Supporting Figures

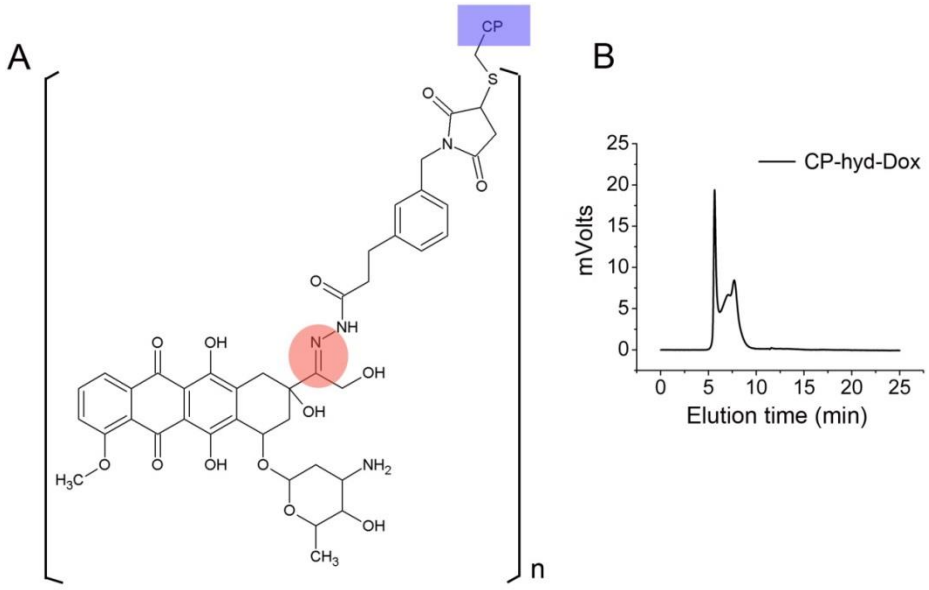

Fig.S1. (A) Structure of the CP-hyd-Dox conjugate (hyd: hydrazone linker). Dox is conjugated to CP (highlighted in blue) by a BMPH crosslinker and forms an acid-labile hydrazone bond (highlighted in red) between Dox and CP. (B) Purity verification of the CP-hyd-Dox conjugate measured by HPLC equipped with a size exclusion column. The elution buffer is 30% acetonitrile and 70% PBS. The elution times of the CP-hyd-Dox conjugate and free Dox start at ~5.5 min and ~13.7 min, respectively.

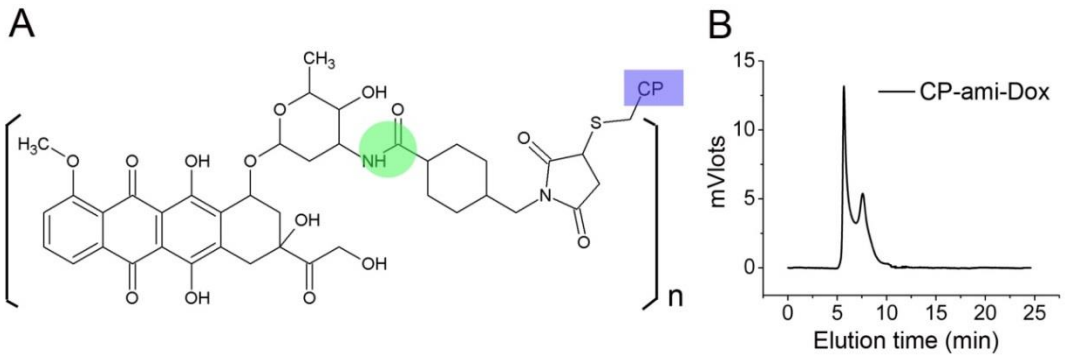

Fig.S2. (A) Structure of the CP-ami-Dox conjugate (ami: amide linker). Dox-SMCC is conjugated to CP (highlighted in blue) and a pH-insensitive amide bond (highlighted in green) forms between Dox and CP. (B) Purity verification of the CP-ami-Dox conjugate measured by HPLC equipped with a size exclusion column. The elution buffer is 30% acetonitrile and 70% PBS. The elution times of the CP-ami-Dox conjugate and free Dox start at ~5.5 min and ~13.7 min, respectively.

