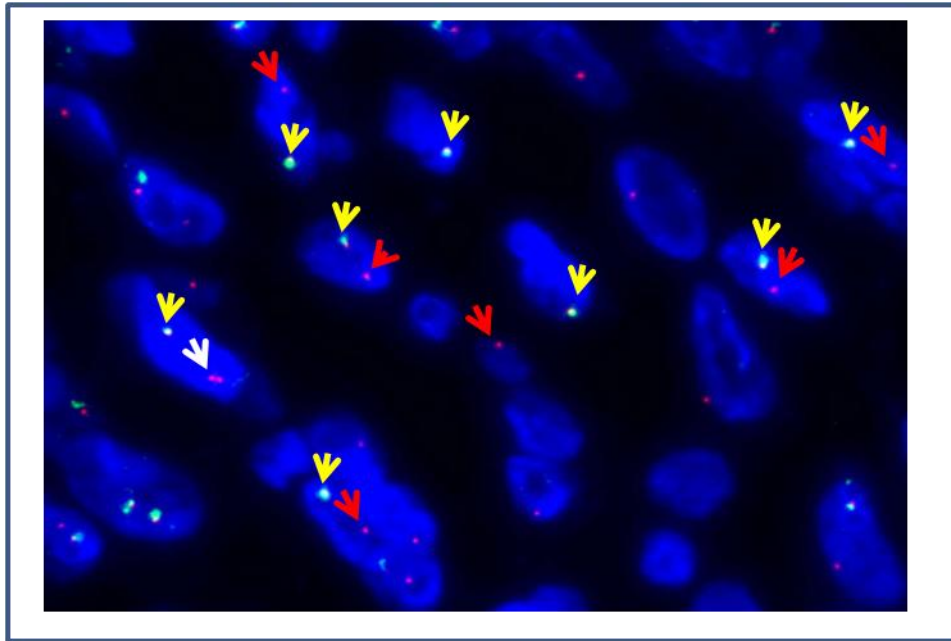


Supplementary Figure 1. ALK unbalanced translocation



The ALK unbalanced translocation in Epithelioid Rhabdomyosarcoma (sample n3, table 1) was demonstrated by FISH ALK break-apart fusion. The intact ALK alleles are indicated by the yellow arrows (green plus red signals) while red arrows indicate isolated red signals corresponding to 3' ALK derivatives. ALK Break Apart Probe was purchased from Vysis (cat: o8N16-001, Abbot Park, Illinois USA) and were hybridized according to manufacturer instructions

Supplementary Table 1. Table showing the probes used for FISH experiments

Gene	Company	Probe Name
<i>ALK</i>	Abbott Molecular	Vysis ALK Dual Color, Break Apart FISH Probe
<i>SS18</i>	Abbott Molecular	Vysis SS18 Dual Color, Break Apart Probe
<i>EWS</i>	Abbott Molecular	Vysis EWSR1 Dual Color, Break Apart Probe
<i>ROS1</i>	Zytovision	ZytoLight SPEC ROS1 Dual Color, Break Apart Probe
<i>EWSR1-FLI1</i>	Zytovision	ZytoLight PEC EWSR1/FLI1 TriChec Probe
<i>FGFR2</i>	Zytovision	ZytoLight SPEC FGFR2 Dual Color, Break Apart Probe
<i>FUS</i>	Zytovision	ZytoLight SPEC FUS Dual Color, Break Apart Probe
<i>RET</i>	Zytovision	ZytoLight SPEC RET Dual Color, Break Apart Probe
<i>SS18 SSX1</i>	Zytovision	ZytoLight SPEC SS18/SSX1 TriCheck Probe
<i>TFE3</i>	Zytovision	ZytoLight SPEC TFE3 Dual Color, Break Apart Probe
<i>YWHAE</i>	Zytovision	ZytoLight SPEC YWHAE Dual Color, Break Apart Probe

<i>ATF1</i>	BAC. In house labelled	RP11-834H3, RP11-787D7, RP11-991C19
<i>BCOR</i>	BAC. In house labelled	RP11-429N5, RP11-320G24
<i>CIC</i>	BAC. In house labelled	RP11-317E13, RP11-374A11
<i>CREB1</i>	BAC. In house labelled	RP11-703O8, RP11-237A5
<i>ETV6</i>	BAC. In house labelled	RP11-75N23, RP11-418C2
<i>NTRK3</i>	BAC. In house labelled	RP11-846G5, RP11-285I14, RP11-121M4
<i>NR4A3</i>	BAC. In house labelled	RP11-30L7, RP11-30N20
<i>NTRK1</i>	BAC. In house labelled	RP11-180B22, RP11-1038N13
<i>NCOA2</i>	BAC. In house labelled	RP11-183D21, RP11-774K7, RP11-479K21
<i>PHF1</i>	BAC. In house labelled	RP11-602P21, RP11-175A4.
<i>WWTR1</i>	BAC. In house labelled	RP11-941L15, RP11-1151O19

