

## **Online Supplemental Material**

### **Supplemental Materials and Methods**

#### **Western blot analysis for histone H3**

Plasma was analyzed by Western blotting for histone H3. Plasma was mixed with Laemmli-Buffer supplemented with 5% beta-mercaptoethanol (Biorad). After 3 min at 95°C, samples were subjected to 15% (w/v) SDS-polyacrylamide gel electrophoresis, followed by immunoblotting procedure with a polyclonal rabbit anti-histone H3 (Abcam, product number ab1791) as primary antibody and goat-anti-rabbit-IgG conjugated to horseradish peroxidase (Biorad) as secondary antibody. Detection was carried out with a Pierce ECL Western Blotting Substrate (Thermo Scientific). Human recombinant histone H3 (New England Biolabs) served as a positive control.

#### **Isolation and visualization of plasma DNA**

DNA was isolated from plasma using a DNA isolation kit according to manufacturer's instructions (Omega bio-tek, Norcross, GA), subjected to 2% agarose gel electrophoresis in the presence of ethidium bromide and visualized using a gel documentation system (BioRad, Hercules, CA).

## **Legends to Supplemental Figures and Tables**

**Supplemental Table 1. Learning cohort. Summary of demographic and diagnostic data from TMA patients and healthy controls.** The learning cohort included plasma from 10 healthy controls (Ctrl), 4 patients with D+HUS, 8 patients with tumor-associated TMA, 11 patients with TMA of unknown etiology and 6 patients with acute acquired TTP before PEX therapy. From 5 of these TTP patients samples were available from the time when they were in remission. The table displays sample number, diagnosis, gender (Male/Female), age at first plasma sampling (years), ADAMTS13 activity (%) measured by FRETs-VWF73 assay, ADAMTS13 inhibitor (Bethesda units/ml), plasma DNA (ng/ml), plasma nucleosomes (fold increase over control), plasma LDH activity (OD/min), plasma myeloperoxidase (fold increase over control), plasma S100A8/A9 (fold increase over control), plasma histone H3 (number of bands detected by Western blot). The data are also displayed in figure 1 and supplemental Fig. 1.

