Supplemental Data

RAF gene fusion breakpoints in pediatric brain tumors are characterized by significant enrichment of sequence microhomology

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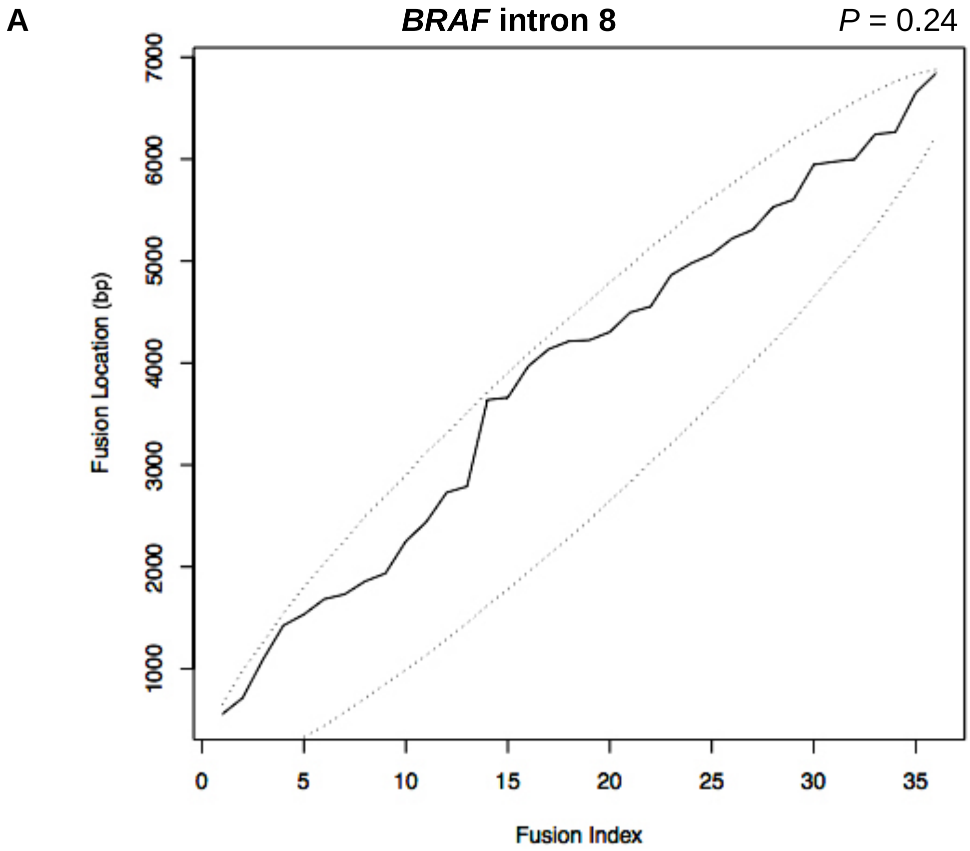
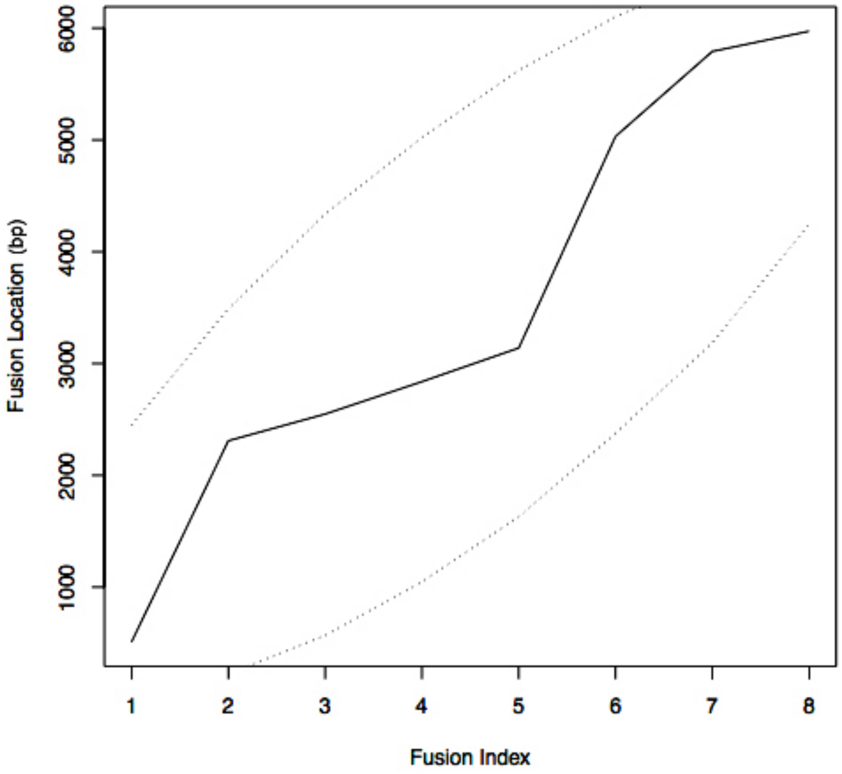
Supplemental Figure S1 Distribution of breakpoint positions in introns.

Dotted lines represent 95% confidence intervals for expected location of successive breakpoints, solid lines represent location of actual breakpoints in introns. Plots for introns with fewer than five breakpoints are not shown. p-values are the results of Kolmogorov-Smirnov tests against the null hypothesis that the locations are drawn from a uniform distribution across the intron. Fusion location is represented as the number of base pairs from the start of each intron; the fusion index is the cumulative number of fusion events at each location.

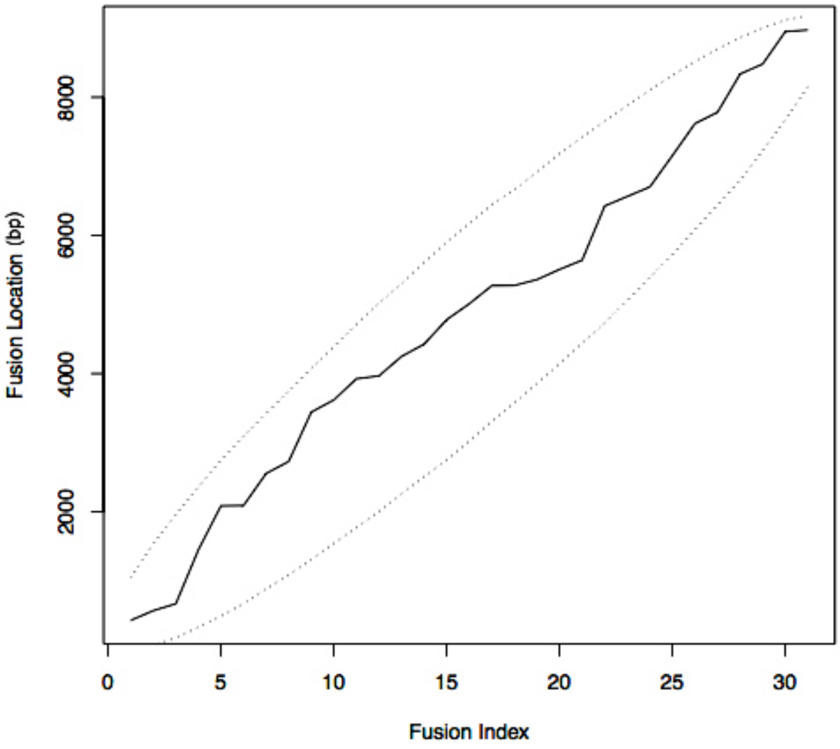
(*A*) *BRAF* intron 8; (*B*) *KIAA1549* intron 15; (*C*) *KIAA1549* intron 16.



