SUPPLEMENTARY INFORMATION

Biomimetic nanoparticles with enhanced affinity towards activated endothelium as a versatile tool for theranostic drug delivery

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SUPPLEMENTARY FIGURES

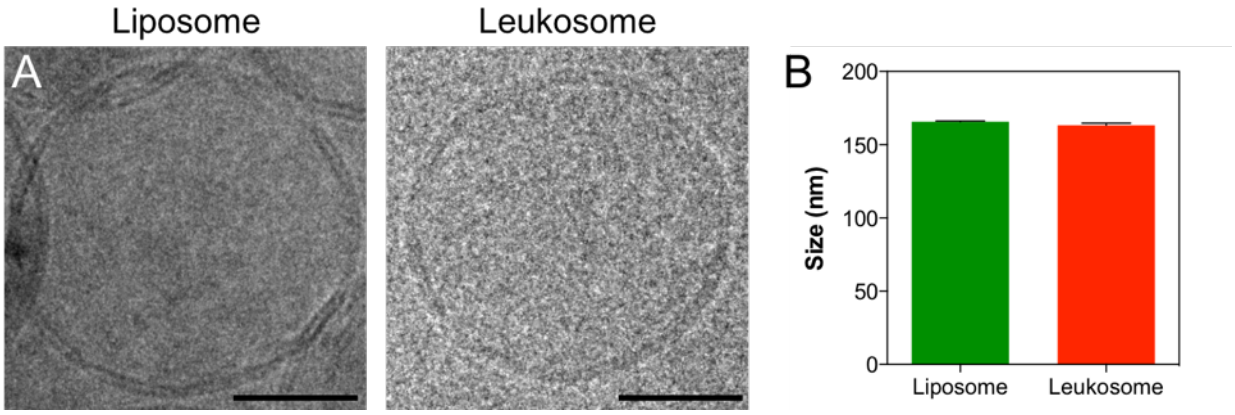

Figure S1. Cyro-TEM and size of NP. A) Representative cyro-TEM images of liposomes and leukosomes. Scale bar = 50nm. B) Size results from dynamic light scattering comparing liposomes and leukosomes.

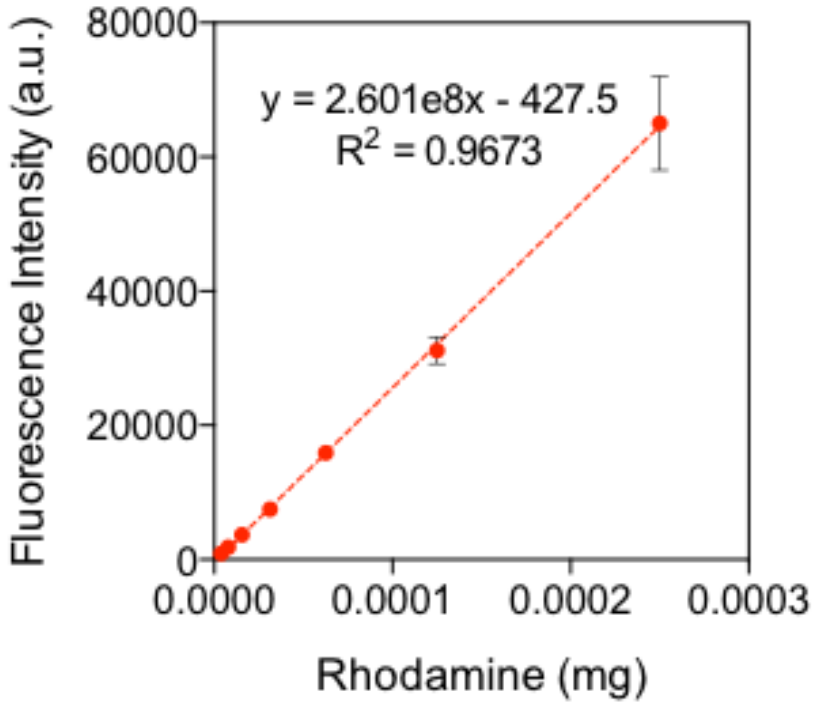

Figure S2. Standard curve of Rhodamine. The fluorescence intensity of various amounts of rhodamine was measured using a plate reader. This information and the equation generated was used to determine the amount of rhodamine incorporated into NPs (Fig. 2E). Values represent the mean with error bars as s.e.m.

