

## Supporting Information

**DNA-based microparticle tension sensors ( $\mu$ TS) for measuring cell mechanics in non-planar geometries and for high-throughput quantification**

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**Materials and Methods****1. Materials****1.1 Reagents**

All reagents were purchased from Sigma-Aldrich (St. Louis, MO) unless otherwise specified. Cy3B NHS ester (Cat# PA63101) was purchased from GE Healthcare Life Sciences (Pittsburgh, PA). Azido-PEG4-NHS ester (Cat# AZ103-100) and Alkyne-PEG4-NHS ester (Cat# TA103-100) were purchased from Click Chemistry Tools (Scottsdale, AZ). 6-Azidosulfonylhexyltriethoxysilane (Cat# SIA0780.0) was purchased from Gelest (Morrisville, PA). SMCC (succinimidyl 4-(N-maleimidomethyl)cyclohexane-1-carboxylate) (Cat# 22360), Azide-NHS ester (Cat# 88902) and Bond-Breaker™ TCEP Solution (Cat# 77720) were purchased from Thermo Fisher Scientific (Waltham, MA). No. 2 round glass coverslips (Cat# 48382-085) were purchased from VWR (Radnor, PA). P2 size exclusion gel (Cat#1504118) was purchased from Bio-Rad (Hercules, CA). Silica-NH<sub>2</sub> beads (Cat# SA05000) were purchased from Bangs Laboratories, Inc (Fishers, IN). Red blood cell lysis buffer (Cat# 420301) and biotinylated anti-mouse CD3 $\epsilon$  (Cat# 100243) were purchased from Biolegend (San Diego, CA). Biotinylated pMHC ovalbumin (SIINFEKL) was obtained from the NIH Tetramer Core Facility at Emory University. Midi MACS (LS) startup kit (Cat# 130-042-301) (separator, columns, stand), and mouse CD8+ T cell isolation kit (Cat# 130-104-075) were purchased from Miltenyi Biotec (Bergisch Gladbach, Germany). Cyclo[Arg-Gly-Asp-D-Phe-Lys(PEG-PEG)] (Cat# PCI-3696-PI) and Cyclo(Arg-Ala-Asp-D-Phe-Lys) (Cat# PCI-3883-PI) were obtained from Peptides International. SiR-Actin (cat# CY-SC001) was purchased from Cytoskeleton.

**1.2 Oligonucleotides**

All oligonucleotides were purchased from Integrated DNA Technologies (Coralville, IA) except for BHQ2 strands, which were custom synthesized by Biosearch Technologies (Novato, CA).

**List of oligonucleotides used**

<b>Name</b>	<b>Sequence (5' to 3')</b>
Unmodified top Strand	CAC AGC ACG GAG GCA CGA CAC

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Alkyne-BHQ2 top strand	/5Hexynyl/ - CAC AGC ACG GAG GCA CGA CAC - /3BHQ2/
Biotin-BHQ2 top Strand	/5Biosg/ - CAC AGC ACG GAG GCA CGA CAC - /3BHQ2/
12 pN bottom strand	/5UniAmM/ - GTG TCG TGC CTC CGT GCT GTG TTT TT - /3ThioMC3-D/
56 pN bottom strand	/5ThioMC6-D/ - TTT TT/iUniAmM/ GTG TCG TGC CTC CGT GCT GTG

### 1.3 Instruments

Barnstead Nanopure water purifying system (Thermo Fisher), High-performance liquid chromatography (Agilent 1100), Nanodrop 2000 UV-Vis Spectrophotometer (Thermo Scientific), Matrix-assisted laser desorption/ionization time-of-flight mass spectrometer (MALDI-TOF-MS, Voyager STR), Nikon Eclipse Ti microscope equipped with Evolve electron multiplying charge coupled device (Photometrics), an Intensilight epifluorescence source (Nikon), a CFI Apo 100X NA 1.49 objective and TIRF launcher. Nikon confocal microscope with a 60x oil objective and a C2si scanhead. BD LSR II flow cytometer. Beckman coulter CytoFLEX flow cytometer. Amnis ImageStream<sup>X</sup> Mk II imaging flow cytometer.

## 2. Methods

### 2.1 OT-1 T-cell harvesting and purification

OT-1 T cell receptor transgenic mice were housed and bred in the Division of Animal Resources Facility at Emory University in accordance with the Institutional Animal Care and Use Committee. OT-1 T cells express the CD8 co-receptor and specifically recognize chicken ovalbumin epitope 257–264 (SIINFEKL) in the context of the MHC allele H-2K<sup>b</sup>. Naïve OT-1 T cells were enriched from the spleen using magnetic activated cell sorting according to manufacturer instructions provided with the CD8+ T cell Isolation Kit (Miltenyi Biotec, Germany). Briefly, a single cell suspension of splenocytes was obtained and incubated with biotinylated antibodies specific for unwanted splenic cell populations. These populations were separated from the OT-1 T cells following incubation with anti-biotin magnetic beads and enrichment on a magnetic column. Purified T cells were washed and resuspended in HBSS solution and kept on ice before experiment.

### 2.2 Preparation of mouse platelets

Blood from C57Bl/6J mice was collected by cardiac puncture, anticoagulated with acid citrate dextrose, added to equal volumes modified Tyrode's buffer (140mM NaCl, 2.7mM KCl, 0.4mM NaH<sub>2</sub>PO<sub>4</sub>, 10mM NaHCO<sub>3</sub>, 5mM Dextrose, 10mM HEPES) containing 3U apyrase and centrifuged at 200xg for 5min. The platelet fraction was removed and to it added 1U apyrase and 1uM prostaglandin E1. Platelets were centrifuged at 700xg for 5min and resuspended in Walsh buffer (137mM NaCl, 2.7mM KCl, 1mM MgCl<sub>2</sub>, 3.3mM NaH<sub>2</sub>PO<sub>4</sub>, 20mM HEPES, pH 7.4, 0.1% glucose, 0.1% bovine serum albumin) at a concentration of 1x10<sup>9</sup> platelets/mL.

### 2.3 DNA strand modification

*Labeling DNA strands with dyes:* Aminated bottom strand (either 12 pN or 56 pN) (100 μM) was mixed with excess Cy3B-NHS ester / Atto647-NHS ester (500 μg/mL) and allowed to react in aqueous solution (pH=9) for 3 hours at room temperature. The mixture was then filtered by P2 gel to remove salts and unreacted dyes and purified by HPLC. (solvent A: 0.1 M TEAA, solvent B: 100% MeCN; initial condition was 10% B with a gradient of 1% per min, flow rate: 0.5 mL/min). The product was characterized by MALDI-TOF mass spectrometry.

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**Labeling DNA strands with RGD/RAD ligand:** 100 nmols of Cyclo[Arg-Ala-Asp-D-Phe-Lys(PEG-PEG)] (Cat# PCI-3696-PI) or Cyclo(Arg-Ala-Asp-D-Phe-Lys) peptide was reacted with 150 nmoles of azido-NHS ester in 20  $\mu$ L DMSO overnight. Afterwards, the azido RGD/RAD product was purified by HPLC. (solvent A 0.1 M TEAA, solvent B: 100% MeCN; initial condition was 10% B with a gradient of 0.5% per min, flow rate: 1 mL/min). Azido cRGD/RAD was then conjugated to alkyne DNA strand via copper catalyzed click reaction. Briefly, a mixture of azido-RGD from last step, Alkyne DNA strand (100  $\mu$ M), CuSO<sub>4</sub> (400  $\mu$ M), THPTA (2 mM) and sodium ascorbate (10 mM) in 1X PBS solution was allowed to react in room temperature for 2 hours. The mixture was then subjected to P2 gel filtration and purified by HPLC. (solvent A: 0.1 M TEAA, solvent B: 100% MeCN; initial condition was 10% B with a gradient of 1% per min, flow rate: 0.5 mL/min). The product was characterized by MALDI-TOF mass spectrometry following drying.

**2.4 Preparation of  $\mu$ TSs presenting pMHC**

First, maleimide particles were synthesized by mixing 3 mg of 5  $\mu$ m aminated silica beads (Bangs Laboratories) with 1 mg SMCC crosslinker in 1 mL DMSO and reacting on a shaker for 2 hours. Afterwards, the particles were centrifuged down at 5000 rpm for 5 minutes. The supernatant was discarded, and the particles were resuspended to DMSO. This process was repeated for four times to remove unreacted SMCC. Afterwards, the beads were washed with PBS three times to remove excess DMSO to prepare the beads for the following step of DNA conjugation.

During the preparation of maleimide coated particles, the tension probes were assembled in 1M NaCl by mixing the fluorophore labeled 12/56 pN bottom strands (500 nM) and biotin-quencher strand (1  $\mu$ M) in the ratio of 1:2. The mixture was heat annealed at 95  $^{\circ}$ C for 5 min and cool down to 25  $^{\circ}$ C for 30 min. 1  $\mu$ L 5 mM TCEP (100-fold) was added to 100  $\mu$ L of the assembled probes to activate thiol group on bottom strands. After 15 min, the probe was diluted with 400  $\mu$ L PBS to 100 nM and added to maleimide coated beads. The reaction was incubated for 1 hour and the resulting DNA conjugated particles were purified by centrifugation. Specifically, the particles were centrifuged in BSA blocked microcentrifuge tubes at 800 rpm for 5 min, after which the supernatant was discarded and the particles were resuspended into 1 mL PBS. This process was repeated four times. The particles were then resuspended into 0.1% BSA and incubated for 30 min to block the particle surface, followed by washing with PBS for three times. The beads were then incubated with 10  $\mu$ g/mL streptavidin and 10  $\mu$ g/mL biotinylated OVA-N4 sequentially for 45 min at room temperature with thrice PBS washes in between (**Figure S1**). Finally, particles were buffer exchanged with HBSS and ready for imaging with T-cells.

