

1 **Mechano-Cas12a Assisted Tension Sensor (MCATS) for Massively Amplified Cell Traction Force**
2 **Measurements**

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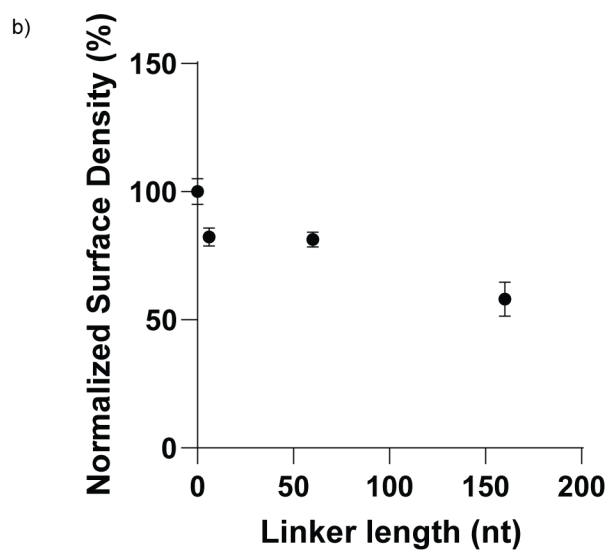
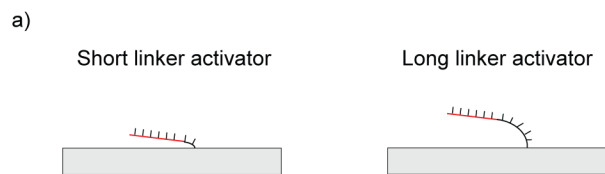
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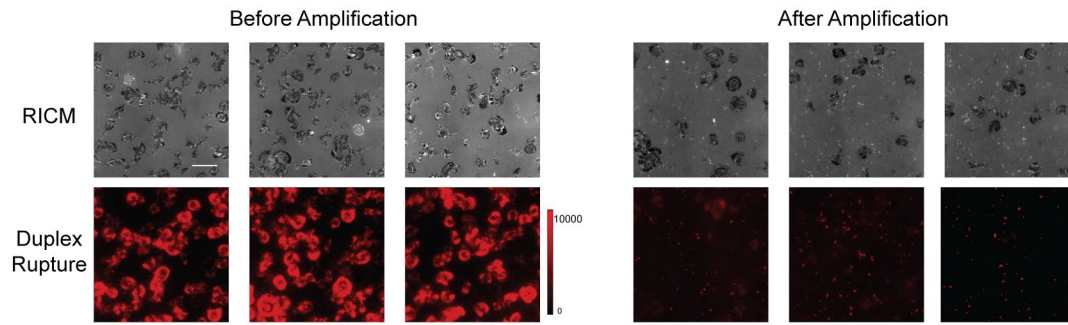
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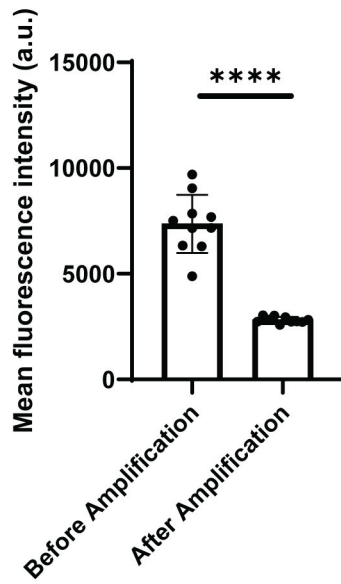


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 38 **Figure S1. Comparison of surface density with different length of linkers.** A) Scheme showing
 39 activators with different linker length on the surface. B) Plots of normalized surface density vs linker
 40 length. All activators are incubated on surface for 1hr at 100nM. Surface density is normalized to activator
 41 without linker. Error bar represents S.E.M from five independent surfaces.

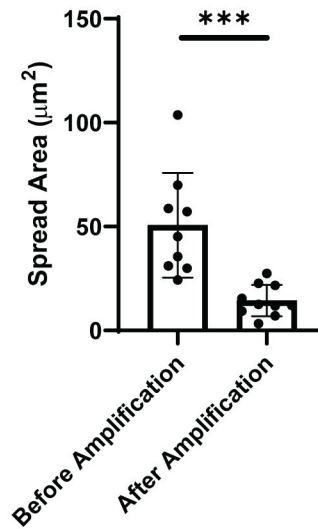
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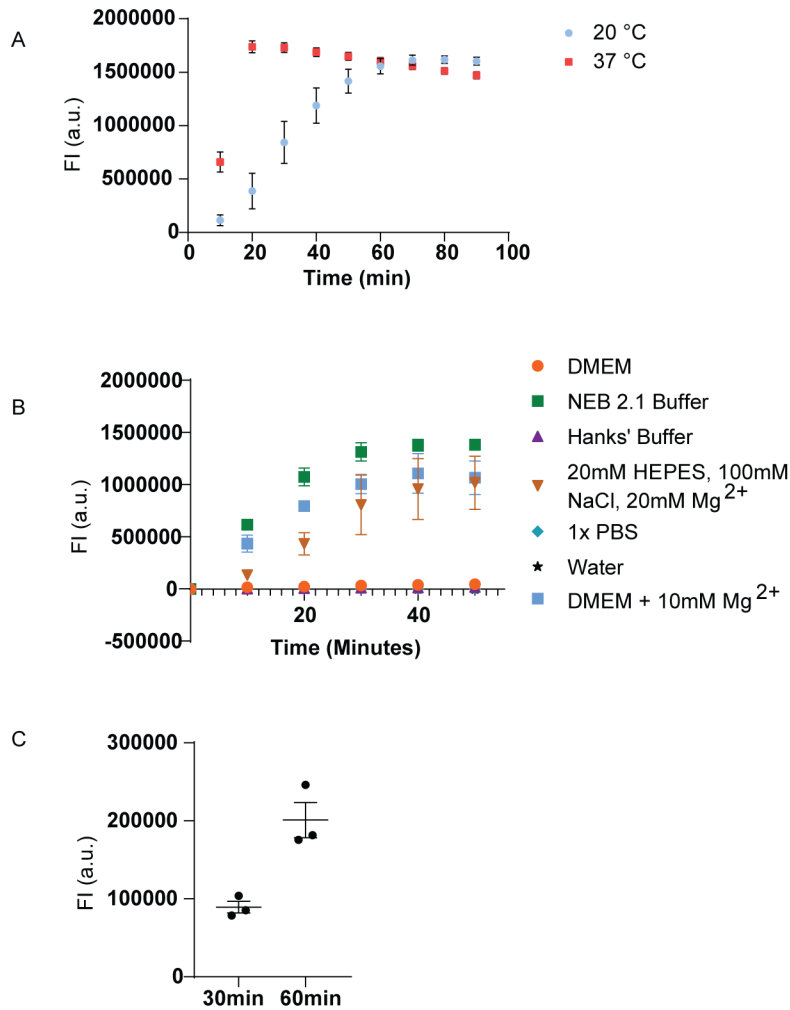


