doi:10.1038/nature09626

Suppl. Table 1. Sequencing primers.

B-RAF	**Forward**	**Reverse**
Exon1	CGGCGACTTCTCGTCGTCTC	CTGCATGACGGAGAGGGACA
Exon2	CTGGCAGTTACTGTGATGTAGTTG	CTTCCCAAATCTATTCCTAATCCCACC
Exon3	GGACCATCTAGATATCACATATG	CATTCCTGTATGACATGGATGCCTC
Exon4	GTAGAAATGGTGTTGTATCTGACC	GATCAAAGTAACAAACCCTACAGTC
Exon5	CGATGGAATATTAGGGAGCCAAACC	CTAAACAAATGTTGGCCTCTAGG
Exon6	GTTGTCAAGCTTGAAATCAGTTGC	CTGTATAGCTGAACCAGCATTAC
Exon7	CTGAGAATGGAATTTGATCTC	GCATGTCACTGAAGAGCAGAAGTC
Exon8	CTTGAGCAAAGCAGCTTTGGC	CAGAAGCTTTTCTGATTTGTGATTC
Exon9	GTGTCCACTTGTTCTTATCATTCAGC	GTTTCTCTACACATTTTTCTCTGTG
Exon10	GTATGTGTCTATGTCTATCATC	GAATTCTGTGTCACATATGGAC
Exon11	CTCTTCCTGTATCCCTCTCAGGC	GGTAGGAGTCCCGACTGCTGTGAAC
Exon12	CTAGTACAGGAATATCATTGTTAG	CTATCAGCCATACCATATAACATTGC
Exon13	GTAGGAGGTTAGACTTGGCAATTGC	GTTAGCATCCTTATGTTCCTGGAC
Exon14	CTTGACTGGAGTGAAAGGTTTG	CAGGCTGTGGTATCCTGCTCTCC
Exon15	GACTCTAAGAGGAAAGATGAAGTAC	GTTGAGACCTTCAATGACTTTCTAG
Exon16	CTGTCTCTTTCTGAGTATGTAG	CTATCCTTCACGCTTACCCAGGAG
Exon17	GTGGGTTTCCCACCATCTATGATG	GAGTCTGCACATAGAATCCAAACTC
Exon18	GATTTCAGGTGCTTTCTTGTAAAGTG	CCACACAAGTGTTCTTTGGTTC
PDGFRβ	**Forward**	**Reverse**
Primer 1	AGAGGGCAGTAAGGAGGACTTCC	ACCTCCCTGTCCCCAATGGTGG
Primer 2	ACAGACCCACAGCTGGTGGTG	TCTGCCACCTTCACGCGAACC
Primer 3	ACCGCCCACTGTCCTGTGGTTC	AGTCATAGGGCAGCTGCATGGG
Primer 4	TGATCTCAGCCATCCTGGCCC	TAGTTGGAGGACTCGATGTCTGC
Primer 5	ATGTGTCCTTGACCGGGGAGAG	AGTCTCTCGAGAAGCAGCACCAG
Primer 6	TACCCAGAGCTGCCCATGAACG	AGAAGGGGACAGCTGATAAGGGC
N-RAS	**Forward**	**Reverse**
Exon1	TAAAGTACTGTAGATGTGGCTCGCC	ACAGAATATGGGTAAAGATGATCCGAC
Exon2	GGCTTGAATAGTTAGATGCTTATTTAACCTTGGC	GCTCTATCTTCCCTAGTGTGGTAACCTC
Exon3	CCACTGTACCCAGCCTAATC	AAGAGACAGAGGCTGCAGTG
Exon4	ACACCAGCCCGTTTATGGCT	TGTGCAGAAGAGGATAGGCAGA
K-RAS	**Forward**	**Reverse**
Exon1	AAGGTACTGGTGGAGTATTTG	GTACTCATGAAAATGGTCAGAG
Exon2	TGAAGTAAAAGGTGCACTGTAATAATCCAG	CATTTATAAAACAGGGATATTACCTACCTC
Exon3	TGACAAAAGTTGTGGACAGGT	GCAATGCCCTCTCAAGAGACAA
Exon4	ACAAAACACCTATGCGGATGACA	AACAGTCTGCATGGAGCAGG
H-RAS	**Forward**	**Reverse**
Exon1	GGCTGAGCAGGGCCCTCCTTGGCAGG	GCCCTATCCTGGCTGTGTCCTGGGC
Exon2	GGTACCAGGGAGAGGCTGGCTGTGTGAAC	CAGCGGCATCCAGGACATGCGCAG
Exon3	TACAGGTGAACCCCGTGAGG	GGAGAGGGTCAGTGAGTGCT
Exon4	ACCTTTGAGGGGCTGCTGTA	CACAAGGGAGGCTGCTGAC

