Supporting Information

MMP-2-Controlled Transforming Micelles for Heterogeneic Targeting and Programmable Cancer Therapy

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Pharmacokinetics studies

For in vivo pharmacokinetic study, six-week-old mice were intravenously injected with free DOX, NF@DOX and HEKM@DOX at the same DOX dosage of 10 mg/kg of mouse body weight (n =3 for each group), respectively. After injection, blood samples were harvested at predetermined time points (0.25, 0.5, 1, 2, 4, 8, 12, 24 and 48 h). Then the plasma samples were collected immediately, repeatedly freeze-thawed and ultrasonicated for 3 min. After the plasma samples were subjected to centrifugation at 3000 rpm for 5min. Finally, the fluorescence of supernatant was examined by fluorescence spectroscopy (Ex, 480 nm; Em, 560-590 nm). Standard curves for DOX in blood were generated by the addition of free DOX at different concentrations to whole blood followed by extraction and quantification as mentioned above.

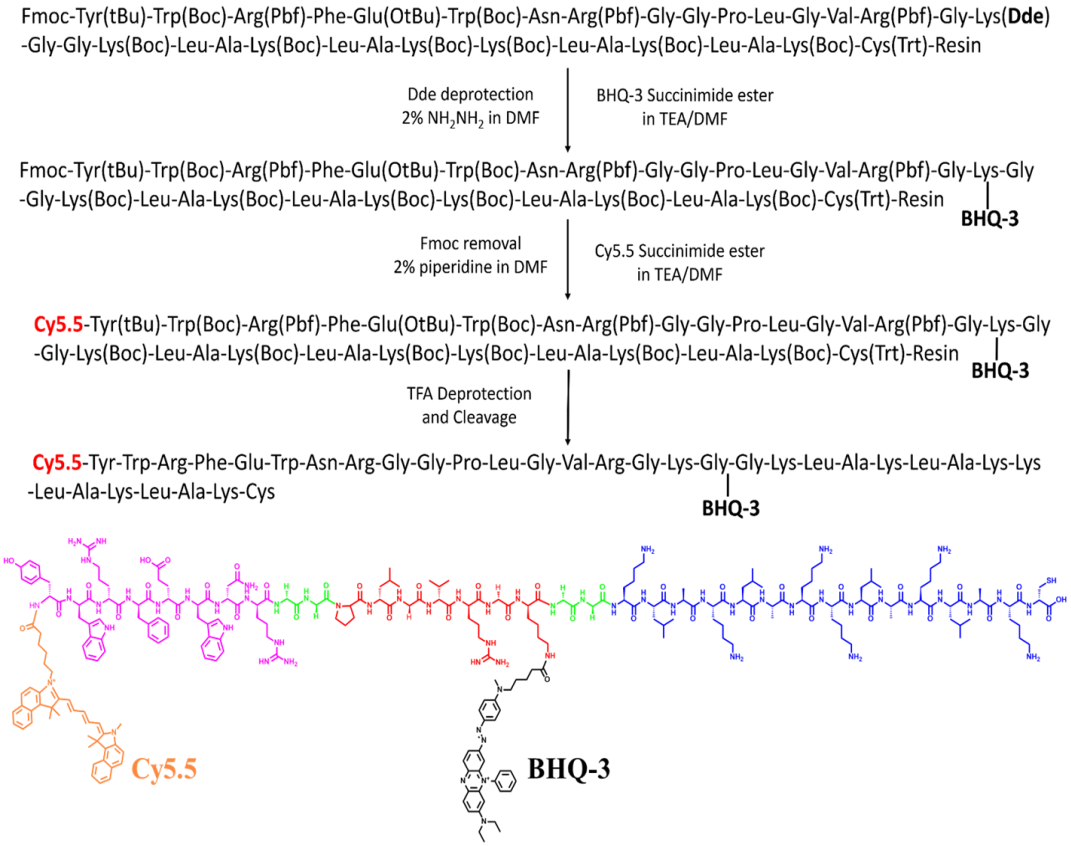
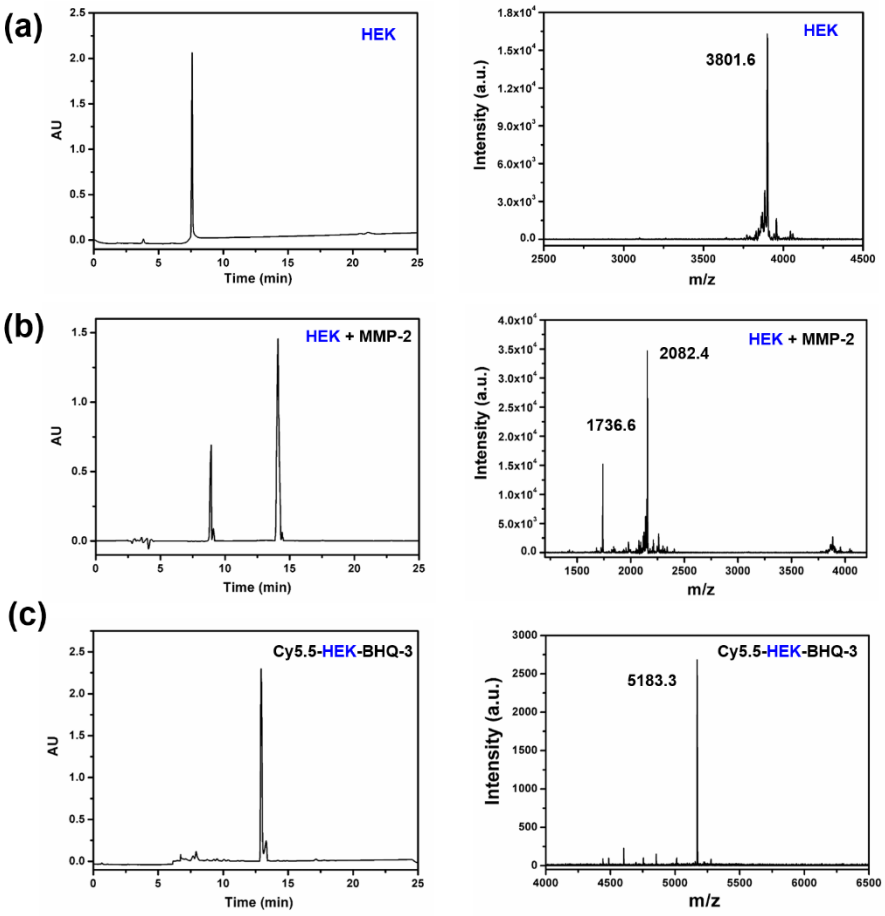


