Supplementary Figure 1. Patients with *CD74-ROS1* fusion and non-*CD74-ROS1* fusion had a comparable frequency of treatment-emergent *ROS1* point mutations.
Supplementary Figure 2. Patients with intracranial-only progression and extracranial-only progression had a comparable distribution of *CD74-ROS1* and non-*CD74-ROS1*. 
Supplementary Table 1. Detailed clinical and molecular information for all the 49 patients treated with first-line crizotinib who were included in this study.

| Patient number | Age | Sex  | Smoking history | Tumor histology | Clinical stage | Baseline brain metastasis status | ROS1 fusion detected at baseline by DNA-NGS | Best objective response with crizotinib | PFS on crizotinib therapy (months) |
|----------------|-----|------|-----------------|-----------------|----------------|----------------------------------|------------------------------------------|----------------------------------------|----------------------------------|
| P1             | 42  | Male | No              | Adenocarcinoma  | IV             | No                               | CD74-ROS1                               | PR                                    | 3                                |
| P2             | 45  | Male | Yes             | Adenocarcinoma  | IV             | Yes                              | SDC4-ROS1                               | PR                                    | 7                                |
| P3             | 65  | Male | Yes             | Adenocarcinoma  | IV             | No                               | CD74-ROS1                               | SD                                    | 5                                |
| P4             | 40  | Male | No              | Adenocarcinoma  | IV             | No                               | CD74-ROS1                               | PR                                    | 27                               |
| P5             | 58  | Male | Yes             | Adenocarcinoma  | IV             | Yes                              | CD74-ROS1                               | SD                                    | 10                               |
| P6             | 51  | Female | No             | Adenocarcinoma  | IV             | No                               | SDC4-ROS1                               | PR                                    | 22                               |
| P7             | 49  | Male | No              | Adenocarcinoma  | IV             | No                               | SDC4-ROS1                               | PR                                    | 15                               |
| P8             | 61  | Female | No             | Adenocarcinoma  | IV             | No                               | CD74-ROS1                               | PR                                    | 13                               |
| P9             | 58  | Male | Yes             | Adenocarcinoma  | IV             | Yes                              | CD74-ROS1                               | PR                                    | 24                               |
| P10            | 66  | Female | No             | Adenocarcinoma  | IV             | No                               | CCDC6-ROS1                              | PR                                    | 47                               |
| P11            | 49  | Female | No             | Adenocarcinoma  | IV             | No                               | SDC4-ROS1                               | PR                                    | 27                               |
| P12            | 53  | Male | No              | Adenocarcinoma  | IV             | No                               | CD74-ROS1                               | PR                                    | 34                               |
| P13            | 63  | Female | No             | Adenocarcinoma  | IV             | No                               | CD74-ROS1                               | PR                                    | 44                               |
| P14            | 64  | Female | No             | Adenocarcinoma  | IV             | No                               | CD74-ROS1                               | PR                                    | 33.5                             |
| P15            | 57  | Male | Yes             | Adenocarcinoma  | IV             | No                               | SDC4-ROS1                               | PR                                    | 14                               |
| P16            | 47  | Female | No             | Adenocarcinoma  | IV             | No                               | TPM3-ROS1                               | PR                                    | 15                               |
| P17            | 63  | Female | No             | Adenocarcinoma  | IV             | Yes                              | EZR-ROS1                                | PR                                    | 17                               |
| P18            | 56  | Male | Yes             | Adenocarcinoma  | IV             | Yes                              | SDC4-ROS1                               | PR                                    | 12.3                             |
| P19            | 49  | Male | Yes             | Adenocarcinoma  | IV             | Yes                              | EZR-ROS1                                | PR                                    | 13                               |
| P20            | 47  | Female | No             | Adenocarcinoma  | IV             | Yes                              | CD74-ROS1                               | SD                                    | 8                                |
| P21            | 49  | Male | No              | Adenocarcinoma  | IV             | No                               | EZR-ROS1                               | PR                                    | 47                               |
Crizotinib progression

