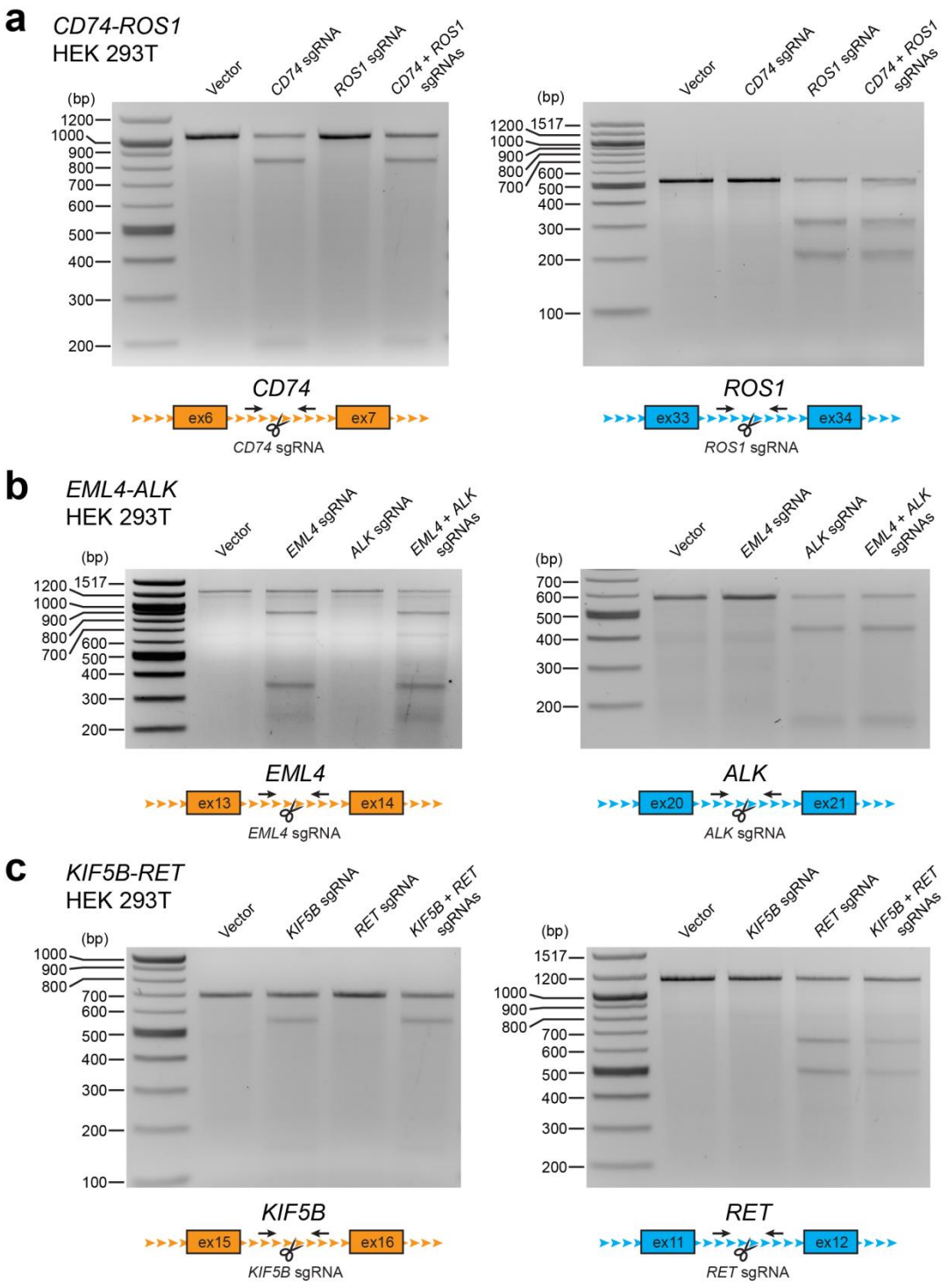


Supplementary Figure 1 – Surveyor assay for each targeted genomic region. a-c) HEK 293T cells were transfected with constructs expressing Cas9 alone (vector) or with a targeting sgRNA. For each sgRNA target site, the surrounding genomic region was amplified by PCR and subjected to the Surveyor assay to assess the formation of indels.