B ***KIAA1549* intron 15** *P* = 0.75

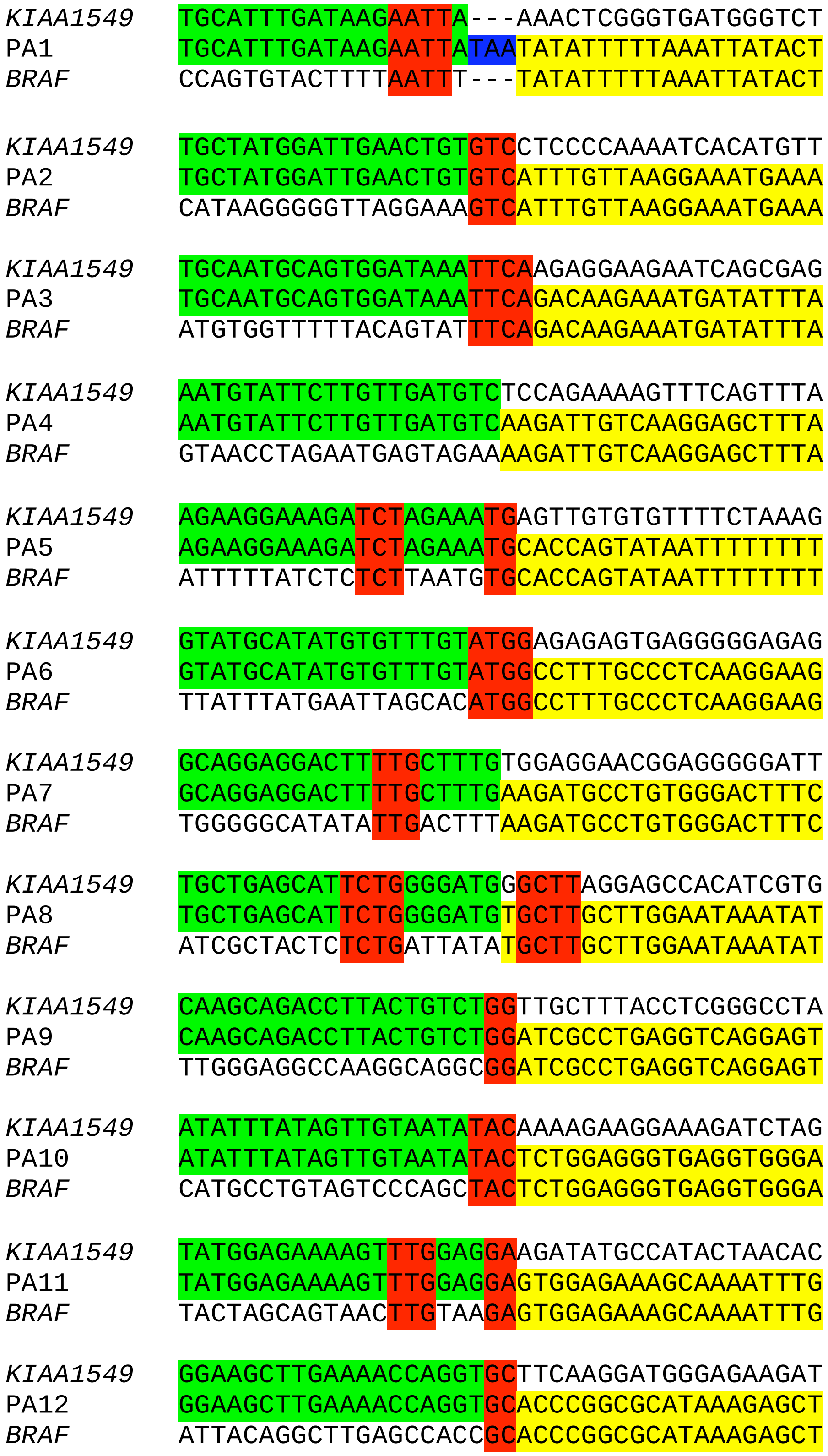


C ***KIAA1549* intron 16** *P* = 0.75



Supplemental Figure S2 Sequence alignment for 40 simple breakpoints.

Fusion sequences aligned with *KIAA1549* and *BRAF* are highlighted in green and yellow respectively. Nucleotides which align to neither reference gene (insertions) are in blue. Nucleotides which align to both reference genes (microhomology) are in red.



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KIAA1549   TGCATTTGATAAGAATTA---AAACTCGGGTGATGGGTCT
PA1        TGCATTTGATAAGAATTATAATATATTTTTAAATTATACT
BRAF       CCAGTGTACTTTTAATTT---TATATTTTTAAATTATACT

KIAA1549   TGCTATGGATTGAACTGTGTCCTCCCCAAAATCACATGTT
PA2        TGCTATGGATTGAACTGTGTCATTTGTTAAGGAAATGAAA
BRAF       CATAAGGGGGTTAGGAAAGTCATTTGTTAAGGAAATGAAA

KIAA1549   TGCAATGCAGTGGATAAATTCAAGAGGAAGAATCAGCGAG
PA3        TGCAATGCAGTGGATAAATTCAGACAAGAAATGATATTTA
BRAF       ATGTGGTTTTTACAGTATTTCAGACAAGAAATGATATTTA

KIAA1549   AATGTATTCTTGTTGATGTCTCCAGAAAAGTTTCAGTTTA
PA4        AATGTATTCTTGTTGATGTCAAGATTGTCAAGGAGCTTTA
BRAF       GTAACCTAGAATGAGTAGAAAAGATTGTCAAGGAGCTTTA

KIAA1549   AGAAGGAAAGATCTAGAAATGAGTTGTGTGTTTTCTAAAG
PA5        AGAAGGAAAGATCTAGAAATGCACCAGTATAATTTTTTTT
BRAF       ATTTTTATCTCTCTTAATGTGCACCAGTATAATTTTTTTT

KIAA1549   GTATGCATATGTGTTTGTATGGAGAGAGTGAGGGGGAGAG
PA6        GTATGCATATGTGTTTGTATGGCCTTTGCCCTCAAGGAAG
BRAF       TTATTTATGAATTAGCACATGGCCTTTGCCCTCAAGGAAG

KIAA1549   GCAGGAGGACTTTTGCTTTGTGGAGGAACGGAGGGGGATT
PA7        GCAGGAGGACTTTTGCTTTGAAGATGCCTGTGGGACTTTC
BRAF       TGGGGGCATATATTGACTTTAAGATGCCTGTGGGACTTTC

KIAA1549   TGCTGAGCATTCTGGGGATGGGCTTAGGAGCCACATCGTG
PA8        TGCTGAGCATTCTGGGGATGTGCTTGCTTGGAATAAATAT
BRAF       ATCGCTACTCTCTGATTATATGCTTGCTTGGAATAAATAT

KIAA1549   CAAGCAGACCTTACTGTCTGGTTGCTTTACCTCGGGCCTA
PA9        CAAGCAGACCTTACTGTCTGGATCGCCTGAGGTCAGGAGT
BRAF       TTGGGAGGCCAAGGCAGGCGGATCGCCTGAGGTCAGGAGT