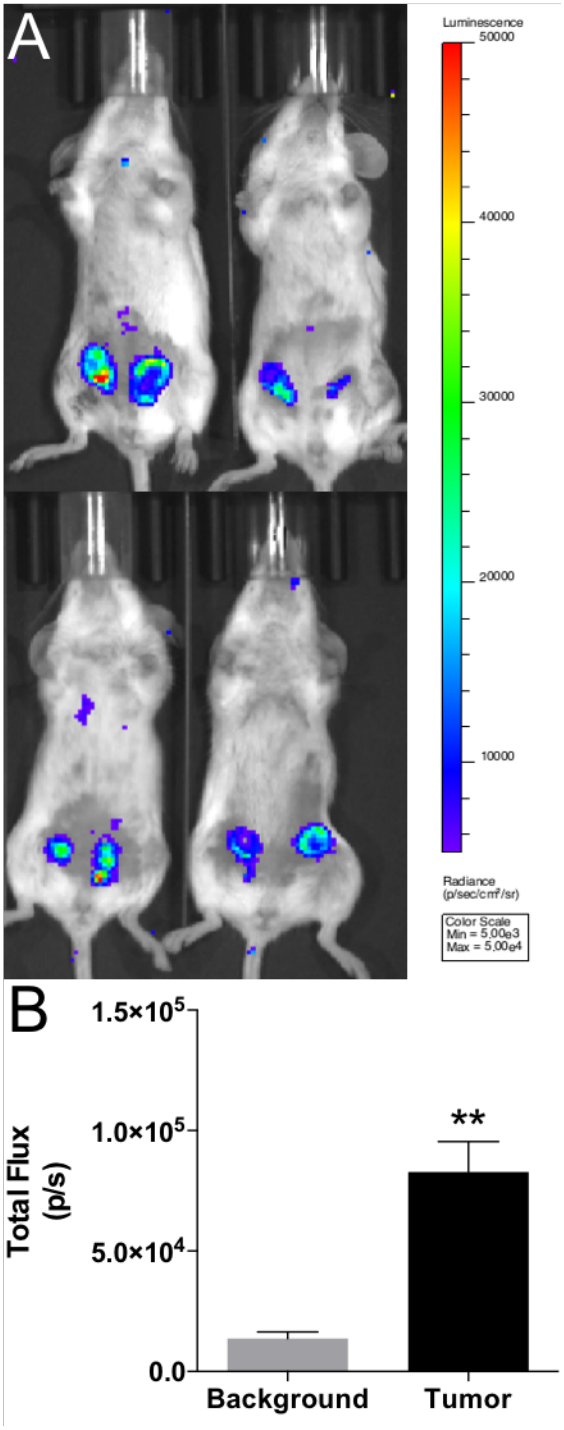

Figure S3. Bioluminescent imaging of luminol in 4T1 model. A) Bioluminescent imaging using luminol of mice bearing 4T1 tumors (2 per mouse). B) Quantification of signal comparing signals observed at tumor site versus signals in the chest (i.e., background). For B: ** = $p < 0.01$ and values represent the mean with error bars as s.e.m.

