Supplementary data

Supplementary Figure Legends:

Supplementary Figure S1 miR-493 expression inhibits liver metastasis of colon cancer cells. (A) Effects of the expression of the candidate miRNAs on in vitro growth of HCT116 cells. HCT116/GFP cells were transfected with the indicated miRNAs, and mixed with HCT116/RFP cells as described in Figure 2A. The mixture of cells was cultivated in vitro under normal culture conditions for 2 weeks, and the number of GFP- or RFP-positive cells was counted, and the inhibitory effect of each miRNA on the GFP/RFP ratio was evaluated as described in Figure 2B. (B) Effects of the expression of miRNA mimics on proliferation of HCT116 cells in the spleen. The inhibitory effects of each miRNA were calculated as described in Figure 2G. (C) Effects of the expression of miRNA mimics on in vitro growth of HCT116 cells. The inhibitory effects of each miRNA were calculated as described above. Average values of two independent experiments are shown.
Supplementary Figure S2 miR-493 expression induces cell death of colon cancer cells in metastasized liver. (A) HE stain of metastasized liver on day 2 after splenic injection of HCT116/GFP cells. Arrows show 2 examples of metastasized cells. (B) Annexin V assays of HCT116 cells transfected with miR-493 in vitro. HCT116/RFP cells were transfected with the indicated miRNA mimic, cultivated under normal culture conditions, and incubated with alexa 488-Annexin V as described in Figure 3C. The fraction of liver-metastasized HCT116/RFP cells that were positive for Annexin V staining was quantified under fluorescent microscope. (C) Invasion assays of HCT116 cells transfected with miRNA. HCT116 cells transfected with the indicated miRNA mimics were incubated with medium containing 0.1% serum overnight, and used for standard 24-transwell Matrigel invasion assays.

Supplementary Figure S3 IGF1R is a direct target of miR-493 and
partially mediates the inhibition of liver metastasis by miR-493. (A) Transient luciferase assays. The reporter plasmid with IGF1R 3'UTR or the control plasmid was transfected into HCT116 cells together with the indicated miRNA mimic, and the luciferase activity was measured 2 days after the transfection. (B) Western blot analyses of DLD-1 cells infected with the miR-493-expressing lentiviruses or the control viruses. (C) HCT116/GFP cells were infected with the control lentiviruses or viruses that express IGF1R, transfected with control or miR-493 mimics, and used for Western blot analyses with the indicated antibodies. (D) The HCT116/GFP cells described above were used for liver metastasis assays as described in Figure 2B. Relative GFP/RFP ratios were calibrated based on the values of the cells transfected with the control RNA.

Supplementary Figure S4 A high level of miR-493 expression is associated with the absence of liver metastasis of colon cancer. (A-E)
RT-qPCR analyses of the indicated miRNAs were performed with the samples from the three groups of primary colon cancer, and presented as described in Figures 5A-5C. (F, G) The 44 specimens of the primary tumors were classified into 26 cases without lung metastasis, 7 cases with metachronous lung metastasis, and 11 cases with synchronous lung metastasis. RT-qPCR analyses of miR-493 (F) or miR-493* (G) were performed as described in Figure 5A or 5B, respectively. Green lines indicate average values.

Supplementary Figure S5 miR-493 expression is induced during carcinogenesis in a subset of colon cancers. (A, C) RT-qPCR analyses of (A) miR-34a and (C) miR-125b in 27 primary tumors (14 cases without liver metastasis and 13 cases with synchronous liver metastasis) and the corresponding non-cancerous specimens. (B, D) Ratios of (B) miR-34a and (D) miR-125b levels between tumor and non-cancerous specimens analyzed in (A) and (C), respectively.
Supplementary Table Legends:

**Supplementary Table 1.** List of Cy5/Cy3 ratios of miRNAs included in the miRNA library. HCT116 cells infected with the human miRNA library were used to generate liver metastasis via splenic injection. Library-introduced cells were recovered from the spleen and liver, and the integrated miRNAs were amplified by PCR and labeled with Cy3 and Cy5, respectively. The Cy5/Cy3 ratio of each miRNA is presented. Experiments were repeated four times, and average dropout ratios and p-values were calculated.

**Supplementary Table 2.** Overlap of *in silico*-predicted miR-493 targets and genes whose levels were reduced by miR-493 expression. Lists of *in silico*-predicted genes as target genes of miR-493 (Targetscan, miRanda, PITA), overlapped genes among the *in silico* programs (*in silico* overlaps), and genes whose levels were reduced by 2-fold after miR-493 expression (2-fold reduction) are shown. Eight genes shared by
the “*in silico* overlaps” and “2-fold reduction” are shown in the far right column (total overlap).

**Supplementary Table 3.** Clinical data for colon cancer patients. Patient clinical data at the time of surgery. Numbers in brackets indicate percentages in each group.
Okamoto et al., Supplementary Figure S1

A

B

C
Okamoto et al., Supplementary Figure S3

A

B

C

D

relative luciferase activity

psiCHECK
psiCHECK-IGF1R-UTR

lentivirus
control
miR-493

IGF-1R
Actin

lentiviruses
cont
IGF1R

oligonucleotide mimics
control
miR-493

relative GFP/RFP ratio

psiCHECK
psiCHECK-IGF1R-UTR

lentivirus
control
miR-493

IGF-1R
Actin

lentiviruses
cont
IGF1R

oligonucleotide mimics
control
miR-493

relative luciferase activity

mRNA
control
miR-493
miR-493
let-7e
miR-125b
miR-34a
miR-668

psiCHECK
psiCHECK-IGF1R-UTR

lentivirus
control
miR-493

IGF-1R
Actin

lentiviruses
cont
IGF1R

oligonucleotide mimics
control
miR-493

relative GFP/RFP ratio

control
IGF1R

miR-493

p<0.05
Okamoto et al., Supplementary Figure S4

A

miR-125b

B

miR-128a

C

miR-657

D

miR-659

E

miR-668

F

miR-493

G

miR-493∗

Relative miRNA expression
Okamoto et al., Supplementary Figure S5

A

miR-34a

B

Induction during carcinogenesis

C

miR-125b

D

Induction during carcinogenesis

w/o metastasis

synchronous metastasis

n.s.

p<0.05
**Supplementary Table 3**

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<th>all patients</th>
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<tr>
<td></td>
<td>66.3±10.3</td>
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<tr>
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