Electronic Supplementary Material (ESI) for Molecular BioSystems.
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Supporting Information

Staphylococcus aureus Extracellular Vesicles: Surface-Binding Antagonists of Biofilm Formation

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Table S1. Organisms used in this study.

Strain	Designation
Enterococcus faecium **(E.f.)**	**ATCC 19434/KACC 11954**
Staphylococcus aureus **(S.a.)**	**ATCC 25923**
Klebsiella pneumoniae **(K.p.)**	**ATCC 20755/KACC 1954**
Acinetobacter baumannii **(A.b.)**	**Clinical Isolate**
Pseudomonas aeruginosa **(P.a.)**	**PAO1**
E. coli **(E.c.)**	**MG1655**

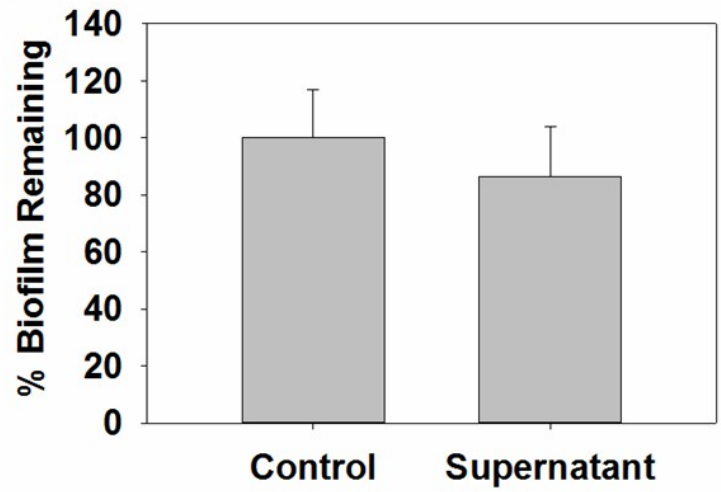

Figure S1. Impact of the *S. aureus* supernatant on preformed biofilms of an *A. baumannii* clinical isolate. Preformed *A. baumannii* biofilms were not removed when incubated with the *S. aureus* supernatant for 24 h.

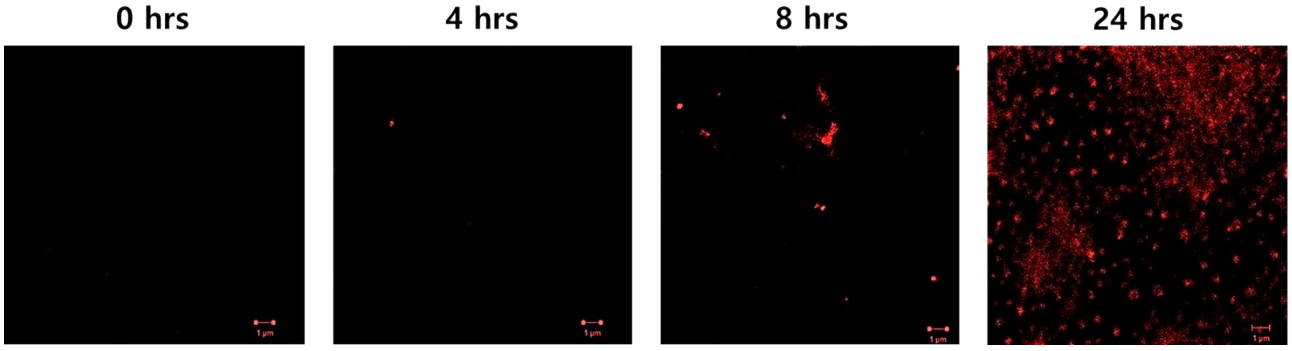

Figure S2. Confocal image showing the presence of cell debris and EVs on the polystyrene surface after exposure to the *S. aureus* ATCC 25923 supernatants. The supernatants were taken at the times listed from growing cultures of *S. aureus* ATCC 25923, as described in Figure 2.