MEK1	**Forward**	**Reverse**
Exon2	GCTTTCTTTCCATGATAGGAGTAC	ATCAGTCTTCCTTCTACCCTGG
Exon3	CCTGTTTCTCCTCCCTCTACC	ACACCCACCAGGAATACTGC

Suppl. Table 2. Patient characteristics, available patient-matched tumor samples and tumor-matched short-term cultures, and summary of *B-RAF/RAS* sequencing and PDGFRβ expression.

Patient ID	Sex	Age	Stage	Best overall response	Progression free survival (days)	Biopsy & culture ID	Site of biopsy	*B-RAF* exons 1-14, 16-18	*B-RAF* exon 15	*N-RAS*	*K-RAS*	*H-RAS*	PDGFRβ
Pt23	M	62	M1a	57%	466	Pt23 baseline	SC mass-thigh	N/D	V600E	N/D	N/D	N/D	–
						Pt23 resistant	Large bowel (cecum)	WT^	V600E	WT	WT	WT	+
Pt43	M	52	M1c	83%	161	Pt43 baseline	Pelvic bone metastasis	N/D	V600E	N/D	N/D	N/D	–
						Pt43 resistant	Bowel	WT^	V600E	WT	WT	WT	–
Pt48	M	30	M1b	14%	113	Pt48 baseline	SC mass, shoulder	N/D	V600E	N/D	N/D	N/D	–
						Pt48 resistant	Heart	WT	V600E	WT	WT	WT	+
						Pt48 R	Heart	WT	V600E	WT	WT	WT	+
Pt55	F	65	M1c	37%	270	Pt55 baseline	Femoral node	N/D	V600E	WT	N/D	N/D	–
						Pt55 resistant DP1	Inguinal node*	WT	V600E	Q61K	WT	WT	–
						Pt55 R	Inguinal node*	WT	V600E	Q61K	WT	WT	–
						Pt55 resistant DP2	Small bowel	WT	V600E	Q61R	WT	WT	N/D
						Pt55 R2	Small bowel	WT	V600E	Q61R	WT	WT	N/D
Pt56	F	45	M1c	53%	106	Pt56 baseline	Right pubic area	N/D	V600E	N/D	N/D	N/D	–
						Pt56 resistant	Right pubic (labial)	WT^	V600E	WT	WT	WT	–
Pt81	M	44	M1a	73%	141	Pt81 baseline	SC	N/D	V600E	N/D	N/D	N/D	–
						Pt81 resistant	Small bowel	WT^	V600E	WT	WT	WT	–
Pt84	M	60	M1c	84%	113	Pt84 baseline	Cutaneous	N/D	V600E	N/D	N/D	N/D	–
						Pt84 resistant	Cutaneous	WT	V600E	WT	WT	WT	–
Pt92	M	46	M1a	32%	149	Pt92 day 15	Axillary node	N/D	V600E	N/D	N/D	N/D	–
						Pt92 resistant	Abdominal mass	WT	V600E	WT	WT	WT	+
Pt111-001	F	66	M1b	53%	137	Pt111-001 baseline	Cutaneous, left clavicular**	N/D	V600E	WT	WT	WT	–
						Pt111-001 resistant DP1	Cutaneous, left lateral neck**	WT#	V600E	WT	WT	WT	–
						Pt111-001 resistant DP2	Cutaneous, left clavicular**	WT#	V600E	WT	WT	WT	–

						Pt111-001 resistant DP3	Cutaneous, left shoulder**	WT#	V600E	WT	WT	WT	–
Pt111-005	M	72	M1c	42%	126	Pt111-005 baseline	N/A	N/A	N/A	N/A	N/A	N/A	N/A
						Pt111-005 resistant	Adrenal gland	WT	V600E	WT	WT	WT	N/D
						Pt111-001 R	Adrenal gland	WT	V600E	WT	WT	WT	–
Pt111-010	M	48	M1c	24%	126	Pt111-010 baseline	Lymph node, left inguinal	N/D	V600E	N/D	N/D	N/D	–
						Pt111-010 resistant DP1	Cutaneous, left anterior thigh, superior	WT#	V600E	WT	WT	WT	N/D
						Pt111-010 resistant DP2	Cutaneous, left anterior thigh, inferior	WT^#	V600E	WT	WT	WT	–
						Pt111-010 R	Cutaneous, left anterior thigh, inferior	WT	V600E	WT	WT	WT	–
Pt104-004	M	54	M1c	75%	84	Pt104-004 baseline	Lung	N/D	V600E	N/D	N/D	N/D	–
						Pt104-004 resistant	Pelvic mass	WT	V600E	WT	WT	WT	+