**2.5 Preparation of  $\mu$ TSs presenting cyclized RGD**

First, maleimide particles were synthesized by mixing 3 mg of 5  $\mu$ m aminated silica beads (Bangs Laboratories) with 1 mg SMCC crosslinker in 1 mL DMSO and reacting on the shaker for 2 hours. Afterwards, the particles were centrifuged down at 5000 rpm for 5 minutes. The supernatant was discarded, and the particles were resuspended to DMSO. This process was repeated four times to remove unreacted SMCC. Afterwards, the beads were washed in PBS three times to remove DMSO for the following DNA conjugation.

During the preparation of maleimide coated particles, the tension probes were assembled in 1M NaCl by mixing the fluorophore labeled 12/56 pN bottom strands (500 nM), RGD conjugated quencher strand (1  $\mu$ M) in the ratio of 1:2. The mixture was heat annealed at 95  $^{\circ}$ C for 5 min and then cooled down to 25  $^{\circ}$ C for 30 min. 100  $\mu$ L of the assembled probes were reacted with 100-fold excess TCEP for 15 min to activate thiol groups on the bottom strands. Afterwards, the probe was diluted with 400  $\mu$ L PBS to 100 nM and added to the above maleimide conjugated bead and incubated for 1 hour at room temperature, followed by 3X PBS washes. Particles were finally buffer exchanged with Tyrode buffer for platelet experiments (**Figure S1**).

**2.6 Preparation of TGT probes on planar glass surfaces**

No. 1.5H glass coverslips (Ibidi) were sequentially sonicated in MilliQ water (18.2 M $\Omega$  cm<sup>-1</sup>) and 200 proof ethanol, 10 min each. The glass coverslips were rinsed copiously with MilliQ water and immersed

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in freshly prepared piranha solution (3:1 sulfuric acid:H<sub>2</sub>O<sub>2</sub>) for 30 min to remove organic adsorbates and activate hydroxyl groups on the surface (**CAUTION: Piranha is highly reactive and explosive on contact with organics!**). The cleaned substrates were rinsed with MilliQ water in a 200 mL beaker at least 6 times and further washed with ethanol thrice. Slides were then transferred to a 200 mL beaker containing 3% APTES in ethanol for 1 h, washed with ethanol thrice and baked in an oven (~110°C) for 30 min. After cooling, the slides were incubated with 10 mg/mL SMCC for one hour, then washed with ethanol thrice and water one time and finally dried under nitrogen. The slides were then mounted to 6-channel microfluidic cells (Sticky-Slide VI 0.4, ibidi).

Subsequently, TGT probes were assembled in 1M NaCl by mixing the fluorophore labeled 12/56 pN bottom strands (100 nM) and quencher strand (200 nM) in a ratio of 1:2. The mixture was heat annealed at 95 °C for 5 min and then cooled down to 25 °C for 30 min. 500 µL of the assembled probe was reacted with 100-fold TCEP (5µL x 1mM) for 15 min to activate the thiol group and then added to channels and incubated for 1 hour at room temperature, which was then followed by 3X PBS washes. Then 10 µg/mL streptavidin was added to the substrates and incubated for 45 min at room temperature followed by 3X PBS washes. Next, 10 µg/mL biotinylated OVA-N4 was added to the substrates, incubated for 45 min at room temperature and washed thrice with PBS (**Figure S3**). Surfaces were buffer exchanged with HBSS buffer before imaging.

### 2.7 DNA density measurement

Aminated silica beads were incubated with 1 mg/mL SMCC in DMSO for 2 hours to yield maleimide coated bead. Beads were then centrifuged down and washed with DMSO for four times and PBS three times. DNA duplexes were assembled in 1M NaCl by mixing unmodified 12 pN TGT bottom strands and top strand in a ratio of 1:2. The mixture was heat annealed at 95 °C for 5 min and then cooled down to 25 °C for 30 min. 100-fold excess TCEP was added to the assembled probes to activate thiol groups on bottom strands. After 15 min, 100 nM DNA was mixed with maleimide coated beads and incubated at room temperature for 1 hour. Afterwards, the particles were washed with PBS 4 times, resuspended into 1 mL 30 mM NaOH to denature the DNA duplexes on the particle surface. After 20 min, particles in the suspension were counted using a hemocytometer and centrifuged down at 15000 rpm for 5 min. The supernatant was then collected for quantitating single strand DNA through Oligreen Kit. The density was calculated given the number of DNA strands, number of particles and particle surface area (**Figure S2**).

### 2.8 Immobilizing µTS onto glass coverslips

#### Method 1: copper catalyzed alkyne-azide click reaction

##### Step 1 (glass coverslip modification):

No. 2 round glass coverslips (VMR) were sequentially sonicated in MilliQ water (18.2 MΩ cm<sup>-1</sup>) and 200 proof ethanol, 10 min each. The glass coverslips were rinsed copiously with MilliQ water and immersed in freshly prepared piranha solution (3:1 sulfuric acid:H<sub>2</sub>O<sub>2</sub>) for 30 min to remove organic adsorbates and activate hydroxyl groups (**CAUTION: Piranha is highly reactive and explosive on contact with organics!**). The glass coverslips were then washed six times with water, twice with ethanol and dried under nitrogen. Afterwards, the substrates were incubated with 50 mg/mL 6-Azidosulfonylhexyltriethoxysilane in DMSO for 2 hours, washed with ethanol thrice and baked in oven (~110°C) for 30 min.

##### Step 2 (bead modification and immobilization):

3 mg aminated silica beads were incubated with 2mg/mL Alkyne-PEG4-NHS ester and 2 mg/mL SMCC in DMSO for overnight. On the next day, the beads were then centrifuged down at 15000 rpm for 5 min and washed with DMSO three times and PBS two times. Next, particles were mixed with 55 µL DMSO, 10 µL 2M TEAA, 10 µL 10mM CuTBTA, 15 µL water, 10 µL 18 mg/mL ascorbic acid and then added to azide coated glass coverslips prepared in step 1. Another coverslip was put on top to make a "sandwich" and left overnight. On the next day, unfixed beads were washed away gently in a beaker filled with PBS. Fluorophore labeled 12/56 pN bottom strands and quencher strand were mixed and annealed at a ratio



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of 1:2 in 1 M NaCl at a 100 nM concentration. The DNA was added to bead coated coverslip and allowed to be immobilized onto bead for 1 hour. After washing away excess DNA with PBS, 10  $\mu\text{g}/\text{mL}$  streptavidin was added to the substrates and incubated for 45 min at room temperature followed by 3X PBS washes. Next, 10  $\mu\text{g}/\text{mL}$  OVA-N4 was added to the substrates, incubated for 45 at room temperature and washed thrice with PBS (**Figure S5**). Substrates were buffer exchanged with HBSS before imaging.

### Method 2: copper free DBCO-azide click reaction

#### Step 1 (glass coverslip modification):

No. 2 round glass coverslips (VMR) were sequentially sonicated in MilliQ water ( $18.2 \text{ M}\Omega \text{ cm}^{-1}$ ) and 200 proof ethanol, 10 min each. The glass coverslips were rinsed copiously with MilliQ water and immersed in freshly prepared piranha solution (3:1 sulfuric acid:H<sub>2</sub>O<sub>2</sub>) for 30 min to remove organic adsorbates and activate hydroxyl groups (**CAUTION: Piranha is highly reactive and explosive on contact with organics!**). The glass coverslips were then washed six times with water, twice with ethanol and dried under nitrogen. Afterwards, the substrates were incubated with 3% APTES in ethanol for 1 h, washed with ethanol thrice and baked in oven ( $\sim 110^\circ\text{C}$ ) for 30 min. 50  $\mu\text{L}$  5 mg/mL NHS-PEG4-DBCO (50% water pH=9, 50% DMSO) was added onto coverslip. Another coverslip was put on top to make a “sandwich” and left for 2 hours.

#### Step 2 (bead modification and immobilization):

3 mg aminated silica beads were incubated with 2mg/mL Azide-PEG4-NHS ester and 2 mg/mL SMCC in DMSO for overnight. On the next day, the beads were then centrifuged down at 15000 rpm for 5 min, washed with DMSO three times and resuspend into 50  $\mu\text{L}$  DMSO. Bead suspension was then added onto DBCO coated glass in step 1. Another coverslip was put on top to make a “sandwich” and left overnight. On the next day, unfixed beads were washed away gently in a beaker filled with PBS. Fluorophore labeled 12/56 pN bottom strands and quencher strand were mixed and annealed at a ratio of 1:2 in 1 M NaCl at a 100 nM concentration. The DNA was added to bead coated coverslip and allowed to be immobilized onto bead after 1 hour. After washing away excess DNA with PBS, 10  $\mu\text{g}/\text{mL}$  streptavidin was added to the substrates and incubated for 45 min at room temperature followed by 3X PBS washes. Next, 10  $\mu\text{g}/\text{mL}$  OVA-N4 was added to the substrates, incubated for 45 at room temperature and washed thrice with PBS (**Figure S5**). Substrates were buffer exchanged with HBSS before imaging.

### 2.9 Immunostaining

After tension imaging, the cells on surfaces were fixed using 4% formaldehyde in PBS for 10 min. The surfaces were then gently washed with PBS thrice to prevent cell detachment. Afterwards, cells were permeabilized in 0.1% Triton 100X in PBS for 5 min followed by washing with PBS. The surfaces were then rinsed with 2% BSA and incubated overnight at  $4^\circ\text{C}$ . On the next day, the surfaces were washed with PBS thrice. 1  $\mu\text{M}$  SiR-Actin was added to the surface and incubated for 1 hour at room temperature. Afterwards, the surfaces were washed with PBS thrice and imaged on microscope.

### 2.10 MALDI-TOF characterization and sample preparation

MALDI-TOF was performed on a Bruker Daltonics ultraflex II TOF/TOF. Purified oligonucleotides in nanopure water were spotted in a 1:1 (v/v) ratio with saturated matrix solution. To prepare the matrix, 3-hydroxypicolinic acid was added in excess to a solution containing 50% acetonitrile and 0.1% trifluoroacetic acid. Dried samples were measured using the linear negative mode. If samples displayed a low signal-to-noise ratio, multiple spectra were collected using the summation function. Spectra were analyzed in Bruker's Flex Analysis 3.4 software.

