



Supporting Information

A Multiplexed Cell-Free Assay to Screen for Antimicrobial Peptides in Double Emulsion Droplets

*N. Nuti, P. Rottmann, A. Stucki, P. Koch, S. Panke, P. S. Dittrich**

Methods

Solutions

The buffer used for vesicle production and storage and as outer aqueous phase for all double emulsion experiments is composed of 500 mM tris(hydroxymethyl)aminomethane (TRIS HCl) pH 7.5 buffer, prepared by dissolving TRIS in Milli-Q water, followed by pH adjustment with hydrochloric acid 37%. When used as outer aqueous phase for double emulsion production, the buffer was supplemented with 2 volume % polyvinyl alcohol (PVA) (87–90% hydrolyzed, molecular weight 30'000–70'000). The oil phase for double emulsion production is composed of HFE-7500 (3M) supplemented with 2 volume % FluoroSurfactant (Ran Biotech, USA). The self-quenching solutions encapsulated within the LUVs were composed of either 20 mM sulforhodamine B sodium salt in 500 mM TRIS pH 7.5, or 50 mM 5(6)-carboxyfluorescein in 500 mM TRIS pH 7.5 buffer.

Microfluidic chip fabrication and surface treatment

Our microfluidic setup comprises two different units, one for double emulsion production, the other for double emulsion trapping, incubation, and image acquisition. The patterned master molds for the preparation of the chips were fabricated as follows. A 100 cm diameter silicon wafer (Si-Mat, Germany) was dehydrated for 10 min at 200 °C, spin coated with SU-8 2015 for 30 s at 1750 rpm, and soft-baked at 95 °C for 240 s. Using a MA6 mask aligner (Süss MicroTec, Germany), and a transparency photomask (Micro Lithography Services, UK), the wafer was exposed to a UV light dose of 150 mJ cm⁻² at 365 nm. Then, the wafer was post-baked at 95 °C for 300 s and developed using mr-Dev 600 developer for 4 min revealing the 20 µm high features. The wafer was then rendered hydrophobic by exposing it to 1H,1H,2H,2H-perfluorodecyltrichlorosilane at 100 mbar for 12 h. The microfluidic chips were realized by pouring liquid PDMS (poly(dimethylsiloxane), oligomer to curing agent ratio of 10:1) on the silicon wafer mold to a height of 5 mm and curing it at 80 °C for 3 h. PDMS and curing agent (Sylgard 184) were purchased from Dow Corning. After punching holes in the PDMS with a 0.5 mm inner diameter Rapid-Core biopsy puncher, the PDMS and a #1.5 glass coverslip were exposed to oxygen plasma for 45 s using a PDC-32G plasma cleaner (Harrick Plasma), and immediately bonded to each other. The chips were then baked at 80 °C for 20 min. The production chip channel carrying the outer aqueous phase was coated with a solution of 50 mg ml⁻¹ polyvinyl alcohol (PVA) (87–90% hydrolyzed, molecular weight 30'000–70'000, Sigma Aldrich) to increase its hydrophilicity. Positive air pressure was used to prevent the coating of the other channels, preserving their hydrophobicity. After about 3 minutes, the PVA solution was aspirated, and the chip was baked at 120°C for 20 min.

Microfluidic chip operation for double emulsion production

All liquids were injected in the microfluidic device using a neMESYS syringe pump (Cetoni, Germany). 1 ml Plastipak syringes (BD, Germany) were connected to the chip using PTFE microtubing (0.56 × 1.07 mm; Fisher Scientific) and metal connector tips (0.013" inner diameter, New England Small Tube). The on-chip double emulsion production was monitored using an inverted microscope equipped with a Miro M110 high-speed camera (Vision Research, New Jersey, USA). At flow rates of 1-2 µl min⁻¹ inner aqueous phase, 1-1.5 µl min⁻¹ oil, and 3-5 µl min⁻¹ outer aqueous phase, we created DE droplets at a frequency of 0.3-1 kHz, with an inner aqueous droplet diameter of ~28 µm and an outer oil shell diameter of ~32 µm. The inner aqueous phase consisted of cell-free extract and DNA to which the LUVs were directly added. After quantifying

the LUV concentration by determining the total phospholipid content in the LUV suspension, the vesicles were added to the inner aqueous phase to a final phospholipid concentration of 20 mM. The trapping chip was mounted on an automated inverted microscope (Nikon Ti-Eclipse) and filled with buffer. The double emulsions were then flushed in the chip and got trapped in the geometric traps. While imaging, a steady flow of buffer was maintained to avoid bubble formation. Each double emulsion was imaged via brightfield and fluorescence microscopy using a 20x objective (S Plan Fluor ELWD 20x). The fluorescent excitation was performed using a Lumencor Spectra X LED light source equipped with the appropriate optical filters and dichroic mirrors (6-FAM measurement: cyan LED, 475/28 excitation filter, 495 dichroic, 525/50 emission filter; SRB measurement: green LED, 549/15 excitation filter, 562 dichroic, 593/40 emission filter). Images were recorded using a Hamamatsu Orca Flash 4 camera. The image acquisition was controlled via the NIKON NIS-Elements Advanced Research software, and the focus was kept via the Nikon Perfect Focusing System. The recorded fluorescence images were analyzed using ImageJ 2.0.0 (National Institute of Health, USA).

Vesicle preparation

Large unilamellar vesicles were prepared with different combination of lipids and fluorescent dyes, but always following the same protocol. A total of 20 μ mol of the lipids of choice were dissolved in 2 ml chloroform, and vacuum dried on the bottom of a 10 ml round glass flask using a rotary evaporator at a pressure of 200 mbar and a water bath heated to 50 °C. The lipid film was then hydrated with 0.5 ml of a solution of 20 mM SRB or 50 mM 6-FAM in TRIS pH 7.5 buffer, for 3 h under gentle swirling of the flask (orbital shaker on its lowest speed setting). The resulting suspension, composed mostly of multilamellar vesicles, was frozen and thawed for 11 times in a bath of liquid nitrogen, and then in a water bath heated to 50 °C. The resulting suspension, now composed of mostly unilamellar vesicles, was extruded twice using the Avanti Mini Extruder (Avanti Polar Lipids), to decrease and homogenize the size of the vesicles, and further decrease their lamellarity. The extruder was assembled with two 10 mm PE drain discs and a polycarbonate membrane with the desired pore size, both from Whatman. The lipids were extruded 11 times using first a polycarbonate membrane with 400 nm pores, and then 11 times using a membrane with 100 nm pores. The resulting suspension contained both encapsulated and unencapsulated dye, and it was dialyzed using a 0.5 ml 20K MWCO Slide-A-Lyzer Dialysis Cassettes (Thermo Fisher), immersed in a 200 ml bath of 500 mM TRIS HCl pH 7.5. This was repeated three times, twice for 2 h and once overnight. The resulting suspension was analyzed using dynamic light scattering (DLS) on a ZetaSizer Nano (Malvern) using disposable polystyrene semimicro cuvettes (BRAND) with a path length of 1 cm (Supplementary Figure 7). 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC), cholesterol, 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phospho-(1'-rac-glycerol) (sodium salt) (POPG), 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphoethanolamine (POPE), 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine-N-[methoxy(polyethylene glycol)-2000] (ammonium salt) (DSPE-PEG(2000)), were all purchased from Avanti Polar Lipids.

Table 1. LUV composition

Mammalian-like	48% POPC	48% Cholesterol	4% DSPE-PEG(2000)
Bacteria-like	72% POPE	24% POPG	4% DSPE-PEG(2000)

Cloning and plasmids

In general, all genes for peptides were provided on high copy plasmids (pUC18 origin of replication) and expression in cell-free extract was initiated from a phage T7 promoter. Plasmids

were produced in cultures with cells (such as *E. coli* Top10) which did not provide the phage T7 gene 1 (encoding T7 RNA polymerase), thus avoiding premature peptide gene expression. Plasmid construction followed standard molecular biology methods and correct construction was confirmed by Sanger sequencing (Microsynth, Switzerland). The genes for pneumolysin and delta-hemolysin (Table 1, Supplementary Information) were chemically synthesized at IDT DNA (Belgium) and Gibson-assembled with plasmid backbone obtained by PCR (see Table 2, Supplementary Information, for primers) and plasmid pUC18_T7_sfGFP as the template. The gene for Cathelicidin-BF was synthesized by Twist-Bioscience, PCR amplified, and digested with BamHI and NdeI for ligation into the equally digested plasmid pUC18_T7_sfGFP. The genes for PepG1, Ascaphin-6, Cecropin P1, Oxyopinin 2b, and Meucin 25 were ordered with T7 promoters as plasmid constructs in pUC18 plasmid backbones from Twist Bioscience. The alpha-hemolysin encoding plasmid, which contained a theophylline riboswitch in addition to the T7 promoter, was a gift from Sheref Mansy (Addgene plasmid #53116). When used, the plasmid required the addition of 1.5 mM theophylline to activate the riboswitch. Plasmid production for cell-free protein expression was performed by overnight incubation of a 100 ml LB culture with ampicillin at 37°C and constant agitation of 200 rpm. The plasmid was purified according to the PureLink HiPure Plasmid Midiprep Kit (Thermo Fischer Scientific) according to the manufacturer's instructions. The concentration of the purified plasmid DNA was measured with a nanodrop device (Thermo Fischer Scientific).

