METHODS

Cell culture, lentiviral constructs and infections. All cell lines were maintained in DMEM with 10% or 20% (short-term cultures) heat-inactivated FBS (Omega Scientific) and 2 mmol l^{-1} glutamine in humidified, 5% CO_2 incubator. To derive PLX4032-resistant sub-lines, M229 and M238 were seeded at low cell density and treated with PLX4032 at 1 μM every 3 days for 4–6 weeks and clonal colonies were then isolated by cylinders. M249 R was derived by successive titration of PLX4032 up to 10 μM. PLX4032-resistant sub-lines and short-term cultures were replenished with 1 μM PLX4032 every 2 to 3 days. shRNAs were sub-cloned into the lentiviral vector pLL3.7. N-RAS(Q61K) mutant overexpression construct was made by PCR-amplifying from M249 R4 cDNA and sub-cloning into the lentiviral vector (UCLA Vector Core), creating pRRLsin.cPPT.CMV.hTERT.IRES.GFP-Flag-^{Q61K}NRAS. Wild-type PDGFRβ overexpression construct was PCR-amplified from cDNA and sub-cloned into a lentiviral vector (Clontech), creating pLVX-Tight-Puro-PDGFRβ-Myc. Lentiviral constructs were co-transfected with three packaging plasmids into HEK293T cells. Infections were carried out with protamine sulphate.

Cellular proliferation, drug treatments and siRNA transfections. Cell proliferation experiments were performed in a 96-well format (five replicates), and baseline quantification performed at 24 h after cell seeding along with initiation of drug treatments (72 h). Stocks and dilutions of PLX4032 (Plexxikon), AZD6244 (Selleck Chemicals)and U0126 (Promega) were made in DMSO. siRNA pool (Dharmacon) transfections were carried out in 384-well format. TransIT transfection reagent (Mirus) was added to each well and incubated at 37 °C for 20 min. Subsequently, cells were reverse transfected, and the mixture was incubated for 51–61 h at 37 °C. Cells were quantified using CellTiter 96 Aqueous One Solution (Promega) or CellTiter-GLO Luminescence (Promega) following the manufacturer's recommendations.

Protein detection. Cell lysates for western blotting were made in RIPA (Sigma) with protease inhibitor cocktail (Roche) and phosphatase inhibitor cocktails I and II (Santa Cruz Biotechnology). Western blots were probed with antibodies against p-MEK1/2 (S217/221), total MEK1/2, p-ERK1/2 (T202/Y204), total ERK1/2, PDGFRβ, and EGFR (all from Cell Signaling Technologies), B-RAF and N-RAS (Santa Cruz Biotechnology), pan-RAS (Thermo Scientific) and tubulin (Sigma). p-RTK arrays were performed according to the manufacturer's recommendations (Human Phospho-RTK Array Kit, R&D Systems). For PDGFRβ immunohistochemistry, paraffin-embedded formalin fixed tissue sections were subjected to antigen retrieval and incubated with a rabbit monoclonal anti-PDGFRβ antibody (Cell Signaling Technology) followed by labelled anti-rabbit polymer horseradish peroxidase (Envision System, Dako Cytomation). Immunocomplexes were visualized using the DAB (3,3′-diaminobenzidine) peroxidase method and nuclei haematoxylin-counterstained.

***In vitro* kinase assay.** Cells were harvested and protein lysates prepared in a NP40-based buffer before subjected to immunoprecipitation (IP). IP beads were then resuspended in ADBI buffer (with Mg/ATP cocktail) and incubated with an inactive, recombinant MEK1 or a truncated RAF-1 (positive control) (Millipore), and with DMSO or 1 μM PLX4032 for 30 min at 30 °C. The beads were subsequently pelleted and the supernatant resuspended in sample buffer for western blotting to detect p-MEK and total MEK.

Activated RAS pull-down assay. Melanoma lysates were incubated with glutathione agarose beads coupled to 80 μg GST-RAF-1-RBD (Thermo) for 1 h at 4 °C. As controls, Pt48 R lysate was pre-incubated with either 0.1 mM GTPγS (positive control) or 1 mM GDP (negative control) in the presence of 10 mM EDTA (pH 8.0) at 30 °C for 15 min. Reactions were terminated by adding 60 mM $MgCl_2$. After washing with Wash Buffer (Thermo), proteins bound to beads were eluted by protein sample buffer. RAS or NRAS levels were detected by immunoblotting.

