Materials Figure for

Rapid multi-dynamic algorithm for gray image analysis of the stroma percentage on colorectal cancer

Supplementary Figure
Supplementary Figure 1. The regions with tissues segmented by the MATLAB for the calculation of TSP-cad.

(A) Original Red-Green-Blue(RGB) style (B) Image was annotated using tumor, stroma and necrosis labels.

The result was num1 = 0.0863  num2 = 0.5459  num3 = 0.368

we considered the tumor and stroma proportions to be complementary.

TSP = Stroma/(Tumor + Stroma)*100% = num3/(num2 + num3)*100% = 0.368/(0.5459 + 0.368) * 100% = 40.27%
Supplementary Figure 2. Kaplan-Meier survival analysis curves of SGH chort for TSP-low verse TSP-high. (A) represent the OS of the left sided and rectum tumors (B) represent the OS of the right sided tumors.
Supplementary Figure 3. Kaplan-Meier survival analysis for overall survival of TSP-low versus TSP-high. Results for SGH cohort (A,B), Training cohort (C,D), Testing cohort (E,F), according to the TSP-cad(50%) and TSP-cad(medium) classifier stratified by clinicopathological risk factors. P-values were calculated by log-rank test.
Materials Table for

Rapid multi-dynamic algorithm for gray image analysis of the stroma percentage on colorectal cancer

Supplementary Table
**Supplementary Table 1** Clinicopathological data for TMA cohort in relation to TSP-manual

| Variable                        | TSP-manual<sup>a</sup> | N  | TSP-low(%) | TSP-high(%) | p value |
|--------------------------------|-------------------------|----|------------|-------------|---------|
| Age(years)                     |                         |    |            |             |         |
| <65                             | 55                      | 37 (59.7%) | 18 (40.9%) | 0.057      |
| ≥65                             | 51                      | 25 (40.3%) | 26 (59.1%) |           |
| Gender                          |                         |    |            |             | 0.947   |
| Male                            | 55                      | 32 (51.6%) | 23 (52.3%) |           |
| Female                          | 51                      | 30 (48.4%) | 21 (47.7%) |           |
| TNM Stage                       |                         |    |            |             | <0.001*  |
| I+II                            | 51                      | 41 (66.1%) | 10 (22.7%) |           |
| III+IV                          | 55                      | 21 (33.9%) | 34 (77.3%) |           |
| Depth of invasion               |                         |    |            |             | 0.991    |
| T1+T2                           | 12                      | 7 (11.3%)  | 5 (11.4%)  |           |
| T3+T4                           | 94                      | 55 (88.7%) | 39 (88.6%) |           |
| Lymph node metastasis           |                         |    |            |             | <0.001*  |
| N0                              | 49                      | 39 (62.9%) | 10 (22.7%) |           |
| N1+N2                           | 57                      | 23 (37.1%) | 34 (77.3%) |           |
| Nerve invasion                  |                         |    |            |             | 0.720    |
| NO                              | 79                      | 47 (75.8%) | 32 (73.7%) |           |
| YES                             | 27                      | 15 (24.2%) | 12 (27.3%) |           |
| Vessel invasion                 |                         |    |            |             | 0.061    |
| NO                              | 66                      | 34 (54.8%) | 32 (72.7%) |           |
| YES                             | 40                      | 28 (45.2%) | 12 (27.3%) |           |
## Supplementary Table 2
Clinicopathological data for TCGA cohort in relation to TSP-manual

| Variable                    | TSP-manual |   |   | p value |
|-----------------------------|------------|---|---|---------|
|                            | N          | TSP-low(%) | TSP-high(%) |         |
| Age(years)                  |            |             |             | 0.697   |
| <65                         | 200        | 160(46.2%)  | 40(44.0%)   |         |
| ≥65                         | 237        | 186(53.8%)  | 51(56.0%)   |         |
| Gender                      |            |             |             | 0.647   |
| Male                        | 235        | 188(54.3%)  | 47(51.6%)   |         |
| Female                      | 202        | 158(45.7%)  | 44(48.4%)   |         |
| TNM stage                   |            |             |             | 0.024*  |
| I+II                        | 238        | 198(57.2%)  | 40(44.0%)   |         |
| III+IV                      | 199        | 148(42.8%)  | 51(56.0%)   |         |
| Depth of invasion           |            |             |             | 0.021*  |
| T1+T2                       | 91         | 80(23.1%)   | 11(12.1%)   |         |
| T3+T4                       | 346        | 266(76.9%)  | 80(87.9%)   |         |
| Lymph node metastasis       |            |             |             | 0.028*  |
| N0                          | 246        | 204(59.0%)  | 42(46.2%)   |         |
| N1+N2                       | 191        | 142(41.0%)  | 49(53.8%)   |         |
| Lymphatic invasion          |            |             |             | 0.237   |
| NO                          | 287        | 232(67.1%)  | 55(60.4%)   |         |
| YES                         | 150        | 114(32.9%)  | 36(39.6%)   |         |
| Vessel invasion             |            |             |             | 0.61    |
| NO                          | 340        | 271(78.3%)  | 69(75.8%)   |         |
| YES                         | 97         | 75(21.7%)   | 22(24.2%)   |         |

