Supporting Information for

"Bull's Eye" Janus Particles as Artificial Antigen-Presenting Cells for T Cell Activation

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Experimental Section

1. Reagents and cells

Silica particles (3 μm in diameter, 5% w/v) were purchased from Spherotech Inc (Lake Forest, IL). Bovine serum albumin (BSA) and biotinylated BSA (BSA-biotin) were purchased from Thermo Scientific (Waltham, MA). Biotin N-hydroxysuccinimide ester (biotin-NHS) was purchased from Sigma-Aldrich (St. Louis, MO). Anti-human CD3 (anti-CD3) OKT antibody was purchased from eBioscience (San Diego, CA) and conjugated with biotin-NHS. Phalloidin Alexa Fluor-647 conjugate was purchased from Cell Signaling Technology (Danvers, MA). Fibronectin (human) was obtained from BD Biosciences (San Jose, CA) and further conjugated with Alexa Fluor-488 carboxylic acid, succinimidyl ester from Life Technologies (Grand Island, NY). Protein kinase C (PKC)-θ antibody was purchased from Santa Cruz Biotech (Dallas, Texas). Fluo-4 AM and Alexa Fluor-647 chicken anti-goat IgG (H+L) were purchased from Life Technologies (Grand Island, NY). Polydimethylsiloxane (PDMS; Sylgard 184) was obtained from Dow Corning (Midland, MI) and used at 2:1 (w:w) base-to-curing-agent ratio. Jurkat T cells (clone E6-1) were a gift from Prof. Jay T. Groves (University of California, Berkeley) and originally purchased from ATCC (Manassas, VA). Jurkat T cells were cultured in RPMI 1640 complete growth media supplemented with 10 % fetal bovine serum (FBS), 1 mM sodium pyruvate, 100 units/mL penicillin, and 100 μg/mL streptomycin. Ultrapure water (resistivity of 18.2 MΩ·cm) was used. Calcium-containing imaging buffer (121 mM NaCl, 6 mM $NaHCO_3$, 5.4 mM KCl, 5.5 mM D-glucose, 0.8 mM $MgCl_2$, 25 mM HEPES, 1.8 mM $CaCl_2$, pH 7.4) was used for live-cell imaging experiments. Live-cell imaging chambers and temperature controller were purchased from Bioptechs (Butler, PA).

2. Microcontact printing of Janus particles

Silica particles and glass microscope slides were treated with piranha solution (H_2SO_4:30% H_2O_2 3:1) at 75 °C for 15 minutes (*Caution: Piranha solution is extremely corrosive*) and rinsed with ultrapure water. Monolayers of particles were made on the glass miscroslide sides via a solution evaporation method.[1] Sylgard 184 base and curing agent were mixed at 2:1 (w:w) ratio in a plastic cup, poured into a flat petridish, degassed in vacuum until no more bubbles were visible, and cured at 65 °C for 12 hours. Small sections of the PDMS stamp (1 cm × 1 cm) were cut out and treated with piranha solution to make the surface hydrophilic. To generate the reverse "bull's eye" pattern in which a fibronectin patch is surrounded by anti-CD3 molecules, the top surface of a PDMS stamp was incubated with fibronectin Alexa Fluor-488 conjugate solution (2 μg/mL) for at least 20 minutes, dried under a stream of filtered air, and immediately pressed against a monolayer of silica particles at a pressure of 1.5×10^4 Pa. After 3 minutes, the stamp with embedded silica particles was peeled off from the substrate and incubated with BSA-biotin Alexa Fluor-568 conjugate solution (16.5 μg/mL) for 1.5 hours. Particles were sonicated off the PDMS stamp and harvested in 1× PBS buffer containing BSA (0.005 %, w/v). Janus particles with the native "bull's eye" pattern were prepared through the same procedure using a PDMS stamp inked with BSA-biotin Alexa Fluor-568 conjugate. We noticed that many native "bull's eye" particles exhibited a gap between the BSA-biotin patch and fibronectin-covered surface after incubation with fibronectin, likely because fibronectin molecules were prevented from adsorbing onto the particle surface near the PDMS stamp due to the large size. The gap was filled by an additional step of incubation in a diluted fibronectin solution (1 μg/mL in 1× PBS buffer) for 2 hours. The Janus particles were further functionalized with streptavidin (100 nM) and then biotinylated anti-CD3 (20 nM).