BA: break apart, F: fusion

Supplementary Table 2. Table showing the comparison between the fusions called by ADx, ARR and SFU in samples investigated by sarcoma panel

Samples	Sarcoma panel ADx	Sarcoma panel ARR	Sarcoma panel SFU	FISH
1	<i>ETV6-NTRK3</i>	<i>PLAG1-CHCHD7-ACACB*</i>	<i>FUS-GPT2*</i>	<i>ETV6-NTRK3</i> confirmed
2	<i>EWSR1-PATZ1</i>	<i>EWSR1-PATZ1</i>	<i>RAB7A-MFSD4*</i>	<i>EWSR1</i> translocated
3	<i>EWSR1-TFCP2</i>	<i>SLC34A2-ROS1</i>	<i>SLC34A2-ROS1</i>	<i>EWSR1</i> translocated
	<i>ALK Δ2-18</i>			<i>ALK</i> unbalanced translocation
4	<i>EWSR1-NR4A3</i>	no fusions	no fusions	<i>EWSR1-NR4A3</i> confirmed
5	<i>EWSR1-FLI1</i>	no fusions	<i>FUS-MFSD4*</i>	<i>EWSR1-FLI1</i> confirmed
6	<i>EWSR1-CREM</i>	<i>EWSR1-CREM</i>	no fusions	<i>EWSR1</i> translocated
7	<i>YWHAE-NUT2</i>	<i>HMGA2-HRK*</i>	no fusions	<i>YWHAE</i> translocated
8	<i>SS18-SSX4</i>	<i>SS18-SSX4</i>	<i>EWSR1-MFSD4*</i>	<i>SS18</i> intragenic rearrangement
9	<i>SS18-SSX1</i>	<i>SS18-SSX1</i>	<i>FUS-MFSD4*</i>	<i>SS18</i> not translocated
10	<i>CIC-DUX4</i>	<i>CIC-DUX4</i>	<i>FUS-MFSD4*</i>	<i>CIC</i> not translocated
11	<i>EWSR1-CREB3L2</i>	<i>EWSR1-CREB3L2</i>	no fusions	Not done
12	<i>EWSR1-FLI1</i>	<i>EWSR1-FLI1</i>	<i>FUS-DAOA-AS1*</i>	Not done
13	<i>NAB2-STAT 6</i>	no fusions	no fusions	Not detectable by FISH
14	<i>ETV6-NTRK3</i>	<i>ETV6-NTRK3</i>	<i>ETV6-NTRK3</i>	Not done
15	no fusions	no fusions	<i>FUS-MFSD4*</i> <i>FUS-DAOA-AS1*</i> <i>FUS-PRPSAP1*</i>	<i>SS18</i> , <i>EWSR1</i> , <i>FUS</i> negative
16	no fusions	no fusions	no fusions	<i>WWTR1</i> negative ^a
17	no fusions	no fusions	no fusions	<i>SS18</i> , <i>BCOR</i> , <i>CIC</i> negative
18	no fusions	no fusions	no fusions	<i>EWSR1</i> , <i>FUS</i> negative ^a
19	no fusions	no fusions	<i>FUS-PRPSAP1*</i>	<i>SS18</i> negative
20	no fusions	no fusions	<i>FUS-PRPSAP1*</i>	<i>EWSR1</i> , <i>FUS</i> , <i>ATF1</i> , <i>CREB1</i> negative

21	no fusions	no fusions	no fusions	Not done
22	no fusions	no fusions	no fusions	<i>EWSR1, BCOR, FUS, CIC, NCOA2</i> negative ^a
23	no fusions	no fusions	no fusions	Not done
24	no fusions	no fusions	no fusions	Not done
25	no fusions	<i>SLC34A2-ROS1</i>	<i>FUS-MFSD4</i> *	FUS negative ^a
26	no fusions	no fusions	no fusions	Not done
27	no fusions	no fusions	no fusions	ALK negative
28	no fusions	<i>FUS-KCNAB1</i> *	<i>FUS-MFSD5</i> *	<i>SS18, EWSR1, BCOR, CIC, TFE3</i> negative
29	not evaluable	<i>RREB1-MKL2</i> ^b	<i>RREB1-MKL2</i> ^b	<i>PHF1, FUS, CIC, BCOR, EWSR1, NR4A3</i> negative
30	not evaluable	<i>CTD-HMGA2</i> *	no fusions	<i>EWSR1, NCOA2, CIC, SS18, BCOR</i> negative
31	not evaluable	<i>FUS-RP11-541P9.3</i> *	no fusions	Not done
Total	14 fusions	16 fusions	17 fusions	