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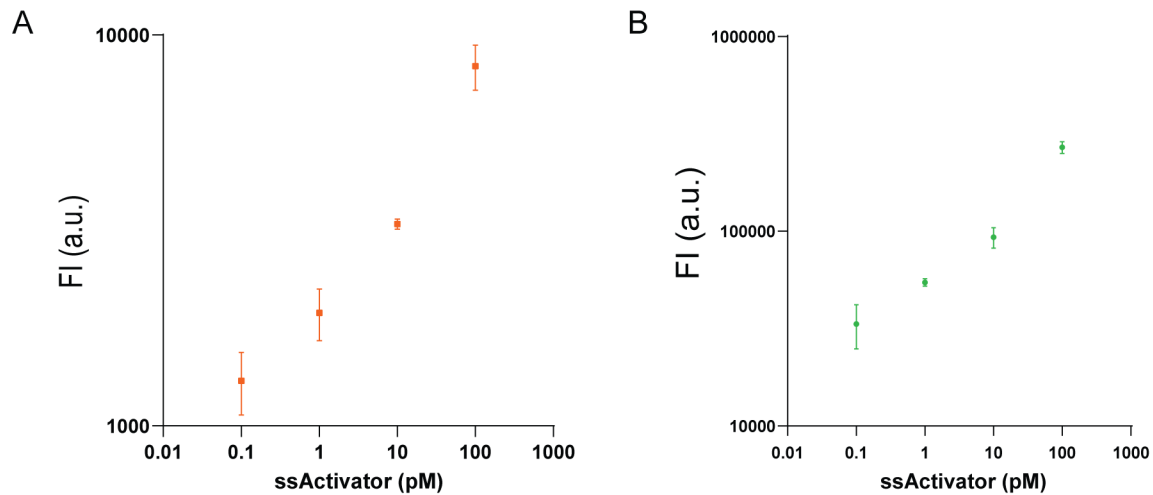


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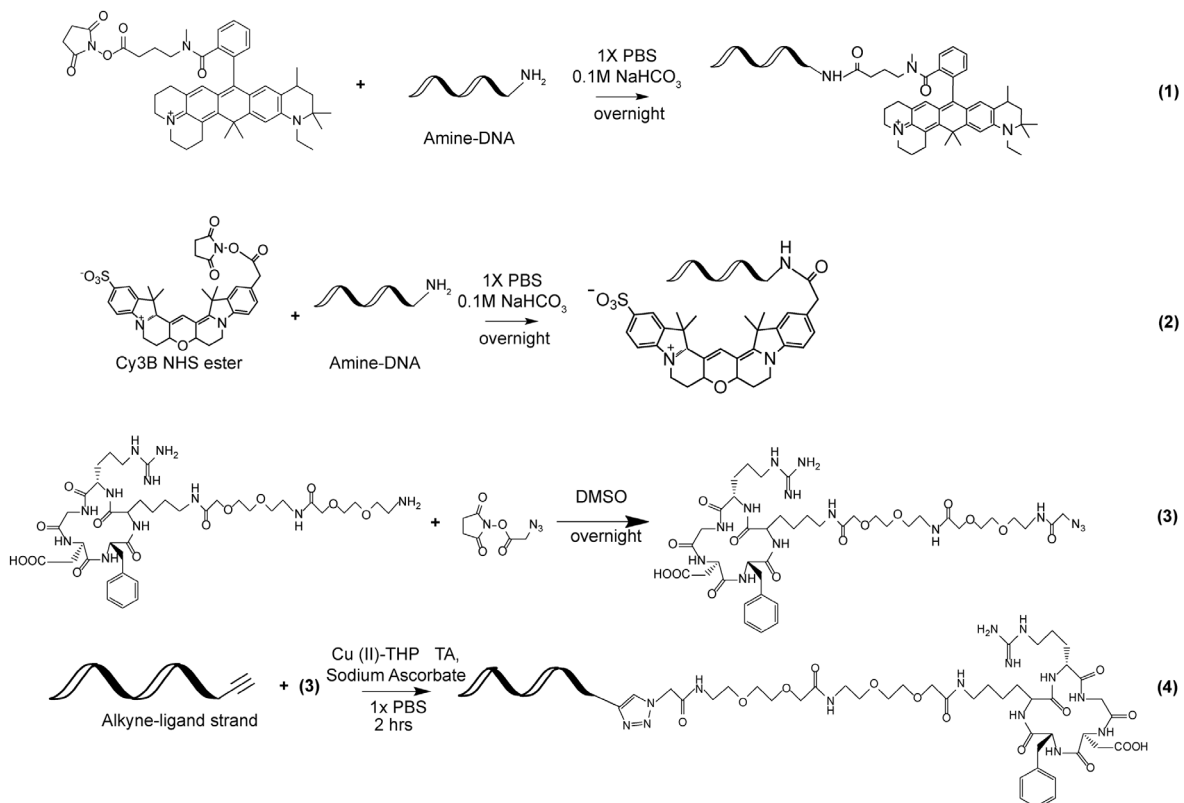
Figure S2. Cas12a auto-cleavage of surface-tethered activator after activation. A) Representative RICM, and duplex rupture (red) fluorescence images after platelets were incubated on concealed activator surface. The images compare the RICM cell spread area and duplex rupture signals before and after Cas12a/gRNA and reporter DNA were added for 1hr. Scale bar = 12 μm . B) Plot of mean fluorescence beneath platelets individual before and after amplification. Significance is calculated with two tailed student t test, $P < 0.0001$. Value is not background subtracted. Error bar represents S.D from 10 individual platelets C) Plot of spread area beneath individual platelets determined from RICM before and after amplification. Significance is calculated with student t test, $P = 0.0004$. Error bar represents S.D from 10 individual platelets



