

Supplemental Material

Supplemental Table 1. Baseline medication of individuals stratified by low and high monocyte platelet aggregate (MPA) levels.

Baseline Medications, <i>n</i> (%)	All <i>n</i> =149	MPA ^{low} <i>n</i> =28	MPA ^{high} <i>n</i> =32	<i>P</i> -value
Statin	30 (20.1)	4 (14.3)	8 (25.0)	0.349
Ezetimibe	4 (2.7)	2 (7.1)	0 (0.0)	0.214
Oral antidiabetic medication				
Metformin	47 (31.5)	11 (39.3)	9 (28.1)	0.522
SGLT2 inhibitor	12 (8.1)	5 (17.9)	2 (6.2)	0.235
DPP4 inhibitor	5 (3.4)	0 (0.0)	1 (3.1)	1
Antidiabetic injectables				
GLP1 agonist	29 (19.5)	7 (25.0)	4 (12.5)	0.318
Insulin	22 (14.8)	5 (17.9)	3 (9.4)	0.454

Supplemental Table 2. Platelet aggregation in participants stratified by low and high monocyte platelet aggregate (MPA) levels.

% aggregation, median [IQR]	All <i>n</i> =149	MPA ^{low} <i>n</i> =28	MPA ^{high} <i>n</i> =32	<i>P</i> -value
ADP, 2 μ M	89.2 [48.6, 92.2]	90.5 [68.1, 93.5]	85.1 [63.4, 91.6]	0.171
ADP, 1 μ M	32.6 [15.3, 87.8]	33.6 [21.5, 84.0]	28.4 [15.3, 88.6]	0.461
ADP, 0.2 μ M	5.6 [3.6, 10.6]	7.7 [3.8, 10.8]	5.8 [3.5, 10.1]	0.755
AA 1600 μ M	90.4 [88.1, 92.2]	90.2 [87.3, 92.2]	89.8 [88.8, 92.3]	0.637
AA, 160 μ M	7.7 [3.0, 89.6]	7.0 [2.4, 39.8]	36.6 [3.8, 91.3]	0.238
collagen 1 μ g/ml	85.5 [10.7, 85.5]	85.9 [63.4, 89.6]	86.3 [63.3, 89.4]	0.951
collagen 0.2 μ g/ml	5.7 [2.9, 9.7]	6.40 [4.0, 10.2]	5.20 [3.2, 10.4]	0.778
serotonin 10 μ M	5.8 [4.6, 10.3]	6.25 [4.6, 10.7]	5.70 [4.6, 9.6]	0.82
epinephrine 1 μ M	89.9 [83.4, 92.9]	90.3 [84.2, 93.9]	89.1 [79.6, 91.8]	0.447
epinephrine 0.4 μ M	87.6 [60.0, 92.3]	87.0 [20.2, 90.1]	84.9 [40.8, 90.8]	0.72
epinephrine 0.1 μ M	15.6 [7.4, 83.8]	15.4 [9.7, 87.7]	14.3 [7.8, 36.4]	0.273

AA, arachidonic acid; ADP, adenosine diphosphate; IQR, interquartile range; MPA, monocyte platelet aggregate.

Supplemental Table 3. Demographics and clinical characteristics of patients included in the RNAseq analysis.

