

# Supplementary Information

## Lipid nanoparticle encapsulated large peritoneal macrophages migrate to the lungs via the systemic circulation in a model of clodronate-mediated lung-resident macrophage depletion

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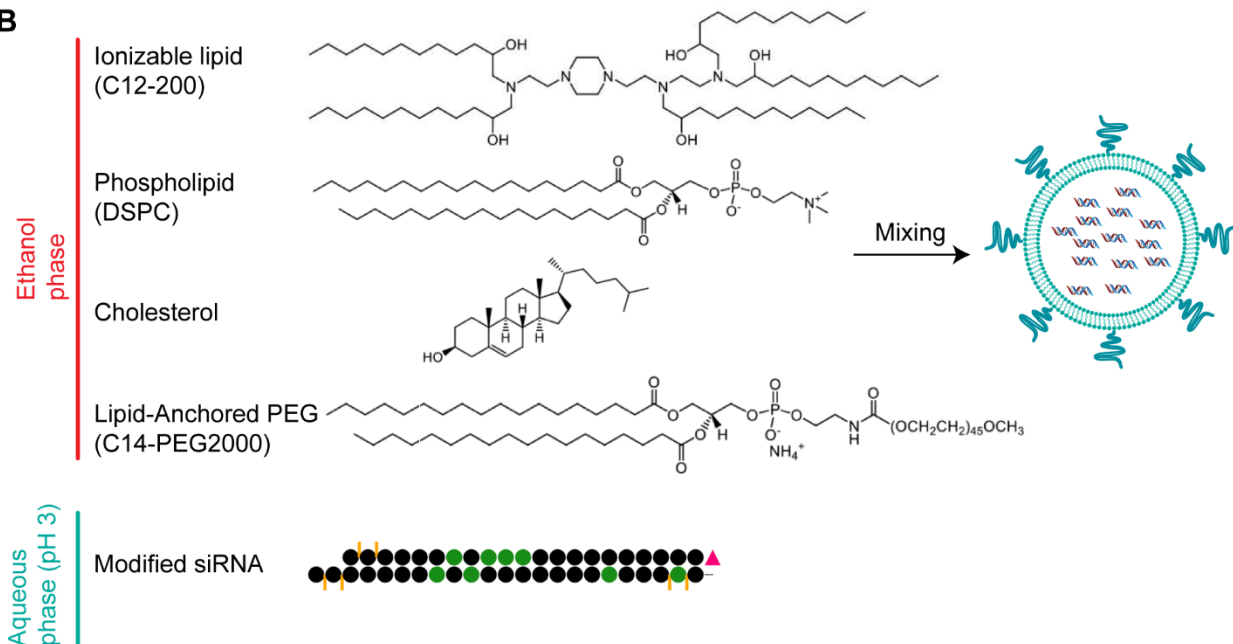
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**A**



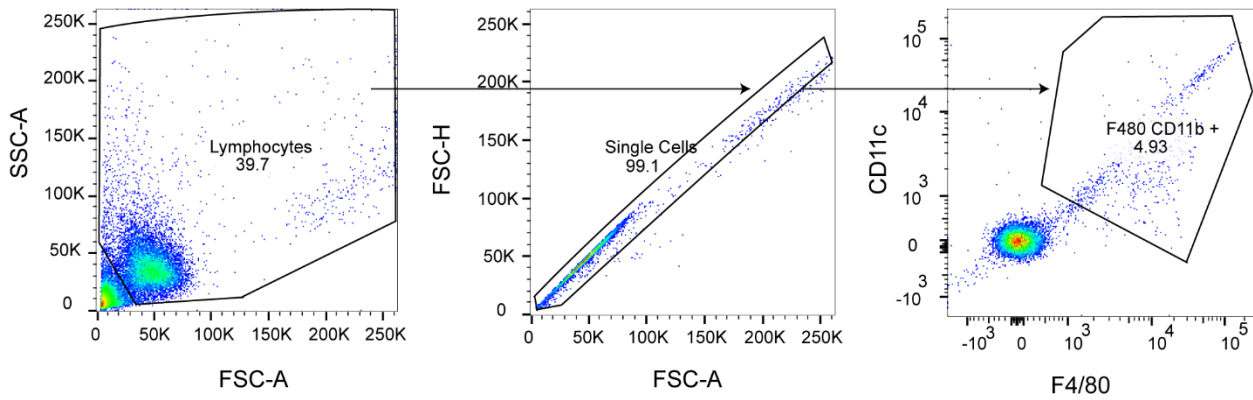
**B**



18 **Supplementary Figure 1: siRNA design and schematics of C12-200 lipid nanoparticle**  
19 **formulation. A.** siRNA design. A typical double-stranded siRNA design with 21 oligonucleotides  
20 on the sense strand and 23 oligonucleotides on the anti-sense strand designed against mouse  
21 CD-45 transcript, bases were modified by F- and O-Me at the 2'- positions wherever shown to  
22 avoid susceptibility to endo- and exo-nucleases. Cy5.5 was labeled to the 5'end of the sense

23 strand. **B.** Schematics for C12-200-based LNP formulation and siRNA encapsulation. Details  
24 described under methods and materials.

