

Supplementary Tables S1–S4

Supplementary Table S1. TaqMan Advanced miRNA Assays for qRT-PCR.

| Assay Name | Assay ID | Context Sequence |
|-------------------|-----------------|-------------------------|
| hsa-miR-129-1-3p | 480873_mir | AAGCCCUUACCCCAAAAAGUAU |
| hsa-miR-566 | 479373_mir | GGGCGCCUGUGAUCCCAAC |
| hsa-miR-598-5p | 479080_mir | GCGGUGAUCCCGAUGGUGUGAGC |
| hsa-miR-4669 | 478925_mir | UGUGUCCGGGAAGUGGAGGAGG |
| hsa-miR-6756-5p | 480284_mir | AGGGUGGGGCUGGAGGUGGGGCU |

Supplementary Table S2. Urinary miRNA biomarker in the patients with well to poorly differentiated CRC.

| | miR-129-1-3p ($2^{-\Delta Ct}$, Median, IQR) | <i>p</i> Value | miR-566 ($2^{-\Delta Ct}$, Median, IQR) | <i>p</i> Value |
|---------------------------|--|----------------|---|----------------|
| HC (<i>n</i> = 203) | 0.00040 (0.00016–0.00096) | | 0.048 (0.026–0.140) | |
| CRC tub (<i>n</i> = 186) | 0.00145 (0.00086–0.00292) | < 0.001* | 0.200 (0.082–0.473) | < 0.001* |
| por (<i>n</i> = 17) | 0.00152 (0.00081–0.00262) | < 0.001* | 0.136 (0.042–0.343) | 0.064* |

Ct, threshold cycle; IQR, interquartile range; HC, healthy control; CRC, colorectal cancer; tub, well to moderate differentiated adenocarcinoma; por, poorly differentiated adenocarcinoma

* *P* values were calculated in comparison with HC.

Supplementary Table S3. Expression of miR-129-1-3p and miR-566 in serum sample.

| | | HC (n = 24) | CRC (n = 24) | p Value |
|---------------------|--------------------------------------|--|---|----------------|
| miR-129-1-3p | 2^{-ΔCt}, Median, IQR | 0.00259 (0.00081–0.00756) | 0.00445 (0.00292 –0.00740) | 0.071 |
| miR-566 | 2^{-ΔCt}, Median, IQR | 0.259 (0.113–1.976) | 1.304 (0.471–1.984) | 0.027 |

