM/ATR-Substrate Chk2-Interacting Zn ²⁺ -finger protein;ASCIZ	protein kinase (NRS/TK)
<i>AURKA</i>	6790	serine/threonine kinase 6	protein kinase (NRS/TK)
<i>AURKB</i>	9212	aurora kinase B	protein kinase (NRS/TK)
<i>AURKC</i>	6795	aurora kinase C	protein kinase (NRS/TK)
<i>AXL</i>	558	AXL receptor tyrosine kinase	protein kinase (RTK)
<i>BCKDK</i>	10295	branched chain alpha-ketoacid dehydrogenase kinase	protein kinase
<i>BLK</i>	640	B lymphoid tyrosine kinase	protein kinase (NRTK)
<i>BMP2K</i>	55589	BMP2 inducible kinase	protein kinase (NRS/TK)
<i>BMP2KL</i>	347359	BMP2 inducible kinase-like	protein kinase (NRS/TK)
<i>BMPR1A</i>	657	bone morphogenetic protein receptor, type IA	protein kinase (RS/TK)
<i>BMPR1B</i>	658	bone morphogenetic protein receptor, type IB	protein kinase (RS/TK)
<i>BMPR2</i>	659	bone morphogenetic protein receptor, type II (serine/threonine kinase)	protein kinase (RS/TK)
<i>BMX</i>	660	BMX non-receptor tyrosine kinase	protein kinase (NRTK)
<i>BRAF</i>	673	v-raf murine sarcoma viral oncogene homolog B1	protein kinase (NRS/TK)
<i>BRD3</i>	8019	bromodomain containing 3	protein kinase (NRS/TK)
<i>BRD4</i>	23476	bromodomain containing 4	protein kinase (NRS/TK)
<i>BRSK1</i>	84446	KIAA1811 protein	protein kinase (NRS/TK)
<i>BRSK2</i>	9024	serine/threonine kinase 29	protein kinase (NRS/TK)
<i>BTK</i>	695	Bruton agammaglobulinemia tyrosine kinase	protein kinase (NRTK)
<i>BUB1</i>	699	BUB1 budding uninhibited by benzimidazoles 1 homolog (yeast)	protein kinase (NRS/TK)
<i>BUB1B</i>	701	BUB1 budding uninhibited by benzimidazoles 1 homolog beta (yeast)	protein kinase (NRS/TK)
<i>C1orf57</i>	84284	chromosome 1 open reading frame 57;C1orf57	kinase related
<i>C9orf95</i>	54981	chromosome 9 open reading frame 95;C9orf95	kinase related
<i>C9orf98</i>	158067	chromosome 9 open reading frame 98;C9orf98	nucleotide kinase
<i>CABC1</i>	56997	chaperone, ABC1 activity of bc1 complex like (S. pombe)	protein kinase
<i>CALM1</i>	801	calmodulin 1 (phosphorylase kinase, delta)	kinase related

hGENE	GENE ID	DESCRIPTION	KINASE CLASS
CALM2	805	calmodulin 2 (phosphorylase kinase, delta)	kinase related
CALM3	808	calmodulin 3 (phosphorylase kinase, delta)	kinase related
CAMK1	8536	calcium/calmodulin-dependent protein kinase I	protein kinase (NRS/TK)
CAMK1D	57118	calcium/calmodulin-dependent protein kinase ID	protein kinase (NRS/TK)
CAMK1G	57172	calcium/calmodulin-dependent protein kinase IG	protein kinase (NRS/TK)
CAMK2A	815	calcium/calmodulin-dependent protein kinase (CaM kinase) II alpha	protein kinase (NRS/TK)
CAMK2B	816	calcium/calmodulin-dependent protein kinase (CaM kinase) II beta	protein kinase (NRS/TK)
CAMK2D	817	calcium/calmodulin-dependent protein kinase (CaM kinase) II delta	protein kinase (NRS/TK)
CAMK2G	818	calcium/calmodulin-dependent protein kinase (CaM kinase) II gamma	protein kinase (NRS/TK)
CAMK4	814	calcium/calmodulin-dependent protein kinase IV	protein kinase (NRS/TK)
CAMKK1	84254	calcium/calmodulin-dependent protein kinase kinase 1, alpha	protein kinase (NRS/TK)
CAMKK2	10645	calcium/calmodulin-dependent protein kinase kinase 2, beta	protein kinase (NRS/TK)
CAMKV	79012	hypothetical protein MGC8407	protein kinase (NRS/TK)
CARD11	84433	caspase recruitment domain family, member 11;CARD11	nucleotide kinase
CARKL	23729	carbohydrate kinase-like	carbohydrate kinase
CASK	8573	calcium/calmodulin-dependent serine protein kinase (MAGUK family)	nucleotide kinase
CCL2	6347	chemokine (C-C motif) ligand 2;CCL2	protein kinase
CCL4	6351	chemokine (C-C motif) ligand 4;CCL4	protein kinase (RTK)
CCRK	23552	cell cycle related kinase	protein kinase (NRS/TK)
CD2	914	CD2 antigen (p50), sheep red blood cell receptor;CD2	protein kinase
CDC2	983	cell division cycle 2, G1 to S and G2 to M	protein kinase (NRS/TK)
CDC2L1	984	cell division cycle 2-like 1 (PITSLRE proteins)	protein kinase (NRS/TK)
CDC2L2	985	cell division cycle 2-like 2 (PITSLRE proteins)	protein kinase (NRS/TK)
CDC2L6	23097	cell division cycle 2-like 6 (CDK8-like)	protein kinase (NRS/TK)
CDC42BP G	55561	CDC42 binding protein kinase gamma (DMPK-like)	protein kinase (NRS/TK)
CDC7	8317	CDC7 cell division cycle 7 (S. cerevisiae)	protein kinase

hGENE	GENE ID	DESCRIPTION	KINASE CLASS
			(NRS/TK)
<i>CDK10</i>	8558	cyclin-dependent kinase (CDC2-like) 10	protein kinase (NRS/TK)
<i>CDK2</i>	1017	cyclin-dependent kinase 2	protein kinase (NRS/TK)
<i>CDK3</i>	1018	cyclin-dependent kinase 3	protein kinase (NRS/TK)
<i>CDK4</i>	1019	cyclin-dependent kinase 4	protein kinase (NRS/TK)
<i>CDK5</i>	1020	cyclin-dependent kinase 5	protein kinase (NRS/TK)
<i>CDK5R1</i>	8851	cyclin-dependent kinase 5, regulatory subunit 1 (p35)	protein kinase (NRS/TK)
<i>CDK6</i>	1021	cyclin-dependent kinase 6	protein kinase (NRS/TK)
<i>CDK7</i>	1022	cyclin-dependent kinase 7 (MO15 homolog, <i>Xenopus laevis</i> , cdk-activating kinase)	protein kinase (NRS/TK)
<i>CDK8</i>	1024	cyclin-dependent kinase 8	protein kinase (NRS/TK)
<i>CDK9</i>	1025	cyclin-dependent kinase 9 (CDC2-related kinase)	protein kinase (NRS/TK)
<i>CDKL1</i>	8814	cyclin-dependent kinase-like 1 (CDC2-related kinase)	protein kinase (NRS/TK)
<i>CDKL2</i>	8999	cyclin-dependent kinase-like 2 (CDC2-related kinase)	protein kinase (NRS/TK)
<i>CDKL3</i>	51265	cyclin-dependent kinase-like 3	protein kinase (NRS/TK)
<i>CDKL4</i>	344387	cyclin-dependent kinase-like 4	protein kinase (NRS/TK)
<i>CDKL5</i>	6792	cyclin-dependent kinase-like 5	protein kinase (NRS/TK)
<i>CHEK1</i>	1111	CHK1 checkpoint homolog (<i>S. pombe</i>)	protein kinase (NRS/TK)
<i>CHEK2</i>	11200	CHK2 checkpoint homolog (<i>S. pombe</i>)	protein kinase (NRS/TK)
<i>CHKA</i>	1119	choline kinase alpha	kinase related
<i>CIB1</i>	10519	calcium and integrin binding 1 (calmyrin);CIB1	kinase related
<i>CIB4</i>	130106	calcium and integrin binding family member 4;CIB4	kinase related
<i>CKB</i>	1152	creatine kinase, brain	kinase related
<i>CKM</i>	1158	creatine kinase, muscle	kinase related
<i>CKMT1A</i>	548596	creatine kinase, mitochondrial 1A;CKMT1A	kinase related
<i>CKMT2</i>	1160	creatine kinase, mitochondrial 2 (sarcomeric)	kinase related
<i>CKS1B</i>	1163	CDC28 protein kinase regulatory subunit 1B	protein kinase
<i>CKS2</i>	1164	CDC28 protein kinase regulatory subunit 2	protein kinase

hGENE	GENE ID	DESCRIPTION	KINASE CLASS
<i>CLK1</i>	1195	CDC-like kinase 1	protein kinase (NRS/TK)
<i>CLK2</i>	1196	CDC-like kinase 2	protein kinase (NRS/TK)
<i>CLK3</i>	1198	CDC-like kinase 3	protein kinase (NRS/TK)
<i>COASY</i>	80347	Coenzyme A synthase;COASY	kinase related
<i>COL4A3BP</i>	10087	collagen, type IV, alpha 3 (Goodpasture antigen) binding protein;COL4A3BP	protein kinase
<i>CRKL</i>	1399	v-crkl sarcoma virus CT10 oncogene homolog (avian)-like;CRKL	kinase related
<i>CSF1R</i>	1436	colony stimulating factor 1 receptor, formerly McDonough feline sarcoma viral (v-fms) oncogene homolog	protein kinase (RTK)
<i>CSK</i>	1445	c-src tyrosine kinase	protein kinase (NRTK)
<i>CSNK1A1</i>	1452	casein kinase 1, alpha 1	protein kinase (NRS/TK)
<i>CSNK1A1L</i>	122011	casein kinase 1, alpha 1-like	protein kinase (NRS/TK)
<i>CSNK1D</i>	1453	casein kinase 1, delta	protein kinase (NRS/TK)
<i>CSNK1E</i>	1454	casein kinase 1, epsilon	protein kinase (NRS/TK)
<i>CSNK1G1</i>	53944	casein kinase 1, gamma 1	protein kinase (NRS/TK)
<i>CSNK1G2</i>	1455	casein kinase 1, gamma 2	protein kinase (NRS/TK)
<i>CSNK1G3</i>	1456	casein kinase 1, gamma 3	protein kinase (NRS/TK)
<i>CSNK2A1</i>	1457	casein kinase 2, alpha 1 polypeptide	protein kinase (NRS/TK)
<i>CSNK2B</i>	1460	casein kinase 2, beta polypeptide	protein kinase (NRS/TK)
<i>DAK</i>	26007	dihydroxyacetone kinase 2 homolog (<i>S. cerevisiae</i>);DAK	kinase related
<i>DAPK1</i>	1612	death-associated protein kinase 1	protein kinase (NRS/TK)
<i>DAPK2</i>	23604	death-associated protein kinase 2	protein kinase (NRS/TK)
<i>DAPK3</i>	1613	death-associated protein kinase 3	protein kinase (NRS/TK)
<i>DCAKD</i>	79877	dephospho-CoA kinase domain containing;DCAKD	kinase related
<i>DCAMKL2</i>	166614	hypothetical protein MGC45428	protein kinase (NRS/TK)
<i>DCK</i>	1633	deoxycytidine kinase	nucleotide kinase
<i>DDR1</i>	780	discoidin domain receptor family, member 1	protein kinase (RTK)
<i>DDR2</i>	4921	discoidin domain receptor family, member 2	protein kinase (RTK)

hGENE	GENE ID	DESCRIPTION	KINASE CLASS
<i>DGKA</i>	1606	diacylglycerol kinase, alpha 80kDa	kinase related
<i>DGKB</i>	1607	diacylglycerol kinase, beta 90kDa	kinase related
<i>DGKG</i>	1608	diacylglycerol kinase, gamma 90kDa	kinase related
<i>DGKK</i>	139189	diacylglycerol kinase, kappa;DGKK	kinase related
<i>DGKZ</i>	8525	diacylglycerol kinase, zeta 104kDa	kinase related
<i>DGUOK</i>	1716	deoxyguanosine kinase	nucleotide kinase
<i>DKFZp434B1231</i>	91156	eEF1A2 binding protein;DKFZp434B1231	protein kinase (NRS/TK)
<i>DKFZp761P0423</i>	157285	hypothetical protein DKFZp761P0423	protein kinase (RS/TK)
<i>DLG1</i>	1739	discs, large homolog 1 (Drosophila);DLG1	nucleotide kinase
<i>DLG3</i>	1741	discs, large homolog 3 (neuroendocrine-dlg, Drosophila);DLG3	nucleotide kinase
<i>DTYMK</i>	1841	deoxythymidylate kinase (thymidylate kinase)	nucleotide kinase
<i>DYRK1A</i>	1859	dual-specificity tyrosine-(Y)-phosphorylation regulated kinase 1A	protein kinase (NRS/TK)
<i>DYRK1B</i>	9149	dual-specificity tyrosine-(Y)-phosphorylation regulated kinase 1B	protein kinase (NRS/TK)
<i>DYRK2</i>	8445	dual-specificity tyrosine-(Y)-phosphorylation regulated kinase 2	protein kinase (NRS/TK)
<i>DYRK3</i>	8444	dual-specificity tyrosine-(Y)-phosphorylation regulated kinase 3	protein kinase (NRS/TK)
<i>DYRK4</i>	8798	dual-specificity tyrosine-(Y)-phosphorylation regulated kinase 4	protein kinase (NRS/TK)
<i>EEF2K</i>	29904	eukaryotic elongation factor-2 kinase	protein kinase (NRS/TK)
<i>EGFR</i>	1956	epidermal growth factor receptor (erythroblastic leukemia viral (v-erb-b) oncogene homolog, avian)	protein kinase (RTK)
<i>EIF2AK1</i>	27102	heme-regulated initiation factor 2-alpha kinase	protein kinase (NRS/TK)
<i>EIF2AK4</i>	415116	serine/threonine-protein kinase pim-3	protein kinase (RS/TK)
<i>EPHA1</i>	2041	EPH receptor A1	protein kinase (RTK)
<i>EPHA2</i>	1969	EPH receptor A2	protein kinase (RTK)
<i>EPHA3</i>	2042	EPH receptor A3	protein kinase (RTK)
<i>EPHA4</i>	2043	EPH receptor A4	protein kinase (RTK)
<i>EPHA6</i>	285220	EPH receptor A6	protein kinase (RTK)
<i>EPHB1</i>	2047	EPH receptor B1	protein kinase (RTK)
<i>EPHB4</i>	2050	EPH receptor B4	protein kinase (RTK)
<i>EPHB6</i>	2051	EPH receptor B6	protein kinase (RTK)
<i>ERBB2</i>	2064	v-erb-b2 erythroblastic leukemia viral oncogene homolog 2, neuro/glioblastoma derived oncogene homolog (avian)	protein kinase (RTK)
<i>ERBB3</i>	2065	v-erb-b2 erythroblastic leukemia viral oncogene homolog 3 (avian)	protein kinase (RTK)
<i>ERBB4</i>	2066	v-erb-a erythroblastic leukemia viral oncogene homolog 4 (avian)	protein kinase (RTK)

hGENE	GENE ID	DESCRIPTION	KINASE CLASS
<i>ERN1</i>	2081	endoplasmic reticulum to nucleus signalling 1	protein kinase
<i>ETNK1</i>	55500	ethanolamine kinase 1	kinase related
<i>ETNK2</i>	55224	ethanolamine kinase 2	kinase related
<i>EXOSC10</i>	5394	exosome component 10;EXOSC10	protein kinase (NRS/TK)
<i>FASTK</i>	10922	FAST kinase	protein kinase (NRS/TK)
<i>FASTKD1</i>	79675	FAST kinase domains 1;FASTKD1	protein kinase
<i>FASTKD2</i>	22868	FAST kinase domains 2;FASTKD2	protein kinase
<i>FASTKD3</i>	79072	FAST kinase domains 3;FASTKD3	protein kinase
<i>FASTKD5</i>	60493	FAST kinase domains 5;FASTKD5	protein kinase
<i>FER</i>	2241	fer (fps/fes related) tyrosine kinase (phosphoprotein NCP94)	protein kinase (NRTK)
<i>FES</i>	2242	feline sarcoma oncogene	protein kinase (NRTK)
<i>FGFR1</i>	2260	fibroblast growth factor receptor 1 (fms-related tyrosine kinase 2, Pfeiffer syndrome)	protein kinase (RTK)
<i>FGFR2</i>	2263	fibroblast growth factor receptor 2 (craniofacial dysostosis 1, Crouzon, Pfeiffer and Jackson-Weiss syndrome)	protein kinase (RTK)
<i>FGFR3</i>	2261	fibroblast growth factor receptor 3 (achondroplasia, thanatophoric dwarfism)	protein kinase (RTK)
<i>FGFRL1</i>	53834	fibroblast growth factor receptor-like 1;FGFRL1	protein kinase (RTK)
<i>FGR</i>	2268	Gardner-Rasheed feline sarcoma viral (v-fgr) oncogene homolog	protein kinase (NRTK)
<i>FLJ10986</i>	55277	hypothetical protein FLJ10986;FLJ10986	carbohydrate kinase
<i>FLJ23356</i>	84197	hypothetical protein FLJ23356	protein kinase
<i>FLJ25006</i>	124923	hypothetical protein FLJ25006	protein kinase (NRS/TK)
<i>FLJ40852</i>	285962	hypothetical protein FLJ40852	protein kinase
<i>FLT1</i>	2321	fms-related tyrosine kinase 1 (vascular endothelial growth factor/vascular permeability factor receptor)	protein kinase (RTK)
<i>FLT3</i>	2322	fms-related tyrosine kinase 3	protein kinase (RTK)
<i>FLT4</i>	2324	fms-related tyrosine kinase 4	protein kinase (RTK)
<i>FN3K</i>	64122	fructosamine 3 kinase	kinase related
<i>FN3KRP</i>	79672	fructosamine-3-kinase-related protein	kinase related
<i>FRK</i>	2444	fyn-related kinase	protein kinase (NRTK)
<i>FUK</i>	197258	fucokinase	kinase related
<i>FXN</i>	2395	frataxin;FXN	kinase related
<i>FYN</i>	2534	FYN oncogene related to SRC, FGR, YES	protein kinase (NRTK)
<i>GALK1</i>	2584	galactokinase 1	carbohydrate kinase
<i>GALK2</i>	2585	galactokinase 2	carbohydrate kinase
<i>GCK</i>	2645	glucokinase (hexokinase 4, maturity onset diabetes of the young 2)	carbohydrate kinase
<i>GK</i>	2710	glycerol kinase	carbohydrate kinase
<i>GK2</i>	2712	glycerol kinase 2	carbohydrate kinase
<i>GK5</i>	256356	hypothetical protein MGC40579;MGC40579	carbohydrate kinase

hGENE	GENE ID	DESCRIPTION	KINASE CLASS
<i>GLYCTK</i>	132158	glycerate kinase	carbohydrate kinase
<i>GNE</i>	10020	glucosamine (UDP-N-acetyl)-2-epimerase/N-acetylmannosamine kinase	carbohydrate kinase
<i>GRK6</i>	2870	G protein-coupled receptor kinase 6	protein kinase (NRS/TK)
<i>GRK7</i>	131890	G protein-coupled receptor kinase 7	protein kinase (NRS/TK)
<i>GSG2</i>	83903	haspin	protein kinase (RS/TK)
<i>GSK3A</i>	2931	glycogen synthase kinase 3 alpha	protein kinase (NRS/TK)
<i>GTF2H1</i>	2965	general transcription factor IIH, polypeptide 1, 62kDa;GTF2H1	protein kinase (NRS/TK)
<i>GUK1</i>	2987	guanylate kinase 1	nucleotide kinase
<i>HCK</i>	3055	hemopoietic cell kinase	protein kinase (NRTK)
<i>HIPK1</i>	204851	homeodomain interacting protein kinase 1	protein kinase (NRS/TK)
<i>HIPK2</i>	28996	homeodomain interacting protein kinase 2	protein kinase (NRS/TK)
<i>HIPK3</i>	10114	homeodomain interacting protein kinase 3	protein kinase (NRS/TK)
<i>HIPK4</i>	147746	homeodomain interacting protein kinase 4	protein kinase (NRS/TK)
<i>HK1</i>	3098	hexokinase 1	carbohydrate kinase
<i>HK2</i>	3099	hexokinase 2	carbohydrate kinase
<i>HK3</i>	3101	hexokinase 3 (white cell)	carbohydrate kinase
<i>HKDC1</i>	80201	hexokinase domain containing 1;HKDC1	carbohydrate kinase
<i>HSPB8</i>	26353	heat shock 22kDa protein 8	protein kinase (NRS/TK)
<i>IGF1R</i>	3480	insulin-like growth factor 1 receptor	protein kinase (RTK)
<i>IHPK1</i>	9807	inositol hexaphosphate kinase 1	kinase related
<i>IHPK2</i>	51447	inositol hexaphosphate kinase 2	Lipid Kinase
<i>IHPK3</i>	117283	inositol hexaphosphate kinase 3	Lipid Kinase
<i>IKBKE</i>	9641	inhibitor of kappa light polypeptide gene enhancer in B-cells, kinase epsilon	protein kinase (NRS/TK)
<i>ILK</i>	3611	integrin-linked kinase	protein kinase (NRS/TK)
<i>INSRR</i>	3645	insulin receptor-related receptor	protein kinase (RTK)
<i>IPMK</i>	253430	inositol polyphosphate multikinase	Lipid Kinase
<i>IPPK</i>	64768	inositol 1,3,4,5,6-pentakisphosphate 2-kinase;IPPK	Lipid Kinase
<i>IRAK2</i>	3656	interleukin-1 receptor-associated kinase 2	protein kinase (RS/TK)
<i>IRAK3</i>	11213	interleukin-1 receptor-associated kinase 3	protein kinase (RS/TK)
<i>IRAK4</i>	51135	interleukin-1 receptor-associated kinase 4	protein kinase (RS/TK)
<i>ITGB1BP3</i>	27231	integrin beta 1 binding protein 3;ITGB1BP3	kinase related

hGENE	GENE ID	DESCRIPTION	KINASE CLASS
<i>ITK</i>	3702	IL2-inducible T-cell kinase	protein kinase (NRTK)
<i>ITPKB</i>	3707	inositol 1,4,5-trisphosphate 3-kinase B	kinase related
<i>JAK1</i>	3716	Janus kinase 1 (a protein tyrosine kinase)	protein kinase (NRTK)
<i>JAK2</i>	3717	Janus kinase 2 (a protein tyrosine kinase)	protein kinase (NRTK)
<i>JAK3</i>	3718	Janus kinase 3 (a protein tyrosine kinase, leukocyte)	protein kinase (NRTK)
<i>KDR</i>	3791	kinase insert domain receptor (a type III receptor tyrosine kinase)	protein kinase (RTK)
<i>KHK</i>	3795	ketohehexokinase (fructokinase)	carbohydrate kinase
<i>KIAA0999</i>	23387	KIAA0999 protein	protein kinase (RS/TK)
<i>KIAA2002</i>	79834	KIAA2002 protein	protein kinase (RS/TK)
<i>KSR</i>	8844	kinase suppressor of ras	protein kinase (NRS/TK)
<i>KSR2</i>	283455	kinase suppressor of Ras-2	protein kinase (NRS/TK)
<i>LATS1</i>	9113	LATS, large tumor suppressor, homolog 1 (Drosophila)	protein kinase (NRS/TK)
<i>LATS2</i>	26524	LATS, large tumor suppressor, homolog 2 (Drosophila)	protein kinase (NRS/TK)
<i>LCK</i>	3932	lymphocyte-specific protein tyrosine kinase	protein kinase (NRTK)
<i>LIMK1</i>	3984	LIM domain kinase 1	protein kinase (NRS/TK)
<i>LIMK2</i>	3985	LIM domain kinase 2	protein kinase (NRS/TK)
<i>LMTK2</i>	22853	lemur tyrosine kinase 2	protein kinase (RTK)
<i>LOC220686</i>	220686	hypothetical protein LOC220686;LOC220686	kinase related
<i>LOC340156</i>	340156	hypothetical protein LOC340156	protein kinase (NRS/TK)
<i>LOC340371</i>	340371	hypothetical protein LOC340371	protein kinase (NRS/TK)
<i>LOC375133</i>	375133	similar to phosphatidylinositol 4-kinase alpha	Lipid Kinase
<i>LOC388957</i>	388957	similar to BMP2 inducible kinase	protein kinase (NRS/TK)
<i>LOC389599</i>	389599	similar to amyotrophic lateral sclerosis 2 (juvenile) chr. region, candidate 2; ILP-interacting protein ILPIPA	protein kinase
<i>LOC390877</i>	390877	similar to adenylate kinase (EC 2.7.4.3), cytosolic - common carp	nucleotide kinase
<i>LOC442075</i>	442075	similar to serine/threonine kinase, establishes embryonic polarity	protein kinase (NRS/TK)
<i>LOC54103</i>	54103	hypothetical protein LOC54103;LOC54103	protein kinase (RTK)
<i>LOC646505</i>	646505	similar to Dual specificity protein kinase CLK3 (CDC-like kinase 3);unassigned	protein kinase (NRS/TK)

hGENE	GENE ID	DESCRIPTION	KINASE CLASS
LOC647279	647279	similar to MAP/microtubule affinity-regulating kinase 3;unassigned	protein kinase (NRS/TK)
LOC648152	648152	similar to ataxia telangiectasia and Rad3 related protein;unassigned	protein kinase (NRS/TK)
LOC649288	649288	similar to Adenylate kinase isoenzyme 4, mitochondrial (Adenylate kinase 3-like 1)	nucleotide kinase
LOC650122	650122	similar to choline kinase alpha isoform a;unassigned	kinase related
LOC652722	652722	similar to PTK2 protein tyrosine kinase 2 isoform a;unassigned	protein kinase (NRTK)
LOC652799	652799	similar to Mast/stem cell growth factor receptor precursor (SCFR) (c-kit) (CD117 antigen);unassigned	protein kinase (RTK)
LOC653052	653052	similar to Homeodomain-interacting protein kinase 2 (hHIPk2);unassigned	protein kinase (NRS/TK)
LOC653155	653155	similar to PRP4 pre-mRNA processing factor 4 homolog B;unassigned	protein kinase (NRS/TK)
LOC727761	727761	similar to deoxythymidylate kinase (thymidylate kinase);unassigned	nucleotide kinase
LOC730000	730000	similar to testis-specific serine kinase 6;unassigned	protein kinase (NRS/TK)
LOC732306	732306	similar to vaccinia related kinase 2;unassigned	protein kinase (NRS/TK)
LOC91461	91461	hypothetical protein BC007901	protein kinase (NRTK)
LOC91807	91807	myosin light chain kinase (MLCK)	protein kinase (NRS/TK)
LRGUK	136332	leucine-rich repeats and guanylate kinase domain containing;LRGUK	nucleotide kinase
LRPPRC	10128	leucine-rich PPR-motif containing;LRPPRC	protein kinase (RS/TK)
LRRK2	120892	leucine-rich repeat kinase 2	protein kinase (NRS/TK)
LYK5	92335	protein kinase LYK5	protein kinase
LYN	4067	v-yes-1 Yamaguchi sarcoma viral related oncogene homolog	protein kinase (NRTK)
MAGI1	9223	membrane associated guanylate kinase, WW and PDZ domain containing 1;MAGI1	nucleotide kinase
MAK	4117	male germ cell-associated kinase	protein kinase (NRS/TK)
MAP2K1	5604	mitogen-activated protein kinase kinase 1	protein kinase
MAP2K1IP1	8649	mitogen-activated protein kinase kinase 1 interacting protein 1	protein kinase
MAP2K2	5605	mitogen-activated protein kinase kinase 2	protein kinase
MAP2K5	5607	mitogen-activated protein kinase kinase 5	protein kinase
MAP2K6	5608	mitogen-activated protein kinase kinase 6	protein kinase
MAP2K7	5609	mitogen-activated protein kinase kinase 7	protein kinase

