1   < Electronic supporting information >

2 Tumor-binding prodrug micelles of polymer-drug

3 conjugates for anticancer therapy in HeLa cells

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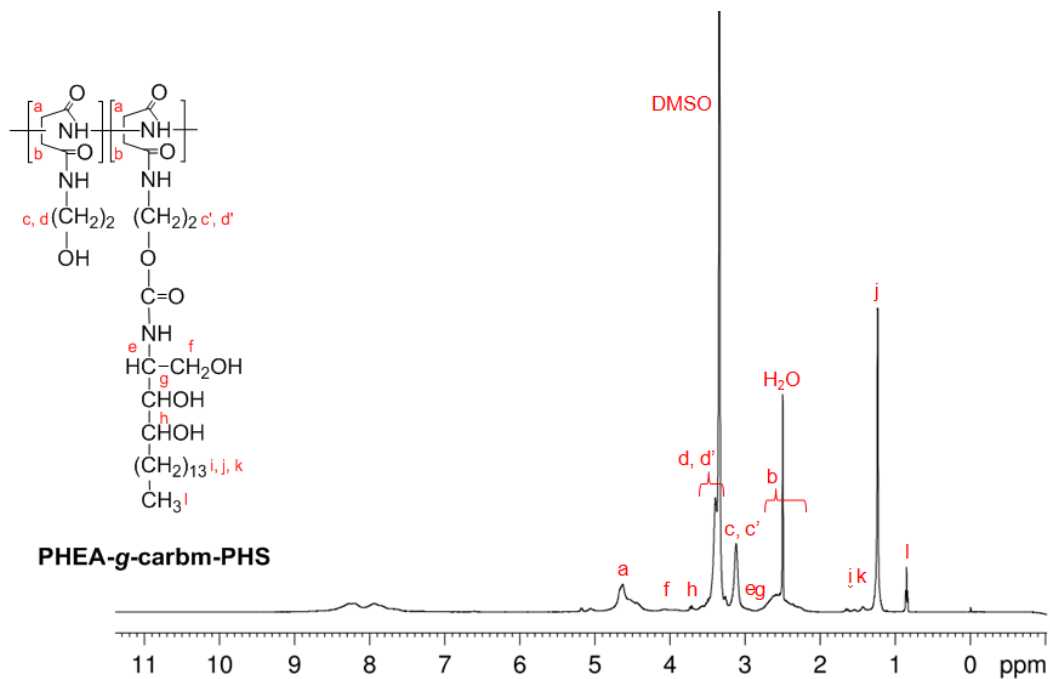
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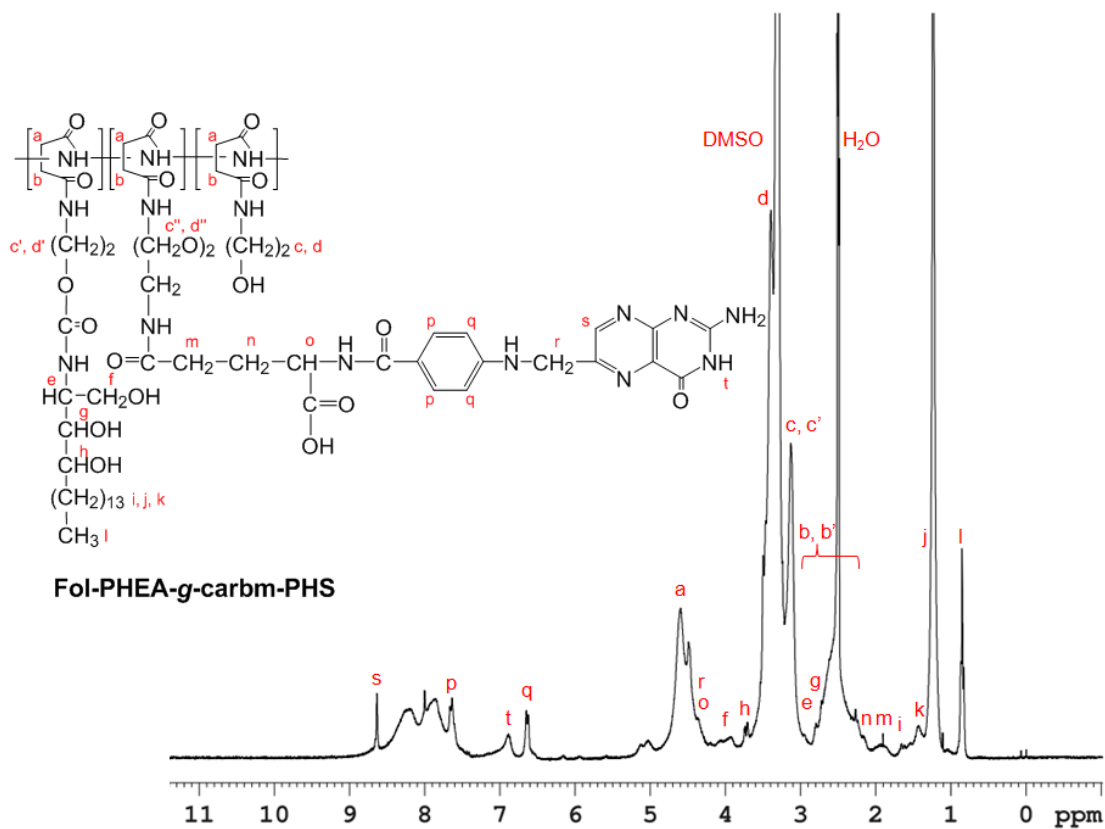17 [‡] These authors contributed equally to this work.