| Sites for Progression             | Rebiopsy sample for NGS at progression | ROS1 fusion detected at progression by DNA-NGS | ROS1 point mutation detected at crizotinib progression and were undetected at baseline | Subsequent treatment after crizotinib resistance |
|-----------------------------------|----------------------------------------|-----------------------------------------------|-----------------------------------------------------------------------------------------|-----------------------------------------------|
| Pleural Metastasis                | Pleural effusion                       | CD74-ROS1                                     | ROS1 G2032R                                                                              | Chemotherapy                                  |
| Pleural Metastasis                | Plasma                                  | SDC4-ROS1                                     | ROS1 G2032R                                                                              | Clinical trial                                |
| Brain Metastasis                  | Plasma                                  | CD74-ROS1                                     | none                                                                                    | Chemotherapy                                  |
| Brain Metastasis                  | Cerebrospinal fluid and Plasma          | CD74-ROS1                                     | ROS1 G2032R                                                                              | Clinical trial                                |
| Leptomeningeal Metastasis         | Plasma                                  | CD74-ROS1                                     | none                                                                                    | Clinical trial                                |
| Lung nodules enlargement          | Lung nodules                            | SDC4-ROS1                                     | ROS1 G2032K                                                                              | Clinical trial                                |
| Lung new nodules                  | Plasma                                  | SDCC6-ROS1                                    | none                                                                                    | Alectinib                                     |
| Lung nodules enlargement          | Lung nodules                            | CD74-ROS1                                     | ROS1 G2032K                                                                              | Chemotherapy                                  |
| Lung new nodules                  | Lung nodules                            | CD74-ROS1                                     | ROS1 G2032R                                                                              | Clinical trial                                |
| Lung+Brain Metastasis             | Lung nodules and Cerebrospinal fluid    | SDC4-ROS1                                     | ROS1 L1174F                                                                              | Clinical trial                                |
| New occurring Lung nodules        | Plasma                                  | TPM3-ROS1                                     | none                                                                                    | Chemotherapy                                  |
| Brain Metastasis                  | Cerebrospinal fluid and Plasma          | EZR-ROS1                                      | none                                                                                    | Ceritinib + Radiotherapy                      |
| Lung nodules enlargement          | Lung nodules                            | SDC4-ROS1                                     | ROS1 G2032K                                                                              | Ceritinib                                     |
| Lung nodules enlargement          | Lung Metastasistissue                   | CD74-ROS1                                     | ROS1 L2026M                                                                              | Chemo+ Radiotherapy                           |
| Brain Metastasis                  | Cerebrospinal fluid and Plasma          | EZR-ROS1+ROS1-MRAS                             | ROS1 L2026M                                                                              | Clinical trial                                |
| Brain Metastasis                  | Brain Metastasistissue                  | EZR-ROS1                                      | none                                                                                    | Chemo+ Radiotherapy                           |
| Lung nodules enlargement          | Brain Metastasistissue                  | EZR-ROS1                                      | ROS1 L2026M                                                                              | Clinical trial                                |
| ID | Age | Gender | Stage | Her2 | Tissue Type | Score | Genes                          |
|----|-----|--------|-------|------|-------------|-------|-------------------------------|
| P22| 50  | Female | No    | No   | Adenocarcinoma | IV    | Yes                           |
| P23| 37  | Female | No    | No   | Adenocarcinoma | IV    | No                            |
| P24| 58  | Female | No    | No   | Adenocarcinoma | IIIb  | No                            |
| P25| 51  | Male   | Yes   | No   | Adenocarcinoma | IV    | Yes                           |
| P26| 42  | Female | No    | No   | Adenocarcinoma | IV    | No                            |
| P27| 50  | Female | No    | No   | Adenocarcinoma | IV    | No                            |
| P28| 46  | Male   | No    | No   | Adenocarcinoma | IV    | Yes                           |
| P29| 39  | Female | No    | No   | Adenocarcinoma | IV    | No                            |
| P30| 35  | Female | No    | No   | Adenocarcinoma | IV    | No                            |
| P31| 53  | Female | No    | No   | Adenocarcinoma | IV    | No                            |
