Supporting Information

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SI Text

SI Materials and Methods

Microfluidic Device Fabrication. Microfluidic devices used to measure leukocyte migration in response to chemoattractant gradients were manufactured using standard microfabrication techniques. Two layers of photoresist (SU8; Microchem), the first one 10 µm thin (corresponding to the migration channels) and the second one 70 µm thick (corresponding to the FCCs) were patterned on one silicon wafer sequentially using two photolithographic masks and processing cycles according to the instructions from the manufacturer. The wafer with patterned photoresist was used as a mold to produce polydimethylsiloxane (PDMS) (Fisher Scientific) devices, which were then bonded to the base of glass-bottom 12- or 24-well plates, using an oxygen plasma machine (Nordson-March).

Preparation of Humanized NPRMs. Human MPs were prepared (1) and added to thin lipid films in glass flasks (after organic solvent removal by rotary evaporation; 10 min; 25 °C) containing fluorescent 1,2-dioleoyl-*sn*-glycero-3-phospho-L-serine-*N*-7-nitro-2-1, 3-benzoxadiazol-4-yl (100 µg; Avanti Polar Lipids) and 7*S*,8*R*, 17*S*-trihydroxy-docosa-4*Z*,9*E*,11*E*,13*Z*,15*E*,19*Z*-hexaenoic acid resolvin D1 (RvD1), 1 µg (Cayman Chemical), or *O*-[9,12]-benzo-ω6-epi-lipoxin A_4 (LXA_4 analog) (1 µg) (2) prepared for these studies by custom synthesis (Avanti Polar Lipids). Intercalation of RvD1 or LXA_4 analog and fluorescent phospholipid was performed by aqueous energy dissemination using a sonic dismembrator (output power, 15 W; 15 min; 25 °C; Fisher Scientific). Humanized NPRMs were layered on Sephadex G50 columns (Sigma-Aldrich), and fractions were collected in 0.2-µm-filtered PBS. Incorporation of RvD1 and LXA_4 analog was determined using LC-MS/MS, and fluorescent phospholipid content was confirmed by flow cytometry (FACSCanto II; BD Biosciences). NPRMs were sized using calibration beads (Corpuscular) by flow cytometry. Primary human neutrophils and monocytes were pretreated for 15 min at 37 °C with two lipid mediators, LXA_4 (10 nM) and RvD1 (10 nM), known to be anti-inflammatory and proresolving. In a similar fashion, cells were pretreated with the NPRM at a ratio of 2:1 NP per cell.

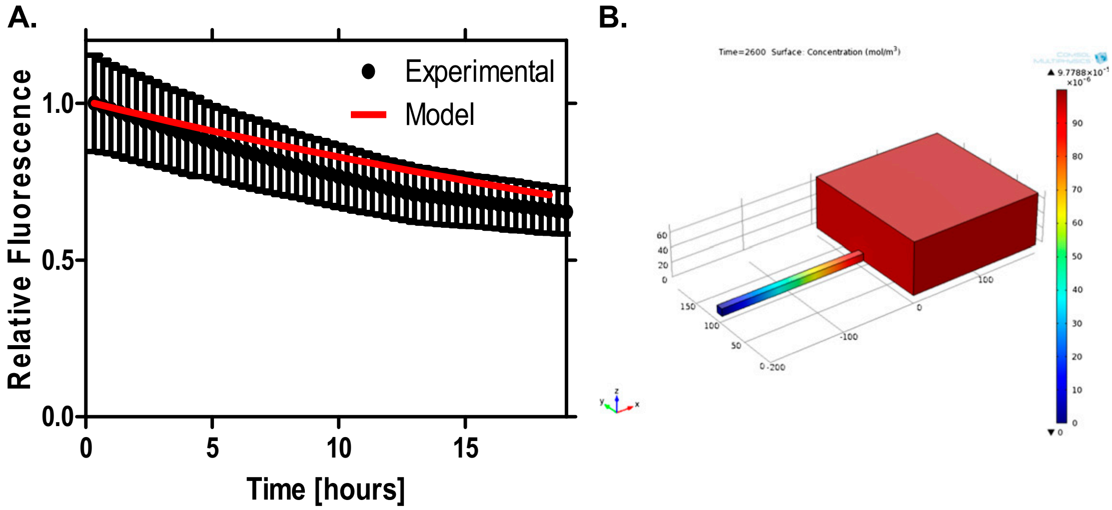
Primary Human Neutrophil and Monocyte Isolation. De-identified, fresh human blood samples from healthy volunteers, aged 18 y and older, who were not receiving immunosuppressants, were purchased from Research Blood Components, LLC. Peripheral blood was drawn in tubes containing a final concentration of 5 mM EDTA (Vacutainer; Becton Dickinson) and processed within 2 h of blood collection. Using a sterile technique, neutrophils and monocytes were isolated from whole blood using HetaSep and a Ficoll-Paque gradient, respectively, followed by the EasySep Human Neutrophil and Monocyte Enrichment Kits (STEMCELL Technologies) following the manufacturer's protocol. The purity of neutrophils and monocytes was assessed to be >98% using the Sysmex KX-21N Hematology Analyzer (Sysmex America, Inc.). Neutrophils were stained with Hoechst (32.4 µM) (Sigma), and monocytes were stained with the PKH26 Red Fluorescent Cell Linker Kit for Phagocytic Cell Labeling (4 µM) (Sigma) following the manufacture's protocols. The final aliquots of neutrophils and monocytes were resuspended in RPMI plus 10% FBS (stock 50 mL FBS/450 mL RPMI) (Sigma-Aldrich) at a concentration of 20,000 cells/2 µL and kept at 37 °C during pretreatment with different proresolving molecules and NPRMs. Cells were then immediately introduced into the microfluidic device for the chemotactic assay. The ratio between neutrophils and monocytes for the heterogeneous samples was 2:1. All experiments were repeated at least three times with neutrophils and monocytes from three different donors.

