Supporting Information

Silica shelled and block copolymer encapsulated red-emissive AIE nanoparticles with 50% quantum yield for two-photon excited vascular imaging

Junlong Geng,[ab] Chi Ching Goh,[c] Wei Qin,[d] Rongrong Liu,[b] Nikodem Tomczak,[b] Lai Guan Ng,[c] Ben Zhong Tang,[de] and Bin Liu*[ab]

[a] *Department of Chemical and Biomolecular Engineering, National University of Singapore, Singapore 117585*
E-mail: cheliub@nus.edu.sg
[b] *Singapore Immunology Network (SIgN), A*STAR (Agency for Science, Technology and Research), Biopolis, Singapore 138648*
[c] *Institute of Materials Research and Engineering, 3 Research Link, Singapore 117602*
[d] *Department of Chemistry, Division of Biomedical Engineering, The Hong Kong University of Science and Technology, Clear Water Bay, Kowloon, Hong Kong, China*
[e] *SCUT-HKUST Joint Research Laboratory, Guangdong Innovative Research Team, State Key Laboratory of Luminescent Materials & Devices, South China University of Technology, Guangzhou 510640, China*

Experimental Section

Materials

2,3-Bis(4-(phenyl(4-(1,2,2-triphenylvinyl)phenyl)amino)phenyl)-fumaronitrile (TPETPAFN) was synthesized according to the previous report.[1] QD655 was purchased from Invitrogen. Poly(ethylene oxide)-*b*-poly(propylene oxide)-*b*-poly(ethylene oxide), tetraethyl orthosilicate (TEOS), diethoxydimethylsilane (DEDMS), 4-(dicyanomethylene)-2-methyl-6-(p-dimethylamino-styryl)-4H-pyran (DCM), tetraphenylethylene (TPE), Evans Blue, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT), penicillin-streptomycin solution, fetal bovine serum (FBS), trypsin-EDTA solution, tetrahydrofuran (THF), Evans Blue, and methanol were purchased from Sigma-Aldrich. Milli-Q water was supplied by

Milli-Q Plus System. NIH/3T3 fibroblast cells were provided by American Type Culture Collection.

Characterization

The UV-vis absorption and fluorescence spectra of AIE NPs were measured using a Shimadzu UV-1700 spectrophotometer and a fluorometer (LS-55, Perkin Elmer, USA), respectively. Their surface morphologies were studied with field emission transmission electron microscopy (JEM-2010F, JEOL, Japan). The average particle size was analyzed by dynamic light scattering (DLS) with particle size analyzer (90 Plus, Brookhaven Instruments Co. USA) at room temperature. Fluorescence lifetime measurements were performed on a Fluorolog-3 iHR spectro-fluorometer with an excitation wavelength of 488 nm. The fluorescence quantum yields for TPETPAFN-F127-SiO2 NPs and TPETPAFN-F127 NPs were measured using 4-(dicyanomethylene)-2-methyl-6-(p-dimethylamino-styryl)-4H-pyran in methanol as the reference.

TPA measurements

TPA spectra were recorded according to the procedure reported in the litereature.[2] The samples were excited with laser pulses of 100 fs in the spectral range 800-920 nm, using Rhodamine 6G in methanol as the reference.. The TPA cross section was calculated using the reported equation.[3]

Wide-field microscopy imaging

Fluorescence imaging of individual NP and QD655 was conducted using a Wide-Field Microscope (WFM) based on a Nikon ECLIPSE Ti-U inverted microscope frame. The procedures follow those reported in the literature.[2] The sample was prepared by depositing a droplet of NP solution on a glass cover slide, and dried under N_2 flow. Fluorescence intensity time-traces were collected by acquiring 1000 consecutive frames at a rate of 10 frames per second and extracting the number of counts per particle for each frame. Statistical analysis of the data was further performed using a custom analysis software.

