nature biomedical engineering

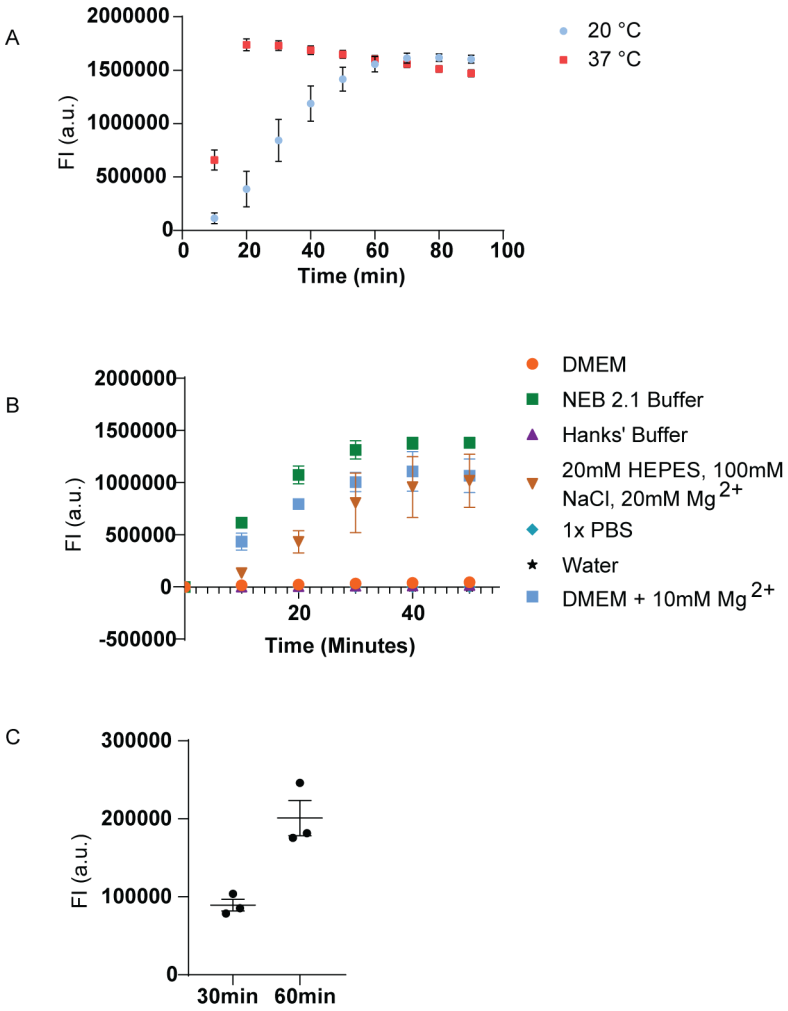
Article

https://doi.org/10.1038/s41551-023-01114-1

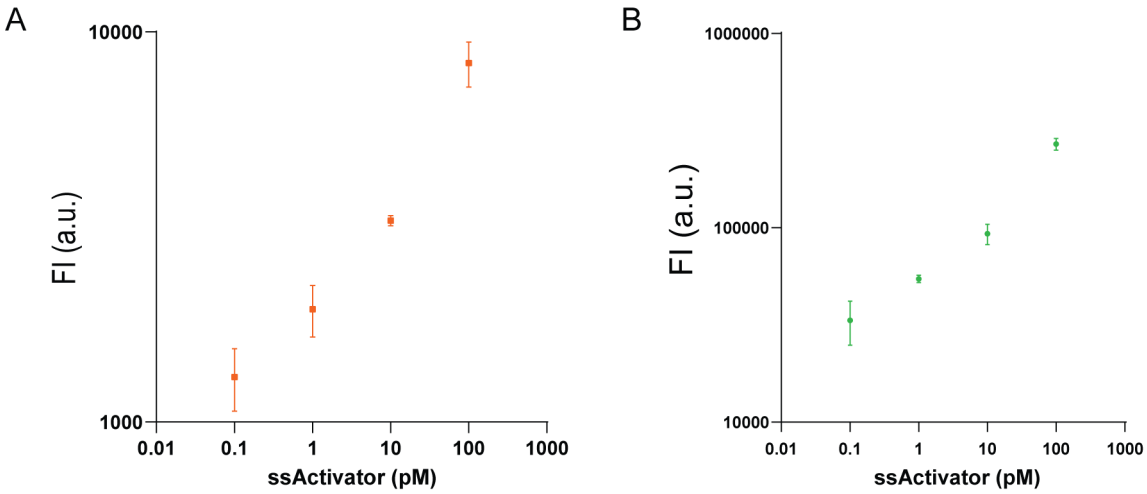
Detection of cellular traction forces via the force-triggered Cas12a-mediated catalytic cleavage of a fluorogenic reporter strand