| P32| 33  | Male   | Yes   | No   | Adenocarcinoma | IV    | No                            |
| P33| 58  | Female | No    | No   | Adenocarcinoma | IV    | No                            |
| P34| 49  | Female | No    | No   | Adenocarcinoma | IV    | No                            |
| P35| 60  | Male   | Yes   | No   | Adenocarcon    | IV    | Yes                           |
| P36| 54  | Male   | Yes   | No   | Adenocarcinoma | IV    | No                            |
| P37| 44  | Male   | Yes   | No   | Adenocarcinoma | IV    | No                            |
| P38| 55  | Male   | No    | No   | Adenocarcinoma | IV    | No                            |
| P39| 36  | Female | No    | No   | Adenocarcinoma | IV    | No                            |
| P40| 63  | Male   | No    | No   | Adenocarcinoma | IV    | No                            |
| P41| 55  | Female | No    | No   | Adenocarcinoma | IV    | No                            |
| P42| 54  | Male   | No    | No   | Adenocarcinoma | IV    | No                            |
| P43| 40  | Female | No    | No   | Adenocarcinoma | IV    | No                            |
| P44| 45  | Female | Yes   | No   | Adenocarcinoma | IV    | No                            |
| P45| 49  | Female | No    | No   | Adenocarcinoma | IV    | No                            |
| P46| 46  | Female | No    | No   | Adenocarcinoma | IV    | No                            |
| P47| 26  | Female | No    | No   | Adenocarcinoma | IV    | Yes                           |
| P48| 59  | Female | No    | No   | Adenocarcinoma | IV    | No                            |
| P49| 52  | Female | No    | No   | Adenocarcinoma | IV    | No                            |
| Condition                        | Sample Type              | Biomarker | Mutation | Treatment          |
|---------------------------------|--------------------------|-----------|----------|--------------------|
| Lung+Brain Metastasis          | Plasma                   | TPM3      | ROS1     | Ceritinib          |
| Lung new nodules               | Lung nodules and         | CD74      | ROS1     | Chemotherapy       |
| Lung+Brain Metastasis          | Cerebrospinal fluid and  | CD74      | ROS1     | Clinical trial     |
| Lung+Brain Metastasis          | Brain Metastasis         | CD74      | ROS1     | Ceritinib          |
| Lung nodules enlargement       | Lung nodules             | EZR       | ROS1     | Chemotherapy       |
| Lung nodules enlargement       | Lung nodules             | SLC34A2   | ROS1     | Clinical trial     |
| Lung nodules enlargement       | Lung nodules             | CD74      | ROS1     | Ceritinib          |
| Lung nodules enlargement       | Lung nodules             | CD74      | ROS1     | Chemotherapy       |
| Brain Metastasis               | Brain Metastasis tissue  | CD74      | ROS1     | Clinical trial     |
| Lung nodules enlargement       | Lung nodules             | SLC34A2   | ROS1     | Ceritinib          |
| Lung nodules enlargement       | Lung nodules             | CD74      | ROS1     | Clinical trial     |
| Lung nodules enlargement       | Lung nodules             | CD74      | ROS1     | Ceritinib          |
| Lung nodules enlargement       | Lung nodules             | CD74      | ROS1     | Chemotherapy       |
| Brain Metastasis               | Brain Metastasis tissue  | CD74      | ROS1     | Clinical trial     |
| Lung nodules enlargement       | Lung nodules             | SLC34A2   | ROS1     | Ceritinib          |
| Lung nodules enlargement       | Lung nodules             | CD74      | ROS1     | Clinical trial     |
| Lung nodules enlargement       | Lung nodules             | CD74      | ROS1     | Ceritinib          |
| Lung nodules enlargement       | Lung nodules             | CD74      | SDC4     | Clinical trial     |
| Lung nodules enlargement       | Lung nodules             | CD74      | ROS1     | Chemotherapy       |
| Lung nodules enlargement       | Lung nodules             | SDC4      | ROS1     | Clinical trial     |
| Lung nodules enlargement       | Lung nodules             | CD74      | ROS1     | Clinical trial     |
| Lung nodules enlargement       | Lung nodules             | CD74      | ROS1     | Clinical trial     |
| Back Metastasis                | Lung nodules             | SDC4      | ROS1     | Clinical trial     |
| Lung+Brain Metastasis          | Lung nodules and         | SDC4      | ROS1     | Clinical trial     |
| Pleural and Brain Metastasis   | Cerebrospinal fluid and  | CD74      | ROS1     | Clinical trial     |
| Lung nodules enlargement       | Lung nodules             | SDC4      | ROS1     | Clinical trial     |
| Lung nodules enlargement       | Lung nodules             | CD74      | ROS1     | Clinical trial     |
| Lung nodules enlargement       | Lung nodules             | CD74      | ROS1     | Clinical trial     |
| Lung nodules enlargement       | Lung nodules             | CD74      | SDC4     | Clinical trial     |
| Lung nodules enlargement       | Lung nodules             | CD74      | SDC4     | Clinical trial     |
| Lung nodules enlargement       | Lung nodules             | CD74      | ROS1     | Clinical trial     |
| Lung nodules enlargement       | Lung nodules             | CD74      | ROS1     | Clinical trial     |
| Lung nodules enlargement       | Lung nodules             | CD74      | ROS1     | Clinical trial     |
| Lung nodules enlargement       | Lung nodules             | CD74      | ROS1     | Clinical trial     |
| Lung nodules enlargement       | Lung nodules             | CD74      | ROS1     | Clinical trial     |
| Lung nodules enlargement       | Lung nodules             | CD74      | ROS1     | Clinical trial     |
| Lung nodules enlargement       | Lung nodules             | CD74      | ROS1     | Clinical trial     |
| Lung nodules enlargement       | Lung nodules             | CD74      | ROS1     | Clinical trial     |
Supplementary Table 2. Clinical characteristics of the cohort according to mode of disease progression (n=49).

