Electronic Supplementary Material (ESI) for Lab on a Chip.

Enrichment of Extracellular Vesicles with Lipid Nanoprobe Functionalized Nanostructured Silica

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Supplementary Information

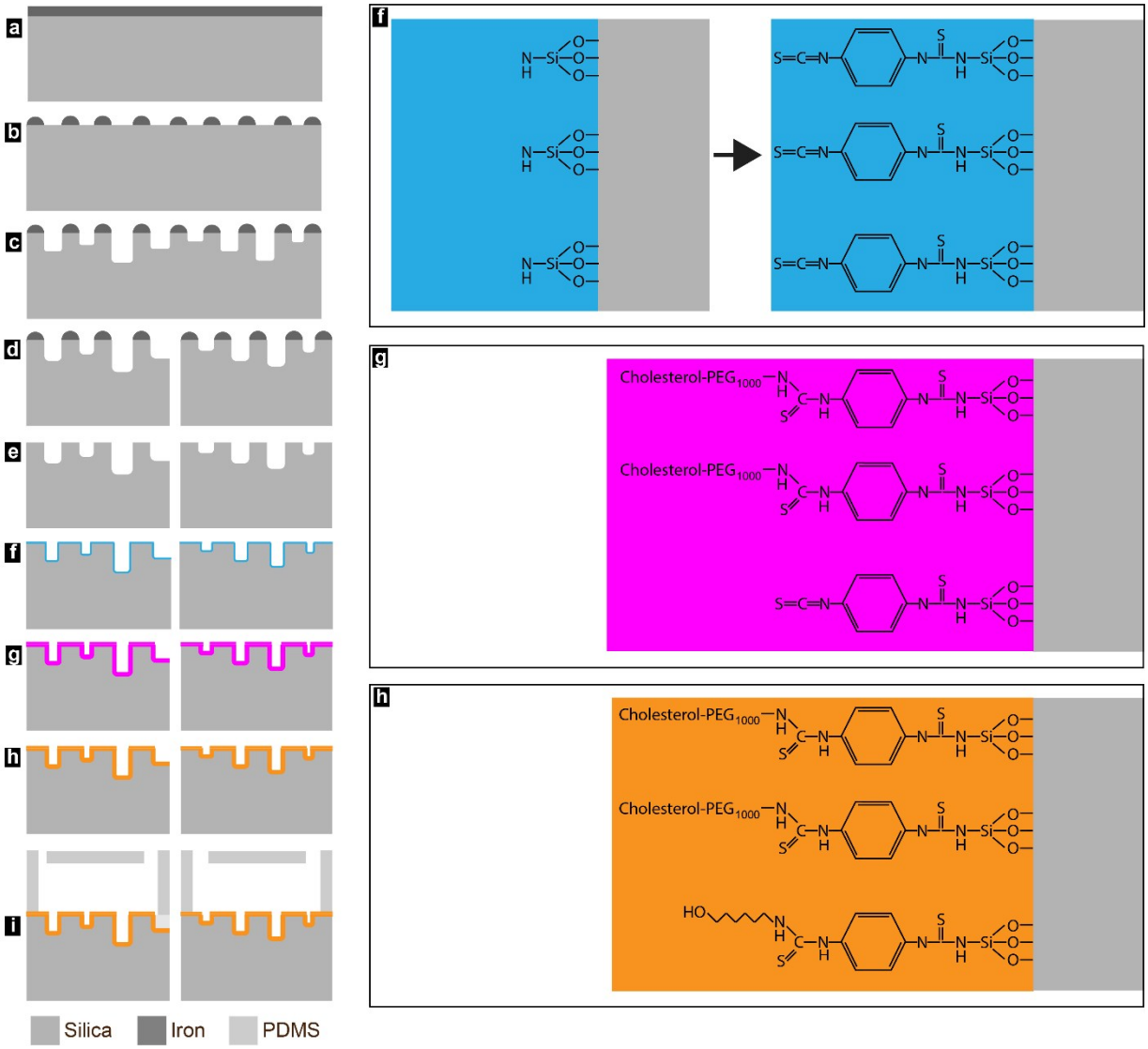

Figure S1. Fabrication Process for Nanostructured Substrate Integrated Micromixer. a) bare silica is coated with electron-beam deposited with several nanometer-thick iron. b) iron is annealed to form iron nanoparticles. c) silica is reactive ion etched with iron nanoparticle mask. d) wafer is diced e) remaining oxidized iron nanoparticle mask is stripped with piranha etch. f) nanostructured surface is silanized (left) and functionalized with PDITC (right). g) nanostructured surface is functionalized with cholesterol lipid nanoprobe. h) lipid nanoprobe nanostructured surface is deactivated with 6-amino-1-hexanol. i) PDMS micromixer is bonded to nanostructured substrate via PDMS glue and heat curing.