In the format provided by the authors and unedited

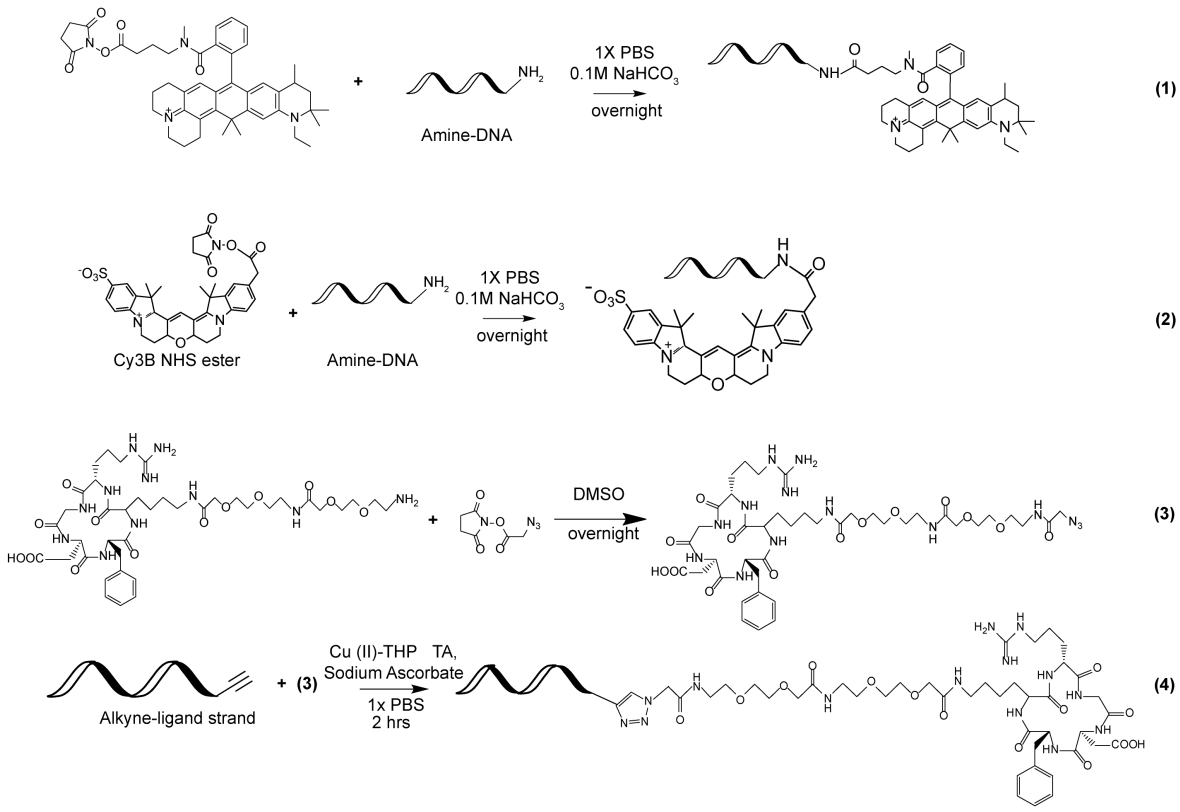
Contents



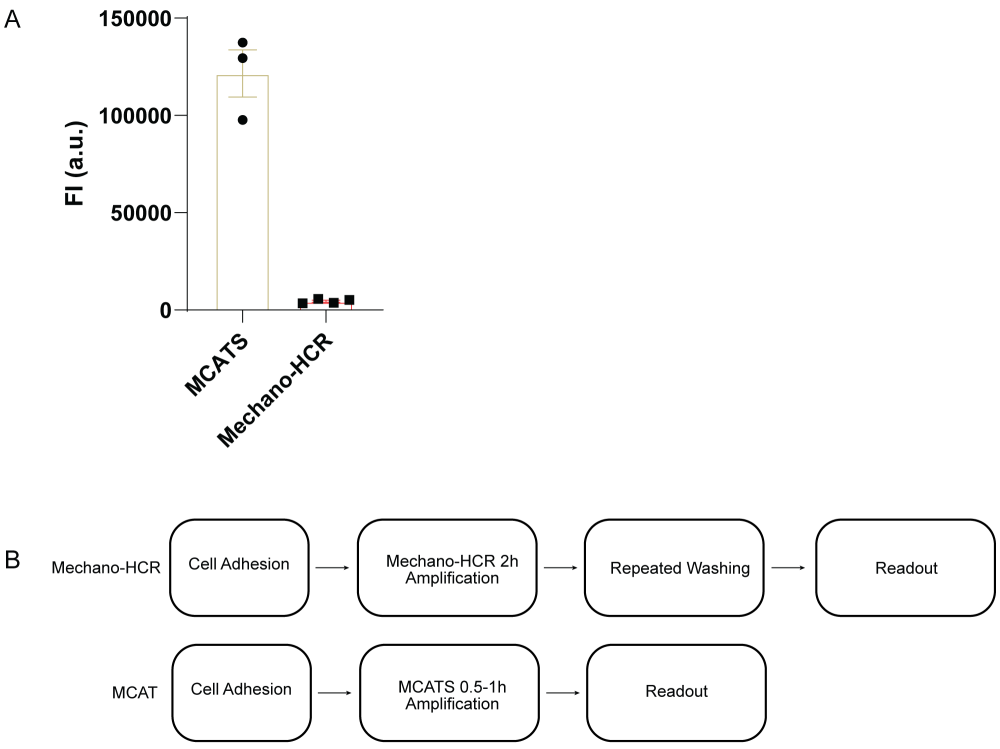
Supplementary Fig. 1 l Optimization of MCATS parameters of temperature, buffer, and reaction time. A) Plot showing time-dependent fluorescence for Cas12a mediated hydrolysis of reporter DNA at 20 and 37 °C. The reaction was run with 100 nM soluble activator, 20 nM gRNA/Cas12a complex, and 100 nM reporter DNA. Results show that Cas12a activity is greater at 37 °C. Error bar represents S.E.M. obtained from three independent experiments. B) Plots of Cas12a activity in different buffers. We found that Cas12a is markedly less active in standard cell culture media compared to that of NEB buffer 2.1 which is likely due to decreased Mg ion concentration in cell media. Spiking cell culture media with 10 mM Mg2+ rescued Cas12a activity. Error bar represents S.E.M. obtained from three independent experiments. C) Plots of fluorescence signal at 30 and 60 min after triggering MCATS with Cas12a and reporter determined from human platelets (2*10^6). Error bar represents S.E.M. obtained from three independent experiments. Results indicate that 1hr amplification provides improved signal in cell experiments.



