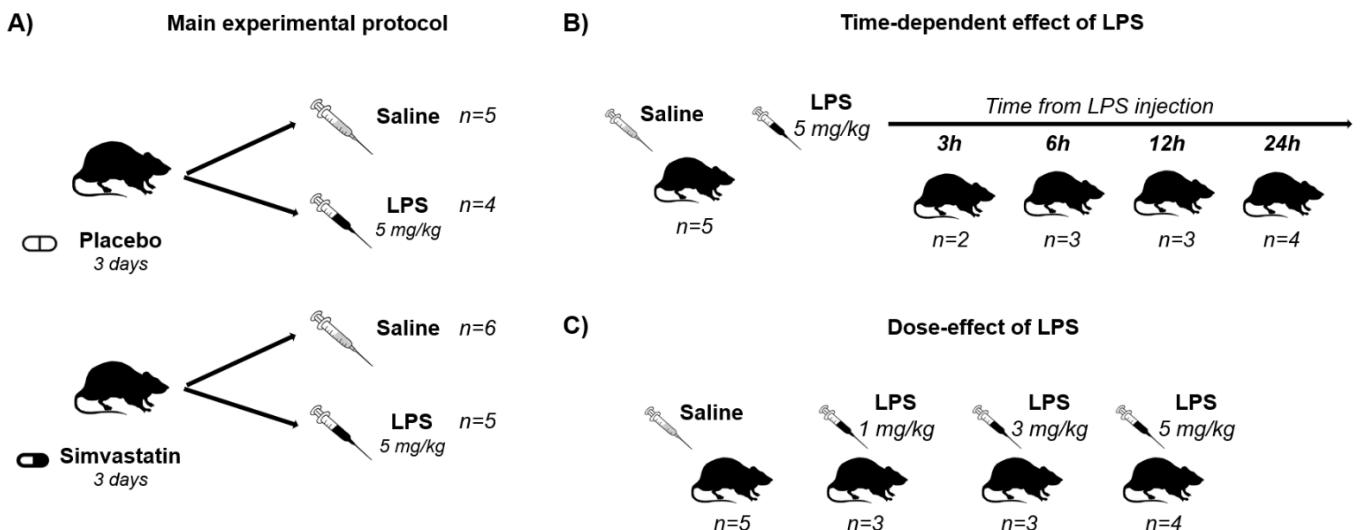


Simvastatin preserves the antithrombotic environment of the liver and prevents sepsis-associated coagulopathy in a rat model of endotoxemia

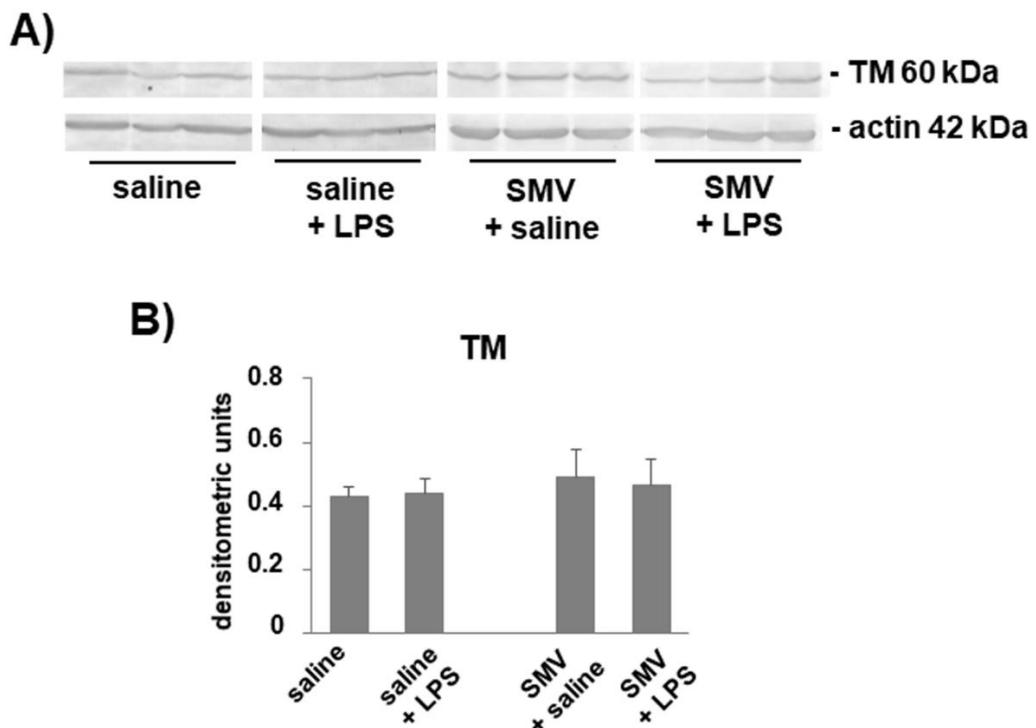
Vincenzo La Mura ^{1,2,3}, Nicoletta Gagliano³, Francesca Arnaboldi³, Patrizia Sartori³, Patrizia Procacci³, Luca Denti³, Eleonora Liguori³, Niccolò Bitto¹, Giuseppe Ristagno^{4,5}, Roberto Latini⁴, Daniele Dondossola^{3,6}, Francesco Salerno³, Armando Tripodi¹, Massimo Colombo⁷, Flora Peyvandi^{1,5}.

