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SUPPLEMENTARY INFORMATION

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Nanoparticle orientation to control RNA loading and ligand display on extracellular vesicles for cancer regression

Fengmei Pi[1,2,3,4], Daniel W. Binzel[1,2,3,4], Tae Jin Lee[3,5,8], Zhefeng Li[1,2,3,4], Meiyan Sun[6], Piotr Rychahou[7], Hui Li[1,2,3,4], Farzin Haque[1,2,3,4], Shaoying Wang[1,2,3,4], Carlo M. Croce[3,5], Bin Guo[6], B. Mark Evers[7] and Peixuan Guo[1,2,3,4,5]*

[1]College of Pharmacy, The Ohio State University, Columbus, OH, USA. [2]Center for RNA Nanobiotechnology and Nanomedicine, The Ohio State University, Columbus, OH, USA. [3]Comprehensive Cancer Center, The Ohio State University, Columbus, OH, USA. [4]Dorothy M. Davis Heart and Lung Research Institute, The Ohio State University, Columbus, OH, USA. [5]Department of Cancer Biology and Genetics, College of Medicine, The Ohio State University, Columbus, OH, USA. [6]Department of Pharmacological and Pharmaceutical Sciences, College of Pharmacy, University of Houston, Houston, TX, USA. [7]Markey Cancer Center, Department of Surgery, University of Kentucky, Lexington, KY, USA. Present address: [8]Department of Neurosurgery, McGovern Medical School, University of Texas Health Science Center at Houston, Houston, TX, USA. *e-mail: guo.1091@osu.edu

Nanoparticle Orientation to Control RNA Surface Display on Extracellular Vesicles for Cancer Regression

*Fengmei Pi[1], Daniel W. Binzel[1], Meiyan Sun[2], Taejin Lee[4], Piotr Rychahou[3], Farzin Haque[1], Hui Li[1], Shaoying Wang[1], Carlo M. Croce[4], Bin Guo[2], B. Mark Evers[3], and Peixuan Guo[1]**

Supplementary Materials

Fig. S1 Physical properties of PSMA/EV/siSurvivin nanoparticles

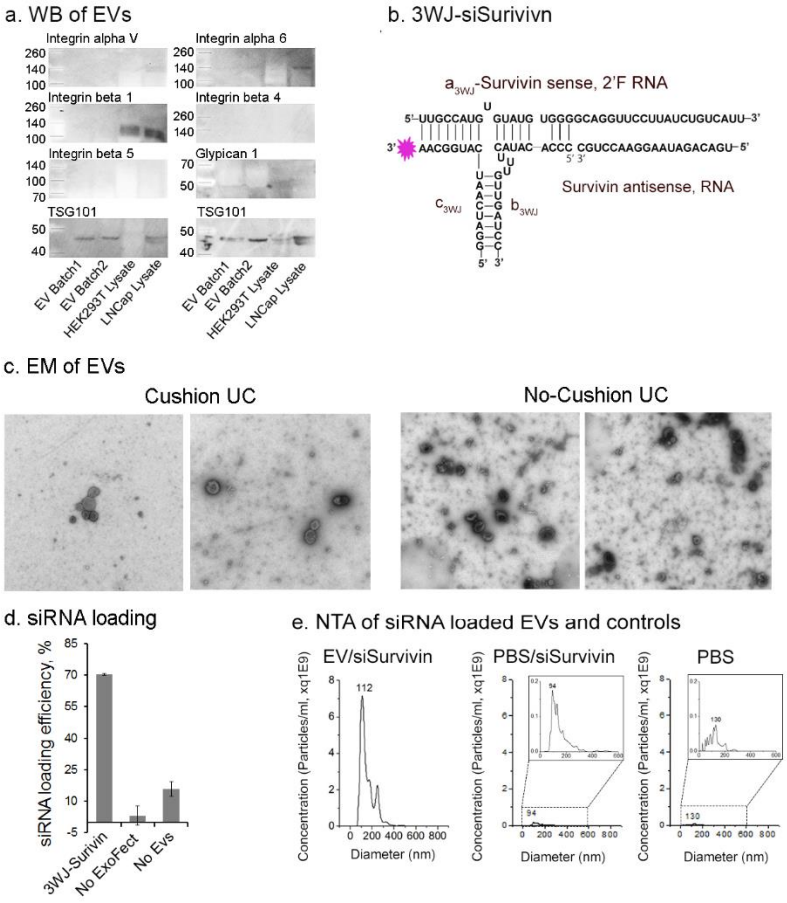

Fig. S1: Physical properties of PSMA$_{apt}$/EV/siSurvivin nanoparticles. **(a)** Western blot assay to test the presence of EV marker TSG101 from the purified HEK293T EVs. EVs were detected as negative for integrin α5, integrin α6, integrin β1, integrin β4, integrin β5 and glypican1 expression. HEK293T cell lysate and LNCaP cell lysate were used as controls. Equal amount of