hGENE	GENE ID	DESCRIPTION	KINASE CLASS
<i>MAP3K11</i>	4296	mitogen-activated protein kinase kinase kinase 11	protein kinase (NRS/TK)
<i>MAP3K12</i>	7786	mitogen-activated protein kinase kinase kinase 12	protein kinase (NRS/TK)
<i>MAP3K14</i>	9020	mitogen-activated protein kinase kinase kinase 14	protein kinase
<i>MAP3K15</i>	389840	FLJ16518 protein	protein kinase
<i>MAP3K2</i>	10746	mitogen-activated protein kinase kinase kinase 2	protein kinase
<i>MAP3K5</i>	4217	mitogen-activated protein kinase kinase kinase 5	protein kinase
<i>MAP3K6</i>	9064	mitogen-activated protein kinase kinase kinase 6	protein kinase
<i>MAP3K7</i>	6885	mitogen-activated protein kinase kinase kinase 7	protein kinase (NRS/TK)
<i>MAP3K8</i>	1326	mitogen-activated protein kinase kinase kinase 8	protein kinase
<i>MAP4K1</i>	11184	mitogen-activated protein kinase kinase kinase kinase 1	protein kinase
<i>MAP4K2</i>	5871	mitogen-activated protein kinase kinase kinase kinase 2	protein kinase
<i>MAP4K3</i>	8491	mitogen-activated protein kinase kinase kinase kinase 3	protein kinase
<i>MAP4K4</i>	9448	mitogen-activated protein kinase kinase kinase kinase 4	protein kinase
<i>MAP4K5</i>	11183	mitogen-activated protein kinase kinase kinase kinase 5	protein kinase
<i>MAPK1</i>	5594	mitogen-activated protein kinase 1	protein kinase (NRS/TK)
<i>MAPK10</i>	5602	mitogen-activated protein kinase 10	protein kinase (NRS/TK)
<i>MAPK12</i>	6300	mitogen-activated protein kinase 12	protein kinase (NRS/TK)
<i>MAPK13</i>	5603	mitogen-activated protein kinase 13	protein kinase (NRS/TK)
<i>MAPK14</i>	1432	mitogen-activated protein kinase 14	protein kinase (NRS/TK)
<i>MAPK15</i>	225689	extracellular signal-regulated kinase 8	protein kinase (NRS/TK)
<i>MAPK3</i>	5595	mitogen-activated protein kinase 3	protein kinase (NRS/TK)
<i>MAPK4</i>	5596	mitogen-activated protein kinase 4	protein kinase (NRS/TK)
<i>MAPK6</i>	5597	mitogen-activated protein kinase 6	protein kinase (NRS/TK)
<i>MAPK8</i>	5599	mitogen-activated protein kinase 8	protein kinase (NRS/TK)
<i>MAPK9</i>	5601	mitogen-activated protein kinase 9	protein kinase (NRS/TK)
<i>MAPKAP K2</i>	9261	mitogen-activated protein kinase-activated protein kinase 2	protein kinase (NRS/TK)
<i>MAPKAP K3</i>	7867	mitogen-activated protein kinase-activated protein kinase 3	protein kinase (NRS/TK)
<i>MAPKAP</i>	8550	mitogen-activated protein kinase-activated protein kinase 5	protein kinase

hGENE	GENE ID	DESCRIPTION	KINASE CLASS
<i>K5</i>			(NRS/TK)
<i>MARK2</i>	2011	MAP/microtubule affinity-regulating kinase 2	protein kinase (NRS/TK)
<i>MARK3</i>	4140	MAP/microtubule affinity-regulating kinase 3	protein kinase (NRS/TK)
<i>MAST1</i>	22983	microtubule associated serine/threonine kinase 1	protein kinase (NRS/TK)
<i>MAST2</i>	23139	microtubule associated serine/threonine kinase 2	protein kinase (NRS/TK)
<i>MASTL</i>	84930	microtubule associated serine/threonine kinase-like	protein kinase (NRS/TK)
<i>MATK</i>	4145	megakaryocyte-associated tyrosine kinase	protein kinase (NRTK)
<i>MERTK</i>	10461	c-mer proto-oncogene tyrosine kinase	protein kinase (RTK)
<i>MET</i>	4233	met proto-oncogene (hepatocyte growth factor receptor)	protein kinase (RTK)
<i>MGC16169</i>	93627	hypothetical protein MGC16169	protein kinase
<i>MGC42105</i>	167359	hypothetical protein MGC42105	protein kinase (NRS/TK)
<i>MINK1</i>	50488	misshapen/NIK-related kinase	protein kinase
<i>MKNK1</i>	8569	MAP kinase interacting serine/threonine kinase 1	protein kinase (NRS/TK)
<i>MKNK2</i>	2872	MAP kinase interacting serine/threonine kinase 2	protein kinase (NRS/TK)
<i>MORN2</i>	378464	MORN repeat containing 2;MORN2	kinase related
<i>MOS</i>	4342	v-mos Moloney murine sarcoma viral oncogene homolog	protein kinase (NRS/TK)
<i>MPP1</i>	4354	membrane protein, palmitoylated 1, 55kDa;MPP1	protein kinase
<i>MPP2</i>	4355	membrane protein, palmitoylated 2 (MAGUK p55 subfamily member 2);MPP2	nucleotide kinase
<i>MPP3</i>	4356	membrane protein, palmitoylated 3 (MAGUK p55 subfamily member 3);MPP3	nucleotide kinase
<i>MPP4</i>	58538	membrane protein, palmitoylated 4 (MAGUK p55 subfamily member 4);MPP4	nucleotide kinase
<i>MPP5</i>	64398	membrane protein, palmitoylated 5 (MAGUK p55 subfamily member 5);MPP5	nucleotide kinase
<i>MPP6</i>	51678	membrane protein, palmitoylated 6 (MAGUK p55 subfamily member 6);MPP6	nucleotide kinase
<i>MPP7</i>	143098	membrane protein, palmitoylated 7 (MAGUK p55 subfamily member 7);MPP7	nucleotide kinase
<i>MST1R</i>	4486	macrophage stimulating 1 receptor (c-met-related tyrosine kinase)	protein kinase (RTK)
<i>MUSK</i>	4593	muscle, skeletal, receptor tyrosine kinase	protein kinase (RTK)
<i>MVK</i>	4598	mevalonate kinase (mevalonic aciduria)	kinase related
<i>MYLK2</i>	85366	myosin light chain kinase 2, skeletal muscle	protein kinase

hGENE	GENE ID	DESCRIPTION	KINASE CLASS
			(NRS/TK)
<i>MYO3B</i>	140469	myosin IIIB	protein kinase (NRS/TK)
<i>NADK</i>	65220	NAD kinase	kinase related
<i>NAGK</i>	55577	N-acetylglucosamine kinase	kinase related
<i>NEK10</i>	152110	NIMA (never in mitosis gene a)- related kinase 10	protein kinase
<i>NEK11</i>	79858	NIMA (never in mitosis gene a)- related kinase 11	protein kinase
<i>NEK2</i>	4751	NIMA (never in mitosis gene a)-related kinase 2	protein kinase
<i>NEK3</i>	4752	NIMA (never in mitosis gene a)-related kinase 3	protein kinase
<i>NEK4</i>	6787	NIMA (never in mitosis gene a)-related kinase 4	protein kinase
<i>NEK5</i>	341676	NIMA (never in mitosis gene a)-related kinase 5	protein kinase
<i>NEK6</i>	10783	NIMA (never in mitosis gene a)-related kinase 6	protein kinase
<i>NEK7</i>	140609	NIMA (never in mitosis gene a)-related kinase 7	protein kinase
<i>NEK8</i>	284086	NIMA (never in mitosis gene a)- related kinase 8	protein kinase
<i>NEK9</i>	91754	NIMA (never in mitosis gene a)- related kinase 9	protein kinase
<i>NJMU-R1</i>	64149	protein kinase Njmu-R1	protein kinase
<i>NLK</i>	51701	nemo like kinase	protein kinase (NRS/TK)
<i>NME1</i>	4830	nucleoside-diphosphate kinase 1	nucleotide kinase
<i>NME1-NME2</i>	654364	NME1-NME2 protein;NME1-NME2	nucleotide kinase
<i>NME2</i>	4831	nucleoside-diphosphate kinase 2	nucleotide kinase
<i>NME3</i>	4832	nucleoside-diphosphate kinase 3	nucleotide kinase
<i>NME4</i>	4833	nucleoside-diphosphate kinase 4	nucleotide kinase
<i>NME5</i>	8382	non-metastatic cells 5, protein expressed in (nucleoside-diphosphate kinase)	nucleotide kinase
<i>NME6</i>	10201	non-metastatic cells 6, protein expressed in (nucleoside-diphosphate kinase)	nucleotide kinase
<i>NME7</i>	29922	non-metastatic cells 7, protein expressed in (nucleoside-diphosphate kinase)	nucleotide kinase
<i>NPR2</i>	4882	natriuretic peptide receptor B/guanylate cyclase B (atrionatriuretic peptide receptor B)	protein kinase
<i>NRBP</i>	29959	nuclear receptor binding protein	protein kinase (NRS/TK)
<i>NTRK1</i>	4914	neurotrophic tyrosine kinase, receptor, type 1	protein kinase (RTK)
<i>NTRK2</i>	4915	neurotrophic tyrosine kinase, receptor, type 2	protein kinase (RTK)
<i>NTRK3</i>	4916	neurotrophic tyrosine kinase, receptor, type 3	protein kinase (RTK)
<i>NUAK2</i>	81788	likely ortholog of rat SNF1/AMP-activated protein kinase	protein kinase (NRS/TK)
<i>NUP62</i>	23636	nucleoporin 62kDa;NUP62	protein kinase (NRS/TK)
<i>NYD-SP25</i>	89882	protein kinase NYD-SP25	protein kinase

hGENE	GENE ID	DESCRIPTION	KINASE CLASS
<i>OXSR1</i>	9943	oxidative-stress responsive 1	protein kinase
<i>PAK1</i>	5058	p21/Cdc42/Rac1-activated kinase 1 (STE20 homolog, yeast)	protein kinase
<i>PAK2</i>	5062	p21 (CDKN1A)-activated kinase 2	protein kinase
<i>PAK3</i>	5063	p21 (CDKN1A)-activated kinase 3	protein kinase
<i>PAK4</i>	10298	p21(CDKN1A)-activated kinase 4	protein kinase
<i>PAK6</i>	56924	p21(CDKN1A)-activated kinase 6	protein kinase
<i>PAK7</i>	57144	p21(CDKN1A)-activated kinase 7	protein kinase
<i>PANK2</i>	80025	pantothenate kinase 2 (Hallervorden-Spatz syndrome)	kinase related
<i>PANK3</i>	79646	pantothenate kinase 3	kinase related
<i>PANK4</i>	55229	pantothenate kinase 4	kinase related
<i>PAPSS1</i>	9061	3'-phosphoadenosine 5'-phosphosulfate synthase 1;PAPSS1	kinase related
<i>PAPSS2</i>	9060	3'-phosphoadenosine 5'-phosphosulfate synthase 2;PAPSS2	kinase related
<i>PBK</i>	55872	T-LAK cell-originated protein kinase	protein kinase
<i>PCK2</i>	5106	phosphoenolpyruvate carboxykinase 2 (mitochondrial)	kinase related
<i>PCTK1</i>	5127	PCTAIRE protein kinase 1	protein kinase (NRS/TK)
<i>PCTK2</i>	5128	PCTAIRE protein kinase 2	protein kinase (NRS/TK)
<i>PCTK3</i>	5129	PCTAIRE protein kinase 3	protein kinase (NRS/TK)
<i>PDGFRA</i>	5156	platelet-derived growth factor receptor, alpha polypeptide	protein kinase (RTK)
<i>PDGFRB</i>	5159	platelet-derived growth factor receptor, beta polypeptide	protein kinase (RTK)
<i>PDGFRL</i>	5157	platelet-derived growth factor receptor-like;PDGFRL	protein kinase (RTK)
<i>PDIK1L</i>	149420	PDLIM1 interacting kinase 1 like	protein kinase (NRS/TK)
<i>PDK1</i>	5163	pyruvate dehydrogenase kinase, isoenzyme 1	protein kinase
<i>PDK2</i>	5164	pyruvate dehydrogenase kinase, isoenzyme 2	protein kinase
<i>PDK3</i>	5165	pyruvate dehydrogenase kinase, isoenzyme 3	protein kinase
<i>PDK4</i>	5166	pyruvate dehydrogenase kinase, isoenzyme 4	protein kinase
<i>PDPK1</i>	5170	3-phosphoinositide dependent protein kinase-1	protein kinase
<i>PDXK</i>	8566	pyridoxal (pyridoxine, vitamin B6) kinase	kinase related
<i>PFKFB1</i>	5207	6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 1	carbohydrate kinase
<i>PFKFB2</i>	5208	6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 2	carbohydrate kinase
<i>PFKFB3</i>	5209	6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 3	carbohydrate kinase
<i>PFKL</i>	5211	phosphofructokinase, liver	carbohydrate kinase
<i>PFKM</i>	5213	phosphofructokinase, muscle	carbohydrate kinase
<i>PFKP</i>	5214	phosphofructokinase, platelet	carbohydrate kinase
<i>PFTK1</i>	5218	PFTAIRE protein kinase 1	protein kinase (NRS/TK)
<i>PGK1</i>	5230	phosphoglycerate kinase 1	carbohydrate kinase
<i>PGK2</i>	5232	phosphoglycerate kinase 2	carbohydrate kinase
<i>PHKA1</i>	5255	phosphorylase kinase, alpha 1 (muscle)	protein kinase (NRS/TK)

hGENE	GENE ID	DESCRIPTION	KINASE CLASS
<i>PHKA2</i>	5256	phosphorylase kinase, alpha 2 (liver)	protein kinase (NRS/TK)
<i>PHKB</i>	5257	phosphorylase kinase, beta	protein kinase (NRS/TK)
<i>PHKG1</i>	5260	phosphorylase kinase, gamma 1 (muscle)	protein kinase (NRS/TK)
<i>PHKG2</i>	5261	phosphorylase kinase, gamma 2 (testis)	protein kinase (NRS/TK)
<i>PI4K2B</i>	55300	phosphatidylinositol 4-kinase type-II beta	Lipid Kinase
<i>PI4KII</i>	55361	phosphatidylinositol 4-kinase type II	Lipid Kinase
<i>PIK3C2G</i>	5288	phosphoinositide-3-kinase, class 2, gamma polypeptide	Lipid Kinase
<i>PIK3C3</i>	5289	phosphoinositide-3-kinase, class 3	Lipid Kinase
<i>PIK3CA</i>	5290	phosphoinositide-3-kinase, catalytic, alpha polypeptide	Lipid Kinase
<i>PIK3CBP</i>	5291	phosphoinositide-3-kinase, catalytic, beta polypeptide	Lipid Kinase
<i>PIK3CG</i>	5294	phosphoinositide-3-kinase, catalytic, gamma polypeptide	Lipid Kinase
<i>PIK3R1</i>	5295	phosphoinositide-3-kinase, regulatory subunit 1 (p85 alpha)	Lipid Kinase
<i>PIK3R3</i>	8503	phosphoinositide-3-kinase, regulatory subunit 3 (p55, gamma)	Lipid Kinase
<i>PIK3R4</i>	30849	phosphoinositide-3-kinase, regulatory subunit 4, p150	Lipid Kinase
<i>PIK3R5</i>	23533	phosphoinositide-3-kinase, regulatory subunit 5, p101	Lipid Kinase
<i>PIK4CA</i>	5297	phosphatidylinositol 4-kinase, catalytic, alpha polypeptide	Lipid Kinase
<i>PIK4CB</i>	5298	phosphatidylinositol 4-kinase, catalytic, beta polypeptide	Lipid Kinase
<i>PIM1</i>	5292	pim-1 oncogene	protein kinase (RS/TK)
<i>PIM2</i>	11040	pim-2 oncogene	protein kinase (RS/TK)
<i>PINK1</i>	65018	PTEN induced putative kinase 1	protein kinase (RS/TK)
<i>PIP5K1A</i>	8394	phosphatidylinositol-4-phosphate 5-kinase, type I, alpha	Lipid Kinase
<i>PIP5K1B</i>	8395	phosphatidylinositol-4-phosphate 5-kinase, type I, beta	Lipid Kinase
<i>PIP5K2A</i>	5305	phosphatidylinositol-4-phosphate 5-kinase, type II, alpha	Lipid Kinase
<i>PIP5K2C</i>	79837	phosphatidylinositol-4-phosphate 5-kinase, type II, gamma	kinase related
<i>PIP5K3</i>	200576	phosphatidylinositol-3-phosphate/phosphatidylinositol 5-kinase, type III	Lipid Kinase
<i>PIP5KL1</i>	138429	phosphatidylinositol-4-phosphate 5-kinase-like 1	Lipid Kinase
<i>PKLR</i>	5313	pyruvate kinase, liver and RBC	carbohydrate kinase
<i>PKM2</i>	5315	pyruvate kinase, muscle	carbohydrate kinase
<i>PKMYT1</i>	9088	membrane-associated tyrosine- and threonine-specific cdc2-inhibitory kinase	protein kinase (NRS/TK)
<i>PLAU</i>	5328	plasminogen activator, urokinase	kinase related
<i>PLK1</i>	5347	polo-like kinase 1 (Drosophila)	protein kinase (NRS/TK)
<i>PLK2</i>	10769	polo-like kinase 2 (Drosophila)	protein kinase (NRS/TK)
<i>PLK4</i>	10733	polo-like kinase 4 (Drosophila)	protein kinase (NRS/TK)
<i>PLXNA3</i>	55558	plexin A3;PLXNA3	protein kinase (RTK)

hGENE	GENE ID	DESCRIPTION	KINASE CLASS
<i>PLXNA4B</i>	91584	plexin A4, B;PLXNA4B	protein kinase (RTK)
<i>PLXNB2</i>	23654	plexin B2;PLXNB2	protein kinase (RTK)
<i>PMVK</i>	10654	phosphomevalonate kinase	kinase related
<i>PNCK</i>	139728	pregnancy upregulated non-ubiquitously expressed CaM kinase	protein kinase (NRS/TK)
<i>PNKP</i>	11284	polynucleotide kinase 3'-phosphatase	nucleotide kinase
<i>PRKAA1</i>	5562	protein kinase, AMP-activated, alpha 1 catalytic subunit	protein kinase (NRS/TK)
<i>PRKAA2</i>	5563	protein kinase, AMP-activated, alpha 2 catalytic subunit	protein kinase (NRS/TK)
<i>PRKAB1</i>	5564	protein kinase, AMP-activated, beta 1 non-catalytic subunit	protein kinase (NRS/TK)
<i>PRKAB2</i>	5565	protein kinase, AMP-activated, beta 2 non-catalytic subunit	protein kinase (NRS/TK)
<i>PRKACA</i>	5566	protein kinase, cAMP-dependent, catalytic, alpha	protein kinase (NRS/TK)
<i>PRKACB</i>	5567	protein kinase, cAMP-dependent, catalytic, beta	protein kinase (NRS/TK)
<i>PRKACG</i>	5568	protein kinase, cAMP-dependent, catalytic, gamma	protein kinase (NRS/TK)
<i>PRKAG1</i>	5571	protein kinase, AMP-activated, gamma 1 non-catalytic subunit	protein kinase (NRS/TK)
<i>PRKAG2</i>	51422	protein kinase, AMP-activated, gamma 2 non-catalytic subunit	protein kinase (NRS/TK)
<i>PRKAG3</i>	53632	protein kinase, AMP-activated, gamma 3 non-catalytic subunit	protein kinase (NRS/TK)
<i>PRKAR1A</i>	5573	protein kinase, cAMP-dependent, regulatory, type I, alpha (tissue specific extinguisher 1)	protein kinase (NRS/TK)
<i>PRKAR1B</i>	5575	protein kinase, cAMP-dependent, regulatory, type I, beta	protein kinase (NRS/TK)
<i>PRKAR2A</i>	5576	protein kinase, cAMP-dependent, regulatory, type II, alpha	protein kinase (NRS/TK)
<i>PRKAR2B</i>	5577	protein kinase, cAMP-dependent, regulatory, type II, beta	protein kinase (NRS/TK)
<i>PRKCA</i>	5578	protein kinase C, alpha	protein kinase (NRS/TK)
<i>PRKCB1</i>	5579	protein kinase C, beta 1	protein kinase (NRS/TK)
<i>PRKCE</i>	5581	protein kinase C, epsilon	protein kinase (NRS/TK)
<i>PRKCG</i>	5582	protein kinase C, gamma	protein kinase (NRS/TK)
<i>PRKCH</i>	5583	protein kinase C, eta	protein kinase (NRS/TK)

hGENE	GENE ID	DESCRIPTION	KINASE CLASS
<i>PRKCI</i>	5584	protein kinase C, iota	protein kinase (NRS/TK)
<i>PRKCQ</i>	5588	protein kinase C, theta	protein kinase (NRS/TK)
<i>PRKCZ</i>	5590	protein kinase C, zeta	protein kinase (NRS/TK)
<i>PRKD1</i>	5587	protein kinase D1	protein kinase (NRS/TK)
<i>PRKD2</i>	25865	protein kinase D2	protein kinase (NRS/TK)
<i>PRKD3</i>	23683	protein kinase D3	protein kinase (NRS/TK)
<i>PRKG1</i>	5592	protein kinase, cGMP-dependent, type I	protein kinase (NRS/TK)
<i>PRKG2</i>	5593	protein kinase, cGMP-dependent, type II	protein kinase (NRS/TK)
<i>PRKR</i>	5610	protein kinase, interferon-inducible double stranded RNA dependent	protein kinase (NRS/TK)
<i>PRKX</i>	5613	protein kinase, X-linked	protein kinase (NRS/TK)
<i>PRKY</i>	5616	protein kinase, Y-linked	protein kinase (NRS/TK)
<i>PRPF4B</i>	8899	PRP4 pre-mRNA processing factor 4 homolog B (yeast)	protein kinase (NRS/TK)
<i>PRPS1</i>	5631	phosphoribosyl pyrophosphate synthetase 1;PRPS1	kinase related
<i>PRPS1L1</i>	221823	phosphoribosyl pyrophosphate synthetase 1-like 1;PRPS1L1	kinase related
<i>PRPS2</i>	5634	phosphoribosyl pyrophosphate synthetase 2;PRPS2	kinase related
<i>PSKH1</i>	5681	protein serine kinase H1	protein kinase (NRS/TK)
<i>PTK2</i>	5747	PTK2 protein tyrosine kinase 2	protein kinase (NRTK)
<i>PTK2B</i>	2185	PTK2B protein tyrosine kinase 2 beta	protein kinase (NRTK)
<i>PTK6</i>	5753	PTK6 protein tyrosine kinase 6	protein kinase (NRTK)
<i>PTK7</i>	5754	PTK7 protein tyrosine kinase 7	protein kinase (RTK)
<i>PTK9</i>	5756	PTK9 protein tyrosine kinase 9	protein kinase (NRTK)
<i>PXK</i>	54899	PX domain containing serine/threonine kinase	kinase related
<i>RAF1</i>	5894	v-raf-1 murine leukemia viral oncogene homolog 1	protein kinase (NRS/TK)
<i>RAGE</i>	5891	renal tumor antigen	protein kinase (NRS/TK)
<i>RBKS</i>	64080	ribokinase	nucleotide kinase
<i>RET</i>	5979	ret proto-oncogene (multiple endocrine neoplasia and medullary thyroid carcinoma 1, Hirschsprung disease)	protein kinase (RTK)
<i>RFK</i>	55312	riboflavin kinase	kinase related
<i>RIOK1</i>	83732	RIO kinase 1 (yeast)	protein kinase

hGENE	GENE ID	DESCRIPTION	KINASE CLASS
			(NRS/TK)
<i>RIOK2</i>	55781	RIO kinase 2 (yeast)	protein kinase (NRS/TK)
<i>RIOK3</i>	8780	RIO kinase 3 (yeast)	protein kinase (NRS/TK)
<i>RIPK1</i>	8737	receptor (TNFRSF)-interacting serine-threonine kinase 1	protein kinase (NRS/TK)
<i>RIPK2</i>	8767	receptor-interacting serine-threonine kinase 2	protein kinase (NRS/TK)
<i>RIPK5</i>	25778	receptor interacting protein kinase 5	protein kinase (NRS/TK)
<i>RKHD3</i>	84206	ring finger and KH domain containing 3;RKHD3	protein kinase
<i>ROR2</i>	4920	receptor tyrosine kinase-like orphan receptor 2	protein kinase (RTK)
<i>RP2</i>	6102	retinitis pigmentosa 2 (X-linked recessive);RP2	nucleotide kinase
<i>RP6-213H19.1</i>	51765	Mst3 and SOK1-related kinase	protein kinase
<i>RPS6KA1</i>	6195	ribosomal protein S6 kinase, 90kDa, polypeptide 1	protein kinase (NRS/TK)
<i>RPS6KA2</i>	6196	ribosomal protein S6 kinase, 90kDa, polypeptide 2	protein kinase (NRS/TK)
<i>RPS6KA3</i>	6197	ribosomal protein S6 kinase, 90kDa, polypeptide 3	protein kinase (NRS/TK)
<i>RPS6KA4</i>	8986	ribosomal protein S6 kinase, 90kDa, polypeptide 4	protein kinase (NRS/TK)
<i>RPS6KA5</i>	9252	ribosomal protein S6 kinase, 90kDa, polypeptide 5	protein kinase (NRS/TK)
<i>RPS6KA6</i>	27330	ribosomal protein S6 kinase, 90kDa, polypeptide 6	protein kinase (NRS/TK)
<i>RPS6KB1</i>	6198	ribosomal protein S6 kinase, 70kDa, polypeptide 1	protein kinase (NRS/TK)
<i>RPS6KB2</i>	6199	ribosomal protein S6 kinase, 70kDa, polypeptide 2	protein kinase (NRS/TK)
<i>RPS6KC1</i>	26750	ribosomal protein S6 kinase, 52kDa, polypeptide 1	protein kinase (NRS/TK)
<i>RPS6KL1</i>	83694	ribosomal protein S6 kinase-like 1	protein kinase (NRS/TK)
<i>SCYL1</i>	57410	SCY1-like 1 (<i>S. cerevisiae</i>)	protein kinase (NRTK)
<i>SCYL2</i>	55681	hypothetical protein FLJ10074	protein kinase (NRTK)
<i>SCYL3</i>	57147	ezrin-binding partner PACE-1	protein kinase (NRTK)
<i>SEPHS2</i>	22928	selenophosphate synthetase 2;SEPHS2	kinase related
<i>SGK</i>	6446	serum/glucocorticoid regulated kinase	protein kinase (NRS/TK)
<i>SGK2</i>	10110	serum/glucocorticoid regulated kinase 2	protein kinase (NRS/TK)

hGENE	GENE ID	DESCRIPTION	KINASE CLASS
<i>SGK3</i>	23678	serum/glucocorticoid regulated kinase-like	protein kinase (NRS/TK)
<i>SH3BP4</i>	23677	SH3-domain binding protein 4;SH3BP4	protein kinase (NRTK)
<i>SH3BP5</i>	9467	SH3-domain binding protein 5 (BTK-associated);SH3BP5	kinase related
<i>SH3BP5L</i>	80851	SH3-binding domain protein 5-like;SH3BP5L	kinase related
<i>SLAMF6</i>	114836	SLAM family member 6;SLAMF6	protein kinase (RTK)
<i>SNF1LK</i>	150094	SNF1-like kinase	protein kinase (NRS/TK)
<i>SNRK</i>	54861	SNF-1 related kinase	protein kinase (NRS/TK)
<i>SNX16</i>	64089	sorting nexin 16;SNX16	kinase related
<i>SPHK1</i>	8877	sphingosine kinase 1	carbohydrate kinase
<i>SPHK2</i>	56848	sphingosine kinase 2	carbohydrate kinase
<i>SRC</i>	6714	v-src sarcoma (Schmidt-Ruppin A-2) viral oncogene homolog (avian)	protein kinase (NRTK)
<i>SRPK1</i>	6732	SFRS protein kinase 1	protein kinase (NRS/TK)
<i>SRPK2</i>	6733	SFRS protein kinase 2	protein kinase (NRS/TK)
<i>SRPK3</i>	26576	serine/threonine kinase 23	protein kinase (NRS/TK)
<i>STK11</i>	6794	serine/threonine kinase 11 (Peutz-Jeghers syndrome)	protein kinase (NRS/TK)
<i>STK16</i>	8576	serine/threonine kinase 16	protein kinase (NRS/TK)
<i>STK17B</i>	9262	serine/threonine kinase 17b (apoptosis-inducing)	protein kinase (NRS/TK)
<i>STK19</i>	8859	serine/threonine kinase 19	protein kinase (NRS/TK)
<i>STK24</i>	8428	serine/threonine kinase 24 (STE20 homolog, yeast)	protein kinase
<i>STK25</i>	10494	serine/threonine kinase 25 (STE20 homolog, yeast)	protein kinase
<i>STK3</i>	6788	serine/threonine kinase 3 (STE20 homolog, yeast)	protein kinase
<i>STK32A</i>	202374	serine/threonine kinase 32A	protein kinase (NRS/TK)
<i>STK32B</i>	55351	serine/threonine kinase 32B	protein kinase (NRS/TK)
<i>STK32C</i>	282974	serine/threonine kinase 32C	protein kinase (NRS/TK)
<i>STK33</i>	65975	serine/threonine kinase 33	protein kinase (NRS/TK)
<i>STK36</i>	27148	serine/threonine kinase 36 (fused homolog, Drosophila)	protein kinase (RS/TK)
<i>STK38</i>	11329	serine/threonine kinase 38	protein kinase (NRS/TK)

