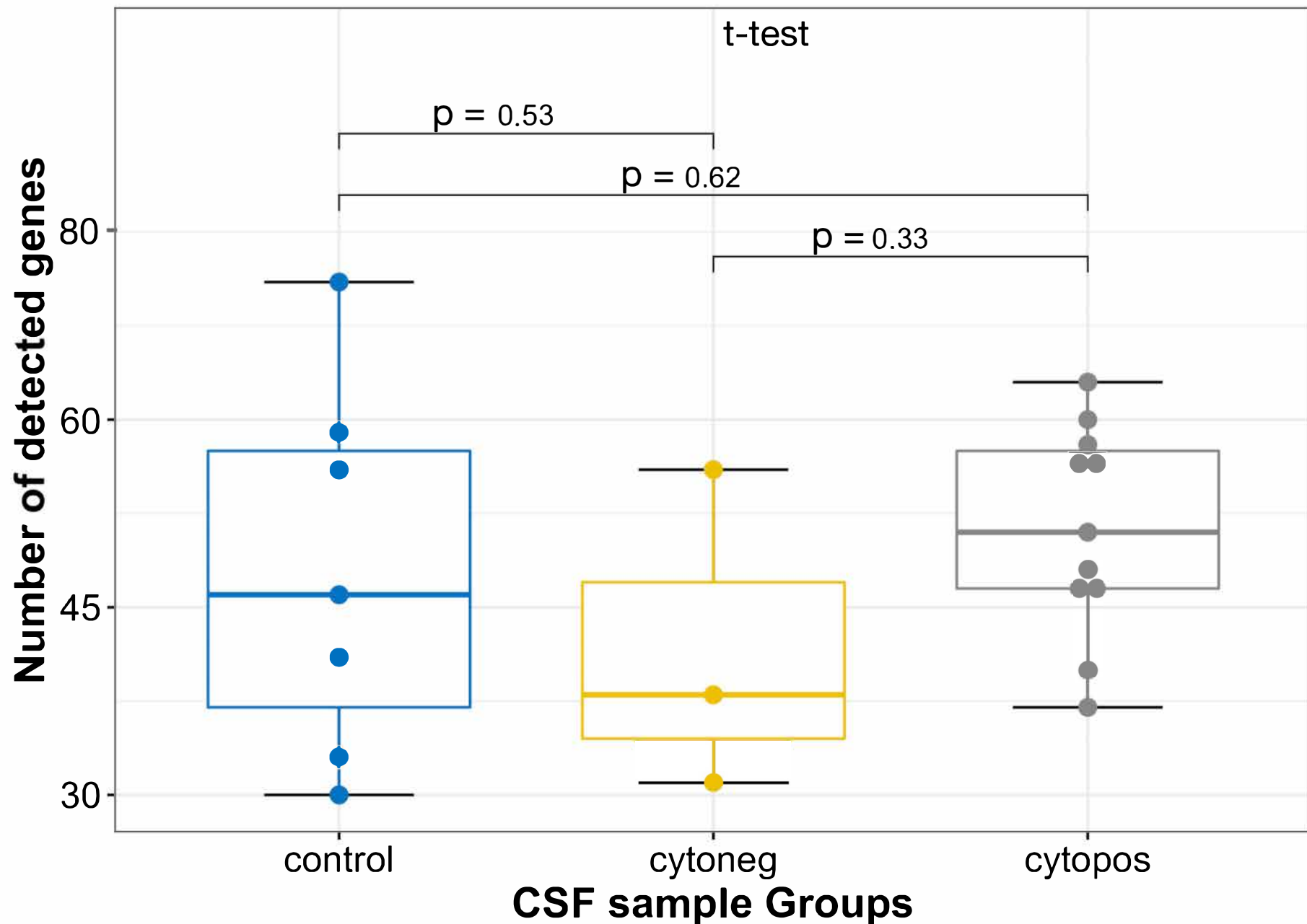
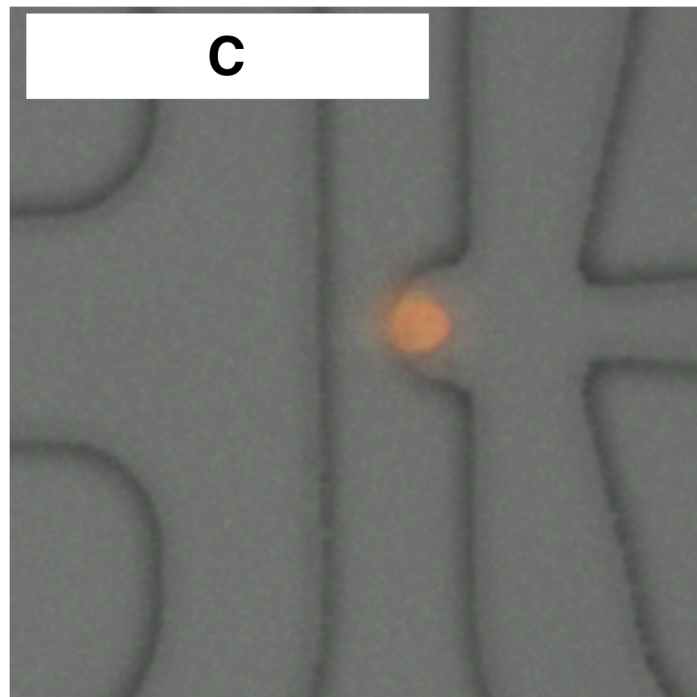