Figure S1. Synthetic scheme and chemical structure of the peptide HEK.


(a)
HEK
AU
Time (min)
HEK
3801.6
Intensity (a.u.)
m/z
(b)
HEK + MMP-2
AU
Time (min)
HEK + MMP-2
2082.4
1736.6
Intensity (a.u.)
m/z
(c)
Cy5.5-HEK-BHQ-3
AU
Time (min)
Cy5.5-HEK-BHQ-3
5183.3
Intensity (a.u.)
m/z

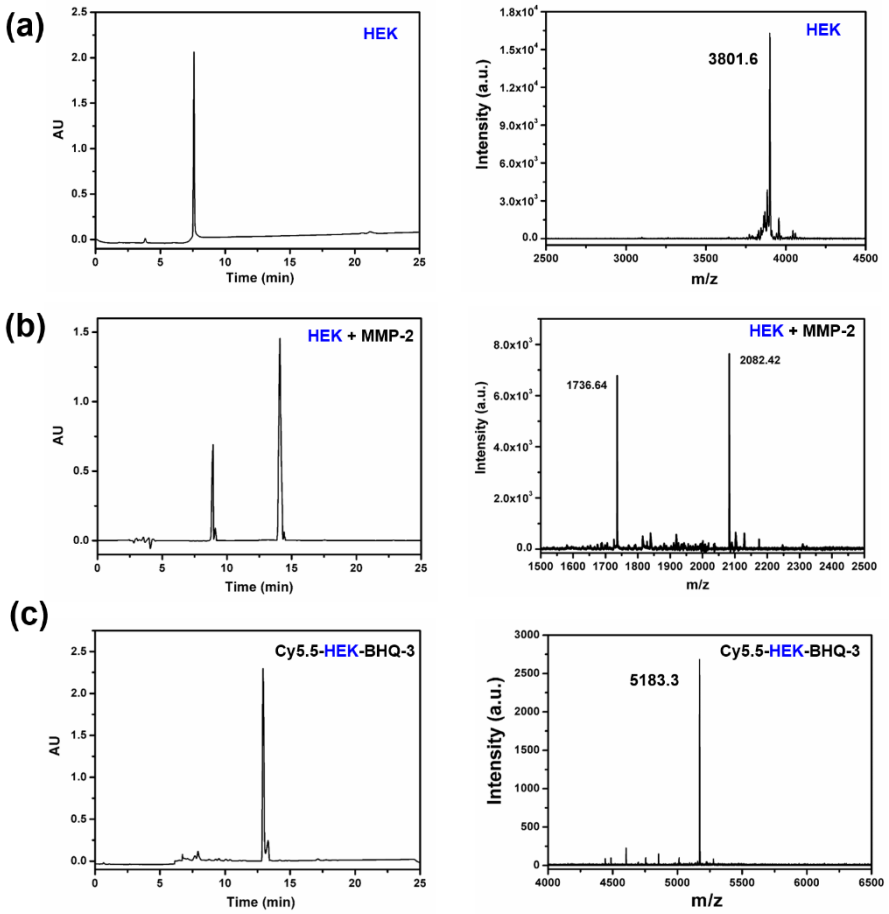


Figure S2. (a) HPLC and MALDI-TOF-MS characterization of HEK peptide. (b) MALDI-TOF-MS and HPLC monitoring of the HEK peptide after incubation with MMP-2 (50 nmol/L). (c) HPLC and MALDI-TOF-MS analysis of Cy5.5-HEK(-BHQ-3).