Mutation status of *B-RAF* and *RAS* genes and PDGFRβ expression status are summarized for a collection of PLX4032 clinical trial biopsy samples and tumor-matched short-term cultures. Samples labeled as resistant are from tumors that initially responded to (PR 30% by RECIST criteria) and then progressed on PLX4032. Abbreviations and symbols: M, male; F, female; SC, subcutaneous; Pt# R, short-term culture derived from tissue directly above; DP, disease progression; N/D, not done; N/A, not available; * shown in Suppl. Fig. 4a; ** shown in Suppl. Fig. 4b; ^ shown by ultradeep sequencing (Suppl. Fig 6) in addition to Sanger sequencing; # shown by deep sequencing (Suppl. Fig. 7) as well as Sanger sequencing. Blue, pertinent negative results. Red, pertinent positive results. PDGFRβ expression determined by immunohistochemistry (IHC) for tissues and immunoblotting for short-term cultures (relative to PLX4032-sensitive parental and PLX4032-resistant sub-lines). PDGFRβ IHC is performed only for the available elevan, baseline/resistant, patient-paired, tumor samples and defined as positive if specific immuno-reactivity exceeds 20% within representative tumor sections (example shown in Suppl. Fig. 13).

Suppl. Table 3. Gene Set Enrichment Analysis of differential expressed genes in M229 P *vs*. R5 (top) and M238 P *vs*. R1 (bottom).

Molecular Signature Database Gene Set	# of Differentially Expressed Genes in the Set	# of Genes in the Set	p-value for Enrichment (Fisher's Exact Test)	List of Differentially Expressed Genes in the Set
HSA04512_ECM_RECEPTOR_INTERACTION	31	86	1.48E-013	COL11A1, COL11A2, COL5A1, LAMA1, ITGA5, LAMB3, SDC3, SDC1, ITGA2, SDC2, ITGA3, COL6A2, COL6A3, COL5A2, COL6A1, COL1A2, ITGA10, LAMA5, LAMA4, COL1A1, ITGA11, ITGA6, COL3A1, HSPG2, COL4A2, ITGB1, CD44, FN1, COL4A1, ITGA9, DAG1
HSA04510_FOCAL_ADHESION	45	198	6.39E-011	**PDGFRB**, COL11A1, COL11A2, PDGFC, LAMA1, ITGA5, RAC2, FARP2, ITGA2, ITGA6, MYLK, ITGA3, **FLT1**, CAV1, LAMA5, COL4A1, VCL, COL6A2, COL6A3, COL5A2, COL6A1, COL1A2, ITGA10, PAK3, LAMA4, LAMB3, COL1A1, ITGA11, **EGFR**, MYL9, JUN, **MET**, COL4A2, ITGB1, COL3A1, FN1, FLNC, PRKCA, FLNB, ITGA9, COL5A1, VEGFC, VEGFA, CAPN2, TLN2
BREAST_CANCER_ESTROGEN_SIGNALING	24	100	6.24E-007	RAC2, TNFAIP2, SERPINE1, TGFA, NGFR, CLU, **TIE1**, STC2, F3, FGF1, COL6A1, **KIT**, PAPPA, GSN, AZGP1, IGFBP2, **EGFR**, ITGA6, JUN, CTSB, PLAU, SERPINA3, CD44, IL6
HSA04610_COMPLEMENT_AND_COAGULATION_CASCADES	19	68	7.63E-007	BDKRB1, F2R, C4A, BDKRB2, PROS1, C1S, C1R, C5, CFI, CD55, PLAU, PLAT, A2M, TFPI, F3, SERPINE1, SERPIND1, SERPINA5, PLAUR
HSA04360_AXON_GUIDANCE	27	127	1.66E-006	GNAI1, CXCR4, RAC2, SEMA5A, SEMA6A, EFNB2, EFNA5, SEMA3D, **NRP1**, L1CAM, SEMA3E, PAK3, UNC5B, NFATC1, NTN4, **MET**, SEMA3B, PLXNA2, ITGB1, **EPHA3**, SEMA6D, SEMA3G, **EPHB2**, **EPHB3**, SLIT3, LIMK2, ABLIM3
CARDIACEGFPATHWAY	8	17	1.86E-005	PRKCA, ADAM12, FOS, **EGFR**, JUN, EDN1, EDNRB, NFKB1
HSA03320_PPAR_SIGNALING_PATHWAY	15	68	2.14E-004	DBI, GK, FADS2, SORBS1, MMP1, ACSL5, FABP7, ACSL3, FABP5, RXRG, LPL, CYP27A1, ACSL1, SCD, CPT1A
IL1RPATHWAY	9	32	6.06E-004	IL1RAP, IL1R1, MAP3K1, IL6, TGFB1, TGFB3, JUN, IRAK2, NFKB1
PROSTAGLANDIN_SYNTHESIS_REGULATION	8	28	1.08E-003	ANXA1, PTGER2, ANXA3, PTGER4, ANXA8, PLA2G4A, EDN1, EDNRB
LYSINE_DEGRADATION	8	28	1.08E-003	ALDH1A1, PLOD2, AADAT, SHMT1, ALDH1B1, PLOD3, ALDH1A3, AASS

