Supplemental data for:

Revealing Dynamics of Accumulation of Systemically Injected Liposomes in the Skin by Intravital Microscopy

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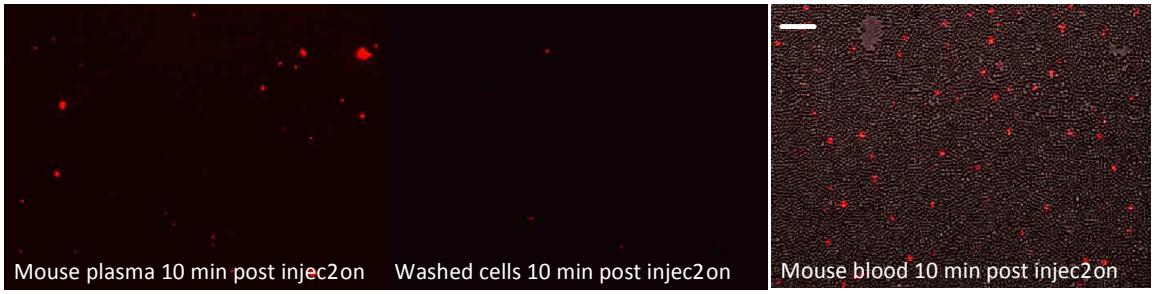

Fig. S1. Aggregates of EPC liposomes in blood collected 10 min' post-injection
Size bar=50 μm. Most of the aggregates were in the plasma fraction.