KIAA1549   ATATTTATAGTTGTAATATACAAAAGAAGGAAAGATCTAG
PA10       ATATTTATAGTTGTAATATACTCTGGAGGGTGAGGTGGGA
BRAF       CATGCCTGTAGTCCCAGCTACTCTGGAGGGTGAGGTGGGA

KIAA1549   TATGGAGAAAAGTTTGGAGGAAGATATGCCATACTAACAC
PA11       TATGGAGAAAAGTTTGGAGGAGTGGAGAAAGCAAAATTTG
BRAF       TACTAGCAGTAACTTGTAAGAGTGGAGAAAGCAAAATTTG

KIAA1549   GGAAGCTTGAAAACCAGGTGCTTCAAGGATGGGAGAAGAT
PA12       GGAAGCTTGAAAACCAGGTGCACCCGGCGCATAAAGAGCT
BRAF       ATTACAGGCTTGAGCCACCGCACCCGGCGCATAAAGAGCT
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KIAA1549	CTTTAAAAACCCATACTGTGCCGAAAGCACCAGCACTGTG
PA13	CTTTAAAAACCCATACTGTGCTAAATATATATATAGTTTT
BRAF	ACCATCGTTGTTATAAAGAGCTAAATATATATATAGTTTT

KIAA1549	TCCACAAGGTTCCACATGACAGCCAAGGGATGGAAGCAG
PA14	TCCACAAGGTTCCACATGACCAGCCAAGGGACAATTGTTT
BRAF	CAGGCGTGAGCCACTGCACCCAGCCAAGGGACAATTGTTT

KIAA1549	GGCAGGAGGTGTGAGGGAGAGGAGAGATTTGAAAGACCTA
PA15	GGCAGGAGGTGTGAGGGAGAGCAAAATTTGGTTAATCCAT
BRAF	AACTTGTAAGAGTGGAGAAAGCAAAATTTGGTTAATCCAT

KIAA1549	GATGAATTTTTAGCAGTAATTTTTGGCAGGTTCGTGTTGT
PA16	GATGAATTTTTAGCAGTAATTTCATCCTCATAAAAACTCA
BRAF	CTTTACGTAGACTTTCTTATTTCATCCTCATAAAAACTCA

KIAA1549	ACCTGCTGTGTCGCAGG-----CACTCTGCTTAGGTGCTG
PA17	ACCTGCTGTGTCGCAGGTGCTTTGCATTGTGTTAGGCAAT
BRAF	CTAATAACTGTGGGCTA-----TGCATTGTGTTAGGCAAT

KIAA1549	TTCCTTAAATCATGTGGCATATTGTTTTAGTCAAAGAACA
PA18	TTCCTTAAATCATGTGGCATTCTCAGCTCACTGCAACCTC
BRAF	GCTGGAGTGCAGTGGTGCAATCTCAGCTCACTGCAACCTC

KIAA1549	GCTCTTGCTATGGATTGAACTGTGTCCTCCCCAAAATCAC
PA19	GCTCTTGCTATGGATTGAACTACCAAAAATAAAAAATTAG
BRAF	ACATGATGAAACCCCATCTCTACCAAAAATAAAAAATTAG

KIAA1549	AATTCGCTGGGCGTGGT------GGCGGGCACCTGTAGTC
PA20	AATTCGCTGGGCGTGGTTTGAATCATGTTTCATGATTCAA
BRAF	TTAAATTATACTATAAA------CATGTTTCATGATTCAA

KIAA1549	CCATTCTCCTGCCTCAGCCTCCCGAGTAGCTGGGATTATA
PA21	CCATTCTCCTGCCTCAGCCTGTAATCCCAGCACTTTGGGA
BRAF	GGTTGCGGCGGCTTATGCATGTAATCCCAGCACTTTGGGA

KIAA1549	CTTGTAATAAGGGTGACCATTATAATTCAGGCCTTGGTAG
PA22	CTTGTAATAAGGGTGACCATAGTTCCTTCCACCTGAAAGA
BRAF	GGATATGGCCTTTGTCTTGTAGTTCCTTCCACCTGAAAGA

KIAA1549	GAGAAATATCCTCAAGGTTTTTTTCCTTCTCCCTAGTCAG
PA23	GAGAAATATCCTCAAGGTTTTGAGCAGAGAAGGGACAACC
BRAF	CTGGTTGGATCTAGGAACATTGAGCAGAGAAGGGACAACC

KIAA1549	TGATTTTTTTAAATGATTGAGTTTTGTATGGGGAGGAACC
PA24	TGATTTTTTTAAATGATTGATTGATTAGGACTAATAATTG
BRAF	CCTGAAATGGACATCAACATTTGATTAGGACTAATAATTG

KIAA1549	TGTAAAATAGCACAACCACTTTGAAAAACAGTTTGGCAGT
PA25	TGTAAAATAGCACAACCACTTTCTTCTTAGAAGTCACAGC
BRAF	TCTTTTTTTCTCTGAATTCTTTCTTCTTAGAAGTCACAGC

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KIAA1549    AATTCGCTGGGCGTGGTGGCGGGCACCTGTAGTCCCAGCT
PA26        AATTCGCTGGGCGTGGTGGCAGGCATAAGGTAATGTACTT
BRAF        TCTCTTCCTGTATCCCTCTCAGGCATAAGGTAATGTACTT

KIAA1549    CTGTATATCTTGGGGGGATAAATTCATGAAGTTTGAAAAT
PA29        CTGTATATCTTGGGGGGATAATGTAAGAGACATTAGTATA
BRAF        CCAAGGGACAATTGTTTTTAATGTAAGAGACATTAGTATA

KIAA1549    AGTTACTGTTCTTCTGAAA-TATCTAATTCCAAAAGGAAC
PA32        AGTTACTGTTCTTCTGAAACAAGGAAAACAAGGTTTGCAG
BRAF        CACAGTTTGTTAAATACCA-AAGGAAAACAAGGTTTGCAG

KIAA1549    TTAAAAAGTTAAACATATA--TCCTACCGTATGAACCAGG
PA33        TTAAAAAGTTAAACATATACAGGCTCATGCCTGTAATCCC
BRAF        ATTTCAGGCCAGGCATAGT--GGCTCATGCCTGTAATCCC

KIAA1549    GCTTGCAGTGAGCTGAGAT--AGTGCCACTGCAGTCCGGC
PA34        GCTTGCAGTGAGCTGAGATTGTATGATTGTAACCAGTGTA
BRAF        CCTTATCCATCCATATTTT--TATGATTGTAACCAGTGTA

KIAA1549    ACCAAGATGGTTGACTTAAATCCAGCCTTATCAATAATCA
PA35        ACCAAGATGGTTGACTTAAATTTTTTTACACTTCTAGCAT
BRAF        TCCTTCTGCAACTGTCAGAATTTTTTTACACTTCTAGCAT

KIAA1549    ACACCATTATTCAATGGTTTTCTCATTGTGGACTTTTGAT
PA36        ACACCATTATTCAATGGTTTTAACTAGCAAGGTTCACCTC
BRAF        TTTCTAGTACTAGTTTTCCTTAACTAGCAAGGTTCACCTC