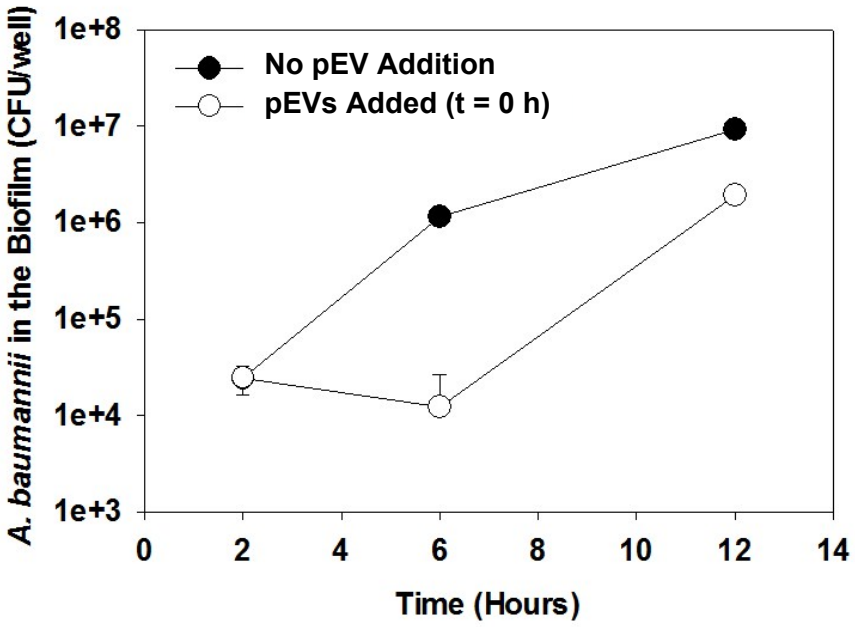

Figure S3. Impact of *S. aureus* pEVs on the number of surface-attached *A. baumannii* cells. Both the pEVs (12.5 µg/ml) and *A. baumannii* were mixed together at 0 h. The resulting *A. baumannii* population attached to the polystyrene surfaces was determined after 2, 6 and 12 hours. For comparison, a control *A. baumannii* culture without pEV addition was also evaluated. The presence of the pEVs led to a clear and significant reduction in the number of *A. baumannii* attached to the surface after 6 h when compared with the control, demonstrating that the pEVs act to inhibit the establishment of this pathogen on the surface.

