Supplementary Table 1. The clinical characteristics of patients with CRLM in this study.

| Characteristics                                | (n = 91)                      |
|------------------------------------------------|-------------------------------|
| Age at liver resection (years)                 | Median 59 (34 - 66)           |
| Gender                                         |                               |
| Male                                           | 47 (51.6%)                    |
| Female                                         | 44 (48.4%)                    |
| Primary tumor location                         |                               |
| Right-sided                                    | 18 (19.8%)                    |
| Left-sided                                     | 73 (80.2%)                    |
| Nodal involvement of primary tumor             |                               |
| N1-2                                           | 59 (64.8%)                    |
| N0                                             | 32 (35.2%)                    |
| Time between primary tumor and liver metastases|                               |
| Synchronous                                    | 71 (78.0%)                    |
| Metachronous                                   | 20 (22.0%)                    |
| Preoperative CEA (ng/mL)                       |                               |
| ≤ 5                                            | 20 (22.0%)                    |
| > 5                                            | 71 (78.0%)                    |
| Preoperative chemotherapy                      |                               |
| Chemotherapy alone                             | 47 (51.6%)                    |
| Chemotherapy + Cetuximab                       | 23 (25.3%)                    |
| Chemotherapy + Bevacizumab                     | 14 (15.4%)                    |
| No preoperative chemotherapy                   | 7 (7.7%)                      |
| Best Response before surgery                   |                               |
| PR                                             | 43 (47.3%)                    |
| SD                                             | 41 (45.1%)                    |
| NA                                             | 7 (7.7%)                      |
| Postoperative chemotherapy | Count | Percentage |
|----------------------------|-------|------------|
| Chemotherapy alone         | 65    | 71.4%      |
| Chemotherapy + Cetuximab   | 15    | 16.5%      |
| Chemotherapy + Bevacizumab | 3     | 3.3%       |
| No postoperative chemotherapy | 8    | 8.8%       |

| Concomitant ablation | Count | Percentage |
|----------------------|-------|------------|
| Yes                  | 27    | 29.7%      |
| No                   | 64    | 70.3%      |

| Number of liver metastasis | Count | Percentage |
|----------------------------|-------|------------|
| = 1                        | 25    | 27.5%      |
| > 1                        | 66    | 72.5%      |

| Extent of liver metastases | Count | Percentage |
|----------------------------|-------|------------|
| Unilateral                 | 40    | 44.0%      |
| Bilateral                  | 51    | 56.0%      |

| Diameter of the largest liver metastasis (cm) | Median 4.3 (1.0 – 15.7) |
|-----------------------------------------------|--------------------------|

| CRS | Count | Percentage |
|-----|-------|------------|
| Low risk (Score 0-2) | 33 | 36.3% |
| High risk (Score 3-5) | 58 | 63.7% |

CRLM, colorectal liver metastasis
CEA, carcinoembryonic antigen
PR, partial response
SD, stable disease
CRS, clinical risk score
Supplementary Table 2. The clinical characteristics of patients with CRLM at four time points.

| Characteristics                                      | Baseline ctDNA (n = 53) | Pre-operation ctDNA (n = 65) | Post-operation ctDNA (n = 82) | Post-ACT ctDNA (n = 49) |
|------------------------------------------------------|-------------------------|------------------------------|------------------------------|-------------------------|
| Age at liver resection (years)                       | Median 56 (20 - 73)     | Median 56 (20 - 73)          | Median 56 (20 - 75)          | Median 56 (20 - 73)     |
| Gender                                               |                         |                              |                              |                         |
| Male                                                 | 32 (60.4%)              | 43 (66.2%)                   | 53 (64.6%)                   | 30 (61.2%)              |
| Female                                               | 21 (39.6%)              | 22 (33.8%)                   | 29 (35.4%)                   | 19 (38.8%)              |
| Primary tumor location                               |                         |                              |                              |                         |
| Right-sided                                          | 9 (17.0%)               | 11 (16.9%)                   | 16 (19.5%)                   | 9 (18.4%)               |
| Left-sided                                           | 44 (83.0%)              | 54 (83.1%)                   | 66 (80.5%)                   | 40 (81.6%)              |
| Nodal involvement of primary tumor                   |                         |                              |                              |                         |
| N1-2                                                 | 34 (64.2%)              | 43 (66.2%)                   | 57 (69.5%)                   | 32 (65.3%)              |
| N0                                                   | 19 (35.8%)              | 22 (33.8%)                   | 25 (30.5%)                   | 17 (34.7%)              |
| Prior resection of the primary tumor                 |                         |                              |                              |                         |
| No                                                   | 33 (62.3%)              | 36 (55.4%)                   | 49 (59.8%)                   | 31 (63.3%)              |
| Yes                                                  | 20 (37.7%)              | 29 (44.6%)                   | 33 (40.2%)                   | 18 (36.7%)              |
| Time between primary tumor and liver metastases      |                         |                              |                              |                         |
| Synchronous                                          | 42 (79.2%)              | 53 (81.5%)                   | 66 (80.5%)                   | 41 (83.7%)              |
| Metachronous                                         | 11 (20.8%)              | 12 (18.5%)                   | 16 (19.5%)                   | 8 (16.3%)               |
| Preoperative CEA (ng/mL)                             |                         |                              |                              |                         |
| ≤ 5                                                  | 22 (41.5%)              | 27 (41.5%)                   | 32 (39.0%)                   | 22 (44.9%)              |
| > 5                                                  | 31 (58.5%)              | 38 (58.5%)                   | 50 (61.0%)                   | 27 (55.1%)              |
| Preoperative chemotherapy                            |                         |                              |                              |                         |
| Yes                                                  | 51 (96.2%)              | 64 (98.5%)                   | 76 (92.7%)                   | 44 (89.8%)              |
| No                                                   | 2 (3.8%)                | 1 (1.5%)                     | 6 (7.3%)                     | 5 (10.2%)               |
| Extend of liver metastases                           |                         |                              |                              |                         |
| Solitary                                             | 18 (34.0%)              | 18 (27.7%)                   | 23 (28.0%)                   | 11 (22.4%)              |
| Multifocal                                           | 35 (66.0%)              | 47 (72.3%)                   | 59 (72.0%)                   | 38 (77.6%)              |
| Unilateral                                           | 27 (50.9%)              | 28 (43.1%)                   | 36 (43.9%)                   | 20 (40.8%)              |
|                  | 1st Clinic (26.4%) | 2nd Clinic (32.7%) | 3rd Clinic (30.8%) | 4th Clinic (29.9%) |
|------------------|--------------------|--------------------|--------------------|--------------------|
| Bilateral        | 26 (49.1%)         | 37 (56.9%)         | 46 (56.1%)         | 29 (59.2%)         |
| Concomitant ablation |                  |                    |                    |                    |
| Yes              | 39 (73.6%)         | 20 (30.8%)         | 25 (30.5%)         | 11 (22.4%)         |
| No               | 14 (26.4%)         | 45 (69.2%)         | 57 (69.5%)         | 38 (77.6%)         |
| Postoperative chemotherapy |            |                    |                    |                    |
| Yes              | 47 (88.7%)         | 58 (89.2%)         | 76 (92.7%)         | 47 (95.9%)         |
| No               | 6 (11.3%)          | 7 (10.8%)          | 6 (7.3%)           | 2 (4.1%)           |
| CRS              |                    |                    |                    |                    |
| Low risk (Score 0-2) | 21 (39.6%)         | 18 (27.7%)         | 30 (36.6%)         | 21 (42.9%)         |
| High risk (Score 3-5) | 32 (60.4%)         | 47 (72.3%)         | 52 (63.4%)         | 28 (57.1%)         |

