**Supplementary Figure legend**

**Supplementary Figure 1** CryoEM images of doxorubicin-loaded liposomes and leukosomes. CryoEM images reveal the typical coffee-bean shape for both nanovesicles. Scale bar 100 nm.

**Supplementary Figure 2** Release profile of doxorubicin from leukosomes at different pH conditions. Release experiments revealed a faster release of doxorubicin from leukosomes when incubated in an acidic (pH 6.5) environment compared to a physiologic (pH 7.4) one. Experiments are conducted in 50% FBS to mimic physiologic conditions.

**Supplementary Figure 3** Physical stability of doxorubicin-loaded leukosomes in simulated serum. Leuko DOX were incubated in a mixture of FBS:PBS (50:50 v/v) to mimic in vivo conditions. Average size and zeta potential (surface charge) were evaluated up to 19h of incubation.

**Supplementary Figure 4** Organ biodistribution of leukosomes. Organ biodistribution of liposomes and leukosomes after 1, 6 and 24 h from i.v. injection. Mice (n=5 for each group) received 2 mg of rhodamine-labeled liposomes and leukosomes.

**Supplementary Figure 5** Tumor accumulation of liposomes and leukosomes. Tumor accumulation of liposomes and leukosomes after 1, 6 and 24 h from i.v. injection was evaluated by spectrofluorometer analysis. Mice (n=5 for each group) received 2 mg of rhodamine-labeled liposomes and leukosomes. At all the time points, leukosomes showed higher accumulation. **p<0.01.

**Supplementary Figure 6** Tumor blood vessel distribution of co-injected liposomes and leukosomes in a breast cancer animal model. Leukosomes (red) were administered 4 min later than control liposomes (green) (administered at t = 0 min) and images were collected using IVM immediately following the injection. Both liposomes and leukosomes tend to accumulate towards the vessel wall. Liposomes then fill the whole vessel while leukosomes keep a preferential and prolonged distribution towards the vessel walls showing a persistent higher fluorescence. Scale bar = 200 μm.
Supplementary Figure 2

The figure shows the release of doxorubicin over time (in hours) under different conditions. The x-axis represents time (in hours) ranging from 0 to 48, and the y-axis represents the percentage of doxorubicin release ranging from 0 to 100.

Two conditions are depicted:
- **FBS 50% pH 6.6**
  - Solid line with square markers
- **FBS 50% pH 7.4**
  - Dashed line with triangle markers
Supplementary Figure 5

![Bar chart showing % ID/g organ at 1 h, 6 h, and 24 h with Liposome and Leukosome categories.](image-url)
Supplementary Figure 6

A (t = 0 min – Liposome injection) (t = 4 min – Leukosome injection)

B

(t = 1 min)

(t = 2 min)

(t = 3 min)

(t = 5 min)

(t = 6 min)

(t = 7 min)

Liposome: GREEN

Leukosome: RED