Supplementary Data

SUPPLEMENTARY MATERIALS AND METHODS

Reagents

CATEGORY	REAGENT or RESOURCE	SOURCE	IDENTIFIER
Cell culture			
	D-MEM/Ham's F-12 with L-Glutamine and Phenol Red	Wako	048-29785
	D-MEM (Low Glucose) with L-Glutamine and Phenol Red	Wako	044-33555
	D-MEM (High Glucose) with L-Glutamine and Phenol Red	Wako	044-29765
	RPMI-1640 with L-Glutamine and Phenol Red	Wako	189-02025
	Fetal Bovine Serum	Japan Bioserum	S1760-500
	Penicillin-Streptomycin Solution (x100)	Wako	168-23191
	0.5w/v% Trypsin-5.3mmol/l EDTA・4Na Solution with Phenol Red (x10)	Wako	206-17291
Solution			
	Disodium hydrogen phosphate dodecahydrate	Wako	196-02835
	Potassium chloride	Wako	163-03545
	Potassium dihydrogen phosphate	Wako	169-04245
	Sodium chloride	Wako	191-01665
	Paraformaldehyde	Wako	162-16065
	Sodium hydroxide	Wako	198-13765
	Hydrochloric acid	Wako	080-01066
Staining reagent			
	Calcein-AM (3',6'-Di(O-acetyl)-4',5'-bis[N,N-bis(carboxymethyl)aminomethyl]fluorescein, tetraacetoxymethyl ester)	Wako	349-07201
Anti-cancer drug			
	Doxorubicin	Wako	040-21521

Stock Solutions

1) Phosphate buffered saline (PBS): 137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, and 1.8 mM KH_2PO_4, pH7.2. Stored at room temperature.

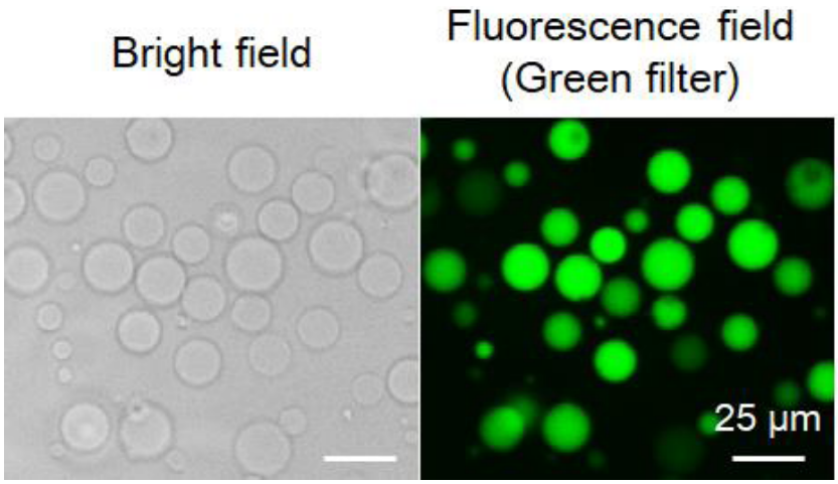
2) 4 mM calcein-acetoxymethyl ester (calcein-AM) (Takara) in dimethyl sulfoxide (DMSO). Stored at -20^{o}C.

3) 2 mM doxorubicin (Wako) in dimethyl sulfoxide (DMSO). Stored at -20^{o}C.