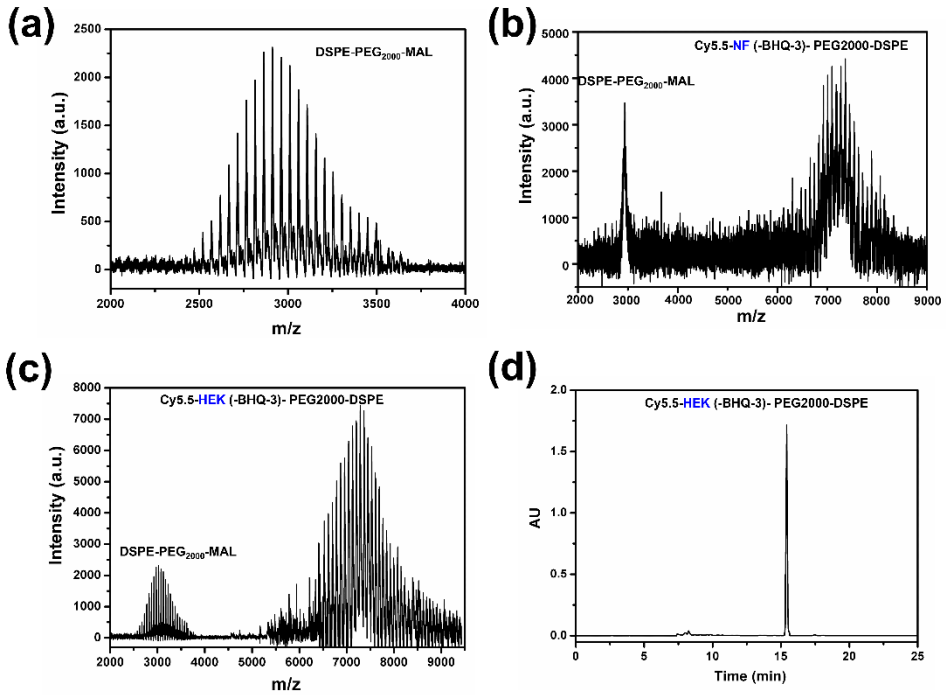


Figure S3. (a-b) MALDI-TOF-MS analysis of the conjugation of NF with DSPE-PEG2000-MAL. (c-d) MALDI-TOF-MS and HPLC analysis of the Cy5.5-HEK(-BHQ-3)-PEG2000-DSPE. α-cyano-4-hydroxycinnaminic acid (CHCA) was used as matrix.

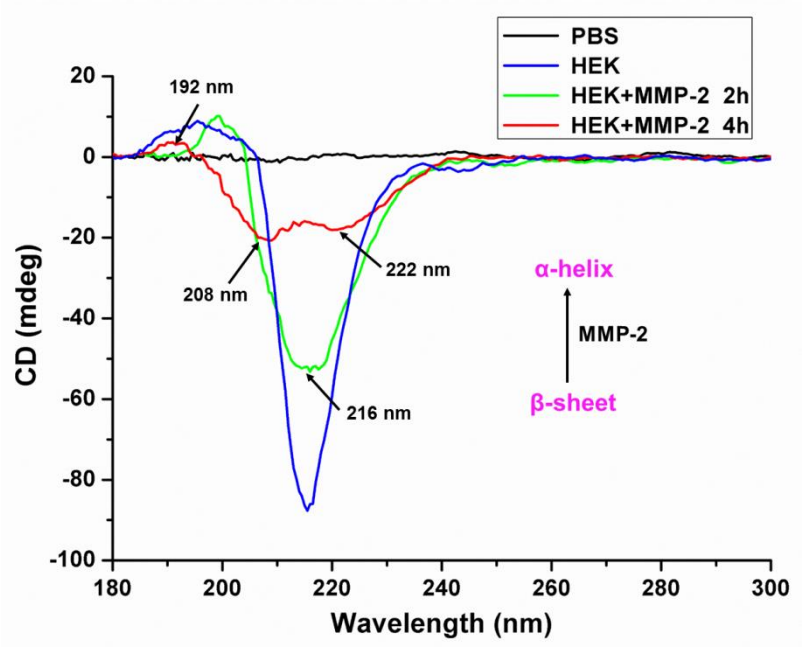


Figure S4. Circular dichroism (CD) spectra analysis of the HEK peptides (0.5 mg/mL) secondary structure transformation process in the absence/presence of MMP-2 in PBS solutions (50 nmol/L).