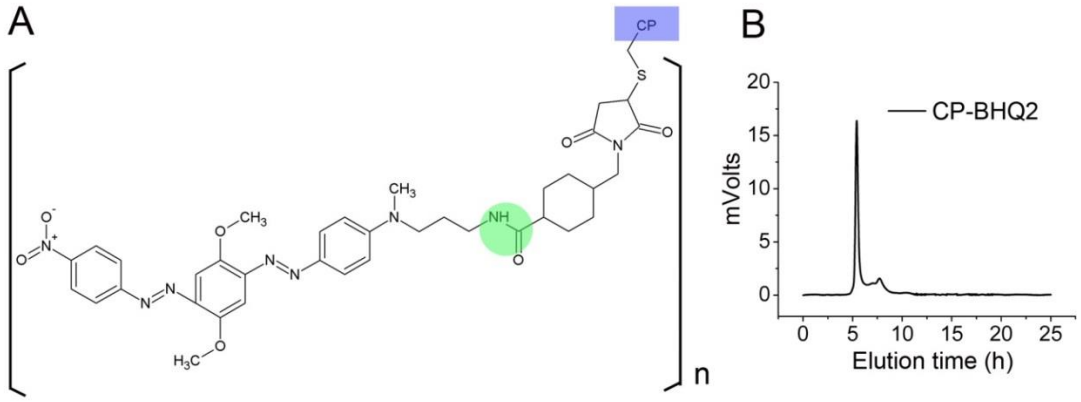

Fig.S3. (A) Structure of the CP-BHQ2 conjugate. BHQ2 is conjugated to CP (highlighted in blue) by a sulfo-SMCC crosslinker and forms a pH-insensitive amide linker (highlighted in green) between BHQ2 and CP. (B) Purity verification of the CP-BHQ2 conjugate measured by HPLC equipped with a size exclusion column. The elution buffer is 30% acetonitrile and 70% PBS. The elution times of the CP-BHQ2 conjugate and free BHQ2 start at ~5.5 min and ~12 min, respectively.

