Supplemental Methods

LumeNEXT Fabrication

The LumeNEXT devices were prepared using standard photolithography and soft lithography techniques. First, silicon masters were created using photolithography. The device patterns were designed with Adobe Illustrator and printed on high-resolution photomasks in which the desired features were transparent and the remaining area was black. The device layers were created by spin-coating SU-8, a negative photoresist, onto silicon wafers. Following a soft-bake of the spin-coated wafer, the pattern was transferred by shining UV light through the photomask onto the photoresist, causing it to cure. A postexposure hard bake of the wafer was done on a hot plate. The remaining uncured SU-8 was removed by washing with an SU-8 developer, propylene glycol methyl ether acetate (Sigma) and washed with acetone and isopropyl alcohol.

The devices were then prepared using soft lithography. PDMS (polydimethylsiloxane) was poured over the silicon master on a hot plate and baked at 80°C for 4 hours. PDMS is widely used for the fabrication of microfluidic devices for a number of reasons, it is bioinert and non-toxic as well as optically clear, making it ideal for live-imaging purposes. PDMS rods were fabricated using 23 gauge needles resulting in lumens with an inner diameter of about 340µm.

The top and bottom layers of the chamber were combined and the PDMS rods were inserted into the unit creating a complete device. The devices were bonded to the glass surface by creating a hydrophilic environment using oxygen-plasma treatment.

Device and Collagen Preparation

LumeNEXT devices were prepared as previously described.[19] Briefly, devices were treated with 1% polyethylenimine, then 0.1% glutaraldehyde (Sigma-Aldrich), and washed with DI water. Collagen I (Corning) was neutralized to pH 7.2 (5mg/mL) by mixing 1:1 with 7.7 pH 2xPBS/4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) solution (ThermoFisher). The collagen was pipetted into the device and polymerized in an incubator at 37°C for at least 2h. Drops of media were added to the device's side ports and the PDMS rod was pulled from the chambers leaving behind a media filled lumen. Lumens were then incubated with 30µg/mL bovine fibronectin (Sigma-Aldrich). iECs were resuspended at $2x10^4$ cells/µL and loaded into the lumen. The devices were incubated for 2h and flipped every 30min. Unadhered cells were aspirated and replaced with fresh media. Lumens were cultured for two days with media exchanges twice daily.