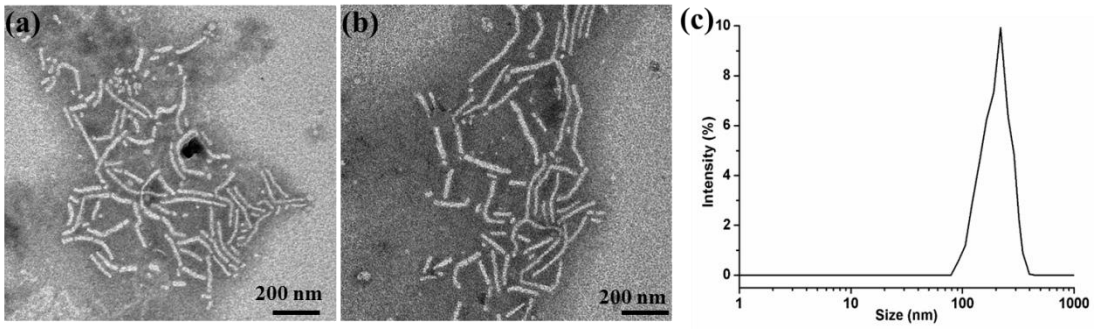


Figure S5. (a) TEM images of NFM without MMP-2. (b) TEM images of NFM incubated with MMP-2 for 4 h. (c) Hydrodynamic size of NFM incubated with MMP-2 for 4 h.

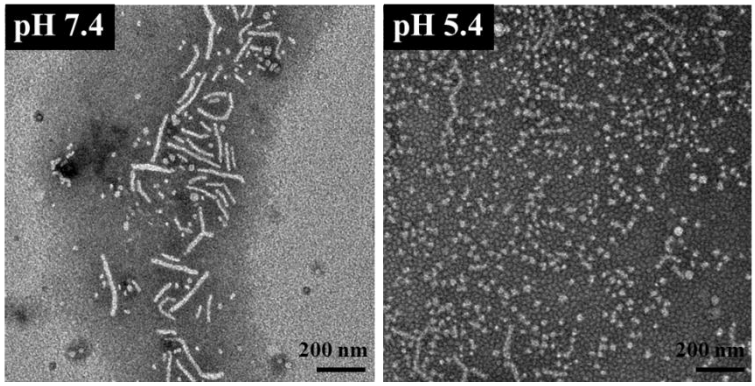


Figure S6. TEM images of HEKM morphologies after immersed in PBS buffer (pH 7.4 and pH 5.4) with 8 h. Scale bars, 200 nm.

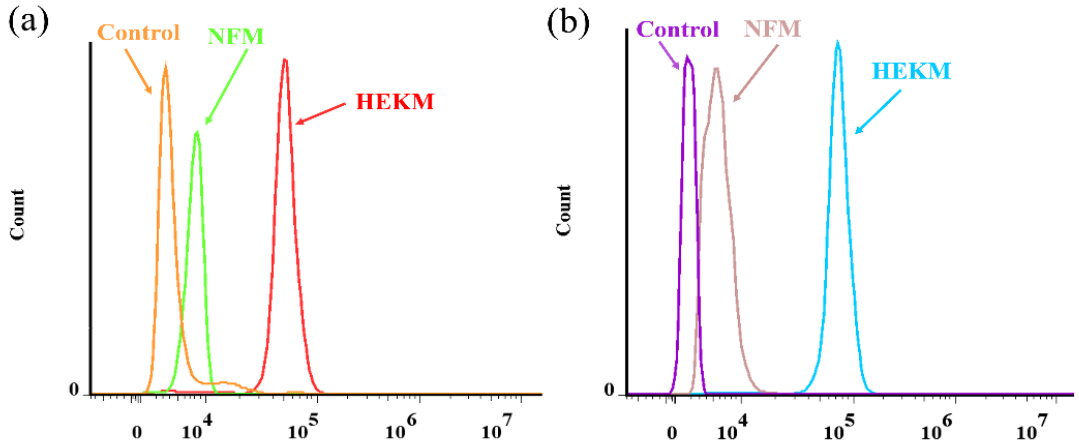


Figure S7. Flow cytometry assay of DOX uptake by (a) SKBR-3 and (b) MDA-MB-468 cell after incubated with $HEKM_{DOX}$ and NFM_{DOX}.

