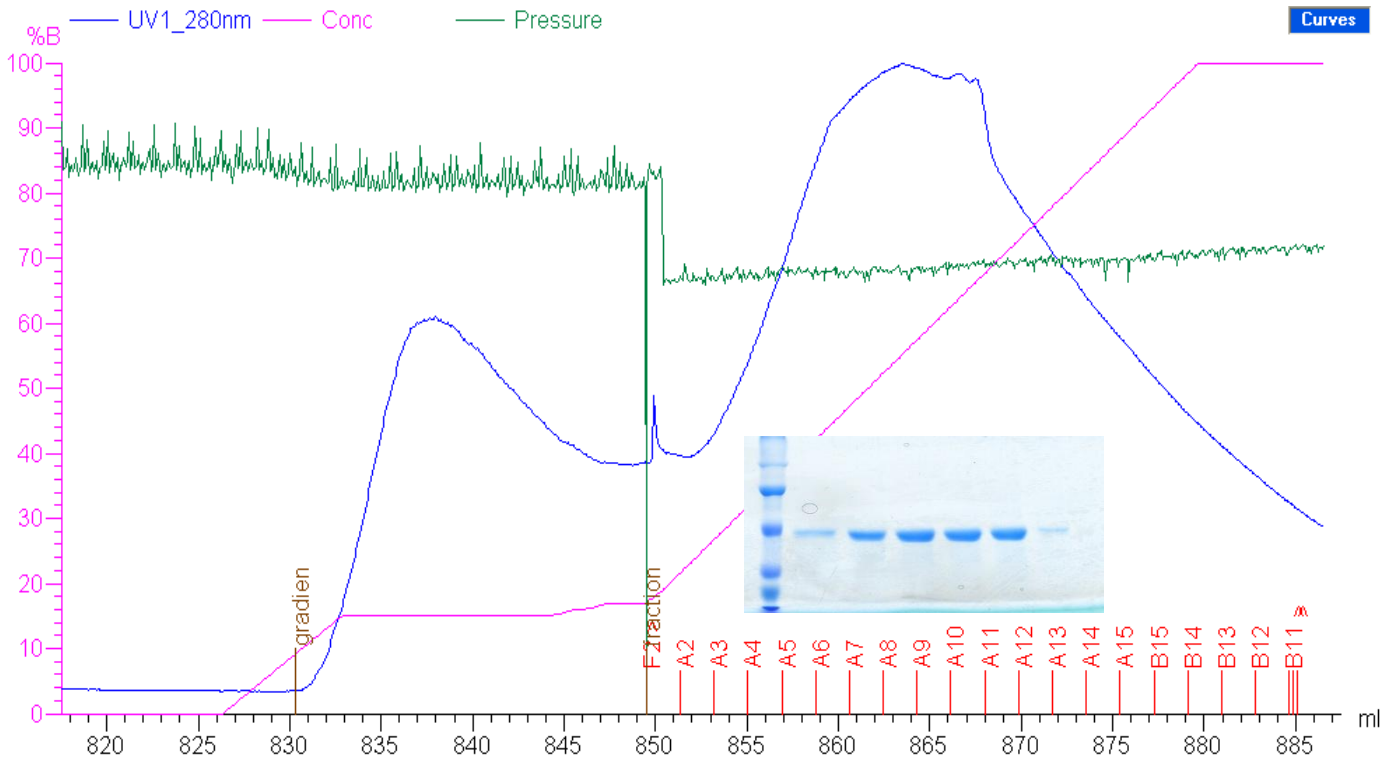