KIAA1549    TAGACATGAGGCAGGAGAGGAAAAGCAAGAAGCCGATGGT
PA37        TAGACATGAGGCAGGAGAGGATTGTAAGCTCTCAAGAATG
BRAF        TGCATCTTACTTCCCATGAGATTGTAAGCTCTCAAGAATG

KIAA1549    AACTTTTAAGCAATTTTTTGTAAAGATGGGGGTCTTGCTA
PA38        AACTTTTAAGCAATTTTTTGCATTTTTATCTCTCTTAATG
BRAF        ACAGAATCAAAGGATATGAACATTTTTATCTCTCTTAATG

KIAA1549    CGATCCTCCTGCCTTGGCCTCCCACATTTCTGGGATTATA
PA39        CGATCCTCCTGCCTTGGCCTAGTTATAGATATTAGAGCAG
BRAF        GCTAATATTAATATGCAAATAGTTATAGATATTAGAGCAG

KIAA1549    AAGATTGCCCCAAAATAAAAATCTTCCTCAGATTTTGAAT
PA40        AAGATTGCCCCAAAATAAAAATGTTGAAGACTTTATATGA
BRAF        ATTTGATTTTTCTGTAGAAAATGTTGAAGACTTTATATGA

KIAA1549    TTGTTTTAGGCCACCATTTCTAAGGTATTTTGTTTTGGCA
PA41        TTGTTTTAGGCCACCATTTCTTAAATTTAAGTTTTGTATT
BRAF        TCCTAATATTGACTTCAGTCTTAAATTTAAGTTTTGTATT

KIAA1549    TGCCTAGCATAGCATAAACTTTCAATAAATATTAGCTGCC
PA42        TGCCTAGCATAGCATAAACTTTTCATGAGTCACTTTTTAA
BRAF        CAAGTGGCATTTCTTGTCCTTTTCATGAGTCACTTTTTAA

KIAA1549    CTTCTTCGTTGGTTCCTGGTTTTGTCTCTTAACAGTTTAC
PMA1        CTTCTTCGTTGGTTCCTGGTTGGGCCCATATTTTTTCACT
BRAF        TACTTATTACCGCTGCCATTTGGGCCCATATTTTTTCACT
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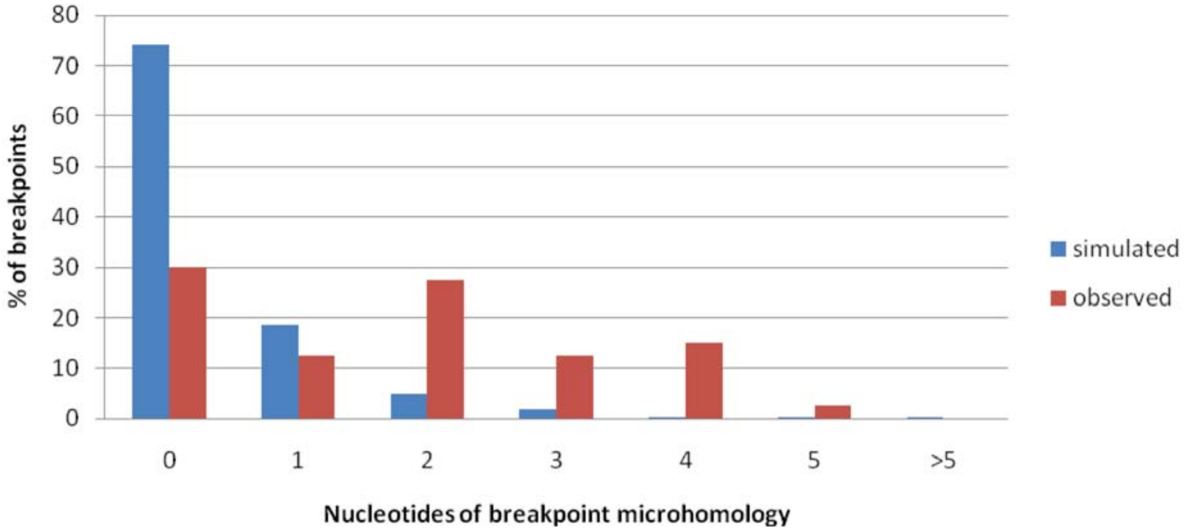
KIAA1549	TTTGATCTTTTAAGGGTATGTCAGACTATTTTCTAATAGT
PMG4	TTTGATCTTTTAAGGGTATGTCTATTTGCAGAGACTATTC
BRAF	AGCCTGTAAACAAAAAGTTGTCTATTTGCAGAGACTATTC

Supplemental Figure S3 Observed and expected distribution of breakpoint microhomology.



Supplemental Figure S4 Effect of alternative alignments on flanking microhomology.

(*A*) Perfect alignment of sequence results in 3 bp of flanking microhomology for PA14.
(*B*) An alternative alignment with 1 bp mismatch results in 9 bp of flanking microhomology.

A

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KIAA1549    TCCACAAGGTTCCACATGACAGCCAAGGGGATGGAAGCAG
PA14        TCCACAAGGTTCCACATGACCAGCCAAGGGACAATTGTTT
BRAF        CAGGCGTGAGCCACTGCACCCAGCCAAGGGACAATTGTTT
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B

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KIAA1549    CAAGGTTCCACATGAC-AGCCAAGGGGATGGAAGCAGAGA
PA14        CAAGGTTCCACATGACCAGCCAAGGGACAATTGTTTTTAA
BRAF        CGTGAGCCACTGCACCCAGCCAAGGGACAATTGTTTTTAA
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Supplemental Table S1 Clinical and breakpoint information for individual tumor samples.