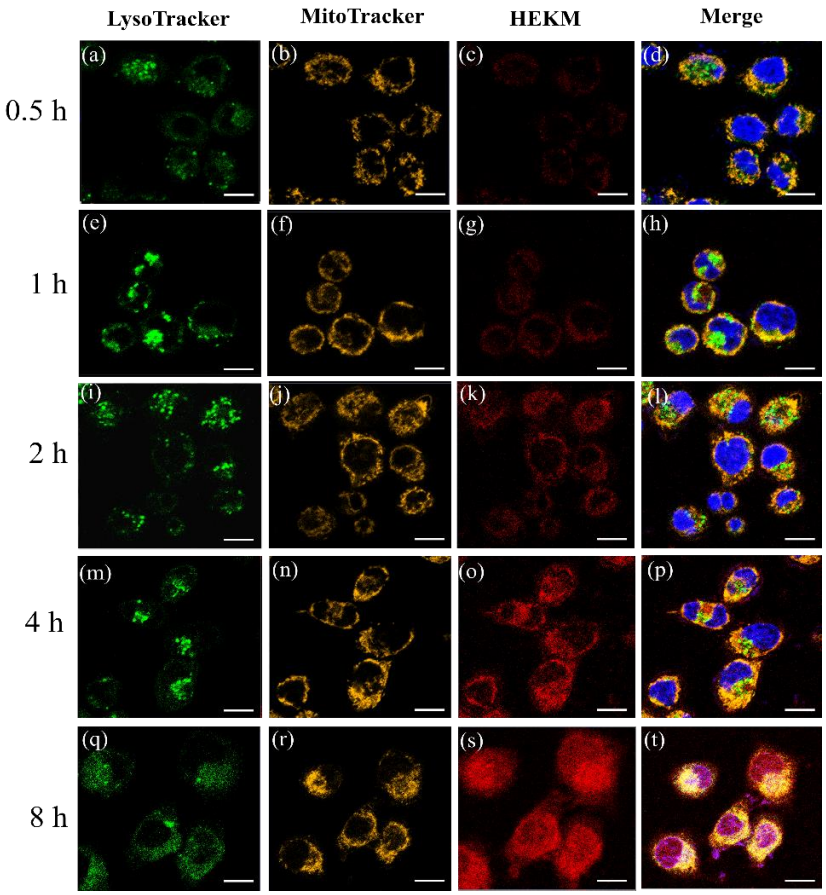
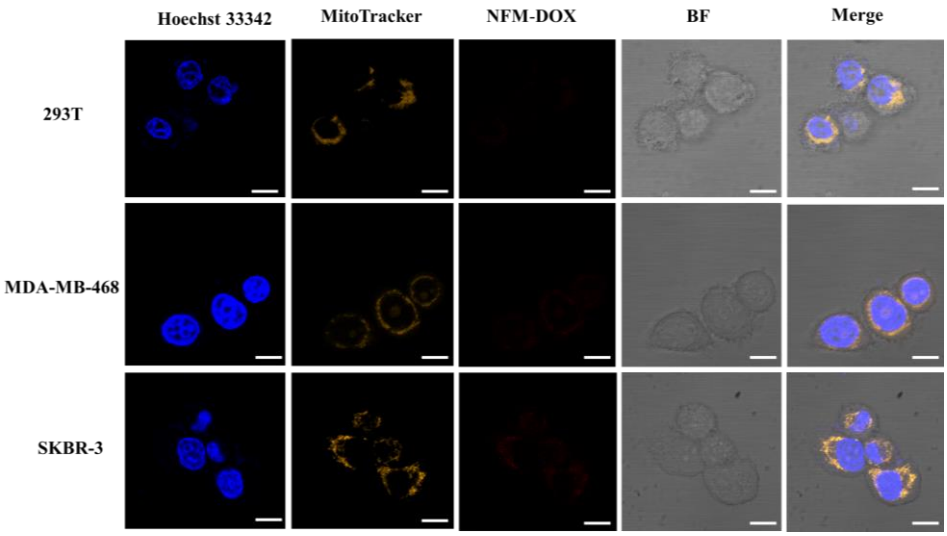


Figure S8. Confocal images of time-dependent uptake of targeting $HEKM_{DOX}$ in SKBR-3 cells. The nuclei, lysosomes and mitochondrial of the cells were stained with Hoechst 33342 (blue), LysoTracker (Green), MitoTracker (orange), respectively. Scale bar is 20 μm.



