

<b>A) EVs subset a</b>	IFN- $\gamma$ 1	IFN- $\gamma$ 2	TNF- $\alpha$ 1	TNF- $\alpha$ 2	IP-10 1	IP-10 2
neg-EVs	< LLOD	< LLOD	< LLOD	< LLOD	12,1	< LLOD
HDV-EVs	< LLOD	< LLOD	< LLOD	< LLOD	< LLOD	< LLOD

<b>B) EVs subset b</b>	IFN- $\gamma$ 1	IFN- $\gamma$ 2	TNF- $\alpha$ 1	TNF- $\alpha$ 2	IL-6 1	IL-6 2
neg-EVs	< LLOD	< LLOD	173,6	13,0	< LLOD	< LLOD
HDV-EVs	< LLOD	< LLOD	54,4	18,1	< LLOD	< LLOD
UV-HDV-EVs	< LLOD	< LLOD	59,6	49,2	< LLOD	< LLOD

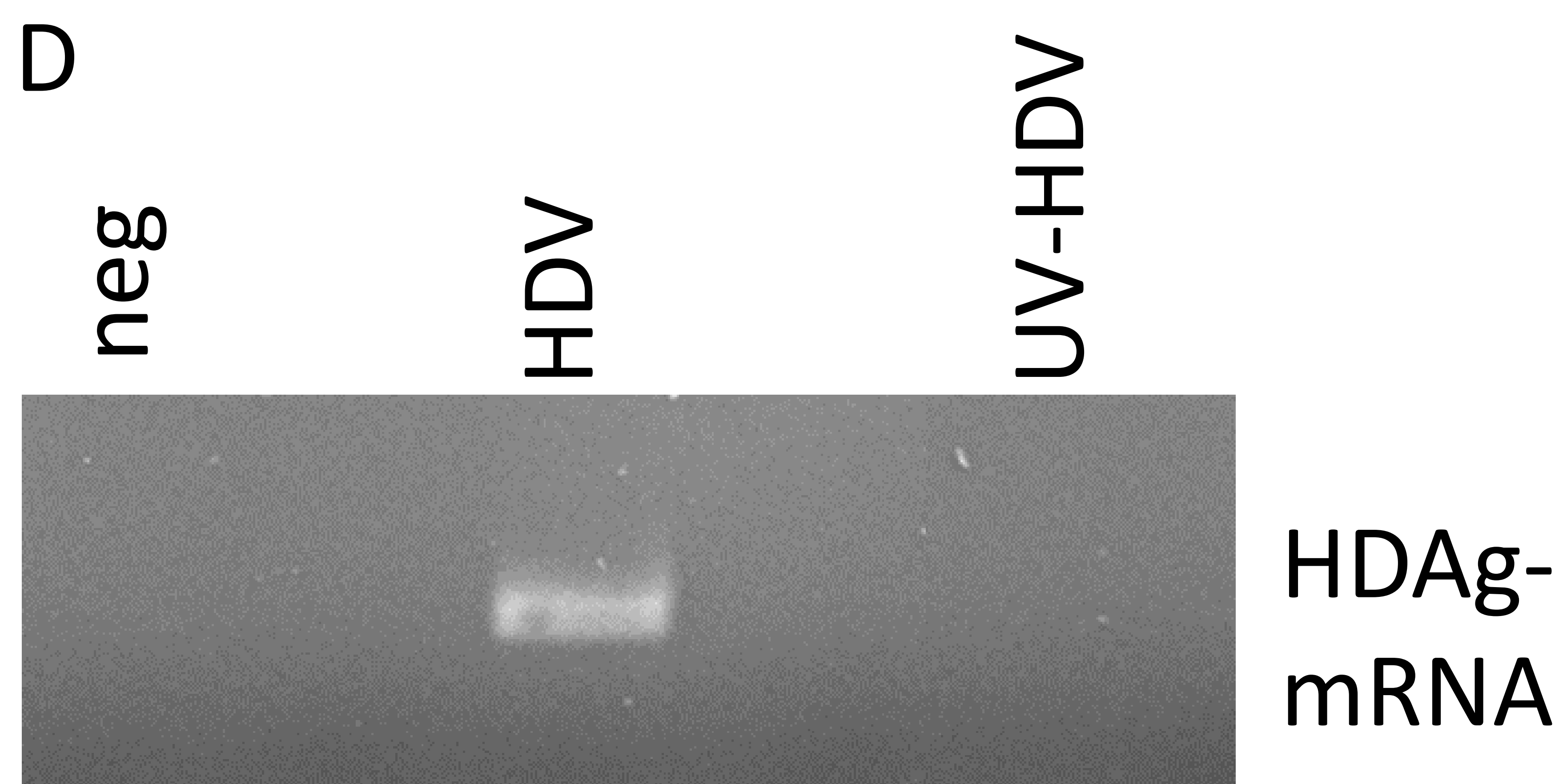
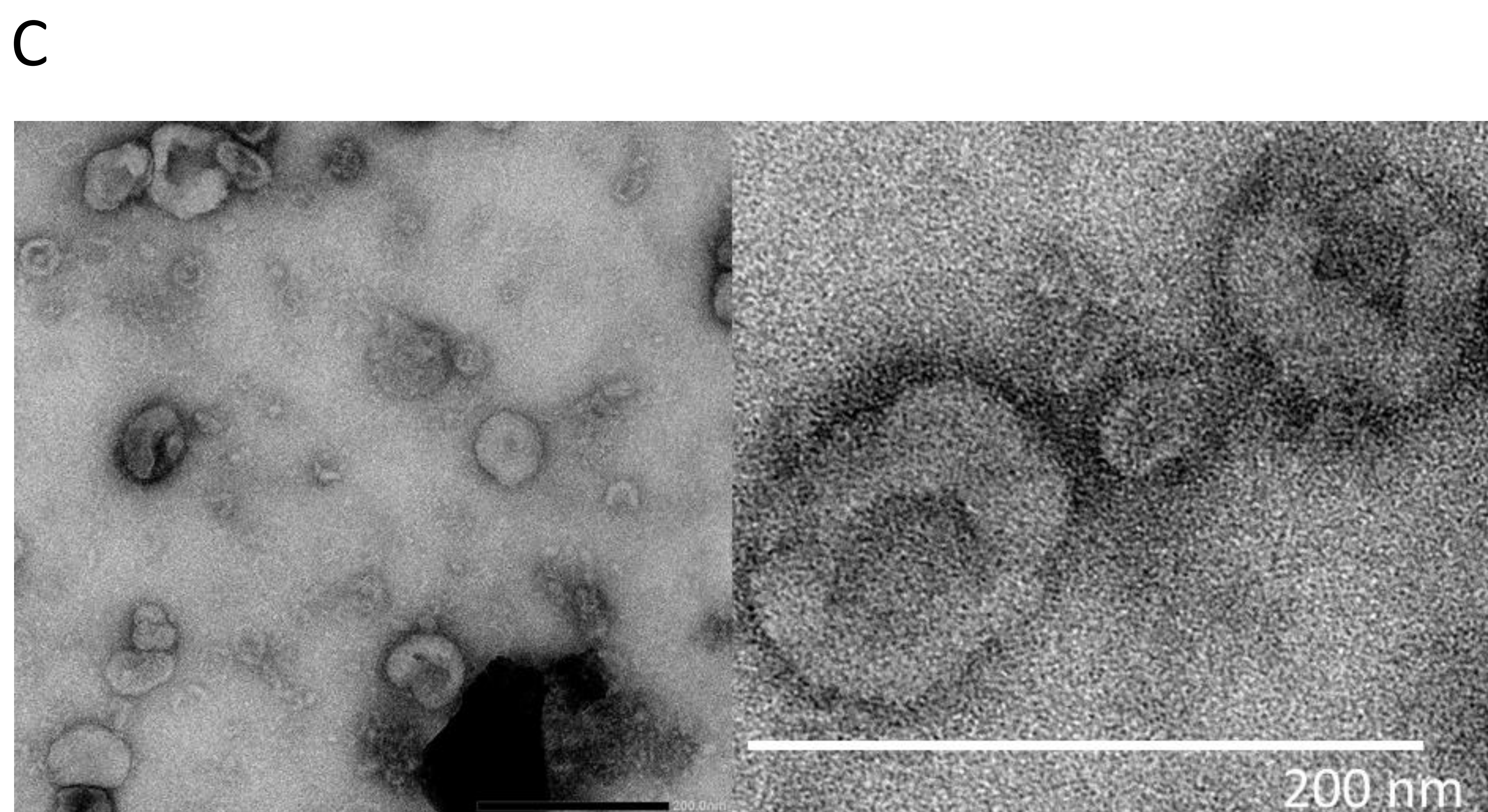
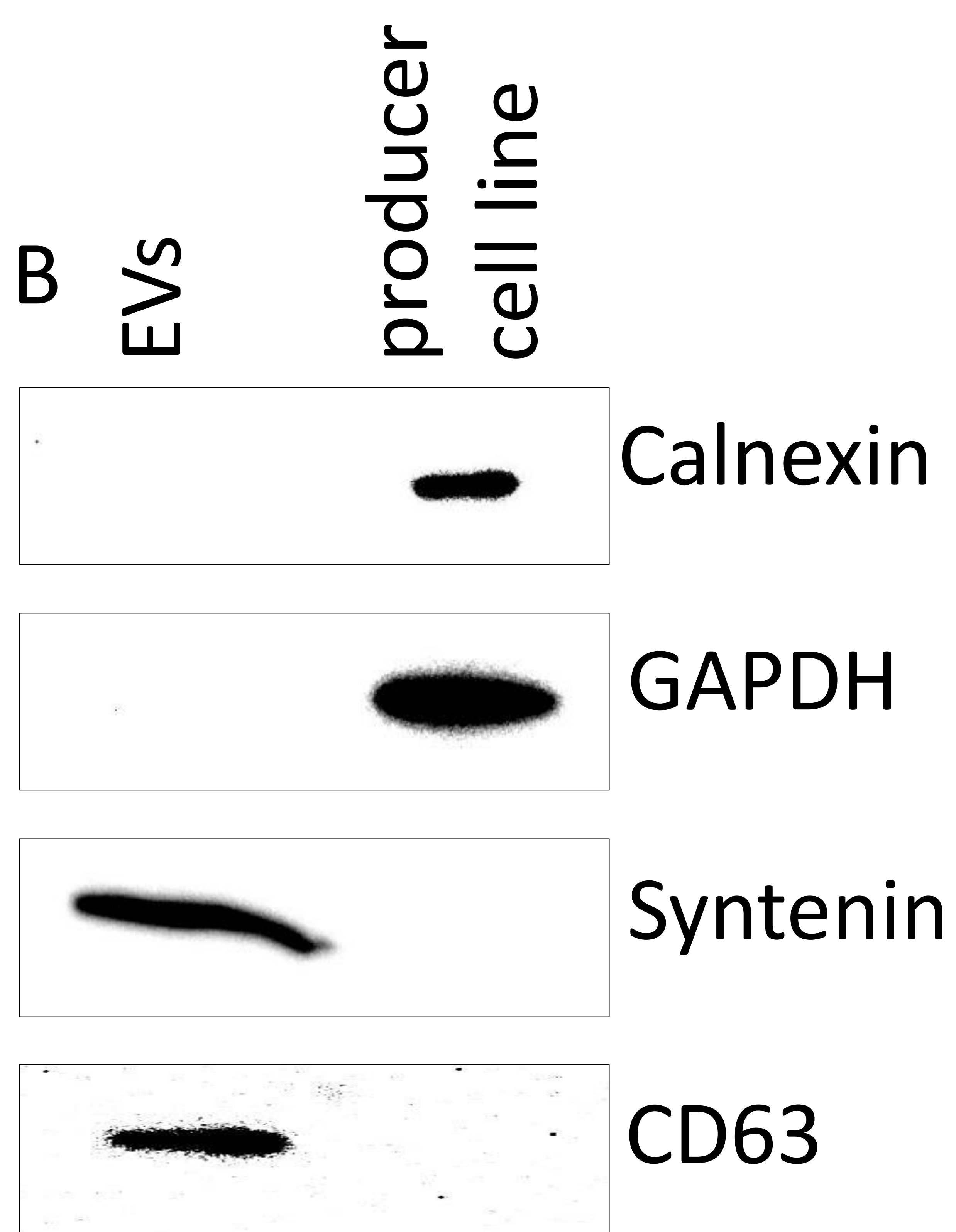
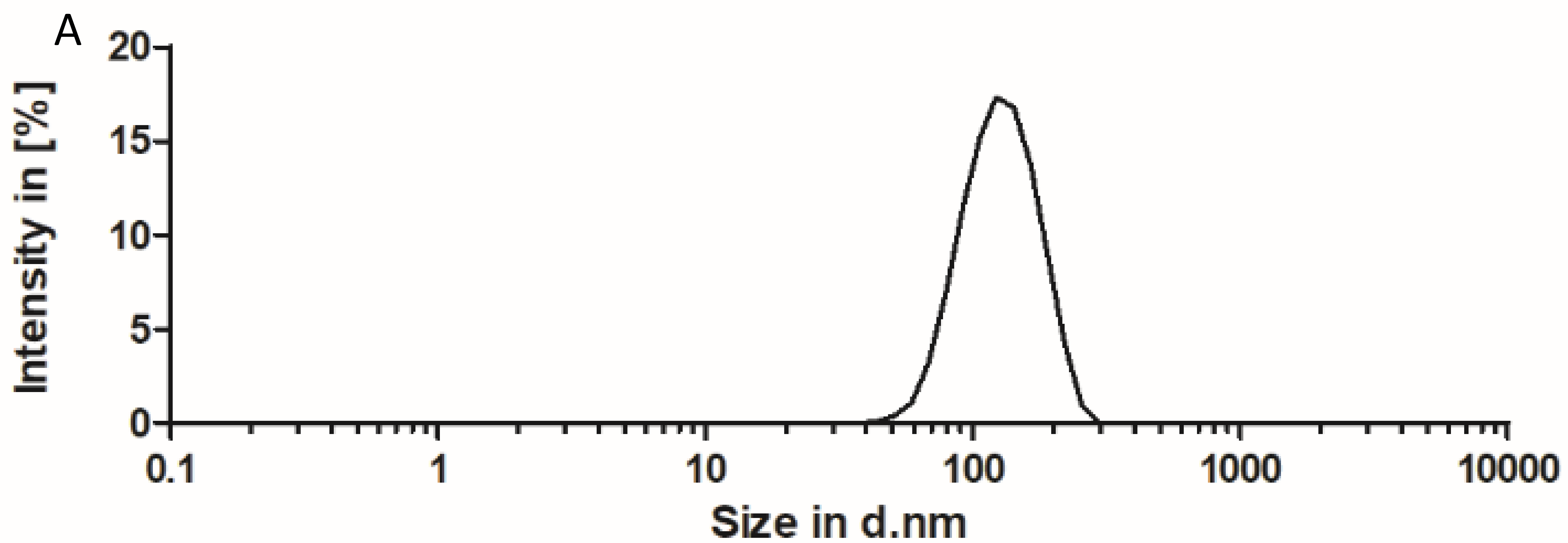
<b>C) EVs subset c</b>	IFN- $\gamma$ 1	IFN- $\gamma$ 2	TNF- $\alpha$ 1	TNF- $\alpha$ 2	IL-6 1	IL-6 2
neg-EVs	< LLOD	< LLOD	< LLOD	< LLOD	< LLOD	< LLOD
HDV-EVs	< LLOD	< LLOD	< LLOD	< LLOD	< LLOD	< LLOD
UV-HDV-EVs	< LLOD	< LLOD	< LLOD	< LLOD	< LLOD	< LLOD
IFN- $\beta$ -EVs	< LLOD	< LLOD	< LLOD	< LLOD	< LLOD	< LLOD

