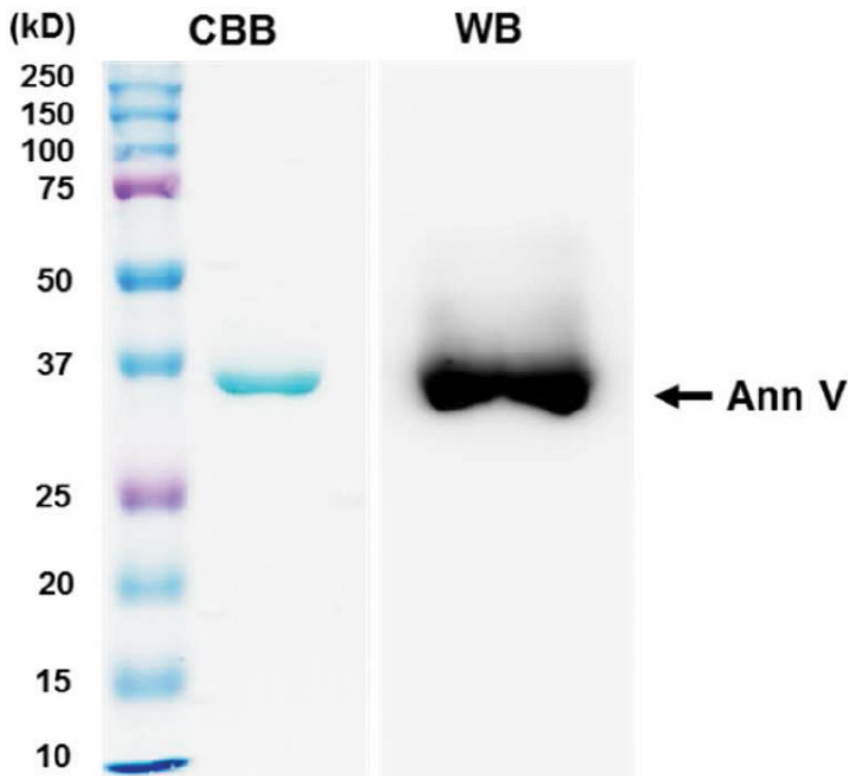


Supplemental Data

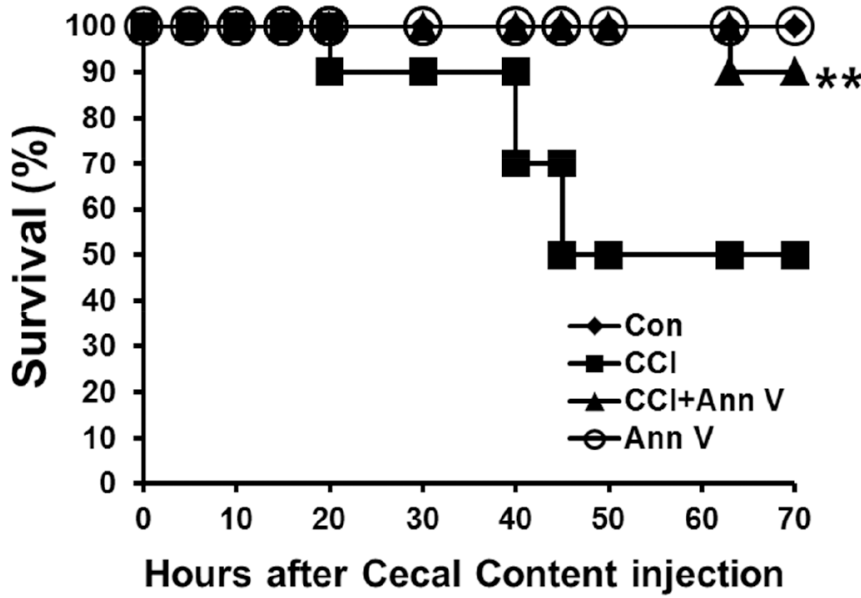
**Annexin A5 Increases Survival in Murine Sepsis Model by Inhibiting HMGB1-Mediated Proinflammation and Coagulation**

