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Supporting Information for:

Histamine-Functionalized Polymer Micelles as a Drug Delivery System in 2D and 3D Models of Breast Cancer

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Table ESI 1. Characterization of NPs.

Codes*	Materials	Histamine vs other thiol	NPs				DOX-NPs		
			pH 5.0	pH 7.4			pH 7.4		
			CMC	PdI	Intensity Mean	Volume Mean	PdI	Intensity Mean	Volume Mean
		(%)	μg mL^{-1}		d.n.m	d.nm		d.n.m	d.n.m
LL0	$PAGE_{22}$-PEO_{246}	-	12	0.06 ± 0	33 ± 0	27 ± 0	0.72 ± 0.05	107 ± 11	33 ± 7
LL1	$POGE_{22}$-PEO_{246}	0	3.7	0.27 ± 0.06	227 ± 50	26 ± 1	0.24 ± 0.01	116 ± 3	100 ± 3
LL2	P(HGE_{7}-OGE_{15})-PEO_{246}	32	6.9	0.14 ± 0.02	25 ± 2	18 ± 0	0.53 ± 0.02	425 ± 78	290 ±189
LL3	P(HGE_{11}-OGE_{11})-PEO_{246}	48	7.9	0.06 ± 0.01	40 ± 1	33 ± 0	0.78 ± 0.20	605 ± 31	510 ± 109
LL4	P(HGE_{18}-OGE_{4})-PEO_{246}	80	8.3	0.12 ± 0.0	71 ± 2	50 ± 0	0.59 ± 0.05	67 ± 2	65 ± 2
LL5	$PHGE_{22}$-PEO_{246}	100	63	0.18 ± 0.04	20 ± 1	18 ± 1	0.87 ± 0.24	1239± 600	1045 ± 714
B1	$PBGE_{22}$-PEO_{246}	0	13	0.30 ± 0.03	177 ± 43	70 ± 16	0.23 ± 0.01	76 ± 8	47 ± 2
B2	P(HGE_{10}-BGE_{12})-PEO_{246}	44	7.1	0.14 ± 0.02	30 ± 1	23 ± 0	0.54 ± 0.14	417 ± 101	118± 52
LH0	$PAGE_{20}$-PEO_{430}	-	26	0.03 ± 0.01	44 ± 1	38 ± 2	0.71 ± 0.06	570 ± 84	351 ± 109
LH1	$POGE_{20}$-PEO_{430}	0	8.7	0.09 ± 0.01	94 ± 2	74 ± 1	0.32 ± 0.05	244 ± 149	235 ± 161
LH2	P(HGE_{4}-OGE_{16})-PEO_{430}	22	10	0.10 ± 0.01	78 ± 1	61 ± 1	0.46 ± 0.11	262 ± 32	132 ± 29
LH3	P(HGE_{10}-OGE_{10})-PEO_{430}	51	8.6	0.08 ± 0.01	59 ± 1	47 ± 2	0.89 ± 0.11	634 ± 58	447 ± 88
LH4	P(HGE_{16}-OGE_{4})-PEO_{430}	82	12	0.80 ± 0.11	332 ± 58	65 ± 2	0.46 ± 0.06	274 ± 18	80 ± 9
LH5	$PHGE_{20}$-PEO_{430}	100	410	0.27 ± 0.06	227 ± 54	26 ± 2	0.86 ± 0.13	705 ± 260	47 ± 32
HH0	$PAGE_{41}$-PEO_{486}	-	16	0.02 ± 0.02	51 ± 1	44 ± 1	0.50 ± 0.12	63 ± 11	53. ± 4
HH1	$POGE_{41}$-PEO_{486}	0	4.7	0.72 ± 0.02	962 ± 58	1450 ± 126	0.22 ± 0.01	125 ± 20	112 ± 12
HH2	P(HGE_{19}-OGE_{22})-PEO_{486}	45	9.1	0.20 ± 0.0	155 ± 26	85 ± 13	0.40 ± 0.05	252 ± 29	119 ± 32
HH3	$PHGE_{41}$-PEO_{486}	100	-	0.16 ± 0.06	37 ± 2	31 ± 0	0.59 ± 0.08	238 ± 81	64 ± 32

Note: *LL = low PXGE M_w and low PEG M_w group; LH = low PXGE M_w and high PEG M_w group; HH = high PXGE M_w and high PEG M_w group; (X in PXGE = A, O, H). B = BGE unit group.