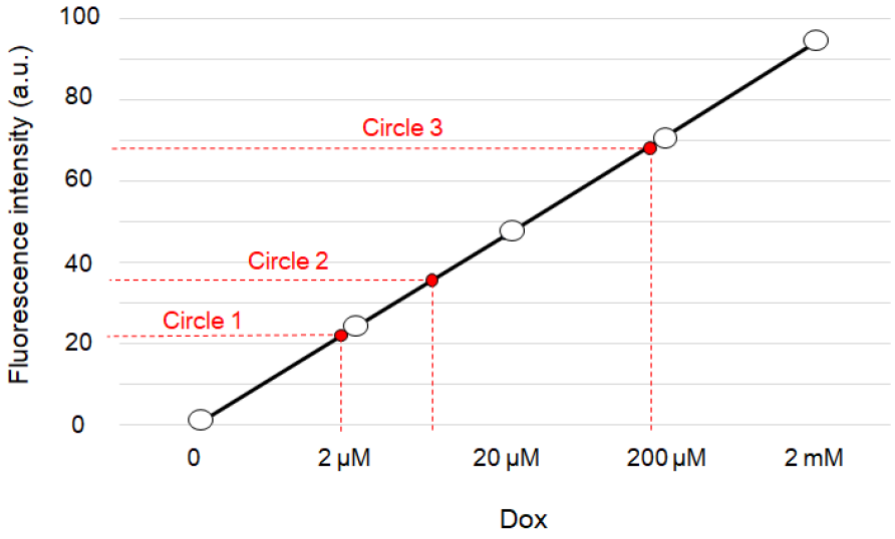
Cell Lines and Culture Conditions

Human cervical cancer cell line HeLa, human liver cancer-derived cell line Huh-7, human gastric cancer-derived cell line KE-39, human pancreatic cancer-derived cell line PK-59, human colon cancer-derived cell line colo320 and mouse ascites-derived macrophage-like cell line J774-1 were obtained from RIKEN cell bank (Tsukuba, Japan). Mouse fibroblast cell line C2C12 was kindly provided by Dr. Yuki Nakayama (Kumamoto University, Japan), mouse spermatogonia cell line GC-1 was kindly provided by Dr. Ko Eto (Kumamoto University, Japan) and human embryonic kidney cell line 293 was kindly provided by Dr. Terumasa Ikeda (Kumamoto University, Japan). HeLa cells, colo320 cells and GC-1 cells were maintained in Dulbecco's modified Eagle's medium (D-MEM) /Ham's F-12 nutrient mixture containing 5% fetal bovine serum (FBS) and 1 % penicilin-streptomycin solution (PS). Huh-7 cells were maintained in D-MEM (Low Glucose) containing 10 % FBS and 1 % PS. C2C12 cells and 293 cells were maintained in D-MEM (High Glucose) containing 10 % FBS and 1 % PS. KE-39 cells, PK-59 cells and J774-1 cells were maintained in RPMI-1640 containing 10 % FBS and 1 % PS. Cells were cultured at 37 °C in a 5% CO2 incubator.