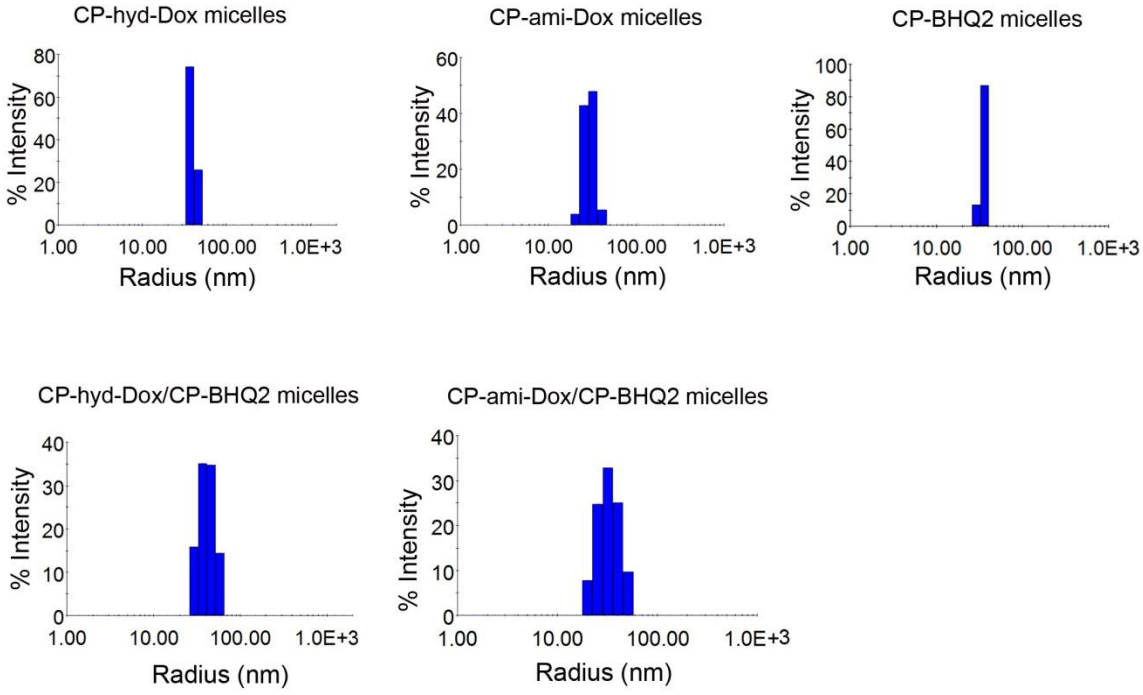

Fig.S4. Hydrodynamic radii of CP-hyd-Dox micelles, CP-ami-Dox micelles, CP-BHQ2 micelles, CP-hyd-Dox/CP-BHQ2 mixed micelles and CP-ami-Dox/CP-BHQ2 mixed micelles measured by dynamic light scattering. (hyd: hydrazone linker, ami: amide linker). All of them self-assemble to ~40 nm radius micelles in aqueous solution, respectively.

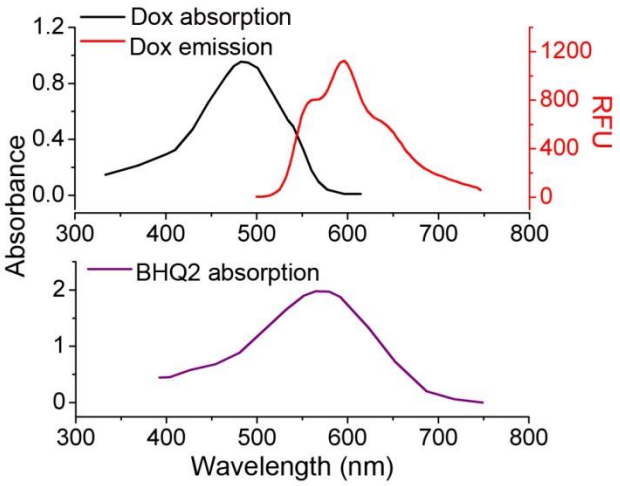

Fig.S5. The absorption and fluorescence emission spectra of Dox and the absorption spectrum of BHQ2. The absorption peak of Dox is 490 nm and its emission peak is 590 nm. The absorption spectrum of BHQ2 overlaps the fluorescence emission spectrum of Dox from 530 nm to 670 nm.