Table ESI 2. Molecular weight and Zeta potential of NPs.

Codes*	Materials	Histamine content [%]	Molecular weight by ^{1}H-NMR [Da]	Zeta potential ζ [mV]
LL0	$PAGE_{22}$-PEO_{246}	-	10800	-0.6±1
LL1	$POGE_{22}$-PEO_{246}	0	16700	-0.4±1
LL2	$P(HGE_{7}$-$OGE_{15})$-PEO_{246}	30	17100	-1.8±1
LL3	$P(HGE_{11}$-$OGE_{11})$-PEO_{246}	50	17400	0.4±0.7
LL4	$P(HGE_{18}$-$OGE_{4})$-PEO_{246}	80	17900	0.9±2
LL5	$PHGE_{22}$-PEO_{246}	100	18100	1.5±1
B1	$PBGE_{22}$-PEO_{246}	0	16200	-0.6±0.9
B2	$P(HGE_{10}$-$BGE_{12})$-PEO_{246}	44	17100	-0.9±3
LH0	$PAGE_{20}$-PEO_{430}	-	18900	-1±0.9
LH1	$POGE_{20}$-PEO_{430}	0	24200	-1.3±2
LH2	$P(HGE_{4}$-$OGE_{16})$-PEO_{430}	20	24500	-0.2±0.5
LH3	$P(HGE_{10}$-$OGE_{10})$-PEO_{430}	50	24900	-1.0±1
LH4	$P(HGE_{16}$-$OGE_{4})$-PEO_{430}	80	25300	-0.3±0.4
LH5	$PHGE_{20}$-PEO_{430}	100	25600	0.3±2
HH0	$PAGE_{41}$-PEO_{486}	-	21400	-0.1±3
HH1	$POGE_{41}$-PEO_{486}	0	32200	-0.2±0.4
HH2	$P(HGE_{19}$-$OGE_{22})$-PEO_{486}	45	33400	0.1±1
HH3	$PHGE_{41}$-PEO_{486}	100	34900	1.6±2

Note: *LL = low PXGE M_w and low PEG M_w group; LH = low PXGE M_w and high PEG M_w group; HH = high PXGE M_w and high PEG M_w group; (X in PXGE = A, O, H). B = BGE unit group.

