**Supplementary Information, Data S1**

## Materials and Methods

Mice: C57BL/6J-ApcMin/J mice ($Apc^{Min/+}$) were purchased from Jackson Laboratories (Bar Harbor, ME, USA) but were bred and maintained at the Mouse Core Facility of the Center for Colon Cancer Research at the University of South Carolina (USC), Columbia, SC. All aspects of the animal experiments were conducted in accordance with the guidelines and approval of the USC Institutional Animal Care and Use Committee. The $Apc^{Min/+}$ mouse model of colon cancer is a genetic model of the disease. These mice are relatively healthy and long lived, in contrast to orthotopic models of colon cancer, which quickly succumb to the disease. $Apc^{Min/+}$ mice do not start to get sick until about 18 weeks of age, at which point they become anemic. They develop muscle wasting at about 20 weeks of age and typically die when about six months old. Our treatment regimen (see below) ended long before $Apc^{Min/+}$ mice show any signs of illness.

Tumor suppressor miRNAs and total plant RNA: MiR-34a, miR-143, and miR-145 synthesized to have the 2'-O--methylation at the 3'-end, which is characteristic of miRNAs made in plants, were purchased from Integrated DNA Technologies. These three miRNAs are highly conserved in mammals and the sequences are the same in mice as in humans. These miRNAs have been validated as tumor suppressors in many studies (Trang, Wiggins et al. 2011, Okada, Lin et al. 2014, Panza, Votino et al. 2014, Wang, Shi et al. 2014). Total plant RNA for gavage was isolated from flash frozen *Arabidopsis thaliana*, ecotype Columbia, using TRIzol reagent (Life Technologies)

according to the manufacturer's instructions, but with two additional ethanol precipitations to remove any traces of the reagent. Total plant RNA contains all high and low molecular weight RNA species present in the plant. Therefore, it contains all endogenous plant RNAs, including, for example, mRNAs, tRNAs and rRNA, as well as the entire set of endogenous plant miRNAs. In general, there is no homology between plant and animal miRNAs, although one bioinformatics study indicated that plants and animals share members of the miR854 family (Millar and Waterhouse 2005, Arteaga-Vazquez, Caballero-Perez et al. 2006, Jones-Rhoades, Bartel et al. 2006).

Experimental protocol: Five week old $Apc^{Min/+}$ mice were divided into three treatment groups of seven mice each. The treatment groups corresponded to daily gavage with either 1) total plant RNA (from *Arabidopsis thaliana*, ecotype Columbia) spiked with a cocktail of tumor suppressor miRNAs commercially synthesized to have the 3'-methylation characteristic of plant miRNAs, 2) total plant RNA alone, or 3) water (all RNA preparations were dissolved in water). Gavage with 150 µl of the preparations indicated above began when the mice were five weeks old and continued daily for four weeks. This time frame is a standard preventive regimen for experiments using $Apc^{Min/+}$ mice. The daily doses in the RNA treatment groups corresponded to 61 µg total plant RNA alone or plus 4.5 to 7.4 µg (corresponding to 0.7 to 1.0 nmole) of each species of tumor suppressor miRNA. No weight loss was observed in any of the mice during the entire 28 days of treatment. Because one of the best signs of toxicity of therapeutic treatments has been loss of weight within 3-5 days of treatment, the absence of weight loss in our animals indicates that our treatments had no obvious toxicity. In addition, the

animals did not develop anemia during the experiment, as indicated by normal (as opposed to pale) coloration of extremities, further arguing against toxic side effects.

Six hours after the last gavage treatment, mice were humanely sacrificed by cervical dislocation after administration of anesthesia using isoflurane by inhalation. The small and large intestines were removed, flushed with phosphate buffered saline, and sliced longitudinally. The small intestine was divided into four equal segments with the colon treated as the fifth segment. Sections for RNA isolation were flash frozen on dry ice, and segments for determination of tumor burden were fixed in 10% formalin and stained with 0.002% methylene blue. Tumors were counted under a dissecting microscope by a single highly experienced investigator, who was blinded to the treatments.

Statistical analysis: We chose to use the Kolmogorov-Smirnov (K-S) test to analyze the tumor number data because the test is sensitive to any differences in the distributions, including differences in shape, spread or median and is relatively insensitive to outliers, thereby providing a robust test of whether the distributions are significantly different. The graphical presentation of the data in Figure 1A, which shows the basis of the K-S test, enabled the insightful observation that six out of seven of the mice in the tumor suppressor miRNA-treated group had fewer tumors than the mouse with the fewest tumors in the water-treated group. The one-sided K-S test is appropriate in our case because we are interested in whether the number of tumors in the tumor suppressor miRNA-treated group is less than that in the water-treated group, not just whether the number of tumors is different in those two groups. Finally, the Mann-Whitney (M-W)

directional test, another commonly used non-parametric statistic, gave a similar p-value for the difference between the miRNA-treated and water treated groups (M-W p = 0.0075, K-S p = 0.0058).

