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Supplemental information

Functional genome-wide short hairpin RNA library screening identifies key molecules for extracellular vesicle secretion from microglia

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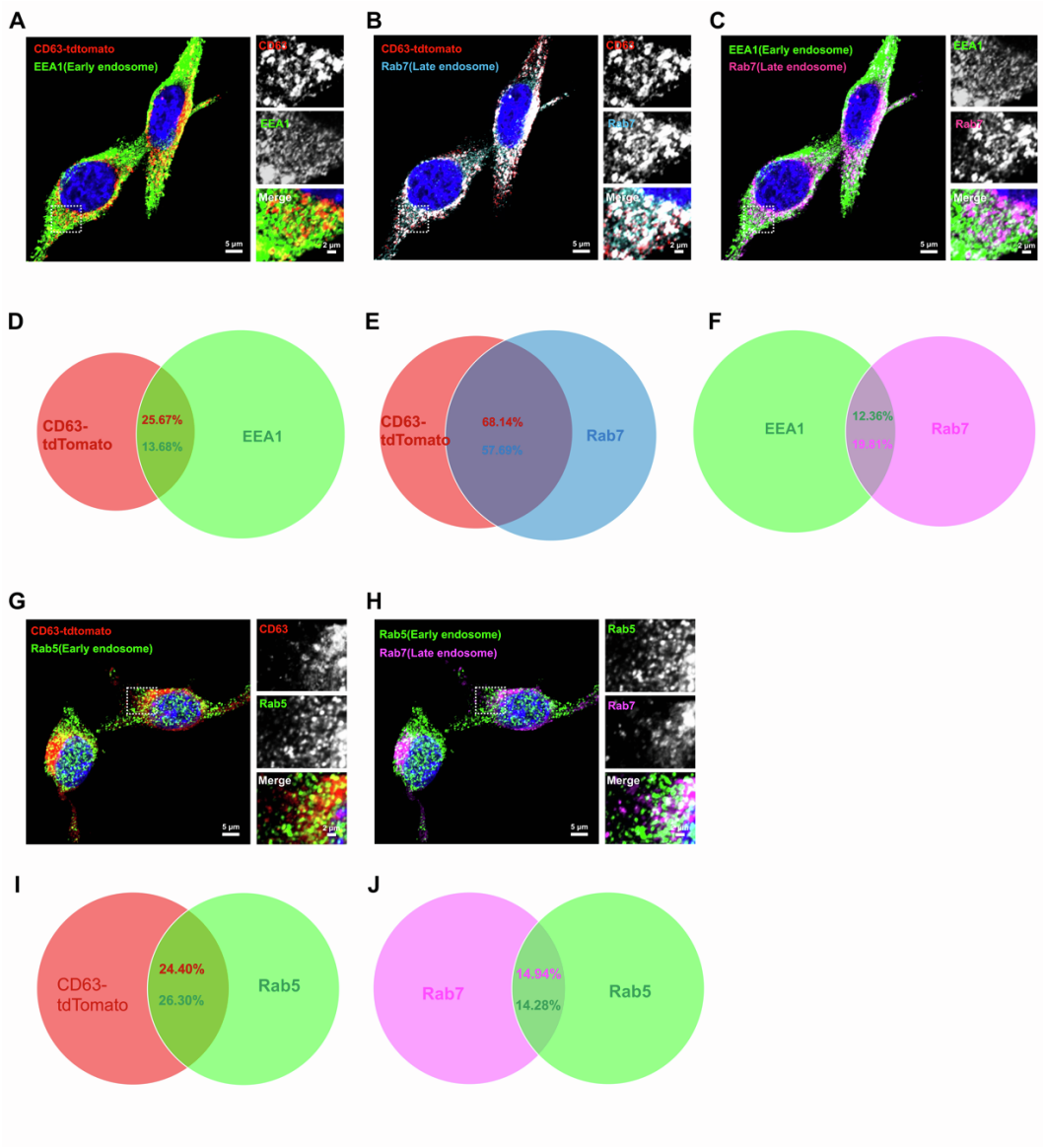
Supplemental Table S1. Number of barcodes and range of sequence frequencies identified in uninfected-, tdTomato^{high}- TurboGFP^{high} cells.