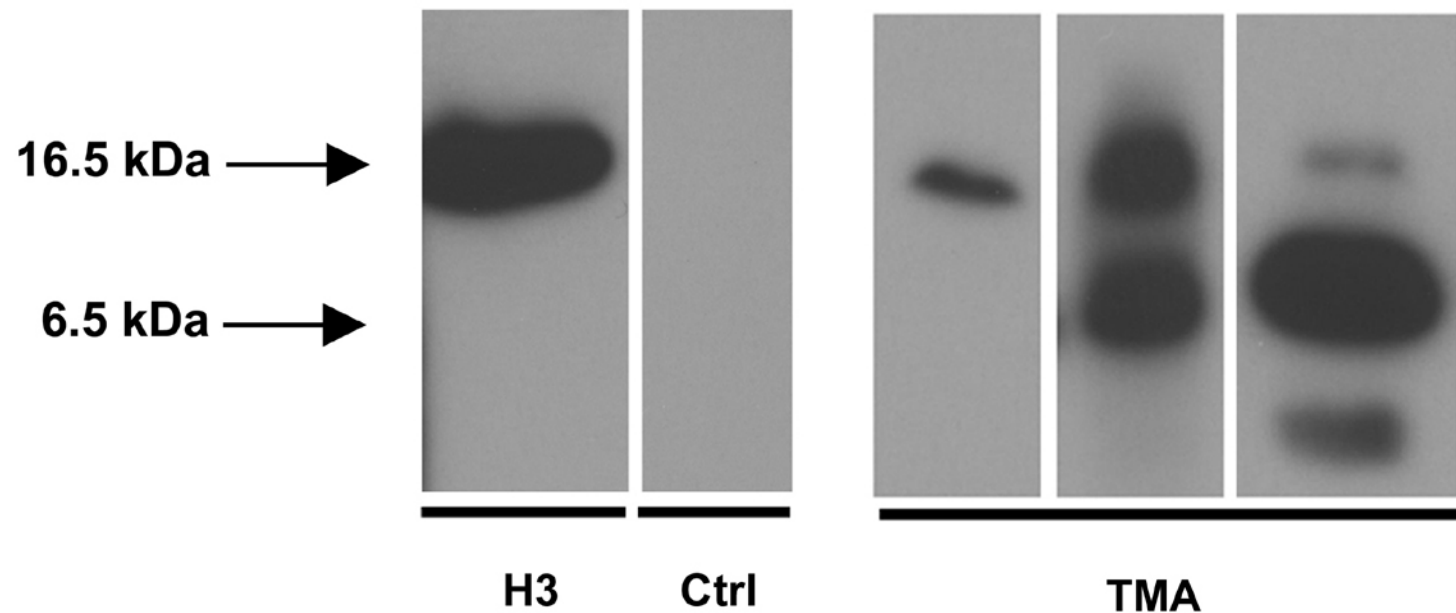
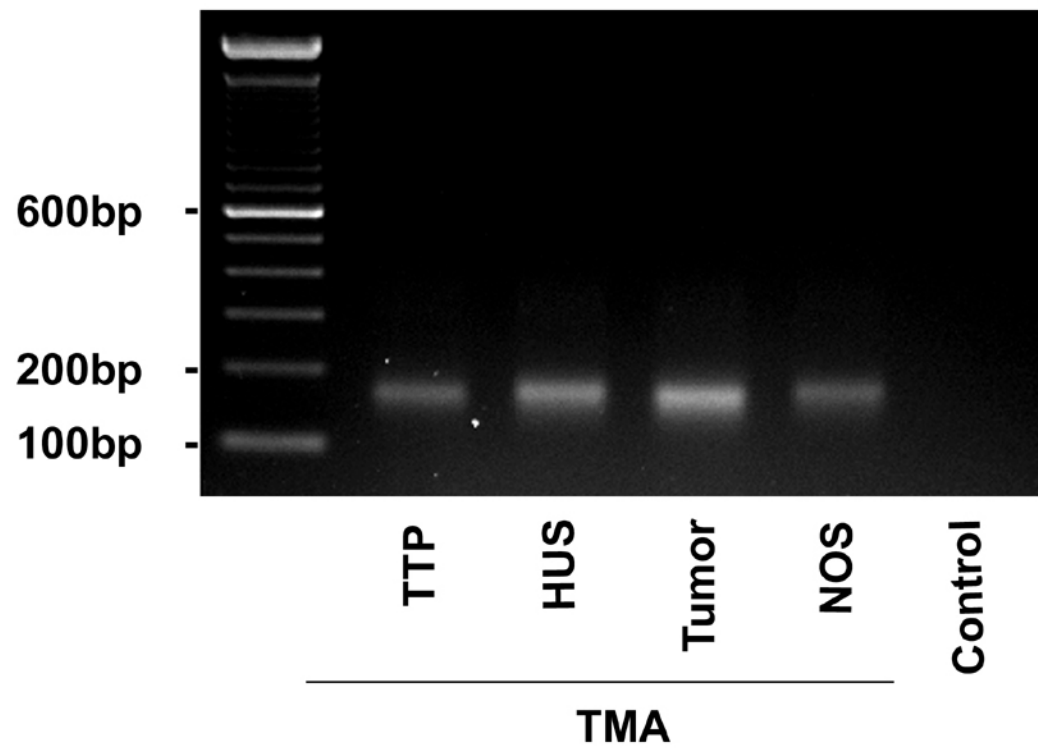
**Supplemental Figure 1. Circulating Histones and DNA are fragmented in patients with acute TMA.** (A) Immunoblot of histone H3. Recombinant histone H3 was used as a positive control (H3). Histone H3 of approximately 16kDa as well as 2 fragments of histone H3 were detected in 17 of 29 patients. The number of histone H3 bands detected in each patient is listed in Supplemental Table 1. TMA patient samples #31 (left), #35 (middle) and #17 (right) are shown as examples. All controls investigated (n = 10) were negative for Histone H3. (B) Gel electrophoresis of plasma DNA isolated from 4 patients with acute acquired TTP, D+HUS (HUS), tumor associated TMA (Tumor), a TMA of unknown etiology (not otherwise specified [NOS]) and from a healthy control (Ctrl). Patients, but not the control showed a single band of approximately 180 bp.