hGENE	GENE ID	DESCRIPTION	KINASE CLASS
<i>STK38L</i>	23012	serine/threonine kinase 38 like	protein kinase (NRS/TK)
<i>STK40</i>	83931	Ser/Thr-like kinase	protein kinase
<i>STYK1</i>	55359	protein kinase STYK1	protein kinase (RTK)
<i>SYK</i>	6850	spleen tyrosine kinase	protein kinase (NRTK)
<i>TAOK3</i>	51347	TAO kinase 3	protein kinase
<i>TBK1</i>	29110	TANK-binding kinase 1	protein kinase (NRS/TK)
<i>TEC</i>	7006	tec protein tyrosine kinase	protein kinase (NRTK)
<i>TESK1</i>	7016	testis-specific kinase 1	protein kinase (NRS/TK)
<i>TESK2</i>	10420	testis-specific kinase 2	protein kinase (NRS/TK)
<i>TGFBR1</i>	7046	transforming growth factor, beta receptor I (activin A receptor type II-like kinase, 53kDa)	protein kinase (RS/TK)
<i>TGFBR2</i>	7048	transforming growth factor, beta receptor II (70/80kDa)	protein kinase (RS/TK)
<i>TGFBR3</i>	7049	transforming growth factor, beta receptor III (betaglycan, 300kDa);TGFBR3	protein kinase (RS/TK)
<i>TIE1</i>	7075	tyrosine kinase with immunoglobulin-like and EGF-like domains 1	protein kinase (RTK)
<i>TK1</i>	7083	thymidine kinase 1, soluble	nucleotide kinase
<i>TLK1</i>	9874	tousled-like kinase 1	protein kinase (NRS/TK)
<i>TLK2</i>	11011	tousled-like kinase 2	protein kinase (NRS/TK)
<i>TNK1</i>	8711	tyrosine kinase, non-receptor, 1	protein kinase (NRTK)
<i>TNNI3K</i>	51086	TNNI3 interacting kinase	protein kinase
<i>TP53RK</i>	112858	TP53 regulating kinase	protein kinase (NRS/TK)
<i>TPK1</i>	27010	thiamin pyrophosphokinase 1	kinase related
<i>TPR</i>	7175	translocated promoter region (to activated MET oncogene);TPR	protein kinase (RTK)
<i>TRIB1</i>	10221	tribbles homolog 1 (Drosophila)	protein kinase
<i>TRIB2</i>	28951	tribbles homolog 2 (Drosophila)	protein kinase
<i>TRIB3</i>	57761	tribbles homolog 3 (Drosophila)	protein kinase
<i>TRIM27</i>	5987	tripartite motif-containing 27;TRIM27	protein kinase (RTK)
<i>TRPM7</i>	54822	transient receptor potential cation channel, subfamily M, member 7	protein kinase (NRS/TK)
<i>TSSK1</i>	83942	serine/threonine kinase 22D (spermiogenesis associated)	protein kinase (NRS/TK)
<i>TSSK2</i>	23617	serine/threonine kinase 22B (spermiogenesis associated)	protein kinase (NRS/TK)
<i>TSSK3</i>	81629	serine/threonine kinase 22C (spermiogenesis associated)	protein kinase (NRS/TK)
<i>TSSK6</i>	83983	serine/threonine protein kinase SSTK	protein kinase

hGENE	GENE ID	DESCRIPTION	KINASE CLASS
			(NRS/TK)
<i>TTK</i>	7272	TTK protein kinase	protein kinase
<i>TWF2</i>	11344	PTK9L protein tyrosine kinase 9-like (A6-related protein)	protein kinase (NRTK)
<i>TXK</i>	7294	TXK tyrosine kinase	protein kinase (NRTK)
<i>TXNDC3</i>	51314	thioredoxin domain containing 3 (spermatozoa);TXNDC3	nucleotide kinase
<i>TYK2</i>	7297	tyrosine kinase 2	protein kinase (NRTK)
<i>TYRO3</i>	7301	TYRO3 protein tyrosine kinase	protein kinase (RTK)
<i>UCK2</i>	7371	uridine-cytidine kinase 2	nucleotide kinase
<i>UHMK1</i>	127933	U2AF homology motif (UHM) kinase 1	protein kinase (NRS/TK)
<i>ULK2</i>	9706	unc-51-like kinase 2 (C. elegans)	protein kinase (NRS/TK)
<i>ULK3</i>	25989	DKFZP434C131 protein	protein kinase (NRS/TK)
<i>ULK4</i>	54986	hypothetical protein FLJ20574	protein kinase
<i>VRK1</i>	7443	vaccinia related kinase 1	protein kinase (NRS/TK)
<i>VRK2</i>	7444	vaccinia related kinase 2	protein kinase (NRS/TK)
<i>VRK3</i>	51231	vaccinia related kinase 3	protein kinase (NRS/TK)
<i>WNK1</i>	65125	protein kinase, lysine deficient 1	protein kinase (NRS/TK)
<i>WNK4</i>	65266	protein kinase, lysine deficient 4	protein kinase (NRS/TK)
<i>XRCC6B P1</i>	91419	XRCC6 binding protein 1;XRCC6BP1	protein kinase
<i>XYLB</i>	9942	xylulokinase homolog (H. influenzae)	carbohydrate kinase
<i>YES1</i>	7525	v-yes-1 Yamaguchi sarcoma viral oncogene homolog 1	protein kinase (NRTK)
<i>YSK4</i>	80122	hypothetical protein FLJ23074	protein kinase
<i>ZAK</i>	51776	sterile alpha motif and leucine zipper containing kinase AZK	protein kinase (NRS/TK)
<i>ZAP70</i>	7535	zeta-chain (TCR) associated protein kinase 70kDa	protein kinase (NRTK)

Table 4: Ranking of average differential proliferation (1 μ M PLX4720/control) for 597 kinase-related ORFs, relative to MEK DD

RANK	GENE	AVERAGE	STDEV.	Z-SCORE	NOTES
-	ALL ORFS	44.26%	7.19%	-	
1	MEK DD	100.00%	6.54%	7.75	(+) Control
2	RAF1	86.80%	18.00%	5.92	
3	PRKCE	74.76%	8.78%	4.24	
4	MAP3K8	67.51%	10.03%	3.23	
5	PRKCH	67.37%	17.56%	3.21	
6	FGR	65.03%	20.76%	2.89	
7	CRKL	64.74%	19.66%	2.85	
8	PAK3	60.33%	13.59%	2.23	
9	AXL	59.75%	10.23%	2.15	
10	LCK	59.47%	15.66%	2.11	Lethal
11	ERBB2	59.32%	9.39%	2.09	2 SD = 58.64%
12	PRKCQ	55.40%	11.97%	1.55	
13	NME3	55.10%	15.46%	1.51	
14	MOS	55.04%	11.84%	1.50	
15	KHK	55.03%	14.75%	1.50	
16	TIE1	54.65%	15.26%	1.44	Proliferative
17	PRKAG2	54.23%	7.15%	1.39	
18	LOC91461	53.75%	12.45%	1.32	
19	TYRO3	53.64%	10.57%	1.30	
20	CDK3	53.55%	13.92%	1.29	
21	PIM2	53.36%	10.78%	1.27	
22	CIB4	53.31%	12.21%	1.26	
23	PRPS2	53.24%	14.53%	1.25	
24	PRKCB1	53.12%	7.74%	1.23	
25	ACVR1B	53.08%	9.53%	1.23	
26	ETNK2	52.85%	12.63%	1.19	
27	STK36	52.85%	15.28%	1.19	
28	DDR1	52.78%	15.54%	1.19	
29	PRKCA	52.47%	13.38%	1.14	
30	AURKB	52.22%	11.80%	1.11	
31	CAMK4	52.14%	9.09%	1.10	
32	DAPK1	51.94%	11.43%	1.07	
33	AURKC	51.89%	11.23%	1.06	Proliferative
34	IGF1R	51.35%	10.70%	0.99	
35	FN3K	51.27%	12.65%	0.97	
36	LOC646505	50.97%	16.89%	0.93	
37	PIK3CB	50.90%	12.87%	0.92	
38	YES1	50.68%	9.51%	0.89	

RANK	GENE	AVERAGE	STDEV.	Z-SCORE	NOTES
39	LOC340156	50.59%	14.63%	0.88	
40	MAP4K5	50.57%	35.45%	0.88	
41	ABL1	50.55%	19.09%	0.87	
42	MAP2K1IP1	50.47%	11.54%	0.86	
43	PRKAR1B	50.34%	8.31%	0.84	
44	RPS6KA1	50.12%	12.28%	0.81	
45	TSSK6	50.10%	11.70%	0.81	
46	NEK9	50.04%	14.37%	0.80	
47	DLG1	49.98%	12.07%	0.80	
48	PTK6	49.93%	10.93%	0.79	
49	SCYL2	49.85%	12.51%	0.78	
50	STK11	49.82%	12.10%	0.77	
51	C9orf98	49.82%	10.38%	0.77	
52	PCK2	49.77%	12.93%	0.77	
53	NTRK2	49.77%	10.02%	0.77	
54	TRIM27	49.69%	10.38%	0.75	
55	FN3KRP	49.69%	11.83%	0.75	
56	CAMK2D	49.61%	11.93%	0.74	
57	ALPK2	49.56%	10.41%	0.74	
58	LOC652722	49.52%	13.10%	0.73	
59	LATS2	49.49%	12.45%	0.73	
60	STK3	49.48%	10.15%	0.73	
61	CDC42BPG	49.44%	10.19%	0.72	
62	HKDC1	49.44%	11.53%	0.72	
63	CDK7	49.42%	14.45%	0.72	Proliferative
64	WNK4	49.37%	14.07%	0.71	
65	PRPF4B	49.36%	12.18%	0.71	
66	MAPKAPK2	49.30%	12.83%	0.70	
67	MEK WT	49.29%	14.33%	0.70	(-) Control
68	PRPS1	49.28%	12.09%	0.70	
69	BTK	49.22%	14.59%	0.69	
70	MYO3B	49.19%	11.05%	0.69	
71	TESK1	49.18%	12.19%	0.68	
72	PLXNA3	49.11%	9.04%	0.67	
73	CLK2	49.09%	8.01%	0.67	
74	PFKFB1	49.02%	14.04%	0.66	
75	DAK	49.00%	12.68%	0.66	
76	ITPKB	48.99%	16.62%	0.66	
77	CHEK1	48.98%	14.41%	0.66	
78	MERTK	48.94%	12.88%	0.65	
79	IKBKE	48.83%	12.46%	0.63	
80	CARD11	48.83%	15.02%	0.63	

RANK	GENE	AVERAGE	STDEV.	Z-SCORE	NOTES
81	PANK3	48.78%	9.70%	0.63	
82	TRIB2	48.76%	10.71%	0.63	
83	CDC7	48.74%	9.83%	0.62	
84	PIK3C3	48.69%	12.05%	0.62	
85	LRGUK	48.60%	11.29%	0.60	
86	SCYL3	48.58%	10.86%	0.60	
87	MAP3K14	48.57%	8.10%	0.60	
88	LOC730000	48.57%	13.36%	0.60	
89	LOC442075	48.55%	10.75%	0.60	
90	LOC54103	48.50%	10.77%	0.59	
91	NPR2	48.49%	13.29%	0.59	
92	COL4A3BP	48.49%	11.10%	0.59	
93	MYLK2	48.49%	11.83%	0.59	
94	CSNK2B	48.48%	11.52%	0.59	
95	CARKL	48.47%	9.19%	0.58	
96	TP53RK	48.44%	14.25%	0.58	Proliferative
97	PRKCG	48.42%	8.31%	0.58	
98	ALK	48.39%	14.07%	0.57	
99	ETNK1	48.37%	11.25%	0.57	
100	DYRK2	48.36%	11.80%	0.57	
101	DGKA	48.31%	10.71%	0.56	
102	ADPGK	48.30%	13.36%	0.56	
103	PRKAB2	48.29%	10.75%	0.56	
104	ULK3	48.28%	11.18%	0.56	
105	KIAA2002	48.28%	12.39%	0.56	
106	IRAK3	48.26%	10.96%	0.56	
107	TYK2	48.24%	10.88%	0.55	
108	MAP2K7	48.23%	12.06%	0.55	
109	NRBP	48.18%	13.40%	0.54	
110	CDK2	48.14%	14.65%	0.54	
111	MORN2	47.98%	11.36%	0.52	
112	EPHB4	47.92%	12.52%	0.51	
113	PRKCZ	47.88%	12.68%	0.50	
114	RFK	47.85%	10.44%	0.50	
115	RAGE	47.83%	10.46%	0.50	
116	LOC91807	47.80%	11.25%	0.49	
117	PIK3CG	47.76%	11.25%	0.49	
118	PIK3C2G	47.74%	10.75%	0.48	
119	ARSG	47.71%	10.26%	0.48	
120	CSNK1A1L	47.66%	10.26%	0.47	
121	ALS2CR7	47.65%	11.56%	0.47	
122	FLJ40852	47.62%	12.34%	0.47	

RANK	GENE	AVERAGE	STDEV.	Z-SCORE	NOTES
123	<i>LOC390877</i>	47.61%	11.43%	0.46	
124	<i>TRIB1</i>	47.59%	12.91%	0.46	
125	<i>TPR</i>	47.58%	13.00%	0.46	
126	<i>RPS6KL1</i>	47.57%	12.33%	0.46	
127	<i>CCRK</i>	47.56%	10.28%	0.46	
128	<i>PDPK1</i>	47.53%	10.42%	0.45	
129	<i>FASTKD5</i>	47.52%	10.70%	0.45	
130	<i>CDKL3</i>	47.49%	13.14%	0.45	
131	<i>DTYMK</i>	47.49%	10.60%	0.45	
132	<i>MPP3</i>	47.47%	10.65%	0.45	
133	<i>HSPB8</i>	47.46%	11.73%	0.44	
134	<i>NME6</i>	47.44%	14.48%	0.44	
135	<i>NEK3</i>	47.42%	15.46%	0.44	
136	<i>PIK3R4</i>	47.41%	11.14%	0.44	
137	<i>MGC16169</i>	47.41%	13.90%	0.44	
138	<i>OXSRI</i>	47.40%	11.68%	0.44	
139	<i>MASTL</i>	47.35%	10.28%	0.43	
140	<i>PNCK</i>	47.35%	10.15%	0.43	
141	<i>ADCK2</i>	47.32%	15.57%	0.43	
142	<i>SNX16</i>	47.26%	12.76%	0.42	
143	<i>HCK</i>	47.24%	23.41%	0.41	
144	<i>CDKL2</i>	47.21%	10.60%	0.41	
145	<i>NEK11</i>	47.20%	14.68%	0.41	
146	<i>BMP2K</i>	47.15%	11.18%	0.40	
147	<i>BUB1</i>	47.15%	11.76%	0.40	
148	<i>PINK1</i>	47.13%	11.38%	0.40	
149	<i>RBKS</i>	47.08%	8.73%	0.39	
150	<i>LOC732306</i>	47.03%	8.82%	0.39	
151	<i>PKLR</i>	47.03%	12.10%	0.38	
152	<i>DYRK1A</i>	47.01%	12.69%	0.38	
153	<i>RPS6KA4</i>	47.01%	11.70%	0.38	
154	<i>DGKG</i>	46.94%	12.71%	0.37	
155	<i>CDK5R1</i>	46.93%	9.40%	0.37	
156	<i>FLJ25006</i>	46.87%	11.64%	0.36	
157	<i>PBK</i>	46.86%	14.05%	0.36	
158	<i>ACVR1C</i>	46.82%	9.98%	0.35	
159	<i>FLT1</i>	46.78%	10.42%	0.35	
160	<i>PLXNA4B</i>	46.76%	9.39%	0.35	
161	<i>MVK</i>	46.71%	9.26%	0.34	
162	<i>LIMK2</i>	46.70%	11.29%	0.34	
163	<i>DYRK1B</i>	46.68%	20.75%	0.34	
164	<i>MARK3</i>	46.67%	11.71%	0.34	Proliferative

RANK	GENE	AVERAGE	STDEV.	Z-SCORE	NOTES
165	<i>BMPR1B</i>	46.66%	12.24%	0.33	
166	<i>NUP62</i>	46.63%	9.92%	0.33	
167	<i>JAK2</i>	46.62%	12.50%	0.33	
168	<i>MAPK12</i>	46.55%	9.98%	0.32	
169	<i>DDR2</i>	46.54%	7.99%	0.32	
170	<i>MAK</i>	46.52%	15.42%	0.31	
171	<i>GLYCTK</i>	46.50%	13.19%	0.31	
172	<i>AK1</i>	46.47%	9.68%	0.31	
173	<i>MAPK15</i>	46.47%	11.14%	0.31	
174	<i>MAST1</i>	46.45%	10.77%	0.30	
175	<i>PAPSS2</i>	46.39%	11.53%	0.30	
176	<i>CSF1R</i>	46.34%	12.86%	0.29	
177	<i>TPK1</i>	46.29%	9.52%	0.28	
178	<i>PAK4</i>	46.24%	9.98%	0.28	
179	<i>NAGK</i>	46.21%	12.34%	0.27	
180	<i>CDK8</i>	46.20%	7.68%	0.27	
181	<i>STK40</i>	46.19%	12.92%	0.27	
182	<i>CIB1</i>	46.10%	10.92%	0.26	
183	<i>PLK1</i>	46.08%	9.99%	0.25	
184	<i>FLJ23356</i>	46.04%	10.45%	0.25	
185	<i>LOC220686</i>	46.04%	9.31%	0.25	
186	<i>PRKAB1</i>	46.03%	12.00%	0.25	
187	<i>JAK3</i>	46.02%	10.17%	0.24	
188	<i>NME1</i>	46.02%	13.01%	0.24	
189	<i>NME5</i>	46.00%	11.53%	0.24	
190	<i>FER</i>	45.97%	9.92%	0.24	
191	<i>AK3L1</i>	45.97%	10.39%	0.24	
192	<i>PRKG2</i>	45.90%	13.24%	0.23	
193	<i>RP6-213H19.1</i>	45.88%	12.83%	0.22	
194	<i>AKT3</i>	45.87%	11.32%	0.22	
195	<i>PSKH1</i>	45.86%	10.16%	0.22	
196	<i>PRKAR2B</i>	45.84%	13.05%	0.22	
197	<i>FUK</i>	45.81%	10.94%	0.22	
198	<i>ADCK4</i>	45.79%	11.25%	0.21	
199	<i>TEC</i>	45.78%	11.46%	0.21	
200	<i>PRKAG1</i>	45.77%	11.08%	0.21	
201	<i>FXN</i>	45.77%	12.03%	0.21	
202	<i>AAK1</i>	45.68%	11.49%	0.20	
203	<i>CAMK2B</i>	45.67%	19.84%	0.20	
204	<i>COASY</i>	45.64%	11.07%	0.19	
205	<i>PRKAG3</i>	45.63%	10.21%	0.19	
206	<i>NME7</i>	45.58%	10.18%	0.18	

RANK	GENE	AVERAGE	STDEV.	Z-SCORE	NOTES
207	<i>LMTK2</i>	45.58%	12.15%	0.18	
208	<i>PANK2</i>	45.57%	11.72%	0.18	
209	<i>PRKD3</i>	45.47%	13.14%	0.17	
210	<i>PHKA2</i>	45.47%	10.92%	0.17	
211	<i>SLAMF6</i>	45.46%	9.67%	0.17	
212	<i>SRPK1</i>	45.46%	12.54%	0.17	
213	<i>HIPK1</i>	45.42%	12.59%	0.16	
214	<i>EPHA1</i>	45.39%	10.59%	0.16	
215	<i>WNK1</i>	45.38%	13.79%	0.15	
216	<i>PDIK1L</i>	45.33%	12.31%	0.15	
217	<i>BMP2KL</i>	45.33%	10.08%	0.15	
218	<i>MAP3K5</i>	45.32%	14.13%	0.15	
219	<i>EPHA2</i>	45.30%	12.87%	0.14	
220	<i>CCL2</i>	45.29%	9.71%	0.14	
221	<i>CDKL1</i>	45.25%	10.13%	0.14	
222	<i>NJMU-R1</i>	45.23%	8.13%	0.13	
223	<i>LOC652799</i>	45.18%	10.30%	0.13	
224	<i>GUK1</i>	45.15%	12.23%	0.12	
225	<i>NME4</i>	45.12%	13.03%	0.12	
226	<i>YSK4</i>	45.11%	13.47%	0.12	
227	<i>NEK2</i>	45.11%	9.38%	0.12	
228	<i>C9orf95</i>	45.10%	9.38%	0.12	
229	<i>CDC2</i>	45.05%	11.95%	0.11	
230	<i>FGFR2</i>	45.01%	11.23%	0.10	
231	<i>IPMK</i>	44.98%	10.67%	0.10	
232	<i>STK32C</i>	44.98%	12.10%	0.10	
233	<i>PIP5K2A</i>	44.92%	14.55%	0.09	
234	<i>PRKX</i>	44.90%	10.44%	0.09	
235	<i>TRPM7</i>	44.80%	10.36%	0.07	
236	<i>FLJ10986</i>	44.74%	10.95%	0.07	
237	<i>SNF1LK</i>	44.74%	12.70%	0.07	
238	<i>MAP3K6</i>	44.71%	9.75%	0.06	
239	<i>LOC653052</i>	44.70%	14.56%	0.06	
240	<i>IRAK2</i>	44.69%	11.61%	0.06	
241	<i>XYLB</i>	44.68%	11.47%	0.06	
242	<i>PTK7</i>	44.67%	14.55%	0.06	
243	<i>PKMYT1</i>	44.66%	13.74%	0.05	
244	<i>SPHK2</i>	44.63%	10.35%	0.05	
245	<i>NME2</i>	44.63%	11.57%	0.05	
246	<i>PRKAA1</i>	44.62%	15.03%	0.05	
247	<i>MAPK14</i>	44.60%	10.77%	0.05	
248	<i>NTRK3</i>	44.58%	13.43%	0.04	

RANK	GENE	AVERAGE	STDEV.	Z-SCORE	NOTES
249	PRKACB	44.58%	9.30%	0.04	
250	LOC650122	44.56%	11.24%	0.04	
251	CDK6	44.51%	10.74%	0.03	
252	AMHR2	44.48%	11.11%	0.03	
253	IPPK	44.42%	10.63%	0.02	
254	AK7	44.41%	10.36%	0.02	
255	PIP5KL1	44.36%	11.74%	0.01	
256	LOC340371	44.30%	16.04%	0.01	
257	DKFZp434B1231	44.29%	12.21%	0.00	
258	PRKAA2	44.28%	11.42%	0.00	
259	FASTKD1	44.28%	10.73%	0.00	
260	ARAF	44.27%	14.40%	0.00	RAF Family
261	HK3	44.26%	14.83%	0.00	
262	KSR2	44.25%	10.28%	0.00	
263	PAK7	44.24%	18.45%	0.00	
264	GTF2H1	44.23%	10.90%	0.00	
265	RPS6KB2	44.23%	9.32%	-0.01	
266	PAK6	44.21%	13.83%	-0.01	
267	IRAK4	44.18%	10.27%	-0.01	
268	NLK	44.18%	12.92%	-0.01	
269	FYN	44.14%	12.43%	-0.02	
270	BMPR2	44.14%	12.20%	-0.02	
271	CDK4	44.13%	10.47%	-0.02	
272	STK32A	44.07%	13.93%	-0.03	
273	TNK1	44.06%	10.80%	-0.03	
274	STK24	43.99%	10.79%	-0.04	
275	CSNK2A1	43.99%	11.97%	-0.04	
276	AK3	43.98%	11.16%	-0.04	
277	TTK	43.93%	10.47%	-0.05	
278	PGK2	43.91%	13.31%	-0.05	
279	GALK1	43.89%	10.14%	-0.05	
280	DYRK3	43.89%	9.32%	-0.05	
281	EIF2AK4	43.83%	11.00%	-0.06	
282	PDK1	43.79%	13.31%	-0.07	
283	EIF2AK1	43.79%	12.02%	-0.07	
284	PRKACG	43.79%	11.37%	-0.07	
285	BUB1B	43.74%	10.69%	-0.07	
286	PKM2	43.74%	14.46%	-0.07	
287	LRPPRC	43.64%	10.87%	-0.09	
288	EPHA6	43.63%	12.60%	-0.09	
289	HK2	43.58%	12.55%	-0.10	
290	MET	43.55%	10.99%	-0.10	

RANK	GENE	AVERAGE	STDEV.	Z-SCORE	NOTES
291	CKS2	43.53%	7.83%	-0.10	
292	LOC375133	43.53%	10.05%	-0.10	
293	LIMK1	43.52%	12.43%	-0.10	
294	TWF2	43.50%	11.71%	-0.11	
295	PCTK2	43.47%	12.02%	-0.11	
296	DCAKD	43.43%	11.61%	-0.12	
297	PLXNB2	43.41%	11.85%	-0.12	
298	MST1R	43.35%	11.99%	-0.13	
299	PRKAR1A	43.35%	9.03%	-0.13	
300	SEPHS2	43.32%	9.68%	-0.13	
301	SPHK1	43.31%	9.87%	-0.13	
302	CCL4	43.30%	11.83%	-0.13	
303	KIAA0999	43.28%	9.68%	-0.14	
304	PDGFRL	43.23%	11.93%	-0.14	
305	BRSK1	43.21%	14.17%	-0.15	
306	BRAF	43.21%	12.56%	-0.15	RAF Family
307	MUSK	43.20%	11.03%	-0.15	
308	TNNI3K	43.20%	10.46%	-0.15	
309	GK2	43.18%	15.15%	-0.15	
310	CKB	43.14%	9.45%	-0.16	
311	DGKB	43.12%	11.62%	-0.16	
312	LOC648152	42.99%	11.15%	-0.18	
313	RPS6KA5	42.87%	11.23%	-0.19	
314	CASK	42.87%	12.05%	-0.19	
315	PHKA1	42.86%	10.10%	-0.20	
316	AK2	42.84%	9.83%	-0.20	
317	PIM1	42.81%	14.09%	-0.20	
318	ZAP70	42.81%	12.10%	-0.20	
319	PNKP	42.79%	12.95%	-0.20	
320	CDK10	42.78%	9.77%	-0.21	
321	CHEK2	42.77%	12.86%	-0.21	
322	CAMK2A	42.72%	11.68%	-0.21	
323	CAMK2G	42.70%	9.66%	-0.22	
324	ADRBK2	42.70%	12.56%	-0.22	
325	NEK8	42.69%	9.88%	-0.22	
326	PRKR	42.69%	10.65%	-0.22	
327	CHKA	42.66%	12.84%	-0.22	
328	ACVRL1	42.65%	10.85%	-0.22	
329	PRKY	42.63%	11.80%	-0.23	
330	TRIB3	42.62%	11.45%	-0.23	
331	PRKD2	42.59%	10.48%	-0.23	
332	PIP5K3	42.57%	9.78%	-0.24	

