

Supplementary Material

Multiplexed transcriptome analysis to detect *ALK*, *ROS1* and *RET* rearrangements in lung cancer

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Supplementary Table 1. Percent agreement metrics

| Positive percent agreement:PPA | Negative percent agreement: NPA | Overall percent agreement: OPA | | | | |
|--------------------------------|---------------------------------|--------------------------------|-----------------|-------------|-------------|-------------|
| FISH | | | | | | |
| | ALK | Positive | Negative | PPA | NPA | OPA |
| Nanostring | Positive | 15 | 1 | 94% | 97% | 96% |
| | Negative | 1 | 31 | | | |
| | Total | 16 | 32 | | | |
| | RET | Positive | Negative | PPA | NPA | OPA |
| Nanostring | Positive | 0 | 0 | N/A | 100% | 100% |
| | Negative | 0 | 48 | | | |
| | Total | 0 | 48 | | | |
| | ROS | Positive | Negative | PPA | NPA | OPA |
| Nanostring | Positive | 1 | 0 | 100% | 100% | 100% |
| | Negative | 0 | 47 | | | |
| | Total | 1 | 47 | | | |
| | All fusions | Positive | Negative | PPA | NPA | OPA |
| Nanostring | Positive | 16 | 1 | 94% | 97% | 96% |
| | Negative | 1 | 30 | | | |
| | Total | 17 | 31 | | | |

| | | | | | | |
|--------------|--------------------|-----------------|-----------------|-------------|-------------|-------------|
| FISH | | | | | | |
| | ALK | Positive | Negative | PPA | NPA | OPA |
| Agena | Positive | 16 | 1 | 94% | 97% | 96% |
| | Negative | 1 | 31 | | | |
| | Total | 17 | 32 | | | |
| | RET | Positive | Negative | PPA | NPA | OPA |
| Agena | Positive | 1 | 0 | 100% | 100% | 100% |
| | Negative | 0 | 48 | | | |
| | Total | 1 | 48 | | | |
| | ROS | Positive | Negative | PPA | NPA | OPA |
| Agena | Positive | 2 | 1 | 100% | 94% | 98% |
| | Negative | 0 | 46 | | | |
| | Total | 2 | 47 | | | |
| | All fusions | Positive | Negative | PPA | NPA | OPA |
| Agena | Positive | 19 | 2 | 95% | 93% | 94% |
| | Negative | 1 | 27 | | | |
| | Total | 20 | 29 | | | |

| | | | | | | |
|-------------------------|--------------------|-----------------|-----------------|-------------|------------|------------|
| FISH | | | | | | |
| | ALK | Positive | Negative | PPA | NPA | OPA |
| ThermoFisher NGS | Positive | 16 | 4 | 94% | 88% | 90% |
| | Negative | 1 | 30 | | | |
| | Total | 17 | 34 | | | |
| | RET | Positive | Negative | PPA | NPA | OPA |
| ThermoFisher NGS | Positive | 0 | 1 | N/A | 98% | 98% |
| | Negative | 0 | 50 | | | |
| | Total | 0 | 51 | | | |
| | ROS | Positive | Negative | PPA | NPA | OPA |
| ThermoFisher NGS | Positive | 2 | 1 | 100% | 98% | 98% |
| | Negative | 0 | 48 | | | |
| | Total | 2 | 49 | | | |
| | All fusions | Positive | Negative | PPA | NPA | OPA |
| ThermoFisher NGS | Positive | 18 | 5 | 90% | 84% | 86% |
| | Negative | 2 | 26 | | | |
| | Total | 20 | 31 | | | |