Microfluidic Assay Preparation and Analysis. To prime the microfluidic devices, 15 min before neutrophils were introduced, each CLC was filled with and surrounded by 10 µL of the chemoattractant of interest and extracellular matrix protein. The device was then placed in a vacuum for 15 min. The chemoattractant filled all of the FCCs as the air was displaced. The devices were then vigorously washed three times with 1× PBS to remove any residual chemoattractant that was outside of the FCCs. The device was then submerged in 0.5 mL of cell media. Neutrophils and or monocytes (20,000 cells/2 µL) were then pipetted into the CLC using a gel-loading pipette tip. Neutrophil migration into the migration channel toward the FCC started immediately and was recorded using time-lapse imaging on a fully automated Nikon TiE microscope (10× magnification) with biochamber heated to 37 °C with 5% carbon dioxide gas. Monocyte migration began after a 2-h delay. Image analysis was performed using Elements software (Nikon). Cell counts in the FCC were determined automatically using Elements software.

Functional Single-Cell Elastase Assay. To determine whether monocytes recruited to the FCC were phlogistic or nonphlogistic, we implemented a functional assay in the FCC. We used EnzChekElastase Assay Kit from Molecular Probes, in which a DQ elastin, a soluble bovine neck ligament elastin, has been labeled with BODIPY FL dye such that the conjugate's fluorescence is quenched. The DQ elastin is added to the chemokine solution before the device is primed. This nonfluorescent substrate can be digested by elastase secreted by proinflammatory neutrophils and monocytes to yield highly fluorescent fragments. Digestion products from the substrate have adsorption maxima at ∼505 nm and fluorescence emission maxima at ∼515 nm. Time-lapse imaging (Nikon with biochamber) measured the increase in fluorescence, and because the assay is continuous, kinetic single-cell data could be obtained. Elements software was used to quantify the halo of fluorescence surrounding phlogistic monocytes. The signal from neutrophils was strong, so an average intensity of the entire FCC was measured for the duration of the experiment. Calibration curves relating fluorescence to elastase activity (units per milliliter) for 1-, 2-, 5-, and 8-h incubation times were created by priming the device with a known concentration of porcine pancreatic elastase. We created a calibration curve to correlate fluorescence units to elastase activity (units per milliliter) (R^2 = 0.9992). Neutrophils were used as a direct positive control and were measured with 2,500 fluorescence units after 5 h of culture.

1. Norling LV, et al. (2011) Cutting edge: Humanized nano-proresolving medicines mimic inflammation-resolution and enhance wound healing. *J Immunol* 186(10):5543–5547.

2. Petasis NA, et al. (2008) Design and synthesis of benzo-lipoxin A4 analogs with enhanced stability and potent anti-inflammatory properties. *Bioorg Med Chem Lett* 18 (4):1382–1387.