RANK	GENE	AVERAGE	STDEV.	Z-SCORE	NOTES
333	LOC727761	42.56%	14.17%	-0.24	
334	PTK2B	42.50%	15.72%	-0.25	
335	MPP2	42.48%	10.73%	-0.25	
336	CSNK1A1	42.47%	13.54%	-0.25	
337	PTK9	42.42%	11.31%	-0.26	
338	STK25	42.41%	12.54%	-0.26	
339	PFKL	42.39%	12.01%	-0.26	
340	STK19	42.35%	11.20%	-0.27	
341	PI4KII	42.30%	14.04%	-0.27	
342	HK1	42.26%	12.73%	-0.28	
343	AURKA	42.21%	14.80%	-0.29	
344	GK5	42.21%	10.98%	-0.29	
345	MAP3K7	42.20%	13.13%	-0.29	
346	PFTK1	42.18%	12.49%	-0.29	
347	ERN1	42.17%	13.74%	-0.29	
348	STK33	42.17%	13.22%	-0.29	
349	SYK	42.13%	12.42%	-0.30	
350	GALK2	42.13%	11.34%	-0.30	
351	TSSK1	42.12%	11.98%	-0.30	
352	MAPK6	42.11%	10.58%	-0.30	
353	ASCIZ	42.07%	10.61%	-0.30	
354	PFKP	42.07%	14.99%	-0.31	
355	PXK	42.05%	14.69%	-0.31	
356	PI4K2B	42.05%	11.84%	-0.31	
357	PIP5K2C	42.02%	9.50%	-0.31	
358	PFKFB3	42.00%	12.79%	-0.31	
359	TSSK3	41.97%	14.82%	-0.32	
360	MPP6	41.97%	12.68%	-0.32	
361	MPP4	41.87%	12.27%	-0.33	
362	LOC653155	41.85%	11.50%	-0.34	
363	ALPK1	41.82%	12.37%	-0.34	
364	CDK9	41.76%	10.32%	-0.35	
365	PDK3	41.66%	10.87%	-0.36	
366	CKMT2	41.66%	12.57%	-0.36	
367	CAMK1G	41.63%	12.06%	-0.37	
368	MAPKAPK3	41.61%	13.19%	-0.37	
369	PIK4CB	41.55%	14.29%	-0.38	
370	PRPS1L1	41.54%	11.08%	-0.38	
371	FASTK	41.49%	12.56%	-0.39	
372	CAMK1D	41.49%	11.54%	-0.39	
373	MARK2	41.48%	14.28%	-0.39	
374	PDK4	41.43%	13.43%	-0.39	

RANK	GENE	AVERAGE	STDEV.	Z-SCORE	NOTES
375	NEK7	41.36%	9.57%	-0.40	
376	MAPK4	41.34%	10.83%	-0.41	
377	PIK4CA	41.32%	11.99%	-0.41	
378	JAK1	41.31%	11.56%	-0.41	
379	PDXK	41.30%	11.93%	-0.41	
380	TGFB2	41.25%	15.85%	-0.42	
381	PHKB	41.25%	10.81%	-0.42	
382	ULK2	41.24%	15.35%	-0.42	
383	MKNK1	41.21%	13.08%	-0.42	
384	CDC2L6	41.17%	10.91%	-0.43	
385	CSNK1G3	41.13%	10.53%	-0.44	
386	CAMK1	41.08%	11.68%	-0.44	
387	DGKZ	41.07%	12.69%	-0.44	
388	SGK	40.91%	11.92%	-0.47	
389	TBK1	40.83%	14.81%	-0.48	
390	ILK	40.81%	12.62%	-0.48	
391	STK32B	40.80%	11.79%	-0.48	
392	TXNDC3	40.78%	12.24%	-0.48	
393	RPS6KB1	40.72%	10.13%	-0.49	
394	ZAK	40.71%	10.32%	-0.49	
395	DYRK4	40.66%	12.59%	-0.50	
396	ITGB1BP3	40.57%	11.99%	-0.51	
397	MPP1	40.53%	10.94%	-0.52	
398	HIPK2	40.49%	11.06%	-0.52	
399	MAPK13	40.46%	12.06%	-0.53	
400	TK1	40.39%	11.04%	-0.54	
401	SH3BP4	40.36%	12.11%	-0.54	
402	CKM	40.29%	12.70%	-0.55	
403	FGFRL1	40.23%	16.94%	-0.56	
404	MAPK10	40.23%	12.29%	-0.56	
405	CALM2	40.16%	8.64%	-0.57	
406	CALM3	40.12%	11.85%	-0.58	
407	STYK1	40.09%	11.47%	-0.58	
408	CDKL4	40.08%	11.01%	-0.58	
409	NADK	40.08%	11.89%	-0.58	
410	MPP7	40.06%	11.72%	-0.59	
411	CAMKV	40.03%	12.16%	-0.59	
412	EXOSC10	40.02%	12.78%	-0.59	
413	CDKL5	39.98%	10.10%	-0.60	
414	STK38	39.90%	9.61%	-0.61	
415	INSRR	39.90%	15.08%	-0.61	
416	DCK	39.86%	10.23%	-0.61	

RANK	GENE	AVERAGE	STDEV.	Z-SCORE	NOTES
417	TLK2	39.80%	14.66%	-0.62	
418	PCTK3	39.66%	10.48%	-0.64	
419	LOC388957	39.65%	10.97%	-0.64	
420	PAPSS1	39.59%	13.48%	-0.65	
421	ACVR2B	39.56%	14.70%	-0.65	
422	NME1-NME2	39.53%	12.01%	-0.66	
423	NEK10	39.50%	13.78%	-0.66	
424	MAP3K11	39.47%	9.33%	-0.67	
425	PMVK	39.46%	10.19%	-0.67	
426	MAPK9	39.45%	12.74%	-0.67	
427	MKNK2	39.39%	13.54%	-0.68	
428	GRK7	39.34%	14.98%	-0.68	
429	RIOK2	39.27%	11.79%	-0.69	
430	DGKK	39.27%	9.02%	-0.69	
431	ACVR1	39.15%	13.96%	-0.71	
432	TLK1	39.11%	15.46%	-0.72	
433	LATS1	39.05%	10.46%	-0.73	
434	SCYL1	39.01%	11.41%	-0.73	
435	TESK2	38.93%	13.30%	-0.74	
436	DGUOK	38.90%	14.54%	-0.75	
437	PGK1	38.87%	12.26%	-0.75	
438	MAGI1	38.87%	12.66%	-0.75	
439	SNRK	38.79%	11.61%	-0.76	
440	CALM1	38.65%	10.26%	-0.78	
441	RIOK1	38.58%	12.39%	-0.79	
442	EEF2K	38.56%	11.96%	-0.79	
443	MAPK8	38.45%	10.51%	-0.81	
444	CSNK1D	38.44%	15.60%	-0.81	
445	ULK4	38.42%	12.02%	-0.81	
446	STK38L	38.33%	12.63%	-0.83	
447	RIOK3	38.21%	12.80%	-0.84	
448	MINK1	38.00%	14.94%	-0.87	
449	ROR2	37.95%	15.32%	-0.88	
450	PTK2	37.93%	10.59%	-0.88	
451	PIK3R5	37.84%	13.17%	-0.89	
452	ALDH18A1	37.81%	15.66%	-0.90	
453	NYD-SP25	37.79%	13.52%	-0.90	
454	MAP4K3	37.76%	9.58%	-0.90	
455	AGK	37.61%	13.89%	-0.92	
456	GSK3A	37.56%	14.44%	-0.93	
457	BMPR1A	37.56%	16.10%	-0.93	
458	STK16	37.53%	11.09%	-0.94	

RANK	GENE	AVERAGE	STDEV.	Z-SCORE	NOTES
459	<i>FASTKD2</i>	37.50%	8.53%	-0.94	
460	<i>MAP4K4</i>	37.39%	15.68%	-0.96	
461	<i>LRRK2</i>	37.38%	10.11%	-0.96	
462	<i>TGFBR3</i>	37.33%	11.04%	-0.96	
463	<i>PDGFRB</i>	37.29%	16.90%	-0.97	
464	<i>DLG3</i>	37.09%	12.08%	-1.00	
465	<i>PFKFB2</i>	36.99%	10.37%	-1.01	
466	<i>AKT1</i>	36.66%	13.43%	-1.06	
467	<i>PRKCI</i>	36.54%	11.44%	-1.07	
468	<i>NEK4</i>	36.50%	13.19%	-1.08	
469	<i>PRKD1</i>	36.45%	14.57%	-1.09	
470	<i>SRPK2</i>	36.37%	12.38%	-1.10	
471	<i>SH3BP5</i>	36.37%	17.17%	-1.10	
472	<i>CLK1</i>	36.06%	11.13%	-1.14	
473	<i>GK</i>	35.86%	17.33%	-1.17	
474	<i>IHPK1</i>	35.53%	12.15%	-1.21	
475	<i>IHPK3</i>	35.48%	9.30%	-1.22	
476	<i>PLAU</i>	35.36%	13.10%	-1.24	
477	<i>TAOK3</i>	35.29%	14.78%	-1.25	
478	<i>PAK2</i>	35.20%	12.51%	-1.26	
479	<i>BMX</i>	35.12%	12.94%	-1.27	
480	<i>DAPK2</i>	35.01%	13.92%	-1.29	
481	<i>CSNK1E</i>	34.83%	15.21%	-1.31	
482	<i>MAPK3</i>	34.75%	19.93%	-1.32	Lethal
483	<i>MAP3K15</i>	34.40%	16.76%	-1.37	Lethal
484	<i>MPP5</i>	34.35%	11.80%	-1.38	
485	<i>MAST2</i>	34.08%	16.10%	-1.42	
486	<i>GRK6</i>	34.04%	15.82%	-1.42	
487	<i>DKFZp761P0423</i>	33.93%	13.58%	-1.44	
488	<i>VRK1</i>	33.87%	9.28%	-1.45	
489	<i>DCAMKL2</i>	33.77%	11.96%	-1.46	
490	<i>IHPK2</i>	33.75%	9.56%	-1.46	
491	<i>MATK</i>	33.44%	10.25%	-1.51	
492	<i>RP2</i>	33.20%	13.41%	-1.54	
493	<i>MAP2K2</i>	32.98%	15.34%	-1.57	
494	<i>UCK2</i>	32.82%	13.75%	-1.59	
495	<i>NEK6</i>	32.74%	15.85%	-1.60	
496	<i>PRKG1</i>	32.52%	14.60%	-1.63	
497	<i>MAP3K12</i>	32.49%	15.33%	-1.64	
498	<i>PCTK1</i>	32.48%	13.21%	-1.64	
499	<i>MGC42105</i>	32.36%	13.85%	-1.66	
500	<i>MAP2K6</i>	32.32%	11.92%	-1.66	

RANK	GENE	AVERAGE	STDEV.	Z-SCORE	NOTES
501	<i>SH3BP5L</i>	32.11%	15.55%	-1.69	
502	<i>FES</i>	32.07%	11.04%	-1.70	
503	<i>RKHD3</i>	31.89%	12.45%	-1.72	
504	<i>PRKACA</i>	31.76%	18.45%	-1.74	Lethal
505	<i>MAP4K1</i>	31.51%	13.69%	-1.77	
506	<i>CSNK1G1</i>	30.32%	14.90%	-1.94	
507	<i>MAPKAPK5</i>	30.15%	13.47%	-1.96	
508	<i>CSK</i>	29.83%	14.11%	-2.01	Lethal
509	<i>BRD3</i>	29.16%	8.83%	-2.10	
510	<i>ADRBK1</i>	29.00%	16.93%	-2.12	
511	<i>BRSK2</i>	28.64%	13.09%	-2.17	
512	<i>ADCK1</i>	28.30%	13.52%	-2.22	
513	<i>CSNK1G2</i>	26.81%	14.42%	-2.43	
514	<i>CKMT1A</i>	26.80%	12.42%	-2.43	
515	<i>TSSK2</i>	25.66%	13.30%	-2.59	Lethal
516	<i>CD2</i>	25.01%	14.99%	-2.68	
517	<i>PIP5K1A</i>	24.76%	15.49%	-2.71	
518	<i>PHKG1</i>	23.31%	14.24%	-2.91	
519	<i>ABL2</i>	Low pLX DNA yield			
520	<i>ACVR2A</i>	Low pLX DNA yield			
521	<i>BLK</i>	Low pLX DNA yield			
522	<i>CDC2L1</i>	Low pLX DNA yield			
523	<i>EGFR</i>	Low pLX DNA yield			
524	<i>EPHA3</i>	Low pLX DNA yield			
525	<i>EPHB1</i>	Low pLX DNA yield			
526	<i>ERBB4</i>	Low pLX DNA yield			
527	<i>FASTKD3</i>	Low pLX DNA yield			
528	<i>FGFR1</i>	Low pLX DNA yield			
529	<i>FLT3</i>	Low pLX DNA yield			
530	<i>FLT4</i>	Low pLX DNA yield			
531	<i>HIPK3</i>	Low pLX DNA yield			
532	<i>KSR</i>	Low pLX DNA yield			
533	<i>LYN</i>	Low pLX DNA yield			
534	<i>PANK4</i>	Low pLX DNA yield			
535	<i>PDGFRA</i>	Low pLX DNA yield			
536	<i>PLK4</i>	Low pLX DNA yield			
537	<i>RET</i>	Low pLX DNA yield			
538	<i>SGK3</i>	Low pLX DNA yield			
539	<i>SRC</i>	Low pLX DNA yield			
540	<i>TXK</i>	Low pLX DNA yield			
541	<i>BRD4</i>	Inf. Effic. < 70%			
542	<i>CAMKK2</i>	Inf. Effic. < 70%			

RANK	GENE	AVERAGE	STDEV.	Z-SCORE	NOTES
543	<i>CKS1B</i>		Inf. Effic. < 70%		
544	<i>CLK3</i>		Inf. Effic. < 70%		
545	<i>DAPK3</i>		Inf. Effic. < 70%		
546	<i>EPHA4</i>		Inf. Effic. < 70%		
547	<i>EPHB6</i>		Inf. Effic. < 70%		
548	<i>FGFR3</i>		Inf. Effic. < 70%		
549	<i>FRK</i>		Inf. Effic. < 70%		
550	<i>GCK</i>		Inf. Effic. < 70%		
551	<i>GNE</i>		Inf. Effic. < 70%		
552	<i>HIPK4</i>		Inf. Effic. < 70%		
553	<i>ITK</i>		Inf. Effic. < 70%		
554	<i>KDR</i>		Inf. Effic. < 70%		
555	<i>LOC389599</i>		Inf. Effic. < 70%		
556	<i>LOC649288</i>		Inf. Effic. < 70%		
557	<i>MAP3K2</i>		Inf. Effic. < 70%		
558	<i>NEK5</i>		Inf. Effic. < 70%		
559	<i>NTRK1</i>		Inf. Effic. < 70%		
560	<i>PAK1</i>		Inf. Effic. < 70%		
561	<i>PHKG2</i>		Inf. Effic. < 70%		
562	<i>PIK3CA</i>		Inf. Effic. < 70%		
563	<i>PIK3R3</i>		Inf. Effic. < 70%		
564	<i>PIP5K1B</i>		Inf. Effic. < 70%		
565	<i>RIPK1</i>		Inf. Effic. < 70%		
566	<i>RIPK2</i>		Inf. Effic. < 70%		
567	<i>RPS6KA2</i>		Inf. Effic. < 70%		
568	<i>RPS6KC1</i>		Inf. Effic. < 70%		
569	<i>STK17B</i>		Inf. Effic. < 70%		
570	<i>TGFBF1</i>		Inf. Effic. < 70%		
571	<i>UHMK1</i>		Inf. Effic. < 70%		
572	<i>XRCC6BP1</i>		Inf. Effic. < 70%		
573	<i>BCKDK</i>		Replicate STDEV		
574	<i>C1orf57</i>		Replicate STDEV		
575	<i>CAMKK1</i>		Replicate STDEV		
576	<i>CDC2L2</i>		Replicate STDEV		
577	<i>CDK5</i>		Replicate STDEV		
578	<i>ERBB3</i>		Replicate STDEV		
579	<i>GSG2</i>		Replicate STDEV		
580	<i>LOC647279</i>		Replicate STDEV		
581	<i>LYK5</i>		Replicate STDEV		
582	<i>MAPK1</i>		Replicate STDEV		Lethal
583	<i>MAP2K5</i>		Replicate STDEV		
584	<i>MAP4K2</i>		Replicate STDEV		

RANK	GENE	AVERAGE	STDEV.	Z-SCORE	NOTES
585	<i>NUAK2</i>	Replicate STDEV			
586	<i>PDK2</i>	Replicate STDEV			Lethal
587	<i>PFKM</i>	Replicate STDEV			
588	<i>PIK3R1</i>	Replicate STDEV			
589	<i>PLK2</i>	Replicate STDEV			
590	<i>PRKAR2A</i>	Replicate STDEV			
591	<i>RPS6KA3</i>	Replicate STDEV			
592	<i>RPS6KA6</i>	Replicate STDEV			
593	<i>SGK2</i>	Replicate STDEV			
594	<i>SRPK3</i>	Replicate STDEV			
595	<i>VRK1</i>	Replicate STDEV			
596	<i>VRK2</i>	Replicate STDEV			
597	<i>VRK3</i>	Replicate STDEV			

A375

SKMEL28

Table 5: Results of a secondary screen quantifying the change in PLX4720 GI₅₀ induced by the top 9 candidate resistance ORFs

Secondary Screen							
A375				SKMEL28			
Gene	GI ₅₀ (μ M)	Fold Change GI ₅₀	Rank	Gene	GI ₅₀ (μ M)	Fold Change GI ₅₀	Rank
<i>MAP3K8</i>	>100.0	598	1	<i>MAP3K8</i>	>100.0	~100	1
<i>RAF1</i>	$\geq$ 100.0	598	2	<i>RAF1</i>	$\geq$ 10.0	$\geq$ 10	2
<i>CRKL</i>	>10.0	59.8	3	<i>CRKL</i>	9.7	9.7	3
<i>FGR</i>	>10.0	59.8	4	<i>FGR</i>	5	5	4
<i>PRKCE</i>	4.41	26.4	5	<i>PRKCH</i>	2.26	2.26	5
<i>PRKCH</i>	4.14	24.7	6	<i>PRKCE</i>	1.91	1.91	6
<i>ERBB2</i>	1.33	7.95	7	<i>AXL</i>	1.18	1.18	7
<i>AXL</i>	1	5.98	8	<i>ERBB2</i>	1	1	8
<i>PAK3</i>	0.4934	2.95	9	<i>PAK3</i>	0.9041	0.9041	9
Controls				Controls			
<i>Mock</i>	0.1528	0.91		<i>Mock</i>	0.5287	1.89	
<i>MEK1</i>	0.1671	1	(-)	<i>MEK1</i>	1	1	(-)
<i>MEK DD</i>	4.8	28	(+)	<i>MEK DD</i>	8.29	8.29	(+)

Table 6: Patient characteristics

Patient	Tissue	Age (years)	Gender	Biopsy Location	Time from primary diagnosis (years)	Time from metastatic disease diagnosis (years)	Response	BRAF status (pre- treatment)	BRAF status (on- treatment/ post- relapse)	NRAS status	KRAS status	pMEK status	pERK status
Pt 1	Tissue Biopsy	49	F	Back	5	3	Partial Response	V600E	N/A	N/A	N/A	N/A	N/A
Pt 2	Tissue Biopsy	67	M	Back	2	0	Partial Response	V600E	N/A	N/A	N/A	N/A	N/A
Pt 3	Tissue Biopsy	68	M	Leg	11	0	Partial Response	V600E	N/A	N/A	N/A	N/A	N/A
MM-R	Tissue Biopsy	38	M	Axilla	0	0	Partial Response	V600E	V600E	WT	WT	*	*
M307	Short- term Culture	59	M	Axillary Lymph node	4	4	Stable Disease	V600E	V600E	N/A	N/A	Ref. (14)	**

* See Figure 20

** See Figure 3f

What is claimed is:

1. Use of a RAF inhibitor and a MEK inhibitor to treat cancer in a subject, wherein the subject has cancer cells comprising a B-RAF^{V600E} mutation and wherein cancer cells obtained from the subject exhibit one or more symptoms selected from the group consisting of: increased gene copy number, an alteration in mRNA expression, protein overexpression, and phosphorylation of one or more kinase targets selected from the group consisting of *MAP3K8* (TPL2/COT), *CRKL* (CrkL), *FGR* (Fgr), *PRKCE* (Prkce), *PRKCH* (Prkch), *ERBB2* (ErbB2), *AXL* (Axl), and *PAK3* (Pak3) in comparison to cells obtained from a subject without the cancer.
2. The use of claim 1, wherein the RAF inhibitor is selected from the group consisting of: RAF265, sorafenib, SB590885, PLX 4720, PLX4032, GDC-0879 and ZM 336372.
3. The use of claim 1, wherein the MEK inhibitor is selected from the group consisting of: CI-1040/PD184352, AZD6244, PD318088, PD98059, PD334581, RDEA119, 6-Methoxy-7-(3-morpholin-4-yl-propoxy)-4-(4-phenoxy-phenylamino)-quinoline-3-carbonitrile and 4-[3-Chloro-4-(1-methyl-1H-imidazol-2-ylsulfanyl)-phenylamino]-6-methoxy-7-(3-morpholin-4-yl-propoxy)-quinoline-3-carbonitrile.
4. The use of claim 1, wherein the subject has innate resistance to the RAF inhibitor or is likely to develop resistance to the RAF inhibitor.
5. The use of claim 1, wherein the cancer is selected from the group consisting of melanoma, breast cancer, colorectal cancers, glioma, lung cancer, ovarian cancer, sarcoma, and thyroid cancer.
6. The use of claim 1, wherein the cancer is melanoma.
7. The use of claim 1, wherein the subject has innate resistance to the MEK inhibitor or is likely to develop resistance to the MEK inhibitor.

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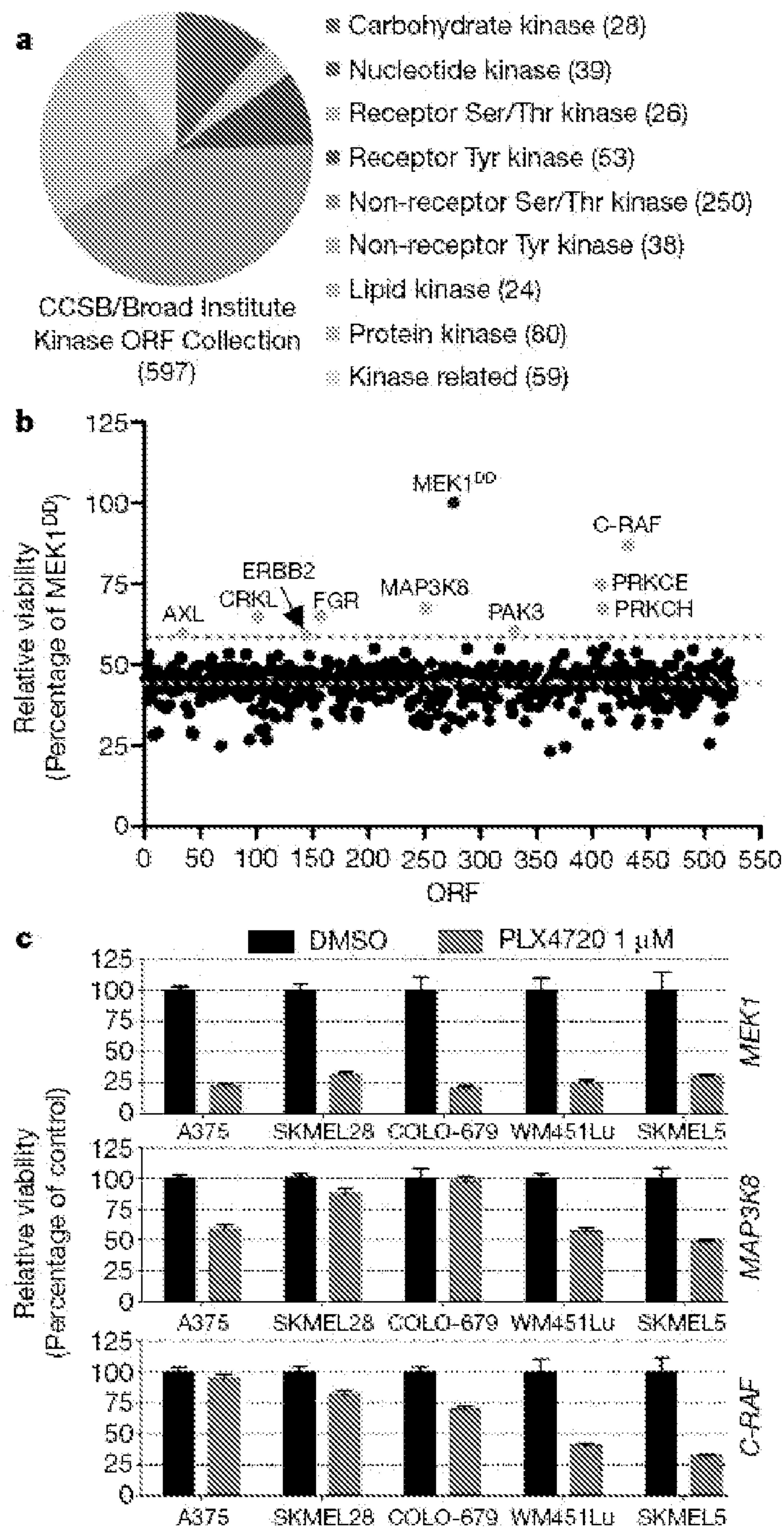


Figure 1

Secondary Screen							
A375				SKMEL28			
Gene	GI50 (µM)	Fold Change	Rank	Gene	GI50 (µM)	Fold Change	Rank
-	0.1528	0.91		-	0.4751	0.4791	
MEK1	0.1671	1	(-)	MEK1	1.0	1	(-)
MEK DD	4.8	28	(+)	MEK DD	8.29	8.29	(+)
MAP3K8	>100.0	~600	1	MAP3K8	>100.0	~100	1
RAF1	≥100.0	~600	2	RAF1	≥100.0	~100	2
CRKL	>10.0	~60	3	CRKL	9.70	9.7	3
FGR	>10.0	~60	4	FGR	5.0	5	4
PRKCE	4.41	~26	5	PRKCH	2.26	2.26	5
PRKCH	4.14	~25	6	PRKCE	1.91	1.91	6
ERBB2	1.33	~8	7	AXL	1.18	1.18	7
AXL	1.00	~6	8	ERBB2	1.00	1.00	8
PAK3	0.4934	~3	9	PAK3	0.9041	0.9041	9