Supplemental Table S2. Functional annotation clusters identified by DAVID bioinformatics resource among hits enriched in cells that resisted ATP stimulation.

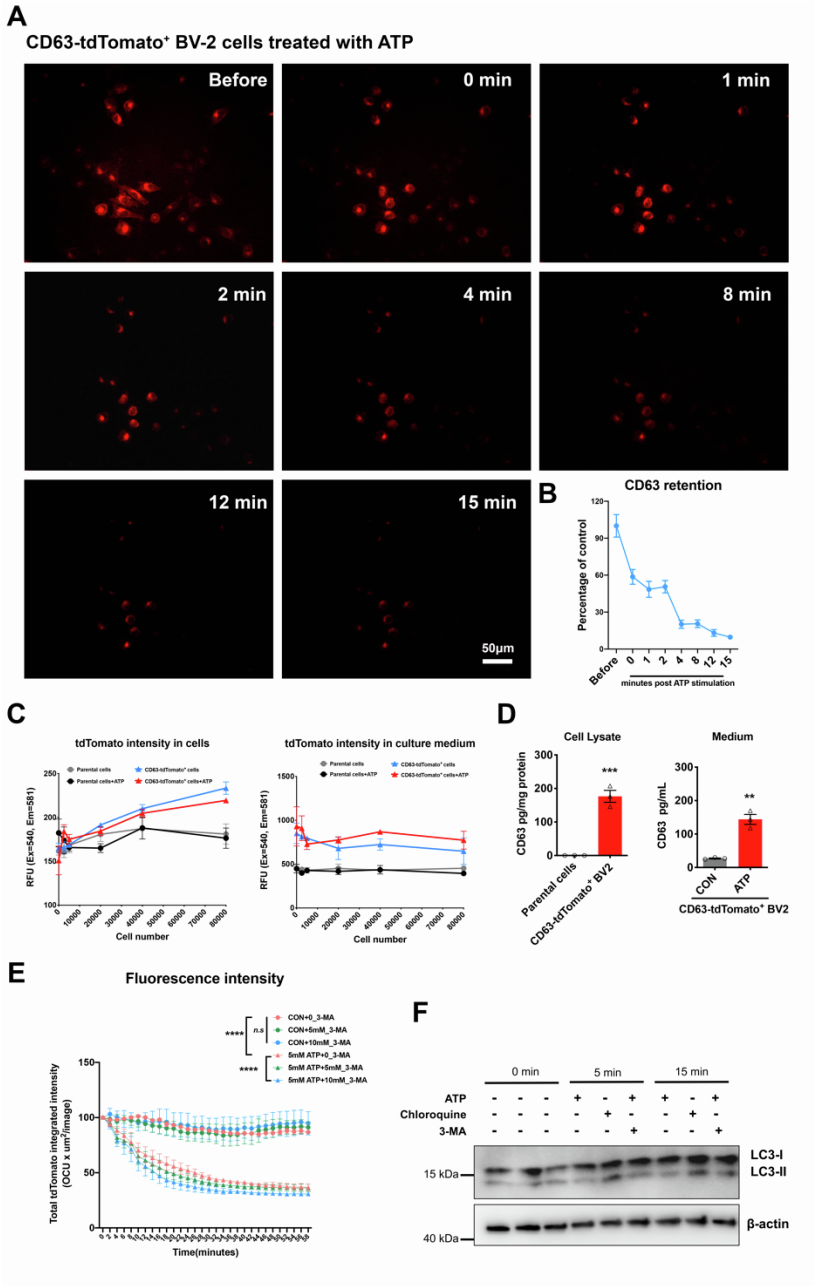
Supplemental Table S3. Hits belonging to the integral component of the membrane and plasma membrane cluster identified with the DAVID bioinformatics database.

Supplemental Table S4. The sequence information of lentivirus packaged shRNAs were used in this study.

Supplemental Table S5. The quantification of the colocalization of CD63-tdtomato and early or late endosome markers.