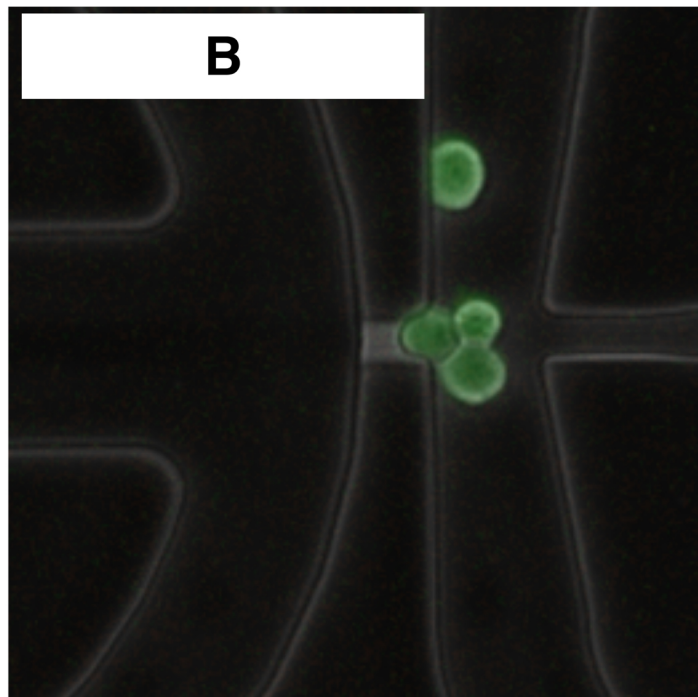
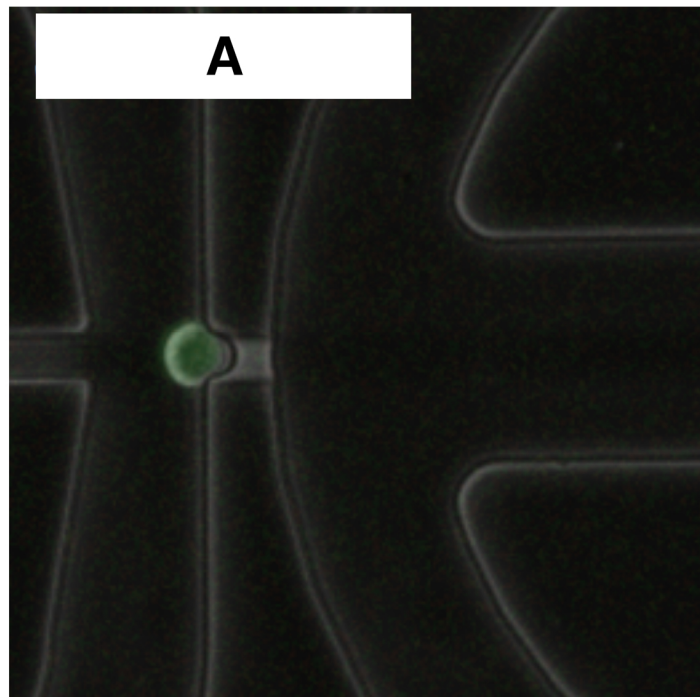