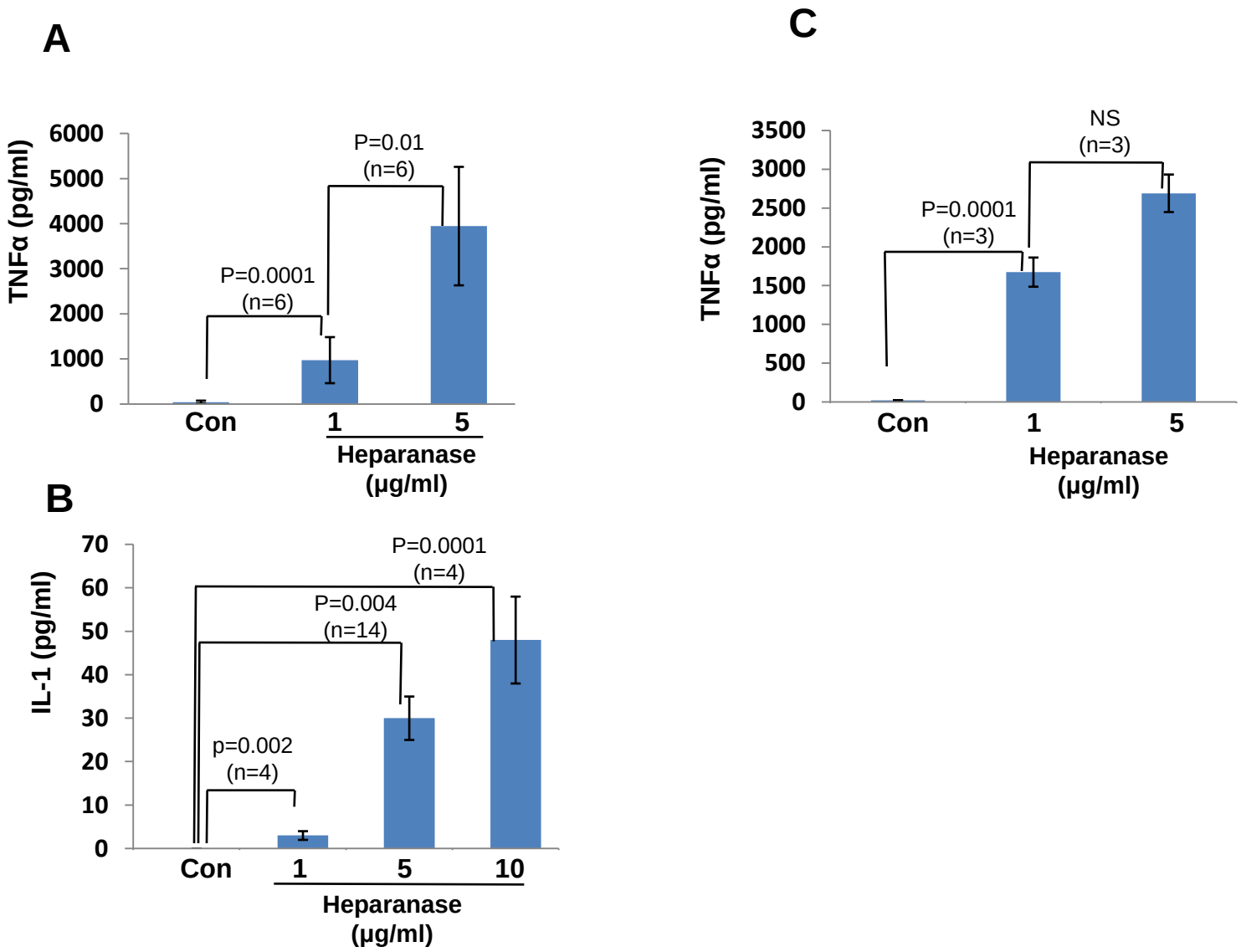
