

## Supplementary Information

### **Circulating endothelial cell-derived extracellular vesicles mediate the acute phase response and sickness behavior associated with CNS inflammation**

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## Supplementary

### Figure Legends

**Figure S1.** IL-1 $\beta$  protein measured in rat brain homogenate, plasma or EV fractions by ELISA against (a) human IL-1 $\beta$  or (b) rat IL-1 $\beta$ . Values are expressed as pg/ml IL-1 $\beta$ . n.d. = not detectable. Data are mean  $\pm$ SEM (n=3).

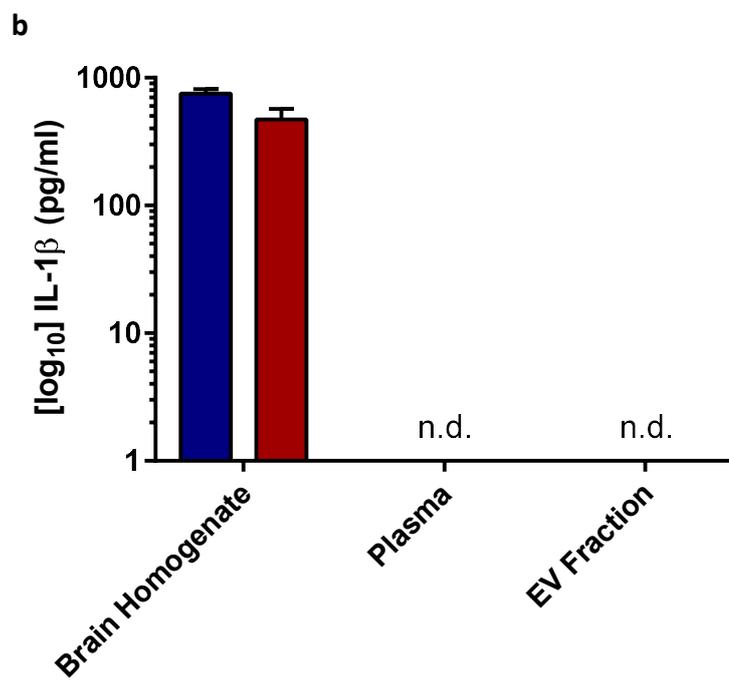
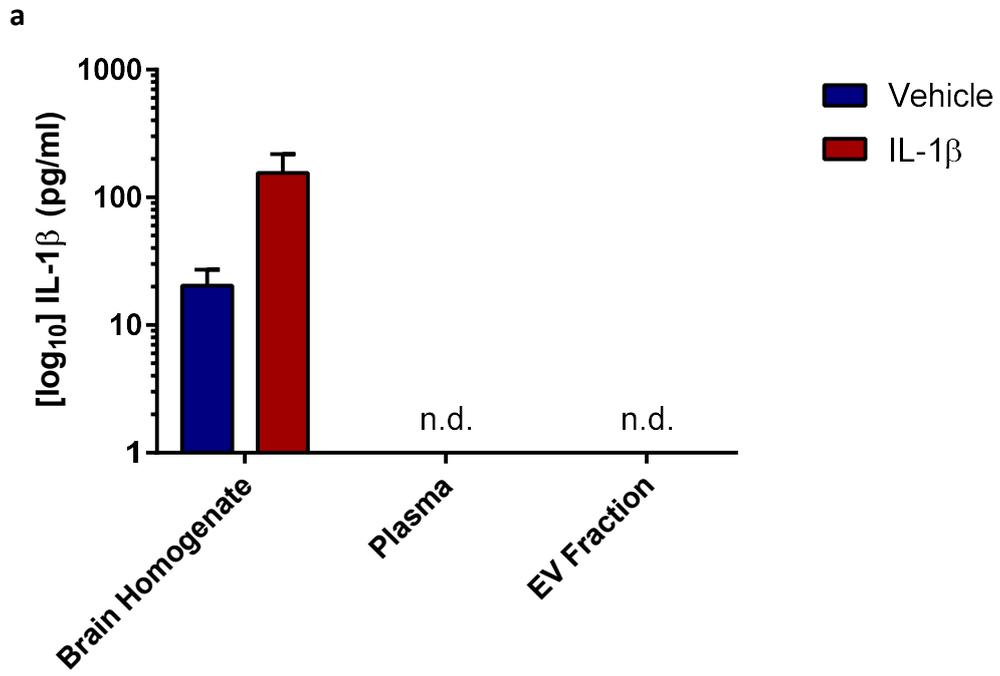
**Figure S2.** Western blot analysis of EVs isolated from the blood of sham and IL-1 $\beta$ -injected animals. (a) CD31 and CD41 (platelet marker) expression as a ratio of Tsg101 expression in the mixed vesicle sample. Note that while the platelet marker (CD41) increases in the IL-1 $\beta$  -challenged animals compared to controls, the increase is significantly greater for the CD31-positive EV population. The dissociation between CD31, which is also expressed in large EVs from platelets, and CD41 expression confirms the enrichment of endothelial cell derived EVs. (b) Photographs of the immunostained blots of CD31, CD41, ALIX and TSG101 EV samples isolated from the plasma of control (saline) and IL-1 $\beta$  injected animals. (c) Electron micrographs of the isolated EVs fraction and EV-free plasma. Data are mean  $\pm$ SD (n=3); \*\*\* P=0.0007 CD31 compared to CD41 for IL-1 $\beta$  EVs, +++++ P=<0.0001 & +++ P=0.0002 Sham compared to IL-1 $\beta$ .