Figures S9. Confocal images of NFM-DOX in 293T, MDA-MB-468 and SKBR-3 cells. The nuclei, mitochondrial of the cells were stained with Hoechst 33342 (blue) and MitoTracker (orange), respectively. Scale bar is 20 μm.

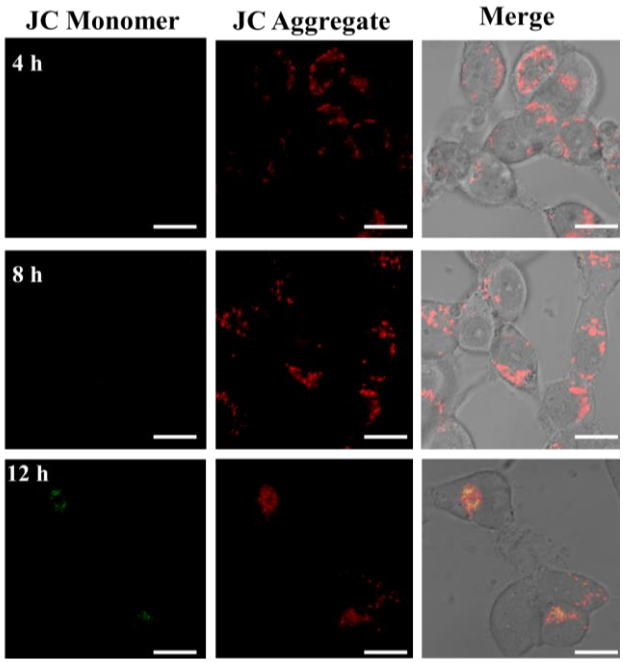


Figure S10. Mitochondrial potential changes of HEKM treated 293T cells measured by JC-1 assay. Scale bar is 20 μm.

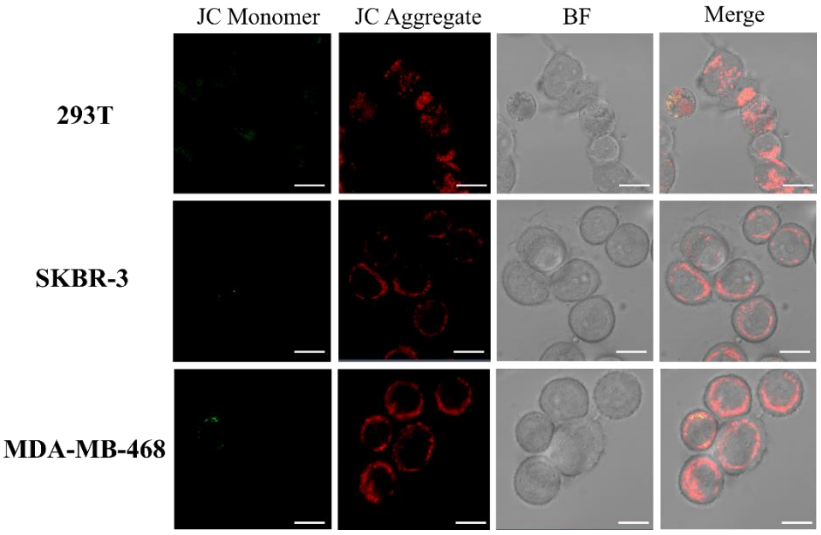


Figure S11. Mitochondrial potential changes of NFM treated SKBR-3, MDA-MB-468 and 293T cells measured by JC-1 assay. Scale bar is 20 μm.

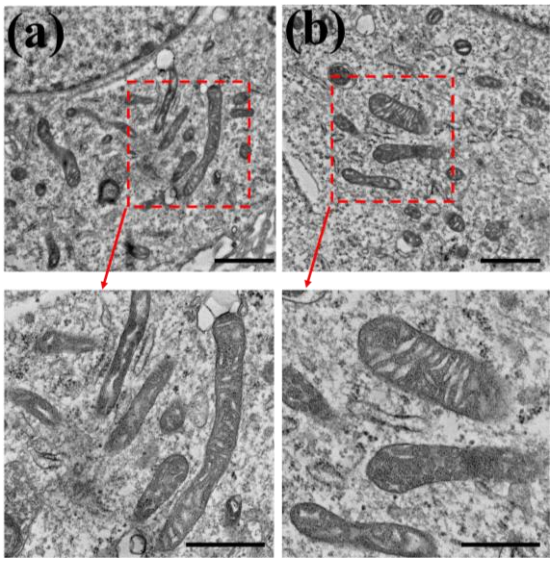


Figure S12. TEM images showing representative mitochondrial morphologies of SKBR-3 cells treated by NFM for 0 h and 12 h, respectively. Scale bar is 500 nm. Magnified images of the areas are shown arrowheads below each image, respectively. Scale bar is 200 nm.