Figure 1d

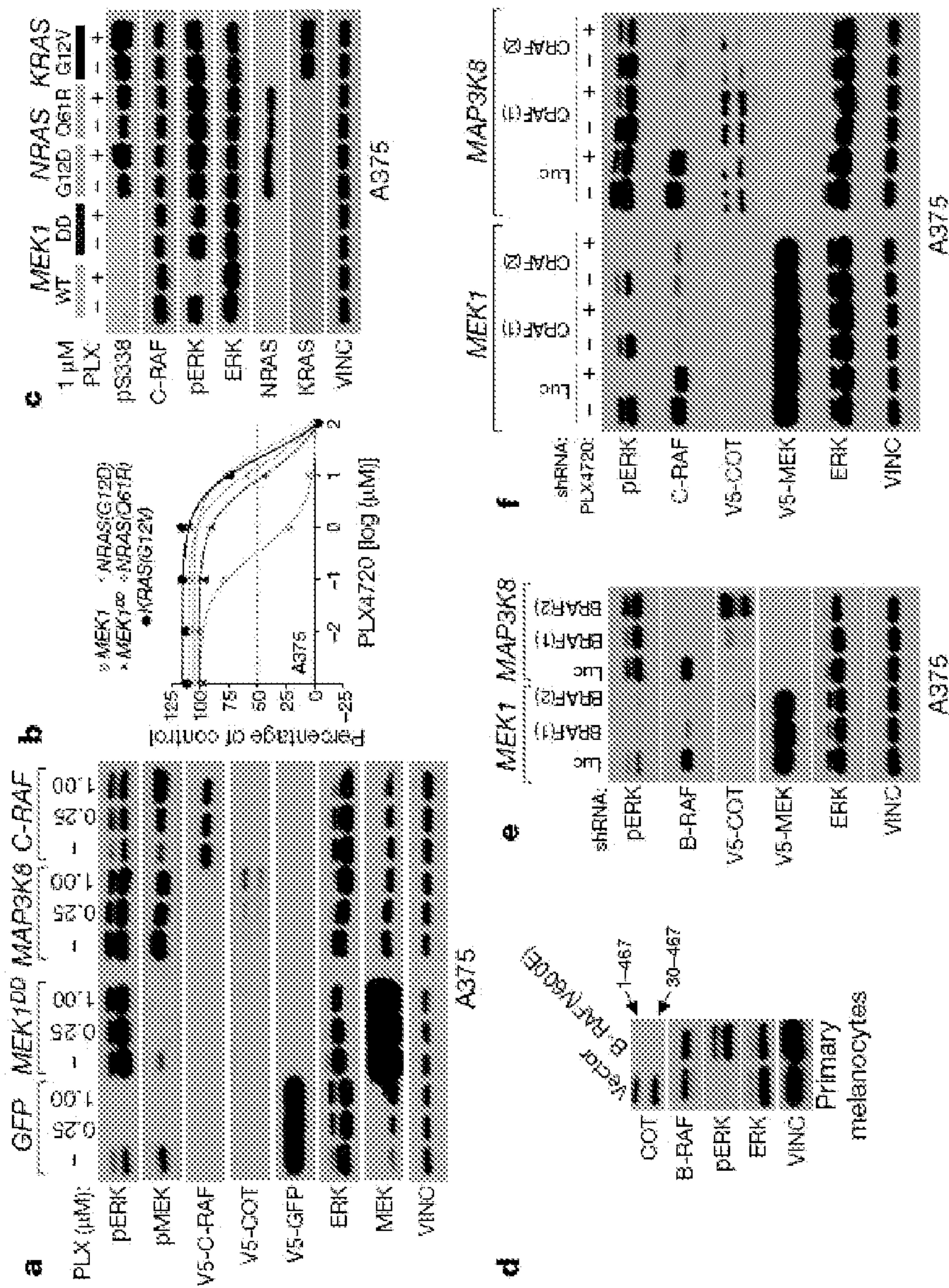


Figure 2

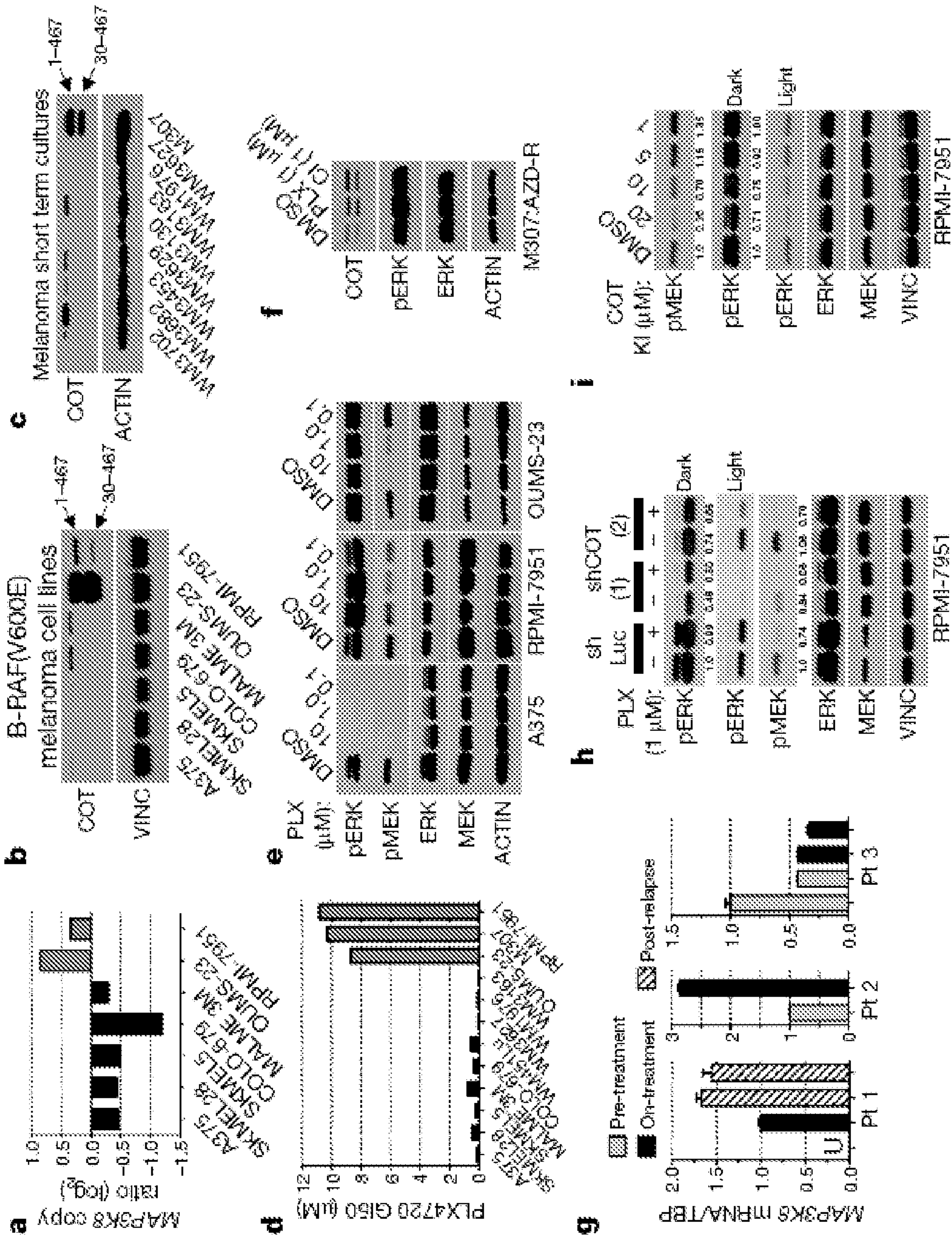
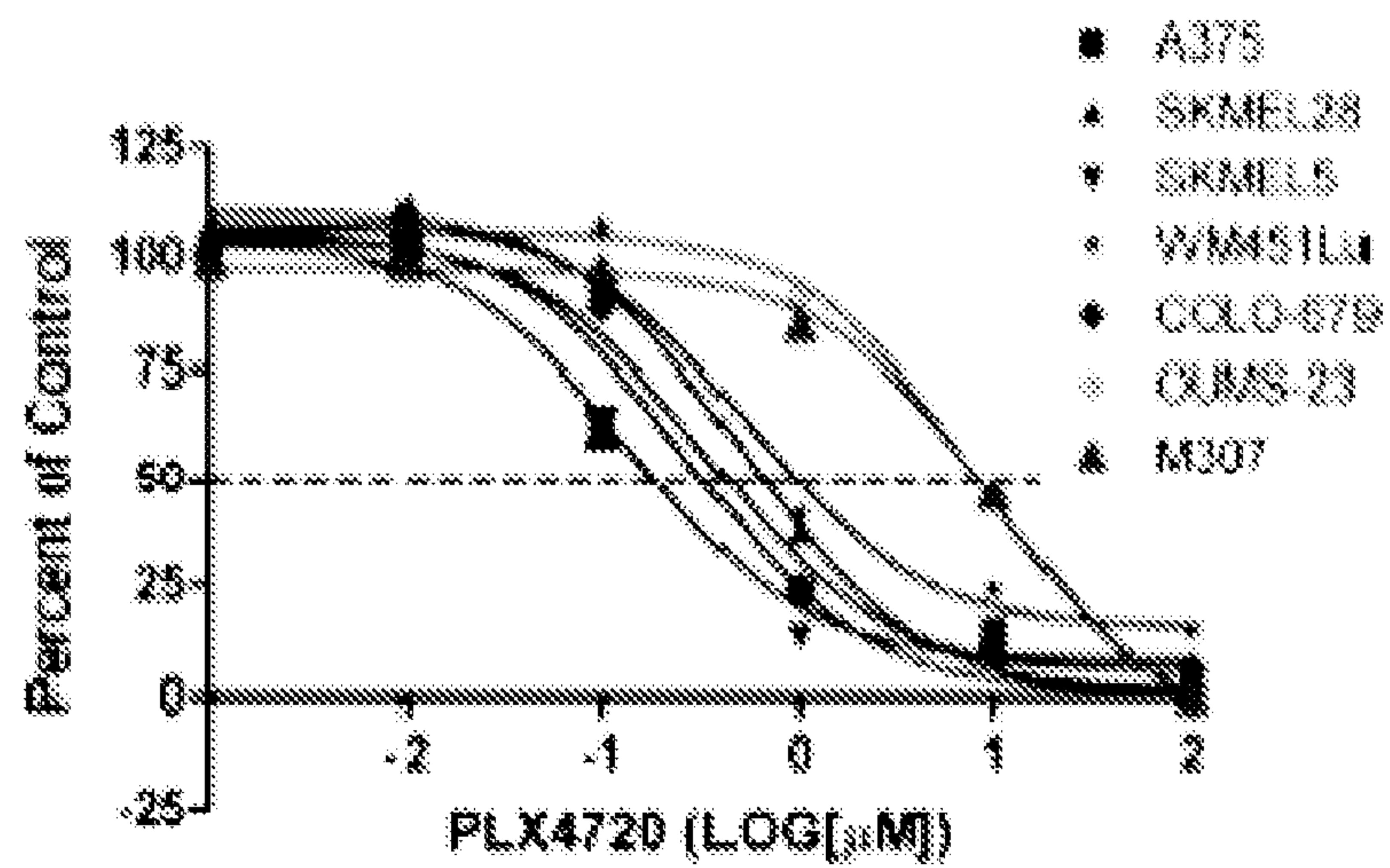


Figure 3

j

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k

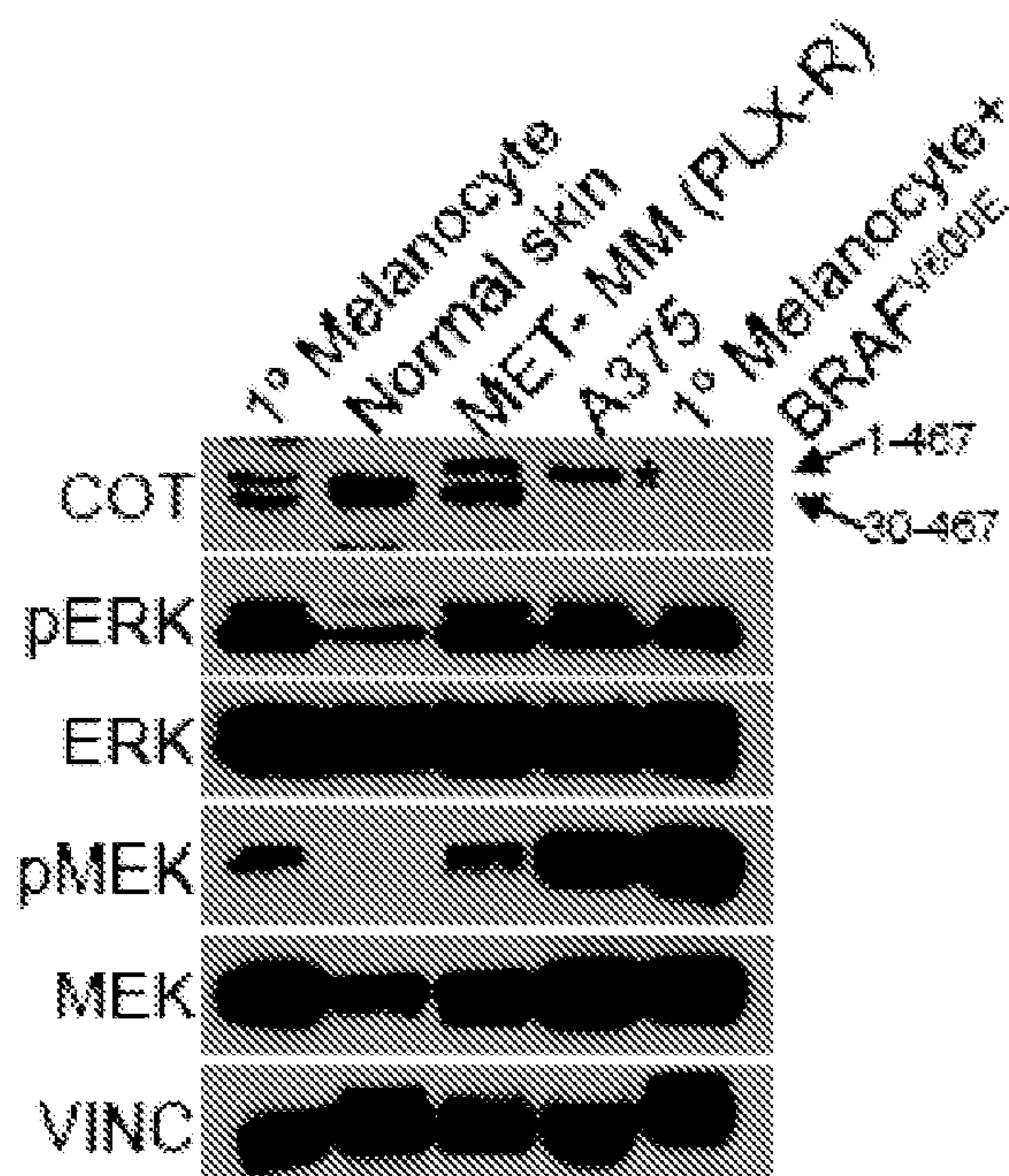


Figure 3

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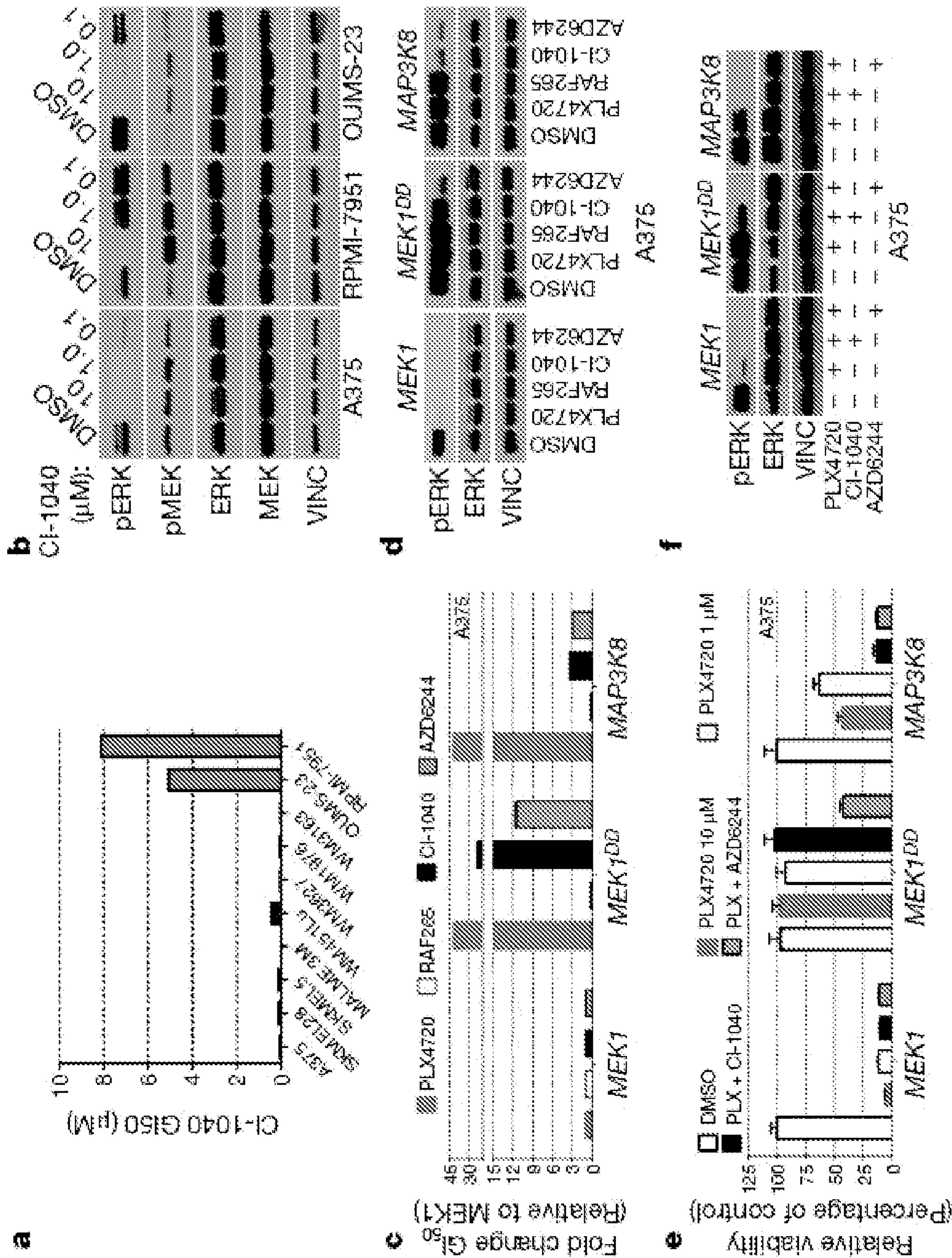
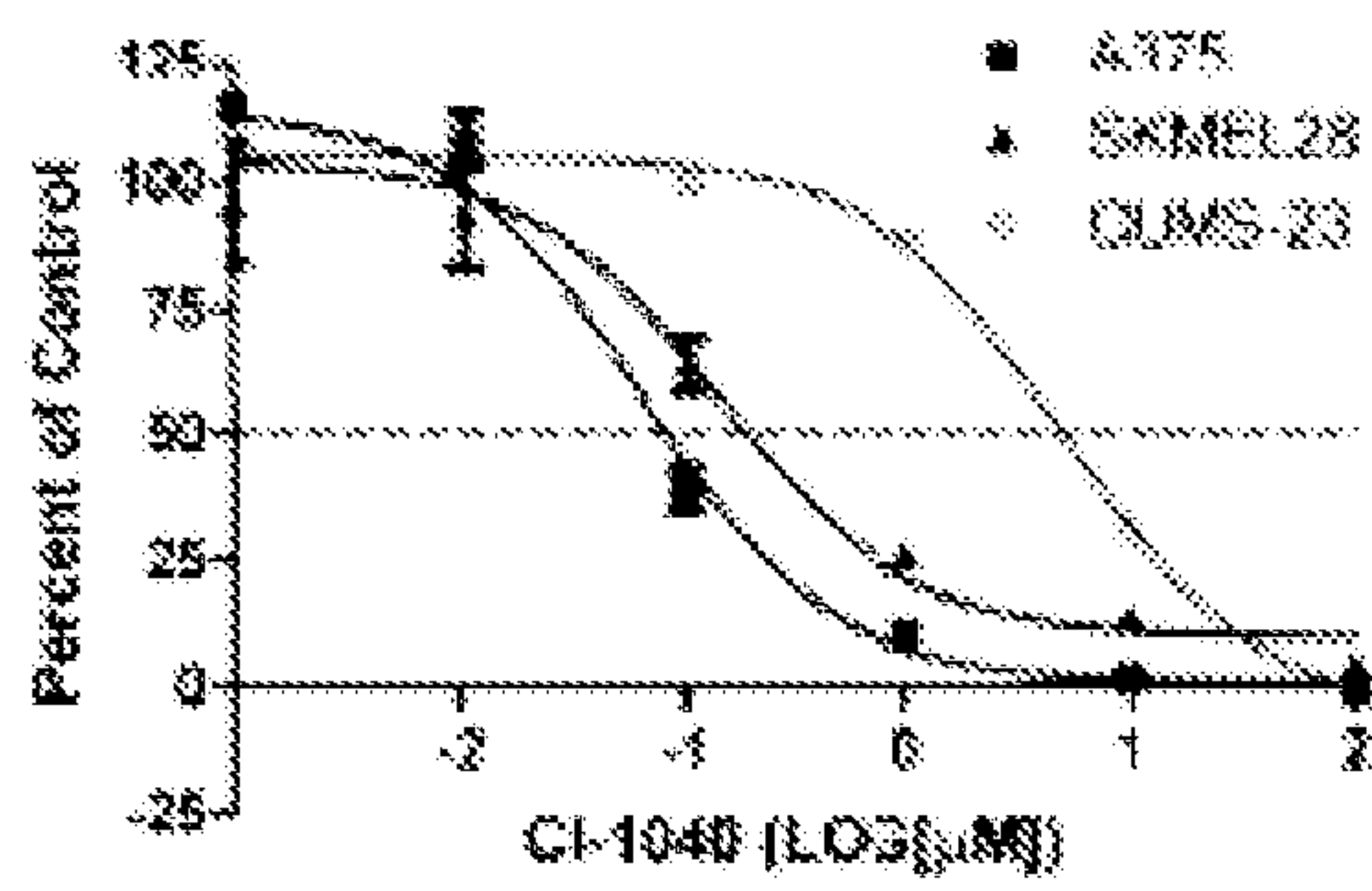