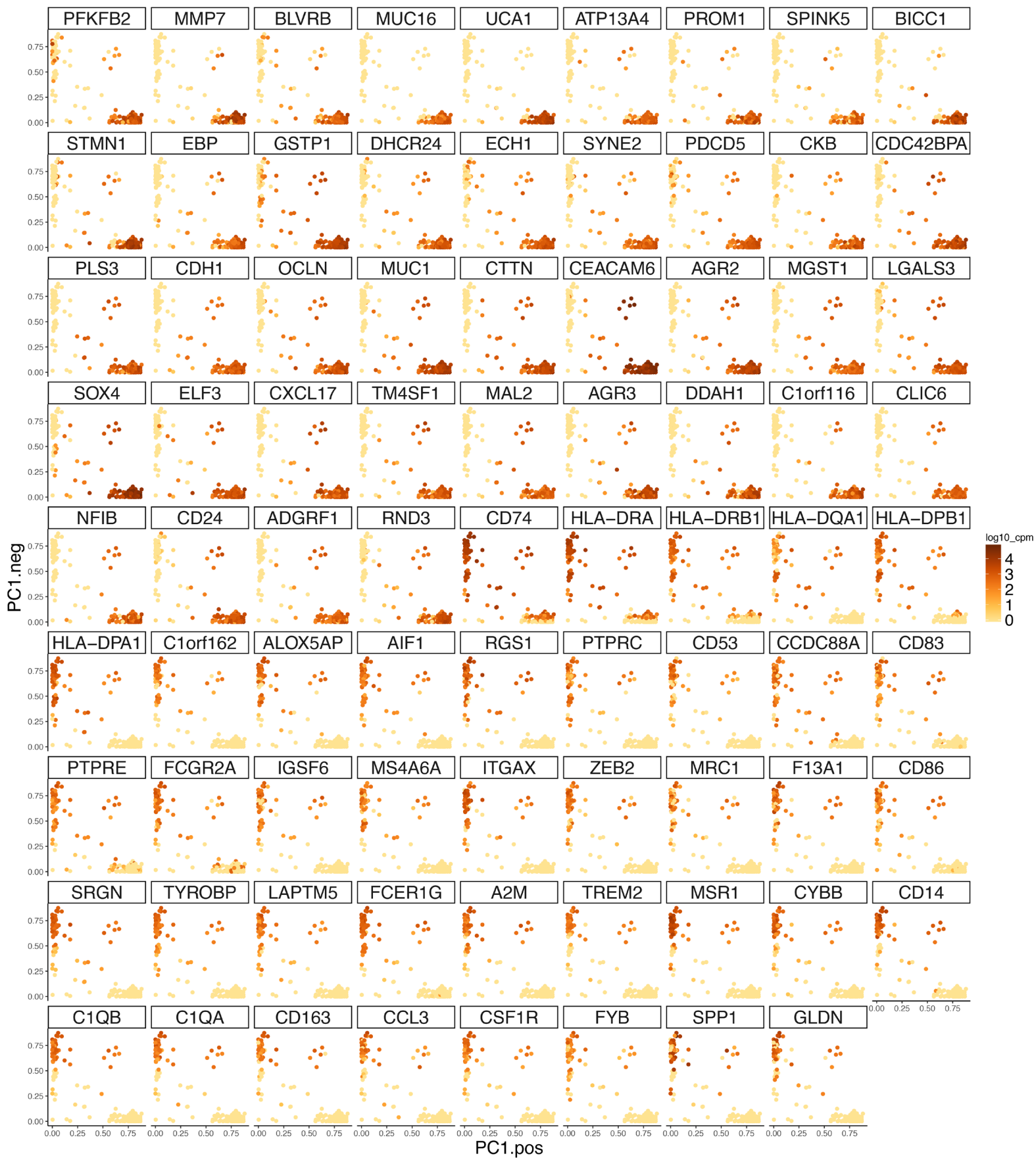
**Supplementary Figure 1.** This heatmap represents relative expression in all 96 genes tested by high throughput qPCR.