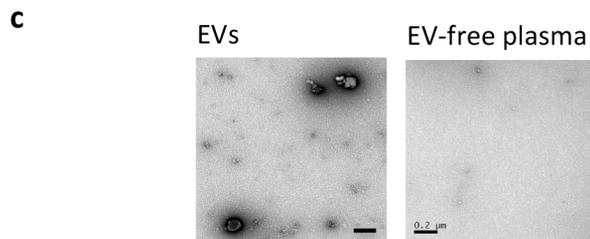
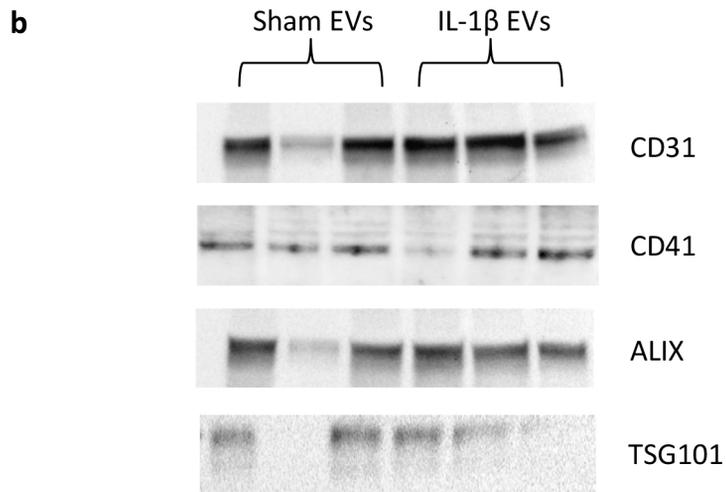
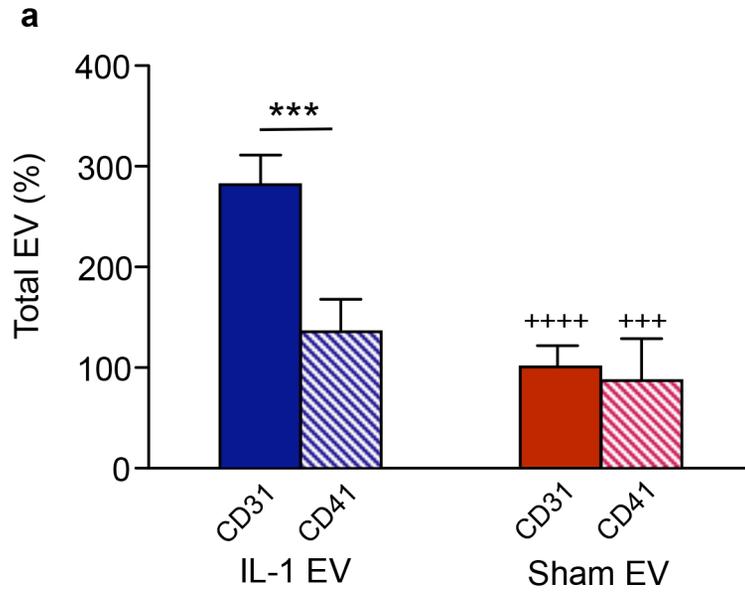
**Figure S3.** Lung and spleen neutrophil numbers in animals receiving EVs isolated from GP-8 supernatant. Total neutrophil numbers were counted in fixed tissue sections from (a) lung and (b) spleen). Data are mean  $\pm$ SEM (n=4).

