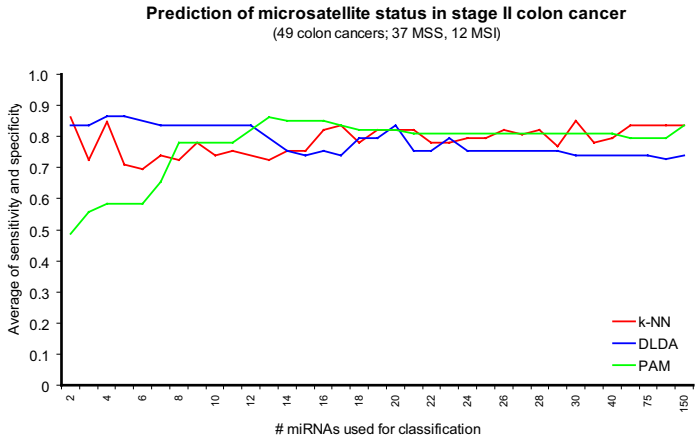
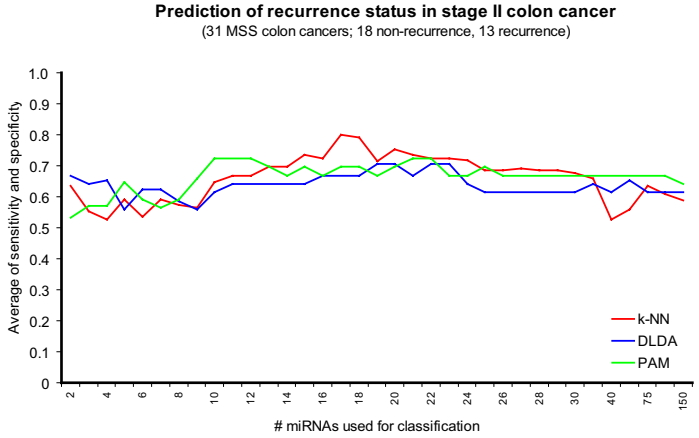
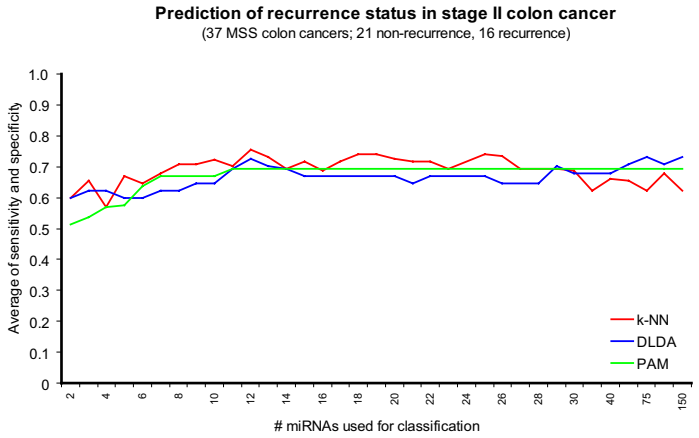
A