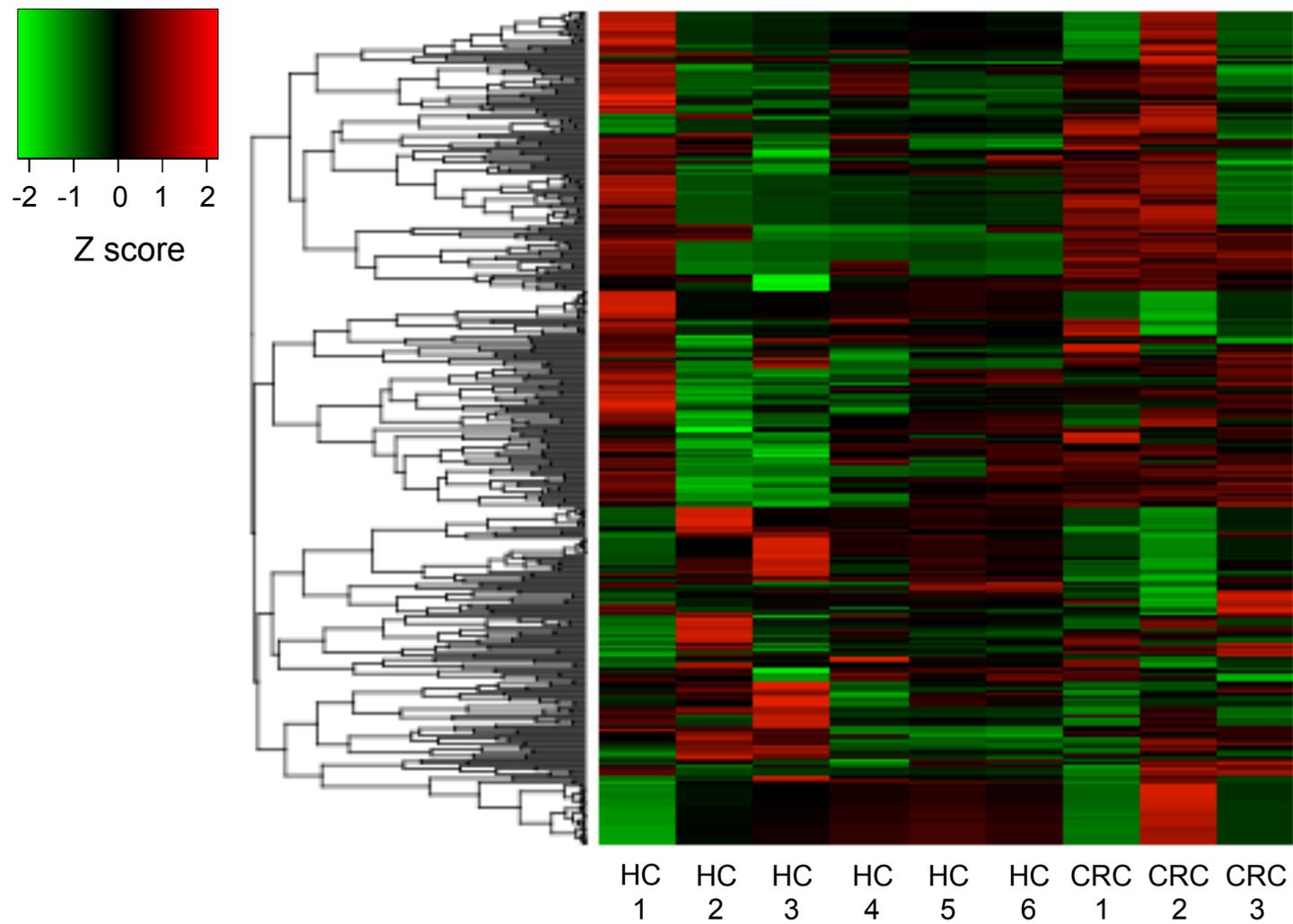
HC, healthy control; CRC, colorectal cancer; IQR, interquartile range; Ct, threshold cycle;

Supplementary Table S4. Characteristics of the patients with CRC for tissue miRNA analysis.

| ID | Age (years) | Gender | Location | Stage | Histological grade |
|-----------|--------------------|---------------|-----------------|--------------|---------------------------|
| CRC1 | 55 | Male | Sigmoid | IIIB | moderately differentiated |
| CRC2 | 74 | Female | Cecum | IIA | poorly differentiated |
| CRC3 | 73 | Female | Sigmoid | IIA | well differentiated |
| CRC4 | 44 | Male | Rectum | IIA | well differentiated |
| CRC5 | 38 | Female | Sigmoid | IIA | well differentiated |
| CRC6 | 80 | Female | Sigmoid | IIIB | well differentiated |
| CRC7 | 69 | Female | Ascending | IIIC | poorly differentiated |
| CRC8 | 83 | Female | Transverse | IIA | moderately differentiated |
| CRC9 | 68 | Male | Sigmoid | IIIB | well differentiated |
| CRC10 | 69 | Female | Ascending | IIA | moderately differentiated |
| CRC11 | 52 | Male | Transverse | IIA | moderately differentiated |
| CRC12 | 70 | Female | Sigmoid | IIIB | moderately differentiated |
| CRC13 | 73 | Female | Cecum | IIIB | mucinous |
| CRC14 | 77 | Male | Sigmoid | IIB | well differentiated |
| CRC15 | 78 | Female | Cecum | IIA | well differentiated |
| CRC16 | 74 | Male | Rectum | IIA | moderately differentiated |
| CRC17 | 72 | Male | Rectum | IIA | moderately differentiated |
| CRC18 | 68 | Male | Sigmoid | IIA | well differentiated |
| CRC19 | 52 | Male | Ascending | IIIB | poorly differentiated |
| CRC20 | 70 | Male | Ascending | IIA | well differentiated |
| CRC21 | 75 | Female | Rectum | IIIB | moderately differentiated |