Quantitative real-time PCR for relative RNA levels. Total RNA was extracted using the RiboPure Kit (Ambion), and reverse transcription reactions were performed using the SuperScript First-Strand Synthesis System (Invitrogen). Real-time PCR analyses were performed using the iCycler iQ Real Time PCR Detection System (BioRad) (Supplementary Table 5). To discriminate specific from non-specific cDNA products, a melting curve was obtained at the end of each run. Data were normalized to tubulin and/or *GAPDH* levels in the samples in duplicates. Relative expression is calculated using the delta-*C*t method using the following equations: $\Delta Ct(\text{Sample}) = Ct(\text{Target}) - Ct(\text{Reference})$; relative quantity $= 2^{-\Delta Ct}$.

Quantitative real-time PCR for relative DNA copy numbers. gDNAs were extracted using the FlexiGene DNA Kit (Qiagen) (Human Genomic DNA-Female, Promega). *NRAS* relative copy number was determined by quantitative PCR (cycle conditions available upon request) using the MyiQ single colour Real-Time PCR Detection System (Bio-Rad). Total DNA content was estimated by assaying β-globin for each sample (Supplementary Table 5), and 20 ng of gDNA was mixed with the SYBR Green QPCR Master Mix (Bio-Rad) and 2 pmol l^{-1} of each primer.

Sequencing. gDNAs were isolated using the Flexi Gene DNA Kit (QIAGEN) or the QIAamp DNA FFPE Tissue Kit. *B-RAF* and *RAS* genes were amplified from genomic DNA by PCR. PCR products were purified using QIAquick PCR Purification Kit (QIAGEN) followed by bi-directional sequencing using BigDye v1.1 (Applied Biosystems) in combination with a 3730 DNA Analyzer (Applied Biosystems). *PDGFRβ* was amplified from cDNA by PCR and sequenced (primers listed in Supplementary Table 1).

***B-RAF* ultra-deep sequencing.** Exon-based amplicons were generated using Platinum high-fidelity *Taq* polymerase, and libraries were prepared following the Illumina library generation protocol version 2.3. For each sample, one library was generated with 18 exons pooled at equal molarity and another library was generated for exon 13 only for validation purpose. Each library was indexed with an unique four base long barcode within the custom made Illumina adaptor. All 10 indexed samples were pooled and sequenced on one lane of Illumina GAIIx flow-cell for single-end 76 base pairs. For error rate estimation, phiX174 genome was spiked in. Base-calling was performed by Illumina RTA version 1.8.70. Alignment was performed using the Novocraft Short Read Alignment Package version 2.06 (http://www.novocraft.com/index.html). First, all reads were aligned to the phiX174 reference genome downloaded from the NCBI. The mismatch rates at each position of the reads were calculated to estimate the error rate of the sequencer (set at 1.67% or five standard deviations, SD) based on the phiX genome data (mean error rate = 0.57%, s.d. = 0.22%). Then, the .qseq.txt files were converted into .fastq file using a custom script (available on request) and during this process, the first 5 bases (unique 4-base barcode and the T at the fifth position) were stripped off from the reads and concatenated to the read name. The .fastq file was parsed into 10 .fastq files for each barcode and only the reads with the first 5 bases perfectly matching any of the 10 barcodes were included. Each .fastq file was aligned to chromosome 7 fasta file, generated from the Human Genome reference sequence (hg18, March 2006, build 36.1) downloaded from the Broad Institute (ftp://ftp.broadinstitute.org/pub/gsa/gatk_resources.tgz) using the Novoalign program. Base calibration option was used, and the output format was set to SAM. Using SAMtools (http://samtools.sourceforge.net/), the .sam files of each lane were converted to .bam files and sorted, followed by removal of potential PCR duplicates using Picard (http://picard.sourceforge.net/). The true background rate was inferred from analysis of independent exon 13 amplicons. None of the 14 positions within exon 13 that had non-reference allele frequency (NAF) > 1.67% in all-exon-samples were validated in the exon13-only samples and vice versa for the one position in the exon 13-only sample, inferring that the true background error rate could be higher at 4.81% (5s.d., mean error rate = 2.72%, s.d. = 0.4%). In total, 12 positions had NAF > 4.81%, and none of them recurred at the same position. We note that the four sample gDNAs extracted from formalin-fixed paraffin-embedded (FFPE) blocks had 5–6 times more variants with NAF above background than the sample extracted from frozen tissue, and the 12 positions with NAF >4.81% were scattered only across the FFPE samples. The numbers of variants within and outside the kinase domain were not significantly different.