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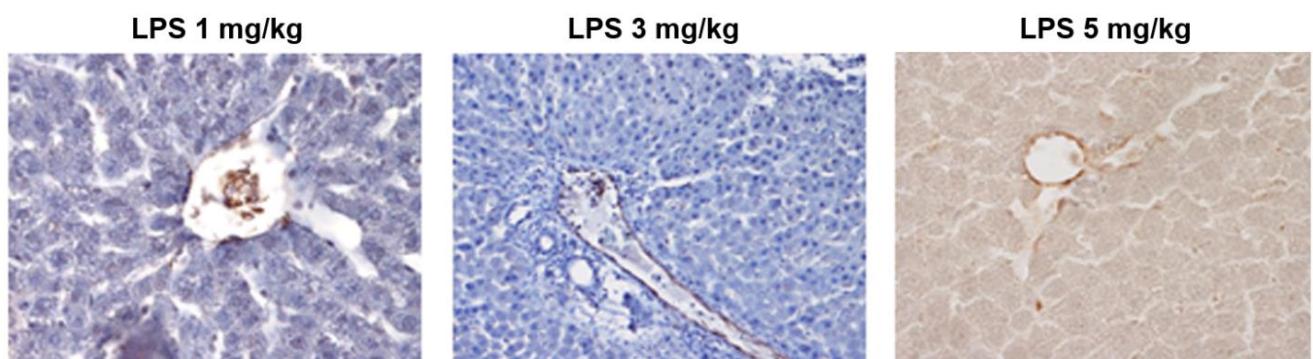
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Supplementary-Figure S1. Schematic representation of experimental groups: A) Main experimental protocol: placebo and 3-day pre-treatment with simvastatin in rats exposed to LPS (5mg/Kg) or saline (24 hours from intraperitoneal injection), B) time-dependent effect protocol, C) dose-effect protocol.



Supplementary-Figure S2. **A)** Representative Western blot analysis showing TM expression in liver homogenates in the main experimental protocol. Data were normalized on actin expression. (SMV=Simvastatin; LPS=Lipopolysaccharide) **B)** Bar graphs showing TM expression after densitometric analysis of immunoreactive bands. Data are reported as mean \pm SD.



Supplementary-Figure S3. Representative micrographs of immunohistochemistry analysis showing VWF expression in the liver of rats after 24 hours from injection of 1-3-5 mg/Kg of lipopolysaccharide (LPS). Increasing doses of LPS did not apparently influence VWF expression intensity and localization. (Original magnification: 20x)

Supplementary-Table S1: morphometry of rats. Comparison of saline lipopolysaccharide (LPS) in placebo and simvastatin (SMV) experimental condition

	PLACEBO			SMV		
	saline	LPS 5mg/Kg	P	saline	LPS 5 mg/Kg	P
Number	5	4		6	5	
Body weight (gr)	313 (306-352)	346 (325-407)	0.063	335 (310-371)	326 (315-333)	0.662
Liver weight (gr)	15 (14-18)	12 (11-15)	0.111	16 (11-19)	12 (11-16)	0.095
Spleen weight (gr)	1.5 (1.1-1.7)	0.9 (0.8-1.3)	0.063	1.1 (0.8-1.5)	1.0 (0.8-1.0)	0.690

Results are presented as median(min-max)

Supplementary-Table S2: morphometry of rats in the experimental groups participating to the analysis of the lipopolysaccharide (LPS) dose-effect response

	Saline	LPS 1 mg/Kg	LPS 3 mg/Kg	LPS 5 mg/Kg	P
Number	5	3	3	4	
Body weight (gr)	313 (306-352)	288 (275-312)	277 (275-312)	346 (325-407)	0.379
Liver weight (gr)	15 (14-18)	14 (12-15)	12 (11-12)	12 (11-15)	0.014
Spleen weight (gr)	1.5 (1.1-1.7)	1.0 (0.9-1.0)	1.1 (1.1-1.1)	0.94 (0.8-1.3)	0.034

Results are presented as median(min-max)

Supplementary-Table S3: morphometry of rats in the experimental groups participating to the analysis of the lipopolysaccharide (LPS) effects at several time point after injection

	saline	LPS 5 mg/Kg 3h	LPS 5 mg/Kg 6h	LPS 5 mg/Kg 12h	LPS 5 mg/Kg 24h	P
number	5	2	3	3	4	
Body weight (gr)	313 (306-352)	323 (318-328)	320 (320-320)	330 (306-333)	346 (325-407)	0.018
Liver weight (gr)	15 (14-18)	15 (14-16)	13 (12-13)	12 (11-14)	12 (11-15)	0.061
Spleen weight (gr)	1.5 (1.1-1.7)	1.1 (1.1-1.1)	1.2 (0.9-1.3)	1.0 (0.8-1.1)	0.94 (0.8-1.3)	0.100