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1   Figure S1. ^{1}H-NMR spectra of (a) PHEA-g-carbm-PHS and (b) Fol-PHEA-g-carbm-PHS in DMSO-

2 d_6.

3   (a)

5   (b)

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1   Figure S2. Morphological transition of self-assembled PHEA-*g*-carbm-PHS as a function of degree of

2   substitution was observed by TEM images; the first column shows (a) spherical micelles (DS 6.8

3   mol%) (b) spherical/worm-like micelles (DS 12.9 mol%) (transitional states of sphere to worm-like

4   micelles were indicated as yellow arrows) and (c) worm-like micelles (DS 26.5 mol%), and the

5   second column indicates magnified images at the region (scale bar is 200 nm)

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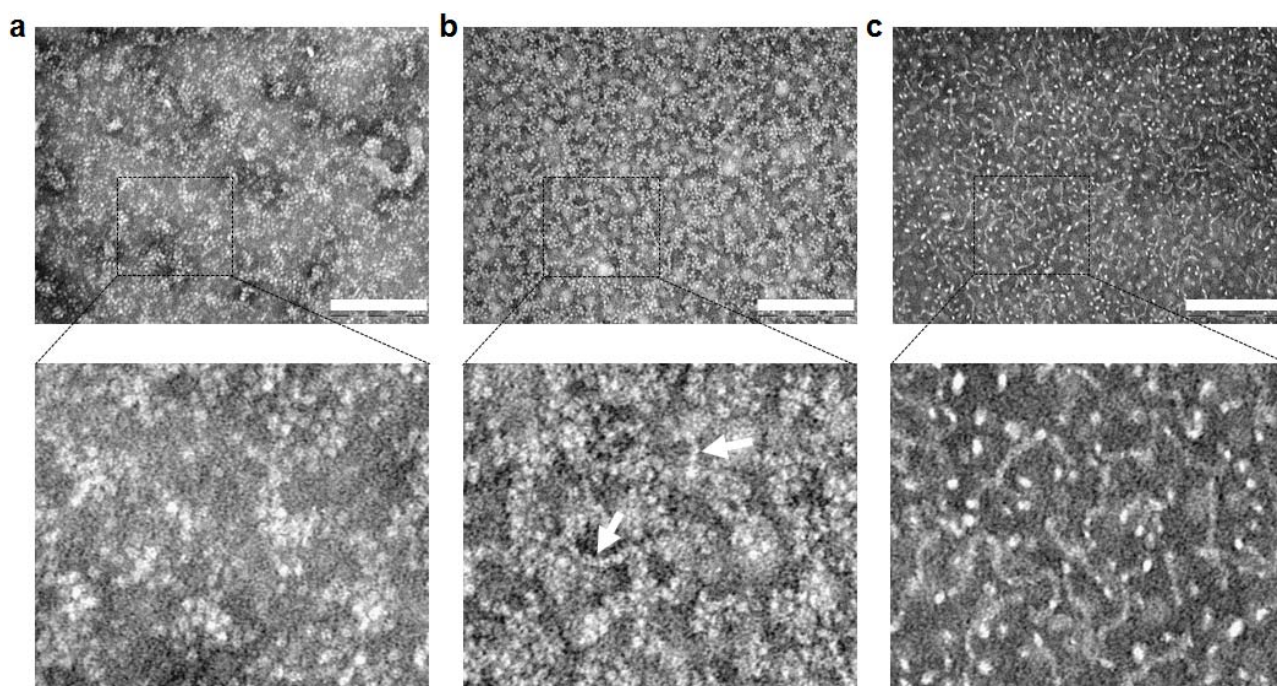
1   Table S1. Summarized IC_{30} value of HeLa cells treated with various formulations for 3 and 6 hr,