Name	Pathology	Site	Age (years)	Sex	*RAF* gene fusion variant	Breakpoint position on chromosome 7*		Breakpoint information		
						KIAA1549	***BRAF***	**Breakpoint microhomology**	**Insertion**	**Flanking microhomology**
PA1	pilocytic astrocytoma	cerebellum	20	F	*KIAA1549-BRAF* exon 16-exon 9	138,545,772	140,488,292	-	TAA	Yes – *KIAA1549*
PA2	pilocytic astrocytoma	cerebellum	4	M	*KIAA1549-BRAF* exon 16-exon 9	138,544,110	140,492,331	GTC	-	No
PA3	pilocytic astrocytoma	cerebellum	7	M	*KIAA1549-BRAF* exon 16-exon 9	138,541,191	140,492,016	TTCA	-	No
PA4	pilocytic astrocytoma	cerebellum	2	M	*KIAA1549-BRAF* exon 16-exon 9	138,545,531	140,490,132	-	-	No
PA5	pilocytic astrocytoma	cerebellum	6	F	*KIAA1549-BRAF* exon 16-exon 9	138,537,229	140,488,002	TG	-	Yes – *KIAA1549*
PA6	pilocytic astrocytoma	cerebellum	5	M	*KIAA1549-BRAF* exon 16-exon 9	138,541,953	140,492,536	ATGG	-	No
PA7	pilocytic astrocytoma	cerebellum	13	M	*KIAA1549-BRAF* exon 16-exon 9	138,545,632	140,491,478	-	-	Yes – *KIAA1549*
PA8	pilocytic astrocytoma	cerebellum	2	M	*KIAA1549-BRAF* exon 16-exon 9	138,539,776	140,487,424	-	-	Yes – BOTH
PA9	pilocytic astrocytoma	cerebellum	14	F	*KIAA1549-BRAF* exon 16-exon 9	138,539,046	140,489,771	GG	-	No
PA10	pilocytic astrocytoma	cerebellum	19	M	*KIAA1549-BRAF* exon 16-exon 9	138,537,253	140,493,553	TAC	-	No
PA11	pilocytic astrocytoma	cerebellum	14	M	*KIAA1549-BRAF* exon 16-exon 9	138,542,582	140,490,053	GA	-	Yes – *KIAA1549*
PA12	pilocytic astrocytoma	cerebellum	14	M	*KIAA1549-BRAF* exon 16-exon 9	138,541,775	140,488,737	GC	-	No
PA13	pilocytic astrocytoma	cerebellum	10	M	*KIAA1549-BRAF* exon 16-exon 9	138,538,583	140,489,045	GC	-	Yes – *KIAA1549*
PA14	pilocytic astrocytoma	cerebellum	10	M	*KIAA1549-BRAF* exon 16-exon 9	138,538,419	140,490,629	C	-	Yes – *BRAF*
PA15	pilocytic astrocytoma	cerebellum	13	M	*KIAA1549-BRAF* exon 16-exon 9	138,540,560	140,490,043	AG	-	No
PA16	pilocytic astrocytoma	cerebellum	10	M	*KIAA1549-BRAF* exon 16-exon 9	138,539,638	140,491,827	ATTT	-	No
PA17	pilocytic astrocytoma	cerebellum	6	M	*KIAA1549-BRAF* exon 16-exon 9	138,544,751	140,492,580	-	TGCTT	Yes – *KIAA1549*
PA18	pilocytic astrocytoma	cerebellum	3	M	*KIAA1549-BRAF* exon 16-exon 9	138,537,721	140,488,961	-	-	Yes – *KIAA1549*
PA19	pilocytic astrocytoma	cerebellum	3	M	*KIAA1549-BRAF* exon 16-exon 9	138,544,115	140,489,713	CT	-	No
PA20	pilocytic astrocytoma	cerebellum	4	M	*KIAA1549-BRAF* exon 16-exon 9	138,540,929	140,488,268	-	TTGAAT	No
PA21	pilocytic astrocytoma	cerebellum	12	F	*KIAA1549-BRAF* exon 16-exon 9	138,543,647	140,488,664	T	-	No
PA22	pilocytic astrocytoma	cerebellum	6	F	*KIAA1549-BRAF* exon 15-exon 9	138,550,036	140,489,962	T	-	No
PA23	pilocytic astrocytoma	cerebellum	3	F	*KIAA1549-BRAF* exon 15-exon 9	138,552,365	140,492,409	TT	-	No
PA24	pilocytic astrocytoma	cerebellum	4	F	*KIAA1549-BRAF* exon 15-exon 9	138,550,565	140,487,615	-	-	No
PA25	pilocytic astrocytoma	cerebellum	4	F	*KIAA1549-BRAF* exon 16-exon 11	138,542,277	140,482,784	CTTT	-	No
PA26	pilocytic astrocytoma	cerebellum	3	F	*KIAA1549-BRAF* exon 16-exon 11	138,540,926	140,481,579	C	-	Yes – *BRAF*
PA27	pilocytic astrocytoma	cerebellum	14	F	*KIAA1549-BRAF* exon 16-exon 11	138,540,694	140,482,042	Complex	Complex	Complex
PA28	pilocytic astrocytoma	cerebellum	7	M	*KIAA1549-BRAF* exon 18-exon 10	Not found	Not found	Unknown	Unknown	Unknown
PA29	pilocytic astrocytoma	cerebellum	19	M	*KIAA1549-BRAF* exon 19-exon 9	138,524,061	140,490,606	TAA	-	No
PA30	pilocytic astrocytoma	cerebellum	13	F	*SRGAP3-RAF1* exon 11-exon 8	-	-	Complex	Complex	Complex

Name	Pathology	Site	Age (years)	Sex	***RAF* gene fusion variant**	**Breakpoint position on chromosome 7**		**Breakpoint information**		
						KIAA1549	***BRAF***	**Breakpoint microhomology**	**Insertion**	**Flanking microhomology**
PA32	pilocytic astrocytoma	brain stem	9	F	*KIAA1549-BRAF* exon 16-exon 9	138,537,866	140,491,538	-	C	No
PA33	pilocytic astrocytoma	cerebellum	9	M	*KIAA1549-BRAF* exon 16-exon 9	138,542,234	140,493,712	-	CA	Yes – *KIAA1549*
PA34	pilocytic astrocytoma	cerebellum	2	F	*KIAA1549-BRAF* exon 16-exon 9	138,540,841	140,488,321	-	TG	No
PA35	pilocytic astrocytoma	cerebellum	3	F	*KIAA1549-BRAF* exon 16-exon 9	138,542,759	140,492,734	AAT	-	No
PA36	pilocytic astrocytoma	cerebral cortex	4	M	*KIAA1549-BRAF* exon 16-exon 9	138,539,500	140,493,175	TT	-	No
PA37	pilocytic astrocytoma	midbrain	7	M	*KIAA1549-BRAF* exon 16-exon 9	138,541,421	140,489,405	GA	-	No
PA38	pilocytic astrocytoma	cerebellum	5	F	*KIAA1549-BRAF* exon 15-exon 9	138,547,082	140,488,023	-	-	No
PA39	pilocytic astrocytoma	cerebellum	6	M	*KIAA1549-BRAF* exon 15-exon 9	138,549,735	140,490,299	T	-	No
PA40	pilocytic astrocytoma	spinal cord	10	F	*KIAA1549-BRAF* exon 15-exon 9	138,550,326	140,489,288	AAAAT	-	No
PA41	pilocytic astrocytoma	cerebellum	9	F	*KIAA1549-BRAF* exon 16-exon 11	138,543,473	140,481,672	TCT	-	No
PA42	pilocytic astrocytoma	cerebellum	14	F	*KIAA1549-BRAF* exon 18-exon 10	138,527,858	140,485,787	CTTT	-	No
PMA1	pilomyxoid astrocytoma	diencephalon	1	F	*KIAA1549-BRAF* exon 15-exon 9	138,547,842	140,492,842	TT	-	No
PMG4	pilomyxoid glioma	spinal cord	12	M	*KIAA1549-BRAF* exon 15-exon 9	138,546,901	140,489,200	TGTC	-	No

* In samples where the breakpoint could not be determined exactly, due to microhomology, it was defined to be located in the middle of the indeterminate region, with the extra nucleotide associated with odd regions arbitrarily assigned to *KIAA1549*.

Supplemental Table S2 Details of five tumor samples with large regions of homology within 300 bp of breakpoint.

Name	***KIAA1549***			***BRAF***		
	Repetitive Element	**Genomic location**	**Position relative to breakpoint**	**Repetitive Element**	**Genomic location**	**Position relative to breakpoint**
PA20	AluY	7:138540766-138541044	-164 to +115	AluSx	7: 140488401-140488688	+133 to +420
PA21	AluY	7:138543498-138543754	-150 to +107	AluSx AluSg	7:140488401-140488688 7:140488730-140489029	-264 to +25 +66 to +365
PA22	AluJb	7:138549693-138549983	-344 to -54	AluSq	7:140489544-140489829	-419 to -134
PA34	AluY	7:138540766-138541044	-76 to +203	AluSx	7:140488401-140488688	+80 to +367
PMG4	AluJo AluJo	7:138546317-138546631 7:138546959-138547279	-585 to -271 +58 to +378	AluSg	7:140488730-140489029	-471 to -172

Supplemental Table S3 Whole gene analysis of repetitive elements and Z-DNA.