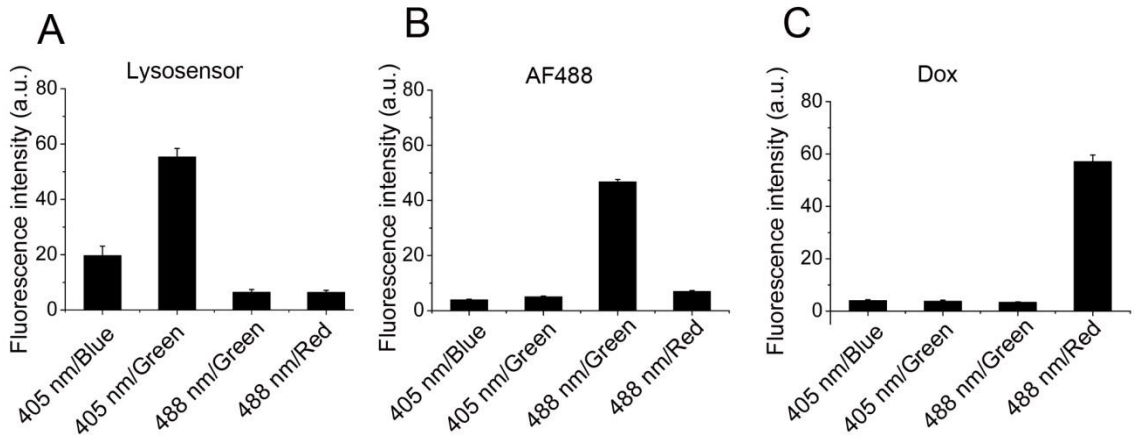

Fig.S6. The fluorescence intensities of Lysosensor, AF488 and Dox in four channels with different excitation and emission measured on a spinning disk confocal microscope. Living cells stained with Lysosensor, AF488, and Dox, respectively, were measured in four channels, which were 405 nm/blue filter (447 ± 30 nm), 405 nm/green filter (525 ± 15 nm), 488 nm/green filter and 488 nm/red filter (607 ± 18 nm). Blue and green filters excited at 405 nm measured the two emission peaks of Lysosensor. Green and red filters excited at 488 nm measured the fluorescence of AF488 and Dox, respectively. There is no fluorescence bleed-through among these three fluorophores in four channels.