|                                      | Extracranial-only progression (n=33) | Intracranial-only progression (n=11) | Extracranial and intracranial progression (n=5) | p value |
|--------------------------------------|--------------------------------------|--------------------------------------|-------------------------------------------------|---------|
| Age at diagnosis (years), median     | 54(40-61)                            | 51(26-66)                            | 55(49-58)                                       | 0.601   |
| (range)                              |                                      |                                      |                                                 |         |
| Sex                                  |                                      |                                      |                                                 | 0.611   |
| Male                                 | 15(45.5)                             | 5(45.5)                              | 1(20.0)                                         |         |
| Female                               | 18(54.5)                             | 6(54.5)                              | 4(80.0)                                         |         |
| Smoking history                      |                                      |                                      |                                                 | 0.842   |
| Yes                                  | 8(24.26)                             | 3(27.3)                              | 4(80)                                           |         |
| No                                   | 25(75.8)                             | 8(72.7)                              | 1(20)                                           |         |
| Histology                            |                                      |                                      |                                                 | 1.000   |
| Adenocarcinoma                       | 33(100)                              | 11(100)                              | 5(100)                                          |         |
| Squamous cell carcinoma              | 0(0)                                 | 0(0)                                 | 0(0)                                            |         |
| ROS1 fusion partner                  |                                      |                                      |                                                 | 0.295   |
| CD74-ROS1                            | 19(57.6)                             | 7(63.6)                              | 2(40)                                           |         |
| Non-CD74-ROS1                        | 14(42.4)                             | 4(36.4)                              | 3(60)                                           |         |
| Brain metastasis at baseline         |                                      |                                      |                                                 | 0.665   |
| Yes                                  | 11(33.3)                             | 4(36.4)                              | 1(20)                                           |         |
| No                                   | 22(66.7)                             | 7(63.6)                              | 4(80)                                           |         |
| Radiographic imaging used for baseline assessment | | | | 0.115 |
| CT                                   | 10(29.4)                             | 1(9.2)                               | 2(40)                                           |         |
| MRI                                  | 23(70.6)                             | 9(81.2)                              | 3(60)                                           |         |
| PET-CT                               | 0(0)                                 | 1(9.2)                               | 0(0)                                            |         |
| Radiographic imaging used for assessing disease progression | | | | 1.000 |
| MRI                                  | 33(100)                              | 11(100)                              | 5(100)                                          |         |
| CT                                   | 0(0)                                 | 1(9.2)                               | 0(0)                                            |         |
| PET-CT                               | 0(0)                                 | 1(9.2)                               | 0(0)                                            |         |
| Samples submitted for NGS at crizotinib progression | | | | 0.425 |
| Lung nodules/ Brain Metastasis/Cerebrospinal fluid samples | 26(78.8) | 7(63.6) | | |
| Plasma/ pleural effusion             | 7(21.2)                              | 4(36.4)                              |                                                 |         |

Abbreviations: CT: computed tomography, MRI: Magnetic resonance imaging, PET-CT: positron emission tomography computed tomography.