Type of element	**Mean number of elements per 100kb (Adjusted p-values)**				
	Control genes (± s.d.)	***KIAA1549***	***BRAF***	***SRGAP3***	***RAF1***
All repetitive elements	182.8 ± 39.99	192.1 (*P* = 1)	224.4 (*P* = 1)	233.6 (*P* = 0.96)	233.2 (*P* = 0.96)
Alu	48.44 ± 33.59	56.02 (*P* = 1)	68.63 (*P* = 1)	42.45 (*P* = 1)	137.7 (*P* = 0.21)
MIR	21.94 ± 13.77	13.34 (*P* = 1)	11.02 (*P* = 1)	43.65 (*P* = 0.68)	16.12 (*P* = 1)
L1	31.87 ± 13.79	42.68 (*P* = 1)	50.60 (*P* = 0.75)	25.18 (*P* = 1)	22.33 (*P* = 1)
L2	16.72 ± 7.808	18.01 (*P* = 1)	20.04 (*P* = 1)	28.78 (*P* = 0.59)	11.16 (*P* = 1)
LTR	15.97 ± 11.23	16.01 (*P* = 1)	10.02 (*P* = 1)	28.78 (*P* = 0.83)	1.240 (*P* = 1)
DNA elements	17.73 ± 6.441	22.01 (*P* = 1)	34.06 (*P* = 0.088)	31.90 (*P* = 0.22)	13.64 (*P* = 1)
Z-DNA	15.30 ± 10.63	10.67 (*P* = 1)	6.011 (*P* = 1)	10.31 (*P* = 1)	12.40 (*P* = 1)

Supplemental Table S4 Analysis of repetitive elements in *KIAA1549* and *BRAF* introns.

Type of element	**Mean number of elements per 100kb**						
	***KIAA1549* intron 15**	***KIAA1549* intron 16**	***KIAA1549* intron 18**	***KIAA1549* intron 19**	***BRAF* intron 8**	***BRAF* intron 9**	***BRAF* intron 10**
All repetitive elements	184.1	214.2	173.4	99.01	208.2	91.12	226.1
Alu	92.05	45.10	49.53	99.01	74.37	45.56	-
MIR	-	11.28	74.29	-	29.75	-	-
L1	15.34	67.65	24.76	-	29.75	-	150.7
L2	-	33.83	-	-	44.62	-	-
LTR	-	45.10	-	-	-	-	-
DNA elements	61.37	-	-	-	-	-	-
Z-DNA	15.34	11.28	-	-	-	-	-

Supplemental Table S5 List of analyzed DNA sequence motifs.

Motif Name	Sequence	Fisher’s exact test adjusted p-value			
		KIAA1549	***BRAF***	**Either**	**Both**
Translin target site (Aoki et al. 1995)	ATGCAG	1	1	1	1
Translin target site (Aoki, et al. 1995)	GCCCWSSW	1	1	1	1
Pur-binding site (Bergemann and Johnson 1992)	GGNNGAGGGAGARRRR	1	1	1	1
Human replication origin consensus (Dobbs et al. 1994)	WAWTTDDWWWDHWGWHMAWTT	1	1	1	1
Heptamer recombination signal (Early et al. 1980)	CACAGTG	1	1	1	1
Nonamer recombination signal (Early, et al. 1980)	ACAAAAACC	1	1	1	1
Murine LTR recombination hotspot (Edelmann et al. 1989)	TGGAAATCCCC	1	1	1	1
ARS consensus *Saccharomyces cerevisiae* (Gale et al. 1992)	WTTTATRTTTW	1	0.22	0.83	1
ARS consensus *Schizosaccharomyces pombe* (Gale, et al. 1992)	WRTTTATTTAW	1	1	1	1
Consensus SAR 1 (Gale, et al. 1992)	AATAAAYAAA	1	1	1	1
Consensus SAR 2 (Gale, et al. 1992)	TTWTWTTWTT	1	0.97	1	1
Consensus SAR 3 (Gale, et al. 1992)	WADAWAYAWW	1	0.36	1	1
Consensus SAR 4 (Gale, et al. 1992)	TWWTDTTWWW	1	1	1	1
Murine parvovirus recombination hotspot (Hogan and Faust 1986)	CTWTTY	1	1	1	1
Human hypervariable minisatellite core sequence (Jeffreys et al. 1985)	GGGCAGGANG	1	1	1	1
Deletion hotspot consensus sequence (Krawczak and Cooper 1991)	TGRRKM	1	1	1	1
Human minisatellite conserved sequence/χ-like element (Krowczynska et al. 1990)	GCWGGWGG	0.040	1	0.58	1
DNA polymerase α frameshift hotspot 1 (Kunkel 1985b)	TCCCCC	1	1	1	1
DNA polymerase α frameshift hotspot 2 (Kunkel 1985b)	CTGGCG	1	1	1	1
DNA polymerase β frameshift hotspot 1 (Kunkel 1985a)	ACCCWR	1	1	1	1
DNA polymerase α/β frameshift hotspot 1 (Kunkel 1985a; Kunkel 1985b)	TGGNGT	1	1	1	1
DNA polymerase α/β frameshift hotspot 2 (Kunkel 1985a; Kunkel 1985b)	ACCCCA	1	1	1	1
Recombination hotspot (Myers et al. 2008)	CCNCCNTNNCCNC	1	1	1	1
Immunoglobulin heavy chain class switch repeat 1 (Ohno 1981; Rabbitts et al. 1981)	GAGCT	1	1	1	1
Immunoglobulin heavy chain class switch repeat 2 (Ohno 1981; Rabbitts, et al. 1981)	GGGCT	1	1	1	1
Immunoglobulin heavy chain class switch repeat 3 (Ohno 1981; Rabbitts, et al. 1981)	GGGGT	1	1	1	1
Immunoglobulin heavy chain class switch repeat 4 (Ohno 1981; Rabbitts, et al. 1981)	TGGGG	1	1	1	1
Immunoglobulin heavy chain class switch repeat 5 (Ohno 1981; Rabbitts, et al. 1981)	TGAGC	1	1	1	1
Mariner transposon-like element (3'end) (Reiter et al. 1996)	GAAAATGAAGCTATTTACCCAGGA	1	1	1	1
XY32 homopurine-pyrimidine H-palindrome motif (Rooney and Moore 1995)	AAGGGAGAARGGGTATAGGGRAAGAGGGAA	1	1	1	1
Drosophila topoisomerase II consensus cleavage site (Sander and Hsieh 1985)	GTNWAYATTNATNNR	1	1	1	1
Vaccinia topoisomerase I consensus cleavage site (Shuman 1991)	YCCTT	1	1	1	1
Vertebrate topoisomerase II consensus cleavage site (Spitzner and Muller 1988)	RNYNNCNNGYNGKTNYNY	1	1	1	1
Ade6-M26 (Schuchert et al. 1991)	ATGACGT	1	1	1	1
Murine MHC recombination hotspot (Steinmetz et al. 1986)	CAGRCAGR	1	1	1	1
Duplication motif 2 (Vissers et al. 2009)	WSCAKVTVBHNYKHV	1	1	1	1
Duplication motif 3 (Vissers, et al. 2009)	RTTYYHYSRSBNHTKGMYHW	1	1	1	1
DNA polymerase arrest site (Weaver and DePamphilis 1982)	WGGAG	1	1	1	1
Retrotransposon (Zimmerer and Passmore 1991)	TCATACACCACGCAGGGGTAGAGGACT	1	1	1	1

Supplemental Table S6 List of primers.

