

SUPPLEMENTAL MATERIAL

Momi et al., <http://www.jem.org/cgi/content/full/jem.20090687/DC1>

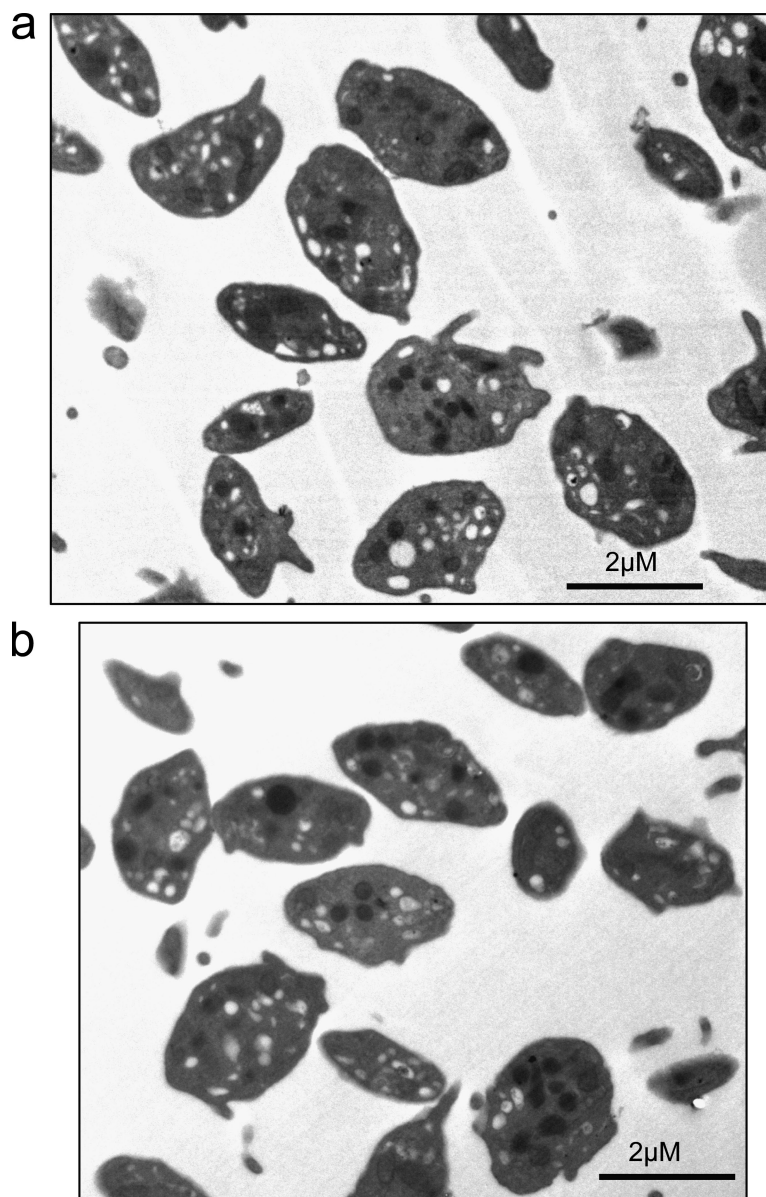
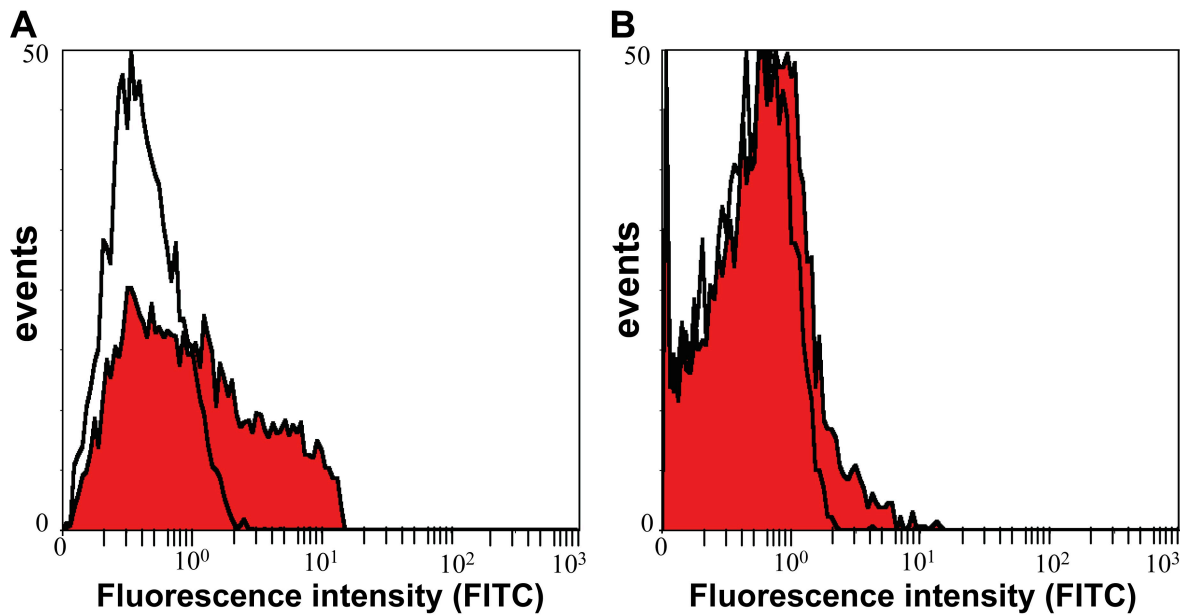