CRLM, colorectal liver metastases
CEA, carcinoembryonic antigen
CRS, clinical risk score
**Supplementary Table 3. Relationship of ctDNA status and clinical characteristics of CRLM.**

| Variables                                | Baseline ctDNA (n = 53) | Pre-operation ctDNA (n = 65) | Post-operation ctDNA (n = 82) | Post-ACT ctDNA (n = 49) |
|------------------------------------------|-------------------------|-----------------------------|-------------------------------|------------------------|
|                                          | Low level (n = 26)      | High level (n = 27)         | Low level (n = 28)            | Low level (n = 27)     |
| Age at liver resection (years)           | 0.928                   | 0.156                       | 0.443                         | 0.553                  |
| < 60                                     | 18                      | 19                          | 22                            | 23                     |
| ≥ 60                                     | 8                       | 8                           | 6                             | 14                     |
| Gender                                   | 0.340                   | 0.420                       | 0.631                         | 0.386                  |
| Female                                   | 12                      | 9                           | 11                            | 11                     |
| Male                                     | 14                      | 18                          | 17                            | 26                     |
| Primary tumor location                   | 0.409                   | 0.318                       | 0.191                         | 0.598                  |
| Right-sided                              | 21                      | 24                          | 26                            | 30                     |
| Left-sided                               | 5                       | 3                           | 2                             | 7                      |
| Nodal involvement of primary tumor       | 0.013                   | 0.182                       | 0.249                         | 0.325                  |
| No                                       | 5                       | 14                          | 12                            | 10                     |
| Yes                                      | 21                      | 13                          | 16                            | 27                     |
| Disease free interval (DFI, months)      | 0.947                   | 0.861                       | 0.476                         | 0.943                  |
| > 12                                     | 5                       | 5                           | 5                             | 6                      |
| < 12                                     | 21                      | 22                          | 23                            | 31                     |
| Diameter of the largest LM (cm)          | <0.001                  | 0.388                       | 0.354                         | 0.586                  |
| < 5                                      | 23                      | 9                           | 16                            | 25                     |
| ≥ 5                                      | 3                       | 18                          | 12                            | 12                     |
| CEA level (ng/mL)                        | 0.280                   | 0.246                       | 0.272                         | 0.390                  |
| < 5                                      | 3                       | 1                           | 3                             | 8                      |
| ≥ 5                                      | 23                      | 26                          | 25                            | 29                     |
| Number of liver metastasis              | 0.497                   | 0.700                       | 0.443                         | 0.207                  |
Low level and high level respectively represent lower-than-median and higher-than-median VAF of baseline ctDNA.

tDNA, circulating tumor DNA
CRLM, colorectal liver metastasis
LM, liver metastasis
CEA, carcinoembryonic antigen
CRS, clinical risk score

|      | < 2 | ≥ 2 | < 2 | ≥ 2 | < 2 | ≥ 2 | < 2 | ≥ 2 |
|------|-----|-----|-----|-----|-----|-----|-----|-----|
| CRS  | 0.008 | 0.069 | 0.503 | 0.407 |
| 0-2  | 15 | 6 | 11 | 7 | 19 | 11 | 13 | 8 |
| 3-5  | 11 | 21 | 17 | 30 | 29 | 23 | 14 | 14 |

| Recurrence | < 2 | ≥ 2 | < 2 | ≥ 2 | < 2 | ≥ 2 | < 2 | ≥ 2 |
|------------|-----|-----|-----|-----|-----|-----|-----|-----|
| No         | 0.132 | 0.122 | < 0.001 | 0.010 |
| Yes        | 15 | 10 | 12 | 23 | 20 | 27 | 11 | 17 |
Supplemental Figure 1. Flow chart of patients enrolled and sample collection at different time points for analysis.