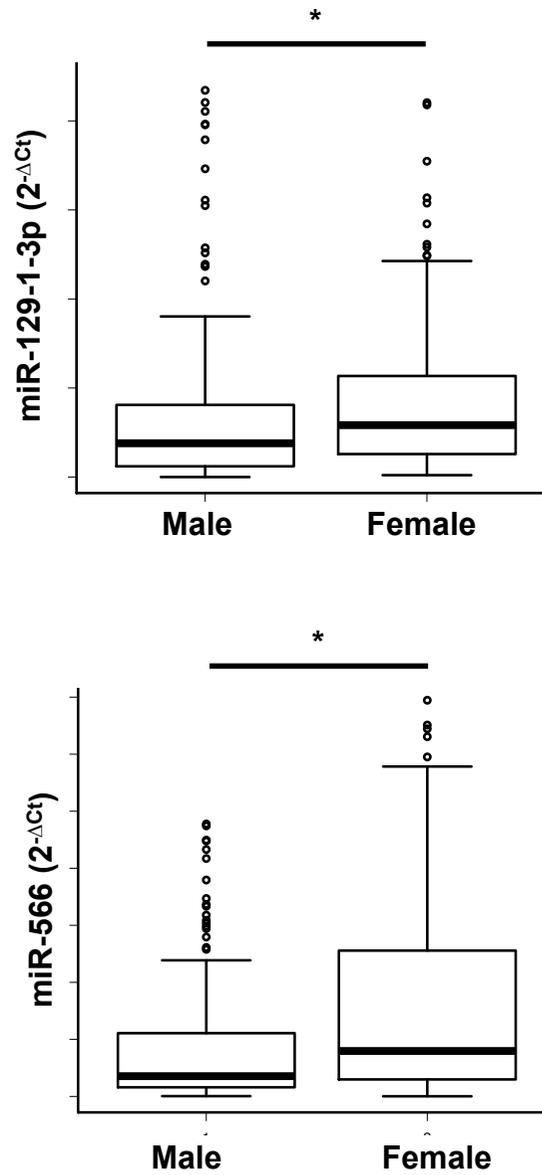
| | | | | | |
|--------------|-----------|-------------|-------------------|------------|----------------------------|
| CRC22 | 69 | Male | Sigmoid | IIA | well differentiated |
| CRC23 | 76 | Male | Transverse | IIA | well differentiated |