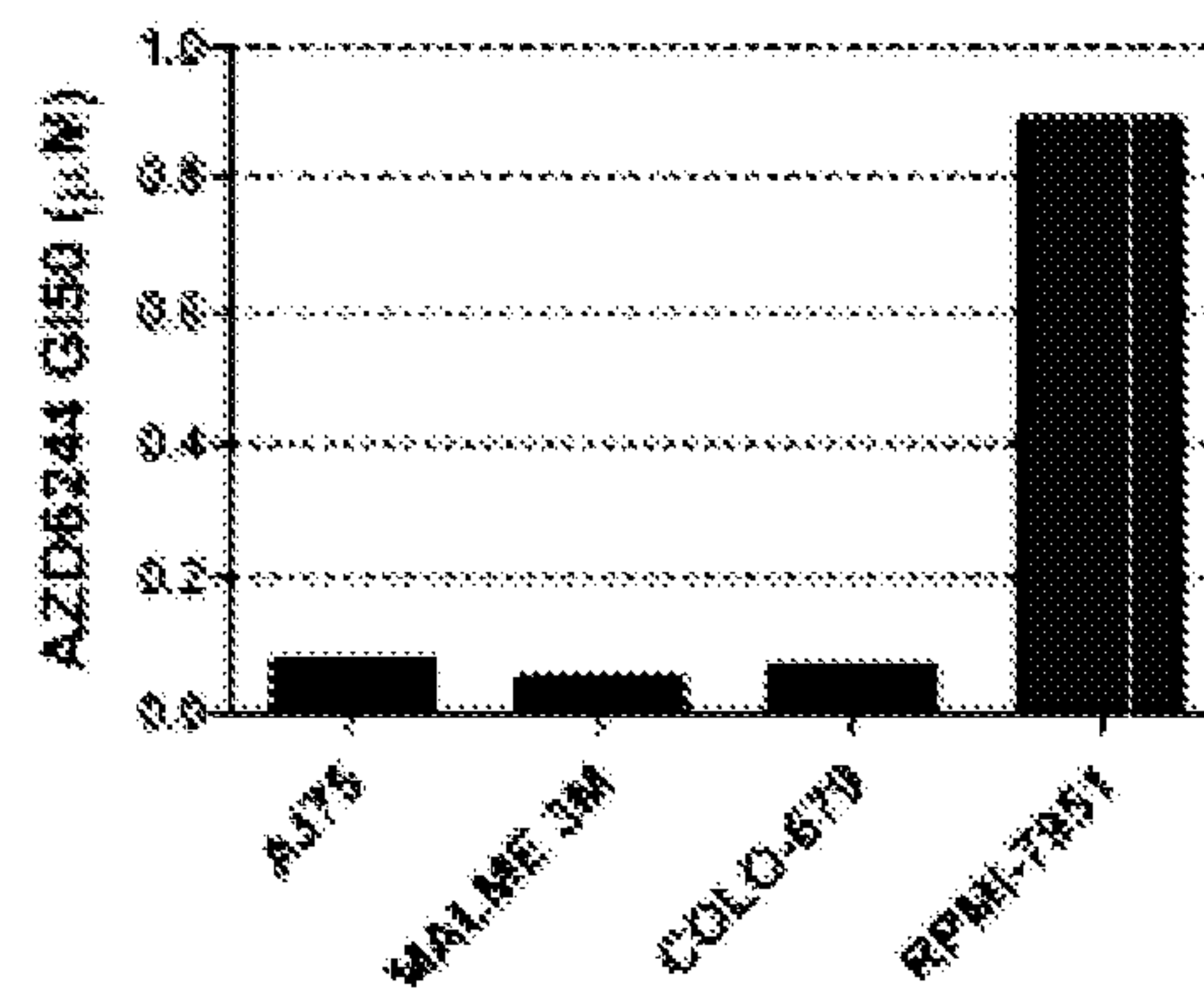
Figure 4

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g



h



i

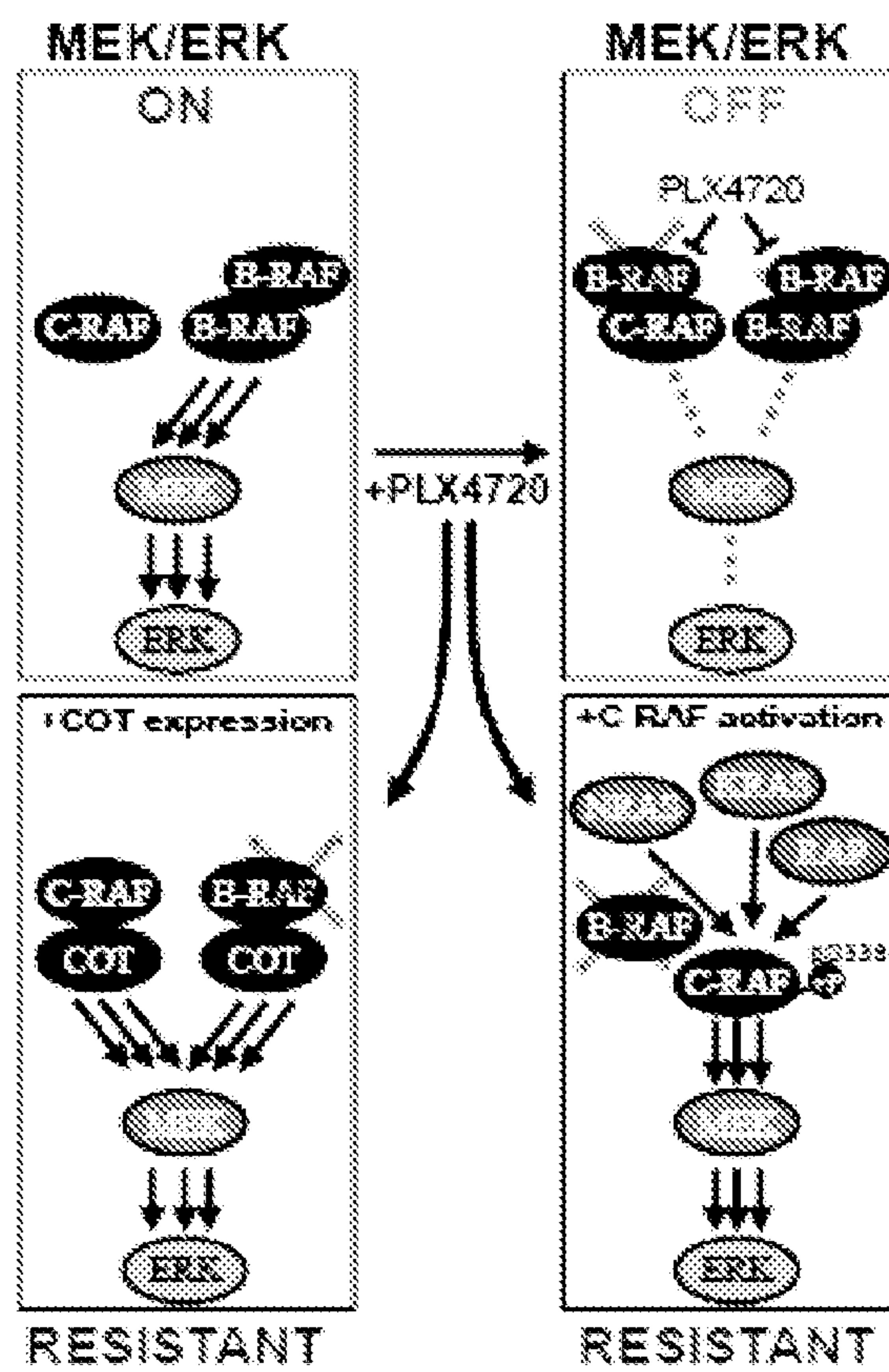


Figure 4

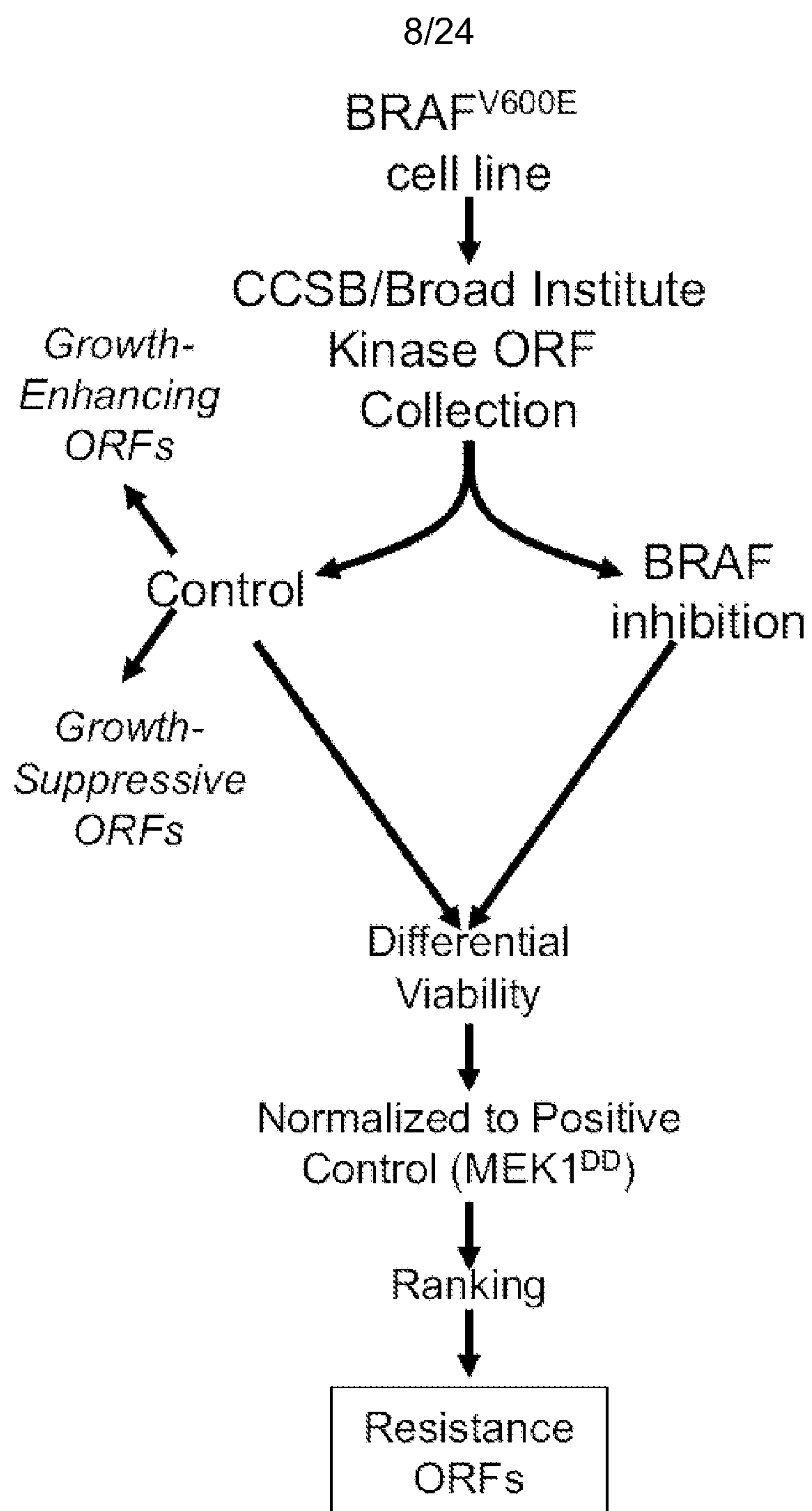


FIG. 5

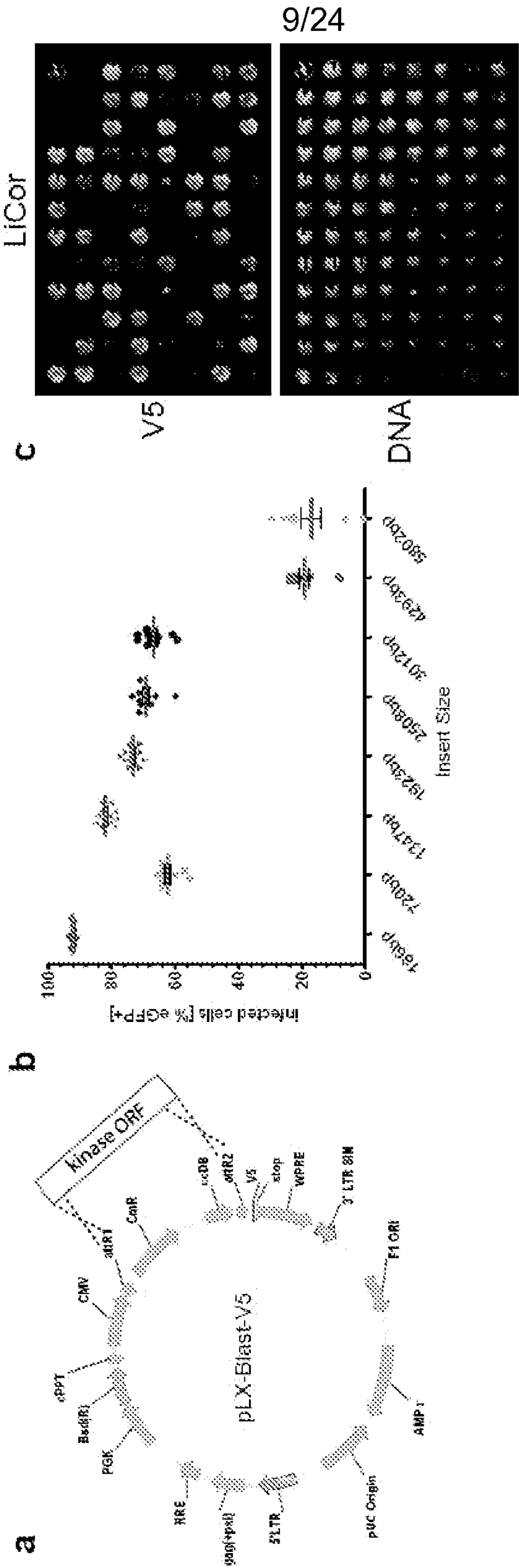


Figure 6

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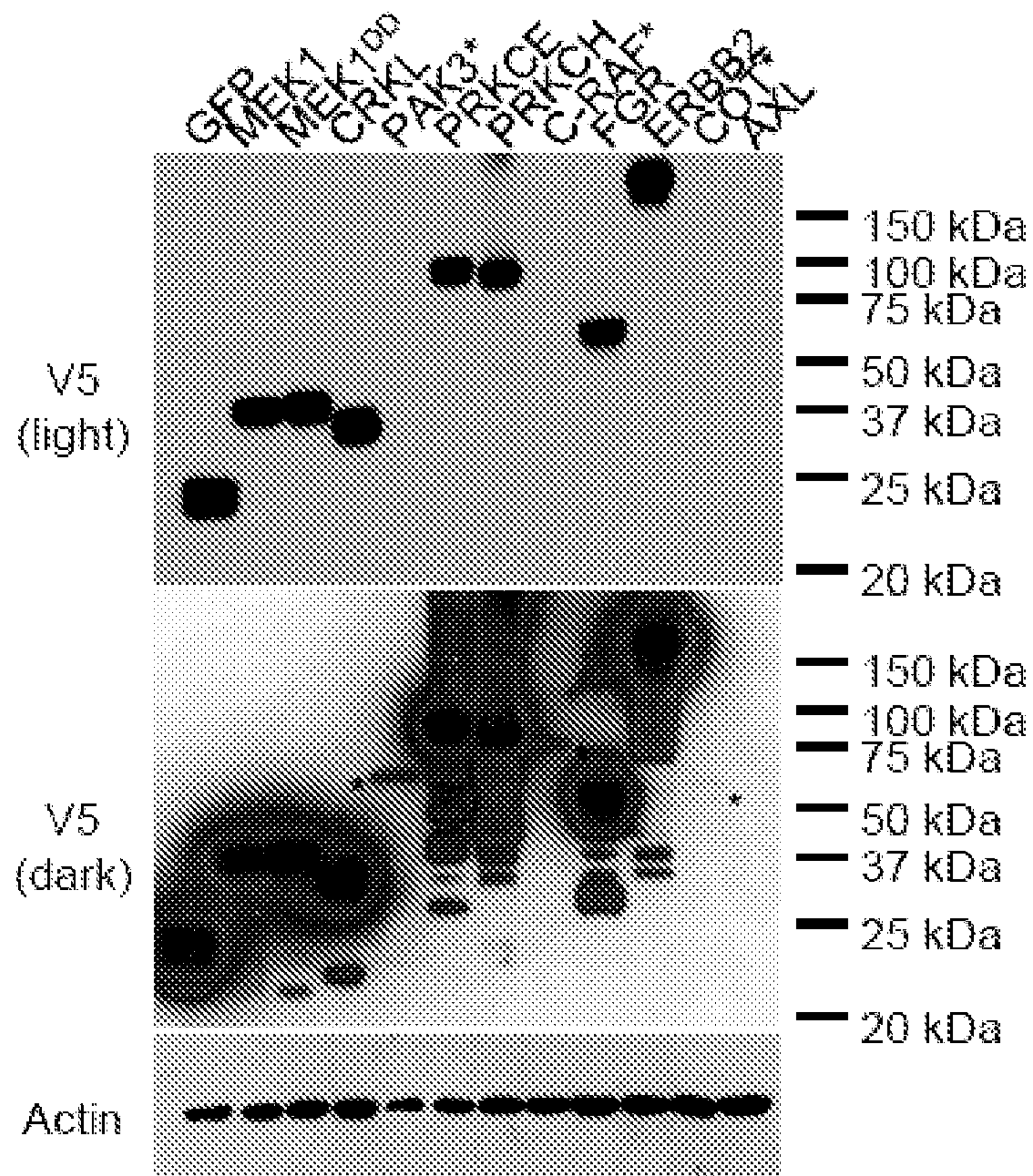


Figure 7

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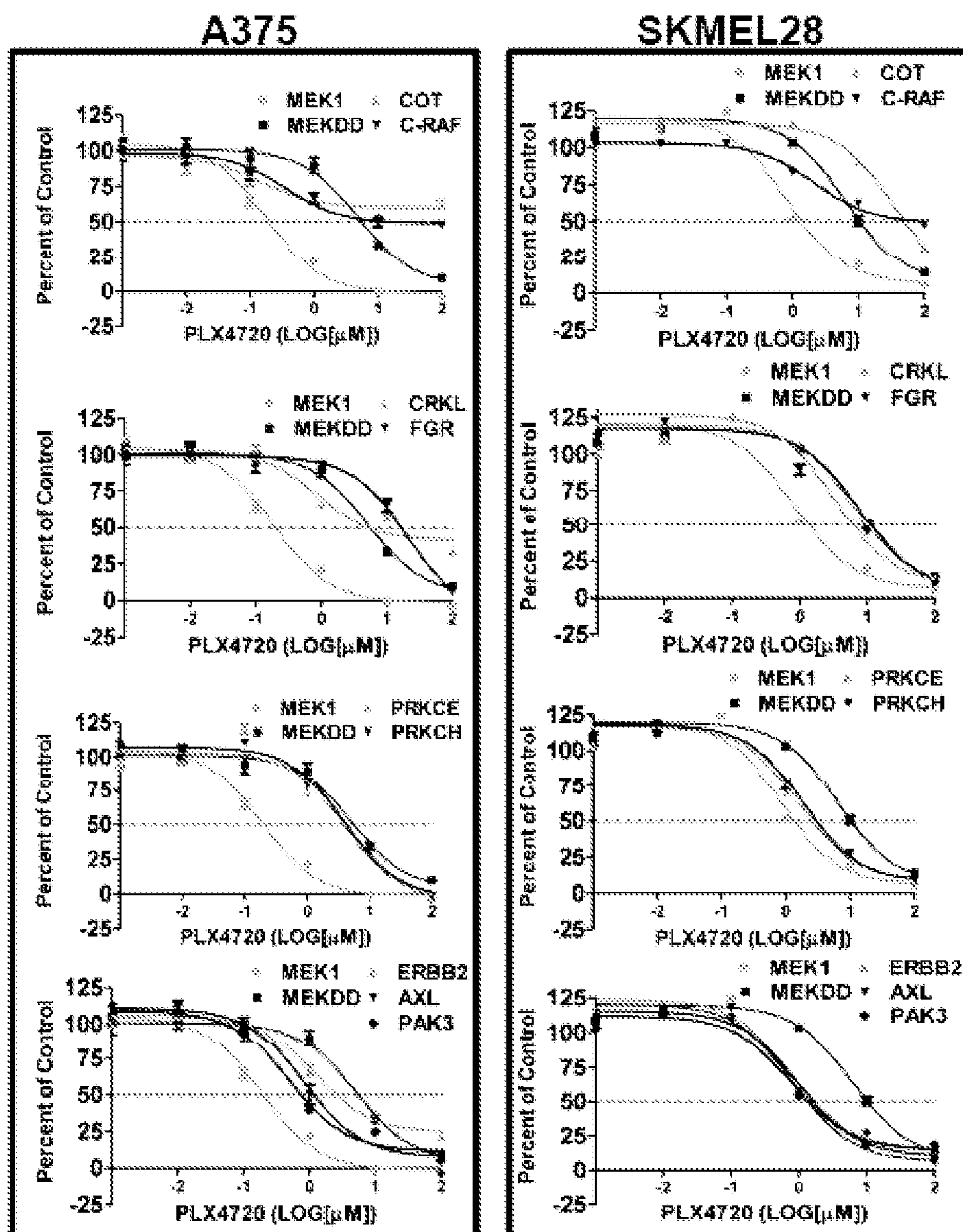


Figure 8

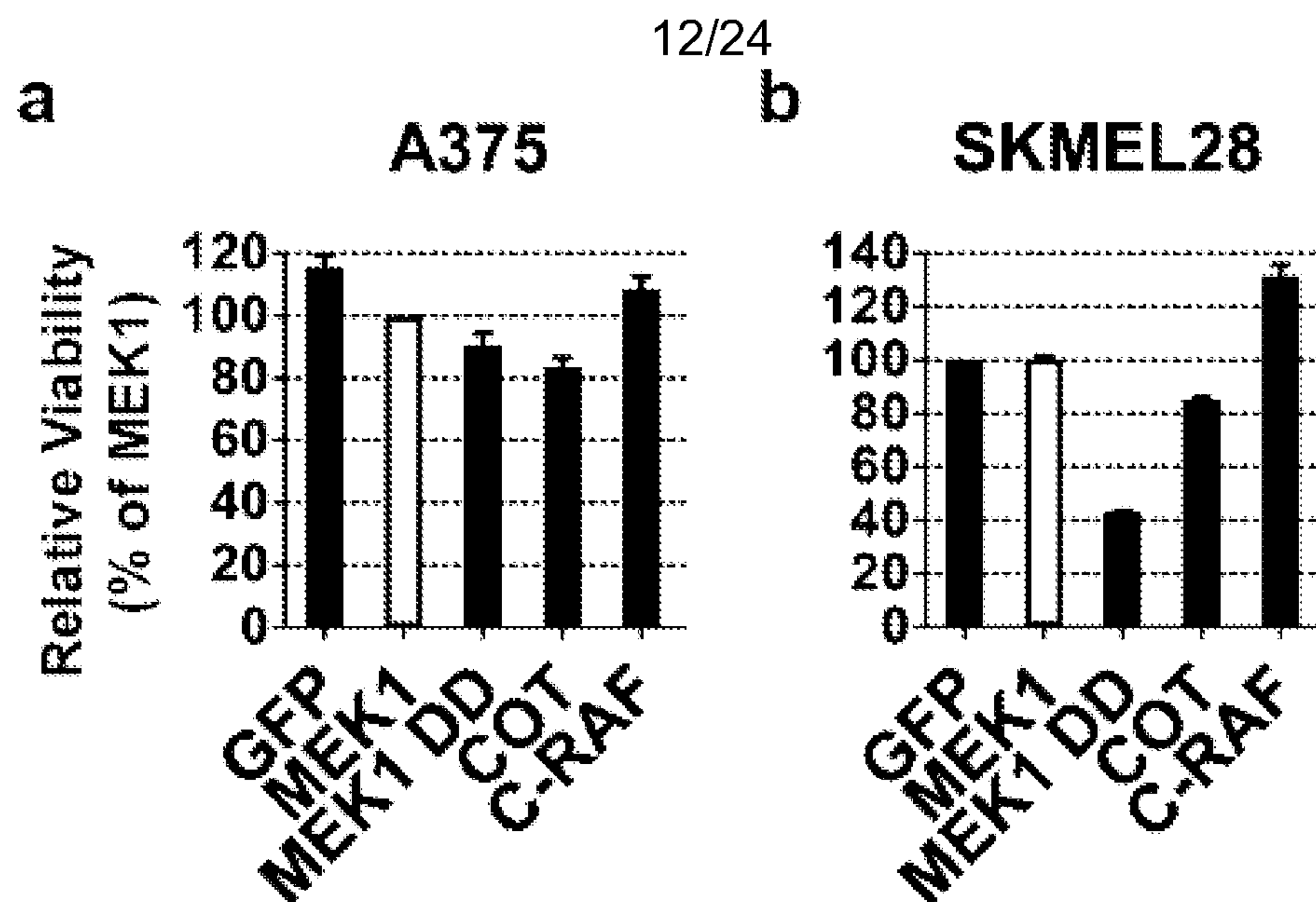


Figure 9

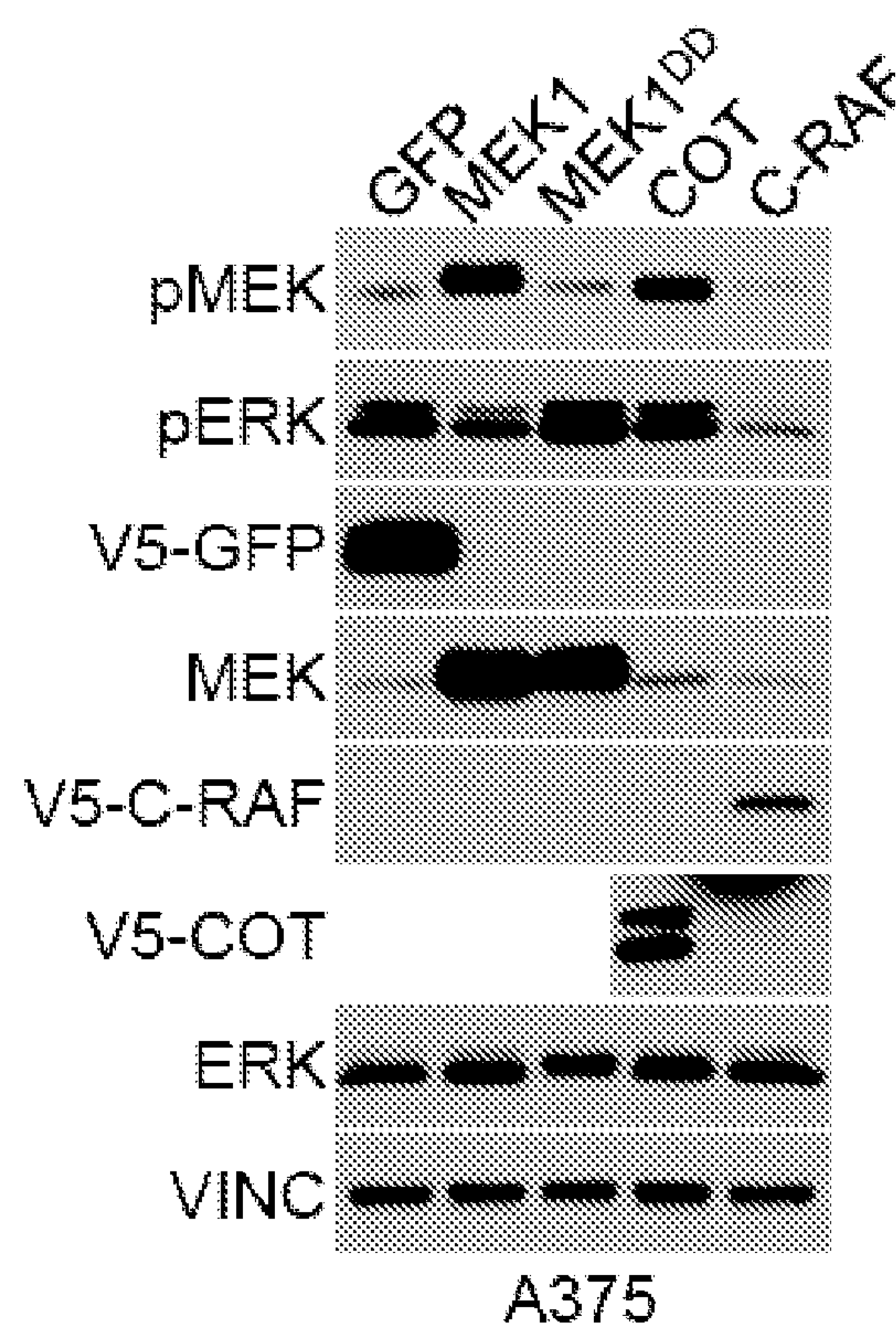


Figure 10

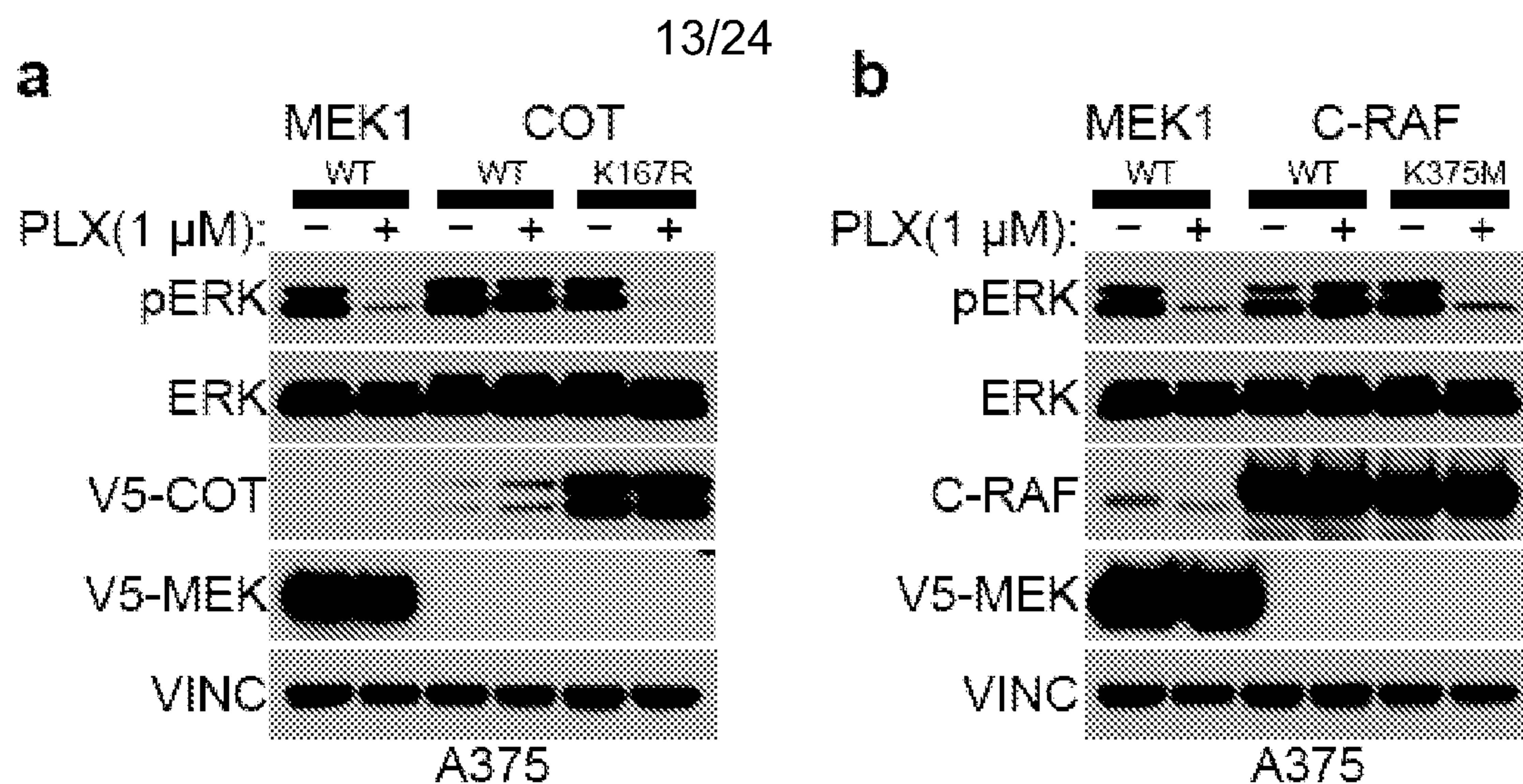


Figure 11

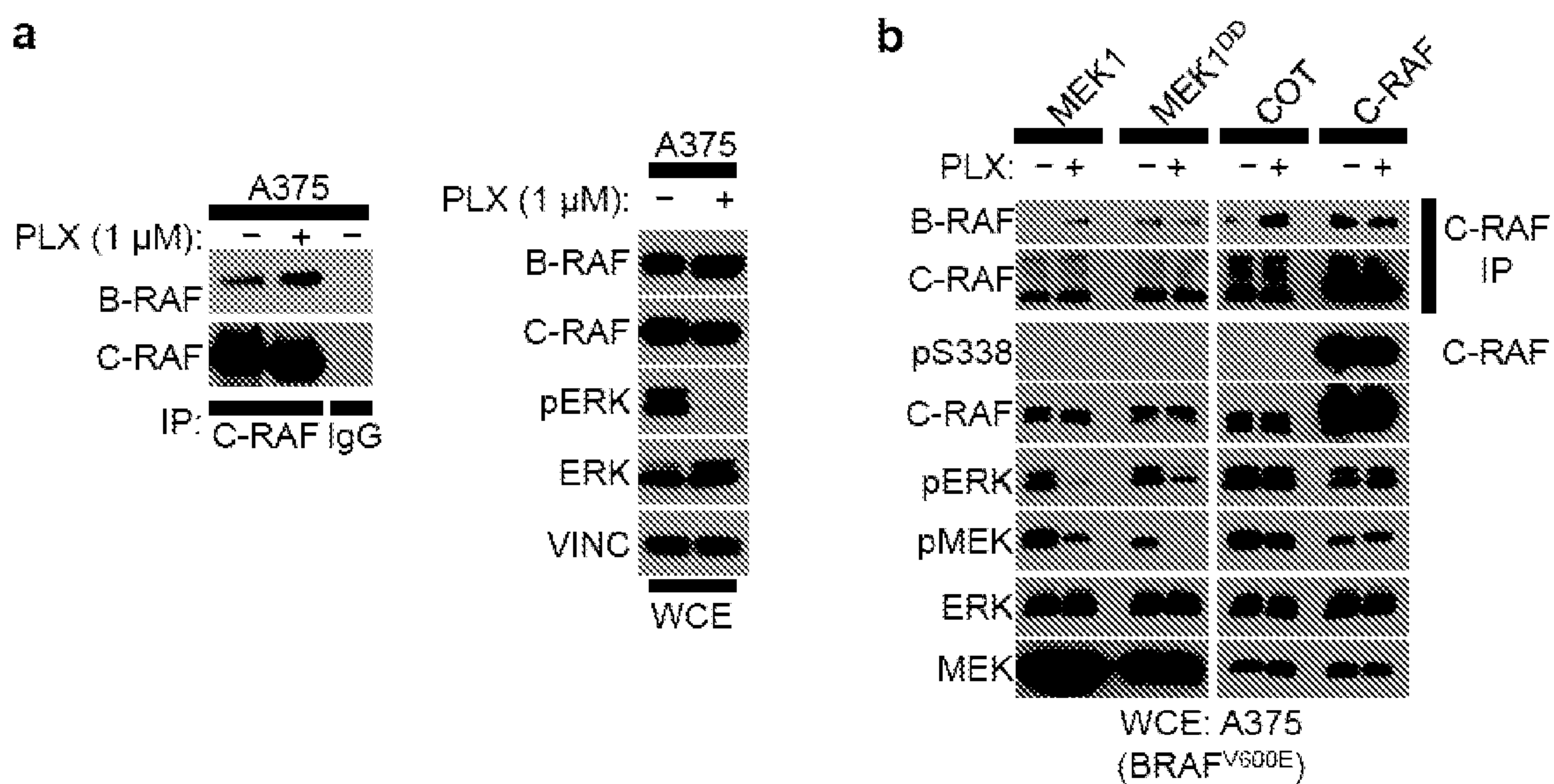


Figure 13

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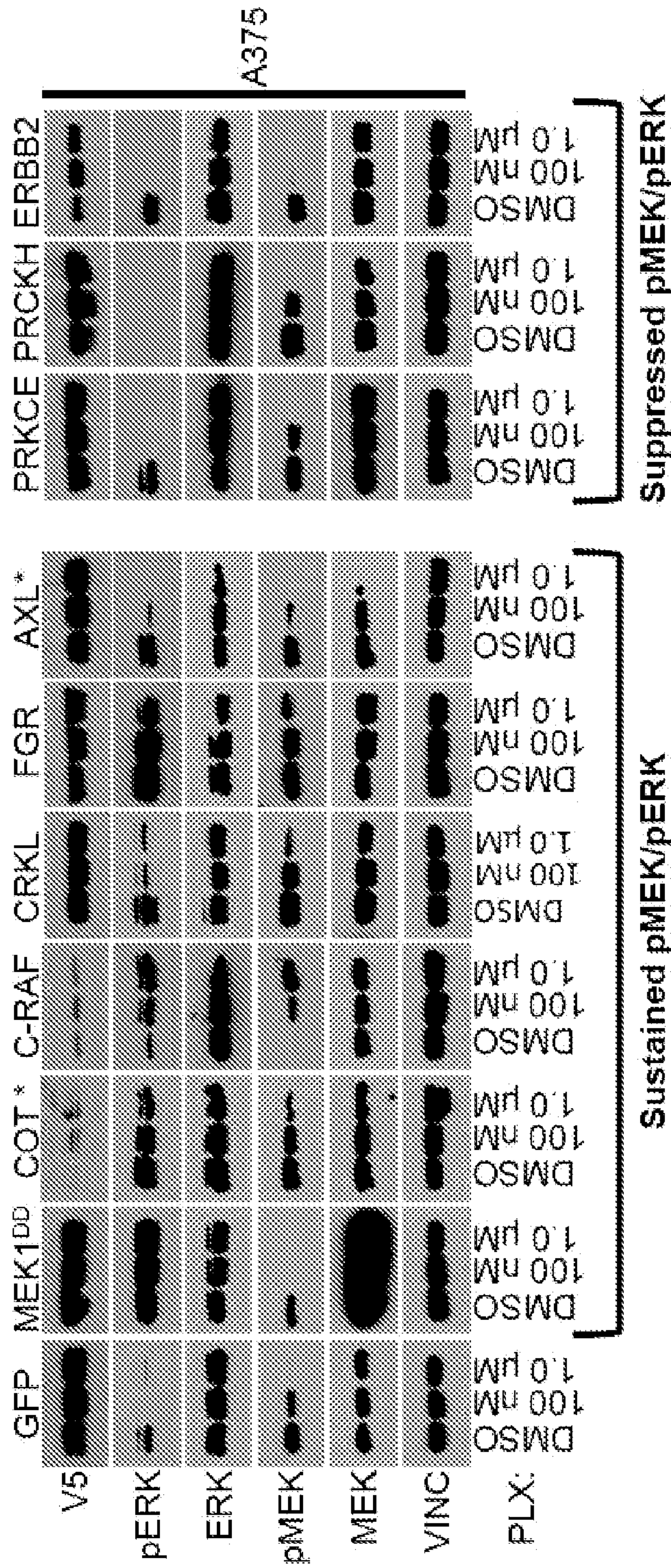