4.5 Synthesis of AIE-F127 NPs and AIE-F127-SiO_2 NPs

AIE-F127 NPs were synthesized by a conventional precipitation approach and AIE-F127-SiO_2 NPs were synthesized using a modified method.[2,4] Firstly, F127 (100 mg) and AIE fluorogens (1 mg) were dissolved in 1.5 mL of THF and stirred for 3 h at room temperature. The reaction mixture was then subject to gentle nitrogen flow to remove THF and yield a solid residue. 1.6 mL of Milli-Q water was subsequently added into the solid F127/AIE mixture under sonication. The AIE loaded F127 NPs were obtained after dialyzing with a membrane (cut-off MW= 14 000) against Milli-Q water for three days. To prepare AIE-F127-SiO_2 NPs, the solid F127/AIE mixture was sonicated in 1.6 mL of 0.85 M hydrochloride solution rather than Milli-Q water to form a homogeneous suspension. Then, 180 μL of TEOS was added into the mixture and the solution was further stirred for 2 h at room temperature. 30 μL of DEDMS was subsequently added to the mixture to terminate the silica shell growth at NP surfaces. The reaction mixture was kept stirring for another 24 h at room temperature. Finally, the solution was dialyzed against Milli-Q water for three days to remove hydrochloride and unreacted reagents. The suspension was then purified using a 0.2 μm syringe filter to yield AIE-F127-SiO_2 NPs.

Photostability of TPETPAFN-F127 NPs and TPETPAFN-F127-SiO_2 NPs

To compare the photostability of TPETPAFN-F127 NPs and TPEPTAFN-F127-SiO_2 NPs, 20 μL of both NPs at same TPETPAFN concentration were spin down on each cover glass and dried in fume hood, followed irradiation with 810 nm laser with 50% confluence. The fluorescence intensity at the designed time interval was measured by the microscopy. The photostability was expressed by the ratio of the fluorescence intensity after excitation for a designated time interval (I) to its initial value as a function of the exposure time (I_0).

Fluorescence stability of TPETPAFN-F127-SiO_2 NPs

The fluorescence stability of TPEPTAFN-F127-SiO_2 NPs incubated in 1 × PBS at 37 °C was studied by a fluorometer. The fluorescence intensity of the NP suspension was recorded with

the fluorometer at designated time interval, and the I/I_0 was evaluated, where I_0 is the fluorescence intensity of freshly prepared TPETPAFN-F127-SiO_2 NP suspension at 641 nm and I is that of NPs after incubation at designated time period, respectively.

Cytotoxicity of TPETPAFN-F127-SiO_2 NPs

NIH/3T3 fibroblast cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) containing 10% FBS and 1% penicillin streptomycin at 37 °C in a humidified environment containing 5% CO_2. The metabolic activity of NIH/3T3 fibroblast cells was determined using methylthiazolyldiphenyl-tetrazolium (MTT) assays. NIH/3T3 cells were seeded in 96-well plates (Costar, IL, USA) at a density of 4×10^4 cells mL^{-1}. After 24 h incubation, the medium was replaced by TPETPAFN-F127-SiO_2 NP suspensions at different NP concentrations, and the cells were then incubated for 24 and 48 h, respectively. After the designated incubation time, each well was washed twice with 1 × PBS and added with 100 μL of freshly prepared MTT (0.5 mg mL^{-1}) solution in cell medium. After 3 h incubation, the MTT medium was carefully removed and each well was added with 100 μL of DMSO to dissolve all the precipitates formed. The absorbance of MTT at 570 nm was measured by the microplate reader (Genios Tecan). Cell viability was expressed by the ratio between the absorbance of the cells incubated with NP suspensions and that of the cells incubated with culture medium only.

Real-time two-photon intravital blood vascular imaging of the tibial muscle

Mice were anesthetized with150 mg/kg ketamine and 10 mg/kg xylazine and placed on a heating pad to maintain a core body temperature of 37 °C throughout the imaging procedure. 150 μL of 200 μg/mL TPETPAFN-F127-SiO_2 NPs or TPETPAFN-F127 NPs at a NP concentration was administered via retro-orbital injection prior to imaging. Fur was removed from the calf and the thigh by shaving. The leg was immobilized in an extended position by gently stretching the leg downwards and fitting the leg into a pre-mould blu-Tack. A small skin incision was made to the right of the lateral saphenous vein, which runs from the ankle to

the posterior tibialis muscle. The connective tissue was gently teased away. A ring of vacuum grease was placed around the incision to hold a drop of PBS and coverslip prior to the start of imaging. As a control, mice without TPETPAFN-F127-SiO_2 were also imaged under the same experimental conditions. All procedures were performed in accordance with the guidelines of Institutional Animal Care and Use Committee. The images were aquired using a TriM Scope II single-beam two-photon microscope with a tunable 680-1080 nm laser.[5] Intensity measurements were calculated by first compressing each 80 μm stack into one z-projected image, and then selecting 5 different vessels from that image to obtain the intensity values. One mouse was used for each condition and three images were obtained from each mouse. TPETPAFN-F127-SiO_2 NPs were excited at 810 nm, and the emission was collected at 655/40 nm.