	All <i>n</i>=102	MPA^{low} <i>n</i>=21	MPA^{high} <i>n</i>=17	<i>P</i>- value
Age , median [IQR]	52 [38, 63]	52 [34.5, 62.5]	54 [39, 65]	0.706
Females , <i>n</i> (%)	58 (56.9)	10 (47.6)	14 (82.4)	0.057
Race^A , <i>n</i> (%)				0.301
White	60 (58.8)	14 (66.7)	12 (70.6)	
Black or African American	23 (22.5)	3 (14.3)	4 (23.5)	
Asian	13 (12.7)	1 (4.8)	1 (5.9)	
Ethnicity , <i>n</i> (%)				0.515
Hispanic or Latino	16 (15.7)	5 (23.8)	2 (11.8)	
BMI (kg/m²) , median [IQR]	27.7 [24.9, 31.7]	28.4 [25.4, 32.0]	27.2 [24.5, 28.3]	0.186
BMI >30 kg/m² , <i>n</i> (%)	34 (33.3)	8 (38.1)	3 (17.6)	0.282
Smoking status , <i>n</i> (%)				0.602
Current	3 (3)	1 (4.8)	1 (5.9)	
Former	25 (24.5)	6 (28.57)	2 (11.8)	
Never	74 (72.6)	14 (66.7)	14 (82)	
Family history of CVD , <i>n</i> (%)	27 (26.5)	7 (33.3)	3 (17.6)	
Hypertension , <i>n</i> (%)	34 (33.3)	8 (38.1)	5 (29.4)	0.734
Cancer , <i>n</i> (%)	14 (13.7)	4 (19.0)	3 (17.6)	1
Diabetes mellitus , <i>n</i> (%)	52 (51)	10 (47.6)	9 (52.9)	0.945

BMI, body mass index; CVD, cardiovascular disease; IQR, interquartile range; MPA, monocyte platelet aggregate.

^ARace and ethnicity were self-reported, with predefined options determined by the investigators. Six participants (5.9%) in the whole RNAseq cohort and 3 participants (14.3%) in the MPA^{low} group did not select any of the predefined racial categories.

Supplemental Table 4. Platelet RNA transcripts included in the Thromboinflammation Platelet Signature (TIPS).

Adjusted <i>P</i> -value <0.1	
Upregulated	Downregulated
<i>S100B</i>	<i>CNKSR3</i>
<i>STIM2-AS1</i>	<i>FP565260.6</i>
<i>AC027287.2</i>	<i>KLF11</i>
<i>PSME1</i>	<i>NPAT</i>
<i>S100A11</i>	<i>L3MBTL2-AS1</i>
<i>ANXA3</i>	<i>CPEB4</i>
<i>G0S2</i>	<i>SPEN</i>
<i>FUNDC2</i>	<i>GRHL1</i>
<i>S100A10</i>	<i>PPP4R3A</i>
<i>HLA-DRA</i>	<i>XIAP</i>
<i>BEX1</i>	<i>IL7</i>
<i>CTSS</i>	<i>CLASP1</i>
<i>SNRPN</i>	<i>HINT3</i>
<i>GTF3A</i>	
<i>TBC1D7</i>	
<i>LCP1</i>	
<i>OSER1</i>	
<i>MTCH2</i>	
<i>AC140479.4</i>	
<i>POLE4</i>	
<i>FCER1G</i>	
<i>AL390728.4</i>	
<i>RNY3P8</i>	
<i>RNY3P1</i>	
<i>AL135938.1</i>	
<i>ATRAID</i>	
<i>NTAN1</i>	
<i>S100A12</i>	
<i>CD96</i>	

P-value adjusted for age, sex, race, and ethnicity.

Supplemental Table 5. Demographics and clinical characteristics of patients in the SLE cohort with MPA available.

	All <i>n</i>=69
Age , median [IQR]	34 [27, 46]
Race^A , <i>n</i> (%)	
White	32 (47)
Black or African American	23 (34)
Asian	13 (19)
Ethnicity , <i>n</i> (%)	
Hispanic	17 (26)

IQR, interquartile range; MPA monocyte platelet aggregates; SLE, systemic lupus erythematosus.

^ARace and ethnicity were self-reported, with predefined options determined by the investigators.

Supplemental Table 6. Demographics and clinical characteristics of patients in the HARP cohort.