### 2.11 Fluorescence imaging

2D imaging was conducted with a Nikon Eclipse Ti inverted microscope driven by the NIS Elements software. The microscope features an Evolve electron multiplying charge coupled device (EMCCD, Photometrics), an Intensilight epifluorescence source (Nikon), a CFI Apo 100 $\times$  NA 1.49 objective (Nikon),

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a TIRF launcher with three laser lines: 488 nm (50 mW), 561 nm (50 mW), and 640 nm (40mW), and a Nikon Perfect Focus System which allows the capture of multipoint and time-lapse images without loss of focus. All of the experiments were performed using bright field, reflection interference contrast microscopy (RICM) and the following Chroma filter cubes: TRITC, FITC, Cy5. Imaging was performed on 96 well plates and glass coverslips using Hank's Balanced Salts supplemented with 0.35 g/L NaHCO<sub>3</sub> and 10 mM HEPES as cell imaging media for T-cells and Tyrode's buffer (134 mM NaCl, 12 mM NaHCO<sub>3</sub>, 2.9 mM KCl, 0.34 mM NaH<sub>2</sub>PO<sub>4</sub>, 5 mM HEPES, 5 mM glucose, 2 mM MgCl<sub>2</sub>, 1 mM CaCl<sub>2</sub>, 0.1% BSA, pH=7.4) for platelets. All imaging data was acquired at room temperature.

3D imaging was conducted with a Nikon confocal microscope with a 60x oil objective and a C2si scanhead. Experiments were performed using three laser lines (488 nm, 561 nm and 640 nm) and the filters with the following bandpasses: 445/50+60LP, 525/50, and 600/50. Z-stack imaging was performed using the ND Acquisition module in Nikon Elements. For 3D reconstructions, a step size of 150 nm was used. Images were reconstructed in ImageJ.

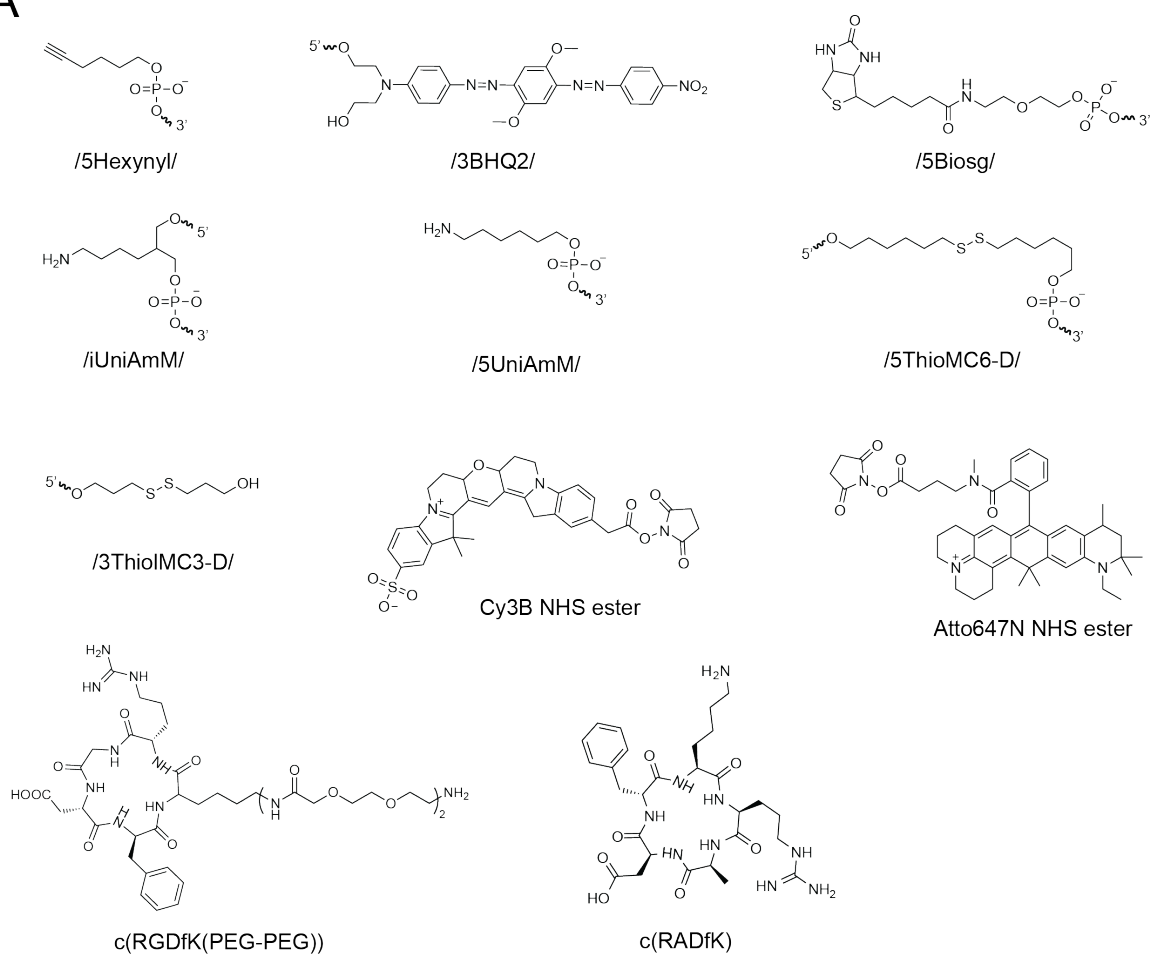
### **2.12 Flow cytometry experiments**

Flow experiments were conducted on BD LSR II flow cytometer with five laser lines: 355 nm, 407 nm, 488 nm, 561nm, 633 nm and Beckman coulter CytoFLEX flow cytometer with two laser lines: 488 nm and 638 nm. Cells and  $\mu$ TS were mixed in desired media in 96 well plate for microscope imaging and transferred to 1 mL microcentrifuge tube for flow cytometry characterization.  $\mu$ TS population was gated out in the side scatter vs forward scatter plots. Tension signals were compared according to the mean fluorescence intensity of populations.

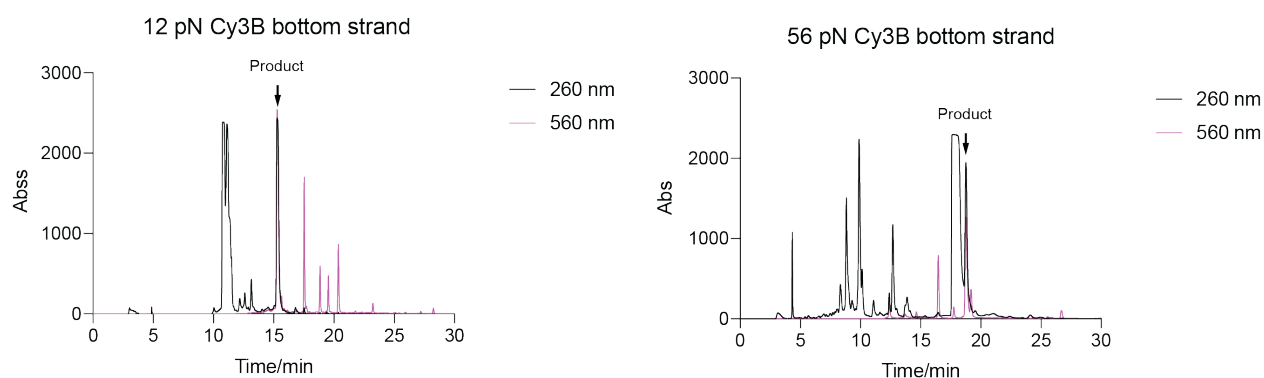
## **Supplemental Figures**

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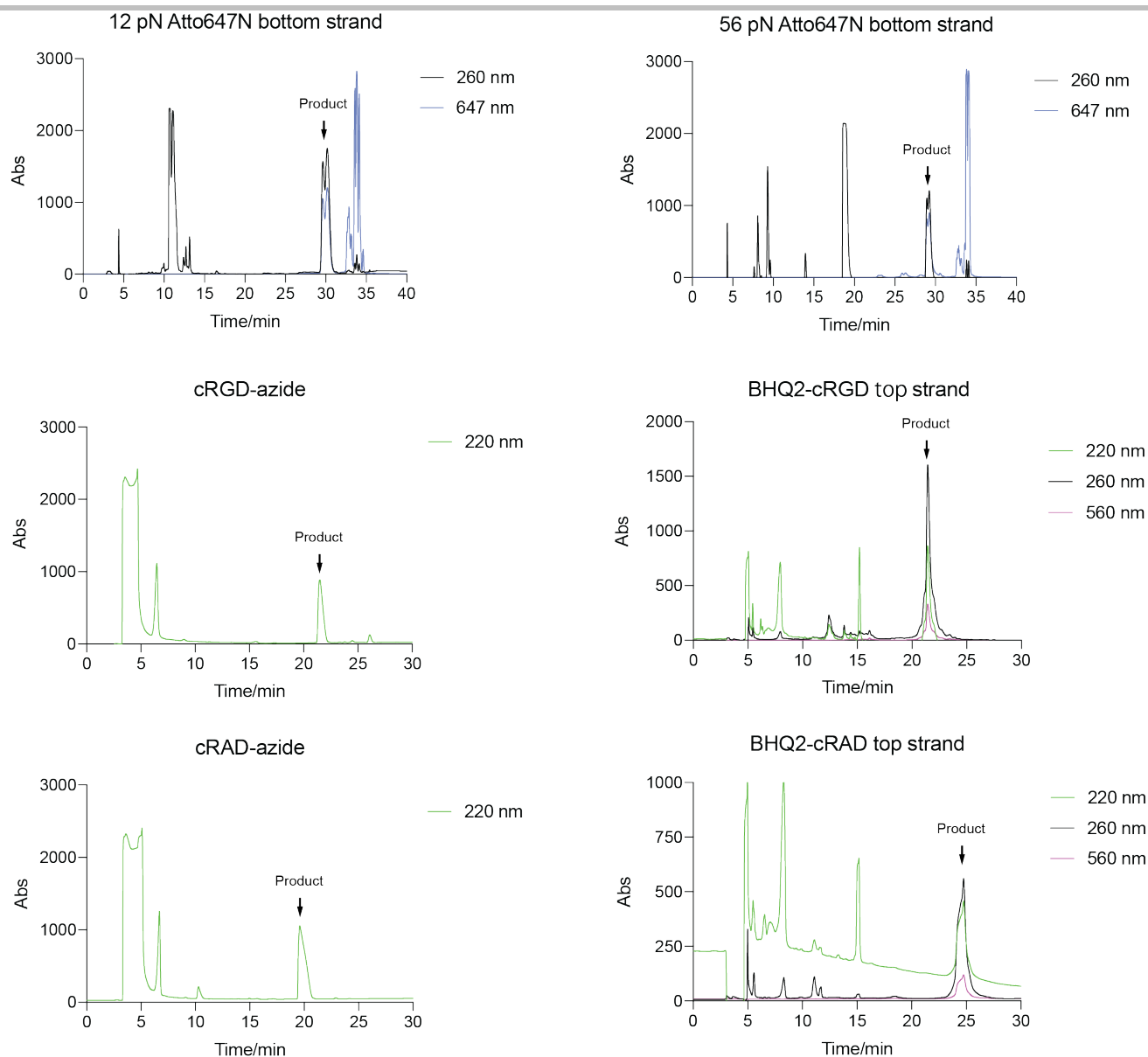
A