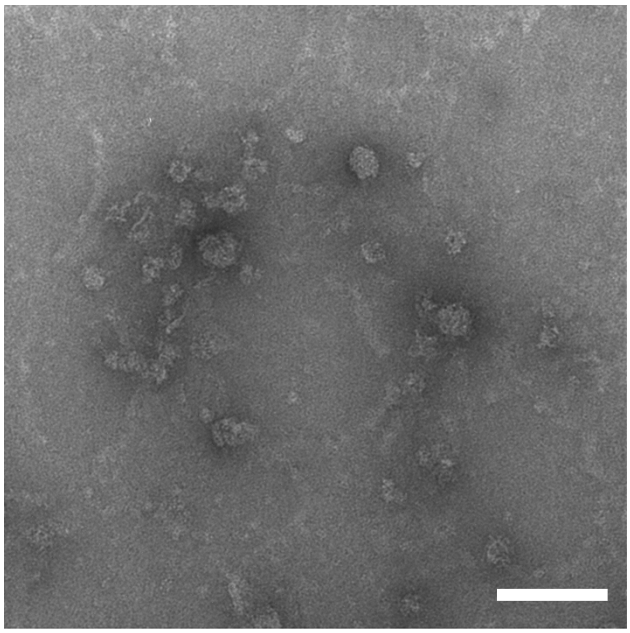

Fig. ESI 1 TEM image of DOX-LL5. Scale bar = 100 nm**.**

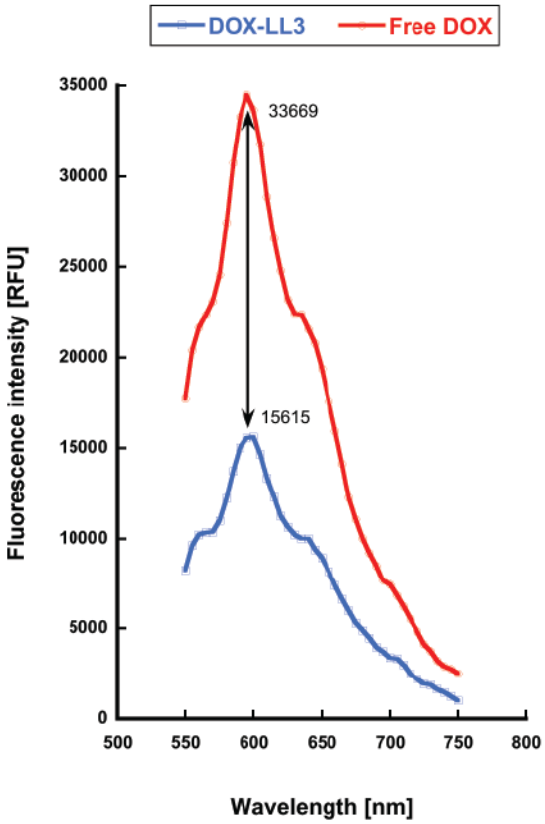

Fig. ESI 2 Verification of DOX loading.

