

## The Role of Extracellular Vesicle Fusion with Target Cells in Triggering Systemic Inflammation

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### Running Title:

Systematic action of extracellular vesicles -mediated cell stimulation

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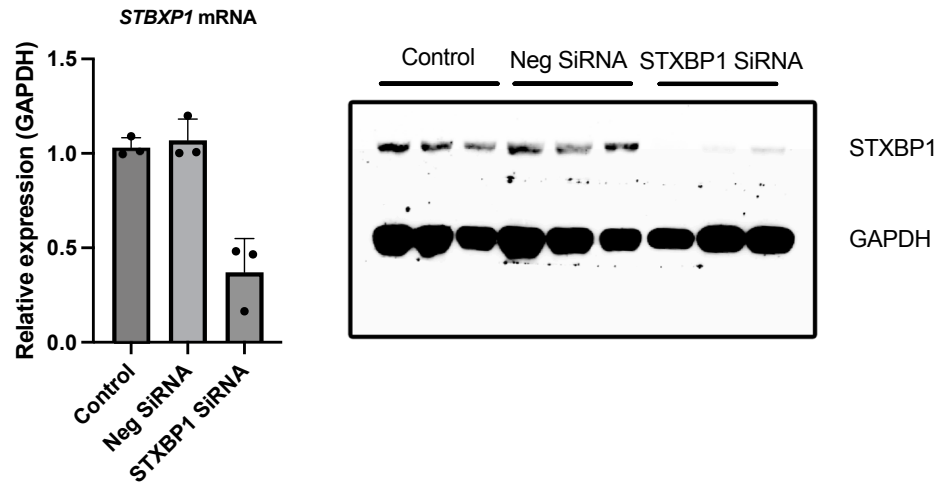
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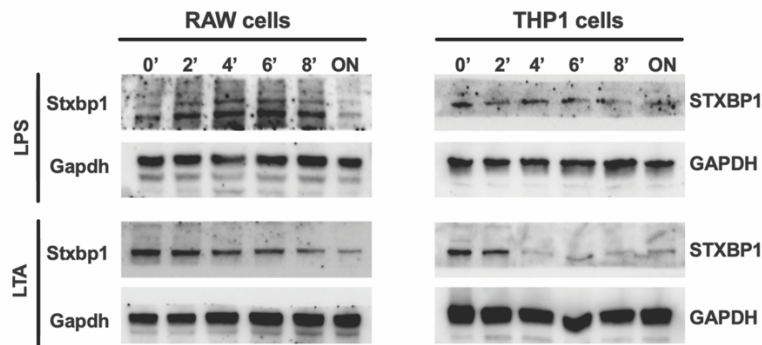
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Supplemental Fig. 1



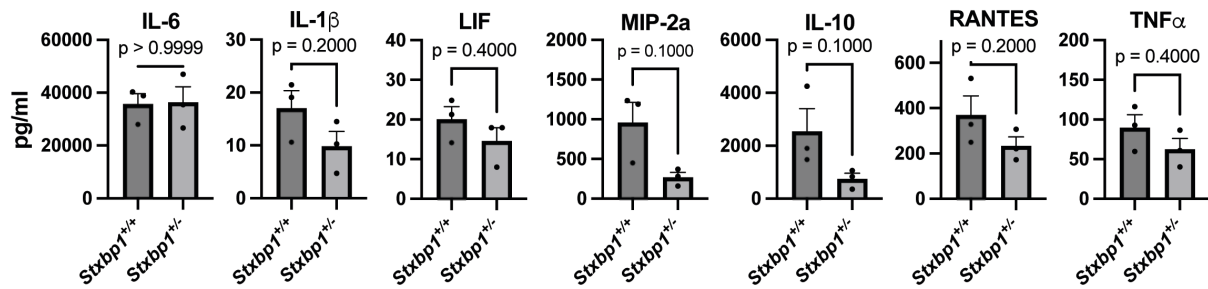
**Supplementary Figure 1:** Expression of *Stxbp1* in SiRNA treatment mice. P-values determined by ordinary one-way ANOVA with Tukey's multiple comparisons test (SEM;  $n=3$ , mice per group).