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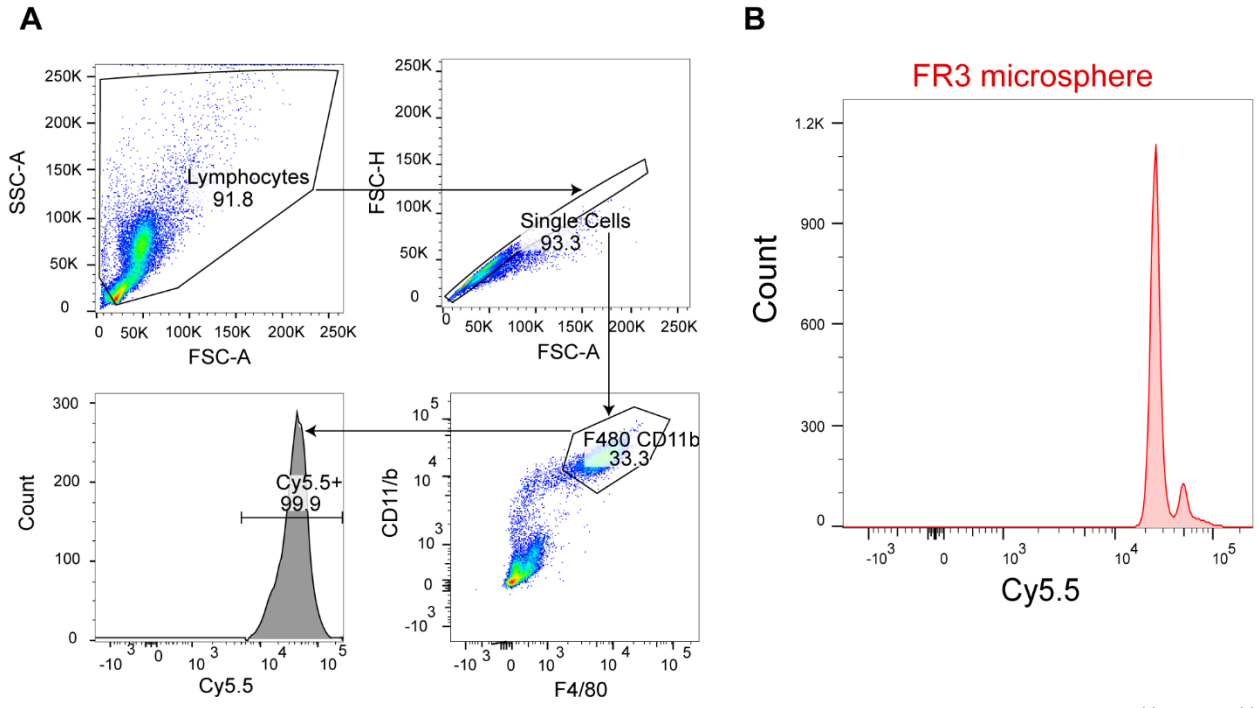