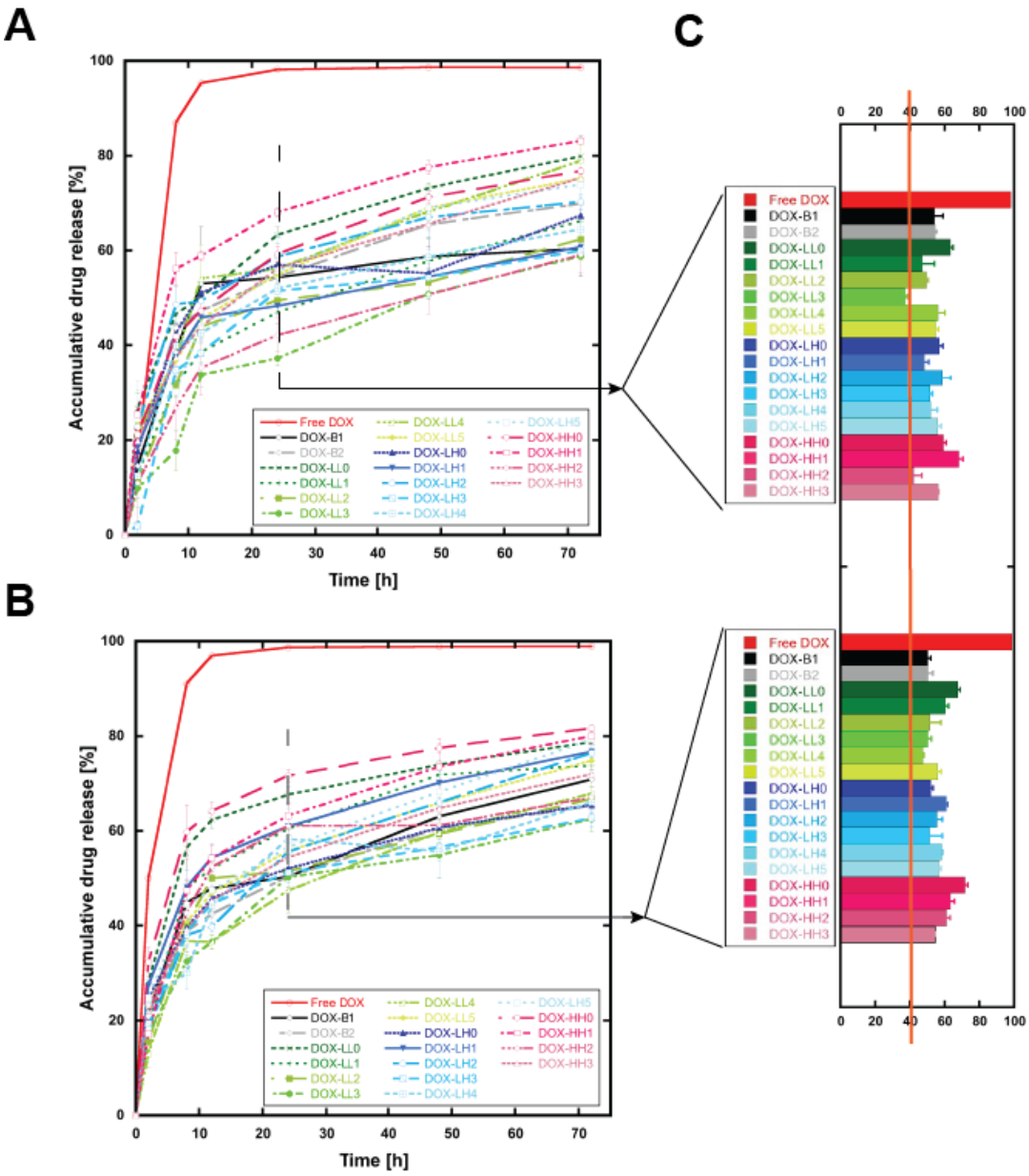

Fig. ESI 3 *In vitro* DOX release at 37°C. (A) DOX release in PBS pH 6.0. (B) DOX release in PBS pH 7.4. (C) Accumulated drug release after 24 h for all of the DOX-loaded materials at two different pHs. Results are shown as mean ± SD (n = 3). The orange line marks the level of drug released from DOX-LL3 in pH 6.0.