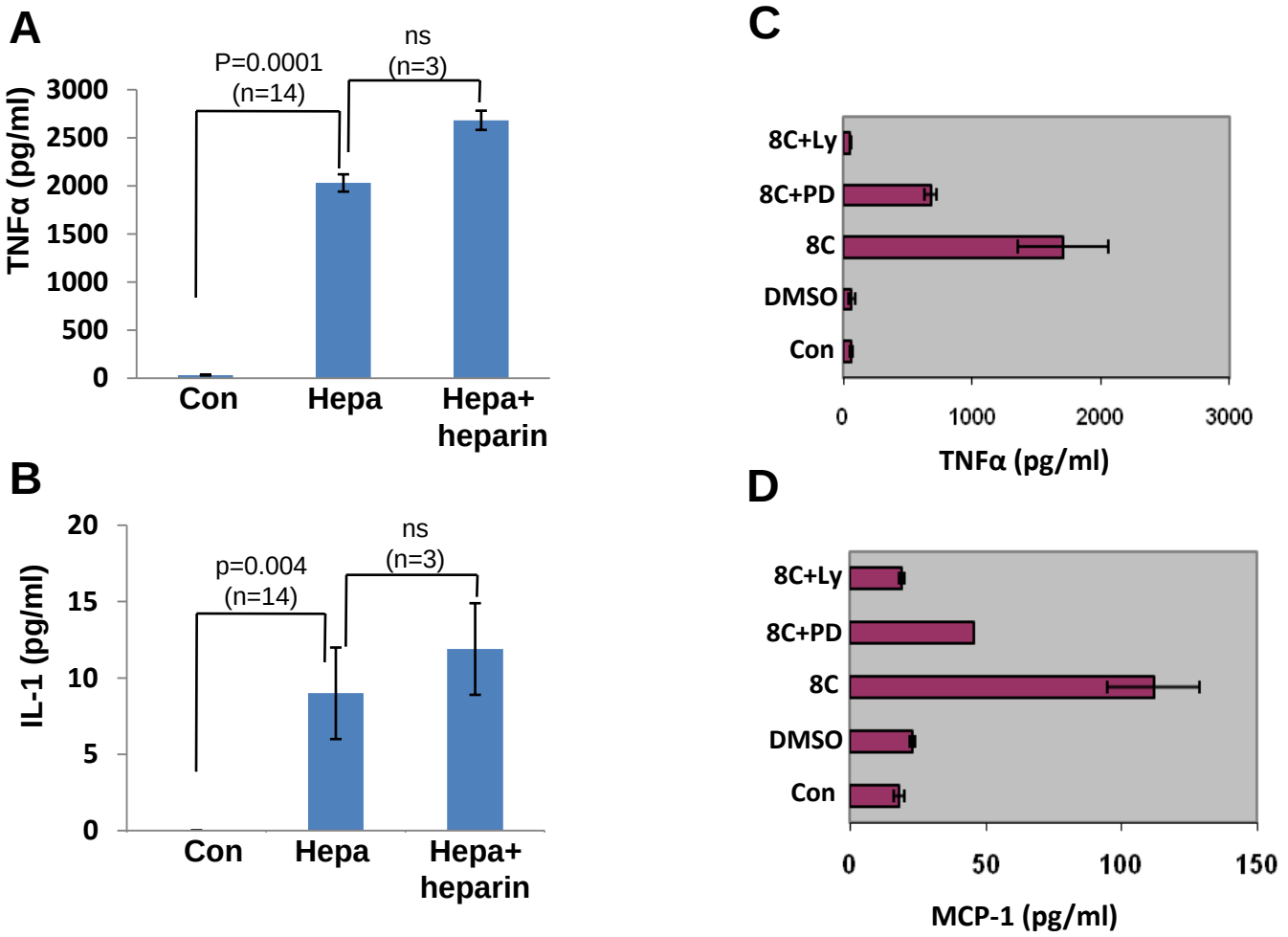
Supplement Material