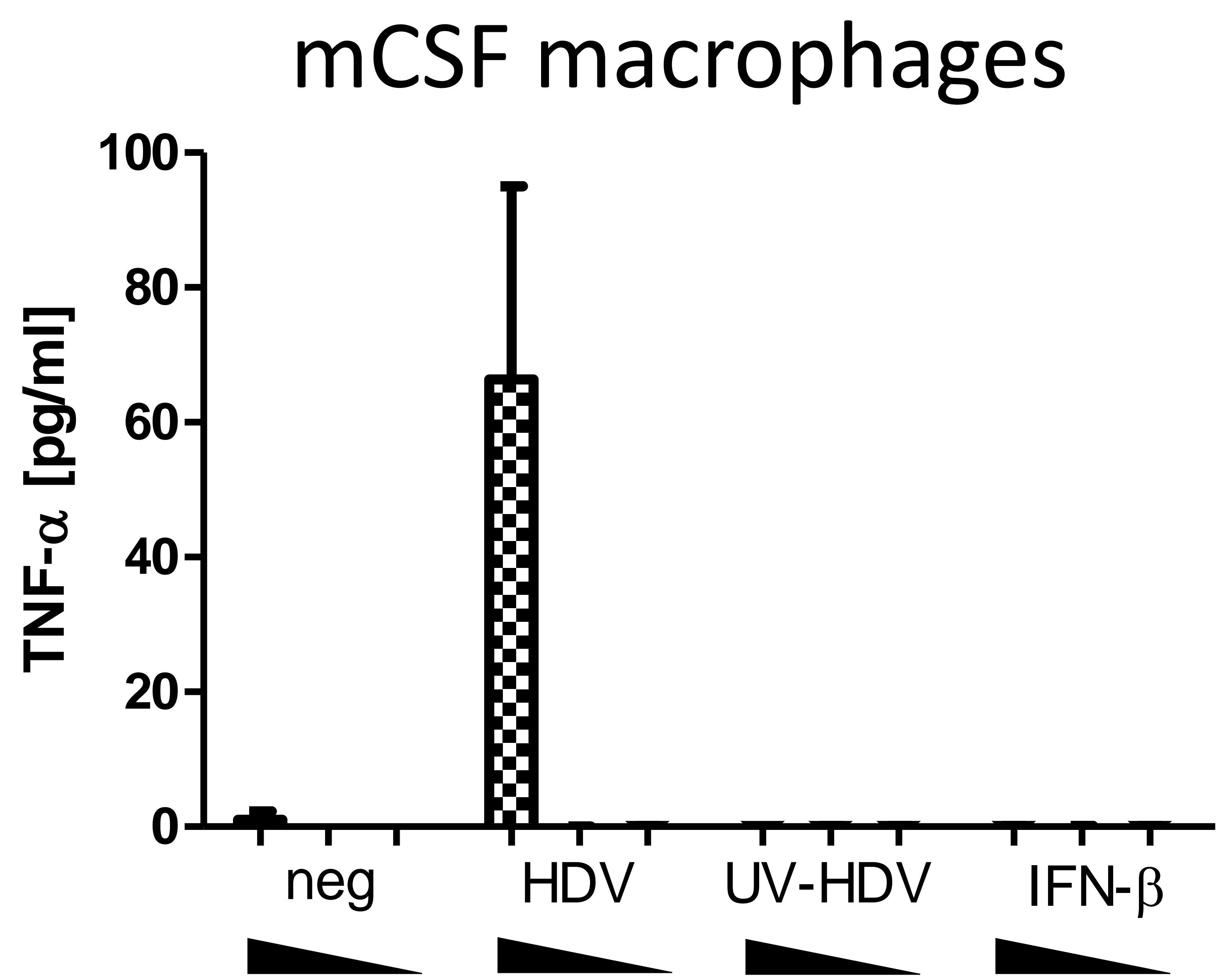
<b>D) supernatants</b>	IFN- $\gamma$ 1	IFN- $\gamma$ 2	TNF- $\alpha$ 1	TNF- $\alpha$ 2	IL-6 1	IL-6 2
neg day 3	< LLOD	< LLOD	< LLOD	< LLOD	< LLOD	< LLOD
HDV day 3	< LLOD	< LLOD	< LLOD	< LLOD	< LLOD	< LLOD
UV-HDV day 3	< LLOD	< LLOD	< LLOD	< LLOD	< LLOD	< LLOD
IFN- $\beta$ day 3	< LLOD	< LLOD	< LLOD	< LLOD	< LLOD	< LLOD
neg day 5	< LLOD	< LLOD	< LLOD	< LLOD	< LLOD	< LLOD
HDV day 5	< LLOD	< LLOD	< LLOD	< LLOD	< LLOD	< LLOD
UV-HDV day 5	< LLOD	< LLOD	< LLOD	< LLOD	< LLOD	< LLOD
IFN- $\beta$ day 5	< LLOD	< LLOD	< LLOD	< LLOD	< LLOD	< LLOD
neg day 8	< LLOD	< LLOD	< LLOD	< LLOD	< LLOD	< LLOD
HDV day 8	< LLOD	< LLOD	< LLOD	< LLOD	< LLOD	< LLOD
UV-HDV day 8	< LLOD	< LLOD	< LLOD	< LLOD	< LLOD	< LLOD
IFN- $\beta$ day 8	< LLOD	< LLOD	< LLOD	< LLOD	< LLOD	< LLOD
neg day 10	< LLOD	< LLOD	< LLOD	< LLOD	< LLOD	< LLOD
HDV day 10	< LLOD	< LLOD	9,8	< LLOD	< LLOD	< LLOD
UV-HDV day 10	< LLOD	< LLOD	< LLOD	< LLOD	< LLOD	< LLOD
IFN- $\beta$ day 10	< LLOD	< LLOD	< LLOD	< LLOD	< LLOD	< LLOD
neg day 12	< LLOD	< LLOD	< LLOD	< LLOD	< LLOD	< LLOD
HDV day 12	< LLOD	< LLOD	< LLOD	< LLOD	< LLOD	< LLOD
UV-HDV day 12	< LLOD	< LLOD	< LLOD	< LLOD	< LLOD	< LLOD
IFN- $\beta$ day 12	< LLOD	< LLOD	< LLOD	< LLOD	< LLOD	< LLOD
neg day 15	< LLOD	< LLOD	< LLOD	< LLOD	< LLOD	< LLOD
HDV day 15	< LLOD	< LLOD	< LLOD	< LLOD	< LLOD	< LLOD
UV-HDV day 15	< LLOD	< LLOD	< LLOD	< LLOD	< LLOD	< LLOD
IFN- $\beta$ day 15	< LLOD	< LLOD	< LLOD	< LLOD	< LLOD	< LLOD