C.

Molecule Type	Molecular Weight [kDa]	Diffusion Coefficient [cm^2/s]	[FCC], t $_{½}$ [hrs]
MCP1	1.1	$3.9*10^{-7}$	34
IL-8	8	$3.5*10^{-7}$	38
fMLP	0.4	10^{-6}	11
LTB_4	0.3	10^{-6}	11
LXA_4	0.3	10^{-6}	11
Elastin	640	10^{-8}	> 20 days
Tropoelastin	70	10^{-7}	8 days
Elastase	29.5	$2*10^{-7}$	4 days

Fig. S1. Diffusion modeling of chemoattractant gradient in device. (*A*) Finite element model (COMSOL) of diffusion of chemoattractant gradient of device and comparison with experimental measurements of small-molecular-mass (1 kDa) fluorescent dye diffusing out of the FCCs. (*B*) Visualization of the gradient in the migration channels as simulated using COMSOL finite element modeling software package. (*C*) Summary of diffusion coefficients for chemoattractants used in experiments and the time for concentration of chemoattractant in the FCC to decrease to half of its original levels.

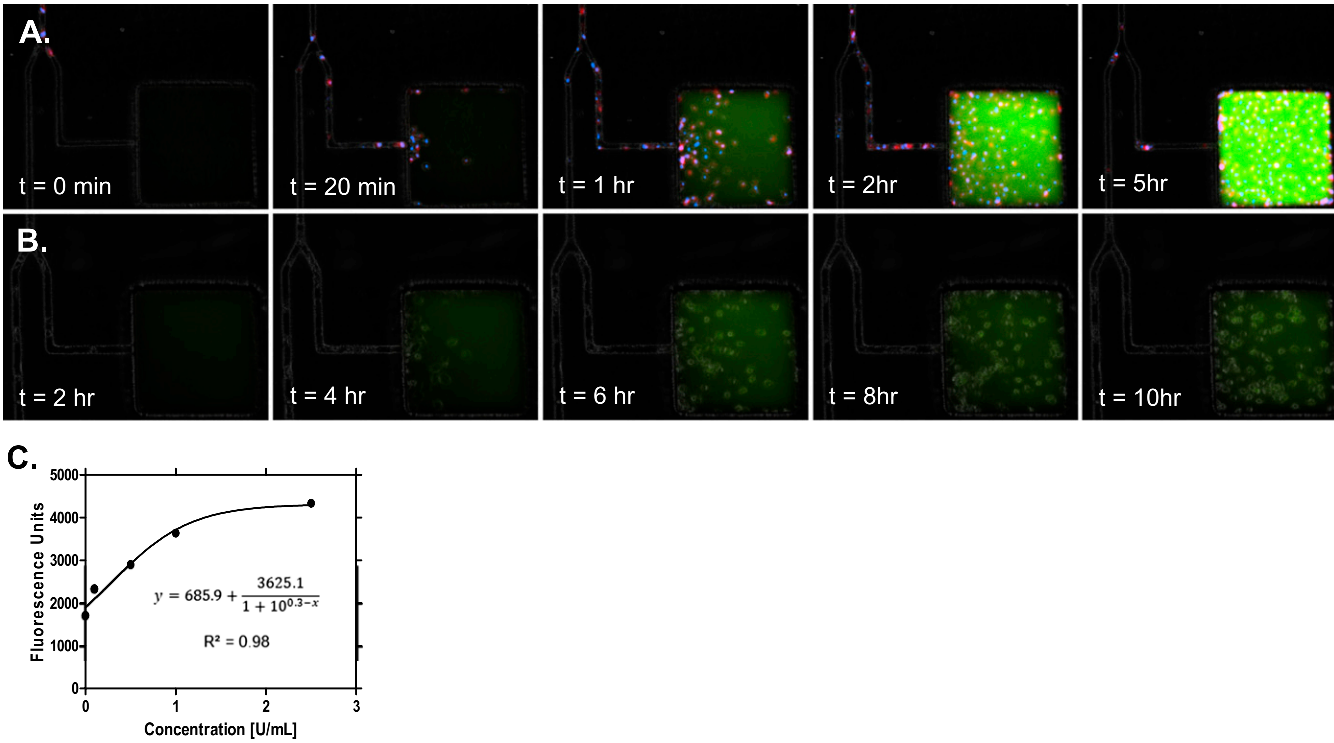