Cell-free production of proteins and antimicrobial peptides

Each construct was expressed with the AccuRapid™ cell-free protein expression kit (Bioneer, South Korea). After the addition of 8 nM plasmid DNA the mixture was either encapsulated in DEs, or loaded in a 384 well plate, and incubated for at least 16 h at room temperature (~25°C). In such conditions the average concentration of protein produced after the incubation is ~0.2 mg ml⁻¹ (~6 μM) which was estimated based on cell-free produced sfGFP.

Plate reader experiments

Plate reader experiments were performed in a Cytation 5 automated plate reader (BioTek), using transparent bottom 384-well polystyrene microplates (Thermo Scientific). Within each well, the aqueous content was overlaid with 20 μl hexadecane to prevent evaporation. The plate reader was equipped with the appropriate optical filters (6-FAM/sfGFP measurement: 484/20 nm excitation filter and 530/25 nm emission filter; SRB measurement: 565/20 nm excitation filter and 606/20 nm emission filter).

Data is presented in terms of “fractional fluorescence”:

$$f_F = 100 * \frac{f_{(t)} - f_{(0)}}{f_{(\infty)} - f_{(0)}}$$

Where $f_{(t)}$ is the fluorescence intensity caused by the newly cell-free produced AMP, $f_{(0)}$ is the background fluorescence intensity of the liposomes before the addition of the AMP plasmid DNA, and $f_{(\infty)}$ is the fluorescence of the LUVs completely lysed by the addition to the mixture of 0.2% Triton X-100 (for such task, Triton X-100 acts as an SDS equivalent).

Double emulsion trapping and observation

After assembling the microfluidic trapping device on an inverted microscope, the double emulsions were injected into the inlet port using a syringe pump at a flow rate of 1 μl min⁻¹. While imaging, a continuous stream of fresh buffer was kept flowing through the chamber at a rate of 0.2-0.5 μl min⁻¹. Each individual trap was imaged via brightfield and fluorescence microscopy, using a fully motorized inverted wide-field microscope (Nikon Ti-Eclipse). As a light source for

fluorescence microscopy, we used a Lumencor Spectra X LED equipped with the appropriate optical filters and dichroic mirrors (6-FAM measurement: cyan LED, 475/28 nm excitation filter, 495 nm dichroic mirror, 525/50 nm emission filter; SRB measurement: green LED, 549/15 nm excitation filter, 562 nm dichroic mirror, 593/40 nm emission filter). Images were recorded using a Hamamatsu Orca Flash 4 camera. The microscopy setup was controlled with the Nikon NIS-Elements Advanced Research software. Fluorescence microscopy images were processed and analyzed using the FIJI software and its particle analysis plugin⁷⁸. After automatically selecting the circular regions of interest (ROIs) via thresholding, their mean grey values were measured. This process was automated using a custom FIJI script. The data was then processed using the R software. Oil droplets and overlapping double emulsions were manually omitted from the analysis.

Flow-cytometric double emulsion analysis and sorting experiment

For each sample, we diluted 1 μl of double emulsions 1:200 in buffer. The sample was then analyzed using an LSRFortessaTM air-cooled multi-laser benchtop flow cytometer (BD), equipped with the appropriate optical configuration (6-FAM measurements: 488 nm laser with 530/30 nm bandpass filter; SRB measurements: 561 nm laser with 586/15 bandpass filter). For each sample 10,000 events were collected. Data analysis was performed using the FlowJo software (TreeStar, USA). For the droplet sorting experiment, meucín-25 and pneumolysin were separately expressed with the AccuRapidTM cell-free protein expression kit (Bioneer) in single emulsions containing bacterial-like and mammalian-like LUVs, as described in section 1.4. After the DE formation, the fluorescence signals of 6-FAM and SRB were analyzed individually for the DEs containing meucín-25 and for the DEs containing pneumolysin. A 1:1 mixture of the DEs was analyzed on the FACS device and the droplet population was sorted according to its increase in either red fluorescence (selection for pneumolysin) or green fluorescence (selection for meucín-25). Additionally, an ungated sample of the population was collected. After selection of 10'000 droplets per gate, the DNA was retrieved by PCR amplification using the KAPA2G HotStart Robust PCR kit (Merck KGaA, Darmstadt) and the primer set oPR178 and oPR179 according to the manufacturers' protocol. The amplified DNA was separated via a 1% agarose gel and the length of its bands compared to a 2-log DNA ladder (NEB, Ipswich, MA, USA).

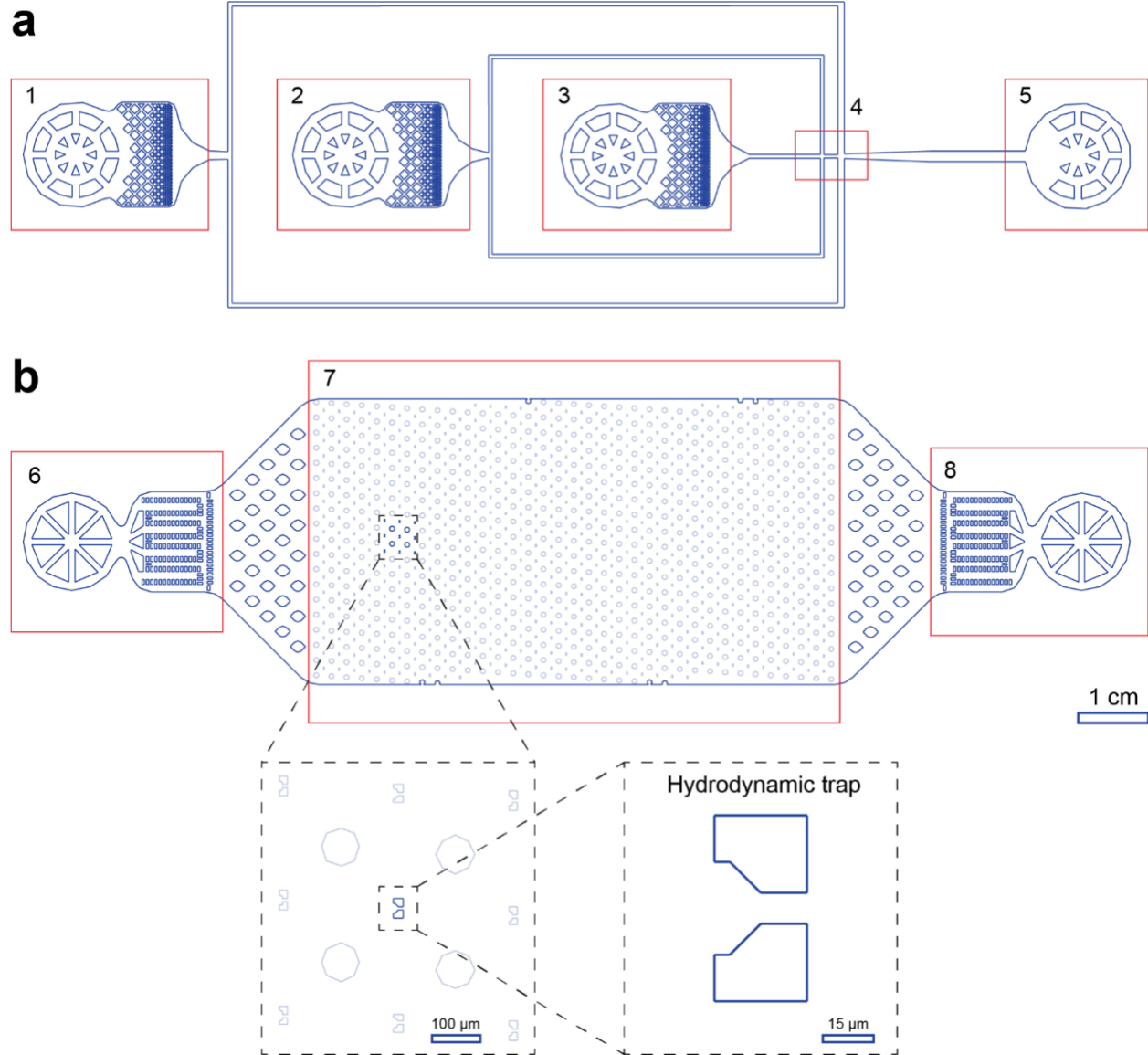
Membrane damage assay

Chemically synthesized Meucín-25 (H-VKLIQIRIWIQYVTVLQMFMSMKTQK-OH) and Cathelicidin-BF (H- KRFKFFKLLKKSVKKRAKFFKKPRVIGVSIPIF-OH) were purchased from Pepscan (Lelystad, NL) in >90% purity and dissolved and stored in DMSO. To measure the membrane damage of both cytoplasmic and outer membrane, we used *E. coli* TOP10 cells transformed with plasmid pSEVA 732, containing the gene for super folder green fluorescent protein (sfGFP) under the control of a strong constitutive promoter BBa_J23118 (BioBricks parts registry, parts.igem.org) and a kanamycin resistance cassette. Overnight pre-cultures were used to inoculate (1:100) fresh Mueller Hinton Broth (MHB; Sigma Aldrich) medium. Cells were grown until exponential phase ($\sim\text{OD}_{600} = 0.4$), then put on ice for 20 min and finally diluted to $6 \cdot 10^5$ cells ml^{-1} with MHB medium containing a final concentration of $1 \mu\text{g ml}^{-1}$ propidium iodide (PI)⁷⁹. PI is a DNA-intercalating dye, indicative of damages occurring at the cytoplasmic membrane⁸⁰. Next, 50 μl of cell suspension were added to microtiter plate wells containing 50 μl MHB containing a 2-fold dilution series of both peptides, using $100 \mu\text{g ml}^{-1}$ as the highest concentration. This was performed in duplicates. Cells and peptides were incubated at room temperature for 30 min. For each sample, the GFP and PI fluorescence of 10,000 cells was measured using a flow cytometer (LSRFortessa, BD Biosciences), equipped with the appropriate optical configuration (488 nm laser with 530/30 nm bandpass filter and 579 nm laser with 610/20 nm bandpass filter). To

determine the membrane damaging properties, we calculated the percentage of GFP retaining and PI acquired cells using the FlowJo V10 software (TreeStar, USA).