a: The FISH were performed on different samples respect to those used for NGS. b: The fusion was confirmed by FISH and RT-PCR that were performed after the *RREB1-MKL2* fusion identification by ARR and SFU.* Unknown/unpublished

Supplementary Table 3: table showing the ROS1, ALK and RET FISH performed in 31 out of 121 ADx negative samples

Samples	FISH		
	<i>ROS1</i>	<i>ALK</i>	<i>RET</i>
61	Translocated	neg	neg
62	Translocated	neg	neg
63	neg	neg	Translocated
64	neg	Not done	neg
65	neg	Not done	Not done
66	neg	neg	Not done
67	neg	Not done	Not done
68	neg	Not done	Not done
69	neg	neg	Not done
70	neg	neg	Not done
71	neg	neg	neg
72	neg	neg	neg
73	neg	neg	Not done
74	Not done	neg	Not done
75	Not done	Not done	neg
76	Not done	neg	Not done
77	neg	Not done	neg
78	neg	Not done	Not done
79	neg	neg	Not done
80	neg	neg	Not done

81	neg	neg	Not done
82	neg	Not done	Not done
83	neg	neg	Not done
84	neg	neg	Not done
85	neg	Not done	Not done
86	neg	Not done	neg
87	neg	neg	Not done
88	neg	Not done	neg
89	neg	Not done	Not done
90	hem del	neg	Not done
91	neg	neg	Not done

total RET:

total samples:31 total ROS1: 28 total ALK: 19 10

All the translocation detected by FISH were unbalanced. neg: Negative; hem del: ROS1 hemizygote deletion.

Supplementary Table 4. Table showing the fusion genes identified by ARR and SFU in the 21 ADx positive samples

Samples	Lung panel ADx	Lung panel ARR	Lung Panel SFU	FISH
32	<i>KIF5C-ALK</i>	<i>KIF5C-ALK</i>	no fusions	<i>ALK</i> confirmed
33	<i>EML4-ALK</i>	<i>EML4-ALK</i>	no fusions	<i>ALK</i> confirmed
34	<i>KIF5B-RET</i>	<i>KIF5B-RET</i>	no fusions	<i>RET</i> confirmed
35	<i>RET-NCOA4</i>	<i>RET-NCOA4</i>	no fusions	<i>RET</i> confirmed
36	<i>KIAA1468-RET</i>	<i>NTRK1- KCNK13*</i> <i>EGFR-FAM194B*</i>	no fusions	<i>RET</i> confirmed
37	<i>TPM3-NTRK1</i>	<i>TPM3-NTRK1</i>	<i>TPM3-NTRK1</i>	<i>NTRK1</i> confirmed
38	<i>FGFR2-TACC3</i>	<i>FGFR2-TACC3</i>	<i>FGFR2-TACC3</i>	<i>FGFR2</i> confirmed
39	<i>ETV6-NTRK3</i>	no fusions	no fusions	<i>ETV6-NTRK3</i> confirmed
40	<i>TPM3 -NTRK1</i>	<i>TPM3 -NTRK1</i>	<i>EGFR-TRIM13*</i>	<i>NTRK1</i> not confirmed
41	<i>KIAA1549-BRAF</i>	<i>KIAA1549-BRAF</i>	<i>BRAF-UBE2L3*</i>	Nd
42	<i>KIAA1549-BRAF</i>	<i>KIAA1549-BRAF</i>	no fusions	Nd
43	<i>EML4-ALK</i>	<i>EML4-ALK</i>	<i>EML4-ALK</i>	Nd
44	<i>TPR-NTRK1</i>	<i>TPR-NTRK1</i>	no fusions	Nd
45	<i>EML4-ALK</i>	<i>EML4-ALK</i>	no fusions	Nd
46	<i>EML4-ALK</i>	no fusions	no fusions	Nd
47	<i>ETV6-NTRK3</i>	<i>ETV6-NTRK3</i>	<i>ETV6-NTRK3</i>	Nd
48	<i>TPM3-NTRK1</i>	<i>TPM3-NTRK1</i>	<i>TPM3-NTRK1</i>	Nd
49	<i>TPM3-NTRK1</i>	<i>TPM3-NTRK1</i>	<i>TPM3-NTRK1</i>	Nd
50	<i>TPR-NTRK1</i>	<i>TPR-NTRK1</i>	no fusions	Nd
51	<i>ETV6-NTRK3</i>	<i>ETV6-NTRK3</i>	<i>ETV6-NTRK3</i>	Nd
52	<i>TFG-NTRK3</i>	<i>TFG-NTRK3</i>	<i>IGH-BRAF^a</i>	Nd