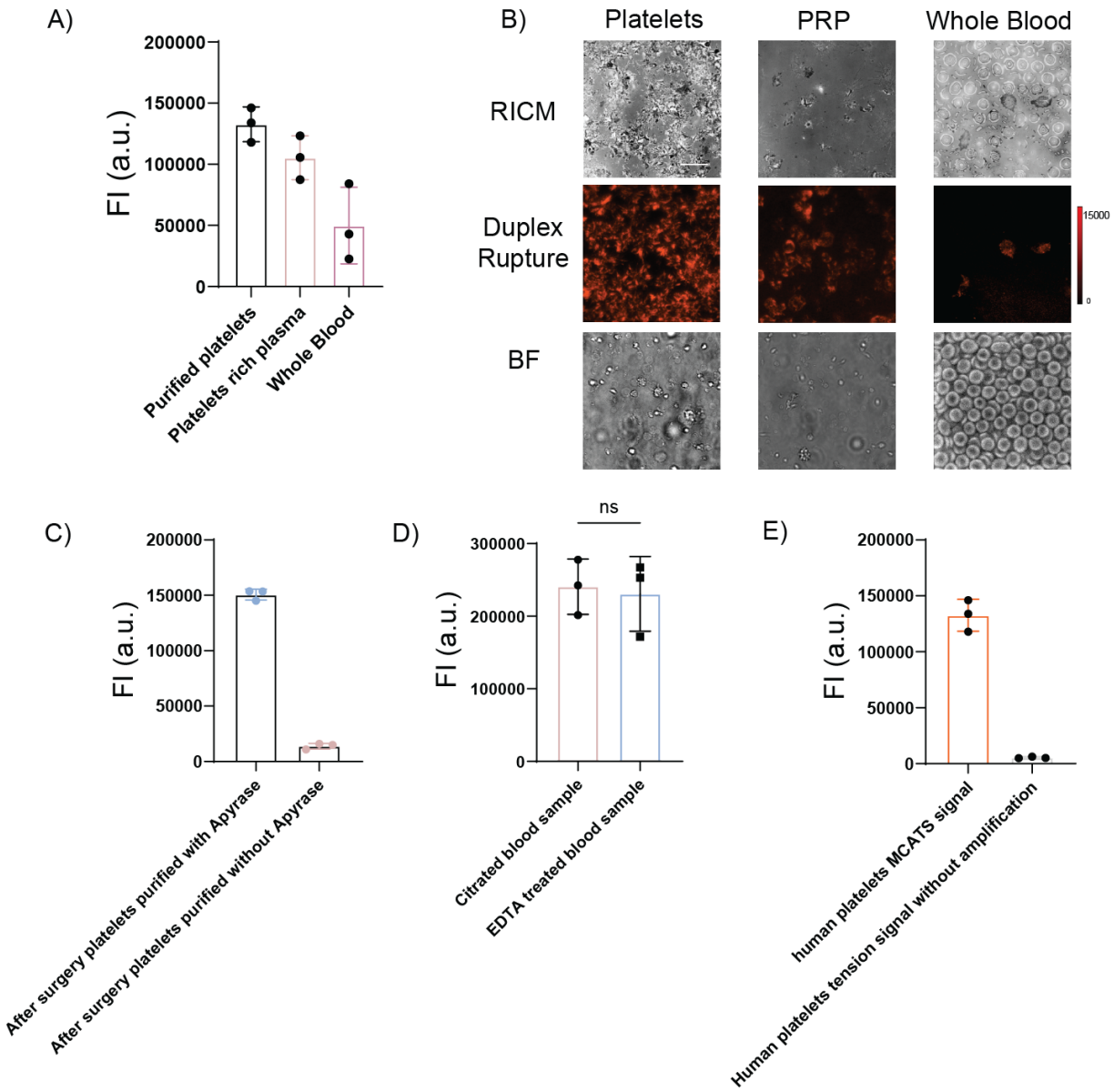
Supplementary Fig. 2 l Comparing MCATS with two fluorogenic ssDNA substrates. A) 20 nM Cas12a-gRNA complex and 100 nM reporter DNA (/5ATTO565N/TT ATT /3BHQ_2/) of was added to various concentration of ssDNA and mixed with concealed activator for a total concentration of 100nM in solution, plot of fluorescence intensity-ssActivator concentration. Error bar represents S.E.M. obtained from three independent experiments. Center represents the average of the three independent experiments. S/N (signal to noise ratio) = 35 for 100pM ssActivator. Noise was calculated from standard deviation of the blank B) 20 nM Cas12a-gRNA complex and 100 nM reporter DNA (/56-FAM/TT ATT /3IABkFQ/) of was added to 100 nM ssDNA and concealed activator in solution, plot of fluorescence intensity-ssActivator concentration. S/N = 18. The better S/N of Atto 565N reporter is chosen for more sensitive detection in later experiments. Error bar represents S.E.M. obtained from three independent experiments. Center represents the average of the three independent experiments.



