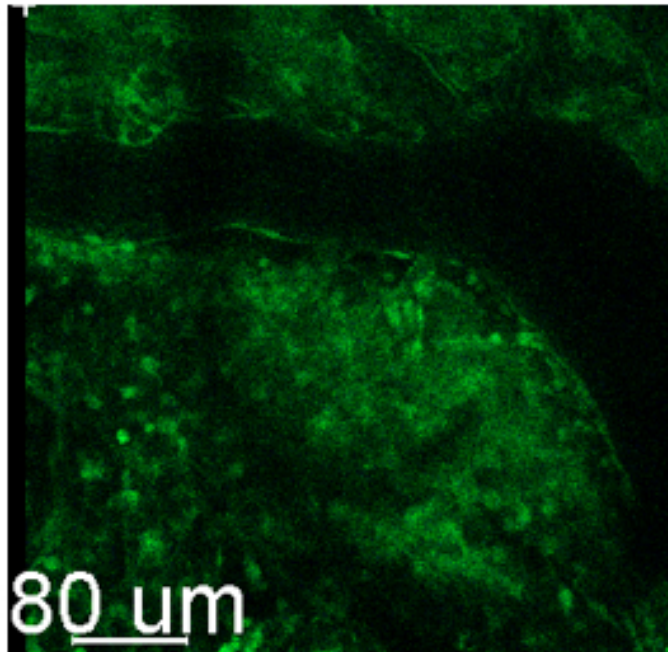


**Supporting Information****Multiple Administrations of Viral Nanoparticles Alter in Vivo Behavior—Insights from Intravital Microscopy**

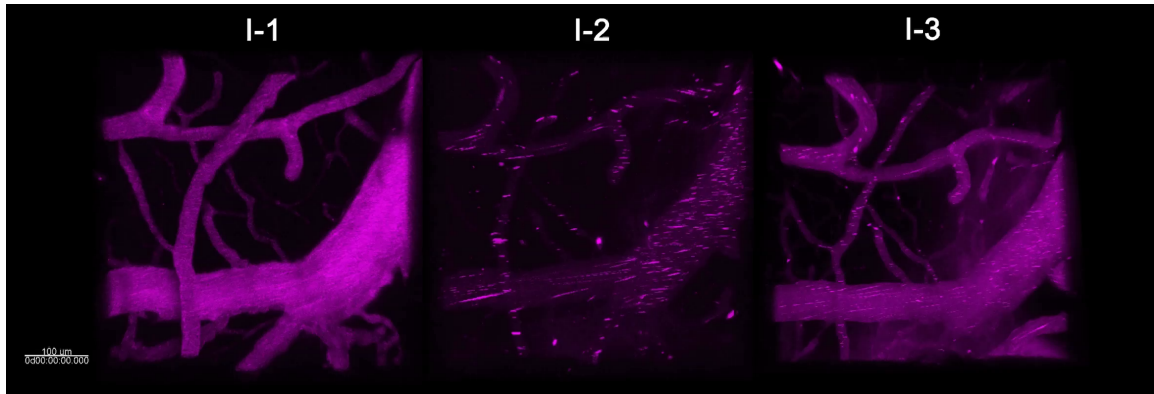
Sourabh Shukla,<sup>†,‡,#</sup> R. Dixon Dorand,<sup>§,#</sup> Jay T. Myers,<sup>//</sup> Sarah E. Woods,<sup>†</sup> Neetu M. Gulati,<sup>⊥</sup> Phoebe L. Stewart,<sup>⊥</sup> Ulrich Commandeur,<sup>◆</sup> Alex Y. Huang,<sup>\*,‡,§,||</sup> and Nicole F. Steinmetz<sup>\*,†,‡,#,∇,○</sup>

<sup>†</sup>Department of Biomedical Engineering, <sup>‡</sup>Case Comprehensive Cancer Center, <sup>§</sup>Pathology, <sup>//</sup> Pediatrics, <sup>⊥</sup>Pharmacology, and Cleveland Center for Membrane and Structural Biology, <sup>#</sup>Radiology, <sup>∇</sup>Macromolecular Science and Engineering, <sup>○</sup>Materials Science and Engineering, Case Western Reserve University, 10900 Euclid Avenue, Cleveland, Ohio 44106, United States.

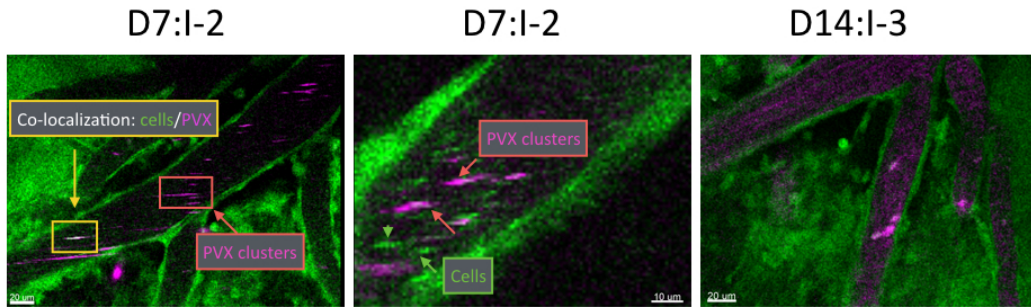
<sup>◆</sup>Institute for Molecular Biotechnology, RWTH Aachen University, Worringer Weg 1, 52074 Aachen, Germany.



**Supporting Figure S1:** C57BL/6-Tg(UBC-GFP) mice express green fluorescent protein (GFP) in all tissues under the control of the human ubiquitin C promoter,<sup>49</sup> which enables visualization of vasculature clearly via absence of fluorescence signal as compared to the surrounding tissue.



**Supporting Figure S2:** Intravital imaging performed over 10 min post-injection of PVX-A647 particles upon the three weekly i.v. administrations (video files). Imaging data shows bright and uniform fluorescence signals (in pink) after first injection (I-1), formation of clusters following the second injection (I-2); fewer clusters are observed after the third injection (I-3).



**Supporting Figure S3:** GFP expressing circulating monocytes showed insignificant co-localization with PVX-A647 clusters on days 7 and 14 following injections I-2 and I-3, respectively, thereby ruling out possibility of cellular phagocytosis as a major cause of cluster formation.