Primer	**Primer sequence**
Primers for *RAF* gene fusion detection in cDNA	
KIAA1549 cDNA nested 1	GAGGGACGCAGGAGATAAGA
KIAA1549 cDNA nested 2	CCAGGAAGAGCTCACGGATA
BRAF cDNA nested 1	AAGTAATCCATGCCCTGTGC
BRAF cDNA nested 2	TGCTGAGGTGTAGGTGCTGT
SRGAP3 cDNA	TGGCAGTAACCTCATCACCA
RAF1 cDNA	GGTTGGGTCGACAACCTTTA
Primers in *KIAA1549* intron 15 for DNA fusion detection	
KIAA1549 intron 15 DNA 1	CGTCCACAACTCAGCCTACA
KIAA1549 intron 15 DNA 2	CAGAGGGTTATTTGGTTTTGTTG
KIAA1549 intron 15 DNA 3	TGGGAGAAAGAGGTTAATGGAA
KIAA1549 intron 15 DNA 4	CCTTATGCAACCAGCCATTT
KIAA1549 intron 15 DNA 5	TTCATTTGCCTGCTTCAGTG
KIAA1549 intron 15 DNA 6	CAGGCCTTGGTAGAAATCCA
KIAA1549 intron 15 DNA 7	AACACCCACAAAGGACTGCT
KIAA1549 intron 15 DNA 8	TACCAGTGGGCTGTTCTTCC
KIAA1549 intron 15 DNA 9	TAGGCCAAGCAAAACCTGTC
KIAA1549 intron 15 DNA 10	CAATTTGAACCTTTGCGTTTT
KIAA1549 intron 15 DNA 11	TGCACACATACGTAGGCTTTTT
KIAA1549 intron 15 DNA 12	TTTGGCTGTTTAGGGGCTTT
Primers in *KIAA1549* intron 16 for DNA fusion detection	
KIAA1549 intron 16 DNA 1	CAGTGGGGGTCCTTCTACAG
KIAA1549 intron 16 DNA 2	AAACCGTGGTTGCATTTGAT
KIAA1549 intron 16 DNA 3	GGGGGATTTTATGGAAGCAT
KIAA1549 intron 16 DNA 4	ACATGAAATTGGGTGGCAAT
KIAA1549 intron 16 DNA 5	TTTCAGGAAATGCATGGTACA
KIAA1549 intron 16 DNA 6	GCTGGGTTTACCTTCCTTCC
KIAA1549 intron 16 DNA 7	ATCAGTTTCTGTTCGTGTGTGTG
KIAA1549 intron 16 DNA 8	AGCACAGATGGGGAAAACTG
KIAA1549 intron 16 DNA 9	CCCCTATTAAAGGGCAGAGC
KIAA1549 intron 16 DNA 10	GCATTCAGTTTATCTGGAAGACA
KIAA1549 intron 16 DNA 11	CCAAAGAAAACAGCAGCACA
KIAA1549 intron 16 DNA 12	GGAGAGAGTGAGGGGGAGAG
KIAA1549 intron 16 DNA 13	AACCAGGTGCTTCAAGGATG
KIAA1549 intron 16 DNA 14	AAGAATCAGCGAGCCTTCAA
KIAA1549 intron 16 DNA 15	GGGGATGAGATGAATCTGGA
KIAA1549 intron 16 DNA 16	GGCACCTGTAGTCCCAGCTA
KIAA1549 intron 16 DNA 17	AAAGAAATGAGGGGATATGCTTC
KIAA1549 intron 16 DNA 18	AGGTGAGAGGGAGCAGTTGA
KIAA1549 intron 16 DNA 19	TTGTTCACTGCTGGTCTCTGA
KIAA1549 intron 16 DNA 20	TTGTTTGTTTCCCCCTTGAC
KIAA1549 intron 16 DNA 21	ACTCTGTCGGCCTGTGTTTT
KIAA1549 intron 16 DNA 22	AACCCATACTGTGCCGAAAG
KIAA1549 intron 16 DNA 23	GCCAGTCTGTGTGACTCCAA
KIAA1549 intron 16 DNA 24	CAACTTCAGAAGCAAGGCTGA
KIAA1549 intron 16 DNA 25	TTCCAAAAGGAACAATTCACAG
KIAA1549 intron 16 DNA 26	GGGACCAGTAAACGGTCAGA
Primers in *KIAA1549* intron 18 for DNA fusion detection	
KIAA1549 intron 18 DNA 1	TGCTGCCAGAGGGATCTACT
KIAA1549 intron 18 DNA 2	CAGCACCTCACGATCAAGAA
KIAA1549 intron 18 DNA 3	GGAAAATGACGTGCCAAGAT

KIAA1549 intron 18 DNA 4	GAATCAAGGCTCAGCGGTAG
KIAA1549 intron 18 DNA 5	AATTGAGGTTGTCGGTCAGG
KIAA1549 intron 18 DNA 6	GCTGGGGGAGTGGTTTTAAT
KIAA1549 intron 18 DNA 7	GGTAGAAGTGCGCAGGAAAG
Primers in *KIAA1549* intron 19 for DNA fusion detection	
KIAA1549 intron 19 DNA 1	GAGCTCAGCCAGTGGAAATC
KIAA1549 intron 19 DNA 2	CCTCCTGGTGTTTTGGCTTA
KIAA1549 intron 19 DNA 3	TTTGCCCAGCTTAGCTTCAT
KIAA1549 intron 19 DNA 4	TATGCGGCAAATATCAGCAA
Primers in *BRAF* intron 8 for DNA fusion detection	
BRAF intron 8 DNA 1	CACCACGAAATCCTTGGTCT
BRAF intron 8 DNA 2	ATGTCCATTTCAGGCCATTC
BRAF intron 8 DNA 3	GTGGCACATGTTGACCTACG
BRAF intron 8 DNA 4	TGGCTTCATCAATCCTTAAAAA
BRAF intron 8 DNA 5	GGCATTTTGACATATTTTGGTT
BRAF intron 8 DNA 6	TGGATGGATAAGGGCACACT
BRAF intron 8 DNA 7	GTGGCGTGATCTTGGCTTAT
BRAF intron 8 DNA 8	GCACGCGACTGTAATCCTAGA
BRAF intron 8 DNA 9	AACGATGGTAGTGGGGACAG
BRAF intron 8 DNA 10	TGAATGGCCCCAAATATCTC
BRAF intron 8 DNA 11	TTGGGGTTTCAGAGATAGGC
BRAF intron 8 DNA 12	CAAACCACTATGGAAGGCAAA
BRAF intron 8 DNA 13	TCCTTCACAGCCAAGCTTCT
BRAF intron 8 DNA 14	TCAAGGTCCTTATATCCAACTGC
BRAF intron 8 DNA 15	TAGTCTCCCTCCCCAAAACA
BRAF intron 8 DNA 16	GGTTGTCCCTTCTCTGCTCA
BRAF intron 8 DNA 17	CCCAACATACCAGGGAACAG
BRAF intron 8 DNA 18	GCCAAGCTTATGATAGCACAAAT
BRAF intron 8 DNA 19	AACTTCTGGGTCAAGCGTTC
Primers in *BRAF* intron 9 for DNA fusion detection	
BRAF intron 9 DNA 1	AGCAAAAAGCAGTGCCGTAG
BRAF intron 9 DNA 2	GAAAGGATGGGCCAAAAAG
BRAF intron 9 DNA 3	CATTGGTTCCTGGAGGCTTA
BRAF intron 9 DNA 4	GCCACTATCTTTCAGATAACAAGGA
BRAF intron 9 DNA 5	TCTAAAGCACCATTACTACATTCAGA
BRAF intron 9 DNA 6	GGCAAGATGAAATGGGAGAA
BRAF intron 9 DNA 7	CAGAAAGCTTGTGGCAGTGA
BRAF intron 9 DNA 8	GGACAAGAAATGCCACTTGC
BRAF intron 9 DNA 9	GGCAGAAGACAACAGGGAAG
Primers in *BRAF* intron 10 for DNA fusion detection	
BRAF intron 10 DNA 1	TGTCACAATGTCACCACATTACA
BRAF intron 10 DNA 2	TGCCTGAGAGGGATACAGGA
BRAF intron 10 DNA 3	TCTCTTAATGCTGTCCCTCTCC
BRAF intron 10 DNA 4	TTTATGGGGTATGAGCAGGAA
BRAF intron 10 DNA 5	GGAGCAAGATAAGTGCTGCT
BRAF intron 10 DNA 6	CTTTGGTAGGGTAAAAGATCCTC
BRAF intron 10 DNA 7	AGCAAAAAGCAGTGCCGTAG