**Supplementary Figure 2.** This box plot compared the number of detected genes in the parallel qPCR test (total genes =96) among control samples (n=7), cytology negative samples (n=3) and cytology positive samples (n=11). No significant difference was observed by



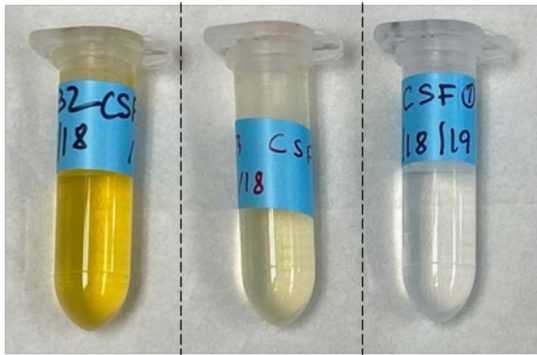
**Supplementary Figure 3.** Examples of single-cell capture. (A) a live single cell; (B) multiple cells; (C) a dead cell.



Supplementary Figure 4. Characteristic genes in each cluster from scRNAseq.

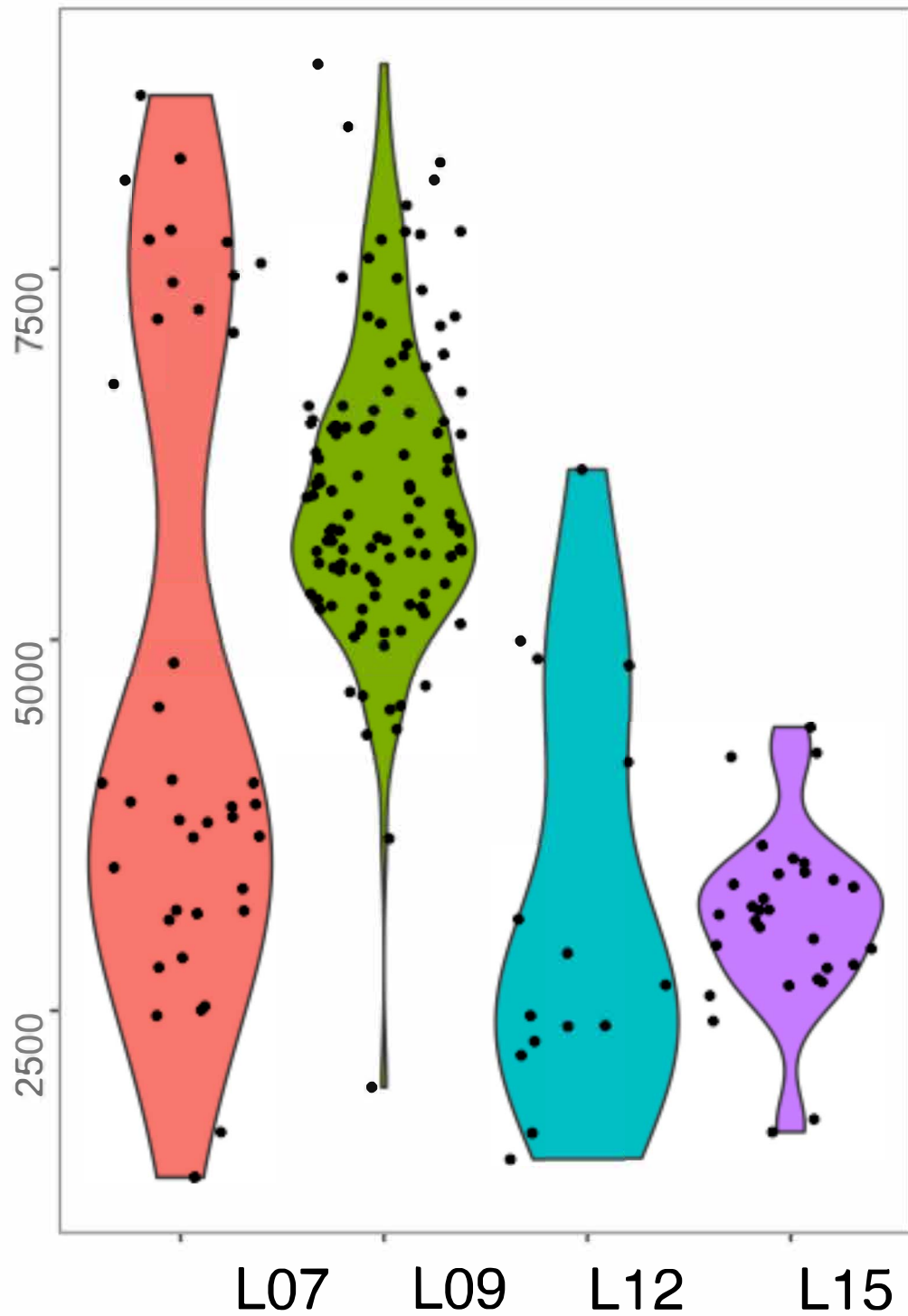


Ptn 13a. | Ptn 13b. | Ptn 14a.