Supplementary Figures S1–S5

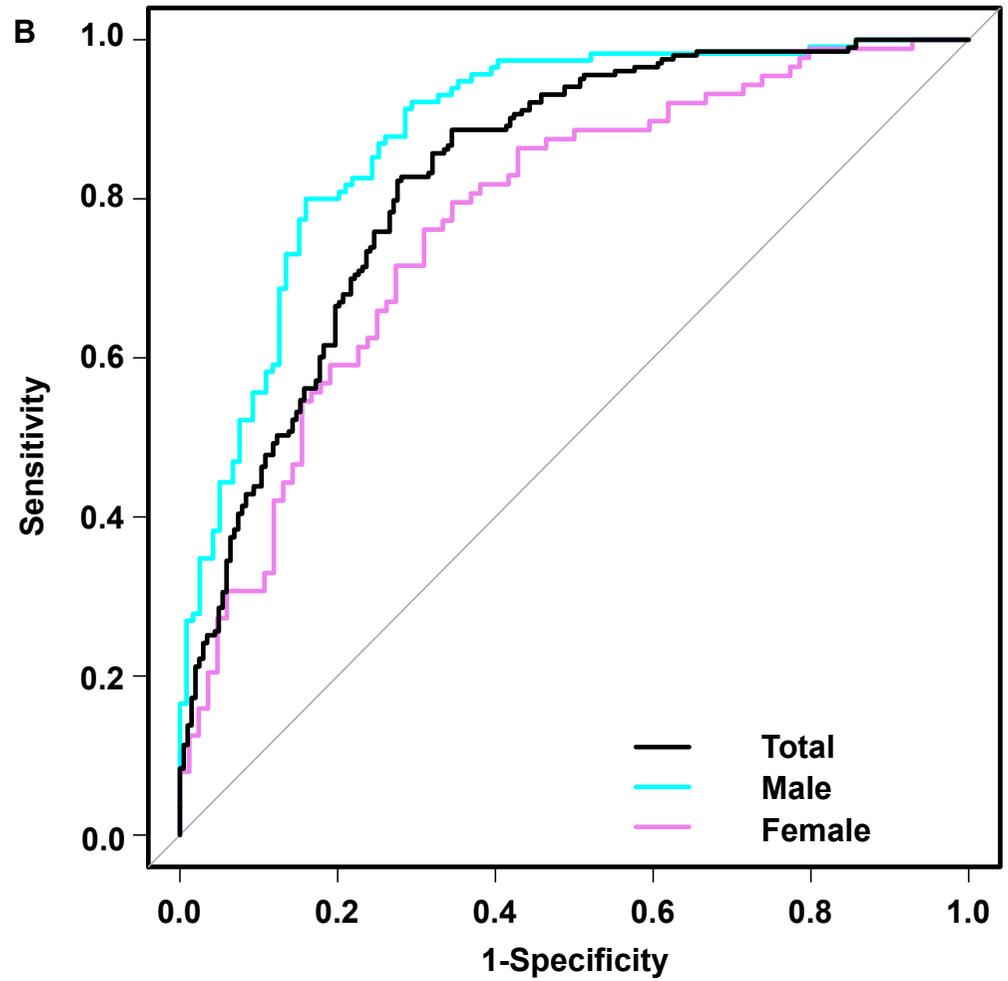


Supplementary Figure S1. Urinary miRNA microarray in the discovery cohort. HC, healthy control; CRC, colorectal cancer