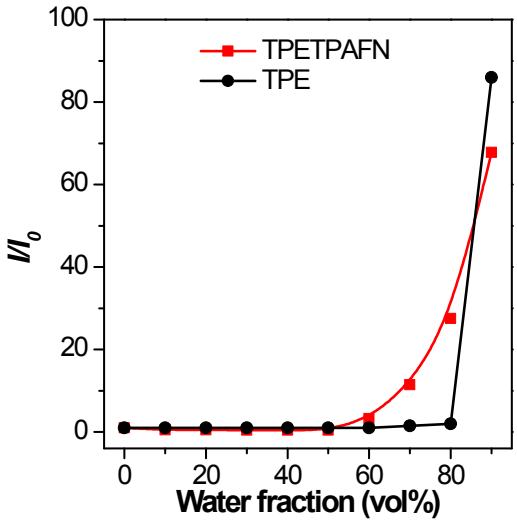

Fig. S1 Fluorescence intensity evolution of TPE and TPETPAFN in water/THF mixture with different water concentration, where I_0 is the fluorescence intensity in pure THF and I is that in different water/THF mixtures, respectively.

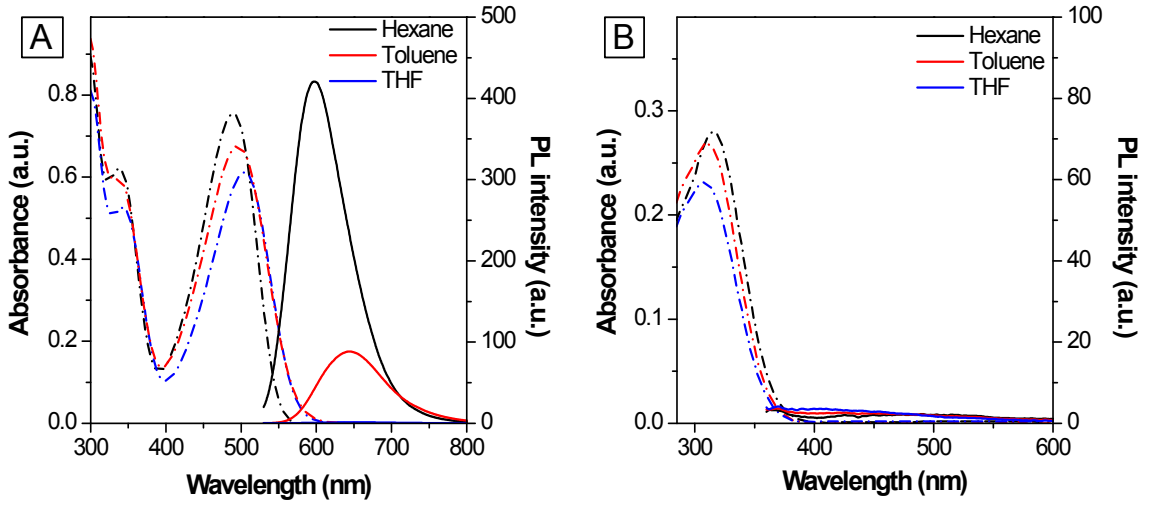

Fig. S2 UV-vis absorption (dash dotted line) and PL (solid line) spectra of TPETPAFN (A) and TPE (B) in Hexane, toluene, and THF upon excitation at 510 nm and 320 nm, respectively.

