Supplemental material

R-type fonticins produced by *Pragia fontium* form large pores with high conductance

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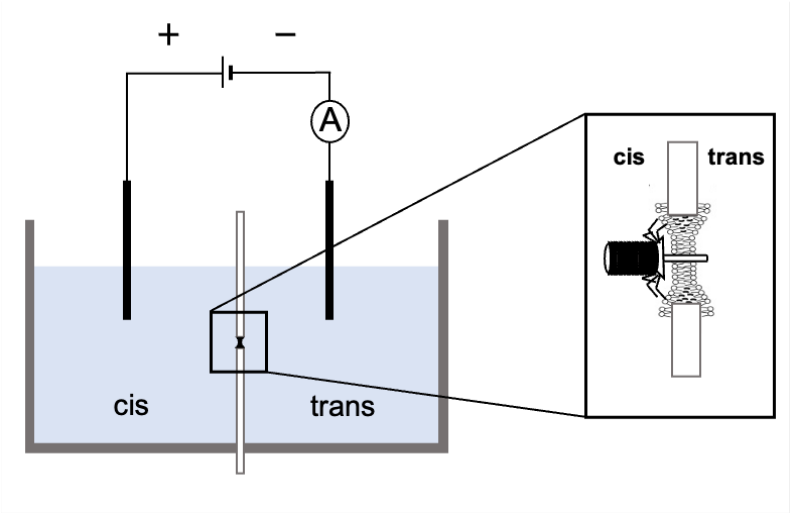
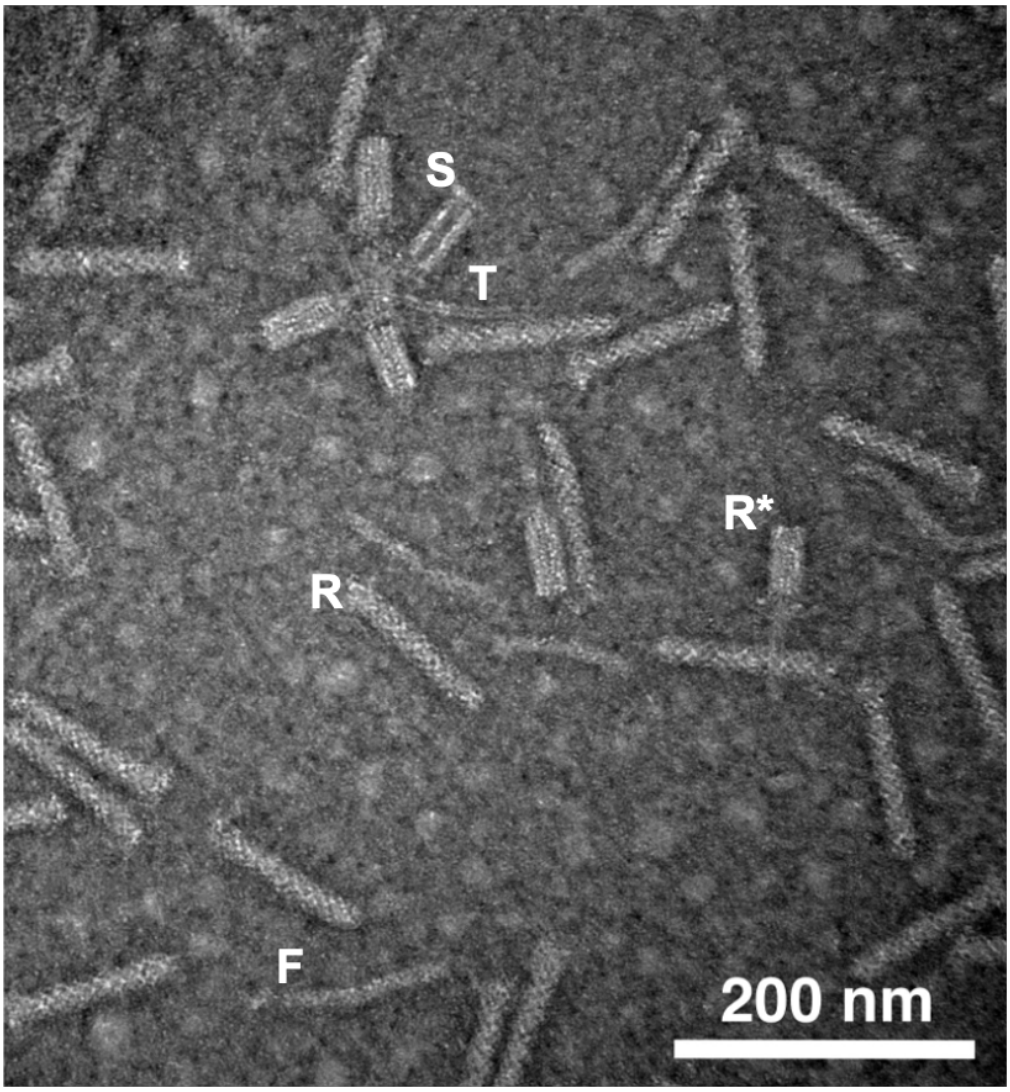
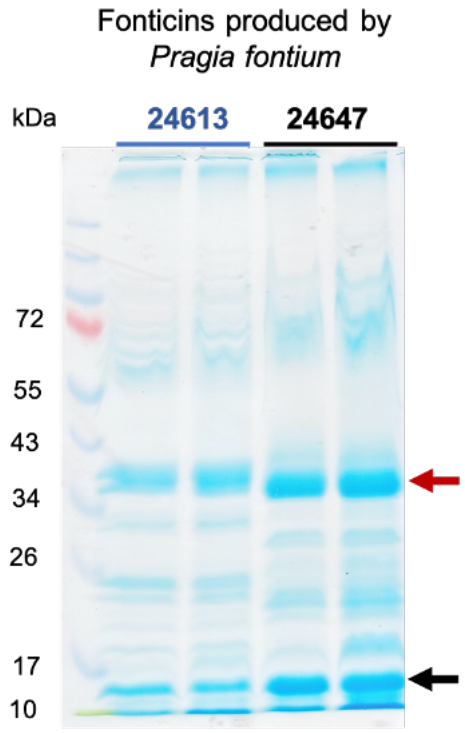


