ONLINE METHODS

Cell culture. HL-60 cells were grown in RPMI 1640 (with L-glutamine, Cellgro) with 25 mM Hepes (Hepes solution, cell culture–tested; Sigma), 10% FBS (certified and heat-inactivated; Invitrogen) and 1% antibiotic-antimycotic (100×, Invitrogen). The cells were differentiated with growth medium containing 1.3% DMSO (Hybri-Max, sterile-filtered and hybridoma tested; Sigma) 5–8 d before the microscopy experiments.

PLGA microparticle fabrication. Polylactic-co-glycolic acid (PLGA) 50/50 had a molecular weight of approximately 10^5 g mol^{-1}, which corresponds to an inherent viscosity of 0.95–1.10 dl g^{-1} in hexafluoroisopropanol (Absorbable Polymers; Durect Corporation). N-formyl-Met-Leu-Phe (fMLP), CD, polyvinylalcohol (PVA, 87–89% hydrolyzed, 30,000–70,000 g mol^{-1}), dimethyl sulfoxide (DMSO), methylene chloride (DCM) had reagent-grade or higher and were used without further purification (all purchased from Sigma-Aldrich).

PLGA microparticles loaded with fMLP or CD were prepared using a solvent evaporation–spontaneous emulsion technique[20]. For particles with 9% nominal loading of fMLP, 10 mg of lyophilized fMLP powder was added directly to a PLGA solution of 100 mg polymer in 2 ml of DCM. For particles with 17% nominal loading of fMLP, 20 mg of lyophilized fMLP powder was added directly to a PLGA solution of 100 mg polymer in 2 ml of DCM. For particles with 1% nominal loading of fMLP, fMLP was dissolved at a concentration of 5 mg ml^{-1} in PBS and 200 µl of this solution was added to a PLGA solution of 100 mg polymer in 2 ml of DCM. For particles with 9% nominal loading of CD, 5 mg of CD were dissolved in 1 ml of DCM and added to a PLGA solution of 50 mg polymer in 1 ml of DCM.

When the fMLP powder was added directly to the PLGA solution, the solution was sonicated on ice twice for 10 s at 38% amplitude (GEX600 600 W ultrasonic processor). This step was omitted when the fMLP or the cytochalasin was dissolved before adding it to the PLGA solution. The PLGA solution was added dropwise under vortexing to 4 ml (2 ml in case of the cytochalasin beads) of 1 wt% PVA aqueous solution. The mixture was sonicated for 2 × 10 s on ice at 38% amplitude. The resulting oil-in-water droplets were hardened by evaporating the DCM in 200 ml (75 ml in the case of the cytochalasin beads) of 0.3 wt% of PVA aqueous solution with magnetic stirring for 3 h at room temperature (20–22 °C). The particles were washed three times by centrifugation at 12,000*g* for 5 min and then recovered by lyophilization and stored at −20 °C.

Scanning electron microscopy. The morphology of the fMLP-loaded PLGA particles was measured by scanning electron microscopy (SEM). Particles were fixed on an aluminum stub using two-sided carbon tape and sputter-coated with gold under vacuum in an argon atmosphere at a sputter current of 40 mA (model 108auto; Cressington). The samples were then imaged using a Philips XL-30 ESEM with 10 kV of accelerating voltage.

Dynamic light scattering. Particle sizes were measured by dynamic light scattering using a Brookhaven ZetaPALS system equipped with a particle sizer (Brookhaven Instruments Corporation). The particles were diluted to 0.01 mg ml^{-1} in DI water to give an average count rate of 200,000 c.p.s. before testing. Correlation functions were collected at a scattering angle of 90° (incident beam wavelength, 532 nm). Then the effective diameter and polydispersity of the particle were calculated using the manufacturer's particle sizing software (version 2.27).

