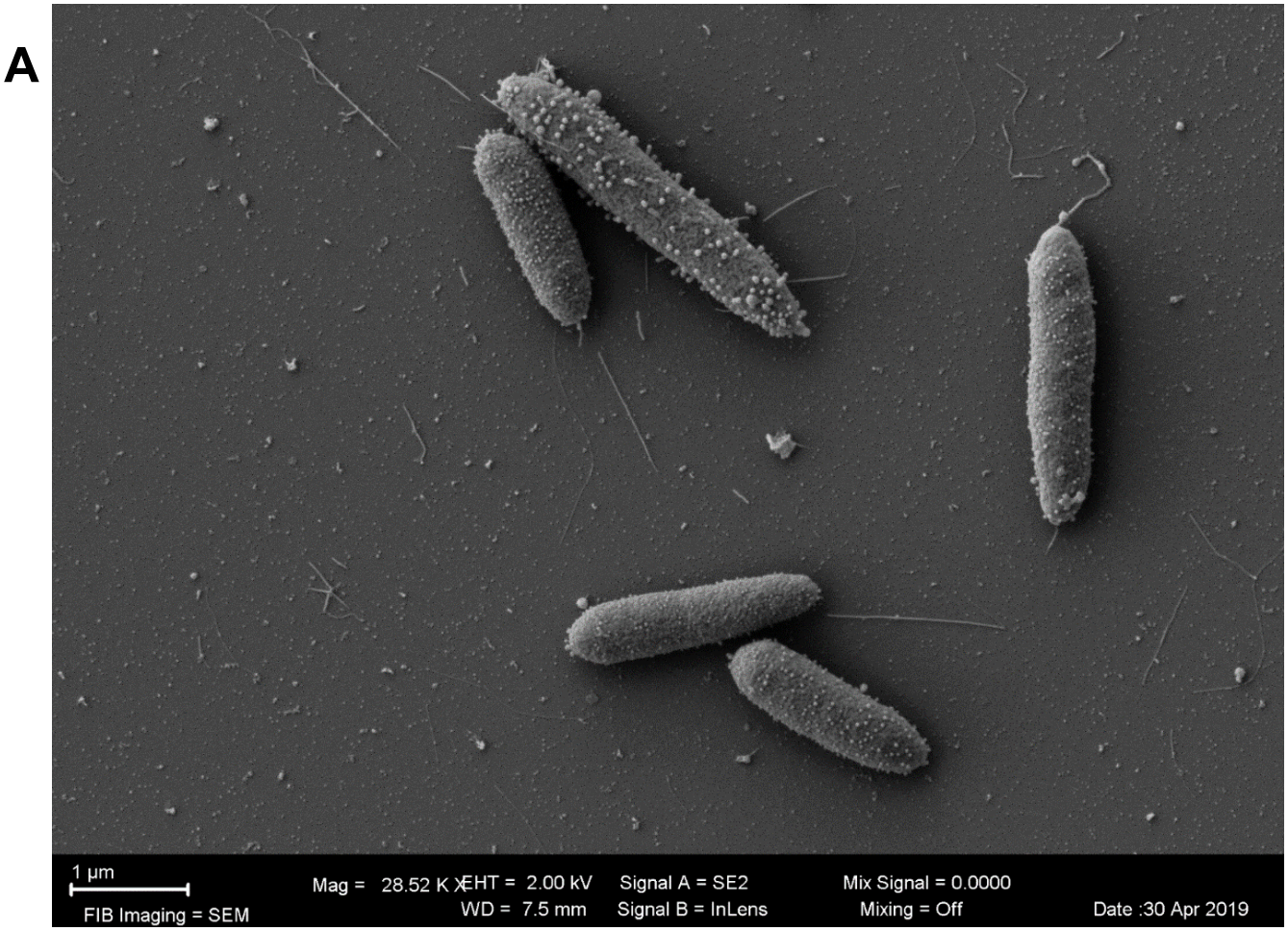
Figure S1



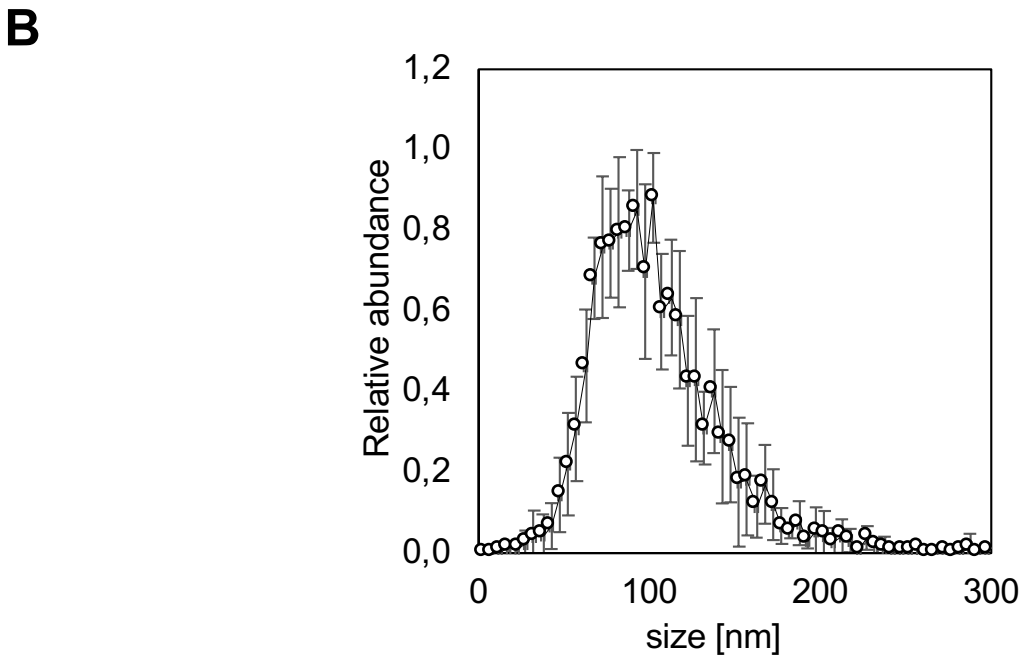


Figure S1. A) The full-size SEM micrograph used in Fig. 1A of *Pto* DC3000 growth in planktonic culture (OD_{600} = 3-4). **B)** Size profile of EVs from *Pto* DC3000 planktonic cultures in fluid samples (OD_{600} = 7.5-11).