B



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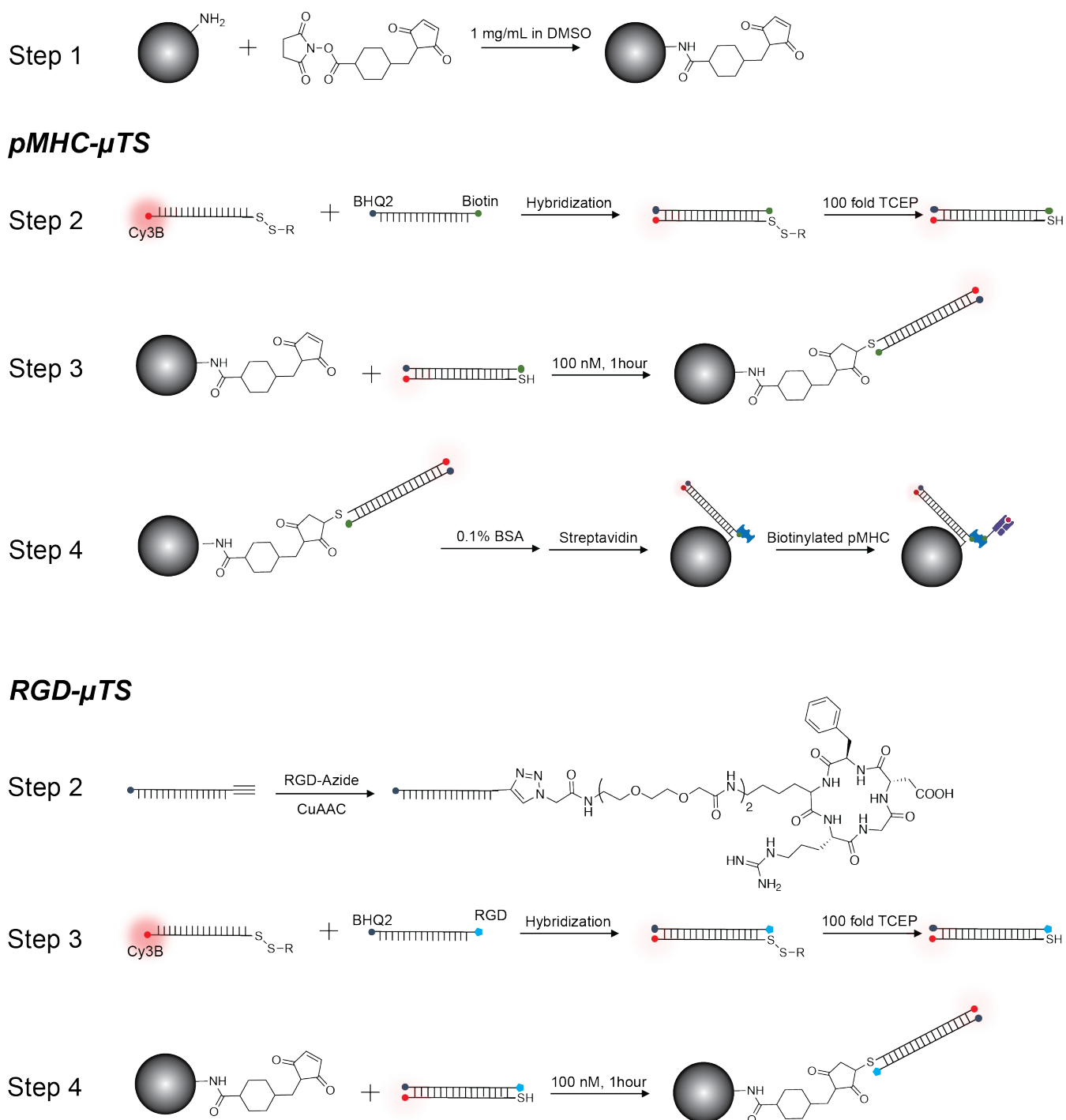


C

Sample	Calculated mass	m/z found (MALDI-TOF)	Error
12 pN Cy3B bottom strand	8953.3	8987.507	0.4%
56 pN Cy3B bottom strand	9037.3	9072.352	0.4%
12 pN Atto647N bottom strand	9138.5	9069.506	0.8%
56 pN Atto647N bottom strand	9223.5	9158.754	0.7%
BHQ2-cRGD top strand	8112.9	8109.8 (ESI-MS)	
BHQ2-cRAD top strand	7836.6	7835.8 (ESI-MS)	

**Figure S1. Characterization of modified oligonucleotides.** (A) Chemical structures of oligonucleotides, dye NHS esters and RGD/RAD peptides. (B) HPLC traces of reaction products. Arrows indicate the peaks associated with the products and that were isolated for mass spectrometry analysis. (C) Calculated mass/charge ratio and mass/charge ratio found using MALDI-TOF and ESI-MS.

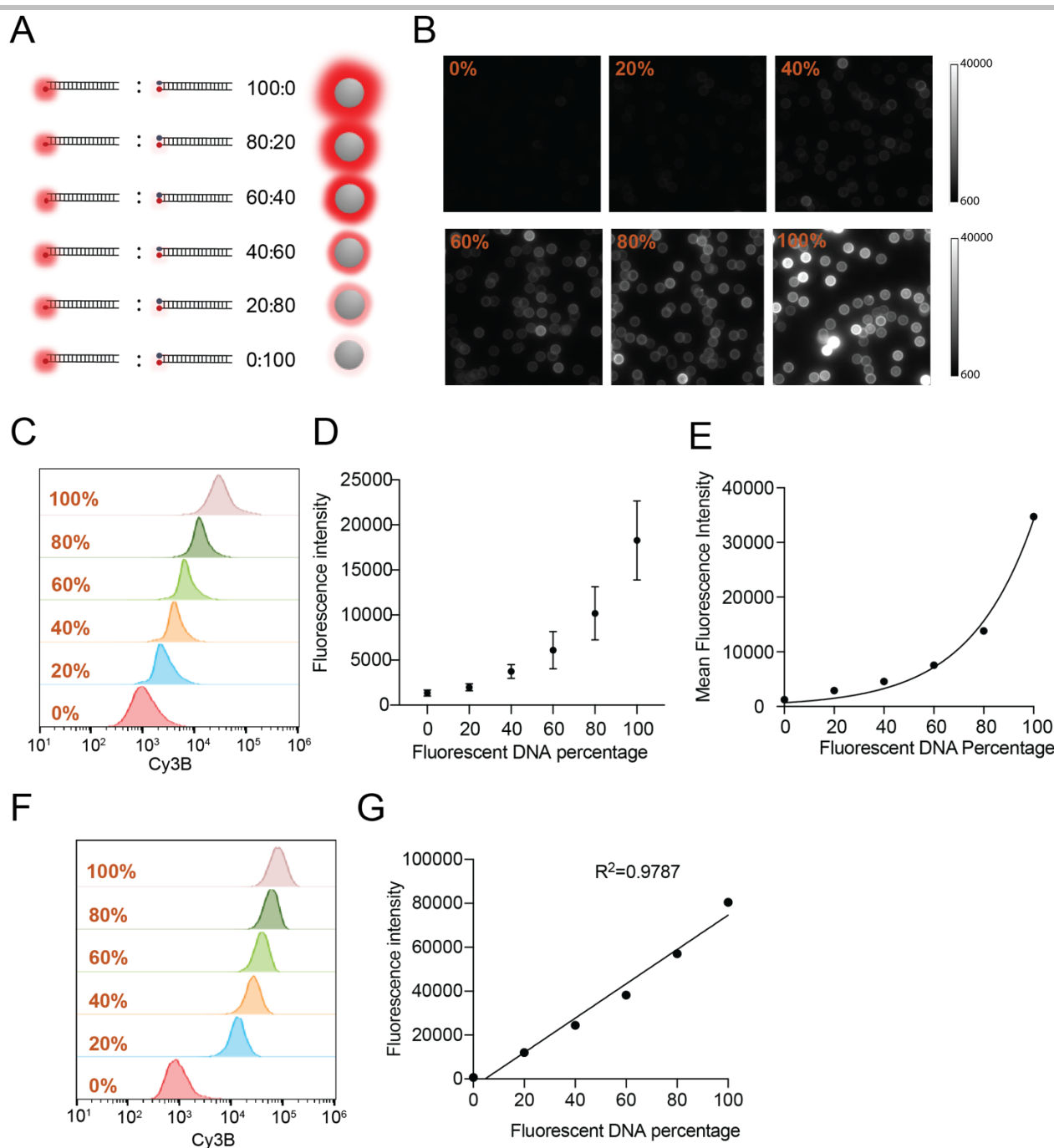
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**Figure S2. Preparation of Microparticle Tension Sensor ( $\mu$ TS) presenting pMHC and cRGD ligands for detecting TCR and integrin forces, respectively.**