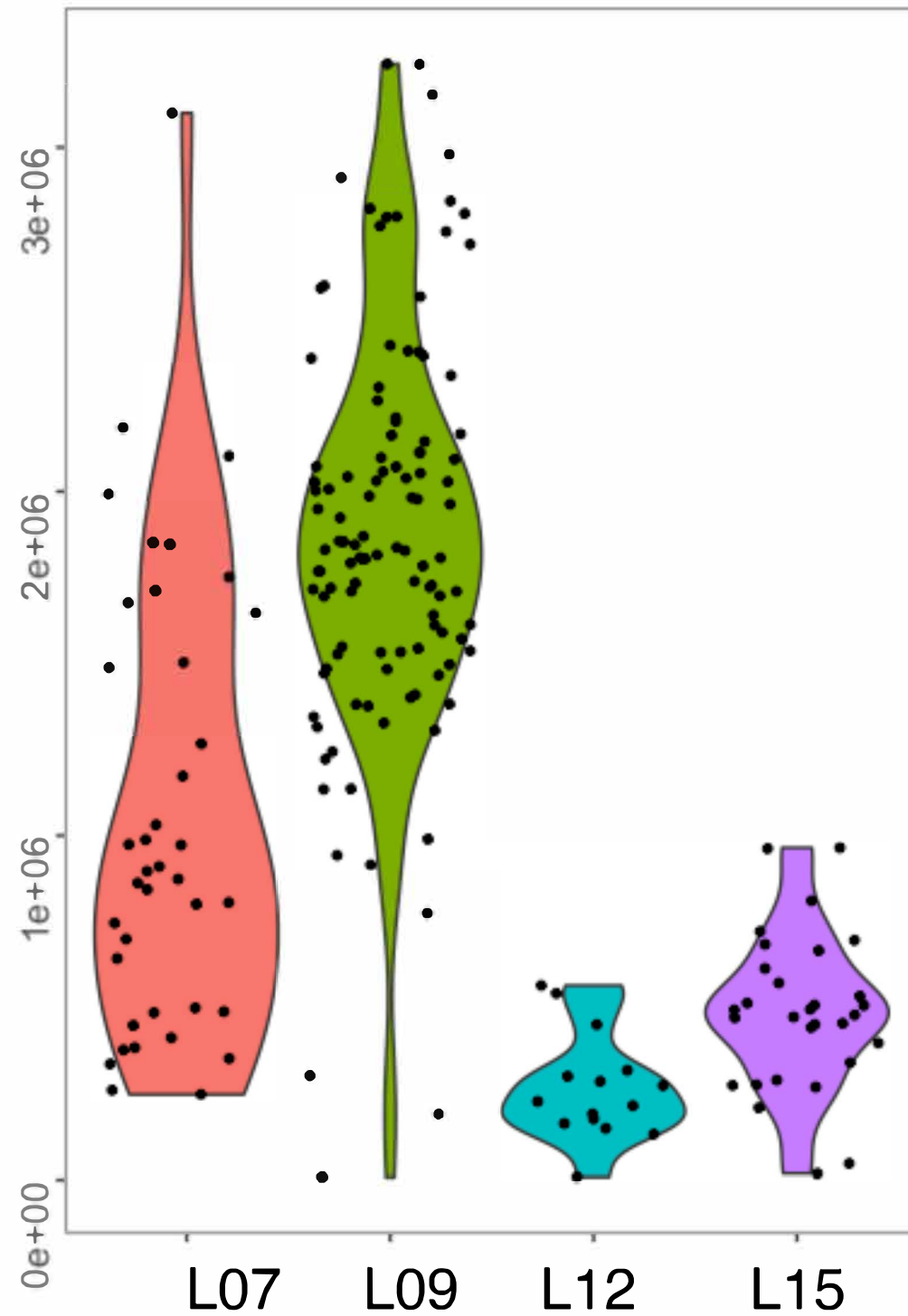


**Supplementary Figure 5.** Two samples of CSF were collected from patient 13 on different dates. CSF samples Ptn 13a. and Ptn 13b. are tinted with yellow as compared to CSF sample Ptn 14a. The color probably is due to hemolysis residues as this patient suffered from intracranial hemorrhage.

# nGene



# nUMI



Supplementary Figure 6. Number of genes and number of UMI from scRNA seq.