Figure 12

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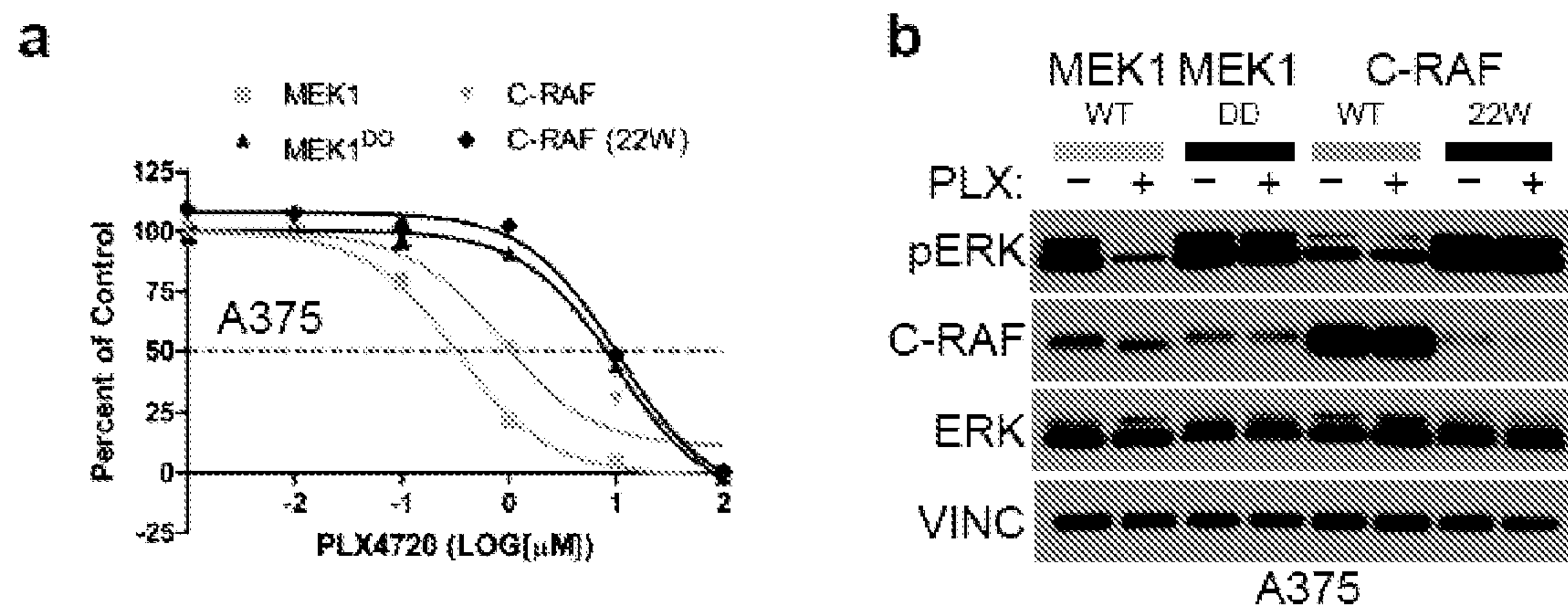


Figure 14

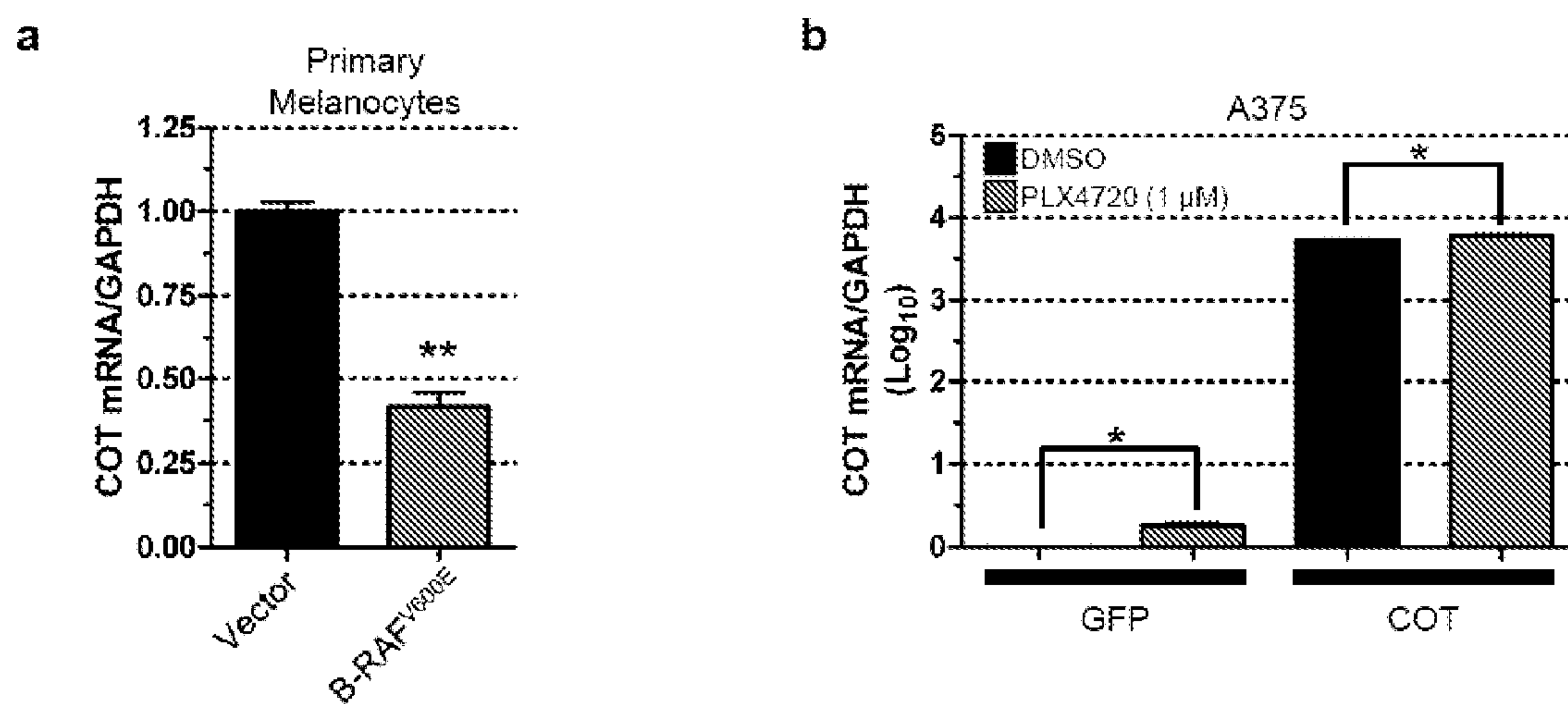


Figure 15

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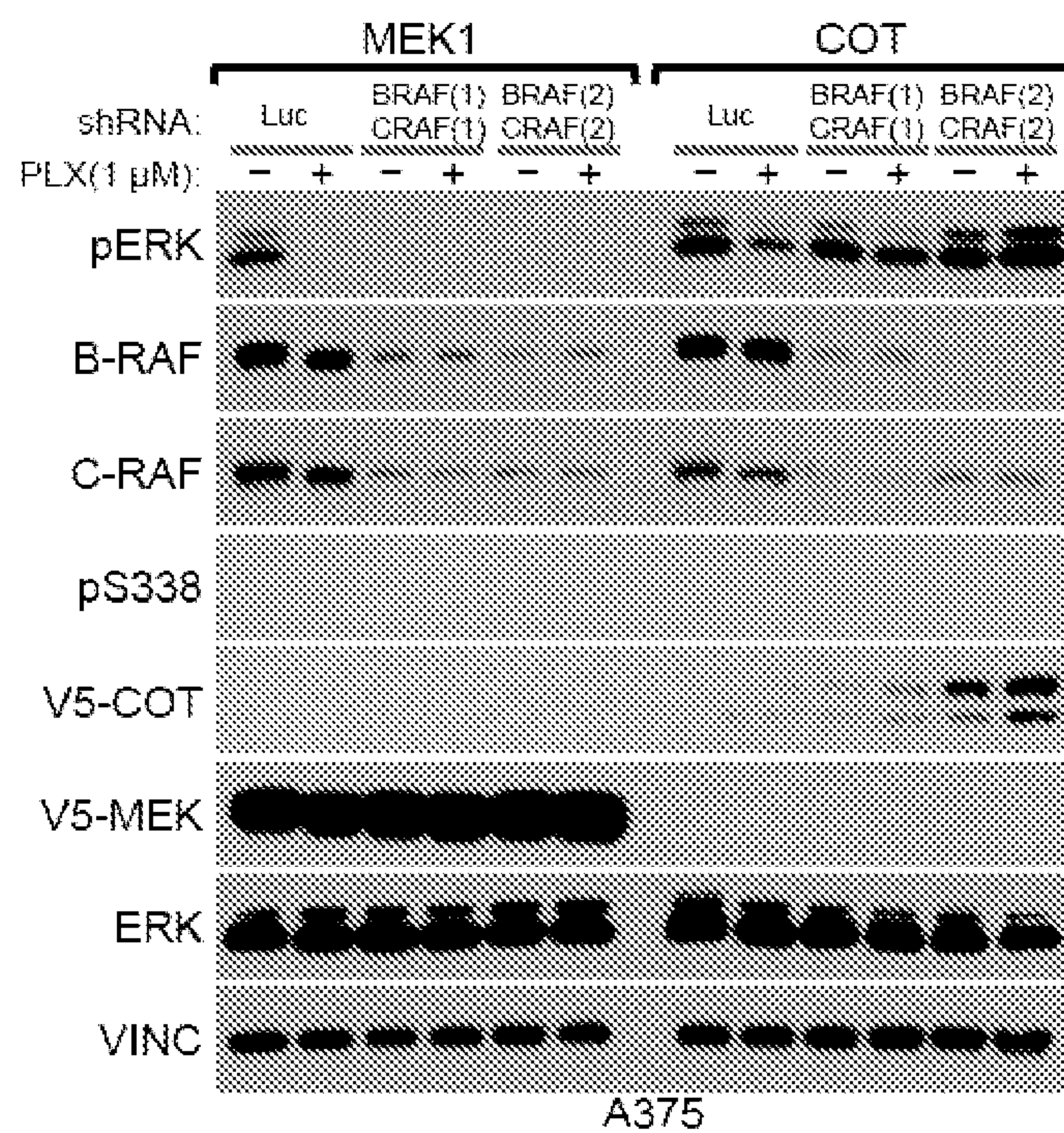


Figure 16

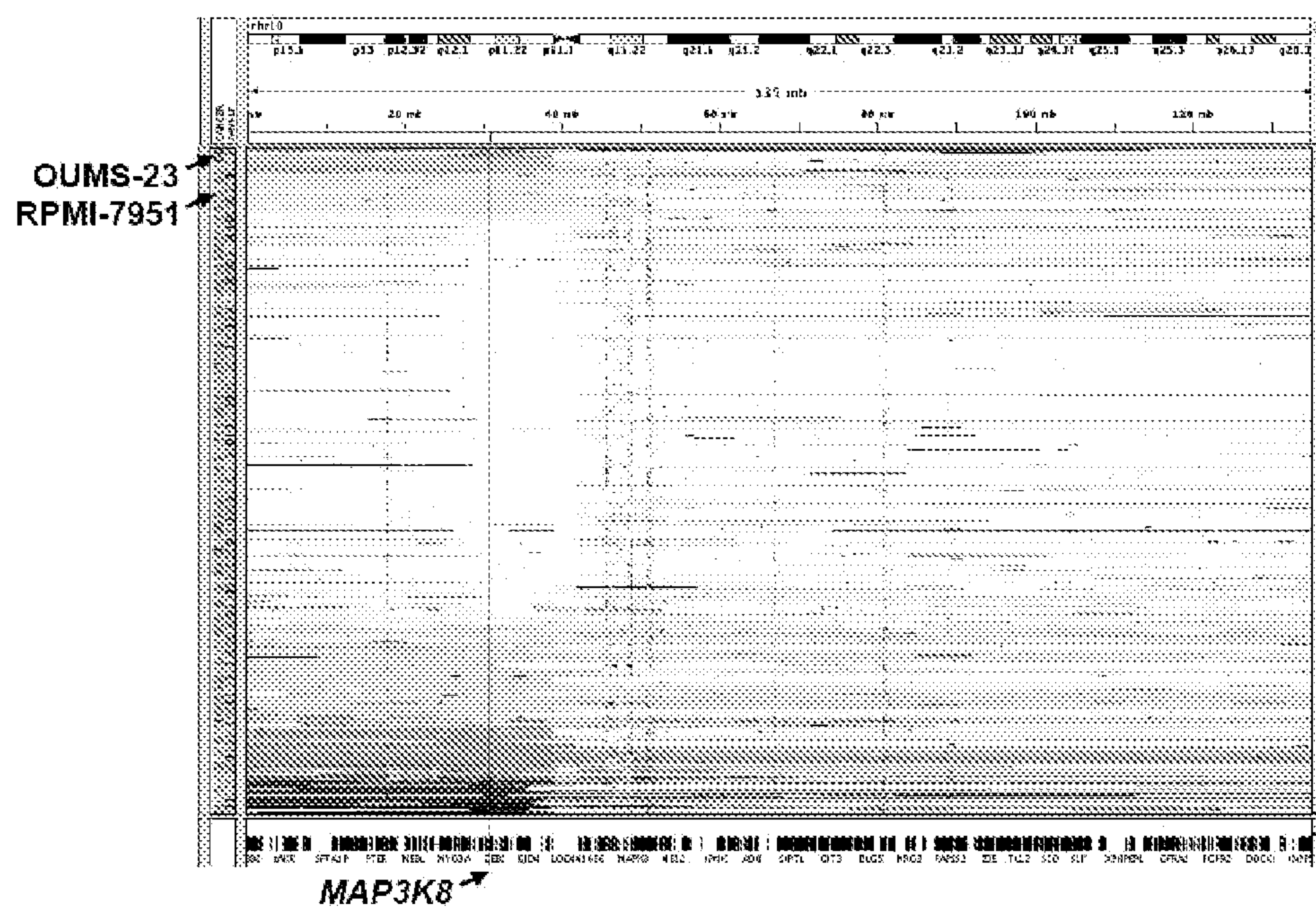
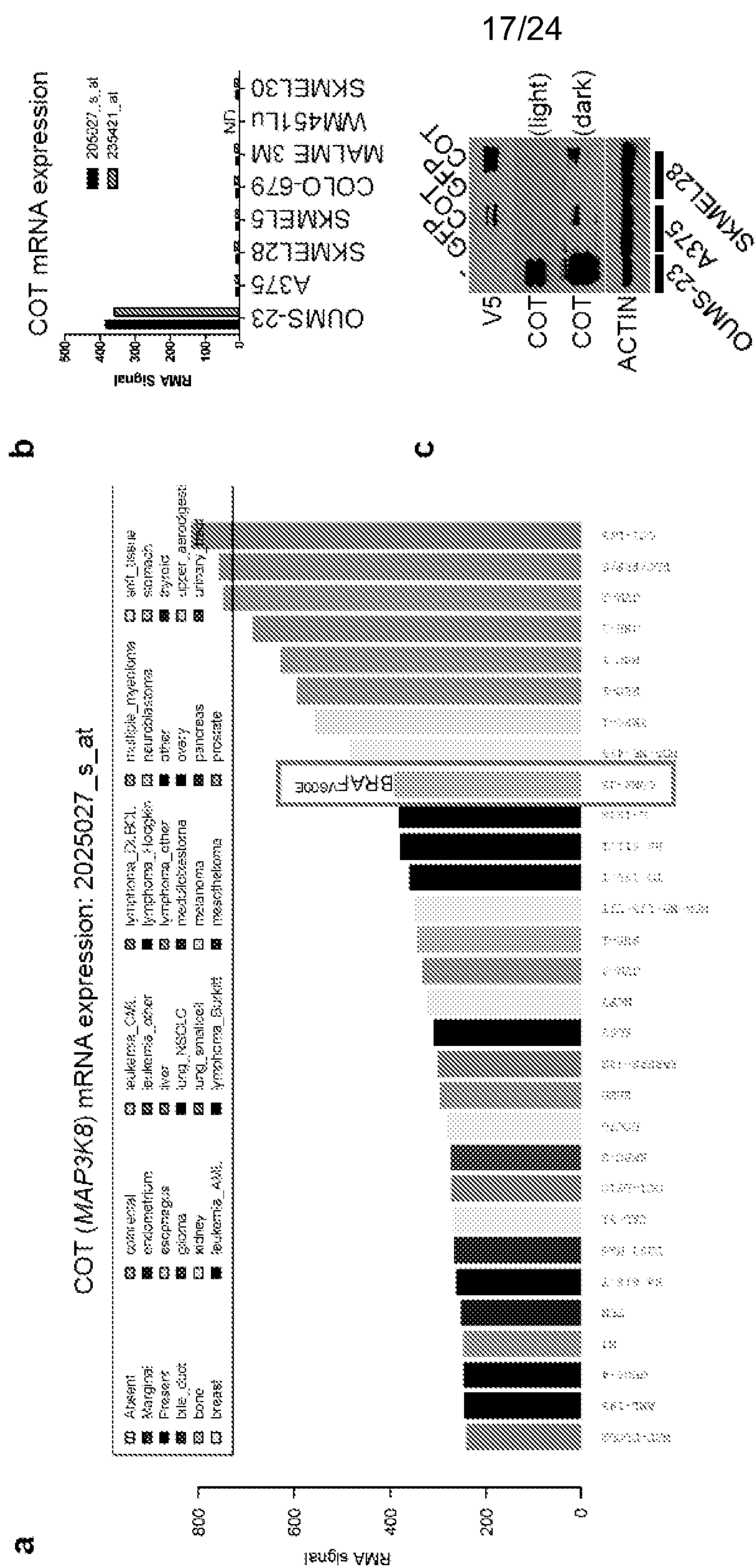


Figure 17



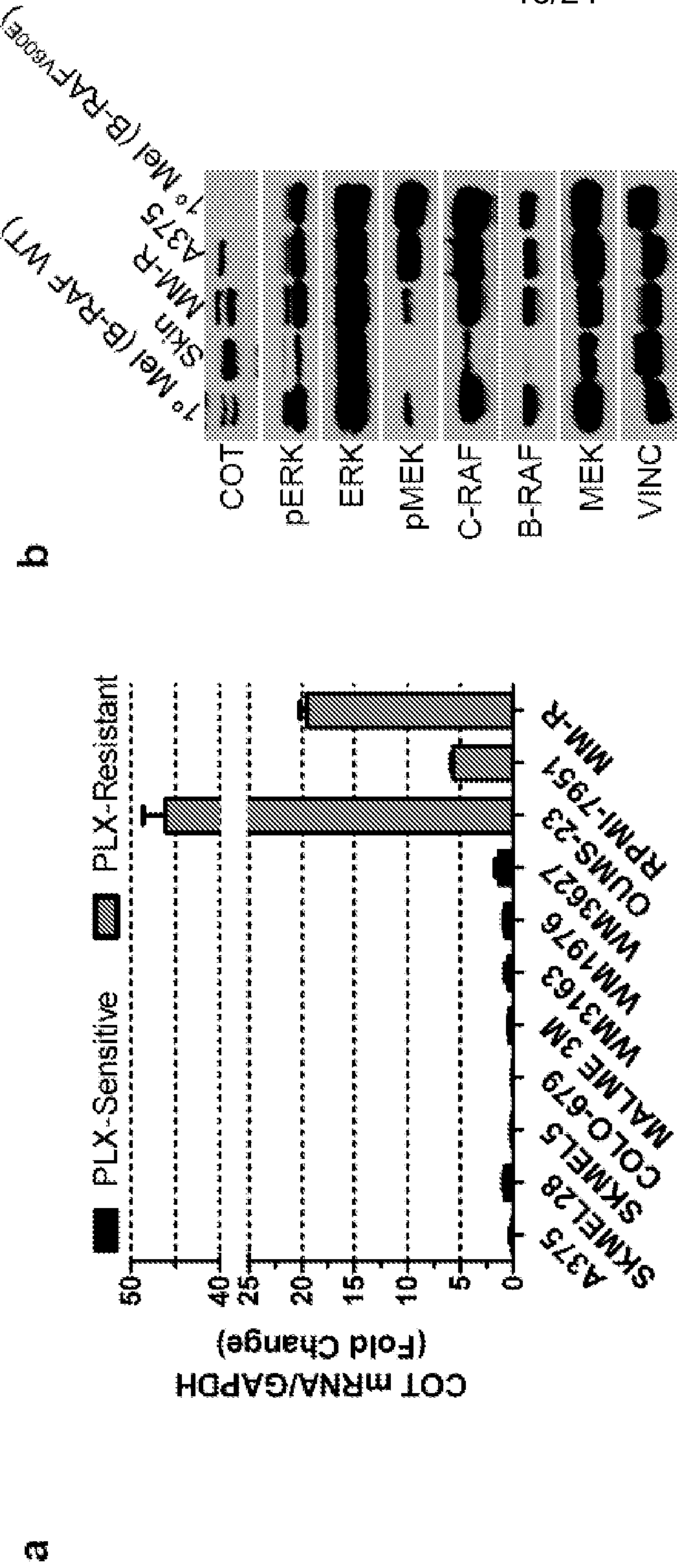


Figure 19

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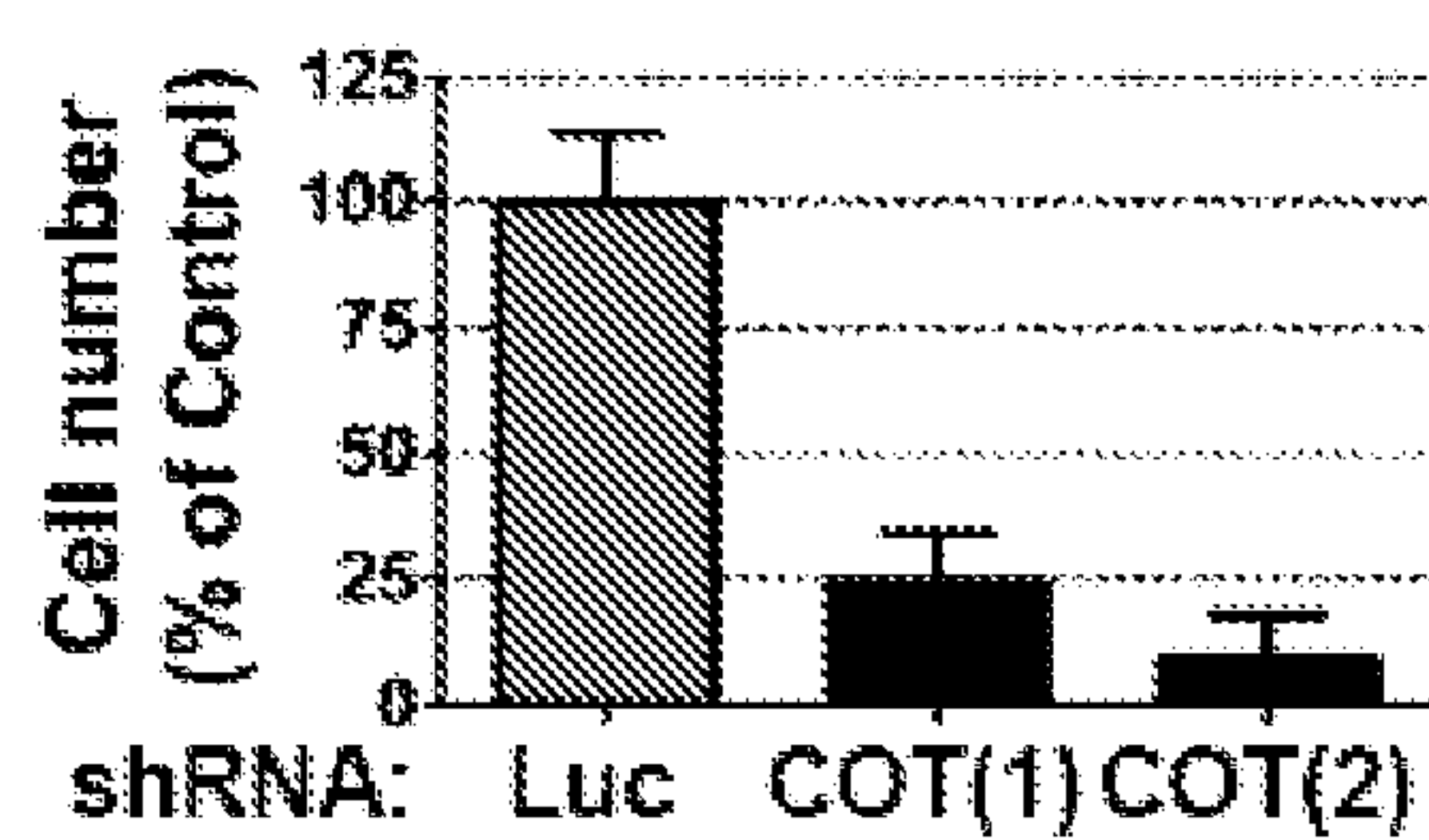
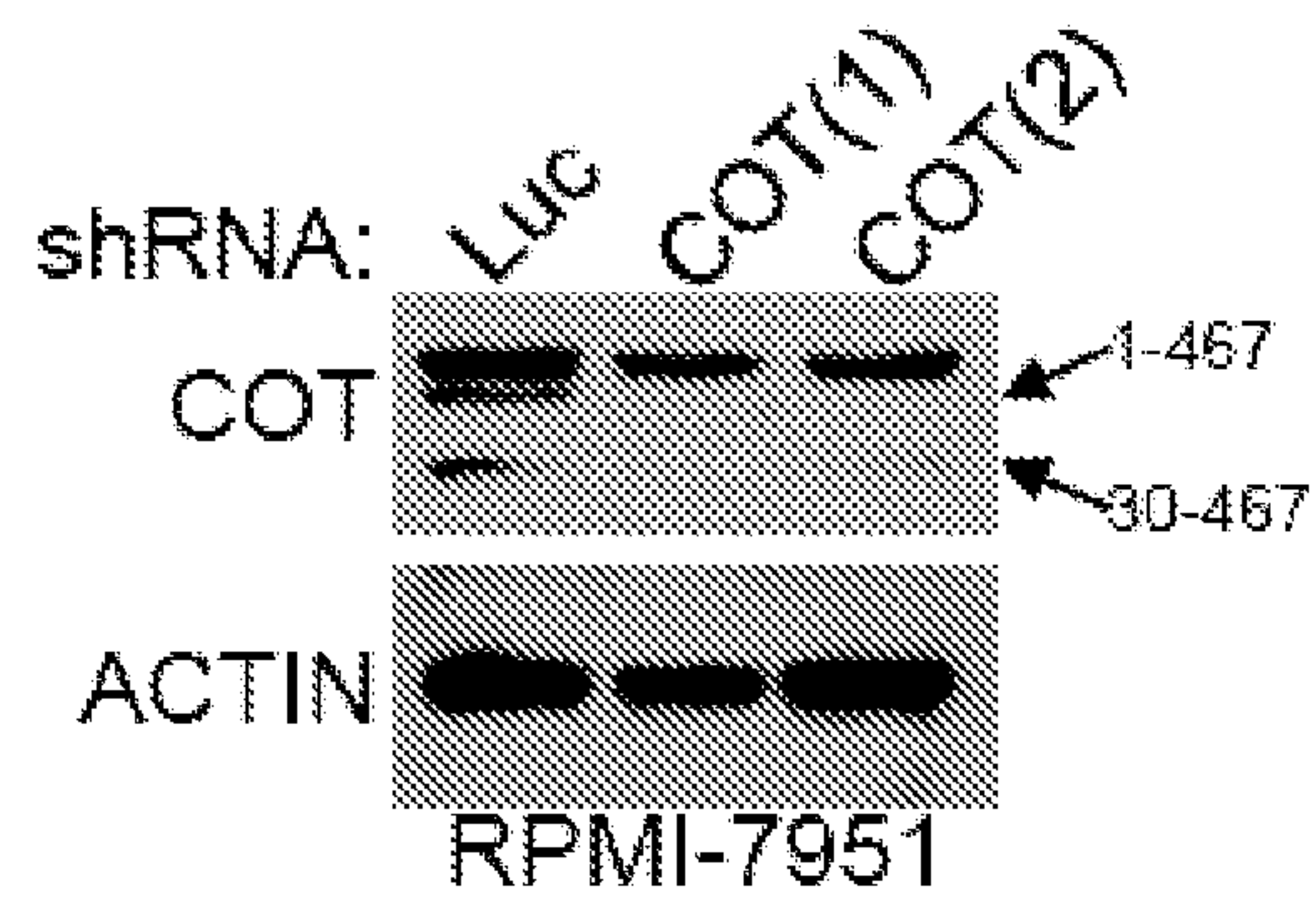
a**b**