3. Calcium imaging and analysis

Jurkat T cells were serum starved in serum-free cell media at 37 °C for 2 hours before imaging. To load cells with the intracellular calcium indicator Fluo-4 AM, 1 million cells were incubated with 5 μg/mL Fluo-4 in serum-free cell media at 37 °C for 30 minutes, washed, and then incubated in serum-containing cell media at 37 °C for another 30 minutes to allow complete de-esterification of Fluo-4. The Fluo-4 loaded T cells were suspended in 1× imaging buffer and added into an imaging chamber at 37 °C after the addition of Janus particles. Concentrations of Janus particles and cells were kept the same in all experiments. Time-lapse multi-channel epi-fluorescence images were immediately acquired on a Nikon Eclipse Ti microscope system equipped with an Andor iXon3 EMCCD Camera and a Nikon Plan Apo 40×/0.95 N.A objective or a Nikon Plan Apo 100×/1.49 N.A TIRF objective. Images were acquired with 100-millisecond exposure time, 2-second interval time and a total duration of 1000 seconds. Imaging parameters such as laser intensity, EMCCD gain and exposure, were all kept exactly the same for each set of imaging experiments. Imaging chambers were maintained at 37 °C with a temperature controller.

A Matlab script was used to quantify the fluorescence intensity of individual cells as a function of time. The algorithm detects the outlines of individual cells and calculates the integrated fluorescence intensity for each cell. Cells not in contact with any particles were removed manually. T cell calcium response was quantified based on a previously reported method.[2] Due to uneven loading of dyes, cells had different basal fluorescent intensity at resting state. A basal intensity for each cell was obtained by averaging the fluorescence intensities of the first 25 imaging frames before the first calcium peaks. The fluorescence intensity of each cell was then normalized against the basal intensity to enable comparison of the calcium signaling between

different samples. For a T cell to be considered activated, its calcium response must meet two criteria: peak intensity of the calcium elevation must be at least 5 times higher than the basal intensity, and each calcium peak must persist longer than 1 minute. The activation percentage (% activation) is the percentage of T cells that are activated. Average fluorescence amplitude was obtained by averaging normalized fluorescence intensities of all frames for each cell. Response fraction was calculated as the fraction of time during which a T cell remained activated.

4. Immunofluorescence staining and confocal fluorescence imaging

Jurkat T cells were serum starved at 37 °C for 2 hours before mixing with particles. 5 million cells were mixed with Janus particles in 1× imaging buffer solution for 4 minutes before fixation. Cells were fixed with 2 % (w/v) paraformaldehyde (PFA) on ice for 15 minutes, permeabilized with 0.01 % Triton X-100 for a few seconds, and blocked with 1 % BSA for 1 hour. To label actin, permeabilized cells were incubated with 0.32 μg/mL phalloidin Alexa Fluor-647 for 30 minutes at room temperature. To label PKC-θ, permeabilized cells were incubated with 1 μg/mL PKC-θ antibody at room temperature for 2 hours, washed with 1× PBS solution for 3 times, blocked with 1% BSA for 30 minutes, and incubated with 1 μg/mL chicken anti-goat IgG (H+L) Alexa Fluor-647 conjugate at room temperature for 1 hour.

Laser scanning confocal fluorescence imaging was done on a Nikon A1R-A1 confocal microscope system equipped with a Nikon 100× oil-immersed objective and a Hamamatsu C11440 camera (Light Microscopy Imaging Center, Indiana University). Alternative scanning mode was used to avoid possible crosstalk between channels. Z-scan stacks were acquired with a 0.15 μm stepsize on Z axis. Images were analyzed with ImageJ software.

