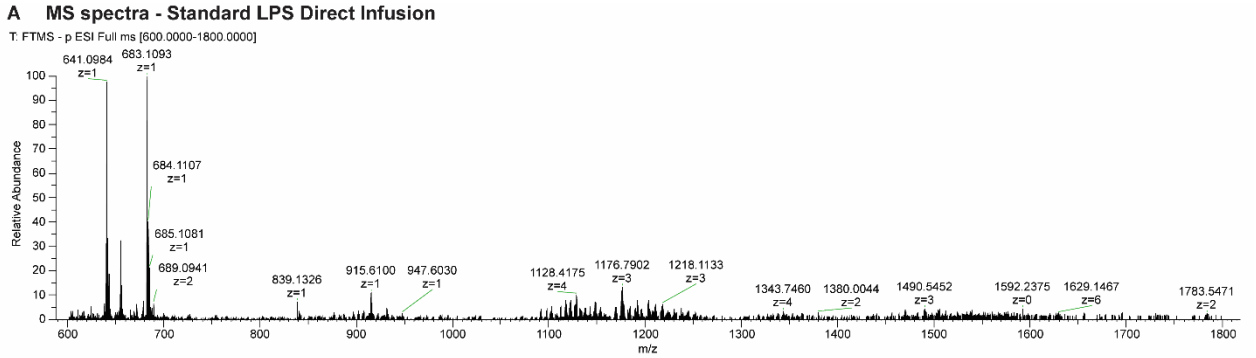
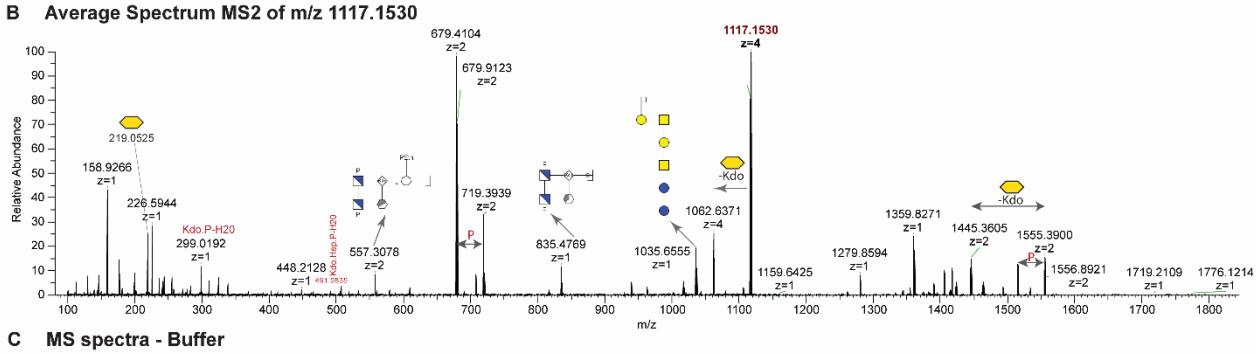
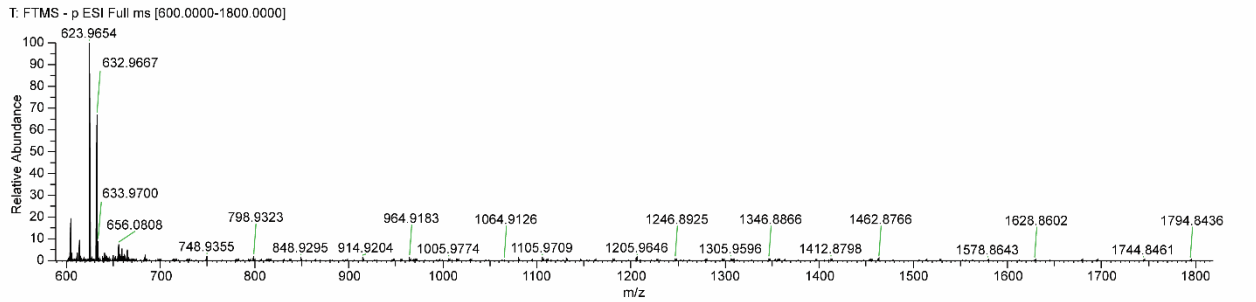
# Appendix