B



C



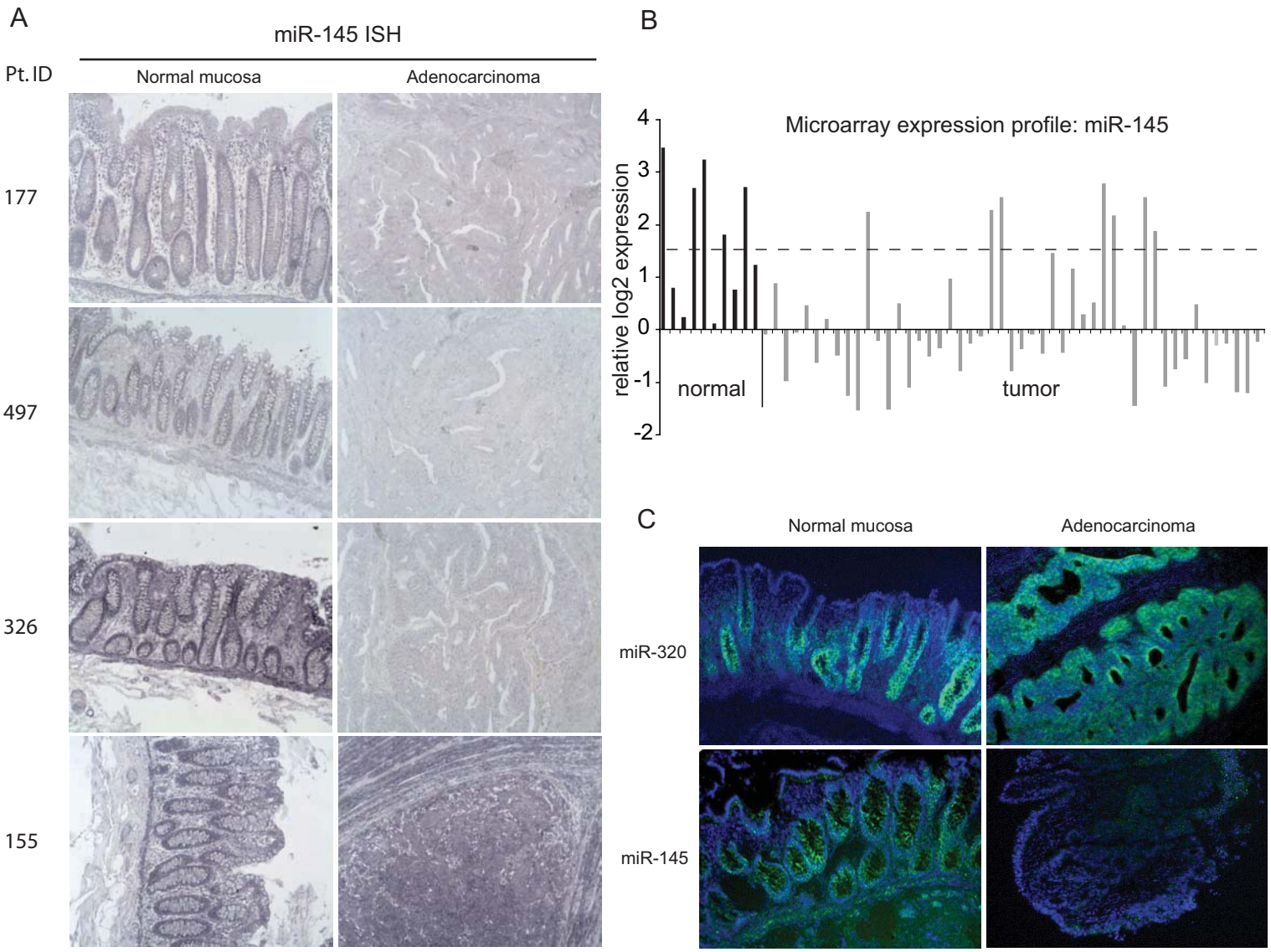
Supplementary figure 1. Prediction of microsatellite and recurrence status in stage II colon cancer

Cross validated prediction performance of three classification algorithms used for predicting microsatellite and recurrence status in a set of stage II colon cancers. The average of sensitivity and specificity was used to evaluate the performance in leave-one-out cross validation. For the k-NN algorithm, the maximum value when k was varied from 1 to 5 was plotted. (A) 49 colon cancers were used for microsatellite status prediction (12 MSI and 37 MSS). (B) Prediction of recurrence status for the MSS colon cancer samples satisfying the following criteria: At least 53 months of disease free survival in the non-recurrence group and recurrence of disease within 53 months after surgery in the recurrence group. 31 MSS samples satisfied these criteria (18 recurrence and 13 non-recurrence).(C) All 37 MSS colon cancer samples were used for recurrence status prediction (21 recurrence and 16 non-recurrence).