Supplemental Figure S1. Confocal microscopic imaging of CD63-tdTomato with endosomal markers in tdTomato-CD63$^+$ BV-2 cells. Related to Figure 1. BV-2 cell clone stably expressing CD63-tdTomato (red) is fixed and immunolabeled for EEA1 or Rab5 (early endosomal marker, green) and Rab7 (late endosomal marker, cyan/purple). The fluorescent images were captured using laser-scanning confocal microscopy (Zeiss 880 AiryScan with 63x objective lens and zoom in 2.0).
A, D: CD63-tdTomato co-localizes limitedly with EEA1.
B, E: CD63-tdTomato co-localizes partially with Rab7.
C, F: EEA1 and Rab7 predominantly mark separate organelles.
G, I: CD63-tdTomato co-localizes limitedly with Rab5. H, J: Rab5 and Rab7 predominantly mark separate organelles.
D-F, I, J: Venn diagrams based on co-localization analysis of labeling combinations in A-C, G-H. Circle size is proportional to total dots detected for a protein, overlap to number of co-localized dots. Images were analyzed by dot detection in two or three channels, after which overlapping dots were classified as colocalized particles. Percentages represent the co-localized fraction of the correspondingly colored protein. See methods for a more detailed description of the analysis, see Supplemental Table S5 for standard deviations, cell numbers. Scale bars 5µm in larger images, 2µm in insets. Total 20 cells were analyzed in D-F in and 32 cells in I, J.



Supplemental Figure S2. Fluorescence intensity change in a tdTomato-CD63 BV-2 stable cell line upon ATP stimulation. Related to Figure 1.

(A) Time course of tdTomato-CD63 signal decline. Scale bar: 50μm.

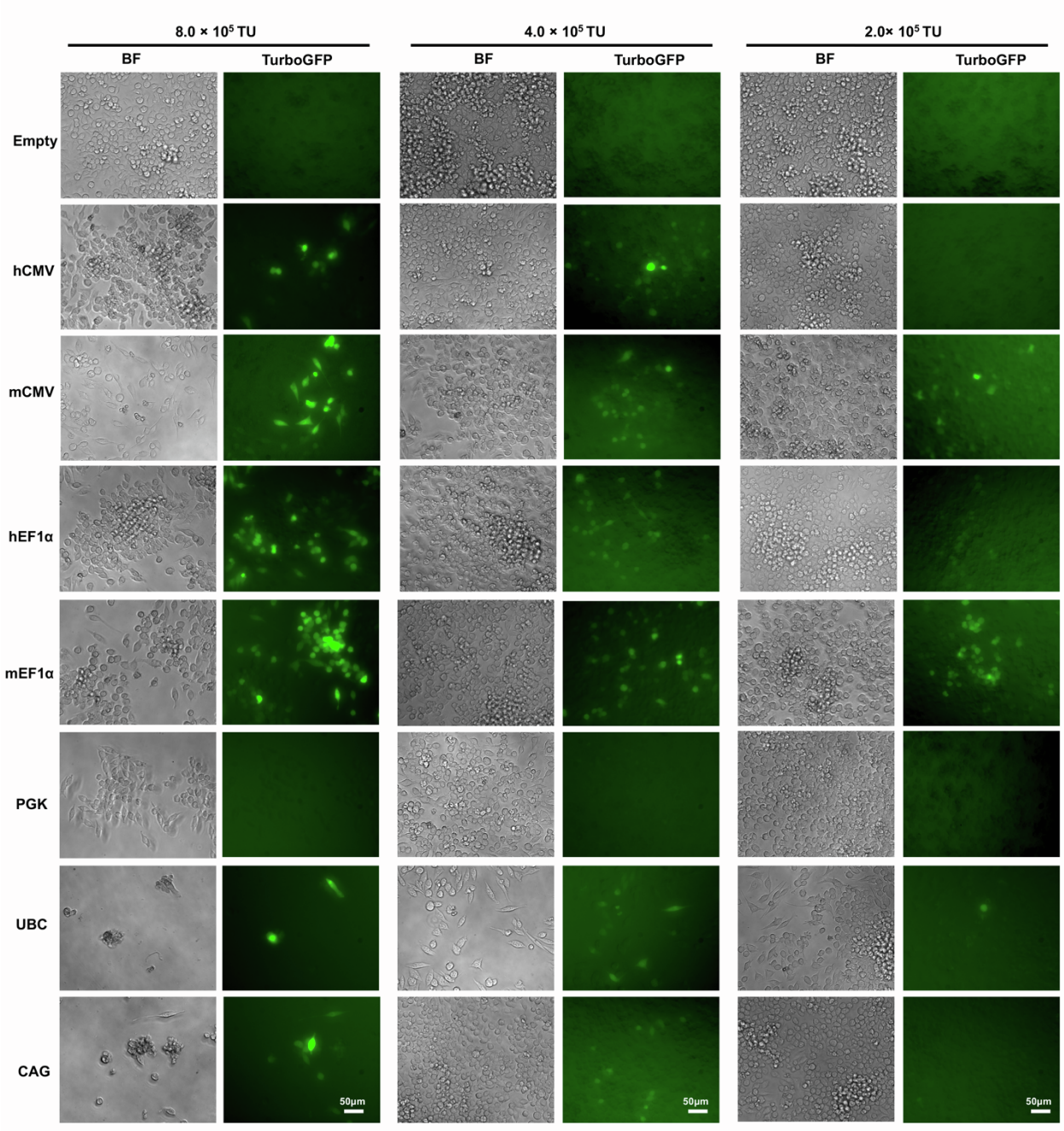
(B) Quantification of tdTomato-CD63 fluorescence signal in the time-course decline. Total 16 cells were analyzed.