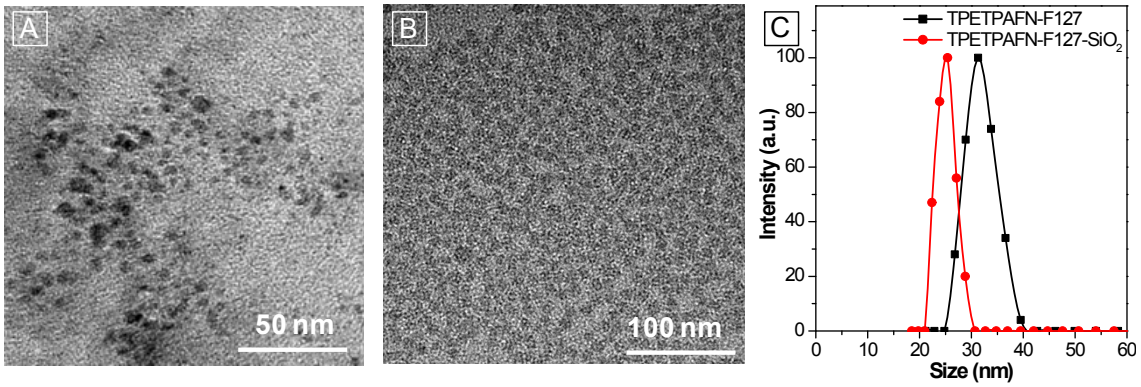

Fig. S3 FE-TEM images of (A) TPETPAFN-F127 NPs and (B) TPETPAFN-F127-SiO_2 NPs and their respective (C) DLS size distributions.

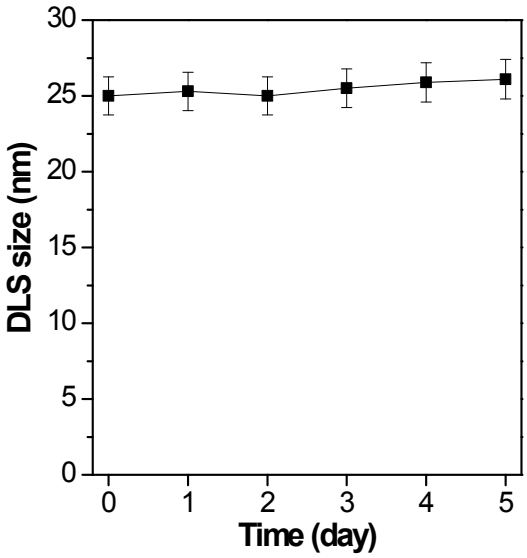

Fig. S4 DLS size evolution of TPETPAFN-F127-SiO_2 NPs at different time.

Table S1 Emission decay components of TPETPAFN-F127 NPs, TPETPAFN-F127-SiO_2 NPs, TPE-F127 NPs and TPE-F127-SiO_2 NPs.

samples	τ_1 (ns)	A_1 (%)	τ_2 (ns)	A_2 (%)	τ_{av}* (ns)	χ^2
TPETPAFN-F127	1.03	63.38	3.16	36.62	1.81	1.00
TPETPAFN-F127-SiO_2	1.94	16.95	4.64	83.05	4.18	0.99
TPE-F127	4.29	80.64	9.14	19.36	5.23	1.00
TPE-F127-SiO_2	2.43	35.63	6.25	65.82	4.99	1.00

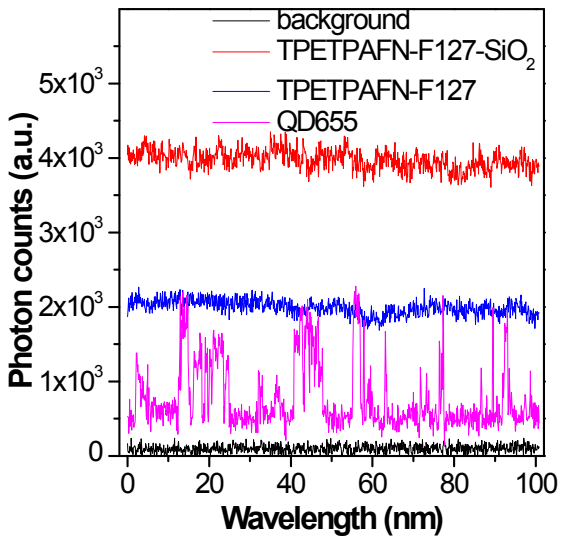

Fig. S5 Representative luminescence intensity time-traces for TPETPAFN-F127-SiO_2 NPs, TPETPAFN-F127 NPs, and QD655 (λ_{ex} = 488 nm).