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 53 **Figure S3. Optimization of MCATS parameters of temperature, buffer, and reaction time.** A) Plot
 54 showing time-dependent fluorescence for Cas12a mediated hydrolysis of reporter DNA at 20 and 37 °C.
 55 The reaction was run with 100 nM soluble activator, 20 nM gRNA/Cas12a complex, and 100 nM reporter
 56 DNA. Results show that Cas12a activity is greater at 37 °C. Error bar represents S.E.M. obtained from
 57 three independent experiments. B) Plots of Cas12a activity in different buffers. We found that Cas12a is
 58 markedly less active in standard cell culture media compared to that of NEB buffer 2.1 which is likely due
 59 to decreased Mg ion concentration in cell media. Spiking cell culture media with 10 mM Mg²⁺ rescued
 60 Cas12a activity. Error bar represents S.E.M. obtained from three independent experiments. C) Plots of
 61 fluorescence signal at 30 and 60 min after triggering MCATS with Cas12a and reporter determined from
 62 human platelets (2*10⁶). Error bar represents S.E.M. obtained from three independent experiments.
 63 Results indicate that 1hr amplification provides improved signal in cell experiments.

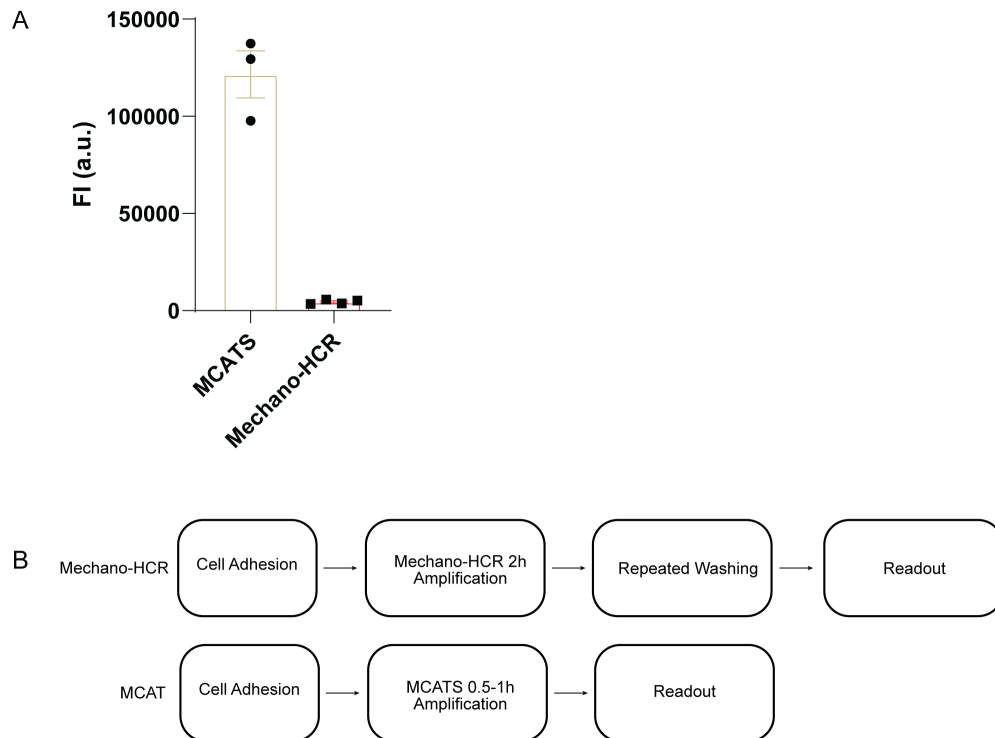


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65 **Figure S4. Comparing MCATS with two fluorogenic ssDNA substrates.** A) 20 nM Cas12a-gRNA
66 complex and 100 nM reporter DNA (/5ATTO565N/TT ATT /3BHQ_2/) of was added to various
67 concentration of ssDNA and mixed with concealed activator for a total concentration of 100nM in solution,
68 plot of fluorescence intensity-ssActivator concentration. Error bar represents S.E.M. obtained from three
69 independent experiments. S/N (signal to noise ratio) = 35 for 100pM ssActivator. Noise was calculated
70 from standard deviation of the blank B) 20 nM Cas12a-gRNA complex and 100 nM reporter DNA (/56-
71 FAM/TT ATT /3IABkFQ/) of was added to 100 nM ssDNA and concealed activator in solution, plot of
72 fluorescence intensity-ssActivator concentration. S/N = 18. The better S/N of Atto 565N reporter is chosen
73 for more sensitive detection in later experiments.

