

## Supplemental information for

# miR-103/107 prolong Wnt/ $\beta$ -catenin signaling and colorectal cancer stemness by targeting Axin2

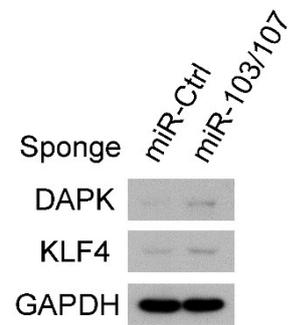
Hsin-Yi Chen<sup>1, 2, 7, 8</sup>, Yaw-Dong Lang<sup>4</sup>, Han-Nan Lin<sup>1</sup>, Yun-Ru Liu<sup>6</sup>, Chun-Chieh

Liao<sup>2,3</sup>, André Wendindondé Nana<sup>1</sup>, Yun Yen<sup>1, 8</sup>, and Ruey-Hwa Chen<sup>2, 3, 5</sup>

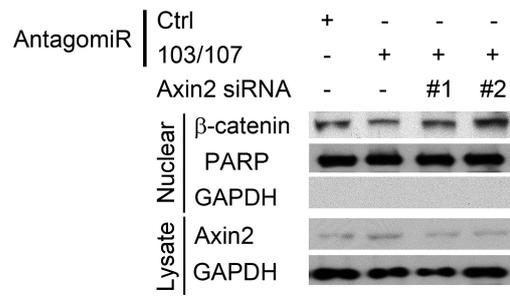
<sup>1</sup>Ph.D Program for Cancer Molecular Biology and Drug Discovery, College of Medical Science and Technology, Taipei Medical University, <sup>2</sup>Institute of Biological Chemistry, Academia Sinica, <sup>3</sup>Institute of Molecular Medicine, College of Medicine, National Taiwan University, <sup>4</sup>Institute of Biomedical Science, Academia Sinica, <sup>5</sup>Institute of Biochemical Sciences, College of Life Science, National Taiwan University, Taipei, Taiwan. <sup>6</sup>Office of Human Research, Taipei Medical University, Taipei, Taiwan. <sup>7</sup>Graduate Institute of Cancer Biology and Drug Discovery, College of Medical Science and Technology, Taipei Medical University, <sup>8</sup>TMU Research Center of Cancer Translational Medicine.

Correspondence to: Hsin-Yi Chen, email: [hyichen@tmu.edu.tw](mailto:hyichen@tmu.edu.tw) ; Ruey-Hwa Chen, email: [rhchen@gate.sinica.edu.tw](mailto:rhchen@gate.sinica.edu.tw)