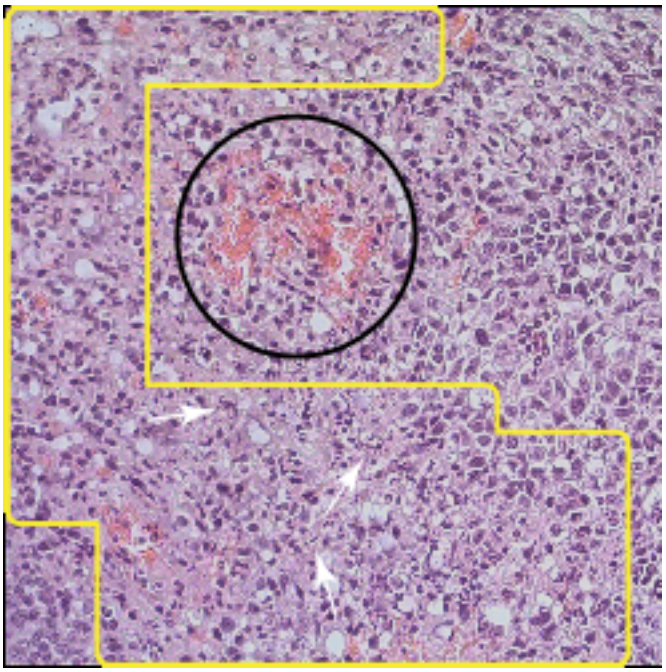

Figure S4. H&E image of 4T1 tumor. This 40x image, along with Fig. 3A, confirms an inflammatory process is present throughout the tumor tissue. A necrotic process is evident corroborated by the presence of cells characterized by retraction of the cytoplasm and fragmentation of nuclei (white arrows). The necrosis is most likely brought upon by the haphazard structure and distribution of blood vessels (Black circles). In addition, there is edema seen throughout tissue samples (yellow line). Both images (Fig. S2 and 3A) were taken from the same tissue focused in different areas to highlight certain structures prevalent throughout the tissue.

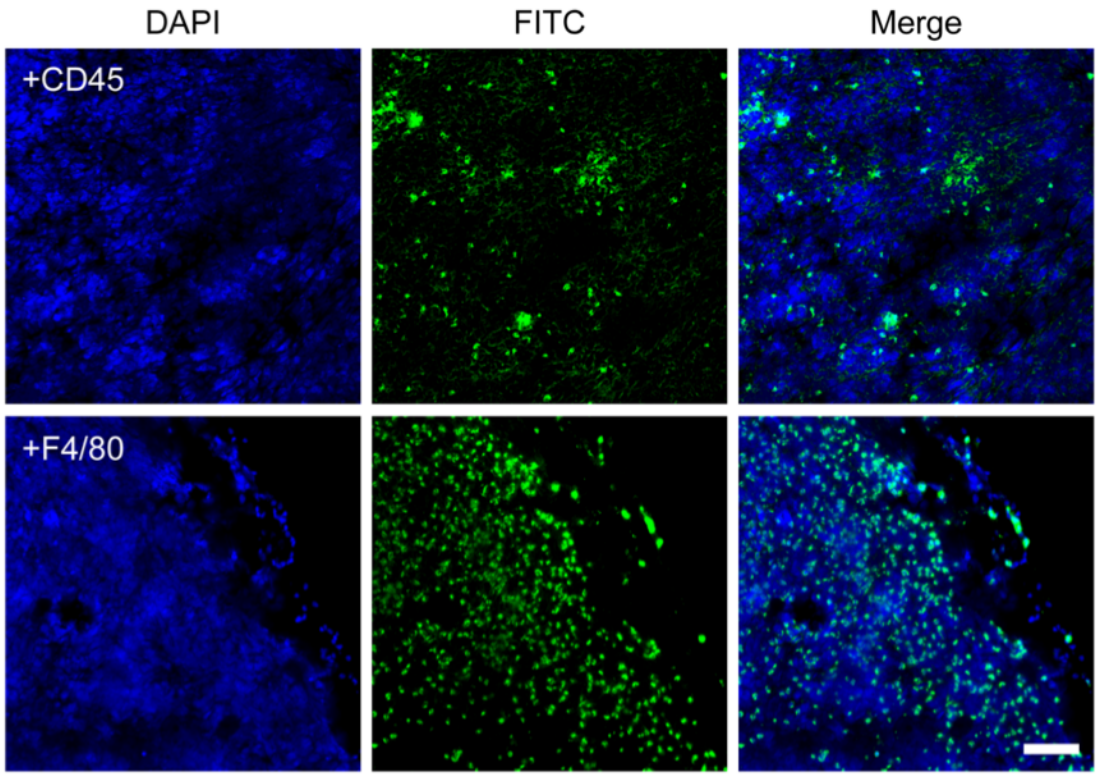

Figure S5. Leukocyte infiltration in 4T1 tumor cells. Representative immunofluorescence images depicting the distribution of CD45 (upper) and F4/80 (lower) on 4T1 tumor sections collected from untreated mice. Nuclei were stained in blue; scale bar: 100 μm.