**Supplemental Figure 2. Correlation of plasma markers of patients with acute TMAs.** Values for DNA (ng/ml), nucleosomes (fold increase over healthy controls), LDH activity (OD<sub>490</sub>/min), MPO (fold increase over healthy controls), S100A8/A9 (fold increase over healthy controls), ADAMTS13 activity (%) from patients with acute TMA (n = 29, see Supplemental Table 1) were correlated to each other using Spearman correlation. The table shows Spearman r and Bonferroni-adjusted significance levels. Results were considered significant at  $p < 0.005$ .

**Supplemental Table 2. Investigational cohort. Summary of demographic and diagnostic data from acquired TTP patients during therapy.** TTP patients were treated as indicated in table 1. Plasma samples were collected at admission, before PEX or as indicated in “Sample Description”. The table displays sample number, patient ID, gender (Male/Female), age at first plasma sampling (years), diagnosis, treatment, days after first admission to hospital, ADAMTS13 activity (%) detected by FRETs-VWF73 or immunoblotting (IB), ADAMTS13 inhibitor (Bethesda units/ml), platelet count (1000/ $\mu$ l), DNA (ng/ml), plasma MPO (fold increase over controls). The data are also displayed in figures 2, 3 and 4.

Sample #	Diagnosis	Acute / Remission	Sex	Age	ADAMTS13 activity (% of Ctrl)	ADAMTS13 inhibitor (BU/ml)	DNA (ng/ml)	Nucleosomes (fold increase)	LDH Activity (OD/min)	Myeloperoxidase (fold increase)	S100A8/A9 (fold increase)	# of H3 Bands [WB]
1	Ctrl		M	59	>100%		8	0.5	0.020	2.4	0.7	0
2	Ctrl		F	52	>100%		26	3.7	0.016	1.2	0.5	0
3	Ctrl		F	31	>100%		43	0.5	0.003	0.1	0.5	0
4	Ctrl		M	39	>100%		46	0.5	0.030	1.6	2.2	0
5	Ctrl		M	42	>100%		13	0.2	0.007	0.4	1.7	0
6	Ctrl		F	43	>100%		29	1.9	0.020	2.2	1.8	0
7	Ctrl		M	49	>100%		57	0.7	0.011	0.8	1.0	0
8	Ctrl		M	52	>100%		45	1.4	0.013	1.0	0.8	0
9	Ctrl		F	32	>100%		28	0.1	0.001	0.2	0.4	0
10	Ctrl		F	45	>100%		16	0.4	0.001	0.1	0.4	0
n = 10	Ctrl			Mean	>100%		31.0	1.0	0.012	1.0	1.0	0/10
11	D+HUS	Acute	M	2	33%		9316	156.7	12.635	575.8	> 100	3
12	D+HUS	Acute	F	6	85%		2661	133.6	13.113	33.2	> 100	2
13	D+HUS	Acute	F	4	77%		1728	453.6	6.339	34.6	> 100	2
14	D+HUS	Acute	M	5	45%		5789	138.2	7.228	127.4	> 100	3
n = 4	D+HUS	Acute		Mean	60%		4873.4	220.5	9.829	192.8	> 100	4/4
15	Tumor-TMA	Acute	F	47	45%		794	39.4	11.097	49.6	> 100	0
16	Tumor-TMA	Acute	M	71	76%		1786	73.3	1.776	15.0	76.3	1