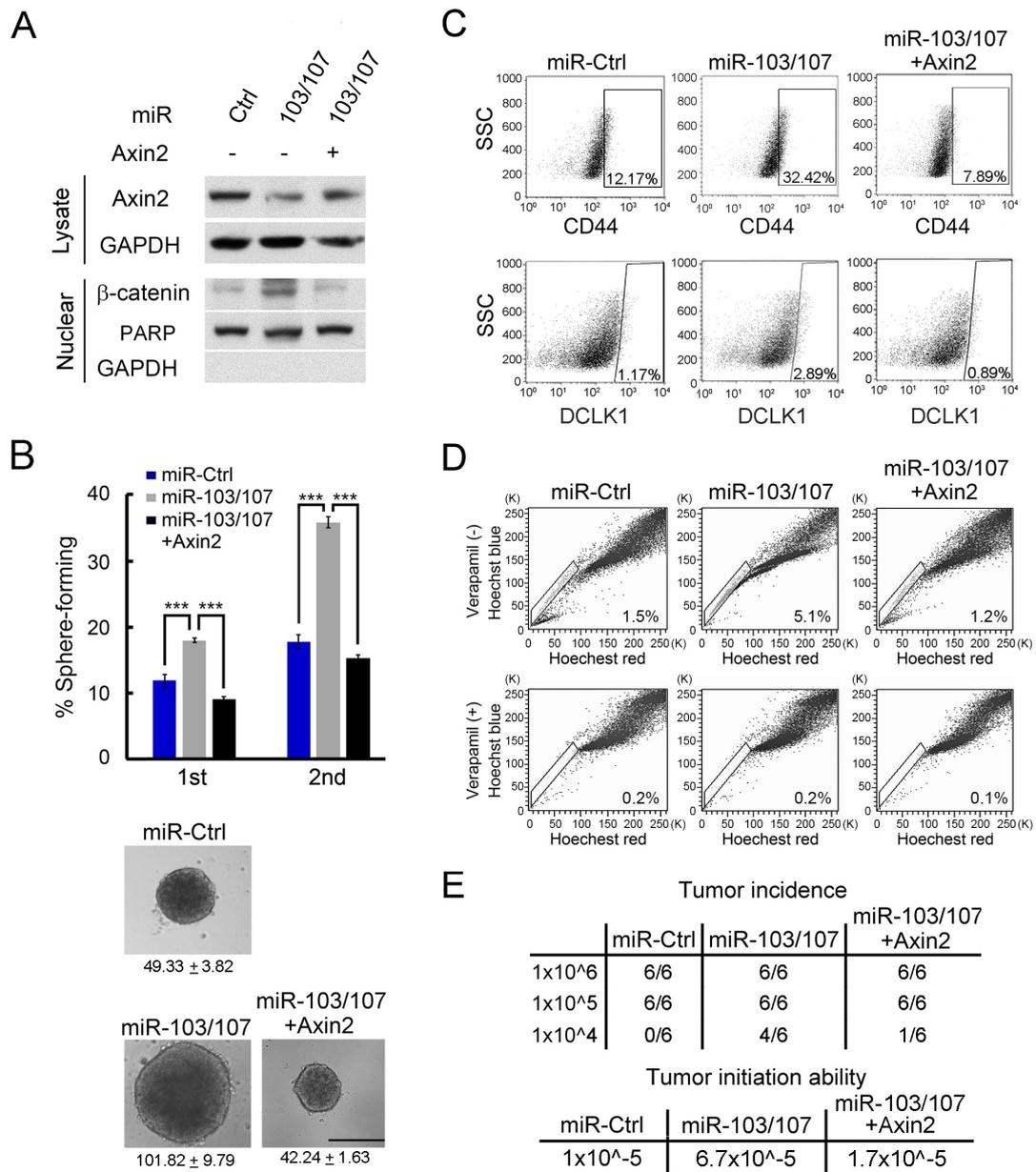
## Supplemental Data



**Figure S1. The efficacy of miR-103/107 sponge.** Immunoblot analysis of DAPK and KLF4 in HCT116 cells stably expressing indicated miRNA sponges. GAPDH was used as a loading control.

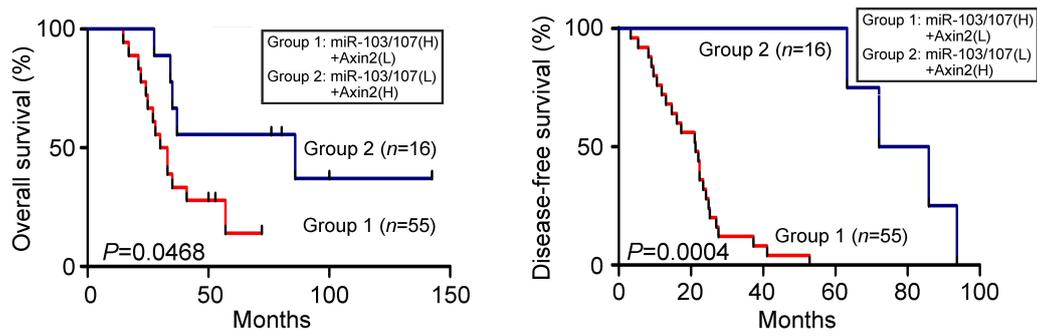


**Figure S2. miR-103/107 induce β-catenin nuclear accumulation via regulating Axin2 mRNA.** Immunoblot analysis of Axin2 and nuclear β-catenin levels in HT29 cells transiently transfected with indicated antagomiRs and/or siRNA.



**Figure S3. Suppression of Axin2 by miR-103/107 promotes CRC stemness. A.** Axin2 and nuclear  $\beta$ -catenin levels in HT29 cells expressing indicated miRNAs and Axin2 construct were assayed by immunoblot analysis. **B.** Tumor sphere-forming abilities of HT29 derivatives as shown in (A). Representative images of spheres and sphere sizes (calculated from 30 spheres per group experiment) are shown on the right

panel. The images were taken at 3 weeks after cell plating. Quantitative data of sphere number are shown on the left panel. Scale bar, 50  $\mu$ m. Data are mean  $\pm$  S.D. \*\*\*:  $P < 0.0005$ ,  $n = 3$ . **C-D.** HT29 derivative as shown in **(A)** were assayed by flow cytometry for cell surface expression of stem-like markers CD44 (upper panel) and DCLK1 (lower panel) **(C)**, and side-population cells **(D)**. **E.** Tumor-initiation ability was measured by subcutaneously implanting HT29 derivatives as shown in **(A)** at indicated cell numbers into nude mice. Seven weeks after transplantation, tumor incidence and tumor-initiating ability were measured.