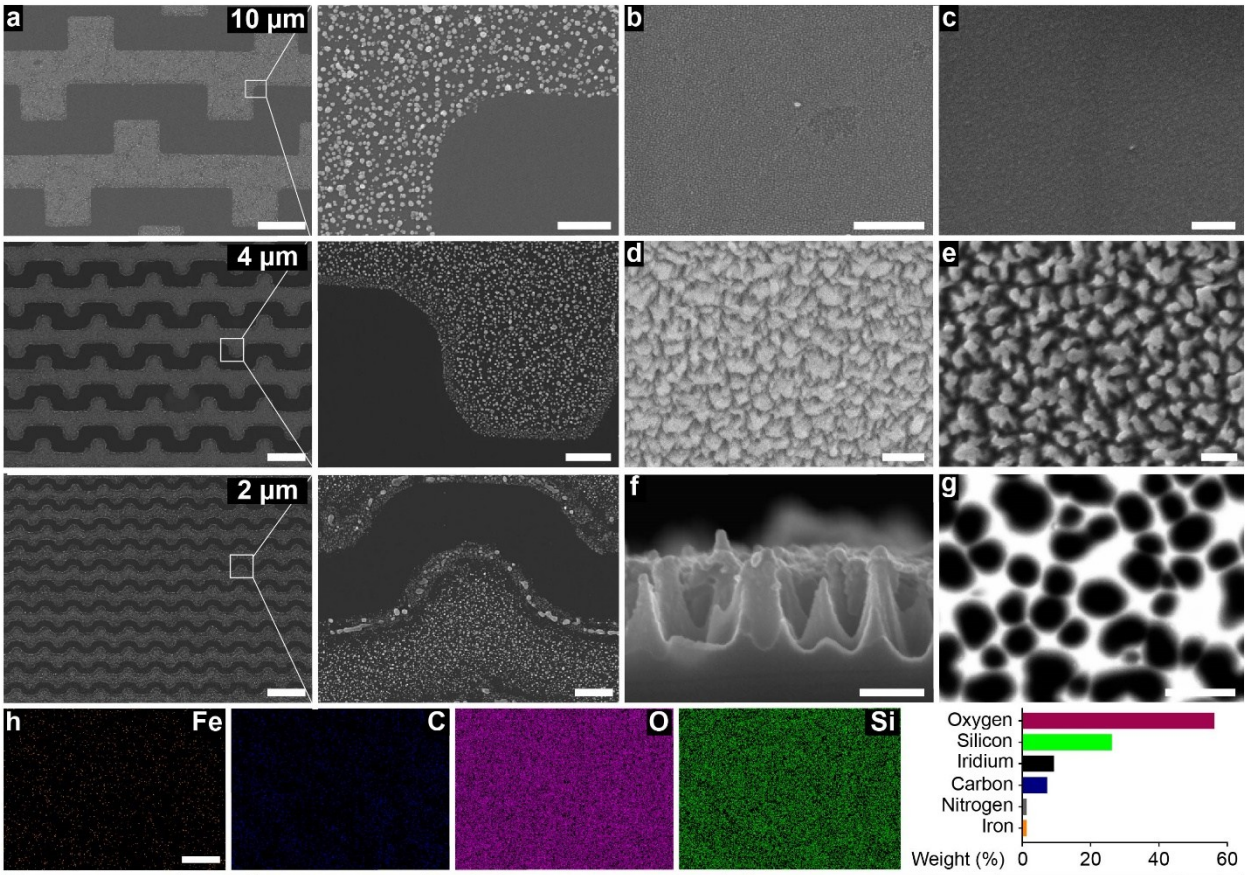

Figure S2. Preparation of nanostructures using FeNPs assisted dry etching. a) patterned FeNPs on substrate surface with line-width resolution ranging from 2 µm to 10 µm. Scale bars in left column and right column are 10 µm and 1 µm respectively. b) SEM image of 3-nm iron film fused in oxygen ambient. Scale bar is 200 nm. c) SEM image of planar silica surface as a negative control. Scale bar is 200 nm. d) SEM image of planar silica surface after etching. Scale bar is 200 nm. e) SEM image in top view of substrate after FeNPs assisted etching. Scale bar is 200 nm. f) SEM image in side view of substrate after FeNPs assisted etching. Scale bar is 200 nm. g) SEM image of bottom after piranha cleaning. Scale bar is 200 nm. h) energy dispersive spectroscopy analysis of surface elements after piranha cleaning and iridium coating. Scale bar is 200 nm.