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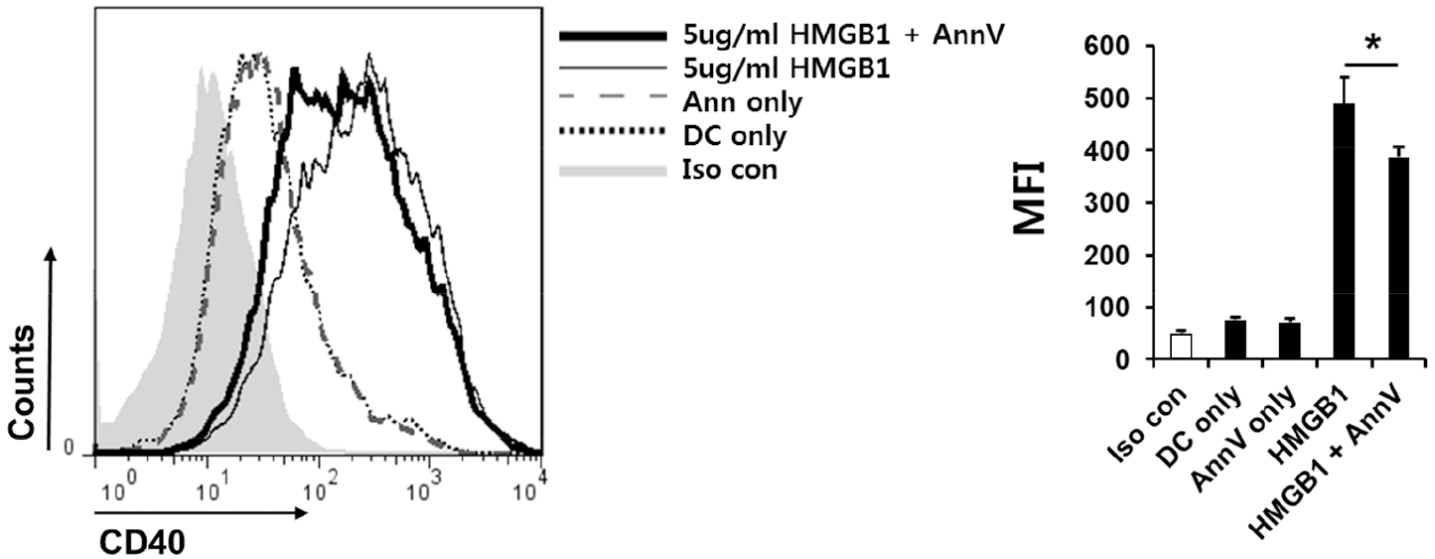
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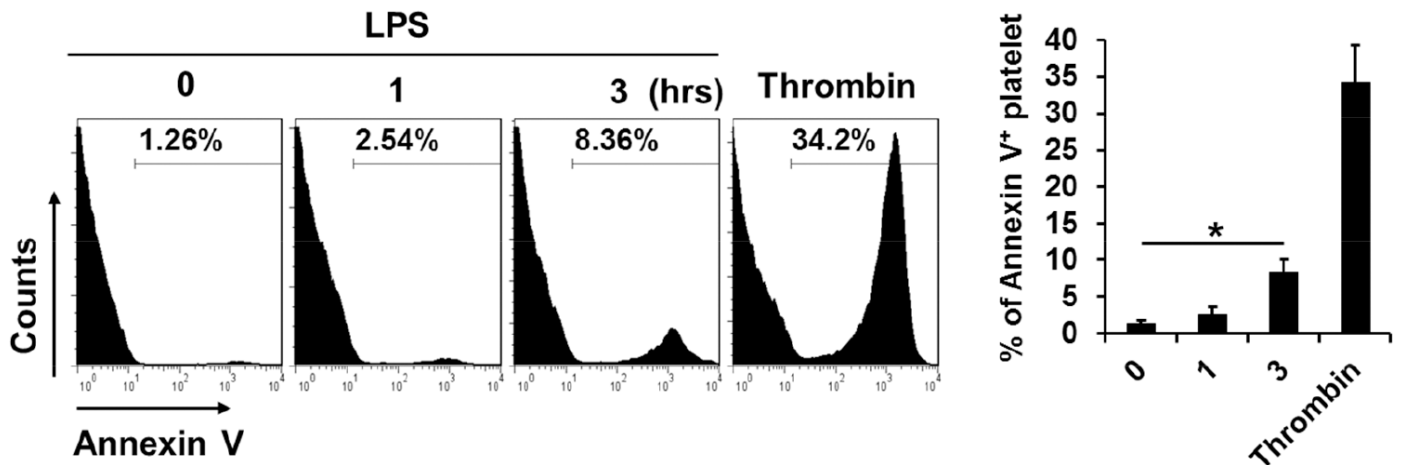
**Supplementary Figure S1.** Characterization of the annexin A5 protein. The annexin A5 protein was successfully expressed. The size and its purity were assessed using 12% gradient SDS-PAGE and Coomassie Brilliant Blue staining (left). Recombinant annexin A5 protein was confirmed by Western blot using anti-annexin A5 antibody (right).



