# *Supplementary Material*

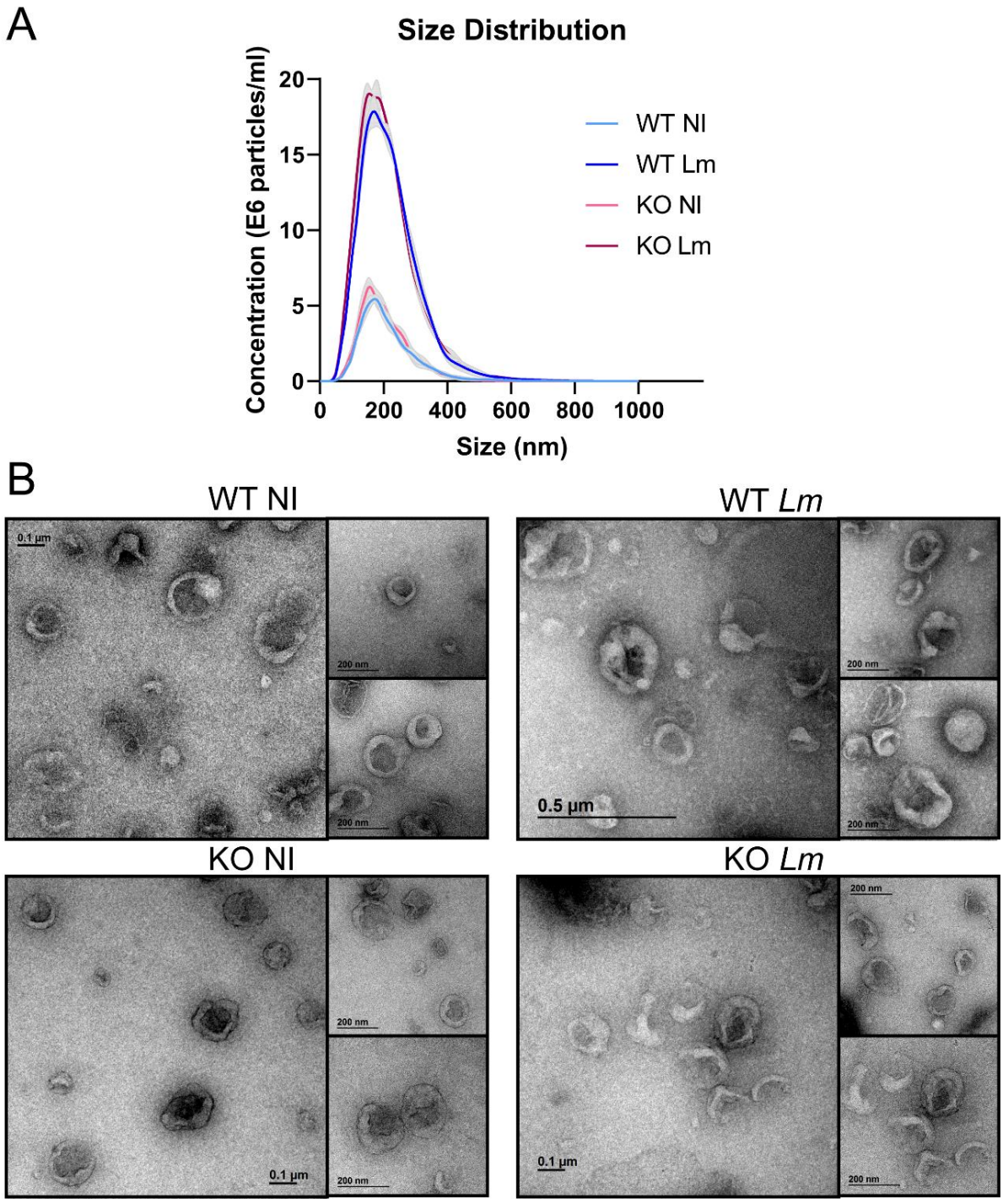
## 1 Supplementary Data

Fig. S1-6

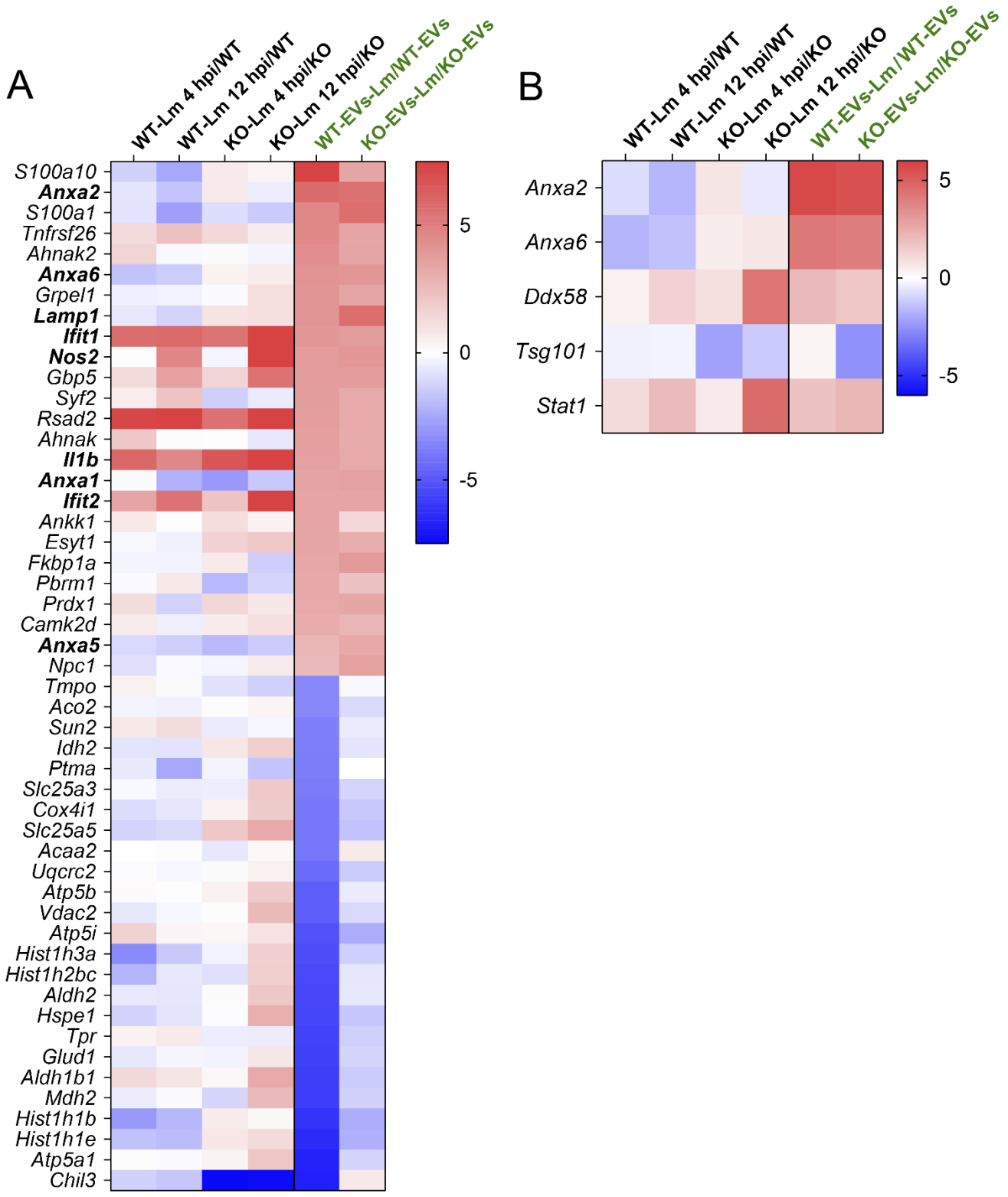
Table S1-8

## 2 Supplementary Figures and Tables

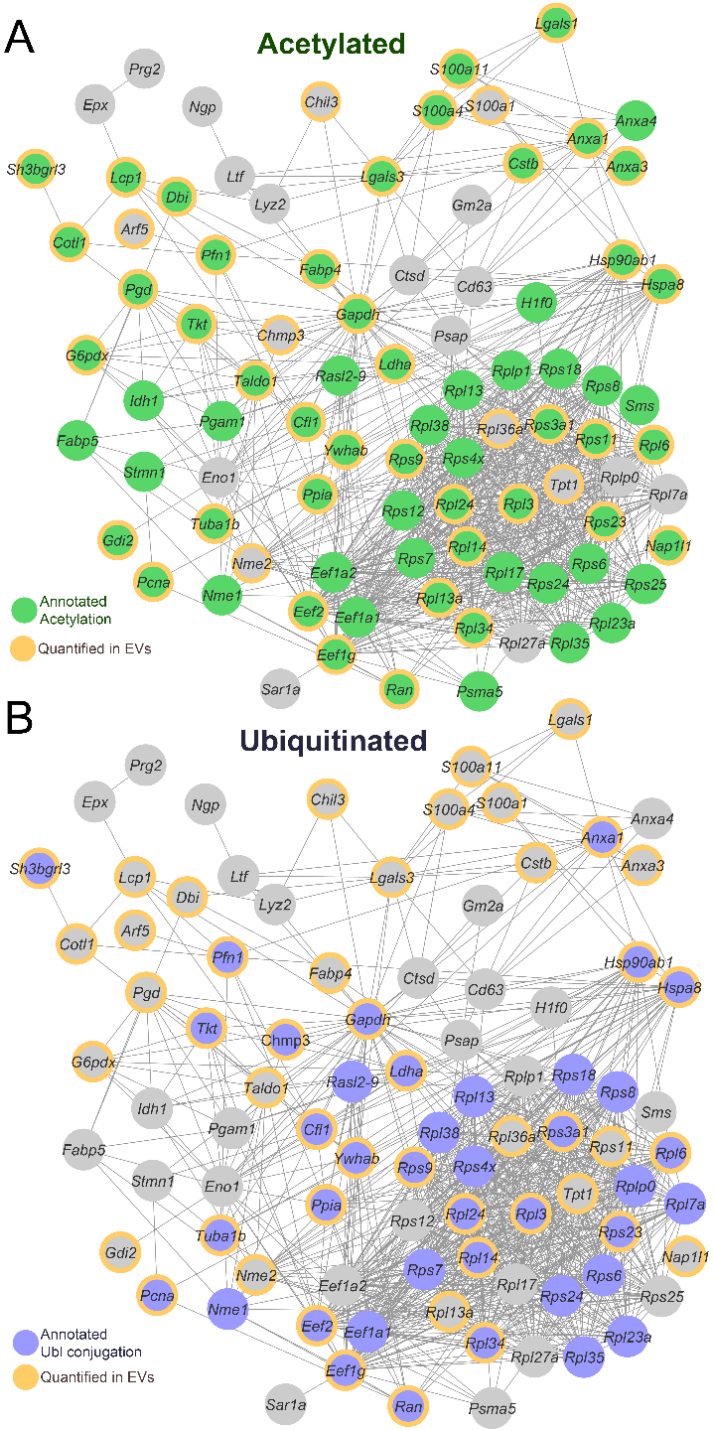
## 2.1 Supplementary Figures



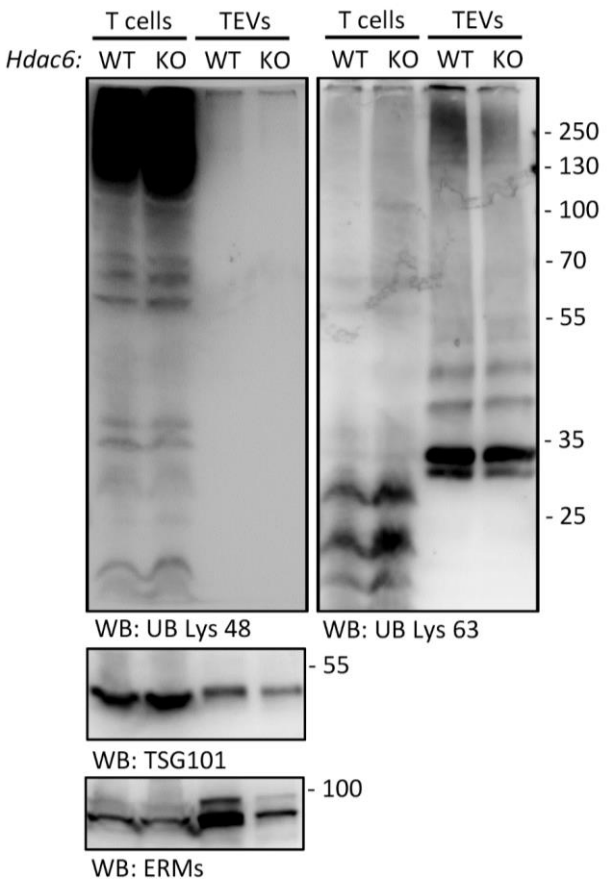