Fig. S2. Elastase assay validation. (*A*) Neutrophils migrate to LTB_4 (100 nM), which stimulates elastase activity. Using the EnzChekElastase Assay Kit from Molecular Probes, a fluorescent signal is produced upon substrate cleavage by the enzyme. The sequence of time-lapse images illustrates the strong elastase activity after 5 h (Movie S4). (*B*) Monocytes migrate to LTB_4 and a halo around the cells is observed identifying enzyme after 10 h (Movie S5). (*C*) Calibration curve created by adding known concentrations of porcine pancreatic elastase to the FCC.

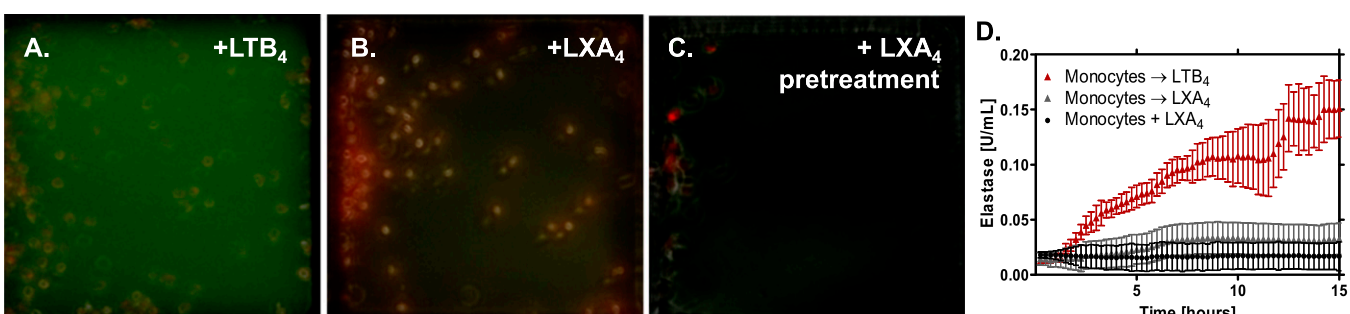


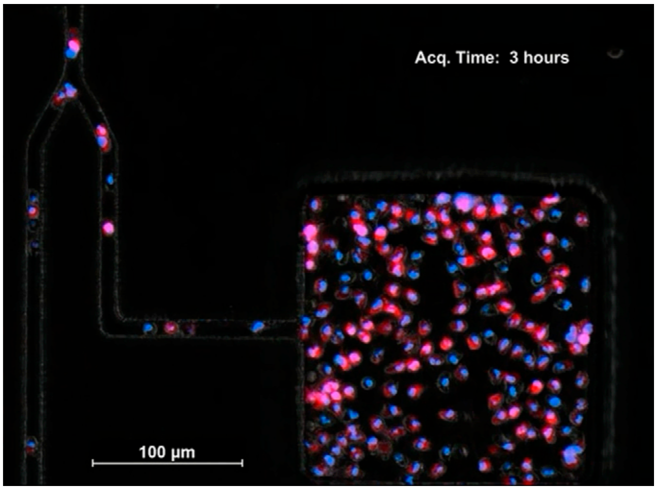
Fig. S3. Monocyte migration and elastase release. (*A*) Monocytes migrating to LTB_4 (100 nM) produce an elastase signal of ~0.1 U/mL after 6 h. (*B*) Monocytes migrate to LXA_4 (100 nM), and the elastase signal is significantly reduced compared with that seen with migration to LTB_4 (100 nM). (*C*) Monocytes are pretreated with LXA_4, and migration dramatically decreases along with the production per cell of elastase. (*D*) Graph of elastase release (units per milliliter) vs. time (hours) for previously described conditions (*A*–*C*).