a *CD74-ROS1* Der(6) genomic breakpoint sequences (293T)

```
EXPECTED   ATTCAGGGTCCTGAAGTAGAAGGT-CACAGGCTGGATTACTTAATCCCT
CLONE 1    ATTCAGGGTCCTGAAGTAGAAGGTTCACAGGCTGGATTACTTAATCCCT
CLONE 2    ATTCAGGGTCCTGAAGTAGAAGGT-CACAGGCTGGATTACTTAATCCCT
CLONE 3    ATTCAGGGTCCTGAAGTAGAAGGT-CACAGGCTGGATTACTTAATCCCT
CLONE 4    ATTCAGGGTCCTGAAGTAGAAGGT-CACAGGCTGGATTACTTAATCCCT
CLONE 5    ATTCAGGGTCCTGAAGTAGAAGGT-CACAGGCTGGATTACTTAATCCCT
CLONE 6    ATTCAGGGTCCTGAAGTAGAAGGT-CACAGGCTGGATTACTTAATCCCT
CLONE 7    ATTCAGGGTCCTGAAGTAGAAGGT-CACAGGCTGGATTACTTAATCCCT
CLONE 8    ATTCAGGGTCCTGAAGTAGAAG--T-CACAGGCTGGATTACTTAATCCCT
CLONE 9    ATTCAGGGTCCTGA--------------------ATTACTTAATCCCT
CLONE 10   ATTCAGG----------------------GTGGATTACTTAATCCCT
```

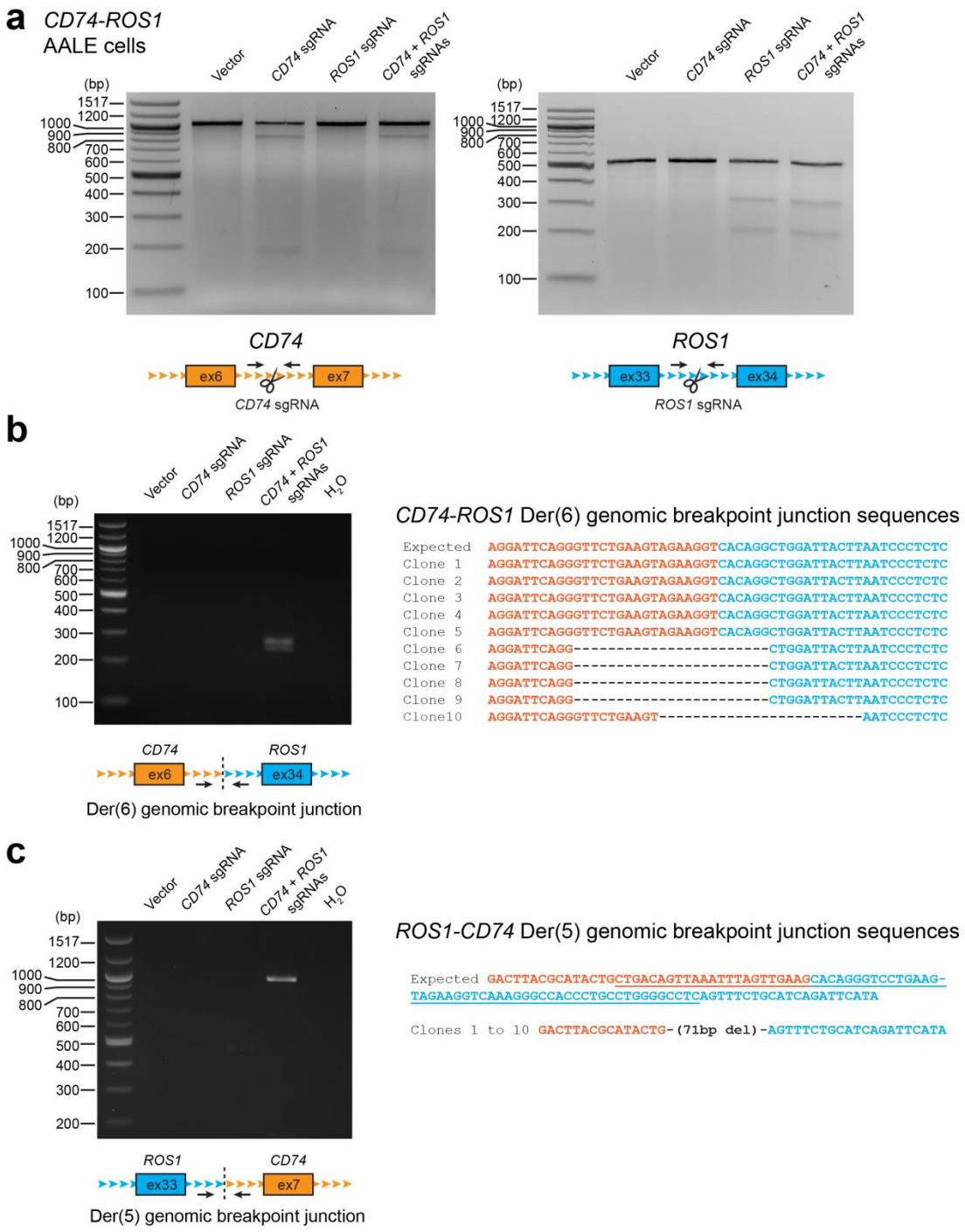
b *ROS1-CD74* Der(5) genomic breakpoint sequences (293T)

```
EXPECTED   ACAGTTAAATTTAGTTGAAG-CAAAGGGCCACCCTGCCTGGGGCCTCAG
CLONE 1    ACAGTTAAATTTAGTTGAAGTCAAAGGGCCACCCTGCCTGGGGCCTCAG
CLONE 2    ACAGTTAAATTTAGTTGAAG-CAAAGGGCCACCCTGCCTGGGGCCTCAG
CLONE 3    ACAGTTAAATTTAGTTGAAG-CAAAGGGCCACCCTGCCTGGGGCCTCAG
CLONE 4    ACAGTTAAATTTAGTTGAAG-CAAAGGGCCACCCTGCCTGGGGCCTCAG
CLONE 5    ACAGTTAAATTTAGTTGAA---------------GGCCTGGGGCCTCAG
CLONE 6    ACAGTTAAATTTAGTTGAA---------------GGCCTGGGGCCTCAG
CLONE 7    ACAGTTAAATTTAGT--------------------GCCTGGGGCCTCAG
CLONE 8    ACAGTTAAATTTAGT--------------------GCCTGGGGCCTCAG
```