**Supplementary Figure 1. Isolated EVs present typical size and topology. (A)** NTA size profile distribution. Grey area indicates standard error of the mean. **(B)** Transmission electron microscopy images from DEVs isolated from non-infected (NI) or *Lm* infected DCs. For each condition, three images are shown. Magnification for the image in the left: × 80,000. Top right image magnification: x120,000. Bottom right image magnification: x150,000. Scale bar is included for each image (0.5µm, 0.1µm or 200nm).



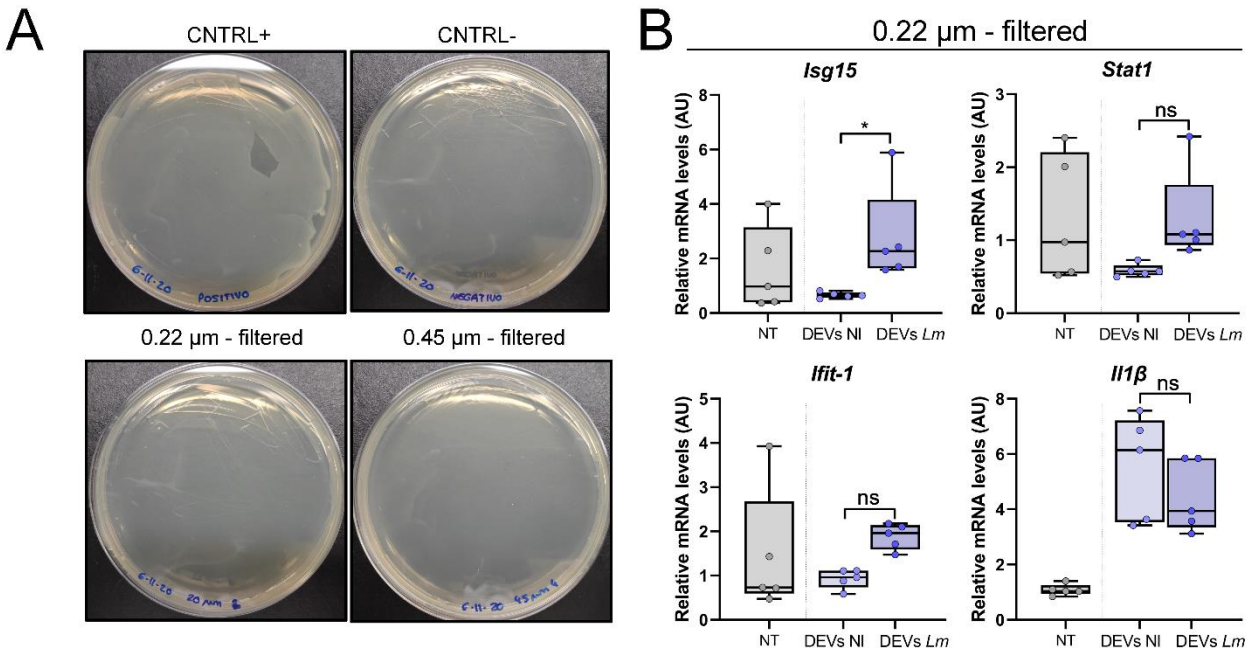

**Supplementary Figure 2. Protein profiling from total cell lysates and their derived EVs from WT and KO-HDAC6 BMDCs. (A)** Significant protein abundance changes shared between the total cell lysate and EVs after *Lm* infection (FDRq <0.05). **(B)** Protein abundance in total cell lysates and EVs observed in western-blot validation. Rows are labeled according to gene name.