Supplementary Figures

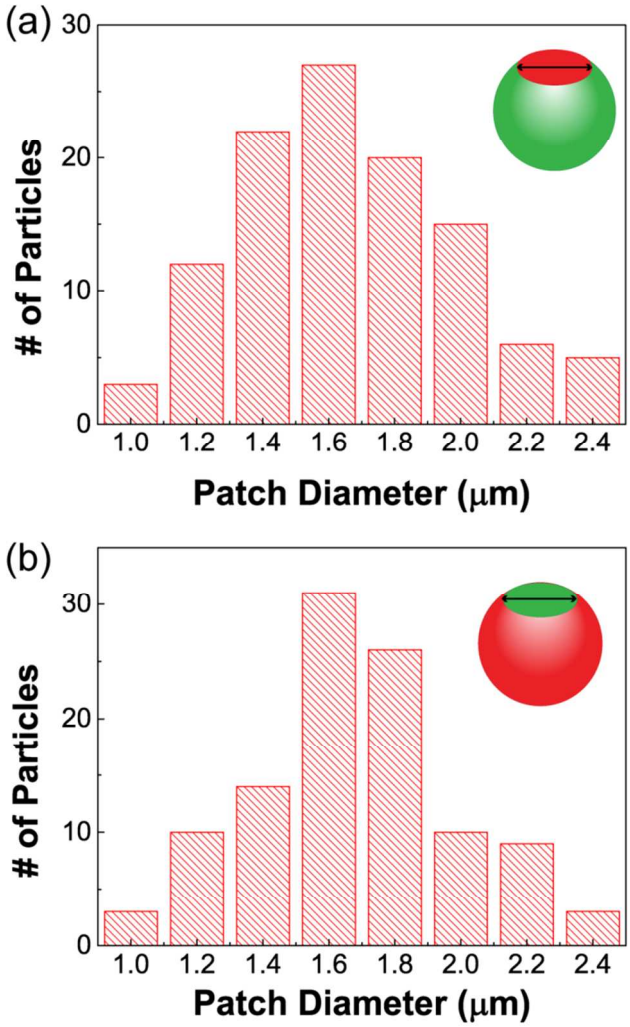

Figure S1. Size distribution of (a) anti-CD3 and (b) fibronectin patches.