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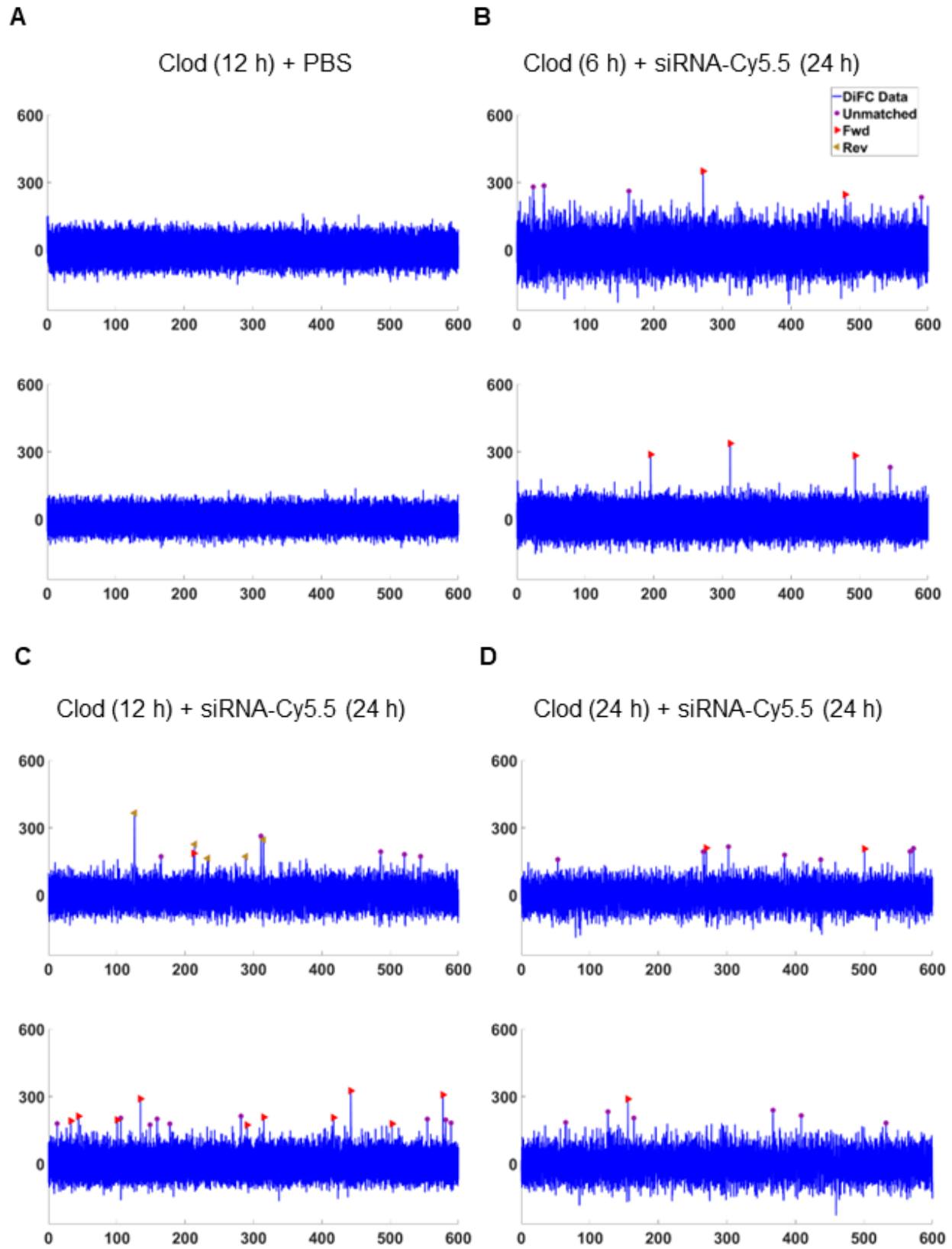
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**Supplementary Figure 2: Representative flow cytometry gating strategy in isolated BALF cells to assess clodronate mediated depletion of F4/80<sup>hi</sup>+ CD11c<sup>hi</sup>+AM population**



29 **Supplementary Figure 3: Flow cytometry gating scheme of A.** Cy5.5 MFI in CD11b<sup>hi</sup> F4/80<sup>hi</sup>  
 30 LPMs obtained from the respective treatment groups along with **B.** Histogram of Flash Red 3  
 31 (FR3) microsphere MFI. Cells were pre-gated on size and viability.

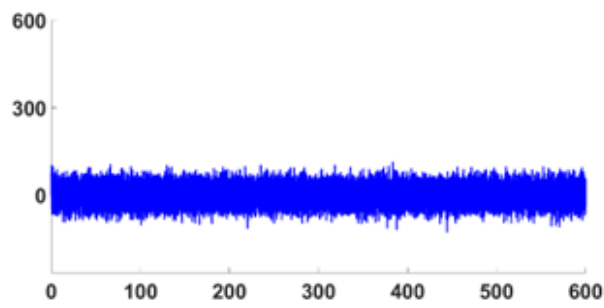
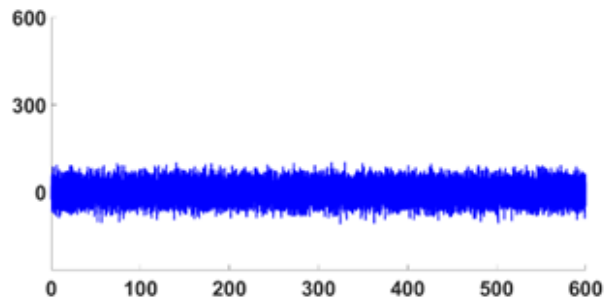