a

	PURE SILICA	0 MIN	2 MIN	4 MIN	6 MIN	8 MIN
R_Q (nm)	0.638 ± 0.738	12.1 ± 1.95	21.8 ± 3.17	25.2 ± 1.40	30.9 ± 1.96	41.7 ± 3.94
R_A (nm)	0.691 ± 0.847	9.43 ± 1.32	17.6 ± 2.60	20.2 ± 1.14	24.6 ± 1.33	33.4 ± 3.13
INCREASE IN SURFACE AREA (%)	0.508 ± 0.168	23.1 ± 2.91	67.5 ± 11.1	71.6 ± 1.85	73.4 ± 6.28	89.5 ± 19.3

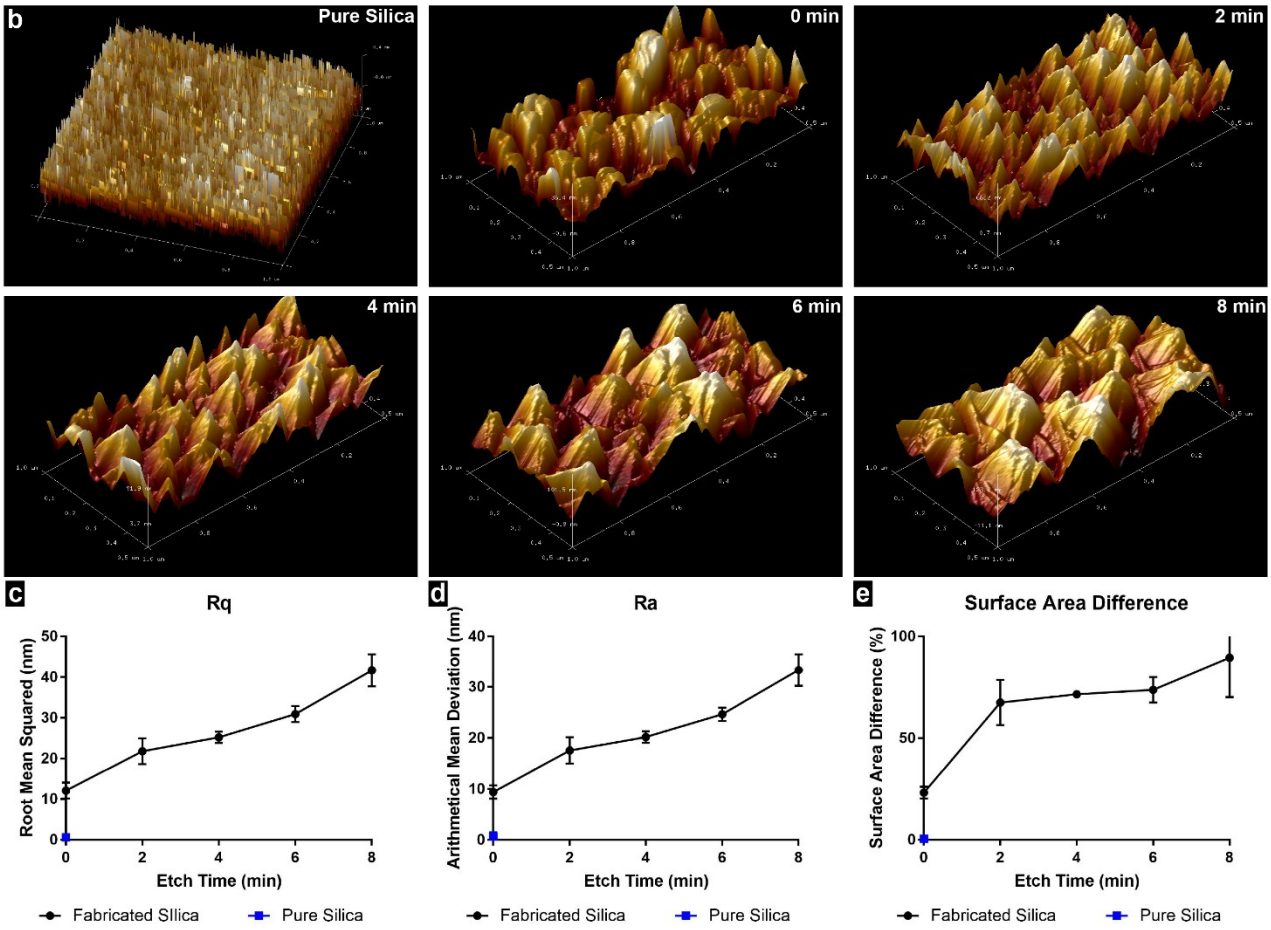

Figure S3. AFM surface roughness and profile characterization of generated nanostructures under various etching time. a) Table of measured roughness parameters R_Q, R_a, and increase in surface area for blank silica (n=2), 0 minutes (n=3), 2 minutes (n=5), 4 minutes (n=5), 6 minutes (n=5), and 8 minutes (n=5). R_Q is the root mean