	All <i>n</i>=85	No MI <i>n</i>=41	MI <i>n</i>=44	<i>P</i>- value
Age , median [IQR]	61 [52, 70]	62 [57, 69]	60 [49, 72]	0.421
Race^A , <i>n</i> (%)				0.162
White	61 (72)	32 (78)	29 (66)	
Black or African American	14 (17)	7 (17)	7 (16)	
Ethnicity , <i>n</i> (%)				0.345
Hispanic or Latino	27 (32)	11 (27)	16 (36)	
BMI (kg/m²) , median [IQR]	27.8 [24.3, 32.1]	29.2 [24.3, 32.8]	27.3 [24.3, 32]	0.340
Smoking status , <i>n</i> (%)				0.605
Current	12 (14)	6 (15)	6 (15)	
Former	25 (29)	14 (34)	11 (19)	
Hypertension , <i>n</i> (%)	54 (64)	30 (73)	24 (55)	0.075
Diabetes mellitus , <i>n</i> (%)	29 (34)	14 (34)	15 (37)	0.996
Hyperlipidemia , <i>n</i> (%)	48 (54)	25 (61)	23 (44)	0.419

BMI, body mass index; IQR, interquartile range; MI, myocardial infarction; SD, standard deviation.

^ARace and ethnicity were self-reported, with predefined options determined by the investigators. Ten participants (9%) in the whole cohort, 2 participants (5%) in the no MI group, and 8 participants (18%) in the MI group, respectively, did not select any of the predefined racial categories.

Supplemental Table 7. Demographics and clinical characteristics of patients in SLE cohort with platelet RNA sequencing available.

	All n=157	Control n=36	SLE n=121	P- value
Age , median [IQR]	32 [25, 46]	32 [25, 46]	37 [30, 49]	0.15
Race^A , n (%)				0.006
White	75 (48)	19 (53)	56 (46)	
Black or African American	52 (33)	11 (31)	41 (34)	
Asian	27 (17)	3 (8)	24 (20)	
Ethnicity , n (%)				
Hispanic or Latino	43 (27)	7 (19)	36 (30)	0.476

IQR, interquartile range; SLE, systemic lupus erythematosus.

^ARace and ethnicity were self-reported, with predefined options determined by the investigators. Three participants in the control group (8%) did not select any of the predefined racial categories.

Supplemental Table 8. Demographics and clinical characteristics of patients in Psoriasis cohort.

	All n=90	Control n=39	Psoriasis n=51	P- value
Age , median [IQR]	47 [33, 60]	35 [28, 63]	47[38, 61]	0.12
Male sex , n (%)	51 (57)	18 (46)	33 (65)	0.08
White^A , n (%)	68 (76)	28 (72)	40 (78)	0.21
Hispanic or Latino , n (%)	16 (18)	6 (15)	10 (20)	0.6

IQR, interquartile range.

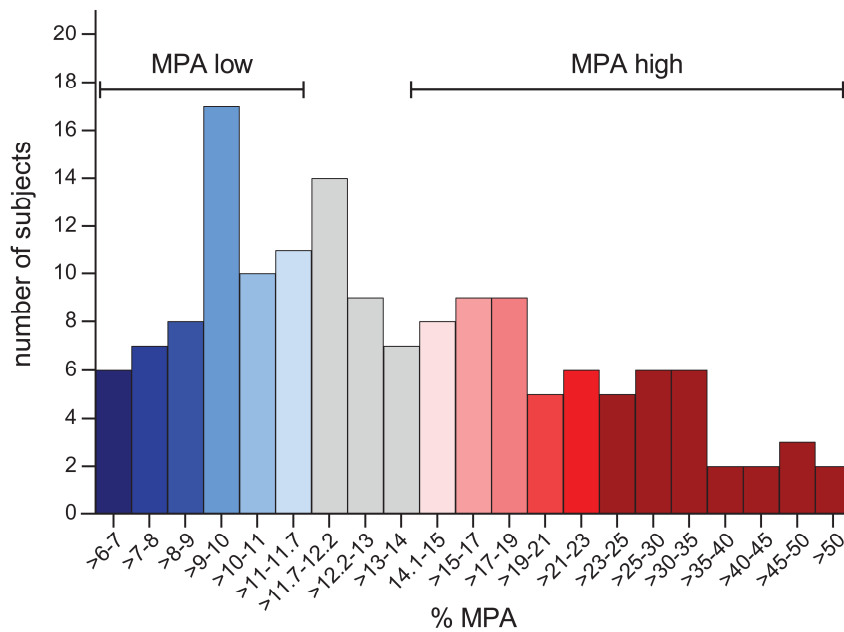
^ARace and ethnicity were self-reported, with predefined options determined by the investigators.