<b>E) supernatants</b>	IFN- $\gamma$ 1	IFN- $\gamma$ 2	IL-6 1	IL-6 2
neg day 4	< LLOD	< LLOD	< LLOD	< LLOD
HDV day 4	< LLOD	< LLOD	< LLOD	< LLOD
UV-HDV day 4	< LLOD	< LLOD	< LLOD	< LLOD
IFN- $\beta$ day 4	< LLOD	< LLOD	9,40	13,52
neg day 7	< LLOD	< LLOD	< LLOD	< LLOD
HDV day 7	< LLOD	< LLOD	< LLOD	< LLOD
UV-HDV day 7	< LLOD	< LLOD	< LLOD	< LLOD
IFN- $\beta$ day 7	< LLOD	< LLOD	< LLOD	< LLOD
neg day 9	< LLOD	< LLOD	< LLOD	< LLOD
HDV day 9	< LLOD	< LLOD	< LLOD	< LLOD
UV-HDV day 9	< LLOD	< LLOD	< LLOD	< LLOD
IFN- $\beta$ day 9	< LLOD	< LLOD	13,70	< LLOD
neg day 11	< LLOD	< LLOD	< LLOD	< LLOD
HDV day 11	< LLOD	< LLOD	< LLOD	< LLOD
UV-HDV day 11	< LLOD	< LLOD	< LLOD	< LLOD
IFN- $\beta$ day 11	< LLOD	< LLOD	< LLOD	< LLOD