Supplemental Figure 2



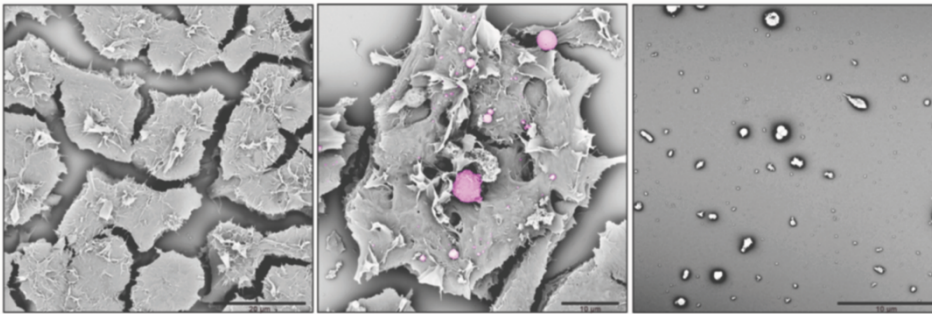
**Supplementary Figure 2:** Raw macrophages and THP1 were stimulated with LPS and LTA. Following the stimulation, *Stxbp1* expression levels were determined at 0 h, 2 h, 4 h, 6 h, 8 h and overnight, with 0 h as a negative control. *Stxbp1* expression levels are compared to GAPDH levels. Data represent a representative experiment from two independent experiments.

### Supplemental figure 3



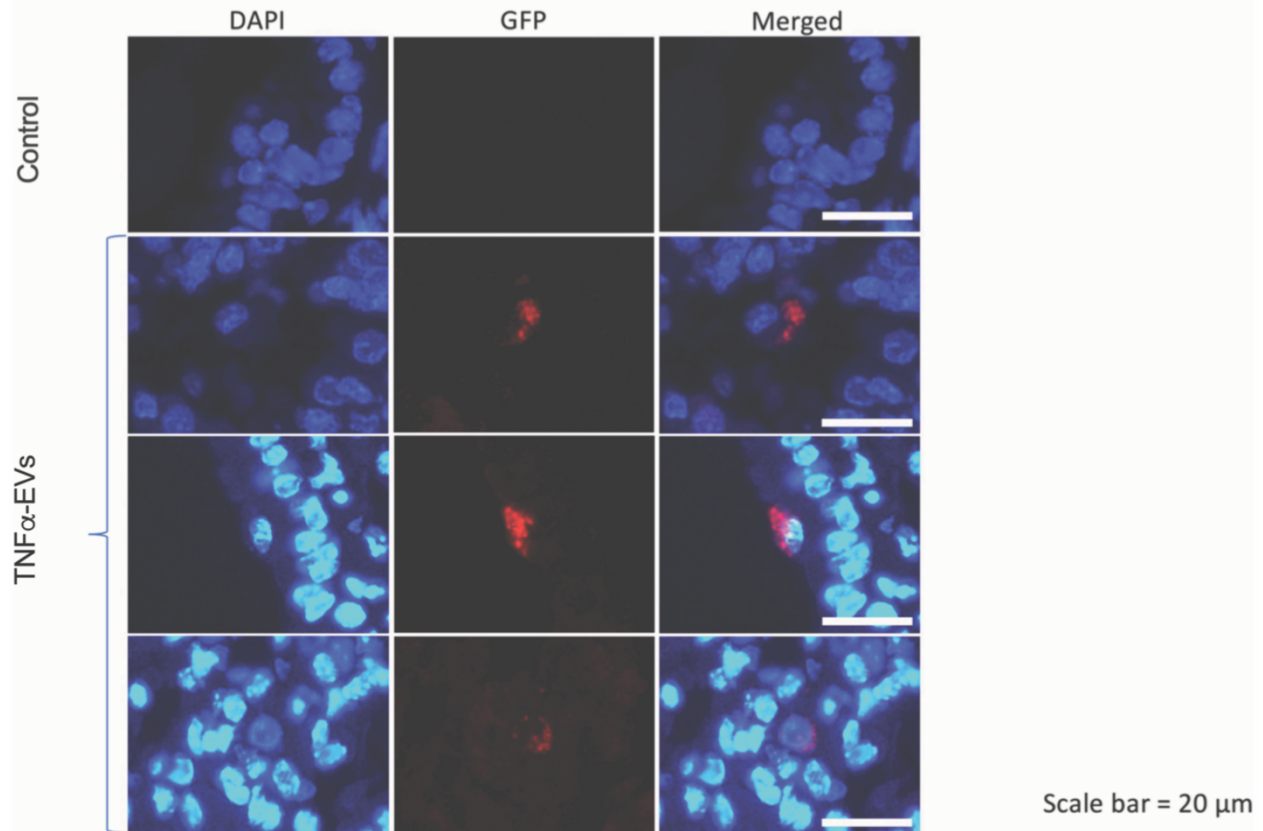
**Supplementary Figure 3:** Polymicrobial sepsis was induced by puncture of the cecum in *Stxbp1*<sup>+/+</sup> and *Stxbp1*<sup>+/-</sup> mice. The release of IL-6, IL-1β, LIF, MIP-2a, IL-10, RANTES, and TNFα was quantified 16 h after CLP operation. P-values determined by ordinary one-way ANOVA with Tukey's multiple comparisons test (SEM; *n*=3, mice per group).