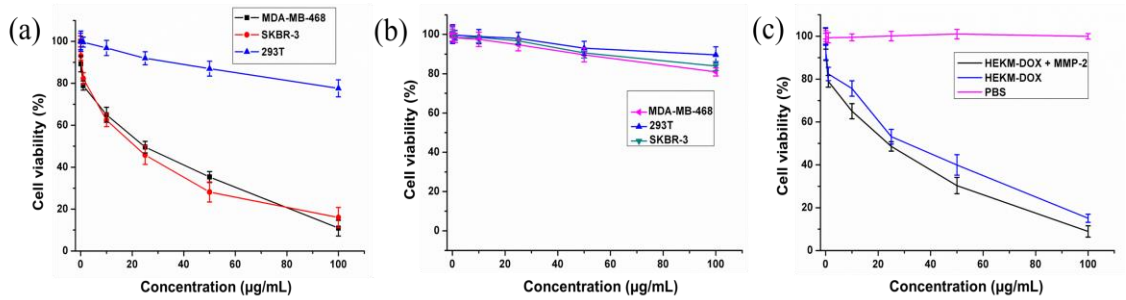


Figure S13. Cell viability of SKBR-3, MDA-MB-468 and 293T cells after incubation with (a) HEKMs and (b) NFMs at varied concentrations for 48 h. (c) The cytotoxicity of SKBR-3 with rod-to-sphere transfoming of the $HEKM_{DOX}$ in the presence of MMP-2.

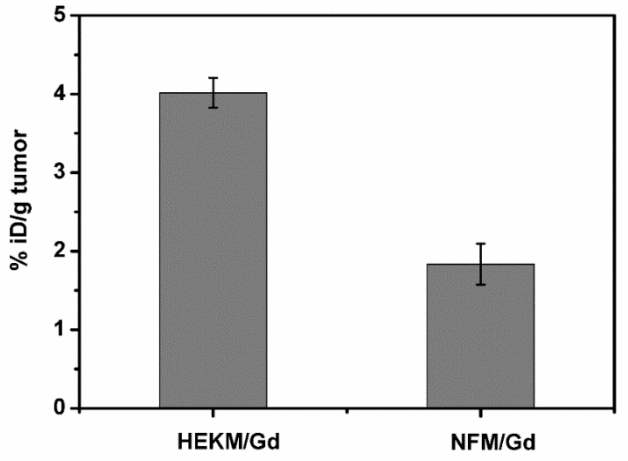


Figure S14. Tumor accumulation of Gd^{3+} at 8 h after injection of HEKM/Gd and NFM/Gd.

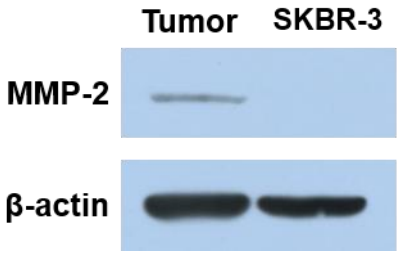


Figure S15. Western blot assay of the SKBR-3 cell and tumor tissue lysate towards the MMP-2 antibody. β-actin was used as a loading control.