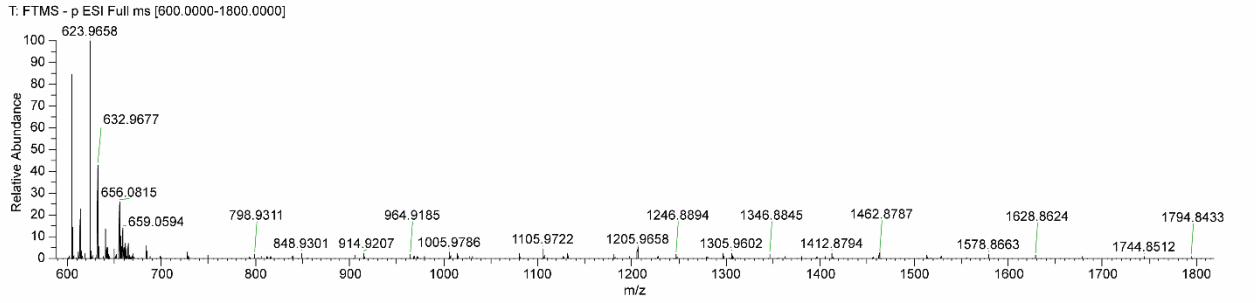
*Streptococcus pyogenes* EVs induce the alternative inflammasome via Caspase-4/-5 in human monocytes