Supplemental Table 9. Demographics and clinical characteristics of patients in the PACE-PAD cohort depending on TIPS tertile.

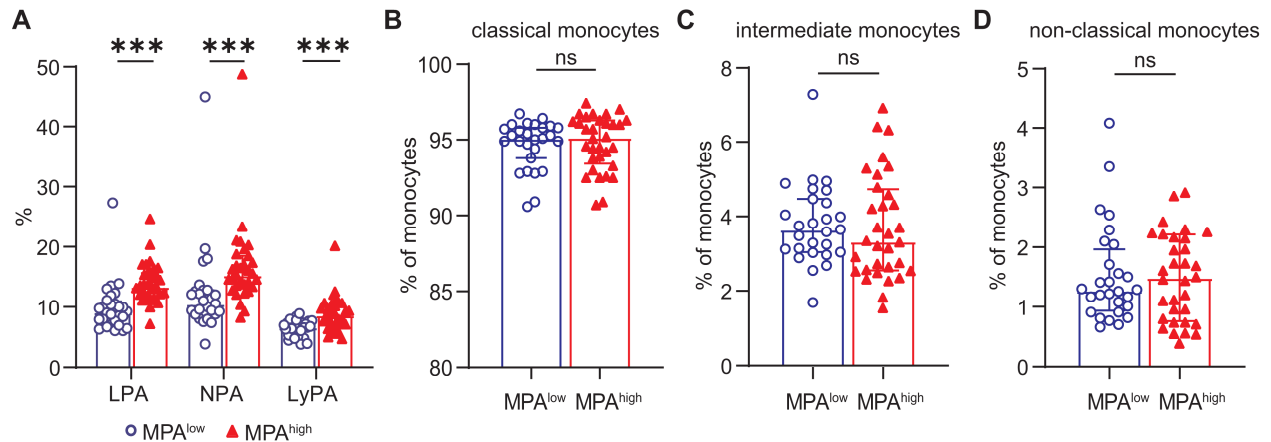
	All n=129	Tertile 1 n=32	Tertile 2 n=48	Tertile 3 n=49	P- value
Age , median [IQR]	70 [65, 79]	72 [67, 80]	71 [1, 79]	70 [65, 77]	0.845
Females , n (%)	45 (35)	13 (41)	12 (25)	20 (41)	0.193
Race , n (%)					0.065
White	82 (64)	28 (88)	29 (60)	25 (51)	
Black or African American	26 (20)	2 (6)	11 (23)	13 (27)	
Asian	3 (2)	0 (0)	1 (2)	2 (4)	
Ethnicity , n (%)					0.255
Hispanic or Latino	25 (19)	3 (9)	11 (23)	11 (22)	
BMI (kg/m²) , mean (SD)	26.9 (5.7)	27.1 (4.5)	27.3 (6.5)	26.3 (5.6)	0.677
Smoking status , n (%)					0.416
Current	19 (15)	6 (18.8)	4 (8.3)	9 (18)	
Former	79 (61)	20 (63)	33 (69)	26 (53)	
Never	31 (24.0)	6 (18.8)	11 (22.9)	14 (29)	
Hypertension , n (%)	109 (85)	29 (91)	39 (81)	41 (84)	0.515
Diabetes mellitus , n (%)	70 (54)	12 (38)	29 (60)	29 (60)	0.089
Hyperlipidemia , n (%)	88 (68)	21 (66)	35 (73)	32 (65)	0.677
Antiplatelet medication , n (%)	118 (92)	31 (97)	44 (92)	43 (88)	0.356
Aspirin	111 (86)	30 (94)	42 (88)	39 (80)	0.186
Clopidogrel	49 (38)	10 (31)	20 (42)	19 (39)	0.636
HbA1c , (%)	6.9 (1.4)	6.5 (1.1)	7.4 (1.5)	6.7 (1.5)	0.081

BMI, body mass index; IQR, interquartile range; PAD, peripheral artery disease; SD, standard deviation.