*P < 0.05
| Tumor location               | TSP-manual | TSP-cad(50%) | TSP-cad(median) |
|-----------------------------|------------|--------------|-----------------|
|                             | TSP-low    | TSP-high     |                 |
| Right                       | 169        | 132(38.2%)   | 37(40.7%)       |
| Left and rectal             | 268        | 214(61.8%)   | 54(59.3%)       |

\*P < 0.05

**Supplementary Table 3**  Cross-tabulation of TSP-manual versus TSP-cad after dichotomisation in the Training cohort

|                      | TSP-manual | TSP-cad(50%) | TSP-cad(median) |
|----------------------|------------|--------------|-----------------|
|                      | TSP-low    | TSP-high     |                 |
| TSP-cad(50%)         | 444        | 50           | 494             |
| K=0.827              | 4          | 205          | 209             |
| total                | 448        | 255          | 703             |
| TSP-cad(median)      | 318        | 31           | 349             |
| K=0.543              | 130        | 224          | 354             |
| total                | 448        | 255          | 703             |
### Supplementary Table 4  Cross-tabulation of TSP-manual versus TSP-cad after dichotomisation in the Testing cohort

|                  | TSP-manual |          |          |
|------------------|------------|----------|----------|
|                  | TSP-low    | TSP-high | total    |
| TSP-cad(50%)     | 190        | 27       | 217      |
| K=0.776          |            |          |          |
| TSP-cad(median)  | 139        | 10       | 149      |
| K=0.575          |            |          |          |

### Supplementary Table 5  Predictive performance comparison among the different TSP in SGH cohort

| SGH cohort      | Linear trend χ² score | LR test |
|-----------------|------------------------|---------|
| Over survival   |                        |         |
| TSP-manual      | 12.025                 | 11.601  |
| TSP-cad(50%)    | 10.953                 | 10.3    |
| TSP-cad(median) | 13.445                 | 13.673  |

LR, likelihood ratio;
**Supplementary Table 6**  Uni- and multivariate Cox regression analysis for overall survival in the Training cohort

|                | Univariate analysis |                  |                  | Multivariate analysis |                  |                  |
|----------------|---------------------|------------------|------------------|----------------------|------------------|------------------|
|                |                     | TSP-manual        | TSP-cad(50%)     | TSP-cad(median)      |                  |                  |
|                | HR                  | 95%CI             | HR               | 95%CI               | HR               | 95%CI            |
| Age(years)     |                     |                  |                  |                      |                  |                  |
| <65            | 1                   |                  |                  |                      |                  |                  |
| ≥65            | 3.508               | 1.804-5.183      |                  |                      |                  |                  |
| Gender         |                     |                  |                  |                      |                  |                  |
| Male           | 1                   |                  |                  |                      |                  |                  |
| Female         | 0.594               | 0.368-0.956      |                  |                      |                  |                  |
| TNM Stage      |                     |                  |                  |                      |                  |                  |
|                | T1+T2       | T3+T4       | T1+T2       | T3+T4       | T1+T2       | T3+T4       | T1+T2       | T3+T4       |
|----------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|
| III+IV         | 3.695       | 2.291-5.959 | 1.818       | 0.563-5.869 | 1.822       | 0.571-5.811 | 1.755       | 0.569-5.411 |
| LN metastasis  |             |             |             |             |             |             |             |             |
| N0             | 1           | 1           | 1           | 1           | 1           | 1           | 1           | 1           |
| N1+N2          | 3.584       | 2.223-5.779 | 1.497       | 0.464-4.830 | 1.494       | 0.469-4.759 | 1.516       | 0.494-4.653 |
| Nerve invasion |             |             |             |             |             |             |             |             |
| NO             | 1           | 1           | 1           | 1           | 1           | 1           | 1           | 1           |
| YES            | 2.165       | 1.359-3.450 | 1.191       | 0.713-1.992 | 1.21        | 0.724-2.021 | 1.217       | 0.729-2.031 |
| Vessel invasion|             |             |             |             |             |             |             |             |
| NO             | 1           | 1           | 1           | 1           | 1           | 1           | 1           | 1           |
| YES            | 3.004       | 1.913-4.716 | 1.981       | 1.190-3.298 | 1.98        | 1.189-3.295 | 1.965       | 1.182-3.267 |
| Differentiation status | 1.511 | 0.610-3.743 |             |             |             |             |             |             |
| Well           | 1           |             |             |             |             |             |             |             |
| Moderate+poor  | 1.159       | 0.670-1.675 |             |             |             |             |             |             |
| Tumor size     |             |             |             |             |             |             |             |             |
| <5cm           | 1           |             |             |             |             |             |             |             |
| ≥5cm           | 1.059       | 0.670-1.675 |             |             |             |             |             |             |
| Tumor location |             |             |             |             |             |             |             |             |
| Right          | 1           |             |             |             |             |             |             |             |
| Left and rectal| 0.564       | 0.360-0.884 | 0.581       | 0.366-0.992 | 0.575       | 0.363-0.913 | 0.567       | 0.358-0.898 |
| TSP-manual     |             |             |             |             |             |             |             |             |
| TSP-low        | 1           |             |             |             |             |             |             |             |
| TSP-high       | 1.61        | 1.030-2.517 | 1.376       | 0.876-2.161 |             |             |             |             |
| TSP-cad(50%)   |             |             |             |             |             |             |             |             |
| TSP-low        | 1           |             |             |             |             |             |             |             |
| TSP-high       | 1.638       | 1.042-2.573 | 1.413       | 0.908-2.256 |             |             |             |             |
| TSP-cad(median)|             |             |             |             |             |             |             |             |
| TSP-low        | 1           |             |             |             |             |             |             |             |
| TSP-high       | 1.759       | 1.101-2.809 |             |             |             |             |             |             |