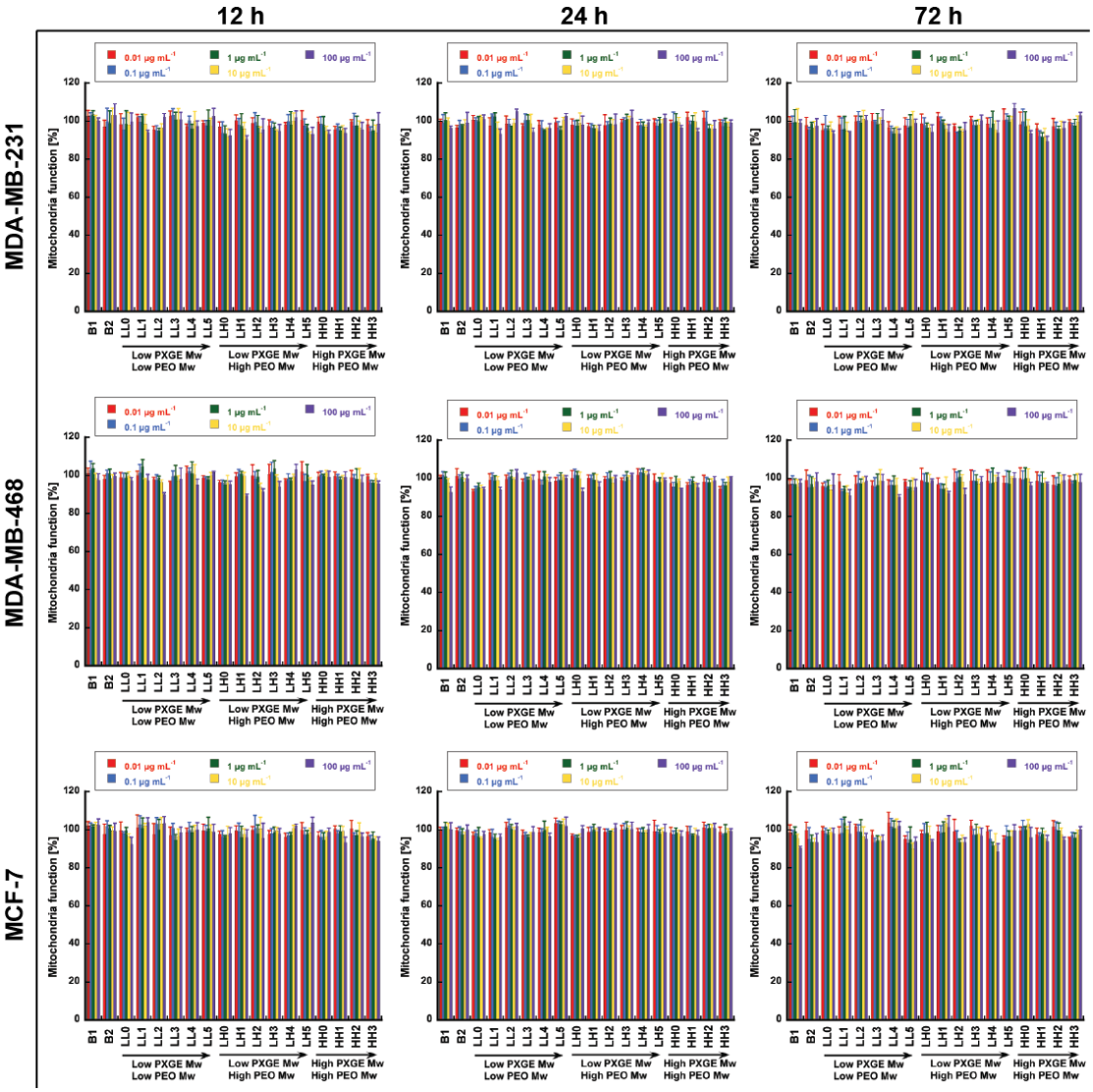

Fig. ESI 4 Evaluation of mitochondrial function (MTT) of the non-loaded polymers for 12 h (A–C), 24 h (D–F) and 72 h (G–I) incubations with breast cancer cell lines MDA-MB-231 (A, D, G), MDA-MB-468 (B, E, H) and MCF-7 (C, F, I).