Supplementary Table 3. Clinical and molecular details of 11 patients with intracranial-only progression

| Patient number | Age  | Sex  | Smoking history | Tumor histology      | Clinical stage | Baseline brain metastasis status | ROS1 fusion        | Rebiopsy sample for NGS at progression | Best objective response with crizotinib | PFS on crizotinib therapy (months) | Resistance mechanisms |
|----------------|------|------|-----------------|----------------------|----------------|----------------------------------|--------------------|----------------------------------------|---------------------------------------|-------------------------------------|----------------------|
| 1              | 65   | Female | No              | Adenocarcinoma      | IV             | No                               | CD74-ROS1          | Plasma                                 | SD                                    | 5                     | unknown              |
| 2              | 40   | Male  | No              | Adenocarcinoma      | IV             | No                               | CD74-ROS1          | Cerebrospinal fluid and Plasma          | PR                                    | 27                    | ROS1 G2032R           |
| 3              | 58   | Male  | Yes             | Adenocarcinoma      | IV             | Yes                              | CD74-ROS1          | Cerebrospinal fluid and Plasma          | SD                                    | 10                    | unknown              |
| 4              | 66   | Female | No              | Adenocarcinoma      | IV             | No                               | CCDC6-ROS1         | Plasma                                 | PR                                    | 47                    | unknown              |
| 5              | 63   | Female | No              | Adenocarcinoma      | IV             | Yes                              | CD74-ROS1          | Cerebrospinal fluid and plasma          | PR                                    | 17                    | ROS1 L2026M           |
| 6              | 47   | Female | No              | Adenocarcinoma      | IV             | Yes                              | CD74-ROS1          | Brain tissue                           | SD                                    | 8                     | unknown              |
| 7              | 49   | Male  | No              | Adenocarcinoma      | IV             | No                               | EZR-ROS1+BTBD9-ROS1 | Cerebrospinal fluid and plasma          | PR                                    | 47                    | unknown              |
| 8              | 51   | Male  | Yes             | Adenocarcinoma      | IV             | Yes                              | CD74-ROS1          | Cerebrospinal fluid and plasma          | PR                                    | 27                    | ROS1 G2032R           |
| 9              | 39   | Female | No              | Adenocarcinoma      | IV             | No                               | SDC4-ROS1          | Cerebrospinal fluid and plasma          | PR                                    | 10.2                  | unknown              |
| 10             | 55   | Female | No              | Adenocarcinoma      | IV             | No                               | CD74-ROS1          | Brain tissue                           | PR                                    | 7                     | unknown              |
| 11             | 26   | Female | No              | Adenocarcinoma      | IV             | Yes                              | SDC4-ROS1          | Cerebrospinal fluid and plasma          | PR                                    | 24                    | unknown              |

Abbreviations: PFS, progression-free survival; PR, partial response; SD, stable disease
### Supplementary Table 4. Clinical and molecular details of 5 patients with both extracranial and intracranial progression

| Patient number | Age | Sex   | Smoking history | Histology       | Clinical stage | Baseline brain metastasis status | ROS1 fusion | Extracranial progression sites       | Best objective response with crizotinib | PFS on crizotinib therapy (months) | Rebiopsy samples for NGS at progression | Resistance mechanisms |
|----------------|-----|-------|----------------|-----------------|----------------|---------------------------------|-------------|--------------------------------------|----------------------------------------|--------------------------------------|--------------------------------------|-------------------------------------|
| 1              | 57  | Male  | Yes            | Adenocarcinoma  | IV             | No                              | SDC4-ROS1   | Lung nodules                        | PR                                     | 14                                   | Lung nodules and Cerebrospinal fluid | ROS1 L1174F                         |
| 2              | 50  | Female| No             | Adenocarcinoma  | IV             | Yes                             | TPM3-ROS1   | Bone and Lung Nodules               | SD                                     | 7                                    | Plasma                              | unknown                            |
| 3              | 58  | Female| No             | Adenocarcinoma  | IIIb           | No                              | CD74-ROS1   | Lung Nodules                        | SD                                     | 28                                   | Lung nodules and Cerebrospinal fluid | unknown                            |
| 4              | 49  | Female| No             | Adenocarcinoma  | IV             | No                              | CD74-ROS1   | Pleural metastasis                  | PR                                     | 30                                   | Lung nodules and Cerebrospinal fluid | unknown                            |
| 5              | 46  | Female| No             | Adenocarcinoma  | IV             | No                              | SDC4-ROS1   | Pleural metastasis and Lung Nodules | PR                                     | 10                                   | Lung nodules and Cerebrospinal fluid | unknown                            |

Abbreviations: PFS, progression-free survival; PR, partial response; SD, stable disease