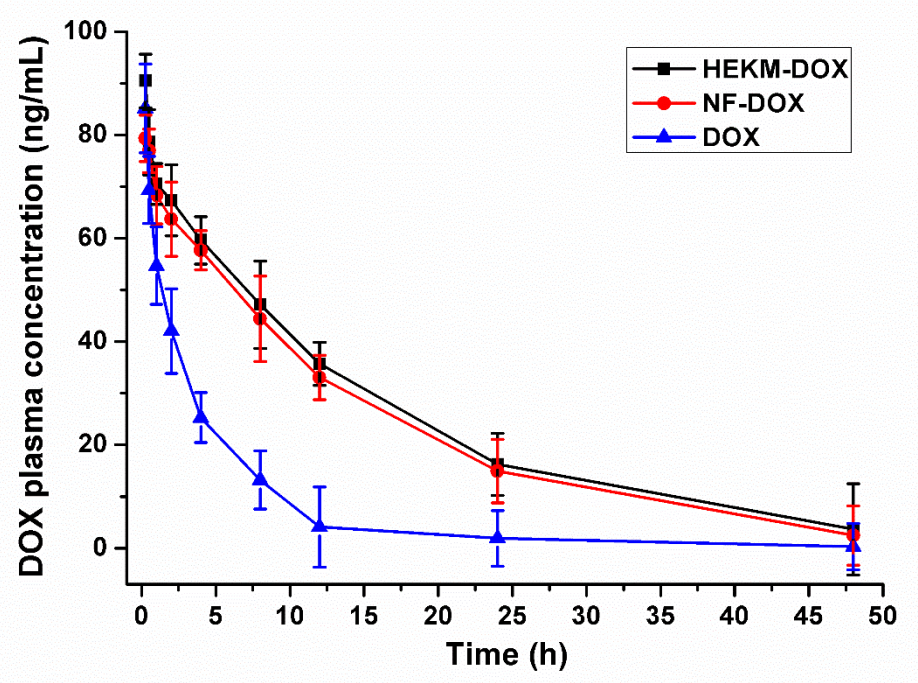


Figure S16. Pharmacokinetics profiles of DOX after intravenous injection of free DOX, NF@DOX and HEKM@DOX at 10 mg/kg dose in mice (mean ± SD, n = 3).

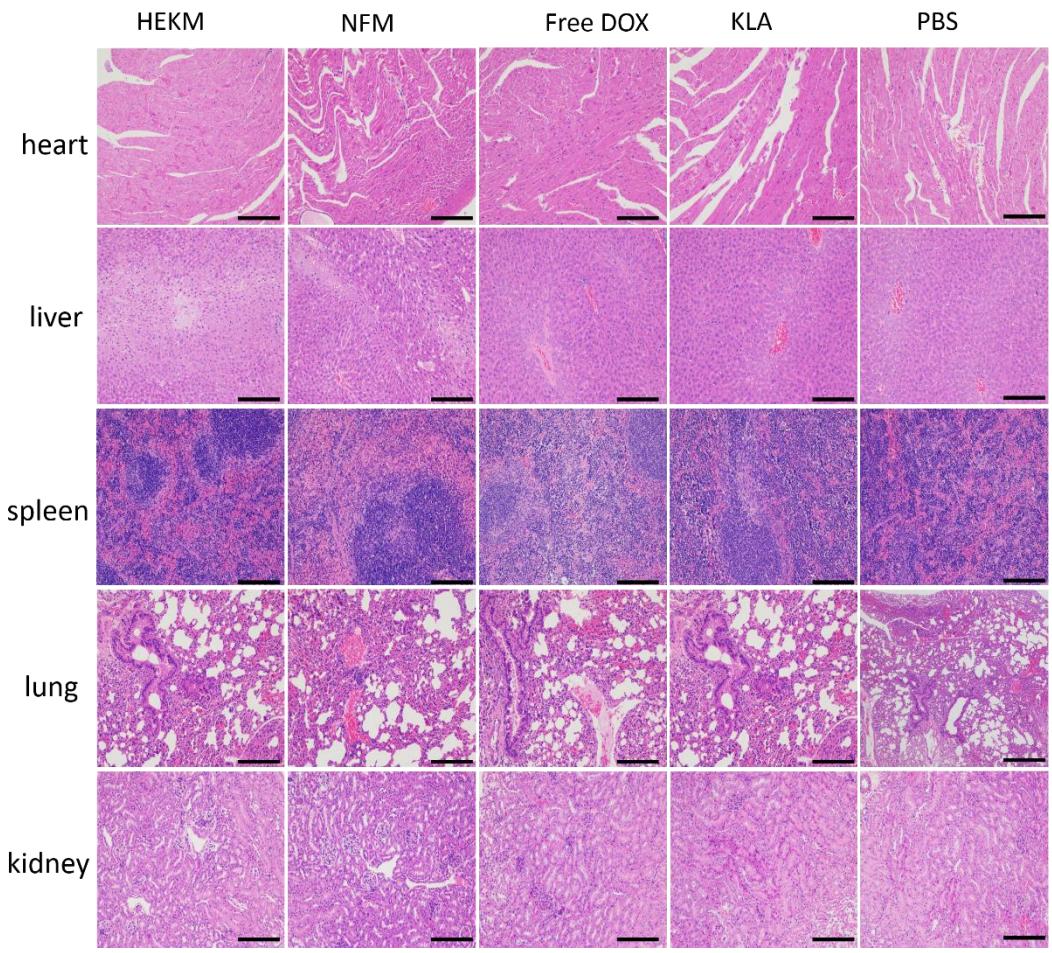


Figure S17. H&E staining analysis of heart, liver, spleen, lung, kidney of SKBR-3 tumor bearing xenograft mice after treatment with PBS, free DOX, $HEKM_{DOX}$, NFM_{DOX} and KLA (scale bar is 100 μm for all images).