Supplemental Figure 1
Scheme of BLM setup.
The Teflon cuvette is divided into two compartments by a Teflon partition with a small aperture in the middle. Phospholipid bilayer is formed across the aperture by painting 3% *E. coli* polar lipids (Avanti Polar Lipids) dissolved in n-decane/butanol (9:1 v/v). Fonticins are added to the *cis* side of the cuvette. Thus, fonticins penetrates the bilayer with the core from the *cis* side to the *trans* side of the bilayer. Note that fonticin and the bilayer in the scheme are not to scale. The membrane potential is maintained by an external power source using Ag/AgCl electrodes and electric current is amplified with an operating amplifier.



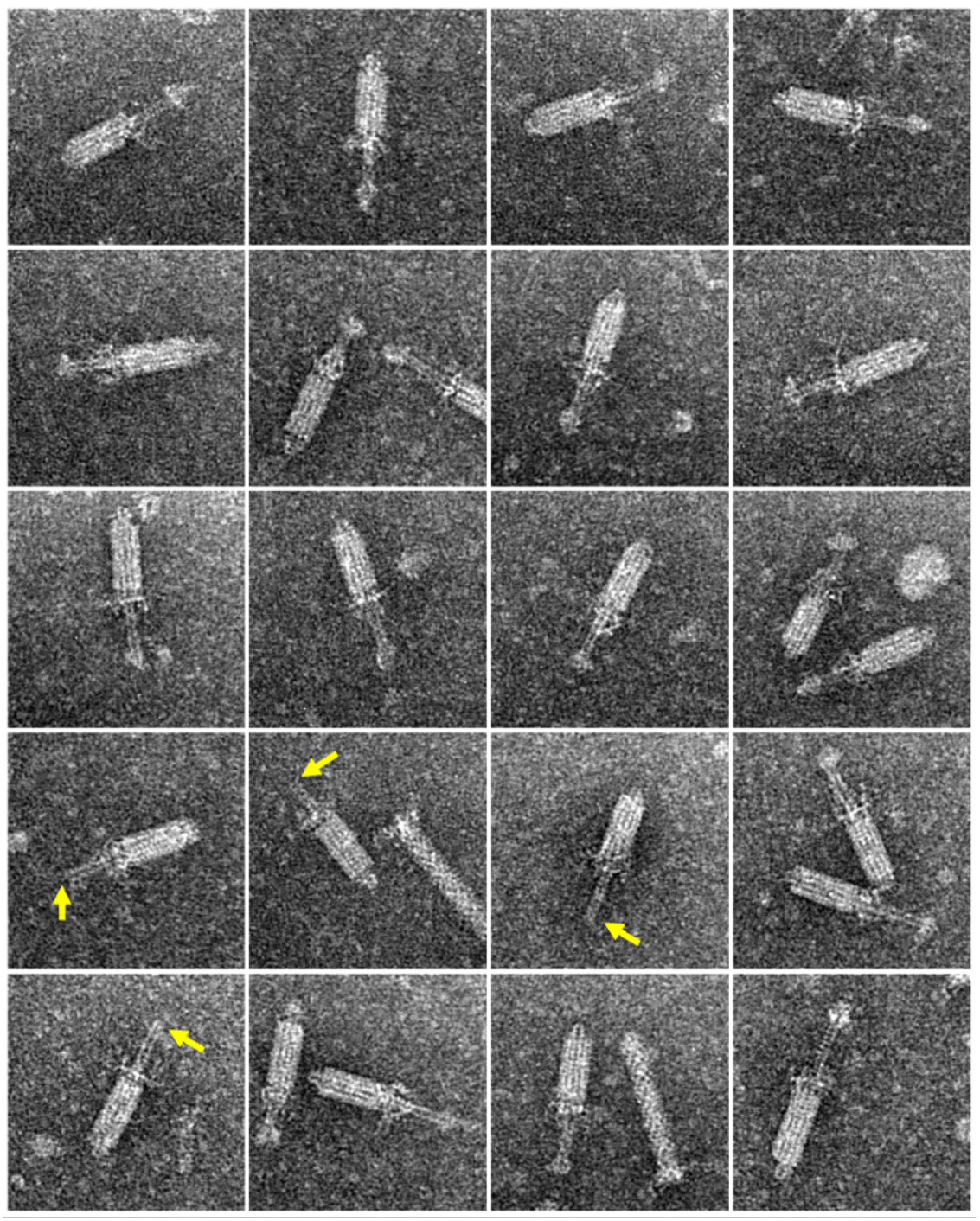
Supplemental Figure 2
Transmission electron micrograph of fonticin particles.
Noncontracted R-type fonticins (R) are the most abundant particles produced by *P. fontium* 24613. Contraction of the R-type particles is mediated mechanically so the contracted fonticins (R*) can be seen by TEM. Relatively common are also production artefacts, like disassembled sheaths (S) and tubes (T). *P. fontium* 24613 produces also F-type fonticins (F), but in a very low amount.