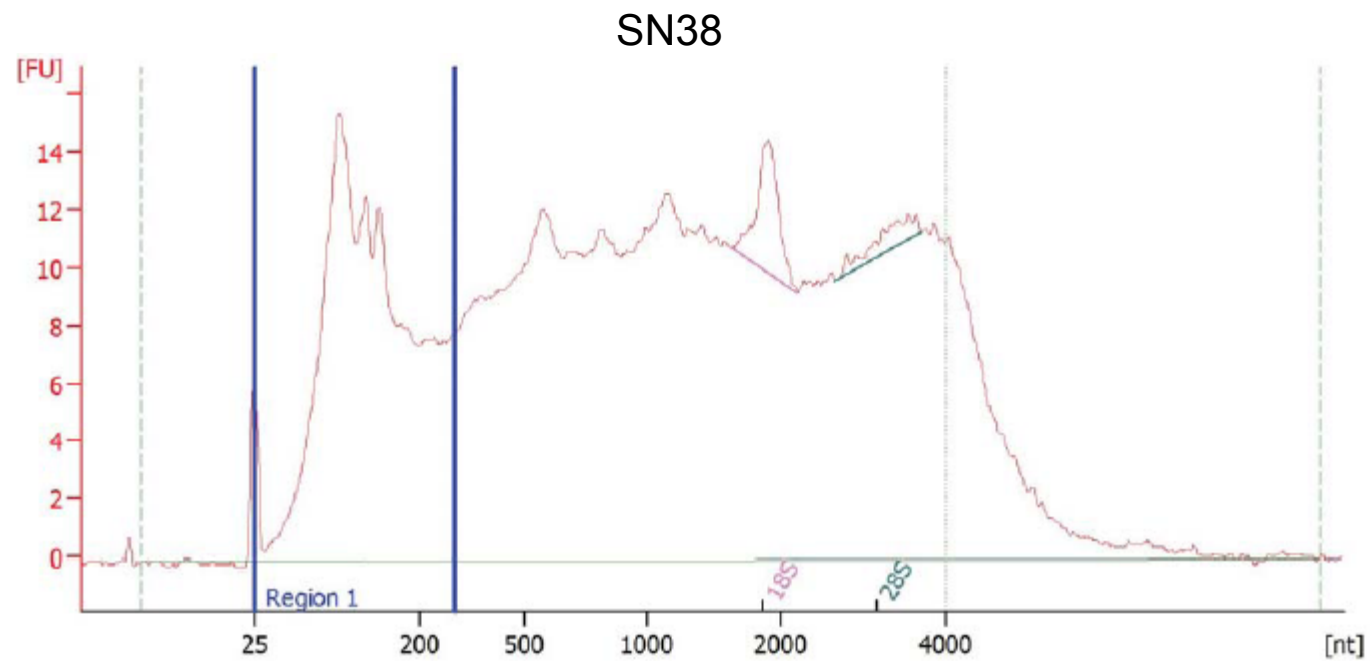
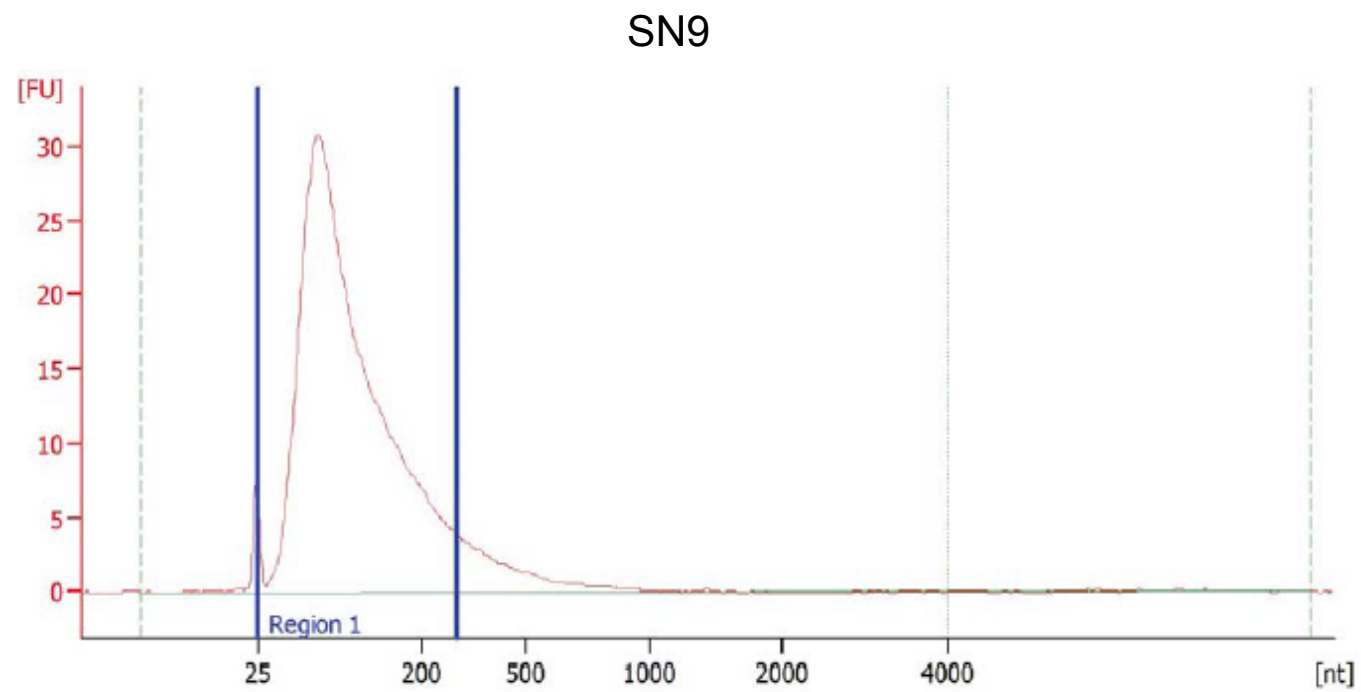
Supplementary Table 2. Description of positive control cell lines.