**Supplementary Table 1.** Test Cohort - Summary of Clinical Sample Information.

|     | Sex | Age | Diagnosis         | scRNA-seq | Cytology | MRI        | Tx before LP   | Known mutations           |
|-----|-----|-----|-------------------|-----------|----------|------------|--|---------------------------|
| C01 | F   | 44  | MVD               |           | NA       | NA         | NA   | NA                        |
| C02 | M   | 74  | MVD               |           | NA       | NA         | NA   | NA                        |
| C03 | M   | 67  | MVD               |           | NA       | NA         | NA   | NA                        |
| C04 | M   | 62  | Pituitary adenoma |           | NA       | NA         | NA   | NA                        |
| C05 | F   | 58  | Pituitary adenoma |           | NA       | NA         | NA   | NA                        |
| C06 | M   | 68  | Pituitary adenoma |           | NA       | NA         | NA   | NA                        |
| C07 | F   | 43  | Pituitary adenoma |           | NA       | NA         | NA   | NA                        |
| L01 | F   | 26  | NSCLC             |           | pos      | Positive   | Radiotherapy, chemotherapy, targeted therapy                             | Unspecified EGFR mutation |
| L02 | M   | 65  | NSCLC             |           | pos      | Positive   | Unknown  | EGFR exon 19 deletion     |
| L03 | M   | 72  | NSCLC             |           | pos      | Positive   | Targeted therapy   | EGFR exon 19 deletion     |
| L04 | F   | 71  | NSCLC             |           | neg      | Negative   | Chemotherapy   | EGFR exon 20 mutation     |
| L05 | F   | 43  | NSCLC             |           | neg      | Suspicious | Radiotherapy chemotherapy, targeted therapy                              | ROS mutation              |
| L06 | M   | 63  | NSCLC             |           | pos      | Negative   | Stereotactic radiosurgery, chemotherapy, targeted therapy                | EGFR 19 deletion          |
| L07 | M   | 58  | NSCLC             | X         | pos      | Suspicious | Stereotactic radiosurgery, chemotherapy, targeted therapy, immunotherapy | HER2 mutation             |

|     |   |    |       |   |            |            |  |  |
|-----|---|----|-------|---|------------|------------|--|--|
| L08 | F | 72 | NSCLC |   | Suspicious | Suspicious | Stereotactic radiosurgery, chemotherapy, targeted therapy, immunotherapy | None   |
| L09 | F | 73 | NSCLC | X | pos        | Positive   | Stereotactic radiosurgery, chemotherapy, targeted therapy                | TP53 R248W, EGFR 19 deletion                                 |
| L10 | M | 84 | NSCLC |   | pos        | Positive   | Chemotherapy, immunotherapy  | EGFR exon 19 deletion  |
| L11 | F | 47 | NSCLC |   | pos        | Suspicious | Stereotactic radiosurgery, targeted therapy                              | EGFR exon 20 mutation, (pos in urine), EGFR exon 21 mutation |
| L12 | F | 40 | NSCLC | X | pos        | Positive   | Chemotherapy, targeted therapy   | ALK translocation  |
| L13 | F | 51 | NSCLC |   | pos        | Positive   | Stereotactic radiosurgery, chemotherapy, targeted therapy                | EGFR exon 21 mutation  |
| L14 | F | 74 | NSCLC |   | neg        | Positive   | Chemotherapy, immunotherapy, targeted therapy                            | EGFR exon 20 mutation  |
| L15 | M | 72 | NSCLC | X | pos        | Positive   | Targeted therapy   | EGFR exon 19 deletion  |

**Supplementary Table 2.** Clinical cytology reports of four patients whose CSF underwent single cell RNA sequencing.

| <b>Item</b>                        | <b>Ref. Range</b>              | <b>L04</b> | <b>L09</b> | <b>L12</b> |           | <b>L15</b> |
|------------------------------------|--------------------------------|------------|------------|------------|-----------|------------|
| <b>CSF tube #</b>                  | Unknown                        | 4          | 3          | 4          | 1         | 3          |
| <b>Color, CSF</b>                  | Latest Ref Range:<br>Colorless | Colorless  | Colorless  | Colorless  | Colorless | Colorless  |
| <b>Turbidity, CSF</b>              | Latest Ref Range:<br>Clear     | Clear      | Clear      | Clear      | Clear     | Clear      |
| <b>Color after centrifuge, CSF</b> | Latest Ref Range:<br>Colorless | Colorless  | Colorless  | Colorless  | Colorless | Colorless  |
| <b>Turb. after centrif., CSF</b>   | Latest Ref Range:<br>Clear     | Clear      | Clear      | Clear      | Clear     | Clear      |
| <b>WBC count, CSF</b>              | Latest Ref Range:<br>0 - 5 /uL | 6 (H)      | 70 (HH)    | 2          | 5         | 6 (H)      |
| <b>RBC count, CSF</b>              | Latest Ref Range:<br>0 - 5 /uL | <1         | <1         | <1         | 116 (H)   | 1          |
| <b>Neutrophils, CSF</b>            | Latest Ref Range:<br>0 - 6 %   | 6          | 0          |            | 2         | 0          |
| <b>Lymphocytes, CSF</b>            | Latest Ref Range:<br>40 - 80 % | 3 (L)      | 24 (L)     |            | 71        | 9 (L)      |
| <b>Monocytoid cells, CSF</b>       | Latest Ref Range:<br>15 - 45 % | 88 (H)     | 16         |            | 24        | 91 (H)     |
| <b>Others. CSF</b>                 | Latest Ref Range:<br>0 %       |            | 60 (H)     |            | 2 (H)     |            |