**Table of Content**



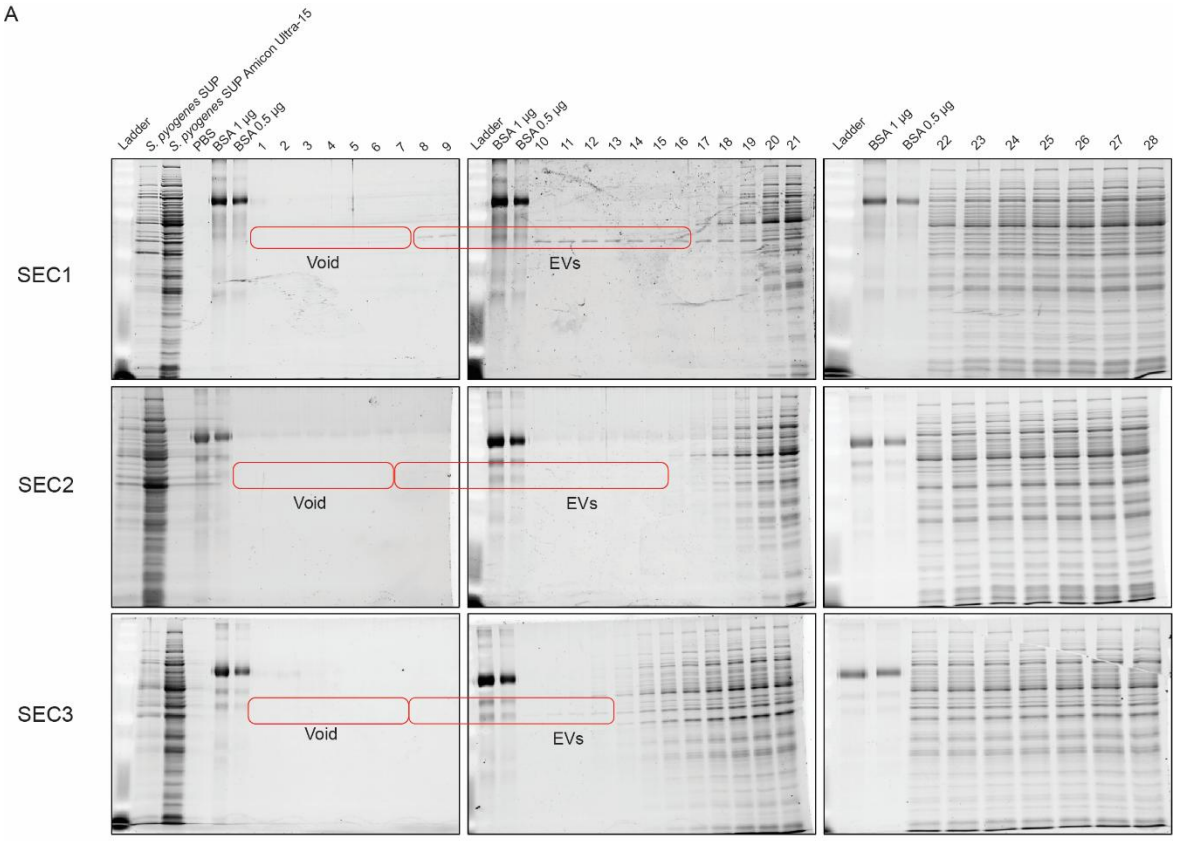



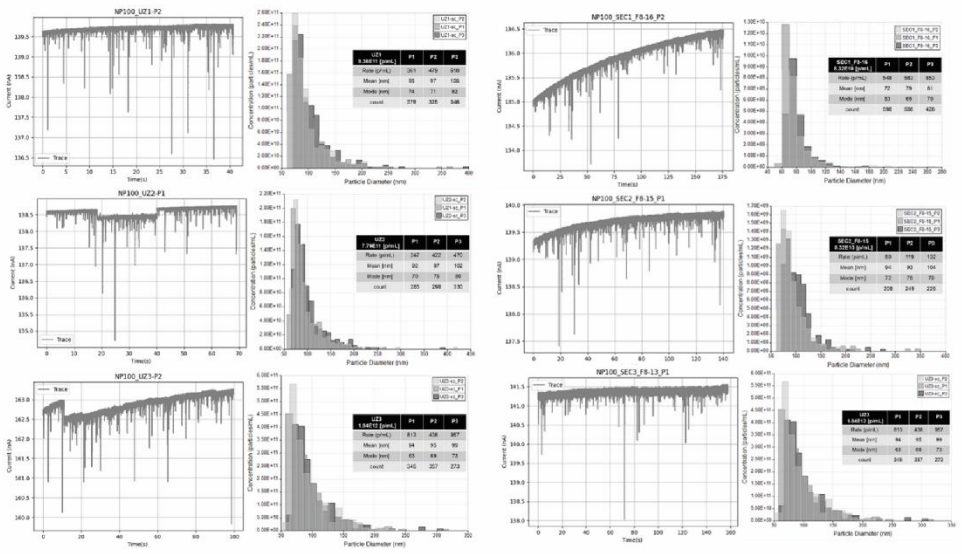

| Mass to charge | Ion (proposed composition) | Type |
|---|---|---|
| 143.0360414 | Z-Hex1-B | BZ |
| 219.0075776 | KDO1-B | B |
| 708.4134473 | ZY-HexN1KDO2P1 | ZY |
| 835.4769324 | ^{3,5}XY-HexN2KDO1P2 | ^{3,5}X_{KDO}Y |
| 939.2908211 | ^{2,5}XZ-HexN2KDO1P2 | ^{2,5}X_{KDO}Z |

**Appendix Figure S1. Direct Infusion ESI HCD MS/MS analysis. (A)** MS-full scan from *E. coli* 055:B5 LPS in positive ionization mode. **(B)** MS/MS scan of precursor m/z 117.1530. The low m/z region of the HCD MS/MS spectra corresponds to the LPS bearing Kdo (m/z 219.05) and phosphates (m/z 290.01). Other ions corresponding to 055:B5 LPS structure are indicated in the MS/MS spectra with proposed composition. Additional annotations are provided in the table below. **(C)** MS-full scan of buffer used for the isolation of bacterial EVs and **(D)** bacterial EV supernatant.