### Supplemental Figure 4



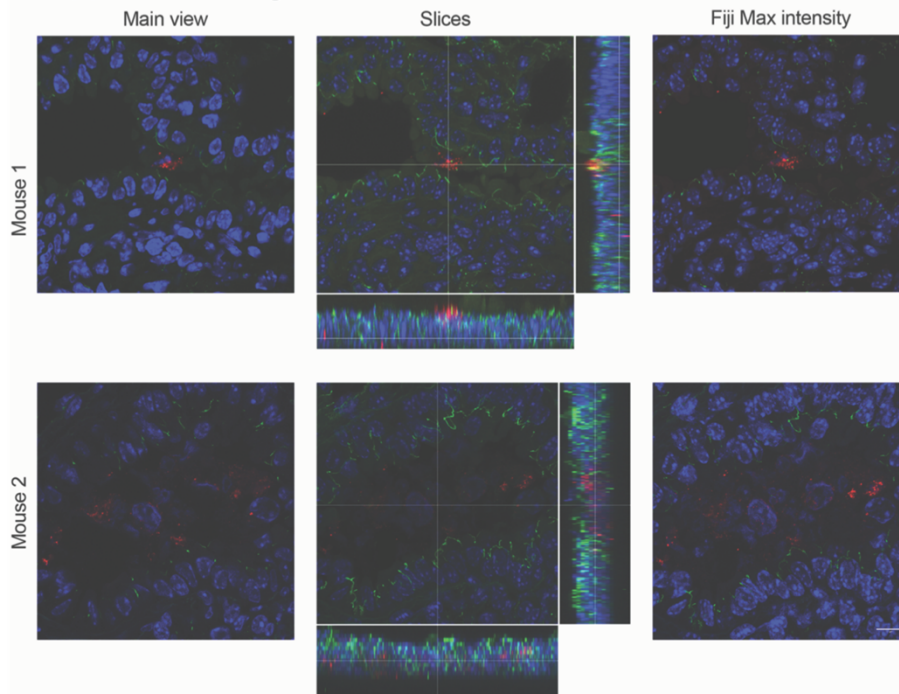
**Supplementary Figure 4:** Representative SEM pictures of HEK293 cells (*left panel*), EVs attaching (*marked in magenta pseudo colors*) to HEK293 cells (*middle panel*), and isolated EVs are shown (*right panel*). Scale bar corresponds to 20 μm (*left panel*) and 10 μm (*middle*) and (*right panel*). (*n*=2 of different measurements).