2   followed by 1 hr postincubation. IC_{30} value was determined by MTT assay.

| Incubation time (hr)	IC_{30} value (µg/ml)			
	PHEA	PHS_M	$^{FOL}PHS_M$	Free PHS
3	N.A.	47.8	39.9	7.5
6	N.A.	39.1	29.9	5.8

3   N.A. indicated "not available".

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6   Figure S3. Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL). Apoptosis

7   induced by PHS delivered into HeLa cells by various formulations for 3 hr: (a) PHEA(control), (b)

8   PHS_M, (c) $^{FOL}PHS_M$, and (d) free PHS. Blue (left): Nucleus (DAPI) Green (right): Fragmented DNA.

9   Arrows indicated significantly TUNLE-positive HeLa cells.
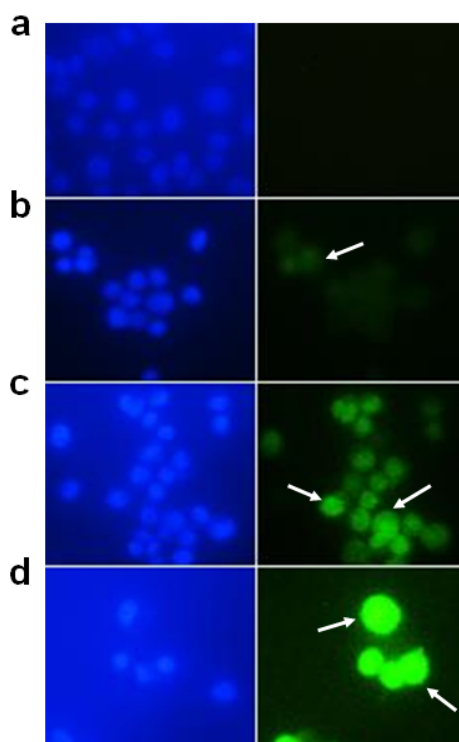
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1   Figure S4. Uptake of DOX-loaded PHS_M and $^{FOL}PHS_M$ in HeLa cells determined by flow cytometer.