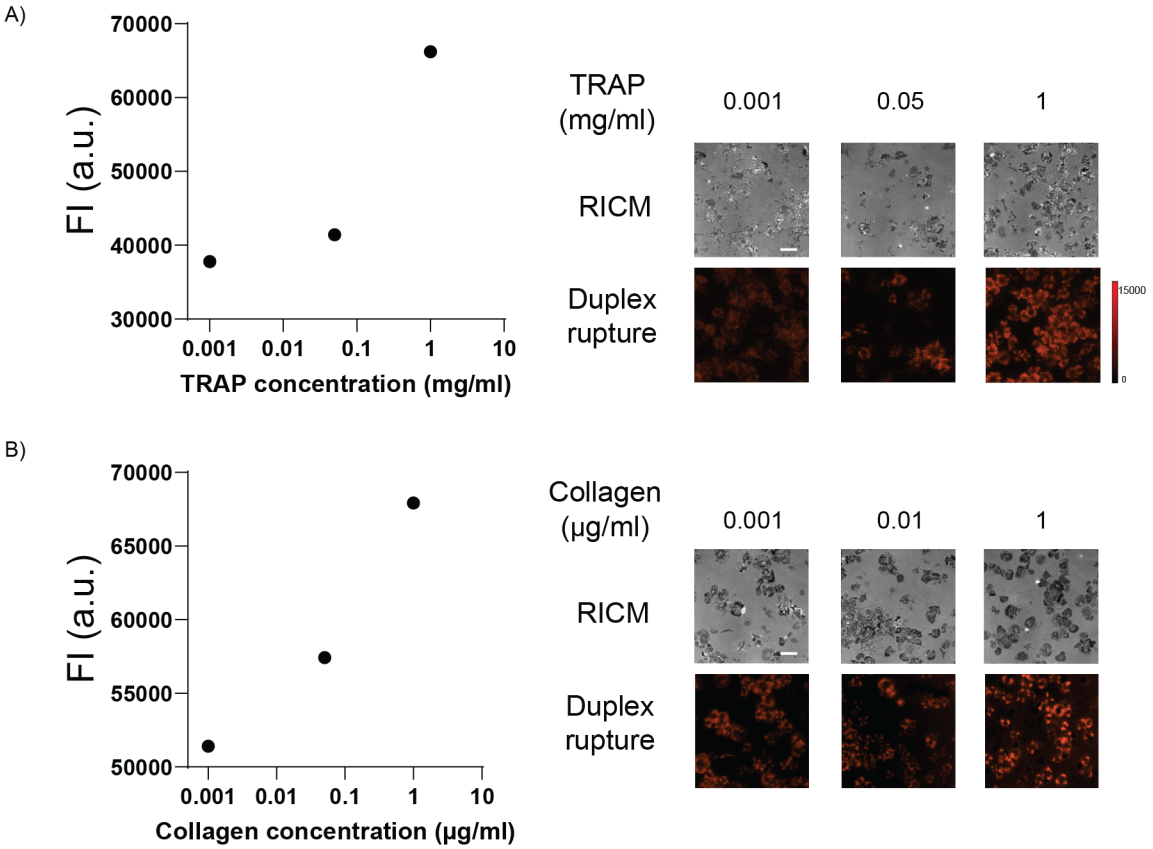
Supplementary Fig. 3 l Modified oligonucleotides. Chemical structures and reactions of oligonucleotides, dye NHS esters and cRGDfk peptides.