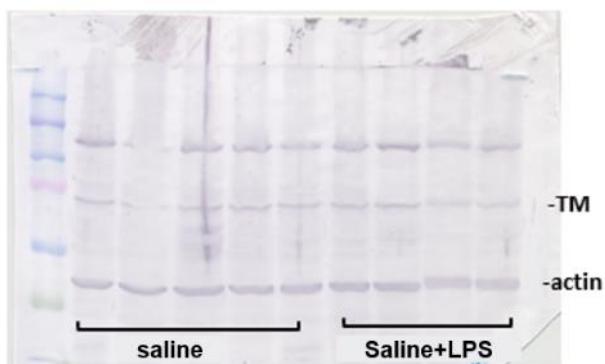
Results are presented as median(min-max)

Supplementary-Table S4: Histological grading for the analysis of liver sections stained with haematoxylin and eosin in the main experimental protocol. (SMV=Simvastatin; LPS=Lipopolysaccharide) Hepatocyte eosinophilia and liver necrosis were graded as follows: - absence, + mild, ++ moderate, +++ severe.

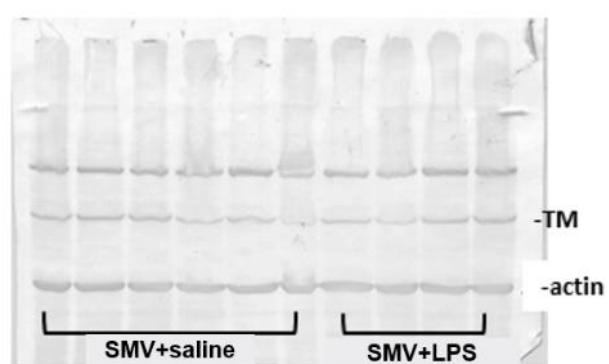
	necrosis	eosinophilia
saline	-	+/-
saline + LPS	++/+++	++
SMV + saline	-	+/-
SMV + LPS	+	+/++

Supplementary-Uncropped Western Blot Images

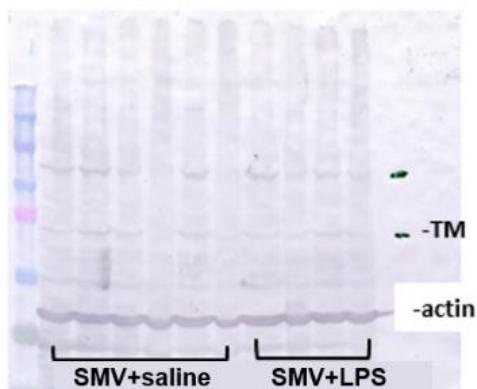
A)



B)



C)



Uncropped Western Blot Images of supplementary Figure S2, showing TM expression in liver homogenates in the main experimental protocol. (SMV=Simvastatin; LPS=Lipopolysaccharide).

Supplementary-Antibodies and DNA/cDNA Clones

Antibodies

Target antigen	Vendor or Source	Catalog #	Working concentration	Lot # (preferred but not required)	Persistent ID / URL
Primary antibodies					
goat anti-fibrinogen GAHu/Fbg/7S	Nordic-MUBio	GAHu/Fbg/7S	dilution 1:400 in PBST		
sheep anti-Thrombomodulin (TM)	R&DSystems	AF3947	1:20 in PBST		
rabbit monoclonal anti-von Willebrand factor (VWF) (,)	Cell Signaling	65707	1:500 in PBST	D8L8G	
mouse monoclonal anti-β-actin	Sigma-Aldrich	A5441	1:7500 in TBST	AC-15	
Secondary antibodies					
rabbit anti-goat HRP conjugated	Thermo Scientific	81-1620	dilution 1:400 in PBST		
rabbit anti-sheep HRP conjugated	Novus	NB7195	1:6000 in TBST		

goat anti-rabbit HRP conjugated	Novus	NB7160	1:6000 in TBST		
anti-mouse HRP conjugated	Sigma-Aldrich	A9044	1:6000 in TBST		
goat anti-rabbit Alexa488 conjugated	Jakson ImmunoResear ch	111-545-045	1:500 in PBST		

DNA/cDNA Clones

Clone Name	Sequence	Source / Repository	Persistent ID / URL
KLF-2 sense	ACTTGCAGCTACACCAACTG	IDT Integrated DNA Technologies, Belgium	
KLF-2 antisense	CTGTGACCCGTGTGCTTG	IDT Integrated DNA Technologies, Belgium	
18s sense	CTGCCCTATCAACTTCGATGGTAG	IDT Integrated DNA Technologies, Belgium	
18s antisense	CCGTTCTCAGGCTCCCTCTC	IDT Integrated DNA Technologies, Belgium	