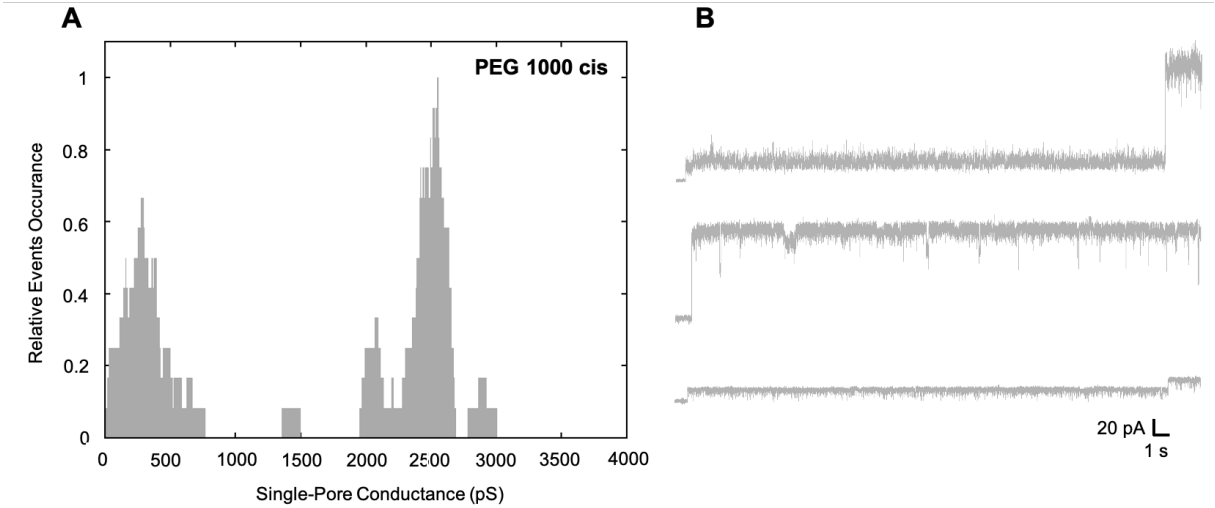
Supplemental Figure 3
SDS-PAGE of fonticins produced by *Pragia fontium* 24613 and 24647.
Protein subunits of fonticins were dissociated by boiling in a sample buffer for 5 min. Fonticins (0.5 mg/ml) were loaded on a 15% Tris-HCl SDS gel. First line is a molecular mass standard (Page Ruler prestained protein ladder, 10 – 180 kDa; Thermo Fisher). The second and the third line show fonticins produced by the strain of *P. fontium* 24613 in duplicate and the last two lines are duplicates of fonticins produced by *P. fontium* 24647. Proteins were visualised by Coomassie blue staining. In both fonticins productions two major protein bands of 38 and 15 kDa were revealed, corresponding to the sheath (red arrow) and tube (black arrow) subunits, respectively. We performed a mass spectroscopy identification that confirmed the presence of major tube protein QQ39_04825 (15.4 kDa) in both strains and predictable sheath protein QQ39_04820 (39.1 kDa) in *P. fontium* 24613. For MS analysis (as described below) the samples were prepared in triplicates as a standard fonticin preparation.
Protein Digestion: Protein samples were resuspended in 100mM TEAB containing 2% SDC. Cysteins were reduced with 10 mM final concentration of TCEP and blocked with 40 mM final concentration of chloroacetamide (60°C for 30 min). Samples were cleaved on beads with 1 µg of trypsine at 37°C overnight. After digestion samples were centrifuged and supernatants were collected and acidified with TFA to 1% final concentration. SDC was removed by extraction to ethylacetate (1). Peptides were desalted using in-house made stage tips packed with C18 disks (Empore) (2).
nLC-MS 2 Analysis: Nano Reversed phase column (EASY-Spray column, 50 cm x 75 µm ID, PepMap C18, 2 µm particles, 100 Å pore size) was used for LC/MS analysis. Mobile phase buffer A was composed of water and 0.1% formic acid. Mobile phase B was composed of acetonitrile and 0.1% formic acid. Samples were loaded onto the trap column (Acclaim PepMap300, C18, 5 µm, 300 Å Wide Pore, 300 µm x 5 mm, 5 Cartridges) for 4 min at 15 µl/min. Loading buffer was composed of water, 2% acetonitrile and 0.1% trifluoroacetic acid. Peptides were eluted with Mobile phase B gradient from 4% to 35% B in 60 min. Eluting peptide cations were converted to gas-phase ions by electrospray ionization and analysed on a Thermo Orbitrap Fusion (Q-OT- qIT, Thermo). Survey scans of peptide precursors from 350 to 1400 m/z were performed at 120K resolution (at 200 m/z) with a 5×10^5 ion count target. Tandem MS was performed by isolation at 1,5 Th with the quadrupole, HCD fragmentation with normalized collision energy of 30, and rapid scan MS analysis in the ion trap. The MS/MS