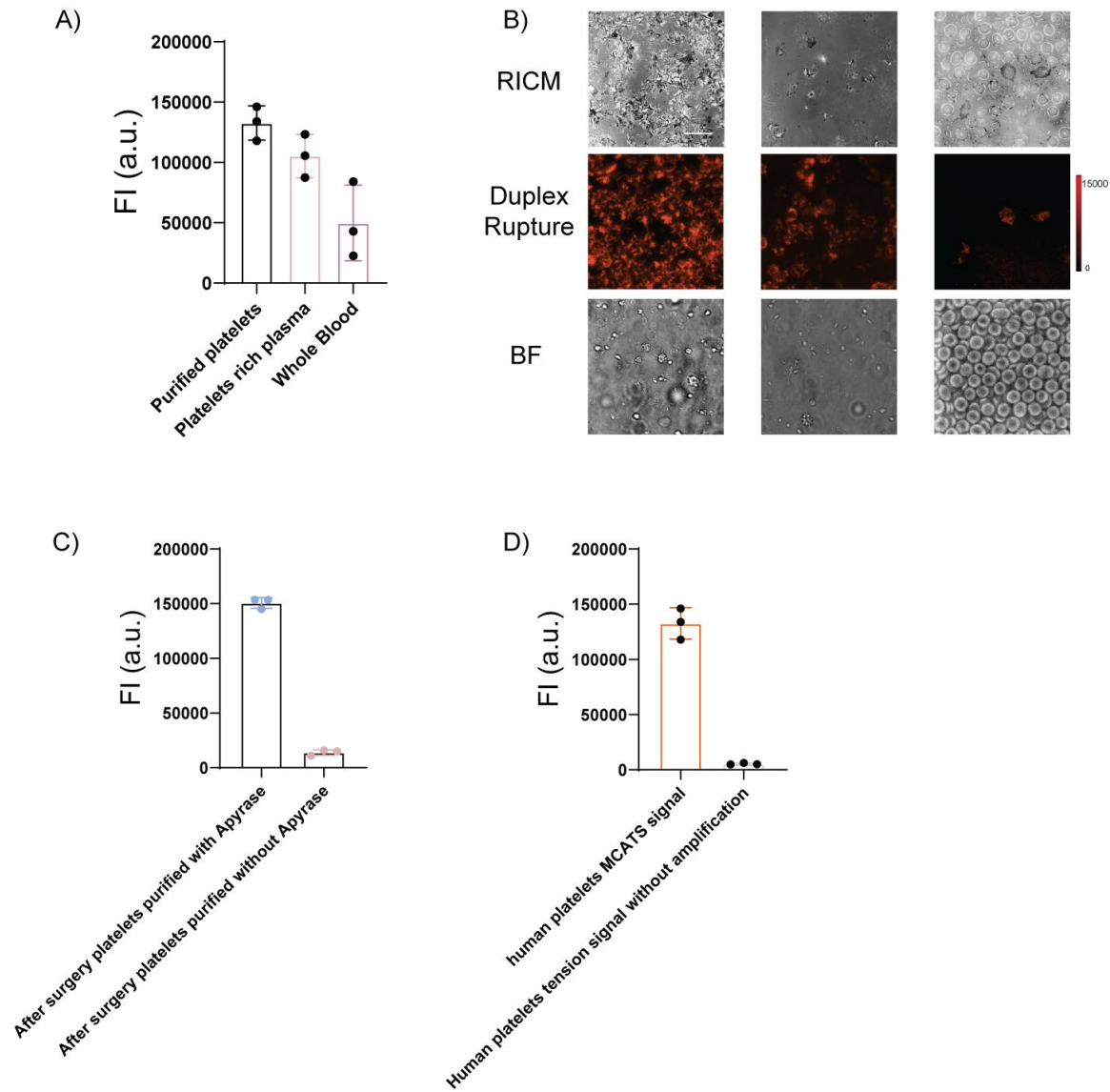


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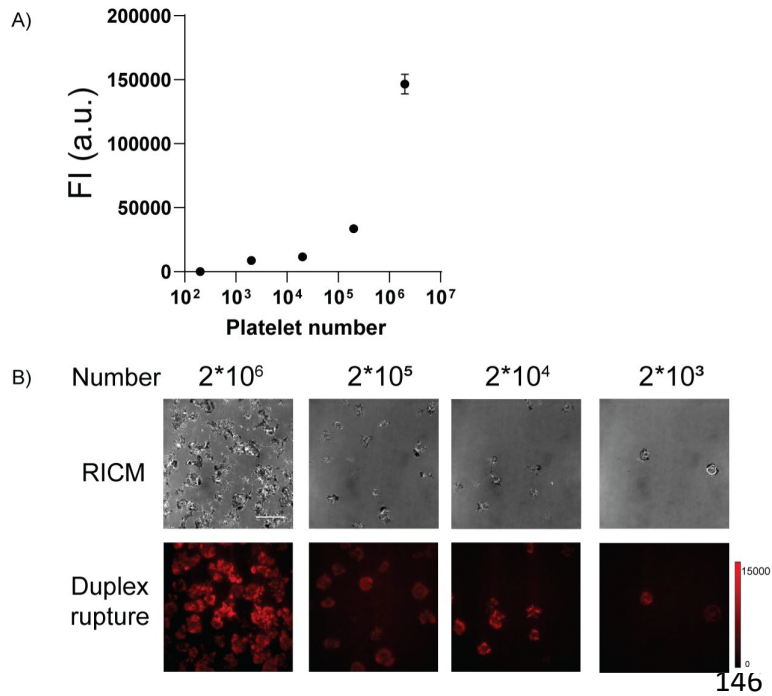
Figure S5: Modified oligonucleotides. Chemical structures and reactions of oligonucleotides, dye NHS esters and cRGDfk peptides.