Release measurements. PLGA beads loaded with fMLP samples were prepared by dispersing in HBSS at a concentration of 5 mg ml^{-1}. We added 200 µl of this solution into a 1.5 ml microcentrifuge tube for each time point (5, 30, 60 min and so forth) of the release curve (**Supplementary Fig. 2b**). At the respective time points, the tube was centrifuged at 2,300*g* for 3 min and 150 µl of the supernatant was collected. The supernatant was then either used undiluted for an ultraviolet (UV) fluorescence spectroscopy measurement of the fMLP concentration or diluted 1:100 for a high-performance liquid chromatography measurement of the fMLP concentration.

High-performance liquid chromatography was done using a Thermo Separation Products Spectra system P4000 pump, ThermoFinnigan Surveyor Plus photodiode array detector (PDA), a YMC-Pack ODS-AQ analytical column (length = 250 mm, inner diameter = 4.6 mm, particle size = 5 µm) and Finnigan LCQ Deca mass spectrometer (HPLC-MS). Mass spectra were obtained using electro-spray ionization (± ESI) with 5kV spray voltage, 275 °C capillary temperature, and a 5.5 mm inner diameter capillary. Sheath and sweep gas flow rates were 40 and 20 (arbitrary units as used by the control software Finnigan Xcaliber V 1.3), respectively. Eluant composition was 0.1% formic acid in ACN (solvent a), 10 mM ammonium formate (solvent b) and 10 mM ammonium formate in 90% ACN (solvent c). The mobile phase elution (1 ml min^{-1}) was isocratic (with a solvent ratio a:b:c of 4:72:24 for 13.5 min, ramped to 4:0:96 over 4.5 min, and isocratic (4:0:96) for 17 min. Aliquots (200 µl) from the fMLP matrix were removed temporally, spiked with 2 µg of *boc*-lysine (2-(*tert*-butoxycarbonylamino)-6-aminohexanoic acid) internal standard, and 20 µl full-loop injections were made. fMLP relative retention was 1.17 ± 0.2 min and the method limit of detection 1.7 ± 2 ng was achieved in negative mode using select ion monitoring of the mass-to-charge ratio (*m/z*) 436 and 499 (**Supplementary Fig. 9**).

The UV light fluorescence spectroscopy detection of fMLP was done with a spectrofluorimeter (FluoroMax-3, HORIBA Jobin Yvon). The excitation wavelength was 240 nm and the fluorescence emission spectrum was recorded in the range from 260 nm to 350 nm (**Supplementary Fig. 10**). The peak region of this spectrum around 284 nm was used for the quantification of the concentration of fMLP.

Gradient chamber assay. Fibronectin (from bovine plasma; Sigma) was dissolved in sterile water at 1 mg/ml concentration for 1 h at room temperature and then diluted 1:5 in sterile filtered calcium and magnesium–free (Ca/Mg-free) PBS (DPBS; VWR). We plated 100 µl of the diluted fibronectin solution on a coverslip and incubated for 1 h at room temperature. Then the fibronectin was aspirated and the coverslips were washed twice with Ca/Mg-free PBS. Then 200 µl of modified HBSS (mHBSS: HBSS (Invitrogen) with 0.2% (wt/vol) BSA (low endotoxin) (Sigma-Aldrich)) were plated on the coverslip for 5 min. Differentiated HL-60 cells were washed once (400*g* for 1 min) in mHBSS and plated on the coverslip and incubated for 20 min at 37 °C in a humid chamber. Unbound cells were washed three times with mHBSS[31].

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The coverslip was mounted in a Zigmond gradient chamber. One reservoir of the chamber was filled with mHBSS buffer containing chemoattractant and the other reservoir with plain mHBSS buffer. As chemoattractant we either used fMLP directly (as purchased from Sigma-Aldrich) or we used fMLP loaded into and subsequently released from PLGA beads. For collecting the released fMLP, PLGA beads loaded with fMLP were dispersed in mHBSS buffer. After a certain amount of time (30 min or 60 min), the beads were centrifuged (at 2,300*g* for 5 min) and the supernatant containing the released fMPL was collected. This supernatant was diluted in buffer (mHBSS) and filled into one reservoir of a Zigmond gradient chamber[22] and pure control buffer (mHBSS) was filled into the other reservoir. The cellular response was recorded with time-lapse microscopy on a Nikon TE-2000 microscope with a ×10 phase contrast lens at a frame rate of six frames per minute.

