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ADVANCED FUNCTIONAL MATERIALS

Supporting Information

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Coupling Nanostructured Microchips with Covalent Chemistry Enables Purification of Sarcoma-Derived Extracellular Vesicles for Downstream Functional Studies

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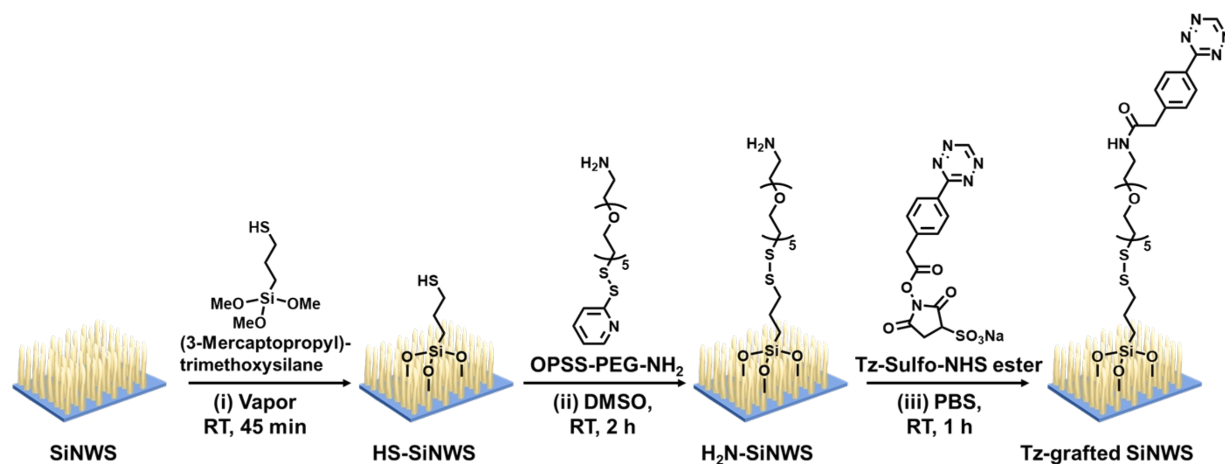

Figure S1. A 3-step Chemical modification process of Si nanowire substrates (SiNWS): (i) vapor deposition of (3-mercaptopropyl) trimethoxysilane to give HS-SiNWS; (ii) incorporation of a disulfide linker via ortho-pyridyl disulfide polyethylene glycol amine (OPSS-PEG-NH_2) to give H_2N-SiNWS; and (iii) NHS ester reaction between Tz-sulfo-NHS ester and the terminal primary amine group to generate the Tz-grafted SiNWS.

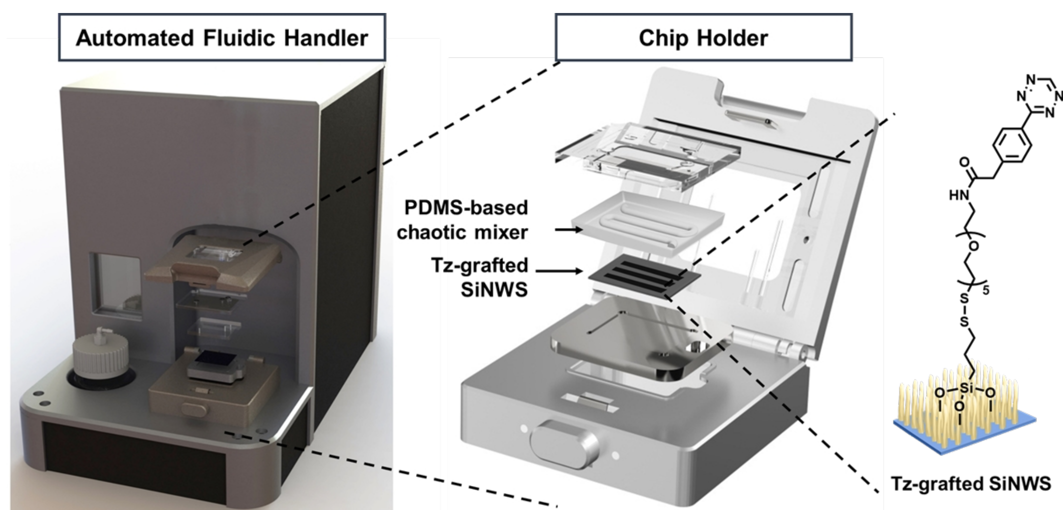

Figure S2. Photograph and schematic of an automated fluidic handler and a chip holder. The fully automated digital fluidic handling system (left) is composed of rotary valves, two PSD4 syringe pumps (Hamilton Robotics), and a controller program with a graphical user interface that was used to control the loading of reagents and test samples. The chip holder (middle) is used to integrate Tz-grafted SiNWS and PDMS-based chaotic mixer to make a complete ES-EV Click chip.