cell lysate was used as negative control. **(b)** Primary sequence and secondary structure of 3WJ harboring surviving siRNA sequences. **(c)** EM image of EVs purified from HEK293T cell culture medium, with either differential ultracentrifugation method or OptiPrep cushion modified ultracentrifugation method. **(d)** Loading efficiency of siRNA into EVs. Control samples without transfection reagent Exo-Fect or EVs were tested. In the "No EVs" control sample, the $Alexa_{647}$ labeled 3WJ-Survivin RNA nanoparticles were treated with ExoFect, and pelleted down after adding ExoTC. Around 15% of $Alexa_{647}$-3WJ-Surivin RNA were detected in the pellets, which might be caused by forming complex with ExoTC. (e) NTA quantifying the particle amount and testing the particle size distribution of 3WJ-survivin siRNA loaded EVs or negative controls without EVs, or PBS only.

Fig. S2: Determine the condition to digest 3WJ-cholesterol 2'F RNA nanoparticles

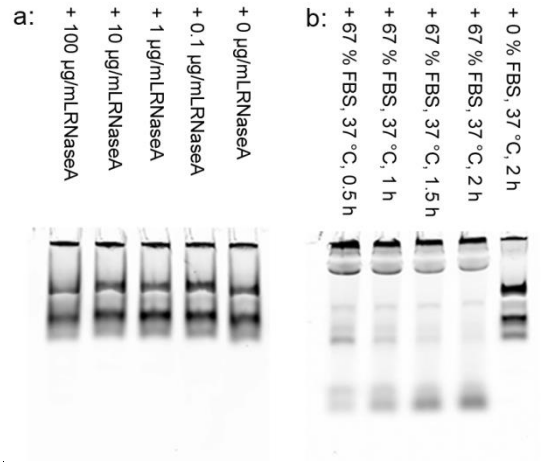

Fig. S2: Test the condition to digest 3WJ-cholesterol 2'F RNA nanoparticles. **(a).** 2'F $Alexa_{647}$-3WJ-cholesterol RNA nanoparticles cannot be digested by RNaseA at the above tested concentrations, **(b).** but it can be digested in 67 % FBS. The native polyacrylamide gels were imaged with Typhoon (GE healthcare) using Cy5 channel. The condition of incubating with 67 % FBS at 37 °C for 2 hours was used for testing whether EVs can protect arrow head or arrow tail cholesterol displaying 3WJ 2'F RNA nanoparticles .

Fig. S3: Specific siRNA delivery to cells *in vitro* using PSMA aptamer-displaying EVs.

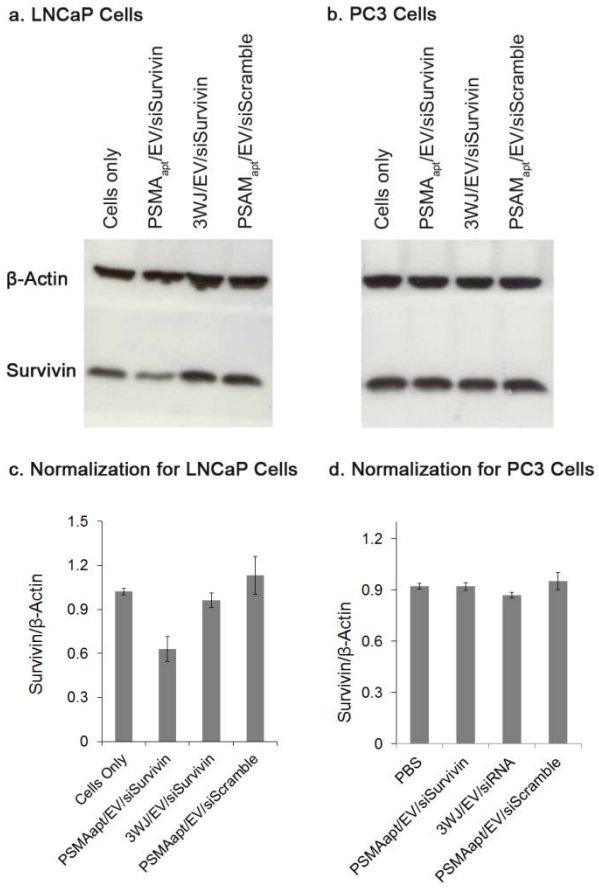

Fig. S3: Specific siRNA delivery to cells *in vitro* using PSMA aptamer-displaying EVs.

Western blot assay for PSMA aptamer-mediated delivery of survivin siRNA by EV to **(a)** PSMA(+) prostate cancer LNCaP cells and **(b)** PSMA(-) prostate cancer PC3 cells. (**c**) and (**d**) quantified the band intensity of 3 independent experiments with Image J software, and normalized the relative survivin protein expression level to β-actin.