**Supplementary Table 3.** Validation Cohort - Summary of clinical sample information in qPCR validation of CEACAM6

|                                | Sex | Age | Diagnosis                     | Cytology | MRI        | Tx before LP   | Known Mutations        |
|--------------------------------|-----|-----|-------------------------------|----------|------------|--|------------------------|
| <b>Ctrl 1</b><br><b>“C01”</b>  | F   | 44  | Microvascular Decompression   | NA       | NA         | NA   | NA                     |
| <b>Ctrl 2</b><br><b>“C04”</b>  | M   | 62  | Pituitary Adenoma             | NA       | NA         | NA   | NA                     |
| <b>Ctrl 3</b>                  | F   | 44  | Meningioma                    | NA       | NA         | NA   | NA                     |
| <b>Ctrl 4</b>                  | M   | 83  | Normal Pressure Hydrocephalus | NA       | NA         | NA   | NA                     |
| <b>Ctrl 5</b>                  | F   | 30  | Chiari Malformation           | NA       | NA         | NA   | NA                     |
| <b>Ptn 1.a</b>                 | M   | 72  | NSCLC                         | Positive | Suspicious | Stereotactic radiosurgery, chemotherapy, targeted therapy                              | EGFR exon 19 deletion  |
| <b>Ptn 1.b</b>                 | M   | 72  | NSCLC                         | Positive | Suspicious | Targeted therapy   | EGFR exon 19 deletion  |
| <b>Ptn 2</b><br><b>“L02”</b>   | M   | 65  | NSCLC                         | Positive | Suspicious | unknown  | EGFR exon 19 deletion  |
| <b>Ptn 3</b>                   | F   | 57  | NSCLC                         | Positive | Positive   | Stereotactic radiosurgery, chemotherapy, targeted therapy                              | EGFR exon 21 mutation  |
| <b>Ptn 4.a</b><br><b>“L07”</b> | M   | 58  | NSCLC                         | Positive | Suspicious | Stereotactic radiosurgery, radiotherapy, chemotherapy, targeted therapy, immunotherapy | Not Known              |
| <b>Ptn 4.b</b><br><b>“L07”</b> | M   | 58  | NSCLC                         | Positive | Positive   | Radiotherapy, chemotherapy, targeted therapy   | Not Known              |
| <b>Ptn 5</b>                   | F   | 75  | NSCLC                         | Positive | Positive   | Radiotherapy, chemotherapy, targeted therapy   | EGFR exon 20 insertion |

|                         | Sex | Age | Diagnosis                                 | Cytology | MRI        | Tx before LP   | Known Mutations               |
|-------------------------|-----|-----|---|----------|------------|--|-------------------------------|
| <b>Ptn 6</b>            | M   | 58  | Inflammation from PD-1 antibody for NSCLC | Negative | Suspicious | Cranial resection, radiotherapy, chemotherapy, targeted therapy, immunotherapy | Negative                      |
| <b>Ptn 7</b>            | F   | 62  | NSCLC                                     | Positive | Positive   | Stereotactic radiosurgery, chemotherapy, immunotherapy                         | EGFR exon 20 insertion        |
| <b>Ptn 8<br/>"L13"</b>  | F   | 51  | NSCLC                                     | Positive | Positive   | Cranial resection, stereotactic radiosurgery, chemotherapy, targeted therapy   | EGFR exon 21 mutation         |
| <b>Ptn 9</b>            | F   | 74  | NSCLC                                     | Negative | Positive   | Chemotherapy, targeted therapy   | EGFR exon 20 mutation         |
| <b>Ptn 10</b>           | F   | 68  | NSCLC                                     | Positive | Positive   | Radiotherapy, chemotherapy, targeted therapy, immunotherapy                    | EGFR exon 19 deletion         |
| <b>Ptn 11</b>           | M   | 60  | NSCLC                                     | Positive | Positive   | Chemotherapy and targeted therapy  | EGFR exon 20 and 21 mutations |
| <b>Ptn 12<br/>"L15"</b> | M   | 72  | NSCLC                                     | Positive | Positive   | Targeted therapy   | EGFR exon 19 deletion         |
| <b>Ptn 13.a</b>         | F   | 54  | NSCLC                                     | Positive | Positive   | Targeted therapy   | ALK positive                  |
| <b>Ptn 13.b</b>         | F   | 54  | NSCLC                                     | Positive | Positive   | Chemotherapy and targeted therapy  | ALK positive                  |
| <b>Ptn 14.a</b>         | M   | 58  | NSCLC                                     | Positive | Negative   | Targeted therapy   | EGFR exon 21 mutation         |
| <b>Ptn 14.b</b>         | M   | 59  | NSCLC                                     | Positive | Negative   | Chemotherapy, targeted therapy   | EGFR exon 21 mutation         |