Figure S1. Electron microscopy of *MMP-2*^{-/-} and WT platelets. Resting washed platelets, were analyzed by electron microscopy. Ultrastructure of resting WT platelets (a; *n* = 5) and resting *MMP-2*^{-/-} platelets (b; *n* = 5). No significant ultrastructural alterations are evident in *MMP-2*^{-/-} platelets. Bars, 2 μm. 10 microscopic fields were analyzed for each sample.

Platelet P-selectin



Platelet active MMP-2

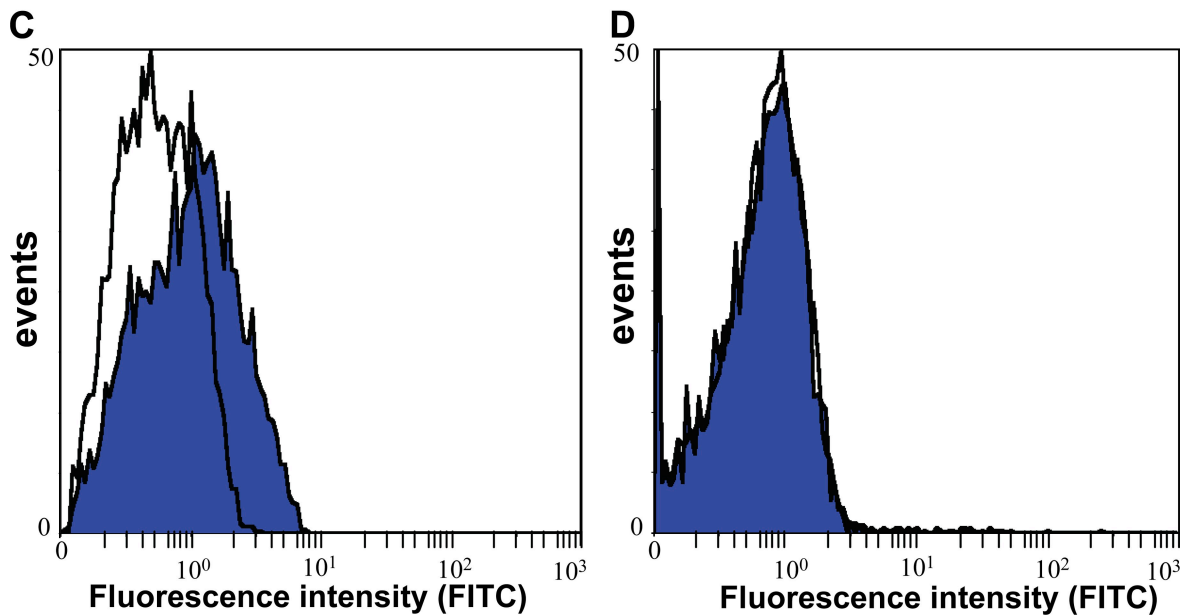


Figure S2. Platelet surface expression of P-selectin and MMP-2. Example of flow cytometry plots of the expression of P-selectin (red) and active MMP-2 (blue) on the platelet surface in WT (A and C) and *MMP-2*^{-/-} (B and D) mice in blood collected at baseline (white) and immediately downstream of an arterial damage. Data are expressed as percentage of positive cells. Results are representative of five independent experiments.

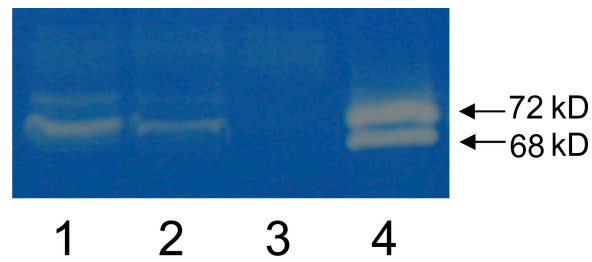


Figure S3. Absence of MMP-2 in *MMP-2*^{-/-} mice. Example of zymography of plasma samples confirming that pro-MMP-2 was present in WT mice (lane 1) and, at an intermediate level, in *MMP-2*^{+/-} mice (lane 2), whereas it was not expressed in *MMP-2*^{-/-} mice (lane 3). Both the pro- (72 kD) and the active (68 kD) form of MMP-2 are present in WT and *MMP-2*^{+/-} mice. Lane 4, standard mouse MMP-2 protein. Results are representative of eight independent experiments.