(C) Quantification of fluorescence signals in the cell and culture medium with or without ATP stimulation. n=3 independent experiments.

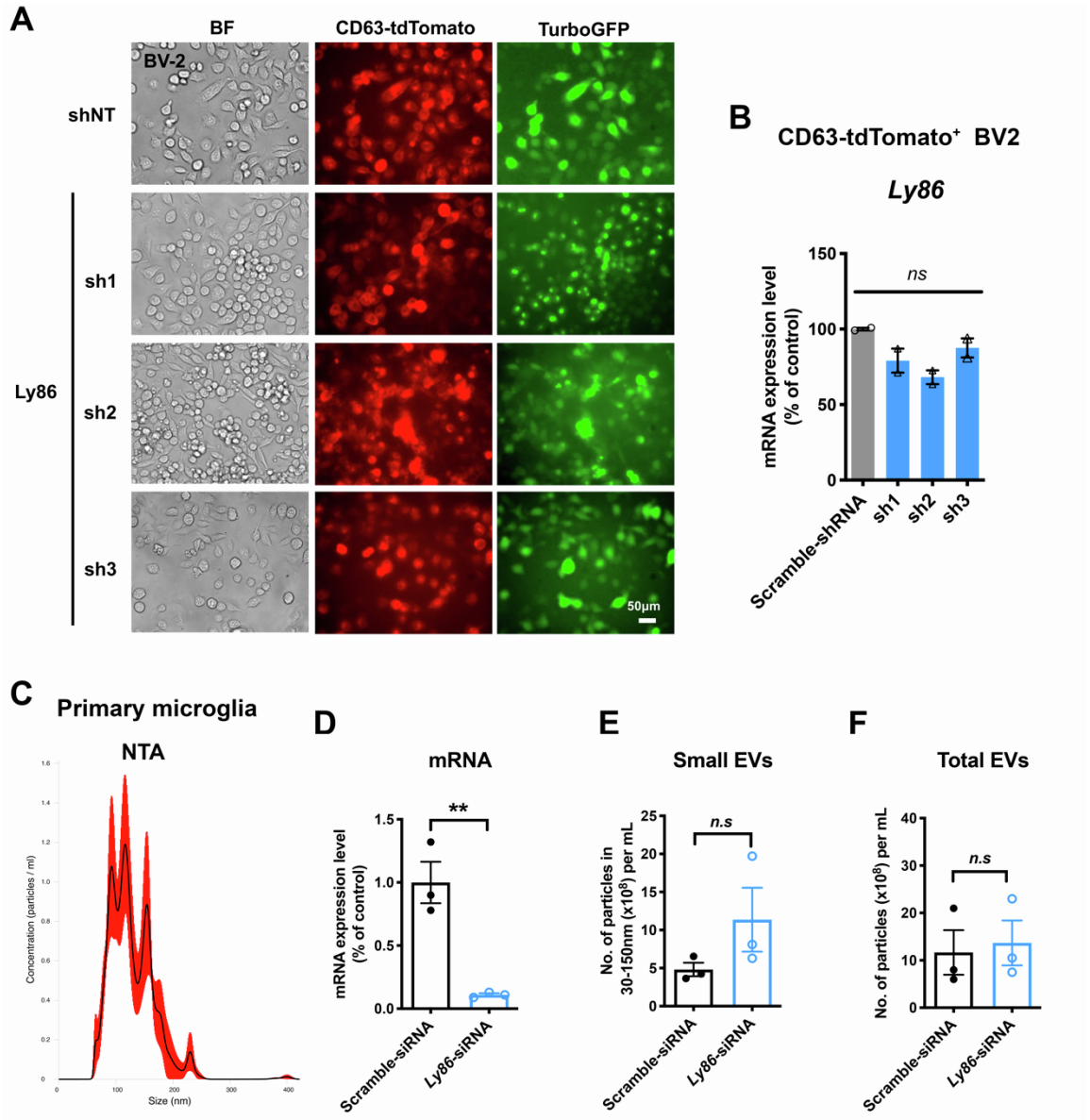
(D) CD63 ELISA analysis of cell lysates and culture medium with or without ATP stimulation. n=3 independent experiments.