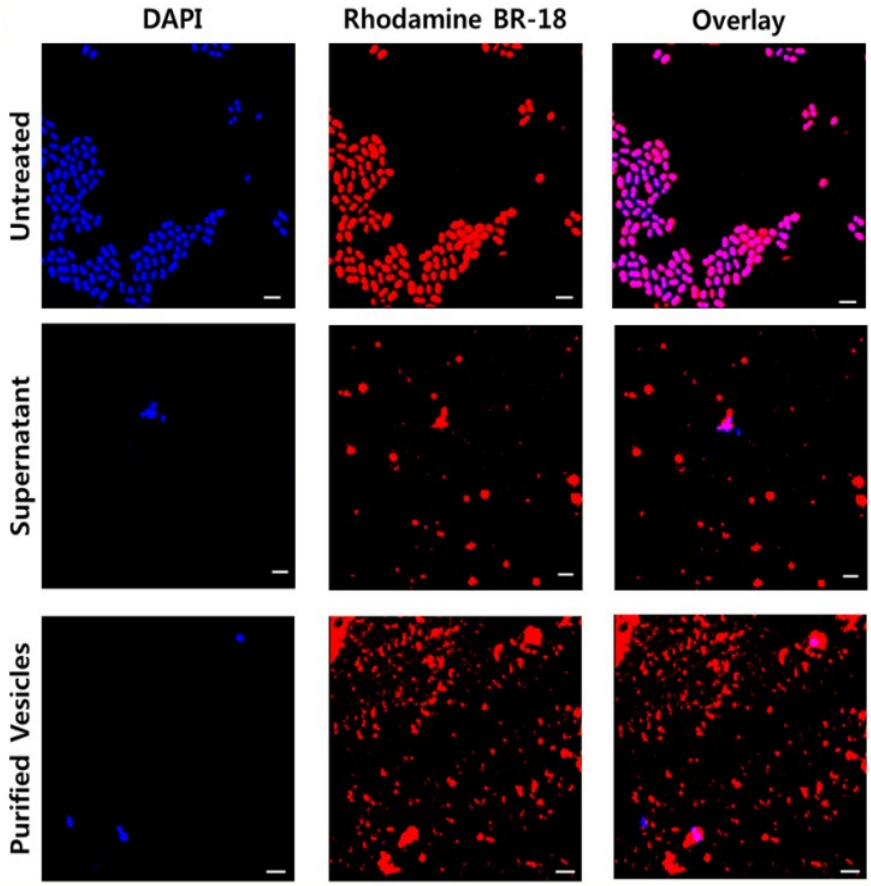

Figure S4. Confocal images showing *A. baumannii* bacterial cells (blue) on the polystyrene substrate surface using DAPI-labeling of the cellular DNA. Rhodamine BR-18 labels all of the cellular membranes red, including those of *A. baumannii* and those within the *S. aureus* EVs. Within the overlay images, the EVs appear as red while the *A. baumannii* are visible as violet. Each of these surfaces were treated as listed on the left, *i.e.*, untreated, with filter-sterilized supernatant from an overnight culture of *S. aureus* or with 12.5 μg/ml purified EVs in HEPES buffer. Treatments were performed for one hour. Note the significantly lower presence of *A. baumannii* when the surface was covered by EVs. The scale bar is 2 μm.

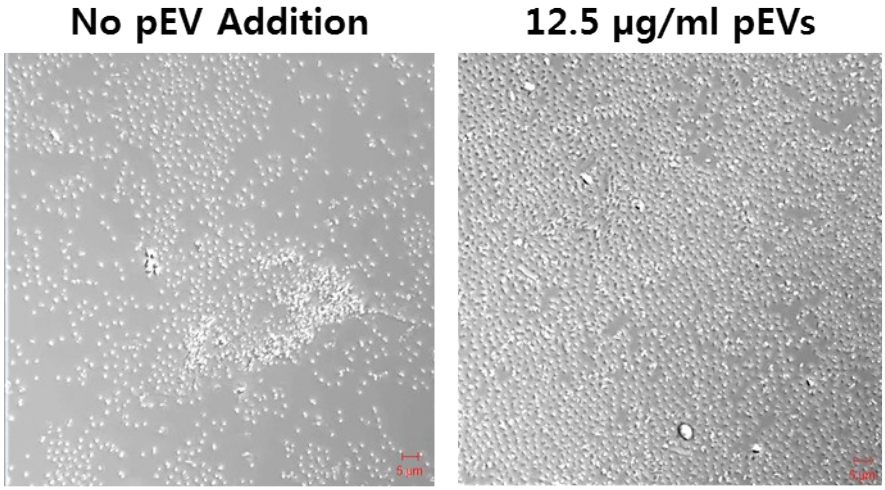

Figure S5. Microscopic images showing the *A. baumannii* biofilm morphologies after an exposure to 12.5 μg/ml *S. aureus* pEVs. The biofilms were formed for 24 h and washed as described in the Materials and Methods. Afterwards, HEPES buffer alone or HEPES buffer with 12.5 μg/ml pEVs was added to cover the surface. The biofilms were then incubated at 37ºC for 24 h before being imaged.

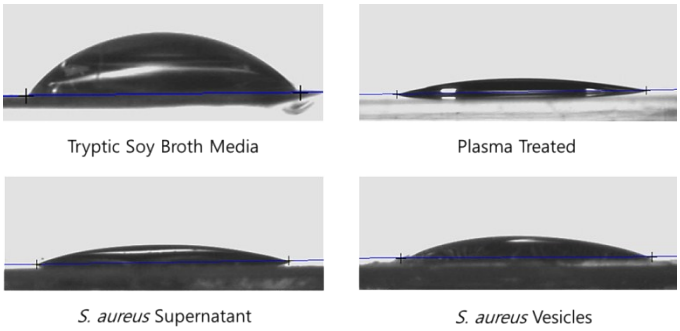

Figure S6. Sessile water droplets showing the effects of treatment on the polystyrene surface. The images obtained show the similar water contact angles from the plasma and *S. aureus* EV treated surfaces. The plasma treatment was performed for 60 s.