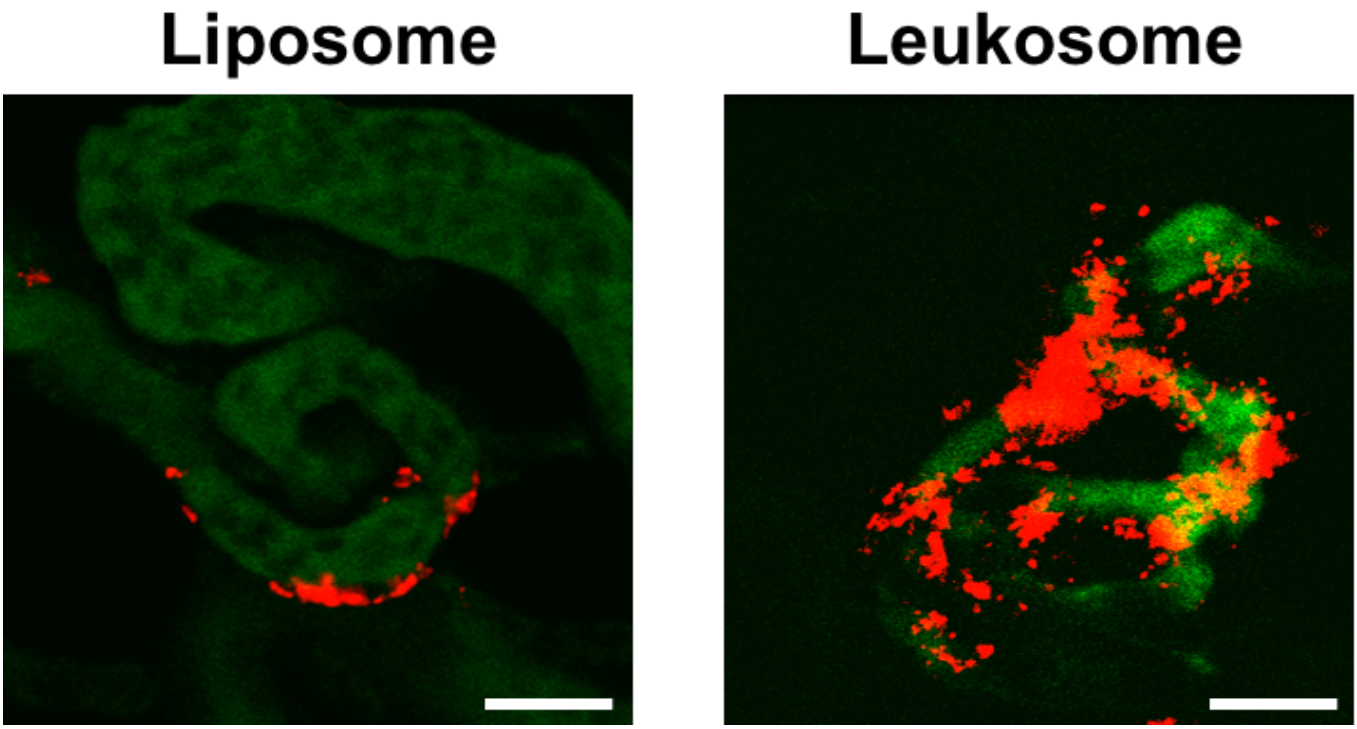

Figure S6. NP accumulation in 4T1 tumors at 1h. Representative IVM images of 4T1 tumor vessels treated with liposomes or leukosomes after 1h of administration. Both NP are in red, vessels in green. Scale bar = 50µm.