(E) Monitoring the fluorescence signal in cells with or without ATP stimulation or treated with the autophagy inhibitor 3-MA for 1 h. n=3 independent experiments.

(F) Representative immunoblots of the typical protein marker LC3 for autophagy in the cell lysate with or without ATP stimulation, or under the condition with the autophagy inhibitor 3-MA.



Supplemental Figure S3. Identifying the optimal promoter for BV-2 cells. Related to Figure 2.
BV-2 cells were cultured in 96-well plate (3,000 cell/well) for 1 d. Then, 30 μL of base medium with 2x Polybrene and serum was added to a Virus plate (25 μL/well), then added to BV-2 cell culture medium. Cells were incubated with a gradient concentration (0.25×10^5–8.0×10^5 TU) of lentivirus overnight, and supplemented with growth medium (100 μL). Cells were visually inspected and TurboGFP fluorescence intensity was assessed daily for 48 to 96 h. Promotor mEF1α showed the strongest activity in the BV-2 cell line.

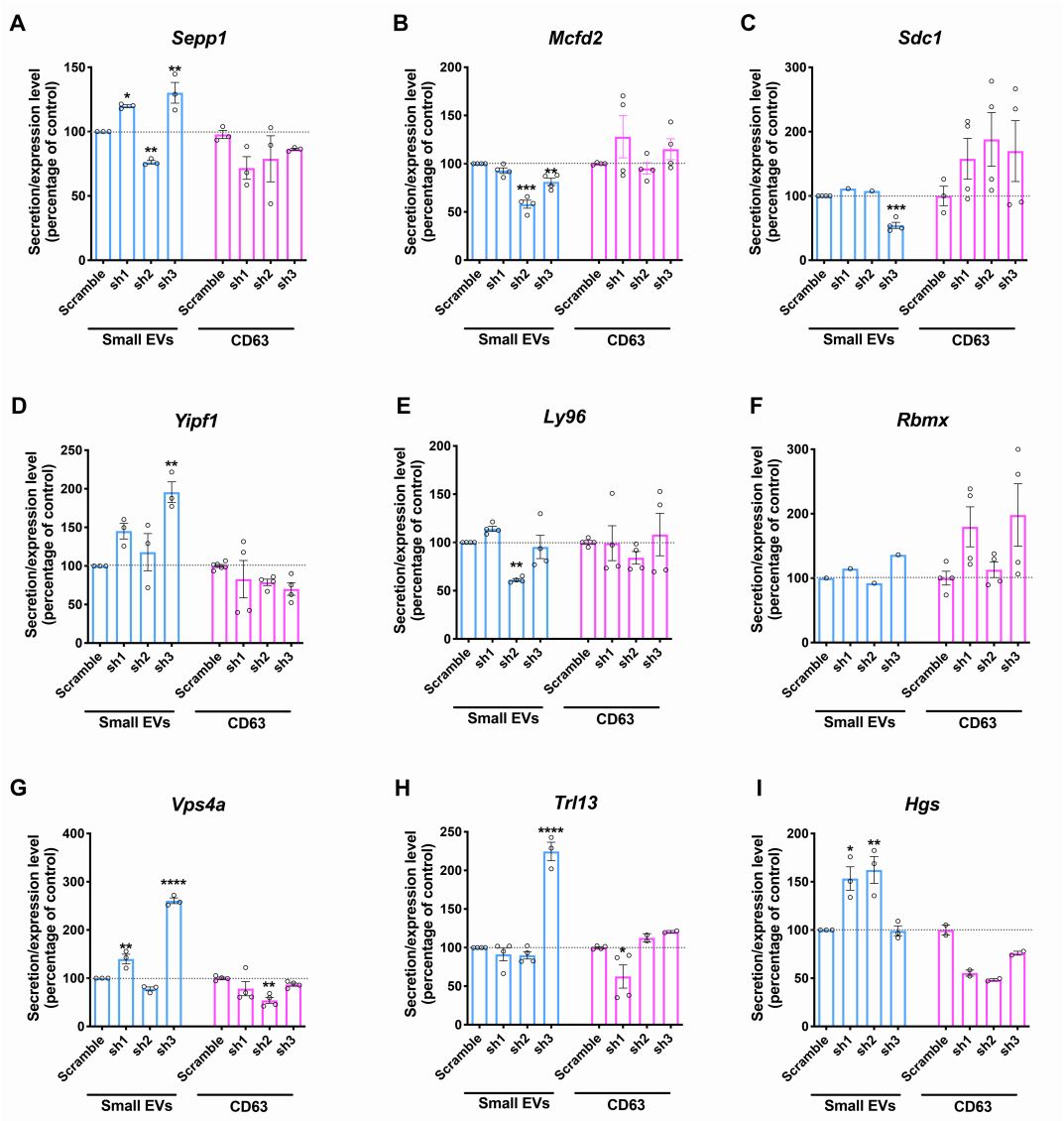


Supplementary Figure S4. Knockdown of Ly86 in primary microglia but not in BV-2 cells. Related to Figures 4 and 5.

(A) CD63-tdTomato+ BV-2 cells were transfected with three lentivirus packaged shRNAs targeting Ly86.

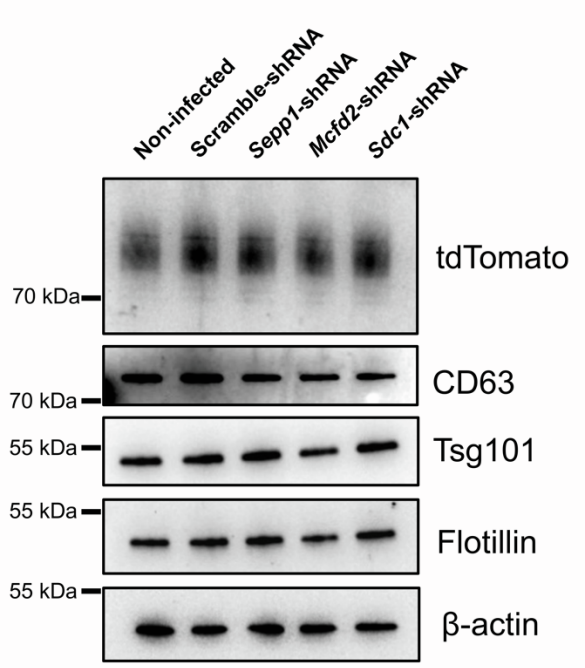
(B) mRNA levels of Ly86 in BV-2 cells after transfection with shRNAs. n=2 independent experiments.

(C-E) Particle distribution(C), specific mRNA levels(D), small EVs(E), and total EVs (F) secretion in primary microglia transduced with Ly86 siRNAs. n=3 independent experiments.