ion count target was set to 10^4 and the max injection time was 35 ms. Only those precursors with charge state 2–6 were sampled for MS/MS. The dynamic exclusion duration was set to 45 s with a 10 ppm tolerance around the selected precursor and its isotopes. Monoisotopic precursor selection was turned on. The instrument was run in top speed mode with 2 s cycles (3).

Data analysis: All data were analysed and quantified with the MaxQuant software (version 1.5.3.8) (4). The false discovery rate (FDR) was set to 1% for both proteins and peptides and we specified a minimum peptide length of seven amino acids. The Andromeda search engine was used for the MS/MS spectra search against the *Pragia fontium* database (downloaded from NCBI in June 2018, containing 12 006 entries). Enzyme specificity was set as C-terminal to Arg and Lys, also allowing cleavage at proline bonds and a maximum of two missed cleavages. Dithiomethylation of cysteine was selected as fixed modification and N-terminal protein acetylation and methionine oxidation as variable modifications. The “match between runs” feature of MaxQuant was used to transfer identifications to other LC-MS/MS runs based on their masses and retention time (maximum deviation 0.7 min) and this was also used in quantification experiments. Quantifications were performed with the label-free algorithms described recently. Data analysis was performed using Perseus 1.5.2.4 software (5).



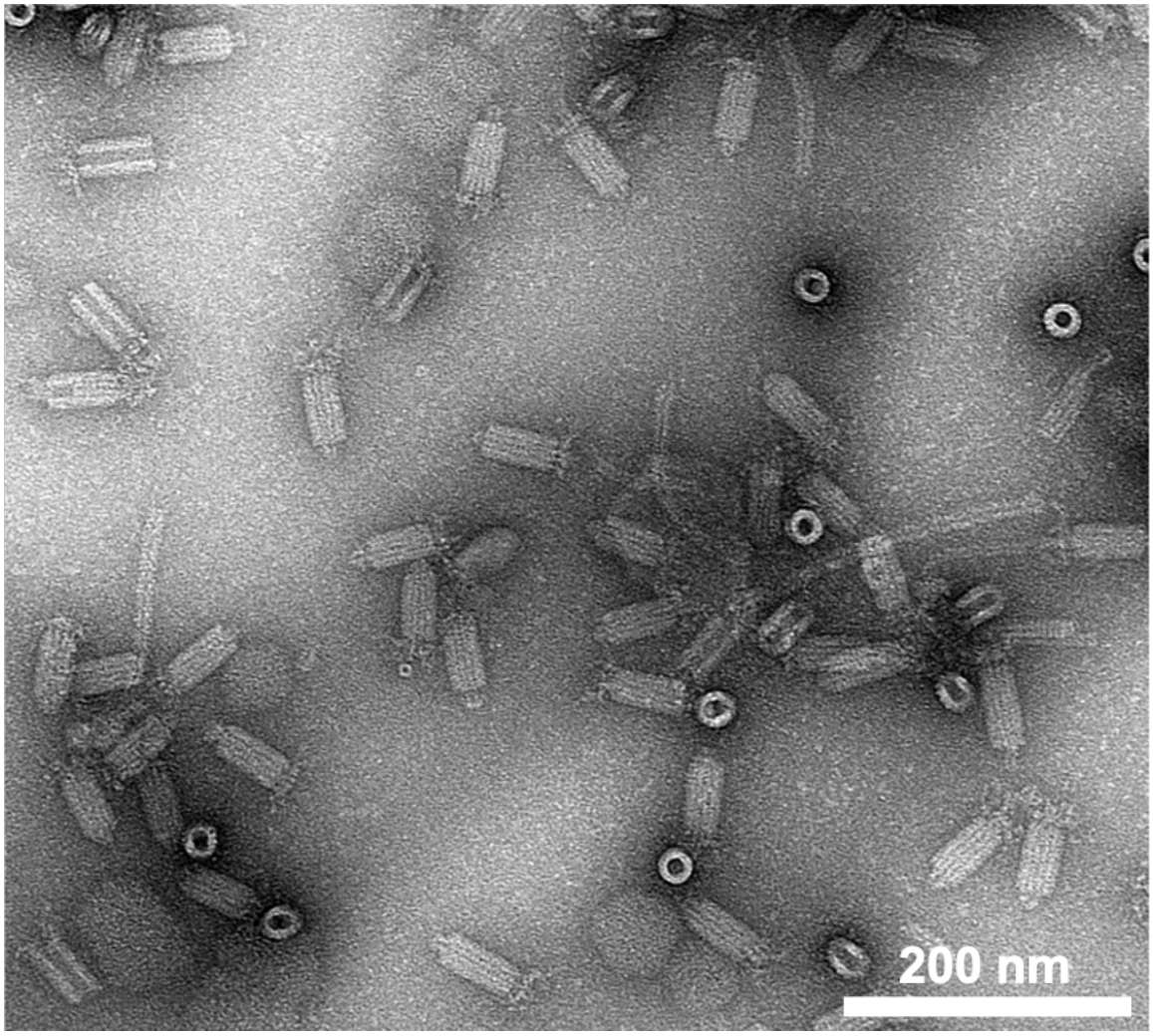
Supplemental Figure 4
Gallery of fonticin particles in the contracted conformation differing in their tube endings.
Vast majority of fonticin particles produced by *P. fontium* 24613 have the tube endings endowed with a terminal membrane piercing protein complex (or spike complex), but also particles with released spike complexes are rarely present in the sample (yellow arrows).