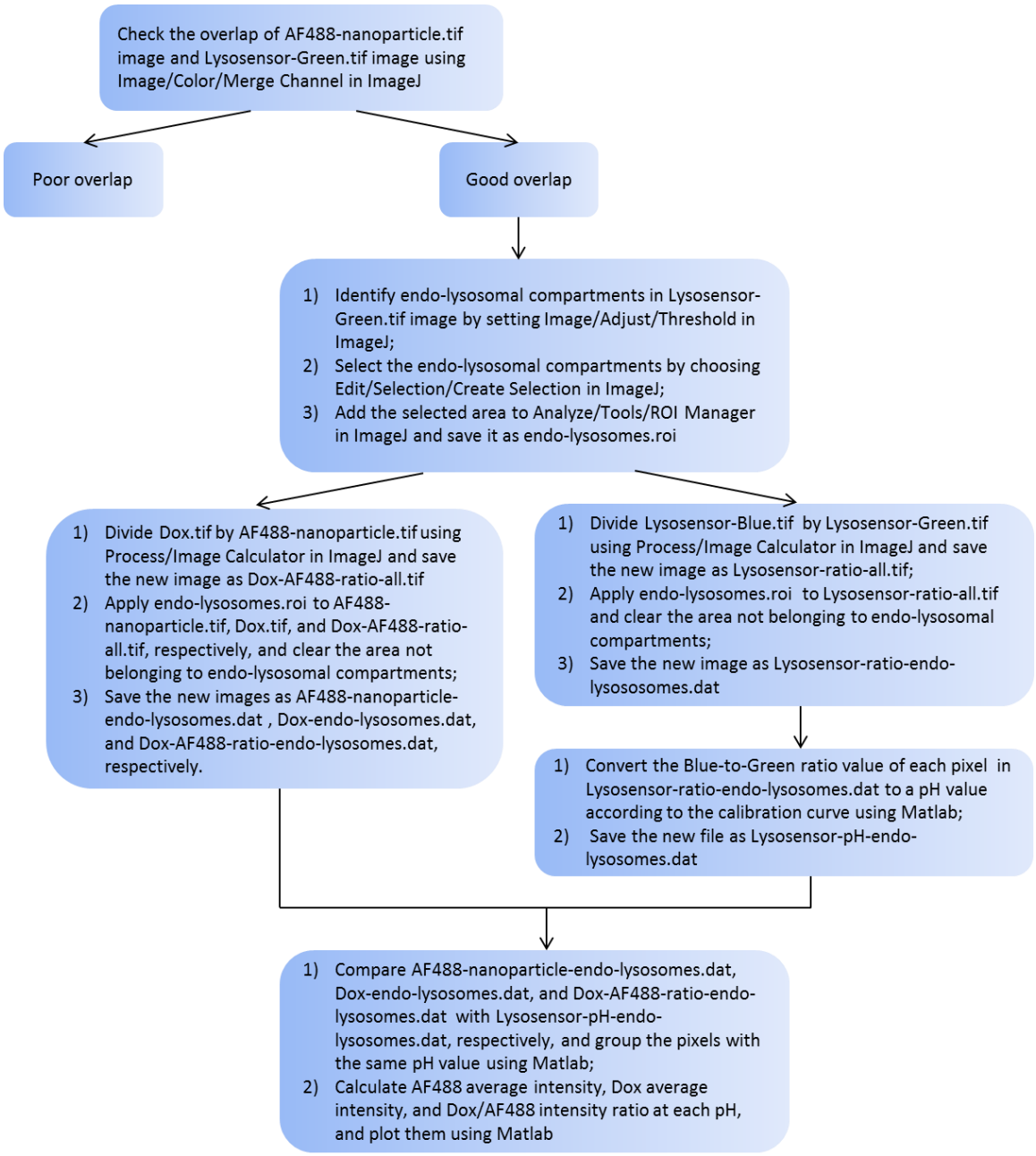

Fig.S7. Flowchart of image analysis process for ratiometric fluorescence imaging. Four fluorescence images, including the green fluorescence image of AF488-labeled nanoparticles (AF488-nanoparticle.tif image), the red fluorescence image of Dox drugs (Dox.tif image) as well as the blue and green fluorescence images of Lysosensor (Lysosensor-Blue.tif image and Lysosensor-Green.tif image) were acquired during ratiometric fluorescence imaging. The calibration curve of Lysosensor's Blue-to-Green ratio against pH was obtained in the calibration experiment. Image analysis was performed with ImageJ and Matlab software.

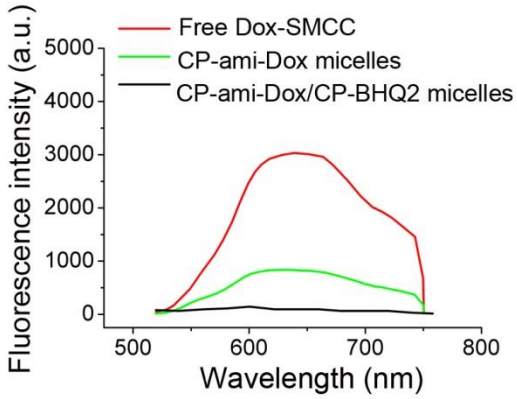

Fig.S8. The Dox fluorescence emission spectra of ~5.3 μM (Dox concentration) free Dox-SMCC, CP-ami-Dox micelles and CP-ami-Dox/CP-BHQ2 mixed micelles (ami: amide linker) measured by a fluorospectrometer excited at 470 nm. The CP-ami-Dox conjugate was synthesized by reacting Dox-SMCC with CP. Compared with free Dox-SMCC, Dox fluorescence is partly quenched in CP-ami-Dox micelles and completely quenched in CP-ami-Dox/CP-BHQ2 mixed micelles.