a: *IGH-BRAF* gene fusion was recently reported in one patient with Hairy Cells Leukemia V600E negative (*Thompson et al, Leukemia e Lymphoma 2020; 61:8, 2024-2026*). *: unknown-unpublished fusions; Nd: not done

Supplementary Table 5. Table showing the gene fusion called by ARR and SFU in ADx negative samples

Samples	Lung panel ADx	Lung panel ARR	Lung panel SFU
61 (supp. Tab2)	no fusions	<i>EGFR-NTRK3</i> *	no fusions
64 (supp. Tab2)	no fusions	<i>BRAF-KIAA^a</i>	<i>BRAF-RASA1</i> *
65 (supp. Tab2)	no fusions	<i>TNS3-ROS1</i> *	no fusions
66 (supp. Tab2)	no fusions	<i>SMARCB1-PDGFR^a</i> *	<i>FGFR3-MFSD4</i> *
69 (supp. Tab2)	no fusions	<i>CHMP2A->CTD-UQCRFS1</i> *	no fusions
		<i>SOX6->BRAF</i> *	
70 (supp. Tab2)	no fusions	<i>RP11-MYOZ2->ROS1</i> *	no fusions
71 (supp. Tab2)	no fusions	<i>PRMT3-EGFR</i> *	no fusions
92	no fusions	<i>JHDM1D-BRAF^b</i>	<i>JHDM1D-BRAF</i>
93	no fusions	<i>NRAS-BRAF</i> *	<i>NRAS-BRAF</i> *
94	no fusions	<i>SLC34A2-ROS1^c</i>	no fusions
95	no fusions	<i>KDR - RET</i> *	no fusions
96	no fusions	<i>MET-FGFR2</i> *	no fusions
97	no fusions	<i>ATM-FGFR2</i> *	no fusions
98	no fusions	<i>FLT3 -SMAD4</i> *	no fusions
99	no fusions	<i>EGFR -MFN2</i> *	no fusions
100	no fusions	<i>MET-SEMA3A</i> *	no fusions
101	no fusions	<i>RP11- SST-BRAF</i> *	no fusions
102	no fusions	<i>CLTCL1-ALK</i> *	no fusions
103	no fusions	<i>TNS3 - ROS1</i> *	no fusions
104	no fusions	<i>RP11-MYOZ2->ROS1</i> *	<i>BAI2-EGFR</i> *
105	no fusions	<i>ZNF609 - KRAS</i> *	no fusions
106	no fusions	<i>RP11-MYOZ2->ROS1</i> *	no fusions
107	no fusions	<i>RP11-MYOZ2->ROS1</i> *	no fusions
108	no fusions	<i>TRIO - FGFR1</i> *	no fusions
109	no fusions	<i>DSCC1-MET</i> *	no fusions
110	no fusions	<i>RP11-FGFR2</i> *	no fusions
		<i>MET->CTNND1- OR5BA1P</i> *	
111	no fusions	<i>ETV6-KRAS</i> *	no fusions
112	no fusions	<i>RP11-MYOZ2->ROS1</i> *	no fusions
113	no fusions	<i>RP11- BRAF</i> *	no fusions
		<i>FGFR2 - GABRG1</i> *	

114	no fusions	<i>HPSE2-ROS1*</i>	no fusions
116	no fusions	no fusions	<i>RAB7A-MFSD4*</i>
117	no fusions	no fusions	<i>RAB7A-MFSD4*</i>
118	no fusions	no fusions	<i>RAB7A-MFSD4*</i>
119	no fusions	no fusions	<i>MET-MFSD4*</i>
120	no fusions	no fusions	<i>RAB7A-MFSD4*</i>
121	no fusions	no fusions	<i>RAB7A-MFSD4*</i>
122	no fusions	no fusions	<i>CCNC-KRAS*</i>
123	no fusions	no fusions	<i>FUS-B3GALT5*</i>
124	no fusions	no fusions	<i>MBD1-MET*</i>
122	no fusions	no fusions	<i>RAB7A-ZNF618*</i>
125	no fusions	no fusions	<i>RORA-BRAF*</i>