2   HeLa cells were incubated with 5 µg/ml of equivalent DOX loaded in $^{FOL}PHS_M$, PHS_M, and control

3   (DPBS) for 0.5 hr and 0.5 hr followed by additional 1 hr postincubation with fresh medium.
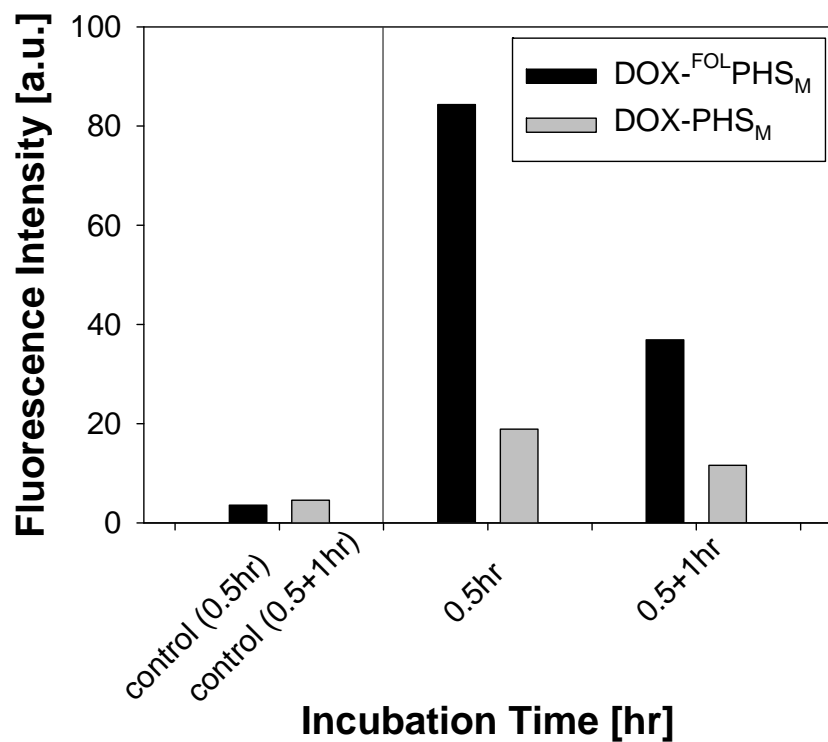
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6   Figure S5. CLSM images with low magnification of HeLa cells incubated with (a) DOX-PHS_M (b)

7   DOX-$^{FOL}PHS_M$, and (c) free DOX for 2 hr, followed by additional 1 hr incubation (Scale bar indicates

8   50 µm).

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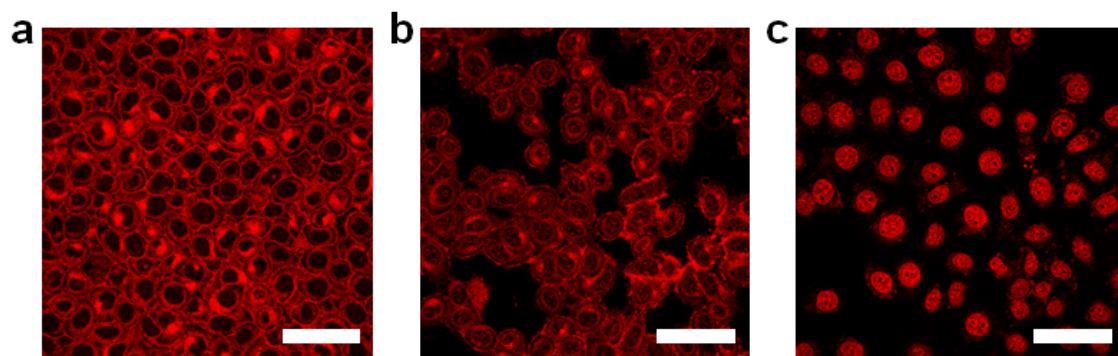
1   Intracellular distribution of DOX-PHS$_M$ and DOX-^{FOL}PHS$_M$ in individual HeLa cells

2   The subcellular distribution of DOX-loaded PHS conjugates was quantitatively evaluated by analysis

3   of confocal images with home-made MATLAB^{TM} code. We defined the accumulated amount of DOX

4   in the nucleus of individual cells as a ratio of total fluorescence intensity of DOX from nucleus to that

5   of background.

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$$Celluar\ uptake = \frac{\sum_i^n I_i/n}{I_b}$$

7   where, n, I, and I$_b$ are the number of cells, total intensity of pixels inside of nucleus, and intensity of

8   background signal, respectively. To do this, we analyzed confocal images as followed process: (1)

9   tracing the edge of nucleus of each cell to obtain boundary coordinates from Hoechst 33342 images

10   (left) (2) Accumulating the total fluorescence intensity of pixels only in the nucleus according to

11   determined boundary in DOX images (3) measuring the background fluorescence intensity at black

12   spot, which is defined as area without DOX traces, and (4) calculating the DOX uptake in nuclei by