| Cell line name | Specific fusion |
|-----------------------|------------------------|
| H2228 (FFPE) | ALK/EML4 v. 3a & 3b |
| RT-122 | FGFR3-TACC3 |
| H3122 | ALK/EML4 v. 1 |
| HCC-78 | SLC34A2-ROS1 |

Supplementary Table 3. Specific fusion detected using the ALK, RET or ROS1 Variant specific Assay by Agena (AbA) Panels

| Gene | Variant |
|----------------|---------------------------|
| EML4;ALK | Ee13;Ae20 |
| | Ee20;Ae20 |
| | Ee6a/b;Ae20 |
| | Ee14;ins11del49Ae20 |
| | Ee2;Ae20 & Ee2;ins117Ae20 |
| | Ee13;ins69Ae20 |
| | Ee14;del12Ae20 |
| | Ee15del19;del20Ae20 |
| | Ee18;Ae20 |
| | |
| KIF;ALK | Ke17;Ae20 |
| | Ke24;Ae20 |
| | |
| KIF5B;RET | Ke15;Re12 |
| | Ke16;Re12 |
| | Ke22;Re12 |
| | Ke23;Re12 |
| | Ke24;Re11 |
| | |
| CCDC6;RET | Ce1;Re12 |
| | |
| FIG(GOPC);ROS1 | Fe7;Re35 |
| | Fe3;Re36 |
| | |
| TPM3;ROS1 | Te8;Re35 |
| | |
| SDCC4;ROS1 | Se2;Re32 |
| | Se4;Re32 |
| | Se4;Re34 |
| | |
| SLC34A2;ROS1 | Se12;Re32 |
| | Se12;Re34 |
| | Se4;Re32 |
| | Se4;Re34 |
| | |
| CD74;ROS1 | Ce6;Re32 |
| | Ce6;Re34 |
| | |
| EZR;ROS1 | Ee10;Re34 |
| | |
| LRIG3;ROS1 | Le16;Re35 |