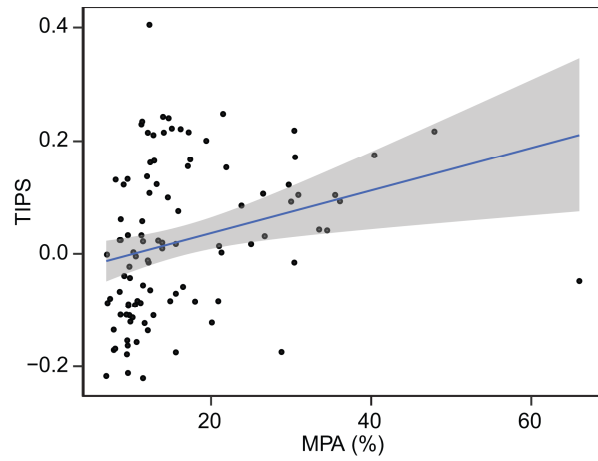
^ARace and ethnicity were self-reported, with predefined options determined by the investigators. Eighteen patients (14%) in the whole cohort, 2 patients (6%) in Tertile 1, 7 patients (14.6%) in Tertile 2, and 9 patients (18%) in Tertile 3, respectively, did not select any of the predefined racial categories.



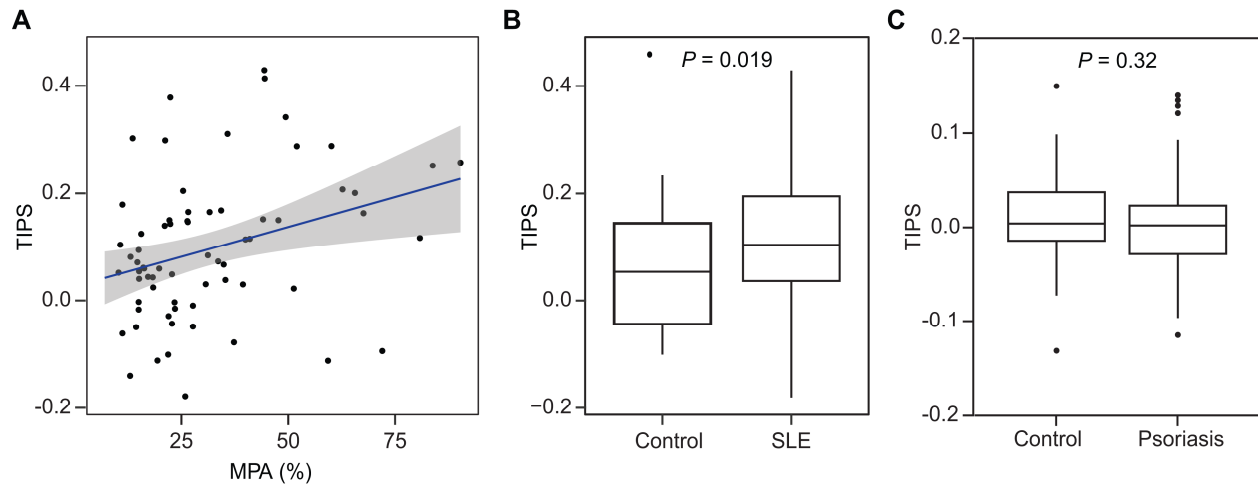
Supplemental Figure 1. MPA levels at baseline. Percentiles of monocyte platelet aggregate (MPA) levels were calculated based on MPA values of $n=149$ participants at two unique timepoints (TP). **(A)** Low MPA levels were defined as $\leq 40^{\text{th}}$ percentile (11.7%) and high MPA levels at $\geq 60^{\text{th}}$ percentile (14.1%).