Table S1. Potentiation of mouse platelet aggregation by 0.5 ng/ml of active MMP-2: time course of the effect

Preincubation time	Increase in platelet aggregation		
	0.8 µg/ml collagen	3 µM U46619	0.05 U/ml α-thrombin
<i>min</i>	%	%	%
2	116.7	101.4	104
5	6.7	37.2	32
10	3.3	24.1	15.4
30	3.3	19.3	15.0

Data are expressed as the percentage of increase of maximal amplitude as compared with platelet aggregation in the absence of MMP-2.

Table S2 – Platelet aggregation, ATP release and P-selectin expression in response to various agonists in WT and *MMP-2*^{-/-} mice.

Parameter	Agonist/concentration	WT	<i>MMP-2</i> ^{-/-}	Reduction in <i>MMP-2</i> ^{-/-} versus WT mice	P-value (WT versus <i>MMP-2</i> ^{-/-})
		%	%	%	
Platelet aggregation	ADP				
	0.2 μ M	25 \pm 1.2	0	100	0.00010
	0.4 μ M	45 \pm 2.6	12 \pm 1.1	73.3	0.00033
	2.0 M	51.7 \pm 16	23 \pm 2.3	55.5	NS
	10 μ M	78 \pm 10	62 \pm 18	20.5	NS
	Collagen				
	2.0 μ g/ml	66 \pm 4.3	1.4 \pm 1.6	97.8	0.0014
	5.0 μ g/ml	75 \pm 7	25 \pm 7.2	66.6	0.0377
	10 μ g/ml	86.5 \pm 16	32 \pm 7.4	63	NS
	U46619				
	3.0 μ M	25 \pm 0.5	0	100	0.0131
	4.0 μ M	64 \pm 14	21.2 \pm 9	33.2	NS
	Arachidonic acid				
	0.1 mM	28 \pm 1.7	0	100	0.00039
	0.4 mM	46.5 \pm 4.9	31.7 \pm 5.3	31.7	0.00346
	0.6 mM	77.6 \pm 4.8	52.7 \pm 0.3	32.1	0.0034
	Epinephrine				
	10 μ M	31.6 \pm 8.3	6 \pm 6	81.1	0.035
	100 μ M	49.6 \pm 12.4	26.6 \pm 13.6	46.4	NS
	α -Thrombin				
	0.05 U/ml	34.2 \pm 3.36	18.2 \pm 1.7	46.8	0.0040
	0.1 U/ml	77.3 \pm 8.2	71.1 \pm 2.5	8.1	NS
ATP release	ADP				
	0.4 μ M	0.7 \pm 0.05	0.3 \pm 0.1	62.8	0.04977
	2.0 μ M	1.9 \pm 0.07	0.7 \pm 0.06	62.6	0.00718
	4.0 μ M	2.3 \pm 0.1	1.9 \pm 0.05	18	NS
	Arachidonic acid				
	0.1 mM	1.1 \pm 0.1	0.3 \pm 0.03	69	0.00259
	0.6 mM	6.6 \pm 1.3	3.1 \pm 0.4	53.4	NS
	Collagen				
	2.0 μ g/ml	5.8 \pm 1.11	0.8 \pm 0.5	86.6	0.0137
	30 μ g/ml	10.4 \pm 3.9	9.6 \pm 1.3	7.7	NS
P-selectin expression	ADP				
	10 μ M	25 \pm 1.2	0	100	<0.001
	20 μ M	45 \pm 2.6	12 \pm 1.1	73.3	<0.001
	α -thrombin				
	0.05 U/ml	40.4 \pm 4.4	22.3 \pm 1.4	45	0.0045
	0.1 U/ml	69.8 \pm 1.1	55.5 \pm 5.3	20.5	0.0294
	Collagen				
	30 μ g/ml	28 \pm 1.7	0	100	<0.001
	Coll + epi				
	0.5 μ g/ml + 1 μ M	24.8 \pm 1.0	10.4 \pm 0.6	58	0.0375
	5 μ g/ml + 10 μ M	34.3 \pm 0.9	16.6 \pm 0.5	51.7	0.0007
	20 μ g/ml + 50 μ M	34.2 \pm 3.8	19.7 \pm 1.1	42	0.0318

Platelet aggregation is expressed as percentage of amplitude; ATP release was expressed as nmol/10⁸ platelets; and P-selectin is expressed as percentage of positive platelets. Data represent means \pm SEM (n = 4 for platelet aggregation; n = 3 for ATP release and P-selectin expression). Coll + epi, collagen + epinephrine.