Table S1. Summary of dynamic constants describing neutrophil and monocyte migration to an array of chemoattractants

Chemoattractant	Function in vivo	Cell type	Delay (h)	Accumulation time (h)	Plateau no. of cells	Rate (cells/h)	$t_{1/2}$ (h)
fMLP	Proinflammatory; derived from bacterial protein degradation or from mitochondrial proteins upon tissue damage; strong chemoattractant induces adherence, degranulation, and production of tissue-destructive oxygen-derived free radicals in phagocytic cells	PMN	0.16	4	285	71	2
		Mono	6	8	17	2.1	7
IL-8	Proinflammatory; produced by macrophages, epithelial, and endothelial cells; induces chemotaxis of granulocytes	PMN	>0.16	3	173	57.7	1
		Mono	Ø	Ø	Ø	Ø	Ø
LTB_4	Proinflammatory; produced from leukocytes in response to inflammatory mediators; induces the adhesion and activation of leukocytes on the endothelium, allowing them to bind to and cross it into the tissue; potent chemoattractant and induces the formation of reactive oxygen species and the release of lysosome enzymes by PMNs	PMN	>0.16	2.5	282	112.8	2
		Mono	4	7	73	10.4	6
MCP-1	Proinflammatory; produced by endothelial cells, mononuclear cells, mast cells, T cells, osteoblasts, and fibroblasts; attracts monocytes and basophils but not neutrophils or eosinophils; may be involved in the recruitment of monocytes into the arterial wall during the disease process of atherosclerosis	PMN	Ø	Ø	Ø	Ø	Ø
		Mono	4	>15	44	4	10
LXA_4	Anti-inflammatory and proresolving; biosynthesized by leukocytes; inhibits platelet activating factor (PAF)- or fMLP-induced neutrophil and eosinophil chemotaxis in vitro	PMN	9	3	5	2.5	9
		Mono	3	9	31	3.4	4

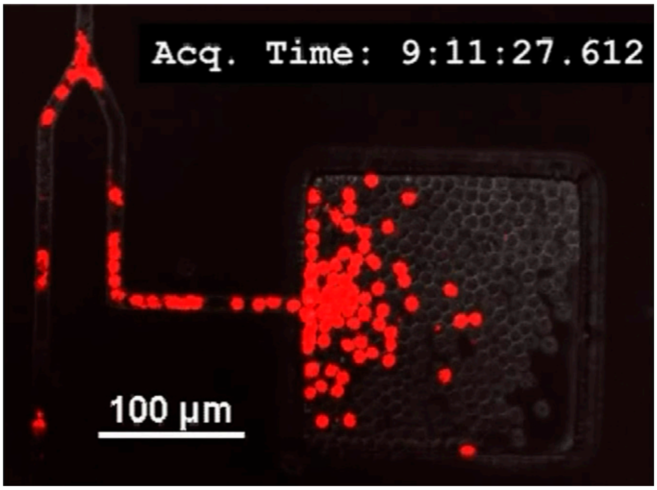
For further detail on the chemoattractant mediators see refs. 1 and 2. Mono, monocyte. Ø, no migration.

1. Cassatella MA (2010) in *Fundamentals of Inflammation*, eds Serhan CN, Ward PA, Gilroy DW (Cambridge University Press, New York), pp 49–64.
2. Serhan CN, Haeggström JZ (2010) in *Fundamentals of Inflammation*, eds Serhan CN, Ward PA, Gilroy DW (Cambridge University Press, New York), pp 153–175.



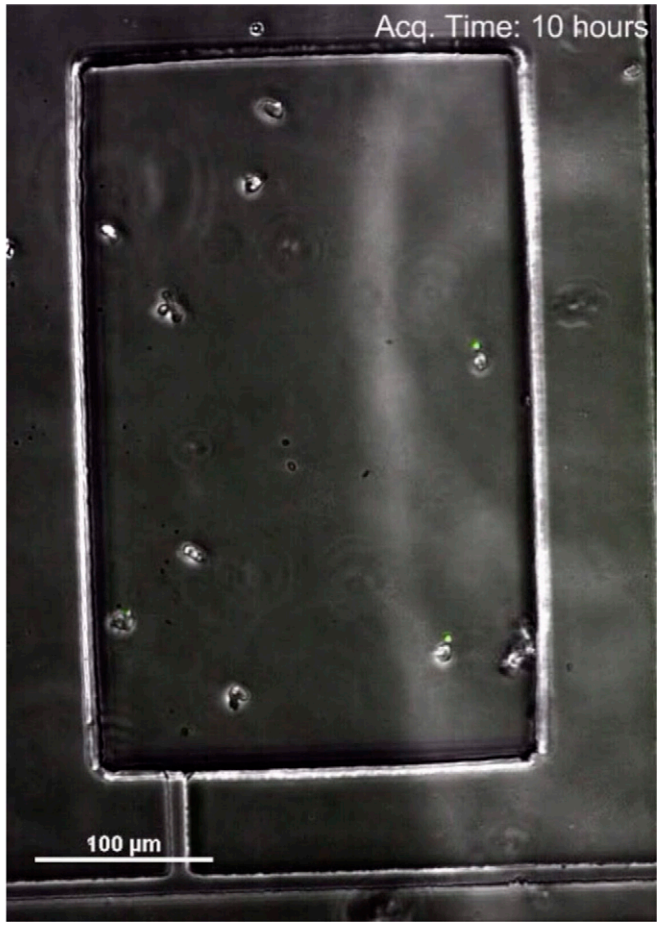
Movie S1. Double-stained (blue, nucleus; red, membrane) neutrophils begin migrating along the chemoattractant gradient after 20 min. Cells continue to migrate into the FCC until the chamber is filled with cells by 5 h. There is no pressure gradient influencing cell migration in microfluidic device.