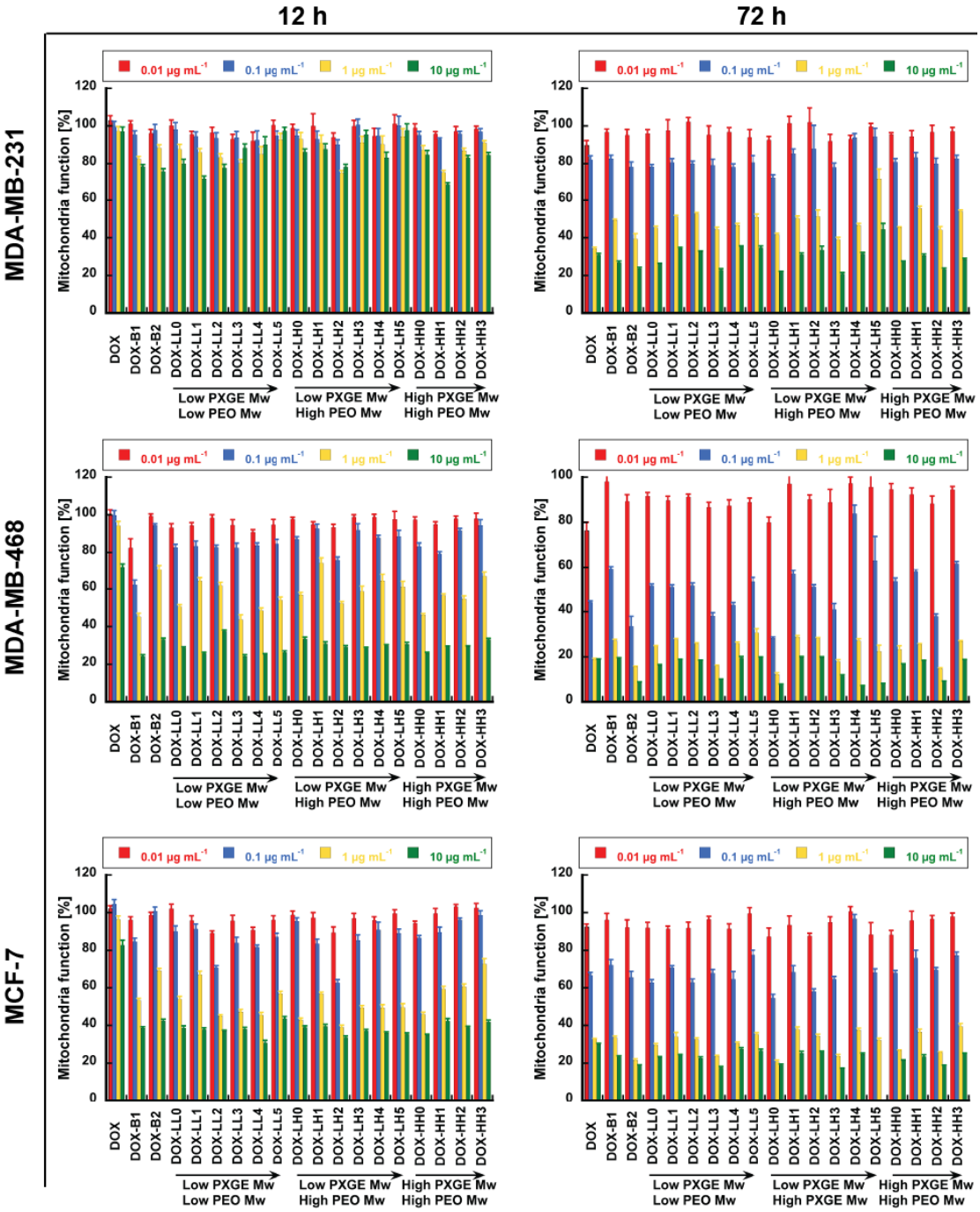

Fig. ESI 5 Impairment of mitochondrial function as a result of treatment with DOX-loaded NPs. MTT assays after incubating DOX-NPs for 12 h or 72 h with breast cancer cell lines MDA-MB-231, MDA-MB-468, and MCF-7. Results are shown as mean ± SD (n = 5).

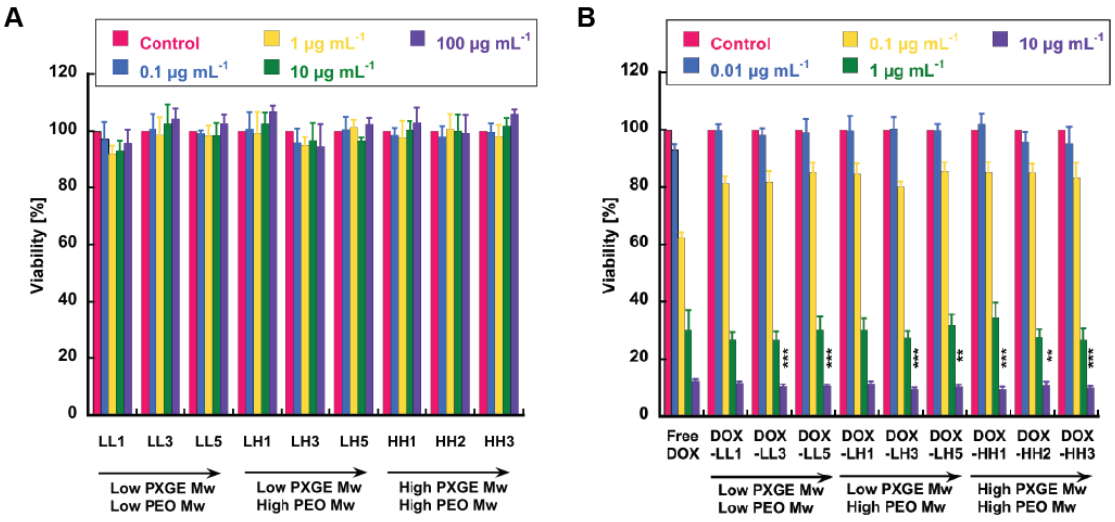

Fig. ESI 6 ATP luminance assays (viability) for MDA-MB-231 cells in a 2D model. Assays were carried out 72 h after cells were incubated with (A) neat NPs or (B) DOX-NPs. Results are shown as mean ± SD (n = 6). *p < 0.05, **p < 0.01 and ***p < 0.001 compared to control.

