

laminin, and no difference on fibronectin. The images shown are representative micrographs of 12-15 independent scans for each integrin ligand. *Bar* = 40 μ m. (B) Quantitation of cell spreading presented as the total surface area covered by cells, in square micrometer, averaged to 300 cells.

Fig. 7. A function blocking mAb to α 2 β 1 completely inhibits cell spreading in both the control cells and the miR31-expressing MDA-MB-231 cells. Wild-type and miR-31-expressing MDA-MB-231 cells were incubated with either an α 2 β 1 monoclonal blocking antibody (LS-C247646) or control IgG and spread on collagen for 1 h. Cells were then fixed, permeabilized and stained for 30 min with Alexa 568-phalloidin to visualize actin filaments. A. The images shown are representative micrographs of 12-15 independent scans for each treatment. *Bar* = 40 μ m. B. Quantitation of cell spreading presented as the total surface area covered by cells, in square micrometer, from 300 cells.

Supplemental Figure legends:

Fig. S1. miR31 overexpression did not affect cell proliferation. Equal numbers of cells were seeded in 6-well plates. At each day, the number of viable cells was counted. Each experiment was performed in triplicates, and the data are representative of three independent experiments.

Fig. S2. Densitometry and statistical analyses of western blot data.

Semi-quantitative digital image analysis was performed with ImageJ software to detect the intensity of integral density of particular bands. The pixel density of each individual protein was calculated on the basis of the pixel density for the β -actin as a housekeeping control protein, the

values of which were equal to 1. The plotted data are the mean \pm s. d. of at least 3 independent western blots. * Student TTest determined that the $p < 0.05$ when compared to either the control or the GFP cells.

Fig. S3. miR-31 inhibits expression of several alpha and beta integrin subunits in a prostate cancer cell line. Real-time qt RT-PCR of the indicated integrin subunits from untransfected LNCaP cells or LNCaP cells transfected of either control microRNA or miR-31.

Fig. S4. miR-31 directly targets the 3'UTR of several integrins subunits and represses their expression. (A) Firefly luciferase reporter plasmid, pmirGlo was transiently co-transfected into MDA-MB-231 cells with either miR-31 of the control miR, and luciferase activities were measured after 24 h. (B) Firefly luciferase reporter plasmids, pmirGlo control or pmirGlo containing the 3'UTR of each indicated integrin subunit, were transfected into MDA-MB-231 cells that were transiently co-transfected with either miR-31 or control miR, and luciferase activities were measured after 24 h. The values were plotted as a ratio of the control microRNA over the miR31 treatment. (C-F) Mutation of miR-31 seed sequences in the 3'UTR of the indicated integrins subunits abrogated the miR-31 effect. MDA-MB-231 cells were transfected with the luciferase reporter plasmids containing the 3'UTR of the indicated integrin subunit with deletion of the miR31 seed sequences, and co-transfected with or without miR31. Luciferase activities were measured after 24 h. For all luciferase activity assays, Renilla luciferase activity was used for normalization. The data are the mean \pm s. d. of at least 3 independent transfections.

Fig. S5. miR-31 directly targets the 3'UTR of ITGA2, ITGA5, ITGAV and ITGB3 integrins and represses their expression in LNCaP cells. The firefly luciferase activity of each construct was normalized to the internal control renilla luciferase activity and plotted a ratio of the control microRNA over the miR31 treatment.

Fig. S6. miR-31 affects cell spreading in a ligand-dependent manner. Wild-type and miR-31-expressing MDA-MB-231 cells spread on collagen, Matrigel, fibronectin and laminin for 30 min, were fixed, permeabilized and stained for 30 min with Alexa 568-phalloidin to visualize actin filaments. miR-31-transfected cells showed significant inhibition of spreading on collagen and Matrigel, less extensive inhibition on laminin and no inhibition on fibronectin, compared to control cells expressing the empty vector. Quantitation of cell spreading presented as the total surface area covered by cells, in square micrometer, from an average of 300 cells.