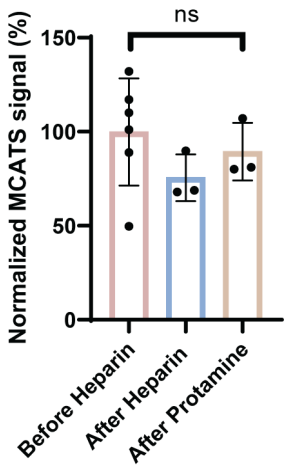
Supplementary Fig. 4 l Comparison between MCATS and Mechano-HCR. A) Comparison of fluorescent signal of MCATS and Mechano-HCR amplified tension signal in the same day experiment with same number of cells (20000 NIH/3T3) seeded on the same surface and measured from same plate reader. Error bar stands for S.E.M. from n=3 independent experiments for MCATS and n=4 independent experiments for mechano-HCR. Center represents the average of the independent measurments. B) Comparison of workflow of MCATS and Mechano-HCR. MCATS requires less steps in sample handling and less time in incubation.



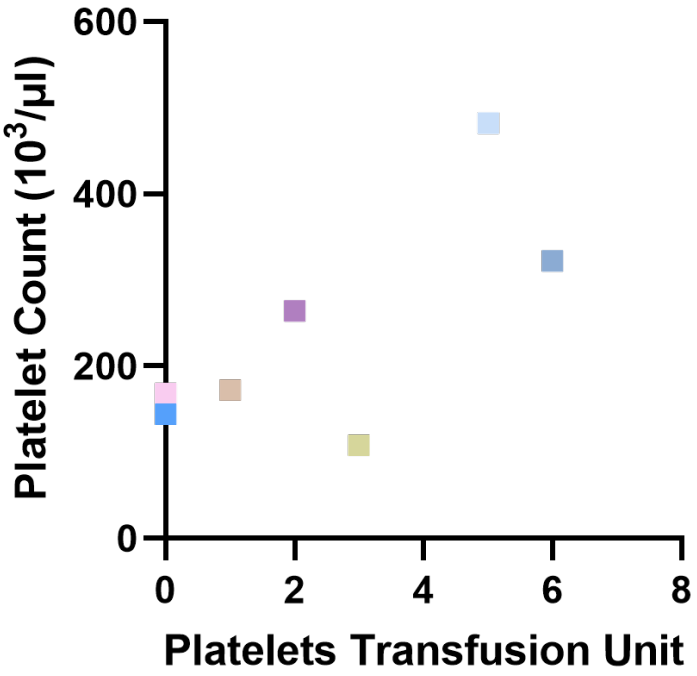
Supplementary Fig. 5 l Platelets handling optimization. A) Comparison of MCATS signal using purified platelets, platelets rich plasma and whole blood. Error bar represents S.D from three wells with same patient sample. Center of error bar means the average of three measurements. B) Representative of RICM, duplex rupture (red) fluorescence images and bright field images of tension signal when seeding different samples on concealed activator surface for 1hr. Scale bar = 12 μm. C) Comparison of MCATS signal in one experiment with platelet purification with/without Apyrase. In some post-surgery samples, patients' blood showed hemolysis during centrifugation, which required Apyrase to prevent platelets aggregation during purification. Error bar represents S.D from three wells with same patient sample. Center of the error bar means the average of three measurements. D) Comparison of MCATS signal with platelets purified from EDTA treated or citrated blood sample. Error bar represents S.D. from three wells with same patient sample. Center of the error bar means the average of three measurements. The *P* value is calculated by two-sided student t test. *P*=0.80. E) Comparison of MCATS and non-amplified duplex rupture signal using plate reader, indicating MCATS amplification is crucial for detecting platelets tension signal. Error bar represents S.E.M. from three independent experiments.