A

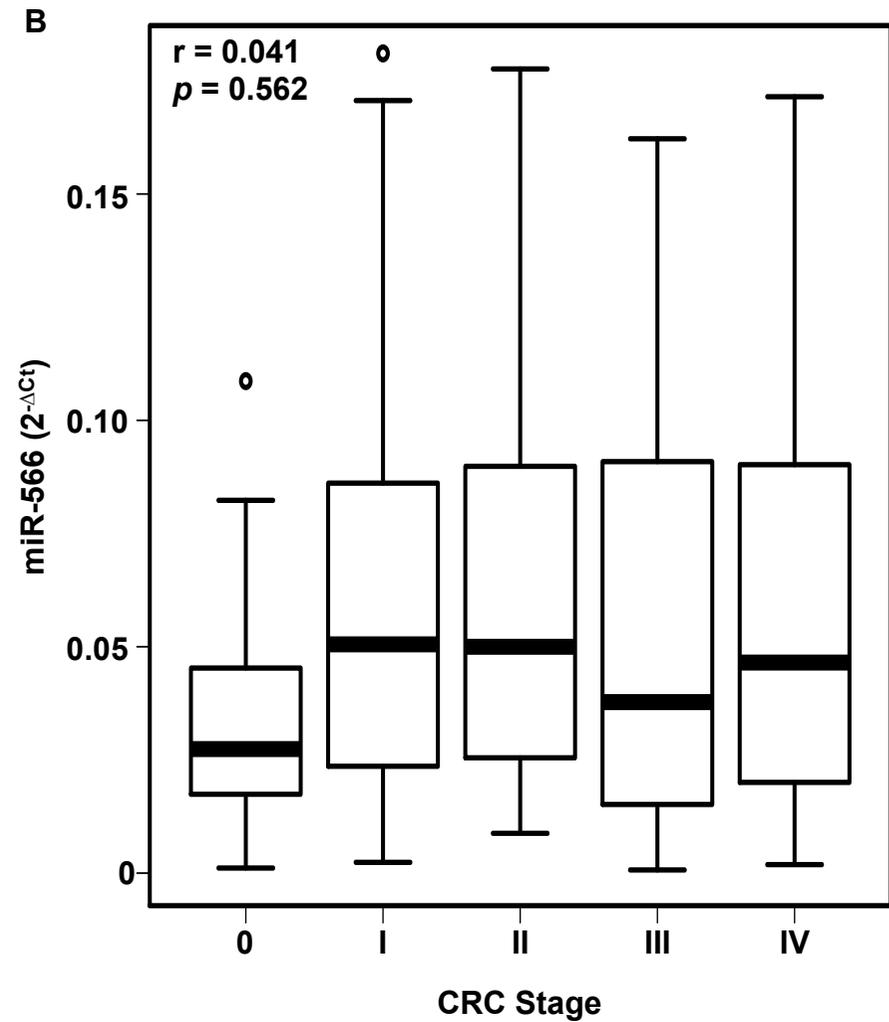
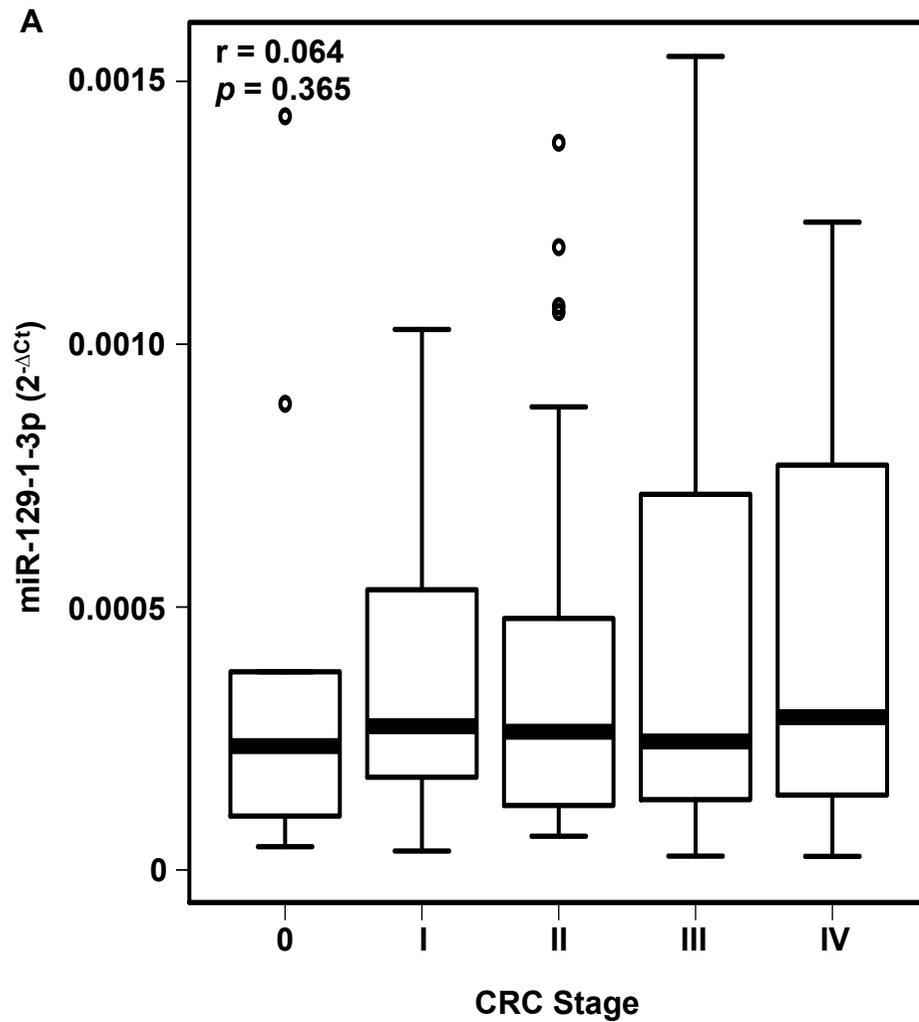