c *CD74-ROS1* fusion transcript sequence (293T)

```
ACACCATGGAGACCATAGACTGGAAGGTCTTTGAGAGCTGGATGCACCATTGGCTCCTGT
TTGAAATGAGCAGGCACTCCTTGGAGCAAAAGCCCACTGACGCTCCACCGAAAGATGATT
TTTGGATACCAGAAACAAGTTTCATACTTACTATTATAGTTGGAATATTTCTGGTTGTTA
CAATCCCACTGACCTTTGTCTGGC
```

Supplementary Figure 2 – Sequences of *CD74-ROS1* genomic breakpoints and cDNA fusion transcripts. a-b) Alignment of sequences from cloned PCR products corresponding to the *CD74-ROS1* Der(6) and *ROS1-CD74* Der(5) genomic breakpoint junctions. c) Sequence of PCR product corresponding to the *CD74-ROS1* cDNA fusion transcript.



Supplementary Figure 3 – *CD74-ROS1* translocation generated in immortalized lung epithelial cells. AALE cells were transfected with constructs expressing Cas9 alone (vector) or with *CD74* sgRNA only, *ROS1* sgRNA only, or both *CD74* and *ROS1* sgRNAs. a) The region surrounding the targeted site was amplified and subjected to the Surveyor assay to assess indel formation. b-c) PCR detection of the b) *CD74-ROS1* Der(6) and c) *ROS1-CD74* Der(5) genomic breakpoint junctions. Also shown are sequences of each PCR product. Data shown are representative results from a total of two independent experiments.

a *EML4-ALK* genomic breakpoint PCR sequence

GTGAACACAGTTGTGTTGTTCAATTTTTAAGGTATTTTTAGATGATAAATATTGATGTAAGTGGAGACAGTTG
ACCTGAACAGCAAGTTCTTAGGCTCCATGGCACCCAGGGTGCTTCCACCCAACCTTCCCTCCCTCCCTCGTTC
ACGTGGGGTTATACTTGCAACACAGTCTGCTGGTTCACCCAGCCTTCCCTGGCTCCCTCCCCATTTCCTCTCA
TGGGCATTTCTCCTAATAAAATCTGCAGACCATATTGGGTCTAATCCCATCTCCAGTCTGCTTCTTGGAGGAA
CCAGACTAACATGACTCTGCCCTATATAATACAAATAATTATTTTCCATATATCTGATTTTTAGCTTTGCATT
TACTTTAAATCATGCTTCAATTAAAGACACACCTTCTTTAATCATTTTATTAGTATTTCTAAGTATGATGGAA
AGGTTCAGAGCTCAGGGGAGGATATGGAGATCCAGGGAGGCTTCCTGTAGGAAGTGGCCTGTGTAGTGCTTCA
AGGGC

b *EML4-ALK* cDNA fusion transcript PCR sequence

GTGGAGTCATGCTTATATGGAGCAAAACTACTGTAGAGCCCACACCTGGGAAAGGACCTAAAGTGTACCGCCG
GAAGCACCAGGAGCTGCAAGCCATGCAGATGGAGCTGCAGAGCCCTGAGTACAAGCTGAGCAAGCTCCGCACC
TCGACCATCATGACCGACTACAACCCCAACTACTGCTTTGCTGGCAAGA