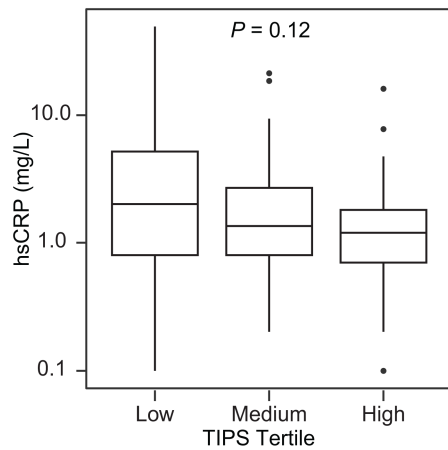
Supplemental Figure 2. Leukocyte platelet aggregates and monocyte subsets in MPA high and low groups. Whole blood was fixed with 1% formalin, stained with antibodies against CD45, C61, CD14 and CD16 and analyzed by flow cytometric analysis. **(A)** Leukocyte platelet aggregates (LPA), neutrophil platelet aggregates (NPA), and lymphocyte platelet aggregates (LyPA) were quantified based on forward side scatter properties and positivity for CD45 and CD61. **(B-D)** Monocyte subsets were defined as CD14⁺CD16⁻ classical monocytes, CD14⁺CD16⁺ intermediate monocytes and CD14⁽⁺⁾CD16⁺ non-classical monocytes. Results were compared between MPA^{high} ($n=32$) versus MPA^{low} ($n=28$) individuals. Graphs show median $\pm$ interquartile range; Man-Whitney-U test was applied, *** $P < 0.001$.



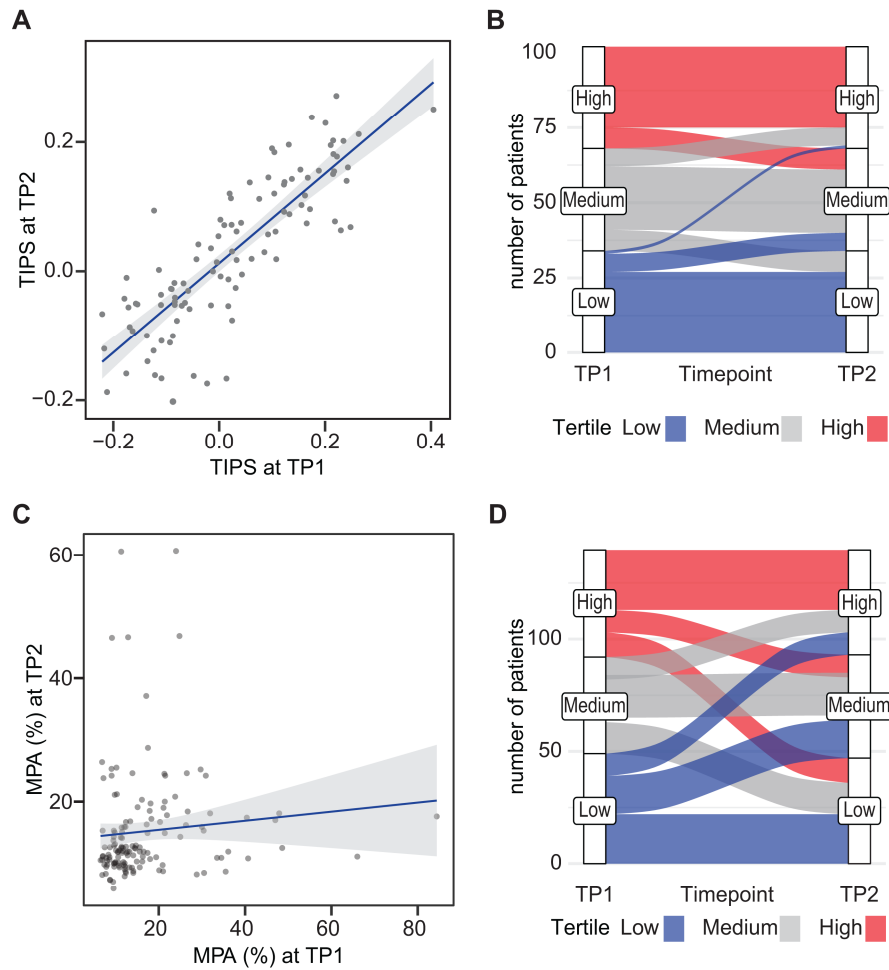
Supplemental Figure 3. Correlation of TIPS and MPA levels. Spearman's correlation of TIPS and monocyte platelet aggregate (MPA) levels in all CHORD study participants with platelet RNA sequencing and MPA levels available at time point 1 ($n=100$) ($r=0.44$, $P=0.03$).