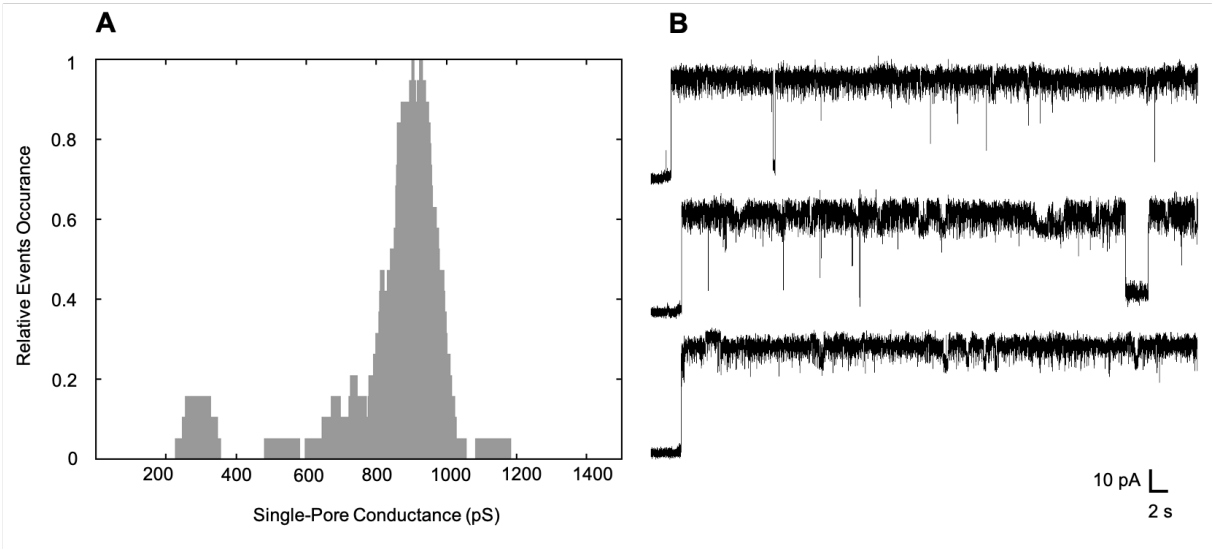
Supplemental Figure 5

Conductance levels mediated by blocking fonticin pore by PEG 1000 added to the *cis* side of the cuvette.

(A) Histogram of single-pore conductance. **(B)** Current traces of fonticin pores blocked by PEG 1000. Except for clearly blocked fonticin pores (single-pore conductance < 700 pS) the unexplained high conductance states appeared (~2.5 nS). The incubation of fonticin with PEG 1000 in the *cis* compartment possibly led to disintegration of some fonticin particles to smaller parts (possibly shorter