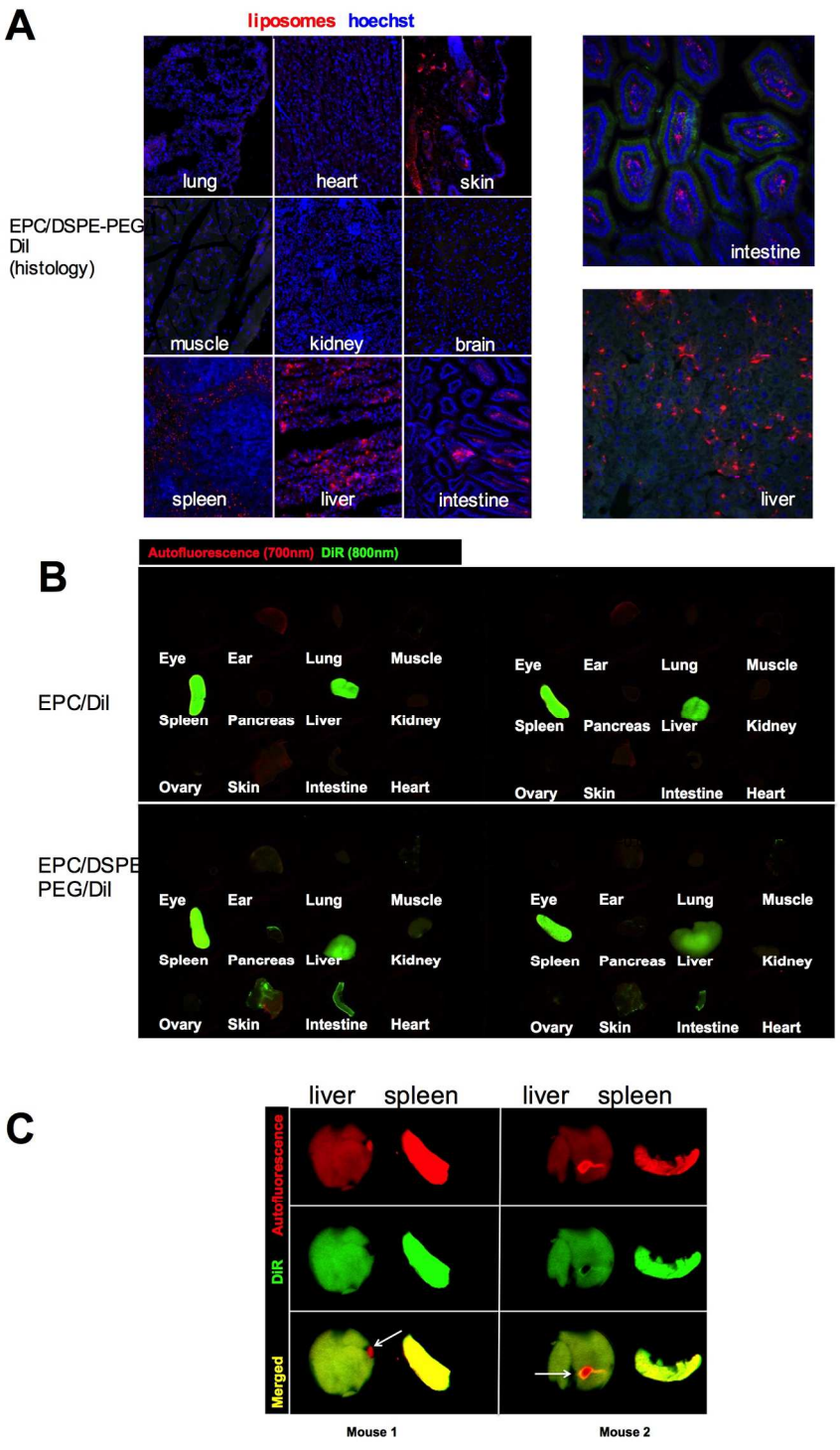

Fig. S2. Organs of mice injected with liposomes

A) histological sections of organs of mice injected with PEGylated DI-labeled liposomes. Right panel shows cross section of microvilli of the small intestine and large liver image with Kupffer cells internalizing the liposomes; B) whole organ images of mice injected with DiR labeled non-PEGylated and PEGylated EPC liposomes. Two mice per group

are shown. Excluding liver and spleen, intestine and skin showed the highest uptake of PEGylated liposomes; C) liver images of mice injected with DiR labeled PEGylated EPC liposomes. The autofluorescence (700nm, red) was enhanced to provide nice contrast to the DiR fluorescence (800nm, green). Gallbladder (red dot, arrow) was completely negative, ruling out the role of bile excretion as a reason for intestinal accumulation of liposomes.

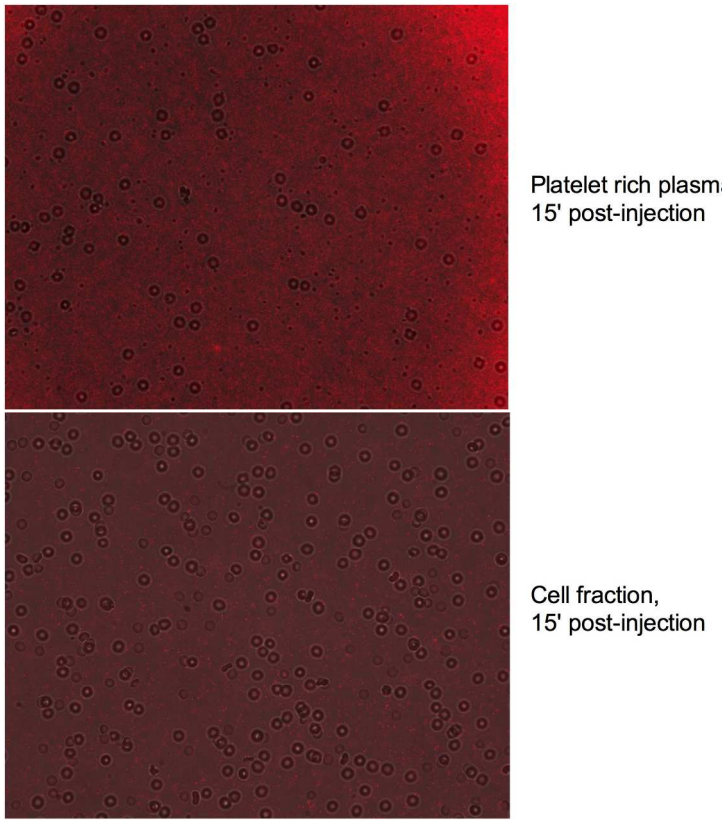

Fig. S3. Lack of aggregation of PEGylated EPC liposomes in mouse blood and serum

Mice were injected with liposomes and the blood was collected 15 min post-injection. There were no visible aggregates of liposomes and no uptake by leukocytes and platelets.