Figure 20

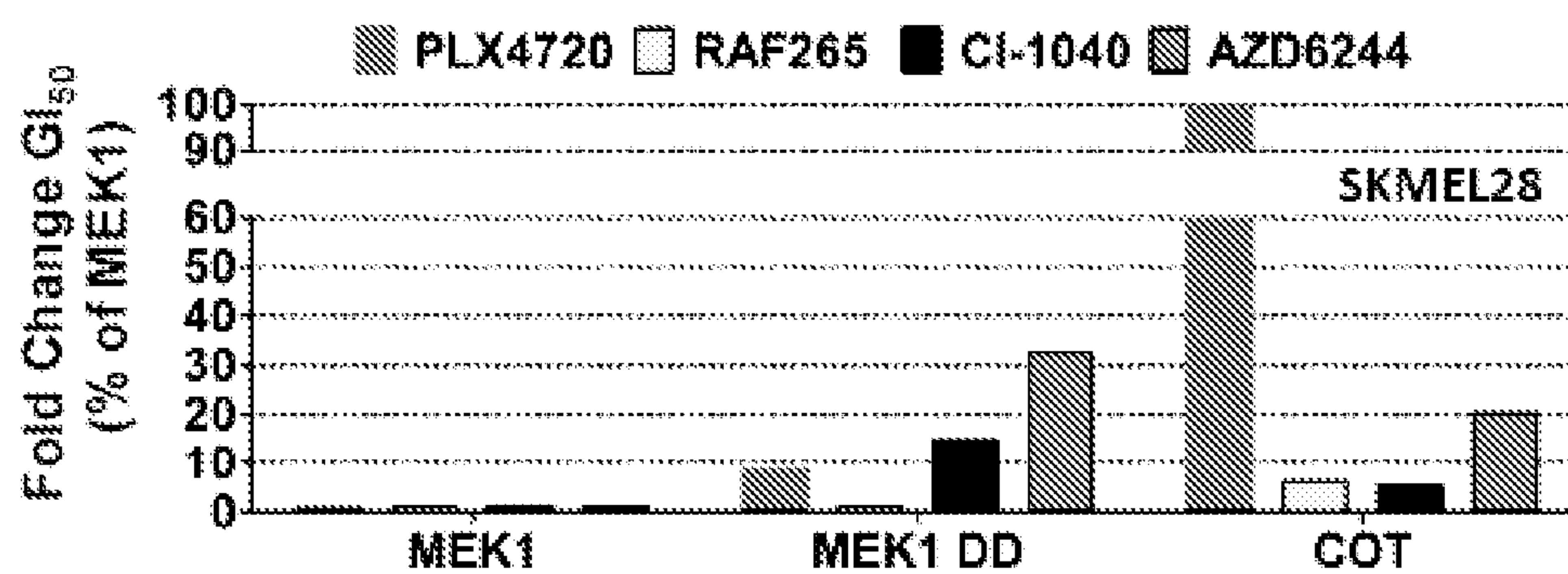


Figure 21

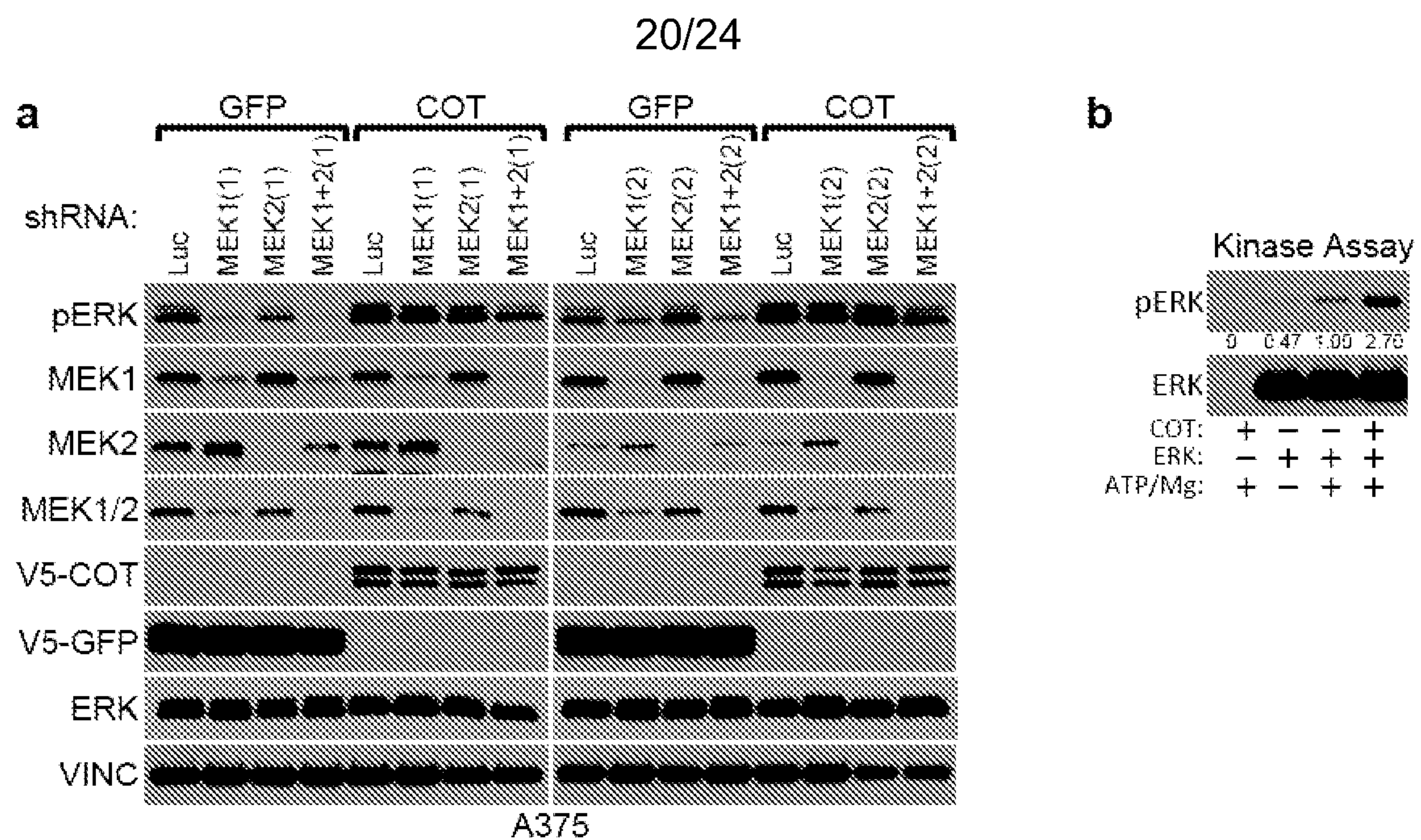


Figure 22

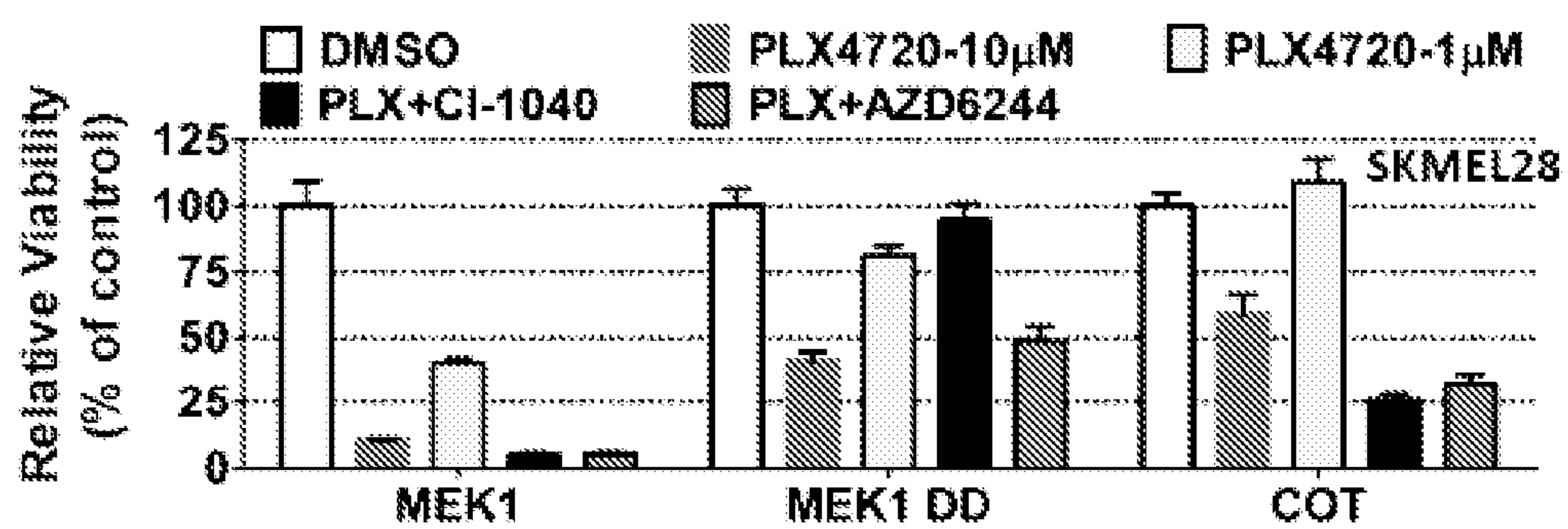


Figure 23

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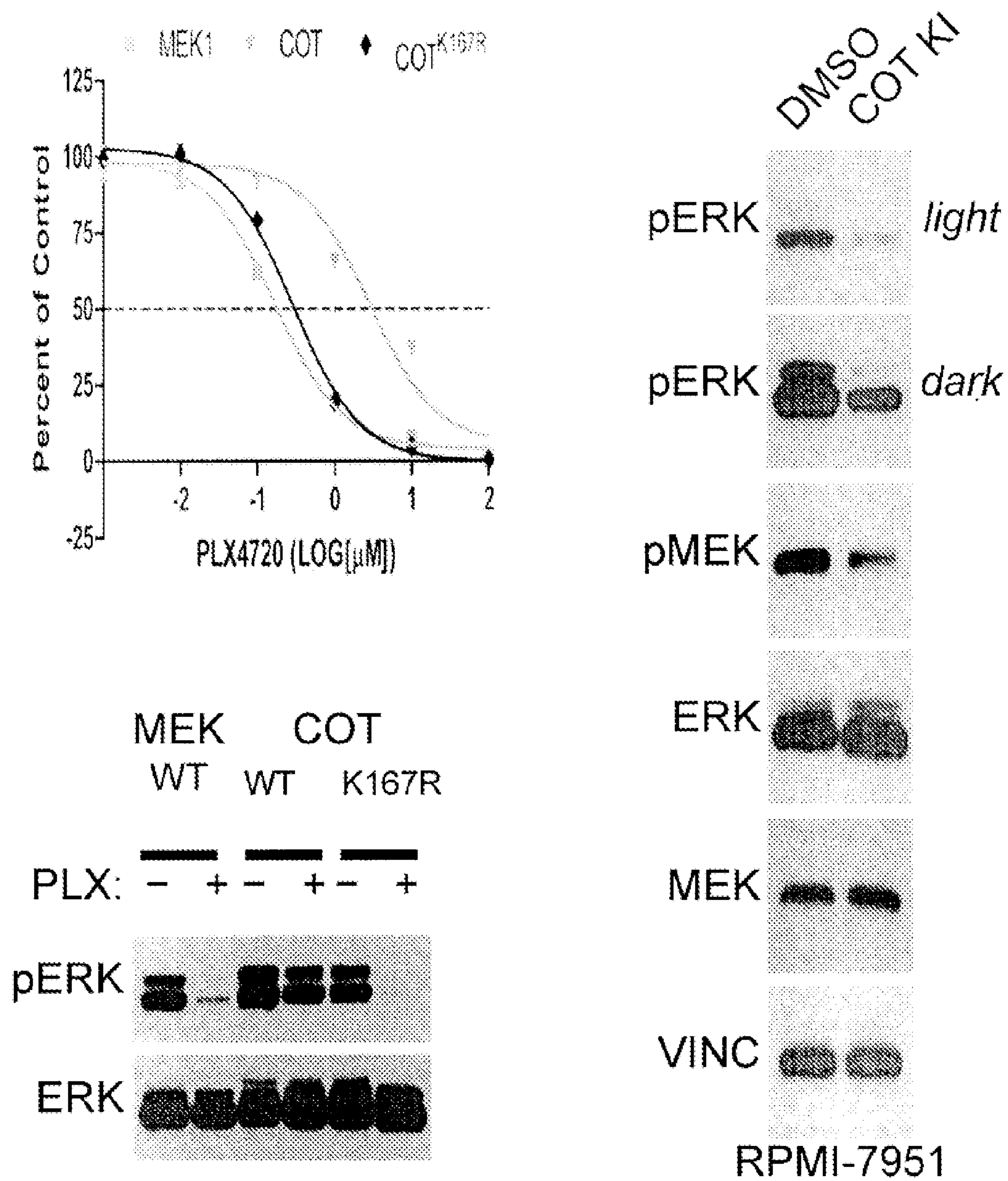


Figure 24

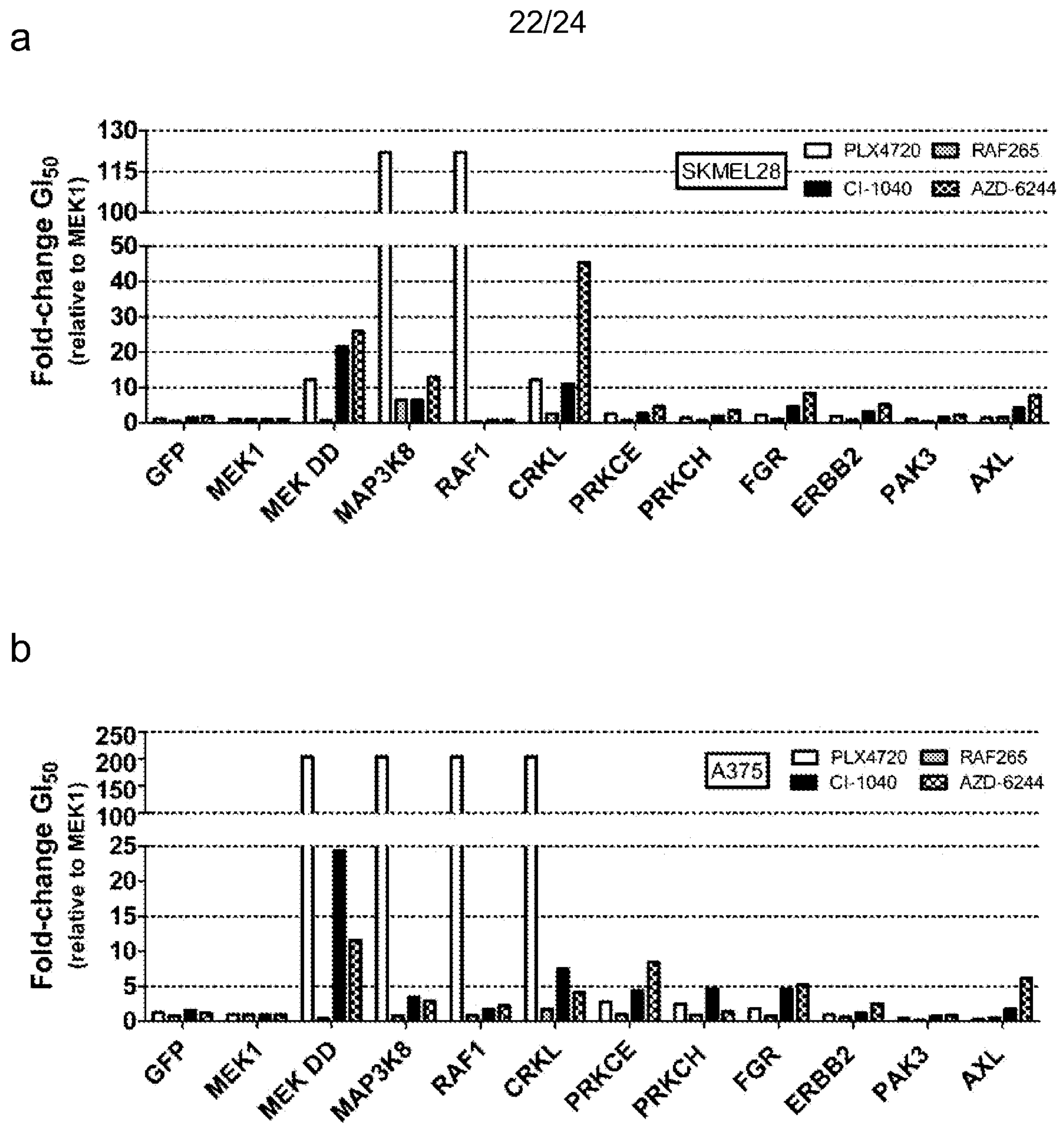


Figure 25

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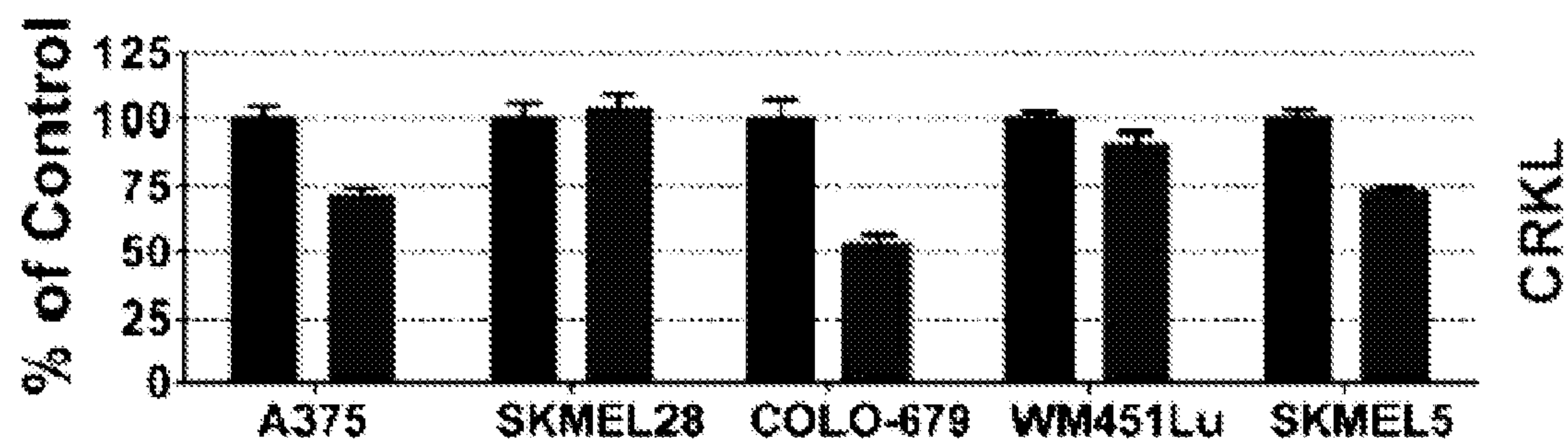


Figure 26

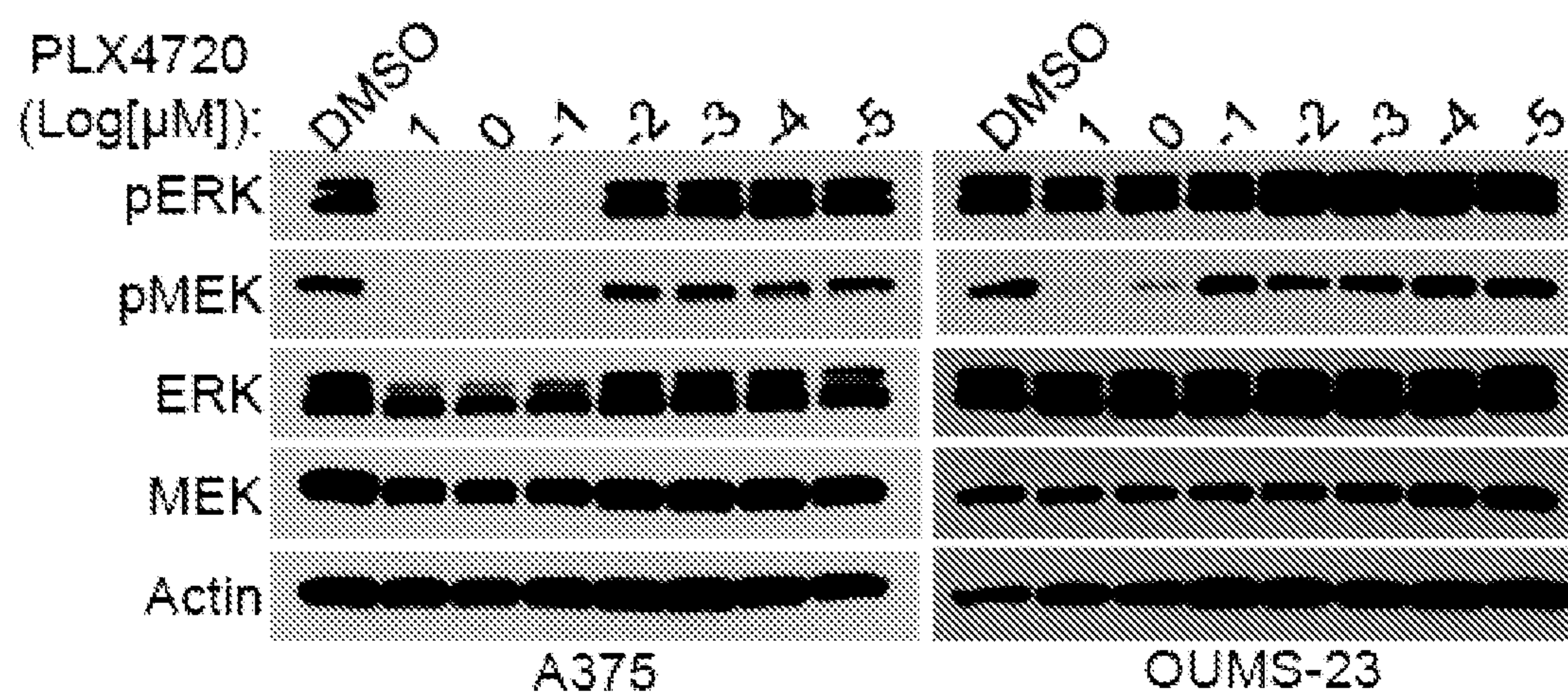


Figure 27

b

Cell line name	BRAF mutational status	AZD8234 sensitivity	Fold sensitivity (rel. to A375)
UACC-62	V600E	0.0243356	0.120
Melme-3M	V600E	0.0534783	0.325
COLO-679	V600E	0.074301	0.354
SK-MEL-5	V600E	0.0748311	0.329
MEL-HO	V600E	0.0847348	0.338
A-375	V600E	0.0874318	0.402
WM-286-4	V600D	0.1284449	0.638
UACC-257	V600E	0.145148	0.724
K129AX	V600E, V600D	0.154652	0.745
3ER-37	V600E	0.1655719	0.816
WM-115	V600D	0.1673357	0.825
LOX IMVI	V600E	0.1731772	0.843
C361	V600E	0.3270947	1.593
Hs 294T	V600E	0.3424192	1.638
A2059	V600E	0.3455313	1.739
Hs 595T	V600E	0.5116367	2.557
SK-MEL-24	V600E	0.6527371	3.357
C32	V600E	0.9826231	4.846
3ER-38	V600E	2.6921518	13.375

a

Cell line name	BRAF mutational status	PLX4720 sensitivity	Fold sensitivity (rel. to A375)
UACC-62	V600E	0.17553526	0.886
A-375	V600E	0.20219493	1.000
SK-MEL-5	V600E	0.21298643	1.050
MEL-HO	V600E	0.25852974	1.275
Melme-3M	V600E	0.27633443	1.352
K029AX	V600E, V600D	0.34965807	1.724
3ER-37	V600E	0.4014448	1.990
COLO-679	V600E	0.4489202	2.219
UACC-257	V600E	0.5370857	3.142
A2059	V600E	0.69652145	3.445
C-361	V600E	0.92425234	4.558
WM-115	V600D	1.0702045	5.217
WM-286-4	V600D	1.2738535	6.301
LOX IMVI	V600E	1.3171776	6.495
C32	V600E	1.6075206	7.927
Hs 294T	V600E	2.3284001	11.462
3ER-38	V600E	2.5939914	12.791
Hs 595T	V600E	2.851989	14.463
SK-MEL-24	V600E	1	38.443

Figure 28