Molecular Signature Database Gene Set	# of Genes in the Set	# of Differentially Expressed Genes	p-value for Enrichment (Fisher's Exact Test)	List of Differentially Expressed Genes in the Set
HSA04510_FOCAL_ADHESION	198	47	7.41E-016	**PDGFRB**, ILK, COL11A1, PDGFC, CAV2, PIK3CG, PDGFB, ITGA5, RAC2, PAK1, FARP2, ARHGAP5, ITGA6, MYLK, ITGA4, ACTN1, ITGA3, COL1A2, **FLT1**, CAV1, ITGB3, **KDR**, ITGB8, PDGFD, VCL, COL6A3, ITGA8, ITGA10, LAMA4, **IGF1R,** TLN1, COL1A1, ITGA11, **EGFR**, MYL9, JUN, **MET**, FIGF, ITGB1, FN1, FLNC, FLNB, ITGA9, COL5A1, VEGFC, **ERBB2**, SPP1
HSA04512_ECM_RECEPTOR_INTERACTION	86	23	1.51E-009	COL1A2, COL11A1, COL5A1, ITGA5, SDC3, SDC1, ITGA6, ITGA4, ITGA3, ITGB3, ITGB8, COL6A3, ITGA8, ITGA10, LAMA4, COL1A1, ITGA11, ITGB1, FN1, SV2C, ITGA9, DAG1, SPP1
BREAST_CANCER_ESTROGEN_SIGNALING	100	21	6.87E-007	AR, AZGP1, NGFR, **KIT**, CLU, PAPPA, GSN, IL6, IGFBP2, IL6R, STC2, **EGFR**, JUN, RAC2, TGFA, SERPINE1, ITGA6, FGF1, SERPINA3, **ERBB2**, PLAU
INTEGRIN_MEDIATED_CELL_ADHESION_KEGG	93	20	8.44E-007	ITGA8, ILK, TLN1, ITGA10, RAC2, ITGB3, TNS1, ITGB1, CAV2, ITGB8, ITGA11, SORBS1, ITGA5, CAV1, PAK1, VCL, ITGA6, ITGA9, ITGA4, ITGA3
HSA04640_HEMATOPOIETIC_CELL_LINEAGE	87	19	1.23E-006	MME, IL1R1, IL1B, **KIT**, IL7R, IL7, IL6, IL6R, CSF2RA, KITLG, CD9, ITGB3, ITGA5, TFRC, ITGA6, CD55, ITGA4, ANPEP, ITGA3
HSA05218_MELANOMA	70	15	2.04E-005	**PDGFRB**, MITF, PDGFC, **IGF1R**, PIK3CG, PDGFB, **EGFR**, PDGFD, FGF14, FGF13, FGF12, FGF1, FGF2, **MET**, FGF5
HSA04520_ADHERENS_JUNCTION	74	15	4.09E-005	SNAI2, TJP1, SNAI1, LEF1, **IGF1R**, **ERBB2**, PTPRJ, SORBS1, **EGFR**, RAC2, FARP2, VCL, **MET**, ACTN1, WASF3
PROSTAGLANDIN_SYNTHESIS_REGULATION	28	8	2.19E-004	ANXA6, HPGD, PTGER2, PTGFR, EDNRA, EDN1, EDNRB, PLA2G4A
ST_DICTYOSTELIUM_DISCOIDEUM_CAMP_CHEMOTAXIS_PATHWAY	32	8	5.94E-004	ITPR3, ITGA9, DAG1, PDE3B, DGKA, ITPR1, PAK1, ANGPTL2
O_GLYCAN_BIOSYNTHESIS	13	5	7.73E-004	GALNT10, GALNT6, GCNT1, ST3GAL4, GALNT7