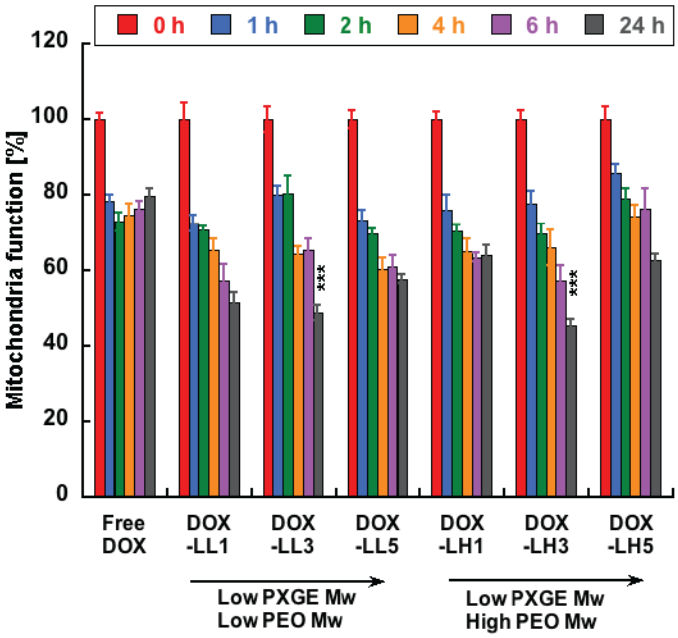

Fig. ESI 7 Drug wash-out MTT assays. MDA-MB-468 cells were treated with 1 $\mu g \cdot mL^{-1}$ DOX-NPs that were removed at the designated time points. New medium was refilled and cells were cultured until 24 h. Results are shown as mean ± SD (n = 6). ***$p < 0.001$ compared to free DOX, DOX-LL1, DOX-LL5, DOX-LH1, and DOX-LH5.