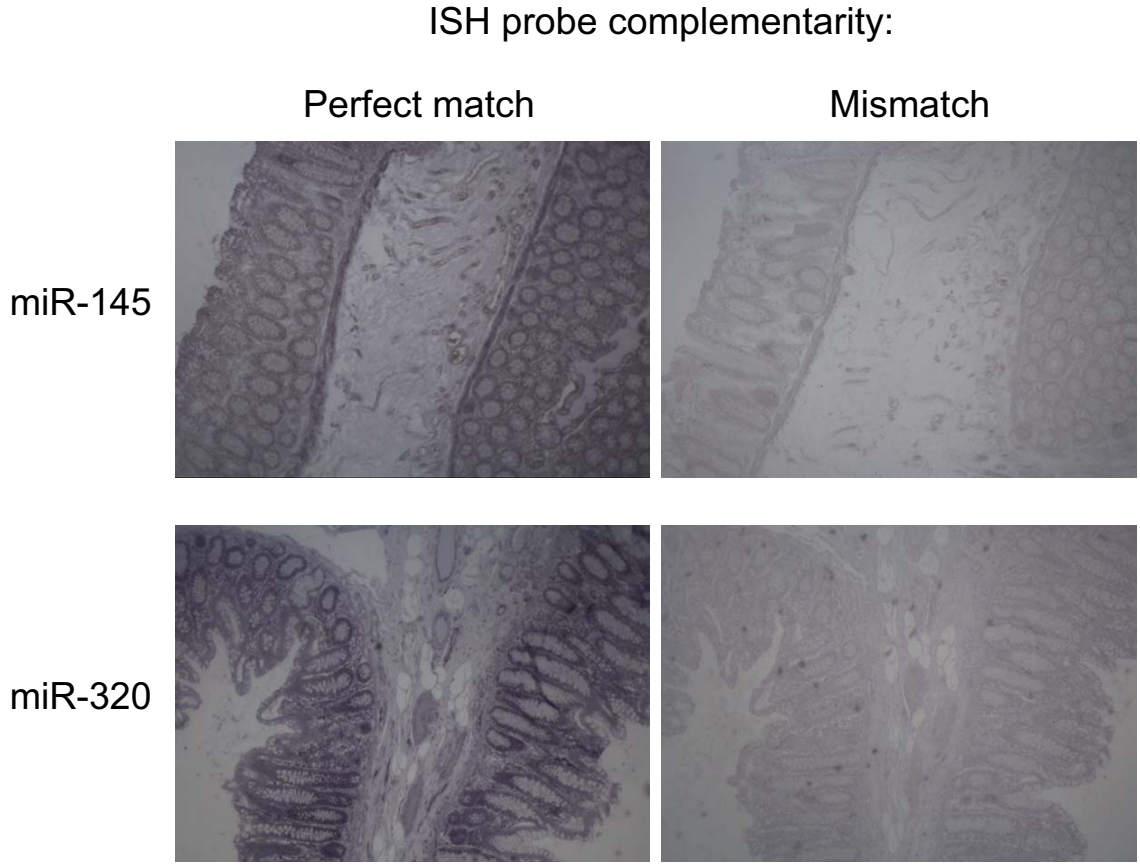
Supplementary Information

Method used for in situ detection of miRNAs in fresh frozen tissue

In situ detection of microRNAs were performed on 10 μm frozen tissue sections from matched normal mucosa and adenocarcinoma material. Sections were fixed in 4% paraformaldehyde, acetylated and pre-hybridized in hybridization solution (50% formamide, 5XSSC, 0.5 mg/ml yeast tRNA, 1x Denhardt's solution) for 30 minutes prior to hybridization. 2.5 pmol probe (LNA-modified and DIG or FITC-labeled oligonucleotides, Exiqon) complementary to miR-145 and miR-320 were hybridized to the sections for 1 hr at 25°C lower than Tm of the probe. Following a series of post-hybridization washes the *in situ* hybridization signals were detected using a signal amplification system based on horseradish peroxidase and FITC-conjugated tyramide (Perkin Elmer) according to the manufacturer's instructions. Slides were mounted in Prolong Gold containing DAPI (Invitrogen) and analyzed with an Olympus MVX10 microscope equipped with a CCD camera and Olympus CellP software.



Supplementary figure 2. ISH analysis of miR145 and miR-320 expression in normal colon mucosa and adenocarcinoma. (A) Colorimetric ISH analysis of miR-145 in formalin fixed and paraffin embedded (FFPE) tissue sections revealed that in three out of four stage II colon adenocarcinomas miR-145 was downregulated. (B) Microarray-based expression profiling of miR-145 in 59 colon specimens (10 normal mucosa and 49 adenocarcinoma) showed that in 86% (42/49) of the adenocarcinoma samples miR-145 expression was below the median expression value in the normal mucosa samples (dashed line). (C) Analysis of miR-145 and miR-320 *in vivo* expression in fresh frozen specimens using fluorescence based ISH. Tissue from two patients were analyzed (other patients than used in (A). ISH images are shown for one patient only, as both patients yielded very similar results. The ISH analyses based on fresh frozen tissue confirmed the results obtained using FFPE tissue. miR-145 and miR-320 were both expressed by the normal crypt epithelial cells. The expression of miR-320 appeared in a gradient declining from the bottom to the top of the crypts, while miR-145 was expressed throughout the colonic crypt. The adenocarcinoma cells showed high expression of miR320, and very weak expression of miR-145.



Supplementary figure 3. Evaluation of ISH-specificity of miR-145 and miR-320 in FFPE sections from normal colon mucosa. Specificity of colorimetric ISH analysis of miR-145 and miR-320 in consecutive formalin fixed and paraffin embedded (FFPE) tissue sections from normal colon mucosa were evaluated using probes that were either perfectly complementary to the miRNA (perfect match) or mismatched at two (miR-145) or three (miR-320) nucleotide positions. The signal obtained with a perfect match probe for each miRNA was drastically reduced when using the corresponding mismatch probe instead. Images were captured at 5x magnification.