Supplementary Figure 1. treRNA expression in human colon cancer samples. treRNA expression was significantly higher in clinical colon cancer samples than normal samples ($p<0.001$). Experiments were carried out in triplicates. Data were presented as mean ± sd.

Supplementary Figure 2. treRNA expression in clinical breast cancer samples and cancer cell lines.

Supplementary Figure 3. treRNA did not affect cell proliferation in MCF7 cells *in vitro*. CellTiter Cell Proliferation assay was used to determine cell growth in MCF7 cells expressing a vector control, a control long noncoding RNA HAR1A or treRNA. Cell proliferation was measured at 490nm at Day 0-5. Data were represented as mean and ± s.d.

Supplementary Figure 4. Primary tumor growth following the transplantation of MCF7 cells in mice. MCF7 cell expressing a luciferase and treRNA or a vector control were transplanted into mouse mammary fat pads. Luciferase signals of primary tumors were quantified. MCF7-luc cells expressing treRNA showed slight higher tumor growth *in vivo* than MCF7 cells expressing a vector control. The graph showed the mean photon flux ± sd of three independent experiments.

Supplementary Figure 5. treRNA expression was suppressed by treRNA siRNAs in A549 cells. TreRNA expression was determined by qRT-PCR in A549 cells transfected with two different treRNA siRNAs or a control siRNA. Data were represented as mean and ± s.d.

Supplementary Figure 6. Knockdown of treRNA did not affect cell proliferation in A549 cells *in vitro*. CellTiter Cell Proliferation assay was used to determine cell growth in A549 cells expressing two different treRNA siRNAs or a control siRNA. Cell proliferation was measured at 490nm at Day 0-3. Data were represented as mean and ± s.d.

Supplementary Figure 7. Knockdown of endogenous treRNA reduced cell migration and invasion *in vitro*. Quantitative analysis of A549 cells expressing two different treRNA siRNAs or a control siRNA in migration and invasion assays. Data were represented as mean and ± s.d.

Supplementary Figure 8. Enforced treRNA expression in MCF7 cells did not affect Snail expression. Snail expression was detected by Western blot in MCF7 cells expressing treRNA or a vector control.

Supplementary Figure 9. Knockdown of treRNA in A549 cells did not affect the RNA levels of E-cadherin, ZO-1 and β–catenin. Quantitative RT-PCR in A549 cells expressing two different treRNA siRNAs or a control siRNA showed no difference in the RNA expression of epithelial markers E-cadherin, ZO-1 and β-catenin. Data were represented as mean and ± s.d.

Supplementary Figure 10. Endogenouse treRNA expression in the cytoplasmic and nucleic fractions of A549 cells. Cytoplasmic and nucleic fractions were isolated from A549 cells. Primers located in the two exons of treRNA were used to amplify spliced treRNA. Cytoplasma specific S14 and nuclear specific U2 snRNA primers were used as controls. The percentage of treRNA expression was determined by Image Pro analysis software. Approximately 75% of spliced treRNA is located in the cytoplasm of A549 cells. The data were collected from three independent experiments and presented as mean ± sd.

Supplementary Figure 11. Scheme of affinity purification of proteins associated with treRNA. TreRNA was tagged with MS2 RNA. A fusion protein consisting of MS2 binding protein and maltose binding protein (MBP) which was bound to amylose beads and was used as an affinity matrix. Cytoplasmic extracts of cells expressing MS2 tagged treRNA or MS2 tag vector control were applied to the beads. Proteins bound to the affinity matrix were isolated, digested with trypsin, and subjected to LC-MS/MS analysis.

Supplementary Figure 12. MS2 tag of treRNA did not affect the translation suppression function of treRNA in MCF7 cells. E-cadherin expression was determined by Western blot in MCF7 cells expressing treRNA or a MS2 tag control vector or MS2 tagged treRNA.

Supplementary Figure 13. TreRNA expression in MCF7 cells did not affect the expression of PUF60, SF3B3, hnRNP K, FXR1 and FXR2. The expressions of these genes were detected by Western blot in MCF7 cells expressing treRNA or a vector control.

Supplementary Figure 14. Short hairpin RNAs (shRNAs) downregulated the expressions of their respective target genes in MCF7 cells. Two different shRNAs for each gene were introduced into MCF7 cells and the expression of their respective target genes were detected by immunoblots.

Supplementary Figure 15. Knockdown of FXR1 or FXR2 did not affect cell proliferation in MCF7-luc cells. Cell proliferation was measured by CellTiter Cell Proliferation assay in MCF7-luc cells expressing FXR1 shRNAs, FXR2 shRNAs or a control shRNA. Cell proliferation was measured at 490nm at Day 0-5. Data were represented as mean and ± s.d.

Supplementary Figure 16. Primary tumor growth following the transplantation of MCF7-luc-treRNA cells in mice. MCF7-luc-treRNA cells expressing shRNAs targeting hnRNP K, FXR1, FXR2 individually or in combination were transplanted into mouse mammary fat pads. Luciferase signals of primary tumors were measured by a bioluminescence Xenogen imaging system. Primary tumor growth showed no difference when FXR2 expression was knocked down compared with the cells expressing a control shRNA. Primary tumor growth was increased when hnRNP K or FXR1 expression was knocked down. Primary tumor growth was significantly decreased in the double knockdown of hnRNP K and FXR2 expression, or triple knockdown of hnRNP K, FXR1 and FXR2. The graph showed the mean photon flux ± sd of three independent experiments.

Supplementary Figure 17. Plasmids expressing treRNA or a vector control were *in vitro* transcribed and labeled with biotin. Biotinylated treRNA was incubated with MCF7 cell extracts and pull-down was performed. E-cadherin level was determined by qRT-PCR. Experiments were carried out in triplicate and data were presented as mean ± sd.