Figure S2

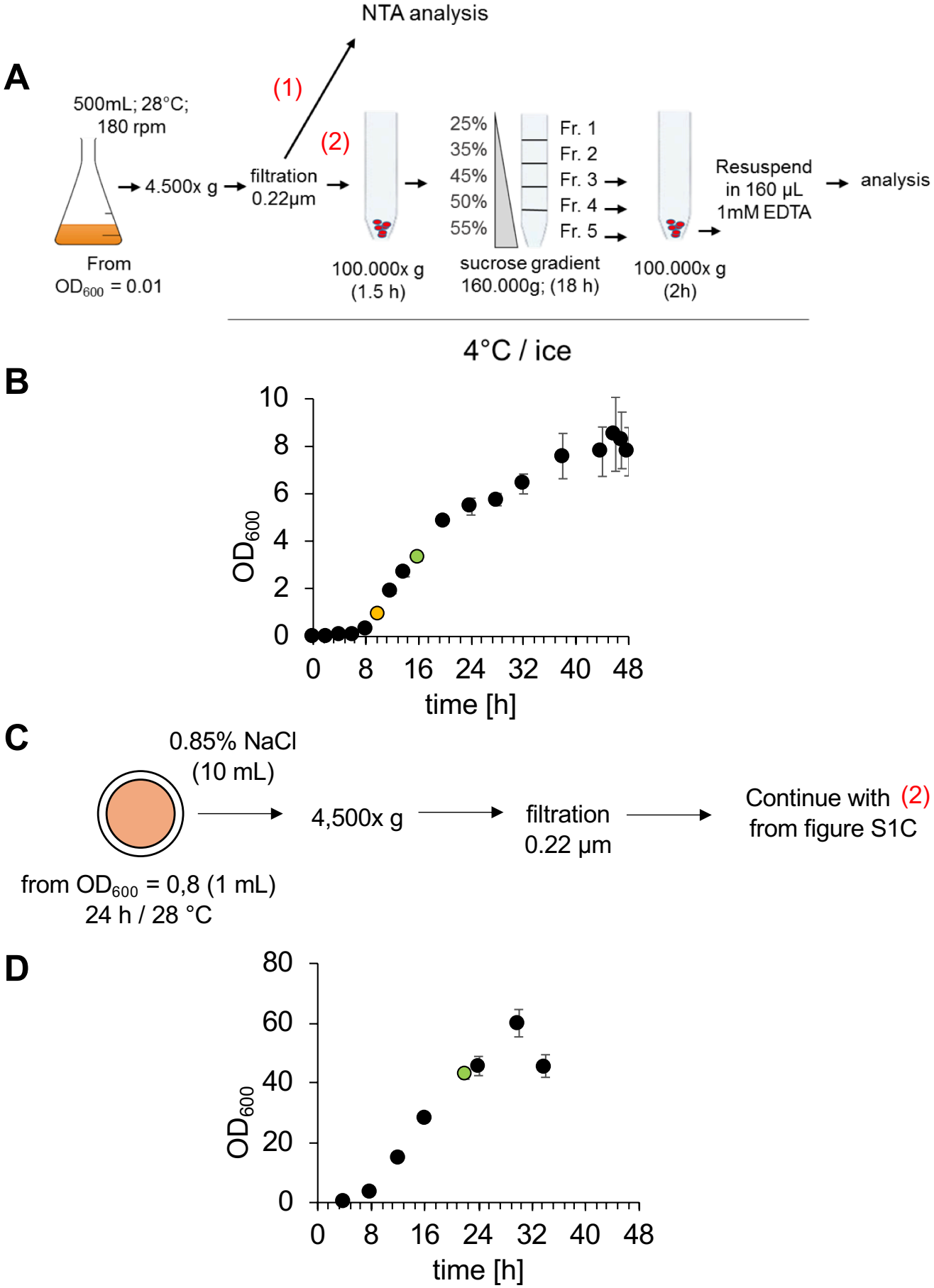


Figure S2. Isolation of *Pto* DC3000 EVs. A) Schematic overview of EVs isolation from planktonic cultures for fluid sample (1) and gradient enriched sample (2) analysis. **B)** Growth measurements of planktonic *Pto* DC3000 cultures. Orange indicates EV isolation from early exponential growth stages (OD_{600} = 1-2); green indicates EV isolation from late exponential growth stages (OD_{600} = 3-4). **C)** Schematic overview of EVs isolation from biofilm cultures. **D)** Growth measurements of biofilm *Pto* DC3000 cultures. The green dot represents the growth stage from which the bacteria were used for experiments.