***B-RAF* deep sequence from whole exome sequence analysis.** Genomic libraries were generated following the Agilent SureSelect Human All Exon Kit Illumina Paired-End Sequencing Library Prep Version 1.0.1 protocol at the UCLA Genome Center. Agilent SureSelect All Exon ICGC version was used for capturing ~50 megabase (Mb) exome. The Genome Analyzer IIx (GAIIx) was run using standard manufacturer's recommended protocols. Base-calling was done by Illumina RTA version 1.6.47. Two lanes of Illumina single end (SE) run were generated for each of Pt111-001 normal, baseline and DP2 samples, and one lane of Illumina paired end (PE) run was generated for each of Pt111-001 DP1, DP3 as well as Pt111-010 normal, baseline, DP1 and DP2 samples. Alignment was performed using the Novocraft Short Read Alignment Package version 2.06. Human Genome reference sequence (hg18, March 2006, build 36.1), downloaded from the UCSC genome database located at http://genome.ucsc.edu and mirrored locally, was indexed using novoindex program (-k 14 -s 3). Novoalign program was used to align each lane's qseq.txt file to the reference genome. Base calibration option and adaptor stripping option for paired-end run were used and the output format was set to SAM. Using SAMtools (http://samtools.sourceforge.net/), the .sam files of each lane were converted to .bam files, sorted and merged for each sample and potential PCR duplicates were removed using Picard (http://picard.sourceforge.net/). The .bam files were filtered for SNV calling and small INDEL calling to reduce the likelihood of using spuriously mis-mapped reads to call the variants. For the .bam file to call SNVs, the last 5 bases were trimmed and only the reads lacking indels were retained. For the .bam file to call small INDELs, only the reads containing one contiguous INDEL but not positioned at the beginning or the end of the read were retained. SOAP consensus-calling model implemented in SAMtools was used to call the variants, both SNVs and indels, and generate the .pileup files for each .bam file. Coding regions ± 2 bp of *B-RAF* gene were extracted

from the .pileup files and the reads were manually examined for rare variants (non reference alleles).

Microarray data generation and analysis. Total RNAs were extracted using the RiboPure Kit (Ambion) from cells (DMSO or PLX4032, 1 μM, 6 h). cDNAs were generated, fragmented, biotinylated, and hybridized to the GeneChip Human Gene 1.0 ST Arrays (Affymetrix). The arrays were washed and stained on a GeneChip Fluidics Station 450 (Affymetrix); scanning was carried out with the GeneChip Scanner 3000 7G; and image analysis with the Affymetrix GeneChip Command Console Scan Control. Expression data were normalized, background-corrected, and summarized using the RMA algorithm implemented in the Affymetrix Expression ConsoleTM version 1.1. Data were log-transformed (base 2) for parametric analysis. Clustering was performed with MeV 4.4, using unsupervised hierarchical clustering analysis on the basis of Pearson correlation and complete/average linkage clustering. Differentially expressed genes were identified using significance analysis of microarrays (SAM) with the R package 'samr' (R 2.9.0; FDR < 0.05; fold change greater than 2). To identify and rank pathways enriched among differentially expressed genes, *P*-values (Fisher's exact test) were calculated for gene sets with at least 20% differentially expressed genes. Curated gene sets of canonical pathways in the Molecular Signatures Database (MSigDB) were used.

Copy number variation analysis. Illumina HumanExon510S-DUO bead arrays (Illumina) were performed following the manufacturer's protocol. Scanned array data were imported into BeadStudio software (Illumina), where signal intensities for samples were normalized against those for reference genotypes. Log_2 ratios were calculated, and data smoothed using the median with window size of 10 and step size of five probes.

Cell cycle and apoptosis analysis. All infected cells were replenished with PLX4032 24 h after infections (M229 R5 treated with AZD6244 to inhibit rebound p-ERK on PDGFRβ KD), fixed, permeabilized, and treated with RNase (Qiagen). Cells were stained with 50 mg ml^{-1} propidium iodide (BD Pharmingen) and the distribution of cell cycle phases was determined by Cell Quest Pro and ModiFit software. For apoptosis, post-infection cells were stained with Annexin V-V450 (BD Pharmingen) and propidium iodide for 15 min at room temperature. Flow cytometry data were analysed by the FACS Express V2 software.

Image acquisition and data processing. Statistical analyses were performed using InStat 3 Version 3.0b (GraphPad Software), and graphical representations using DeltaGraph or Prism (Red Rock Software). An Optronics camera system was used in conjunction with Image-Pro Plus software (MediaCybernetics) and Adobe Photoshop 7.0.