

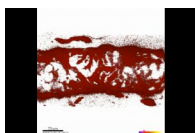
## SUPPLEMENTAL MATERIAL

Proebstl et al., <http://www.jem.org/cgi/content/full/jem.20111622/DC1>

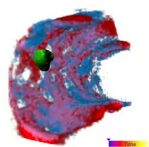
**Video 1. Development of an inflammatory response in a TNF-stimulated tissue.** The video shows a cremasteric venule ( $\sim 50 \mu\text{m}$  diam) of an  $\alpha\text{SMA-RFPcherry} \times \text{Lys-EGFP-ki}$  mouse (exhibiting both *RFPcherry*-labeled pericytes (red) and *EGFP*-labeled leukocytes (green), immunostained in vivo for EC junctions with Alexa Fluor-647-labeled anti-PECAM-1 mAb 390 (blue) and stimulated with TNF (300 ng/mouse i.s.). The video (captured at  $\sim 40\times$ ) shows the development of an inflammatory response (from 2 h after injection of the cytokine), as seen from the luminal side. Images were captured at one stack per minute and the sequence shows a 120-min period. Still images are shown in Fig. 1 B.



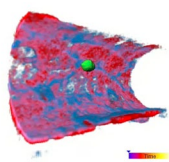
**Video 2. Abluminal neutrophil crawling along pericyte processes (example 1).** The video captures the abluminal crawling of neutrophils along pericytes processes viewed from the extravascular tissue and their subsequent migration into the tissue in a TNF-stimulated cremaster of an  $\alpha\text{SMA-RFPcherry} \times \text{Lys-EGFP-ki}$  mouse (exhibiting both *RFPcherry*-labeled pericytes and *EGFP*-labeled leukocytes in red and green on the video, respectively). The high optical zoom viewed from the extravascular space shows a single neutrophil (in green and isolated from the rest of the inflammatory response by creating an isosurface on it using IMARIS software for clarity of the image) migrating through the pericyte layer after TEM (1 stack per minute, for a total duration of 26 min) until its full migration out into the tissue. Still images of this video are shown in Fig. 2 A.



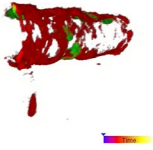
**Video 3. Abluminal neutrophil crawling along pericyte processes (example 1).** The video captures the TEM of the same leukocyte as shown in Video 2 through a paracellular pore (PECAM-1 labeling in blue), followed by abluminal crawling along pericytes processes and subsequent migration into the tissue in a TNF-stimulated cremaster of an  $\alpha\text{SMA-RFPcherry} \times \text{Lys-EGFP-ki}$  mouse (exhibiting both *RFPcherry*-labeled pericytes and *EGFP*-labeled neutrophils in red and green on the video, respectively). The neutrophil is isolated from the rest of the inflammatory response by creating an isosurface using IMARIS software for clarity of the image. At the end of the sequence, the track followed by the neutrophil during migration between the endothelium and pericyte layer is shown, alongside the displacement (arrow) between the site of TEM and final site of extravasation. Still images of this cell migration between the endothelial and pericyte layers are shown in Fig. 2, A and C, example 1.



**Video 4. Abluminal neutrophil crawling along pericyte processes (example 2).** The video shows another example of abluminal crawling of a neutrophil along pericytes processes and subsequent migration into the tissue in a TNF-stimulated cremaster of an  $\alpha\text{SMA-RFPcherry} \times \text{Lys-EGFP-ki}$  mouse. The single neutrophil (*EGFP*, green) is isolated from the rest of the inflammatory response by creating an isosurface on it using IMARIS software for clarity of the image. At the end of the sequence the track followed by the neutrophil during migration between the endothelium and pericyte layer (*RFPcherry*, red) is shown, alongside the displacement (arrow) between the site of TEM and final site of extravasation. Still images of the track followed by this cell between the endothelial and pericyte layers are shown in Fig. 2, B and C, example 2.



**Video 5. Abluminal neutrophil crawling along pericyte processes (example 3).** The video is a third example of TEM of a neutrophil (*EGFP*, green) through a paracellular pore (PECAM-1 labeling in blue), followed by abluminal crawling of pericytes processes (*RFPcherry*, red) and subsequent migration into the tissue in a TNF-stimulated cremaster of an  $\alpha\text{SMA-RFPcherry} \times \text{Lys-EGFP-ki}$  mouse. The neutrophil is isolated from the rest of the inflammatory response by creating an isosurface using IMARIS software for clarity of the image. At the end of the sequence, both the track followed by the neutrophil during migration between the endothelium and pericyte layer and the displacement (arrow) between site of TEM and final site of extravasation through the venular wall are shown. A still image of the track followed by this cell between the endothelial and pericyte layers is shown in Fig. 2 C, example 3.



**Video 6. Hot spot of neutrophil emigration through the pericyte gaps.** The video captures several neutrophils migrating through the same gap (area of  $\sim 12 \mu\text{m}^2$ ) between adjacent pericytes (hot spot) in a TNF-stimulated cremaster of an  $\alpha\text{SMA-RFPcherry} \times \text{Lys-EGFP-ki}$  mouse (exhibiting both *RFPcherry* pericytes [red] and *EGFP* leukocytes [green]). The optical zoom is viewed from the extravascular side, image stacks have been taken every minute for a period of 2 h; and still images of this sequence are shown in Fig. 4 B.