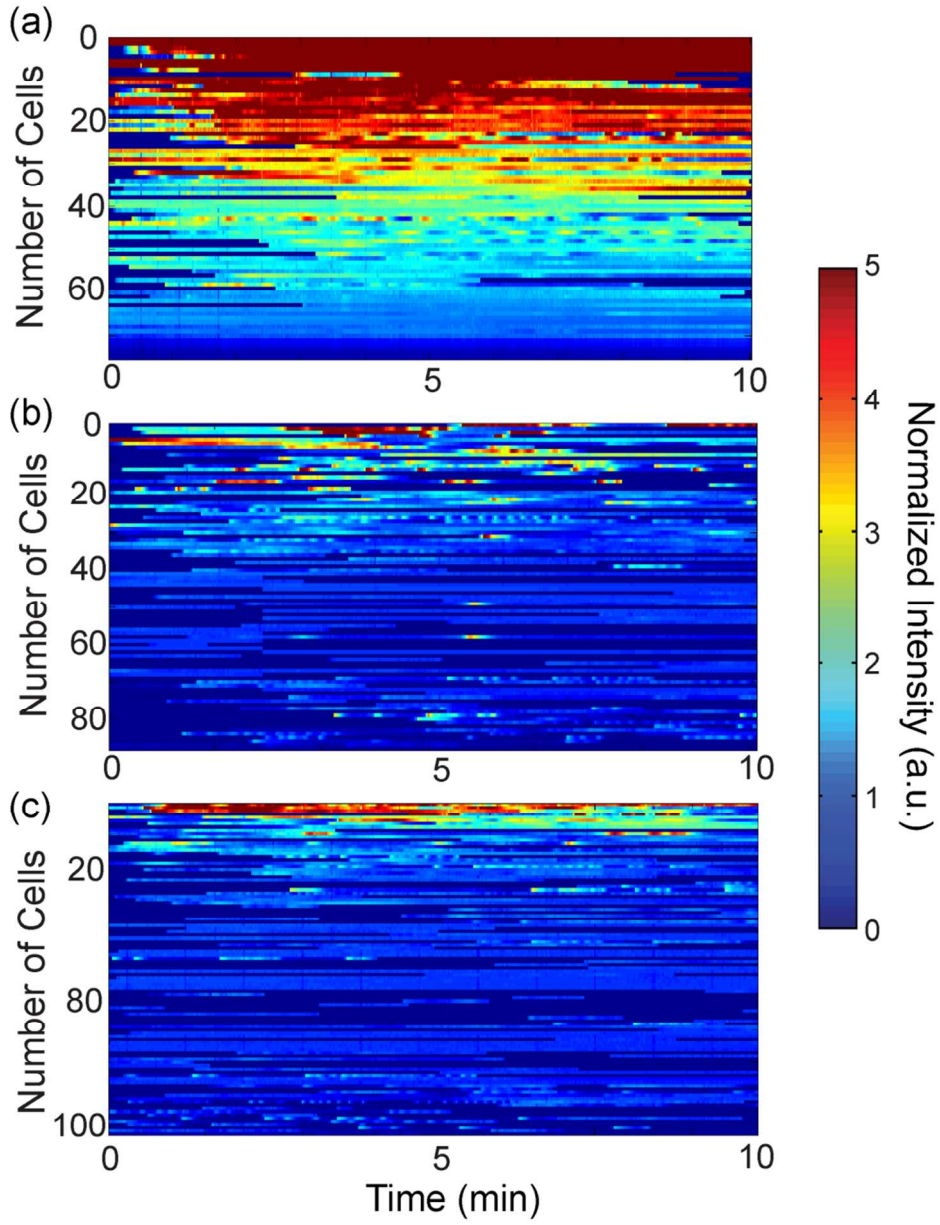

Figure S2. Intracellular calcium elevation in Jurkat T cells that are stimulated by particles coated uniformly with (a) anti-CD3, (b) fibronectin and (c) BSA. Jurkat T cells were loaded with calcium-sensitive dye, Fluo-4, whose fluorescence intensity increases with intracellular [Ca^{2+}]. Normalized fluorescence intensities of individual cells are shown on a color scale. Cells are sorted based on the fluorescence intensity of the first peak. Time zero is defined as the time when T cells are flown into imaging chambers.

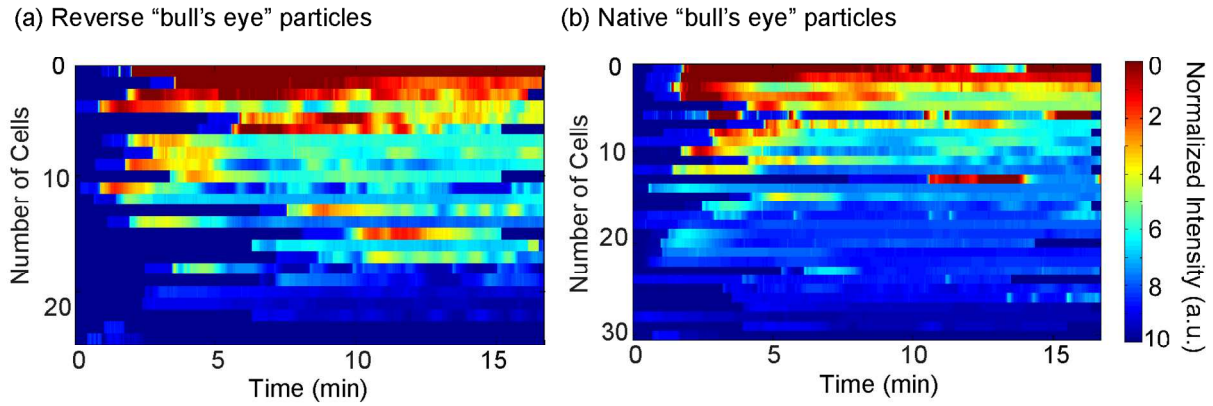

Figure S3. Calcium response of T cells that are in direct contact with the “bull’s eye” patterns. Normalized fluorescence intensities of individual cells are shown on a color scale. Cells are sorted based on the fluorescence intensity of the first peak. Time zero is defined as the time when T cells are flown into imaging chambers.