**Figure S4. miR-103/107-Axin2 axis correlates with poor prognosis of CRC patients.** Kaplan–Meier analysis of overall survival and disease-free survival of CRC patients with the corresponding expression profiles. Log-Rank test was used for comparison between groups.

Fig. 3C LNA+siRNA      Fig. S2 LNA+siRNA      Fig. 5A HCT116      Fig. S3 HT29

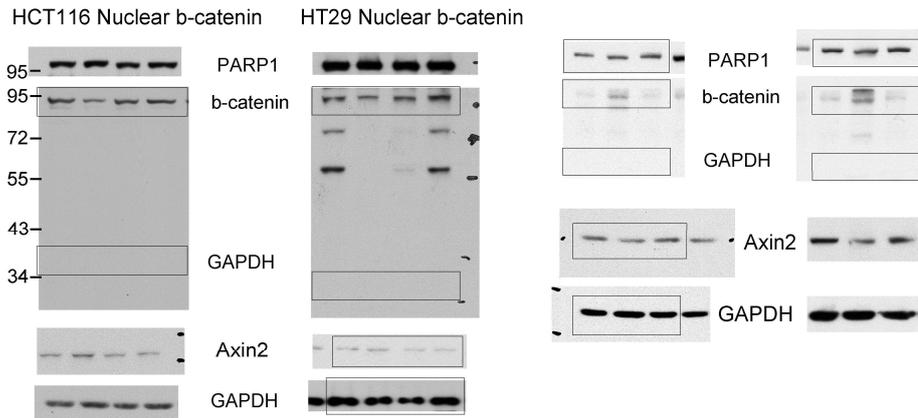


Fig.4A Wnt 3a treatment

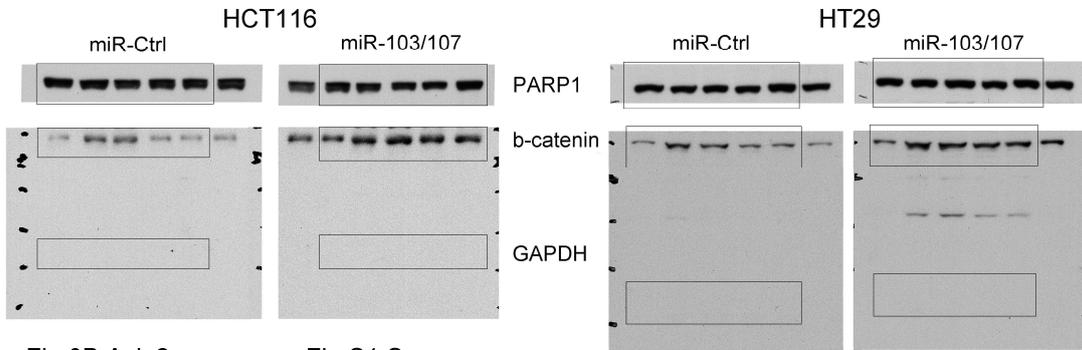


Fig.3B Axin2

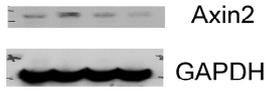


Fig.S1 Sponge

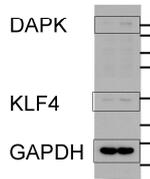


Fig.3F Flag-Axin2

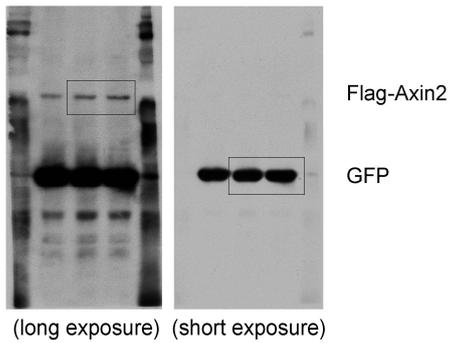


Fig.2B Wnt signal

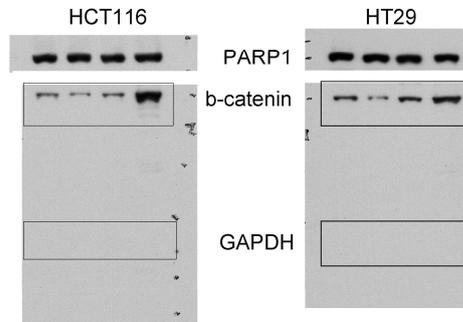


Figure S5. Full scans of western blot data shown in Fig. 2-5, S1-S3.