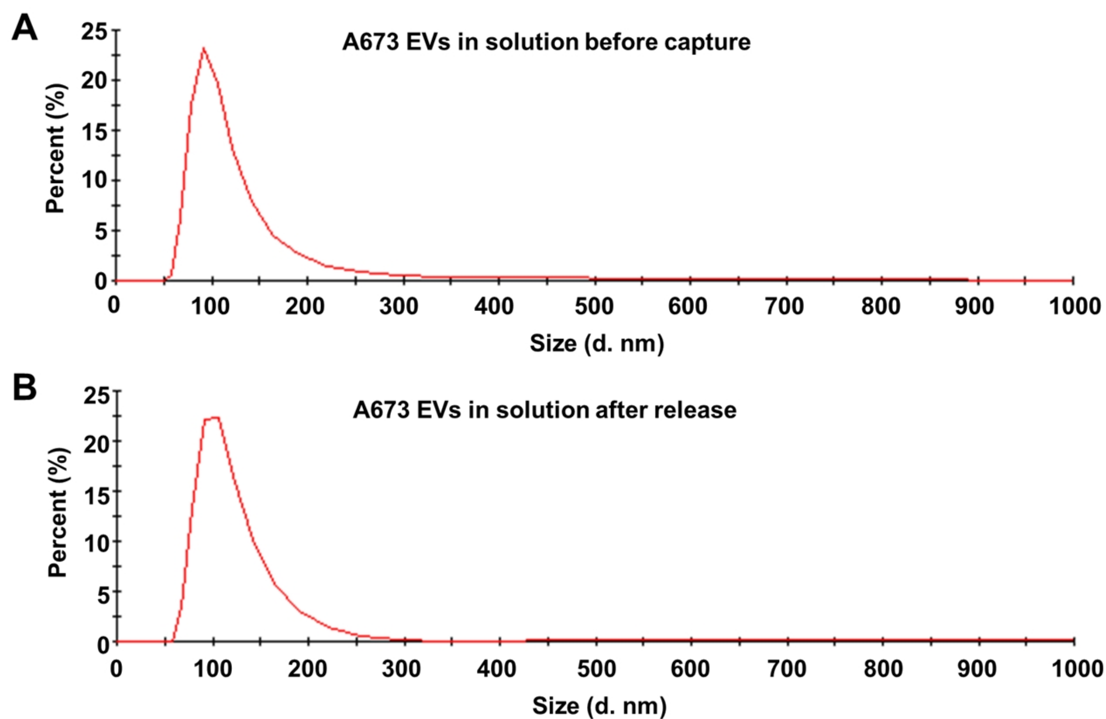

Figure S3. Size distributions of EVs before capture and after release measured by dynamic light scattering (DLS). (A) The size distribution of A673 EVs in solution before capture study. (B) The size distribution of A673 EVs in solution after release.

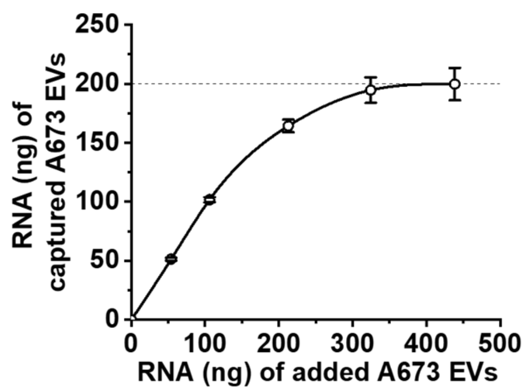

Figure S4. Capture capacity of ES-EV Click Chips was examined by introducing different amounts of A673 EVs into the devices, followed by the quantification of EV-derived RNA. The ES-EV Click Chips were saturated after capturing a quantity of A673 EVs, which were lysed to obtain 200 ng of EV-derived RNA.