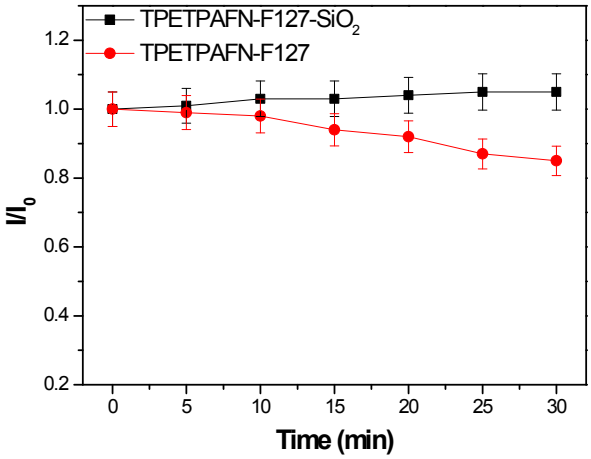

Fig. S6 Photostability of TPETPAFN-F127-SiO_2 and TPEPAFN-F127 NPs upon continuous laser excitation at 810 nm for 30 min. I_0 is the initial fluorescence intensity and I is the fluorescence intensity of the sample at different time points after irradiation.

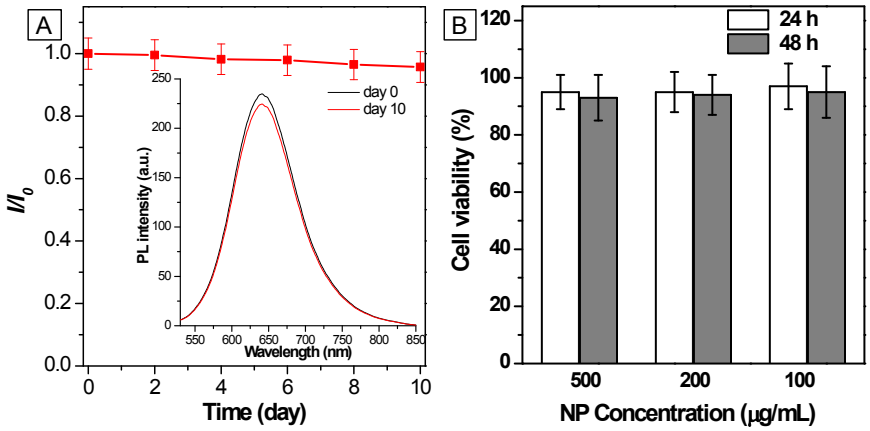

Fig. S7 (A) PL intensity evolution of TPETPAFN-F127-SiO_2 NPs upon incubation with 1 × PBS at 37 °C for different time, where I_0 is the fluorescence intensity at 641 nm for the fresh NP suspension and I is that for NPs after incubation for different time, respectively. The inset shows the PL spectra of freshly prepared TPETPAFN-F127-SiO_2 NPs (black) and that after 10 days incubation with 1 × PBS at 37 °C (red). (B) Metabolic viability of NIH/3T3 fibroblast cells after incubation with TPETPAFN-F127-SiO_2 NP suspensions at various NP concentrations for 24 h and 48 h, respectively.

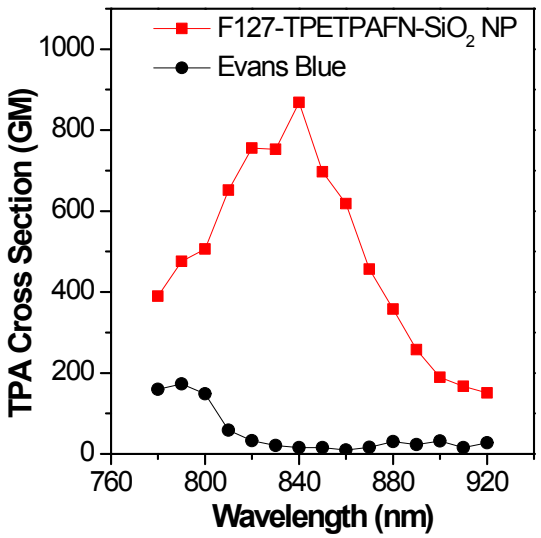

Fig. S8 TPA spectra of TPETPAFN-F127-SiO_2 NPs (based on NP concentration) and Evans Blue in water.