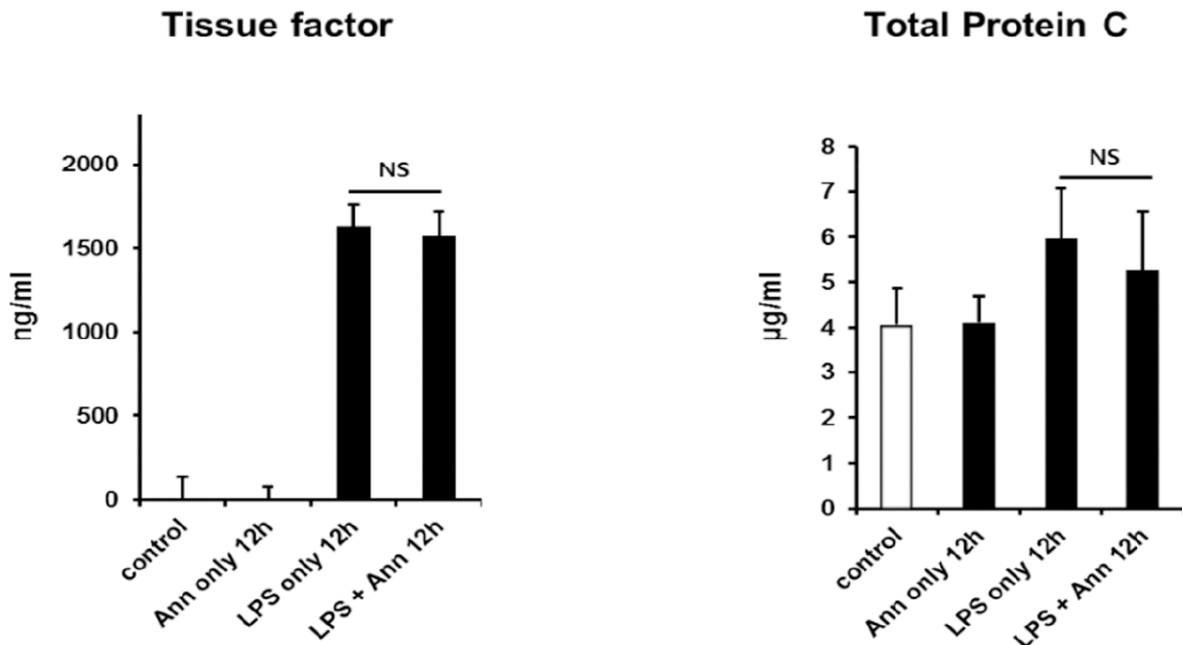
**Supplementary Figure S2.** Annexin A5 provides protection against LD<sub>50</sub> cecal content injection. C57BL/6 mice were intraperitoneally injected with 15mg/mouse of cecal contents. Each mouse was intravenously injected with 500 μg (in 100 μL PBS) annexin A5 protein concurrently with CCI challenge and monitored for 70 h. \*\* - indicates significant differences ( $P < 0.005$ ) from data obtained during the CCI challenge. The line graph illustrates survival of C57BL/6 mice in different treatment groups over time. We monitored survived mice in each group for another 3 wks, no unexpected deaths were observed. Each group consisted of 10 mice and the experiments were performed twice. Annexin A5 is abbreviated as "Ann V" and control group as "con."