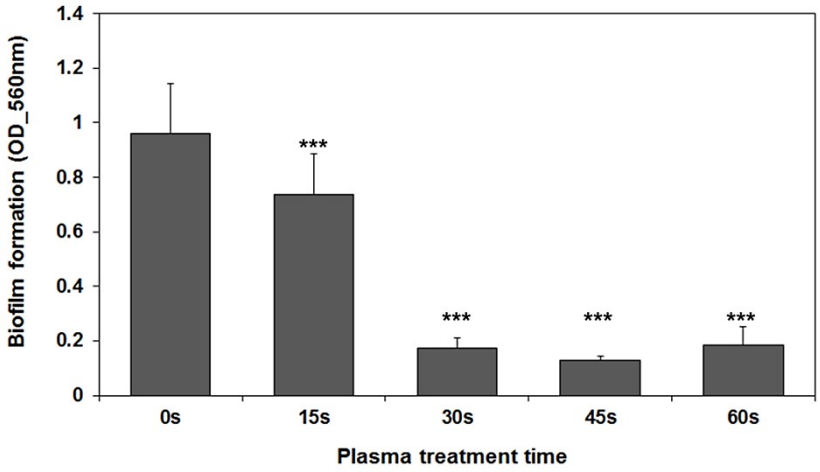

Figure S7. Biofilm formation by a clinical isolate of *A. baumannii* within oxygen plasma treated polystyrene wells. The 96-well plates were treated for 0, 15, 30, 45 or 60 s (*** indicates $p < 0.001$).

