SUPPLEMENTAL DATA

Platelets can enhance vascular permeability

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Supplemental Figure 1. Microspheres can be employed to estimate the presence of joint permeability in arthritic mice. Non-arthritic control (left) and arthritic (right) mice were injected intravenously with 0.09 µm or 0.22 µm diameter fluorescent microspheres and the fluorescence visualized 5 minutes later using an in vivo imaging system. The control and arthritic mice received the same concentration of fluorescent microspheres. Data representative of 10 mice.
Supplemental Figure 2
Supplemental Figure 2. Microspheres can localize to joints during arthritis and upon serotonin injection independently of neutrophil transportation. (A) Non-arthritis controls and arthritic mice were injected intravenously with 0.45 µm, 0.84 µm, 3.2 µm or 10.2 µm diameter fluorescent microspheres and the fluorescence visualized 5 minutes later using an in vivo imaging system. Control mice non-arthritis and the arthritic mice all received the same concentration of fluorescent microspheres. Radiant efficiency quantifications in externalized organs (lung, heart, liver, spleen and kidney) and ankle joints were performed 45 minutes after microsphere injections. The quantification of fluorescence in organs of arthritic mice relative to non-arthritis control is portrayed. (B and C) Gr1 cell-depleted (>98%) and their isotypic control arthritic or non-arthritic control mice were injected with 0.45 µm (B) or 0.84 µm (C) microspheres and the ankle joints localization of microspheres monitored by an in vivo imaging system. Radiant efficiency quantifications in the ankle joints for all mice are presented. *** p<0.0001 (D) Gr1 cell-depleted (>98%) and their isotypic controls mice were intravenously injected with serotonin or diluent. The fluorescent microspheres (0.45 µm) were next administrated to all mice and the fluorescence in ankle joints was quantified by an in vivo imaging system. Radiant efficiency quantifications in the ankle joints for all mice are presented. ** p=0.0013
Supplemental Figure 3. A single administration of fluoxetine is not sufficient to impact the gap formation in arthritic mice. (A) Arthritic and non-arthritic mice were intraperitoneally injected with fluoxetine (2.5 mg/kg) one hour prior to the 0.45 µm microsphere injection. The fluorescence in ankle joints was monitored 5 minutes later using an in vivo imaging system. Radiant efficiency quantifications in the ankle joints for all mice are presented. (B) The serotonin concentrations in platelets isolated from mice that received a bolus administration of fluoxetine were measured by ELISA.
Supplemental Table 4

Supplemental Table 4. The gap formation in joint vasculature is independent of SERT expression during initial events of arthritis. (A) The platelet-depleting antibody or its isotype control were administrated to mice 18 hours prior K/BxN injections (100 µL). (B) WT and Slc6a4 \(^{-/-}\) mice were injected with 100 µL of K/BxN serum. (A-B) Five minutes after K/BxN injection, 0.45 µm microspheres were injected and the fluorescence was monitored 5 minutes later using an in vivo imaging system. The radiant efficiency quantifications in the ankle joints for all mice are presented. (A) * p=0.0208 (B) *** p=0.0005
Supplemental Figure 5. The gap formation in joint vasculature is independent of mast cells during arthritis. Control non-arthritic or arthritic Kit<sup>W-sh</sup> mice (day 7 post K/BxN serum transfer) and C57BL/6J mice were injected intravenously with 0.45 μm microspheres and the fluorescence was monitored 5 minutes later using an in vivo imaging system. The radiant efficiency quantifications in the ankle joints for all mice are presented. * p=0.0141 (C57BL/6) and * p=0.0335 (Kit<sup>W-sh</sup> mice)
**Supplemental video 1. Intravital visualization of the joint vasculature permeability.** Non-arthritic (A) and arthritic (B) LysM-eGFP mice were injected intravenously with dextran-fluorescein and 0.45 µm Nile-Red microspheres and imaged using 2-photon microscopy. The microsphere efflux from the vascular circulation toward the collagen matrix occurs independently of leukocyte transportation, is visible during arthritis only and evidences the presence of gaps. Red: microspheres; Green: blood vessel; Cyan blue: neutrophils and Indigo blue: collagen. Scale bar: 25 µm.