B



| miR-129-1-3p + miR-566 | AUC [95% CI] |
|------------------------|---------------------|
| Total | 0.828 [0.789–0.868] |
| Male | 0.882 [0.839–0.925] |
| Female | 0.773 [0.703–0.843] |

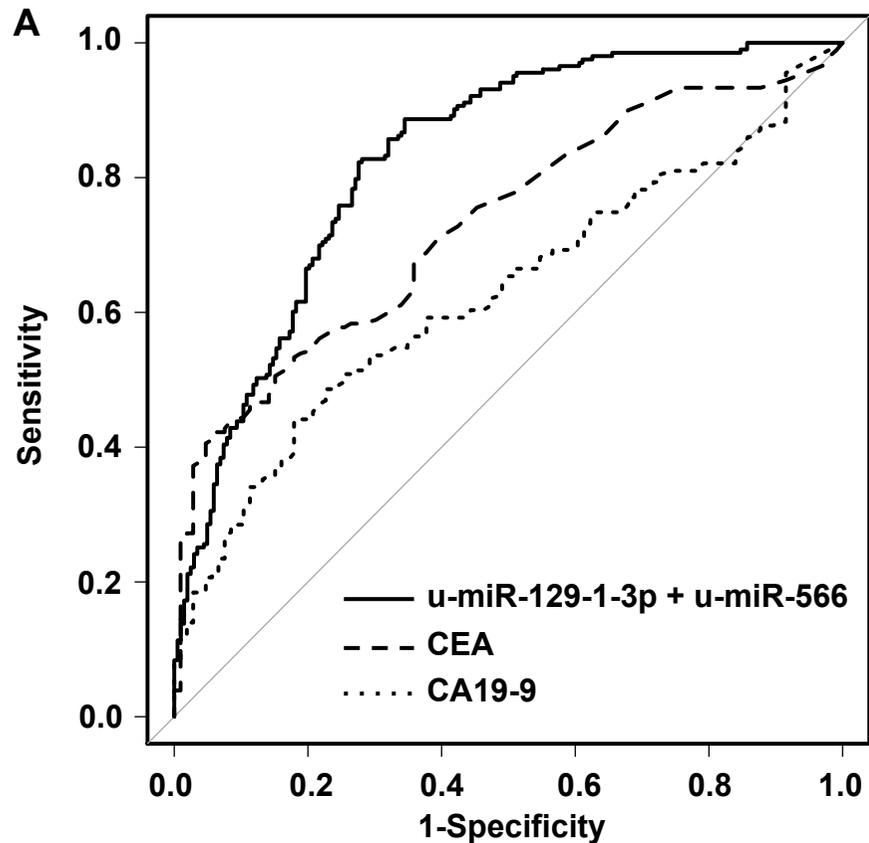
Supplementary Figure S2. Effect of gender on urinary biomarker. A. Boxplots according to genders, B. Receiver operator characteristics curve in the whole cohort.
 AUC, area under the curve; 95% CI, 95% confidence interval; * $p < 0.001$