squared, and R_A is the arithmetical mean deviation of the assessed profile. b) Sample AFM 3D profiles used to quantify roughness and profile. c) Increase in R_q measurement with respect to etch time. d) Increase in R_a measurement with respect to etch time. e) Increase in surface area with respect to etch time.

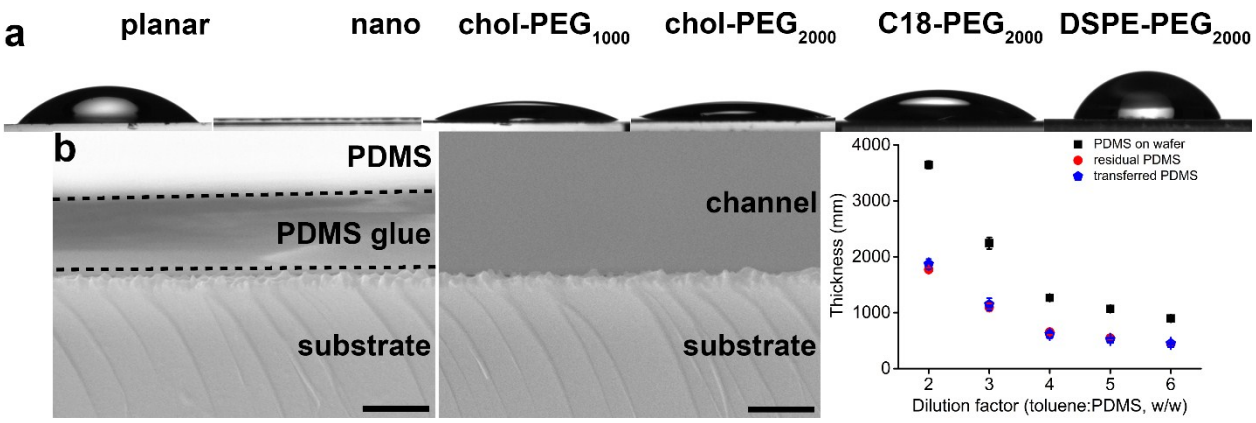

Figure S4. Surface grafting lipid nanoprobes and device assembly. a) respective contact angles measured from various lipid nanoprobes-functionalized surfaces. b) optimization of mix ratio between PDMS prepolymer and toluene and SEM images of binding PDMS micromixer to nanostructured silica substrate use the mixture as a glue. Scale bar is 1μm.