tubes) which might exert higher conductance. The conductance histogram (n > 40) was constructed using Kernel Density Estimation (rectangular kernel with 50 pS width).



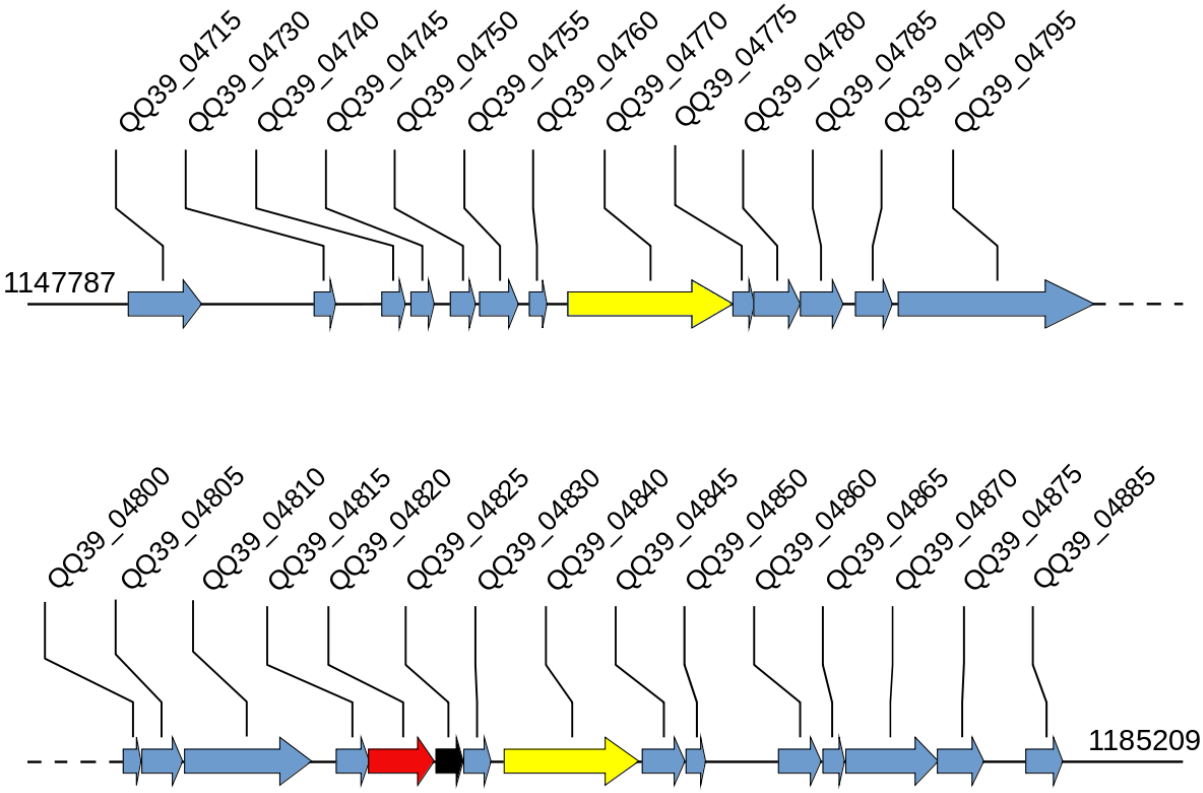
Supplemental Figure 6
Transmission electron micrograph of 25240 fonticin particles.
P. fontium 25240 produces R-type particles that are morphologically identical with 24613 and also form pores with similar conductance on BLM. However, 25240 fonticins tend more to spontaneous contraction which might explain lower BLM activity in comparison with 24613 fonticins.