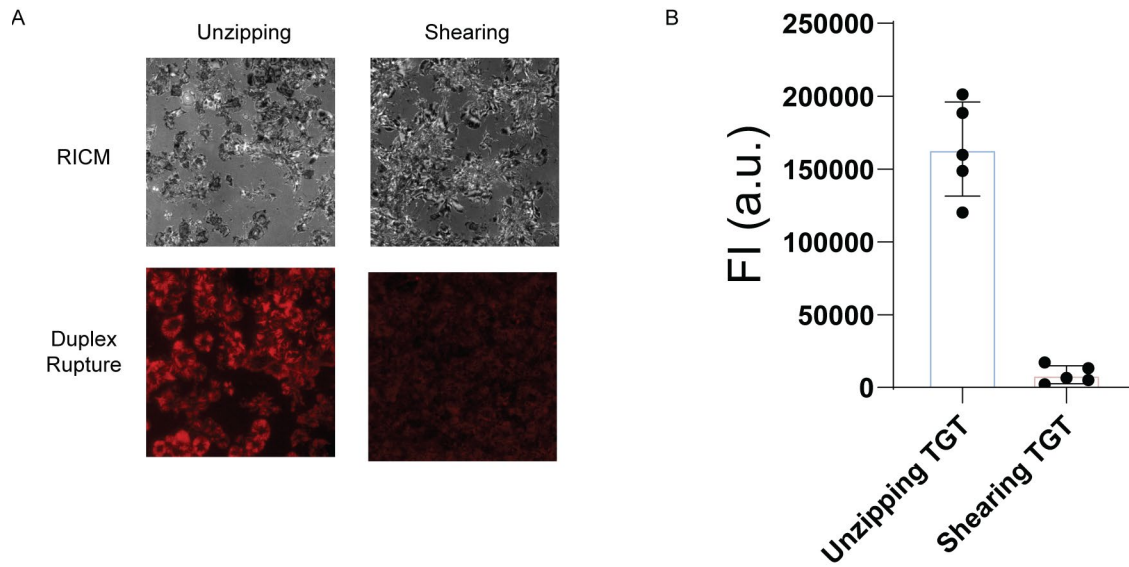
77 **Figure S6: Comparison between MCATS and Mechano-HCR.** A) Comparison of fluorescent signal of
 78 MCATS and Mechano-HCR amplified tension signal in the same day experiment with same number of cells
 79 (20000 NIH/3T3) seeded on the same surface and measured from same plate reader. B) Comparison of
 80 workflow of MCATS and Mechano-HCR. MCATS requires less steps in sample handling and less time in
 81 incubation



114 **Figure S7: Platelets handling optimization.** A) Comparison of MCATS signal using purified platelets,
 115 platelets rich plasma and whole blood. Error bar represents S.D from three wells with same patient sample.
 116 B) Representative of RICM, duplex rupture (red) fluorescence images and bright field images of tension
 117 signal when seeding different samples on concealed activator surface for 1hr. Scale bar = 12 μm. C)
 118 Comparison of MCATS signal in one experiment with platelet purification with/without Apyrase. In some
 119 post-surgery samples, patients' blood showed hemolysis during centrifugation, which required Apyrase to
 120 prevent platelets aggregation during purification. Error bar represents S.D from three wells with same
 121 patient sample. D) Comparison of MCATS and non-amplified duplex rupture signal using plate reader,
 122 indicating MCATS amplification is crucial for detecting platelets tension signal. Error bar represents S.E.M.
 123 from three independent experiments.