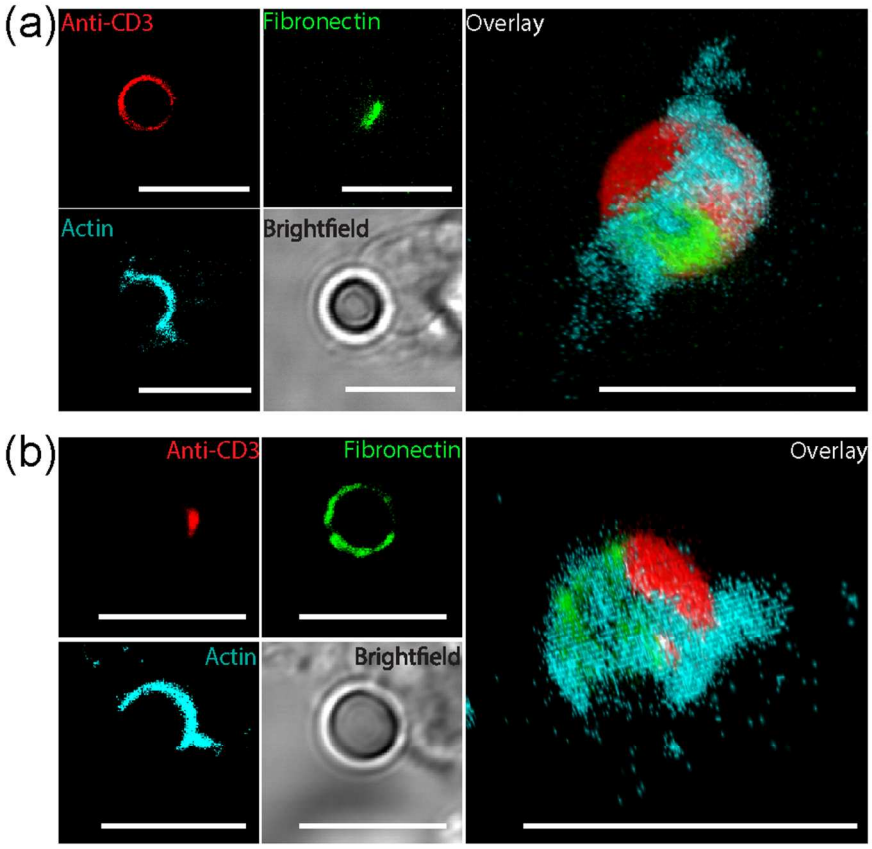

Figure S4. Fluorescence confocal images show diffusive localization of actin in T cells that are stimulated by "bull's eye" particles. The images are representative of 41 cells for the native "bull's eye" particles and 26 for the reverse type particles. Scale bars: 5 μm.

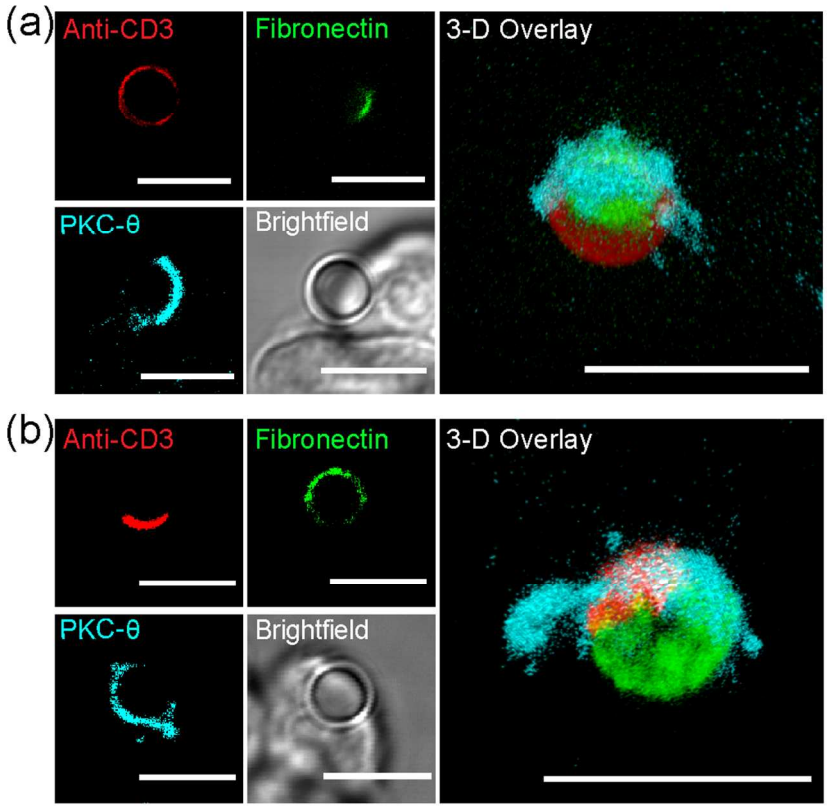

Figure S5. Fluorescence confocal images show diffusive localization of PKC-θ in T cells that are stimulated by "bull's eye" particles. The images are representative of 37 cells for the native "bull's eye" particles and 19 for the reverse type particles. Scale bars: 5 μm.