## Supplemental Figure 5



**Supplementary Figure 5:** Immunofluorescence images of figure 6. Mice were challenged with TNFR1-GFP EVs isolated from TNFR1 GFP stable cells that were stimulated with TNF $\alpha$  overnight (*EVs from 200 million cells/mouse*). Thirty minutes after EV challenge lung samples were collected and analyzed by immunostaining. The nuclei of the cells in the lung tissue of a non-challenged mouse (*left panel*) and lung tissue from a EV challenged mouse (*middle panel*) were counterstained with DAPI (*blue*) and EVs were visualized with anti GFP AF594 (*red*). Scale bar corresponds to 20  $\mu$ m (*n=3 of different measurements*).

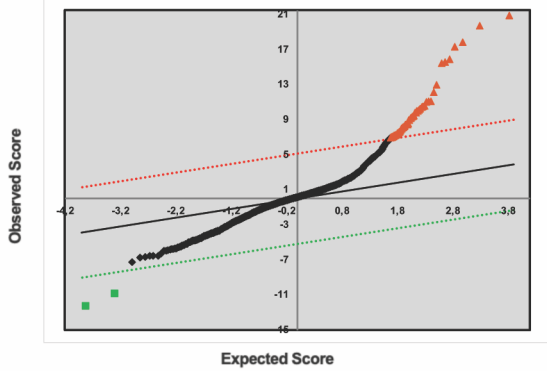
## Supplemental Figure 6



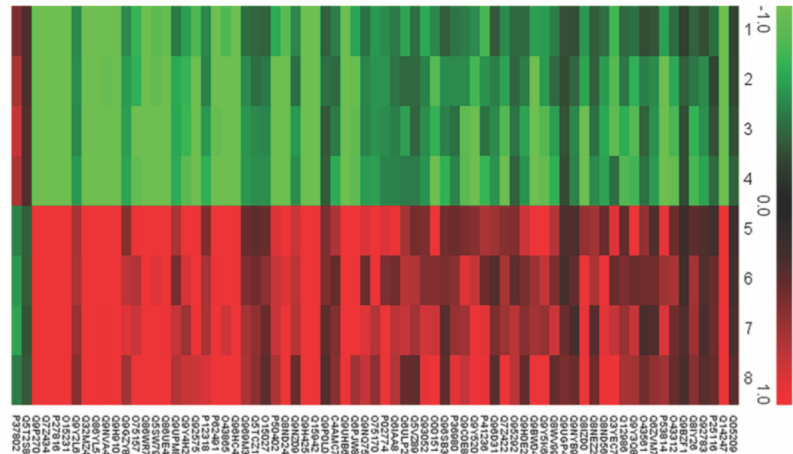
**Supplementary Figure 6:** Confocal images of figure 6. Confocal image (*right panel*) with EVs (*red*) and plasma membrane marker ZO-1 (*green*) (epithelial and endothelial cells). The nuclei are counterstained with DAPI (*blue*). Scale bar corresponds to 10  $\mu\text{m}$  ( $n=3$  of different measurements).

# Supplementary Figure 7

A



B



**Supplementary Figure 7: A.** Significance analysis of microarrays (SAM). Significance analysis of microarrays analysis was performed to compare proteome sample isolates collected from polytrauma and sepsis patients. Over-expressed proteins are illustrated in red, down-regulated proteins are depicted in green. In total 68 proteins were found over-expressed, and 2 proteins were down-regulated (*q-value* 0%, *fold-change* > 4.0). **B.** Heatmap. Red indicates highly expressed proteins and Green indicates lower expressed proteins with 4-fold change. Data points 1-4 are values from EVs isolated from polytrauma patients and 5-8 from EVs isolated from sepsis patients.