Figure S3

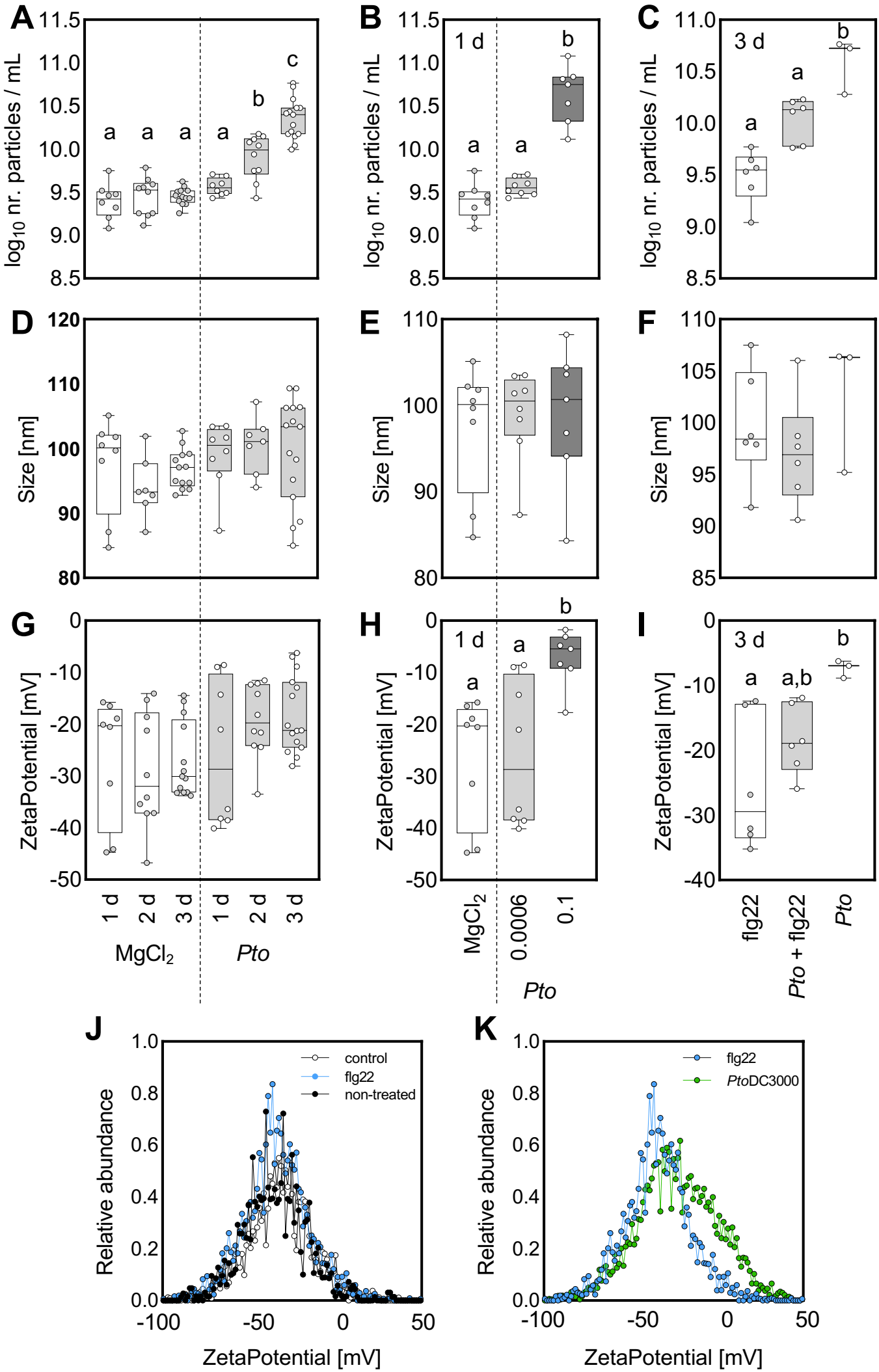

A
B
C
D
E
F
G
H
I
J
K
log10 nr. particles / mL
Size [nm]
ZetaPotential [mV]
1 d
2 d
3 d
MgCl2
Pto
0.0006
0.1
flg22
Pto + flg22
Relative abundance
control
flg22
non-treated
PtoDC3000

Figure S3. Biophysical parameters of particles in apoplastic fluids from *A. thaliana* plants infected with *Pto* DC3000. A, D, G) Particle parameters over days post infection (dpi). **B, E, H)** Particle parameters in response to inoculation with different *Pto* DC3000 densities. **C, F, I)** Particle parameters in response to inoculation with different *Pto* DC3000 and co-treatment with flg22. Each dot represents value of independent samples for size and ζ-potential it represents median. 3-12 independent samples were used for each experiment. **J, K)** The profile of ζ-potential for each particle collected from apoplastic fluids of plants treated as indicated and gradient enriched EVs. Control = 0.2 mM EDTA; flg22 = 100 nM; n.t. = not treated; *Pto* DC3000 OD_{600} = 0.0006. Each treatment was 3 days long. The dots represent the mean across the ζ-potential values from independent samples: n = 8 (control); n = 10 (*Pto* DC3000); n = 6 (flg22); n = 4 (non-treatment).