Supporting Code

```
%%%%%%%%%%% plot control %%%%%%%%%%%
%%% FONT & SIZE %%%
xyLabelFontSize=26;
tickNumberFontSize=22;
legendFontSize=20;
tickLineWidth=1.5;
linesLineWidth=1.5;
pointSize=4;
glbFontName=['Times'];
%%%%%%%%%%% plot control end %%%%%%

% load multi-files of Lysosensor pH-endo-lysosomes
cali(:,:,1)=load('Lysosensor-pH-endo-lysosomes-1.dat');
cali(:,:,2)=load('Lysosensor-pH-endo-lysosomes-2.dat');
cali(:,:,3)=load('Lysosensor-pH-endo-lysosomes-3.dat');
cali(:,:,4)=load('Lysosensor-pH-endo-lysosomes-4.dat');
cali(:,:,5)=load('Lysosensor-pH-endo-lysosomes-5.dat');
cali(:,:,6)=load('Lysosensor-pH-endo-lysosomes-6.dat');
cali(:,:,7)=load('Lysosensor-pH-endo-lysosomes-7.dat');
cali(:,:,8)=load('Lysosensor-pH-endo-lysosomes-8.dat');
cali(:,:,9)=load('Lysosensor-pH-endo-lysosomes-9.dat');
cali(:,:,10)=load('Lysosensor-pH-endo-lysosomes-10.dat');


% load multi-files of nanoparticle-endo-lysosomes
ratio(:,:,1)=load('AF488-nanoparticle-endo-lysosomes-1.dat');
ratio(:,:,2)=load('AF488-nanoparticle-endo-lysosomes-2.dat');
ratio(:,:,3)=load('AF488-nanoparticle-endo-lysosomes-3.dat');
ratio(:,:,4)=load('AF488-nanoparticle-endo-lysosomes-4.dat');
ratio(:,:,5)=load('AF488-nanoparticle-endo-lysosomes-5.dat');
ratio(:,:,6)=load('AF488-nanoparticle-endo-lysosomes-6.dat');
ratio(:,:,7)=load('AF488-nanoparticle-endo-lysosomes-7.dat');
ratio(:,:,8)=load('AF488-nanoparticle-endo-lysosomes-8.dat');
ratio(:,:,9)=load('AF488-nanoparticle-endo-lysosomes-9.dat');
ratio(:,:,10)=load('AF488-nanoparticle-endo-lysosomes-10.dat');


[ratiosize1,ratiosize2,ratiosize3]=size(ratio);
% calculate ph using cali
ph(:,:,:)=(cali(:,:,:)+0.87)/0.25;

% round the ph to 0.1
ph=roundn(ph,-1);

% set the ph range
phrange=0:0.1:14;

% get the size of ph range
[temp,phrangesize]=size(phrange);

% do the loop, i:j=count:k
for l=1:ratiosize3
   for k=1:phrangesize % if k=1, ph=3.0, if k=2, ph=3.1, and so on
      count=1;    % count number, how many pixels for each ph value
      for i=1:512
         for j=1:512

            if ph(i,j,l)==phrange(k)  % check to store ratio value at certain ph
               ph_ratio(count,k,l)=ratio(i,j,l);
               count=count+1;
```

```
            end

          end
        end
        if count==1
          ph_ratio(:,k,l)=0; % we need to do this to prevent if we do not have ratio at some ph
        end
    end
end

[phratiosize1,phratiosize2,phratiosize3]=size(ph_ratio);

% merge multi files ratio into one
for k=1:phratiosize3
    iorg=1;
    for i=phratiosize1*(k-1)+1:phratiosize1*k
        ph_ratio_multifiles(i,:)=ph_ratio(iorg,:,k);
        iorg=iorg+1;
    end
end


% plot Average AF488 intensity (ratio_mean)vs pH
h1=figure('visible','off');  % do not show the fig
hold on;
for i=1:phratiosize2
    ph_ratio_mean_multifiles(i)=sum(ph_ratio_multifiles(:,i))./sum(ph_ratio_multifiles(:,i)~=0);
end

% delete 0 for the plot
phrange_mean=phrange;
locate0=find(isnan(ph_ratio_mean_multifiles));
ph_ratio_mean_multifiles(locate0)=[];
phrange_mean(locate0)=[];

plot(phrange_mean(:),ph_ratio_mean_multifiles(:),'ro','MarkerFaceColor','r');
set (gca,'LineWidth',tickLineWidth,'FontName',glbFontName,'FontSize',tickNumberFontSize); % set line width and font
xlabel ('pH');
ylabel ('Average AF488 Intensity');
set (gca,'XTick',[3:0.5:8])
set (gca,'XLim',[3 8])
hold off;
print (gcf,'Average AF488 intensity-pH','-dtiff','-r300'); % save the figure to a file
close (h1);  % close the fig

% write to dat files
M2f=fopen('Average AF488 intensity-pH.dat','w');
ovarM(1,:)=phrange_mean(:);
ovarM(2,:)=ph_ratio_mean_multifiles(:);
fprintf(M2f,'%f %f\n',ovarM);
fclose(M2f);
```