|               | <b>Sex</b> | <b>Age</b> | <b>Diagnosis</b> | <b>Cytology</b> | <b>MRI</b> | <b>Tx before LP</b>   | <b>Known Mutations</b> |
|---------------|------------|------------|------------------|-----------------|------------|---|------------------------|
| <b>Ptn 15</b> | M          | 59         | NSCLC            | Negative        | Positive   | Chemotherapy, targeted therapy, immunotherapy               | EGFR exon 19 deletion  |
| <b>Ptn 16</b> | M          | 81         | NSCLC            | Positive        | Positive   | Radiotherapy, chemotherapy, targeted therapy, immunotherapy | ALK positive           |

\* a. and b. refer to CSF sample collected from same patient on different dates.

\* CSF samples Ctrl 1, Ctrl 2, Ptn 2, Ptn 4, Ptn 8 and Ptn 12 were also used in “test cohort” (Supplementary Table 1).

**Supplementary Table 4.** Left table - Contingency table describes the correlation between the detection of *CEACAM6* gene in CSF samples and cytology test of LM diagnosis, n=20,  $P < 0.01$  (Fisher's exact test). Right table – Sensitivity, Specificity, Positive Prediction Value (PPV), Negative Prediction Value (NPV) and Accuracy parameters to detect *CEACAM6* gene in CSF samples indicative of LM diagnosis.

| Contingency      |                   |                   |
|------------------|-------------------|-------------------|
| $P < 0.01$       |                   |                   |
|                  | Cytology positive | Cytology negative |
| CEACAM6 positive | 15                | 0                 |
| CEACAM6 negative | 2                 | 3                 |
|                  |                   |                   |
|                  |                   |                   |

| Sensitivity |       |
|-------------|-------|
|             | %     |
| Sensitivity | 88.2  |
| Specificity | 100.0 |
| PPV         | 100.0 |
| NPV         | 60.0  |
| Accuracy    | 94.1  |

**Supplementary Table 5.** Number of solid parenchymal brain metastases at time of lumbar puncture sample collection.

| <b>Pt Number</b> | <b>Cytology</b> | <b>MRI</b> | <b>Parenchymal Brain Mets Y/N</b> | <b>Number of Parenchymal Brain Mets at LP</b> |
|------------------|-----------------|------------|-----------------------------------|---|
| Ctrl 1 “C01”     | NA              | NA         | NA                                | NA  |
| Ctrl 2 “C04”     | NA              | NA         | NA                                | NA  |
| Ctrl 3           | NA              | NA         | NA                                | NA  |
| Ctrl 4           | NA              | NA         | NA                                | NA  |
| Ctrl 5           | NA              | NA         | NA                                | NA  |
| Ptn 1.a          | Positive        | Suspicious | Y                                 | 3   |
| Ptn 1.b          | Positive        | Suspicious | Y                                 | 3   |
| Ptn 2 “L02”      | Positive        | Suspicious | Y                                 | 10  |
| Ptn 3            | Positive        | Positive   | Y                                 | 4   |
| Ptn 4.a “L07”    | Positive        | Suspicious | Y                                 | 8   |
| Ptn 4.b “L07”    | Positive        | Positive   | Y                                 | >20   |
| Ptn 5            | Positive        | Positive   | N                                 | 0   |
| Ptn 6            | Negative        | Suspicious | Y                                 | >20   |
| Ptn 7            | Positive        | Positive   | Y                                 | 1   |
| Ptn 8 “L13”      | Positive        | Positive   | Y                                 | >20   |
| Ptn 9            | Negative        | Positive   | N                                 | 0   |
| Ptn 10           | Positive        | Positive   | Y                                 | 4   |
| Ptn 11           | Positive        | Positive   | Y                                 | >20   |
| Ptn 12           | Positive        | Positive   | N                                 | 0   |
| Ptn 13.a         | Positive        | Positive   | Y                                 | >20   |
| Ptn 13.b         | Positive        | Positive   | Y                                 | >20   |
| Ptn 14.a         | Positive        | Negative   | N                                 | 0   |
| Ptn 14.b         | Positive        | Negative   | N                                 | 0   |
| Ptn 15           | Negative        | Positive   | N                                 | 0   |
| Ptn 16           | Positive        | Positive   | N                                 | 0   |

\* a. and b. refer to CSF sample collected from same patient on different dates.

\* CSF samples Ctrl 1, Ctrl 2, Ptn 2, Ptn 4, Ptn 8 and Ptn 12 were also used in “test cohort” (Supplementary Table 1).