13   dividing the total DOX intensity by background fluorescence intensity to compensate signal to noise.
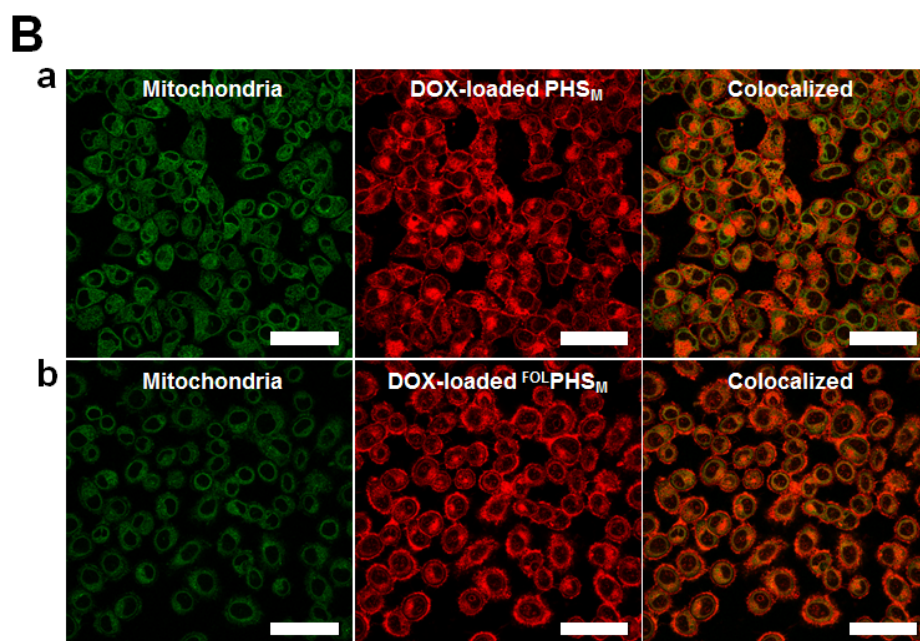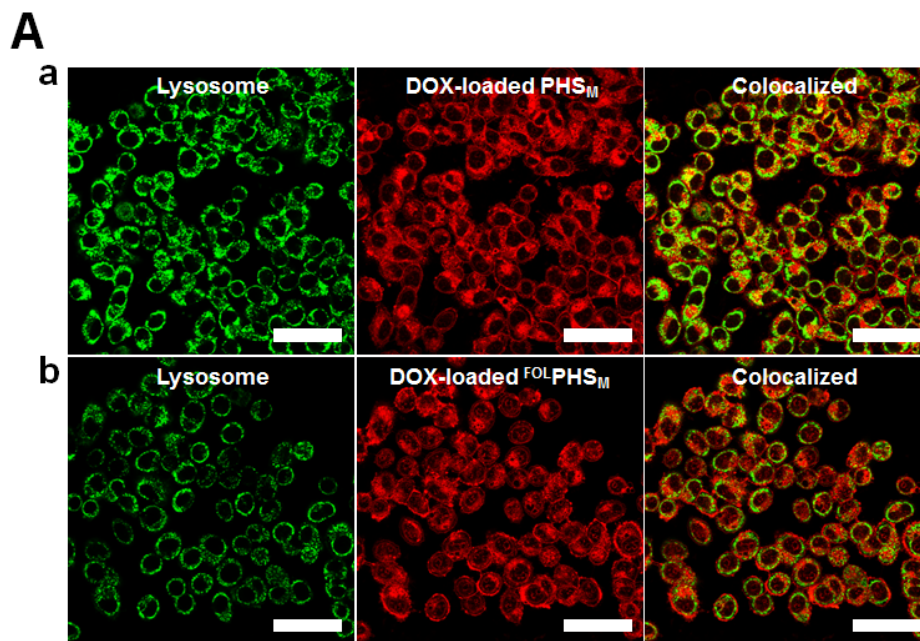
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1   Figure S6. CLSM images of HeLa cells treated with (a) free DOX, (b) DOX-PHS$_M$, and (c) DOX-

2   ^{FOL}PHS$_M$ to quantify the relative amount of DOX in nuclear region. Each top panel displays CLSM

3   images from Hoechst 33342 (left), colocalized (middle), and DOX (right). Bottom panel demonstrates

4   black and white images, of which the counted pixels from nucleus were changed into white color for

5   debugging purpose in order to confirm all the pixels inside of nucleus are considered. Among them,

6   "*" denotes the nucleus of HeLa cells, of which fluorescence intensity are taken into account for