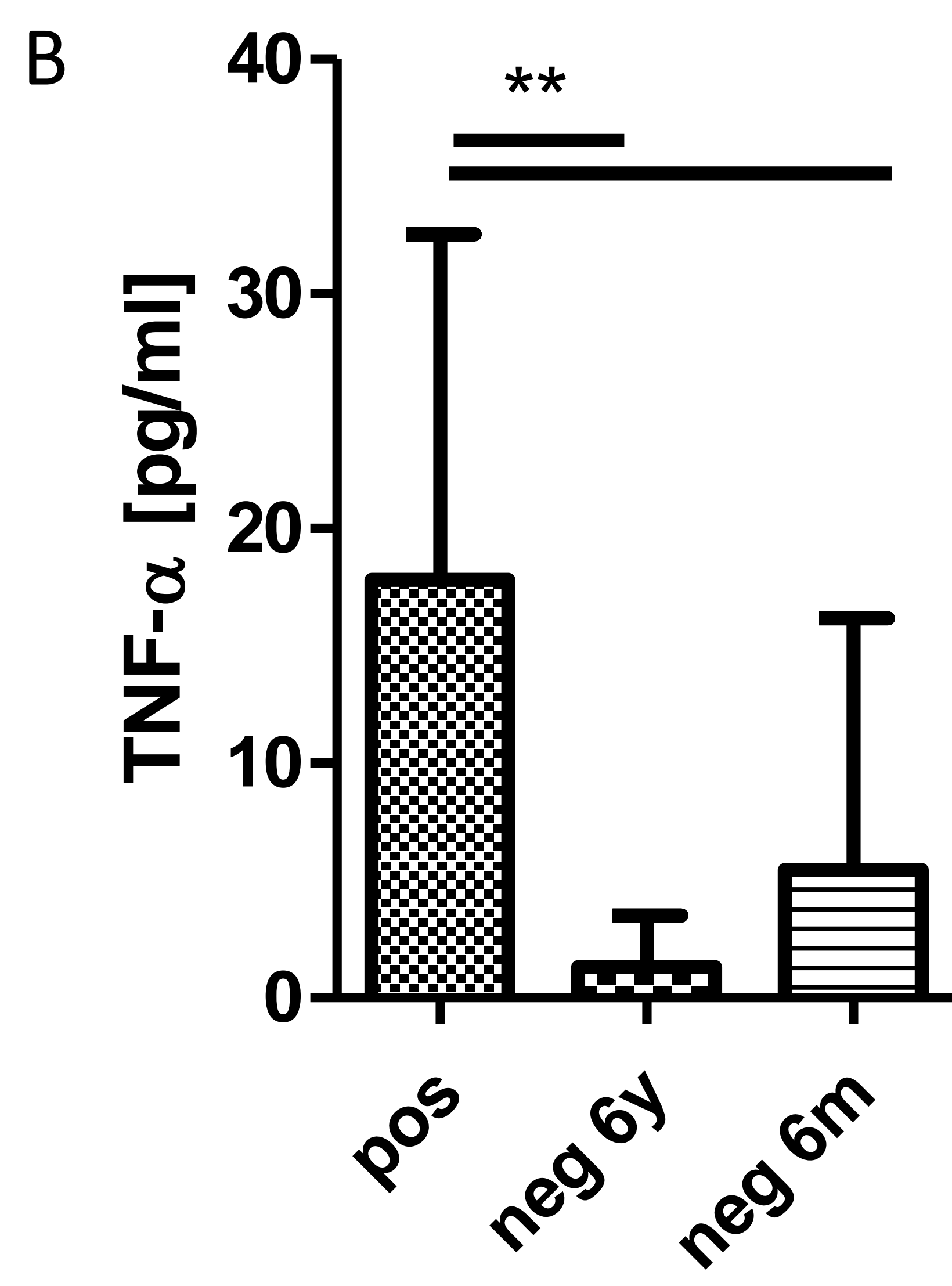
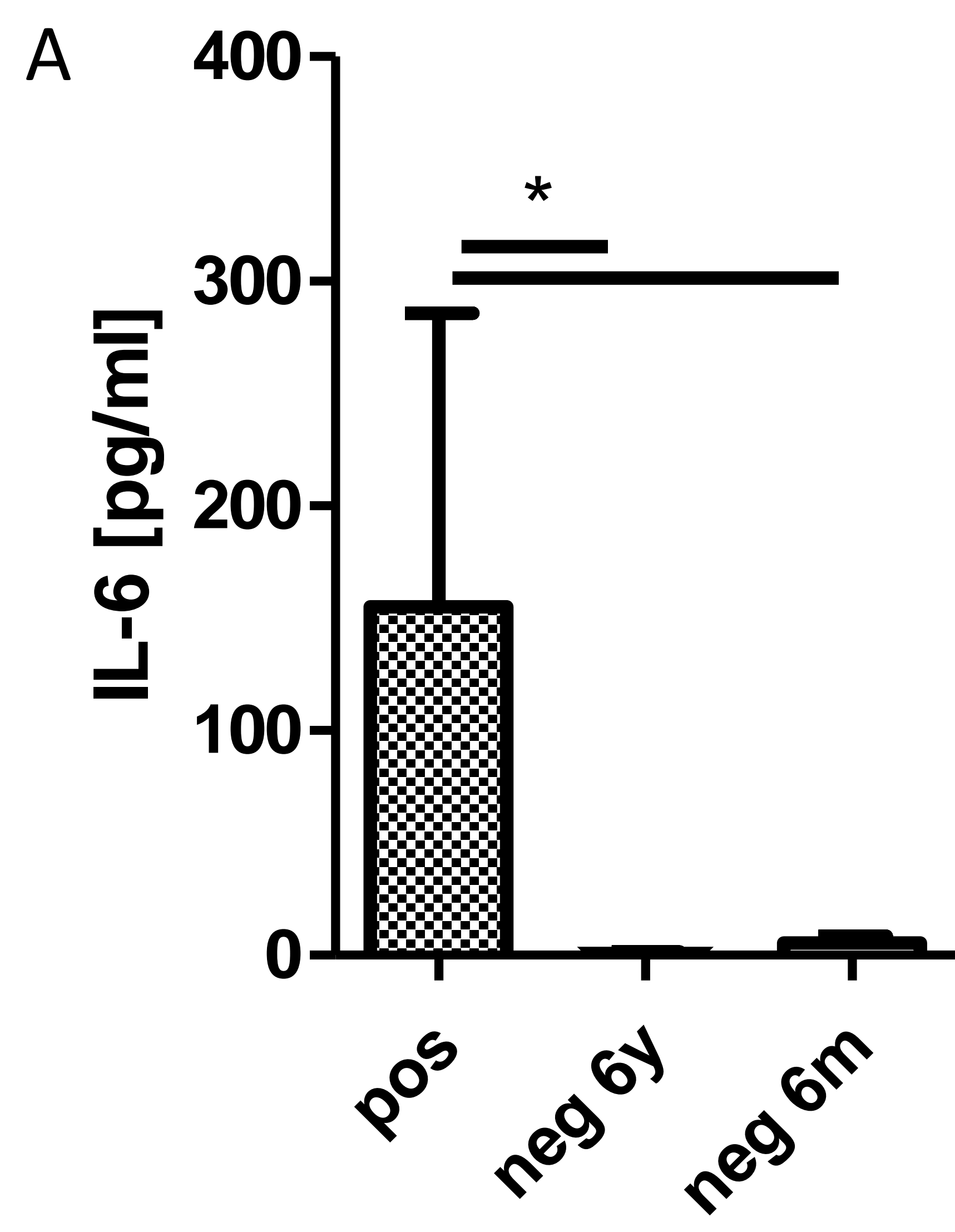
<b>F) supernatants</b>	TNF- $\alpha$ 1	TNF- $\alpha$ 2	IL-6 1	IL-6 2
neg day 5	< LLOD	< LLOD	< LLOD	< LLOD
HDV day 5	28,9	29,8	< LLOD	< LLOD
UV-HDV day 5	< LLOD	< LLOD	< LLOD	< LLOD
neg day 7	< LLOD	< LLOD	< LLOD	16,3
HDV day 7	< LLOD	< LLOD	< LLOD	< LLOD
UV-HDV day 7	< LLOD	< LLOD	< LLOD	< LLOD
neg day 11	< LLOD	< LLOD	< LLOD	< LLOD
HDV day 11	< LLOD	< LLOD	< LLOD	< LLOD
UV-HDV day 11	< LLOD	0,6	< LLOD	< LLOD
neg day 13	31,1	14,9	< LLOD	< LLOD
HDV day 13	9,5	12,5	< LLOD	< LLOD
UV-HDV day 13	< LLOD	< LLOD	< LLOD	< LLOD