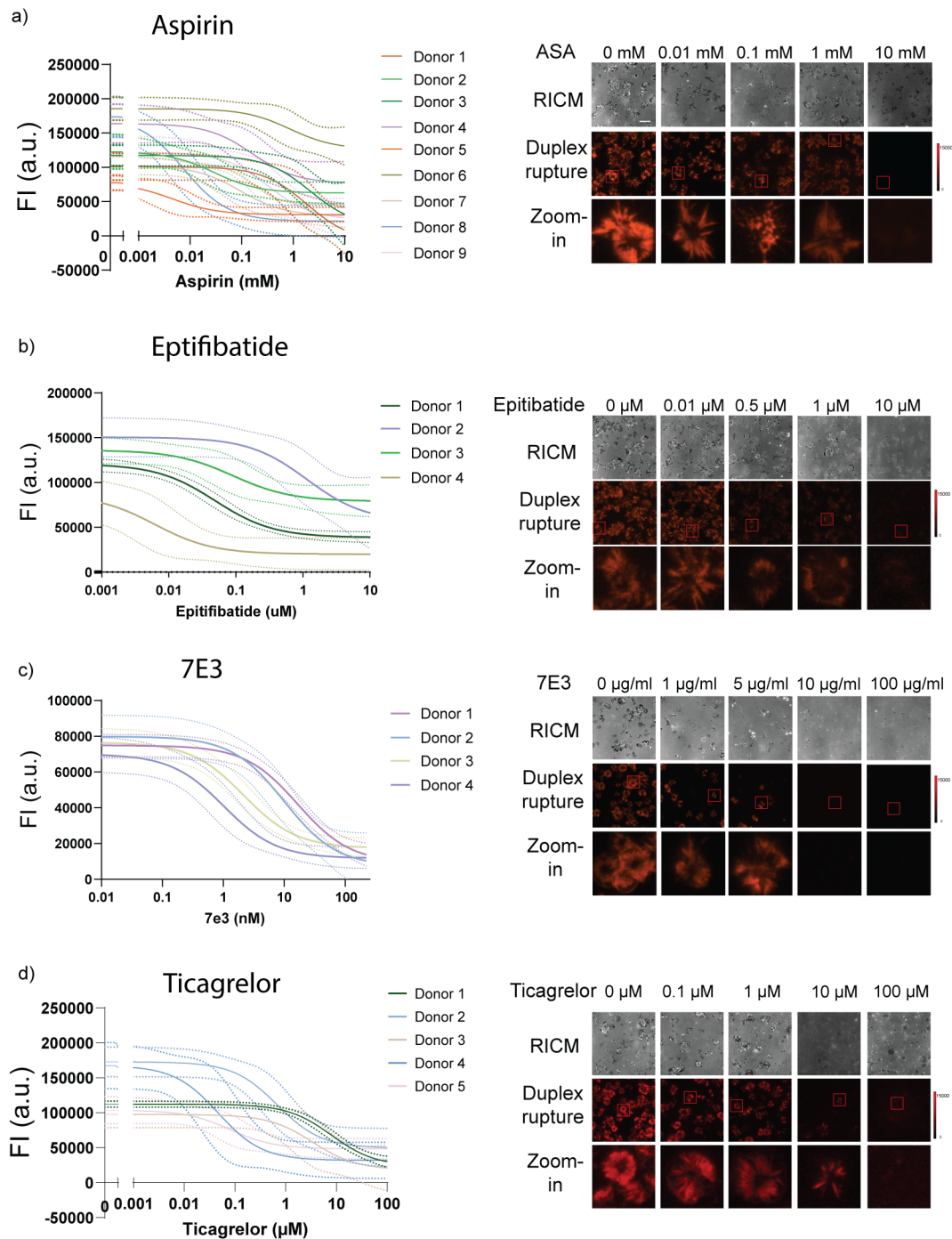


147 **Figure S8: Measuring MCATS signal with different number of platelets seeded on surface.** A)
 148 Comparison of MCATS signal using purified platelets, platelet rich plasma, and whole blood. Error bar
 149 represents S.E.M. from three independent experiments. B) Representative of RICM, duplex rupture (red)
 150 fluorescence images and bright field images of tension signal when seeding different number of cells on
 151 concealed activator surface. Scale bar = 10 μ m



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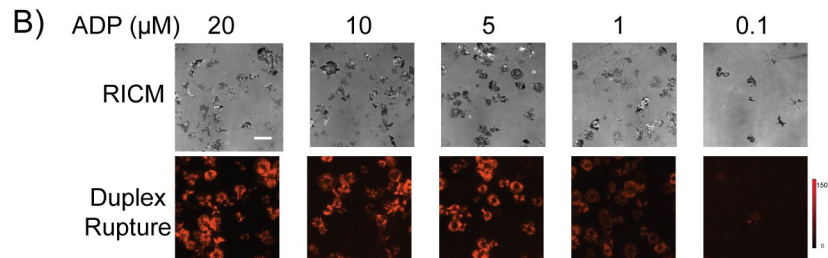
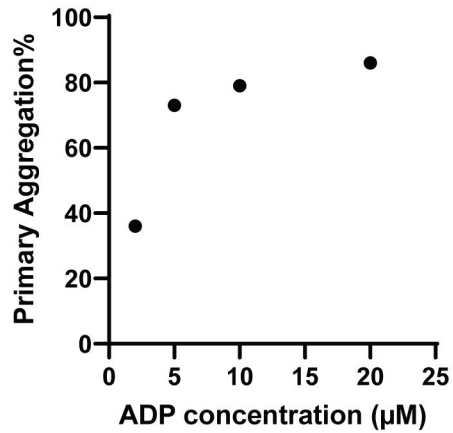
Figure S9: Measuring human platelets tension with shearing and unzipping DNA tension probes. A) Representative RICM and fluorescence images of cells cultured on $T_{\text{tol}} = 12$ pN and $T_{\text{tol}} = 56$ pN surfaces for 1hr. Scale bar = 12 μm . The red color is emission from Atto647N tagging the activator. The intensity bar next to each fluorescence image shows the absolute signal intensity for each image. B) Plots showing MCATS signal of same number of cells seeded on $T_{\text{tol}} = 12$ pN and $T_{\text{tol}} = 56$ pN surfaces. Error bars represent S.E.M. from n=3 independent experiments.