Single-cell stimulation experiments. Fibronectin (from bovine plasma, Sigma-Aldrich) was dissolved in sterile water at 1 mg ml^{-1} concentration for 1 h at room temperature and then diluted 1:5 in sterile filtered Ca/Mg-free PBS (DPBS; VWR). We plated 125 µl of the diluted fibronectin solution on a coverslip and incubated for 1 h at room temperature. Afterwards the fibronectin was aspirated and the coverslips were washed twice with RPMI 1640 medium (RPMI; Cellgro) containing 1% FBS and 25 mM Hepes. 100 µl of differentiated HL-60 cells at a concentration of about 1.2×10^6 cells ml^{-1} were plated on the coverslip and incubated for 20 min at 37 °C in a humid chamber. Unbound cells were washed three times with RPMI. PLGA particles loaded with fMLP were dispersed in RPMI. The size distribution of the beads was narrowed by the following washing procedure: 1 ml of the RPMI medium containing 1 mg of beads was centrifuged in 1.5 ml tubes at 1,000*g* for 3 min. The supernatant was discarded and the pellet was redispersed in 1 ml RPMI. This solution was then diluted 1:500 in RPMI and plated on the coverslip with the cells. The coverslips were mounted into the Nikon TE-2000 microscope equipped with holographic optical tweezers. Individual PLGA particles were trapped and manipulated at laser powers of around 5 mW in the focal plane.

We imaged our samples using differential interference contrast (DIC) microscopy using an inverted microscope (TE2000, Nikon) with an oil immersion objective lens (Apochromat TIRF ×100, NA 1.49; Nikon). The images were recorded with a charge-coupled device (CCD) camera (ORCA ER; Hamamatsu) at a frame rate of 1 Hz. To manipulate the microparticles, we used holographic optical tweezers. A liquid crystal spatial light modulator (HEO 1080P, Holoeye) was used as a diffractive optical element for a laser beam with a wavelength of 1,064 nm (Compass 4W CW laser; Coherent). The spatial light modulator was placed in a conjugate focal plane of the microscope objective. The computer-controlled holographic patterns on the spatial light modulator dephased the laser beam spatially to allow the creation of multiple laser spots in the focal plane of the microscope. The real-time manipulation of the microparticles and the image acquisition was controlled by software implemented in Matlab (Mathworks).

Microthermometry and spinning disk confocal microscopy. PLGA particles were suspended in a solution of Rhodamine B (0.05 mM). Rhodamine B is a temperature-sensitive fluorescent dye that shows a decrease in fluorescence by 2.3% per 1 °C temperature increase. The sample was excited with a 561 nm laser and the emitted fluorescence was imaged with a spinning disk confocal microscope (Nikon Eclipse Ti microscope equipped with a spinning disk confocal head and an Andor iXon EM-CCD camera). Ratiometric imaging was done by dividing an image of a particle in an optical trap at high laser power (230 mW in focal plane) by a reference image at 0 mW laser power. The resulting ratio image was subtracted from 1 and then multiplied by a factor of −44 to derive the temperature distribution around the bead. An offset was added to normalize the background temperature to the room temperature of 24 °C.

YFP actin transfection. YFP-actin was amplified by PCR (polymerase chain reaction) using the following sets of primers: 5′-AACGAATTCATGGTGAGCAAGGGCGAGGA-3′ and 5′-GCGGAATTCCTAGAAGCATTTGCGGTGGACG-3′ before it was inserted into pCMV vector to generate pCMV-YFP-actin plasmid. For transient transfection of HL-60 cells, 2 million DMSO-differentiated HL-60 cells were electroporated with 1.5 µg endotoxin-free plasmids (pCMV-YFP-actin) using the Nucleofector Kit V with the Amaxa Nucleofector system (Lonza) according to the manufacturer's instructions. The cells were then cultured for 24 h in Iscove's Modified DMEM containing 20% FBS before imaging.

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