32 **Supplementary Figure 4: Representative graphs of DiFC scans for additional mice depicted**

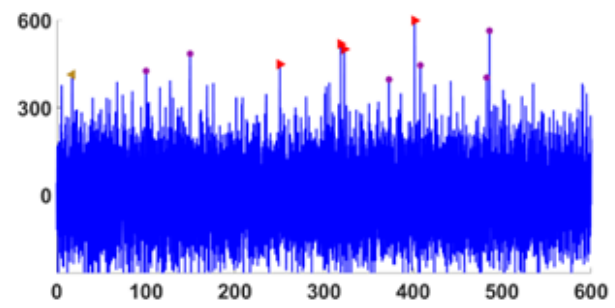
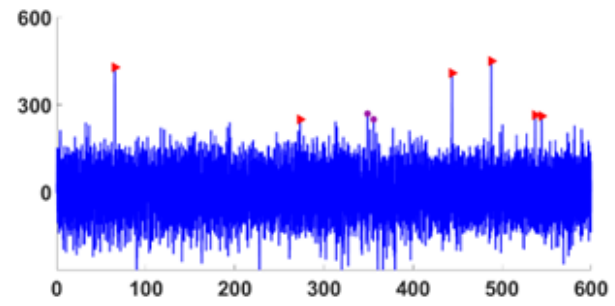
33 **as number of peaks detected over 600 s from 2 mice per group from an n = 4/group with**  
34 **the indicated treatments. A.** Clod (12 h) + PBS **B.** Clod (6 h) + siRNA-Cy5.5 (24 h) **C.** Clod (12  
35 h) + siRNA-Cy5.5 (24 h) **D.** Clod (24 h) + siRNA-Cy5.5 (24 h). The top and bottom panels are  
36 graphs for individual mice; data shown for 2 mice for the respective treatment groups. Graphs are  
37 representative snapshots of a 10 min scan period from a total scanning time of 45 min per mouse.  
38 Each peak (arrowhead) represents a circulating cell labeled with siRNA-Cy5.5 (C12-200) in  
39 systemic circulation, depicted as signal versus time.

**A**

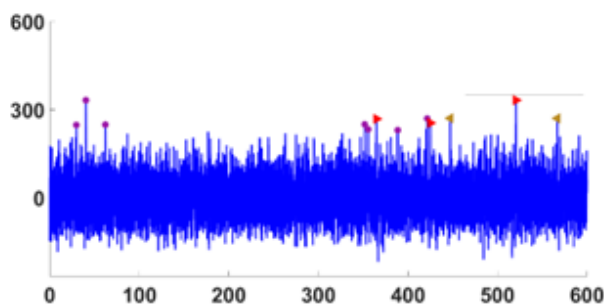
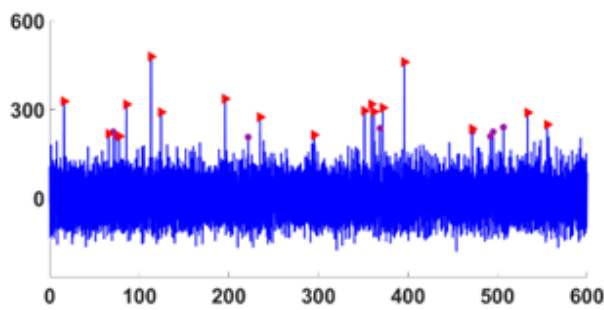
No Clod + siRNA-Cy5.5 (24 h)

**B**

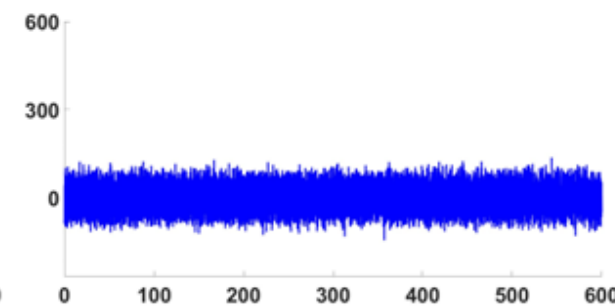
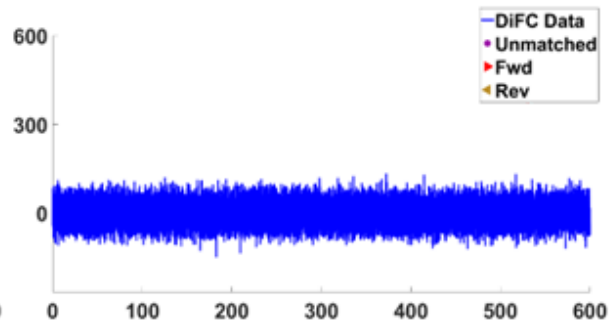
Clod (12 h) + siRNA-Cy5.5 (3 h)