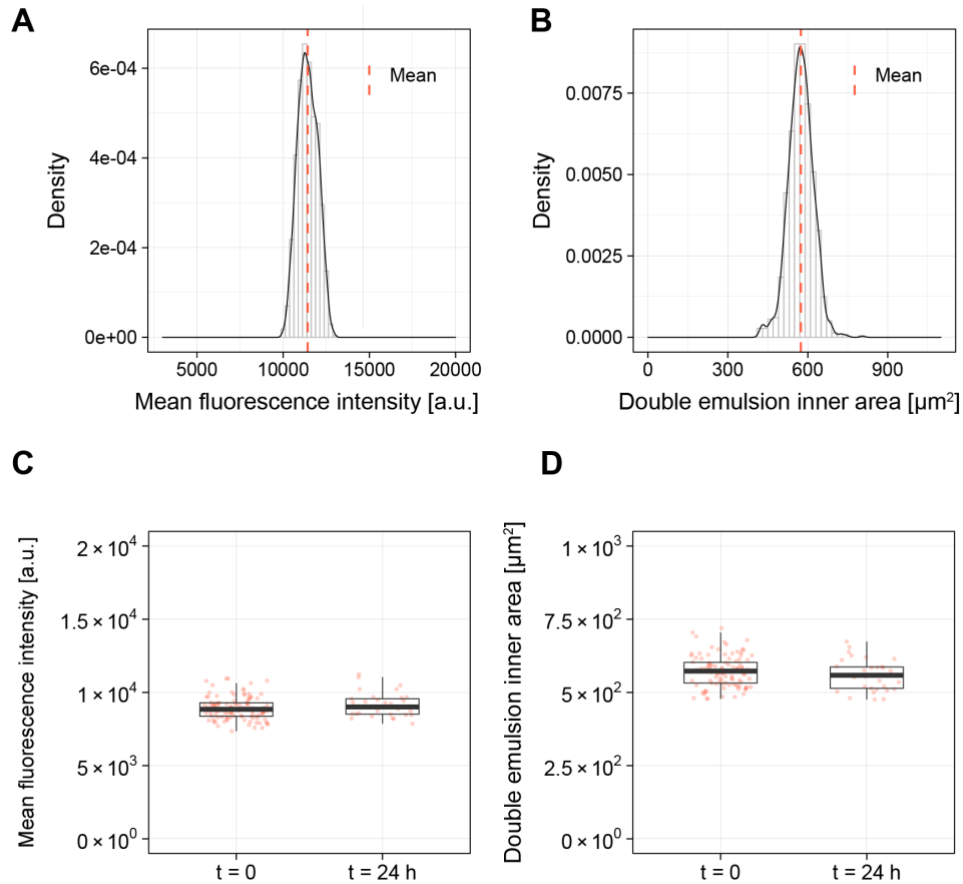
Statistical methods

In all displayed boxplots the middle line represents the median, the boxes define the 25th and 75th percentiles, and the whiskers represent the standard deviation. Single data points are overlaid on the boxplot.

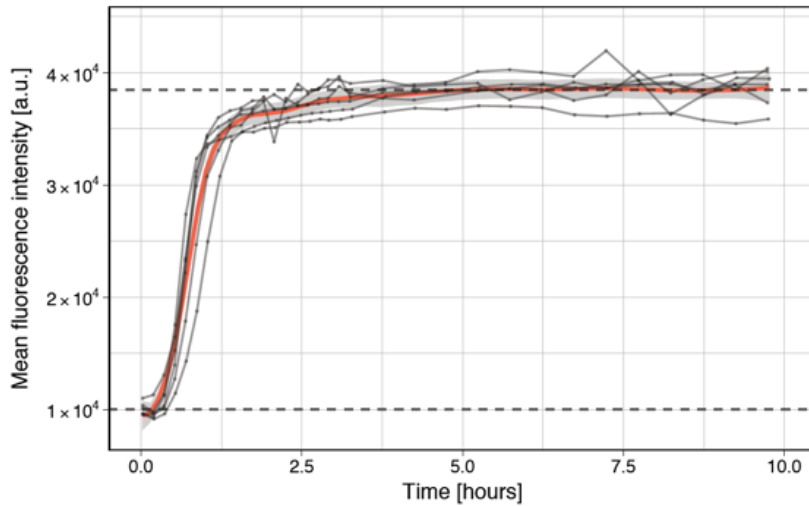


Supplementary Figure 1. Layout of the microfluidic devices. (a) Microfluidic device for double emulsion droplet production. The device consists of three inlets for oil (2) and aqueous phases (1, 3) with filters. At the first junction (4), water-in-oil droplets are formed and immediately pushed into a stream of outer aqueous phase, forming water-in-oil-in-water double emulsion droplets. The droplets flow out of the outlet (5) and can be collected in an Eppendorf tube. (b) The trapping array device consists of an inlet (6) and an outlet (7), separated by a single chamber containing an array of 612 hydrodynamic traps (7). The double emulsions are physically trapped by the flow

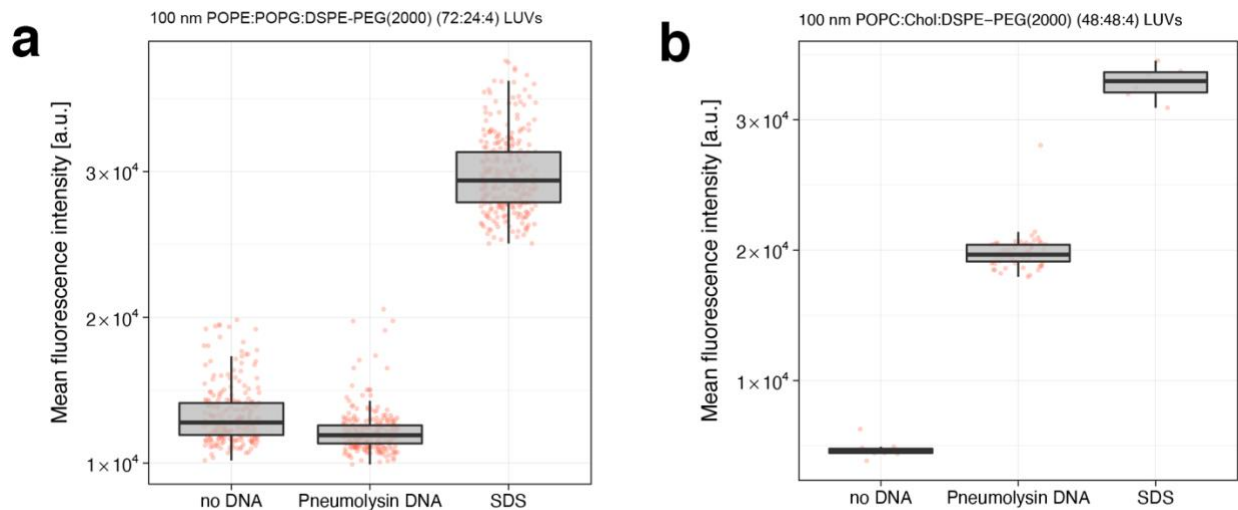
of buffer within the narrow-gapped microposts and can be released by reversing the flow direction. Both chips had structures of 20 μm in height. See also Supplementary Video 1.



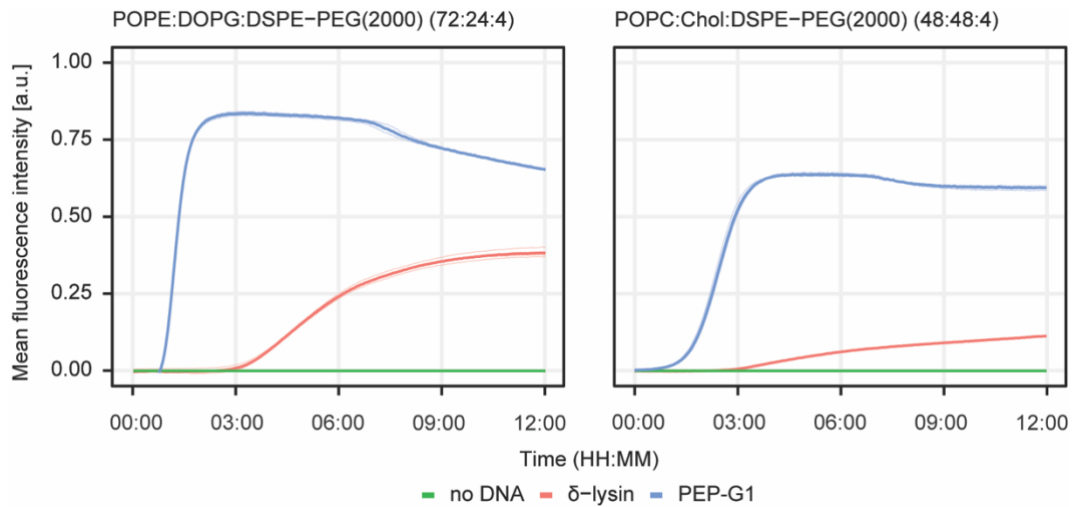
Supplementary Figure 2. Stability of the double emulsions containing LUVs. (a) Distribution of background fluorescence between different double emulsions containing LUVs, indicating a homogeneous encapsulation ($n = 1072$). (b) Distribution of the double emulsions' inner diameter (inner aqueous phase) between different double emulsions containing LUVs, indicating a homogeneous size distribution ($n = 1072$). (c) Stability of the LUVs background fluorescence over time, indicating dye retention ($n = 107$ and $\text{CV}\% = 8.75$ for $t = 0$, $n = 33$ and $\text{CV}\% = 8.89$ for $t = 24$ h). (d) Stability of the double emulsions' inner diameter (inner aqueous phase) over time ($n = 107$ and $\text{CV}\% = 9.09$ for $t = 0$, $n = 33$ and $\text{CV}\% = 8.75$ for $t = 24$ h). The analyzed double emulsions contained mammalian-like LUVs loaded with a self-quenching concentration of SRB.