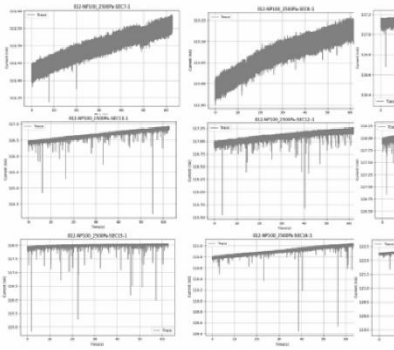
A




B

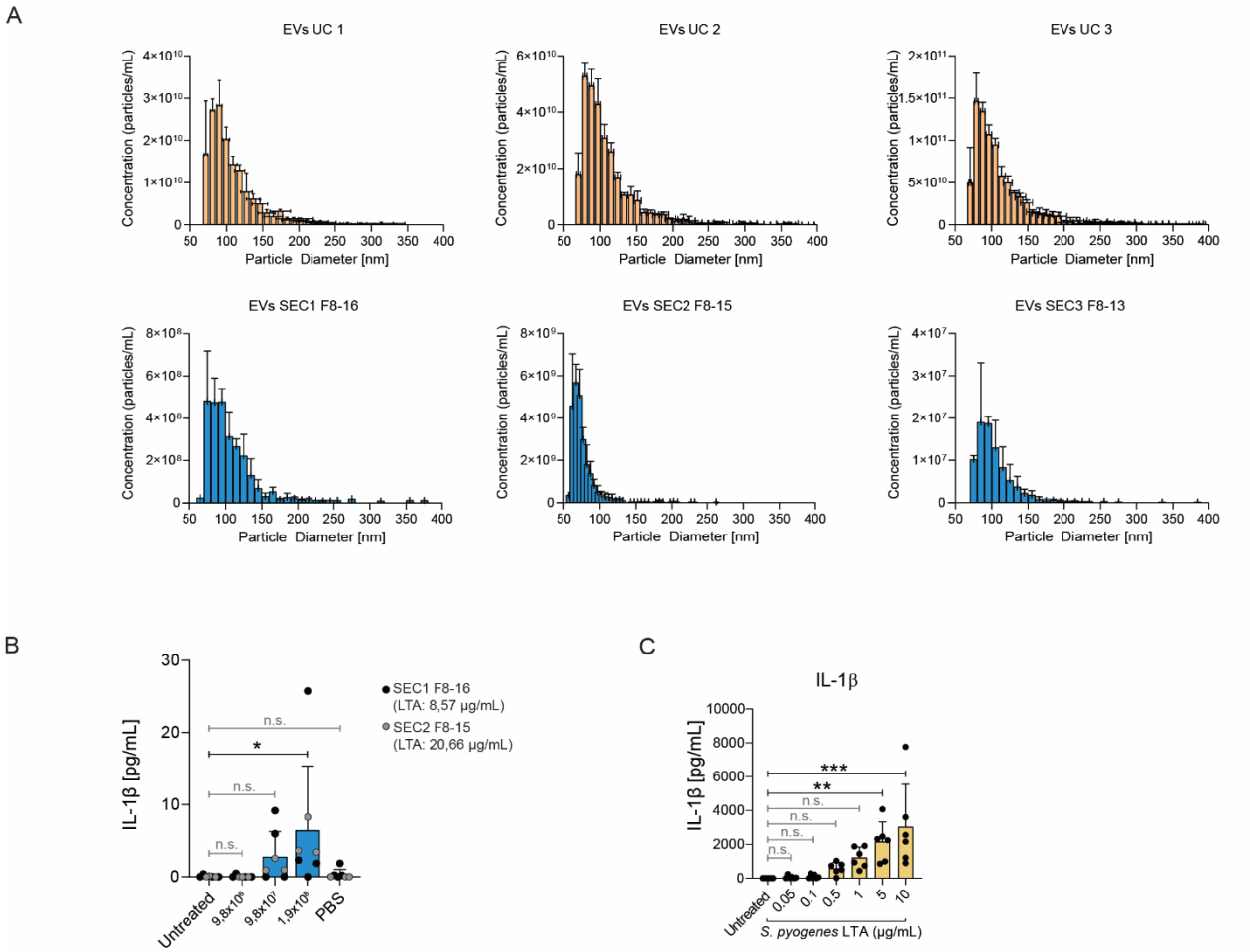



C



| SEC_indivdual fractions | Rate (p/mL) | Mean [nm] | Mode [nm] | count | Conc [p/mL] |
|---|---|---|---|---|---|
| F7 | 1.9 | NaN | NaN | 2 | NaN |
| F8 | 1.9 | NaN | NaN | 2 | NaN |
| F9 | 56 | 78 | 71 | 56 | 5.3E09 |
| F10 | 75 | 79 | 70 | 79 | 7.5E09 |
| F11 | 61 | 79 | 63 | 64 | 6.1E09 |
| F12 | 242 | 85 | 67 | 253 | 2.4E10 |
| F13 | 72 | 88 | 103 | 103 | 3.2E10 |
| F14 | 383 | 85 | 68 | 402 | 1.1E11 |
| F15 | 339 | 85 | 67 | 354 | 1.0E11 |
| F16 | 62 | 87 | 72 | 65 | 1.8E10 |
| F17 | 173 | 89 | 71 | 182 | 5.1E10 |
| F18 | 282 | 93 | 82 | 296 | 3.4E9 |

**Appendix Figure S2. *Spy* EV isolation using Size exclusion chromatography (SEC). (A)** SYPRO Ruby stained polyacrylamide gels (12%) of 28 SEC fractions eluted from ultrafiltrated *S. pyogenes* culture supernatants (SUP, Amicon Ultra-15 concentrate). **(B)** UC- and pooled SEC-EV-quantification with TPRS/Exoid. Plots of representative blockage events over time, termed traces with size & concentration histograms and quantification results inscribed in respective tables (UC-EVs on the left, pooled SEC-EVs on the right). Note that with the Exoid, traces are acquired at 3 different pressures (P1,2,3). P1 is 200Pa lower than P2. P3 is 300Pa higher than P2 in one duty cycle. Appropriately diluted samples were measured with a NP100 and 47mm stretch and CPC100 calibration particles. **(C)** Characterization of Individual SEC-fractions. Trace-plots of individual SEC-fractions at one pressure (2500Pa, 1200mV, 60sec on NP100) with available quantification results summarized in a table.




**Appendix Figure S3. SEC EV quantification and IL-1β response (A)** Size and concentration of particles present in EV UC as well as SEC EV batches. Bars represent the mean ±SD of three dilutions measured. **(B)** IL-1β released by human monocytes in response to *Spy* EVs isolated using SEC. Bars represent the mean ±SD of seven biological replicates with pooled EV fractions from 2 independent SECs (black circles: SEC1 F8-16; grey circles: SEC2 F8-15). *P=0.0354.* **(C)** IL-1β released by human monocytes stimulated with increasing amounts of *Spy* LTA for 18 h. Bars represent the mean ±SD of six biological replicates. *P=0.0073* (5 µg/mL)*, P=0.0002* (10 µg/mL). **Data information:** (BC) One-way ANOVA was applied with Holm-Šídák correction for multiple comparisons. *p≤0.05, **p≤0.01, ***p≤0.001, n.s. not significant