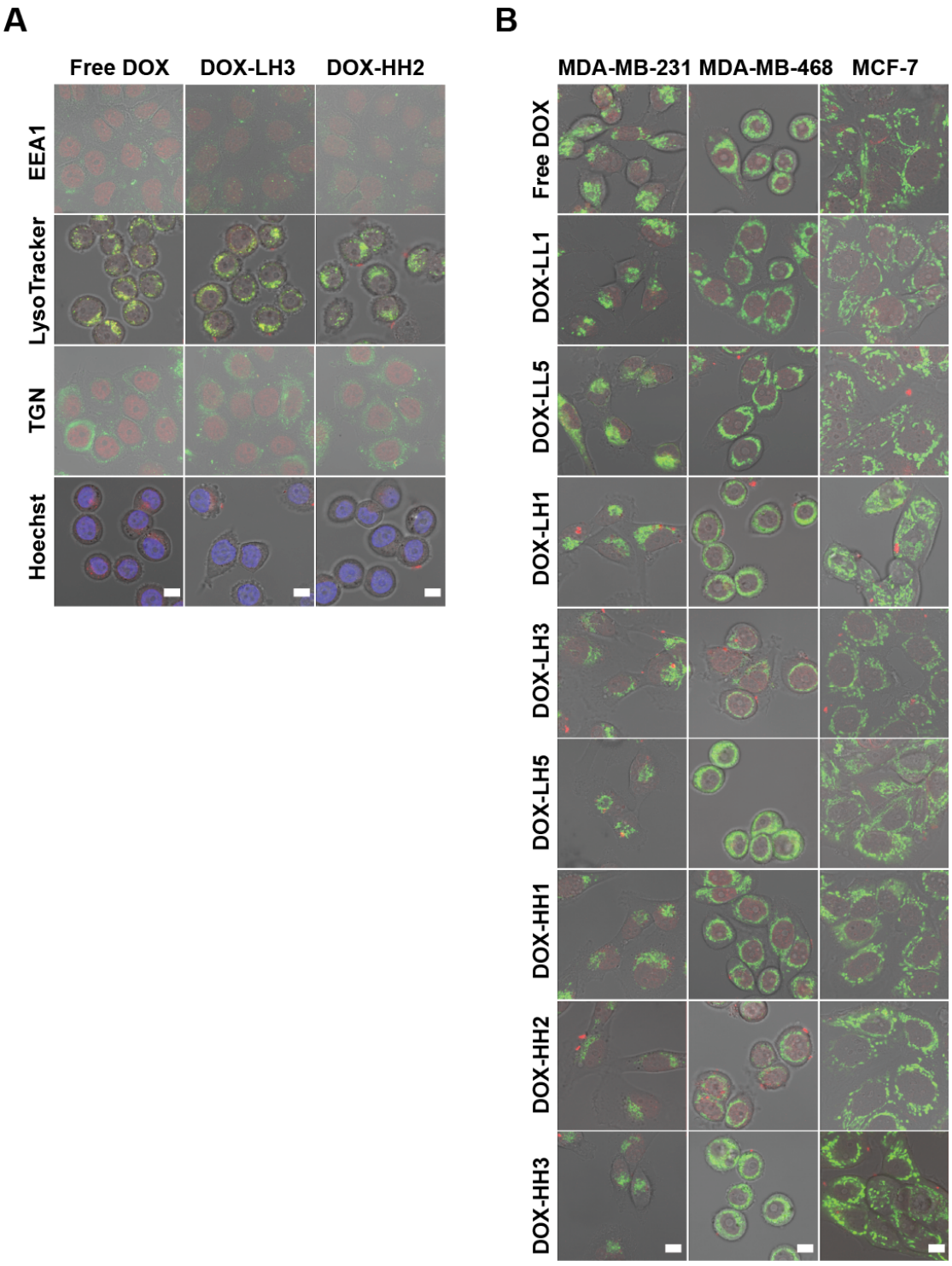

Fig. ESI 8 Confocal images forming the basis for the heat maps. **(A)** DOX-NP localization tracking in MDA-MB-468 cells. **(B)** DOX-NP co-localization in mitochondria in three breast cancer cell lines. Scale bar = 10 μm.

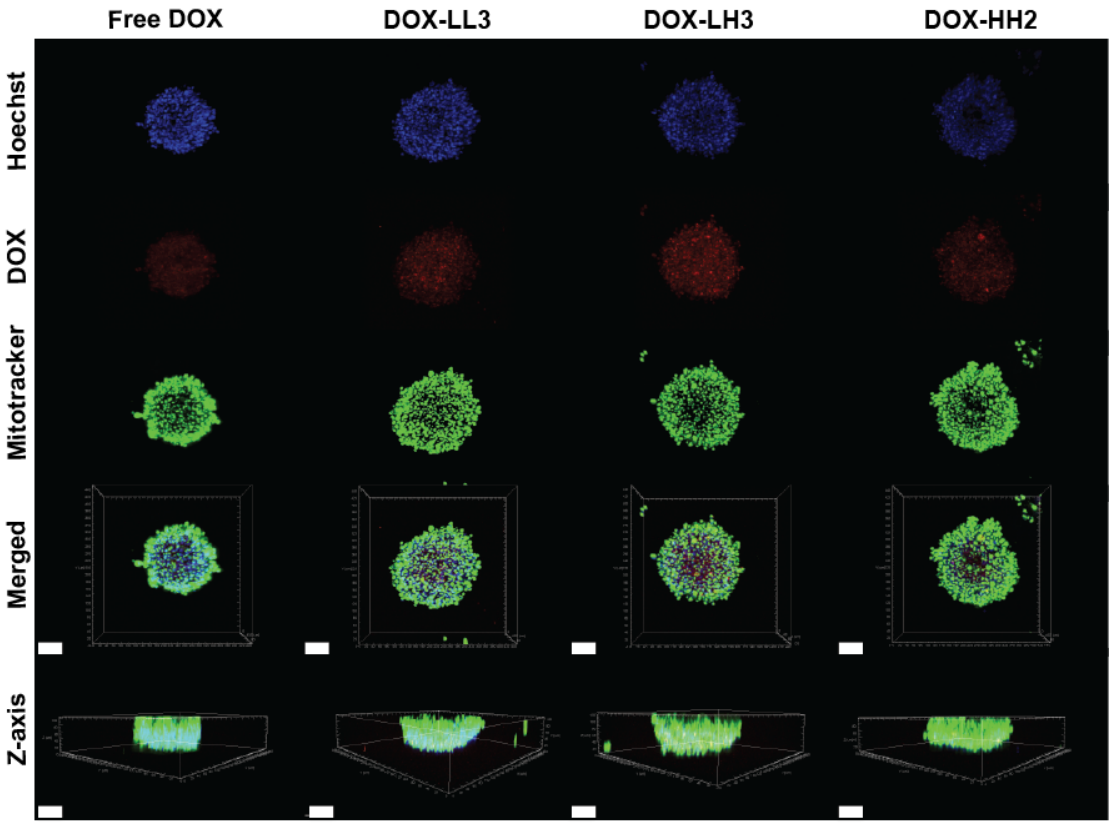

Fig. ESI 9 DOX-mitochondria co-localization study in 3D spheroids. MDA-MB-231 spheroids were treated with 2 μg·mL^{-1} DOX or DOX-NPs for 4 h. Nuclei were stained with Hoechst and mitochondria were stained with MitoTracker and represented as a false green color. Images were analyzed and reconstructed with Imars software. Scale bar = 70 μm.