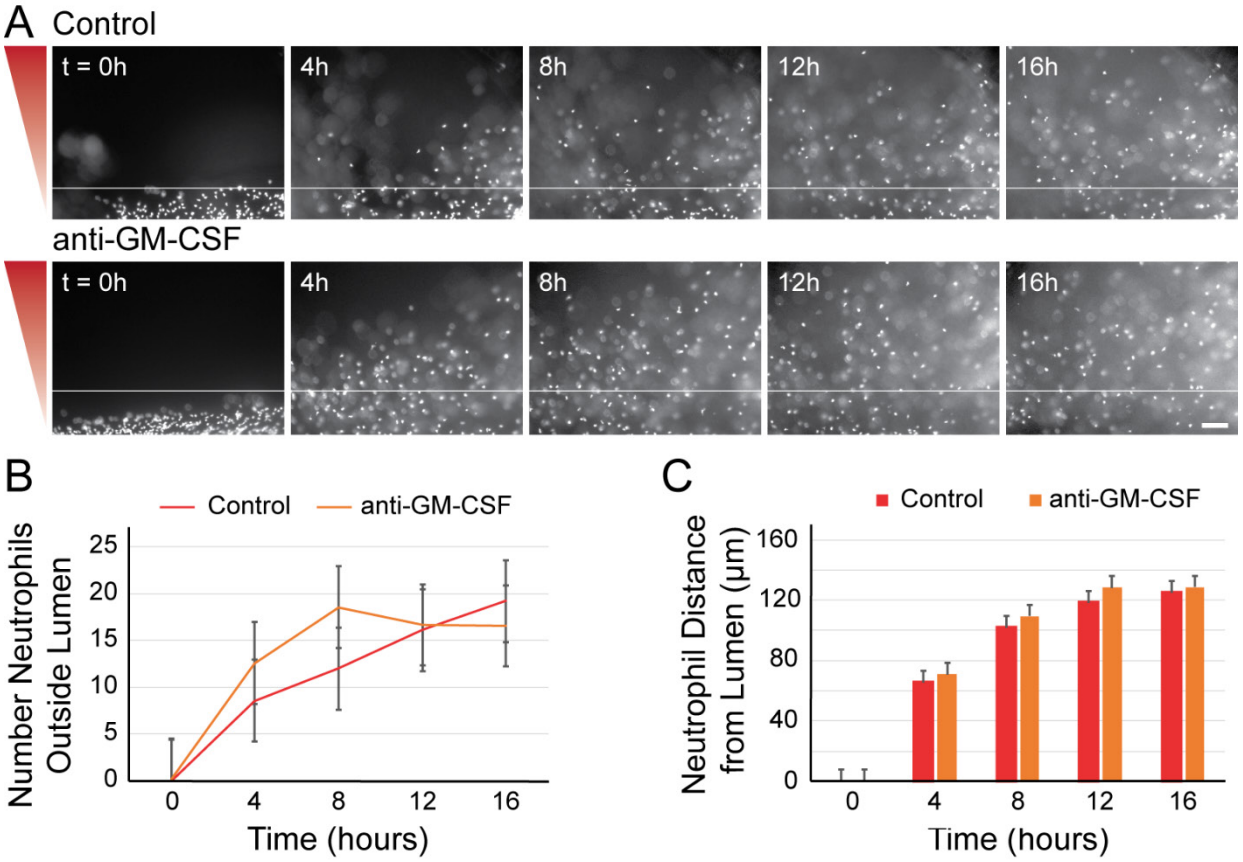
Neutrophil Isolation

Neutrophils were isolated using the MACSxpress Neutrophil Isolation Kit. Blood was drawn into Potassium/EDTA vacutainer tubes to prevent coagulation. The lyophilized MACSxpress Neutrophil Isolation Cocktail pellet was reconstituted in 2mL Buffer A. For magnetic labeling, the final cocktail was prepared by combining 1 part reconstituted pellet with 1 part Buffer B and 4 parts whole blood in a 15mL conical. The tube was then incubated for 5 minutes on the MACSmix Tube Rotator at 12rpm. The tube was then placed in the magnetic field of the MACSxpress Separator for 15 minutes. The supernatant which contains the neutrophils was collected into a fresh 15mL conical. Residual erythrocytes were removed by magnetic depletion.

Erythrocyte binding magnetic beads were added to the supernatant and incubated on the tube rotator for 5 minutes. The tube was then incubated for 10 minutes in the MACsxpress Separator. The erythrocyte-free supernatant was then collected into a fresh 15mL conical tube. The cells were pelleted at 200rcf for 5 minutes. Finally, the cells were resuspended in 1mL PBS, counted, and stained. The entire isolation protocol was performed at room temperature.

Image Processing and Data Analysis

To determine the number of migrated neutrophils, phase and fluorescent images from each experiment were opened in FIJI. The fluorescent images were leveled individually using the Adjust Window/Level function in FIJI. The cells were then outlined using the Find Edges function of FIJI. Binary images, which are required for the particle analysis plugin, were then created using the Threshold function in FIJI. Finally, a box was drawn from the edge of the lumen towards the source of *P. aeruginosa* to select an area of the image equal to 100µm for regions analysis or 400µm for total migrated neutrophil analysis. The Analyze Particles function in FIJI was used to count the number of cells in the selected region that were between 10 and 150 $pixels^2$ in area to eliminate particles that were artifacts of the image processing. The particles selected by FIJI as cells were confirmed by visualizing the outlines of counted particles. The percent migrated neutrophils was calculated by dividing the number of neutrophils in each region at each timepoint by the maximum number of total migrated neutrophils (the sum of all regions) at one timepoint in the endothelium plus neutrophils and *P. aeruginosa* condition.



Supplemental Figure 1. Neutrophil migration does not require GM-CSF signaling
Neutrophils were pre-incubated with PBS (control) or anti-GM-CSF blocking antibody for 30 minutes before starting the migration experiment and the experiment was run in the continued presence of the antibody. **(A)** Representative images of neutrophil migration. Neutrophils stained with calcein am. Bacterial gradient direction shown in red. White line indicates the edge of the endothelial lumen. Scale bar represents 100µm. **(B)** The number of neutrophils outside the lumen was counted at four-hour intervals using particle analysis in FIJI. **(C)** The distance from the lumen edge was measured for all in-focus neutrophils outside the lumen at four-hour intervals. Each bar represents the mean plus SEM. Data quantified from 9 lumens (Control) or 8 lumens (anti-GM-CSF) across 3 independent experiments.

Supplemental Video 1. *P. aeruginosa* induce neutrophil extravasation and migration
Neutrophils migrate out of an endothelial lumen to *P. aeruginosa* (bottom) but not iEC media alone (top). Phase and fluorescent images shown separately on left and right, respectively. Neutrophils stained with calcein am. Bacterial or iEC media gradient starts at the top of the frame. Imaged for 16 hours. Scale bar represents 100µm.

Supplemental Video 2. Migration to *P. aeruginosa* requires an endothelium
More neutrophils migrate further and for longer out of an endothelial lumen (bottom) compared to an unlined lumen (top). Phase and fluorescent images shown separately on left and right, respectively. Neutrophils stained with calcein am. Bacterial gradient starts at the top of the frame. Imaged for 16 hours. Scale bar represents 100μm.

Supplemental Video 3. Neutrophil migration requires IL-6 signaling
Incubation anti-IL-6R (bottom) resulted in a reduction in the number of neutrophils migrating and the distance migrated compared to uninhibited neutrophils (top). Phase and fluorescent images shown separately on left and right, respectively. Neutrophils stained with calcein am. Bacterial gradient starts at the top of the frame. Imaged for 16 hours. Scale bar represents 100μm.