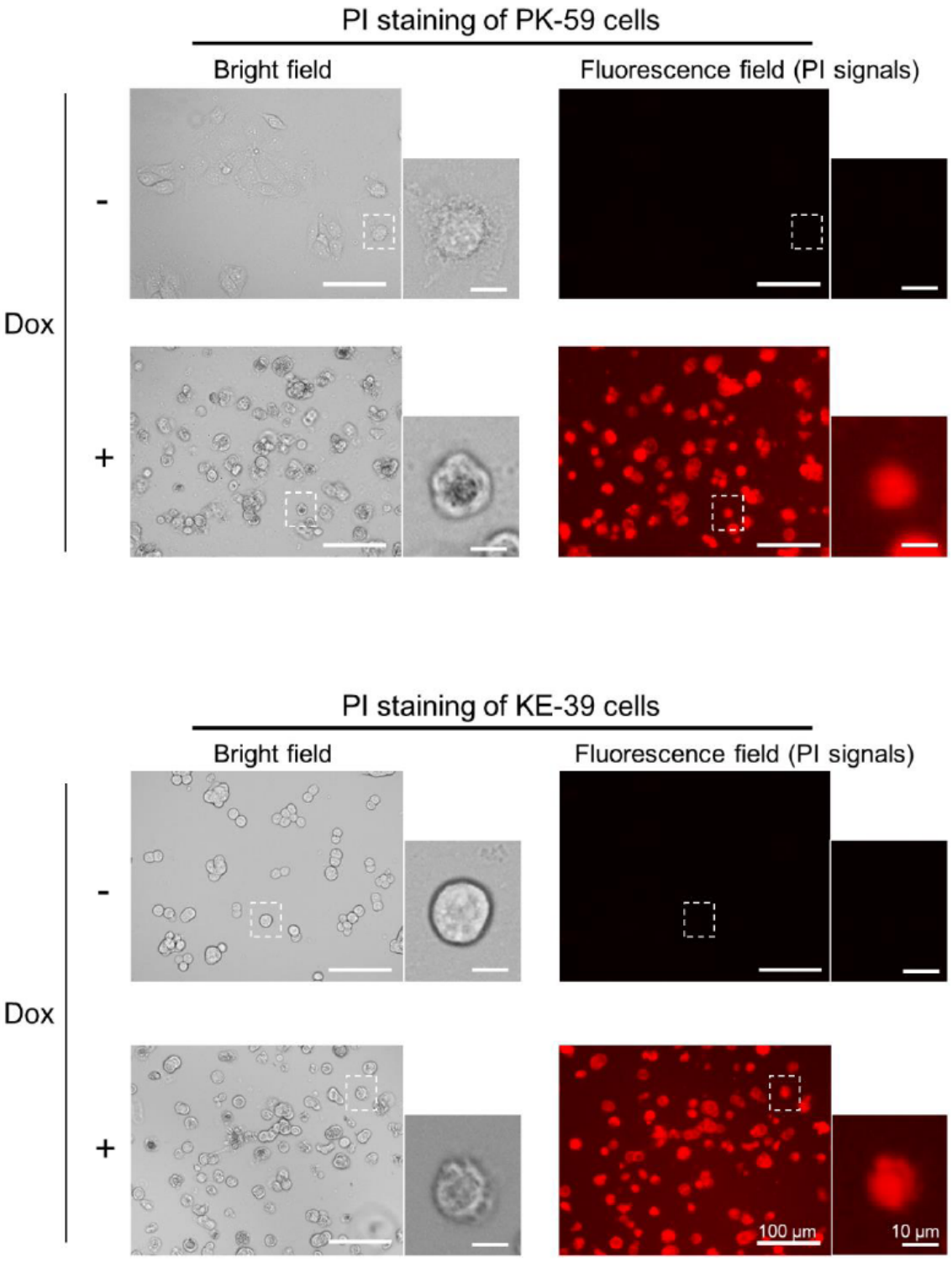
SUPPLEMENTARY FIGURES



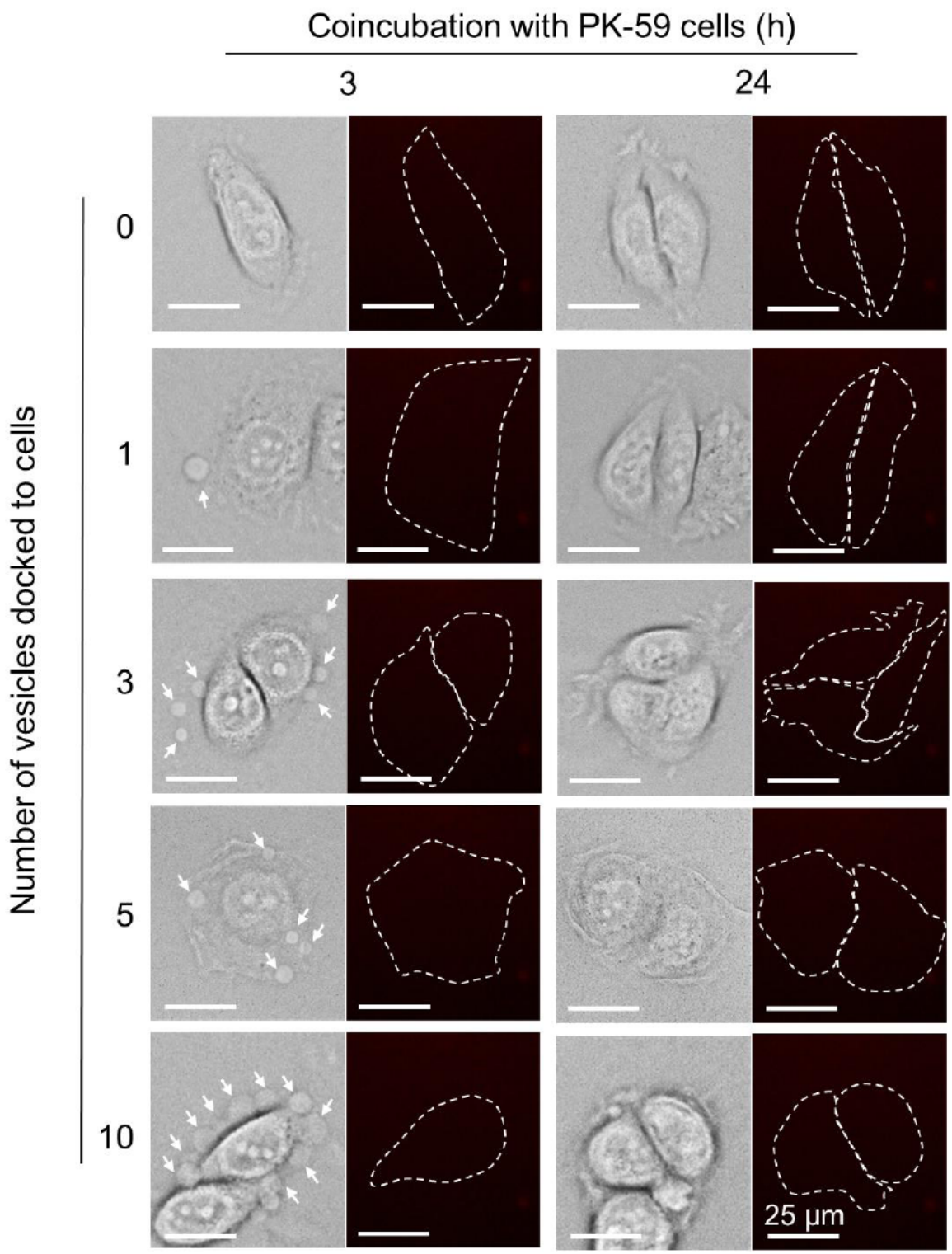
Supplementary Figure S1: HeLa cell-derived giant membrane vesicles were incubated with calcein-AM, an indicator of esterase activity, followed by fluorescence microscopy. Bar = 25 μm.