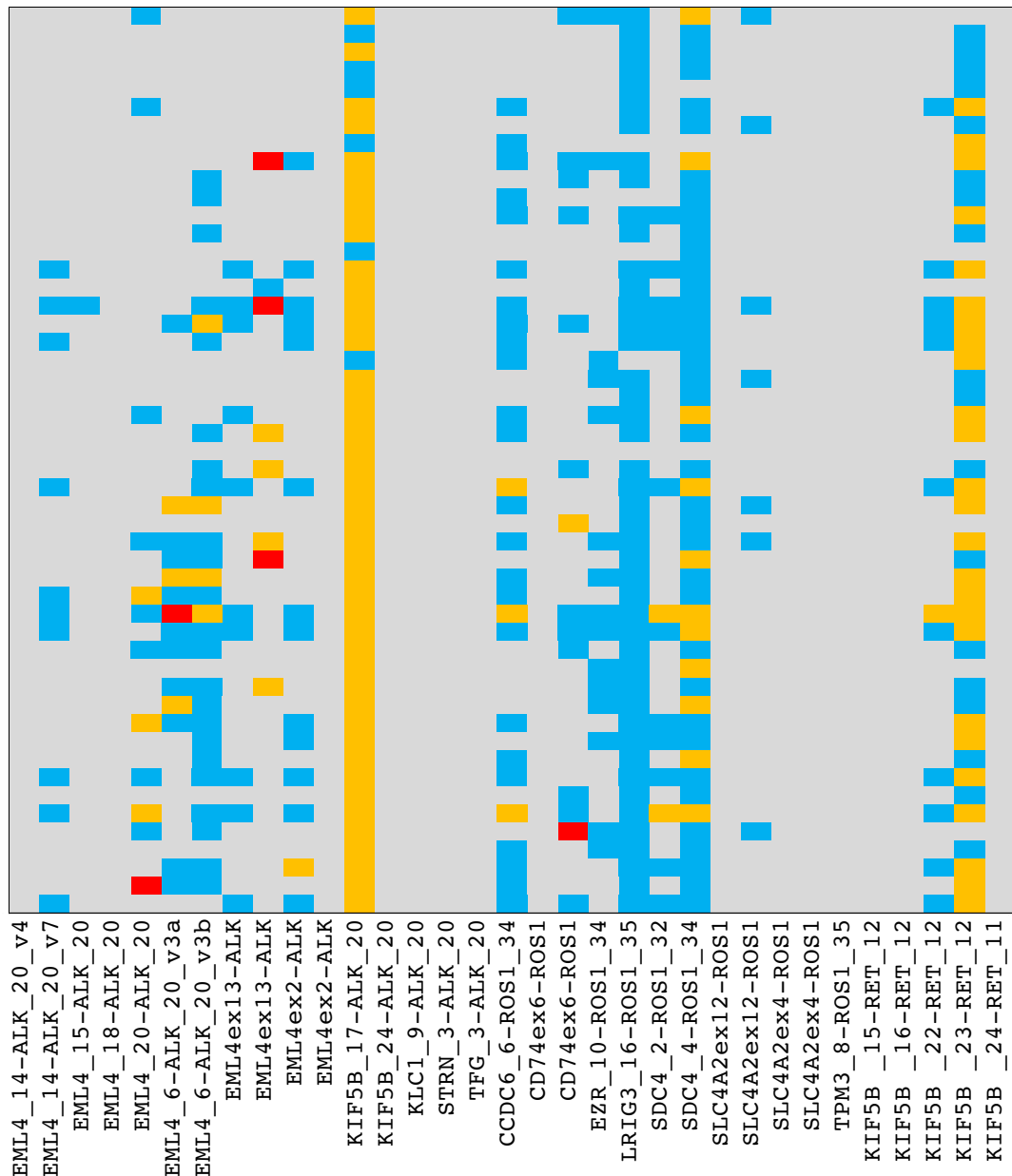
* **Ee13;Ae20** = ELM4 exon 13 fused with ALK exon 20