7   calculation of relative total amount of DOX.
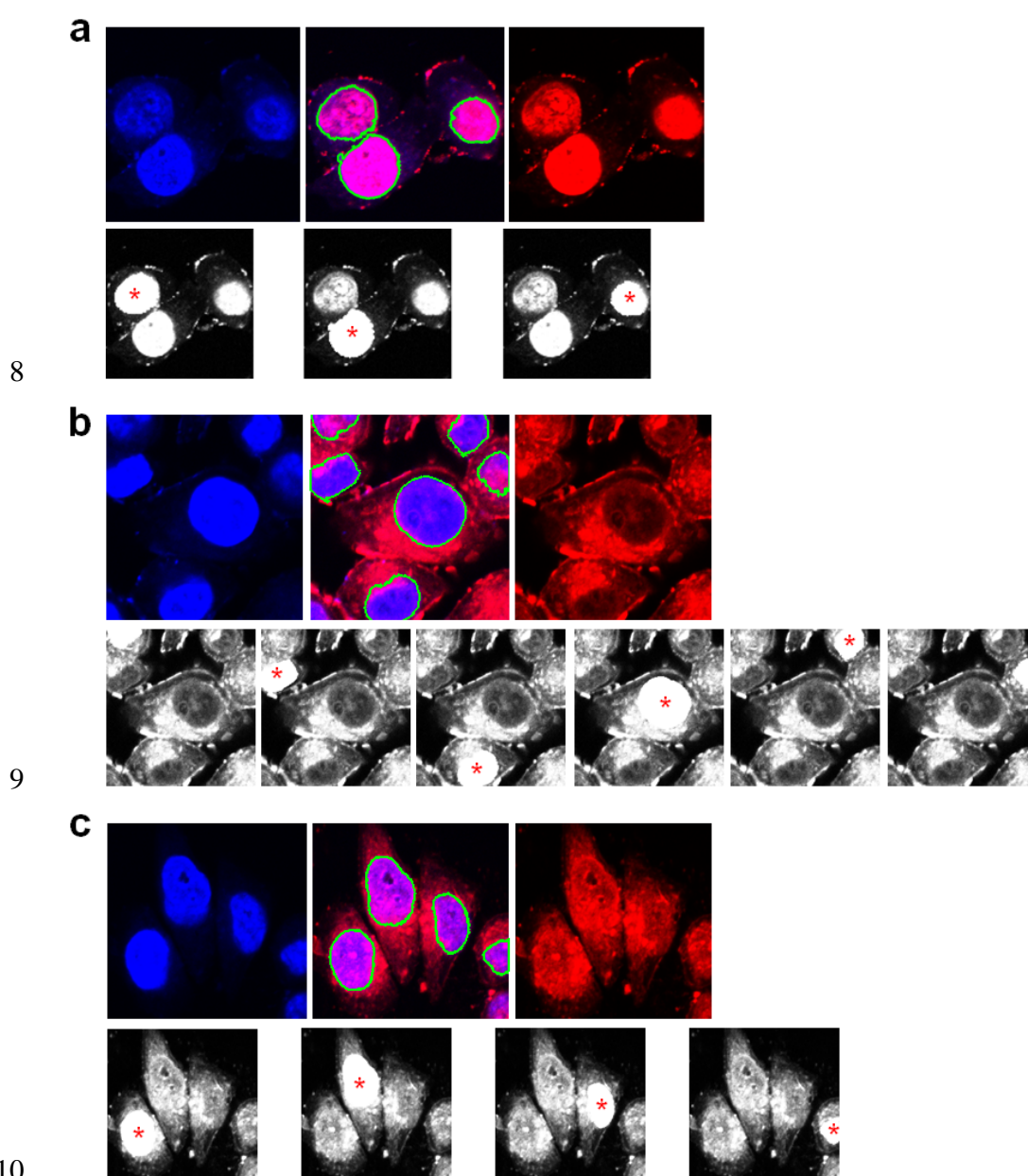
1   Figure S7. Low magnification CLSM images of (A) lysosomal and (B) mitochondrial distribution of

2   (a) DOX-PHS$_M$ and (b) DOX-^{FOL}PHS$_M$ in HeLa cells. HeLa cells were stained with either

3   LysoTracker$^®$ green (A) and MitoTracker$^®$ green (B) (Scale bar: 50 µm).

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