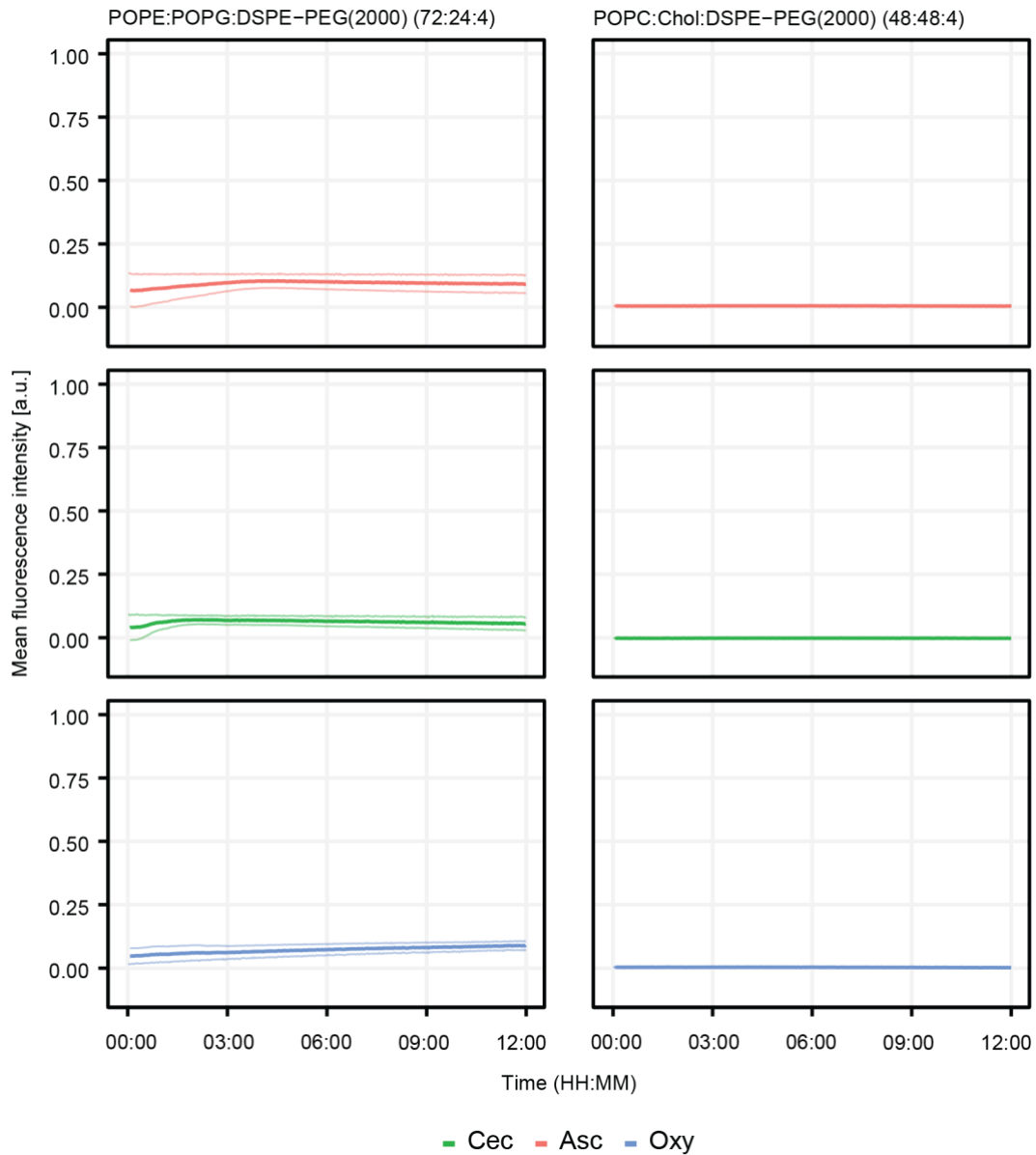
Supplementary Figure 3. Effect of SDS on LUVs encapsulated in double emulsions over time. SDS penetrates through the oil shell into the inner aqueous phase of the double emulsion, where it can lyse the fluorophore-laden LUVs. The fluorescence intensity increases and reaches a plateau when all the vesicles have been disrupted ($n = 8$). The vesicles are encapsulated in cell-free extract and were lysed by the addition of 0.5wt% SDS in buffer on the outside of the double emulsions, followed by an incubation at room temperature ($\sim 25^{\circ}\text{C}$).



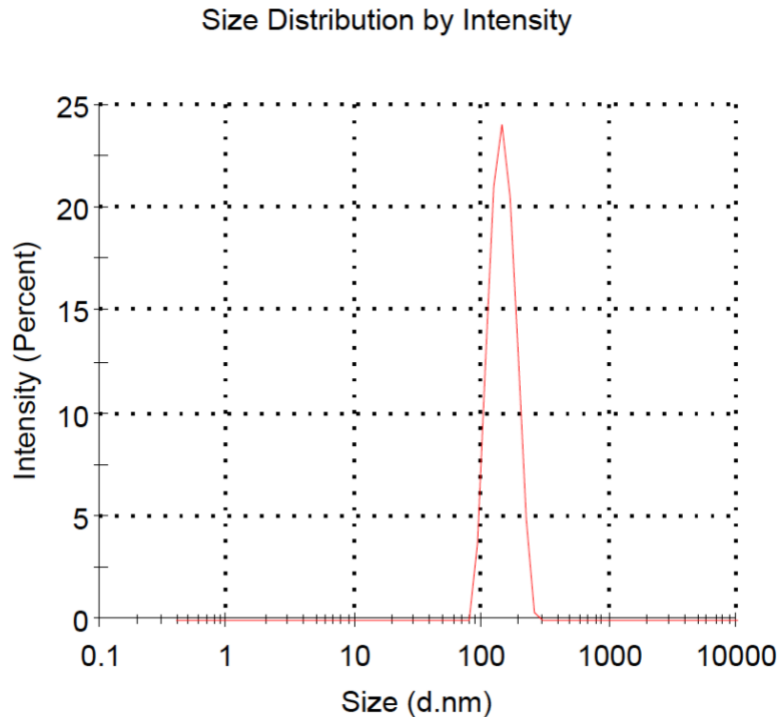
Supplementary Figure 4. Pneumolysin effect on different membrane compositions. Effect of pneumolysin on bacteria-like LUVs (a), and mammalian-like LUVs (b). Mean fluorescence intensities are measured via fluorescence microscopy after incubating the double emulsions at room temperature for 16 hours. The label *no DNA* indicates double emulsions without any addition of external plasmid DNA (panel a: $n = 278$, panel b: $n = 10$), *Pneumolysin DNA* indicates double emulsions with 8 nM pneumolysin plasmid DNA (panel a: $n = 267$, panel b: $n = 61$), *SDS* indicates double emulsions without any addition of external plasmid DNA, exposed to a solution of 0.5% SDS in buffer throughout the incubation (panel a: $n = 315$, panel b: $n = 6$).



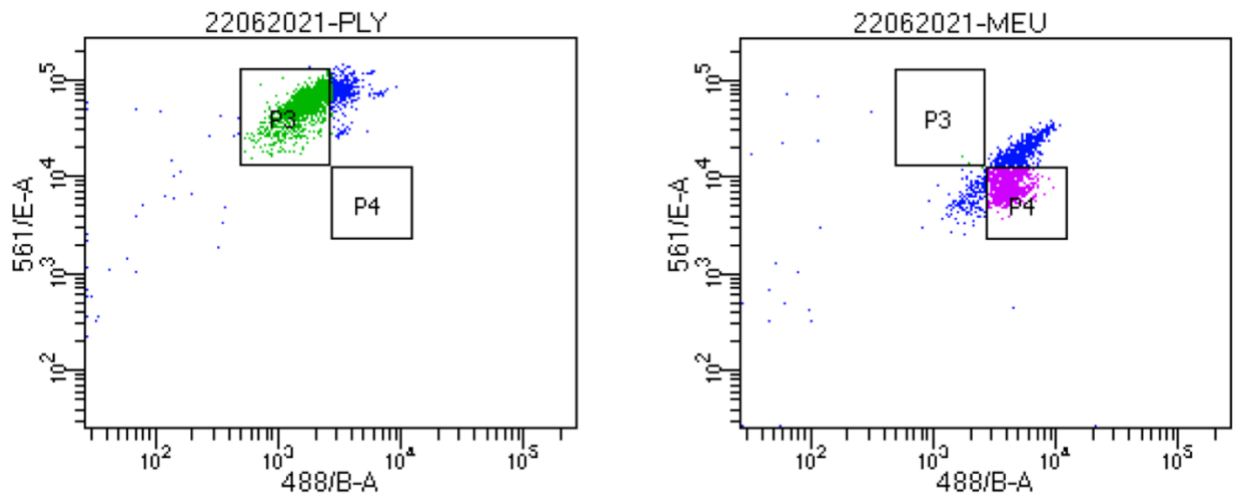
Supplementary Figure 5. Fluorophore leakage kinetic from bacteria-like LUVs loaded with a self-quenching concentration of 6-FAM (left), and mammalian-like LUVs loaded with a self-quenching concentration of SRB (right), induced by the cell-free expression of delta-lysin and pepG1 (sequences in Supplementary Table 1), in 384 well-plates, starting at time 0. Solid lines represent the average of three technical replicates visible below.