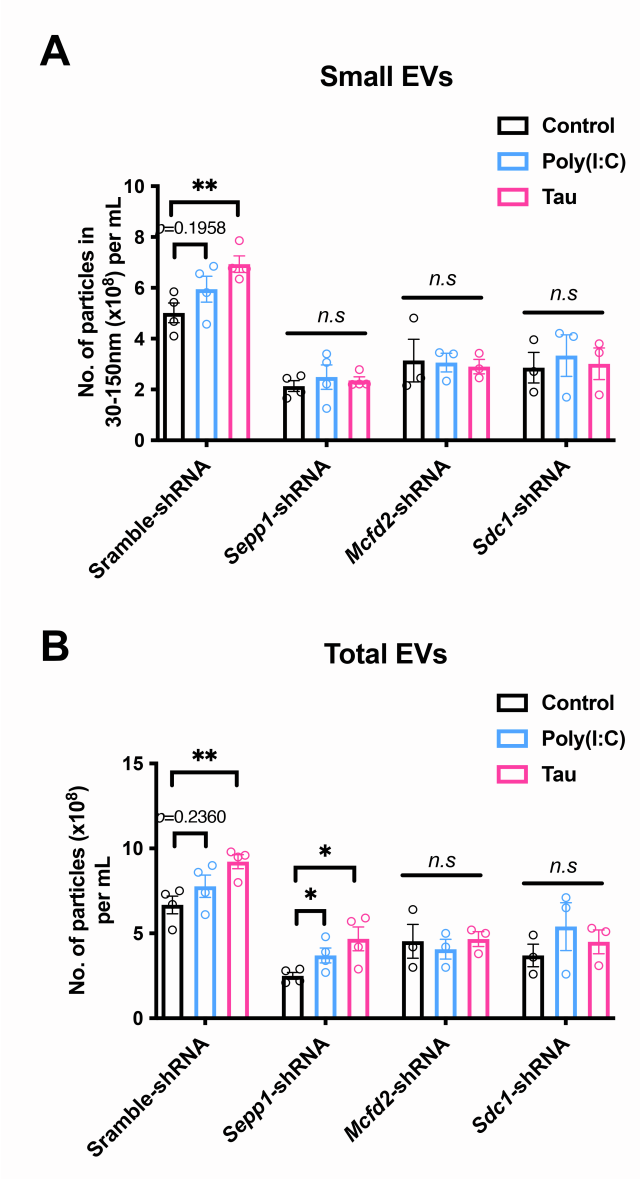


Supplementary Figure S5. Limit effect of potential hits on EV release under physiological condition. Related to Figure 4.

(A-I) Small EVs and CD63 secretion in cells transduced with lentiviral shRNA clones targeting *Sepp1* (A), *Mcfd2* (B), *Sdc1* (C), *Yipf1* (D), *Ly96* (E), *Rbmx* (F), *Vps4a* (G), *Tlr13* (H), or *Hgs* (I). $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ and $^{****}p < 0.0001$ compared with scramble-shRNA group, as determined by one-way ANOVA (alpha = 0.05) and Tukey's *post-hoc*; n=3-4 independent experiments, graphs indicate mean ± s.e.m.



Supplementary Figure S6. Immunoblotting of EV markers in the EV fraction isolated from culture conditional medium in lentiviral shRNA infected cell clones. Related to Figure 4. tdTomato, CD63, Tsg101, flotillin and b-actin was tested by immunoblotting using qEV-isolated EV fraction from clonal cell lines after lentiviral infection of shRNA targeting scramble, *Sepp1, Mcfd2* or *Sdc1* and single cell sorting.



Supplementary Figure S7. The effect of silencing target genes on EV release after stimulation of gene-silenced BV-2 reporter cells with (poly(I:C) or tau aggregates. Related to Figure 6. Quantification of secreted small EVs (A) and total EVs (B) after treatment of gene-silenced BV-2 reporter cells with 20 µg/mL high molecular weight poly(I:C) or 10 µg/mL tau aggregates. Two-tailed *t*-test, $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$, *ns*, no statistical significance, n=3-4 independent experiments.