Abbreviations: HR: hazard ratio; CI: confidence interval; LN metastasis: Lymph node metastasis

*p < 0.05
**Supplementary Table 7** Uni- and multivariate Cox regression analysis for overall survival in the Testing cohort

|                | Univariate analysis | Multivariate analysis |
|----------------|---------------------|-----------------------|
|                | HR 95%CI            | TSP-manual            | TSP-cad(50%)         | TSP-cad(median) |
| Age (years)    |                     |                       |                      |                |
| <65            | 1                   | HR 95%CI              | HR 95%CI            | HR 95%CI       |
| ≥65            | 2.368 1.646-3.407   | HR 95%CI              | HR 95%CI            | HR 95%CI       |
| Gender         |                     |                       |                      |                |
| Male           | 1                   | HR 95%CI              | HR 95%CI            | HR 95%CI       |
| Female         | 0.613 0.413-0.909   | HR 95%CI              | HR 95%CI            | HR 95%CI       |
| TNM Stage       | I+II   | 1       | 1       | 1       | 1       |
|-----------------|--------|---------|---------|---------|---------|
| III+IV          | 3.54   | 2.404-5.212* | 1.171 | 0.443-3.096 | 1.197  | 0.46-3.114 | 1.214  | 0.486-3.169 |

| Depth of invasion | T1+T2 | 1       | 1       | 1       | 1       |
|-------------------|--------|---------|---------|---------|---------|
| T3+T4            | 2.878  | 1.504-5.506* | 1.343 | 0.681-2.65 | 1.343  | 0.68-2.651 | 1.315  | 0.666-2.597 |

| LN metastasis    | N0     | 1       | 1       | 1       | 1       |
|------------------|--------|---------|---------|---------|---------|
| N1+N2            | 3.724  | 2.516-5.512* | 2.334 | 0.869-6.268 | 2.305  | 0.872-6.092 | 2.186  | 0.841-5.678 |

| Nerve invasion   | NO     | 1       | 1       | 1       | 1       |
|------------------|--------|---------|---------|---------|---------|
| YES              | 2.298  | 1.575-3.353* | 1.38  | 0.96-2.103 | 1.41   | 0.927-2.145 | 1.398  | 0.919-2.126 |

| Vessel invasion  | NO     | 1       | 1       | 1       | 1       |
|------------------|--------|---------|---------|---------|---------|
| YES              | 2.684  | 1.858-3.875* | 1.608 | 1.055-2.451* | 1.593  | 1.045-2.427* | 1.593  | 1.046-2.425* |

| Differentiation status | Well     | 1       | 1       | 1       | 1       |
|------------------------|----------|---------|---------|---------|---------|
| Moderate+poor          | 2.574    | 1.050-6.312* | 2.613 | 0.874-5.352 | 2.19   | 0.884-5.423 | 2.198  | 0.888-5.443 |

| Tumor size | <5cm | 1       | 1       | 1       | 1       |
|------------|------|---------|---------|---------|---------|
| ≥5cm       | 1.287 | 0.889-1.862 |

| Tumor location | Right | 1       | 1       | 1       | 1       |
|----------------|--------|---------|---------|---------|---------|
| Left and rectal | 0.502  | 0.348-0.723* | 0.467 | 0.320-0.682* | 0.461  | 0.316-0.673* | 0.456  | 0.312-0.664* |

| TSP-manual     | TSP-low | 1       | 1       | 1       | 1       |
|----------------|----------|---------|---------|---------|---------|
| TSP-high       | 1.75     | 1.214-2.523* | 1.512 | 1.045-2.187* |

| TSP-cad(50%)   | TSP-low | 1       | 1       | 1       | 1       |
|----------------|----------|---------|---------|---------|---------|
| TSP-high       | 1.659    | 1.145-2.405* | 1.443 | 0.993-2.097 |

| TSP-cad(median) | TSP-low | 1       | 1       | 1       | 1       |
|-----------------|----------|---------|---------|---------|---------|
| TSP-high       | 1.823   | 1.239-2.683* | 1.632 | 1.105-2.410* |

Abbreviations: HR: hazard ratio; CI: confidence interval; LN metastasis: Lymph node metastasis
*P < 0.05