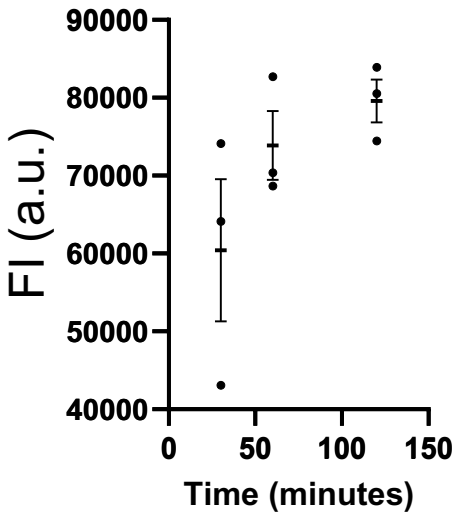
Supplementary Fig. 6 l TRAP and collagen agonist test. A) Plots of MCATS signal against different concentration of TRAP. Data point represents average from n=2 measurements. Representative RICM, duplex rupture fluorescence (red) images for one donor with TRAP concentration ranging from 0.001 mg/ml to 1mg/ml. Scale bar = 10 µm. B) Plots of MCATS signal against different concentration of Collagen. Data point represents average 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.



Supplementary Fig. 7 l 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 for after heparin and protamine group and n=6 for before heparin group. Significance is calculated with two-sided student t-test with P= 0.14.



Supplementary Fig. 8 l Plot of platelets transfusion unit vs platelet count of 7 CPB patients. Platelet count is measured in the clinic before surgery. The correlation analysis shows a positive correlation that was not statistically significant (Pearson's correlation coefficient r= 0.72, two-tailed *P*=0.07)



Supplementary Fig. 9 l 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.

Supplementary Table 1 | List of oligonucleotides.

Name	Sequence (5' to 3')
No Spacer Cas12a Bottom Strand	/5AmMC6/CCTCCTCAAGGAACAACATTGC/3Bio/
Cas12a Top Strand	/5Hexynyl/GCAATGTTGTTCCTTGAGGAGG/3BHQ_2/
6T Spacer Cas12a Bottom Strand	/5AmMC6/CCTCCTCAAGGAACAACATTGCTTTTTT/3Bio/
60T Spacer Cas12a Bottom Strand	/5AmMC6/GTGTCGTGCCTCCGTGCTGTGTTTTTTTTTTTTTTTTTTTT TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT/3Bio/
60T Spacer Shearing mode Cas12a Bottom Strand	/5BiosG/TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT TTTTTTTTTTTTTTT/iAmMC6T/CCTCCTCAAGGAACAACATTGC
160T Spacer Cas12a Bottom Strand	/5AmMC6/GTGTCGTGCCTCCGTGCTGTGTTTTTTTTTTTTTTTTTTT TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT/3Bio/
Control Scramble Bottom Strand	CGCATCTGTGCGGTATTTCAC/3Bio/
Control Scramble top Strand	GCTGGGCTACGTGGCGCTCTT
ATTO 565N Reporter DNA	/5ATTO565N/TTATT/3BHQ_2/
FAM reporter DNA	/56-FAM/TTATT/3IABkFQ/
MCATS crRNA	UAA UUU CUA CUA AGU GUA GAU GCA AUG UUG UUC CUU GAG GA

Supplementary Table 2 | 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

Supplementary Table 3 | 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 mcL-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 mcL-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	0	0