Supplemental Figure 7

Pore-forming activity of fonticins produced by *Pragia fontium* 25240.

The *P. fontium* 25240 strain, which produces only R-type fonticins, was used to show that pore-forming activity of only R-type fonticins have very similar effect on BLM as fonticins produced by strain 24613 (R- and F-type). **(A)** Histogram of single-pore conductance. Measurements were performed at a constant potential of 50 mV in 1 M NaCl, 10 mM Tris, 8 mM $MgCl_2$, pH 7.3 with *E. coli* lipids. Fonticins (50 µg/ml) were added to the *cis* side of the cuvette. The mean single-pore conductance of fonticins produced by *Pragia fontium* 25240 was 904 ± 75 pS. The conductance histogram (n > 40) was constructed using Kernel Density Estimation (rectangular kernel with 50 pS width). **(B)** Representative current traces of fonticin single pores.



Supplemental Figure 8
One of the gene clusters responsible for production of fonticins of *P. fontium* 24613.
Highlighted – red: predicted sheath protein, black: major tube protein, yellow: proteins containing tape measure domain. The completely finished genome of *P. fontium* 24613 (GenBank acc. no. CP010423, (6)) contains several candidate gene clusters which could be potentially responsible for fonticin production:

i) between coordinates 1147787-1185209 (QQ39_04710-04910)
ii) 1204718-1233504 (QQ39_05000-05250)
iii) 1479070-1511795 (QQ39_06360-06650)
iv) 1515876-1528104 (QQ39_06665-06745)
v) 2901088-2922872 (QQ39_12620-12735)

Table 1
Proteins identified by MS in fonticin samples of *P. fontium* 24613 within the first gene cluster.

protein ID	locus tag	start	-	stop	function
AKJ41470.1	QQ39_04730	1152335	-	1152694	phage antitermination protein Q (hypothetical protein)
AKJ41475.1	QQ39_04755	1155032	-	1155691	phage tail protein
AKJ41478.1	QQ39_04770	1156424	-	1159072	phage tail tape measure protein (hypothetical protein)
AKJ41479.1	QQ39_04775	1159072	-	1159416	phage tail protein
AKJ41480.1	QQ39_04780	1159424	-	1160173	phage minor tail protein L
AKJ41482.1	QQ39_04790	1161064	-	1161696	phage tail assembly protein
AKJ41486.1	QQ39_04810	1165905	-	1167974	tail fiber domain-containing protein (hypothetical protein)
AKJ41488.1	QQ39_04820	1168863	-	1169945	predicted sheath protein
AKJ41489.1	QQ39_04825	1169963	-	1170400	major tube protein (DUF3383 family)
AKJ41492.1	QQ39_04840	1171105	-	1173213	tape measure domain-containing protein (hypothetical protein)
AKJ41499.1	QQ39_04885	1179188	-	1179781	tail assembly chaperone (hypothetical protein)

References

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