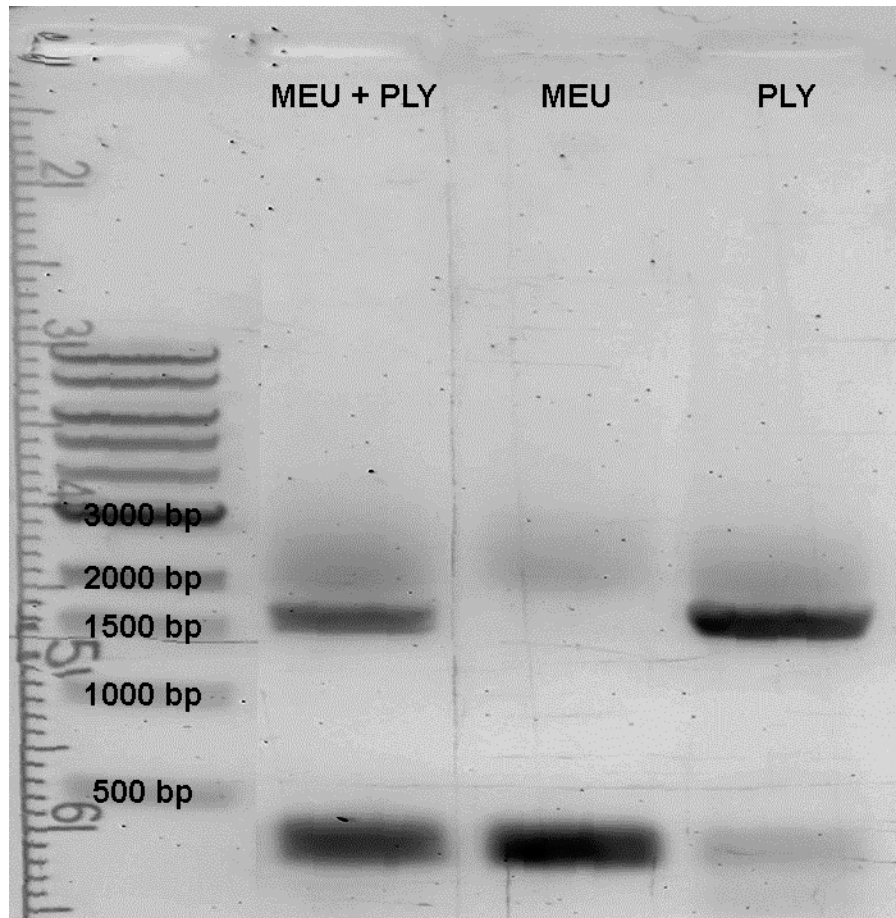
Supplementary Figure 6. Antimicrobial peptides with low hydrophobicity appear not to be salt-resistant. Fluorophore leakage kinetic from bacteria-like LUVs loaded with a self-quenching concentration of 6-FAM (left), and mammalian-like LUVs loaded with a self-quenching concentration of SRB (right), induced by the cell-free expression of Cecropin P1 (Cec), Ascaphin-6 (Asc), Oxyopinin 2b (Oxy) (sequences in Supplementary Table 1), in 384 well-plates, starting at time 0. Solid lines represent the average of two independent reactions visible below. For all three experiments, alpha-hemolysin and Meucin-25 were used as positive controls.



Supplementary Figure 7. Dynamic light scattering (DLS) size measurement of LUVs. Representative plot for the size distribution measurement of LUVs. In the graph shown here, POPC:Chol:DSPE-PEG(2000) (48:48:4) LUVs in 350 mM Tris-HCl buffer (pH 7.5) were measured on a ZetaSizer Nano (Malvern) using disposable polystyrene semimicro cuvettes (BRAND) with a path length of 1 cm.



Supplementary Figure 8. FACS light scatter gates of a mixture of DEs containing either meucini-25 or pneumolysin (10⁷000 events). The gate labelled P3 is meant to select pneumolysin DEs showing an increase in 561 nm fluorescence but no increase in 561 nm fluorescence. The gate labelled P4 is meant to select meucini DEs showing an increase in 488 nm fluorescence but no increase in 561 nm fluorescence.



Supplementary Figure 9. Agarose gel electrophoresis after sorting. DEs containing a bacterial specific AMP (meucin-25) were mixed with DEs containing a mammalian specific AMP (pneumolysin). The lane labelled MEU+PLY contains the PCR amplified DNA retrieved from the mixtures of Meucin and Pneumolysin double emulsions that were not sorted (the DEs passed through the FACS device without any active sorting). Meucin DNA is visible at 333 bp and Pneumolysin DNA at 1605 bp. The lane labelled MEU contains the PCR amplification of droplets gated for an increase in 488 nm fluorescence but no increase in 561 nm fluorescence. The amplified product has a length of 333 bp and indicates an efficient selection of droplets containing only Meucin-25. The lane labelled PLY contains the PCR amplification of droplets gated for an increase in 561 nm fluorescence but no increase in 488 nm fluorescence. The amplified product has a length of 1605 bp and indicates an efficient selection of droplets containing mainly pneumolysin (a very faint band is visible at ~333 bp).

Gene fragments and primers

Supplementary Table 1 | DNA sequences used in this work. Start and stop codons are indicated in bold. The phage T7 promoter sequence is underlined and bold.

Name	Sequence 5' □ 3'
Pneumo- lysin	<u>TAATACGACTCACTATAGGG</u> AGACCCTCTAGAACTGCAGTAATTTTGT AACTTTAAGAAGGAGATATACAT ATGG CAAACAAAGCAGTGAATGACTTCA TTCTGGCAATGAATTATGATAAAAAAAAAACTCCTGACTCATCAAGGGGAGA GTATCGAAAATCGTTTTATCAAGGAGGGTAATCAGTTGCCGGACGAGTTC GTCGTCATTGAACGTA AAAACGTTCACTGAGCACTAACACGAGCGATATT TCCGTCACGGCCACCAATGACTCACGTCTCTATCCAGGGGCGCTGTTGGT GGTGGATGAAACCCTGCTGGAAAATAATCCAACCCTGCTGGCGGTGGAC CGCGCACCGATGACTTATTCTATCGATTTGCCGGGCCTTGCGAGTAGCGA CTCCTTTCTGCAAGTTGAAGATCCGAGCAACTCGTCCGTGCGCGGCC GTGAACGACCTGTTGGCAAATGGCACCAGGACTATGGGCAGGTTAATAA TGTCCCGGCGCGGATGCAATATGAGAAGATTACGGCACACTCAATGGAG CAGCTTAAAGTGAAGTTCGGCTCGGACTTCGAGAAAACGGGTAACCTCCT GGACATTGATTTTAATTCAGTGCATAGTGGCGAAAAGCAAATCCAGATCGT AAATTTTAAACAAATCTATTATACGGTCAGCGTTGATGCCGTGAAAACCC GGGAGATGTGTTTCAGGATACGGTGACCGTTGAAGACCTGAAACAGCGA GGTATCAGTGCGGAACGTCCACTCGTGTATATTTCCAGTGTGGCTTATGG TCGTCAAGTGTACCTCAAACCTTGA AACACCTCGAAATCTGACGAGGTTG AGGCGGCATTTGAAGCCTTAATCAAAGGCGTTAAAGTGGCGCCCAGACT GAGTGGAAACAAATCCTGGACAATACCGAAGTGAAGCAGTAATTTTAGG CGGCGATCCGAGCTCCGGCGCCCGCGTGGTTACTGGTAAGGTGGATATG GTGGAGGATCTCATTCAAGAGGGTTCACGTTTTACGGCCGATCACCCCGG GCTGCCGATTTCTTATACCACCTCTTTTTTACGCGACAATGTGGTTGCCAC CTTCCAGAATAGCACTGATTACGTGGAGACCAAGGTGACTGCATATCGTA ATGGAGACCTTCTTCTGGACCATAGCGGGGCCTATGTGGCGCAGTATTAT ATCACTTGGGATGAACTGTTCGTATGATCACCAGGTAAGGAAGTGCTGAC TCCGAAGGCATGGGACCGCAATGGCCAGGACCTGACTGCGCATTTCACC ACGAGTATCCCCTTAAGGGTAACGTGCGTAATTTGAGCGTGAAAATTCG CGAATGCACCGGACTGGCATGGGAATGGTGGCGTACGGTTTATGAAAAG ACCGATCTGCCACTGGTGC GGAAACGTACCATTTGATTTGGGGCACCAC GTTATATCCCCAAGTGGAGGATAAAGTTGAAAATGATTAAT TAAGG ATCCTA GCATAACCCCTTGGGGCCTCTAAACGGGTCTTGAGGGGTTTTTTG
Delta-lysin	<u>TAATACGACTCACTATAGGG</u> AGACCCTCTAGAACTGCAGTAATTTTGT AACTTTAAGAAGGAGATATACAT ATGG CGGCGGATATTATTAGCACCATTG GCGATCTGGTGAATGGATTATTGATACCGTGAACAAATTTAAAT TAAT TTT TCTGAAAAAATTTTAGAGTTATGTTTGATTATAGGAGAATCTGATTATTCCA CGTAAGCTTTGTGCAGGGATCCTAGCATAACCCCTTGGGGCCTCTAAACG GGTCTTGAGGGGTTTTTTG
Ascaphin-6	<u>TAATACGACTCACTATAGGG</u> AGACCCTCTAGAACTGCAGTAATTTTGT AACTTTAAGAAGGAGATATACAT ATGG GCTTTAAAGATTGGATTAAGGCG CGGCGAAAAAACTGATTA AAACCGTGGCGAGCAGCATTGCGAACGAAT AA TTTTTCTGAAAAAATTTTAGAGTTATGTTTGATTATAGGAGAATCTGATTATT CCACGTAAGTGTGCAGGGATCCTAGCATAACCCCTTGGGGCCTCTAAACG GGTCTTGAGGGGTTTTTTG
Meucin-25	<u>TAATACGACTCACTATAGGG</u> AGACCCTCTAGAACTGCAGTAATTTTGT AACTTTAAGAAGGAGATATACAT ATGG TGAAACTGATTCAGATTCGCATTT GGATTCAGTATGTGACCGTGTTACAGATGTTTAGCATGAAAACCAAACAGT