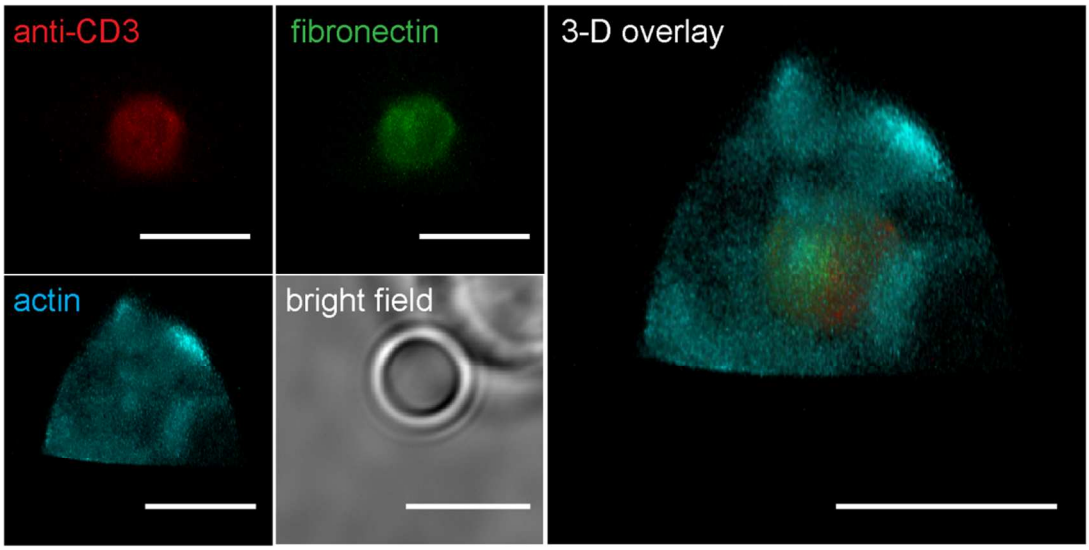

Figure S6. Fluorescence confocal images show intracellular clustering of actin in a T cell stimulated by a control particle that is uniformly coated with anti-CD3 and fibronectin. The images are representative of 36 cells. Scale bars: 5 μm.

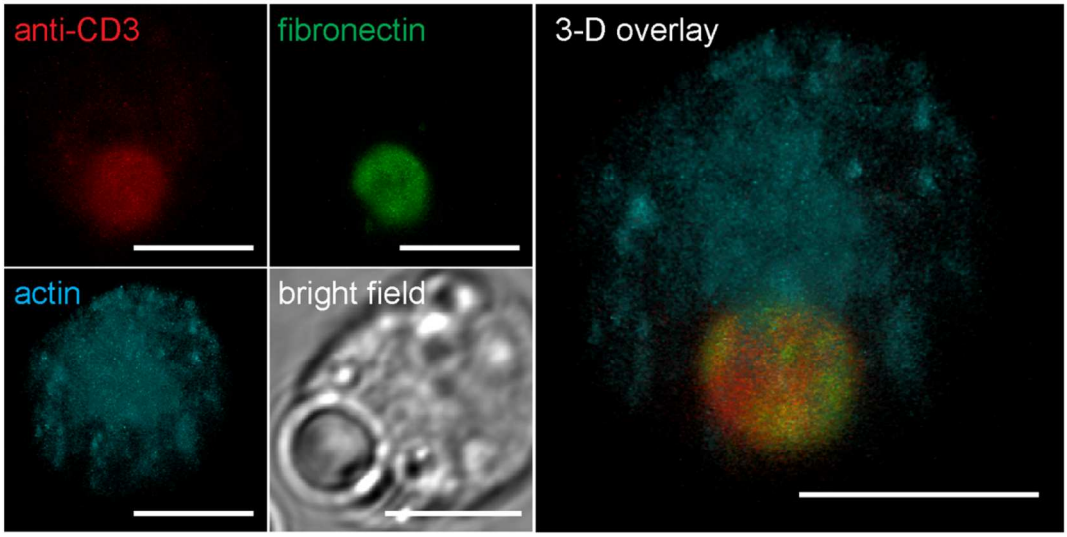

Figure S7. Fluorescence confocal images show intracellular clustering of PKC-θ in a T cell stimulated by a control particle that was uniformly coated with anti-CD3 and fibronectin. The images are representative of 30 cells. Scale bars: 5 μm.

References

(1) Jiang, S.; Granick, S. A Simple Method to Produce Trivalent Colloidal Particles. *Langmuir* **2009**, *25*, 8915-8918.

(2) Salles, A.; Billaudeau, C.; Sergé, A.; Bernard, A.-M.; Phélipot, M.-C.; Bertaux, N.; Fallet, M.; Grenot, P.; Marguet, D.; He, H.-T.; Hamon, Y. Barcoding T Cell Calcium Response Diversity with Methods for Automated and Accurate Analysis of Cell Signals (MAAACS). *PLoS Comput. Biol.* **2013**, *9*, e1003245.