**C**

Clod (12 h) + siRNA-Cy5.5 (6 h)

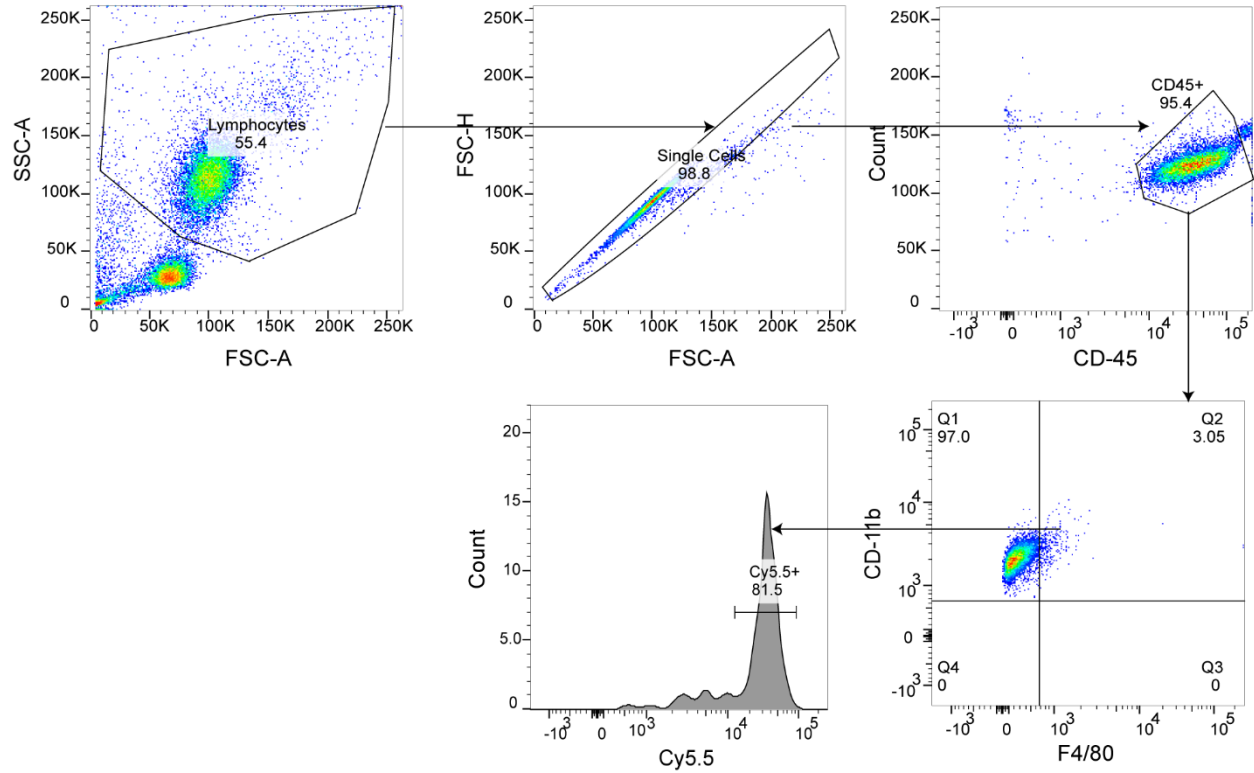
**D**

Clod (12 h) + PBS



41 **Supplementary Figure 5: Representative graphs of DiFC scans for additional mice depicted**  
42 **as number of peaks detected over 600 s from 2 mice per group from an n = 4/group with**  
43 **the indicated treatments. A. No Clod + siRNA-Cy5.5 (24 h) B. Clod (12 h) + siRNA-Cy5.5 (3 h)**  
44 **C. Clod (12 h) + siRNA-Cy5.5 (6 h) D. Clod (12 h) + PBS.** The top and bottom panels are graphs  
45 for individual mice; data shown for 2 mice for the respective treatment groups. Graphs are  
46 representative snapshots of a 10 min scan period from a total scanning time of 45 min per mouse.  
47 Each peak (arrowhead) represents a circulating cell labeled with siRNA-Cy5.5 (C12-200) in  
48 systemic circulation, depicted as signal versus time.





49 **Supplementary Figure 6: Representative flow cytometry gating strategy to assess**  
 50 **percentage of Cy5.5+ F4/80<sup>hi</sup> CD11b<sup>hi</sup> macrophage population in whole blood PBMCs.**

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