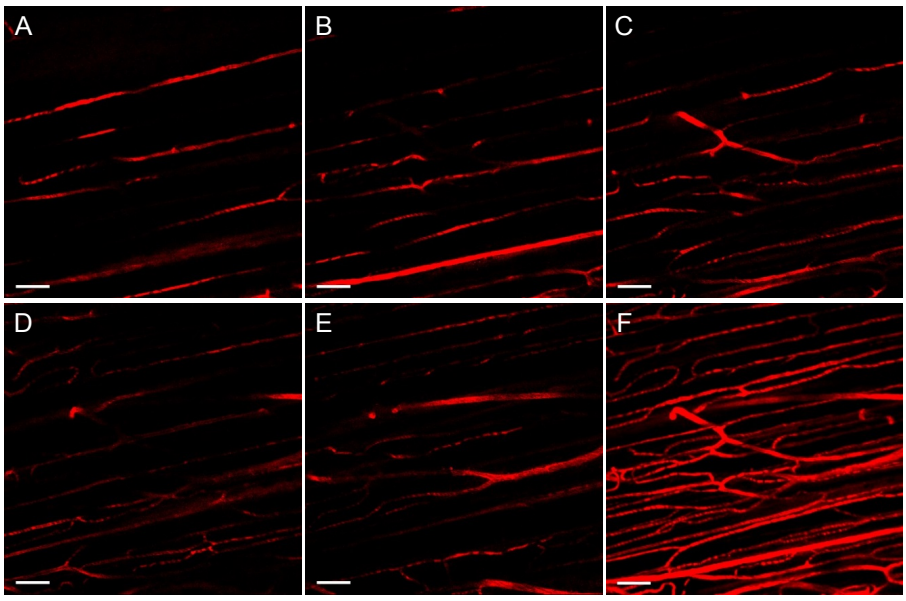

Fig. S9 Intravital TPFI for blood vessels of mouse tibial muscle stained with TPETPAFN-F127 NPs at depths of (A) 0, (B) 20, (C) 40, (D) 60, (E) 80 μm and (F) the respective Z-projected image. The scale bar is 50 μm.

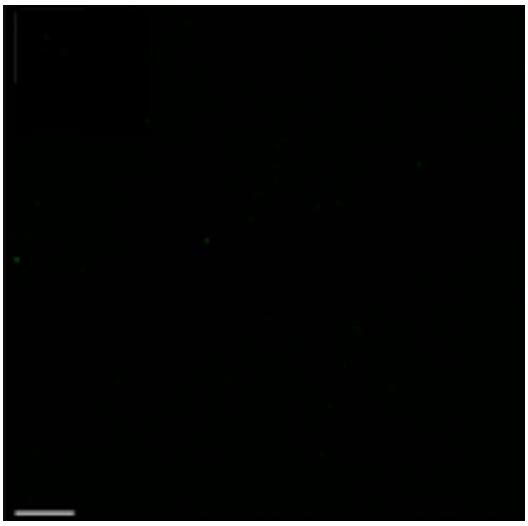

Fig. S10 The image of intravital TPFI for blood vessels of mouse tibial muscle without TPETPAFN-F127-SiO_2 NP injection; the scale bar is 50 μm.

References

1. K. Li, W. Qin, D. Ding, N. Tomczak, J. L. Geng, R. R. Liu, J. Z. Liu, X. H. Zhang, H. W. Liu, B. Liu, B. Z. Tang, *Sci. Rep.,* 2013, **3**, 1150.
2. J. L. Geng, C. C. Goh, N. Tomczak, J. Liu, R. R. Liu, L. Ma, L. G. Ng, G. G. Gurzadyan, B. Liu, *Chem. Mater.,* 2014, **26**, 1874.
3. D. A. Oulianov, I. V. Tomov, A. S. Dvornikov, P. M. Rentzepis, *Opt. Commun.,* 2001, **191**, 235.
4. Q. S. Huo, J. Liu, L. Q. Wang, Y. B. Jiang, T. N. Lambert, E. Fang, *J. Am. Chem. Soc.,* 2006, **128**, 6447.
5. S. Devi, Y. Wang, W. K. Chew, R. Lima, N. A-González, C. N. Z. Mattar, S. Z. Chong, A. Schlitzer, N. Bakocevic, S. Chew, J. L. Keeble, C. C. Goh, J. L. Y. Li, M. Evrard, B. Malleret, A. Larbi, L. Renia, M. Haniffa, S. M. Tan, J. K. Y. Chan, K. Balabanian, T. Nagasawa, F. Bachelerie, A. Hidalgo, F. Ginhoux, P. Kubes, L. G. Ng, *J. Exp. Med.,* 2013, **210**, 2321.