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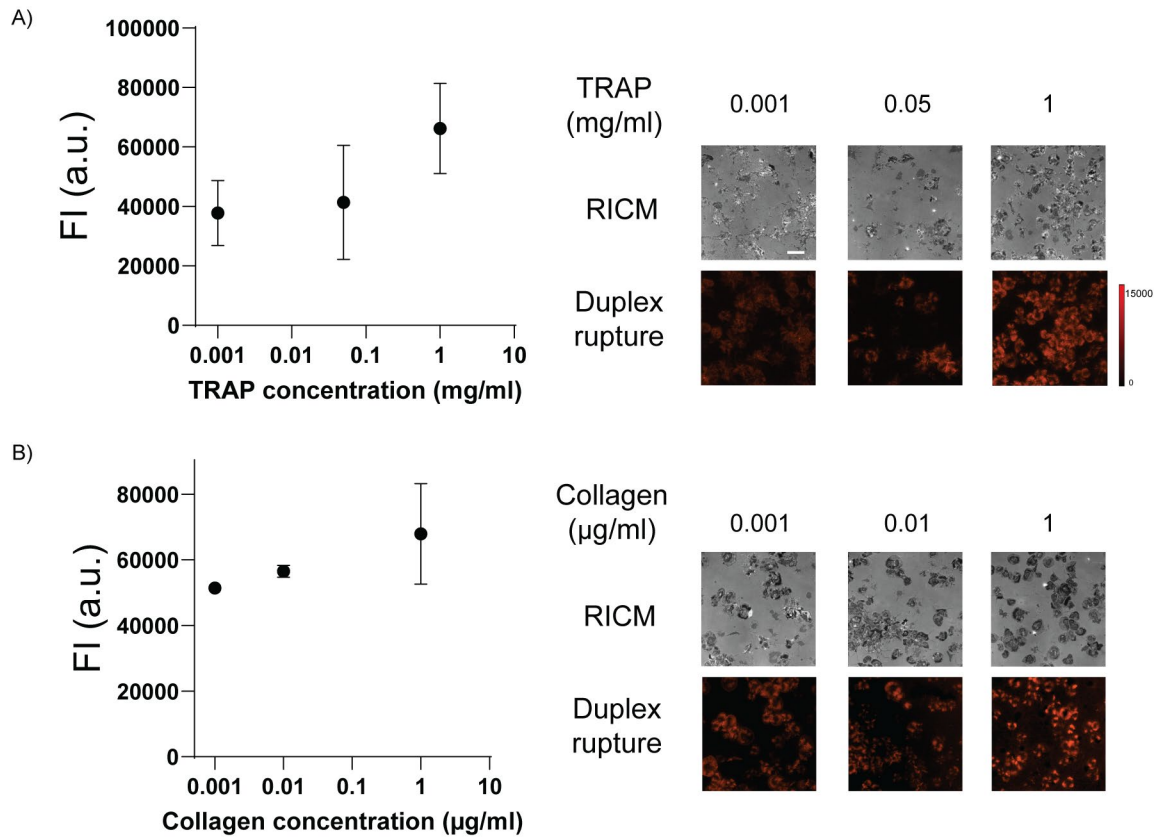
Figure S10: MCATS used to measure dose-response curves for inhibitors of platelets. a-d) Plots of [Aspirin], [Eptifibatide], [7E3], [Ticagrelor] vs MCATS signal for different donors. A dose-response titration of six drug concentrations, for each drug for individual donors is measured with MCATS and all measurements were performed in duplicate or triplicate. Mechano-IC50 for each donor was calculated by fitting plot to a standard dose-response function: $\text{Signal} = \text{Bottom} + (\text{Top} - \text{Bottom}) / (1 + ([\text{drug}] / \text{IC}_{50}))$. Solid line represents the fitting and dashed line represents 95% CI. Representative RICM, duplex rupture fluorescence (red) and zoom-in fluorescence images for one donor with drug concentration ranging from 0 mM to 10mM. Scale bar = 10 μm .

A) **Light Transmission Aggregometry**



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Figure S11: LTA data for ADP agonist test. A) Light Transmission aggregometry data for primary aggregation value against different concentration of ADP. B) Representative RICM, duplex rupture fluorescence (red) images for one donor with ADP concentration ranging from 0.1 mM to 20mM. Scale bar = 10 µm.



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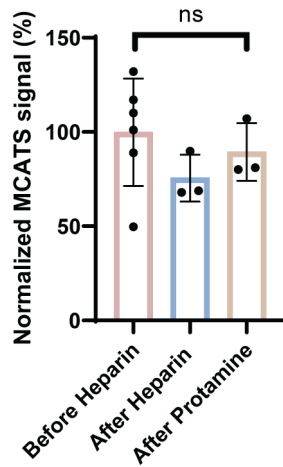
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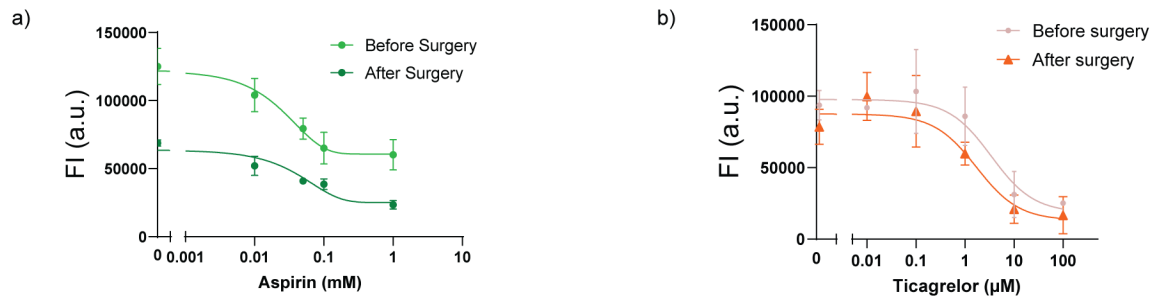
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Figure S12: TRAP and collagen agonist test. A) Plots of MCATS signal against different concentration of TRAP. Error bar represents SD from n=2 measurements. Representative RICM, duplex rupture fluorescence (red) images for one donor with TRAP concentration ranging from 0.001 mg/ml to 1mg/ml. Scale bar = 10 µm. B) Plots of MCATS signal against different concentration of Collagen. Error bar represents SD from n=2 measurements. Representative RICM, duplex rupture fluorescence (red) images for one donor with TRAP concentration ranging from 0.001 µg/ml to 1µg/ml. Scale bar = 10 µm.



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Figure S13: Heparin and protamine influence on platelets tension. Plots of normalized MCATS signal for purified platelets from non-heparin treated blood, purified platelets from 2hr heparin (0.25U/ml) treated blood, and purified platelets from heparin treated and protamine neutralized (1mg/100U) blood. Results indicating heparin influence on platelets was fully reversed after protamine addition. Error bar represents S.E.M. from n=3 independent experiments.