Blich et al
Suppl. Fig. I



Blich et al
Suppl. Fig. II



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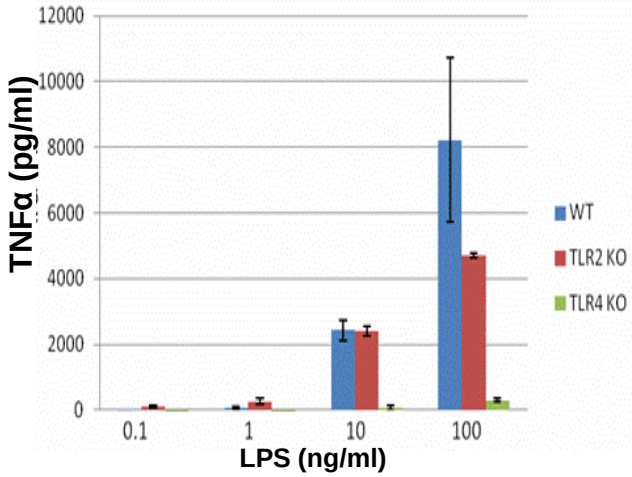
Suppl. Fig. III

A



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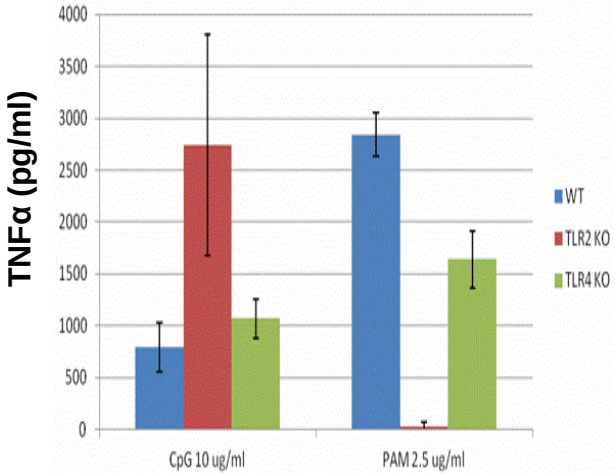
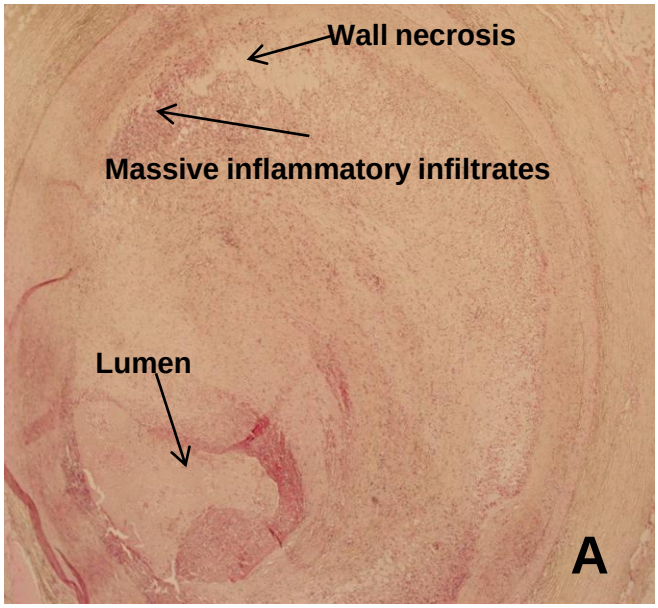
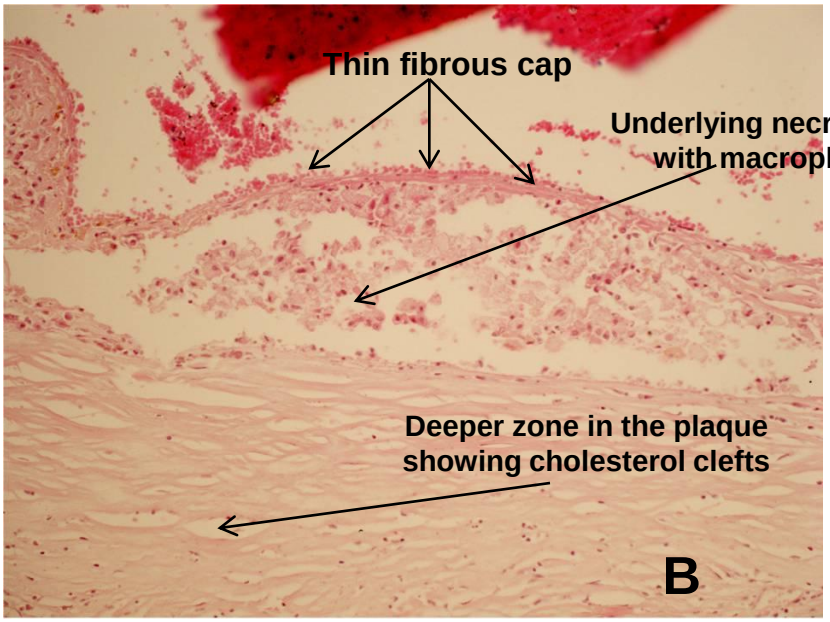
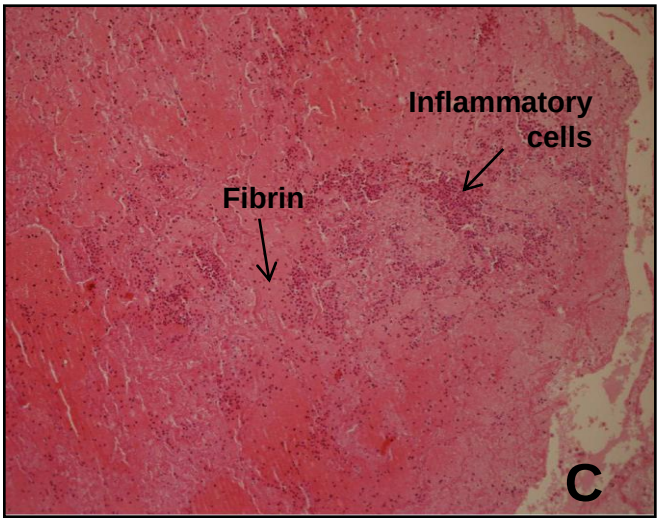