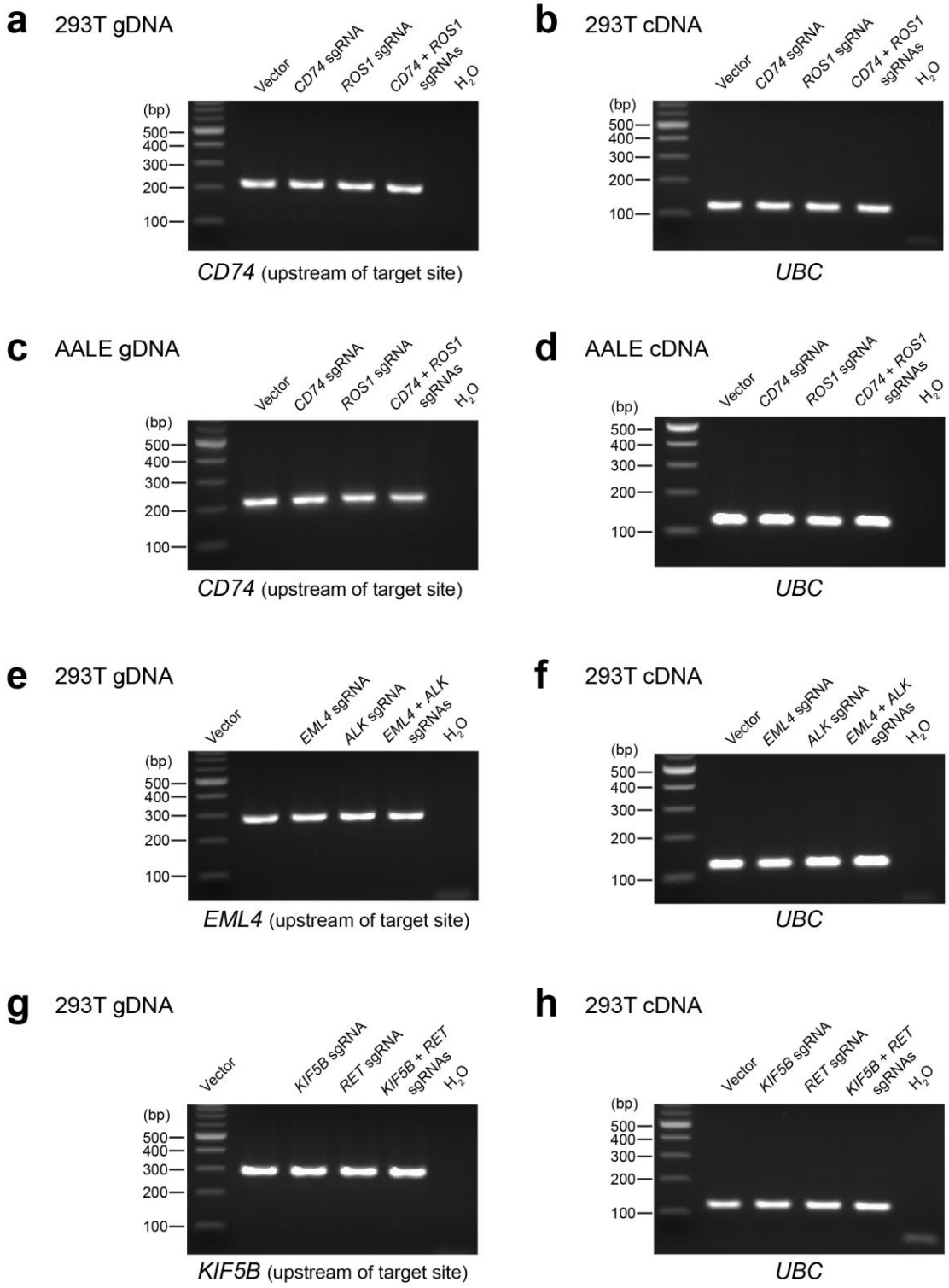
c *KIF5B-RET* genomic breakpoint PCR sequence