RNA isolation from mouse tissue: Flash frozen intestinal sections for RNA isolation were stored at -70$^{o}$C. The frozen tissues were disrupted with a hand-held polytron at maximum speed in the presence of 10 ml of TRIzol reagent (Life Technologies) per gram of tissue, and total RNA was isolated according to manufacturer instructions.

Periodate treatment: We used periodate treatment of the RNA from mouse tissue in order to specifically detect the administered miRNAs. In preparation for oxidation with sodium periodate, 40 μg of each total RNA sample from mouse intestine was brought up to 100 μl in sterile double-distilled water and then ethanol precipitated (-20$^{o}$C overnight in three volumes ethanol and 1/10 volume 3 M sodium acetate). Siliconized microcentrifuge tubes were used to minimize losses. Ethanol precipitates were spun down, washed in 75% ethanol, allowed to dry at room temperature, and resuspended in 80 μl of an 8.3 pM solution (in sterile double-distilled water) of the spike-in normalization control miRNA, *C. elegans* miR-39 (2'-O-methylated at the 3'-end and purchased from Integrated DNA Technologies). Methylated *C. elegans* miR-39 was added to all samples before oxidation because endogenous RNA controls are all un-methylated and will be oxidized by periodate, rendering them useless for normalization. After being resuspended, each sample was divided into two equal (20 μg) portions, one to be oxidized and the other to serve as un-oxidized control. Quantitative real-time PCR of

the un-oxidized control showed that the concentration of the spiked-in miR-39 relative to the endogenous normalization control RNU6-B was the same in all samples, indicating that use of the spiked-in, methylated miR-39 as the normalization control for the oxidized samples wouldn't introduce a systematic bias into the measurements.

Each 20 μg aliquot for oxidation was ethanol precipitated overnight as above and resuspended in 20 μl of borax/boric acid buffer pH 8.6 (0.06 M borax, 0.06 M boric acid). Then 1/8 volume (= 2.5 μl) 200 mM sodium periodate was added to each, and the samples were incubated for one hour at room temperature in the dark. Next, 2 μl 100% glycerol was added to each, and incubation in the dark was continued for 40 minutes more. Finally, the samples were ethanol precipitated as above, two times, after which each was resuspended in 40 μl sterile double-distilled water. Resuspended RNA samples were stored frozen at -20$^{o}$C.

Reverse transcription and quantitative real-time PCR: We used the miScript-PCR system (Qiagen), including the miScript II RT Kit, miScript SYBR Green PCR Kit, and miScript primer assays for miR-34a, miR-143, miR-145, miR-100, and miR-39 according to the manufacturer's instructions. The reverse transcription (RT kit) reactions were performed using miScript HiSpec buffer and 1.5 μg RNA. The resulting cDNA was diluted 11-fold in sterile double-distilled water (the minimum amount specified in the kit protocol), aliquoted, and stored at -20$^{o}$C. Quantitative real-time PCR reactions were performed in triplicate using an Applied Biosystems 7300 machine and the reaction and cycling conditions specified in the miScript-PCR protocol. 2.5 μl 11-fold diluted cDNA was used per reaction, which is the maximum amount of cDNA specified in the kit

protocol. The normalization control quantitative real-time PCR reactions were always performed in the same run as the reactions for the miRNA of interest.

References

Arteaga-Vazquez, M., et al. (2006). "A family of microRNAs present in plants and animals." Plant Cell **18**(12): 3355-3369.

Jones-Rhoades, M. W., et al. (2006). "MicroRNAS and their regulatory roles in plants." Annu Rev Plant Biol **57**: 19-53.

Millar, A. A. and P. M. Waterhouse (2005). "Plant and animal microRNAs: similarities and differences." Funct Integr Genomics **5**(3): 129-135.

Okada, N., et al. (2014). "A positive feedback between p53 and miR-34 miRNAs mediates tumor suppression." Genes Dev **28**(5): 438-450.

Panza, A., et al. (2014). "Peroxisome proliferator-activated receptor gamma-mediated induction of microRNA-145 opposes tumor phenotype in colorectal cancer." Biochim Biophys Acta **1843**(6): 1225-1236.

Trang, P., et al. (2011). "Systemic delivery of tumor suppressor microRNA mimics using a neutral lipid emulsion inhibits lung tumors in mice." Mol Ther **19**(6): 1116-1122.

Wang, L., et al. (2014). "MiR-143 acts as a tumor suppressor by targeting N-RAS and enhances temozolomide-induced apoptosis in glioma." Oncotarget **5**(14): 5416-5427.