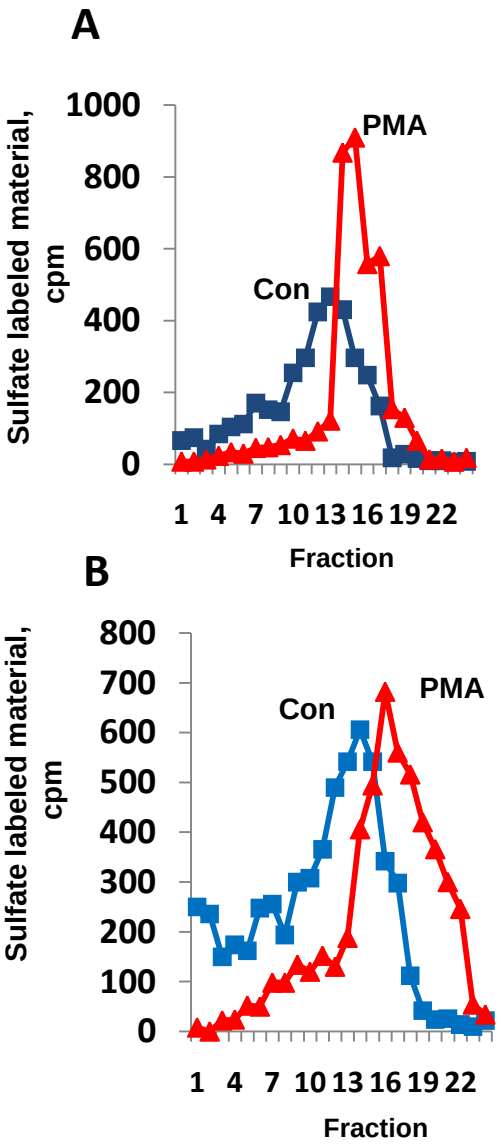
Blich et al,
Suppl. Fig. IV







Blich et al,
Suppl. Fig. V



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Suppl. Fig. VI

Suppl. Table I. Figure 3B and Suppl. Figure IIIC, D summary of statistics

TNF-α		**p**	**n**
	Con vs. Hepa	0.0001	14
	Con vs. 8c	0.0001	5
	PD+Hepa vs. Hepa	0.005	3
	PD+8c vs. 8c	0.005	3
	LY+Hepa vs. Hepa	0.03	3
	LY+8c vs. 8c	0.03	3
MCP-1		**p**	**n**
	Con vs. Hepa	0.05	3
	Con vs. 8c	0.02	3
	PD+Hepa vs. Hepa	0.07	3
	PD+8c vs. 8c	0.05	3
	LY+Hepa vs Hepa	0.05	3
	LY+8c vs. 8c	0.02	3
IL-1		**p**	**n**
	Con vs. Hepa	0.0001	5
	PD+Hepa vs. Hepa	0.09	4
	LY+Hepa vs. Hepa	0.03	4
MMP-9		**p**	**n**
	Con vs. Hepa	0.0001	6
	PD+Hepa vs. Hepa	0.01	4
	LY+Hepa vs. Hepa	0.01	4

See figure legend for more details.

Suppl. Table II. Figure 4A summary of statistics

TNF-α		**p**	**n**
	Con vs. Hepa	0.0005	6
	Con vs. Hepa+bay	N/S	6
	Hepa vs. Hepa+bay	0.0005	6
MMP-9		**p**	**n**
	Con vs. Hepa	0.005	3
	Con vs. Hepa+bay	N/S	3
	Hepa vs.Hepa+bay	0.007	3
IL-1		**p**	**n**
	Con vs. Hepa	0.003	3
	Con vs. Hepa+bay	0.004	3
	Hepa vs. Hepa+bay	0.006	3
MCP-1		**p**	**n**
	Con vs. Hepa	0.0002	3
	Con vs. Hepa+bay	0.002	3
	Hepa vs. Hepa+bay	0.0001	3

N/S-non significant

See figure legend for more details.

Suppl. Table III. Figure 4D summary of statistics

		p	n
TNF-α	Hepa TLR2-/- vs. WT Hepa	0.0001	4
	DM TLR2-/- vs. WT DM	0.002	3
	Hepa TLR4-/- vs. WT Hepa	0.0001	4
	DM TLR4-/- vs. WT DM	0.001	3
	Hepa TLR2,4-/- vs. WT Hepa	0.0001	4
	DM TLR2,4-/- vs. WT DM	0.001	3
		p	**n**
MMP-9	Hepa TLR2-/- vs. WT Hepa	0.0001	4
	DM TLR2-/- vs. WT DM	0.02	3
	Hepa TLR4-/- vs. WT Hepa	0.003	4
	DM TLR4-/- vs. WT DM	N/S	3
	Hepa TLR2,4-/- vs. WT Hepa	0.004	4
	DM TLR2,4-/- vs. WT DM	0.02	3
		p	**n**
MCP-1	Hepa TLR2-/- vs. WT Hepa	0.0001	4
	DM TLR2-/- vs. WT DM	0.009	3
	Hepa TLR4-/- vs. WT Hepa	N/S	4
	DM TLR4-/- vs. WT DM	N/S	3
	Hepa TLR2,4-/- vs. WT Hepa	0.0001	4
	DM TLR2,4-/- vs. WT DM	0.008	3

N/S-non significant

See figure legend for more details

Suppl. Table IV. Morphometric analysis of heparanase staining in specimens of VP compared to specimens of SP and control (N)