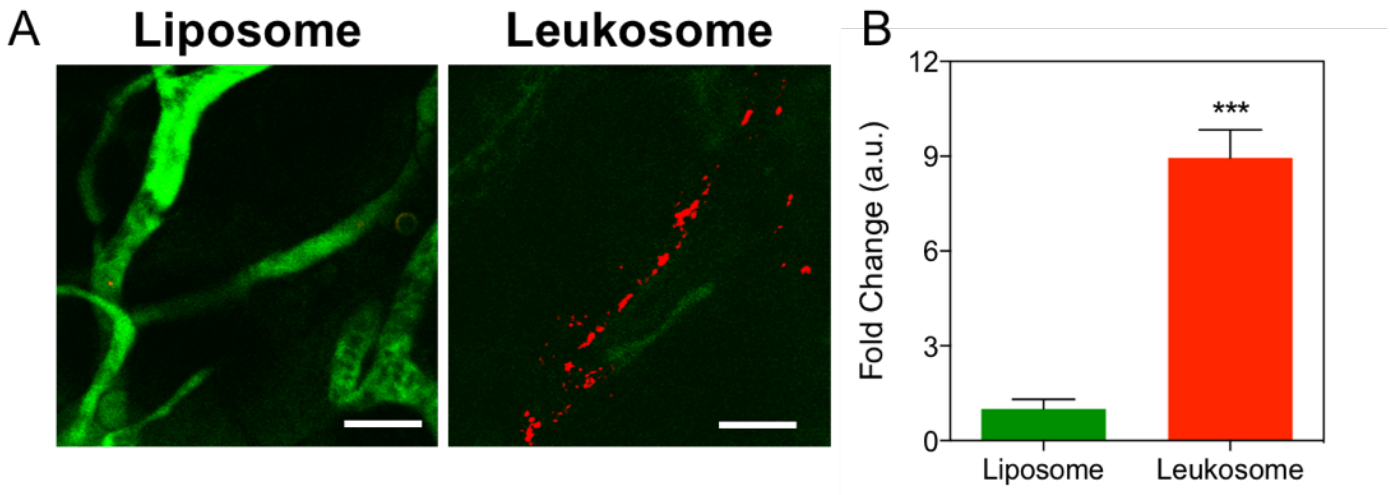

Figure S7. NP accumulation in 4T1 tumors at 6h. A) Representative IVM images of 4T1 tumor vessels treated with liposomes or leukosomes after 6h of administration. Both NP are in red, vessels in green. Scale bar = 50μm. B) Quantification of liposome and leukosomes accumulation in tumors at 6h. Values shown are normalized with respect to liposome accumulation. For B: *** = $p < 0.001$ and values represent the mean with error bars as s.e.m.