Figure S4

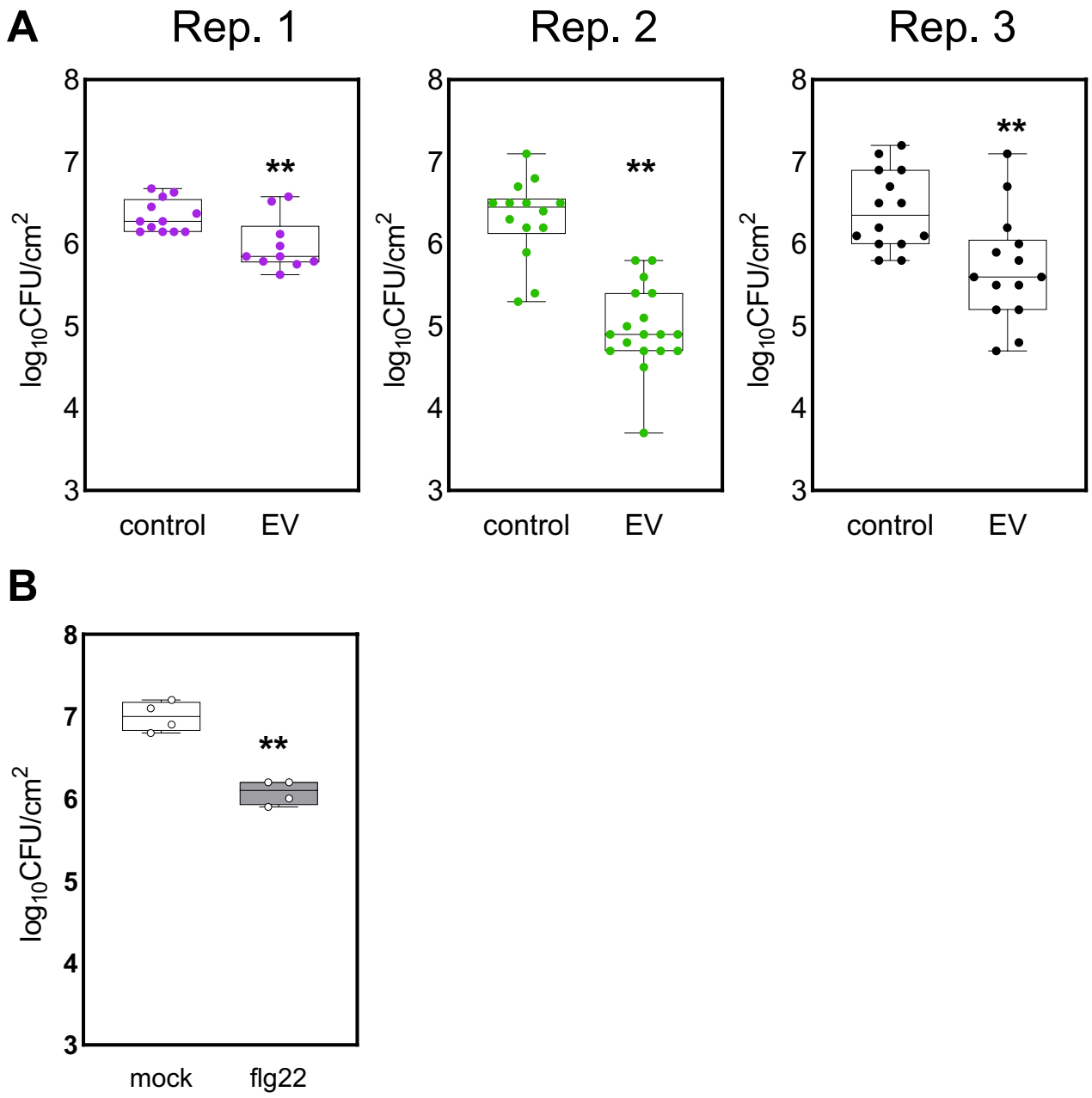


Figure S4. Pre-treatment with *Pto* DC3000 EVs induces resistance against subsequent *Pto* DC3000 infection. A) Three individual biological repeats of *Pto* DC3000 growth (CFU) after infection into leaves of *A. thaliana* without and with EV pre-treatment at 3 dpi (control = 0.02 mM EDTA). Each biological repeat consists of 12 independent samples. **B)** *Pto* DC3000 growth (CFU) after infection (3 dpi) into leaves of *A. thaliana* without and with 100 nM flg22 1 day pre-treatment (mock = 10 mM $MgCl_2$) n = 4. Asterisks represent the statistical difference between the treated and control samples (two tailed Student t-test $p < 0.01$).