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190 **Figure S14: Sensitivity of aspirin and ticagrelor for patients before and after surgery.** (A) Plots of
191 [Aspirin] vs MCATS signal for patient before and after Surgery. Error Bar representative three replicate
192 wells in the same day experiment. Mechano-IC₅₀ for each donor was calculated by fitting plot to a standard
193 dose-response function: $\text{Signal} = \text{Bottom} + (\text{Top} - \text{Bottom}) / (1 + ([\text{drug}] / \text{IC}_{50}))$. The difference of calculated IC₅₀
194 before and after surgery for two patients is non-significance with two tailed student T test, $P = 0.65$. (B) Plots
195 of [Ticagrelor] vs MCATS signal for patient before and after Surgery. Error Bar represent two replicate wells
196 in the same day experiment. Mechano-IC₅₀ was calculated by fitting plot to a standard dose-response
197 function: $\text{Signal} = \text{Bottom} + (\text{Top} - \text{Bottom}) / (1 + ([\text{drug}] / \text{IC}_{50}))$.

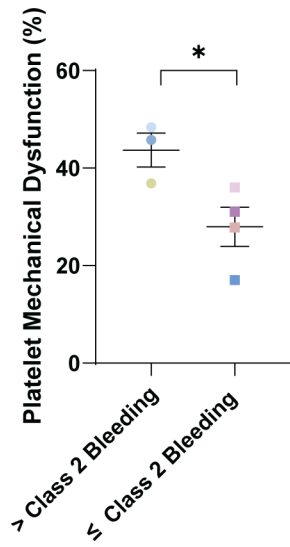
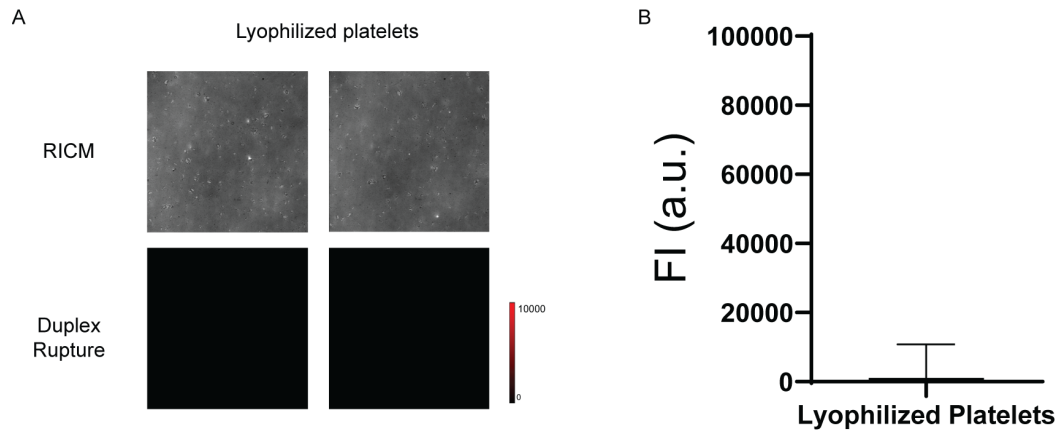


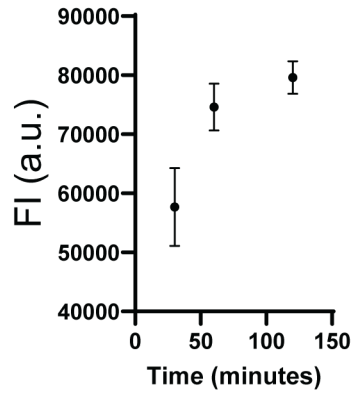
Figure S15: Patient bleeding severity is correlated with platelet mechanical dysfunction.

198 Plot of reduction in MCATS signal (%) for subjects binned into two groups: mild/insignificant bleeding
199 (class 1 and 2) or moderate/severe/massive bleeding (class 3, 4, and 5). Significance is calculated with
200 two tailed student t-test with $P=0.03$. Error bars represent S.E.M.



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Figure S16: Tension measurement of lyophilized platelets. A) Representative RICM, duplex rupture fluorescence (red) images for lyophilized platelets on tension probes. B) Plots of MCATS signal of lyophilized platelets seeded on concealed DNA tension probes for 1hr. Results indicating lyophilized platelets have no active mechanical signal.



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Figure S17: Amplification time influence on MCATS signal. Plots of MCATS signal at different time of amplification in platelet tension detection experiment. Error bar represents SEM of three independent experiments.

Table S2: ESI-Mass result

Strand Name	Expected Mass	Measured Mass
Cy3B 60T Cas12a Bottom Strand	26059.7	26059.3
Atto 647N 60T Cas12a Bottom Strand	26145.7	26143.3
cRGDfk BHQ2 Cas12a Strand	8514	8514.7
Cy3B 6T Bottom Strand	9633.2	9632.8
Cy3B No spacer Cas12a Bottom Strand	7808	7807.5

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Table S3: Demographics, laboratory values, TEG data and surgery Note for CPB Patients

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5
Age	61	22	79	25	68
Sex	F	M	F	F	M
Operation	MVR/TVR	PVR	Myectomy + AVR	Nephrectomy	Bentall
Hematology*					
Platelet count (cells mL-1)	322	264	172	482	168
Medication*					
Aspirin	Y	Y	N	N	N
ADPI	N	N	N	N	N
TEG6S MA (mm)	68.6	69	64	66	63
Operation Note					
CPB time (min)	314	84	73	204	182
Blood Products Transfused within 24h of Operation					
RBC	3	0	0	18	0
PLT	6	2	1	5	0
FFP	4	0	0	19	0
Cryo	15	5	0	50	0
	Patient 6	Patient 7			
Age	71	39			
Sex	M	M			
Operation	CABG x 3	AVR/MVR			
Hematology*					
Platelet count (cells mL-1)	144	108			
Medication*					
Aspirin	N	N			
ADPI	N	N			
TEG6S MA (mm)	65.4	68.1			
Operation Note					
CPB time (min)	116	405			
Blood Products Transfused within 24h of Operation					
RBC	0	6			
PLT	0	3			
FFP	0	7			

Cryo

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