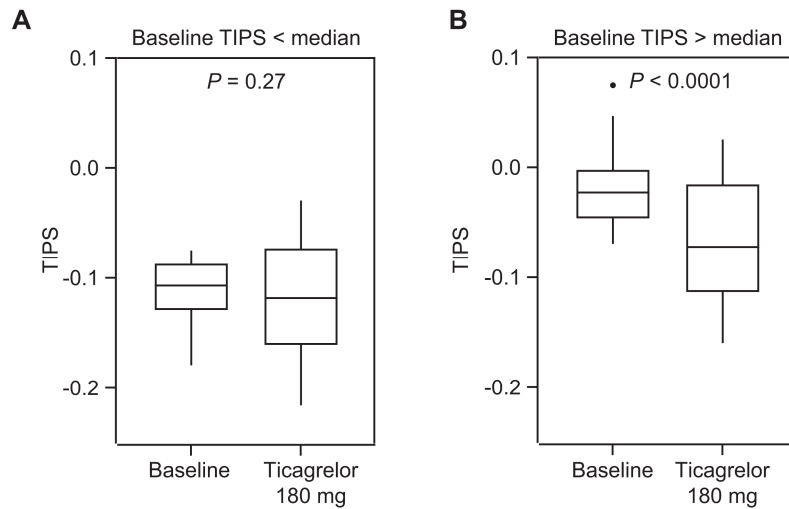
Supplemental Figure 4. TIPS in autoimmune disease cohorts. TIPS was calculated in a cohort of women with systemic lupus erythematosus (SLE) ($n=121$) and healthy controls ($n=36$) with MPA levels available for $n=57$ patients with SLE and $n=12$ healthy controls, and in a cohort of patients with psoriasis ($n=52$) and healthy controls ($n=40$). **(A)** Spearman's correlation of TIPS and MPA in the SLE cohort ($r=0.33$, $P=0.006$). **(B)** TIPS was compared between healthy controls and SLE patients and **(C)** between healthy controls and patients with psoriasis. Boxes show the interquartile range (IQR) with the median line; whiskers extend to $1.5 \times$ IQR, points beyond that range are shown as outliers. Mann-Whitney U test was used.



Supplemental Figure 5. Association of TIPS and high sensitive C-reactive protein. Platelet RNA sequencing for TIPS calculation and high sensitive C-reactive protein (hsCRP) were available for $n=102$ participants at timepoint 1. HsCRP was compared in participants stratified by TIPS tertile. Boxes show the interquartile range (IQR) with the median line; whiskers extend to $1.5 \times \text{IQR}$, points beyond that range are shown as outliers. Kruskal-Wallis test was applied.



Supplemental Figure 6. Reproducibility of TIPS and circulating MPA. Platelet RNA sequencing for TIPS calculation ($n=102$) and MPA levels measured by flow cytometry ($n=140$) were available at two time points (TP) with a four-week interval. (A) Spearman's correlation of TIPS at TP1 and TP2 ($r=0.84$, $P<0.001$). (B) Change in TIPS tertile at TP1 and TP2. (C) Spearman correlation of MPA levels at TP1 and TP2 ($r=0.25$, $P=0.003$). (D) Change in MPA tertiles at TP1 and TP2.



Supplemental Figure 7. Effect of ticagrelor on TIPS. TIPS was calculated in a cohort of healthy subjects that had blood collected for platelet RNA isolation at baseline (>4 weeks without antiplatelet medication) and after four weeks of treatment with 180 mg ticagrelor ($n=50$). The effects of ticagrelor on TIPS are shown for study participants with **(A)** baseline TIPS below the median and **(B)** baseline TIPS above the median. Paired t-test was applied.