Figure S5

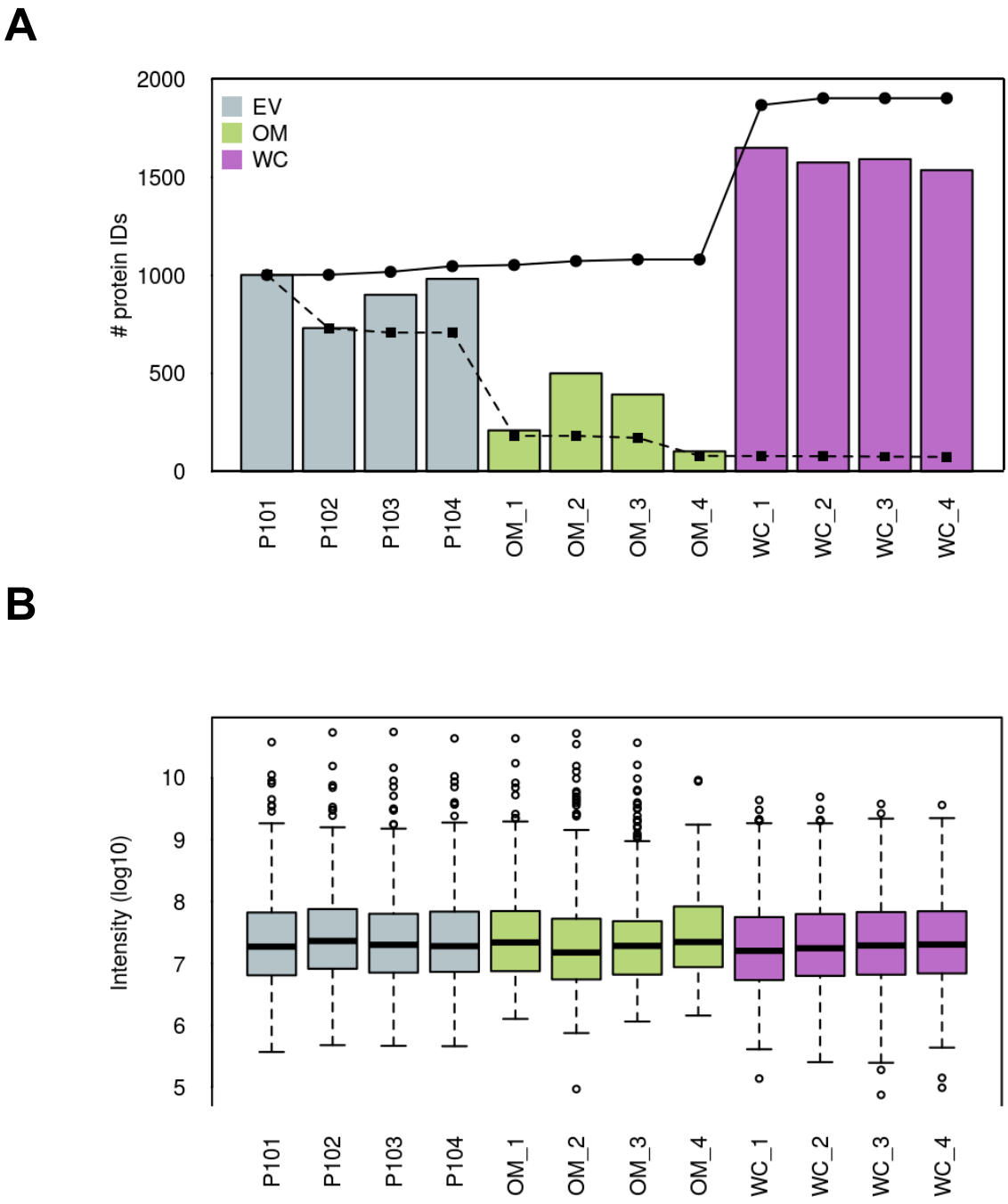


Figure S5. Characteristics of the proteomic analysis. A) Barplot shows the number of identified proteins in each replicate. The solid line indicates the cumulative protein IDs and dashed line shows the shared protein IDs. **B)** Boxplot shows a comparable distribution of protein intensities from each replicate.