a: *BRAF-KIAA* (sample 64) was identified in pilocytic astrocitoma (*Balasubramanian et al J Clin Neurosci. 2020 ;79:269-271*). b: *JHDM1D-BRAF* (sample 92) was previously reported in gastric cancer [*Sase et al Mol Cancer Ther. 2018;17(10):2217-2225*]. c: while *SLC34A2-ROS1* (case 94) has been reported in carcinomas [*Lee et al, Mod Pathol. 2015;(4):468-79 and Davies et al. Clin Cancer Res. 2012;18(17):4570-9*]. The prevalence of *RAB7A* and *MFSD4* genes in the fusions identified by SFU, strongly suggest that were false positive results. Supp. Tab2: sample already reported in Supplementary Table 3. * All these fusions were previously unreported/unpublished

Supplementary Table 6. Gene fusion identified by ARR and SFU in the 12 ADx not evaluable samples

Samples	Lung panel ADx	Lung panel ARR	Lung panel SFU
N63 (supp tab2)	not evaluable	<i>SLC34A2-ROS1</i>	<i>SLC34A2-ROS1</i>
N73 (supp.tab2)	not evaluable	<i>BRAF-RDX*</i>	no fusions
		<i>ABHD11-CLDN3->NTRK2*</i>	
		<i>MET->CTD-TMEM86B*</i>	
		<i>AL356154.1-NRG3->NTRK3*</i>	
		<i>PLSCR4-RP11->EGFR*</i>	
N88 (supp.tab2)	not evaluable	no fusions	no fusions
N89 (supp.tab2)	not evaluable	no fusions	no fusions
126	not evaluable	<i>SLC34A2-ROS1</i>	<i>SLC34A2-ROS1</i>
127	not evaluable	<i>PSMD6,RP11-129B22.1-NRG1*</i>	no fusions
128	not evaluable	<i>NRG1->CSNK1A1L-RN7SKP1*</i>	no fusions
		<i>NTRK2->RP11- RP11*</i>	

129	not evaluable	<i>RET-KIF5B</i> ^a	no fusions
130	not evaluable	no fusions	no fusions
131	not evaluable	no fusions	no fusions
132	not evaluable	no fusions	no fusions
133	not evaluable	no fusions	no fusions

a: The *RET-KIF5B* translocation (case n 129) was previously published (*Velcheti V et al, Thorac Oncol. 2017;12(7):e98-e99.v*) in a lung adenocarcinoma patient. Supp. Tab2: sample already reported in Supplementary Table 3. *: all these fusions were previously unreported/unpublished

The parameters used for Arriba:

```

export version=1.8.1
export sample=$PATIENT.fastq
export folder=$PATIENT
STAR --runThreadN 8
      --genomeDir
      $HOME/genome/GRCh37_gencode_v19_CTAT_lib_Apr032020.plugin-play/ctat_ge
      nome_lib_build_dir/ref_genome.fa.star.idx
      --genomeLoad NoSharedMemory
      --readFilesIn $HOME/fastq/$sample
      --outTmpDir $HOME/NGSeqINT/temp$folder
      --outStd BAM_Unsorted
      --outSAMtype BAM Unsorted
      --outSAMunmapped Within
      --outBAMcompression 0
      --outFilterMultimapNmax 1
      --outFilterMismatchNmax 3
      --chimSegmentMin 10
      --chimOutType WithinBAM SoftClip
      --chimJunctionOverhangMin 10
      --chimScoreMin 1
      --chimScoreDropMax 30
      --chimScoreJunctionNonGTAG 0
      --chimScoreSeparation 1
      --alignSJstitchMismatchNmax 5 -1 5 5
      --chimSegmentReadGapMax 3 |
arriba -x /dev/stdin
      -o $HOME/analysis/$folder/ARRIBA.fusions.tsv
      -O $HOME/analysis/$folder/ARRIBA.fusions.discarded.tsv

```

-a

\$HOME/genome/GRCh37_gencode_v19_CTAT_lib_Apr032020.plug-n-play/ctat_genome_lib_build_dir/ref_genome.fa

-g

\$HOME/genome/GRCh37_gencode_v19_CTAT_lib_Apr032020.plug-n-play/ctat_genome_lib_build_dir/ref_annot.gtf

-b

\$HOME/arriba/arriba_v2.0.0/database/blacklist_hg19_hs37d5_GRCh37_v2.0.0.tsv.gz

z