	VP (n= 10)	SP (n= 4)	N (n= 6)	p
Staining Percent (mean± SD)	3.7 ± 2.5	0.6 ± 0.4	1.2 ± 1.8	*p =0.02 ** p= 0.04
Optical density (mean ± SD)	156 ± 15.5	131 ± 18.8	153 ± 12.04	*p =0.02

VP- vulnerable plaque; SP- stable plaque, N- normal coronaries. *p= VP vs. SP
**p= VP vs. N

Supplementary Figure Legends

Suppl. Figure I. Heparanase purification. Latent 65 kDa heparanase was purified from serum-free medium conditioned by heparanase-transfected CHO cells. Cells were grown to confluence and were then cultured in serum-free medium for 24 h. Medium was collected, filtered (0.45 micron) and applied onto a HiTrap heparin column (Pharmacia) equilibrated with 20 mM phosphate buffer, pH 6.0. Following washes (15 column volumes), bound material was eluted with a linear gradient of NaCl in 20 mM phosphate buffer (pH 6.0). Eluted fractions were analyzed by gradient SDS-PAGE followed by Gelcode (Pierce, Rockford, IL, USA) staining. A single, highly purified protein band was obtained in fractions eluted with 0.7-1 M NaCl (A7-A12) and used for all subsequent experiments.

Suppl. Figure II. Dose response. J774 cells (A, B) or human monocytes isolated from peripheral blood of healthy volunteers (C) were left untreated (Con) or were incubated with the indicated concentration of heparanase. Medium was collected after 24 h and the levels of TNFα (A, C) and IL-1 (B) were quantified by ELISA.

Suppl. Figure III. A, B. Heparin treatment. J774 cells were left untreated (Con) or were incubated with heparanase (5 μg/ml) in the absence (Hepa) or presence of heparin (50 μg/ml; Hepa+heparin). Medium was collected after 24 h and the levels of TNFα (A) and IL-1 (B) was quantified by ELISA. **C, D.** MAPK and PI3-K inhibitors. J774 cells were pretreated with LY 294002 (Ly; 20 μM) or PD 98059 (PD; 30 μM), selective inhibitors of PI 3-kinase and MAPK, respectively, for 30 min prior to the addition of heparanase C-domain (8C). Vehicle (DMSO) or medium alone (Con) were included as controls. Culture medium was collected after 20 h and TNFα (C) and MCP-1 (D) levels were quantified by ELISA.

Suppl. Figure IV. TLR2- and 4-deficient cells still respond to appropriate TLR ligands. Macrophages isolated from thioglycolate-treated wild type (WT, blue), $TLR2^{-/-}$ (red), and $TLR4^{-/-}$ (green) mice were incubated ($37^{o}C$, 24 h) with the indicated concentrations of LPS and TNFα levels were quantified by ELISA (A). Cells were similarly treated with CpG (10 μg/ml) or PAM (2.5 μg/ml) and TNFα levels were quantified as above (B). Note marked increase in TNFα in $TLR2^{-/-}$, but not in $TLR4^{-/-}$ cells, by LPS and an opposite effect following PAM treatment.

Suppl. Figure V. Histological feature of VP. **A-C**, Hematoxilin and Eosin staining. Shown are representative photomicrographs of VP obtained post mortem from patients who died from acute MI. Original magnification A x4; B x20; C x10.

Suppl. Figure VI. Macrophages exhibit heparanase activity. Macrophages were isolated from wild type mice and left untreated (Con; ■) or were stimulated with PMA (PMA; 100 ng/ml, ▲) for 24 h. Cell lysate (A) and medium (B) samples were then incubated (20 h, 37^{o}C) with ^{35}S-labeled ECM and labeled degradation products released into the incubation medium were analyzed by gel filtration, as described under 'Materials and Methods'. Note increased heparanase activity (higher amounts and small size of HS degradation fragments) in the cell lysate and conditioned medium following PMA stimulation.