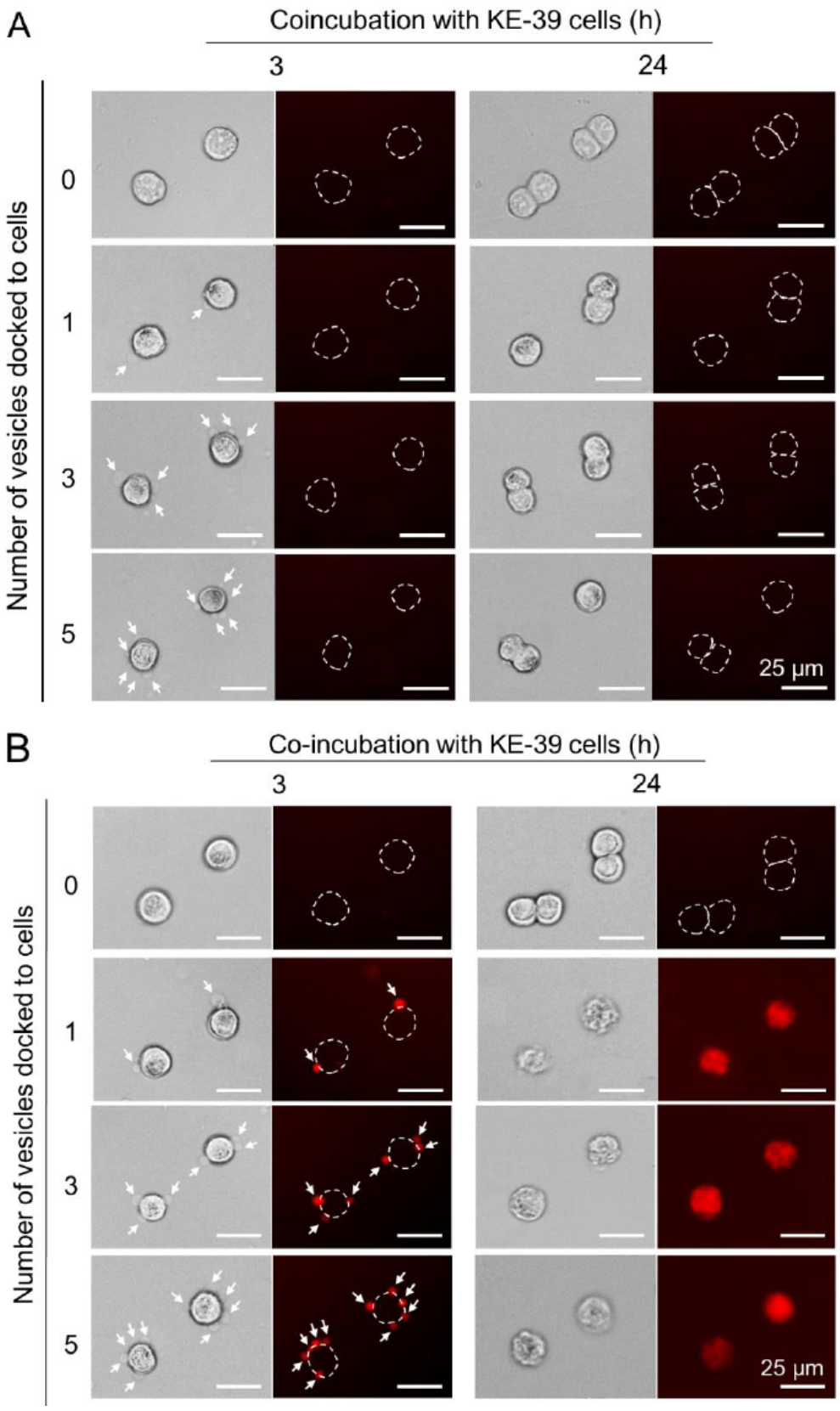
Supplementary Figure S2: Standard curve to calculate the Dox concentration. On the basis of the fluorescence intensity in the vesicle 1, 2 and 3 shown in Fig. 1A (see the vesicles indicated by dashed-line-circles in Fig. 1A), the concentration of Dox in the vesicle 1, 2 and 3 was estimated, respectively.



Supplementary Figure S3: Co-incubation of the Dox-enclosed vesicles (+) with PK-59 (*upper*) and KE-39 (*lower*) cancer cells generated multiple cells exhibiting abnormal morphology with propidium iodide (PI)-positive staining, indicative of cells undergoing cell death. In contrast, co-incubation of Dox-free vesicles (-) with cells exhibited no morphological changes with PI-negative staining. The area in the white boxes are enlarged to show morphology of the cells.



Supplementary Figure S4: Microscopic observation of KE-39 cells at a fixed time point after addition of Dox-free vesicles. Cells docked with no (zero), one, three, five, and 10 vesicles are shown. Images were obtained at the indicated time under the red fluorescence filter to visualize autofluorescent signals from Dox. Vesicles docked to the cellular cortex are indicated by arrows. White-dashed lines indicate the cellular cortex of the observed cells. Bar = 25 μm.



Supplementary Figure S5: Microscopic observation of PK-59 cells at a fixed time point after addition of Dox-enclosed (**B**) or Dox-free vesicles (**A**). Cells docked with no (zero), one, three and five vesicles are shown. Images were obtained at the indicated time under the red fluorescence filter to visualize autofluorescent signals from Dox. Vesicles docked to the cellular cortex are indicated by arrows. White-dashed lines indicate the cellular cortex of the observed cells. Bar = 25 μm.