17	Tumor-TMA	Acute	F	74	63%		3778	85.0	1.256	13.9	9.4	3 (Figure S1A)
18	Tumor-TMA	Acute	F	47	67%		934	31.2	1.672	20.7	10.4	2
19	Tumor-TMA	Acute	M	29	32%		108	9.6	0.411	10.8	54.2	0
20	Tumor-TMA	Acute	M	74	64%		2632	84.5	2.688	39.9	> 100	2
21	Tumor-TMA	Acute	F	57	51%		101	0.6	0.191	3.5	0.2	0
22	Tumor-TMA	Acute	F	73	32%		468	124.8	5.153	23.4	> 100	2
n = 8	Tumor-TMA	Acute		Mean	54%		1325.4	56.1	3.030	22.1	-	5/8
23	NOS-TMA	Acute	F	72	72%		416	13.3	3.389	9.3	> 100	0
24	NOS-TMA	Acute	F	18	65%		316	25.2	5.251	18.5	> 100	0
25	NOS-TMA	Acute	M	77	67%		1215	50.2	1.672	115.3	> 100	2
26	NOS-TMA	Acute	M	21	63%		1865	53.6	5.153	134.7	> 100	2
27	NOS-TMA	Acute	M	26	71%		266	10.0	0.094	48.9	5.4	0
28	NOS-TMA	Acute	M	83	36%		33	3.8	0.058	10.5	0.9	0
29	NOS-TMA	Acute	M	63	65%		128	0.6	0.031	32.6	1.1	0
30	NOS-TMA	Acute	F	34	49%		46	4.5	0.042	11.1	1.1	0
31	NOS-TMA	Acute	M	26	61%		123	28.7	3.802	11.1	> 100	1 (Figure S1A)
32	NOS-TMA	Acute	F	50	>100%		41	2.6	0.010	2.3	0.8	0
33	NOS-TMA	Acute	F	28	72%		245	24.1	3.078	4.9	> 100	0
n = 11	Tumor-TMA	Acute		Mean	62%		426.7	19.7	2.053	36.3	-	3/11
34	TTP #1	Acute	F	25	<5%	>>2	2860	12.9	1.282	30.3	> 100	2
35	TTP #2	Acute	F	28	<5%	1	1625	53.5	4.345	32.9	> 100	2 (Figure S1A)
36	TTP #3	Acute	M	57	<5%	1.7	145	5.1	0.554	7.9	2.5	0
37	TTP #4	Acute	F	26	<5%	>2	182	13.7	2.125	17.6	> 100	1
38	TTP #5	Acute	F	57	<5%	1.7	908	57.0	6.459	32.5	> 100	2
39	TTP #6	Acute	M	29	<5%	1.8	348	12.5	2.849	13.8	> 100	1
n = 6	TTP	Acute		Mean	<5%		1011.3	25.8	2.936	22.5	-	5/6
40	TTP #1	Remission			92%		28	1.0	0.042	1.7	2.1	0
41	TTP #2	Remission			67%		36	2.0	0.062	1.8	1.5	0
42	TTP #3	Remission			73%		34	0.9	0.046	5.2	1.7	0
43	TTP #4	Remission			94%		14	0.9	0.076	6.6	2.2	0
44	TTP #5	Remission			80%		59	1.1	0.11	3.6	2.4	0
n = 5	TTP	Remission		Mean	81%		34.0	1.2	0.067	3.8	2.0	0/5

**Supplemental Table 1**

**A****B****Supplemental Figure 1**

<b>DNA</b>	-					
<b>Nucleosomes</b>	0.84 ( $<0.0001$ )	-				
<b>LDH</b>	0.61 (0.0004)	0.79 ( $<0.0001$ )	-			
<b>MPO</b>	0.71 ( $<0.0001$ )	0.64 (0.0002)	0.55 (0.002)	-		
<b>S100A8/A9</b>	0.58 (0.001)	0.66 ( $<0.0001$ )	0.84 ( $<0.0001$ )	0.52 (0.004)	-	
<b>ADAMTS13</b>	0.04 (0.85)	0.10 (0.60)	-0.06 (0.76)	-0.01 (0.95)	-0.15 (0.45)	-
<b>Spearman r (p - Value)</b>	<b>DNA</b>	<b>Nucleosomes</b>	<b>LDH</b>	<b>MPO</b>	<b>S100A8/A9</b>	<b>ADAMTS13</b>

**Supplemental Figure 2**