	AATTTTTCTGAAAAAATTTTAGAGTTATAGTGACTTAGGCTATTCATAAGTT TGATTATAGGAGTGTGCAATCCTAGCATAACCCCTTGGGGCCTCTAAACG GGTCTTGAGGGGTTTTTTG
Cathelidini-BF	<u>TAATACGACTCACTATAGGG</u> AGACCCTCTAGAAATAATTTTGTTAACTTT AAGAAGGAGATATACATATGAAACGCTTTAAAAAATTTTTAAAAAACTGAA AAAAAGCGTGAAAAAACGCGCGAAAAAATTTTTAAAAAACGCGCGTGAT TGGCGTGAGCATTCCGTTTTAAATATTGATGTATCACTTACAACACGCACGT AAGCTTTGTGCAGGGATCCTAGCATAACCCCTTGGGGCCTCTAAACGGGT CTTGAGGGGTTTTTTG
Oxyopinin-2B	<u>TAATACGACTCACTATAGGG</u> AGACCCTCTAGAACTGCAGTAATTTTGTTT AACTTTAAGAAGGAGATATACATATGAGCTATATTCCGTGCGGCGAAAGC TGC GTGTATATTCCGTGCACCGTGACCGCGCTGCTGGGTTGCTCGTGCA GCAACAAAGTGTGCTATAAAAACTAAATTTTTCTGAAAAAATTTTAGAGTTAT GTTTGATTATAGGAGTGTGCAATCCTAGCATAACCCCTTGGGGCCTCTAA ACGGGTCTTGAGGGGTTTTTTG
PepG1	<u>TAATACGACTCACTATAGGG</u> AGACCCTCTAGAACTGCAGTAATTTTGTTT AACTTTAAGAAGGAGATATACATATGCTGGTGACCCTGAGCGCGATGTTA CAGTTTGGCATTCTGATTGCGTTTATTGGCCTGGTGATTGATCTGATT AACTGAGCCAGAAAAAATAATTTTTCTGAAAAAATTTTAGAGTTATGTTT ATTATAGGAGTGTGCAGGGATCCTAGCATAACCCCTTGGGGCCTCTAAAC GGGTCTTGAGGGGTTTTTTG
Cecropin P1	<u>TAATACGACTCACTATAGGG</u> AGACCCTCTAGAACTGCAGTAATTTTGTTT AACTTTAAGAAGGAGATATACATATGAGTTGGCTGAGTAAAACCGCGAAA AAGTTAGAAAATAGCGCCAAAAAACGGATTAGCGAGGGTATTGCGATTGC GATTC AAGGGGGCCCTCGTTAAATTTTTCTGAAAAAATTTTAGAGTTATAGT GACTTAGGCTATTCATAAGTTTGATTATAGGAGTGTGCAATCCTAGCATAA CCCCTTGGGGCCTCTAAACGGGTCTTGAGGGGTTTTTTG
JF001A (Alpha - hemolysin)	AAT <u>TAATACGACTCACTATAGGG</u> TGATACCAGCATCGTCTTGATGCCCTT GGCAGCACCCCTGCTAAGGTAACAACAAGATGGATTCTGATATCAATATCA AAACCGGCACCACCGATATCGGCTCCAATACCACCGTTAAAACCGGTGAT CTGGTGACCTATGATAAAGAAAACGGTATGCATAAAAAAGTGTTTTACTCG TTTATTGACGATAAAAACCATAACAAAAAACTGCTGGTCATCCGCACCAAA GGCACCATTGCGGGTCAATACCGTGTGTACTCCGAAGAAGGTGCGAACA AAAGCGGTCTGGCTTGGCCGTCTGCCTTTAAAGTGCAGCTGCAACTGCC GGATAATGAAGTGGCGCAGATTTT CAGATTATTATCCGCGTAATAGCATCG ATACCAAAGAATATATGAGTACCCTGACCTATGGTTTTAATGGCAATGTTA CCGGTGATGATACGGGTAAAATTGGCGGTCTGATTGGCGCCAATGTGTCC ATTGGTCATACGCTGAAATACGTGCAACCGGATTTCAAACCATTCTGGAA AGTCCGACCGATAAAAAAGTGGGTTGGAAAGTTATCTTCAACAACATGGT GAATCAGA ACTGGGGTCCGTACGATCGCGATTCTGGAATCCGGTTTTATG GCAATCAGCTGTTTATGAAAACCCGCAACGGTAGTATGAAAGCGGCGGAT AATTTTCTGGACCCGAACAAAGCCTCAAGCCTGCTGTCCAGCGGTTTTAG CCCGGATTTTGCCACGGTTATTACCATGGATCGCAAAGCCAGCAAACAGC AGACCAACATTGATGTGATCTACGAACGTGTGCGTGATGATTATCAACTG CATTGGACCTCAACCAATTGGAAAGGCACCAATACCAAAGATAAATGGAC GGATCGCAGTTCAGAACGCTACAAAATTGATTGGGAAAAAGAAGAAATGA CCA ACT AACTCGAGCACCACCACCACCACCCTGAGATCCGGCTGCTAA CAAAGCCCGAAAGGAAGCTGAGTTGGCTGCTGCCACCGCTGAGCAATAA CTAGCATAACCCCTTGGGGCCTCTAAACGGGTCTTGAGGGGTTTTTTGCT GAAAGGAGGAACTATATCCGGATTGGCGAATGGGACGCGCCCTGTAGCG

