Supplementary information

Supplementary Materials and Methods

Clinical samples

All patients were clearly identified as having colorectal cancer based on the clinicopathologic criteria described by the Japanese Society for Cancer of the Colon and Rectum. Resected cancer tissues were immediately cut and embedded in Tissue-Tek OCT medium (Sakura), frozen in liquid nitrogen, and maintained at -80°C until RNA extraction. Frozen tissue specimens were homogenized in guanidium thiocyanate, and total RNA was obtained by ultracentrifugation through a cesium chloride cushion. Clinicopathological factors and clinical stage were classified by the criteria of the Japanese Society for Cancer of the Colon and Rectum. All sample data, including age, gender, tumor size and depth, lymphatic invasion, lymph node metastasis, vascular invasion, liver metastasis, distant metastasis, clinical stage and histological grade were obtained from the clinical and pathologic records.

Laser micro-dissection (LMD)

For LMD, five micron frozen sections were fixed in 70% ethanol for 30 sec, stained with hematoxylin and eosin, and dehydrated as follows: five sec each in 70%, 95%, and 100% ethanol and a final five min in xylene. Sections were air-dried, then micro-dissected with the LMD system. Target cells were excised, at least 100 cells per

section, and bound to the transfer film, and total RNA extracted.

Quantitative real-time reverse transcriptase-polymerase chain reaction (qRT-PCR)

For *miR-25* and *miR-92a* qRT-PCR, cDNA was synthesized from ten ng of total RNA using TaqMan™ miRNA hsa-*miR-25*- or *92a*-specific primers (Applied Biosystems) and a TaqMan™MicroRNA Reverse Transcription Kit (Applied Biosystems). PCR was performed in a LightCyclerTM 480 System (Roche Applied Science) using the LightCyclerTM 480 Probes Master kit (Roche Applied Science). The following temperature profile was used: initial denaturation at 95°C for ten min, followed by 45 cycles of denaturation at 95°C for ten s, annealing at 60°C for ten s, and extension at 65°C for ten s. Expression levels of *miR-25* and miR-*92a* were normalized to that of the small nuclear RNA RNU6B (Applied Biosystems) transcript. Each assay was performed three times to verify the results, and the mean normalized value of expression was used for subsequent analyses.

Supplementary Figure legends

Supplementary Figure S1

Tissue section stained with hematoxylin and eosin before (left) and after (right) laser microdissection in six representative samples of colorectal cancer.

Supplementary Figure S2

Two-dimensional hierarchical cluster analysis of upregulated miRNAs and samples was performed to generate a heatmap. The distance between every pair of miRNAs was calculated as 1.0 - (Pearson's correlation coefficient in expression signal between these two miRNAs). The heatmaps indicate a high (red) or low (green) level of expression. The Ward's linkage algorithm was used for cluster analysis.

A: Results of unsupervised hierarchical clustering of four normal and thirteen cancer stromal samples based on expression of miRNAs. Each column represents a sample and each row a probe set. **B:** Cluster Dendrogram. All components of the miR-17-92a cluster and the miR-106b-25 cluster except miR-92a is classified in Cluster 2 (middle). On the other hand, miR-92a was classified in Cluster 3(Right).