Fig. S4. Design of EGFR$_{apt}$/3WJ/Cholesterol and FA/3WJ/Cholesterol

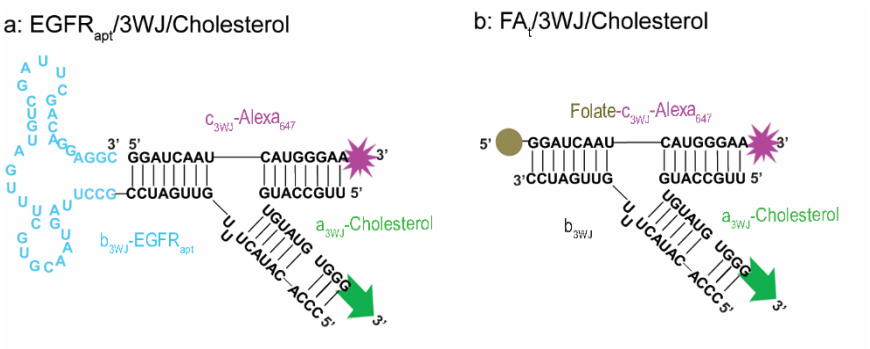

Fig. S4: Primary sequence and secondary structure of RNA nanoparticles (a). EGFR$_{apt}$/3WJ/Cholesterol RNA nanoparticle for breast cancer study (b). FA/3WJ/Cholesterol RNA nanoparticle for colorectal cancer study.

Fig. S5. Survivin expression in CRC patients.

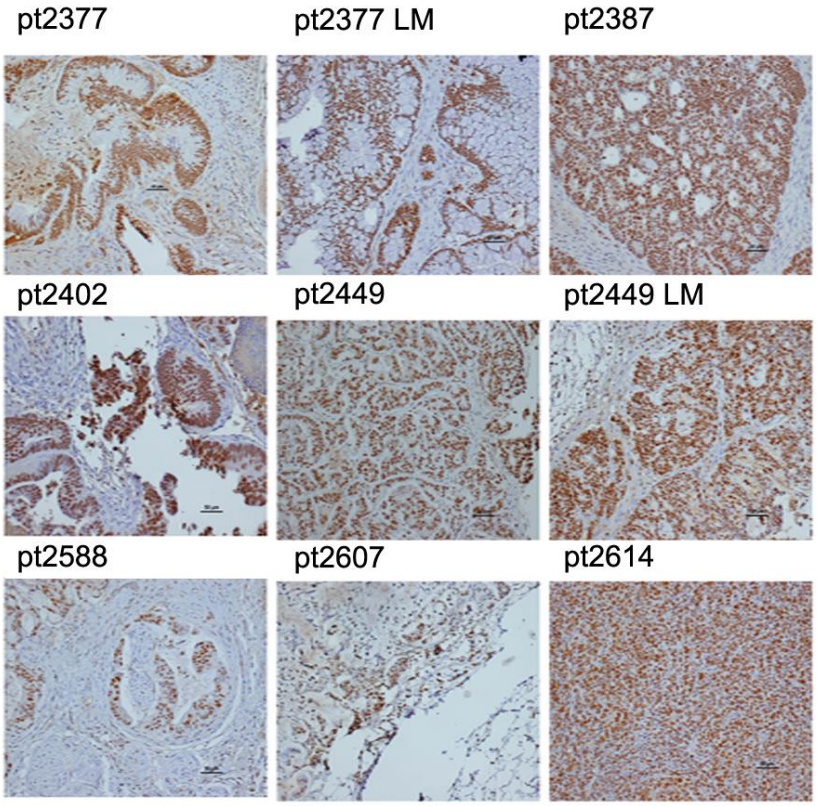

Fig. S5: Analysis of survivin expression in CRC Patients. Examples of immunohistochemical staining for survivin (Survivin (71G4B7) Rabbit mAb #2808; Cell Signaling, 1:500) (n=9 patient samples).

Fig. S6. Cell gating method for flowcytometry analysis.

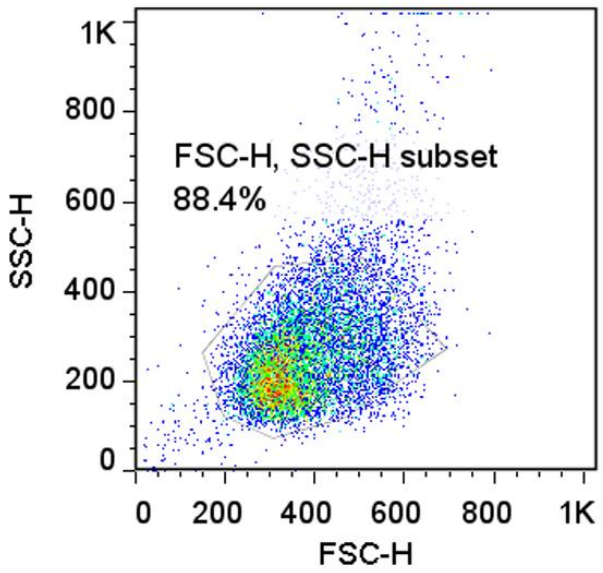

Fig. S6: Gating method for flowcytometry analysis. Examples of gating cells for flow cytometry analysis study by size with forward scatter (FSC) and side scatter (SSC). Data is analyzed by FlowJo 7.6.2.