Primers in *SRGAP3* intron 10 for DNA fusion detection	
SRGAP3 intron 10 DNA 1	CCTGAGCTCCACTCTTGGAC
SRGAP3 intron 10 DNA 2	TTCTCCCTTTTCACCCAGTG
SRGAP3 intron 10 DNA 3	CCTGGCCTCAAGTGATTCTC
SRGAP3 intron 10 DNA 4	CCCAGGAGTTGGAGGTTACA
SRGAP3 intron 10 DNA 5	GCCCAGGCTTCTTCCTCTAT
SRGAP3 intron 10 DNA 6	GGTGTTCTTCCTGCCCTACA
SRGAP3 intron 10 DNA 7	TTTCCCCTGCAAAGAAAAGT
Primers in *SRGAP3* intron 11 for DNA fusion detection	
SRGAP3 intron 11 DNA 1	CCAGGTGAGAGTGGGAGAGT
SRGAP3 intron 11 DNA 2	CTTTTGGGGCTGCTGTGT
SRGAP3 intron 11 DNA 3	CAGACCAGAGCCAGTGAACA
SRGAP3 intron 11 DNA 4	AAGGGAGTTGGGCAAAGTCT
SRGAP3 intron 11 DNA 5	CCCACTTAGACACCCTGCAT
SRGAP3 intron 11 DNA 6	CCCTCGTGGTCTGGATTAGA
SRGAP3 intron 11 DNA 7	TCCTTTGCTTTCTGGATTGG
SRGAP3 intron 11 DNA 8	GGCTCACTGGCCTTGTATGT
SRGAP3 intron 11 DNA 9	TGATTGGCTGCATAGTCTGG
SRGAP3 intron 11 DNA 10	TGGGGTAGATGAGTCCTTGG
SRGAP3 intron 11 DNA 11	TAGCTGGGTGTCGTGGTACA
Primers in *RAF1* intron 7 for DNA fusion detection	
RAF1 intron 7 DNA 1	CAGTTGGAGTGCAATGGCTA
RAF1 intron 7 DNA 2	TTTTCCTATTGTTCCTGGAGTC
RAF1 intron 7 DNA 3	CCACGATTGACTGAGCATGT
RAF1 intron 7 DNA 4	CCCTGCTCCCAAAACATAAT
RAF1 intron 7 DNA 5	TCTCAGCCCTCAGCTCTTTC
RAF1 intron 7 DNA 6	AAGAGGAGGAGGATGTGCAA
Primers for sequencing unaffected alleles of *KIAA1549*, *BRAF*, *SRGAP3* and *RAF1*	
KIAA1549 intron 16 DNA 1 R	CCTAAATTGCGGGGATTACA
KIAA1549 intron 16 DNA 2 R	TGAAACTTTTCTGGAGACATCAA
KIAA1549 intron 16 DNA 3 R	GTACTTCCCACCACCAGGAA
KIAA1549 intron 16 DNA 4 R	GCAGCTTTAAGTTGGGGCTA
KIAA1549 intron 16 DNA 5 R	TGTCTTCCAGATAAACTGAATGC
KIAA1549 intron 16 DNA 6 R	TGCAGTGGCACTATCTCAGC
KIAA1549 intron 16 DNA 7 R	CCCTCCTCCTTCCCATTAAA
KIAA1549 intron 16 DNA 8 R	CCCTGCCACATTTCTTCTGT
KIAA1549 intron 16 DNA 9 R	AACACGAACCTGCCAAAAAT
KIAA1549 intron 16 DNA 10 R	CCCTGGGCCTATTCCTTTAT
KIAA1549 intron 16 DNA 11 R	TGCCACATGATTTAAGGAAACA
KIAA1549 intron 16 DNA 12 R	GCAGCACGTTCTGTTTCTGA
KIAA1549 intron 16 DNA 13 R	TTAGCATGTTACCGGGCACT
BRAF intron 8 DNA 1 R	CATTGGCAAGTGCTTCAAAA
BRAF intron 8 DNA 2 R	GGCGACAGAGCAACTGAGTC
BRAF intron 8 DNA 3 R	TAGCGACTGTCCCCACTACC
BRAF intron 8 DNA 4 R	GAGATATTTGGGGCCATTCA
BRAF intron 8 DNA 5 R	TGATGAAGGGAAGGGATGTAA
BRAF intron 8 DNA 6 R	CCTCTCGAGTAGCTGCGATT
BRAF intron 8 DNA 7 R	GGTTCAAACCTGGCCTTTCT

BRAF intron 8 DNA 8 R	GCATCATCTGTTCTCTTGTTCC
BRAF intron 8 DNA 9 R	AAGGACTGATAGAAGCCCTTGA
BRAF intron 10 DNA 1 R	TTTTTCCATCCTAATATTGACTTCAG
BRAF intron 10 DNA 2 R	GGGGAGAGGGACAGCATTAA
BRAF intron 10 DNA 3 R	CATGAAGAAATTTAGCACTGATTGA
SRGAP3 intron 10 DNA 1 R	AGCGGGCTGTAGTTTGAGAA
SRGAP3 intron 11 DNA 1 R	CAATACTGTCTCACCCGATGG
SRGAP3 intron 11 DNA 2 R	TCTGAAAGGGAAGTAAAGTCAGC
SRGAP3 intron 11 DNA 3 R	ACAGCTGTACGGGCTGAACT
SRGAP3 intron 11 DNA 4 R	GTGACACAAGGCCAAAGGTT
RAF1 intron 7 DNA 1 R	TGCTTTAAAAACAAAGAACAGCTT

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