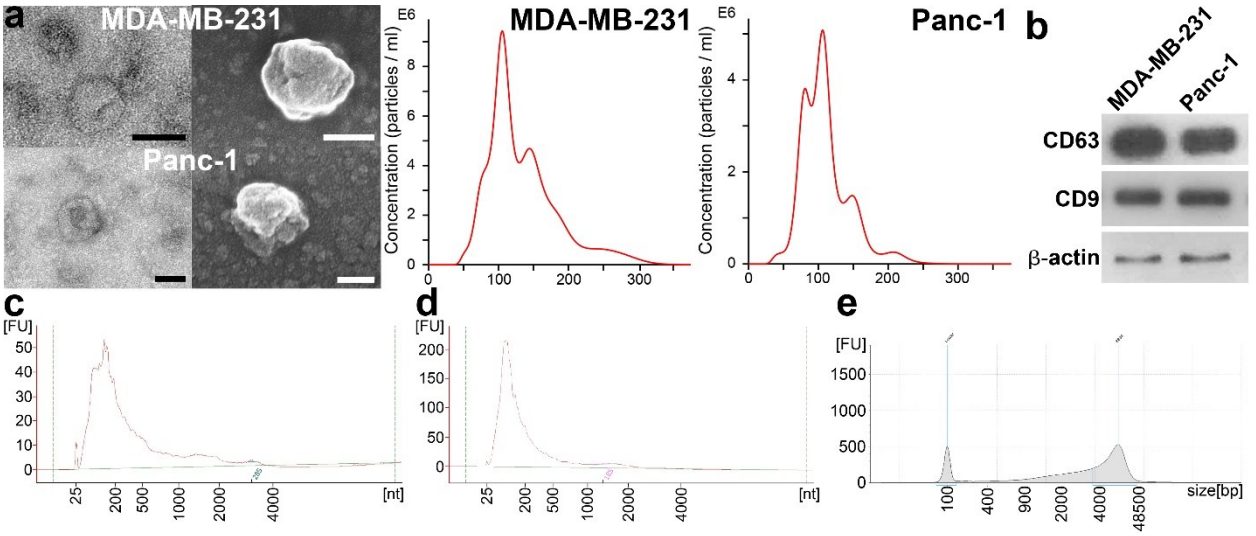

Figure S5. Characterization of nEVs isolated from two cancer cell lines. a) TEM (left) and SEM (right) images of nEVs derived from MDA-MB-231 cells and Panc-1 cells, and respective size distribution determined by Nanosight. Scale bars are 100 nm b) CD63, CD9, and β-actin were extracted and identified from isolated nEVs by Western Blot. c) RNA extracted from total nEVs derived from MDA-MB-231 cells. d) RNA extracted from the flow-through nEVs derived from MDA-MB-231 cells. e) DNA extracted from isolated nEVs derived from MDA-MB-231 cells.

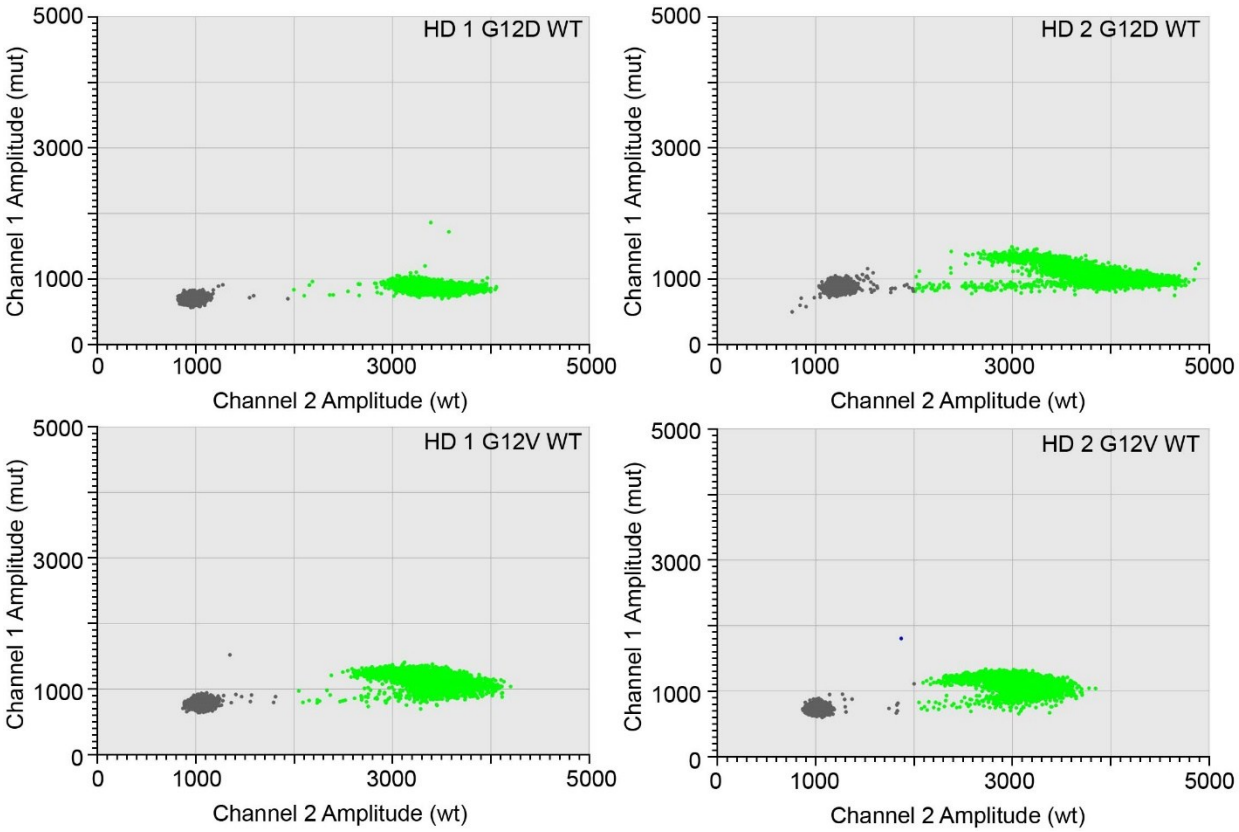

Figure S6. Mutation detection using DNA samples derived from nEVs of Healthy donors (HD).