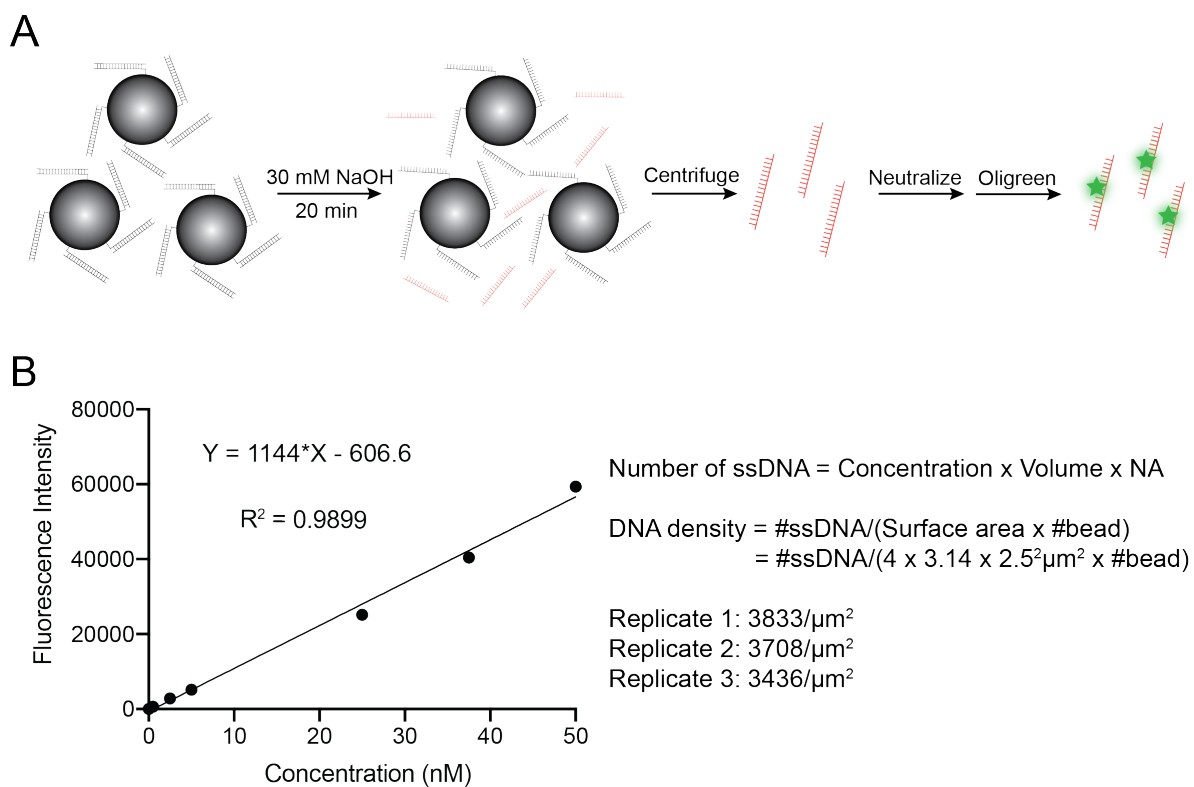


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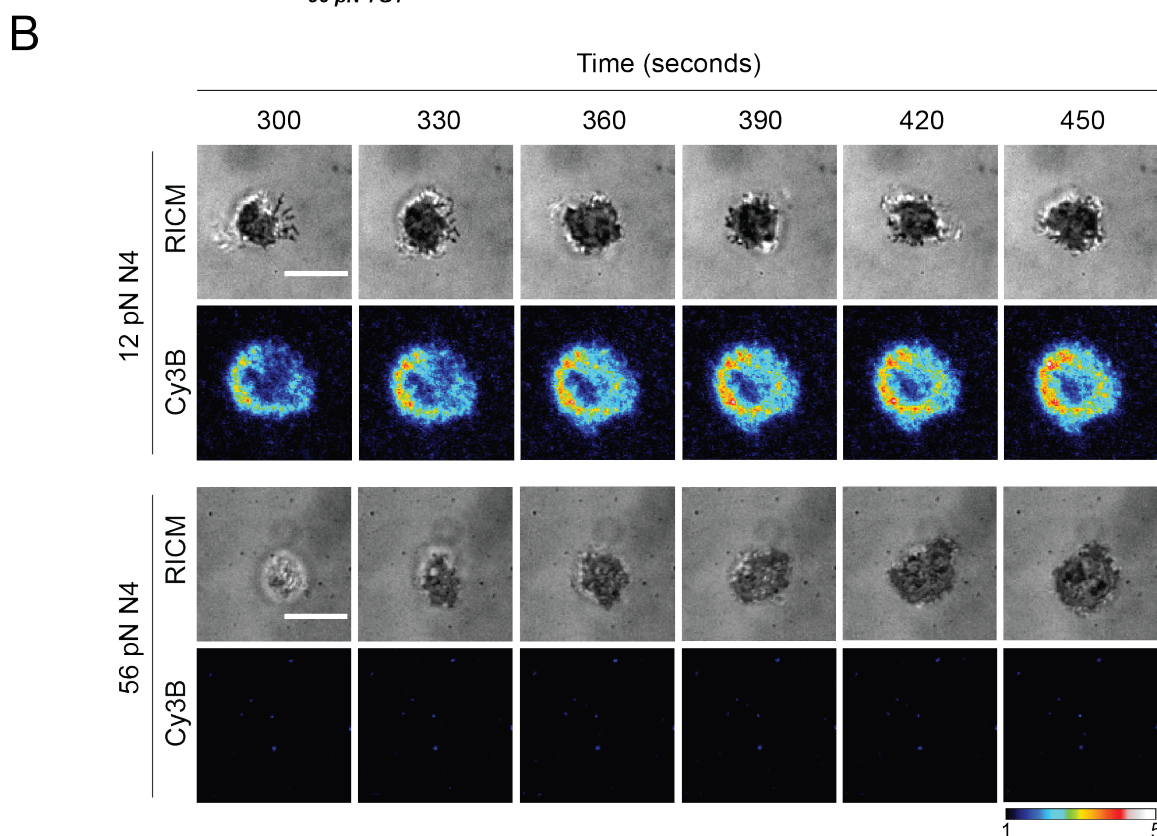
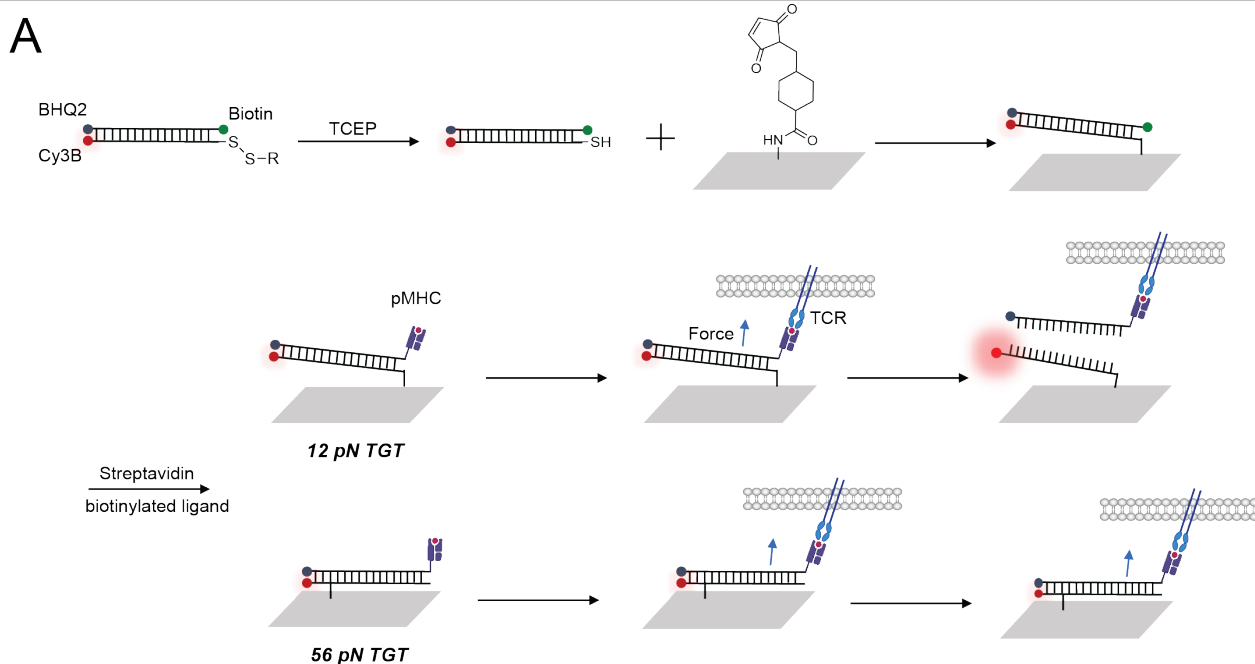
**Figure S3. Determination of probe sensitivity and quenching efficiency.** (A) Particles were modified with binary mixtures of quenched and non-quenched TGTs of different ratios while maintaining a total TGT concentration = 100 nM. (B) Representative Cy3-fluorescence channel images of particles prepared with fluorescent TGT concentrations ranging from 0% to 100%. Microscope images were constructed by selecting max intensity from 21 z-stacks using the Z projection plugin in ImageJ. (C) Intensity histograms of particles (from A) analyzed using flow cytometry. (D-E) Plots showing the fluorescence intensity measured using microscopy and flow cytometry as a function of fluorescent TGT ratio. The function appears non-linear likely due to intermolecular FRET because of the high DNA density on the particle surface. (F) Histograms of particle fluorescence measured using flow cytometry. In this case, the particles were prepared using a low density of DNA. (G) Plots of mean fluorescence intensity of particles obtained from (F) and this shows a linear relation between the fraction of fluorescent DNA and the intensity of a particle. Thus, the plot in (G) supports the premise of intermolecular FRET at high DNA densities.