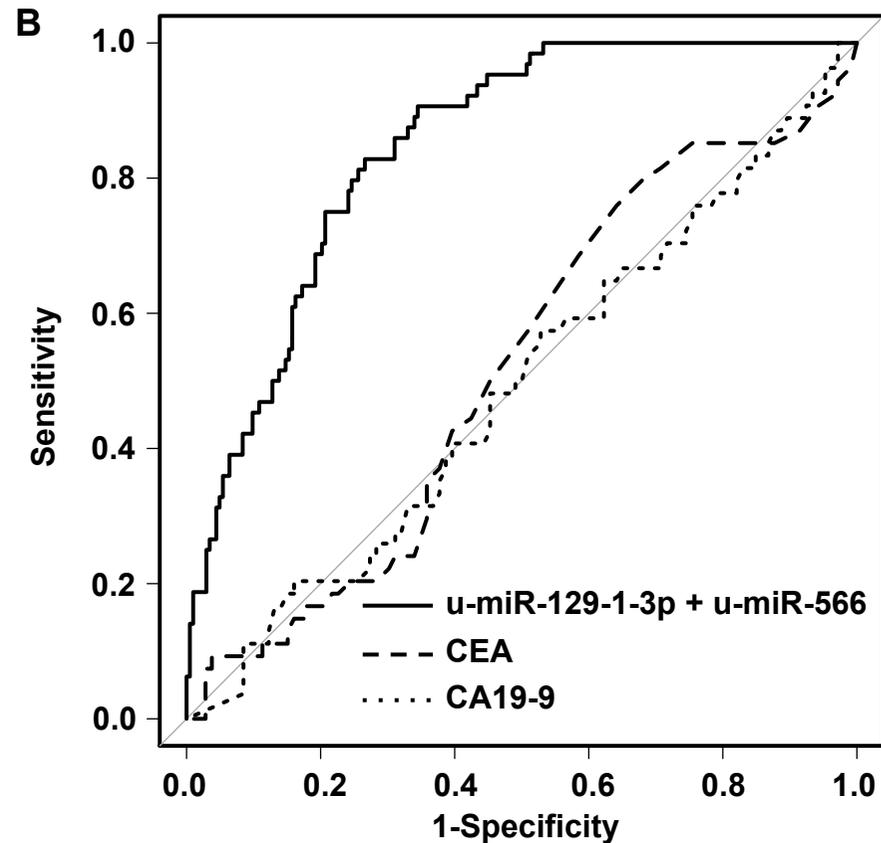
Supplementary Figure S3. Correlation between urinary miRNA level and disease stage. A. miR-129-1-3p. B. miR-566.

Ct, cycle threshold; CRC, colorectal cancer

Data were analyzed using the Spearman rank correlation.



| Biomarker | AUC [95% CI] | Sensitivity % | Specificity % |
|-------------------------------|------------------------|------------------|------------------|
| CEA | 0.731 [0.674–0.789] | 46.7* | 87.7* |
| CA19-9 | 0.627 [0.562–0.691] | 28.9* | 90.6* |
| u-miR-129-1-3p + u-miR-566 | 0.828 [0.789–0.868] | 82.8 | 71.9 |