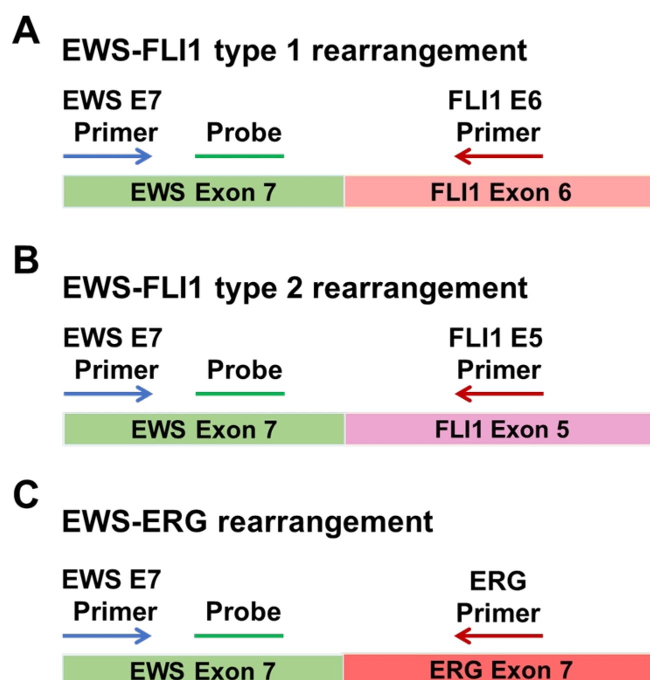

Figure S5. Schematic diagram of EWS rearrangements and RT-ddPCR quantification. (A) EWS-FLI1 type 1 rearrangement in A673 cells and A673 cell-derived EVs. (B) EWS-FLI1 type 2 rearrangement in SK-ES-1 cells and SK-ES-1 cell-derived EVs. (C) EWS-ERG rearrangement in ES-5838 cells and ES-5838 cell-derived EVs.

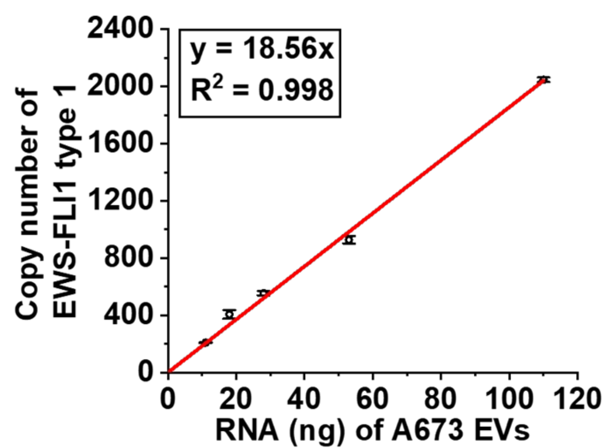

Figure S6. Linear correlation between the copy number of EWS-FLI1 type 1 rearrangement and the amount of A673 EV-derived RNA (n = 3).