Figure S6

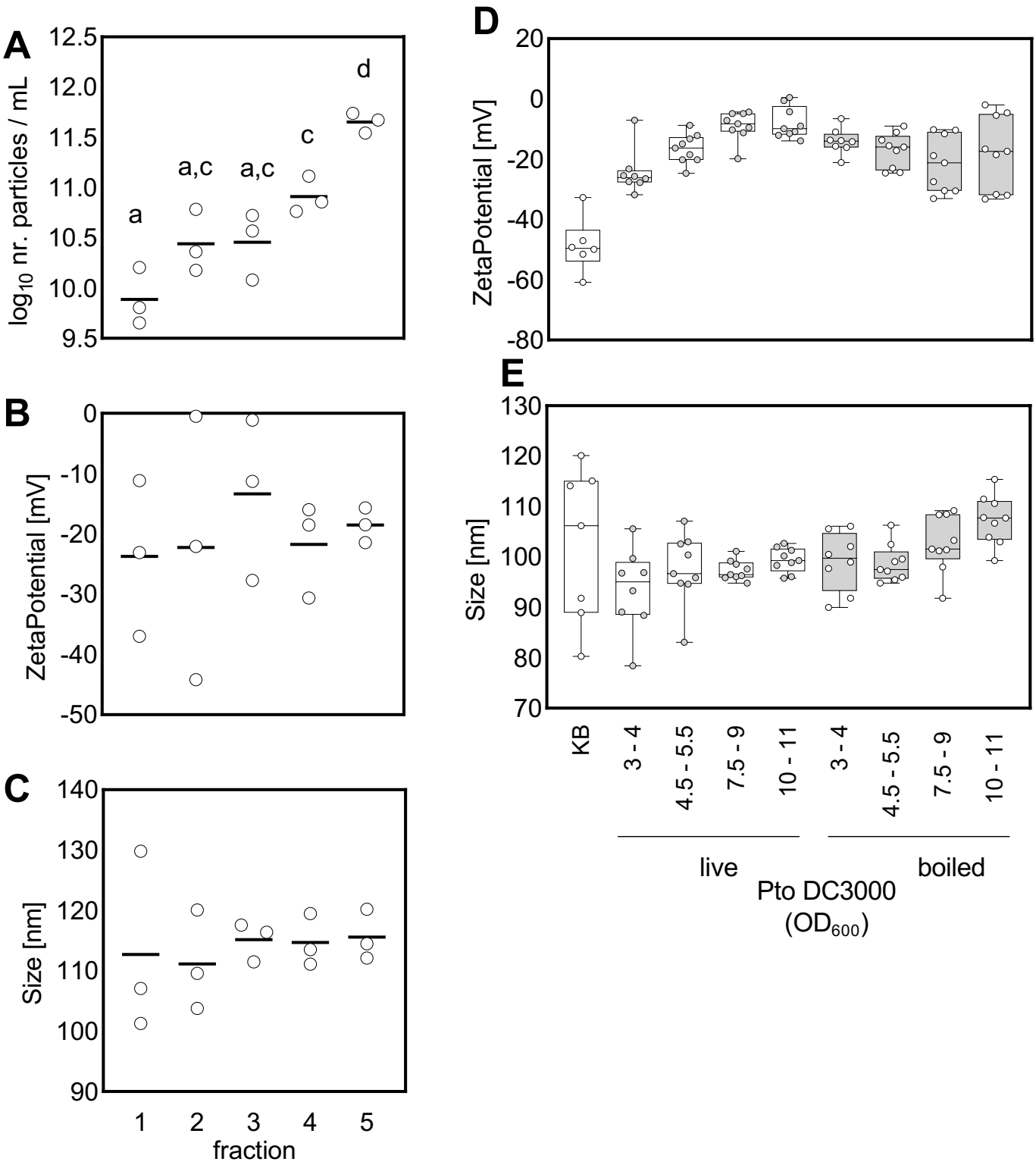


Figure S6. Biophysical parameters of *Pto* DC3000 EVs across isolation methods. A-C) NTA measurements of particle concentration (A), ζ-potential (B) and size (C) of *Pto* DC3000 EVs collected from each step of gradient enrichment. **D-E)** NTA analysis of particle ζ-potential (D) and size (E) of *Pto* DC3000 EVs from fluid samples before (live) and after boiling. Each dot represents an independent sample.