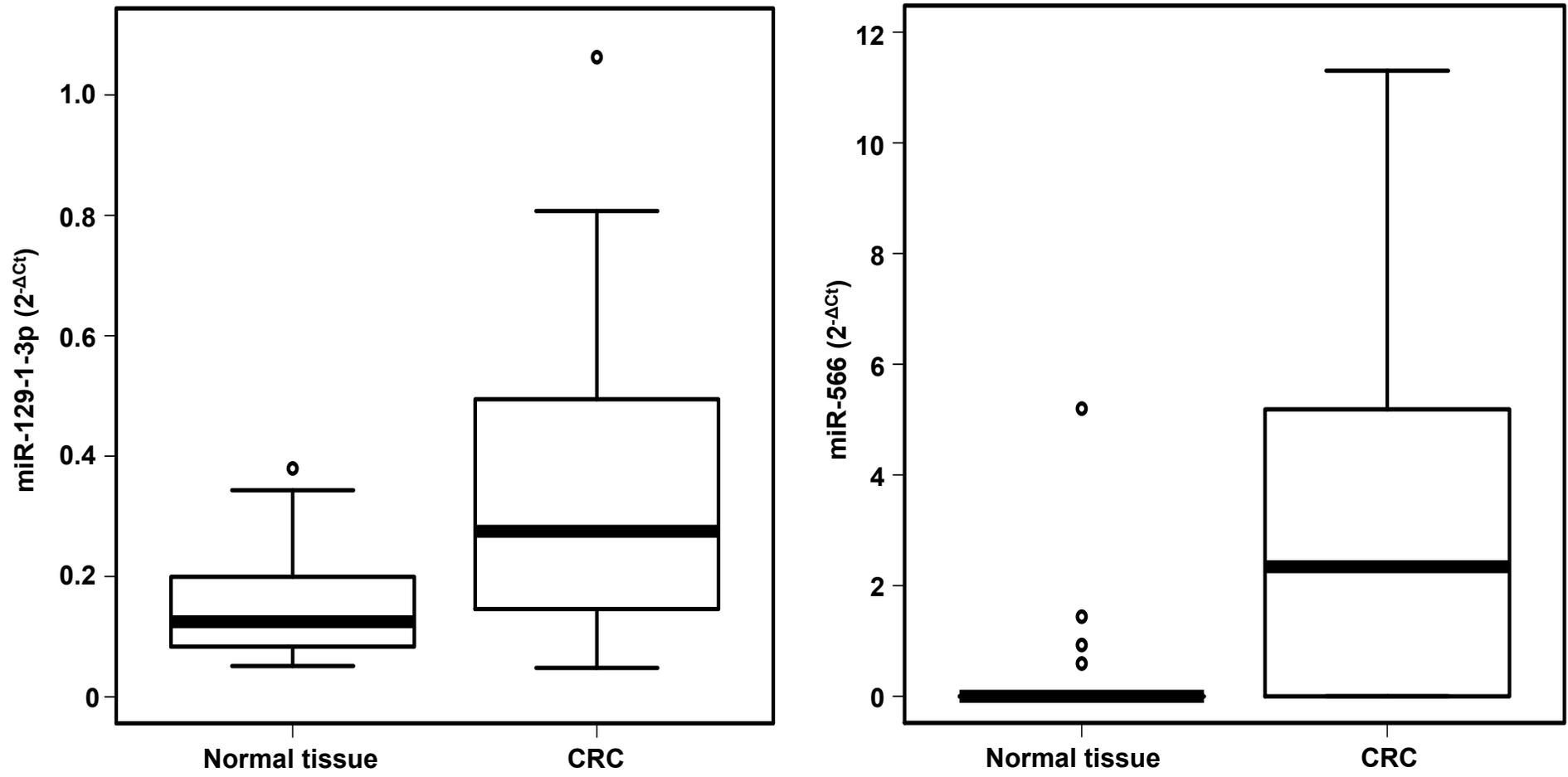
| Biomarker | AUC [95% CI] | Sensitivity % | Specificity % |
|-------------------------------|------------------------|------------------|------------------|
| CEA | 0.519 [0.425–0.613] | 11.1* | 87.7* |
| CA19-9 | 0.490 [0.395–0.586] | 11.1* | 90.6* |
| u-miR-129-1-3p + u-miR-566 | 0.845 [0.798–0.893] | 82.8 | 73.4 |

Supplementary Figure S4 Comparison between urinary biomarker and serum CEA/CA19-9.

A. All Stage CRC. B. Stage 0/I CRC

* The cut-off values for CEA and CA19-9 were 5ng/mL and 37U/mL, respectively.

u-miR-129-1-3p, urinary miR-129-1-3p; u-miR-566, urinary miR-566; AUC, area under the curve; 95% CI, 95% confidence interval



| | $2^{-\Delta Ct}$ (Median, IQR) | | <i>p</i> Value |
|--------------|--------------------------------|------------------------|----------------|
| | Normal tissue (<i>n</i> = 23) | CRC (<i>n</i> = 23) | |
| miR-129-1-3p | 0.125 (0.083-0.199) | 0.275 (0.146-0.494) | 0.008 |
| miR-566 | 0 (0-0) | 2.342 (0-5.185) | 0.002 |

Supplementary Figure S5. Expression of miR-129-1-3p and miR-566 in tissue sample.
 Ct, cycle threshold; CRC, colorectal cancer; IQR, interquartile range