GCGCATTAAAGCGCGGGCGGGTGTGGTGGTTACGCGCAGCGTGACCGCTA
CACTTGCCAGCGCCCTAGCGCCCGCTCCTTTTCGCTTTCTTCCCTTCCTTT
CTCGCCACGTTTCGCCGGCTTTCCCGTCAAGCTCTAAATCGGGGGCTCC
CTTTAGGGTTCCGATTTAGTGCTTTACGGCACCTCGACCCCAAAAACTTG
ATTAGGGTGATGGTTCACGTAGTGGGCCATCGCCCTGATAGACGGTTTTT
CGCCCTTTGACGTTGGAGTCCACGTTCTTTAATAGTGGACTCTTGTTCCAA
ACTGGAACAACACTCAACCCTATCTCGGTCTATTCTTTTGATTTATAAGGG
ATTTTGCCGATTTTCGGCCTATTGGTTAAAAAATGAGCTGATTTAACAAAAAT
TTAACGCGAATTTTAACAAAAATTAACGTTTACAATTTAGGTGGCACTTT
TCGGGGAAATGTGCGCGGAACCCCTATTTGTTTATTTTTCTAAATACATTC
AAATATGTATCCGCTCATGAGACAATAACCCTGATAAATGCTTCAATAATAT
TGAAAAAGGAAGAGTATGAGTATTCAACATTTCCGTGTCGCCCTTATTCCC
TTTTTTGCGGCATTTTGCCTTCCTGTTTTTGTCTACCCAGAAACGCTGGTG
AAAGTAAAAGATGCTGAAGATCAGTTGGGTGCACGAGTGGGTACATCGA
ACTGGATCTCAACAGCGGTAAGATCCTTGAGAGTTTTTCGCCCGAAGAAC
GTTTTCCAATGATGAGCACTTTTAAAGTTCTGCTATGTGGCGCGGTATTAT
CCCGTATTGACGCCGGGCAAGAGCAACTCGGTGCGCCGATACACTATTCT
CAGAATGACTTGGTTGAGTACTCACCAGTCACAGAAAAGCATCTTACGGA
TGGCATGACAGTAAGAGAATTATGCAGTGCTGCCATAACCATGAGTGATA
ACACTGCGGCCAACTTACTTCTGACAACGATCGGAGGACCGAAGGAGCT
AACCGCTTTTTTGCACAACATGGGGGATCATGTAACCTGCCTTGATCGTT
GGGAACCGGAGCTGAATGAAGCCATACCAAACGACGAGCGTGACACCAC
GATGCCTGCAGCAATGGCAACAACGTTGCGCAAACTATTAACCTGGCGAAC
TACTTACTCTAGCTTCCCGGCAACAATTAATAGACTGGATGGAGGCGGAT
AAAGTTGCAGGACCACTTCTGCGCTCGGCCCTTCCGGCTGGCTGTTTTAT
TGCTGATAAATCTGGAGCCGGTGAGCGTGGGTCTCGCGGTATCATTGCA
GCACTGGGGCCAGATGGTAAGCCCTCCCGTATCGTAGTTATCTACACGAC
GGGGAGTCAGGCAACTATGGATGAACGAAATAGACAGATCGCTGAGATA
GGTGCCTCACTGATTAAGCATTGGTAACCTGTCAGACCAAGTTTACTCATAT
ATACTTTAGATTGATTTAAAACCTTCATTTTAATTTAAAAGGATCTAGGTGA
AGATCCTTTTTGATAATCTCATGACCAAATCCCTTAACGTGAGTTTTCGTT
CCACTGAGCGTCAGACCCCGTAGAAAAGATCAAAGGATCTTCTTGAGATC
CTTTTTTTCTGCGCGTAATCTGCTGCTTGCAAACAAAAAACCACCGCTAC
CAGCGGTGGTTTTGTTGCCGGATCAAGAGCTACCAACTCTTTTTCCGAAG
GTAACCTGGCTTCAGCAGAGCGCAGATACCAAATACTGTCTTCTAGTGTA
GCCGTAGTTAGGCCACCACTTCAAGAACTCTGTAGCACCGCCTACATACC
TCGCTCTGCTAATCCTGTTACCAGTGGCTGCTGCCAGTGGCGATAAGTCG
TGTCTTACCGGGTTGGACTCAAGACGATAGTTACCGGATAAAGGCGCAGC
GGTCGGGCTGAACGGGGGGTTCGTGCACACAGCCCAGCTTGGAGCGAA
CGACCTACACCGAACTGAGATACCTACAGCGTGAGCTATGAGAAAGCGC
CACGCTTCCCGAAGGGAGAAAGGCGGACAGGTATCCGGTAAGCGGCAG
GGTCGGAACAGGAGAGCGCACGAGGGAGCTTCCAGGGGGAAACGCCTG
GTATCTTTATAGTCCTGTTCGGGTTTCGCCACCTCTGACTTGAGCGTCGATT
TTTGTGATGCTCGTCAGGGGGGCGGAGCCTATGGAAAAACGCCAGCAAC
GCGGCCTTTTTACGGTTCCTGGCCTTTTGTGGCCTTTTGTCTACATGTTT
TTTCTGCGTTATCCCCTGATTCTGTGGATAACCGTATTACCGCCTTTGAG
TGAGCTGATACCGCTCGCCGACCCGAACGACCGAGCGCAGCGAGTCA
GTGAGCGAGGAAGCGGAAGAGCGCCTGATGCGGTATTTTCTCCTTACGC
ATCTGTGCGGTATTTACACCCGCATATATGGTGCCTCTCAGTACAATCTG
CTCTGATGCCGCATAGTTAAGCCAGTATACTCCGCTATCGCTACGTGA
CTGGGTGATGGCTGCGCCCGACACCCGCCAACACCCGCTGACGCGCC

CTGACGGGCTTGTCTGCTCCCGGCATCCGCTTACAGACAAGCTGTGACC
GTCTCCGGGAGCTGCATGTGTCAGAGGTTTTACCGTCATCACCGAAACG
CGCGAGGCAGCTGCGGTAAAGCTCATCAGCGTGGTCGTGAAGCGATTCA
CAGATGTCTGCCTGTTTCATCCGCGTCCAGCTCGTTGAGTTTTCTCAGAAG
CGTTAATGTCTGGCTTCTGATAAAGCGGGCCATGTTAAGGGCGGTTTTTT
CCTGTTTTGGTCACTGATGCCTCCGTGTAAGGGGGATTTCTGTTTCATGGGG
GTAATGATACCGATGAAACGAGAGAGGATGCTCACGATACGGGTTACTGA
TGATGAACATGCCCGGTTACTGGAACGTTGTGAGGGTAAACAACCTGGCG
GTATGGATGCGGCGGGACCAGAGAAAAATCACTCAGGGTCAATGCCAGC
GCTTCGTTAATACAGATGTAGGTGTTCCACAGGGTAGCCAGCAGCATCCT
GCGATGCAGATCCGGAACATAATGGTGCAGGGCGCTGACTTCCGCGTTT
CCAGACTTTACGAAACACGGAAACCGAAGACCATTTCATGTTGTTGCTCAG
GTCGCAGACGTTTTGCAGCAGCAGTCGCTTACGTTCCGCTCGCGTATCG
GTGATTCATTCTGCTAACCAGTAAGGCAACCCCGCCAGCCTAGCCGGGTC
CTCAACGACAGGAGCACGATCATGCGCACCCGTGGGGCCGCCATGCCG
GCGATAATGGCCTGCTTCTCGCCGAAACGTTTGGTGGCGGGACCAGTGA
CGAAGGCTTGAGCGAGGGCGTGCAAGATTCCGAATACCGCAAGCGACAG
GCCGATCATCGTCGCGCTCCAGCGAAAGCGGTCTCGCCGAAAATGACC
CAGAGCGCTGCCGGCACCTGTCCTACGAGTTGCATGATAAAGAAGACAG
TCATAAGTGCGGCGACGATAGTCATGCCCCGCGCCACCGGAAGGAGCT
GACTGGGTTGAAGGCTCTCAAGGGCATCGGTGCAGATCCCGGTGCCTAA
TGAGTGAGCTAACTTACATTAATTGCGTTGCGCTCACTGCCCGCTTTCCA
GTCGGGAAACCTGTGCTGCCAGCTGCATTAATGAATCGGCCAACGCGCG
GGGAGAGGCGGTTTTGCGTATTGGGCGCCAGGGTGGTTTTTTCTTTTACCA
GTGAGACGGGCAACAGCTGATTGCCCTTACCGCCTGGCCCTGAGAGAG
TTGCAGCAAGCGGTCCACGCTGGTTTGCCCCAGCAGGCGAAAATCCTGT
TTGATGGTGGTTAACGGCGGGATATAACATGAGCTGTCTTCGGTATCGTC
GTATCCCACTACCGAGATATCCGCACCAACGCGCAGCCCGGACTCGGTA
ATGGCGCGCATTGCGCCCAGCGCCATCTGATCGTTGGCAACCAGCATCG
CAGTGGGAACGATGCCCTCATTACGATTTGCATGGTTTGTGAAAACCG
GACATGGCACTCCAGTCGCCTTCCCGTTCCGCTATCGGCTGAATTTGATT
GCGAGTGAGATATTTATGCCAGCCAGCCAGACGCAGACGCGCCGAGACA
GAACTTAATGGGCCCGCTAACAGCGCGATTTGCTGGTGACCCAATGCGA
CCAGATGCTCCACGCCAGTCGCGTACCGTCTTCATGGGAGAAAATAATA
CTGTTGATGGGTGTCTGGTCAGAGACATCAAGAAATAACGCCGGAACATT
AGTGCAGGCAGCTTCCACAGCAATGGCATCCTGGTCATCCAGCGGATAG
TTAATGATCAGCCCACTGACGCGTTGCGCGAGAAGATTGTGCACCGCCG
CTTTACAGGCTTCGACGCCGCTTCGTTCTACCATCGACACCACCACGCTG
GCACCCAGTTGATCGGCGCGAGATTTAATCGCCGCGACAATTTGCGACG
GCGCGTGCAGGGCCAGACTGGAGGTGGCAACGCCAATCAGCAACGACT
GTTTGGCCGCCAGTTGTTGTGCCACGCGGTTGGGAATGTAATTCAGCTCC
GCCATCGCCGCTTCCACTTTTTCCCGGTTTTTCGCAGAAACGTGGCTGGC
CTGGTTACACGCGGGAAACGCTCTGATAAGAGACACCGGCATACTCT
GCGACATCGTATAACGTTACTGGTTTTACATTCACCACCCTGAATTGACTC
TCTTCCGGGCGCTATCATGCCATACCGCGAAAGGTTTTGCGCCATTTCGAT
GGTGTCCGGGATCTCGACGCTCTCCCTTATGCGACTCCTGCATTAGGAAG
CAGCCCAGTAGTAGGTTGAGGCCGTTGAGCACCGCCGCCGCAAGGAATG
GTGCATGCAAGGAGATGGCGCCCAACAGTCCCCCGGCCACGGGGCCTG
CCACCATACCACGCCGAAACAAGCGCTCATGAGCCCAGAGTGGCGAGC
CCGATCTTCCCATCGGTGATGTCGGCGATATAGGCGCCAGCAACCGCA
CCTGTGGCGCCGGTGATGCCGGCCACGATGCGTCCGGCGTAGAGGATC