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**Figure S4. Quantifying DNA probe density on  $\mu$ TS** (A) Schematic shows denaturation of DNA in high pH and further quantification of DNA strand via the Oligreen kit. (B) Standard curve of DNA top strand and equations showing how to convert concentration of top strand in the supernatant to DNA density on particles (average 3660/μm<sup>2</sup>).

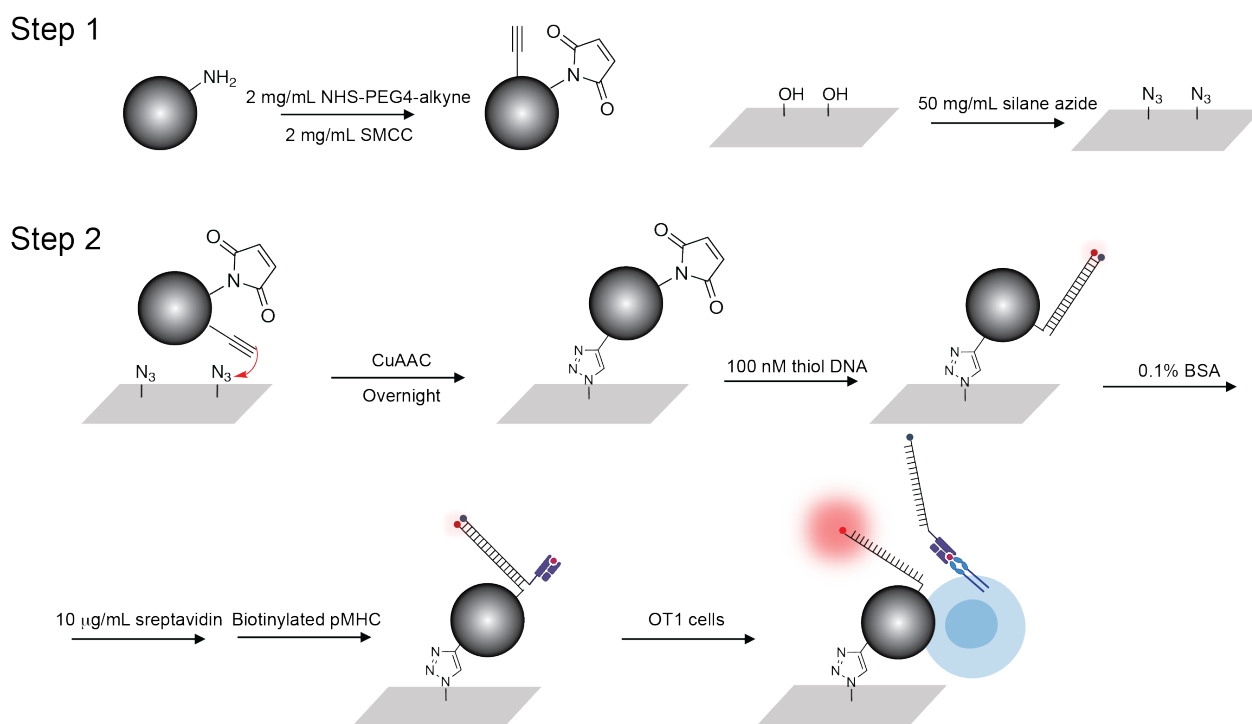
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**Figure S5. Visualizing TCR forces on planar surfaces.** (A) Schematic showing the preparation and response of TGT tension probes presenting pMHC in the 12 pN and 56 pN geometries. (B) Representative RICM and Cy3B time-lapse images showing T-cell spreading and force generation (fluorescence intensity fold change) on 12 and 56 pN TGT surfaces starting at 5 min after cell plating. Scale bar = 5  $\mu$ m

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## Method 1 Copper catalyzed Alkyne-azide click reaction



## Method 2 Copper free DBCO-azide click reaction

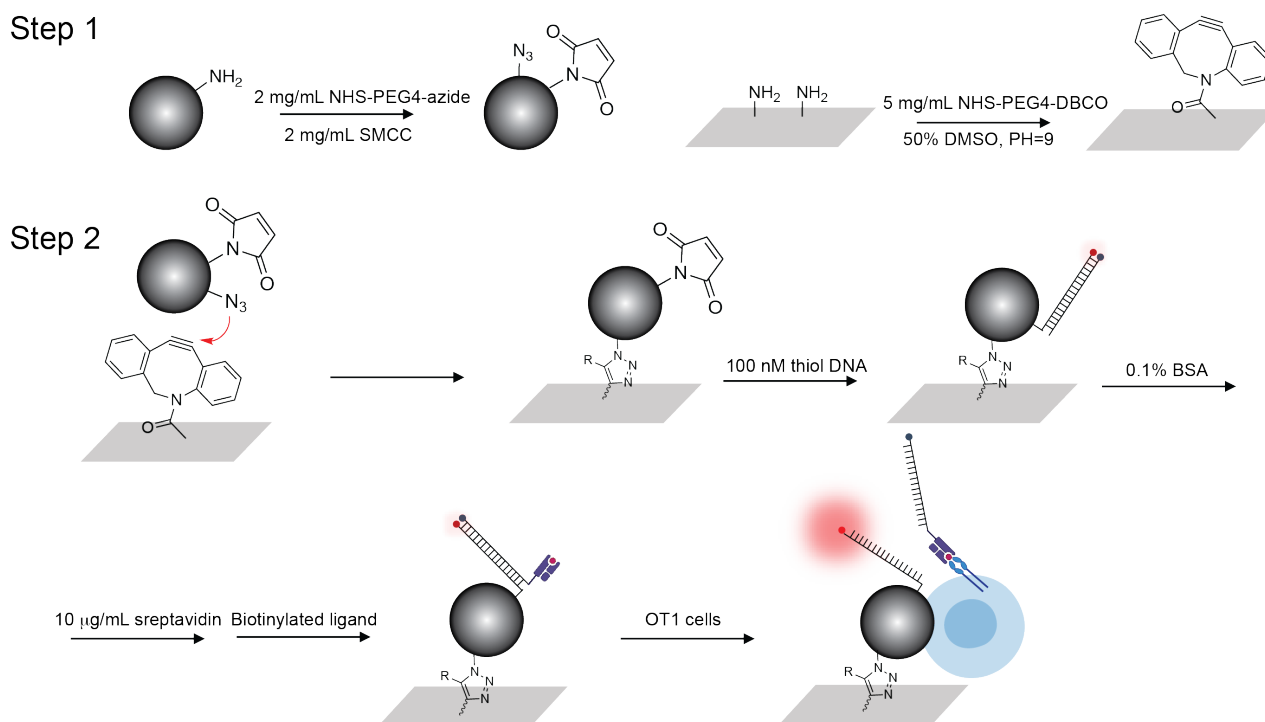
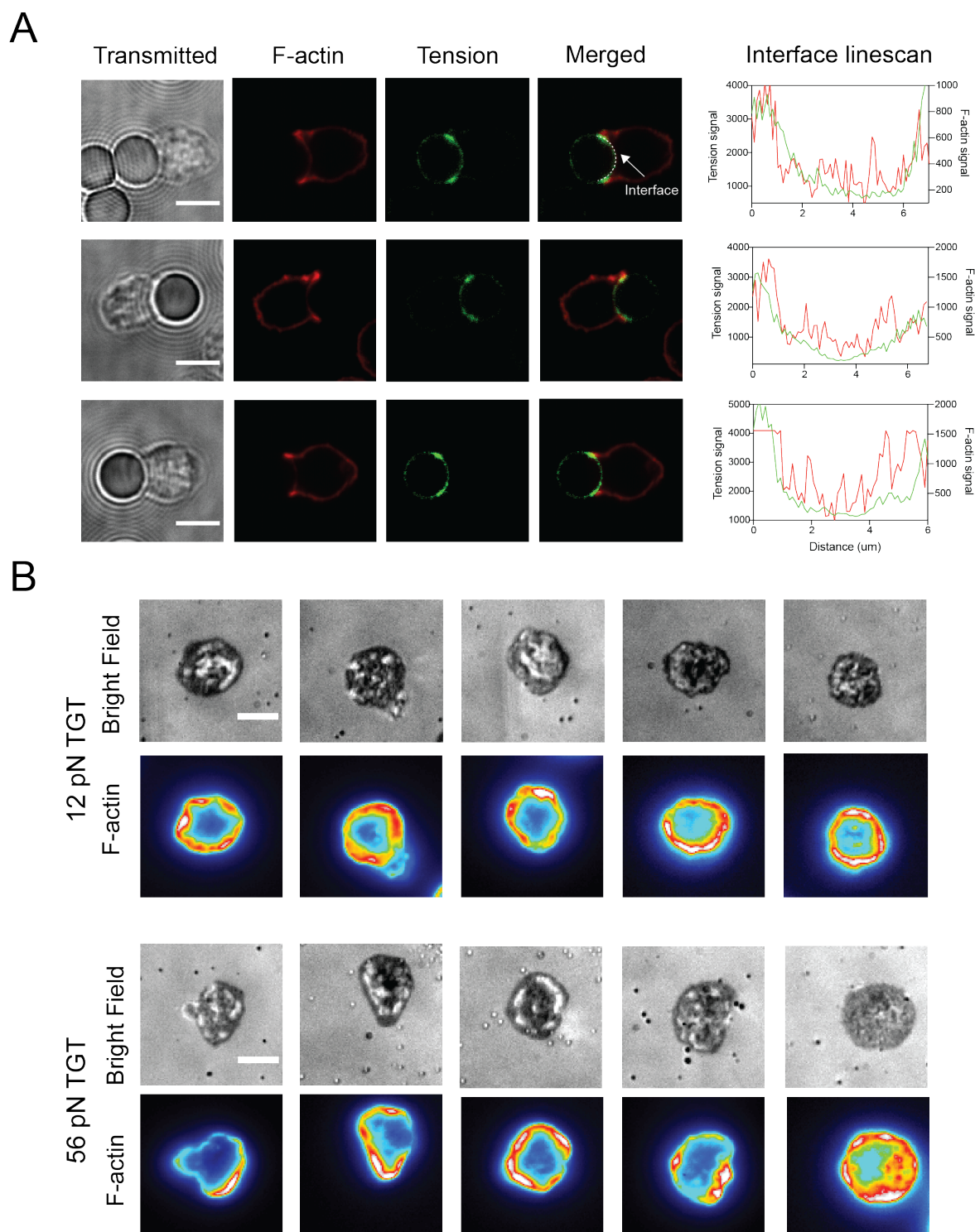