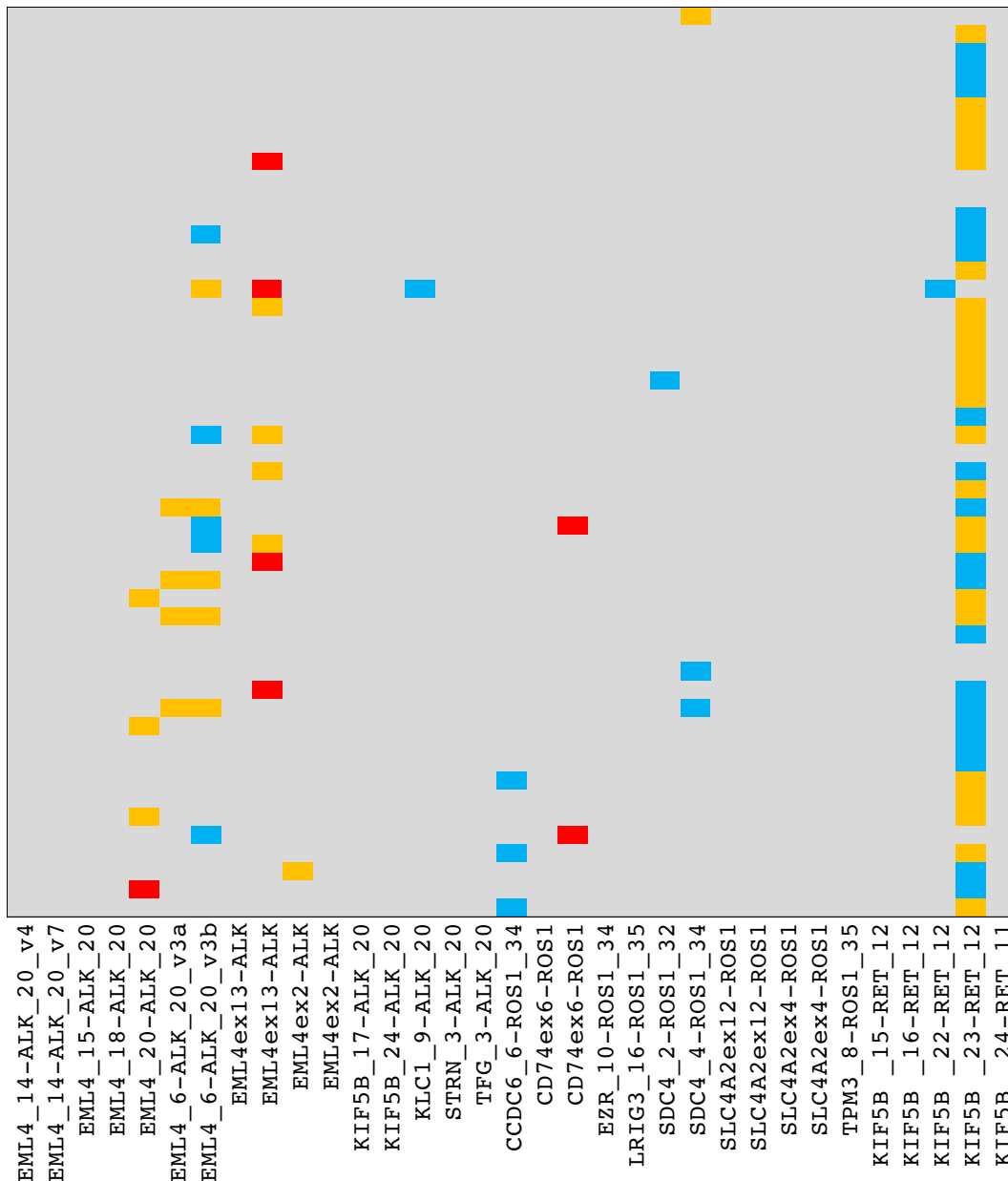
Supplementary Figure 1. Representative Bioanalyser images for two clinical NCSLC samples, i.e. SN9 representing a poor quality RNA sample and SN38 representing a good quality sample.