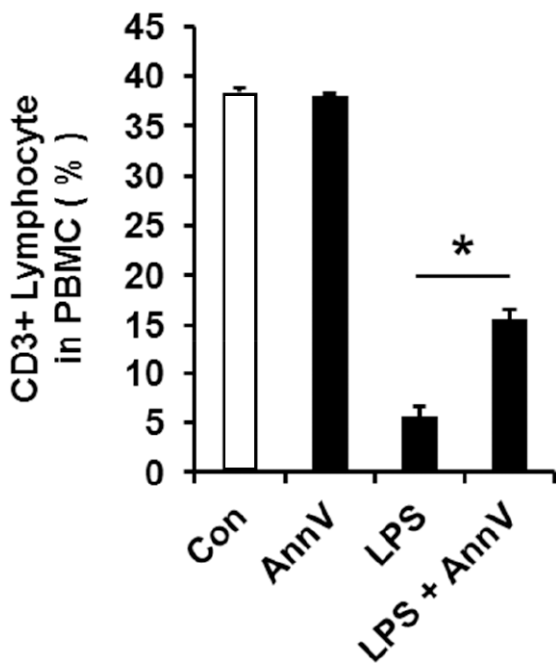
**Supplementary Figure S3.** Annexin A5 reduces rHMGB1 induced DC maturation level. Bone marrow DCs ( $1 \times 10^6$ ) from C57BL6 mice were stimulated with 5 ug/mL HMGB1, followed by 50 ug annexin A5 treatment. All groups were incubated at 37°C for 18 h. Eighteen hours after the treatment, maturation surface markers CD40 on DCs were measured using flow cytometry. The bar graph illustrates the mean fluorescence intensity (MFI) of each surface marker. HGMB1 treatment was used as a positive control and immature DCs served as a negative control. \* - indicates significant differences ( $P < 0.05$ ) from data obtained during the HMGB1 only challenge.



**Supplementary Figure S4.** Annexin A5 binds to LPS-activated platelet *in vivo*. C57BL/6 mice were injected with LPS and binding of annexin A5 was detected using flow cytometry immediately after and in 0, 1 and 3 h following the LPS treatment. Platelet groups were selected using an anti-CD41 antibody. The bar graph illustrates the percentage of annexin A5-positive platelets in the total platelet population. Thrombin was used as a positive control. Data represent the mean  $\pm$  S.E.M. of three independent experiments. \* - indicates significant differences ( $P < 0.05$ ) from data obtained during the LPS only challenge.



**Supplementary Figure S5.** Annexin A5 does not alter tissue factor and protein C level during sepsis. C57BL/6 mice were challenged with 50 mg/kg LPS, followed by simultaneous intravenous injection of 500 µg (in 100 µL PBS) annexin A5 protein. Whole blood was collected by cardiac puncture and serum was extracted 12 h after the treatment. Serum concentrations of tissue factor and total protein C were determined using ELISA. The concentrations are illustrated as line plots. Data represent the mean  $\pm$  S.E.M. of three independent experiments. No significant differences were observed at each time point between the LPS only and annexin A5 + LPS treatments.



**Supplementary Figure S6.** Annexin A5 may attenuate LPS-induced lymphopenia. C57BL/6 mice were challenged with 50 mg/kg LPS, followed by intravenous injection of 500  $\mu$ g (in 100  $\mu$ L PBS) annexin A5 protein. Twelve hours after the treatment, whole blood was collected by cardiac puncture and red blood cell was removed using 5 mL of ACK lysing buffer. Pan T-cell marker, CD3, positive lymphocytes were measured using flow cytometry. The bar graph illustrates the percentage of CD3 + lymphocyte population in PBMC. \* - Indicates significant differences ( $P < 0.05$ ) from data obtained during the LPS only challenge. Data represent the mean  $\pm$  S.E.M. of three independent experiments.