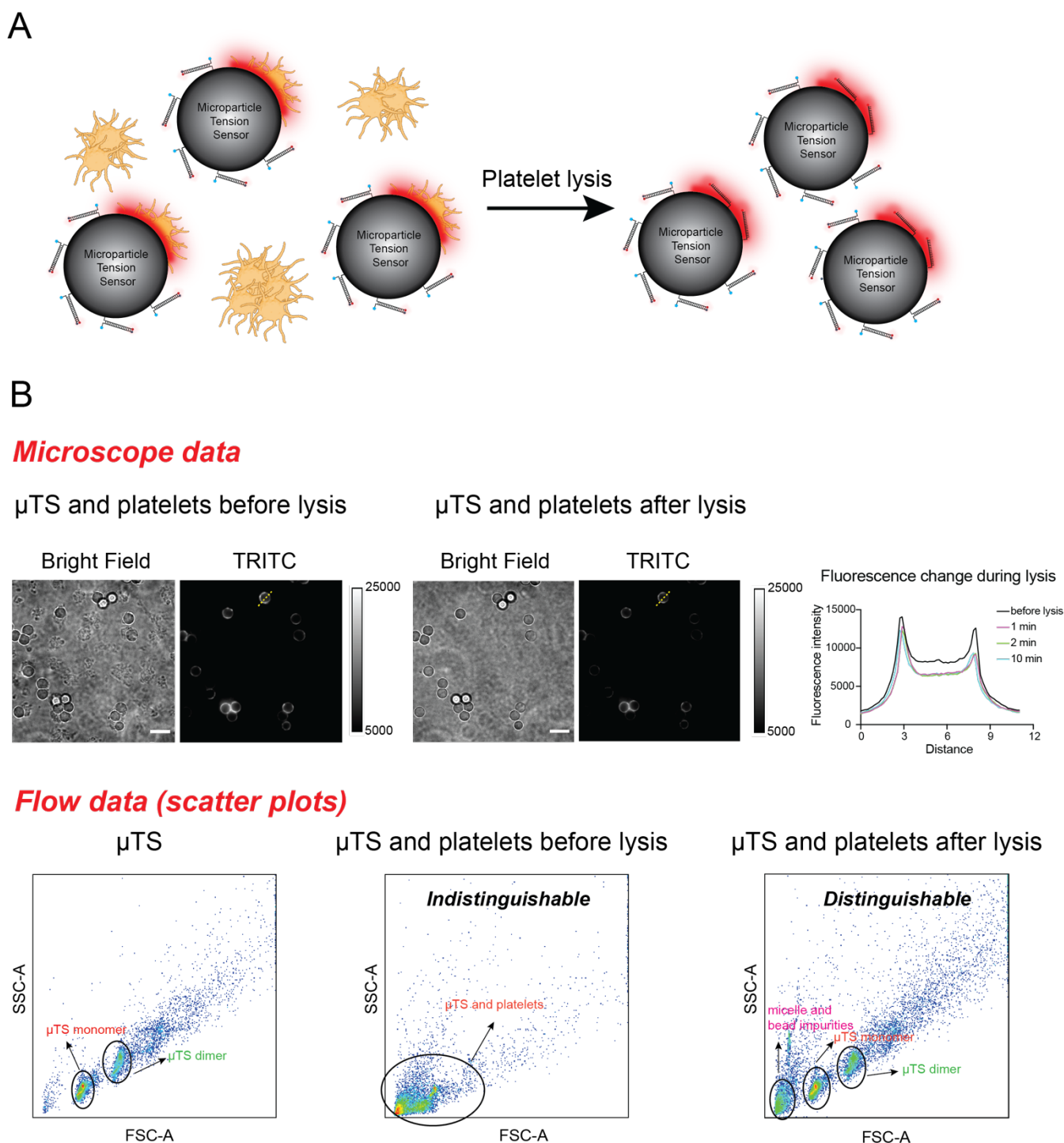
Figure S6. Schematic showing immobilization of  $\mu\text{TS}$  onto glass coverslips through click chemistry.



**Figure S7. Colocalization between F-actin and tension on non-planar and planar surfaces.** (A) Representative confocal microscope images of T cell-12 pN  $\mu$ TS contacts. Images of transmitted light and fluorescence images of F-actin staining and tension signal show colocalization between F-actin and tension. Plots show intensity profiles of F-actin stain and tension signal along the dotted white line, and confirm the high colocalization between these two signals. (B) Representative TIRF images showing F-actin enrichment of T-cells plated on 12 pN and 56 pN TGT planar surfaces. Scale bar = 5  $\mu$ m

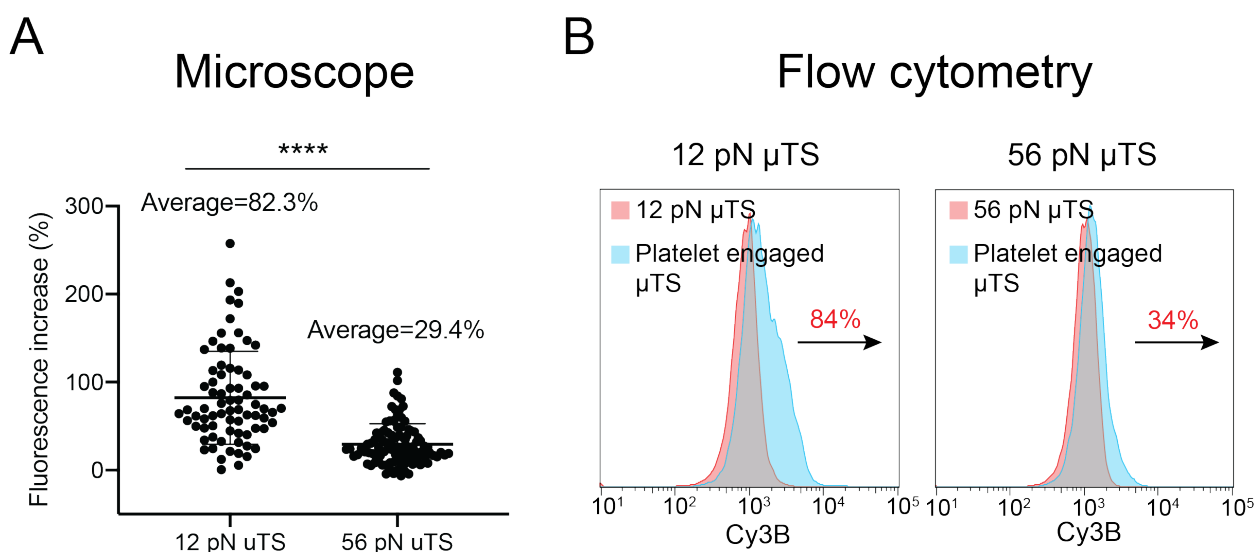


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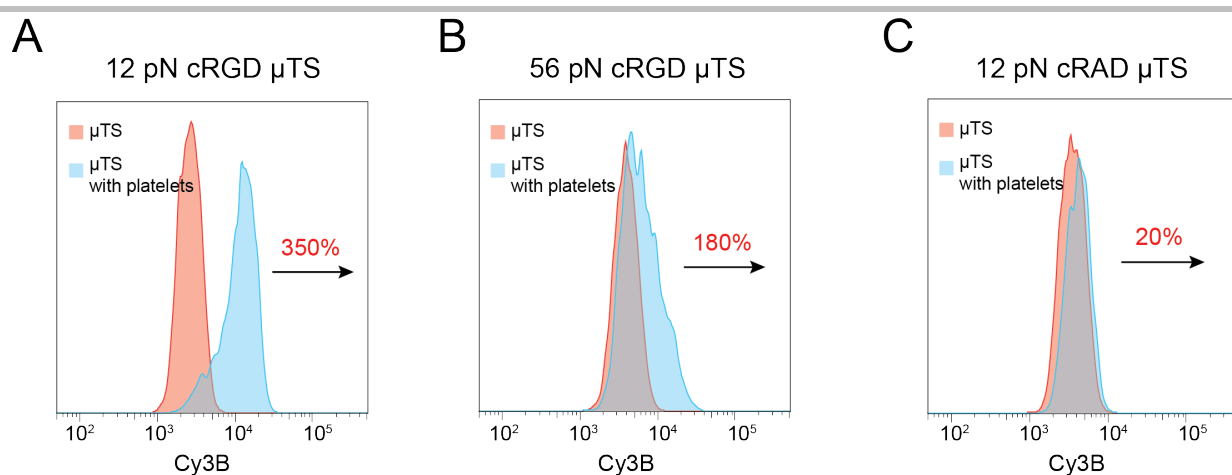
**Figure S8. Lysing platelets in  $\mu$ TS-platelets suspension for flow cytometry characterization.** (A) Schematic showing force signal at the junction between  $\mu$ TS and platelets. To lyse the platelets, platelets were incubated with lysis buffer (10 mM Tris HCl, 150 mM NaCl, 0.5 mM EDTA, 0.5% NP 40) for 15 min. (B) (top) Representative bright field and fluorescence images showing changes after adding lysis buffer. Platelets were completely lysed after 1 min. Fluorescence of  $\mu$ TS subtly changed after adding lysis buffer and remained stable, indicating the lysis buffer did not denature DNA. Line scan is shown for the dotted yellow line in the images. (bottom) Flow data: forward and side-scatter plots of  $\mu$ TS and platelet- $\mu$ TS mixed samples. Platelet population and  $\mu$ TS population are indistinguishable prior to adding lysis buffer. Scale bar = 5  $\mu$ m.