Movie S1



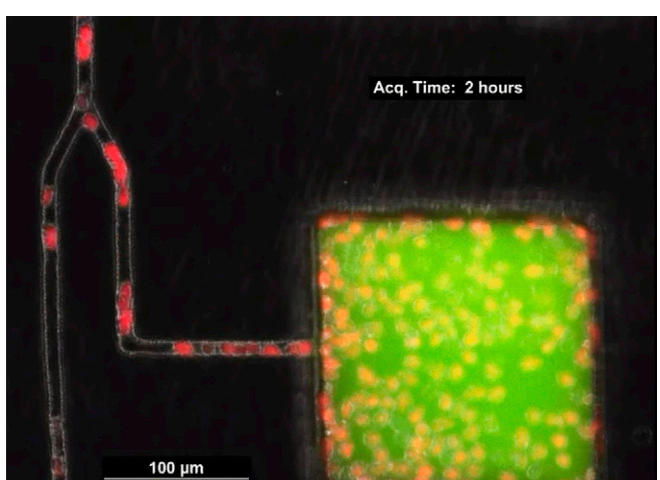
Movie S2. Neutrophils (bright field) and monocytes (red) dynamically migrate to LTB_4 (100 nM). Neutrophils were imaged every 10 min, and PKH26 fluorescently tagged monocytes every hour. This in vitro model correlates to the dynamics seen in vivo where neutrophils migrate to afflicted site after injury or microbial infection. This is followed by migration of monocytes to site of inflammation.

Movie S2



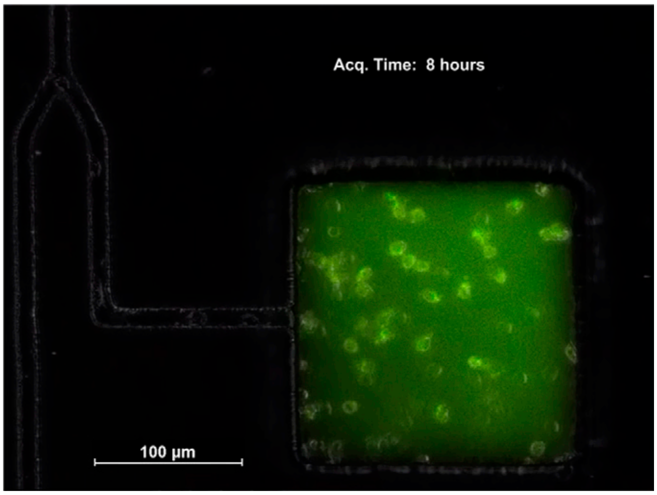
Movie S3. Monocyte migration after interacting with a fluorescently labeled (green) proresolving nanomedicine particle (NPRM). Images were acquired every 20 min.

Movie S3



Movie S4. Neutrophils migrate to LTB_4 (100 nM), which stimulates elastase activity. Using the EnzChekElastase Assay Kit from Molecular Probes, a fluorescent signal is produced upon substrate cleavage by the enzyme. The movie illustrates the strong elastase activity after 5 h.

Movie S4



Movie S5. Monocytes migrate to LTB_4, which stimulates elastase activity. Using the EnzChekElastase Assay Kit from Molecular Probes, a fluorescent halo around the cells is observed identifying enzyme after 10 h.

Movie S5