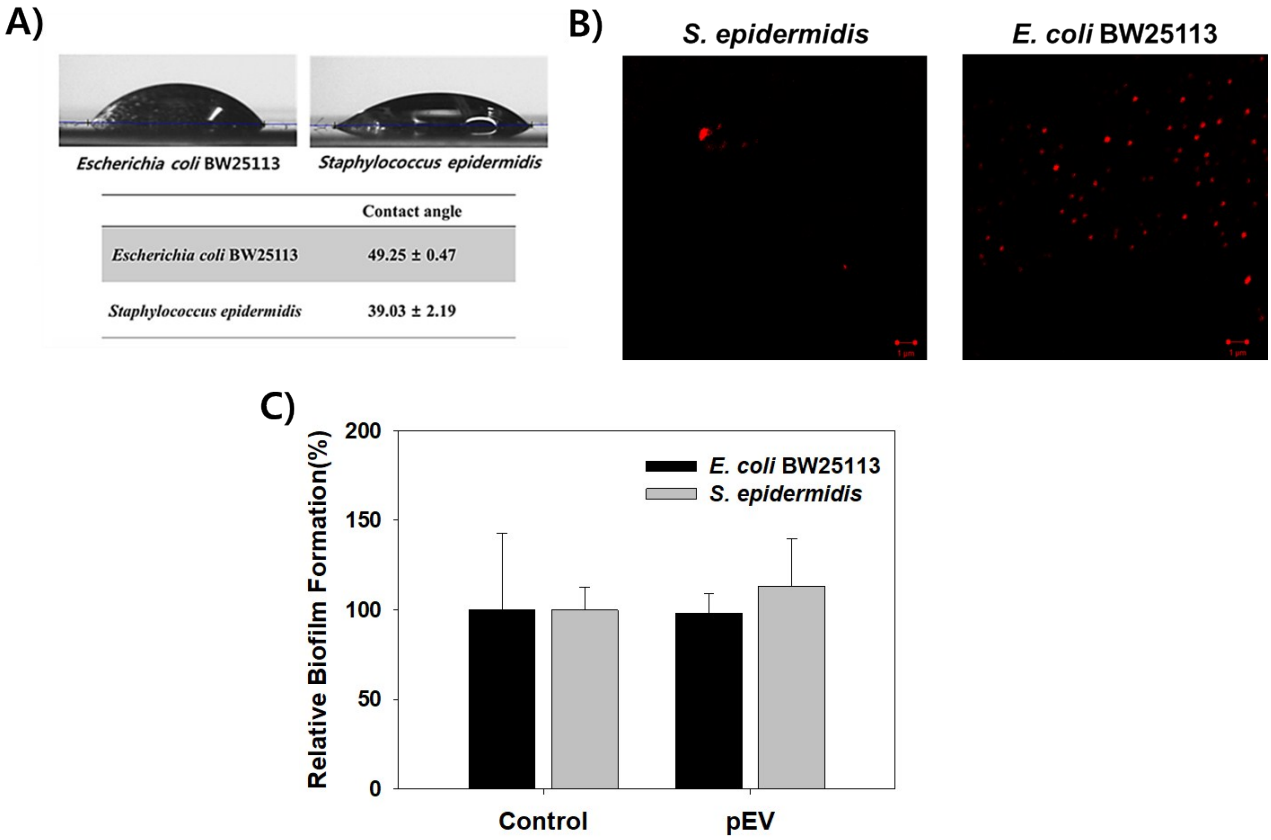

Figure S8. A) Images of sessile water drop and water contact angles of different substrates with *E. coli* BW25113 and *S. epidermidis* EV-coated substrate. B) Confocal images of different strain EV coated polystyrene surface. The concentration of pEVs used was 12.5μg/ml, the same as with *S. aureus* ATCC 25923. C) Biofilm formation by *Acinetobacter baumannii* on the *E. coli* or *S. epidermidis* EV-treated substrates, showing their lack of activity against this strain.

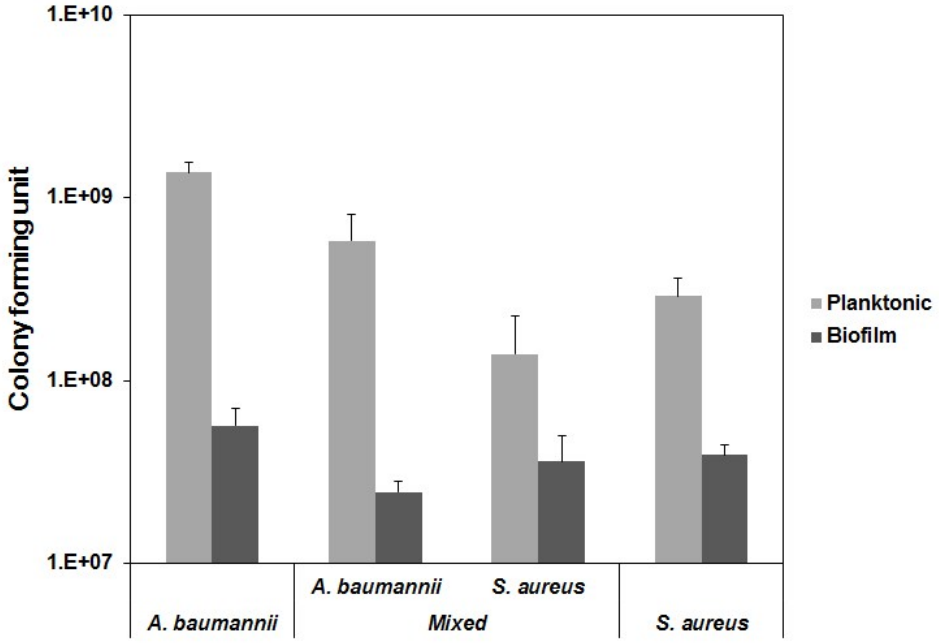

Figure S9. Viable numbers of the clinical *A. baumannii* and *S. aureus* ATCC 25923 strains in both the planktonic and biofilm populations after growth and biofilm formation. Note the decrease in the planktonic populations of both strains when co-cultured even though the biofilms from both the individual and co-cultures contained similar numbers of *S. aureus*. In contrast, the *A. baumannii* population in the mixed biofilm decreased significantly.