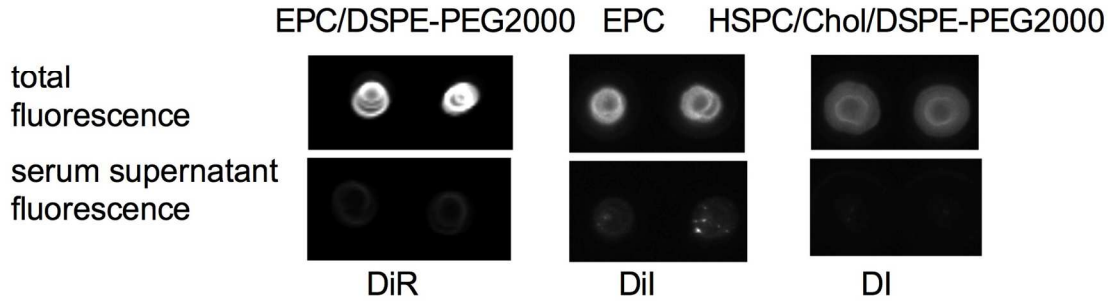

Fig. S4. Liposomes show minimal transfer of the membrane dye to serum
Liposomes were incubated for 1h in mouse sera (20nmol+30 µl sera) and centrifuged at 400,000g for 30 min. The liposome/serum mix before centrifugation (total fluorescence) and the supernatant after the centrifugation (dissociated fluorescence) were applied on a nitrocellulose membrane and scanned in parallel with Li-COR Odyssey (EPC/DSPE-PEG-2000/DiR) or Bio-Rad gel imager (EPC/DiI and HSPC/Chol/DSPE-PEG-2000/DiI. Some aggregates of EPC (center) ended in the supernatant because of difficulty in pelleting low-density liposomes in serum.

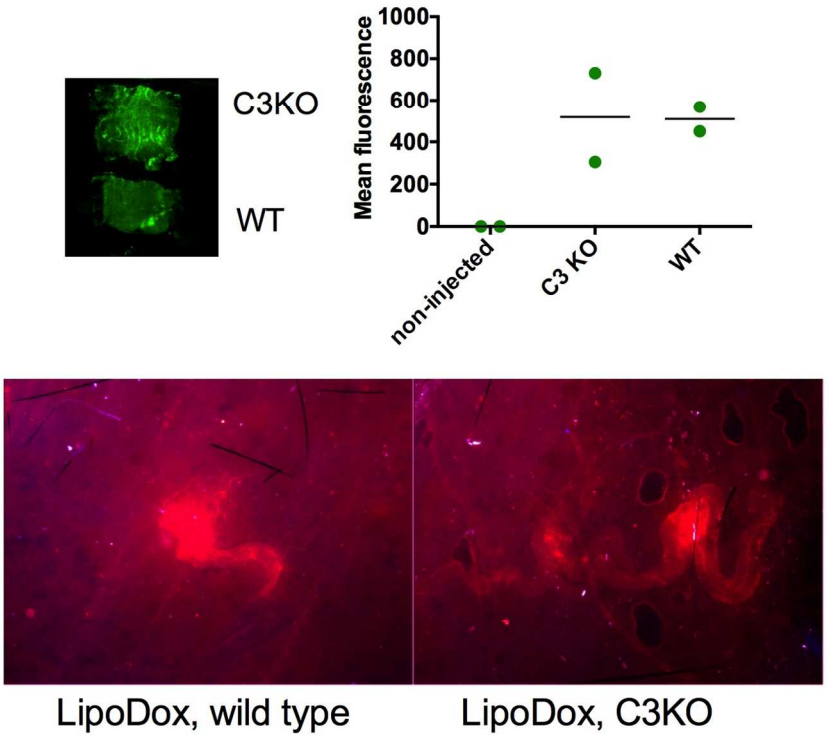

Fig. S5. Lack of effect of complement C3 deficiency on skin accumulation
PEGylated DiR labeled EPC liposomes were injected into C3 deficient or wild type mice (C57/BL6 background). There was no decrease in skin accumulation in C3 deficient mice as determined by LiCOR imaging of NIR fluorescence in the skin (upper image and signal quantification). The same result was observed for LipoDox (lower panel) fluorescence under low magnification microscope (much smaller imaging area than Li-COR).