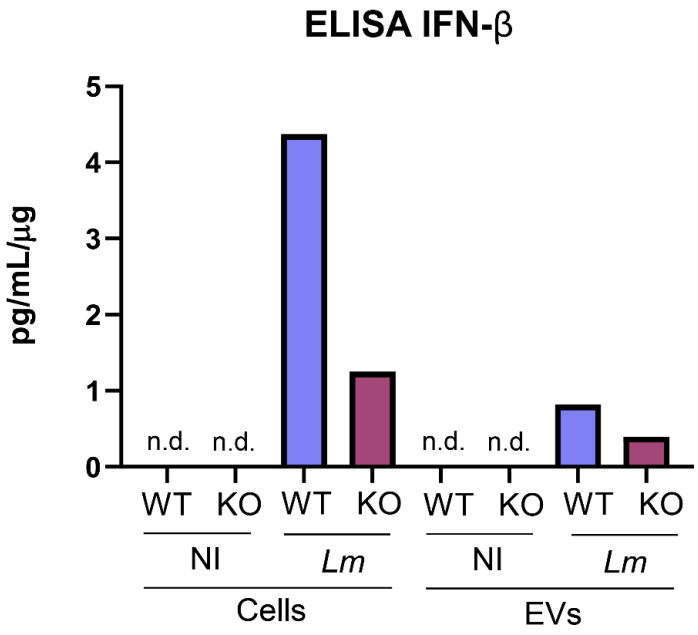

**Supplementary Figure 3. Enrichment in acetylated and ubiquitinated DC proteins upon *Lm* infection.** The 95 proteins identified with at least two peptides showing increased abundance (Zq > 2) in *Hdac6*$^{+/+}$ after 12 hours of *Lm* infection were submitted to STRING functional enrichment analysis. Results indicate significant overrepresentation of **(A),** acetylated (UniProt KW0007-Acetylation, FDRq = 2.64e-35) and **(B)**, ubiquitinated (UniProt KW0832-Ubl conjugation, FDRq=6.37e-14) proteins. Proteins annotated as Acetylation and Ubl conjugation are indicated in green and purple, respectively. DC proteins that were also quantified in the EVs are outlined in yellow. Proteins are labeled according to gene name.




**Supplementary Figure 4. Ubiquitination in K-48 and K-63 state in T lymphoblast total cell lysates and their derived EVs.** Protein extracts were prepared from EVs and *Hdac6*$^{+/+}$ (WT) or *Hdac6*$^{-/-}$ (KO) T cell lymphoblasts samples isolated from spleen. Cells and EVs were blotted with an antibody against polyubiquitin chains linked through Lys48 ubiquitin (UB) residue (left panel) or Lys63 UB residue (right panel). TSG101 and ERMs were used as EVs markers. Both panels corresponds to same sample.




**Supplementary Figure 5. Pore filtration methods restrain *Lm* and do not induce strong anti-pathogenic responses. (A)** BHI-Agar plates with *Lm* BHI liquid culture (CNTRL+, upper left image), BHI medium (CNTRL-, upper right image) and *Lm* filtered through 0.22 µm (lower left image) or 0.45 µm (lower right image) cultured for over 3 days**. (B)** qPCR analysis of the expression levels of *Isg15*, *Stat1*, *Ifit-1* and *Il1β* in DCs treated with vehicle (NT), DEVs isolated from non-infected DCs (NI) or *Lm*-infected (Lm). DEVs were filtered through 0.22 µm ($n$=5, one-way analysis of variance (ANOVA) test with Tukey's posttest; *$P$ < 0.05 and ns, not significant).




**Supplementary Figure 6. IFN-β is detected following *Lm* infection.** HDAC6 WT or KO whole DCs (Cells) or dendritic derived extracellular vesicles (EVs) lysate under non-infection (NI), or Lm-infection (*Lm*) conditions were subjected to LumiKine™ Xpress mIFN-β 2.0 assay. Detected pg/mL were plot based on total amount of proteins in the lysate (µg). Non-detectable samples are indicated as n.d.