	GAGATCTCGATCCCGCGA
pUC18_T7 _sfGFP	AACGACGGCCAGTGCCAAGCTTGCGAAATT <u>TAATACGACTCACTATAGGG</u> AGACCCCTCTAGAAACTGCAGTAATTTTGTAACTTTAAGAAGGAGATATA CATATGAGCAAAGGAGAAGAACTTTTCACTGGAGTTGTCCCAATTCCTGTT GAATTAGATGGTGATGTTAATGGGCACAAATTTTCTGTCCGTGGAGAGGG TGAAGGTGATGCTACAAACGGAAACTCACCCCTTAAATTTATTTGCACTAC TGGAAACTACCTGTTCCGTGGCCAACACTTGTCACTACTCTGACCTATG GTGTTCAATGCTTTTTCCCGTTATCCGGATCACATGAAACGGCATGACTTTT TCAAGAGTGCCATGCCCGAAGGTTATGTACAGGAACGCACTATATCTTTT AAAGATGACGGGACCTACAAGACGCGTGCTGAAGTCAAGTTTGAAGGTGA TACCCTTGTTAATCGTATCGAGTTAAAGGGTATTGATTTTAAAGAAGATGG AAACATTCCTGGACACAACTCGAGTACAACCTTAACTCACACAATGTATA CATCACGGCAGACAAACAAAAGAAATGGAATCAAAGCTAACTTCAAATTCG CCACAACGTTGAAGATGGTTCGTTCACTAGCAGACCATTATCAACAAA TACTCCAATTGGCGATGGCCCTGTCCTTTTACCAGACAACCATTACCTGTC GACACAATCTGTCCTTTGAAAGATCCCAACGAAAAGCGTGACCACATGG TCCTTCTTGAGTTTGTAACTGCTGCTGGGATTACACATGGCATGGATGAG CTCTACAAATAATAAGGATCCTAGCATAAACCCTTGGGGCCTCTAAACGG GTCCTTGAGGGGTTTTTTGAATTCGTAATCATGGTCATAGCTGTTTCCTGTG TGAAATTGTTATCCGCTCACAAATTCACACAACATAACGAGCCGGAAGCATA AAGTGTAAGCCTGGGGTGCCTAATGAGTGAGCTAACTCACATTAATTGC GTTGCGCTCACTGCCCCTTTCCAGTCGGGAAACCTGTCGTGCCAGCTG CATTAATGAATCGGCCAACGCGCGGGGAGAGGGCGGTTTGCATATTGGGC GCTCTTCCGCTTCTCGCTCACTGACTCGCTGCGCTCGGTGCTTCCGGCTG CGGCGAGCGGTATCAGCTCACTCAAAGGCGGTAATACGGTTATCCACAG AATCAGGGGATAACGCAGGAAAGAACATGTGAGCAAAGGCCAGCAAAA GGCCAGGAACCGTAAAAGGCCGCGTTGCTGGCGTTTTTCCATAGGCTC CGCCCCCTGACGAGCATCACAAAATCGACGCTCAAGTCAGAGGTGGC GAAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCTGGAAGCTCC CTCGTGCGCTCTCCTGTTCCGACCCTGCCGTTACCGGATACCTGTCCGC CTTTCTCCCTTCGGGAAGCGTGCGCCTTTCTCAAAGCTCACGCTGTAGGT ATCTCAGTTCGGTGTAGGTGCTTCGCTCCAAGCTGGGCTGTGTGCACGAA CCCCCGTTCAGCCCGACCCTGCGCCTTATCCGGTAACTATCGTCTTGA GTCCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGT AACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAA GTGGTGGCCTAACTACGGCTACACTAGAAGAACAGTATTTGGTATCTGCG CTCTGCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTGATCC GGCAAACAACCACCGCTGGTAGCGGTGGTTTTTTTTGTTTGAAGCAGCA GATTACGCGCAGAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTAC GGGGTCTGACGCTCAGTGGAACGAAAACCTCACGTTAAGGGATTTTGGTCA TGAGATTATCAAAAAGGATCTTACCTAGATCCTTTTAAATTAATAAATGAAG TTTTAAATCAATCTAAAGTATATATGAGTAACTTGGTCTGACAGTTACCAA TGCTTAATCAGTGAGGCACCTATCTCAGCGATCTGTCTATTTTCGTTTCATCC ATAGTTGCCTGACTCCCCGTCGTGTAGATAACTACGATACGGGAGGGCTT ACCATCTGGCCCCAGTGCTGCAATGATACCGCGAGACCCACGCTCACCG GCTCCAGATTTATCAGCAATAAACCAGCCAGCCGGAAGGGCCGAGCGCA GAAGTGGTCCTGCAACTTTATCCGCCTCCATCCAGTCTATTAATTGTTGCC GGGAAGCTAGAGTAAGTAGTTCGCCAGTTAATAGTTTGCACAACGTTGTT GCCATTGCTACAGGCATCGTGGTGTACGCTCGTCGTTTGGTATGGCTTC ATTCAGCTCCGGTTCCCAACGATCAAGGCGAGTTACATGATCCCCCATGT

TGTGCAAAAAGCGGTTAGCTCCTTCGGTCCTCCGATCGTTGTCAGAAGT
AAGTTGGCCGCAGTGTTATCACTCATGGTTATGGCAGCACTGCATAATTCT
CTTACTGTCATGCCATCCGTAAGATGCTTTTCTGTGACTGGTGAGTACTCA
ACCAAGTCATTCTGAGAATAGTGTATGCGGCGACCGAGTTGCTCTTGCCC
GGCGTCAATACGGGATAATACCGCGCCACATAGCAGAACTTTAAAAGTGC
TCATCATTGAAAACGTTCTTCGGGGCGAAAACCTCTCAAGGATCTTACCG
CTGTTGAGATCCAGTTCGATGTAACCCACTCGTGCACCCAACTGATCTTC
AGCATCTTTTACTTTACCCAGCGTTTCTGGGTGAGCAAAAACAGGAAGGC
AAAATGCCGCAAAAAGGGAATAAGGGCGACACGGAAATGTTGAATACTC
ATACTCTTCCTTTTTCAATATTATTGAAGCATTTATCAGGGTTATTGTCTCA
TGAGCGGATACATATTTGAATGTATTTAGAAAAAT**AA**ACAAATAGGGGTTT
CGCGCACATTTCCCCGAAAAGTGCCACCTGACGTCTAAGAAACCATTATT
ATCATGACATTAACCTATAAAAATAGGCGTATCACGAGGCCCTTTCGTCTC
GCGCGTTTCGGTGATGACGGTGAAAACCTCTGACACATGCAGCTCCCGG
AGACGGTCACAGCTTGTCTGTAAGCGGATGCCGGGAGCAGACAAGCCCG
TCAGGGCGCGTCAGCGGGTGTGGCGGGTGTCCGGGCTGGCTTAACTAT
GCGGCATCAGAGCAGATTGTAAGTACTGAGAGTGCACCATATGCGGTGTGAAAT
ACCGCACAGATGCGTAAGGAGAAAATACCGCATCAGGCGCCATTCGCCA
TTCAGGCTGCGCAACTGTTGGGAAGGGCGATCGGTGCGGGCCTCTTCGC
TATTACGCCAGCTGGCGAAAGGGGGATGTGCTGCAAGGCGATTAAGTTG
GGTAACGCCAGGGTTTTCCAGTCACGACGTTGTAA

Supplementary Table 2 | Primer list for plasmids construction of delta-hemolysin and pneumolysin.

Name	Sequence 5' → 3'	Description
oPR 146	GATATACATATGGCGGCGGATAT TATTAG	Fwd. primer amplification delta-hemolysin gene fragment
oPR 147	GGATCCCCTAGGGCGCACGTGG AATAATCAGATTCTC	Rev. primer amplification of delta-hemolysin gene fragment
oPR 148	GGTCATATGGCAAACAAAGC	Fwd. primer amplification of pneumolysin gene fragment
oPR 149	TGCGGATCCTTATTAATCATTTT	Rev. primer amplification of pneumolysin gene fragment
oPR 15	CTTTGTTTGCCATATGTATATCTC C	Rev. primer for amplification of pUC18_T7 backbone fragment based on the pUC18_T7_sfGFP plasmid and Gibson assembly with the pneumolysin gene fragment
oPR 152	TAATATCCGCCGCCATATGTATAT CT	Rev. primer for amplification of pUC18_T7 backbone fragment based on the pUC18_T7_sfGFP plasmid and Gibson assembly with the delta-hemolysin gene fragment
oPR 160	TGATTAATAAGGATCCTAGCATAA C	Fwd. primer for amplification of pUC18_T7 backbone fragment based on the pUC18_T7_sfGFP plasmid and Gibson assembly with the pneumolysin gene fragment
oPR 161	CTAGGGGATCCTAGCATAAC	Fwd. primer for amplification of pUC18_T7 backbone fragment based on the pUC18_T7_sfGFP plasmid and Gibson assembly with the delta-hemolysin gene fragment
oPR 178	ATTTGATGCCTTTAATTAACCAAG CTTGCGAAATTAATACG	Fwd. primer amplification of DNA retrieved after FACS sorting of DEs
oPR 179	AACAGGAGTCCAAGACTAGTGAA TTCAAAAACCCCTCAAG	Rev. primer amplification of DNA retrieved after FACS sorting of DEs
oPR 222	ATGGGATCCCTGCACAAAGCTTA CGTG	Rev. primer amplification of cathelicidin-BF gene fragment
oPR 221	GCCCATATGAAACGCTTTAAAAAA TTTTTTAAAAAACTG	Fwd. primer amplification of cathelicidin-BF gene fragment