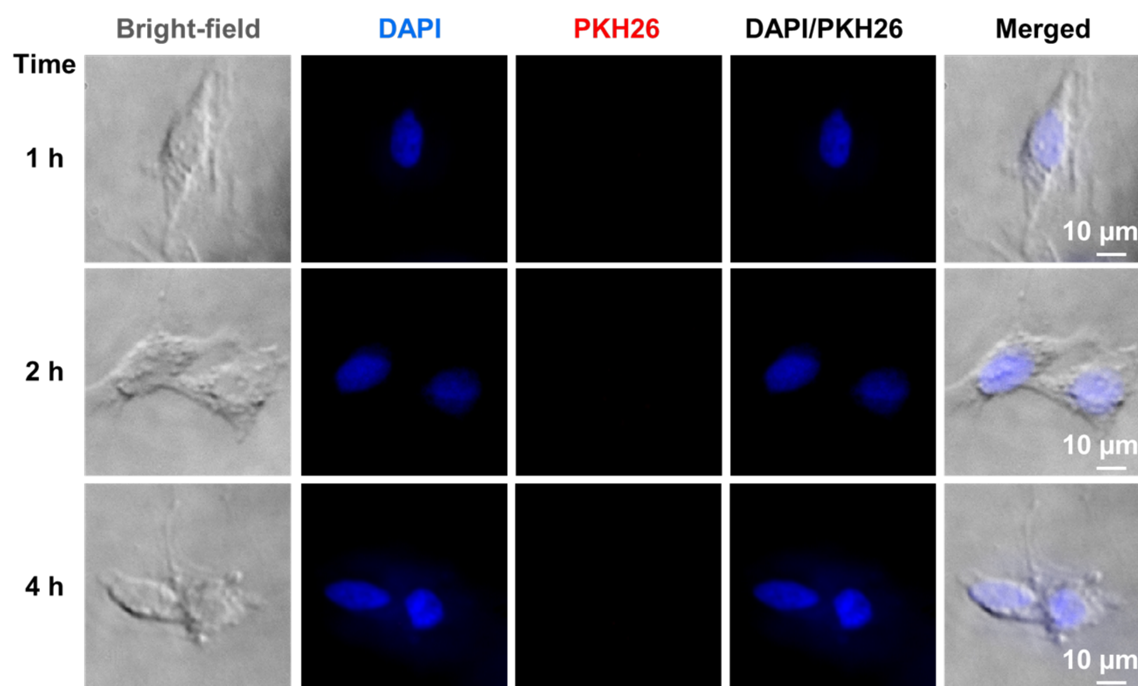

Figure S7. Fluorescence images of negative controls of EV uptake study. The PKH26 negative control samples (without ES-5838 EVs) were purified by ES-EV Click Chips and co-cultured with A673 cells for 1, 2, and 4 h, respectively. Nuclei (blue) of A673 cells are stained with DAPI. No red fluorescence signal was observed in these A673 cells.

Table S1. DNA sequence of primers and probes for the detection of EWS rearrangements.

EWS rearrangements	Amplicon length (bp)	Primers /probes	DNA sequence (5'→3')
EWS-FLI1 type 1	76	Forward	CTA CAG CCA AGC TCC AAG TC
		Reverse	GAC TGA GTC ATA AGA AGG GTT CTG
		Probe	TAG CCA ACA GAG CAG CAG
EWS-FLI1 type 2	104	Forward	CTA CAG CCA AGC TCC AAG TC
		Reverse	GTG AGG ATT GGT CGG TGT G
		Probe	TAG CCA ACA GAG CAG CAG
EWS-ERG	97	Forward	CTA CAG CCA AGC TCC AAG TC
		Reverse	ACC GGT CCA GGC TGA T
		Probe	TAG CCA ACA GAG CAG CAG

Table S2. Standard deviation (SD) and coefficient of variation (CV) of ES-EV Click Chips.

Evaluation index	Test 1	Test 2	Test 3	Test 4	Test 5	Mean	SD	CV%
Isolation efficiency	88%	87%	93%	90%	97%	91%	3.6%	4.0

Table S3. Clinical characteristics and EV-based detection of EWS rearrangements of the Ewing sarcoma (ES) patients and healthy donors (HDs).

Patient No.	Gender	Age[a]	Clinical stage	FISH test on tissue	Gene status in EVs[b]	EWS rearrangement in EVs (copies)[c]	Blood drawing timepoint
ES1	Male	19 (Y)	IV	EWS rearrangement	EWS-FLI1 (Type 1)	216	Treatment-naïve
ES2	Male	12 (Y)	IV	EWS rearrangement	EWS-FLI1 (Type 2)	35	Under salvage treatment
ES3	Male	10 (M)	IV	EWS rearrangement	EWS-FLI1 (Type 2)	82	Treatment-naïve
ES4	Male	18 (Y)	IV (Recurrent)	EWS rearrangement	EWS-FLI1 (Type 2)	36	Under salvage treatment
HD1	Male	18 (Y)	N/A[b]	N/A	N/A	0	N/A
HD2	Male	20 (Y)	N/A	N/A	N/A	0	N/A
HD3	Male	21 (Y)	N/A	N/A	N/A	0	N/A
HD4	Male	31 (Y)	N/A	N/A	N/A	0	N/A

[a]Y: years. M: months. [b]N/A: not available. [c]per 0.3 mL plasma.

ES Patient Enrollment and Blood Sample Collection. We enrolled four ES patients (stages IV) and four HDs in May 2018 and June 2020 at UCLA under IRB #00000173. This study was approved by the Ethics Committee of UCLA and written informed consent for this study was obtained for each patient. A 10-mL peripheral venous blood sample was collected in a BD Vacutainer™ Plastic Blood Collection Tube with K2EDTA (BD Medical, Cat. #366643, Thermo Fisher Scientific). Blood samples were centrifuged at 300 g for 5 min and subsequently at 2,000 g for 5 min at 4 °C. The plasma was collected, aliquoted, and stored at −80 °C. For each study, 0.3 mL of plasma was centrifuged at 3,000 g for 15 min to remove cell debris and run through the ES-EV Click Chip under the optimal condition.