## 2.2 Supplementary Tables

| Reagent | Application | Use | Reference | Manufacturer |
|---|---|---|---|---|
| TOPRO-3 | Flow cytometry | 1:10000 | T3605 | Invitrogen |
| LIVE/DEAD® Fixable Yellow Dead Cell Stain | Flow cytometry | 1:1000 | L34968 | Invitrogen |
| CD11b-PECy7 | Flow cytometry | 1:200 | 25-0112-81 | Invitrogen |
| CD11c-BV421 | Flow cytometry | 1:200 | 565452 | BD |
| GR-1-V450 | Flow cytometry | 1:200 | 75-5931 | Tonbo Biosciences |
| MHCII-APCFire750 | Flow cytometry | 1:400 | 107652 | BioLegend |
| Annexin A2 | Western-blot | 1:1000 | ab185957 | Abcam |
| Annexin A6 | Western-blot | 1:1000 | 686921 | R&D Systems |
| Calnexin | Western-blot | 1:500 | ab10286 | Abcam |
| ERMs | Western-blot | 1:4000 | | Rabbit ascitis |
| NLRP3/NALP3 | Western-blot | 1:500 | AG-20B-0014-C100 | AdipoGen Life Sciences |
| MFG-E8 | Western-blot | 1:500 | AF2805 | R&D Systems |
| RIG-I | Western-blot | 1:500 | 3743S | Cell Signaling Technology |
| STAT1 | Western-blot | 1:1000 | 9172 | Cell Signaling Technology |
| TSG101 | Western-blot | 1:1000 | ab83 | Abcam |
| UB Lys 48 | Western-blot | 1:500 | ab140601 | Abcam |
| UB Lys 63 | Western-blot | 1:1000 | 05-1308 | Sigma-Aldrich |
| α-Tubulin | Western-blot | 1:1000 | T6199 | Sigma-Aldrich |
| Anti-mouse-HRP | Western-blot | 1:5000 | 31446 | Invitrogen |
| Anti-rabbit-HRP | Western-blot | 1:5000 | 31460 | Invitrogen |
| Anti-goat-HRP | Western-blot | 1:5000 | 31402 | Invitrogen |

**Table S1.** List of antibodies used for Western-blot and Flow Cytometry and the used dilution.

| Gene | Forward Sequence | Reverse Sequence |
|---|---|---|
| *Ywhaz* | CGTTGTAGGAGCCCGTAGGTCAT | TCTGGTTGCGAAGCATTGGG |
| *β-Actin* | CAGAAGGAGATTACTGCTCTGGCT | TACTCCTGCTTGCTGATCCACATC |
| *Isg15* | CTAGAGCTAGAGCCTGCAG | AGTTAGTCACGGACACCAG |
| *Stat1* | CGCGCATGCAACTGGCATATAACT | ATGCTTCCGTTCCCACGTAGACTT |
| *Ifit1* | CTGAGATGTCACTTCACATGGAA | GTGCATCCCCAATGGGTTCT |
| *Il1b* | AAAGACGGCACACCCACCCTGC | TGTCCTGACCACTGTTGTTTCCCAG |
| *Il12 p40* | TGGTTTGCCATCGTTTTGCTG | ACAGGTGAGGTTCACTGTTTCT |
| *Cd40* | GACTCAGGCGAATTCTCAGC | GTCTCAGTGGCCATCTCCAT |
| *Cd86* | CTGGACTCTACGACTTCACAATG | AGTTGGCGATCACTGACAGTT |
| *Cxcl10* | CCAGTGCTGCCGTCATTTTC | GGCTCGCAGGGATGATTTC |

**Table S2.** List of primers, with their corresponding sequence, used for qPCR.

**Table S3:** Protein quantification in total cell lysates

**Table S4:** IPA analysis of total cell lysates: canonical pathways and diseases and functions category

**Table S5:** Protein quantification in EVs

**Table S6:** IPA analysis of EVs: diseases and functions category

**Table S7:** Ubiquitinated and acetylated peptides in total cell lysates and EVs

**Table S8:** Enrichment analysis of ubiquitinated and acetylated proteins