GCGTGTGCCACCACGCCTGGATAATTTTGTTTTTGTATTTTTAGTAGAGATGGAGTTTCACCGTGTTAGCCAGG
ATGGTCTTGATCTCCTGACCGGAGGTCCTGGGCTGGGCCTGGTGCTCATTTAGTCCTGGGGCAGGGGTCAGGGG
AGACAGTAGACCAGGAACCAGAGAGGGTCGAAGTAC

d *KIF5B-RET* cDNA fusion transcript PCR sequence

GGCATCTTTACTAAAAGACCTTGCAGAAATAGGAATTGCTGTGGGAAATAATGATGTAAAGGAGGATCCAAAGT
GGGAATTCCCTCGGAAGAACTTGGTTCTTGGAAAAACTCTAGGAGAAGGCGAATTTGGA

Supplementary Figure 4 – Sequences of *EML4-ALK* and *KIF5B-RET* genomic breakpoints and cDNA fusion transcripts. PCR products from detection of the *EML4-ALK* and *KIF5B-RET* genomic breakpoints (a,c) and cDNA fusion transcripts (b,d) were directly sequenced.



Supplementary Figure 5 – Control PCR reactions for all genomic DNA and cDNA samples. For each genomic DNA sample, a unique set of primers were designed to amplify a region upstream of one of the targeted sites to serve as a control PCR reaction (a,c,e,g). For all cDNA samples, the housekeeping gene *UBC* was amplified as the control (b,d,f,h). Primers are listed in Supplementary Table 1.

Supplementary Table 1 – Primers used in this study

Oligos for cloning of sgRNAs		
sgRNA target	**Forward primer (5' >> 3')**	**Reverse primer (5' >> 3')**
EML4	CACCGACCTGAACAGCAAGTTTGT	AAACACAAACTTGCTGTTCAGGTC
ALK	CACCGGCCTTGCTGAAACTTCCTT	AAACAAGGAAGTTTCAGCAAGGCC
KIF5B	CACCGGTGGGCAGATAAAGAGGTC	AAACGACCTCTTTATCTGCCCACC
RET	CACCGACACTTCCACTGTAGTCAG	AAACCTGACTACAGTGGAAGTGTC
CD74	CACCGTCCTGAAGTAGAAGGTCAA	AAACTTGACCTTCTACTTCAGGAC
ROS1	CACCGTTAAATTTAGTTGAAGCAC	AAACGTGCTTCAACTAAATTTAAC
Primers to amplify genomic breakpoint		
Target	**Forward primer (5' >> 3')**	**Reverse primer (5' >> 3')**
EML4-ALK	GTCCTCCCTCTCGTGGTAAC	GCCCTTGAAGCACTACACAG
KIF5B-RET	CTCACTATGGGGCCACTTGAC	GTACTTCGACCCTCTCTGGTTC
CD74-ROS1 Der(6)	CCAAGAGAGCCTTGGGCGTT	AAGACCTCACATGCCACAAAGAAG
ROS1-CD74 Der(5)	ACCACATCAATTCCATGCTCCTAA	TCTTCTGACACTCAAAGCCCATT
Primers to amplify cDNA fusions		
Target	**Forward primer (5' >> 3')**	**Reverse primer (5' >> 3')**
EML4-ALK	GTGCAGTGTTTAGCATTCTTGGGG	TCTTGCCAGCAAAGCAGTAGTTGG
KIF5B-RET	AGGAAATGACCAACCACCAG	TCCAAATTCGCCTTCTCCTA
CD74-ROS1	ATGCACCTGCTCCAGAATGC	GCCAGACAAAGGTCAGTGGG
Primers to amplify targeted region for surveyor assay		
Target	**Forward primer (5' >> 3')**	**Reverse primer (5' >> 3')**
EML4	TGGCAGGCAGTGTAAACTTGC	CAGATAGTGGTGATGGCTGCAC
ALK	CACCCTCAAATCCACTGCTG	GGCCCTTGAAGCACTACACAG
KIF5B	GGGTTCATGCCATTCTCCCA	CACCATGCTCAGCCTACACA
RET	CGCCCTCATGTGCTTATTGC	CTCGCTCTGCTTCTCTAGGC
CD74	GCTGCAGAGTATGTGGGGTT	AGCAATGGGCACCTTGGTAA
ROS1	CCAGCCTTATGACCACTCCT	GCAAACACAGGGCCAAAGAC
Primers for control PCRs		
Target	**Forward primer (5' >> 3')**	**Reverse primer (5' >> 3')**
EML4 upstream	GGAGCGGCAATTCACTAACA	CAGGTGTGGGCTCTACAGTA
KIF5B upstream	GAGCAGCTGAGATGATGGCA	AGGTCCAATAAATGCCGCCT
CD74 upstream	GCTGCAGAGTATGTGGGGTT	CAAACAGGAGCCAATGGTGC
UBC transcript	CGGGATTTGGGTCGCAGTTCTTG	CGATGGTGTCACTGGGCTCAAC