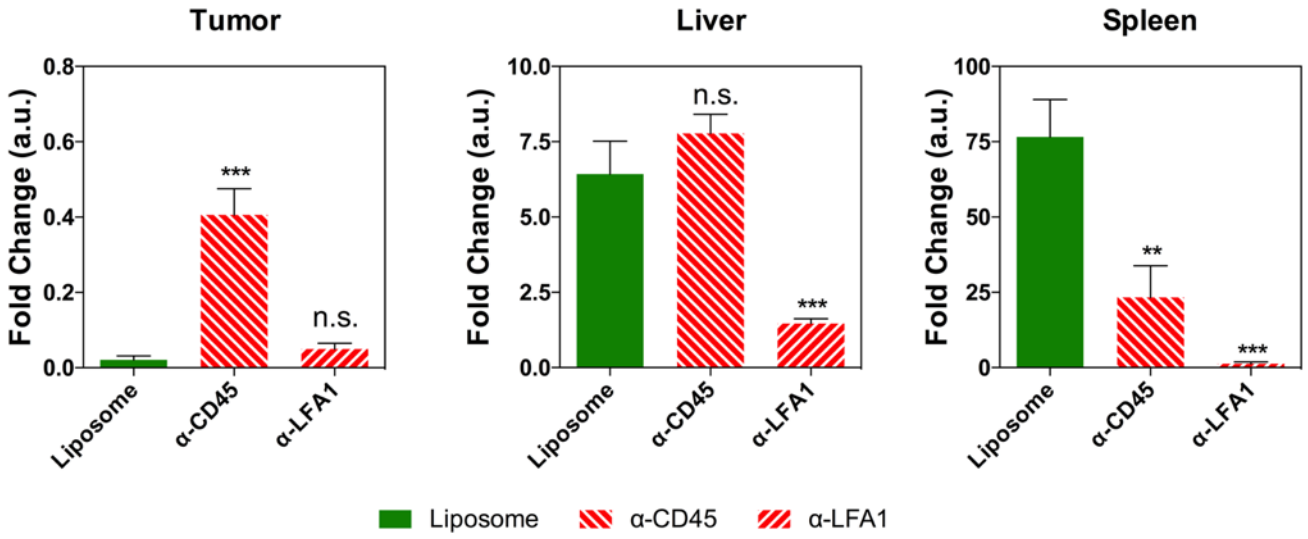

Figure S8. Comparing liposome accumulation to blocked leukosomes. A-C) Quantification of the NP present in the tumor (A), liver (B), and spleen (C) of 4T1-bearing mice after 1h of administration. This data goes along with Figure 4 and compares the accumulation in these organs to liposomes (as opposed to leukosomes in Fig. 4). Values shown are normalized to accumulation of leukosomes and statistics are shown compared to the liposome group. For all graphs: ** = $p < 0.01$, *** = $p < 0.001$, n.s. = not significant ($p > 0.05$) and values represent the mean with error bars as s.e.m.