**Figure S4.** Fluorescence imaging of EVs. PHK-67 (green) labelled EVs were found in the spleen, lung, but not heart. However, compared to Liver (figure 3) the number of PHK-67 labelled EVs was far less prominent. Nuclei are labelled with DAPI (blue).

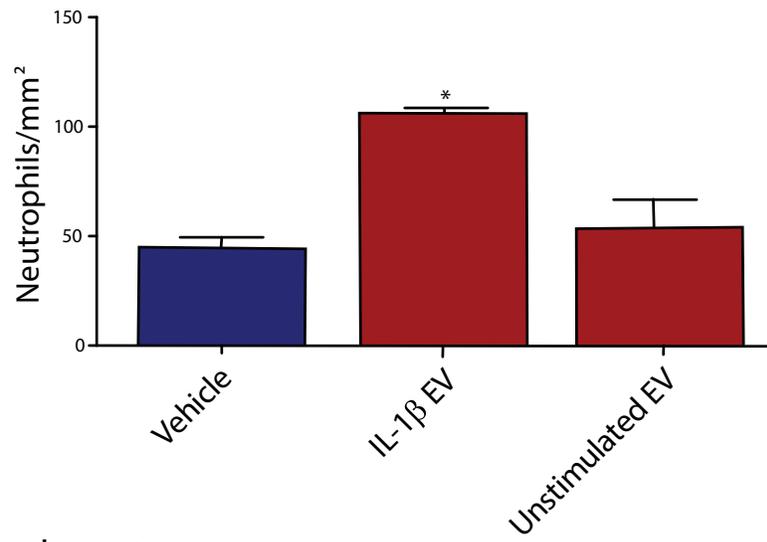
**Supplementary Videos.** NanoSight Tracking Analysis videos of blank PBS, EVs isolated from the plasma of animals after an intracranial injection of either saline or IL-1 $\beta$  and the supernatant/EV-free fraction from the EV isolation process. It should be noted that EV yield in the supernatant/EV-free

fraction was  $<10^6$ /ml and particles were an average size of  $<100$ nm and as such are likely to be small liposomes, rather than exosomes or microvesicles.

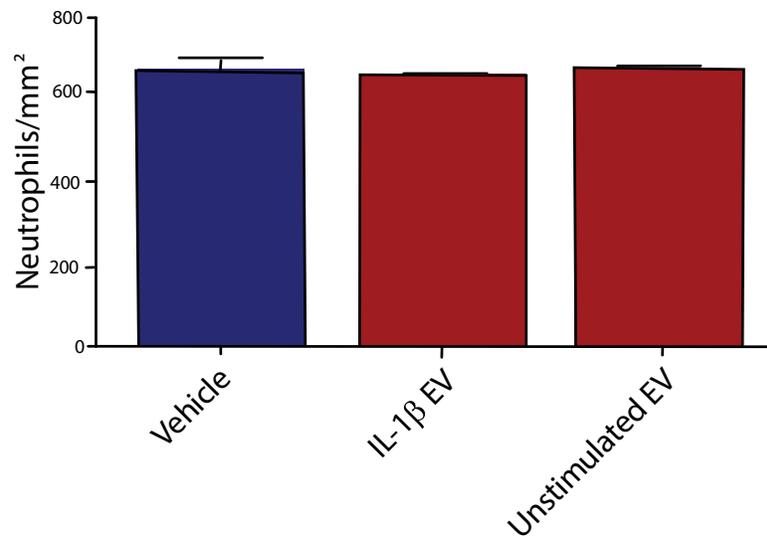




**a Lung**



**b Spleen**



PKH-67-Labelled EVs

Control