Samples

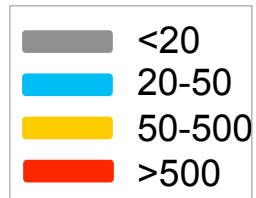
Raw data



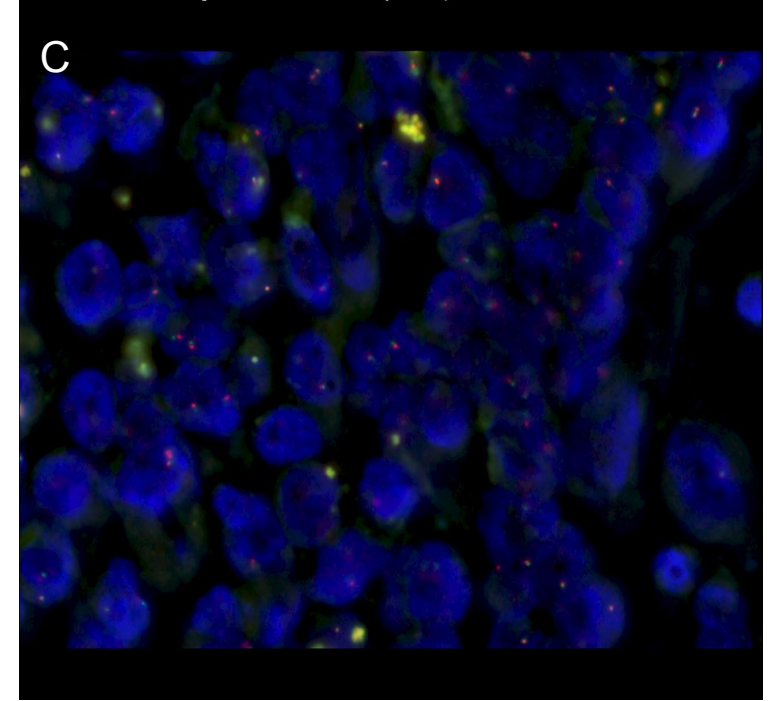
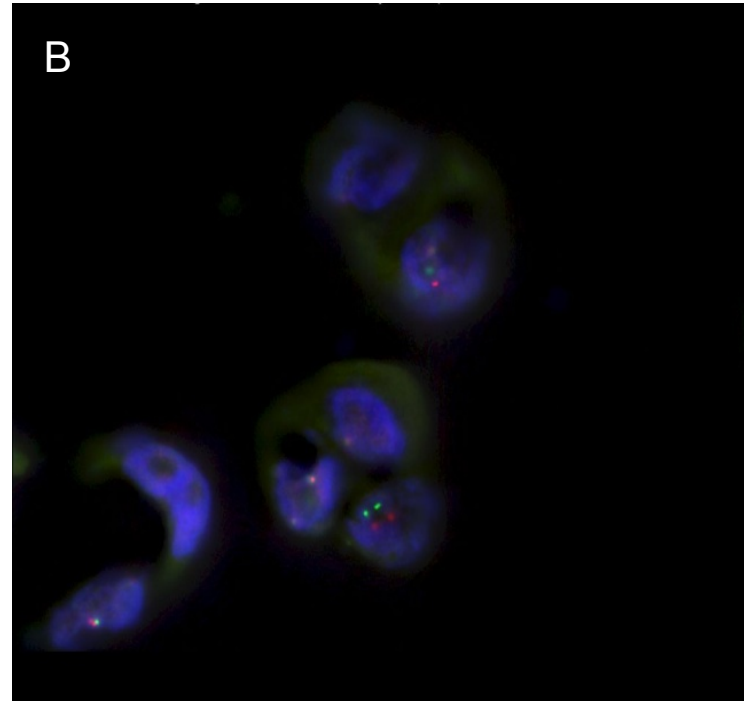
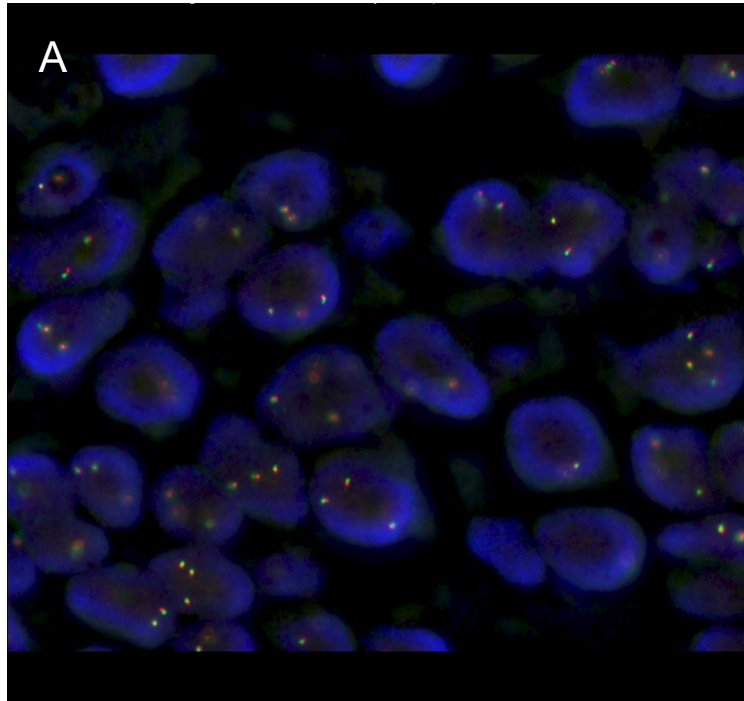
Normalised data



Counts



Supplementary Figure 2. Raw vs. normalised values in the Nanostring analysis. After normalisation, counts above 50 were considered positive.



Supplementary Figure 3. *ALK* signal patterns observed by FISH. (A) A non-*ALK*-rearranged tumor. (B) An *ALK*-rearranged tumor with a split (greater than two signal widths apart). (C) Sample SN10, An atypical *ALK* FISH result with many narrow breaks and non-specific staining.