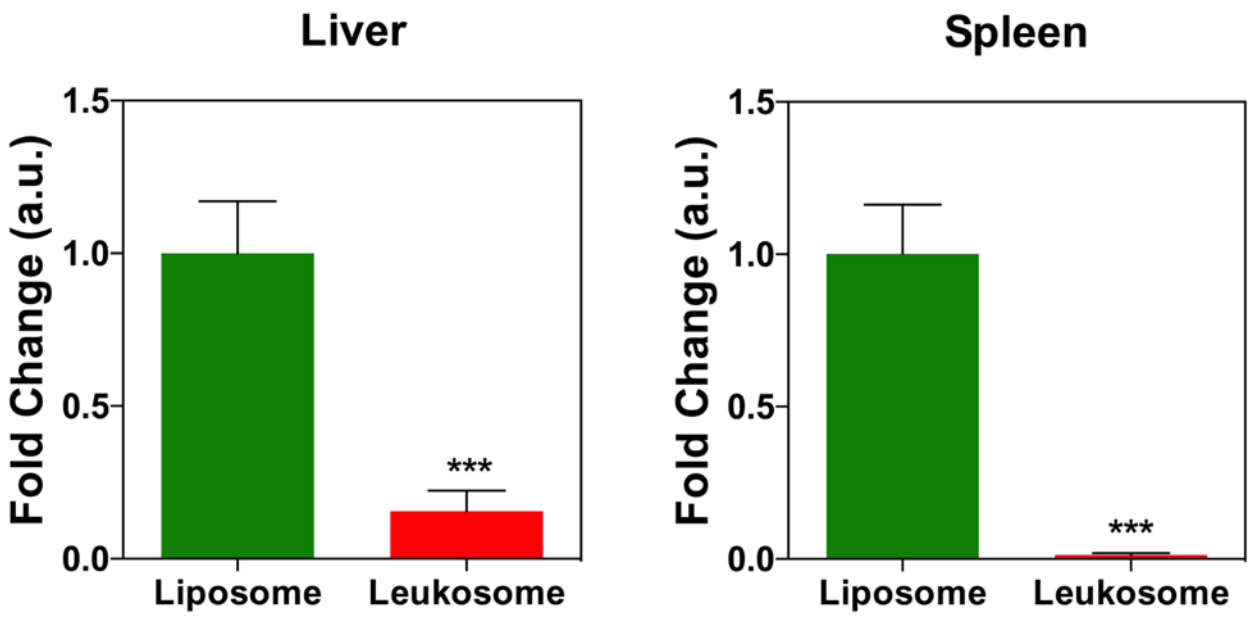

Figure S9. NP accumulation in liver and spleen. The liver and spleen of 4T1-bearing mice were collected to analyze the accumulation of NP after 1h. In both organs, leukosomes demonstrated a significant reduction in accumulation compared to liposomes confirming their ability to avoid rapid sequestration from the MPS. Values shown are normalized with respect to liposome accumulation. For both graphs: *** = $p < 0.001$ and values represent the mean with error bars as s.e.m.

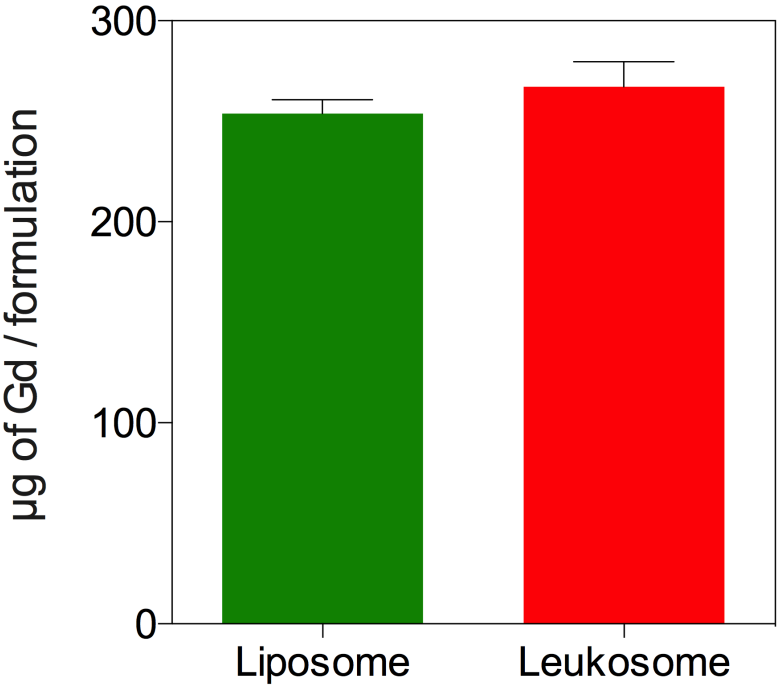

Figure S10. Gd incorporation into NP. Quantification of the amount of Gd integrated into NPs. For all graphs: values represent the mean with error bars as s.e.m.

SUPPLEMENTARY MOVIE CAPTIONS

Supplementary Movie S1. IVM of liposomes's distribution in 4T1 breast tumors. Multiple z-stack positions of tumor-associated vasculature in 4T1 tumors shows that very few liposomes remain present after six hours. Liposomes that remain are localized within the lumen of the vessel. Scale bar = 20 μm. Leukosomes shown in red, vasculature shown in green.

Supplementary Movie S2. IVM of leukosome's distribution in 4T1 breast tumors. Multiple z-stack positions of tumor-associated vasculature in 4T1 tumors show leukosomes are localization at the vessel wall six hours after administration. Furthermore, several leukosomes are observed to extravasate into the tumor parenchyma. Scale bar = 20 μm. Leukosomes shown in red, vasculature shown in green.

SUPPLEMENTARY MATERIALS AND METHODS

Statistical Analysis:

Statistics were calculated with GraphPad Prism 6 software. A one-way ANOVA with a Dunnet post-hoc test to correct for multiple comparisons was used to analyze the antibody blocking experiments. For the comparison of NP accumulation in tumors at 6h and in liver and spleen after 1hr, a two-tailed unpaired t-tests assuming populations with the same scatter. In all cases: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. Unless otherwise noted, all values are represented as mean ± s.e.m.