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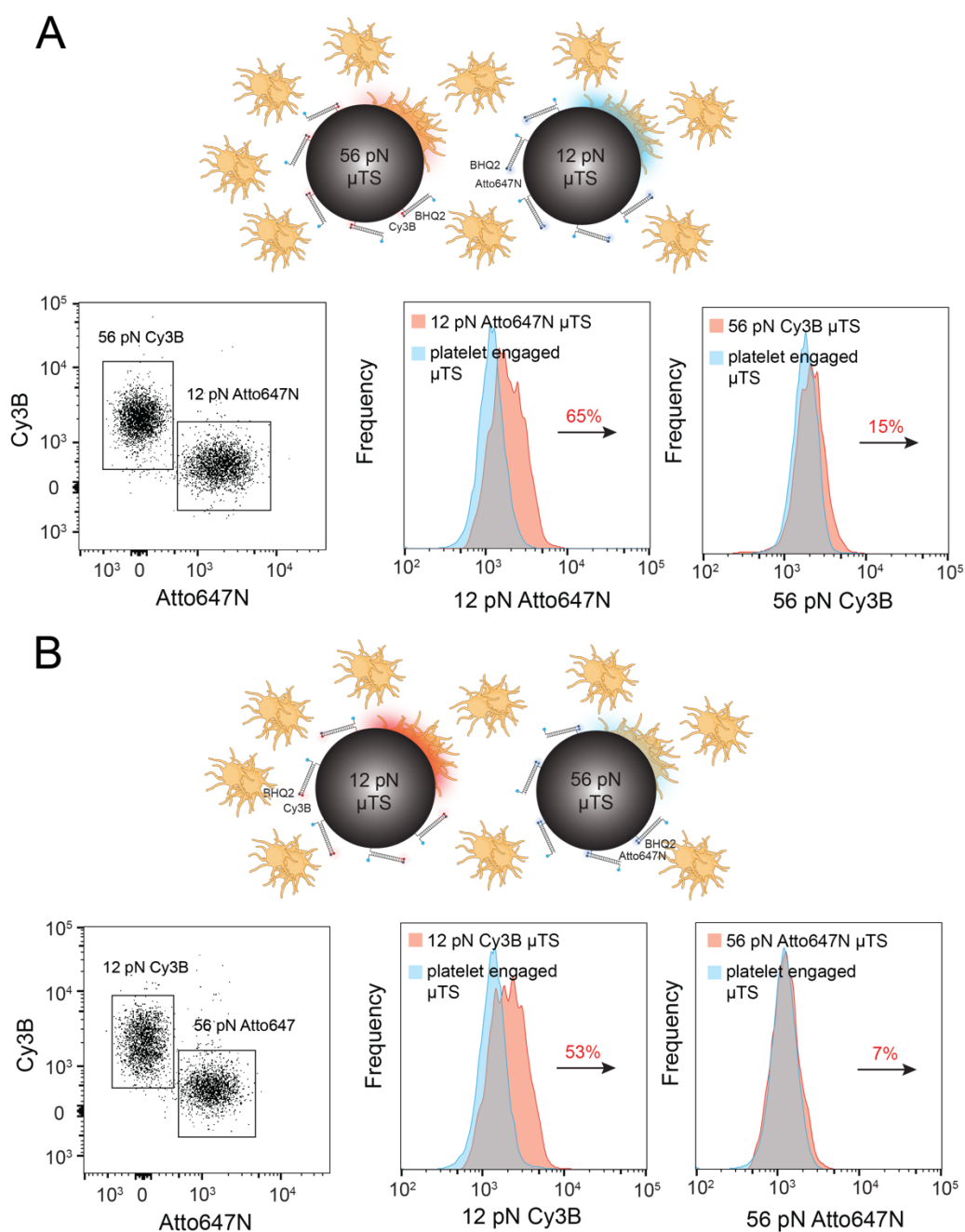
**Figure S9. Comparison of force signal for 12 and 56 pN  $\mu$ TS as measured by microscopy and flow cytometry.** (A) Mean fluorescence increase normalized to the background of the  $\mu$ TS. The signal was measured at the mid-plane of the  $\mu$ TS for particles that strongly engaged platelets. 12 pN  $\mu$ TS showed an 82.3% fluorescence increase while 56 pN  $\mu$ TS exhibited a 29.4% increase. Each data point represents a single  $\mu$ TS particle and there were  $n = 80$  particles in each group. The error bar shows the standard deviation. (B) Mean fluorescence intensity (MFI) of 12 pN and 56 pN  $\mu$ TS increased 84% and 34%, respectively after engaging platelets for 1 hr at room temperature.

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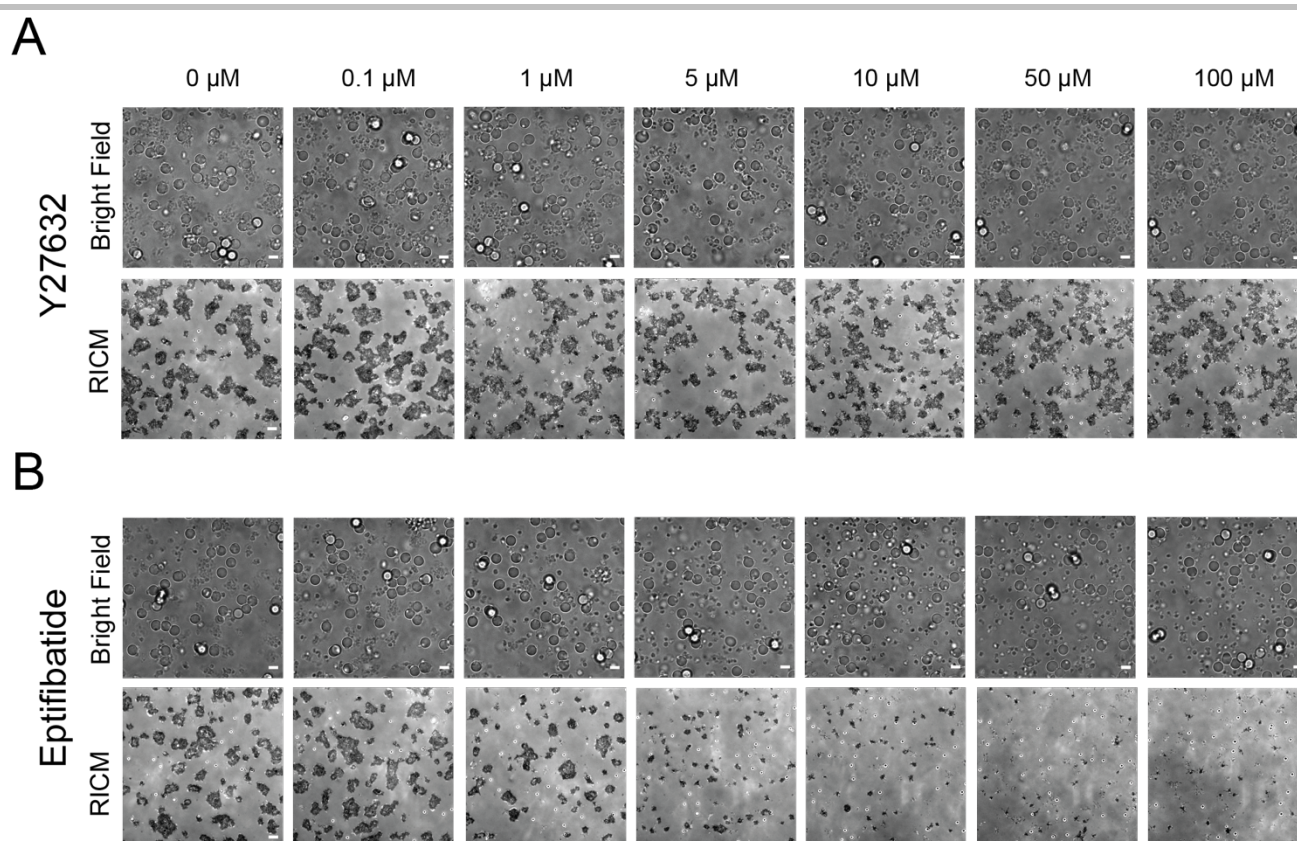
**Figure S10.  $\mu$ TS presenting RAD as a negative control to confirm force dependent fluorescence change.** (A) Mean fluorescence intensity (MFI) of 12 pN cRGD  $\mu$ TS increased 3.5-fold after engaging platelets for 1 hr at room temperature. (B) MFI of 56 pN cRGD  $\mu$ TS increased 1.8-fold after engaging platelets for 1 hr. (C) MFI of 12 pN cRAD  $\mu$ TS increased 0.2-fold after engaging platelets. Note: the intensity values shown in these experiments are different from the ones in the main figures because this platelet- $\mu$ TS samples were allowed to engage for 1 hr, and these experiments were conducted on a different type of flow cytometer (BD LSR II flow cytometer) with different voltage settings. The total number of events analyzed in each experiment was approximately 10,000 events.

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**Figure S11. Demonstration of spectrally barcoding  $\mu$ TS tension threshold magnitude.** (A) Multiplexing the 12 pN Atto647N  $\mu$ TS and 56 pN Cy3B  $\mu$ TS probes. (B) Multiplexing the 12 Cy3B  $\mu$ TS and 56 pN Atto647N  $\mu$ TS. In both experiments, the mixture of two  $\mu$ TS beads were simultaneously added to the platelet suspension. After 1 hour of incubation, lysis buffer was added to lyse platelet aggregates to allow for flow analysis.  $\mu$ TSs with different force thresholds were gated out according to Cy3B vs Atto647N scatter plots. MFI of these  $\mu$ TSs were then compared to  $\mu$ TS that were not incubated with platelets. 12 pN  $\mu$ TS showed a higher fluorescence change than 56 pN  $\mu$ TS. Similar results was observed after swapping the spectral encoding.

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**Figure S12. Representative bright field and RICM images showing platelet aggregation and adhesion changes with increasing concentrations of Y27632 (A) and eptifibatide (B). Mouse platelets were incubated with the drug for 30 min and then add to 96 well plates for 1 hr and then imaged. Scale bar = 5  $\mu\text{m}$ .**