Suppl. Table 4. shRNA sequences based on siRNA sequences.

shRNA ID	**Oligonucleotide sequence**
shRNACONTROL (sense)	TGGAATCTCATTCGATGCATACTTCAAGAGAGTATGCATCGAATGAGATTCCTTTTTTC
shRNACONTROL (antisense)	TCGAGAAAAAAGGAATCTCATTCGATGCATACTCTCTTGAAGTATGCATCGAATGAGATTCCA
shPDGFRβ2 (sense)	TGAGCGACGGTGGCTACATGTTCAAGAGACATGTAGCCACCGTCGCTCTTTTTTC
shPDGFRβ2 (antisense)	TCGAGAAAAAAGAGCGACGGTGGCTACATGTCTCTTGAACATGTAGCCACCGTCGCTCA
shPDGFRβ3 (sense)	TGAAGCCACGTTACGAGATCTTCAAGAGAGATCTCGTAACGTGGCTTCTTTTTTC
shPDGFRβ3 (antisense)	TCGAGAAAAAAGAAGCCACGTTACGAGATCTCTCTTGAAGATCTCGTAACGTGGCTTCA
shPDGFRβ4(sense)	TGGTGGGCACACTACAATTTCCACACCAAATTGTAGTGTGCCCACCTTTTTTC
shPDGFRβ4 (antisense)	TCGAGAAAAAAGGTGGGCACACTACAATTTGGTGTGGAAATTGTAGTGTGCCCACCA
shNRAS1 (sense)	TGAGCAGATTAAGCGAGTAATTCAAGAGATTACTCGCTTAATCTGCTCTTTTTTC
shNRAS1 (antisense)	TCGAGAAAAAAGAGCAGATTAAGCGAGTAATCTCTTGAATTACTCGCTTAATCTGCTCA
shNRAS2 (sense)	TGAAATACGCCAGTACCGAATTCAAGAGATTCGGTACTGGCGTATTTCTTTTTTC
shNRAS2 (antisense)	TCGAGAAAAAAGAAATACGCCAGTACCGAATCTCTTGAATTCGGTACTGGCGTATTTCA
shNRAS3 (sense)	TGTGGTGATGTAACAAGATATTCAAGAGATATCTTGTTACATCACCACTTTTTTC
shNRAS3 (antisense)	TCGAGAAAAAAGTGGTGATGTAACAAGATATCTCTTGAATATCTTGTTACATCACCACA
shNRAS4 (sense)	TGCACTGACAATCCAGCTAATTCAAGAGATTAGCTGGATTGTCAGTGCTTTTTTC
shNRAS4 (antisense)	TCGAGAAAAAAGCACTGACAATCCAGCTAATCTCTTGAATTAGCTGGATTGTCAGTGCA

Suppl. Table 5. Quantifying mRNA and gDNA copy numbers: genes, primers and real-time PCR conditions.

mRNA	**Forward**	**Reverse**
N-RAS	ACAGTGCCATGAGAGACCAA	TCGCTTAATCTGCTCCCTGT
B-RAF	ATGTTGAATGTGACAGCACC	CTCACACCACTGGGTAACAA
PDGFRβ	TTCCATGCCGAGTAACAGAC	CGTTGGTGATCATAGGGGAC
Tubulin	GACAGCTCTTCCACCCAGAG	TGAAGTCCTGTGCACTGGTC
GAPDH	CAATGACCCCTTCATTGACC	GACAAGCTTCCCGTTCTCAG
gDNA	**Forward**	**Reverse**
N-RAS	TTGGATTGTGTCCGTTGAGC	ACCCTGAGTCCCATCATCAC
Globin	AATTCACCCCACCAGTGCAG	CTTCCCGTTCTCAGCCTTGA

A single step at 95°C for 10 min preceded 40 cycles of amplification (95°C for 30 s, 52°C for 30 s, and 72°C for 30 s). Subsequently, melting curve analysis was performed as follows: 95°C for 10 s, 52°C for 10 s, and 95°C for 10 s.