Supplementary 1





supplementary 3



supplementary 4

## Supplementary information

### Supplementary data 1: Extracellular vesicle subsets and conditioned media do not contain proinflammatory cytokines in response to HDV infection.

EV preparations and conditioned media used to purify EVs were subjected to ELISA measurement. NTCP-expressing HepG2-cells were used as producer cell line.

Detectable values are highlighted in grey, values below lower limit of detection (7,8 pg cytokine/ml) are marked with < LLOD.

- A) IFN- $\gamma$ , TNF- $\alpha$  and 10 kDa interferon gamma-induced protein 10 (IP-10) content in size-exclusion chromatography (SEC)-purified EV preparation. Each column represents the duplicate measurement of a single sample.
- B) IFN- $\gamma$ , TNF- $\alpha$  and IL-6 content in SEC-purified EV preparation. Each column represents the duplicate measurement of a single sample.
- C) IFN- $\gamma$ , TNF- $\alpha$  and IL-6 content in SEC-purified EV preparation. Each column represents the duplicate measurement of a single sample. NTCP-expressing HepG2-cells were used as producer cell line.
- D) IFN- $\gamma$ , TNF- $\alpha$  and IL-6 content in conditioned media collected on different days p.i.. Each column represents the duplicate measurement of a single sample. NTCP-expressing HepG2-cells were used as producer cell line.
- E) IFN- $\gamma$  and IL-6 content in conditioned media collected on different days p.i.. Each column represents the duplicate measurement of a single sample. NTCP-expressing Huh7-cells were used as producer cell line.
- F) TNF- $\alpha$  and IL-6 content in conditioned media collected on different days p.i.. Each column represents the duplicate measurement of a single sample. NTCP-expressing HepG2-cells were used as producer cell line.

### Supplementary data 2: Quality control of size-exclusion chromatography purified extracellular vesicles

- A) EVs purified from media of Na<sup>+</sup> taurocholate *cotransporting polypeptide* (NTCP)-expressing hepatoma cells (shown: HepG2) were subjected to dynamic light scattering (DLS) measurement as size control.
- B) Lysates obtained from purified EVs and the NTCP-expressing producer cell line Huh7 were subjected to SDS-polyacrylamide gel electrophoresis and visualized by Western blotting and subsequent antibody staining to control for EV purity (Thery, Witwer et al. 2018). As characteristic proteins deprived from EV, Calnexin and Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) were stained. As proteins typically enriched in EVs, Syntenin and Cluster of Differentiation (CD)63 were stained.
- C) EVs were collected from media of NTCP-expressing HepG2-cells, fixed in 2% PFA and analysed by negative-stain electron microscopy. Scale bars 200 nm.
- D) EVs were collected from media of untreated NTCP-expressing HepG2-cells (neg), HDV-infected cells (HDV) or cells infected with UV-inactivated HDV (UV-HDV). RNA was isolated and presence of HDV-Antigen mRNA (HDAG mRNA) was shown by nested PCR and agarose gelelectrophoresis.

### Supplementary data 3: Impact of HepG2-derived HDV-primed EVs on primary human macrophages

Macrophage-colony stimulating factor differentiated macrophages were stimulated with EVs purified from cell culture medium by Size-exclusion chromatography (SEC). Cell culture medium was collected

either from untreated NTCP-expressing HepG2-cells (neg), HDV-infected cells (HDV), cells infected with UV-inactivated HDV (UV-HDV) or interferon  $\beta$  (IFN- $\beta$ ). The inoculum was normalized to the number of secreting cells and added in 5-fold dilutions. Supernatants collected from macrophages were analysed by ELISA for TNF- $\alpha$  content. Graph shows dose-dependent results from one individual experiment.

**Supplementary data 4: Size-exclusion chromatography purifies immunostimulatory entities from patient sera**

Human macrophage-colony stimulating factor differentiated macrophages (mCSF M $\phi$ ) were stimulated with EVs purified from patient sera by Size-exclusion chromatography (SEC). Sera contained at least  $1,61 \cdot 10^5$  Genom equivalents HDV RNA / ml (pos), were HDV-negative for 6 years (neg 6y) or HDV-negative for 6 months (neg 6m). The inoculum was normalized to the plasma volume and supernatants collected from mCSF M $\phi$  were analysed by ELISA. Mean  $\pm$ SD from three independent experiments is given. Statistical analysis was done using Mann Whitney test. \*  $p < 0.05$ , \*\*  $p < 0.01$ . A) Interleukin-6 (IL-6) released by mCSF M $\phi$  B) TNF- $\alpha$  released by mCSF M $\phi$ .